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Table of Contents

Acknowledgments.....	i
Table of Contents	iii
Abstract	1
Zusammenfassung	3
1 Introduction	5
1.1 Smc complexes maintain and structure the genome.	5
1.2 Cohesin's functions	6
1.3 The Cohesin complex	7
1.4 Cohesin entraps chromatin.....	8
1.5 Cohesin's roles in the cell cycle.....	9
1.6 Cohesin's ATPase activity	11
1.7 Loading complex	12
1.8 Cohesin loading onto chromatin	13
1.9 The role of Cohesin and Nipbl/Mau2 in diseases.....	15
2 Aim of the study.....	16
3 Results	17
3.1 Cohesin spontaneously loads onto plasmid DNA <i>in vitro</i>	17
3.2 Purification of the human loading complex.....	19
3.3 Recombinant loading complex is active in <i>Xenopus</i> egg extract.....	22
3.4 The loading complex stimulates Cohesin's ATPase activity.	23
3.5 Nipbl exclusively enhances Cohesin's ATPase	28
3.6 The N-terminus of Nipbl is dispensable for enhancing Cohesin's ATPase domain.	32
3.7 Nipbl-PA mutant	36
3.8 Cohesin's interaction with its loading complex	45
4 Discussion	59
4.1 Nipbl potentially interacts with all Cohesin subunits.....	60
4.2 Nipbl enhances Cohesin's ATPase activity.	63
4.3 Nipbl ^{1,183-2,804} is sufficient to stimulate Cohesin's ATPase activity <i>in vitro</i>	64
4.4 Scc1 and the addition of DNA might regulate the Nipbl-dependent stimulation of Cohesin's ATPase activity.	65

5	Material and methods.....	70
5.1	Cloning.....	70
5.2	Generation of baculoviruses.....	70
5.3	Expression of proteins in insect cells.....	71
5.4	Purifications.....	72
5.4.1	Purification of Condensin, Cohesin and Cohesin subcomplexes	72
5.4.2	Purification of His-Pds5A/B, His-SA1/2, His-Smc1, Flag-Smc1 or Flag-Nipbl	73
5.4.3	Purification of the loading complex	74
5.4.4	E.coli expression and purification of Scc1 fragments	75
5.4.5	Protein separation and analysis	76
5.5	ATPase assays	77
5.5.1	Data Analysis	78
5.6	Cohesin bulk loading assay	78
5.7	Binding assays.....	79
5.7.1	Binding to Cohesin.....	80
5.7.2	Binding to loading complex.....	80
5.8	<i>Xenopus leavis</i> egg extract	80
5.9	Cross-linking mass spectrometry	80
5.9.1	Chemical cross-linking: Mau2 in complex with Nipbl	81
5.9.2	Chemical cross-linking: Nipbl in complex with Cohesin	81
5.9.3	Chemical cross-linking: Cohesin	82
5.9.4	Reduction, Alkylation & Digestion of the cross-linked sample	82
5.9.5	Strong Cation Exchange Chromatography	82
5.9.6	Nano LC-MS Analysis	82
5.9.7	Identification of cross-linked peptides.....	83
5.10	Bioinformatics.....	84
6	References	85
7	Appendix.....	92
7.1	Cross-linking mass spectrometry	94
7.1.1	Cross-linked peptides: Mau2 in complex with Nipbl	94
7.1.2	Cross-linking mass spectrometry of Nipbl in complex with Cohesin	103
7.1.3	Cross-linking mass spectrometry of Cohesin	138
7.2	List of abbreviations.....	143

Table of Contents

7.3	List of figures	145
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Abstract

Members of the structural maintenance of chromosomes (Smc) proteins are a very well conserved protein family. There are 3 Smc complexes in humans. All of them have important roles in the cell cycle to maintain genome organization and integrity. All Smc proteins have an elongated structure and two dimerization domains far apart from each other. Thereby, and with the help of other proteins, a ring-like structure is created to entrap chromatin and thus physically link DNA double-strands together.

In my thesis, I focus on the Smc complex Cohesin. This complex fulfils diverse cellular functions such as gene expression, sister chromatid cohesion and genome organization. Despite advances in understanding Cohesins' functions on a cellular level, it remains unclear how Cohesin can associate and entrap DNA. Studies in cell systems revealed that this DNA-“loading” reaction is dependent on the Cohesin loading complex which consists of Mau2 and Nipbl, a HEAT-repeat containing protein, as well as Cohesin's intrinsic ATPase activity. Aberrant functioning of Cohesin leads to diverse diseases. Among them is the Cornelia de Lange Syndrome that is also caused by mutations in Nipbl. To better understand how Cohesin and the loading complex interact with each other, we decided to study both proteins *in vitro*.

For this, I set out to purify full length human loading complex. To learn more about the interaction of Cohesin with its loading complex, I performed protein binding assays and a mass spectrometry approach in which I made use of chemical cross-linking of nearby lysines to thereby generate a proximity map of protein-protein interactions. Our initial data suggests that Nipbl potentially interacts with all Cohesin subunits, showing no direct binding interface.

Furthermore, I made some progress in understanding some aspects of the Cohesin loading reaction. In my experiments, Nipbl stimulates Cohesin's ATPase activity, a reaction that is boosted by the addition of DNA. To understand this reaction better, we tested mutated and truncated forms of Nipbl as well as mutated Cohesin and Cohesin subcomplexes. In summary, we have some hints that Cohesin's kleisin subunit Scc1 and

Abstract

potentially DNA are interacting with the HEAT repeat domain of Nipbl which seems to be important for the Nipbl-dependent stimulation of Cohesin's ATPase activity.

Zusammenfassung

Mitglieder der Proteinfamilie mit dem Namen Structural Maintenance of Chromosome kurz Smc Proteine, sind eine evolutionär sehr stark erhaltene Gruppe von Proteinen. In humanen Zellen gibt es 3 Smc-Komplexe. Sie alle erfüllen spezifische Aufgaben in der Aufrechterhaltung von chromosomalen Strukturen. Die Smc Proteine machen sich dafür ihre langgezogene Struktur zu Nutze, die an beiden Enden durch Proteindomänen zusammengehalten werden. Dadurch und mit der Hilfe von weiteren Proteinen, wird eine Ringstruktur aufgebaut, wodurch sie in der Lage sind in ihrem Inneren Chromatinstränge zu beherbergen.

In meiner Doktorarbeit habe ich mich hauptsächlich mit dem Smc Komplex Cohesin beschäftigt. Dieser ist unter anderem wichtig für die Aufrechterhaltung der Geneexpression, den Zusammenhalt von Schwesterchromatiden und im Aufbau der Chromatinstruktur. Trotz vieler neuer Erkenntnisse auf zellulärer Ebene, weiß man wenig darüber wie Cohesin überhaupt an die DNA „geladen“ wird. Durch Versuche in Zellen ist bekannt, dass Cohesin dafür den sogenannten Ladung-Komplex, bestehend aus den Proteinen Mau2 und Nipbl, das sogenannte HEAT-Motive Proteindomänen enthält, und Cohesins intrinsische ATPase Aktivität benötigt. Sollte Cohesin nicht einwandfrei funktionieren, können Krankheiten wie z.B. das Cornelia de Lange Syndrom, dem auch Mutationen in Nipbl zu Grunde liegen, auftreten. Um das Zusammenspiel von Cohesin und seinem Ladefaktor besser zu verstehen, haben wir beschossen beide Proteinkomplexe in einem zellfreien System zu untersuchen.

Um dies zu erreichen, habe ich den menschlichen Ladungskomplex aufgereinigt. Um zu verstehen welche Protein-Protein Wechselwirkungen Cohesin mit seinem Ladekomplex hat, habe ich Bindungsversuche und chemische Cross-Linking Massenspektrometrie, eine Methode, die Lysine in räumlicher Nachbarschaft kovalent miteinander verknüpft, umso mögliche Protein-Proteininteraktionen zu entdecken, verwendet. Die vorläufigen Ergebnisse deuten darauf hin, dass alle Cohesinuntereinheiten mit Nipbl in Kontrakt stehen und kein spezifisches Bindemotiv gefunden werden konnte.

Desweiteren haben wir uns einen wichtigen Teilaspekt des Cohesinladevorgangs angeschaut. Ich habe Experimente durchgeführt, die zeigen konnten, dass Nipbl Cohesins ATPaseaktivität selektiv verstärkt. Um diese Reaktion besser zu verstehen, haben wir Experimente mit mutierten und gekürzten Versionen von Nipbl, aber auch mit mutierten Cohesinkomplexen und Subkomplexe durchgeführt. Zusammenfassend kann man sagen, dass wir vermuten, dass Cohesin's Kleisinuntereinheit, Scc1, und vermutlich DNA mit den HEAT-Motiven von Nipbl reagieren. Diese Interaktion könnte für die selektive Beschleunigung der ATP-Hydrolyse von Cohesin durch Nipbl wichtig sein.

1 Introduction

1.1 Smc complexes maintain and structure the genome.

Humans have in total 46 individual strings of DNAs that associate with proteins to form chromosomes. The chromosomes need to be structured because although the chromosomes encode for all genes, not every protein is needed at a given time. For this, some parts of the genome are accessible and serves as templates for transcription, followed by translation to produce proteins. Other parts of genomes are silenced, and gene expression is repressed. The structure of the chromosomes is not only modulated for the regulation of gene expression but also during the cell cycle. Large parts of the DNA are accessible during interphase where many genes are expressed to prepare for the cell cycle. In S-phase the DNA is replicated and sister chromatids remain connected till mitosis, where the chromatids get compacted. The compacted chromatids are then separated into two daughter cells. This process is very important because each daughter cells should end up with the correct number of chromosomes and the DNA content should not be altered. For this, the cells have established control mechanisms that can stop the cell cycle and e.g. damaged DNA can be repaired by several pathways (Sirbu and Cortez, 2013).

The structural maintenance of chromosomes (Smc) proteins act as guardians for the chromosomes. They contribute to most processes, mentioned above, and protect the structure and integrity of the DNA. The Smc proteins heterodimerize and form together with other proteins three distinct complexes in humans (Wells et al., 2017). The Cohesin complex contains Smc1 and Smc3, the Condensin complex Smc2 and Smc4, and the unnamed Smc5/6 complex Smc5 and Smc6. The Smc complexes are functional ATPases and have a ring structure that enables them to interact with DNA (Hirano, 2006). Each Smc complex has its own but also overlapping functions with other Smc complexes. Cohesin is involved in many processes such as in structuring the genome, sister chromatid cohesion, DNA recombination, DNA repair, gene regulation, in mitotic, post-mitotic but also in meiotic cells (Nasmyth and Haering, 2009, Peters et al., 2008). Condensin is a major player in chromosome condensation in mitosis, but is also implicated in gene regulation during interphase (Aragon et al., 2013). The Smc5/6 complex is the least studied Smc complex, that functions in DNA-repair (Diaz and Pecinka, 2018).

1.2 Cohesin's functions

The Cohesin complex is an important factor for cell division because of its crucial role in sister chromatid cohesion (Michaeli et al., 1997, Guacci et al., 1997). DNA is replicated in S-phase and Cohesin physically connects the two sister chromatids till they are separated in the end of mitosis (Uhlmann and Nasmyth, 1999). This gives rise to two daughter cells with the same genetic information (detailed description in chapter 1.5).

Cohesin also plays a role in DNA repair. The Cohesin complex is mainly involved in a pathway called homologous recombination to repair double strand breaks (Peters et al., 2008, Mehta et al., 2013). For this, Cohesin accumulates at sites of double strand breaks which is mediated by the Cohesin loading complex (Strom et al., 2004). There, Cohesin brings the sister chromatids in vicinity so that double strand break repair proteins can repair the break using the unscathed sister chromatid as template.

Cohesin is a regulator of gene expression in mitotic and post-mitotic cells – a process that is independent of its role in the cell cycle (Wendt et al., 2008). Cohesin's involvement in gene regulation, was first suggested by a study in *Drosophila*. There a mutation in the Cohesin loading factor, Nipped-B (homolog of human Nipbl) resulted in a deficient activation of homeobox genes that are crucial for development (Robert A. Rollins, 1999). Further evidence is provided by the Cornelia de Lange syndrome (CdLS), a congenital disorder, that is often caused by heterozygote mutations in the Nipbl gene. Cells derived from CdLS patients show normal sister chromatid cohesion but the genome-wide distribution of Cohesin is changed, leading to developmental defects due to differential gene expression pattern (Deardorff et al., 2012, Liu and Krantz, 2008). Another example is the DNA-binding protein CCCTC-binding factor (CTCF), that co-localizes with Cohesin on chromatin. CTCF and Cohesin are implicated in insulating gene expression by regulating promotor/enhancer interactions (Wendt et al., 2008). How, Cohesin regulates gene expression is not well understood but there are some evidence mostly coming from chromosome conformation capture experiments suggesting that Cohesin structures chromosomes and thereby brings distant sequences together (Schwarzer et al., 2016, van Ruiten and Rowland, 2018).

The Cohesin complex is not just active in mitosis, but also in meiosis (McNicoll et al., 2013, Nasmyth, 2001). To create a haploid cell, replicated germ cells undergo two consecutive rounds of chromosome segregation (meiosis I and II) but without having a round of DNA-replication in between. Cohesin is implicated in the separation of homologues and sister chromatids, but also in DNA repair of programmed double strand breaks and homologues recombination (Mehta et al., 2013). In meiosis, some Cohesin's core-subunits are replaced by meiosis specific subunits. The replaced subunits share sequence similarity with each other (McNicoll et al., 2013).

1.3 The Cohesin complex

The Cohesin complex consists of four core subunits which form a proteinous ring of approximately 50 nm (Gruber et al., 2003, Anderson et al., 2002, Haering et al., 2002). Cohesin contains a heterodimer of two structural maintenance of chromosomes proteins Smc1 and Smc3. Both proteins are highly elongated and dimerize at two domains that are far apart. One of those domains is the central hinge domain that is formed by folding back of the poly-peptide chain. The hinge domain is followed by a 35 nm long extended coiled-coil structure and a second dimerization domain, the head domain that is formed by the N- and C-termini (Haering et al., 2002). This domain is also called the nucleotide binding domain because together the Smc1/3 head domains form ATP-binding cassette (ABC) - like reaction centres. Both head domains are bridged by the kleisin subunit Scc1 (Gruber et al., 2003). Scc1 is mainly unstructured but its N-terminus forms a helix bundle that binds to the coiled-coil domain proximal to the head domain of Smc3 (Gligori et al., 2014, Schleiffer et al., 2003). The C-terminus of Scc1 folds into a winged helix domain and associates with the head domain of Smc1 (Haering et al., 2004). The fourth Cohesin core subunit is the SA protein, SA1 or SA2 in higher eukaryotes (Sumara et al., 2000, Losada et al., 2000). The SA protein interacts with Scc1 (Haering et al., 2002).

1.4 Cohesin entraps chromatin.

Cohesin forms a tripartite ring (Gruber et al., 2003, Haering et al., 2002). This ring structure was confirmed using biochemical approaches but also visualized by low resolution rotary shadowing electron microscopy and more recently by negative stain electron microscopy, showing an open ring and a closed, rod-shaped Cohesin, respectively (Anderson et al., 2002, Huis in 't Veld et al., 2014, Hons et al., 2016). Both techniques show that the coiled-coil domains of Cohesin are discontinuous and display kinks (Figure 1).

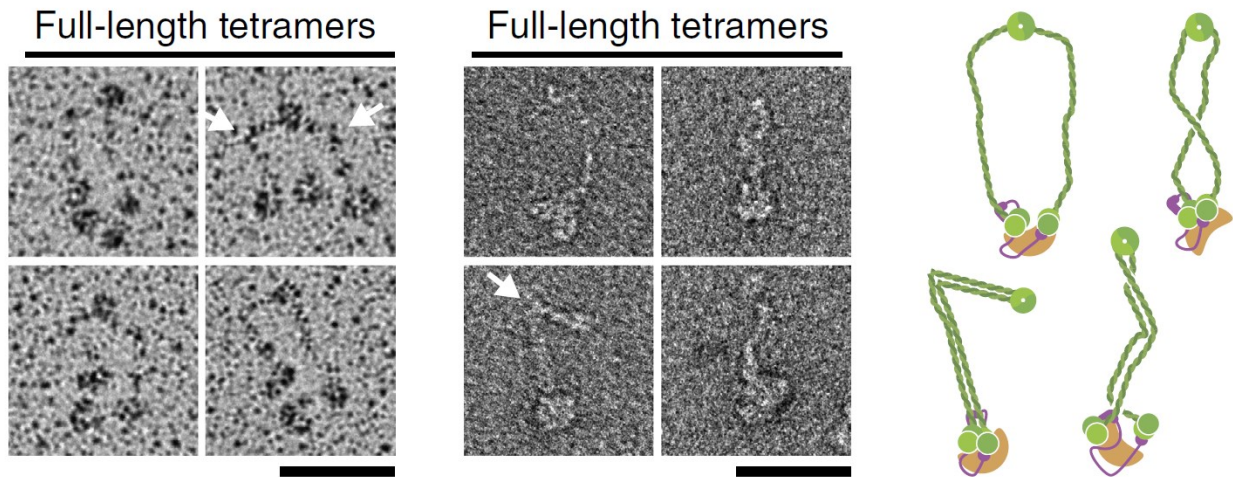


Figure 1: Recombinant Cohesin displays different conformations depending on the visualization method.

Recombinant human Cohesin shows an open ring structure using rotary shadowing electron microscopy (left panel) and a closed rod-like structure using negative stain electron microscopy (middle panel). Schematics of possible Cohesin conformations derived from both visualization methods are shown. Reproduced from Hons et al, 2016.

Cohesin interacts with chromatin presumably by topological entrapment. This mode of interaction was studied *in vivo* in *S. cerevisiae* where purified Cohesin was still bound to circular minichromosomes. This interaction was destroyed either by cleavage of the Cohesin ring or of the minichromosome (Ivanov and Nasmyth, 2005). More sophisticated experiments could show that an engineered Cohesin complex in which the Smc3:Smc1, Smc3:Scc1 and Smc1:Scc1 interfaces were all covalently linked would still hold replicated minichromosomes together even under denaturing conditions (Haering et al., 2008). A topological Cohesin:DNA interaction that was sensitive to either cleavage of the Cohesin ring or plasmid DNA was also shown *in vitro* using purified fission yeast proteins

(Murayama and Uhlmann, 2014). Those experiments are consistent with the co-called “ring-model” where Cohesin is entrapping one or more strands of chromatin. However, there are also alternative models (Huang et al., 2005, Nasmyth, 2011). It is still unclear if the Cohesin:chromatin interaction is established by multiple Cohesin complexes or a single Cohesin complex (Weitzer et al., 2003, Gruber et al., 2003, Haering et al., 2002).

1.5 Cohesin’s roles in the cell cycle

Cohesin fulfils many different functions in the cell cycle – most likely by entrapment of either one or two chromatin strands inside its ring-structure (Ivanov and Nasmyth, 2005, Haering et al., 2008). Cohesin’s association with chromatin is highly regulated and fine-tuned. In interphase, there are two populations of Cohesin on chromatin, one population with a residence time of seconds and another of minutes (Gerlich et al., 2006, Ladurner et al., 2014). The dynamic way of Cohesin’s interaction with chromatin is mediated by its loading and unloading factors. Cohesin loading onto chromatin is facilitated by the loading complex (Ciosk et al., 2000). Cohesin’s dissociation in turn is facilitated by the unloading factors Pds5 and Wapl (Tedeschi et al., 2013, Eichinger et al., 2013, Kueng et al., 2006). Humans express two Pds5 isoforms: Pds5A and B, both interact with Cohesin (Sumara et al., 2000, Losada et al., 2005). Presumably, the Cohesin ring stays intact while it is unloaded and loaded from chromatin. To achieve this, Cohesin interfaces need to transiently open and close. While the hinge domain is implicated in the loading process, the Smc3:Scc1N interface is implicated in unloading (Gruber et al., 2006, Huis in 't Veld et al., 2014, Eichinger et al., 2013). This suggests that Cohesin has a separate entry and exit gate that are controlled by different regulatory proteins. The dynamic behaviour of Cohesin changes completely during S-phase where a subpopulation of Cohesin is stably bound to chromatin to hold sister chromatids together most likely by entrapment of two chromatin fibres (Uhlmann and Nasmyth, 1999). In humans, cohesive Cohesin is created upon binding of Sororin to the unloading factors Wapl-Pds5 and thereby displacing Wapl from opening the Cohesin exit gate (Nishiyama et al., 2010). In addition to Sororin, two conserved lysines in Smc3 (105/106) are acetylated by Esco1 (Ben-Shahar et al., 2008, Zhang et al., 2008). In yeast cells, acetylation is sufficient to stabilize Cohesin on chromatin. In human mitotic cells, the stable bound Cohesin is removed in two steps. In prophase, the bulk of Cohesin first dissociates from the chromosome arms in the “prophase pathway” leaving

behind Cohesin that still connects the sister centromeres (Waizenegger et al., 2000). The prophase pathway leads to the characteristic X-shape of the mitotic chromosomes and the removed Cohesin remains in an intact ring-form. The residual Cohesin stays till all sister chromatids are aligned at the metaphase plate. Then, Cohesin's subunit Scc1 is cleaved by the protease Separase, in a highly controlled manner (Uhlmann et al., 1999, Uhlmann et al., 2000). This frees the chromatids and they are pulled towards the two opposing cell poles, giving rise to two daughter cells with identical genetic material (Figure 2).

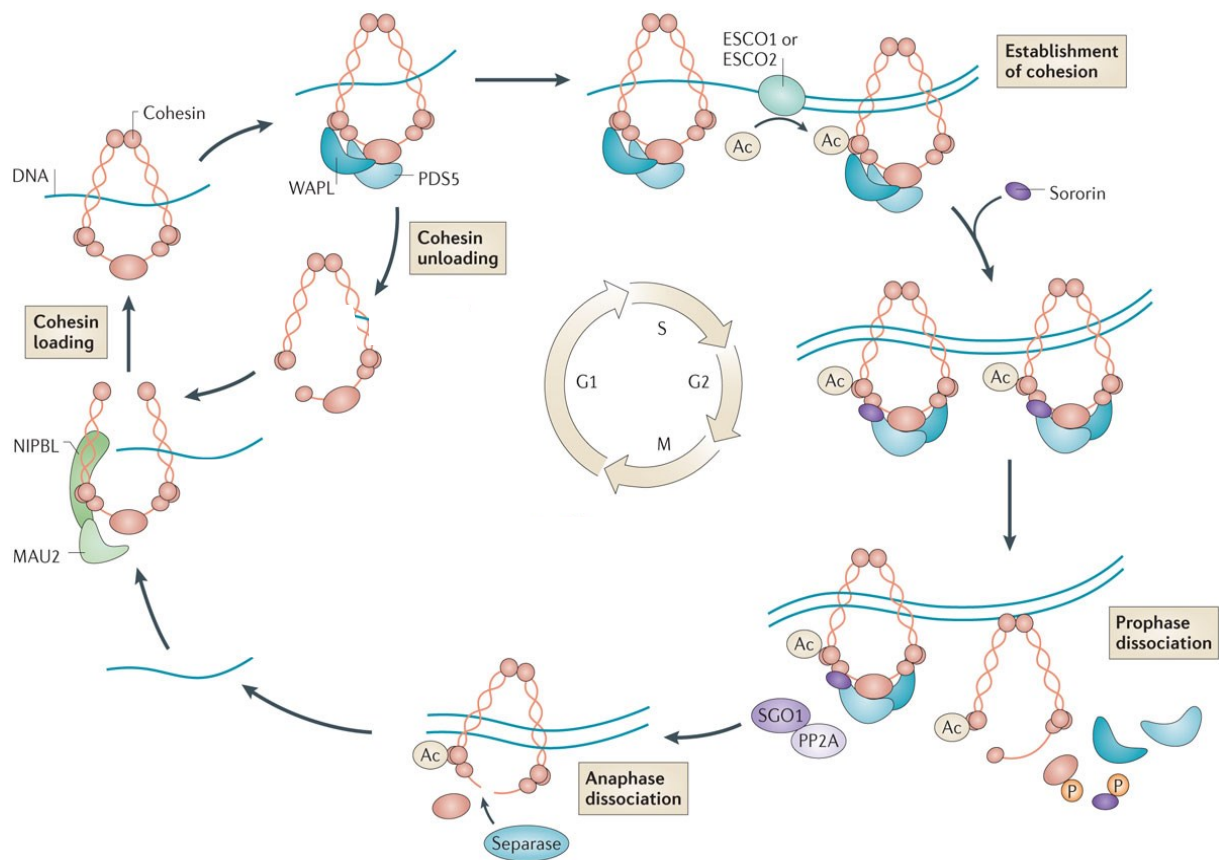


Figure 2: Cohesin during the cell cycle.

Cohesin association and dissociation on chromatin is mediated by the loading complex or Wapl-Pds5, respectively. In S-phase, sister chromatid cohesion is established by acetylation of Cohesin and binding of Sororin to the release factors Pds5-Wapl. The bulk of Cohesin is removed in prophase and the residual Cohesin is cleaved in anaphase prior to cell division (adapted from Losada, 2014).

1.6 Cohesin's ATPase activity

Cohesin is an ATPase. The catalytical sites are formed by the interaction of its Smc1 and Smc3 subunits. The N- and C-termini of each Smc protein forms a head domain. Upon ATP-binding, the head domain of Smc1 dimerizes with the one of Smc3. This creates two active sites for ATP hydrolysis. Like other ABC-like ATPase, the catalytic domain of Cohesin has a 2-fold symmetry, meaning that if one Smc head domain binds ATP using their walker A and walker B motif, then the other Smc head contacts the ATP with their signature motif or vice versa (Figure 3) (Arumugam et al., 2003). For this reason, two ATP molecules are bound. Biochemical experiments using purified *S. cerevisiae* Smc1/3 head domains only or full length Smc1/3, implied that the binding of the two ATP molecules happens in a cooperative manner, while hydrolysis does not (Arumugam et al., 2006).

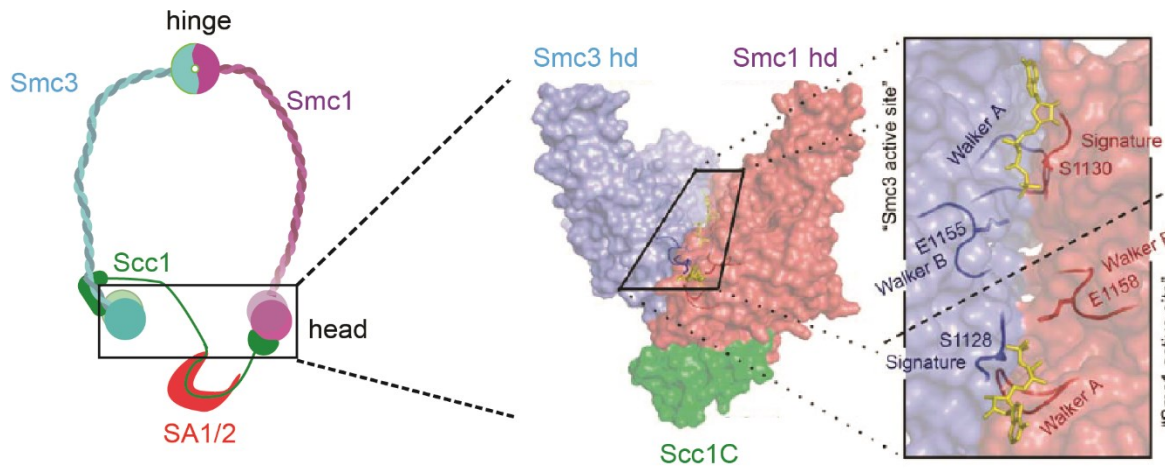


Figure 3: The head domains of Cohesin's Smc1/3 subunits form 2 active sites for ATP hydrolysis.

Schematic of the Cohesin core-subunits: Smc1/3, Scc1 and SA1/2. Smc1/3 are elongated proteins that dimerize at their hinge and head domain. Scc1 connects the Smc1/3 heads by interacting with the coiled-coil region emerging from Smc3's head domain and the head domain of Smc1. The SA protein binds to Scc1. The model shows that the Smc1/3 heads need to be connected to form two active sites for ATP hydrolysis. Figure adapted from Arumugam et al., 2006.

The ATPase activity of the Smc1/3 dimer is enhanced by full length Scc1, but also by the addition of a C-terminal fragment of Scc1 (Arumugam et al., 2006). The ATPase activity of the fission yeast, *S. pombe*, Cohesin trimer Psm1^{Smc1}/Psm3^{Smc3} in complex with Rad21^{Scc1} is slightly enhanced by the addition of Psc3^{SA}. Experiments in *S. cerevisiae* have shown that Cohesin complexes that are deficient in ATP hydrolysis bind transiently to chromatin, while stable binding depends on the ATPase activity (Arumugam et al.,

2003, Hu et al., 2011). *In vivo* experiments clearly showed that the loading complex facilitates Cohesin's association with chromatin and that this depends on catalytically active Cohesin (Arumugam et al., 2003, Weitzer et al., 2003, Ciosk et al., 2000). How the loading complex mediates the Cohesin:chromatin interaction is poorly understood. Interestingly, recent *in vitro* experiments using fission yeast proteins showed that Cohesin's ATPase activity was strongly stimulated by its Cohesin loading complex in the presence of plasmid DNA. Furthermore, it was shown that the loading complex enhanced Cohesin's association with plasmid DNA in a bulk Cohesin loading assay (Murayama and Uhlmann, 2014). These experiments confirmed a link between Cohesin's ATPase activity and the loading complex in the loading reaction *in vitro*.

1.7 Loading complex

The Cohesin loading complex consists of two proteins Nipbl and Mau2, orthologs of *S. cerevisiae* Scc2 and Scc4, respectively (Ciosk et al., 2000). Human Nipbl is a large protein with over 316 kDa and exists in two isoforms. This thesis focuses on isoform A which is the longer version with 2,804 aa. Isoform B (2,697aa) is a splice variant with the main difference of a missing C-terminus of Nipbl^{2,698-2,804}. Nipbl is a highly conserved protein and orthologs have been identified in a wide range of organisms. Structure predictions suggests that Nipbl contains multiple Huntingtin, Elongation factor 3 (EF3), Protein Phosphatase (PP2A), and the yeast PI3-kinase IOR1 (HEAT) - repeat motifs made of α -helices (Bermudez et al., 2012). HEAT-repeats are often involved in protein-protein interactions. In contrast to Nipbl, Mau2 (67 kDa, 652 aa) orthologs are highly divergent, but share a common protein fold consisting of α -helices that form tetratripeptide repeats (TPRs) (Watrin et al., 2006). Immunoprecipitation (IP) experiments in *Xenopus* egg extract as well as structural studies using yeast proteins showed that Scc4/Mau2 binds to the N-terminus of Nipbl and that this binding persists throughout the cell cycle (Takahashi et al., 2008, Ciosk et al., 2000, Hinshaw et al., 2015, Chao et al., 2015). The loading complex Nipbl/Mau2 can be found on chromatin or in the nucleoplasm, depending on the cell cycle state (Watrin et al., 2006). In *Xenopus* egg extracts, the loading complex dissociates from chromatin in mitosis (Takahashi et al., 2004). *In vivo*, chromatin association of fission yeast Mis4^{Nipbl}/Ssl3^{Mau2} and the loading of Cohesin onto chromosomes depends on the presence of Mau2. In contrast to this, *in vitro* Mau2 is dispensable for the Cohesin loading

reaction in a bulk loading assay, showing that Mis4^{Nipbl} alone can facilitate this reaction (Chao et al., 2015). This goes in line with the notion that purified fission yeast Nipbl binds to double-stranded DNA (dsDNA), in a sequence independent manner and is sufficient to enhance Cohesin's ATPase activity (Murayama and Uhlmann, 2014). Nevertheless, Mau2 is clearly important for the Cohesin:DNA interaction *in vivo* (Watrin et al., 2006). It has been speculated that Mau2 might serve as a chromatin adapter that opens the chromatin rather than being involved in the actual loading process (Chao et al., 2015). Alternatively, Mau2 binding might be needed for the stability of Nipbl, as it might protect Nipbl from aggregation but also seems to be important to localize Cohesin loading to the centromere as has been shown for budding yeast Cohesin (Hinshaw et al., 2015, Woodman et al., 2013).

1.8 Cohesin loading onto chromatin

The Cohesin loading complex Nipbl/Mau2 mediates Cohesin's association with chromatin (Ciosk et al., 2000). The timing of Cohesin loading varies with species. In humans, Cohesin loading onto chromosomes starts already in telophase (Shintomi and Hirano, 2009). In *Xenopus* egg extracts, Cohesin loading is linked to licensing of DNA replication in S-phase (Takahashi et al., 2004). In most organisms, Cohesin can also be loaded in multiple cell cycle phases, but sister chromatin cohesion is only established during S-phase (Takahashi et al., 2008, Uhlmann and Nasmyth, 1999).

Cohesin associates with chromatin by topological entrapment. Presumably, the loading complex loads Cohesin onto chromatin, a reaction that depends on Cohesin's ATPase activity. Cohesin and the loading complex do not co-localize in the genome. Therefore, it was thought that their interaction is very transient (Hu et al., 2011). This notion is also supported by immunoprecipitation experiments from human and yeast cell extracts in which the loading complex rarely bound to Cohesin (Watrin et al., 2006, Arumugam et al., 2003). Therefore, it was not known how the loading complex would contact Cohesin. *In vitro* experiments suggested that human Nipbl interacts with the Cohesin Smc1/3 dimer while peptide array assays using fission yeast proteins suggested that Mis4^{Nipbl}/Ssl3^{Mau2} might interact with multiple Cohesin subunit including the Rad21^{Scc1} and Psc3^{SA} subunit (Murayama and Uhlmann, 2014, Bermudez et al., 2012). Still a Cohesin binding site was not identified and it is also not known how Nipbl stimulates Cohesin's ATPase activity in

detail. A study in fission yeast has shown that the loading complex stimulates Cohesin's ATPase activity and that this facilitated Cohesin's association with plasmid *in vitro*. However, the mechanism on which Cohesin is loaded onto DNA *in vitro* and on chromatin *in vivo* is poorly understood. Presumably Cohesin complexes are already assembled before they associate with chromatin. To entrap DNA, pre-assembled Cohesin rings need to open transiently one interface to allow chromatin to be embraced. The head domains of Smc1/3 are dimerizing in an ATP-dependent and dynamic manner and it was thought that this motion might be important for entrapment of DNA. But interestingly, artificially fusing Cohesin's Smc3:Scc1 or Scc1:Smc1 interfaces showed no loading defects *in vivo* (Gruber et al., 2006). Unexpectedly, Cohesin was not loaded properly anymore by artificially tethering the Smc1/3 hinge domain together (Gruber et al., 2006). This was surprising because the hinge interaction is thought to be very stable in contrast to the dynamic motions of the Smc1/3 head domain. More indications that the hinge domain might be important for the DNA:Cohesin interaction came from a study in *S. cerevisiae*, where Cohesin with mutations that neutralize the positive charged hinge interface still associated with chromatin, but was no longer able to establish cohesion in S-phase (Kurze et al., 2011). Both studies, indicated that the hinge domain might be the Cohesin entry gate. So, if the hinge domain would be opened to allow DNA entry by stimulation Cohesin's ATPase activity in the head domain, then it could be that the ATPase reaction somehow transduce a signal through the coiled-coil domain and thereby open the Cohesin hinge, so that DNA can enter the ring (Nasmyth, 2011). It is also conservable that Cohesin folds back on itself and brings the hinge in proximity to the heads domains where the loading complex could act on both the same time (Gruber et al., 2006).

1.9 The role of Cohesin and Nipbl/Mau2 in diseases

Mutations of Cohesin are linked to multiple human diseases. Cohesion defects in meiosis can give rise to aneuploidy which mostly results in miscarriage due to lethality. However, there are some exceptions such as trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), and trisomy 13 (Patau syndrome) (Hunt, 2001, Peters et al., 2008). Advanced maternal age is associated with a higher risk of giving birth to children with chromosomal abnormalities. This phenomenon correlates with the loss of Cohesin in oocytes and is often referred as the maternal age effect (Jessberger, 2012).

There is accumulating evidence suggesting a role of Cohesin in some cancer types such as Ewing Sarcoma, glioblastoma, urothelial carcinoma, acute megakaryoblastic leukaemia, acute myeloid leukaemia (Solomon et al., 2014, Losada, 2014). Interestingly, the Cohesin core subunit SA2 is among the 12 most prominent hits in genes mutated in cancer (Brennan et al., 2013). Some cancer studies implied that sister chromatid segregation defects and chromosome instability, while others suggested Cohesin's role in gene expression as being the cause of the diseases (Losada, 2014, Solomon et al., 2014). However, how Cohesin malfunction contributes to the development of cancer is currently not well understood.

There is another spectrum of diseases called "Cohesinopathies" which are derived from germline mutations in Cohesin's core subunits or regulatory proteins, namely the Warsaw Breakage syndrome, Robert Syndrome and Cornelia de Lange syndrome (Peters et al., 2008). The Cornelia de Lange Syndrome (CdLS) is a severe disorder showing facial malformations, limb defects, growth and cognitive retardation. This syndrome is especially interesting because in approximately 60% of all cases Nipbl is heterozygotic mutated. These mutations in Nipbl are ranging from frame shifts, large deletions, insertions and miss sense mutations but can also be caused by mutations in Smc1A, Scc1, and Hdac8 (histone and Cohesin deacetylase) and rarely in Smc3 (Mannini et al., 2013). CdLS is not linked to cohesion defects but is presumably caused by an altered distribution of Cohesin on chromosomes that causes gene expression misregulation. The altered gene expression pattern are especially problematic during development (Liu and Krantz, 2008, Deardorff et al., 2012).

2 Aim of the study

Cohesin is a fascinating protein complex that is involved in diverse cellular processes. If Cohesin or its regulatory subunits are dysregulated or mutated, it can lead to a several severe diseases. Therefore, it is crucial to study Cohesin's function and regulation mechanisms to then be able to understand disease much better. Through elegant studies it became clear that Cohesin forms a tripartite ring that embraces chromatin. The loading of Cohesin onto chromatin is mediated by the Cohesin loading complex in an ATP-dependent reaction. However, the loading reaction itself is poorly understood. Very little is known about how the loading complex interacts with Cohesin in the first place. Cohesin is a large protein complex with an inner diameter of 40 nm (Haering et al., 2002). The two interfaces that are implicated in the loading reaction are far apart, namely its head domains that hydrolyse ATP and the putative entry gate which might be formed by the hinge domain (Arumugam et al., 2003, Gruber et al., 2006). Previous studies showed that the human loading complex interacts with the Cohesin Smc1/3 dimer (Bermudez et al., 2012). A peptide array assay probing for *S. pombe* Cohesin:loading complex interactions, suggested that there are multiple interactions surfaces all over Cohesin (Murayama and Uhlmann, 2014). Both results are interesting, but a direct binding surface is not identified yet.

The aims of this study were to obtain insights into the physical interaction of Cohesin subunits with its loading complex subunits and to understand the relationship between the structure and function *in vitro*. For this, I set out to generate recombinant human loading complex Nipbl in complex with Mau2. Through binding assays in combination with cross-linking mass spectrometry, a method to detect putative protein-protein interaction with high precision, I aimed to find potential Cohesin:Nipbl/Mau2 binding sites. I also aimed to reconstitute the stimulatory effect of Nipbl on Cohesin's ATPase activity in the presence of DNA to understand the regulation of this process that is linked to Cohesin loading better. For this, I generated and tested truncated and mutated forms of Nipbl as well as used Cohesin mutants and Cohesin subcomplexes. Lastly, I aimed to get a better understanding in the interplay of Cohesin, DNA and the Loading complex.

3 Results

3.1 Cohesin spontaneously loads onto plasmid DNA *in vitro*.

Cohesin fulfils multiple roles in the cell cycle. To perform those functions, Cohesin must interact with chromatin, most likely by topological entrapment of the DNA. It was recently shown that purified fission yeast Cohesin entraps plasmid DNA in an *in vitro* loading assay (Murayama and Uhlmann, 2014). Importantly, the reaction was facilitated by the loading complex and ATP-dependent. Establishing such an assay would be great tool to study Cohesin at a molecular level as this is still poorly understood.

At that time, the loading complex could not be purified yet. However, I wanted to test whether recombinant human Cohesin interacted spontaneously with DNA in a topological manner. For this, I adapted the *in vitro* loading assay that had been established in the Uhlmann lab. Cohesin was incubated with circular, nicked plasmid DNA in the presence of ATP for 1 h at 32 °C, prior to a Cohesin pull down using Scc1-antibody presenting beads. To enrich for topological associated Cohesin, high salt washes were used to eliminate unspecific Cohesin-DNA interactions. Cohesin was peptide eluted and then degraded by ProteinaseK. The recovered DNA was analyzed by agarose gel electrophoresis and stained with GelRed. In this assay, Cohesin bound 1 - 10 % of the DNA (Figure 4). The amount of pulled down DNA was dependent on the elution method: most DNA could be recovered if the Scc1-antibody beads were directly treated with ProteinaseK (Figure 4 E). This elution method consumed a large quantity of self-made Scc1 antibody. To reuse the beads, I first eluted with Scc1 peptide and added ProteinaseK, subsequently.

To confirm the observed DNA:Cohesin interaction was topological, I tested whether Cohesin would also pull-down the same plasmid but in a linear form. Interestingly, Cohesin bound almost exclusively to the circular plasmid, even when the amounts of linearized plasmid were increased (Figure 4 A, B). *In vivo*, the Cohesin ring structure is destroyed in the end of mitosis by enzymatic cleavage of its subunit Scc1 by the protease Separase. This is a very important process, to ensure that all chromosomes are ready to be divided into two daughter cells. To mimic this reaction, a recombinant Cohesin that contains an

Results

engineered tobacco etch virus (TEV) protease recognition site in its Scc1 subunit was used (Huis in 't Veld et al., 2014). I performed a loading assay comparing wild type Cohesin to a Cohesin in which Scc1 subunit had been pre-cleaved during purification. And indeed, DNA was only recovered if the Cohesin ring was intact (Figure 4 C, D).

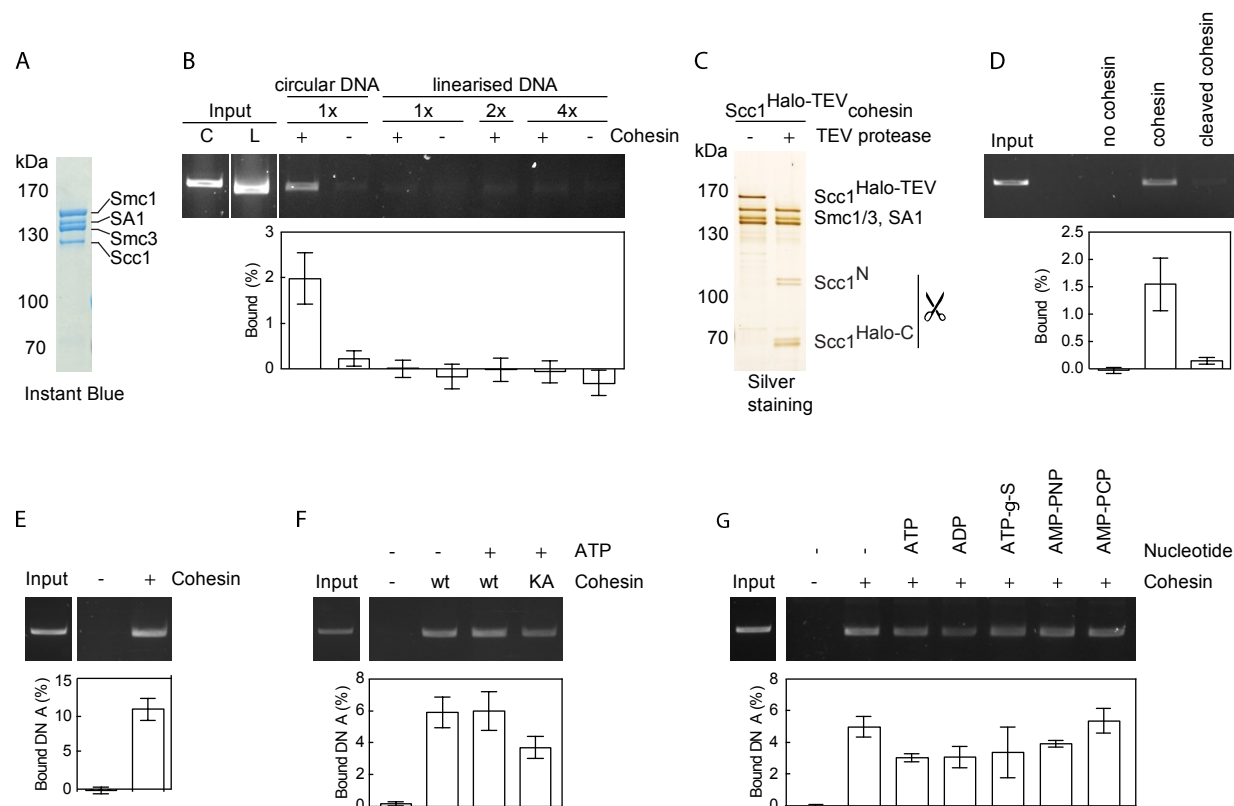


Figure 4: Spontaneous loading of Cohesin onto plasmid DNA *in vitro* is topological and independent of Cohesin's ATPase activity.

(A) Tetrameric Cohesin complex analyzed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS- PAGE) and Instant Blue staining (B) *In vitro* Cohesin loading assay testing different DNA substrates. Cohesin shown in (A) was incubated with nicked, circular plasmid or with 1 x, 2 x or 4 x linearized plasmid DNA in the presence of ATP, then immunoprecipitated with Scc1-antibody presenting beads. Cohesin and any associated DNA was eluted with a Scc1 peptide followed by protein digestion using ProteinaseK. The recovered DNA was separated by agarose gel electrophoresis and stained with GelRed. 7 % of the input DNA is shown. Values represent the mean $\pm$ SEM for 3 independent experiments. (C) Silver stained SDS PAGE of non-cleaved and TEV protease cleaved Scc1^{TEV-Halo} Cohesin used in experiment (D) is shown. (D) *In vitro* loading assay of non-cleaved and cleaved Scc1^{TEV-Halo} Cohesin. Both protein complexes were incubated with nicked circular plasmid and ATP. 4 % of the input DNA is shown. n=4 (E) *In vitro* loading assay of Cohesin WT. GelRed stained agarose gel of recovered DNA, after directly eluting the DNA with ProteinaseK, skipping the Scc1 peptide elution. n=3 (F) Loading reaction of Cohesin composed of Smc1/3 wild type (WT) or K39A (KA), an ATP binding deficient form of Cohesin in the presence or absence of ATP. (n=3) (G) Cohesin loading assay of WT Cohesin in the absence or presence of ATP and various nucleotide analogs (n=3). Figure adapted from Davidson et al, 2016.

These experiments implied that Cohesin spontaneously interacted with DNA in a topological manner. *In vivo*, not only the loading complex but also Cohesin's ATPase activity are important for Cohesin to associate with chromatin (Ladurner et al., 2014, Arumugam et al., 2003). Therefore, I tested whether the presence of ATP and Cohesin's ATPase activity were required for this spontaneous loading. I addressed this by adding ATP, derivatives of ATP or no ATP to the Cohesin loading assay. The reactions were not influenced by ATP, although some ATP analogs had a small effect (Figure 4 F). In addition, I tested a Cohesin complex harbouring K39A mutations in both Smc heads which abolished ATP-binding. This complex bound less DNA in comparison to wild type Cohesin (Figure 4 G).

Taken together, Cohesin loaded inefficiently, but in a topological manner onto plasmid DNA. This reaction was independent on the loading complex and seemed to be ATP-independent. These results were published in Davidson et al, 2016. Complementary data from this study showed that fluorescently labelled Cohesin loaded spontaneously onto lambda DNA in a total internal reflection (TIRF) microscope setup where Cohesin's dynamical behaviour was observed. Consistent with my own experiments, the reaction was ATP- independent and not mediated by the loading complex but sensitive to cleavage of either the Cohesin ring or the DNA.

3.2 Purification of the human loading complex.

In vitro Cohesin loaded spontaneously onto plasmid DNA – most-likely in a topological manner (chapter 3.1). However, in cells, Cohesin's association with chromatin is mediated by the Cohesin loading complex Nipbl/Mau2, in a process that involves Cohesin's ATPase activity. To reconstitute Cohesin's function in a more physiological way, I set out to purify the human loading complex Nipbl/Mau2.

The loading complex consists of Nipbl, a large protein with a molecular mass of 316 kDa, and Mau2 with 67 kDa. The large size of the loading complex made recombinant protein production challenging. For this, I chose the insect cells expression system because of its high expression levels in comparison to other mammalian expression systems. In addition, insect cells have eukaryotic chaperones and post-translational modification systems that facilitate folding of proteins (Nettleship et al., 2010). This system also allows the

generation of mutant complexes. Expression vectors for sole or co-expression of Nipbl and Mau2 were generated. Both proteins were tagged with various affinity-tags at their amino-termini for purification. **Table 1** lists all tagged variations of Nipbl and Mau2 that had been tested for purification.

Table 1: Various forms of tagged Nipbl and Mau2 alone or in complex.

Nipbl	Mau2	Co-expression
Flag-Nipbl	Mau2	Flag-Nipbl, His-Mau2
His-Nipbl	Flag-Mau2	His-Nipbl, Flag-Mau2
Flag2-his-Nipbl	His-Mau2	GST-Nipbl, His-Mau2
Flag-Halo-Nipbl	Flag2-his-Mau2	Flag-Halo-Nipbl, Mau2
Flag-GFP-Nipbl	His-Halo-Mau2	Flag-GFP-Nipbl, Mau2

Affinity purification of Flag- or His-Nipbl enriched for full length Nipbl (Figure 5 A, B, C). The preparations showed a high degree of unrelated proteins and Nipbl degradation products. The degradation products had been especially obvious, once Flag-tetramethyl-rhodamine (TMR)-Halo-Nipbl, that could be excited by UV light, was pulled down with M2-Flag beads and analyzed by SDS-PAGE and UV excitation (Figure 5 C). Mau2 was co-purified, but seemed to be sub-stoichiometric. This was most-likely due to C-terminal degradation of Nipbl as Mau2 interacts with the amino-terminus of Nipbl. A two-step affinity purification using first an amino-terminal Flag- epitope on Nipbl and then Ni-NTA beads for His-Mau2 binding (Figure 5 B) were performed but did not improve the purification. To enrich for full length Nipbl and enhance the overall sample purity, Nipbl was simultaneously tagged at its N- and C-termini (Table 2).

Table 2: In insect cells expressed, N- and C-terminally tagged Nipbl alone or in complex with Mau2.

Nipbl	Nipbl, Mau2
Twin-strep-Nipbl-his	Twin-strep-Nipbl-his, Mau2
Twin-strep-flag-Nipbl-his	Twin-strep-flag-Nipbl-his, Mau2
Flag-Halo-Nipbl-his	Flag-Halo-Nipbl-his, Mau2

This tagging strategy resulted in preparations with increased sample purity and highly enriched for either full length Nipbl alone or in complex with stoichiometric amounts of

Results

untagged Mau2 (Figure 5 D). The identity of Nipbl and Mau2 were confirmed by mass spectrometry (Figure 22 B) and western blot analysis (Figure 6 A). Size exclusion chromatography confirmed folded Nipbl alone and in complex with Mau2 (data not shown). Furthermore, gel filtration of His/Flag-Mau2 only preparations showed aggregated proteins.

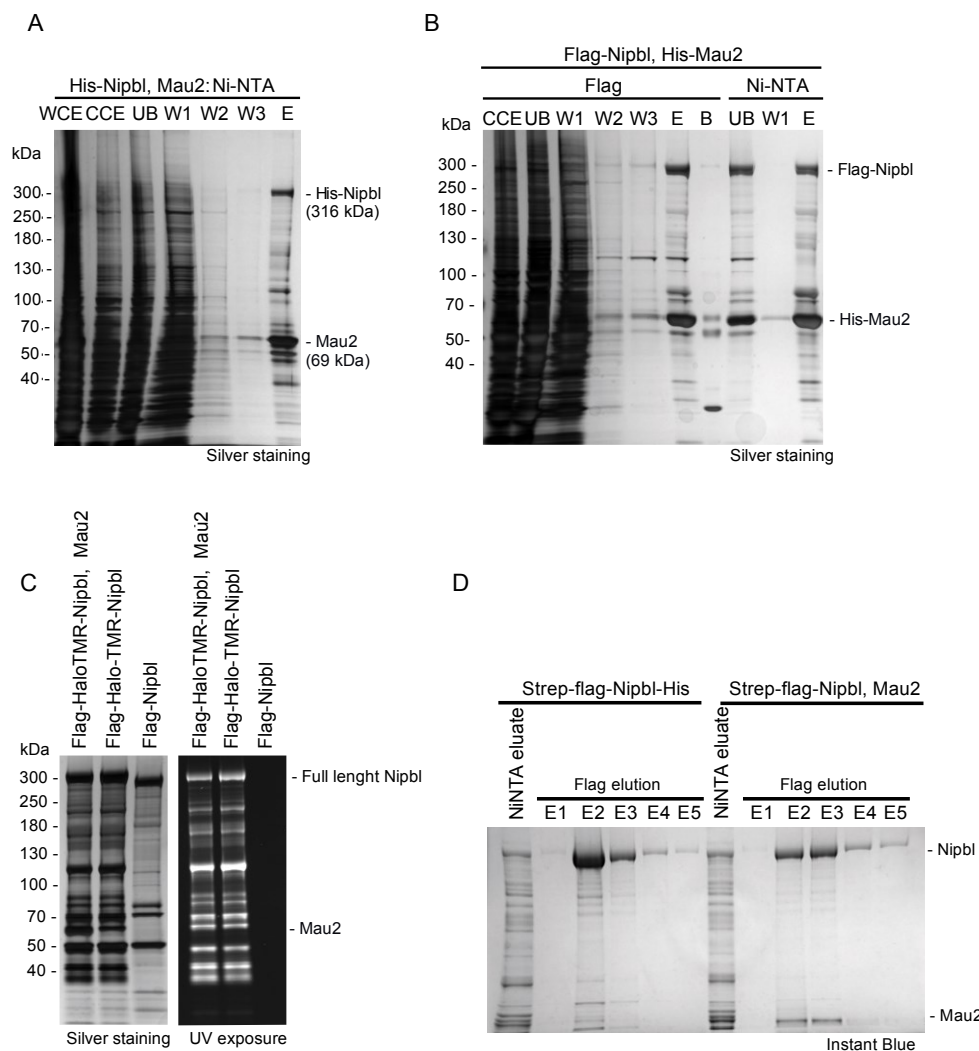


Figure 5: Preparations of full length Nipbl alone or in complex with Mau2.

(A) Silver stained SDS PAGE to analyze a Ni-NTA pull-down of His-Nipbl co-expressed with Mau2. Shown are whole cell extract after cell lysis (WCE), crude cell extract (CCE) after clearing the extract, unbound protein (UB) after incubation of CCE with Ni-beads, 3 consecutive washing steps (W1-3) and elution with imidazole (E) (B) Silver stained SDS PAGE to analyze preparations of affinity purified Flag-Nipbl co-expressed with His-Mau2 using Flag-M2-beads followed by Ni-NTA beads (C) UV exposed (to visualize the Halo-tag) and silver stained SDS-PAGE gel of a Flag-M2 pull-down of various forms of Flag-tagged Nipbls. (D) Instant Blue stained SDS PAGE gel of fractions from the sequential two step purification of Twin-strep-flag-Nipbl-his either alone or co-expressed with Mau2. Eluted fraction after initial purification using Ni-NTA beads, followed by a second step using Flag-M2-beads (E1-E5).

First a meiotic *Xenopus* egg extract, arrested in G1-phase, was prepared (Gillespie et al., 2012). The extract was either mock-depleted or depleted of the *Xenopus* loading complex using anti-Nipbl-antibody-beads. Then, S-phase and thereby Cohesin recruitment to chromatin, was initiated by the addition of *Xenopus* sperm chromatin and CaCl_2 . Chromatin isolation after 90 min followed by a western blot analysis detecting Nipbl/Mau2 and Cohesin core-subunits showed that Cohesin loading was abolished in extract depleted of the loading complex while adding-back recombinant human Nipbl/Mau2 restored Cohesin loading. Mock depletion had no effect on Cohesin levels on chromatin. Interestingly, the add-back of recombinant Nipbl alone mediated Cohesin loading onto chromatin as well (Figure 6). This showed that the purified recombinant Nipbl alone or in complex with Mau2 was functional in *Xenopus* egg extract system.

The *S. pombe* loading complex, $\text{Mis4}^{\text{Nipbl}}/\text{Ssl3}^{\text{Mau2}}$, stimulated topological Cohesin loading onto plasmid DNA *in vitro* (Murayama and Uhlmann, 2014). In chapter 3.1, we observed that Cohesin spontaneously loaded onto DNA in a bulk assay (Figure 4) or in a TIRF microscopy set up (Davidson et al., 2016). To test, whether the human recombinant loading complex stimulated Cohesin loading onto plasmid DNA, I added the loading complex to the Cohesin bulk loading assay. Indeed, I observed a stimulatory effect of Cohesin, in the presence of ATP. This effect was reproduced seven times, but then this activity was no longer detected in further assays. Despite testing many parameters, the reason for this change was not determined (data not shown).

3.4 The loading complex stimulates Cohesin's ATPase activity.

Studying how Cohesin interacts with its loading complex, will help to better understand its function in cells. As described previously, the purified loading complex was functional in *Xenopus* egg extract, and initially stimulated Cohesin loading onto plasmid DNA *in vitro* in the presence of ATP. It was recently shown using fission yeast proteins that Nipbl stimulated Cohesin's ATPase activity *in vitro* (Murayama and Uhlmann, 2014) but it's not clear how this reaction is regulated.

Results

To study the influence of the human loading complex on Cohesin's ATPase activity, I used thin layer chromatograph (TLC) based ATPase assays that had been previously established in the lab (Ladurner et al., 2014). ATP assays showed that a purified and frozen tetrameric Cohesin-SA1 complex hydrolyzed 1.77 ± 0.6 ATP/min at 32 °C. This reaction was stimulated 4-fold in the presence of Nipbl and was further enhanced 2.3-fold by the addition of λ DNA. The presence of λ DNA alone had no effect on Cohesin's ATPase activity (Figure 7).

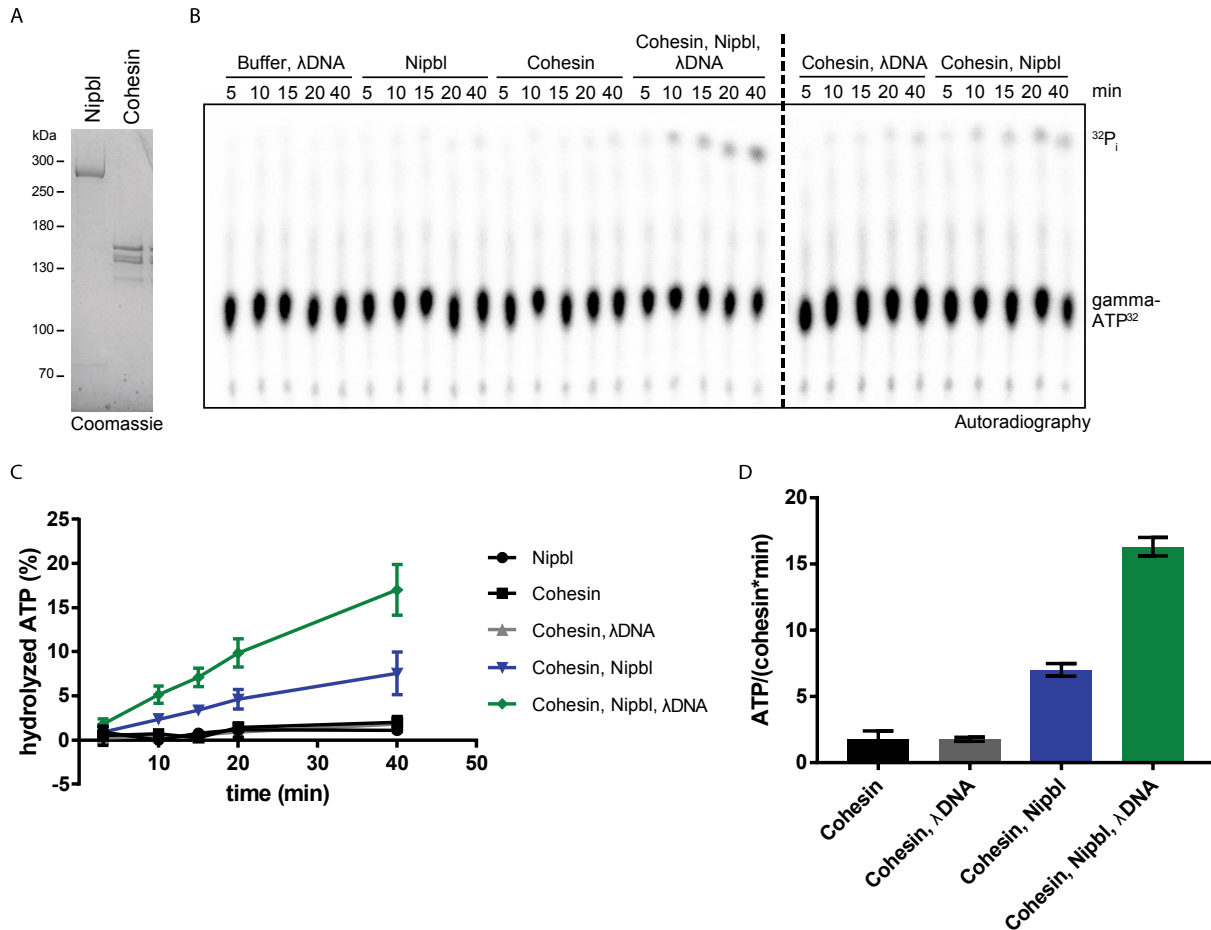


Figure 7: Nipbl stimulates Cohesin's ATPase activity – a reaction boosted by the presence of DNA.

(A) Coomassie stained SDS PAGE analysis of the preparations of purified Cohesin and Nipbl used in (B + C). (B) Representative thin layer chromatograph used to separate hydrolyzed $\gamma^{32}\text{P}_i$ from radioactive ATP. ATPase assay showing hydrolysis stimulated by various combinations of proteins and λ DNA indicated in the figure. (C) Quantification of ATPase assays shown in (B) (D) Bar graph showing Cohesin's rate of hydrolysis in the presence of either DNA, Nipbl or both. Values represent the mean $\pm$ SD for 3 experiments.

Results

To test whether the stimulatory effect of Nipbl is due to Cohesin's ATPase activity, I compared wild type Cohesin with two "ATPase dead" Cohesin mutants. Cohesin has two active sites for ATP hydrolysis and mutations in both the walker A motifs (Smc1/3 K39A) or in the walker B motifs (Smc1 E1157Q and Smc3 E1144Q) have been shown to abolish ATP-binding or hydrolysis, respectively (Arumugam et al., 2003). Indeed, a direct comparison between wild type and the walker A/B mutated Cohesin showed that the stimulatory effect of Nipbl was dependent on catalytically active Cohesin complexes (Figure 8).

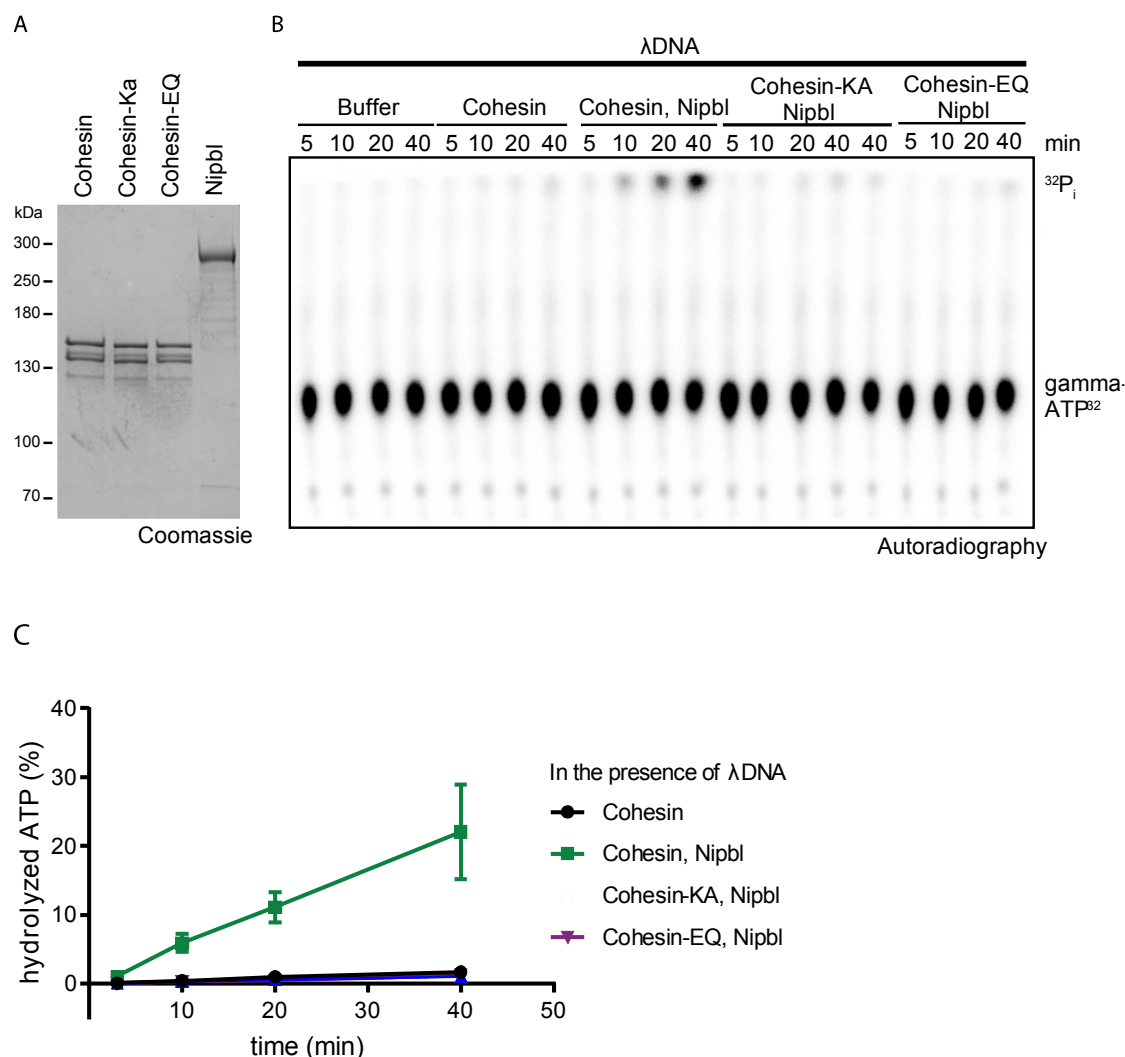


Figure 8: Nipbl's stimulation of Cohesin's ATPase activity depends on catalytically active forms of Smc1 and Smc3.

(A) Coomassie stained SDS PAGE of purified preparations of Nipbl and Cohesin composed of either wild type or ATPase "dead" forms (KA and EQ) of Smc1 and Smc3. (B) Representative TLC comparing Nipbl's ability to stimulate the ATPase activity of Cohesin WT, KA and EQ in the presence of λ DNA. (C) Quantification of experiment shown in (B) and two additional experiments. Values represent the mean ± SD of three independent experiments.

Results

To characterize the stimulatory effect of Nipbl better, I decided to test whether the ionic strength of the buffer had an influence on this reaction. Nipbl¹¹⁸³⁻²⁸⁰⁴, a Nipbl fragment described in more detail in chapter 3.6, was incubated with Cohesin and λ DNA in buffers with increasing salt concentrations ranging from 50 mM to 500 mM NaCl. Cohesin showed the strongest activity at 50 mM and 100 mM NaCl. This activity decreased 6-fold at 150 mM NaCl and even more at 200 mM NaCl. There was no influence of Nipbl and no ATP hydrolysis at all detected at 250 mM and 500 mM NaCl, respectively (Figure 9 A). Nipbl influenced Cohesin's ATP activity at ionic strengths ranging from 50 mM – 150 mM, irrespective if NaCl or KCl was present in the buffer (Figure 9 B). KCl, compared to NaCl, enhanced the reaction slightly more strongly at 50 mM and 100 mM. Note that full length Halo-Nipbl/Mau2 had been used to compare KCl with NaCl and that this gave a different stimulation pattern than using Nipbl¹¹⁸³⁻²⁸⁰⁴. 50 mM salt stimulated the reaction with full length Nipbl/Mau2 best while the activity dropped almost to the same level at 100 mM and 150 mM salt, in contrast to the experiment with Nipbl¹¹⁸³⁻²⁸⁰⁴ (Figure 9 A). The difference might be explained by the nature of Nipbl that was used to enhance the reaction and not by the presence of Mau2 as it seemed to be dispensable for Nipbl's stimulatory effect (Figure 22 D, E).

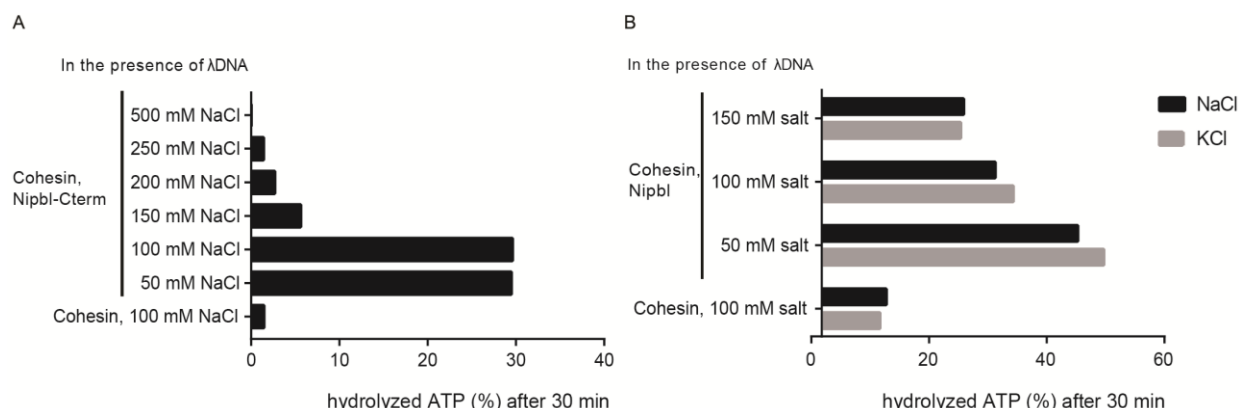


Figure 9: Nipbl's stimulation of Cohesin's ATPase activity is salt dependent.

ATPase assays testing the dependency of ionic strength on the stimulatory effect of Nipbl to Cohesin's ATPase activity in the presence of DNA. (A) ATPase assay testing increasing salt concentrations ranging from 50 mM to 500 mM NaCl. The reactions had been carried out with Nipbl¹¹⁸³⁻²⁸⁰⁴, called Nipbl-Cterm. (B) ATPase assay of Cohesin- Scc1^{TEV-Halo-TMR} and Halo-Nipbl/Mau2 comparing buffers containing 50 mM, 100 mM and 150 mM NaCl or KCl.

Results

To investigate what Nipbl to Cohesin ratio enhanced Cohesin's ATPase activity most strongly, I used a fixed concentration of 100 nM Cohesin and titrated Nipbl ranging from 50 nM to 5 μ M. These experiments had been performed with Nipbl^{1,182-2,804} / Nipbl-Cterm (chapter 3.6) because of its high yields after purification, in contrast to full length Nipbl. Titrating the Nipbl concentration showed that even sub-stoichiometric amounts of Nipbl enhanced the ATPase activity of Cohesin. The velocity of the reaction peaked at a 1:1 protein ratio. Enhancing Nipbls concentration further decreased the reaction again (Figure 10 A). The presence of λ DNA and Nipbl together stimulated Cohesin's ATPase activity more than 9-fold (Figure 7). Substituting λ DNA with equal amounts a 3.3 kbp plasmid either in a linearized or nicked form resulted in a similar stimulatory effect. Super-coiled plasmid enhanced Cohesin's ATPase activity slightly better than these other forms (Figure 10 B).

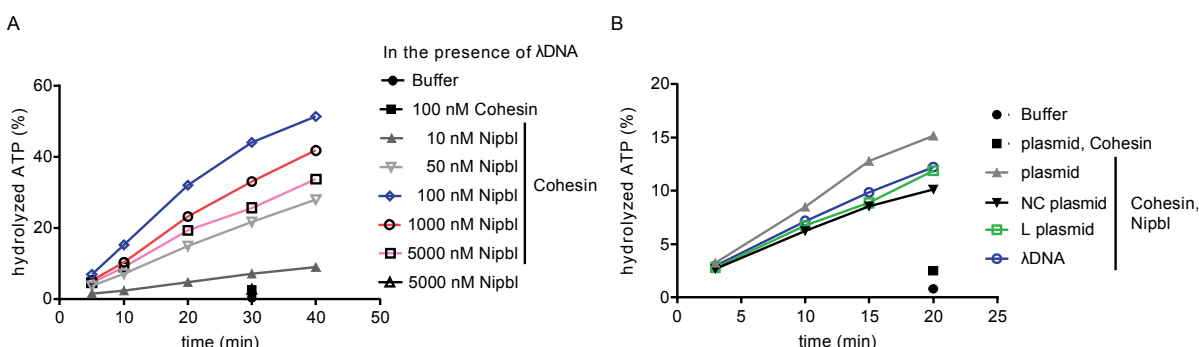


Figure 10: A 1:1 stoichiometric ratio of Cohesin to Nipbl results in the highest stimulation of ATPase activity. Stimulation by DNA is independent of its source.

(A) ATPase assay of Cohesin and Nipbl^{1,183-2,804} / Nipbl-Cterm in the presence of λ DNA. Reactions were carried out with various concentrations of Nipbl, ranging from 10 – 5,000 nM. (B) ATPase assay of Cohesin Scc1^{TEV}-Halo-TMR and Halo-Nipbl/Mau2 comparing the effect of various types of DNA. The same plasmid was used to generate nicked (NC), linearized (L) and supercoiled forms (plasmid).

Taken together, the salt concentration was crucial for the reaction and both Nipbl and DNA enhanced Cohesin's ATPase activity ranging from 50 - 150 mM salt. Titration experiments suggested that the reaction velocity peaked at a 1:1 ratio of Nipbl:Cohesin. The addition of various forms of dsDNA had little effect on the reaction. For this, I performed ATPase experiments at 100 mM salt using a stoichiometric protein ratio of 100 nM Cohesin:Nipbl in the presence of λ DNA.

3.5 Nipbl exclusively enhances Cohesin's ATPase

Cohesin interacts with three HEAT-repeat containing proteins. One of them is the SA protein (SA1/2 in mitotic cells) that binds to Scc1 and is considered as a core-subunit (Hara et al., 2014). The function of the SA protein is currently not well understood. Nipbl and Pds5A/B are regulatory subunits of Cohesin. Nipbl mediates Cohesin's loading onto chromatin in a ATP dependent reaction while Pds5A/B appears to act as an unloading factor. Recent crystal structures from Nipbl, Pds5A/B and SA2 showed that all three proteins have an overall similar hook-like structure, partially formed by the HEAT-repeat domain (Figure 11 A) (Lee et al., 2016, Hara et al., 2014, Chao et al., 2017, Kikuchi et al., 2016). Due to the structural similarity of Nipbl and Pds5 and as they are both implicated in Cohesin's association with chromatin, I decided to compare how Pds5 and Nipbl interacted with Cohesin *in vitro*. For this, I expressed His-Pds5A and Pds5A-His in Sf9 insect cells and purified them by performing a Ni-NTA pull-down.

An analytical SDS-PAGE gel stained with Coomassie showed a successful purification and western blot analysis confirmed the identity of the proteins by using specific antibodies against Pds5A (Figure 11 B). I examined the association of Pds5A, Nipbl/Mau2 and additionally Wapl with Cohesin. For this, Cohesin-Smc3-flag was bound to M2-Flag beads and then incubated with Pds5A, Wapl or Nipbl/Mau2. Analysis of the FLAG peptide eluted proteins by SDS PAGE, followed by silver staining or western blotting showed that Cohesin formed complexes with Pds5A, Wapl and Nipbl-Mau2 (Figure 11 C, D). To test, if Pds5A interacted in a similar way as Nipbl with Cohesin, I compared both proteins in ATPase assays. Pds5A did not enhance Cohesin's ATPase activity in the presence of DNA, in contrast to Nipbl. Interestingly, adding Nipbl and Pds5A in equal amounts to the ATPase reactions reduced Nipbl's stimulating effect on Cohesin's ATPase to 85 % of the original velocity. Increasing the Pds5A concentration 10 x slowed down the reaction to 68 % (Figure 11 E, F). Although, Nipbl and Pds5A both formed complexes with Cohesin, Nipbl solely stimulated its ATPase activity *in vitro*.

Results

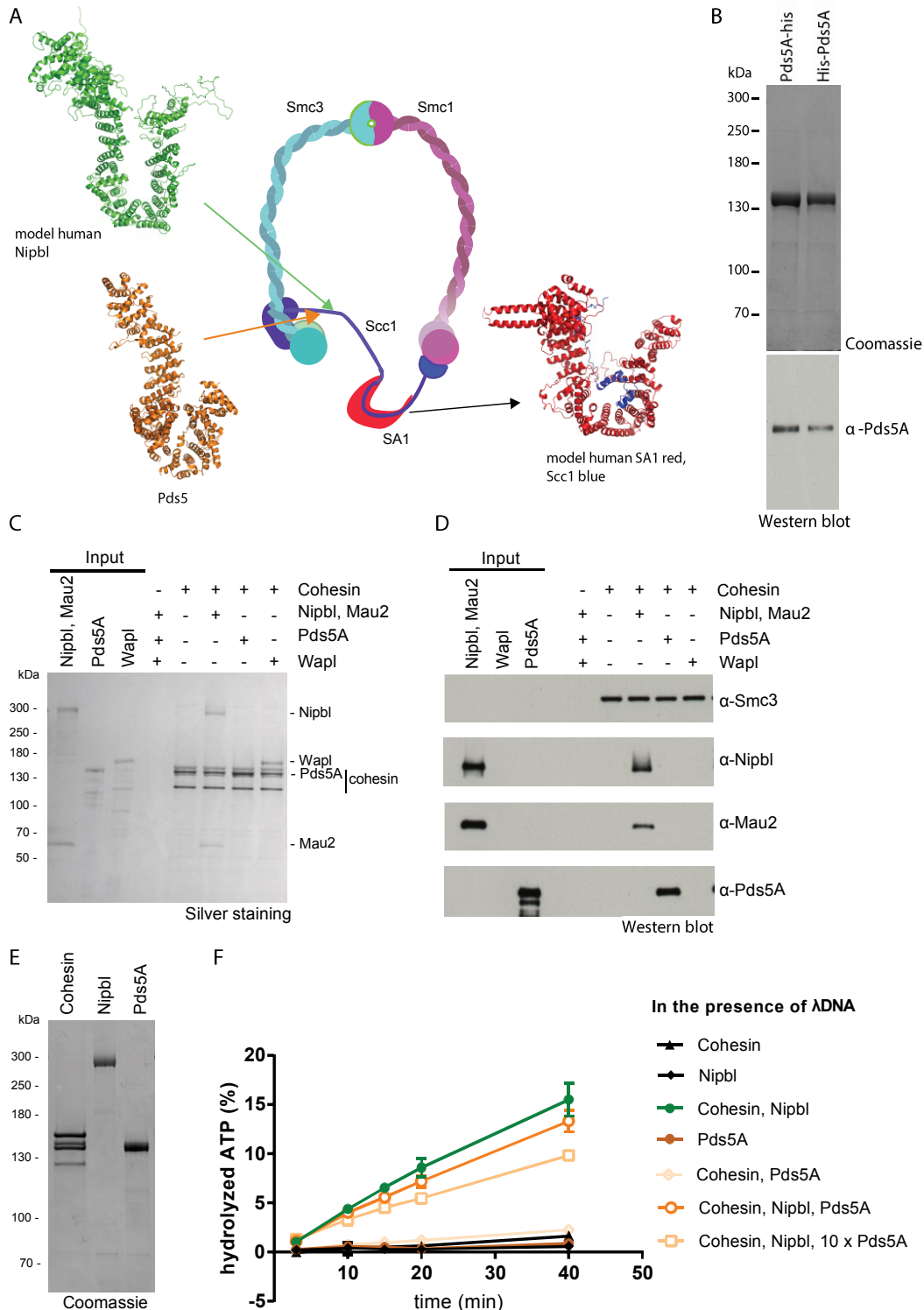


Figure 11: Pds5A interacts with Cohesin but does not boost its ATPase activity.

(A) Schematic of human Cohesin and cartoons of three HEAT-repeat domain containing Scc1-binding proteins: *Lachancea thermotolerans* Pds5 (orange, PDB: 5F0N), a model of human Nipbl (green, based on PDB: 5T8V), model of human SA1 (red) bound to Scc1 (blue) (based on PDB: 4PK7, modelling by Alexander Schleiffer) (B) Analytical Coomassie stained SDS PAGE of N- and C-terminally tagged purified preparations. Western blot analysis was used to confirm the identity of preparations as human Pds5A. >>

>> 12: Pds5A interacts with Cohesin but does not boost its ATPase activity.

(C) Binding assay testing Cohesin's ability to interact with His-Wapl, His-Pds5A and Twin-strep-flag-Nipbl-His/ Mau2 complex. Cohesin was immobilized on Flag-M2-Beads and incubated with the various recombinant protein samples. The immobilized proteins bound to beads were analyzed by SDS PAGE and visualized by silver staining. For comparison with the eluted samples, 20 % of the input protein is shown. (D) The samples in (C) were analyzed by western blotting to detect the presence of Nipbl, Mau2, Smc3, Pds5A in either the eluted (17%) or the input (10 %) samples. As Wapl was detected in the silver gel, no western blotting was performed for this protein. (E) Coomassie stained SDS Page of protein preparations used in ATPase assays. (F) Influence of His-Pds5A on the ATPase activity of Cohesin. Values represent the mean $\pm$ SD. Proteins used are described in the legend. (n=3).

Humans have three distinct Smc complexes: Cohesin, Condensin and the Smc5/6 complex (Hirano, 2006). All are functional ATPases and interact with DNA. Condensin and Cohesin associate with proteins that contain HEAT-repeat domains (Wells et al., 2017). To test whether Nipbl was specifically enhancing Cohesin's ATPase activity, recombinant Condensin II was generated. The Condensin II complex was expressed using a single virus and successfully purified using a similar strategy as for Cohesin (Figure 13 B). Condensin was prepared by Dr. Iain Davidson and Dr. Kota Nagasaka. ATPase assays showed that the recombinant Condensin II was an active ATPase and hydrolyzed ATP 23 x faster than equal amounts of Cohesin in the presence of λ DNA. Neither Condensin's nor Cohesin's ATPase was affected by the addition of λ DNA (Figure 13 C, D, E, Figure 7 C). Unlike Cohesin, Condensin II was not stimulated by the addition of Nipbl alone or together with DNA, showing that Nipbl specifically acted on Cohesin's ATPase.

Results

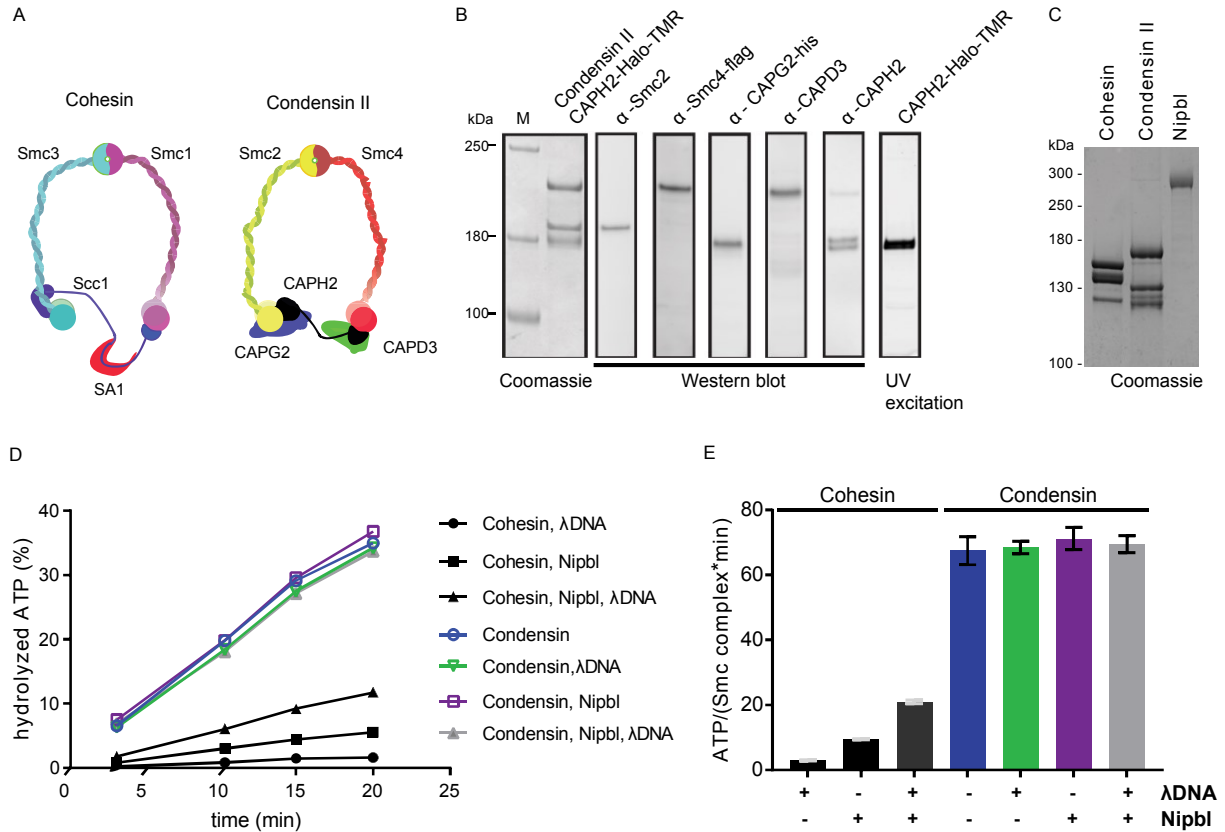


Figure 13: Nipbl does not stimulate the ATPase activity of a related Smc complex, Condensin II.

(A) Schematic diagram of structurally homologous Smc protein complexes: human Cohesin and human Condensin II. Both complexes are ring shaped and are assembled from Smc ATPases (Huis in 't Veld et al., 2014). (B) Coomassie stained SDS PAGE of the purified Condensin II preparation. Analytical western blots probed using specific antibodies recognizing each Condensin II subunit. SDS PAGE and UV exposure of Condensin II to excite the TMR labelled CAPH2-Halo-TMR subunit. (C) Coomassie stained SDS PAGE of purified Cohesin, Condensin II and Nipbl used in for the ATPase assays shown in (D) and (E). (D) ATPase assay comparing equal amount of Cohesin and Condensin II in their ability to be stimulated by either Nipbl, DNA or both together. (E) Hydrolysis rate of Cohesin and Condensin II derived from experiment shown in (E) and another not shown experiment.

3.6 The N-terminus of Nipbl is dispensable for enhancing Cohesin's ATPase domain.

Human Nipbl is a large 316 kDa protein. There are two isoforms in humans which are splice variants. In this thesis, I used exclusively the longer isoform A with 2,804 aa. Multiple protein sequence alignments ranging from fungi till human proteins showed that some parts of human Nipbl were very well conserved (analyzed by Alexander Schleiffer) (Figure 14 A). Nipbl¹⁻¹¹⁵ showed high sequence conservation and most-likely corresponds to the Mau2-binding site. This binding site was followed by an intrinsically unstructured domain, Nipbl^{115-1,182}, that was absent in lower eukaryotes. Nipbl^{1,183-2,804} was in contrast, very well conserved and contained the typical HEAT repeat domain. The role of the various domains of Nipbl had not been extensively studied and, for example, the Cohesin binding site was unknown. To shed some light on which domains participate in enhancing Cohesin's ATPase activity, I set out to produce Nipbl fragments. The generation of Nipbl fragments was challenging as there was no crystal structure yet available. I decided on the fragment boundaries based on previous studies, multiple protein sequence alignments to find potential conserved domains as well as *in silico* secondary structure predictions. Figure 14 A illustrates all fragments that I generated and shows the crystal structure that was not determined at that time. All fragments were N-terminally tagged with a His- and with a Twin-strep-his-tag and expressed in Sf9 insect cells. Affinity purification using Strep-Tac-sepharose beads or Ni-NTA beads pulled down all fragments (Figure 14 B ,C), but subsequent gel filtration revealed that Nipbl^{497-1,745}, Nipbl^{1,745-2,357} and Nipbl^{1,745-2,804} had been aggregated (data not shown). Gel filtration of Nipbl¹⁻⁴⁹⁷ in complex with Mau2 confirmed properly folded protein complexes and binding assays showed that they were binding to Cohesin (Figure 15 A).

Results

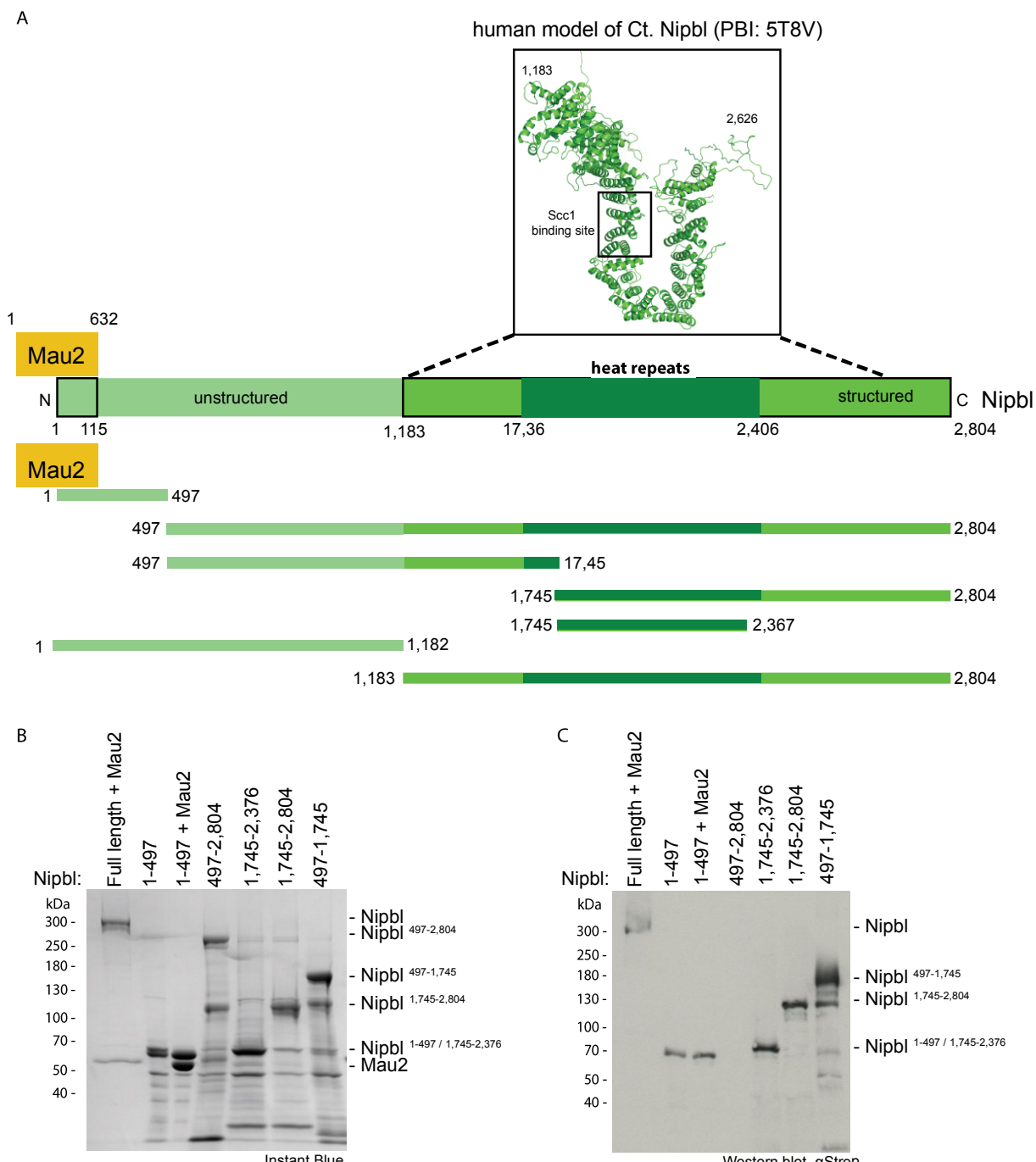


Figure 14: Preparation of truncated forms of Nipbl.

(A) Schematic of human Nipbl showing the Mau2 binding site, an unstructured domain (light green) and a structured region (green) that includes HEAT repeat motifs (dark green). The recently crystalized *Chaetomium thermophilum* Nipbl^{385-1,840} (PBD: 5T8V) was used to model human Nipbl (done by Alexander Schleiffer). A cartoon of this model is shown with the Scc1 binding site enclosed in a box (Kikuchi et al., 2016). Schematics of all N-terminally His- and Twin-strep-his- tagged Nipbl truncations made in this thesis are shown. (B) Coomassie stained SDS PAGE to analyzes a Ni-NTA pull down of some Nipbl truncations. (C) Western blot analysis using strep antibody for detection of the N-terminal Twin-strep tag in the samples shown in (B). His-Nipbl^{497-2,804} cannot be detected, because of the missing Twin-strep tag.

Most Nipbl fragments were not expressed very well, in contrast to Nipbl^{1,183-2,804} (also referred to Nipbl-Cterm) where high protein yields had been obtained using affinity purifications. Nipbl^{1-1,182} (also referred to Nipbl-Nterm) in complex with Mau2 was pulled-down too, but with lower yield. One test experiment was performed to analyze if these Nipbl fragments formed a complex with Cohesin in an *in vitro* binding assay. For this, purified Cohesin was either incubated with affinity purified full length Nipbl, Nipbl^{1-1,182}/Mau2 or Nipbl^{1,183-2,804}. The Nipbl-fragments were pulled down using their amino terminal Twin-strep-tag. Eluted proteins were analyzed by SDS PAGE stained with Coomassie. This showed that Nipbl^{1-1,182}/Mau2 was not pulled-down, suggesting that the protein might have aggregated during the experiment. In contrast, Nipbl full length and Nipbl^{1,183-2,804} formed complexes with Cohesin (Figure 15 B). Next, I performed ATPase assays to test whether the Nipbl fragment Nipbl^{1,183-2,804} contained the protein domain responsible for stimulating Cohesin's ATPase activity. Interestingly, Nipbl^{1,183-2,804} stimulated Cohesin's ATPase activity more than 2-fold better than full length Nipbl. I used two differently tagged full length Nipbl proteins that both showed a similar stimulatory effect (Figure 15 D, E). Budding yeast Nipbl and Cohesin both bound to *dsDNA in vitro* (Murayama and Uhlmann, 2014) and this binding might be helpful to load Cohesin onto DNA. Therefore, I tested if Nipbl^{1,183-2,804}:Cohesin interaction was stimulated additionally by λ DNA. Interestingly, DNA enhanced the interaction of Nipbl^{1,183-2,804} with Cohesin 5-fold/ 3-fold (n=2) while full length Nipbl:Cohesin binding was enhanced 2-fold. Note that Nipbl^{1,183-2,804} and full length Nipbl stimulated Cohesin's ATPase activity similarly in the absence of DNA (Figure 15 F, G).

Results

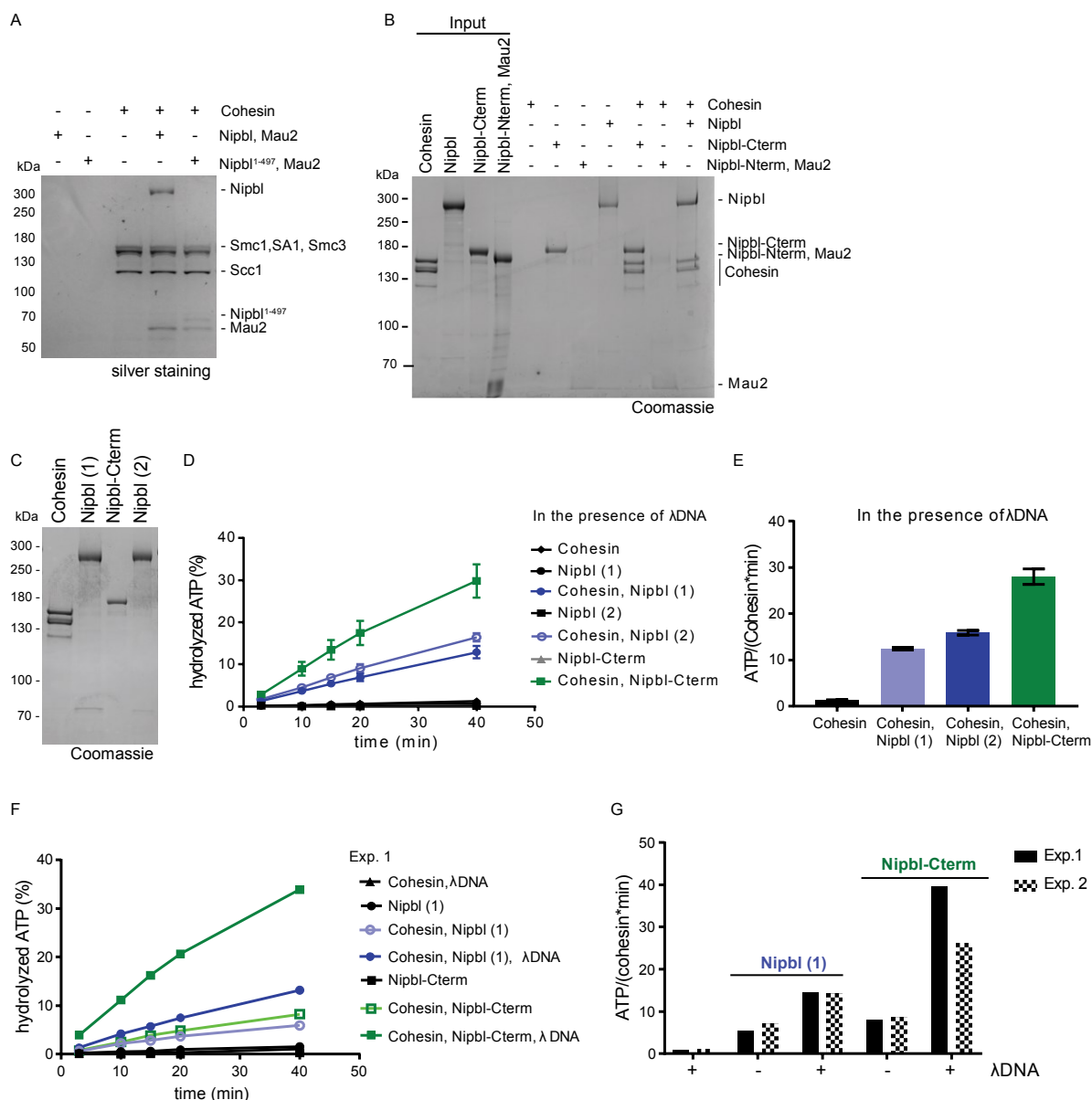


Figure 15: A Nipbl C-terminal fragment interacts with Cohesin and boosts its ATPase activity.

(A) Coomassie stained SDS PAGE shows that Nipbl¹⁻⁴⁹⁷/Mau2 binds like wt Nipbl to Cohesin, pre-bound to flag-beads. (B) Binding assay testing Cohesin's interaction with Twin-strep-Nipbl¹¹⁸³⁻²⁸⁰⁴ (Nipbl-Cterm), Nipbl-Nterm (Twin-strep-Nipbl¹⁻¹¹⁸²-his) in complex with Mau2 and full-length Twin-strep-flag-Nipbl-his. Proteins were incubated in solution and complexes were isolated using Strep-Tacin beads. Coomassie stained SDS PAGE shows 80 % of the eluted fraction and 50 % of the proteins used as input. (C) Coomassie stained SDS PAGE of proteins used in ATPase assay shown in (D) Twin-strep-Nipbl-his (Nipbl (1)), Twin-strep-flag-Nipbl-his (Nipbl (2)), Twin-strep-Nipbl-Cterm-his and Cohesin. (D) Quantification of ATPases comparing Nipbl-Cterm and full length Nipbl (1,2) in their abilities to stimulate Cohesin's ATPase in the presence of λ DNA. (n=3) (E) ATP hydrolysis rates derived from time-course analysis shown in (D). (F) Quantification of a representative ATPase assay testing a stimulatory effect of λ DNA on the Nipbl-Cterm - Cohesin interaction. (G) ATP hydrolysis rates derived from 2 time-course experiments: Experiment 1 shown in (F) and another experiment 2 (data not shown) to compare the stimulatory effect of DNA of full length Nipbl with Nipbl-Cterm.

3.7 Nipbl-PA mutant

During the course of my study, *Chaetomium thermophilum* (Ct) Nipbl^{385–1,840} was crystallized (Kikuchi et al., 2016). The Nipbl fragment bound specifically to Ct Scc1^{126–230}. This interaction was reduced by introducing single point mutations into Nipbl.

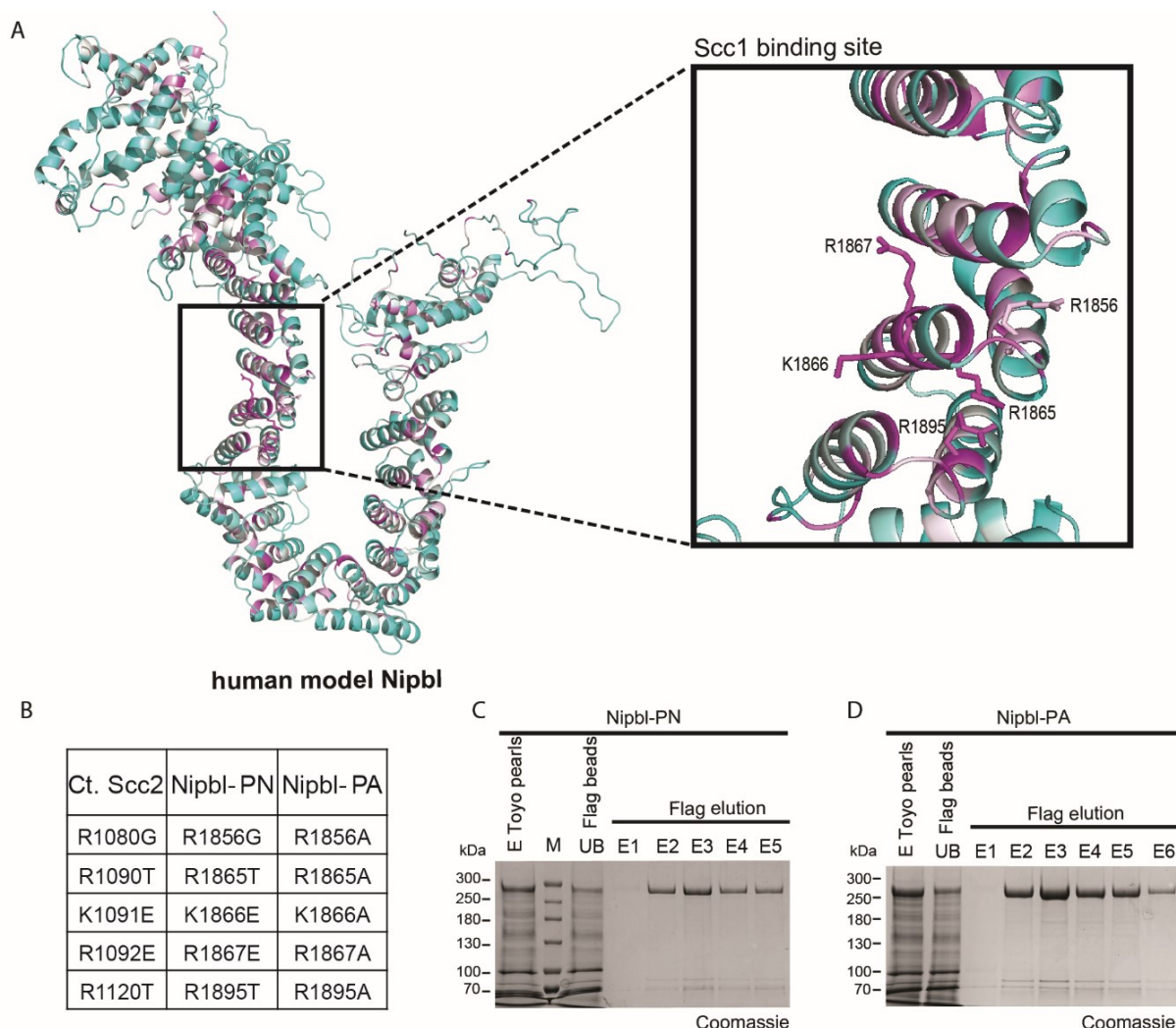
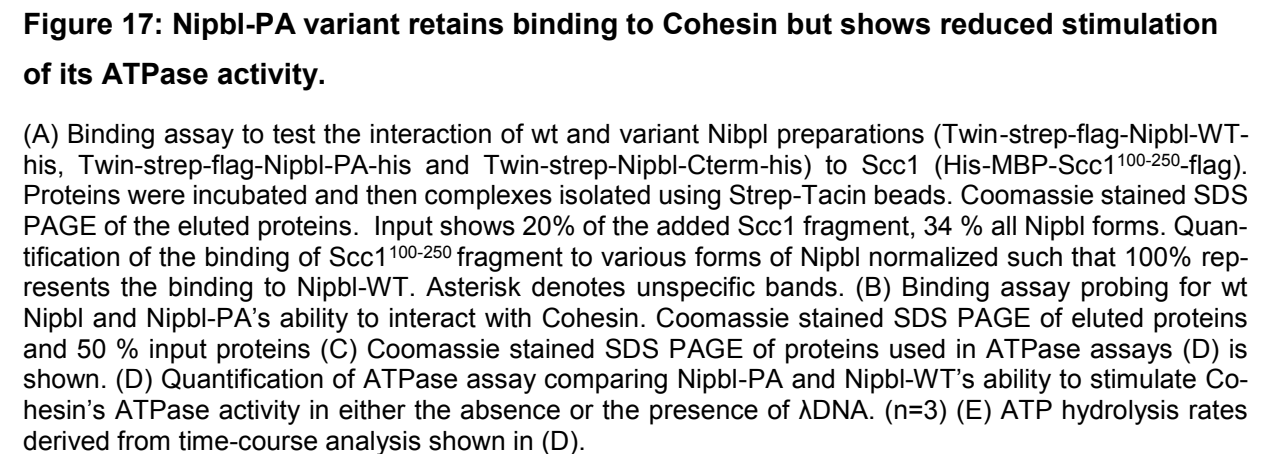


Figure 16: Purification of Nipbl complexes with mutations in its HEAT repeat domain.

(A) Structure of human Nipbl^{1187–2636} (modelled by Alexander Schleiffer and based on the crystal structure of *Chaetomium thermophilum* Nipbl^{385–1,840} (PDB: 5T8V)) showing the distribution of conserved amino acids. Regions of low (cyan) and high (magenta) conservation are indicated based on multiple sequence alignments of proteins found from fungi to vertebrates. Blow up of a potential Scc1 binding site first described in (Kikuchi et al., 2016). (B) Table describing single point mutations in *Chaetomium thermophilum* Nipbl (Kikuchi et al., 2016) and the corresponding residues in the human protein. Two Nipbl mutants were generated: Nipbl-PN with five residues mutated to the same amino acid as previously described or Nipbl-PA with only alanine substitutions. Coomassie stained SDS PAGE of two step affinity purification preparations of Twin-strep-flag-Nipbl-PN-his (C) and Twin-strep-flag-Nipbl-PA-his(D).

The mutated residues were chosen because of their conservation, positive correlation to mutations in human CdLS patients and electro static interactions. Five of those mutations weakened the Scc1:Nipbl interaction, without influencing the folding of Nipbl. All those mutations localized to the HEAT repeat domain of Nipbl (Kikuchi et al., 2016). As this the was first report proposing a Nipbl:Cohesin binding site, I decided to determine if those five residues would also affect the human loading complex and substituted the same residues in human Nipbl to either the published amino acids (Nipbl-PN) or to alanines (Nipbl-PA) (Kikuchi et al., 2016). For this, multiple sequence alignments were used to identify the corresponding residues in the human protein (Figure 16 A, B). Nipbl-PN and Nipbl-PA were expressed in Sf9 insect cells and affinity purifications were performed. Nipbl-PN was not stable and precipitated but Nipbl-PA behaved well during purification and storage (Figure 16 C, D). To reproduce the published data and to see if this binding site might be conserved from human to fungi, I wanted to performed binding assays with Nipbl and Scc1. I unsuccessfully tried to purify full length human Scc1 alone or in complex with SA1. Because of this, I aligned Scc1 orthologs from fungi to humans to identify conserved regions and then generate fragments containing the proposed Nipbl binding site (Kikuchi et al., 2016). In addition, I performed *in silico* structure predictions of Scc1 using bioinformatic online tools to take any secondary structures into account. I expressed His-MBP-C3-Scc1⁶⁵⁻²¹⁵-flag, His-MBP-C3-Scc1¹⁰⁰⁻²⁵⁰-flag and His-MBP-C3-Scc1¹²⁰⁻²¹⁰-flag in *E. coli*. The maltose binding protein (MBP) was added to increase solubility and could be cleaved off by the PreScission protease. The fragments were purified using affinity purification, ion exchange chromatography and gel filtration chromatography to ensure non-aggregated proteins. Then, I performed a binding assay and tested whether the Nipbl-PA mutant was able to bind the Scc1¹⁰⁰⁻²⁵⁰ fragment. Nipbl-PA, WT Nipbl and Nipbl^{1,183-2,804} were incubated with Scc1¹⁰⁰⁻²⁵⁰ fragment prior to a Nipbl pull-down. This analysis showed that the binding of this Scc1 fragment was reduced in the Nipbl-PA mutant (Figure 17 A). To check if this Scc1 binding site might be the only one in Cohesin, I investigated if Nipbl-PAs forms a complex with tetrameric Cohesin and, interestingly, it did (Figure 17 B).

Nipbl-PA bound to the Cohesin complex but showed a reduced binding to a Scc1 fragment. To test whether this Scc1:Nipbl interface might be important for stimulating Cohesin's ATPase, I performed ATPase assays. In these experiments, Nipbl-PA still enhanced



38

Cohesin, showed both a diminished ability to stimulate its ATPase activity and also reduced binding to Scc1 pointing to the importance of Scc1 in mediating Nipbl's stimulatory effect on Cohesin. To investigate this further, I purified Cohesin sub-complexes such as Smc3-flag, dimeric Cohesin Smc1/3 that has shown to be an active ATPase, trimeric Cohesin Smc1/3 in complex with Scc1 and analyzed if their ATPase activity was stimulated by Nipbl in the presence of DNA. Nipbl had no effect on the ATPase activity of Smc3 alone or in complex with Smc1 (Figure 18), although the dimer formed a complex with Nipbl *in vitro* (see chapter 3.8, Figure 23 A, B). In contrast, the Cohesin trimer showed a strong stimulatory effect pointing again to the important of Scc1 in mediating the interaction with Nipbl. The weaker stimulation of the Cohesin trimer compared to tetramer containing SA1, could be explained by the lower amount of Scc1, as judged by analysis of the Cohesin preparation on SDS-PAGE, stained with Coomassie (Figure 18 A, B).

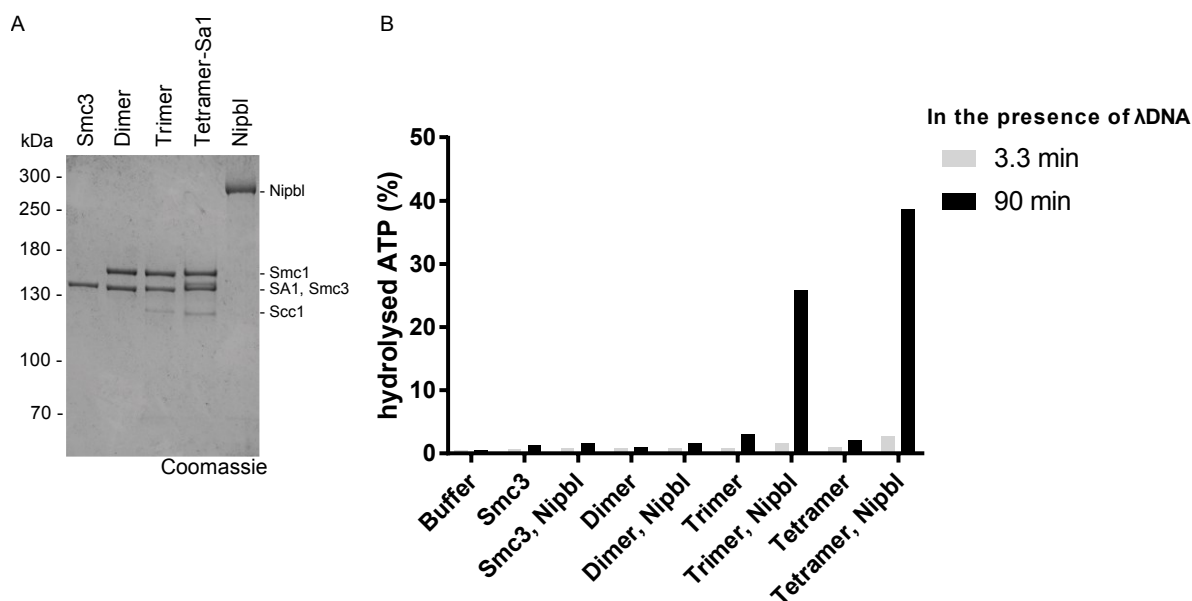


Figure 18: Scc1 is required for Nipbl's stimulatory effect on Cohesin's ATPase activity.

ATPase assay testing the influence of Nipbl on various Cohesin subcomplexes. (A) Coomassie stained SDS PAGE of protein preparations used in the ATPase assay shown in (B) (B) Quantification of ATPase assay. Levels of ATP hydrolysis at 3.3 min and 90 min.

To investigate whether the addition of SA1 would further enhance the Cohesin:Nipbl reaction, I performed ATPase assays with trimeric Cohesin and tested whether the addition of SA protein would further stimulate this reaction. In this set up, all complexes must have had the same amount of Scc1. For this, I set out to produce recombinant SA1 and SA2. His-SA1 and His-SA2 were expressed in insect cells and purified using Ni-NTA pull-down. The protein identity was confirmed by western blot analysis (Figure 19 A). To evaluate their functional activity, I tested whether the SA proteins would bind specifically to their proposed Scc1 binding site (Hara et al., 2014). For this, I generated the corresponding fragment, His-MBP-Scc1³⁰⁶⁻⁴¹⁰-flag, and used as control the Scc1-fragment with the Nipbl binding site His-MBP-Scc1¹⁰⁰⁻²⁵⁰-flag. Both fragments had been expressed in bacteria and purified using Ni-NTA pull-down, ion exchange chromatography and finally gel filtration chromatography. SA1/2 were incubated with either Scc1¹⁰⁰⁻²⁵⁰ or with Scc1³⁰⁶⁻⁴¹⁰ prior to a flag pull-down. The eluted proteins were analyzed with SDS PAGE stained with Coomassie. This showed that both SA1 and SA2 bound specifically to the proposed SA binding site, Scc1³⁰⁶⁻⁴¹⁰, and not the control Scc1¹⁰⁰⁻²⁵⁰ fragment (Figure 19 B). The SA proteins also formed a complex with trimeric Cohesin, shown by incubation of the SA proteins with trimeric Cohesin that was pre-bound to flag beads (Figure 19 C). To finally investigate, if the SA proteins triggered the Nipbl:Cohesin interaction, an ATPase assay was performed either with trimeric Cohesin alone or with equal amounts of recombinant SA1 added. Nipbl stimulated the ATPase activity of the trimeric Cohesin 7-fold and this was boosted further by the addition of DNA (Figure 19 D, E). The addition of SA1 did not affect this reaction indicating that Nipbl's stimulation of Cohesin's ATPase activity is largely independent of the SA proteins (Figure 19 E).

Results

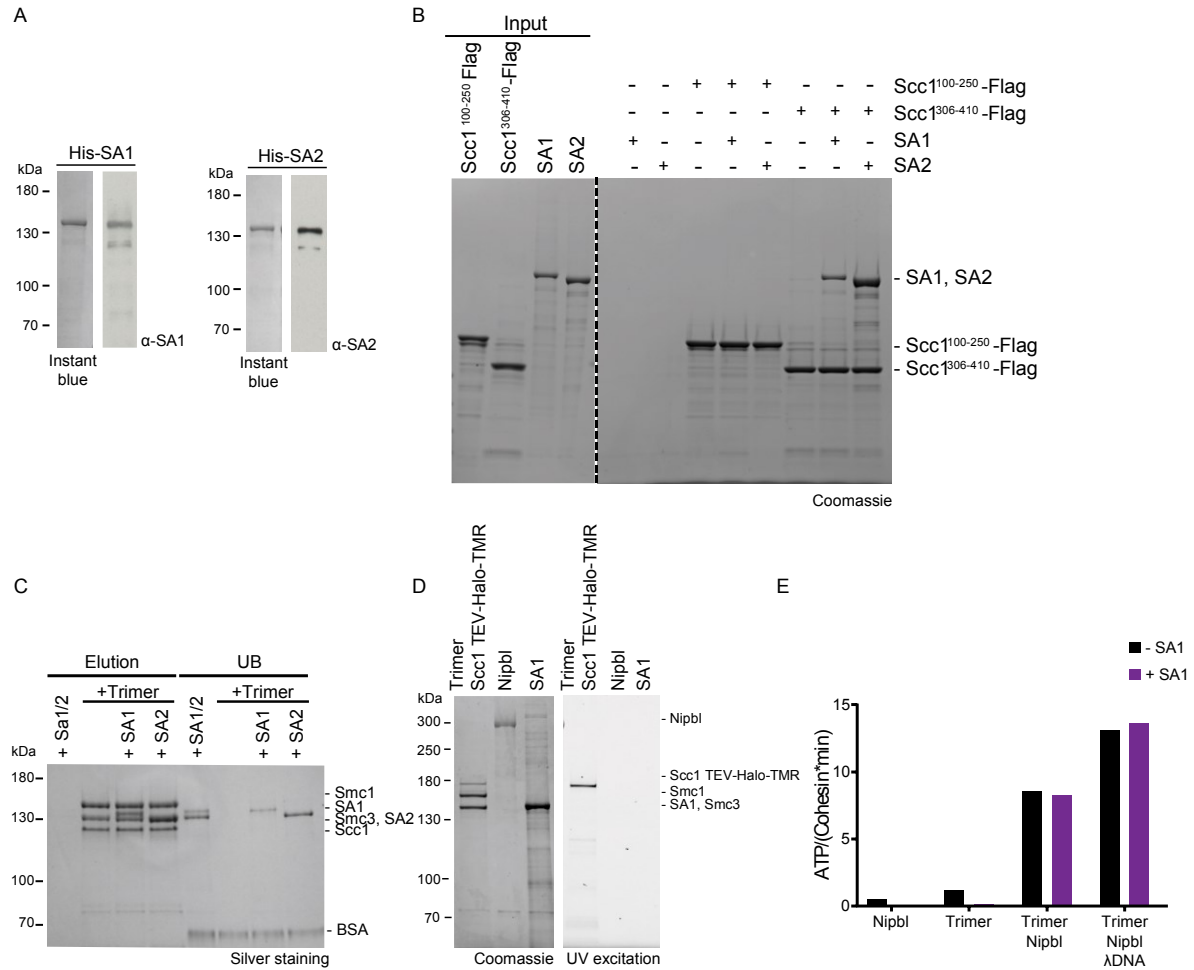


Figure 19: Addition of SA1/2 did not further boost the stimulated ATPase activity of the trimeric Cohesin.

(A) SDS PAGE of purified SA1 and SA2 preparations stained with Coomassie and analyzed by western blot (B) Binding assay to test whether the purified SA1 and SA2 can interact with a proposed Scc1 binding site. Either SA1 or SA2 were incubated with Scc1 fragments (His-MBP-Scc1¹⁰⁰⁻²⁵⁰ flag or His-MBP-Scc1³⁰⁶⁻⁴¹⁰flag) prior to a pulldown using flag-M2-beads. SDS PAGE stained with Coomassie showing 50 % of the eluted protein and 20 % input from Scc1-fragments and 33 % input from SA1/2. (C) Binding assay testing SA1's and SA2's ability to form a complex with trimeric Cohesin pre-bound to Flag-M2-beads. Silver stained SDS-PAGE shows 50 % of the eluted protein and 5% of the proteins in the unbound fraction (UB) (D) UV-exposed (to detect the Halo tag) and Coomassie stained SDS PAGE analysis of trimeric Cohesin (Scc1-TEV-Halo-TMR), Nipbl and SA1 preparations used in the ATPase assays in (E) (E) ATP hydrolysis rates derived from time-course analysis testing SA1's contribution to Nipbl's stimulatory effect on Cohesin's ATPase in the absence and presence of DNA.

Results

To check whether Scc1's ability to tether the Smc1/3 heads was important for this reaction, I tested if only intact Cohesin rings, with uncleaved Scc1 subunits, were targeted by Nipbl. Previous experiments established the importance of intact Cohesin rings for entrapping DNA in the bulk *in vitro* loading assay both in the absence of (chapter 3.1) and also the presence of Nipbl (Murayama and Uhlmann, 2014). To test this, I used a trimeric Cohesin that contained an engineered TEV site in its Scc1 subunit (Scc1-TEV-Halo). The Scc1-TEV subunits were fully cleaved by TEV protease treatment as judged by an analytical SDS-PAGE stained with Coomassie and UV-exposure (excitation of Halo-TMR at Scc1C-terminus). Interestingly, a comparison of equal amounts of trimeric Cohesin composed of either cleaved or intact Scc1 showed no detectable difference in ATPase assays (Figure 20 B, C). The rates of hydrolysis for both forms were enhanced by Nipbl and were further enhanced by the addition of DNA. Showing that Nipbl acted equally well on a complex containing the cleaved Scc1 subunit.

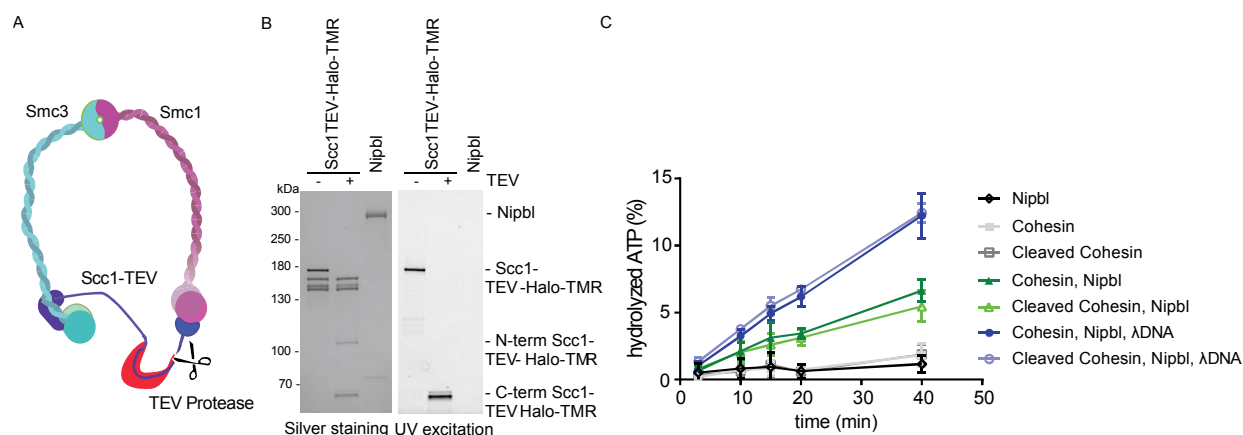


Figure 20: Cleavage of Scc1, the subunit spanning the Smc heads in Cohesin, does not influence the ability of Nipbl to activate Cohesin's ATPase activity.

(A) Schematic of Cohesin showing the location of an artificially inserted TEV site in Scc1, a subunit that spans the ATPase domains of Smc1 and Smc3. (B) UV excited and then Coomassie stained SDS PAGE of protein samples used in ATPase assay. Cohesin-Scc1^{TEV-Halo-TMR} was either cleaved or not cleaved by incubation with TEV protease during purification. TEV cleavage is apparently complete under these conditions. (C) ATPase assay testing Nipbl's influence on Cohesin containing a cleaved Scc1 subunit. Cohesin and Nipbl were both present at 75 nM. (n=3)

Results

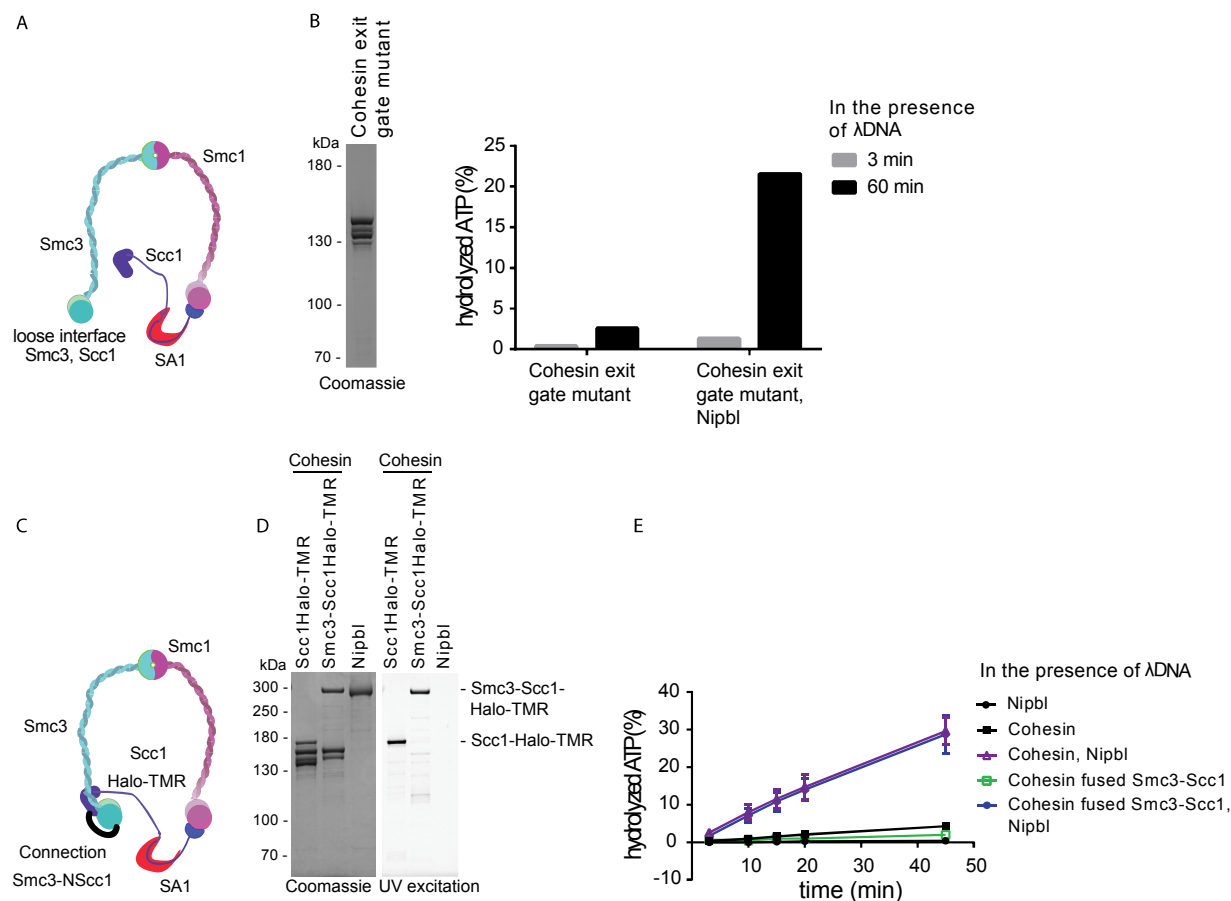


Figure 21: Altering the Smc3:Scs1 interface has little or no influence of the ability of Nipbl to stimulate Cohesin's ATPase activity.

(A) Schematic of an "exit gate mutant" of Cohesin showing a loose Smc3-Scs1 interface due to a mutant form of Scs1 with four altered residues. (B) Coomassie stained SDS PAGE of exit "gate mutant" Cohesin preparation. Assay testing Nipbl's influence on the exit gate mutant Cohesin ATPase activity. ATP hydrolysis after 3 and 60 min incubation of Cohesin with ATP. The level of "exit gate" ATPase activity, while not compared directly in this experiment, is similar to that typically seen for Cohesin wild type. (C) Schematic of Cohesin with a covalently connected Smc3-Scs1 interface. (D) UV excited and then Coomassie stained SDS PAGE of protein samples used in the ATPase assay shown in (E) (E) Quantification of ATPase assay testing the influence of a fused Smc3-Scs1 interface. (n=3)

The Scs1 subunit seemed to be very important for mediating Nipbl's stimulatory effect on Cohesin' ATPase activity. The N-terminus of Scs1 forms a helix bundle with the first coiled-coil proximal to the Smc3 head domain (Gligori et al., 2014), thereby creating the exit gate, an important interface for Cohesin unloading from DNA. In our Nipbl:Cohesin cross-linking mass spectrometry experiments, we detected some putative protein-protein interactions between this exit gate and Nipbl (see chapter 3.8). To investigate how an altered NScs1:Smc3 interface would influence ATPase reaction of Nipbl:Cohesin, I made use of a previously published Cohesin mutant, termed "exit gate mutant". This mutant had

a weakened Scc1-Smc3 interface due to 4-point mutations in Scc1^{L53E, L64E, Y60E, L74D} (Huis in 't Veld et al., 2014), resulting in a partially open Cohesin ring, as shown by rotary shadowing electron microscopy. I addressed whether the loading complex still interacted with this partially open Cohesin, by performing an ATPase assay in the presence of DNA. This assay demonstrated that the ATPase activity of the exit gate mutant Cohesin was stimulated by Nipbl, indicating that this loose interface was not eliminating Nipbl's ability to stimulate Cohesin's ATPase (Figure 21 A, B).

To test whether a covalent linkage of Scc1-Smc3 interface had an influence on Nipbl stimulatory effect, I performed an ATPase assays with recombinant Cohesin containing fusion protein of Smc3 to Scc1 and compared it to wild type Cohesin (generated and purified by Dr. Iain Davidson). These assays also showed no difference between the wild type and fused mutant complexes (Figure 21 D, E).

3.8 Cohesin's interaction with its loading complex

To study how the loading complex interacts with Cohesin, I used protein binding assays and cross-link mass spectrometry. Cross-linking mass spectrometry (XLMS) is a powerful method to detect protein to protein interactions because it relies on connecting proximal amino acids such as lysines using chemical reagents. The connected/"cross-linked" lysines are detected by standard trypsin digest of the cross-linked proteins, subsequent identification of un- and cross-linked peptides using mass spectrometry and extensive data analysis to finally create proximity maps of putative protein-interactions. The cross-linking experiments were performed in collaboration with the Karl Mechtler group, IMBA, Vienna. Unless indicated, the cross-linked protein samples were analyzed by Dr. Rebecca Beveridge.

First, I investigated whether Cohesin stably interacted with Nipbl/Mau2 *in vitro* because *in vivo* this interaction was thought to be transient (Arumugam et al., 2003, Watrin et al., 2006). To compare Cohesin-SA1 with Cohesin-SA2, I purified Cohesin-SA1 and generated a vector for co-expression of all Cohesin-SA2 subunits using a cloning method, called BiGBac (Weissmann et al., 2016). The same tagging and purification strategy as for Cohesin-SA1 was used to obtain tetrameric Cohesin-SA2. Then, I performed binding assays using pre-purified Nipbl alone or in complex with Mau2 and incubated these either with Cohesin-SA1 or Cohesin-SA2. Nipbl was immobilized making use of an affinity tag on its N-terminus. Analysis of eluted proteins by silver stained SDS PAGE and by western blotting showed that Nipbl bound to Cohesin. There was no detectable difference between Cohesin-SA1 and Cohesin-SA2 (Figure 22 B,C). Furthermore, Mau2 was dispensable of the Cohesin:Nipbl interaction (Figure 22 B,C).

Furthermore, I performed a preliminary binding assay using Cohesin subcomplexes hoping to identify Cohesin core proteins that act as direct binding partners of Nipbl. For this, Nipbl was incubated with either pre-purified SA1, SA2, a dimeric complex of Smc1/3, a trimeric complex of Smc1/3 in complex with Scc1 or a tetrameric Cohesin complex with all 4 core subunits in solution. Nipbl was then pulled-down and subsequent analysis of the eluted proteins by silver stained SDS PAGE and western blotting showed that Nipbl formed complexes with all tested Cohesin subunits, except the SA1 subunit (Figure 23).

Results

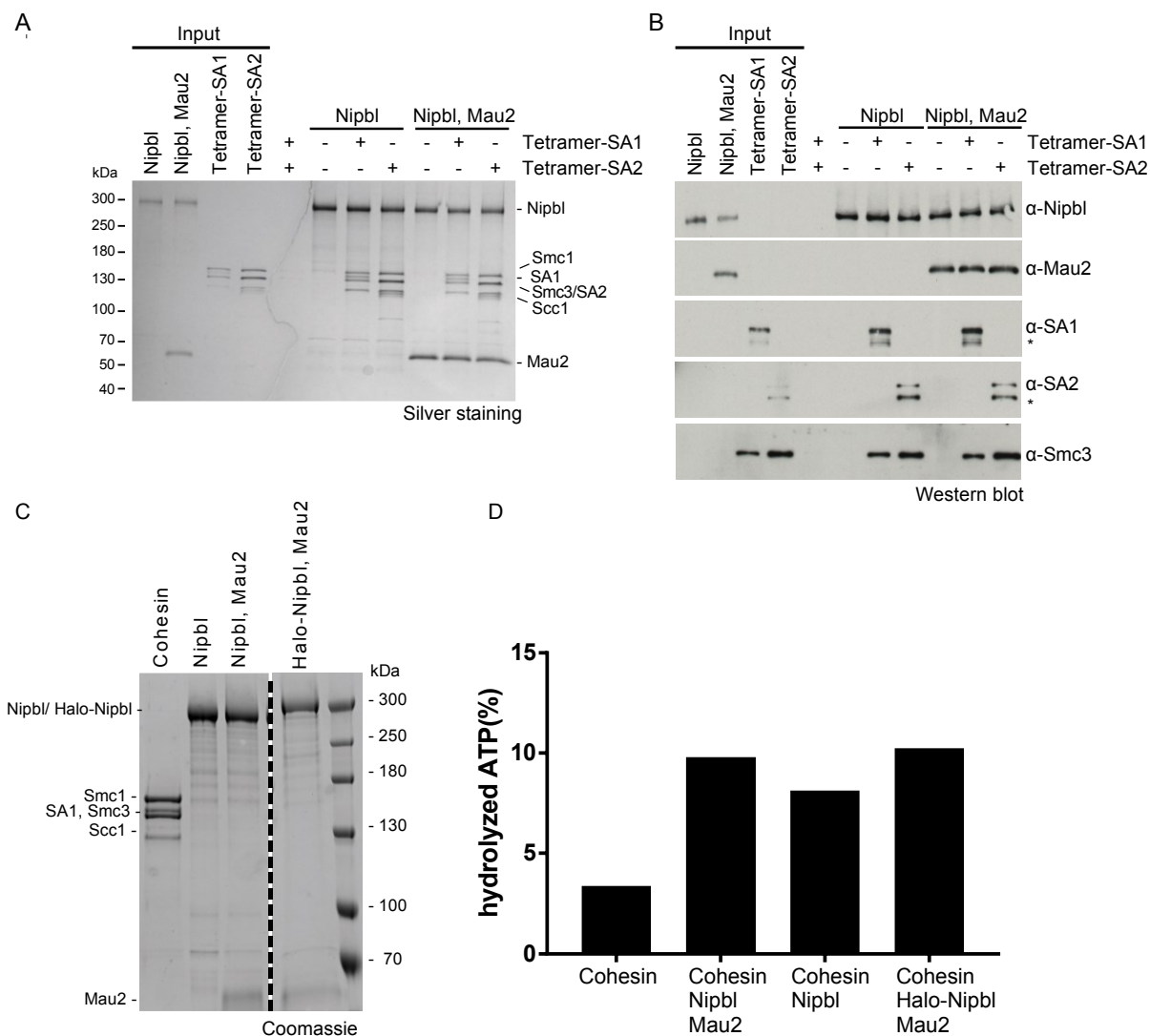


Figure 22: Mau2 is dispensable for the Cohesin – Nipbl interaction, while SA1 or SA2 is interchangeable.

(A) Schematic of the ring-shaped Cohesin complex (Huis in 't Veld et al., 2014). The Smc1/3 head domains are binding each other and thereby creating two ATPase reactions centre. Scc1 is connecting the two heads and is bound by either SA1 or SA2. (B) Pre-purified Cohesin tetramer in complex with either SA1 or SA2 was incubated with purified Twin-strep-flag-Nipbl-his either alone or in complex with Mau2. Nipbl was isolated using Strep-tacin-beads. A representative silver stained SDSPAGE gel of the eluted proteins is shown. Signal from 10 % of the input proteins used for this experiment are indicated (input). (C) The samples shown in (B) were analyzed by Western blotting to detect Nipbl, Mau2, SA1, SA2 and Smc3. Samples analyzed represent 4% of the input and 17% of the eluted fraction. Asterisks denote protein degradation bands. (D) Coomassie stained SDS PAGE of protein preparations used in the ATPase assay shown in (E). (E). Quantification of an ATPase assay comparing Nipbl either alone or in complex with Mau2 amount of hydrolyzed ATP (%) after 20 min is shown.

Results

Note that although less dimeric complex was used in comparison to trimeric or tetrameric Cohesin, Smc1/3 still bound to Nipbl. To exclude that the purified SA1 protein was not folded properly, I performed additional binding experiments demonstrating specific binding to a Scc1 fragment (Scc1³⁰⁶⁻⁴¹⁰), containing its proposed binding site (Hara et al., 2014), as well as to the Cohesin trimer (see chapter 3.7, Figure 19 B, C).

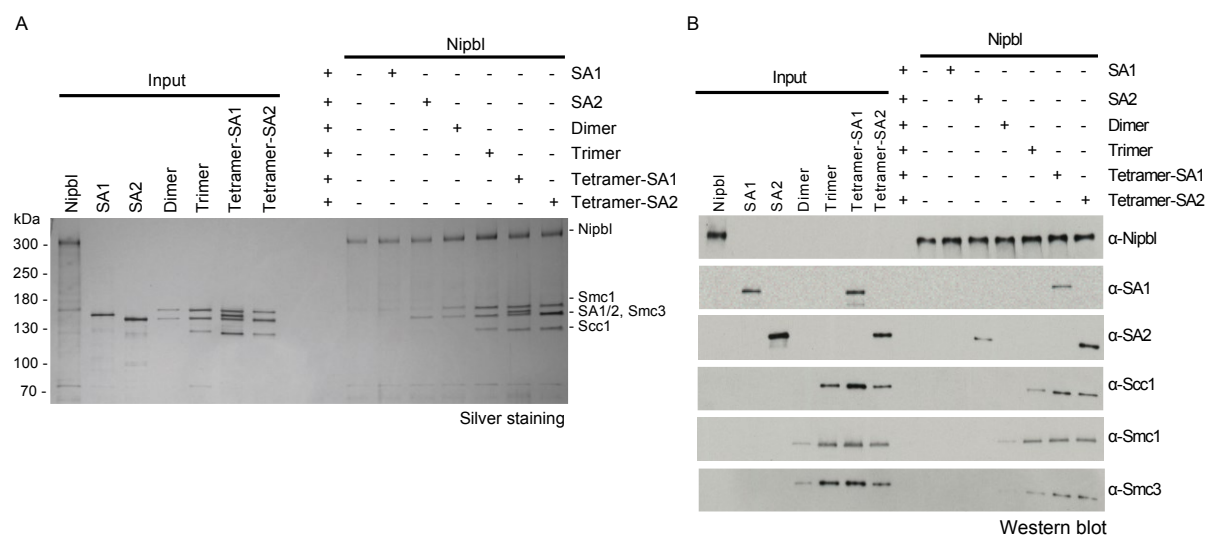


Figure 23: Nipbl interacts with SA2 alone as well as Smc1/3 dimers, Scc1-Smc1/3 trimers and SA1 or 2-Scc1-Smc1/3 tetramers.

(A) Binding assay testing Nipbl's ability to interact with Cohesin sub-complexes. Proteins were incubated in solution prior to a Twin-strep-flag-Nipbl-his pull down and elution. Silver stained SDS PAGE showing 50 % of the elution and 20 % of proteins used as input. (B) Analytical western blot analysis of samples from experiment shown in (A). 20 % of eluted proteins and 20 % of input proteins were detected with specific antibodies against Nipbl, Smc3, Smc1, SA1, SA2 and Scc1.

Nipbl alone enhanced Cohesin's ATPase activity to a similar level as when complexed to Mau2 (Figure 22 E) suggesting that Mau2 may, rather than directly mediate loading complex:Cohesin interaction, perform other important functions in the cellular context. That is why, I decided to omit Mau2 and only perform cross-linking mass spectrometry with Nipbl in complex with Cohesin-SA1 which remained a complex sample of more than 800 kDa. Cross-linking mass spectrometry had been performed previously with human Cohesin-SA1 and was thus preferred over Cohesin-SA2 (Huis in 't Veld et al., 2014).

The loading complex had never been analyzed by cross linking spectrometry, in contrast to Cohesin. Therefore, I performed a test experiment with Nipbl:Mau2. This complex had

a molecular mass of around 400 kDa and a known Nipbl:Mau2 binding interface. For this, Twin-strep-flag-Nipbl-his/Mau2 was purified using two consecutive affinity purification steps. Cross-linking was performed in solution with the cross-linking reagent disuccinimidyl suberate (DSS) (Figure 24 A, B) that covalently connects proximal lysines. Extensive data analysis, identified 530 intra-protein cross-links in Mau2 and Nipbl as well as 2 inter-protein cross-links between Nipbl and Mau2 (Figure 24 C). Mau2 cross-linked with the N-terminal affinity tag of Nipbl, confirming that Mau2 binds to the N-terminus of Nipbl. 527 intra-protein cross-links of Nipbl had been found showing that Nipbl contained free lysines that might be able to detect the Cohesin's binding site. Nipbl 300-1100 formed many cross-links due to a high frequency of lysines (Figure 24 C) but also as this Nipbl region is unstructured and thus more solvent accessible (Figure 24 A).

Results

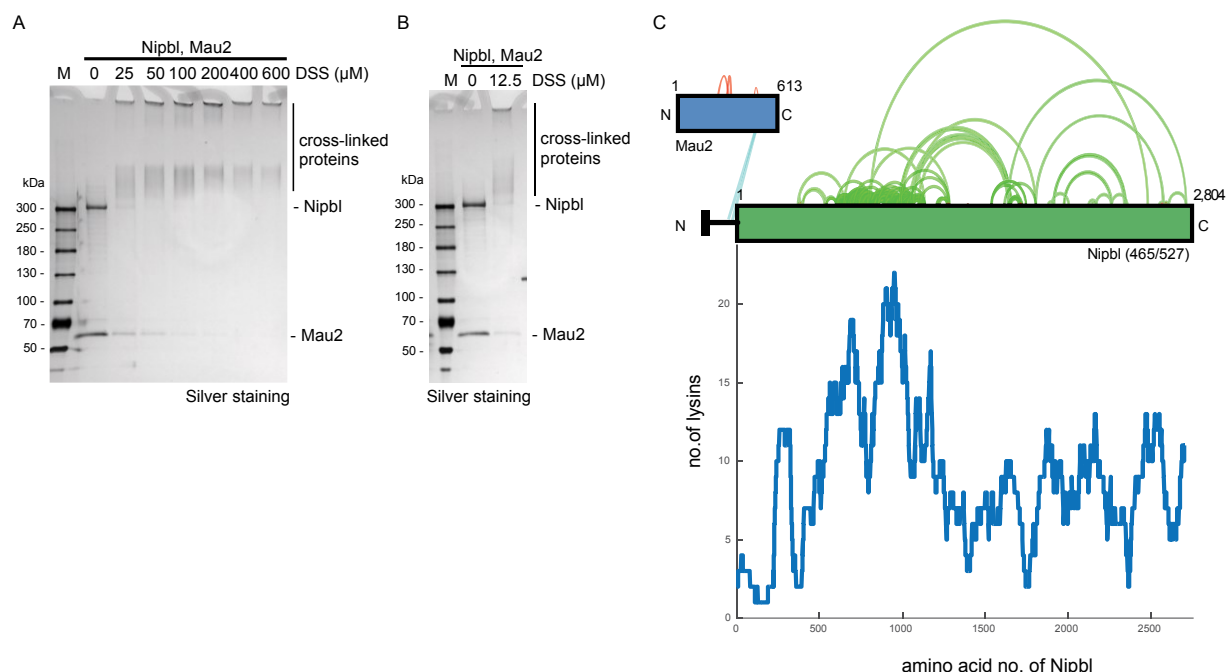


Figure 24: Cross-linking mass spectrometry confirmed Mau2's interaction with the N-terminus of Nipbl.

(A) Silver stained SDS PAGE of an Nipbl/Mau2 complex after incubation with increasing amounts of DSS, a chemical reagent cross-linking proximal lysines. (B) Silver stained SDS PAGE of the final cross-linking reaction used for analysis by mass spectrometry. (C) Bar plot showing all uniquely determined intra-molecular cross-links found in Mau2 (red) and Nipbl (green) as well as inter-molecular cross-links (between Mau2 and Nipbl, in blue). Of the intra-molecular cross-links in Nipbl, 465 out of 527 were uniquely determined while 62 cross-links are ambiguously as they can be assigned to at least 2 different locations in the protein. Two lysines in Mau2 were cross-linked to the Flag-tag at the N-terminus of Nipbl. Frequency distribution of existing lysines along the length of Nipbl: no of lysines in every 100 amino acids of Nipbl (frequency distribution analysis done by Dr. Benedikt Bauer).

As, we successfully detected cross-links in the Mau2:Nipbl sample, I performed cross-linking mass spectrometry with a more complex sample: Nipbl in complex with Cohesin. For this, I purified the Cohesin-SA1 complex and Nipbl separately using two affinity purification steps for each. The pre-purified Cohesin complex was then incubated with Nipbl that was still bound to strep-tacin-sepharose beads, in the absence of ATP and in a buffer containing 100 mM NaCl. This salt concentration was used in all binding as well as ATPase assays. The Nipbl:Cohesin complex was eluted and proximal lysines were cross-linked with DSS (Figure 25 A). Finding cross-linked peptides was challenging but we collected 2 independent data sets with Cohesin:Nipbl cross-links. Experiment 1 with a total of 1638 cross-links and experiment 2 with 188 cross-links. An overview about the distribution of intra- and inter-protein cross-links identified in both experiments is shown in

Table 3. A proximity map of all putative protein-protein interactions in experiment 1 is shown in Figure 25 B and for experiment 2 in appendix Figure 30 B.

Table 3: Nipbl:Cohesin cross-links (XLs) found in experiment 1 and 2.

	Number of XLs (exp.1)	Number of XLs (exp.2)
Nipbl - Cohesin	278	11
Nipbl-Smc3	122	4
Nipbl-Smc1	55	5
Nipbl-Scc1	32	1
Nipbl-SA1	69	1
Cohesin inter XLs	188	40
Smc1-Smc3	150	38
Smc1-Scc1	7	0
Smc1-SA1	7	0
Scc1-SA1	11	1
Scc1-Smc3	8	0
Smc3-SA1	5	1
Intra XLs	1172	137
Nipbl-Nipbl	856	70
Smc1-Smc1	160	37
Smc3-Smc3	126	28
Scc1-Scc1	4	0
SA1-SA1	26	2
Total XLs	1638	188

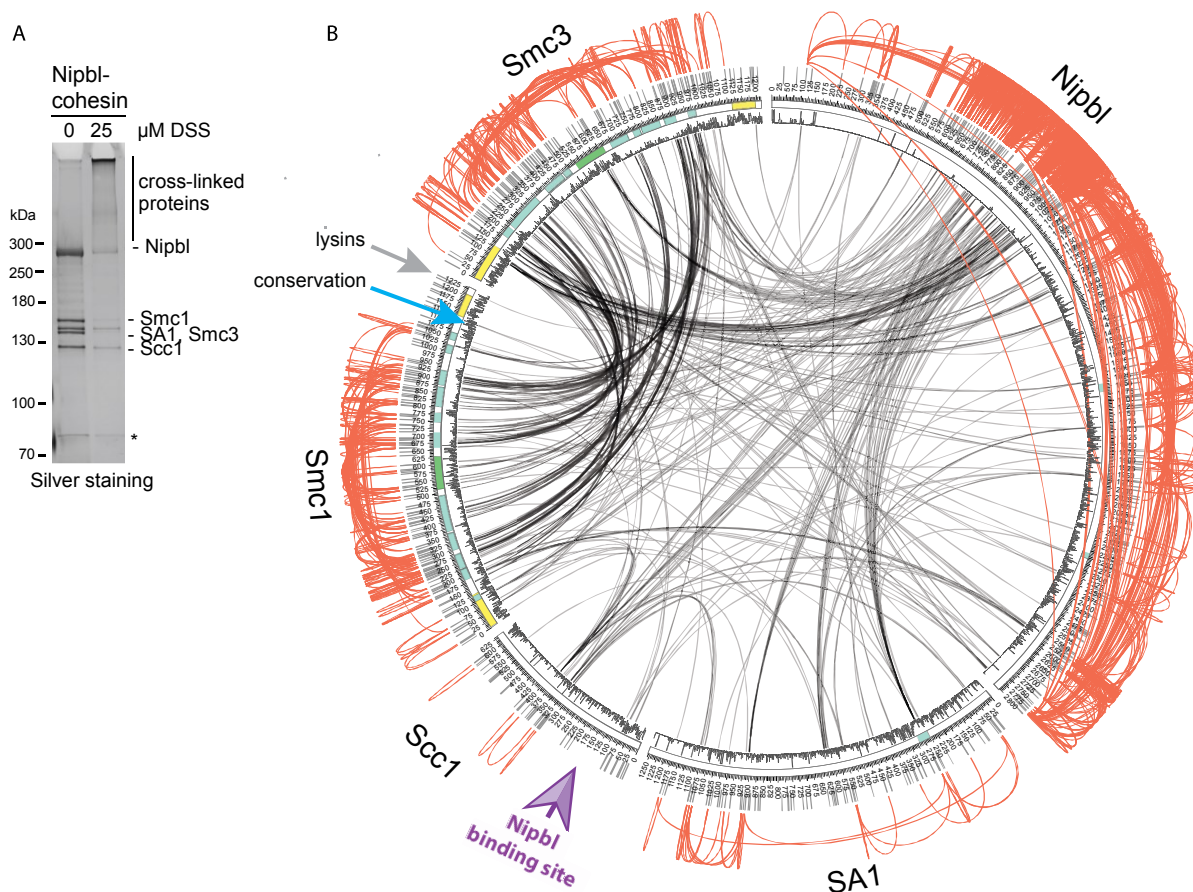


Figure 25: Crosslinking mass spectrometry indicates that Nipbl contacts all four Cohesin subunits.

(A) Cross linking mass spectrometry experiment to detect protein-protein interactions between-20 Nipbl and Cohesin subunits. Silver stained SDS PAGE of Nipbl bound to Cohesin in solution before and after incubation with the cross-linking reagent DSS. (B) Circos blot of all cross-linked peptides ($n=1638$) found in samples from experiment shown in (A). Intra cross-links ($n=1172$) are shown in red while inter cross-links ($n=278$) are depicted in grey. All potential lysine cross-linking sites are designated by grey sticks (grey arrow pointing to an example) and conservation is blotted (blue arrow points to an example). A magenta arrow highlights the potential Scc1 binding site proposed by (Kikuchi et al., 2016). Important domains in Smc1/3 are marked: green hinge domain, yellow head domains, light green coiled-coil domain.

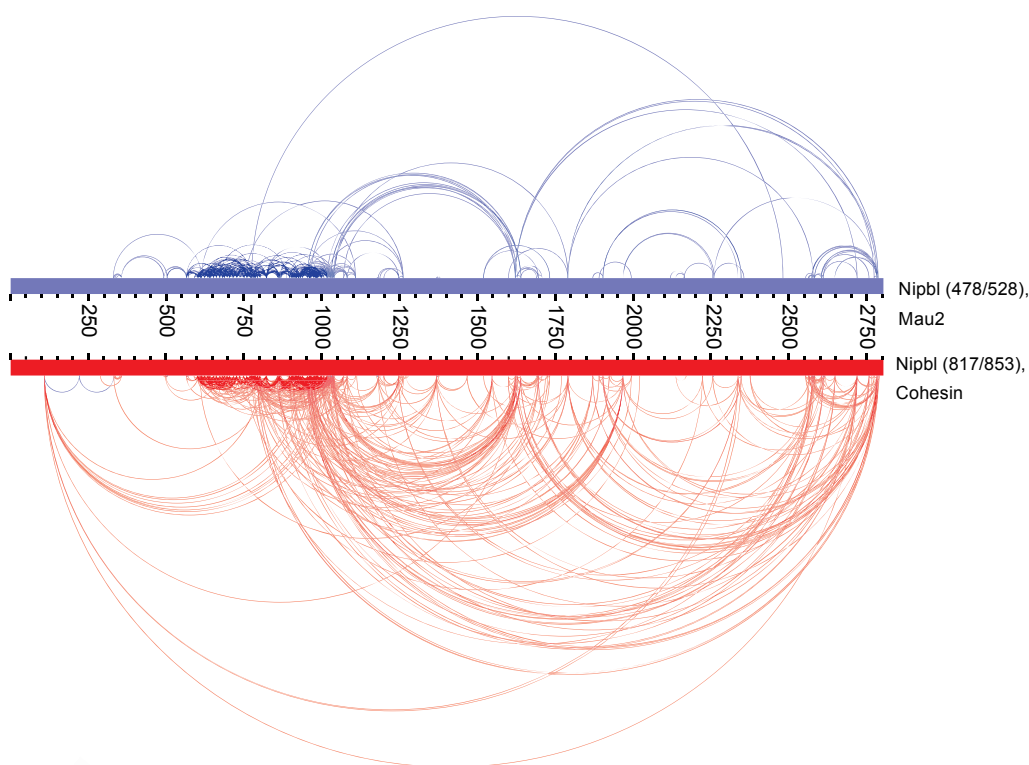
Both Nipbl:Cohesin data sets had been validated, however, the validation process is only presented for experiment 1 where 20 times (compared to Exp. 2) more inter-protein cross-links between Nipbl and Cohesin were detected. 38 % of those crosslinks were formed with the well conserved part of Nipbl ^{1,183-2,804}. To establish the validity of the database, each cross-linked peptide was mapped onto an existing crystal structures and the distance between the C α atoms of the cross-linked lysines was measured. A cross-link was considered valid based on the length of the cross-linker DSS, that was used in all

experiments, and if the distance between the C α atoms of the cross-linked lysines from the native structure was less than 30 Å (Merkley et al., 2014). In the case where structures were determined for proteins from other species, protein alignments were used to identify the corresponding human residue. In total 74 % of all mapped cross-links (n=125) fit those distance constraints (Figure 26 C) including 6 out of 7 of the SA1 intra-protein and 5 out of 5 of the Smc3:Scc1 inter-protein cross-links. The highest percentage, where 32 % of cross-links (16/51) did not fit the constraints, had been measured in the *Ct. Nipbl* crystal shown in Figure 26. This may point to an intrinsic flexibility in Nipbl's hook structure. A second case with a large fraction of unexpectedly large distances, was the hinge domain formed by the central domains of Smc1/3 where 2 out of 5 of the crosslinks spanned residues more than 30 Å apart (Figure 26 C). The 2 cross-linked peptides had been found at the corner of the crystal (at the beginning of the coiled-coil domains) which might be due to a crystallization artefact. Strikingly, 32 % of cross-links (13/40) located at the Cohesin exit gate Smc3:NScc1 did not match the validation constraints. Importantly, these invalid cross-links were situated at accessible protein surfaces and were not buried. In conclusion, 74 % of measured distances of intra- and inter-protein cross-links (n=125) matched the expected distance constraints based on the length of the cross-linker and available atomic resolution protein structures.

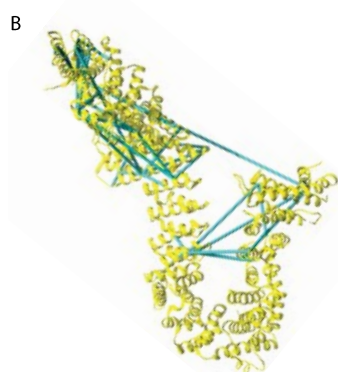
In addition, I compared the Nipbl intra-protein cross-links from the Mau2:Nipbl (n=523) and Nipbl:Cohesin data sets (exp.1, n = 856). 63 % of intra-protein cross-links from the Nipbl:Mau2 were also identified in the Nipbl:Cohesin set (exp. 1). The overall distribution of these intra-protein cross-links appeared to be similar in both data sets (Figure 26 A). Nearly 85 % (Mau2-Nipbl data set) and 62 % (Nipbl:Cohesin data set, exp.1) of protein-protein interaction had been detected in the mainly unstructured domain of Nipbl^{1-1,182}. This was expected due to the high frequency of lysines located in this region (300-1100) and as this region most-likely is unstructured and thus solvent accessible (Figure 24 B). 2.3 % (Mau2-Nipbl) and 9.6 % (Nipbl:Cohesin) of the interactions were detected between the unstructured part of Nipbl (715-1177) and the more conserved, structured part of Nipbl (1183-2804). 13 % (Nipbl:Mau2) and 29 % (Nipbl:Cohesin, exp. 1) of intra-protein cross-links were located in the conserved part of Nipbl^{1,183-2,804}.

Results

A



B



51 Nipbl intraprotein cross-links

C

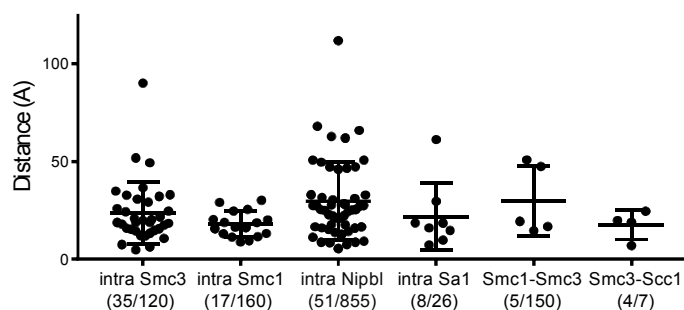


Figure 26: Validation of cross-links identified in Cohesin:Nipbl experiment 1.

Analysis of the cross-linking mass spectrometry experiment of Nipbl/Mau2 complex or Nipbl/Cohesin complex. (A) Bar blot comparing all uniquely determined Nipbl intra-molecular cross-links from the Nipbl-Mau2 cross-linking experiment (in blue) with those found in the Nipbl:Cohesin cross-linking experiment (in red, Figure 24 C). Number of uniquely determined and the total number of cross-links (including ambiguous cross-links) are indicated in parenthesis at the right (unique/total). (B) Cartoon of *Ct* Nipbl (PBD: 5T8V) is shown in yellow. Intra-molecular cross-links are marked with cyan lines. (C) Overview of measured distances of all inter/intra-molecular cross-links that could be assigned to available structural models. In the case where structures were determined for proteins from other species, protein alignments were used to identify the corresponding human residue.

A striking feature of the distribution of cross-links in the Nipbl samples were 14 intra-protein contacts found between Nipbl residue 110, and residues 222, 802, a region between

927 – 1018, 1622, 2337, 2347 and 2719 in the Nipbl:Cohesin data set. These widely distributed contacts were not detected in the Nipbl:Mau2 data set, as residue 110 resides in the Mau2 binding site of Nipbl (mapped between residues 1-115) and, accordingly would likely be blocked when the site is occupied by Mau2.

The validation of the identified cross-links in Nipbl:Cohesin exp. 1 and exp. 2 data sets indicated that both data sets contain protein-protein interactions with physiological relevance. Overall the Cohesin intra- and inter-protein cross-links were consistent with those found in previous studies (Chao et al., 2017, Huis in 't Veld et al., 2014, Hons et al., 2016). Previously, protein interactions between all domains of Smc1 and Smc3 had been identified. Also known binding surfaces such as the exit gate formed by NScc1:CSmc3, the SA1:Scc1 interface as well as the Smc1:SA1 binding site had been observed.

Both data sets suggested that Nipbl forms contacts with all Cohesin subunits. In total, 11 putative Nipbl:Cohesin interactions had been detected in the Nipbl:Cohesin exp. 2. Nipbl region 787-865 cross-links with 3 regions in the coiled coil domain of Smc1/3, close to the hinge (Smc1 677 and 420, Smc3 743). Another putative interaction was detected from Nipbl 328 and 500 with Smc1 294 and Smc3 218 (both coiled-coil domain), respectively. Nipbl 974 and 965 interacted with SA1 905 and Scc1 364, respectively, both residues were located at the SA1:Scc1 binding interface. Lastly, Nipbl 1200 and 1261, residues located in the structured part of Nipbl, were cross-linked to two critical lysines in Smc3 (105/6): the acetylation of these residues correlates with stable binding of Cohesin to DNA, an important mechanism ensuring that sister chromatids stay connected from S-phase until the end of mitosis.

A total of 278 cross-links between Nipbl and Cohesin subunits were detected in the Cohesin:Nipbl experiment 1 data set. These cross-links are distributed all along the Cohesin ring circumference, and some residues, surprisingly, cross-link multiple regions frequently (Figure 24 B). Such frequently cross-linked peptides often detect protein interactions that would be physically impossible – that is they could not form at the same time in a single, isolated complex. In total, Nipbl formed 32 cross-links to Scc1. There were 3 cross-links at the exit gate - Scc1 72 cross-linked to Nipbl 1791 and 2574 while Scc1 82 connected

to Nipbl 1548 – and these three Nipbl residues are found far apart in the crystal structure. Many other wide ranging and fairly promiscuous cross-linking interactions were found including: (1) 29 putative Scc1:Nipbl protein-protein interactions detected at the Scc1:SA1 interface (2) Scc1 residues 317 to 387 cross-linked to 23 residues in Nipbl's unstructured domain (Nipbl 726 - 1034) as well as 9 residues in the more structured domain (Nipbl 1548, 1791, 1968, 2563-2783) (3) 69 cross-links between SA1 and Nipbl were detected, revealing no obvious binding-pattern (4) 55 putative Smc1:Nipbl interactions were detected and these ranged over all the Smc1 proteins domains (13 cross-links at the head domain, 35 coiled-coils and 7 at the hinge domain). (5) Of the 122 total Smc3:Nipbl cross-links detected, 52 localized to Smc3 head domain, 54 along the coiled-coils and 16 to the hinge domain. Interestingly, a total of 10 % of all Scc1 as well as 15 % of all Smc3 inter-cross-links from Scc1 and Smc3 to Nipbl, localized to the exit gate of Cohesin. The Nipbl:Scc1 binding site, proposed by Kikuchi et al 2016, was detected in neither data set, likely due to the absence of available lysines in this Scc1 region (Figure 25 B).

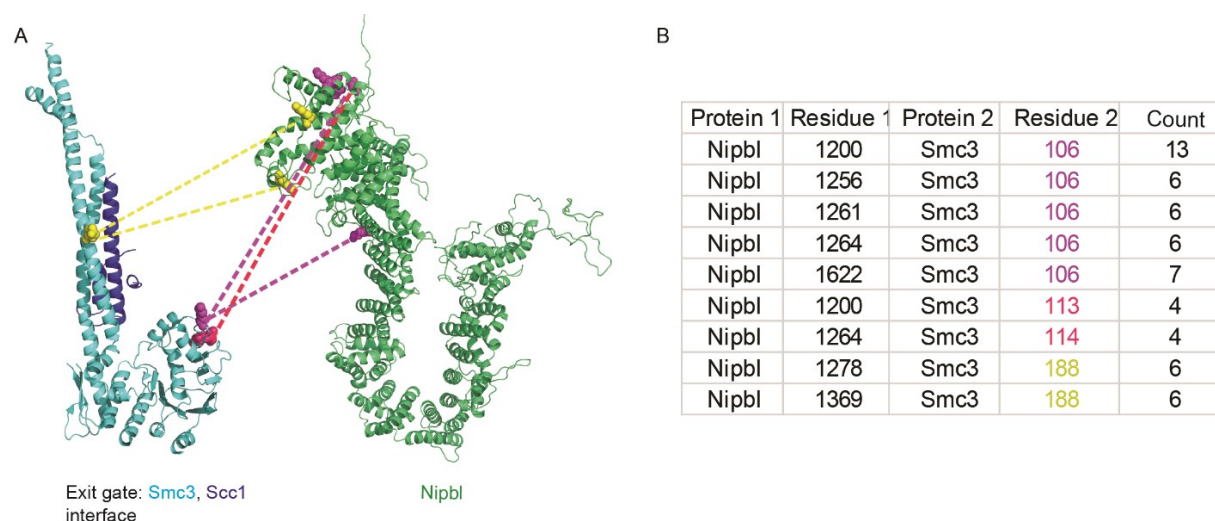


Figure 27: Frequently identified cross-links localized at the acetyl loop and exit gate of Smc3.

(A) Proximal lysines that form cross-links are mapped into the cartoon representation of the crystal structure of the Smc3-Scc1 interface (PDB: U4X3) and are connected to their counterpart in the crystal structure of Nipbl (human model, PDB: 5T8V). (B) Table of all cross-links identified and used for visualization in (A) (Count = CSM value).

Due to the high number of Nipbl:Cohesin in the cross-linking mass spectrometry experiment 1, I filtered the data set using the cross-link spectrum match (CSM) value (Figure

27 A, B). The CSM describes the number of MS/MS spectra that were matched to the cross-link peptide sequence. This reflects the abundance of a given cross-link and can be seen as a label-free method for semi-quantification. 77 % of Nipbl: Cohesin cross-links had a CSM value of 1, while 3.4 % of Nipbl:Cohesin cross-links had a CMS value between 5 - 10 and just 1 interaction, Nipbl¹²⁰⁰ to Smc3¹⁰⁶, had 13. Focusing on the Nipbl:Cohesin cross-links that had a CSM value greater than 4, showed that solely two important domains in Smc3 cross-linked with Nipbl 1200-1622. (1) Smc3 residue 188, located in the exit gate and (2) Smc3 residues 106 and 114, located in a loop, implicated in stabilizing Cohesin's interaction with chromatin (Figure 27 A, B).

Only Smc1 and Smc3 intra- and interprotein and Nipbl intraprotein cross-links had CMS values greater than 9. Most Smc1/3 cross-links were in accordance to previously published human Cohesin cross-linking data showing that the coiled-coil domains of both Smc proteins are frequently in proximity to each other (Hons et al., 2016, Huis in 't Veld et al., 2014, Chao et al., 2017). Interestingly, a subset of cross-links was indicating that the coiled-coil domain of Cohesin not just interacts with each other to form a rod like-structure. They could be much more flexible and even fold-back on itself in a way that the head and the hinge domain would come closer together (Figure 28 A). Examples were cross-links from Smc1 551 at the hinge domain to Smc3 936, a residue situated in the coiled-coil domain proximal to the head domain. Cross-links from Smc1 72 at the head domain to Smc3 820 in the middle of the coiled-coil domain. Smc3 308 to Smc3 729 or Smc3 459 to Smc3 308, both residues were in the coiled-coils but too far apart to be cross-linked if the coiled-coil domain would be an open ring or rod-like structure. Important to note is that the discussed putative interactions between the Smc1/3 coiled-coil domains and the coiled-coil domain to hinge domain had been frequently found, as judged by the CSM value. However, there had been many more of these interactions, including head to hinge interactions, in the non-filtered data set (appendix Figure 30). To exclude, experimental difference and to get a hint if the binding of Nipbl induce a folded-back Cohesin conformation, I performed a control experiment where I purified Cohesin and performed cross-linking in solution.

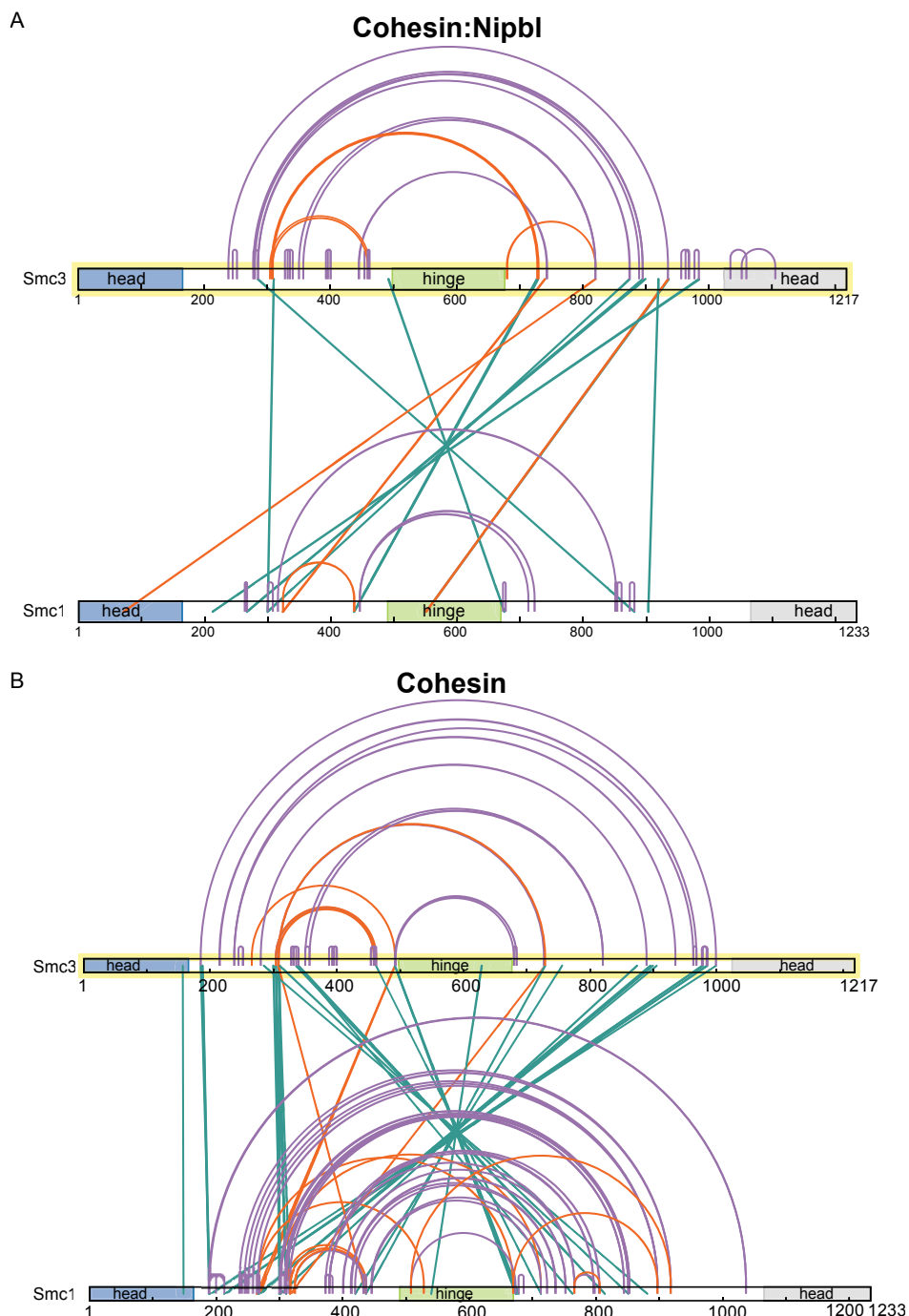


Figure 28: Repeatedly found cross-links in Nipbl:Cohesin or only Cohesin sample indicate that Cohesin folds the coiled-coil back on themselves.

The poly peptide chains of Smc1 and Smc3 form a central hinge domain, that dimerized. The hinge domain is followed by an antiparallel discontinuous coiled-coil domain that end in a head domain that is formed by their N- and C-termini. Both head domains are dimerizing to form the ATPase centres. Proximity maps of Smc1 and Smc3 intraprotein (purple) and interprotein (green) links. Cross-links that are not in agreement with a rod shaped coiled-coil formation, but rather indicate a back-folded structure are depicted in orange. (A) Cross-links with a CSM value greater than 9, from the Nipbl:Cohesin exp. 1 (n=82). (B) Cross-link from the Cohesin experiment (n=97).

The cross-link experiment was performed in collaboration with Zsuzsanna Orban-Nemeth. In total 142 putative interactions had been found. A proximity map of all putative interactions is shown in appendix Figure 31). Overall, the cross-links were in accordance to previously published data (Hons et al., 2016, Huis in 't Veld et al., 2014, Chao et al., 2017). Less cross-links had been detected and that why I compared all detected intra- and inter-protein cross-links to the filtered Nipbl:Cohesin data set. Interestingly, Cohesin alone showed putative protein-protein interactions that are consistent with very flexible coiled-coil conformations of Cohesin as well (Figure 28 B). In conclusion, there was no indication that Nipbl induced a certain Cohesin conformation upon binding.

4 Discussion

Cohesin loading onto chromatin is mediated by the loading complex, Nipbl/Mau2, and involves Cohesin's ATPase activity. Recent studies suggested that Cohesin's ATPase activity might not only be important for the initial loading step, but could also contribute to Cohesin's function while it is associated with chromatin. It has been hypothesized that Cohesin forms small DNA loops and actively enlarges them by extruding DNA through its ring and thereby structures chromosomes (Nasmyth, 2001). This mechanism, often referred as "loop extrusion", could explain Cohesin's role in gene regulation because it would allow Cohesin to bring distant enhancers and promoters in vicinity. Furthermore, this looping mechanism of Cohesin might also help during sister chromatid cohesion by individualizing sister chromatids prior to cell division. During my studies, sophisticated chromatin conformation capture experiments indeed showed that the depletion of Cohesin affects the higher order structure of chromosomes (Schwarzer et al., 2016, Wutz et al., 2017, Fudenberg et al., 2016). Upon depletion of the Cohesin release factor Wapl in HAP1 cells, longer DNA loops were detected in HiC-experiments, which correlated with the presence of Nipbl/Mau2 (Haarhuis et al., 2017). In another recent study scientists have managed to visualize the related Smc complex Condensin in an *in vitro* TIRF microscopy setup while it was spooling DNA through its ring (Ganji et al., 2018). For these and other experiments in yeast, it has been speculated that Cohesin might structure chromosomes through loop extrusion and that Nipbl could be the factor that drives Cohesin's ATPase function in this process (Nasmyth, 2001, Haarhuis et al., 2017).

These recent findings make it even more interesting to study the interplay between Cohesin and its loading complex. So far, it is unclear how Nipbl stimulates Cohesin's ATPase activity and how this relates to Cohesin loading and potentially to loop extrusion. Binding assays and chemical cross-linking mass spectrometry of Nipbl:Cohesin implied that all Cohesin subunits interact with Nipbl. This finding is consistent with studies performed in yeast (Chao et al., 2017, Murayama and Uhlmann, 2014). My experiments show that Nipbl^{1,183-2,804} is sufficient and even more potent than full length Nipbl in stimulating Cohesin's ATPase activity *in vitro*, while Mau2 and Nipbl^{1-1,882} are dispensable. Additional experiments suggested that mutations in the HEAT-repeat domain of Nipbl^{R1,856A, R1,865A,}

K1,866A, R1,867, R1,895A, referred to as Nipbl-PA, reduce Nipbl's stimulatory effect on Cohesin's ATPase activity. The reaction was also not responsive to the addition of DNA anymore. Preliminary experiments also show that the Nipbl-PA mutant experiences a reduced binding to the Cohesin subunit Scc1. This factor was identified to be crucial for Nipbl's ability to stimulate Cohesin's ATPase activity. These results together with recent publications suggest that Nipbl might contact Scc1 and potentially also DNA via its HEAT repeat domain (Kikuchi et al., 2016, Petela et al., 2017). This might trigger Cohesin loading onto chromatin and/or loop extrusion.

4.1 Nipbl potentially interacts with all Cohesin subunits.

It is still elusive how the loading complex interacts with Cohesin to load Cohesin onto chromatin and how this might lead to loop extrusion. In the literature, there are several hypotheses trying to understand how Cohesin loading is achieved. The extended conformation of *Ashyba gossypii* Scc2/4, orthologs of human Nipbl/Mau2, was measured to be around 28 nm (Chao et al., 2015). Nipbl orthologs from fungus are smaller than human orthologs. However, the inner diameter of Cohesin is around 40 nm (Haering et al., 2002). Given that the hinge domain and the ATPase domain of Cohesin are located at opposite ends of the complex and both are believed to participate in the loading process, there are multiple possible mechanisms on how loading could be achieved (Gruber et al., 2006, Arumugam et al., 2003, Weitzer et al., 2003). Firstly, upon hydrolysing ATP conformational changes could occur that are transmitted to the coiled-coil domains and thereby open the Cohesin hinge, so that DNA can enter the ring (Nasmyth, 2011). Secondly, Cohesin could adopt a conformation that would bring the head and the hinge domain in close proximity. Nipbl could then open the hinge domain and activate Cohesin's ATPase activity. This could be achieved by the discontinuous coiled-coil domains of Smc1/3 as they might fold back (Gruber et al., 2006). Thirdly, recently it was proposed that the Cohesin's exit gate formed by Smc3:Scc1N could also serve as an entry gate (Chao et al., 2017). The exit gate and the Smc1/3 heads would then be in close proximity. Lastly, the postulated loop extrusion mechanisms would not even require an entry gate, because Cohesin could simply spool DNA through its ring (Petela et al., 2017). To shed some light on these hypothesis, we performed binding assays in combination with chemical cross-linking mass spectrometry to map potential binding sites between Nipbl and Cohesin. Although, we

obtained a high number of cross-links between Cohesin and Nipbl, our initial data suggested that Nipbl interacts with all Cohesin subunits, showing no direct binding interface that we could inspect more closely. Nevertheless, we have identified some cross-links at the DNA exit gate Smc3:NScc1 and tested two Cohesin mutants of this region. One of them has a covalently closed exit gate and the other one an exit gate that is weakened by mutations in Scc1 (Huis in 't Veld et al., 2014). Both complexes were still stimulated by Nipbl and therefore did not reveal a striking defect. These experiments neither confirm nor exclude the model that postulates Cohesin's exit gate might act as entry gate. Cohesin loading could be a step wise process and ATP-hydrolysis could be uncoupled from opening a gate.

My cross-linking data obtained from human proteins agrees with cross-linking mass spectrometry experiments and a peptide array assay performed with budding and fission yeast Cohesin and Scc2^{Nipbl}/Scc4^{Mau2}, respectively. Both studies identified potential protein-protein interactions around the whole Cohesin ring circumference (Chao et al., 2017, Murayama and Uhlmann, 2014). Interestingly, the cross-linking experiments with *S. pombe* Mis4^{Nipbl}/Ssl3, detected some putative protein-protein interactions between Mau2 and Cohesin subunits, namely with the coiled-coil domain of Smc1 residue 764 and Smc3 residue 387, the head domain of Smc3 residue 1010 and with Scc1 residue 387 (Chao et al., 2017). Mau2 was excluded from our cross-linking mass spectrometry experiments, because it was dispensable for enhancing Cohesin's ATPase activity as well as for the formation of a Nipbl:Cohesin complex.

The fact that a lot of putative Cohesin to Nipbl are detected by the cross-linking experiments, might not seem surprising. In negative stain electron microscopy Cohesin and its loader seem to be flexible (Hons et al., 2016, Chao et al., 2015). It appears reasonable that most cross-links are a result of being in proximity by mere chance. It is also conceivable that the cross-linking mass spectrometry experiments show a lot of possible conformations that Cohesin and Nipbl can adopt. To get a clearer result, I would repeat the cross-linking mass spectrometry experiments, but use the Nipbl^{1,183-2,804} fragment, that is missing over 1,182 aa of unstructured regions, but still enhances Cohesin's ATPase activity. In addition, I would use a non-hydrolysable form of ATP, with the aim of locking

Cohesin and Nipbl in a certain conformation. Cohesin:Nipbl cross-linking has been performed in solution. It would also be interesting to load Cohesin:Nipbl onto DNA and to then perform cross-linking mass spectrometry.

It is still unclear what conformation Cohesin adopts in solution before loading, while it associates with chromatin and bound to other regulatory proteins. Tetrameric Cohesin has shown to display different conformations, depending on the visualization method. Rotary shadowing electron microscopy experiments detected mainly open-ring structures while negative stain electron microscopy rod-like conformations. Both visualization methods showed that the coiled-coil domain displayed kinks. These kinks remind of the fission yeast Cohesin dimer, Pms1^{Smc1} and Pms3^{Smc3}, that adopt mainly a bend conformation in atomic force microscopy (Sakai et al., 2003). In addition, *S. cerevisiae* Smc2-Smc4 dimers, subunits from the related Smc complex Condensin, show a very dynamic behaviour and adopt multiple conformations in high speed atomic force microscopy in solution (Eeftens et al., 2016). The hinge domain of the Smc2/4 dimer frequently contacted the heads domain (Eeftens et al., 2016). Previous cross-linking mass spectrometry of tetrameric Cohesin have detected putative protein-protein interactions between the coiled-coil domain of Smc1 and Smc3, being consistent with Cohesin being an open-ring or a rod-shaped complex. The cross-linking mass spectrometry experiments carried out in this study suggests that Cohesin can adopt even more conformations. Putative interactions from hinge to head domains have been detected, as well as cross-links in the coiled-coil domain that are neither consistent with a rod-shaped nor an open conformation. These cross-links suggest that the coiled-coil domains are very flexible and can adopt lots of conformations. It is unlikely that these putative interactions had been formed by multiple Cohesin complexes being in proximity by mere chance because those conformations have also been detected in a data set where I filtered for high abundant cross-links (in the Nipbl:Cohesin experiment 1). Previous cross-linking mass spectrometry experiments on human Cohesin have been performed while Cohesin was bound to beads which might favour an open/rod shaped Cohesin conformation (Hons et al., 2016). In this study, the Cohesin:Nipbl complex or Cohesin alone was cross-linked in solution. It is conceivable this high degree of flexibility is due to the cross-linking in solution. The head to hinge and the various interactions in the coiled-coil domain of Smc1 and Smc3, have been found in the Nipbl:Cohesin but also

in the Cohesin only experiment. This implies that this interaction might not be induced upon binding of Nipbl to Cohesin. However, an *in vivo* study carried out in budding yeast, detected a putative interaction between the hinge domain of Smc1 with Pds5 that binds to Scc1 using fluorescence resonance energy transfer (FRET) pairs (Intyre et al., 2007). This suggests that the discontinues coiled-coil domains of Smc1/3 might allow conformations where head and hinge are in proximity *in vivo*.

4.2 Nipbl enhances Cohesin's ATPase activity.

I studied Nipbl's stimulatory effect on Cohesin's ATPase activity *in vitro*, by performing ATPase assays based on thin layer chromatography. Human tetrameric Cohesin complexes hydrolyse 1.77 ± 0.6 ATP/min at 32 °C. This activity is in accordance with previous studies where wild type human dimers and trimers hydrolyzed 0.3 ATP/min and 2.1 ATP/min, respectively, *S. cerevisiae* trimeric Cohesin 2.1 ATP/mol and *S. pombe* tetrameric complexes 3.4 ATP/min (Murayama and Uhlmann, 2014, Ladurner et al., 2014, Arumugam et al., 2006). Some variations in the specific activities can be explained because different Cohesin subcomplexes had been tested. The rate of hydrolysis of the *S. cerevisiae* and human Smc1/3 dimers were shown to be enhanced by the presence of Scc1 (Arumugam et al., 2006, Ladurner et al., 2014). Variations in the specific activities of Cohesin can also be due to differences in buffer composition and species specific. Most importantly, I showed that the recombinant human Nipbl stimulates Cohesin's ATPase activity and this effect is enhanced by the addition of DNA. DNA alone has no effect on Cohesin's ATPase activity. I was also able to abrogate Nipbl's stimulatory effect by mutations in the catalytic centres of Cohesin. Nipbl's stimulatory effect to Cohesin's ATPase activity are in accordance with studies in *S. cerevisiae* and *S. Pombe* (Murayama and Uhlmann, 2014, Petela et al., 2017).

4.3 Nipbl^{1,183-2,804} is sufficient to stimulate Cohesin's ATPase activity *in vitro*.

Human Nipbl is a large protein with 316 kDa. Most parts of its N-terminus of Nipbl are unstructured. Nipbl^{1,183-2,804} contains a HEAT repeat domain. A recent study proposed that Scc1 would bind to this region (Kikuchi et al., 2016). I set out to generate smaller Nipbl fragments with the aim to find a minimal Nipbl interface that still interacts with Cohesin and enhance its ATPase activity. Secondary structure predictions and previous studies were used to design the Nipbl fragments. Affinity purification and gel filtration chromatography revealed that most of the newly designed Nipbl fragments aggregated. Mapping the Nipbl truncations onto the crystal structure suggested that some fragments might have had problems to fold properly because of missing secondary structures. Another possible reason for the aggregation might originate from the fact that Nipbl's N-terminus is inherently unstructured. N-terminally Nipbl fragments might therefore be prone to aggregation, if the structured C-terminus is missing.

Nipbl¹⁻⁴⁹⁷ in complex with Mau2 is not aggregated and forms a complex with Cohesin *in vitro*. At first, this result was surprising due to the fact that initial binding experiments comparing Nipbl alone and in complex with Mau2 indicated that Mau2 is dispensable for this interaction. However, this result is in accordance with Chao et al., 2017. Their cross-linking mass spectrometry experiments detected multiple putative interaction sites between *S. cerevisiae* Mau2 and Smc1, Smc3 and Scc1 (Chao et al., 2017).

Nipbl^{1,183-2,804} is another fragment that was generated. It contains the HEAT repeat domain that has been implicated in Scc1 binding (Kikuchi et al., 2016). Nipbl^{1,183-2,804} stimulates Cohesin's ATPase activity. Cohesin's specific activities are enhanced to a similar extent by either full length Nipbl or Nipbl¹¹⁸³⁻²⁸⁰⁴ in the absence of DNA. The addition of DNA enables Nipbl^{1,183-2,804} to further boost the activity of Cohesin exceeding the capabilities of full length Nipbl. This Nipbl fragment was generated because it aligns to *S. pombe* Mis4^{Nipbl192-1,587} (Chao et al., 2015) The *S. pombe* Mis4^{Nipbl} fragment loaded Cohesin onto plasmid DNA in an *in vitro* loading assay. *In vivo*, Cohesin did not associate with chromatin anymore, most likely due to the missing Ssl3^{Mau2} (Chao et al., 2015). Furthermore, a very similar human Nipbl fragment (deletion upstream of exon 11) was generated in the

Rowland lab (Haarhuis et al., 2017). This fragment still load Cohesin to chromatin in HAP1 cells *in vivo* and overcame the need for Mau2 (Haarhuis et al., 2017). Taken together, my experiments together with previous studies, imply that the C-terminus of Nipbl is responsible for stimulating Cohesin's ATPase activity and that this facilitates the loading of Cohesin onto DNA *in vitro* and *in vivo* (Chao et al., 2015, Haarhuis et al., 2017).

4.4 Scc1 and the addition of DNA might regulate the Nipbl-dependent stimulation of Cohesin's ATPase activity.

Human Nipbl forms a complex with the Cohesin dimer Smc1/3, an interaction that had been previously detected by Bermudez et al., 2012. Although, Nipbl binds to the dimer, its ATPase activity is not enhanced. Trimeric Cohesin, Smc1/3 in complex with Scc1, on the other hand is stimulated by Nipbl. This suggests that Scc1 either regulates this interaction directly or simply brings the head domain together more frequently by connecting the head domains and thus boost the activity of Smc1/3 (Haering et al., 2004, Gruber et al., 2003). Interestingly, tetrameric Cohesin, with a cleaved Scc1 subunit behaves like non-cleaved Scc1. Cross-linking mass spectrometry showed a lot of putative Cohesin:Nipbl interactions. It might well be that there are so many Cohesin:Nipbl interactions that this can overcome a cleaved Scc1 subunits and Nipbl can enhance Cohesin's ATPase activity. The stimulation is not due to the presence of SA1 because preliminary results indicate that both trimeric and tetrameric Cohesin have the same specific activity. This suggests that Scc1 itself is important for the stimulation of Cohesin's ATPase activity.

Human Cohesin trimeric and tetrameric complexes behave differently to what was published for budding and fission yeast Cohesin. The ATPase activity of *S. pombe* tetrameric Cohesin was only stimulated by the addition of both DNA and Mis4^{Nipbl}/Ssl3^{Mau2} (Murayama and Uhlmann, 2014). This contrasts with human and *S. cerevisiae* tetrameric Cohesin that is stimulated by Nipbl^{Scc2} alone – a reaction that is further boosted upon addition of DNA (Petela et al., 2017). Trimeric Cohesin also shows a different activation pattern in the three species. As mentioned before, human trimeric and tetrameric Cohesin complexes are stimulated by Nipbl alone and the addition of DNA enhances this reaction. *S. cerevisiae* trimeric Cohesin is only stimulated by the addition of both DNA and Nipbl, while tetrameric Cohesin is already stimulated by Nipbl and further enhanced by the DNA

(Petela et al., 2017). *S. pombe*, trimeric Cohesin was shown to be stimulated by both plasmid DNA and Mis4^{Nipbl}/Ssl3^{Mau2} (Murayama and Uhlmann, 2014). Mis4^{Nipbl} alone was not tested. This stimulation was enhanced upon addition of the Psc3^{SA} protein. The boosted ATPase activity correlated with an increase in Cohesin loading onto plasmid DNA in the bulk *in vitro* loading assay. Mutations in Psc3^{SA} reduced the loading efficiency but also the specific ATPase activity (Murayama and Uhlmann, 2014). The differences in the activation pattern are interesting to note, but might be explained by species variations or contamination with residual DNA, while purification of the proteins.

Preliminary results from studies with the human Cohesin complex, suggested that Scc1 might be important for the Cohesin:Nipbl interaction. This finding became interesting when the structures of the Nipbl orthologs *Chaetomium thermophilum* (C.t) Scc2^(Nipbl)385–1,840 and *Ashyba gossypii* Scc2^(Nipbl)378–1,479 had been solved by Kikuchi et al., 2016 and Chao et al., 2017, respectively. Both studies identified residues in the HEAT-repeats of Nipbl as being important for Cohesin's function. Kikuchi et al., 2017 showed that *C. t.* Scc2^(Nipbl)385–1,840 interacts with *in vitro* transcribed-translated *Ct.* Scc1¹²⁶⁻²³⁰. The authors introduced single point mutations into Nipbl, based on electro static potential, conservation and comparison with human CdLS patients. Some point mutations led to a weakened binding between Nipbl and *Ct.* Scc1¹²⁶⁻²³⁰. The authors concluded that five residues in *Ct.* Nipbl, namely Scc2^(Nipbl)R1,089G, Scc2^(Nipbl)R1,090T, Scc2^(Nipbl)K1,091E, Scc2^(Nipbl)R1,092E, Scc2^(Nipbl)R1120T are crucial for Scc1-binding, but are not interfering with protein folding (Kikuchi et al., 2016). I mutated these five residues in human Nipbl to alanine, Nipbl^{R1,856A, R1,865A, K1,866A, R1,867, R1,895A}, referred to as Nipbl-PA. Nipbl-PA seems to interact less well with human Scc1¹⁰⁰⁻²⁵⁰ as compared to wild type Nipbl. This finding implies that the Scc1:Nipbl binding site might be conserved from *C.t.* to humans (Kikuchi et al., 2016). Interestingly, despite its reduced binding to Scc1, Nipbl-PA formed a complex with tetrameric Cohesin. This agrees with Chao et al., 2017 because they introduced 2-point mutations to budding yeast Scc2^(Nipbl)K788A, K792A, corresponding to human Nipbl^{K1,866, K1,870}. Importantly, one of the two introduced mutations is the same amino acid, Scc2^(Nipbl)K788A, as in human Nipbl-PA^{K1,866A}. Substitution of wild type Scc2^(Nipbl) with Scc2^(Nipbl)K788A, K792A in *S. cerevisiae* reduced viability (Chao et al., 2017). It also reduced Cohesin levels at known genomic loci, measured by ChIP-qPCR. The mutated Nipbl still bound to Cohesin *in vitro* and *in vivo* tested by co-

sedimentation in glycerol gradients and co-immunoprecipitation from cell lysates, respectively (Chao et al., 2017). Another recent study in budding yeast showed that deletions in Scc1 have a major effect on Cohesin (Petela et al., 2017). *S. cerevisiae* Scc1^{Δ152-256} was still able to form a trimeric Cohesin ring, but led to a loading defect in yeast cells *in vivo*. The Nipbl-dependent ATP hydrolysis was reduced *in vitro* (Petela et al., 2017). Together, these data point towards a functional relationship between Nipbl and Cohesin's Scc1 subunit. This is still consistent with the cross-linking mass spectrometry data obtained in this study as well as the reported data for budding yeast (Chao et al., 2017). Multiple putative Scc1:Nipbl interactions had been detected but not all interactions necessarily need to play an important role in enhancing Cohesin's ATPase activity.

Not only Scc1 but also the addition of DNA seems to be an important for the Nipbl-dependent stimulation of Cohesin's ATPase activity. Indeed, the human Nipbl-PA stimulates Cohesin's ATPase activity less efficiently than wild type Nipbl. In addition, the Nipbl-PA mutant is not responsive to the addition of DNA anymore. All residues mutated in Nipbl-PA/R1,856A, R1,865A, K1,866A, R1,867, R1,895A are very well conserved. Strikingly, almost all of them are basic amino acids. So, it might well be that those residues are binding to the negatively charged DNA which could be an important mechanism. There are three main observations in the literature that make this result interesting. First, the Nipbl-PA data is consistent with a study in *S. cerevisiae* (Petela et al., 2017). They substituted only two of the five residues from human Nipbl-PA mutant, namely Scc2^(Nipbl)K1866, R1867. This *S. cerevisiae* Scc2^(Nipbl)K788E, H789E supported Scc1 association with the centromeric Cohesin loading sites in yeast. However Cohesin was not found any more at other loci as measured by calibrated Chip-seq (Petela et al., 2017). Scc2^(Nipbl)K788E, H789E showed a reduced stimulatory effect toward Cohesin's ATPase activity *in vitro*. Strikingly, there was no response to the addition of DNA anymore (Petela et al., 2017). Second, the crystal structure of *S. cerevisiae* Ycg1, a HEAT repeat containing protein, that binds to the kleisin subunit of Condensin, was solved in complex with DNA. Nipbl adopts a very similar structure as Ycg1 and therefore might also bind to Scc1 and DNA in the same fashion (Kschonsak et al., 2017). Third, fission yeast Mis4^{Nipbl} binds to dsDNA *in vitro* (Murayama and Uhlmann, 2014). So, it would be interesting to test if Nipbl-PA is still able to interact with DNA *in vitro*.

In conclusion, experiments shown in this thesis are overall consistent with previous studies in fission/budding yeast and human cell lines. Together, the data support the notion that Nipbl might contact multiple Cohesin subunits, as shown by cross-linking mass spectrometry, binding assays and peptide array assays by myself and others (Chao et al., 2017, Murayama and Uhlmann, 2014). The Nipbl^{1,183-2,804} seems to be the part of Nipbl that is responsible for stimulating Cohesin's ATPase activity. In *S. pombe*, a similar Mis4^(Nipbl) mutant stimulated Cohesin loading onto plasmid *in vitro* (Chao et al., 2015). *In vivo*, this part of Nipbl was shown to be important for Cohesin loading in human HAP1 cells (Schwarzer et al., 2016). Nipbl^{1,183-2,804} is still 160 kDa. Truncating this protein further might help to nail down important functional parts of Nipbl or one might be able to distinguish multiple functional sites. The available crystal structures will help to generate these fragments (Kikuchi et al., 2016, Chao et al., 2017). Especially the HEAT-repeat region of Nipbl certainly deserves a further investigation. This study and previous studies have shown that the Nipbl-dependent stimulation of Cohesin's ATPase activity is reduced *in vitro* by mutations in the HEAT repeat region of Nipbl (Petela et al., 2017). *In vivo*, mutations in this area lead to defects in Cohesin's association with chromatin, shown for *S. cerevisiae* (Chao et al., 2017, Petela et al., 2017). *In vitro* and *in vivo* experiments suggested that the HEAT-repeat domain might interact with either Scc1, DNA or both. This needs to be investigated further, by testing whether also the human Nipbl-PA mutant can bind to dsDNA *in vitro*. The HEAT repeat domain of Nipbl is implicated in contacting Scc1. By comparing the binding of wild type Nipbl with the Nipbl-PA mutant, preliminary results suggest that Nipbl interacts with some residues in a region of Scc1¹⁰⁰⁻²⁵⁰ and this leads to an enhanced activity of Cohesin's ATPase. The proposed *S. cerevisiae* consensus sequences that interacts with Nipbl, could not be detected in human Scc1 (Petela et al., 2017). Therefore, it would be interesting to investigate this further.

The results of my study, together with previous studies are important steps towards a better understanding of the Cornelia de Lange syndrome (CdLS) (Deardorff et al., 2012, Liu and Krantz, 2008, Chao et al., 2017, Kikuchi et al., 2016, Petela et al., 2017). CdLS is a congenital disorder, that is often caused by heterozygote mutations in the Nipbl gene. Cells derived from CdLS patients display normal sister chromatid cohesion but the

genome-wide distribution of Cohesin is changed. This leads to developmental defects due to differential gene expression pattern (Deardorff et al., 2012, Liu and Krantz, 2008). The mutated residues in the Nipbl-PA mutant, that had been investigated in this study, correlate with mutations found in patients. My own experiments and previous studies have shown that Cohesin can spontaneously load onto DNA in the absence of Nipbl/Mau2 and independent of ATP-hydrolysis (Davidson et al., 2016, Stigler et al., 2016, Murayama and Uhlmann, 2014). The Nipbl-PA mutant enhances Cohesin's ATPase activity less well than wild type Nipbl. The reaction is not responsive to the addition of DNA anymore. So, it might be that Cohesin still loads onto chromosomes *in vivo* in the presence of Nipbl-PA mutant and Cohesin might still be able to perform sister chromatid cohesion. It is known that very little Cohesin complex are needed for this process. Instead, Cohesin's function in loop extrusion might be impaired, leading to an altered Cohesin distribution in the genome. This in turn could cause structural changes in the chromosomes, resulting in changed gene expression pattern as seen in CdLS patients.

5 Material and methods

5.1 Cloning

Standard Gibson assembly cloning was used to introduce cDNAs into the multibac library vector plasmid (pLIB). The pLib vector contains a polyhedrin promoter and terminator sequence for insect cell expression of recombinant proteins. The biGBac cloning system described in (Weissmann et al., 2016) was used to generate co-expression vectors of tetrameric Cohesin-SA2, tetrameric/trimeric Cohesin-Scc1^{TEV-Halo} and Cohesin-Scc1-^{TEV-mKikGR}. For co-expression of various tagged Nipbl with Mau2, pLib Nipbl was digested with AvrII and the expression cassette of Mau2 introduced using Gibson assembly. pET21A vectors were used for bacterial expression in *E.coli*. Different tags for purification were added through primers. Oligonucleotides were ordered from Sigma or mostly from Microsynth. High fidelity Phusion or hot start Phusion polymerase (Thermo Scientific) was used for PCR reactions. A typical PCR reaction contained 20 µl 5x HF or GC buffer, 2 µl dNTPs (stock 10 mM each), 0.5 µl each primer (100 µM), 50 - 200 ng template DNA, 1 µl Phusion polymerase and water in a total volume of 100 µl. PCR products were gel extracted using a gel extraction kit (IMP/IMBA services, Qiagen). PCR products were then inserted into digested vectors using Gibson assembly and transformed into electrocompetent DH10 EMBacY *E. coli* (IMP/IMBA services) using electroporation. The plasmids were extracted using miniprep or maxiprep kits (Qiagen or IMP/IMBA services), analyzed by restriction analysis and sequenced by sanger sequencing IMP/IMBA facility. Lists of all plasmids generated in this study can be found in appendix Table 6, Table 7, Table 8 and Table 9. The cloning strategy were designed in clone manager.

5.2 Generation of baculoviruses

Plasmids with expression cassettes for insect cell expression were transformed into a DH10Bac *E. coli*. The *E. coli* strain contains a bacmid in which the plasmid will be inserted through a transposon that is encoded on a helper plasmid (Invitrogen Bac-to-Bac manual). To ensure that the plasmid was inserted into the right place of the bacmid gene classical blue/ white selection was used. For this, the transformed bacteria were recovered for 4 - 6 h at 37 °C and plated on LB-Agar containing multiple antibiotics such as 7 µg/ml

gentamycin, 50 µg/ml kanamycin 10 µg/ml tetracycline, 0.1 mM IPTG (Fermentas) and 100 µg/ml X-gal (Fermentas). After 48 h positive white colonies were picked and miniprep cultures were seeded overnight at 37 °C. The bacmid was then isolated using standard isopropanol extraction. The isolated DNA was washed with 70 % v/v ethanol, air-dried and finally resuspended with 50 µl sterile water in a cell culture hood to ensure a sterile environment.

10 µl of the extracted DNA was transfected to 1×10^6 adherent Sf9 insect cells on a 6-well plate (Nunc) in serum-free medium (Sf900 IISFM, Gibco) using the transfection reagent Cellfectin (Invitrogen) or FuGENE 6 (Roche) according to the Bac-to-Bac manual. After 3 d, the initial virus (V0) was harvested and amplified to virus generation 1 (V1) by infecting 100 ml Sf9 suspension culture with the V0. The amplified V1 was harvested upon YFP expression, because the YFP expression was an indirect measure of protein expression and served as signal for amplified virus. The virus containing supernatant was sterile-filtered and stored at 4 °C. All baculoviruses generated in this study are listed in the appendix Table 6, Table 7 and Table 8.

5.3 Expression of proteins in insect cells

Most proteins were expressed in Sf9 insect cells (Thermo Scientific). The cells were cultivated in suspension using Grace Insect Cell Medium (Thermo Scientific prepared by IMP/IMBA services) supplemented with 1 % v/v Gibco L-Glutamine (200 mM, Thermo Scientific), 10 % FCS (Invitrogen), 1 % v/v Pen/Strep (stock 10,000 U/mL Penicillin and 10 mg/mL Streptomycin, Sigma) and 1 % v/v Pluronic-F68 (100 x, Invitrogen) at 27 °C. For protein expression, freshly amplified SF9 cells were split to 1×10^6 cells/ml in 500 ml and 5-8 ml V1 or V2 culture was added for 48 h – 36 h at 27 °C or for reducing protein degradation 12 h at 27 °C and reducing the temperature to 21 °C for 12 h – 48 h. A successful protein production was indirectly measured by checking a YFP signal that was expressed independently using a fluorescent cell counter (Luna FL Dual Fluorescence Cell Counter, Logos Biosystems).

To harvest the insect cells, the suspension culture was spun down for 20 min at 2,500 rpm with a Beckmann JS-4.2 rotor at 4 °C. The cell pellets were resuspended in ice-cold

PBS, transferred to a 15 or 50 ml falcon tubes and again pelleted for 10 min, 2,500 rpm with a Hereaus 3SR+ swing-out centrifuge at 4 °C. The cell pellet was frozen in liquid nitrogen and stored at -80 °C.

5.4 Purifications

5.4.1 Purification of Condensin, Cohesin and Cohesin subcomplexes

Complexes were purified using two consecutive affinity steps: Ni-NTA beads (Quiagen) followed by Flag-M2-beads. Pentameric Condensin II contained the following subunits: Smc2, Smc4-flag CAPG2-His, CAPH2-Halo, CAPD3. Tetrameric Cohesin contained His-SA2/1, Smc3-flag, Smc1, Scc1 (various mutants). Trimeric Cohesin contained no SA protein but His-Scc1 and dimeric Cohesin His-Smc1 and Smc3-flag. Single Smc proteins were one step purified using His-Smc1 or Smc3-flag.

Insect cell pellets containing expressed Condensin, Cohesin and Cohesin subcomplexes were thawed on ice and resuspended in lysis buffer (35 mM Hepes pH 7.5, 250 mM NaCl, 5 % glycerol, 3 mM β -mercaptoethanol, 50 mM imidazole, 0.02 % Tween-20, 1 tablet/50 ml Roche complete EDTA-free protease inhibitors, 10 μ g/ml pepstatin 10 μ g/ml chymostatin, 10 μ g/ml leupeptin, 0.1 mM PMSF) on ice. The cells were lysed by dounce homogenization (tight pestle, Wheaton douncer) 4 x 8 strokes on ice prior to clearing by centrifugation at 47,810 x g for 40 min at 4 °C with a SS34 rotor and binding proteins on equilibrated Ni-NTA beads (Quiagen, 1 ml resin/10 ml cell pellet) in batch for 1 h at 4 °C. The resin was washed 3 x with at least 20 bed volumes of washing buffer (35 mM Hepes pH 7.5, 250 mM NaCl, 5 % glycerol, 3 mM β -mercaptoethanol, 50 mM imidazole, 0.02 % Tween-20). The proteins were then either eluted in batch or transferred first into a column (Poly-Prep Chromatography Columns, BioRad) and then eluted with 35 mM Hepes pH 7.5, 150 mM NaCl, 5 % glycerol, 400 mM imidazole, 0.02 % Tween-20, 0.1 mM PMSF using 2 x 2 bed volumes for 30 min, or 10 x 0.5 bead volume, respectively. For Cohesin binding assay, the proteins were dialyzed for 3 h and stored at -80 °C. Optional, proteins were concentrated using Amicon100K MWCO centrifugal filter units (Millipore) before storing.

For a 2 steps purification, the eluted proteins were rebound using equilibrated M2-Flag beads (Sigma, 0.5 ml beads/10 ml cell pellet) for 1 h at 4 °C in batch. Then, the beads were transferred to a gravity flow column (Poly-Prep Chromatography Columns, BioRad) and washed with at least 20 bed volumes (35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 0.02 % Tween-20), prior to elution in multiple small volumes using a buffer containing 0.5 mg/ml flag peptide (IMBA/IMP services) 35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol). The proteins were pooled, optionally concentrated as previously described, aliquoted and stored at -80 °C.

Recombinant Condensin II production and western blot analysis shown in this thesis had been performed by Dr. Iain Davidson and Dr. Kota Nagasaka.

5.4.2 Purification of His-Pds5A/B, His-SA1/2, His-Smc1, Flag-Smc1 or Flag-Nipbl

His-Pds5A/B, His-SA1/2, His-Smc1, Smc3-Flag, Flag-/His-Nipbl alone or in complex with Mau2 were purified using a single affinity step. Cell lysis and pulldown was performed like described in section 5.4.1, Purification of Condensin, Cohesin and Cohesin subcomplexes, step 1. All buffers and type of beads used are listed in Table 4.

Table 4: Buffer conditions and beads used for single step purification.

Protein	Lysis buffer	Type of beads	Washing buffer	Elution buffer
His-Pds5A His-Pds5B His-SA1/2 His-Smc1 His-Nipbl	35 mM Hepes pH 7.5 200 mM NaCl 5 % glycerol 3 mM β -mercaptoethanol 50 mM imidazole 0.02 % Tween-20 1 tablet/50 ml Roche complete EDTA-free 0.1 mM PMSF	Ni-NTA beads (Qiagen), 1 ml bead/4 ml cell pellet	35 mM Hepes pH 7.5 200 mM NaCl 5 % glycerol 3 mM β -mercaptoethanol, 50 mM imidazole 0.02 % Tween-20	35 mM Hepes pH 7.5 200 mM NaCl 5 % glycerol 300 mM imidazole Dialysis buffer: 35 mM Hepes pH 7.5 200 mM NaCl 5 % glycerol
Smc3-flag	35 mM Hepes pH 7.5 150 mM NaCl 5 % glycerol 0.02 % Tween-20 1 tablet/50 ml Roche complete EDTA-free	Flag-M2-beads (Sigma) 0.5 ml bead/2 ml cell pellet	35 mM Hepes pH 7.5 150 mM NaCl 5 % glycerol 0.02 % Tween-20	35 mM Hepes pH 7.5 150 mM NaCl 5 % glycerol 0.5 mg/ml flag peptide

Material and methods

	0.1 mM PMSF			
Flag-Nipbl (+/- His Mau2)	20 mM Tris pH8 400 mM NaCl 5 % glycerol 0.5 mM DTT 0.02 % Tween-20 1 tablet/50 ml Roche complete EDTA-free 0.1 mM PMSF 10 µg/ml pepstatin 10 µg/ml chymostatin 10 µg/ml leupeptin	Flag-M2-beads (Sigma) 1 ml bead/10 ml cell pellet	20 mM Tris pH8 400 mM NaCl 5 % glycerol 0.5 mM DTT 0.02 % Tween-20	20 mM Tris pH8 200 mM NaCl 5 % glycerol 0.5 mg/ml flag peptide

5.4.3 Purification of the loading complex

Twin-strep-(flag)-Nipbl-his alone or in complex with untagged Mau2 were purified using two consecutive affinity purification steps machining use of the N- and C-terminal tags of Nipbl to retain full length protein. First, the proteins were pulled down using Ni-NTA beads that bind to the His-tag and eluted. This is followed by rebinding Nipbl either to Strep-Tacin Sepharose beads (IBA) or M2-flag-beads (Sigma), depending on the assay they were purified for and the existing tags.

Cell lysis and clearing of the lysates is described in detail in chapter 5.4.1 using a lysis buffer containing 35 mM Hepes pH 7.5, 250 mM NaCl, 5 % glycerol, 3 mM β -mercaptoethanol, 50 mM imidazole, 0.02 % Tween-20, 1 tablet/50 ml Roche complete EDTA-free protease inhibitors, 10 µg/ml pepstatin 10 µg/ml chymostatin, 10 µg/ml leupeptin, 0.1 mM PMSF, 10 µg/ml aprotinin. The cleared lysate was bound to equilibrated Toyo pearls (1 ml resin/10 ml cell pellet) in batch for 1 h at 4 °C. The resin was washed 1 x in batch and then transferred to a column (Poly-Prep Chromatography Columns, BioRad) and further washed with 20 bed volume lysis buffer. The proteins were eluted with 35 mM Hepes pH 7.5, 150 mM NaCl, 5 % glycerol, 400 mM imidazole, 0.02 % Tween-20, 0.1 mM PMSF) 5 x 1 bead volume.

In cases where equilibrated Strep-Tacin sepharose beads (IBA, 0.5 ml beads/10 ml cell pellet) were used, Nipbl was rebound to beads in a column (Poly-Prep Chromatography Columns, BioRad) by gravity flow. The beads were washed with 20 x bead volumes (35 mM Hepes pH 7.5, 150 mM NaCl, 5 % glycerol, 0.02 % Tween-20) and eluted in small

volumes using 10 mM desthiobiotin, 35 mM Hepes pH 7.5, 150 mM NaCl, 5 % glycerol. Fractions were analyzed by Coomassie stained SDS PAGE and directly aliquoted, pooled and optionally concentrated using Amicon100K MWCO centrifugal filter units (Millipore) before storing.

If Nipbl was further purified using M2-Flag beads (Sigma, 10 ml cell pellet, 0.5 ml flag beads), then preparation was incubated in batch with resin for 1 h at 4 °C, before transferring it a column (Poly-Prep Chromatography Columns, BioRad). The beads were washed with 20 bead volumes (35 mM Hepes pH 7.5, 150 mM NaCl, 5 % glycerol, 0.02 % Tween-20) and the proteins were eluted with small volumes of 0.5 mg/ml flag peptide, 35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol. The preparations were further treated as for the Strep-Tacin- bead elutions.

5.4.4 *E.coli* expression and purification of Scc1 fragments

E. coli BL21 (DE3) had been transformed with inducible pET21A expression vectors containing His-MBP-Scc1 Fragment-flag. 2 l of bacteria culture in log phase of growth were induced with 1 mM IPTG at 21 °C, overnight. The cells were pelleted by centrifugation for 30 min and 2 or 2.5 ml cell pellets had been obtained and stored at -80 C.

For purification, cells had been thawed in lysis buffer containing 50 mM Tris pH 8, 300 mM NaCl, 5 % glycerol, 10 mM imidazole, 2.5 U/ml Benzonase, 1 protein inhibitor tablet EDTA free/ 50 ml (Roche), 10 µg/ml pepstatin 10 µg/ml chymostatin, 10 µg/ml leupeptin, 0.1 mM PMSF, 10 µg/ml aprotinin and 2 U/ml Lysozyme. The cells had been transferred to a Rosetta glass tube and sonicated for 5 min, 50 % strength with a 2 sec on and off rate on ice. The lysate had been cleared by centrifugation at 47,810 x g for 60 min at 4 °C with a SS34 rotor. The cleared lysate was then bound to 2.5 ml Ni-NTA beads (Quiagen) prior to 3 x 5 min washes (buffer: 20 mM Tris pH 7.5, 200 mM NaCl, 5 % glycerol, 1 mM PMSF, 40 mM imidazole) and elution with 12.5 ml 20 mM Tris pH 7.5, 50 mM NaCl, 5 % glycerol, 400 mM imidazole. The elution was pooled and diluted to 50 ml using the sample buffer without imidazole. The dilution was then loaded onto a HiTrap Q sepharose FF (GE Healthcare), 5 ml column and eluted with a salt gradient starting from 50 mM to 1 M NaCl in the buffer. Peak fractions were analyzed and concentrated to 1 ml using Amicon10K MWCO centrifugal filter units (Millipore). The concentrated proteins had been loaded to a Superdex 200 16 60 (GE Healthcare, buffer: 35 mM Hepes pH 7.5, 150 mM NaCl, 2.5 %

glycerol). Peak fractions had been analyzed by SDS PAGE and Coomassie staining and were pooled. After concentrating, 2.5 % glycerol was added to the preparations for storage. Preparations had been snap frozen and stored at – 80 C.

5.4.5 Protein separation and analysis

To analyze proteins, a sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS PAGE) was performed. Depending on the protein size, precast 4-12 % or 6 % Tris-Glycine or 4-12 % Bis-Tris NuPAGE Novex gels (Life Technologies) were used to separate the proteins. Various protein ladders (Thermo Scientific) and BSA or β -Galactosidase were used as standards to compare protein size and yield, respectively. The separated proteins were analyzed by silver staining, Coomassie or Instant Blue staining or immunoblotting/western blotting.

For **silver staining**, the gels were fixed for 1 h with a solution containing 10 % v/v acetic acid and 45 % v/v methanol. After washing with water for 1 h, the gel was sensitized with a solution containing 900 μ M $\text{Na}_2\text{S}_2\text{O}_3$ (Sigma) for 1 min. The gel washed for 1 min and incubated with staining solution (0.05 % v/v formaldehyde (37 % stock), 6% w/v Na_2CO_3 (Sigma), 0.2 % w/v AgNO_3 (Sigma)) for 20 min. After 1 min washing with water, the silver stain was developed using a solution containing 6 % w/v Na_2CO_3 (Sigma), 0.05 % v/v formaldehyde (stock 37 %). A solution with 10 % v/v acetic acid was used to stop the reaction.

Proteins were also visualized by adding **Coomassie** (0.25 % w/v Coomassie Brilliant Blue, 10 % v/v acetic acid) for 60 min or by adding **Instant Blue** (Expedeon) for 20 min.

To analyze proteins by immunoblotting/**western blotting**, the separated proteins were transferred from SDS PAGE gels to 0.45 μ M PVDF membranes (GE Immobilon P 0.45 μ m, GE Healthcare) using a wet transfer system (Bio-Rad Mini Trans Blot chambers). The wet transfer was performed for 1 h at 100 V at 4 °C using a buffer containing 25 mM Tris pH 8.3, 192 mM glycine and freshly added 10 % methanol. After the transfer, the membrane was rinsed with TBS-Tween-20 (50 mM Tris pH 7.6, 150 mM NaCl, 0.05 % Tween-20) and incubated with blocking solution (TBS-Tween-20, 5% milk powder) for 30 min at

room temperature (RT). Then, the membrane was incubated with specific primary antibodies (typically 0.5-2 µg/ml) in blocking solution for 1 h, at RT or overnight at 4 °C. The membranes were washed 3 x 5 min with TBS-Tween-20 prior to incubation with a secondary antibody that was coupled to HRPQ, in TBS-Tween-20, 5 % milk powder for 30 min at RT. The secondary antibody was chosen depending on the primary antibody and diluted according to the manufacturer manual. The membranes were washed 3 x 5 min with TBS-Tween-20 and developed using ECL solution (GE Healthcare) and directly exposed with ChemiDoc™ XRS+ (Biorad) or first exposed on a film (Amsterdam Hyperfilm ECL, GE Healthcare) for 1 sec – 40 min in the dark. Specific antibodies listed in Table 5.

Table 5: List of antibodies used in this study.

Antibody-ID	Peptide no.	Antigen	raised in	Supplier
	444	SA1	rabbit	Gramsch
	447	SA2	rabbit	Gramsch
010702F01		Nipbl	rat	Absea
A974	974	Mau2	rabbit	Gramsch
	727	Smc3	rabbit	Gramsch
A683		Smc3 acetylated	mouse	Katsu Shirahiges
955	647	Pds5A	rabbit	Gramsch
900	623	Scc1	rabbit	Gramsch
A300-055A		Smc1	rabbit	Abcam
sc-8654		H3	goat	Santa Cruz
34850		Strep	mouse	Qiagen

5.5 ATPase assays

ATPase reaction was carried out in 25 µl reactions, using 100 nM Cohesin, 100 nM Nipbl (either alone or in complex with Mau2) and 0.5 µl Lambda DNA (stock 0.5 mg/ml, NEB) in a final buffer containing 35 mM Hepes pH 7.5, 75 mM NaCl, 25 mM KCl, 2 mM MgCl₂, 5 % glycerol, 0.1 mg/ml ultra-pure BSA, 1 mM DTT). The mix was kept on ice, till the reaction was initiated by addition of 0.2 µM ATP (Sigma), spiked in with [γ-³²P]-ATP (PerkinElmer, specific activity 3000Ci/mmol) and transferring the reaction to 32 °C. Time points were taken by mixing 4 µl of the reaction with 1 µl stop buffer (50 mM EDTA, 5 % w/v SDS). Thin layer chromatography (TLC) was used to separate hydrolyzed ³²P_i from [γ-³²P]-ATP. For this, 0.5 -1 µl of the terminated reactions were spotted on polyethylenimine cellulose F sheets (Merck), dried for 30 min and run for 40 min using 0.75 M KH₂PO₄ pH 3.4 as

mobile phase. The sheets were dried for 40 min, enwrapped with saran wrap and used to expose an erased phosphor screens for 1 – 24 h. The phosphor screens were scanned using a phosphoimager (BioRad). The separated spots represented [γ - ^{32}P]-ATP and the faster migrating released radioactive inorganic phosphate were quantified using ImageJ. The signal was normalized to background and subtracted with the buffer condition. The addition of 100 nM Condensin II, 100 nM His-Pds5A, 100 nM His-SA1 was mentioned in the figure legends.

5.5.1 Data Analysis

Identification of peptides and proteins

To verify the presence of Cohesin, Nipbl and possible other proteins in the sample, the RAW-files were loaded into Proteome Discoverer (version 1.4.0.288, Thermo Scientific). All MS/MS spectra were searched using MS AMANDA (Dorfer et al., 2014) against the human Swissprot protein sequence database. The following search parameters were used: Carbamidomethylation on cysteine was set as a fixed modification, and oxidation on methionine was set as a variable modification. Monoisotopic masses were searched for tryptic peptides allowing for up to two missed cleavages. The peptide mass tolerance was set to ± 5 ppm, the fragment mass tolerance to ± 20 Da. The result was filtered to 1% FDR using Percolator algorithm integrated in Proteome Discoverer. Additional filters were applied: search engine rank was set to 1 and minimum peptide length was set to 5.

5.6 Cohesin bulk loading assay

Nicked, circular plasmid pSP64 (C DNA, 3.3 kb) was prepared by digestion with the nicking enzyme Nt.BspQI (New England BioLabs) for 1 h at 37° C, followed by gel extraction using a gel extraction kit (Qiagen). A linearized plasmid pSP64 (L DNA, 3.3 kb) was obtained by restriction digestion with BamHI (New England BioLabs) and gel extraction (kit Quiagen).

The *in vitro* bulk loading reaction was adapted from previously described loading assay described in (Murayama and Uhlmann, 2014). The reactions were carried out in 20 μl . For DNA cleavage experiments, 150 nM Cohesin and either 3.3 nM nicked plasmid or increasing amounts 1 x, 2 x or 4 x of linearized plasmid were mixed to a final reaction buffer of 35 mM Tris pH7.5, 56 mM NaCl, 19 mM KCl, 1 mM MgCl_2 , 1 mM TCEP, 10 % glycerol,

0.03 % Triton-X 100, 0.5 mM ATP and incubated for 1 h at 32 °C. To dissolve non-topological DNA Cohesin interactions, 180 µl stop buffer (35 mM Tris pH 7.5, 500 mM NaCl, 20 mM EDTA, 0.35 % Triton-X 100, 5 % glycerol) was added for 5 min at 32 °C. The reaction was then transferred to 15 µl anti-Scc1 antibody agarose beads (Biorad, antibody ID A900) (Waizenegger et al., 2000) in 200 µl low salt buffer (35 mM Tris pH 7.5, 150 mM NaCl, 0.35 % triton-X 100, 5 % glycerol) and rocked for 2 h at 4 C°. The beads were washed twice with high salt buffer (35 mM Tris pH 7.5, 750 mM NaCl, 0.35 % triton-X 100, 5 % glycerol) and once with low salt buffer. Then, the beads were peptide eluted in 30 µl buffer (1 mg/ml Scc1 peptide, IMBA facility, 35 mM Tris pH 7.5, 150 mM NaCl, 0.35 % triton-X 100, 5 % glycerol) for 30 min at 4 C°. 1 mg/ml ProteinaseK was then added to 20 µl of the elution for 20 min at 50 C°. The recovered DNA was then analyzed by 0.8 % agarose gel electrophoresis stained with GelRed (Biotium) and recorded with Chemi-Doc™ XRS+ (Biorad). Band intensities were quantified using ImageJ, subtracted from background and normalized to C bead control. Values represent mean +/- standard error of the mean for 3 or 4 independent experiments.

Cleavage of Cohesin Scc1^{Halo-biotin-TEV} was carried out during the purification using TEV protease (IMBA services) for 3 h at 16 C°. 150 nM cleaved and non-cleaved Cohesin was incubated with 3.3 nM circular nicked DNA for 20 min at 37 C. The experiment was further carried out as described above, except that Tris was substituted with HEPES in all buffers.

To test the influence of ATP on the bulk loading reaction, 150 nM Cohesin smc1/3 wild type was compared with Cohesin smc1/3 K38A (KA), mutations inhibiting the hydrolysis of ATP either in the presence or absence of ATP. In addition, wild type Cohesin was challenged with 0.5 mM ATP or 0.5 mM ATP derivatives such as ADP, ATP-γS (Jena Bioscience), AMP-PNP (Jena Bioscience), AMP-PC (Jena Bioscience) and incubated for 90 min.

To test the influence of Nipbl, 100 nM Nipbl was incubated with 120 nM Cohesin in the presence of 3.3 nM nicked plasmid DNA and 0.1 mM ATP at 32 °C for 1 h and then proceeded as described. Various salt and protein concentrations had been tested.

5.7 Binding assays

5.7.1 Binding to Cohesin

In insect cell expressed trimeric or tetrameric Cohesin were purified using Ni-NTA beads (5.4.1.). The eluted proteins were dialyzed and stored. To perform a binding assay, excess of this Cohesin was re-bound to 15 ul Flag-M2-beads in a final buffer consisting of 35 mM Hepes pH 7.5, 150 mM NaCl, 5% glycerol, 0.01 % Tween-20 for 1h at 4 °C. The resin was washed 3 x 5 min with 500 ul washing buffer (35 mM Hepes pH 7.5, 150 mM NaCl, 5% glycerol, 0.01 % Tween-20). 100 nM of various proteins (Pds5A, Nipbl-Mau2, SA1/2) added in 400 ul final buffer (35 mM Hepes pH 7.5, 150 mM NaCl, 5 % glycerol, 0.02 % Tween-20, 0.1 mg/ml BSA) for 1 h at 4 °C. The samples were carefully washed 3 x for 5 min, prior to peptide eluting Cohesin using 45 ul elution buffer (0.5 mg/ml flag peptide, 35 mM Hepes, 150 mM NaCl, 5% glycerol). Proteins were analyzed SDS PAGE and silver staining or western blotting (5.4.5).

5.7.2 Binding to loading complex

250 nM Twin-strep-flag-Nipbl-His/ Nipbl-PA or Nipbl-Cterm were incubated with equal amounts of proteins of His-MBP-Scclfragments-flag in 50 ul (final buffer: 35 mM Hepes pH7.5, 100 mM NaCl, 5 % glycerol, 0.02 % Tween-20, 0.5 mg/ml BSA) for 1 h at 4 °C. Or 100 nM Twin-strep-flag-Nipbl-(or PA)-His were incubated with equal amounts of Cohesin in 200 ul (final buffer: 35 mM Hepes pH7.5, 100 mM NaCl, 5 % glycerol, 0.02 % Tween-20, 0.5 mg/ml BSA) for 1 h at 4 °C. Nipbl pulled down by incubation with 15 - 20 ul strep-tacin beads for 45 min, 4 °C, prior to 3 x 5 min washes (buffer: 35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 0.02 % Tween-20) and eluting using 45 ul elution buffer (35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 10 mM desthiobiotin (IBA)).

5.8 *Xenopus leavis* egg extract

Xenopus leavis egg extract preparation was performed by Dr. Iain Davidson and had been previously described (Huis in 't Veld et al., 2014, Davidson et al., 2016)

5.9 Cross-linking mass spectrometry

Cross-linking mass spectrometry was performed in collaboration with Dr. Rebecca Beveridge, Zsuzsanna Orban-Nemeth and the IMBA Mass Spectrometry facility (supervisor

Karl Mechtler). The cross-linked proteins samples had been prepared by me and mass spectrometry analysis was done by Dr. Rebecca Beveridge for all samples except the Cohesin only experiment that had been performed by Zsuzsanna Orban-Nemeth. The sections (5.9.4, 5.9.5, 5.9.6, 5.5.1, 5.9.7) of this thesis had been written by Rebecca Beveridge.

5.9.1 Chemical cross-linking: Mau2 in complex with Nipbl

Twin-strep-flag-Nipbl-his was co-expressed with Mau2 and purified with two consecutive affinity steps: Ni-NTA beads, followed by strep -tacin beads (5.4.3). Neither glycerol nor Tween-20 were used in the last washing and elution buffers. Then the proteins were eluted in column in small volumes (elution buffer 35 mM Hepes, 10 mM desthiobiotin, 100 mM NaCl). Fractions with high amount of protein were pooled and then cross-linked. For this, disuccinimidyl suberate (DSS, Sigma) was resolved in freshly in DMSO. Depending on a test titration or experience, 12.5 or 25 μ M DSS was added to the proteins sample incubated for 20 min at room temperature to cross-link the proteins. The reaction was then quenched by adding 1:40 of 2 M ammonium bicarbonate for 10 min at room temperature. Then, the samples had been stored at 4 °C till they were further processed for mass spectrometry.

5.9.2 Chemical cross-linking: Nipbl in complex with Cohesin

Twin-strep-flag-Nipbl-his and Cohesin were expressed separately. Cohesin was purified as described in 5.4.1 with two consecutive affinity steps and eluted with 35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 0.02% Tween-20. At the same time, Twin-strep-flag-Nipbl-his was purified with Ni-NTA beads (described in 5.4.3) and then rebound to Strep-Tactin Sepharose beads (IBA) in batch. After 2 washes with washing buffer (35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 0.02% Tween-20), the purified Cohesin and to 0.1 mg/ml BSA was added for 1 h at 4 C. The sample was washed 5 times (washing buffer without glycerol and Tween-20) with a large volume, prior to eluting the proteins in batch 2 x in 1 bead volume 35 mM Hepes pH 7.5, 100 mM NaCl, 10 mM desthiobiotin (IBA) for 20 min at 4 °C. Cross-linking was performed describes in 5.9.1.

5.9.3 Chemical cross-linking: Cohesin

Cohesin (Smc3-flag, His-Scc1, Smc3, Twin-strep-SA1) was purified using Ni-NTA beads as described in 5.4.1. Cohesin was eluted with 35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 300 mM imidazole, 0.02% Tween-20 and rebound to Strep-Tacin-sepharose beads (IBA) in batch. After 2 washes using a buffer with 35 mM Hepes pH 7.5, 100 mM NaCl, 5 % glycerol, 0.02% Tween, followed by 3 washes with 35 mM Hepes pH 7.5, 100 mM NaCl, Cohesin was eluted in 2 x 1 bead volume in batch for 20 min using elution buffer (35 mM Hepes pH 7.5, 100 mM NaCl, 10 mM desthiobiotin). Cross-linking was performed using 25 μ M DSS (as described in 5.9.1).

5.9.4 Reduction, Alkylation & Digestion of the cross-linked sample

All reactions were carried out at 37 °C, unless stated otherwise. The samples were first reduced with Tris-(2-carboxyethyl)-phosphine (TCEP, 5 mM) for 30 minutes and subsequently alkylated by Iodoacetamide (10 mM) for 30 minutes at room temperature. Overnight digestion was performed with trypsin (500 ng), the digestion was quenched by the addition of 10% trifluoroacetic acid, and the sample was desalted (*via* a Sep-Pak C18 3 cc Vac Cartridge, Waters). The eluate was evaporated to dryness and resuspended in SCX Buffer A for strong cation exchange separation.

5.9.5 Strong Cation Exchange Chromatography

Digested Peptides were loaded onto a TSKgel SP-2SW column (250 mm x 4.6 mm x 5 μ m). SCX Buffer A consisted of 5 mM phosphate pH 2.7 15% acetonitrile, Buffer B consisted of 5 mM phosphate pH 2.7 1 M NaCl 5% acetonitrile and Buffer C consisted of 5mM phosphate pH 6 15% acetonitrile. The gradient was as follows. 0-10 min (100% A); 10-40 min (to 40% A/ 50% B/ 10% C); 40-50 min (to 25% A/ 50% B/ 25% C); 50-55min (to 50% B/ 50% C); 55-70min (to 50% B/ 50% C); 70-72 min (to 100% A); 72-150min (100% A). An UltiMate 3000 HPLC μ flow system was used for detection, monitoring the wavelength at 214 nm. Fractions were collected every 2 minutes between minutes 40 and 100, evaporated to dryness and resuspended in 0.1% TFA prior to LC-MS/MS measurement.

5.9.6 Nano LC-MS Analysis

Each fraction was measured using an UltiMate 3000 HPLC RSLC nano system (Thermo Scientific) coupled to a Q Exactive Plus mass spectrometer (Thermo Scientific), equipped

with a Proxeon nanospray source (Thermo Scientific). Peptides were loaded onto a trap column (Thermo Fisher Scientific, Amsterdam, Netherlands, PepMap C18, 5 mm × 300 µm ID, 5 µm particles, 100 Å pore size) at a flow rate of 25 µL min⁻¹ using 0.1 % TFA as mobile phase. After 10 min, the trap column was switched in line with the analytical column (Thermo Fisher Scientific, Amsterdam, Netherlands, PepMap C18, 500 mm × 75 µm ID, 2 µm, 100 Å). Peptides were eluted using a flow rate of 230 nL min⁻¹, and a binary 1 h gradient. The following elution steps were employed (buffer A: H₂O/ FA = 99.9/0.1; buffer B: H₂O/ ACN/ FA = 19.92/ 80/ 0.08). 0-10 minutes (2% B); 10-70 min (to 35% B); 70-75 min (to 90% B); 75-80 min, (90% B); 80-82 min (to 2% B); 82-105 min (2% B).

The Q Exactive HF mass spectrometer was operated in data-dependent mode, selecting up to 10 precursors from the MS1 scan (resolution of 60 000) in the range of m/z 350 to 2000 for higher-energy collisional dissociation (HCD). Fragment ions were analyzed with a resolution of 30 000 and an ion threshold of 1e6, excluding singly and doubly charged ions. HCD was performed with 28% normalised collision energy and an isolation window of 1 m/z. The underfill ratio was set to 10% and the dynamic exclusion to 30s. Ion target values were 3e6 (maximum injection: 60 ms) for full scans and 1e6 (maximum injection: 250 ms) for MS/MS scans.

5.9.7 Identification of cross-linked peptides

For cross-link searches, Thermo Xcalibur raw files were converted into mgf input files for pLink (version 1.21) analysis *via* Thermo Proteome Discoverer. In the pLink file xlink.ini, the DSS cross-linker was configured (lysine reactive, cross-linker mass of 138.0681 u). In instrument.ini, the standard instrument parameters for HCD fragmentation were selected. In the pLink.ini file the crosslinker for analysis was selected, the modifications were chosen (as in MS AMANDA search) and the FDR filter was set to 5%. Crosslinked peptide hits that were identified by three PSMs or lower were additionally verified (or discarded) by manual inspection and validation.

5.10 Bioinformatics

ImageJ (1.51p) had been used to quantify the bulk Cohesin loading assay and the ATPase assays. Cross-linked peptides were visualized using xVis (Grimm et al., 2015), xiNET (Comb et al., 2015) and CX-Circos (Krzywinski et al., 2009). Protein sequences to perform multiple sequence alignments were collected using the databases Uniprot and blastP (v2.2.27) (Altschul et al., 1997). The program Jalview 2.10.2 and Mafft were used to calculate multiple sequence alignments (Waterhouse et al., 2009, Katoh and Toh, 2008). JPREPRED was used to predict the secondary structure of proteins (Drozdetskiy et al., 2015). The human Nipbl structure was modelled with MODELLER based on the *Chaetomium thermophilum* crystal structure PDB:5T8V using an alignment created by HHPRED (Webb and Sali, 2014, Zimmermann et al., 2017). To visualize and measure amino acid distances in crystal structures, PDB files and PyMOL 1.6.0.0 was used (DeLano Scientific).

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7 Appendix

Table 6: List of library vectors (pLIB) for single or co-expression in insect cells.

pLIB	Vector name
Smc1A	c6
Smc3-flag	605A
Scc1	c18
Scc1-TEV	13M
Scc1-TEV-mkikGR	300c
Scc1-TEV-Halo	44B
His-Scc1-TEV-Halo	576D
His-Scc1-TEV-EGFP	578A
His-SA1	l17
His-SA2	c30
Pds5A-his	60B
Twin-Strep	822A or 822C
Twin-Strep-SA1	837B
Myc3-Scc1-his	904 A, 904 B, 904 C
pLib-MBP-C3-SacI-his	903C
Mau2	190B
Flag-Mau2	192C
His-Mau2	212E
Flag-his-Mau2	LC28C
His-EGFP-Mau2	LC37A
His-Halo-Mau2	LC38B
Nipbl	142D
Flag-Nipbl	150F
His-Nipbl	144M
GST-Nipbl	148C
His-flag2-Nipbl	LC27D
Flag-EGFP-C3-Nipbl	LC35A
Flag-Halo-C3-Nipbl-his	LC46E
Flag-EGFP-C3-Nipbl-his	LC48G
Nipbl-his	705K
Twin-strep-Flag-Nipbl-his	712C
Twin-strep-Nipbl-his	721C
Twin-strep-flag-Nipbl-PN-his	838F
Twin-strep-flag-Nipbl-PA-his	839A
Twin-strep-Nipbl 1183-2804-his	860A
Twin-strep-Nipbl 1-1182-his	862B
Twin-strep-Nipbl-CDLS1-his	902A
His-Nipbl 1-497	EGA1b
Twin-strep-his-Nipbl 1-497	EGA10a
His-Nipbl 1-497, Mau2	EGA15a
Twin-strep-his-Nipbl 1-497, Mau2	EGA18c
His-Nipbl 497-2804	EGA5gR
Twin-strep-his-Nipbl 497-2804	EGA20f
His-Nipbl 1745-2376	EGA4b
Twin-strep-his-Nipbl 1745-2376	EGA11d
His-Nipbl 1745-2804	EGA9a
Twin-strep-his-Nipbl 1745-2804	EGA16d
His-Nipbl 497-1745	EGA12a
His-Nipbl 2474-2804	EGA21c
pLIB: Co-expression vectors	

Appendix

Twin-strep-flag-Nipbl-PA-his, Mau2	858 A
Twin-strep-flag-Nipbl-PA-his, Mau2	589 A
Flag-Halo-C3-Nipbl-his, Mau2	LC50A
Flag-EGFP-Nipbl-his	LC51B
Twin-strep-flag-Nipbl-his, Mau2	LC715B
Twin-step-Nipbl-his, Mau2	LC722A
Nipbl-his, Mau2	LC718B
His-Nipbl, Mau2	LC719A

Table 7: Trimeric and tetrameric Cohesin complexes for insect cell expression, cloned in this study.

Indicated are the clones where Expression cassette (EC) 1 or 2 were PCR'd from. Step 1 assemblies (pnnGA1AB or CD) were combined to Big2 vectors for co-expression.

BiGbac expression vectors step 1 assembly	EC1	EC 2	Vector name
pnnGA1 AB_Smc1, Smc3-flag	c6	605A	18c
pnnGA1 CB_Scc1, His-SA2	c18	c30	16 J
pnnGA1 CB_Scc1, His-SA2	c18	c30	16 D
pnnGA1 CB_Scc1-TEV-mKikGR, His-SA1	300c	l17	17K
pnnGA1 CB_Scc1-TEV-Halo, His-SA1	44B	l17	L20
pnnGA1 CB_His-Scc1-TEV-Halo			S13A
pnnGA1 CB_His-Scc1-TEV-EGFP			S14A
BiGbac expression vectors, step 2 assemblies	pnnGA1_AB	pnnGA1_BC	Final vector
Tetramer: Big2_ Smc3-flag, Smc1, Scc1-TEV-mKikGR, His-SA1	18c	17K	S8B
Tetramer: Big2_ Smc3-flag, Smc1, Scc1-TEV-Halo, His-SA1	18c	20L	S10A
Tetramer: Big2_ Smc3-flag, Smc1, Scc1, His-SA2	18c	16J	TB1
Trimer: Big2_ Smc3-flag, Smc1, His-Scc1-TEV-Halo	18c	S13A	S15C
Trimer: Big2_ Smc3-flag, Smc1, His-Scc1-TEV-EGFP	18c	S14C	S16C

Table 8: List of Cohesin complexes for insect cell expression used in this study.

	Plasmid number	Reference/ generated by
Smc3-Flag, Smc1, His-SA1, Scc1	CA1	Weissmann et al., 2016
Smc3 (K39A) -Flag, Smc1 (K39A), His-SA1, Scc1	CA2	Weissmann et al., 2016
Smc1 E1157Q, Smc3 E1144Q-Flag, His-SA1, Scc1	CA3	Weissmann et al., 2016
Smc3-Flag, Smc1, Scc1	58	Pim Huis in't Veld
Smc3-Flag, Smc1, His-SA1, Scc1L53E, L64E, Y60E, L74D	222	Huis in 't Veld et al., 2014
Scc1-Smc3-Flag, Smc1, His-SA1	ID152	Iain Davidson
Smc2, Smc4-flag CAPG2-His, CAPH2-Halo, CAPD3	Condensin II	Iain Davidson, Kota Nagasaka

Table 9 : PET21A plasmids for inducible expression in *E.coli*.

pET21A -His-MBP-Scc1-Fragment-flag	Vector name
pET21A -His-MBP-Scc1 65-215 -flag	827A
pET21A -His-MBP-Scc1 100-250 -flag	828B
pET21A -His-MBP-Scc1 120-210 -flag	829B
pET21A-His-MBP-Scc1 306 -402-flag	830F

7.1 Cross-linking mass spectrometry

7.1.1 Cross-linked peptides: Mau2 in complex with Nipbl

Table 10: List of Twin-strep-flag-Nipbl-his and Mau2 intra- and interprotein cross-links.

Protein1	Residue 1	Protein2	Residue	Peptide sequence
Mau2	479	Nipbl	31	YNEAKR(5) - GGGSGGGSGGSAWSHPQFEKSGGSDYKDDDDK(20)
Mau2	479	Nipbl	38	YNEAKR(5)-SGGSDYKDDDDK(7)
Mau2	250	Mau2	311	SVKPCLK(3)-AQKYTDK(3)
Mau2	311	Mau2	322	AQKYTDK(3)-ALMQLEKLK(7)
Mau2	479	Mau2	487	YNEAKR(5)-ETLKMSNAEDLNR(4)
Nipbl	378	Nipbl	405	MKLKG(2)-DSTKLTLR(4)
Nipbl	378	Nipbl	662	KMKLGK(3)-SEMKSSES(4)
Nipbl	381	Nipbl	405	LGKDEK(3)-DSTKLTLR(4)
Nipbl	384	Nipbl	405	LGKDEKEQSEK(6)-DSTKLTLR(4)
Nipbl	389	Nipbl	405	DSTKLTLR(4)-EQSEKAAMYDISSPSK(5)
Nipbl	405	Nipbl	541	DSTKLTLR(4)-FSKEVQDK(3)
Nipbl	405	Nipbl	551	DSTKLTLR(4)-DKPLKK(5)
Nipbl	405	Nipbl	754/787	DSTKLTLR(4)-QKGESRPETPK(2)
Nipbl	541	Nipbl	613	FSKEVQDK(3)-VDSQASITQSDSIKKPEEIK(15)
Nipbl	541	Nipbl	614	FSKEVQDK(3)-KPEEIK(1)
Nipbl	541	Nipbl	754/787	FSKEVQDK(3)-QKGESRPETPK(2)
Nipbl	551	Nipbl	613	DKPLKK(5)-VDSQASITQSDSIKKPEEIK(15)
Nipbl	551	Nipbl	614	DKPLKK(5)-KPEEIK(1)
Nipbl	613	Nipbl	651	VDSQASITQSDSIKKPEEIK(15)-KSDPELSK(1)
Nipbl	613	Nipbl	662	VDSQASITQSDSIKKPEEIK(15)-SEMKSSES(4)
Nipbl	613	Nipbl	710	VDSQASITQSDSIKKPEEIK(15)-TTECKQNESTIVEPK(5)
Nipbl	613	Nipbl	823	VDSQASITQSDSIKK(15)-PSTEKK(5)
Nipbl	614	Nipbl	710	KPEEIK(1)-TTECKQNESTIVEPK(5)
Nipbl	614	Nipbl	649	KPEEIK(1)-STPENHPETPKK(11)
Nipbl	614	Nipbl	651	KPEEIK(1)-KSDPELSK(2)
Nipbl	614	Nipbl	658	KPEEIK(1)-SDPELSKSEM(7)
Nipbl	614	Nipbl	682	KPEEIK(1)-LVETKSSENK(5)
Nipbl	614	Nipbl	754/787	KPEEIK(1)-QKGESRPETPK(2)
Nipbl	619	Nipbl	649	KPEEIKQCNDAPVSVLQEDIVGSLK(6)-STPENHPETPKK(11)
Nipbl	619	Nipbl	651	KPEEIKQCNDAPVSVLQEDIVGSLK(6)-KSDPELSK(2)
Nipbl	619	Nipbl	710	KPEEIKQCNDAPVSVLQEDIVGSLK(6)-TTECKQNESTIVEPK(5)
Nipbl	638	Nipbl	651	QCNDAPVSVLQEDIVGSLKSTPENHPETPKK(19)-KSDPELSK(1)
Nipbl	638	Nipbl	662	QCNDAPVSVLQEDIVGSLKSTPENHPETPK(19)-SEMKSSES(4)
Nipbl	638	Nipbl	672	QCNDAPVSVLQEDIVGSLKSTPENHPETPK(19)-LAESKPNENR(5)
Nipbl	649	Nipbl	658	STPENHPETPKK(11)-SDPELSKSEM(7)
Nipbl	649	Nipbl	662	STPENHPETPKK(11)-SEMKSSES(4)
Nipbl	649	Nipbl	672	STPENHPETPKK(11)-LAESKPNENRLVETK(5)
Nipbl	649	Nipbl	682	STPENHPETPKK(11)-LVETKSSENK(5)

Appendix

Nipbl	649	Nipbl	687	STPENHPETPKK(11)-SSENKLETK(5)
Nipbl	649	Nipbl	691	STPENHPETPKK(11)-LETKVETQTEELK(4)
Nipbl	649	Nipbl	700	STPENHPETPKK(11)-VETQTEELKQNESR(9)
Nipbl	649	Nipbl	710	STPENHPETPKK(11)-TTECKQNESTIVEPK(5)
Nipbl	649	Nipbl	745	STPENHPETPKK(11)-SETTKSRPETPK(5)
Nipbl	651	Nipbl	672	KKSDPELSK(2)-LAESKPENRLVETK(5)
Nipbl	651	Nipbl	682	KKSDPELSK(2)-LVETKSENK(5)
Nipbl	651	Nipbl	687	KSDPELSK(1)-SSENKLETK(5)
Nipbl	651	Nipbl	700	KSDPELSK(1)-VETQTEELKQNESR(9)
Nipbl	651	Nipbl	710	KSDPELSK(1)-TTECKQNESTIVEPK(5)
Nipbl	658	Nipbl	672	SDPELSKSEMK(7)-LAESKPENNR(5)
Nipbl	658	Nipbl	682	SDPELSKSEMK(7)-LVETKSENK(5)
Nipbl	658	Nipbl	687	KSDPELSKSEMK(8)-SSENKLETK(5)
Nipbl	658	Nipbl	700	SDPELSKSEMK(7)-VETQTEELKQNESR(9)
Nipbl	658	Nipbl	730	SDPELSKSEMK(7)-LSDTKPNDNK(5)
Nipbl	662	Nipbl	682	SEMKQSESR(4)-LVETKSENK(5)
Nipbl	662	Nipbl	687	SEMKQSESR(4)-SSENKLETK(5)
Nipbl	662	Nipbl	691	SEMKQSESR(4)-LETKVETQTEELK(4)
Nipbl	662	Nipbl	700	SEMKQSESR(4)-VETQTEELKQNESR(9)
Nipbl	662	Nipbl	754/787	SEMKQSESR(4)-QKGESRPETPK(2)
Nipbl	662	Nipbl	1157	SEMKQSESR(4)-HKKDDDK(3)
Nipbl	672	Nipbl	687	LAESKPENRLVETK(5)-SSENKLETKVETQTEELK(5)
Nipbl	672	Nipbl	691	LAESKPENNR(5)-LETKVETQTEELK(4)
Nipbl	672	Nipbl	700	LAESKPENNR(5)-VETQTEELKQNESR(9)
Nipbl	672	Nipbl	720	LAESKPENNR(5)-QNESTIVEPKQENNR(10)
Nipbl	672	Nipbl	710	LAESKPENNR(5)-TTECKQNESTIVEPK(5)
Nipbl	672	Nipbl	730	LAESKPENNR(5)-LSDTKPNDNK(5)
Nipbl	672	Nipbl	735	LAESKPENNR(5)-LSDTKPNDNKQNNGR(10)
Nipbl	672	Nipbl	745	LAESKPENNR(5)-SETTKSRPETPK(5)
Nipbl	672	Nipbl	752	LAESKPENNR(5)-SRPETPKQK(7)
Nipbl	672	Nipbl	765	LAESKPENNR(5)-QKSDGHPETPK(2)
Nipbl	672	Nipbl	807	LAESKPENNR(5)-NEGRPETPKHR(9)
Nipbl	682	Nipbl	700	LVETKSENK(5)-VETQTEELKQNESR(9)
Nipbl	682	Nipbl	720	LVETKSENK(5)-QNESTIVEPKQENNR(10)
Nipbl	682	Nipbl	710	LVETKSENK(5)-TTECKQNESTIVEPK(5)
Nipbl	682	Nipbl	730	LVETKSENK(5)-LSDTKPNDNK(5)
Nipbl	682	Nipbl	735	LVETKSENK(5)-LSDTKPNDNKQNNGR(10)
Nipbl	682	Nipbl	745	LVETKSENK(5)-SETTKSRPETPK(5)
Nipbl	682	Nipbl	754/787	LVETKSENK(5)-QKGESRPETPK(2)
Nipbl	682	Nipbl	763/796	LVETKSENK(5)-GESRPETPKQK(9)
Nipbl	682	Nipbl	765	LVETKSENK(5)-QKSDGHPETPK(2)
Nipbl	682	Nipbl	807	LVETKSENK(5)-NEGRPETPKHR(9)
Nipbl	682	Nipbl	831	LVETKSENK(5)-HKQDTK(2)
Nipbl	687	Nipbl	710	SSENKLETK(5)-TTECKQNESTIVEPK(5)
Nipbl	687	Nipbl	720	SSENKLETK(5)-QNESTIVEPKQENNR(10)
Nipbl	687	Nipbl	730	SSENKLETK(5)-LSDTKPNDNK(5)
Nipbl	687	Nipbl	735	SSENKLETK(5)-LSDTKPNDNKQNNGR(10)
Nipbl	687	Nipbl	745	SSENKLETK(5)-SETTKSRPETPK(5)
Nipbl	687	Nipbl	752	QKGESRPETPK(2)-DSTKLTLR(4)
Nipbl	687	Nipbl	754/787	SSENKLETK(5)-QKGESRPETPK(2)
Nipbl	687	Nipbl	776	SSENKLETK(5)-QKGDGRPETPK(2)
Nipbl	687	Nipbl	807	SSENKLETK(5)-NEGRPETPKHR(9)
Nipbl	691	Nipbl	710	LETKVETQTEELK(4)-TTECKQNESTIVEPK(5)
Nipbl	691	Nipbl	720	LETKVETQTEELK(4)-QNESTIVEPKQENNR(10)
Nipbl	691	Nipbl	735	LETKVETQTEELK(4)-LSDTKPNDNKQNNGR(10)
Nipbl	691	Nipbl	754/787	LETKVETQTEELK(4)-QKGESRPETPK(2)
Nipbl	691	Nipbl	807	LETKVETQTEELK(4)-NEGRPETPKHR(9)
Nipbl	700	Nipbl	720	VETQTEELKQNESR(9)-QNESTIVEPKQENNR(10)

Appendix

Nipbl	700	Nipbl	730	VETQTEELKQNESR(9)-LSDTKPNDNK(5)
Nipbl	700	Nipbl	745	VETQTEELKQNESR(9)-SETTKSRPETPK(5)
Nipbl	700	Nipbl	752	TTECKQNESTIVEPK(5)-SRPETPKQK(7)
Nipbl	700	Nipbl	754/787	VETQTEELKQNESR(9)-QKGESRPETPK(2)
Nipbl	700	Nipbl	765	VETQTEELKQNESR(9)-QKSDGHPETPK(2)
Nipbl	700	Nipbl	831	VETQTEELKQNESR(9)-HKQDTK(2)
Nipbl	710	Nipbl	730	TTECKQNESTIVEPK(5)-LSDTKPNDNK(5)
Nipbl	710	Nipbl	735	TTECKQNESTIVEPK(5)-LSDTKPNDNKQNNGR(10)
Nipbl	710	Nipbl	745	TTECKQNESTIVEPK(5)-SETTKSRPETPK(5)
Nipbl	710	Nipbl	752	TTECKQNESTIVEPK(5)-SRPETPKQK(7)
Nipbl	710	Nipbl	754/787	TTECKQNESTIVEPK(5)-QKGESRPETPK(2)
Nipbl	710	Nipbl	765	TTECKQNESTIVEPK(5)-QKSDGHPETPK(2)
Nipbl	710	Nipbl	776	TTECKQNESTIVEPK(5)-QKGDGRPETPK(2)
Nipbl	710	Nipbl	807	TTECKQNESTIVEPK(5)-NEGRPETPKHR(9)
Nipbl	710	Nipbl	829	TTECKQNESTIVEPK(5)-KPEVSKHK(6)
Nipbl	720	Nipbl	730	QNESTIVEPKQENR(10)-LSDTKPNDNK(5)
Nipbl	720	Nipbl	745	QNESTIVEPKQENR(10)-SETTKSRPETPK(5)
Nipbl	720	Nipbl	752	QNESTIVEPKQENR(10)-SRPETPKQK(7)
Nipbl	720	Nipbl	754/787	QNESTIVEPKQENR(10)-QKGESRPETPK(2)
Nipbl	720	Nipbl	763/796	QNESTIVEPKQENR(10)-GESRPETPKQK(9)
Nipbl	720	Nipbl	765	QNESTIVEPKQENR(10)-QKSDGHPETPK(2)
Nipbl	720	Nipbl	776	QNESTIVEPKQENR(10)-QKGDGRPETPK(2)
Nipbl	720	Nipbl	807	QNESTIVEPKQENR(10)-NEGRPETPKHR(9)
Nipbl	720	Nipbl	824	QNESTIVEPKQENR(10)-KPEVSK(1)
Nipbl	720	Nipbl	935	QNESTIVEPKQENR(10)-PSSGEQKSR(7)
Nipbl	730	Nipbl	745	LSDTKPNDNK(5)-SETTKSRPETPK(5)
Nipbl	730	Nipbl	752	LSDTKPNDNK(5)-SRPETPKQK(7)
Nipbl	730	Nipbl	754/787	LSDTKPNDNK(5)-QKGESRPETPK(2)
Nipbl	730	Nipbl	763	LSDTKPNDNK(5)-GESRPETPKQK(9)
Nipbl	730	Nipbl	765	LSDTKPNDNK(5)-QKSDGHPETPK(2)
Nipbl	730	Nipbl	774	LSDTKPNDNK(5)-SDGHPETPKQK(9)
Nipbl	730	Nipbl	776	LSDTKPNDNK(5)-QKGDGRPETPK(2)
Nipbl	730	Nipbl	798	LSDTKPNDNK(5)-QKNEGRPETPK(2)
Nipbl	730	Nipbl	807	LSDTKPNDNK(5)-NEGRPETPKHR(9)
Nipbl	730	Nipbl	824	LSDTKPNDNK(5)-KPEVSKHK(1)
Nipbl	730	Nipbl	850	LSDTKPNDNK(5)-AEALKQRPDGR(5)
Nipbl	735	Nipbl	765	LSDTKPNDNKQNNGR(10)-QKSDGHPETPK(2)
Nipbl	735	Nipbl	752	LSDTKPNDNKQNNGR(10)-SRPETPKQK(7)
Nipbl	735	Nipbl	754/787	LSDTKPNDNKQNNGR(10)-QKGESRPETPK(2)
Nipbl	735	Nipbl	818	LSDTKPNDNKQNNGR(10)-DSGKPSTEK(4)
Nipbl	745	Nipbl	754/787	SETTKSRPETPK(5)-QKGESRPETPK(2)
Nipbl	745	Nipbl	763/796	SETTKSRPETPK(5)-GESRPETPKQK(9)
Nipbl	745	Nipbl	765	SETTKSRPETPK(5)-QKSDGHPETPK(2)
Nipbl	745	Nipbl	774	SETTKSRPETPK(5)-SDGHPETPKQK(9)
Nipbl	745	Nipbl	776	SETTKSRPETPK(5)-QKGDGRPETPK(2)
Nipbl	745	Nipbl	785	SETTKSRPETPK(5)-GDGRPETPKQK(9)
Nipbl	745	Nipbl	807	SETTKSRPETPK(5)-NEGRPETPKHR(9)
Nipbl	745	Nipbl	818	SETTKSRPETPK(5)-DSGKPSTEK(4)
Nipbl	745	Nipbl	905	SETTKSRPETPK(5)-NEHGKSDSSK(6)
Nipbl	752	Nipbl	763/796	SRPETPKQK(7)-GESRPETPKQK(9)
Nipbl	752	Nipbl	765	SRPETPKQK(7)-QKSDGHPETPK(2)
Nipbl	752	Nipbl	774	SRPETPKQK(7)-SDGHPETPKQK(9)
Nipbl	752	Nipbl	776	SRPETPKQK(7)-QKGDGRPETPK(2)
Nipbl	752	Nipbl	785	SRPETPKQK(7)-GDGRPETPKQK(9)
Nipbl	752	Nipbl	798	SRPETPKQK(7)-QKNEGRPETPK(2)
Nipbl	752	Nipbl	807	SRPETPKQK(7)-NEGRPETPKHR(9)
Nipbl	752	Nipbl	818	SRPETPKQK(7)-DSGKPSTEK(4)
Nipbl	752	Nipbl	831	SRPETPKQK(7)-HKQDTK(2)

Appendix

Nipbl	752	Nipbl	869	SRPETPKQK(7)-DHDNKQK(5)
Nipbl	754/787	Nipbl	765	PETPKQKGESR(7)-QKSDGHPETPK(2)
Nipbl	754/787	Nipbl	754/787	QKGESRPETPK(2)-QKGESR(2)
Nipbl	754/787	Nipbl	763/796	QKGESRPETPK(2)-GESRPETPKQK(9)
Nipbl	754/787	Nipbl	774	QKGESRPETPK(2)-SDGHPETPKQK(9)
Nipbl	754/787	Nipbl	776	QKGESRPETPK(2)-QKGDGRPETPK(2)
Nipbl	754/787	Nipbl	798	QKGESRPETPK(2)-QKNEGRPETPK(2)
Nipbl	754/787	Nipbl	807	QKGESRPETPK(2)-NEGRPETPKHR(9)-
Nipbl	754/787	Nipbl	818	QKGESRPETPK(2)-DSGKPSTEK(4)
Nipbl	754/787	Nipbl	824	QKGESRPETPK(2)-KPEVSK(1)
Nipbl	754/787	Nipbl	829	QKGESRPETPK(2)-KPEVSKHK(6)
Nipbl	754/787	Nipbl	831	QKGESRPETPK(2)-HKQDTK(2)
Nipbl	754/787	Nipbl	842	QKGESRPETPK(2)-LKSER(2)
Nipbl	754/787	Nipbl	850	QKGESRPETPK(2)-AEALKQRPDGR(5)
Nipbl	754/787	Nipbl	869	QKGESRPETPK(2)-DHDNKQK(5)
Nipbl	754/787	Nipbl	944	QKGESRPETPK(2)-VKQGDSNK(2)
Nipbl	754/787	Nipbl	964	QKGESR(2)-LGFKSPTSKDDK(9)
Nipbl	754/787	Nipbl	975	QKGESR(2)-SKVDTNK(2)
Nipbl	754/787	Nipbl	1066	QKGESRPETPK(2)-LIKDREDK(3)
Nipbl	763/796	Nipbl	774	GESRPETPKQK(9)-SDGHPETPKQK(9):0
Nipbl	763/796	Nipbl	776	GESRPETPKQK(9)-QKGDGRPETPK(2)
Nipbl	763/796	Nipbl	785	GESRPETPKQK(9)-GDGRPETPKQK(9)
Nipbl	763/796	Nipbl	798	GESRPETPKQK(9)-QKNEGRPETPK(2)
Nipbl	763/796	Nipbl	807	GESRPETPKQK(9)-NEGRPETPKHR(9)
Nipbl	763/796	Nipbl	818	GESRPETPKQK(9)-DSGKPSTEK(4)
Nipbl	763/796	Nipbl	829	GESRPETPKQK(9)-KPEVSKHK(6)
Nipbl	763/796	Nipbl	831	GESRPETPKQK(9)-HKQDTK(2)
Nipbl	763/796	Nipbl	850	GESRPETPKQK(9)-AEALKQRPDGR(5)
Nipbl	763/796	Nipbl	869	GESRPETPKQK(9)-DHDNKQK(5)
Nipbl	763/796	Nipbl	910	GESRPETPKQK(9)-SDSSKTDK(5)
Nipbl	765	Nipbl	785	QKSDGHPETPK(2)-GDGRPETPKQK(9)
Nipbl	765	Nipbl	798	QKSDGHPETPK(2)-QKNEGRPETPK(2)
Nipbl	765	Nipbl	807	QKSDGHPETPK(2)-NEGRPETPKHR(9)
Nipbl	765	Nipbl	818	QKSDGHPETPK(2)-RDSGKPSTEK(5)
Nipbl	765	Nipbl	831	QKSDGHPETPK(2)-HKQDTK(2)
Nipbl	765	Nipbl	850	QKSDGHPETPK(2)-AEALKQRPDGR(5)
Nipbl	774	Nipbl	798	QKNEGRPETPK(2)-SDGHPETPKQK(9)
Nipbl	774	Nipbl	807	SDGHPETPKQK(9)-NEGRPETPKHR(9)
Nipbl	774	Nipbl	818	SDGHPETPKQK(9)-DSGKPSTEK(4)
Nipbl	774	Nipbl	823	SDGHPETPKQK(9)-PSTEKKPEVSK(5)
Nipbl	774	Nipbl	831	SDGHPETPKQK(9)-HKQDTK(2)
Nipbl	774	Nipbl	842	SDGHPETPKQK(9)-LKSER(2)
Nipbl	776	Nipbl	798	QKGDGRPETPK(2)-QKNEGRPETPK(2)
Nipbl	776	Nipbl	807	QKGDGRPETPK(2)-NEGRPETPKHR(9)
Nipbl	776	Nipbl	818	QKGDGRPETPK(2)-DSGKPSTEK(4)
Nipbl	776	Nipbl	824	QKGDGRPETPK(2)-KPEVSK(1)
Nipbl	776	Nipbl	842	QKGDGRPETPK(2)-LKSER(2)
Nipbl	776	Nipbl	869	QKGDGRPETPK(2)-DHDNKQK(5)
Nipbl	785	Nipbl	798	GDGRPETPKQK(9)-QKNEGRPETPK(2)
Nipbl	785	Nipbl	807	GDGRPETPKQK(9)-NEGRPETPKHR(9)
Nipbl	785	Nipbl	818	GDGRPETPKQK(9)-DSGKPSTEK(4)
Nipbl	798	Nipbl	818	QKNEGRPETPK(2)-DSGKPSTEK(4)
Nipbl	798	Nipbl	824	QKNEGRPETPK(2)-KPEVSK(1)
Nipbl	798	Nipbl	829	QKNEGRPETPK(2)-KPEVSKHK(6)
Nipbl	798	Nipbl	831	QKNEGRPETPK(2)-HKQDTK(2)
Nipbl	798	Nipbl	850	QKNEGRPETPK(2)-AEALKQRPDGR(5)
Nipbl	798	Nipbl	869	QKNEGRPETPK(2)-DHDNKQK(5)
Nipbl	798	Nipbl	871	QKNEGRPETPK(2)-QKSDDRGESER(2)

Appendix

Nipbl	807	Nipbl	818	NEGRPETPKHR(9)-DSGKPSTEK(4)
Nipbl	807	Nipbl	823	NEGRPETPKHR(9)-PSTEKKPEVSK(5)
Nipbl	807	Nipbl	824	NEGRPETPKHR(9)-KPEVSK(1)
Nipbl	807	Nipbl	829	NEGRPETPKHR(9)-KPEVSKHK(6)
Nipbl	807	Nipbl	831	NEGRPETPKHR(9)-HKQDTK(2)
Nipbl	807	Nipbl	835	NEGRPETPKHR(9)-QDTKSDSPR(4)
Nipbl	807	Nipbl	842	NEGRPETPKHR(9)-LKSER(2)
Nipbl	807	Nipbl	850	NEGRPETPKHR(9)-AEALKQRPDGR(5)
Nipbl	807	Nipbl	869	NEGRPETPKHR(9)-DHDNKQK(5)
Nipbl	807	Nipbl	910	NEGRPETPKHR(9)-SDSSKTDK(5)
Nipbl	807	Nipbl	1309	NEGRPETPKHR(9)-LSTDKTVK(5)
Nipbl	818	Nipbl	831	DSGKPSTEK(4)-HKQDTK(2)
Nipbl	818	Nipbl	835	DSGKPSTEK(4)-HKQDTKSDSPR(6)
Nipbl	818	Nipbl	842	DSGKPSTEK(4)-LKSER(2)
Nipbl	818	Nipbl	850	DSGKPSTEK(4)-AEALKQRPDGR(5)
Nipbl	818	Nipbl	869	DSGKPSTEK(4)-DHDNKQK(5)
Nipbl	823	Nipbl	2529	DSGKPSTEK(9)-KSSPSK(1)
Nipbl	824	Nipbl	835	KPEVSK(1)-HKQDTKSDSPR(6)
Nipbl	824	Nipbl	842	KPEVSK(1)-LKSER(2)
Nipbl	824	Nipbl	850	KPEVSK(1)-AEALKQRPDGR(5)
Nipbl	824	Nipbl	869	KPEVSK(1)-DHDNKQK(5)
Nipbl	824	Nipbl	944	KPEVSK(1)-VKQGDSNK(2)
Nipbl	829	Nipbl	842	KPEVSKHK(6)-LKSER(2)
Nipbl	829	Nipbl	850	KPEVSKHK(6)-AEALKQRPDGR(5)
Nipbl	829	Nipbl	869	KPEVSKHK(6)-DHDNKQK(5)
Nipbl	829	Nipbl	871	KPEVSKHK(6)-QKSDDRGESER(2)
Nipbl	829	Nipbl	910	KPEVSKHK(6)-SDSSKTDK(5)
Nipbl	829	Nipbl	913	KPEVSKHK(6)-TDKLERK(3)
Nipbl	829	Nipbl	944	KPEVSKHK(6)-VKQGDSNK(2)
Nipbl	829	Nipbl	975	KPEVSKHK(6)-SKVDTNK(2)
Nipbl	831	Nipbl	842	HKQDTK(2)-LKSERAEALK(2)
Nipbl	831	Nipbl	850	HKQDTK(2)-AEALKQRPDGR(5)
Nipbl	831	Nipbl	869	HKQDTK(2)-DHDNKQK(5)
Nipbl	831	Nipbl	871	HKQDTK(2)-QKSDDRGESER(2)
Nipbl	835	Nipbl	850	QDTKSDSPR(4)-AEALKQRPDGR(5)
Nipbl	835	Nipbl	869	HKQDTKSDSPR(6)-DHDNKQK(5)
Nipbl	842	Nipbl	869	LKSER(2)-DHDNKQK(5)
Nipbl	871	Nipbl	842	LKSER(2)-QKSDDRGESER(2)
Nipbl	850	Nipbl	869	AEALKQRPDGR(5)-DHDNKQK(5)
Nipbl	850	Nipbl	871	AEALKQRPDGR(5)-QKSDDRGESER(2)
Nipbl	850	Nipbl	905	AEALKQRPDGR(5)-SSSRNEHGKSDSSK(10)
Nipbl	850	Nipbl	910	AEALKQRPDGR(5)-SDSSKTDK(5)
Nipbl	850	Nipbl	944	AEALKQRPDGR(5)-VKQGDSNK(2)
Nipbl	869	Nipbl	905	DHDNKQK(5)-SSSRNEHGKSDSSK(10)
Nipbl	869	Nipbl	910	DHDNKQK(5)-SDSSKTDK(5)
Nipbl	869	Nipbl	913	DHDNKQK(5)-TDKLERK(3)
Nipbl	869	Nipbl	917	DHDNKQK(5)-KRRHESGDSR(1)
Nipbl	869	Nipbl	944	DHDNKQK(5)-VKQGDSNK(2)
Nipbl	869	Nipbl	950	DHDNKQK(5)-QGDSNKS(6)
Nipbl	869	Nipbl	955	DHDNKQK(5)-SRSDKLGF(5)
Nipbl	869	Nipbl	973	DHDNKQK(5)-RTEGNKSK(6)
Nipbl	869	Nipbl	975	DHDNKQK(5)-SKVDTNK(2)
Nipbl	869	Nipbl	1002	DHDNKQK(5)-SGALKNFVIPK(5)
Nipbl	869	Nipbl	1036	DHDNKQK(5)-DKVEKIGLVEDLNK(5)
Nipbl	869	Nipbl	1071	DHDNKQK(5)-DREDKSR(5)
Nipbl	871	Nipbl	905	QKSDDRGESER(2)-SSSRNEHGKSDSSK(10)
Nipbl	871	Nipbl	910	QKSDDRGESER(2)-SDSSKTDK(5)
Nipbl	871	Nipbl	913	QKSDDRGESER(2)-TDKLERK(3)

Appendix

Nipbl	871	Nipbl	917	QKSDDRGESER(2)-KHRHESGDSR(1)
Nipbl	871	Nipbl	955	QKSDDRGESER(2)-SRSDKLGFK(5)
Nipbl	905	Nipbl	917	SSSRNEHGIKSDSSK(10)-KHRHESGDSR(1)
Nipbl	905	Nipbl	944	SSSRNEHGIKSDSSK(10)-VKQGDSNK(2)
Nipbl	905	Nipbl	950	NEHGIKSDSSK(6)-QGDSNKS(6)
Nipbl	905	Nipbl	973	NEHGIKSDSSK(6)-RTEGNKSK(6)
Nipbl	910	Nipbl	917	SDSSKTDK(5)-KHRHESGDSR(1)
Nipbl	910	Nipbl	944	SDSSKTDK(5)-VKQGDSNK(2)
Nipbl	910	Nipbl	955	SDSSKTDK(5)-SRSDKLGFK(5)
Nipbl	910	Nipbl	1022	SDSSKTDK(5)-DGNVTQETKK(9)
Nipbl	913	Nipbl	944	TDKLERK(3)-VKQGDSNK(2)
Nipbl	913	Nipbl	955	TDKLERK(3)-SRSDKLGFK(5)
Nipbl	913	Nipbl	959	TDKLERK(3)-LGFKSPTSK(4)
Nipbl	913	Nipbl	973	TDKLERK(3)-RTEGNKSK(6)
Nipbl	913	Nipbl	1066	TDKLERK(3)-LIKDREDK(3)
Nipbl	917	Nipbl	944	KHRHESGDSR(1)-VKQGDSNK(2)
Nipbl	917	Nipbl	964	KHRHESGDSR(1)-SPTSKDDK(5)
Nipbl	917	Nipbl	975	KHRHESGDSR(1)-SKVDTNK(2)
Nipbl	944	Nipbl	955	VKQGDSNK(2)-SDKLGFK(3)
Nipbl	944	Nipbl	959	VKQGDSNK(2)-LGFKSPTSK(4)
Nipbl	944	Nipbl	973	VKQGDSNK(2)-RTEGNKSK(6)
Nipbl	944	Nipbl	975	VKQGDSNK(2)-SKVDTNK(2)
Nipbl	944	Nipbl	980	VKQGDSNK(2)-SKVDTNKAHPDNK(7)
Nipbl	944	Nipbl	986	VKQGDSNK(2)-AHPDNKAEFPSYLLGGR(6)
Nipbl	944	Nipbl	1008	VKQGDSNK(2)-NFVIPKIK(6)
Nipbl	944	Nipbl	1022	VKQGDSNK(2)-DGNVTQETKK(9)
Nipbl	944	Nipbl	1033	VKQGDSNK(2)-DKVEK(2)
Nipbl	944	Nipbl	1036	VKQGDSNK(2)-DKVEKIGLVEDLNK(5)
Nipbl	944	Nipbl	1088	VKQGDSNK(2)-SNKGSIDQSVLK(3)
Nipbl	950	Nipbl	959	QGDSNKS(6)-LGFKSPTSK(4)
Nipbl	950	Nipbl	967	QGDSNKS(6)-DDKRTEGNK(3)
Nipbl	950	Nipbl	973	QGDSNKS(6)-RTEGNKSK(6)
Nipbl	950	Nipbl	975	QGDSNKS(6)-SKVDTNK(2)
Nipbl	950	Nipbl	980	QGDSNKS(6)-SKVDTNKAHPDNK(7)
Nipbl	950	Nipbl	1022	QGDSNKS(6)-DGNVTQETKK(9)
Nipbl	955	Nipbl	967	SRSDKLGFK(5)-DDKRTEGNK(3)
Nipbl	955	Nipbl	973	SRSDKLGFK(5)-RTEGNKSK(6)
Nipbl	955	Nipbl	975	SRSDKLGFK(5)-SKVDTNK(2)
Nipbl	955	Nipbl	980	SRSDKLGFK(5)-SKVDTNKAHPDNK(7)
Nipbl	955	Nipbl	986	SDKLGFK(3)-AHPDNKAEFPSYLLGGR(6)
Nipbl	955	Nipbl	1013	SRSDKLGFK(5)-RDKDGNVTQETK(3)
Nipbl	955	Nipbl	1022	SRSDKLGFK(5)-DGNVTQETKK(9)
Nipbl	959	Nipbl	967	LGFKSPTSK(4)-DDKRTEGNK(3)
Nipbl	959	Nipbl	973	LGFKSPTSK(4)-RTEGNKSK(6)
Nipbl	959	Nipbl	975	LGFKSPTSK(4)-SKVDTNK(2)
Nipbl	959	Nipbl	980	LGFKSPTSK(4)-SKVDTNKAHPDNK(7)
Nipbl	959	Nipbl	986	LGFKSPTSK(4)-AHPDNKAEFPSYLLGGR(6)
Nipbl	964	Nipbl	975	SPTSKDDK(5)-SKVDTNK(2)
Nipbl	964	Nipbl	980	SPTSKDDK(5)-VDTNKAHPDNK(5)
Nipbl	964	Nipbl	986	SPTSKDDK(5)-AHPDNKAEFPSYLLGGR(6)
Nipbl	964	Nipbl	1022	SPTSKDDK(5)-DGNVTQETKK(9)
Nipbl	967	Nipbl	975	SPTSKDDK(8)-SKVDTNK(2)
Nipbl	967	Nipbl	980	DDKRTEGNK(3)-SKVDTNKAHPDNK(7)
Nipbl	973	Nipbl	986	RTEGNKSK(6)-AHPDNKAEFPSYLLGGR(6)
Nipbl	973	Nipbl	1002	RTEGNKSK(6)-SGALKNFVIPK(5)
Nipbl	973	Nipbl	1008	RTEGNKSK(6)-NFVIPKIK(6)
Nipbl	973	Nipbl	1013	RTEGNKSK(6)-DKDGNVTQETK(2)
Nipbl	973	Nipbl	1022	RTEGNKSK(6)-DGNVTQETKK(9)

Appendix

Nipbl	973	Nipbl	1033	RTEGNKSK(6)-DKVEK(2)
Nipbl	973	Nipbl	1036	RTEGNKSK(6)-DKVEKIGLVEDLNK(5)
Nipbl	973	Nipbl	1048	RTEGNKSK(6)-GAKPVVVLQK(3)
Nipbl	975	Nipbl	1002	SKVDTNK(2)-SGALKNFVIPK(5)
Nipbl	975	Nipbl	1008	SKVDTNK(2)-NFVIPKIK(6)
Nipbl	975	Nipbl	1013	SKVDTNK(2)-RDGDGNTQETK(3)
Nipbl	975	Nipbl	1022	SKVDTNK(2)-DGNVTQETKK(9)
Nipbl	975	Nipbl	1036	SKVDTNK(2)-DKVEKIGLVEDLNK(5)
Nipbl	975	Nipbl	1045	SKVDTNK(2)-IGLVEDLNKGAK(9)
Nipbl	975	Nipbl	1048	SKVDTNK(2)-GAKPVVVLQK(3)
Nipbl	980	Nipbl	1002	SKVDTNKAHPDNK(7)-SGALKNFVIPK(5)
Nipbl	980	Nipbl	1008	SKVDTNKAHPDNK(7)-NFVIPKIK(6)
Nipbl	980	Nipbl	1013	SKVDTNKAHPDNK(7)-DKDGNVTQETK(2)
Nipbl	980	Nipbl	1033	SKVDTNKAHPDNK(7)-DKVEK(2)
Nipbl	980	Nipbl	1036	VDTNKAHPDNK(5)-DKVEKIGLVEDLNK(5)
Nipbl	980	Nipbl	1022	VDTNKAHPDNK(5)-DGNVTQETKK(9)
Nipbl	980	Nipbl	1023	SKVDTNKAHPDNK(7)-KMEMK(1)
Nipbl	980	Nipbl	1045	SKVDTNKAHPDNK(7)-IGLVEDLNKGAK(9)
Nipbl	980	Nipbl	1048	SKVDTNKAHPDNK(7)-GAKPVVVLQK(3)
Nipbl	980	Nipbl	1077	SKVDTNKAHPDNK(7)-SSLKPIK(4)
Nipbl	980	Nipbl	1088	SKVDTNKAHPDNK(7)-SNKGSIDQSVLK(3)
Nipbl	986	Nipbl	1002	AHPDNKAEFPSYLLGGR(6)-SGALKNFVIPK(5)
Nipbl	986	Nipbl	1008	AHPDNKAEFPSYLLGGR(6)-NFVIPKIK(6)
Nipbl	986	Nipbl	1013	AHPDNKAEFPSYLLGGR(6)-DKDGNVTQETK(2)
Nipbl	986	Nipbl	1022	AHPDNKAEFPSYLLGGR(6)-DGNVTQETKK(9)
Nipbl	986	Nipbl	1031	AHPDNKAEFPSYLLGGR(6)-GEPKDK(4)
Nipbl	986	Nipbl	1033	AHPDNKAEFPSYLLGGR(6)-DKVEK(2)
Nipbl	986	Nipbl	1048	AHPDNKAEFPSYLLGGR(6)-GAKPVVVLQK(3)
Nipbl	986	Nipbl	1066	AHPDNKAEFPSYLLGGR(6)-LIKDREDK(3)
Nipbl	986	Nipbl	1088	AHPDNKAEFPSYLLGGR(6)-SNKGSIDQSVLK(3)
Nipbl	1002	Nipbl	1013	SGALKNFVIPK(5)-RDGDGNTQETK(3)
Nipbl	1002	Nipbl	1022	SGALKNFVIPK(5)-DGNVTQETKK(9)
Nipbl	1002	Nipbl	1023	SGALKNFVIPK(5)-KMEMK(1)
Nipbl	1002	Nipbl	1031	SGALKNFVIPK(5)-GEPKDK(4)
Nipbl	1002	Nipbl	1033	SGALKNFVIPK(5)-DKVEK(2)
Nipbl	1002	Nipbl	1036	SGALKNFVIPK(5)-DKVEKIGLVEDLNK(5)
Nipbl	1002	Nipbl	1045	SGALKNFVIPK(5)-IGLVEDLNKGAK(9)
Nipbl	1002	Nipbl	1048	SGALKNFVIPK(5)-GAKPVVVLQK(3)
Nipbl	1002	Nipbl	1066	SGALKNFVIPK(5)-LIKDREDK(3)
Nipbl	1002	Nipbl	1071	SGALKNFVIPK(5)-DREDKSR(5)
Nipbl	1002	Nipbl	1077	SGALKNFVIPK(5)-SSLKPIK(4)
Nipbl	1002	Nipbl	1082	SGALKNFVIPK(5)-NKPSKSNK(2)
Nipbl	1002	Nipbl	1088	SGALKNFVIPK(5)-SNKGSIDQSVLK(3)
Nipbl	1002	Nipbl	1157	SGALKNFVIPK(5)-KDDDK(1)
Nipbl	1002	Nipbl	1309	SGALKNFVIPK(5)-LSTDKTVK(5)
Nipbl	1002	Nipbl	1670	SGALKNFVIPK(5)-KDAVTSK(1)
Nipbl	1002	Nipbl	1676	SGALKNFVIPK(5)-DAVTSKMDQGSIER(6)
Nipbl	1002	Nipbl	1687	SGALKNFVIPK(5)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1008	Nipbl	1013	NFVIPKIK(6)-DKDGNVTQETK(2)
Nipbl	1008	Nipbl	1022	NFVIPKIK(6)-DGNVTQETKK(9)
Nipbl	1008	Nipbl	1023	NFVIPKIK(6)-KMEMK(1)
Nipbl	1008	Nipbl	1027	NFVIPKIK(6)-KMEMKGEPK(5)
Nipbl	1008	Nipbl	1031	NFVIPKIK(6)-GEPKDK(4)
Nipbl	1008	Nipbl	1033	NFVIPKIK(6)-DKVEK(2)
Nipbl	1008	Nipbl	1036	NFVIPKIK(6)-DKVEKIGLVEDLNK(5)
Nipbl	1008	Nipbl	1045	NFVIPKIK(6)-IGLVEDLNKGAK(9)
Nipbl	1008	Nipbl	1048	NFVIPKIK(6)-GAKPVVVLQK(3)
Nipbl	1008	Nipbl	1066	NFVIPKIK(6)-LIKDREDK(3)

Appendix

Nipbl	1008	Nipbl	1071	NFVIPKIK(6)-DREDKSR(5)
Nipbl	1010	Nipbl	1023	IKRDK(2)-KMEMK(1)
Nipbl	1010	Nipbl	1033	IKRDK(2)-DKVEK(2)
Nipbl	1013	Nipbl	1023	DKDGNVTQETK(2)-KMEMK(1)
Nipbl	1013	Nipbl	1027	RDKDGNVTQETK(3)-KMEMKGEPK(5)
Nipbl	1013	Nipbl	1033	RDKDGNVTQETK(3)-DKVEK(2)
Nipbl	1013	Nipbl	1036	DKDGNVTQETK(2)-DKVEKIGLVEDLNK(5)
Nipbl	1013	Nipbl	1045	DKDGNVTQETK(2)-IGLVEDLNKGAK(9)
Nipbl	1013	Nipbl	1048	DKDGNVTQETK(2)-GAKPVVVLQK(3)
Nipbl	1013	Nipbl	1055	DKDGNVTQETK(2)-PVVVLQKLSLDDVQK(7)
Nipbl	1013	Nipbl	1066	RDKDGNVTQETK(3)-LIKDREDK(3)
Nipbl	1013	Nipbl	1071	DKDGNVTQETK(2)-DREDKSR(5)
Nipbl	1013	Nipbl	1088	RDKDGNVTQETK(3)-SNKGSIDQSVLK(3)
Nipbl	1013	Nipbl	1687	DKDGNVTQETK(2)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1022	Nipbl	1031	DGNVTQETKK(9)-GEPKDK(4)
Nipbl	1022	Nipbl	1033	DGNVTQETKK(9)-DKVEK(2)
Nipbl	1022	Nipbl	1036	DGNVTQETKK(9)-DKVEKIGLVEDLNK(5)
Nipbl	1022	Nipbl	1045	DGNVTQETKK(9)-IGLVEDLNKGAK(9)
Nipbl	1022	Nipbl	1048	DGNVTQETKK(9)-GAKPVVVLQK(3)
Nipbl	1022	Nipbl	1055	DGNVTQETKK(9)-PVVVLQKLSLDDVQK(7)
Nipbl	1022	Nipbl	1063	DGNVTQETKK(9)-LSLDDVQKLIK(8)
Nipbl	1022	Nipbl	1066	DGNVTQETKK(9)-LIKDREDK(3)
Nipbl	1022	Nipbl	1071	DGNVTQETKK(9)-DREDKSR(5)
Nipbl	1022	Nipbl	1077	DGNVTQETKK(9)-SRSSLKPIK(6)
Nipbl	1022	Nipbl	1085	DGNVTQETKK(9)-NKPSKSNK(5)
Nipbl	1022	Nipbl	1088	DGNVTQETKK(9)-SNKGSIDQSVLK(3)
Nipbl	1023	Nipbl	1033	DKVEK(2)-KMEMK(1)
Nipbl	1023	Nipbl	1036	KMEMK(1)-DKVEKIGLVEDLNK(5)
Nipbl	1023	Nipbl	1045	KMEMK(1)-IGLVEDLNKGAK(9)
Nipbl	1023	Nipbl	1048	KMEMK(1)-GAKPVVVLQK(3)
Nipbl	1027	Nipbl	1048	KMEMKGEPK(5)-GAKPVVVLQK(3)
Nipbl	1031	Nipbl	1045	GEPKDK(4)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	1031	Nipbl	1063	GEPKDK(4)-LSLDDVQKLIK(8)
Nipbl	1033	Nipbl	1055	DKVEK(2)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	1033	Nipbl	1063	DKVEK(2)-LSLDDVQKLIK(8)
Nipbl	1033	Nipbl	1066	DKVEK(2)-LIKDREDK(3)
Nipbl	1033	Nipbl	1071	DKVEKIGLVEDLNK(2)-DREDKSR(5)
Nipbl	1033	Nipbl	1077	DKVEK(2)-SSLKPIK(4)
Nipbl	1033	Nipbl	1088	DKVEK(2)-SNKGSIDQSVLK(3)
Nipbl	1036	Nipbl	1048	DKVEKIGLVEDLNK(5)-GAKPVVVLQK(3)
Nipbl	1036	Nipbl	1066	DKVEKIGLVEDLNK(5)-LIKDREDK(3)
Nipbl	1036	Nipbl	1071	DKVEKIGLVEDLNK(5)-DREDKSR(5)
Nipbl	1036	Nipbl	1077	DKVEKIGLVEDLNK(5)-SSLKPIK(4)
Nipbl	1036	Nipbl	1082	DKVEKIGLVEDLNK(5)-NKPSK(2)
Nipbl	1036	Nipbl	1085	DKVEKIGLVEDLNK(5)-NKPSKSNK(5)
Nipbl	1036	Nipbl	1088	DKVEKIGLVEDLNK(5)-SNKGSIDQSVLK(3)
Nipbl	1045	Nipbl	1066	IGLVEDLNKGAK(9)-LIKDREDK(3)
Nipbl	1045	Nipbl	1071	IGLVEDLNKGAK(9)-DREDKSR(5)
Nipbl	1045	Nipbl	1077	IGLVEDLNKGAK(9)-SSLKPIK(4)
Nipbl	1045	Nipbl	1082	IGLVEDLNKGAK(9)-NKPSK(2)
Nipbl	1045	Nipbl	1088	IGLVEDLNKGAK(9)-SNKGSIDQSVLK(3)
Nipbl	1048	Nipbl	1066	GAKPVVVLQK(3)-LIKDREDK(3)
Nipbl	1048	Nipbl	1071	GAKPVVVLQK(3)-DREDKSR(5)
Nipbl	1048	Nipbl	1085	GAKPVVVLQK(3)-NKPSKSNK(5)
Nipbl	1048	Nipbl	1088	GAKPVVVLQK(3)-SNKGSIDQSVLK(3)
Nipbl	1048	Nipbl	1130	GAKPVVVLQK(3)-STVNEKPK(6)
Nipbl	1063	Nipbl	1077	LSLDDVQKLIK(8)-SRSSLKPIK(6)
Nipbl	1063	Nipbl	1085	LSLDDVQKLIK(8)-NKPSKSNK(5)

Appendix

Nipbl	1063	Nipbl	1088	LSLDDVQKLIK(8)-SNKGSIDQSVLK(3)
Nipbl	1063	Nipbl	1157	LSLDDVQKLIK(8)-KDDDK(1)
Nipbl	1066	Nipbl	1077	LIKDREDK(3)-SSLKPIK(4)
Nipbl	1066	Nipbl	1080	LIKDREDK(3)-SSLKPIKNK(7)
Nipbl	1066	Nipbl	1085	LIKDREDK(3)-NKPSKSNK(5):
Nipbl	1066	Nipbl	1088	LIKDREDK(3)-SNKGSIDQSVLK(3)
Nipbl	1066	Nipbl	1130	LIKDREDK(3)-RSTVNEKPK(7)
Nipbl	1066	Nipbl	1309	LIKDREDK(3)-LSTDKTVK(5)
Nipbl	1066	Nipbl	1687	LIKDREDK(3)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1071	Nipbl	1080	DREDKSR(5)-PIKNKPSK(3)
Nipbl	1071	Nipbl	1082	DREDKSR(5)-NKPSK(2)
Nipbl	1071	Nipbl	1085	DREDKSR(5)-NKPSKSNK(5)
Nipbl	1071	Nipbl	1088	DREDKSR(5)-SNKGSIDQSVLK(3)
Nipbl	1071	Nipbl	1130	DREDKSR(5)-RSTVNEKPK(7)
Nipbl	1077	Nipbl	1088	SRSSLKPIK(6)-SNKGSIDQSVLK(3)
Nipbl	1077	Nipbl	1130	SSLKPIK(4)-RSTVNEKPK(7)
Nipbl	1077	Nipbl	1157	SSLKPIK(4)-KDDDK(1)
Nipbl	1077	Nipbl	1670	SSLKPIK(4)-LRKDAVTSK(3)
Nipbl	1077	Nipbl	1687	SSLKPIK(4)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1082	Nipbl	1130	NKPSK(2)-RSTVNEKPK(7)
Nipbl	1085	Nipbl	1130	NKPSKSNK(5)-RSTVNEKPK(7)
Nipbl	1085	Nipbl	1157	NKPSKSNK(5)-KDDDK(1)
Nipbl	1085	Nipbl	1687	NKPSKSNK(5)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1088	Nipbl	1130	SNKGSIDQSVLK(3)-RSTVNEKPK(7)
Nipbl	1088	Nipbl	1676	SNKGSIDQSVLK(3)-DAVTSKMDQGSIER(6)
Nipbl	1088	Nipbl	1687	SNKGSIDQSVLK(3)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1088	Nipbl	1839	SNKGSIDQSVLK(3)-TKAMK(2)
Nipbl	1118	Nipbl	1157	VKMNK(2)-KDDDK(1)
Nipbl	1118	Nipbl	1670	VKMNK(2)-KDAVTSK(1)
Nipbl	1130	Nipbl	1157	RSTVNEKPK(7)-KDDDK(1)
Nipbl	1130	Nipbl	1295	STVNEKPK(6)-HQLNELGSESAKIK(12)
Nipbl	1225	Nipbl	1230	KEKQK(3)-RKAYEPK(2)
Nipbl	1230	Nipbl	1295	RKAYEPK(2)-HQLNELGSESAKIK(12)
Nipbl	1235	Nipbl	1295	AYEPLTPEEMMDSSTFK(5)-HQLNELGSESAKIK(12)
Nipbl	1248	Nipbl	1309	LTPEEMMDSSTFKR(13)-LSTDKTVK(5)
Nipbl	1297	Nipbl	1309	IKAMGIMDK(2)-LSTDKTVK(5)
Nipbl	1417	Nipbl	1422	LDPHGGGLSSKAK(12)-AKCSTHK(2)
Nipbl	1417	Nipbl	1427	LDPHGGGLSSKAK(12)-CSTHKQR(5)
Nipbl	1567	Nipbl	1744	DSNAEEDSNKK(10)-SQKDEESSEGTHHAK(3)
Nipbl	1567	Nipbl	1780	DSNAEEDSNKK(10)-SIKTTSPSQFSTLK(4)
Nipbl	1568	Nipbl	1780	KIDQDVVITNSYETAMR(1)-SIKTTSPSQFSTLK(4)
Nipbl	1647	Nipbl	1839	LLVHQFSNKSTEMALR(9)-TKAMK(2)
Nipbl	1670	Nipbl	1687	LRKDAVTSK(3)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1670	Nipbl	2767	KDAVTSK(1)-YKDRPQIAR(2)
Nipbl	1670	Nipbl	2822	KDAVTSK(1)-ESDIIYKK(7)
Nipbl	1670	Nipbl	2835	KDAVTSK(1)-LTNKVVQTLR(4)
Nipbl	1676	Nipbl	1738	DAVTSKMDQGSIER(6)-DTTLETEKAMK(8)
Nipbl	1676	Nipbl	1744	DAVTSKMDQGSIER(6)-SQKDEESSEGTHHAK(3)
Nipbl	1676	Nipbl	1756	DAVTSKMDQGSIER(6)-DEESSEGTHHAKEIETTQIMHR(12)
Nipbl	1687	Nipbl	1744	ILKQVSGGEDEIQQLQK(3)-SQKDEESSEGTHHAK(3)
Nipbl	1738	Nipbl	1756	DTTLETEKAMK(8)-DEESSEGTHHAKEIETTQIMHR(12)
Nipbl	1780	Nipbl	1567	DSNAEEDSNKK(10)-SIKTTSPSQFSTLK(4)
Nipbl	1839	Nipbl	2626	TKAMK(2)-VYDKAINR(4)
Nipbl	1839	Nipbl	2831	TKAMK(2)-IALTSANKLTNK(8)
Nipbl	1839	Nipbl	2835	TKAMK(2)-LTNKVVQTLR(4)
Nipbl	1918	Nipbl	1952	VIKILR(3)-KLVNETFQK(1)
Nipbl	1939	Nipbl	2043	ITEMCVKMIR(7)-YEESLADSDNKGVNNSGR(11)
Nipbl	1951	Nipbl	2385	RVNDEEGIKK(9)-NKADQQLVEIDKK(2)

Appendix

Nipbl	1951	Nipbl	2395	RVNDEEGIKK(9)-DQQLVEIDKK(10)
Nipbl	1952	Nipbl	2395	KLVNETFQK(1)-ADQQLVEIDKK(10)
Nipbl	2016	Nipbl	2304	SEEDSSYKPVKK(11)-MQQADRDWKK(9)
Nipbl	2016	Nipbl	2308	SEEDSSYKPVKK(11)-VAKQEDLK(3)
Nipbl	2167	Nipbl	2214	YYGAISKLK(7)-GNSKVNK(4)
Nipbl	2185	Nipbl	2214	SQHQEDPNNTSLLTNKPALLR(16)-GNSKVNK(4)
Nipbl	2212	Nipbl	2308	VNIKDK(4)-VAKQEDLK(3)
Nipbl	2308	Nipbl	2404	VAKQEDLK(3)-YAGFIHMKAVAGMK(8)
Nipbl	2308	Nipbl	2831	VAKQEDLK(3)-IALTSANKLTNK(8)
Nipbl	2602	Nipbl	2631	QHLKNLCGFSDSK(4)-KTGVHFHPK(1)
Nipbl	2611	Nipbl	2626	NLCGFSDSKIQK(9)-VYDKAINRK(4)
Nipbl	2611	Nipbl	2631	NLCGFSDSKIQK(9)-KTGVHFHPK(1)
Nipbl	2614	Nipbl	2631	IQKYSPSESAK(3)-KTGVHFHPK(1)
Nipbl	2622	Nipbl	2631	YSPSESAKVYDK(8)-KTGVHFHPK(1)
Nipbl	2622	Nipbl	2831	YSPSESAKVYDK(8)-IALTSANKLTNK(8)
Nipbl	2622	Nipbl	2835	YSPSESAKVYDK(8)-LTNKVVQTLR(4)
Nipbl	2631	Nipbl	2653	KTGVHFHPK(1)-SDMANSKITEEVK(7)
Nipbl	2631	Nipbl	2659	KTGVHFHPK(1)-ITEEVKR(6)
Nipbl	2631	Nipbl	2831	KTGVHFHPK(1)-IALTSANKLTNK(8)
Nipbl	2631	Nipbl	2835	KTGVHFHPK(1)-LTNKVVQTLR(4)
Nipbl	2653	Nipbl	2806	SDMANSKITEEVK(7)-KLVFWVDTIK(1)
Nipbl	2653	Nipbl	2831	SDMANSKITEEVK(7)-IALTSANKLTNK(8)
Nipbl	2653	Nipbl	2835	SDMANSKITEEVK(7)-LTNKVVQTLR(4)
Nipbl	2659	Nipbl	2767	ITEEVKR(6)-YKDRPQIAR(2)
Nipbl	2659	Nipbl	2831	ITEEVKR(6)-IALTSANKLTNK(8)
Nipbl	2696	Nipbl	2831	NKAITSLLGGGSPK(2)-IALTSANKLTNK(8)
Nipbl	2696	Nipbl	2835	NKAITSLLGGGSPK(2)-LTNKVVQTLR(4)
Nipbl	2822	Nipbl	2835	ESDIYKK(7)-LTNKVVQTLR(4)

7.1.2 Cross-linking mass spectrometry of Nipbl in complex with Cohesin

Table 11 : List of all intraprotein cross-links Nipbl:Cohesin (exp. 1).*

Protein1	Residue1	Pro-	Residue2	Peptide
Smc1	13	Smc1	59	LIEIENFKSYK(8)-VKTLR(2)
Smc1	16	Smc1	72	DLIHGAPVGKPAANR(10)-SYKGR(3)
Smc1	72	Smc1	106	DLIHGAPVGKPAANR(10)-VIVGGSSEYKINN(10)
Smc1	72	Smc1	1022	DLIHGAPVGKPAANR(10)-VLTFDLTKYPDANPNNEQ(8)
Smc1	59	Smc1	72	DLIHGAPVGKPAANR(10)-VKTLR(2)
Smc1	59	Smc1	106	VKTLR(2)-VIVGGSSEYKINN(10)
Smc1	170	Smc1	172	SGELAQEYDKR(10)-KKEMVK(1)
Smc1	170	Smc1	173	SGELAQEYDKR(10)-KEMVK(1)
Smc1	170	Smc1	177	SGELAQEYDKR(10)-EMVKAEDTQFNYHR(4)
Smc1	170	Smc1	1053	SGELAQEYDKR(10)-AKKAK(2)
Smc1	189	Smc1	1037	KKNIAAER(1)-DKFQETSDEFEAAR(2)
Smc1	190	Smc1	200	KKNIAAER(2)-EAKQEKEEADR(3)
Smc1	190	Smc1	1030	KKNIAAER(2)-AMEKLESVR(4)
Smc1	190	Smc1	1037	KNIAAER(1)-DKFQETSDEFEAAR(2)
Smc1	203	Smc1	213	EAKQEKEEADR(6)-LKDEVVR(2)
Smc1	213	Smc1	939	LKDEVVR(2)-MQDIKLPLSK(5)
Smc1	213	Smc1	1026	LKDEVVR(2)-IAAPNMKAMEK(7)
Smc1	213	Smc1	1030	LKDEVVR(2)-AMEKLESVR(4)
Smc1	213	Smc1	1037	LKDEVVR(2)-DKFQETSDEFEAAR(2)
Smc1	237	Smc1	245	LYHNEVEIEKLNK(10)-ELASKNK(5)
Smc1	237	Smc1	918	LYHNEVEIEKLNK(10)-EVTAIETKLEQK(8)

Appendix

Smc1	237	Smc1	922	LYHNEVEIEKLNK(10)-LEQKR(4)
Smc1	240	Smc1	247	LNKELASK(3)-NKEIEK(2)
Smc1	240	Smc1	251	LNKELASK(3)-EIEKDKK(4)
Smc1	245	Smc1	251	ELASKNK(5)-EIEKDKK(4)
Smc1	245	Smc1	918	ELASKNKEIEK(5)-EVTAIETKLEQK(8)
Smc1	247	Smc1	258	NKEIEK(2)-MDKVEDELKEK(3)
Smc1	251	Smc1	258	NKEIEKDK(6)-MDKVEDELKEK(3)
Smc1	258	Smc1	267	MDKVEDELKEK(3)-KKELGK(1)
Smc1	258	Smc1	324	MDKVEDELKEK(3)-SLQNAQKHYKK(7)
Smc1	258	Smc1	897	MDKVEDELKEK(3)-KLGGANK(1)
Smc1	264	Smc1	267	VEDELKEK(6)-KKELGK(1)
Smc1	264	Smc1	268	MDKVEDELKEK(9)-KKELGK(2)
Smc1	264	Smc1	896	MDKVEDELKEK(9)-KKLGGANK(1)
Smc1	266	Smc1	268	VEDELKEKK(8)-KELGK(1)
Smc1	267	Smc1	881	KKELGK(1)-KSEVNDKNHEMEEIR(1)
Smc1	267	Smc1	903	KKELGK(1)-LGGANKEMTHLQK(6)
Smc1	268	Smc1	887	KELGKMMR(1)-SEVNDKNHEMEEIRK(6)
Smc1	272	Smc1	528	KELGKMMR(5)-LIDLQOPTQKK(10)
Smc1	272	Smc1	880	ELGKMMR(4)-NQHLAKK(6)
Smc1	272	Smc1	887	ELGKMMR(4)-SEVNDKNHEMEEIR(6)
Smc1	282	Smc1	287	EQQQIEKEIK(7)-EKDSELNQK(2)
Smc1	285	Smc1	294	EQQQIEKEIEK(10)-DSELNQKRPQYIK(7)
Smc1	285	Smc1	677	EIKEK(3)-EKKER(2)
Smc1	285	Smc1	874	EIKEKDSELNQK(3)-IIDETMAQLQDLKNQHLAK(13)
Smc1	285	Smc1	881	EIKEKDSELNQK(3)-KSEVNDKNHEMEEIR(1)
Smc1	287	Smc1	881	EIKEKDSELNQK(5)-KSEVNDKNHEMEEIR(1)
Smc1	294	Smc1	854	DSELNQKRPQYIK(7)-LKKEEQR(3)
Smc1	294	Smc1	300	EKDSELNQK(9)-PQYIKAK(5)
Smc1	300	Smc1	308	RPQYIKAK(6)-ENTSHKIK(6)
Smc1	300	Smc1	311	RPQYIKAK(6)-KLEAAK(1)
Smc1	300	Smc1	713	RPQYIKAK(6)-LKYSQSDLEQTK(2)
Smc1	300	Smc1	851	RPQYIKAK(6)-KDENEIEKLK(8)
Smc1	302	Smc1	853	AKENTSHK(2)-LKKEEQR(2)
Smc1	308	Smc1	311	ENTSHKIK(6)-KLEAAK(1)
Smc1	308	Smc1	844	ENTSHKIK(6)-KDENEIEK(1)
Smc1	308	Smc1	851	ENTSHKIK(6)-KDENEIEKLK(8)
Smc1	310	Smc1	317	IKKLEAAK(2)-KSLQNAQK(1)
Smc1	311	Smc1	317	KLEAAK(1)-KSLQNAQK(1)
Smc1	311	Smc1	851	KLEAAK(1)-KDENEIEKLK(8)
Smc1	311	Smc1	853	LKKEEQR(2)-KLEAAK(1)
Smc1	311	Smc1	844	KLEAAK(1)-KDENEIEK(1)
Smc1	316	Smc1	437	LEAAK(5)-IEKLEEYITTSK(3)
Smc1	316	Smc1	851	KLEAAK(6)-KDENEIEKLK(8)
Smc1	316	Smc1	853	LEAAK(5)-LKKEEQR(2)
Smc1	317	Smc1	433	KSLQNAQK(1)-EIEENQKR(7)
Smc1	317	Smc1	437	KSLQNAQK(1)-IEKLEEYITTSK(3)
Smc1	317	Smc1	729	KSLQNAQK(1)-FKASR(2)
Smc1	324	Smc1	330	SLQNAQKHYK(7)-KGDMELEK(1)
Smc1	324	Smc1	433	SLQNAQKHYK(7)-EIEENQKR(7)
Smc1	324	Smc1	437	SLQNAQKHYK(7)-IEKLEEYITTSK(3)
Smc1	324	Smc1	734	SLQNAQKHYK(7)-HLALNLQEKSK(9)
Smc1	327	Smc1	437	SLQNAQKHYKK(10)-RIEKLEEYITTSK(4)
Smc1	345	Smc1	752	EMLSVEKAR(7)-INDIKR(5)
Smc1	345	Smc1	806	EMLSVEKAR(7)-KRLEFENQK(1)
Smc1	345	Smc1	814	EMLSVEKAR(7)-LEFENQKTR(7)
Smc1	373	Smc1	379	DLTLEENQVKK(10)-LKEEASK(2)
Smc1	373	Smc1	384	DLTLEENQVKK(10)-LKEEASKR(7)
Smc1	373	Smc1	796	DLTLEENQVKK(10)-EFEEKVKR(6)

Appendix

Smc1	379	Smc1	752	LKEEASK(2)-INDIKR(5)
Smc1	379	Smc1	766	LKEEASK(2)-DLKEK(3)
Smc1	379	Smc1	796	LKEEASKR(2)-EFEEKVKR(6)
Smc1	379	Smc1	798	LKEEASK(2)-EFEEKVKR(8)
Smc1	379	Smc1	805	LKEEASKR(2)-QNEIAKK(6)
Smc1	384	Smc1	395	EEASKRAATLAQELEK(5)-AATLAQELEKFNR(10)
Smc1	401	Smc1	763	DQKADQDRDLLEER(3)-EREMKDLK(5)
Smc1	401	Smc1	766	DQKADQDRDLLEER(3)-EMKDLKEK(6)
Smc1	401	Smc1	798	DQKADQDRDLLEER(3)-EFEEKVKR(8)
Smc1	401	Smc1	805	DQKADQDRDLLEER(3)-QNEIAKK(6)
Smc1	401	Smc1	806	DQKADQDRDLLEER(3)-KRLEFENQK(1)
Smc1	413	Smc1	736	KKVETEA(1)-SKLESELANFGPR(2)
Smc1	413	Smc1	752	KKVETEA(1)-INDIKR(5)
Smc1	414	Smc1	734	KKVETEA(2)-HLALNLQEKSK(9)
Smc1	414	Smc1	752	KVETEA(1)-INDIKR(5)
Smc1	414	Smc1	897	KVETEA(1)-KLGGANKEMTHLQK(1)
Smc1	420	Smc1	736	VETEA(1)-SKLESELANFGPR(2)
Smc1	433	Smc1	437	EIEENQKR(7)-IEKLEEYITTSK(3)
Smc1	433	Smc1	723	LREIEENQKR(9)-YSQSDLEQTKTR(10)
Smc1	437	Smc1	723	RIEKLEEYITTSK(4)-YSQSDLEQTKTR(10)
Smc1	446	Smc1	723	LEEYITTSKQSLEEQK(9)-YSQSDLEQTKTR(10)
Smc1	446	Smc1	713	LEEYITTSKQSLEEQK(9)-LKYSQSDLEQTK(2)
Smc1	461	Smc1	713	NKKDELQSER(2)-LKYSQSDLEQTK(2)
Smc1	500	Smc1	528	KAEIMESIKR(1)-LIDLCQPTQKK(10)
Smc1	500	Smc1	540	KAEIMESIKR(1)-VLGKNMDAIIVDSEK(4)
Smc1	500	Smc1	660	KAEIMESIKR(1)-SGVISGGASDLKAK(12)
Smc1	508	Smc1	660	KAEIMESIKR(9)-SGVISGGASDLKAK(12)
Smc1	508	Smc1	673	KAEIMESIKR(9)-AVDKLK(4)
Smc1	508	Smc1	903	KAEIMESIKR(9)-LGGANKEMTHLQK(6)
Smc1	508	Smc1	910	KAEIMESIKR(9)-EMTHLQKEVTAIETK(7)
Smc1	508	Smc1	918	KAEIMESIKR(9)-EVTAIETKLEQK(8)
Smc1	528	Smc1	540	LIDLCQPTQKK(10)-VLGKNMDAIIVDSEK(4)
Smc1	528	Smc1	660	LIDLCQPTQKK(10)-SGVISGGASDLKAK(12)
Smc1	528	Smc1	662	LIDLCQPTQKK(10)-SGVISGGASDLKAKAR(14)
Smc1	528	Smc1	673	LIDLCQPTQKK(10)-AVDKLK(4)
Smc1	528	Smc1	677	LIDLCQPTQKK(10)-LKEKK(4)
Smc1	529	Smc1	589	KYQIAVTK(1)-ELKGAK(3)
Smc1	529	Smc1	592	KYQIAVTK(1)-GAKLVIDVIR(3)
Smc1	536	Smc1	660	YQIAVTKVLGK(7)-SGVISGGASDLKAK(12)
Smc1	536	Smc1	662	YQIAVTKVLGK(7)-SGVISGGASDLKAKAR(14)
Smc1	540	Smc1	660	VLGKNMDAIIVDSEK(4)-SGVISGGASDLKAK(12)
Smc1	584	Smc1	589	GEPETFLPLDYLEVKPTDEKLR(20)-ELKGAK(3)
Smc1	589	Smc1	606	ELKGAK(3)-YEPPIKK(7)
Smc1	637	Smc1	660	HKTVALDGTLFQK(2)-SGVISGGASDLKAK(12)
Smc1	669	Smc1	896	WDEKAVDK(4)-KKLGGANK(1)
Smc1	669	Smc1	897	WDEKAVDK(4)-KLGGANK(1)
Smc1	669	Smc1	903	WDEKAVDK(4)-LGGANKEMTHLQK(6)
Smc1	673	Smc1	677	AVDKLK(4)-EKKER(2)
Smc1	673	Smc1	881	AVDKLK(4)-KSEVNDKNHEMEEIR(1)
Smc1	675	Smc1	686	AVDKLKEK(6)-LTEELKEQMK(6)
Smc1	675	Smc1	881	AVDKLKEK(6)-KSEVNDKNHEMEEIR(1)
Smc1	677	Smc1	686	EKKER(2)-LTEELKEQMK(6)
Smc1	678	Smc1	686	EKKER(3)-LTEELKEQMK(6)
Smc1	686	Smc1	694	LTEELKEQMK(6)-RKEAELR(2)
Smc1	686	Smc1	881	LTEELKEQMK(6)-KSEVNDKNHEMEEIR(1)
Smc1	690	Smc1	694	LTEELKEQMKAK(10)-KEAELR(1)
Smc1	723	Smc1	729	YSQSDLEQTKTR(10)-FKASR(2)
Smc1	723	Smc1	734	YSQSDLEQTKTR(10)-HLALNLQEKSK(9)

Appendix

Smc1	734	Smc1	752	INDIKR(5)-HLALNLQEKSK(9)
Smc1	752	Smc1	763	INDIKR(5)-EREMKDLK(5)
Smc1	752	Smc1	766	INDIKR(5)-DLKEK(3)
Smc1	752	Smc1	806	INDIKR(5)-KRLEFENQK(1)
Smc1	763	Smc1	806	EMKDLK(3)-KRLEFENQK(1)
Smc1	766	Smc1	798	EMKDLKEK(6)-EFFFFEKVKR(8)
Smc1	766	Smc1	806	DLKEK(3)-KRLEFENQK(1)
Smc1	766	Smc1	1037	EMKDLKEK(6)-DKFQETSDEFEAAR(2)
Smc1	796	Smc1	805	EFFFFEKVK(6)-RQNEIAKK(7)
Smc1	796	Smc1	806	NIREFFFFEKVK(9)-QNEIAKKR(7)
Smc1	798	Smc1	805	EFFFFEKVKR(8)-QNEIAKK(6)
Smc1	798	Smc1	806	EFFFFEKVKR(8)-KRLEFENQK(1)
Smc1	834	Smc1	844	EDQDKVHMWEQTVK(5)-KDENEIEK(1)
Smc1	851	Smc1	854	KDENEIEKLK(8)-KEEQR(1)
Smc1	853	Smc1	861	LKKEEQR(2)-HMKIIDETMAQLQDLK(3)
Smc1	854	Smc1	861	KEEQR(1)-HMKIIDETMAQLQDLK(3)
Smc1	874	Smc1	881	IIDETMAQLQDLKNQHLAK(13)-KSEVNDK(1)
Smc1	874	Smc1	897	IIDETMAQLQDLKNQHLAK(13)-KLGGANK(1)
Smc1	880	Smc1	887	NQHLAKK(6)-SEVNDKNHEMEEIR(6)
Smc1	897	Smc1	910	KLGGANK(1)-EMTHLQKEVTAIETK(7)
Smc1	934	Smc1	944	HNLLQACKMQDIK(8)-LPLSKGTMDDISQEEGSSQGEDSVSGSQR(5)
Smc1	1026	Smc1	1037	IAAPNMKAMEK(7)-LESVRDKFQETSDEFEAAR(7)
Smc1	1030	Smc1	1037	AMEKLESVR(4)-DKFQETSDEFEAAR(2)
Smc3	26	Smc3	621	DQTIVDPFSSKHNVIVGR(11)-FDKAFK(3)
Smc3	105	Smc3	113	VIGAKK(5)-DQYFLDKK(7)
Smc3	105	Smc3	114	VIGAKK(5)-KMTVK(1)
Smc3	105	Smc3	140	VIGAKK(5)-SNPYIVKQGGK(8)
Smc3	106	Smc3	114	KDQYFLDK(1)-KMTVK(1)
Smc3	113	Smc3	140	KDQYFLDKK(8)-SNPYIVKQGGK(8)
Smc3	114	Smc3	140	KMTVK(1)-SNPYIVKQGGK(8)
Smc3	140	Smc3	157	SNPYIVKQGGK(8)-LKLLR(2)
Smc3	140	Smc3	185	SNPYIVKQGGK(8)-ETEGKR(5)
Smc3	143	Smc3	157	QGGKINQMATAAPDSQR(3)-LKLLR(2)
Smc3	157	Smc3	185	LKLLR(2)-ETEGKR(5)
Smc3	157	Smc3	968	LKLLR(2)-KLEQCNTLKK(1)
Smc3	172	Smc3	185	KEESISLMK(1)-ETEGKR(5)
Smc3	180	Smc3	188	KEESISLMKETEGK(9)-REKINELLK(3)
Smc3	180	Smc3	357	EESISLMKETEGKR(8)-EKEER(2)
Smc3	185	Smc3	188	ETEGKR(5)-EKINELLK(2)
Smc3	185	Smc3	429	EESISLMKETEGKR(13)-EKNLEQYNKLDQDLNEVK(2)
Smc3	185	Smc3	486	ETEGKR(5)-EENAEQQALAAKR(12)
Smc3	185	Smc3	999	ETEGKR(5)-EKLIK(2)
Smc3	188	Smc3	968	EKINELLK(2)-KLEQCNTLKK(1)
Smc3	188	Smc3	999	EKINELLK(2)-EKLIK(2)
Smc3	207	Smc3	968	LHTLEEEKEELAQYQK(8)-KLEQCNTLKK(1)
Smc3	215	Smc3	968	EELAQYQKWDK(8)-KLEQCNTLKK(1)
Smc3	218	Smc3	997	WDKMR(3)-ALDQFVNFSEQKEK(12)
Smc3	218	Smc3	999	WDKMR(3)-EKLIK(2)
Smc3	238	Smc3	745	AKLDELSAK(2)-EKRRQSEK(2)
Smc3	238	Smc3	924	AKLDELSAK(2)-ELEKMTNR(4)
Smc3	238	Smc3	934	AKLDELSAK(2)-QGMILLK(6)
Smc3	238	Smc3	935	AKLDELSAK(2)-KKEECMK(1)
Smc3	238	Smc3	936	AKLDELSAK(2)-KKEECMKK(1)
Smc3	245	Smc3	252	LDELSAKR(7)-ETSGEKSR(6)
Smc3	245	Smc3	905	AKLDELSAKR(9)-WKNMEK(2)
Smc3	245	Smc3	924	LDELSAKR(7)-ELEKMTNR(4)
Smc3	245	Smc3	935	LDELSAKR(7)-KKEECMK(1)
Smc3	252	Smc3	920	ETSGEKSR(6)-EHMDAINHDTKELEK(11)

Appendix

Smc3	252	Smc3	924	ETSGEKS(6)-ELEKMTNR(4)
Smc3	266	Smc3	492	DKMEDIER(2)-REDLEKK(6)
Smc3	266	Smc3	889	DKMEDIER(2)-SEDLDNSIDKTEAGIK(10)
Smc3	266	Smc3	899	DKMEDIER(2)-ELQKS(4)
Smc3	278	Smc3	285	ELKTK(3)-ISAMKEEK(5)
Smc3	278	Smc3	895	ELKTK(3)-SEDLDNSIDKTEAGIKELQK(16)
Smc3	278	Smc3	899	ELKTK(3)-ELQKS(4)
Smc3	278	Smc3	905	ELKTK(3)-WKNMEK(2)
Smc3	280	Smc3	288	ELKTKISAMK(5)-EEKEQLSAERQEQIK(3)
Smc3	280	Smc3	486	TKISAMKEEK(2)-EENAEQQALAAKR(12)
Smc3	280	Smc3	889	TKISAMK(2)-SEDLDNSIDKTEAGIK(10)
Smc3	285	Smc3	895	ISAMKEEK(5)-SEDLDNSIDKTEAGIKELQK(16)
Smc3	300	Smc3	304	QEQIKQR(5)-TKLELK(2)
Smc3	300	Smc3	308	QEQIKQR(5)-TKLELKAK(6)
Smc3	300	Smc3	462	QEQIKQR(5)-NKKDELQSER(3)
Smc3	304	Smc3	310	TKLELK(2)-AKDLQDELQSER(2)
Smc3	304	Smc3	459	TKLELK(2)-YYEVKNK(5)
Smc3	304	Smc3	461	TKLELK(2)-NKKDELQSER(2)
Smc3	304	Smc3	462	TKLELK(2)-KDELQSER(1)
Smc3	304	Smc3	727	TKLELKAK(2)-KFKASR(1)
Smc3	308	Smc3	328	TKLELKAK(6)-LLKER(3)
Smc3	308	Smc3	459	LELKAK(4)-YYEVKNK(5)
Smc3	308	Smc3	462	TKLELKAK(6)-NKKDELQSER(3)
Smc3	308	Smc3	727	TKLELKAK(6)-KFKASR(1)
Smc3	308	Smc3	729	LELKAK(4)-FKASR(2)
Smc3	328	Smc3	332	LLKER(3)-QKLLEK(2)
Smc3	328	Smc3	336	LLKER(3)-LLEKIEEK(4)
Smc3	332	Smc3	340	QKLLEK(2)-IEEKQK(4)
Smc3	336	Smc3	342	LLEKIEEK(4)-QKELAETEPK(2)
Smc3	340	Smc3	820	IEEKQK(4)-IKLEGIITR(2)
Smc3	342	Smc3	1025	QKELAETEPK(2)-KYEAIQLTFK(1)
Smc3	350	Smc3	357	ELAETEPKFNSVK(8)-EKEER(2)
Smc3	350	Smc3	820	ELAETEPKFNSVK(8)-IKLEGIITR(2)
Smc3	355	Smc3	409	FNSVKEKEER(5)-SLDQAINDKKR(9)
Smc3	355	Smc3	756	FNSVKEKEER(5)-TFMPKQR(5)
Smc3	355	Smc3	820	FNSVKEKEER(5)-IKLEGIITR(2)
Smc3	357	Smc3	820	EKEER(2)-IKLEGIITR(2)
Smc3	378	Smc3	409	TDLYAKQGR(6)-SLDQAINDKKR(9)
Smc3	388	Smc3	396	GSQFTSKEER(7)-DKWIKK(5)
Smc3	393	Smc3	400	DKWIK(2)-ELKSLDQAINDK(3)
Smc3	396	Smc3	400	DKWIKK(5)-ELKSLDQAINDK(3)
Smc3	400	Smc3	934	ELKSLDQAINDK(3)-QGMLLKK(6)
Smc3	409	Smc3	756	SLDQAINDKK(9)-TFMPKQR(5)
Smc3	410	Smc3	418	SLDQAINDKKR(10)-QIAAIHKDLEDTEANKEK(7)
Smc3	427	Smc3	436	DLEDTEANKEK(9)-NLEQYNKLDQDLNEVK(7)
Smc3	427	Smc3	743	DLEDTEANKEK(9)-MLKEK(3)
Smc3	427	Smc3	756	DLEDTEANKEK(9)-TFMPKQR(5)
Smc3	445	Smc3	727	LDQDLNEVKAR(9)-KFKASR(1)
Smc3	445	Smc3	729	LDQDLNEVKAR(9)-FKASR(2)
Smc3	445	Smc3	740	LDQDLNEVKAR(9)-DSILSEMMLK(8)
Smc3	445	Smc3	743	LDQDLNEVKAR(9)-MLKEK(3)
Smc3	454	Smc3	461	KYYEVK(1)-NKKDELQSER(2)
Smc3	454	Smc3	462	KYYEVK(1)-NKKDELQSER(3)
Smc3	459	Smc3	462	YYEVKNK(5)-KDELQSER(1)
Smc3	486	Smc3	684	EENAEQQALAAKR(12)-KAEELGELEAK(1)
Smc3	486	Smc3	493	EENAEQQALAAKR(12)-KQQLLR(1)
Smc3	492	Smc3	503	REDLEKK(6)-AATGKAILNGIDSINK(5)
Smc3	492	Smc3	673	REDLEKK(6)-KSRLELQK(1)

Appendix

Smc3	492	Smc3	684	REDLEKK(6)-KAEELGELEAK(1)
Smc3	493	Smc3	503	KQQLLR(1)-AATGKAILNGIDSINK(5)
Smc3	493	Smc3	673	KQQLLR(1)-KSRLELQK(1)
Smc3	493	Smc3	680	KQQLLR(1)-LELQKDVR(5)
Smc3	493	Smc3	684	KQQLLR(1)-KAEELGELEAK(1)
Smc3	612	Smc3	621	DTAYPETNDAIPMISKLR(16)-FDKAFK(3)
Smc3	612	Smc3	935	DTAYPETNDAIPMISKLR(16)-KKEECMK(1)
Smc3	680	Smc3	684	LELQKDVR(5)-KAEELGELEAK(1)
Smc3	680	Smc3	820	LELQKDVR(5)-IKLEGIITR(2)
Smc3	727	Smc3	740	KFKASR(1)-DSILSEMMLK(8)
Smc3	740	Smc3	751	DSILSEMMLK(8)-QQSEKTFMPK(5)
Smc3	743	Smc3	751	MLKEK(3)-QQSEKTFMPK(5)
Smc3	743	Smc3	756	MLKEK(3)-TFMPKQR(5)
Smc3	871	Smc3	874	ETEGGTVLTATTSELEAINKR(20)-VKDTMAR(2)
Smc3	889	Smc3	899	SEDLNSIDKTEAGIK(10)-ELQKSMER(4)
Smc3	895	Smc3	905	SEDLNSIDKTEAGIKELQK(16)-WKNMEK(2)
Smc3	899	Smc3	905	ELQKSMER(4)-WKNMEK(2)
Smc3	899	Smc3	956	ELQKSMERWK(4)-ELGSLPQEAPEKYQTLNLSK(12)
Smc3	934	Smc3	936	QGMMLK(6)-KKEECMK(1)
Smc3	935	Smc3	968	KKEECMK(1)-KLEQCNTLKK(1)
Smc3	956	Smc3	968	ELGSLPQEAPEKYQTLNLSK(12)-KLEQCNTLKK(1)
Smc3	963	Smc3	968	YQTLNLSKQLFR(7)-KLEQCNTLKK(1)
Smc3	968	Smc3	978	KLEQCNTLKK(1)-KYSHVNKK(1)
Smc3	968	Smc3	984	KLEQCNTLKK(1)-YSHVNKK(6)
Smc3	977	Smc3	984	LEQCNTLKK(9)-YSHVNKK(6)
Smc3	977	Smc3	985	KLEQCNTLKK(10)-KALDQFVNFSEQK(1)
Smc3	977	Smc3	997	KLEQCNTLKK(10)-ALDQFVNFSEQKEK(12)
Smc3	978	Smc3	985	KYSHVNKK(1)-KALDQFVNFSEQK(1)
Smc3	984	Smc3	999	YSHVNKK(6)-EKLIK(2)
Smc3	1034	Smc3	1059	YEAQLTFKQVSK(9)-KGDVEGSQSQDEGEGSGESER(1)
Smc3	1038	Smc3	1052	QVSKNFSEVFQK(4)-LVPGGKATLVMK(6)
Smc3	1052	Smc3	1059	LVPGGKATLVMK(6)-KGDVEGSQSQDEGEGSGESER(1)
Smc3	1052	Smc3	1105	LVPGGKATLVMK(6)-VSFTGKQGMER(6)
SA1	52	SA1	905	KSPGEK(1)-YYNDYGDIIKETLSK(10)
SA1	52	SA1	273	KSPGEK(1)-LELLQKR(7)
SA1	57	SA1	273	SPGEKSR(5)-LELLQKR(7)
SA1	172	SA1	349	KMTTEFDEDSGDYPLTMPGPQWKK(23)-LKCLK(2)
SA1	257	SA1	273	NKMIGK(2)-LELLQKR(7)
SA1	261	SA1	273	LELLQKR(7)-MIGKR(4)
SA1	273	SA1	558	LELLQKR(7)-KTQIDDR(1)
SA1	273	SA1	1177	LELLQKR(7)-TGMNMYMVR(7)
SA1	453	SA1	555	VLTAKEK(5)-HDPQAEALAKR(11)
SA1	555	SA1	558	VLTAKEK(5)-KTQIDDR(1)
SA1	905	SA1	916	YYNDYGDIIKETLSK(10)-QIDKIQCAK(4)
SA1	905	SA1	1071	YYNDYGDIIKETLSK(10)-MSVNSGSSSSKTSSVR(11)
SA1	910	SA1	916	ETLSKTR(5)-QIDKIQCAK(4)
SA1	910	SA1	969	ETLSKTR(5)-FALTFGLDQIKTR(11)
SA1	916	SA1	969	QIDKIQCAK(4)-FALTFGLDQIKTR(11)
SA1	916	SA1	1071	QIDKIQCAK(4)-MSVNSGSSSSKTSSVR(11)
SA1	916	SA1	1079	QIDKIQCAK(4)-KGRPPLHK(1)
SA1	953	SA1	1071	TAHVSGIKELAR(9)-MSVNSGSSSSKTSSVR(11)
SA1	979	SA1	1071	EAVATLHKDGEFAFK(8)-MSVNSGSSSSKTSSVR(11)
SA1	1020	SA1	1071	KTVHSYLEK(1)-MSVNSGSSSSKTSSVR(11)
SA1	1020	SA1	1079	KTVHSYLEK(1)-KGRPPLHK(1)
SA1	1020	SA1	1086	KTVHSYLEK(1)-GRPPLHKK(7)
SA1	1071	SA1	1079	MSVNSGSSSSKTSSVR(11)-KGRPPLHK(1)
SA1	1071	SA1	1086	MSVNSGSSSSKTSSVR(11)-GRPPLHKK(7)
SA1	1071	SA1	1087	MSVNSGSSSSKTSSVR(11)-KRVEDESLDNTWLNLR(1)

Appendix

SA1	1168	SA1	1177	LEDLNRKDR(7)-TGMNYMKVR(7)
Scc1	317	Scc1	323	ETKAK(3)-KLIVDSVK(1)
Scc1	387	Scc1	406	LLKLFTTR(3)-KGGEADNLDEFLK(1)
Scc1	335	Scc1	387	LLKLFTTR(3)-ELDSKTIR(5)
Scc1	591	Scc1	604	KQAAAK(1)-FYSFLVLKK(8)
Nipbl	110	Nipbl	222	EKSMQNR(2)-NIHDNKVSGPLSGNSANHHADNPR(6)
Nipbl	110	Nipbl	802	EKSMQNR(2)-AEALKQRPDGR(5)
Nipbl	110	Nipbl	927	EKSMQNR(2)-SKVDTNK(2)
Nipbl	110	Nipbl	938	EKSMQNR(2)-AHPDNKAEFPSYLLGGR(6)
Nipbl	110	Nipbl	960	EKSMQNR(2)-NFVIPKIK(6)
Nipbl	110	Nipbl	988	EKSMQNR(2)-DKVEKIGLVEDLNK(5)
Nipbl	110	Nipbl	1000	EKSMQNR(2)-GAKPVVVLQK(3)
Nipbl	110	Nipbl	1007	EKSMQNR(2)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	110	Nipbl	1018	EKSMQNR(2)-LIKDREDK(3)
Nipbl	110	Nipbl	1040	EKSMQNR(2)-SNKGSIDQSVLK(3)
Nipbl	110	Nipbl	1622	EKSMQNR(2)-KDAVTSK(1)
Nipbl	110	Nipbl	2337	EKSMQNR(2)-NKADQQLVEIDKK(2)
Nipbl	110	Nipbl	2347	EKSMQNR(2)-NKADQQLVEIDKK(12)
Nipbl	110	Nipbl	2719	EKSMQNR(2)-YKDRPQIAR(2)
Nipbl	222	Nipbl	333	NIHDNKVSGPLSGNSANHHADNPR(6)-LGKDEKEQSEK(3)
Nipbl	289	Nipbl	357	SPQPVCSPAGSEGTPKGSRPPLILQSQSLPCSSPR(16)-DSTKLTLR(4)
Nipbl	333	Nipbl	353	LGKDEKEQSEK(3)-AAMYDISSPSKDSTK(12)
Nipbl	333	Nipbl	357	LGKDEK(3)-DSTKLTLR(4)
Nipbl	333	Nipbl	821	LGKDEKEQSEK(3)-DHDNKQK(5)
Nipbl	336	Nipbl	353	LGKDEKEQSEK(6)-AAMYDISSPSKDSTK(12)
Nipbl	336	Nipbl	357	LGKDEKEQSEK(6)-DSTKLTLR(4)
Nipbl	493	Nipbl	503	FSKEVQDK(3)-DKPLKK(5)
Nipbl	498	Nipbl	503	EVQDKDK(5)-PLKKR(3)
Nipbl	498	Nipbl	504	FSKEVQDKDK(8)-PLKKR(4)
Nipbl	498	Nipbl	506	EVQDKDKPLK(5)-KQDSYPQEAGGATGGNRPASQETGSTGNGSR(1)
Nipbl	498	Nipbl	662	EVQDKDKPLK(5)-TTECKQNESTIVEPK(5)
Nipbl	500	Nipbl	603	EVQDKDKPLK(7)-KSDPELSKSEMK(1)
Nipbl	503	Nipbl	506	DKPLKK(5)-KQDSYPQEAGGATGGNRPASQETGSTGNGSR(1)
Nipbl	565	Nipbl	571	VDSQASITQSDSIKK(15)-KP EEIKQCNDAPVSVLQEDIVGSLK(6)
Nipbl	565	Nipbl	601	VDSQASITQSDSIKKP EEIK(15)-STPENHPETPKK(11)
Nipbl	566	Nipbl	602	VDSQASITQSDSIKKP EEIK(16)-KKSDPELSK(1)
Nipbl	566	Nipbl	717	KP EEIKQCNDAPVSVLQEDIVGSLK(1)-QKSDGHPETPK(2)
Nipbl	571	Nipbl	601	KP EEIKQCNDAPVSVLQEDIVGSLK(6)-STPENHPETPKK(11)
Nipbl	590	Nipbl	602	QCNDAPVSVLQEDIVGSLKSTPENHPETPK(19)-KKSDPELSK(1)
Nipbl	590	Nipbl	624	QCNDAPVSVLQEDIVGSLKSTPENHPETPK(19)-LAESKPENR(5)
Nipbl	601	Nipbl	603	STPENHPETPKK(11)-KSDPELSK(1)
Nipbl	601	Nipbl	610	STPENHPETPKK(11)-SDPELSKSEMK(7):
Nipbl	601	Nipbl	614	STPENHPETPKK(11)-SEMKQSESR(4)
Nipbl	601	Nipbl	624	STPENHPETPKK(11)-LAESKPENR(5)
Nipbl	601	Nipbl	634	STPENHPETPKK(11)-LVETKSENKLETK(5)
Nipbl	601	Nipbl	639	STPENHPETPKK(11)-SSENKLETK(5)
Nipbl	601	Nipbl	652	STPENHPETPKK(11)-VETQTEELKQNESR(9)
Nipbl	601	Nipbl	662	STPENHPETPKK(11)-TTECKQNESTIVEPK(5)
Nipbl	601	Nipbl	697	STPENHPETPKK(11)-SETTKSRPETPK(5)
Nipbl	601	Nipbl	759	STPENHPETPKK(11)-NEGRPETPKHR(9)
Nipbl	602	Nipbl	610	STPENHPETPKK(12)-SDPELSKSEMK(7)
Nipbl	602	Nipbl	614	KKSDPELSK(1)-SEMKQSESR(4)
Nipbl	602	Nipbl	624	KKSDPELSK(1)-LAESKPENR(5)
Nipbl	602	Nipbl	634	KKSDPELSK(1)-LVETKSENKLETK(5)
Nipbl	602	Nipbl	652	KKSDPELSK(1)-VETQTEELKQNESR(9)
Nipbl	602	Nipbl	662	KKSDPELSK(1)-TTECKQNESTIVEPK(5)
Nipbl	603	Nipbl	614	KSDPELSK(1)-SEMKQSESR(4)
Nipbl	603	Nipbl	624	KSDPELSK(1)-LAESKPENR(5)

Appendix

Nipbl	603	Nipbl	634	KSDPELSK(1)-LVETKSSSENKLETK(5)
Nipbl	603	Nipbl	652	KSDPELSK(1)-VETQTEELKQNESR(9)
Nipbl	603	Nipbl	662	KSDPELSK(1)-TTECKQNESTIVEPK(5)
Nipbl	603	Nipbl	672	KSDPELSK(1)-QNESTIVEPKQENR(10)
Nipbl	603	Nipbl	687	KSDPELSK(1)-LSDTKPNDNKQNNR(10)
Nipbl	610	Nipbl	624	KSDPELSKSEM(8)-LAESKPENR(5)
Nipbl	610	Nipbl	634	SDPELSKSEM(7)-LVETKSSSENKLETK(5)
Nipbl	610	Nipbl	639	SDPELSKSEM(7)-SSENKLETK(5)
Nipbl	610	Nipbl	652	SDPELSKSEM(7)-VETQTEELKQNESR(9)
Nipbl	610	Nipbl	662	SDPELSKSEM(7)-TTECKQNESTIVEPK(5)
Nipbl	610	Nipbl	687	SDPELSKSEM(7)-LSDTKPNDNKQNNR(10)
Nipbl	610	Nipbl	697	SDPELSKSEM(7)-SETTKSRPETPK(5)
Nipbl	610	Nipbl	704	SDPELSKSEM(7)-SRPETPKQK(7)
Nipbl	610	Nipbl	794	SDPELSKSEM(7)-LKSER(2)
Nipbl	610	Nipbl	802	SDPELSKSEM(7)-AEALKQRPDGR(5)
Nipbl	610	Nipbl	1696	SDPELSKSEM(7)-SQKDEESSEGTHHAKEIETTQIMHR(3)
Nipbl	614	Nipbl	624	SEMKSSES(4)-LAESKPENR(5)
Nipbl	614	Nipbl	634	SEMKSSES(4)-LVETKSSSENKLETK(5)
Nipbl	614	Nipbl	639	SEMKSSES(4)-SSENKLETK(5)
Nipbl	614	Nipbl	652	SEMKSSES(4)-VETQTEELKQNESR(9)
Nipbl	614	Nipbl	737	SEMKSSES(4)-GDGRPETPKQK(9)
Nipbl	624	Nipbl	634	LAESKPENR(5)-LVETKSSSENK(5)
Nipbl	624	Nipbl	639	LAESKPENR(5)-SSENKLETK(5)
Nipbl	624	Nipbl	652	LAESKPENR(5)-VETQTEELKQNESR(9)
Nipbl	624	Nipbl	662	LAESKPENR(5)-TTECKQNESTIVEPK(5)
Nipbl	624	Nipbl	672	LAESKPENR(5)-QNESTIVEPKQENR(10)
Nipbl	624	Nipbl	682	LAESKPENR(5)-LSDTKPNDNKQNNR(5)
Nipbl	624	Nipbl	687	LAESKPENR(5)-LSDTKPNDNKQNNR(10)
Nipbl	624	Nipbl	704	LAESKPENR(5)-SRPETPKQK(7)
Nipbl	624	Nipbl	706 or 739	LAESKPENR(5)-QKGESRPETPK(2)
Nipbl	624	Nipbl	715 or 748	LAESKPENR(5)-GESRPETPKQK(9)
Nipbl	624	Nipbl	770	LAESKPENR(5)-DSGKPSTEK(4)
Nipbl	624	Nipbl	794	LAESKPENR(5)-LKSER(2)
Nipbl	634	Nipbl	643	LVETKSSSENK(5)-LETKVETQTEELK(4)
Nipbl	634	Nipbl	652	LVETKSSSENK(5)-VETQTEELKQNESR(9)
Nipbl	634	Nipbl	662	LVETKSSSENK(5)-TTECKQNESTIVEPK(5)
Nipbl	634	Nipbl	672	LVETKSSSENKLETK(5)-QNESTIVEPKQENR(10)
Nipbl	634	Nipbl	682	LVETKSSSENKLETK(5)-LSDTKPNDNKQNNR(5)
Nipbl	634	Nipbl	687	LVETKSSSENKLETK(5)-LSDTKPNDNKQNNR(10)
Nipbl	634	Nipbl	697	LVETKSSSENKLETK(5)-SETTKSRPETPK(5)
Nipbl	634	Nipbl	704	LVETKSSSENKLETK(5)-SRPETPKQK(7)
Nipbl	634	Nipbl	706 or 739	LVETKSSSENKLETK(5)-QKGESRPETPK(2)
Nipbl	634	Nipbl	726	LVETKSSSENKLETK(5)-SDGHPETPKQK(9)
Nipbl	634	Nipbl	728	LVETKSSSENKLETK(5)-QKGDGRPETPK(2)
Nipbl	634	Nipbl	759	LVETKSSSENKLETK(5)-NEGRPETPKHR(9)
Nipbl	634	Nipbl	783	LVETKSSSENKLETK(5)-HKQDTK(2)
Nipbl	634	Nipbl	857	LVETKSSSENKLETK(5)-NEHGKSDSSK(6)
Nipbl	639	Nipbl	652	SSENKLETK(5)-VETQTEELKQNESR(9)
Nipbl	639	Nipbl	662	SSENKLETK(5)-TTECKQNESTIVEPK(5)
Nipbl	639	Nipbl	672	SSENKLETK(5)-QNESTIVEPKQENR(10)
Nipbl	639	Nipbl	682	SSENKLETK(5)-LSDTKPNDNK(5)
Nipbl	639	Nipbl	687	SSENKLETK(5)-LSDTKPNDNKQNNR(10)
Nipbl	639	Nipbl	697	SSENKLETK(5)-SETTKSRPETPK(5)
Nipbl	639	Nipbl	706 or 739	SSENKLETK(5)-QKGESRPETPK(2)
Nipbl	639	Nipbl	717	SSENKLETK(5)-QKSDGHPETPK(2)
Nipbl	639	Nipbl	802	SSENKLETK(5)-AEALKQRPDGR(5)
Nipbl	643	Nipbl	672	LETKVETQTEELKQNESR(4)-QNESTIVEPKQENRLSDTK(10)
Nipbl	652	Nipbl	662	VETQTEELKQNESR(9)-TTECKQNESTIVEPK(5)

Appendix

Nipbl	652	Nipbl	672	VETQTEELKQNESR(9)-QNESTIVEPKQENR(10)
Nipbl	652	Nipbl	682	VETQTEELKQNESR(9)-LSDTKPNDNK(5)
Nipbl	652	Nipbl	687	VETQTEELKQNESR(9)-LSDTKPNDNKQNNGR(10)
Nipbl	652	Nipbl	697	VETQTEELKQNESR(9)-SETTKSRPETPK(5)
Nipbl	652	Nipbl	704	VETQTEELKQNESR(9)-SRPETPKQK(7)
Nipbl	652	Nipbl	717	VETQTEELKQNESR(9)-QKSDGHPETPK(2)
Nipbl	652	Nipbl	726	VETQTEELKQNESR(9)-SDGHPETPKQK(9)
Nipbl	652	Nipbl	728	VETQTEELKQNESR(9)-QKGDGRPETPK(2)
Nipbl	652	Nipbl	737	VETQTEELKQNESR(9)-GDGRPETPKQK(9)
Nipbl	652	Nipbl	759	VETQTEELKQNESR(9)-NEGRPETPKHR(9)
Nipbl	662	Nipbl	682	TTECKQNESTIVEPK(5)-LSDTKPNDNK(5)
Nipbl	662	Nipbl	687	TTECKQNESTIVEPK(5)-LSDTKPNDNKQNNGR(10)
Nipbl	662	Nipbl	697	TTECKQNESTIVEPK(5)-SETTKSRPETPK(5)
Nipbl	662	Nipbl	704	TTECKQNESTIVEPK(5)-SRPETPKQK(7)
Nipbl	662	Nipbl	706 or 739	TTECKQNESTIVEPK(5)-QKGESRPETPK(2)
Nipbl	662	Nipbl	715 or 748	TTECKQNESTIVEPK(5)-GESRPETPKQK(9)
Nipbl	662	Nipbl	717	TTECKQNESTIVEPK(5)-QKSDGHPETPK(2)
Nipbl	662	Nipbl	726	TTECKQNESTIVEPK(5)-SDGHPETPKQK(9)
Nipbl	662	Nipbl	759	TTECKQNESTIVEPK(5)-NEGRPETPKHR(9)
Nipbl	662	Nipbl	770	TTECKQNESTIVEPK(5)-DSGKPSTEK(4)
Nipbl	662	Nipbl	783	TTECKQNESTIVEPK(5)-HKQDTK(2)
Nipbl	662	Nipbl	802	TTECKQNESTIVEPK(5)-AEALKQRPDGR(5)
Nipbl	662	Nipbl	927	TTECKQNESTIVEPK(5)-SKVDTNK(2)
Nipbl	672	Nipbl	682	QNESTIVEPKQENR(10)-LSDTKPNDNK(5)
Nipbl	672	Nipbl	687	QNESTIVEPKQENR(10)-LSDTKPNDNKQNNGR(10)
Nipbl	672	Nipbl	697	QNESTIVEPKQENR(10)-SETTKSRPETPK(5)
Nipbl	672	Nipbl	704	QNESTIVEPKQENR(10)-SRPETPKQK(7)
Nipbl	672	Nipbl	706 or 739	QNESTIVEPKQENR(10)-QKGESRPETPK(2)
Nipbl	672	Nipbl	715 or 748	QNESTIVEPKQENR(10)-GESRPETPKQK(9)
Nipbl	672	Nipbl	783	QNESTIVEPKQENR(10)-HKQDTK(2)
Nipbl	682	Nipbl	697	LSDTKPNDNK(5)-SETTKSRPETPK(5)
Nipbl	682	Nipbl	704	LSDTKPNDNK(5)-SRPETPKQK(7)
Nipbl	682	Nipbl	706 or 739	LSDTKPNDNKQNNGR(5)-QKGESRPETPK(2)
Nipbl	682	Nipbl	717	LSDTKPNDNKQNNGR(5)-QKSDGHPETPK(2)
Nipbl	682	Nipbl	728	LSDTKPNDNKQNNGR(5)-QKGDGRPETPK(2)
Nipbl	682	Nipbl	802	LSDTKPNDNKQNNGR(5)-AEALKQRPDGR(5)
Nipbl	682	Nipbl	896	LSDTKPNDNKQNNGR(5)-VKQGDSNK(2)
Nipbl	687	Nipbl	697	LSDTKPNDNKQNNGR(10)-SETTKSRPETPK(5)
Nipbl	687	Nipbl	704	LSDTKPNDNKQNNGR(10)-SRPETPKQK(7)
Nipbl	687	Nipbl	706 or 739	LSDTKPNDNKQNNGR(10)-QKGESRPETPK(2)
Nipbl	687	Nipbl	717	LSDTKPNDNKQNNGR(10)-QKSDGHPETPK(2)
Nipbl	687	Nipbl	802	LSDTKPNDNKQNNGR(10)-AEALKQRPDGR(5)
Nipbl	687	Nipbl	821	LSDTKPNDNKQNNGR(10)-DHDNKQK(5)
Nipbl	697	Nipbl	704 or 715	SETTKSR(5)-PETPKQK(5)
Nipbl	697	Nipbl	706 or 739	SETTKSRPETPK(5)-QKGESRPETPK(2)
Nipbl	697	Nipbl	614	SETTKSRPETPK(5)-SEMKSSES(4)
Nipbl	697	Nipbl	715 or 748	SETTKSRPETPK(5)-GESRPETPKQK(9)
Nipbl	697	Nipbl	624	SETTKSRPETPK(5)-LAESKPNENR(5)
Nipbl	697	Nipbl	717	SETTKSRPETPK(5)-QKSDGHPETPK(2)
Nipbl	697	Nipbl	726	SETTKSRPETPK(5)-SDGHPETPKQK(9)
Nipbl	697	Nipbl	728	SETTKSRPETPK(5)-QKGDGRPETPK(2)
Nipbl	697	Nipbl	737	SETTKSRPETPK(5)-GDGRPETPKQK(9)
Nipbl	697	Nipbl	750	SETTKSRPETPK(5)-QKNEGRPETPK(2)
Nipbl	697	Nipbl	759	SETTKSRPETPK(5)-NEGRPETPKHR(9)
Nipbl	697	Nipbl	783	SETTKSRPETPK(5)-HKQDTK(2)
Nipbl	697	Nipbl	787	SETTKSRPETPK(5)-HKQDTKSDSPR(6)
Nipbl	697	Nipbl	802	SETTKSRPETPK(5)-AEALKQRPDGR(5)
Nipbl	697	Nipbl	862	SETTKSRPETPK(5)-SDSSKTDKLER(5)

Appendix

Nipbl	697	Nipbl	954	SETTKSRPETPK(5)-SGALKNFVIPK(5)
Nipbl	704	Nipbl	715 or 748	SRPETPKQK(7)-GESRPETPKQK(9)
Nipbl	704	Nipbl	717	SRPETPKQK(7)-QKSDGHPETPK(2)
Nipbl	704	Nipbl	726	SRPETPKQK(7)-SDGHPETPKQK(9)
Nipbl	704	Nipbl	728	SRPETPKQK(7)-QKGDGRPETPK(2)
Nipbl	704	Nipbl	737	SRPETPKQK(7)-GDGRPETPKQK(9)
Nipbl	704	Nipbl	759	SRPETPKQK(7)-NEGRPETPKHR(9)
Nipbl	706 or 739	Nipbl	715 or 748	QKGESRPETPK(2)-GESRPETPKQK(9)
Nipbl	706 or 739	Nipbl	717	QKGESRPETPK(2)-QKSDGHPETPK(2)
Nipbl	706 or 739	Nipbl	726	QKGESRPETPK(2)-SDGHPETPKQK(9)
Nipbl	706 or 739	Nipbl	737	QKGESRPETPK(2)-GDGRPETPKQK(9)
Nipbl	706 or 739	Nipbl	750	QKGESRPETPK(2)-QKNEGRPETPK(2)
Nipbl	706 or 739	Nipbl	770	QKGESRPETPK(2)-DSGKPSTEK(4)
Nipbl	706 or 739	Nipbl	802	QKGESRPETPK(2)-AEALKQRPDGR(5)
Nipbl	706 or 739	Nipbl	857	QKGESRPETPK(2)-NEHGIKSDSSK(6)
Nipbl	706 or 739	Nipbl	862	QKGESRPETPK(2)-SDSSKTDKLER(5)
Nipbl	706 or 739	Nipbl	927	QKGESRPETPK(2)-SKVDTNK(2)
Nipbl	715 or 748	Nipbl	726	GESRPETPKQK(9)-SDGHPETPKQK(9)
Nipbl	715 or 748	Nipbl	728	GESRPETPKQK(9)-QKGDGRPETPK(2)
Nipbl	715 or 748	Nipbl	737	GESRPETPKQK(9)-GDGRPETPKQK(9)
Nipbl	715 or 748	Nipbl	750	GESRPETPKQK(9)-QKNEGRPETPK(2)
Nipbl	715 or 748	Nipbl	759	GESRPETPKQK(9)-NEGRPETPKHR(9)
Nipbl	715 or 748	Nipbl	770	GESRPETPKQK(9)-DSGKPSTEK(4)
Nipbl	715 or 748	Nipbl	783	GESRPETPKQK(9)-HKQDTK(2)
Nipbl	715 or 748	Nipbl	794	GESRPETPKQK(9)-LKSER(2)
Nipbl	715 or 748	Nipbl	802	GESRPETPKQK(9)-AEALKQRPDGR(5)
Nipbl	715 or 748	Nipbl	821	GESRPETPKQK(9)-DHDNKQK(5)
Nipbl	715 or 748	Nipbl	1639	GESRPETPKQK(9)-ILKQVSGGEDEIQQLQK(3)
Nipbl	717	Nipbl	728	QKSDGHPETPK(2)-QKGDGRPETPK(2)
Nipbl	717	Nipbl	750	QKSDGHPETPK(2)-QKNEGRPETPK(2)
Nipbl	717	Nipbl	759	QKSDGHPETPK(2)-NEGRPETPKHR(9)
Nipbl	717	Nipbl	802	QKSDGHPETPK(2)-AEALKQRPDGR(5)
Nipbl	726	Nipbl	728	QKGDGRPETPK(2)-SDGHPETPKQK(9)
Nipbl	726	Nipbl	737	SDGHPETPKQK(9)-GDGRPETPKQK(9)
Nipbl	726	Nipbl	759	SDGHPETPKQK(9)-NEGRPETPKHR(9)
Nipbl	726	Nipbl	776	SDGHPETPKQK(9)-KPEVSK(1)
Nipbl	726	Nipbl	787	SDGHPETPKQK(9)-HKQDTKSDSPR(6)
Nipbl	726	Nipbl	802	SDGHPETPKQK(9)-AEALKQRPDGR(5)
Nipbl	728	Nipbl	776	QKGDGRPETPK(2)-KPEVSK(1)
Nipbl	728	Nipbl	979	QKGDGRPETPK(2)-MEMKGEPK(4)
Nipbl	737	Nipbl	750	GDGRPETPKQK(9)-QKNEGRPETPK(2)
Nipbl	737	Nipbl	770	GDGRPETPKQK(9)-DSGKPSTEK(4)
Nipbl	737	Nipbl	783	GDGRPETPKQK(9)-HKQDTK(2)
Nipbl	737	Nipbl	794	GDGRPETPKQK(9)-LKSER(2)
Nipbl	737	Nipbl	802	GDGRPETPKQK(9)-AEALKQRPDGR(5)
Nipbl	737	Nipbl	896	GDGRPETPKQK(9)-VKQGDSNK(2)
Nipbl	737	Nipbl	1040	GDGRPETPKQK(9)-SNKGSIDQSVLK(3)
Nipbl	750	Nipbl	770	QKNEGRPETPK(2)-DSGKPSTEK(4)
Nipbl	750	Nipbl	783	QKNEGRPETPK(2)-HKQDTK(2)
Nipbl	750	Nipbl	794	QKNEGRPETPK(2)-LKSER(2)
Nipbl	750	Nipbl	802	QKNEGRPETPK(2)-AEALKQRPDGR(5)
Nipbl	750	Nipbl	821	QKNEGRPETPK(2)-DHDNKQK(5)
Nipbl	759	Nipbl	770	NEGRPETPKHR(9)-DSGKPSTEK(4)
Nipbl	759	Nipbl	776	NEGRPETPKHR(9)-KPEVSK(1)
Nipbl	759	Nipbl	781	NEGRPETPKHR(9)-KPEVSKHK(6)
Nipbl	759	Nipbl	783	NEGRPETPKHR(9)-HKQDTK(2)
Nipbl	759	Nipbl	787	NEGRPETPKHR(9)-HKQDTKSDSPR(6)
Nipbl	759	Nipbl	794	NEGRPETPKHR(9)-LKSER(2)

Appendix

Nipbl	759	Nipbl	802	NEGRPETPKHR(9)-AEALKQRPDGR(5)
Nipbl	759	Nipbl	862	NEGRPETPKHR(9)-SDSSKTDKLER(5)
Nipbl	759	Nipbl	865	NEGRPETPKHR(9)-TDKLER(3)
Nipbl	759	Nipbl	927	NEGRPETPKHR(9)-SKVDTNK(2)
Nipbl	759	Nipbl	1639	NEGRPETPKHR(9)-ILKQVSGGEDEIQQLQK(3)
Nipbl	770	Nipbl	776	DSGKPSTEK(4)-KPEVSK(1)
Nipbl	770	Nipbl	781	DSGKPSTEK(4)-KPEVSKHK(6)
Nipbl	770	Nipbl	783	DSGKPSTEK(4)-HKQDTK(2)
Nipbl	770	Nipbl	787	DSGKPSTEK(4)-HKQDTKS DSPR(6)
Nipbl	770	Nipbl	794	DSGKPSTEK(4)-LKSER(2)
Nipbl	770	Nipbl	821	DSGKPSTEK(4)-DHDNKQK(5)
Nipbl	770	Nipbl	896	DSGKPSTEK(4)-VKQGDSNK(2)
Nipbl	775	Nipbl	781	DSGKPSTEKK(9)-PEVSKHK(5)
Nipbl	775	Nipbl	783	DSGKPSTEKK(9)-PEVSKHKQDTK(7)
Nipbl	776	Nipbl	783	KPEVSK(1)-HKQDTK(2)
Nipbl	776	Nipbl	787	KPEVSK(1)-HKQDTKS DSPR(6)
Nipbl	776	Nipbl	794	KPEVSK(1)-LKSER(2)
Nipbl	776	Nipbl	802	KPEVSK(1)-AEALKQRPDGR(5)
Nipbl	776	Nipbl	896	KPEVSK(1)-VKQGDSNK(2)
Nipbl	776	Nipbl	2022	KPEVSK(1)-PQLMVKHAMTMQPYLTTK(6)
Nipbl	781	Nipbl	787	KPEVSKHK(6)-QDTKS DSPR(4)
Nipbl	781	Nipbl	794	KPEVSKHK(6)-LKSER(2)
Nipbl	781	Nipbl	802	KPEVSKHK(6)-AEALKQRPDGR(5)
Nipbl	781	Nipbl	821	KPEVSKHK(6)-DHDNKQK(5)
Nipbl	783	Nipbl	794	HKQDTK(2)-LKSER(2)
Nipbl	783	Nipbl	802	HKQDTK(2)-AEALKQRPDGR(5)
Nipbl	783	Nipbl	821	HKQDTK(2)-DHDNKQK(5)
Nipbl	783	Nipbl	823	PEVSKHKQDTK(7)-QKSDDRGESER(2)
Nipbl	783	Nipbl	857	HKQDTK(2)-NEHGIKSDSSK(6)
Nipbl	783	Nipbl	865	HKQDTK(2)-TDKLER(3)
Nipbl	783	Nipbl	965	HKQDTK(2)-DKDGNVTQETK(2)
Nipbl	783	Nipbl	988	HKQDTK(2)-DKVEKIGLVEDLNK(5)
Nipbl	783	Nipbl	1000	HKQDTK(2)-GAKPVVVLQK(3)
Nipbl	783	Nipbl	2574	HKQDTK(2)-YSPSESAKVYDK(8)
Nipbl	787	Nipbl	794	QDTKS DSPR(4)-LKSER(2)
Nipbl	787	Nipbl	802	HKQDTKS DSPR(6)-AEALKQRPDGR(5)
Nipbl	787	Nipbl	821	HKQDTKS DSPR(6)-DHDNKQK(5)
Nipbl	787	Nipbl	857	HKQDTKS DSPR(6)-NEHGIKSDSSK(6)
Nipbl	787	Nipbl	862	HKQDTKS DSPR(6)-SDSSKTDKLER(5)
Nipbl	787	Nipbl	865	HKQDTKS DSPR(6)-SDSSKTDKLER(8)
Nipbl	787	Nipbl	896	HKQDTKS DSPR(6)-VKQGDSNK(2)
Nipbl	787	Nipbl	1018	HKQDTKS DSPR(6)-LIKDREDK(3)
Nipbl	787	Nipbl	1082	HKQDTKS DSPR(6)-STVNEKPK(6)
Nipbl	794	Nipbl	802	LKSER(2)-AEALKQR(5)
Nipbl	794	Nipbl	821	LKSER(2)-DHDNKQK(5)
Nipbl	794	Nipbl	857	LKSER(2)-NEHGIKSDSSK(6)
Nipbl	794	Nipbl	862	LKSER(2)-SDSSKTDKLER(5)
Nipbl	794	Nipbl	865	LKSER(2)-SDSSKTDKLER(8)
Nipbl	794	Nipbl	896	LKSER(2)-VKQGDSNK(2)
Nipbl	794	Nipbl	902	LKSER(2)-QGDSNKS R(6)
Nipbl	794	Nipbl	907	LKSER(2)-SDKLGFK(3)
Nipbl	794	Nipbl	821	LKSER(2)-RDHDNKQK(6)
Nipbl	794	Nipbl	938	LKSER(2)-AHPDNKAFFPSYLLGGR(6)
Nipbl	794	Nipbl	960	LKSER(2)-NFVIPKIK(6)
Nipbl	794	Nipbl	965	LKSER(2)-DKDGNVTQETK(2)
Nipbl	794	Nipbl	1000	LKSER(2)-GAKPVVVLQK(3)
Nipbl	794	Nipbl	1007	LKSER(2)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	794	Nipbl	1015	LKSER(2)-LSLDDVQKLIK(8)

Appendix

Nipbl	794	Nipbl	1029	LKSER(2)-SSLKPIK(4)
Nipbl	794	Nipbl	1082	LKSER(2)-STVNEKPK(6)
Nipbl	794	Nipbl	1264	LKSER(2)-TVKVLNILEK(3)
Nipbl	794	Nipbl	1696	LKSER(2)-SQKDEESSEGTHHAK(3)
Nipbl	794	Nipbl	2223	LKSER(2)-NLYNNILSDKNSSVNLK(10)
Nipbl	794	Nipbl	2783	LKSER(2)-IALTSANKLTNK(8)
Nipbl	794	Nipbl	2787	LKSER(2)-LTNKVVQTLR(4)
Nipbl	802	Nipbl	770	AEALKQRPDGR(5)-DSGKPSTEK(4)
Nipbl	802	Nipbl	821	AEALKQRPDGR(5)-DHDNKQK(5)
Nipbl	802	Nipbl	823	AEALKQRPDGR(5)-QKSDDRGESER(2)
Nipbl	802	Nipbl	857	AEALKQRPDGR(5)-NEHGIKSDSSK(6)
Nipbl	802	Nipbl	862	AEALKQRPDGR(5)-SDSSKTDKLER(5)
Nipbl	802	Nipbl	865	AEALKQRPDGR(5)-TDKLER(3)
Nipbl	802	Nipbl	896	AEALKQRPDGR(5)-VKQGDSNK(2)
Nipbl	802	Nipbl	907	AEALKQRPDGR(5)-SDKLGFK(3)
Nipbl	802	Nipbl	965	AEALKQRPDGR(5)-DKDGNVTQETK(2)
Nipbl	802	Nipbl	991	AEALKQRPDGR(5)-LSTDKTVK(5)
Nipbl	802	Nipbl	1018	AEALKQRPDGR(5)-LIKDREDK(3)
Nipbl	802	Nipbl	1369	AEALKQRPDGR(5)-LDPHGGGLSSKAK(12)
Nipbl	802	Nipbl	1547	AEALKQRPDGR(5)-TAQNFLSIFLKK(11)
Nipbl	802	Nipbl	1622	AEALKQRPDGR(5)-LRKDAVTSK(3)
Nipbl	802	Nipbl	2783	AEALKQRPDGR(5)-IALTSANKLTNK(8)
Nipbl	821	Nipbl	857	DHDNKQK(5)-NEHGIKSDSSK(6)
Nipbl	821	Nipbl	865	DHDNKQK(5)-TDKLER(3)
Nipbl	821	Nipbl	896	DHDNKQK(5)-VKQGDSNK(2)
Nipbl	821	Nipbl	907	DHDNKQK(5)-SDKLGFK(3)
Nipbl	821	Nipbl	911	DHDNKQK(5)-LGFKSPTSK(4)
Nipbl	821	Nipbl	938	DHDNKQK(5)-AHPDNKAEFPSYLLGGR(6)
Nipbl	821	Nipbl	954	DHDNKQK(5)-SGALKNFVIPK(5)
Nipbl	821	Nipbl	960	DHDNKQK(5)-NFVIPKIK(6)
Nipbl	821	Nipbl	985	DHDNKQK(5)-DKVEKIGLVEDLNK(2)
Nipbl	821	Nipbl	988	DHDNKQK(5)-DKVEKIGLVEDLNK(5)
Nipbl	821	Nipbl	1000	DHDNKQK(5)-GAKPVVVLQK(3)
Nipbl	821	Nipbl	1029	DHDNKQK(5)-SSLKPIK(4)
Nipbl	821	Nipbl	1247	DHDNKQK(5)-HQLNELGSESAKIK(12)
Nipbl	821	Nipbl	1628	DHDNKQK(5)-DAVTSKMDQGSIER(6)
Nipbl	821	Nipbl	1870	DHDNKQK(5)-VIKILR(3)
Nipbl	823	Nipbl	862	QKSDDRGESER(2)-SDSSKTDKLER(5)
Nipbl	857	Nipbl	865	NEHGIKSDSSK(6)-TDKLER(3)
Nipbl	857	Nipbl	896	NEHGIKSDSSK(6)-VKQGDSNK(2)
Nipbl	857	Nipbl	907	NEHGIKSDSSK(6)-SDKLGFK(3)
Nipbl	857	Nipbl	938	NEHGIKSDSSK(6)-AHPDNKAEFPSYLLGGR(6)
Nipbl	857	Nipbl	960	NEHGIKSDSSK(6)-NFVIPKIK(6)
Nipbl	857	Nipbl	965	NEHGIKSDSSK(6)-DKDGNVTQETK(2)
Nipbl	857	Nipbl	974	NEHGIKSDSSK(6)-DKDGNVTQETKK(11)
Nipbl	857	Nipbl	1029	NEHGIKSDSSK(6)-SSLKPIK(4)
Nipbl	857	Nipbl	1040	NEHGIKSDSSK(6)-SNKGSIDQSVLK(3)
Nipbl	857	Nipbl	1628	NEHGIKSDSSK(6)-DAVTSKMDQGSIER(6)
Nipbl	857	Nipbl	1639	NEHGIKSDSSK(6)-ILKQVSGGEDEIQQLQK(3)
Nipbl	857	Nipbl	1725	NEHGIKSDSSK(6)-KKFLR(2)
Nipbl	862	Nipbl	869	SDSSKTDK(5)-LERKHR(4)
Nipbl	862	Nipbl	896	SDSSKTDKLER(5)-VKQGDSNK(2)
Nipbl	862	Nipbl	902	SDSSKTDKLER(5)-QGDSNKS(6)
Nipbl	862	Nipbl	907	SDSSKTDKLER(5)-SDKLGFK(3)
Nipbl	862	Nipbl	911	SDSSKTDKLER(5)-SDKLGFKSPTSK(7)
Nipbl	862	Nipbl	916	SDSSKTDKLER(5)-SPTSKDDKR(5)
Nipbl	862	Nipbl	927	SDSSKTDKLER(5)-SKVDTNK(2)
Nipbl	862	Nipbl	932	SDSSKTDKLER(5)-SKVDTNKAHPDNK(7)

Appendix

Nipbl	862	Nipbl	938	SDSSKTDKLER(5)-AHPDNKAEFPSYLLGGR(6)
Nipbl	862	Nipbl	954	SDSSKTDKLER(5)-SGALKNFVIPK(5)
Nipbl	862	Nipbl	965	SDSSKTDKLER(5)-DKDGNVTQETK(2)
Nipbl	862	Nipbl	974	SDSSKTDKLER(5)-DKDGNVTQETKK(11)
Nipbl	862	Nipbl	985	SDSSKTDKLER(5)-DKVEK(2)
Nipbl	862	Nipbl	988	SDSSKTDKLER(5)-DKVEKIGLVEDLNK(5)
Nipbl	862	Nipbl	1018	SDSSKTDKLER(5)-LIKDREDK(3)
Nipbl	862	Nipbl	1040	SDSSKTDKLER(5)-SNKGSIDQSVLK(3)
Nipbl	862	Nipbl	1968	SDSSKTDKLER(5)-SEEDSSYKPVKK(11)
Nipbl	862	Nipbl	2648	SDSSKTDKLER(5)-NKAITSLGGGSPK(2)
Nipbl	865	Nipbl	869	SDSSKTDKLER(8)-KHRHESGDSR(1)
Nipbl	865	Nipbl	896	TDKLER(3)-VKQGDSNK(2)
Nipbl	865	Nipbl	902	SDSSKTDKLER(8)-QGDSNKS(6)
Nipbl	865	Nipbl	907	TDKLER(3)-DKLGFK(3)
Nipbl	865	Nipbl	927	TDKLER(3)-SKVDTNK(2)
Nipbl	865	Nipbl	911	TDKLER(3)-SDKLGFKSPTSK(7)
Nipbl	865	Nipbl	916	TDKLER(3)-SPTSKDDKR(5)
Nipbl	865	Nipbl	938	TDKLER(3)-AHPDNKAEFPSYLLGGR(6)
Nipbl	865	Nipbl	954	TDKLER(3)-SGALKNFVIPK(5)
Nipbl	865	Nipbl	960	TDKLER(3)-NFVIPKIK(6)
Nipbl	865	Nipbl	974	SDSSKTDKLER(8)-DKDGNVTQETKK(11)
Nipbl	865	Nipbl	1007	TDKLER(3)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	865	Nipbl	1968	SDSSKTDKLER(8)-SEEDSSYKPVKK(11)
Nipbl	869	Nipbl	896	KHRHESGDSR(1)-VKQGDSNK(2)
Nipbl	887	Nipbl	896	ERPSSGEQKSR(9)-PDSRPVKQGDSNK(7)
Nipbl	896	Nipbl	907	VKQGDSNK(2)-SDKLGFK(3)
Nipbl	896	Nipbl	911	VKQGDSNK(2)-LGFKSPTSK(4)
Nipbl	896	Nipbl	916	VKQGDSNK(2)-SPTSKDDKR(5)
Nipbl	896	Nipbl	919	VKQGDSNK(2)-DDKRTEGNK(3)
Nipbl	896	Nipbl	925	VKQGDSNK(2)-TEGNKSK(5)
Nipbl	896	Nipbl	927	VKQGDSNK(2)-SKVDTNK(2)
Nipbl	896	Nipbl	932	VKQGDSNK(2)-VDTNKAHPDNK(5)
Nipbl	896	Nipbl	938	VKQGDSNK(2)-AHPDNKAEFPSYLLGGR(6)
Nipbl	896	Nipbl	960	VKQGDSNK(2)-NFVIPKIK(6)
Nipbl	896	Nipbl	974	VKQGDSNK(2)-DKDGNVTQETKK(11)
Nipbl	896	Nipbl	983	VKQGDSNK(2)-GEPKDKVEK(4)
Nipbl	896	Nipbl	988	VKQGDSNK(2)-DKVEKIGLVEDLNK(5)
Nipbl	896	Nipbl	1023	DREDKSR(5)-VKQGDSNK(2)
Nipbl	896	Nipbl	1639	VKQGDSNK(2)-ILKQVSGEDEIQLQK(3)
Nipbl	896	Nipbl	1968	VKQGDSNK(2)-SEEDSSYKPVKK(11)
Nipbl	896	Nipbl	2719	VKQGDSNK(2)-YKDRPQIAR(2)
Nipbl	902	Nipbl	907	QGDSNKS(6)-SDKLGFK(3)
Nipbl	902	Nipbl	911	QGDSNKS(6)-LGFKSPTSK(4)
Nipbl	902	Nipbl	919	QGDSNKS(6)-SPTSKDDKR(8)
Nipbl	902	Nipbl	925	QGDSNKS(6)-TEGNKSK(5)
Nipbl	902	Nipbl	927	VKQGDSNKS(8)-SKVDTNK(2)
Nipbl	902	Nipbl	954	QGDSNKS(6)-SGALKNFVIPK(5)
Nipbl	902	Nipbl	960	QGDSNKS(6)-NFVIPKIK(6)
Nipbl	907	Nipbl	916	SDKLGFK(3)-SPTSKDDKR(5)
Nipbl	907	Nipbl	919	SDKLGFK(3)-DDKRTEGNK(3)
Nipbl	907	Nipbl	925	SDKLGFK(3)-TEGNKSK(5)
Nipbl	907	Nipbl	927	SDKLGFK(3)-SKVDTNK(2)
Nipbl	907	Nipbl	932	SDKLGFK(3)-SKVDTNKAHPDNK(7)
Nipbl	907	Nipbl	954	SDKLGFK(3)-SGALKNFVIPK(5)
Nipbl	907	Nipbl	960	SDKLGFK(3)-NFVIPKIK(6)
Nipbl	907	Nipbl	1000	SDKLGFK(3)-GAKPVVVLQK(3)
Nipbl	907	Nipbl	1007	SDKLGFK(3)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	907	Nipbl	1018	SDKLGFK(3)-LIKDREDK(3)

Appendix

Nipbl	907	Nipbl	1622	SDKLGFK(3)-KDAVTSK(1)
Nipbl	907	Nipbl	1968	SDKLGFK(3)-SEEDSSYPVKK(11)
Nipbl	907	Nipbl	2719	SDKLGFK(3)-YKDRPQIAR(2)
Nipbl	911	Nipbl	919	SDKLGFKSPTSK(7)-DDKRTEGNK(3)
Nipbl	911	Nipbl	925	SDKLGFKSPTSK(7)-TEGNKSK(5)
Nipbl	911	Nipbl	927	LGFKSPTSK(4)-SKVDTNK(2)
Nipbl	911	Nipbl	932	SDKLGFKSPTSK(7)-VDTNKAHPDNK(5)
Nipbl	911	Nipbl	938	LGFKSPTSK(4)-AHPDNKAEFPSYLLGGR(6)
Nipbl	911	Nipbl	954	SDKLGFKSPTSK(7)-SGALKNFVIPK(5)
Nipbl	911	Nipbl	960	LGFKSPTSK(4)-NFVIPKIK(6)
Nipbl	911	Nipbl	965	LGFKSPTSK(4)-DKDGNVTQETK(2)
Nipbl	911	Nipbl	1018	SDKLGFKSPTSK(7)-LIKDREDK(3)
Nipbl	911	Nipbl	1029	SDKLGFKSPTSK(7)-SSLKPIK(4)
Nipbl	911	Nipbl	1040	SDKLGFKSPTSK(7)-SNKGSIDQSVLK(3)
Nipbl	911	Nipbl	2648	SDKLGFKSPTSK(7)-NKAITSLLGGGSPK(2)
Nipbl	916	Nipbl	925	SPTSKDDKR(5)-TEGNKSKVDTNK(5)
Nipbl	916	Nipbl	927	SPTSKDDKR(5)-SKVDTNK(2)
Nipbl	916	Nipbl	932	SPTSKDDKR(5)-VDTNKAHPDNK(5)
Nipbl	916	Nipbl	938	SPTSKDDKR(5)-AHPDNKAEFPSYLLGGR(6)
Nipbl	916	Nipbl	954	SPTSKDDKR(5)-SGALKNFVIPK(5)
Nipbl	916	Nipbl	965	SPTSKDDKR(5)-DKDGNVTQETK(2)
Nipbl	916	Nipbl	1029	SPTSKDDKR(5)-SSLKPIK(4)
Nipbl	919	Nipbl	925	SPTSKDDKR(8)-TEGNKSK(5)
Nipbl	919	Nipbl	927	DDKRTEGNK(3)-SKVDTNK(2)
Nipbl	919	Nipbl	932	SPTSKDDKR(8)-VDTNKAHPDNK(5)
Nipbl	919	Nipbl	938	DDKRTEGNK(3)-AHPDNKAEFPSYLLGGR(6)
Nipbl	919	Nipbl	960	SPTSKDDKR(8)-NFVIPKIK(6)
Nipbl	919	Nipbl	974	SPTSKDDKR(8)-DKDGNVTQETK(11)
Nipbl	919	Nipbl	988	SPTSKDDKR(8)-DKVEKIGLVEDLNK(5)
Nipbl	925	Nipbl	932	TEGNKSK(5)-VDTNKAHPDNK(5)
Nipbl	925	Nipbl	938	TEGNKSK(5)-AHPDNKAEFPSYLLGGR(6)
Nipbl	925	Nipbl	954	TEGNKSK(5)-SGALKNFVIPK(5)
Nipbl	925	Nipbl	960	TEGNKSK(5)-NFVIPKIK(6)
Nipbl	925	Nipbl	965	TEGNKSK(5)-DKDGNVTQETK(2)
Nipbl	925	Nipbl	974	TEGNKSK(5)-DKDGNVTQETK(11)
Nipbl	925	Nipbl	983	TEGNKSK(5)-GEPKDKVEK(4)
Nipbl	925	Nipbl	988	TEGNKSK(5)-DKVEKIGLVEDLNK(5)
Nipbl	925	Nipbl	997	TEGNKSK(5)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	925	Nipbl	1000	RTEGNKSK(6)-GAKPVVVLQK(3)
Nipbl	925	Nipbl	1007	TEGNKSK(5)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	927	Nipbl	938	SKVDTNK(2)-AHPDNKAEFPSYLLGGR(6)
Nipbl	927	Nipbl	954	SKVDTNK(2)-SGALKNFVIPK(5)
Nipbl	927	Nipbl	960	SKVDTNK(2)-NFVIPKIK(6)
Nipbl	927	Nipbl	965	SKVDTNK(2)-DKDGNVTQETK(2)
Nipbl	927	Nipbl	1968	SKVDTNK(2)-SEEDSSYPVKK(11)
Nipbl	927	Nipbl	974	SKVDTNK(2)-DKDGNVTQETK(11)
Nipbl	927	Nipbl	979	SKVDTNK(2)-KMEMKGEPK(5)
Nipbl	927	Nipbl	988	SKVDTNK(2)-DKVEKIGLVEDLNK(5)
Nipbl	927	Nipbl	997	SKVDTNK(2)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	927	Nipbl	1007	SKVDTNK(2)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	927	Nipbl	1015	SKVDTNK(2)-LSLDDVQKLIK(8)
Nipbl	927	Nipbl	1018	SKVDTNK(2)-LIKDREDK(3)
Nipbl	927	Nipbl	1029	SKVDTNK(2)-SSLKPIK(4)
Nipbl	927	Nipbl	1696	SKVDTNK(2)-SQKDEESSETHHAK(3)
Nipbl	932	Nipbl	954	VDTNKAHPDNK(5)-SGALKNFVIPK(5)
Nipbl	932	Nipbl	960	VDTNKAHPDNK(5)-NFVIPKIK(6)
Nipbl	932	Nipbl	965	VDTNKAHPDNK(5)-DKDGNVTQETK(2)
Nipbl	932	Nipbl	974	VDTNKAHPDNK(5)-DKDGNVTQETK(11)

Appendix

Nipbl	932	Nipbl	983	VDTNKAHPDNKAEFPSYLLGGR(5)-GEPKDKVEK(4)
Nipbl	932	Nipbl	1000	VDTNKAHPDNK(5)-GAKPVVVLQK(3)
Nipbl	932	Nipbl	1007	VDTNKAHPDNK(5)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	932	Nipbl	1995	SKVDTNKAHPDNK(7)-YEESLADSDNKGVNSGR(11)
Nipbl	938	Nipbl	927	AHPDNKAEFPSYLLGGR(6)-SKVDTNK(2)
Nipbl	938	Nipbl	954	AHPDNKAEFPSYLLGGR(6)-SGALKNFVIPK(5)
Nipbl	938	Nipbl	960	AHPDNKAEFPSYLLGGR(6)-SNKGSIDQSVLK(3)
Nipbl	938	Nipbl	965	AHPDNKAEFPSYLLGGR(6)-DKDGNVTQETK(2)
Nipbl	938	Nipbl	974	AHPDNKAEFPSYLLGGR(6)-DKDGNVTQETKK(11)
Nipbl	938	Nipbl	975	AHPDNKAEFPSYLLGGR(6)-KMEMK(1)
Nipbl	938	Nipbl	983	AHPDNKAEFPSYLLGGR(6)-GEPKDKVEK(4)
Nipbl	938	Nipbl	985	AHPDNKAEFPSYLLGGR(6)-DKVEK(2)
Nipbl	938	Nipbl	1023	AHPDNKAEFPSYLLGGR(6)-EDKSR(3)
Nipbl	938	Nipbl	1037	AHPDNKAEFPSYLLGGR(6)-NKPSKSNK(5)
Nipbl	938	Nipbl	1070	AHPDNKAEFPSYLLGGR(6)-VKMNK(2)
Nipbl	938	Nipbl	1628	AHPDNKAEFPSYLLGGR(6)-DAVTSKMDQGSIER(6)
Nipbl	938	Nipbl	1791	AHPDNKAEFPSYLLGGR(6)-TKAMK(2)
Nipbl	938	Nipbl	1968	AHPDNKAEFPSYLLGGR(6)-SEEDSSYKPVKK(11)
Nipbl	954	Nipbl	965	SGALKNFVIPK(5)-DKDGNVTQETK(2)
Nipbl	954	Nipbl	974	SGALKNFVIPK(5)-DKDGNVTQETKK(11)
Nipbl	954	Nipbl	975	SGALKNFVIPK(5)-KMEMK(1)
Nipbl	954	Nipbl	979	SGALKNFVIPK(5)-KMEMKGEPK(5)
Nipbl	954	Nipbl	983	SGALKNFVIPK(5)-GEPKDKVEK(4)
Nipbl	954	Nipbl	985	SGALKNFVIPK(5)-DKVEK(2)
Nipbl	954	Nipbl	988	SGALKNFVIPK(5)-DKVEKIGLVEDLNK(5)
Nipbl	954	Nipbl	1007	SGALKNFVIPK(5)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	954	Nipbl	1018	SGALKNFVIPK(5)-LIKDR(3)
Nipbl	954	Nipbl	1023	SGALKNFVIPK(5)-DREDKSR(5)
Nipbl	954	Nipbl	1029	SGALKNFVIPK(5)-SSLKPIK(4)
Nipbl	954	Nipbl	1034	SGALKNFVIPK(5)-NKPSK(2)
Nipbl	954	Nipbl	1037	SGALKNFVIPK(5)-NKPSKSNK(5)
Nipbl	954	Nipbl	1070	SGALKNFVIPK(5)-VKMNK(2)
Nipbl	954	Nipbl	1369	SGALKNFVIPK(5)-LDPHGGGLSSKAK(12)
Nipbl	954	Nipbl	1548	SGALKNFVIPK(5)-KCGSK(1)
Nipbl	954	Nipbl	1628	SGALKNFVIPK(5)-DAVTSKMDQGSIER(6)
Nipbl	954	Nipbl	1639	SGALKNFVIPK(5)-ILKQVSGGEDEIQQLQK(3)
Nipbl	954	Nipbl	1719	SGALKNFVIPK(5)-YKDRPQIAR(2)
Nipbl	954	Nipbl	1870	SGALKNFVIPK(5)-VIKILR(3)
Nipbl	954	Nipbl	1891	SGALKNFVIPK(5)-ITEMCVKMIR(7)
Nipbl	954	Nipbl	2337	SGALKNFVIPK(5)-NKADQQLVEIDKK(2)
Nipbl	954	Nipbl	2574	SGALKNFVIPK(5)-YSPSESAKVYDK(8)
Nipbl	954	Nipbl	2578	SGALKNFVIPK(5)-VYDKAINR(4)
Nipbl	954	Nipbl	2611	SGALKNFVIPK(5)-ITEEVKR(6)
Nipbl	960	Nipbl	965	NFVIPKIK(6)-DKDGNVTQETKK(2)
Nipbl	960	Nipbl	974	NFVIPKIK(6)-DKDGNVTQETKK(11)
Nipbl	960	Nipbl	975	NFVIPKIK(6)-KMEMK(1)
Nipbl	960	Nipbl	979	NFVIPKIK(6)-MEMKGEPK(4)
Nipbl	960	Nipbl	983	NFVIPKIK(6)-GEPKDKVEK(4)
Nipbl	960	Nipbl	985	NFVIPKIK(6)-DKVEK(2)
Nipbl	960	Nipbl	988	NFVIPKIK(6)-DKVEKIGLVEDLNK(5)
Nipbl	960	Nipbl	1000	NFVIPKIK(6)-GAKPVVVLQK(3)
Nipbl	960	Nipbl	1029	NFVIPKIK(6)-SSLKPIK(4)
Nipbl	960	Nipbl	1034	NFVIPKIK(6)-NKPSK(2)
Nipbl	960	Nipbl	1037	NFVIPKIK(6)-NKPSKSNK(5)
Nipbl	960	Nipbl	1040	NFVIPKIK(6)-SNKGSIDQSVLK(3)
Nipbl	960	Nipbl	2611	NFVIPKIK(6)-ITEEVKR(6)
Nipbl	960	Nipbl	2787	NFVIPKIK(6)-LTNKVVQTLR(4)
Nipbl	965	Nipbl	975	DKDGNVTQETK(2)-KMEMK(1)

Appendix

Nipbl	965	Nipbl	979	DKDGNVTQETK(2)-KMEMKGEPK(5)
Nipbl	965	Nipbl	985	DKDGNVTQETK(2)-DKVEK(2)
Nipbl	965	Nipbl	988	DKDGNVTQETK(2)-DKVEKIGLVEDLNK(5)
Nipbl	965	Nipbl	997	DKDGNVTQETK(2)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	965	Nipbl	1000	DKDGNVTQETK(2)-GAKPVVVLQK(3)
Nipbl	965	Nipbl	1015	DKDGNVTQETK(2)-LSLDDVQKLIK(8)
Nipbl	965	Nipbl	1018	DKDGNVTQETK(2)-LIKDR(3)
Nipbl	965	Nipbl	1029	DKDGNVTQETK(2)-SSLKPIK(4)
Nipbl	965	Nipbl	1040	DKDGNVTQETK(2)-SNKGSIDQSVLK(3)
Nipbl	965	Nipbl	1070	DKDGNVTQETK(2)-VKMNR(2)
Nipbl	965	Nipbl	1628	DKDGNVTQETK(2)-DAVTSKMDQGSIER(6)
Nipbl	965	Nipbl	1639	DKDGNVTQETK(2)-ILKQVSGGEDEIQQLQK(3)
Nipbl	965	Nipbl	1690	DKDGNVTQETK(2)-DTTLETEKAMK(8)
Nipbl	965	Nipbl	1732	DKDGNVTQETK(2)-SIKTTSPSQFSTLK(4)
Nipbl	965	Nipbl	1923	DKDGNVTQETK(2)-LWFTPTPHNDKEAMTR(11)
Nipbl	965	Nipbl	1968	DKDGNVTQETK(2)-SEEDSSYKPVKK(11)
Nipbl	965	Nipbl	2337	DKDGNVTQETK(2)-NKADQQLVEIDKK(2)
Nipbl	965	Nipbl	2565	DKDGNVTQETK(2)-KTGVHFHPK(1)
Nipbl	965	Nipbl	2574	DKDGNVTQETK(2)-YSPSESAKVYDK(8)
Nipbl	965	Nipbl	2783	DKDGNVTQETK(2)-IALTSANKLTNK(8)
Nipbl	974	Nipbl	979	DKDGNVTQETKK(11)-MEMKGEPK(4)
Nipbl	974	Nipbl	983	DKDGNVTQETKK(11)-GEPKDK(4)
Nipbl	974	Nipbl	985	DKDGNVTQETKK(11)-DKVEK(2)
Nipbl	974	Nipbl	988	DKVEKIGLVEDLNK(5)-DKDGNVTQETKK(11)
Nipbl	974	Nipbl	997	DKDGNVTQETKK(11)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	974	Nipbl	1000	DGNVTQETKK(9)-GAKPVVVLQK(3)
Nipbl	974	Nipbl	1007	DKDGNVTQETKK(11)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	974	Nipbl	1015	DKDGNVTQETKK(11)-LSLDDVQKLIK(8)
Nipbl	974	Nipbl	1018	DKDGNVTQETKK(11)-LIKDREDK(3)
Nipbl	974	Nipbl	1023	DKDGNVTQETKK(11)-DREDKSR(5)
Nipbl	974	Nipbl	1029	DKDGNVTQETKK(11)-LSLDDVQKLIK(8)
Nipbl	974	Nipbl	1034	DKDGNVTQETKK(11)-NKPSK(2)
Nipbl	974	Nipbl	1037	DKDGNVTQETKK(11)-NKPSKSNK(5)
Nipbl	974	Nipbl	1040	DKDGNVTQETKK(11)-SNKGSIDQSVLK(3)
Nipbl	974	Nipbl	1622	DKDGNVTQETKK(11)-KDAVTSK(1)
Nipbl	974	Nipbl	1628	DKDGNVTQETKK(11)-DAVTSKMDQGSIER(6)
Nipbl	974	Nipbl	1870	DKDGNVTQETKK(11)-VIKILR(3)
Nipbl	974	Nipbl	1923	DKDGNVTQETKK(11)-LWFTPTPHNDKEAMTR(11)
Nipbl	974	Nipbl	1968	DKDGNVTQETKK(11)-SEEDSSYKPVKK(11)
Nipbl	974	Nipbl	2783	DKDGNVTQETKK(11)-IALTSANKLTNK(8)
Nipbl	975	Nipbl	983	KMEMK(1)-GEPKDKVEK(4)
Nipbl	975	Nipbl	985	KMEMK(1)-DKVEK(2)
Nipbl	975	Nipbl	988	KMEMK(1)-DKVEKIGLVEDLNK(5)
Nipbl	975	Nipbl	997	KMEMK(1)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	975	Nipbl	1000	KMEMK(1)-GAKPVVVLQK(3)
Nipbl	975	Nipbl	1007	KMEMK(1)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	975	Nipbl	1015	KMEMK(1)-LSLDDVQKLIK(8)
Nipbl	975	Nipbl	1018	KMEMK(1)-LIKDREDK(3)
Nipbl	975	Nipbl	1029	KMEMK(1)-SSLKPIK(4)
Nipbl	975	Nipbl	1968	KMEMK(1)-SEEDSSYKPVKK(11)
Nipbl	979	Nipbl	988	MEMKGEPK(4)-DKVEKIGLVEDLNK(5)
Nipbl	979	Nipbl	1000	KMEMKGEPK(5)-GAKPVVVLQK(3)
Nipbl	979	Nipbl	1007	KMEMKGEPK(5)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	979	Nipbl	1040	KMEMKGEPK(5)-SNKGSIDQSVLK(3)
Nipbl	983	Nipbl	997	GEPKDKVEK(4)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	983	Nipbl	1000	GEPKDKVEK(4)-GAKPVVVLQK(3)
Nipbl	983	Nipbl	1007	GEPKDKVEK(4)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	983	Nipbl	1015	GEPKDKVEK(4)-LSLDDVQKLIK(8)

Appendix

Nipbl	983	Nipbl	1018	GEPKDKVEK(4)-LIKDREDK(3)
Nipbl	983	Nipbl	1029	GEPKDKVEK(4)-SSLKPIK(4)
Nipbl	983	Nipbl	1034	GEPKDKVEK(4)-NKPSK(2)
Nipbl	983	Nipbl	1037	GEPKDKVEK(4)-NKPSKSNK(5)
Nipbl	983	Nipbl	1040	GEPKDKVEK(4)-SNKGSIDQSVLK(3)
Nipbl	985	Nipbl	997	DKVEK(2)-IGLVEDLNKGAKPVVVLQK(9)
Nipbl	985	Nipbl	1000	DKVEK(2)-IGLVEDLNKGAKPVVVLQK(12)
Nipbl	985	Nipbl	1007	DKVEK(2)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	985	Nipbl	1015	DKVEK(2)-LSLDDVQKLIK(8)
Nipbl	985	Nipbl	1018	DKVEK(2)-LIKDR(3)
Nipbl	985	Nipbl	1029	DKVEK(2)-SSLKPIK(4)
Nipbl	985	Nipbl	1547	DKVEK(2)-TAQNFLSIFLKK(11)
Nipbl	988	Nipbl	1000	VEKIGLVEDLNK(3)-GAKPVVVLQK(3)
Nipbl	988	Nipbl	1018	DKVEKIGLVEDLNK(5)-LIKDR(3)
Nipbl	988	Nipbl	1023	DKVEKIGLVEDLNK(5)-DREDKSR(5)
Nipbl	988	Nipbl	1029	DKVEKIGLVEDLNK(5)-SSLKPIK(4)
Nipbl	988	Nipbl	1034	DKVEKIGLVEDLNK(5)-NKPSK(2)
Nipbl	988	Nipbl	1040	DKVEKIGLVEDLNK(5)-SNKGSIDQSVLK(3)
Nipbl	988	Nipbl	1070	DKVEKIGLVEDLNK(5)-VKMNKR(2)
Nipbl	988	Nipbl	1261	DKVEKIGLVEDLNK(5)-LSTDKTVM(5)
Nipbl	988	Nipbl	1968	DKVEKIGLVEDLNK(5)-SEEDSSYPVKK(11)
Nipbl	997	Nipbl	1018	IGLVEDLNKGAKPVVVLQK(9)-LIKDREDK(3)
Nipbl	997	Nipbl	1023	IGLVEDLNKGAKPVVVLQK(9)-DREDKSR(5)
Nipbl	997	Nipbl	1029	IGLVEDLNKGAKPVVVLQK(9)-SSLKPIK(4)
Nipbl	997	Nipbl	1034	IGLVEDLNKGAKPVVVLQK(9)-NKPSK(2)
Nipbl	997	Nipbl	1037	IGLVEDLNKGAKPVVVLQK(9)-NKPSKSNK(5)
Nipbl	997	Nipbl	1791	IGLVEDLNKGAKPVVVLQK(9)-TKAMK(2)
Nipbl	1000	Nipbl	1000	GAKPVVVLQKLSLDDVQK(3)-GAKPVVVLQK(3)
Nipbl	1000	Nipbl	1007	GAKPVVVLQK(3)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	1000	Nipbl	1018	GAKPVVVLQK(3)-LIKDR(3)
Nipbl	1000	Nipbl	1023	GAKPVVVLQK(3)-EDKSR(3)
Nipbl	1000	Nipbl	1034	GAKPVVVLQK(3)-NKPSK(2)
Nipbl	1000	Nipbl	1037	GAKPVVVLQK(3)-NKPSKSNK(5)
Nipbl	1000	Nipbl	1040	GAKPVVVLQK(3)-SNKGSIDQSVLK(3)
Nipbl	1000	Nipbl	1070	GAKPVVVLQK(3)-VKMNKR(2)
Nipbl	1000	Nipbl	1082	GAKPVVVLQK(3)-STVNEKPK(6)
Nipbl	1000	Nipbl	1182	GAKPVVVLQK(3)-KAYEPK(1)
Nipbl	1000	Nipbl	1247	GAKPVVVLQK(3)-HQLNELGSESAK(12)
Nipbl	1000	Nipbl	1369	GAKPVVVLQK(3)-LDPHGGGLSSKAK(12)
Nipbl	1000	Nipbl	1628	GAKPVVVLQK(3)-DAVTSKMDQGSIER(6)
Nipbl	1000	Nipbl	1639	GAKPVVVLQK(3)-ILKQVSGEDEIQQLQK(3)
Nipbl	1000	Nipbl	1968	GAKPVVVLQK(3)-SEEDSSYPVKK(11)
Nipbl	1007	Nipbl	1018	GAKPVVVLQKLSLDDVQK(10)-LIKDR(3)
Nipbl	1007	Nipbl	1023	GAKPVVVLQKLSLDDVQK(10)-DREDKSR(5)
Nipbl	1007	Nipbl	1029	GAKPVVVLQKLSLDDVQK(10)-SSLKPIK(4)
Nipbl	1007	Nipbl	1034	GAKPVVVLQKLSLDDVQK(10)-NKPSK(2)
Nipbl	1007	Nipbl	1037	GAKPVVVLQKLSLDDVQK(10)-NKPSKSNK(5)
Nipbl	1007	Nipbl	1040	GAKPVVVLQKLSLDDVQK(10)-SNKGSIDQSVLK(3)
Nipbl	1007	Nipbl	1070	GAKPVVVLQKLSLDDVQK(10)-VKMNKR(2)
Nipbl	1007	Nipbl	1082	GAKPVVVLQKLSLDDVQK(10)-STVNEKPK(6)
Nipbl	1007	Nipbl	1622	GAKPVVVLQKLSLDDVQK(10)-KDAVTSK(1)
Nipbl	1007	Nipbl	2611	GAKPVVVLQKLSLDDVQK(10)-ITEEVKR(6)
Nipbl	1015	Nipbl	1018	LSLDDVQKLIK(8)-LIKDREDK(3)
Nipbl	1015	Nipbl	1023	LSLDDVQKLIK(8)-EDKSR(3)
Nipbl	1015	Nipbl	1029	LSLDDVQKLIK(8)-SSLKPIK(4)
Nipbl	1015	Nipbl	1034	LSLDDVQKLIK(8)-NKPSK(2)
Nipbl	1015	Nipbl	1037	LSLDDVQKLIK(8)-NKPSKSNK(5)
Nipbl	1015	Nipbl	1070	LSLDDVQKLIK(8)-VKMNKR(2)

Appendix

Nipbl	1015	Nipbl	1109	LSLDDVQKLIK(8)-KDDDK(1)
Nipbl	1015	Nipbl	1791	LSLDDVQKLIK(8)-TKAMK(2)
Nipbl	1015	Nipbl	1968	LSLDDVQKLIK(8)-SEEDSSYPVKK(11)
Nipbl	1018	Nipbl	1023	LIKDR(3)-EDKSR(3)
Nipbl	1018	Nipbl	1029	LIKDR(3)-SSLKPIK(4)
Nipbl	1018	Nipbl	1034	LIKDR(3)-NKPSK(2)
Nipbl	1018	Nipbl	1037	LIKDREDK(3)-NKPSKSNK(5)
Nipbl	1018	Nipbl	1040	LIKDREDK(3)-SNKGSIDQSVLK(3)
Nipbl	1018	Nipbl	1070	LIKDREDK(3)-VKMNK(2)
Nipbl	1018	Nipbl	1082	LIKDREDK(3)-STVNEKPK(6)
Nipbl	1018	Nipbl	1182	LIKDREDK(3)-KAYEPK(1)
Nipbl	1018	Nipbl	1247	LIKDREDK(3)-HQLNELGSESAKIK(12)
Nipbl	1018	Nipbl	1261	LIKDREDK(3)-LSTDKTVK(5)
Nipbl	1018	Nipbl	1771	LIKDREDK(3)-VIKILR(3)
Nipbl	1023	Nipbl	1029	EDKSR(3)-SSLKPIK(4)
Nipbl	1023	Nipbl	1034	EDKSR(3)-NKPSK(2)
Nipbl	1023	Nipbl	1037	DREDKSR(5)-NKPSKSNK(5)
Nipbl	1023	Nipbl	2719	DREDKSR(5)-YKDRPQIAR(2)
Nipbl	1029	Nipbl	1034	SSLKPIK(4)-NKPSK(2):
Nipbl	1029	Nipbl	1040	SSLKPIK(4)-SNKGSIDQSVLK(3)
Nipbl	1029	Nipbl	1070	SSLKPIK(4)-VKMNK(2)
Nipbl	1029	Nipbl	1182	SSLKPIK(4)-KAYEPK(1)
Nipbl	1029	Nipbl	1247	SSLKPIK(4)-HQLNELGSESAKIK(12)
Nipbl	1029	Nipbl	1369	SSLKPIK(4)-LDPHGGGLSSKAK(12)
Nipbl	1029	Nipbl	1547	SSLKPIK(4)-TAQNFLSIFLKK(11)
Nipbl	1029	Nipbl	1622	SSLKPIK(4)-KDAVTSK(1)
Nipbl	1029	Nipbl	1639	SSLKPIK(4)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1029	Nipbl	1891	SSLKPIK(4)-ITEMCVKMIR(7)
Nipbl	1032	Nipbl	1037	SSLKPIKNK(7)-PSKSNK(3)
Nipbl	1034	Nipbl	1040	NKPSK(2)-SNKGSIDQSVLK(3)
Nipbl	1034	Nipbl	1082	NKPSK(2)-STVNEKPK(6)
Nipbl	1034	Nipbl	1247	NKPSK(2)-HQLNELGSESAKIK(12)
Nipbl	1034	Nipbl	1547	NKPSK(2)-TAQNFLSIFLKK(11)
Nipbl	1034	Nipbl	1639	NKPSK(2)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1037	Nipbl	1070	NKPSKSNK(5)-VKMNK(2)
Nipbl	1037	Nipbl	1247	NKPSKSNK(5)-HQLNELGSESAKIK(12)
Nipbl	1037	Nipbl	1628	NKPSKSNK(5)-DAVTSKMDQGSIER(6)
Nipbl	1037	Nipbl	1639	NKPSKSNK(5)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1037	Nipbl	2563	NKPSKSNK(5)-NLGFSDSKIQK(9)
Nipbl	1037	Nipbl	2648	NKPSKSNK(5)-NKAITSLGGGSPK(2)
Nipbl	1040	Nipbl	1070	SNKGSIDQSVLK(3)-VKMNK(2)
Nipbl	1040	Nipbl	1182	SNKGSIDQSVLK(3)-KAYEPK(1)
Nipbl	1040	Nipbl	1247	SNKGSIDQSVLK(3)-HQLNELGSESAKIK(12)
Nipbl	1040	Nipbl	1369	SNKGSIDQSVLK(3)-LDPHGGGLSSKAK(12)
Nipbl	1040	Nipbl	1622	SNKGSIDQSVLK(3)-KDAVTSK(1)
Nipbl	1040	Nipbl	1628	SNKGSIDQSVLK(3)-DAVTSKMDQGSIER(6)
Nipbl	1040	Nipbl	1639	SNKGSIDQSVLK(3)-ILKQVSGGEDEIQQLQK(3)
Nipbl	1040	Nipbl	2583	SNKGSIDQSVLK(3)-KTGVHFHFK(1)
Nipbl	1070	Nipbl	1082	VKMNK(2)-STVNEKPK(6)
Nipbl	1070	Nipbl	1622	VKMNK(2)-KDAVTSK(1)
Nipbl	1070	Nipbl	1870	VKMNK(2)-VIKILR(3)
Nipbl	1082	Nipbl	1109	STVNEKPK(6)-KDDDKAWYEER(1)
Nipbl	1082	Nipbl	1182	STVNEKPK(6)-KAYEPK(1)
Nipbl	1082	Nipbl	1369	STVNEKPK(6)-LDPHGGGLSSKAK(12)
Nipbl	1082	Nipbl	1622	STVNEKPK(6)-KDAVTSK(1)
Nipbl	1082	Nipbl	2719	STVNEKPK(6)-YKDRPQIAR(2)
Nipbl	1113	Nipbl	1628	KDDDKAWYEER(5)-KDAVTSKMDQGSIER(7)
Nipbl	1177	Nipbl	1182	KEKQK(3)KAYEPK(1)-

Appendix

Nipbl	1177	Nipbl	1247	EKQKK(2)-HQLNELGSESAKIK(12)
Nipbl	1179	Nipbl	1182	EKQKK(4)-KAYEPK(1)
Nipbl	1182	Nipbl	1200	KAYEPK(1)-LTPEEMMDSSTFKR(13)
Nipbl	1182	Nipbl	1247	KAYEPK(1)-HQLNELGSESAKIK(12)
Nipbl	1182	Nipbl	1369	KAYEPK(1)-LDPHGGGLLSSKAK(12)
Nipbl	1182	Nipbl	1548	KAYEPK(1)-KCGSK(1)
Nipbl	1182	Nipbl	1622	KAYEPK(1)-LRKDAVTSK(3)
Nipbl	1182	Nipbl	2719	KAYEPK(1)-YKDRPQIAR(2)
Nipbl	1187	Nipbl	1247	AYEPKLTPEEMMDSSTFK(5)-HQLNELGSESAKIK(12)
Nipbl	1200	Nipbl	1256	LTPEEMMDSSTFKR(13)-AMGIMDKLSTDK(7)
Nipbl	1200	Nipbl	1261	LTPEEMMDSSTFKR(13)-LSTDKTVK(5)
Nipbl	1200	Nipbl	1622	LTPEEMMDSSTFKR(13)-KDAVTSK(1)
Nipbl	1247	Nipbl	1256	HQLNELGSESAKIK(12)-AMGIMDKLSTDK(7)
Nipbl	1247	Nipbl	1261	HQLNELGSESAKIK(12)-LSTDKTVK(5)
Nipbl	1247	Nipbl	1271	HQLNELGSESAKIK(12)-VLNILEKNIQDGSK(7)
Nipbl	1247	Nipbl	1369	HQLNELGSESAKIK(12)-LDPHGGGLLSSKAK(12)
Nipbl	1247	Nipbl	1548	HQLNELGSESAKIK(12)-KCGSK(1)
Nipbl	1247	Nipbl	2578	HQLNELGSESAKIK(12)-VYDKAINRK(4)
Nipbl	1247	Nipbl	2611	HQLNELGSESAKIK(12)-ITEEVKR(6)
Nipbl	1249	Nipbl	1261	IKAMGIMDK(2)-LSTDKTVK(5)
Nipbl	1261	Nipbl	1278	LSTDKTVK(5)-NIQDGSKLSTLLNHNNDTEEEER(7)
Nipbl	1271	Nipbl	1369	VLNILEKNIQDGSK(7)-LDPHGGGLLSSKAK(12)
Nipbl	1369	Nipbl	1374	LDPHGGGLLSSKAK(12)-AKCSTHK(2)
Nipbl	1369	Nipbl	1379	LDPHGGGLLSSKAK(12)-CSTHKQR(5)
Nipbl	1369	Nipbl	1467	LDPHGGGLLSSKAK(12)-LPTSKR(5)
Nipbl	1369	Nipbl	1622	LDPHGGGLLSSKAK(12)-KDAVTSK(1)
Nipbl	1369	Nipbl	1791	LDPHGGGLLSSKAK(12)-TKAMK(2)
Nipbl	1369	Nipbl	2719	LDPHGGGLLSSKAK(12)-YKDRPQIAR(2)
Nipbl	1369	Nipbl	2787	LDPHGGGLLSSKAK(12)-LTNKVVQTLR(4)
Nipbl	1374	Nipbl	1467	LPTSKR(5)-AKCSTHK(2)
Nipbl	1467	Nipbl	1791	LPTSKR(5)-TKAMK(2)
Nipbl	1520	Nipbl	1732	KIDQDVVITNSYETAMR(1)-SIKTTSPSQFSTLK(4)
Nipbl	1547	Nipbl	1622	TAQNFLSIFLKK(11)-KDAVTSK(1)
Nipbl	1547	Nipbl	1968	TAQNFLSIFLKK(11)-SEEDSSYKPVKK(11)
Nipbl	1548	Nipbl	2783	KCGSK(1)-IALTSANKLTNK(8)
Nipbl	1599	Nipbl	1622	LLVHQFSNKSTEMALR(9)-KDAVTSK(1)
Nipbl	1599	Nipbl	1696	LLVHQFSNKSTEMALR(9)-SQKDEESSEGETHHAK(3)
Nipbl	1599	Nipbl	1791	LLVHQFSNKSTEMALR(9)-TKAMK(2)
Nipbl	1599	Nipbl	2578	LLVHQFSNKSTEMALR(9)-VYDKAINR(4)
Nipbl	1599	Nipbl	2783	LLVHQFSNKSTEMALR(9)-IALTSANKLTNK(8)
Nipbl	1622	Nipbl	1639	KDAVTSK(1)-ILKQVSGGEDEIQQQLQK(3)
Nipbl	1622	Nipbl	1690	KDAVTSK(1)-DTTLETEKAMK(8)
Nipbl	1622	Nipbl	1794	KDAVTSK(1)-AMKCLSEVVAVDPSILAR(3)
Nipbl	1622	Nipbl	2719	KDAVTSK(1)-YKDRPQIAR(2)
Nipbl	1622	Nipbl	2758	KDAVTSK(1)-KLVPWVDTIK(1)
Nipbl	1622	Nipbl	2767	KDAVTSK(1)-LVPWVDTIKESDIIYK(9)
Nipbl	1622	Nipbl	2783	KDAVTSK(1)-IALTSANKLTNK(8)
Nipbl	1622	Nipbl	2787	LRKDAVTSK(3)-LTNKVVQTLR(4)
Nipbl	1628	Nipbl	1639	DAVTSKMDQGSIER(6)-ILKQVSGGEDEIQQQLQK(3)
Nipbl	1628	Nipbl	1690	DAVTSKMDQGSIER(6)-DTTLETEKAMK(8)
Nipbl	1628	Nipbl	1696	DAVTSKMDQGSIER(6)-SQKDEESSEGETHHAK(3)
Nipbl	1628	Nipbl	1708	DAVTSKMDQGSIER(6)-SQKDEESSEGETHHAKEIETTQIMHR(15)
Nipbl	1628	Nipbl	2758	DAVTSKMDQGSIER(6)-KLVPWVDTIK(1)
Nipbl	1639	Nipbl	1690	ILKQVSGGEDEIQQQLQK(3)-DTTLETEKAMK(8)
Nipbl	1639	Nipbl	1696	ILKQVSGGEDEIQQQLQK(3)-SQKDEESSEGETHHAK(3)
Nipbl	1639	Nipbl	1968	ILKQVSGGEDEIQQQLQK(3)-SEEDSSYKPVKK(11)
Nipbl	1639	Nipbl	2719	ILKQVSGGEDEIQQQLQK(3)-YKDRPQIAR(2)
Nipbl	1639	Nipbl	2783	ILKQVSGGEDEIQQQLQK(3)-IALTSANKLTNK(8)

Appendix

Nipbl	1639	Nipbl	2787	ILKQVSGGEDEIQQLQK(3)-LTNKVVQTLR(4)
Nipbl	1622	Nipbl	2783	KDAVTSK(1)-IALTSANKLTNK(8)
Nipbl	1690	Nipbl	1696	DTTLETEKAMK(8)-SQKDEESSEGTHHAK(3)
Nipbl	1690	Nipbl	1708	DTTLETEKAMK(8)-SQKDEESSEGTHHAKIETTQGIMHR(15)
Nipbl	1693	Nipbl	1628	AMKSQKDEESSEGTHHAK(3)-DAVTSKMDQGSIER(6)
Nipbl	1693	Nipbl	1708	AMKSQK(3)-DEESSEGTHHAKIETTQGIMHR(12)
Nipbl	1693	Nipbl	1724	AMKSQKDEESSEGTHHAK(3)-KKFLR(1)
Nipbl	1693	Nipbl	2783	AMKSQKDEESSEGTHHAK(3)-IALTSANKLTNK(8)
Nipbl	1696	Nipbl	1725	SQKDEESSEGTHHAK(3)-KKFLR(2)
Nipbl	1696	Nipbl	2719	SQKDEESSEGTHHAK(3)-YKDRPQIAR(2)
Nipbl	1696	Nipbl	2758	SQKDEESSEGTHHAK(3)-KLVWVDTIK(1)
Nipbl	1696	Nipbl	2783	SQKDEESSEGTHHAK(3)-IALTSANKLTNK(8)
Nipbl	1696	Nipbl	2790	SQKDEESSEGTHHAK(3)-LTNKVVQTLR(4)
Nipbl	1724	Nipbl	1732	KKFLR(1)-SIKTTSPQFSTLK(4)
Nipbl	1725	Nipbl	1732	KKFLR(2)-SIKTTSPQFSTLK(4)
Nipbl	1791	Nipbl	2578	TKAMK(2)-VYDKAINR(4)
Nipbl	1791	Nipbl	2583	TKAMK(2)-KTGVHFHPK(1)
Nipbl	1791	Nipbl	2648	TKAMK(2)-NKAITSLLGGGSPK(2)
Nipbl	2791	Nipbl	2719	TKAMK(2)-YKDRPQIAR(2)
Nipbl	1791	Nipbl	2758	TKAMK(2)-KLVWVDTIK(1)
Nipbl	1791	Nipbl	2783	TKAMK(2)-IALTSANKLTNK(8)
Nipbl	1791	Nipbl	2805	TKAMK(2)-LTNKVVQTLR(4)
Nipbl	1870	Nipbl	1904	VIKILR(3)-KLVNETFQK(1)
Nipbl	1870	Nipbl	2783	VIKILR(3)-IALTSANKLTNK(8)
Nipbl	1870	Nipbl	2787	VIKILR(3)-LTNKVVQTLR(4)
Nipbl	1891	Nipbl	1904	ITEMCVKMIR(7)-KLVNETFQK(1)
Nipbl	1891	Nipbl	1968	ITEMCVKMIR(7)-SEEDSSYPVKK(11)
Nipbl	1891	Nipbl	1995	ITEMCVKMIR(7)-YEESLADSDNKGVSNGR(11)
Nipbl	1903	Nipbl	1923	VNDEEGIKK(8)-LWFTPTPHNDKEAMTR(11)
Nipbl	1903	Nipbl	2337	VNDEEGIKK(8)-NKADQQLVEIDKK(2)
Nipbl	1903	Nipbl	2347	VNDEEGIKK(8)-NKADQQLVEIDKK(12)
Nipbl	1903	Nipbl	2578	VNDEEGIKK(8)-VYDKAINR(4)
Nipbl	1903	Nipbl	2611	RVNDEEGIKK(9)-ITEEVKR(6)
Nipbl	1904	Nipbl	2347	KLVNETFQK(1)-NKADQQLVEIDKK(12)
Nipbl	1904	Nipbl	2605	KLVNETFQK(1)-SDMANSKITEEVKR(7)
Nipbl	1904	Nipbl	2719	KLVNETFQK(1)-YKDRPQIAR(2)
Nipbl	1904	Nipbl	2787	KLVNETFQK(1)-LTNKVVQTLR(4)
Nipbl	1923	Nipbl	1995	LWFTPTPHNDKEAMTR(11)-YEESLADSDNKGVSNGR(11)
Nipbl	1929	Nipbl	1968	KILNITDVAAACR(1)-SEEDSSYPVKK(11)
Nipbl	1965	Nipbl	1969	SEEDSSYPVKK(8)-KACTQLVDNLVEHILK(1)
Nipbl	1968	Nipbl	1791	SEEDSSYPVKK(11)-TKAMK(2)
Nipbl	1968	Nipbl	2223	SEEDSSYPVKK(11)-NLNNILSDKNSSVNLK(10)
Nipbl	1968	Nipbl	2256	SEEDSSYPVKK(11)-MQQADRDWKK(9)
Nipbl	1968	Nipbl	2758	SEEDSSYPVKK(11)-KLVWVDTIK(1)
Nipbl	1968	Nipbl	2783	SEEDSSYPVKK(11)-IALTSANKLTNK(8)
Nipbl	2137	Nipbl	2170	SQHQPNTSLLTNKPALLR(16)-VNIKDK(4)
Nipbl	2137	Nipbl	2337	SQHQPNTSLLTNKPALLR(16)-NKADQQLVEIDKK(2)
Nipbl	2223	Nipbl	2719	NLNNILSDKNSSVNLK(10)-YKDRPQIAR(2)
Nipbl	2256	Nipbl	2260	MQQADRDWKK(9)-VAKQEDLK(3)
Nipbl	2256	Nipbl	2337	MQQADRDWKK(9)-NKADQQLVEIDKK(2)
Nipbl	2256	Nipbl	2783	MQQADRDWKK(9)-IALTSANKLTNK(8)
Nipbl	2337	Nipbl	2348	NKADQQLVEIDK(2)-KYAGFIHMK(1)
Nipbl	2337	Nipbl	2566	NKADQQLVEIDKK(2)-IQKSPSESASAK(3)
Nipbl	2337	Nipbl	2611	NKADQQLVEIDKK(2)-ITEEVKR(6)
Nipbl	2337	Nipbl	2719	NKADQQLVEIDKK(2)-YKDRPQIAR(2)
Nipbl	2337	Nipbl	2758	NKADQQLVEIDKK(2)-KLVWVDTIK(1)
Nipbl	2347	Nipbl	2611	NKADQQLVEIDKK(12)-ITEEVKR(6)
Nipbl	2376	Nipbl	2605	MSYQVQQAINTCLKDPVR(14)-SDMANSKITEEVKR(7)

Appendix

Nipbl	2376	Nipbl	2719	MSYQVQQAINCLKDPVR(14)-YKDRPQIAR(2)
Nipbl	2376	Nipbl	2783	MSYQVQQAINCLKDPVR(14)-IALTSANKLTNK(8)
Nipbl	2554	Nipbl	2566	QHLKNLCGFSDSK(4)-IQKYSPSESAK(3)
Nipbl	2554	Nipbl	2574	QHLKNLCGFSDSK(4)-YSPSESAKVYDK(8)
Nipbl	2554	Nipbl	2583	QHLKNLCGFSDSK(4)-KTGVHFHPK(1)
Nipbl	2554	Nipbl	2648	QHLKNLCGFSDSK(4)-NKAITSLLGGGSPK(2)
Nipbl	2554	Nipbl	2783	QHLKNLCGFSDSK(4)-IALTSANKLTNK(8)
Nipbl	2563	Nipbl	2574	NLCGFSDSKIQK(9)-YSPSESAKVYDK(8)
Nipbl	2563	Nipbl	2578	NLCGFSDSKIQK(9)-VYDKAINR(4)
Nipbl	2563	Nipbl	2583	NLCGFSDSKIQK(9)-KTGVHFHPK(1)
Nipbl	2563	Nipbl	2719	NLCGFSDSKIQK(9)-YKDRPQIAR(2)
Nipbl	2566	Nipbl	2578	IQKYSPSESAK(3)-VYDKAINR(4)
Nipbl	2566	Nipbl	2583	IQKYSPSESAK(3)-KTGVHFHPK(1)
Nipbl	2566	Nipbl	2611	IQKYSPSESAK(3)-ITEEVKR(6)
Nipbl	2566	Nipbl	2783	IQKYSPSESAK(3)-IALTSANKLTNK(8)
Nipbl	2566	Nipbl	2605	IQKYSPSESAK(3)-SDMANSKITEEVKR(7)
Nipbl	2566	Nipbl	2616	IQKYSPSESAK(3)-SIVKQYLDFK(4)
Nipbl	2566	Nipbl	2719	IQKYSPSESAK(3)-YKDRPQIAR(2)
Nipbl	2566	Nipbl	2758	IQKYSPSESAK(3)-KLVWVDTIK(1)
Nipbl	2574	Nipbl	2578	YSPSESAKVYDK(8)-VYDKAINRK(4)
Nipbl	2574	Nipbl	2583	YSPSESAKVYDK(8)-KTGVHFHPK(1)
Nipbl	2574	Nipbl	2605	YSPSESAKVYDK(8)-SDMANSKITEEVKR(7)
Nipbl	2574	Nipbl	2611	YSPSESAKVYDK(8)-ITEEVKR(6)
Nipbl	2574	Nipbl	2719	YSPSESAKVYDK(8)-YKDRPQIAR(2)
Nipbl	2574	Nipbl	2758	YSPSESAKVYDK(8)-KLVWVDTIK(1)
Nipbl	2574	Nipbl	2787	YSPSESAKVYDK(8)-LTNKVVQTLR(4)
Nipbl	2578	Nipbl	2583	VYDKAINR(4)-KTGVHFHPK(1)
Nipbl	2578	Nipbl	2605	VYDKAINRK(4)-SDMANSKITEEVKR(7)
Nipbl	2578	Nipbl	2611	VYDKAINRK(4)-ITEEVKR(6)
Nipbl	2578	Nipbl	2616	VYDKAINRK(4)-SIVKQYLDFK(4)
Nipbl	2578	Nipbl	2719	VYDKAINR(4)-YKDRPQIAR(2)
Nipbl	2578	Nipbl	2783	VYDKAINR(4)-IALTSANKLTNK(8)
Nipbl	2578	Nipbl	2787	VYDKAINR(4)-LTNKVVQTLR(4)
Nipbl	2583	Nipbl	2611	KTGVHFHPK(1)-ITEEVKR(6)
Nipbl	2583	Nipbl	2783	KTGVHFHPK(1)-IALTSANKLTNK(8)
Nipbl	2583	Nipbl	2787	KTGVHFHPK(1)-LTNKVVQTLR(4)
Nipbl	2605	Nipbl	2719	SDMANSKITEEVKR(7)-YKDRPQIAR(2)
Nipbl	2611	Nipbl	2616	ITEEVKR(6)-SIVKQYLDFK(4)
Nipbl	2611	Nipbl	2719	ITEEVKR(6)-YKDRPQIAR(2)
Nipbl	2616	Nipbl	2775	SIVKQYLDFK(4)-KIALTSANK(1)
Nipbl	2616	Nipbl	2648	SIVKQYLDFK(4)-NKAITSLLGGGSPK(2)
Nipbl	2648	Nipbl	2719	NKAITSLLGGGSPK(2)-YKDRPQIAR(2)
Nipbl	2648	Nipbl	2783	NKAITSLLGGGSPK(2)-IALTSANKLTNK(8)
Nipbl	2719	Nipbl	2783	YKDRPQIAR(2)-IALTSANKLTNK(8)
Nipbl	2719	Nipbl	2787	YKDRPQIAR(2)-LTNKVVQTLR(4)
Nipbl	2758	Nipbl	2774	KLVWVDTIK(1)-ESDIYKK(7)
Nipbl	2758	Nipbl	2775	KLVWVDTIK(1)-KIALTSANK(1)
Nipbl	2774	Nipbl	2787	ESDIYKK(7)-LTNKVVQTLR(4)
Nipbl	2775	Nipbl	2787	KIALTSANK(1)-LTNKVVQTLR(4)

* For simplicity, Nipbls N-terminal Twin-strep-flag-linker (48 amino acids) was subtracted from the residue number.

Table 12: List of all interprotein cross-links Nipbl:Cohesin (exp. 1).*

Protein 1	Residue	Protein 2	Residue	Peptide
Smc1	59	Smc3	140	VKTLR(2)-SNPYIVKQGK(8)
Smc1	59	Smc3	486	TSNLRVKTLR(7)-EENAEQQALAAKREDLEK(12)
Smc1	59	Smc3	1058	VKTLR(2)-ATLVMKK(6)
Smc1	72	Smc3	820	DLIHGAPVGKPAANR(10)-IKLEGIITR(2)
Smc1	170	Smc3	180	SGELAQEYDKR(10)-KEESISLMKETEGK(9)
Smc1	172	Smc3	172	KKEMVK(1)-KEESISLMK(1)
Smc1	172	Smc3	180	KKEMVK(1)-EESISLMKETEGKR(8)
Smc1	189	Smc3	185	KKNIAAER(1)-ETEGKR(5)
Smc1	189	Smc3	188	KKNIAAER(1)-EKINELLK(2)
Smc1	189	Smc3	999	KKNIAAER(1)-EKLIK(2)
Smc1	190	Smc3	185	KNIAAER(1)-ETEGKR(5)
Smc1	190	Smc3	188	KNIAAER(1)-EKINELLK(2)
Smc1	198	Smc3	188	KKNIAAER(1)-EKINELLK(2)
Smc1	213	Smc3	963	LKDEVVR(2)-YQTLCLKQLFR(7)
Smc1	213	Smc3	968	LKDEVVR(2)-KLEQCNTLKK(1)
Smc1	213	Smc3	977	LKDEVVR(2)-LEQCNTLKK(9)
Smc1	213	Smc3	978	LKDEVVR(2)-KYSHVKNK(1)
Smc1	213	Smc3	984	LKDEVVR(2)-YSHVNKK(6)
Smc1	213	Smc3	985	LKDEVVR(2)-KALDQFVNFSEQK(1)
Smc1	245	Smc3	924	ELASKNK(5)-ELEKMTNR(4)
Smc1	258	Smc3	278	MDKVEDELKEK(3)-QVRELKTK(6)
Smc1	258	Smc3	492	MDKVEDELKEK(3)-EDLEKK(5)
Smc1	264	Smc3	905	MDKVEDELKEK(9)-WKNMEK(2)
Smc1	267	Smc3	278	KKELGK(1)-ELKTK(3)
Smc1	267	Smc3	895	KKELGK(1)-SEDLDNSIDKTEAGIKELQK(16)
Smc1	267	Smc3	899	KKELGK(1)-ELQKSMER(4)
Smc1	267	Smc3	905	KKELGK(1)-WKNMEK(2)
Smc1	267	Smc3	909	KKELGK(1)-NMEKEHMDAINHDTK(4)
Smc1	268	Smc3	895	KKELGK(2)-SEDLDNSIDKTEAGIKELQK(16)
Smc1	268	Smc3	899	KELGK(1)-ELQKSMER(4)
Smc1	272	Smc3	621	FDKAFK(3)-ELGKMMR(4)
Smc1	272	Smc3	889	ELGKMMR(4)-SEDLDNSIDKTEAGIK(10)
Smc1	272	Smc3	895	ELGKMMR(4)-SEDLDNSIDKTEAGIKELQK(16)
Smc1	282	Smc3	889	EQQQIEKEIK(7)-SEDLDNSIDKTEAGIK(10)
Smc1	282	Smc3	895	EQQQIEKEIK(7)-SEDLDNSIDKTEAGIKELQK(16)
Smc1	282	Smc3	899	EQQQIEKEIK(7)-ELQKSMER(4)
Smc1	285	Smc3	895	EIKEKDSLNQK(3)-SEDLDNSIDKTEAGIKELQK(16)
Smc1	300	Smc3	304	RPQYIKAK(6)-TKLELK(2)
Smc1	300	Smc3	310	RPQYIKAK(6)-AKDLQDELGNSEQR(2)
Smc1	300	Smc3	874	RPQYIKAK(6)-VKDTMAR(2)
Smc1	302	Smc3	300	AKENTSHK(2)-QEIQQR(5)
Smc1	302	Smc3	304	AKENTSHK(2)-TKLELK(2)
Smc1	308	Smc3	310	ENTSHKIK(6)-AKDLQDELGNSEQR(2)
Smc1	308	Smc3	889	AKENTSHKIK(8)-SEDLDNSIDKTEAGIK(10)
Smc1	310	Smc3	308	IKKLEAAK(2)-TKLELKAK(6)
Smc1	311	Smc3	304	KLEAAK(1)-TKLELK(2)
Smc1	311	Smc3	308	KLEAAK(1)-LELKAK(4)
Smc1	311	Smc3	310	KLEAAK(1)-AKDLQDELGNSEQR(2)
Smc1	317	Smc3	308	KSLQNAQK(1)-TKLELKAK(6)
Smc1	317	Smc3	727	KSLQNAQK(1)-KFKASR(1)
Smc1	317	Smc3	729	KSLQNAQK(1)-FKASR(2)
Smc1	317	Smc3	740	KSLQNAQK(1)-DSILSEMMLK(8)
Smc1	324	Smc3	740	SLQNAQKHYK(7)-DSILSEMMLK(8)
Smc1	395	Smc3	400	AATLAQELEKFNRDQK(10)-ELKSLDQAINDK(3)
Smc1	395	Smc3	409	AATLAQELEKFN(10)-SLDQAINDKKR(9)

Appendix

Smc1	395	Smc3	410	AATLAQELEKFNR(10)-SLDQAINDKKR(10)
Smc1	401	Smc3	409	DQKADQDRDLLEER(3)-SLDQAINDKK(9)
Smc1	401	Smc3	756	DQKADQDRDLLEER(3)-TFMPKQR(5)
Smc1	413	Smc3	409	KKVETEA(1)-SLDQAINDKKR(9)
Smc1	413	Smc3	418	KKVETEA(1)-QIAAIHKDLEDTEANKEK(7)
Smc1	413	Smc3	751	KKVETEA(1)-RQQSEKTFMPK(6)
Smc1	413	Smc3	756	KKVETEA(1)-TFMPKQR(5)
Smc1	420	Smc3	745	VETEA(6)-MLKEK(5)
Smc1	420	Smc3	756	VETEA(6)-TFMPKQR(5)
Smc1	424	Smc3	745	IKQKLR(4)-MLKEK(5)
Smc1	433	Smc3	727	EIEENQKR(7)-KFKASR(1)
Smc1	433	Smc3	740	EIEENQKR(7)-DSILSEMKMLK(8)
Smc1	437	Smc3	454	IEKLEEYITTSK(3)-KYYEVK(1)
Smc1	437	Smc3	462	IEKLEEYITTSK(3)-NKKDELQSER(3)
Smc1	437	Smc3	727	RIEKLEEYITTSK(4)-KFKASR(1)
Smc1	437	Smc3	729	IEKLEEYITTSK(3)-FKASR(2)
Smc1	454	Smc3	429	KLEGELTEEVEMAKR(1)-EKNLEQYNKLDQDLNEVK(2)
Smc1	508	Smc3	934	KAEIMESIKR(9)-QGMLLKK(6)
Smc1	508	Smc3	935	KAEIMESIKR(9)-KKEECMK(1)
Smc1	508	Smc3	936	KAEIMESIKR(9)-KKEECMK(1)
Smc1	529	Smc3	936	KYQIAVTK(1)-KKEECMK(2)
Smc1	540	Smc3	629	VLGKNMDAIIVDSEK(4)-HVFGLTLICR(5)
Smc1	551	Smc3	172	NMDAIIVDSEK(11)-KEESISLMK(1)
Smc1	551	Smc3	935	NMDAIIVDSEK(11)-KKEECMK(1)
Smc1	551	Smc3	936	NMDAIIVDSEK(11)-KKEECMK(1)
Smc1	584	Smc3	114	KMVT(1)-GEPETFLPLDYLEVKPTDEKLR(20)
Smc1	589	Smc3	113	ELKGAK(3)-DQYFLDKK(7)
Smc1	660	Smc3	503	SGVISGGASDLKAK(12)-AATGKAILNGIDSINK(5)
Smc1	662	Smc3	503	SGVISGGASDLKAKAR(14)-AATGKAILNGIDSINK(5)
Smc1	673	Smc3	278	AVDKLK(4)-ELKTK(3)
Smc1	673	Smc3	462	AVDKLKEK(4)-KDELQSER(1)
Smc1	675	Smc3	266	AVDKLKEK(6)-DKMEDIER(2)
Smc1	675	Smc3	278	AVDKLKEK(6)-ELKTK(3)
Smc1	675	Smc3	492	AVDKLKEK(6)-EDLEKK(5)
Smc1	675	Smc3	612	AVDKLKEK(6)-DTAYPETNDAIPMISKLR(16)
Smc1	677	Smc3	492	EKKER(2)-REDLEKK(6)
Smc1	678	Smc3	492	EKKER(3)-REDLEKK(6)
Smc1	686	Smc3	278	LTEELKEQMK(6)-ELKTK(3)
Smc1	686	Smc3	280	LTEELKEQMK(6)-TKISAMKEEK(2)
Smc1	686	Smc3	285	LTEELKEQMK(6)-ISAMKEEK(5)
Smc1	686	Smc3	492	LTEELKEQMK(6)-REDLEKK(6)
Smc1	694	Smc3	300	RKEAELR(2)-QEIQQR(5)
Smc1	713	Smc3	454	LKYSQSDLEQTK(2)-KYYEVK(1)
Smc1	713	Smc3	462	LKYSQSDLEQTK(2)-KDELQSER(1)
Smc1	713	Smc3	612	LKYSQSDLEQTK(2)-DTAYPETNDAIPMISKLR(16)
Smc1	713	Smc3	727	LKYSQSDLEQTK(2)-KFKASR(1)
Smc1	723	Smc3	727	YSQSDLEQTKTR(10)-KFKASR(1)
Smc1	723	Smc3	729	YSQSDLEQTKTR(10)-KFKASR(3)
Smc1	736	Smc3	118	SKLESELANFGPR(2)-KMVTKNDVMNLLSAGFSR(5)
Smc1	736	Smc3	418	SKLESELANFGPR(2)-QIAAIHKDLEDTEANKEK(7)
Smc1	736	Smc3	745	SKLESELANFGPR(2)-MLKEK(5)
Smc1	736	Smc3	956	SKLESELANFGPR(2)-ELGSLPQEAPEKYQTLSLK(12)
Smc1	752	Smc3	756	TFMPKQR(5)-INDIKR(5)
Smc1	805	Smc3	355	QNEIAKK(6)-FNSVKEKEER(5)
Smc1	805	Smc3	820	QNEIAKK(6)-IKLEGIITR(2)
Smc1	814	Smc3	336	RLEFENQKTR(8)-LLEKIEEK(4)
Smc1	814	Smc3	340	RLEFENQKTR(8)-IEEKQKELAEETEPK(4)
Smc1	814	Smc3	820	LEFENQKTR(7)-IKLEGIITR(2)

Appendix

Smc1	829	Smc3	332	NQLKEDQDKVHMWEQTVK(4)-QKLEK(2)
Smc1	829	Smc3	336	NQLKEDQDKVHMWEQTVK(4)-LLEKIEEK(4)
Smc1	844	Smc3	310	KDENEIEK(1)-AKDLQDELAGNSEQR(2)
Smc1	844	Smc3	941	KDENEIEK(1)-KEECMKK(6)
Smc1	851	Smc3	310	KDENEIEKLK(8)-AKDLQDELAGNSEQR(2)
Smc1	853	Smc3	936	LKKEEQR(2)-KEECMKK(1)
Smc1	874	Smc3	278	IIDETMAQLQDLKNQHLAK(13)-ELKTK(3)
Smc1	874	Smc3	285	IIDETMAQLQDLKNQHLAK(13)-ISAMKEEK(5)
Smc1	881	Smc3	278	KSEVNDK(1)-ELKTK(3)
Smc1	881	Smc3	285	KSEVNDK(1)-ISAMKEEK(5)
Smc1	881	Smc3	895	KSEVNDK(1)-SEDLNSIDKTEAGIKELQK(16)
Smc1	881	Smc3	899	KSEVNDKNHEMEEIR(1)-ELQKSMER(4)
Smc1	897	Smc3	252	KLGGANKEMTHLQK(1)-ETSGEKS(6)
Smc1	903	Smc3	245	LGGANKEMTHLQK(6)-LDELSAKR(7)
Smc1	903	Smc3	252	LGGANKEMTHLQK(6)-ETSGEKS(6)
Smc1	903	Smc3	905	LGGANKEMTHLQK(6)-WKNMEK(2)
Smc1	903	Smc3	909	LGGANKEMTHLQK(6)-NMEKEHMDAINHDTKELEK(4)
Smc1	903	Smc3	920	LGGANKEMTHLQK(6)-EHMDAINHDTKELEK(11)
Smc1	903	Smc3	924	LGGANKEMTHLQK(6)-ELEKMTNR(4)
Smc1	910	Smc3	245	EMTHLQKEVTAIETK(7)-LDELSAKR(7)
Smc1	910	Smc3	252	EMTHLQKEVTAIETK(7)-RETSGEKS(7)
Smc1	910	Smc3	920	EMTHLQKEVTAIETK(7)-EHMDAINHDTKELEK(11)
Smc1	910	Smc3	924	EMTHLQKEVTAIETK(7)-ELEKMTNR(4)
Smc1	918	Smc3	238	EVTAIETKLEQK(8)-AKLDELSAK(2)
Smc1	918	Smc3	245	EVTAIETKLEQK(8)-AKLDELSAKR(9)
Smc1	918	Smc3	934	EVTAIETKLEQK(8)-QGMLLKK(6)
Smc1	922	Smc3	245	LEQKR(4)-AKLDELSAKR(9)
Smc1	922	Smc3	285	LEQKR(4)-ISAMKEEKEQLSAER(5)
Smc1	922	Smc3	934	LEQKR(4)-QGMLLKK(6)
Smc1	922	Smc3	963	LEQKR(4)-YQTLSLKQLFR(7)
Smc1	934	Smc3	935	HNLLQACKMQDIK(8)-KKEECMK(1)
Smc1	1026	Smc3	207	IAAPNMKAMEK(7)-LHTLEEEKEELAQYQK(8)
Smc1	1026	Smc3	977	IAAPNMKAMEK(7)-KLEQCNTLKK(10)
Smc1	1026	Smc3	978	IAAPNMKAMEK(7)-KYSHVNKK(1)
Smc1	1026	Smc3	984	IAAPNMKAMEK(7)-YSHVNKK(6)
Smc1	1030	Smc3	188	AMEKLESVR(4)-EKINELLK(2)
Smc1	1037	Smc3	188	DKFQETSDEFEAAR(2)-EKINELLK(2)
Smc1	16	Scc1	387	SYKGR(3)-LLKLFTR(3)
Smc1	59	Scc1	387	VKTLR(2)-LLKLFTR(3)
Smc1	72	Scc1	387	DLIHGAPVGKPAANR(10)-LLKLFTR(3)
Smc1	106	Scc1	387	VIVGSSEYKINN(10)-LLKLFTR(3)
Smc1	173	Scc1	387	KEMVK(1)-LLKLFTR(3)
Smc1	190	Scc1	70	KNIAAER(1)-KAKYLLADCNEAFIK(1)
Smc1	880	Scc1	371	IIDETMAQLQDLKNQHLAKK(19)-ETGGVEKLFSLPAQPLWNNR(7)
Smc3	105	Scc1	387	VIGAKK(5)-LLKLFTR(3)
Smc3	106	Scc1	335	KDQYFLDKK(1)-ELDSKTIR(5)
Smc3	113	Scc1	335	KDQYFLDKK(8)-ELDSKTIR(5)
Smc3	185	Scc1	72	ETEGKR(5)-AKYLLADCNEAFIK(2)
Smc3	188	Scc1	86	EKINELLK(2)-IKMAFRPGVVDLPEENR(2)
Smc3	968	Scc1	387	KLEQCNTLKK(1)-LLKLFTR(3)
Smc3	1034	Scc1	50	YEAQLTFKQVSK(9)-VKMALR(2)
Smc3	1038	Scc1	50	QVSKNFSEVFQK(4)-VKMALR(2)
Smc1	106	SA1	555	VIVGSSEYKINN(10)-VLTAKER(5)
Smc1	146	SA1	558	NFLVFQGAVESIAMKNPK(15)-KTQIDDR(1)
Smc1	190	SA1	273	KNIAAER(1)-LELLQK(7)
Smc1	267	SA1	626	KKELGK(1)-FVVEKHVESDVLEACSK(5)
Smc1	413	SA1	1071	KKVETEA(1)-NSLVTTGGEDDRMSVNSGSSSSKTSVR(22)
Smc1	713	SA1	618	LKYSQSDLEQTK(2)-HLDALLKQIK(7)

Appendix

Smc1	1037	SA1	611	DKFQETSDEFEAAR(2)-MEKHL DALLK(3)
Smc3	157	SA1	257	LKLLR(2)-NKMIGK(2)
Smc3	194	SA1	52	INELLYIEER(6)-KSPGEK(1)
Smc3	612	SA1	1177	DTAYPETNDAIPMISKLR(16)-TGMNYMKVR(7)
Smc3	935	SA1	273	KKEECMK(1)-LELL LQKR(7)
Smc3	968	SA1	273	KLEQCNTLKK(1)-LELL LQKR(7)
SA1	52	Scc1	323	KSPGEK(1)-KLIVDSVK(1)
SA1	57	Scc1	317	KSPGEKSR(6)-ETKAK(3)
SA1	57	Scc1	323	KSPGEKSR(6)-KLIVDSVK(1)
SA1	555	Scc1	558	VLTAKE(5)-KTQIDDR(1)
SA1	916	Scc1	406	QIDKIQCAK(4)-KGGEADNLDEFLK(1)
SA1	1071	Scc1	323	NSLVTGGEDDRMSVNSGSSSKTSSVR(22)-KLIVDSVK(1)
SA1	1079	Scc1	323	KGRPPLHK(1)-KLIVDSVK(1)
SA1	1086	Scc1	323	GRPPLHKK(7)-KLIVDSVK(1)
SA1	1086	Scc1	335	GRPPLHKK(7)-ELDSKTIR(5)
SA1	1087	Scc1	317	KRVEDESLDNTWLN(1)-ETKAK(3)
SA1	1087	Scc1	323	KRVEDESLDNTWLN(1)-KLIVDSVK(1)
Nipbl	726	Scc1	359	SDGHPETPKQK(9)-KLMMWKETGGVEK(1)
Nipbl	794	Scc1	387	LKSER(2)-LLKL FTR(3)
Nipbl	802	Scc1	335	AEALKQRPDGR(5)-ELDSKTIR(5)
Nipbl	802	Scc1	387	AEALKQRPDGR(5)-LLKL FTR(3)
Nipbl	821	Scc1	335	DHDNKQK(5)-ELDSKTIR(5)
Nipbl	821	Scc1	387	DHDNKQK(5)-LLKL FTR(3)
Nipbl	857	Scc1	387	NEHGIKSDSSK(6)-LLKL FTR(3)
Nipbl	862	Scc1	387	SDSSKTDKLER(5)-LLKL FTR(3)
Nipbl	865	Scc1	335	TDKLER(3)-ELDSKTIR(5)
Nipbl	865	Scc1	387	TDKLER(3)-LLKL FTR(3)
Nipbl	896	Scc1	387	VKQGDSNK(2)-LLKL FTR(3)
Nipbl	907	Scc1	387	SDKLGFK(3)-LLKL FTR(3)
Nipbl	925	Scc1	387	TEGNKSK(5)-LLKL FTR(3)
Nipbl	927	Scc1	387	SKVDTNK(2)-LLKL FTR(3)
Nipbl	954	Scc1	364	SGALKNFVIPK(5)-LMMWKETGGVEK(5)
Nipbl	954	Scc1	387	SGALKNFVIPK(5)-LLKL FTR(3)
Nipbl	960	Scc1	323	NFVIPKIK(6)-KLIVDSVK(1)
Nipbl	960	Scc1	335	NFVIPKIK(6)-ELDSKTIR(5)
Nipbl	974	Scc1	387	DKDGNVTQETKK(11)-LLKL FTR(3)
Nipbl	988	Scc1	335	DKVEKIGLVEDLNK(5)-ELDSKTIR(5)
Nipbl	1007	Scc1	335	GAKPVVVLQKLSLDDVQK(10)-ELDSKTIR(5)
Nipbl	1029	Scc1	387	SSLKPIK(4)-LLKL FTR(3)
Nipbl	1034	Scc1	387	NKPSK(2)-LLKL FTR(3)
Nipbl	1548	Scc1	86	KCGSK(1)-IKMAFRPGVVDLPEENR(2)
Nipbl	1791	Scc1	72	TKAMK(2)-AKYLLADCNEAFIK(2)
Nipbl	1968	Scc1	317	SEEDSSYKPVKK(11)-ETKAK(3)
Nipbl	1968	Scc1	387	SEEDSSYKPVKK(11)-LLKL FTR(3)
Nipbl	2563	Scc1	387	NLCGFSDSKIQK(9)-LLKL FTR(3)
Nipbl	2574	Scc1	72	YSPSESAKVYDK(8)-AKYLLADCNEAFIK(2)
Nipbl	2574	Scc1	387	YSPSESAKVYDK(8)-LLKL FTR(3)
Nipbl	2578	Scc1	387	VYDKAINR(4)-LLKL FTR(3)
Nipbl	2783	Scc1	387	IALTSANKLTNK(8)-LLKL FTR(3)
Nipbl	110	Smc1	106	EKSMQNR(2)-VIVGGSSEYKINN(10)
Nipbl	110	Smc1	1222	EKSMQNR(2)-VLTFDLTKYPDANPNPNEQ(8)
Nipbl	289	Smc1	454	NAASFPLRSPQPVCSGSEGTPKGS(24)-KLEGELTEEVE-
Nipbl	289	Smc1	529	SPQPVCSGSEGTPKGS(16)-LIDLCQPTQKKYQIAVTK(11)
Nipbl	325	Smc1	414	QKKQK(3)-KVETAK(1)
Nipbl	333	Smc1	686	LGKDEK(3)-LTEELKEQMK(6)
Nipbl	500	Smc1	267	DKPLK(2)-KKELGK(1)
Nipbl	500	Smc1	453	DKPLK(2)-QSLEEQKK(7)
Nipbl	500	Smc1	675	DKPLK(2)-AVDKLKEK(6)

Appendix

Nipbl	566	Smc1	240	KPEEIK(1)-LYHNEVEIEKLNKELASK(13)
Nipbl	643	Smc1	763	LETKVETQTEELKQNESR(4)-EMKDLKEK(3)
Nipbl	643	Smc1	918	LETKVETQTEELKQNESR(4)-EMTHLQKEVTAIETKLEQK(15)
Nipbl	662	Smc1	106	TTECKQNESTIVEPK(5)-VIVGGSSEYKINN(10)
Nipbl	662	Smc1	713	TTECKQNESTIVEPK(5)-LKYSQSDLEQTK(2)
Nipbl	662	Smc1	844	TTECKQNESTIVEPK(5)-KDENEIEK(1)
Nipbl	687	Smc1	540	PNDNKQNNGR(5)-VLGKNMDAIIVDSEKTGR(4)
Nipbl	697	Smc1	106	SETTKSRPETPK(5)-VIVGGSSEYKINN(10)
Nipbl	704	Smc1	106	SRPETPKQK(7)-VIVGGSSEYKINN(10)
Nipbl	706 or 739	Smc1	106	QKGESRPETPK(2)-VIVGGSSEYKINN(10)
Nipbl	706 or 739	Smc1	1026	QKGESRPETPK(2)-IAAPNMKAMEK(7)
Nipbl	726	Smc1	896	SDGHPETPKQK(9)-NHEMEEIRKK(9)
Nipbl	770	Smc1	106	DSGKPSTEK(4)-VIVGGSSEYKINN(10)
Nipbl	775	Smc1	763	DSGKPSTEKK(9)-EMKDLK(3)
Nipbl	776	Smc1	213	PSTEKKPEVSK(6)-LKDEVVR(2)
Nipbl	787	Smc1	106	HKQDTKSDSPR(6)-VIVGGSSEYKINN(10)
Nipbl	821	Smc1	678	DHDNKQK(5)-EKKEK(3)
Nipbl	865	Smc1	245	TDKLERK(3)-LNKELASKNK(8)
Nipbl	954	Smc1	736	SGALKNFVIPK(5)-SKLESELANFGPR(2)
Nipbl	965	Smc1	106	DKDGNVTQETK(2)-VIVGGSSEYKINN(10)
Nipbl	965	Smc1	713	DKDGNVTQETK(2)-LKYSQSDLEQTK(2)
Nipbl	1000	Smc1	968	GAKPVVVLQK(3)-KLEQCNTLKK(1)
Nipbl	1037	Smc1	170	PSKSNK(3)-SGELAQEYDKRK(10)
Nipbl	1182	Smc1	589	KAYEPK(1)-ELKGAK(3)
Nipbl	1247	Smc1	251	HQLNELGSESAKIK(12)-EIEKDK(4)
Nipbl	1256	Smc1	589	AMGIMDKLSTDK(7)-ELKGAK(3)
Nipbl	1264	Smc1	606	TVKVLNILEK(3)-YEPPIKK(7)
Nipbl	1708	Smc1	854	DEESSEGTTHAKEIETTQIMHR(12)-KEEQR(1)
Nipbl	1891	Smc1	1056	ITEMCVKMIRR(7)-AKQAFEQIK(2)
Nipbl	1965	Smc1	528	SEEDSSYKPVK(8)-LIDLQCTQKK(10)
Nipbl	2170	Smc1	1120	VNIKDK(4)-NSSAQAFGLPENPEEPYLDGINYNVAPGKR(30)
Nipbl	2260	Smc1	1120	VAKQEDLK(3)-NSSAQAFGLPENPEEPYLDGINYNVAPGKR(30)
Nipbl	2337	Smc1	662	NKADQQLVEIDK(2)-SGVISGGASDLKAKAR(14)
Nipbl	2566	Smc1	189	IQKYSPSESAK(3)-KKNIAAER(1)
Nipbl	2574	Smc1	189	YSPSESAKVYDK(8)-KKNIAAER(1)
Nipbl	2566	Smc1	190	
Nipbl	2574	Smc1	185	YSPSESAKVYDK(8)-ETEGKR(5)
Nipbl	2574	Smc1	190	YSPSESAKVYDK(8)-KNIAAER(1)
Nipbl	2578	Smc1	190	VYDKAINRK(4)-KKNIAAER(2)
Nipbl	2611	Smc1	723	ITEEVKR(6)-LKYSQSDLEQTKTR(12)
Nipbl	2719	Smc1	213	YKDRPQIAR(2)-LKDEVVR(2)
Nipbl	2719	Smc1	606	YKDRPQIAR(2)-YEPPIKK(7)
Nipbl	2758	Smc1	213	KLVPWVDTIK(1)-LKDEVVR(2)
Nipbl	2783	Smc1	213	IALTSANKLTNK(8)-LKDEVVR(2)
Nipbl	2783	Smc1	922	IALTSANKLTNK(8)-LEQKR(4)
Nipbl	2783	Smc1	968	IALTSANKLTNK(8)-KLEQCNTLKK(1)
Nipbl	-10	Smc3	1207	SGGSDYKDDDDK(7)-VSHIDVITAEMAKDFVEDDTTHG(13)
Nipbl	500	Smc3	895	SEDLDNSIDKTEAGIKELQK(16)-DKPLK(2)
Nipbl	624	Smc3	968	LAESKPENR(5)-KLEQCNTLKK(1)
Nipbl	643	Smc3	889	LETKVETQTEELKQNESR(4)-SEDLDNSIDKTEAGIKELQK(10)
Nipbl	643	Smc3	895	LETKVETQTEELKQNESR(4)-SEDLDNSIDKTEAGIKELQK(16)
Nipbl	687	Smc3	459	LSDTKPNDNKQNNGR(10)-YYEVKNKK(5)
Nipbl	687	Smc3	751	LSDTKPNDNKQNNGR(10)-QQSEKTFMPK(5)
Nipbl	687	Smc3	899	LSDTKPNDNKQNNGR(10)-ELQKSMER(4)
Nipbl	697	Smc3	172	SETTKSRPETPK(5)-VYDERKEESISLMK(6)
Nipbl	717	Smc3	612	QKSDGHPETPK(2)-DTAYPETNDAIPMISKLR(16)
Nipbl	717	Smc3	751	QKSDGHPETPKQK(2)-QQSEKTFMPK(5)
Nipbl	775	Smc3	418	PSTEKK(5)-QIAAIHKDLEDTEANK(7)

Appendix

Nipbl	783	Smc3	612	HKQDTK(2)-DTAYPETNDAIPMISKLR(16)
Nipbl	787	Smc3	968	HKQDTKSDSPR(6)-KLEQCNTLKK(1)
Nipbl	802	Smc3	157	AEALKQRPDGR(5)-LKLLR(2)
Nipbl	802	Smc3	454	AEALKQRPDGR(5)-KYYEVK(1)
Nipbl	802	Smc3	968	AEALKQRPDGR(5)-KLEQCNTLKK(1)
Nipbl	821	Smc3	612	DHDNKQK(5)-DTAYPETNDAIPMISKLR(16)
Nipbl	857	Smc3	612	NEHGIKSDSSK(6)-DTAYPETNDAIPMISKLR(16)
Nipbl	862	Smc3	612	SDSSKTDKLER(5)-DTAYPETNDAIPMISKLR(16)
Nipbl	865	Smc3	157	TDKLER(3)-LKLLR(2)
Nipbl	887	Smc3	492	PSSGEQKSRPDSPR(7)-REDLEKK(6)
Nipbl	896	Smc3	612	VKQGDSNK(2)-DTAYPETNDAIPMISKLR(16)
Nipbl	907	Smc3	612	SDKLGFK(3)-DTAYPETNDAIPMISKLR(16)
Nipbl	907	Smc3	1194	SDKLGFK(3)-NKVSHIDVITAEMAK(2)
Nipbl	927	Smc3	612	SKVDTNK(2)-DTAYPETNDAIPMISKLR(16)
Nipbl	938	Smc3	968	AHPDNKAEFPSYLLGGR(6)-KLEQCNTLKK(1)
Nipbl	954	Smc3	157	SGALKNFVIPK(5)-LKLLR(2)
Nipbl	954	Smc3	427	SGALKNFVIPK(5)-DLEDTEANKEK(9)
Nipbl	954	Smc3	612	SGALKNFVIPK(5)-DTAYPETNDAIPMISKLR(16)
Nipbl	954	Smc3	621	SGALKNFVIPK(5)-FDKAFK(3)
Nipbl	954	Smc3	680	SGALKNFVIPK(5)-LELQKDVR(5)
Nipbl	954	Smc3	935	SGALKNFVIPK(5)-KKEECMK(1)
Nipbl	954	Smc3	1059	SGALKNFVIPK(5)-KGDVEGSQSQDEGESESER(1)
Nipbl	965	Smc3	968	DKDGNVTQETK(2)-KLEQCNTLKK(1)
Nipbl	974	Smc3	157	DKDGNVTQETTK(11)-LKLLR(2)
Nipbl	974	Smc3	612	DKDGNVTQETTK(11)-DTAYPETNDAIPMISKLR(16)
Nipbl	974	Smc3	935	DKDGNVTQETTK(11)-KKEECMK(1)
Nipbl	974	Smc3	968	DKDGNVTQETTK(11)-KLEQCNTLKK(1)
Nipbl	975	Smc3	612	KMEMK(1)-DTAYPETNDAIPMISKLR(16)
Nipbl	979	Smc3	999	
Nipbl	988	Smc3	935	DKVEKIGLVEDLNK(5)-KKEECMK(1)
Nipbl	1000	Smc3	106	GAKPVVVLQK(3)-KDQYFLDKK(1)
Nipbl	1000	Smc3	140	GAKPVVVLQK(3)-SNPYIVKQGK(8)
Nipbl	1000	Smc3	157	GAKPVVVLQK(3)-LKLLR(2)
Nipbl	1000	Smc3	612	GAKPVVVLQK(3)-DTAYPETNDAIPMISKLR(16)
Nipbl	1000	Smc3	1059	GAKPVVVLQK(3)-KGDVEGSQSQDEGESESER(1)
Nipbl	1007	Smc3	105	GAKPVVVLQKSLDDVQK(10)-VIGAKK(5)
Nipbl	1015	Smc3	105	LSLDDVQKLIK(8)-VIGAKK(5)
Nipbl	1015	Smc3	968	LSLDDVQKLIK(8)-KLEQCNTLKK(1)
Nipbl	1018	Smc3	140	LIKDREDK(3)-SNPYIVKQGK(8)
Nipbl	1018	Smc3	157	LIKDREDK(3)-LKLLR(2)
Nipbl	1018	Smc3	612	LIKDREDK(3)-DTAYPETNDAIPMISKLR(16)
Nipbl	1023	Smc3	188	DREDKSR(5)-EKINELLK(2)
Nipbl	1029	Smc3	140	SSLKPIK(4)-SNPYIVKQGK(8)
Nipbl	1029	Smc3	188	SSLKPIK(4)-EKINELLK(2)
Nipbl	1034	Smc3	157	NKPSK(2)-LKLLR(2)
Nipbl	1034	Smc3	1194	NKPSKSNK(2)-NKVSHIDVITAEMAK(2)
Nipbl	1037	Smc3	140	NKPSKSNK(5)-SNPYIVKQGK(8)
Nipbl	1037	Smc3	157	NKPSKSNK(5)-LKLLR(2)
Nipbl	1037	Smc3	612	NKPSKSNK(5)-DTAYPETNDAIPMISKLR(16)
Nipbl	1040	Smc3	105	SNKGSIDQSVLK(3)-VIGAKK(5)
Nipbl	1040	Smc3	157	SNKGSIDQSVLK(3)-LKLLR(2)
Nipbl	1040	Smc3	188	SNKGSIDQSVLK(3)-EKINELLK(2)
Nipbl	1040	Smc3	935	SNKGSIDQSVLK(3)-KKEECMK(1)
Nipbl	1070	Smc3	140	VKMNR(2)-SNPYIVKQGK(8)
Nipbl	1070	Smc3	157	VKMNR(2)-LKLLR(2)
Nipbl	1082	Smc3	105	STVNEKPK(6)-VIGAKK(5)
Nipbl	1082	Smc3	106	STVNEKPK(6)-KDQYFLDKK(1)
Nipbl	1082	Smc3	140	STVNEKPK(6)-SNPYIVKQGK(8)

Appendix

Nipbl	1082	Smc3	157	STVNEKPK(6)-LKLLR(2)
Nipbl	1082	Smc3	185	STVNEKPK(6)-ETEGKR(5)
Nipbl	1082	Smc3	188	STVNEKPK(6)-EKINELLK(2)
Nipbl	1182	Smc3	106	KAYEPK(1)-KDQYFLDKK(1)
Nipbl	1182	Smc3	140	KAYEPK(1)-SNPYYIVKQGK(8)
Nipbl	1182	Smc3	157	KAYEPK(1)-LKLLR(2)
Nipbl	1200	Smc3	105	LTPEEMMDSSTFKR(13)-VIGAKK(5)
Nipbl	1200	Smc3	106	LTPEEMMDSSTFKR(13)-KDQYFLDK(1)
Nipbl	1200	Smc3	113	LTPEEMMDSSTFKR(13)-DQYFLDKK(7)
Nipbl	1256	Smc3	106	AMGIMDKLSTDK(7)-KDQYFLDK(1)
Nipbl	1261	Smc3	106	LSTDKTvk(5)-KDQYFLDK(1)
Nipbl	1261	Smc3	113	LSTDKTvk(5)-KDQYFLDKK(8)
Nipbl	1264	Smc3	105	TVKVLNILEK(3)-VIGAKK(5)
Nipbl	1264	Smc3	106	TVKVLNILEK(3)-KDQYFLDKK(1)
Nipbl	1264	Smc3	606	LSTDKTvkVLNILEK(8)-YEPPIKK(7)
Nipbl	1264	Smc3	113	TVKVLNILEK(3)-KDQYFLDKK(8)
Nipbl	1264	Smc3	114	TVKVLNILEK(3)-KMTVK(1)
Nipbl	1278	Smc3	188	NIQDGSKLSTLLNHNNDTEEEER(7)-EKINELLK(2)
Nipbl	1369	Smc3	106	LDPHGGGLSSKAK(12)-KDQYFLDKK(1)
Nipbl	1369	Smc3	172	LDPHGGGLSSKAK(12)-KEESISLMK(1)
Nipbl	1369	Smc3	180	LDPHGGGLSSKAKR(12)-KEESISLMKETEGK(9)
Nipbl	1369	Smc3	188	LDPHGGGLSSKAK(12)-EKINELLK(2)
Nipbl	1369	Smc3	985	LDPHGGGLSSKAK(12)-KALDQFVNFSEQK(1)
Nipbl	1379	Smc3	188	CSTHKQR(5)-EKINELLK(2)
Nipbl	1447	Smc3	985	YEKHR(3)-KALDQFVNFSEQK(1)
Nipbl	1622	Smc3	105	KDAVTSK(1)-VIGAKK(5)
Nipbl	1622	Smc3	106	KDAVTSK(1)-KDQYFLDK(1)
Nipbl	1622	Smc3	185	KDAVTSK(1)-ETEGKR(5)
Nipbl	1696	Smc3	1772	SQKDEESSEGETHAK(3)-KKFLR(1)
Nipbl	1732	Smc3	157	SIKTTSPSQFSTLK(4)-LKLLR(2)
Nipbl	1791	Smc3	185	TKAMK(2)-ETEGKR(5)
Nipbl	1870	Smc3	105	VIKILR(3)-VIGAKK(5)
Nipbl	1903	Smc3	105	VNDEEGIKK(8)-VIGAKK(5)
Nipbl	1903	Smc3	106	VNDEEGIKK(8)-KDQYFLDKK(1)
Nipbl	2256	Smc3	140	MQQADRDWKK(9)-SNPYYIVKQGK(8)
Nipbl	2347	Smc3	106	NKADQQLVEIDKK(12)-KDQYFLDKK(1)
Nipbl	2474	Smc3	288	ESMVKDKR(5)-EEKEQLSAER(3)
Nipbl	2563	Smc3	188	NLCGFSDSKIQK(9)-EKINELLK(2)
Nipbl	2566	Smc3	188	IQKYSSESASAK(3)-EKINELLK(2)
Nipbl	2574	Smc3	188	YSPSESASVYDK(8)-EKINELLK(2)
Nipbl	2611	Smc3	340	ITEEVKR(6)-IEEKQK(4)
Nipbl	2719	Smc3	188	YKDRPQIAR(2)-EKINELLK(2)
Nipbl	2719	Smc3	968	YKDRPQIAR(2)-KLEQCNTLKK(1)
Nipbl	2719	Smc3	984	YKDRPQIAR(2)-YSHVNKK(6)
Nipbl	2758	Smc3	968	KLVPWVDTIK(1)-KLEQCNTLKK(1)
Nipbl	2783	Smc3	984	IALTSANKLTNK(8)-YSHVNKK(6)
Nipbl	2783	Smc3	985	IALTSANKLTNK(8)-KALDQFVNFSEQKEK(1)
Nipbl	2337	Smc3	105	NKADQQLVEIDKK(2)-VIGAKK(5)
Nipbl	2787	Smc3	188	LTNKVVQTLR(4)-EKINELLK(2)
Nipbl	2787	Smc3	207	LTNKVVQTLR(4)-LHTLEEEKEELAQYQK(8)
Nipbl	2787	Smc3	935	LTNKVVQTLR(4)-KKEECMK(1)
Nipbl	2578	Smc3	188	VYDKAINRK(4)-EKINELLK(2)
Nipbl	-46	SA1	48	SAWSHPQFEK(1)-PPSTNKK(6)
Nipbl	-46	SA1	1078	SAWSHPQFEK(1)-NKKGR(2)
Nipbl	110	SA1	273	EKSMQNR(2)-LELLQKR(7)
Nipbl	333	SA1	366	LGKDEKEQSEK(3)-ELFPKLELFTNR(5)
Nipbl	336	SA1	366	LGKDEKEQSEK(6)-ELFPKLELFTNR(5)
Nipbl	706 or 739	SA1	273	QKGESRPETPK(2)-LELLQKR(7)

Appendix

Nipbl	715 or 748	SA1	618	GESRPETPKQK(9)-HLDALLKQIK(7)
Nipbl	737	SA1	273	GDGRPETPKQK(9)-LELLQKR(7)
Nipbl	770	SA1	273	DSGKPSTEK(4)-LELLQKR(7)
Nipbl	776	SA1	273	KPEVSK(1)-LELLQKR(7)
Nipbl	783	SA1	273	HKQDTK(2)-LELLQKR(7)
Nipbl	783	SA1	626	HKQDTK(2)-FVVEKHVESDVLEACSK(5)
Nipbl	794	SA1	273	LKSER(2)-LELLQKR(7)
Nipbl	794	SA1	611	LKSER(2)-MEKHL DALLK(3)
Nipbl	794	SA1	618	LKSER(2)-HLDALLKQIK(7)
Nipbl	794	SA1	626	LKSER(2)-FVVEKHVESDVLEACSK(5)
Nipbl	821	SA1	626	DHDNKQK(5)-FVVEKHVESDVLEACSK(5)
Nipbl	821	SA1	273	DHDNKQK(5)-LELLQKR(7)
Nipbl	821	SA1	905	DHDNKQK(5)-YYNDYGDIKETLSK(10)
Nipbl	821	SA1	979	DHDNKQK(5)-EAVATLHKDGIEFAFK(8)
Nipbl	857	SA1	618	NEHGIKSDSSK(6)-HLDALLKQIK(7)
Nipbl	857	SA1	905	NEHGIKSDSSK(6)-YYNDYGDIKETLSK(10)
Nipbl	862	SA1	273	SDSSKTDKLER(5)-LELLQKR(7)
Nipbl	862	SA1	618	SDSSKTDKLER(5)-HLDALLKQIK(7)
Nipbl	862	SA1	626	SDSSKTDKLER(5)-FVVEKHVESDVLEACSK(5)
Nipbl	862	SA1	905	SDSSKTDKLER(5)-YYNDYGDIKETLSK(10)
Nipbl	865	SA1	273	TDKLER(3)-LELLQKR(7)
Nipbl	896	SA1	273	VKQGDSNK(2)-LELLQKR(7)
Nipbl	896	SA1	905	VKQGDSNK(2)-YYNDYGDIKETLSK(10)
Nipbl	907	SA1	438	SDKLGFK(3)-KLFSR(1)
Nipbl	907	SA1	273	SDKLGFK(3)-LELLQKR(7)
Nipbl	925	SA1	273	TEGNKSK(5)-LELLQKR(7)
Nipbl	925	SA1	905	TEGNKSK(5)-YYNDYGDIKETLSK(10)
Nipbl	927	SA1	273	SKVDTNK(2)-LELLQKR(7)
Nipbl	927	SA1	626	SKVDTNK(2)-FVVEKHVESDVLEACSK(5)
Nipbl	927	SA1	905	SKVDTNK(2)-YYNDYGDIKETLSK(10)
Nipbl	954	SA1	261	SGALKNFVIPK(5)-MIGKR(4)
Nipbl	960	SA1	438	NFVIPK(6)-KLFSR(1)
Nipbl	965	SA1	626	DKDGNVTQETK(2)-FVVEKHVESDVLEACSK(5)
Nipbl	965	SA1	905	DKDGNVTQETK(2)-YYNDYGDIKETLSK(10)
Nipbl	1037	SA1	273	NKPSKSNK(5)-LELLQKR(7)
Nipbl	1070	SA1	273	VKMNKR(2)-LELLQKR(7)
Nipbl	1374	SA1	273	AKCSTHK(2)-LELLQKR(7)
Nipbl	1968	SA1	555	SEEDSSYPVKK(11)-VLTAKER(5)
Nipbl	1968	SA1	558	SEEDSSYPVKK(11)-KTQIDDR(1)
Nipbl	1968	SA1	1020	SEEDSSYPVKK(11)-KTVHSYLEK(1)
Nipbl	974	SA1	611	DKDGNVTQETTK(11)-MEKHL DALLK(3)
Nipbl	974	SA1	626	DKDGNVTQETTK(11)-FVVEKHVESDVLEACSK(5)
Nipbl	974	SA1	905	DKDGNVTQETTK(11)-YYNDYGDIKETLSK(10)
Nipbl	975	SA1	905	KMEMK(1)-YYNDYGDIKETLSK(10)
Nipbl	983	SA1	273	GEPKDKVEK(4)-LELLQKR(7)
Nipbl	983	SA1	905	GEPKDKVEK(4)-YYNDYGDIKETLSK(10)
Nipbl	985	SA1	273	DKVEK(2)-LELLQKR(7)
Nipbl	985	SA1	905	DKVEK(2)-YYNDYGDIKETLSK(10)
Nipbl	1007	SA1	257	GAKPVVVLQKLSLDDVQK(10)-NKMIGK(2)
Nipbl	1029	SA1	273	SSLKPIK(4)-LELLQKR(7)
Nipbl	1029	SA1	905	SSLKPIK(4)-YYNDYGDIKETLSK(10)
Nipbl	1034	SA1	273	NKPSK(2)-LELLQKR(7)
Nipbl	1040	SA1	273	SNKGSIDQSVLK(3)-LELLQKR(7)
Nipbl	1082	SA1	273	STVNEKPK(6)-LELLQKR(7)
Nipbl	1182	SA1	273	KAYEPK(1)-LELLQKR(7)
Nipbl	1622	SA1	618	KDAVTSK(1)-HLDALLKQIK(7)
Nipbl	1791	SA1	618	TKAMK(2)-HLDALLKQIK(7)
Nipbl	1791	SA1	453	TKAMK(2)-HDPQAEALAKR(11)

Appendix

Nipbl	1791	SA1	611	TKAMK(2)-MEKHL DALLK(3)
Nipbl	1791	SA1	618	TKAMK(2)-HLDALLKQIK(7)
Nipbl	1904	SA1	555	KLVNETFQK(1)-VLTAKER(5)
Nipbl	2260	SA1	273	VAKQEDLK(3)-LELLQKR(7)
Nipbl	2574	SA1	273	YSPSESAKVYDK(8)-LELLQKR(7)

* For simplicity, Nipbls N-terminal Twin-strep-flag-linker (48 amino acids) was subtracted from the residue number.

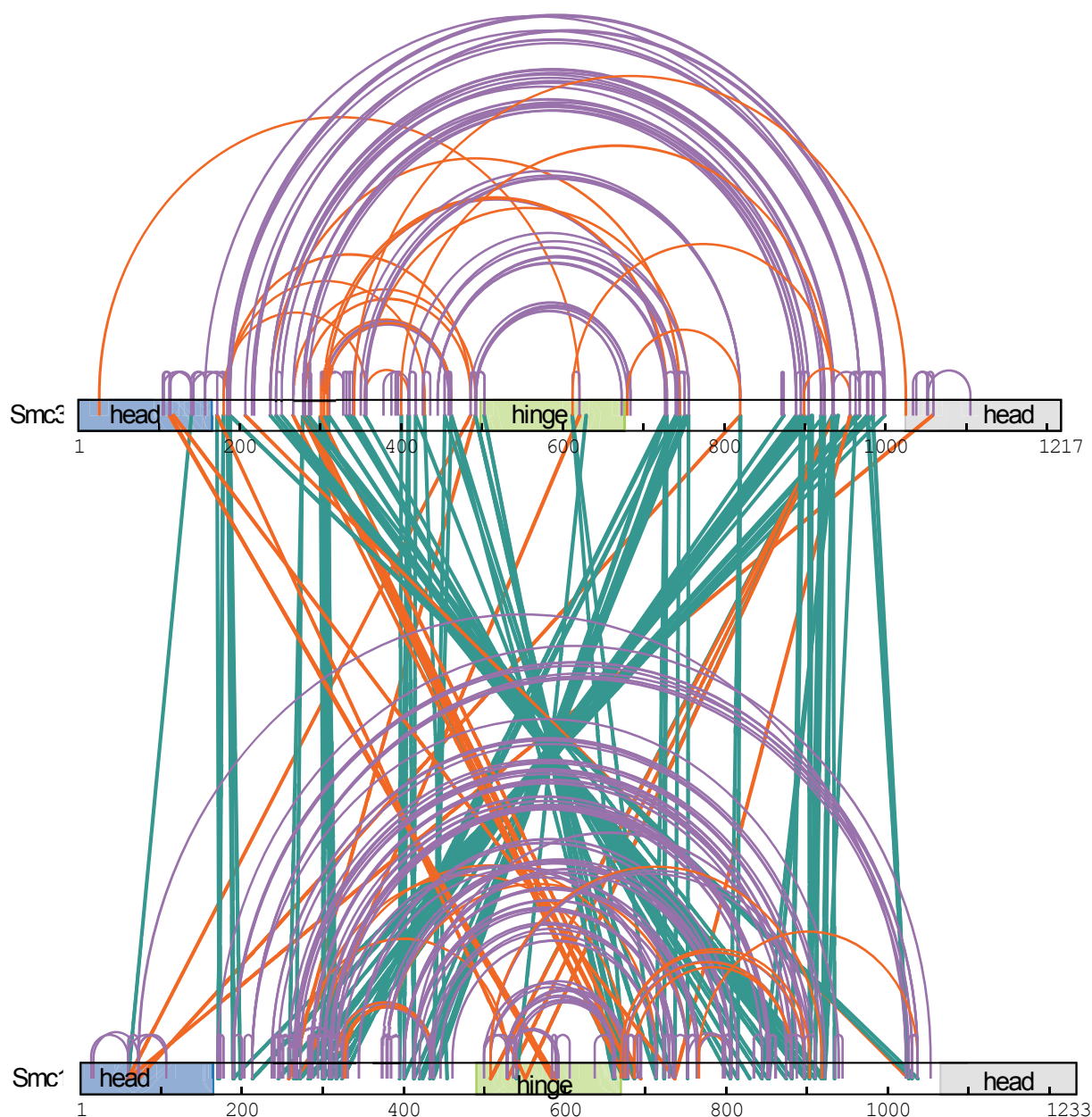


Figure 29: Proximity map of all Smc1 and Smc3 intra and interprotein cross-links from Nipbl:Cohesin XLMS, exp. 1.

Smc1 and Smc3 intra (purple) and interprotein (green) cross-links. Cross-links that are not in agreement with a rod shaped coiled-coil formation, but rather indicate a back-folded structure are depicted in orange.

Appendix

Table 13: Cross-links with a CSM value, greater than 5 from Nipbl:Cohesin cross-link experiment 1*.

Protein 1	Residue 1	Protein 2	Residue2	CSM Count
Nipbl	1200	Smc3	106	13
Nipbl	1256	Smc3	106	6
Nipbl	1261	Smc3	106	6
Nipbl	1264	Smc3	106	6
Nipbl	1622	Smc3	106	7
Nipbl	1200	Smc3	113	4
Nipbl	1264	Smc3	114	4
Nipbl	1278	Smc3	188	6
Nipbl	1369	Smc3	188	6

* For simplicity, Nipbls N-terminal Twin-strep-flag-linker (48 amino acids) was subtracted from the residue number.

Table 14: Smc3 and Smc1 intra and interprotein cross-links with a CSM value greater than 9 from Nipbl:Cohesin cross-link exp. 1*.

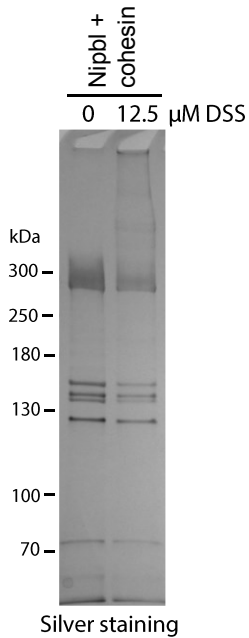
Protein1	Residue1	Protein2	Residue2	CSM count	Location
Smc3	956	SMC3	968	85	
Smc1	72	Smc3	820	15	coiled-coil
Smc3	238	Smc3	935	14	
Smc3	245	Smc3	252	11	
Smc1	264	Smc1	267	11	
Smc3	285	Smc3	278	15	
Smc1	300	Smc3	874	47	
Smc1	300	Smc1	308	27	
Smc3	308	Smc3	729	13	coiled-coil
Smc3	310	Smc1	300	16	
Smc3	336	Smc3	328	13	
Smc3	340	Smc3	332	10	
Smc3	350	Smc3	820	35	
Smc3	357	Smc3	820	18	
Smc3	400	Smc3	393	15	
Smc3	400	Smc3	396	10	
Smc1	437	Smc3	729	31	
Smc1	437	Smc3	727	20	
Smc1	437	Smc1	324	10	coiled-coil
Smc3	445	Smc3	743	38	
Smc1	446	Smc1	713	37	
Smc1	446	Smc1	723	12	
Smc3	454	Smc3	461	15	
Smc3	459	Smc3	308	15	coiled-coil
Smc3	461	Smc3	454	21	
Smc3	462	Smc3	304	11	coiled-coil
Smc3	462	Smc3	459	11	
Smc1	551	Smc3	936	20	hinge to head
Smc1	551	Smc3	935	14	
Smc1	675	Smc3	492	24	
Smc1	677	Smc1	673	14	
Smc3	740	Smc1	324	18	coiled-coil
Smc3	820	Smc3	680	52	coiled-coil
Smc2	851	Smc1	854	10	
Smc1	853	Smc1	316	18	

Appendix

Smc1	861	Smc1	854	14	
Smc1	874	Smc1	881	41	
Smc3	874	Smc3	285	10	
Smc1	881	Smc3	285	16	
Smc3	889	Smc3	280	19	
Smc3	895	Smc1	267	19	
Smc3	895	Smc3	278	11	
Smc3	895	Smc3	285	10	
Smc3	899	Smc1	267	15	
Smc3	920	Smc1	903	11	
Smc3	963	Smc3	968	43	
Smc3	977	Smc3	984	12	
Smc3	984	Smc1	213	25	
Smc3	1059	Smc3	1034	10	
Smc3	1105	Smc3	1052	14	

- Cross-links that indicate flexible coiled-coil domain are marked in yellow.

A



B

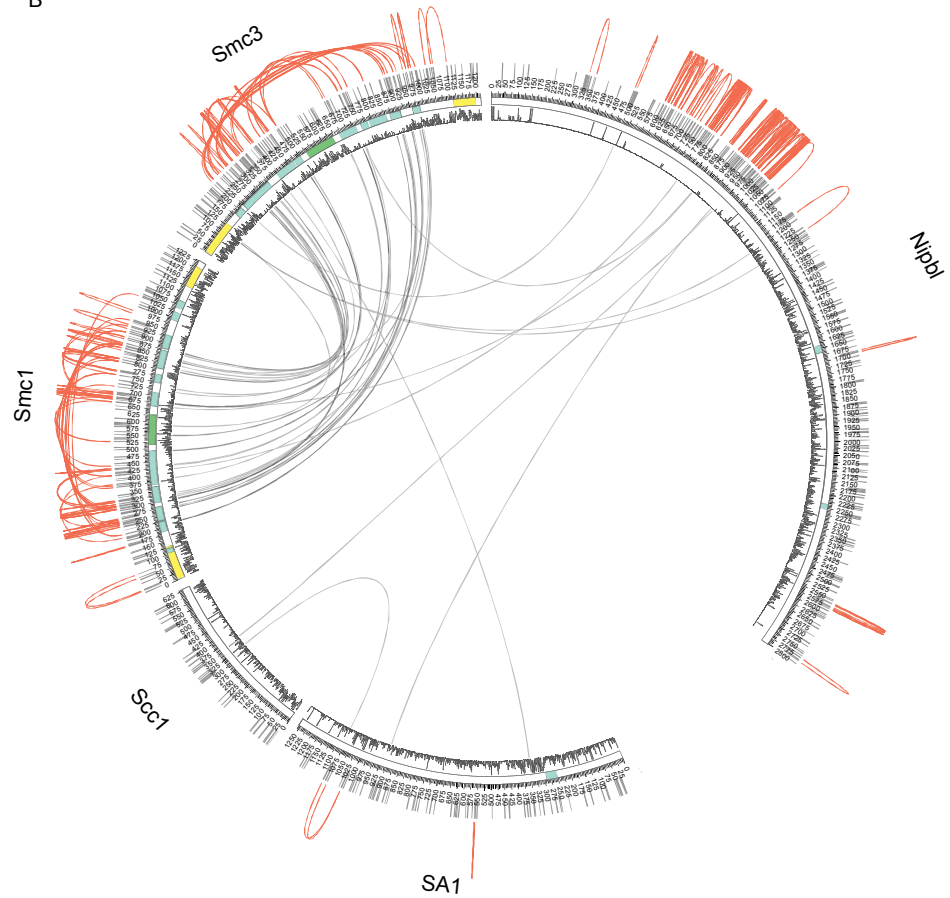


Figure 30: Nipbl potentially contacts all four Cohesin subunits (exp. 2).

(A) Cross-linking mass spectrometry experiment to detect protein-protein interactions between Nipbl and Cohesin subunits. Silver stained SDS PAGE of Nipbl bound to Cohesin in solution before and after incubation with the cross-linking reagent DSS. (B) Proximity map of all intra- (red) and interprotein (grey) links Cohesin:Nipbl, experiment 2 (n=188). All potential lysine cross-linking sites are designated by grey sticks and conservation is blotted. Important domains in Smc1/3 are marked: green hinge domain, yellow head domains, light green coiled-coil domain.

Table 15: List of intraprotein cross-links Nipbl:Cohesin (exp. 2).*

Protein1	Residue 1	Protein2	Residue 2	Peptide sequence
Smc1	13	Smc1	59	LIEIENFKSYK(8)-VKTLR(2)
Smc1	16	Smc1	72	SYKGR(3)-DLIHGAPVGKPAANR(10)
Smc1	203	Smc1	414	EAKQEKEEADR(6)-KVETEA(1)
Smc1	213	Smc1	939	MQDIKLPLSK(5)-LKDEVVR(2)
Smc1	213	Smc1	1026	LKDEVVR(2)-IAAPNMKAMEK(7)
Smc1	245	Smc1	918	ELASKNK(5)-EVTAIETKLEQK(8)
Smc1	251	Smc1	258	EIEKDK(4)-MDKVEDELK(3)
Smc1	253	Smc1	258	EIEKDKK(6)-MDKVEDELK(3)
Smc1	258	Smc1	384	RMDKVEDELK(4)-EEASKR(5)
Smc1	258	Smc1	896	RMDKVEDELK(4)-KKLGGANK(1)
Smc1	258	Smc1	897	MDKVEDELK(3)-KLGGANK(1)
Smc1	300	Smc1	311	RPQYIKAK(6)-KLEAAK(1)
Smc1	300	Smc1	851	RPQYIKAK(6)-KDENEIEKLK(8)
Smc1	302	Smc1	853	AKENTSHK(2)-LKKEEQ(2)
Smc1	308	Smc1	844	AKENTSHK(8)-KDENEIEK(1)
Smc1	308	Smc1	851	ENTSHK(6)-KDENEIEKLK(8)
Smc1	311	Smc1	844	KLEAAK(1)-KDENEIEK(1)
Smc1	316	Smc1	853	KLEAAKK(6)-LKKEEQ(2)
Smc1	317	Smc1	437	KSLQNAQK(1)-IEKLEEYITTSK(3)
Smc1	324	Smc1	433	SLQNAQKHYK(7)-LREIEENQKR(9)
Smc1	324	Smc1	437	SLQNAQKHYK(7)-IEKLEEYITTSK(3)
Smc1	327	Smc1	330	SLQNAQKHYKK(10)-KGDMDELEK(1)
Smc1	379	Smc1	805	LKEEASK(2)-RQNEIAKK(7)
Smc1	413	Smc1	752	KKVETEA(1)-INDIKR(5)
Smc1	414	Smc1	752	KVETEA(1)-INDIKR(5)
Smc1	446	Smc1	713	LEEYITTSKQSLQEEQK(9)-LKYSQSDLEQTK(2)
Smc1	528	Smc1	660	LIDLQPTQKK(10)-SGVISGGASDLKAK(12)
Smc1	528	Smc1	673	LIDLQPTQKK(10)-AVDKLK(4)
Smc1	660	Smc1	673	SGVISGGASDLKAK(12)-AVDKLK(4)
Smc1	669	Smc1	881	WDEKAVDK(4)-KSEVNDK(1)
Smc1	669	Smc1	897	WDEKAVDK(4)-KLGGANK(1)
Smc1	690	Smc1	1037	EQMKAKR(4)-DKFQETSDEFEAARK(2)
Smc1	752	Smc1	806	INDIKR(5)-KRLEFENQK(1)
Smc1	763	Smc1	806	EREMKDLK(5)-KRLEFENQK(1)
Smc1	766	Smc1	796	DLKEK(3)-NIREFEEVK(9)
Smc1	766	Smc1	806	DLKEK(3)-KRLEFENQK(1)
Smc1	851	Smc1	310	KDENEIEKLK(8)-AKDLQDELGNSEQR(2)
Smc3	207	Smc3	968	LHTLEEEKEELAQYQKWDK(8)-KLEQCNTLKK(1)
Smc3	215	Smc3	968	EELAQYQKWDK(8)-KLEQCNTLKK(1)
Smc3	218	Smc3	984	WDKMR(3)-YSHVNNK(6)
Smc3	218	Smc3	999	WDKMR(3)-EKLK(2)
Smc3	238	Smc3	924	AKLDELSAK(2)-ELEKMTNR(4)
Smc3	238	Smc3	935	AKLDELSAK(2)-KKEECMK(1)
Smc3	245	Smc3	924	LDELSAKR(7)-ELEKMTNR(4)
Smc3	244	Smc3	492	DKMEDIER(2)-REDLEKK(6)
Smc3	278	Smc3	895	ELKTK(3)-SEDLDNSIDKTEAGIKELQK(16)
Smc3	278	Smc3	905	ELKTK(3)-WKNMEK(2)
Smc3	280	Smc3	889	TKISAMK(2)-SEDLDNSIDKTEAGIK(10)
Smc3	285	Smc3	895	ISAMKEEK(5)-SEDLDNSIDKTEAGIKELQK(16)
Smc3	304	Smc3	462	TKLELK(2)-KDELQSER(1)
Smc3	304	Smc3	459	TKLELK(2)-YYEVKNKK(5)
Smc3	308	Smc3	459	LELKAK(4)-YYEVKNK(5)
Smc3	308	Smc3	727	TKLELKAK(6)-KFKASR(1)
Smc3	308	Smc3	729	LELKAK(4)-FKASR(2)
Smc3	328	Smc3	336	RLLKER(4)-LLEKIEEK(4)

Appendix

Smc3	340	Smc3	820	IEEKQK(4)-IKLEGIITR(2)
Smc3	350	Smc3	820	ELAETEPKFNSVK(8)-IKLEGIITR(2)
Smc3	357	Smc3	820	EKEER(2)-IKLEGIITR(2)
Smc3	427	Smc3	743	DLEDTEANKEK(9)-MLKEK(3)
Smc3	445	Smc3	743	LDQDLNEVKAR(9)-MLKEK(3)
Smc3	492	Smc3	673	REDLEKK(6)-KSRLELQK(1)
Smc3	493	Smc3	680	KQQLLR(1)-LELQKDVR(5)
Smc3	956	Smc3	968	ELGSLPQEAFEKYQTLCLK(12)-KLEQCNTCLK(1)
Smc3	1034	Smc3	1059	KYEAQLTFKQVSK(10)-KGDVEGSQSQDEGESESER(1)
Smc3	1052	Smc3	1105	LVPGGKATLVCK(6)-VSFTGKQGEMR(6)
SA1	1020	SA1	1071	KTVHSYLEK(1)-MSVNSGSSSSKTSVR(11)
SA1	1020	SA1	1086	KTVHSYLEK(1)-GRPPLHKK(7)
Nipbl	333	Nipbl	357	LGKDEK(3)-DSTKLTLR(4)
Nipbl	614	Nipbl	634	SEMKQSESR(4)-LVETKSSNKLETK(5)
Nipbl	624	Nipbl	639	LAESKPENR(5)-SSNKLETK(5)
Nipbl	624	Nipbl	652	LAESKPENR(5)-VETQTEELKQNESR(9)
Nipbl	624	Nipbl	662	LAESKPENR(5)-TTECKQNESTIVEPK(5)
Nipbl	634	Nipbl	652	LVETKSSNKLETK(5)-VETQTEELKQNESR(9)
Nipbl	639	Nipbl	652	SSNKLETK(5)-VETQTEELKQNESR(9)
Nipbl	662	Nipbl	682	TTECKQNESTIVEPK(5)-LSDTKPNDNK(5)
Nipbl	662	Nipbl	697	TTECKQNESTIVEPK(5)-SETTKSRPETPK(5)
Nipbl	682	Nipbl	697	LSDTKPNDNK(5)-SETTKSRPETPK(5)
Nipbl	682	Nipbl	704	LSDTKPNDNK(5)-SRPETPKQK(7)
Nipbl	715	Nipbl	759	GESRPETPKQK(9)-NEGRPETPKHR(9)
Nipbl	717	Nipbl	2566	QKSDGHPETPK(2)-IQKYSPSESAK(3)
Nipbl	759	Nipbl	770	NEGRPETPKHR(9)-DSGKPSTK(4)
Nipbl	759	Nipbl	776	NEGRPETPKHR(9)-KPEVSK(1):0
Nipbl	759	Nipbl	783	NEGRPETPKHR(9)-HKQDTK(2)
Nipbl	759	Nipbl	787	NEGRPETPKHR(9)-HKQDTKSDSPR(6)
Nipbl	759	Nipbl	794	NEGRPETPKHR(9)-LKSER(2)
Nipbl	770	Nipbl	783	DSGKPSTK(4)-HKQDTK(2)
Nipbl	776	Nipbl	794	KPEVSK(1)-LKSER(2)
Nipbl	776	Nipbl	802	KPEVSK(1)-AEALKQRPDGR(5)
Nipbl	776	Nipbl	821	KPEVSK(1)-DHDNKQK(5)
Nipbl	781	Nipbl	794	KPEVSKHK(6)-LKSER(2)
Nipbl	781	Nipbl	802	KPEVSKHK(6)-AEALKQRPDGR(5)
Nipbl	783	Nipbl	794	HKQDTK(2)-LKSER(2)
Nipbl	783	Nipbl	802	HKQDTK(2)-AEALKQRPDGR(5)
Nipbl	783	Nipbl	821	HKQDTK(2)-DHDNKQK(5)
Nipbl	787	Nipbl	794	HKQDTKSDSPR(6)-LKSER(2)
Nipbl	787	Nipbl	802	HKQDTKSDSPR(6)-AEALKQRPDGR(5)
Nipbl	794	Nipbl	802	LKSER(2)-AEALKQRPDGR(5)
Nipbl	794	Nipbl	821	LKSER(2)-DHDNKQK(5)
Nipbl	802	Nipbl	821	AEALKQRPDGR(5)-DHDNKQK(5)
Nipbl	857	Nipbl	869	NEHGIKSDSSK(6)-KRRHESGDSR(1)
Nipbl	896	Nipbl	907	VKQGDSNK(2)-SDKLGFK(3)
Nipbl	896	Nipbl	911	VKQGDSNK(2)-LGFKSPTSK(4)
Nipbl	896	Nipbl	927	VKQGDSNK(2)-SKVDTNK(2)
Nipbl	896	Nipbl	954	VKQGDSNK(2)-SGALKNFVIPK(5)
Nipbl	907	Nipbl	925	SRSDKLGFK(5)-RTEGNKSK(6)
Nipbl	911	Nipbl	927	LGFKSPTSK(4)-SKVDTNK(2)
Nipbl	916	Nipbl	925	SPTSKDDK(5)-TEGNKSKVDTNK(5)
Nipbl	916	Nipbl	927	SPTSKDDK(5)-SKVDTNK(2)
Nipbl	919	Nipbl	927	SPTSKDDKR(8)-SKVDTNK(2)
Nipbl	925	Nipbl	938	RTEGNKSK(6)-AHPDNKAEFPSYLLGGR(6)
Nipbl	927	Nipbl	954	SKVDTNK(2)-SGALKNFVIPK(5)
Nipbl	927	Nipbl	960	SKVDTNK(2)-NFVIPKIK(6)
Nipbl	927	Nipbl	965	SKVDTNK(2)-DKDGNVTQETK(2)

Appendix

Nipbl	938	Nipbl	965	AHPDNKAEFPSYLLGGR(6)-DKDGNVTQETK(2)
Nipbl	938	Nipbl	974	AHPDNKAEFPSYLLGGR(6)-DGNVTQETKK(9)
Nipbl	954	Nipbl	974	SGALKNFVIPK(5)-DGNVTQETKK(9)
Nipbl	954	Nipbl	985	SGALKNFVIPK(5)-DKVEK(2)
Nipbl	960	Nipbl	965	NFVIPKIK(6)-DKDGNVTQETK(2)
Nipbl	960	Nipbl	974	NFVIPKIK(6)-DGNVTQETKK(9)
Nipbl	960	Nipbl	985	NFVIPKIK(6)-DKVEK(2)
Nipbl	965	Nipbl	985	DKDGNVTQETK(2)-DKVEK(2)
Nipbl	974	Nipbl	988	DKVEKIGLVEDLNK(5)-DGNVTQETKK(9)
Nipbl	974	Nipbl	1000	DKDGNVTQETKK(11)-GAKPVVVLQK(3)
Nipbl	975	Nipbl	985	KMEMK(1)-DKVEK(2)
Nipbl	985	Nipbl	1007	DKVEK(2)-GAKPVVVLQKLSLDDVQK(10)
Nipbl	988	Nipbl	1040	DKVEKIGLVEDLNK(5)-SNKGSIDQSVLK(3)
Nipbl	1000	Nipbl	1018	GAKPVVVLQK(3)-LIKDREDK(3)
Nipbl	1000	Nipbl	1023	GAKPVVVLQK(3)-DREDKSR(5)
Nipbl	1015	Nipbl	1034	LSLDDVQKLIK(8)-NKPSK(2)
Nipbl	1018	Nipbl	1029	LIKDREDK(3)-SSLKPIK(4)
Nipbl	1018	Nipbl	1040	LIKDREDK(3)-SNKGSIDQSVLK(3)
Nipbl	1018	Nipbl	1082	LIKDREDK(3)-STVNEKPK(6)
Nipbl	1182	Nipbl	1247	KAYEPK(1)-HQLNELGSESAKIK(12)
Nipbl	2563	Nipbl	2574	NLCGFSDSKIQK(9)-YSPSESAKVYDK(8)
Nipbl	2563	Nipbl	2578	NLCGFSDSKIQK(9)-VYDKAINR(4)
Nipbl	2563	Nipbl	2583	NLCGFSDSKIQK(9)-KTGVHFHFK(1)
Nipbl	2774	Nipbl	2787	ESDIYKK(7)-LTNKVVQTLR(4)

* For simplicity, Nipbls N-terminal Twin-strep-flag-linker (48 amino acids) was subtracted from the residue number.

Table 16: List of interprotein cross-links Nipbl:Cohesin, experiment 2.*

Protein 1	Residue	Protein 2	Residue 2	Peptide sequence
Scc1	364	Nipbl	974	LMMWKETGGVEK(5)-DKDGNVTQETKK(11)
Smc1	245	Nipbl	865	LNKELASKNK(8)-TDKLERK(3)
Smc1	420	Nipbl	787	KVETEAKIK(7)-HKQDTKSDSPR(6)
Smc1	677	Nipbl	865	EKKER(2)-TDKLERK(3)
Smc1	1026	Nipbl	706 or 739	IAAPNMKAMEK(7)-QKGESRPETPK(2)
Smc3	106	Nipbl	1200	KDQYFLDK(1)-LTPEEMMDSSTFKR(13)
Smc3	106	Nipbl	1261	KDQYFLDK(1)-LSTDKTVK(5)
Smc3	218	Nipbl	500	WDKMR(3)-DKPLKK(2)
Smc3	388	Nipbl	1073	GSQFTSKEER(7)-MNKRK(3)
Smc3	743	Nipbl	862	MLKEKR(3)-NEHGIKSDSSKTDK(11)
SA1	905	Nipbl	965	YYNDYGDIIKETLSK(10)-DKDGNVTQETK(2)
Smc1	213	Smc3	977	LKDEVVR(2)-KLEQCNTLKK(10)
Smc1	213	Smc3	978	LKDEVVR(2)-KYSHVNKK(1)
Smc1	213	Smc3	984	LKDEVVR(2)-YSHVNKK(6)
Smc1	267	Smc3	899	KKELGK(1)-ELQKSMER(4)
Smc1	267	Smc3	905	KKELGK(1)-WKNMEK(2)
Smc1	272	Smc3	936	KELGKMMR(5)-KEECMKK(1)
Smc1	282	Smc3	895	EQQQIEKEIK(7)-SEDLNDSIDKTEAGIKELQK(16)
Smc1	282	Smc3	899	EQQQIEKEIK(7)-ELQKSMER(4)
Smc1	285	Smc3	881	EIKEK(3)-KSEVNDK(1)
Smc1	300	Smc3	310	RPQYIKAK(6)-AKDLQDELAGNSEQR(2)
Smc1	300	Smc3	874	RPQYIKAK(6)-VKDTMAR(2)
Smc1	302	Smc3	300	AKENTSHK(2)-QEIQQR(5)
Smc1	302	Smc3	304	AKENTSHK(2)-TKLELK(2)
Smc1	311	Smc3	308	KLEAAKK(1)-TKLELKAK(6)
Smc1	317	Smc3	729	KSLQNAQK(1)-FKASR(2)

Appendix

Smc1	324	Smc3	740	SLQNAQKHYYK(7)-DSILSEMKMLK(8)
Smc1	384	Smc3	968	EEASKR(5)-KLEQCNTLTK(1)
Smc1	433	Smc3	729	LREIEENQKR(9)-FKASR(2)
Smc1	437	Smc3	729	IEKLEEYITTSK(3)-FKASR(2)
Smc1	528	Smc3	673	LIDLCQPTQKK(10)-AVDKLK(4)
Smc1	551	Smc3	935	NMDAIVDSEKTGR(11)-KKEECMK(1)
Smc1	551	Smc3	936	NMDAIVDSEKTGR(11)-KKEECMK(1)
Smc1	589	Smc3	113	ELKGAK(3)-KDQYFLDKK(8)
Smc1	675	Smc3	492	LKEKK(2)-REDLEKK(6)
Smc1	677	Smc3	492	LKEKK(4)-REDLEKK(6)
Smc1	678	Smc3	492	EKKER(3)-REDLEKK(6)
Smc1	678	Smc3	728	EKKER(3)-ELKTK(3)
Smc1	723	Smc3	729	YSQSDLEQTKTR(10)-FKASR(2)
Smc1	736	Smc3	429	SKLESELANFGPR(2)-EKNLEQYNK(2)
Smc1	805	Smc3	355	RQNEIAKK(7)-FNSVKEK(5)
Smc1	814	Smc3	340	RLEFENQKTR(8)-IEEKQK(4)
Smc1	829	Smc3	336	NQLKEDQDK(4)-LLEKIEEK(4)
Smc1	844	Smc3	310	KDENEIEK(1)-AKDLQDELGNSEQR(2)
Smc1	844	Smc3	941	KDENEIEK(1)-KKEECMKK(6)
Smc1	874	Smc3	285	IIDETMAQLQDLKNQHLAK(13)-ISAMKEEK(5)
Smc1	881	Smc3	278	KSEVNDK(1)-ELKTK(3)
Smc1	881	Smc3	285	KSEVNDK(1)-ISAMKEEK(5)
Smc1	910	Smc3	924	EMTHLQKEVTAIETK(7)-ELEKMTNR(4)
Scc1	323	SA1	1079	KLIVDSVK(1)-KGRPPLHK(1)
Smc3	388	SA1	349	GSQFTSKEER(7)-LKCLK(2)

* For simplicity, Nipbls N-terminal Twin-strep-flag-linker (48 amino acids) was subtracted from the residue number.

7.1.3 Cross-linking mass spectrometry of Cohesin

Table 17: Overview intra- and interprotein cross-links of Cohesin.

	Cohesin
inter XLs	51
Smc1-Smc3	36
Smc1-Scc1	3
Smc1-SA1	7
Scc1-SA1	0
Scc1-Smc3	5
Smc3-SA1	0
Intra XLs	92
Smc1-Smc1	54
Smc3-Smc3	31
Scc1-Scc1	0
SA1-SA1	7
Total XLs	144

Table 18: List of interprotein cross-links Nipbl:Cohesin, experiment 2.*

Protein 1	Residue	Protein 2	Residue 2	Peptide sequence
Scc1	364	Nipbl	974	LMMWKETGGVEK(5)-DKDGNVTQETKK(11)
Smc1	245	Nipbl	865	LNKELASKNK(8)-TDKLERK(3)
Smc1	420	Nipbl	787	KVETEAKIK(7)-HKQDTKSDSPR(6)
Smc1	677	Nipbl	865	EKKER(2)-TDKLERK(3)

Appendix

Smc1	1026	Nipbl	706 or 739	IAAPNMKAMEK(7)-QKGESRPETPK(2)
Smc3	106	Nipbl	1200	KDQYFLDK(1)-LTPEEMMDSSTFKR(13)
Smc3	106	Nipbl	1261	KDQYFLDK(1)-LSTDKTVK(5)
Smc3	218	Nipbl	500	WDKMR(3)-DKPLKK(2)
Smc3	388	Nipbl	1073	GSQFTSKEER(7)-MNKRK(3)
Smc3	743	Nipbl	862	MLKEKR(3)-NEHGIKSDSSKTDK(11)
SA1	905	Nipbl	965	YYNDYGDIIKETLSK(10)-DKDGNVTQETK(2)
Smc1	213	Smc3	977	LKDEVVR(2)-KLEQCNTLKK(10)
Smc1	213	Smc3	978	LKDEVVR(2)-KYSHVNKK(1)
Smc1	213	Smc3	984	LKDEVVR(2)-YSHVNKK(6)
Smc1	267	Smc3	899	KKELGK(1)-ELQKSMEK(4)
Smc1	267	Smc3	905	KKELGK(1)-WKNMEK(2)
Smc1	272	Smc3	936	KELGKMMR(5)-KEECMKK(1)
Smc1	282	Smc3	895	EQQQIEKEIK(7)-SEDLNSIDKTEAGIKELQK(16)
Smc1	282	Smc3	899	EQQQIEKEIK(7)-ELQKSMEK(4)
Smc1	285	Smc3	881	EIKEK(3)-KSEVNDK(1)
Smc1	300	Smc3	310	RPQYIKAK(6)-AKDLQDELGNSEQR(2)
Smc1	300	Smc3	874	RPQYIKAK(6)-VKDTMAR(2)
Smc1	302	Smc3	300	AKENTSHK(2)-QEIQQR(5)
Smc1	302	Smc3	304	AKENTSHK(2)-TKLELK(2)
Smc1	311	Smc3	308	KLEAAKK(1)-TKLELKAK(6)
Smc1	317	Smc3	729	KSLQNAQK(1)-FKASR(2)
Smc1	324	Smc3	740	SLQNAQKHYYK(7)-DSILSEMMLK(8)
Smc1	384	Smc3	968	EEASKR(5)-KLEQCNTLKK(1)
Smc1	433	Smc3	729	LREIEENQKR(9)-FKASR(2)
Smc1	437	Smc3	729	IEKLEEYITTSK(3)-FKASR(2)
Smc1	528	Smc3	673	LIDLQOPTQKK(10)-AVDKLK(4)
Smc1	551	Smc3	935	NMDAIIVDSEKTGR(11)-KKEECMK(1)
Smc1	551	Smc3	936	NMDAIIVDSEKTGR(11)-KKEECMK(1)
Smc1	589	Smc3	113	ELKGAK(3)-KDQYFLDKK(8)
Smc1	675	Smc3	492	LKEKK(2)-REDLEKK(6)
Smc1	677	Smc3	492	LKEKK(4)-REDLEKK(6)
Smc1	678	Smc3	492	EKKER(3)-REDLEKK(6)
Smc1	678	Smc3	728	EKKER(3)-ELKTK(3)
Smc1	723	Smc3	729	YSQSDLEQTKTR(10)-FKASR(2)
Smc1	736	Smc3	429	SKLESELANFGPR(2)-EKNLEQYNK(2)
Smc1	805	Smc3	355	RQNEIAKK(7)-FNSVKEK(5)
Smc1	814	Smc3	340	RLEFENQKTR(8)-IEEKQK(4)
Smc1	829	Smc3	336	NQLKEDQDK(4)-LLEKIEEK(4)
Smc1	844	Smc3	310	KDENEIEK(1)-AKDLQDELGNSEQR(2)
Smc1	844	Smc3	941	KDENEIEK(1)-KEECMKK(6)
Smc1	874	Smc3	285	IIDETMAQLQDLKNQHLAK(13)-ISAMKEEK(5)
Smc1	881	Smc3	278	KSEVNDK(1)-ELKTK(3)
Smc1	881	Smc3	285	KSEVNDK(1)-ISAMKEEK(5)
Smc1	910	Smc3	924	EMTHLQKEVTAIETK(7)-ELEKMTNR(4)
Scc1	323	SA1	1079	KLIVDSVK(1)-KGRPPLHK(1)
Smc3	388	SA1	349	GSQFTSKEER(7)-LKCLK(2)

* For simplicity, Nipbls N-terminal Twin-strep-flag-linker (48 amino acids) was subtracted from the residue number.

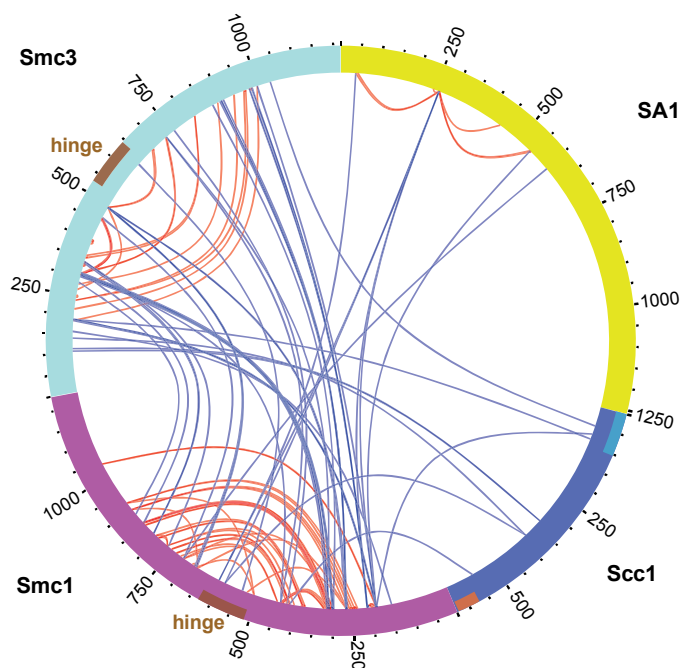


Figure 31: Proximity map of Cohesin interaction.

Proximity map of intra- (red) and interprotein (blue) cross-links of Cohesin in solution (n=144).

Table 19: Intra- and interprotein cross-links Cohesin.

Protein1	Position1	Protein2	Position2	CSMs	Peptide
Smc1	189	Smc1	213	1	KKNIAAER(1)-LKDEVVR(2)
Smc1	189	Smc1	1037	1	KKNIAAER(1)-DKFQETSDEFEAAR(2)
Smc1	190	Smc1	200	3	KKNIAAER(2)-EAKQEKEEADR(3)
Smc1	190	Smc1	213	3	KKNIAAER(2)-LKDEVVR(2)
Smc1	190	Smc1	1037	5	KKNIAAER(2)-DKFQETSDEFEAAR(2)
Smc1	200	Smc1	213	6	EAKQEKEEADR(3)-LKDEVVR(2)
Smc1	237	Smc1	918	3	LYHNEVEIEKLNK(10)-EVTAIETKLEQK(8)
Smc1	240	Smc1	247	16	LNKELASK(3)-NKEIEKDK(2)
Smc1	240	Smc1	251	4	LNKELASK(3)-NKEIEKDK(6)
Smc1	245	Smc1	918	1	ELASKNKEIEKDK(5)-EVTAIETKLEQK(8)
Smc1	251	Smc1	258	8	NKEIEKDK(6)-MDKVEDELKEK(3)
Smc1	251	Smc1	897	2	EIEKDKK(4)-KLGGANK(1)
Smc1	258	Smc1	897	4	MDKVEDELKEK(3)-KLGGANK(1)
Smc1	264	Smc1	267	14	MDKVEDELKEK(9)-KKELGK(1)
Smc1	264	Smc1	268	3	VEDELKEK(6)-KKELGK(2)
Smc1	266	Smc1	528	1	LKEKK(4)-LIDLCQPTQKK(10)
Smc1	266	Smc1	669	1	LKEKK(4)-WDEKAVDK(4)
Smc1	266	Smc1	897	2	VEDELKEKK(8)-KLGGANK(1)
Smc1	300	Smc1	308	8	RPQYIKAK(6)-ENTSHKIK(6)
Smc1	300	Smc1	311	21	RPQYIKAK(6)-KLEAAK(1)
Smc1	300	Smc1	713	2	RPQYIKAK(6)-LKYSQSDLEQTK(2)
Smc1	300	Smc1	851	4	RPQYIKAK(6)-KDENEIEKLKK(8)
Smc1	302	Smc1	853	2	AKENTSHK(2)-LKKEEQR(2)
Smc1	308	Smc1	311	8	AKENTSHKIK(8)-KLEAAK(1)
Smc1	308	Smc1	844	12	AKENTSHKIK(8)-KDENEIEK(1)
Smc1	308	Smc1	851	2	ENTSHKIK(6)-KDENEIEKLK(8)
Smc1	311	Smc1	317	2	KLEAAK(1)-KSLQNAQK(1)
Smc1	311	Smc1	437	1	KLEAAK(1)-RIEKLEEYITTSK(4)

Appendix

Smc1	311	Smc1	844	4	KLEAAKK(1)-KDENEIEK(1)
Smc1	311	Smc1	851	3	KLEAAK(1)-KDENEIEKLKK(8)
Smc1	316	Smc1	853	11	KLEAAKK(6)-LKKEEQR(2)
Smc1	317	Smc1	433	2	KSLQNAQK(1)-EIEENQKR(7)
Smc1	324	Smc1	433	2	SLQNAQKHYK(7)-EIEENQKR(7)
Smc1	324	Smc1	437	5	SLQNAQKHYK(7)-IEKLEEYITTSK(3)
Smc1	373	Smc1	379	1	DLTLEENQVKK(10)-LKEEASK(2)
Smc1	373	Smc1	384	1	DLTLEENQVKK(10)-LKEEASKR(7)
Smc1	373	Smc1	796	2	DLTLEENQVKK(10)-EFEEEEKVKR(6)
Smc1	379	Smc1	766	2	LKEEASK(2)-DLKEK(3)
Smc1	379	Smc1	796	3	LKEEASK(2)-EFEEEEKVKR(6)
Smc1	379	Smc1	805	2	LKEEASK(2)-RQNEIAKK(7)
Smc1	401	Smc1	766	6	FNRDQKADQDR(6)-DLKEK(3)
Smc1	413	Smc1	736	1	KKVETEA(1)-SKLESELANFGPR(2)
Smc1	413	Smc1	752	3	KKVETEA(1)-INDIKR(5)
Smc1	414	Smc1	752	9	KKVETEA(2)-INDIKR(5)
Smc1	420	Smc1	736	15	KKVETEA(8)-SKLESELANFGPR(2)
Smc1	433	Smc1	437	4	LREIEENQKR(9)-IEKLEEYITTSK(3)
Smc1	446	Smc1	713	2	IEKLEEYITTSKQSLEEQK(12)-
Smc1	446	Smc1	723	1	IEKLEEYITTSKQSLEEQK(12)-
Smc1	508	Smc1	673	1	KAEIMESIKR(9)-AVDKLK(4)
Smc1	508	Smc1	918	3	KAEIMESIKR(9)-EVTAIETKLEQK(8)
Smc1	669	Smc1	897	8	WDEKAVDK(4)-KLGGANK(1)
Smc1	678	Smc1	686	4	EKKER(3)-LTEELKEQMK(6)
Smc1	766	Smc1	798	1	DLKEK(3)-EFEEEEKVKR(8)
Smc1	766	Smc1	806	2	DLKEK(3)-KRLEFENQK(1)
Smc3	185	Smc3	999	4	ETEGKR(5)-EKLIK(2)
Smc3	215	Smc3	968	24	LHTLEEEKEELAQYQKWDK(16)-
Smc3	238	Smc3	935	4	AKLDELSAK(2)-KKEECMK(1)
Smc3	238	Smc3	963	1	AKLDELSAK(2)-YQTLSLKQLFR(7)
Smc3	245	Smc3	252	3	AKLDELSAKR(9)-ETSGEKSR(6)
Smc3	266	Smc3	492	3	DKMEDIER(2)-REDLEKK(6)
Smc3	280	Smc3	889	2	TKISAMKEEKEQLSAER(2)-SEDLD-
Smc3	304	Smc3	459	9	TKLELK(2)-YYEVKNK(5)
Smc3	304	Smc3	461	4	TKLELK(2)-NKKDELQSER(2)
Smc3	304	Smc3	462	9	TKLELK(2)-NKKDELQSER(3)
Smc3	308	Smc3	459	14	TKLELKAK(6)-YYEVKNK(5)
Smc3	308	Smc3	727	2	TKLELKAK(6)-KFKASR(1)
Smc3	308	Smc3	729	5	TKLELKAK(6)-KFKASR(3)
Smc3	328	Smc3	332	1	LLKER(3)-QKLEK(2)
Smc3	328	Smc3	336	65	LLKER(3)-LLEKIEEK(4)
Smc3	332	Smc3	340	3	QKLEK(2)-IEEKQK(4)
Smc3	350	Smc3	357	8	ELAETEPKFNSVK(8)-EKEER(2)
Smc3	350	Smc3	820	2	QKELAETEPKFNSVK(10)-IKLEGIITR(2)
Smc3	357	Smc3	820	20	FNSVKEKEER(7)-IKLEGIITR(2)
Smc3	388	Smc3	396	2	GSQFTSKEER(7)-DKWIKK(5)
Smc3	393	Smc3	400	7	DKWIKK(2)-ELKSLDQAINDK(3)
Smc3	396	Smc3	400	5	DKWIKK(5)-ELKSLDQAINDK(3)
Smc3	454	Smc3	461	28	KYYEVK(1)-NKKDELQSER(2)
Smc3	459	Smc3	462	48	YYEVKNK(5)-KDELQSER(1)
Smc3	492	Smc3	684	2	REDLEKK(6)-KAEELGELEAK(1)
Smc3	493	Smc3	680	5	KQQLLR(1)-LELQKDVR(5)
Smc3	680	Smc3	684	3	SRLELQKDVR(7)-KAEELGELEAK(1)
Smc3	963	Smc3	968	3	YQTLSLKQLFR(7)-KLEQCNTLKK(1)
Smc3	977	Smc3	984	2	KLEQCNTLKK(10)-YSHVNKK(6)
Smc3	977	Smc3	985	1	KLEQCNTLKK(10)-KALDQFVNFSEQK(1)
Smc3	978	Smc3	985	2	KYSHVNK(1)-KALDQFVNFSEQK(1)
Twin-Strep-Sa1	87	Twin-Strep-Sa1	308	3	KSPGEK(1)-LELLLQKR(7)

Appendix

Twin-Strep-Sa1	92	Twin-Strep-Sa1	308	3	SPGEKSR(5)-LELLQKR(7)
Twin-Strep-Sa1	292	Twin-Strep-Sa1	308	1	NKMIGK(2)-LELLQKR(7)
Twin-Strep-Sa1	308	Twin-Strep-Sa1	488	1	LELLQKR(7)-HDPQAEALAKR(11)
Twin-Strep-Sa1	308	Twin-Strep-Sa1	590	1	LELLQKR(7)-VLTAKER(5)
Twin-Strep-Sa1	308	Twin-Strep-Sa1	593	1	LELLQKR(7)-KTQIDDR(1)
Twin-Strep-Sa1	590	Twin-Strep-Sa1	593	40	VLTAKER(5)-KTQIDDR(1)
Scc1	72	Smc1	189	1	AKYLLADCNEAFIK(2)-KKNIAAER(1)
Scc1	387	Smc1	589	1	LLKLFTR(3)-ELKGAK(3)
Scc1	558	Smc1	414	5	RWNKR(4)-KKVETEA(2)
Scc1	50	Smc3	1034	3	VKMALR(2)-KYEAIQLTFKQVSK(10)
Scc1	86	Smc3	188	6	IKMAFRPGVVDLPEENR(2)-EKINELLK(2)
Scc1	335	Smc3	106	2	ELDSKTIR(5)-KDQYFLDKK(1)
Scc1	335	Smc3	113	3	ELDSKTIR(5)-KDQYFLDKK(8)
Scc1	387	Smc3	140	3	LLKLFTR(3)-SNPYIVKQGK(8)
Smc1	149	Smc3	157	1	NPKER(3)-LKLLR(2)
Smc1	189	Smc3	188	4	KKNIAAER(1)-EKINELLK(2)
Smc1	190	Smc3	185	5	KKNIAAER(2)-ETEGKR(5)
Smc1	190	Smc3	188	2	KKNIAAER(2)-EKINELLK(2)
Smc1	190	Smc3	999	1	KKNIAAER(2)-EKLIK(2)
Smc1	213	Smc3	977	2	LKDEVVR(2)-KLEQCNTLKK(10)
Smc1	213	Smc3	978	2	LKDEVVR(2)-KYSHVVK(1)
Smc1	213	Smc3	984	31	LKDEVVR(2)-YSHVVK(6)
Smc1	213	Smc3	985	1	LKDEVVR(2)-KALDQFVNFSQK(1)
Smc1	264	Smc3	492	1	LKEKK(2)-REDLEKK(6)
Smc1	267	Smc3	895	4	KKELGK(1)-SEDLDNSIDKTEAGIKELQK(16)
Smc1	267	Smc3	899	2	KKELGK(1)-ELQKSMER(4)
Smc1	267	Smc3	905	2	KKELGK(1)-WKNMEK(2)
Smc1	268	Smc3	492	1	KKELGK(2)-REDLEKK(6)
Smc1	268	Smc3	899	2	KKELGK(2)-ELQKSMER(4)
Smc1	300	Smc3	304	1	RPQYIKAK(6)-TKLELK(2)
Smc1	300	Smc3	310	9	RPQYIKAK(6)-AKDLQDELAGNSEQR(2)
Smc1	300	Smc3	874	79	RPQYIKAK(6)-VKDTMAR(2)
Smc1	302	Smc3	300	6	AKENTSHK(2)-QEIQQR(5)
Smc1	302	Smc3	304	4	AKENTSHK(2)-TKLELK(2)
Smc1	311	Smc3	304	1	KLEAAK(1)-TKLELK(2)
Smc1	311	Smc3	308	6	KLEAAK(1)-TKLELKAK(6)
Smc1	317	Smc3	308	2	KSLQNAQK(1)-TKLELKAK(6)
Smc1	317	Smc3	729	2	KSLQNAQK(1)-KFKASR(3)
Smc1	420	Smc3	756	2	KVETEA(7)-TFMPKQR(5)
Smc1	437	Smc3	308	2	IEKLEEYITTSK(3)-TKLELKAK(6)
Smc1	437	Smc3	729	2	IEKLEEYITTSK(3)-KFKASR(3)
Smc1	540	Smc3	629	1	VLGKNMDAIIVDSEK(4)-HVFGKTLICR(5)
Smc1	673	Smc3	492	5	AVDKLKEK(4)-REDLEKK(6)
Smc1	675	Smc3	492	69	AVDKLKEK(6)-EDLEKK(5)
Smc1	678	Smc3	492	5	EKKER(3)-REDLEKK(6)
Smc1	713	Smc3	462	1	LKYSQSDLEQTK(2)-NKKDELQSER(3)
Smc1	763	Smc3	340	1	EMKDLK(3)-IEEKQK(4)
Smc1	814	Smc3	336	2	RLEFENQKTR(8)-LLEKIEEK(4)
Smc1	814	Smc3	340	1	RLEFENQKTR(8)-IEEKQK(4)
Smc1	851	Smc3	310	1	KDENEIEKLKK(8)-AKDLQDELAGNSEQR(2)
Smc1	881	Smc3	285	1	KSEVNDK(1)-ISAMKEEK(5)
Smc1	190	Twin-Strep-Sa1	308	3	KNIAAER(1)-LELLQKR(7)
Smc1	190	Twin-Strep-Sa1	590	1	KNIAAER(1)-VLTAKER(5)
Smc1	213	Twin-Strep-Sa1	308	6	LKDEVVR(2)-LELLQKR(7)
Smc1	589	Twin-Strep-Sa1	308	2	ELKGAK(3)-LELLQKR(7)
Smc1	606	Twin-Strep-Sa1	87	1	YEPPIKK(7)-KSPGEK(1)
Smc1	606	Twin-Strep-Sa1	308	1	YEPPIKK(7)-LELLQKR(7)
Smc1	713	Twin-Strep-Sa1	653	3	LKYSQSDLEQTK(2)-HLDALLKQIK(7)

7.2 List of abbreviations

Å	Angstrom
aa	amino acid
ABC -like	ATP-binding cassette -like
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
°C	Celsius
CCE	crude cell extract
CdLS	Cornelia de Lange syndrome
ChIP	chromatin immunoprecipitation
Ct	Chaetomium thermophilum
CTCF	CCCTC-binding factor
CTCF	CCCTC-binding factor
C-terminus	Carboxyl-terminus
DNA	Deoxyribonucleic acid
dsDNA	double strand DNA
DSS	Disuccinimidyl suberate
DTT	Dithiothreitol
E	elution
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
exp	experiment
FRET	fluorescence resonance energy transfer
G1 phase	gap1-phase of the cell cycle
G2 phase	gap2-phase of the cell cycle
GST	Glutathione S-transferase
h	hour
HEAT	alpha helical protein repeat; first identified in Huntingtin, Elongation factor3, PR65/A and TOR
HeLa	cervix carcinoma cells from Henrietta Lacks
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His tag	Poly-histidine tag
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kbp	kilo base pair
kDa	kilo dalton
min	minute
mol	mole
MW	molecular weight

Appendix

MWCO	molecular weight cut-off
NBD	nucleotide binding domain
Ni-NTA beads	nickel ion chelated to agarose nitrilotriacetic acid
nm	nano meter
N-terminus	Amino-terminus
PBS	phosphate buffered saline
PCR	polythermal cycling reaction
PDB	protein database
PSMF	phenylmethane sulfonyl fluoride
PVDF	polyvinylidene fluoride
RNA	Ribonucleic acid
rpm	rotations per minute
RT	room temperature
s	second
SDS PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
Smc	Structural maintenance of chromosomes
S-Phase	synthesis phase of the cell cycle
T	temperature
TBS	TRIS buffered saline
TBST	TRIS buffered saline with 0.05% Tween-20
TCEP	Tris-(2-carboxyethyl)-phosphine
TEV protease	tobacco etch virus (TEV) protease
TIRF microscopy	total internal reflection microscopy
TLC	thin layer chromatography
TMR	tetramethyl-rhodamine
TPR	tetratripeptide repeats
UB	unbound
UV light	Ultraviolet light
v/v	volume per volume
w/v	weight per volume
WCE	whole cell extract
wt	wild type
XLMS	Cross-linking mass spectrometry
XLs	cross-links

7.3 List of figures

FIGURE 1: RECOMBINANT COHESIN DISPLAYS DIFFERENT CONFORMATIONS DEPENDING ON THE VISUALIZATION METHOD.	8
FIGURE 2: COHESIN DURING THE CELL CYCLE.	10
FIGURE 3: THE HEAD DOMAINS OF COHESIN'S SMC1/3 SUBUNITS FORM 2 ACTIVE SITES FOR ATP HYDROLYSIS.	11
FIGURE 4: SPONTANEOUS LOADING OF COHESIN ONTO PLASMID DNA <i>IN VITRO</i> IS TOPOLOGICAL AND INDEPENDENT OF COHESIN'S ATPASE ACTIVITY.	18
FIGURE 5: PREPARATIONS OF FULL LENGTH NIPBL ALONE OR IN COMPLEX WITH MAU2.....	21
FIGURE 6: THE PURIFIED RECOMBINANT FULL LENGTH HUMAN COHESIN LOADER, NIPBL, RECONSTITUTES COHESIN LOADING IN <i>XENOPUS</i> EGG EXTRACTS.....	22
FIGURE 7: NIPBL STIMULATES COHESIN'S ATPASE ACTIVITY – A REACTION BOOSTED BY THE PRESENCE OF DNA.	24
FIGURE 8: NIPBL'S STIMULATION OF COHESIN'S ATPASE ACTIVITY DEPENDS ON CATALYTICALLY ACTIVE FORMS OF SMC1 AND SMC3.....	25
FIGURE 9: NIPBL'S STIMULATION OF COHESIN'S ATPASE ACTIVITY IS SALT DEPENDENT.	26
FIGURE 10: A 1:1 STOICHIOMETRIC RATIO OF COHESIN TO NIPBL RESULTS IN THE HIGHEST STIMULATION OF ATPASE ACTIVITY. STIMULATION BY DNA IS INDEPENDENT OF ITS SOURCE.....	27
FIGURE 11: Pds5A INTERACTS WITH COHESIN BUT DOES NOT BOOST ITS ATPASE ACTIVITY.	29
>> 12: Pds5A INTERACTS WITH COHESIN BUT DOES NOT BOOST ITS ATPASE ACTIVITY.	30
FIGURE 13: NIPBL DOES NOT STIMULATE THE ATPASE ACTIVITY OF A RELATED SMC COMPLEX, CONDENSIN II.	31
FIGURE 14: PREPARATION OF TRUNCATED FORMS OF NIPBL.	33
FIGURE 15: A NIPBL C-TERMINAL FRAGMENT INTERACTS WITH COHESIN AND BOOSTS ITS ATPASE ACTIVITY.....	35
FIGURE 16: PURIFICATION OF NIPBL COMPLEXES WITH MUTATIONS IN ITS HEAT REPEAT DOMAIN.....	36
FIGURE 17: NIPBL-PA VARIANT RETAINS BINDING TO COHESIN BUT SHOWS REDUCED STIMULATION OF ITS ATPASE ACTIVITY.	38
FIGURE 18: SCC1 IS REQUIRED FOR NIPBL'S STIMULATORY EFFECT ON COHESIN'S ATPASE ACTIVITY.....	39
FIGURE 19: ADDITION OF SA1/2 DID NOT FURTHER BOOST THE STIMULATED ATPASE ACTIVITY OF THE TRIMERIC COHESIN.	41
FIGURE 20: CLEAVAGE OF SCC1, THE SUBUNIT SPANNING THE SMC HEADS IN COHESIN, DOES NOT INFLUENCE THE ABILITY OF NIPBL TO ACTIVATE COHESIN'S ATPASE ACTIVITY.....	42
FIGURE 21: ALTERING THE SMC3:SCC1 INTERFACE HAS LITTLE OR NO INFLUENCE OF THE ABILITY OF NIPBL TO STIMULATE COHESIN'S ATPASE ACTIVITY.	43
FIGURE 22: MAU2 IS DISPENSABLE FOR THE COHESIN – NIPBL INTERACTION, WHILE SA1 OR SA2 IS INTERCHANGEABLE.	46
FIGURE 23: NIPBL INTERACTS WITH SA2 ALONE AS WELL AS SMC1/3 DIMERS, SCC1-SMC1/3 TRIMERS AND SA1 OR 2-SCC1-SMC1/3 TETRAMERS.	47
FIGURE 24: CROSS-LINKING MASS SPECTROMETRY CONFIRMED MAU2'S INTERACTION WITH THE N-TERMINUS OF NIPBL.	49
FIGURE 25: CROSSLINKING MASS SPECTROMETRY INDICATES THAT NIPBL CONTACTS ALL FOUR COHESIN SUBUNITS.	51
FIGURE 26: VALIDATION OF CROSS-LINKS IDENTIFIED IN COHESIN:NIPBL EXPERIMENT 1.....	53
FIGURE 27: FREQUENTLY IDENTIFIED CROSS-LINKS LOCALIZED AT THE ACETYL LOOP AND EXIT GATE OF SMC3.	55
FIGURE 28: REPEATEDLY FOUND CROSS-LINKS IN NIPBL:COHESIN OR ONLY COHESIN SAMPLE INDICATE THAT COHESIN FOLDS THE COILED-COIL BACK ON THEMSELVES.	57

Appendix

FIGURE 29: PROXIMITY MAP OF ALL SMC1 AND SMC3 INTRA AND INTERPROTEIN CROSS-LINKS FROM NIPBL:COHESIN XLMS, EXP. 1....	132
FIGURE 30: NIPBL POTENTIALLY CONTACTS ALL FOUR COHESIN SUBUNITS (EXP. 2).....	134
FIGURE 31: PROXIMITY MAP OF COHESIN INTERACTION.....	140

List of tables

TABLE 1: VARIOUS FORMS OF TAGGED NIPBL AND MAU2 ALONE OR IN COMPLEX.....	20
TABLE 2: IN INSECT CELLS EXPRESSED, N- AND C-TERMINALLY TAGGED NIPBL ALONE OR IN COMPLEX WITH MAU2.	20
TABLE 3: NIPBL:COHESIN CROSS-LINKS (XLS) FOUND IN EXPERIMENT 1 AND 2.	50
TABLE 4: BUFFER CONDITIONS AND BEADS USED FOR SINGLE STEP PURIFICATION.	73
TABLE 5: LIST OF ANTIBODIES USED IN THIS STUDY.	77
TABLE 6: LIST OF LIBRARY VECTORS (PLIB) FOR SINGLE OR CO-EXPRESSION IN INSECT CELLS.....	92
TABLE 7: TRIMERIC AND TETRAMERIC COHESIN COMPLEXES FOR INSECT CELL EXPRESSION, CLONED IN THIS STUDY.....	93
TABLE 8: LIST OF COHESIN COMPLEXES FOR INSECT CELL EXPRESSION USED IN THIS STUDY.	93
TABLE 9 : PET21A PLASMIDS FOR INDUCIBLE EXPRESSION IN E.COLI.	94
TABLE 10: LIST OF TWIN-STREP-FLAG-NIPBL-HIS AND MAU2 INTRA- AND INTERPROTEIN CROSS-LINKS.....	94
TABLE 11 : LIST OF ALL INTRAPROTEIN CROSS-LINKS NIPBL:COHESIN (EXP. 1).*	103
TABLE 12: LIST OF ALL INTERPROTEIN CROSS-LINKS NIPBL:COHESIN (EXP. 1).*	124
TABLE 13: CROSS-LINKS WITH A CSM VALUE, GREATER THAN 5 FROM NIPBL:COHESIN CROSS-LINK EXPERIMENT 1*.....	133
TABLE 14: SMC3 AND SMC1 INTRA AND INTERPROTEIN CROSS-LINKS WITH A CSM VALUE GREATER THAN 9 FROM NIPBL:COHESIN CROSS-LINK EXP. 1*.....	133
TABLE 15: LIST OF INTRAPROTEIN CROSS-LINKS NIPBL:COHESIN (EXP. 2).*	135
TABLE 16: LIST OF INTERPROTEIN CROSS-LINKS NIPBL:COHESIN, EXPERIMENT 2.*	137
TABLE 17: OVERVIEW INTRA- AND INTERPROTEIN CROSS-LINKS OF COHESIN.....	138
TABLE 18: LIST OF INTERPROTEIN CROSS-LINKS NIPBL:COHESIN, EXPERIMENT 2.*	138
TABLE 19: INTRA- AND INTERPROTEIN CROSS-LINKS COHESIN.	140