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Generation of genetic tools to investigate the trophoblast function of MORC4

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Zusammenfassung

Die Plazenta ist ein essentielles Organ für die embryonale Entwicklung und besteht aus drei Hauptlinien der Trophoblasten nämlich den Zytotrophoblasten, Synzytiotrophoblasten sowie den extravillösen Trophoblasten. Ihre zellulären Identitäten werden durch spezifische Genexpressionsprogramme definiert und anschließend durch Transkriptionsfaktoren sowie cis-regulatorische Elemente gesteuert. Ein Faktor, der spezifisch im Trophoblasten exprimiert wird, ist MORC4, wobei dessen Funktion jedoch weitgehend unbekannt ist. Unsere unveröffentlichten Laborergebnisse zeigen, dass MORC4 mit den Korepressor-Komplexen NCoR1 und NCoR2 interagiert. Um die Funktion von MORC4 während der Plazentaentwicklung zu untersuchen, nutzte ich die Umwandlung humaner pluripotenter Stammzellen über einen naiven Zustand zu Trophoblast-Stammzellen, die die Linien des Zytotrophoblasten, Synzytiotrophoblasten und extravillösen Trophoblasten modellieren. Zu diesem Zweck reduzierte ich MORC4 auf DNA-, RNA- und Proteinebene. Auf genomischer Ebene erzeugte ich induzierbare MORC4-Knockout-Stammzelllinien mithilfe der CRISPR/Cas9-Technologie. Auf Transkriptionsebene stellte ich induzierbare shRNA-vermittelte Knockdown-Stammzelllinien für MORC4 sowie für Untereinheiten von NCoR1 und NCoR2 her. Auf Proteinebene etablierte ich eine MORC4-Degron-Stammzelllinie. Anschließend führte ich die Umwandlung der genannten Stammzelllinien in den naiven Zustand durch, welche durch SUSD2-positive Zellen mit erhöhten Spiegeln der Pluripotenzmarker NANOG, OCT4 und SOX2 gekennzeichnet sind. Diesbezüglich versuchte ich die Generierung von Blastoiden. Zukünftige Experimente werden sich auf die Differenzierung zu Trophoblast-Stammzellen sowie auf nachgelagerte Analysen der Auswirkungen der MORC4-Depletion auf Linienspezifikation und Plazentaentwicklung konzentrieren.

Zusammenfassend habe ich somit eine Reihe genetisch veränderter Zelllinien erzeugt, die wertvolle Instrumente zur Untersuchung der MORC4-Funktion und dessen Beitrag zur menschlichen Plazentaentwicklung bereitstellen.

Abstract

The placenta is an essential organ for embryonic development, and it consists of three major trophoblast lineages: cytotrophoblast (CT), syncytiotrophoblast (ST), and extravillous trophoblast (EVT). Their cellular identities are defined by specific gene expression programs and controlled by transcription factors and cis-regulatory elements. One factor specifically expressed in the trophoblast is MORC4, but its function remains largely unknown. Our unpublished lab findings indicate that MORC4 interacts with the NCoR1 and 2 co-repressor complexes. To address the MORC4 function during placental development, I employed the conversion of human pluripotent stem cells (hPSCs), via a naive state, to trophoblast stem cells that model the CT, ST and EVT lineages. I depleted MORC4 on three levels: DNA, RNA and protein. On the genomic level, I created inducible *MORC4*-KO hPSC lines using the CRISPR/Cas9 technology. On the transcript level, I generated inducible shRNA knockdown PSC lines of *MORC4* and *NCOR1/2* subunits. On the protein level, I established a MORC4 degron hPSC cell line. Next, I performed conversion of the aforementioned hPSC lines to the naive state, indicated by SUSD2-positive cells with increased levels of pluripotency markers *NANOG*, *OCT4* and *SOX2* and attempted generation of blastoids. Future experiments will focus on differentiation to trophoblast stem cells and downstream analysis of the effects of MORC4 depletion on lineage specification and placental development.

Together, I generated a set of genetically engineered cell lines providing useful tools to dissect MORC4 function and its contribution to human placental development.

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1. Introduction

The placenta is a transient organ essential for fetal development and a successful pregnancy. Despite having the same role, it is morphologically the most diverse organ among mammals (Garratt et al., 2013). Functions of the placenta include the bidirectional exchange of gases, nutrients and metabolites between the mother and her fetus, production of pregnancy hormones and modulation of the mother's immune system (Turco & Moffett, 2019). Defective placental development is associated with many pregnancy complications such as preeclampsia, preterm labour, and late spontaneous abortion (Brosens et al., 2011). To perform these functions, the placenta consists of various specialised cell types, with the trophoblast being the most important fetal-derived cell type. It includes the progenitor CT that differentiates into the multinucleated ST or the invasive EVT. These cellular identities are defined by gene expression programs and controlled by transcription factors and cis-regulatory elements. One factor specifically expressed in the trophoblast is MORC4, but its function remains unknown.

1.1. Microorchidia family of proteins

MORC4 belongs to the Microorchidia family, which in mammals includes MORC1,2,3 and 4. They are CW-type zinc finger nuclear proteins and share the following structural domains: the GHKL-type ATPase domain, CW-type zinc finger (ZF) domain and one or more coiled-coil domains (Figure 1.1) (D.-Q. Li et al., 2013). GHKL domain (named after DNA gyrase, hsp90, histidine kinase and DNA mismatch repair enzyme MutL) is found on the N-terminus and functions as a catalytically active ATPase (Dutta & Inouye, 2000; D.-Q. Li et al., 2013; Tencer et al., 2020). The ZF-CW domain comprises about 60 amino acids with at least four cysteine (C) and two tryptophan (W) residues. It has a role in recognising different epigenetic marks found on the DNA, linking MORCs with chromatin remodelling and epigenetic modulation (He et al., 2010; D.-Q. Li et al., 2013). The coiled-coil domain consists of two to five α -

helices wrapped around each other into a super-helical structure, and it is important for the regulation of protein-protein and protein-DNA interactions, protein stability, localisation and signalling transduction. While each of the MORC proteins has a distinct function, they are generally involved in transcriptional silencing of transposable genetic elements via chromatin compaction.

MORC1 is specifically expressed in the male testis, where it silences transposable elements in the developing germline. Mouse homozygous mutant *Morc1* males are infertile because insufficient transposon regulation leads to chromatin instability and meiotic failure (Inoue, 1999; Pastor et al., 2014; Watson et al., 1998). A similar phenotype can be observed in mice with defective DNA methylation or insufficient transposon repression pathways (Bourc'his & Bestor, 2004; Kaneda et al., 2004; Shoji et al., 2009). MORC2 is recruited to the Human Silencing Hub (HUSH) complex, where it acts as a chromatin remodeler. Targets of the HUSH complex include intronless elements such as LINE-1 retrotransposons, endogenous retroviruses (ERVs), and the HIV provirus. The HUSH-mediated silencing is achieved through the recognition and deposition of a repressive trimethylation of histone 3 lysine 9 (H3K9me3) by the SETDB1 methyltransferase (Seczynska et al., 2022; Spencley et al., 2023). In humans, mutations in the ATPase domain of MORC2 and the loss of chromatin decompaction cause a neurological Charcot-Marie-Tooth disorder (Tchasovnikarova et al., 2017). MORC3 functions as an epigenetic silencer of transposable elements in mouse embryonic stem cells (ESCs) via chromatin compaction. In mice, *Morc3* knockout is lethal and results in upregulation of transposable elements, specifically ERVs, and leads to chromatin instability (Desai et al., 2021). It has also been identified as a key part of the monocyte immune system, and acts as a negative regulator of anti-viral type I interferon. MORC3 is a repressor of MORC3-regulated DNA elements (MRE) upstream of key anti-viral genes. Upon viral infection, it is degraded by virulence factors, resulting in the derepression of MREs and activation of the secondary immune response (Gaidt et al., 2021). According to Human Protein Atlas, MORC4 is highly expressed in the trophoblast, with

lower expression levels in the testis, intestine and the appendix; however, its function remains unknown (*MORC4 Protein Expression Summary - The Human Protein Atlas*, n.d.). It has been associated with inflammatory diseases, such as pancreatitis, Crohn's disease and ulcerative colitis, and is upregulated in certain colorectal and breast cancers (Liang et al., 2023; Luo et al., 2020). MORC4 acts as a homodimer with its CW-type zinc finger domains binding histone 3 lysine 4 trimethylation (H3K4me3), and its ATPase activity is DNA-binding-dependent (Tencer et al., 2020). The molecular mechanisms underlying MORC4 function, especially in the trophoblast and early stages of human development, have yet to be elucidated.

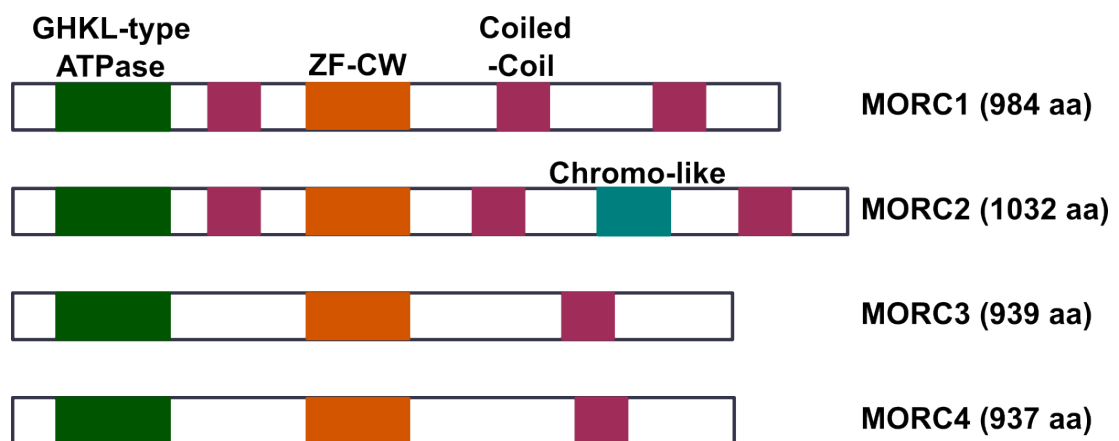


Figure 1.1. **Members of the MORC family and their functional domains.** Adapted from (D.-Q. Li et al., 2013).

1.2. Early human development

At the beginning of human development, the zygote undergoes cleavage divisions, followed by compaction at the 10-cell stage (Figure 1.2.). In the morula, the first cell fate decisions between the inner cell mass (ICM) and the trophectoderm (TE) are taking place (Gerri et al., 2020, 2023; Shahbazi & Pasque, 2024). The outer cells become apicobasally polarised because of the difference in the cell-cell contact. The side of these cells facing the environment accumulates apical polarity determinants, such as atypical protein kinase C (aPKC), while the inner side becomes basal. Because of the lack of cell-cell contact, Hippo signalling is inactive in the outer cells, where the

yes-associated protein 1 (YAP1) translocates to the nucleus. There, it drives expression of TEA-domain family member 4 (*TEAD4*), and activates the TE gene regulatory network (GRN), including genes such as caudal type homeobox 2 (*CDX2*) and GATA binding protein 3 (*GATA3*) (Gerri et al., 2020, 2023). While the role of *HIPPO*, *TEAD4*, *CDX2* and *GATA3* is conserved between human and mouse, a well-known ICM marker SRY-box transcription factor 2 (*SOX2*) shows a differential expression pattern between the two species. In the mouse, it is restricted to the cells of the ICM, whereas in the human, it is expressed in all nuclei and becomes restricted to the ICM at the early blastocyst stage (Gerri et al., 2020, 2023). At the 20-cell stage, a blastocel is formed, and the blastocyst undergoes maturation. Before implantation, the cells of the ICM also need to execute the second cell fate decision between epiblast and hypoblast, also known as primitive endoderm. Pre-implantation epiblast consists of naive pluripotent cells, meaning that they have no lineage-specific bias and are able to give rise to all three germ layers and the germ line. These cells have high expression of the pluripotency GRN core genes such as *SOX2*, Octamer-Binding Protein 4 (*OCT4*), *NANOG*, Kruppel-Like Factor 4 (*KLF4*) and *KLF17* and of the Transforming Growth Factor-beta (TGF- β) signalling factors (Blakeley et al., 2015; Shahbazi & Pasque, 2024). Cells of the hypoblast will later form the yolk sac. They are marked by the high expression of *SOX17*, *GATA4*, *GATA6* and *FOXA2*. For the specification of the hypoblast, Fibroblast Growth Factor (FGF) signalling is crucial (Blakeley et al., 2015; Dattani et al., 2024). A mature blastocyst consists of TE on the outside and defined epiblast and hypoblast on the inside. During implantation, the polar TE found directly above the epiblast attaches to the maternal endometrium and fuses to form the primary syncytium (Figure 1.2) (Molè et al., 2025).

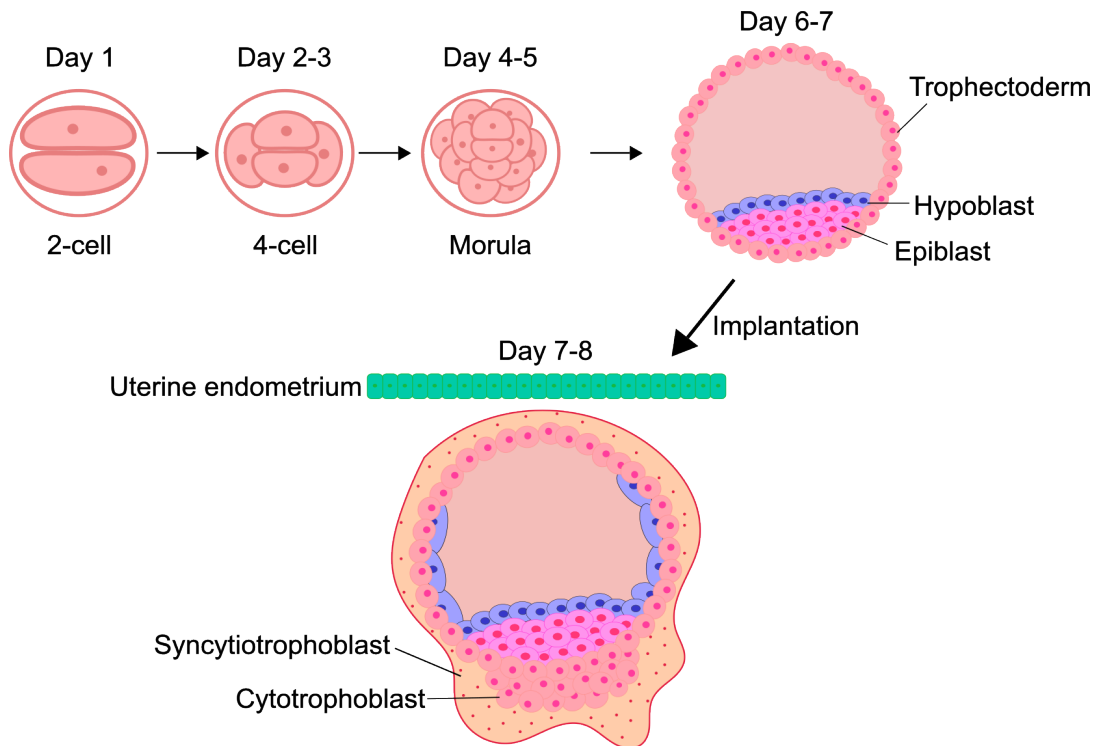


Figure 1.2. **Human embryonic development from day 0 to implantation.** Schematic representation of the cell types and structure of the human embryo during the first week of development.

1.3. Development of the placenta

After the initial implantation, the primary syncytium invades the endometrium, triggering its decidualisation. The cytotrophoblast cells are the progenitor cells of the growing placenta, initially not in direct contact with maternal tissue. These cells are rapidly proliferating, forming primary villous projections through the primary syncytium (Turco & Moffett, 2019). Inside the syncytium, fluid-filled cavities start to appear and fuse, giving rise to the intervillous space that will be filled with maternal blood. The extraembryonic mesenchymal (ExM) cells penetrate through the villi to form the secondary villi. To facilitate gas and nutrient supply, fetal capillaries are also formed from the ExM inside these villi. The CTs can differentiate into two specialised cell types: the fused syncytiotrophoblast or invading extravillous trophoblast (Turco & Moffett, 2019). ST is a multinucleated outer lining of the placental villi. Since it is in direct contact with the maternal blood within the intervillous space, it is the site of gas, nutrient and metabolite exchange between the two blood streams.

It produces crucial pregnancy hormones such as the human chorionic gonadotropin or human placental lactogen, and sustains progesterone production. On the other hand, EVT's are highly migratory cells that invade the maternal decidua and remodel the maternal spiral arteries (Sato et al., 2012). By the end of the first trimester, the villous tree has been established, and supplies the developing fetus with gases, nutrients and other essential substances.

1.4. In vitro models for studying human development

The study of early human development was largely limited by the lack of reliable and physiologically relevant in vitro models. This changed with the emergence of human stem cell research that provides insight into certain aspects of human embryogenesis. These studies have deepened our understanding of key signalling pathways, cell fate decisions and gene regulatory networks governing the earliest stages of life.

Naive PSCs represent the pre-implantation epiblast with unrestricted developmental potential (Theunissen et al., 2016). They can be isolated from the ICM of the blastocyst and were first propagated on a feeder layer with MEK/ERK and PKC inhibitors and Wnt signalling activator, and human leukaemia inhibitory factor (hLIF) (Guo et al., 2016). With time, different naive media compositions have been developed, with PXGL being one of the most commonly used. It contains inhibitors of MEK/ERK, PKC and Wnt signalling and hLIF (Guo et al., 2017; Theunissen et al., 2014). Naive PSCs can be established by episomal expression of NANOG and KLF2 or SOX2 and KLF4 (MacCarthy et al., 2024; Okita et al., 2013; Takashima et al., 2014). Transient overexpression of *super-SOX2* and *KLF4* boosts the developmental potential of primed PSCs, i.e. converts the cells to the naive state. SOX2-A16V, also known as super-SOX, has enhanced SOX2/OCT4 dimerisation ability as a result of the swap between one alanine to valine amino acid residue (MacCarthy et al., 2024). In vitro, they form bright dome-shaped colonies and are able to differentiate into any embryonic cell type. Because of less lineage restriction in human blastocysts compared to mice, naive PSCs are also able

to differentiate into extra-embryonic cell types (Dong et al., 2020; Guo et al., 2021; Shahbazi & Pasque, 2024). Primed PSCs transcriptionally resemble the post-implantation epiblast, meaning that they represent a later developmental stage compared to naive PSCs. They express core pluripotency markers such as NANOG, OCT4 and SOX2, can be isolated from the blastocyst ICM and are maintained in media supplemented with TGF- β and FGF (Thomson et al., 1998; Vallier et al., 2005). Induced PSCs are derived from somatic cells by transient expression of *OCT3/4*, *SOX2*, *C-MYC*, and *KLF4* (Takahashi et al., 2007; J. Yu et al., 2007). Unlike naive, the primed PSCs have an epithelial morphology and can be grown feeder-free. In vitro, they can differentiate into the three germ layers, but not into the extra-embryonic cell types (Guo et al., 2021).

Trophoblast SCs (TSC) can be derived from a blastocyst or CT layer of first trimester placenta (Okoe et al., 2018). Molecularly, they most closely resemble the CT and are maintained in vitro in conditions of active Wnt and EGF, and inhibited ROCK and TGF- β signalling pathways. In addition, TSCs can be derived from naive PSCs in vitro (Dong et al., 2020; Io et al., 2021). They differentiate into the ST with the addition of a cAMP agonist, or into EVT with EGF activation and TGF- β inhibition in the presence of Matrigel and Neuregulin-1 (Okoe et al., 2018).

Organoids are 3D culture systems, mostly stem cell-derived, that recapitulate aspects of tissue development, architecture, functions and disorders (Artegiani & Hendriks, 2025). They form from either tissue-resident or pluripotent stem cells and provide a platform for more complex readouts. In vitro naive PSCs can self-organise into 3D structures that closely resemble the blastocyst (Kagawa et al., 2021; Yanagida et al., 2021; L. Yu et al., 2021). These structures, termed blastoids, are of the developmentally appropriate size, morphology, cell number and composition as the developing blastocyst. Single-cell RNA-seq data show they contain analogues of all the lineages present in the pre-implantation blastocyst (Kagawa et al., 2021; Yanagida et al., 2021; L. Yu et al., 2021). Interestingly, blastoids are able to implant in vitro into endometrial organoids (Kagawa et al., 2021; Molè et al., 2025).

1.5. Inducible genetic tools for molecular perturbations

Loss-of-function experiments are fundamental for studying functions of genes and proteins, and genetic perturbations in hPSCs provide important insight into human development. However, genomic interference studies of hPSCs were limited by the lack of methods to conditionally manipulate gene expression in both hPSCs and hPSC-derived cells. For this reason, inducible systems bring many advantages. They enable the study of stage-specific gene function in dynamic situations such as development or in cases of strong differentiation or apoptotic phenotypes. We took advantage of the single-step optimised inducible gene knockdown or knockout (sOPTiKD or sOPTiKO) platforms as previously described in Bertero *et al*, 2016. This system utilises so-called safe genomic harbours as a site for the knock-in of the transgene for a stable expression even after transdifferentiation of the hPSCs (Sadelain *et al.*, 2011). In this case, the safe genomic harbour is the AAVS1 locus, and it is targeted with two target-optimised zinc-finger nucleases. sOPTiKO combines CRISPR/Cas9 technology and the tetracycline-derepressable system (Bertero *et al.*, 2016; Pawlowski *et al.*, 2017). One AAVS1 allele is targeted with a Cas9 constitutively-expressing cassette. While the second AAVS1 allele carries a cassette with tetracycline-inducible expression of the sgRNA. This is achieved by having a tet operator (tetO2) sequence inside the H1 RNA polymerase III promoter, bound by the constitutively expressed tetracycline-sensitive repressor (OPTtetR) (Pawlowski *et al.*, 2017). Tetracycline treatment causes a conformational change of the repressor and induces the expression of the sgRNA. Consequently, tetracycline treatment results in the KO of the gene of interest. The sOPTiKD system targets only one AAVS1 locus with the previously mentioned zinc-finger nucleases. Only in this case, the transgene carries a tetracycline-inducible expression of the shRNA based on the same mechanism. To facilitate the selection of homologous recombinants, the AAVS1 targeting transgenes carry antibiotic resistance driven by the endogenous promoter. The Cas9 expressing cassette has a neomycin resistance, and the sgRNA/shRNA cassettes have a puromycin resistance included.

Inducible protein of interest (POI) degradation systems, also known as degrons, are based on tags recognisable by E3 ligases (Zhang et al., 2025). Usually, the recognition is induced by small-molecule ligands. Compared to the RNA or DNA level approaches, degron allows for higher specificity, sensitivity and monitoring of immediate effects. We have decided to utilise the dTAG system based on a modified FKBP12^{F36V} protein tag that is recognised by the E3 ubiquitin ligase receptor CRBN after the addition of the dTAG ligand molecule (Nabet et al., 2018). The endogenous locus of POI, using CRISPR/Cas9 technology and microhomology-mediated end joining, is tagged with the dTAG sequence in-frame on either N- or C-terminus (Brand & Winter, 2019). Because of its small size, the dTAG should not interfere with the function of the protein, and in normal conditions, it should not yield any phenotype. By treating the cells with a small molecule ligand, the POI will be recognised by the E3 ligase, leading to the ubiquitination of the POI, and subsequent proteasomal degradation.

1.6. Aim of the project

In order to shed light on the function of MORC4 in the trophoblast lineage during development, I aim to develop three genetic tools for loss-of-function experiments. These tools include previously mentioned inducible knockdown, knockout and degron in hPSC lines. My goal is to investigate the function of MORC4 in early developmental stages using these tools.

2. Materials and Methods

2.1. Tissue culture

Human H9 (WA09, RRID: CVCL_9773, female) and WTC11 (GM25256, RRID: CVCL_Y803, male) pluripotent stem cell lines were cultivated in E8 medium (Chen et al., 2011) (Table 1) without antibiotics and supplemented with 5 μ M Y-27632 (R&D/Tocris, 1254). Cells were cultured on TPP tissue culture-treated plates/dishes (TPP Techno Plastic Products AG) coated for 30 min at RT with Vitronectin XF (Stem Cell Technologies, 07180) dissolved in DPBS (Gibco, 14190169) to a concentration of 10 μ g/mL. Cells were passaged every 3 days at 70% confluency using TrypLE Express Enzyme (GIBCO, 12605010) for dissociation. The cells were cultured at 37°C, 5% CO₂, and the medium was changed daily.

Human trophoblast stem cell line (CT30) was cultivated in TS full medium (Table 2) on Fibronectin (Merck, FC010) coated TC-treated plates. Fibronectin was reconstituted in DPBS to a concentration of 10 μ g/ml, and plates/dishes were coated at 37°C for 60 min. The cells were passaged every 3 days at 80% confluency using TrypLE Express Enzyme. The cells were cultured at 37°C, 5% CO₂, and the medium was changed every second day. Human TSCs were differentiated into syncytiotrophoblast cells using ST differentiation media (Table 2). All cell lines were regularly tested for mycoplasma contamination.

Table 1. **Composition of E8 medium**

Medium reagents	Final concentration
Dulbecco's Modified Eagle Medium:Nutrient Mixture F-12 with HEPES (Gibco, 11330057)	1x
L-ascorbic acid-2-phosphate magnesium (Sigma Aldrich, A8960-5G)	64 μ l/ml
Insulin-Transferrin-Selenium (Gibco, 51500056)	2x

Sodium bicarbonate (Gibco, 25080094)	540 µl/ml
Recombinant human TGFβ1 protein (R&D/Tocris, 240-B-010)	1.8 ng/ml
BSA (Catus Biotech, 1.0)	5 mg/ml
Recombinant FGF2-G3 (Qkine, Qk053)	5.5 ng/ml

Table 2. **Composition of TS full medium and ST differentiation medium**

Medium reagents	TS medium	ST medium	Final concentration
Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Gibco, 11320074)	x	x	1x
Insulin-Transferrin-Selenium-Ethanolamine (Gibco, 51500056)	x	x	1x
Antibiotic-Antimycotic (Gibco, 15240062)	x	x	1x
2-Mercaptoethanol (Gibco, 31350010)	x	x	0.1 mM
L-ascorbic acid (Sigma-Aldrich, A4403)	x	x	1.5 µg/mL
EGF (R&D/Tocris, 236-EG)	x		100 ng/mL
CHIR99021 (R&D/Tocris, 4423)	x		3 µM
A83-01(R&D/Tocris, 2939)	x		1 µM
Y27632 (R&D/Tocris, 1254)	x		8 µM
Fetal bovine serum (PAA laboratories, A15-108)	x		0.2%
Y27632 (R&D/Tocris, 1254)		x	10 µM
KnockOut Serum Replacement (Gibco, 10828010)		x	4%
Forskolin (BioGems, 6652995)		x	2 µM

2.2. Cloning of inducible sgRNA targeting vector

A 20 bp gRNA sequence targeting the human *MORC4* gene was identified using vbc-score.org (Table 3). To create the AarI overhangs, the “top” oligonucleotide was designed by adding 5'-TCCC-3' to the 5' end of the gRNA sequence, and the “bottom” oligonucleotide was designed by adding 5'-AAAC-3' to the 5' end of the reverse complement of the gRNA target sequence. Oligonucleotides were resuspended in nuclease-free ddH₂O to a final concentration of 100 µM. Top and bottom oligos were annealed in a 20 µL reaction containing 9 µL of each oligo and 2 µL of 10x oligo annealing buffer (1 mM Tris-HCl pH = 8, 0.02 mM EDTA, 0.1 M NaCl). The annealing reaction was incubated in a thermocycler for 5 min at 95 °C, followed by slow descent to 4 °C by 0.1 °C per second, and diluted 1:500 in nuclease-free ddH₂O. The backbone plasmid pAAV-Puro_siKO (Addgene #86696) was digested using AarI (Thermo Scientific, ER1581) enzyme. In a 100 µL reaction containing 5 µg of backbone plasmid, 5 µL of AarI (2 U/µL), 2 µL of provided oligonucleotide and 10 µL of 10x AarI digestion buffer. Digestion reaction was incubated overnight at 37 °C. The digested pAAV-Puro_siKO backbone and annealed gRNA oligonucleotides were ligated using T4 DNA ligase (NEB, M0202). 10 µL ligation reaction contained 50 ng of digested plasmid, 4 µL of annealed diluted oligonucleotides, 1 µL of 10x T4 DNA ligase buffer and 1 µL of T4 DNA ligase and was incubated at 4 °C overnight. The following day, the whole ligation reaction was transformed into 50 µL chemically competent *E. coli* cells (XL1-Blue) and plated on LB-agar plates containing 100 µg/mL ampicillin (Amp). After overnight incubation at 37 °C, two colonies from each plate were picked and inoculated into 3 mL of LB-Amp media. The following day, plasmid isolation was done using Mini-PEx kit according to the manufacturer's instructions and sent for Sanger sequencing using the following primer 5'-CGAACGCTGACGTCATCAACC-3'. Clones positive for the gRNA were grown in 100 mL LB-Amp media overnight at 37 °C. Plasmids were isolated using the QIAGEN Midi prep kit (12145X4) following the manufacturer's instructions.

Table 3. Human *MORC4* targeting gRNA sequences

Targeting site	Sequence
Exon 1	5'-GCCCCGGACCTACCGTGCTC-3'
Exon 2	5'-TGGCACTGAAGGGTCGCGTG-3'

2.3. Generation of CRISPR/Cas9 *MORC4* inducible knock-out hPSC lines

H9 cells used for nucleofection were grown in E8 medium until 70% confluency. For electroporation, the Neon™ transfection system (Thermo Fisher, MPK10096) was used. Firstly, the media was removed, and cells were washed with DPBS. The cells were dissociated using TrypLE, resuspended in E8 media and centrifuged for 5 min at 100 g. An aliquot of cell suspension was counted with Trypan Blue staining. After the centrifugation, the supernatant was removed, and the cells were resuspended in DPBS. Aliquot containing 2 million cells was transferred into an Eppendorf tube and centrifuged again. The pellet was resuspended in 100 µL of R buffer with 3 combinations of vector constructs indicated in Table 4. The cell suspension was aspirated into a 100 µL gold tip and inserted into an electroporation cuvette containing 3 mL of E2 buffer. Electroporation was done under the following settings: 1150 V, 20 ms width and 2 pulses. The whole cell suspension was plated into one Vitronectin XF-coated well of a TPP tissue culture-treated 6-well plate with E8 media supplemented with 5 µM Y-27632. Antibiotic selection with 0.2 µg/mL puromycin (Sigma Aldrich, P8833-25MG) and 70 µg/mL neomycin G418 (Sigma Aldrich, A1720) was started 24 h after electroporation and was going on for 72h. After selection, individual colonies were picked under the microscope using a sterile 20 µL micropipette tip and transferred to 24-well Vitronectin XF TPP tissue culture-treated plates with E8 media supplemented with 5 µM Y-27632. Genotyping PCR was performed with OneTaq Quick-Load 2X Master Mix with Standard Buffer (NEB, M0486) according to the manufacturer's protocol. Firstly, all clones were screened for the presence of

the antibiotic resistance cassettes. Positive clones were further genotyped using primers described in Table 5. PCR reactions 1 and 2 detect the integration of sgRNA, while reactions 3 and 4 detect the Cas9 integration. Clones yielding the expected PCR product were further expanded.

Table 4. Electroporation reaction set up

Vector name	Antibiotic resistance	Amount	Reaction
pZFN-AAVS1-L-ELD (Addgene #159297)		4 µg	1,2 and 3
pZFN-AAVS1-R-KKR (Addgene #159298)		4 µg	1,2 and 3
AAVSI-Neo-Cag-Cas9 (Addgene #86698)	neomycin	2 µg	1,2 and 3
AAVSI-Puro-EGFP-gRNA	puromycin	2 µg	1
AAVSI-Puro-MORC4-exon1	puromycin	2 µg	2
AAVSI-Puro-MORC4-exon2	puromycin	2 µg	3

Table 5. Genotyping PCR primers

PCR reaction	Primer	Sequence (5'-3')	T _a	PCR product size
1	Neo_fw	TTGTCAAGACCGACCTGTCC	55 °C	313 bp
	Neo_rev	AGACCGGCTTCCATCCGAGT		
2	Puro_fw	ACCGAGCTGCAAGAACTCTTCC	55 °C	279 bp
	Puro_rv	CAGACCCTTGCCCTGGTGGT		
3	Locus_fw	CTGTTTCCCCTTCCCAGGCA GGTCC	63 °C	991 bp
	Puro_rv	CAGACCCTTGCCCTGGTGGT		
4	Locus_rv	GGAACGGGGCTCAGTCTGA	55 °C	1447 bp
	OPTTetR_fw	CCACCGAGAAGCAGTACGAG		
5	Locus_rv	find above	55 °C	2151 bp
	Cas9_fw	AAACGGCCGGAAGAGAATG		

6	Locus_fw	find above	60 °C	1032 bp
	Neo_rv	GGAACGGGGCTCAGTCTGA		

2.4. Cloning of inducible shRNA-containing vectors

ShRNA sequences targeting human *MORC4*, *NCOR1*, *NCOR2*, *HDAC3* and *TBL1X* were selected from the RNAi Consortium shRNA library (Table 6). ShRNA sequence consists of a 21 bp sense target shRNA, 6 bp loop (5'-CTCGAG-3') and 21 bp of antisense target shRNA. To create the appropriate overhangs, the “top” oligonucleotide was designed by adding 5'-GATCCC-3' to the 5' end of the shRNA sequence, and 5'-TTTTTTG-3' to the 3' end. The “bottom” oligonucleotide was designed by adding 5'-TCGAGAAAAA-3' to the 5' end, and 5'-GG-3' to the 3' end of the reverse complement of the shRNA sequence. Oligonucleotides were resuspended in nuclease-free ddH₂O to a final concentration of 100 µM. Top and bottom oligonucleotides were annealed in a 20 µL reaction containing 9 µL of each oligo and 2 µL of 10x oligo annealing buffer. The thermocycler annealing program is indicated in Table 7. Afterwards, the annealed oligos were diluted 1:500 in nuclease-free ddH₂O.

Table 6. **Selected shRNA sequences**

Target gene	Sequence (5'-3')	Binding region
<i>MORC4</i>	AGGAGTACCGGCTAACAATAA	CDS
<i>MORC4</i>	GAATAGGGAGGTTTCAATT	CDS
<i>MORC4</i>	GAGTACCGGCTAACAATAA	CDS
<i>NCOR1</i>	GCCCACAGATGATGAAGAAAT	3'UTR
<i>NCOR1</i>	GCCATCAAACACAATGTCAAA	CDS
<i>NCOR2</i>	GCCTGTCTAAAGCCTTAATA	3'UTR
<i>NCOR2</i>	GCTTCACAACACAGGCATGAA	CDS

<i>HDAC3</i>	GGCACCCAATGAGTTCTATGAT	3'UTR
<i>HDAC3</i>	GATAGCTATCTGGGACATTA	3'UTR
<i>TBLX1</i>	GCGTAGTAAGTGCTTTATGTA	3'UTR

Table 7. Thermocycler program for shRNA oligonucleotide annealing

94 °C	5 min
93 °C	20 sec
Reduce by 1°C/cycle for 12 cycles	
80 °C	4 min
79 °C	20 sec
Reduce by 1°C/cycle for 3 cycles	
75 °C	4 min
74 °C	20 sec
Reduce by 1°C/cycle for 3 cycles	
70 °C	4 min
69 °C	20 sec
Reduce by 1°C/cycle for 60 cycles	
10 °C	Hold

Backbone plasmid p-sOPTiKD (Addgene #86695) was sequentially digested using BglII (10 U/μL, NEB, R0144) and Sall-HF (20 U/μL, NEB, R3138). In a 20 μL reaction containing 1 μg of backbone plasmid, 1 μL of each restriction enzyme and 2 μL of 10x NEB r3.1 and 10x CutSmart buffer, respectively. Digestion reactions were incubated for 1h at 37°C. The digested plasmid was extracted from an agarose gel using the MiniPEX kit following the manufacturer's instructions. Digested p-sOPTiKD plasmid and annealed shRNA oligos were ligated in a 3:1 insert: vector molar ratio with T4 DNA ligase in a 20 μL reaction overnight at 4 °C. The following day, the whole ligation reaction was transformed into 50 μL chemically competent *E. coli* cells (XL1-Blue) and plated on LB-agar plates containing 100 μg/mL Amp. Colony PCR

was performed using OneTaq Quick-Load 2X Master Mix with Standard Buffer. Primers used were AAVsinglKD_FW (5'-CGAACGCTGACGTCATCAACC-3') and AAVsingleKD_REV (5'-GGGCTATGAACTAATGACCCCG-3'). Clones positive for the shRNA were sent for Sanger sequencing with AAVsinglKD_FW primer. Plasmids were isolated using the QIAGEN Midi prep kit following the manufacturer's instructions.

2.5. Generation of inducible knockdown hPSC lines

H9 cells used for nucleofection were grown in E8 medium until 70% confluency. For electroporation, the Neon™ transfection system (Thermo Fisher, MPK10096) was used. Firstly, the media was removed, and cells were washed with DPBS. The cells were dissociated using TrypLE, resuspended in E8 media and centrifuged for 5 min at 100 g. After the centrifugation, the supernatant was removed, and the cells were resuspended in DPBS. Aliquot containing 2 million cells was transferred into an Eppendorf tube and centrifuged again. The pellet was resuspended in 100 µL of R buffer with 4 µg of pZFN-AAVS1-L-ELD, 4 µg of pZFN-AAVS1-R-KKR and 4 µg of shRNA-containing plasmid (Table 6). The cell suspension was aspirated into a 100 µL gold tip and inserted into an electroporation cuvette containing 3 mL of E2 buffer. Electroporation was done under the following settings: 1150 V, 20 ms width and 2 pulses. The whole cell suspension was plated into a Vitronectin XF-coated 15 cm TPP dish with E8 media supplemented with 5 µM Y-27632. Antibiotic selection with 0.2 µg/mL puromycin (Sigma Aldrich, P8833-25MG) was started 24 h after electroporation and was going on for 72h. After selection, individual colonies were picked under the microscope using a sterile micropipette tip and transferred to 24-well Vitronectin XF TPP tissue culture-treated plates with E8 media supplemented with 5 µM Y-27632. Genotyping PCR was performed with OneTaq Quick-Load 2X Master Mix with Standard Buffer (NEB, M0486) according to the manufacturer's protocol. Firstly, all clones were screened for the presence of the puromycin resistance cassette with the following primers: 5'-ACCGAGCTGCAAGAACTCTTCC-3' and 5'-CAGACCCTTGCCCTGGT GGT-3'. Positive clones were further genotyped for

the appropriate site of integration: 5'-CTGTTTCCCCTTCCCAGGCAGGTCC-3' and 5'-GAGGC GCACCGTGGGCTTGTTTCG-3'. Clones yielding the expected PCR product were further expanded.

2.6. Cloning of degron cutting vectors

A 20 bp gRNA sequence targeting the N-terminus and C-terminus of the human *MORC4* gene was identified using vbc-score.org (Table 8). To create appropriate overhangs, “top” oligonucleotide was designed by adding 5'-CACCG-3' to the 5' end of the gRNA. The “bottom” oligonucleotide was designed by adding 5'-AAAC-3' to the 5' end, and 5'-C-3' to the 3' end of the reverse complement of the gRNA target sequence. Oligonucleotides were resuspended in nuclease-free ddH₂O to a final concentration of 100 µM. The top and bottom oligonucleotides were annealed and phosphorylated by mixing 1 µL each oligo, 1 µL of 10x T4 ligase buffer, 6.5 µL nuclease-free ddH₂O, and 0.5 µL T4 Polynucleotide kinase (NEB, M0201). The reaction was incubated in the thermocycler for 30 min at 37 °C, followed by 5 min at 95 °C. After, the temperature was ramped down to 25 °C at the speed of 5 °C/min. Annealed oligonucleotides were diluted in nuclease-free ddH₂O 1:200.

Table 8. Human *MORC4* targeting gRNA sequences

Targeted terminus	gRNA sequence (5'-3')
N-terminus	AAAAAAATGCTCCTGTACCG
N-terminus	CGGCGGGGGCCCCTCGGTAC
C-terminus	GCTGGAGGCAAATCACATAC
C-terminus	GATCCTGTACACGGTGCTGG

PITCh Cutting Vector backbone (pX330A-sgX-sgPITCh) (Brand & Winter, 2019) was digested using BbsI-HF (NEB, R3539). The digestion reaction consisted of 10 µg of pX330A-sgX-sgPITCh vector, 10 µL of 10x CutSmart buffer and 2 µL of BbsI-HF (20 U/µL). The reaction was split into two 50 µL reactions and incubated for 1h at 37 °C. Afterwards, the digested

plasmid was dephosphorylated by adding 2 μ L of Shrimp alkaline phosphatase (NEB, M0371S) to each digestion reaction for 30 min at 37 °C. Finally, the plasmid backbone was extracted from an agarose gel. Previously annealed gRNA oligonucleotides and the digested plasmid were ligated using T4 DNA ligase. The 20 μ L ligation reaction contained 100 ng of digested plasmid, 2 μ L of annealed diluted oligonucleotides, 2 μ L of 10x T4 DNA ligase buffer and 1 μ L of T4 DNA ligase and was incubated at 4 °C overnight. The following day, the whole ligation reaction was transformed into 50 μ L chemically competent *E. coli* cells (XL1-Blue) and plated on LB-agar plates containing 100 μ g/mL Amp. After overnight incubation at 37 °C, two colonies from each plate were picked and inoculated into 3 mL of LB-Amp media. The following day, plasmid isolation was performed using Mini-PEx kit according to the manufacturer's instructions and sent for Sanger sequencing with the following primer: 5'-GCTGGCCTTTTGCTCACATG-3'. Clones positive for the gRNA were grown in 100 mL LB-Amp media overnight at 37°C. Plasmids were isolated using the QIAGEN Midi prep kit following the manufacturer's instructions.

2.7. Cloning of degron repair vectors

N- and C-terminal dTAG cassettes were amplified from pCRIS-PITChv2 plasmids containing blasticidin (Addgene #91792, #91795) and puromycin (Addgene #91793, #91796) resistance, respectively. Primers were designed with target-specific microhomology regions. The PCR reaction is outlined in Table 9, and the PCR thermocycler programme in Table 10. The PCR product was extracted and purified.

Table 9. **PCR reaction for amplifying the dTAG cassette**

10 μ L	5x NEB Q5 buffer
10 μ L	5x NEB GC enhancer buffer
1 μ L	dNTP mix (10 mM)
2.5 μ L	Primer mix (10 μ M each of PITCh fwd and PITCh rev)

50 ng	pCRIS-PITChv2
0.5 µL	Q5 High-Fidelity DNA Polymerase (NEB, M0491)
to 50 µL	Nuclease-free ddH ₂ O

Table 10. **Thermocycler protocol for amplifying dTAG cassette**

98 °C	30 s	
98 °C	10 s	5 x
55 °C	30 s	
72 °C	45 s	
98 °C	10 s	25 x
68 °C	30 s	
72 °C	45 s	
72 °C	2 min	

The backbone plasmid pCRIS-PITChv2-Puro-dTAG (Addgene #91793) was linearised with MluI-HF (NEB, R3198). 10 µg of pCRIS-PITChv2-Puro-dTAG was mixed with 10 µL of 10x CutSmart Buffer, 2 µL of MluI-HF (20 U/µL). The reaction was split into 50 µL reactions and incubated for 1h at 37 °C. Linearised plasmid was extracted from an agarose gel. The previously amplified dTAG cassettes were inserted into the digested plasmid via Gibson assembly (NEB, E2621). Reaction indicated in Table 11 was incubated at 50 °C for 30 min. Afterwards, the assembly reaction was diluted with 20 µL of nuclease-free ddH₂O, and 2 µL were transformed into 50 µL of chemically competent *E. coli* cells (XL1-Blue). For selection, transformed cells were plated on LB-agar plates containing 100 µg/mL Amp. After overnight incubation at 37 °C, two colonies from each plate were picked and inoculated into 3 mL of LB-Amp media. The following day, plasmid isolation was done using Mini-PEx kit according to the manufacturer's instructions and sent for Sanger sequencing using pPITChv2_seq fwd primer: 5'-GGGTCATTAGTTCATAGCCC-3'. Positive clones were grown in 100 mL LB-Amp media overnight at 37°C.

Plasmids were isolated using the QIAGEN Midi prep kit following the manufacturer's instructions.

Table 11. **Gibson assembly of PITCh Repair Vector**

MluI-digested vector backbone	30 ng
dTAG cassette	20 ng
Nuclease-free ddH ₂ O to	3 µL
2x NEBuilder HiFi Master Mix	3 µL

2.8. Generation of MORC4 degron dTAG hPSC lines with PITCh system vectors

WTC11 hPSCs used for nucleofection were grown in E8 medium until 70% confluency. For electroporation, the Neon™ transfection system was used. Firstly, the media was removed, and cells were washed with DPBS. The cells were dissociated using TrypLE, resuspended in E8 media and centrifuged for 5 min at 100 g. An aliquot of the cell suspension was counted with Trypan Blue staining. After the centrifugation, the supernatant was removed, and the cells were resuspended in DPBS. Aliquot containing 2 million cells was transferred into an Eppendorf tube and centrifuged again. The pellet was resuspended in 100 µL of R buffer. Each nucleofection reaction contained 5 µg of PITCh system plasmids, as indicated in Table 12.

Table 12. **Electroporated PITCh system plasmids**

Plasmid	Reaction	dTAG insertion site
pCRIS-PITChv2-Puro-dTAG_N_MORC4	1	N-terminus of MORC4
pX330A-sgN_MORC4-1-sgPITCh		
pCRIS-PITChv2-dTAG-Puro_C_MORC4_1a	2	C-terminus of MORC4
pX330A-sgC_MORC4-1-sgPITCh		

The cell suspension was aspirated into a 100 μ L gold tip and inserted into an electroporation cuvette containing 3 mL of E2 buffer. Electroporation was done under the following settings: 1150 V, 20 ms width and 2 pulses. The cells were plated into Vitronectin XF-coated 15 cm TPP tissue culture-treated dish with E8 media supplemented with 5 μ M Y-27632. Individual colonies were picked under the microscope using a sterile micropipette tip and transferred to 24-well plates with E8 media supplemented with 5 μ M Y-27632. DNA was extracted using the DirectPCR DNA extraction system (VIAGEN BIOTECH, 732-3259). Genotyping PCR was performed using OneTaq Quick-Load 2X Master Mix with Standard Buffer. Primers used for screening of the appropriate dTAG cassette integration: N-terminus 5'-CCGCAACCTCCCCTTCTACG-3'/5'-AGAGAAGGTCCCGACCCG-3', C-terminus: 5'-ACTTCTGTCCTCCCTC ACTTGGA-3'/ 5'-GTCCGGATCGACGGTGTGG-3'

2.9. Generation of MORC4 degron dTAG hPSC cell lines with Alt-R™ system

sgRNAs targeting human *MORC4* endogenous locus together with homology-directed repair donor oligos containing the degron dTAG were designed using Alt-R™ CRISPR HDR Design Tool and ordered from Integrated DNA Technologies (Table 13). WTC11 hPSCs used for nucleofection were grown in E8 medium until 70% confluency. For electroporation, the Neon™ transfection system was used. Firstly, the media was removed, and cells were washed with DPBS. The cells were dissociated using TrypLE, resuspended in E8 media and centrifuged for 5 min at 100 g. After, the supernatant was removed and the cells were resuspended in DPBS. Aliquot containing 2 million cells was transferred into an Eppendorf tube and centrifuged again. The pellet was resuspended in 100 μ L of R buffer. Each nucleofection reaction contained 5 μ g of Alt-R™ S.p. Cas9 Nuclease V3 (IDT, 1081058), 5 μ g of HDR donor oligos and 3.5 μ L of sgRNA (100 μ M).

Table 13. **Alt-R system sgRNAs and HDR donor oligos**

hMORC4_N-term sgRNA mA*mA*mA*rArArUrGrCrUrCrCrUrGrUrArCrCrGrArGrGrUrUrUrArGrArGrCrUrArGrArAr ArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGr ArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
hMORC4_N-term_dTAG+ 5'GTCGTACCGACTGGTAGATGACAGCAAACCGCAGCCCCGGCGCGCCAGGGCCGGC GGGGGCCCCCTCGGTAAGATCCGCCGCCACCCGACCCACCACCGCCCCGAGCCACCG CCACCTTCCAGTTTTAGAAAGCTCCACATCGAAGACGAGAGTGGCATGTGGTGGGATG ATGCCTGGGTGCCCAGTGGCACCATAGGCATAATCTGGAGATATAGTCAGTTTGGCT CTCTGACCCACACTCATCTGGGCAACCCCTTCTTCCCAGCCTCGGATCACCTCCTGC TTGCCTAGCATAAACTTAAAGGGCTTGTTTCTGTCCCGGGAGGAATCAACTTTCTTTT CATCTTCAAGCATCCCGGTGTAGTGACACACGCAGGTCTGGCCGCGCTTGGGGAAG GTGCGCCCGTCTCCTGGGGAGATGGTTTTCCACCTGCACTCCGTACAGGAGCATTTTT TTGGCCGCCACGGTACCCGTGATCCGAAAACGGGCGTATAGTCGAGACC-3'
hMORC4_C-term sgRNA mG*mC*mU*rGrGrArGrGrCrArArArUrCrArCrArUrArCrGrUrUrUrArGrArGrCrUrArGrArAr ArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGr ArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
hMORC4_C-term_dTAG+ 5'GTCGTACCGACTGGTAGATGACAGCAAACCGAGAGAAGGGTATACAGTCTGGTGCT CAATCCAGTATTTCCAGTTTTAGAAAGCTCCACATCGAAGACGAGAGTGGCATGTGGT GGGATGATGCCTGGGTGCCCAGTGGCACCATAGGCATAATCTGGAGATATAGTCAG TTTGGCTCTCTGACCCACACTCATCTGGGCAACCCCTTCTTCCCAGCCTCGGATCAC CTCCTGCTTGCTAGCATAAACTTAAAGGGCTTGTTTCTGTCCCGGGAGGAATCAAC TTTCTTTCCATCTTCAAGCATCCCGGTGTAGTGACACACGCAGGTCTGGCCGCGCTT GGGGAAGGTGCGCCCGTCTCCTGGGGAGATGGTTTTCCACCTGCACTCCAGATCCGC CGCCACCCGACCCACCACCGCCCCGAGCCACCGCCACCTATGTGATTTGCCTCCAGC ACCGTGTACAGGATCCCAGATCCGAAAACGGGCGTATAGTCGAGACC-3'

The cell suspension was aspirated into a 100 μ L gold tip and inserted into an electroporation cuvette containing 3 mL of E2 buffer. Electroporation was done under the following settings: 1150 V, 20 ms width and 2 pulses. The cells were plated into Vitronectin XF-coated 15 cm TPP tissue culture-treated dish with E8 media supplemented with 5 μ M Y-27632. Individual colonies were picked under the microscope using a sterile micropipette tip and transferred to 24-well plates with E8 media supplemented with 5 μ M Y-27632. DNA was extracted using the DirectPCR DNA extraction system (VIAGEN BIOTECH, 732-3259). Genotyping PCRs were performed using GoTaq G2 DNA Polymerase (Promega, M7841) according to manufacturers protocol. The screening strategy is described in Table 14. Positive clones were further expanded, and the insertion site was amplified using Q5 High-Fidelity 2X Master Mix (NEB, M0492) and sent for Sanger sequencing.

Table 14. **Genotyping PCR screen of MORC4 degron clones**

Targeted terminus	Primer sequence (5'-3')	Positive PCR product length
dTAG cassette only	GTGGAAGTGAGCGAGGGCTA	322 bp
	CACACCTCGTTTCCTCCGTT	
N-terminus	AAGTGAGCGAGGGCTAGTCCCG	260 bp
	TCGGATCACCTCCTGCTTGCC	
N-terminus	AAGTGAGCGAGGGCTAGTCCCG	657 bp
	AGAGAAGGTCCCGACCCG	
C-terminus	AAACTGGCCTCTTTCTCCACACC	349 bp
	CCAGATGAGTGTGGGTCAGAGAGC	
C-terminus	ACTTCTGTCCTCCCTCACTTGA	681 bp
	AAACTGGCCTCTTTCTCCACACC	

2.10. Immunofluorescence

Cells were grown on Fibronectin or Vitronectin glass coverslips. Cells were fixed with 4% paraformaldehyde/ PBS for 20 min at 4 °C and afterwards washed 2 times with PBS. The cells were permeabilised with PBS-0.1%TritonX at RT for 15 minutes and blocked in 4% donkey serum-PBS-0.1%TritonX at room temperature for 30 minutes. The cells were stained with primary antibodies in 4% donkey serum-PBS-0.1%TritonX at 4°C overnight. The next day, the cells were stained with a secondary antibody in 4% donkey serum-PBS-0.1%Tween20 at RT for 1-2 hours. Glass coverslips were mounted on microscope slides using VECTASHIELD Vibrance Antifade Mounting Medium with DAPI (Vector Laboratories, H-1700-2).

2.11. Protein extraction and western blot

Cells were pelleted and resuspended in Hunt lysis buffer (20 mM Tris/HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40) with 1x Protease inhibitor (PI) cocktail (Sigma-Aldrich, 5056489001). They were snap frozen/thawed three times in liquid nitrogen and centrifuged at 21000 x g for 30 min at 4 °C. Protein concentration was determined using Protein Assay Dye Reagent Concentrate (Bio-rad, 5000006). The lysates were boiled at 95 °C in 1x Laemmli buffer (0.25M Tris pH6.8, 0.28M SDS, 40% Glycerol, bromophenol blue, 10% 2-mercapto-ethanol) for 5 minutes. The boiled lysates were loaded on SDS-PAGE gels and run in SDS running buffer (0.25M Tris base, 1.925M Glycine, 0.035M SDS, pH adjusted to 8.3). The stacking was done under 20 mA, and the separation under 40 mA. Transfer was carried out on ice in an ice-cold transfer buffer (25mM Tris, 192 mM Glycine, 10% methanol) at 250mA for 120 minutes onto Immobilon-PVDF Membrane (Merck Millipore, IPVH00010). The membranes were blocked and stained with primary antibody in 5%Milk-PBS-0.1%Tween20 overnight. The next day, the membranes were stained with secondary antibody in 5%Milk-PBS-0.1%Tween20 at RT for 1h and imaged using Clarity Western ECL Substrate (Bio-rad, 1705061) and Fusion-FX (Vilber Lourmat).

2.12. Lentivirus production and transduction

Lentiviral particles were produced in the HEK293T cell line. The cells were seeded in a TC-treated 6-well plate at a 1:6 ratio following passaging at 80 % confluency, one day before transfection. Transfection was performed using Lipofectamine 3000 Transfection Reagent (ThermoFisher, L3000008). Solution A was prepared by mixing 250 µL Opti-MEM (Gibco, 31985070), 5 µL P3000 reagent, 1.375 µg psPAX packaging vector (Addgene, #12260), 1.375 µg envelope plasmid pMD2 (Addgene, #12259), and 2.5 µg pLKO.1-shRNA construct (Table 6). Solution B contained 250 µL Opti-MEM and 7.5 µL Lipofectamine 3000. Solutions A and B were combined (1:1) and incubated for 20 minutes at RT. The medium was aspirated from HEK293T cells and

replaced with 1 mL of Opti-MEM. Subsequently, 500 μ L of the transfection complex was added dropwise per well. Cells were incubated overnight at 37°C, 5 % CO₂. The following day, the medium was replaced with TS Base media containing 0.2 % fetal bovine serum. After 36 hours, virus-containing supernatants were collected and filtered through a 0.45 μ m filter. hTSCs seeded 24 hours earlier were transduced with 2 mL of viral particles mixed with 1 mL of 3 $\times$ growth factor TS full medium (Table 2). The selection was done with 350 μ g/mL neomycin G418 (Sigma Aldrich, A1720).

2.13. Co-Immunoprecipitation

Cells were pelleted and resuspended in Hunt lysis buffer (20 mM Tris/HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40) with 1x PI cocktail (Sigma-Aldrich, 5056489001). They were snap frozen/thawed three times in liquid nitrogen and centrifuged at 21000 x g for 30 min at 4 °C. Protein concentration was determined using Protein Assay Dye Reagent Concentrate (Bio-rad, 5000006). 500-1000 μ g of protein lysate was incubated with 2-10 μ g of antibody overnight at 4 °C, rotating. The following day, Protein G Dynabeads (Invitrogen, 10004D) were washed three times with Hunt lysis buffer with 1xPI cocktail. 50 μ L of beads were added to the antibody-coupled protein extracts and incubated for 3h at 4 °C, rotating. The beads were washed twice with 1xTBS for 5 min and once for 15 min, rotating at 4°C. The purified proteins were eluted from the beads in 1 $\times$ Laemmle buffer (see Section 2.11) by heat incubation at 95°C for 5 min. The eluate was used for Western blotting.

2.14. Naive induction

H9 cells used for nucleofection were grown in E8 medium until 70% confluency. Day prior to electroporation, irradiated mouse embryonic fibroblasts (iMEF) were seeded on 0,1% bovine gelatin (Sigma Aldrich, G1393) coated TPP tissue culture-treated plate with MEF medium (Table 15). For electroporation, the Neon™ transfection system (Thermo Fisher, MPK10096) was used. Firstly, the media was removed, and cells were washed with DPBS.

The cells were dissociated using TrypLE, resuspended in E8 media and centrifuged for 5 min at 100 g. A 10 μ L aliquot of cell suspension was counted with Trypan Blue staining. Cells were resuspended in DPBS, and an aliquot containing 2 million cells was transferred into an Eppendorf tube and centrifuged again. The pellet was resuspended in 100 μ L of R buffer with 6 μ g of pCXLE-Sox2AV-T2A-Klf4-E2A-Myc (Addgene #210017) and 1 μ g pCXWB-EBNA1 (Addgene #37624). The cell suspension was aspirated into a 100 μ L gold tip and inserted into an electroporation cuvette containing 3 mL of E2 buffer. Electroporation was done under the following settings: 1150 V, 20 ms width and 2 pulses. The whole cell suspension was plated into iMEF coated TPP tissue culture-treated plate with E8 media supplemented with 5 μ M Y-27632. Two days post-electroporation and onwards media was exchanged every second day for PXGL medium (Table 17). One week after electroporation, the SUSD2+ cells were FACS sorted (BioLegend, 327408). Naive cells were cultivated on a feeder layer with PXGL media, in a humidified incubator with 5% CO₂, 3% O₂ hypoxic conditions. Cells were passaged every 2/3 days using TrypLE Express Enzyme (GIBCO, 12605010).

Table 15. **MEF medium**

Media reagents	Final concentration
Dulbecco's Modified Eagle Medium, high glucose (Gibco, 11965092)	1x
Fetal bovine serum (PAA laboratories, A15-108)	10%
GlutaMAX Supplement (Gibco, 35050061)	1x
Sodium Pyruvate (Thermo Scientific, 11360039)	0.1 mM
MEM Non-Essential Amino Acids (Gibco, 11140050)	1x
Penicillin-Streptomycin (Gibco, 15140122)	10 U/mL

Table 16. **N2B27 medium**

Media reagent	Final concentration
Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Gibco, 11320074)	0.5x
Neurobasal Medium (Gibco, 21103049)	0.5x
N-2 Supplement (Gibco, 17502048)	0.5x
B-27 Supplement (Gibco, 17504044)	0.5x
GlutaMAX Supplement (Gibco, 35050061)	1x
Sodium Pyruvate (Thermo Scientific, 11360039)	1x
MEM Non-Essential Amino Acids (Gibco, 11140050)	1x
HEPES (1M) (Gibco, 15630080)	10 mM
2-Mercaptoethanol (Gibco, 31350010)	0.1 mM
Penicillin-Streptomycin (Gibco, 15140122)	5 U/mL

Table 17. **PXGL medium**

Media reagent	Final concentration
N2B27 medium	1x
PD 0325901 (Tocris/R&D, 4192)	1 μ M
XAV 939 (Tocris/R&D, 3748)	2 μ M
Go 6983 (Tocris/R&D, 2285)	2 μ M
Recombinant human LIF protein (Qkine, Qk036)	20 ng/mL

2.15. Human blastoid generation

Generation of human blastoids was performed as previously described with minor changes (Heidari Khoei et al., 2023). For MEF exclusion, naive hPSCs were dissociated using TrypLE (GIBCO, 12605010) and seeded in PXGL medium supplemented with 5 μ M Y-27632 on 0,1% bovine gelatin

(Sigma Aldrich, G1393) coated TPP tissue culture-treated plate. After a 45 min incubation, the supernatant containing naive hPSCs was carefully collected and centrifuged for 5 min at 200 g. The cells were counted and resuspended in aggregation medium (N2B27, 0.3% BSA, 10 μ M Y-27632) to the desired seeding concentration. For the 96-well Elplasia plate (Corning, cat. no. 4442), the recommended seeding density is 8-10k cells in a 100 μ L volume per one well. 100 μ L of cell suspension was added per well and incubated in a humidified incubator at 37 °C with 5% CO₂ and 5% O₂. 20 min after seeding, an additional 100 μ L of aggregation medium was added to each well. The following day, 100 μ L of media was aspirated and changed for 100 μ L 2x PALLY medium (N2B27, 20 μ M Y-27632, 6 μ M 1-Oleoyl lysophosphatidic acid (LPA), 2 μ M PD0325901, 2 μ M A83-01, 20 ng/ml hLIF). The next day, 150 μ L of media were changed for PALLY medium (N2B27, 10 μ M Y-27632, 3 μ M LPA, 1 μ M PD0325901, 1 μ M A83-01, 10 ng/ml hLIF). Afterwards, the blastoids were maintained in N2B27 medium supplemented with 500 nM LPA and 10 μ M Y-27632.

2.16. Naive to trophoblast cell transdifferentiation

Naive hPSC lines were transdifferentiated to hTSCsA as described previously (Dong et al., 2020). Briefly, naive hPSC lines were seeded onto 5 μ g/ml Collagen IV (Fisher Scientific, 10376931) pre-coated TPP tissue culture-treated 6-well plates in TS media (Table 2). The media was exchanged every second day, and cells were passaged every 4-5 days using TrypLE (GIBCO, 12605010). To verify the purity of cultures, the cells were FACS sorted using EGFR (Biozym, B352906) and ITGA6 (Thermo Fisher, 11-0495-82) antibodies.

2.17. Real-time quantitative PCR

Total RNA was isolated using InnuPREP miniRNA kit according to the manufacturer's protocol (Innuscreen, 845-KS-2040010). 1–3 μ g of RNA was reverse-transcribed using Random Hexamer Primers (Thermo Scientific,

SO142) and RevertAid Reverse Transcriptase (Thermo Scientific, EP0442). Reverse transcription was carried out under the following conditions: 25°C for 10 minutes, 40°C for 60 minutes, and 70°C for 10 minutes. Quantitative PCR was performed using GoTaq® qPCR Master Mix (Promega, A6002) in technical triplicates, with PBGD serving as the reference gene.

2.18. Luciferase assay plasmid cloning

Genomic elements of interest were PCR amplified from hTSC genomic DNA with Q5 High-Fidelity 2X Master Mix (NEB, M0492). Sequences of the selected genomic regions, including primer sequences, are stated in Table 18. Plasmid backbone pGL4.26[luc2/minP/Hygro] (Promega, E844A) and PCR products were digested with NheI-HF (NEB, R3131) and HindIII-HF (NEB, R104). Afterwards, they were ligated using T4 DNA ligase (NEB, M0202) in a 1:3 vector: insert molar ratio, overnight at 4°C. The whole ligation reaction was transformed into chemically competent *E. coli* bacteria and plated on LB-agar plates containing 100 µg/mL Amp. After overnight incubation at 37 °C, four colonies from each plate were picked and inoculated into 3 mL of LB-Amp media. The following day, plasmid isolation was done using the Mini-PEx kit according to the manufacturer's instructions and digested using NheI-HF and HindIII-HF to confirm the presence of the desired element. Positive colonies were sent for Sanger sequencing using RV3 primer (5'-CTAGCAAATAGGCTGTCC-3'). Positive clones were grown in 100 mL LB-Amp media overnight at 37 °C. Plasmids were isolated using the QIAGEN Midi prep kit (12145X4) following the manufacturer's instructions.

Table 18. **Genomic elements selected for luciferase assay**

Gene	Target sequence (5'-3')	Primer sequence (5'-3')
<i>KRT7</i>	TTGCCTTGAGGCCCTGGACATGTCACTGC CCCTCCCTGGGCCATGGATTTTACCCA AGAT	CTAGAGGAATCTCACTGCCCTCC T
		AGCTTAGTGCTGGCCTGTGAATG G

<i>SDC1</i>	GTCCCTAAAAACAATTTGTTCCCTCAGTTC GGCTGGCTTATCCCTCTGCAATCTTGTTA CACAACAAGCTCTTAAGCAAATGTTTTCTC CACTGAAACCAGCTCCCTGGCCTCCGGG GGCCTCTCCCTCCCTGGGCCTTCAGTCT CCACTAAGGGGACAGGCTAGAGAGACCT CCCAAATCTTCTCCAGCTTGAATCTTCTGG GA	CTAGCATCCAAGGACTCTGCCCT G
		AGCTGTCTCACCAGAAACTCCGG G
<i>SDC1</i>	TTTTTTTTGAGACAAGGTCTCACTCTGTCA CCCATGCTGGGGTAGTGACGTGACGTACA TAATCACAGCTCACTGCAACCTCTGCCTC CTGGGCTCAGGTGATCTTCCCCACCCAGC CTCCACAGGCACATACCACCATGCCTGGC TAATTTGTGTATTTTTGTAGAGATGGGGT TTGCCATGTTGCCAG	CTAGCCACCTCACCTGCTTTGCT A
		AGCTCTGCCTCTCCCAGTGAATC C
<i>ENDOU</i>	ACAGCATAGCAGAGAGGTCAAGAACCTAG ACTCTGGCGCCAACTGCTTGAGGCTGGA ATTCTGGTTCTACTGCTTTCTAGCTATGTG ATCTTGGGCAAGTTATTTGAACCTCTGTGC GTTGGTTTTCGTATCTGTAAAAGTGGGATG ATAATGGAGCCTATCTCATAGAATATTTGT AAGAATTAATGAGTTAATATATGTAAAGC ACTTAGGACAGCAAGTGACACATAGTAGT ACCATAGAAGTATTTGTT	CTAGTGACATTGCGGGTTGAGGA A
		AGCTCAAGGGATGGCCCTGTTG AA
<i>GCM1</i>	GCCCAGTGTGGTAGGGTGTGCCTGCAGT CCTAGCAACTCAGGAGGCTGAGGTGGGA GGATGGCTTGAGGCCAGGGGTTCAAGAC CAGCCTGGGCAACACAGCAAGACCCCTTC CTAAAAACTCAAAATAAA	CTAGTCTGACAGTGGGATCAGC GA
		AGCTTCTCACTATCCCGCCACAA TG

2.19. Antibody list

Table 19. **List of used antibodies, WB - western blot, IF - immunofluorescence, Co-IP - co-immunoprecipitation, FACS - Fluorescence-Activated Cell Sorting**

Antibody	Producer	Raised in	Application
anti-MORC4	Sigma Aldrich, HPA015547	rabbit	WB, IF, Co-IP
anti-NCOR2/SMRT	Abcam, ab5802	rabbit	WB, Co-IP
anti-TBL1X	Proteintech, 13540-1-AP	rabbit	WB, Co-IP
anti-HDAC3	Abcam, ab32369	rabbit	WB
anti-E-CADHERIN	BD Biosciences, 610182	mouse	IF
anti-GAPDH	Abcam, ab8245	mouse	WB

anti-VINCULIN	Santa Cruz Biotechnology, sc-25336	mouse	WB
anti-SOX2	R&D Systems, AF2018	goat	IF
anti-OCT3/4	Santa Cruz Biotechnology, sc-5279	mouse	IF
APC anti-human SUSD2	BioLegend, 327408	rabbit	FACS
anti-mouse IgG light- chain specific	Dianova, 115-035-174	goat	WB
anti-rabbit IgG light- chain specific	Dianova, 211-032-171	mouse	WB
normal rabbit IgG	Sigma-Aldrich, NI01	rabbit	Co-IP control
anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 488	Invitrogen, A-21206	donkey	IF
anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 488	Invitrogen, A-21202	donkey	IF
anti-Goat IgG (H+L) Secondary Antibody, Alexa Fluor® 488	Invitrogen, A-11055	donkey	IF
anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 594	Invitrogen, A-21207	donkey	IF
anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 594	Invitrogen, A-21203	donkey	IF

3. Results

3.1. MORC4 is expressed in the self-renewing hTSCs

According to the Human Protein Atlas, MORC4 is highly expressed in the trophoblast, and I first set out to examine which trophoblast cell types it is present in. To analyse the MORC4 expression pattern, I performed immunofluorescence (IF) stainings in hPSCs, self-renewing (SR) hTSCs and differentiated ST (Figure 3.1A). MORC4 is detected only in the nuclei of the SR hTSCs, and it is not expressed in either hPSCs or differentiated ST. Additionally, I carried out IF of a known epithelial marker, E-CADHERIN (Figure 3.1A). Its expression levels are highest in the self-renewing hTSCs, and upon differentiation and cell fusion, it is downregulated in the ST. It is also expressed in the primed hPSCs in accordance with their epithelial morphology. To confirm the staining results, I performed a western blot (Figure 3.1B). As expected, MORC4 expression is detected only in undifferentiated hTSCs. MORC4 protein has a molecular weight of about 100 kDA and two known isoforms: isoform a (937 aa) and isoform b (900 aa). On the membrane, we can see two bands which likely represent the two isoforms. MORC4 protein is not detected in hPSCs or differentiated ST. I performed further characterisation of the hPSCs with IF stainings. As expected, the primed hPSCs express high levels of key pluripotency markers OCT3/4 and SOX2 (Figure 1C). This also confirms that, under the culture conditions used, cells remained undifferentiated. In conclusion, MORC4 is only expressed in the SR hTSCs.

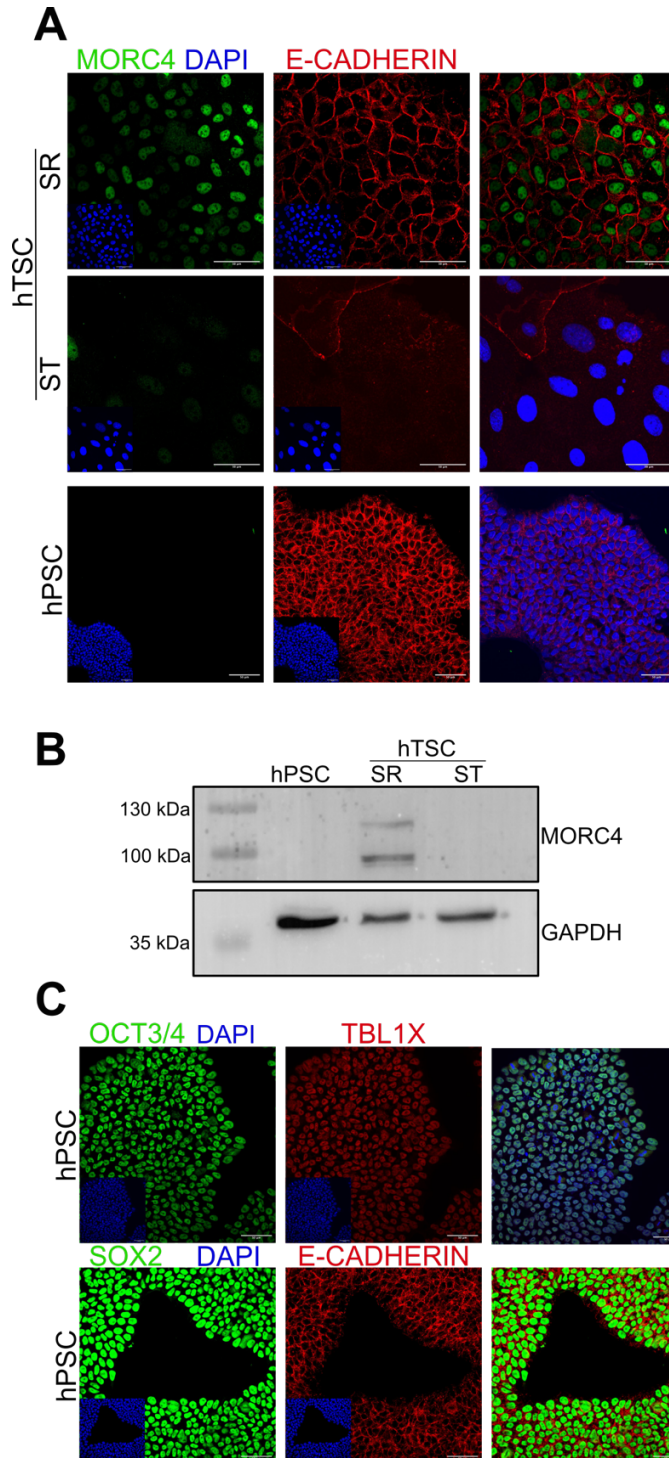


Figure 3.1. **MORC4 is expressed in the self-renewing hTSCs.** **A.** Immunofluorescence stainings of SR hTSCs, ST hTSCs (3 days differentiation) and hPSCs for MORC4 (green) and E-CADHERIN (red), counterstained with DAPI (blue). The rightmost panel shows the merged overlay of all three channels. **B.** Western blot analysis of MORC4 in hPSCs, SR and ST hTSCs. GAPDH serves as a loading control. **C.** Immunofluorescence stainings of hPSCs for OCT3/4 (green), TBL1X (red), SOX2 (green), and E-CADHERIN (red), counterstained with DAPI (blue). The rightmost panel shows the merged overlay of all three channels.

3.2. CRISPR/Cas9 inducible *MORC4*-iKO hPSC lines

To address the functional role of *MORC4* in hTSCs, we set out to examine the effects of *MORC4* depletion. To test this, we first aimed to deplete *MORC4* on a DNA level by generating inducible KO cell lines using CRISPR/Cas9 technology. We decided to utilise hPSCs instead of hTSCs as our starting model system for the generation of *MORC4* iKO cell lines, primarily due to the low efficiency in inducing genetic modifications in hTSCs.

To generate *MORC4*-iKO hPSC lines, I used the OPTimized inducible gene KnockOut method (Figure 3.2A) (Bertero et al., 2016). In this system, the sgRNA is under doxycycline-inducible expression, and the cells are genetically modified to carry constitutively expressed Cas9 and the inducible sgRNA in the *AAVS1* genomic safe harbour locus. The OPTiKO system relies on 4 vectors: the vector carrying the inducible sgRNA targeting gene of interest with puromycin resistance (Figure 3.2B), the vector carrying Cas9 with neomycin resistance (Figure 3.2C), and two vectors carrying zinc-finger nucleases targeting the *AAVS1* locus (Figure 3.2D). After successful integration of transgenes, the KO of the gene of interest can be induced by the addition of tetracycline or doxycycline.

I cloned vectors carrying 3 independent *MORC4* sgRNAs. To produce the transgenic cell lines, I started with nucleofection of the hPSCs with the four vectors (Figure 3.2B-D). After nucleofection and selection with puromycin and neomycin, single clones were picked and genotyped (Figure 3.3A). Genotyping was done in two phases (Figure 3.3B). Firstly, I screened all clones for the presence of the 300 bp neomycin resistance cassette (Figure 3.3C). Afterwards, the positive clones were expanded and screened for the integration of Cas9 and sgRNA at the respective *AAVS1* safe harbour locus (Figure 3.3D). The expected size of the sgRNA band is 919 bp, and of the Cas9 cassette is 1032 bp. Only the clones positive for appropriate integration of both the Cas9 and sgRNA were expanded for further experiments. In cases where a clone was only Cas9 positive, it was also expanded to be used as a “mother” cell line. These cells can, in the future, be targeted at the second allele with different sgRNAs. When working with single clones, we experienced

a high occurrence of spontaneous differentiation, colony collapse or death, and for this reason, we screened around 200 individual clones in total. In the end, I successfully generated two independent cell lines for each of the three *MORC4* sgRNAs.

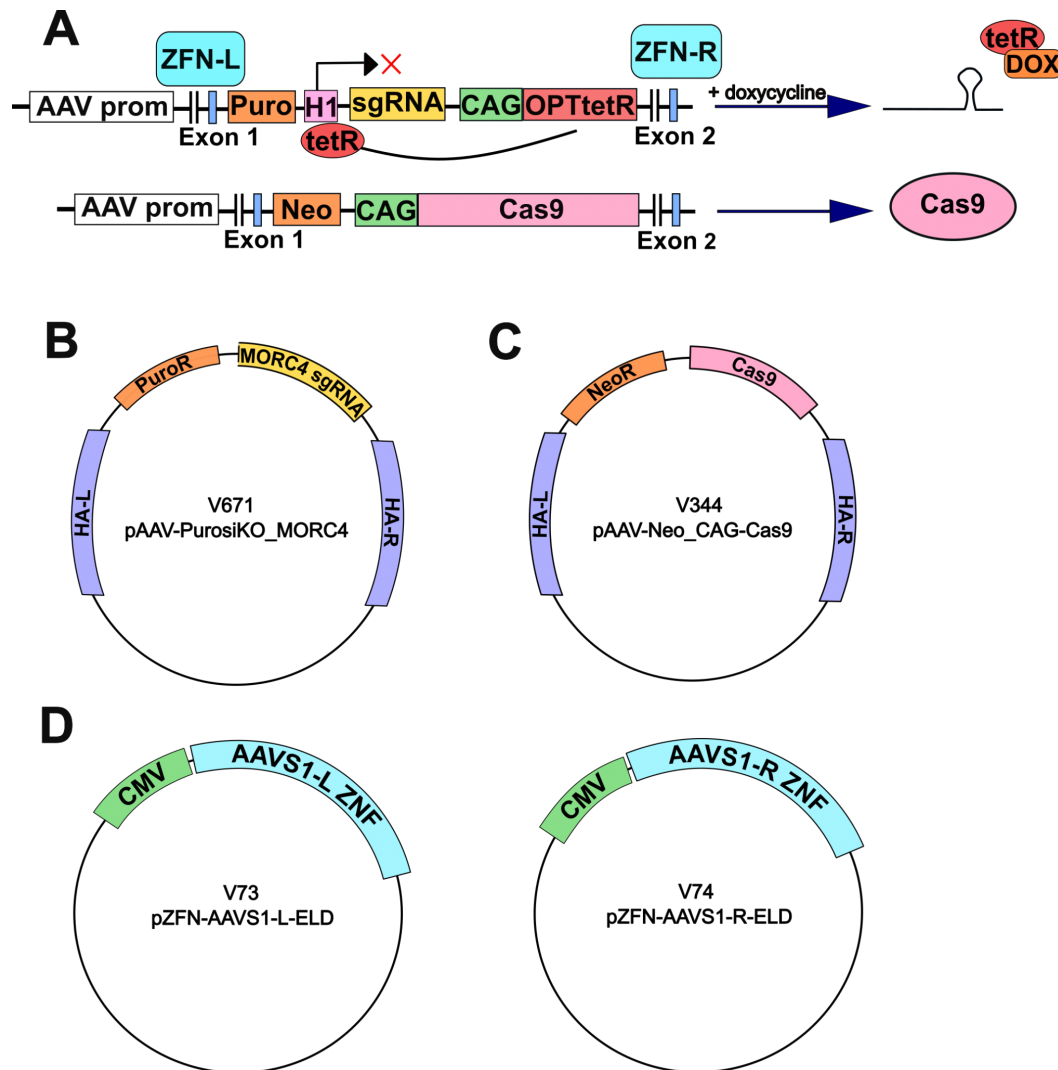


Figure 3.2. **OPTimized Inducible KnockOut system.** **A.** Schematic representation of the *AAVS1* transgenic alleles. ZFN, zinc-finger nuclease; H1, H1 promoter; tetR, tetracycline-sensitive repressor; Puro, puromycin resistance; Neo, neomycin resistance; DOX, doxycycline; CAG, CAG promoter. **B.** Inducible *MORC4* sgRNA cassette carrying vector. HA - *AAVS1* homology arms. **C.** Cas9 cassette carrying vector. **D.** Left and right zinc-finger vectors. CMV, cytomegalovirus promoter.

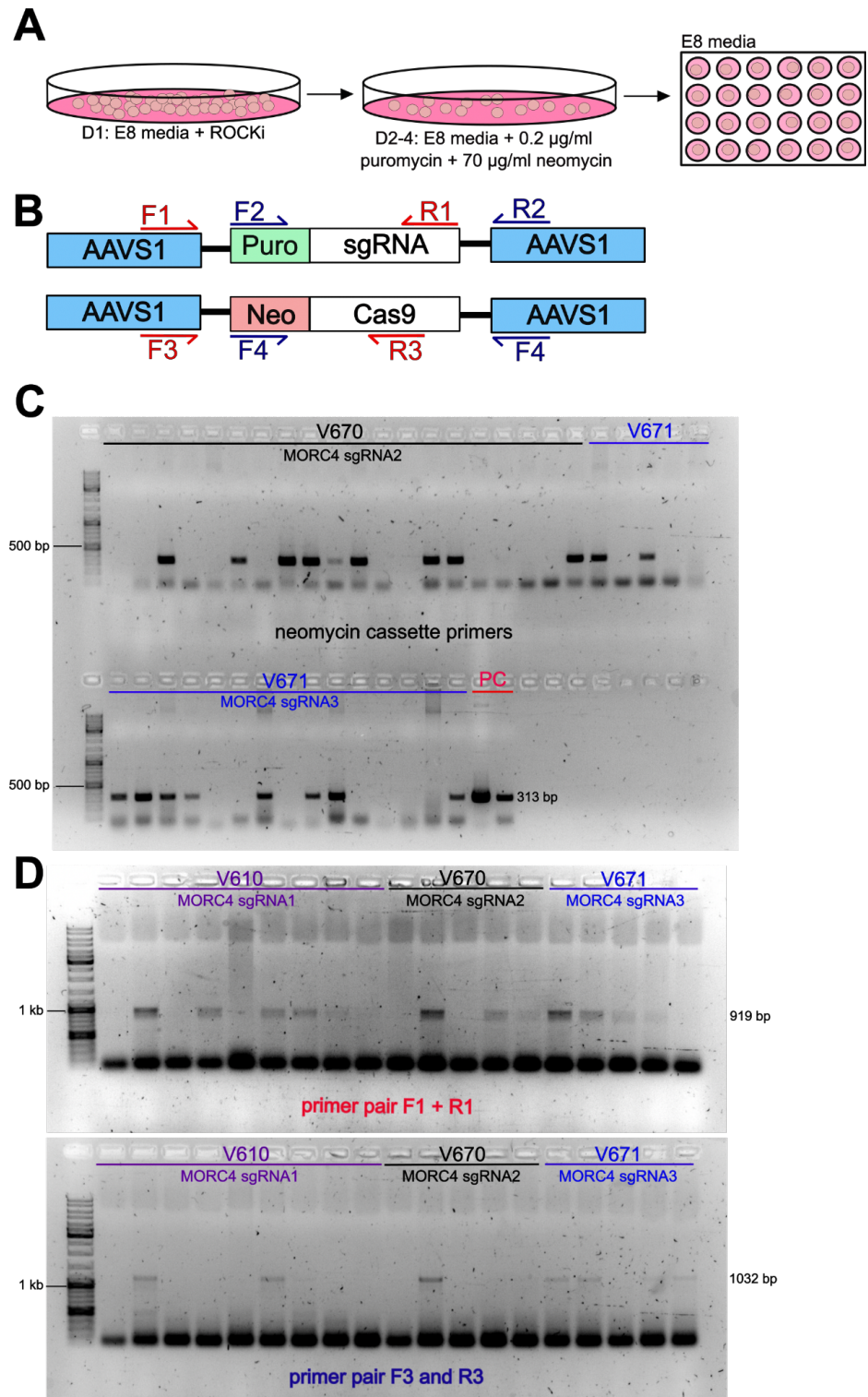


Figure 3.3. **Generation of CRISPR/Cas9 *MORC4*-iKO hPSC lines.** **A.** Schematic representation of the experiment workflow. **B.** Genotyping strategy. F, forward primer; R, reverse primer. **C.** Representative genotyping PCR for the presence of the neomycin resistance cassette. Positive control band size is 313 bp. **D.** Representative genotyping PCR of both transgenic AAVS1 alleles. Positive band for primer pairs F1/R1 is 919 bp, and for F3/R3 is 1032 bp.

3.3. Naive conversion of primed hPSCs produced a SUSP2-positive cell population

To investigate the function of MORC4 in trophoblast identity and development, we had to transdifferentiate the newly generated *MORC4*-iKO hPSC lines into hTSCs. Conventionally cultured primed hPSCs cannot directly differentiate into extraembryonic cell types such as hTSCs, but they first have to be converted into the naive pluripotency state (Figure 3.4A). Afterwards, naive hPSCs can be transdifferentiated into hTSCs, in which we can test the effects of MORC4 depletion (Dong *et al.*, 2020; Guo *et al.*, 2021). We decided to utilise an integration-free naive induction system, meaning that no transgenes were introduced into the endogenous genome. I electroporated wild-type hPSCs, *MORC4*-iKO and iKO empty control lines with a vector containing *SOX2-A16V*, *KLF4*, *MYC* and *EBNA1* (Figure 3.4B). *SOX2-A16V*, also known as super-SOX, has enhanced SOX2/OCT4 dimerisation ability as a result of swapping one alanine for a valine amino acid residue (MacCarthy *et al.*, 2024; Okita *et al.*, 2013). Transient overexpression of *super-SOX2* and *KLF4* boosts the developmental potential of hPSCs, i.e. converts the cells to the naive state, and EBNA1 functions to enhance electroporation efficiency and expression of episomal plasmids (Okita *et al.*, 2013).

Unlike primed, naive hPSCs are cultured on a feeder layer in a PXGL medium (Figure 3.4C). As cells change their identity from primed to naive, they also change their morphology. In contrast to the epithelial phenotype of primed, naive hPSCs, which grow in dense, dome-shaped colonies (Figure 3.4D). They have to be passaged every three days to prevent cells from detaching, which can occur as a result of colony size. In order to get a more homogenous cell population, on day 7 after electroporation, cells were sorted for the naive surface marker Sushi Domain Containing 2 (SUSD2) (Figure 3.5A). Despite the same initial number of cells, post-induction cell line-specific differences appeared. On average, more than 90% of sorted single cells were SUSD2 positive (SUSD2+) (Figure 3.5A).

To confirm the naive identity of sorted cells, I performed preliminary RT-qPCR analysis of the expression levels of key pluripotency markers in primed hPSCs, two naive cell lines (wild type and *MORC4*-iKO) and SR hTSCs (Figure 3.5B). Both naive cell lines show higher expression of *NANOG* and *OCT4* compared to their primed counterpart. Interestingly, the naive *MORC4*-iKO had lower levels of *SOX2* mRNA compared to the primed and naive wt hPSCs. As expected, expression of pluripotency genes was practically undetectable in the hTSCs, while the trophectoderm marker *TEAD4* was highly upregulated. *TEAD4* transcripts were also present in naive cells, suggesting partial spontaneous differentiation to the trophectodermal fate. This experiment will be performed in biological replicates to assess the statistical significance of these results. In short, the SUSD2+ population of cells have upregulated core pluripotency genes, indicating successful naive conversion.

As blastoids recapitulate aspects of preimplantation events and lineage specification, I aimed to use this system to examine whether *MORC4* knockout affects in vitro blastoid formation, and thereby, its role in early human development. In that case, impaired blastoid formation or cavitation would suggest that *MORC4* has a function in TE establishment. But first, I needed to test the blastoid-generating ability of the naive cell lines I previously produced (Figure 3.6). Therefore, I seeded PXGL naive hPSCs in an Elplasia Microcavity plate in N2B27 media supplemented with BSA and Y-27632. The following day, the cells formed tight aggregates at the bottom of the microwell. Blastoid formation is induced for 2 days with the addition of PALLY medium, which contains PD0325901, A83-0, 1-Oleoyl lysophosphatidic acid (LPA), leukaemia inhibitory factor (LIF) and Y-27632 (Heidari Khoei et al., 2023; Kagawa et al., 2021). By the end of day 2, aggregates should initiate cavity formation. While we could observe aggregates growing in size and becoming more compact in the first two days, by the third day, they did not cavitate (Figure 3.6B). Potential reasons for this can be an insufficient concentration or activity of growth factors, suboptimal initial cell number or naive cell population heterogeneity. The homogeneity of the starting cell population seems to be the critical factor for blastoid formation, and it can be improved with an additional round of cell

sorting and propagation. Additionally, different seeding concentrations will be tested.

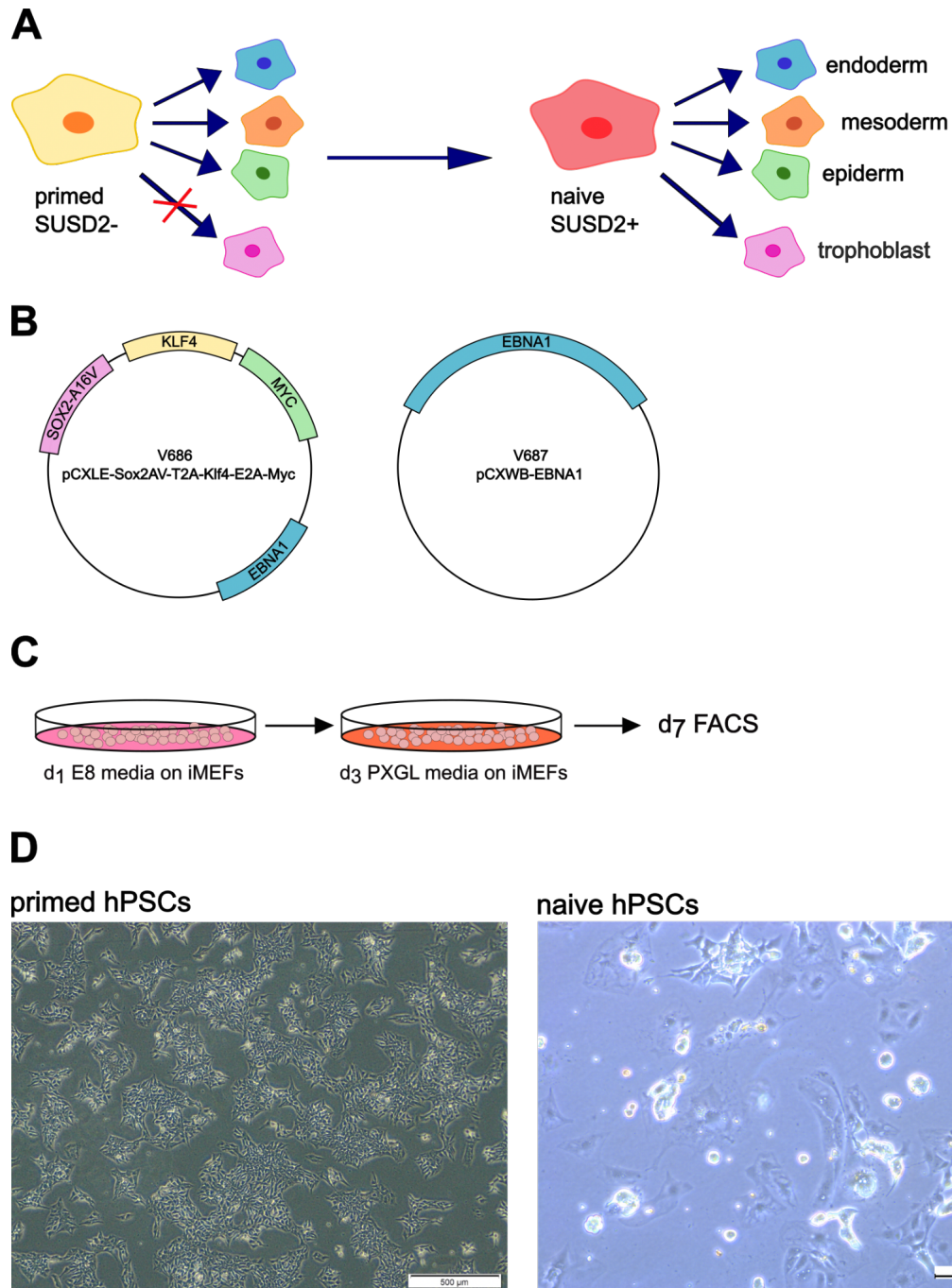


Figure 3.4. Integration-free naive induction of hPSCs. **A.** Differentiation potential of primed and naive hPSCs. **B.** Schematic representation of naive induction vectors. **C.** Naive conversion experimental workflow. **D.** Representative live-cell images of wild-type primed and naive (passage 10 after FACS sorting) hPSCs.

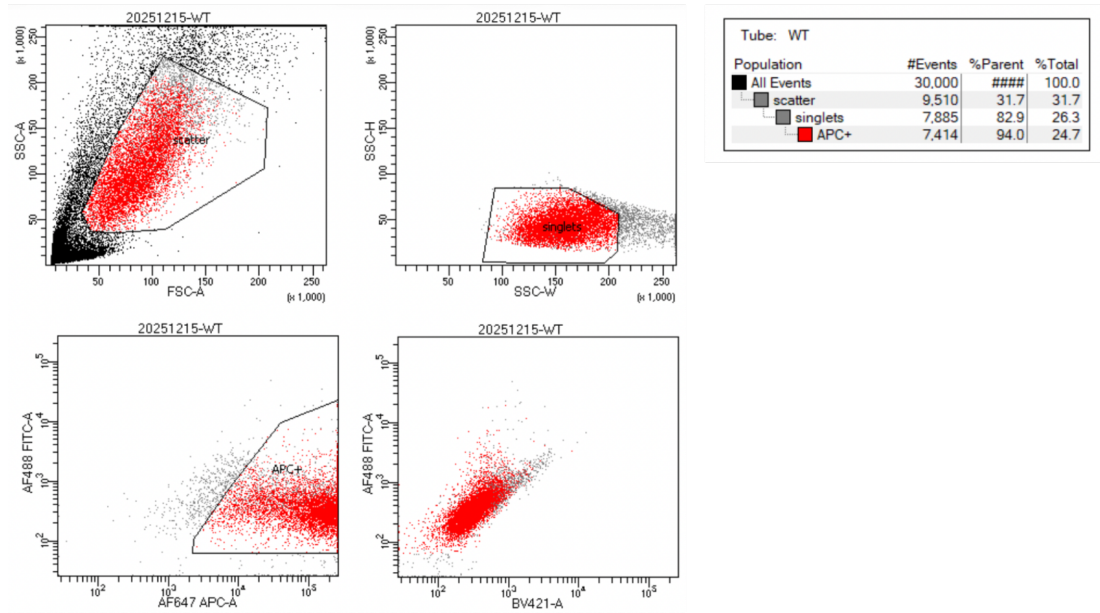
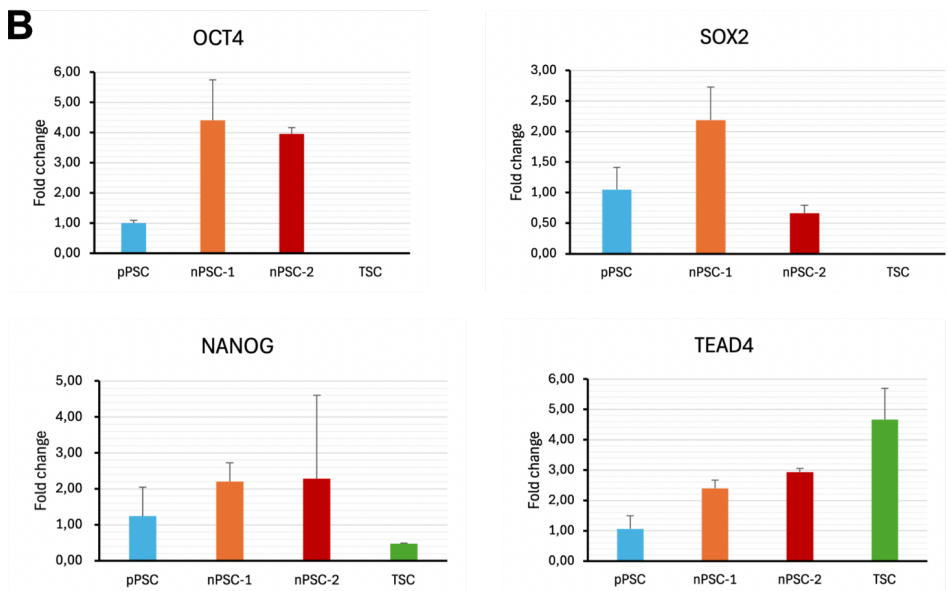
A**B**

Figure 3.5. *SUSD2*⁺ naive hPSCs show high expression of key pluripotency genes. A. Fluorescence-activated cell sorting plots of *SUSD2*⁺ wild-type hPSCs 7 days after naive induction. **B.** Preliminary RT-qPCR analysis of primed hPSCs (pPSC), wild-type H9 naive hPSCs (nPSC-1), *MORC4*-iKO naive hPSCs (nPSC-2) and self-renewing hTSCs. Expression levels were normalised to *PBGD* and calculated using the $2^{-\Delta\Delta CT}$ method (n=1).

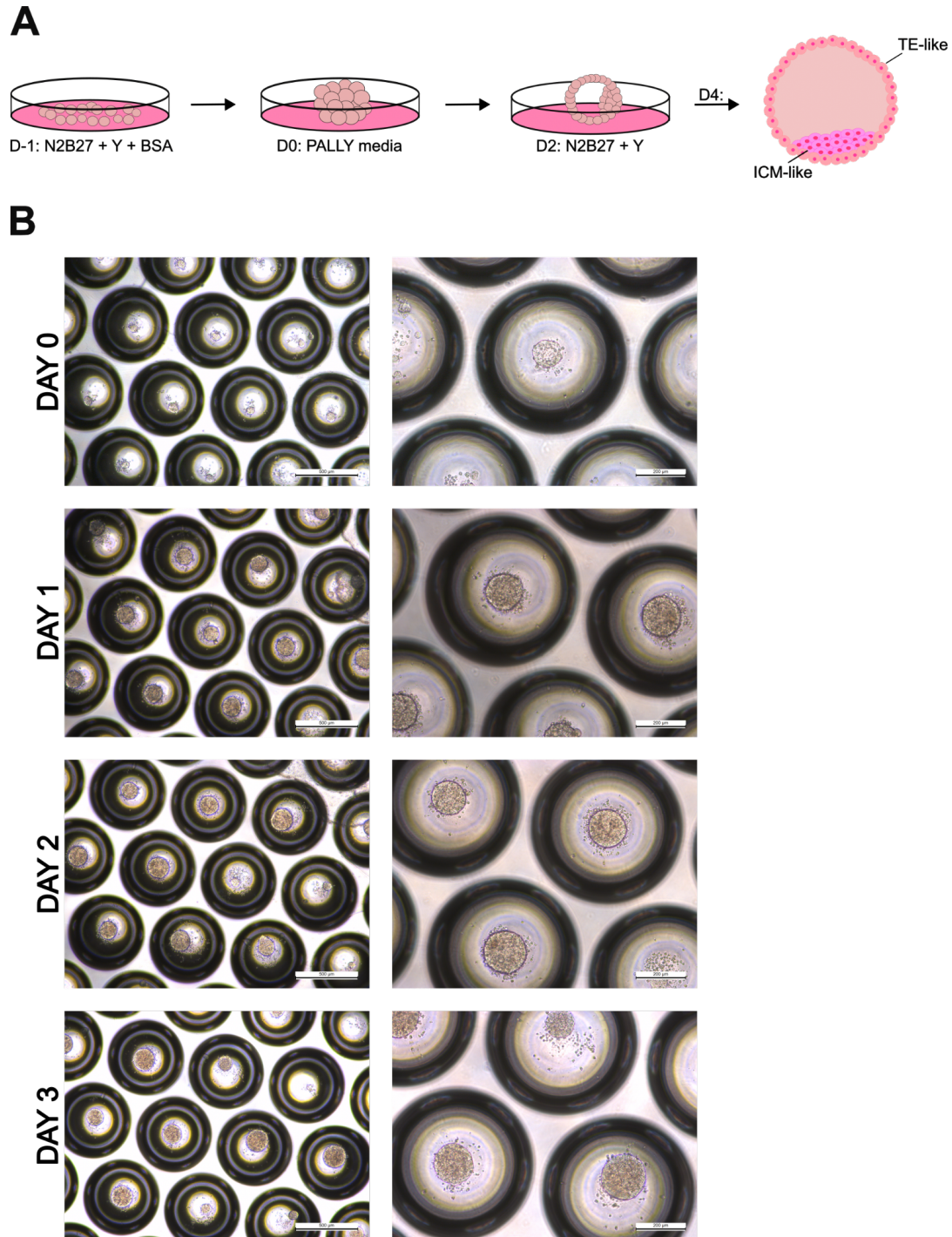


Figure 3.6. **Naive hPSCs aggregated but did not cavitate.** **A.** Schematic representation of human blastoid development protocol according to Kagawa *et al*, 2021. **B.** Representative live-cell images of the blastoid generation attempt in the Elplasia 96-well plate. Microwell size is 500 μ m, and 8000 cells were seeded per well.

3.4. Inducible *MORC4*-iKD and *NCOR1/2*-iKD hPSC lines

Unpublished data from the Latos lab has demonstrated that *MORC4* interacts closely with the Nuclear receptor co-repressor (NCoR) 1 and 2 complexes. Both NCoR1 and NCoR2, also known as SMRT, complexes are essential for development, cell fate decisions, differentiation, and lipid metabolism (Hermanson et al., 2002; P. Li et al., 2011; Mottis et al., 2013; Nofsinger et al., 2008; Zhuang et al., 2018). In addition to *NCOR1* and *NCOR2*, their canonical subunits are the G protein pathway suppressor 2 (*GPS2*), transducin beta like 1 X-linked (*TBL1X*), *TBL1X/Y* related 1 (*TBL1XR1*) and histone deacetylase 3 (*HDAC3*) (Fischle et al., 2002; Guenther et al., 2001; Mottis et al., 2013; Oberoi et al., 2011a; Sun et al., 2013).

Based on this finding, we wanted to examine whether this *MORC4*-NCoR interaction is essential for its trophoblast function. To test this, I aimed to generate iKD cell lines for each of the complex components in addition to *MORC4*. I used the OPTimized inducible gene KnockDown method (Figure 3.7A) (Bertero et al., 2016). The system works on the same principle as the inducible knockout. The cells are genetically modified to carry the tetracycline-inducible shRNA in the *AAVS1* genomic safe harbour. It relies on 3 vectors: the vector carrying the inducible shRNA targeting gene of interest with puromycin resistance (Figure 3.7B) and two vectors carrying zinc-finger nucleases targeting the *AAVS1* locus (Figure 3.7D).

I cloned 2 different shRNA-carrying vectors targeting each of the *NCOR1/2* components: *NCOR1*, *NCOR2*, *TBL1X*, *HDAC3* and 3 shRNAs for *MORC4*. To generate the transgenic cell lines, I nucleofected the hPSCs, followed by two day puromycin selection (Figure 3.8A). I picked and genotyped single clones. To identify recombinants with the desired integration site, I designed primers that bind inside the transgenic cassette and in the *AAVS1* locus (Figure 3.8B, C). Positive clones should yield a 480 bp long PCR product, as can be seen on the agarose gel (Figure 3.8C). In total, we screened 2600 individual clones and generated 35 independent cell lines, at least two for each shRNA construct, including an empty vector and *GFP* shRNA control lines (Figure 3.8D).

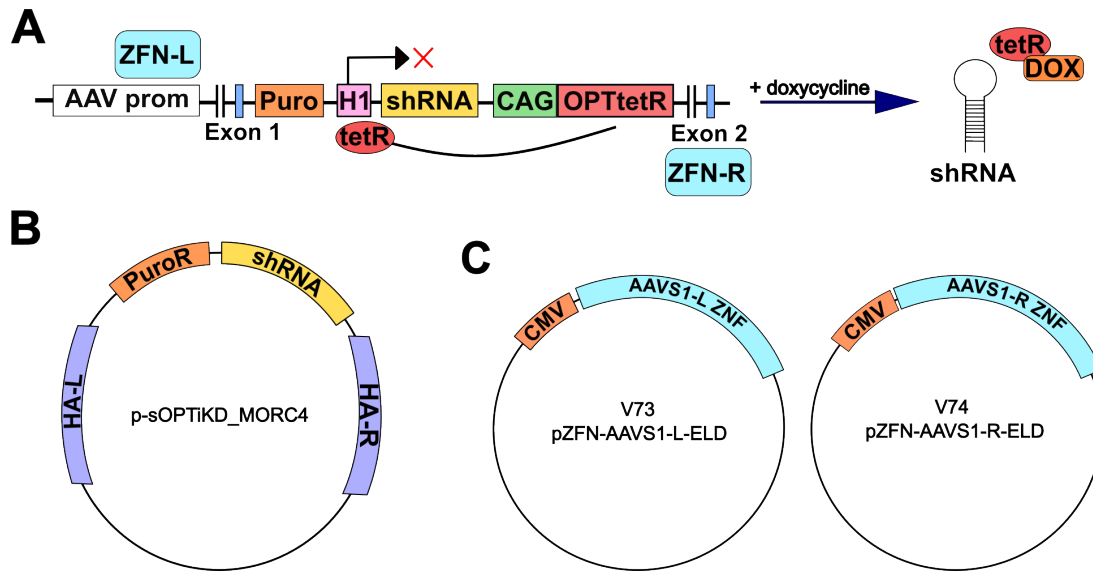


Figure 3.7. **OPTimized Inducible KnockDown system.** **A.** Schematic representation of the AAVS1 transgenic allele. ZFN, zinc-finger nuclease; H1, H1 promoter; tetR, tetracycline-sensitive repressor; Puro, puromycin resistance; DOX, doxycycline; CAG, CAG promoter. **B.** Inducible shRNA-carrying vector. HA, AAVS1 homology arms. **C.** Left and right zinc-finger vectors. CMV, cytomegalovirus promoter.

Before starting naive induction of these newly generated cell lines, I wanted to test the efficiency and robustness of the system. Therefore, I treated the cells for 24 and 48h with doxycycline and performed the preliminary RT-qPCR analysis of the *NCOR1*, *NCOR2* and *HDAC3* transcript levels. As a control, I used a cell line with the iKD cassette that is missing a shRNA sequence, and the fold change in expression levels was compared to the untreated control cell line, i.e. homeostatic levels. Doxycycline treatment caused about 40% upregulation of *NCOR1/2* and 50-60% of *HDAC3* in the control. Untreated *NCOR1* and *NCOR2* iKD lines showed the same levels of target transcripts as in the control, and knockdown induction caused 50% downregulation after 24h and more than 60% after 48h. In the *HDAC3* iKD cells, the 24h drug treatment resulted in 50% reduction and almost 80% after 48h. While these are only preliminary results, they show that the iKD system used is robust and selected shRNAs are efficient at eliminating target transcripts.

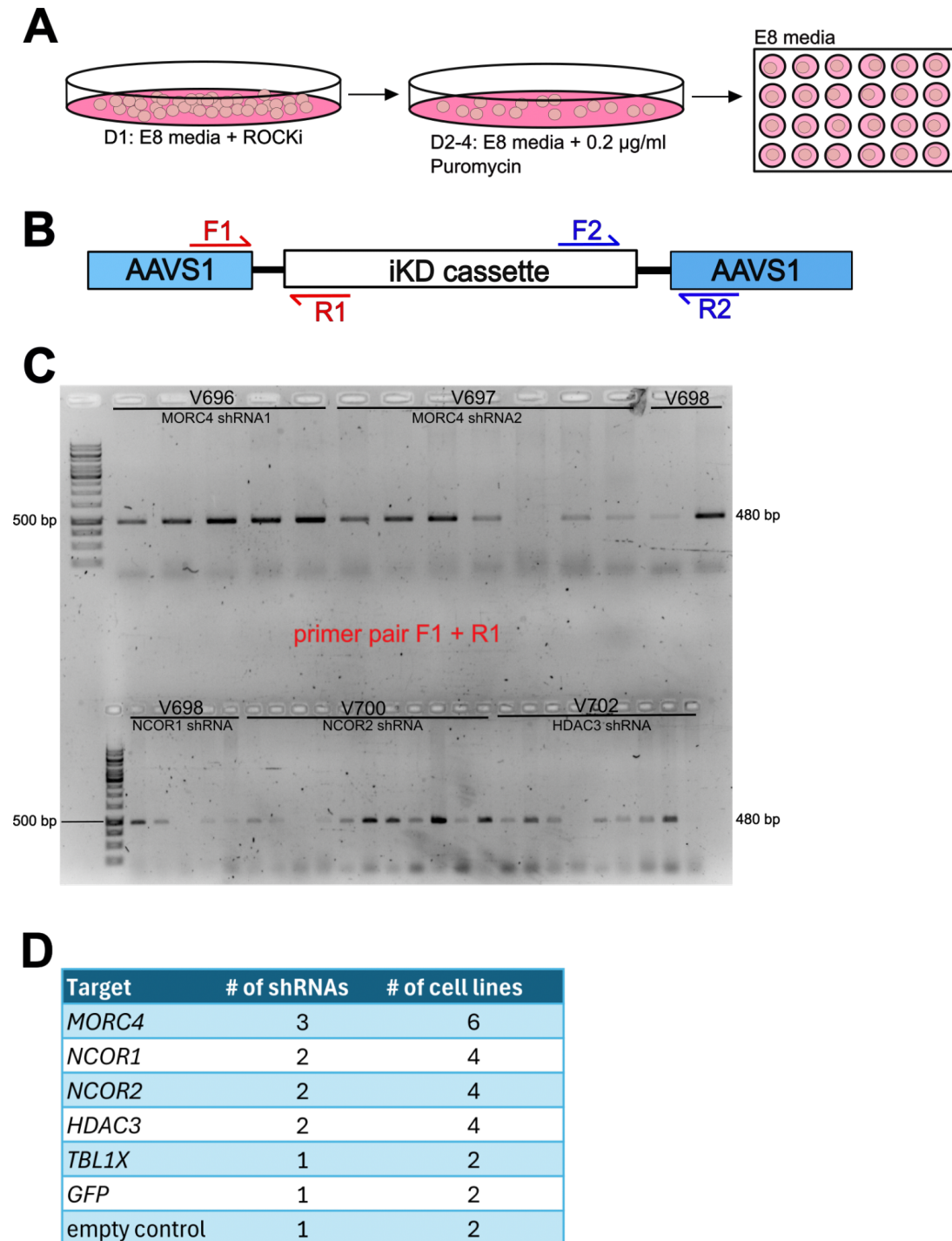


Figure 3.8. **Generation of iKD hPSC lines.** **A.** Experimental workflow scheme. **B.** Genotyping strategy. F, forward primer; R, reverse primer. **C.** Representative genotyping PCR of the transgenic AAVS1 allele. The positive band size for primer pairs F1/R1 is 480 bp. **D.** Summary table of generated iKD hPSC lines.

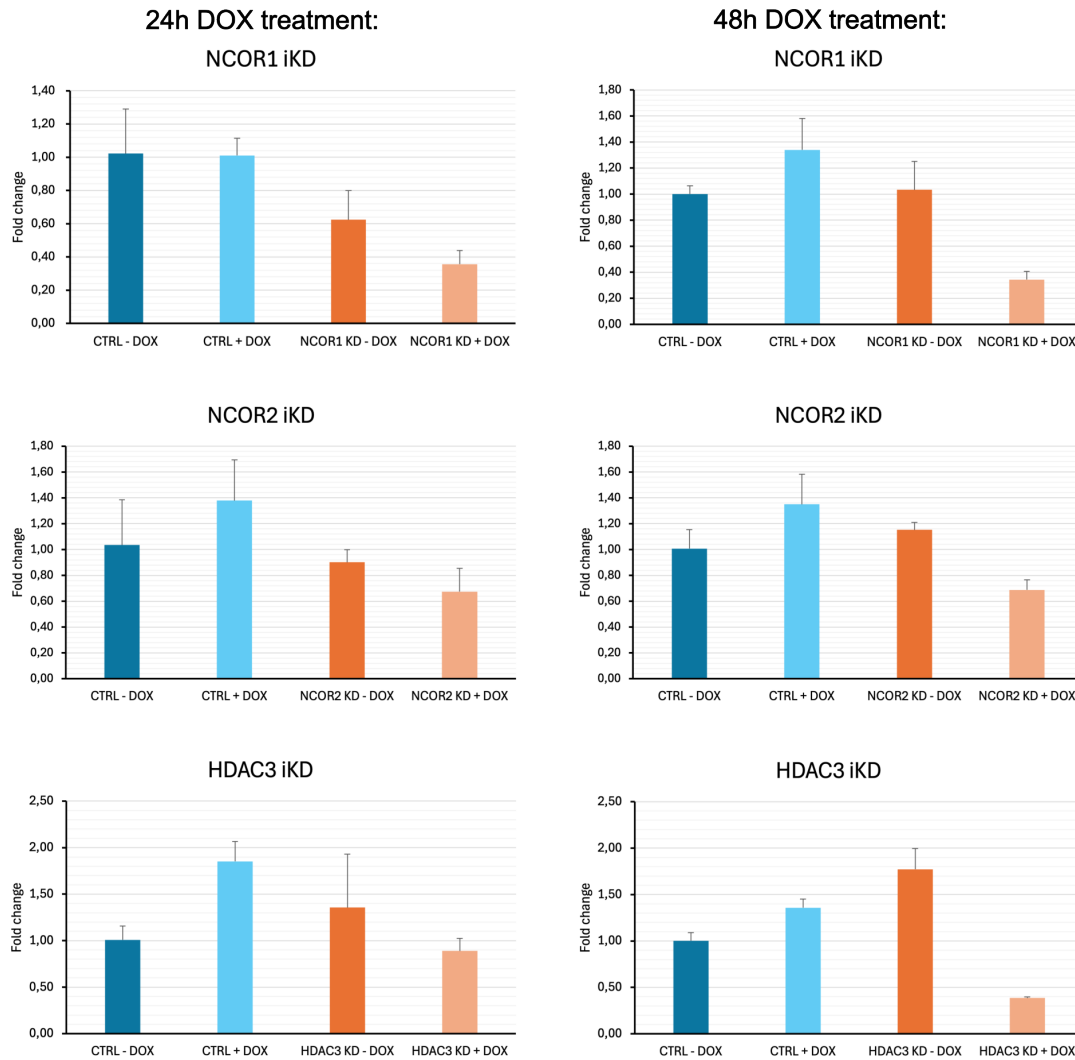


Figure 3.9. Inducible knockdown results in the downregulation of target mRNAs. Preliminary RT-qPCR analysis of the control, NCOR1, NCOR2 and HDAC3 iKD cell lines upon 24 (L) and 48h (R) doxycycline treatment. DOX, doxycycline. Expression levels were normalised to *PBGD* and calculated using the $2^{-\Delta\Delta CT}$ method (n=1).

3.5. Generation of MORC4 degron hPSC lines

Compared with RNA or DNA level loss-of-function approaches, inducible protein degradation systems offer greater temporal precision and reversibility, enabling rapid depletion and clearer linkage between protein loss and observed phenotypes. For this reason, I utilised the degradation tag (dTAG) system to generate the MORC4-degron hPSC lines (Brand & Winter, 2019; Nabet et al., 2018). The dTAG system is based on tagging a protein of interest in frame with a small protein FKBP12^{F36V} mutant. Upon treatment with the dTAG ligand, the protein of interest is recognised by the endogenous E3 ligase receptor CRBN, leading to subsequent ubiquitination and protein degradation (Figure 3.10B). The PITCh system is based on co-transfection of two vectors: a repair vector containing the dTAG cassette flanked by 20 bp microhomology regions and synthetic PITCh-sgRNA recognition sites, and a cutting vector carrying *Cas9* and two sgRNAs (Figure 3.10C). One targeting the genomic locus of interest and the other targeting the synthetic PITCh sites on the repair vector. Upon transfection, the genome is cut at the locus of interest and the dTAG cassette is integrated via homology-directed repair (HDR) (Figure 3.10A). The transgene also contains an integrated antibiotic resistance (puromycin or blasticidin) driven by the endogenous promoter of the protein of interest (POI). Unfortunately, since *MORC4* is not expressed in the hPSCs, antibiotic resistance was not relevant in our experimental setup.

I cloned two sgRNAs for both the N- and C-terminal tagging of the endogenous MORC4 locus into the degron cutting vector. I started by digesting the cutting vector backbone with BbsI-HF restriction endonuclease. The size of the backbone is 8.9 kbp, and the corresponding bands can be seen on the agarose gel (Figure 3.10D). Next, I digested the repair vector backbone with MluI. The size of the desired plasmid is 4.5 kbp, and the size of the excised dTAG cassette is 1000 bp (Figure 3.10E). I amplified the 1 kb dTAG cassette using sgRNA-specific, microhomology-containing PCR primers (Figure 3.10F). After successful cloning of 4 cutting and 4 repair vectors, I electroporated the hPSCs and plated them at very low densities. Because of a lack of antibiotic selection, I had to genotype a large number of single clones (Figure 3.11A).

Since this approach did not yield positive results after multiple reruns, we decided to change our strategy.

I designed a smaller knock-in cassette in the form of an HDR oligonucleotide that contained only the dTAG without the antibiotic resistance cassette. The genotyping strategy was based on having one primer inside the dTAG cassette and one primer inside the MORC4 locus (Figure 3.11B, C). The expected band size of dTAG-positive clones is around 300 bp for both N- and C-terminal tagging. Positive clones were regentyped with a longer PCR with both primers binding inside the MORC4 locus. The size of the wt band is around 300 bp, and the knock-in cassette is 300 bp long (Figure 3.11D). With the modified approach, I screened 4500 clones, detected 20 positive clones and successfully expanded 1 N-terminal tagged cell line. Since MORC4 is not expressed in the hPSCs, the next step is to transdifferentiate this MORC4-degron cell line into hTSCs. Afterwards, hTSCs will be treated with the dTAG ligand and protein levels will be analysed on a western blot to assess system efficiency.

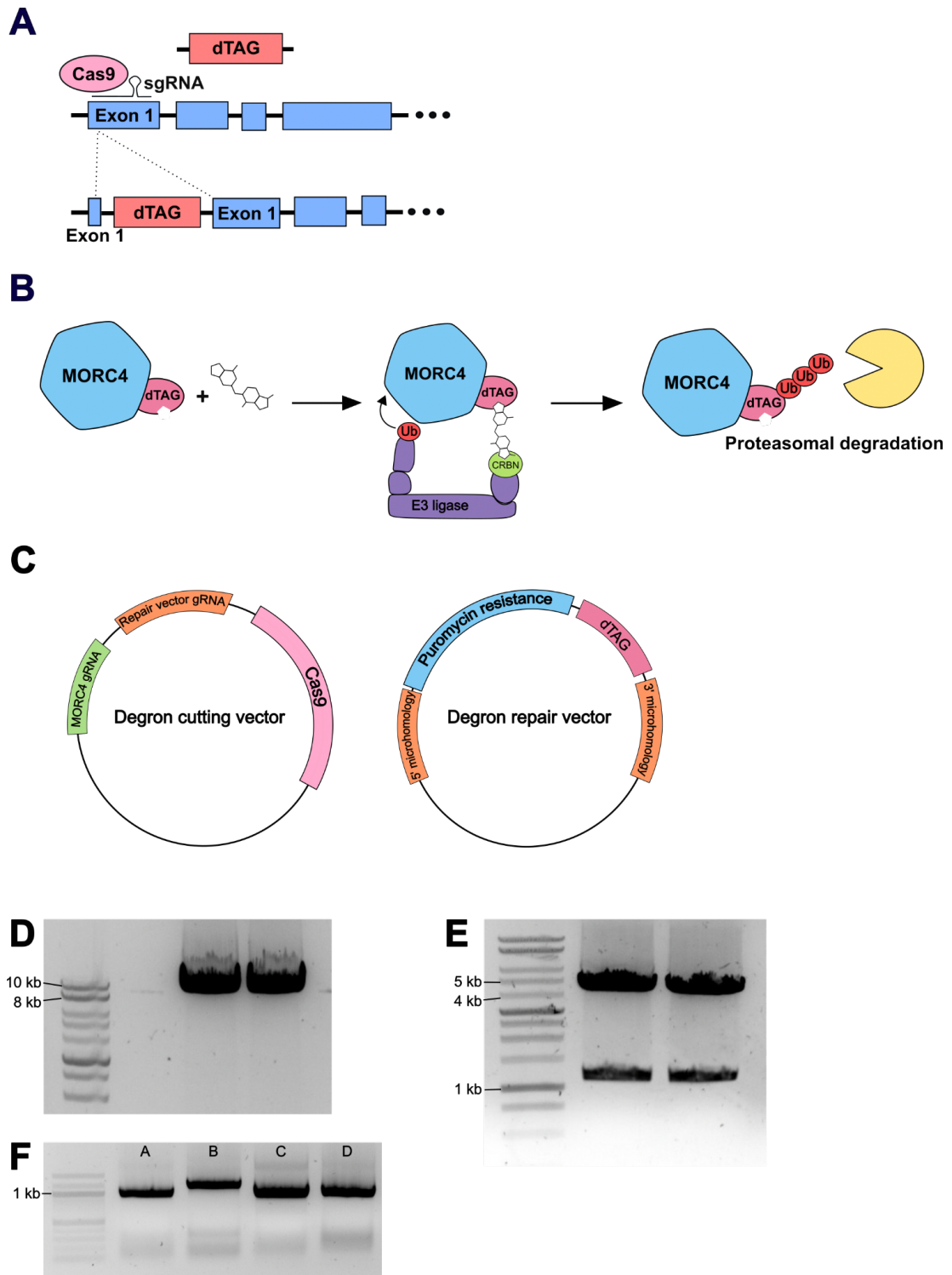


Figure 3.10. **Inducible protein degradation dTAG system.** **A.** Schematic representation of the endogenous locus tagging strategy. **B.** Inducible protein degradation system mode of action scheme. Ub, ubiquitin. **C.** PITCH dTAG system vectors **D.** Restriction digestion of degron cutting vector (9 kb) with BbsI-HF stained with SYBR Green. **E.** Restriction digestion of degron repair vector (4.5 kb) with MluI stained with SYBR Green. **F.** PCR amplification of the dTAG cassette. A/ B - N-terminal tag, C/D - C-terminal tag.

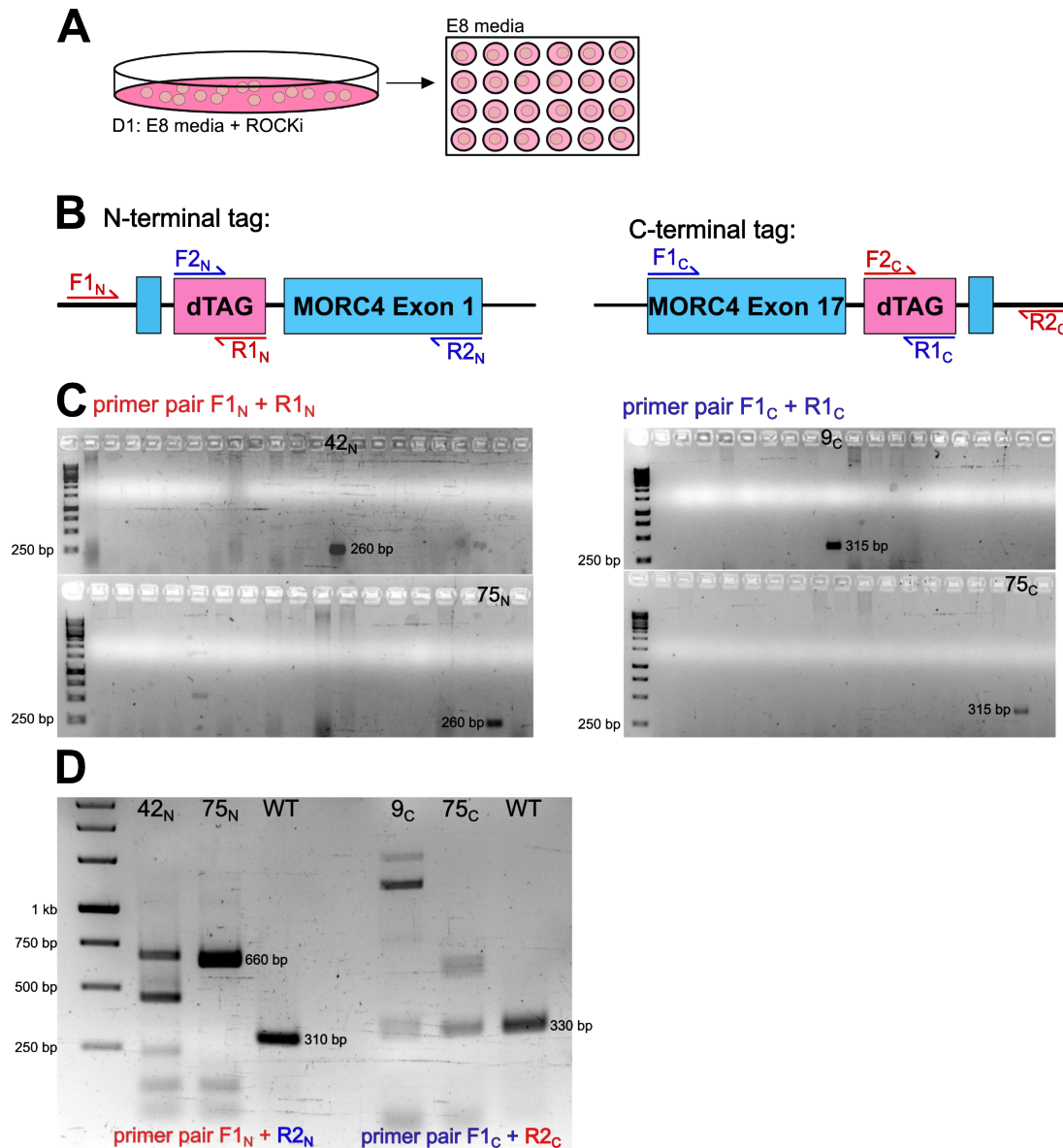


Figure 3.11. **Generation of degron dTAG hPSC lines.** **A.** Scheme of the experimental setup. **B.** Genotyping strategy of N- and C-terminal dTAG clones. F, forward primer; R, reverse primer. **C.** Genotyping PCR of the MORC4 locus. Positive band size for primer pairs $F1_N/R1_N$ is 260 bp and for $F1_C/R1_C$ is 315 bp. **D.** Longer genotyping PCR of dTAG N-/C-terminal dTAG clones and hPSC wt. *MORC4* wt PCR product size is 310/330 bp. dTAG-containing *MORC4* locus size is 660 bp.

3.6 MORC4 interacts with the NCoR1/2 complexes

As previously mentioned, MORC4 has been identified as an interactor of the NCoR1 and 2 complexes in the self-renewing hTSCs. For this reason, I wanted to test the stability of the complex and its interaction with MORC4 upon depletion of different subunits using the shRNA-mediated knockdown. shRNA-containing vectors were transfected into HEK293 cells for lentiviral particle generation. hTSCs were transduced with the aforementioned viral particles and maintained in the self-renewal medium with neomycin selection. Finally, the cells were harvested for protein analysis.

I examined the KD efficiency via a western blot (Figure 3.12A). MORC4 had been successfully depleted with both of the 2 shRNAs tested, and shRNA 1 was selected for further experiments. The depletion of other components of the NCoR1/2 complexes did not result in the downregulation of MORC4. *TBL1X*-targeting shRNA reduced TBL1X protein levels, whereas KD of other subunits had no effect. Unfortunately, I was unable to detect NCOR1 and 2 protein levels due to the lack of a good commercially-available antibody.

To investigate the interactions of MORC4 with the NCoR1 and 2 complexes, I decided to perform a co-immunoprecipitation experiment with TBL1X as the bait (Figure 3.12B, C). Previous structural studies have determined that TBL1X serves as the structural scaffold for the assembly of the SMRT/NCoR complexes, making it the perfect target for identifying the complex interactors (Oberoi *et al*, 2011). As expected, with TBL1X I pulled down MORC4 and HDAC3, which is a known subunit of the complex (Figure 3.12C). Surprisingly, upon NCOR2 and TBL1X KDs there are lower MORC4 levels in the input. This could be a secondary effect of the general KD phenotype, which is the reduction in cell proliferation and high levels of apoptosis (Figure 3.12B). Consequently, the amount of detected MORC4 in the TBL1X IP is lower in NCOR2 and TBL1X KDs compared to the empty vector control (Figure 3.12B).

On the other hand, NCOR1 KD did not affect MORC4 levels, neither in the input nor in the TBL1X IP. Suggesting that TBL1X interacts with MORC4 regardless of other subunits, consistent with its role as the scaffold. HDAC3

levels and its interaction with TBL1X are unaffected by MORC4 and NCOR1 KDs. In conclusion, MORC4 interacts with the NCoR1/2 complexes in hTSCs, but its depletion does not affect core complex assembly. The TBL1X subunit acts as a scaffold and is most likely the direct binder of MORC4.

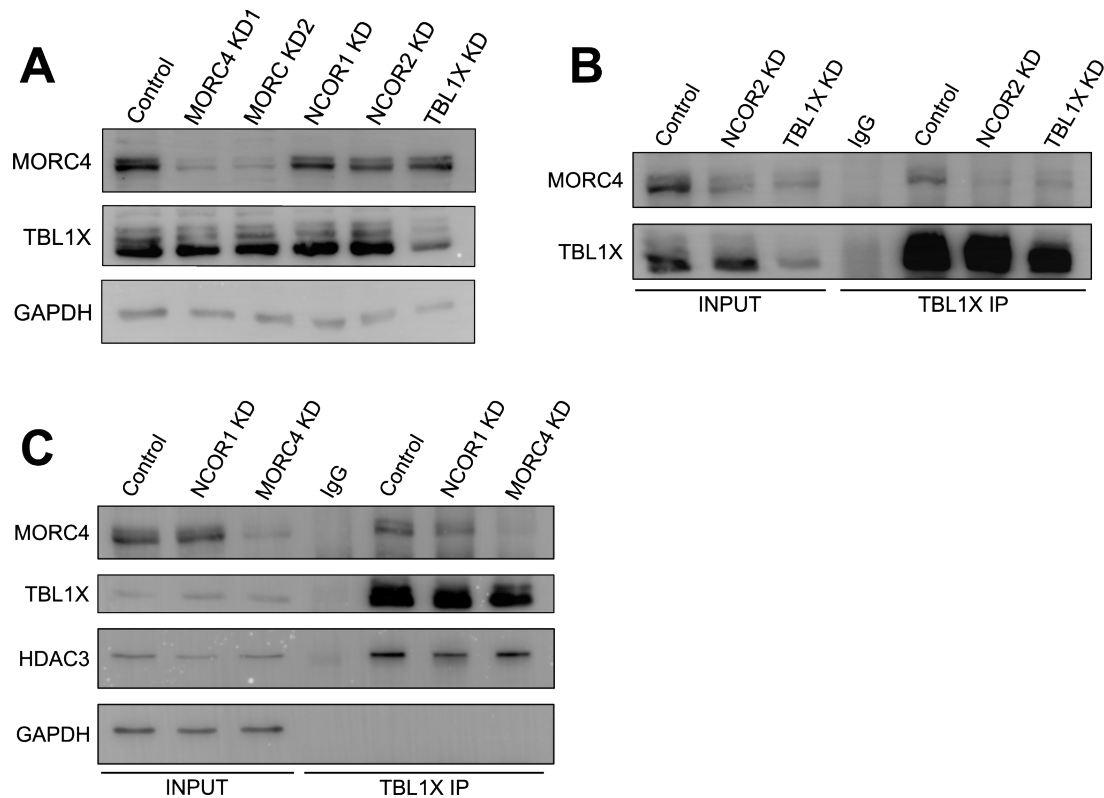


Figure 3.12. **MORC4 interacts with the NCoR complex in the self-renewing hTSCs.** **A.** Western blot analysis of the knockdown efficiency of MORC4, NCOR1, NCOR2 and TBL1X. An empty vector was used as a control, and GAPDH was used as a loading control. **B.** Anti-TBL1X co-immunoprecipitation in SR hTSCs after NCOR2 or TBL1X shRNA-mediated knockdown with empty vector control, analysed by western blot. IgG serves as the antibody control. **C.** Anti-TBL1X co-immunoprecipitation in SR hTSCs after NCOR1 or MORC4 shRNA-mediated knockdown with empty vector control, analysed by western blot. IgG serves as the antibody control. GAPDH serves as the input loading control.

4. Discussion

4.1. MORC4 as a novel epigenetic regulator

Using IF stainings and western blot analysis, I demonstrated that MORC4 is readily detectable in SR hTSCs but absent in ST. Unpublished data from the Latos lab showed that MORC4 is expressed in CT of the first-trimester placental tissue but not in ST or EVT. Given that hTSCs are established in vitro models of placental CT, my observations are consistent with these in vivo findings. MORC4 expression has also been reported in spermatogonia, which are the diploid, progenitor cells that differentiate to spermatozoa (*MORC4 Protein Expression Summary - The Human Protein Atlas*, n.d.). The restricted expression of MORC4 to the progenitor populations of CT and spermatogonia, but not their differentiated counterparts (ST, EVT, and spermatozoa, respectively), raises the possibility that in both contexts MORC4 could act as a temporal, developmental repressor that prevents premature differentiation of the progenitor pool.

Another feature shared by these two lineages is the silencing of endogenous retroviruses (ERVs) in CT and spermatogonia, and the activation of some of these elements as the cells differentiate (Frost et al., 2023; Lavialle et al., 2013; Sakashita et al., 2020; Vcelkova & Latos, 2025). The appropriate regulation of transposable elements is essential for both placental development and spermatogenesis. In the placenta, many ERVs have been co-opted and serve as cis-regulatory elements essential for trophoblast differentiation (Frost et al., 2023; Lavialle et al., 2013; Vcelkova & Latos, 2025). In the testis, ERVs act as species-specific enhancers that drive the expression of germline genes and contribute to transcriptome diversification in mammals (Sakashita et al., 2020). Notably, other members of the MORC family have been shown to play essential roles in the stable repression of ERVs. It is tempting to speculate that MORC4 could operate as a developmentally regulated repressor in trophoblast and germline contexts. MORC4, likely together with multiprotein repressor complexes, would ensure silencing of

these elements in progenitor cells. As cells differentiate and MOR4 is downregulated, the repression would be released, permitting their activation.

Proteins of the MORC family are known epigenetic regulators across many cell types. They are most notably associated with transcriptional silencing of transposable genetic elements by mediating chromatin compaction (Desai et al., 2021; Inoue, 1999; D.-Q. Li et al., 2013; Pastor et al., 2014; Seczynska et al., 2022; Spencley et al., 2023). The HUSH complex recruits MORC2 and SETDB1 to silence intronless elements via chromatin compaction and deposition of the repressive epigenetic mark H3K9me3, respectively (Seczynska et al., 2022; Spencley et al., 2023; Tchasovnikarova et al., 2017). A genome-wide CRISPR screen identified MORC2 as essential for HUSH-mediated silencing, while the loss of MORC2 results in chromatin decompaction and reduction in H3K9me3 deposition (Tchasovnikarova et al., 2017). In the mouse testis, Morc1 and Morc2 form a complex that silences transposable elements, most likely by recruiting DNA methyltransferases, and MORC2 silences X chromosome-linked genes through association with SETDB1 and deposition of H3K9me3 (Kang et al., 2025). Additionally, Morc1 independently recruits SETDB1 to silence transposable elements in the mouse germline (Inoue, 1999; Isota et al., 2025; Pastor et al., 2014; Watson et al., 1998). Similarly, Morc3 has been shown to be involved in ERV transcriptional silencing, as Morc3 knock-out cells display de-repression, reduced H3K9me3 and increased chromatin accessibility (Groh et al., 2021). Interestingly, the unpublished data from the Latos lab show that MORC4 does not interact with the SETDB1 methyltransferase; instead, it cooperates with the NCoR1 and SMRT/NCoR2 co-repressor complexes. Both NCoR1 and NCoR2 complexes play essential roles in development, cell fate decisions, differentiation, and lipid metabolism (Hermanson et al., 2002; P. Li et al., 2011; Mottis et al., 2013; Nofsinger et al., 2008; Zhuang et al., 2018). Their repressive function relies on the histone deacetylase activity of the HDAC3 subunit (Fischle et al., 2002; Guenther et al., 2001; Oberoi et al., 2011b; Sun et al., 2013). Since MORC4 has a catalytically active ATPase domain and has been shown to bind nucleosomes in vitro (Tencer et al., 2020), I speculate that MORC4 closely

cooperates with the NCoR1/2 complexes and serves to compact chromatin deacetylated by the HDAC3, resulting in the silencing of the target genes. In contrast to the stable, long-standing silencing caused by Morc1, MORC2/Morc2, Morc3 and SETDB1/Setdb1, the silencing brought about by MORC4 and NCoR1/2 complexes would be temporarily and developmentally regulated. It would prevent premature differentiation of progenitor cells, ensuring coordinated placental development.

Interestingly, HDAC3 deacetylase activity is dependent on binding the deacetylase-activating domain (DAD) of SMRT/NCoR1, but it also has a deacetylase-independent gene-regulating function (Guenther et al., 2001; Sun et al., 2013). Based on the Co-IP experiments I performed, it seems that MORC4 is recruited to the core complex by TBL1X, but it is not essential for complex assembly. It is worth mentioning that Morc4 has been shown to preferentially bind H3K4me3 in in vitro assays (Tencer et al., 2020). Further studies in the context of NCoR1/2 complexes are necessary to clarify this observation.

Together, MORC4 interacts with the NCoR1/2 co-repressor complexes, where it is likely responsible for chromatin compaction and transcriptional silencing of the target genes in the placental context.

4.2. Inducible genetic tools to investigate the trophoblast function of MORC4

To investigate the function of MORC4 in trophoblast development, I set forth to generate tools for conditional genetic perturbations. Inducible genetic tools enable us to study stage-specific gene function in dynamic situations, such as differentiation. They are especially useful in the stem cell field because they enable us to investigate genes essential for self-renewal, cell cycle and cell fate decisions, since their depletion usually results in strong differentiation or apoptotic phenotypes (Bertero et al., 2016; Zafarana et al., 2009). As is the case with MORC4 shRNA-mediated KD in TSCs, which resulted in loss of proliferation and ambiguous differentiation phenotypes. To address this

problem, I aimed to inducibly deplete the MORC4 on three levels: DNA, RNA and protein.

In the scope of this thesis, I successfully generated six *MORC4*-iKO hPSC lines, two independent cell lines for each of the three *MORC4* sgRNAs. Additionally, I produced the iKD cell lines of *MORC4*, *NCOR1*, *NCOR2*, *HDAC3* and *TBL1X*. In total, I generated 35 independent cell lines, at least two for each shRNA construct, including an empty vector and *GFP* shRNA control lines, and I tested the efficiency and robustness of the chosen iKD system.

I aimed at producing a *MORC4*-degron hPSC line using the dTAG system. This goal proved to be the most difficult one in this project, on account of the lack of antibiotic selection of tagged clones. Since *MORC4* is not expressed in the hPSCs, I had to screen a large pool of clones. At the start, I used the PITCH system, but due to the low efficiency, I switched to using a synthetic HDR oligonucleotide. With this new approach, I was able to generate one cell line with the N-terminally tagged *MORC4* endogenous locus. A potential explanation for why I was less successful with C-terminal tagging is that there are differences in chromatin accessibility between the two ends of protein-coding regions. Promoters found upstream of the first exon are generally more accessible to the transcriptional machinery and other chromatin-binding factors, which makes this region more accessible for the tagging cassette knock-in (Klemm et al., 2019; Mansisidor & Risca, 2022). Additionally, the *MORC4* locus is found on the X chromosome, which makes it even harder to target because of X chromosome inactivation (Wutz, 2011).

Lastly, I established two novel methods in the lab, which are the naive conversion and blastoid generation. I performed the naive conversion of the aforementioned iKO lines, identified a *SUSD2*⁺ population of cells and confirmed their transcriptional profile with RT-qPCR. They showed significantly higher expression of pluripotency-related genes such as *NANOG*, *OCT4* and *SOX2* compared to primed PSCs. I wanted to test their blastoid-forming capacity, which is one of the hallmarks of naive PSCs (Kagawa et al., 2021; Shahbazi & Pasque, 2024; Yanagida et al., 2021; L. Yu et al., 2021). While the cells did form tight aggregates that grew in the first three days, they did not

cavitate or form proper blastoids. Authors of the used protocol report >97% blastoid-forming efficiency with the same culture conditions, and have identified three main parameters that determine appropriate cellular commitment and formation of the blastoids: initial hPSCs state, aggregate size and the molecular environment the aggregates are exposed to (Heidari Khoei et al., 2023; Kagawa et al., 2021). Only naive PSCs have enough transcriptional plasticity to commit to TE cell fate, and the number of potential TE cells depends on the surface-to-volume ratio of the aggregates (Guo et al., 2021). Thus, to improve our experimental setup, we plan to sort the naive cells again and test different seeding numbers. While functional experiments on human blastoids are still novel in the scientific community, they hold great potential to help us gain a better understanding of early development (Q. Li et al., 2025; Molè et al., 2025).

4.3. Limitations and outlook

This thesis lays the groundwork for understanding the trophoblast function of MORC4 and epigenetic regulation of cell fate decisions in the developing placenta. While the development of genetic tools is an experimental endeavour in itself, functional experiments performed on these models are the source of new knowledge. With that in mind, several future directions become apparent.

To gain deeper insight into transcriptional regulation of the self-renewing state of hTSCs, a detailed analysis of the MORC4-KO phenotype and its associated effects is intended. After transdifferentiating the MORC4-iKO naive cell lines into TSCs and inducing the KO, we plan to investigate global transcriptional changes using the Quant-seq method and changes in chromatin compaction via ATAC-seq. Integration of differentially expressed genes with MORC4 and NCoR1/2 ChIP-seq data should reveal direct targets of MORC4-NCoR1/2 complexes. This can be further validated with the inducible KD of complex subunits. Furthermore, after establishing the effects of the MORC4 depletion, it would be exciting to test if overexpression rescues

the phenotype, and the cloning of the inducible *MORC4* overexpression constructs is currently ongoing.

Another interesting avenue of research using the models generated in this thesis is to explore the function of *MORC4* in earlier developmental stages, such as the trophectoderm. Testing the effect of *MORC4* or *NCOR1/2* KD on blastoid formation and maturation could shed light on mechanisms of TE establishment. Even though the naive cells that I tested so far did not yield cavitated blastoids, the RT-qPCR analysis of their transcriptional profile shows promising results, which makes us confident that the naive cell population will become more homogenous with further passaging.

In conclusion, this thesis is the beginning of a comprehensive analysis of the *MORC4* role in the developing placenta. Here, I established in vitro inducible systems for investigating the trophoblast function of *MORC4* together with the *NCoR1/2* complexes. Based on preliminary results, it seems that *MORC4* acts as a transcriptional suppressor of ERVs, protecting the self-renewing state of progenitor cells. Understanding mechanisms governing cell fate decisions is paramount for the improvement of medical care and treatments for developmental disorders and infertility.

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