Inventory of Supplemental Information

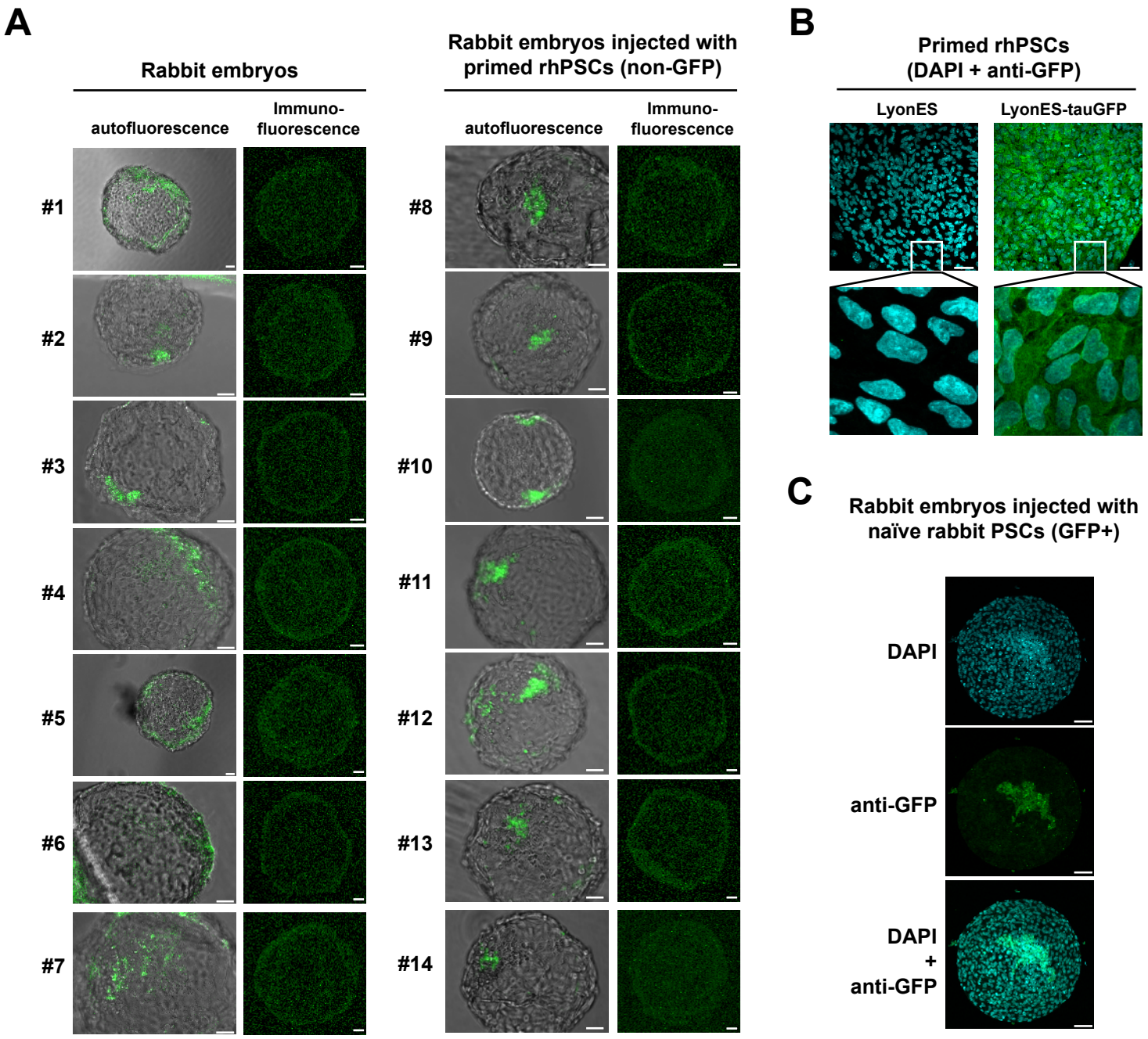

Figure S1: Evaluation of autofluorescence in rabbit embryos. Related to Figure 1. (A) Epifluoresence imaging and immunostaining of GFP in uninjected rabbit embryos (left panel) and in rabbit embryos injected with primed rhesus PSCs not expressing GFP (n = 40). **(B)** Immunostaining of GFP in primed LyonES-tGFP-(S3) rhesus PSCs. **(C)** Immunostaining of GFP in a late blastocyst-stage (E5) rabbit embryo after microinjection of rabbit naïve PSCs into morula-stage (E2) embryos (confocal imaging; scale bars: 50 μm).

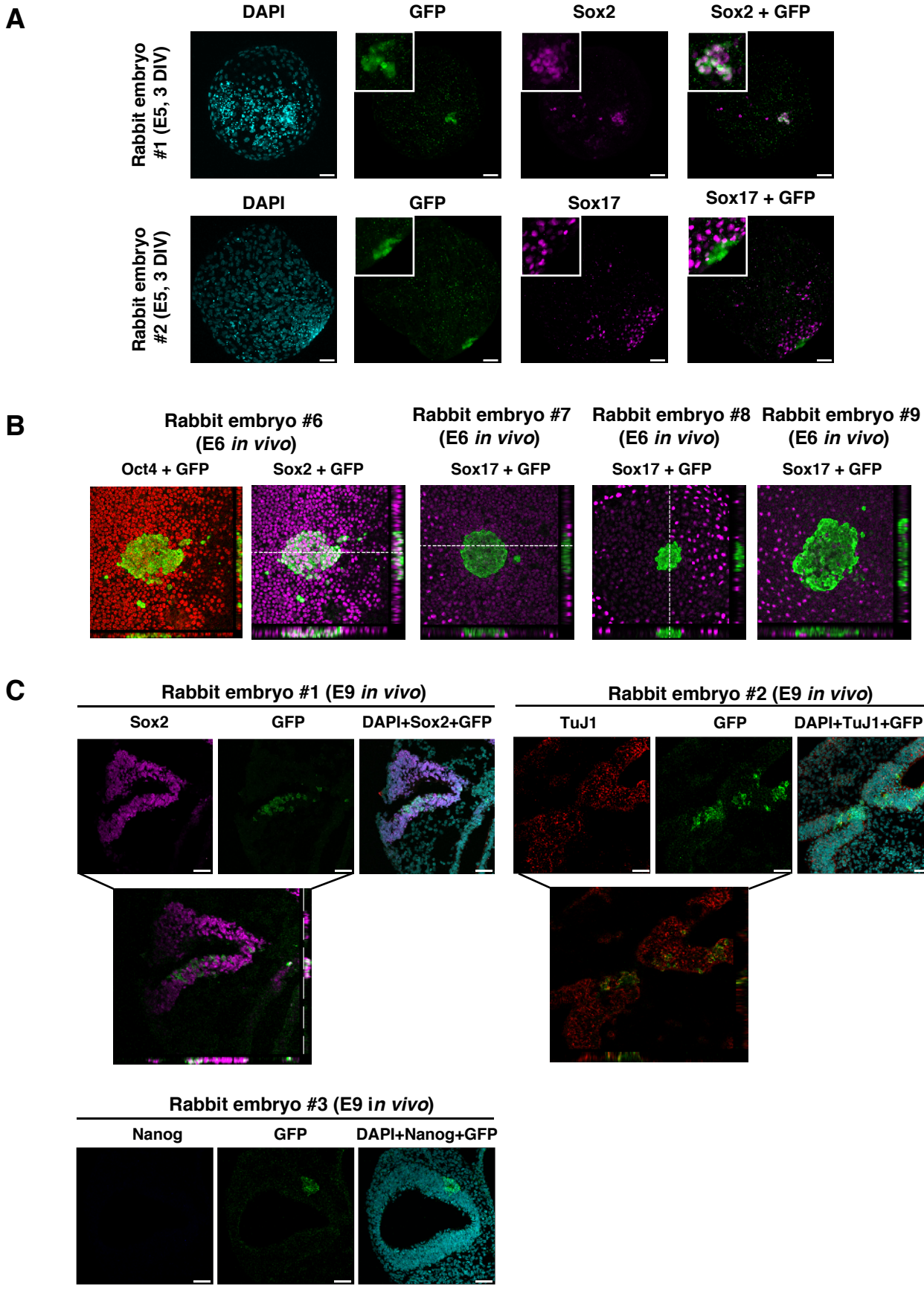

Figure S3: Colonization of rabbit embryos by mouse ESCs. Related to Figure 1. (A) Immunostaining of GFP, Sox2 and Sox17 in rabbit embryos at the late blastocyst-stage (E5) after microinjection of mESC-GFP-2i/LIF cells into morula-stage embryos (confocal imaging; scale bars: 50 μm) (n = 29). **(B)** Orthogonal sections of immunostaining of GFP, Oct4, Sox2 and SOX17 performed on rabbit embryos at the pre-gastrula stage (E6) after microinjection of mESC-GFP cells into morula-stage (E2) embryos and transfer to a surrogate mother (confocal imaging; scale bars: 50 μm). **(C)** Immunostaining of GFP, Sox2, TuJ1 and Nanog in rabbit embryos at the post-implantation stage (E9) after microinjection of mESC-GFP cells into morula-stage embryos (confocal imaging; scale bars: 50 μm).

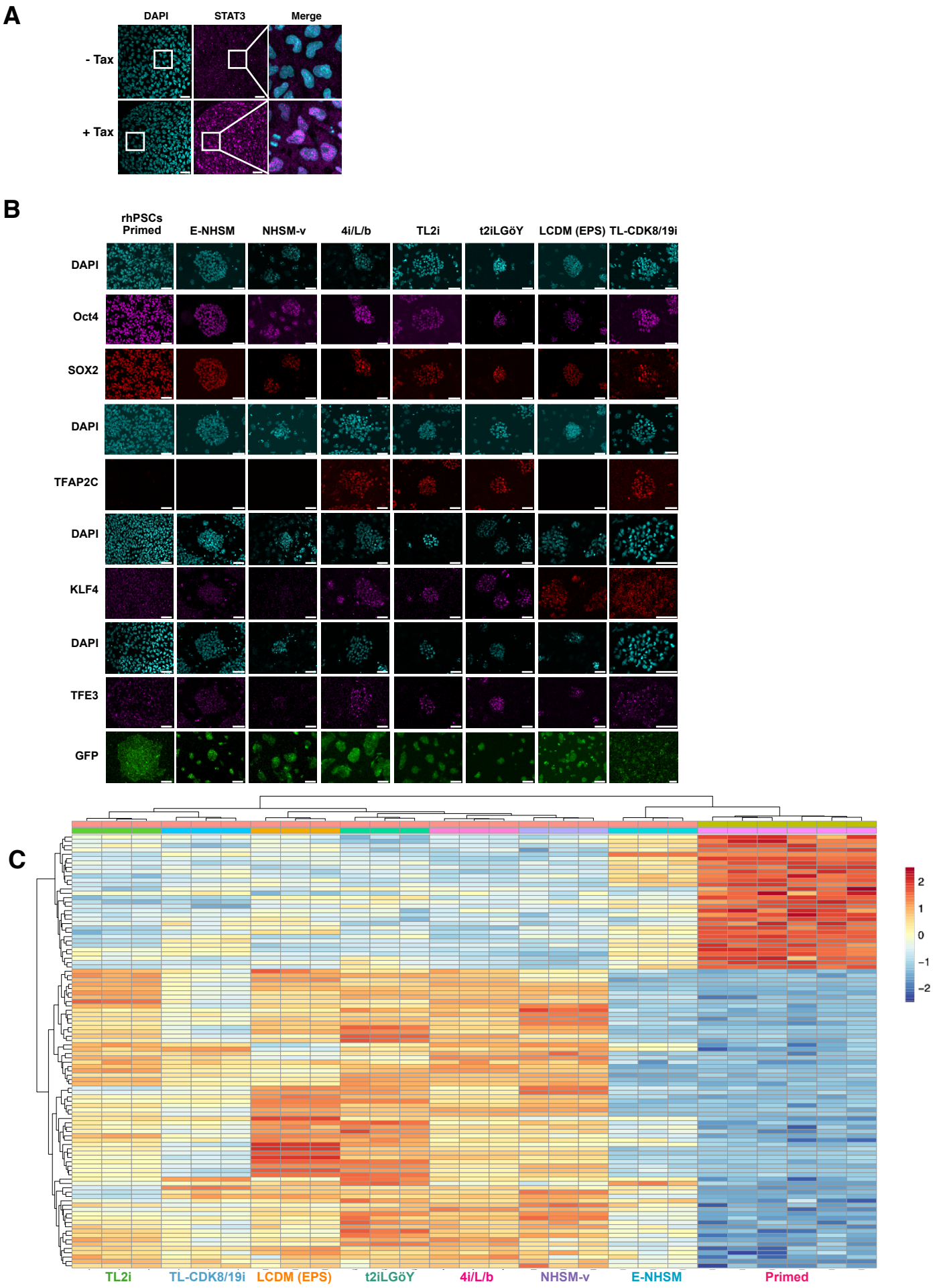

Figure S4: Characterization of LyonES-tGFP-(S3) cells. Related to Figure 3. (A) Immunolabeling of STAT3 in LyonES-tGFP-(S3) cells, before and after treatment with 250 nM tamoxifen for 48 h (confocal imaging; scale bars: 50 μm). **(B)** Immunolabeling of GFP in LyonES-tGFP-(S3) cells, before and after applying culture protocols for naïve conversion (confocal imaging; scale bars: 50 μm). **(C)** Heatmap of transcriptome data (mean values/cell category most differentially expressed 100 genes, listed in Table S2) using Pearson correlation coefficient as a measure of Euclidian distance between rows and between columns.

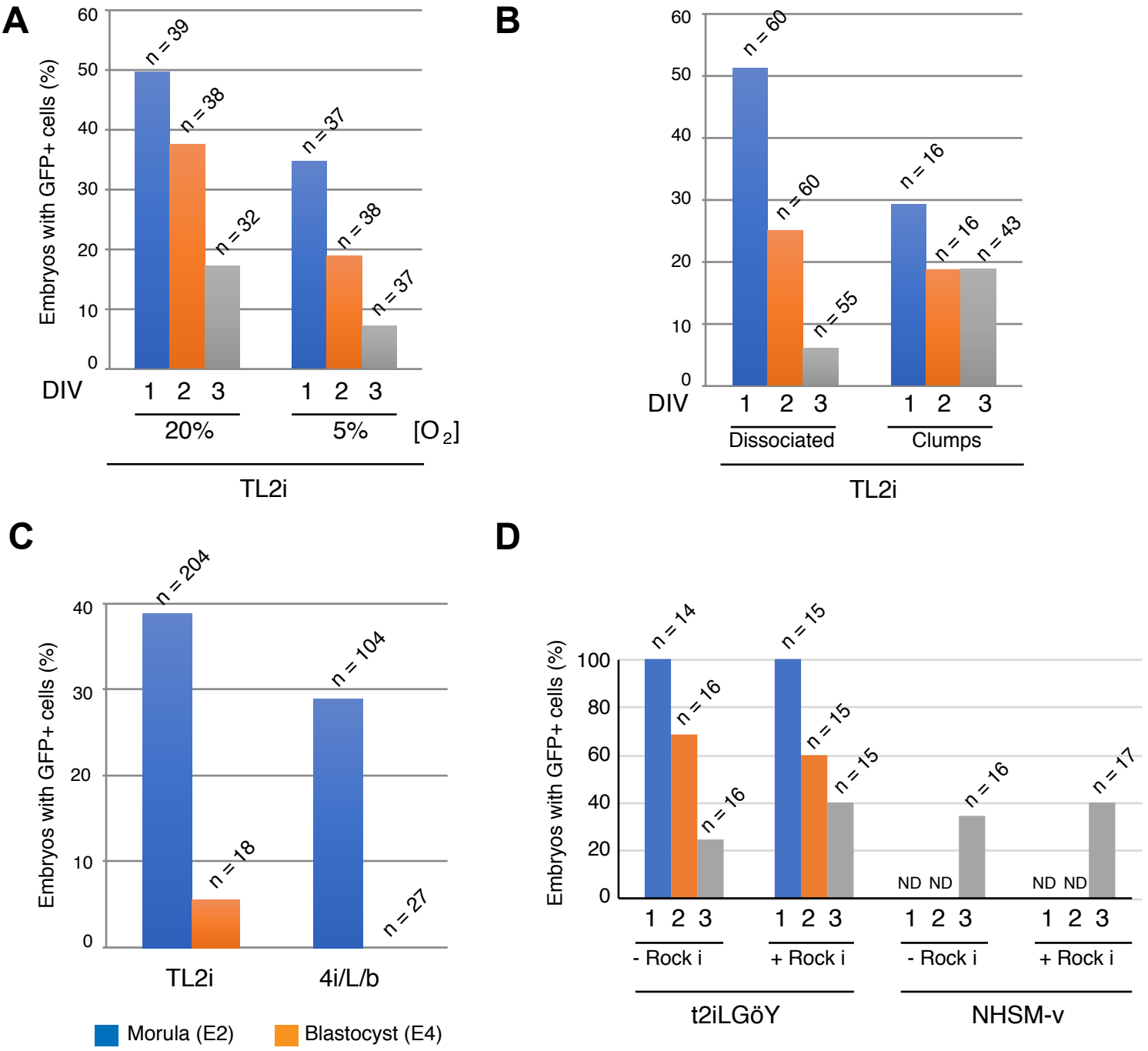

Figure S9: Optimization of the conditions for microinjection of rhesus PSCs into rabbit embryos. **Related to Figure 4. (A)** Histogram of percentage of rabbit embryos with GFP+ cells after microinjection of rhesus TL2i cells at the morula-stage (E2) under normoxic (20%) and hypoxic (5%) conditions. Analyses were performed at 1, 2, and 3 DIV (n = 221). **(B)** Histogram of the percentage of rabbit embryos with GFP+ cells after microinjection of dissociated or clumps of rhesus TL2i cells into morula-stage (E2) embryos. Analyses were performed at 1, 2, and 3 DIV (n = 250). **(C)** Histogram of the percentage of rabbit embryos with GFP+ cells after microinjection of rhesus 4i/L/b and TL2i cells into morula-stage (E2) or blastocyst-stage (E4) embryos. Analyses were performed at 3 DIV (n = 353). **(D)** Histogram of the percentage of rabbit embryos with GFP+ cells after microinjection of rhesus t2iLGöY and E-NHSM cells into morula-stage (E2) embryos, in the presence or absence of ROCK inhibitor (n = 124). Analyses were performed at 3 DIV.

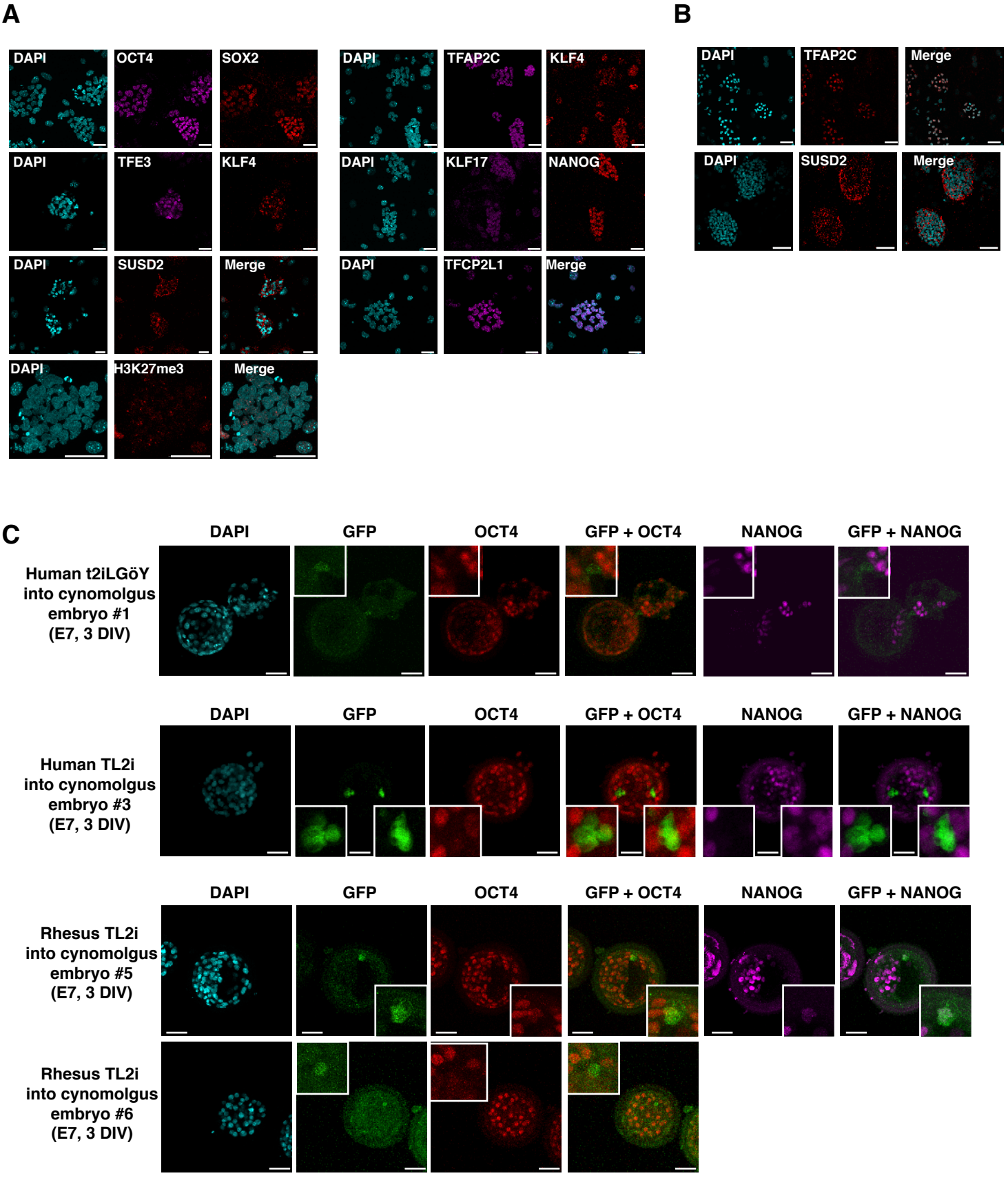

Figure S10: Colonization of cynomolgus embryos by human and rhesus PSCs. Related to Figure 5. (A) Immunostaining of OCT4, SOX2, TFAP2C, KLF4, TFE3, KLF17, NANOG, SUSD2, TFCP2L1, and H3K27me3 in NCRM2-t2iLGöY cells (confocal imaging; scale bars: 50 μm). **(B)** Immunostaining of TFAP2C and SUSD2 in IR7.1-TL2i cells (confocal imaging; scale bars: 50 μm). **(C)** Immunolabeling of GFP, OCT4, and NANOG in late blastocyst-stage cynomolgus embryos (E7) after microinjection of NCRM2-t2iLGöY cells, IR7.1-TL2i cells, and LyonES-tGFP-(S3)-TL2i cells into morula-stage (E4) cynomolgus embryos (confocal imaging; scale bars: 50 μm) (n = 29).

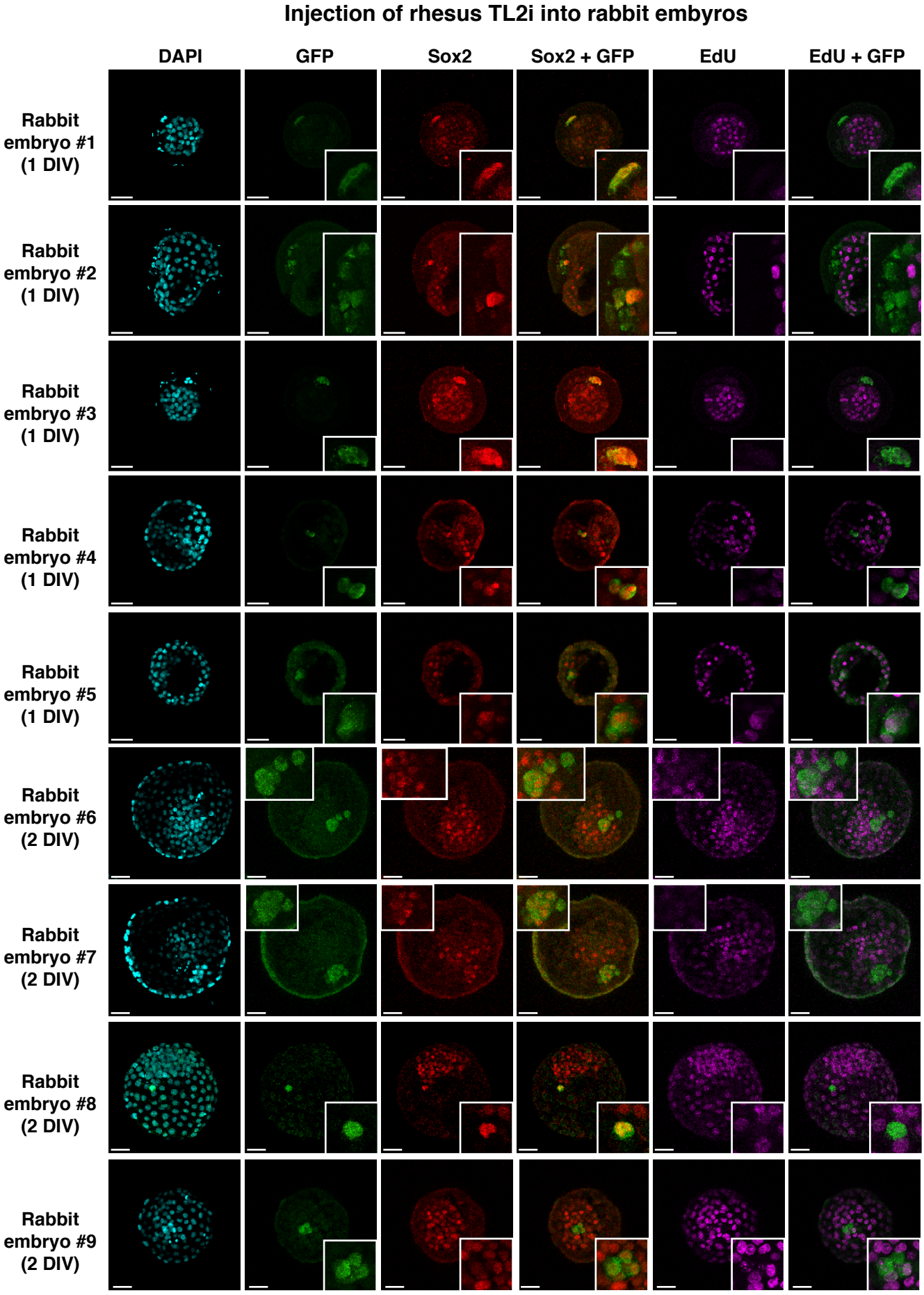

Figure S11: DNA replication of Rhesus PSCs after microinjection into rabbit embryos. Related to Figure 7. Immunostaining of GFP, Sox2, and EdU in late blastocyst-stage rabbit embryos (E5, 3 DIV) after microinjection of rhesus TL2i cells into morula-stage (E2) embryos (confocal imaging; scale bars: 50 μm).

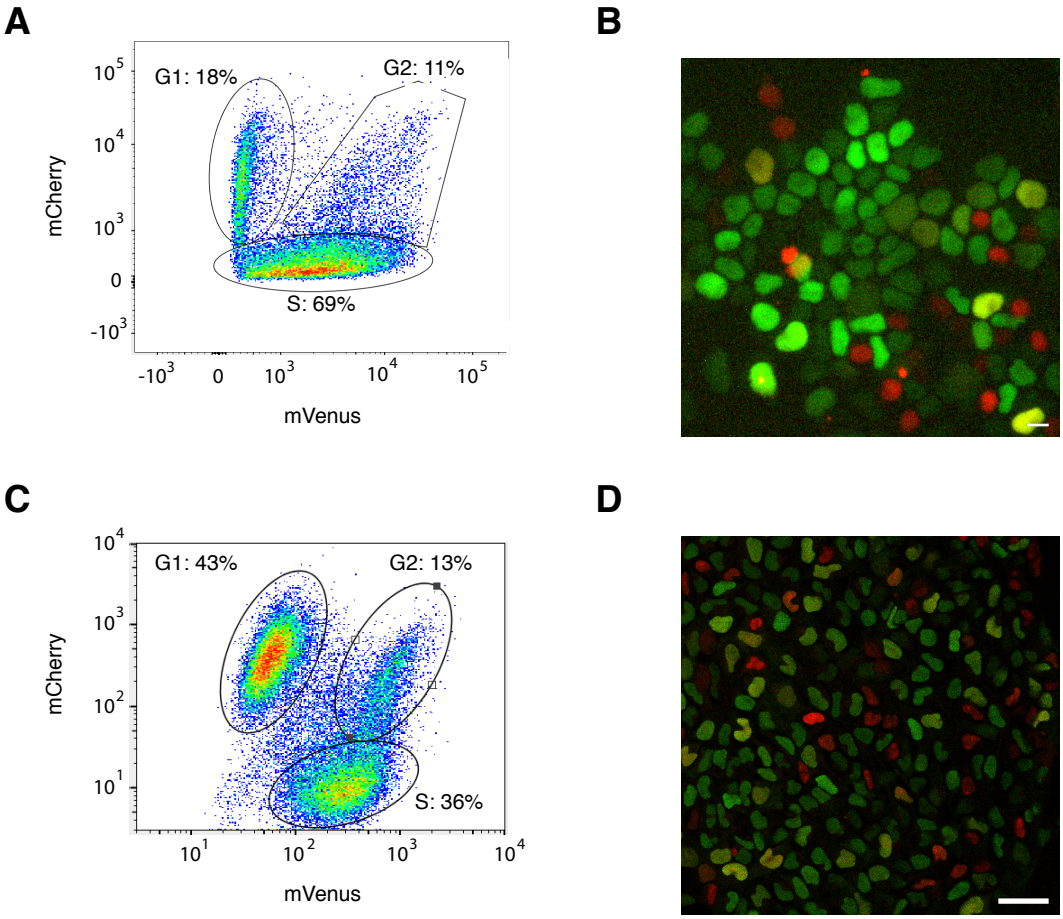

Figure S12: Characterization of mouse ESCs and rhesus PSCs harboring the FUCCI(CA) cell cycle reporter. Related to Figure 7. (A) Flow cytometry analysis and **(B)** epifluorescence imaging of mESCs-FUCCI (CA) cells. **(C)** Flow cytometry analysis and **(D)** epifluorescence imaging of rhesus primed-FUCCI (CA) cells (scale bars: 50 μm).

	Rabbit host		
	E3 (1 DIV)	E4 (2 DIV)	E5 (3 DIV)
Rhesus PSCs			
Primed			
GFP+ embryos/injected emb.	10/104	0/89	0/90
NANOG+ embryos/GFP+ emb.	0/7		
SOX2+ embryos/GFP+ emb.	0/2		
GATA6+ embryos/GFP+ emb.	0/1		
TL2i			
GFP+ embryos/injected emb.	109/141	78/145	79/204
NANOG+ embryos/GFP+ emb.	14/16	7/12	2/7
SOX2+ embryos/GFP+ emb.	45/53	30/52	8/40
GATA6+ embryos/GFP+ emb.	6/31	8/22	14/33
4i/L/b			
GFP+ embryos/injected emb.	45/59	32/65	30/104
NANOG+ embryos/GFP+ emb.	5/7	3/12	1/7
SOX2+ embryos/GFP+ emb.	3/5	0/1	1/6
GATA6+ embryos/GFP+ emb.	4/33	5/19	6/17
t2iLGoY			
GFP+ embryos/injected emb.	92/96	72/97	69/114
NANOG+ embryos/GFP+ emb.	10/40	0/21	0/11
SOX2+ embryos/GFP+ emb.	14/43	6/31	3/10
GATA6+ embryos/GFP+ emb.	3/39	10/38	8/20
LCDM (EPS)			
GFP+ embryos/injected emb.	66/89	36/89	44/157
NANOG+ embryos/GFP+ emb.	0/19	2/12	10/17
SOX2+ embryos/GFP+ emb.	20/31	11/15	5/20
GATA6+ embryos/GFP+ emb.	1/24	0/13	2/19
E_NHSM			
GFP+ embryos/injected emb.	15/71	1/70	0/71
NANOG+ embryos/GFP+ emb.	0/24		
SOX2+ embryos/GFP+ emb.	0/20		
GATA6+ embryos/GFP+ emb.	2/27		
NHSM_v			
GFP+ embryos/injected emb.	11/68	8/84	10/118
NANOG+ embryos/GFP+ emb.	0/3		
SOX2+ embryos/GFP+ emb.	1/3	0/3	0/7
GATA6+ embryos/GFP+ emb.	0/5	0/5	1/3
TL_CDK8/19ii			
GFP+ embryos/injected emb.	43/79	19/80	10/101
NANOG+ embryos/GFP+ emb.	10/20	2/7	1/4
SOX2+ embryos/GFP+ emb.	2/12	2/11	1/5
GATA6+ embryos/GFP+ emb.	6/15	1/3	0/1
Human PSCs			
hiPSCs_t2iLGoY			
GFP+ embryos/injected emb.	27/29	20/31	43/130
NANOG+ embryos/GFP+ emb.			3/13
SOX2+ embryos/GFP+ emb.	24/29	11/20	7/12
GATA6+ embryos/GFP+ emb.			2/10
hiPSCs_TL2i			
GFP+ embryos/injected emb.	33/39	18/36	11/39
NANOG+ embryos/GFP+ emb.	15/15	3/7	0/4
SOX2+ embryos/GFP+ emb.	9/11	4/8	0/6
GATA6+ embryos/GFP+ emb.	1/7	2/3	1/2
Mouse ESCs			
Serum + LIF			
GFP+ embryos/injected emb.			235/238
NANOG+ embryos/GFP+ emb.			77/77
SOX2+ embryos/GFP+ emb.			58/58
SOX17+ embryos/GFP+ emb.			0/30
2iLIF			
GFP+ embryos/injected emb.			14/19
SOX2+ embryos/GFP+ emb.			7/7
SOX17+ embryos/GFP+ emb.			0/7
Total	**775**	**786**	**1395**
Total injected embryos	**2956**		

	Rabbit host	
	E6	E9
Mouse ESCs		
GFP+ embryos/injected emb.	9/9	12/20
SOX2+ embryos/GFP+ emb.	6/6	
SOX17+ embryos/GFP+ emb.	3/3	
Rhesus TL2i		
GFP+ embryos/injected emb.		0/16
Total	**18**	**36**
Total injected embryos	**54**	

	Cynomolgus host
	E7
Rhesus TL2i	
GFP+ embryos/injected emb.	2/7
NANOG+ embryos/GFP+ emb.	0/2
hiPSCs_TL2i	
GFP+ embryos/injected emb.	2/7
NANOG+ embryos/GFP+ emb.	0/2
hiPSCs_t2iLGoY	
GFP+ embryos/injected emb.	2/15
NANOG+ embryos/GFP+ emb.	1/2
Mouse ESCs	
GFP+ embryos/injected emb.	3/5
NANOG+ embryos/GFP+ emb.	1/1
SOX2+ embryos/GFP+ emb.	2/2
Total injected embryos	**36**

Table S1: Summary of intespecies chimera experiments. Related to Figures 1, 2, 4 and 5.

Gene name	Naïve vs Primed
DGKK	DOWN
SSB	DOWN
ZNF114	DOWN
SEPHS1	DOWN
KIAA1551	DOWN
CTTNBP2	DOWN
ZNF732	DOWN
ATXN7L1	DOWN
BCOR	DOWN
BAHD1	DOWN
ISOC1	DOWN
DDHD1	DOWN
ZFHX2	DOWN
DNMT3A	DOWN
FAM118B	DOWN
EIF2S1	DOWN
LTV1	DOWN
MESDC2	DOWN
HSPA8	UP
SLTM	UP
KIAA0368	UP
SEC63	UP
SERPINE2	UP
ZBTB2	UP
QSER1	UP
RCC2	UP
HNRNPM	UP
STAG2	UP
HNRNPAB	UP
PSMC2	UP
SS18	UP
C20H16orf58	UP
MAPK3	UP
CABIN1	UP
TRAK1	UP
STAT2	UP
MGAT5	UP
KIAA0556	UP
CCDC93	UP
ZC2HC1A	UP
TSC22D3	UP

RGS2	UP
CORO7	UP
ZNF516	UP
10 ARL	UP
LCA5	UP
SEMA4F	UP
PLCD1	UP
MEST	UP
CKB	UP
RIN2	UP
SLC1A4	UP
BMP7	UP
GAS7	UP
MTUS1	UP
WSCD1	UP
CRIP2	UP
ELOVL2	UP
SORCS3	UP
DTX4	UP
IGFBPL1	UP
NKD1	UP
PRSS35	UP
CRB2	UP
FAM49A	UP
WLS	UP
NEFL	UP
TUBB3	UP
AXIN2	UP
SHANK1	UP
PLXNA4	UP
ADGRG1	UP
APLP1	UP
SDK2	UP
CFB	UP
TPPP3	UP
CNTFR	UP
DLL1	UP
WNT9A	UP
DCLK2	UP
COL2A1	UP
NCALD	UP
APC2	UP

RSPO3	UP
DCX	UP
SLC1A2	UP
Septin5	UP
A2M	UP
CACNG4	UP
POU3F2	UP
ZIC1	UP
C2	UP
LEF1	UP
SLIT1	UP
SSUH2	UP
INSM1	UP
BMP5	UP
ZIC4	UP
DCC	UP

Table S2: List of genes presented in the heatmap. Related to Figure S4C.

Supplemental Experimental Procedures

Cell lines, media composition, and culture

mESC lines were routinely cultured in Glasgow's modified Eagle's medium (Gibco) supplemented with 10% fetal calf serum (CRC0406; PerbioScience) and 1,000 U/mL LIF on gelatin-coated dishes. They were also cultured in N2B27 supplemented with 1,000 U/mL LIF, 1 μM PD0325901, and 3 μM CHIR99021 (2i/LIF), as indicated. Both the LyonES-tGFP and Lyon-ES-tGFP-(S3) rhesus PSC lines (Wianny et al., 2008, Chen et al., 2015a) and the human iPSC line IR7.1 (Chen et al., 2015a), (Ng et al., 2012) cell lines were routinely cultured at 37 °C in 5% CO_2 and 5% O_2 in knockout Dulbecco's modified Eagle's medium (KO-DMEM) supplemented with 20% knockout serum replacement (KOSR), 1 mM glutamine, 0.1 mM β-mercaptoethanol (Sigma), 1% non-essential amino acid (Gibco), and 4–8 ng/mL FGF2 (Gibco) on growth-inactivated murine embryonic fibroblasts.

For primed to naïve conversion of primate cells, primed cells were dissociated and plated on fresh feeder cells for 24 h before shifting to the naïve culture media. Fresh medium was added daily to the culture plates. The medium composition for E-NHSM reprogramming (Gafni et al., 2013) was as follows: Neurobasal: DMEM-F12 (1:1) supplemented with N2B27 (Gibco), L-ascorbic acid, 0.5% KOSR, 10 ng/mL human LIF (Pepeotech), 5 μM IWR1 (Sigma), 1.5 μM CHIR99021 (Miltenyi Biotec), 1 μM PD0325901 (Miltenyi Biotec), 2 μM BIRB796 (Axon), 5 μM SP600125 (Tocris), 2 μM Gö6983 (Tocris), 20 ng/mL activin A (Peprotech), and 1 μM CGP77675 (Axon). Cells were passaged every 3–4 days by single-cell dissociation using Accutase (Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h. The NHSM-v protocol is a modified version of the NHSM protocol applied to cynomolgus macaque ESCs (Chen et al., 2015b). These cells were cultured in KO-DMEM supplemented with 20% KOSR, 4 ng/mL FGF2 (Gibco), 10 ng/mL hLIF (Peprotech), 3 μM CHIR99021 (Miltenyi Biotec), 0.5 μM PD0325901 (Miltenyi Biotec), 5μM SP600125 (Tocris), and 10 μM SB203580 (Tocris). Cells were passaged every 3–4 days by single-cell dissociation using 1 mg/mL Accutase (Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h. The 4i/L/b media was adapted from the protocol published by Fang *et al.* (Fang et al., 2014). Cells were cultured in KO-DMEM supplemented with 20% KOSR (Gibco), 0.5 μM PD0325901 (Miltenyi Biotec), 3 μM CHIR99021 (Miltenyi Biotec), 10 μM SP600125 (Tocris), 100 μM SB203580 (Tocris), 10 ng/mL hLIF (Peprotech), and 2.5 ng/mL FGF2 (Gibco). Cells were passaged every 3–4 days by single-cell dissociation using 0.05% Trypsin-ethylenediaminetetraacetic acid (Trypsin-EDTA; Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h. Primed cells were converted to 5i/LA state using the following medium composition (Theunissen et al., 2014): DMEM-F12 (1:1) medium supplemented with N2 (Gibco), B27 (Gibco), 0.5% KOSR (Gibco), 1 μM PD0325901 (Miltenyi Biotec), 1 μM IM-12 (Enzo), 0.5 μM SB590885 (Tocris), 1 μM WH-4-023 (Tocris), and 20 ng/mL activin A (Peprotech). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h. Primed cells were converted to the t2iLGöY state using the following medium composition (Guo et al., 2017): first in cRM1 media for three days with 1 μM PD0325901 (Miltenyi Biotec), 10 ng/mL hLIF (Peprotech) and 1mM Valproic acid (VPA), followed by two days in cRM2 media with DMEM-F12 (1:1) medium supplemented with N2, B27 (Gibco), 1 μM PD0325901 (Miltenyi Biotec) and 250 μM Vitamin C (Sigma-Aldrich) and then the final media containing DMEM-F12 (1:1) medium supplemented with N2, B27 (Gibco), 1 μM PD0325901 (Miltenyi Biotec), 1,000 U/mL LIF, 1 μM Gö6983 (Bio-techne), 2 μM XAV939 (Sigma). Cells were passaged every 4–5 days by single-cell dissociation using Tryple (Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h. For reprogramming to the TL2i state, PSCs expressing the inducible fusion protein STAT3-ER^{T2} were cultivated in the TL2i medium composed of KO-DMEM (Gibco), 20% KOSR (Gibco), 10,000 U/mL LIF, 3 μM CHIR99021 (Miltenyi Biotec), 1 μM PD0325901 (Miltenyi Biotec), and 250 nM 4′-Hydroxy-Tamoxifen (4′OHT; Calbiochem). Cells were passaged every 3–4 days by single-cell dissociation using 0.05% Trypsin-EDTA (Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h. For TL-CDK8/19i protocol, cells were cultured in medium composed of KO-DMEM (Gibco), 20% KOSR (Gibco), 10,000 U/mL LIF, and 1 μM CNIO-47799. Cells were passaged every 3–4 days by single-cell dissociation using 0.05% Trypsin-EDTA (Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h.Primed cells were converted to the LCDM (EPS) (Yang et al., 2017) state using medium with the following composition: DMEM-F12 (1:1), N2 (Gibco), B27 (Gibco), 5% KOSR (Gibco), 10 ng/mL hLIF (Peprotech), 1 μM CHIR99021 (Miltenyi Biotec), 2 μM (S)-(+)-Dimethindene maleate (Tocris), 2 μM minocycline hydrochloride (Santa Cruz Biotechnology), and 0.75 μM IWR1 (Sellekchem). Cells were passaged every 3–4 days by single-cell dissociation using 0.05% Trypsin-EDTA (Gibco). ROCK inhibitor (Y-27632; Miltenyi Biotec) was added during passaging for 24 h.

Generation of FUCCI reporter rhesus PSCs and mESCs

The *PB-Puro^R-CAG-mVenus : hGeminin-IRES-mCherry : hCdt1* plasmid was generated as follows. First, an EcoRI/XbaI DNA fragment from the *mCherry-hCdt1(1/100)Cy(-)/pcDNA3* plasmid (Sakaue-Sawano et al., 2017) containing the mCherry : hCdt1 cassette was sub-cloned between the EcoRI and XbaI restriction sites in *PB-CAG-cHA-IRES-hyg* to generate *PB-Hygro-mcherry : hCdt1*. Second, a AlfII/XbaI fragment from the *mVenus-*

hGeminin(1/110)/pcDNA3 plasmid (Sakaue-Sawano et al., 2017) containing the mVenus : hGeminin cassette was subcloned between the SapI and AgeI restriction sites in *PB-Hygro-mcherry : hCdt1* to generate *PB-Hygro-mcherry : hCdt1-mVenus : hGeminin*. Third, a SapI/AgeI fragment containing the *Puro*^{R} gene and the CAG promoter was sub-cloned between the SapI and AgeI restriction sites in *PB-Hygro-mcherry : hCdt1-mVenus : hGeminin* to generate the *PB-Puro*^{R}*-CAG-mVenus : hGeminin-IRES-mCherry : hCdt1* plasmid. Rhesus PSCs and mESCs were transfected using the NEON transfection system according to the instructions provided by the manufacturer. Briefly, cells were dissociated and resuspended at a density of 10.10^{6} cells/mL. For transfection, 100 µL of the cell suspension was mixed with 2.5 µg of the *PB-Puro*^{R}*-CAG-mVenus : hGeminin-IRES-mCherry : hCdt1* vector and 2.5 µg of *PBase* plasmid. Transfection parameters used for rhesus PSCs were 1,050 V, 20 ms, and 2 pulses. Cells were plated on growth-inactivated murine embryonic fibroblasts in medium supplemented with 10 µM ROCK inhibitor (Y-27632; Miltenyi Biotec) and selected in 250 ng/mL G418. For mESCs, the parameters used were 1,200 V, 20 ms, and 2 pulses. Cells were analyzed using a FACS LSR II (Becton-Dickinson) equipped with 355, 488, and 561 nm lasers. Data were acquired and analyzed using the DiVa software.

Histology, histochemistry, and immunostaining

Monkey (E7) and rabbit (E3, E4, E5, and E6) pre-implantation embryos were fixed in 2% paraformaldehyde (PFA) for 20 min at room temperature. After three washes in phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBS-0.1%T), embryos were permeabilized in PBS-1%T overnight at 4 °C on a rotating shaker. Embryos were subsequently placed in blocking solution (PBS-0.1%T) supplemented with 5% donkey serum for 1 h at room temperature. There were incubated with primary antibodies diluted in blocking solution overnight at 4°C (antibodies used in this study are listed below). After four washes (3 × 5 min + 1 × 30 min) in PBS-0.1%T, embryos were incubated in secondary antibodies diluted in blocking solution at a concentration of 1:300 for 1 h at room temperature and transferred through several washes of PBS-0.1%T before staining the nuclei with 4′,6-diamidino-2-phenylindole (DAPI; 0.5 µg/mL). Embryos were analyzed by confocal imaging (DM 6000 CS SP5; Leica). Acquisitions were performed using a water immersion objective (25×/1.25 0.75, PL APO HCX; Leica).

E8 rabbit post-implantation embryos were fixed in 2% PFA at 4 °C for 1 h. For cryoprotection, embryos were placed in 10% sucrose for 1 h followed by 30% sucrose overnight at 4 °C. Embryos were embedded in NEG50 compound (Invitrogen) and frozen at −80 °C. Sections of 15–20 µm were generated using a Microm HM550 cryostat and maintained at −80 °C until immunostaining was performed. For immunostaining, sections were thawed for 30 min at room temperature, saturated with PBS for 15 min at room temperature, and permeabilized (as necessary) with three baths of PBS containing 0.5% Triton X100 (Sigma-Aldrich). Sections were incubated in blocking solution: PBS with 0.1% Triton X100 and 10% donkey serum (Jackson Immunoresearch Laboratory) for 30 min. Subsequently, the sections were incubated with primary antibodies overnight at 4 °C. After several washes, the sections were incubated with the appropriate fluorescence-conjugated secondary antibodies at room temperature for 1 h. Nuclei were stained with DAPI (0.5 µg/mL). Sections were mounted on coverslips with Fluoromount G (Thermo Fisher Scientific). Tiled scans were automatically acquired using the LAS AF software (Leica).

Cells were fixed with 2% PFA in PBS at room temperature for 20 min, washed thrice (10 min each) with PBS and permeabilized in PBS containing 0.5% Triton X100. Non-specific binding sites were blocked using PBS supplemented with 10% donkey serum for 1 h at room temperature. The cells were incubated overnight at 4 °C with primary antibodies. After three rinses (10 min each) with PBS, the cells were incubated with secondary antibodies at room temperature for 1 h. The nuclei were stained with DAPI (0.5 µg/mL). The cells were mounted on coverslips by using the mounting medium M1289 (Sigma). Cells and embryo sections were analyzed by confocal imaging (DM 6000 CS SP5; Leica). Acquisitions were performed using an oil immersion objective (45×/1.25 0.75, PL APO HCX; Leica).

EdU staining was performed using the Click-iT^{TM} Edu Imaging kit (Fisher Scientific). Briefly, embryos microinjected with PSCs were incubated for 1 h with 10 µM EdU in RDH medium. Embryos were fixed with 2–4% PFA, and immunostaining was performed according to the instructions provided by the manufacturer. Embryos were analyzed by confocal imaging (DM 6000 CS SP5; Leica), and acquisitions were performed using a water immersion objective (25×/1.25 0.75, PL APO HCX; Leica).

Two-photon recording

Embryos microinjected with rhesus and mESCs were placed in drops (5 µL) of medium and covered with liquid paraffin (Origio). For recording, embryos were cultured under the inverted Axio-Observer Z1 (Zeiss) two-photon microscope for 3 days, until they reach the E5 stage. Images were acquired using the Zeiss Zen software, and image analysis was performed using the ImageJ software.

Flow cytometry analysis of cell cycle distribution
Cells were labeled for 15 minutes with 10μM EdU in the culture medium using the Click-it Plus EdU flow cytometry kit (ThermoFischer). Cells were then washed and processed for the detection of incorporated EdU according to the manufacturer's instructions. After immunostaining, cells were incubated for 20 min with 1 mg/ml RNAse in PBS-0.13 mM EGTA. Propidium iodide (1 □g/ml) was added just before analysis. Cells were analyzed using a FACS LSR II (Becton-Dickinson) equipped with 355, 488, and 561 nM lasers. Data were acquired and analyzed using DiVa software.

Bioinformatics analysis
RNA-seq data obtained from rhesus primed and naïve cell lines were analyzed using the R software. PCA, differential expression analysis (with false discovery rate <0.1), and hierarchical clustering were performed with DESeq2 (Love et al., 2014). For cell lines, PCA was performed using the top 500 genes selected by highest row variance. For the comparison of cell lines with embryo data, we used the Seurat R package (version 3.1). Briefly, the two compendia of datasets were transformed to a Seurat object and merged. The transcript counts were subsequently log-transformed and normalized. Finally, the variable genes (3,000) were used as input for PCA.

List of antibodies

Target	Supplier	Reference	Host species	Dilution for cells	Dilution for pre-implantation embryo	Dilution for cryosection
GFP	Thermo Fischer	A10262	Chicken	1:300	1:300	1:300
mCherry	Thermo Fischer	M11217	Rat	1:300	1:300	NA
OCT4	Santa Cruz	sc-9081	Rabbit	1:300	1:300	1:300
NANOG	R&D Systems	AF1997	Goat	1:100	1:100	1:100
NANOG	R&D Systems	AF2729	Goat	NA	1:100	1:100
SOX2	R&D Systems	AF2018	Goat	1:100	1:100	1:100
SOX17	R&D Systems	AF1924	Goat	1:100	1:100	NA
GATA6	R&D Systems	AF1700	Goat	1:100	1:100	NA
SUSD2-PE	BioLegend	327406	Mouse	1:100	NA	NA
KLF4	Tebu-Bio	09-0021	Mouse	1:100	NA	NA
TFE3	Thermo Fischer	PA5-21615	Rabbit	1:250	NA	NA
KLF17	Sigma-Aldrich	HPA024629	Rabbit	1:100	NA	NA
HuN	Sigma-Aldrich	MAB1281	Mouse	NA	1:100	NA
TuJ1	Sigma-Aldrich	T2200	Rabbit	NA	NA	1:100
H3K27me3	Cell Signalling	9733	Rabbit	1:1000	NA	NA
TFAP2C	R&D Systems	AF5059	Goat	1:100	NA	NA
TFCP2L1	R&D Systems	AF5726	Goat	1:100	NA	NA

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