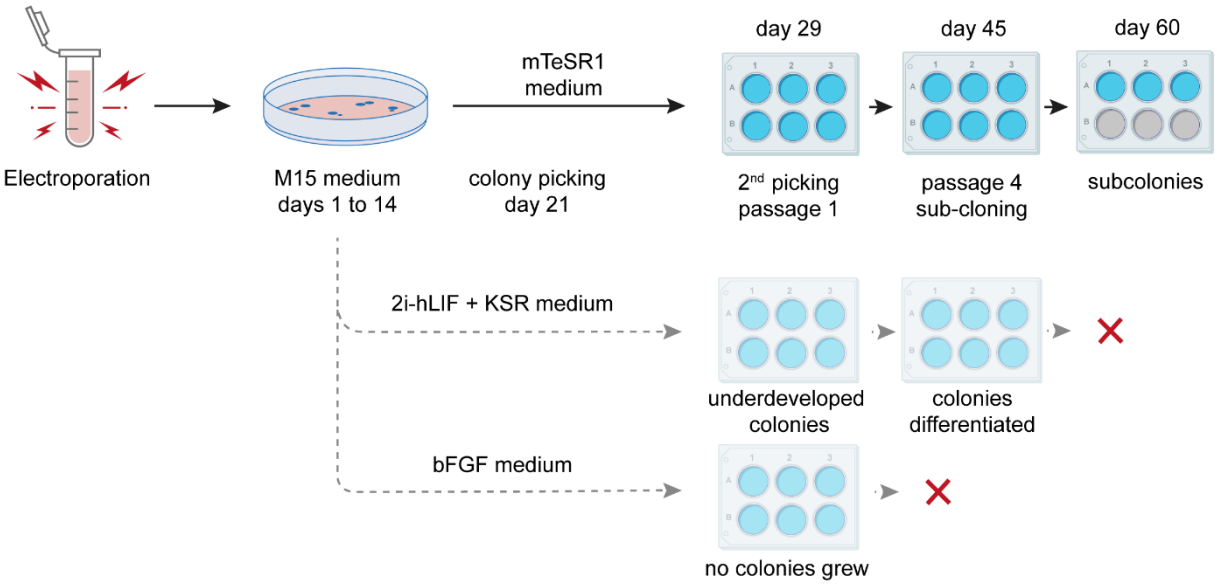
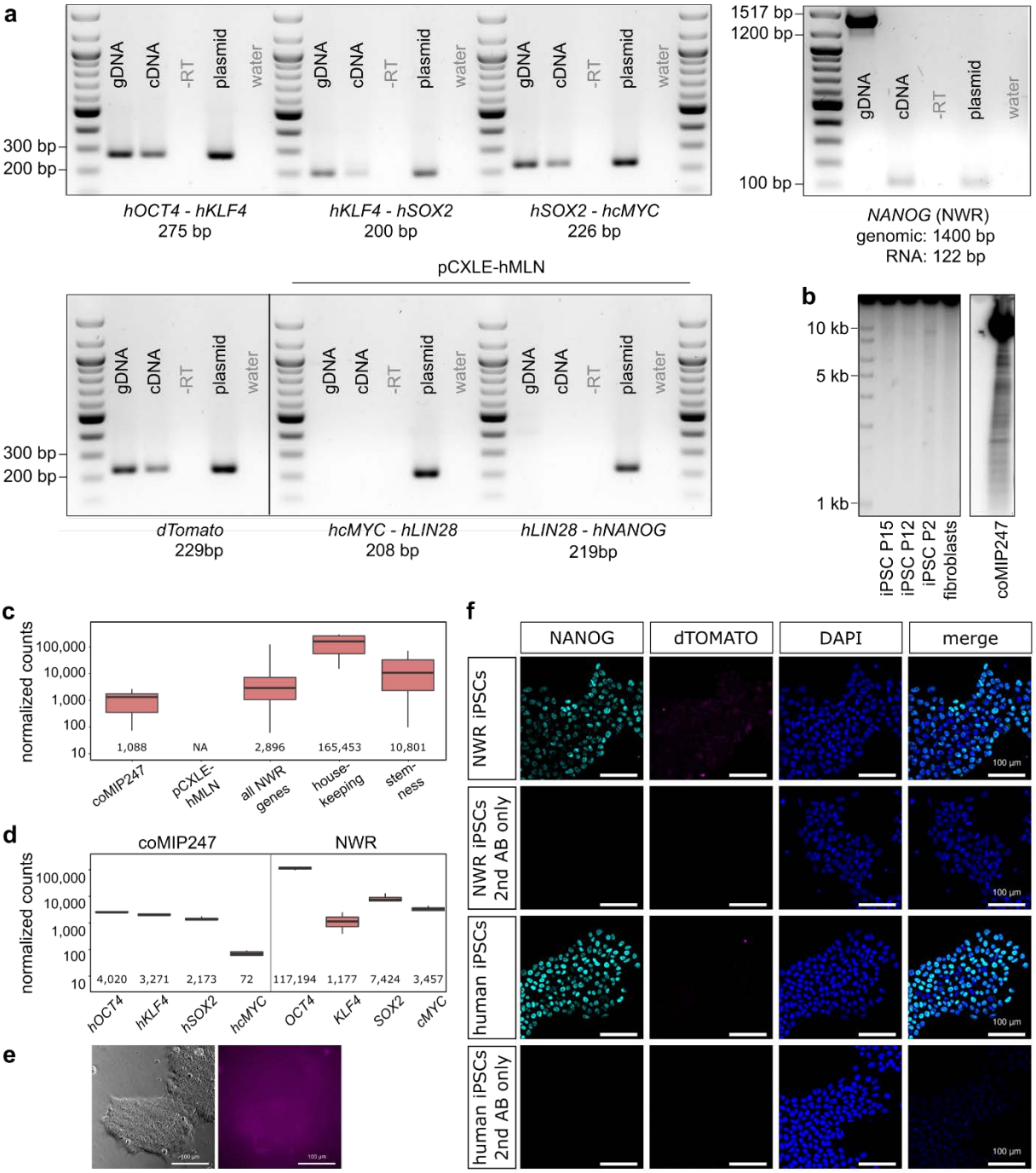


Supplementary Figure S1. Overview of tested reprogramming conditions to generate NWR iPSCs. Related to Figure 1.

After electroporation, cells were grown in M15 reprogramming medium containing LIF. After two weeks, the medium was changed to pluripotency supporting conditions, and at day 21 single colonies were picked. In total, three different media were tested: mTeSR1 (high bFGF medium) resulted in colony formation and successful line establishment as represented in Figure 1d; 2i-hLIF medium led to colony formation, but post picking no lines could be established (experiment terminated at day 45); (low) bFGF medium did not enable colony formation (experiment terminated at day 40).



Supplementary Figure S2. Integration and expression of the reprogramming vector coMIP247. Related to Figure 1.

a) Gel-electrophoresis of PCR amplified genomic DNA (gDNA) and complementary DNA (cDNA). Primer spanned linker regions within the reprogramming vectors coMIP247 and pCXLE-hMLN to disable amplification of endogenous sequences. As negative controls, water and reverse transcription reaction lacking reverse transcriptase (-RT) were used.

Plasmid DNA served as positive control. Amplification of endogenous NWR *NANOG* using exon spanning primers confirmed purity of gDNA and cDNA.

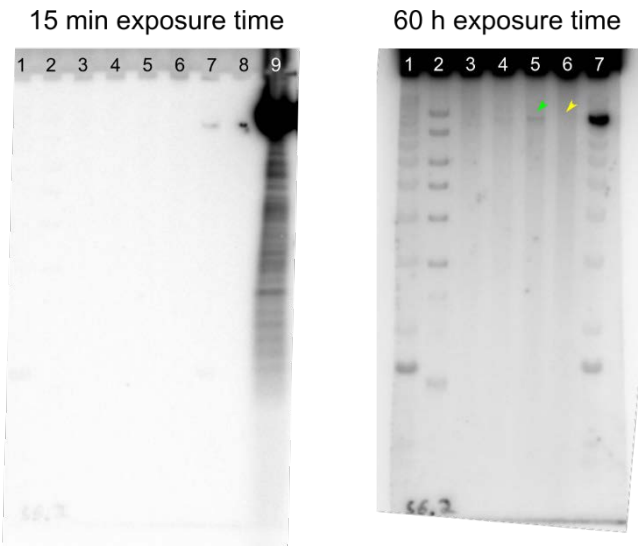
b) Southern blot of SPE1-HF digested genomic DNA isolated from NWR fibroblasts and iPSCs at passage P2, P12 and P15 revealed a single band in iPSCs but not fibroblasts indicating integration of coMIP247 at one side in the genome (left panel, exposure time: 60 hours). As control, digested coMIP247 DNA was loaded (right panel, exposure time: 15 minutes). Full-length blots are presented in Supplementary Figure S3.

c) In RNA-sequencing experiments, reads mapping to coMIP247, but not pCXLE-hMLN, were detected confirming the expression of coMIP247 in NWR iPSCs at approximately 1/3 and 1/10 of all expressed NWR and endogenous NWR genes associated with stemness, respectively. Numbers represent the median per gene category. Number of samples: four. Sample type: NWR iPSCs cultured in primed conditions (mTeSR1).

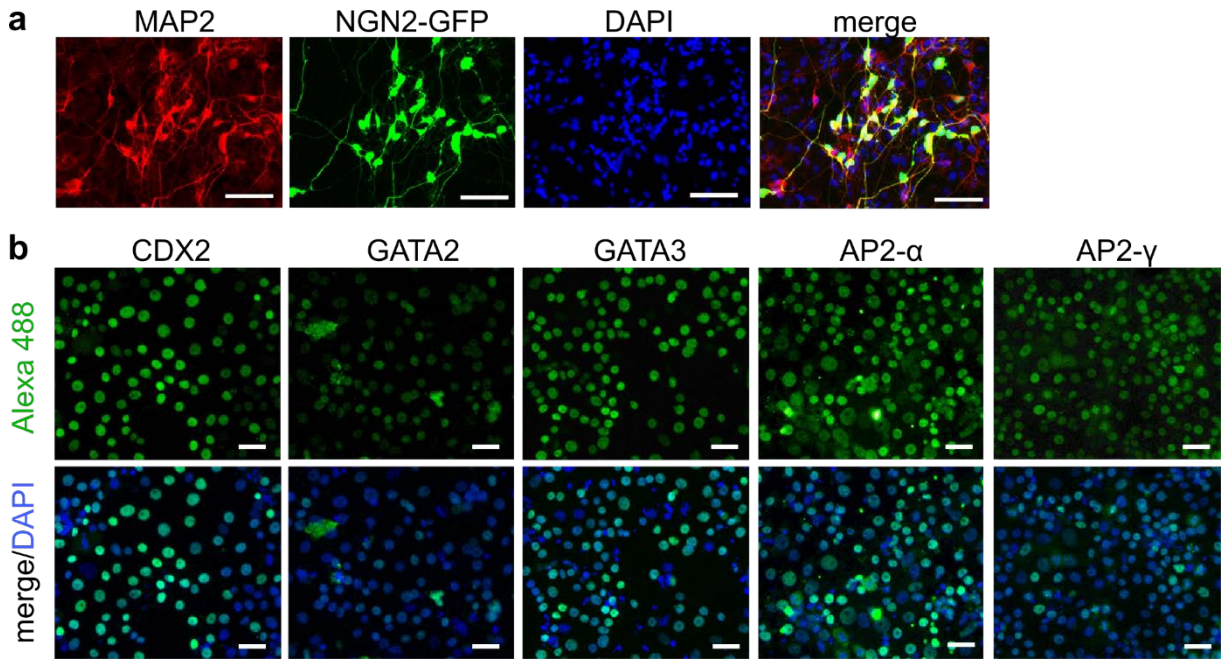
d) Expression of reprogramming factors encoded by coMIP247 in comparison to the expression of the corresponding endogenous NWR genes. RNA-sequencing data, numbers represent the median per gene. Number of samples: four. Sample type: NWR iPSCs cultured in primed conditions (mTeSR1).

e) Live imaging of NWR iPSCs. Left: phase contrast, right: dTOMATO expression, exposure time: 999ms. Scale bars: 100 µm.

f) NWR iPSCs stained for NANOG, dTOMATO, and secondary antibody only. As control, human iPSCs were stained in parallel. Scale bars: 100 µm.

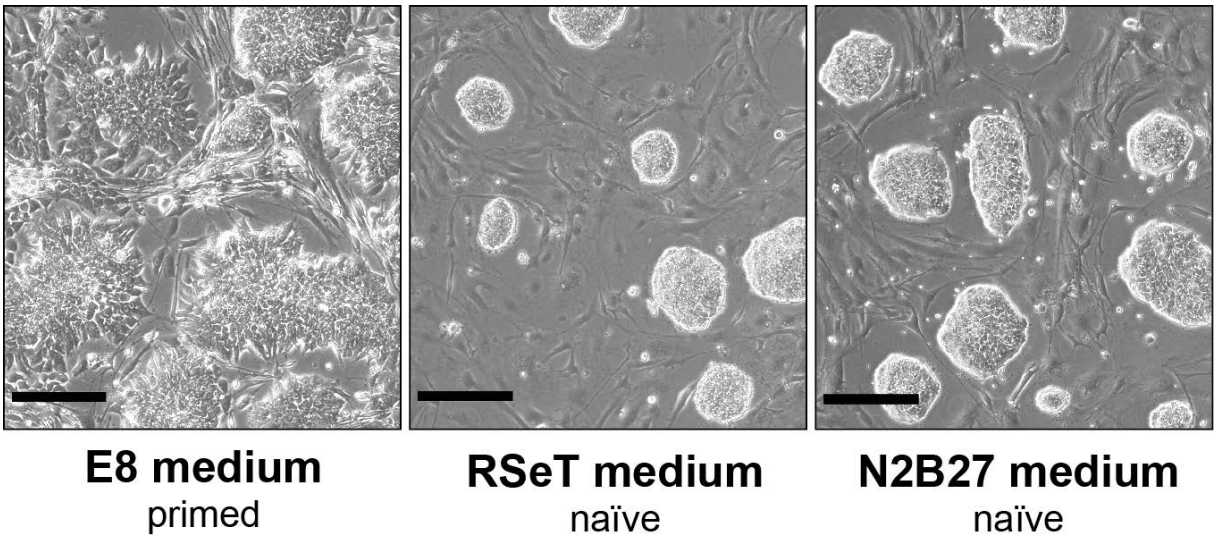


Supplementary Figure S3. Full-length southern blot. Related to Supplementary Figure S2b. To test, if the reprogramming plasmid coMIP247 integrated into the NWR genome, we performed southern blotting of SPE1-HF digested DNA. Lanes were loaded as follows: Invitrogen 1 kb DNA Ladder (1), ThermoFisher MassRuler DNA Ladder (2), NWR iPSCs passage P15 (3), P12 (4), P2 (5), NWR fibroblasts (6), Invitrogen 1 kb DNA Ladder (7), empty lane (8), coMIP247 plasmid (9). coMIP247 gave a strong signal after 15 minutes exposure time (left). To enable longer exposure time, we cut the membrane between lane 7 and 8. A single band in NWR iPSCs (green arrow), but not NWR fibroblasts (yellow arrow), became visible after 60 h exposure time (right). Signal in lane 7 (Invitrogen 1kb DNA Ladder) is probably an artefact from spilled coMIP247 plasmid (lane 9). As no signal was observed in lane 6 (NWR fibroblasts), we concluded that the signal in lane 5 (NWR iPSCs) is specific.



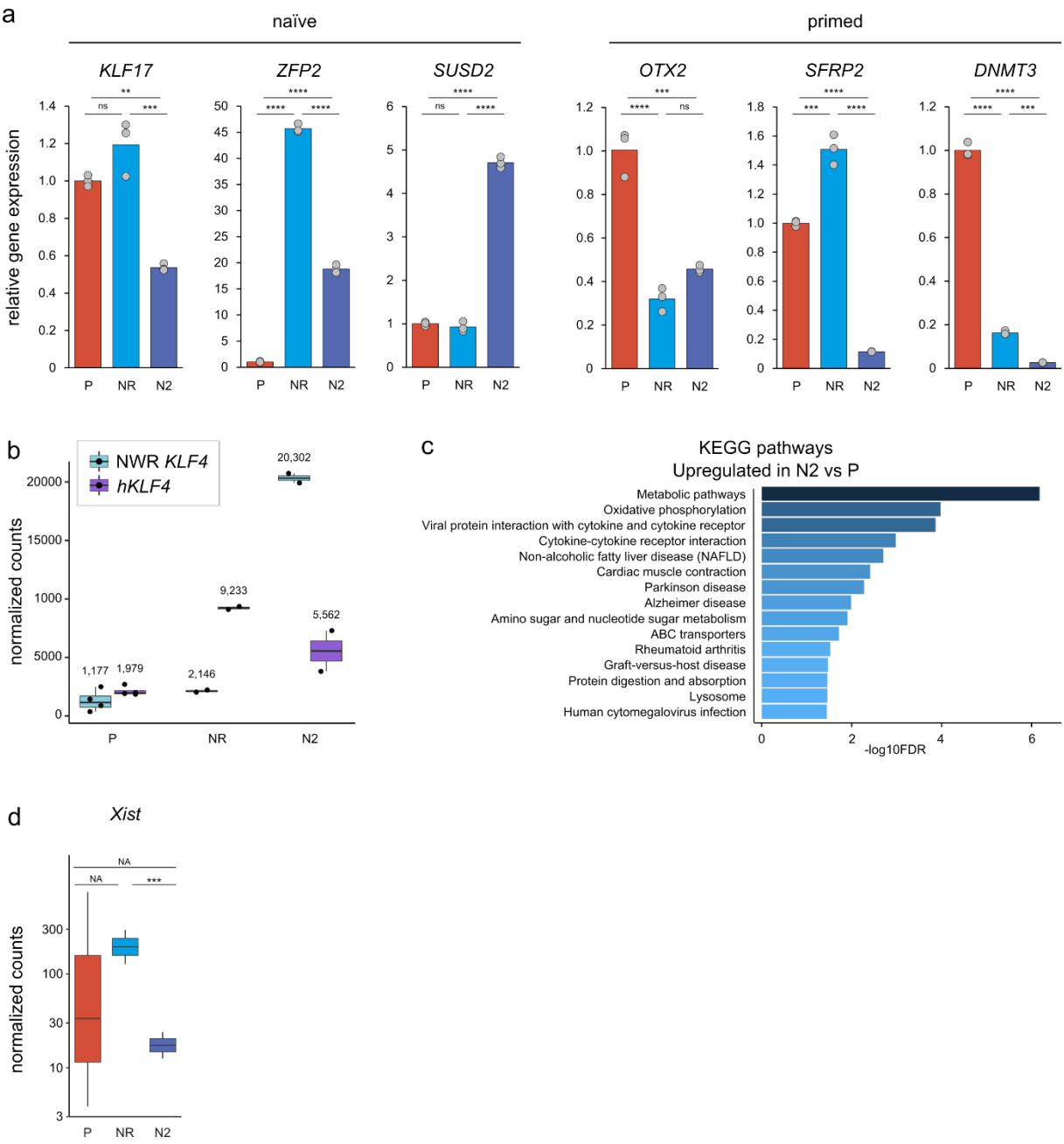
Supplementary Figure S4. Immunostainings of NWR iPSCs differentiated into forebrain-like neurons (using ectopic NGN2-GFP expression) **(a)**, and trophoblast progenitors **(b)**. Related to Figure 2. Scale bars: 50 µm **(a)** and 25 µm **(b)**.

Supplementary Movie M1. NWR iPSCs differentiate into beating cardiomyocytes. Related to Figure 2.



Supplementary Figure S5. Morphology of human iPSCs in primed and naïve culturing conditions. Related to Figure 3.

Human iPSCs (line BIHi005A) grown on mouse embryonic fibroblasts in primed (E8, left), and naïve conditions: RSeT (middle) and N2B27 (right). Scale bars: 100 μm.



Supplementary Figure S6. Characterization of naïve-like pluripotency in NWR iPSCs.

Related to Figures 3 and 5.

a) Relative expression of naïve and primed marker genes was measured by RT-qPCR. In 15/18 comparisons (P vs. NR, P vs. N2, NR vs. N2), results agreed with RNA-sequencing data (see Figure 3d). Bars represent means. *, **, ***, **** P value >0.05, 0.01, 0.001, 0.0001, respectively (one-way ANOVA followed by Bonferroni's post-hoc test, $\alpha < 0.05$).

b) Expression of endogenous NWR *KLF4* and coMIP-encoded exogenous human *KLF4* (*hKLF*) in primed and naïve culturing conditions. RNA-sequencing data, numbers above bars represent the median.

c) Differential gene expression analysis revealed 15 KEGG pathways, which were significantly (DESeq2 adjusted P value < 0.05) upregulated in naïve-like NWR iBCL2-GFP-iPSCs (N2, N2B27 protocol) as compared to primed NWR iPSCs (P, mTeSR1).

d) The long non-coding RNA *Xist* is substantially and significantly lower expressed in N2 as compared to P and NR samples, respectively. The difference NR vs. N2 is significant according to DESeq2 (adjusted P value = 6.2×10^{-10}). As *Xist* expression was not determined in one P sample and the variation was large in P samples in general, DESeq2 could not calculated P values for the comparisons P vs. NR and P vs. N2.

Abbreviations: ns: not significant; na: no answer; NR, N2: NWR iBCL2-GFP-iPSCs, naïve conditions, RSeT and N2B27 protocols, respectively; P: NWR iPSCs primed conditions (mTeSR1).

Supplementary Table T1: List of key marker genes. Related to Figures 3 and 5.

Marker genes associated with pluripotency, stemness, naïve and primed state, and for PGCs were selected from literature (i.a., [1–4]). Additionally, seven common housekeeping genes were added to the list. Listed genes (60) were consistently detected in all NWR samples and used for comparison of gene categories across culturing conditions (Figure 3c). For gene expression comparison of NWR, SWR, human and mouse PSCs by principal component analysis only orthologous genes (excluding PGC marker genes), which were detected in all analyzed species, were used (46/60, black font, Figure 5d).

gene_name	gene_id	category
ACTB	*ACTB*	housekeeping
CTNNB1	*CTNNB1*	housekeeping
EEF1A1	*EEF1A1*	housekeeping
GAPDH/JH767797.1/-	*GAPDH*	housekeeping
PPIA/JH767794.1/+	*PPIA*	housekeeping
RAF1	*RAF1*	housekeeping
RPLP0/JH767752.1/+	*RPLP0*	housekeeping
CFC1	*CRIPTO*	stemness
DNMT3A	*DNMT3A*	stemness
FGF4	*FGF4*	stemness
FGF5	*FGF5*	stemness
FZD5	*FZD5*	stemness
GRB7	*GRB7*	stemness
JARID2	*JARID2*	stemness
LIN28A	*LIN28A*	stemness
LIN28B	*LIN28B*	stemness
NODAL	*NODAL*	stemness
NOG	*NOG*	stemness
PTEN	*PTEN*	stemness
REST/JH767724.1/+	*REST*	stemness
SALL4	*SALL4*	stemness
TCF3	*TCF3*	stemness
TDGF1	*TDGF1*	stemness
ZFP42	*ZFP42*	naïve
DNMT3L	*DNMT3L*	naïve
DPPA5	*DPPA5*	naïve
ESRRB	*ESRRB*	naïve
IL6ST	*IL6ST*	naïve
KLF17	*KLF17*	naïve
KLF2	*KLF2*	naïve

KLF4	*KLF4*	naïve
KLF5	*KLF5*	naïve
TFCP2L1	*TFCP2L1*	naïve
SUSD2	*SUSD2*	naïve
BEX1	*BEX1*	primed
CD24	*CD24*	primed
DNMT3B	*DNMT3B*	primed
OCT6	*POU3F1*	primed
OTX2/JH767740.1/-	*OTX2*	primed
SFRP2	*SFRP2*	primed
TEAD2	*TEAD2*	primed
ZIC2/JH767732.1/+	*ZIC2*	primed
CD9	*CD9*	pluripotency
FOXD3	*FOXD3*	pluripotency
FUT4	*FUT4*	pluripotency
GABRB3	*GABRB3*	pluripotency
LEFTY1	*LEFTY1*	pluripotency
LIFR	*LIFR*	pluripotency
MYC	*MYC*	pluripotency
NANOG	*NANOG*	pluripotency
PODXL/JH767754.1/+	*PODXL*	pluripotency
POU5F1	*POU5F1*	pluripotency
SOX2	*SOX2*	pluripotency
TERF1	*TERF1*	pluripotency
TERT	*TERT*	pluripotency
TFAP2C	*TFAP2C*	PGCs
BLIMP1	*BLIMP1*	PGCs
PRDM14	*PRDM14*	PGCs
NANOS3	*NANOS3*	PGCs
STELLA	*STELLA*	PGCs

Supplementary Table T2. Overview of blastocyst injections followed by retransfer of embryos into pseudo-pregnant foster mice. Related to Figure 4.

Depicted are the number of retransferred embryos, which were either injected with NWR iPSCs or non-injected (control). Pseudo-pregnant foster mice were sacrificed at the indicated developmental stages (E7.5, E8.5 or E9.5). The number of deciduae reflects how many of the retransferred blastocysts implanted, and gives information about the success of retransfer. The number of isolated embryos shows how many embryos developed and were analyzed for GFP expressing cells. Embryos containing GFP positive cells were considered NWR-mouse chimeras. In one experiment (NWRN-iGFP-iPSCs, primed, E7.5), two embryos were isolated from one decidua.

	Embryonic Day	Retransferred Embryos	Deciduae	Isolated Embryos	Chimeras	Success of retransfer (% Deciduae/ Transferred Embryos)	Development (% Isolated Embryos/ Deciduae)	Chimerism (% Chimeras/ Isolated Embryos)
Control (non-injected)	7,5	23	17	12	-	73,9	70,6	0,0
	8,5	25	20	17	-		85,0	0,0
	9,5	48	41	26	-	87,5	61,9	0,0
NWR iGFP-iPSCs primed mTeSR1 P1	7,5	12	9	10	-	75,0	111,1	0,0
	8,5	12	12	9	1	100,0	75,0	11,1
	9,5	24	20	12	1	83,3	60,0	8,3
NWR iBCL2-GFP-iPSCs primed mTeSR1 P2	7,5	12	9	6	1	75,0	66,7	16,7
	8,5	12	12	11	-	100,0	91,7	0,0
	9,5	24	19	13	1	79,2	68,4	7,7
NWR iBCL2-GFP-iPSCs naïve N2B27 N2	7,5	12	8	4	2	66,7	50,0	50,0
	8,5	12	11	4	4	91,7	36,4	100,0
	9,5	24	21	6	1	87,5	28,6	16,7
NWR iBCL2-GFP-iPSCs naïve RSeT NR	7,5	12	9	4	-	83,3	40,0	0,0
	8,5	12	10	5	1	91,7	45,5	20,0
	9,5	12	8	2	-	66,7	25,0	0,0

Supplementary Table T3. Published datasets of human and mouse ESCs.

Overview of RNA-sequencing datasets obtained from human and mouse ESCs, cultured in naïve and primed conditions [5–10].

Supplementary Table T4. Antibodies used for Immunofluorescence.

Antibody	Marker for	Catalog #	Company	Dilution
rabbit isotype IgG	Control	GTX35035	GeneTex	1:200
mouse isotype IgG	Control	16-4714-85	eBioscience	1:200
TFAP2A [AP-α]	Trophoblast	sc-184X	santa cruz	1:100
TFAP2C [AP-γ]	Trophoblast	sc-12762X	santa cruz	1:100
GATA2	Trophoblast	sc-9008X	santa cruz	1:100
GATA3	Trophoblast	sc-268	santa cruz	1:200
CDX2	Trophoblast	D11D10	Cell Signaling	1:40
SOX2	Pluripotency	CS1002	Millipore	1:100
OCT4	Pluripotency	2840 (C30A3)	Cell Signaling	1:100
NANOG	Pluripotency	PA1-097 AF1997	Thermo Fisher R&D Systems	1:100-1:200 1:100
SSEA3	Pluripotency	MA1-020	Thermo Fisher	1:100
ACTN2 (α-Actinin)	Cardiomyocytes	A7811	Sigma-Aldrich	1:600
TNNT2 (Troponin T)	Cardiomyocytes	A25973 (Human Cardiomyocyte Immunocyt-ochemistry Kit)	ThermoFisher	1:1000
GATA4	Endoderm	D3A3M	Cell Signaling	1:100
GATA6	Endoderm	D61E4	Cell Signaling	1:100
SOX17	Endoderm	09-038	Millipore	1:100
PAX6	Neural stem cells	A24354 (Human Neural Stem Cell Immunocyto-chemistry Kit)	Thermo Fisher	1:50
NESTIN	Neural stem cells			1:50
SOX1	Neural stem cells			1:50
SOX2	Neural stem cells			1:50
MAP2	iNeurons	188004	Synaptic Systems GmbH	1:500
dTOMATO/RFP	Transgene	biorbyt	orb334992	1:1000
Alexa Fluor(R) 594 donkey anti-rabbit IgG (H+L)	2nd Antibody	A25970	ThermoFisher	1:200
Alexa Fluor(R) 488 donkey anti-mouse IgG (H+L)	2nd Antibody	A25972	ThermoFisher	1:200
Alexa Fluor(R) 488 goat anti-mouse IgG (H+L)	2nd Antibody	A11001	ThermoFisher	1:1000
Alexa Fluor(R) 488 goat Anti-Rabbit IgG (H+L)	2nd Antibody	A11008	ThermoFisher	1:1000

Supplementary Table T5. Primers.

Primer name	Target	Sequence (5'-3')	Product size (bp)
hOCT-KLF4_F	coMIP247 Plasmid	ACTTCACCGCCCTGTACAG	275
hOCT4-KLF4_R		CTCCCGCCATCTGTTGTTAG	
hKLF4-SOX2_F		GGCACTACAGAAAGCACACC	200
hKLF4-SOX2_R		CTTCAGCTCGGTTTCCATCA	
hSOX2-cMYC_F		ATGAGCCAGCACTACCAGAG	226
hSOX2-cMYC_R		CCTCCTCGTCGCAGTAGAAA	
dTOM_F		GCCCCGTAATGCAGAAGAAG	229
dTOM_R		GTGTAGTCCTCGTTGTGGGA	
hcMYC-LIN28_F	pCXLE-hMLN Plasmid	GGAAACGACGAGAACAGTTGA	208
hcMYC-LIN28_R		GCCTCTTCTGCCGCCTTG	
hLIN28-NANOG_F		CCTAGTGCACAGGGAAAGCC	219
hLIN28-NANOG_R		ACAAGCTGGATCCACACTCA	
NANOG_ES_F	NWR NANOG	TCCAGCAGATGCAAGAACTTT	122 (cDNA) 1400 (gDNA)
NANOG_ES_R		GCAAGTCTTTGGCCAGTTGT	
PPIA_F	NWR PPIA	GTCCAGGAATGGCAAGACCA	142
PPIA_R		TCAAGCAGACGGGGGTAAAG	
ARBP_F	NWR ARBP	CATGCTGAACATCTCGCCCT	113
ARBP_R		AGCGAGAATGCAGAGCTTCC	
OTX2_F	NWR OTX2	TGGGCTGGACATTCCAGTTT	102
OTX2_R		TCCTTCTATGCCTCCGGGAA	
SFRP2_F	NWR SFRP2	GTGGCTCAAAGACAGCTTGC	91
SFRP2_R		CTCCCCACCCACTTTCTGTC	
DNMT3B_F	NWR DNMT3B	TGGAATACGAAGCCCCCAAG	104
DNMT3B_R		GACCAAGTACCCTGTCGCAA	
KLF17_F	NWR KLF17	TGTCTCCCTCCCAACCAAGA	100
KLF17_R		TTCCCATCAAAGGTCCTGGC	
SUSD2_F	NWR SUSD2	TTGGGACTTGTGCACTGTGT	129
SUSD2_R		CACTTTGTTGGGGTGCACAG	
ZFP42_F	NWR SFP42	CGAAGGGTGCGGAAAACGAT	97
ZFP42_R		GCCTTCAACGGGACACACAA	

Supplementary References

1. Habekost, M., Jørgensen, A. L., Qvist, P. & Denham, M. Transcriptomic profiling of porcine pluripotency identifies species-specific reprogramming requirements for culturing iPSCs. *Stem Cell Res.* **41,** 101645 (2019).
2. International Stem Cell Initiative *et al.* Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat. Biotechnol.* **25,** 803–816 (2007).
3. Messmer, T. *et al.* Transcriptional Heterogeneity in Naive and Primed Human Pluripotent Stem Cells at Single-Cell Resolution. *Cell Rep.* **26,** 815–824.e4 (2019).
4. Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S. & Saitou, M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell* **146,** 519–532 (2011).
5. Atlasi, Y. *et al.* The translational landscape of ground state pluripotency. *Nat. Commun.* **11,** 1617 (2020).
6. Chan, Y.-S. *et al.* Induction of a human pluripotent state with distinct regulatory circuitry that resembles preimplantation epiblast. *Cell Stem Cell* **13,** 663–675 (2013).
7. Dirks, R. A. M. *et al.* Allele-specific RNA-seq expression profiling of imprinted genes in mouse isogenic pluripotent states. *Epigenetics Chromatin* **12,** 14 (2019).
8. Factor, D. C. *et al.* Epigenomic comparison reveals activation of “seed” enhancers during transition from naive to primed pluripotency. *Cell Stem Cell* **14,** 854–863 (2014).
9. Rostovskaya, M., Stirparo, G. G. & Smith, A. Capacitation of human naïve pluripotent stem cells for multi-lineage differentiation. *Development* **146,** (2019).
10. Takashima, Y. *et al.* Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell* **158,** 1254–1269 (2014).