

Fig. S1. Principal component analysis matrix using the first five principal components for the top 12,000 most variable expressed genes in day 5, 6 and 7 TE samples. (B) RT-qPCR analysis for the detection of exogenous *KLF5*, *GATA2*, *GATA3* and *MYC* in 5F-hESCs after 48 h of doxycycline treatment. Relative expression is reflected as fold change over uninduced 5F-hESCs cultured normalized to *GAPDH*. (C) Immunofluorescence analysis for the detection of TFAP2C in 5F-hESCs after 48 h of doxycycline. (D) Time-course qRT-PCR analysis for the detection of endogenous *GATA2*, *GATA3*, *TP63*, *NR2F2*, *ENPEP*, *EGFR* and *ITGA6* in 5F-hESCs across 20 days of dox-induction in hTSC media. Relative expression is reflected as fold change over 5F-hESCs at day 0 plated in mTSeR pluripotency media, normalized to *GAPDH*. (E) Immunofluorescence analysis for the detection of GATA3, TP63 and KRT18 (green) and DAPI nuclear expression (blue) in doxycycline-induced and uninduced 5F-hESCs on day 20. Scale bars: 50 μ m. (F) Schematic representation of the strategy for combinatorial transdifferentiation experiments using modified mRNAs.

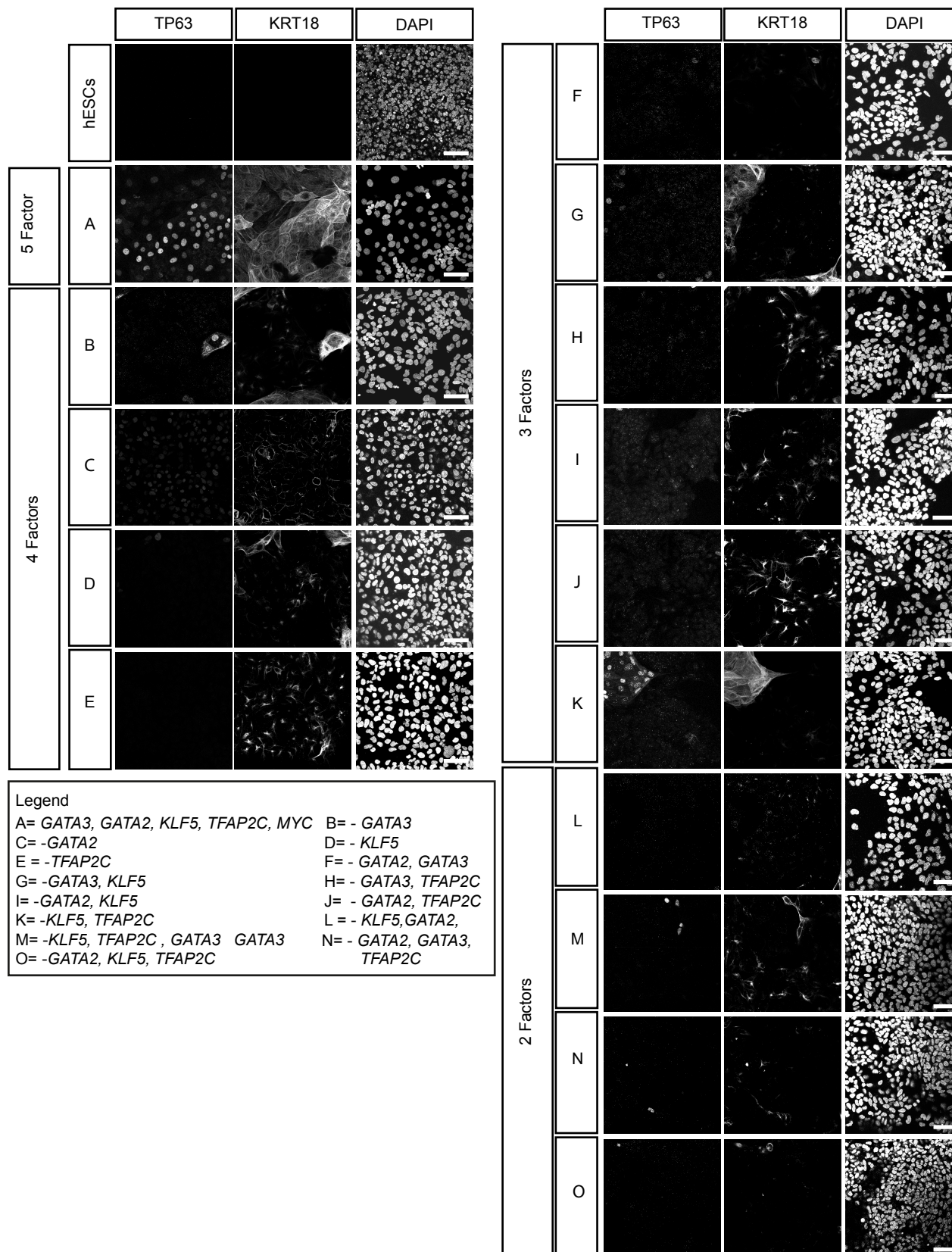


Fig. S2. Extended immunofluorescence analysis for the detection of TP63, KRT18 and DAPI nuclear expression in transfected cells. Key for mRNA cocktail combinations: A, Five factors positive controls; B, *GATA2, TFAP2C, KLF5* and *MYC* (*GATA3* omitted); C, *GATA3, TFAP2C, KLF5* and *MYC* (*GATA2* omitted); D, *GATA2, GATA3, TFAP2C* and *MYC* (*KLF5* omitted); E, *GATA2, GATA3, KLF5* and *MYC* (*TFAP2C* omitted); F, *TFAP2C, KLF5* and *MYC* (*GATA2* and *GATA3* omitted); G, *GATA2, TFAP2C* and *MYC* (*GATA3* and *KLF5* omitted); H, *GATA2, KLF5* and *MYC* (*GATA3* and *TFAP2C* omitted); I, *GATA3, TFAP2C* and *MYC* (*GATA2* and *KLF5* omitted); J, *GATA3, KLF5* and *MYC* (*GATA2* and *TFAP2C* omitted); K, *GATA2, GATA3* and *MYC* (*TFAP2C* and *KLF5* omitted); L, *TFAP2C, KLF5* and *MYC* (*KLF5, GATA2* and *GATA3* omitted); M, *GATA2* and *MYC* (*GATA3, TFAP2C* and *KLF5*); N, *KLF5* and *MYC* (*GATA2, GATA3* and *TFAP2C* omitted). hESCs cultured in mTeSR1 media are included as a negative control. Scale bars: 50 μ m.

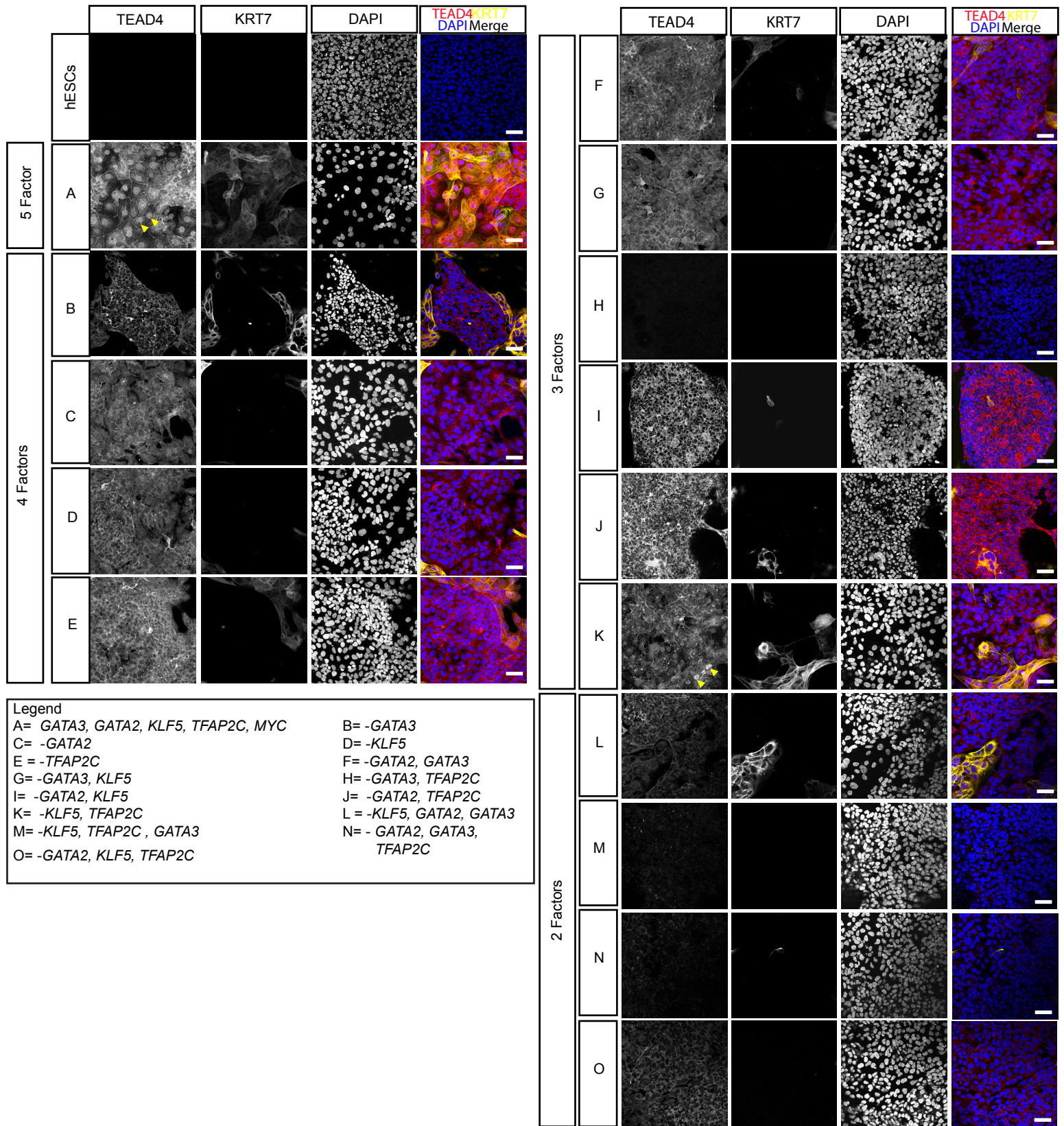


Fig. S3. Immunofluorescence analysis for the detection of TEAD4 (red), KRT7 (yellow) and DAPI (blue) nuclear expression in transfected cells. Key for mRNA cocktail combinations: A, Five factors positive controls; B, *GATA2*, *TFAP2C*, *KLF5* and *MYC* (*GATA3* omitted); C, *GATA3*, *TFAP2C*, *KLF5* and *MYC* (*GATA2* omitted); D, *GATA2*, *GATA3*, *TFAP2C* and *MYC* (*KLF5* omitted); E, *GATA2*, *GATA3*, *KLF5* and *MYC* (*TFAP2C* omitted); F, *TFAP2C*, *KLF5* and *MYC* (*GATA2* and *GATA3* omitted); G, *GATA2*, *TFAP2C* and *MYC* (*GATA3* and *KLF5* omitted); H, *GATA2*, *KLF5* and *MYC* (*GATA3* and *TFAP2C* omitted); I, *GATA3*, *TFAP2C* and *MYC* (*GATA2* and *KLF5* omitted); J, *GATA3*, *KLF5* and *MYC* (*GATA2* and *TFAP2C* omitted); K, *GATA2*, *GATA3* and *MYC* (*TFAP2C* and *KLF5* omitted); L, *TFAP2C*, *KLF5* and *MYC* (*KLF5*, *GATA2* and *GATA3* omitted); M, *GATA2* and *MYC* (*GATA3*, *TFAP2C* and *KLF5*); N, *KLF5* and *MYC* (*GATA2*, *GATA3* and *TFAP2C* omitted). hESCs cultured in in mTeSR1 media are included as a negative control.

Table S1. Bulk RNA-sequencing analysis of TE across human embryo development. RNA-seq data is normalised using the RPKM method.

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<https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.202778#supplementary-data>

Table S2. Genes expressed in common or unique to a specific stage of TE development. Genes were considered to be expressed if RPKM>5.

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Table S3. Transcription factors detected in human TE by RNA-seq analysis.

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Table S4. DESeq2 analysis was used to calculate the probability of differential expression between human TE and EPI. RPKM values and log₂-fold change difference in expression are noted.

DESeq2 analysis				
	TE mean	EPI mean	Log₂FC	p-value
GATA2	4528.6	16.1	8.1	2.20E-19
GATA3	8457.3	255.1	5	4.06E-13
KLF5	4609.4	1931.3	2.4	0.08
TFAP2C	1525.7	1135.1	0.4	0.9
MYC	3830.4	1405.5	1.4	0.6

RPKM values at time-points analysed

	Day 5	Day 6	Day 7
GATA2	19.3	65.6	52.5
GATA3	93.8	120.4	96.8
KLF5	45.2	45.2	46.7
TFAP2C	644.2	98.8	393.7
MYC	32.9	50.9	39.5

Table S5. Oligonucleotide sequences used for qRT-PCR analysis.

<u>Oligo ID</u>	<u>Sequence</u>	<u>Reference</u>	<u>Oligo ID</u>	<u>Sequence</u>
GAPDH_F	GATGACATCAAGAAGGTGGTG		GATA3_3UTR_F	GGTGTCTGTGTTCCAACCAC
GAPDH_R	GTCTACATGGCAACTGTGAGG		GATA3_3UTR_R	GTGGCCAGTGAAAGGAAACA
TP63_F	CTGGAAAACAATGCCCAGA	Soncin et al. 2018	GATA3_F	CCGCCCTACTACGGAAACTC
TP63_R	AGAGAGCATCGAAGGTGGAG	Soncin et al. 2018	GATA3_R	TCTTGAGAAGGGGCTGAGA
EGFR_F	CTAAGATCCCGTCCATCGCC	Soncin et al. 2018	GATA2_3UTR_F	AGGCCACTGACCATGAAGAA
EGFR_R	GGAGCCCAGCACTTTGATCT	Soncin et al. 2018	GATA2_3UTR_R	CGACGTCCATCTGTTCCCTA
ITGA6_F	GGCGGTGTTATGTCTGAGTC		GATA2_F	GACTACAGCAGCGGACTCTT
ITGA6_R	AATCGCCCATCACAAAAGCTC		GATA2_R	GTTGTCGTCTGACAATTTGC
ENPEP_F	AATTTATGTCCAGCCAGAGC			
ENPEP_R	GTGATGAGTCCCCAGTTCTC			
NR2F2_F	GCCATAGTCTGTTCACCTCA	lo et al. 2021		
NR2F2_R	AATCTCGTCGGCTGGTTG	lo et al. 2021		
ENDOU_F	ACAGGGCAGACACCAACAAA			
ENDOU_R	AGGAGGTTGATGAAGCTGC			
GCM1_F	TGCTGTCTGCTTCTCCGTAA			
GCM1_R	CACCTATTCGACTCCCCCTCA			
PSG3_F	TCGTAAAGCGAGGTGATGGG	lo et al. 2021		
PSG3_R	AAGCTCACAGCCTCCATGTC	lo et al. 2021		
SDC1_F	CTTCACACTCCCCACACAGA	lo et al. 2021		
SDC1_R	GTATTCTCCCCGAGGTTTC	lo et al. 2021		
HLAG_F	CCACCACCCTGTCTTTGACTAT	lo et al. 2021		
HLAG_R	ACGTCCTGGGTCTGGTCTT	lo et al. 2021		
LRR32_F	GCTGCACAACACCAAGACAAA	lo et al. 2021		
LRR32_R	GATCAAGGGTCTCAGTGTCTGG	lo et al. 2021		
LVRN_F	GGGAGGGACTCTTCTCAAC	lo et al. 2021		
LVRN_R	GGGAAAACATACCTGGCAAA	lo et al. 2021		
MMP2_F	CCCTGTGTCTTCCCCTTAC			
MMP2_R	ATCGTAGTTGGCTGTGGTCTG			
NOTUM_F	TTTGCTACAAGGTCTACCCG	lo et al. 2021		
NOTUM_R	TCAAACAGCCACTGCACCAC	lo et al. 2021		
GABRP_F	GCCCTAACAGAGCCTCAACA	Rostovkaya et al. 2022		
GABRP_R	CCCTGGATGCACATCCTCTC	Rostovkaya et al. 2022		
HEY1_F	GCTGGTACCCAGTGCTTTGAG	lo et al. 2021		
HEY1_R	CAAGGGCGTGCGGTCAAAGTA	lo et al. 2021		
ISL1_F	TCTCCGGATTTGGAATGGCA	Yang et al. 2021		
ISL1_R	CCTTGCACCGCTTGTGTTGA	Yang et al. 2021		
WNT6_F	CAGCCCCTGGTTATGGACC			
WNT6_R	TCTCCGAATGCCTGTTGC			
MYC_3UTR_F	ACCCTTCGCTATCATGCCTT			
MYC_3UTR_R	TCTTGGGCATGTGGATGAGT			
MYC_F	CGTCCTCGGATTCTCTGCTC			
MYC_R	GCTGCGTAGTTGTGCTGATG			
KLF5_3UTR_F	GGGCTCCCTCAAATGACAGA			
KLF5_3UTR_R	CCACCCCTTACCCATGTTGA			
KLF5_F	CCACCACCTGCCAGTTAAC	Takeda et al. 2022		
KLF5_R	TAAACTTTGTGCAACCAGGGTAA	Takeda et al. 2022		

Table S6. Antibodies used for immunofluorescence analysis.

Antibody	Species	Supplier	Catalogue Number
OCT4	Mouse IgG2b	Santa Cruz	SC-5279
NANOG	Goat	R&D	AF1997
GATA3	Goat	R&D	AF2605
TFAP2C	Goat	R&D	AF5059
GATA2	Rabbit	Santa Cruz	SC-9008
NANOG	Rabbit	Abcam	ab21624
KLF5	Rabbit	Abcam	ab137676
CYTOKERATIN18	Mouse IgG1	Abcam	ab668
TP63	Mouse	Biocare	3066
GCM1	Rabbit	Sigma	HPA011343
HAND1	Rabbit	Abcam	ab196622
TBX3	Goat	Santa Cruz	SC-17871
SOX2	Rat	eBioscience	14-9811-82
TEAD4	Mouse IgG2a	Abcam	ab58310
CYTOKERATIN7	Mouse	Agilent Dako	GA61961-2
Alexa Fluor 488 Phalloidin		ThermoFischer	A12381

Table S7. Annotated sequence files of constructs used to generate modified mRNAs. T7 promoters and T7 terminators are in bold font and underlined. 5'UTR and 3'UTR sequences are in bold font.

GATA2 isoform 1

TAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGAGGTGGC
GCCCGAGCAGCCGCGCTGGATGGCGCACCCGGCCGTGCTGAATGCGCAGCACCCCGACTCACACCACCCGGGCCCT
GGCGCACAACTACATGGAACCCGCGCAGCTGCTGCCTCCAGACGAGGTGGACGCTCTTCTTCAATCACCTCGACTC
GCAGGGCAACCCCTACTATGCCAACCCCGCTCACGCGCGGGCGCGCTCTCTACAGCCCCGCGCACGCCCGCCT
GACCGGAGGCCAGATGTGCCGCCACACTTGTTCACAGCCCGGTTTGGCCTGGCTGGACGGGGGCAAAGCAGC
CCTCTCTGCCGCTGCGGCCACCACCACAACCCCTGGACCGTGAGCCCTTCTCCAAGACGCCACTGCACCCCTC
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AGAAGTGTCTCCTGACCCTAGCACACCAGGGGGCTGCGTCTCCAGCCTCATCTTCCGCGGGGGTAGTGCAGCCCG
AGGAGAGGACAAGGACGGCGTCAAGTACCAGGTGTCACTGACGGAGAGCATGAAGATGGAAAGTGGCAGTCCCTT
GCGCCAGGCCTAGCTACTATGGGCACCCAGCCTGCTACACACCACCCCATCCCCACCTACCCCTCCTATGTGCC
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CAAGAAGAGCAAGAAAGGGGCGGAGTGCTTCGAGGAGCTGTCAAAGTGCATGCAGGAGAAGTCATCCCCCTTCAG
TGCAGCTGCCCTGGCTGGACACATGGCACCTGTGGGCCACCTCCCGCCCTTCAGCCACTCCGGACACATCCTGCC
CACTCCGACGCCCATCCACCCCTCCTCCAGCCTCTCCTTCGGCCACCCCAACCCGTCAGCATGGTGACCGCCAT
GGGCTAG**GCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTTCCCTTGACCTGTACCTCTTGGT**
CTTTGAATAAAGCCTGAGTAGGAAGTAGCATAACCCCTTGGGGCTCTAAACGGGTCTTGAGGGGTTTTTTG

GATA3 isoform 1

TAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGAGGTGAC
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GGGCCTCAGCCACTCCTACATGGACGCGGCGCAGTACCCGCTGCCGGAGGAGGTGGATGTGCTTTTTTAACATCGA
CGGTCAAGGCAACCACGTCCC GCCCTACTACGAAACTCGGTGAGGGCCACGGTGCAGAGGTACCCCTCCGACCCA
CCACGGGAGCCAGGTGTGCCGCCCGCCTCTGCTTCATGGATCCCTACCCCTGGCTGGACGGCGGCAAAGCCCTGGG
CAGCCACCACACCCGCTCCCCCTGGAATCTCAGCCCTTCTCCAAGACGTCCATCCACCACGGCTCCCCGGGGCC
CCTCTCCGTCTACCCCGGCCCTCGTCTCCTCCTGTGCGGGGGCCACGCCAGCCCGCACCTCTTACCTTCCC
GCCCACCCCGCCGAAGGACGTCTCCCCGACCCATCGCTGTCCACCCAGGCTCGGCCGGCTCGGCCGGCAGGA
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CATGACCGCCCTGGGTGGAGCCTCCTCGTTCGACCCACCACCCCATCACCACTACCCGCCCTACGTGCCCGAGTA
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CAAGCGAAGGCTGTCTGCAGCCAGGAGAGCAGGGACGTCTGTGCGAAGTGTGACACCACCAACCACACTCTG
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GCTGACTCACTGGAGGACTTCCCCAAGAACAGCTCGTTTAAACCGGCCCTTCCAGACACATGTCCCTCCCT
GAGCCACATCTCGCCCTCAGCCACTCCAGCCACATGCTGACCACGCCACGCCGATGCACCCGCCATCCAGCCT
GTCCTTTGGACCACACCACCCCTCCAGCATGGTCAACCGCATGGGTAG**GCTGCCTTCTGCGGGGCTTGCCCTTCT**
GGCCATGCCCTTCTTCTTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTAGCATAA
CCCCTTGGGGCCTCTAAACGGGTCTTGAGGGTTTTTTG

KLF5 isoform 1

TAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCTACAAG
GGTGTGAGCATGAGCGCCCGCCTGGGACCCGTGCCCCAGCCGCCGCGCCGAGGACGAGCCGGTGTTCGCGCA
GCTCAAGCCGGTGTGGGGCGCCGGAATCCGGCCCCGCGACGCGCGCTCTTCCCCGGCGAGGAGCTGAAGCACGC
GCACCACCGCCCGCAGGCGCAGCCCGCGCCCGCGCAGGCCCGCAGCCGGCCAGCCGCCCGCCACCGGCCCGCG
GCTGCCTCCAGAGGACCTGGTCCAGACAAGATGTGAAATGGAGAAGTATCTGACACCTCAGCTTCCCTCCAGTTCC
TATAATTCCAGAGCATAAAAAGTATAGACGAGACAGTGCCTCAGTCGTAGACCAGTTCTTCACTGACACTGAAGG
GTTACCTTACAGTATCAACATGAACGTCTTCCCTCCCTGACATCACTCACCTGAGAAGTGGCCTTACAAATCCCA
GAGACCGTGCCTAACACACATCAAGACAGAACCTGTTGCCATTTTCAGCCACCAGAGTGAAACGACTGCCCCCTCC

