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Supplemental Information

Direct Induction of the Three Pre-implantation Blastocyst Cell Types from Fibroblasts

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Supplementary Materials

Figure S1

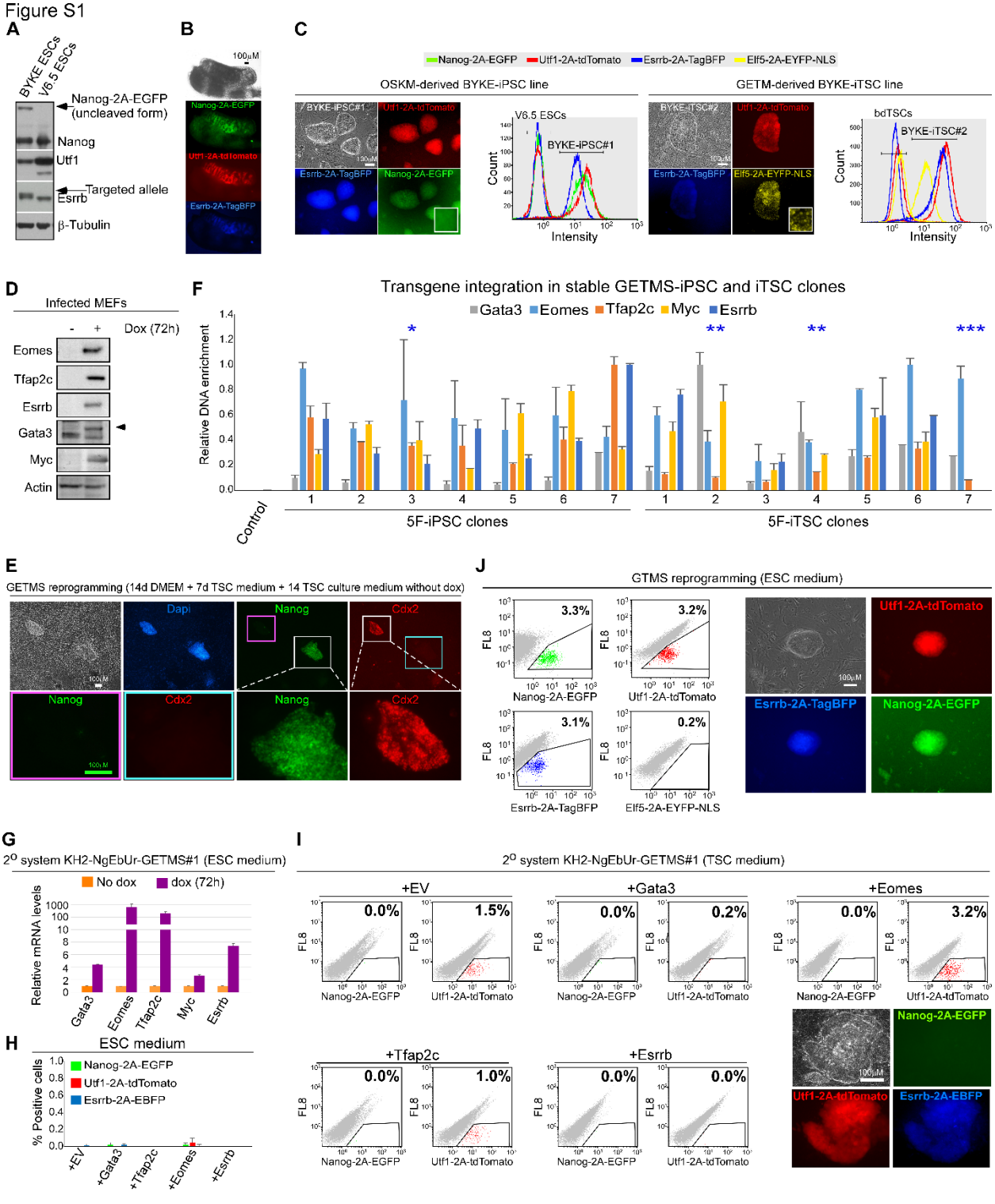

Figure S1. Related to Figure 1.

Esrrb overexpression drives pluripotency fate while Eomes overexpression drives TE fate in GETMS reprogramming combination. (A) Western Blot analysis depicting the protein levels of Nanog, Utf1, Esrrb and β-tubulin in the BYKE ESC system and V6.5 ESC control. **(B)** Bright field and fluorescence images of a 13.5 dpc chimeric male gonad, following BYKE ESC injection, showing the activity of the 3 pluripotency reports. **(C, Left)** Bright field, fluorescence images and FACS analysis of the 3 pluripotent reporters on isolated BYKE-iPSC clone. **(C, Right)** Bright field, fluorescence images and FACS analysis of the 3 TSC reporters on isolated BYKE-iTSC clone. **(D)** Western blot analysis of the five reprogramming factors (Gata3, Tfap2c, Eomes, Myc and Esrrb) and the housekeeping control protein β-actin, 72h post dox exposure in MEFs infected with GETMS. **(E)** Cells were reprogrammed with GETMS in a protocol that promotes the generation of both iPSCs and iTSCs (Figure 1F) in the same dish. Bright field and immunostaining images of endogenous Nanog (green), endogenous Cdx2 (red) and Dapi (blue) showing mutually exclusive expression of Nanog and Cdx2 in their corresponding iPSC and iTSC colonies. Bottom row shows a magnification of the depicted rectangles from the top row. **(F)** qPCR showing the genomic integrations of each of the five factors into seven 5F-iPSC colonies and seven 5F-iTSC colonies. Single asterisk indicates a colony without Gata3 integration, two asterisks indicate colonies without Esrrb integration and three asterisks indicate a colony without Esrrb and Myc integration. Uninfected MEFs were used as control. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(G)** qPCR of the GETMS (5F) genes normalized to the *Gapdh* housekeeping gene in KH2-NgEbUr-GETMS#1 2^{o}MEFs after 72 hours of dox induction. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(H)** Graph summarizing FACS analysis for the 3 pluripotency reporters in KH2-NgEbUr-GETMS#1 2^{o}MEFs that were infected with additional single factor and were reprogrammed for 20 days followed by 10 days of dox removal in ESC medium. **(I)** FACS analysis for Nanog-2A-EGFP and Utf1-2A-tdTomato reporters in KH2-NgEbUr-GETMS#1 2^{o}MEFs that were infected with additional single factor and were reprogrammed for 20 days followed by 10 days of dox removal in TSC medium. For Eomes-infected cells, bright field and fluorescence images of the 3 reporters are shown for one representative iTSC colony. **(J, Left)** FACS analysis for the 4 reporters in BYKE-MEFs infected with GTMS factors that were reprogrammed for 20 days followed by 10 days of dox removal in ESC medium. **(J, Right)** Representative bright field and fluorescence images of GTMS reprogrammed BYKE-MEFs showing triple-positive iPSC colony.

Figure S2

A

Experiment number	Cell line	Culture medium	# of recipient mice	# of injected embryos	# of 13.5 d.p.c recovered embryos/placentas (%)	# of 13.5 d.p.c chimeric embryos/placentas (%)
1	GTMS^{tdTomato}-iPSC#5	S/Lif	2	22	11(50)	7(32)
2	GTMS^{tdTomato}-iPSC#6	S/Lif	2	20	1(5)	1(5)
3	GTMS^{tdTomato}-iPSC#1	S/Lif	1	15	4(26)	4(26)
4	GETMS^{tdTomato}-iPSC#7	S/Lif	3	34	12(27)	3(14)
5	GETMS^{tdTomato}-iPSC#7	70/30	2	24	11(46)	3(12.5)
6	GETMS^{tdTomato}-iPSC#7	2i/Lif	2	21	10(48)	10(48)
7	GETMS^{tdTomato}-iPSC#8	S/Lif	1	15	12(80)	7(47)
8	GETMS^{tdTomato}-iPSC#1	S/Lif	2	56	25(66)	17(30)
9	GETMS^{tdTomato}-iPSC#21	S/Lif	2	27	9(30)	4(15)
10	GETMS^{tdTomato}-iPSC#25	70/30	1	13	5(38)	5(38)
11	GETMS^{tdTomato}-iPSC#5	S/Lif	1	18	9(50)	8(44)
12	GETMS^{tdTomato}-iPSC#4	S/Lif	2	26	2(8)	2(8)
13	GETMS^{tdTomato}-iPSC#6	S/Lif	2	26	4(15)	1(4)
14	GETMS^{tdTomato}-iPSC#2	S/Lif	1	18	6(30)	5(28)
15	OSKM^{tdTomato}-iPSC#4	S/Lif	2	22	12(55)	10(45)
16	ESC^{tdTomato}#7.6	S/Lif	3	43	9(21)	6(14)
17	GETMS^{EGFP}-iTSC#1	70:30	2	26	2(8)	1(4)*
18	GETM^{EGFP}-iTSC#7	70:30	3	39	17(44)	12(31)*
19	GETM^{EGFP}-iTSC#8	70:30	2	21	12(57)	8(38)*
20	GETMS^{EGFP}-iTSC#3	70:30 +RI	2	25	9(36)	2 (8)* + 1 (4)
21	GET^{EGFP}-iTSC#6	70:30 +RI	1	14	3(21)	1 (7)* + 1(7)
22	bdTSC^{EGFP}#2	70:30	7	100	23 (23)	10 (10)* + 1(1)

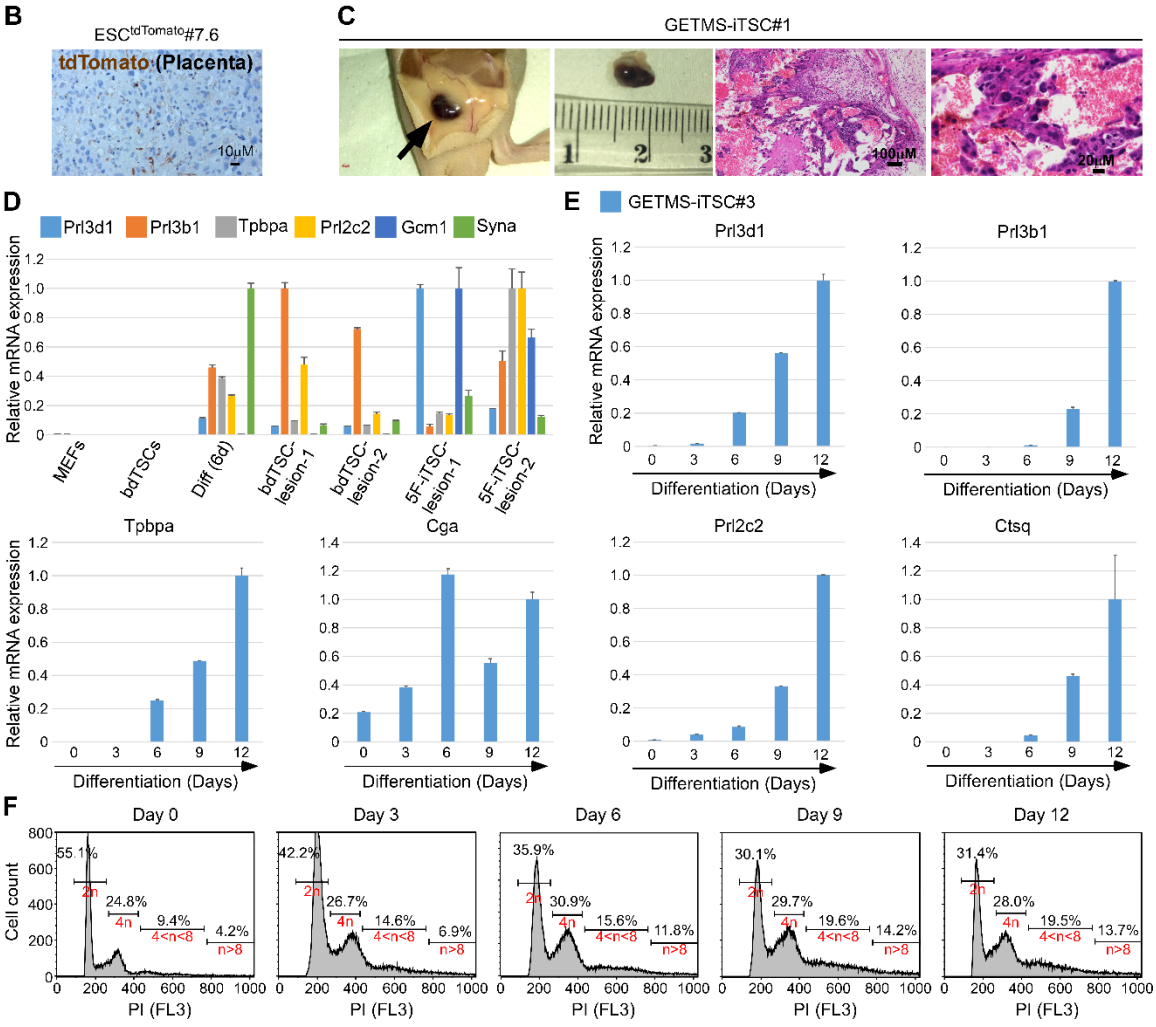

Figure S2. Related to Figure 2.

5F-iTSCs are functional and are capable of differentiating into the various trophoblast lineages and of generating hemorrhagic lesions. (A) A table summarizing the generation of chimeric embryos/placentas with GETMS-iPSC, GETMS-iTSC clones and controls following blastocyst injection. All embryos and placentas were examined at 13.5 dpc. S- stands for serum, RI- stands for Rock inhibitor, 70:30- stands for TSC culturing medium, 2i- stands for two inhibitors (PD0325901 and CHIR99021) and *-stands for low contribution. **(B)** Contribution of tdTomato ESCs to 13.5 dpc placenta. A clear tdTomato signal in endothelial cells of placental blood vessels was detected by immunohistochemistry. **(C, left)** Hemorrhagic lesions 6-7 days following subcutaneous injection of GETMS-iTSC#1 line into nude mice. **(C, right)** H&E staining of paraffin sections of hemorrhagic lesions, showing necrotic tissue with blood and scattered trophoblastic cells. **(D)** qPCR of the indicated trophoblast lineage markers normalized to the *Gapdh* housekeeping gene in the indicated hemorrhagic lesions and controls (i.e. MEFs, bdTSCs and 6 days of bdTSC differentiation). Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(E)** qPCR of the indicated trophoblast lineage markers normalized to the *Gapdh* housekeeping control gene in GETMS-iTSCs#3, grown in differentiation media for the indicated time points. The expression level of these trophoblast differentiation markers was comparable to that of bdTSCs after differentiation (Benchetrit et al. 2015). Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(F)** Flow cytometric analysis of GETMS-iTSCs#3 grown in differentiation medium for the indicated time points, followed by propidium iodide (PI) staining. Scale bars show staining intensities; representative of chromosome copy number. The percentage of cells from each sample in every phase is indicated.

Figure S3

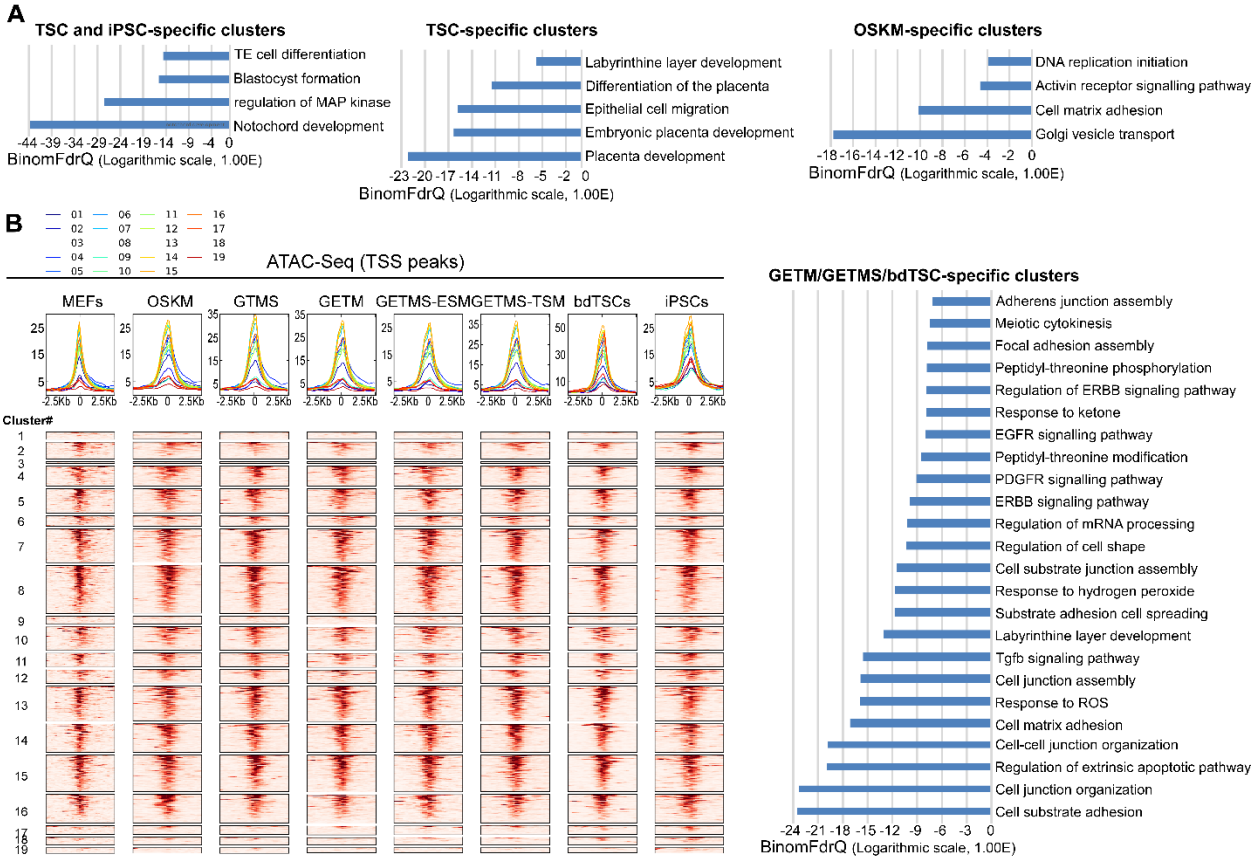
A
TSC and iPSC-specific clusters
TE cell differentiation
Blastocyst formation
regulation of MAP kinase
Notochord development
-44 -39 -34 -29 -24 -19 -14 -9 -4 0
BinomFdrQ (Logarithmic scale, 1.00E)
TSC-specific clusters
Labyrinthine layer development
Differentiation of the placenta
Epithelial cell migration
Embryonic placenta development
Placenta development
-23 -20 -17 -14 -11 -8 -5 -2 0
BinomFdrQ (Logarithmic scale, 1.00E)
OSKM-specific clusters
DNA replication initiation
Activin receptor signalling pathway
Cell matrix adhesion
Golgi vesicle transport
-18 -16 -14 -12 -10 -8 -6 -4 -2 0
BinomFdrQ (Logarithmic scale, 1.00E)
B
ATAC-Seq (TSS peaks)
MEFs
OSKM
GTMS
GETM
GETMS-ESM
GETMS-TSM
bdTSCs
iPSCs
Cluster#
GETM/GETMS/bdTSC-specific clusters
Adherens junction assembly
Meiotic cytokinesis
Focal adhesion assembly
Peptidyl-threonine phosphorylation
Regulation of ERBB signaling pathway
Response to ketone
EGFR signalling pathway
Peptidyl-threonine modification
PDGFR signalling pathway
ERBB signaling pathway
Regulation of mRNA processing
Regulation of cell shape
Cell substrate junction assembly
Response to hydrogen peroxide
Substrate adhesion cell spreading
Labyrinthine layer development
Tgfb signaling pathway
Cell junction assembly
Response to ROS
Cell matrix adhesion
Cell-cell junction organization
Regulation of extrinsic apoptotic pathway
Cell junction organization
Cell substrate adhesion
-24 -21 -18 -15 -12 -9 -6 -3 0
BinomFdrQ (Logarithmic scale, 1.00E)
C
Motif enrichment in GETMS/GTMS vs GETM
Sp1
(p-value= 1e-175)
YY1
(p-value= 1e-146)
Elk1/4
(p-value= 1e-141)
Nrf1
(p-value= 1e-138)
D
H3K27ac
H3K4me2
All XEN genes
MEFs + S
GTMS
GETMS
Average read count
-7.0
TSS TES
7.0Kb
E
Myh6
Pou5f1 (Oct4)
ATAC
bdTSCs
iPSCs
H3K4me2
H3K27ac
MEFs + S
GTMS
GETMS
FPKM
MEFs: 0
GTMS: 47
GETM: 2
GETMS-ESM: 26
bdTSCs: 0
ESCs: 0
MEFs: 0
GTMS: 0
GETM: 0
GETMS-ESM: 0
bdTSCs: 3
ESCs: 1069

Figure S3. Related to Figure 3.

Distal and intronic ATAC-Seq peak analysis in the various reprogramming combinations revealed a strong biased toward the PrE/XEN lineage in combination that harbor Esrrb. (A) Eighteen ATAC-Seq clusters (Figure 3D) were grouped into 9 blocks, based on their similarity, and tested for functional annotations using GREAT. Graphs showing FDR values of GO annotation of biological processes that are enriched for each group in the indicated clusters. **(B)** Metagene plots (top) and heatmaps (bottom) show the ATAC–Seq signal of the 19 gene clusters found in (Figure 3C), for MEFs, bdTSC, iPSC, and indicated reprogramming factor combination lines. Each plot shows a 5Kb fragment at the promoter region (i.e. 2.5Kb upstream and downstream to the transcription start site (TSS)). Cluster ID is color coded. As shown, the promoter region is characterized by strong ATAC-Seq signal, which is generally ubiquitous to all conditions. However, some clusters, (e.g. cluster #17, 18 and 19), do show some matching between promoter ATAC-Seq signal and expression levels (Figure 3C)). **(C)** Motifs were identified by comparing the top distal and intronic ATAC-Seq peaks in GETMS and GTMS to those in GETM treated cells (see also Table S4). **(D)** Average read count over the entire XEN-signature genes (n=225) for H3K27ac and H3K4me2 histone modifications in the indicated samples at the depicted genomic regions. TSS- transcription start site, TES- Transcription end site. **(E)** Genomic visualization of ATAC-Seq profiles (top), ChIP-Seq data of H3K4me2 and H3K27ac (middle) and RNA-Seq levels in FPKM (bottom; color coded: white- no expression, blue- intermediate expression and red- strong expression) in MEFs overexpressing either Esrrb alone (denoted “MEFs + S”), GTMS, or GETMS for 3 days. Red rectangles indicate H3K4me2 peak regions and green rectangles indicate H3K27ac peak regions. *Myh6* and *Oct4 loci* are presented.

Figure S4

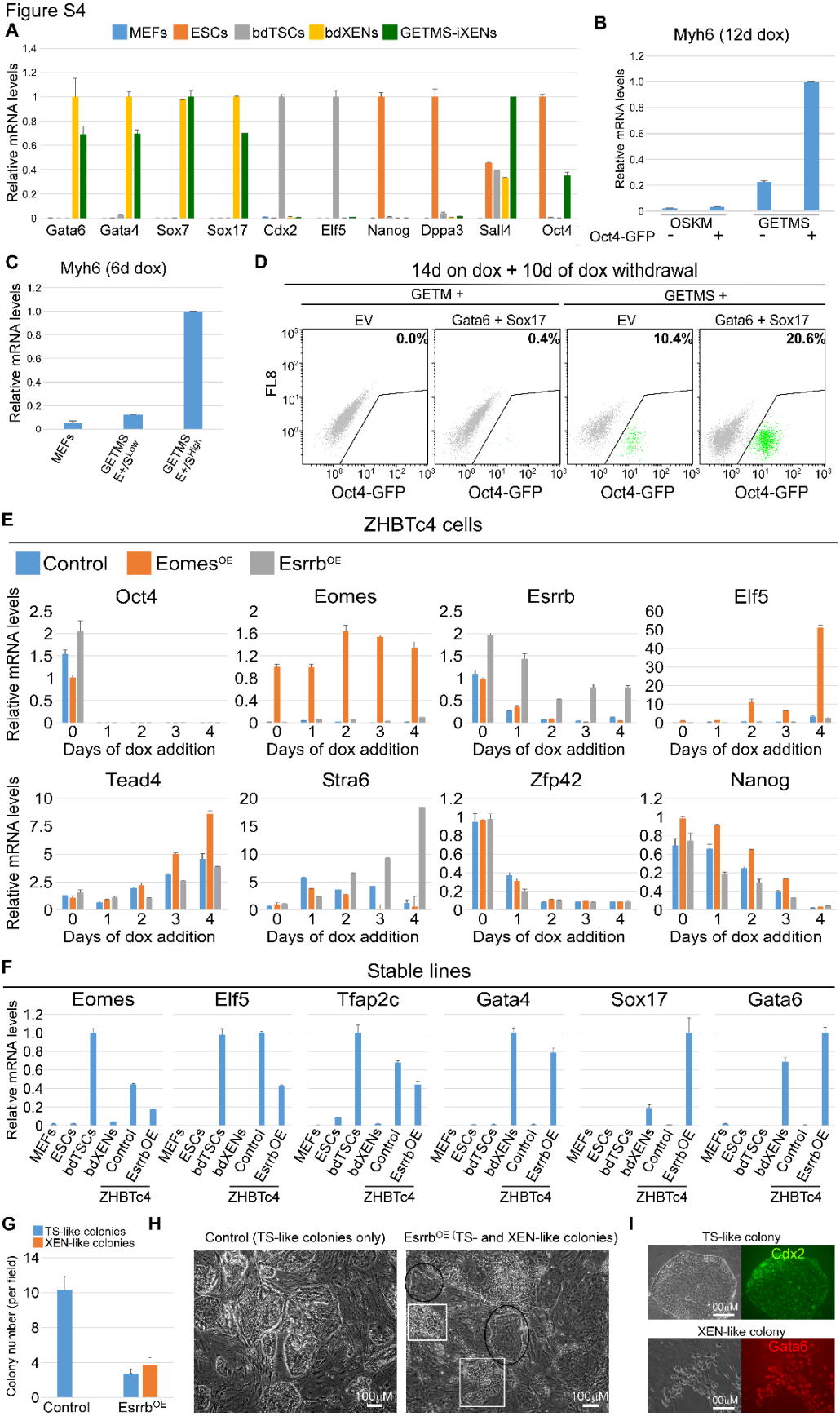
A
MEFs
ESCs
bdTSCs
bdXENs
GETMS-iXENs
Relative mRNA levels
Gata6
Gata4
Sox7
Sox17
Cdx2
Elf5
Nanog
Dppa3
Sall4
Oct4
B
Myh6 (12d dox)
Relative mRNA levels
Oct4-GFP
OSKM
GETMS
C
Myh6 (6d dox)
Relative mRNA levels
MEFs
GETMS E+iS^low
GETMS E+iS^high
D
14d on dox + 10d of dox withdrawal
GETM +
GETMS +
EV
Gata6 + Sox17
0.0%
0.4%
10.4%
20.6%
FL8
Oct4-GFP
E
ZHBTc4 cells
Control
Eomes^OE
Esrrb^OE
Oct4
Eomes
Esrrb
Elf5
Tead4
Stra6
Zfp42
Nanog
Relative mRNA levels
Days of dox addition
F
Stable lines
Eomes
Elf5
Tfap2c
Gata4
Sox17
Gata6
Relative mRNA levels
MEFs
ESCs
bdTSCs
bdXENs
Control
EsrrbOE
ZHBTc4
G
TS-like colonies
XEN-like colonies
Colony number (per field)
Control
Esrrb^OE
H
Control (TS-like colonies only)
Esrrb^OE (TS- and XEN-like colonies)
100μM
I
TS-like colony
Cdx2
XEN-like colony
Gata6
100μM

Figure S4. Related to Figure 4.

Esrrb induces a XEN-like state during GETMS reprogramming and following ESC-TSC transdifferentiation. (A) qPCR of the indicated genes normalized to the *Gapdh* housekeeping gene in MEFs, iPSCs, bdTSCs, bdXENs and GETMS-iXENs. For each gene the highest expressing sample was set to 1. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(B)** Oct4-GFP MEFs were infected with dox-inducible GETMS or OSKM factors. 12 days post dox induction, Oct4-GFP-positive and negative cells were sorted from each combination of factors and used for qPCR analysis. A graph depicting the mRNA levels of *Myh6* normalized to the *Gapdh* housekeeping gene in the indicated samples is shown. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(C)** Oct4-GFP MEFs were infected with dox-inducible GETMS factors, with Eomes marked by GFP and Esrrb marked by tdTomato. 6 days post dox induction, Eomes-GFP positive and Esrrb-TdTomato-Low cells (GETMS E+/S^{Low}) and Eomes-GFP positive and Esrrb-tdTomato-High cells (GETMS E+/S^{High}), were sorted and used for qPCR analysis. A graph depicting the mRNA levels of *Myh6* normalized to the *Gapdh* housekeeping gene in the indicated samples is shown. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(D)** FACS analysis showing the percentage of Oct-GFP-positive cells for Oct4-GFP MEFs that were infected with either GETM or GETMS in combination with either empty vector (EV) or Gata6 and Sox17 and were reprogrammed for 14 days followed by 10 days of dox removal in 2i/L ESC medium. **(E)** qPCR of the indicated genes normalized to the *Gapdh* housekeeping gene during transdifferentiation of ZHBTc4 ESCs into TS-like cells after infection with either constitutively active Eomes or Esrrb or non-infected cells control. d0 refers to pre-dox addition. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(F)** qPCR of the indicated genes normalized to the *Gapdh* housekeeping gene in stable colonies that were generated during ESC-TSC transdifferentiation in ZHBTc4 control (Esrrb^{WT}) and in ZHBTc4 Esrrb-overexpressing cells (Esrrb^{OE}) and in MEFs, ESCs, bdTSCs and bdXENs. **(G)** Quantification of the number of stable TS-like colonies and stable Xen-like colonies in transdifferentiated ZHBTc4 control and Esrrb-overexpressing cells. Error bars presented as a mean ± standard deviation of two duplicate runs from a typical experiment out of 3 independent experiments. **(H)** Bright field images depicting the morphology of stable TS-like cells (circled) and Xen-like cells (squared) generated during ESC-TSC transdifferentiation of ZHBTc4 Esrrb-overexpressing (Esrrb^{OE}) and control cells. **(I)** Immunofluorescence images for Cdx2 (Green) and Gata6

(Red) in stable TS-like and XEN-like colony generated during ESC-TSC transdifferentiation in ZHBTc4 Esrrb-overexpressing cells ($Esrrb^{OE}$).