Cell Reports, Volume *38*

Supplemental information

dCas9 fusion to computer-designed PRC2 inhibitor reveals functional TATA box in distal promoter region

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Figure S1

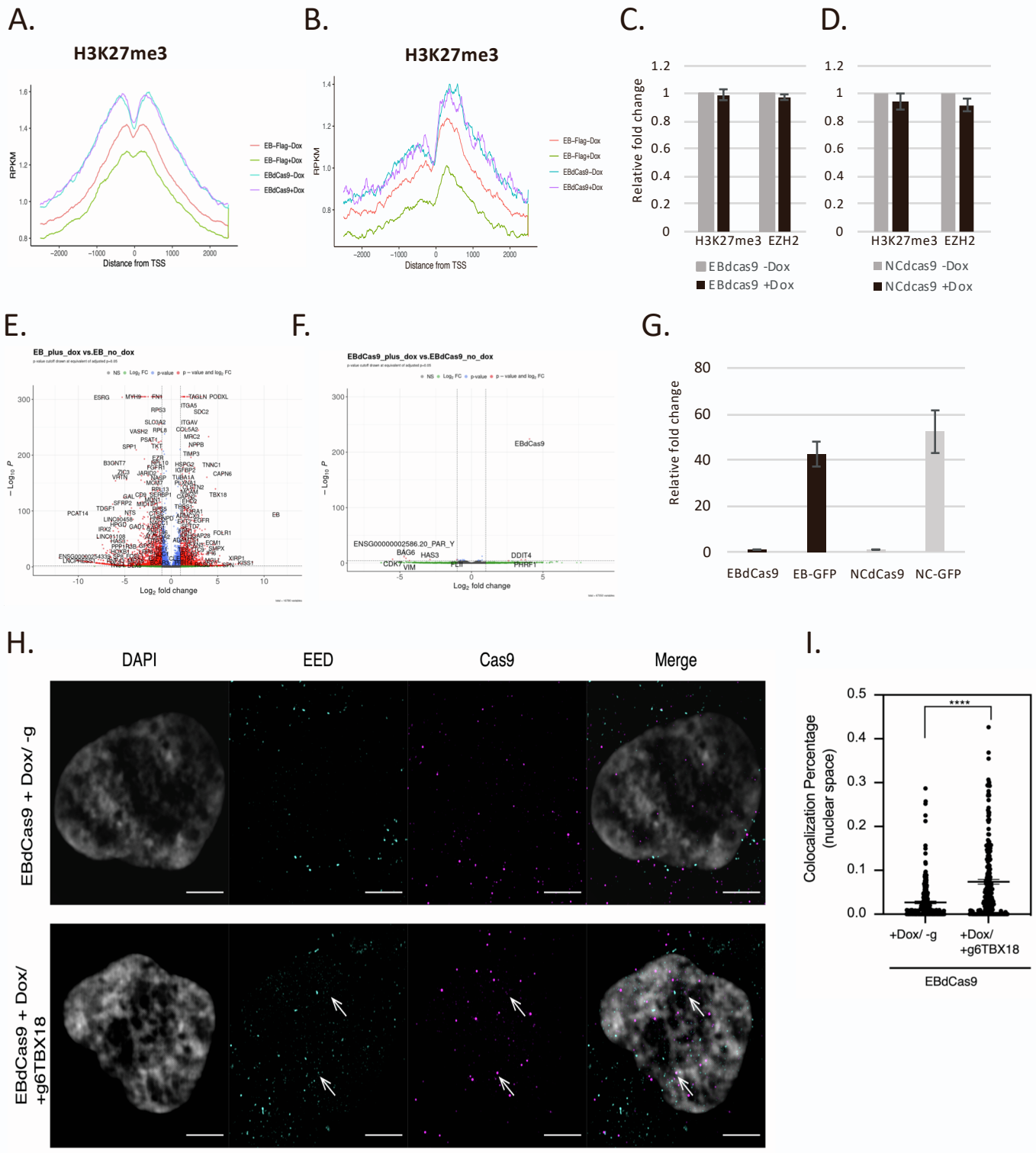

A.
H3K27me3
RPKM
Distance from TSS
EB–Flag–Dox
EB–Flag+Dox
EBdCas9–Dox
EBdCas9+Dox
B.
H3K27me3
RPKM
Distance from TSS
EB–Flag–Dox
EB–Flag+Dox
EBdCas9–Dox
EBdCas9+Dox
C.
Relative fold change
H3K27me3
EZH2
EBdcas9 -Dox
EBdcas9 +Dox
D.
H3K27me3
EZH2
NCdcas9 -Dox
NCdcas9 +Dox
E.
EB_plus_dox vs.EB_no_dox
Log2 fold change
F.
EBdCas9_plus_dox vs.EBdCas9_no_dox
EBdCas9
Log2 fold change
G.
Relative fold change
EBdCas9
EB-GFP
NCdCas9
NC-GFP
H.
DAPI
EED
Cas9
Merge
EBdCas9 + Dox/ -g
EBdCas9 + Dox/ +g6TBX18
I.
Colocalization Percentage (nuclear space)
****
+Dox/ -g
+Dox/ +g6TBX18
EBdCas9

Figure S1: EBdCas9 upregulates *TBX18* gene expression. Related to Figure #1

A-B. H3K27me3 ChIPseq analysis of WTC EB-Flag -Dox, EB-Flag +Dox (3 days), EBdCas9 -Dox, and EBdCas9 +Dox (3 days). (**A**)Genome-wide analysis of H3K27me3 reads mapped 2 kb around transcription start sites (TSSs). Reads were normalized via RPKM and aggregate read signal for all genes in a 2k window centered at the TSS. (**B)** Developmentally regulated genes (~1800) analysis of H3K27me3 in a 2kb window centered at the TSS. Reads were normalized via RPKM and aggregate read signal for developmental genes.

C-D. Quantification of H3K27me3 and EZH2 protein analysis. (**C)** for EBdCas9 and (**D)** for NCdCas9; (n = 3, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t test).

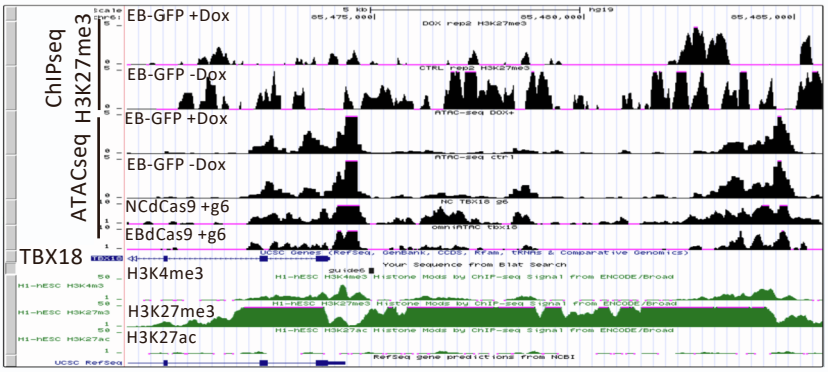
E-F. RNAseq analysis of EBdCas9 compared to EB-Flag**. (E)**. Volcano plot representation of bulk mRNA of differentially expressed genes (DEGs) in naïve hESC Elf1 EB22.2-FLAG 3D+Dox compared to no Dox from (Moody et al., 2017) (**F**) Volcano plot representation of bulk mRNA of differentially expressed genes (DEGs) in iPSC (WTC) EBdCas9 treated with 3D+Dox compared to no Dox . Genes with adjusted p-value <0.05 are in blue, and genes with Log 2FC<-1 or Log2FC>1 and adjusted p-value <0.05 are in red. Genes with known roles in development or pluripotency are labeled.

G. mRNA expression level of EB-GFP, NC-GFP, EBdCas9 and NCdCas9. EB-GFP, NC-GFP, EBdCas9, and NCdCas9 were all induced with Dox for 3D and harvest for EB or NC transcript expression and measured using RT-qPCR. Beta-actin was used for normalization and all samples were compared to their no Dox cell lines counterparts.

H-I. Quantifying colocalization of EED and EBdCas9 (Cas9) in fixed cells. **(H)** Representative maximum intensity projection images of nuclei in each condition. Difference of Gaussian (sigma 2 - sigma 5) was performed to remove background from images prior to max projection. Arrows in EBdCas9 +Dox/+g6*TBX18* condition demonstrates one example of colocalized spots in a nucleus. Scale bars equal 5 µm. **(I)** Scatter plot showing the percent colocalization between EED and EBdCas9 (Cas9). Each dot represents the colocalization percentage in a single cell volume slice. 10 cells were imaged for each condition in a 2-3.5 µm volume with images taken every 0.125 µm. Error bars are mean ± SEM. (n = 2, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001, two-tailed t test).

Figure S2

A.



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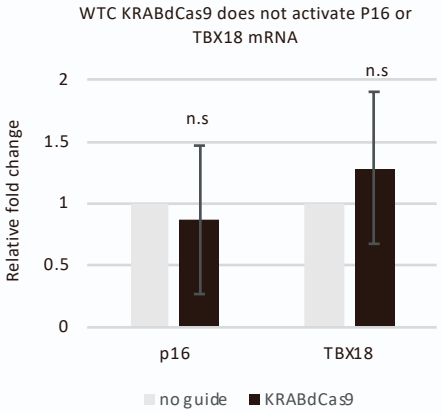
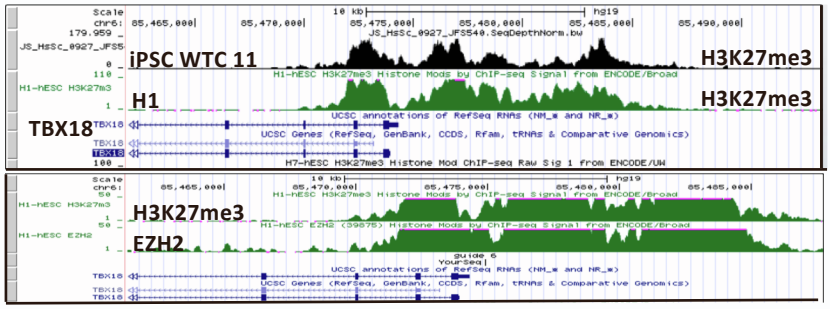


C.



D.

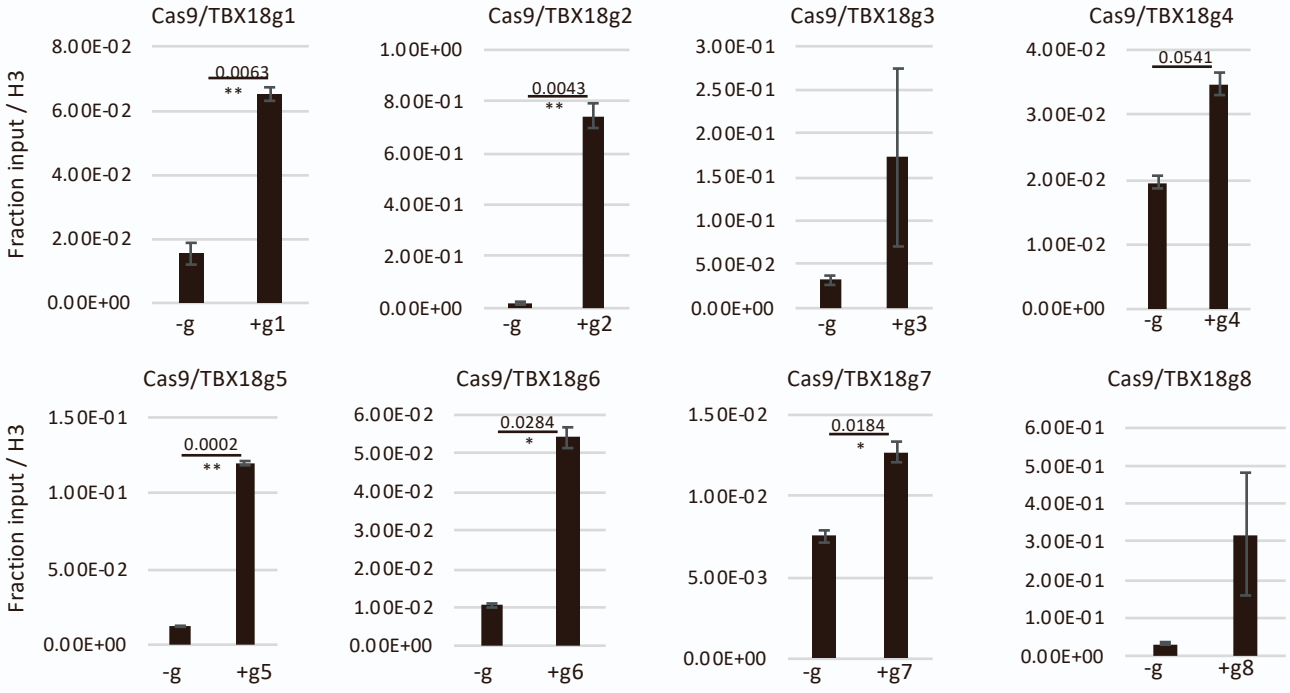


Figure S2: EBdCas9 upregulates *TBX18* gene expression. Related to Figure #1

A. Genome browser view of H3K27me3 ChIPseq analysis for EB-GFP+Dox and EB-GFP-Dox (Moody et al., 2017) and ATACseq analysis for EB-GFP+Dox, EB-GFP-Dox, NCdCas9/g6, and EBdCas9/g6.

B. WTC KRAB-dCas9 does not activate TBX18 or p16 after TBX18/g6 and p16/g1gRNAs transfection. RT-qPCR analysis of TBX18 or p16 expression for KRABdCas9 normalized to beta-Actin and calculated as relative fold change compared to no guide (induced with Dox) of the respected cell line.

C. Genome browser view of H3K27me3 tracks for iPSC WTC 11 EBdCas9 cell line compared to H1 human embryonic stem cell line. The lower panel displays genome bowser view of EZH2 and H3K27me3 tracks of *TBX18* promoter region.

D. ChIP-qPCR of induced (+Dox) EBdCas9 after 3D transfection with TBX18 gRNAs 1-8 or no transfected cells (-g) using Cas9 Ab. Normalized to fraction input /H3.

Figure S3

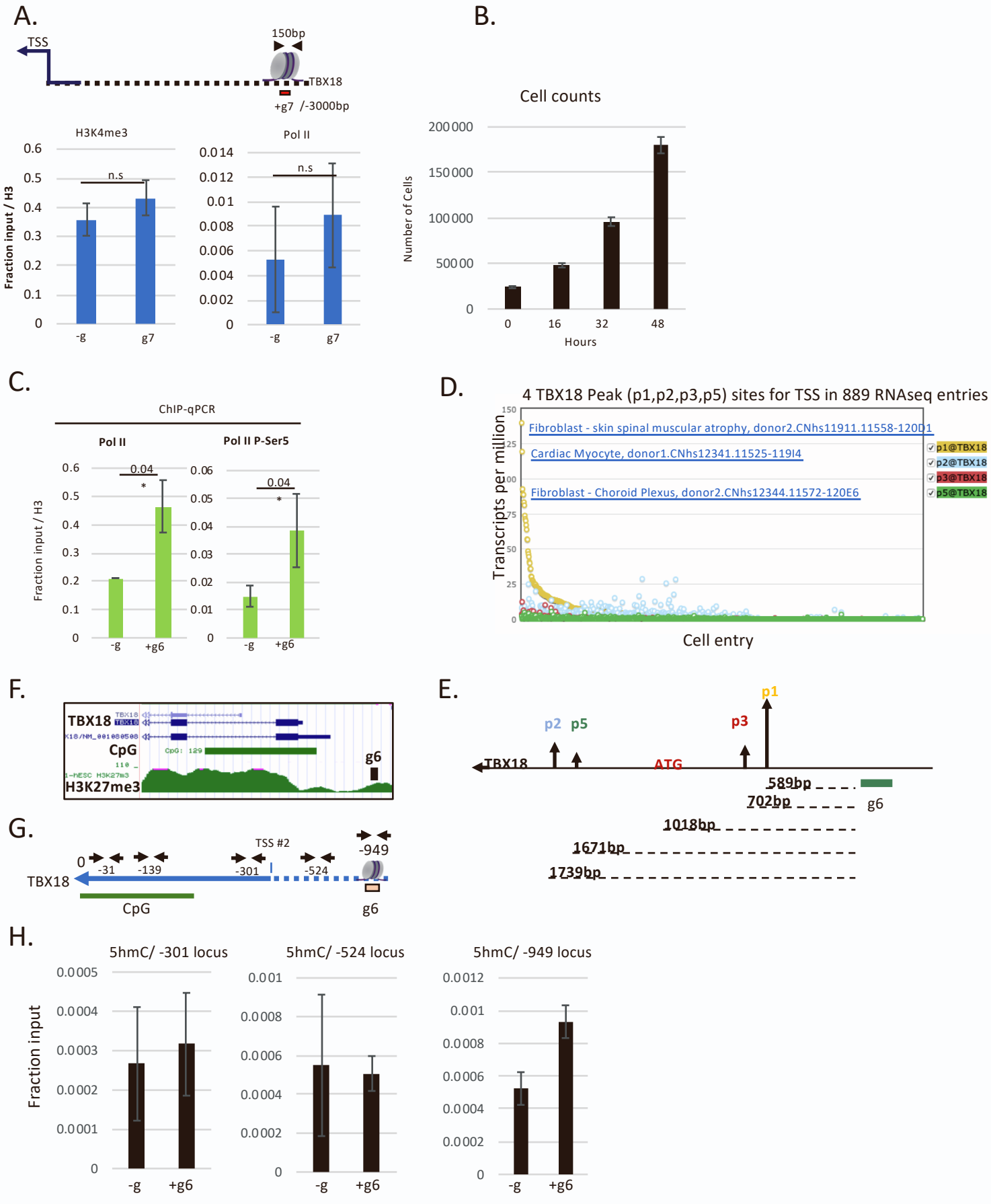

A.
TSS
150bp
TBX18
+g7 /-3000bp
H3K4me3
Pol II
Fraction input / H3
n.s
n.s
-g
g7
B.
Cell counts
Number of Cells
Hours
C.
ChIP-qPCR
Pol II
Pol II P-Ser5
Fraction input / H3
0.04
0.04
-g
+g6
D.
4 TBX18 Peak (p1,p2,p3,p5) sites for TSS in 889 RNAseq entries
Transcripts per million
Fibroblast - skin spinal muscular atrophy, donor2.CNhs11911.11558-120D1
Cardiac Myocyte, donor1.CNhs12341.11525-119I4
Fibroblast - Choroid Plexus, donor2.CNhs12344.11572-120E6
p1@TBX18
p2@TBX18
p3@TBX18
p5@TBX18
Cell entry
F.
TBX18
CpG
H3K27me3
g6
E.
p1
p2
p5
p3
TBX18
ATG
589bp
702bp
g6
1018bp
1671bp
1739bp
G.
TSS #2
0
-31
-139
-301
-524
-949
TBX18
CpG
g6
H.
5hmC/ -301 locus
5hmC/ -524 locus
5hmC/ -949 locus
Fraction input
-g
+g6

Figure S3: No epigenetic remodeling for EBdCas9/ *TBX18* g7. Related to Figure #2. And, *TBX18* TSS is predominantly 600bp downstream of guide 6. Related to Figure #3

A. ChIP-qPCR of TBX18 relative fold change after 3D EBdCas9 Dox induction and g7 RNA transfection. Normalized to fraction input/H3. Antibodies used for ChIP are listed above the graphs (H3K4me3, and Pol II) and the genomic region analyzed by qPCR includes TBX18 g7 flanking region locus. n=3 biological replicates.

B. EBdCas9 doubling time is 16h. Cells were counted over a period of 48h and measured using nucleocounter.

C. ChIP-qPCR of 3D induced EBdCas9 with *TBX18* g6 RNA (+g6) transfected or not (-g) using Pol II CTD and Pol II P-Ser5 Abs and normalizing fraction input / H3 (n = 3, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t test).

D. *TBX18* input in FANTOM5-CAGE reveals 4 TSS peaks (p1, p2, p3, p5) measured by transcript expression (transcripts per million) among 889 human RNAseq datasets. The 3 top expressed datasets are displayed on the graph.

E. Depiction of *TBX18* 4 TSS peaks with respect to guide6 region.

F. *TBX18* CpG region snap shot from UCSC Genome Browser (GRCh36/hg36) Assembly with respect to g6 locus.

G. Illustration of *TBX18* TSS and CpG region. “0” denotes first Met relative to guide 6 (g6) region in bp. Small black arrows annotate the loci for qPCR amplification in F.

H. MedIP-qPCR of induced (+Dox) EBdCas9 after 3D transfection with *TBX18* g6 RNA (+g6) or no transfection (-g) using 5 hydroxymethylcytosine (5hmC) Ab. Normalized to fractioned input.

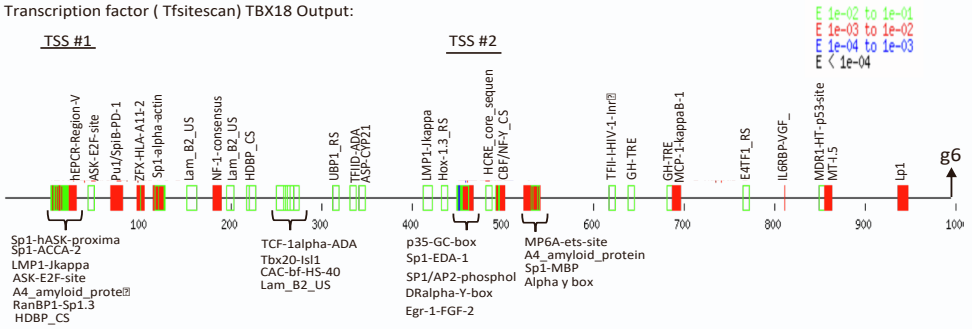
Figure S4

A. Quadruplex forming G-Rich Sequences (QGRS) *TBX18* Output:

```
000001 TACCAAACAT ATATATATAT ATTTGTTAAT CCTAACAAAA AGTCGAGTTG AAACTACAAA ACAAATTGTA CAAATATATA TGTTTGGTGT CATTTAATTA
000101 AAAAGCACTG ATTTTATTAA ATTTGTTTTA AATACATCAA TGGACTCCTT AAAAGCCTGA AGTAACGATT GTAAATTGTC AACAGTAGGA CAAATATAAA
000201 CTTACTTAAA TCACAGAGAA GTTTTTCAAA TGGGGAATTT TATTGCGTCT TCATACTTAT TTCATTTTGC GAAGTTTGGG GGGGTTTTAA AGTTGTCCAT
000301 ATCGTTAAAG ACGTACAGAA TGAAAATCCC ATGACCTACG TTTTAAAATA ACTACCACTC TTTGAAAGAA AAGGTGGGCT GCGGAAAGGG CGTGCTTTTT
000401 CTGACATTTA TATGACGGAC TGAGCGTTAA CGTAACATTT TGCACTCCTA AAGGATTTAC ATATTTCTTA ACCTGGTATC CCCTAAGAAA AAAATCACGG
000501 AGTAAAGTTT AAATAAGCCT AAGCCATATC TGGCGCAGAC TGAGTGCAGG AACATTTAAA TGAGAAAGGA GGGGCCTGCG AATTCTTCCC AGCTTCCCAA
000601 TATTCTGAGT TGTAGCAACA TTAAGCGATA CGGAAGTGGG ACGGCAATTT ACGGAACCTT GAAAGATTCC TGATTTTCTA ACTTGTTGAA CGTCTAGTTT
000701 AAAGCCGGGA ACTTCCGCCT TTCCAGGTCC TGGTGAGGAA AGCTGGAGAC TACGGGGCTG GACTCAGCTA CGGACGTCAG AGAGACAGAG TTCAGCGTCC
000801 GTGTGGCTGG CACATCCAAG CCAGACAGCG GCGCTTTTCA CTCCTGGTTT TTCGCTACCT TGTAAGGAAG TGGGCGCGGG CTGCGCGGAG TCGGCGCCTC
000901 CTGATTGGCC GGCCGTCTGG GGTGATGGACA GGGACCCGGG CTCCGCCCCC GCCGCTTTAT TGACACTAAT GAGCAAGTTC TTCCCACCGC TCTCCTGCCT
001001 GGAAGTGCTG ACAGATCAAG GCAACAAATT TCAATTACAA TCCCTAATTT GTGTCCACAG AGTGTTTTTT ACCCATGTTA GTCCTCTCTG GCGCATCAGT
001101 TGAGGCAGAC CTCGGAGCAG CAGGAGGAGG TGGAAGGGGT GGGAGCAAAG GAGTGCATCA GTGAGAGAGC GCGCGAGAGA GACCCAGGAA AGGAGACTTG
001201 GCCGGCGCGT CGCCGGTTCC TGGATCCCAA CACAAGCGAG AAAGCGGAAA CGCCAAATCT GTTTTTGCCC CGCGGTGGGG AAGGGGGCAG ATCTCGGGAG
001301 GCCCCGAGAG CCTTTTAGTT TTTGGTGGGG GAAGAGCGAG AGCGCGCGTG TGCCCGCGTG AGTGTATATG AGAGAGGGGC GGGCGGGCGC GGGGCGGGGG
001401 G
```

B.

Transcription factor (Tfsitescan) TBX18 Output:



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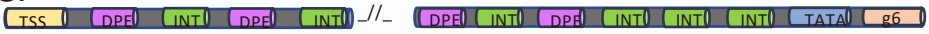


ElemeNT *TBX18* Output:

A possible combination, input sequence position 719 to 754:
ttagtcctctctggcgcatcagttgaggcagacct

A possible combination, input sequence position 685 to 720:
ctaatttgtgtccacagagtgtttttaccccatgt

A possible combination, input sequence position 50 to 90:
tatatgacggactgagcgttaacgtaacattttgcactcc

A possible combination, input sequence position 462 to 497:
ccagacagcggcgcttttcactcctggttttctcgc

A possible combination, input sequence position 220 to 255:
cgaatttcttcccagcttccccaatatttctgagttgt

A possible combination, input sequence position 625 to 660:
ccaccgctctcctgcctggaagtgctgacagatca

A possible combination, input sequence position 50 to 83:
tatatgacggactgagcgttaacgtaacatttt

A possible combination, input sequence position 197 to 232:
ttaaatgagaaaggagggcctgcgaattcttccc

A possible combination, input sequence position 50 to 81:
tatatgacggactgagcgttaacgtaacatt

A possible combination, input sequence position 193 to 228:
acatttaaatgagaaaggaggggctgcgaattct

A possible combination, input sequence position 320 to 355:
taacttgttgaacgtctagtttaaagccgggaact

A possible combination, input sequence position 286 to 321:
caatttacggaaccttgaaagattcctgattttct

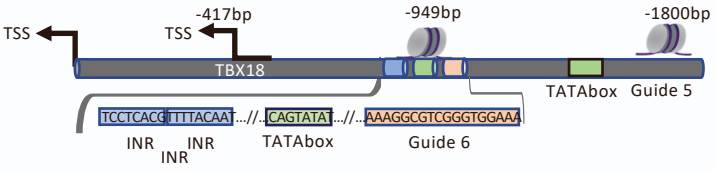
Element	Start position	Sequence	PWM score	Consensus Match
TATA box	50	tatatgac	0.0357	6 out of 8
Mammalian Initiator	479	tcactcc	0.8000	7 out of 7
Mammalian Initiator	894	ccaaatc	0.3693	7 out of 7
Mammalian Initiator	165	ccatatc	0.3409	7 out of 7
Mammalian Initiator	719	ttagtcc	0.2143	7 out of 7
DPE	748	agacct	0.3864	6 out of 6
Bridge 1	738	cagtt	0.0338	2 out of 5
Bridge 2	750	acct	0.0338	3 out of 4
Mammalian Initiator	364	ccaggtc	0.1705	6 out of 7
Mammalian Initiator	335	ctagttt	0.1607	7 out of 7
Mammalian Initiator	737	tcagttg	0.1500	6 out of 7
Mammalian Initiator	457	ccaagcc	0.1477	6 out of 7
Mammalian Initiator	685	ctaattt	0.1393	7 out of 7
DPE	714	ccatgt	0.0331	5 out of 6

distance to Mammalian Initiator A+1 (position 83) is 36
distance to Mammalian Initiator A+1 (position 76) is 29
distance to Mammalian Initiator A+1 (position 74) is 27

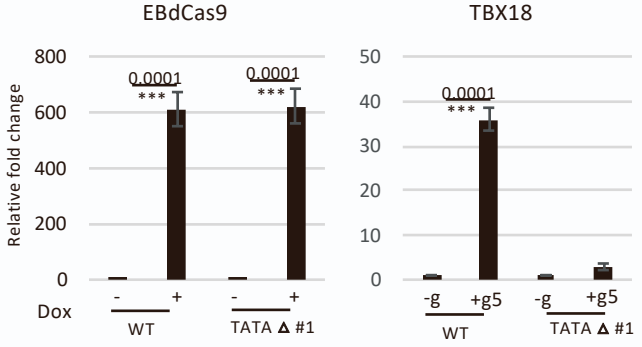
Functionally recommended scores are ≥

Element	Score
TATA box	0.01
BRE upstream	0.05
BRE downstream	0.5
GAGA	0.01
Ohler Motif1	0.1
Mammalian Initiator	0.01
Drosophila Initiator	0.01
BBCABW Initiator	0.1
MTE	0.01
DPE	0.01
Bridge	0.01
Human TCT	0.1
Drosophila TCT	0.1
XCPE 1	0.01
XCPE 2	0.01
Pause button	0.1
TFIIA response element	0.1

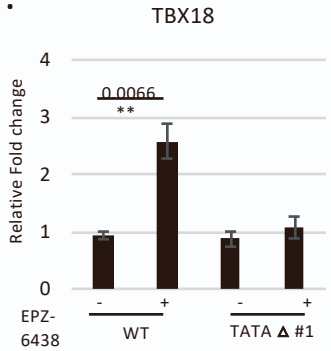
D.



E.



F.



G.

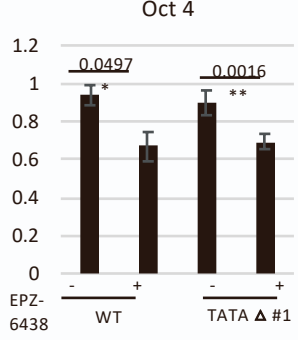


Figure S4: *TBX18* promoter region is decorated with transcription elements. Related to Figure #4

A. Snapshot of TBX18 1.0kb promoter region using the QGRS mapper tool. Highlighted in yellow are the G-quadruplex elements in 5' to 3' direction.

B. Snapshot of TBX18 (1.0kb) promoter region using transcription factor site scan using Tfsitescan tool. g6, TSS#1 and TSS#2 are labeled on the axis.

C. Snapshot of TBX18 TATA box and other transcriptional prediction elements using ElemeNT software tool.

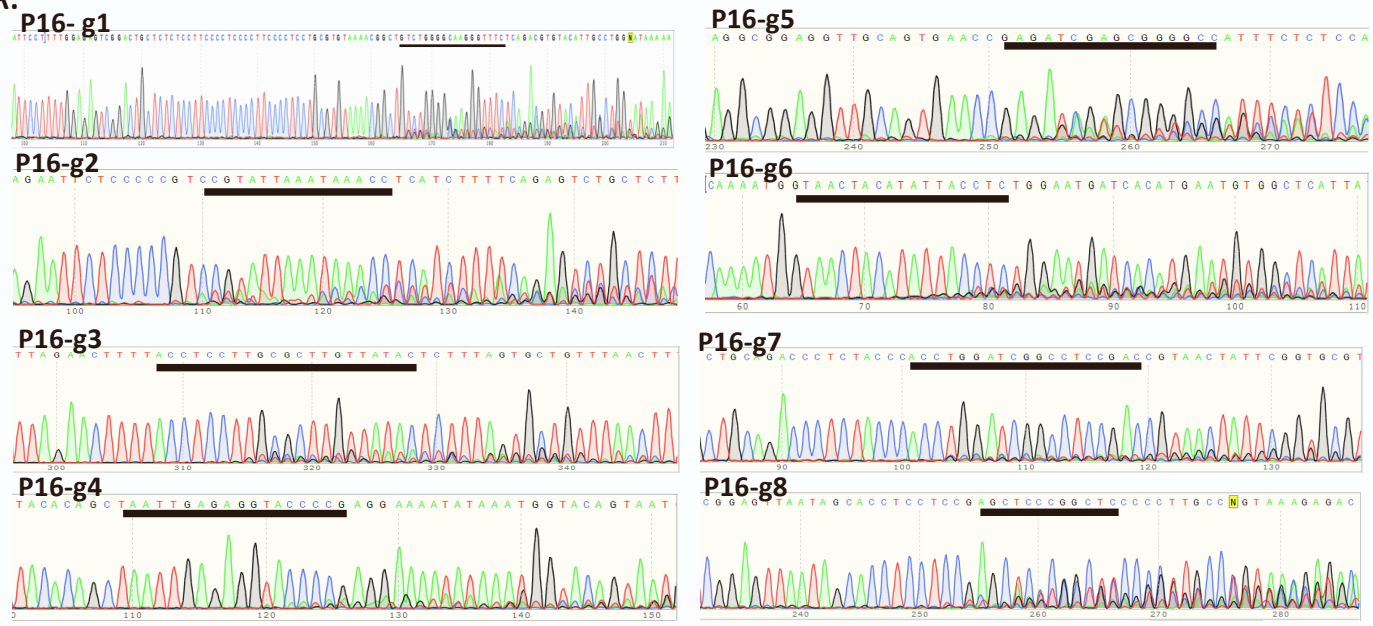
D. TATA box and initiator (INR) representation with respect to TBX18 guide 5 locus using Element Navigation Tool for detection of core promoter elements.

E. RT-qPCR of EBdCas9 and TBX18 relative fold change after 3 days Dox induction and *TBX18* g5 RNA transfection; normalized to beta-Actin and compared to no guide of each respected cell line (WT- EBdCas9 and TATA Δ #1) ($n = 3$, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed *t* test).

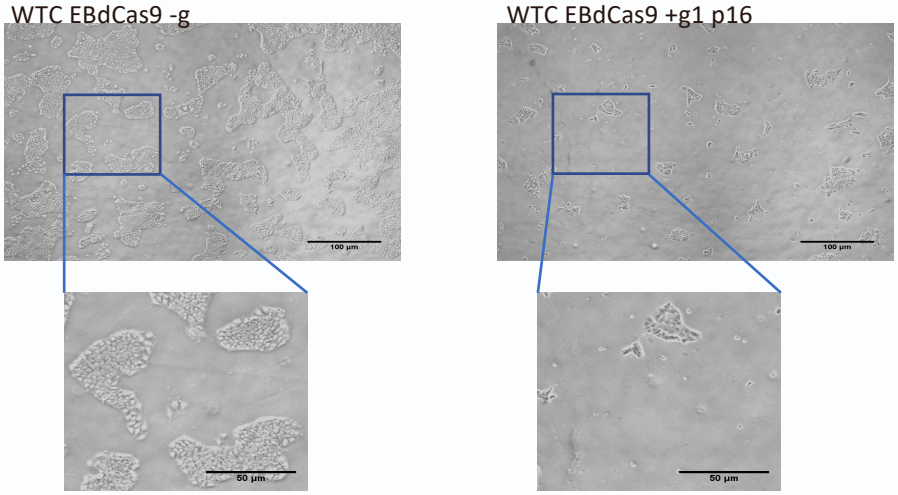
F-G. TBX18 (**F**) and Oct 4 (**G**) mRNA relative fold change after 3 days treatment of EPZ-6438 (5 μM) (+) compared to untreated cells (DMS0) (-) using RT-qPCR; normalized to beta-Actin and compared to no guide of each respected cell line (WT- EBdCas9 and TATA Δ #1) ($n = 3$, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed *t* test).

Figure S5

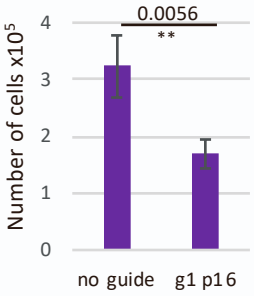
A.



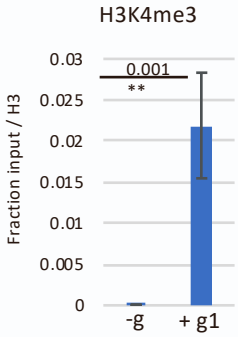
B.



C.



D.



E.

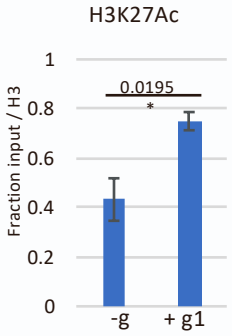


Figure S5. EBdCas9 upregulates *p16* expression. Related to Figure #5

A. Indels of p16 gRNAs (1-8) measuring DNA accessibility using hESC Elf iCas9 cell line(Ferreccio et al., 2018) and Sanger sequencing. Black underlines denote gRNA region.

B-C. EBdCas9/*p16* g1 transfection results in cell proliferation reduction. (**B**) EBdCas9 brightfield after 3D *p16* g1 transfection or no transfection (-g). Scale bar is 50μm. (**C**) Total cell count of EBdCas9 after 3D *p16* g1 transfection or no transfection (-g) in 9cm^2 area field. n=3 biological replicates.

D-E. ChIP-qPCR analysis of (**D**) H3K4me3 or (**E**) H3K27ac marks after 3D transfection with *p16* g1 RNA (+g1) or no transfection (-g). Normalized to fraction Input/ H3 ; (n = 3, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t test).

Figure S6

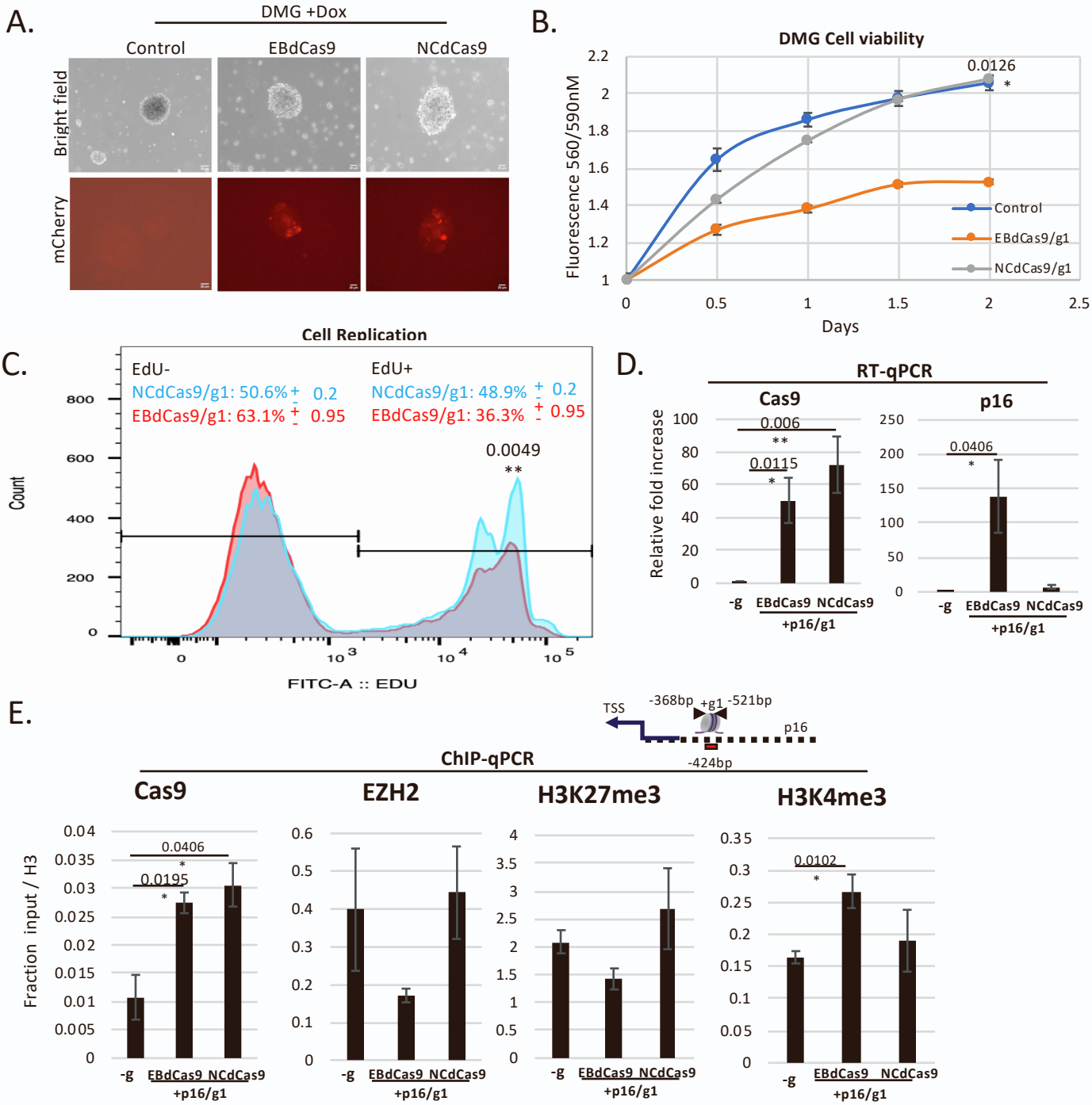

A.
DMG +Dox
Control
EBdCas9
NCdCas9
Bright field
mCherry
B.
DMG Cell viability
Fluorescence 560/590nM
Days
0.0126
*
Control
EBdCas9/g1
NCdCas9/g1
C.
Cell Replication
EdU-
NCdCas9/g1: 50.6% ± 0.2
EBdCas9/g1: 63.1% ± 0.95
EdU+
NCdCas9/g1: 48.9% ± 0.2
EBdCas9/g1: 36.3% ± 0.95
0.0049
**
Count
FITC-A :: EDU
D.
RT-qPCR
Cas9
p16
Relative fold increase
0.006
**
0.0115
*
0.0406
*
-g
EBdCas9
NCdCas9
+p16/g1
E.
TSS
-368bp
+g1
-521bp
p16
-424bp
ChIP-qPCR
Cas9
EZH2
H3K27me3
H3K4me3
Fraction input / H3
0.0406
*
0.0195
*
0.0102
*
-g
EBdCas9
NCdCas9
+p16/g1

Figure S6. EBdCas9 upregulates *p16* expression. Related to Figure #5

A. DMG transient transfection of either EBdCas9 or NCdCas9 after 3D induction with (2µg/ml) Dox.

B-C. Targeting EBdCas9/ g1 to p16 reduces cell viability and cell replication in DMG cells. (**D)**. Cell viability assay of control (+Dox and transfected reagents) DMG or transiently transfected DMG cells with EBdCas9/g1 or NCdCas9/g1 . Times points are every 12h measured by fluorecin using Alamar Blue (n = 3, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t test). (**E)**. EBdCas9/p16+g1 (red) EdU incorporation after 3D of transient transfection to DMG cells compared to NCdCas9/g1 (blue) and measured by FACS. Data represent mean data ± SEM for independent experiments performed in duplicate. (n = 2, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t test).

D. RT-qPCR of dCas9 or p16 relative fold change after 3D Dox induction (dCas9) or p16 g1 RNA transfection (p16) of DMG cells; normalized to DMG housekeeping gene, RLP13A, and compared to control DMG no guide (+Dox) cells.

E. ChIP-qPCR of induced (+Dox) EBdCas9 and NCdCas9 after 3D transfection with p16 g1 RNA (+g1) or not transfected (-g). Normalized to fraction input /H3. Antibodies used for ChIP are listed above the graphs (Cas9, EZH2, H3K27me3 and H3K4me3); (n = 3, SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t test).

Figure S7

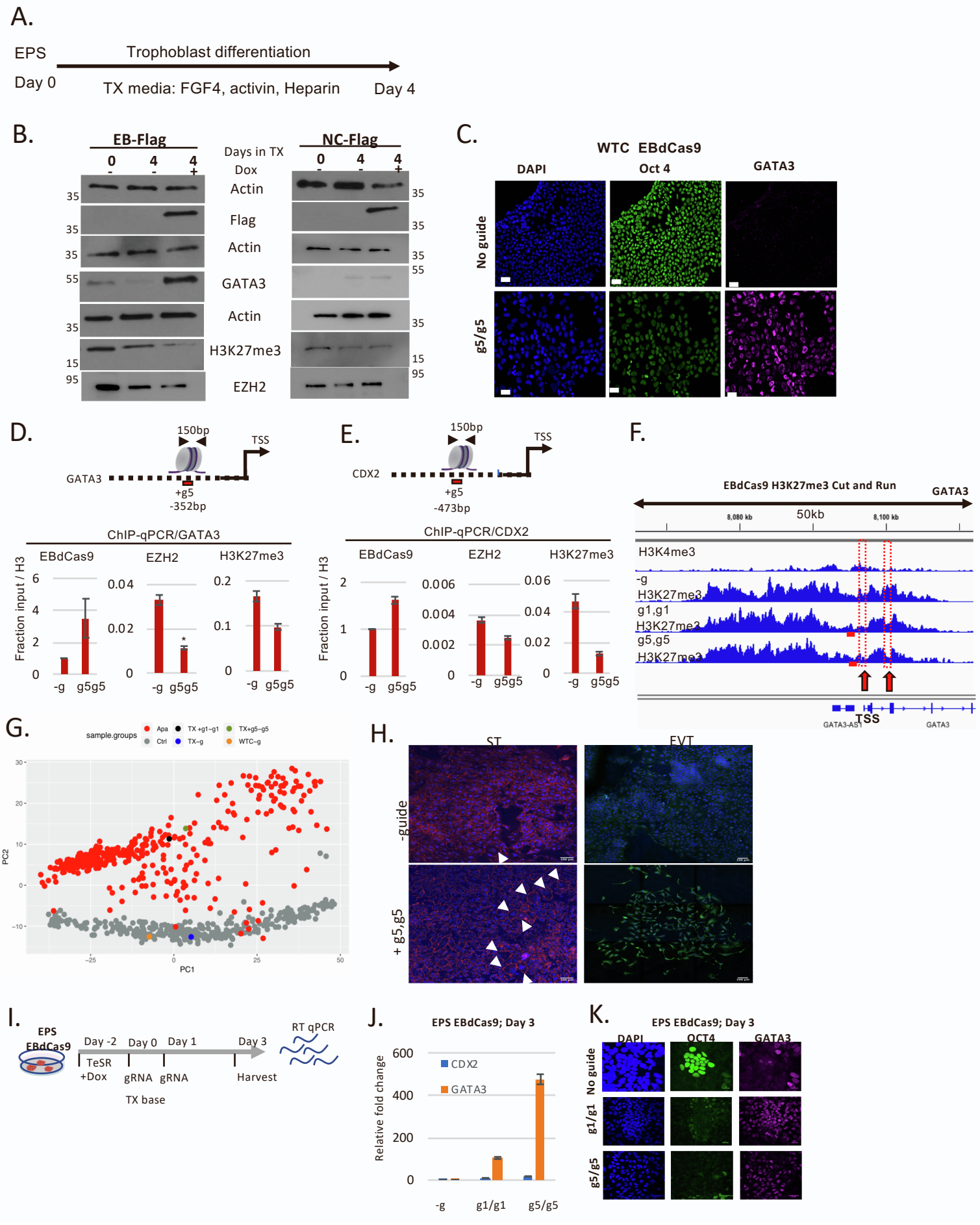

A.
EPS
Day 0
Trophoblast differentiation
TX media: FGF4, activin, Heparin
Day 4
B.
EB-Flag
NC-Flag
Days in TX
Dox
Actin
Flag
GATA3
H3K27me3
EZH2
C.
WTC EBdCas9
DAPI
Oct 4
GATA3
No guide
g5/g5
D.
150bp
TSS
GATA3
+g5
-352bp
ChIP-qPCR/GATA3
EBdCas9
EZH2
H3K27me3
Fraction input / H3
-g
g5g5
E.
CDX2
-473bp
ChIP-qPCR/CDX2
F.
EBdCas9 H3K27me3 Cut and Run
GATA3
50kb
H3K4me3
H3K27me3
g1,g1
g5,g5
TSS
GATA3-AS1
G.
sample.groups
PC1
PC2
H.
ST
EVT
-guide
+ g5,g5
I.
EPS EBdCas9
Day -2
Day 0
Day 1
Day 3
TeSR +Dox
gRNA
gRNA
Harvest
RT qPCR
TX base
J.
EPS EBdCas9; Day 3
Relative fold change
CDX2
GATA3
-g
g1/g1
g5/g5
K.
EPS EBdCas9; Day 3
DAPI
OCT4
GATA3
No guide
g1/g1
g5/g5

Figure S7. EBdCas9 *CDX2/GATA3* promotes trophoblast trans-differentiation. Related to Figure #6

A. EPS EB-Flag and NC- Flag trophoblast differentiation time line.

B. Immunoblot of EPS EB-Flag and NC- Flag with or without Dox after 4D of trophoblast differentiation in TX media or at EPS stage.

C. Immunofluorescent imaging of 3D WTC EBdCas9 with (+) or without (-g) *CDX2* and *GATA3* g1g1 or g5g5 co-transfection. Blue-Dapi, Green-Oct4, Far red- GATA3; scale bar is 50μm.

D-E. ChIP-qPCR analysis of 3D EBdCas9 co- transfected with g5/g5 *CDX2/GATA3*. Normalized to Input/ H3 and compared to -g relative fold change. Antibodies that were used for ChIP are listed above the graphs (Cas9, EZH2, H3K27me3) and the genomic region analyzed by ChIP-qPCR includes *GATA3* g5 (**D**) and *CDX2* g5 (**E**) loci.

F. Cut and Run H3K27me3 analysis of 3 days EBdCas9 co-transfected with g5/g5 *CDX2/GATA3* or g1/g1 *CDX2/GATA3*. H3K27me3 tracks are normalized to IgG and displayed on Integrated Genome Viewer (IGV); 50kb *GATA3* window viewer. Red dots demonstrate *GATA3* TSS and exon 3 regions.

G. PCA analysis of 3 days EBdCas9 cells after co-transfection with g5/g5 *CDX2/GATA3 (green)* or g1/g1 *CDX2/GATA3 (black)*. As controls we used EBdCas9 grown in TX media without guides (Tx-g; blue) or WTC grown in TeSR without guides (WTC-g; orange). RNAseq samples were compared to single cell RNAseq dataset from (Krendl et al., 2017) where APA (Aminopeptidase A) was used as a selective cell surface marker for trophoblast progenitor cells.

H. Immunofluorescence of EBdCas9 3D post *CDX2/GATA3* g5/g5 RNA transfection compared to no guide, and further 6 days (6D) differentiation to either EVT using 7.5mM TGFbi and 100ng/ml (NRG1) or ST using 2mM forskolin. Dapi- blue,ZO-1-red and (chorionic gonadotropin beta) CGB-green. Scale bar is 100μm.

I. WTC EBdCas9 cell line was reprogrammed to EPS, induced with Dox for 2D in TeSR and then switch to TX media (+Dox, no factors) while transfected with gRNA and harvest at 3D.

J. RT-qPCR analysis of EBdCas9 EPS cells after co-transfection with g1/g1 *CDX2/GATA3* or g5/g5 *CDX2/GATA3* compared to no guide (-g) transfection. Normalized to beta-Actin.

K. Immunofluorescent imaging of 3D EPS EBdCas9 with (+) or without (-g) CDX2 and *GATA3* g1g1 or g5g5 co-transfection in TX media. Blue-Dapi, Green-Oct4, Far red- GATA3; scale bar is 50μm.