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Supplemental information

Chromatin remodeling is restricted by transient GATA6 binding during iPSC differentiation to definitive endoderm

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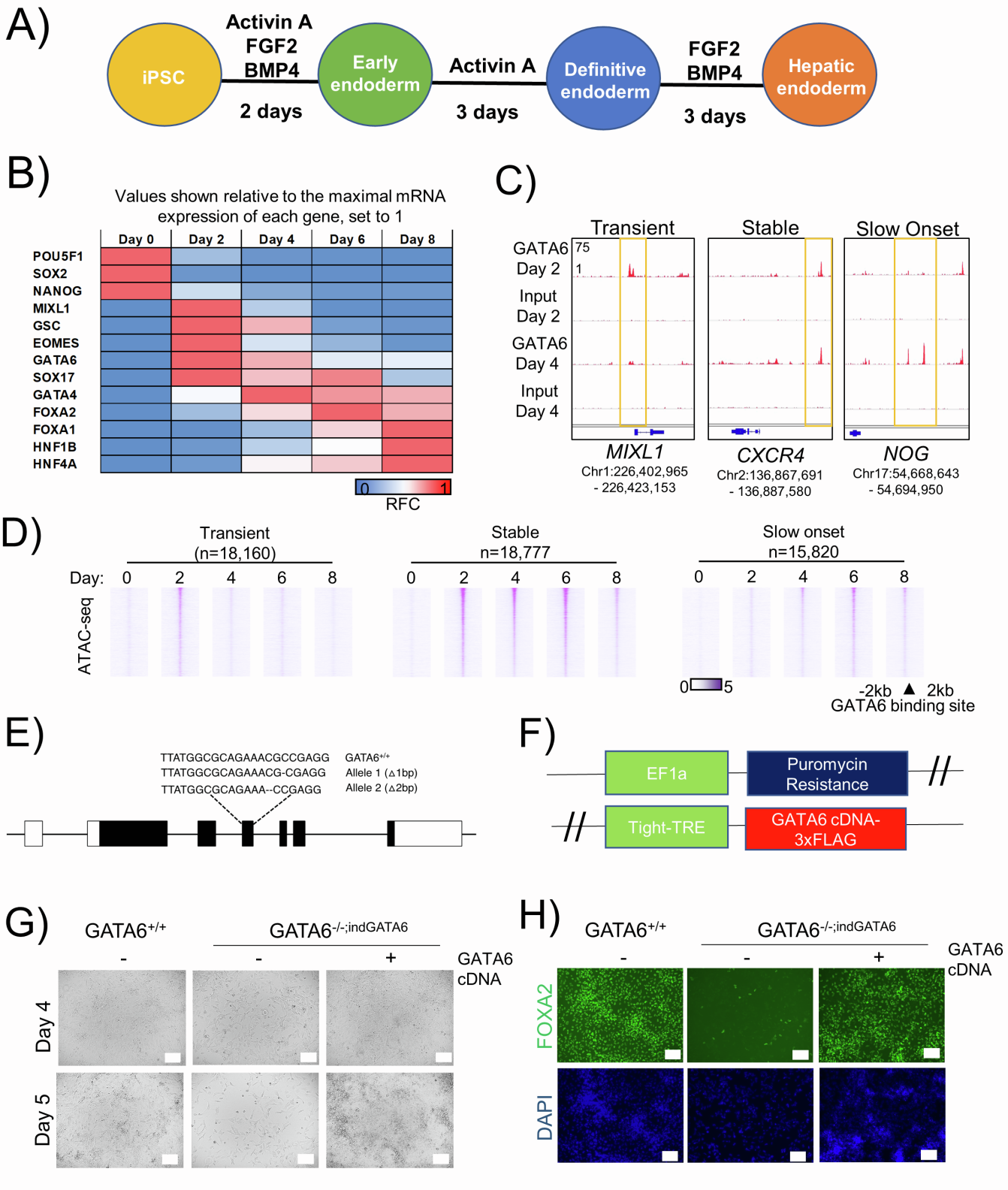
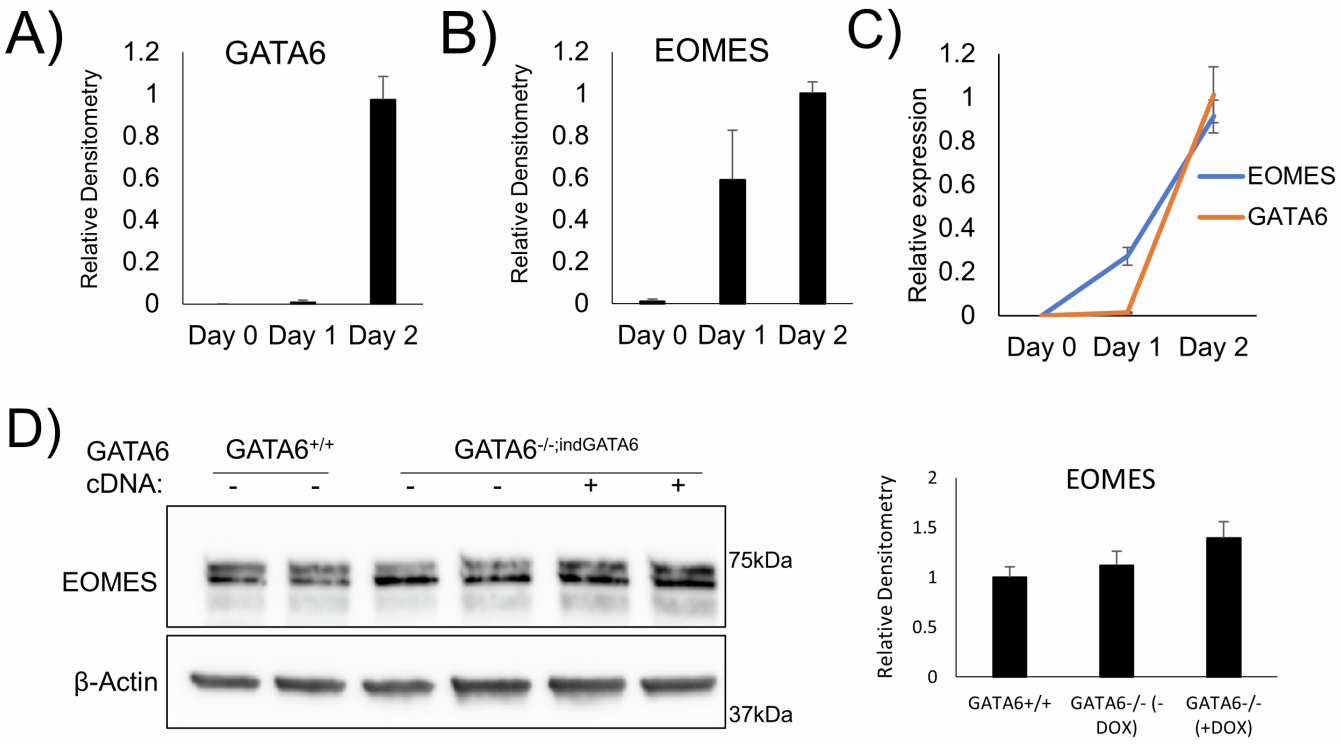

A)
iPSC
Activin A
FGF2
BMP4
2 days
Early endoderm
Activin A
3 days
Definitive endoderm
FGF2
BMP4
3 days
Hepatic endoderm
B)
Values shown relative to the maximal mRNA expression of each gene, set to 1
Day 0
Day 2
Day 4
Day 6
Day 8
POU5F1
SOX2
NANOG
MIXL1
GSC
EOMES
GATA6
SOX17
GATA4
FOXA2
FOXA1
HNF1B
HNF4A
0
1
RFC
C)
Transient
Stable
Slow Onset
GATA6 Day 2
Input Day 2
GATA6 Day 4
Input Day 4
75
1
MIXL1
Chr1:226,402,965 - 226,423,153
CXCR4
Chr2:136,867,691 - 136,887,580
NOG
Chr17:54,668,643 - 54,694,950
D)
Transient (n=18,160)
Stable n=18,777
Slow onset n=15,820
Day:
0
2
4
6
8
ATAC-seq
0
5
-2kb
2kb
GATA6 binding site
E)
TTATGGCGCAGAAACGCCGAGG
TTATGGCGCAGAAACG-CGAGG
TTATGGCGCAGAAA--CCGAGG
GATA6+/+
Allele 1 (Δ1bp)
Allele 2 (Δ2bp)
F)
EF1a
Puromycin Resistance
Tight-TRE
GATA6 cDNA-3xFLAG
G)
GATA6+/+
GATA6-/-;indGATA6
-
-
+
GATA6 cDNA
Day 4
Day 5
H)
GATA6+/+
GATA6-/-;indGATA6
-
-
+
GATA6 cDNA
FOXA2
DAPI

Figure S1: Further characterization of *GATA6*$^{+/+}$ and *GATA6*$^{-/-}$ iPSCs. Related to Figure 2. A) The hepatic endoderm differentiation protocol; B) mRNA expression of key endoderm-enriched transcription factor expression during the commitment of iPSCs to hepatic endoderm. Values shown relative to the peak expression of each transcription factor, set to 1. Data derived from RNA-seq samples, n=2 mean; C) Genome viewer representation of selected regions that display transient, stable and slow onset GATA6 binding profiles during endoderm formation in *GATA6*$^{+/+}$ cells; D) Heatmaps depicting ATAC-seq signal intensity at different subsets of GATA6 binding during hepatic endoderm formation; E) CRISPR/Cas9 targeting of GATA6 exon 4; F) Doxycycline-inducible *GATA6-3xFLAG cDNA* vector inserted into *GATA6*$^{-/-}$ cells; G) Phase contrast images of *GATA6*$^{+/+}$ and *GATA6*$^{-/-}$ cells ± doxycycline cells at day 4 and 5 of the hepatic endoderm differentiation protocol. Sale bar: 100μM; H) Immunofluorescence analysis of *GATA6*$^{+/+}$ and *GATA6*$^{-/-}$ cells ± doxycycline cells for FOXA2 at day 4 of differentiation. Sale bar: 100μM.


A)
GATA6
Relative Densitometry
1.2
1
0.8
0.6
0.4
0.2
0
Day 0 Day 1 Day 2
B)
EOMES
Relative Densitometry
1.2
1
0.8
0.6
0.4
0.2
0
Day 0 Day 1 Day 2
C)
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
Day 0 Day 1 Day 2
EOMES
GATA6
D)
GATA6 cDNA:
GATA6+/+
GATA6-/-;indGATA6
- - - - + +
EOMES
75kDa
β-Actin
37kDa
EOMES
Relative Densitometry
2
1.5
1
0.5
0
GATA6+/+
GATA6-/- (-DOX)
GATA6-/- (+DOX)

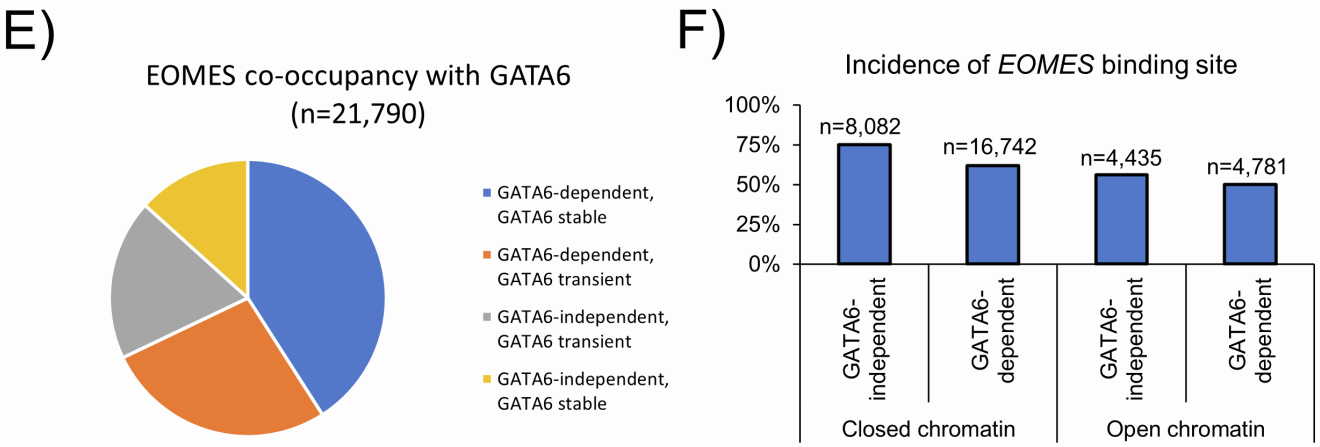

E)
EOMES co-occupancy with GATA6
(n=21,790)
GATA6-dependent, GATA6 stable
GATA6-dependent, GATA6 transient
GATA6-independent, GATA6 transient
GATA6-independent, GATA6 stable
F)
Incidence of EOMES binding site
100%
75%
50%
25%
0%
n=8,082
n=16,742
n=4,435
n=4,781
GATA6-independent
GATA6-dependent
GATA6-independent
GATA6-dependent
Closed chromatin
Open chromatin

Figure S2: Characterization of EOMES binding and chromatin opening in *GATA6*$^{+/+}$ and *GATA6*$^{-/-}$ early endoderm. Related to Figures 3 and 4. A) Quantification of GATA6 western blots normalized to β-actin, shown relative to day 2 value, n=3 mean ± SD; B) Quantification of EOMES western blots normalized to β-actin, shown relative to day 2 value, n=3 mean ± SD; C) RT-qPCR analysis of *GATA6* and *EOMES* mRNA levels during early endoderm formation. Values normalized to the housekeeping mRNA *RPL13*a and shown relative to day 2 values. n=3 mean ± SD; D) EOMES expression in *GATA6*$^{+/+}$ and *GATA6*$^{-/-}$ cells ± doxycycline at day 2 of differentiation, measured by western blot for EOMES and β-actin (left panel). Quantification of blots (right panel), normalized to β-actin and shown relative to wildtype values, n=2 mean ± SD; E) Pie chart representing the regions of EOMES co-occupancy with GATA6, divided by sites that occur at transient or stable GATA6 binding subsets and whether the EOMES binding is GATA6-independent or GATA6-dependent; F) Graph depicting incidence of the canonical EOMES motif at sites of EOMES occupancy in open and closed chromatin that is either GATA6-independent and GATA6-dependent;

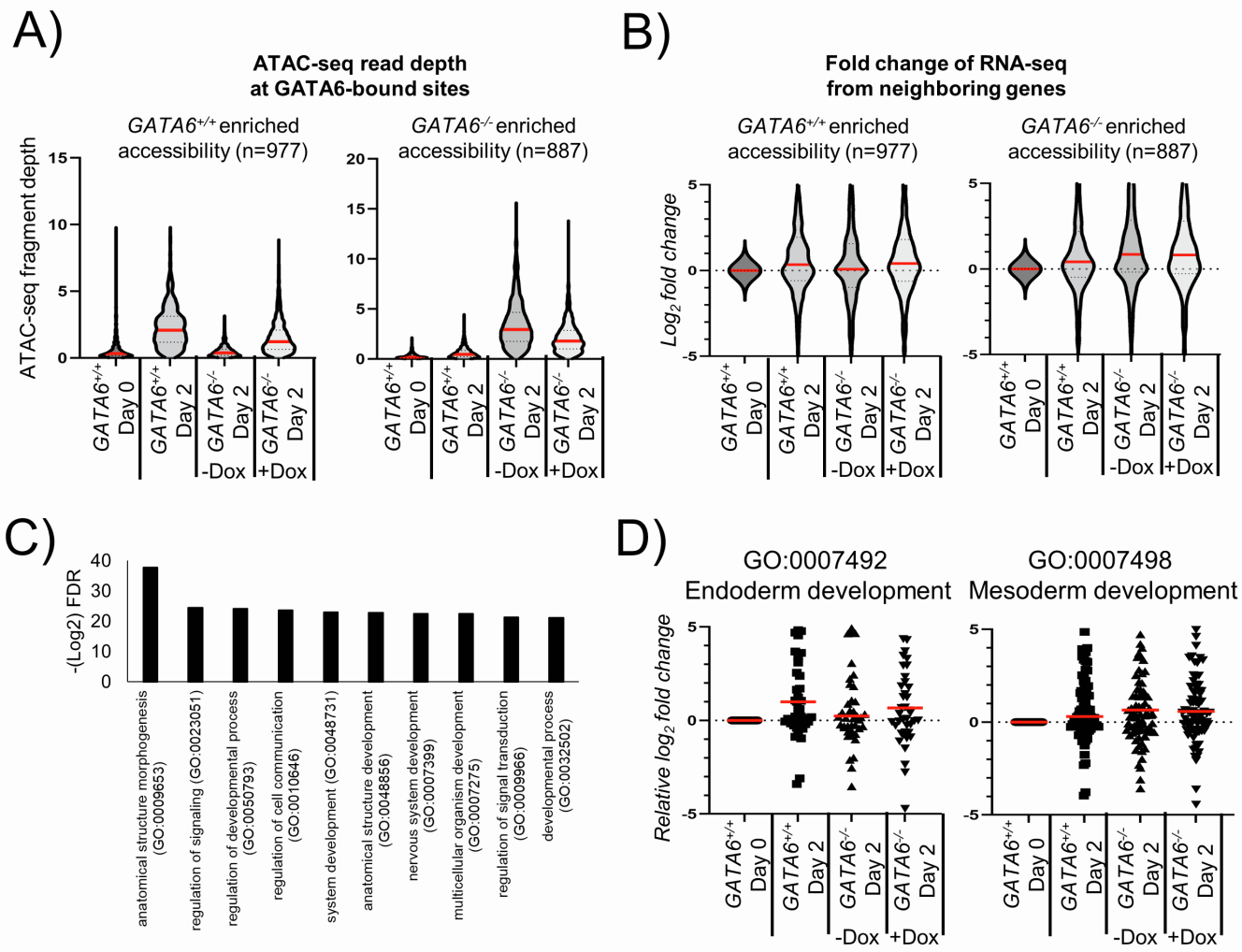
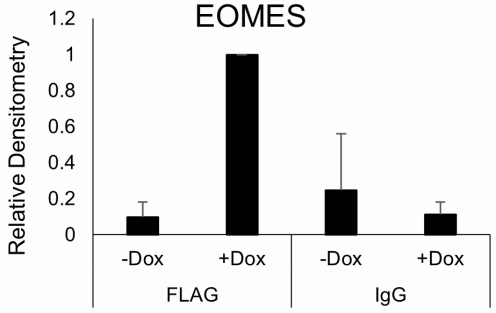
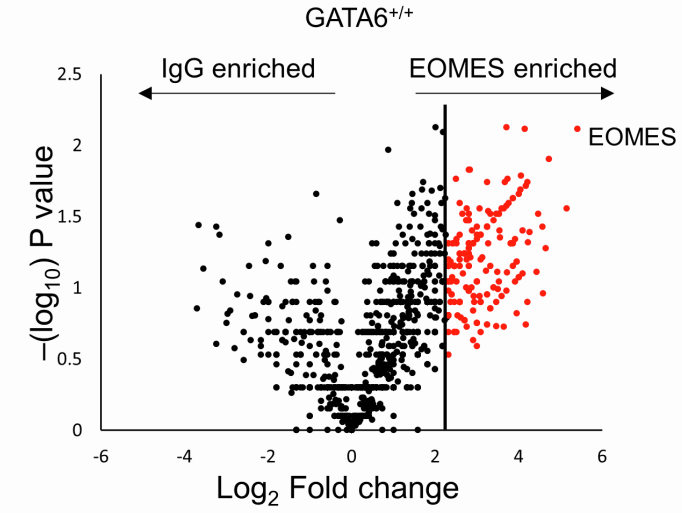

A)
ATAC-seq read depth
at GATA6-bound sites
GATA6+/+ enriched
accessibility (n=977)
GATA6-/- enriched
accessibility (n=887)
ATAC-seq fragment depth
B)
Fold change of RNA-seq
from neighboring genes
Log2 fold change
GATA6+/+ Day 0
GATA6+/+ Day 2
GATA6-/- Day 2
GATA6-/- Day 2
-Dox
+Dox
C)
-(Log2) FDR
anatomical structure morphogenesis (GO:0009653)
regulation of signaling (GO:0023051)
regulation of developmental process (GO:0050793)
regulation of cell communication (GO:0010646)
system development (GO:0048731)
anatomical structure development (GO:0048856)
nervous system development (GO:0007399)
multicellular organism development (GO:0007275)
regulation of signal transduction (GO:0009966)
developmental process (GO:0032502)
D)
GO:0007492
Endoderm development
GO:0007498
Mesoderm development
Relative log2 fold change

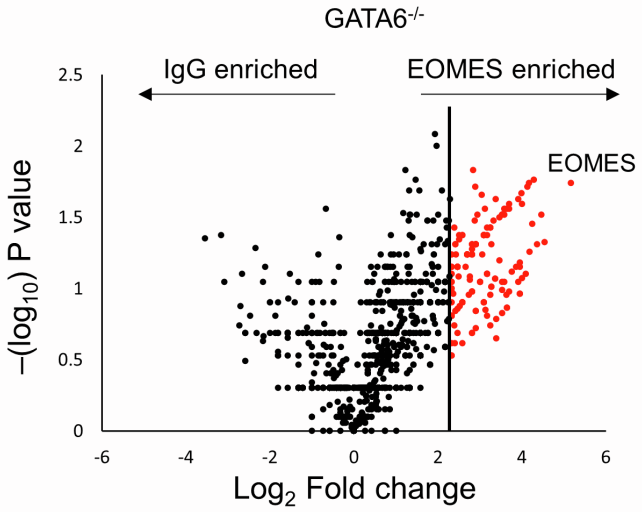
Figure S3: GATA6-dependent gene expression at sites neighboring GATA6-dependent remodeling. Related to Figure 4. A, B) Violin plots depicting the relationship between regions of A) differentially accessible chromatin and B) the mRNA expression of the neighboring genes. ATAC-seq data displayed as fragment depth, mRNA displayed as the log_2 fold change of each neighboring gene relative to day 0 values. Red line indicates median value; C) GO biological process analysis. The top ten significantly enriched pathways related to the genes that neighbor regions of chromatin of chromatin uniquely accessible in *GATA6*$^{-/-}$ cells; D) mRNA expression of genes found uniquely within the endoderm and mesoderm development GO biological processes gene sets, genes found in both sets were removed. mRNA displayed as individual values of the log_2 fold change of each neighboring gene relative to day 0 values. Red line indicates median value.

A)


EOMES
Relative Densitometry
1.2
1
0.8
0.6
0.4
0.2
0
-Dox
+Dox
-Dox
+Dox
FLAG
IgG

B)


GATA6+/+
IgG enriched
EOMES enriched
EOMES
−(log10) P value
Log2 Fold change


GATA6-/-
IgG enriched
EOMES enriched
EOMES
−(log10) P value
Log2 Fold change

C)

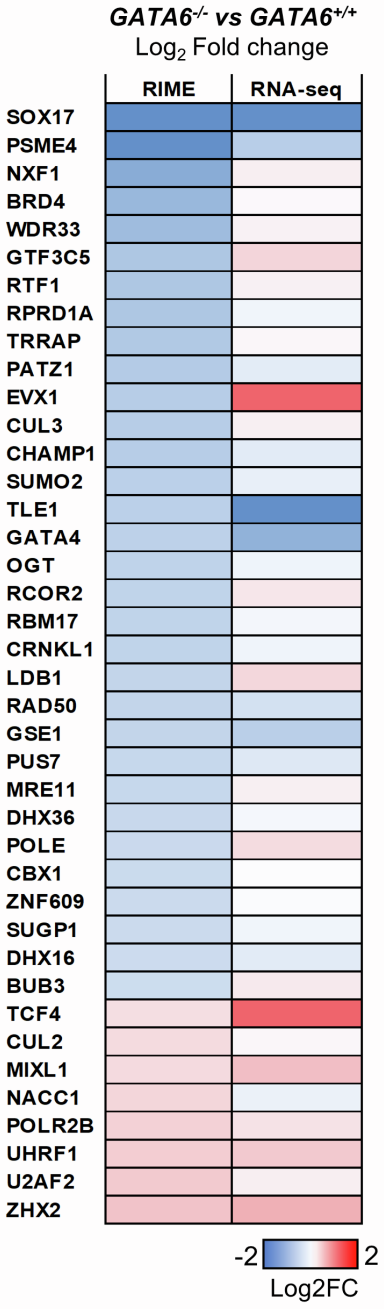

GATA6-/- vs GATA6+/+
Log2 Fold change
RIME
RNA-seq
SOX17
PSME4
NXF1
BRD4
WDR33
GTF3C5
RTF1
RPRD1A
TRRAP
PATZ1
EVX1
CUL3
CHAMP1
SUMO2
TLE1
GATA4
OGT
RCOR2
RBM17
CRNKL1
LDB1
RAD50
GSE1
PUS7
MRE11
DHX36
POLE
CBX1
ZNF609
SUGP1
DHX16
BUB3
TCF4
CUL2
MIXL1
NACC1
POLR2B
UHRF1
U2AF2
ZHX2
-2
2
Log2FC

Figure S4: Additional RIME analysis. Related to Figure 5. A) Quantification of EOMES western blots following FLAG or IgG immunoprecipitation, n=3 mean ± SD; B) Proteins enriched within the EOMES interactome in *GATA6*$^{+/+}$ or *GATA6*$^{-/-}$ cells as determined by RIME analysis at day 2 of differentiation; C) Heatmaps representing the relative $\log_2$ fold change in protein enrichment and mRNA expression corresponding to proteins with differential enrichment (-0.3> $\log_2$ fold change >0.3) in *GATA6*$^{-/-}$ compared to *GATA6*$^{+/+}$ cells at day 2 of differentiation.