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Supplemental Information

Suppressing Nodal Signaling Activity Predisposes Ectodermal Differentiation of Epiblast Stem Cells

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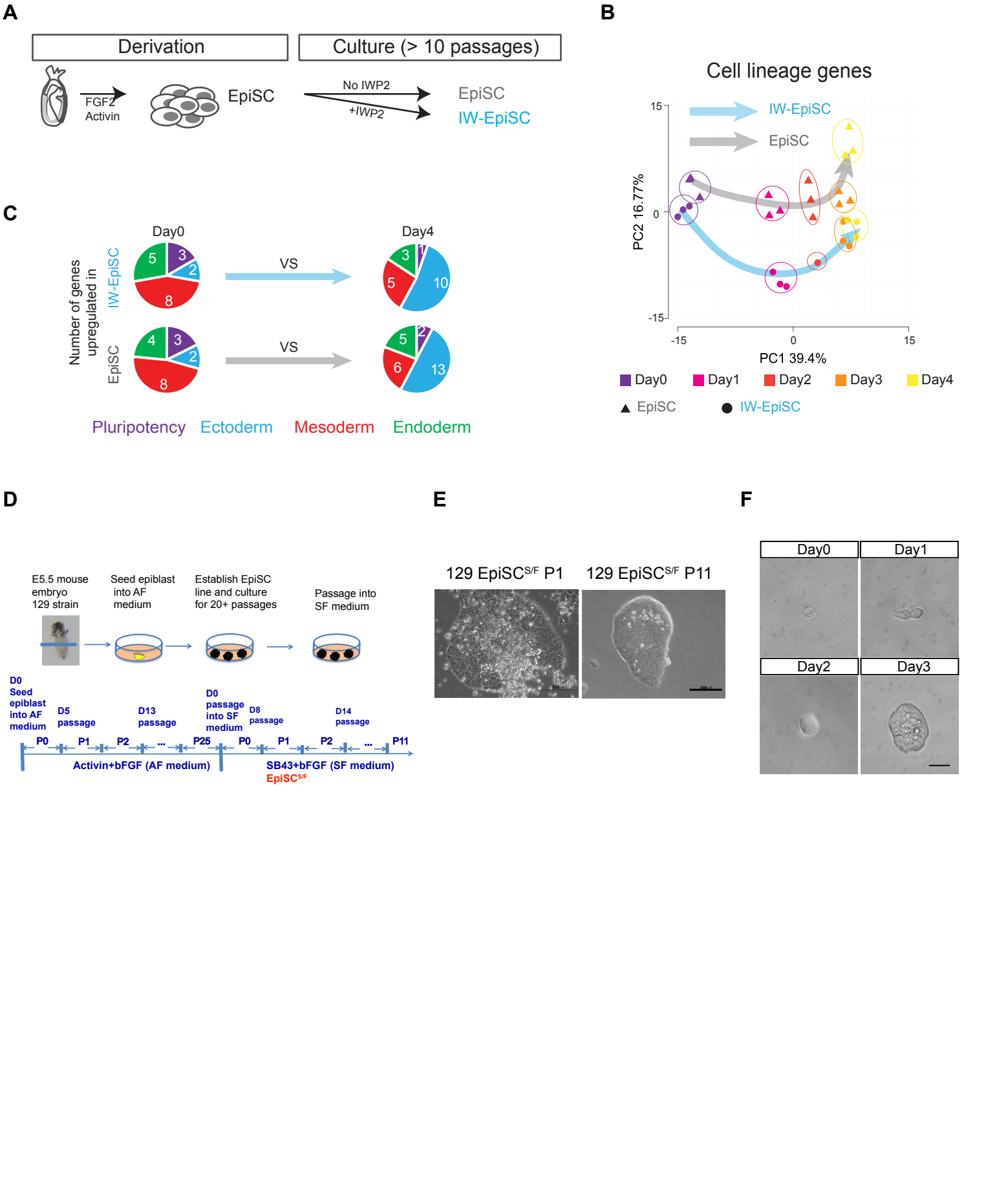

A
Derivation
Culture (> 10 passages)
FGF2
Activin
EpiSC
No IWP2
+IWP2
EpiSC
IW-EpiSC
B
Cell lineage genes
IW-EpiSC
EpiSC
PC2 16.77%
PC1 39.4%
15
0
-15
Day0
Day1
Day2
Day3
Day4
EpiSC
IW-EpiSC
C
Day0
Day4
Number of genes upregulated in
IW-EpiSC
EpiSC
VS
VS
Pluripotency
Ectoderm
Mesoderm
Endoderm
D
E5.5 mouse embryo 129 strain
Seed epiblast into AF medium
Establish EpiSC line and culture for 20+ passages
Passage into SF medium
D0 Seed epiblast into AF medium
D5 passage
D13 passage
D0 passage into SF medium
D8 passage
D14 passage
P0
P1
P2
P25
P11
Activin+bFGF (AF medium)
SB43+bFGF (SF medium)
EpiSC S/F
E
129 EpiSC S/F P1
129 EpiSC S/F P11
F
Day0
Day1
Day2
Day3

Figure S1

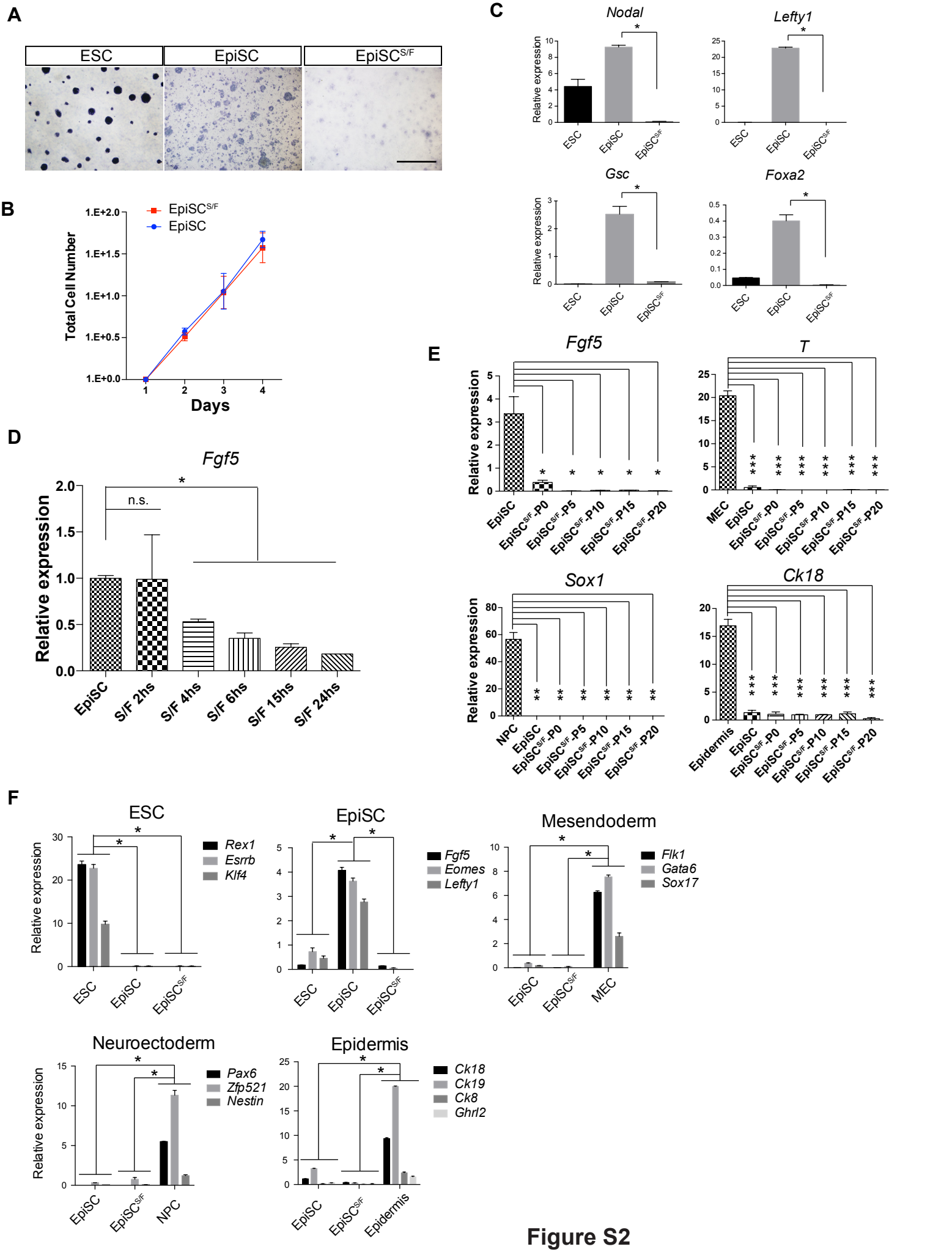

A
ESC
EpiSC
EpiSC$^{S/F}$
B
EpiSC$^{S/F}$
EpiSC
Total Cell Number
1.E+2.0
1.E+1.5
1.E+1.0
1.E+0.5
1.E+0.0
1
2
3
4
Days
C
Nodal
Lefty1
Gsc
Foxa2
Relative expression
ESC
EpiSC
EpiSC$^{S/F}$
D
Fgf5
Relative expression
n.s.
EpiSC
S/F 2hs
S/F 4hs
S/F 6hs
S/F 15hs
S/F 24hs
E
Fgf5
T
Sox1
Ck18
Relative expression
MEC
NPC
Epidermis
EpiSC
EpiSC$^{S/F}$-P0
EpiSC$^{S/F}$-P5
EpiSC$^{S/F}$-P10
EpiSC$^{S/F}$-P15
EpiSC$^{S/F}$-P20
F
ESC
Rex1
Esrrb
Klf4
EpiSC
Fgf5
Eomes
Lefty1
Mesendoderm
Flk1
Gata6
Sox17
Neuroectoderm
Pax6
Zfp521
Nestin
Epidermis
Ck18
Ck19
Ck8
Ghrl2
Relative expression
ESC
EpiSC
EpiSC$^{S/F}$
NPC
MEC
Epidermis

Figure S2

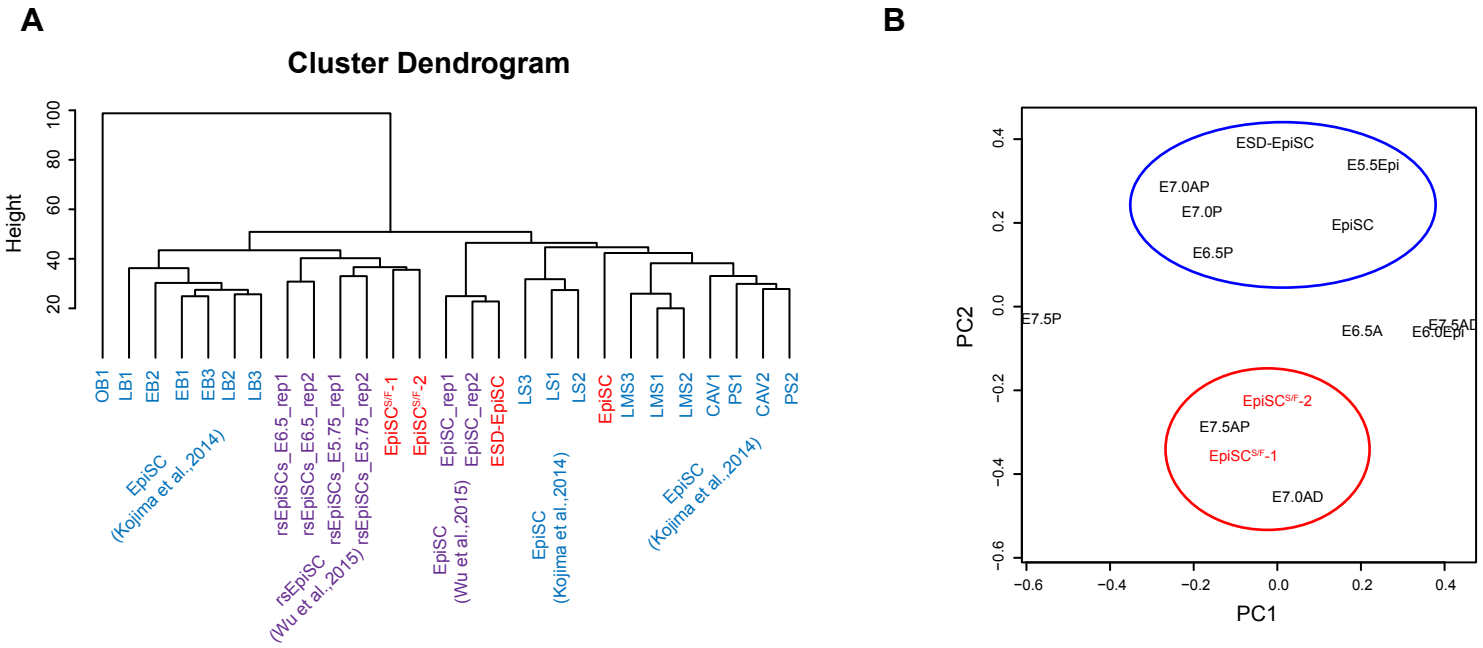
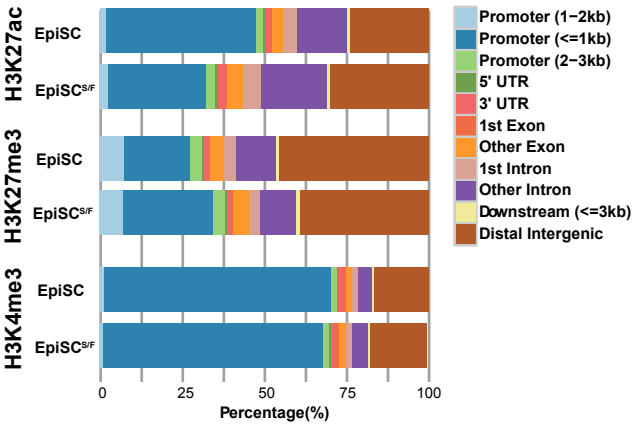

A
Cluster Dendrogram
Height
100
80
60
40
20
OB1
LB1
EB2
EB1
EB3
LB2
LB3
rsEpiSCs_E6.5_rep1
rsEpiSCs_E6.5_rep2
rsEpiSCs_E5.75_rep1
rsEpiSCs_E5.75_rep2
EpiSC$^{S/F}$-1
EpiSC$^{S/F}$-2
EpiSC_rep1
EpiSC_rep2
ESD-EpiSC
LS3
LS1
LS2
EpiSC
LMS3
LMS1
LMS2
CAV1
PS1
CAV2
PS2
EpiSC (Kojima et al.,2014)
rsEpiSC (Wu et al.,2015)
EpiSC (Wu et al.,2015)
EpiSC (Kojima et al.,2014)
EpiSC (Kojima et al.,2014)
B
PC2
PC1
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.6
−0.4
−0.2
0.0
0.2
0.4
ESD-EpiSC
E5.5Epi
E7.0AP
E7.0P
EpiSC
E6.5P
E7.5P
E6.5A
E7.5AD
E6.0Epi
EpiSC$^{S/F}$-2
E7.5AP
EpiSC$^{S/F}$-1
E7.0AD

Figure S3

A


H3K27ac
EpiSC
EpiSC^S/F
H3K27me3
EpiSC
EpiSC^S/F
H3K4me3
EpiSC
EpiSC^S/F
0
25
50
75
100
Percentage(%)
Promoter (1−2kb)
Promoter (<=1kb)
Promoter (2−3kb)
5' UTR
3' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (<=3kb)
Distal Intergenic

B

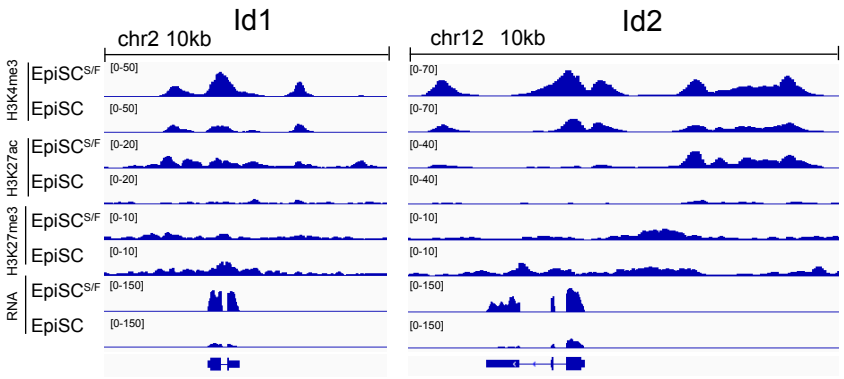

Id1
chr2 10kb
Id2
chr12 10kb
H3K4me3
H3K27ac
H3K27me3
RNA
EpiSC^S/F
EpiSC
[0-50]
[0-70]
[0-20]
[0-40]
[0-10]
[0-150]

Figure S4

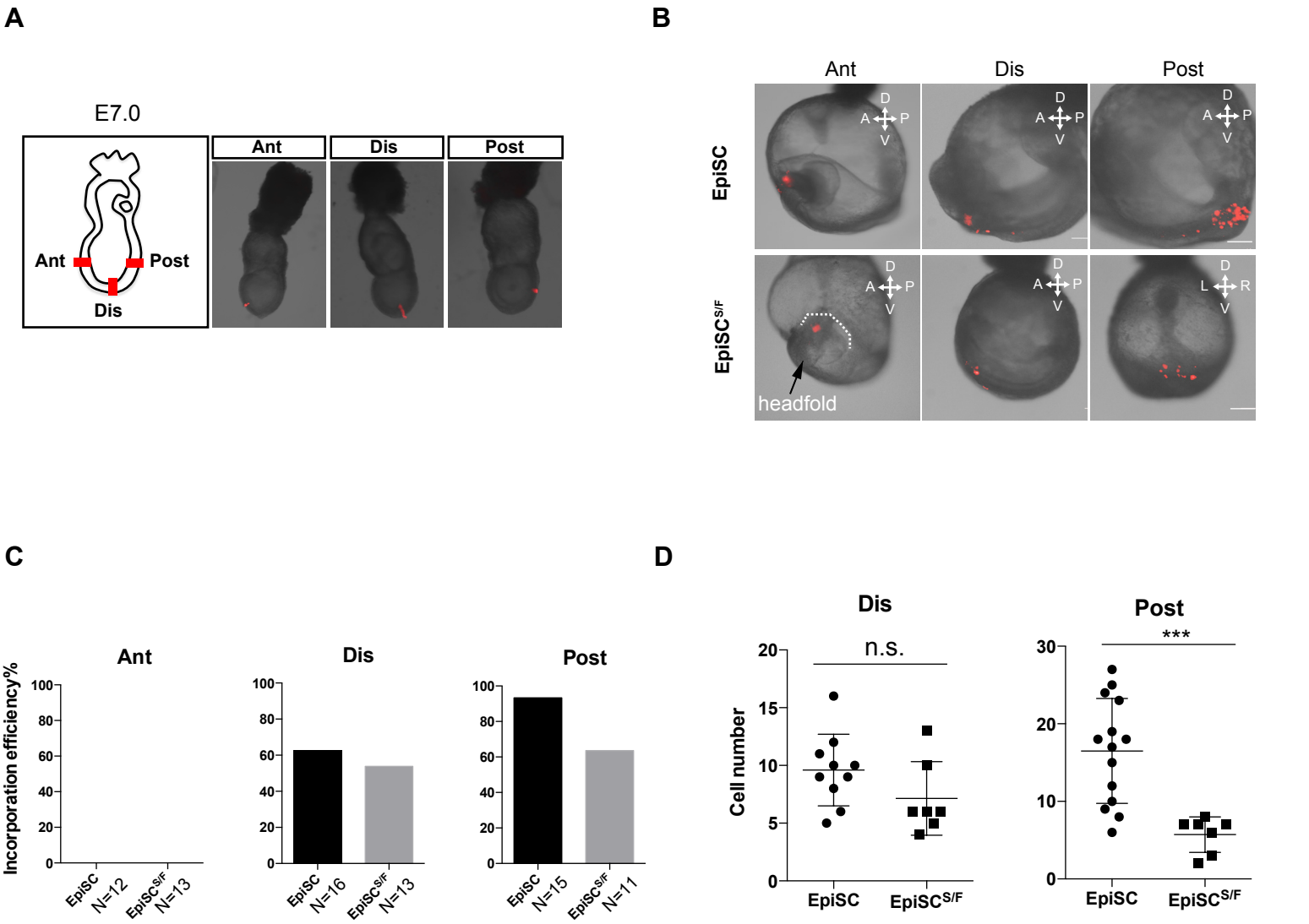

A
E7.0
Ant
Post
Dis
Ant
Dis
Post
B
Ant
Dis
Post
EpiSC
EpiSC^S/F
D
A
P
V
L
R
headfold
C
Ant
Dis
Post
Incorporation efficiency%
100
80
60
40
20
0
EpiSC N=12
EpiSC^S/F N=13
EpiSC N=16
EpiSC^S/F N=13
EpiSC N=15
EpiSC^S/F N=11
D
Dis
n.s.
Post
***
Cell number
20
15
10
5
0
30
EpiSC
EpiSC^S/F

Figure S5

Supplementary Figure Legends

Figure S1. Derivation of EpiSC$^{S/F}$, relate to Figure 1.

(A) Experimental design. EpiSC lines were derived in FGF2 and Activin A-supplemented medium. After 10 passages, EpiSC were maintained in the same medium, or with additional supplement of IWP2 for more than ten passages to generate the IW-EpiSC.

(B) PCA display of the trajectory of lineage differentiation (arrows) of EpiSC and IW-EpiSC based on gene expression profiles at Day 0-4 of differentiation (colour-coded) assessed by reporter card assay for "cell lineage" in 3 lines for each type of EpiSCs.

(C) Pie chart display of the number of up-regulated reporter card genes at Day 0 versus (VS) Day 4 of differentiation of EpiSC and IW-EpiSC. Colour identifies the pluripotency and germ layer associated genes.

(D) Experimental design. 129 EpiSC lines were derived in Activin A and bFGF-supplemented medium from E5.5 129 strain mouse embryo. After 25 passages, EpiSC were maintained in SB43 plus bFGF condition for more than ten passages to generate the EpiSC$^{S/F}$.

(E) Morphology of EpiSC$^{S/F}$ derived from 129 EpiSC. Scale bar, 200μm.

(F) Morphology of EpiSC$^{S/F}$ clone derived from single EpiSC. Scale bar, 50μm.

Figure S2. Characterization of EpiSC$^{S/F}$, relate to Figure 2.

(A) Bright field images showing alkaline phosphatase expression by cytochemical staining of ESCs, EpiSCs and EpiSCs$^{S/F}$. Scale bar, 500 μm.

(B) The profile of population growth of EpiSC$^{S/F}$ and EpiSC, data are mean ±SD.

(C) Q-PCR analysis of the expression of Nodal downstream genes: *Nodal*, *Lefty1*, *Gsc* and *Foxa2* in ESC, EpiSC and EpiSC$^{S/F}$. Data are mean ±SD, n=3 for each cell type, Statistical analysis was performed using Student's t tests (*p <0.05).

(D) Q-PCR analysis of the expression of marker genes of epiblast (*Fgf5*) in EpiSCs under SB43 plus bFGF treatment from 2 hours to 24 hours. Data are mean ±SD, n=3 for each assay. Statistical analysis was performed between control cell (first column) and cells under treatment from 2 hours to 24 hours using Student's t tests (*p <0.05, ** p <0.01, *** p <0.001).

(E) Q-PCR analysis of the expression of marker genes of epiblast (*Fgf5*), mesendoderm (*T*), neuroectoderm (*Sox1*) and epidermis (*Ck18*) in EpiSCs$^{S/F}$. Data are mean ±SD, n=3 for each assay at passage 0 to 20. Statistical analysis was performed between control cell (first column) and EpiSCs$^{S/F}$ of passage 0 to 20 using Student's t tests (*p <0.05, ** p <0.01, *** p <0.001).

(F) Q-PCR analysis of expression level, relative to GAPDH, of markers of ESC (*Rex1*, *Esrrb* and *Klf4*), EpiSC (*Fgf5*, *Eomes* and *Lefty1*), mesendoderm (*Flk1*, *Gata6* and *Sox17*), neuroectoderm (*Pax6*, *Zfp521* and *Nestin*) and epidermis (*Ck18*, *Ck19*, *Ck8* and *Grhl2*) in EpiSC$^{S/F}$ and EpiSC, data are mean ±SD, n=3 samples each. Statistical analysis was performed using Student's t tests (*p <0.05).

Figure S3. Global transcriptome of EpiSCs$^{S/F}$, relate to Figure 3.
(A) Hierarchical clustering of EpiSCs (red: this study, blue: (Kojima et al., 2014), purple: rsEpiSCs (Wu et al., 2015)).
(B) PCA display of RNA-seq data of EpiSCs$^{S/F}$, EpiSCs and embryonic tissue samples.

Figure S4. Epigenetic signature of EpiSCs$^{S/F}$, relate to Figure 4.
(A) Comparison of H3K27ac, H3K27Me3 and H3K4Me3 pattern between EpiSC and EpiSC$^{S/F}$. The peaks with the unique genomic feature are annotated in the color bar diagrams.
(B) The ChIP-seq signal and the expression of ectoderm-related genes (*Id1 and Id2*). In each gene, first panel shows H3K4me3 signal, second panel shows H3K27ac signal, third panel shows H3K27me3 signal around transcription start site in EpiSC and EpiSC$^{S/F}$. The last panel shows RNA expression level in EpiSC and EpiSC$^{S/F}$.

Figure S5. Differentiation of EpiSC$^{S/F}$ in E7.0 host chimeras, relate to Figure 5.
(A) RFP-expressing EpiSC$^{S/F}$ and EpiSCs grafted to anterior (Ant), distal (Dis) and posterior (Post) regions of E7.0 embryo.
(B) Distribution of graft-derived cells in host embryo 24 hours after transplantation of RFP-expressing cells to anterior (Ant), distal (Dis) and posterior (Post) regions of E7.0 embryo. Scale bar, 200μm.
(C) Percentage of embryos showing incorporation of graft-derived cells transplanted to anterior (Ant), distal (Dis) and posterior (Post) regions of E7.0 embryo. N=12 embryos for EpiSC, and N=13 embryos for EpiSC$^{S/F}$ in anterior (Ant) grafted group. N=16 embryos for EpiSC, and N=13 embryos for EpiSC$^{S/F}$ in distal (Dis) grafted group. N=15 embryos for EpiSC, and N=11 embryos for EpiSC$^{S/F}$ in posterior (Post) grafted group.
(D) Number of EpiSC and EpiSC$^{S/F}$- derived cells in the host embryo following cell transplantation to distal (Dis) and posterior (Post) regions of E7.0 embryo. Data are mean ±SD, N=10 embryos for EpiSC, and N=7 embryos for EpiSC$^{S/F}$ in distal (Dis) grafted group. N=14 embryos for EpiSC, and N=7 embryos for EpiSC$^{S/F}$ in posterior (Post) grafted group. Statistical analysis was performed using Student's t tests (*** $p < 0.001$).