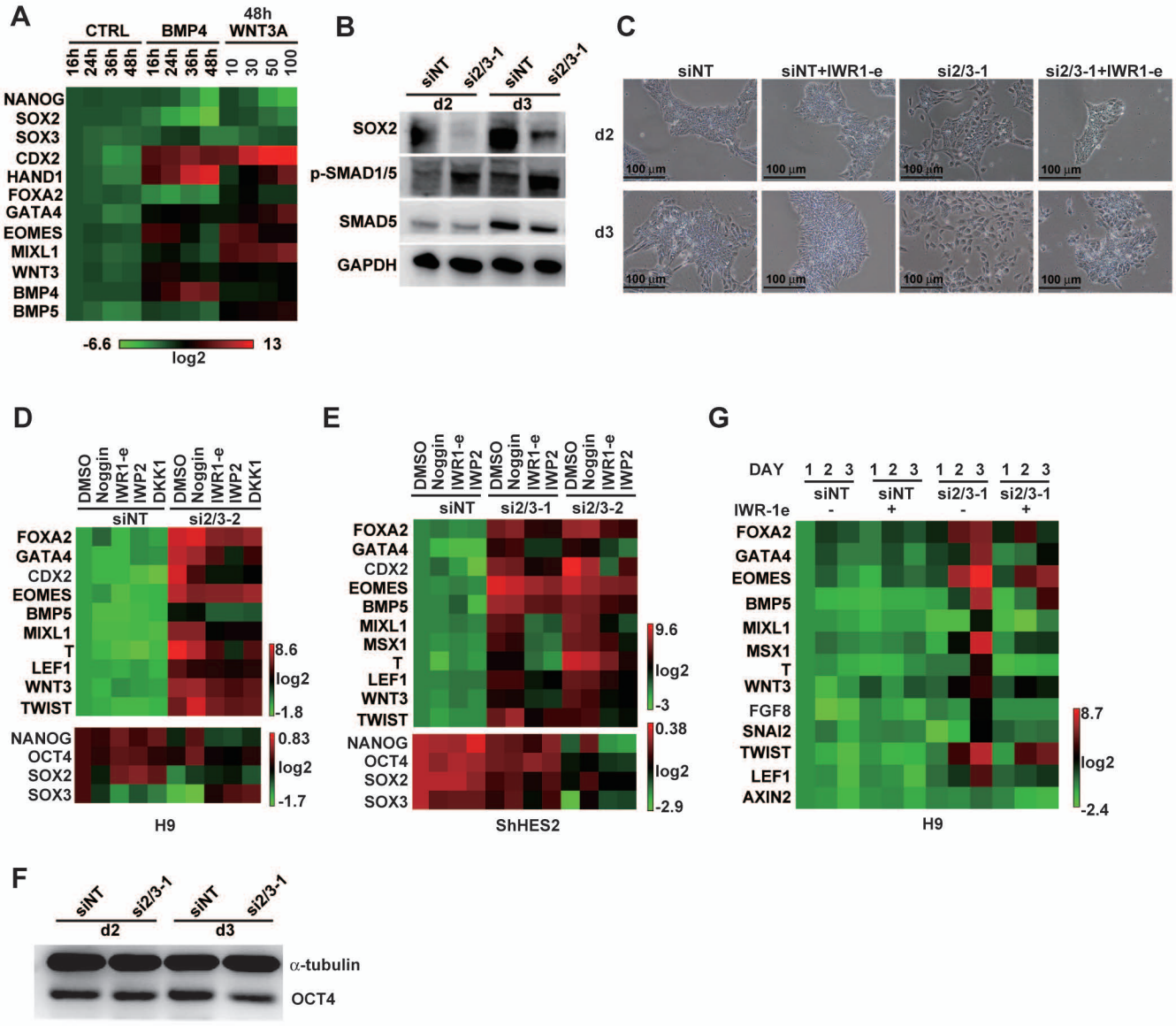
A
CTRL
BMP4
48h WNT3A
16h 24h 36h 48h 16h 24h 36h 48h 10 30 50 100
NANOG
SOX2
SOX3
CDX2
HAND1
FOXA2
GATA4
EOMES
MIXL1
WNT3
BMP4
BMP5
-6.6
13
log2
B
siNT si2/3-1 siNT si2/3-1
d2 d3
SOX2
p-SMAD1/5
SMAD5
GAPDH
C
siNT
siNT+IWR1-e
si2/3-1
si2/3-1+IWR1-e
d2
d3
100 μm
D
DMSO Noggin IWR1-e IWP2 DKK1 DMSO Noggin IWR1-e IWP2 DKK1
siNT si2/3-2
FOXA2
GATA4
CDX2
EOMES
BMP5
MIXL1
T
LEF1
WNT3
TWIST
8.6
log2
-1.8
NANOG
OCT4
SOX2
SOX3
0.83
log2
-1.7
H9
E
DMSO Noggin IWR1-e IWP2 DMSO Noggin IWR1-e IWP2 DMSO Noggin IWR1-e IWP2
siNT si2/3-1 si2/3-2
FOXA2
GATA4
CDX2
EOMES
BMP5
MIXL1
MSX1
T
LEF1
WNT3
TWIST
9.6
log2
-3
NANOG
OCT4
SOX2
SOX3
0.38
log2
-2.9
ShHES2
G
DAY 1 2 3 1 2 3 1 2 3 1 2 3
siNT siNT si2/3-1 si2/3-1
IWR-1e - + - +
FOXA2
GATA4
EOMES
BMP5
MIXL1
MSX1
T
WNT3
FGF8
SNAI2
TWIST
LEF1
AXIN2
8.7
log2
-2.4
H9
F
siNT si2/3-1 siNT si2/3-1
d2 d3
α-tubulin
OCT4

Figure S3. Knock down of *SOX2* leads to enhanced non-neural lineage differentiation by activating the Wnt pathway. Related to Figure 4.

(A) RT-qPCR results of lineage and pluripotency markers after BMP4 (50 ng/ml) treatment for 16, 24, 36 and 48 hrs, or with increasing dosages of WNT3A treatment (10, 30, 50 and 100 ng/ml) for 48 hrs in hESCs. Scale bar represents $\log_2$ of expression levels.

(B) Western blot results of p-SMAD1/5 and total SMAD5 after *SOX2/3* knock down in H9 hESCs at day 2 and day 3.

(C) Morphology of H9 hESCs after *SOX2/3* knock down for 2 days and 3 days. IWR1-e (10 μM) treatment rescues the differentiation phenotype caused by *SOX2/3* KD.

(D and E) RT-qPCR results of pluripotency and lineage markers after *SOX2/3* knock down for 3 days in H9 hESCs using si2/3-2 (D) and in SHhES2 hESCs using si2/3-1 and si2/3-2 (E). DMSO, Noggin (200 ng/ml), IWR1-e (10 μM), IWP2 (10 μM), DKK1 (100 ng/ml) were used to rescue *SOX2/3* KD caused differentiation. Scale bar represents $\log_2$ of expression levels.

(F) Western blot for OCT4 protein levels after *SOX2/3* knock down for 2 and 3 days, showing OCT4 protein levels did not decrease till day 3.

(G) RT-qPCR analysis showing increase of lineage markers after *SOX2/3* knock down in H9 hESCs for 1, 2 and 3 days, with the most significant increase occurring on day 3. IWR1-e (10 μM) treatment attenuated the increase of lineage markers.