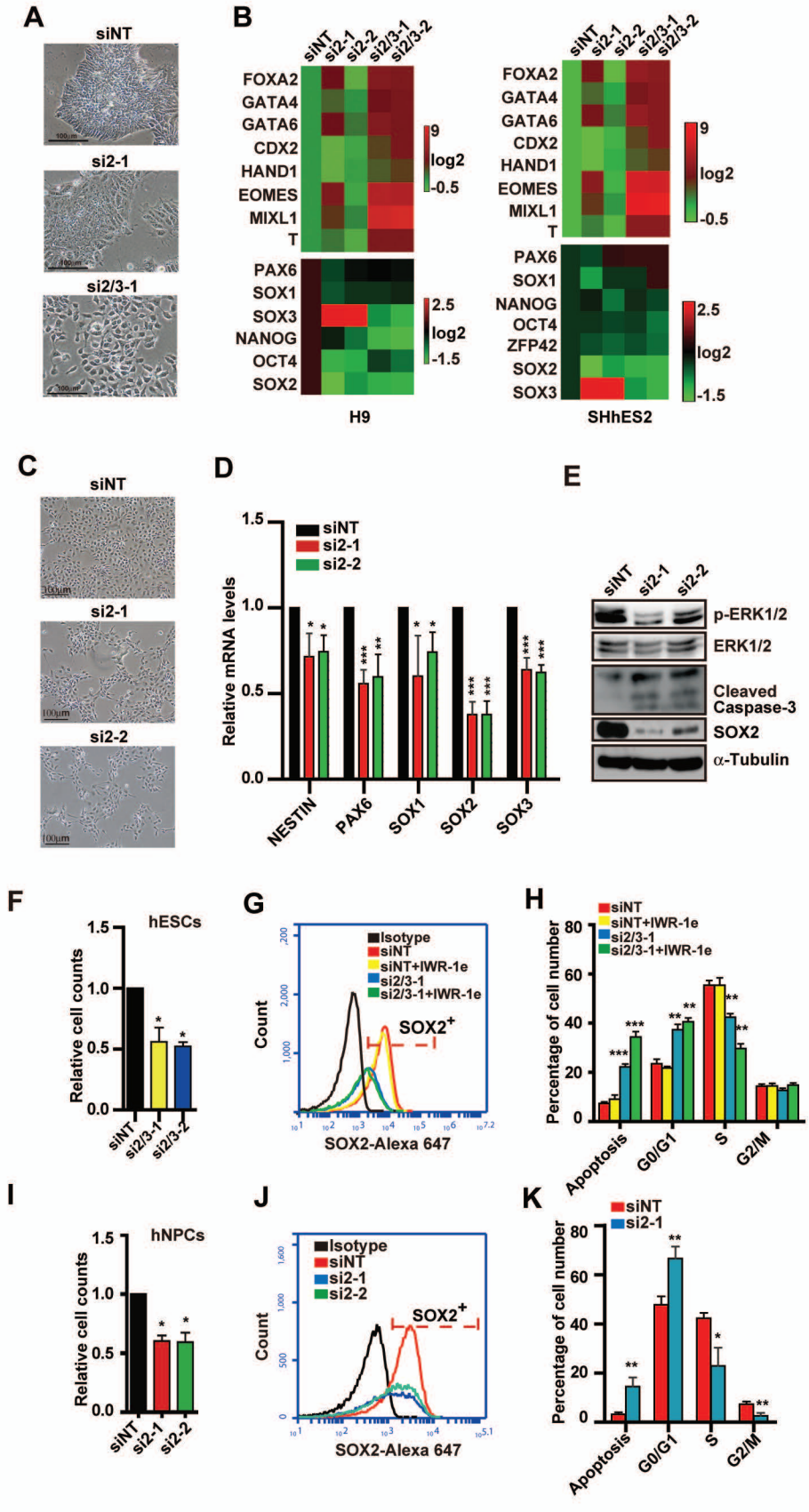
A
siNT
si2-1
si2/3-1
B
siNT
si2-1
si2-2
si2/3-1
si2/3-2
FOXA2
GATA4
GATA6
CDX2
HAND1
EOMES
MIXL1
T
PAX6
SOX1
SOX3
NANOG
OCT4
SOX2
ZFP42
9
log2
-0.5
2.5
-1.5
H9
SHhES2
C
siNT
si2-1
si2-2
100μm
D
Relative mRNA levels
1.5
1.0
0.5
0.0
NESTIN
PAX6
SOX1
SOX2
SOX3
E
p-ERK1/2
ERK1/2
Cleaved Caspase-3
SOX2
α-Tubulin
F
hESCs
Relative cell counts
siNT
si2/3-1
si2/3-2
G
Isotype
siNT
siNT+IWR-1e
si2/3-1
si2/3-1+IWR-1e
SOX2+
Count
SOX2-Alexa 647
H
Percentage of cell number
Apoptosis
G0/G1
S
G2/M
I
hNPCs
si2-1
si2-2
J
K

Figure S2. SOX2 regulates self-renewal and protects survival, proliferation and cell cycle in hESCs and hESC-derived hNPCs. Related to Figure 3.

(A) Morphology of hESCs (H9) transfected with siRNAs of Non-targeting (siNT), *SOX2* (si2-1) or *SOX2/3* (si2/3-1).

(B) RT-qPCR results for germ layer and pluripotency markers after transfection with indicated siRNA oligos for 3 days in two hESC lines, H9 and SHhES2. Scale bar represents $\log_2$ of expression level. si2-1, *SOX2* siRNA-1; si2/3-1, *SOX2/3* siRNA-1.

(C) Morphology of H9 hESC-derived hNPC transfected with indicated siRNA oligos.

(D) RT-qPCR results showing decreased NPC stemness markers after *SOX2* KD in hNPCs.

(E) Western blot results decreased p-ERK1/2 and increased active caspase3 upon *SOX2* KD in hNPCs.

(F) Relative cell numbers decreased in H9 2 days after *SOX2* knock down.

(G) FACS analysis showing efficient knock down of SOX2 in H9 hESCs transfected with siNT and si2/3-1 for 3 days. IWR1-e (10 μM) does not restore SOX2 expression.

(H) BrDU/7AAD FACS analysis was performed. Quantification of FACS results showing proliferation defects and increased apoptosis upon *SOX2/3* knock down in hESCs. Percentages of subG1 apoptotic cells, cells in G0/G1, S and G2/M phases were shown. IWR1-e (10μM) treatment does not rescue proliferation defects and apoptosis.

(I) Relative cell numbers decreased in H9-derived hNPCs 2 days after *SOX2* knock

down.

(J) FACS analysis showing efficient knock down of SOX2 in H9 derived hNPC transfected with siNT, si2-1 and si2-2.

(K) BrDU/7AAD FACS analysis was performed. Quantification of FACS results showing proliferation defects and increased apoptosis upon *SOX2* knock down in hNPCs. Percentages of subG1 apoptotic cells, cells in G0/G1, S and G2/M phases were shown.