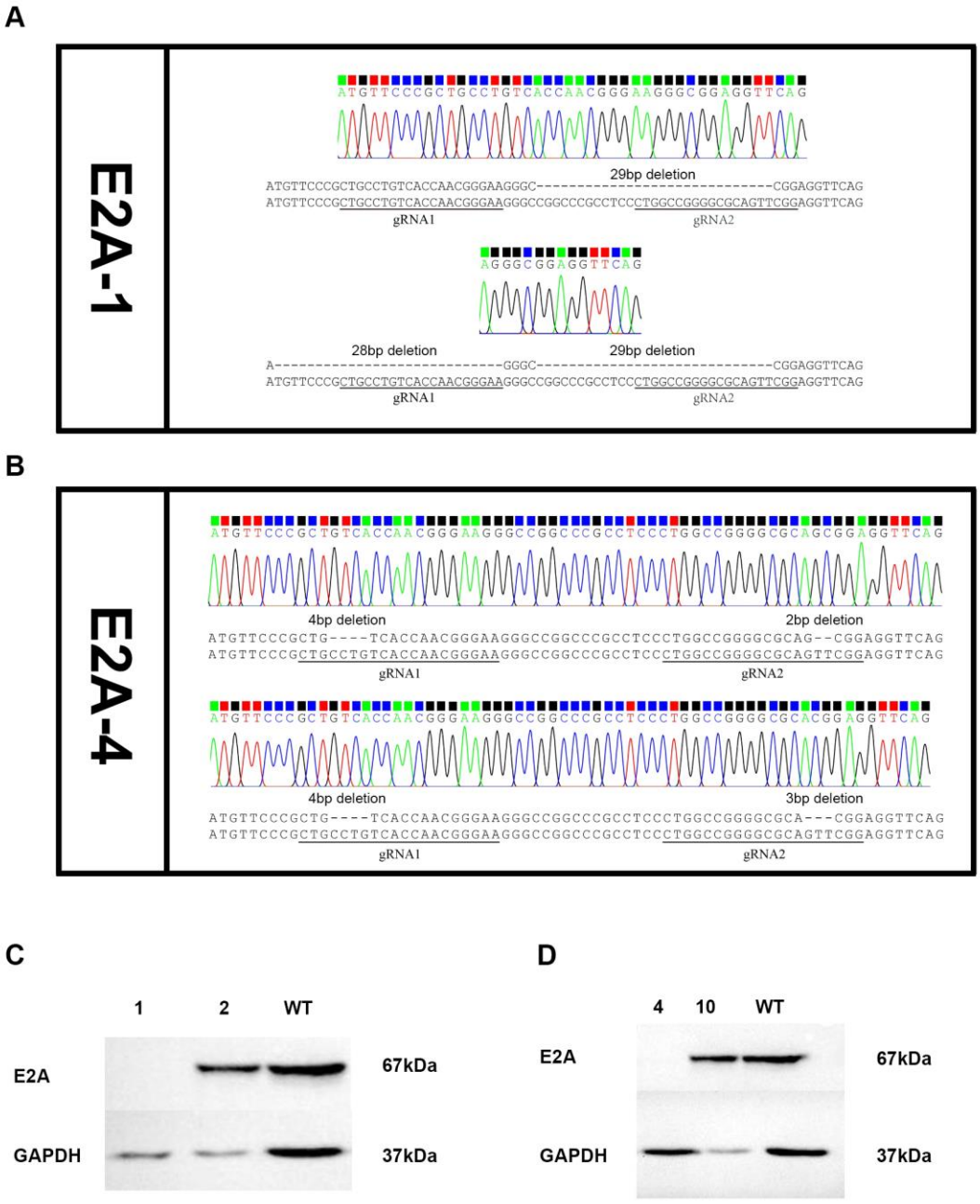

Fig. S1. Generation of E2A KO hESCs lines KO-1 and KO-4 by CRISPR-Cas9 targeting of the E2A gene locus.

A-B. Sanger sequencing of genomic DNA from two E2A KO clones (KO-1 and KO-4) comparing the sequences to WT.

C-D. Western blot analysis of E2A protein expression in wild type and E2A KO hESCs for both KO-1 and KO-4.

Images in (A), (B), (C), (D) are representative of three independent experiments.

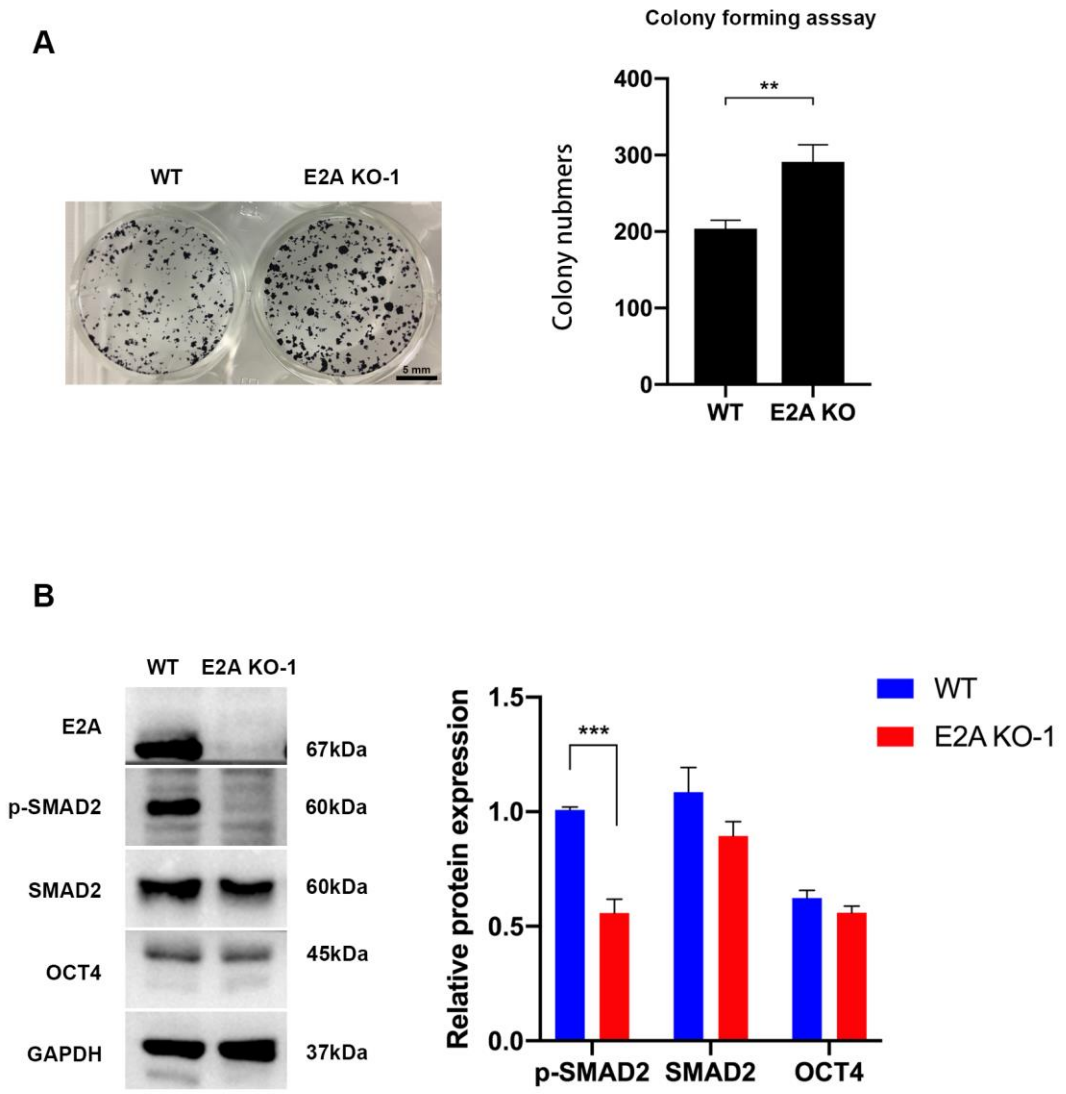

Fig. S2 Comparison of colony forming ability and nodal signalling pathway expression in WT and E2A KO hESCs.

A. Colony forming and quantitative analysis of WT and E2A KO hESCs.

B. Western blot and quantitative analysis of p-SMAD2, SMAD2 and OCT4 in WT and E2A KO hESCs.

Images and graphs in (A), (B) are representative of three independent experiments. Error bars represent mean ± SD (n = 3 independent experiments). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ by Student's test. Scale bars in (A) 5mm

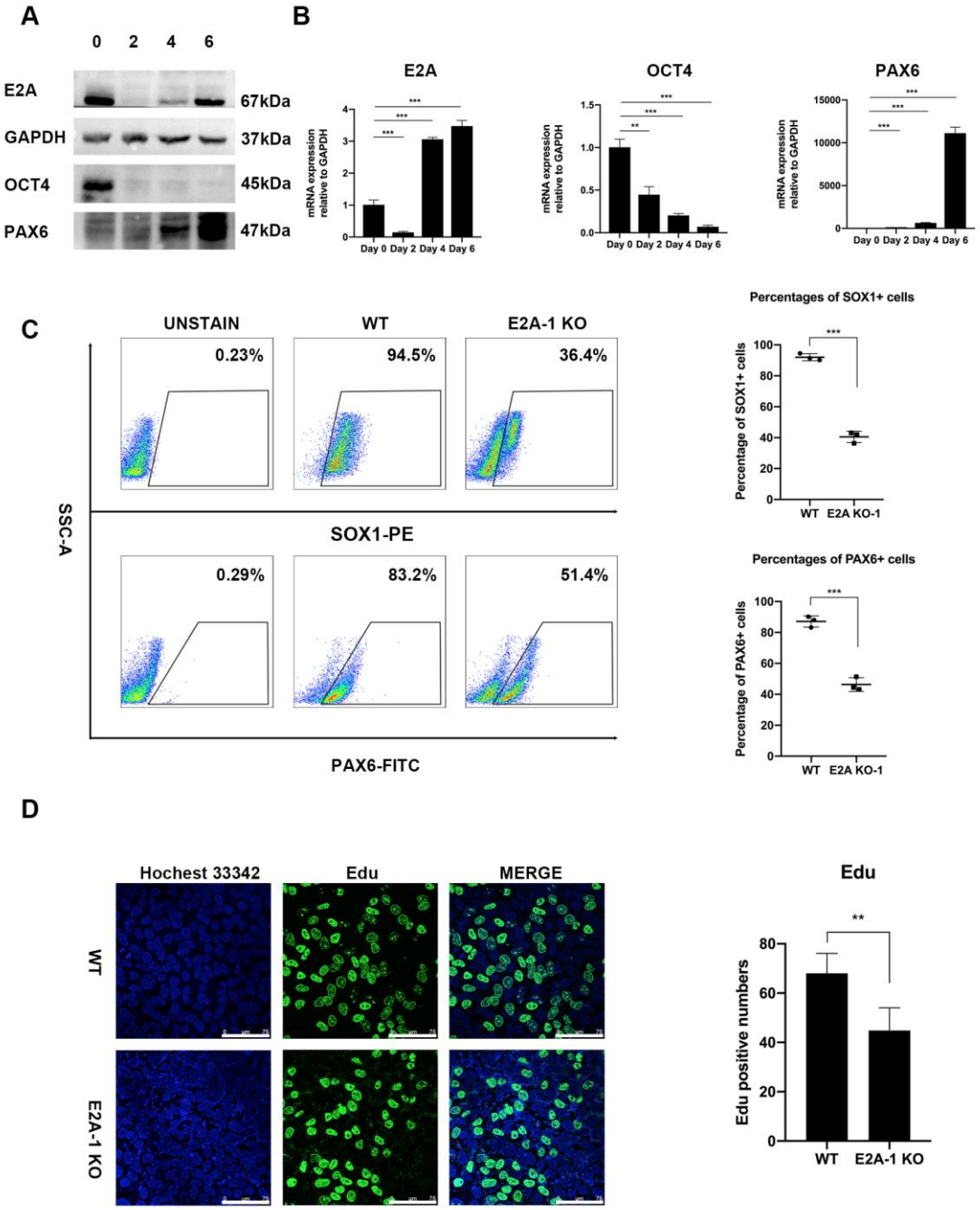

Fig. S3 The function of E2A in neural ectoderm differentiation.

A. Western blot analysis of E2A, OCT4 and PAX6 in neural induction period.

B. qPCR analysis of E2A, OCT4 and PAX6 in neural induction period.

C. Flow-cytometric analysis and percentage of SOX1 and PAX6 expression on d9 neural differentiated cells.

D. Edu staining and quantitative analysis of positive cells on neural differentiated WT and E2A KO cells.

Images in (A), (C), (D) and graphs in (B), (C), (D) are representative of three independent experiments. Error bars represent mean ± SD (n = 3 independent experiments). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ by Student's test. Scale bars in (D) 75um

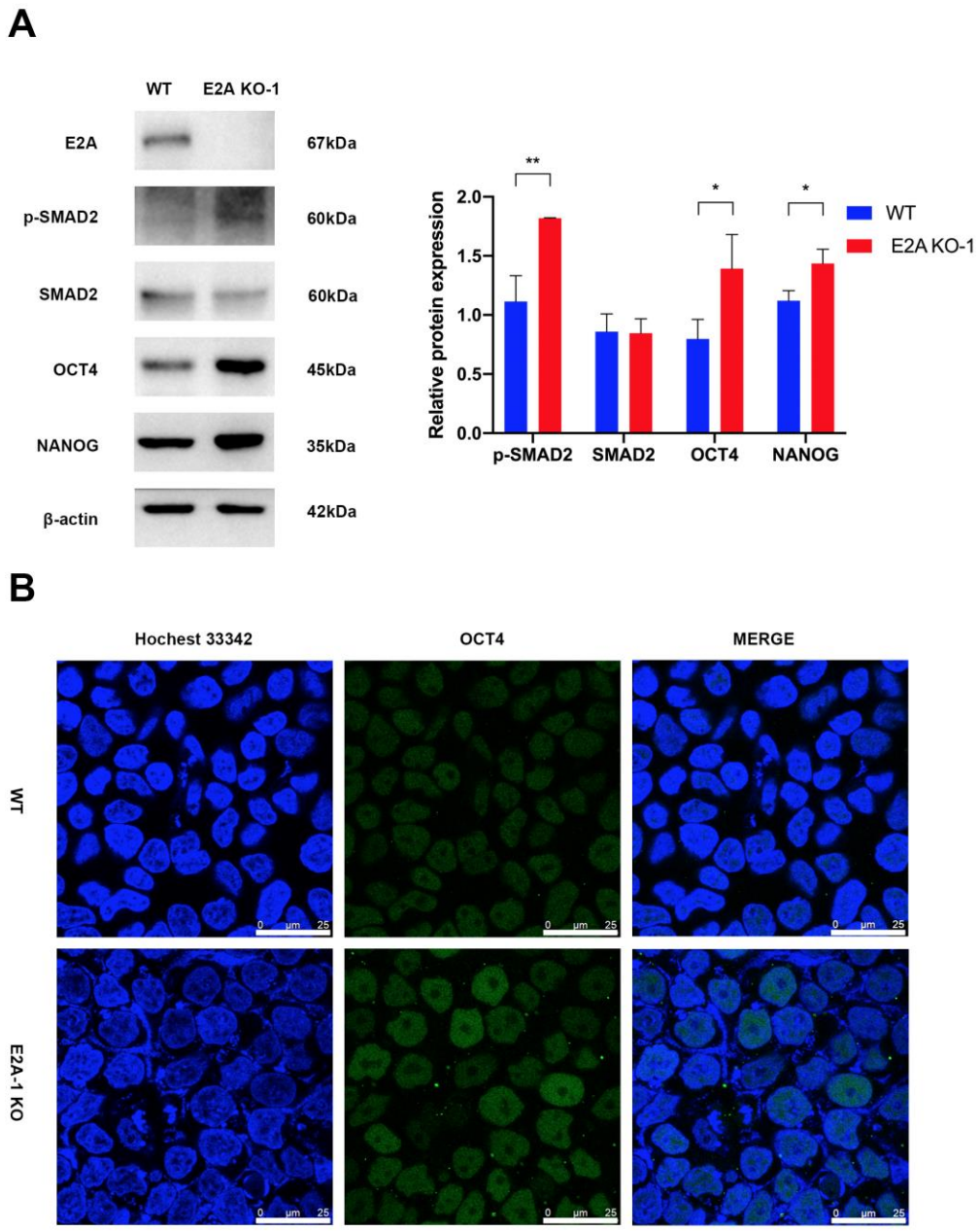

Fig. S4 The function of E2A in NODAL signaling and pluripotency markers during neural ectoderm differentiation.

A Western blot and quantitative analysis of p-SMAD2, SMAD2, OCT4 and NANOG on d3 neural differentiated cells.

B. Immunofluorescent staining of OCT4 on d3 neural differentiated cells.

Images in (A), (B) and graphs in (A) are representative of three independent experiments. Error bars represent mean ± SD (n = 3 independent experiments). *$p < 0.05$, ** $p < 0.01$ by Student's test. Scale bars in (D) 25um

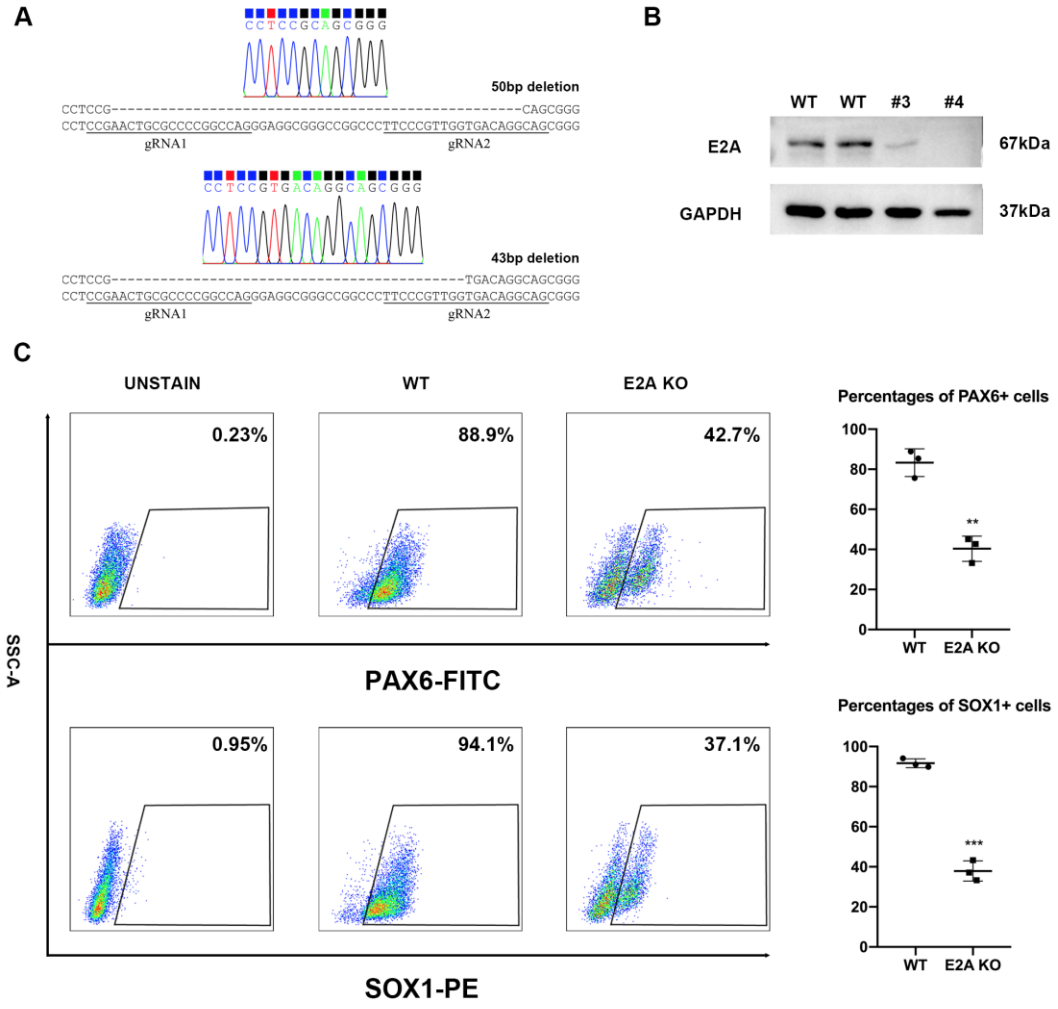

Fig. S5. A. Experimental results comparing WT and KO-4 E2A$^{-/-}$ H9 hESC line.

A. Sanger sequencing of genomic DNA from E2A KO clone (KO-4 H9) comparing the sequences to WT.

B. Western blot analysis of E2A protein expression in WT and E2A KO H9 hESCs for KO-4.

C. Flow-cytometric analysis and percentage of SOX1 and PAX6 expression on d6 neural differentiation of WT and E2A KO-4 H9 hESCs.

Images in (A), (B) and graphs in (C) are representative of three independent experiments. Error bars represent mean ± SD (n = 3 independent experiments). ** $p < 0.01$, *** $p < 0.005$ by Student's test.

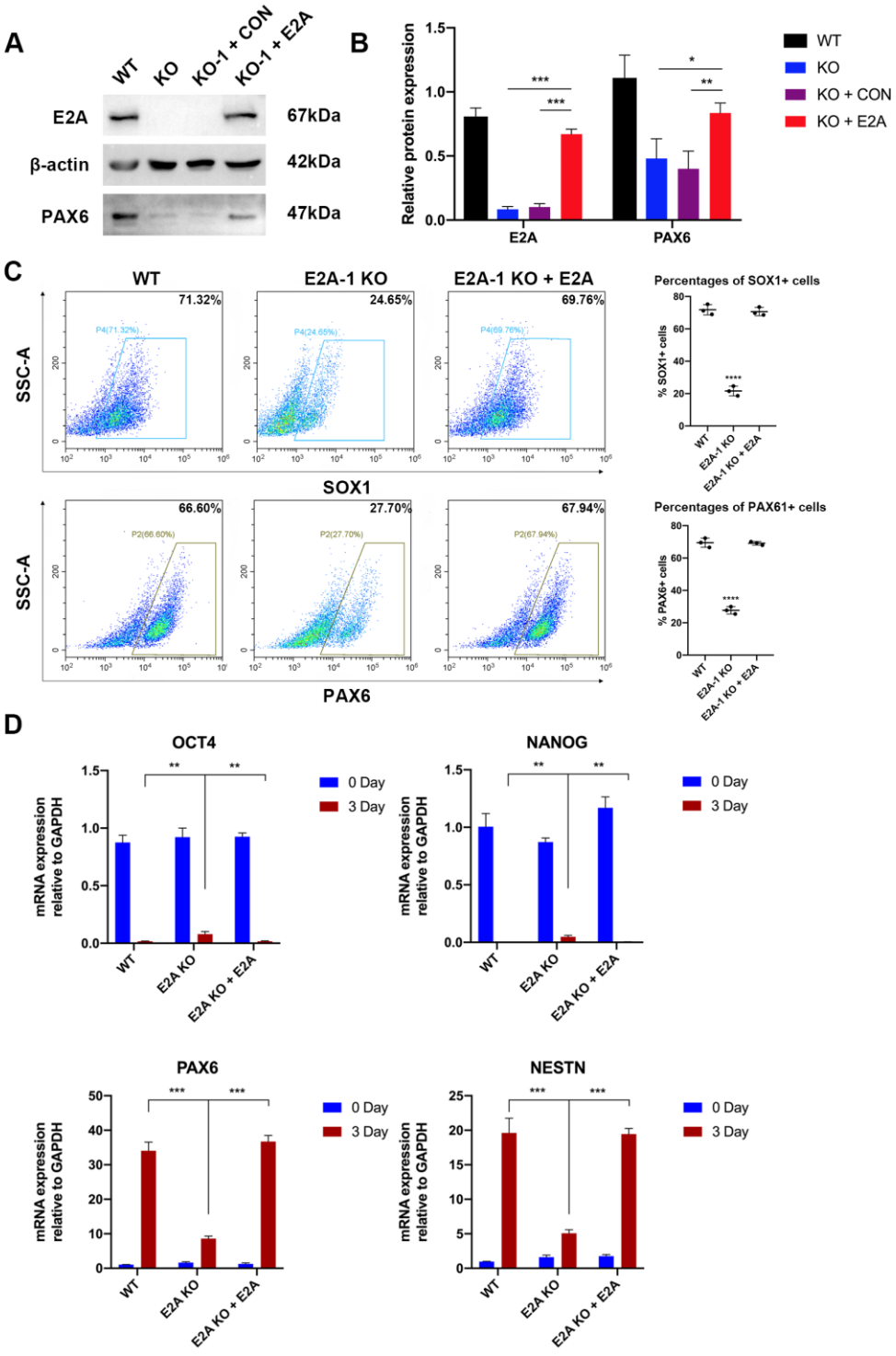

Fig. S6. Ectopic E2A rescues E2A KO hESCs neural differentiation defects.

A. Western blot analysis of E2A and PAX6 in wild type, E2A KO, E2A KO+hOE, E2A KO+ CON cells after 3 days' neural differentiation.

B. Quantitative analysis of western blot results in Fig. S6A.

C. Flow cytometric analysis the percentage of SOX1 and PAX6 positive rate in wild type, E2A KO, E2A KO+hOE cells after 3 days' neural differentiation. **** $p < 0.0001$.

D. qPCR analysis for expression of pluripotency and differentiation markers in undifferentiated hESCs (day 0) and d3 neural differentiation cells. ** $p < 0.01$, *** $p < 0.005$.

Images in (A), (B), plots in (C) and graphs in (C), (D) are representative of three independent experiments. Error bars represent mean ± SD (n = 3 biological replicates). ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$ by Student's test.

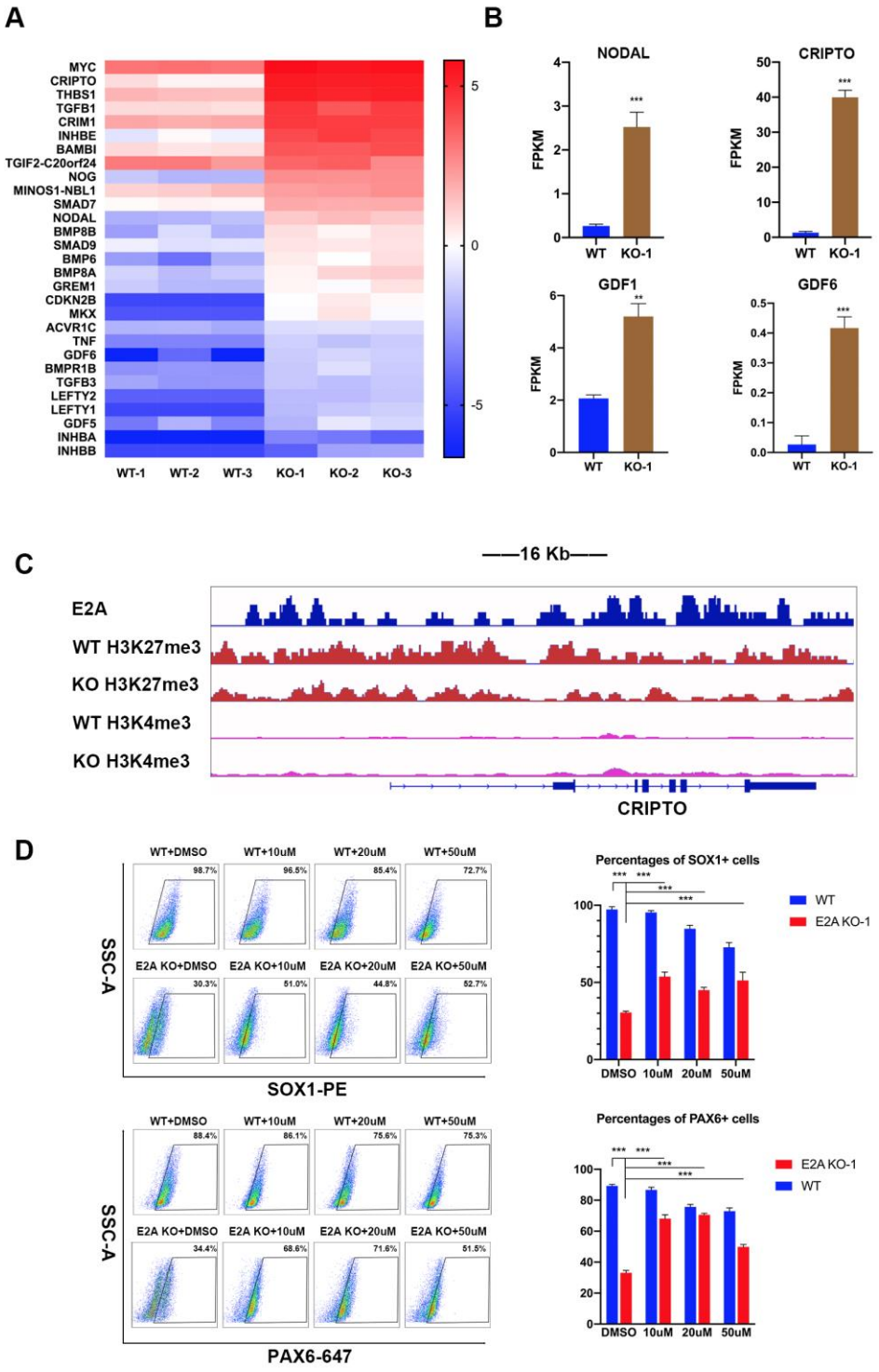

Fig. S7. SB431542 partially rescues E2A KO hESCs neural differentiation defects.

A. Heatmap of NODAL signalling related different expressing genes on d3 wild type neural differentiated cells.

B. Expression of NODAL signalling related genes after 3 days' neural differentiation, as determined by RNA-seq. FPKM, fragments per kilobase of exon per million fragments mapped.

C. The binding of E2A, H3K27me3 and H3K4me3 on NODAL signalling agonist CRIPTO.

D. Flow cytometric analysis and percentage of SOX1 and PAX6 positive expression in wild or E2A KO hESCs at day 3 after neural differentiation. The cells were treated with DMSO and SB431542 during neural differentiation.

Plots and graphs in (D) are representative of three independent experiments. Error bars represent mean ± SD (n = 3 biological replicates). * p <0.05, ** p < 0.01, *** p < 0.005 by Student's test.

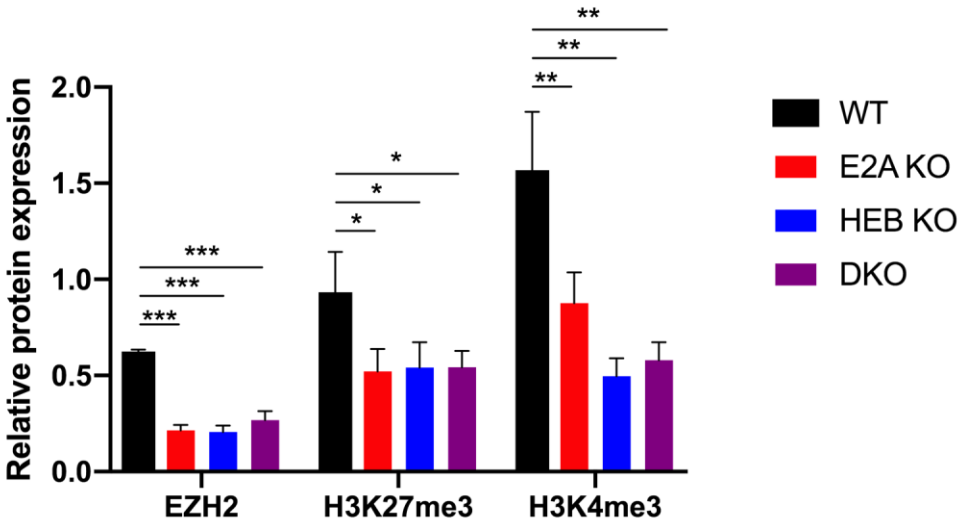

Fig. S8 Quantitative analysis of EZH2, H3K27me3 and H3K4me3 expression in Fig. 6B by image J software.

Graph in Fig. S8 are representative of three independent experiments. Error bars represent mean ± SD (n = 3 biological replicates). * p <0.05, ** p < 0.01, *** p < 0.005 by Student's test.

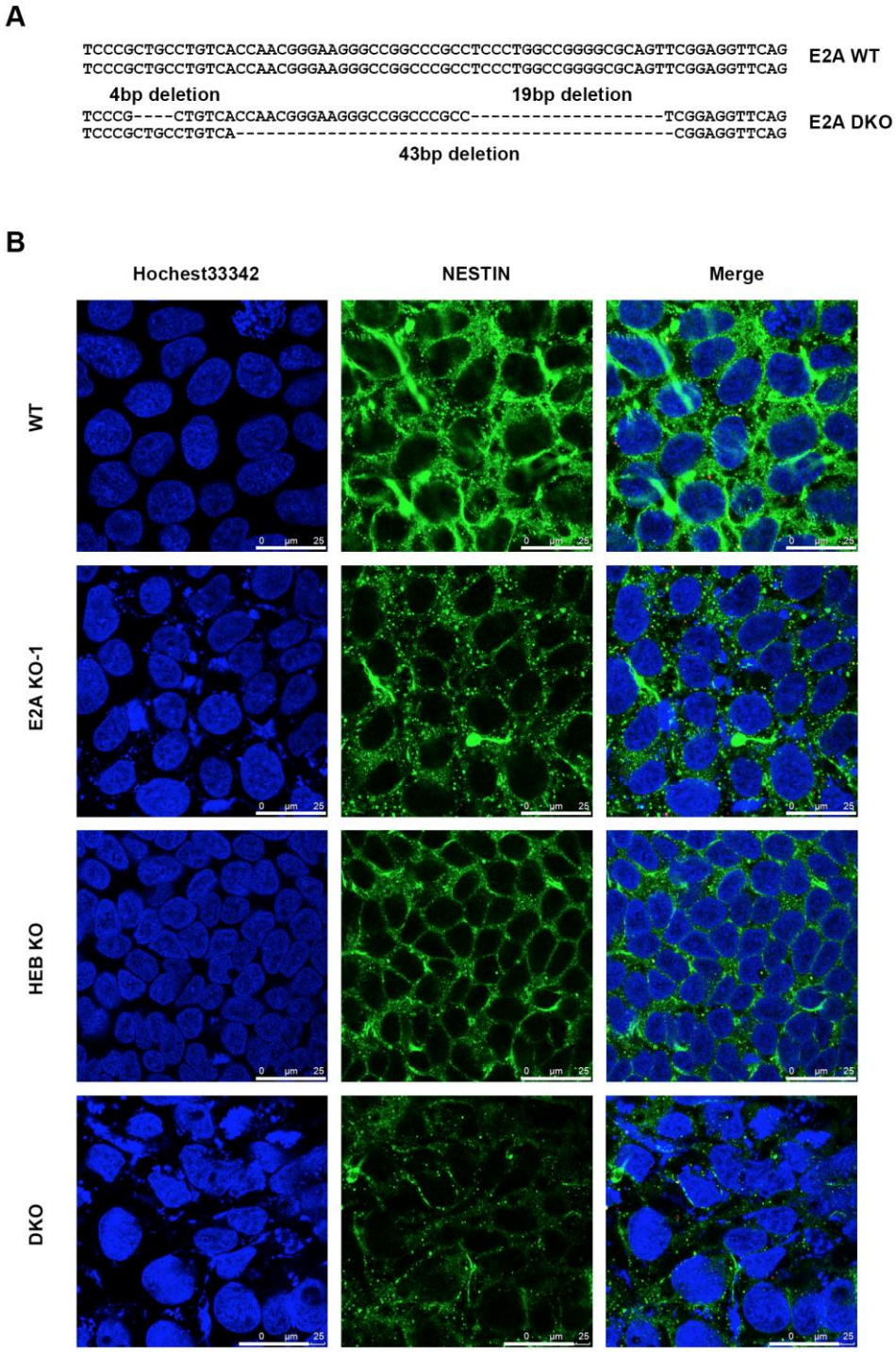

Fig. S9 E2A/HEB double knockout (DKO) hESCs display a more severe neural differentiation defects.

A. Sanger sequencing of genomic DNA from E2A/HEB double knockout cells on E2A alleles comparing the sequences to WT.

B. Immunofluorescence analysis of NESTIN in wild type, E2A KO, HEB KO, E2A KO/HEB KO double knockout (DKO) hESC after 3days' neural differentiation.

Images in (A), (B) are representative of three independent experiments. Scale bars in (D) 25um

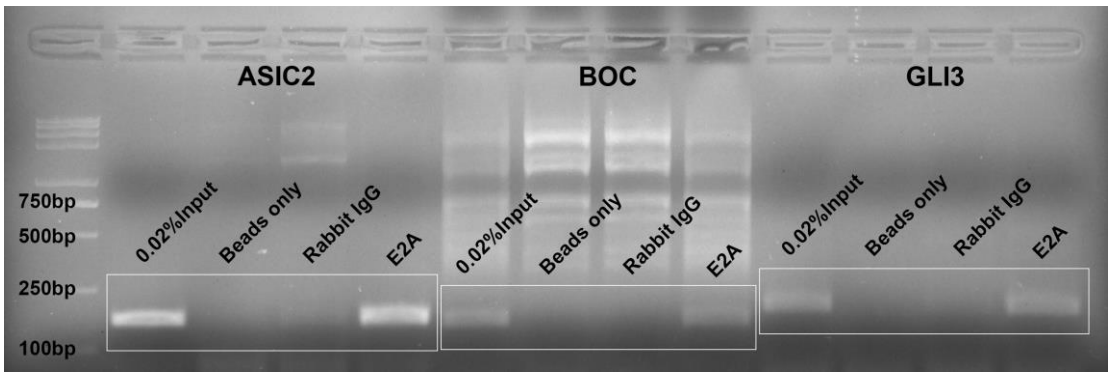

Fig. S10 The uncropped representative image of ChIP-PCR related to Fig. 5E

Images are representative of three independent experiments.

Table S1 Antibodies used in this study

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Table.S2 ChIP-PCR and qPCR primers used in this study

Gene	Primer forward	Primer reverse
E2A	GGCCCTGAGCCTCCGTTCTC	TCGAGGGCCG CCTCTCGCCG
T	TATGAGCCTCGAATCCACATAGT	CCTCGTTCTGATAAGCAGTCAC
MESP1	TCGAAGTGGTTCCTTGG	TGCTTGCCTCAAAGTGTC
OCT4	CTTGAATCCCGAATGGAAAGGG	GTGTATATCCCAGGGTGATCCTC
NANOG	ACAACTGGCCGAAGAATAGCA	GGTTCCCAGTCGGGTTCAC
ASIC2	AAGCCGAAGGATGTACAGAAGG	GCTGAGCCGCGCTAAA
BOC	ATCGTCACCAAAGGCCAGAG	TAGGTGCCTGAGTCCTCCTC
LEF1	TCCCGTGAAGAGCAGGCTAA	AGGCAGCTGTCATTCTTGGAC
LMX1A	GCTCAGATCCCTTCCGACAG	GAGGTGTCGTCGCTATCCAG
NRP2	TGCAGTGGACATCCCAGAAA	TTTCTTTGTCGGTCGAGGGG
GLI3	GTGAGCGAGAAAGCCGTTG	TCGTCACTCGATGTTGAAGGT
SOX2	CCGTTCATCGACGAGGCTAA	ATGTGCGCGTAACTGTCCAT
GATA4	GTGTCCCAGACGTTCTCAGTC	GGGAGACGCATAGCCTTGT
GATA6	CTGCGGGCTCTACAGCAAG	GTTGGCACAGGACAATCCAAG
SOX17	CCTTCACGTGTACTACGGCG	GTTCAAATTCCGTGCGGTCC
SOX1	CAACCAGGACCGGGTCAAAC	CCTCGGACATGACCTTCCACT
PAX6	CGAGACTGGCTCCATCAGAC	CTTTTCGCTAGCCAGGTTGC
NESTIN	AAGAGACTCAACAGCGACGG	TCTTGTCCCGCAGACTTCAG
GAPDH	TCTCCTCTGACTTCAACAGCGAC	CCCTGTTGCTGTAGCCAAATTC
ASIC2(ChIP-PCR)	ACCTGGTTTAAGCCGGACAA	CGTTGGATTTCTGGCTTCCT
BOC(ChIP-PCR)	CTCCTGGGGTTTTTGTTGATGT	CCCTCAGCATTCTCCCATCAC
GL3(ChIP-PCR)	CGTCCTGTCTGCTCCCATC	GGGGATGGCTTTGGGAAAATG

Table S3. All significant differences in gene expression between WT and E2A$^{-/-}$ hESCs as determinated by RNA-seq.

Click here to Download Table S3

Table S4. The E2A ChIP-Seq binding peaks in WT hESCs.

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Table S5. GO analysis of E2A ChIP-Seq in hESCs.

Click here to Download Table S5

Table S6. All significant differences in gene expression between WT and E2A$^{-/-}$ NPCs as determinated by RNA-seq.

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Table S7. The E2A ChIP-Seq binding peaks in WT NPCs.

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Table S8. GO and mSigDB analysis of E2A directly regulated genes.

Click here to Download Table S8

Table S9. H3K4me3 and H3K27me3 binding peaks in wild type and E2A^{KO} NPCs

Click here to Download Table S9

Table S10. H3K4me3 and H3K27me3 binding peaks in wild type and E2A^{KO} NPCs

Click here to Download Table S10