SUPPLEMENTAL DATA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Apoptosis assay–Ncoa3 knockdown or control ESCs and day 2 EB cells were stained with fluorescein isothiocyanate (FITC)-Annexin V and propidium iodide (PI) simultaneously, followed by data collection using a BD FACSCalibur and data analyzation with the Cellquest software (BD).

Table S1: Primers for gene expression analysis

Gene	Forward primer	Reverse primer
β-Actin	CAGAAGGAGATTACTGCTCTGGCT	TACTCCTGCTTGCTGATCCACATC
Ncoa3	GCCTGGCTTTGAAGACATAATCCG	TCTTGATAGTGACGCTTCTGGGAC
Nanog	TACAAGGGTCTGCTACTGAGATGC	TTGGGACTGGTAGAAGAATCAGGG
Oct4	ATCAGCTTGGGCTAGAGAAGGATG	AAAGGTGTCCCTGTAGCCTCATAC
Sox2	GCGGAGTGGAAACTTTTGTCC	CGGGAAGCGTGTACTTATCCTT
Klf4	AGCCACCCACACTTGTGACTAT	AGTGGTAAGGTTTCTCGCCTGT
Nestin	CTGGATCTGGAAGTCAACAGAGGT	ATCCTCAGTTTCCACTCCTGTAGC
Pax6	TAACGGAGAAGACTCGGATGAAGC	GGGCAAACACATCTGGATAATGGG
Gata4	GCTATGCATCTCCTGTCACTCAGA	CCAAGTCCGAGCAGGAATTTGAAG
Gata6	CTTCTCCTTCTACACAAGCGACCA	ATACTTGAGGTCACTGTTCTCGGG
Sox17	CCCAACACTCCTCCCAAAGTATCT	TCTCTGTCTTCCCTGTCTTGGTTG
Hand1	AAGGATGCACAAGCAGGTGAC	TTTAATCCTCTTCTCGCCGGG
Nkx2.5	ACTATGCCCTGTCCCTCAGATTTC	TCCTAGTGTGGAATCCGTCGAAAG
T	CATCGGAACAGCTCTCCAACCTAT	TACCATTGCTCACAGACCAGAGAC
Flk1	CAGGAAACTACACGGTCATCCTCA	AGGAATCCATAGGCGAGATCAAGG
Bmp4	ACAGCGGTCCAGGAAGAAGAAT	TGCACAATGGCATGGTTGGT
Cdx2	CAGTCCCTAGGAAGCCAAGTGAAA	AAGTGAAACTCCTTCTCCAGCTCC

Table S2: Primers for ChIP analysis

Gene	Forward primer	Reverse primer
Oct4	GCCCATTCAAGGGTTGAGTACTTG	GACAATGGCCTTGGCTGGACAAT
ActB	CGTGTGACAAAGCTAATGAGGCTG	CTAAGTTCAGTGTGCTGGGAGTCT
Nanog	GGATGTCTTTAGATCAGAGGATGCCC	CAGGGTCCACCATGGACATTGTAA
Sox2	GCGAGTGGTTAAACAGAGCTTTCC	CCTTATGGAAATGAAGGCGAACGG

Table S3: 19-nt targeting sequences for shRNAs

Gene	**Sequences**
shGFP	GAACGGCATCAAGGTGAAC
shN1-1	CCGACAACAGCGACAACAG
shN1-2	AAGAAACGATGAGAAGGCC
shN1-3	ACCAGCCCTTTCTCTCAAC
shN2-1	GCGAGCAGGAGAATAAGTA
shN2-2	AGTCCTCGCATCCCACCCA
shN2-3	CAGCGGCCAAATTACACCA
shN3-1	GTGTGTCAGTCAAACAGCA
shN3-2	GTGAGACCAAGGACCAACA
shN3-3	TCTGGCCAAGAGAAAGACC
shEs-1	GATTCGATGTACATTGAGA

shEs-2	GTGGATGCTCCTGCTAGAG
shNr5-1	GCAAGTGTCTCAATTTAAA
shNr5-2	GCAGAAGACTACCTGTACT

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. The expression levels of differential markers in shGFP control and stable Ncoa3 knockdown ESCs and EBs. RNA samples purified from shGFP control and stable Ncoa3 knockdown ESCs (day 0) and EBs (day 2) were subjected to quantitative RT-PCR. Averages and standard deviations from three independent experiments are plotted. *, $p<0.05$; **, $p<0.01$.

Figure S2. Cell death analysis of shGFP control and stable Ncoa3 knockdown ESCs and day 2 EBs. shGFP control and stable Ncoa3 knockdown ESCs and day 2 EBs were stained with Annexin V and PI, followed by flow-cytometry analysis. A representative plot was shown for each condition. The numbers in up-right and lower-right panels are the percentage of necrotic and apoptotic cells (averages and standard deviations from three independent experiments), respective.

Figure S3. WT and mutant Ncoa3 are expressed at a similar level. (A) Ncoa3 mRNA expression in V6.5 ESCs described in Figure 6A was measured by quantitative RT-PCR. Averages and standard deviations from three independent experiments are plotted. (B) Ncoa3 protein levels in V6.5 ESCs described in Figure 6A were detected by Western blot. The ΔN (133 kDa) and ΔC (156 kDa) mutants could not be separated from the endogenous WT Ncoa3 (the upper band marked with a triangle). The ΔAD (104 kDa) and ΔNRID (118 kDa) mutants moved faster in the gel (the lower band pointed by an arrow). A fast moving band was also detected in the cells expressing the ΔC mutant. This might be caused by protein degradation. Quantification results of Western blot were shown below corresponding bands as average ± standard deviation (n=3). For ΔAD, ΔC and ΔNRID, the expression value is the sum of two bands.

Figure S1

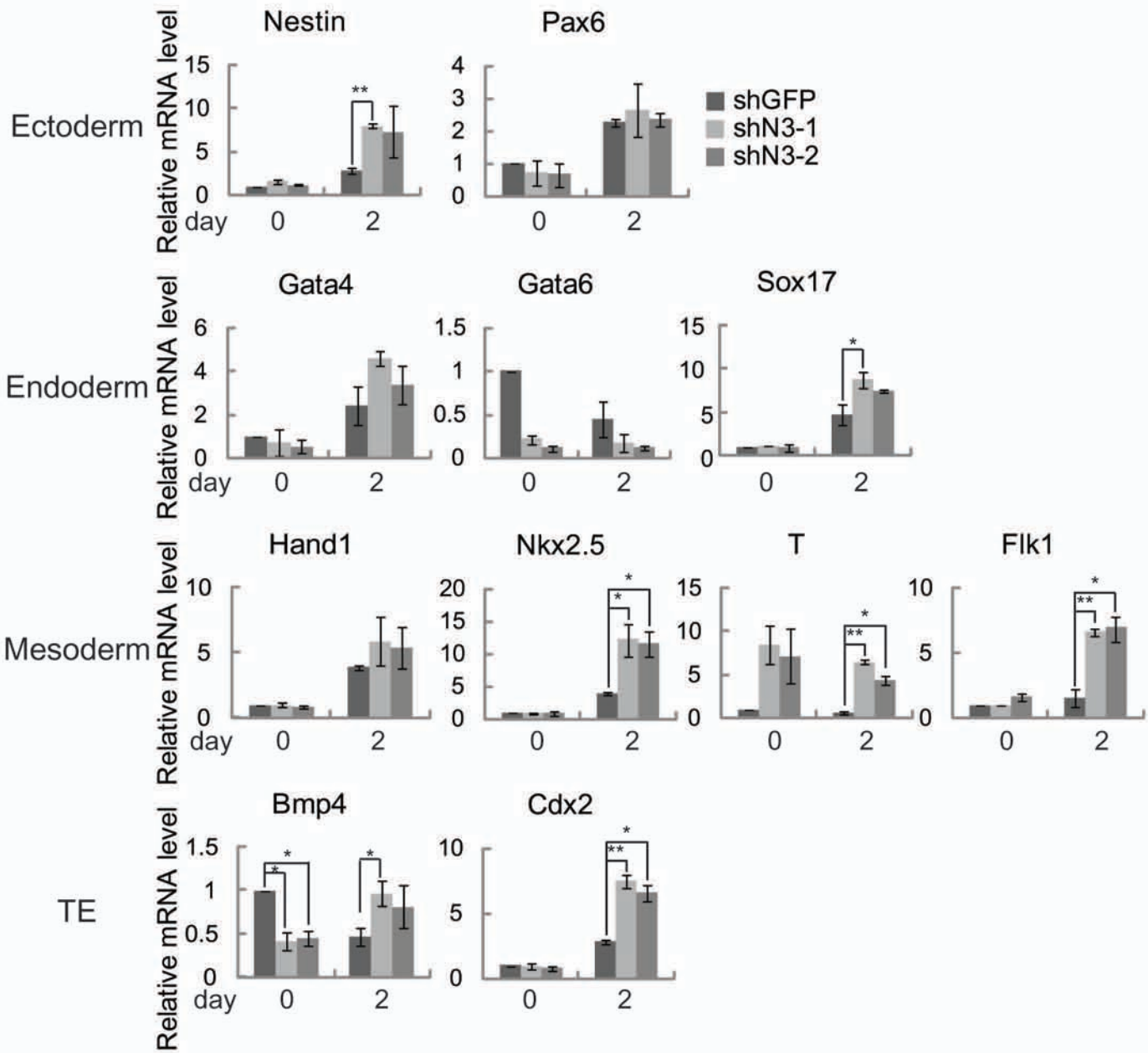

Ectoderm
Endoderm
Mesoderm
TE
Relative mRNA level
Nestin
Pax6
Gata4
Gata6
Sox17
Hand1
Nkx2.5
T
Flk1
Bmp4
Cdx2
shGFP
shN3-1
shN3-2
day
0
2

Figure S2

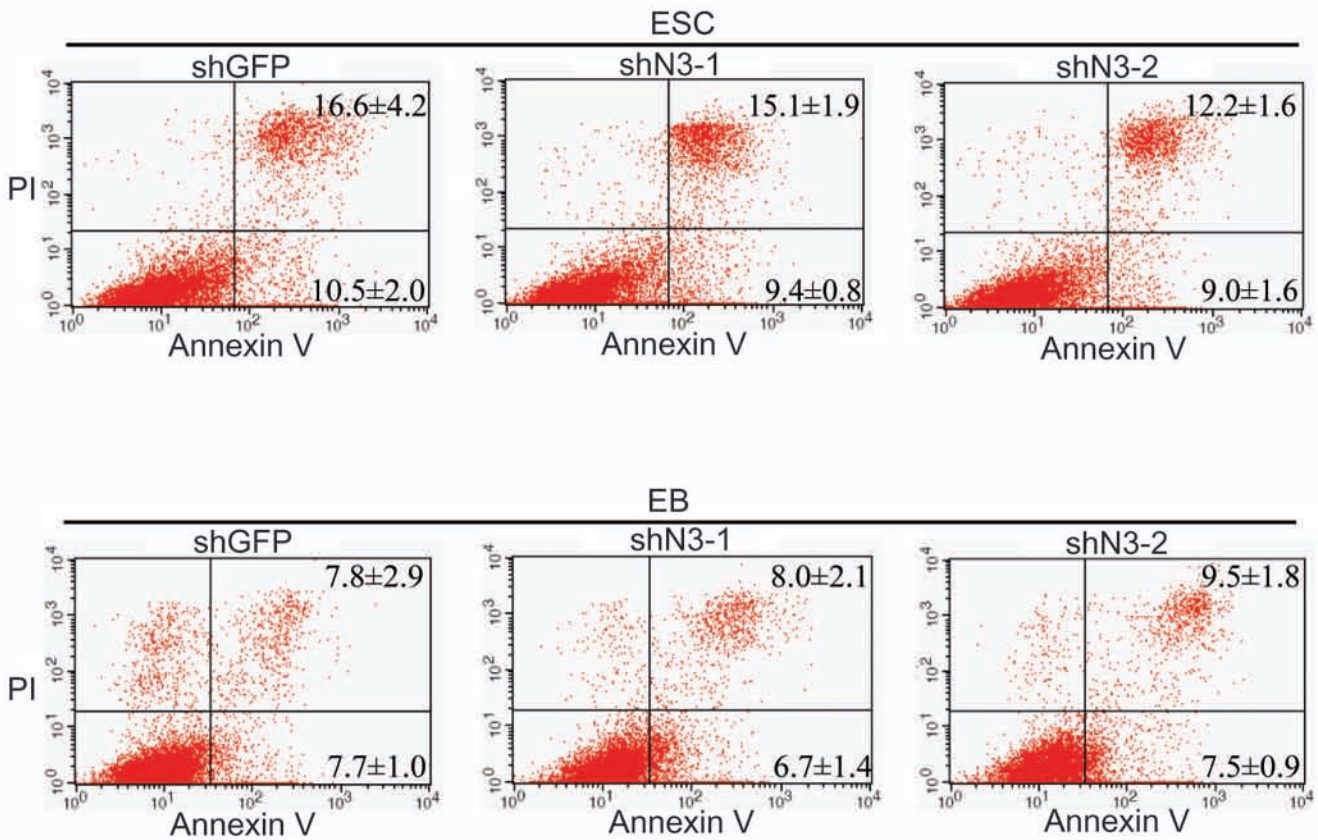
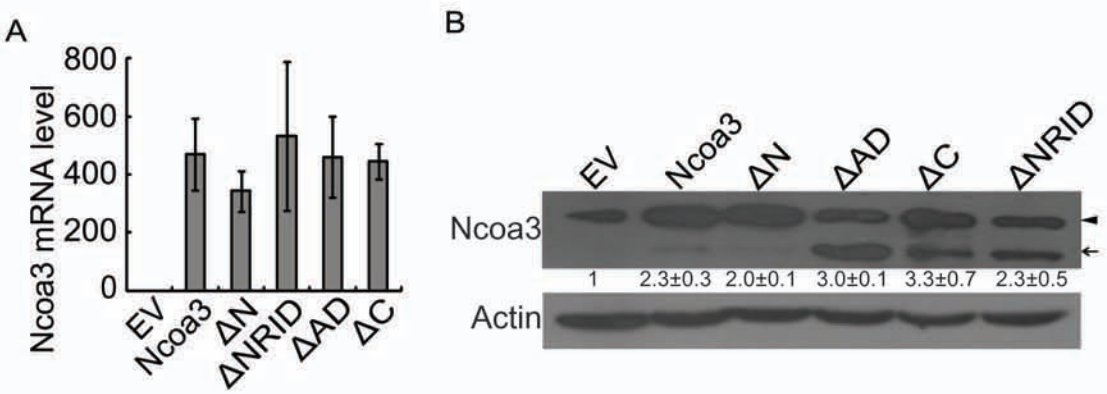

ESC
shGFP
shN3-1
shN3-2
16.6±4.2
10.5±2.0
15.1±1.9
9.4±0.8
12.2±1.6
9.0±1.6
PI
Annexin V
EB
shGFP
shN3-1
shN3-2
7.8±2.9
7.7±1.0
8.0±2.1
6.7±1.4
9.5±1.8
7.5±0.9
PI
Annexin V

Figure S3


A
Ncoa3 mRNA level
800
600
400
200
0
EV
Ncoa3
ΔN
ΔNRID
ΔAD
ΔC
B
EV
Ncoa3
ΔN
ΔAD
ΔC
ΔNRID
Ncoa3
1
2.3±0.3
2.0±0.1
3.0±0.1
3.3±0.7
2.3±0.5
Actin