

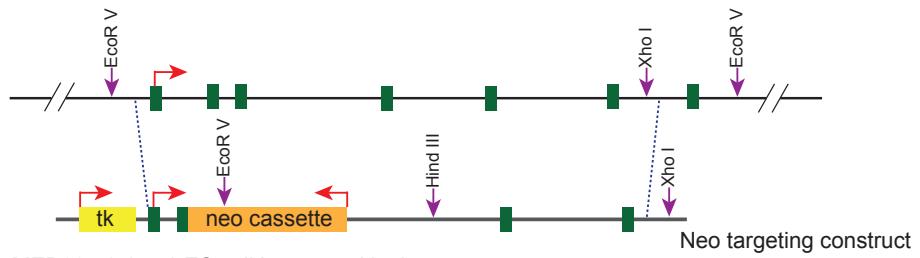
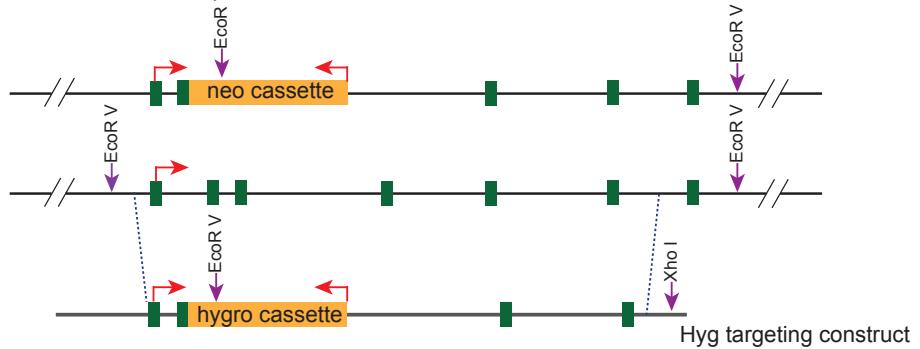
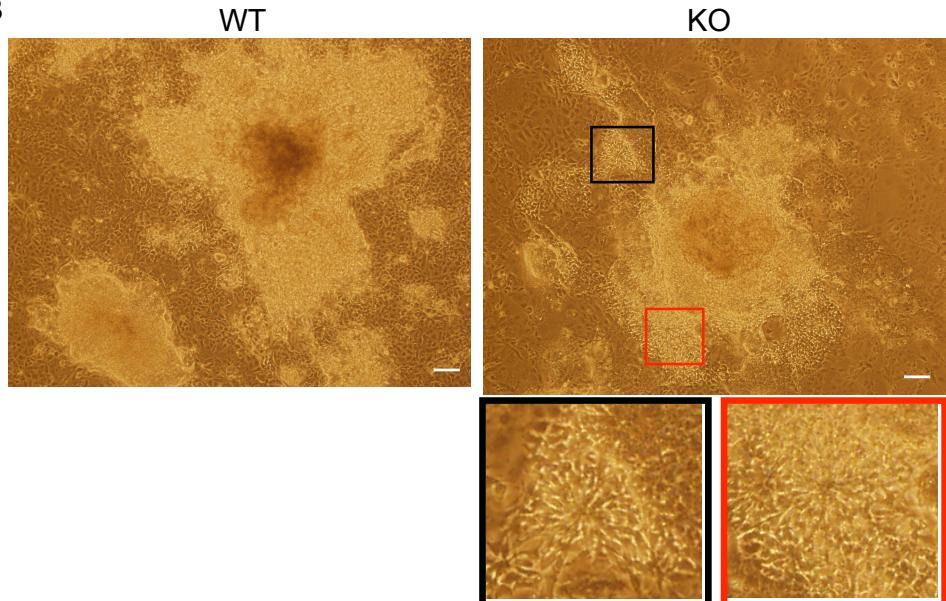
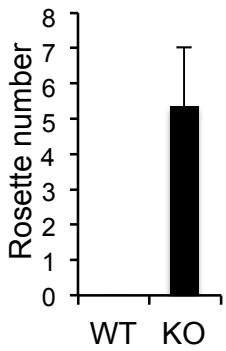
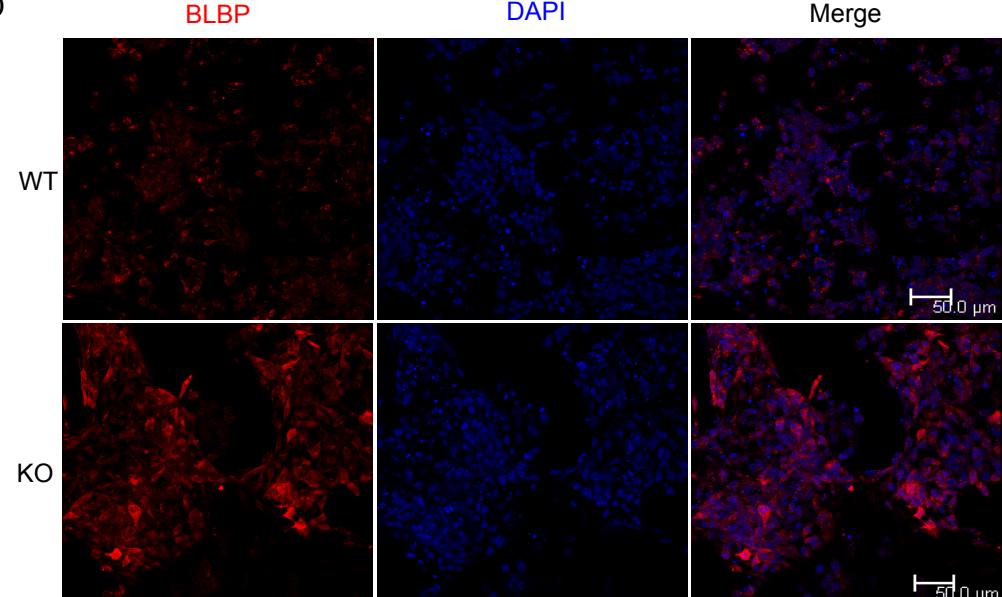
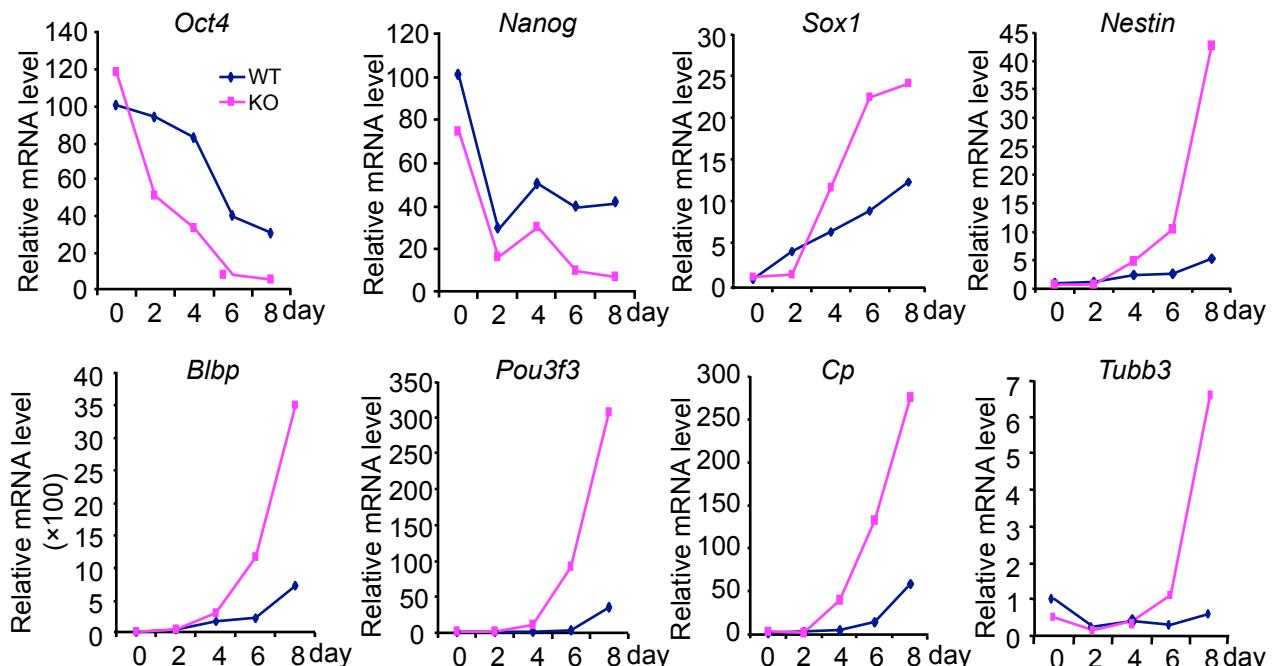
A Step 1: Wild-type MED23 locus is targeted by neo-cassette**Step 2: MED23 +/- (neo) ES cell is targeted by hyg-cassette****B****C****D**

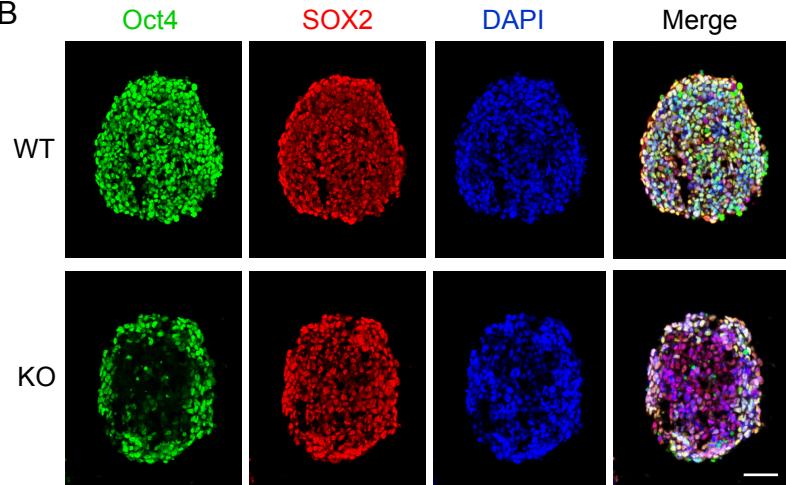
Figure S1. Deletion of *Med23* enhances neural differentiation

- (A) Scheme of *Med23* -/- ES cell generation.
- (B) Frequently neural rosettes (indicated by white dotted circle) appear in the spontaneous differentiation culture of the *Med23* KO EBs at day 9. Scale bar=50 μ m.
- (C) Rosette number from (A); for both of KO and WT, n>=10; “n” indicates random field imaging.
- (D) Immunostaining for BLBP (marker of mature neural precursors) in WT and KO cells at day 7 of monolayer culture. Scale bars=50 μ m.

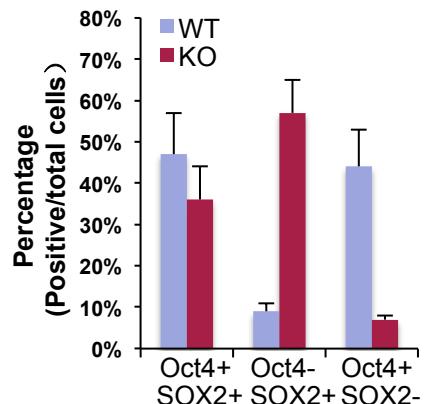
A



B



C

**Figure S2. Med23 deficiency facilitates neural differentiation in SFEB culture**

(A) ESCs were cultured in serum-free suspension conditions with KSR (SFEB).

Expression levels of marker genes for ESC self-renewal and neural precursor cells were measured by quantitative real-time PCR. *Gapdh* mRNA expression was used for normalization.

(B) Immunostaining for Oct4 and SOX2 in WT and KO cells at day 6 of SFEB culture.

Scale bars=50 μ m.

(C) Percentage of cell types from (B), Oct4+/SOX2+, Oct4-/SOX2- and Oct4+/SOX2+; for WT, n=7 sections from 7 different EBs; for KO, n=6 sections from 6 different EBs; "n" indicates sample number.

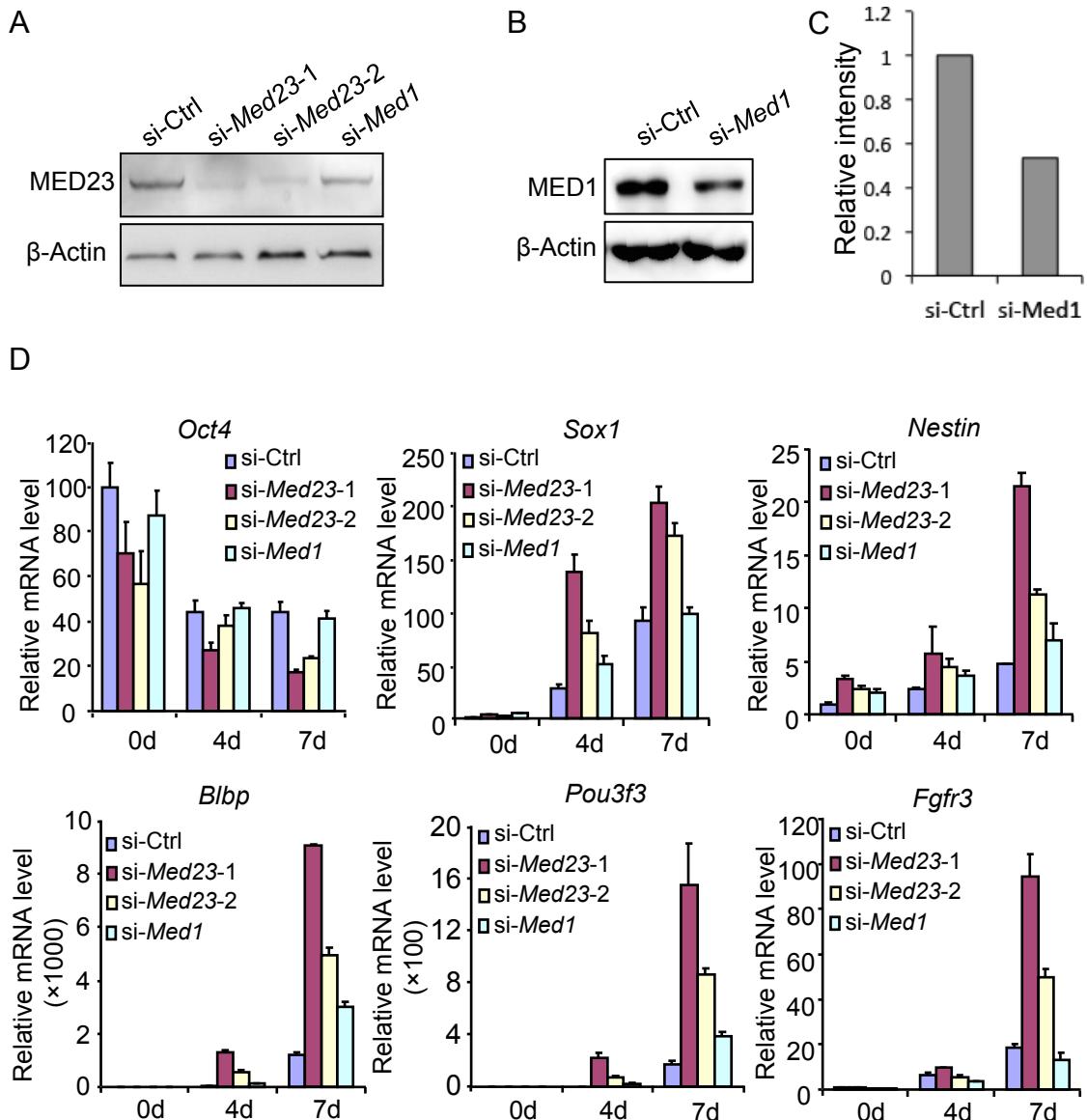
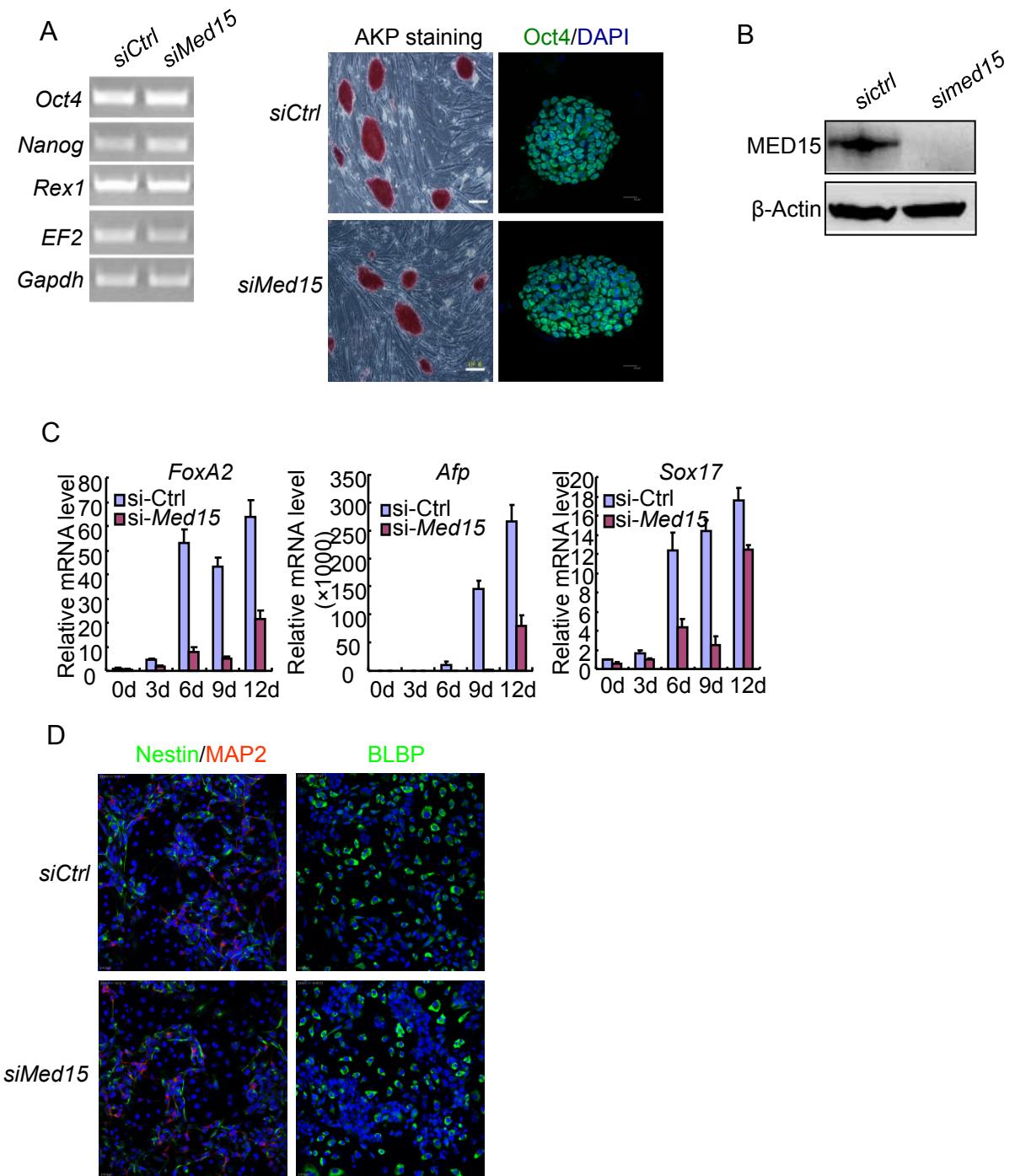
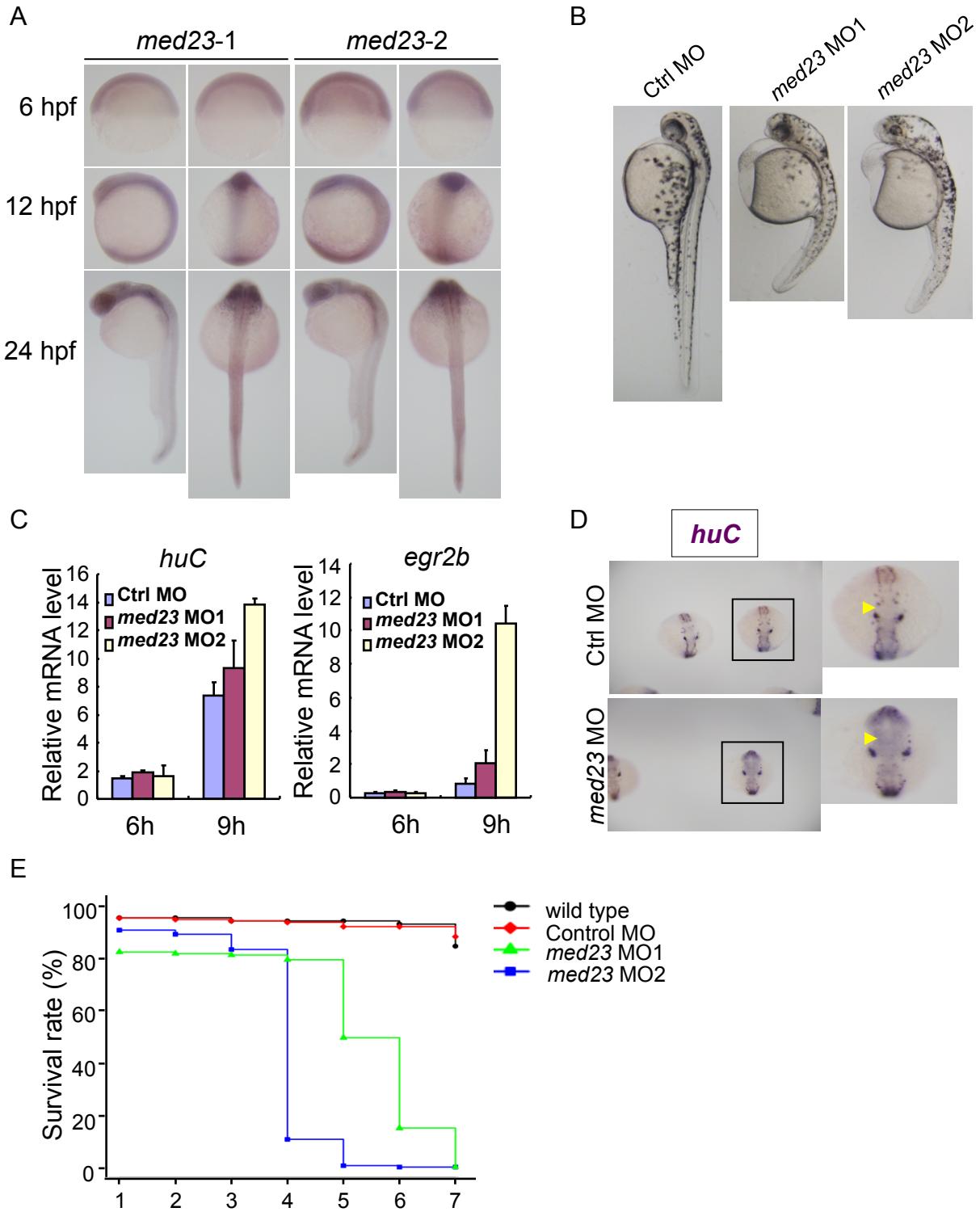


Figure S3. Knockdown of *Med23*, but not *Med1*, promotes neural differentiation in R1 ESCs

- (A) Immunoblot of *Med23* K/D and *Med1* K/D R1 ES cells.
- (B) *Med1* expression levels in *Med23* K/D and *Med1* K/D R1 ESCs.
- (C) *Med1* mRNA levels in *Med23* K/D and *Med1* K/D R1 ESCs by Real-time PCR
- (D) R1 ESCs were cultured under monolayer differentiation condition with N2B27 medium. Marker genes expression of ES self-renewal and neural precursor were measured by real-time PCR at indicated time points. *Gapdh* mRNA was used as internal control.

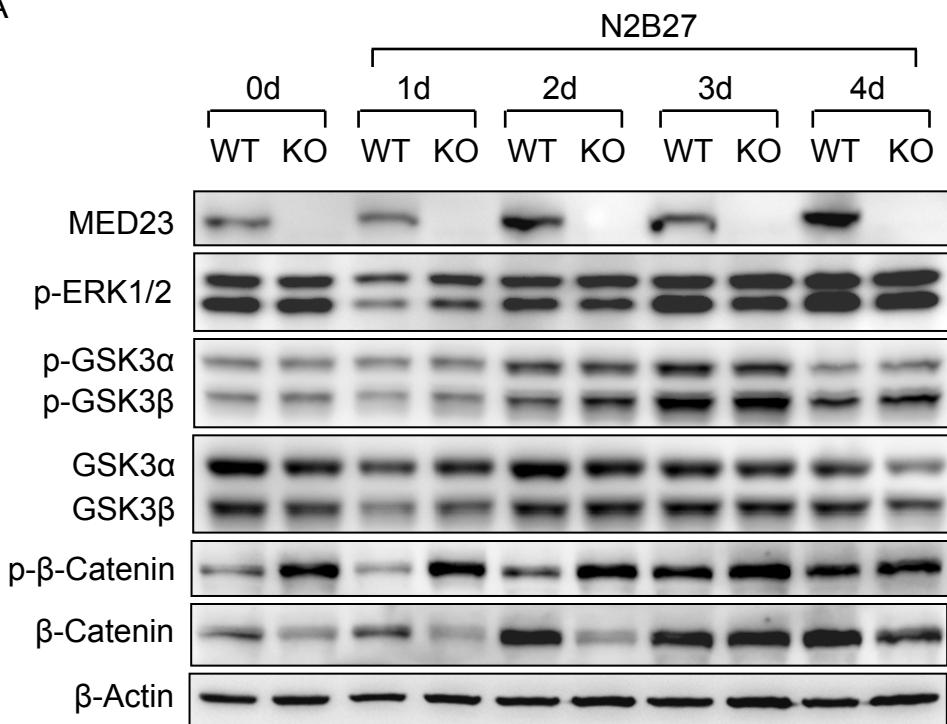
**Figure S4. Knockdown of *Med15* impairs endoderm differentiation**

- (A) Characterization of *Med15* K/D ESCs by RT-PCR, alkaline phosphatase activity test and immunochemistry.
- (B) Immunoblot of *Med15* K/D ES cells.
- (C) Endoderm gene expression of *Med15* K/D ESCs during spontaneous differentiation.
- (D) Immunostaining of Nestin, MAP2 and BLBP in control group and *Med15* K/D cells by monolayer culture.

**Figure S5. Characterization of *med23* MO embryos.**

- (A) Expression of *med23* in zebrafish embryos.
- (B) Phenotypes of *med23* MO embryos at 48 hpf.
- (C) qRT-PCR to measure the mRNA levels of *huC* and *egr2b* at 6 hpf and 9 hpf.
- (D) *In situ* hybridization of *huC* mRNA levels in control MO zebrafish and *med23* morphant at 12 hpf.
- (E) Survival rates of *med23* MO embryos.

A



B

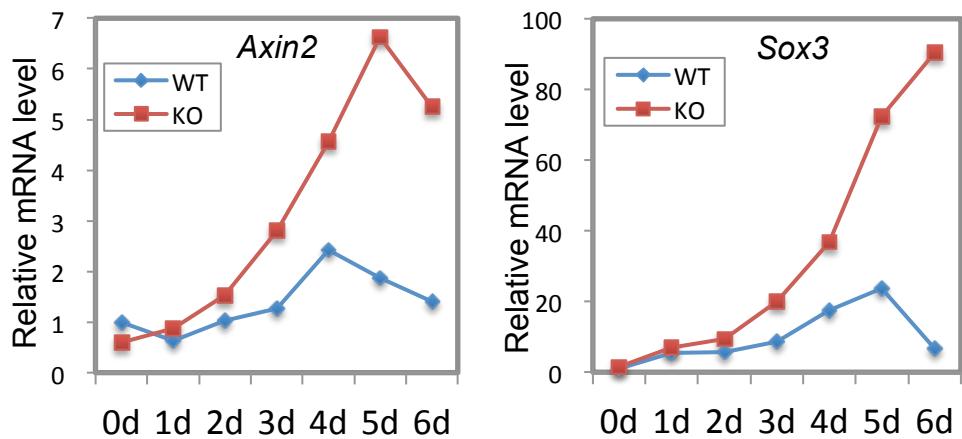


Figure S6. Characterization of signaling pathways during neural differentiation

- (A) Western blots to analyze the protein levels of MED23, ERK1/2, GSK3 and β -Catenin in wild type and *Med23* KO cells.
- (B) qRT-PCR to measure the mRNA levels of *Axin2* and *Sox3* in wild type and *Med23* KO cells.

Table S1. Primers used in real-time PCR, RNAi, ChIP and cloning assays

Primer sequences used for real-time PCR analysis:	
Oct4-F	5'- GATCACTCACATGCCAATCA -3'
Oct4-R	5'- CTGTAGCCTCATACTCTTCGTT -3'
Nanog-F	5'- CAGCCCTGATTCTTCTACCA -3'
Nanog-R	5'- GACCTTGTTCTCCTCCTCCT -3'
Sox2-F	5'- CACAACCTGGAGATCAGCAA -3'
Sox2-R	5'- CTCCGGGAAGCGTGTACTTA -3'
Sox1-F	5'- GCCGAGTGGAAAGGTATGTC -3'
Sox1-R	5'- TGTAATCCGGGTGTTCTTCAT -3'
Blbp-F	5'- GGGTAAGACCCGAGTTCCCTC -3'
Blbp-R	5'- ATCACCACTTGCCACCTTC -3'
Nestin-F	5'- CTGCTCTGCCTCTAGCACAC -3'
Nestin-R	5'- TTAAGGGTAGCACATGTCTG -3'
Pou3f3-F	5'- CACCAGCATTGACAAGATCG -3'
Pou3f3-R	5'- CGTCATGCCTTTCCCTTT -3'
Cp-F	5'- GCCTTGGCAAGAGATAAGCA -3'
Cp-R	5'- CAGGGCCTAAAAACCCCTAGC -3'
Fgfr2-F	5'- CACCAACTGCACCAATGAAC -3'
Fgfr2-R	5'- GGCTGGGTGAGATCCAAGTA -3'
Fgfr3-F	5'- CATCCGGCAGACATACACAC -3'
Fgfr3-R	5'- TTCACTTCCACGTGCTTCAG -3'
Tubb3-F	5'- GCGCTTTGGACACCTATTCA -3'
Tubb3-R	5'- TTCCGCACGACATCTAGGACTG -3'
Sma-F	5'- CTGCTCTGCCTCTAGCACAC -3'
Sma-R	5'- TTAAGGGTAGCACATGTCTG -3'
Gsc-F	5'- GCAAGGACTTGCACAGACAG -3'
Gsc-R	5'- CAGCTAGCTCCTCGTTGCTT -3'
Flk1-F	5'- CAGCTCCAAGTGGCTAAGG -3'
Flk1-R	5'- CAGAGAACACACCGAAAGA -3'
Foxa2-F	5'- GCCCAGTCACGAACAAAGC -3'
Foxa2-R	5'- CCCAAAGTCTCCACTCAGCCTC -3'
Afp-F	5'- TGCTGCAAATTACCCATGAT -3'
Afp-R	5'- AAGGTTGGGTGAGTTCTTG -3'
Sox17-F	5'- AAGAAACCTAAACACAAACAGCG -3'
Sox17-R	5'- TTTGTGGGAAGTGGGATCAAGAC -3'
Axin2-F	5'- GCAGGAGCCTCACCCCTTC -3'
Axin2-R	5'- TGCCAGTTCTTGCGCTTT -3'
Sox3-F	5'- CGCTGGCTCTGACCACT -3'
Sox3-R	5'- GCAAACACCCACAGCGATT -3'
Med1-F	5'- CCTTCTTCTCCGCAGTCAC -3'
Med1-R	5'- GGAAGAGCAGCGTAAATCG -3'

BMP4-F	5'-TGATACCTGAGACCAGGGAAAG-3'
BMP4-R	5'-AGCCGGTAAAGATCCCTCAT-3'
Id1-F	5'-TTTGTGGCCAGTGGTTAAATATG-3'
Id1-R	5'-AAGGTGGATGGCTTTATGGAATT-3'
Id2-F	5'-CCGCTGACCACCCCTGAAC-3'
Id2-R	5'-ACATAAGCTCAGAAGGGAAATTCAGAT-3'
EF2-F	5'-AAAAGTATGAGTGGGACGTTGC-3'
EF2-R	5'-CCTTGATCTCATTCAAGGTACTGC-3'
Gapdh-F	5'-GACTTCAACAGCAACTCCCAC-3'
Gapdh-R	5'-TCCACCACCCCTGTTGCTGTA-3'
Bmp2a-F for zebrafish	5'-GCGCTAATGGTTCTGATGGT-3'
Bmp2a-R for zebrafish	5'-TGGGTCTGCTAACCTGCTCT-3'
Bmp2b-F for zebrafish	5'-CCAGCAGAGCAAACACGATA-3'
Bmp2b-R for zebrafish	5'-AGCCACTCGTACTGGCATCT-3'
Bmp4-F for zebrafish	5'-GAGGCACAACACCTCCAAAT-3'
Bmp4-R for zebrafish	5'-CGTAGCTGGTCCCACCTCTTC-3'
Bmp6-F for zebrafish	5'-GAGAATTGAGCTGGCAGGAC-3'
Bmp6-R for zebrafish	5'-TTGGGACGTTCTCTGGATTG-3'
Bmp7a-F for zebrafish	5'-GAAGGGAGCTGTACCTGCTG-3'
Bmp7a-R for zebrafish	5'-CATTCTGCACCATGTGAGG-3'
Egr2b-F for zebrafish	5'-TCCTCGTCCAAGGAAATAC-3'
Egr2b-R for zebrafish	5'-TCCCTCATGCATATCCGACA-3'
Anf-F for zebrafish	5'-ACAGCCCGTCTGTGTTACC-3'
Anf-R for zebrafish	5'-CTCTCACATCCACTGGAGCA-3'
Flh-F for zebrafish	5'-GCGGAGATGAGAGAACGAAAC-3'
Flh-R for zebrafish	5'-TCGCCAGTACTGCATCTTG-3'
HuC(Elavl3)-F for zebrafish	5'-CGGTCAGAACCCACTAGGAG-3'
HuC(Elavl3)-R for zebrafish	5'-TCAATGGTTATGGGGGAGAA-3'
Hoxb1a-F for zebrafish	5'-AGGCTGGATACCAACCACCTG-3'
Hoxb1a-R for zebrafish	5'-CAGGCCTGTGCCCCATAACT-3'
Val-F for zebrafish	5'-CAGAAGAACCAACCTGGAGGA-3'
Val-R for zebrafish	5'-TGTATCCATCAAACGCTCCA-3'
Six3a-F for zebrafish	5'-AGCATGAATCCATCCTACGC-3'
Six3a-R for zebrafish	5'-TTCTCGGCCTCCTGATAGTG-3'
Cyp26b1-F for zebrafish	5'-CAGGAGCTCAAGGAGTCCAC-3'
Cyp26b1-R for zebrafish	5'-CAGACAGCCGTATGAAGAA-3'
Otx2-F for zebrafish	5'-CGACCTGCCAAAAAGAAGAG-3'
Otx2-R for zebrafish	5'-TCATGGGGTAGGATCTTG-3'
Rbl13a-F for zebrafish	5'-TCTGGAGGACTGTAAGAGGTATGC-3'
Rbl13a-R for zebrafish	5'-AGACGCACAATCTTGAGAGCAG-3'
Primer sequences for luciferase assay of Bmp4 promoter	
FL(full length) -F	5'-AACTCGAGTGTAAATTGGCTGTGTTAGA-3'
FL(full length) -R	5'-AAAAGCTTAATCGCCTCCCTCTGGGGAT-3'

FL-A -F	5' - AACTCGAGTTGAGAGCTACCTGAAGCTC-3'
FL-A -R	5' - AAAAGCTTAATGCCTCCCTCTGGGAT -3'
FL-B -F	5' - AACTCGAGTGTAAATTGGCTGTGTTAGA -3'
FL-B -R	5' - AAAAGCTTGCTCACCTGGGACCACGTG-3'
FL-AB -F	5' - AACTCGAGTTGAGAGCTACCTGAAGCTC -3'
FL-AB -R	5' - AAAAGCTTGCTCACCTGGGACCACGTG-3'
Sequence of oligonucleotides	
si-Med23-1	5' - TGAAGCCCAGGTTGTTAT -3'
si-Med23-2	5' - GAGATAAGTAAGTTACATG -3'
si-Med1	5' - AGTAACATCTTGCCAGCA -3'
si-Med15	5' - GATCAACAAAGATCGACAAG -3'
si-Ctrl	5' - GTGCGCTGCTGGTGCCAAC -3'
Primer sequences for ChIP assay	
Bmp4 (A)-F	5' - CCAAGAACCGGAAATTGCTA -3'
Bmp4 (A)-R	5' - TACAAACGAGGATGGGAAG -3'
Bmp4 (B)-F	5' - CTGCTCACAGCCTGTTCAA -3'
Bmp4 (B)-R	5' - TGGGCTCCCTGAGTTAGA -3'
Bmp4 (C)-F	5' - CGATTAAGGGAGGAGGGAAAG -3'
Bmp4 (C)-R	5' - TCTTAGGCTGGGTCTCTCA -3'
Sequence of Morpholinos	
med23 MO-1	5' CATCCTCAGGGCTGTCCATGAACAT 3'
med23 MO-2	5' AAACGCCTCCTCGATCACCTCCGTT 3'
Control MO	5' CCTCTTACCTCAGTTACAATTATA 3'
Primer sequences for the PCR-cloned WISH probes	
HuC-F	5' - AAGGATCCATGGTTACTATAATTAGCAC -3'
HuC-R	5' - AAAAGCTTCAAGCCTGTGCTGCTTGC -3'
Egr2b-F	5' - AAGAATTCATGACAGCTAAACTTGGAG -3'
Egr2b-R	5' - AAAAGCTTCACTGGTTGAACGGACG -3'
Primer sequences for the PCR-cloned cDNA	
Bmp4-F for zebrafish	5' - AAAAGCTTATGATTCCCTGGTAATCGAATG -3'
Bmp4-R for zebrafish	5' - AAGAATTCTAGCGGCAGCCACACCCCTC -3'

*F: forward primer, R: reverse primer