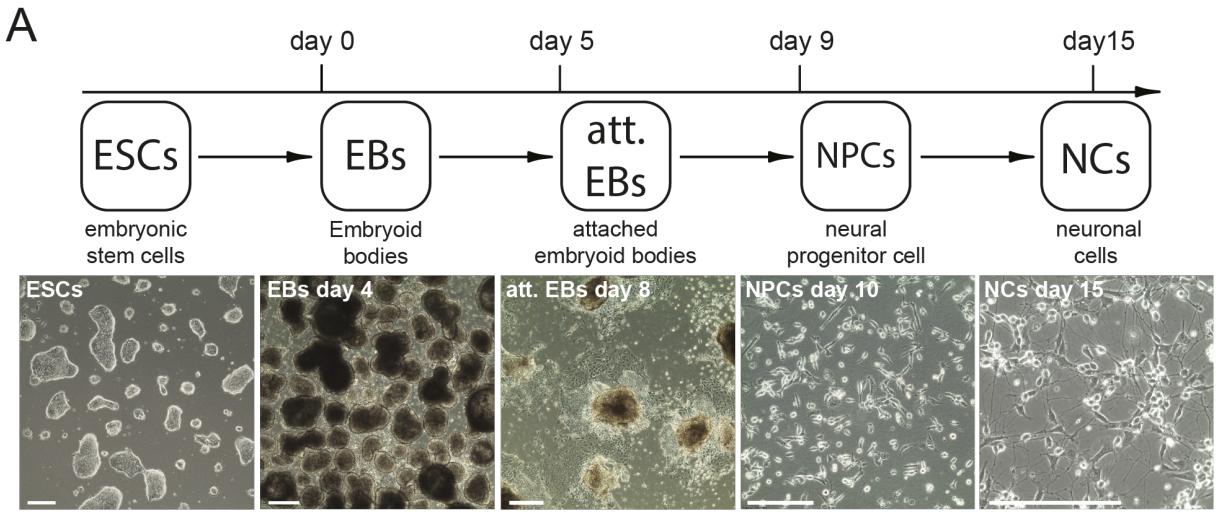

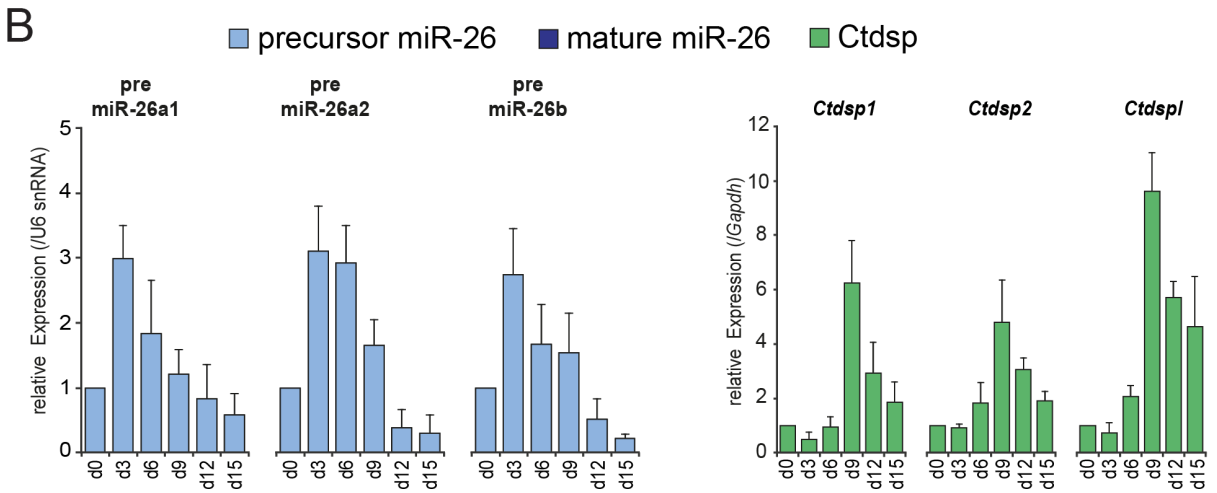

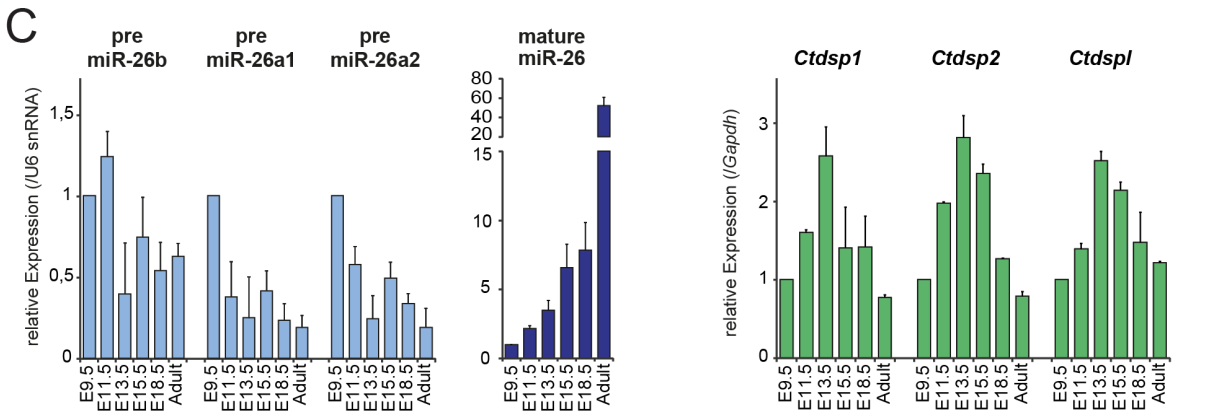

Figure S1. Expression of miR-26 family members and Ctdsp host genes in differentiating ESCs and during mouse development.

(**A**) Schematic representation of the differentiation protocol. Scale bars: 250µm (**B**) Expression pattern of pre-miR-26a1, -26a2, -26b and *Ctdsp1*, *Ctdsp2* and *CtdspL* during WT ESC differentiation. n=4 biological replicates, mean ± SD. (**C**) qRT-PCR analyses of precursor and mature miR-26 and *Ctdsp1/2/L* in the brain of mice during embryonal development and in adult brains, n = 4 biological replicates, mean ± SD.

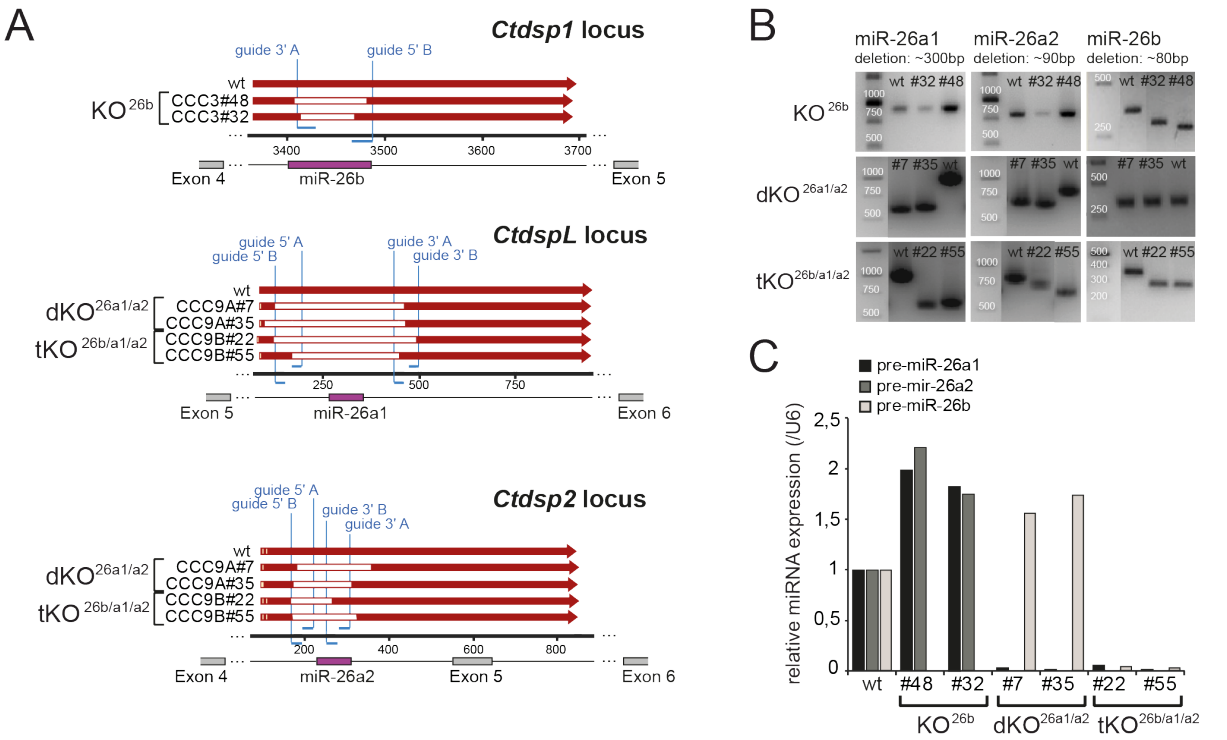

Figure S2. Generation and validation of miR-26 KO ES cell lines. (**A**) Shown are the *Ctdsp* loci, the location of miR-26 family members and the guide RNAs for Cas9-nickase deletion. WT sequences are represented by a red solid arrow, deletions by open areas. Individual ESC clone names are indicated. (**B**) miR- 26b-, miR-26a1- and miR-26a2-specific PCR analysis on genomic DNAs of WT and of miR-26 KO ESC clones. Predicted sizes of the deletions are indicated. (**C**) qRT-PCR analysis specific for pre-miR-26a1, pre-miR-26a2 and pre-miR-26b using RNA samples of WT and of KO ESCs clones. (**D**) Screen shots of *Ctdsp1*, *CtdspL* and *Ctdsp2* loci and detected reads using RNA-Seq data of WT and miR-26 KO ESC clones.

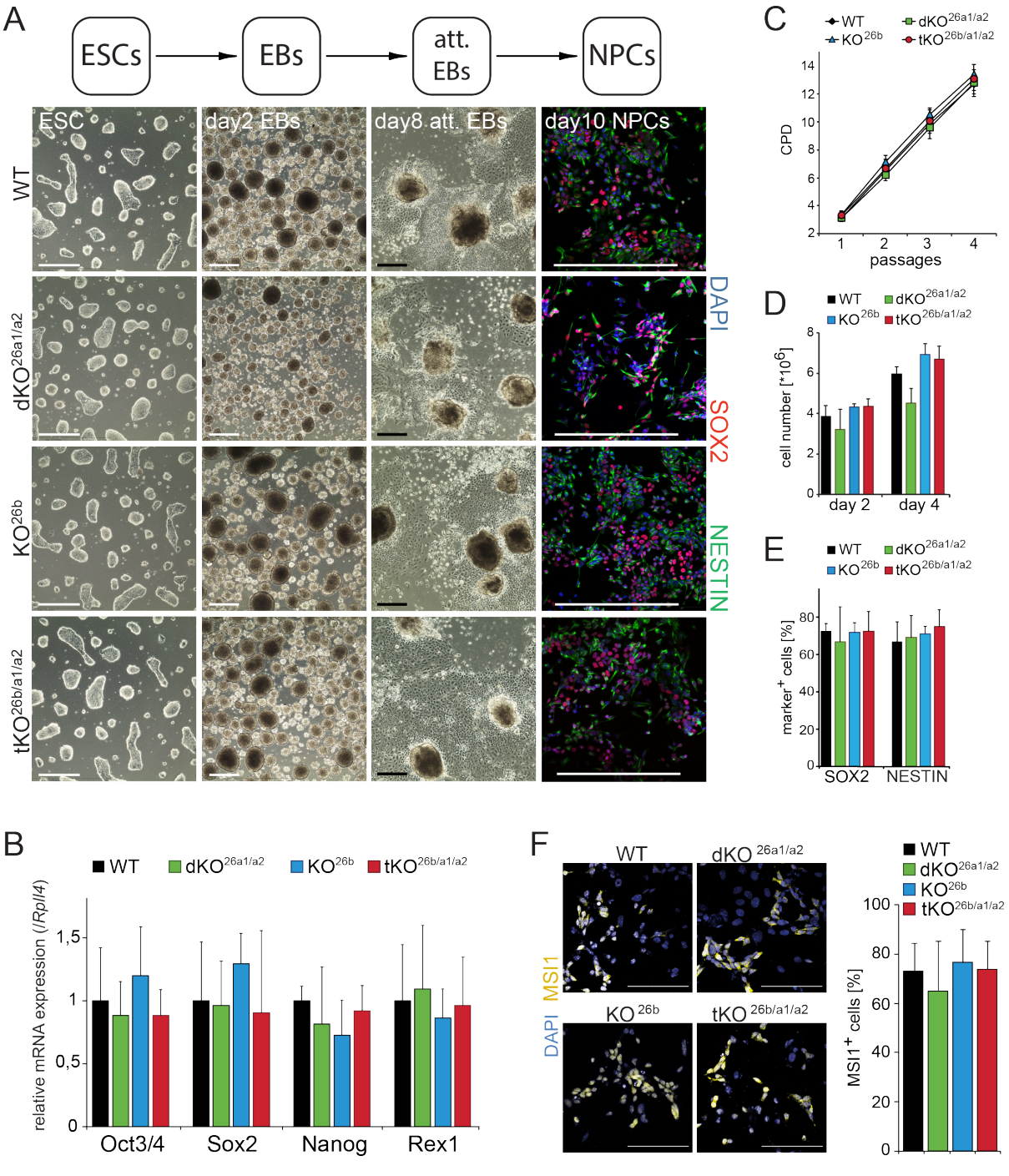

Figure S3. KO of miR-26 family members leaves differentiation of ESCs to neural progenitor cells (NPCs) unaffected. (**A**) Scheme of individual stages of *in vitro* differentiation from ESCs to NPCs (top). Phase contrast panels show representative morphological changes of WT and miR-26 KO cultures during neural differentiation from undifferentiated ESCs via embryoid bodies (EBs) to attached EBs (att. EBs). Also shown are SOX2- and NESTIN-specific immunostainings of WT, KO^{26b}, $dKO^{26a1/a2}$ and $tKO^{26b/a1/a2}$ NPC cultures (day 10, right column). Scale bars: 250µm. (**B**) qRT-PCR analysis specific for *Oct3/4*, *Sox2*, *Nanog* and *Rex1* in WT and miR-26 KO ESCs, n=3 biological replicates, mean ± SD. (**C**) Quantification of cumulative population doublings (CPD) of WT and miR-26 KO cultures ESCs throughout 4 passages (ESCs re-plated every 2nd day), n = 3 biological replicates, mean ± SD. (**D**) Single cell suspensions of WT and miR-26 KO EBs on day 2 and 4 were prepared and average cell numbers were determined (1×10^6 ESCs plated at day 0), n = 3 biological replicates, mean ± SD. (**E**) Quantification of immunostainings shown in panel A (right column). Frequencies of $SOX2^+$ and $NESTIN^+$ cells are indicated, n = 3 biological replicates, mean ± SD. (**F**) MSI1-specific immunostainings of WT and miR-26 KO NPC cultures (day 10) and their quantification (right), n = 3 biological replicates, mean ± SD. Scale bars: 100 µm.

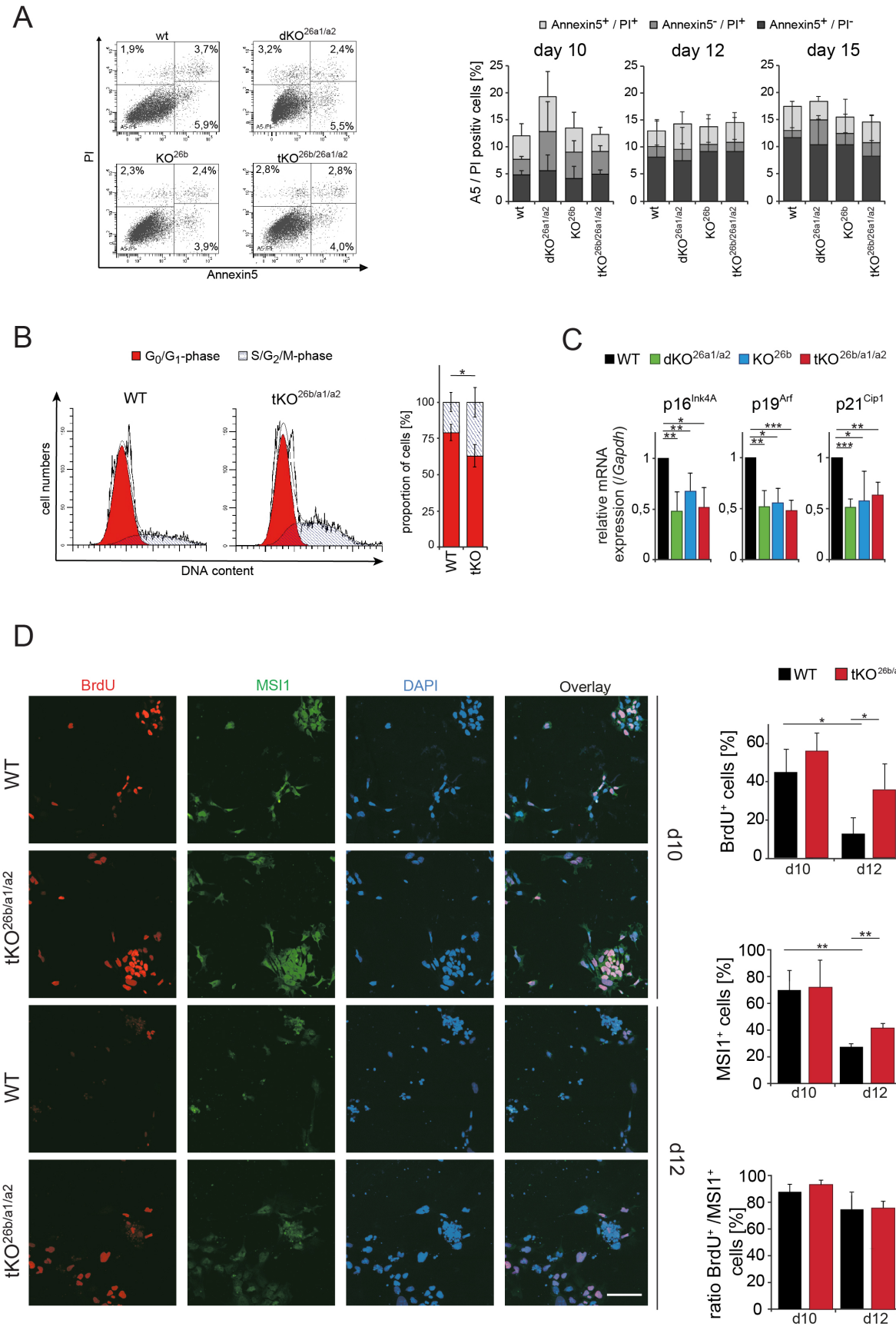

Figure S4. Apoptosis and cell cycle regulation in NPC cultures

A) Flow cytometric apoptosis and cell death analysis in WT and miR-26 KO NC cultures. Representative FACS plot (Annexin5 vs. PI) at day 15 of differentiation (left) and quantification of Annexin5- and Propidiumiodide-positive cells at day 10, 12 and 15 of differentiation (right), n = 3 biological replicates, mean ± SD. (**B**) Cell cycle phase analyses of WT and $tKO^{26b/a1/a2}$ NC cultures (day 15). The frequencies of G_0/G_1, $S/G_2/M$-type cells are shown in the bar chart (right). n=3 biological replicates, * $p < 0,05$ (t-test). (**C**) qRT-PCR analysis of the cell cycle regulators $p16^{INK4A}$, $p19^{Arf}$ and $p21^{Cip1}$ in cell cultures at day 15 of differentiation. n = 3 biological replicates, mean ± SD, * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$ (ANOVA). (**D**) Representative immunostaining of BrdU incorporation and costaining with MSI1 of cell cultures at d10 and d12 of neural differentiation. Frequencies of marker+ cell are shown on the right, n=3 biological replicates, mean ± SD. Scale bars. 100 µm, * $p < 0,05$, ** $p < 0,01$ (ANOVA).

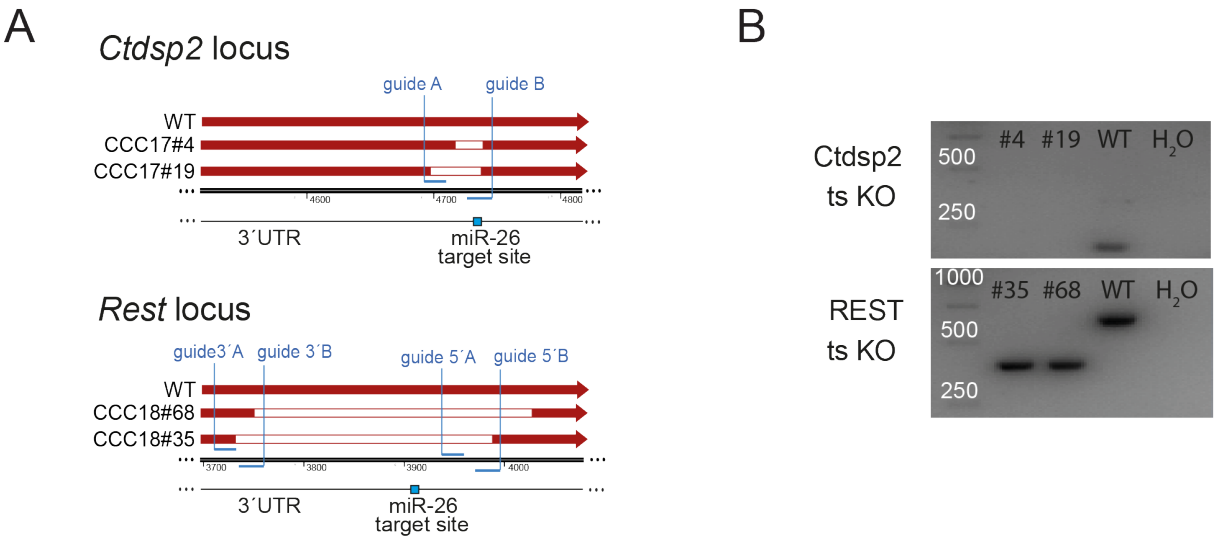

Figure S5. Generation and validation of Ctdsp2 and Rest 3`UTR miR-26 ts KO ES cell lines. (**A**) Schematic representation of the genomic Ctdsp2 and the Rest loci, the location of miR-26 target sites and the guide RNAs for Cas9-nickase deletion are indicated. WT sequences are represented by a red solid arrow, deletions by open areas. Individual ESC clone names are indicated on the left. (**B**) miR-26 target site-specific PCR analysis on genomic DNAs of WT and of miR-26 target site KO ESC clones.

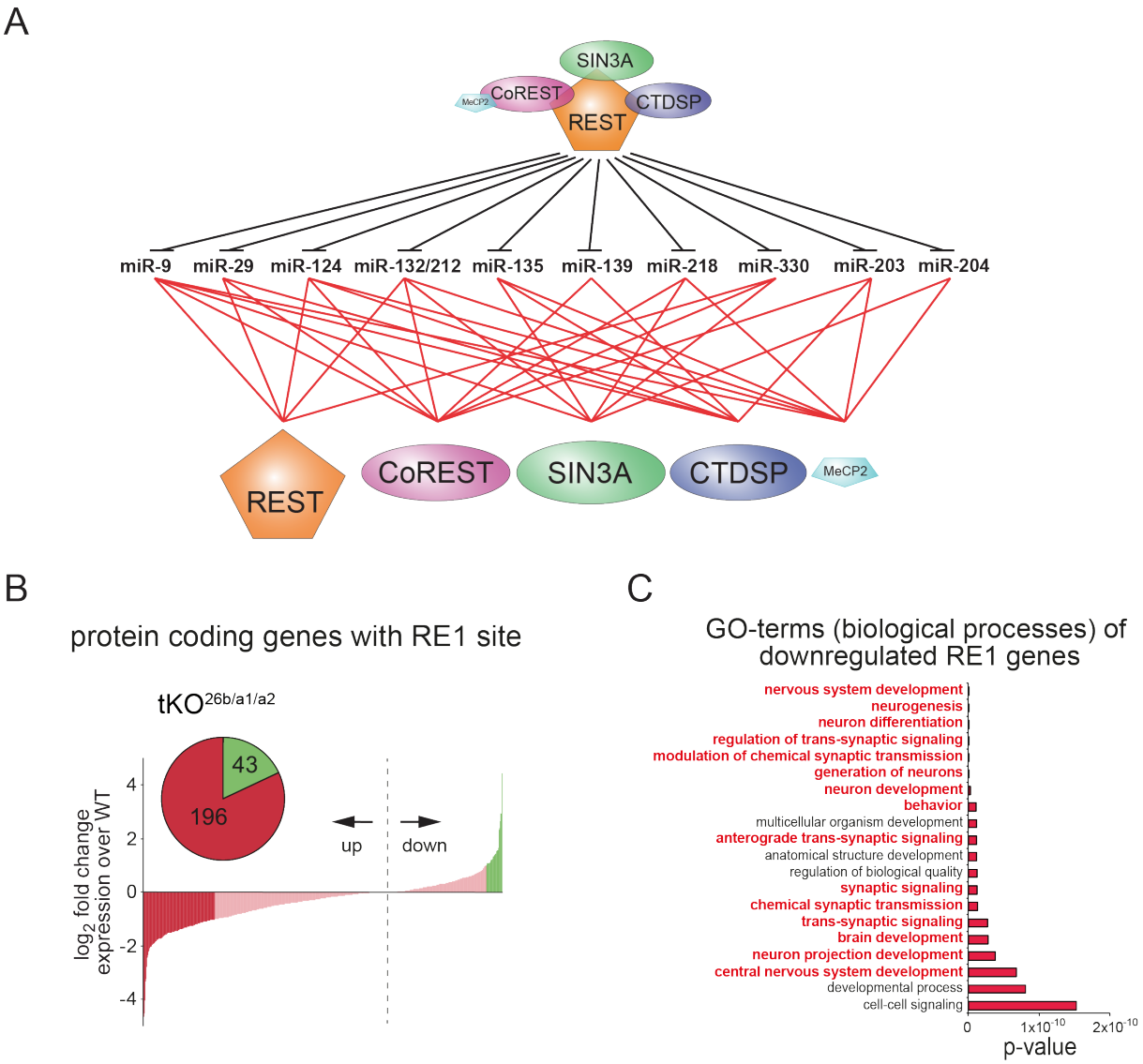

Figure S6. REST/miR network and the global downregulation of RE1 protein-coding genes in miR-26 KO cells

(**A**) Scheme of REST to miR / miR to REST regulation. REST regulated miRs and their predicted targeting to REST complex members are shown (http://www.targetscan.org/mmu_71/).

(**B**) Global down- (red) and upregulated (green) protein-coding genes with a RE1 sequence 10 kb upstream and 10 kb downstream of transcriptional start site in tKO$^{26b/a1/a2}$ cells compared to WT cells at day 15 of differentiation. Pie chart shows numbers of down- and up-regulated transcripts. (**C**) GO Term analyses of downregulated genes shown in A. No GO terms were retrieved for upregulated genes.

Table S1: Genomic target sequences and CRISPR oligonucleotides (Cas9 D10A nickase)

Name	Sequence (5' – 3'), PAM sequences underlined
miR-26b 5'A target	**GAATTACTTGAACTGGGTCCCGG**
miR26b 5'A F	CACCGAATTACTTGAACTGGGTCC
miR26b 5'A R	AAACGGACCCAGTTCAAGTAATTC
miR-26b 3'B target	**TTCTCCATTACTTGGCTCGGGGG**
miR26b 3'B F	CACCGTTCTCCATTACTTGGCTCGG
miR26b 3'B R	AAACCCGAGCCAAGTAATGGAGAAC
miR-26a1 5'A target	**CTGCACTCCGGACGTGCTTGTGG**
miR26a1 5'A F	CACCGCTGCACTCCGGACGTGCTTG
miR26a1 5'A R	AAACCAAGCACGTCCGGAGTGCAGC
miR-26a1 5'B target	**TCTTTGGCAGTAGACACCCCGGG**
miR26a1 5'B F	CACCGTCTTTGGCAGTAGACACCCC
miR26a1 5'B R	AAACGGGGTGTCTACTGCCAAAGAC
miR-26a1 3'A target	**CAAGCTTGGCTACAGGCAAAGGG**
miR26a1 3'A F	CACCGCAAGCTTGGCTACAGGCAAA
miR26a1 3'A R	AAACTTTGCCTGTAGCCAAGCTTGC
miR-26a1 3'B target	**TCCCGGAGACTCAGGACCGGAGG**
miR26a1 3'B F	CACCGTCCCGGAGACTCAGGACCGG
miR26a1 3'B R	AAACCCGGTCCTGAGTCTCCGGGAC
miR-26a2 5'A target	**CGGCTTGTGTAGGTCCCATCTGG**
miR-26a2 5'A F	CACCGCGGCTTGTGTAGGTCCCATC
miR-26a2 5'A R	AAACGATGGGACCTACACAAGCCGC
miR-26a2 5'B target	**CTGCTGGAATCCCGTACAGAAGG**
miR-26a2 5'B F	CACCGCTGCTGGAATCCCGTACAGA
miR-26a2 5'B R	AAACTCTGTACGGGATTCCAGCAGC
miR-26a2 3'A target	**TGGACGGACACAGCCTATCCTGG**
miR-26a2 3'A F	CACCGTGGACGGACACAGCCTATCC
miR-26a2 3'A R	AAACGGATAGGCTGTGTCCGTCCAC
miR-26a2 3'B target	**TCTTGATTACTTGTTTCTGGAGG**
miR-26a2 3'B F	CACCGTCTTGATTACTTGTTTCTGG
miR-26a2 5'B R	AAACCCAGAAACAAGTAATCAAGAC
Ctdsp2 A ts target	**TATCAAATCATGAAGCAAGGTGG**
Ctdsp2 A ts F	CACCGTATCAAATCATGAAGCAAGG
Ctdsp2 A ts R	AAACCCTTGCTTCATGATTTGATAC

Ctdsp2 3'B target	**TATTCAAAAACTTGAACTGTAGG**
Ctdsp2 B ts F	CACCGTATTCAAAAACTTGAACTGT
Ctdsp2 B ts R	AAACACAGTTCAAGTTTTTGAATAC
Rest ts 3´A target	**TCCTCTTACATTAACTCCCGAGG**
Rest ts 3´A F	CACCGTCCTCTTACATTAACTCCCG
Rest ts 3´A R	AAACCGGGAGTTAATGTAAGAGGAC
Rest ts 3´B target	**AGCTCGTGCAGGCAGGTGCAAGG**
Rest ts 3´B F	CACCGAGCTCGTGCAGGCAGGTGCA
Rest ts 3´B R	AAACTGCACCTGCCTGCACGAGCTC
Rest ts 5´A target	**TAACTTAATTTATATAAAGCAGG**
Rest ts 5´A F	CACCGGTAACTTAATTTATATAAAGC
Rest ts 5´A R	AAACGCTTTATATAAATTAAGTTAC
Rest ts 5´B target	**GAAAAAAAAGAGATTTTAATTGG**
Rest ts 5´A F	CACCGGAAAAAAAAGAGATTTTAAT
Rest ts 5´A R	AAACATTAAAATCTCTTTTTTTTC

Table S2: PCR oligonucleotides

A cDNA primer (qRT-PCRs)

Name	Sequence (5' - 3')
Gapdh F	TGGAGAAACCTGCCAAGTATG
Gapdh R	TCATACCAGGAAATGAGCTTGA
Rpl4 F	TTGGGTTGTATTCACTCTGCG
Rpl4 R	CAGACCAGTGCTGAGTCTTGG
Oct4 F	CCGTGAAGTTGGAGAAGGTG
Oct4 R	GAAGCGACAGATGGTGGTCT
Sox2 F	GCGGAGTGGAAACTTTTGTCC
Sox2 R	CGGGAAGCGTGTACTTATCCTT
Nanog F	TCTTCCTGGTCCCCACAG TTT
Nanog R	GCAAGAATAGTTCTCGGGATGAA
Ctdsp1 F	CCCAGTCCAGTACCTGCTTC
Ctdsp1 R	CATCTATCTCCACCGGGATG
Ctdsp2 F	GGAAGGGACCTGAGGAAAAC
Ctdsp2 R	CCTCGAAGACTGGAATCAGG
CtdspL F	GTTGAAATCGACGGAACCAT
CtdspL R	GCCAAGCTGGCAGTAAAGAG
Neurod1 F	ATGACCAAATCATACAGCGAGAG
Neurod1 R	CCAGCGACACTGAGTCCTG
Neurog1 F	CCAGCGACACTGAGTCCTG
Neurog1 R	CGGGCCATAGGTGAAGTCTT
Msi1 F	TAAAGTGCTGGCGCAATCG
Msi1 R	TCTTCGTCCGAGTGACCATCT
Pax6 F	TACCAGTGTCTACCAGCCAAT
Pax6 R	TGCACGAGTATGAGGAGGTCT
Ncam1 F	ACCACCGTCACCACTAACTCT
Ncam1 R	TGGGGCAATACTGGAGGTCA
Tubb3 F	TTCTGGTGGACTTGGAACCT
Tubb3 R	CGCACGACATCTAGGACTGA
Rest F	CATGGCCTTAACCAACGACAT
Rest R	CGACCAGGTAATCGCAGCAG
$p16^{Ink4A}$ F	GTACCCCGATTCAGGTGATG
$p16^{Ink4A}$ R	GGAGAAGGTAGTGGGGTCCT

p19^{ARF} F	GCTCTGGCTTTCGTGAACAT
p19^{ARF} R	CGAATCTGCACCGTAGTTGA
p21^{Cip} F	ACATCTCAGGGCCGAAAAC
p21^{Cip} R	GGCACTTCAGGGTTTTCTCTT

B miRNA primer (qRT-PCRs)

Name	Cat. No.:
snRNA U6	MS00033740
mmu-miR-26a-5p	MS00032613
mmu-miR-26b	MS00001344
mmu-miR-9-5p	MS00012873
mmu-miR-124-3p	MS00029211
mmu-miR-218-3p	MS00006118
mmu-miR-135a-5p	MS00011130
mmu-miR-26a-2_1_PR	MP00005250
mmu-miR-26a-1_1_PR	MP00005243
mmu-miR-26b_1_PR	MP00005257

C genomic DNA primer (Endpoint PCRs)

Name	Sequence (5’ - 3’)
miR-26b del F	GTCTTGTGCAGCCCTCTTTC
miR-26b del R	GCTTAGGGGTGATCCACAAA
miR-26a1 del F	GCGCTGGTTGTTGTGTCTAA
miR-26a1 del R	CAGTGAGAGAAGCCCTGGAG
miR-26a2 del F	CATAGACTGGGTGGCGAGTT
miR-26a2 del R	GTTTTCCTCAGGTCCCTTCC
miR-26ts-CTDSP2 F	AACTGCCCTGCACCATAAGC
miR-26ts-CTDSP2 R	TGGCATCCTACAGTTCAAGTTTT

miR-26ts-REST F	CCTCGGCAGAAGCACCG
miR-26ts-REST R	CTGTTTCAGGGGAAGGGAGATTA

D Primer for cloning luciferase reporter constructs
(lower case letters show mutation sites)

Name	Sequence (5' - 3')
Rest 3´UTR F	GATCCTCGAGCTGAGCCTCGGCAGAAGC
Rest 3´UTR R	GATCGCGGCCGCATGTTTAACTTATCCTACACAATCTTGACC
Ctdsp2 3´UTR F	GATCCTCGAGCCCATGGGTCCCTAATTAAAGAAGTTG
Ctdsp2 3´UTR R	GATCCTCGAGTGGTTGTCCCAAGTGTTGCTGATCGCGGCCG CCAGACTCTTAATGGCATCCTACAG
Rest mut 3'UTR F	ATTGGCTTAGTAAATatCTtaGAGAATTTGCCTGCTT
Rest mut 3'UTR R	AAAGCAGGCAAATTCTctAAGatATTTACTAAGCCAAT
Rest 2.mut 3´UTR F	CTAGTAACTAGTGGTAAcaTTagAATGGTAGCATTCTTTACAGC
Rest 2.mut 3´UTR R	GCTGTAAAGAATGCTACCATTctAAtgTTACCACTAGTTACTAG
Ctdsp2 mut 3'UTR F	TTGTTGTATTCAAAAcaTTagACTGTAGGATGCCATT
Ctdsp2 mut 3'UTR R	AATGGCATCCTACAGTctAAtgTTTTGAATACAACAA