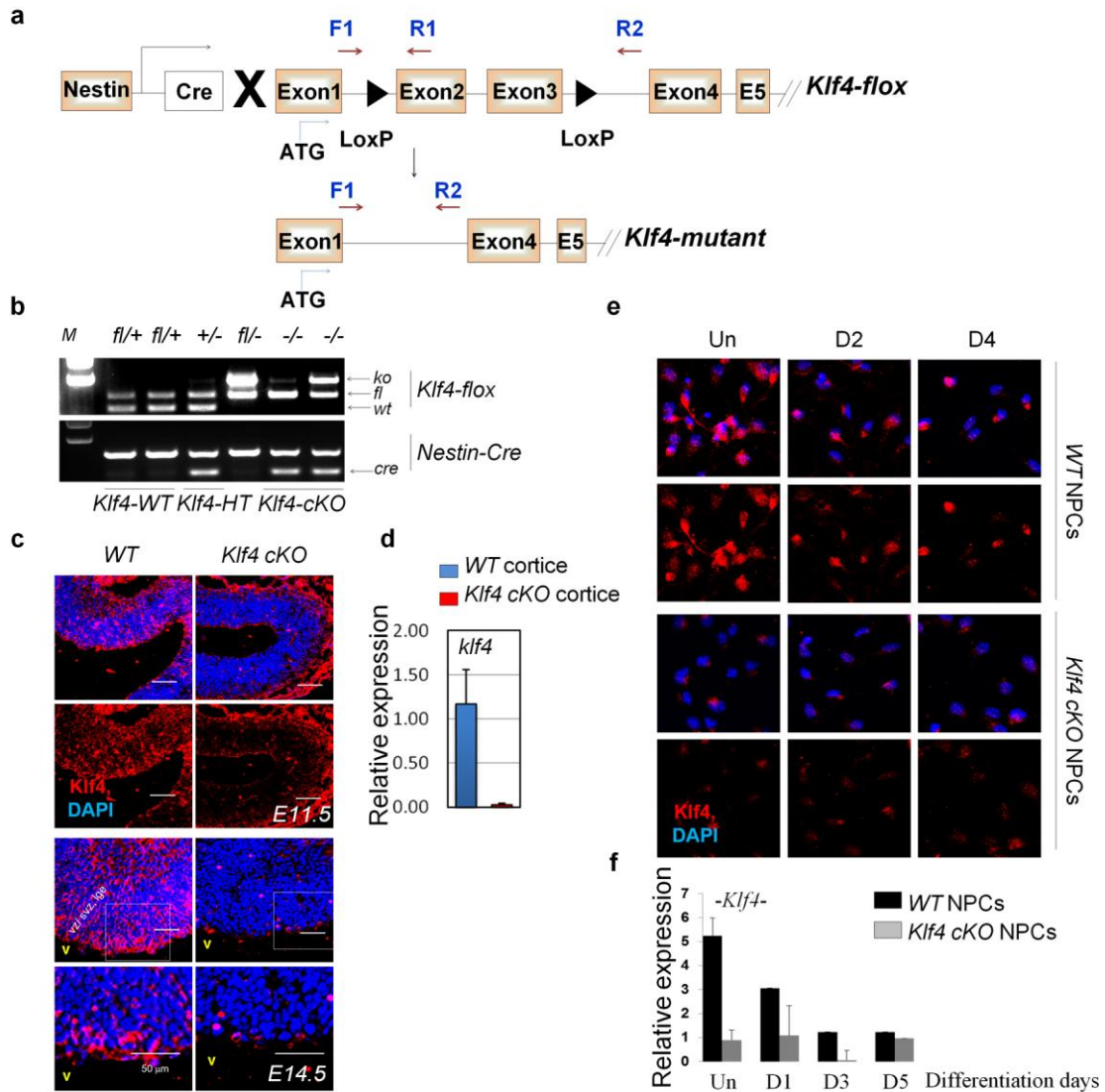
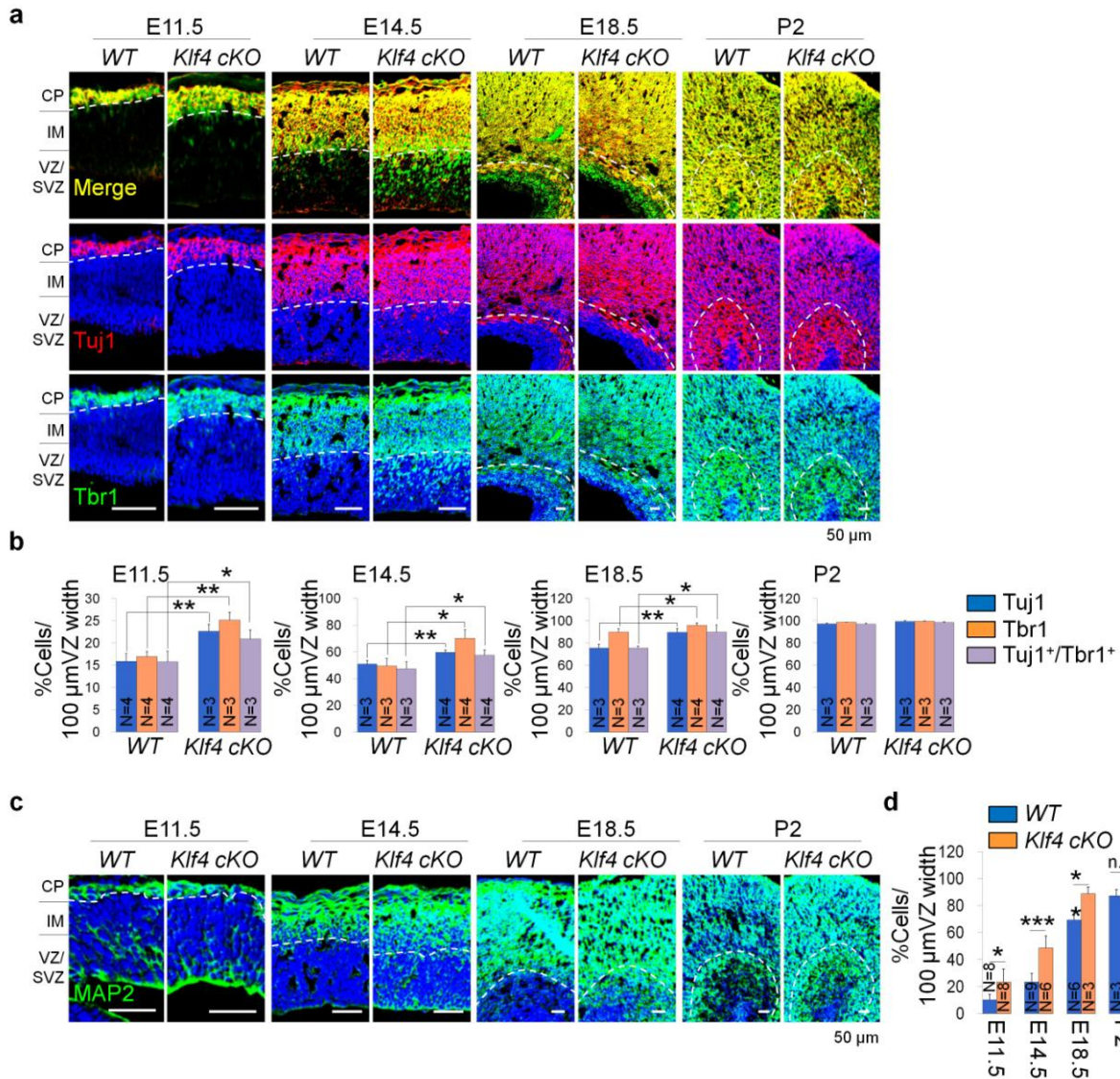


SUPPLEMENTARY FIGURE LEGENDS

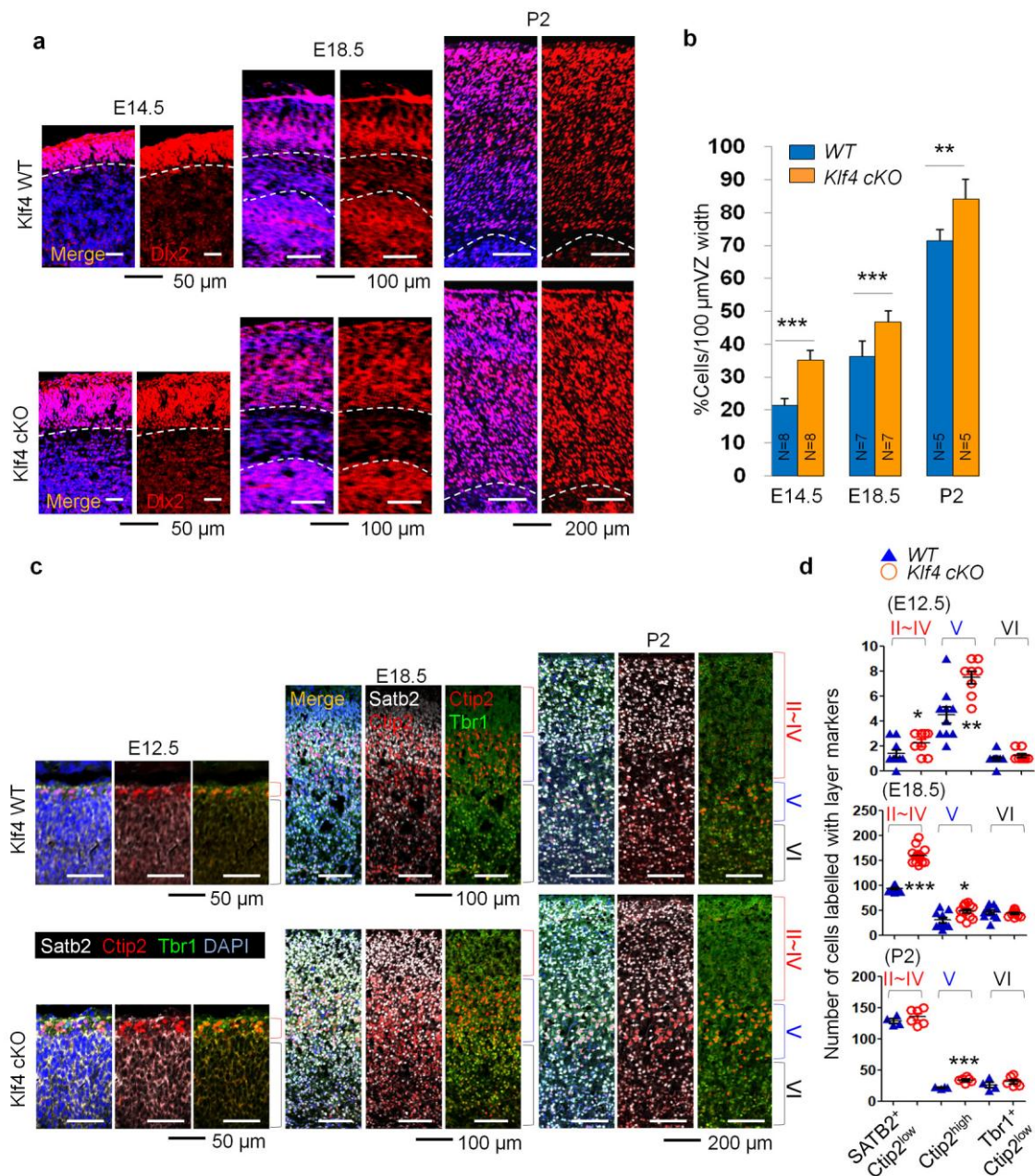


Supplementary Figure 1. Generation of *Klf4* conditional knockout mice. (a, b) *Nestin-cre* mice were crossed with *Klf4*^{fl/+} or *Klf4*^{fl/fl} mice to generate *wild-type* and *Klf4* conditional knockout embryos. Genotyping was performed with indicated primers. (b) Upper band is *Klf4* knockout, middle band is floxed, and lower band indicates wild-type. Left lane (*M*) indicates size

marker. (c) Fixed cyro-embedded coronal sections from E11.5 or E14.5 mouse forebrain stained with antibodies against *Klf4* (red). DAPI (blue). Scale bars, 50 μm . (d) qPCR analysis of *Klf4* mRNA cortices equivalent to those shown in (c, lower panel). (e) Immunostaining to detect *Klf4* expression in *wild-type* and *Klf4 cKO* NPCs. Nuclear staining shown by DAPI (blue). Scale bar, 50 μm . (f) qPCR analysis of *Klf4* levels in *wild-type* and *Klf4* NPCs at indicated days (d) of differentiation (n=2-3).



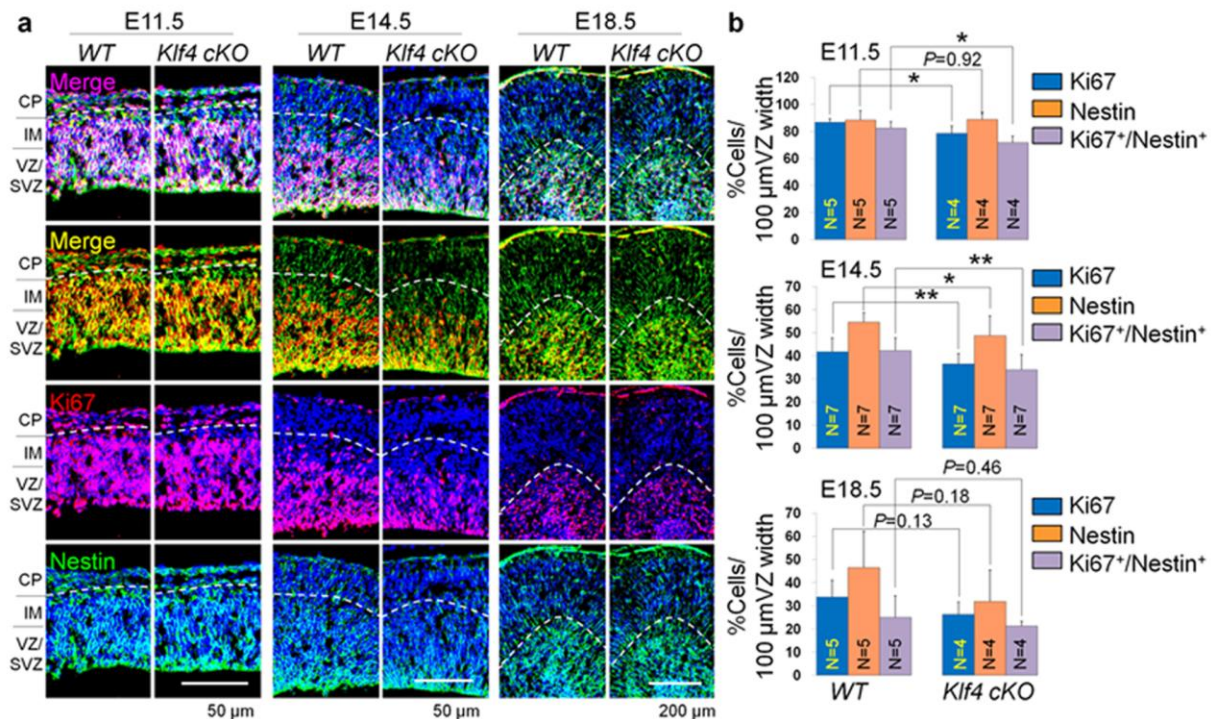
Supplementary Figure 2. Klf4 down-regulation enhances neurogenesis *in vivo*. (a, c) Fixed coronal sections from E11.5, E14.5, E18.5 or P2 mouse forebrain stained with antibodies against Tuj1 (red), Tbr1 (green) or Map2 (green). DAPI (blue). (b, d) Images shown above were quantified in cortex areas of 100 μm ventricular width extending from VZ to the pial surface (E11.5: *Klf4*^{f/+} (WT), n=4-8, *Klf4* cKO, n=3-8, and E14.5: *Klf4*^{f/+} (WT), n=3-9, *Klf4* cKO, n=4-6, E18.5: *Klf4*^{f/+} (WT), n=3-6, *Klf4* cKO, n=3-4, P2: *Klf4*^{f/+} (WT), n=3, *Klf4* cKO, n=3). Scale bars, 50 μm . Values correspond to mean \pm SD. t-tests were performed to calculate significance (*P < 0.05, **P < 0.005, ***P < 0.0005).



Supplementary Figure 3. Klf4 down-regulation enhances deep layer corticogenesis *in vivo*.

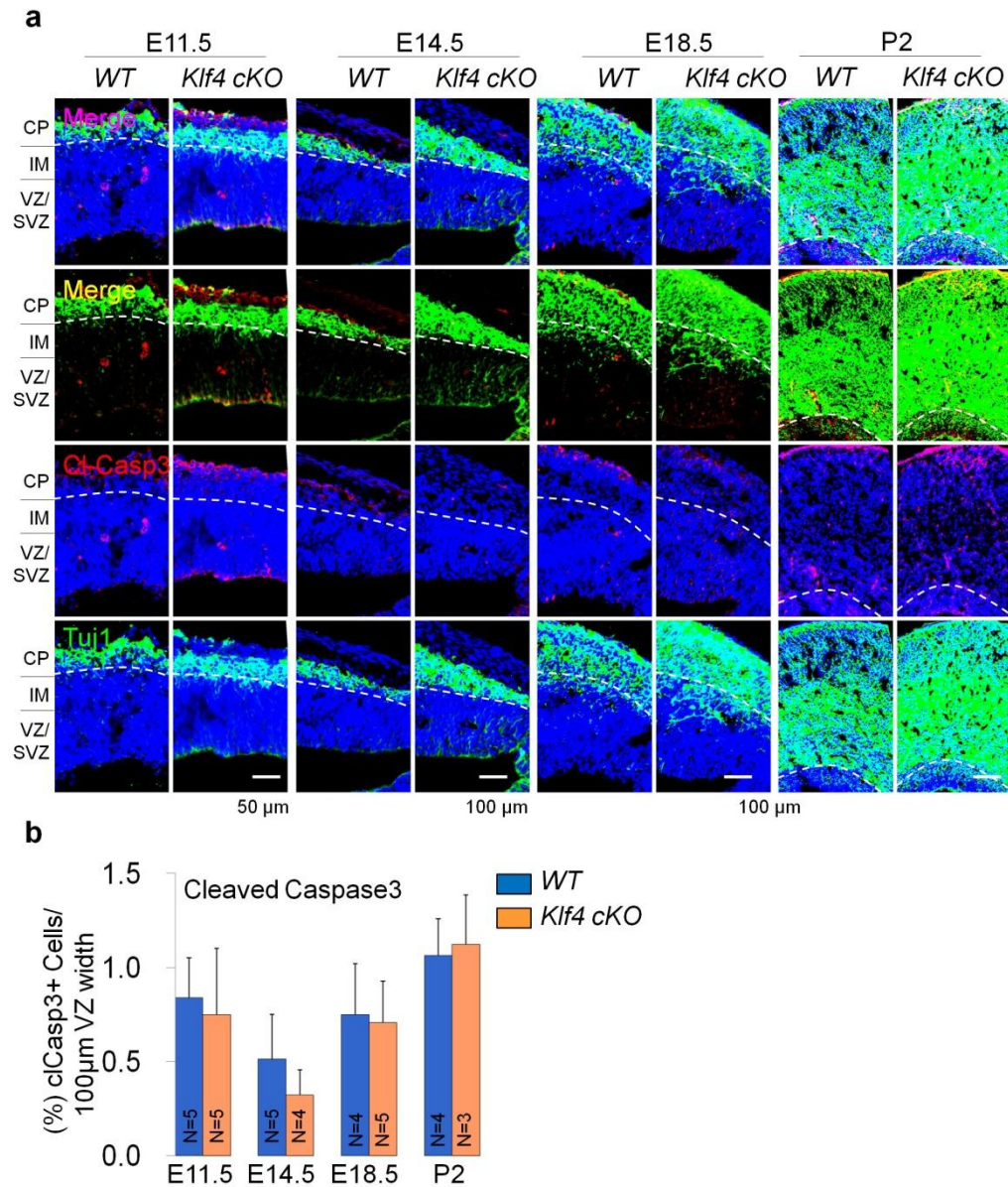
(a) Fixed coronal sections from E14.5, E18.5 or P2 mouse forebrain stained with antibodies against Dlx2 (red). DAPI (blue). (b) Images shown in (a) were quantified in cortex in areas of 100 μm ventricular width extending from VZ to the pial surface in E14.5 (n=8/genotype), E18.5 (n=7/genotype), and P2 (n=5/genotype). (c) Representative immuno-labeling of E12.5, E18.5

and P2 cortical sections with indicated antibodies revealing cortical layering in WT and *Nestin^{cre}:Klf4 cKO* mice. (d) Quantification of (c) numbers over brackets denote cortical layers where the cells were counted. CTIP2^{low} (layer 6) and CTIP2^{high} (layer 5) expressing cells were used to differentiate between Tbr1⁺/CTIP2^{low} cells of layer 5 and CTIP2^{high} cells in layer 5. SATB2⁺/CTIP2⁻ cells were counted above layer of CTIP2^{high} cells in E12.5 (n=8~10/genotype), E18.5 (n=10~12/genotype), and P2 (n=4~6/genotype). Values correspond to mean±SD. t-tests were performed to calculate significance (*P < 0.05, **P < 0.005, ***P < 0.0005).



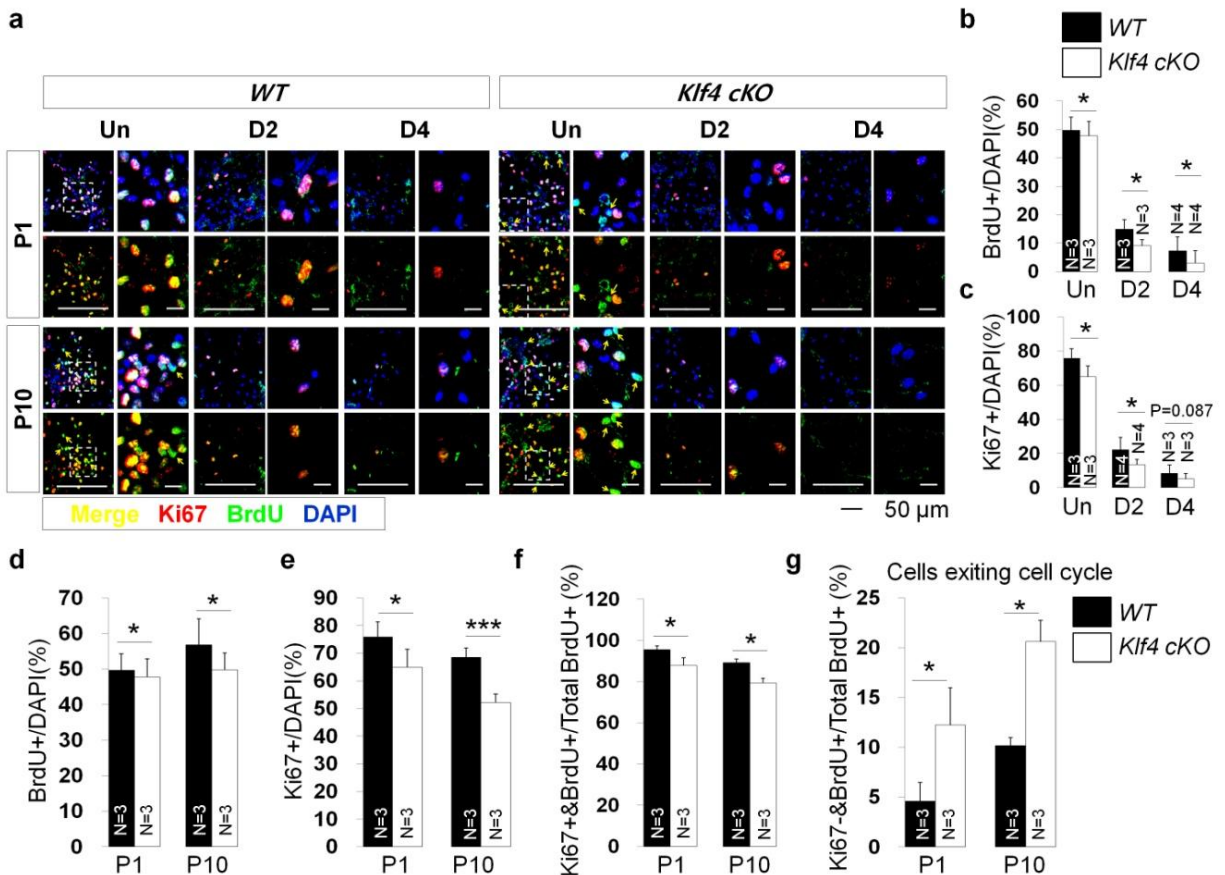
Supplementary Figure 4. Klf4 down-regulation enhances neuronal maturation and decreases proliferative neural progenitor population in developmental neocortex. (a) Fixed coronal sections from E11.5, E14.5, or E18.5 mouse forebrain stained with antibodies against Nestin (green) and Ki67 (red). DAPI (blue). (b) Images shown above were quantified in cortex

in areas of 100 μm ventricular width extending from VZ to the pial surface. Scale bars, 50 μm . Values correspond to mean \pm SD. *t*-tests were performed to calculate significance (**P* < 0.05, ***P* < 0.005).



Supplementary Figure 5. *Klf4* down-regulation does not change cell death of progenitors and neurons in developmental neocortex. (a) Representative immuno-labeling for apoptotic cells with clCasp3 and neuronal marker TuJ1 in coronal telencephalic sections from E11.5, E14.5, or P2 mice. (b) Apoptosis of cells were quantified in cortex in areas of 100 mm ventricular width

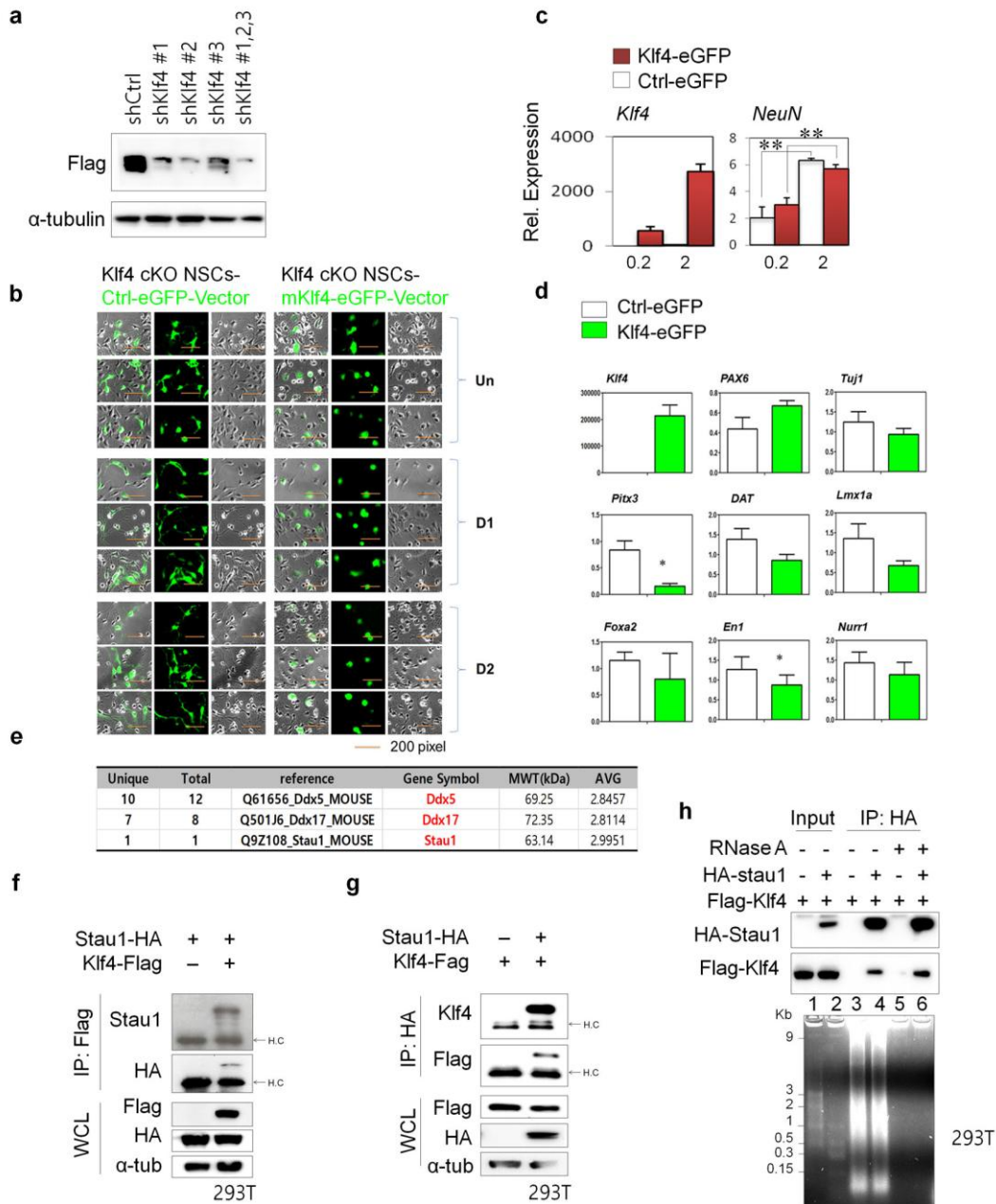
extending from VZ to the pial surface (E11.5: *Klf4*^{fl/+} (WT), n=5, *Klf4* cKO, n=5, and E14.5: *Klf4*^{fl/+} (WT), n=5, *Klf4* cKO, n=4, E18.5: *Klf4*^{fl/+} (WT), n=4, *Klf4* cKO, n=5, P2: *Klf4*^{fl/+} (WT), n=4, *Klf4* cKO, n=3). Scale bars, 50 or 100 μ m. Values correspond to mean \pm SD. *t*-tests were performed to calculate significance.



Supplementary Figure 6. *Klf4* down-regulation decreases proliferative neural progenitor

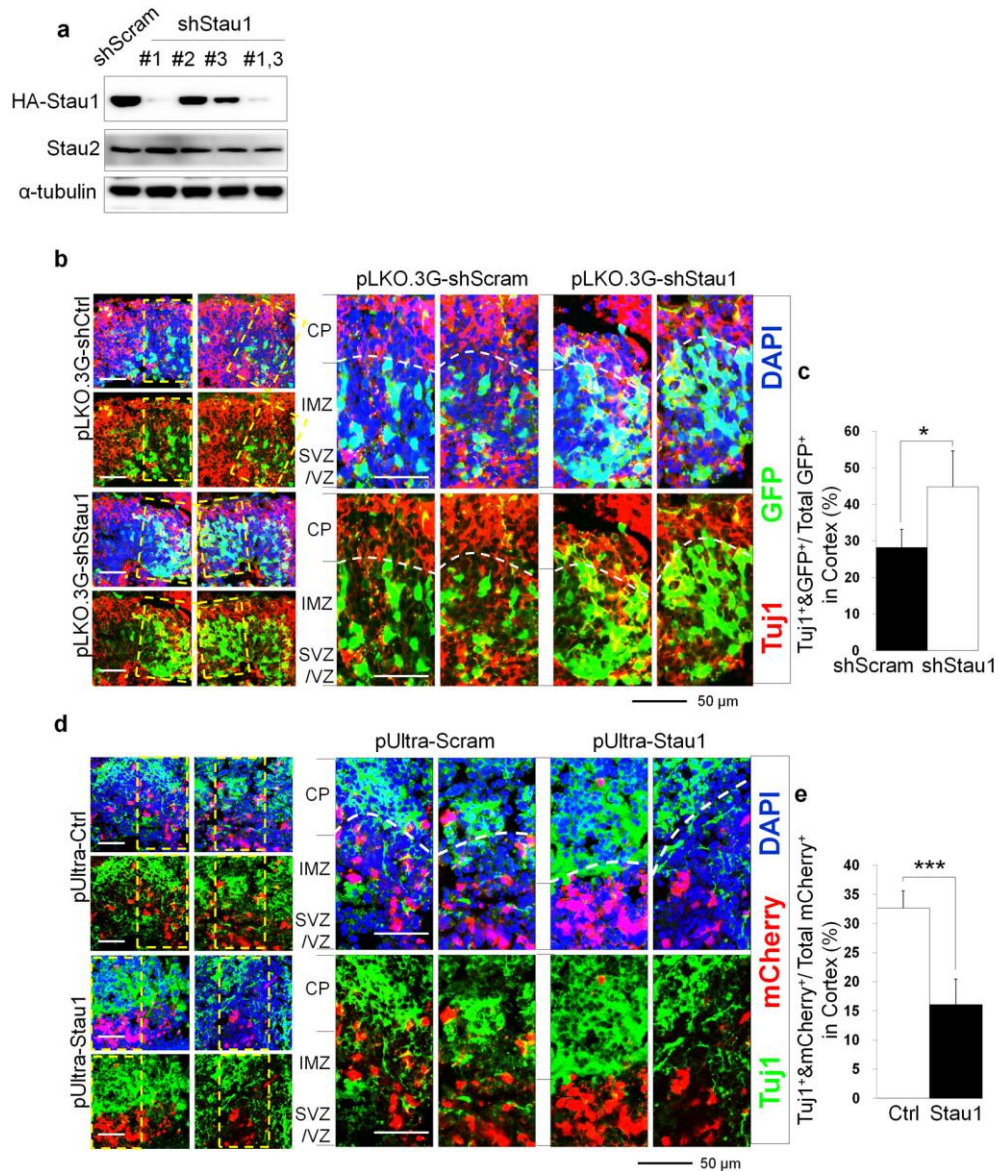
cells and increases cells exiting cell cycle. (a) Representative immuno-labeling for Ki67 and BrdU in the P1 (passage 1) or P10 (passage 10) NPCs pulsed with BrdU (30 μ g/ml) for 5 hrs. The area outlined by dotted lines is magnified in the adjacent images. Un, undifferentiation; D2, differentiation 2 days *in vitro*; D4, differentiation 4 days *in vitro*. Yellow arrows indicate cells exiting cell cycle which are Ki67 negative cells incorporated BrdU in the S phase. (b-e) Quantification of (a). Histogram showing percent stained cells relative to the number of DAPI⁺

nuclei. Scale bar, 50 μ m. D, differentiation days *in vitro*. (f) Fraction of Ki67⁺BrdU⁺ cells in total BrdU⁺ cell population. (g) Fraction of cells exiting the cell cycle defined as fraction of Ki67⁻BrdU⁺ cells in total BrdU⁺ cell population. Scale bars, 50 μ m. Values correspond to mean \pm SD. *t*-tests were performed to calculate significance (**P* < 0.05, ***P* < 0.005, ****P* < 0.0005).



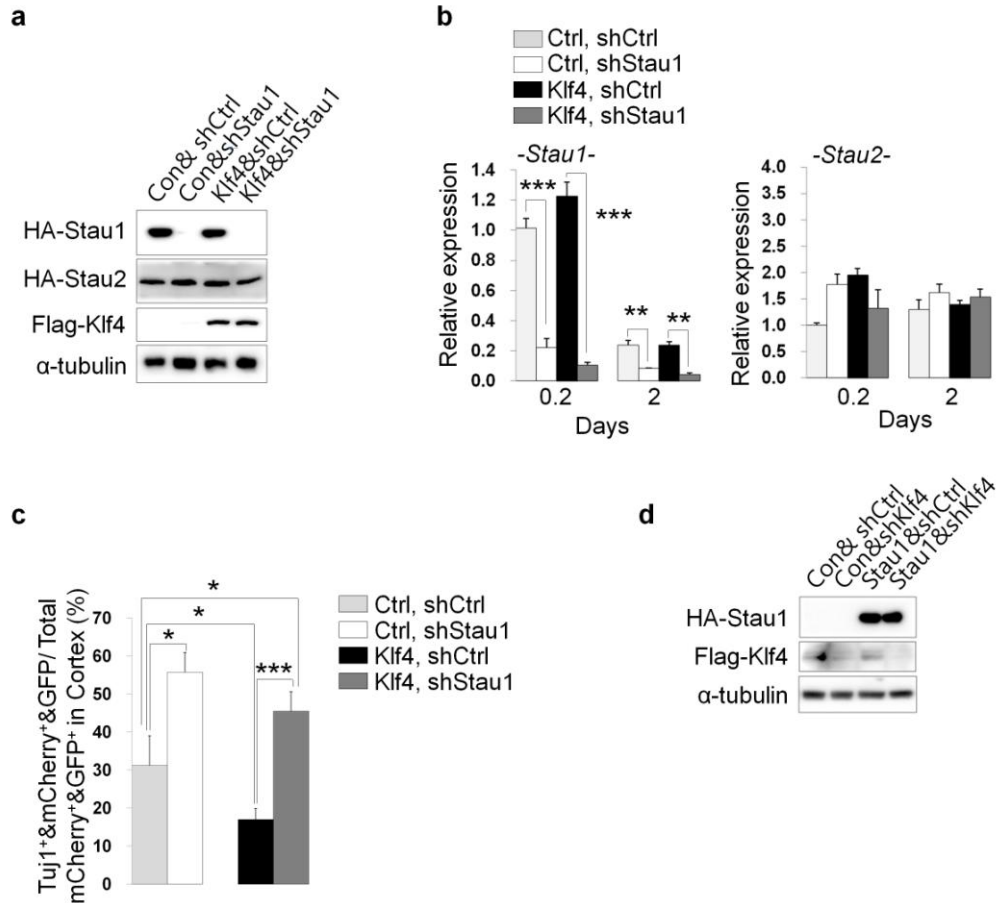
Supplementary Figure 7. Klf4 regulates NPC neuronal differentiation and interacts with post-transcriptional regulators. (a) NPCs expressing Flag-Klf4 were transfected with pLKO.1-

shScramble or pLKO.1-shKlf4 #1, #2, and #3, and one day later, Flag and α -tubulin in lysates were detected by immunoblotting (n=2). **(b)** *Klf4* cKO NPCs transfected with *Control-EGFP* vector or *Klf4-EGFP* vector. GFP⁺ cells were assessed after 1 or 2 days of culture in N2 medium. **(c, d)** qPCR analysis of indicated transcripts in samples equivalent to those shown in (b) (n=3). **(e)** MS spectrometry analysis with Flag-Klf4 in NPCs **(f, g)** Co-Immunoprecipitation (co-IP) of HA-tagged Stau1 or Flag-tagged Klf4 in HEK293T cells transfected with these constructs. **(h)** (upper) Co-IP of above samples with or without RNase A (10 μ g/ml). Values correspond to mean \pm SD. *t*-tests were performed to calculate significance (*P < 0.05, **P < 0.005). (lower) Agarose gel of total RNA in above samples were separated with standard electrophoresis procedures. We used a 7.5 \times 10 cm mini-gel horizontal system (C.B.S. Scientific) to run a 1.5% agarose gel at 100 V (6 V/cm) for 2 h. Marker lanes (1–2) contained commercial RNA ladders (1. New England Bio Labs ssRNA ladder #No362s, 2. NEB low Range ssRNA ladder #No364s). Lane 3 and 4, total RNA in IP lysates without RNase A (10 μ g/ml). Lane 5 and 6, total RNA in IP lysates with RNase A (10 μ g/ml). RNA ladders and each total RNA samples were premixed with 2xRNA loading dye (Thermo Scientific, #Ro641). The bands were visualized using Molecular Imager[®] Gel Doc[™] XR+ (Bio-Rad). See also Supplementary Figs. 19, 20.



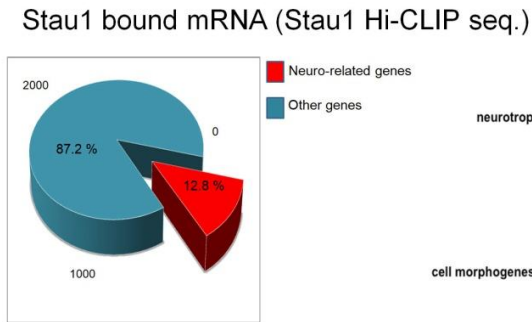
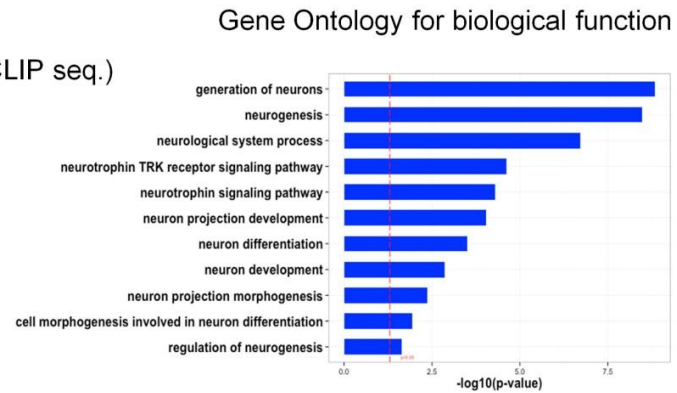
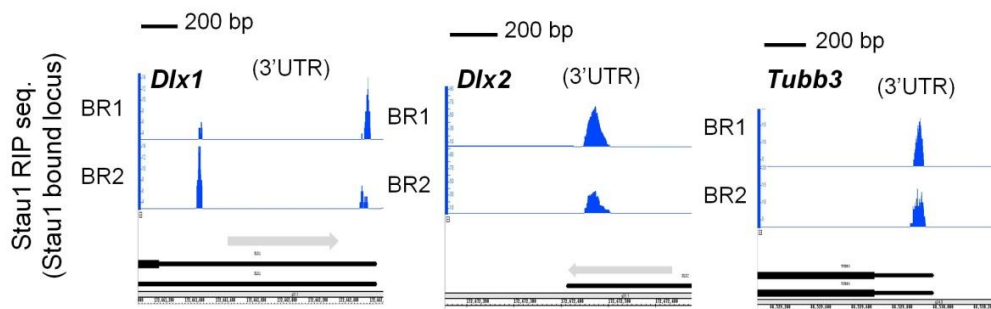
Supplementary Figure 8. Stau1 regulates NPC neuronal differentiation *in vivo*. (a) NPCs were infected with pLKO.1-shScramble or pLKO.1-shStau1 #1, #2, and #3, and one day later, Stau1, Stau2 and α -tubulin in lysates were detected by immunoblotting (n=2). (b) Effect of Stau1 knockdown on embryonic mouse cortex. Green fluorescent images indicate GFP labeled shScramble or shStau1 expression in electroporated embryo brain. CP, cortical plate; IMZ, intermediate zone; SVZ/VZ, (sub)ventricular zone. (c) The effect of Stau1 knockdown on

neurogenesis was evaluated by counting Tuj1 (red)/EGFP (green) double positive cells versus total EGFP-positive cells in cortex. shScramble (n=4), shStau1 (n=6). **(d)** Effect of Stau1 overexpression on embryonic mouse cortex. Red fluorescent images indicates mCherry labeled control or mStau1 expression in electroporated embryo brain. CP, cortical plate; IMZ, intermediate zone; SVZ/VZ, (sub)ventricular zone. **(e)** The effect of Stau1 overexpression on neurogenesis was evaluated by counting Tuj1(green)/mCherry(red) double positive cells versus total mCherry-positive cells in cortex. Control (n=5), Stau1 (n=7). Values correspond to mean \pm SD. *t*-tests were performed to calculate significance (*P < 0.05, **P < 0.005). See also Supplementary Fig. 20.

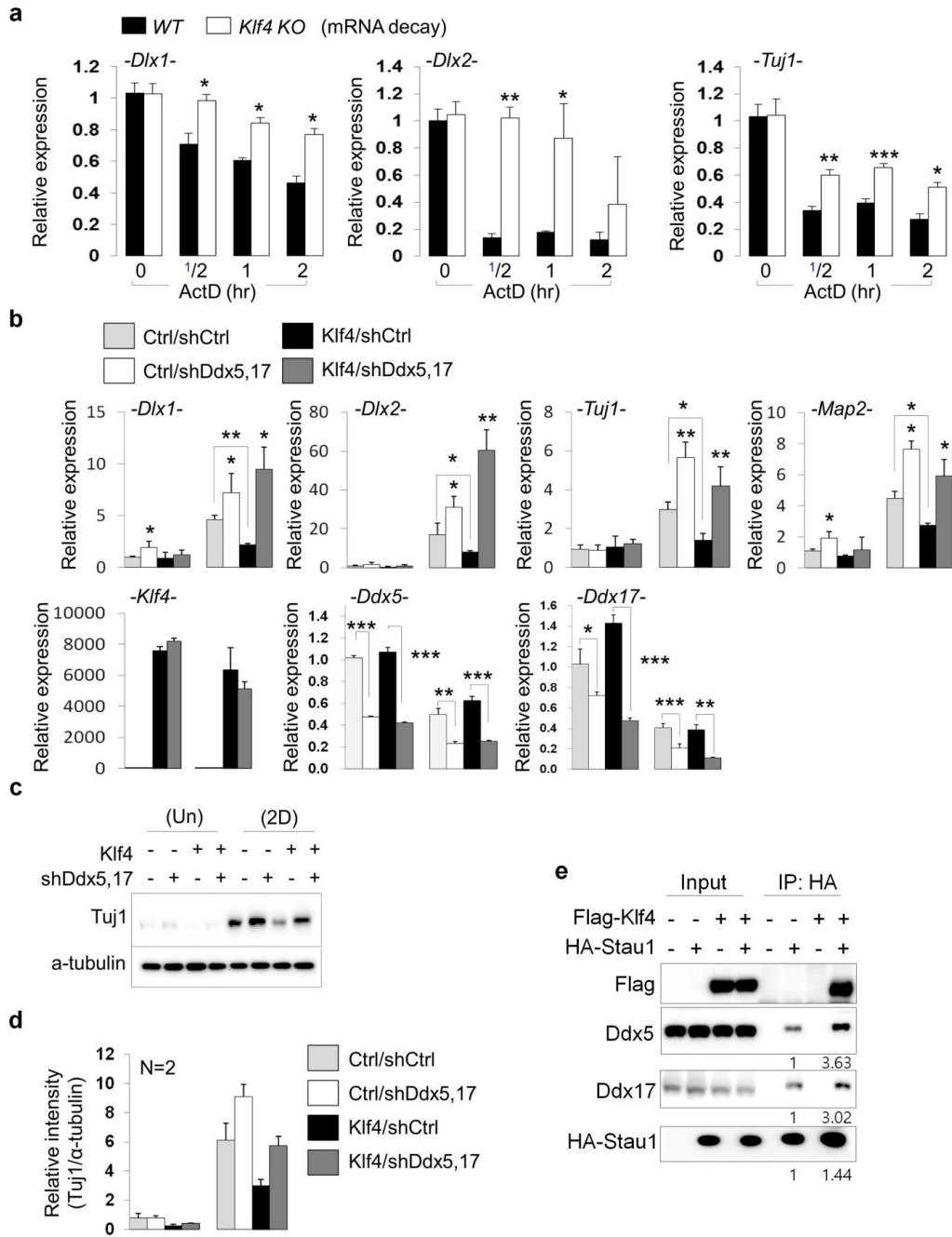


Supplementary Figure 9. Stau1 rescues defect of neurogenesis seen in Klf4 overexpression

in vivo. (a) Immunoblot of indicated antibodies in Klf4-overexpressing or control NPCs infected with pLKO.1 shScramble or pLKO.1 shStau1 lentivirus (n=1-2). (b) RT-qPCR of indicated transcripts in samples shown in (a) (n=3). (c) The rescue effect of Stau1 knockdown on neurogenesis was evaluated by counting Tuj1⁺mCherry⁺GFP⁺ triple positive cells versus total mCherry⁺GFP⁺ cells in electroporated cortex. shScramble/Control (n=3), shStau1/Control (n=3), shScramble/Klf4 (n=3), shStau1/Klf4 (n=6). (d) Immunoblots of indicated antibodies in Stau1-overexpressing or control NPCs infected with pLKO.1 shScramble or pLKO.1 shKlf4 lentivirus (n=1). Values correspond to mean±SD. Anova tests were performed to calculate significance (*P < 0.01, **P < 0.001, ***P < 0.0001). See also Supplementary Table 4, Supplementary Fig. 20.

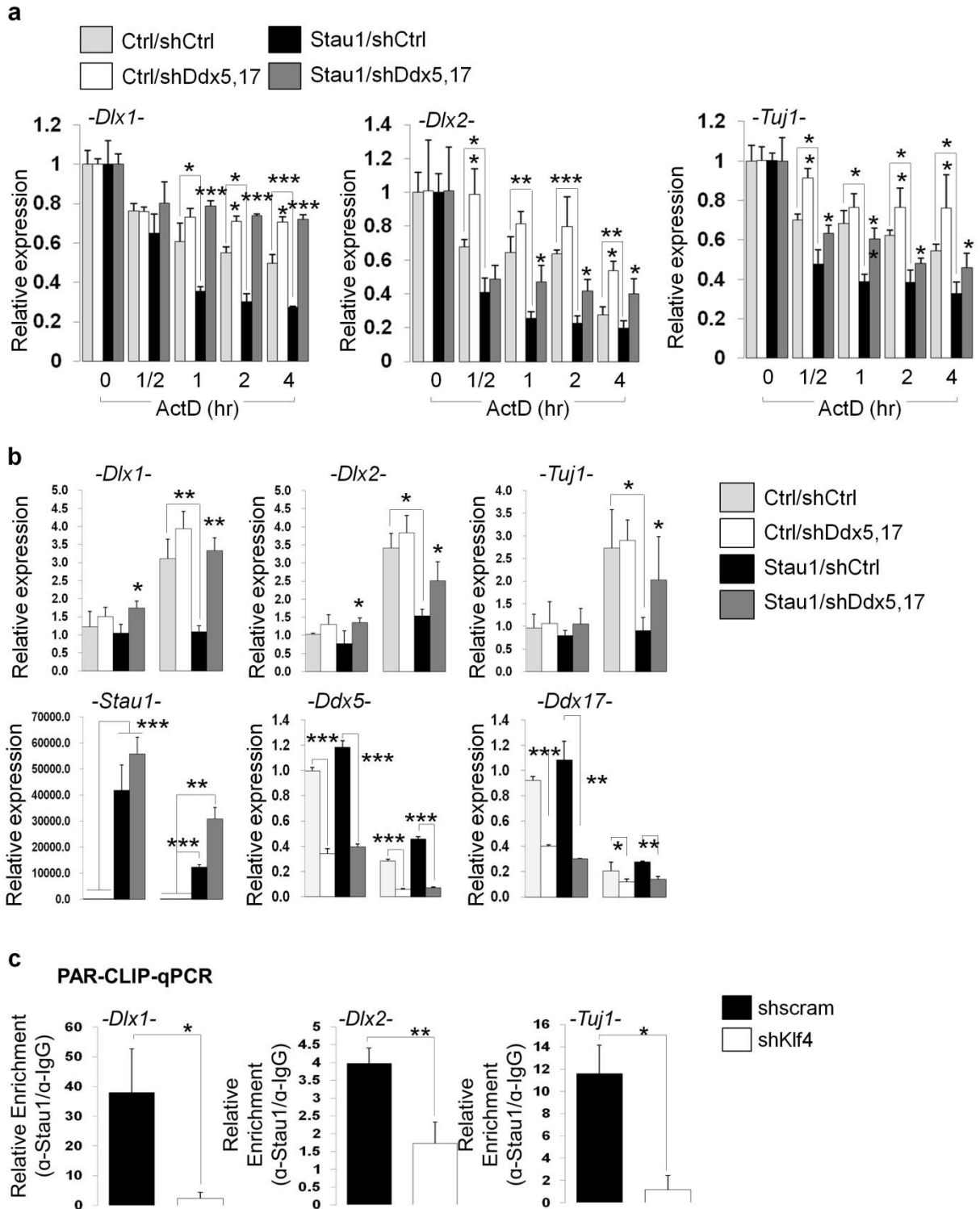
a**b****c**

Supplementary Figure 10. Stau1 specifically binds to neurogenesis-associated mRNAs. (a) Stau1 hi-CLIP sequencing analysis in HEK293 cells (GSE52447)¹. Among genes enriched by Stau1, 12.8% are neuro-related genes. (b) Gene ontology (GO) analysis of annotated genes at Stau1 binding sites. (c) Stau1 RIP sequencing analysis². Distribution of Stau1 binding peaks in HEK293 cells. Stau1 protein was highly enriched in 3'UTRs of *Dlx1*, *Dlx2* and *Tubb3* mRNAs.



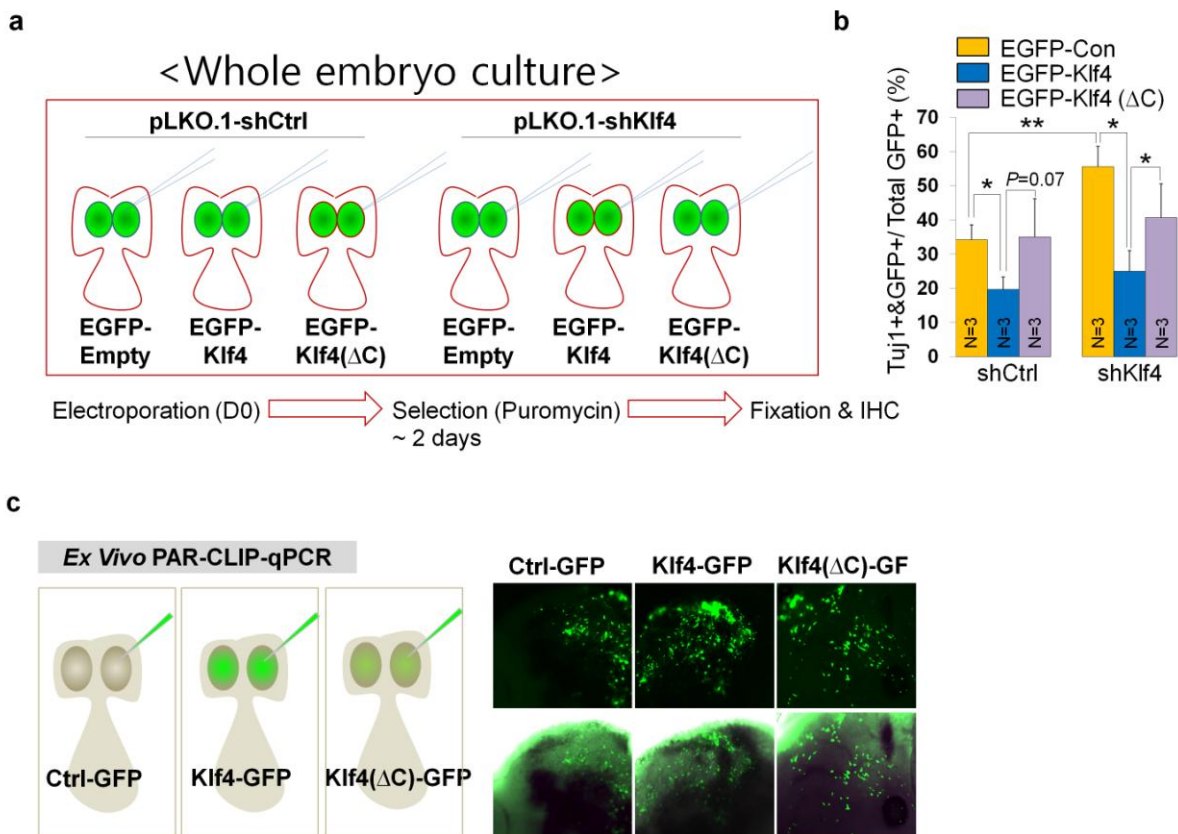
Supplementary Figure 11. *Klf4* promotes mRNA decay and inhibit neurogenesis by cooperating with *Stau1/Ddx5/17* complex. (a) Wild-type and *Klf4* cKO NPCs were treated with actinomycin D (5 μ g/ml) for indicated times and mRNAs were prepared for RNA stability assays (n=3). Values correspond to mean \pm SD. *t*-tests were performed to calculate significance

(*P < 0.05, **P < 0.005, ***P < 0.0005). **(b)** RT-qPCR of indicated transcripts in control- or Klf4-overexpressing NPCs infected with pLKO.1 shScramble or pLKO.1 shDdx5/17 lentivirus cultured for 0.2 or 2 days in differentiation conditions (n=3). Values correspond to mean±SD. Anova tests were performed to calculate significance (*P < 0.01, **P < 0.001, ***P < 0.0001). **(c)** Immunoblot of indicated antibodies in samples shown in (b) (n=2). See Supplementary Table 4. **(d)** Quantification of (c). **(e)** HEK293T cells were transfected with empty or Flag-Klf4 expression plasmids with control or HA-Stau1 vector. At 24 hours after transfection, lysates were immunoprecipitated with anti-IgG or anti-Mbd3 (n=1) and were analyzed by immunoblotting for indicated proteins. See also Supplementary Fig. 21.

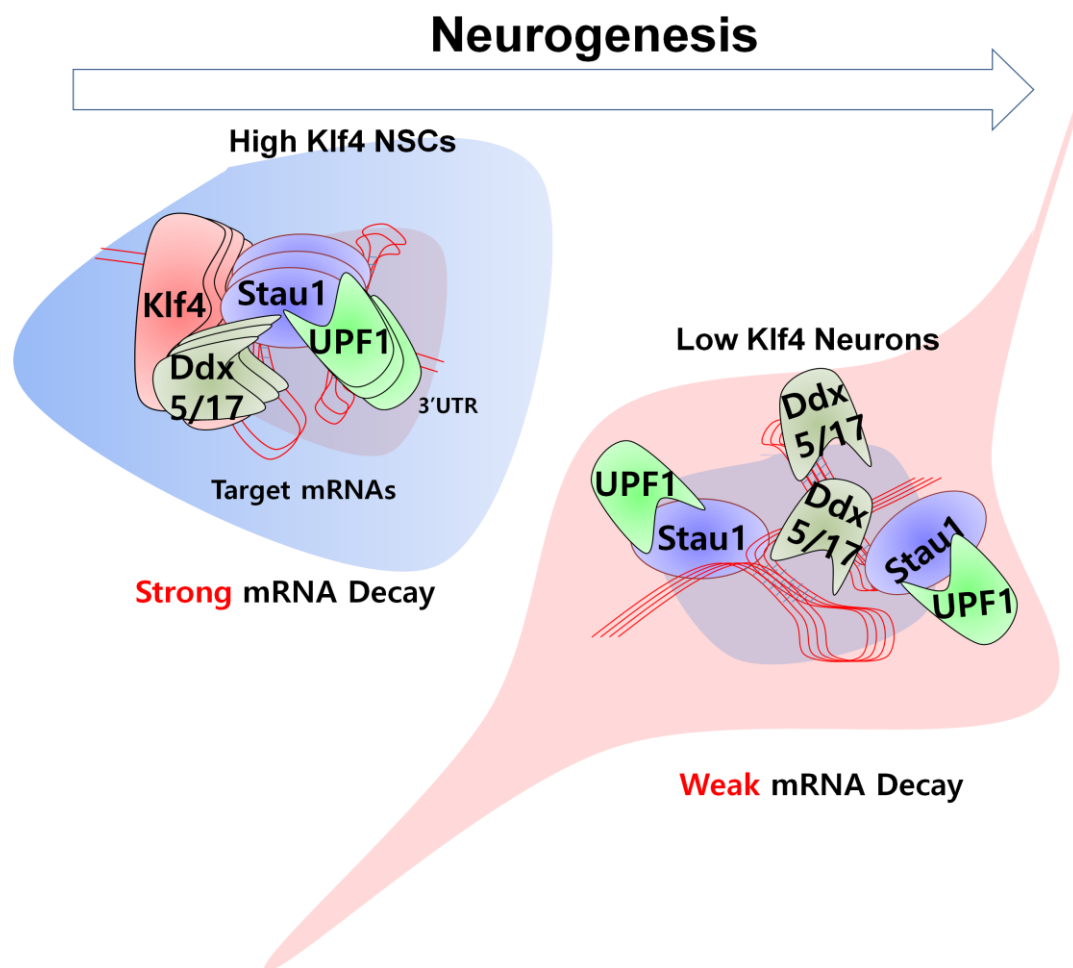


Supplementary Figure 12. Ddx5/17 double stranded RNA helicases are novel components

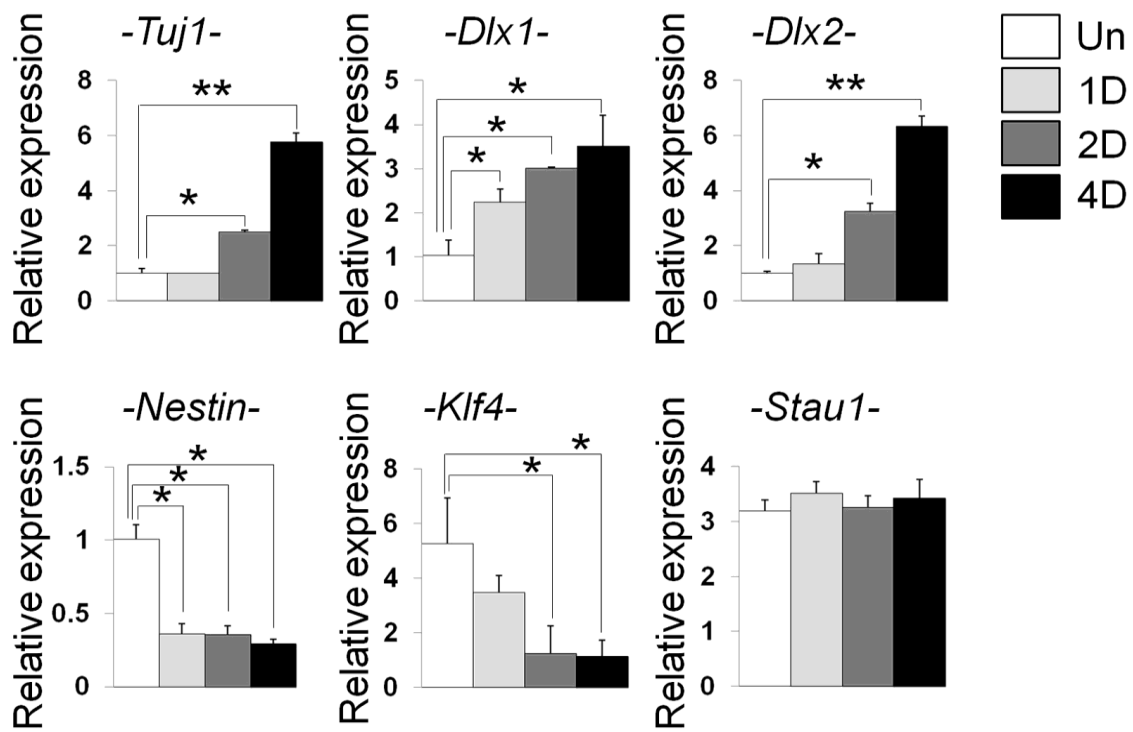
for SMD (a) RT-qPCR to assess the mRNA decay following Stau1 overexpression in NPCs transduced with shScramble or shDdx5/17 knockdown constructs. NPCs were treated with actinomycin D (5 $\mu\text{g/ml}$) for indicated times, and mRNAs were prepared for RNA stability assay (n=3). (b) RT-qPCR of indicated transcripts in control- or Stau1-overexpressing NPCs infected with pLKO.1 shScramble or pLKO.1 shDdx5/17 lentivirus cultured for 0.2 or 2 days in differentiation conditions (n=3). (c) PAR-CLIP qPCR to assess Stau1 enrichment on target mRNAs in NPCs transduced with shKif4 knockdown or shScramble constructs. Each values enriched by Stau1 protein were divided by IgG control. Data is presented as mean \pm SD. Anova tests were performed to calculate statistical significance (*P < 0.01, **P < 0.001, ***P < 0.0001). See Supplementary Table 4.



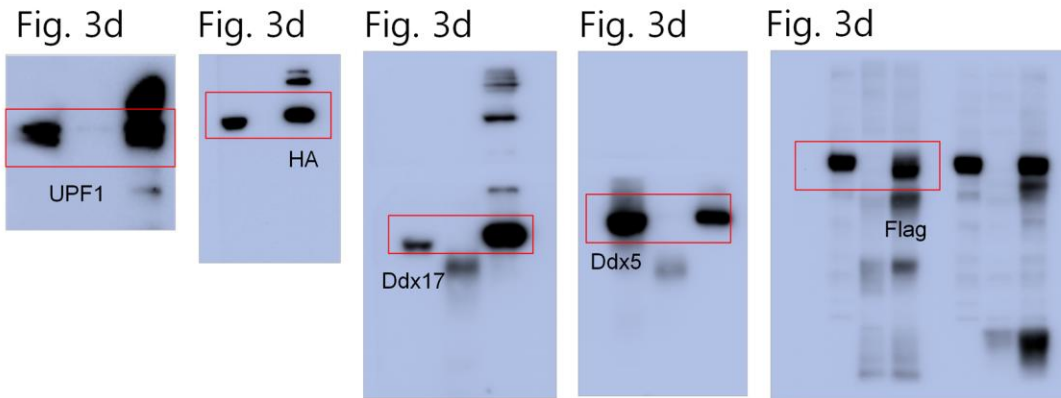
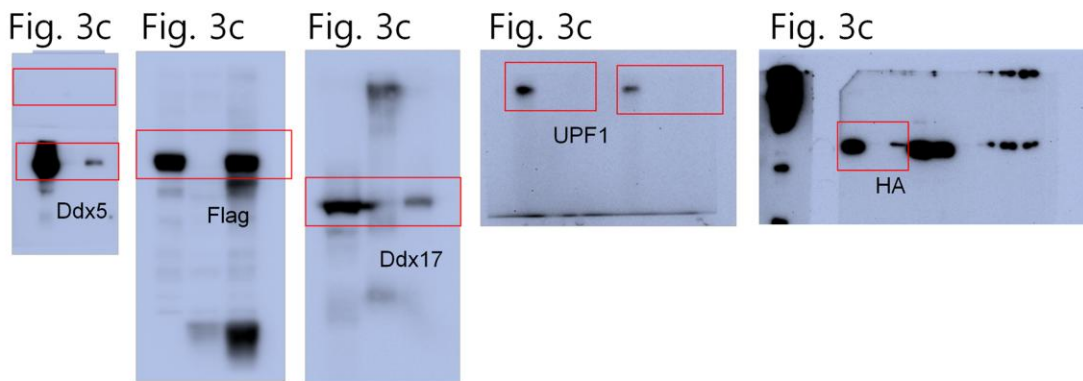
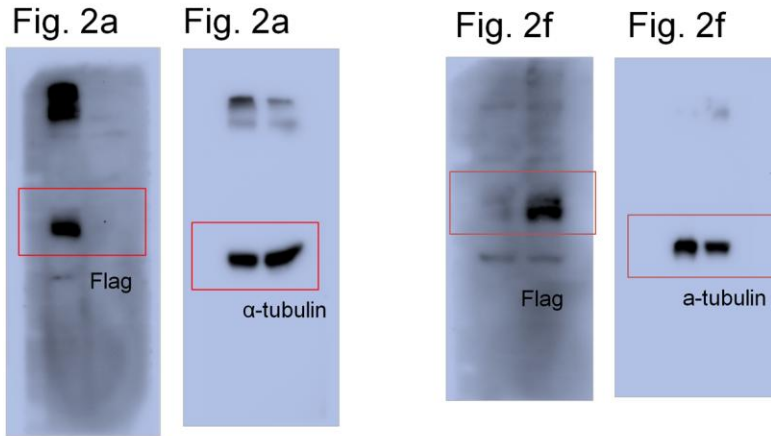
Supplementary Figure 13. Klf4 inhibits neurogenesis through formation of Klf4/Stau1 complex *ex vivo*. (a) Schematic of injection site (green) and experimental process for *ex utero* electroporation. pEGFP-Control, pEGFP-Klf4, or pEGFP-ΔC vectors were electroporated in mouse embryonic cortex together with shscramble or shKlf4 lentiviral vectors. (b) The effect of wild-type Klf4 or its mutant (ΔC) overexpression on neurogenesis was evaluated by counting Tuj1/EGFP double positive cells versus total EGFP-positive cells in VZ/SVZ/IMZ/CP compartments (n=3). (c) Schematic of PAR-CLIP-qPCR experiment in cortices electroporated with pEGFP-Control, pEGFP-Klf4, or pEGFP-ΔC constructs and grown in *ex vivo* condition for 2 days (2D).



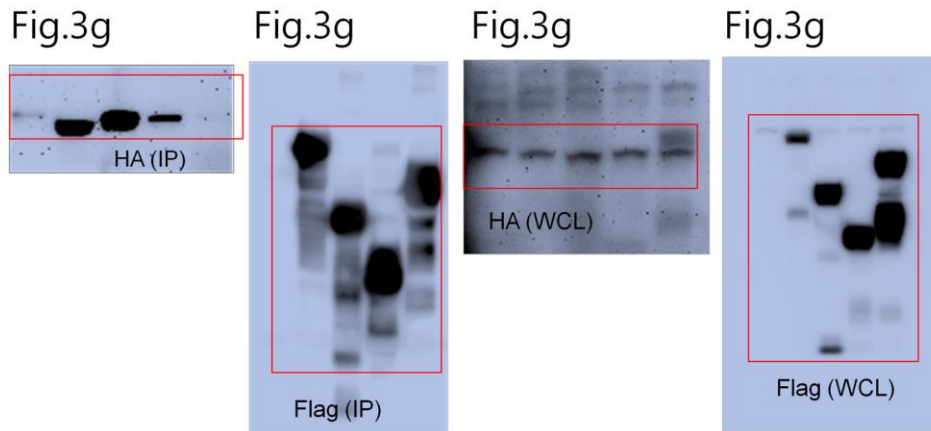
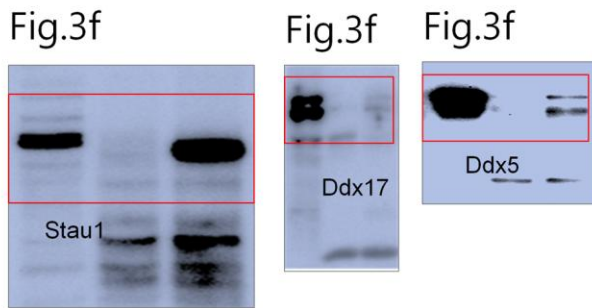
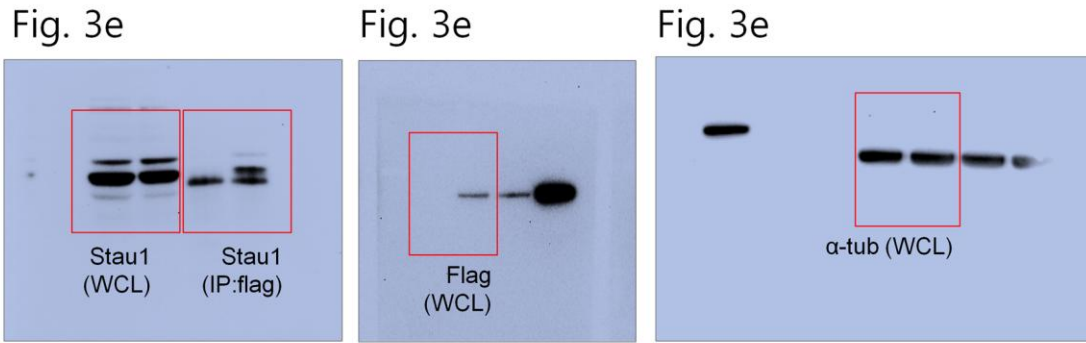
Supplementary Figure 14. Klf4/Ddx5/17 dependent Stau1 mediated mRNA decay (SMD) regulates cortical neurogenesis. A model of proposed function of the Klf4-Ddx5/17-Stau1 axis in NPC cell fate determination during neurogenesis.



Supplementary Figure 15. Klf4, but not Stau1, decreases during neural differentiation of NPCs. qPCR analysis of indicated mRNAs. Values correspond to the mean \pm SD. Diff. (d), days in differentiation. Statistical t-test analysis was performed to calculate significance (*P < 0.05, **P < 0.005, ns, P > 0.05).

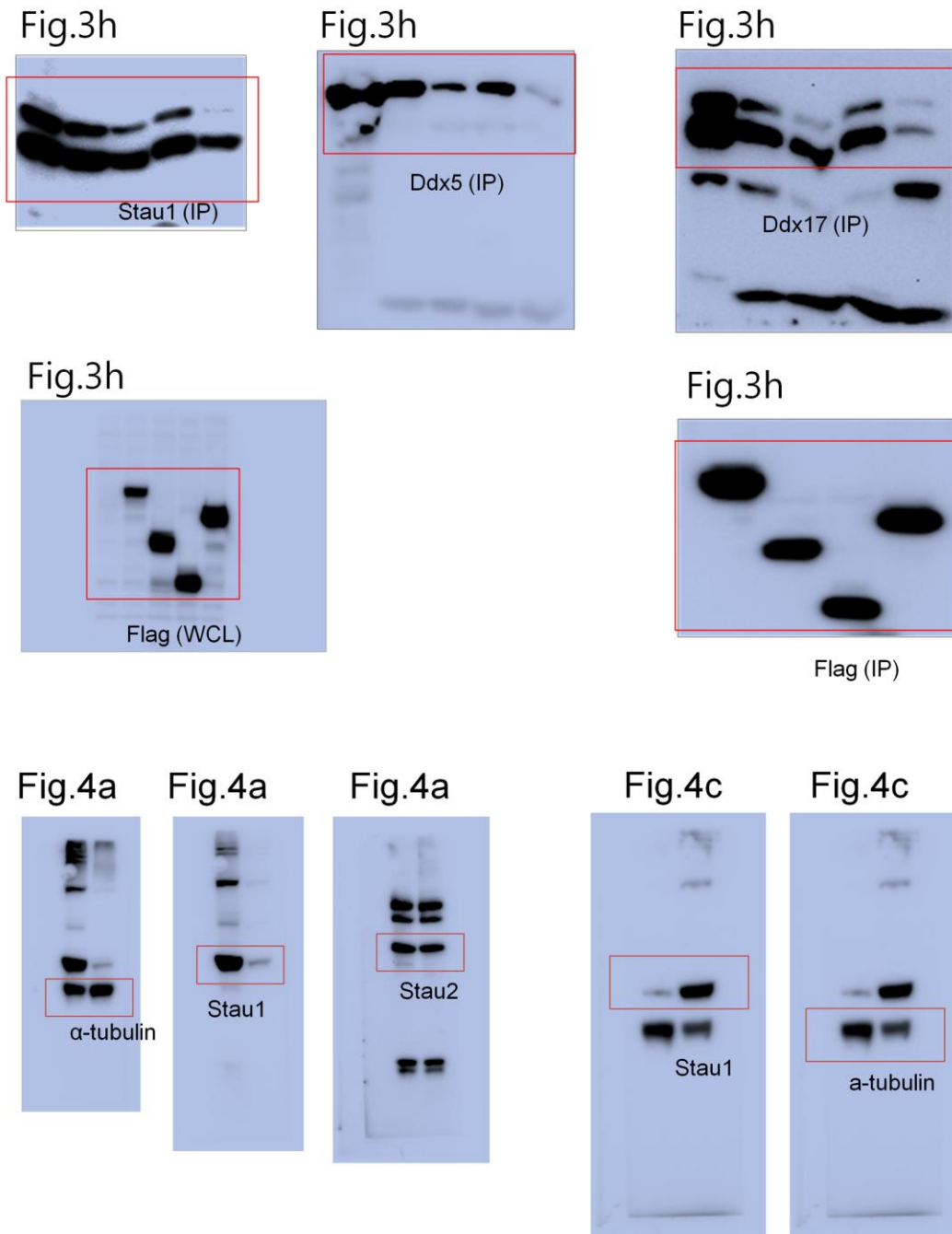


Supplementary Figure 16. Full blots from main figures. The full blots used for Figure 2a, f, 3c, d. The specific bands shown in the main figures are indicated by red boxes.



Supplementary Figure 17. Full blots from main figures. The full blots used for Figure 3e-g.

The specific bands shown in the main figures are indicated by red boxes.



Supplementary Figure 18. Full blots from main figures. The full blots used for Figure 3h, 4a, c. The specific bands shown in the main figures are indicated by red boxes.

Fig. S7a

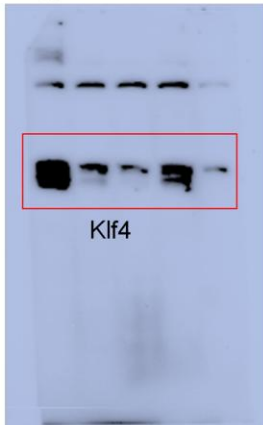


Fig. S7a



Fig. S7f



Fig. S7f

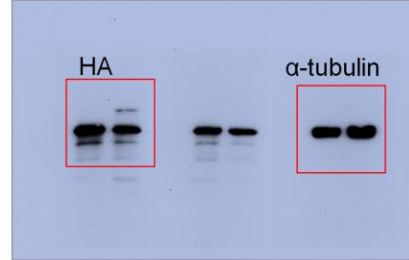


Fig. S7f



Fig. S7g

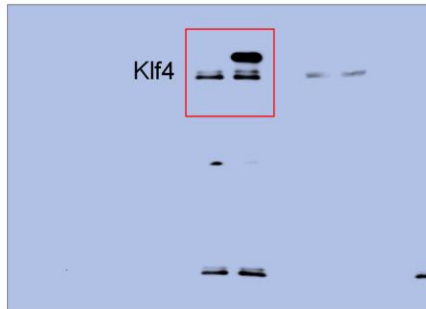


Fig. S7g

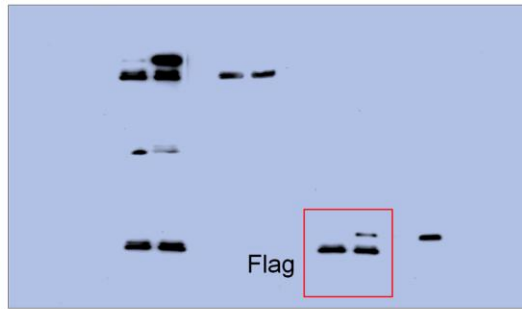


Fig. S7g

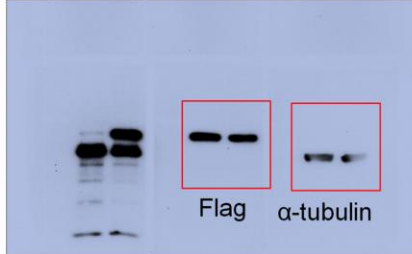
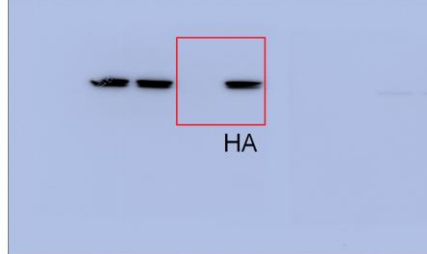
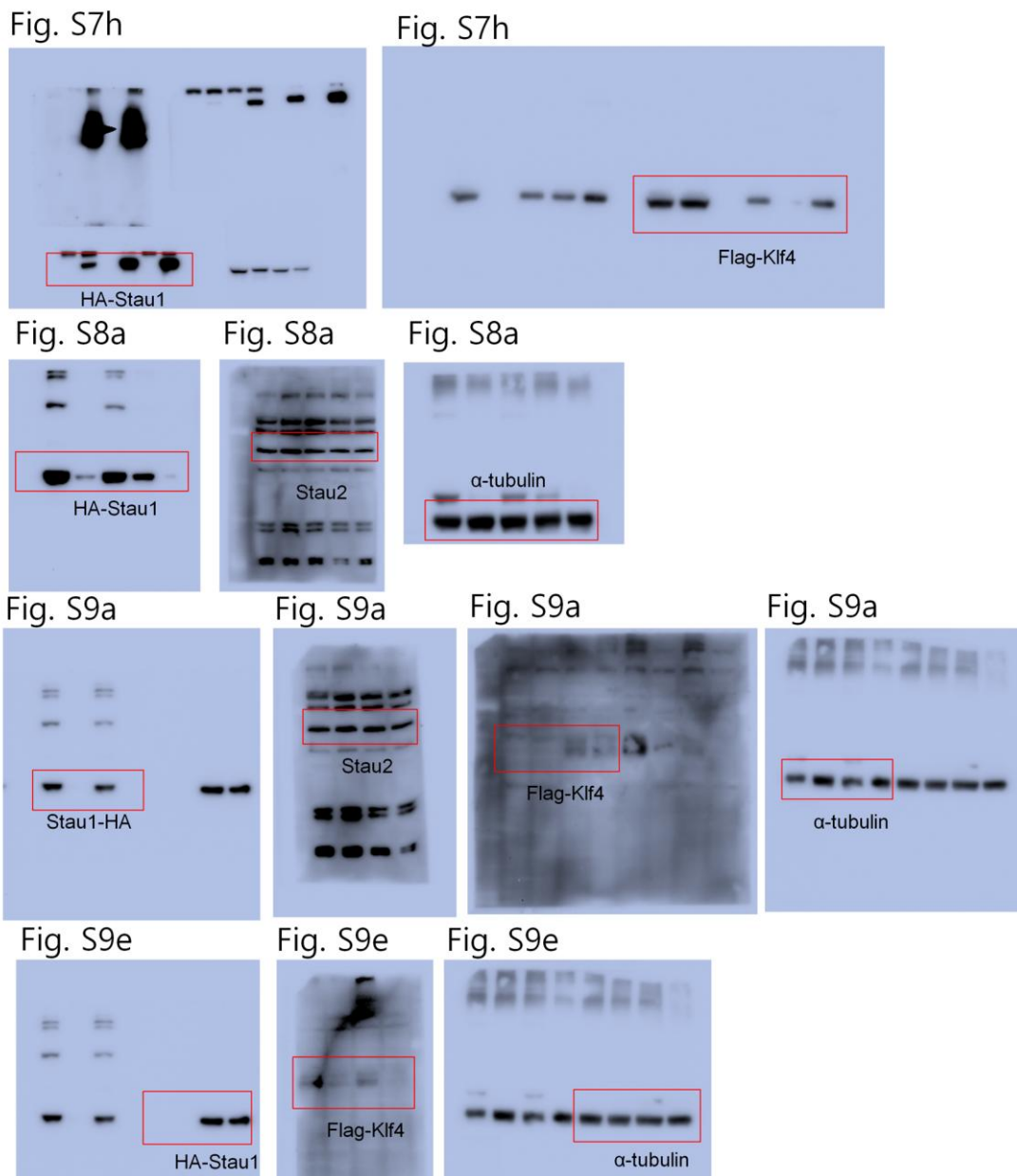


Fig. S7g



Supplementary Figure 19. Full blots from main figures. The full blots used for Supplementary Figure 7a, f, g. The specific bands shown in the main figures are indicated by red boxes.



Supplementary Figure 20. Full blots from main figures. The full blots used for Supplementary Figure 7h, 8a, 9a, e. The specific bands shown in the main figures are indicated by red boxes.

Fig. S11c

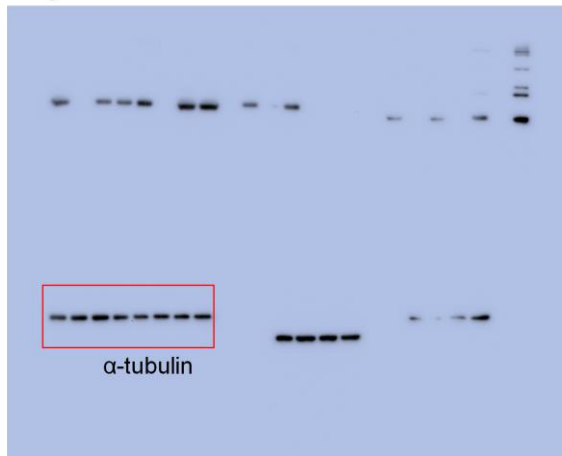


Fig. S11c

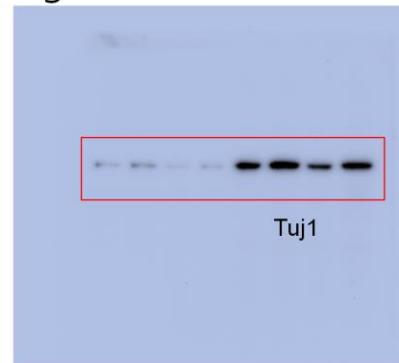


Fig. S11e

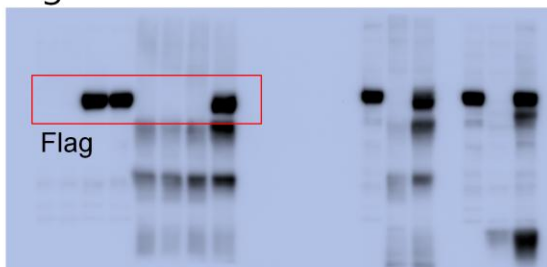


Fig. S11e

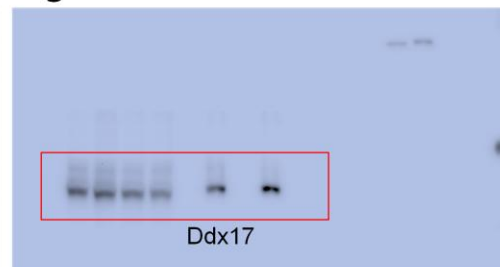
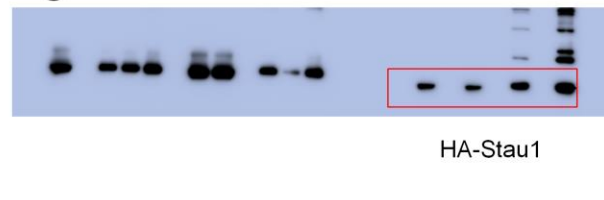


Fig. S11e



Fig. S11e



Supplementary Figure 21. Full blots from main figures. The full blots used for Supplementary Figure 11c, e. The specific bands shown in the main figures are indicated by red boxes.

Supplementary Table 1. Construction of pLKO3G shRNA and target sequences.

To knock down Klf4 and Stau1 expression in NSCs we generated pLKO.3G short hairpin (sh)RNA lentiviral vector system. Target sequences of Klf4 and Stau1 were chosen using RNAi consortium shRNA library (<http://www.broadinstitute.org/rnai/public/>) (three clones targeting different sequences in the three coding regions of Klf4 and Stau1 gene) against Klf4 and Stau1. To generate oligos for cloning, sense and antisense sequences of chosen target sequences were ordered from IDT. Sequence-verified shRNA lentiviral plasmid vectors for mouse Klf4 and Stau1 gene were cloned into the pLKO.3G vector and knockdown efficiency were measured by western blot (see Supplementary Fig. 7a).

For gene transfections we utilized Lipofectamine 3000 and Amaxa nucleofection (Lonza, <http://www.lonzabio.com/cell-biology/transfection/>) for the transfection of mouse NSCs. A range of knockdown efficiencies was observed. The shKlf4 (#1, #2 and #3) clones showed maximum efficiencies and three vectors were used for further experiments (see Fig. 2a). For shStau1 knockdown experiment, Two (we encoded it as shStau1 #1 and #3) out of three clones appeared to be efficiently knocking down Stau1 expression in NSCs (see Fig. 4a and Supplementary Fig. 8a) and both were used for further experiments. For shControl transfection, sequences were chosen based on Sigma-Aldrich shRNA products which had been previously validated as a non-target shRNA control. The plasmids containing the below sequences that does not target any gene, making it useful as a negative control in our experiments and appear no change Klf4 or Stau1 expression (see Supplementary Fig. 7a and 8a).

Gene	shKlf4 #1	Target region
Target sequence	CATGTTCTAACAGCCTAAATG	3' UTR
Ordered oligo	AATTCATGTTCTAACAGCCTAAATGCTCGAGCATTTAGGCTGTTAGAACATGTTTTTTAT	
Gene	shKlf4 #2	
Target sequence	TTGTGGATATCAGGGTATAAA	3' UTR
Ordered oligo	AATTTTGTGGATATCAGGGTATAAACTCGAGTTTATACCCTGATATCCACAATTTTTTTAT	
Gene	shKlf4 #3	
Target sequence	CTCTCTCACATGAAGCGACTT	CDS
Ordered oligo	AATTCCTCTCACATGAAGCGACTTCTCGAGAAGTCGCTTCATGTGAGAGAGTTTTTTAT	
Gene	shStau1 #1	
Target sequence	ATTGCGCTGAAGCGGAATTTG	CDS
Ordered oligo	AATTATTGCGCTGAAGCGGAATTTGCTCGAGCAAATTCGCTTCAGCGCAATTTTTTTAT	
Gene	shStau1 #2	
Target sequence	GCCAAGGGATGAATCCTATTA	CDS
Ordered oligo	AATTGCCAAGGGATGAATCCTATTACTCGAGTAATAGGATTCATCCCTTGGCTTTTTTTAT	
Gene	shStau1 #3	
Target sequence	CCAGGGATTCCAGGTTGAATA	CDS
Ordered oligo	AATTCAGGGATTCCAGGTTGAATACTCGAGTATTCAACCTGGAATCCCTGGTTTTTTAT	

Supplementary Table 2. Primer sequences used for quantitative or RT-PCR analysis.

Genes	Primer Sequences	Tm (°C)
<i>Nestin</i>	5'-CCCTGAAGTCGAGGAGCTG-3'	57.4
	5'-CTGCTGCACCTCTAAGCGA-3'	57.3
<i>Dlx1</i>	5'-ATGCCAGAAAGTCTCAACAGC-3'	60.6
	5'-AACAGTGCATGGAGTAGTGCC-3'	62.4
<i>Dlx2</i>	5'-AAAGAAAGTCCGGAACACG-3'	60.1
	5'-TCTTCTTGAACCTGCATCGGC-3'	60.5
<i>Tuj1</i>	5'-TAGACCCCAGCGGCAACTAT-3'	58.2
	5'-GTTCCAGGTTCCAAGTCCACC-3'	58.0
<i>Gad67</i>	5'-GCCACAAACTCAGCGGCATAGAAA-3'	60.0
	5'-AGACGTCATACTGCTTGTCTGGCT-3'	60.0
<i>NeuN</i>	5'-GAAACCGCAAGCCCTCATTTTC-3'	60.1
	5'-TTGGATGCCTCTTGGTTTGGT-3'	60
<i>Pitx3</i>	5'-TGCGCTGTCGTTATCGGAC-3'	62.4
	5'-GGTAGCGATTCTCTGGAAGG-3'	61.7
<i>Pax6</i>	5'-AACCCACGCAAGATGGCTG-3'	62.9
	5'-GCATCCCAGTGCATAAAAACCA-3'	61.4
<i>Dat</i>	5'-AAATGCTCCGTGGGACCAATG-3'	63
	5'-GTCTCCCGCTCTTGAACCTC-3'	61.9
<i>Lmx1a</i>	5'-ACGGCCTGAAGATGGAGGA-3'	62.3
	5'-CAGAAACCTGTCCGAGATGAC-3'	60.1
<i>Foxa2</i>	5'-CCCTACGCCAACATGAACTCG-3'	63
	5'-GTTCTGCCGGTAGAAAGGGA-3'	61.2
<i>En1</i>	5'-CTAAGGCCCGATTTTCGGTTG-3'	60.8
	5'-GAGTGAACGGGTCTCTACCT-3'	62.4
<i>Klf4</i>	5'-GTGCCCCGACTAACCGTTG-3'	63
	5'-GTCGTTGAACTCCTCGGTCT-3'	61.2
<i>Staufen1</i>	5'-GGACCCTCACTCTCGGATG-3'	60.8
	5'-TTCTGGCAGGGTTCACTCT-3'	62.7
<i>Staufen2</i>	5'-AGTGACCTCTGGCACAACCTCT-3'	62.8
	5'-TGGCTTCAGCAGTAGGAGATG-3'	61.3
<i>Gapdh</i>	5'-AGGTCGGTGTGAACGGATTTG-3'	57.6
	5'-TGTAGACCATGTAGTTGAGGTCA-3'	55.1

Gapdh : Glyceraldehyde-3-phosphate dehydrogenase

Supplementary Table 3. Primer sequences used for mRNA decay and PAR-CLIP qPCR analysis.

Gene	Sequence	Tm (°C)	GC (%)
mTuj1-CLIP(1-F)	AGTTGCTCGCAGCTGGGGTGT	58.2	55
mTuj1-CLIP(1-R)	AAAGCTGGGGGCAGTGTC	59.7	57.9
mTuj1-CLIP(2-F)	CTTCTCACCAGCTCATTAGG	52.5	50
mTuj1-CLIP(2-R)	ACAGAGGTGGCTAAAATGGG	54.9	50
mTuj1-CLIP(3-F)	CCCATTTTAGCCACCTCTGT	54.9	50
mTuj1-CLIP(3-R)	CTGACAGACAGCAGTAAC	50	50
mDlx1-CLIP(1-F)	GGTATCAGTTCGCACTCAC	53.4	50
mDlx1-CLIP(1-R)	AAGCCCTCTGCCTTCACTGTT	54	52
mDlx1-CLIP(2-F)	TGCTTCTCGGAGCCCCTAAAA	58.6	52.4
mDlx1-CLIP(2-R)	AAGATGAGAGTCTGGTCCGG	55.7	55
mDlx1-CLIP(3-F)	CCGGACCAGACTTCTCATCTT	56	55
mDlx1-CLIP(3-R)	GGTCGTAGAATGAGGACAGC	55	55
mDlx1-CLIP(4-F)	CTCCAAAAAGTCACCAGGTCTG	55.7	50
mDlx1-CLIP(4-R)	TCAGCGTCAGATGAAGTCTG	54.3	50
mDlx1-CLIP(5-F)	CAGACTTCATCTGACGCTGA	54.3	50
mDlx1-CLIP(5-R)	ACTTTCCTGTCCTTGTTCCT	54.7	50
mDlx1-CLIP(6-F)	GGAACAAGGACAGGAAAG	50.3	50
mDlx1-CLIP(6-R)	CCAGGTGTTCTTGTCCAAACG	56.4	52.4
mDlx1-CLIP(7-F)	GAACACCTGGCCAAGAAG	53.5	55.6
mDlx1-CLIP(7-R)	GCCGCTGCTTGTGTCTTACT	58	55
mDlx1-CLIP(8-F)	TCGACCACAGAACAACAAGTC	54.7	50
mDlx1-CLIP(8-R)	TCGGGTTTACAGCCACACAC	57.7	55
mDlx1-CLIP(9-F)	GTGTGTGGCTGTAAACCCGA	57.7	55
mDlx1-CLIP(9-R)	CTTATTTACAAAGTTCGCCCC	54.3	45.5
mDlx2-CLIP(1-F)	TCGAGTGGACAGCGTCTGA	57.9	58.2
mDlx2-CLIP(1-R)	AGAAGTGGCTCCACGAC	54.5	58.8
mDlx2-CLIP(2-F)	GTCGTGGAGCCACTTCT	54.5	58.8
mDlx2-CLIP(2-R)	ACCCTGAGGATCACGCTGA	58.3	57.9
mDlx2-CLIP(3-F)	TCAGCGTGATCCTCAGGGT	58.3	57.9
mDlx2-CLIP(3-R)	TGGAGTAGGACCCAGGAG	55.3	61.1
mDlx2-CLIP(4-F)	ACCTCCTGGGTCTACTCCA	59.6	60
mDlx2-CLIP(4-R)	GGTCATCCGCAAAGGCACCTA	59.8	57.1
mDlx2-CLIP(5-F)	TAGGTGCCTTTGCGGATGACC	59.8	57.1
mDlx2-CLIP(5-R)	GGGAAATCTGCACAGACACC	53.6	52.6
mDlx2-CLIP(6-F)	GGTGTCTGTGCAGATTTCCCC	58.3	57.1

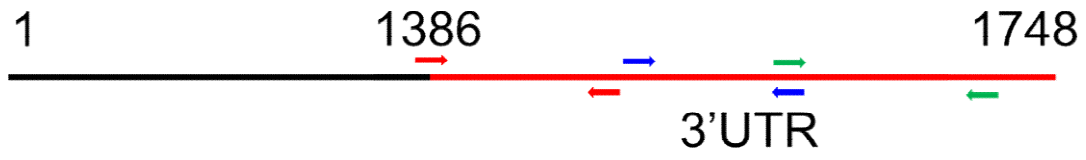
mDlx2-CLIP(6-R)

ATAGGGACTGCTGAGGTCCTG

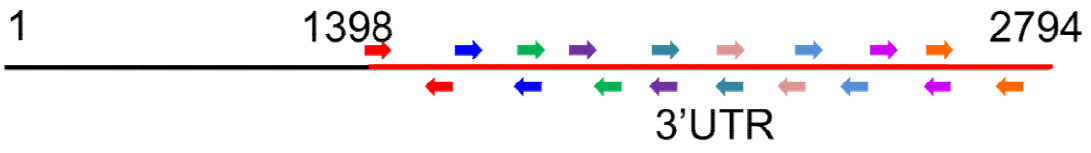
58.2

54.5

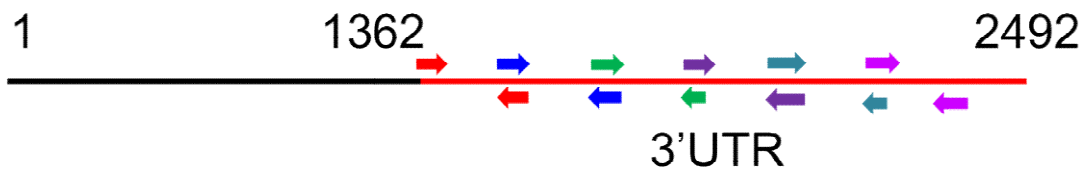
m*Tuj1*-CLIP



m*Dlx1*-CLIP



m*Dlx2*-CLIP



Supplementary Table 4. Values and parameters for the statistical comparisons shown in main and supplementary figures

Fig.1F

Klf6 gene	Table Analyzed	Date 1	Dlx7 gene	Table Analyzed	Date 1	Dlx2 gene	Table Analyzed	Date 1			
One-way analysis of variance			One-way analysis of variance			One-way analysis of variance					
P value	< 0.0001		P value	< 0.0002		P value	< 0.0001				
P value summary	***		P value summary	***		P value summary	***				
Are means signif. different	Yes		Are means signif. different	Yes		Are means signif. different	Yes				
Number of groups	4		Number of groups	4		Number of groups	4				
F	39.81		F	24.67		F	130.1				
R squared	0.9372		R squared	0.9024		R squared	0.9799				
ANOVA Table			ANOVA Table			ANOVA Table					
SS		df	MS			SS		df	MS		
Treatment (between colu)	45.17	3	15.06	Treatment (between colu)	4184	3	1395	Treatment (between colu)	42130	3	14040
Residual (within columns)	3.020	3	0.3783	Residual (within columns)	452.3	8	56.53	Residual (within columns)	863.7	8	108
Total	48.2	11		Total	4636	11		Total	42993	11	
Tukey's Multiple Comparison	Mean Diff.	q	Significant	Summary	95% CI of diff.	Tukey's Multiple Comparison	Mean Diff.	q	Significant	Summary	95% CI of diff.
WT-Uh vs cKO-Uh	4.103	11.59	Yes	***	2.498 to 5.714	WT-Uh vs cKO-Uh	-12.27	2.936	No	ns	-32.41 to 6.913
WT-Uh vs WT-ZDV	0.7079	1.991	No	ns	-0.9003 to 2.316	WT-Uh vs WT-ZDV	-8.86	2.04	No	ns	-28.52 to 10.80
WT-Uh vs cKO-ZDV	4.281	12.09	Yes	***	2.665 to 5.902	WT-Uh vs cKO-ZDV	-48.96	11.26	Yes	***	-68.64 to -29.32
cKO-Uh vs WT-ZDV	-3.389	9.569	Yes	***	-5.690 to -1.700	cKO-Uh vs WT-ZDV	3.881	0.8892	No	ns	-15.77 to 23.55
cKO-Uh vs cKO-ZDV	-0.8737	0.5209	No	ns	-1.420 to -0.776	cKO-Uh vs cKO-ZDV	38.52	8.342	Yes	**	40.96 to -16.57
WT-ZDV vs cKO-ZDV	3.865	10.1	Yes	***	1.978 to 5.194	WT-ZDV vs cKO-ZDV	-40.12	9.245	Yes	***	-59.78 to -20.46

Fig.4E

Dlx7 gene	Table Analyzed	Date 1	Sox9 gene	Table Analyzed	Date 1	Sox9 gene	Table Analyzed	Date 1			
One-way analysis of variance			One-way analysis of variance			One-way analysis of variance					
P value	< 0.0001		P value	< 0.0001		P value	< 0.0001				
P value summary	***		P value summary	***		P value summary	***				
Are means signif. different	Yes		Are means signif. different	Yes		Are means signif. different	Yes				
Number of groups	4		Number of groups	4		Number of groups	4				
F	56.15		F	270		F	66.2				
R squared	0.9547		R squared	0.9902		R squared	0.9613				
ANOVA Table			ANOVA Table			ANOVA Table					
SS		df	MS	SS		df	MS				
Treatment (between colu)	12.43	3	4.142	Treatment (between colu)	2229	3	743.1	Treatment (between colu)	25.2	3	8.601
Residual (within columns)	0.93	3	0.307375	Residual (within columns)	220.2	8	27.52	Residual (within columns)	1.039	8	0.1299
Total	13.37	11		Total	2251.2	11		Total	26.84	11	
Tukey's Multiple Comparison	Mean Diff.	q	Significant	Summary	95% CI of diff.	Tukey's Multiple Comparison	Mean Diff.	q	Significant	Summary	95% CI of diff.
WT-Uh vs cKO-Uh	1.373	3.75	No	ns	-0.8028 to 3.4965	WT-Uh vs cKO-Uh	-0.7716	3.707	No	ns	-1.714 to 0.1710
WT-Uh vs WT-ZDV	-0.6963	4.433	No	*	-1.406 to 0.01424	WT-Uh vs WT-ZDV	-2.136	10.26	Yes	***	-3.079 to -1.914
WT-Uh vs cKO-ZDV	-2.581	16.45	Yes	***	-3.291 to -1.871	WT-Uh vs cKO-ZDV	-3.859	18.54	Yes	***	-4.801 to -2.918
cKO-Uh vs WT-ZDV	-0.4802	3.063	No	ns	-1.190 to 0.2299	cKO-Uh vs WT-ZDV	-1.366	6.957	Yes	**	-2.307 to -0.4221
cKO-Uh vs cKO-ZDV	-2.365	10.09	Yes	***	-3.075 to -1.655	cKO-Uh vs cKO-ZDV	-3.097	14.83	Yes	***	-4.030 to -2.144
WT-ZDV vs cKO-ZDV	-1.885	12.69	Yes	***	-2.695 to -1.075	WT-ZDV vs cKO-ZDV	-1.722	8.276	Yes	**	-2.666 to -0.7798

Fig. 4F

Figure 68

One way Anova test (Tukey's multiple comparison test)									
0101	Site Analyzed	01							
One-way analysis of variance									
F value	0.036								
F value summary	---								
Are means equal (default) (P < 0.05)	Yes								
Number of groups	4								
F	0.0082								
R squared	0.0009								
ANOVA Table	SS	df	MS						
Treatment (between columns)	0.00129	3	0.00043						
Residual (within columns)	0.226	8	0.02825						
Total	0.2341	11							
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff				
01v	01Cin vs 01C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
01v	01Cin vs 01B	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
01v	01Cin vs 01B&01C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
01v	01C vs 01B	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
01v	01C vs 01B&01C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
01v	01B vs 01B&01C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
0102	Site Analyzed	02							
One-way analysis of variance									
F value	0.432								
F value summary	---								
Are means equal (default) (P < 0.05)	Yes								
Number of groups	4								
F	1.072								
R squared	0.0369								
ANOVA Table	SS	df	MS						
Treatment (between columns)	0.00272	3	0.00091						
Residual (within columns)	0.2627	8	0.03287						
Total	0.2654	11							
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff				
02v	02Cin vs 02C	-0.020	2.013	Yes	HA	-0.0410 to 0.0010			
02v	02Cin vs 02B	-0.020	2.013	Yes	HA	-0.0410 to 0.0010			
02v	02Cin vs 02B&02C	-0.020	2.013	Yes	HA	-0.0410 to 0.0010			
02v	02C vs 02B	-0.020	2.013	Yes	HA	-0.0410 to 0.0010			
02v	02C vs 02B&02C	-0.020	2.013	Yes	HA	-0.0410 to 0.0010			
02v	02B vs 02B&02C	-0.020	2.013	Yes	HA	-0.0410 to 0.0010			
0103	Site Analyzed	03							
One-way analysis of variance									
F value	0.922								
F value summary	---								
Are means equal (default) (P < 0.05)	Yes								
Number of groups	4								
F	0.872								
R squared	0.0333								
ANOVA Table	SS	df	MS						
Treatment (between columns)	0.00274	3	0.00091						
Residual (within columns)	0.2647	8	0.03309						
Total	0.2674	11							
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff				
03v	03Cin vs 03C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
03v	03Cin vs 03B	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
03v	03Cin vs 03B&03C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
03v	03C vs 03B	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
03v	03C vs 03B&03C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
03v	03B vs 03B&03C	-0.00011	0.0007	No	HA	-0.0445 to 0.0396			
0104	Site Analyzed	04							
One-way analysis of variance									
F value	0.000								
F value summary	---								
Are means equal (default) (P < 0.05)	Yes								
Number of groups	4								
F	0.0000								
R squared	0.0000								
ANOVA Table	SS	df	MS						
Treatment (between columns)	0.00000	3	0.00000						
Residual (within columns)	0.00000	8	0.00000						
Total	0.00000	11							
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff				
04v	04Cin vs 04C	-0.00000	0.00000	No	HA	-0.00000 to 0.00000			
04v	04Cin vs 04B	-0.00000	0.00000	No	HA	-0.00000 to 0.00000			
04v	04Cin vs 04B&04C	-0.00000	0.00000	No	HA	-0.00000 to 0.00000			
04v	04C vs 04B	-0.00000	0.00000	No	HA	-0.00000 to 0.00000			
04v	04C vs 04B&04C	-0.00000	0.00000	No	HA	-0.00000 to 0.00000			
04v	04B vs 04B&04C	-0.00000	0.00000	No	HA	-0.00000 to 0.00000			

Fig. 6C

Fig. 7A

Table Analyzed	Data 1			
One-way analysis of variance				
P value	< 0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	6			
F	17.49			
R squared	0.862			
ANOVA Table	SS	df	MS	
Treatment (between columns)	8065	5	1613	
Residual (within columns)	1291	14	92.21	
Total	9355	19		
Tukey's Multiple Comparison Test				
	Mean Diff.	q	Significant? Summary	95% CI of diff
Klf4KONSC GFP-control (Un) vs Klf4KONSC GFP-mKlf4(Un)	29.87	6.222	Yes **	7.599 to 52.15
Klf4KONSC GFP-control (Un) vs Klf4KONSC GFP-dC(Un)	12.71	2.647	No ns	-9.566 to 34.98
Klf4KONSC GFP-control (Un) vs Klf4KONSC GFP-control (2D)	52.16	10.06	Yes ***	28.10 to 76.22
Klf4KONSC GFP-control (Un) vs Klf4KONSC GFP-mKlf4(2D)	49.66	9.576	Yes ***	25.60 to 73.72
Klf4KONSC GFP-control (Un) vs Klf4KONSC GFP-dC(2D)	48.35	8.223	Yes ***	21.07 to 75.63
Klf4KONSC GFP-mKlf4(Un) vs Klf4KONSC GFP-dC(Un)	-17.17	3.575	No ns	-39.44 to 5.108
Klf4KONSC GFP-mKlf4(Un) vs Klf4KONSC GFP-control (2D)	22.29	4.297	No ns	-1.771 to 46.34
Klf4KONSC GFP-mKlf4(Un) vs Klf4KONSC GFP-mKlf4(2D)	19.79	3.816	No ns	-4.271 to 43.84
Klf4KONSC GFP-mKlf4(Un) vs Klf4KONSC GFP-dC(2D)	18.48	3.143	No ns	-8.799 to 45.76
Klf4KONSC GFP-dC(Un) vs Klf4KONSC GFP-control (2D)	39.45	7.607	Yes **	15.39 to 63.51
Klf4KONSC GFP-dC(Un) vs Klf4KONSC GFP-mKlf4(2D)	36.95	7.125	Yes **	12.89 to 61.01
Klf4KONSC GFP-dC(Un) vs Klf4KONSC GFP-dC(2D)	35.65	6.062	Yes **	8.367 to 62.92
Klf4KONSC GFP-control (2D) vs Klf4KONSC GFP-mKlf4(2D)	-2.499	0.4508	No ns	-28.22 to 23.22
Klf4KONSC GFP-control (2D) vs Klf4KONSC GFP-dC(2D)	-3.806	0.6141	No ns	-32.56 to 24.95
Klf4KONSC GFP-mKlf4(2D) vs Klf4KONSC GFP-dC(2D)	-1.307	0.2109	No ns	-30.06 to 27.45

Fig.7B

Fig. S9D

shCon&Con OE	mCherry& Tu1&mCherry&GFP	Tuj1&mCherry&GFP	Tuj1&mCherry&GFP/mCherry&GFP	#1	#2	#3	#4	#5	#6
5	2	40	40	25	50	14.28571428	40		
4	2	50	50	25	50	28.57142857	57.14285714	16.66666667	50
7	2	28.57143	28.57143	#3	40	60	20	42.85714286	50
				#4					
				#5					
shStau1&Con OE	4	2	50						40
	7	4	57.14286						50
	5	3	60						50
shCon&Klf4 OE	7	1	14.28571						40
	6	1	16.66667						50
	5	1	20						40
shStau1&Klf4 OE	10	4	40						50
	6	3	42.85714						40
	4	2	50						40
	5	2	40						50
	4	2	50						40

Table Analyzed	Date
1	1

One-way analysis of variance	P value	P value summary	Are means signif. different? (P < 0.05)	Number of groups	F	R squared
One-way analysis of variance	< 0.0001	***	Yes	4	31.19	0.8948

ANOVA Table	SS	df	MS
Treatment (between columns)	2727	3	908.9
Residual (within columns)	320.6	11	29.14
Total	3047	14	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant?	Summary	95% CI of diff
shCon&Con OE vs shStau1&Con OE	-24.52	7.868	Yes	**	-40.74 to -8.311
shCon&Con OE vs shCon&Klf4 OE	14.21	4.558	No	ns	-2.007 to 30.42
shCon&Con OE vs shStau1&Klf4 OE	-14.29	5.293	Yes	*	-28.33 to -0.2447
shStau1&Con OE vs shCon&Klf4 OE	38.73	12.43	Yes	***	22.52 to 54.94
shStau1&Con OE vs shStau1&Klf4 OE	10.24	3.793	No	ns	-3.803 to 24.28
shCon&Klf4 OE vs shStau1&Klf4 OE	-28.49	10.56	Yes	***	-42.53 to -14.45

Fig. S11B

shCon&Con OE	mCherry& Tu1&mCherry&GFP	Tuj1&mCherry&GFP	Tuj1&mCherry&GFP/mCherry&GFP	#1	#2	#3	#4	#5	#6
5	2	40	40	25	50	14.28571428	40		
4	2	50	50	25	50	28.57142857	57.14285714	16.66666667	50
7	2	28.57143	28.57143	#3	40	60	20	42.85714286	50
				#4					
				#5					
shStau1&Con OE	4	2	50						40
	7	4	57.14286						50
	5	3	60						50
shCon&Klf4 OE	7	1	14.28571						40
	6	1	16.66667						50
	5	1	20						40
shStau1&Klf4 OE	10	4	40						50
	6	3	42.85714						40
	4	2	50						40
	5	2	40						50
	4	2	50						40

Table Analyzed	Date
1	1

One-way analysis of variance	P value	P value summary	Are means signif. different? (P < 0.05)	Number of groups	F	R squared
One-way analysis of variance	< 0.0001	***	Yes	4	31.19	0.8948

ANOVA Table	SS	df	MS
Treatment (between columns)	2727	3	908.9
Residual (within columns)	320.6	11	29.14
Total	3047	14	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant?	Summary	95% CI of diff
shCon&Con OE vs shStau1&Con OE	-24.52	7.868	Yes	**	-40.74 to -8.311
shCon&Con OE vs shCon&Klf4 OE	14.21	4.558	No	ns	-2.007 to 30.42
shCon&Con OE vs shStau1&Klf4 OE	-14.29	5.293	Yes	*	-28.33 to -0.2447
shStau1&Con OE vs shCon&Klf4 OE	38.73	12.43	Yes	***	22.52 to 54.94
shStau1&Con OE vs shStau1&Klf4 OE	10.24	3.793	No	ns	-3.803 to 24.28
shCon&Klf4 OE vs shStau1&Klf4 OE	-28.49	10.56	Yes	***	-42.53 to -14.45

Fig. S12A

Supplementary References

1. Sugimoto, Y. *et al.* hiCLIP reveals the in vivo atlas of mRNA secondary structures recognized by Staufen 1. *Nature* **519**, 491-494 (2015).
2. Ricci, E.P. *et al.* Staufen1 senses overall transcript secondary structure to regulate translation. *Nat. Struct. Mol. Biol.* **21**, 26-35 (2014).