Supplementary Figures

Figure S1

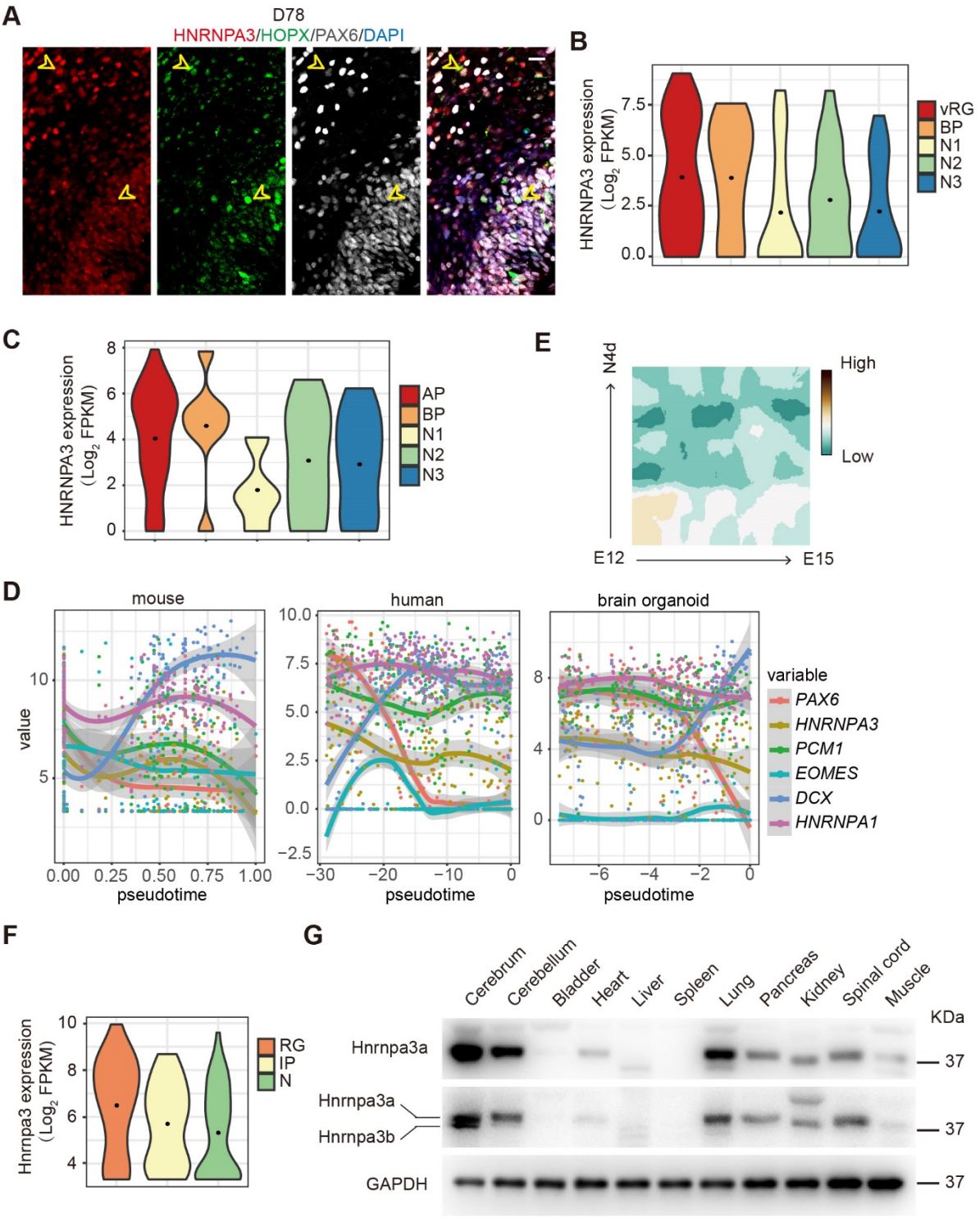

Fig. S1. Gene expression dynamics for *HNRNPA3* at various differentiation status. (**A**) Cerebral organoids at D78 were stained with antibodies against HNRNPA3 (red), HOPX (green) and PAX6 (grey). Arrowheads indicated cells co-labeled by HNRNPA3 and HOPX antibodies. Scale bar, 50 μm. (**B, C, F**) Violin plots showing the expression of *HNRNPA3*

mRNA in various cell types from GW12–13 human cortex (B), cultured human cerebral organoids (C) or developmental mouse cortex (F) from reported scRNA-seq datasets: https://bioinf.eva.mpg.de/shiny/sample-apps/ShinyCortex/. vRG, ventricular RG; AP, apical progenitor; BP, basal progenitor; N1, newborn neuron; N2, intermediate neuron; N3, mature neuron; IP, intermediate progenitor; N, neuron. (**D**) Expression waves of *HNRNPA3* and other indicated genes across developmental pseudotime in the mouse or human fetal brain or cultured human brain organoids. Data were retrieved from the online resource ShinyCortex: https://bioinf.eva.mpg.de/shiny/sample-apps/ShinyCortex/. (**E**) Spatial-temporal expression of *Hnrnpa3* in mouse AP along the birthdate axis (i.e., from E12 to E15) and differentiation axis. N4d, 4-day-old neurons. Data was retrieved from online resource: http://genebrowser.unige.ch/telagirdon/#query_the_atlas. (**G**) Homogenates of indicated tissues from mice at P0 were subjected to IB with antibodies against Hnrnpa3a (top row), Hnrnpa3 (middle row), or GAPDH.

Figure S2

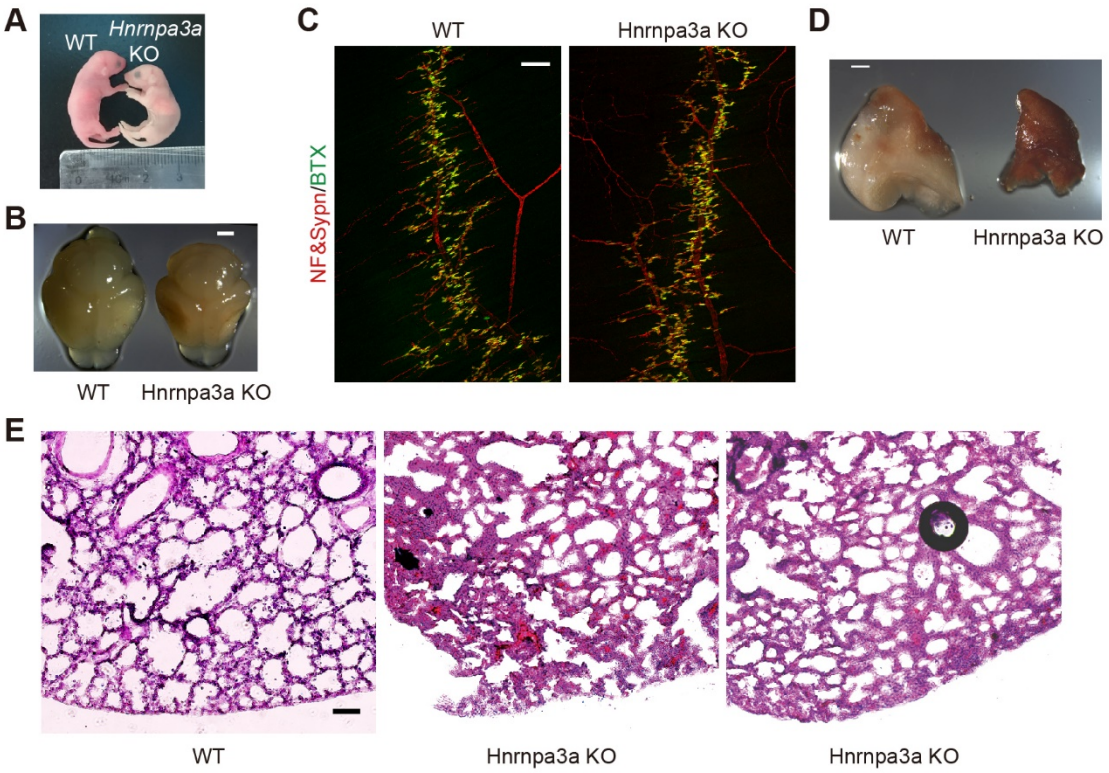

Fig. S2. Hnrnpa3a deletion causes developmental defect in lung. (**A**) Gross appearance of WT and *Hnrnpa3a* KO neonatal pups. (**B**) Brain appearance of WT and *Hnrnpa3a* KO mice at P2. Scale bar, 1 mm. (**C**) Whole-mount immunostaining for the diaphragm of mice at P0 using antibodies against neurofilament (NF) and synapsin-1 (Sypn) to label presynaptic nerves and Rhodamine-labeled alpha-bungarotoxin (BTX) to label postsynaptic AChR on muscle cells. Scale bar, 100 μm. (**D**) Gross appearance of the lung from WT (left) or *Hnrnpa3a* KO mice at P2. Note the remarkable decrease in lung size in KO mice. Scale bar, 1 mm. (**E**) HE staining for lung sections from WT or *Hnrnpa3a* KO mice at P2. Note the abnormal pulmonary alveoli with thickened alveolar epithelium and disrupted septum in KO mice. Scale bar, 100 μm.

Figure S3

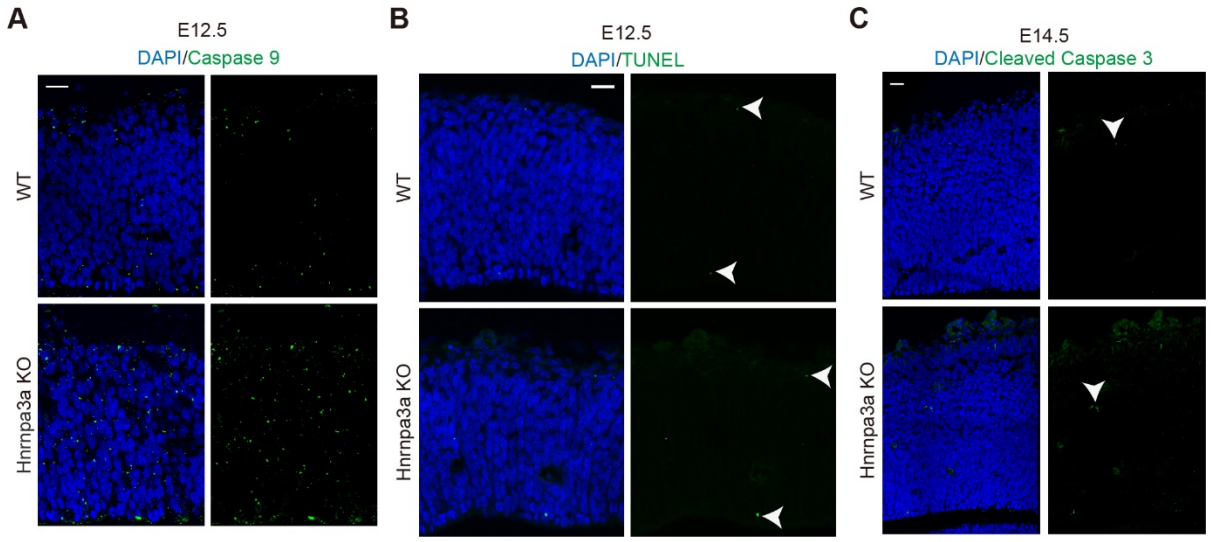

Fig. S3. Hnrnpa3a deficiency does not evoke apoptosis. Brain slices from E12.5 (A, B) or E14.5 (C) mice were subjected to immunostaining for caspase-9 (A), TUNEL (B), or cleaved activated caspase-3 (C), with DAPI marking cell nucleus. Scale bars, 20 μm. Note the comparable minimal TUNEL or cleaved caspase-3 signals in both WT and KO mice. The increase in caspase-9 signals in KO mice might be a reflective of DNA damage response.

Figure S4

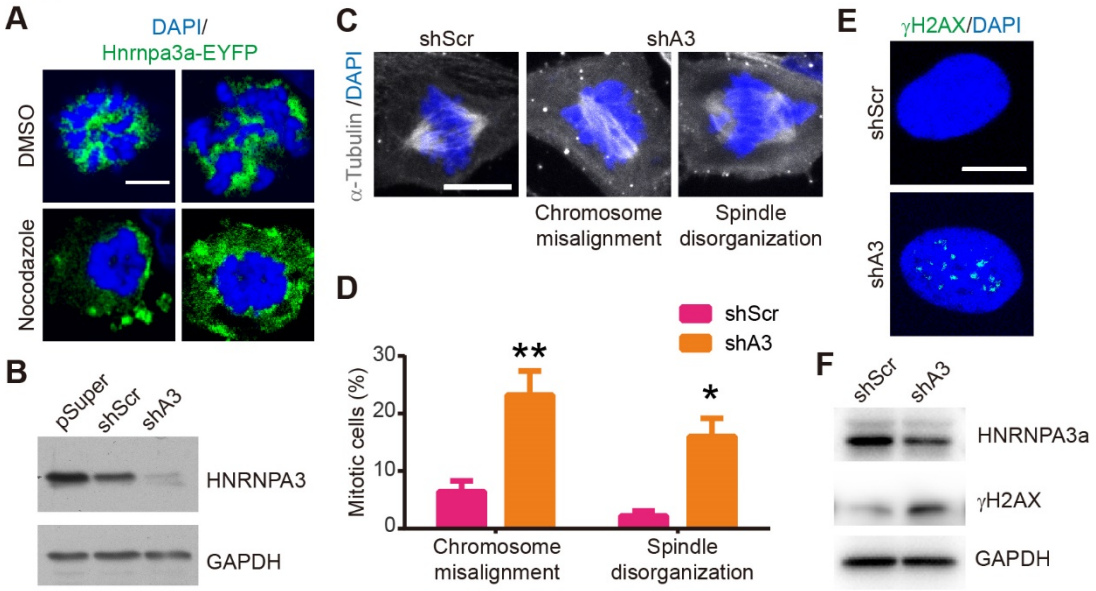

Fig. S4. Role of HNRNPA3 in mitotic division of ReN cells. (**A**) ReN cells transfected with construct encoding Hnrnpa3a-EYFP fusion protein were treated with DMSO or Nocodazole (100 ng/ml) for 6 hr. Scale bar, 5 μm. (**B**) Small interference RNA sequence against HNRNPA3 (shA3) or scrambled sequence (shScr) was cloned into pSuper vector. Efficiency of knockdown was tested in transfected 293T cells by IB. (**C**) ReN cells transfected with shA3 or shScr construct were stained with α-Tubulin to label spindle (grey) and DAPI to label DNA (blue), respectively. Images are representative cases of cells at metaphase. Scale bar, 10 μm. (**D**) Quantification for the percentage of cells with chromosome misalignment or spindle disorganization in shScr and shA3 ReN cells during mitosis. Data are presented as mean ± SEM from 5 independent experiments with ≥100 cells analyzed each time (chromosome misalignment, unpaired t test, $P = 0.0066$; spindle disorganization, unpaired t test with Welch's correction, $P = 0.0101$). (**E**) Immunostaining for γH2AX (green) signals in chromatin labeled by DAPI (blue) in shA3 or shScr-transfected ReN cells. Scale bar, 10 μm. (**F**) IB for the level of γH2AX in shScr or shA3-transfected ReN cells.

Figure S5

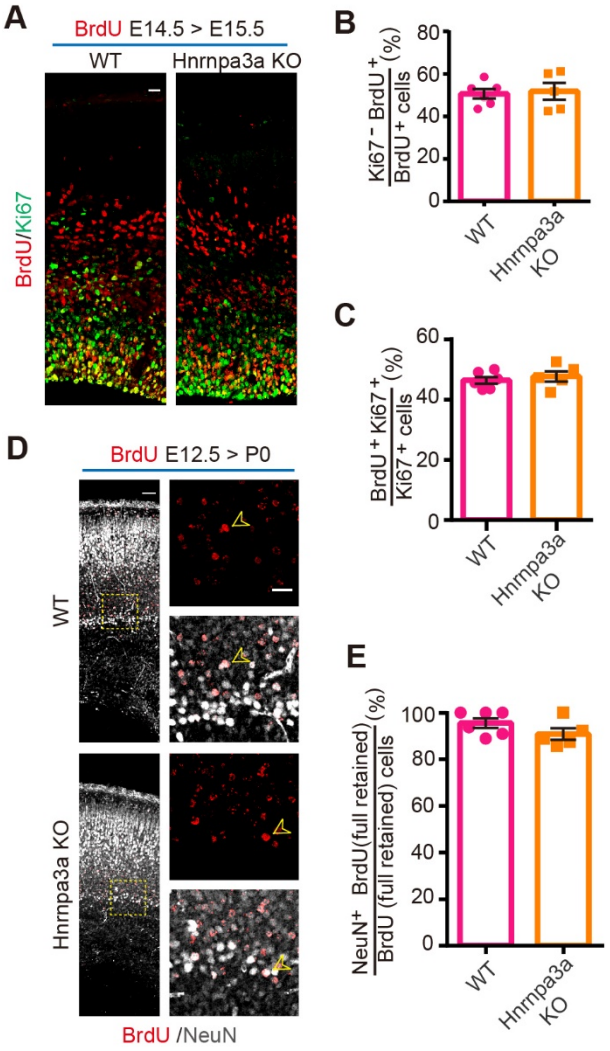

Fig. S5. Hnrnpa3a affects cortical neurogenesis in mice. (**A**) E14.5 mice with indicated genotype were pulse labeled with BrdU and examined one day later by staining with BrdU (red) and Ki67 (green). Scale bar, 20 μm. (**B**) Quantification for the percentage of $BrdU^+Ki67^-$ cells among $BrdU^+$ cells (Unpaired t test, $P = 0.7467$). (**C**) Quantification for the percentage of $BrdU^+Ki67^+$ cells among $Ki67^+$ cells in E15.5 mice with indicated genotype (Unpaired t test, $P = 0.5134$). (**D**) E12.5 mice were subjected to pulse labeling with BrdU and analyzed at P0 by immunostaining with pan-neuronal marker NeuN (grey). Hollow arrowheads indicate $NeuN^+$ cells with fully retained BrdU. Scale bars, 50 (left) and 20 μm (magnified area). (**E**) Quantification for the percentage of $NeuN^+$ cells among cells with fully retained BrdU (Mann-Whiteney test, $P = 0.1537$).

Figure S6

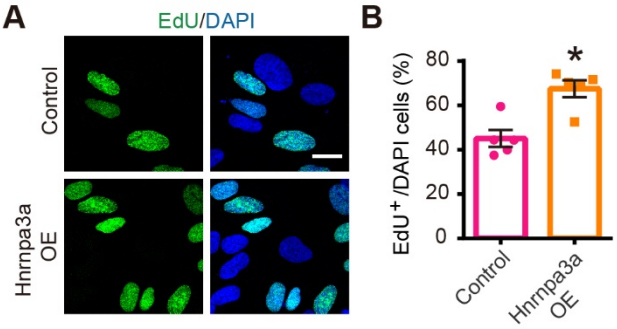

Fig. S6. HNRNPA3 promotes NP proliferation. (**A**) ReN cells without or with HNRNPA3 over-expression (OE) were subjected to EdU pulse labeling, followed with DAPI (blue) staining. Scale bar, 20 μm. (**B**) Quantification for the percentage of EdU^+ cells (Mann Whitney test, $P = 0.0159$).

Figure S7

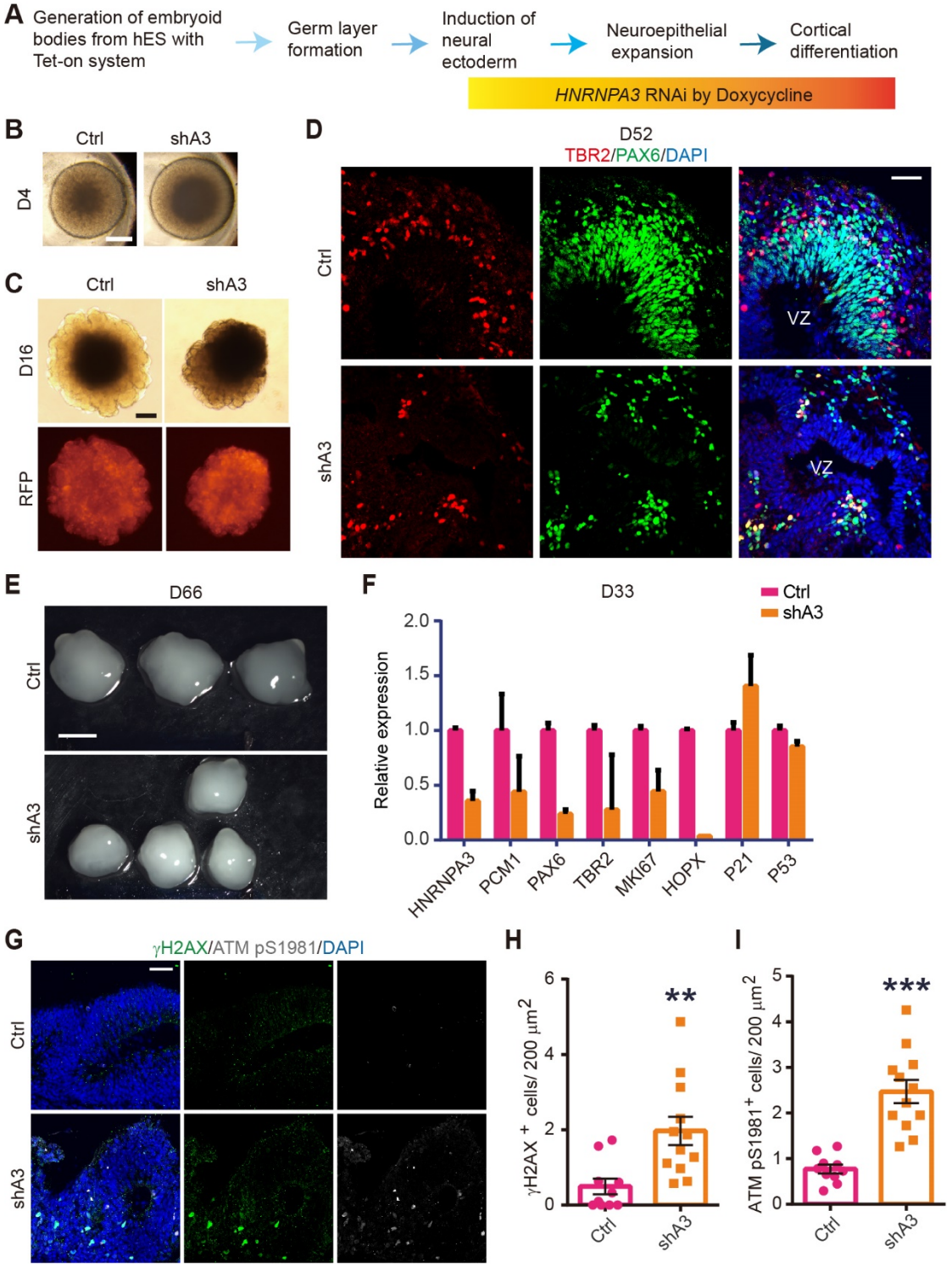

Fig. S7. Role of HNRNPA3 in the development of cultured human cerebral organoids. (**A**) Timeline of human brain organoid culture and *HNRNPA3* interference RNA (RNAi) induction. (**B**) Samples of control (Ctrl) and shA3 embryonic bodies (EB) at D4. Scale bar, 100 μm. (**C**) Images of Ctrl and shA3 organoids at D16. RFP signals indicate the expression

of doxycycline-driven system. Scale bar, 100 μm. (**D**) Slices of control or shA3 organoids at D52 were stained with antibodies against PAX6 (green) and TBR2 (red). Scale bars, 50 μm. (**E**) Representative images of control and shA3 organoids at D66. Scale bar, 200 μm. (**F**) Gene expressions measured by quantitative PCR (qPCR) in organoids at D33. GAPDH was used as the internal control. Gene expression levels are normalized to control groups. (G) Immunostaining for the signals of γH2AX (green) and ATM pS1981 (grey) in D30 organoid slices. Scale bar, 50 μm. (H, I) Quantification for the number of cells positively labeled by γH2AX (H) or ATM pS1981 (I). Unpaired t test with Welch's correction; $P = 0.0031$ in (H) and $P < 0.0001$ in (I).

Table S1. Primers for qPCR analysis.

HOPX	5’-GAGACCCAGGGTAGTGATTTGA-3’ (forward, F)
	5’-AAAAGTAATCGAAAGCCAAGCAC-3’ (reverse, R)
PAX6	5’-ACCCATTATCCAGATGTGTTTGCCCGAG-3’ (F)
	5’-ATGGTGAAGCTGGGCATAGGCGGCAG-3’ (R)
TBR2	5’-CACCGCCACCAAACTGAGAT-3’ (F)
	5’-CGAACACATTGTAGTGGGCAG-3’ (R)
MKI67	5’-ACGCCTGGTTACTATCAAAAGG-3’ (F)
	5’-CAGACCCATTTACTTGTGTTGGA-3’ (R)
P21	5’-ATGTGTCCTGGTTCCCGTTTC-3’ (F)
	5’-CATTGTGGGAGGAGCTGTGA-3’ (R)
TP53	5’-TTCCCTGGATTGGCAGC-3’ (F)
	5’-TCTGAAAATGTTTCCTGACTCAGA-3’ (R)
PCM1	5’-TAGCTGAGGTCGAAAAGGCG-5’ (F)
	5’-TTCCCGCTCCACACTTCAAA-3’ (R)
GAPDH	5’-GTGAAGCAGGCATCTGAGGG-3’ (F)
	5’-GCCGTATTCATTGTCATACCAGG-3’ (R).
HNRNPA3	5’- GGGGGAGGAGGGCCA-3’ (F)
	5’- CCCTCCGCGACCCATAAAAT-3’ (R)

Table S2. Primary antibodies for immunostaining, immunoprecipitation, and immunoblotting.

HNRNPA3(1-50aa)	Abcam, ab50949, 1:1000 (for immunoblotting)
HNRNPA3(1-100aa)	Abcam, ab78300, 1:1000 (for immunostaining and immunoblotting)
cleaved Caspase-3	Cell Signaling, 4370, 1:200 (for immunostaining)
Ctip2	Abcam, ab18465, 1:1000 (for immunostaining)
Cux1	Santa Cruz, sc-13024, 1:200 (for immunostaining)
GFP	Aves Lab, GFP-1020, 1:500 (for immunostaining)
PAX6	Covance, PRB-278P, 1:1000 (for immunostaining)
PAX6	R&D, AF8150, 1:400 (for immunostaining)
Tbr2	ThermoFisher, 14-4875-82, 1:400 (for immunostaining)
TBR2	R&D, AF6166, 1:400 (for immunostaining)
Ki67	Abcam, ab15580, 1:500 (for immunostaining)
KI67	BD, 550609, 1:400 (for immunostaining)
Phospho-Histone H3	Santa Cruz, sc-8656-R, 1:200 (for immunostaining)
Phospho-Histone H3	Cell signaling, 9706S, 1:500 (for immunostaining)
Tbr1	Millipore, ab31940, 1:1000 (for immunostaining)
Phospho-Vimentin	MBL International, D076-3s, 1:500 (for immunostaining)
BrdU	Sigma-Aldrich, B2531, 1:200 (for immunostaining)
BrdU	Abcam, ab6326, 1:1000 (for immunostaining)
SMC1A	Abcam, ab9262, 1:400 (for immunostaining and immunoblotting)
phosphorylated-SMC1A	Abcam, ab240573, 1:400 (for immunostaining and immunoblotting)
HOPX	Sigma, HPA030180, 1:1000 (for immunostaining)
Caspase-9	Abcam, ab32539, 1:200 (for immunostaining)
γH2AX	Abcam, ab81299, 1:500 (for IB)
γH2AX	Cell signaling, 9718, 1:500 (for immunostaining)
ATM pS1981	Cell signaling, 5883S,1:500 (for IB)
ATM pS1981	Rockland, 200-301-400, 1:500 (for immunostaining)
α-Tubulin	Sigma-Aldrich, T5168, 1:500 (for immunostaining)
Satb2	Abcam, ab69995, 1:1000 (for immunostaining)
Neurofilament	Cell Signaling, 2837S, 1:1000 (for immunostaining)
Synpsin-1	Cell Signaling, 5297S, 1:1000 (for immunostaining)
BubR1	Abcam, ab28193, 1:400 (for immunostaining and immunoblotting)
GAPDH	proteintech, 60004-1-Ig, 1:5000 (for immunoblotting)
Dcx	Santa Cruz, sc-8066, 1:200 (for immunostaining)
NeuN	Millipore, MAB377, 1:500 (for immunostaining)