Table S1. Antibodies used for immunostaining and Western Blot					
Antibody	Specie	Manufacturer	Catalog Number	Application	Dilution
Tuj1	Mouse	Sigma	T8660	Immunostaining	1:400
	Rabbit	Biolegend	PRB-435P	Immunostaining	1.:250
GFAP	Rabbit	DAKO	Z0334	Immunostaining	1:1000
RB	Mouse	BD pharmingen	554136	Immunostaining	1:250
	Mouse	Santa Cruz	sc-102	Western blot	1:200
Dcx	Goat	Santa Cruz	sc-8066	Immunostaining	1:500
Cleaved Caspase 3	Rabbit	Cell Signaling	D175	Immunostaining	1:400
Ki67	Rabbit	Thermo Fisher Scientific	RM-9106S	Immunostaining	1:500
Sox2	Goat	Santa Cruz	Y17	Immunostaining	1:500
BrdU	Rat	Accurate	OBT0030	Immunostaining	1:250
Pax6	Rabbit	Biolegend	901301	Immunostaining	1:300
Vimentin	Chicken	Abcam	Ab24525	Immunostaining	1:500
GAPDH	Mouse	Santa Cruz	sc-32233	Westernblot	1:1000
p107	Rabbit	Santa Cruz	sc-318	Western blot	1:200
P130	Rabbit	Santa Cruz	sc-317	Western blot	1:200

Table S2. Secondary antibodies used for immunostaining and Western Blot					
Antibody	**Specie**	**Manufacturer**	**Catalog Number**	**Application**	**Dilution**
Anti-Chicken Cy3	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	703-165-155	Immunostaining	1:250
Anti-Goat FITC	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	705-095-147	Immunostaining	1:250
Anti-Goat Cy3	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	705-165-147	Immunostaining	1:250
Anti-Goat Cy5	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	705-175-147	Immunostaining	1:250
Anti-Mouse FITC	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	715-095-151	Immunostaining	1:250
Anti-Mouse Cy3	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	715-165-151	Immunostaining	1.:250
Anti-Mouse Cy5	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	715-175-151	Immunostaining	1:250
Anti-Mouse HRP	Horse	Cell Signaling	7076S	Western Blot	1:10000
Anti-Rabbit FITC	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	711-095-152	Immunostaining	1:250
Anti-Rabbit Cy3	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	711-165-152	Immunostaining	1:250
Anti-Rabbit Cy5	Donkey	Jackson ImmunoResearch LABORATORIES, INC.	711-175-152	Immunostaining	1:250

Table S3. Primers used for the gene profile analysis by RT-qPCR

Gene		Primer (5'-3')	Length (bp)	
ApoER2	Forward	CTGTGGCGACATTGATGAGTG	119	(Wang and Seed, 2003)
	Reverse	TCTTGGTCAGTAGGTCCATCTC		
BAX	Forward	CAGCTGACATGTTTTCTGACGGCA	118	Designed by Lasergene software
	Reverse	GTTCTGATCAGTTCCGGCACCTTG		
CASP3	Forward	GTGGTACAGAACTGGACTGTGGCATTG	175	Designed by Lasergene software
	Reverse	ATGGCACAAAGCGACTGGATGAAC		
CASP8	Forward	CCCTGCTGAGCACGTGGAGTTAG	158	Designed by Lasergene software
	Reverse	CCAGATCTTCACTGTCCAGTTGTTCC		
CYCLIN A2	Forward	CTAGCGCAGCAGCAGAGGC	91	Designed by Lasergene software
	Reverse	TCCAAGGAGGAACGGTGACAT		
CYCLIN E	Forward	GCTCCCTGATCCCCACACCTG	232	Designed by Lasergene software
	Reverse	AGAAGAATTGCTCGCATTTTTGGCT		
E2F1	Forward	CAAGAAGTCCAAGAACCACATCCAGT	117	Designed by Lasergene software
	Reverse	AGCTGCTGCTCGCTCTCCTG		
GAPDH	Forward	GAAGGTGAAGGTCGGAGTC	226	(Lei et al., 2009)
	Reverse	GAAGATGGTGATGGGATTTC		
p14	Forward	GGTTTTCGTGGTTCACATCCCG	107	Designed by Lasergene software
	Reverse	GCCCTAGACGCTGGCTCCTCA		
p21	Forward	CAGGGGAGCAGGCTGAAGGGT	106	Designed by Lasergene software
	Reverse	ATCAGCCGGCGTTTGGAGTGG		
p53	Forward	AGGCCCATCCTCACCATCATCAC	179	Designed by Lasergene software
	Reverse	AGTGCTCGCTTAGTGCTCCCTGG		
p107	Forward	TCAAAATCCATATGAAGAACCACCAAAG	146	Designed by Lasergene software
	Reverse	CTAAGTCATCCCCAATCATCCGAAA		
p130	Forward	CAGCAGCGAGGAAGGAAACAG	213	Designed by Lasergene software
	Reverse	AAAATTAGGGTTCACAAGTTCTTTACGAT		
VLDLR	Forward	CGAGACTGTCAAAGTACTGCAACTA	177	(Ozcelik et al., 2008)
	Reverse	CACTAAGAGCAAGAGAGGAAGAATG		

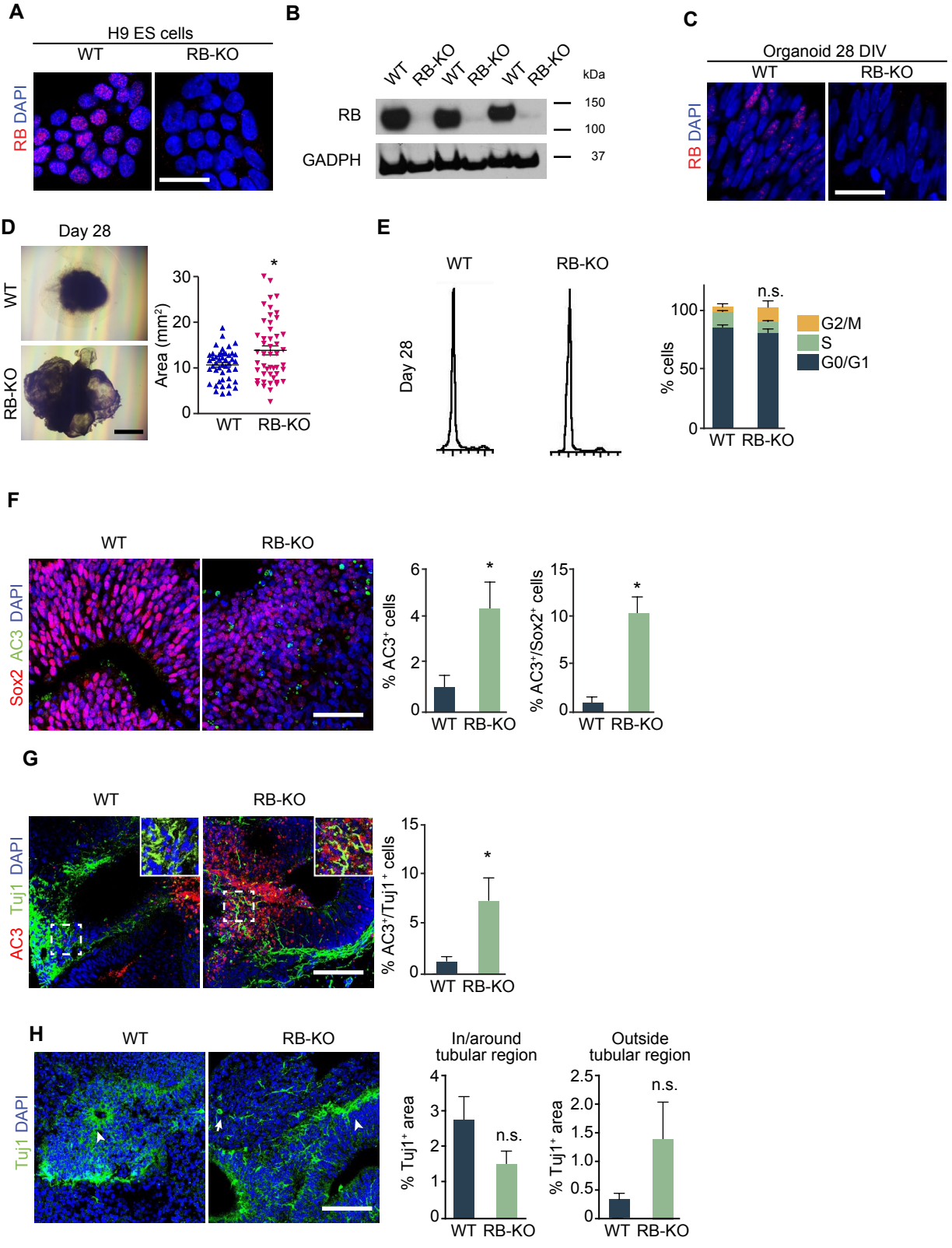

Figure S1: The lack of RB increases the size and number of apoptotic cells in *RB*-KO cerebral organoids from a different clone of human ES cells. We generated a second clone of *RB*-deficient H9 ESC line using CRISPR/Cas9. (A) The images show representative WT and KO-ESCs in culture immunostained against RB and counterstained with DAPI. (B) Western blot was performed to detect RB and GADPH proteins in samples from WT and *RB*-KO ESCs. (C) The images show representative WT and KO-organoids at 28 DIV sectioned and immunostained against RB and counterstained with DAPI. (D) The pictures show representative WT and KO organoids at 28 DIV. The graph shows the area mean of WT and KO organoids at 28 DIV. (E) The graphs show representative WT and KO-organoid cell cycle profiles at 28 DIV and the percentage of cells in each cell cycle phases. (F) The images show representative WT and KO-organoids at 28 DIV sectioned and immunostained against AC3/Sox2 and counterstained with DAPI. The graphs show the percentage of $AC3^+$ cells/DAPI and $AC3^+$ cells/$Sox2^+$ cells. (G) The images show representative WT and KO-organoids at 28 DIV sectioned and immunostained against AC3/Tuj1 and counterstained with DAPI. The graph shows the percentage of $AC3^+$ cells/$Tuj1^+$ cells. (H) The images show representative WT and KO-organoids at 28 DIV sectioned and immunostained against Tuj1 and counterstained with DAPI. Arrowheads in the WT and KO organoid show $Tuj1^+$ cells in/around tubular region. An arrow in the KO organoid shows ectopic $Tuj1^+$ cells outside the tubular region. The graphs show the percentage of Tuj1-stained area in/around and outside the tubular region. The results are the mean ± SEM of 5-48 organoids from 3 independent experiments (* $P<0.05$, paired Student´s t-test and n.s.=not significant). Scale bar: A-C, 25 µm; F-G, 50 µm; enlarged area, 25 µm.

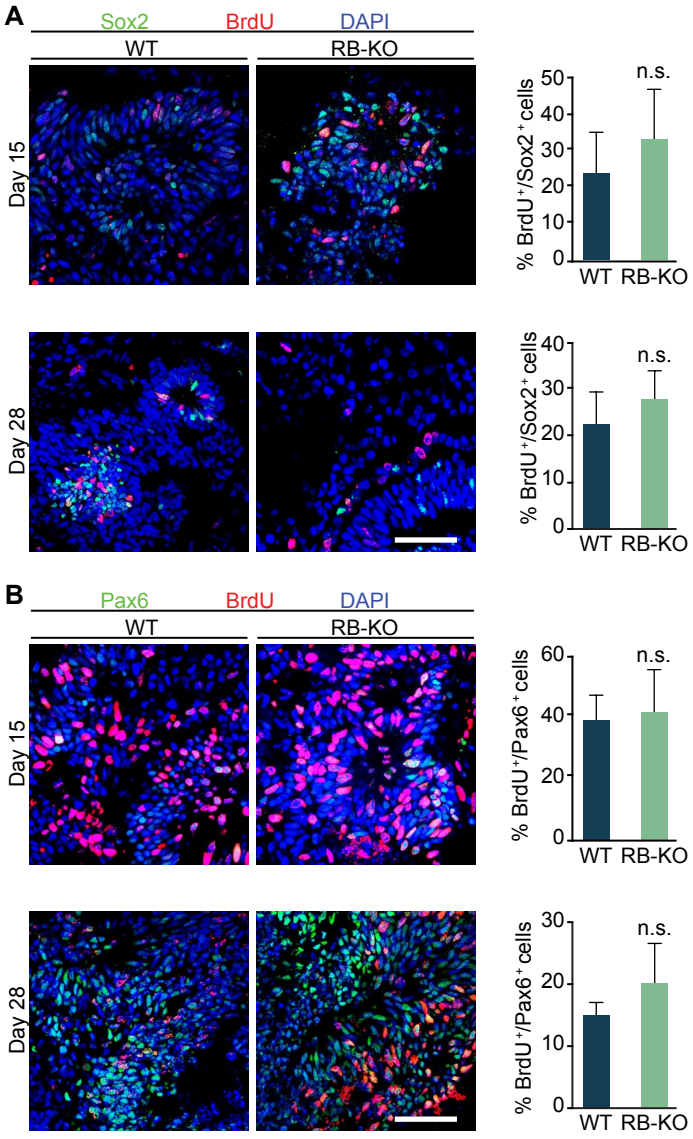

Figure S2: The lack of RB did not affect the proliferation of NSC/progenitor cells in cerebral organoids at 28 DIV. (A) The images show representative WT and KO-organoids at 15 and 28 DIV sectioned and immunostained against BrdU/Sox2 and counterstained with DAPI. The graphs show the percentage of $BrdU^+$ cells/$Sox2^+$ at both ages. (B) The images show representative WT and KO-organoids at 15 and 28 DIV sectioned and immunostained against BrdU/Pax6 and counterstained with DAPI. The graphs show the percentage of $BrdU^+$ cells/$Pax6^+$ at both ages. The results are the mean ± SEM of 5-6 organoids from 3 independent experiments (n.s.=not significant). Scale bar: 50 µm.

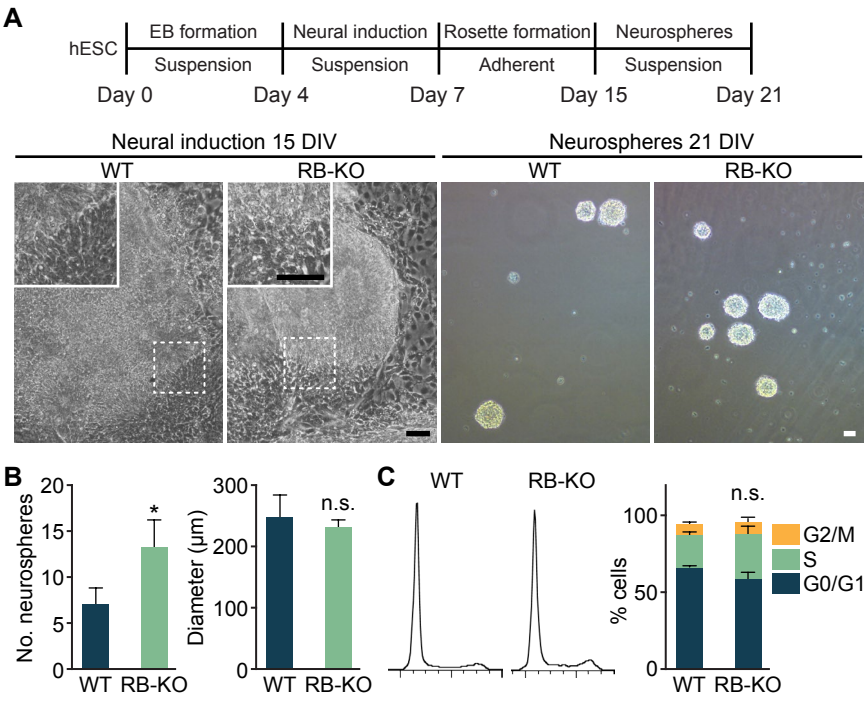

Figure S3: RB deletion promotes the proliferation of neuropheres. (A) Experimental scheme to obtain neuropheres from H9 ESCs at 21 DIV. The images show representative rosettes and neuropheres from WT and KO cultures at 15 and 21 DIV, respectively. Boxed insets in WT and KO cultures show rosette formation. (B) The graphs show the number of neurospheres and their diameter in WT and KO cultures. (C) The graphs on the left show representative WT and KO cell cycle profiles and the graph on the right shows the percentage of cells in each cell cycle phase. The results are the mean ± SEM of neurospheres from 8 independent experiments (* $P<0.05$, paired Student´s t-test and n.s.=not significant). Scale bar: 50 µm.