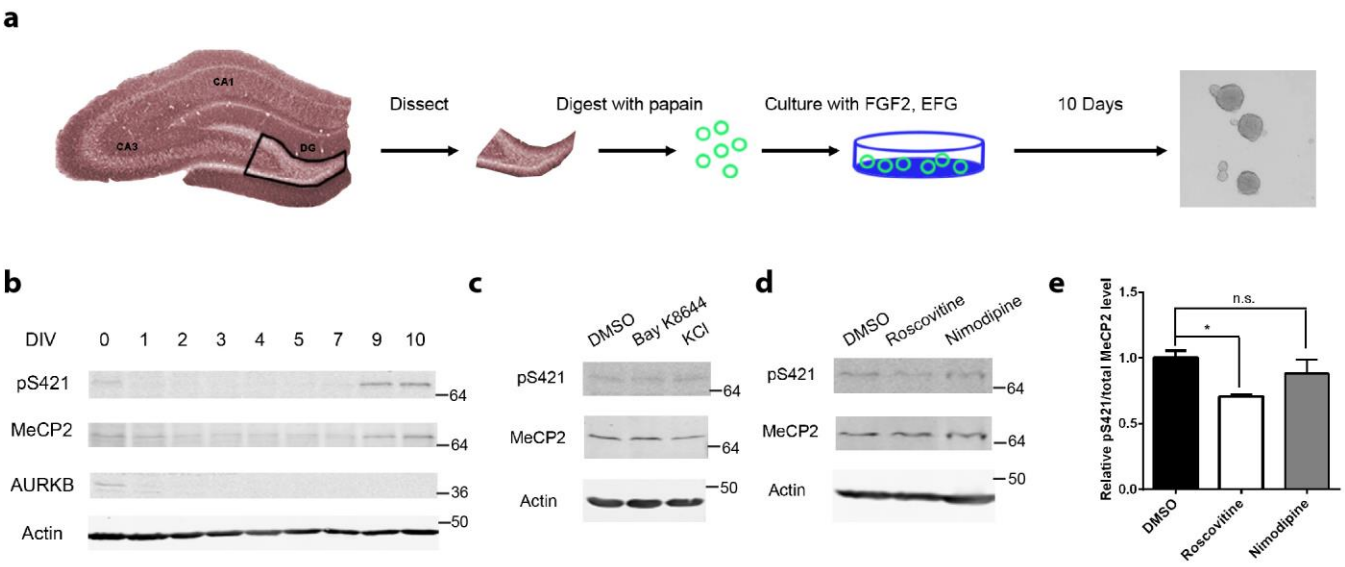
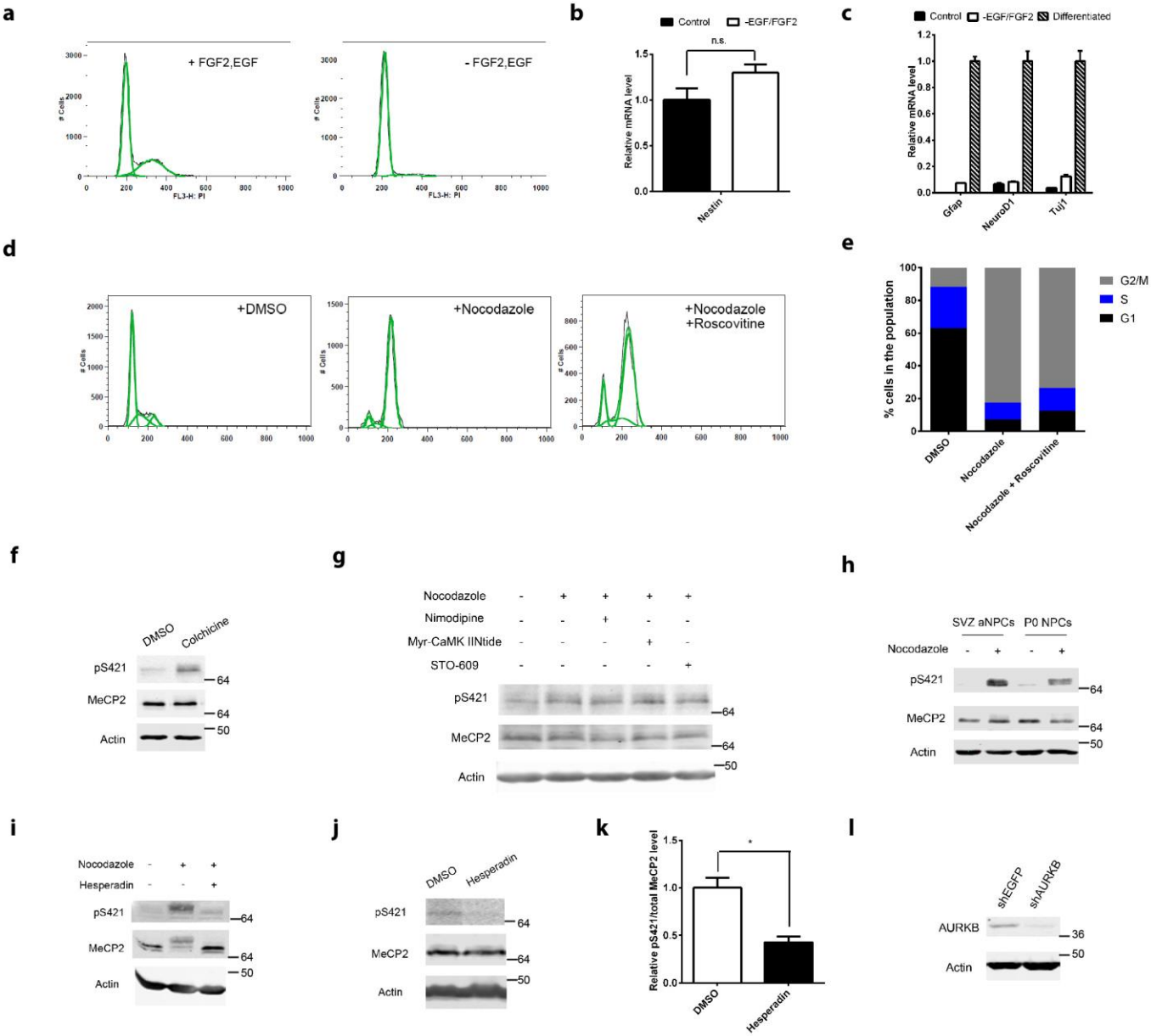
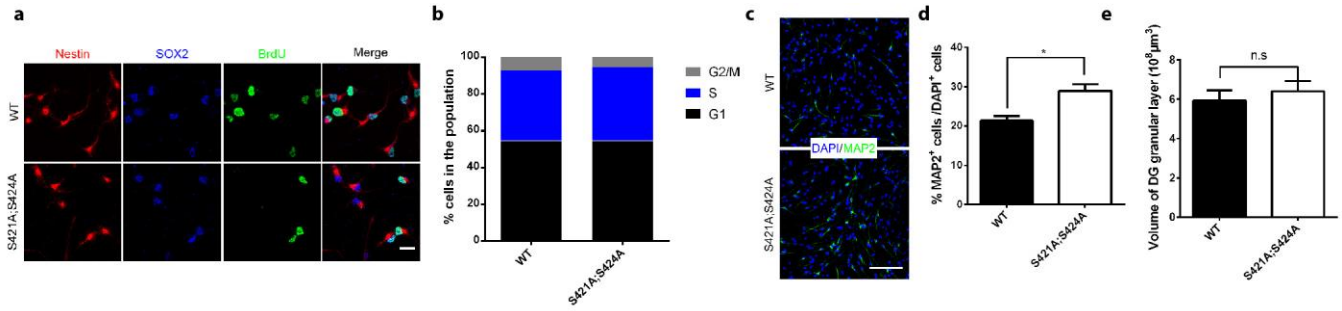
Supplementary Figures:



Supplementary figure 1: MeCP2 Ser421 phosphorylation regulation in aNPCs.
(a) Schematic drawing showing the isolation of aNPCs from the dentate gyrus for culture. **(b)** Time course analysis of S421 phosphorylation in differentiated wild type aNPCs by Western blot. **(c)** Western blot analysis of S421 phosphorylation in wild type aNPCs in the presence of Bay K8644 or KCl. **(d-e)** Western blot analysis and quantification of S421 phosphorylation in wild type aNPCs in the presence of roscovitine or nimodinpine. Numbers next to Western blots are molecular weight markers.

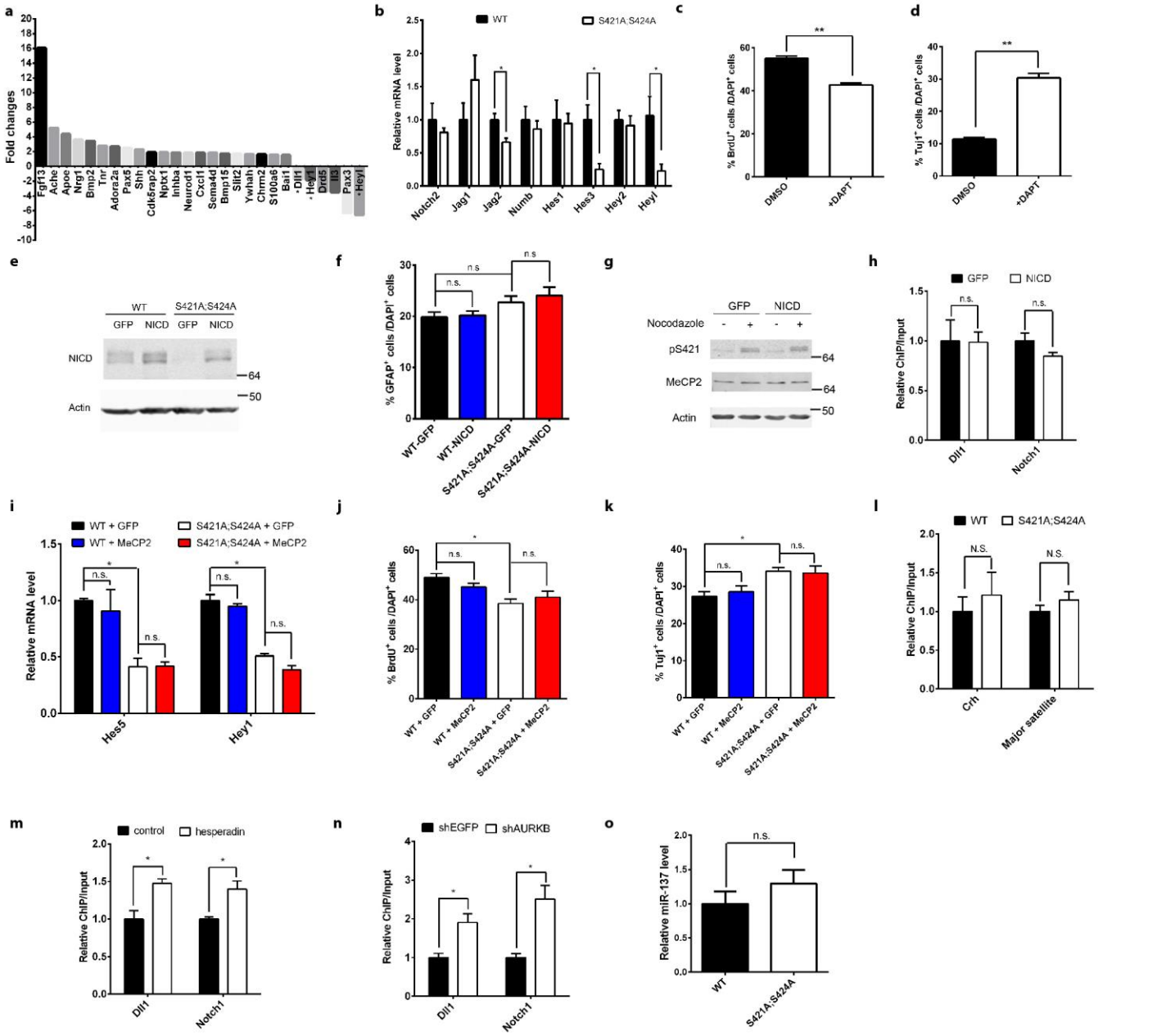


Supplementary figure 2: Cell cycle-linked MeCP2 S421 phosphorylation.
(a) Cell cycle analysis of DNA content of wild type aNPCs under normal condition and FGF2/EGF withdrawal. **(b-c)** RT-qPCR analysis of transcription level of key cell type markers, Nestin, Gfap, NeuroD1 and Tuj1 in aNPCs grown under normal proliferating conditions (control), after growth factor withdrawal, and under differentiating conditions (differentiated). **(d)** Cell cycle analysis of DNA content of wild type aNPCs under treatment conditions: 1) DMSO, 2) 36 hours of nocodazole treatment, 3) 24 hours of roscovitine (25 µM) treatment after pre-synchronization of the cells by nocodazole for 12 hours. **(e)** Quantification of the percentage of cells from (d) at different stages of the cell cycle under each condition. **(f)** Western blot analysis of MeCP2 Ser421 phosphorylation level in aNPCs treated with DMSO or 1µg/µl colchicine. **(g)** Western blot analysis of MeCP2 Ser421 phosphorylation level in aNPCs treated with DMSO, nocodazole or nocodazole coupled with Nimodipine (5µM), Myr-CaMK IINtide (5µM), or STO-609 (1.5µM). **(h)** Western blot analysis of MeCP2 Ser421 phosphorylation level in NPCs isolated from adult subventricular zone or postnatal day 0 brain and treated with nocodazole. **(i)** Western blot analysis of MeCP2 Ser421 phosphorylation level in N2A cells treated with DMSO, nocodazole alone, or nocodazole and hesperidin. **(j-k)** Western blot analysis of MeCP2 Ser421 phosphorylation level in aNPCs treated with DMSO or hesperidin. **(l)** Western blot analysis showing shRNA against aurora kinase B (AURKB), but not shRNA against EGFP, reduces AURKB protein level in wild type aNPCs. Numbers next to Western blots are molecular weight markers.



Supplementary figure 3: Altered neural differentiation of MeCP2 phosphor-mutant aNPC isolated from adult hippocampus.

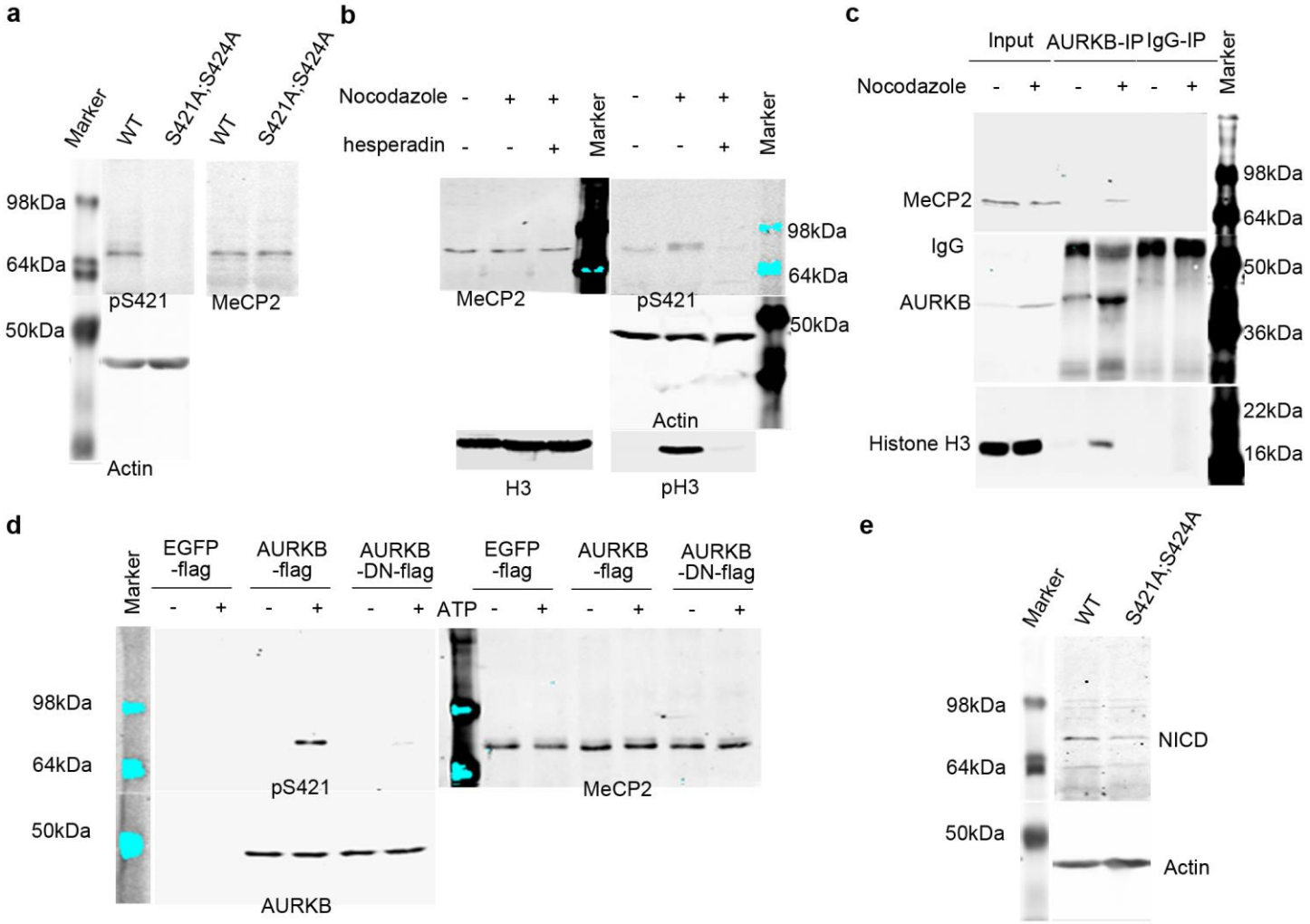
(a) High magnification view of a representative subfield from figure 3a in the main text, showing each individual channel and the overlay. Scale bar=10µm. **(b)** Comparison of the proportion of cells at different stages of the cell cycle between WT and phosphor-mutant aNPCs. **(c)** Representative immunocytochemistry images of MAP2+ neurons differentiated from WT and phosphor-mutant aNPCs. Scale bar=50µm. **(d)** Quantification of the percentage of MAP2+ cells in WT and phosphor-mutant aNPCs under differentiation condition. (n=3 in each group) **(e)** Comparison of the volume of dentate granular layer between WT and phosphor-mutant mice. The bar graghs in this figure show the mean ± s.e.m * $p<0.05$ ** $p<0.01$.



Supplementary figure 4: Decreased Notch signaling in *Mecp2*$^{S421A;S424A/y}$ aNPCs and the underlying mechanisms.

(a) Genes with larger than 1.5 fold changes from Neurogenesis and Neural Stem Cells PCR Array of WT and *Mecp2*$^{S421A;S424A/y}$ aNPCs (Normalized to WT, n=3 in each group). Notch pathway related genes are marked with an asterisk. **(b)** RT-qPCR analysis of the relative mRNA level of other Notch pathway related genes in WT and *Mecp2*$^{S421A;S424A/y}$ aNPCs (n=5 in each group). **(c)** Quantification of the percentage of BrdU-labeled cells in aNPCs treated with DMSO or DAPT (5μM), followed by BrdU pulse labeling. **(d)** Quantification of the percentage of Tuj1+ neurons differentiated from WT and *Mecp2*$^{S421A;S424A/y}$ aNPCs treated with DMSO or DAPT. **(e)** Western blot analysis confirms the overexpression of NICD in WT and *Mecp2*$^{S421A;S424A/y}$ aNPCs infected with NICD-letivirus. **(f)** Analysis of glial differentiation in either WT or phosphor-mutant aNPCs infected with either GFP- or NICD-expressing lentivirus. **(g)** Western blot analysis of S421 phosphorylation in WT aNPCs infected with either GFP- or NICD-expressing lentivirus in the absence or presence of nocodazole. **(h)** Chromatin immunoprecipitation analysis of MeCP2 occupancy at the promoters of *Dll1* and *Notch1* in WT aNPCs infected with either GFP- or NICD-expressing lentivirus. **(i)** RT-qPCR analysis of transcription of Notch target genes *Hes5* and *Hey1* in either WT or phosphor-mutant aNPCs infected with either GFP- or MeCP2-expressing lentivirus. **(j)** Analysis of proliferation in either WT or phosphor-mutant aNPCs infected with either GFP- or MeCP2-expressing lentivirus. **(k)** Analysis of neural differentiation in either WT or phosphor-mutant aNPCs infected with either GFP- or MeCP2-expressing lentivirus. **(l)** Chromatin immunoprecipitation analysis of MeCP2 occupancy at the promoter of Crh and at the major satellite repeats in WT and phosphor-mutant

aNPCs. **(m)** Chromatin immunoprecipitation analysis of MeCP2 occupancy at the promoters of *Dll1* and *Notch1* in WT aNPCs in the absence or presence of aurora kinase B inhibitor hesperidin. **(n)** Chromatin immunoprecipitation analysis of MeCP2 occupancy at the promoters of *Dll1* and *Notch1* in WT aNPCs infected with lentivirus encoding shRNA specifically against either EGFP or AURKB. **(o)** miR137 expression is not different between WT and phosphor-mutant aNPCs. Numbers next to Western blots are molecular weight markers. The bar graghs in this figure show the mean ± s.e.m * $p<0.05$ ** $p<0.01$.



Supplementary figure 5: Full blot images for key Western blots only shown in portion in the main figures. **(a)** Full blot for figure 1a. **(b)** Full blot for figure 2a. **(c)** Full blot for figure 2g. **(d)** Full blot for figure 2h. **(e)** Full blot for figure 4e.

Supplementary Table 1 List of qPCR primers used in the study

Gene Names	Forward	Reverse
Gapdh	aatgggaagcttgtcatcaacg	gaagacaccagtagactccacgacata
Dll1	agcgactgaggtgtaagatg	aacctggttctcagcagcag
Jag1	atacacgtggccatctctgc	aaccgcagcaataagtgagc
Jag2	cttccacaggtctgttggtg	tcctctcacgttctttcctg
Notch1	gaacaacaaggaggagactc	tccatgtgatccgtgatgtc
Notch2	caggacaataaggaagagac	tccatgtggtcagtgatgtc
Hes1	gcacttaagaaagatagctcc	ggtatttccccaacacgctc
Hes3	acactactcacatcagatacg	agagtccttgcagtgagttc
Hes5	gaaacacagcaaagccttcg	tgcagggtcaggaactgtac
Hey1	ctttgagaagcagggatctg	ctccgatagtccatagccag
Hey2	ggtaaaggctactttgatgcc	aggccttccactgagcttag
Heyl	actgcctttgagaaacaggg	atcaaagaaccctgtgccac
Numb	gatgccaagaaagctgagac	catctctgaagatgcagtgc
Tuj1	atctttggtcagagtggtgc	ggcagtcacaattctcacac
NeuroD1	acggatcaatcttctcttcc	cgtgaaagatggcattaagc
GFAP	atcggtctaagtttgcagac	ctccagatcgcaggtcaag
Nestin	ggctacatacaggactctgc	ctggtatcccaaggaaatgc