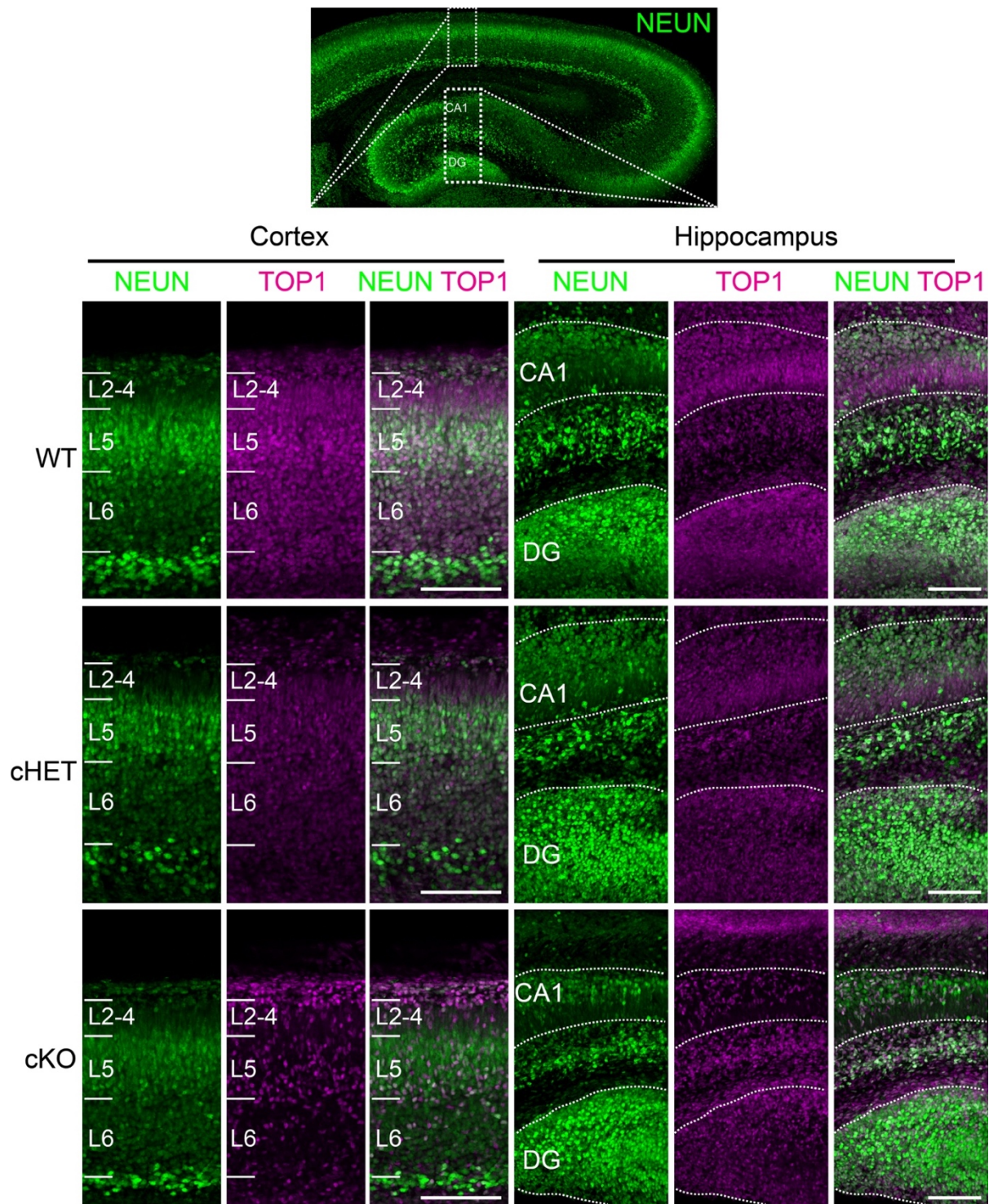


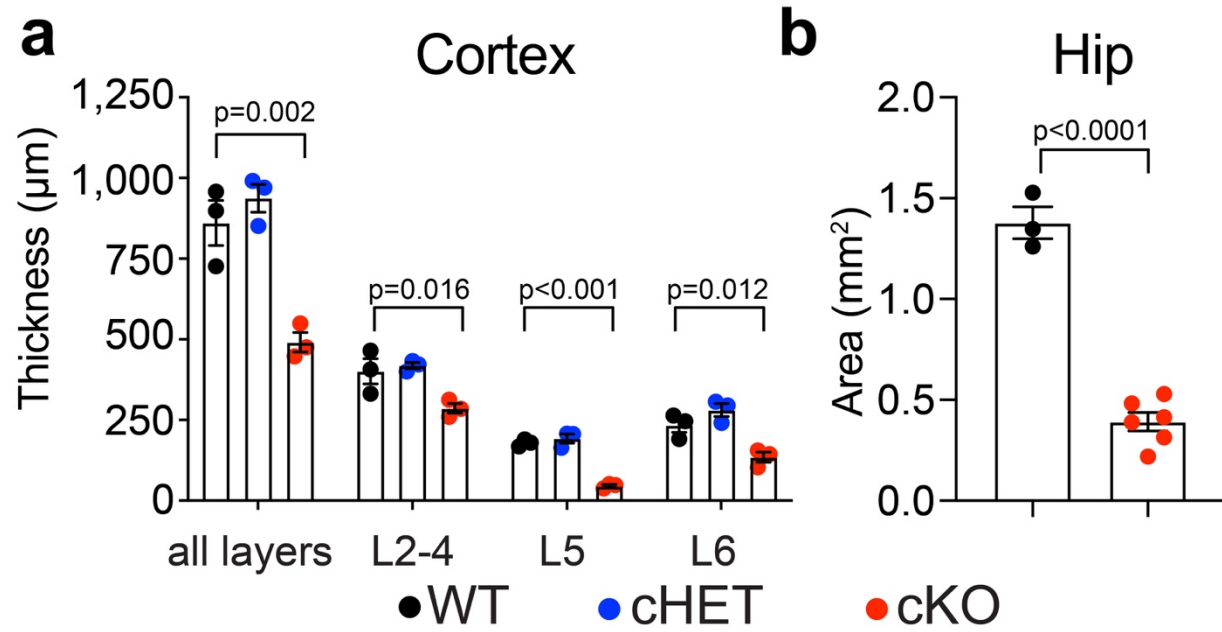
Supplementary Information

Deletion of Topoisomerase 1 in excitatory neurons causes genomic instability and early onset neurodegeneration

Fragola et al.



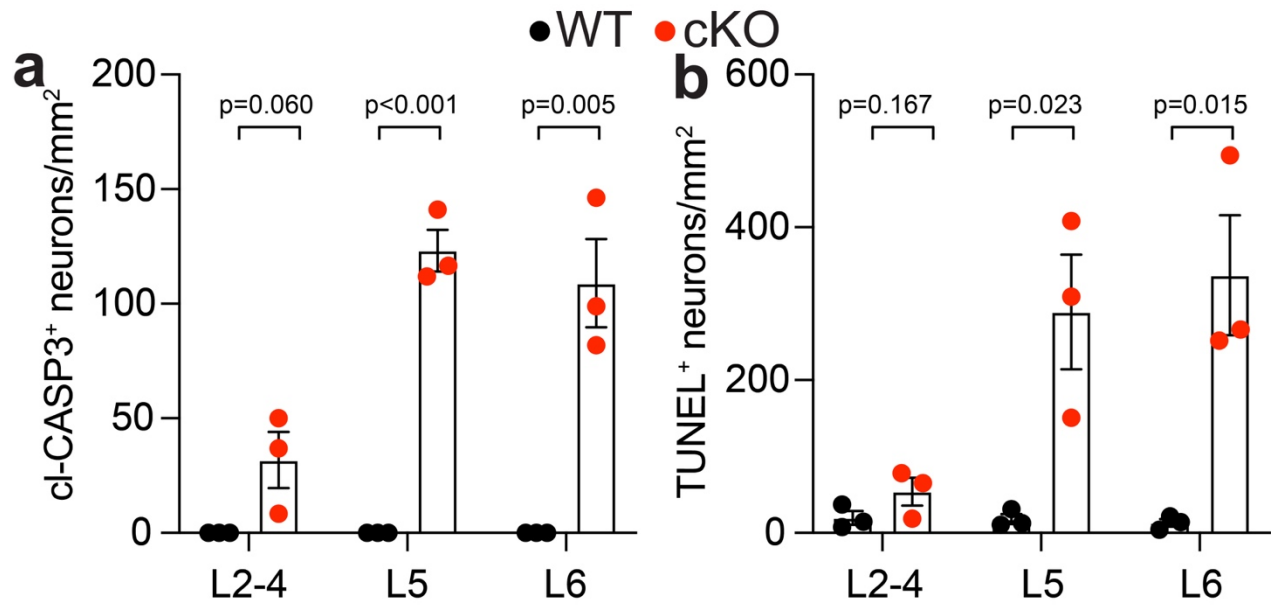
Supplementary Figure 1. TOP1 expression in WT, *Top1* cHET and *Top1* cKO P0 brain. Immunostaining of TOP1 and NEUN in P0 cortex and hippocampus of WT, *Top1* cHET and *Top1* cKO mice. Inset scale bars = 100 μ m. DG = dentate gyrus. Images are representative of 2 independent experiments.



Supplementary Figure 2. Cortical thickness in all layers and hippocampal area are reduced in *Top1* cKO mice at P15.

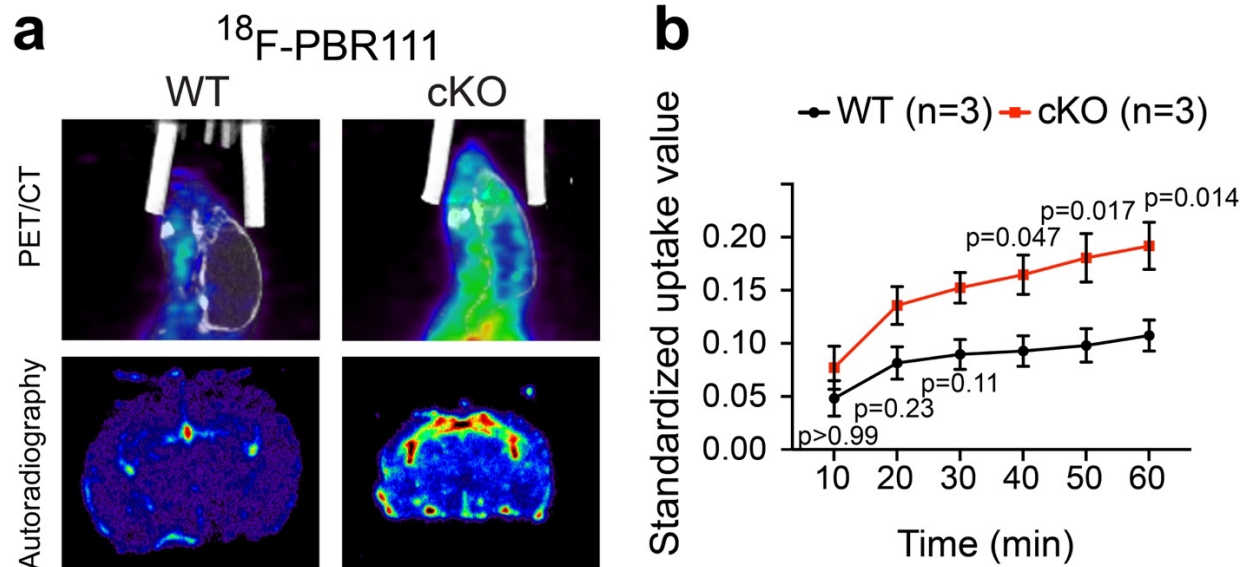
(a) Quantification of cortical thickness in WT, *Top1* cHET and *Top1* cKO (n=3 mice per genotype). One-way ANOVA. **(b)** Quantification of hippocampal (Hip) area.

WT (n=3), cKO (n=6). Two-sided student t-test. Values are mean and error bars are $\pm$ SEM.



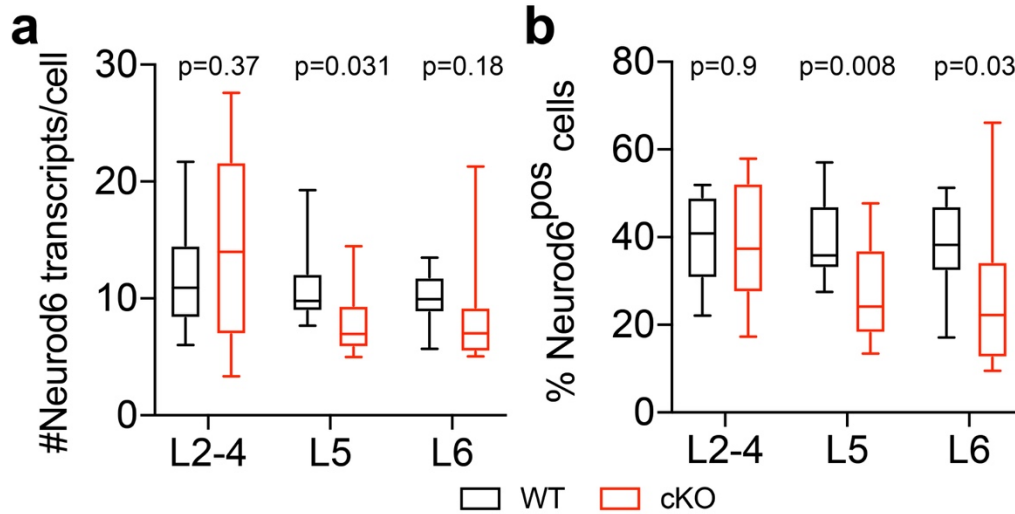
Supplementary Figure 3. Increased apoptosis of L5 and L6 neurons in *Top1* cKO mice.

Quantification of **(a)** cl-CASP3⁺ and **(b)** TUNEL⁺ neurons in cerebral cortex L2-4, L5 and L6 of WT (n=3) and cKO (n=3) P7 mice. n=2 sections per mouse. Two-sided student's t-test. Values are mean and error bars are ± SEM.



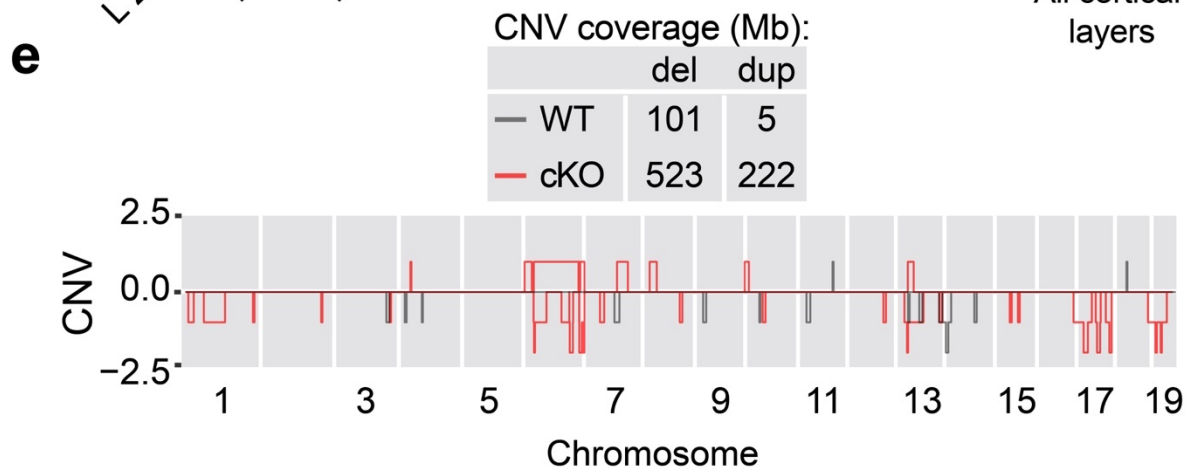
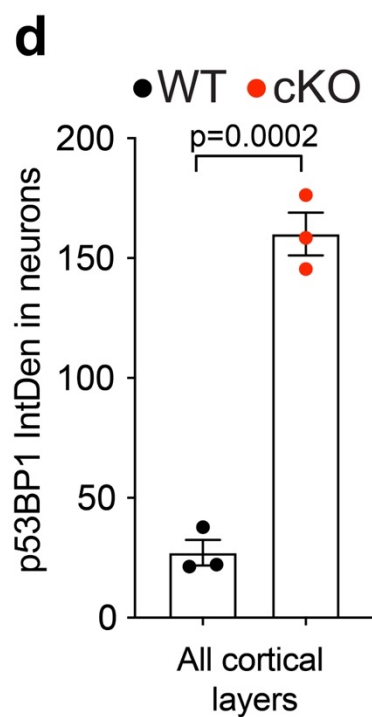
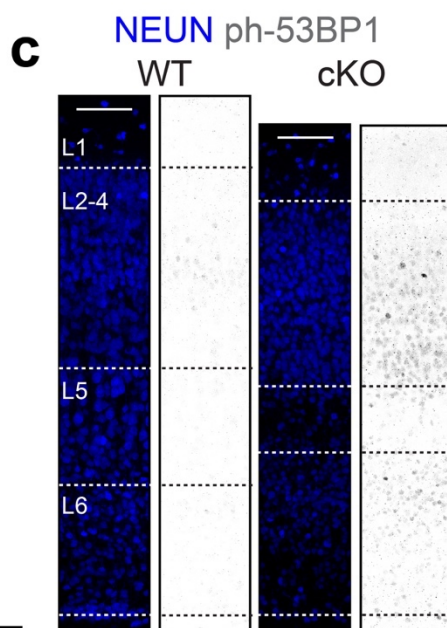
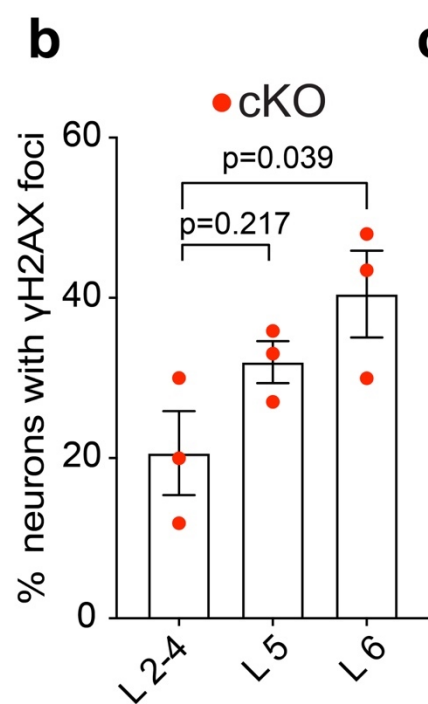
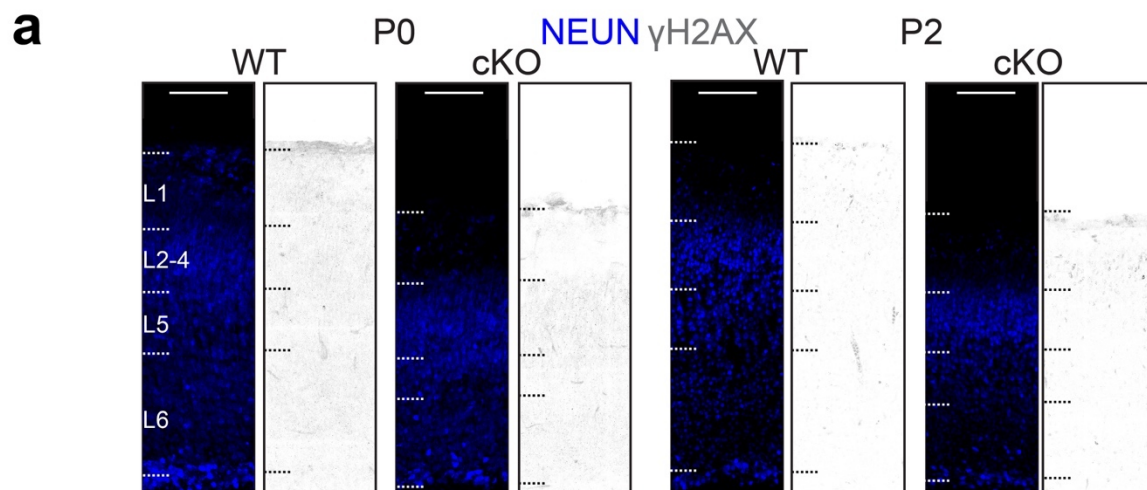
Supplementary Figure 4. Neuroinflammation in *Top1* cKO mice visualized by PET imaging with ^{18}F -PBR111.

(a) PET and computed tomography (CT) images (upper), high resolution autoradiography (lower) of WT and cKO P15 mice injected i.p. with ^{18}F -PBR111. **(b)** Quantification of ^{18}F -PBR111 uptake in the brain of WT and cKO P15 mice. Two-way ANOVA with Bonferroni post hoc test. 95% confidence interval of difference: 10 min (-0.100 to 0.0423), 20 min (-0.125 to 0.0170), 30 min (-0.134 to 0.00840), 40 min (-0.143 to -0.000815), 50 min (-0.154 to -0.0113), 60 min (-0.156 to -0.0132). n=3 mice per genotype. Values are mean and error bars are $\pm$ SEM.



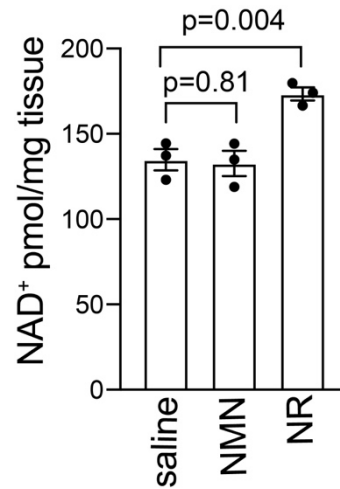
Supplementary Figure 5. Quantification of *Neurod6* transcript levels and percentage of *Neurod6*⁺ neurons in different cortical layers with single-molecule *in situ* hybridization.

(a) Average number of transcripts/cell/section from the indicated cortical layers in P7 WT and *Top1* cKO cortex. **(b)** Mean percentages of neurons with more than 5 transcripts (*Neurod6*⁺) in the different cortical layers of WT and cKO cortex. Box is first to third quartile, line is median, error bars are minimum and maximum values. Two-sided student t-test. n=11 WT sections, n=14 cKO sections. 2 mice/genotype.



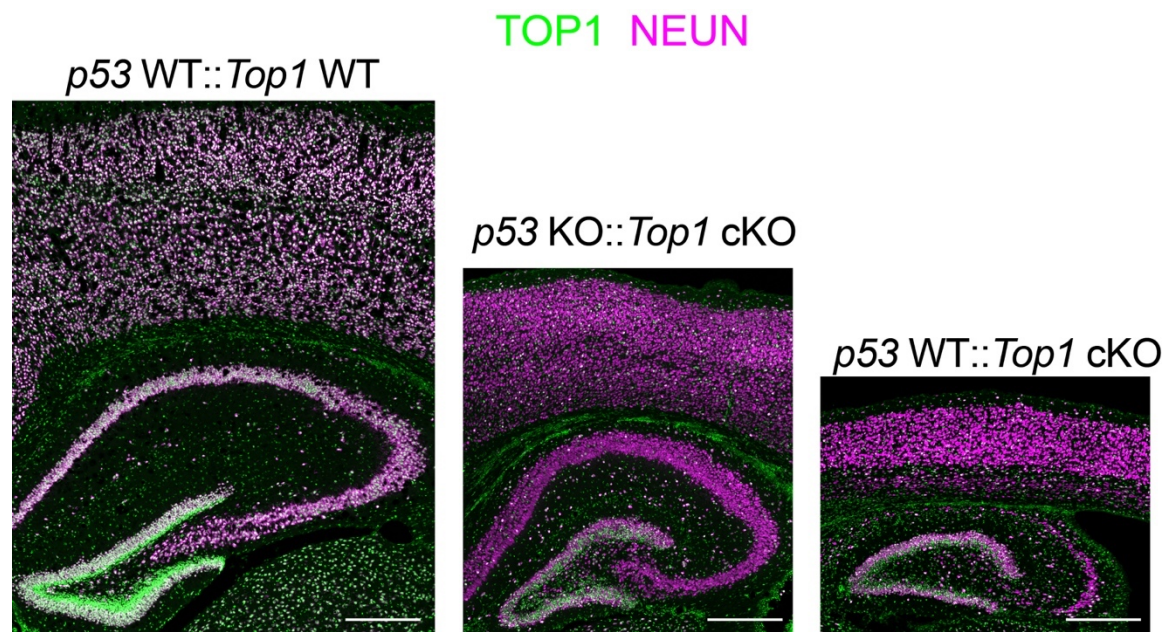
Supplementary Figure 6. Elevated DNA damage and more abundant CNVs in P7 *Top1* cKO mice.

(a) Immunostaining and of γ H2AX and NEUN in P0 and P2 WT and cKO cortex. Images are representative of 3 mice per genotype. Scale bar = 100 μ m. **(b)** Graph showing the percentage of neurons with γ H2AX foci in L2-4, L5 and L6 of *Top1* cKO mice (n=3 mice per genotype). One-way ANOVA with Dunnett's multiple comparison test. **(c)** Immunostaining and **(d)** quantification of ph-53BP1 and NEUN in P7 WT and cKO cortex. (n=3 mice per genotype). IntDen=Integrated density. Scale bar = 100 μ m. Two-sided student t-test. **(e)** Graph showing the overlap of all the CNVs identified in WT and cKO neurons at the different genomic locations. CNV coverage is indicated. Y axis indicates the number of CNVs identified at the correspondent genomic location; above zero are duplications, below zero are deletions. Values are mean and error bars are $\pm$ SEM.



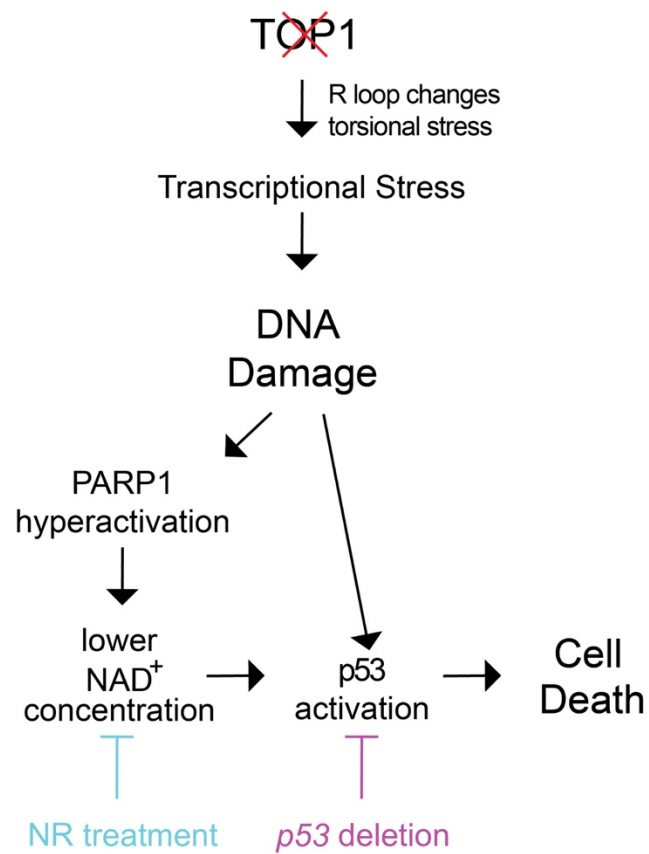
Supplementary Figure 7. Treatment with NR increases NAD⁺ levels in the cerebral cortex of neonatal mice.

Quantification of NAD⁺ levels in cortical lysates of P3 mice collected 1 h after i.p. injection with saline, NR (100 mg/kg) or NMN (100 mg/kg). One-way ANOVA. n=3 animals per condition. Values are mean and error bars are ± SEM.

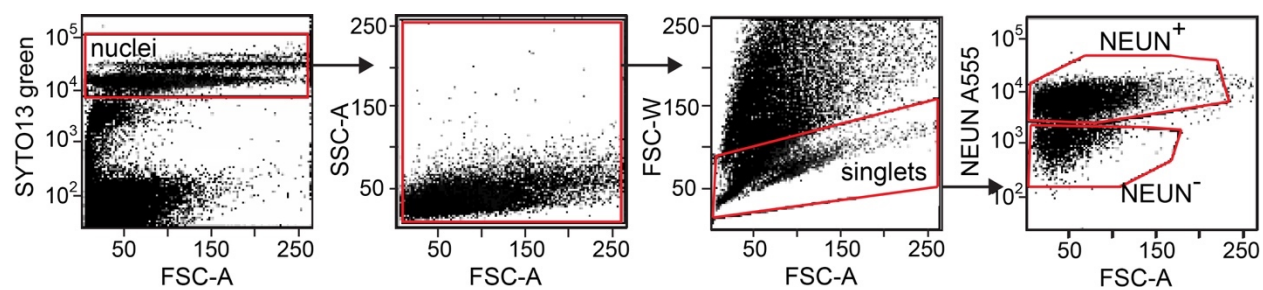


Supplementary Figure 8. Increased neuronal survival in *p53 KO::Top1 cKO* P15 cortex and hippocampus.

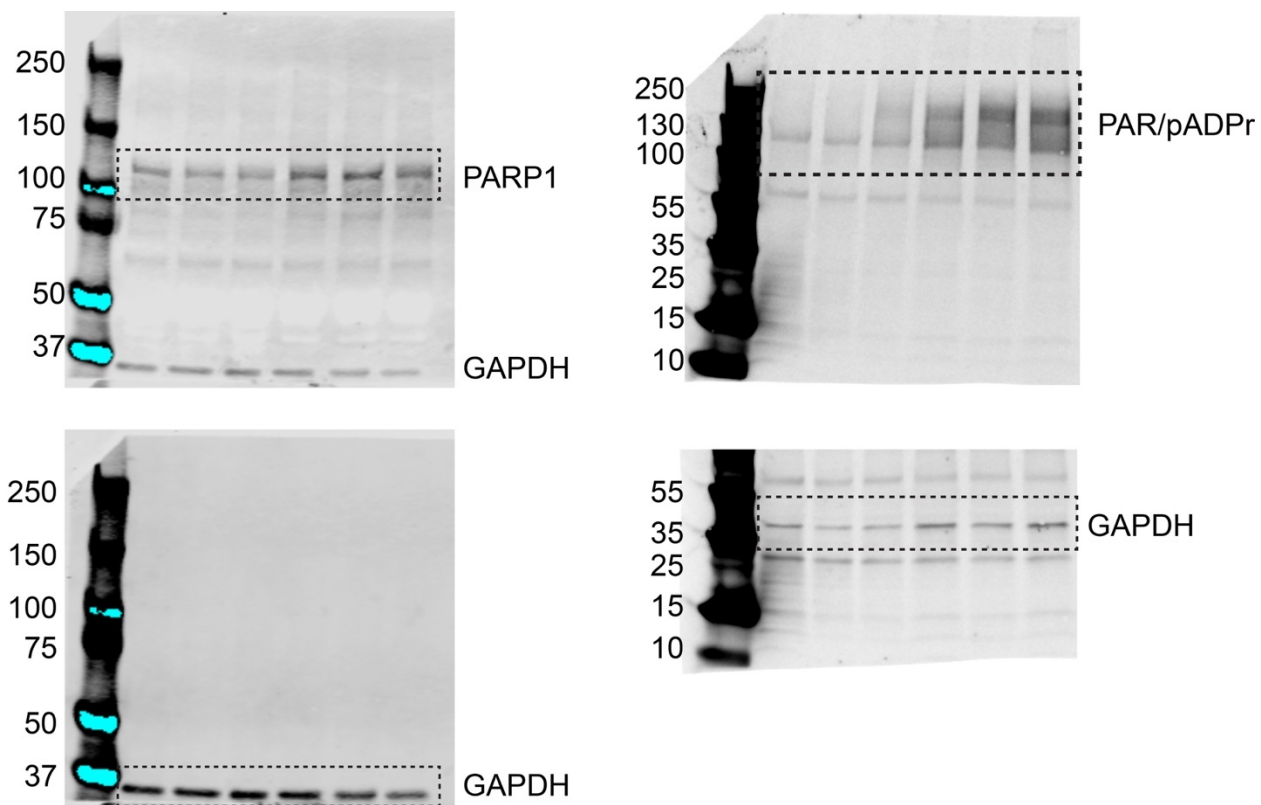
TOP1 and NEUN immunostaining of *p53 WT::Top1 WT*, *p53 KO::Top1 cKO* and *p53 WT::Top1 cKO* P15 cortex. Scale bar = 300 μm. Images are representative of 3 independent experiments.



Supplementary Figure 9. Potential mechanism of DNA damage-induced neuronal death in *Top1*-deficient neurons.



Supplementary Figure 10. Gating strategy used for sorting neurons for Figure 6d and Supplementary Figure 6e.



Supplementary Figure 11. Uncropped immunoblots from Figure 7a.