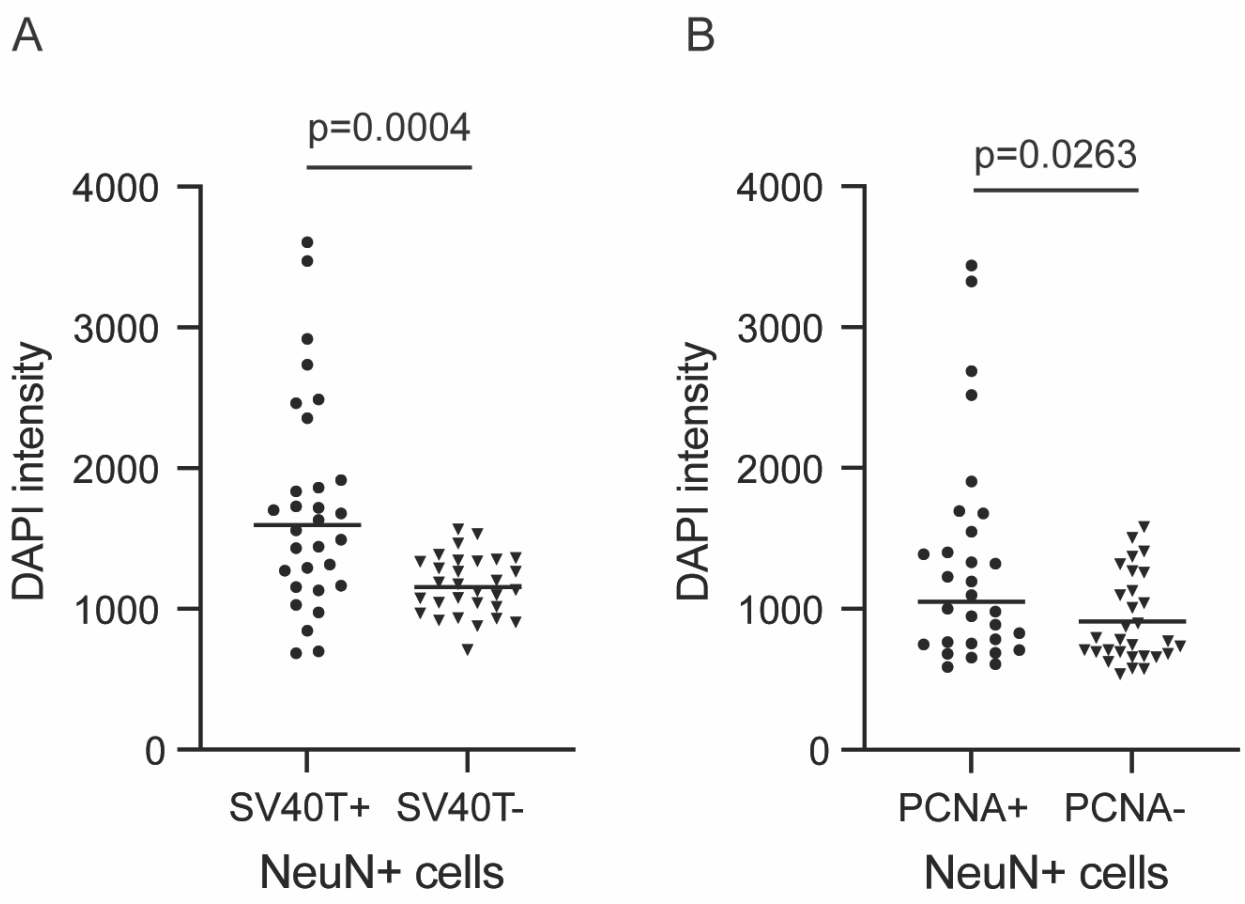
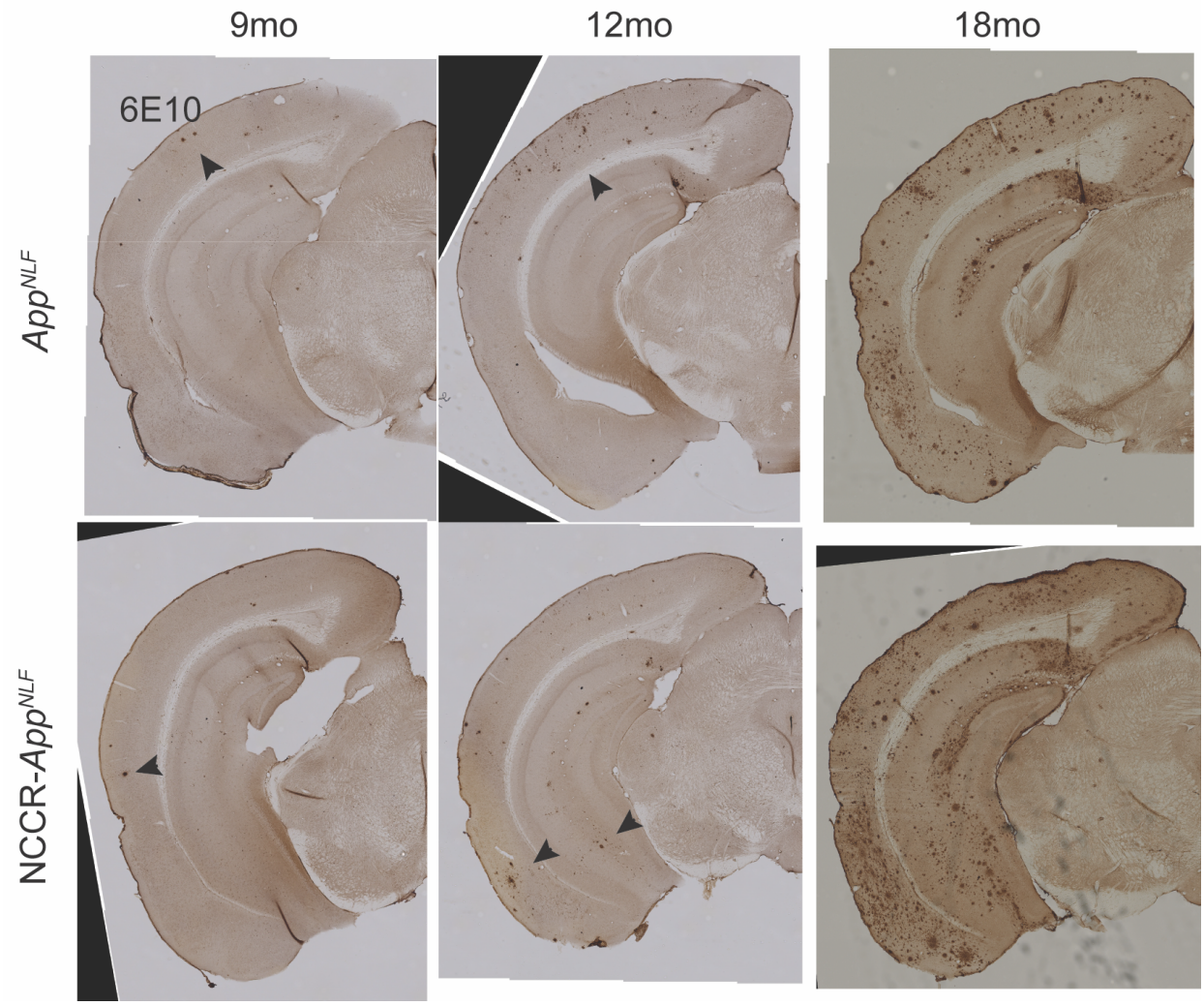
Supplementary Material

Neuronal Cell Cycle Re-Entry Enhances Neuropathological Features in *App*^{NLF} Knock-In Mice

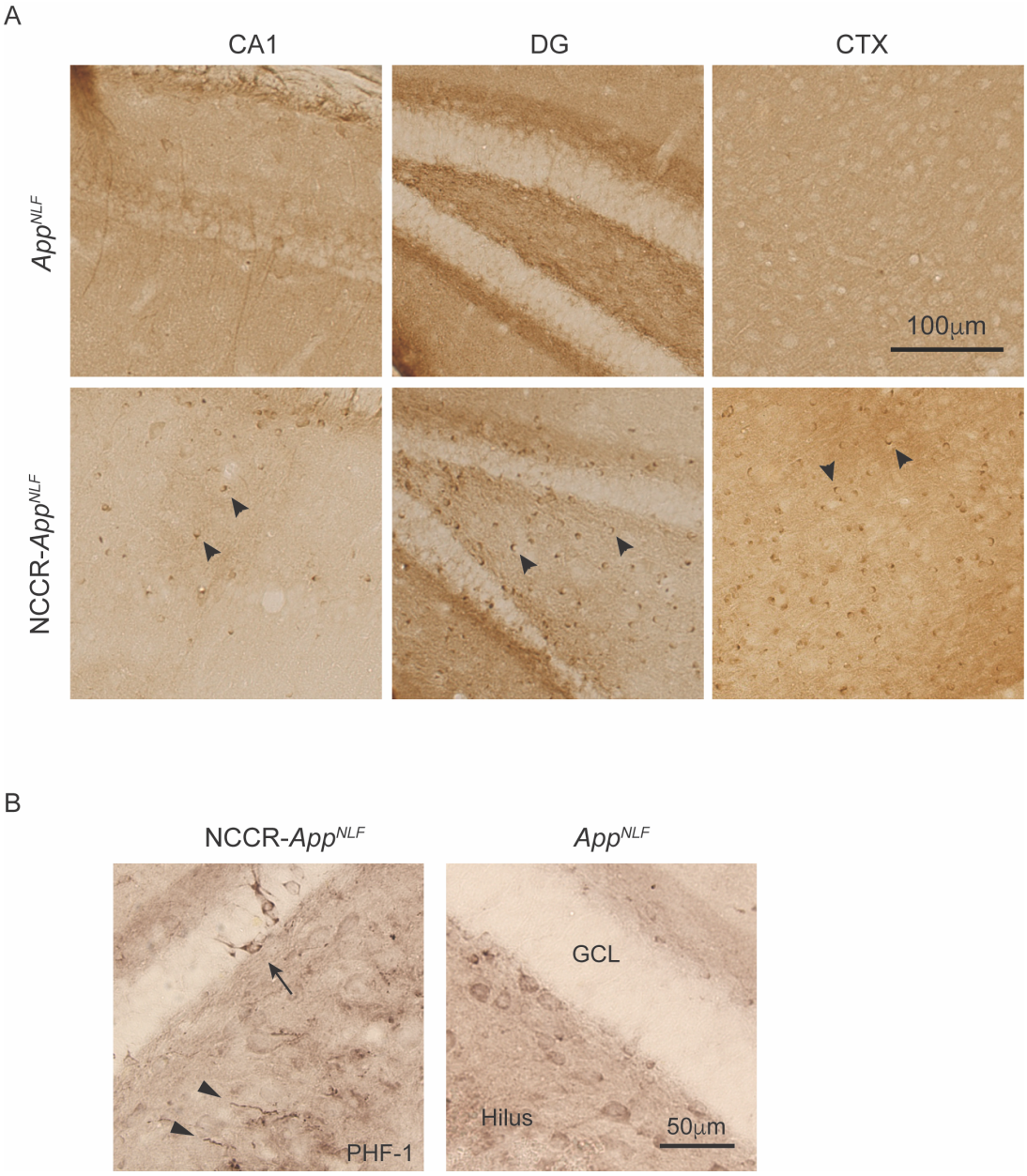
Supplementary Figure 1. DAPI fluorescence intensities are increased in SV40T+/NeuN+ (A) and PCNA+/NeuN+ (B) cells in NCCR-*App*^{NLF} animals. A) DAPI fluorescence intensities were measured in SV40T/NeuN/DAPI immunolabeled brain sections from 9 months, 12 months, and 18 months animals. We measured the intensities from SV40T+/NeuN+ and SV40T-/NeuN+ cells in the cortex (n=10 for each from each time point; n=30 per SV40T+ and SV40T- cells). DAPI intensities are higher in the SV40T+/NeuN+ cells compared to SV40T-/NeuN+ cells (mean values: 1,719 versus 1,164; two-tailed Mann-Whitney U test, p=0.0004). B) Similar measures were done in PCNA/NeuN/DAPI immunolabeled brain sections. DAPI intensities were higher in the PCNA+/NeuN+ cells compared to PCNA-/NeuN+ cells (mean values: 1,313 versus 912.8, two-tailed Mann-Whitney U test, p=0.0263).



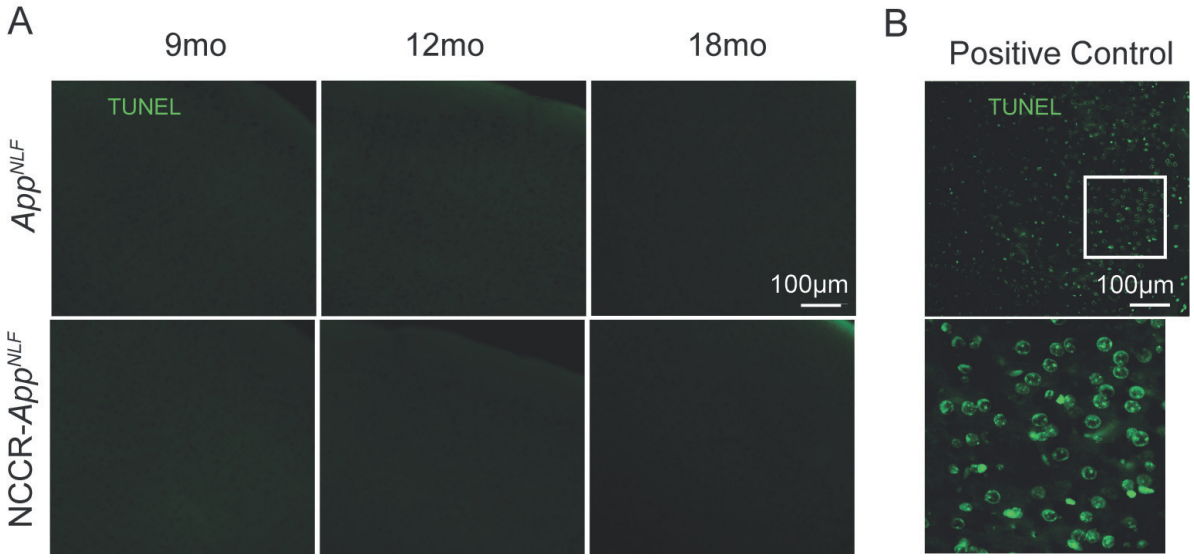
Supplementary Figure 2. Representative images of 6E10 immunolabeled DAB stained brain hemispheres generated from stitched microfield images. 6E10 is a monoclonal antibody targeting aa1-17 of Aβ domain. The images show progressive increase in Aβ plaques between 9 and 18months of age in both *App*^{NLF} and NCCR-*App*^{NLF} animals. Aβ plaques (arrowheads) are detectable starting around 9 months of age and become very abundant at 18 months of age in both genotypes.



Supplementary Figure 3. A) Representative images of PHF-1 phopho-tau DAB stains in CA1, dentate gyrus (DG) and cortex (CTX) of 18-month-old *App*^{NLF} and NCCR-*App*^{NLF} animals. At 18 months of age, NCCR-*App*^{NLF} animals showed a dramatic increase in cellular accumulation of PHF-1 phospho-tau (arrowheads) in the forebrain compared to age-matched *App*^{NLF} mice. B) Representative images of PHF-1 phospho-tau DAB stains in the dentate gyrus of 12-month-old NCCR-*App*^{NLF} and *App*^{NLF} mice. Altered PHF-1 phospho-tau distribution was observed in the NCCR-*App*^{NLF} animals. There were tortuous, dystrophic neurites labeled with PHF-1 phospho-tau in the hilus (arrowheads). We also noticed granule cell layer neurons stained with PHF-1 in the NCCR-*App*^{NLF} mice (arrows). The PHF-1 tau lesions observed in 18-month-old NCCR-*App*^{NLF} animals were not observed in the 12-month-old NCCR-*App*^{NLF} animals (as shown in Fig. 3A).



Supplementary Figure 4. Despite the increase in pan-nuclear γH2AX signal, NCCR-*App*^{NLF} animals do not show DNA fragmentation as shown by TUNEL assay. A) TUNEL staining did not show DNA fragmentation in either NCCR-*App*^{NLF} or *App*^{NLF} animals at 9, 12, and 18 months of age. Scale bar = 100 µm. B) Images of a positive TUNEL control brain section samples treated with DNase run under the same conditions as NCCR-*App*^{NLF} and *App*^{NLF} mice samples. Bottom panel is 63x magnification of the area within the white box.



Supplementary Figure 5. NCCR-*App*^{NLF} animals show enhanced neurodegeneration at 9-, 12-, and 18-months of age compared to *App*^{NLF} animals. Fluoro-jade C staining was used to evaluate neurodegeneration. A) Representative cortical images of Fluoro-Jade C (green) stained brain sections show increased stain in NCCR-*App*^{NLF} animals compared to *App*^{NLF} animals. B) Higher magnification images of inset in (A) shows finer Fluoro-Jade C stain in NCCR-*App*^{NLF} animals. Constellation of punctate stain suggests neuropil degeneration in NCCR-*App*^{NLF} animals which could contribute to the severe cortical atrophy observed at 18 months of age.