Supplemental Figure Legends

Figure S1. Schematic representation of the *brat* locus.

The organization of the *brat* gene and the nature of the nucleotide change at position 37,739 in the *867* mutant fly line are shown. The sequences of the primers used to sequence the coding region are provided.

Figure S2. Progenitor-specific knockdown of *brat* and overexpression of Notch intracellular domain leads to neurodegeneration.

Representative 5-µm paraffin sections of adult brains from 9-11 days-old *Insc-Gal4/+* (controls, n=8), *Insc-Gal4>UAS-brat*$_{RNAi}$ (n=8) *and wor-Gal4> UAS-NICD* (n=7) flies.

Figure S3. Large nuclei are present in regions of proliferating cells in *brat*^{chs} brains.

Confocal microscope images of brains from *brat*^{chs}*; pcna-GFP* flies stained for DAPI (blue), GFP (green) and PH3 (red). The zone containing large nuclei is located above the dashed line. This zone is also highly proliferative as indicated by both PH3 staining and the GFP from the *pcna-GFP* reporter .

Figure S4. Additional validation of *earmuff* gene expression.

qRT-PCR analysis of *erm* mRNA in heads of flies of the indicated genotypes using 2 additional primer pairs. The sequences of the *erm* primer pairs used in

this Figure and in Figure 6 are provided in the Methods. Values shown are mean ± SEM from three biological replicates. ns: not significant, based on non-parametric Mann Whitney U-test.

Figure S5. *R9D11-mCD8-GFP*-positive axonal projections in *brat^{chs}* brains.

A. High magnification view of a tumor from a *brat^{chs}* brain expressing GFP (green) from the *erm* reporter, *mCD8-R9D11-GFP*. The brain has been counterstained with DAPI (blue) to visualize nuclei. Although *erm* normally is expressed in Type II INPs, some of the cells that express *mCD8-R9D11-GFP* make projections that resemble axons (arrows). **B.** A portion of a *brat^{chs}* brain expressing GFP (green) from the *erm* reporter, *mCD8-R9D11-GFP* with tumor regions outlined with dotted lines. This brain was stained for the neuronal protein Elav (red) and GFP (green), and counterstained with DAPI (blue) to visualize nuclei. Two of the tumor regions, indicated with boxes, are shown at higher magnification to the right. A small subset of the GFP-positive cells express the neuronal protein, Elav (arrowheads).

Figure S6. Axonal retraction is not identifiable in mushroom bodies (MB) of *brat^{chs}* brains.

Representative confocal microscope images of brains from 7-11 day old flies of the indicated genotypes stained for Fas II (red) and GFP (green). MB structures are present in controls (*pcna-GFP*, n=8 and *OK107-G4>UAS-mCD8::GFP/+*, n=5) and *brat^{chs}; pcna-GFP* (n=9) brains, but not when *brat* activity is reduced in

MB neurons (*OK107-G4>UAS-mCD8::GFP; UAS-brat*$_{RNAi}$, n=7). MBs are outlines with the dotted rectangles.

Figure S7. Neurodegeneration is not detected in brains of flies exhibiting MB axon retraction.

Representative 5-μm paraffin sections of adult brains from 9-11 days-old *OK107-G4>UAS-mCD8::GFP/+* (controls, n=3) and *OK107-G4>UAS-mCD8::GFP; UAS-brat*$_{RNAi}$ (n=5) flies. Holes in the neuropil are not observable in brains, in which *brat* was knocked down in MB neurons.