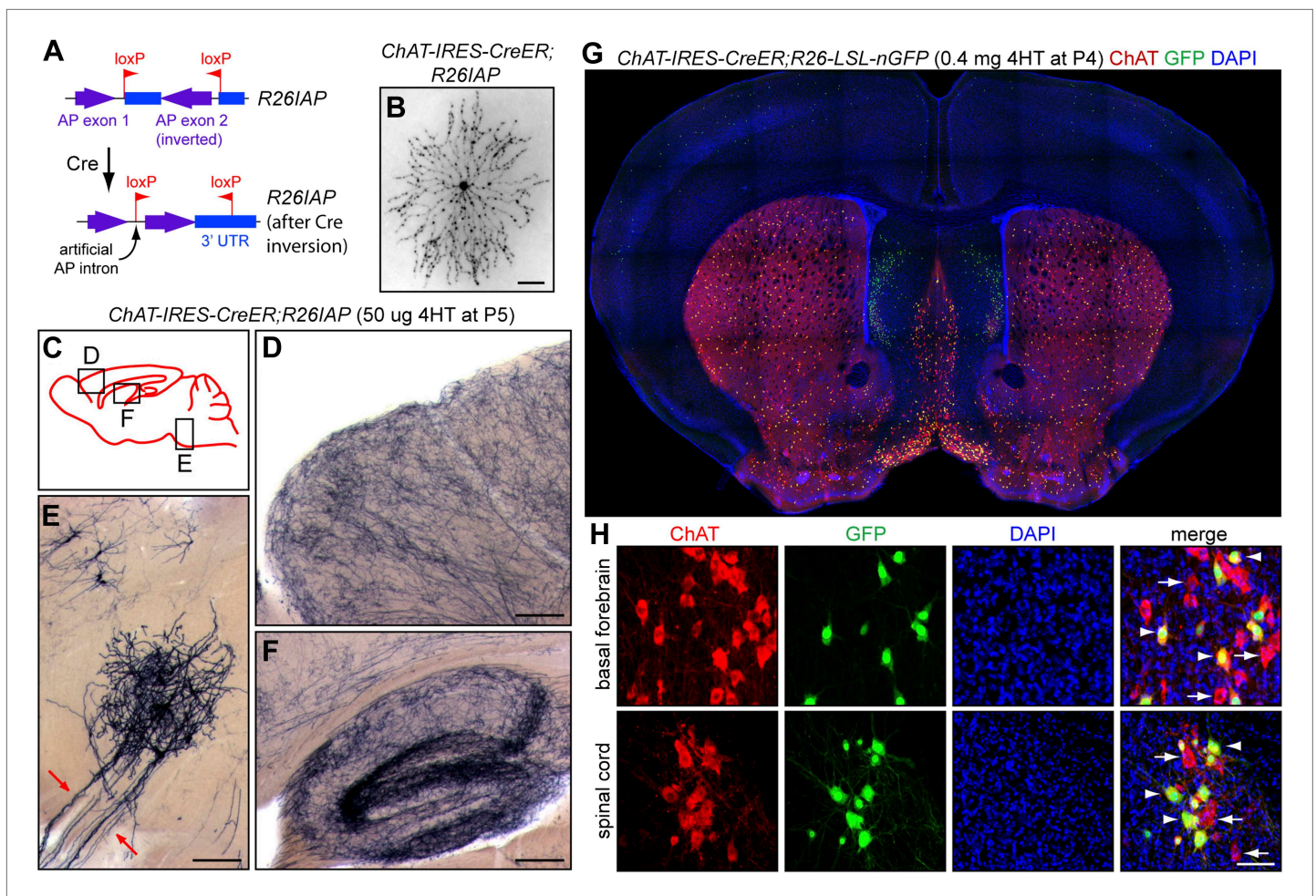# Figures and figure supplements

Complete morphologies of basal forebrain cholinergic neurons in the mouse

Hao Wu, et al.

Figure 1. Cholinergic neuron specificity of Cre-mediated recombination. (**A**) Structure of the *R26IAP* knock-in. In the absence of Cre-mediated recombination, the 3' half of the AP coding region is inverted in the germline configuration. It assumes the correct orientation following Cre-mediated recombination between inverted *loxP* sites. (**B**) P30 retina from *Chat-IRES-CreER;R26IAP* mice treated with 4HT. AP histochemistry labels cholinergic (starburst) amacrine cells. Scale bar, 100 µm. (**C–F**) P30 brain from *Chat-IRES-CreER;R26IAP* mice treated with high dose 4HT at P5. AP histochemistry labels numerous axons throughout the cortex (**D**) and hippocampus (**F**), as well as cranial motor neurons (**E**), the axons of which are seen exiting the brain stem (red arrows). Scale bars in **D–F**, 200 µm. (**G** and **H**) Coronal sections of P30 forebrain from *Chat-IRES-CreER;R26-LSL-nGFP* mice treated with high dose 4HT at P4. Approximately 50% of cholinergic neurons in the basal forebrain, medial septal nucleus, striatum, and spinal cord (visualized with ChAT immunohistochemistry) are GFP+. Medial to the striatum, a distinctive group of GFP+ cell is ChAT−; these cells presumably expressed *Chat* (and, therefore, *Cre*) in the early postnatal period and then repress *Chat* expression in adulthood. In (**H**), arrows point to ChAT+;GFP− neurons and arrowheads point to ChAT+; GFP+ neurons. Scale bar, 50 µm.
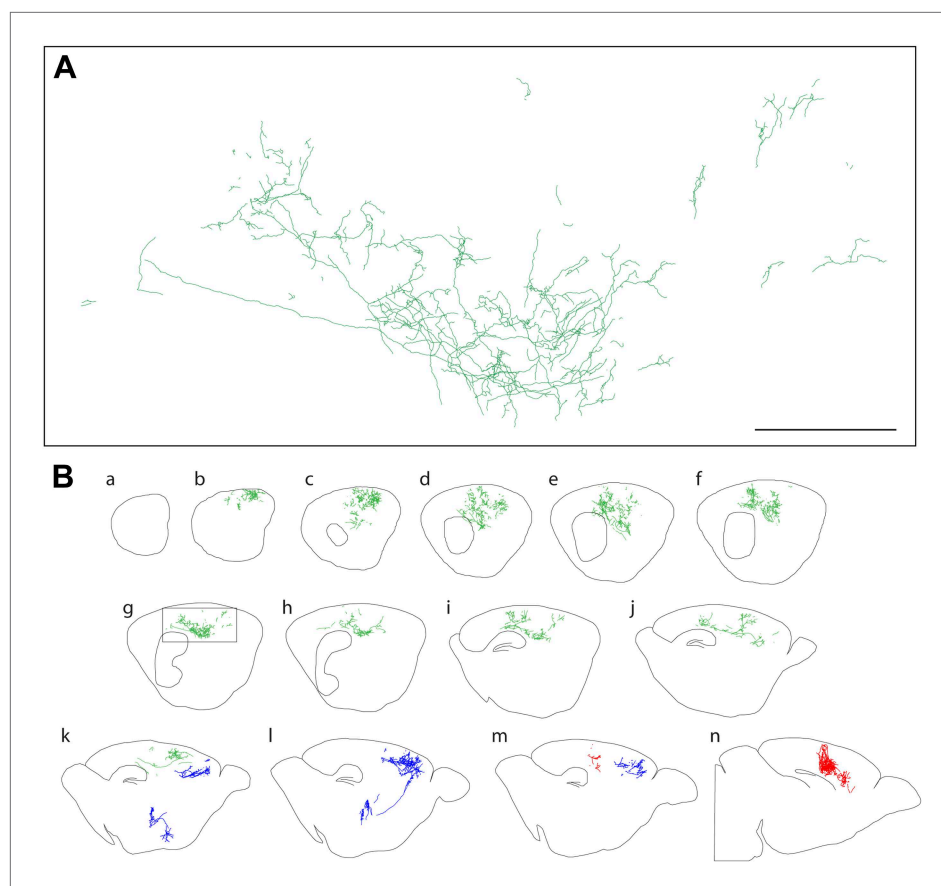
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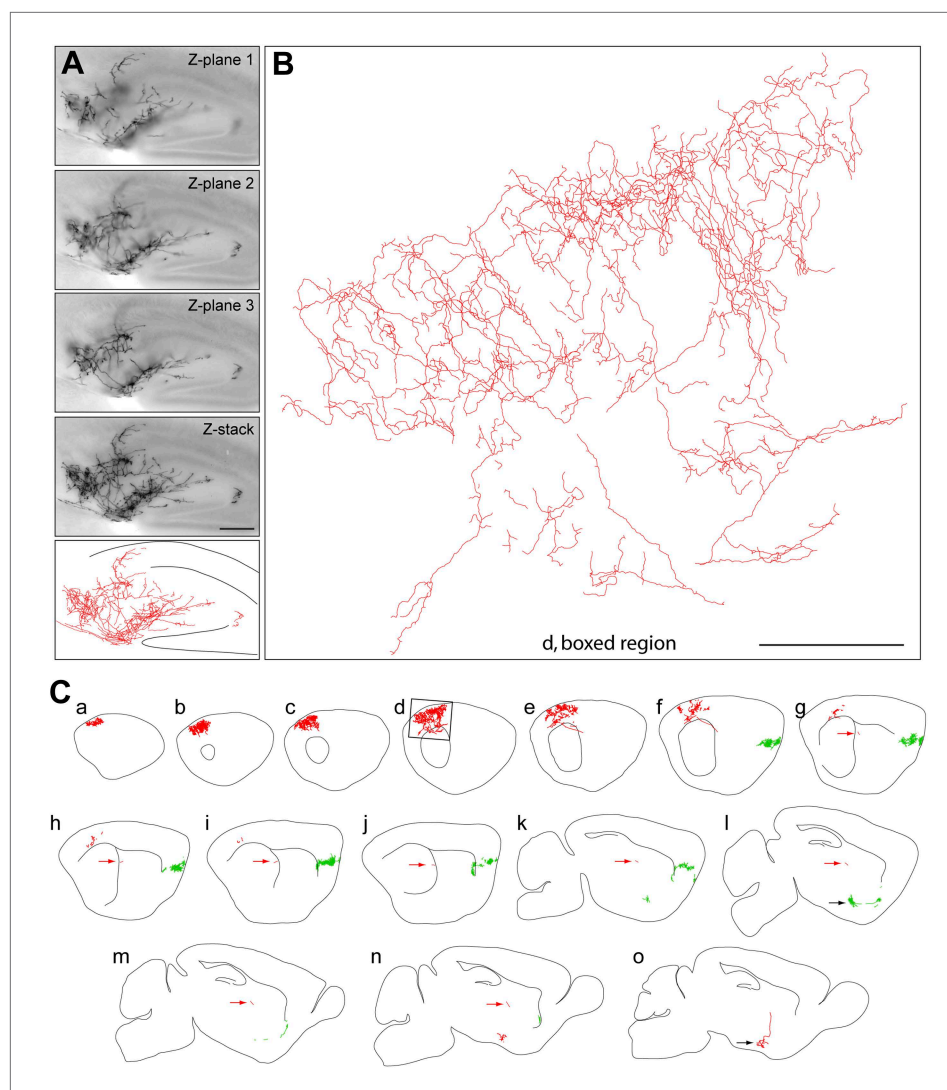
Figure 2. Axon arbors of forebrain cholinergic neurons from P30 *Chat-IRES-CreER;R26IAP* mice visualized with sparse Cre-mediated recombination. (**A**) Part of the arbor of a forebrain cholinergic neuron in a P30 hippocampus visualized in a single 300 μm section at three Z-planes and in a Z-stacked image. Bottom, the traced arbor. Scale bar, 200 μm. (**B** and **C**) Fifteen consecutive 300 μm sagittal sections from a single P30 hemisphere (**C**) with two fully traced AP+ forebrain cholinergic neurons, colored red and green. Black arrows in panels l and o, the two cell bodies. Red arrows in g–n, the proximal axon segment for the red neuron trace. (**B**) An enlarged view of the boxed region of section d in (**C**). Scale bar in (**B**), 500 μm (corrected for tissue shrinkage in BBBA).
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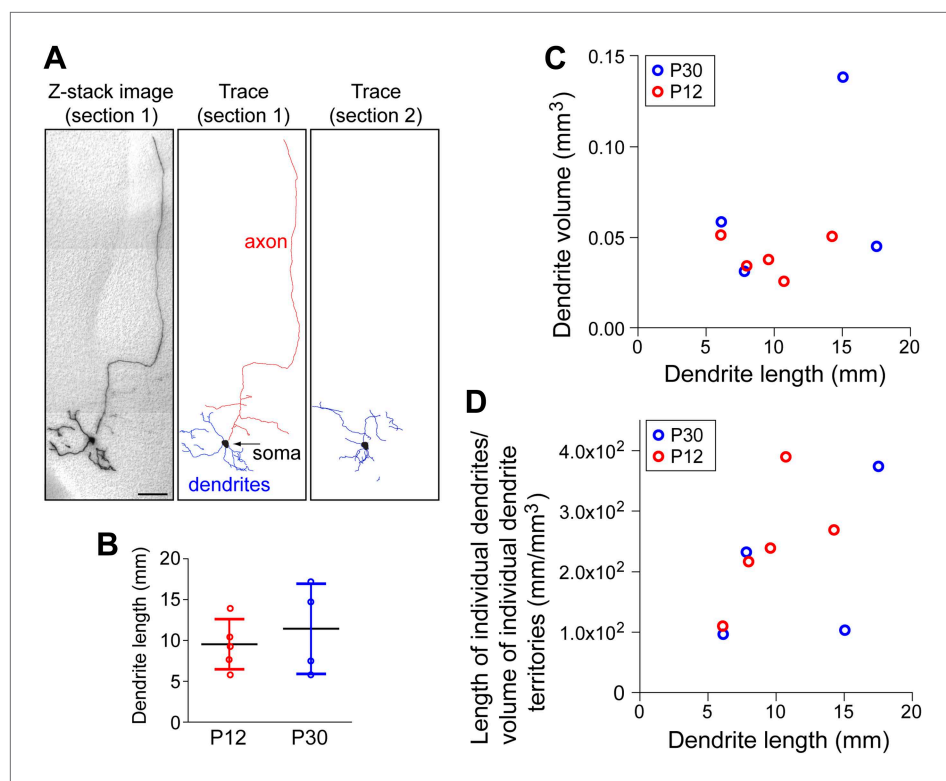
Figure 2—figure supplement 1. Dendrite structure among forebrain cholinergic neurons.

**Figure 3**. Axon arbors of forebrain cholinergic neurons from P12 *Chat-IRES-CreER;R26IAP* mice visualized with sparse Cre-mediated recombination. (**A**) Enlarged view of the boxed region from panel g in (**B**). Scale bar, 500 µm (corrected for tissue shrinkage in BBBA). (**B**) Fourteen consecutive 300 µm sagittal sections from a single P12 hemisphere (a–n) with three traced AP+ cholinergic neurons, colored red, green, and blue. The blue neuron is shown in its entirety, including the cell body and dendrites in the basal forebrain; for the green and red neurons, only the cortical axon arbors are shown.
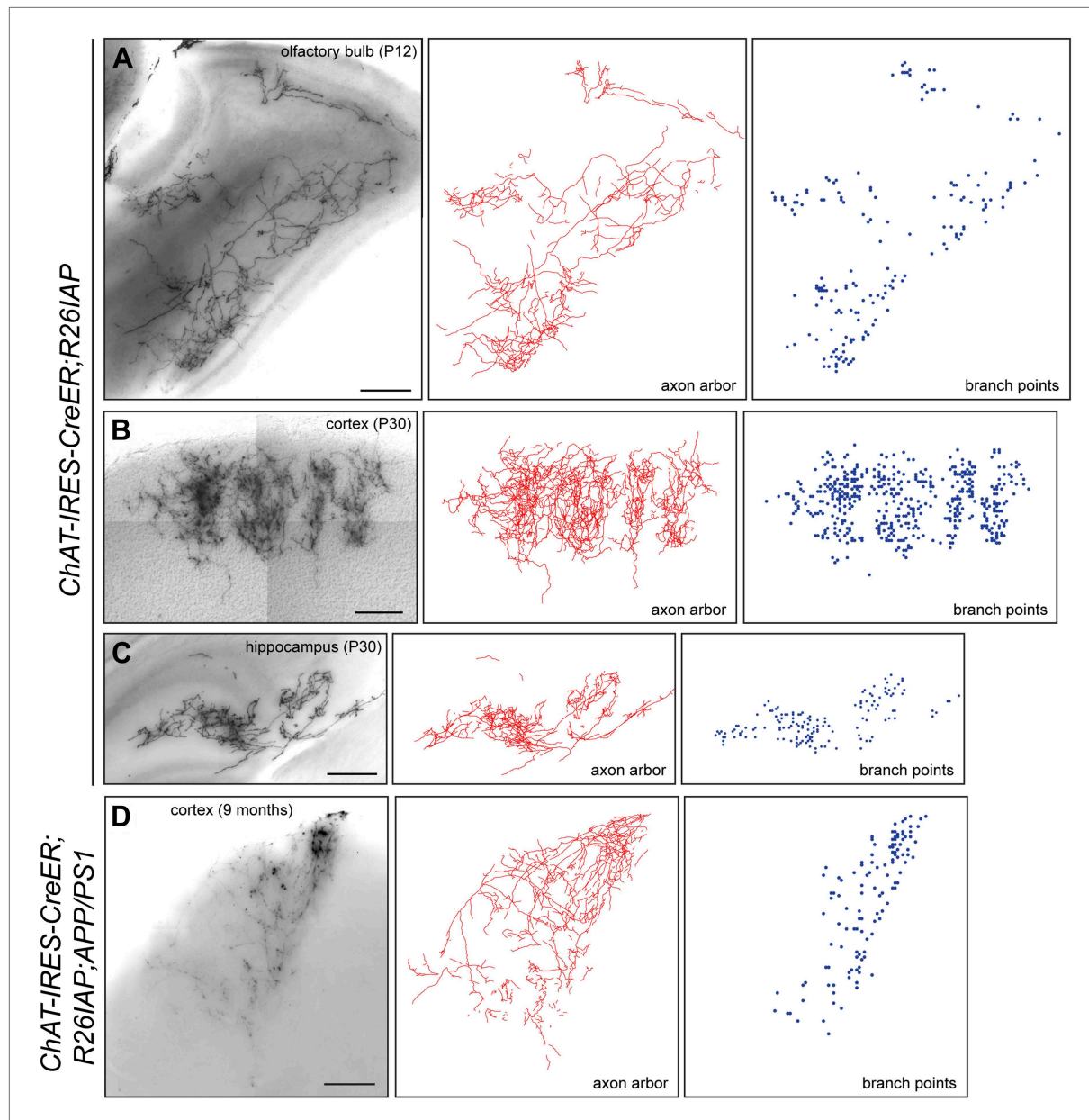DOI: 10.7554/eLife.02444.006
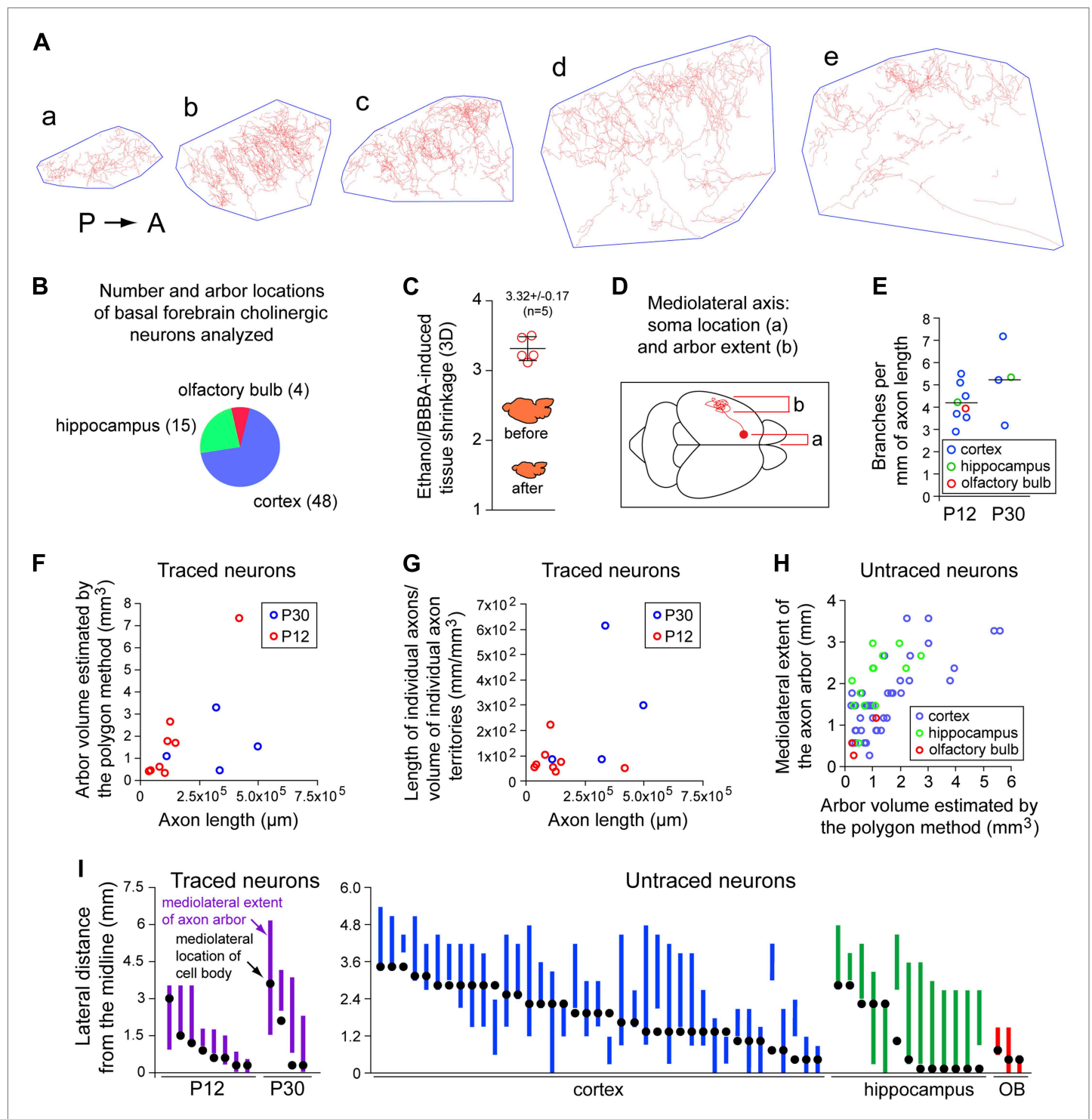
Figure 4. Quantitative analysis of morphologic parameters for cholinergic axon arbors. (**A**) The polygon method for estimating the target area for a single cholinergic axon arbor. Traced axon arbor images are shown for each 300 μm sagittal section from the P30 brain in *Figure 2C*, panels a–e. A minimal convex polygon has been drawn around each trace, providing an upper estimate of the cortical territory that is directly influenced by the arbor. As seen in panels d and e, the polygon method somewhat overestimates the target area by including regions that are relatively far from the axon. P, posterior. A, anterior. (**B**) Axon arbor locations for the 67 basal forebrain cholinergic neurons analyzed. (**C**) Quantification of tissue volume shrinkage due to dehydration in ethanol and BBBA. (**D**) Schematic of a forebrain cholinergic neuron in a dorsal view of the mouse brain showing the mediolateral cell body and arbor locations, the parameters displayed in panels (**H**) and (**I**). (**E**) The number of branch points per mm of axon length for the 12 forebrain cholinergic neurons that were traced. These data were obtained from two 300 μm sections per arbor by measuring the total axon length and counting

Figure 4. Continued on next page

Figure 4. Continued

all branch points for the AP+ arbor within each section. (**F**) Scatter plot of arbor volume (estimated using the polygon method) vs axon length for the 12 forebrain cholinergic neurons that were traced. (**G**) Scatter plot of axon density (length divided by arbor volume) vs axon length for the 12 forebrain cholinergic neurons that were traced. (**H**) Scatter plot of the mediolateral extent (defined in panel **D**) vs arbor volume (estimated using the polygon method) for the 55 forebrain cholinergic arbors that were not traced. (**I**) Mediolateral cell body and arbor locations for the 12 traced neurons (left) and the 55 untraced neurons (right). Black dots represent cell body location and the vertical bar represents the mediolateral extent of the axon arbor. OB, olfactory bulb.

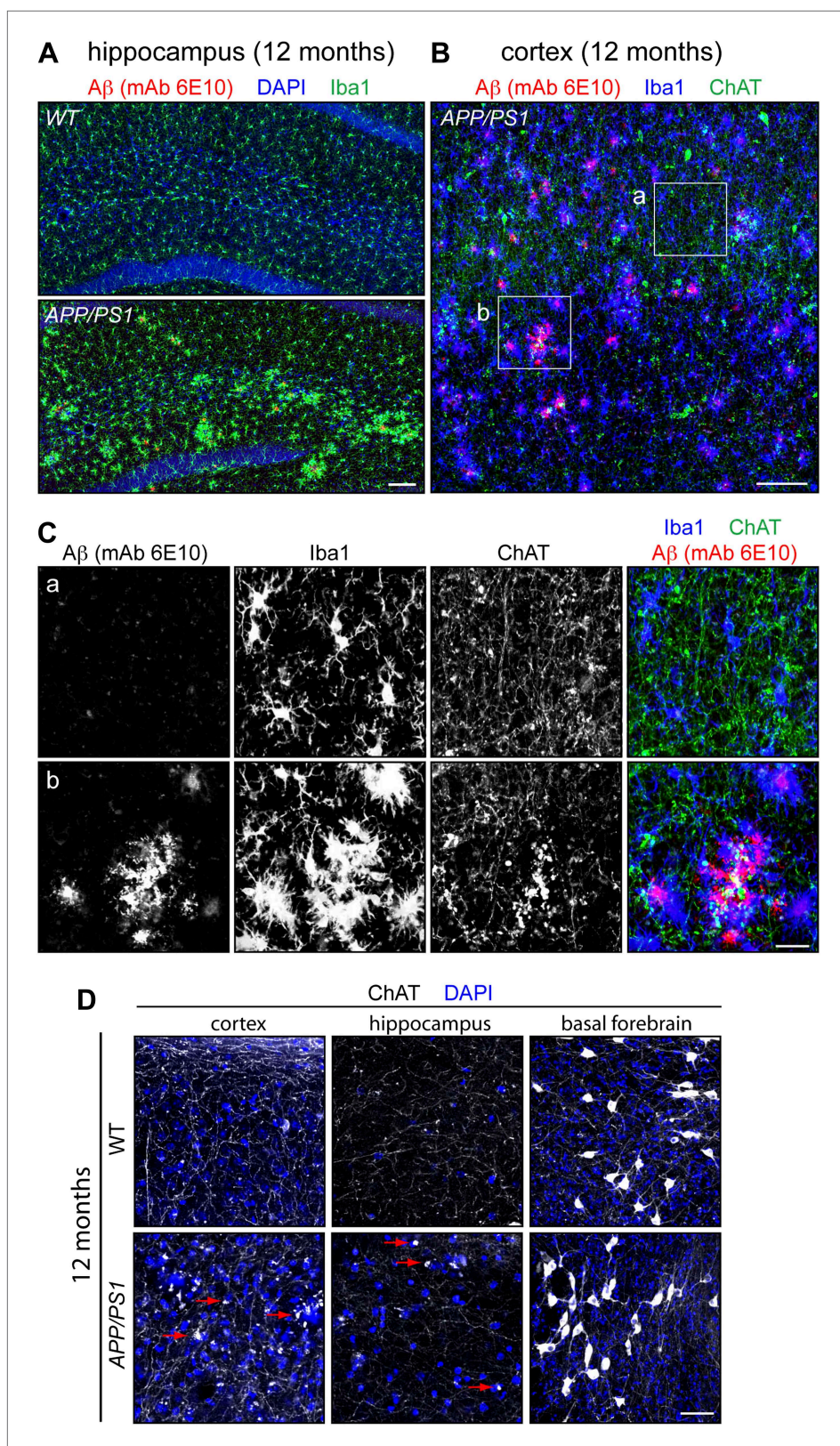**Figure 4—figure supplement 1**. Cholinergic axon arbors: Z-stacked images, traces, and branch point locations.
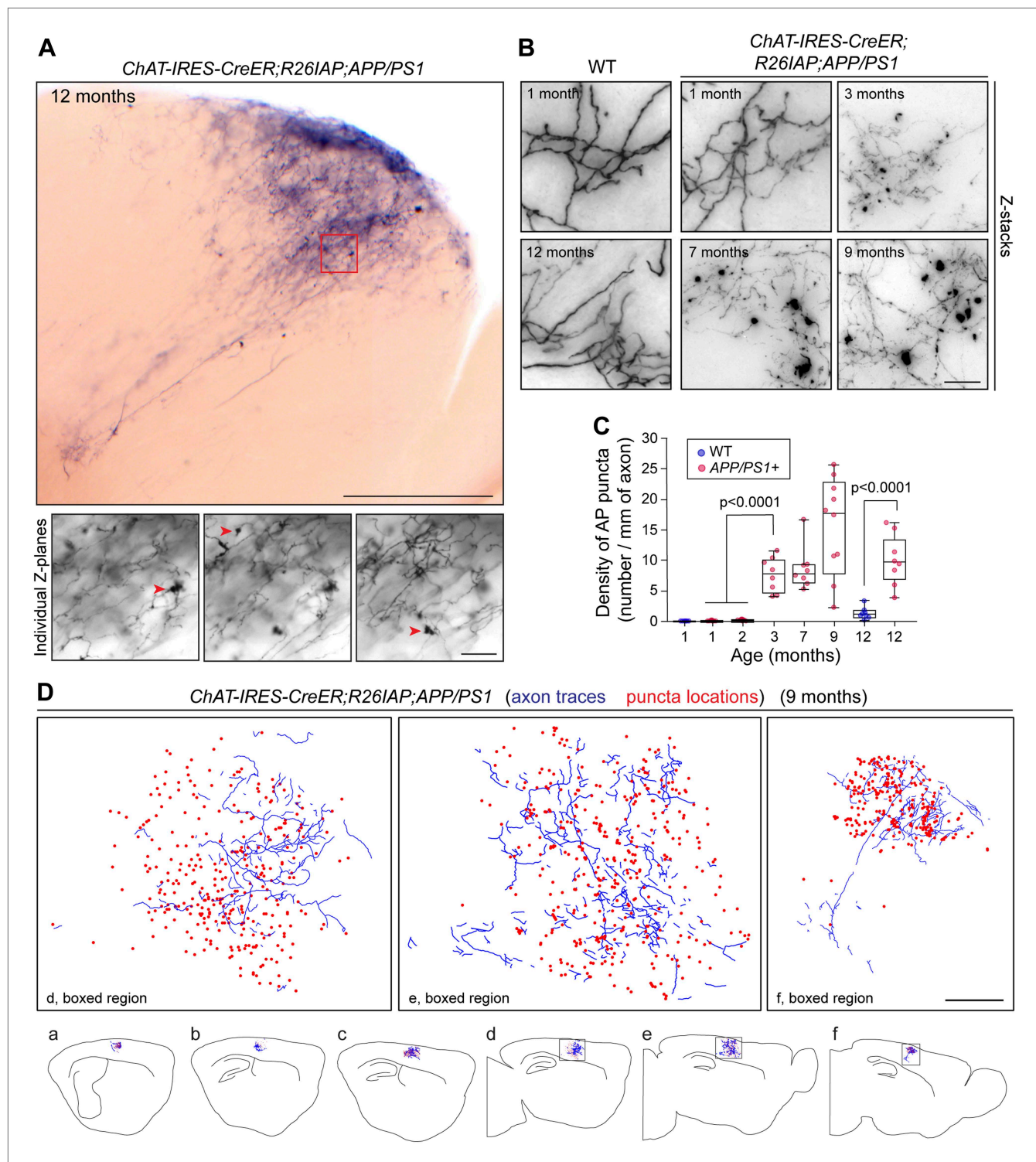
Figure 5. Disruption of cholinergic axon arbors in *Chat-IRES-CreER;R26IAP;APP/PS1* mice. (**A**) Upper panel, 300 μm sagittal section of a 12 month old *Chat-IRES-CreER;R26IAP;APP/PS1* brain showing part of a single AP+ axon arbor. The olfactory bulb is visible at lower right. Lower panels, three Z-planes enlarged from the region enclosed in the red square in the upper panel. Red arrowheads point to clumps of AP+ material (puncta). Scale bars: upper panel, 500 μm; lower panels, 50 μm. (**B**) Comparison of representative regions from forebrain cholinergic axon arbors in the cortex of *Chat-IRES-CreER;R26IAP* brains (WT; left) and *Chat-IRES-CreER;R26IAP;APP/PS1* brains (right), between one and 12 months of age. Structural heterogeneity, including the clumping of AP + material (puncta) and loss of AP staining intensity, increases with age in the *APP/PS1* background. Scale bar, 50 μm. (**C**) Quantification of AP+ puncta in the cortex and hippocampus of *Chat-IRES-CreER;R26IAP* (i.e., WT) and *Chat-IRES-CreER;R26IAP;APP/PS1* mice at

*Figure 5. Continued on next page*

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different ages. Puncta appear at 3 months in *Chat-IRES-CreER;R26IAP;APP/PS1* mice. The box plots indicate the extreme data points (top and bottom bars), the 25–75% interval (box), and the median (central line). p-values, student's *t* test. (**D**) Complete tracing of an AP+ cortical cholinergic arbor (blue) with the locations of AP + puncta (red dots) indicated. Panels a–f show six adjacent 300 μm sagittal sections within which this arbor resides. The three enlarged images above correspond to the boxed regions in panels d–f. Scale bar, 500 μm (corrected for tissue shrinkage in BBBA).
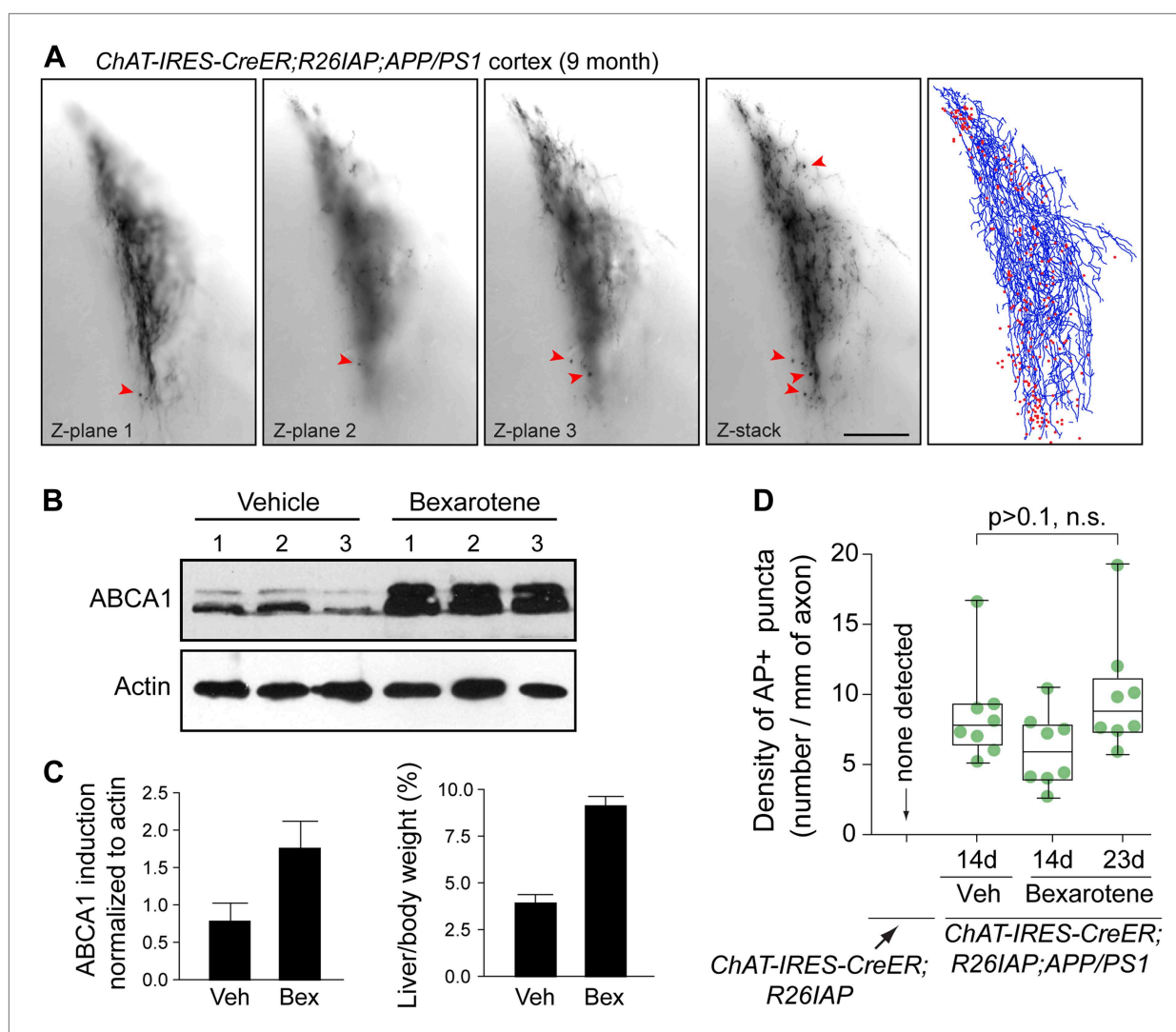
Figure 5—figure supplement 1. Aβ deposition, microglial reorganization, and disorganization of cholinergic fibers in the *APP/PS1* brain.
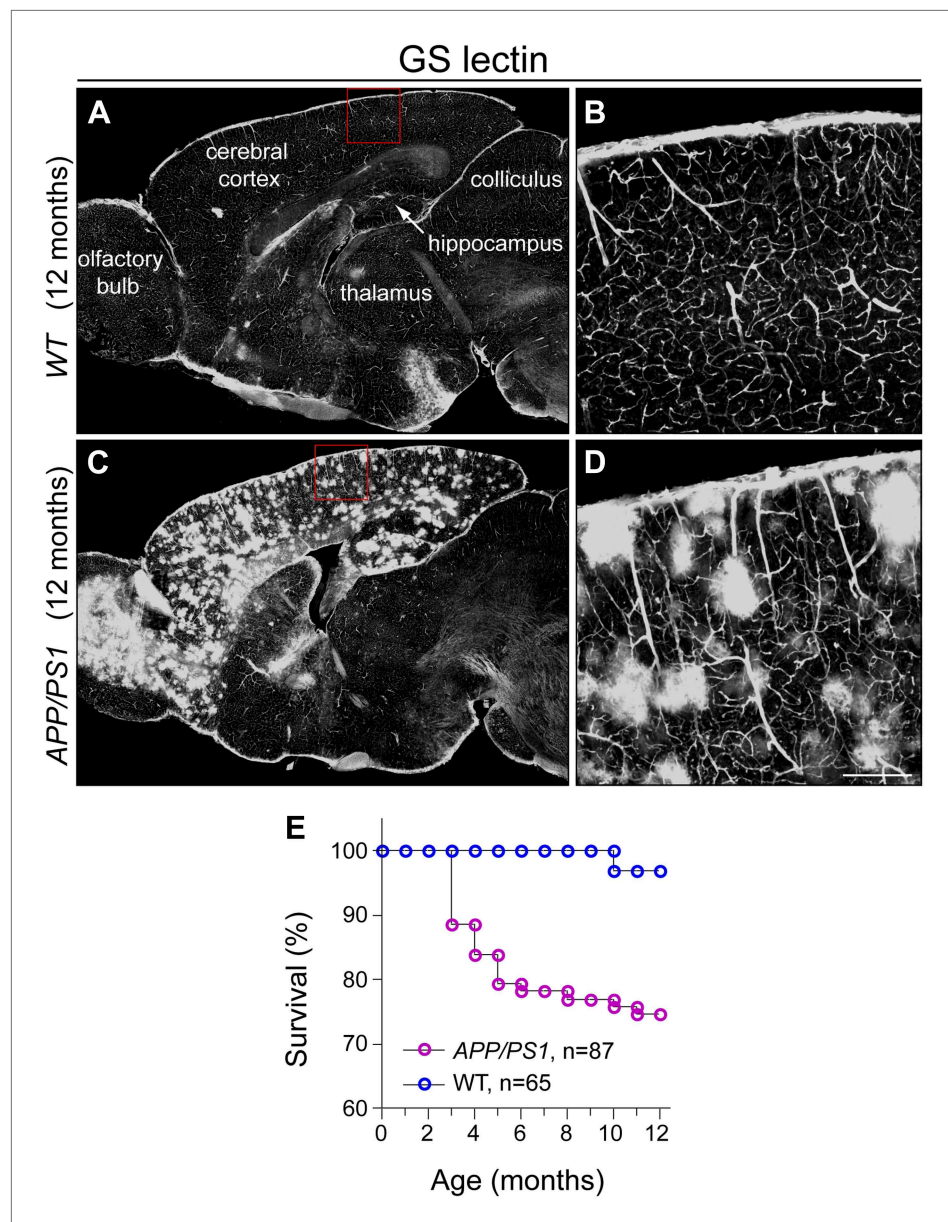
Figure 5—figure supplement 2. Characterization of *APP/PS1* mice: survival and microglial activation.

Figure 5—figure supplement 3. Tracing AP+ axons, and locating and quantifying AP+ puncta with and without bexarotene treatment.
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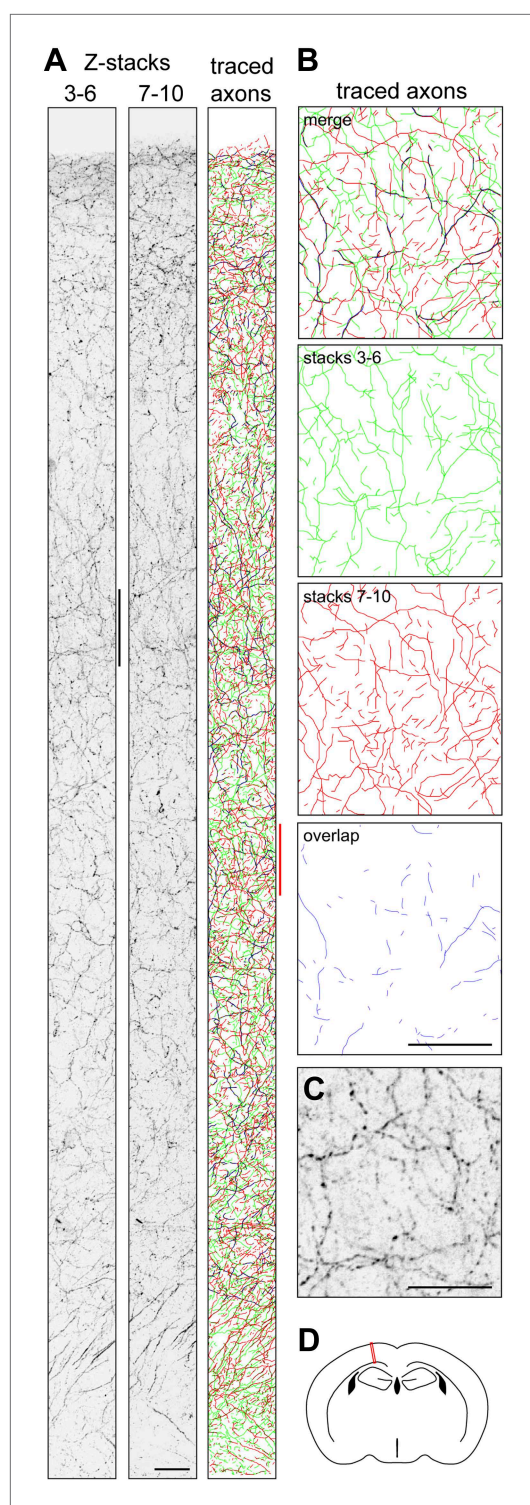
Figure 6. Quantifying ChAT+ axon density in P30 mouse cortex. (**A**) Coronal section of P30 mouse motor cortex following ChAT immunostaining. The cortical surface is at the top; the base of the cortex is at the bottom. Confocal images of the fluorescently immu-nostained tissue (converted to grey scale and inverted) were captured at Z-plane separations of 2 μm. Z-stacks

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encompassing planes 3–6 and 7–10 are shown. The traced axons for both sets of Z-stacks are color-coded with axons in stacks 3–6 in green, axons in stacks 7–10 in red, and regions of overlap in blue. Scale bar, 25 µm. (**B**) Axon tracings corresponding to the region in the right panel in (**A**) that is demarcated by the vertical red line. Scale bar, 25 µm. (**C**) ChAT immunostaining corresponding to the region adjacent to the left panel in (**A**) that is demarcated by the vertical black line. Scale bar, 25 µm. (**D**) The red rectangle shows the region of motor cortex analyzed in (**A**), at approximately Bregma −1.06.
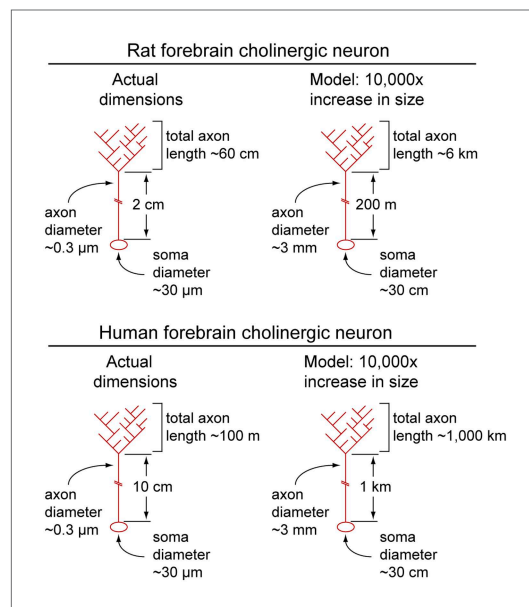
Figure 7. Calculated dimensions of rat and human forebrain cholinergic neurons. Actual dimensions of human and rat forebrain cholinergic neurons, calculated from the data in *Table 1* (left image in each pair). A macroscopic model in which the linear dimensions of each cholinergic neuron have been multiplied 10,000-fold (right image in each pair). Soma and axons are shown; dendrites have been omitted.