Expanded View Figures

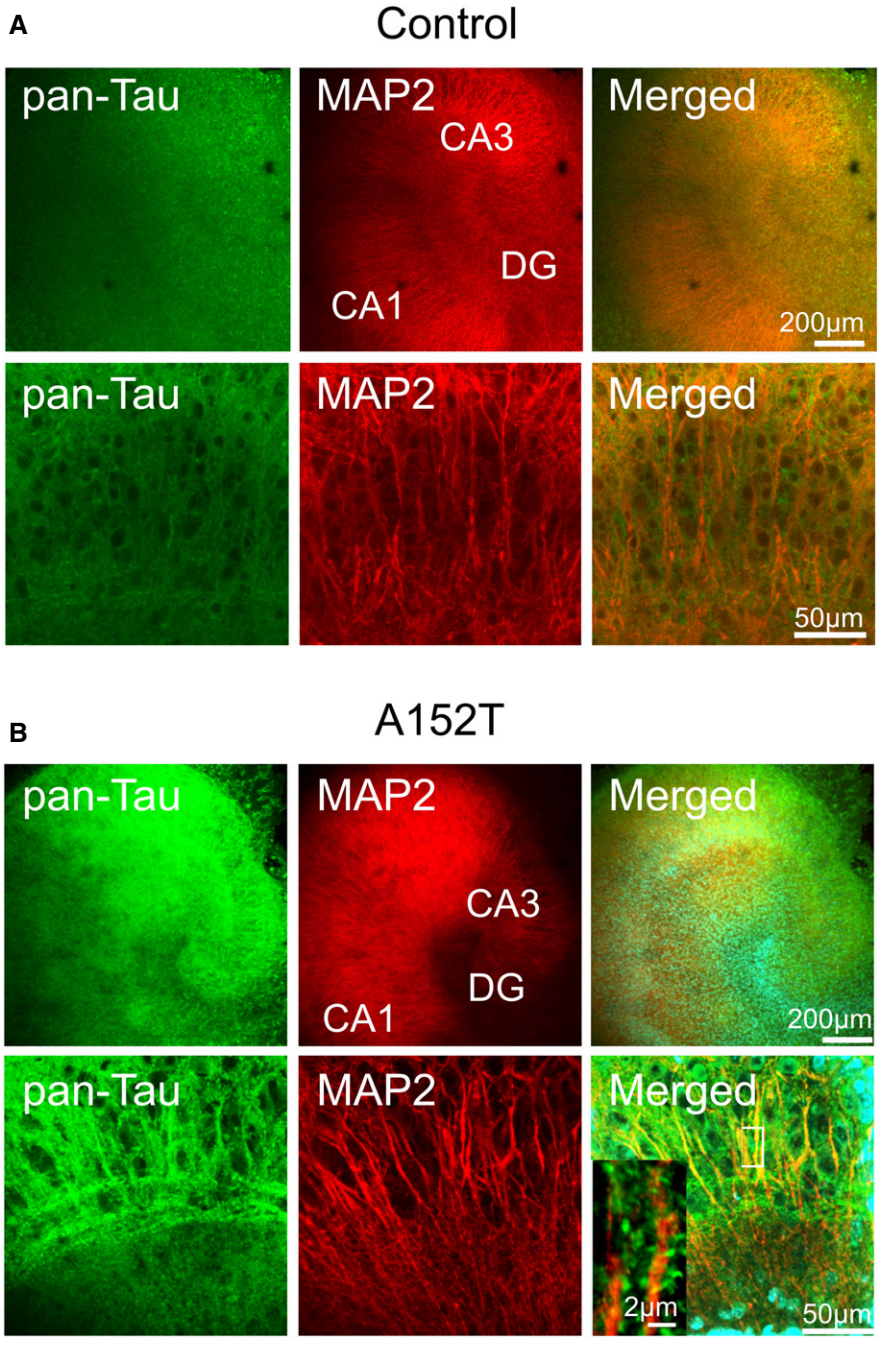

Figure EV1. Expression of hTau^{AT} causes missorting in organotypic hippocampal slice cultures.

A Immunohistochemistry highlighting the dendritic marker MAP2 and the axonal marker Tau protein (by pan-Tau antibody K9JA recognizing both human and mouse Tau) in slice cultures from non-transgenic littermate at DIV 10. Overview images (upper panel) and higher magnification of area CA3 (lower panel). Tau is restricted to axons and not seen in dendrites.

B Immunohistochemistry highlighting the dendritic marker MAP2 and axonal Tau protein (by pan-Tau antibody K9JA recognizing both human and mouse Tau) in slice cultures from hTau^{AT} at DIV 10. Overview images (upper panel) and higher magnification of area CA3 (lower panel). In hTau^{AT} slices, Tau is seen both in axons and dendrites. Higher magnification of apical dendrite (inset) displays Tau in dendritic spines in contrast to MAP2, which is restricted to the dendritic shaft.

Figure EV2. Neuronal counting in young and old slice cultures demonstrating progressive cell loss due to hTau^{AT} expression and CREB shut-off.

A NeuN-positive cell bodies were counted in the DG and within the pyramidal cell layer in areas CA3 and CA1 in slice cultures at DIV 10 in defined regions of interest (ROI). The number of neurons was unchanged in all regions investigated (n = 7 slices; prepared from at least two animals).

B NeuN-positive cell bodies were counted in the DG and within the pyramidal cell layer in area CA3 and CA1 in 60-day-old slice cultures. The number of neurons was strongly reduced in hTau^{AT} slices, both within area DG (**P = 0.0096) and CA3 (***P = 0.0007), whereas the number of neurons in the CA1 region (n.s., P = 0.2893) was only slightly affected (n = 6–9 slices; prepared from at least two animals; Student's t-test).

C Comparison of NeuN mRNA levels in control littermates and hTau^{AT} animals (control = 100%; n = 4 animals). In hTau^{AT} animals, NeuN mRNA is decreased to ~70% of control, indicating neuronal loss (n = 4 animals). Student's t-test; *$P < 0.05$.

D Caspase-3 activity was not significantly changed in hTau^{AT} slices (n = 5 slice homogenates prepared from 6 slices; Student's t-test; n.s., P = 0.4143).

E Slice homogenates of control (Ctrl, lanes 1 + 2) and hTau^{AT} (A152T, lanes 3 + 4) were probed for Western blot analysis with antibodies against CREB and p133CREB. The ratio of p133CREB to CREB was strongly reduced in hTau^{AT} slices (n = 12) in comparison to control littermates (n = 6 slice homogenates, Student's t-test; **P = 0.0064), indicating the activation of the CREB shut-off pathway.

Data information: Error bars indicate mean ± SEM.

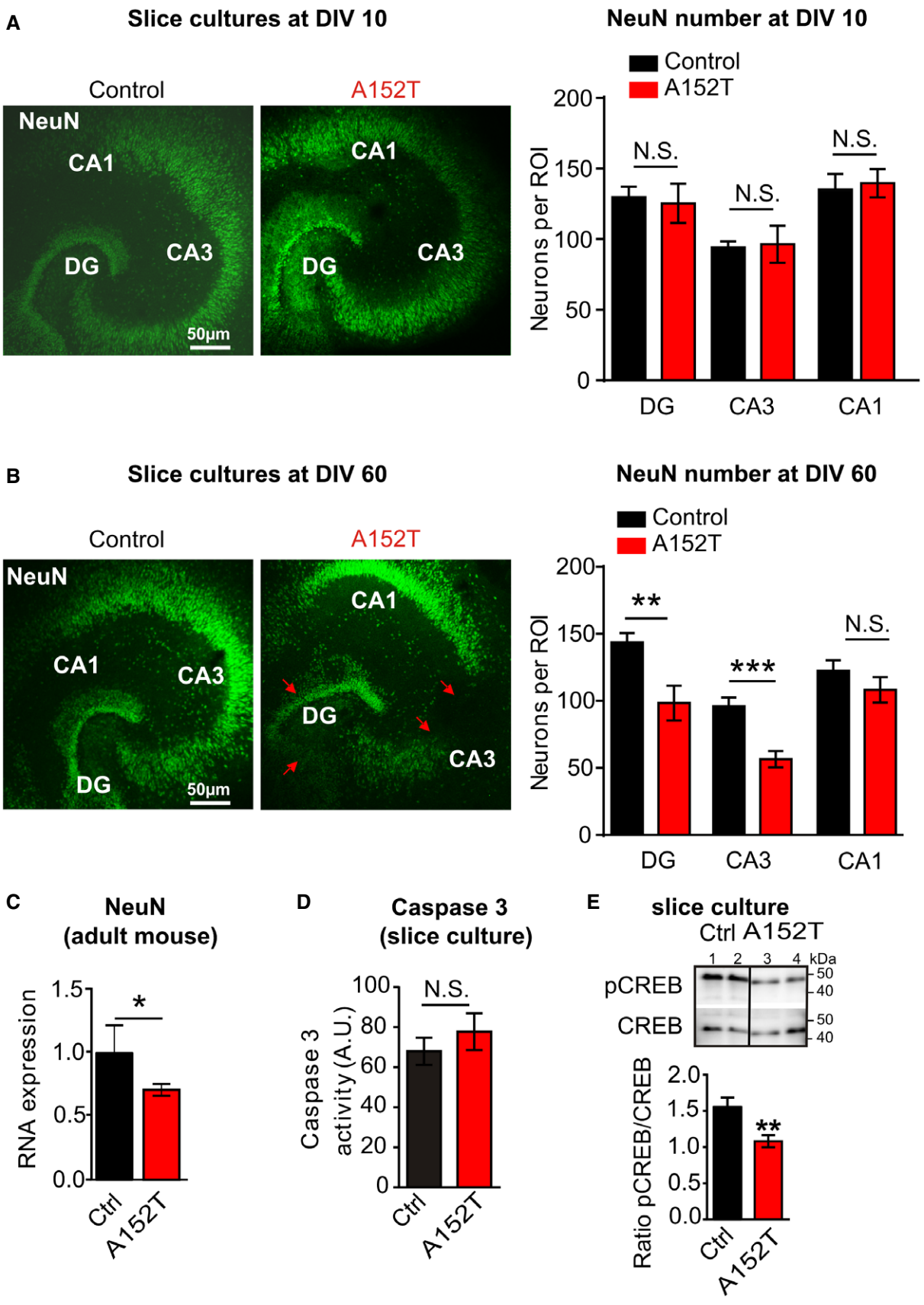

Figure EV2.

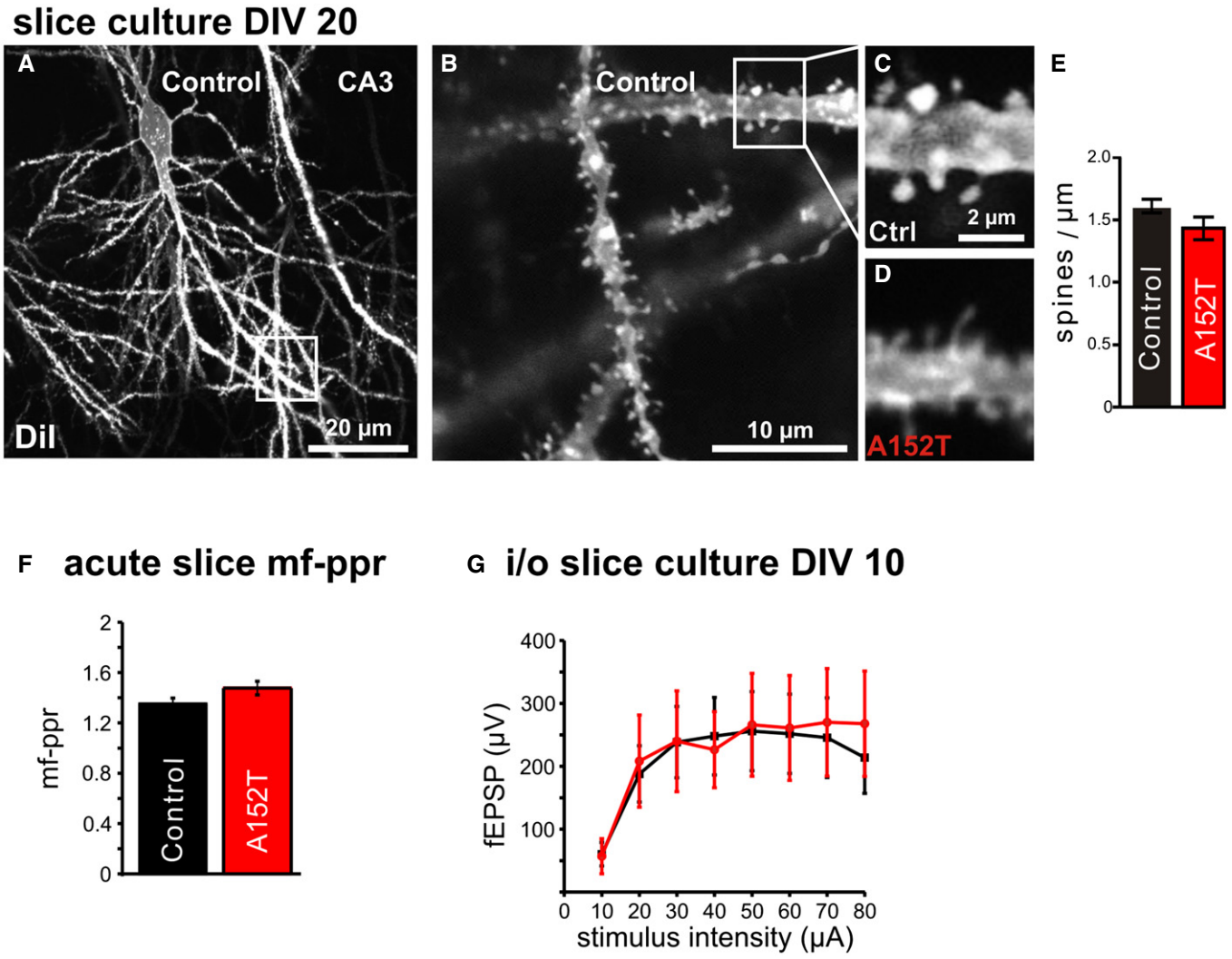

Figure EV3. Dendritic spine density, paired-pulse ratio as well as input–output relation in area CA3 of young slice cultures is unchanged.

A Example of a DiI-labeled neuron in 20-day-old control slice culture.

B Example of soma region and dendritic region where apical dendrites of area CA3 pyramidal neurons were imaged.

C Spines on apical dendrites (~100 µm distance to the cell body) in control littermate slices (magnification of box in B). Most spines are of the "stubby" type.

D Spines on dendrites from hTau^{AT}-expressing slices have a mostly filamentous morphology.

E Quantification of spine density per µm dendrite length did not show differences between control and hTau^{AT}-expressing neurons (Student's *t*-test: $P = 0.110$; $n = 26$ neurons from at least 12 slices (Control); $n = 24$ neurons from at least 12 slices (hTau^{AT})).

F Paired-pulse ratio as a measure for short-term plasticity in the mossy fiber pathway of adult mice shows no differences between control littermates ($n = 29$) and Tau^{AT}-expressing mice ($n = 38$).

G Input–output relation of field excitatory potentials recorded in stratum pyramidale of area CA3 in hippocampal slice cultures. At DIV 10, no change between control littermates ($n = 7$) and hTau^{AT}-expressing mice ($n = 12$) could be detected.

Data information: Error bars represent SEM.

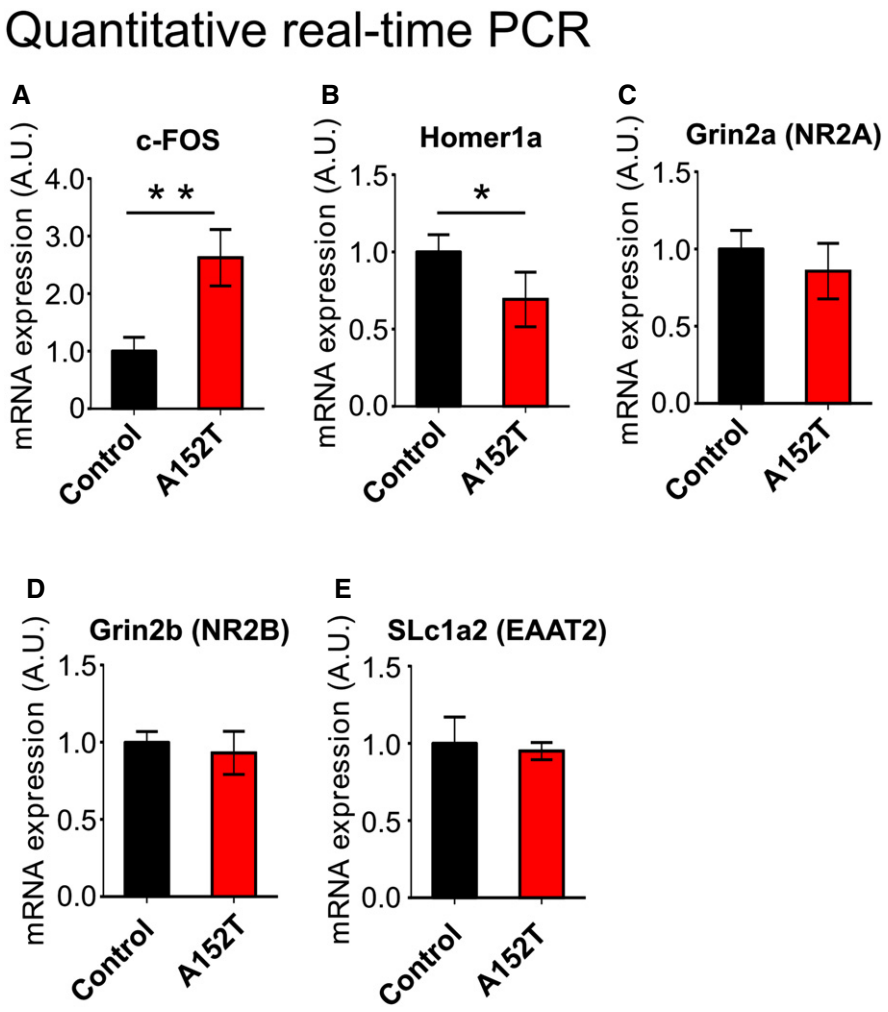

Figure EV4. Quantitative real-time PCR for network activity markers, NMDAR subunits, and glutamate transporter in hippocampi from 16-month-old hTau^{AT}-expressing mice.

A Quantification of c-Fos mRNA in control littermate and hTau^{AT} transgenics. Note the pronounced increase in c-Fos expression in transgenic animals (~250% of control), indicating enhanced neuronal activity.

B Amount of mRNA of the scaffolding protein Homer1a in control littermate mice and hTau^{AT}-expressing mice. Mutant Tau expression decreases expression of Homer1a (~70% of control).

C, D Quantification of Grin2a (C) and Grin2b (D) transcripts. No obvious changes were detected, indicating no translational changes in NR2A and NR2B subunits in hTau^{AT} mice.

E Quantification of SLc1a2 (EAAT2) transcripts. No obvious changes could be detected, indicating that EAAT2 mRNA levels are normal in hTau^{AT} mice.

Data information: Error bars denote SEM; *$P < 0.05$, **$P < 0.01$. All experiments were carried out with 4 animals per group.

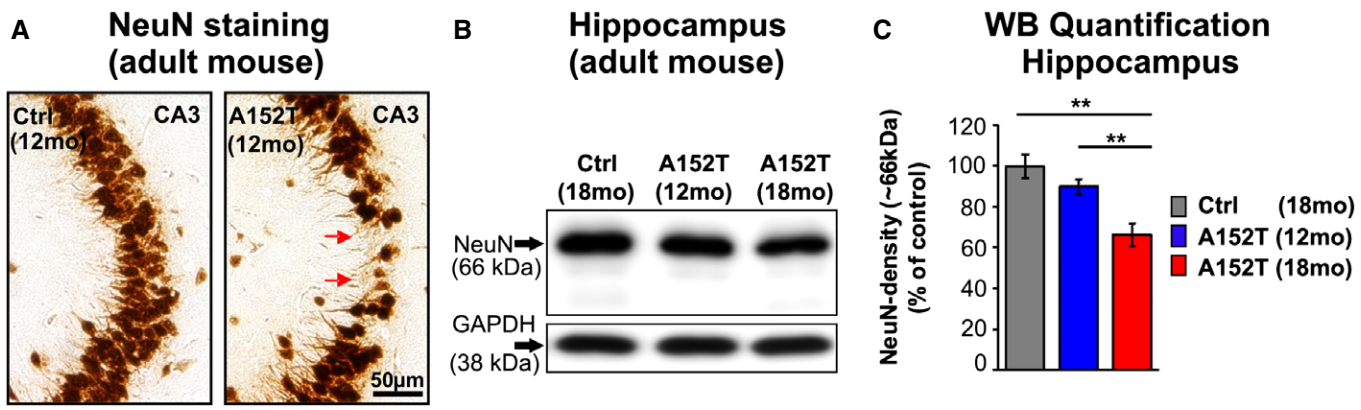

Figure EV5. In adult mice, the expression of hTau^{AT} leads to progressive neuronal loss in the hippocampus.

A Microphotograph of paraffin sections of 12-month-old control littermates and hTau^{AT}-expressing mice demonstrating neuronal loss (red arrows) in area CA3 by anti-NeuN immunochemistry.

B Western blots of hippocampus extracts show NeuN expression levels in 12- and 18-month-old hTau^{AT} and 18-month-old control mice (Ctrl). GAPDH serves as a loading control. Note the dramatic loss of NeuN (~66 kDa) in 18-month-old hTau^{AT} compared to control mice.

C Quantification of (B) normalized to GAPDH. Neuronal loss of ~10% in 12-month-old hTau^{AT} mice (blue bar) and neuronal loss of ~35% in 18-month-old hTau^{AT} mice (red bar) compared to control indicate progressive neurodegeneration in aging hTau^{AT} mice. Bars show mean values ± SEM (n = 4 animals). One-way ANOVA followed by a *post hoc* Newman–Keuls multiple comparison test. Asterisks indicate significant differences (**$P < 0.01$) between control and 18-month-old hTau^{AT} mice and between 12- and 18-month-old hTau^{AT} mice. CA, cornu ammonis; mo, months.