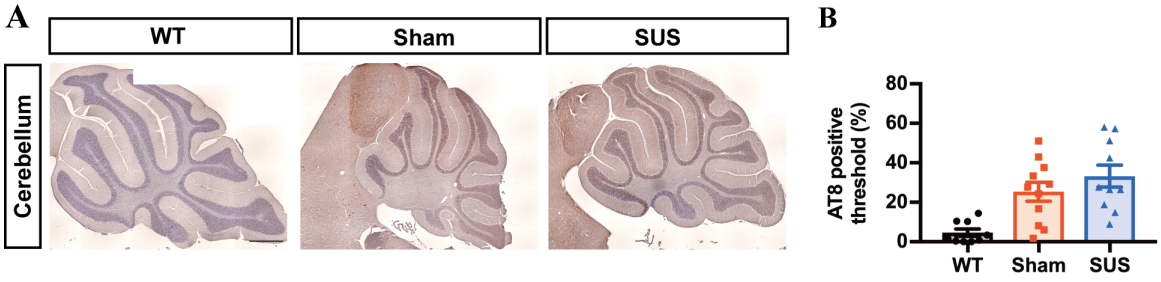
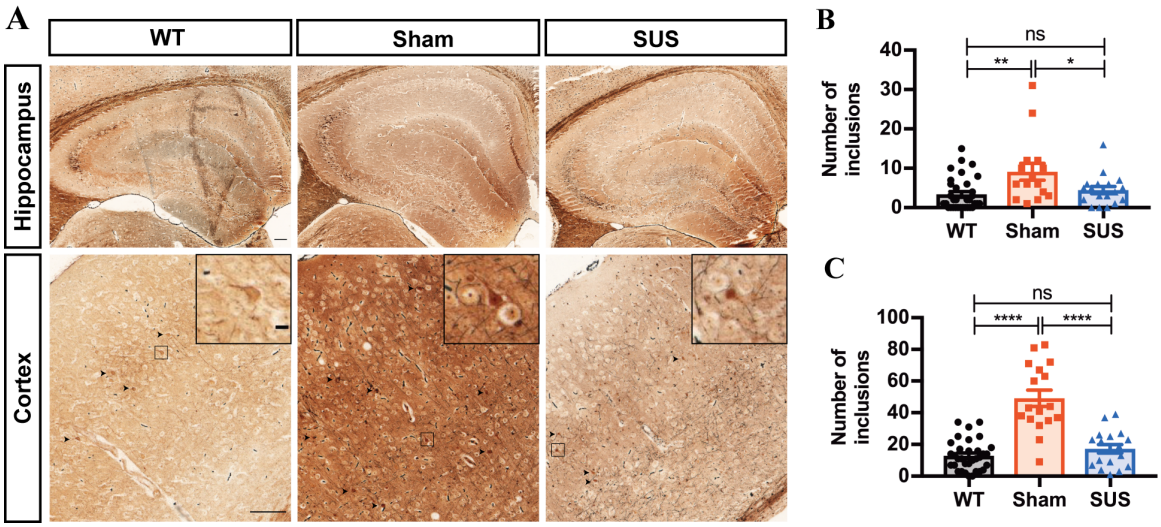
Figure S1



Repeated ultrasound treatment does not impact tau pathology in the cerebellum

(A, B) Immunohistochemistry with the phospho-tau antibody AT8 did not reveal any significant differences in the pathology in the cerebellum. (one-way ANOVA with Tukey's multiple comparison test). This was expected as we did not target the cerebellum with scanning ultrasound.

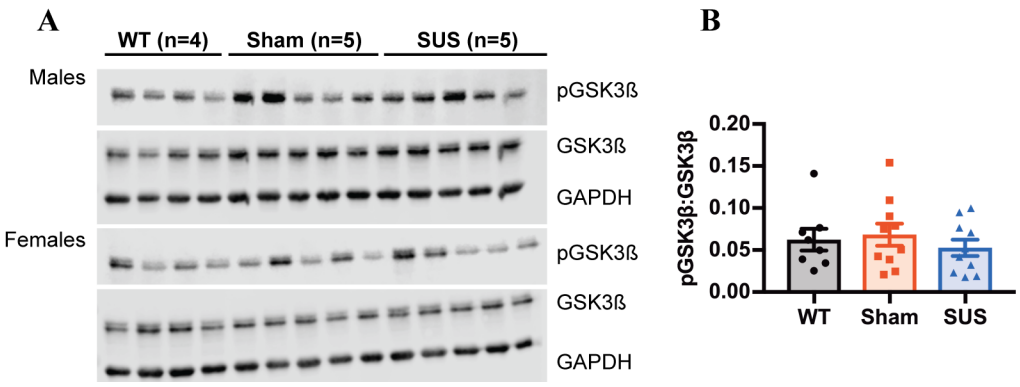
Figure S2



Repeated ultrasound treatments improve tau pathology in K3 mice

(A) Bielschowsky-silver positive NFT-like inclusions (arrowheads) were abundant in the hippocampus and cortex (arrowheads) of K3 mice as seen in the sham mice at 20 weeks of age. Repeated ultrasound treatments significantly reduced the number of these inclusions in both (B) the hippocampus and (C) the cortex (one-way ANOVA with Tukey's multiple comparison test, *$p<0.05$, **$p<0.005$, ****$p<0.0001$). Scale bar: 100µm (inset, scale bar 10µm).

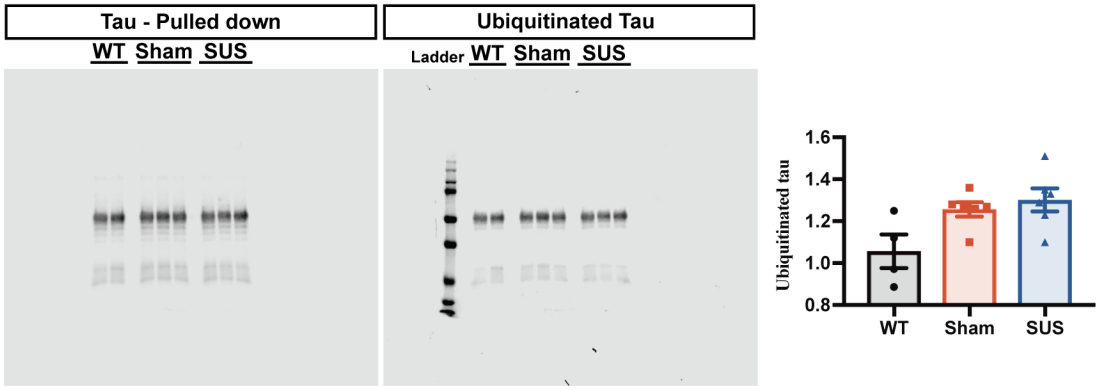
Figure S3



Repeated ultrasound treatments do not affect the activity of the tau kinase GSK3β in the hippocampus

(A) To probe for the mechanism underlying the reduced hyperphosphorylation of tau in the SUS-treated mice, the activity of the kinase GSK3β, which phosphorylates tau at multiple epitopes, was determined using p-GSK3β (pSer9, inactive) and total GSK3β antibodies. (B) There was no change in the activity of the kinase in the hippocampal RAB fractions between the three groups (one-way ANOVA with Tukey's multiple comparison test).

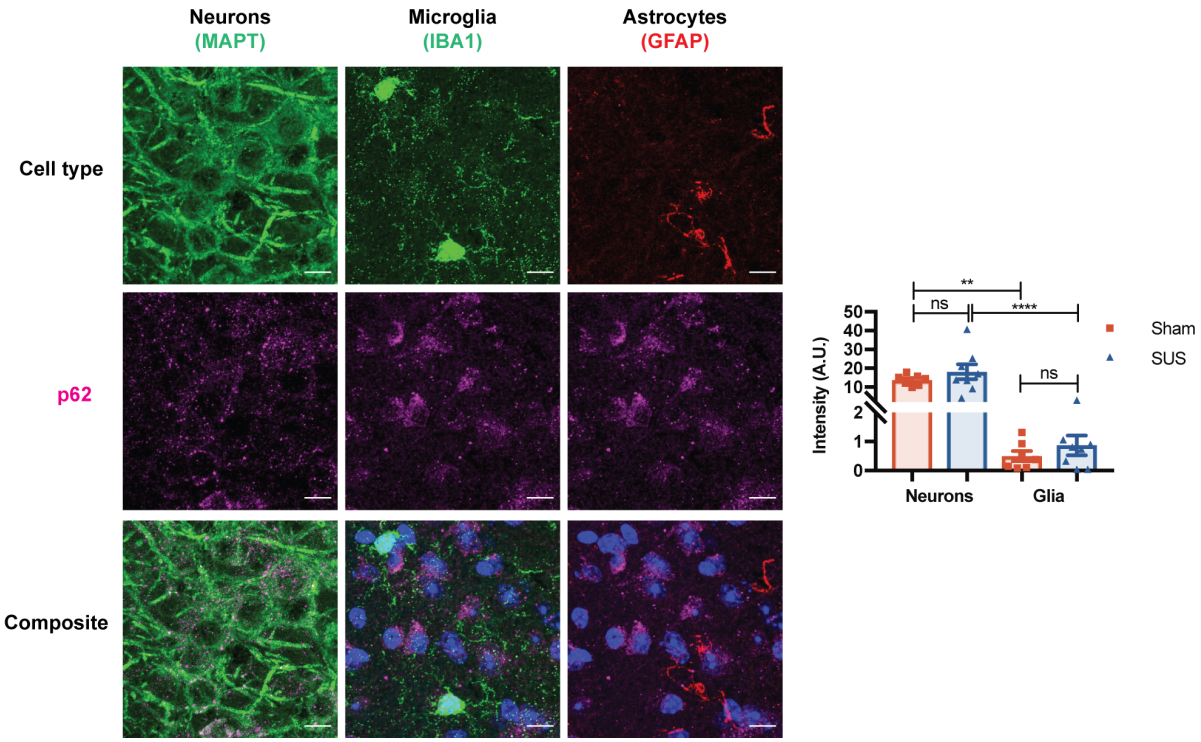
Figure S4



Repeated ultrasound treatments do not alter the ubiquitination of tau in the hippocampus

The effect of repeated ultrasound treatments on the ubiquitination of tau (approximately 55 kDa) and its proteasomal degradation was measured by immunoprecipitating tau and probing for ubiquitination of the tau pulled down in the hippocampal lysates. No difference was observed in the amount of ubiquitin or its profile between the sham and SUS groups (one-way ANOVA with Tukey's multiple comparison test).

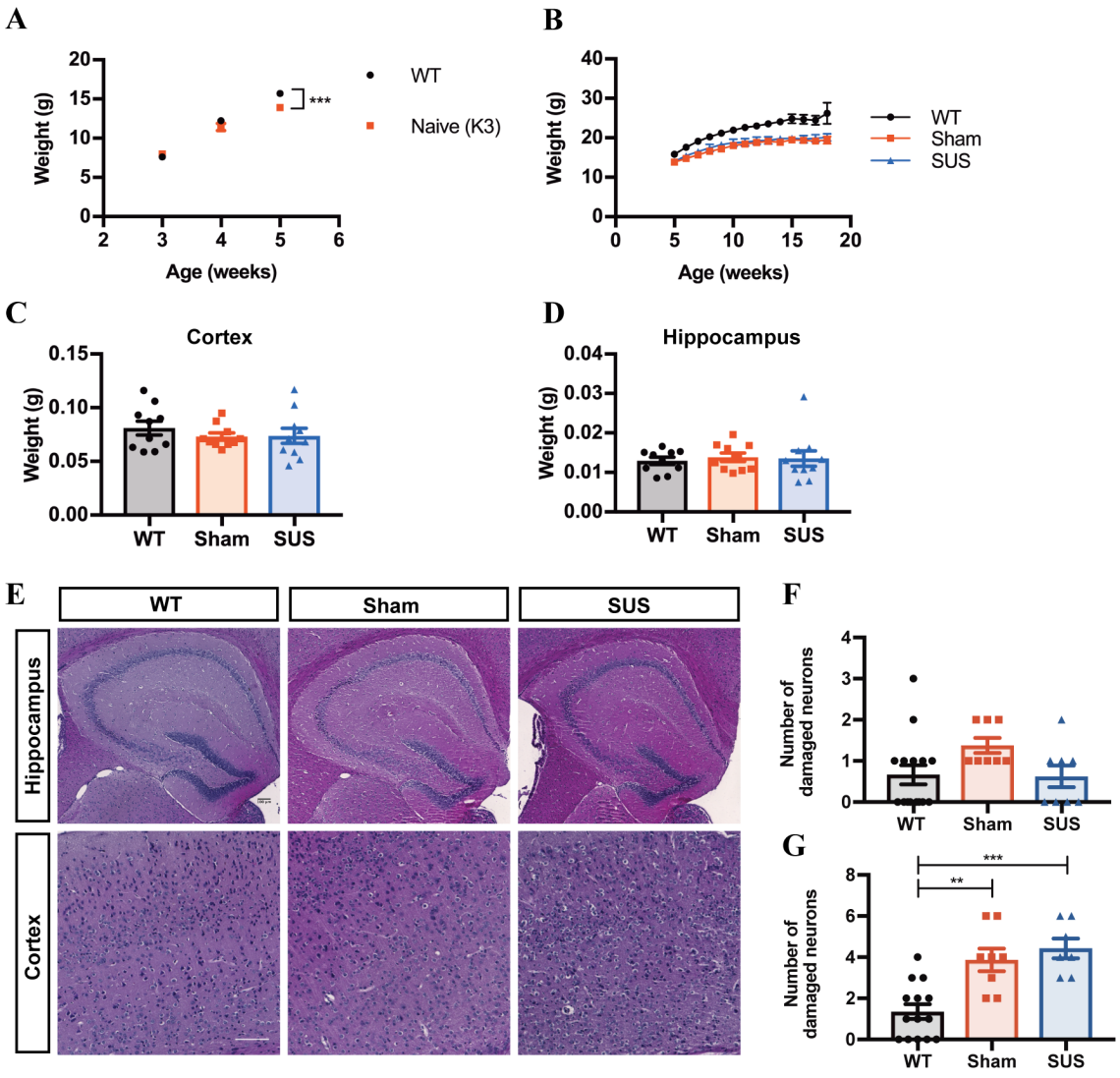
Figure S5



The autophagy marker, p62, is localized to neurons and not glial cells in the hippocampus.

Hippocampal sections were co-stained with p62 and the neuronal marker MAP2, the microglial marker Iba1 and the astrocytic marker GFAP, to analyze the subcellular localization of p62. p62 puncta co-localized with neurons significantly more than with glia (one-way ANOVA with Tukey's multiple comparison test, **p<0.01, ****p<0.0001).

Figure S6



Repeated ultrasound treatments are safely tolerated by K3 mice.

(A) The weights of the K3 mice and their WT littermate controls were monitored prior to the treatment period up to week 5 as were (B) the weights of the SUS- and sham-treated mice and their WT littermates throughout the treatment period. This revealed no difference between the sham (red squares) and SUS groups (blue triangles), both of which had a significantly lower weight than their WT littermates (black circles) throughout the treatment period. (Multiple t-tests, ***$p<0.001$). The weights of (C) cortices and (D) hippocampi were measured *post mortem* and were found to be similar for all three groups.

(E, F) Staining with hematoxylin and eosin to assess neuronal damage and hemorrhaging, revealed no significant difference between the three groups in the hippocampus (one-way ANOVA with Tukey's multiple comparison test). (E, G) In the cortex, the SUS and sham controls had significantly more damage than their WT littermates (one-way ANOVA with Tukey's multiple comparison test, **$p<0.01$, ***$p<0.001$); however, no difference was observed between the two transgenic groups (one-way ANOVA with Tukey's multiple comparison test).