Supplemental Material for:

Caspase-1 inhibition improves cognition without significantly altering amyloid and inflammation in aged Alzheimer disease mice

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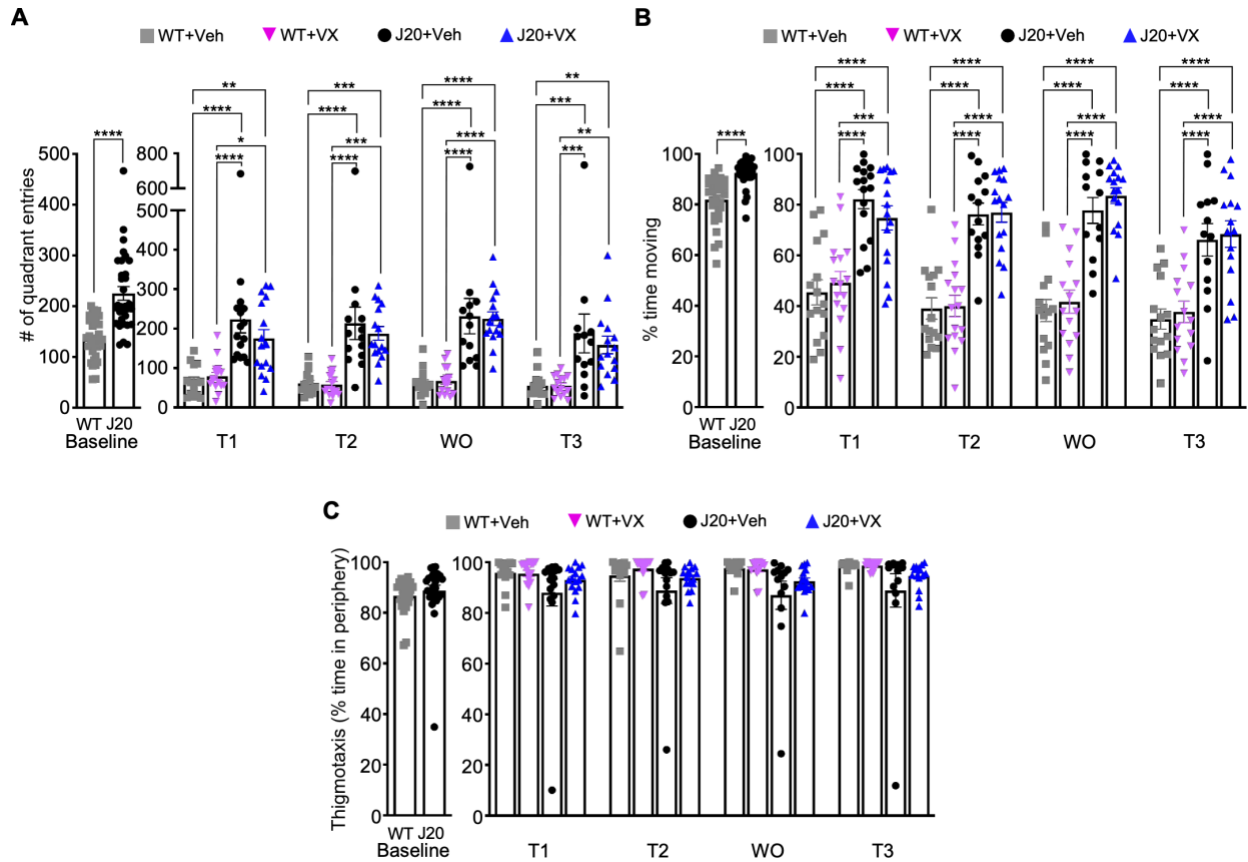
Fig. S1. **VX-765 does not reverse open field behaviour in aged J20 mice. A** Number of quadrant entries in the open field task at baseline [t = 5.91, p < 0.0001, unpaired t-test] and at post-treatment T1 (3 injections), T2 (9 total injections), WO (1 month washout), and T3 (12 total injections) [$F_{time-point}$ = 7.38, p = 0.0001; $F_{treatment}$ = 14.98, p < 0.0001; $F_{interaction}$ = 2.21, p = 0.02, two-way ANOVA, Bonferroni's post-hoc]. **B** Percentage time moving in the open field task at baseline [t = 5.62, p < 0.0001, unpaired t-test] and at post-treatment T1, T2, WO, and T3 [$F_{time-point}$ = 11.26, p < 0.0001; $F_{treatment}$ = 30.13, p < 0.0001, two-way ANOVA, Bonferroni's post-hoc]. **C** Percentage time in the periphery, or thigmotaxis, in the open field task at baseline and at post-treatment T1, T2, WO, and T3. Bars represent mean ± SEM; symbols denote performances of individual mice. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.
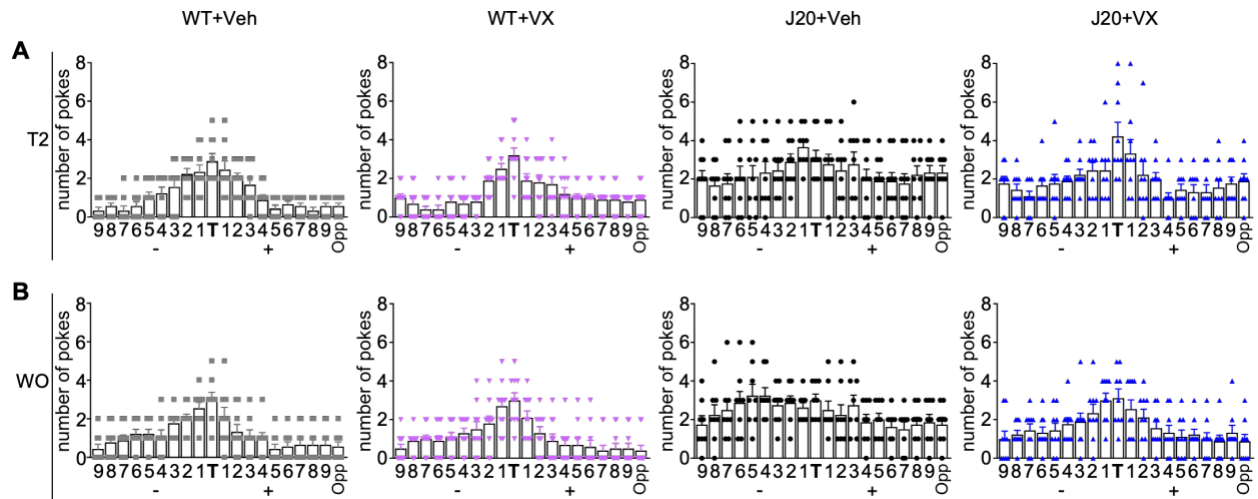
Fig. S2. VX-765 reverses Barnes maze deficits in aged J20 mice. A-B Barnes maze probe at (**A**) T2 and (**B**) WO showing distribution of nose "pokes" for each hole where (T) indicates target hole, +1 to 9 and – 1 to 9 indicates successive holes to the right and left of the target hole, respectively, and (Opp) indicates the hole directly opposite the target hole. Bars represent mean ± SEM; symbols denote performances of individual mice.
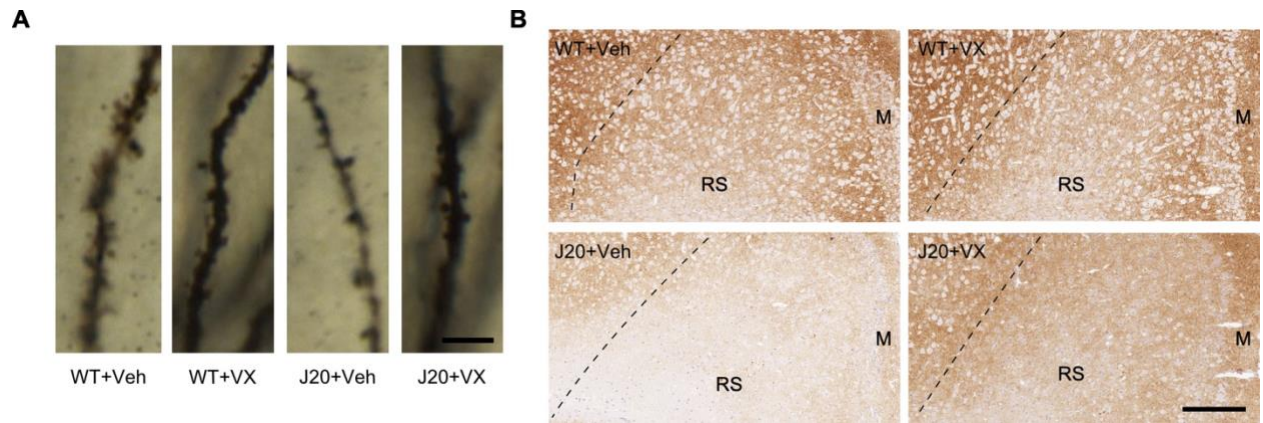
Fig. S3. VX-765 reverses loss of synaptophysin levels in the cortex of 15-month-old J20 mice. A Representative Golgi-Cox-stained dendritic spines in the hippocampal CA1 SR. Scale bar = 5 μm. **B** Representative micrographs of synaptophysin staining from the midline (M) to the retrosplenial (RS) area of the cortex. Scale bar = 200 μm.
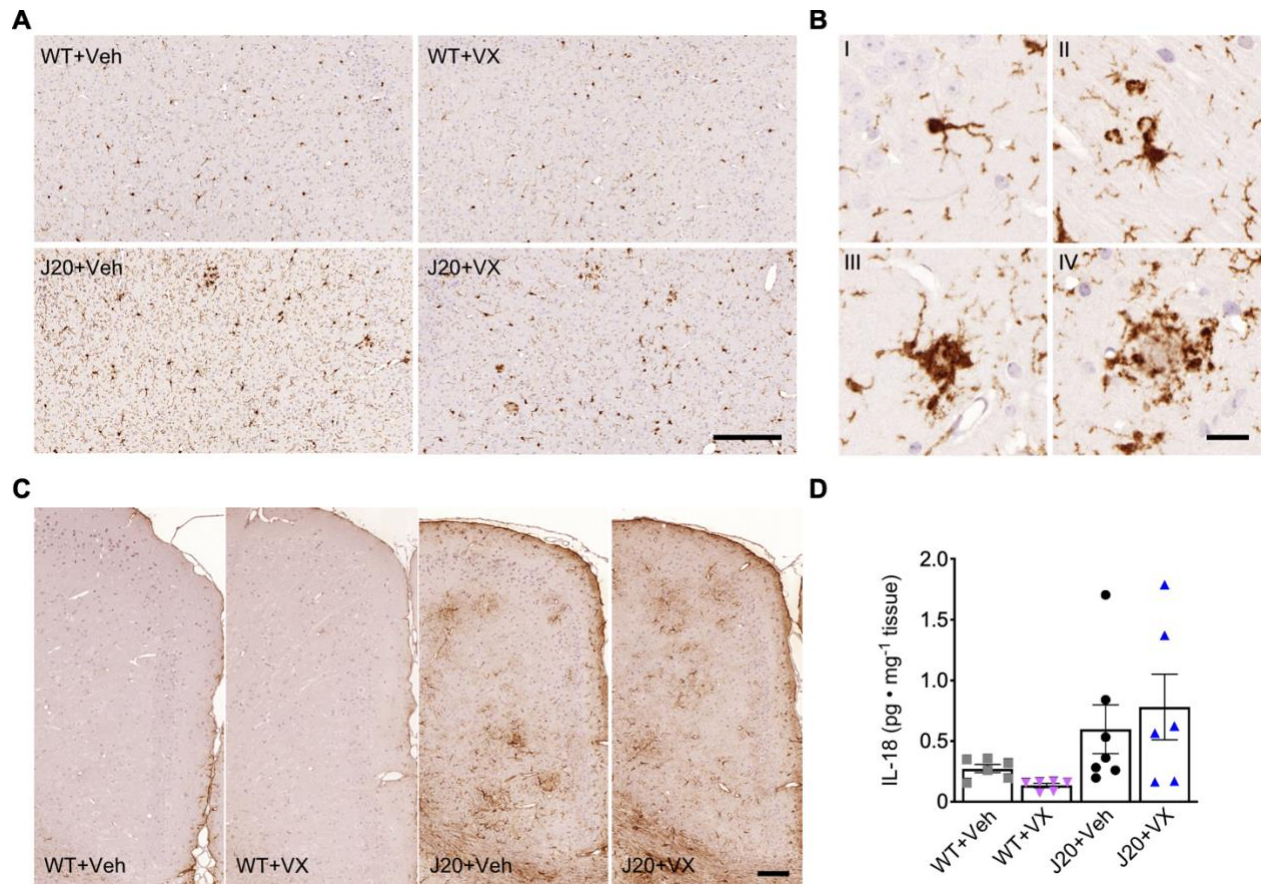
Fig. S4. VX-765 does not reverse inflammation in the cortex of 15-month-old J20 mice. A Representative micrographs of Iba1^{+} microglial staining in the retrosplenial and motor areas of the cortex. Scale bar = 200 μm. **B** Iba1^{+} micrographs representing type I, II, III, and IV microglial subtypes. Scale bar = 20 μm. **C** Representative GFAP[+] astrocyte staining in the retrosplenial cortex. Scale bar = 100 μm. **D** IL-18 cortical protein levels measured with sandwich ELISA. Bars represent mean ± SEM; symbols denote performances of individual mice.
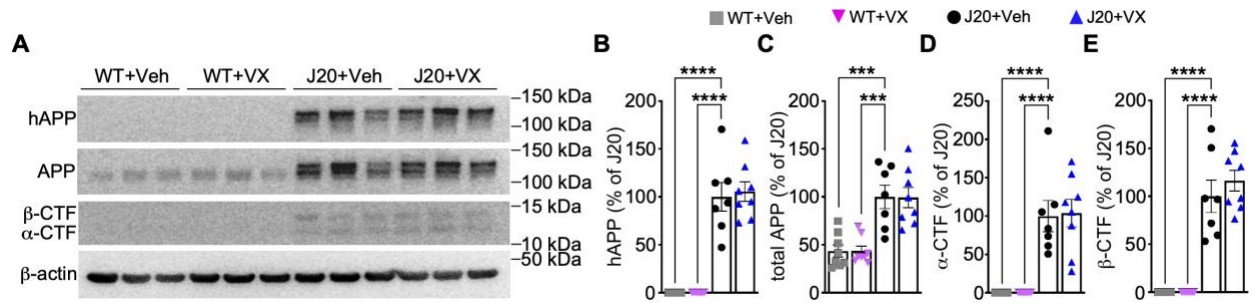
Fig. S5. VX-765 does not affect APP and CTF in the cortex of 15-month-old J20 mice. A Representative western blot images and **B-E** quantification of **B** human APP (hAPP) [$F_{genotype}$ = 146.3, p < 0.0001], **C** total mouse + human APP [$F_{genotype}$ = 40.65, p < 0.0001], **D** alpha (α) [$F_{genotype}$ = 61.32, p < 0.0001] and **E** beta (β) [$F_{genotype}$ = 131.9, p < 0.0001] C-terminal fragments (CTF) in the cortex. Two-way ANOVA, Dunnett's post-hoc compared to J20+Veh for (B-E). Bars represent mean ± SEM; symbols denote performances of individual mice. *** p < 0.001, **** p < 0.0001.
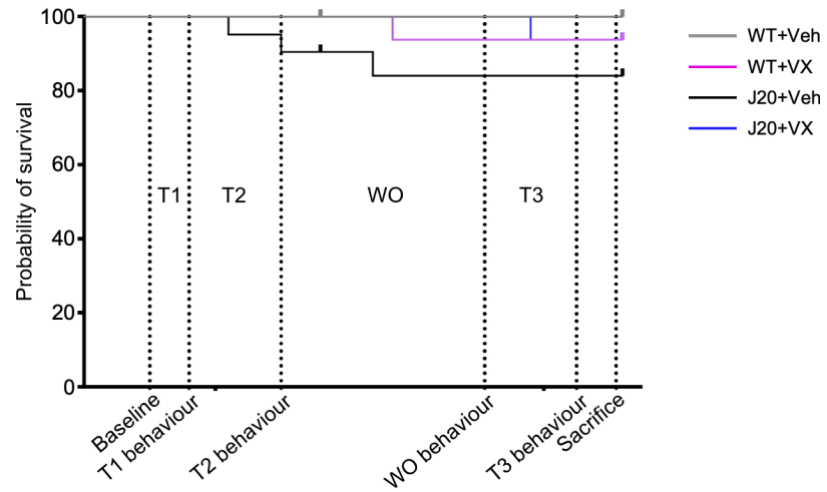
Fig. S6. VX-765 does not affect survival in 15-month-old J20 mice. Survival curve of WT and J20 mice during experimental paradigm. No significant differences found when comparing survival curves of the different treatment groups.