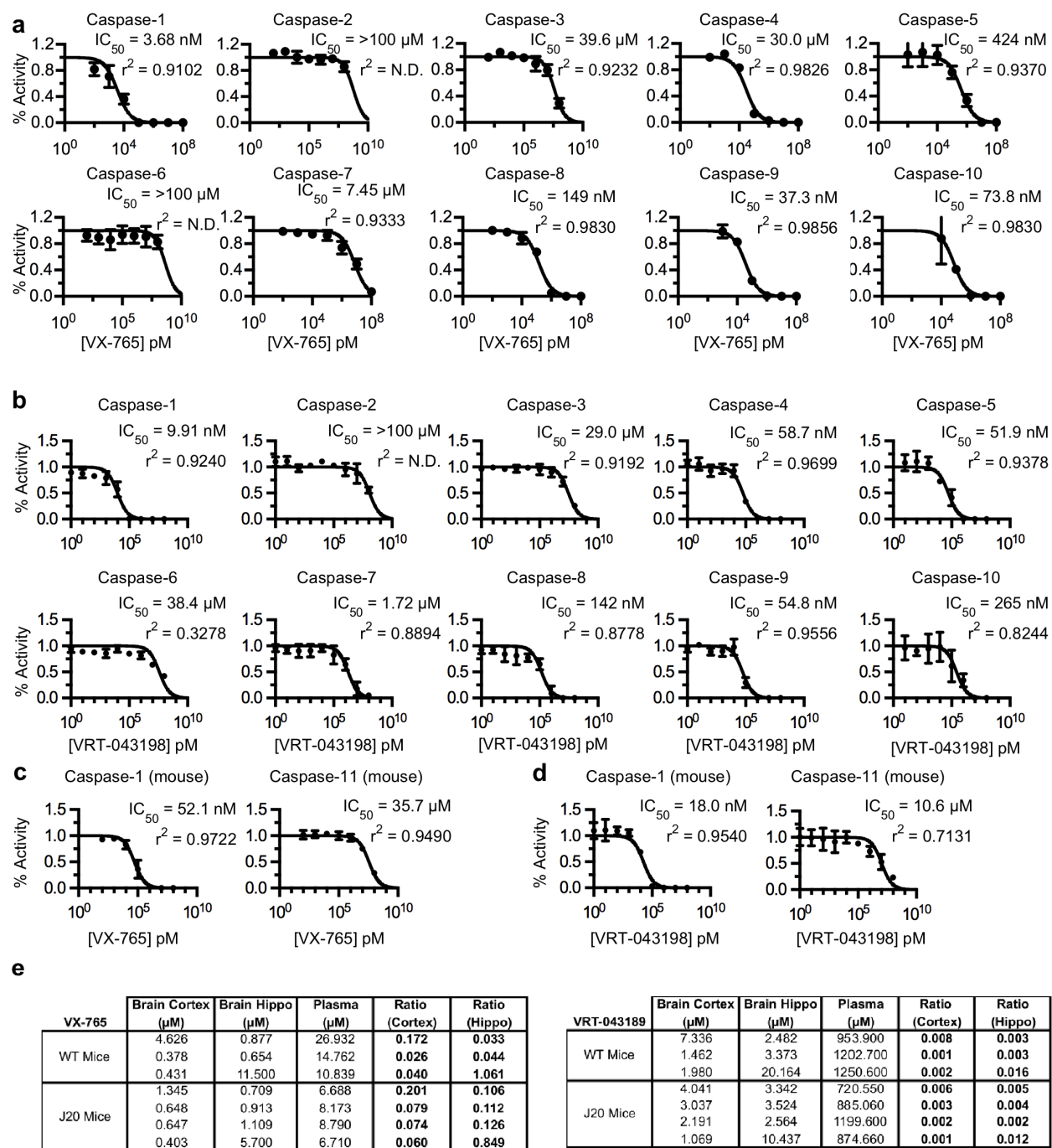


***Caspase-1 inhibition alleviates cognitive impairment, inflammation and amyloid  
accumulation in an Alzheimer's disease mouse model***

***Supplementary Figures***

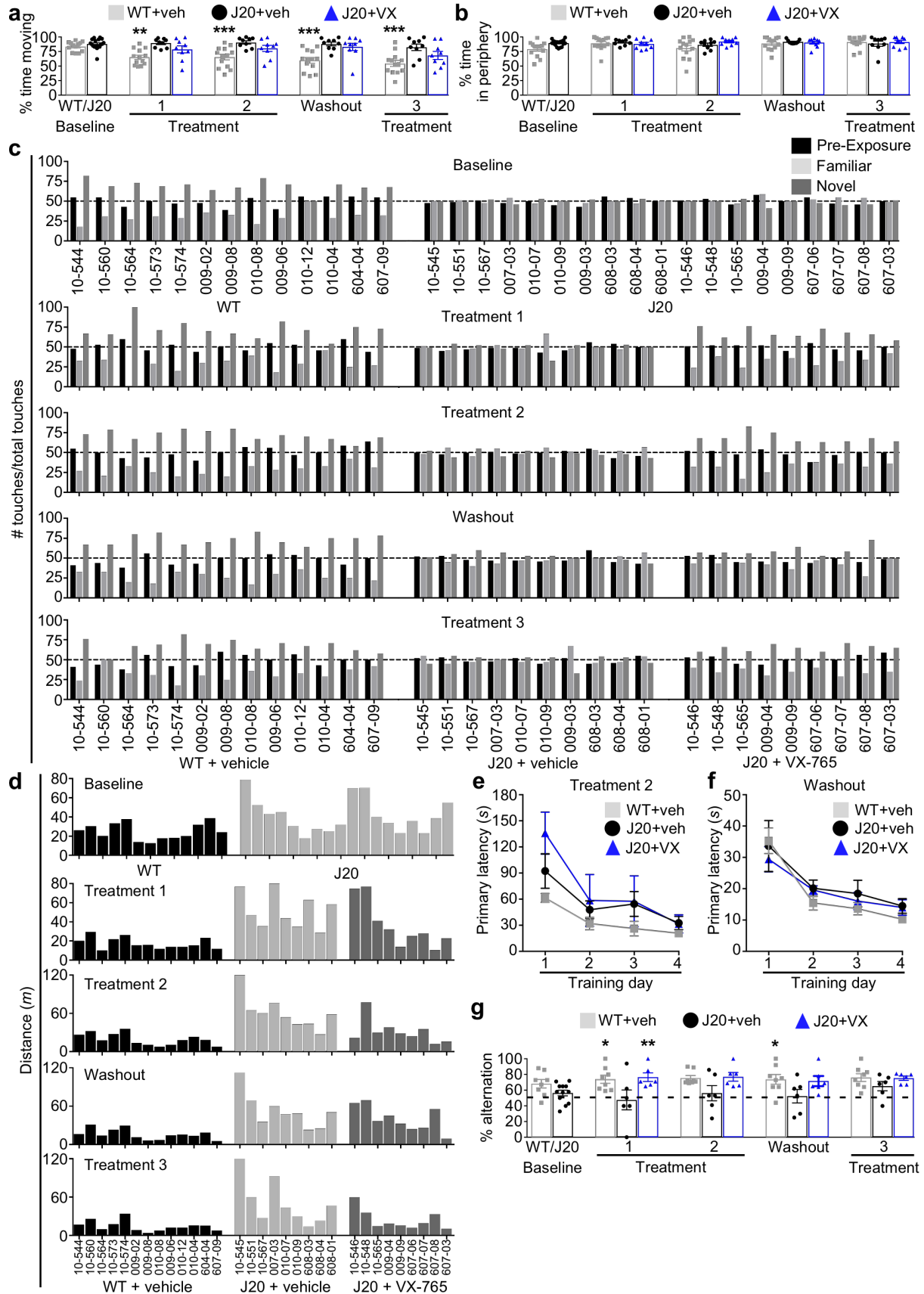
***Flores et al.,***

Supplementary figure 1. Flores et al., Novel treatment against AD



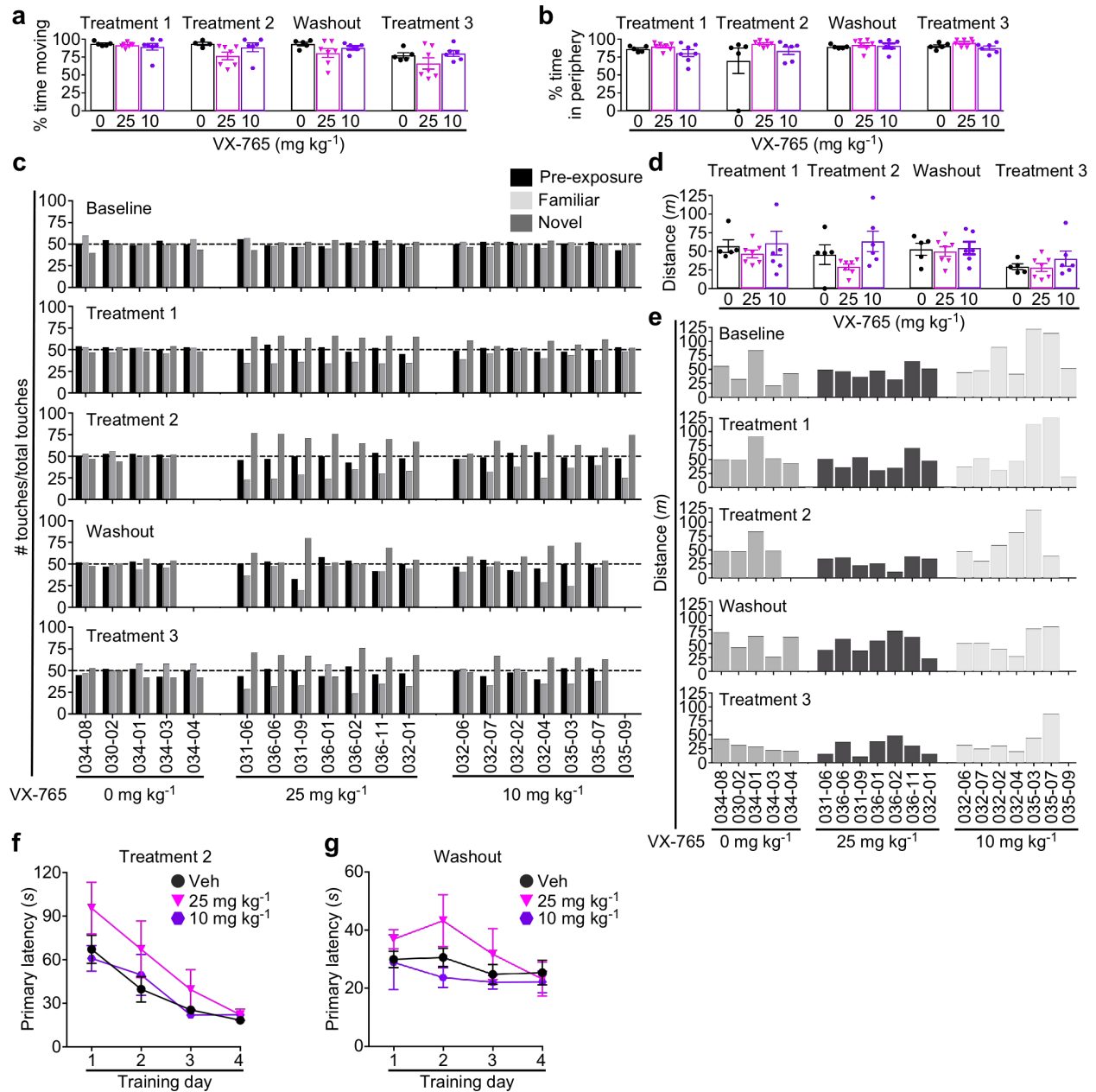
**Supplementary Figure 1. VX-765 and VRT-043198 selectivity and blood brain barrier permeability. (a-b)**  $IC_{50}$  of (a) VX-765 and (b) VRT-043198 against human Casp1-10. (c-d)  $IC_{50}$  of (c) VX-765 and (d) VRT-043198 against mouse Casp1 and Casp11 (equivalent to human Casp4&5). (a-d) Data represents mean of duplicate in vitro experiments and s.e.m. (e) Blood brain barrier permeability of VX-765 and VRT-043198 in 3 WT and 4 J20 mice brain cortex, hippocampus and plasma. Ratios of brain levels to plasma levels are calculated.

Supplementary figure 2. Flores et al., Novel treatment against AD



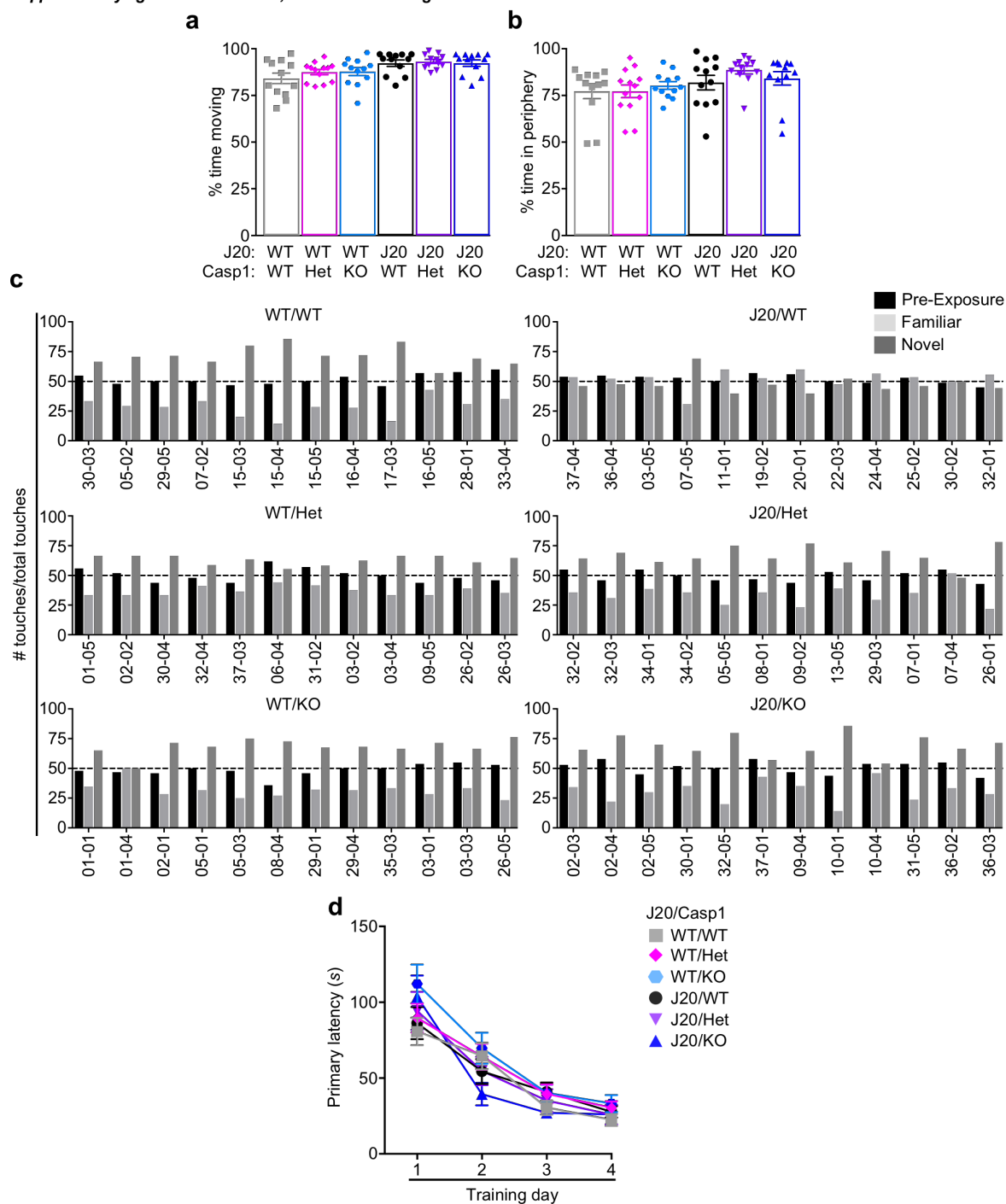
**Supplementary Figure 2. Behavioural assessment of vehicle-treated WT and J20 mice and 50 mg kg<sup>-1</sup> VX-765-treated J20 mice. (a)** % time moving [Treatment,  $F(2,28) = 10.89$ ,  $p=0.0003$ ; Time,  $F(3,84) = 6.644$ ,  $p=0.0004$ , two-way repeated-measures ANOVA, Dunnett's post-hoc versus J20 + vehicle,  $**p<0.01$ ,  $***p<0.001$ ] and **(b)** % time in periphery of vehicle-treated WT (grey squares), vehicle-treated J20 (black circles) and VX-765-treated J20 (blue triangles). Each mouse tested is represented by one symbol. **(c)** NOR # of touches of familiar or novel objects for each individual mouse at each test session. Pre-exposure indicates mice performance with two identical objects, familiar and novel indicates # of touches of familiar and novel objects, respectively. **(d)** Distance travelled in the Open Field task of individual vehicle-treated WT or J20 and VX-765-treated J20. **(e-f)** Barnes maze primary latency to target during learning acquisition at **(e)** T2 and **(f)** WO. **(g)** % alternation in Y-maze apparatus. Two-way repeated measures ANOVA, Dunnett's post-hoc versus J20 + vehicle,  $*p<0.05$ ,  $**p<0.01$ . Data in a,b,e-g represents mean plus s.e.m.

Supplementary figure 3. Flores et al., Novel treatment against AD

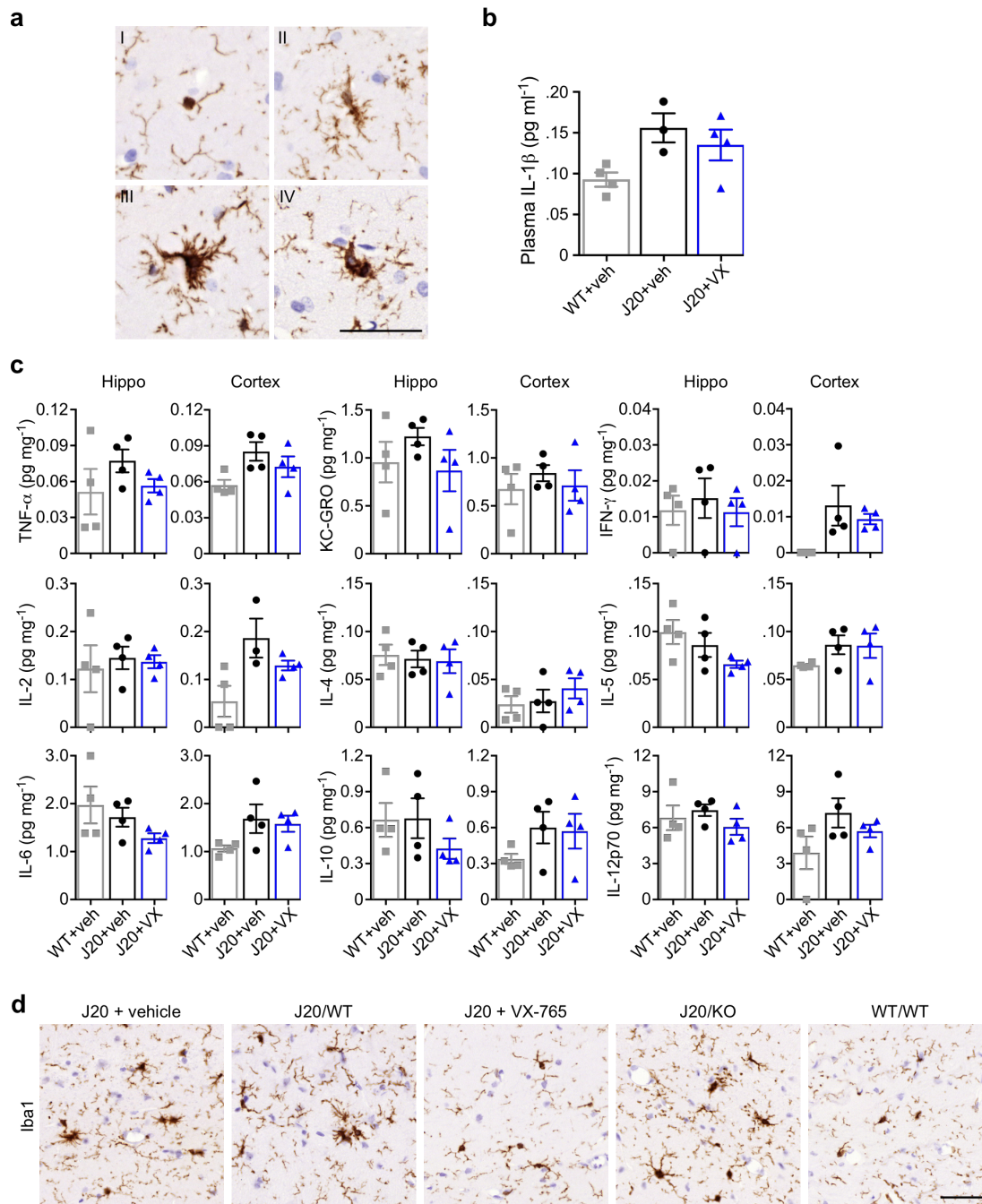


**Supplementary Figure 3. Behavioural assessment of vehicle-, or 25 or 10 mg kg<sup>-1</sup> VX-765-treated J20 mice.** (a) % time moving and (b) % time in periphery of vehicle-treated J20 (black circles), 25 mg kg<sup>-1</sup> (pink triangles), or 10 mg kg<sup>-1</sup> (purple hexagon) VX-765-treated J20 mice. Each mouse tested is represented by one symbol. (c) NOR # of touches of familiar and novel object for each individual mouse. (d) Distance travelled [Treatment 2,  $F(2,14) = 4.106$ ,  $p = 0.0395$ , ANOVA, Dunnett's post-hoc versus J20 + vehicle (0)] and (e) distance travelled by each individual mouse in the Open field task. Two mice (J20 + Vehicle, J20 + 10 mg kg<sup>-1</sup> VX-765) remained immobile during behavioral testing and were removed from the analysis. (f-g) Barnes maze primary latency to target during learning acquisition at (f) T2 and (g) WO. Data in a,b,d,f-g represents mean and s.e.m.

Supplementary figure 4. Flores et al., Novel treatment against AD

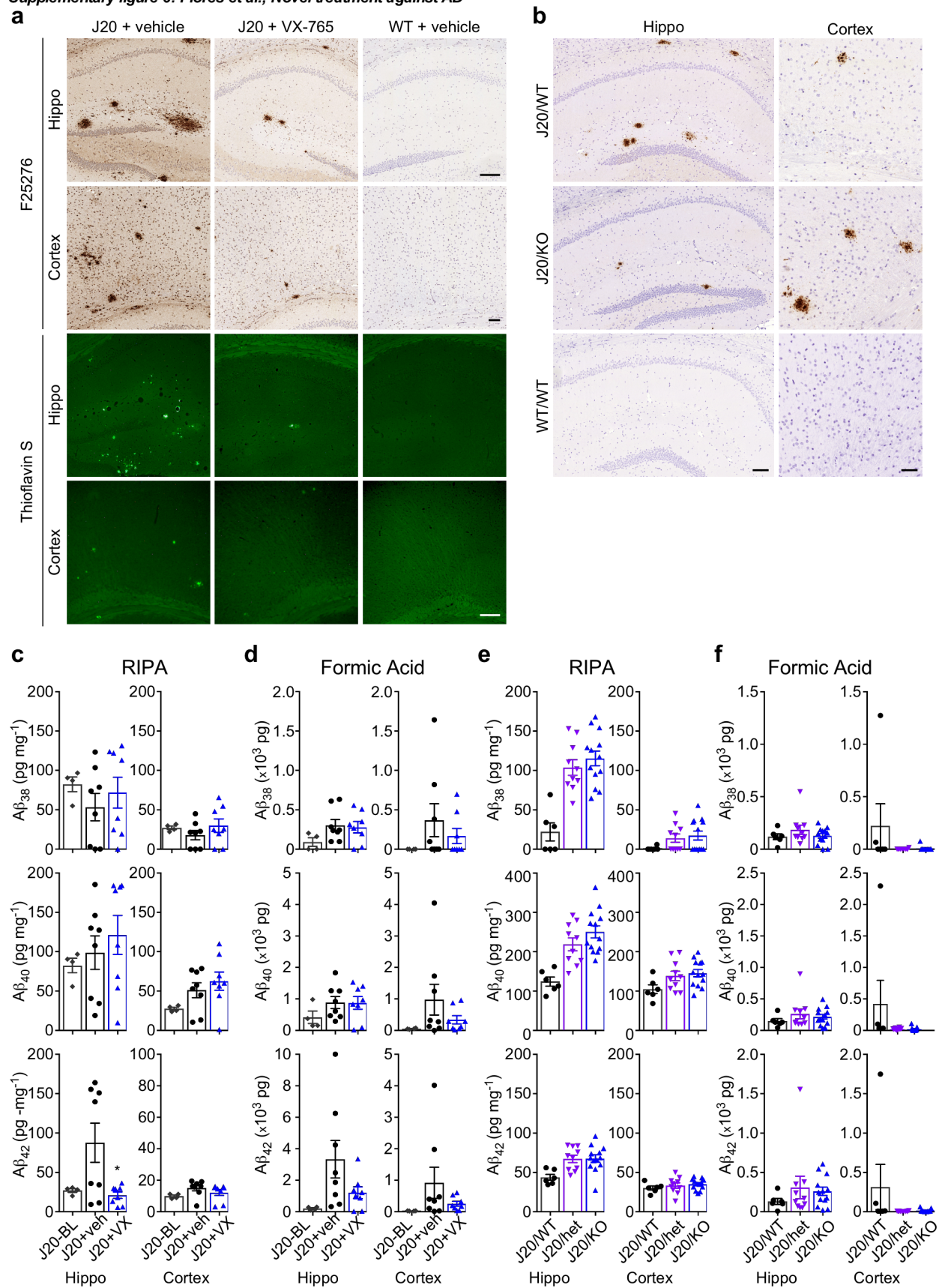


**Supplementary Figure 4. Behavioural assessment of J20/KO mice.** (a) % time moving and (b) % time in periphery of the following genotypes: J20<sup>-/-</sup>/Casp1<sup>+/+</sup> WT/WT (grey squares), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> WT/Het (pink diamond), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> WT/KO (blue hexagons), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> J20/WT (black circles), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> J20/Het (purple triangles), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> J20/KO (blue triangles). Each mouse tested is represented by one symbol. (c) NOR # of touches of familiar and novel object for each individual mouse. (d) Barnes maze primary latency to target during learning acquisition. Data in a,b,d represents mean and s.e.m.



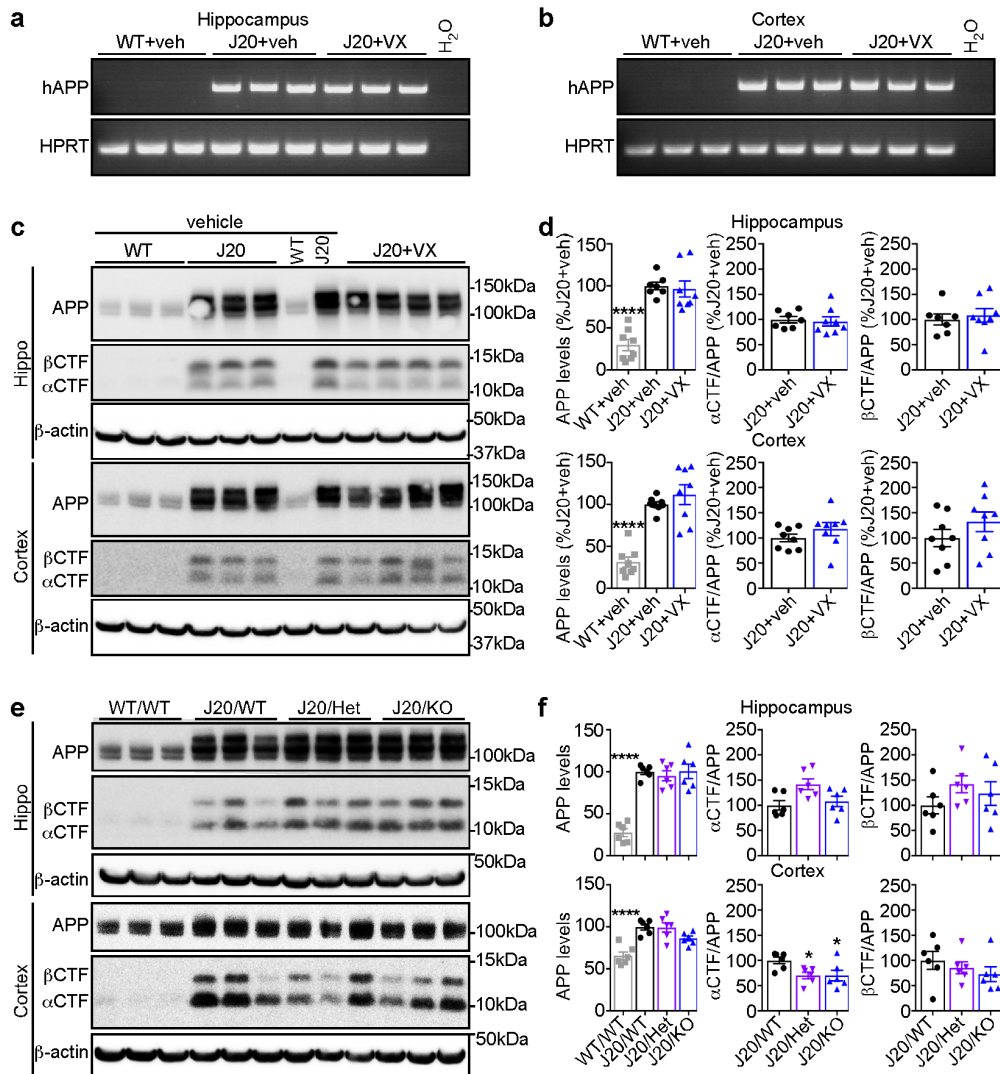
**Supplementary Figure 5. Inflammatory marker analyses of vehicle-treated WT and J20, and VX-765-treated J20.** (a) Micrographs showing Iba1 I, II, III, IV microglial subtypes. (b) IL-1 $\beta$  plasma levels after T3 in periphery of vehicle-treated WT (grey squares), vehicle-treated J20 (black circles) and VX-765-treated J20 (blue triangles). (c) Cytokine levels in vehicle-treated WT and J20 and in VX-765-treated J20 brain hippocampi and cortex (n=4/group). (d) Iba1 immunopositive staining comparing 8-month old vehicle-treated J20, J20/WT, VX-765-treated J20, J20/*Casp1*<sup>-/-</sup> (J20/KO), and WT/WT mice hippocampi. Each mouse tested is represented by one symbol. Data represents mean and s.e.m. Scale bar in (a) = 20  $\mu$ m, (d) = 50  $\mu$ m.

Supplementary figure 6. Flores et al., Novel treatment against AD



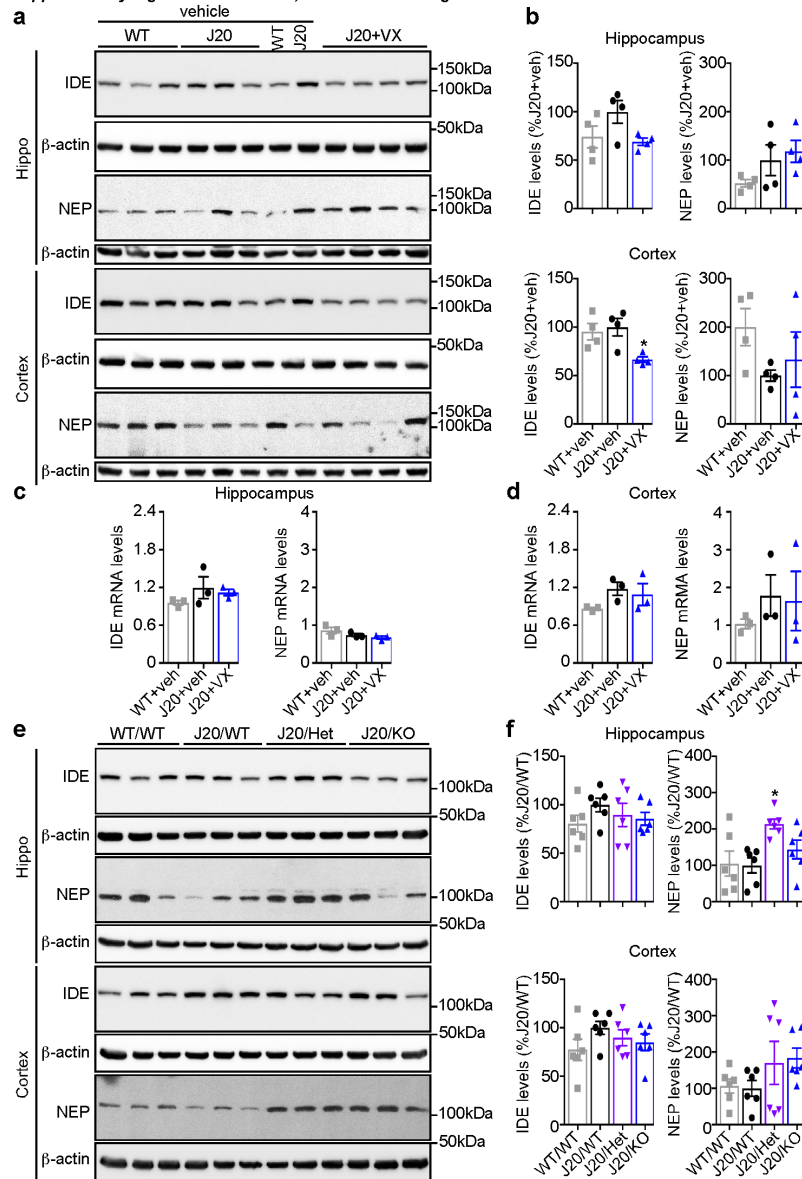
**Supplementary Figure 6: A $\beta$  stained with Thioflavin S or anti-A $\beta_{1-40}$  F25276 antiserum in mice hippocampi or cortex. (a)** Comparison of anti-A $\beta$  F25276 immunostaining and thioflavin S-stained in 8-month old vehicle-treated WT and J20 and in VX-765-treated J20 brain hippocampi and cortex (after T3). **(b)** Anti-A $\beta$  immunopositive staining in 8-month-old J20/WT, J20/*Casp1*<sup>-/-</sup> (J20/KO), and WT/WT mice. **(c)** RIPA-soluble **(d)** and formic acid soluble A $\beta_{38}$ , A $\beta_{40}$ , and A $\beta_{42}$  levels in baseline J20 (dark grey diamonds), vehicle-treated J20 (black circles) and VX-765-treated J20 (blue triangles) brain hippocampi and cortex measured by ELISA [A $\beta_{42}$  RIPA-Hippo, F(2,17) = 4.795, p=0.0223]. **(e)** RIPA-soluble **(f)** and formic acid soluble A $\beta_{38}$ , A $\beta_{40}$ , and A $\beta_{42}$  levels in J20<sup>-/+</sup>/*Casp1*<sup>+/+</sup> J20/WT (black circles), J20<sup>-/+</sup>/*Casp1*<sup>-/-</sup> J20/Het (purple triangles), and J20<sup>-/+</sup>/*Casp1*<sup>-/-</sup> J20/KO (blue triangles). Each mouse tested is represented by one symbol. Data represents mean and s.e.m. **(c-f)** ANOVA, Dunnett's post-hoc versus J20 + vehicle **(c-d)** or J20/WT **(e-f)**, \*p<0.05. Scale bars = 50  $\mu$ m.

Supplementary figure 7. Flores et al., Novel treatment against AD

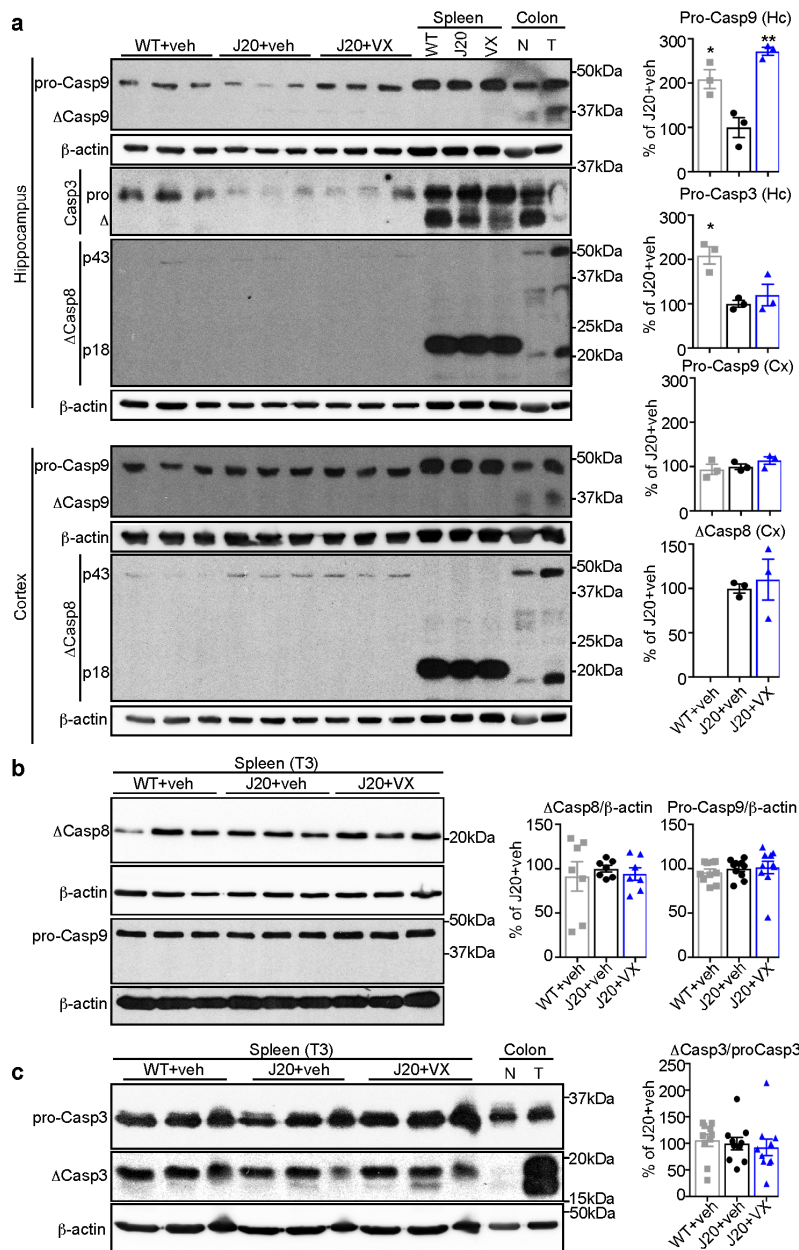


**Supplementary Figure 7: APP and CTFs levels after VX-765 or *Casp1* KO.** (a-b) Ethidium bromide-stained agarose gel of human APP and HPRT RT-PCR amplicons from the (a) hippocampus and (b) cortex of vehicle-treated WT and J20, and VX-765-treated J20. (c-d) APP and CTF (anti-APP C-terminal antibody) western blots, and quantitative analyses in the brain hippocampi and cortex of vehicle-treated WT (grey squares), vehicle-treated J20 (black circles) and VX-765-treated J20 (blue triangles) [APP hippo,  $F(2,20) = 28.81$ ,  $p < 0.0001$ ; APP cortex,  $F(2,20) = 31.2$ ,  $p < 0.0001$ ]. Quantitative results represented as ratios of APP levels over  $\beta$ -actin or CTFs over APP and expressed as percentage of J20 + vehicle. ANOVA, Dunnett's post-hoc versus J20 + vehicle, \*\*\*\* $p < 0.0001$ . (e-f) APP and CTF western blots, and quantitative analyses in the brain hippocampi and cortex of J20<sup>-/-</sup>/*Casp1*<sup>+/+</sup> WT/WT (grey squares), J20<sup>-/-</sup>/*Casp1*<sup>+/+</sup> J20/WT (black circles), J20<sup>-/-</sup>/*Casp1*<sup>+/+</sup> J20/Het (purple triangles), and J20<sup>-/-</sup>/*Casp1*<sup>-/-</sup> J20/KO (blue triangles) [APP hippo,  $F(3,20) = 26.04$ ,  $p < 0.0001$ ; APP cortex,  $F(3,20) = 14.07$ ,  $p < 0.0001$ ;  $\alpha$ CTF cortex,  $F(2,15) = 4.45$ ,  $p = 0.0304$ ]. Quantitative results represented as ratios of APP levels over  $\beta$ -actin or CTFs over APP and expressed as percentage  $\pm$  SEM of J20/WT. ANOVA, Dunnett's post-hoc versus J20/WT, \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ . Each mouse tested is represented by one symbol. Data represents mean and s.e.m.

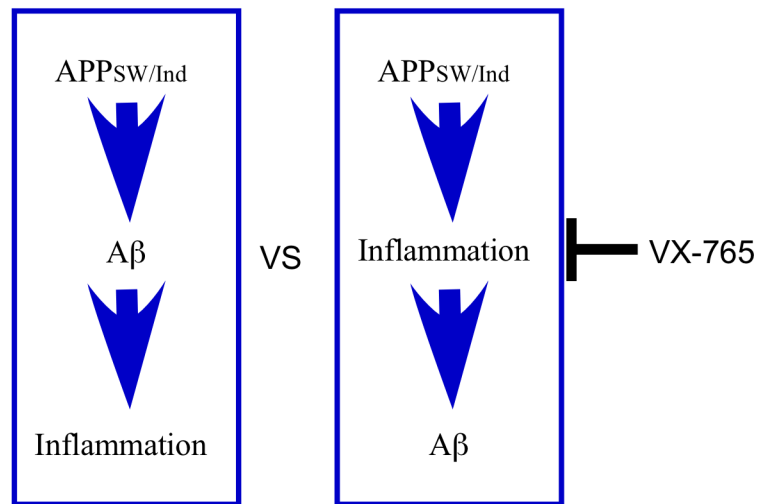
Supplementary Figure 8. Flores et al., Novel treatment against AD.



**Supplementary Figure 8: IDE and NEP levels after VX-765 or Casp1 KO.** (a-b) IDE and NEP western blots, and quantitative analyses in the brain hippocampi and cortex of vehicle-treated WT (grey squares), vehicle-treated J20 (black circles) and VX-765-treated J20 (blue triangles) [IDE cortex,  $F(2,9) = 6.098$ ,  $p=0.0212$ ]. Quantitative results represented as ratios of IDE or NEP levels over  $\beta$ -actin and expressed as percentage of J20 + vehicle. ANOVA, Dunnett's post-hoc versus J20 + vehicle,  $*p<0.05$ . (c-d) mRNA levels of IDE and NEP in the (c) hippocampus and (d) cortex of vehicle-treated WT and J20, and VX-765-treated J20. (e-f) IDE and NEP western blots, and quantitative analyses in the brain hippocampi and cortex of J20<sup>-/-</sup>/Casp1<sup>+/+</sup> WT/WT (grey squares), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> J20/WT (black circles), J20<sup>-/-</sup>/Casp1<sup>+/+</sup> J20/Het (purple triangles), and J20<sup>-/-</sup>/Casp1<sup>-/-</sup> J20/KO (blue triangles) [NEP Hippo,  $F(3,20) = 4.537$ ,  $p=0.0139$ ]. Quantitative results represented as ratios of IDE or NEP levels over  $\beta$ -actin and expressed as percentage of J20/WT. ANOVA, Dunnett's post-hoc versus J20/WT,  $*p<0.05$ . Each mouse tested is represented by one symbol. Data represents mean and s.e.m.



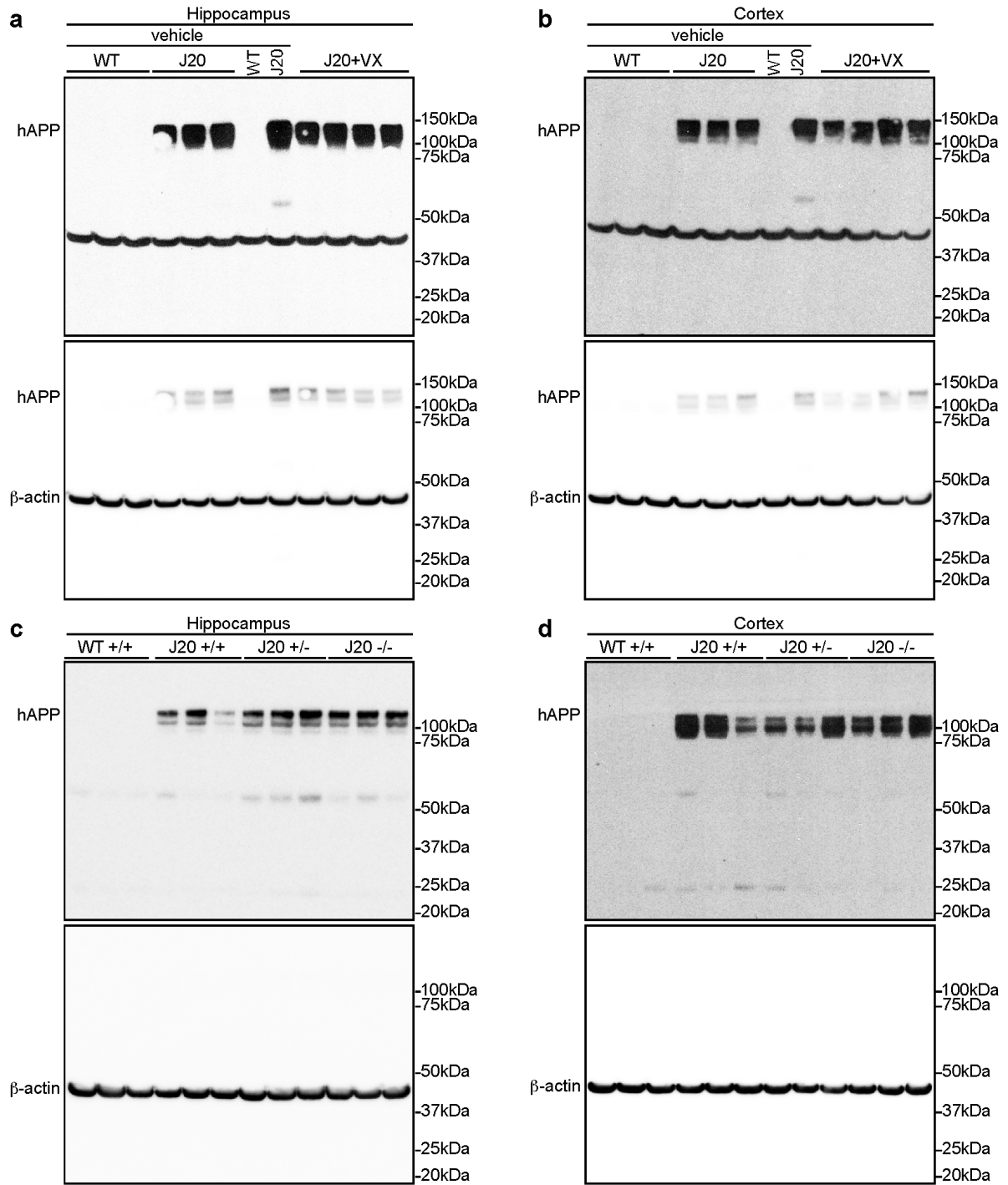
**Supplementary Figure 9. Caspase-3, -8 and -9 activation after VX-765.** (a) Western blot and quantitative analyses of Casp3, Casp8, and Casp9 expression in the hippocampus and cortex of vehicle-treated WT (grey squares), vehicle-treated J20 (black circles) and VX-765-treated J20 (blue triangles). Mouse spleen and colon tissues were used as controls. N: normal, T: tumor. (b-c) Representative (b) cleaved Casp8 (ΔCasp8) and pro-Casp9, and (c) Casp3 western blot and quantitative analyses in the spleen of vehicle-treated WT, J20, and VX-765-treated J20 mice. Quantitative results represented as ratios of (a,b) pro-Casp3, ΔCasp8 or pro-Casp9 over β-actin, or (c) cleaved Casp3 (ΔCasp3) over pro-Casp3 and are expressed as percentage of J20 + vehicle. ANOVA, Dunnett's post-hoc versus J20 + vehicle, \*p<0.05, \*\*p<0.01. Each mouse tested is represented by one symbol. Data represents mean and s.e.m.



**Supplementary Figure 10: Schematic diagram to illustrate pathway inhibited by VX-765.**

The  $APP^{SW/Ind}$  transgene is predicted to increase  $A\beta$  levels, which then generate inflammation through microglial activation (Left panel). Our results are more consistent with a model where the  $APP^{SW/Ind}$  transgene first induces inflammation, possibly in neurons, and then leads to increased  $A\beta$  (Right panel). By inhibiting Casp1, VX-765 blocks inflammation and subsequent  $A\beta$  accumulation.

Supplementary figure 11. Flores et al., Novel treatment against AD



**Supplementary Figure 11: VX-765 prevents progressive A $\beta$  accumulation in J20 mice.** (a-b) Full western blot images of hAPP and  $\beta$ -actin in the (a) hippocampus and (b) cortex of vehicle-treated WT, vehicle-treated J20 and VX-765-treated J20 from Fig. 5l. (c-d) Full western blot images of hAPP and  $\beta$ -actin in the (c) hippocampus and (d) cortex of cortex of J20<sup>-/-</sup>/*Casp1*<sup>+/+</sup> WT/WT, J20<sup>-/-</sup>/*Casp1*<sup>+/+</sup> J20/WT, J20<sup>-/-</sup>/*Casp1*<sup>+/+</sup> J20/Het, and J20<sup>-/-</sup>/*Casp1*<sup>-/-</sup> J20/KO from Fig. 5n.