

**Pimavanserin, a 5HT<sub>2A</sub> receptor inverse agonist, rapidly suppresses A $\beta$  production and related pathology in a mouse model of Alzheimer's disease**

**SUPPLEMENTAL FIGURES**

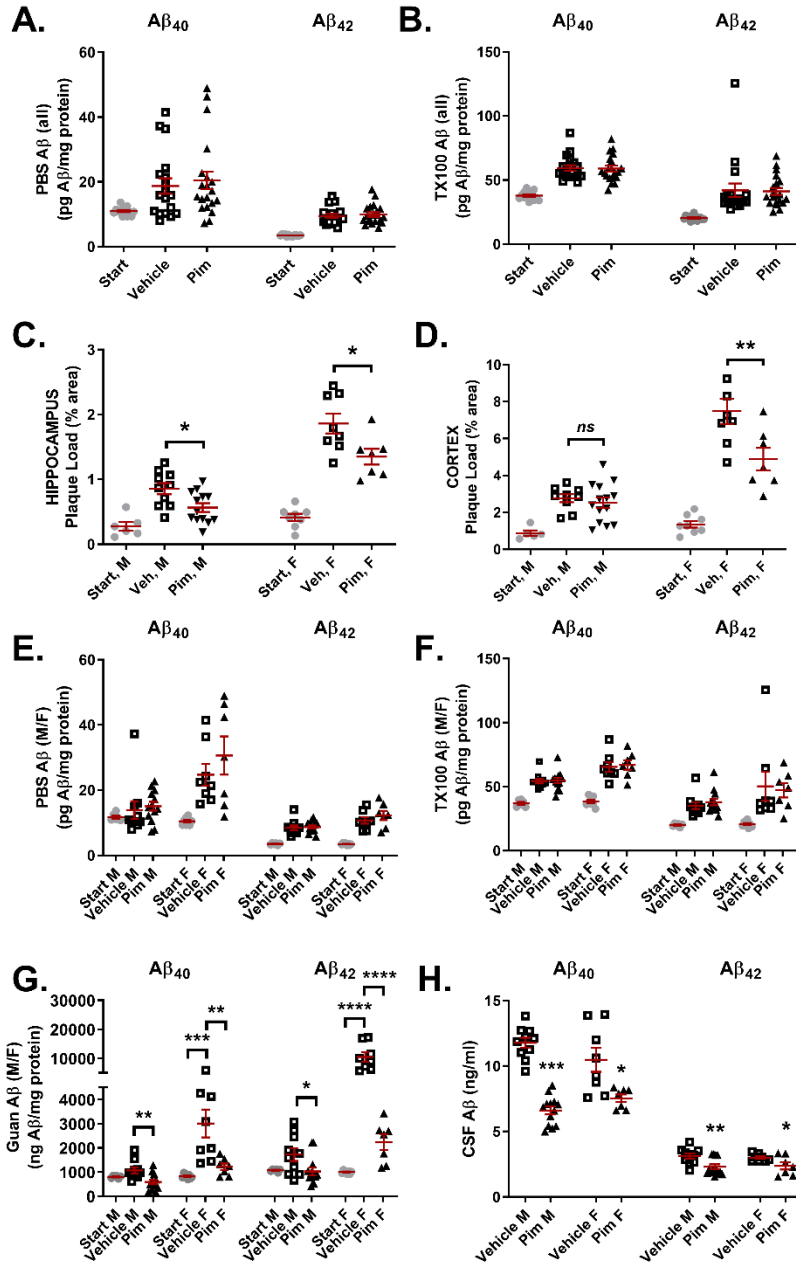
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**Figure S1: Effect of chronic Pimavanserin on soluble A $\beta$  species and sex differences in A $\beta$  pathology** (A,B) In combined males and female mice combined, A $\beta_{40}$  and A $\beta_{42}$  in the PBS-soluble and Triton X-100-soluble fractions was significantly increased in vehicle and Pim groups compared to Start, but Vehicle and Pim were not different from each other (PBS A $\beta_{40}$ : Vehicle  $18.7 \pm 2.42$ , Pim  $20.5 \pm 2.73$ ,  $p=0.838$ ; PBS A $\beta_{42}$ : Vehicle  $9.5 \pm 0.63$ , Pim  $9.9 \pm 0.68$ ,  $p=0.916$ ; TX-100 A $\beta_{40}$ : Vehicle  $59.3 \pm 2.29$ , Pim  $59.0 \pm 2.26$ ,  $p=0.993$ ; TX-100 A $\beta_{42}$ : Vehicle  $41.9 \pm 5.38$ , Pim  $41.1 \pm 2.66$ ,  $p=0.987$ ; data as mg A $\beta$ /mg protein). When separated by sex, A $\beta$  plaque load in the (C) hippocampus and (D) cortex was still significantly elevated in the Vehicle and Pim groups compared to Start ( $n = 5-14$  mice per group). Comparing the Vehicle and Pim groups at the same age, A $\beta$  plaques were universally reduced in mice treated with Pimavanserin, with the only exception of cortical plaque load in male mice which was not significantly different ( $p=0.546$ ). Hippocampi from mice in the Start, Vehicle, and Pimavanserin groups was sequentially homogenized in PBS, then 1% Triton X-100, then 5M guanidine. (E,F) When separated by sex, A $\beta$  levels in the PBS and Triton X-100 soluble fractions still did not

differ between vehicle and Pimavanserin-treated mice. (G) Similar to data for combined sexes, in males,  $A\beta_{40}$  and  $A\beta_{42}$  were reduced by  $44.9 \pm 8.7\%$  ( $p=0.0041$ ) and  $40.3 \pm 15.1\%$  ( $p=0.0061$ ), respectively, and in females were reduced by  $59.5 \pm 4.5\%$  ( $p=0.0355$ ) and  $79.6 \pm 3.1\%$  ( $p<0.0001$ ), respectively. Similar to previous findings, females had substantially more  $A\beta$  compared to male mice. (H) CSF  $A\beta_{40}$  and  $A\beta_{42}$  levels were still significantly reduced in mice treated with Pimavanserin compared to vehicle when sexes were analyzed separately. Data presented as mean  $\pm$  SEM.

### Figure S2. Timeline of chronic Pim administration studies

