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Supplementary Data

Peroxisome Proliferators Reduce Spatial Memory Impairment, Synaptic Failure, and Neurodegeneration in Brains of a Double Transgenic Mice Model of Alzheimer's Disease

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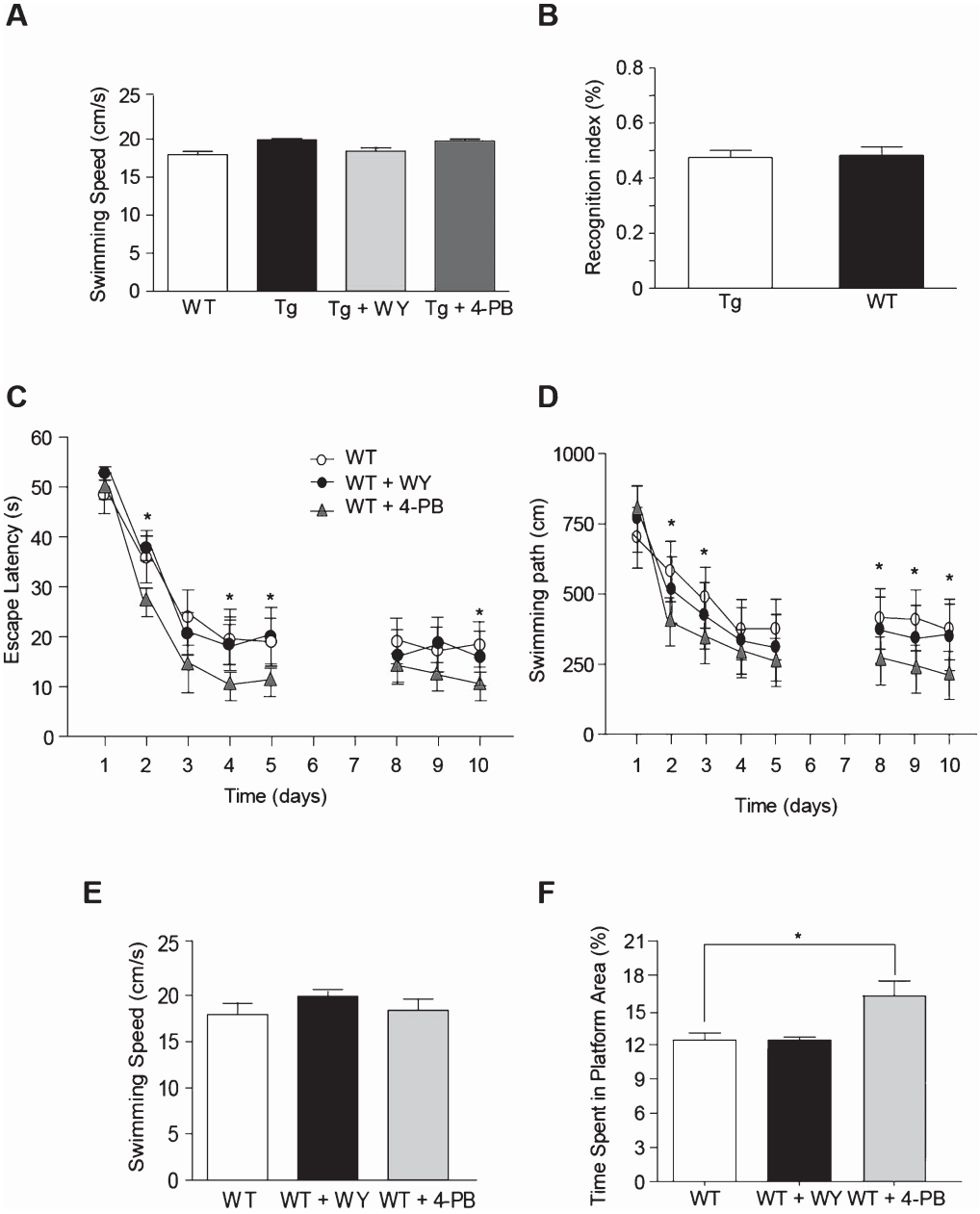
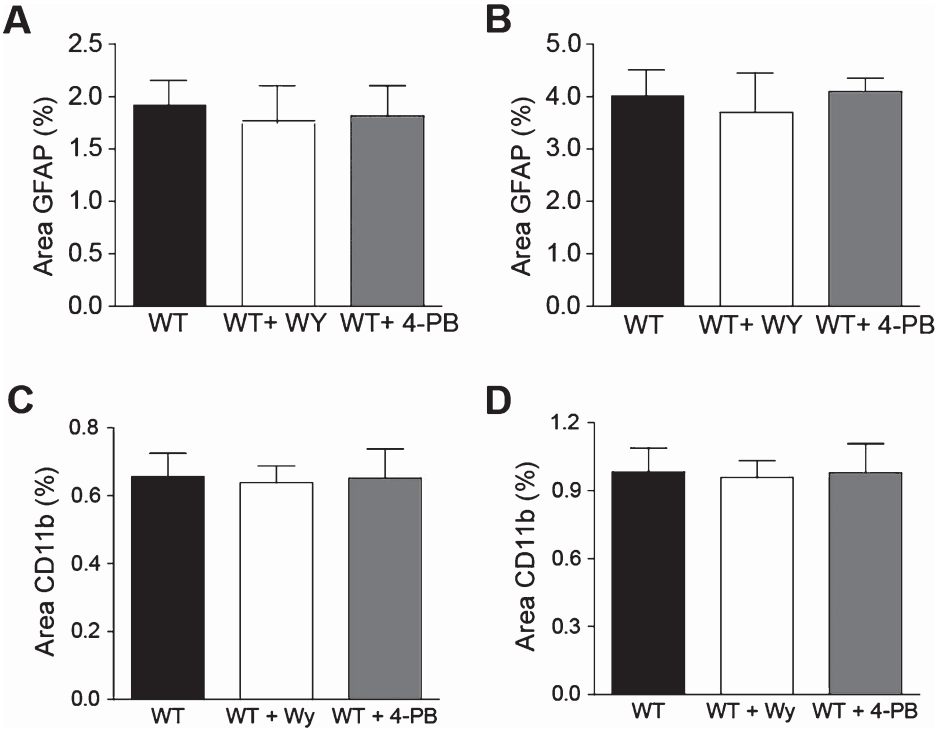
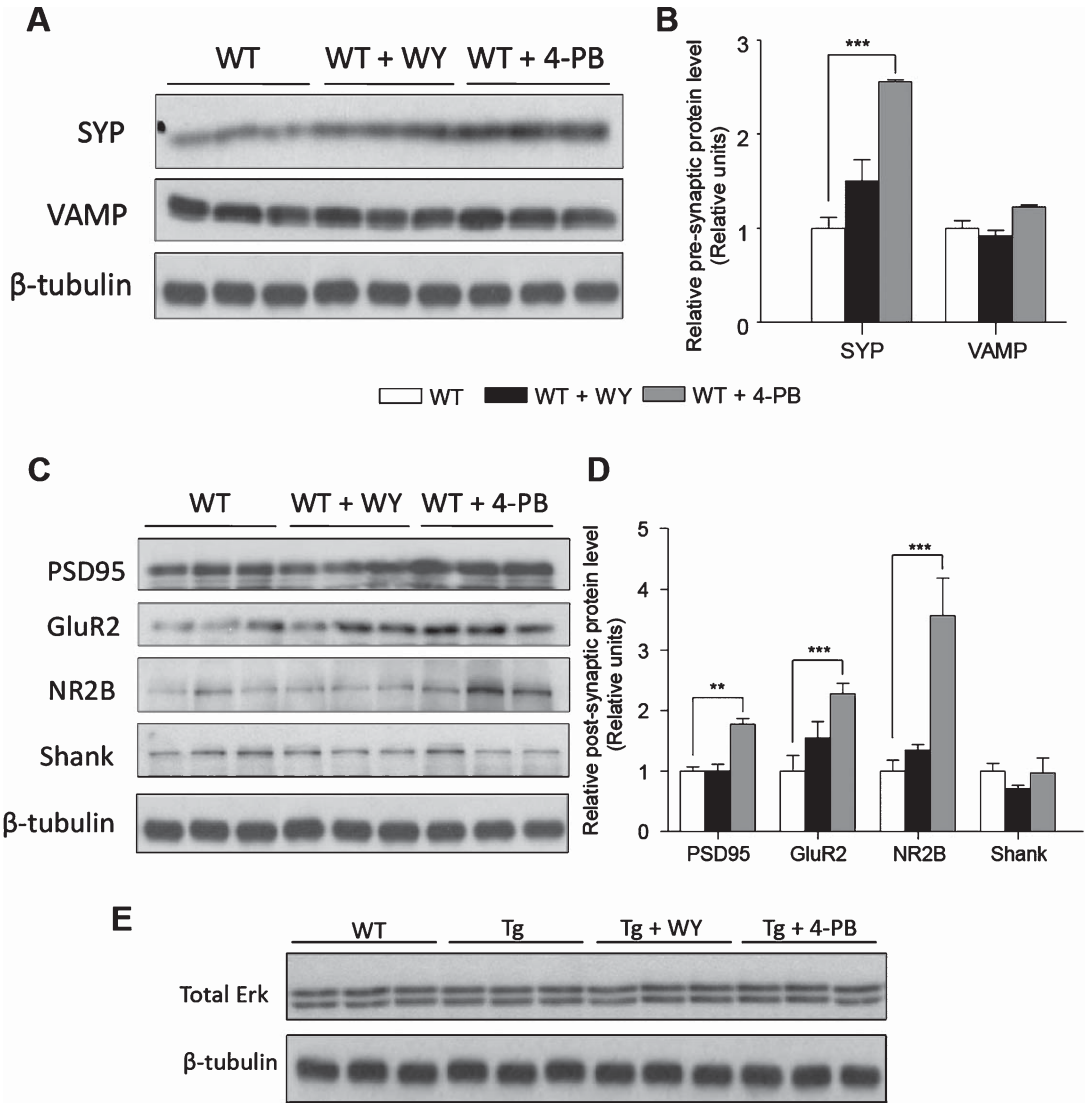


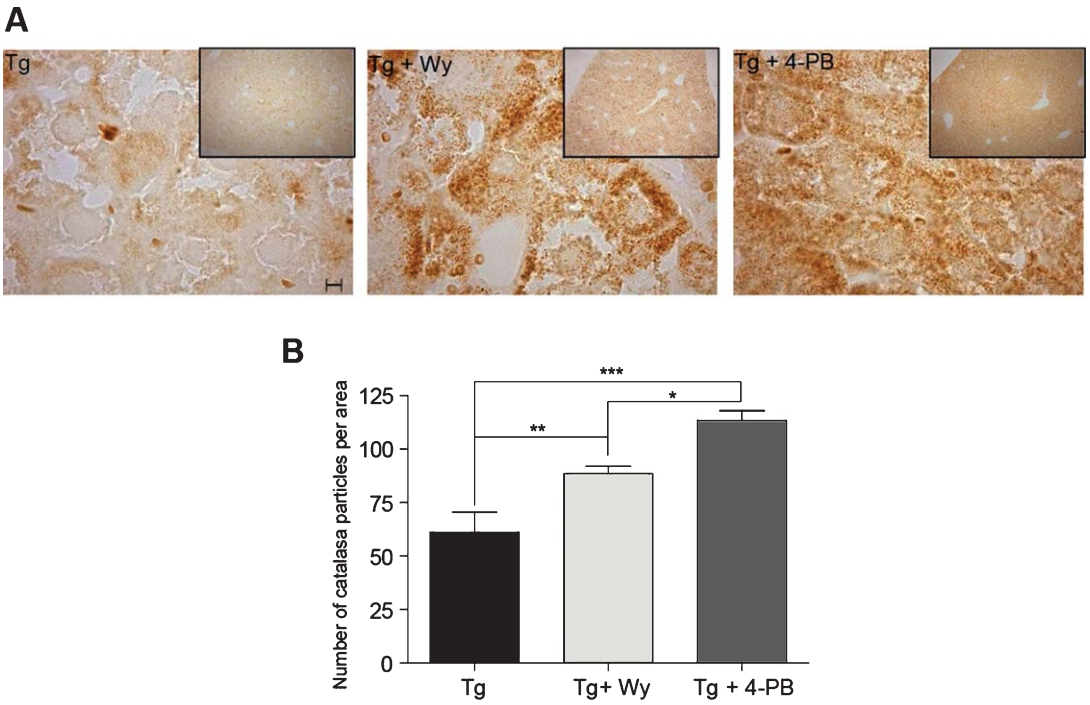
Supplementary Figure 1. Treatment with peroxisome proliferators (PPs) improves the special memory in wild-type (WT) mice. WT animals were treated with Wy-14643 (WY) or 4-phenylbutyrate (4-PB) for 60 days. Spatial memory was evaluated by the Morris water maze task in comparison with WT untreated control mice. A) Analysis of swimming speeds shows no major differences between the experimental groups that could account for the escape latency improvement. B) No statistically significant differences were observed in the recognition index between 5 month old WT and transgenic (Tg) mice (before treatment). C) Escape latency (time to reach the hidden platform) of WT was significantly reduced only by 4-PB treatment (day 10: F 23.54, $d.f.$ 4/11, WT versus WT + 4-PB M –14.09, 95% CI [–24.48, –3.702], $^*p < 0.05$). D) Analysis of the swimming path showed decreased distances to reach the platform in WT mice treated with 4-PB (day 10: F 20.69, $d.f.$ 4/11, WT versus WT + 4-PB M 18.431, 95% CI [5.529, 38.332], $^*p < 0.05$). E) No differences were observed in the swimming speed between control WT mice and treated WT mice. F) Analysis of the time spent by the mice swimming in the area near the platform when it was removed. 4-PB treated wild-type animals showed higher swimming time in the platform area as compared to WT controls (F 5.434, $d.f.$ 2/11, WT versus WT + 4-PB M 24.08, 95% CI [0.1545, 48.01], $^*p < 0.05$). Data are presented as means ± S.E.M. Statistical significant differences were calculated by one-way ANOVA, followed by Bonferroni's *post hoc* test. Time curves were analyzed by Repeated Measures ANOVA.



Supplementary Figure 2. Effects of PP treatment in the neuroinflammation markers in WT mice. Quantification of the GFAP area fraction ((GFAP area/total field area)*100) in cortex (A) and hippocampus (B). Quantification of the CD11b area fraction ((CD11b area/total field area)*100) in cortex (C) and hippocampus (D). In both cases, no statistically significant differences were observed. Data are presented as means ± S.E.M. Statistical significant differences were calculated by one-way ANOVA, followed by Bonferroni's *post hoc* test.



Supplementary Figure 3. Treatments with WY and 4-PB increase the levels of synaptic proteins in WT mice. Immunoblots of presynaptic (A) and postsynaptic (C) proteins from the hippocampus of WT and WY- or 4-PB-treated WT mice. The three bands for each condition come from brain samples of three different mice. B, D) Graphs correspond to the densitometric analysis of the protein bands normalized against total Erk and the WT levels (B: SYP: F 28.54, d.f. 2/8, WT versus WT + 4-PB M –1.555, 95% CI [–2.133, –0.9771], ***$p < 0.001$; D: PSD95: F 22.83, *d.f.* 2/8; WT versus WT + 4-PB M –0.7730, 95% CI [–1.207, –0.3393], **$p < 0.01$); GLUR2: F 41.25, *d.f.* 2/8; WT versus WT + 4-PB M –2.378, 95% CI [–3.122, –1.634], ***$p < 0.001$; NR2B: F 41.45, *d.f.* 2/8; WT versus WT + 4-PB M –2.900, 95% CI [–4.044, –1.756], ***$p < 0.001$). E) Validation of Erk as protein loading control using β-tubulin as reference. Data are presented as means ± S.E.M. Statistical significant differences were calculated by one-way ANOVA, followed by Bonferroni's *post hoc* test.



Supplementary Figure 4. WY and 4-PB treatment induce peroxisome proliferation in AβPP/PS1 mice. A) Catalase activity in liver slices was detected by DAB oxidation. Inset shows a magnification of the indicated zone. Scale bar represents 10 μm. B) For DAB oxidation staining, peroxisome density was quantified as the average number of catalase-positive particles per μm^2 (F 20.27, $d.f.$ 2/42, Tg versus Tg + WY M –27.44, 95% CI [–48.00, –6.889], $^{**}p < 0.01$; Tg versus Tg + 4-PB M –52.19, 95% CI [–72.75, –31.64], $^{***}p < 0.001$; Tg + WY versus Tg + 4-PB M –24.75, 95% CI [–43.30, –6.196], $^{*}p < 0.05$). Data are presented as means ± S.E.M. Statistical significant differences were calculated by one-way ANOVA, followed by Bonferroni´s *post hoc* test.