SUPPLEMENTARY DATA

Myo-Inositol Dampens Apathetic Symptoms in Aged Mice and Is Associated with Sex-Specific Synapse Remodelling

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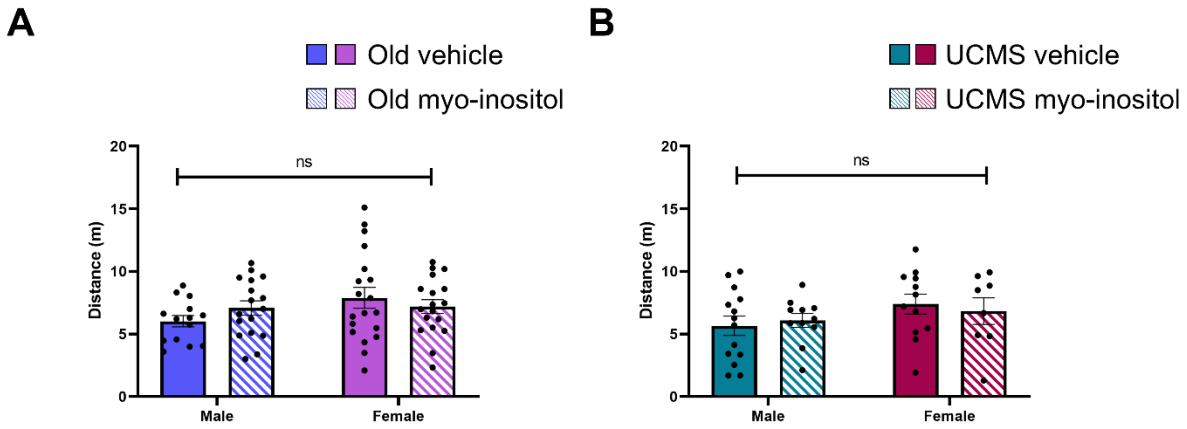
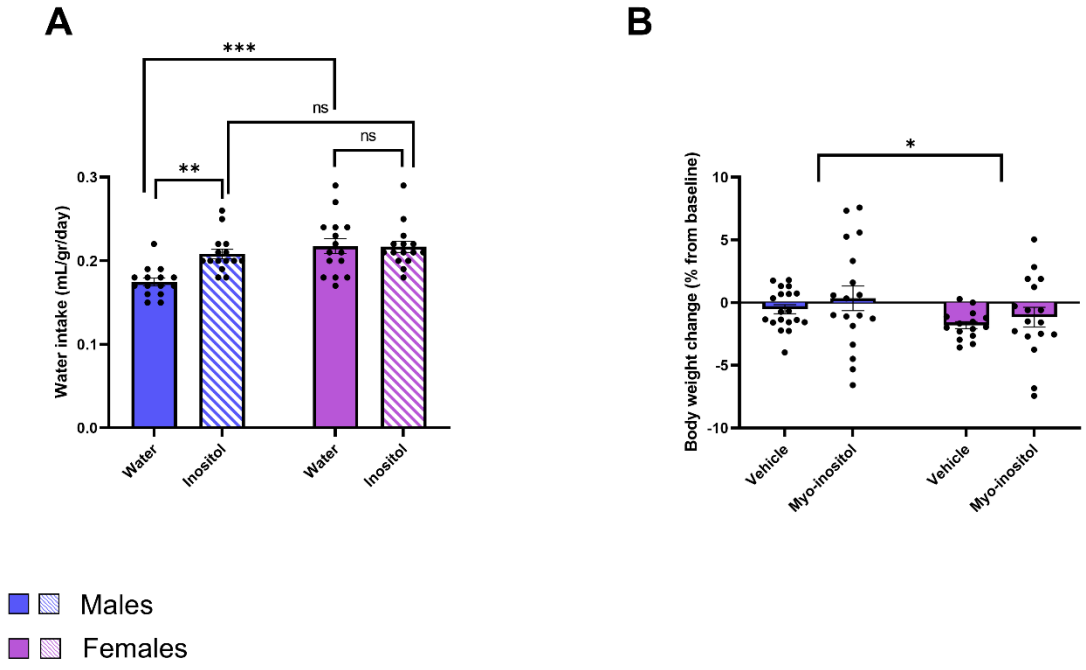


Supplementary Figure 1. Total distance traveled in EPM test in mice treated with vehicle/myo-inositol. Total distance traveled in EPM in male (**A**) and female (**B**) mice.



Supplementary Figure 2. Water intake and body weight changes following myo-inositol administration. (A) Daily water intake normalized to body weight was recorded for all experimental groups included in the experiment shown in Fig. 2. Values represent cage-level estimates obtained by dividing total daily water consumption by the number of animals per cage (n=4 cages per condition), subsequently normalized to average cage body weight. Measurements were collected daily during the last two weeks of treatment. Two-Way ANOVA revealed significant main effects of sex ($F(1,56)=14.62$; $p<0.001$), treatment ($F(1,56)=5.922$; $p=0.018$), and their interaction ($F(1,56)=6.415$; $p=0.014$). Post-hoc analysis (Tukey's) identified the primary source of variance as a baseline sex

SUPPLEMENTARY DATA

difference in vehicle-treated animals (females vs. males: $p<0.001$), consistent with previously reported sex differences in fluid consumption in rodents [1, 2, 3]. Within-sex comparisons revealed that myo-inositol treatment increased water intake in males (0.18 vs. 0.21 ml/g/day; $p=0.004$) but not in females (0.22 vs. 0.22 ml/g/day; $p>0.05$). No significant difference was observed between myo-inositol-treated males and females (0.21 vs. 0.22 ml/g/day; $p>0.05$). Estimated daily doses were comparable across treated groups (females: 1.10 g/kg/day; males: 1.05 g/kg/day). **(B)** Body weight change, expressed as percentage variation between the first and last week of treatment, was monitored weekly throughout the experiment. Two-Way ANOVA revealed a significant effect of sex ($F(1,65)=4.084$; $p=0.047$) but not of treatment or their interaction, indicating that myo-inositol administration did not significantly affect body weight over the four-week treatment period.

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2. McGivern, R. F., Henschel, D., Hutcheson, M., & Pangburn, T. (1996). Sex difference in daily water consumption of rats: effect of housing and hormones. Physiology & behavior, 59(4-5), 653–658. https://doi.org/10.1016/0031-9384(95)02017-9
3. Nair, A. V., Yanhong, W., Paunescu, T. G., Bouley, R., & Brown, D. (2019). Sex-dependent differences in water homeostasis in wild-type and V-ATPase B1-subunit deficient mice. PloS one, 14(8), e0219940. https://doi.org/10.1371/journal.pone.0219940

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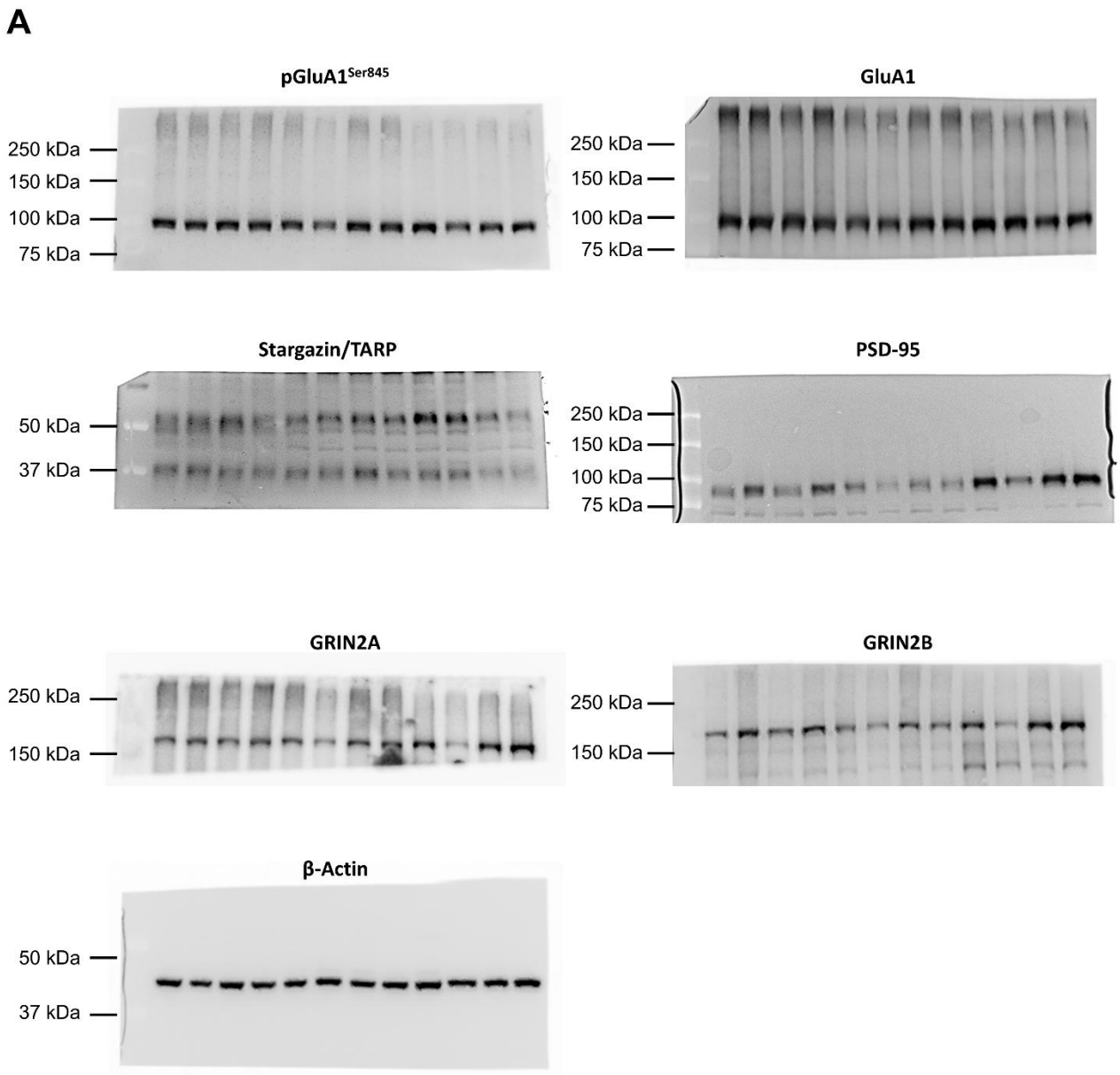

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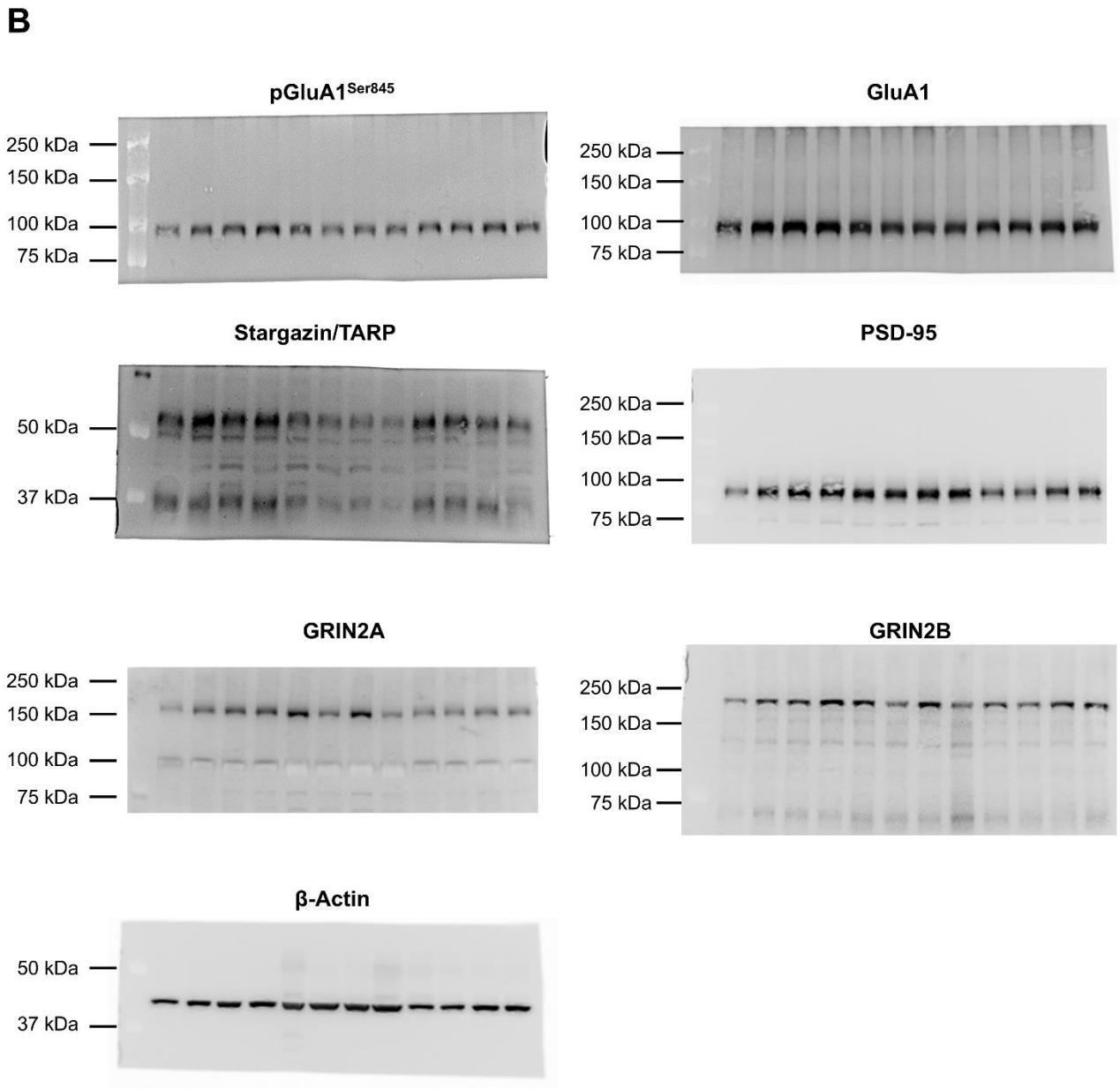

Supplementary Figure 3-4. Uncropped Western blots corresponding to data presented in Figure 4 and 5 of the main manuscript. Uncropped Western blots corresponding to data presented in Figure 4A (**A**) and in Figure 5A (**B**).