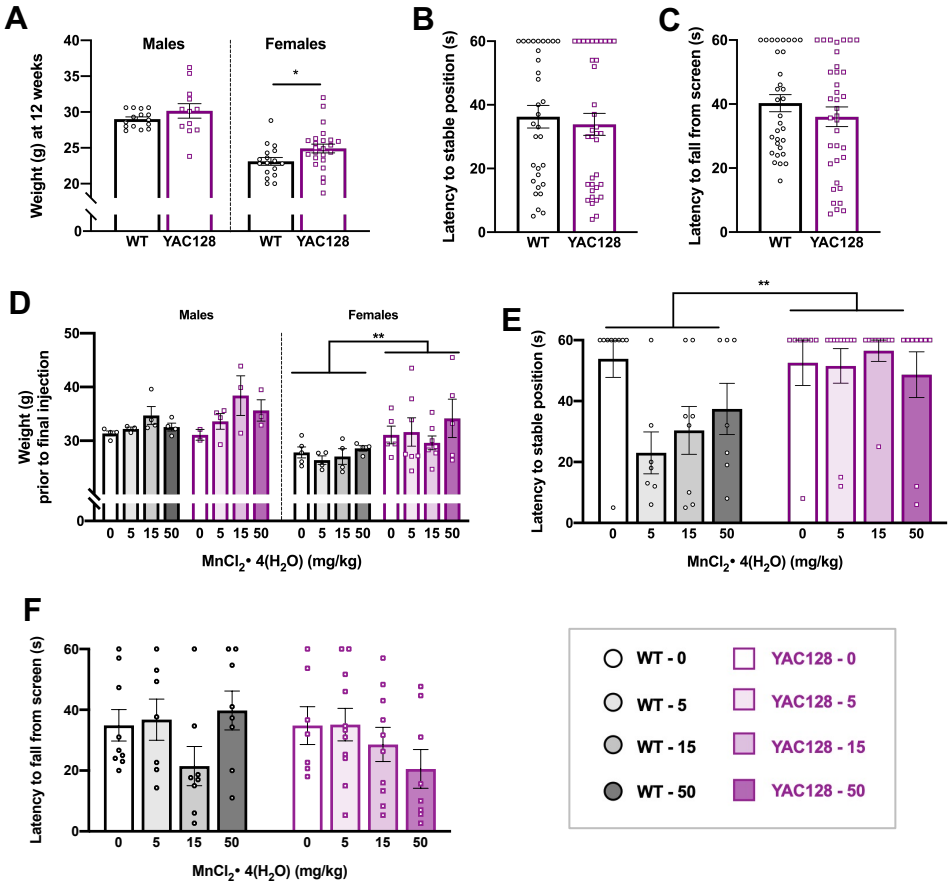
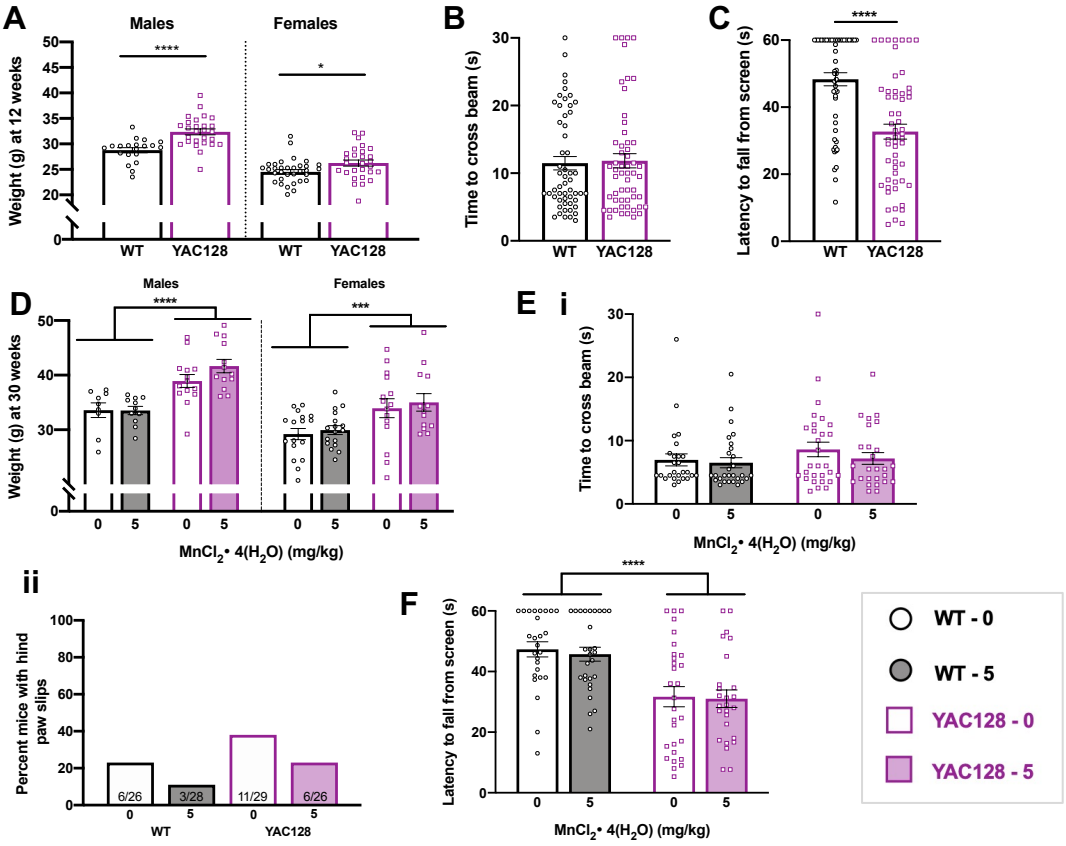


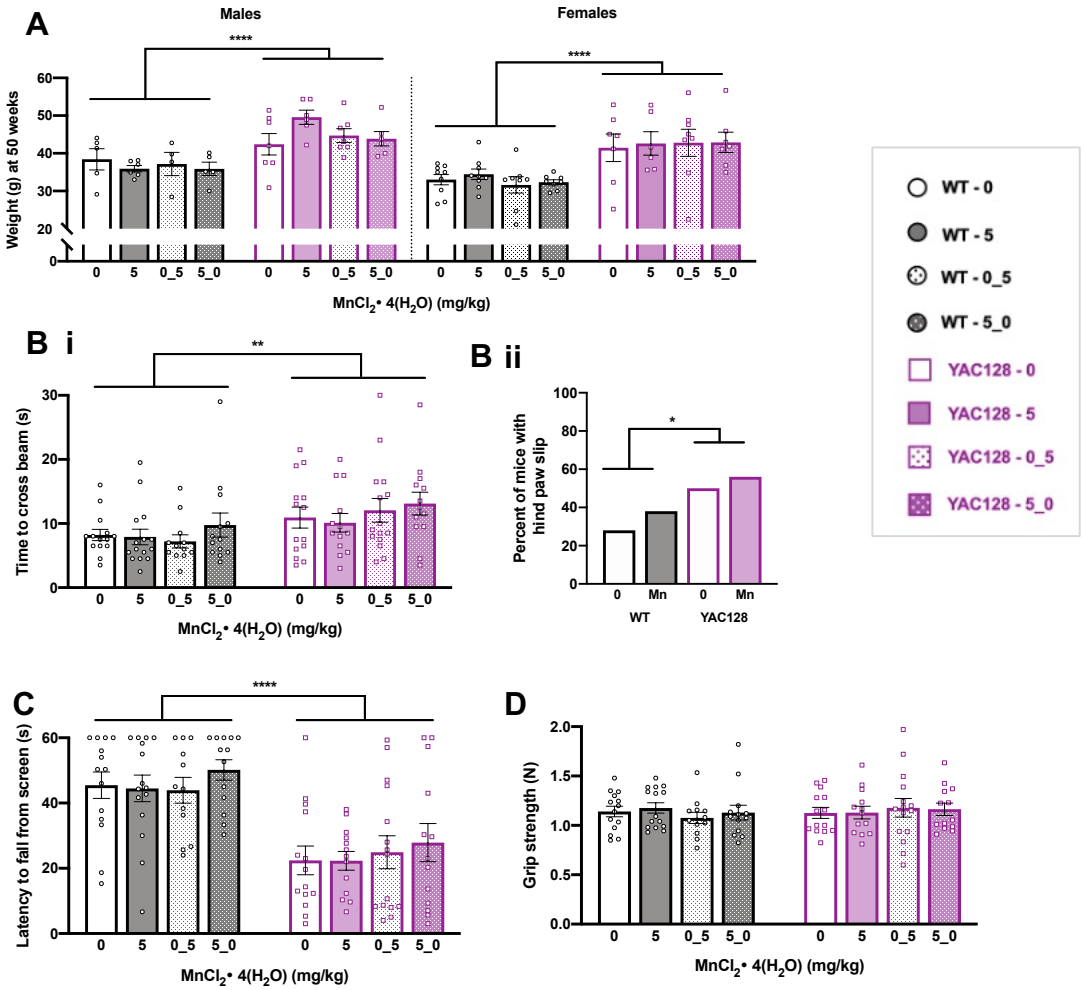
Supplementary Figure 1 Body weights and additional behavioral tasks from Study 1. Mice underwent a week-long baseline behavioral battery at 11 weeks old (A-C) prior to beginning twice weekly subcutaneous injections (0, 5, 15, or 50 mg/kg $MnCl_2 \bullet 4(H_2O)$) from 12 weeks until 32 weeks of age. The behavioral battery was repeated at 30 weeks of age (D-F) to assess effects of chronic Mn exposure and mice were sacrificed and dissected at 32 weeks of age, 24 hours after the final injection. **(A)** Males weighed more than females at 12 weeks of age and YAC128 mice weighed more than WT (Sex $F_{1, 64}$=71.768, P<0.0001, Genotype $F_{1, 64}$=5.058, P=0.028), which were both expected differences[36]. **(B)** Wire hang was performed as an additional assessment of motor coordination. Mice either achieved a stable position (n=24 WT and n=27 YAC128) or fell (n=8 WT and n=9 YAC128) during the task; a fall was assigned a maximum score of 60 s. There was no significant difference between genotypes on the wire hang task at 11 weeks of age (Mann-Whitney U=529.5, P=0.568). **(C)** Limb strength, represented by latency to fall from the inverted screen, was not significantly different between WT and YAC128 mice (Mann-Whitney U=501, P=0.358) despite the observed weight difference between genotypes. **(D)** We monitored the weight of all animals to ensure

appropriate overall health while administering Mn. Consistent with 12-week measurements, males weighed more than females and as expected YAC128 mice weighed more than WT at 32 weeks of age (Sex $F_{1, 52}$= 15.279, P<0.001, Genotype $F_{1, 52}$= 8.341, P=0.006). There was no effect of the 20-week-long Mn exposure on body weight, indicating lack of overt toxicity or general malaise caused by repeated Mn injections. A small number of animals exhibited mild injection site irritation (hair loss, rigid skin) at all Mn doses, but no gross adverse reactions were noted. **(E)** On the wire hang, a mouse either achieved a stable position (n=18 WT and n=9 YAC128) or fell (n=14 WT and n=27 YAC128) during the task. YAC128 mice exhibited a deficit on this task at 30 weeks of age compared to WT, but there were no effects of Mn (Genotype $F_{1, 60}$= 11.70, P=0.001, Treatment $F_{3, 60}$= 1.930, P=0.134). **(F)** Overall limb strength, indicated by latency to fall from the inverted screen was not different between genotypes or Mn exposure groups (Genotype $F_{1, 60}$= 0.6471, P=0.4243, Treatment $F_{3, 60}$= 1.397, P=0.2525), even despite a significant weight difference between genotypes. For all, mean ± S.E.M. plotted unless otherwise noted. Asterisks * indicate genotype effect, pound # indicates Mn effect within genotype. # p <0.05, ## p <0.01, * p<0.05 **p<0.01, **** p<0.0001. n=7-11 per genotype-treatment group with approximately equal number of males and females.



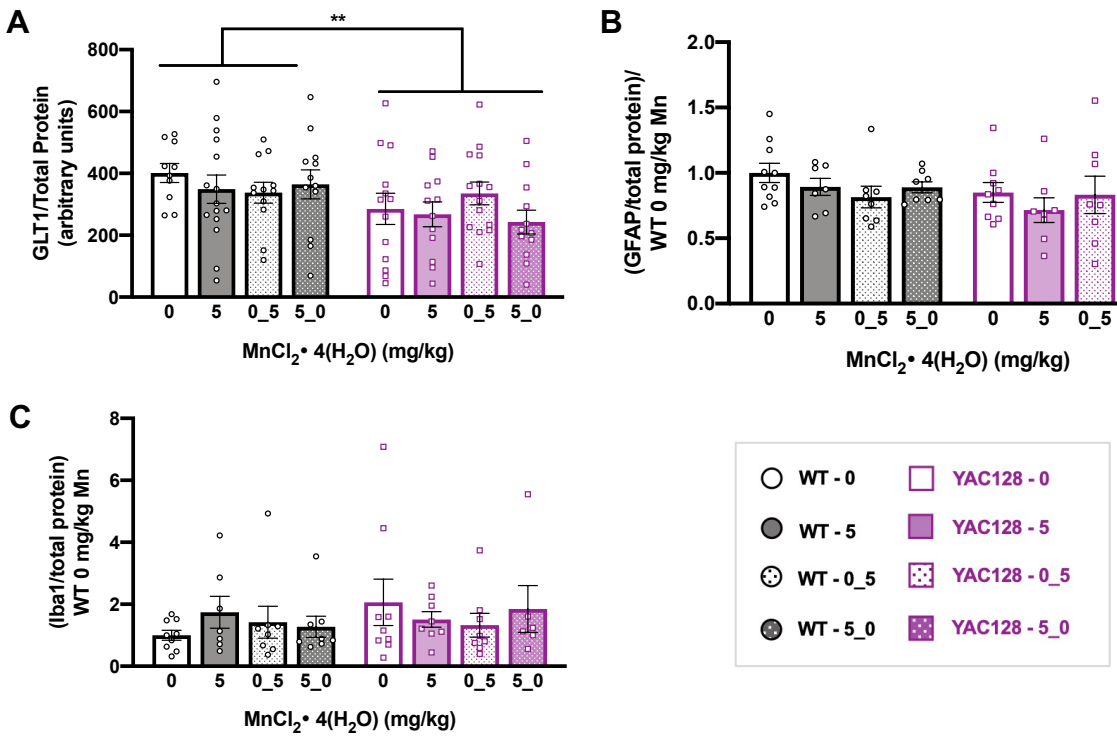
Supplementary Figure 2 Body weight and additional behavioral data from mice in chronic Study 2. Mice underwent a week-long baseline behavioral battery at 11 weeks old (**A-C**) prior to beginning twice weekly subcutaneous injections [0 or 5 mg/kg $MnCl_2 \bullet 4(H_2O)$] at 12 weeks of age. The behavioral battery was repeated at 30 weeks of age **(D-F),** then half of the mice changed exposure groups at 31 weeks of age and behavioral battery was repeated at 50 weeks of age (see manuscript Fig. 5). **(A)** As expected, males weighed more than females and YAC128 mice were significantly heavier than WT at 12 weeks of age prior to beginning Mn exposure (Sex $F_{1, 105}$=90.24, P<0.0001, Genotype $F_{1, 105}$=23.85, P<0.0001). **(B)** As another measure of motor coordination in Study 2, mice performed the balance beam task. The average time to cross the beam (average of two trials; max 30 s) and the total number of hind paw slips (accumulated from both trials) were recorded. At 11 weeks YAC128 mice were not impaired based on the time it took to cross the beam (Mann-Whitney U=1437, P=0.773), nor the percentage of mice that displayed hind paw slips during either trial (not shown; WT =14/54 and YAC128= 19/55 animals; $\chi^2 = 0.959$, P=0.327). **(C)** On the inverted screen task, YAC128 mice fell sooner than WT (t_{107}=5.262, P<0.0001). The significant difference between genotypes on the inverted screen at 11 weeks could suggest a lack of strength in the YAC128 mice, however this difference may be driven by the significantly heavier weight of the YAC128 mice. **(D)** Again,

males weighed more than females and YAC128 mice weighed more than WT but there was no effect of Mn exposure in any group (Sex $F_{1, 101}$=29.138, P<0.0001, Genotype $F_{1, 101}$=41.122, P<0.0001). **(E)** YAC128 mice did not display a motor coordination deficit on the balance beam task at 30 weeks of age, based on the average time to cross the balance beam (Genotype $F_{1, 105}$ =1.436, P=0.236, Treatment $F_{1, 105}$=0.905, P=0.347) **(i)** and the total number of hind paw slips accumulated across both trials (vehicle-treated WT and YAC128 mice χ^2=1.42, P=0.234). Mn also had no impact within WT (χ^2=1.48, P=0.223) or YAC128 (χ^2=1.42, P=0.234) mice on the number of hind paw slips after this length of Mn exposure **(ii)**. **(F)** On the inverted screen, 18-weeks of low-dose Mn had no impact on latency to fall from the screen and YAC128 mice fell sooner than WT mice (Genotype $F_{1, 105}$ =28.95, P<0.0001, Treatment $F_{1, 105}$=0.157, P=0.693). For all, mean ± S.E.M. plotted unless otherwise noted. Asterisks * indicate genotype effect. * p <0.05, **, p<0.01, *** p<0.001, **** p<0.0001. n=26-29 per genotype-treatment group with approximately equal number of males and females.



Supplementary Figure 3 Body weight and additional behavioral data from mice in chronic Study 2 at 50 weeks of age. **(A)** By 50 weeks of age, males continued to weigh more than females and YAC128 mice were significantly heavier than WT, but there was no effect of Mn (Sex $F_{1, 93}$=6.909, P=0.010, Genotype $F_{1, 93}$=52.011, P<0.0001, Treatment $F_{1, 93}$=0.364, P=0.779, all Interactions Ps > 0.05). **(B)** YAC128 mice took significantly longer time (s) to cross the balance beam (Genotype $F_{1, 101}$ =9.329, P=0.003, Treatment $F_{3, 101}$=0.946, P=0.422) **(i)** and displayed more hind paw slips compared to WT **(ii).** For hind paw slips, the three Mn exposure groups were combined in a single Mn group and compared to vehicle groups for each individual genotype, and there was no effect of Mn (P>0.05). For WT, 40% of the "5" treatment group exhibited >1 hind paw slip; 33% of "0_5" WT and 38% of "5_0" mice had hind paw slips. Hind paw slips were present in 46% of YAC128 "5" mice, 67% of YAC128 "0_5" and 53% YAC128 "5_0" mice. Of the YAC128 mice had more hind paw slips than WT regardless of Mn exposure (χ^2=4.127, P=0.042). **(C)** General limb strength as measured by the latency to fall from the inverted screen was decreased in YAC128 mice compared to WT but not changed by any Mn exposure (Genotype $F_{1, 101}$ =49.93, P<0.0001, Treatment

$F_{1, 101}$=0.694, P=0.558, Interaction $F_{1, 101}$=0.087, P=0.967). **(D)** Two paw grip strength was also assessed at 50 weeks as a control measure, and there were no significant differences on this task for genotype or Mn exposure (Genotype $F_{1, 101}$ =0.158, P=0.691, Treatment $F_{3, 101}$=0.065, P=0.978, Interaction $F_{3, 101}$=0.466, P=0.706). For all, mean ± S.E.M. plotted unless otherwise noted. Asterisks * indicate genotype effect, pound # indicates Mn effect within genotype. #/* p <0.05, **, p<0.01, *** p<0.001, **** p<0.0001. n=12-15 per genotype-treatment group with approximately equal number of males and females.



Supplementary Figure 4 Additional post-dissection (52 weeks of age) outcomes for mice used in Study 2. **(A)** GLT-1 protein expression by western blot. No effect of Mn exposure but YAC128 mice had significantly lower overall GLT-1 expression compared to WT (Genotype $F_{1, 92}$= 7.238, P=0.0085, Treatment $F_{3, 92}$= 0.437, P=0.727). n=10-15 per genotype-treatment group. **(B)** Cortical GFAP expression by western blot did not significantly differ among genotype-treatment groups (Genotype $F_{1, 57}$=0.572, P=0.452, Treatment $F_{3, 57}$= 1.13, P=0.344). n=6-10 per genotype-treatment group. **(C)** Cortical Iba1 expression by western blot did not significantly differ among genotype-treatment groups (Genotype $F_{1, 55}$=0.885, P=0.351, Treatment $F_{3, 55}$= 0.094, P=0.963). n=6-10 per genotype-treatment group. Iba1 and GFAP were probed from the same blots, Iba1 followed by GFAP, stripped between probes. For Iba1, one statistical outlier (YAC128 "5_0") was removed (>2 Std. deviations) and one WT-0 band was removed due to presumed poor transfer (undetectable levels). For all, mean ± S.E.M. plotted unless otherwise noted. Asterisks * indicate genotype effect, pound # indicates Mn effect within genotype. #/* p <0.05, **, p<0.01, *** p<0.001, **** p<0.0001 n as marked with approximately equal number males and females per group.