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## Supplementary Information

# Lithium suppression of tau induces brain iron accumulation and neurodegeneration

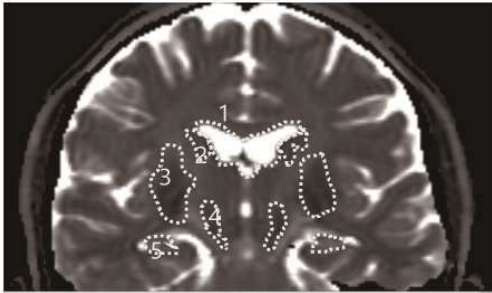
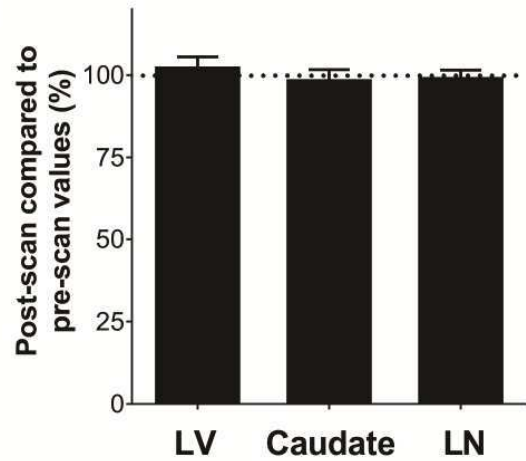
Peng Lei, Scott Ayton, Ambili Thoppuvalappil Appukuttan, Steve Moon, James A. Duce, Irene Volitakis, Robert Cherny, Stephen J. Wood, Mark Greenough, Gregor Berger, Christos Pantelis, Patrick McGorry, Alison Yung, David I. Finkelstein, and Ashley I. Bush

## Inventory of Supplementary Information.

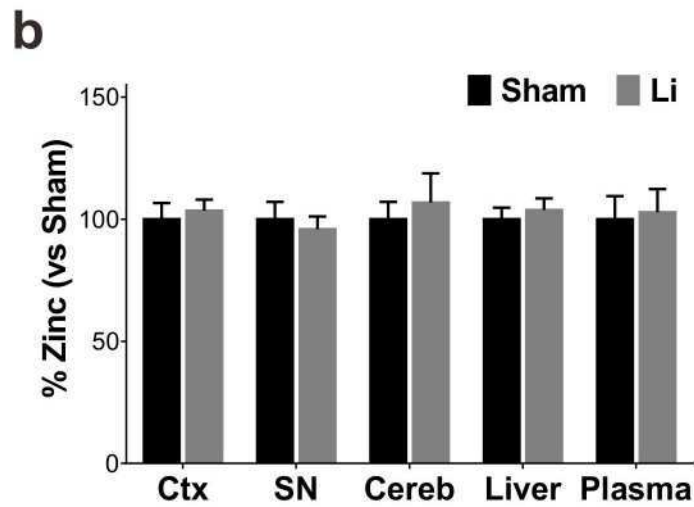
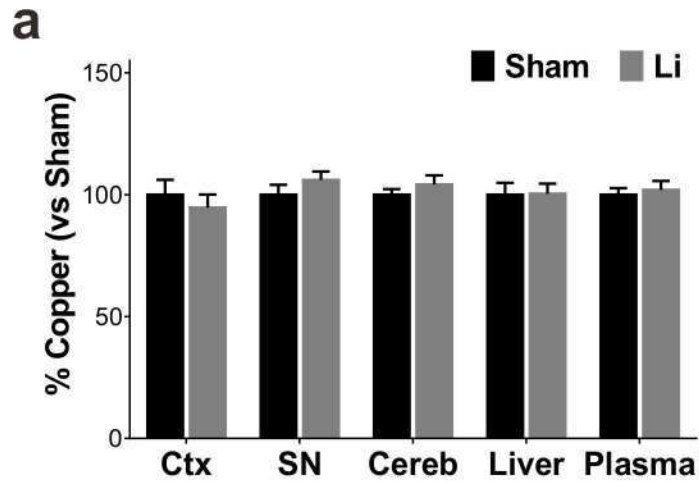
*Supplementary Data*

**Supplementary Figure 1–14**

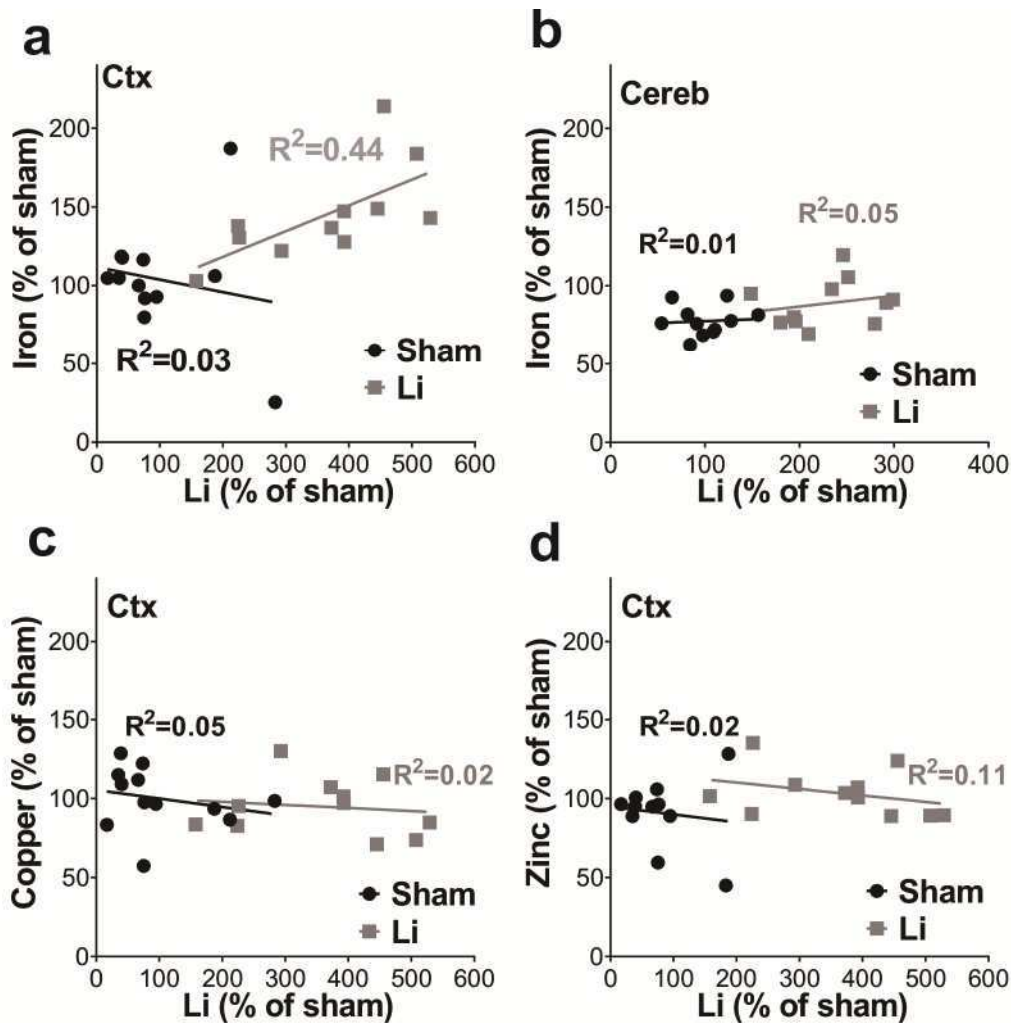
*Reference*

**a****b**

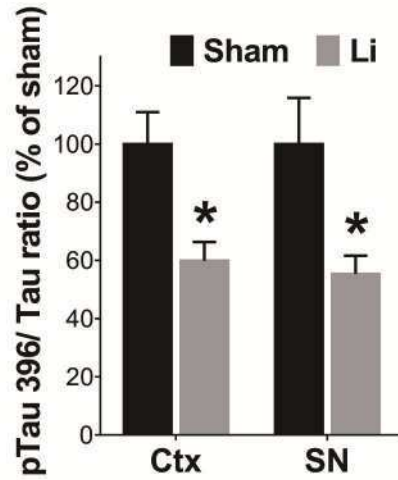
**Supplementary Figure 1.**  $T_2$  relaxation time in brain regions from subjects treated with lithium. **a)** Regions of interest were mapped as illustrated by an operator blinded to treatment group. 1. lateral ventricular area (LV); 2. Caudate; 3. lenticular nucleus (LN); 4. SN; 5. Hippocampus (previously reported<sup>1</sup>). **b)** No change in  $T_2$  relaxation time was found in the Caudate and LN of lithium-treated participants ( $p=0.18$  for Caudate;  $p=0.29$  for LN, two-tailed  $t$ -test). LV area was unaltered by Li treatment ( $p=0.177$ , two-tailed  $t$ -test).  $n$ [treatment as usual]=9,  $n$ [lithium]=11. Means  $\pm$  SEM are shown.



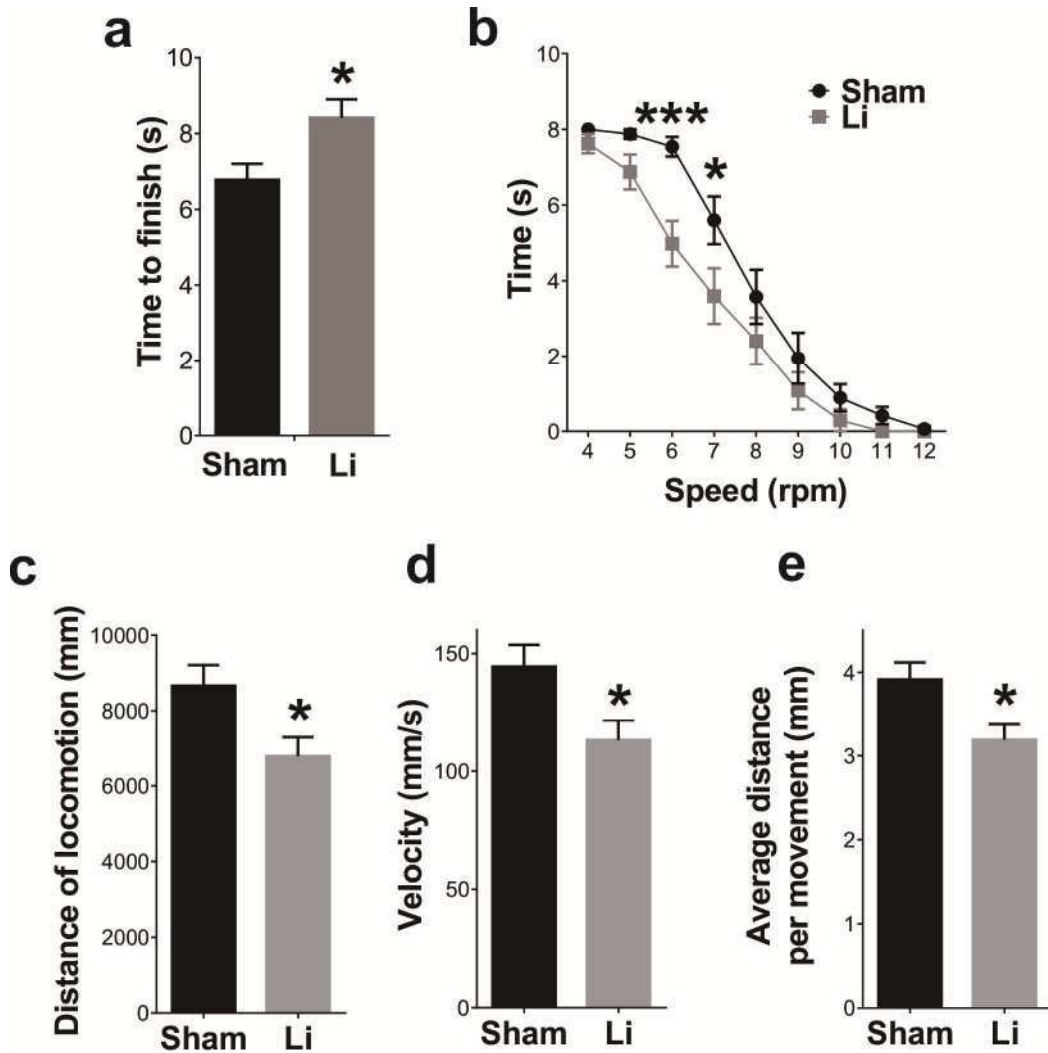
**Supplementary Figure 2.** No changes in copper (a) or zinc (b) levels in tissues of Li-treated mice. Means  $\pm$  SEM are shown, n=12 per treatment group.



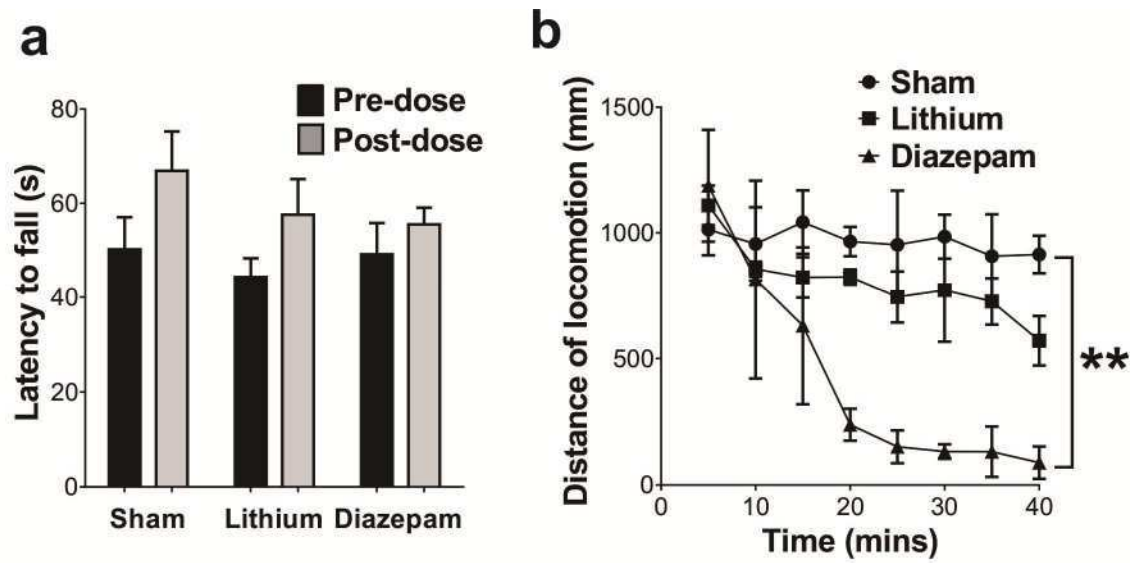
**Supplementary Figure 3.** Correlations between bio-metals and Li in brain regions of Li-treated and sham-treated mice. **a)** Iron levels in cortex correlates with lithium levels in Li-treated mice ( $R^2=0.439$ ,  $p=0.026$ , linear regression) but not sham-treated mice ( $R^2=0.0327$ ,  $p=0.574$ , linear regression). **b)** No correlation between iron and lithium levels in cerebellum. **c-d)** No correlation between copper (**c**) or zinc (**d**) and lithium levels in cortex.  $n=12$  per treatment group.



**Supplementary Figure 4.** Li treatment reduces phosphorylated tau levels in both cortex and SN. Quantification of western blot showed reduction of tau phosphorylation at Ser396 in cortex ( $p=0.034$ , two-tailed t-test) and SN ( $p=0.039$ , two-tailed t-test) of Li-treated mice compared to sham-treated mice. Means  $\pm$  SEM are shown,  $n=12$  per treatment group. \*  $p<0.05$ .

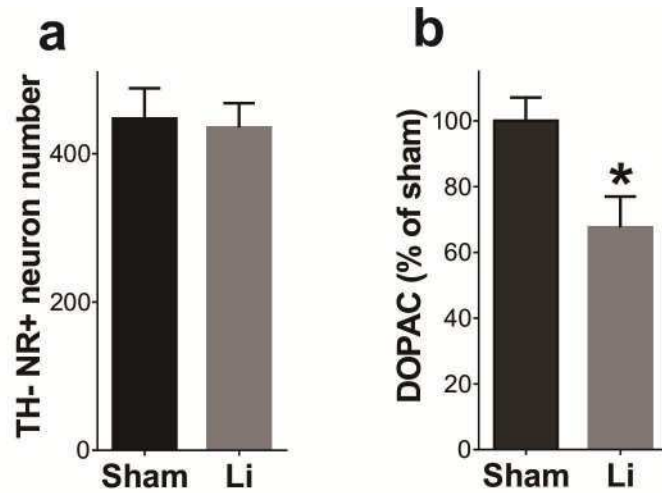


**Supplementary Figure 5.** Additional locomotor deficits induced by Li treatment. **a)** Li-treated mice required longer time to finish ( $p=0.041$ , two-tailed t-test) in the Pole test after 21 days of treatment. **b)** Li-treated mice showed reduced maximum speed in the Rotarod test. Two-way ANOVA with post-hoc Dunnett's test: speed ( $p < 0.001$ ), and treatment ( $p < 0.001$ ) effects but no interaction ( $p = 0.107$ ). **c-e)** Li-treated mice showed reduced distance of locomotion (**c**,  $p=0.022$ , two-tailed t-test), reduced velocity (**d**,  $p=0.023$ ), and reduced average distance per movement (**e**,  $p=0.018$ ) in the Open field test. Means  $\pm$  SEM are shown,  $n=12$  per treatment group. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

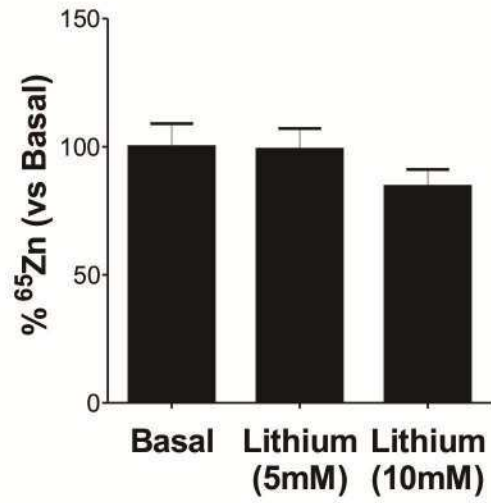


**Supplementary Figure 6.** Li-induced motor impairment is independent of its sedative effect. **a)** Neither a single dose of Li (3.6mg/kg, gavage) nor diazepam (3mg/kg, gavage) significantly altered the performance of mice in the Rotarod test 2 hours post-dose. **b)** Diazepam sedated mice within 20 minutes, evidenced by decreased locomotion in the Open field test 20 minutes after the dose was delivered, however no such effect was found following the lithium dose. Means  $\pm$  SEM are shown,  $n=5$  per treatment group. Both experiments tested for significance with two-way ANOVA and post-hoc Dunnett's test. \*\*  $p=0.0064$ .

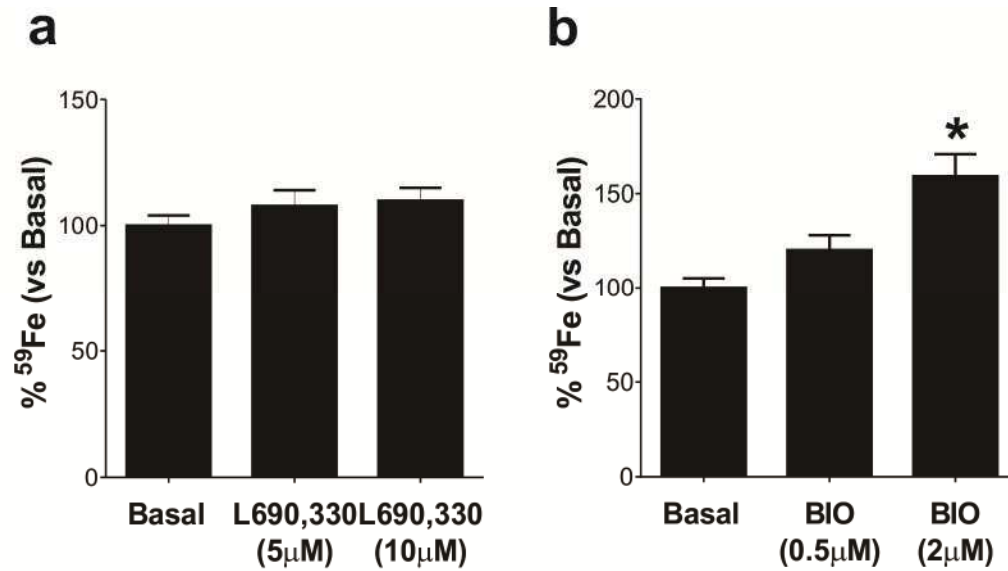




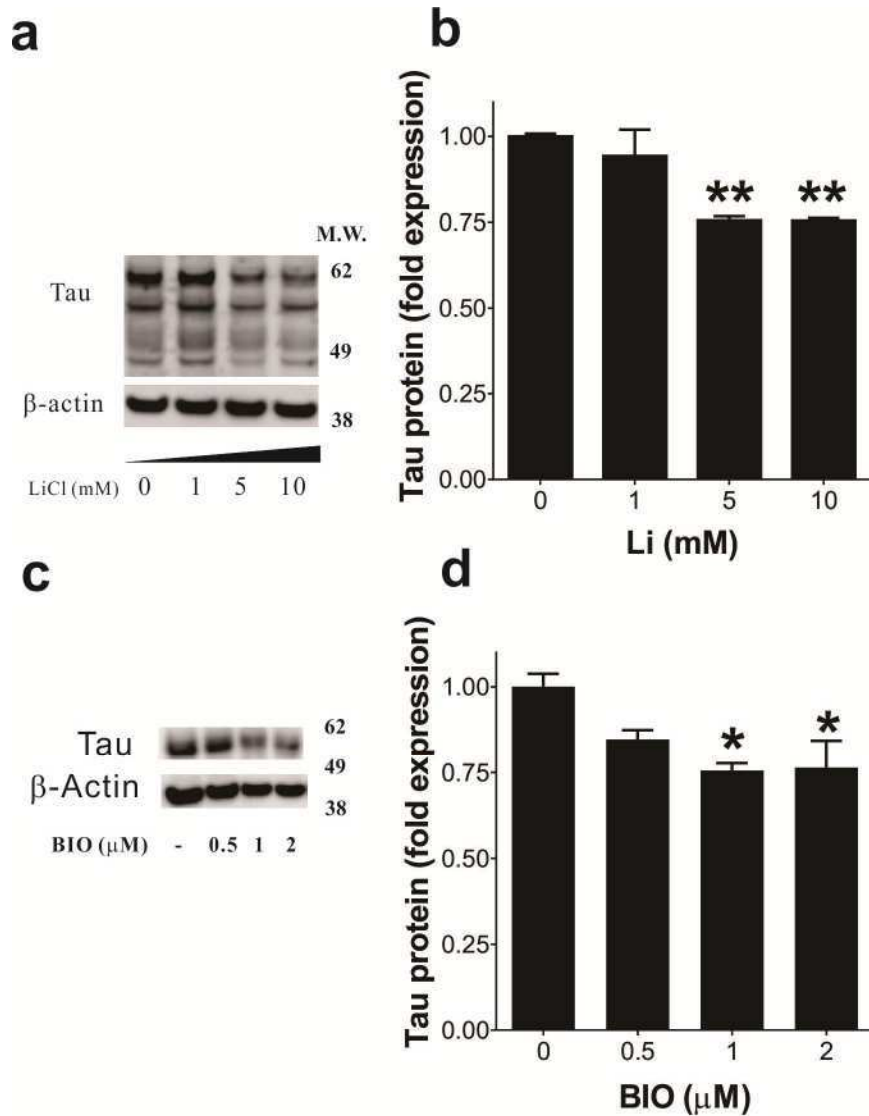
**Supplementary Figure 7.** Li treatment selectively affects dopaminergic neurons in the SN. a) No significant reduction ( $p=0.821$ , two-tailed t-test) was found in TH-negative, Neutral Red-positive neurons in the SN of Li-treated mice.  $n=5$  per treatment group. b) Significant reductions were found in the striatal DOPAC levels ( $p=0.024$ , two-tailed t-test).  $n=12$  per treatment group. Means  $\pm$  SEM are shown. \*  $p<0.05$ .



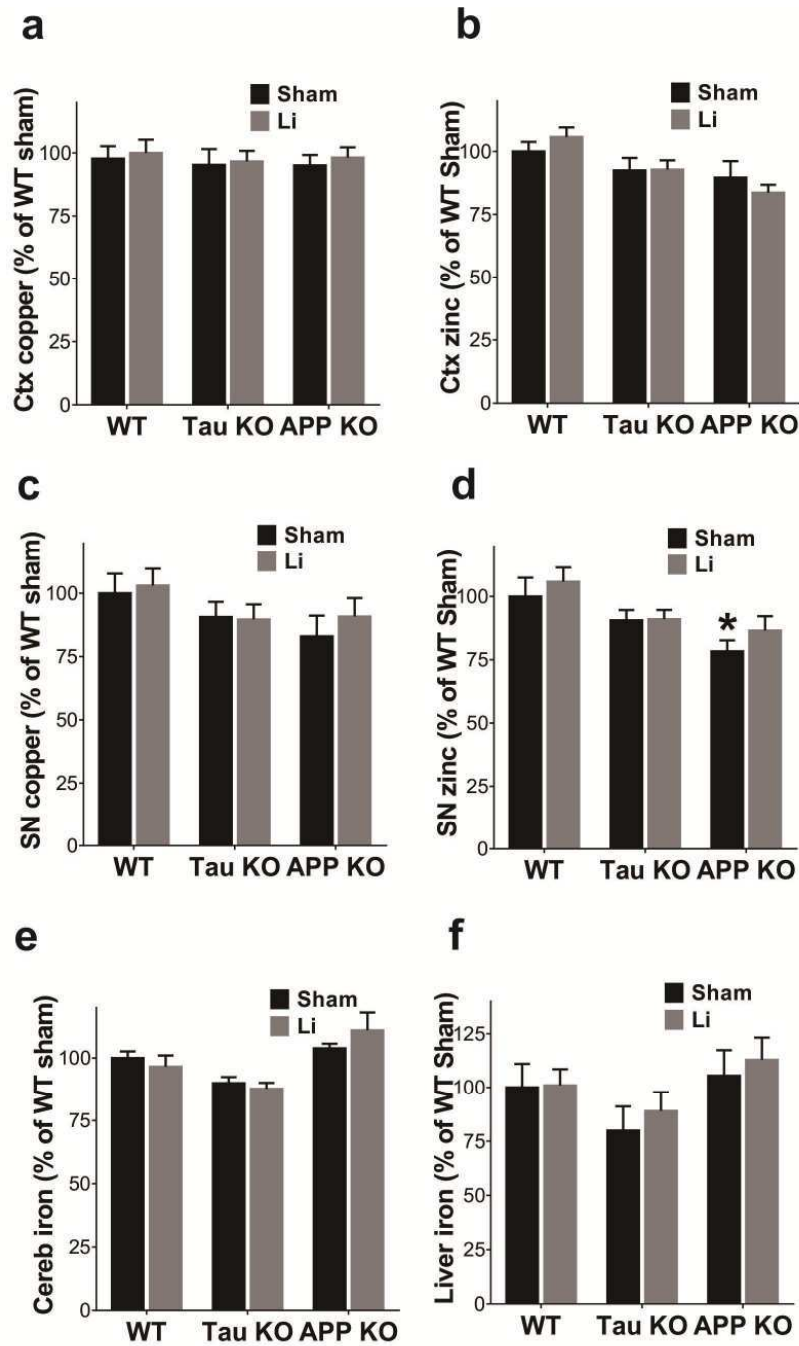
**Supplementary Figure 8.** Lithium treatment does not alter <sup>65</sup>Zn retention in primary neurons ( $p=0.176$  for 10mM Li, two-tailed t-test). Means  $\pm$  SEM are shown,  $n=4$  per group.



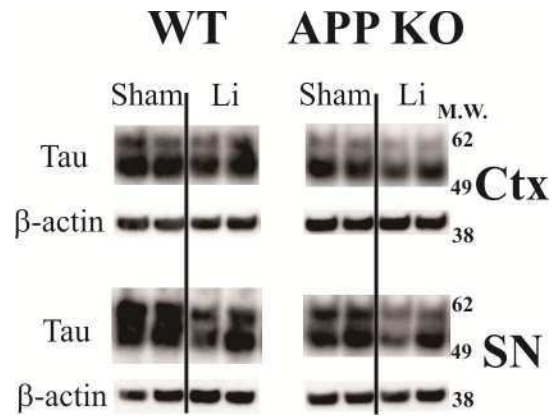
**Supplementary Figure 9.** Iron accumulation is induced GSK-3 inhibition. **a)** No <sup>59</sup>Fe retention was found after L690,330 ( $p=0.106$ , one-way ANOVA with post-hoc Dunnett's test) treatment. **b)** Significant <sup>59</sup>Fe retention was found after BIO treatment ( $p=0.002$ , one-way ANOVA with post-hoc Dunnett's test). Each experiment was independently repeated three times. Means  $\pm$  SEM are shown,  $n=4$  per group. \*  $p<0.05$ .



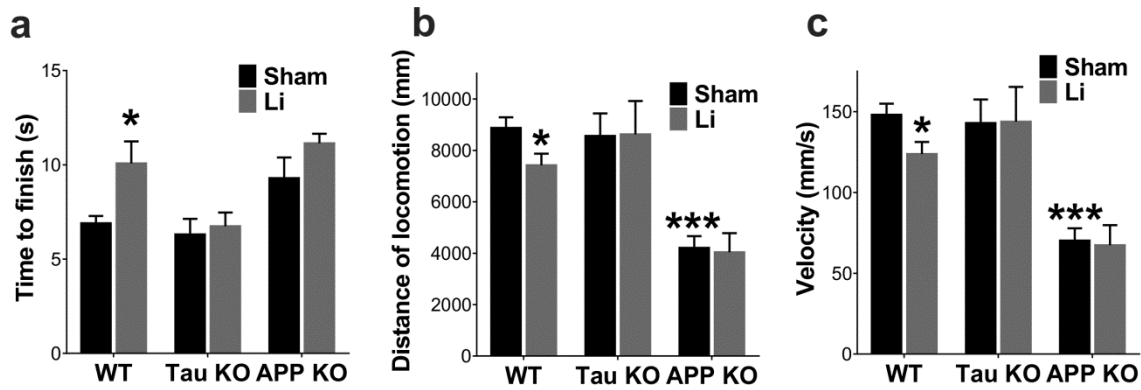
**Supplementary Figure 10.** Li and BIO (18h incubation) suppress tau protein levels. **a)** Representative tau western blots from lithium-treated SH-SY5Y cells. **b)** Quantification of **(a)** showed lowering of tau with 5 mM ( $p=0.003$ , one-way ANOVA with post-hoc Dunnett's test) or 10 mM ( $p=0.002$ ) Li treatment. **c)** Representative western blot for tau from BIO-treated primary neurons. **d)** Quantification of **(c)** showed that BIO lowered tau levels ( $p=0.043$  for 1  $\mu$ M and  $p=0.048$  for 2  $\mu$ M, one-way ANOVA with post-hoc Dunnett's test). Means  $\pm$  SEM are shown,  $n=4$  per group, and the experiments were independently repeated three times. \*  $p<0.05$ , \*\*  $p<0.01$ .



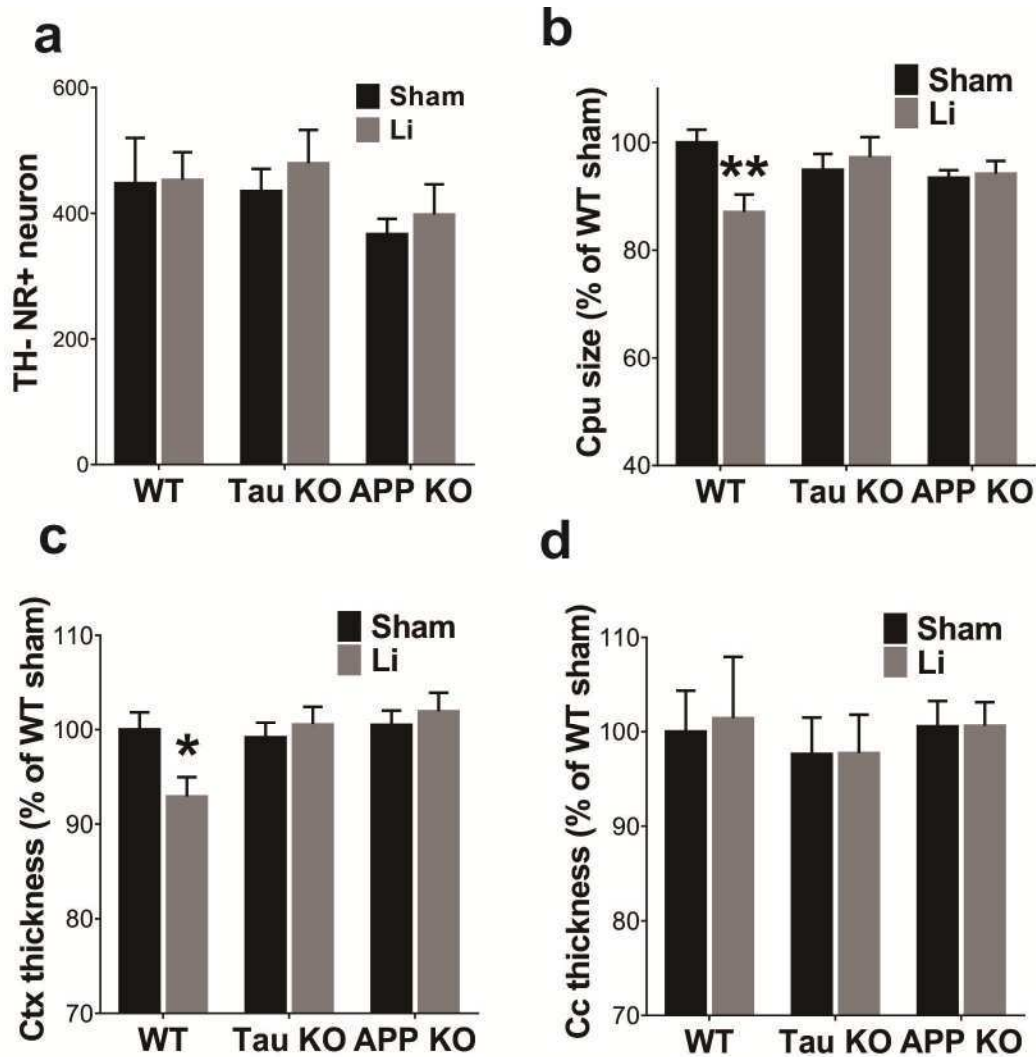
**Supplementary Figure 11.** No changes in (a) cortex copper, (b) cortex zinc, (c) SN copper, (d) SN zinc, (e) cerebellum iron, or (f) liver iron levels in tissues of Li-treated mice, regardless of genotype. Means  $\pm$  SEM are shown, n=9-11 per treatment group.



**Supplementary Figure 12.** Representative western blot images of cortex and SN tau protein in wild type and APP knockout mice.



**Supplementary Figure 13.** Additional motor deficits in wild type mice induced by Li treatment. **a-c)** Li-treated wild type mice required longer time to finish [**a**; two-way ANOVA with post-hoc Dunnett's test: genotype ( $p=0.014$ ), and treatment ( $p<0.001$ ) effects but no interaction ( $p=0.283$ )] in the Pole test, showed reduced distance of locomotion [**b**; genotype ( $p<0.001$ ), but no treatment ( $p=0.368$ ) effects nor interaction ( $p=0.501$ )], and reduced velocity [**c**; genotype ( $p<0.001$ ), but no treatment ( $p=0.361$ ) effects nor interaction ( $p=0.500$ )] in the Open field test, but loss of tau or APP protected against Li-induced motor impairment. APP knockout mice showed motor deficits themselves, evidenced by reduced distance of locomotion ( $p<0.001$ ), and reduced velocity ( $p<0.001$ ). Li treatment did not further worsen the phenotype. Means  $\pm$  SEM are shown,  $n=9-11$  per treatment group. \*  $p<0.05$ , \*\*\*  $p<0.001$ .



**Supplementary Figure 14.** Other neuroanatomical changes induced by Li. **a)** No significant reduction was found in TH-negative, Neutral Red-positive neurons in the SN of Li-treated mice. **b-c)** Li treatment reduced CPu size [**b**; two-way ANOVA with post-hoc Dunnett's test: no genotype ( $p=0.606$ ) or treatment ( $p=0.153$ ) effects, but interaction ( $p=0.017$ )] and cortical thickness [**c**; genotype ( $p=0.041$ ) and interaction ( $p=0.043$ ) effects, but no treatment ( $p=0.371$ ) effect] in wild type mice, but the effect was abolished by loss of tau or APP. **d)** No change in corpus callosum thickness was detected in all three mouse strains. Means  $\pm$  SEM are shown,  $n=9-11$  per treatment group. \*  $p<0.05$ , \*\*  $p<0.01$ .



## Reference

1. Gómez-Sintes R, Lucas JJ. NFAT/Fas signaling mediates the neuronal apoptosis and motor side effects of GSK-3 inhibition in a mouse model of lithium therapy. *J Clin Invest* 2010; **120**(7): 2432-2445.