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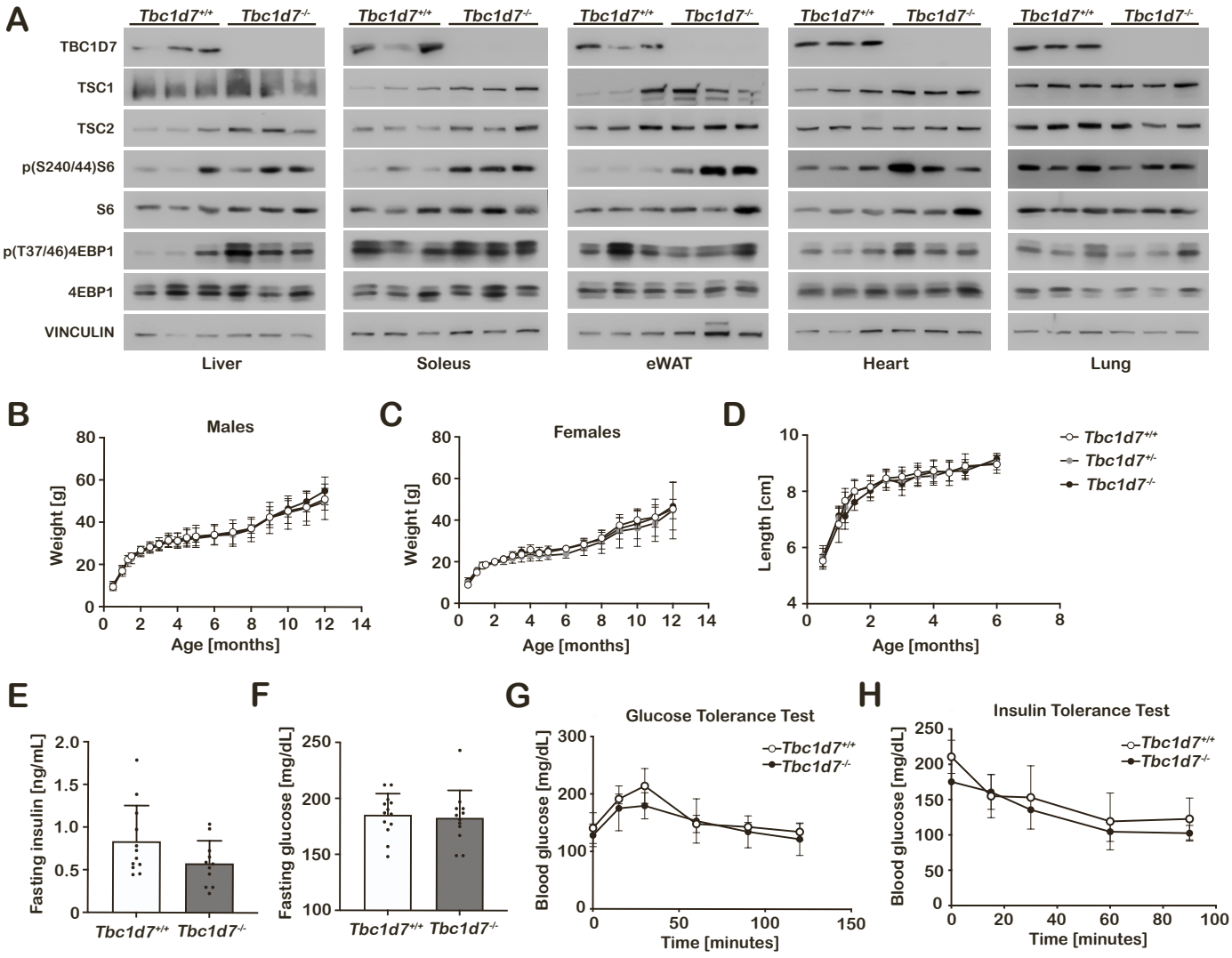
Supplemental information

The non-essential TSC complex component TBC1D7 restricts tissue mTORC1 signaling and brain and neuron growth

Sandra Schrötter, Christopher J. Yuskaitis, Michael R. MacArthur, Sarah J. Mitchell, Aaron M. Hosios, Maria Osipovich, Margaret E. Torrence, James R. Mitchell, Gerta Hoxhaj, Mustafa Sahin, and Brendan D. Manning

supplemental FIGURE 1



Supplemental Figure 1: Related to Figure 1.

(A) Loss of TBC1D7 protein in tissue lysates from *Tbc1d7*$^{-/-}$ mice, with a tissue-specific increase in phospho-S6 and phospho-4EBP1. Sample data from 3 mice of each genotype at 6 weeks of age following a 6-hr daytime fast are shown.

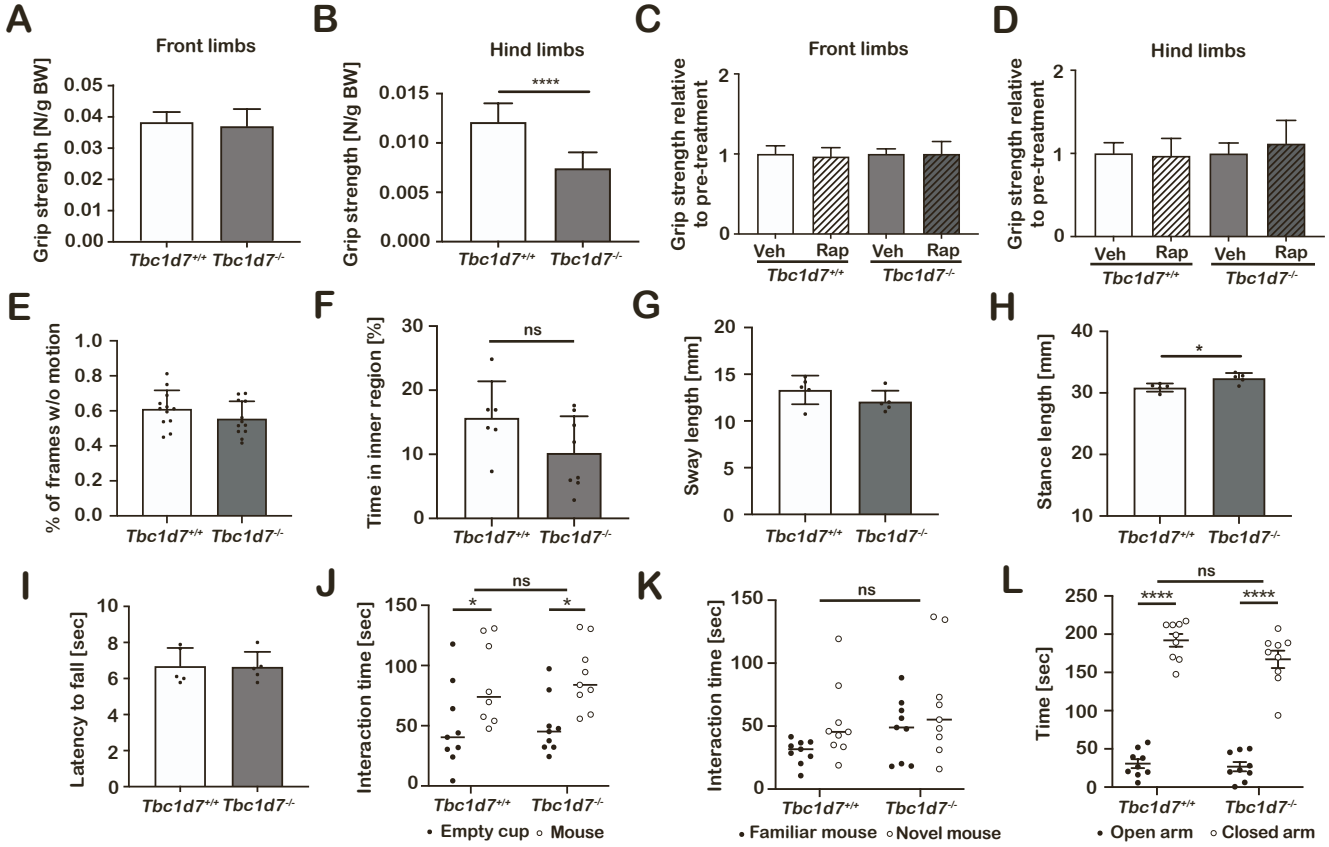
(B,C) Weights from a cohort of littermate offspring, separated by gender, from crosses between *Tbc1d7*$^{+/-}$ mice over 13 months graphed as mean ± S.D. at each age. (B) N= 6 *Tbc1d7*$^{+/+}$, 10 *Tbc1d7*$^{+/-}$ and 4 *Tbc1d7*$^{-/-}$ male mice; (C) N= 7 *Tbc1d7*$^{+/+}$, 9 *Tbc1d7*$^{+/-}$ and 8 *Tbc1d7*$^{-/-}$ female mice. Age 0 is the weight at weaning (21 days).

(D) Length (snout to tail) of mice from a cohort of littermate offspring from crosses between *Tbc1d7*$^{+/-}$ mice over 6 months graphed as mean ± S.D. at each age. N= 13 *Tbc1d7*$^{+/+}$, 19 *Tbc1d7*$^{+/-}$ and 12 *Tbc1d7*$^{-/-}$ mice. Age 0 is the weight at weaning (21 days).

(E,F) Fasting insulin and glucose from 6 month-old mice graphed as mean ± S.D.. N= 12 *Tbc1d7*$^{+/+}$ and 13 *Tbc1d7*$^{-/-}$.

(G,H) Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) in 3 month-old mice graphed as mean ± S.D.. N= 4 *Tbc1d7*$^{+/+}$ and 3 *Tbc1d7*$^{-/-}$.

supplemental FIGURE 2



Supplemental Figure 2: Related to Figure 2.

(A-D) Grip strength measurements of front limbs (A) and hind limbs (B) in 3 month-old mice (Pre-treatment). Data is graphed as mean ± S.D. for 10 mice per genotype. Grip strength measurements of front limbs (C) and hind limbs (D) in 4 month-old mice after a month treatment with vehicle or rapamycin (1 mg/kg, MWF) treatment. Data are graphed as mean ± S.D. relative to pre-treatment strength. N=5 mice per genotype and treatment group. Statistical analysis by Student´s t-test, ****$p < 0.0001$.

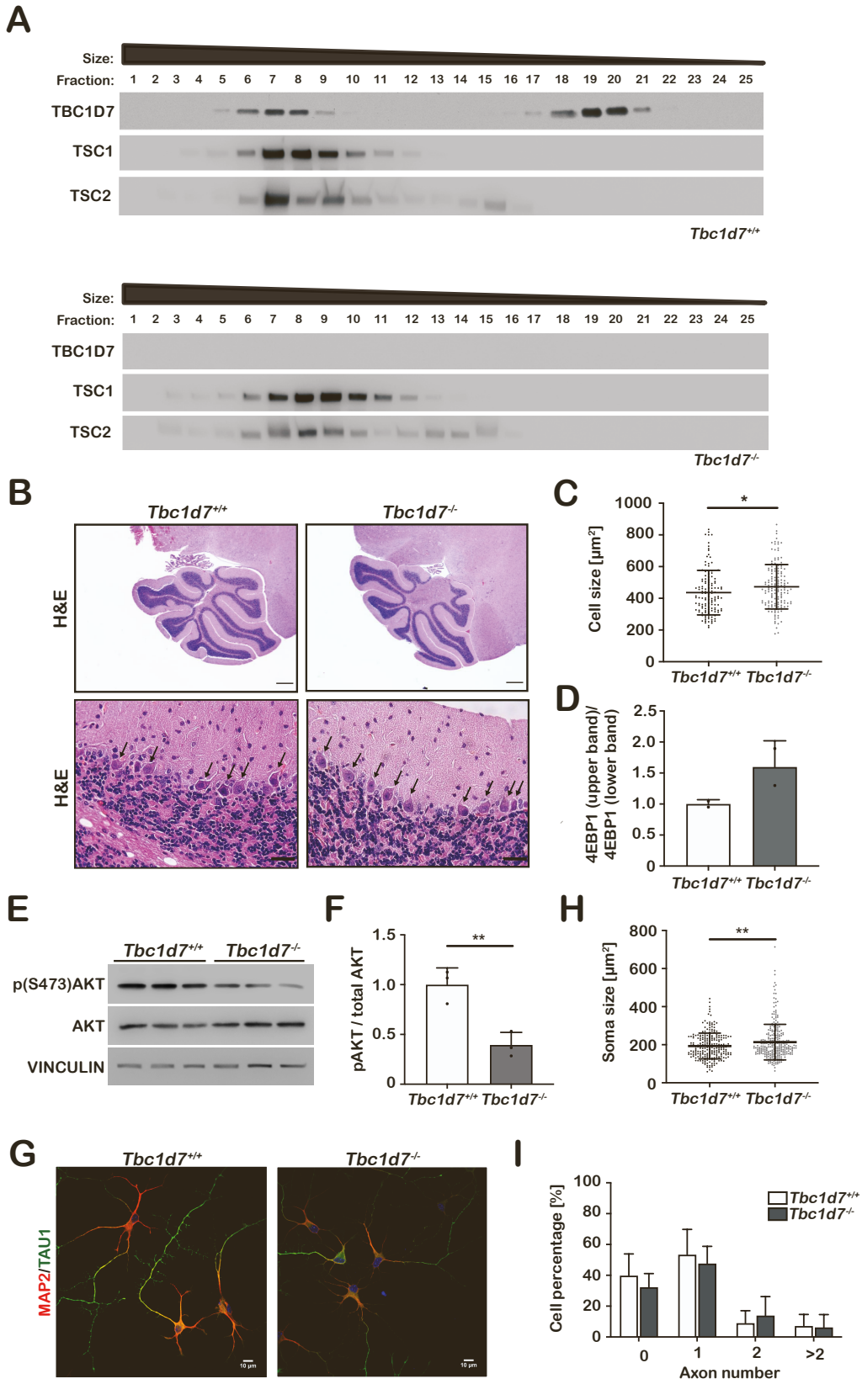
(E,F) Open field test of 6 month-old mice, with percent of time without motion (E) and time spent in the inner region (F), quantified for 12 mice of each genotype and graphed as mean ± S.D..

(G,H) Gait analysis of 6 month-old mice with (G) sway length and (H) stance length quantified for 5 mice of each genotype and graphed as mean ± S.D.. Statistical analysis by Student´s t-test, *$p < 0.05$.

(I) Motor coordination test on an accelerating rotarod for 6 month-old mice. Data are from 9 mice of each genotype, graphed as mean latency to fall over 5 trials per mouse ± S.E.M..

(J-L) Three-chamber test (J, K) and elevated plus-maze test (L) performed on 6 month-old mice for 9 mice of each genotype and graphed as mean ± S.D.. Statistical analysis by two-way ANOVA, *$p < 0.05$, ****$p < 0.0001$.

supplemental FIGURE 3



Supplemental Figure 3: Related to Figure 4.

(A) Immunoblots of size exclusion chromatography fractions of whole brain lysates from mice aged 1 year.

(B,C) Histological analysis of the cerebellum in sagittal sections of brains from 6 week-old mice stained for H&E. (C) The soma area of Purkinje neurons (arrows in B) cell size (C) was measured in sections from 5 mice of each genotype and graphed as mean ± S.D.. Scale bars = 500 µm (top) and, 50 µm (bottom). N= 131 *Tbc1d7*$^{+/+}$ and 148 *Tbc1d7*$^{-/-}$ cells.

(D) Quantification of 4E-BP1 upper to lower band ratio from immunoblot in figure 4D, graphed as mean ± S.D.. N=2.

(E,F) Immunoblot of whole brain lysates from 3 mice of each genotype at 6 weeks of age following a 6-hr daytime fast are shown in (E) and quantified as the mean ratio of P-Akt to total Akt ± S.D. (F).

(G-I) Immunofluorescence imaging of primary hippocampal neurons at DIV4 (G), with soma area (H) and axon number (I) measured and graphed as mean ± S.D. for 2 technical replicates in each of 3 independent experiments. N= 238 *Tbc1d7*$^{+/+}$ and 279 *Tbc1d7*$^{-/-}$ cells.

Statistical analysis (C,F,H) by Student´s t-test, *$p < 0.05$, **$p < 0.01$.