Cell Reports, Volume *25*

Supplemental Information

Sensitive Periods for Cerebellar-Mediated Autistic-like Behaviors

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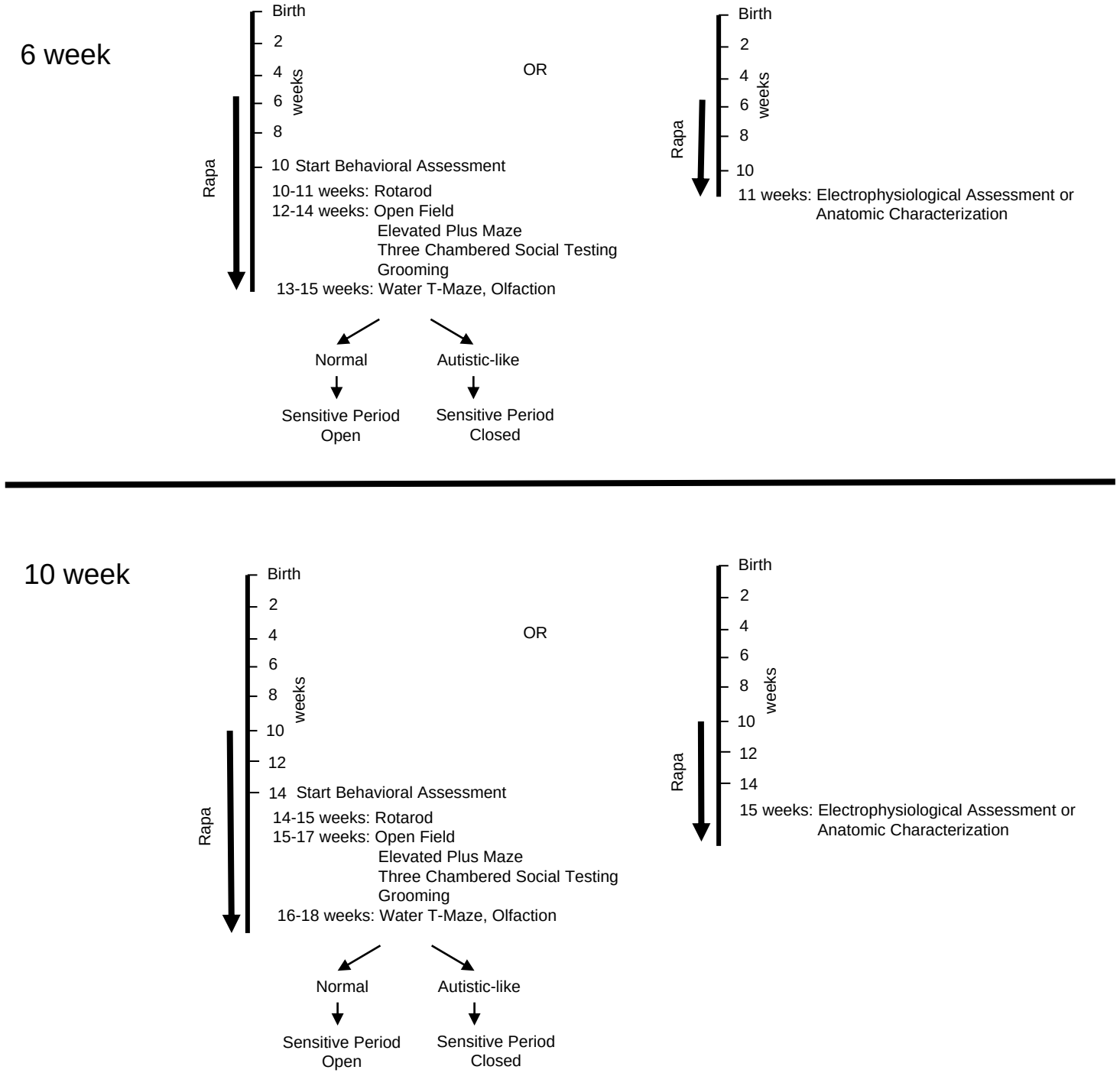
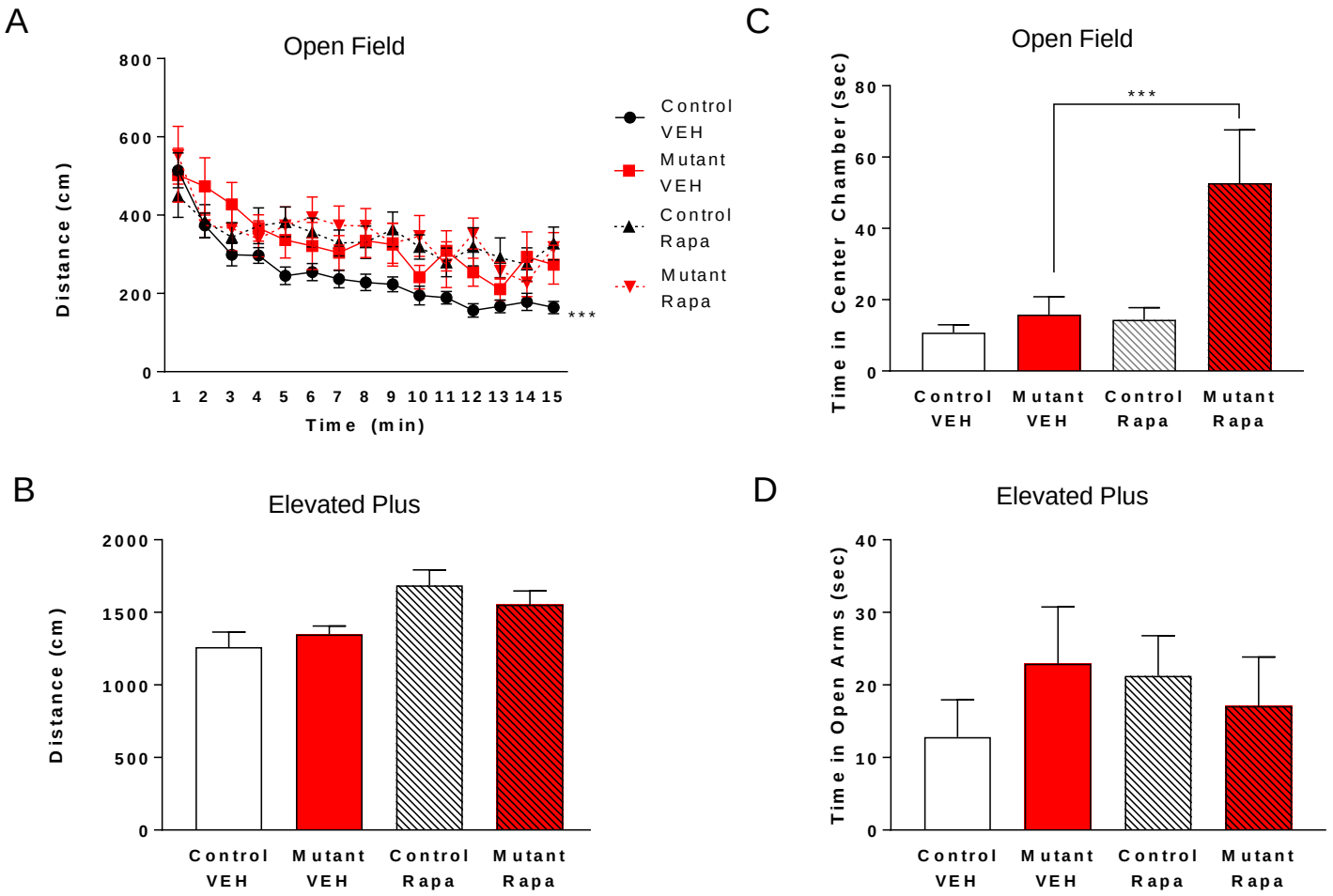
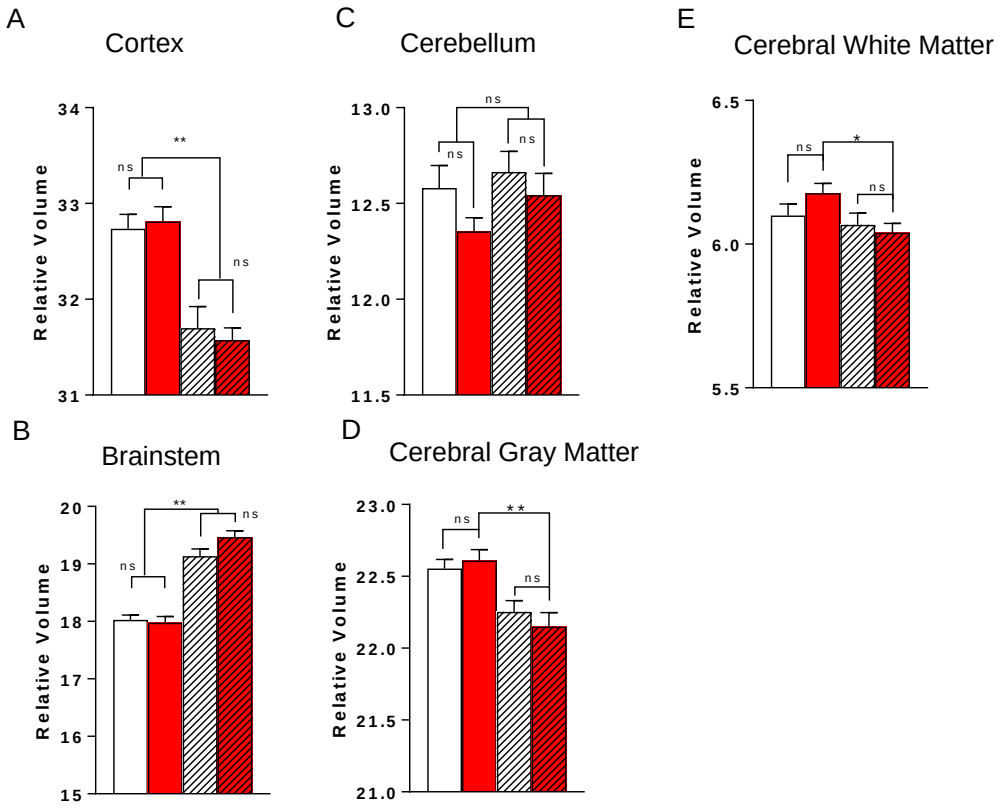


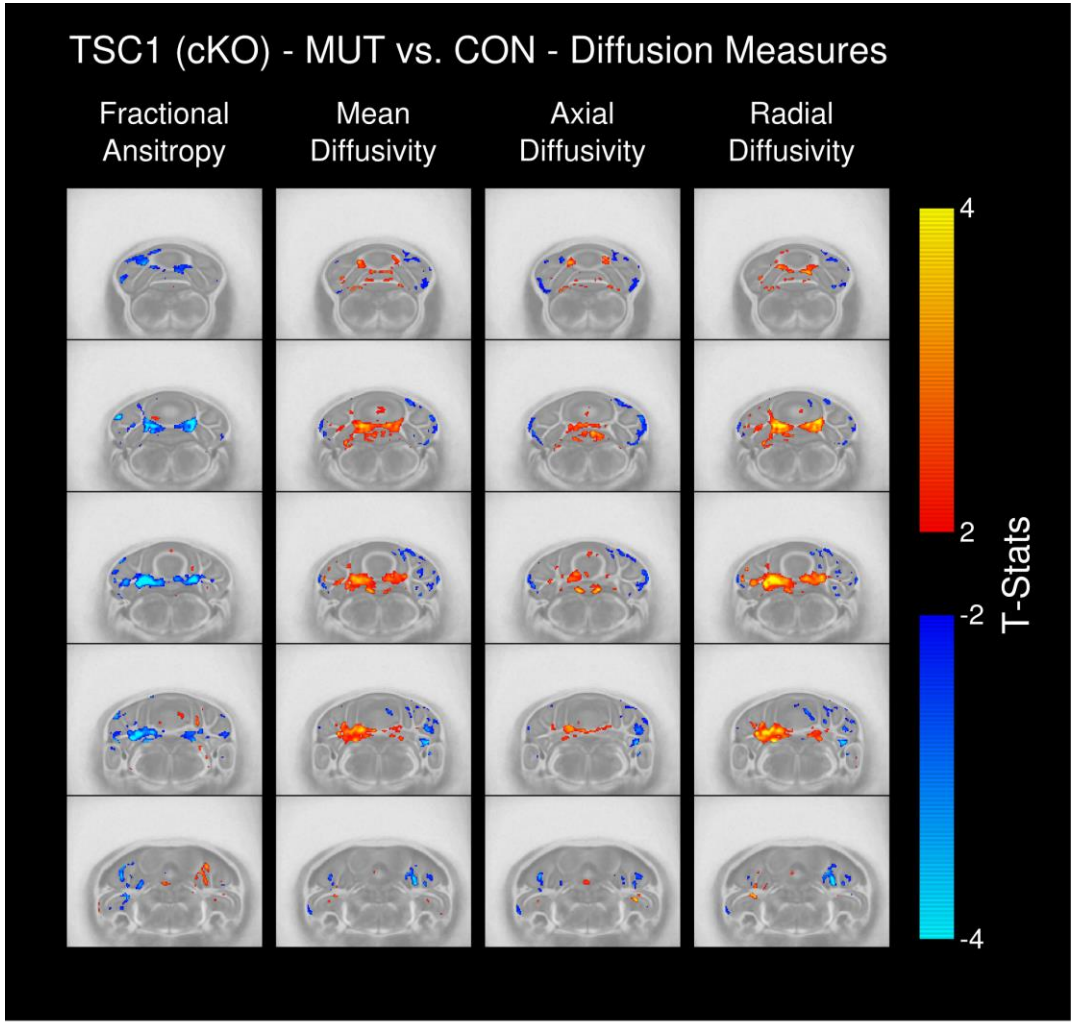
Supplementary Figure 1. Schematic of treatment paradigms and timeline of assessments. Related to Figures 1-5. 6 week cohort (above) and 10 week cohort (below). Arrow indicates timing of Rapamycin (Rapa) initiation and treatment.



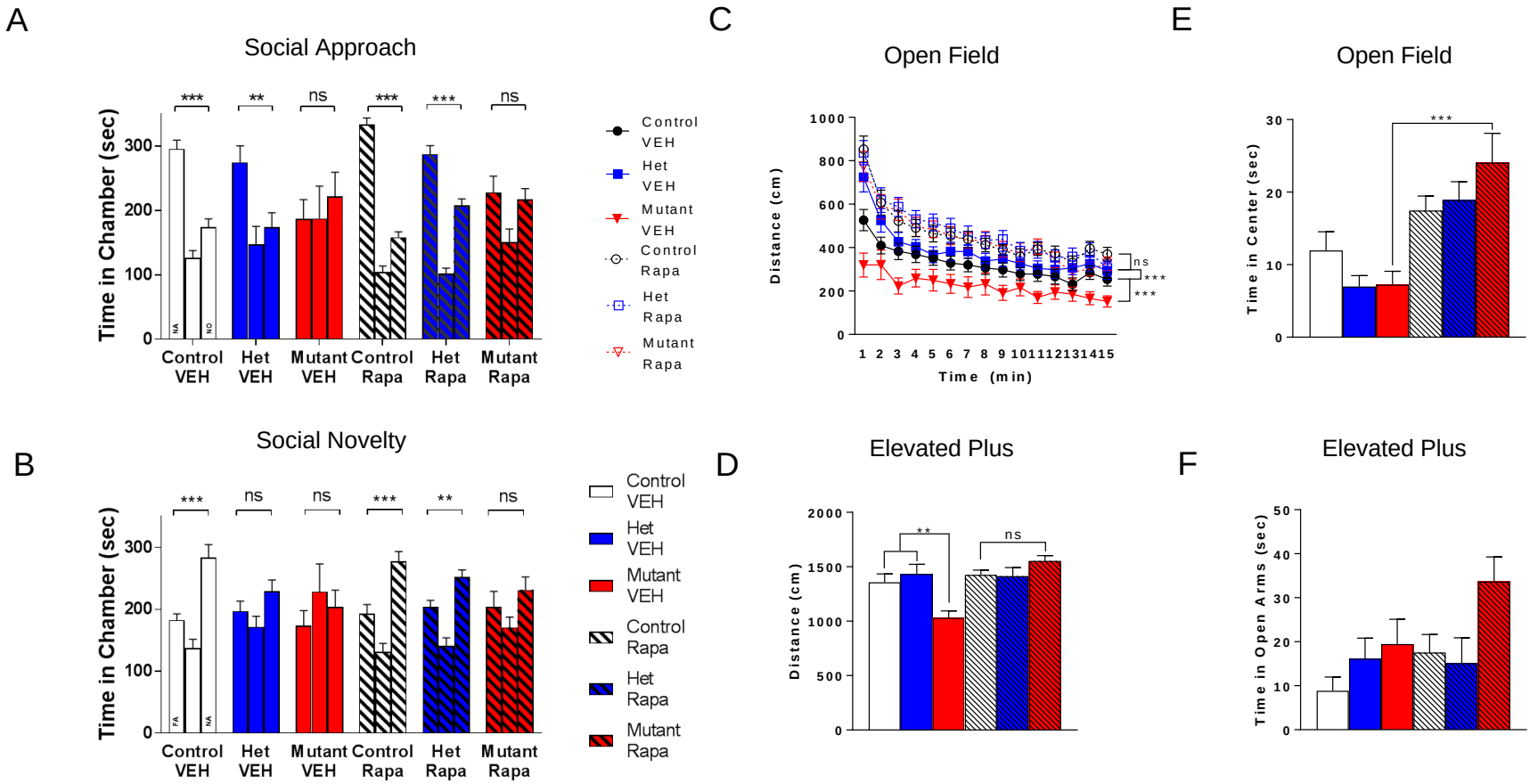
Supplementary Figure 2. Locomotor function and anxiety behaviors in PC *Tsc1* mutants with rapamycin treatment initiated at 6 weeks, related to Figure 1. No change in locomotor behaviors in open field in any group tested in either **A.** open field or **B.** elevated plus maze testing. No significant differences in anxiety behaviors between control and PC *Tsc1* mutants in either **C.** open field or **D.** elevated plus maze testing. Rapamycin induces increase time spent in center of open field in PC mutant mice, a change that is not reflected in amount of open arm time spent in elevated plus maze. One or two way anova, Bonferroni's post hoc testing. ***, $p < 0.001$. All comparison not significant unless otherwise specified. VEH vehicle, Rapa rapamycin. Data are reported as mean ± SEM.



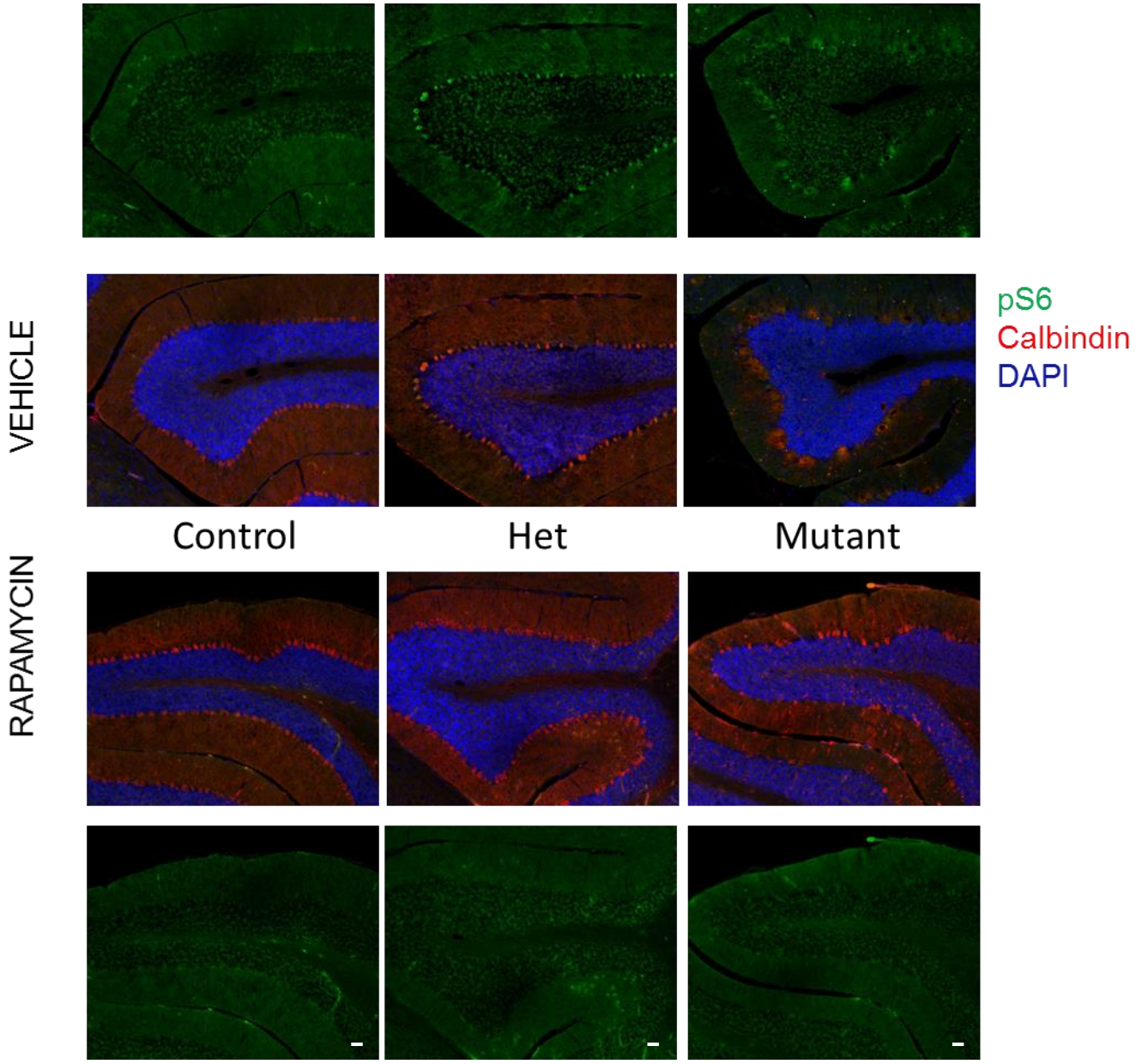
Supplementary Figure 3. Brain volumes of multiple brain regions altered with rapamycin treatment (related to Figure 3). Voxelwise analysis of relative volumes of **A.** Cortex, **B.** brainstem, **C.** Cerebellum, and Cerebral **D.** Gray and **E.** White matter. *, FDR <0.05; **, FDR<0.001; ns, FDR>0.1. VEH: vehicle; Rapa: rapamycin. CON: control; MUT: mutant; VEH: vehicle; RAPA: rapamycin. Data are reported as mean ± SEM.



Supplementary Figure 4. Diffusion studies in PC *Tsc1* mice (cKO) with treatment initiated at 6 weeks, related to Figure 3.
Voxelwise differences in Fractional Anisotropy, Mean Diffusivity, Axial Diffusivity, and Radial Diffusivity between control (CON) and mutant (MUT) mice with vehicle (left) and rapamycin (right) treatment.



Supplementary Figure 5. 10 week Rapamycin Cohort Behaviors (related to Figure 4). Rapamycin initiated at 10 weeks does not rescue social behaviors in three chambered apparatus in both **A.** social approach and **B.** social novelty testing in mutants However, in time spent in chambers, **B.** rapamycin appears to rescue social novelty deficits in hets. NA: novel animal, NO: novel object, FA: familiar animal. Reduced locomotion in 10 week vehicle treated mutant mice in **C.** open field and **D.** elevated plus maze testing. No significant difference in anxiety behaviors between control and PC *Tsc1* mutants in either **E.** open field or **F.** elevated plus maze testing. However, rapamycin induces increased time spent in center of open field in PC mutant mice with trend towards increased time spent in open arm of elevated plus maze (p= 0.15). Two way ANOVA, Bonferroni's post hoc testing. **, p<0.01; ***, p <0.001. All comparisons not significant (ns) unless otherwise specified. Data are reported as mean ± SEM. VEH: vehicle, Rapa: rapamycin.



Supplementary Figure 6, related to Figure 4-5. Purkinje cell (PC) survival in homozygous PC *Tsc1* mutants (mutant) is not rescued by rapamycin treatment initiated at 10 weeks. Heterozygous (Het) mutants do not show reductions in PC numbers. Calbindin staining to label PCs (Red), pS6 (Green), with DAPI counterstain (Blue). pS6 staining reflects elevated mTOR signaling in vehicle treated Hets and mutants; elevated staining is not present with rapamycin treatment. pS6 staining is additionally shown separately for ease of view. Scale bar: 100μm.