

Supplemental Table 1: Results of statistical analyses for 9-11 week-old mice.

Main effects of two-way ANOVA tests across sexes for each behavioral assay are reported as P-values. P<0.05 was considered to be statistically significant; significant effects are bolded. Dash indicates that a pairwise comparison was unable to be completed; RM, repeated measures.

N	Test	Test Details	Post hoc Multiple Comparisons
Elevated Balance Beam			
N=21 Males=11 Females = 10	CN Males & Females Time to Cross (10mm): RM 2-way ANOVA	day x sex: F(3,57)=0.8883, P=0.4528 sex: F(1,19)=0.3849, P=0.5424 day: F(2.592,49.24)=0.8088, P=0.4794	Šídák's, Male vs. Female Day 1: P=0.5957 Day 2: P=0.9999 Day 3: P=0.9954 Day 4: P=0.8159
N=20 Males=10 Females = 10	<i>Tsc1</i> ^{mut/wt} Males & Females Time to Cross (10mm): RM 2-way ANOVA	sex x day: F(3,54)=0.1186, P=0.9488 sex: F(1,18)=1.784, P=0.1983 day: F(2.078,37.41)=1.630, P=0.2089	Šídák's, Male vs. Female Day 1: P=0.8600 Day 2: P=0.9072 Day 3: P=0.5291 Day 4: P=0.7677
N=24 Males=13 Females = 11	<i>Tsc1</i> ^{mut/mut} Males & Females Time to Cross (10mm): RM 2-way ANOVA	sex x day: F(3,66)=1.882, P=0.1412 sex: F(1,22)=2.828, P=0.1068 day: F(2.175,47.86)=1.630, P=0.2089	Šídák's, Male vs. Female Day 1: P=0.1941 Day 2: P=0.1873 Day 3: P=0.8345 Day 4: P=0.9753
N=21 Males=11 Females = 10	CN Males & Females Time to Cross (8mm): RM 2-way ANOVA	sex x day: F(3,57)=0.08166, P=0.9697 sex: F(1,19)=4.365, P=0.0504 day: F(2.530,48.07)=0.1666, P=0.8912	Šídák's, Male vs. Female Day 1: P=0.6696 Day 2: P=0.1831 Day 3: P=0.7248 Day 4: P=0.6880
N=20 Males=10 Females = 10	<i>Tsc1</i> ^{mut/wt} Males & Females Time to Cross (8mm): RM 2-way ANOVA	sex x day: F(3,54)=0.028667, P=0.9940 sex: F(1,18)=1.016, P=0.3269 day: F(2.805,50.49)=1.372, P=0.2627	Šídák's, Male vs. Female Day 1: P=0.4348 Day 2: P=0.9786 Day 3: P=0.7831 Day 4: P=0.9490
N=24 Males=13 Females = 11	<i>Tsc1</i> ^{mut/mut} Males & Females Time to Cross (8mm): RM 2-way ANOVA	sex x day: F(3,66)=1.073, P=0.3667 sex: F(1,22)=0.04588, P=0.8324 day: F(1.938,42.63)=1.330, P=0.2747	Šídák's, Male vs. Female Day 1: P=0.9623 Day 2: P=0.9119 Day 3: P=0.9853 Day 4: P=0.9845
N=21 Males=11 Females = 10	CN Males & Females Foot-slips (10mm): RM 2-way ANOVA	sex x day: F(3,57)=2.184, P=0.0986 sex: F(1,19)=0.3501, P=0.5610 day: F(2.029,38.55)=0.9767, P=0.3867	Šídák's, Male vs. Female Day 1: P=0.8113 Day 2: P>0.9999 Day 3: P=0.5205 Day 4: --

N=20 Males=10 Females = 10	<i>Tsc1</i> ^{mut/wt} Males & Females Foot-slips (10mm): RM 2-way ANOVA	sex x day: F(3,54)=0.8319, P=0.4822 sex: F(1,18)=1.528, P=0.2323 day: F(2,201,39.62)=0.8319, P=0.4527	Šídák's, Male vs. Female Day 1: P>0.9999 Day 2: P=0.5205 Day 3: -- Day 4: P=0.8142
N=24 Males=13 Females = 11	<i>Tsc1</i> ^{mut/mut} Males & Females Foot-slips (10mm): RM 2-way ANOVA	sex x day: F(3,66)=0.3565, P=0.7846 sex: F(1,22)=0.1423, P=0.7056 day: F(2,591,57)=2.834, P=0.0536	Šídák's, Male vs. Female Day 1: P>0.9999 Day 2: P=0.9607 Day 3: P=0.9998 Day 4: P=0.9061
N=21 Males=11 Females = 10	CN Males & Females Foot-slips (8mm): RM 2-way ANOVA	sex x day: F(3,57)=1.182, P=0.3248 sex: F(1,19)=0.3073, P=0.5858 day: F(2,557,48.59)=0.9318, P=0.4204	Šídák's, Male vs. Female Day 1: P=0.5953 Day 2: P=0.7766 Day 3: P=0.9931 Day 4: P=0.6969
N=20 Males=10 Females = 10	<i>Tsc1</i> ^{mut/wt} Males & Females Foot-slips (8mm): RM 2-way ANOVA	sex x day: F(3,54)=0.9649, P=0.4160 sex: F(1,18)=1.477, P=0.2400 day: F(2,6,46.8)=1.076, P=0.3621	Šídák's, Male vs. Female Day 1: P=0.9981 Day 2: P=0.9960 Day 3: P=0.1967 Day 4: P=0.9090
N=24 Males=13 Females = 11	<i>Tsc1</i> ^{mut/mut} Males & Females Foot-slips (8mm): RM 2-way ANOVA	sex x day: F(3,66)=1.160, P=0.3318 sex: F(1,22)=0.02369, P=0.8791 day: F(2,539,55.86)=5.157, P=0.0051	Šídák's, Male vs. Female Day 1: P=0.9985 Day 2: P=0.8292 Day 3: P=0.9969 Day 4: P=0.8586
Social Interaction			
N=21 Males=11 Females = 10	CN Males & Females Direct Contacts (Approach): RM 2-way ANOVA	sex x preference: F(1,19)=0.08960, P=0.7679 sex: F(1,19)=0.04783, P=0.8292 preference: F(1,19)=23.86, P=0.0001	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.0071 Female: P=0.0040
N=20 Males=10 Females = 10	<i>Tsc1</i> ^{mut/wt} Males & Females Direct Contacts (Approach): RM 2-way ANOVA	sex x preference: F(1,18)=1.994, P=0.1750 sex: F(1,18)=0.1828, P=0.6741 preference: F(1,18)=16.01, P=0.0008	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.1605 Female: P=0.0025
N=24 Males=13 Females = 11	<i>Tsc1</i> ^{mut/mut} Males & Females Direct Contacts (Approach): RM 2-way ANOVA	sex x preference: F(1,22)=1.639, P=0.6895 sex: F(1,22)=1.694, P=0.2065 preference: F(1,22)=19.11, P=0.002	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.1627 Female: P=0.0845

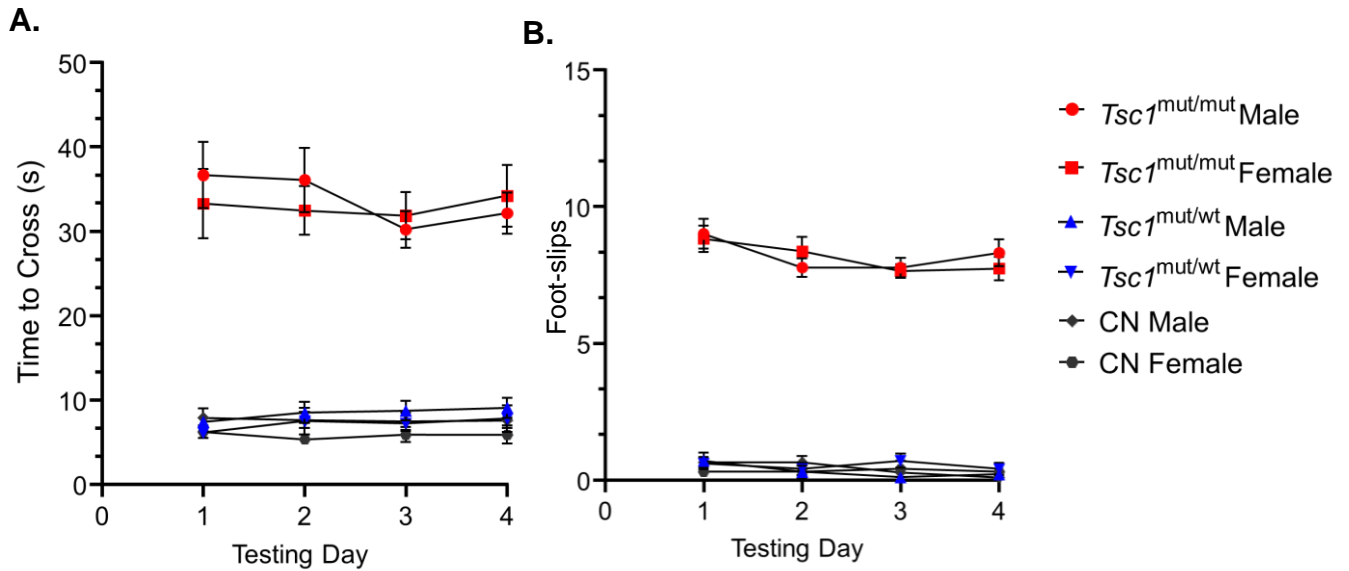
N=21 Males=11 Females = 10	CN Males & Females Direct Contacts (Novelty): RM 2-way ANOVA	sex x preference: F(1,19)=3.604, P=0.0729 sex: F(1,19)=0.009826, P=0.9222 preference: F(1,19)=32.77, P<0.0001	Šídák's, Familiar animal vs. unfamiliar animal Male: P<0.0001 Female: P=0.0318
N=20 Males=10 Females = 10	<i>Tsc1</i> ^{mut/wt} Males & Females Direct Contacts (Novelty): RM 2-way ANOVA	sex x preference: F(1,18)=17.61, P=0.0005 sex: F(1,18)=4.992, P=0.0384 preference: F(1,18)=70.45, P<0.0001	Šídák's, Familiar animal vs. unfamiliar animal Male: P<0.0001 Female: P=0.0164
N=24 Males=13 Females = 11	<i>Tsc1</i> ^{mut/mut} Males & Females Direct Contacts (Novelty): RM 2-way ANOVA	sex x preference: F(1,22)=0.6437, P=0.4310 sex: F(1,22)=5.774, P=0.0251 preference: F(1,22)=30.97, P<0.0001	Šídák's, Familiar animal vs. unfamiliar animal Males: P=0.0002 Females: P=0.0076
N=65 CN=11 Males, 10 Females <i>Tsc1</i> ^{mut/wt} = 10 Males, 10 Females <i>Tsc1</i> ^{mut/mut} = 13 Males, 11 Females	CN, <i>Tsc1</i> ^{mut/wt} , & <i>Tsc1</i> ^{mut/mut} , Males & Females Percentage of Investigation (Approach): 2-way ANOVA	sex x genotype: F(2,59)=0.3772, P=0.6874 genotype: F(2,59)=3.220, P=0.0471 sex: F(1,59)=1.250, P=0.2681	Šídák's, Genotype CN vs. <i>Tsc1</i> ^{mut/wt} : P=0.6523 CN vs. <i>Tsc1</i> ^{mut/mut} : P=0.0424 <i>Tsc1</i> ^{mut/wt} vs. <i>Tsc1</i> ^{mut/mut} : P=0.4173
N=65 CN=11 Males, 10 Females <i>Tsc1</i> ^{mut/wt} = 10 Males, 10 Females <i>Tsc1</i> ^{mut/mut} = 13 Males, 11 Females	CN, <i>Tsc1</i> ^{mut/wt} , & <i>Tsc1</i> ^{mut/mut} , Males & Females Percentage of Investigation (Novelty): 2-way ANOVA	sex x genotype: F(2,59)=2.922, P=0.0617 genotype: F(2,59)=1.239, P=0.2972 sex: F(1,59)=12.18, P=0.0009	Šídák's, Male vs. Female CN: P=0.0055 <i>Tsc1</i> ^{mut/wt} : P=0.0434 <i>Tsc1</i> ^{mut/mut} : P=0.9986
Repetitive Behavior			
N=43 CN=6 Males, 8 Females <i>Tsc1</i> ^{mut/wt} = 6 Males, 5 Females <i>Tsc1</i> ^{mut/mut} = 9 Males, 9 Females	CN, <i>Tsc1</i> ^{mut/wt} , & <i>Tsc1</i> ^{mut/mut} , Males & Females Total Time Grooming: 2-way ANOVA	sex x genotype: F(2,37)=2.414, P=0.1034 genotype: F(2,37)=0.2444, P=0.7844 sex: F(1,37)=4.682, P=0.0370	Šídák's, Male vs. Female CN: P=0.0500 <i>Tsc1</i> ^{mut/wt} : P=0.4180 <i>Tsc1</i> ^{mut/mut} : P=0.9752

Supplemental Table 2: Results of statistical analyses for 16-24 week-old mice.

Main effects of two-way ANOVA tests across sexes for each behavioral assay are reported as P-values. $P < 0.05$ was considered to be statistically significant; significant effects are bolded. Dash indicates that a pairwise comparison was unable to be completed; RM, repeated measures.

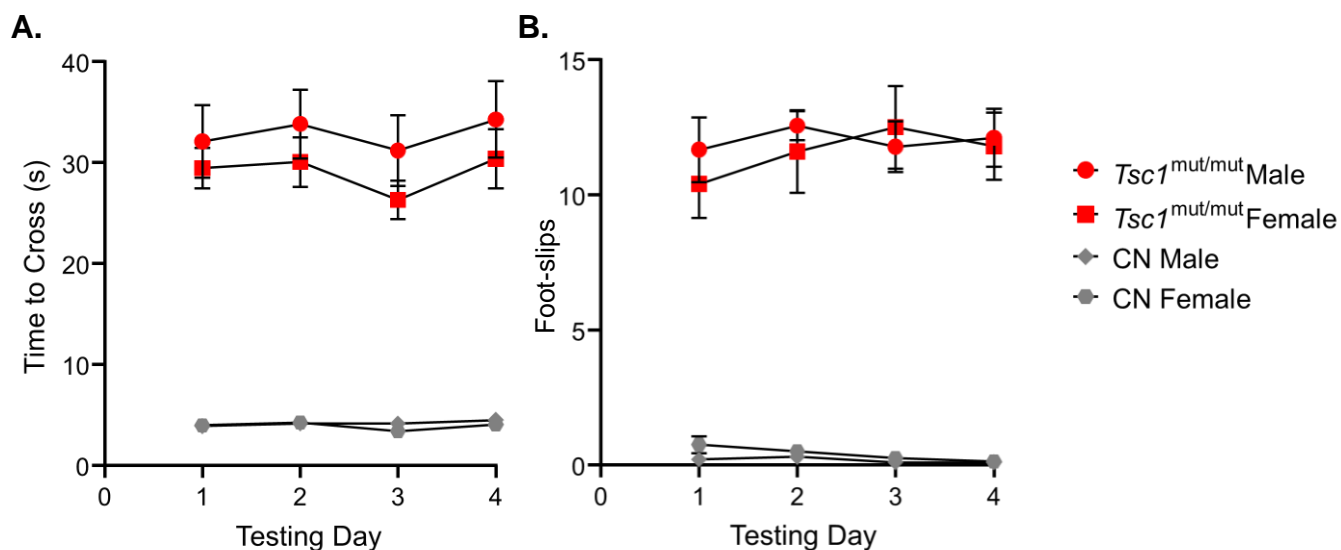
N	Test	Test Details	<i>Post hoc</i> Multiple Comparisons
Elevated Balance Beam			
N=18 Males=10 Females=8	CN Males & Females Time to Cross (10mm): RM 2-way ANOVA	sex x day: $F(3,48)=0.6286$, $P=0.6001$ sex: $F(1,16)=1.279$, $P=0.2930$ day: $F(2.703,43.25)=0.2806$, $P=0.6036$	Šídák's, Male vs. Female Day 1: $P=0.7256$ Day 2: $P=0.9976$ Day 3: $P=0.9871$ Day 4: $P=0.9992$
N=19 Males=9 Males=10	<i>Tsc1</i> ^{mut/mut} Males & Females Time to Cross (10mm): RM 2-way ANOVA	sex x day: $F(3,51)=0.7325$, $P=0.5374$ sex: $F(1,17)=2.595$, $P=0.12556$ day: $F(2.896,49.24)=1.746$, $P=0.1714$	Šídák's, Male vs. Female Day 1: $P=0.3835$ Day 2: $P=0.9159$ Day 3: $P=0.4280$ Day 4: $P=0.6606$
N=18 Males=10 Females=8	CN Males & Females Time to Cross (8mm): RM 2-way ANOVA	sex x day: $F(3,48)=0.77699$, $P=0.5126$ sex: $F(1,16)=0.2006$, $P=0.6602$ day: $F(2.719,43.5)=0.9122$, $P=0.4350$	Šídák's, Male vs. Female Day 1: $P=0.9998$ Day 2: $P>0.9999$ Day 3: $P=0.6919$ Day 4: $P=0.9515$
N=19 Males=9 Males=10	<i>Tsc1</i> ^{mut/mut} Males & Females Time to Cross (8mm): RM 2-way ANOVA	sex x day: $F(3,51)=0.1847$, $P=0.9063$ sex: $F(1,17)=1.010$, $P=0.3290$ day: $F(2.012,34.21)=2.271$, $P=0.1183$	Šídák's, Male vs. Female Day 1: $P=0.9525$ Day 2: $P=0.8574$ Day 3: $P=0.6724$ Day 4: $P=0.8931$
N=18 Males=10 Females=8	CN Males & Females Foot-slips (10mm): RM 2-way ANOVA	sex x day: $F(3,48)=1.183$, $P=0.3260$ sex: $F(1,16)=0.1616$, $P=0.6930$ day: $F(2.395,38.31)=0.3188$, $P=0.7668$	Šídák's, Male vs. Female Day 1: $P=0.8222$ Day 2: $P=0.8142$ Day 3: $P=0.8142$ Day 4: --
N=19 Males=9 Males=10	<i>Tsc1</i> ^{mut/mut} Males & Females Foot-slips (10mm): RM 2-way ANOVA	sex x day: $F(3,51)=1.904$, $P=0.1406$ sex: $F(1,17)=0.04234$, $P=0.8394$ day: $F(2.526,42.94)=1.1055$, $P=0.3510$	Šídák's, Male vs. Female Day 1: $P=0.9978$ Day 2: $P=0.9772$ Day 3: $P=0.8486$ Day 4: $P=0.8643$
N=18 Males=10 Females=8	CN Males & Females Foot-slips (8mm): RM 2-way ANOVA	sex x day: $F(3,48)=0.8063$, $P=0.4966$ sex: $F(1,16)=2.538$, $P=0.1307$ day: $F(2.618,41.89)=1.936$, $P=0.1454$	Šídák's, Male vs. Female Day 1: $P=0.5121$ Day 2: $P=0.9507$ Day 3: $P=0.9081$ Day 4: $P=0.9998$

N=19 Males=9 Males=10	<i>Tsc1</i> ^{mut/mut} Males & Females Foot-slips (8mm): RM 2-way ANOVA	sex x day: F(3,51)=0.9218, P=0.4370 sex: F(1,17)=0.08584, P=0.7731 day: F(2.193,37.29)=1.280, P=0.2918	Šídák's, Male vs. Female Day 1: P=0.9249 Day 2: P=0.9642 Day 3: P=0.9911 Day 4: P=0.9995
Social Interaction			
N=14 Males=8 Females=6	CN Males & Females Direct Contacts (Approach): RM 2-way ANOVA	sex x preference: F(1,12)=1.323, P=0.2725 sex: F(1,12)=2.937, P=0.1123 preference: F(1,12)=35.16, P<0.0001	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.0003 Female: P=0.0163
N=18 Males=9 Males=9	<i>Tsc1</i> ^{mut/mut} Males & Females Direct Contacts (Approach): RM 2-way ANOVA	sex x preference: F(1,16)=8.096, P=0.0117 sex: F(1,16)=1.729, P=0.2071 preference: F(1,16)=58.68, P<0.0001	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.5288 Female: P=0.0025
N=14 Males=8 Females=6	CN Males & Females Direct Contacts (Novelty): RM 2-way ANOVA	sex x preference: F(1,12)=0.2264, P=0.6427 sex: F(1,12)=3.252, P=0.0965 preference: F(1,12)=41.70, P<0.0001	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.0004 Female: P=0.0038
N=18 Males=9 Males=10	<i>Tsc1</i> ^{mut/mut} Males & Females Direct Contacts (Novelty): RM 2-way ANOVA	sex x preference: F(1,16)=8.096, P=0.0117 sex: F(1,16)=1.729, P=0.2071 preference: F(1,16)=7.556, P=0.0143	Šídák's, Empty cup vs. unfamiliar animal Male: P=0.1889 Female: P=0.0942
N=32 CN=8 Males, 6 Females <i>Tsc1</i> ^{mut/mut} =9 Males, 9 Females	CN & <i>Tsc1</i> ^{mut/mut} Males & Females Percentage of Investigation (Approach): 2-way ANOVA	sex x genotype: F(1,27)=5.713, P=0.0241 genotype: F(1,27)=6.065, P=0.0205 sex: F(1,27)=5.848, P=0.0226	Šídák's, Male vs. Female CN: P=0.9998 <i>Tsc1</i> ^{mut/mut} : P=0.0026
N=32 CN=8 Males, 6 Females <i>Tsc1</i> ^{mut/mut} =9 Males, 9 Females	CN & <i>Tsc1</i> ^{mut/mut} Males & Females Percentage of Investigation (Novelty): 2-way ANOVA	sex x genotype: F(1,27)=0.4497, P=0.5082 genotype: F(1,27)=12.92, P=0.0013 sex: F(1,27)=4.472, P=0.0438	Šídák's, Male vs. Female CN: P=0.5644 <i>Tsc1</i> ^{mut/mut} : P=0.0917



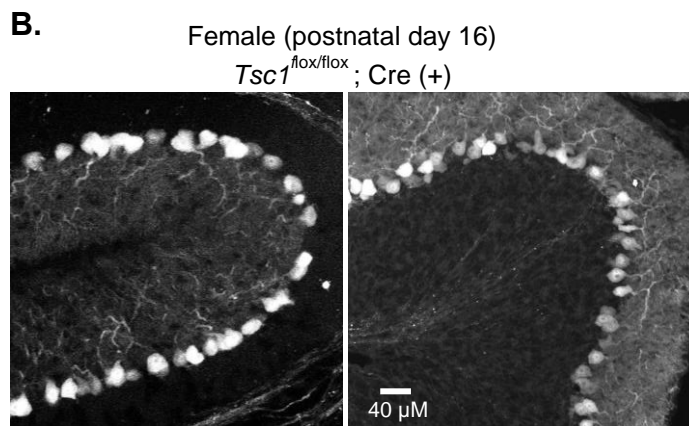
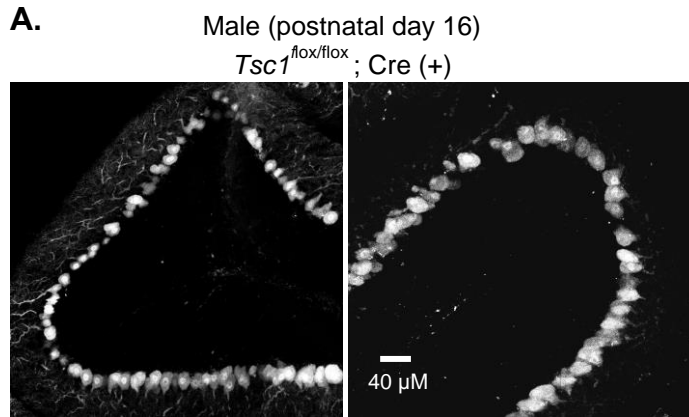
Supplemental Figure 1:

Motor coordination and balance testing of male and female 9–11 week-old *Tsc1*^{mut/mut} (N=13 males and N=11 females), *Tsc1*^{mut/wt} (N=10 males and N=10 females), and control (CN, N=11 males and N=10 females) mice were tested on an elevated balance beam 8mm in width. *Tsc1*^{mut/mut} mice performed significantly ($p < 0.0001$) worse than control animals on across all four testing days, but no difference in the time to cross (panel **A**) or number of hindlimb foot-slips (panel **B**) between sexes within each of the genotypes. Mean \pm SEM values are plotted.



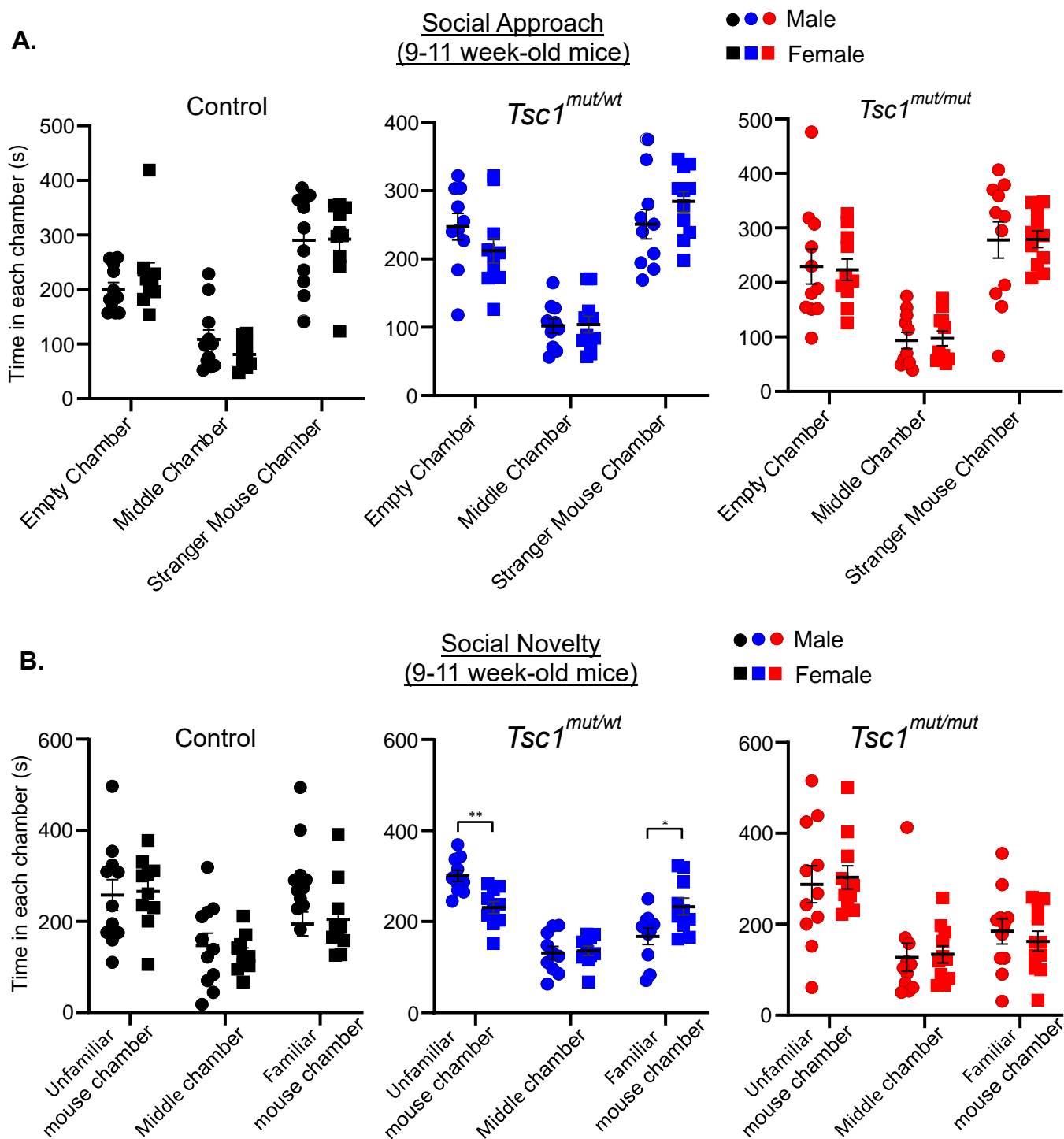
Supplemental Figure 2.

16-24 week-old *Tsc1*^{mut/mut} (N=9 males and N=10 females) and control (N=10 males and N=8 females) mice of both sexes were tested on an elevated balance beam 8mm in width. *Tsc1*^{mut/mut} mice performed significantly ($p < 0.0001$) worse than control animals on across all four testing days, and no difference in the time to cross (panel **A**) or number of hindlimb foot-slips (panel **B**) between sexes was measured for either genotype. Mean \pm SEM values are plotted.



Supplemental Figure 3.

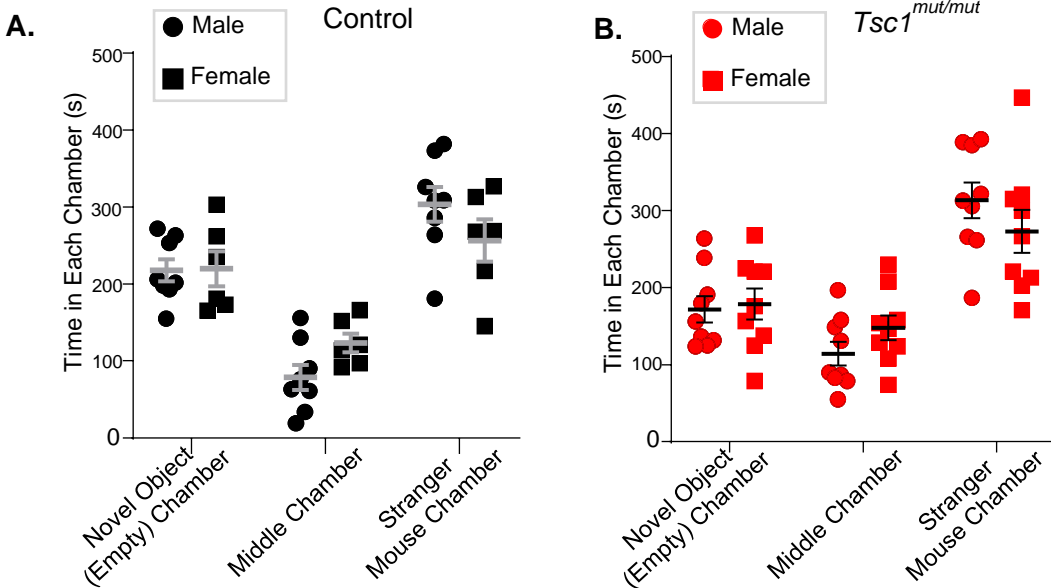
Using the Ai14 tdTomato Cre-reporter line (see Methods) tdTomato immunofluorescence was similarly expressed in the Purkinje neuron layer of parasagittal cerebellar sections taken from P16 male (panel **A.**) and P16 female (panel **B.**) *Tsc1^{flox/flox}*; Cre (+) animals.



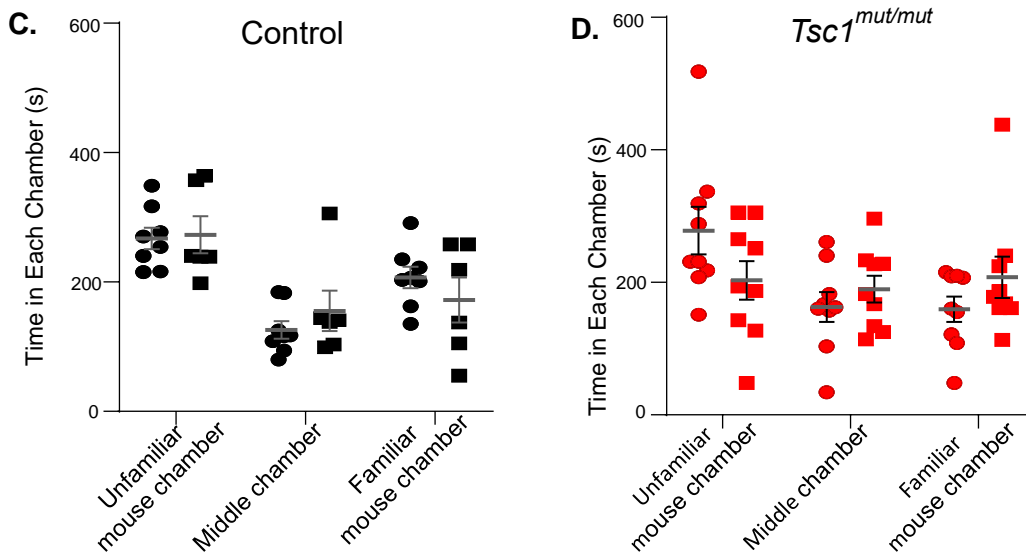
Supplemental Figure 4.

9-11 week-old control (*black*, left), *Tsc1^{mut/wt}* (*blue*, center), and *Tsc1^{mut/mut}* (*red*, right) mice of both sexes performed the 3-chamber social approach (**A.**) and social novelty (**B.**) assays for 10 minutes (presented in Figures 4 and 6). The time male and female mice spent in each chamber are plotted for each type of assay and across the three genotypes. Mean \pm SEM values are plotted as black bars. Within each genotype for the social approach assay, there were no significant differences across male and female animals for the time spent in each chamber (Student's unpaired t-test). In the Social Novelty assay (panel **B.**), male *Tsc1^{mut/wt}* animals spent significantly ($P < .01$) more time in the unfamiliar mouse chamber, and significantly ($P < .05$) less time in the familiar mouse chamber, when compared to female *Tsc1^{mut/wt}* mice (unpaired Student's t-test). Using 3-way ANOVA analyses of time in chamber data (using genotype, chamber, and sex as major factors), we found chamber to be the only significant ($P < .0001$) major factor for each assay. No significant interactions between factors were measured in the social approach (**A.**) and social novelty assays (**B.**).

Social Approach (16-24 week-old mice)



Social Novelty (16-24 week-old mice)



Supplemental Figure 5.

16-24 week-old control (*black*) and $Tsc1^{mut/mut}$ (*red*) mice performed the 3-chamber social approach and social novelty assays (presented in Figures 5 and 6, respectively). The time spent in each chamber during the 10-minute social approach assay (**A. B.**) and the 10-minute social novelty assay (**C. D.**) are plotted for each genotype. Mean \pm SEM values are plotted as grey bars. Within each genotype, there were no significant differences between male and female animals for the time spent in each chamber during social approach or social novelty testing (Student's unpaired t-test). A 3-way ANOVA was used to assess these data across genotypes, using genotype, chamber, and sex as major factors. For social approach, this analysis revealed Chamber to be the only significant ($P < .0001$) major factor. A significant ($P = .023$) interaction was also identified between Chamber and Genotype factors. For social novelty (**C. D.**), three-way ANOVA analysis revealed Chamber to be the only significant ($P < .0001$) major factor with no significant interactions.