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Supplementary Materials for

Postnatal immune activation causes social deficits in a mouse model of tuberous sclerosis: Role of microglia and clinical implications

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Materials

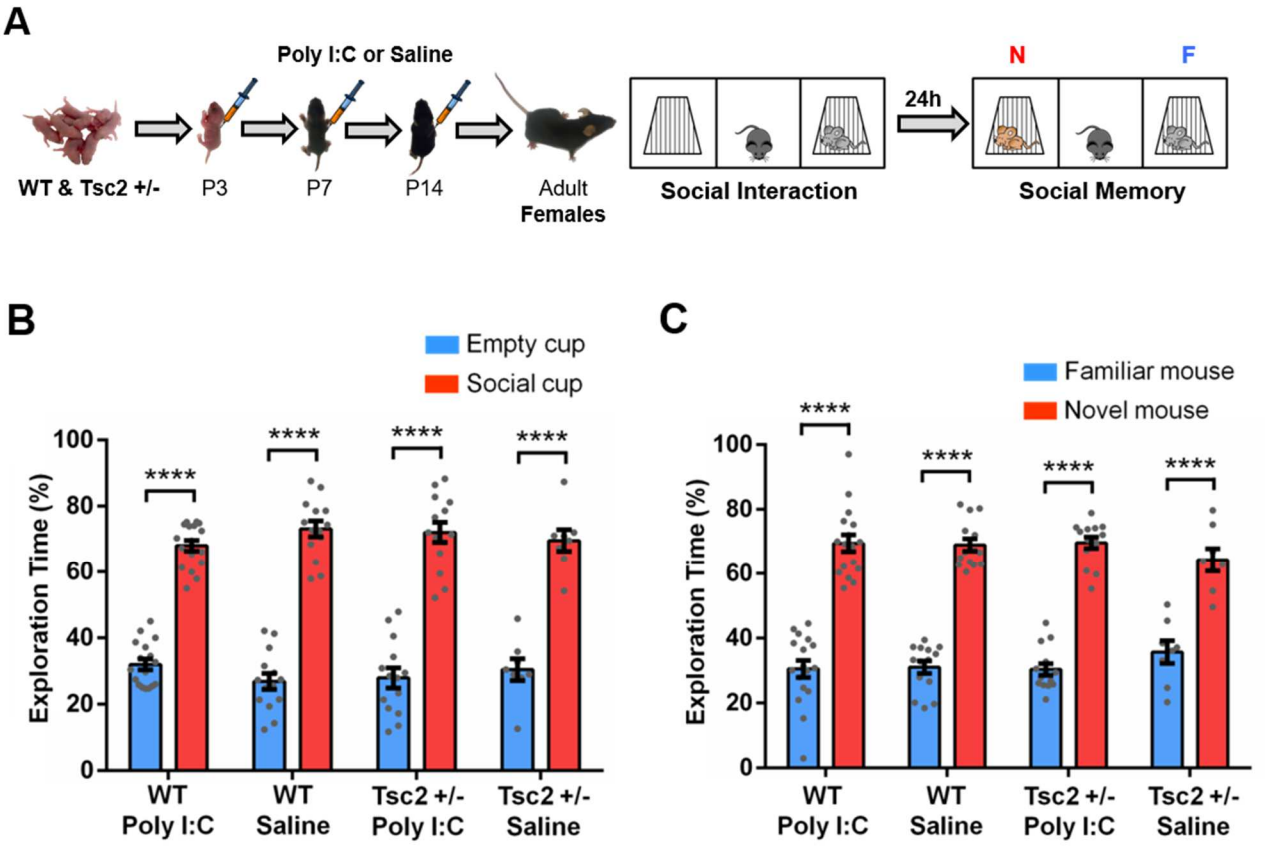

Fig. S1. Early post-natal immune activation does not induce social memory deficits in adult Tsc2$^{+/-}$ female mice.

(**A**) Time line for injections of Poly I:C or Saline and behavior approach. (**B**) Female WT/Poly I:C (n=17; P<0.0001, t=15.69), WT/Saline (n=14; P<0.0001, t=13.55), Tsc2$^{+/-}$/Poly I:C (n=14; P<0.0001, t=10.39) and Tsc2$^{+/-}$/Saline (n=8; P<0.0001, t=8.46) mice show normal social interaction (they spent significantly more time exploring the social cup than the empty cup). Analyses of the data using 2-way ANOVA revealed no significant differences between groups. (**C**) Female WT/Poly I:C (n=17; P<0.0001, t=10.51), WT/Saline (n=14; P<0.0001, t=13.88), Tsc2$^{+/-}$/Poly I:C (n=14; P<0.0001, t=15.60) and Tsc2$^{+/-}$/Saline (n=8; P<0.0001, t=5.78) mice show normal social memory (they spent significantly more time exploring the novel mouse than the familiar mouse).

Analyses of the data using 2-way ANOVA revealed no significant differences between groups. Data represent means ± SEM as well as individual data.

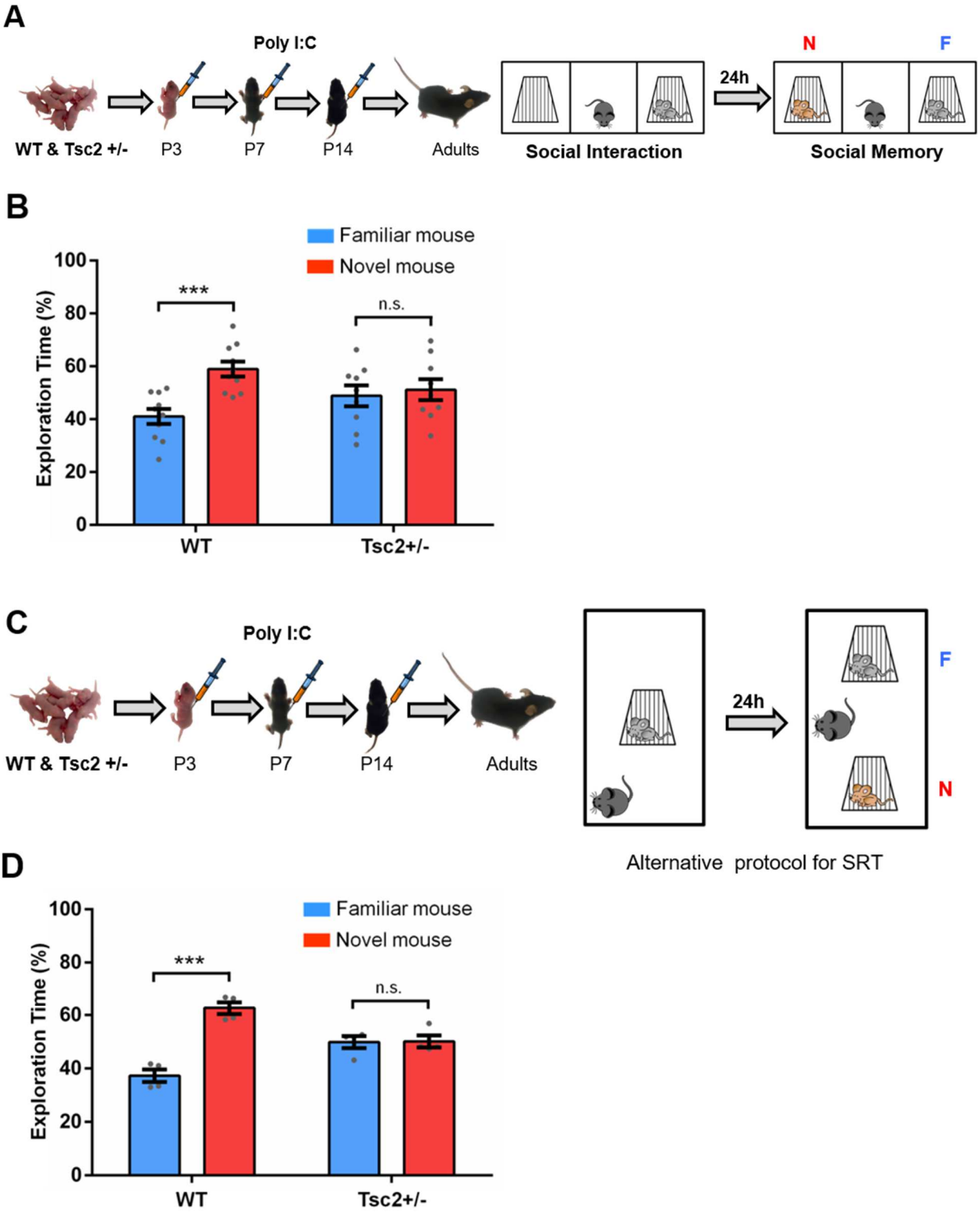

Fig. S2. Confirmation by a second researcher and with an alternative protocol of social memory deficits in $Tsc2^{+/-}$ male mice injected with Poly I:C early post-natally.

(**A** and **C**) Time line for injections of Poly I:C and behavior approach. (**B**) WT/Poly I:C (n=10; P<0.001, t=4.42) show normal social memory. $Tsc2^{+/-}$/Poly I:C (n=9; P=0.68, t=0.41) show social memory deficit. (**D**) WT/Poly I:C (n=4; P<0.001, t=7.62) show normal social memory. $Tsc2^{+/-}$/Poly I:C (n=4; P=0.93, t=0.08) show social memory deficit. Data represent means ± SEM as well as individual data.

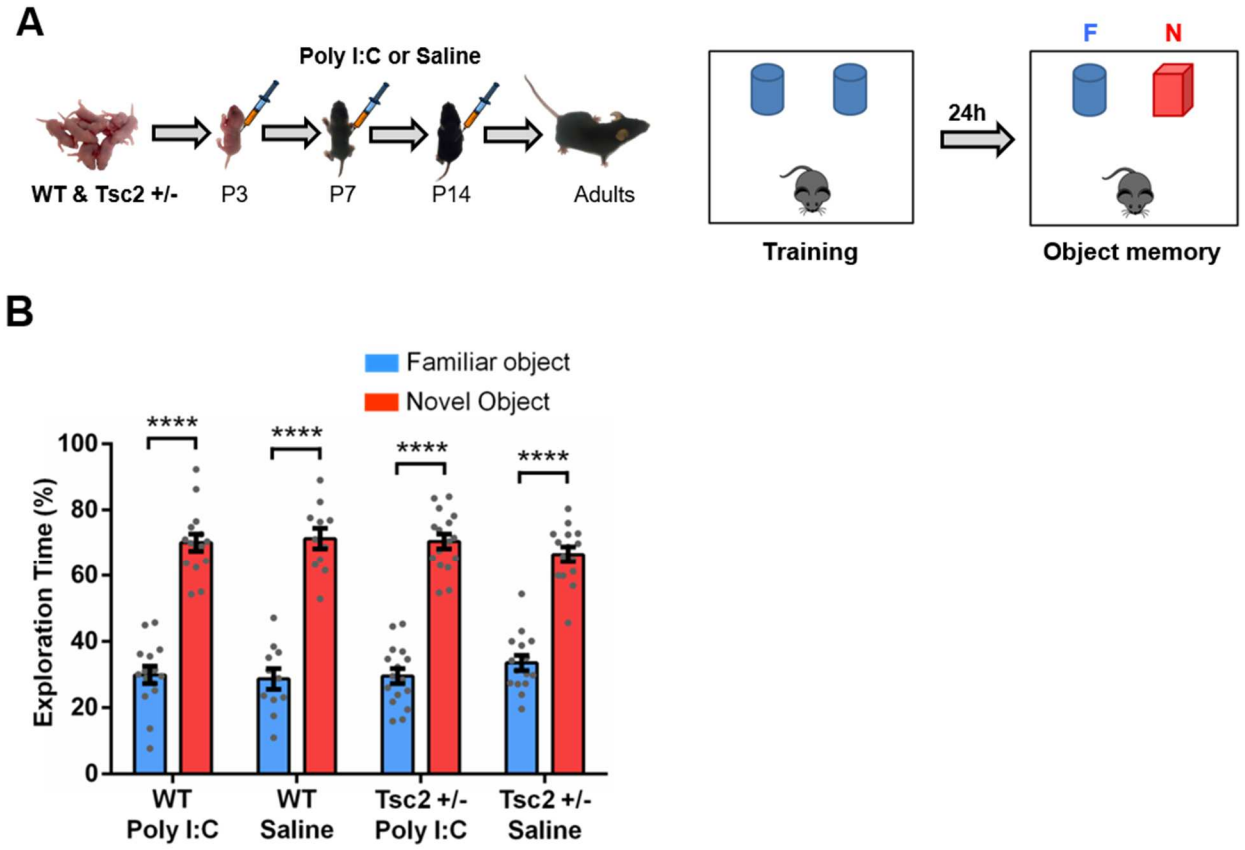

Fig. S3. Early post-natal immune activation does not affect object memory on Tsc2$^{+/-}$ male mice.

(**A**) Time line for injections of Poly I:C or Saline and behavior approach. (**B**) WT/Poly I:C (n=15; P<0.0001, t=10.86), WT/Saline (n=11; P<0.0001, t=9.60), Tsc2$^{+/-}$/Poly I:C (n=16; P<0.0001, t=12.75) and Tsc2$^{+/-}$/Saline (n=15; P<0.0001, t=10.30) mice show normal object memory (they spent significantly more time exploring the novel object than the familiar object). Analyses of the data using 2-way ANOVA revealed no significant differences between groups. Data represent means ± SEM as well as individual data.

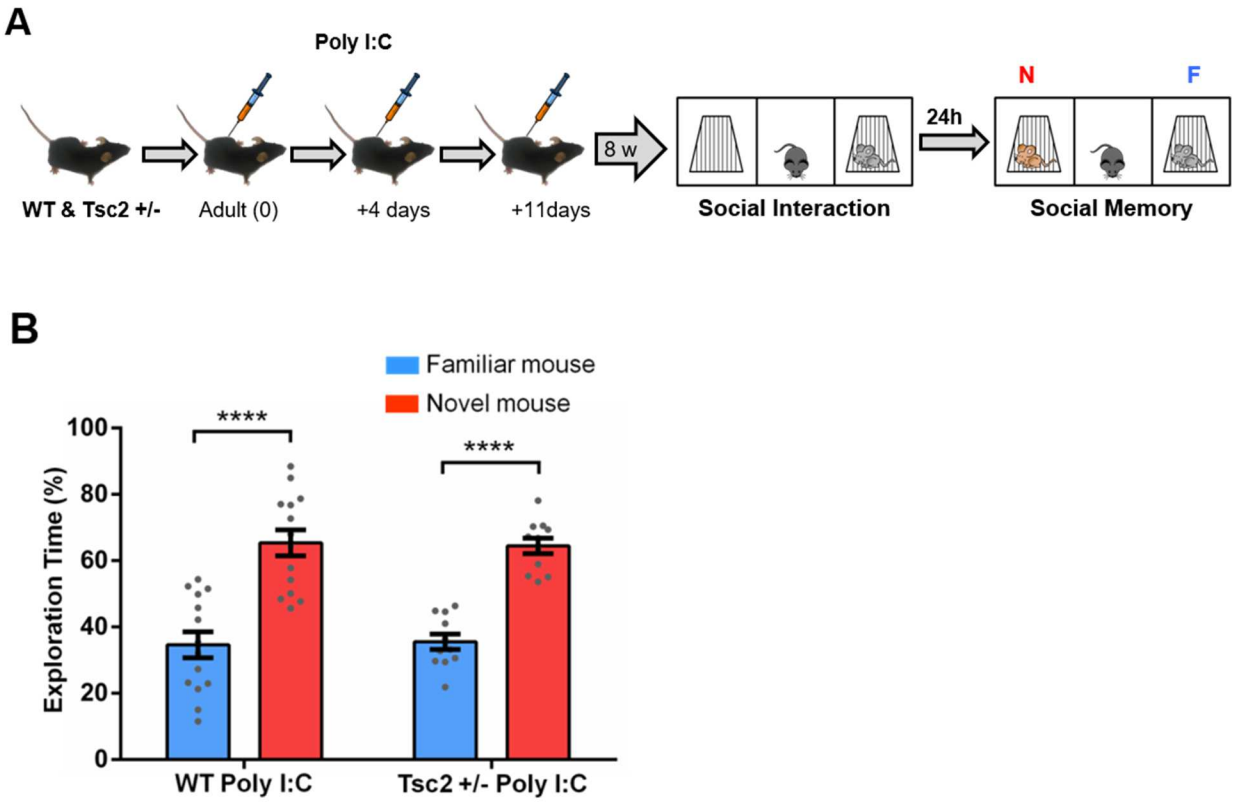

Fig. S4. Immune activation in adult Tsc2$^{+/-}$ male mice does not trigger a long lasting social memory deficit.

(**A**) Time line for injections of Poly I:C and behavior approach. (**B**) Both, WT (n=14; P<0.0001, t=5.51) and Tsc2$^{+/-}$ (n=11; P<0.0001, t=8.66) mice injected with Poly I:C as adults and tested 8 weeks later, show normal social memory. Data represent means ± SEM as well as individual data**.**

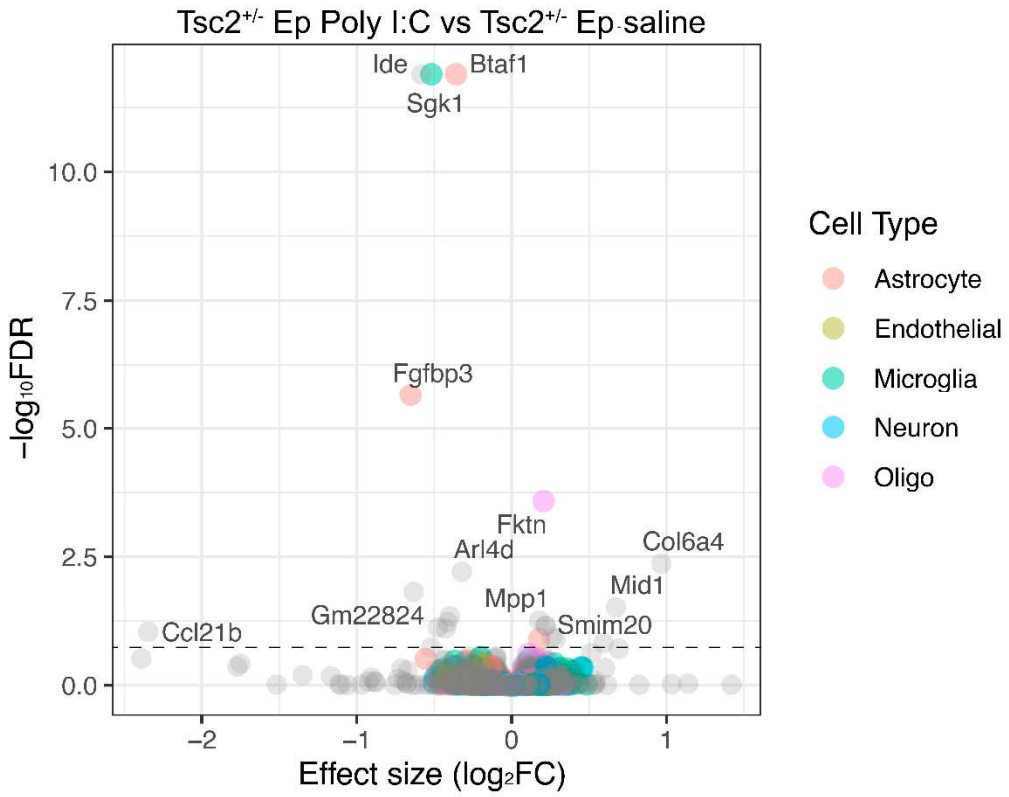

Fig. S5. Gene expression changes in the brain of Tsc2$^{+/-}$ male mice with early post-natal immune activation.

Volcano plot shows differential gene expression for Poly I:C vs saline exposed Tsc2$^{+/-}$ mice at ~4 months age, across prefrontal cortex, hippocampus, and cerebellum. Genes are colored according to cell-type specificity in mouse brain.

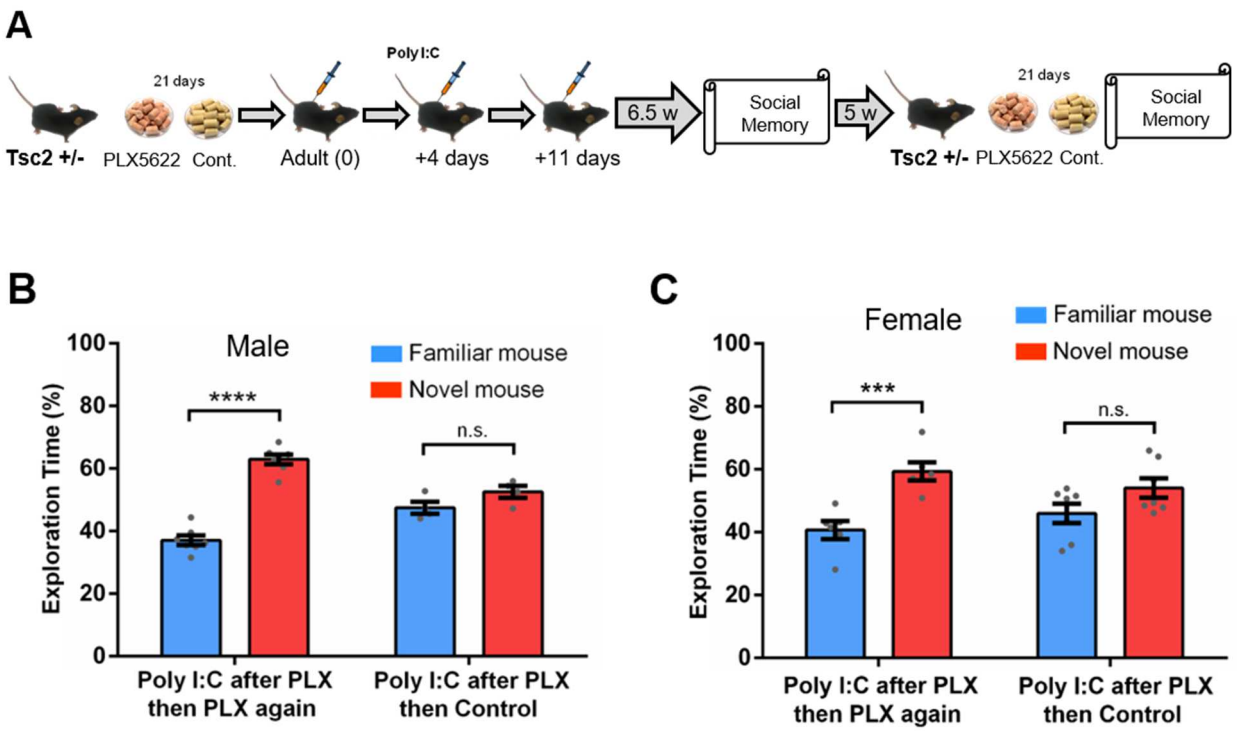

Fig. S6. Depletion of the microglia activated by the Poly I:C injections during microglia repopulation rescued the social memory phenotype of TSC2$^{+/-}$ mice.

(**A**) Time line for injections of Poly I:C, treatment with PLX or control chow and behavior approach. (**B**) Male Tsc2$^{+/-}$/Poly I:C after PLX then PLX again (n=7; P<0.0001, t=12.02) show normal social memory. Tsc2$^{+/-}$/Poly I:C after PLX then Control chow (n=4; P=0.108, t=1.88) show social memory deficit. (**C**) Female Tsc2$^{+/-}$/Poly I:C after PLX then PLX again (n=6; P<0.001, t=4.63) mice show normal social memory. Tsc2$^{+/-}$/Poly I:C after PLX then Control chow (n=7; P=0.089, t=1.84) mice show social memory deficit. Data represent means ± SEM as well as individual data.

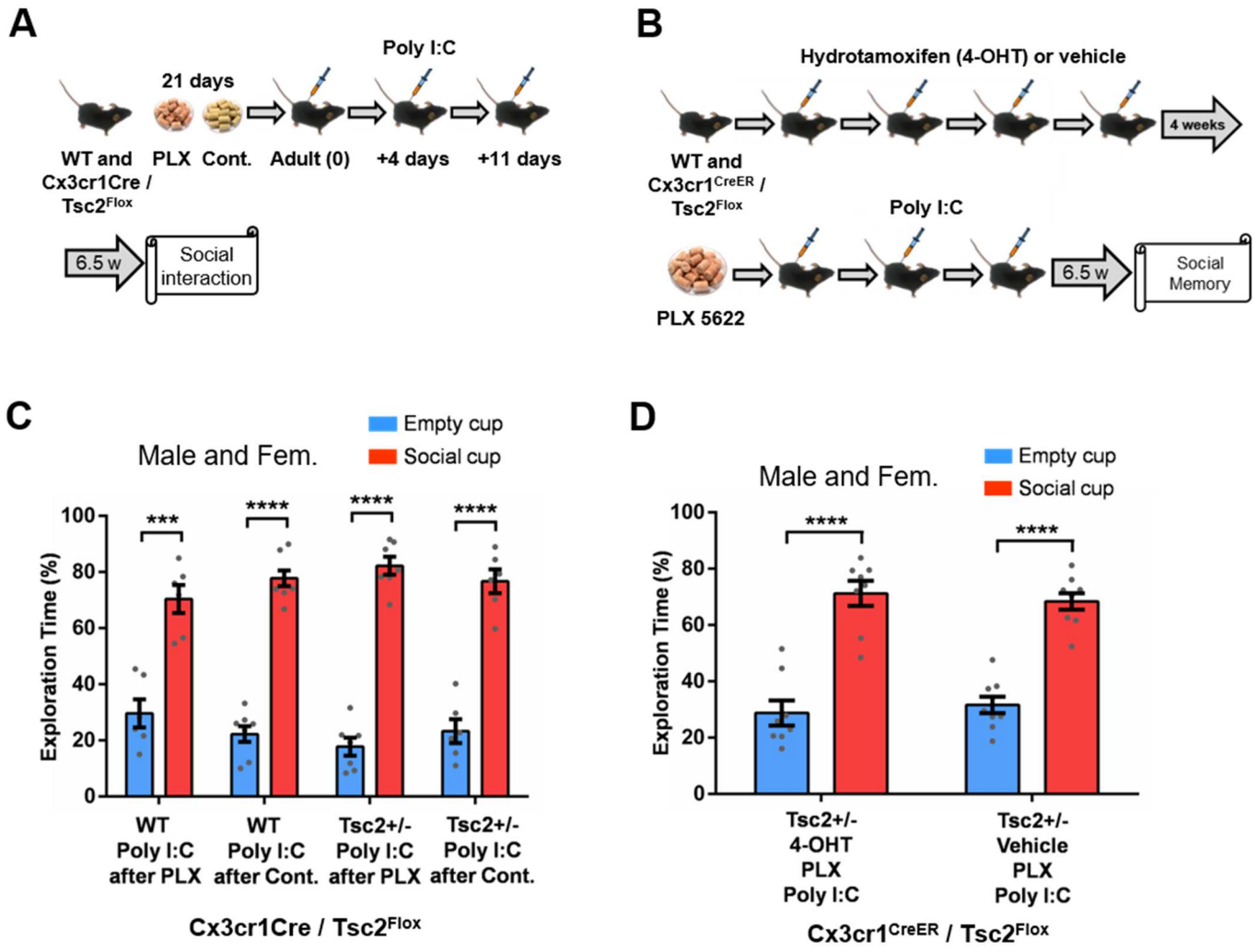

Fig. S7. Immune activation during the repopulation of microglia does not induce Social interaction deficits in Cx3cr1^{Cre} /Tsc2^{Flox} or Cx3cr1 ^{ER}/Tsc2^{Flox} mice

(**A**) Time line for injections of Poly I:C, treatment with PLX5622 (PLX; depletes microglia) or Control chow and behavior approach. (**B**) Time line for injections of 4-Hydroxyramoxifen or vehicle and Poly I:C, treatment with PLX and behavior approach. (**C**) Male and Female WT/Poly I:C after PLX (n=6; P<0.001, t=5.75), WT/Poly I:C after Control chow (n=8; P<0.0001, t=14.12), Tsc2$^{+/-}$/Poly I:C after PLX (n=7; P<0.0001, t=14.38) and Tsc2$^{+/-}$/Poly I:C after Control chow (n=6; P<0.0001, t=8.85) mice show normal social interaction. Analyses of the data using 2-way ANOVA revealed no significant differences between groups. (**D**) Male and Female Cx3cr1^{CreER}/ Tsc2^{Flox} /4-OHT mice injected with Poly I:C after PLX (n=8; P<0.0001, t=6.75) and Cx3cr1^{CreER}/ Tsc2^{Flox}

/Vehicle mice injected with Poly I:C after PLX (n=9; $P<0.0001$, $t=8.96$) show normal social interaction. Data represent means ± SEM as well as data for individual mice. As indicated, in figure (**C**) $Tsc2^{+/-}$ represents $Cx3cr1^{Cre}$-$Tsc2^{Flox}$. In figure (**D**) $Tsc2^{+/-}$ represents $Cx3cr1^{CreER}$/ $Tsc2^{Flox}$.

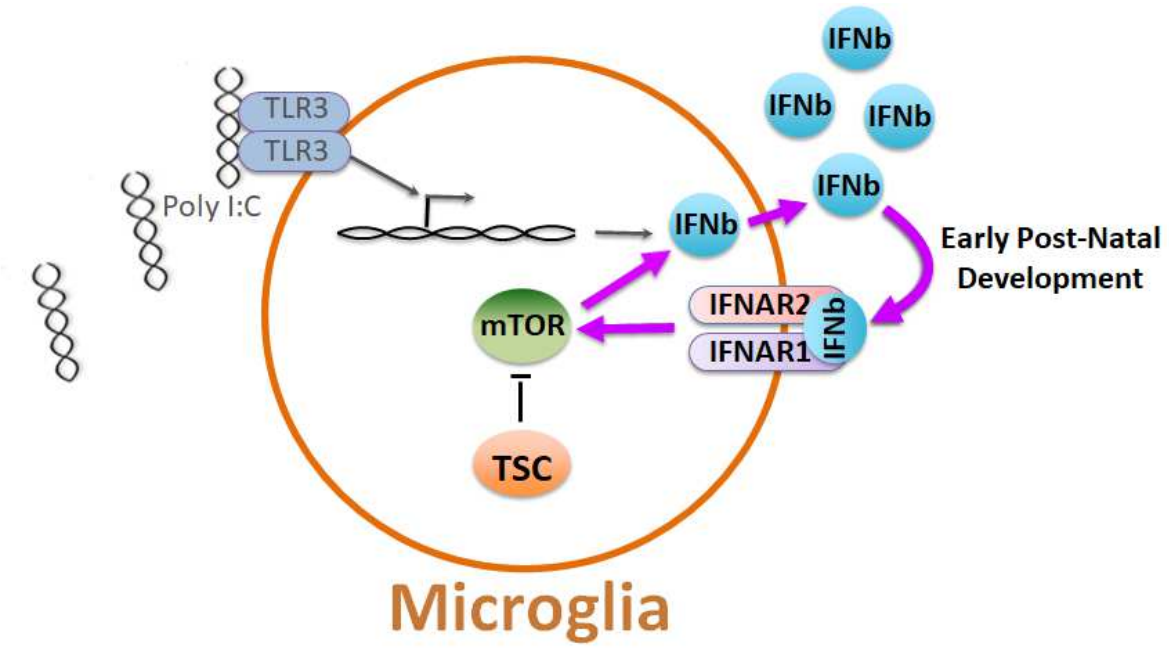

Fig. S8. Model for the mechanism responsible for the social memory deficits in Tsc2$^{+/-}$ Ep mice.

We propose that the Poly I:C injection during early post-natal, trigger an exacerbated production of IFNβ (type I IFN) in Tsc2$^{+/-}$ mice caused by their elevated levels of mTOR. Then, IFNβ activates IFNAR1, which in turn activates the mTOR pathway, and consequently triggers even more IFN production. We propose that this cycle is perpetuated in microglia and it is responsible for the social memory deficits we observed in Tsc2$^{+/-}$ Ep mice. Importantly, blocking this cycle in adults with rapamycin (m-TOR inhibitor) permanently rescues the social memory deficits of the Tsc2$^{+/-}$ Ep mice.

SYMBOL	logFC	AveExpr	t	P.Value	FDR. adjusted. P	Direction
Col6a4	0.96700838	0.25547203	4.47999992	4.21E-05	0.00431595	**upregulated**
Mid1	0.67423705	2.83698473	4.02270745	0.00018995	0.03044891	**upregulated**
RP23-468D17.1	0.21947736	3.21036637	3.75636028	0.00044246	0.06930776	**upregulated**
Smim20	0.21928455	3.75043663	3.75616346	0.00044273	0.06933948	**upregulated**
Fktn	0.20660018	5.67793925	5.0154018	6.73E-06	0.00026002	**upregulated**
Mpp1	0.17756655	4.7553308	3.84432726	0.00033561	0.05522782	**upregulated**

Table S1. Upregulated genes in the brain of $Tsc2^{+/-}$ male mice with early post-natal immune activation.

Known markers of microglia were significantly enriched among upregulated genes in $Tsc2^{+/-}$ Ep male mice.

Data file S1. Full summary statistic for all differential expression results from RNAseq analysis.