Supplemental material

Supplemental material Figure S1. Centrally-Injected TNFα at 50 ng induces all the Major Symptoms of Sickness Behavior in Mice. A. Duration of social exploration. B. Duration of immobility. Mice were treated i.c.v. with one microliter of either aCSF or TNFα (three concentrations of 25, 50 or 100 ng/µl) and features of sickness behavior were measured over a 3 min duration at time 0 and 2, 6 and 24 h post-treatment. Twenty-four mice were allocated to four i.c.v. treatment groups per treatment) matched for mean baseline social exploration time and body weight. Duration of exploration and immobility were analyzed by a two-way ANOVA with treatment (aCSF, TNFα 25, TNFα 50 and TNFα 100) as a between-subject factor and time (0, 2, 6 and 24 h) as a within-subject factor.

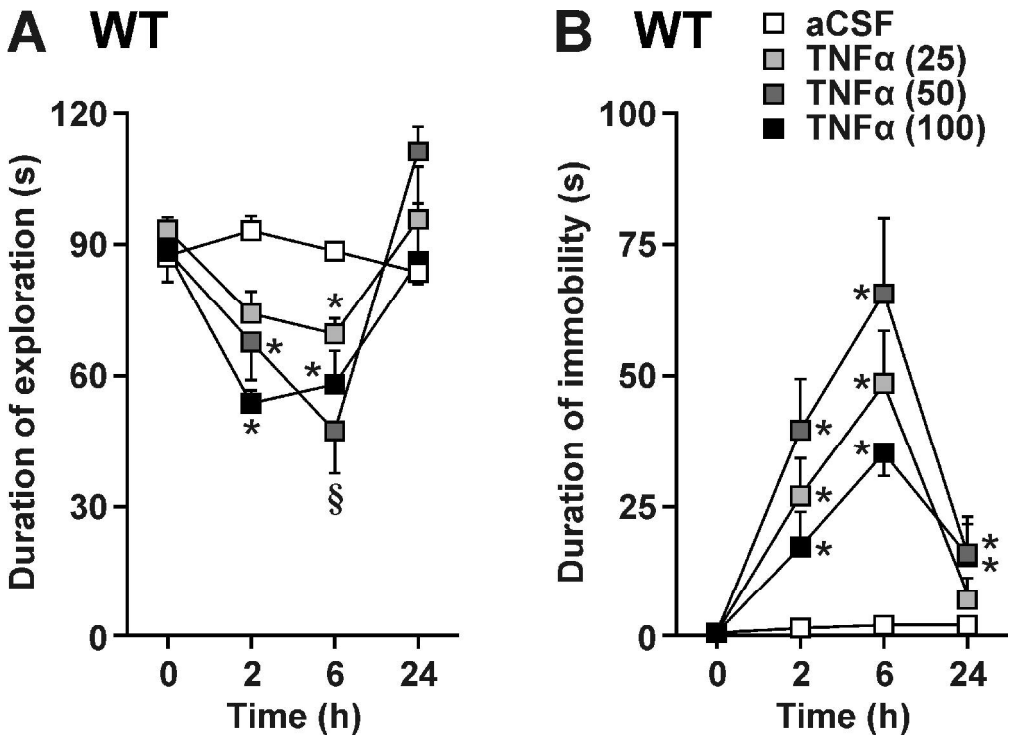
The baseline for duration of social exploration ranged from 87 ± 9 s to 93 ± 4 s over the four treatments (time 0; Supporting Information Fig. S1A). Duration of social exploration was affected by treatments in a time-dependent manner (dose x time interaction; $F(9, 60) = 4.1$, $p<0.01$). Post-hoc comparisons of individual group means revealed that TNFα decreased the duration of social exploration for 25 ng at only 6 h ($p<0.05$), for 50 ng at both 2 ($p<0.05$) and at 6 h ($p<0.001$) and for 100 ng at both 2 ($p<0.05$) and at 6 h ($p<0.05$). Social exploration returned to control levels by 24 h for all TNFα groups. At initiation of the experiment, all mice were fully active. Duration of immobility increased in response to TNFα according to time (dose x time interaction; $F(6, 40) = 3.2$, $p<0.01$] (Supporting Information Fig. S1B). Post-hoc comparisons of individual group means revealed that TNFα increased immobility for 25 ngl at 2 and 6 h ($p<0.05$) and both 50 ng and 100 ng at 2, 6 and 24 h ($p<0.05$). Based upon results of this experiment, we selected a dose of 50 ng TNFα for subsequent experiments since this concentration was able to induce the full spectrum of sickness behavior symptoms for the duration of the test. Each value represents the mean ± SEM. (n=6/group; * $p<0.05$ and § $p<0.01$ compared to the aCSF control at each time).

Supplemental material Table S1. Body weight change induced by i.c.v. injection of TNFα in mice. Body weight change 6 h after treatment in the experiment described above was analyzed by a one-way ANOVA. As expected, body weight was significantly reduced 6 h following i.c.v. injection of TNFα as compared to the aCSF treatment [$F(3, 20) = 19.6$, $p<0.001$] (Supporting Information Table S1). Post-hoc comparisons of individual dose means revealed no difference in body weight loss among the three doses of TNFα.

Supporting Information Table S1. Body weight changes in mice in response to increasing doses of TNFα.

		Δ Body Wt. (g)			
WT	aCSF	1.05	±	0.17	
	TNFα (25 ng)	-0.83	±	0.13	#
	TNFα (50 ng)	-0.78	±	0.28	#
	TNFα (100 ng)	-0.92	±	0.24	#

Body weight changes 6 h after i.c.v. treatment. Values represent means ± SEM (n=6/group; * $p<0.05$, § $p<0.01$ or # $p<0.001$ compared to the appropriate aCSF control).



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