

Supplementary Information

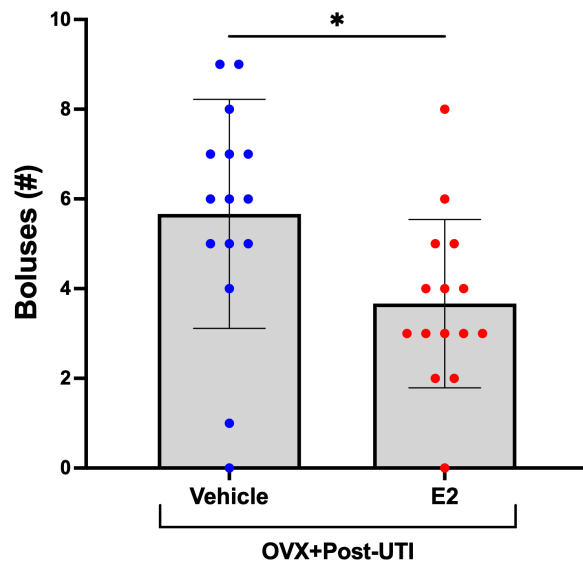


Figure S1. 17 β -estradiol (E2) significantly reduces UTI-induced emotionality. Among oophorectomized and UTI inoculated mice (OVX+UTI), animals treated with E2 defecated statistically significantly less during an open field test compared to vehicle treated (sesame oil) controls (n = 15 / group). Data are expressed as mean $\pm$ SD. *p < 0.05.

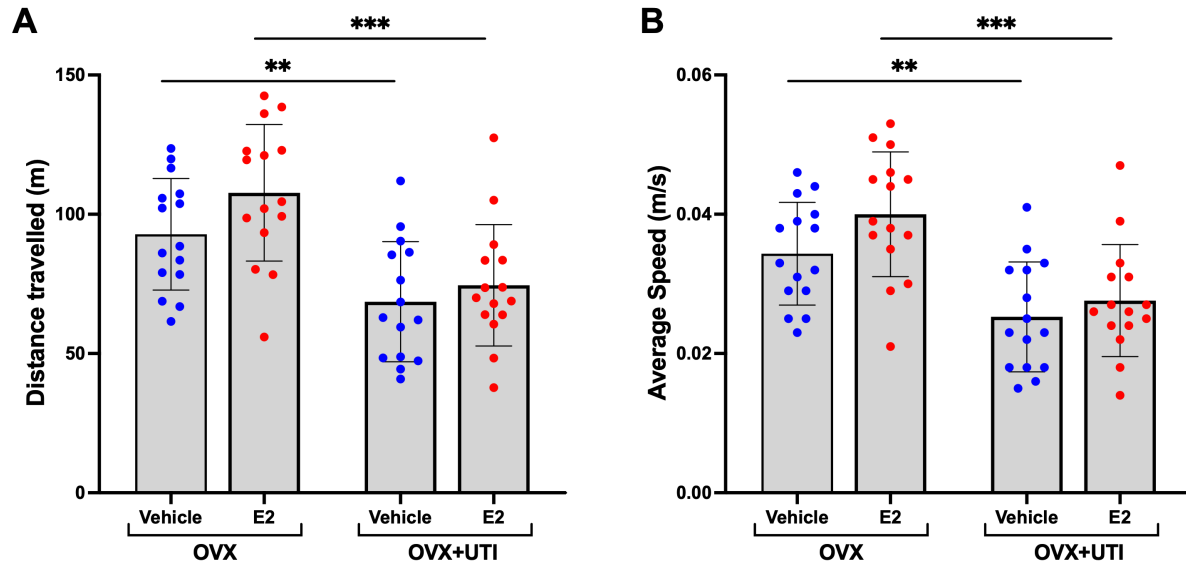


Figure S2. 17β -estradiol (E2) does not significantly alter general locomotor activity behavior. (a, b) Following oophorectomy, but prior to UTI induction and E2 treatment (OVX), there were no statistically significant differences between groups ($n = 15$ / group) in distance traveled (m) or average speed (m/s) in an open field test. Among oophorectomized and UTI inoculated mice (OVX+UTI), individuals treated with E2 did not significantly differ in distance traveled (m) or average speed (m/s) in an open field test compared to vehicle treated (sesame oil). All mice, regardless of treatment group, tended to be less active following UTI induction. Data are expressed as mean $\pm$ SD. ** $p < 0.01$, *** $p < 0.001$.

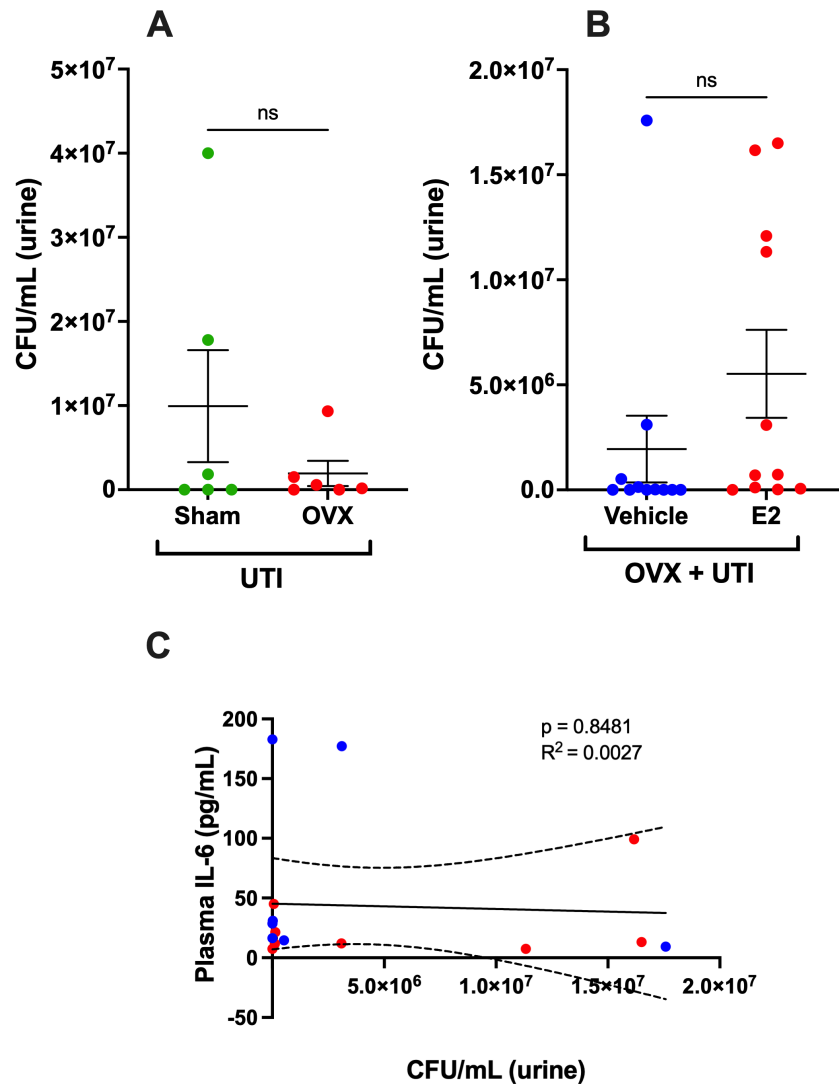


Figure S3. Transurethral inoculation of *E. coli* causes UTI. Bacterial enumeration was quantified 72 hrs. *post* inoculation. Animals for which urine could not be obtained in sufficient volume were excluded from this analysis. **(a)** In the initial experiment (Fig. 1), there was no significant difference in bacterial burden [as measured by colony forming unit (CFU)] between mice with UTI (sham; $n = 6$, mean = 9.9×10^6) and oophorectomized mice with UTI (OVX+UTI; $n = 6$, mean = 1.9×10^6) ($p = 0.2675$; $t = 1.174$, $df = 10$). **(b)** In the primary experiment (Figs. 2-5), oophorectomized mice with UTI (OVX+UTI) treated with 17β -estradiol (E2, $n = 11$, mean = 5.53×10^6) did not have statistically significantly different bacterial burden (CFU) compared to vehicle treated oophorectomized mice with UTI (sesame oil, $n = 11$, mean = 1.9×10^6) ($p = 0.1878$; $t =$

1.364, $df = 20$). (c) Regression analysis revealed that bacterial burden (CFU) 3 days *post* infection did not explain a significant amount of the variability in plasma IL-6 levels. Data are expressed as mean $\pm$ SEM. Dotted lines represent 95 % confidence intervals.

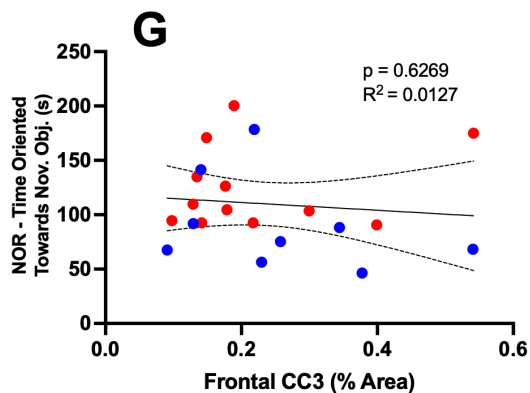
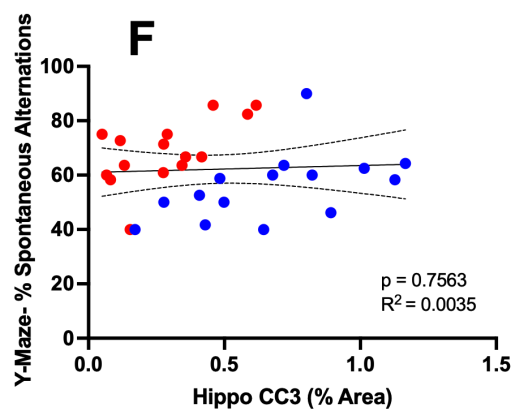
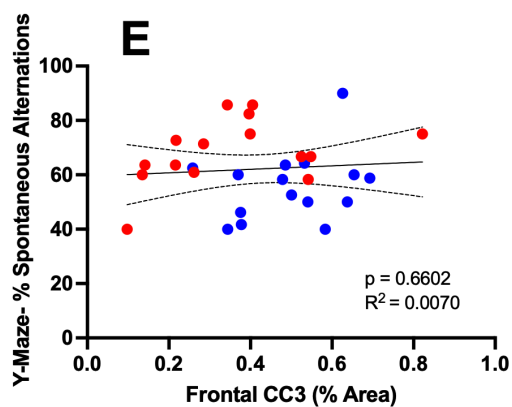
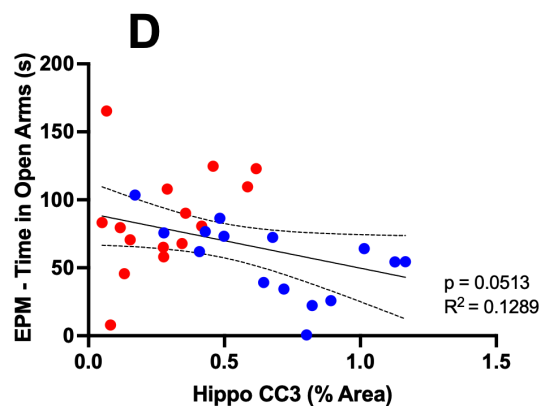
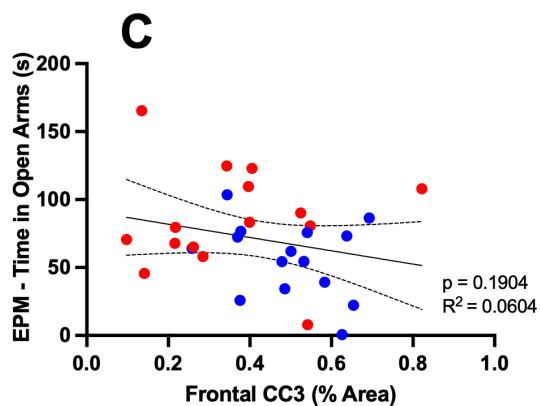
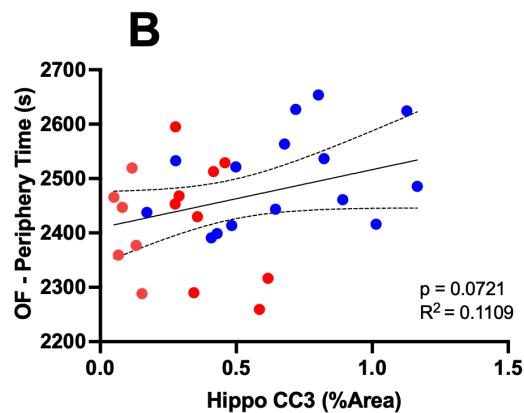
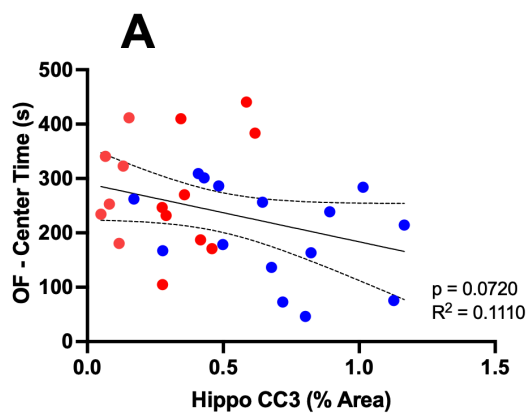


Figure S4. Associations of cleaved caspase-3 (CC3) with UTI-induced behavior. **(a-g)**

Regression analyses across groups (n = 21-30) of relationships between frontal cortical and hippocampal CC3 with delirium-like behaviors. Behavioral tests utilized were open field (OF), elevated plus maze (EPM), novel object recognition test (NOR), and Y-maze. Veh = vehicle treated; E2 = 17 β -estradiol treated. Dotted lines represent 95 % confidence intervals.

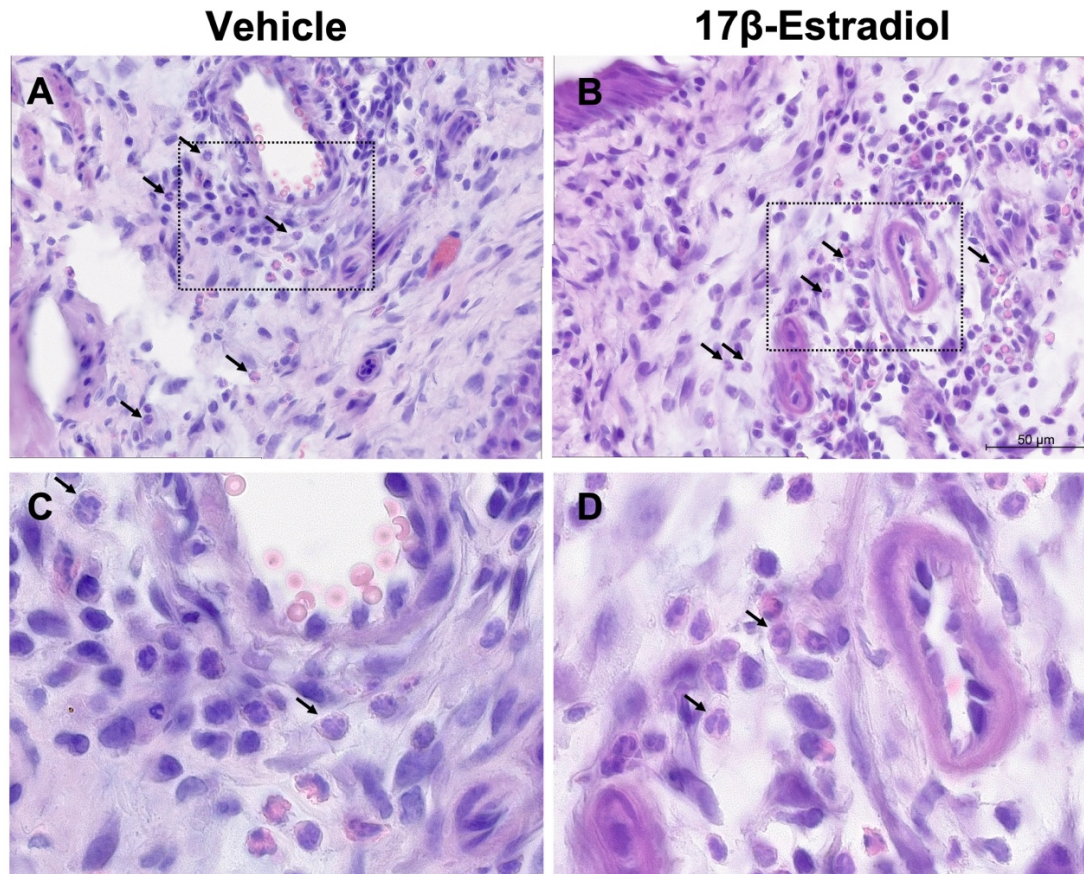


Figure S5. We performed H&E staining of a subset of randomly chosen animals and observed no difference in inflammatory cell infiltrates in bladder tissue (mucosal layer) between E2 and vehicle treated animals. Representative images are displayed. (a) Both vehicle and 17β-estradiol treated mice show evidence of inflammatory cell infiltrates (examples indicated by arrows). Low (a, b) and high (c, d) magnification of representative micrographs of bladder sections (8μm).

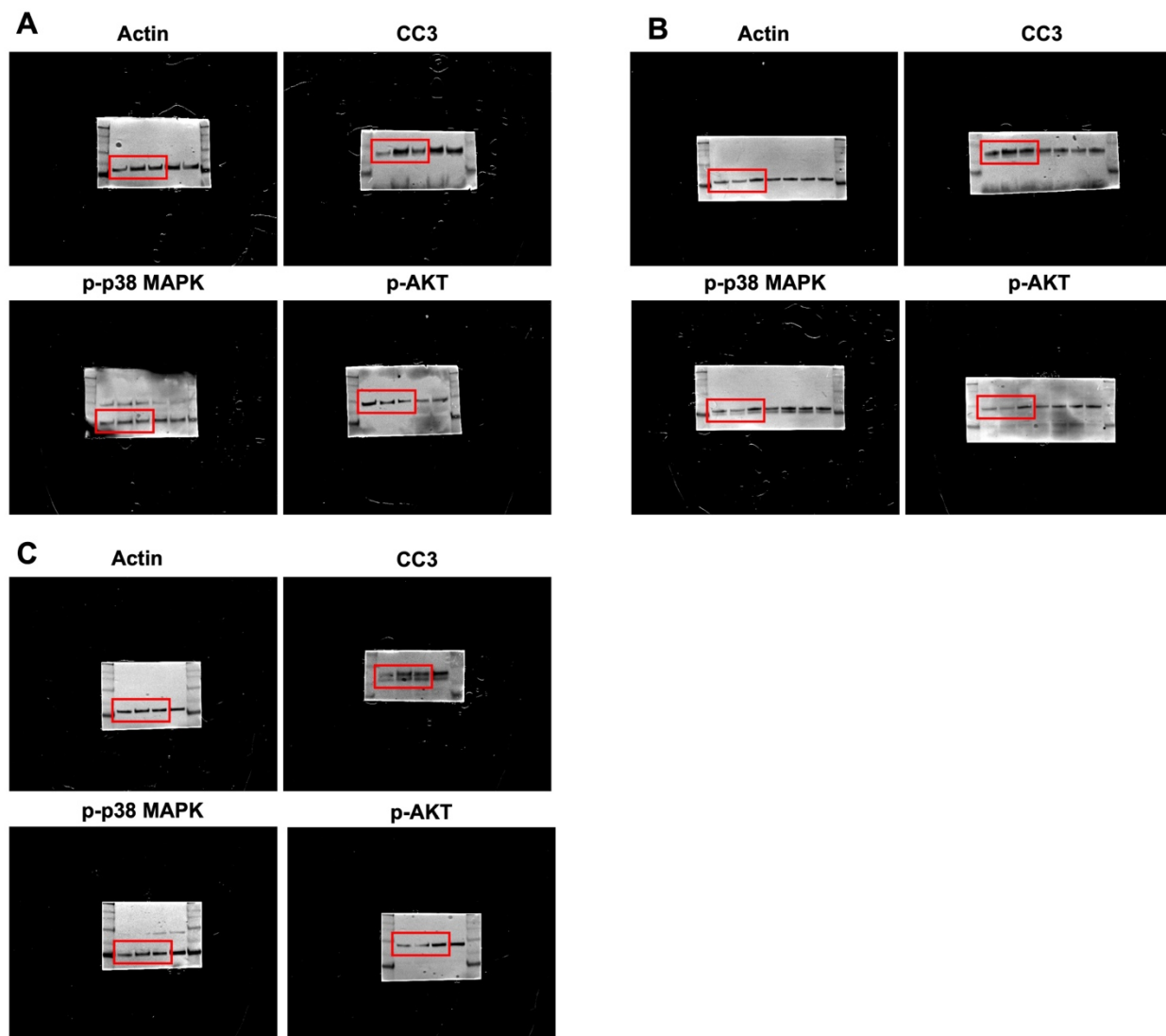


Figure S6. Full unedited gels corresponding to Figure 6b, c. (a-c) represent the 3 replicate experiments – with the replicate depicted in (a) shown in Fig. 6b. For each molecule, we have listed the utilized antibody in Table S1.

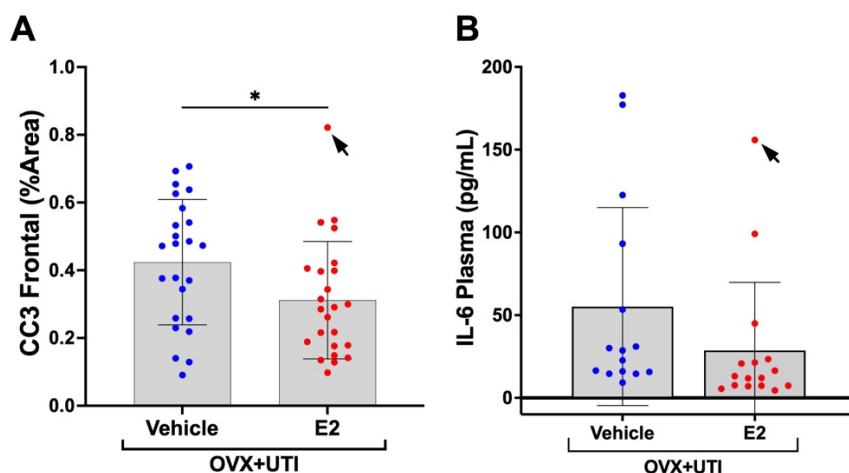


Figure S7. Data corresponding to Fig. 3a and 5a in the main text. (a) For CC3, a single statistical outlier was identified (indicated by arrow) using Grubbs' method with an Alpha = 0.05. Exclusion of the data point yielded $p = 0.0072$ ($t = 2.814$, $df = 45$), while inclusion (depicted here) resulted in $p = 0.0352$ ($t = 2.170$, $df = 46$). For (b) plasma IL-6, a single statistical outlier was identified (indicated by arrow) using Grubbs' method with an Alpha = 0.05. We believe the abnormally high value may have resulted from hemolysis of the sample. This single data point was removed, and an analysis yielded a p-value of 0.0495 ($t = 2.102$, $df = 18.45$). Here we present the figure with the data point included where the analysis resulted in $p = 0.1654$ ($t = 1.430$, $df = 24.66$). Data are expressed as mean $\pm$ SD.

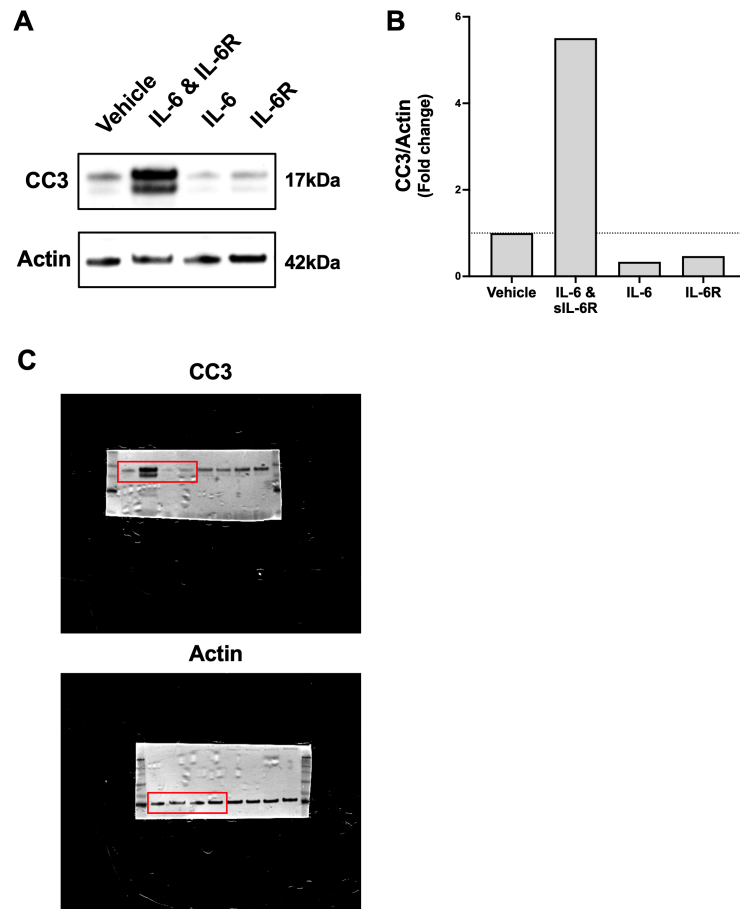


Figure S8. In Fig. 6, IL-6 was always administered with sIL-6R since neurons do not express the IL-6 receptor. As an additional control we administered IL-6 alone and quantified cleaved caspase-3 (CC3) in a single experiment (shown here). In the presence of IL-6 alone there was no statistically significant difference compared to vehicle. The edited (**a**) and unedited (**c**) blots are provided. For (**b**), vehicle (growth medium); IL-6 and sIL-6R (10 ng/mL and 100ng/mL, respectively); IL-6 (10 ng/mL), IL-6R (100 ng/mL).

Table S1. Western blot primary antibodies.

Antigen	Dilution	Vendor	Catalog #
Actin-HRP	WB=50,000	Life Tech.	MA5-15739-HRP
Cleaved Caspase-3	IF=800/WB=600	Cell Sig.	9664
Neurofilament-Light	IF=1000	Life Tech.	PA1-10000
NeuN	IF=600	Life Tech.	MA5-33103
p-AKT (Thr308)	WB=800	Cell Sig.	13038
p-P38 MAPK (Thr180/Tyr182)	WB=800	Cell Sig.	4511