SUPPLEMENTAL TABLE 1. CHRONIC UNPREDICTABLE STRESS SCHEDULE

DAY	STRESSOR 1	STRESSOR 2
1	CROWDING	LIGHT ON
2	PEPPERMINT ODOR	COLD EXPOSURE (4°C for 1h)
3	LIGHT OFF	FOOD DEPRIVATION
4	CAGE TILTING (45°)	IMMOBILIZATION (1h)
5	WATER DEPRIVATION	STROBE
6	CAGE ROTATION	WET BEDDING
7	SWIM (10m)	ISOLATION
8	PEPPERMINT ODOR	COLD EXPOSURE (4°C for 1h)
9	IMMOBILIZATION (1h)	LIGHT ON
10	WATER DEPRIVATION	LIGHT OFF
11	CAGE ROTATION	CROWDING
12	FOOD DEPRIVATION	WET BEDDING
13	SWIM (10m)	CAGE TILTING (45°)
14	COLD EXPOSURE (4°C for 1h)	ISOLATION
15	PEPPERMINT ODOR	WATER DEPRIVATION
16	LIGHT ON	IMMOBILIZATION (1h)
17	LIGHT OFF	STROBE
18	CAGE TILTING (45°)	CAGE ROTATION
19	CROWDING	WET BEDDING
20	SWIM (10m)	FOOD DEPRIVATION
21	LIGHT OFF	LIGHT ON
22	CAGE ROTATION	ISOLATION
23	WATER DEPRIVATION	IMMOBILIZATION (1h)
24	COLD EXPOSURE (4°C for 1h)	CAGE TILTING (45°)
25	STROBE	WET BEDDING
26	SWIM (10m)	ISOLATION
27	PEPPERMINT ODOR	CROWDING
28	COLD EXPOSURE*	ROTATION*

*LAST STRESSORS WERE CONSISTENTLY USED IN COMBINATION ON THE LAST STRESS DAY

SUPPLEMENTAL TABLE 2. STRESS RE-EXPOSURE SCHEDULE

DAY	STRESSOR 1	STRESSOR 2
57	CROWDING	LIGHT ON
58	PEPPERMINT ODOR	FOOD DEPRIVATION
59	LIGHT OFF	IMMOBILIZATION (1h)
60	CAGE TILTING (45°)	STROBE
61	WATER DEPRIVATION	WET BEDDING
62	SWIM (10m)	ISOLATION
63	COLD EXPOSURE (4°C for 1h)	CAGE ROTATION

** SHORT TERM UNPREDICTABLE STRESS PARADIGM USED FOR STRESS RE-EXPOSURE FOLLOWING POST STRESS PERIOD

SUPPLEMENTAL TABLE 3. PRIMER SEQUENCE

GENE	FORWARD	REVERSE
TLR4	CCA GAA TGA GGA CTG GGT GAG AAA	CCA CCA CAA TAA CTT TCC GGC TCT TG
RAGE	TGA CCC TGA CCT GTG CCA TC	CCT CAT CCT CAT GCC CTA CCT C
HMGB1	CAC TGC TGC GGA TGA CAA GC	CCT CCT CGT CGT CTT CCT CTT
HMBS	GGA GGT CCG AGC CAA GGA CCA	CTG GCA CGC TAC AGC CTC CTT CC

Supplemental Figure 1. CUS results in long lasting microglial reactivity in the hippocampus. Sprague Dawley rats were stressed for a total of 3 days (US) or 28days (CUS) and sacrificed 4hrs or 28 days (Post Stress) following the last stress exposure. Immunohistochemistry was conducted for the microglial marker IBA1 in the dorsal hippocampal sub-regions of dentate gyrus (DG), CA1 mossy cell layer and CA3 pyramidal cell layer in controls and following CUS. Representative images are shown for each condition and hippocampal subregion.

Supplemental Figure 2. CUS does not alter hippocampal microglia density in Sprague Dawley rats. (A) Experimental paradigm. (B-C) Microglial density was determined for the dorsal hippocampal sub regions of dentate gyrus (DG), CA1 mossy cell layer and CA3 pyramidal cell layer following (B) CUS (no effect, $F(1,18)=0.58$, $p=0.4562$) and (C) short term stress re-exposure (no effect, $F(3,47)=0.0299$, $p=0.9929$). The results are expressed as the mean ± SEM. N=4 animals per group. Two-way ANOVA followed by performed for CUS and Post stress ± US data set.

Supplemental Figure 3. Reversible effects of CUS on basal corticosterone serum levels and body weight in Sprague Dawley rats. (A) Basal corticosterone levels during stress exposure and stress recovery (interaction of stress and time, $F(5,25)=10.13$, $p< 0.01$). CORT levels were determined from tail blood samples collected at 9:00 am at each time point. (B) Body weight during stress exposure and stress recovery (interaction of stress and time, $F(6,66)=24.41$; $p< 0.01$. Two-way ANOVA repeated measures followed by Bonferroni post hoc test performed for each time point.

Supplemental Figure 4. CUS increases nuclear export of HMGB1 in the hippocampus. (A) Experimental paradigm of CUS and post stress period in Sprague Dawley rats. Nuclear and cytoplasmic HMGB1 levels in total lysates prepared from hippocampal tissue collected (B) 4hrs (interaction of stress and localization, ($F(1,16)=6.346$, $p=0.0228$) (C) 28 days after the last CUS stressor (interaction of prior stress and localization, ($F(1,23)=12.5$, $p=0.0018$). The results are expressed as the mean ± SEM. N=5-8 animals per group. *$p< 0.01$. Two-way ANOVA was performed for CUS and post stress group.

Supplemental Figure 5. Acute stress re-exposure is insufficient to reinstate anhedonic behavior in post stress animals. (A) Hypothalamic-pituitary-adrenal (HPA) response in post stress animals during and after acute (2h) restraint stress re-exposure (main effect of time, $F(5,52)=21.09$, $p<0.0001$, no effect of stress $F(5,52)=3.345$, $p=0.0724$). Corticosterone levels were determined from tail blood samples collected immediately prior (at basal; B), during (15, 30, 60 and 120 minutes during restraint min; 15R, 30R, 60R and 120R respectively) and post acute immobilization (60 minutes post restraint; 60P). (B) Post stress rats were tested for sucrose preference 4h and 24h following acute (2h) restraint re exposure (no effect, $F(1,20)=0.0077$, $p=0.9306$). The results are expressed as the mean ± SEM. N=10-12 animals per group. Two-way ANOVA repeated measures performed for corticosterone and sucrose preference test.

Supplemental Figure 6. Previous CUS exposure alters Inflammasome signaling in rats. (A) Paradigm for CUS plus post stress re-exposure to unpredictable stress. Levels of IL1β and NLRP3 were quantified in enriched microglia or total lysates prepared from hippocampal tissue collected 4hrs after the last stressor. (B-C) mRNA levels were determined in enriched hippocampal microglia by PCR analysis; (B) IL1β (no effect, $F(1,30)=2.011$, $p=0.1665$) and (c) NLRP3 (main effect of stress, $F(1,31)=4.916$, $p=0.0341$; no interaction of stress and prior exposure $F(1,31)=4.916$, $p=0.0341$). (D-E) mRNA in total hippocampal lysates by PCR analysis; (D) IL1β (no effect, $F(1,20)=0.7788$, $p=0.3880$) and (E) NLRP3 (main effect of stress, $F(1,20)=5.228$, $p=0.0333$); no interaction, $F(1,20)=0.0083$, $p=0.9280$). (F-G) Protein levels determined by western blot analysis; (F) IL1β (significant interaction of stress and prior exposure, $F(1,19)=4.415$, $p=0.0492$) and (G) NLRP3 (no effect, $F(1,24)=0.9043$, $p=0.3511$) protein in total hippocampal lysates by western blot analysis. All genes were normalized to housekeeping gene HMBS. IL1β and NLRP3 levels were normalized to β-actin. The results are expressed as the mean ± SEM. N=6-10 animals per group. # $p\leq0.1$. Two-way ANOVA was performed for each gene and protein followed by Bonferroni post hoc test for data sets with significant interaction.

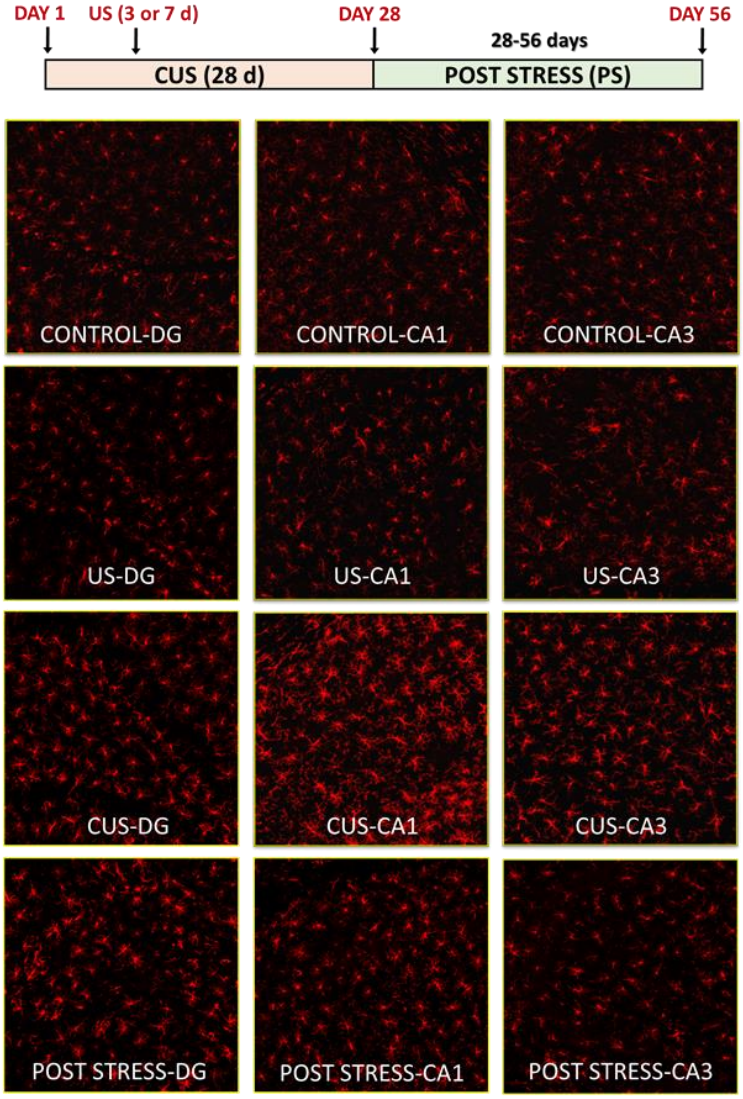


FIGURE S1

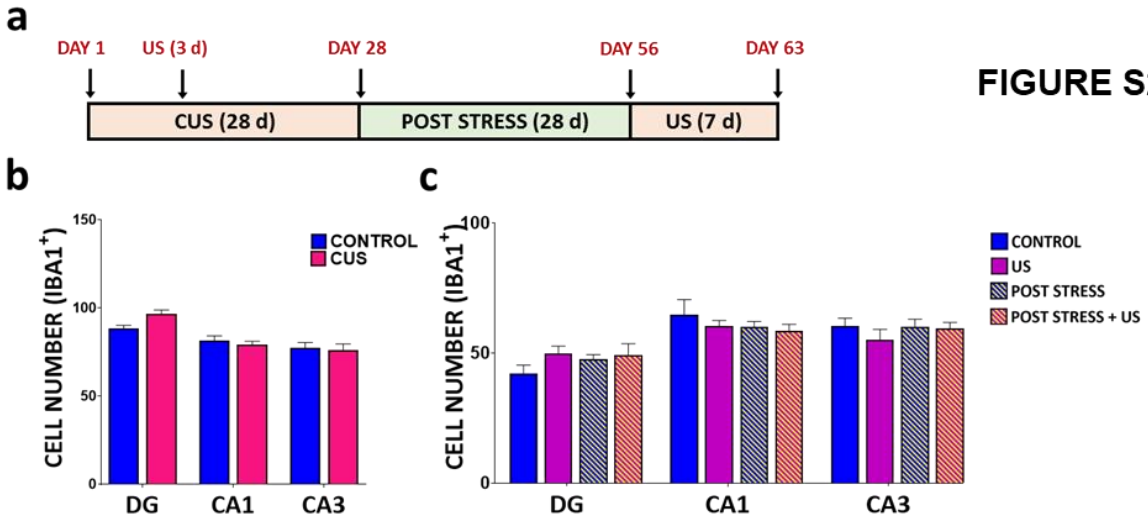


FIGURE S2

FIGURE S3

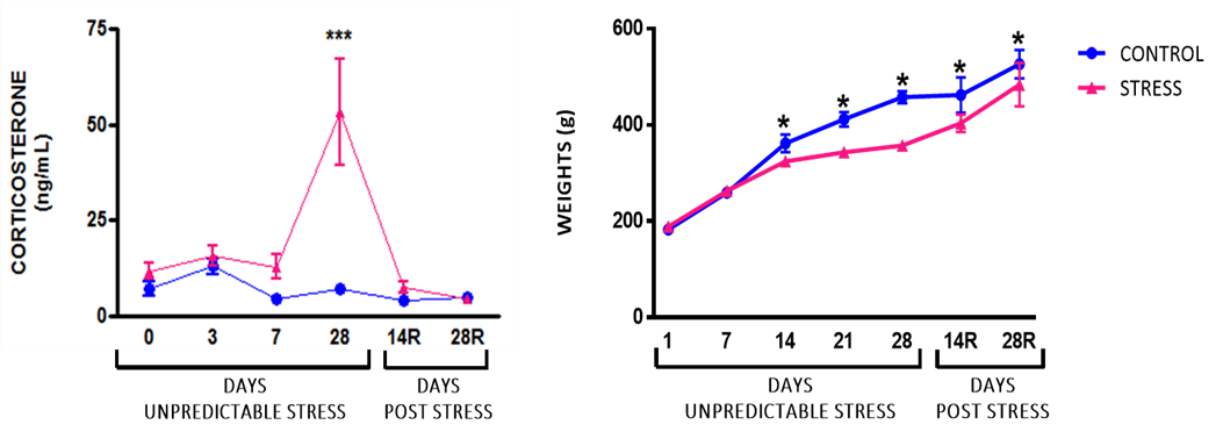

CORTICOSTERONE (ng/mL)
75
50
25
0
***
0
3
7
28
14R
28R
DAYS UNPREDICTABLE STRESS
DAYS POST STRESS
WEIGHTS (g)
600
400
200
0
*
1
7
14
21
28
14R
28R
DAYS UNPREDICTABLE STRESS
DAYS POST STRESS
CONTROL
STRESS

FIGURE S4

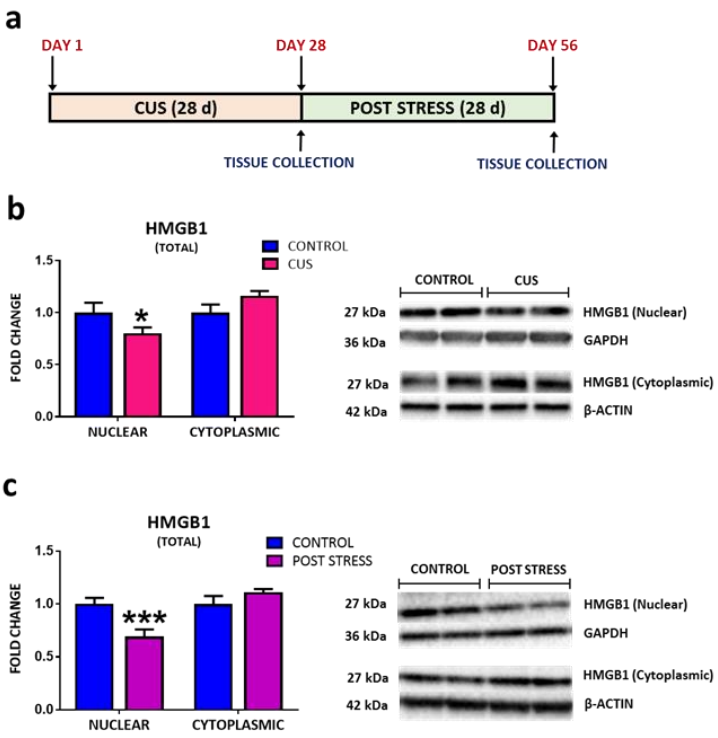
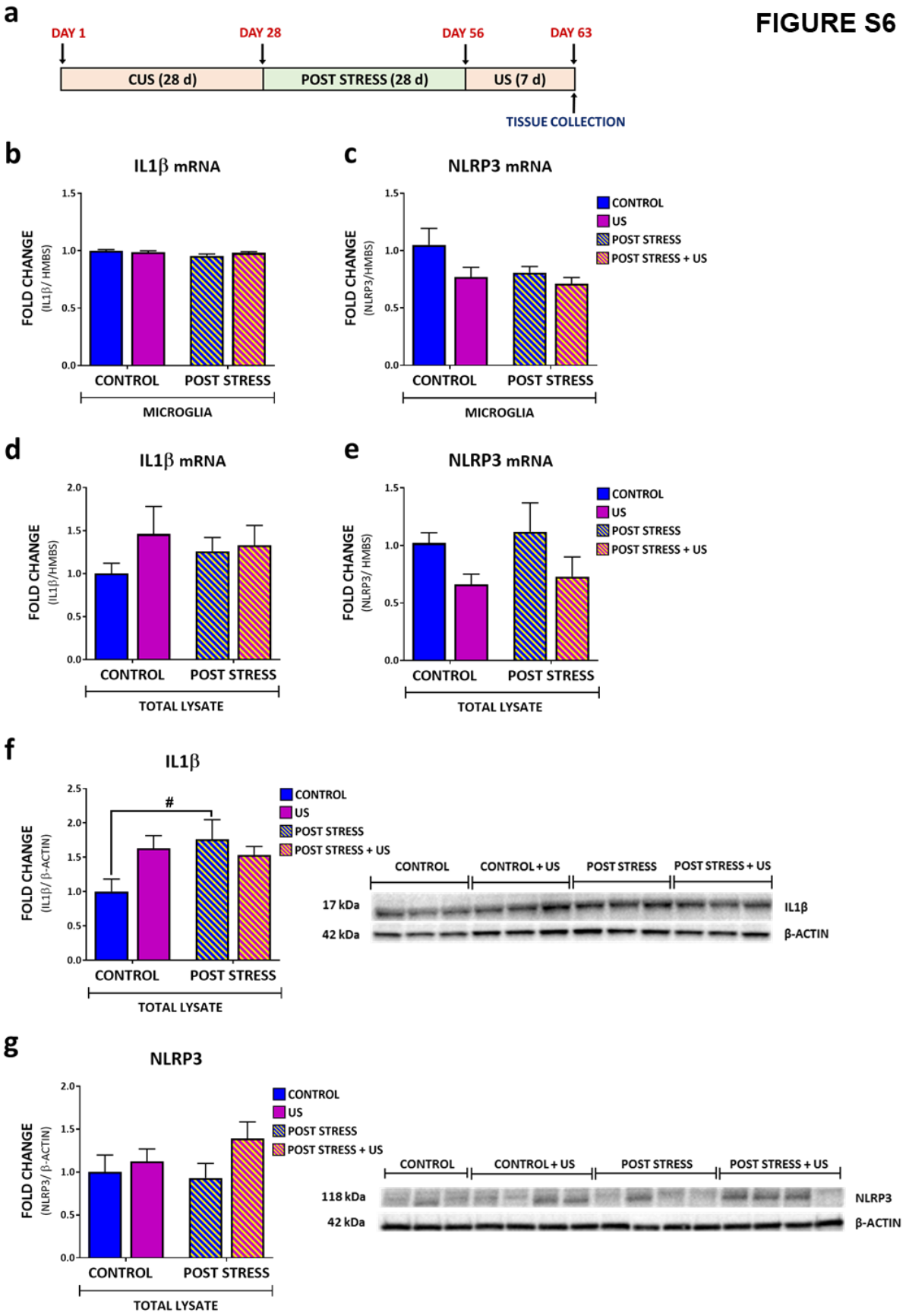


FIGURE S5

FIGURE S6


a
DAY 1
DAY 28
DAY 56
DAY 63
CUS (28 d)
POST STRESS (28 d)
US (7 d)
TISSUE COLLECTION
b
IL1β mRNA
FOLD CHANGE
(IL1β/ HMBS)
CONTROL
POST STRESS
MICROGLIA
c
NLRP3 mRNA
FOLD CHANGE
(NLRP3/HMBS)
CONTROL
US
POST STRESS
POST STRESS + US
CONTROL
POST STRESS
MICROGLIA
d
IL1β mRNA
FOLD CHANGE
(IL1β/HMBS)
CONTROL
POST STRESS
TOTAL LYSATE
e
NLRP3 mRNA
FOLD CHANGE
(NLRP3/ HMBS)
CONTROL
US
POST STRESS
POST STRESS + US
CONTROL
POST STRESS
TOTAL LYSATE
f
IL1β
#
FOLD CHANGE
(IL1β/ β-ACTIN)
CONTROL
US
POST STRESS
POST STRESS + US
CONTROL
POST STRESS
TOTAL LYSATE
CONTROL
CONTROL + US
POST STRESS
POST STRESS + US
17 kDa
42 kDa
IL1β
β-ACTIN
g
NLRP3
FOLD CHANGE
(NLRP3/ β-ACTIN)
CONTROL
US
POST STRESS
POST STRESS + US
CONTROL
POST STRESS
TOTAL LYSATE
CONTROL
CONTROL + US
POST STRESS
POST STRESS + US
118 kDa
42 kDa
NLRP3
β-ACTIN