Stress-induced Neuronal CSF1 Provokes Microglia-mediated Neuronal Remodeling and Depressive-like Behavior

Supplemental Information

Supplemental Methods

Throughout behavioral assessments mice were exposed to daily overnight stressors, but prior to each behavioral test mice were left undisturbed for 2-4 hours:

Open-field test (OF). Mice were placed in a Plexiglas test apparatus (40×40×25 cm) and recorded for 15 min. Activity in the open-field was analyzed using an automated system (ANY-maze, Stoelting Company).

Forced Swim Test (FST). Mice were placed for 10 min in a clear cylinder filled with water (24±1°C, 18 cm depth). Sessions were video-recorded and scored for total immobility time by a blinded experimenter. Time immobile during the 2-6 minute block is reported.

Sucrose Consumption Test (SCT). Mice were single-housed for 24 h and provided bottles containing water for acclimation. The night before testing bottles containing water were placed in cage overnight to measure baseline water consumption. In the morning water bottles were removed and mice were water-deprived for 12 h. Bottles containing water with 1% sucrose were placed in the cage for 1 h and then weighed for sucrose consumption.

Novelty Suppressed Feeding Test (NSFT). Mice were food-deprived for 18 h and placed in a dimly lit, novel environment (24×40×14 cm, fresh bedding) with food in the center. The latency to feed was recorded. Home cage food intake was measured as a control.

Quantitative Immunofluorescence (cont.): Brain slices containing mPFC were selected and bilateral images were obtained for each sample. Cell counts were quantified using standardized parameters with ImageJ software (NIH, Bethesda, Maryland). To quantify GFP inclusions within Iba-1+ microglia, brain slices containing mPFC were selected and bilateral images were obtained at high magnification (100x). Individual confocal stacks were analyzed by a blinded experimenter and the proportion and number of GFP inclusions were recorded. Quantification of dendritic spine

density was performed in deconvolved confocal image stacks (AutoquantX Version 3.0.1, Media Cybernetics, Bethesda, MD) by a blinded experimenter using NeuronStudio software. An average of 20 dendritic segments (15 – 40 um in length) were collected per animal and spine densities per segment were averaged to yield mean values per animal. To quantify knockdown of CSF1 through immunohistology, bilateral confocal images were collected from virally-infected brain slices containing mPFC. Individual neurons were identified by EGFP expression and relative fluorescent intensity of CSF1 immunofluorescence were measured using standardized parameters in ImageJ.

Table S1. List of primer sequences.

Gene	Forward (5'-3')	Reverse (5'-3')
Csf1 (mouse)	CGAGTCAACAGAGCAACCAA	TGTCAGTCTCTGCCTGGATG
CSF1 (human)	CCCAGCAACTTCCTCTCAGCA	AGCAGGGCAGATGGATGGTC
Il34 (mouse)	TACAGCCACCTCTGCTTGTG	GCAAGATACGGCATTTGGTT
IL34 (human)	GCGGTATCTGTGGGTCTTGG	ACATTCAGCAGCAGCGTCTC
Csf1r	GACCCTGAATCTCCCGGAAG	GGTACAACGGTAGGTCCCAG
Cx3cl1	ATCCGCCACATTGGAAGACC	ACAGCAGGACTCGGCCAAAC
Cx3cr1	CCTGCAAGAATCGCAAGAAGG	TGGCTGAACGCCACTGTCTC
Tgfb	CCCTATATTTGGAGCCTGGA	CTTGCGACCCACGTAGTAGA
Tgfbr1	GCGGGGAGAAGAAGTTGCTG	CGTCCATGTCCCATTCTCTTTG
Il1b	CAGGCTCCGAGATGAACAAC	GGTGGAGAGCTTTCAGCTCATAT
Tnfa	CGACGTGGAACTGGCAGAAG	GAGGGAGGCCATTTGGGAAC

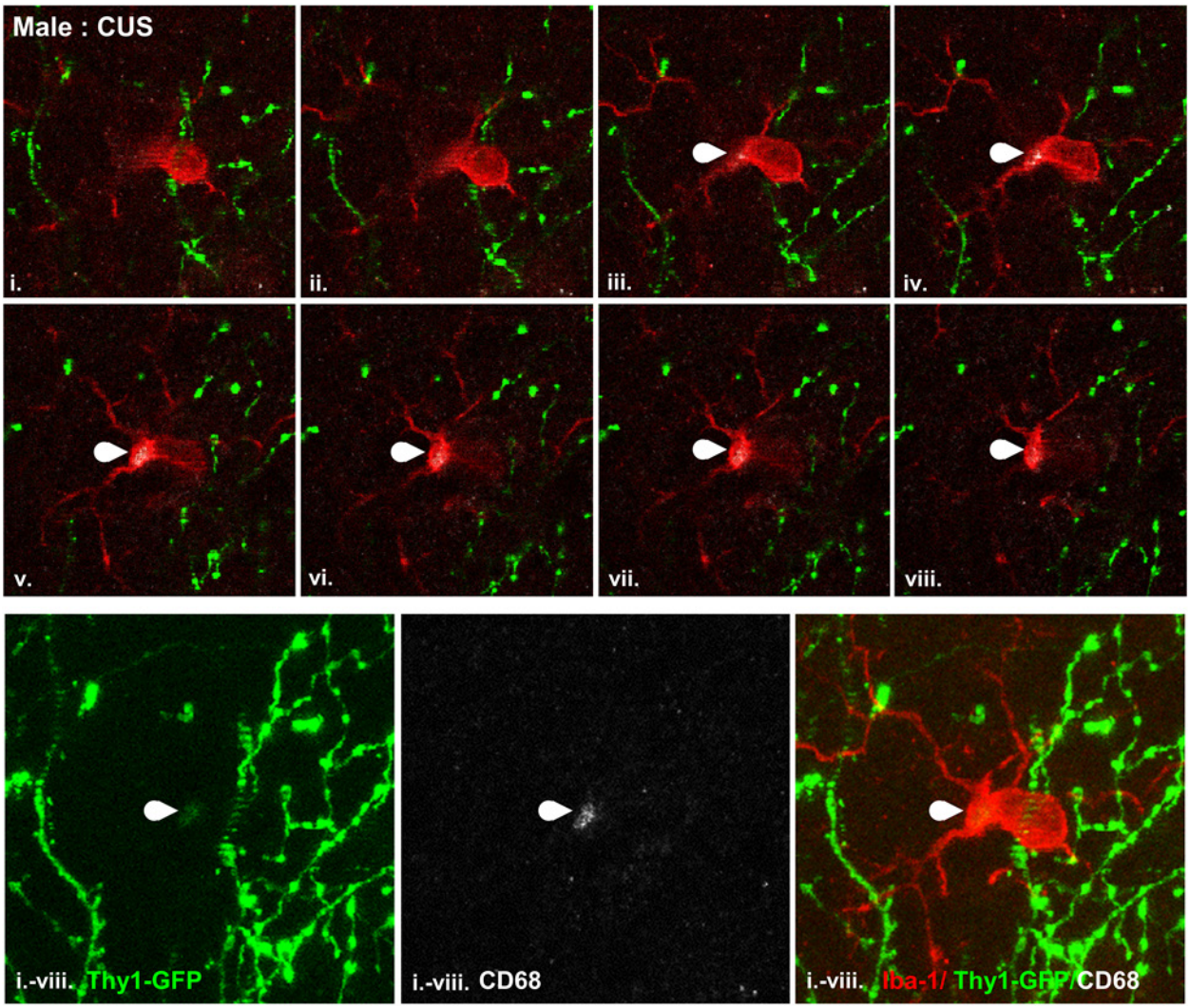

Figure S1. EGFP+ neuronal elements co-localized with lysosomal marker CD68 within microglia in layer I of the medial PFC.

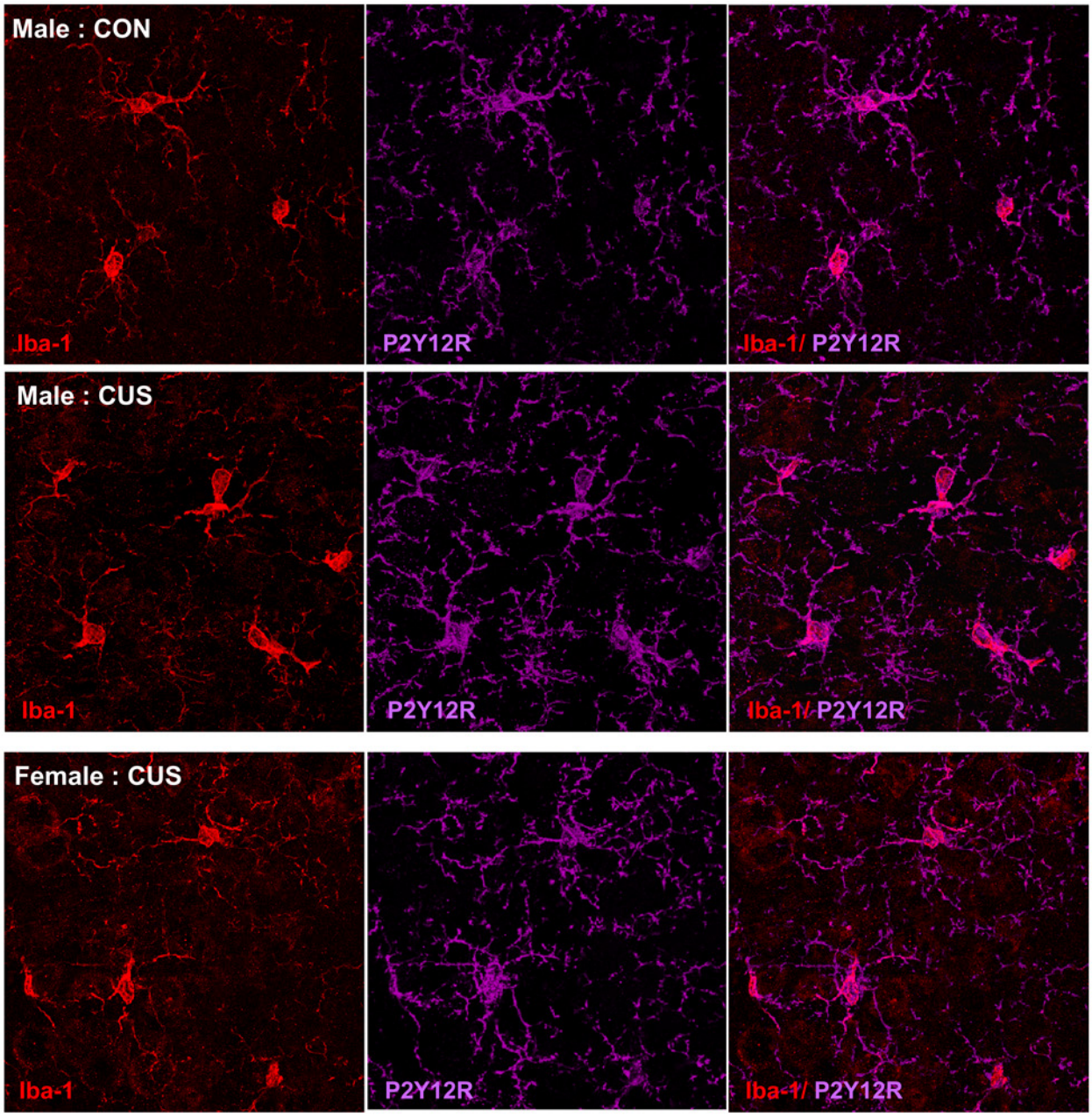

Figure S2. Microglia (Iba-1+) in the medial PFC of control and CUS mice co-localize with resident microglia-specific marker P2Y12 receptor.

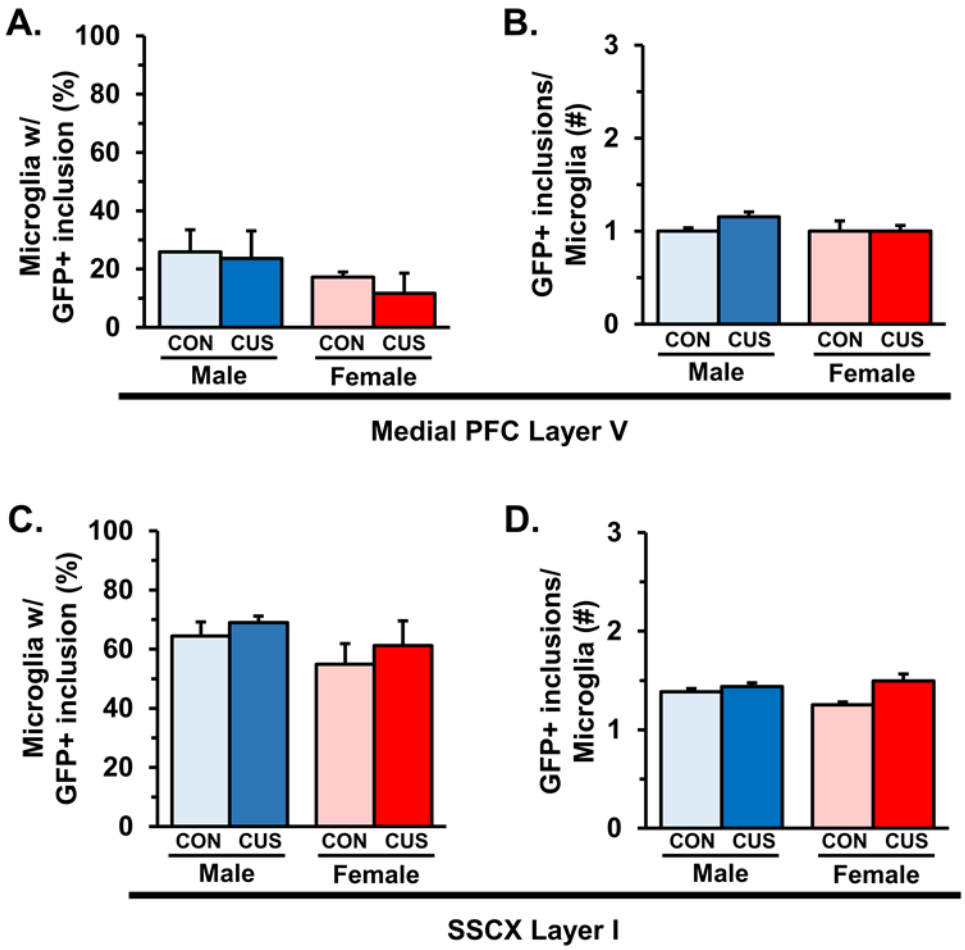

Figure S3. Chronic unpredictable stress did not induce microglia-mediated dendritic remodeling in medial PFC layer V or somatosensory cortex (SSCX) layer I of male or female mice.

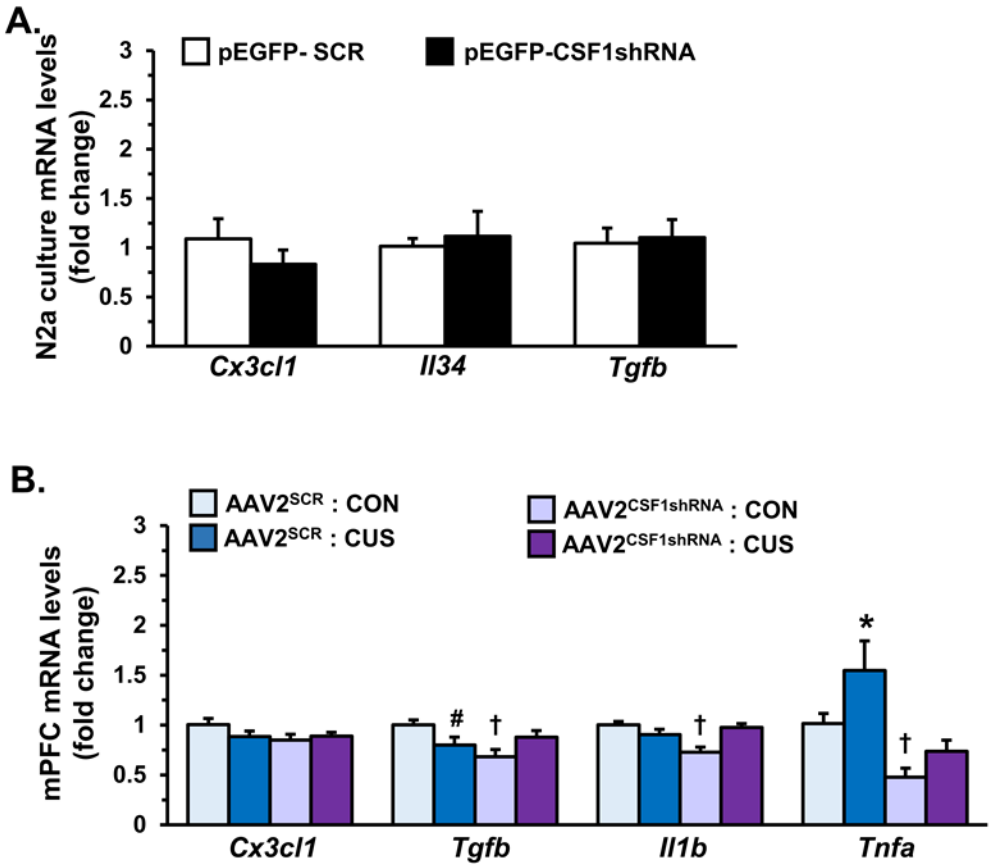

Figure S4. pEGFP-CSF1shRNA did not change expression of neuroimmune factors in N2a cells, but AAV2-CSF1shRNA shifted neuroimmune factors in the medial PFC.

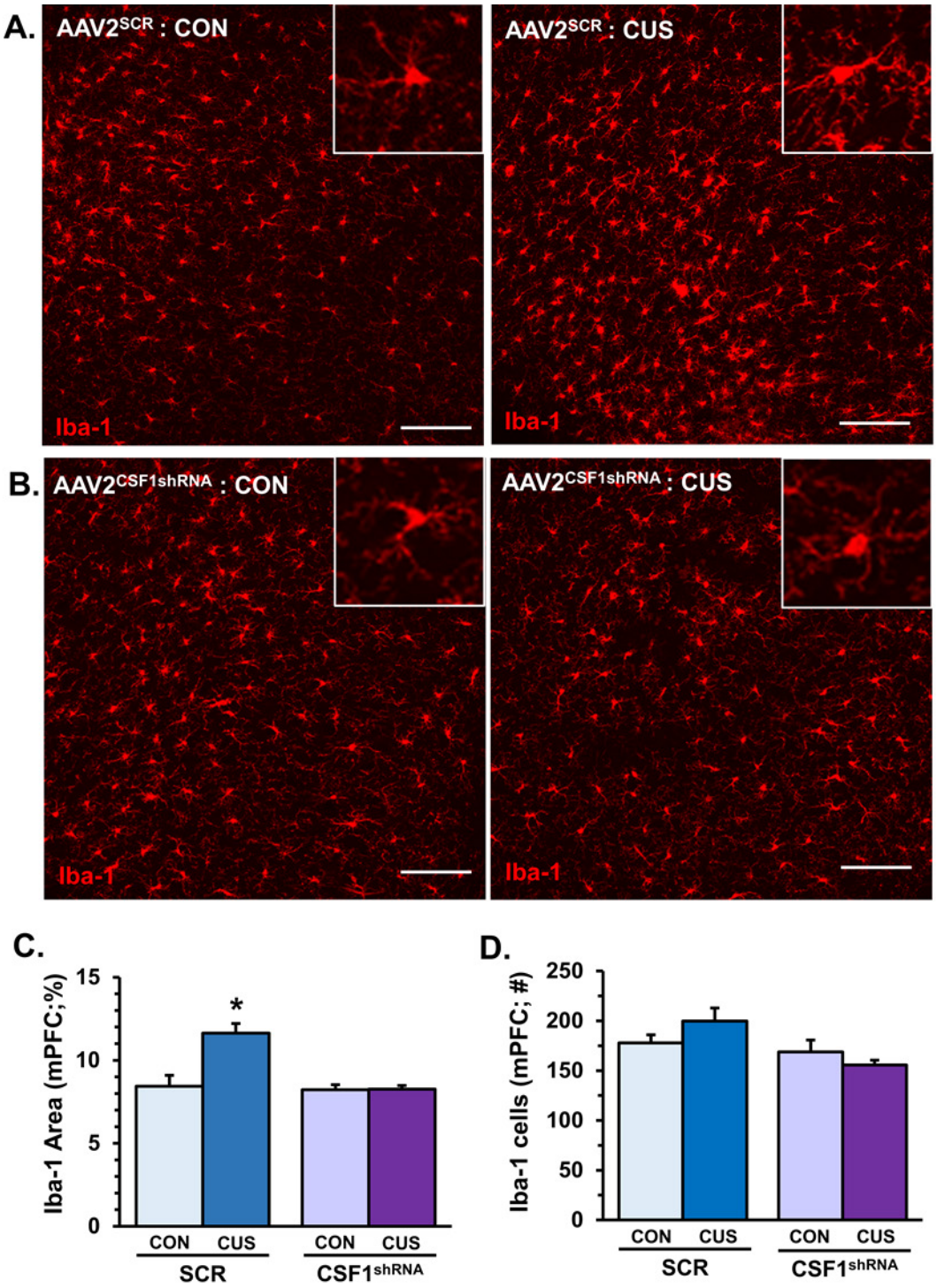

Figure S5. AAV2-CSF1shRNA attenuated alterations in microglia morphology observed in the medial PFC following chronic unpredictable stress.