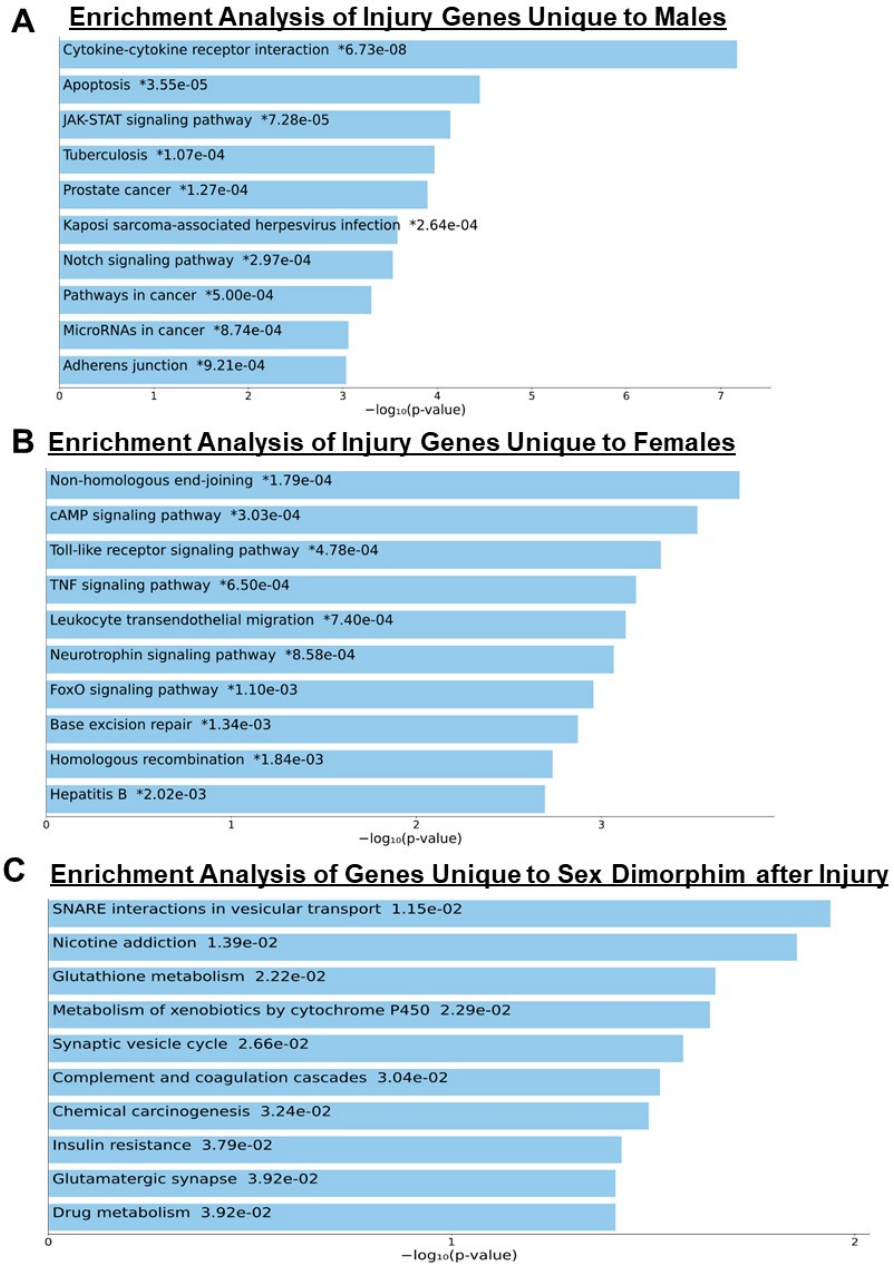
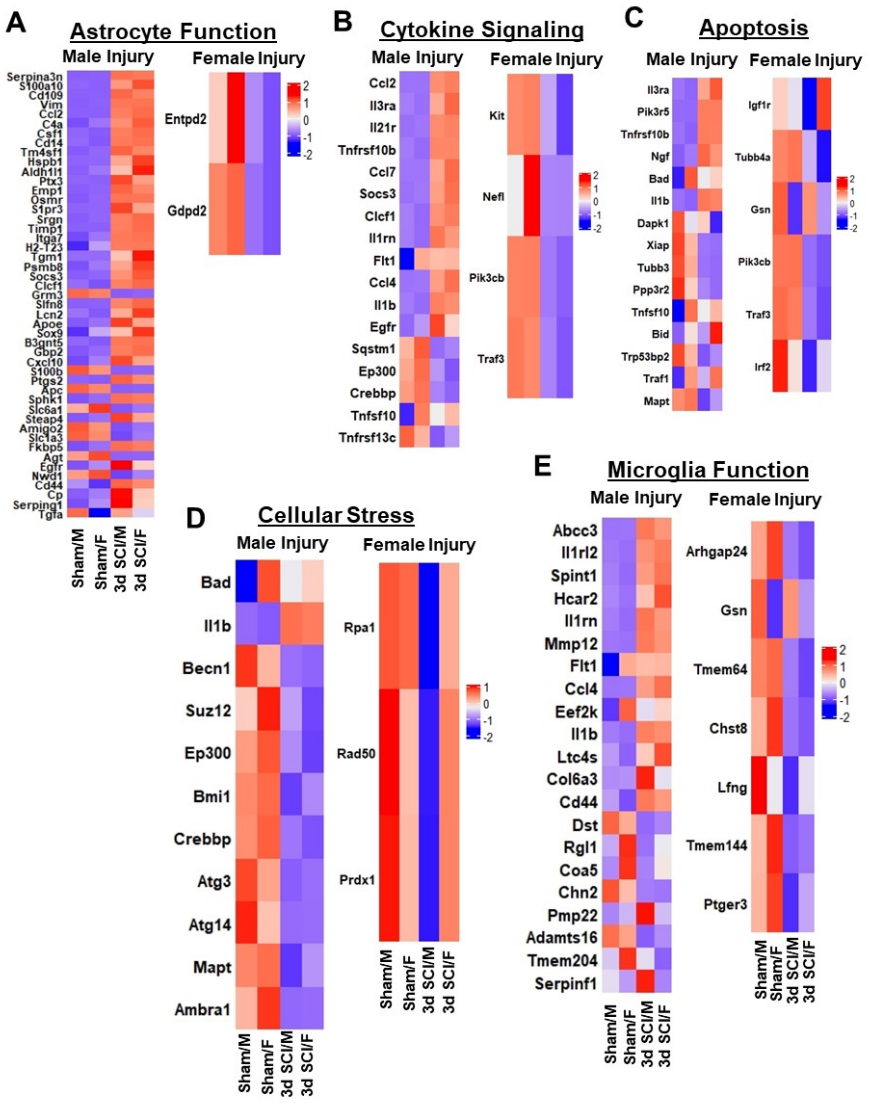
Supplemental information

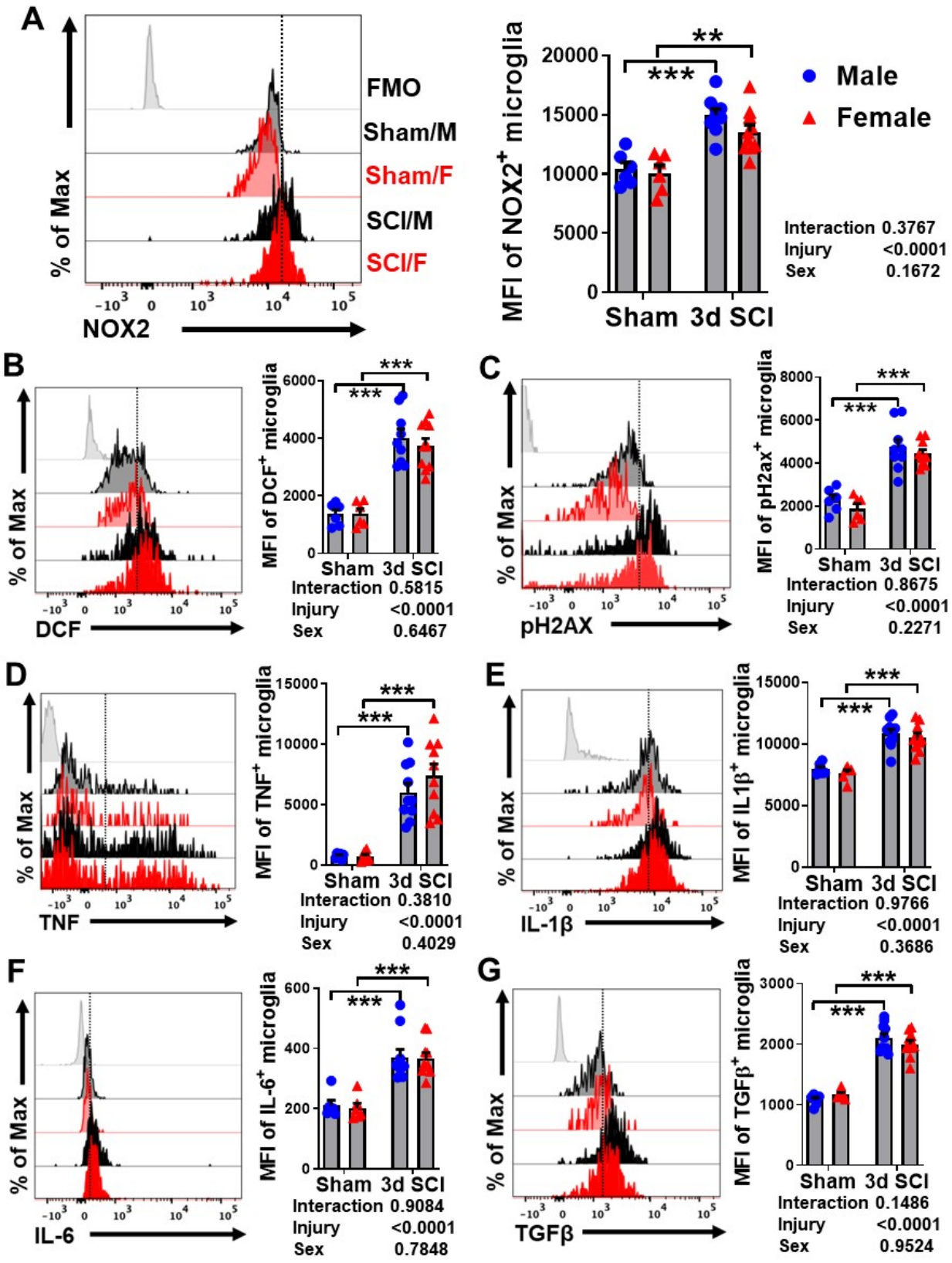
Supplemental Information includes Supplemental six figures and figure legends.



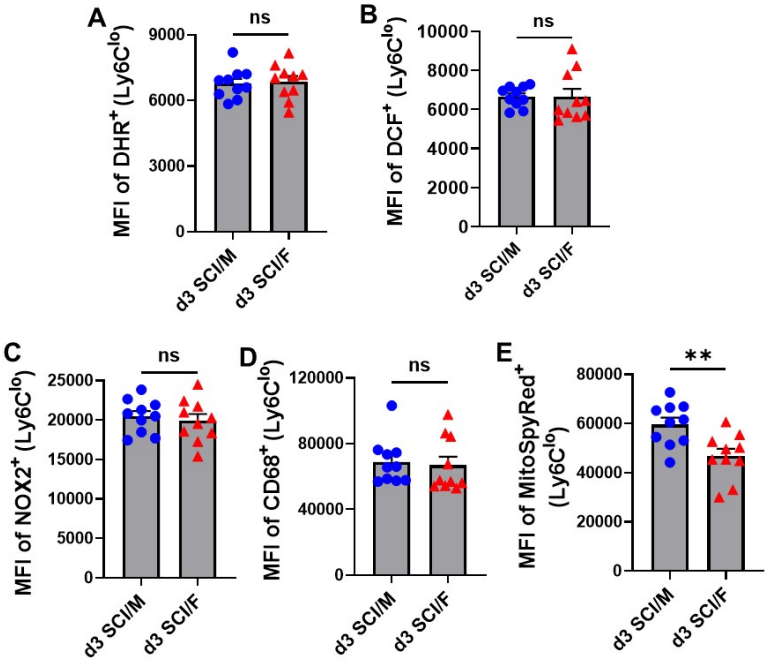
Supplementary Figure 1. Enrichment analysis of genes unique to sex after injury. **(A)** Enrichment analysis of injury genes unique to male mice show a high content of genes related to Cytokine-Cytokine Receptor Interaction and Apoptosis. The length of the bar represents the significance of that specific gene-set or term. Greater statistical significance is denoted by the increased brightness of the bar color. **(B)** Enrichment analysis of injury genes unique to female mice show a high content of genes related to Non-Homologous End-Joining and cAMP Signaling pathway. **(C)** Pathway analysis of genes unique to sex dimorphism after injury. N=3-4 mice/group.



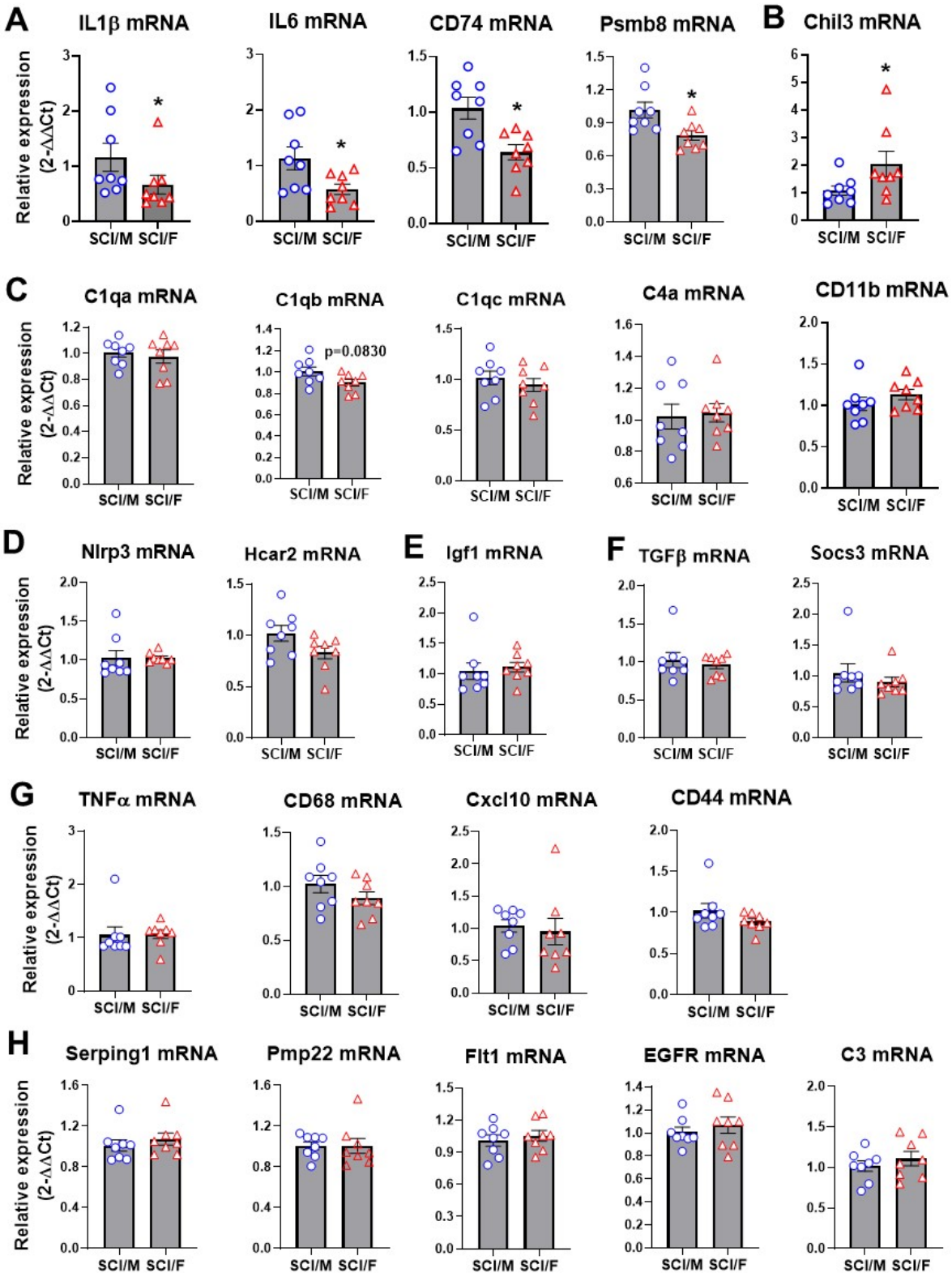
Supplementary Figure 2. Sex dimorphism differentially modifies genes related to astrocyte function, cytokine signaling, cellular stress and microglia function in acute stages of SCI. (**A**) Heatmap of genes related to Astrocyte Function that are DE by injury in male mice but not in females and vice versa. Color coding was based on z-score scaling. (**B**) Heatmap of genes related to Cytokine Signaling that are DE by injury and male specific (left) or female specific (right). (**C**) Heatmap of genes related to Apoptosis that are DE by injury and male specific (left) or female specific (right). (**D**) Heatmap of genes related to Cellular Stress that are DE by injury and male specific (left) or female specific (right). (**E**) Heatmap of genes related to Microglia Function that are DE by injury and male specific (left) or female specific (right). N=3-4 mice/group.


A
FMO
Sham/M
Sham/F
SCI/M
SCI/F
% of Max
NOX2
MFI of NOX2+ microglia
Male
Female
Interaction 0.3767
Injury <0.0001
Sex 0.1672
Sham 3d SCI
B
DCF
MFI of DCF+ microglia
Interaction 0.5815
Injury <0.0001
Sex 0.6467
C
pH2AX
MFI of pH2ax+ microglia
Interaction 0.8675
Injury <0.0001
Sex 0.2271
D
TNF
MFI of TNF+ microglia
Interaction 0.3810
Injury <0.0001
Sex 0.4029
E
IL-1β
MFI of IL1β+ microglia
Interaction 0.9766
Injury <0.0001
Sex 0.3686
F
IL-6
MFI of IL-6+ microglia
Interaction 0.9084
Injury <0.0001
Sex 0.7848
G
TGFβ
MFI of TGFβ+ microglia
Interaction 0.1486
Injury <0.0001
Sex 0.9524

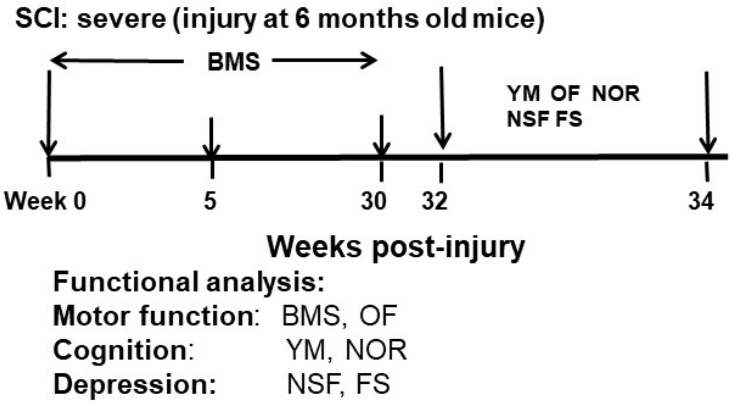
Supplementary Figure 3. Microglial production of reactive oxygen species and inflammatory cytokines during acute spinal cord injury. Microglial activity in male and female mice at 3d post-SCI was measured using flow cytometry. Representative histograms and subsequent MFI quantification of **(A)** NOX2 protein expression, **(B)** reactive oxygen species production, **(C)** DNA damage, and production of **(D)** TNF, **(E)** IL-1β, **(F)** IL-6 and (**G**) TFGβ cytokines in $CD45^{int}CD11b^{+}$ microglia are shown. Significant differences were seen after injury, but not between sexes. For each sex, N=6 mice per sham group, and N=10 mice per SCI group. ***p<0.001 vs. Sham group; Two-way ANOVA following Tukey's multiple comparisons test. Abbreviations: d (day), FMO (fluorescence minus one), Max (maximum), MFI (mean fluorescence intensity), SCI (spinal cord injury), μm (micrometer).



Supplementary Figure 4. Sex differences in infiltrating $CD45^{hi}CD11b^{+}Ly6C^{lo}$ monocytes at 3 days post-injury. (A-B) No sex differences were seen in ROS production as evidenced by both MFI of DHR^{+} and DCF^{+} $Ly6C^{lo}$ monocytes. **(C)** MFI quantification of NOX2 protein expression. **(D)** MFI quantification of CD68-positive $Ly6C^{lo}$ monocytes. **(E)** MFI of mitochondrial membrane potential in $Ly6C^{lo}$ monocytes using $MitoSpyRed^{+}$ was significantly lower in females vs. males after SCI. N=10 mice per SCI group. **p<0.01 vs. SCI/M with unpaired t test.


A
IL1β mRNA
IL6 mRNA
CD74 mRNA
Psmb8 mRNA
B
Chil3 mRNA
Relative expression (2-ΔΔCt)
SCI/M SCI/F
C
C1qa mRNA
C1qb mRNA
p=0.0830
C1qc mRNA
C4a mRNA
CD11b mRNA
D
Nlrp3 mRNA
Hcar2 mRNA
E
Igf1 mRNA
F
TGFβ mRNA
Socs3 mRNA
G
TNFα mRNA
CD68 mRNA
Cxcl10 mRNA
CD44 mRNA
H
Serping1 mRNA
Pmp22 mRNA
Flt1 mRNA
EGFR mRNA
C3 mRNA

Supplementary Figure 5. Middle-age alters inflammatory genes in response to acute SCI. Six month-old male and female mice were subjected to SCI and spinal cord samples (~5-mm segments) were collected at 3 d post-injury and processed for RNA extraction and a list of inflammatory genes qPCR analysis. **(A)** Pro-inflammatory genes (IL1β, IL6, CD74, Psmb8) were downregulated in SCI/F mice compared to SCI/M animals. **(B)** Anti-inflammatory gene Chil3 (Ym1) expression was increased in SCI/F group vs SCI/M mice. **(C-D)** Complement pathway genes (C1qa, C1qb, C1qc, C4a), and Nlrp3, Hcar2 (hydroxycarboxylic acid receptor 2), and CD11b genes that were elevated in young SCI/F mice showed no differential changes between SCI/M and SCI/F in middle-age mice. **(E)** Igf1 (insulin-like growth factor I) mRNA expression that was significantly downregulated in young SCI/F mice showed no alteration between SCI/M and SCI/F in middle-age mice. **(F-H)** There were no differential changes between SCI/F and SCI/M groups in anti-inflammatory genes (TGFβ, Socs3), pro-inflammatory genes (TNFα, CD68, Cxcl10, CD44, Pmp22, C3, Flt1), and astrocyte function (Serping1, Egfr). Gene expression was normalized by GAPDH and expressed as a fold-change relative to sham vehicle control. n=8 mice/group with Mann Whitney test, *p < 0.05.



Supplementary Figure 6. Age-matched male and female C57BL/6 mice at 6 months old were subjected to severe (70 kdyn) SCI followed by 8 months post-injury. Diagram illustrating timepoints by weeks post-injury and behavior experiments conducted after the initial injury. All behavior tests are categorized by the functional outcome that was examined.