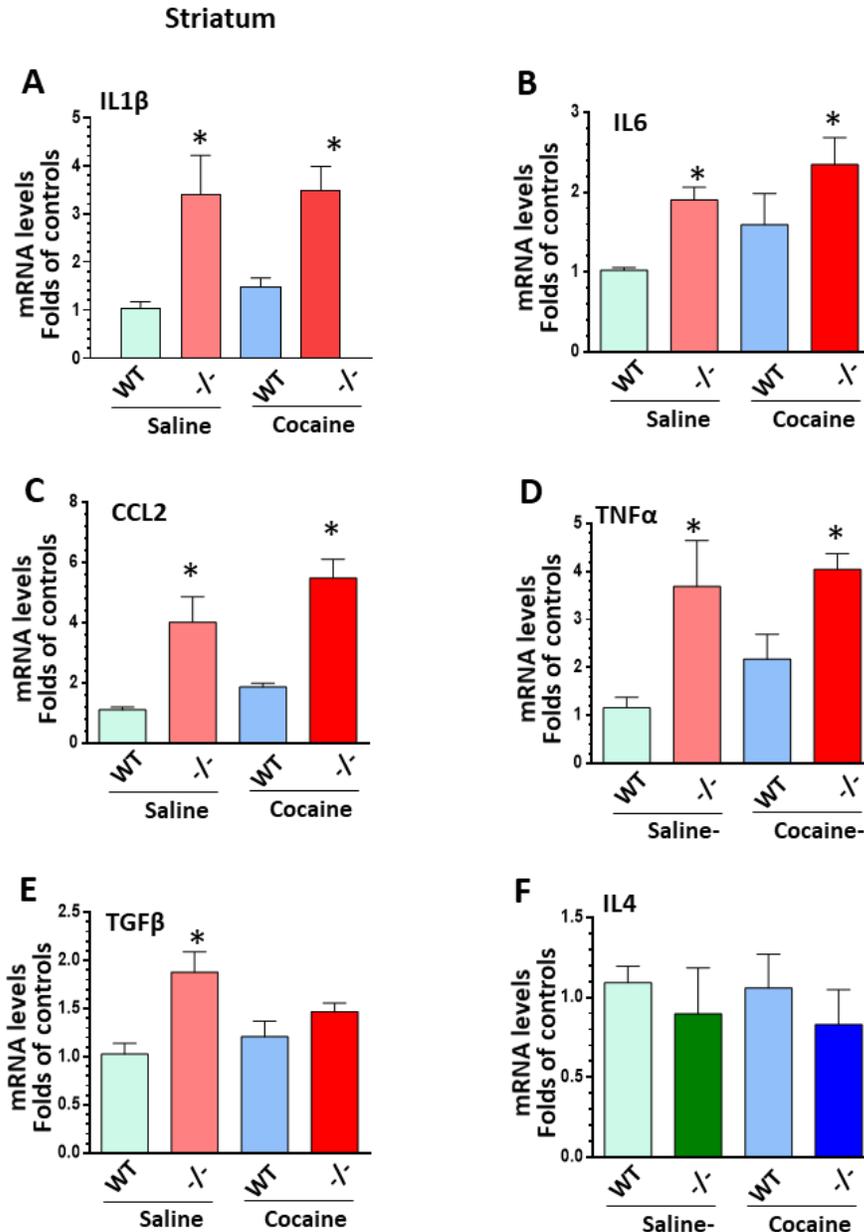
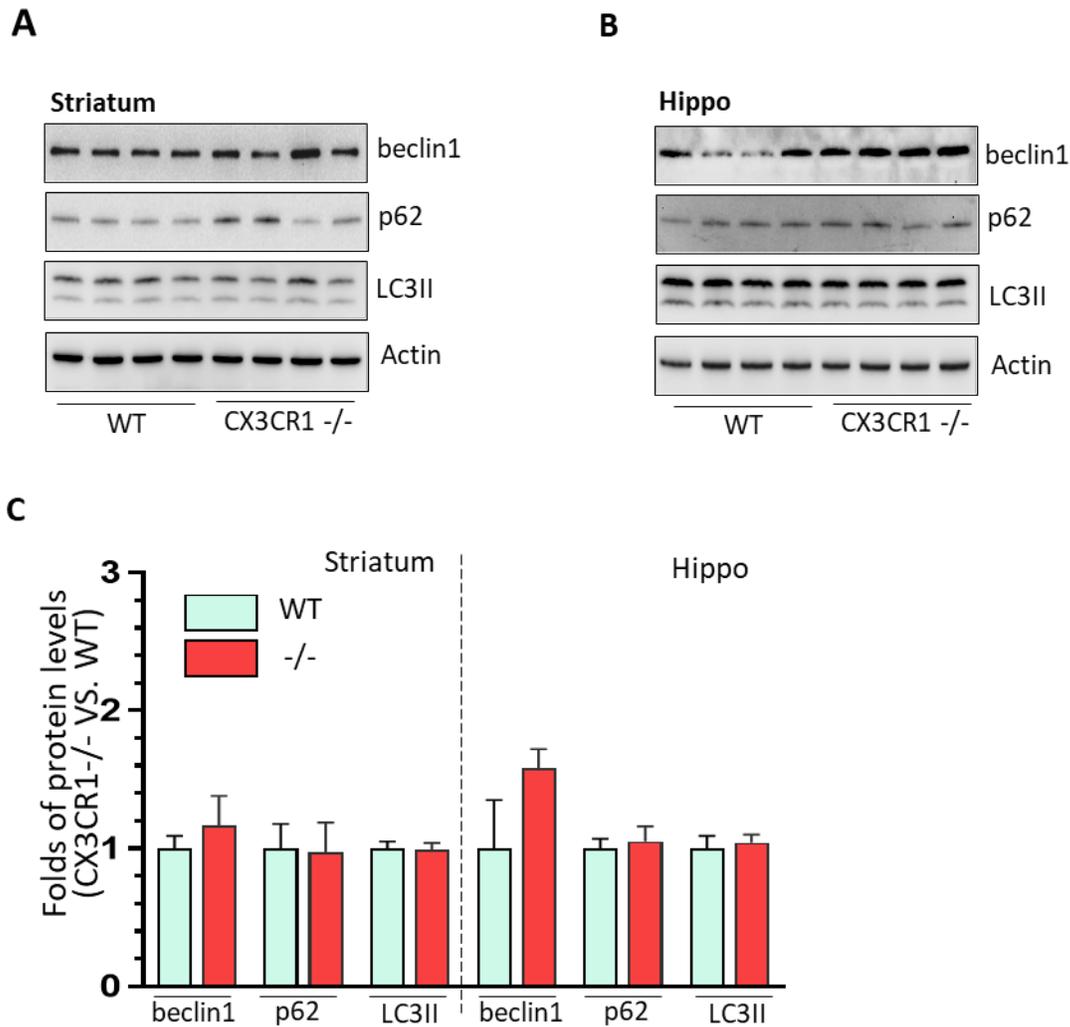


Supplementary Figure S1. One-time cocaine injection did not induce significant difference on the locomotor activity between CX3CR1^{-/-} mice and WT controls (n = 6 - 8; one-way ANOVA analysis. * P < 0.05, group of mice with cocaine vs. group of WT with saline. There was no significant difference between WT mice and CX3CR1^{-/-} mice with one-time injection of cocaine.

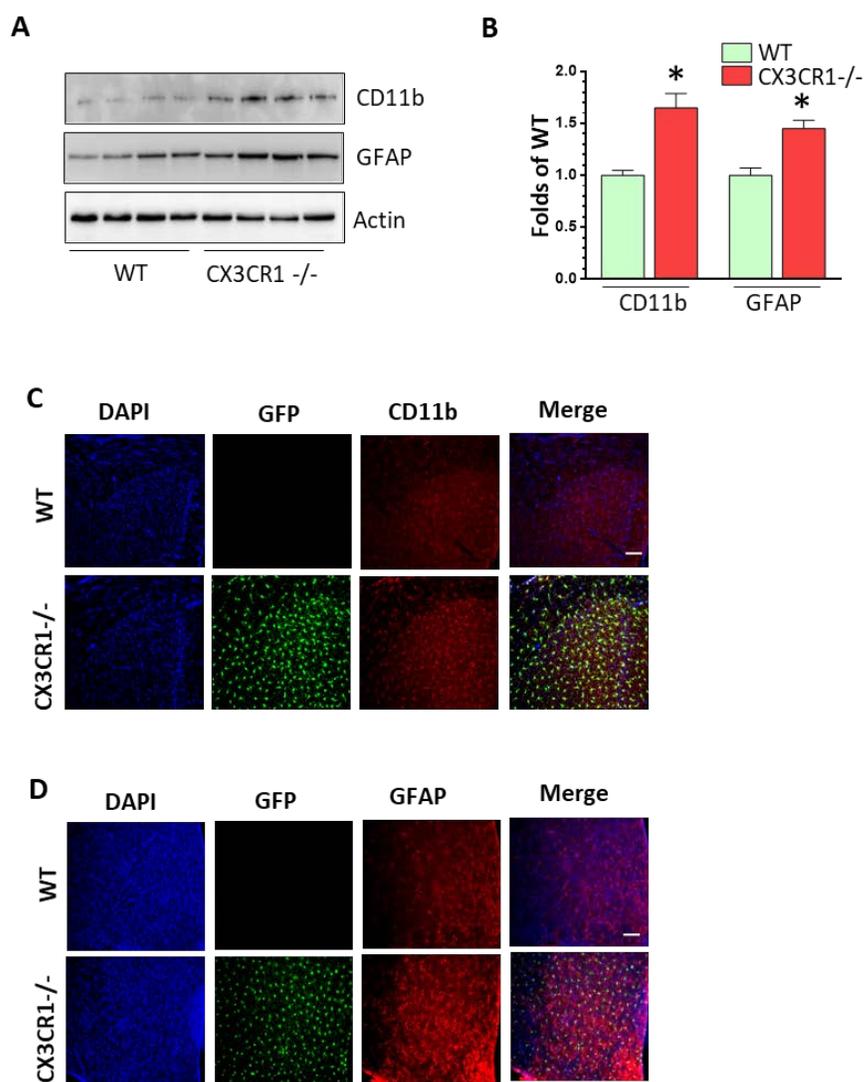


Supplementary Figure S2. The expression levels of pro-inflammatory and anti-inflammatory mediators in the striatum of CX3CR1^{-/-} and WT mice. (A) CX3CR1^{-/-} mice showed increased mRNA levels of IL1 β in both cocaine-treated conditions and basal levels (n = 4 - 5, * P < 0.05). GAPDH was served as internal controls. (B) CX3CR1^{-/-} mice showed increased mRNA levels of IL6 in both cocaine-treated conditions and basal levels (n = 4 - 5, * P < 0.05). GAPDH was served as internal controls. (C) CX3CR1^{-/-} mice showed increased mRNA levels of ccl2 in both cocaine-treated conditions and basal levels (n = 4 - 5, * P < 0.05). GAPDH was served as internal controls. (D) CX3CR1^{-/-} mice showed increased mRNA levels of TNF α in both cocaine-treated conditions and basal levels (n = 4 - 5, * P < 0.05). GAPDH was served as internal controls. (E) CX3CR1^{-/-}

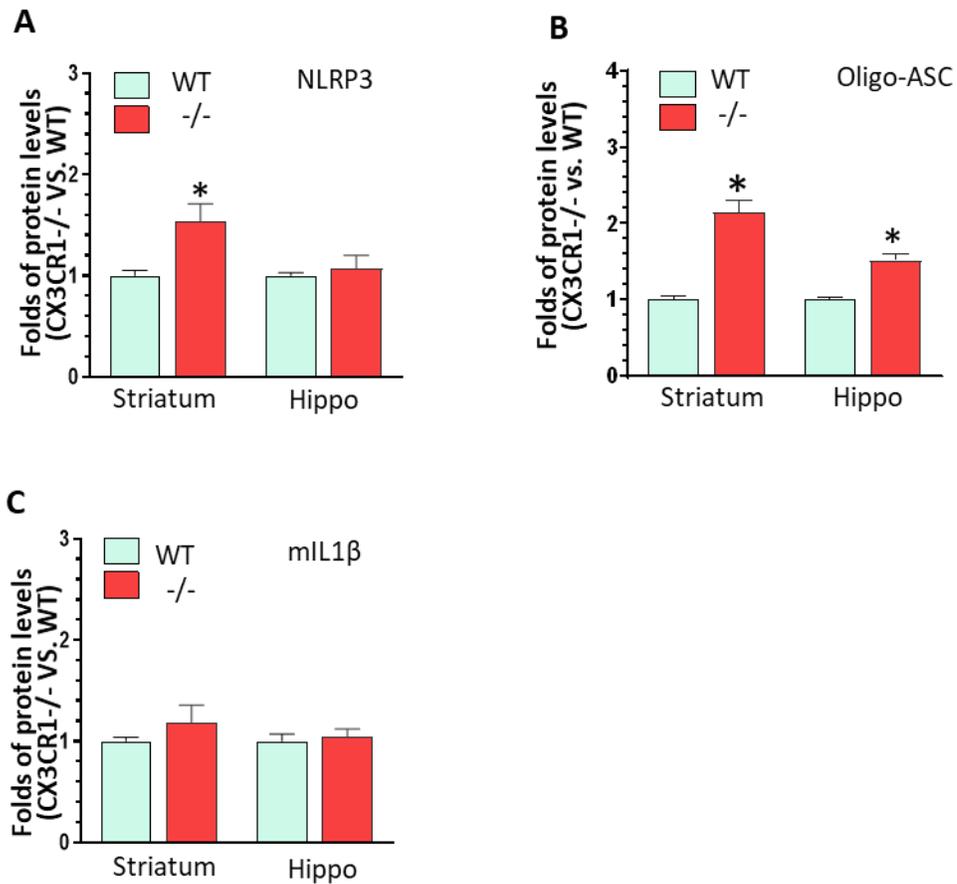
-/- mice showed increased mRNA levels of TGF β in basal levels but not in cocaine-treated conditions (n = 4 - 5, * P < 0.05). GAPDH was served as internal controls. (F) CX3CR1 *-/-* mice showed no significantly changes on mRNA levels of IL4 in both cocaine-treated conditions and basal levels (n = 4 - 5, * P > 0.05). GAPDH was served as internal controls.



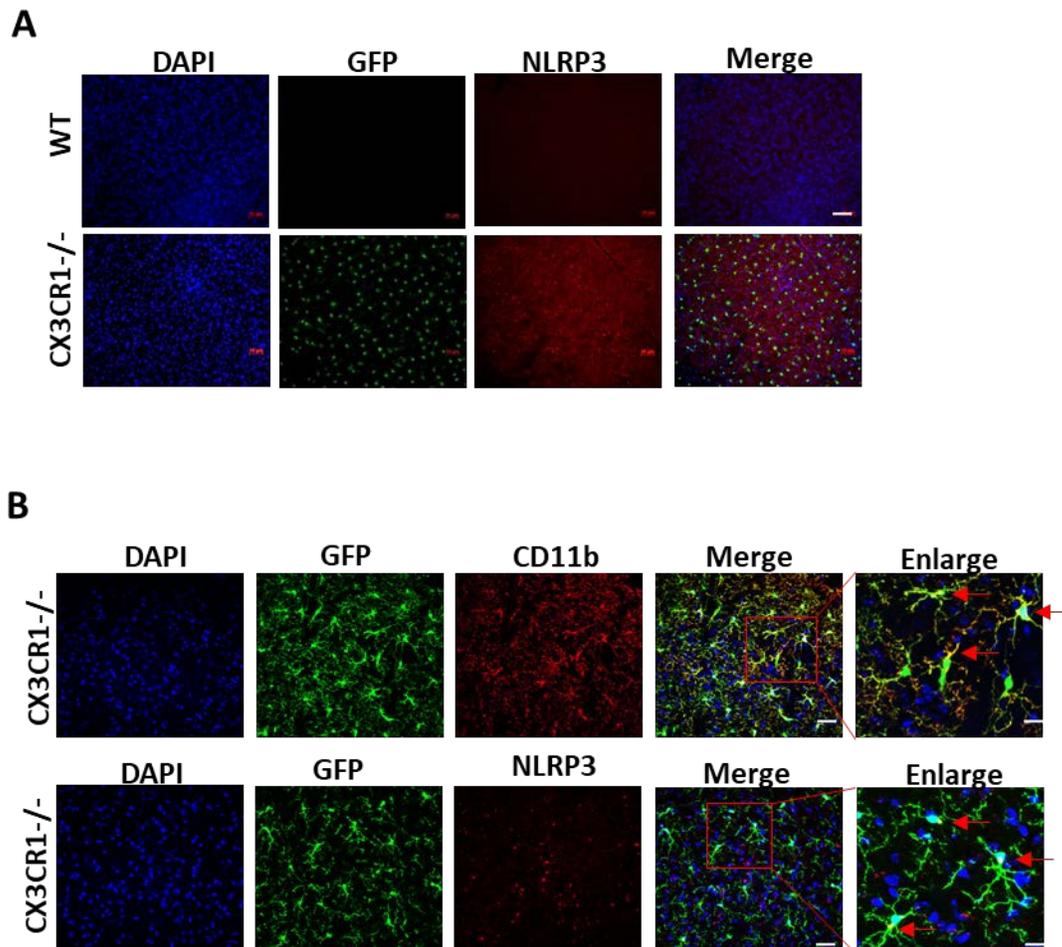
Supplementary Figure S3. The expression levels of autophagic markers in the brains of CX3CR1^{-/-} and WT mice. **(A)** Representative WBs showed no difference on the expression levels of beclin1, p62, and LC3II in the striatum of CX3CR1^{-/-} mice compared to WT mice with cocaine. The levels of β -actin were served as internal controls for equal protein loading (n = 4–6, P > 0.05). **(B)** Representative WBs showed no difference on the expression levels of beclin1, p62, and LC3II in the hippocampus of CX3CR1^{-/-} mice compared to WT mice with cocaine. The levels of β -actin were served as internal controls for equal protein loading (n = 4–6, P > 0.05). **(C)** The statistical results for the expression levels of beclin1, p62, and LC3II in the striatum and hippocampus of CX3CR1^{-/-} and WT mice.



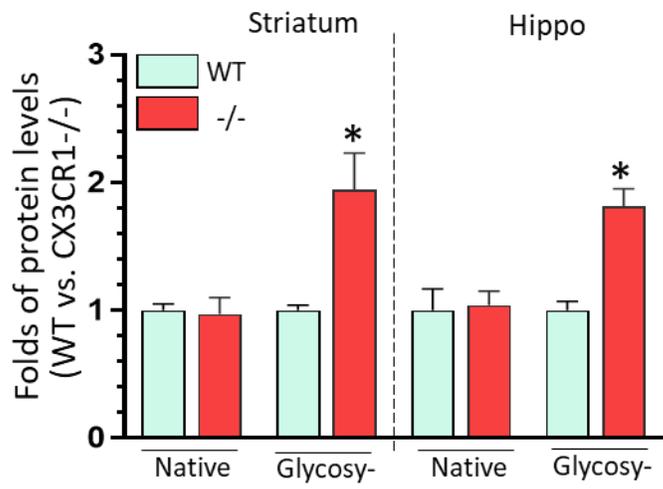
Supplementary Figure S4. CX3CR1^{-/-} mice showed increased microglia and astrocyte activation levels compared to WT mice under basal conditions. (A) Representative WBs showed significant increase on CD11b and GFAP levels in the striatum of CX3CR1^{-/-} mice compared to WT mice without cocaine administration. The levels of β -actin were served as internal controls for equal protein loading (n = 4 - 6, * P < 0.05). (B) Statistic results for WBs shown in (A). (C) Immunostaining results showed increased signal intensity of CD11b in CX3CR1^{-/-} mice compared to WT mice. CD11b was co-localized to GFP signal in CX3CR1^{-/-} mice. Scale bar: 10 μ M. (D) Immunostaining results showed increased signal intensity of GFAP in CX3CR1^{-/-} mice compared to WT mice. GFAP was not co-localized to GFP signal in CX3CR1^{-/-} mice. Scale bar: 10 μ M.



Supplementary Figure S5. CX3CR1 deficiency primed NLRP3 inflammasome in the brain under basal conditions. (A) Statistic results showed that CX3CR1 deficiency increased the NLRP3 levels in the striatum under basal conditions (n = 4 - 6, * P < 0.05). (B) Statistic results showed that CX3CR1 deficiency increased the ASC oligomerization in the striatum and hippocampus under basal conditions (n = 4 - 6, * P < 0.05). (C) Statistic results showed that CX3CR1 deficiency had no impact on the production of mature IL1 β in the striatum and hippocampus under basal conditions (n = 4 - 6, P > 0.05).



Supplementary Figure S6. CX3CR1 deficiency increased NLRP3 levels in the brain under basal conditions. (A) Immunostaining results showed increased signal intensity of NLRP3 in CX3CR1^{-/-} mice compared to WT mice. Scale bar: 10 μ M. (B) Enlarged images confirmed that NLRP3 co-localized with GFP in the brains of CX3CR1^{-/-} mice. Scale bar: 2 μ M. The co-localization of CD11b and GFP was served as positive controls.



Supplementary Figure S7. CX3CR1 deficiency increased glycosylated LAMP2 levels in the brain under basal conditions (n = 4 - 6, * P < 0.05).

Supplementary Table S1. The summary findings of WT and CX3CR1 ^{-/-} mice with saline or cocaine injections.

First hit Second hit	CX3CR1 ^{-/-} vs.WT Saline	CX3CR1 ^{-/-} -VS.WT cocaine
mRNA levels		
IL1beta	up	no change
IL6	up	no change
TNFa	up	no change
ccl2	up	no change
behavioral tests		
locomotor activity	no change	up
CPP	no change	up
NLRP3 signaling		
NLRP3 levels	up (sriatum)	up (both regions)
oligo ASC	up (both regions)	up (both regions)
mCasp 1	no change	up (both regions)
glycosylated LAMP2	up (both regions)	up (both regions)
mCat D	up (sriatum)	up (both regions)
mIL1beta	no change	up (both regions)