Supplementary Information

CX3CR1 deficiency exacerbates immune-mediated hepatitis by increasing NF-κB-mediated cytokine production in macrophage and T cell

Mi et al.

Supplementary Figure1

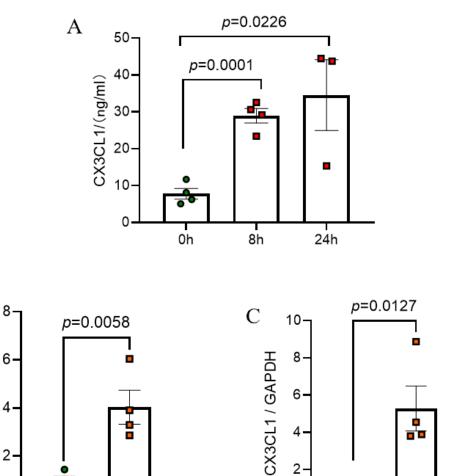
В

CX3CR1 / GAPDH

0

PBS

ConA



Supplementary Figure 1. Serum level of CX3CL1. 15mg/kg body weight ConA were injected in 8 weeks C57BL/6J mice. Serum levels of CX3CL1(A) were measured by ELISA kit, and mRNA level of CX3CL1(B) and CX3CR1(C) were measured by PT-PCR at 8 hours after injection.

2

0

PBS

ConA

BMDMs

Age-matched and sex-matched $Cx3cr1^{+/+}$ and $Cx3cr1^{+/+}$ mice were euthanized by CO_2 . Separate tibiae and femurs carefully, Cut the top end with sterile scissor, flushed the bone marrow with PBS into a sterile tube. Remove red blood cells with 5ml 1x red blood cell Lysis Solution. Adjust the cell density to 2 x 10^6 cells/ml in DMEM contain 20ng/ml CSF-1(PEPROTECH, 315-02). Seed bone marrow cells in 10cm petri dish, incubate at 37°C in 5% CO2 atmosphere. On the next day, Add more 5ml differentiation medium in each dish. The bone marrow derived macrophage was ready on day 7.

Resveratrol treatment before ConA

Resveratrol (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) at 200mg/ml, and store in 4°C. To inhibit the phosphorylation of NF-KB p65, mice was gavaged with resveratrol 30ug per g body weight per day, for 7 days before ConA. Mice was injected with 13 mg/ body weight ConA immediately after the last dose resveratrol, and sacrificed at 24 hours after ConA. Low dose ConA here to reduced mortality.

Supplementary Table 1. Antibodies for flow cytometry

Antibody	Clone	Cat Number	Company
CD45-FITC	30-F11	11-0451-82	eBioscience
CD45-e450	30-F11	48-0451-82	eBioscience
CD11b-PE-Cy7	M1/70	25-0112-81	eBioscience
Ly6C-e450	HK1.4	48593282	eBioscience
Ly6G-APC	1A8-Ly6g	17-9668-80	eBioscience
CD11c-PE	N418	12-0114-81	eBioscience
CD3-APC	17A2	17003282	eBioscience
CD4-e605	RM4-5	83-0042-42	eBioscience
CD8-e450	53-6.7	48-0081-82	eBioscience
NK1.1- FITC	PK136	11-5941-85	eBioscience
NK1.1	PK136	108713	Biolegend
CD1d-PE	1B1	12-0011-82	eBioscience
CCR2-APC	SA203G11	150627	Biolegend
CX3CR1-FITC	SA011F11	149019	Biolegend
CD11b- PE-Cy7	M1/70	25-0112-81	eBioscience
CD19-PE	6D5	115507	Biolegend
F4/80-e605	BM8	123133	Biolegend