

Supplementary information

Adeno-associated Virus Vector-mediated Interleukin-10 Induction Prevents Vascular Inflammation in a Murine Model of Kawasaki Disease

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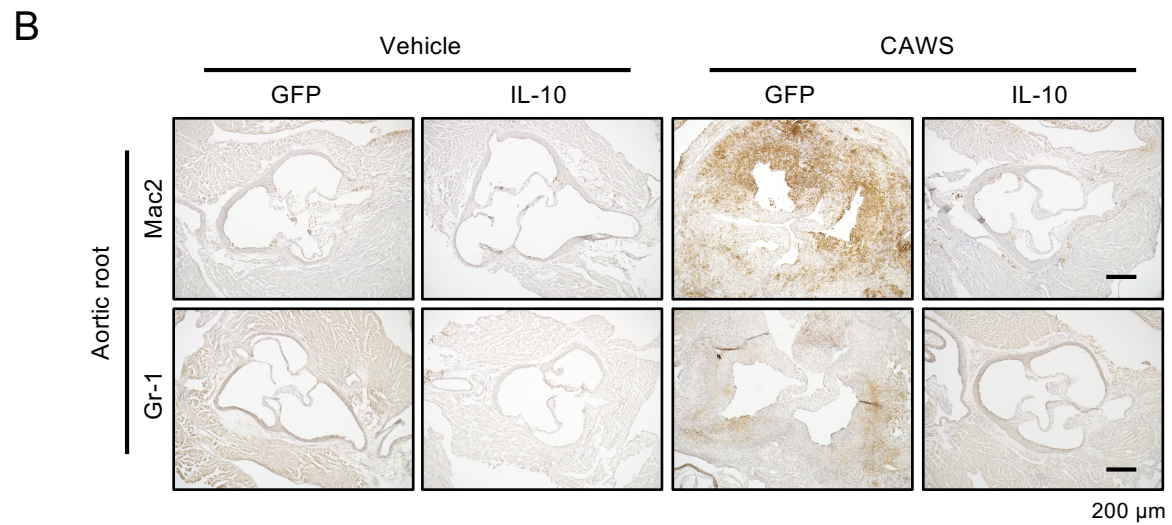
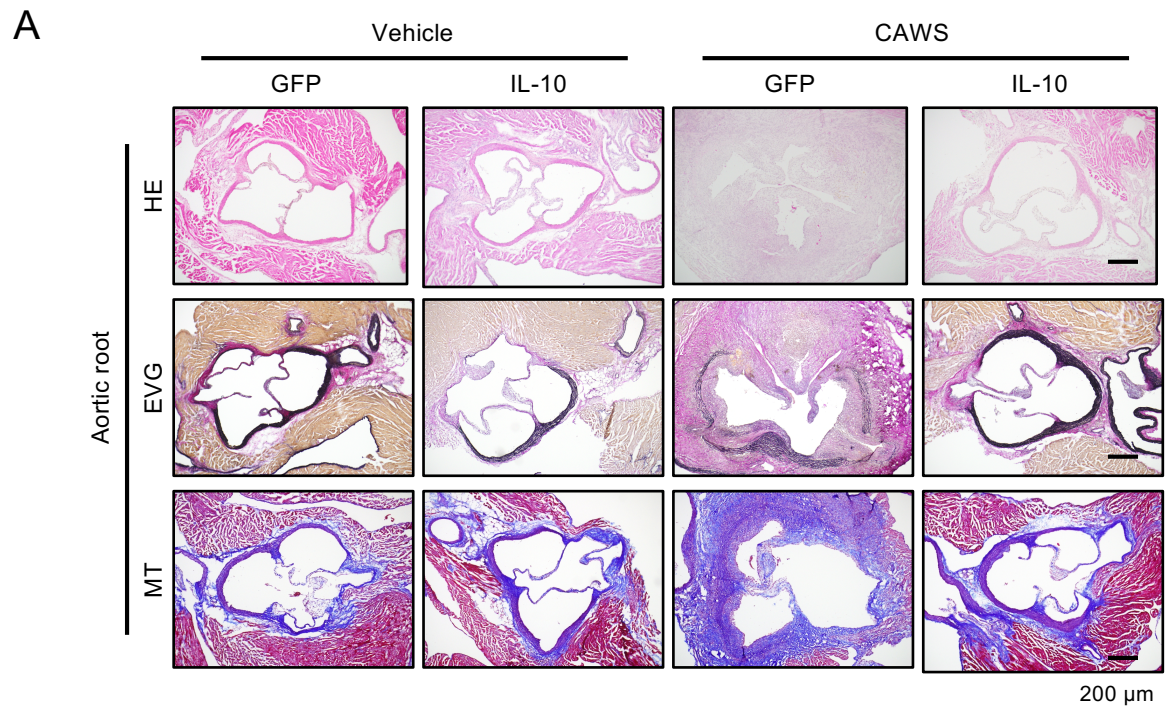
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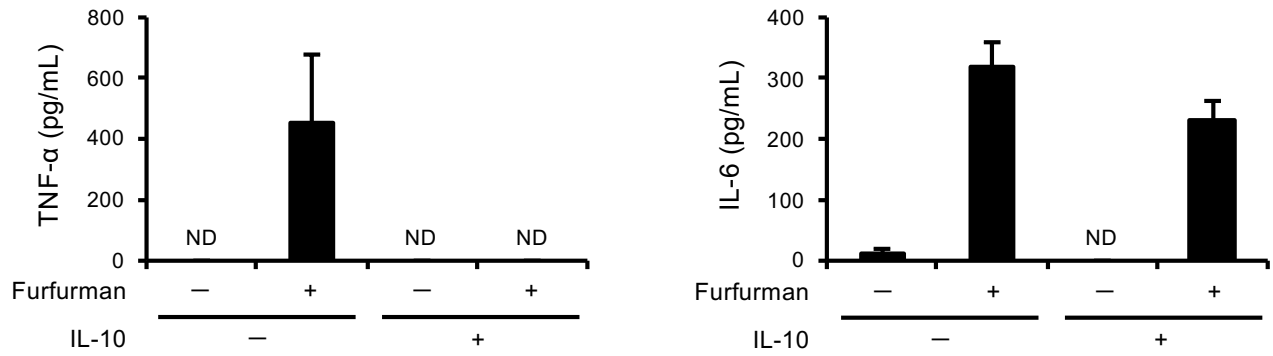
Supplementary table. List of the primers used in this study

Gene	Forward	Reverse
<i>Tnfa</i>	5'-CCCCAAAGGGATGAGAAGTTC-3'	5'-GCTTGTCACCTCGAATTTTGAGAA-3'
<i>Il6</i>	5'-ACAACCACGGCCTTCCCTACTT-3'	5'-CACGATTTCCCAGAGAACATGTG-3'
<i>Il1b</i>	5'-TGAAGTTGACGGACCCCAA-3'	5'-TGATGTGCTGCTGTGAGATT-3'
<i>Il17a</i>	5'-ACGCGCAAACATGAGTCCAG-3'	5'-CTCAGCAGCAGCAACAGCATC-3'
<i>Ccl2</i>	5'-GGCTCAGCCAGATGCAGTTAAC-3'	5'-GCCTACTCATTGGGATCATCTTG-3'
<i>Ccr2</i>	5'-CCTGCAAAGACCAGAAGAGGG-3'	5'-GAGATGTTGATAGTATGCCGTGGA-3'
<i>Mmp2</i>	5'-GACATACATCTTTGCAGGAGACAAG-3'	5'-TCTGCGATGAGCTTAGGGAAA-3'
<i>Mmp9</i>	5'-CCTGGAACACACGACATCTTC-3'	5'-TGGAAACTCACACGCCAGAA-3'
<i>Timp1</i>	5'-GTAAGGCCTGTAGCTGTGCC-3'	5'-AGGTGGTCTCGTTGATTCT-3'
<i>Colla1</i>	5'-GAGCGGAGAGTACTGGATCG-3'	5'-GTTCCGGCTGATGTACCAGT-3'
<i>Col3a1</i>	5'-CCCAACCCAGAGATCCCATT-3'	5'-GAAGCACAGGAGCAGGTGTAGA-3'
<i>Tgfb1</i>	5'-CTCCCGTGGCTTCTAGTGC-3'	5'-GCCTTAGTTTGGACAGGATCTG-3'
<i>Clec4n</i>	5'-AAGCGGAGCAGAATTCATCA-3'	5'-CCATTTGCCATTACCTTGTTGGA-3'
<i>Clec7a</i>	5'-TGCTTTGTGGTAGTAGTGGTTGCT-3'	5'-TGTGGGCTTGTGGTTCTCTTT-3'
<i>Clec4e</i>	5'-CAGTGGCAATGGGTGGATGATTAC-3'	5'-AGTCCCTTATGGTGGCACAGTC-3'
<i>Csf2</i>	5'-TCGTCTCTAACGAGTTCTCCTT-3'	5'-CGTAGACCCTGCTCGAATATCT-3'
<i>Gapdh</i>	5'-TGTGTCCGTCGTGGATCTGA-3'	5'-TTCGTGTTGAAGTCGCAGGAG-3'



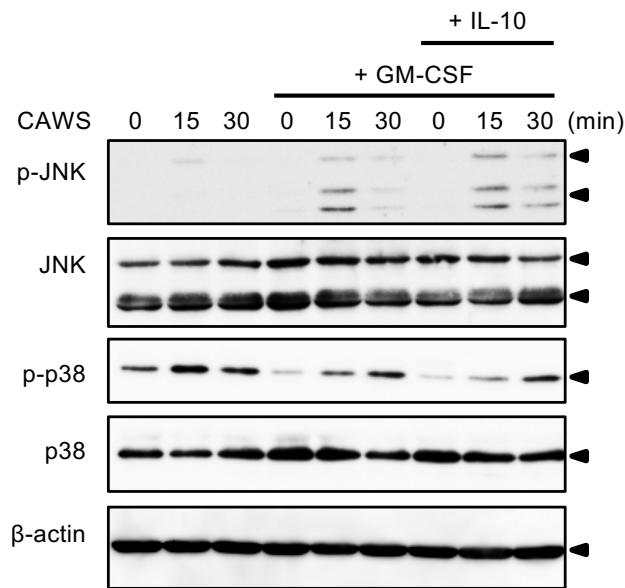
Supplementary figure S1. Histological analysis of the heart sections at 56 days after CAWS treatment

Mice were treated intraperitoneally with CAWS or vehicle, 2 weeks after the injection of AAV-GFP (control) or AAV-IL-10. Heart sections were obtained at day 56 after CAWS or vehicle treatment. (A) HE, EVG, and MT staining. (B) Mac2 and Gr-1 immunohistochemical staining. Representative images of the aortic root and coronary artery are shown.



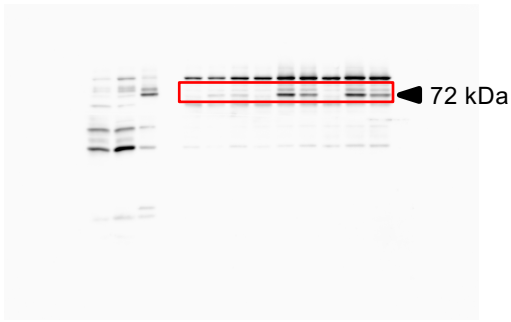
Supplementary figure S2. Effect of furfurman on the production of TNF- α and IL-6 in BMDCs

BMDCs were pretreated with or without IL-10 (40 ng/mL) for 1 h, and then treated with furfurman (1–10 μ g/mL) for 48 h. The TNF- α and IL-6 protein levels in the supernatants were assessed (n = 3).

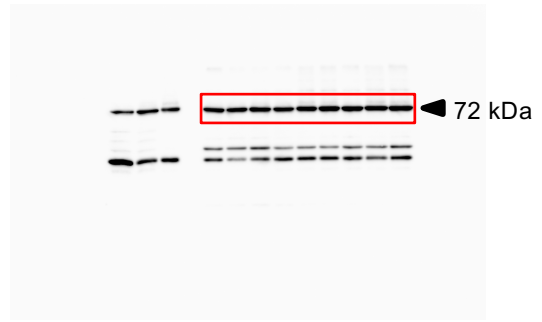


Supplementary figure S3. Effect of CAWS on the activation of JNK and p38

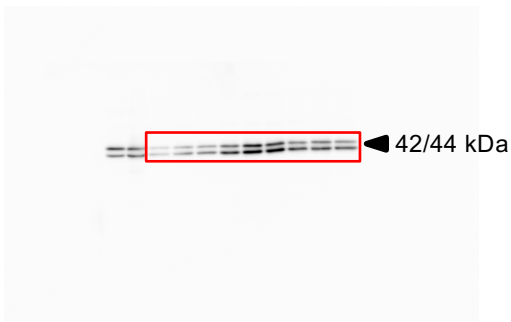
BMMs were treated with or without GM-CSF (1 ng/mL) for 48 h. The cells were pretreated with or without IL-10 (40 ng/mL) for 1 h, and then treated CAWS (10 μg/mL) for the indicated periods. Cell lysates were prepared and analyzed by Western blotting with antibodies against JNK, p-JNK, p38, and p-p38.



IB: anti-p-Syk



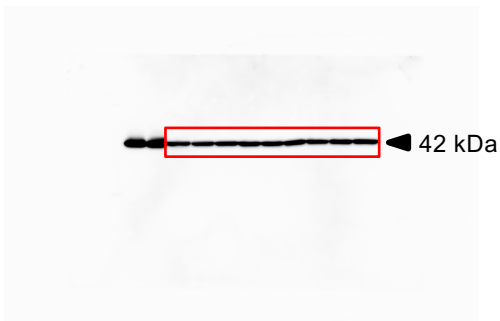
IB: anti-Syk



IB: anti-p-ERK1/2



IB: anti-ERK1/2



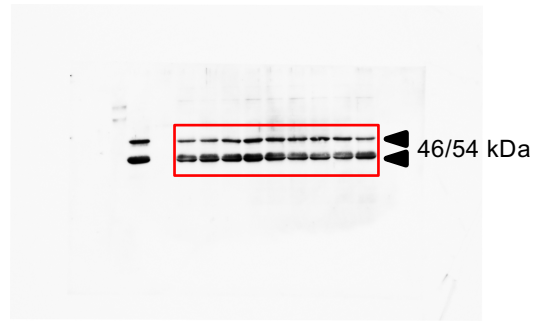
IB: anti-β-actin

Supplemental figure S4. Uncropped images of Western blotting in Figure 7C

Cell lysates were prepared and separated by SDS-PAGE and transferred to PVDH membrane. The membrane was immunoblotted with anti-p-Syk (72 kDa), Syk (72 kDa), p-ERK1/2 (42/44 kDa), ERK1/2 (42/44 kDa), or β-actin (42 kDa) antibodies. The cropped parts indicated by the boxes are shown in Figure 7C.



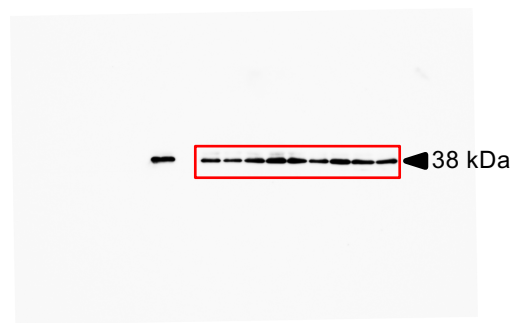
IB: anti-p-JNK



IB: anti-JNK



IB: anti-p-p38



IB: anti-p38



IB: anti- β -actin

Supplemental figure S5. Uncropped images of Western blotting in Supplemental figure S3

Cell lysates were prepared and separated by SDS-PAGE and transferred to PVDF membrane. The membrane was immunoblotted with anti-p-JNK (46/54 kDa), JNK (46/54 kDa), p-p38 (38kDa), p38 (38 kDa), or β -actin (42 kDa) antibodies. The cropped parts indicated by the boxes are shown in Supplemental figure S3.