SUPPLEMENTARY MATERIAL

Melanocortin 3 receptor activation with [D-Trp8]-γ-MSH suppresses inflammation in apolipoprotein E deficient Mice

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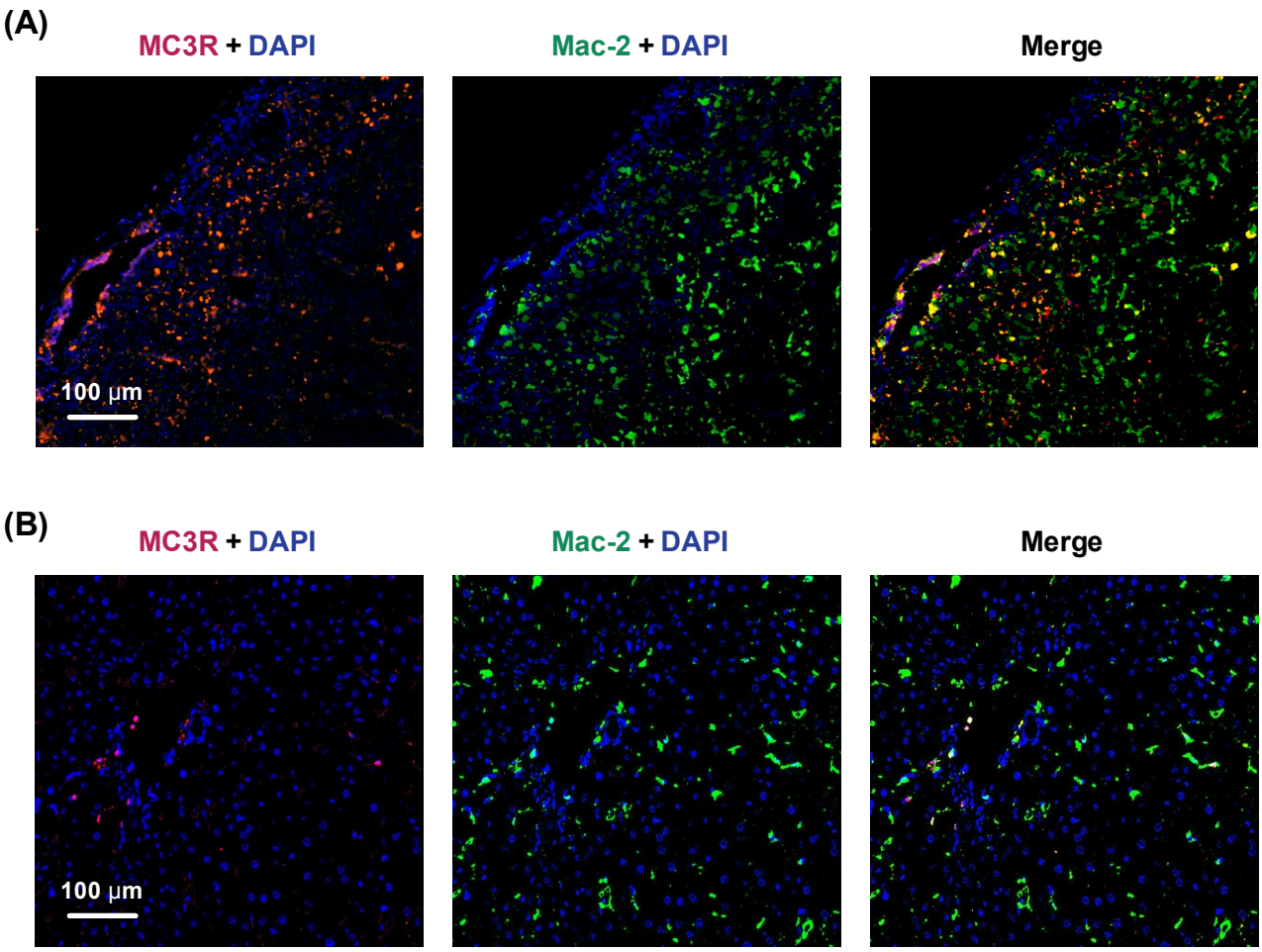
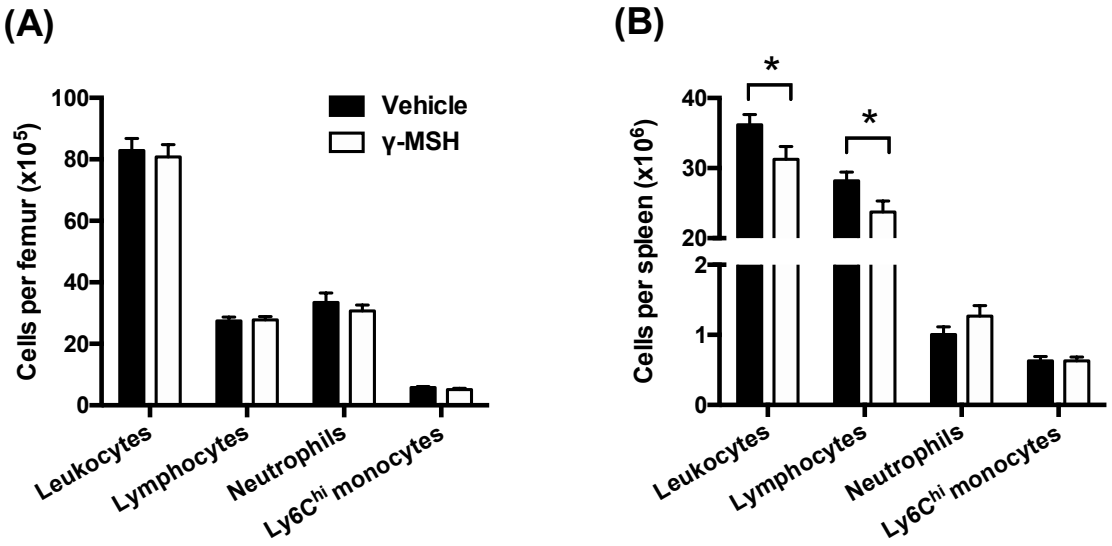
Supplemental Table I. Quantitative RT-PCR primers for mouse genes.

Gene name Accession number	**5'-3' primer sequence**
ACTA2 NM_007392.3	Forward: agattgtgcgcgacatcaaag Reverse: gcagactccataccgataaagga
ACTB NM_007393.5	Forward: tccatcatgaagtgtgacgt Reverse: gagcaatgatcttgatcttca
CCL2 NM_011333.3	Forward: aggtccctgtcatgcttctg Reverse: aaggcatcacagtccgagtc
CCL5 NM_013653.3	Forward: tgctgctttgcctacctctc Reverse: ttggcacacacttggcggtt
CCR1 NM_009912.4	Forward: ggacaaaatactctggaaacacaga Reverse: tgtgaaatctgaaatctccatcc
CCR2 NM_009915.2	Forward: agagagctgcagcaaaaagg Reverse: ggaaagaggcagttgcaaag
CCR5 NM_009917.5	Forward: gagacatccgttccccctac Reverse: gtcggaactgacccttgaaa
COL1A2 NM_007743.3	Forward: tgcagtaacttcgtgcctagc Reverse: acgtggtcctctgtctcca
COL3A1 NM_009930.2	Forward: ctaaaattctgccaccccgaa Reverse: aggatcaacccagtattctccactc
COL4A1 NM_009931.2	Forward: ctggcacaaaagggacgag Reverse: acgtggccgagaatttcacc
CD163 NM_001170395.1	Forward: tccacacgtccagaacagtc Reverse: ccttggaaacagagacaggc
CD206 NM_008625.2	Forward: cctctggtgaacggaatgat Reverse: cttcctttggtcagctttgg
CD62L NM_011346.2	Forward: tgcagagagacccagcaag Reverse: cagacccacagcttcaggat
CD62P NM_011347.2	Forward: gagggaagaaagccagacg Reverse: ggcgtccaggaacctttt
CX3CR1 NM_009987.4	Forward: cagcatcgaccggtacctt Reverse: gctgcactgtccggttgtt
ICAM1 NM_010493.3	Forward: tggccctggtcaccgttgtgat Reverse: aacagttcacctgcacggaccca
IL-1b NM_008361.4	Forward: tgtaatgaaagacggcacacc Reverse: tcttctttgggtattgcttgg
IL-1Ra NM_031167.5	Forward: gcatcttgcagggtcttttc Reverse: gtgagacgttggaaggcagt

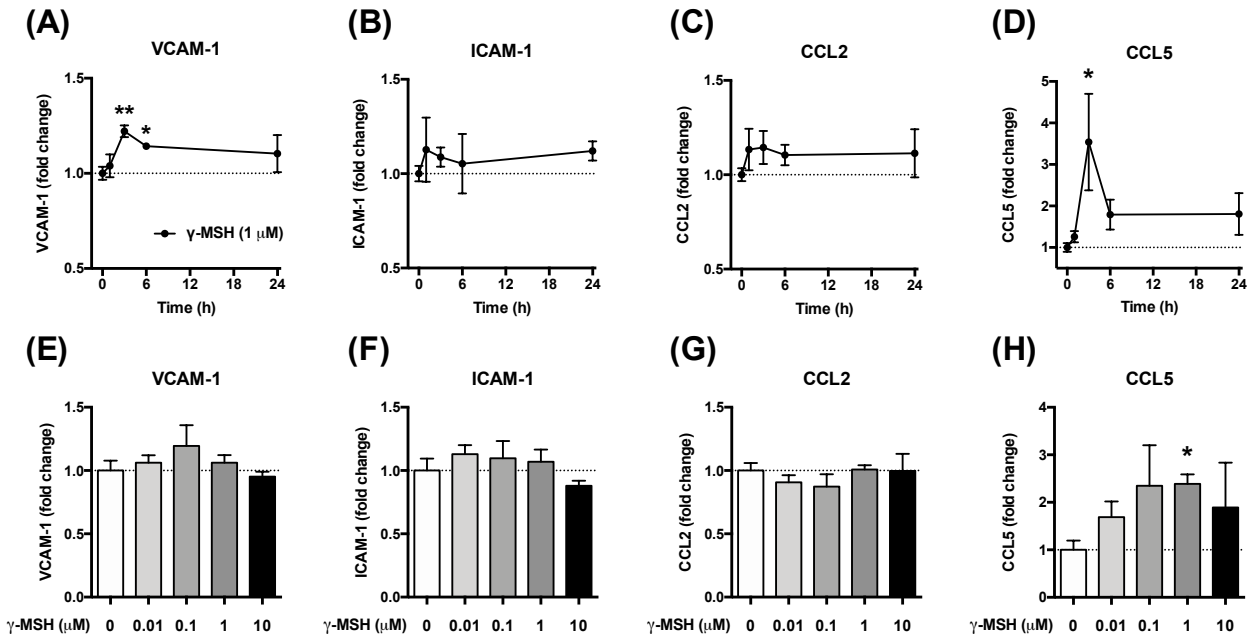
LFA-1 NM_001253872.1	Forward: ccccagacttttgctactgg Reverse: cgtgtgtccaggttgtagctc
MerTK NM_008587.1	Forward: gtggcagtgaagaccatgaagttg Reverse: gaactccgggatagggagtcat
MMP9 NM_013599.4	Forward: ctggacagccagacactaaag Reverse: ctcgcaagtcttcagag
PECAM1 NM_008816.3	Forward: cggtgttcagcgagatcc Reverse: actcgacaggatggaaatcac
S29 NM_009093.2	Forward: atgggtcaccagcagctcta Reverse: agcctatgtccttcgcgtact
SR-BI NM_016741.2	Forward: gcccatcatctgccaact Reverse: tcctgggagccctttttact
TGFb NM_011577.2	Forward: ccgcaacaacgccatctatg Reverse: cccgaatgtctgacgtattgaag
TNFa NM_013693.3	Forward: ctgaacttcggggtgatcgg Reverse: ggcttgtcactcgaattttgaga
VCAM1 NM_011693.3	Forward: ggtcttgggagcctcaacggt Reverse: agggccatggagtcaccgattt
Ym1 NM_009892.3	Forward: gggcatacctttatcctgag Reverse: ccactgaagtcatccatgtc



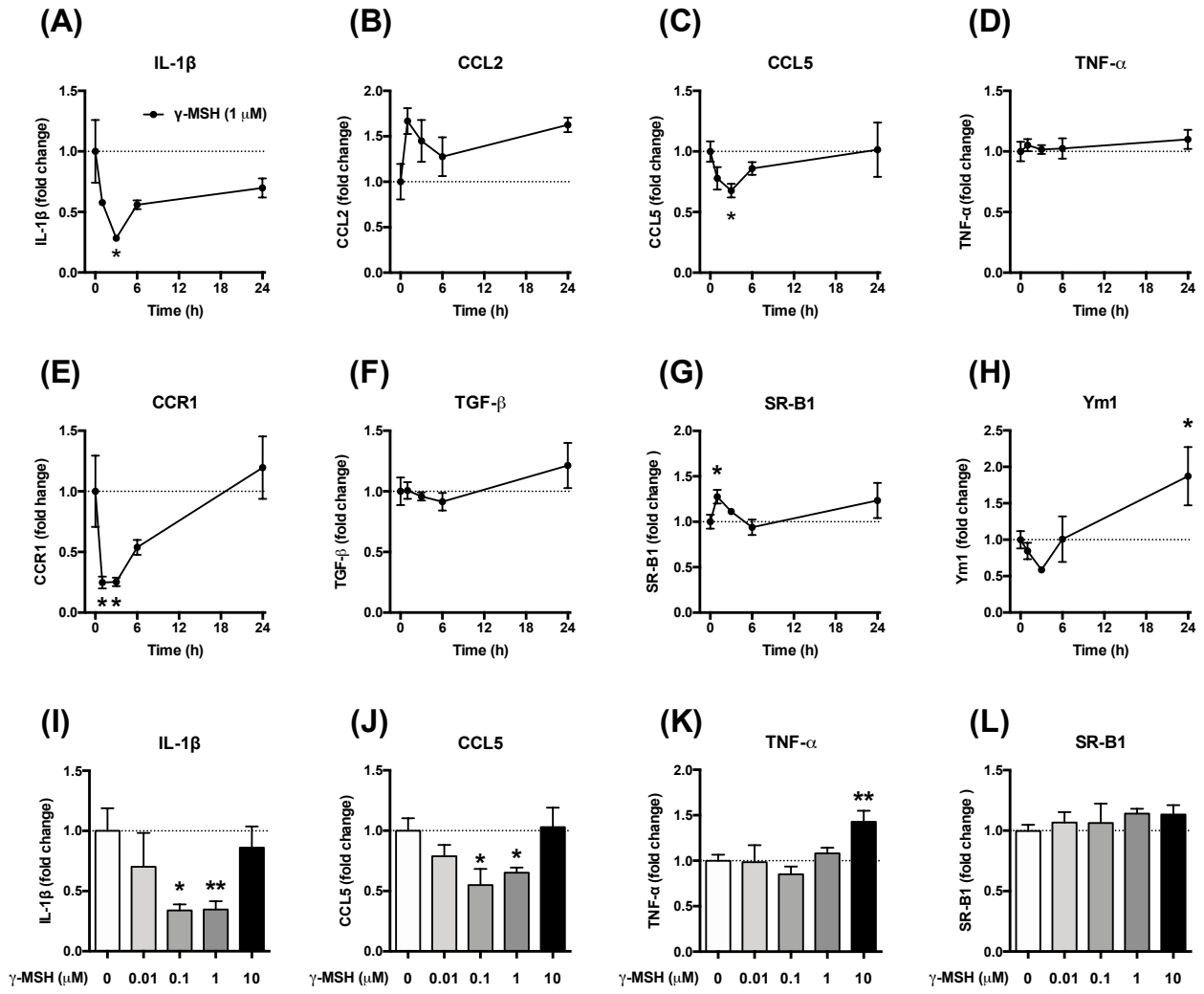
Supplemental Fig. I. Melanocortin MC_3 expression in the spleen and liver. **(A)** Immunofluorescence staining of melanocortin MC_3 receptor (MC3-R, red) and Mac-2 (green) in the spleen of high-fat diet-fed (12 weeks) *ApoE*$^{-/-}$ mouse. **(B)** Immunofluorescence staining of melanocortin MC_3 receptor (MC3-R, red) and Mac-2 (green) in the liver of high-fat diet-fed (12 weeks) *ApoE*$^{-/-}$ mouse. Scale bars, 100 μm.



Supplemental Fig. II. Leukocyte counts in the femur and spleen of γ-MSH-treated mice. Quantification of total leukocytes ($CD45^+$), lymphocytes ($CD45^+$, $CD11b^-$), neutrophils ($CD45^+$, $CD11b^+$, $CD115^-$, $Ly6G^+$) and $LyC6^{high}$ monocytes ($CD45^+$, $CD11b^+$, $CD115^+$, $Ly6G^-$, $Ly6C^{high}$) in the femur (**A**) and spleen (**B**) of vehicle- and γ-MSH-treated mice. Data are mean ± S.E.M., n = 11 (vehicle) and 10 (γ-MSH). *$P < 0.05$ versus vehicle.



Supplemental Fig. III. The effects of γ-MSH on the expression of adhesion molecules and chemokines in aortic endothelial cells. (**A**-**D**) Quantitative RT-PCR analysis of vascular cell adhesion molecule 1 (*VCAM-1*), intercellular adhesion molecule 1 (*ICAM-1*) and chemokine (C-C motif) ligands 2 (*CCL2*) and 5 (*CCL5*) transcript expression in aortic endothelial cells that were treated with 1 μM [D-Trp8]-γ-MSH for 1-24 h or left untreated (control, 0 h time point). (**E**-**F**) Quantitative RT-PCR analysis of *VCAM-1*, *ICAM-1*, *CCL2* and *CCL5* transcripts in aortic endothelial cells that were treated with increasing concentrations of [D-Trp8]-γ-MSH for 3 h. Data are mean ± S.E.M., n = 3-5 per group. *$P < 0.05$ and **$P < 0.01$ versus control.



Supplemental Fig. IV. The effects of γ-MSH on the expression of M1- and M2-type macrophage markers in bone marrow-derived macrophages. (**A**-**H**) Quantitative RT-PCR analysis of interleukin-1β (*IL-1β*), chemokine (C-C motif) ligands 2 (*CCL2*) and 5 (*CCL5*), tumor necrosis factor alpha (*TNF-α*), C-C chemokine receptor type 1 (*CCR1*), transforming growth factor β (*TGF-β*), scavenger receptor class B type 1 (*SR-B1*) and chitinase-like 3 (*Ym1*) transcript expression in mouse bone marrow-derived macrophages that were treated with 1 μM [D-Trp8]-γ-MSH for 1-24 h or left untreated (control, 0 h time point). (**I**-**L**) Quantitative RT-PCR analysis of *IL-1β*, *TNF-α*, *CCL5* and *SR-B1* transcripts in bone marrow-derived macrophages that were treated with increasing concentrations of [D-Trp8]-γ-MSH for 3 h. Data are mean ± S.E.M., n = 3-6 per group. *$P < 0.05$ and **$P < 0.01$ versus control.