Myeloid HO-1 Modulates Macrophage Polarization

and Protects Against Ischemia-Reperfusion Injury

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Supplemental Figure 1

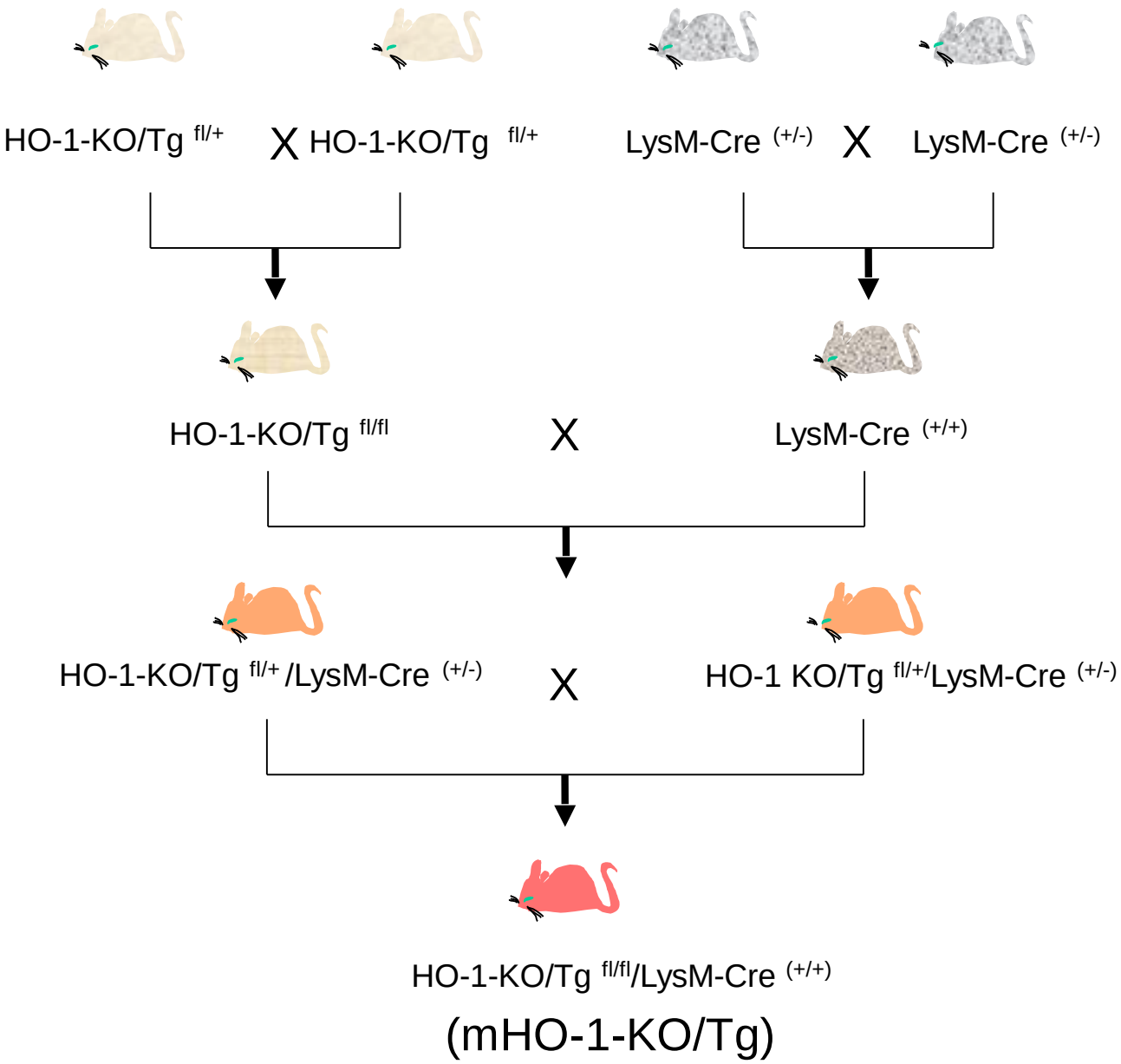

Figure S1. Strategy for the generation of mHO-1-KO mice and mHO-1-Tg mice. Breeding strategy employed to develop myeloid specific HO-1 knockout (mHO-1-KO) mice and myeloid specific HO-1 transgenic (mHO-1-Tg) mice. Both mHO-1-KO and mHO-1-Tg mice were obtained via successive cross-breeding strategy as shown. Homozygous floxed HO-1-KO or Tg (HO-1-KO fl/fl/HO-1-Tg fl/fl) mice and homozygous LysM-Cre (LysM-Cre +/+) mice were crossed to obtain heterozygous mice for both alleles, HO-1-KO/Tg fl/+ and LysM-Cre +/-. Further, heterozygous floxed HO-1-KO/Tg mice (HO-1 fl/+ LysM-Cre +/-) were crossed to obtain homozygous myeloid specific floxed HO-1-KO/Tg mice (HO-1-KO/Tg fl/fl LysM-Cre +/+).

Supplemental Figure 2

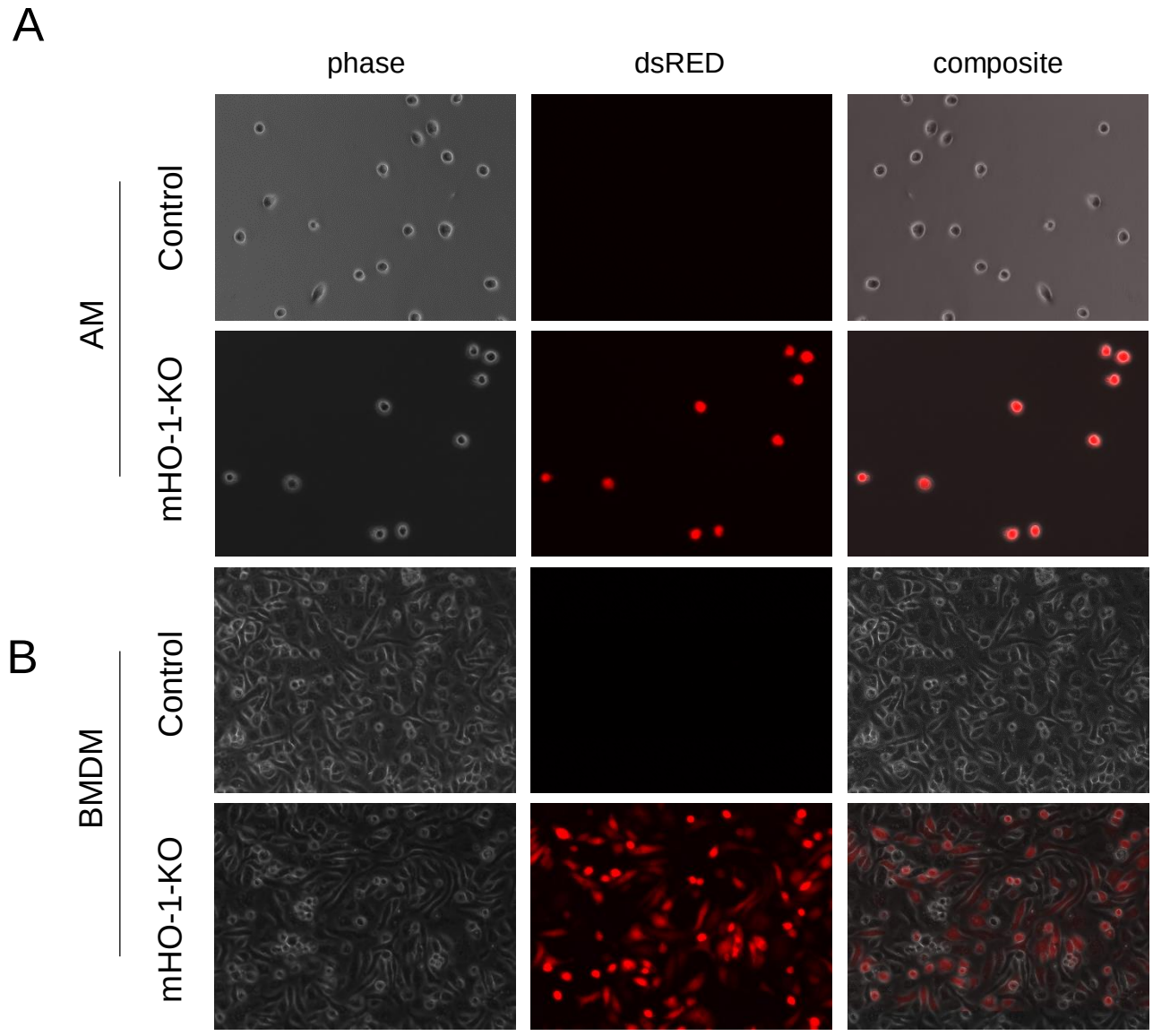

Figure S2. Deletion efficiency of HO-1 in Alveolar and Bone marrow derived macrophages (BMDMs).
dsRED fluorescence detection in macrophages from mHO-1-KO mice. A: Alveolar macrophages (AM). Top: Floxed HO-1 (HO-1-KO fl/fl) controls. Bottom: mHO-1-KO mice. 200X. B: BMDMs. Top: Floxed HO-1 (HO-1-KO fl/fl) controls. Bottom: mHO-1-KO mice. magnification, ×200; Phase: phase contrast. dsRED: red fluorescence channel. Composite: overlay images from phase contrast and red fluorescence channel.

Supplemental Figure 3

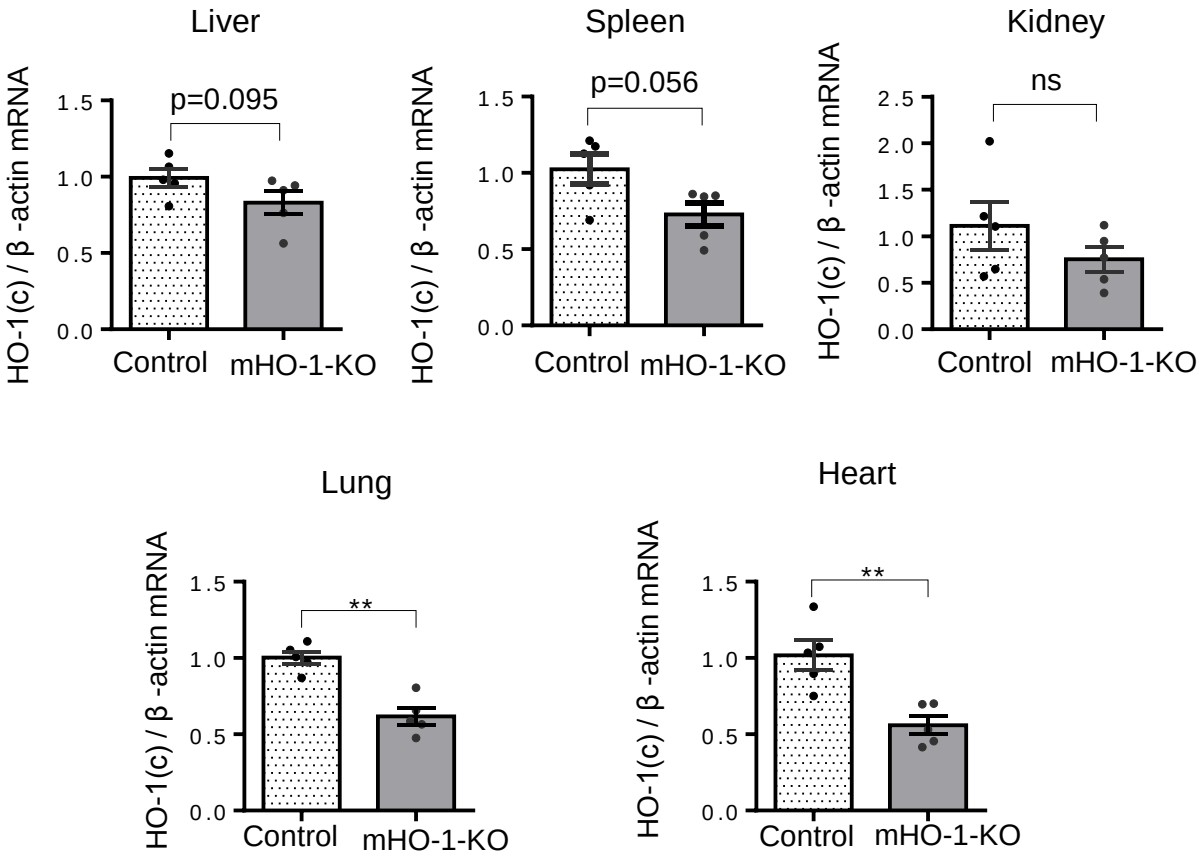

Figure S3. HO-1 mRNA expression in tissues from mHO-1-KO mice vs. controls. HO-1 mRNA levels in tissue homogenate of liver, spleen, lung, kidney and heart from mHO-1-KO mice vs. controls (n=5/group). Data were normalized to β-actin gene expression, and shown mean ± SEM (scatter dot blot). Statistical analyses were done with two-tailed Mann-Whitney U test. ns: not significant; **p < 0.01

Supplemental Figure 4

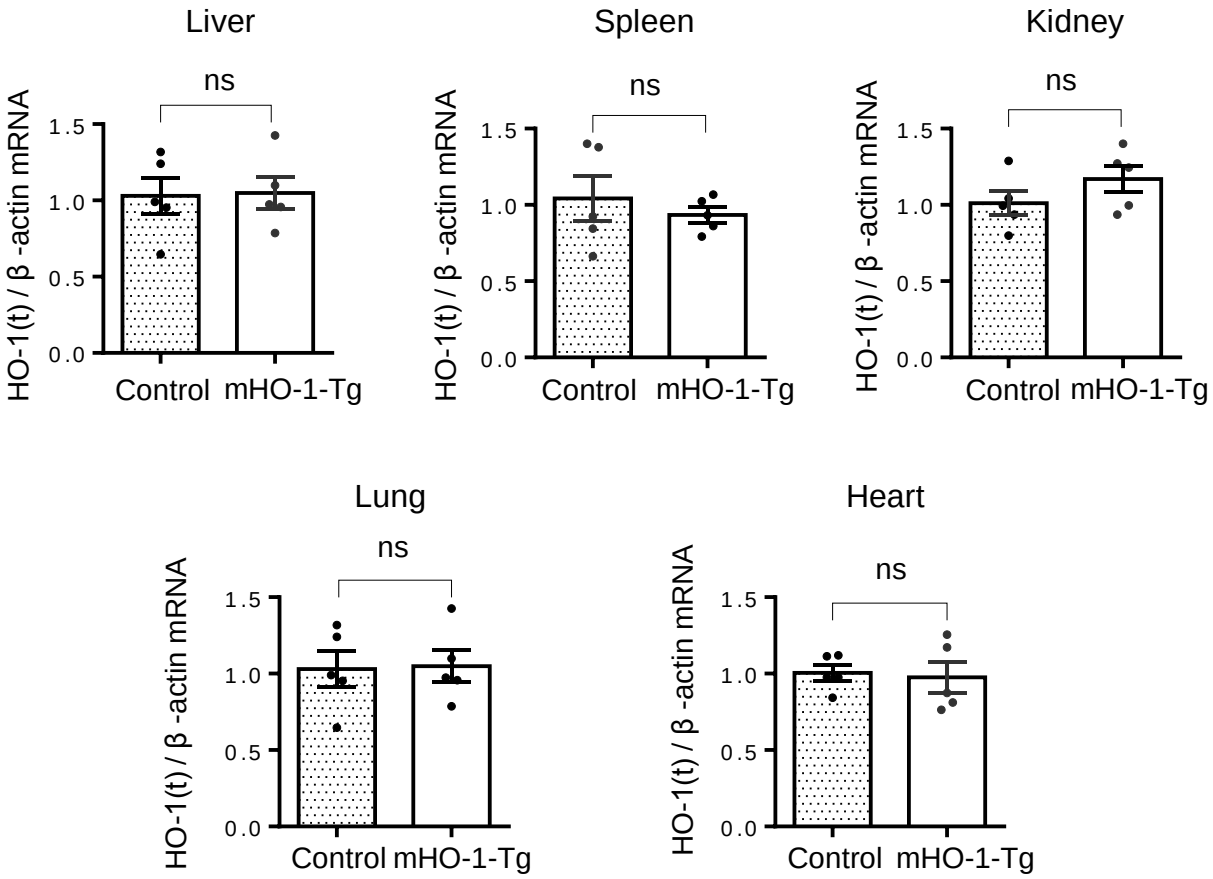

Figure S4. HO-1 mRNA expression in tissues from mHO-1-Tg mice vs. controls.
HO-1 mRNA levels in tissue homogenate of liver, spleen, lung, kidney and heart from mHO-1-Tg mice vs. controls (n=5/group). Data were normalized to β-actin gene expression, and shown as mean ± SEM (scatter dot blot). Statistical analyses were done with two-tailed Mann-Whitney U test. ns: not significant

Supplemental Figure 5

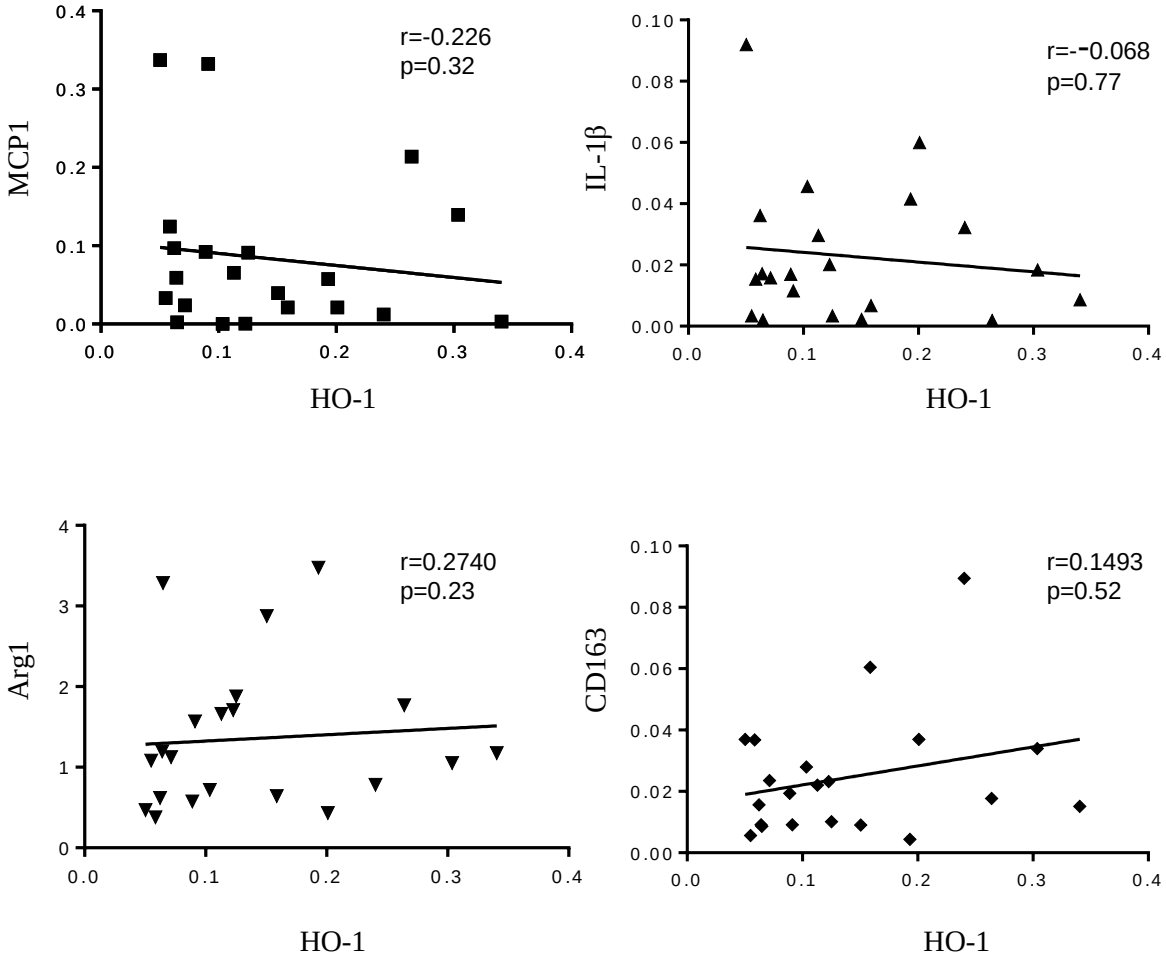

Figure S5. The correlation between human HO-1 and cytokine expression in post transplantation liver biopsies.
The correlations of gene expression between HO-1 and proinflammatory cytokines MCP-1 and IL-1β and anti-inflammatory cytokines Arg1 and CD163 expression in post transplantation liver biopsy samples were analyzed with non-parametric spearman method in Graphpad prism 6 (n = 21, 11 low HO-1 and 10 high HO-1).

Supplemental Figure 6

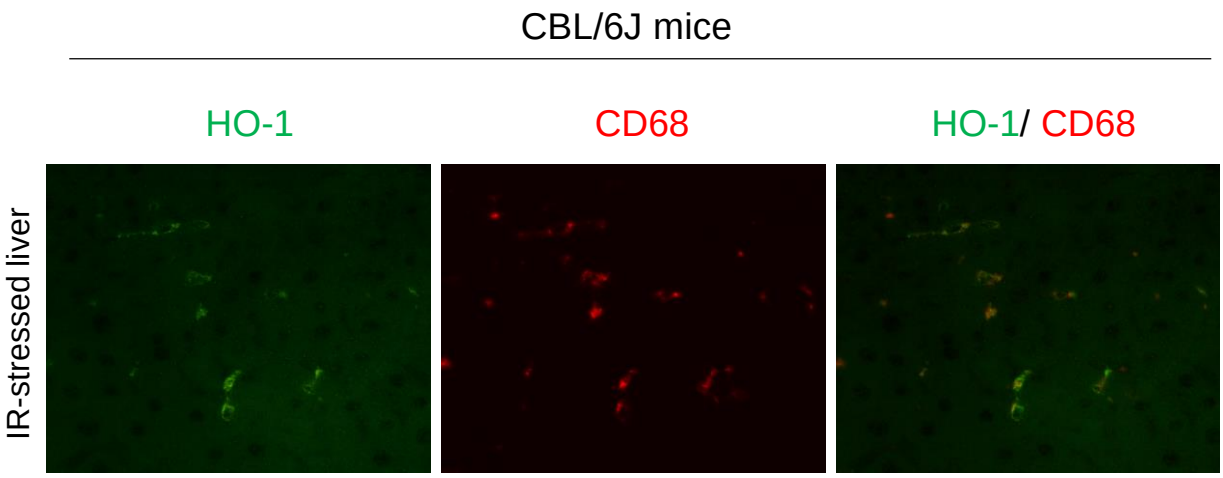

Figure S6. HO-1 localization in IR-stressed liver.
Immunofluorescence staining of HO-1 (green, left column), CD68 for hepatic macrophages (red, middle column) and merged images (right column), IR-stressed livers. Representative of three experiments is shown. Magnification 400X

Supplemental Figure 7

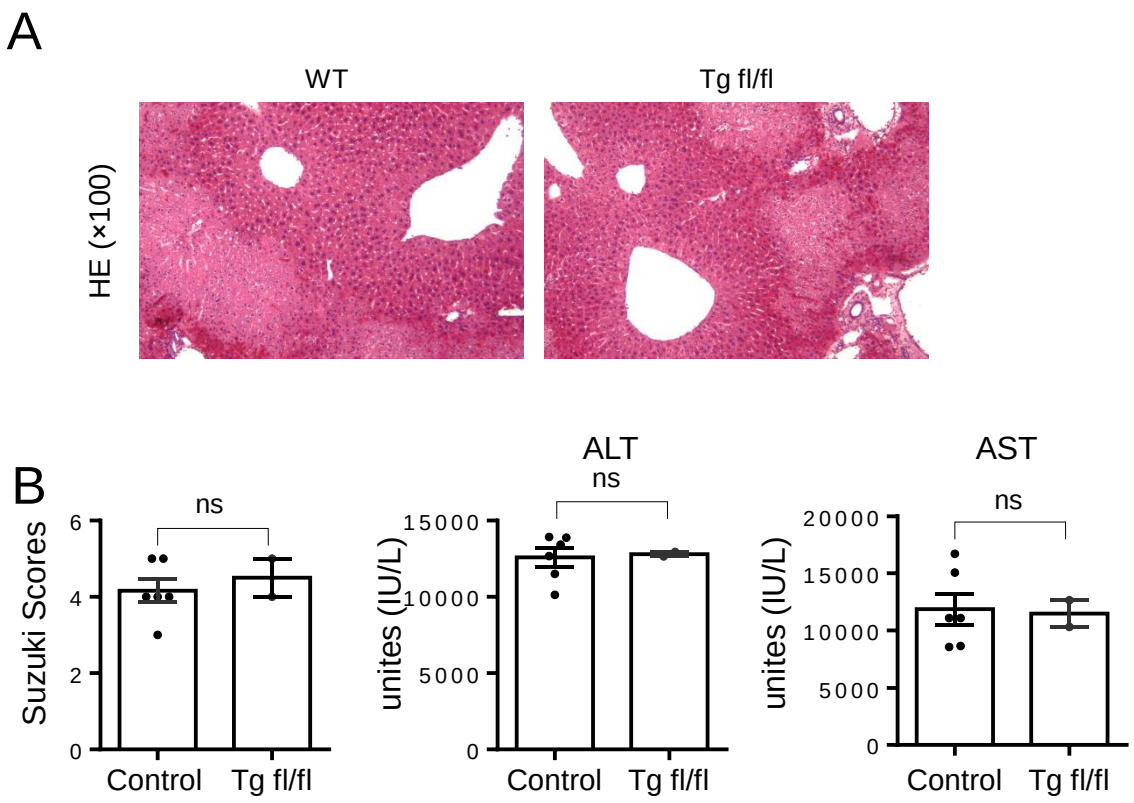

Figure S7. Comparisons of floxed HO-1-Tg and wild-type mice.
A. Liver histology of C57BL/6 wild-type (WT) and floxed HO-1-Tg (Tg fl/fl) after liver IRI. Representative section (magnification, ×100). B. Suzuki's histological grading of liver IRI (left). Serum ALT levels (Middle). Serum AST levels (right). (n=8, 6 control, 2 Tg fl/fl). Data are shown as mean ± SEM (scatter dot blot). Statistical analyses were done with two tailed Mann-Whitney U test. ns: not significant

Table S1: Genotyping primers, using agarose gel electrophoresis

Primer name	Primer sequence
Mouse *Hmox1*-g62145r	5'- GTC TGT AAT CCT AGC ACT CGA A-3'
Mouse *Hmox1*-g61822f	5'- CTC ACT ATG CAA CTC TGT TGG AGG -3'
DsRed-N2	5'- GGA AGG ACA GCT TCT TGT AGT CG -3'
pLysM-FP1	5'- CTT GGG CTG CCA GAA TTT CTC -3'
LysM-RP2	5'- CCT CAC CCC AGC ATC TCT AAT TC -3'
VE-Cad-Cre-R	5'- ATCACTCGTTGCATCGACCGGTAA -3'
Human *HMOX1* 42F	5'- GGG TGA TAG AAG AGG CCA AGA -3'
Human *HMOX1* 81R	5'- CTT GTT GCG CTC AAT CTC CT -3'

Table S2: Genotyping primers using Roche UPL probes and real-time PCR

Primer name	Primer sequence
Human *HMOX1* (101)fp	5' GGC CTC CCT GTA CCA CAT C 3'
Human *HMOX1* (101)rp	5' AGA CTG GGC TCT CCT TGT TG 3'
Mouse *Gja5* (6)fp	5' GGC TAC CAG AAG GTG AGC AG 3'
Mouse *Gja5* (6)rp	5' AGG CAG GGA CAT CGT GTT AT 3'
Mouse *Hmox1* (17)fp	5' AGG CTA AGA CCG CCT TCC T 3
Mouse *Hmox1* (17)rp	5' TGT GTT CCT CTG TCA GCA TCA 3'
uniHO1 (49/134)fp	5'CAT GCC CCA GGA TTT GTC 3'
uniHO1 (49/134)rp	5' TCA TGA ACT CAG CAT TCT C 3'
Mouse *Actb* (56)fp	5' AAG GCC AAC CGT GAA AAG AT 3'
Mouse *Actb* (56)rp	5' GTG GTA CGA CCA GAG GCA TAC 3'

Table S3: Primers used for gene expression

Primer name	Primer sequence
Mouse *Cxcl10fp*	5'-CCAGAATCGAAGGCCATCAA-3'
Mouse *Cxcl10rp*	5'-CATTTCCTTGCTAACTGCTTTCAG-3'
Mouse *Mcp-1 fp*	5'- CATCCACGTGTTGGCTCA-3'
Mouse *Mcp-1 rp*	5'- GATCATCTTGCTGGTGAATGAGT-3'
Mouse *IL1 β fp*	5'-TGTAATGAAAGACGGCACACC-3'
Mouse *IL1 β rp*	5'-TCTTCTTTGGGTATTGCTTGG-3
Mouse *Arg1 fp*	5'-GAATGGAAGAGTCAGTGTGG-3'
Mouse *Arg1 rp*	5'-AATGACACATAGGTCAGGGT-3
Mouse *Cd163 fp*	5'-GACGACAGATTCAGCGACTT-3'
Mouse *Cd163 rp*	5'-CCGAGGATTTCAGCAAGTCCA-3'
Human *CXCL10 fp*	5'-GCTGCCGTCATTTTCTGC-3'
Human *CXCL10 rp*	5'-TCTCACTGGCCCGTCATC-3'
Human *MCP-1 fp*	5'-TTCTGTGCCTGCTGCTCAT-3'
Human *MCP-1 rp*	5'-GGGGCATTGATTGCATCT-3'
Human *IL-1 β fp*	5'-CTGTCCTGCGTGTTGAAAGA-3'
Human *IL-1 β rp*	5'-TTGGGTAATTTTTGGGATCTACA-3'
Human *ARG1 fp*	5'-GGCAAGGTGATGGAAGAAAC-3'
Human *ARG1 rp*	5'-AGTCCGAAACAAGCCAAGGT-3'
Human *CD163 fp*	5'-GGATCTGCTGACTTCAGAAG-3'
Human *CD163 rp*	5'-CTCCTTGTCTGTTCCTCCAA-3'
Human *CD80 fp*	5'- AAACTCGCATCTACTGGCAAA-3'
Human *CD80 rp*	5'- GGTTCTTGTACTCGGGCCATA-3'