Supplemental Data

Manuscript: Tubular GM-CSF Promotes late MCP-1/CCR2-mediated Fibrosis and Inflammation after Ischemia/Reperfusion Injury

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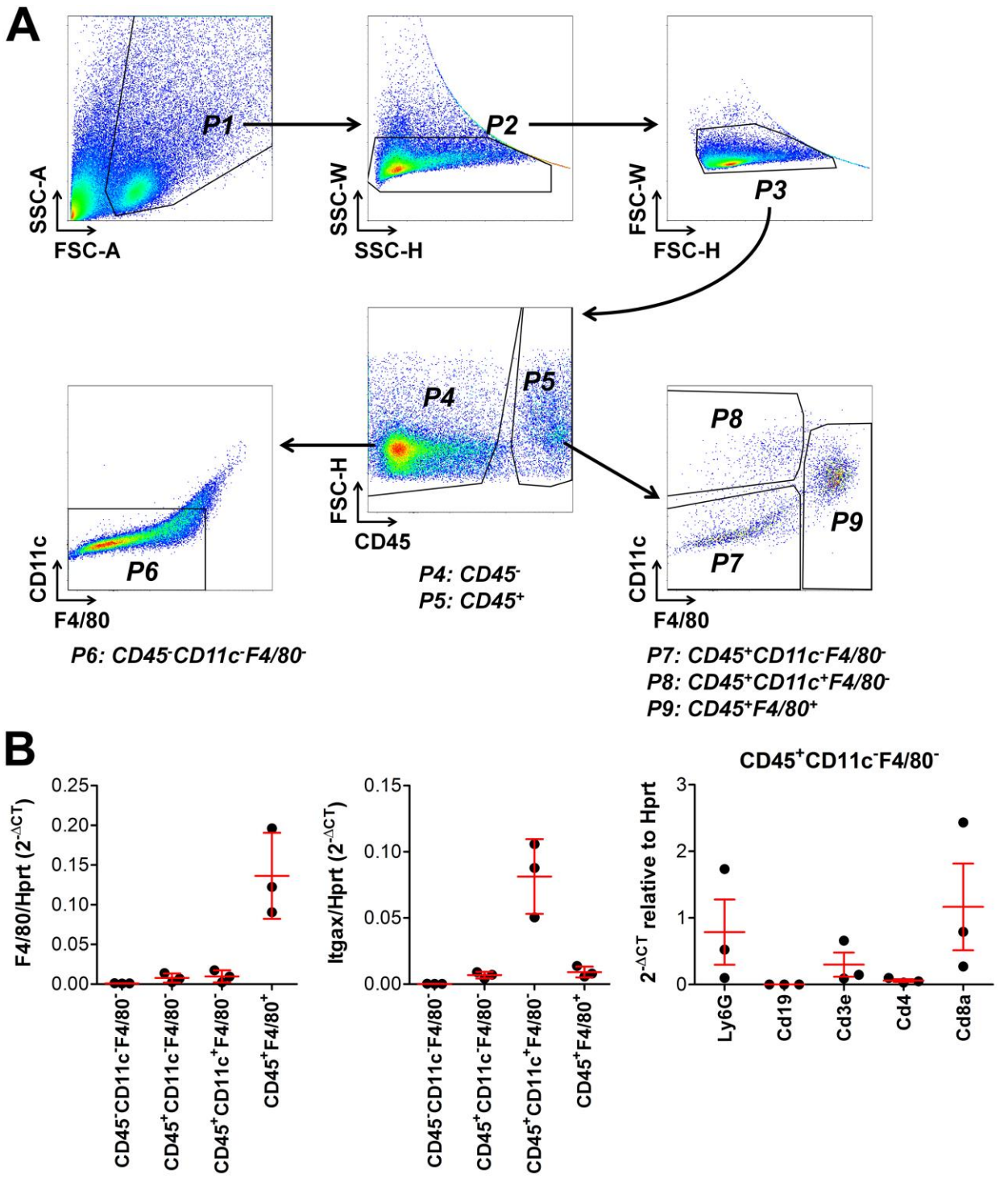
Supplemental Table 1. Mouse experiment design

Strain	# of mice	Surgery	Treatment	Dosing	Termination (after U-IRI)	Outcome measurements
WT	6					FACS sorting for resident and infiltrating/patrolling macrophages from IRI kidneys
WT	6	U-IRI			14 days	
Ccr2$^{-/-}$	6	U-IRI			14 days	
WT	12	U-IRI			14 days	FACS sorting for CD45$^{\pm}$CD11c$^{\pm}$F4/80$^{\pm}$ cells from IRI kidneys
WT	8	U-IRI			14 days	FACS sorting for PDGFRβ^{+} myofibroblasts from IRI kidneys
WT	6				0 day	Studying the correlation between macrophage accumulation and kidney fibrosis during AKI-to-CKD transition
WT	6	U-IRI			1 day	
WT	6	U-IRI			7 days	
WT	6	U-IRI			14 days	
WT	6	U-IRI			30 days	
WT	8	U-IRI			14 days	Studying the importance of MCP-1/CCR2 signaling in macrophage accumulation, kidney fibrosis and inflammation at the late stage of IRI
Ccr2$^{-/-}$	8	U-IRI			14 days	
WT	14	U-IRI			30 days	
Ccr2$^{-/-}$	11	U-IRI			30 days	
WT	8	U-IRI	Vehicle	Every 12 hours for 7 days starting on 7 days after U-IRI	14 days	Studying pharmacological inhibition of MCP-1/CCR2 signaling during AKI-to-CKD transition
WT	8	U-IRI	RS102895		14 days	

Abbreviations: U-IRI, unilateral ischemia/reperfusion injury; FACS, fluorescence-activated cell sorting.

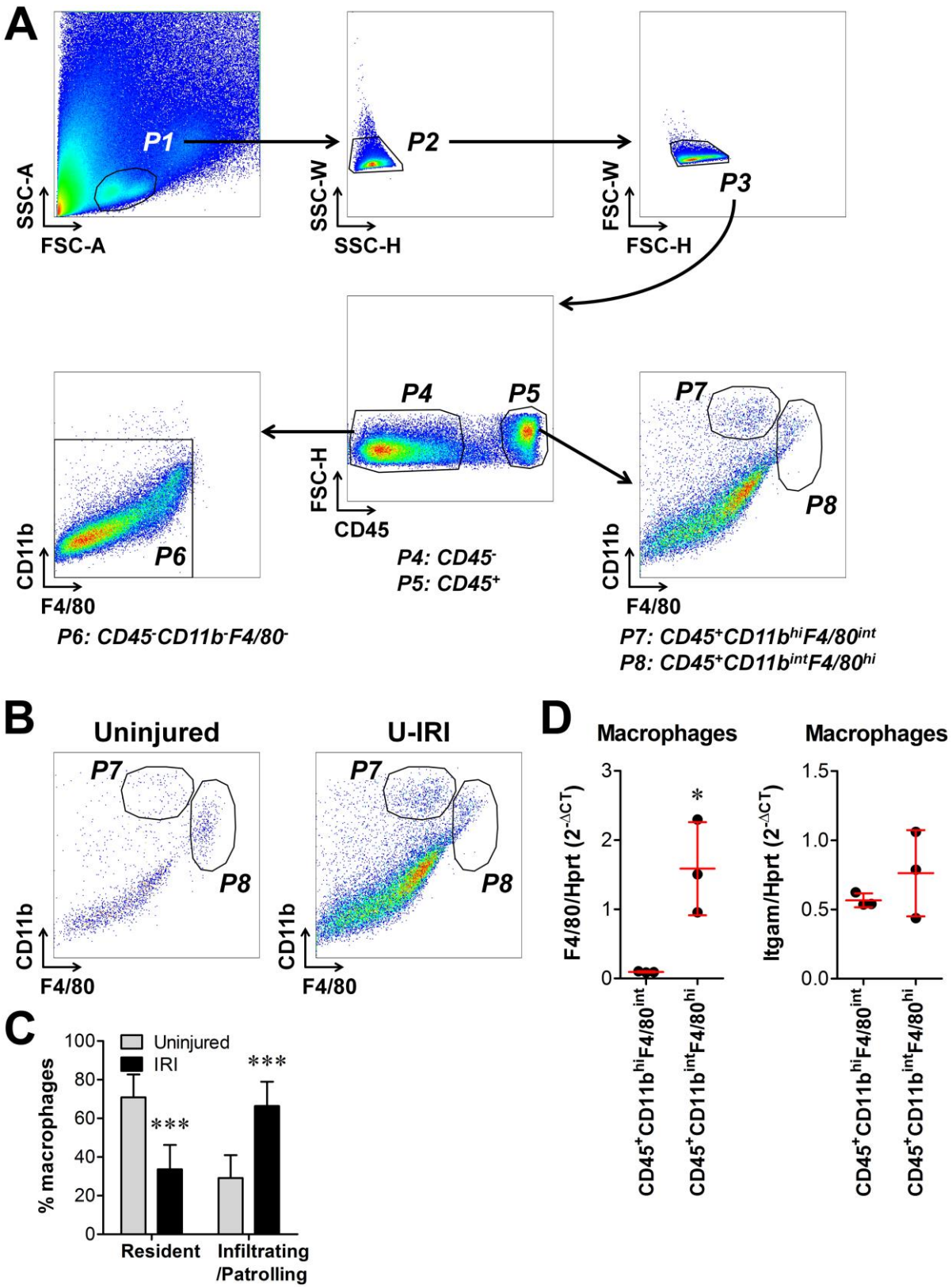
Supplemental Table 2. Primer sequences used for quantitative PCR

Gene	Forward	Reverse
Acta2	TCTGGACGTACAACTGGTATTG	GGCAGTAGTCACGAAGGAATAG
Arg1	CAGAAGAATGGAAGAGTCAG	CAGATATGCAGGGAGTCACC
Ccl2	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCCTTCTTG
Ccl3	TTCTCTGTACCATGACACTCTGC	CGTGGAATCTTCCGGCTGTAG
Ccl8	TCTACGCAGTGCTTCTTTGCC	AAGGGGGATCTTCAGCTTTAGTA
Ccr1	ATACTCTGGAAACACAGACTCAC	CCCACCACTCCAATGATGAA
Ccr2	AGGAGCCATACCTGTAAATGC	GTTGATAGTATGCCGTGGATGA
Ccr3	AGCTATGATGTTTACTACCTGACTG	ATGCCATTCTACTTGTCTCTGG
Ccr5	AAGAGACTCTGGCTCTTGC	CAGGGTGCTGACATACCATAA
Cd3e	GAAAGCTCGAGTGTGTGAGT	GCCTTGGCCTTCCTATTCTT
Cd4	GAGAGTTCCCAGAAGAAGATCAC	AGGCGAACCTCCTCTAATTAATAC
Cd8a	GTGGACCTGGTATGTGAAGTG	TGAAGCCATATAGACAACGAAGG
Cd19	AGCTGTATGTGTGGGCTAAAG	CCACAGTGAGATCTTGGTTGAT
Cd68	ATTGAGGAAGGAACTGGTGTAG	CCTCTGTTCCTTGGGCTATAAG
Chi3l1	CAAGGAACTGAATGCGGAAT	GGCTCCCAGACGTATCATGT
Col1a1	GAAACCCGAGGTATGCTTGA	GGGTCCCTCGACTCCTACAT
Col3a1	ACCAAAAGGTGATGCTGGAC	GACCTCGTGCTCCAGTTAGC
Csf1	GCAGGAGTATTGCCAAGGAG	GTTAGCATTGGGGGTGTTGT
Csf2	TGGTCTACAGCCTCTCAGCA	CCGTAGACCCTGCTCGAATA
Cx3cl1	ACGAAATGCGAAATCATGTGC	CTGTGTCGTCTCCAGGACAA
Cxcr4	GCAGCAGGTAGCAGTGAAA	GTGTATATACTCACACTGATCGGTTC
F4/80	TGAATGGCTCCATTTGTGAA	GATGGCCAAGGATCTGAAAA
Fn1	GATCAGTGGGATAAGCAGCA	ATATGTCCCTCCTCGTGACG
Hprt1	CAGTACAGCCCCAAAATGGT	CAAGGGCATATCCAACAACA
Itgam	ATGGACGCTGATGGCAATACC	TCCCCATTCACGTCTCCCA
Itgax	CAACTGCACAGCAGGAGTGT	TAGCCGAGGCTGTGTATGTG
Kim1	GAGAGTGACAGTGGTCTGTATTG	CGTGTGGGAATCTCTGGTTTA
Lcn2	GACCTAGTAGCTGTGGAAAC	GACGCCATTGGTGGTGTTAA
Ly6G	TTGTGGACTCTCACAGAAGC	GTCTTCACGTTGACAGCATTAC
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
Msr1	AAAGAGATGACAGAGAATCAGAGG	CAAGGAGGTAGAGAGCAATGAG
Nos2	CCAAGCCCTCACCTACTTCC	CTCTGAGGGCTGACACAAGG
Pdgfb	ACAGAGACTCCGTAGATGAAGA	ATCGATGAGGTTCCGAGAGA
Pdgfrb	GGAGTCCATAGGGAGGAAGC	CACCTTCTCCAGTGTGCTGA
Tgfb1	CCACCTGCAAGACCATCGAC	CTGGCGAGCCTTAGTTTGGAC
Tnfa	GAACTGGCAGAAGAGGCACT	AGGGTCTGGGCCATAGAACT



Supplemental Figure 1. FACS sorting of macrophages, dendritic cells, T cells/PMNs and renal cells. (A) Kidney cells were isolated by FACS 14 days after unilateral IRI. $CD45^-$ (P4) and $CD45^+$ (P5) cells were initially sorted from P1 to P3. $CD45^-CD11c^-F4/80^-$ (P6) cells were sorted from P4; whereas $CD45^+CD11c^-F4/80^-$ (P7), $CD45^+CD11c^+F4/80^-$ (P8) and $CD45^+F4/80^+$ (P9) cells were sorted from P5. The regions circled are the gates used to isolate corresponding cells, respectively. P, population; FSC, forward scatter; SSC, side scatter; A, area; H, height; W, width.

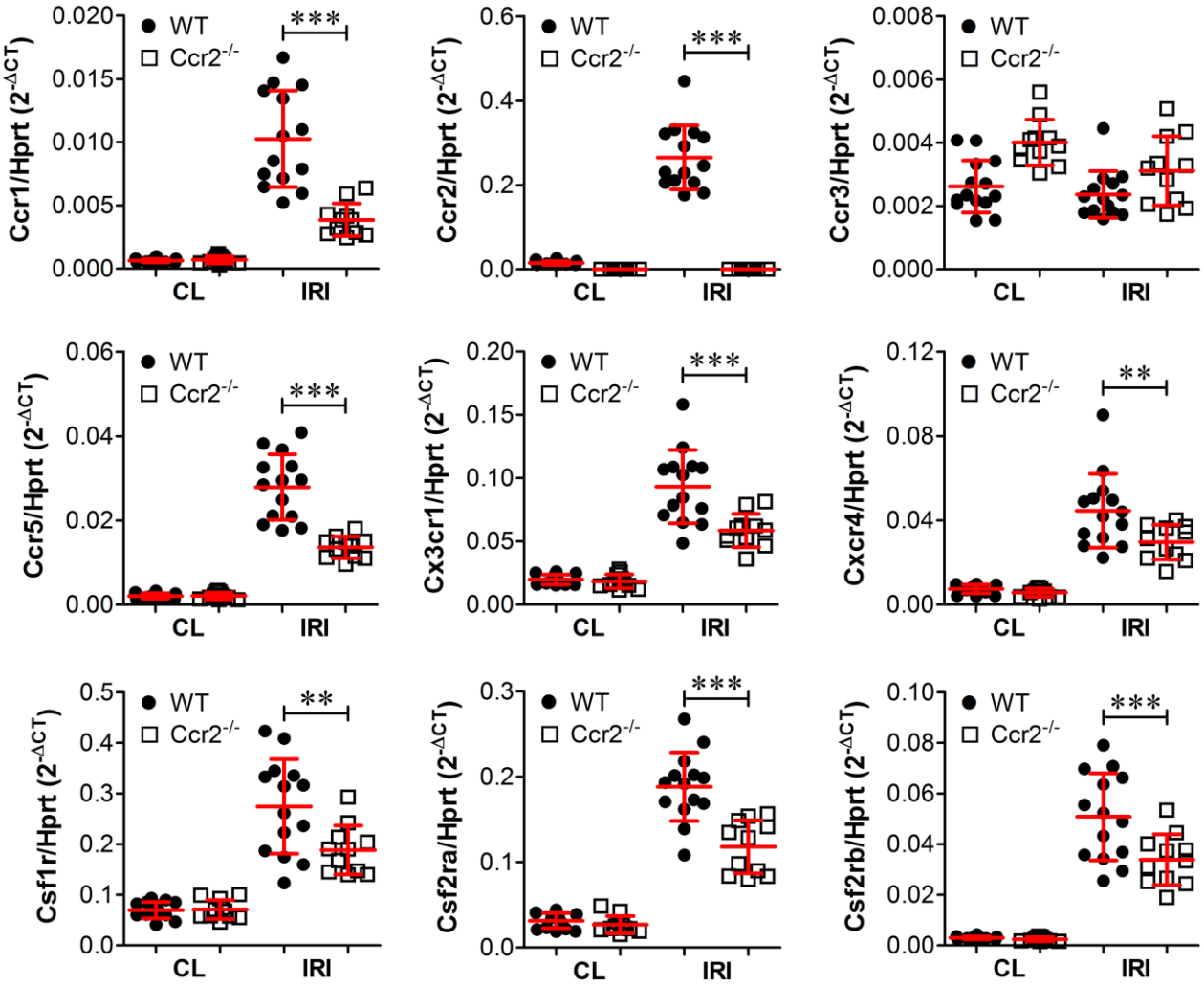
(B) Quantitative PCR analysis of mRNA from the indicated cell populations for *F4/80* (macrophage marker), *Itgax* (dendritic cell marker CD11c), *Ly6G* (PMN marker), *Cd19* (B cell marker), *Cd3e* (general T cell marker), *Cd4* (T helper cell marker) and *Cd8a* (cytotoxic T cell marker). n=3 cell pools of 4 kidneys/pool, 12 kidneys total.



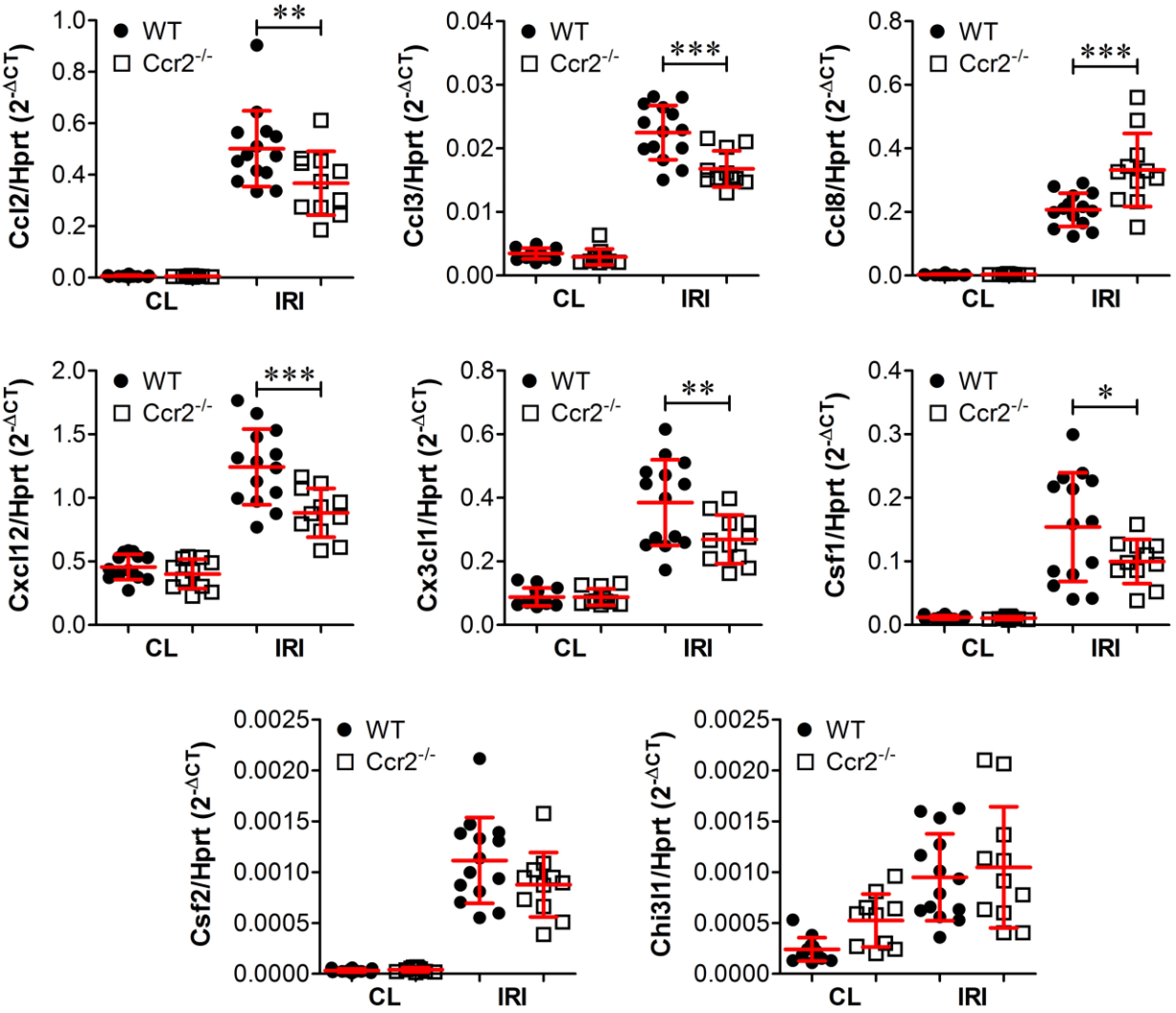
Supplemental Figure 2. FACS sorting of resident and infiltrating/patrolling macrophages. (A) Wild-type kidney cells were isolated by FACS 14 days after unilateral IRI. $CD45^-$ (P4) and $CD45^+$

(P5) cells were initially sorted from P1 to P3. $CD45^{+}CD11b^{hi}F4/80^{int}$ (P7) and $CD45^{+}CD11b^{int}F4/80^{hi}$ (P8) cells were sorted from P5. The regions circled are the gates used to isolate corresponding cells, respectively. P, population; FSC, forward scatter; SSC, side scatter; A, area; H, height; W, width. (B) $CD45^{+}CD11b^{hi}F4/80^{int}$ (P7) and $CD45^{+}CD11b^{int}F4/80^{hi}$ (P8) cells were sorted from uninjured and IRI kidneys. (C) The percentages of resident and infiltrating/patrolling macrophages were determined. ***$p<0.001$ versus uninjured kidney. n=6 kidneys. (D) Quantitative PCR analysis of mRNA from the indicated cell populations for *F4/80* and *Itgam* (*CD11b*). ***$p<0.05$. n=3 cell pools of 2 kidneys/pool, 6 kidneys total.

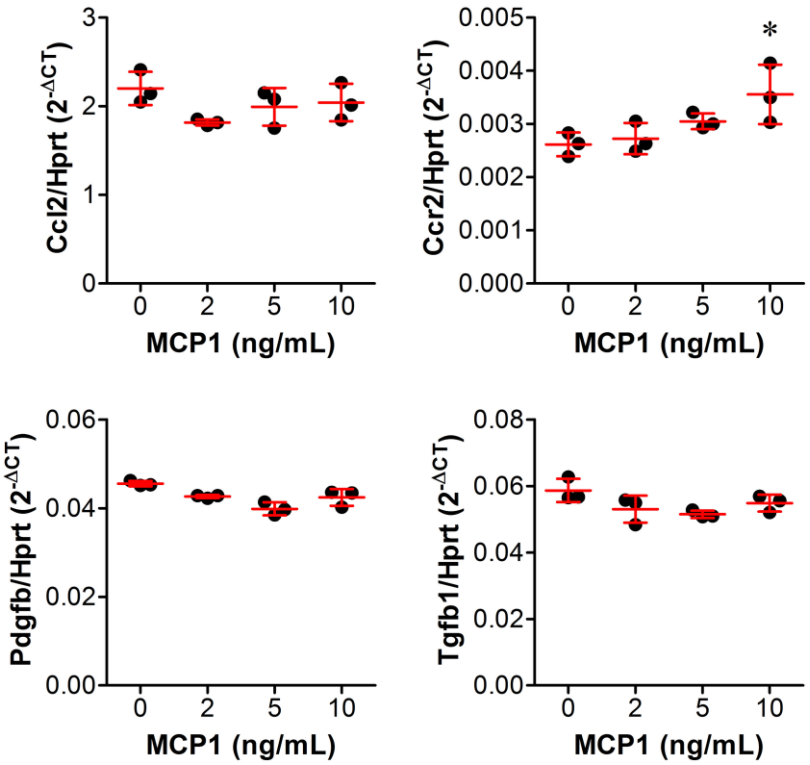
receptors after unilateral IRI.



Supplemental Figure 3. Loss of CCR2 reduces expression of multiple chemoattractant (A) Wild-type (WT) and *Ccr2*$^{-/-}$ mice were subjected to 27 minutes warm unilateral IRI. Unilateral IRI and contralateral (CL) kidneys were harvested at 30 days after unilateral IRI. Quantitative RT-PCR analysis for *Ccr1*, *Ccr2*, *Ccr3*, *Ccr5*, *Cxcr4*, *Cx3cr1*, *Csf1r*, *Csf2ra1* and *Csf2rb1* was performed on whole kidney mRNA. **p<0.01 and ***p<0.001 versus WT IRI kidney. n=14 WT and 11 for *Ccr2*$^{-/-}$ mice.



Supplemental Figure 4. Loss of CCR2 reduces expression of multiple chemoattractants after unilateral IRI. (A) Wild-type (WT) and *Ccr2*$^{-/-}$ mice were subjected to 27 minutes warm unilateral IRI. Unilateral IRI and contralateral (CL) kidneys were harvested at 30 days after unilateral IRI. Quantitative RT-PCR analysis for *Ccl2*, *Ccl3*, *Ccl8*, *Cxcl12*, *Cx3cl1*, *Csf1*, *Csf2* and *Chi3l1* was performed on whole kidney mRNA. *p<0.05, **p<0.01 and ***p<0.001 versus WT IRI kidney. n=14 WT and 11 for *Ccr2*$^{-/-}$ mice.



Supplemental Figure 5. MCP-1 does not activate bone marrow-derived macrophages (BMMs) to express profibrotic signals in vitro. BMMs were treated with recombinant mouse MCP-1 at the indicated concentrations for 24 hours, and cell lysates were harvested for RNA isolation. Quantitative PCR for *Ccl2*, *Ccr2*, *Pdgfb* and *Tgfb1* was performed on BMM mRNA. *p<0.05 vs 0 ng/mL. n=3.