Suppl. information on:

Anne M. Hüsing, Vera C. Wulfmeyer, Svenja Gaedcke, Susanne V. Fleig, Song Rong, David DeLuca, Hermann Haller, Roland Schmitt, and Sibylle von Vietinghoff:

Myeloid CCR2 promotes atherosclerosis after acute kidney injury

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Supplemental Methods

Histology

For immunostaining, rat anti-mouse and human CD11b (M1/70), Armenian hamster anti-mouse CD11c (N418) (both biolegend, San Diego, CA), rabbit anti-human CD3 (polyclonal, Dako, Denmark), rat anti-mouse F4/80 (BM8) (Origene, Rockville, MD) were used with corresponding secondary antibodies: AF488 donkey anti-rat IgG, AF488 donkey anti-rabbit IgG, AF555 goat anti-rat IgG, AF555 donkey anti-rabbit IgG (all Invitrogen, Carlsbad, CA), Cy3 goat anti-Armenian hamster IgG (Jackson Immunoresearch, West Grove, PA) followed by DAPI nuclear counterstain (Dianova, Hamburg, Germany). Images were obtained using a HS All-in-One Fluorescence Microscop BZ-9000 (Keyence Itasca, IL) or AxioObserver Z1 (Carl Zeiss Microscopy GmbH, Jena, Germany). Confocal imaging was performed on a Leica TCS SP8 with a 20x multi-immersion objective using LASX (Leica, Wetzlar, Germany).

RNA isolation and real time PCR

RNA was isolated using NucleoSpin RNA Plus Kit for cells and NucleoSpin RNA Kit for organs (both Macherey-Nagel, Duren, Germany). Yield and purity were determined in a Colibri Microvolume Spectrometer (Titertek-Berthold, Pforzheim, Germany). After reverse transcription (M-MLV-RT, Promega, Madison, Wisconsin, USA), real-time PCR was performed on a LightCycler 96 using SYBR-Green (FastStart Taq DNA Polymerase dNTPack, Roche, Grenzach-Wyhlen, Germany). Products were confirmed by melting curve analysis and gel electrophoresis. Data were analyzed with HPRT as a reference gene using LinRegPCR software.

Primer sequences were as follows (5′-3′):

Ccl2	FP	TTAAAAACCTGGATCGGAACCAA
	RP	GCATTAGCTTCAGATTTACGGGT
Ccr2	FP	ATCCACGGCATACTATCAACATC
	RP	TCGTAGTCATACGGTGTGGTG
Hprt	FP	CAGTCCCAGCGTCGTGATTA
	RP	AGCAAGTCTTTCAGTCCTGTC
Itgax	FP	CTGGATAGCCTTTCTTCTGCTG
	RP	GCACACTGTGTCCGAACTCA

Microarray specification

This study employed a refined version of the Whole Mouse Genome Oligo Microarray 4x44K v2 (Design ID 026655, Agilent Technologies, '026655QM_RCUG_MusMusculus', Design ID 084956) developed by the Research Core Unit Genomics, Hannover Medical School. For aortic mRNA assessment, 150ng total RNA was used. Synthesis of Cy3-labeled cRNA was performed in ¾ reaction volumes with the 'Low Input Quick Amp Labeling Kit

One-Color' (#5190-2305, Agilent Technologies) according to the manufacturer's recommendations. cRNA fragmentation, hybridization and washing steps were carried-out as recommended in the 'One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling Protocol V6.7', 2000-2500ng of labeled cRNA were used for hybridization. For renal mRNA assessment, 200ng of total RNA were used to prepare aminoallyl-UTP-modified (aaUTP) cRNA (Amino Allyl MessageAmp™ II Kit; #AM1753; Thermo Fisher Scientific) applying one-round of amplification as directed by the company, with a twofold downscaling of all reaction volumes. 50% of cDNA was used for aaUTP-cRNA synthesis. The labeling of aaUTP-cRNA was performed by use of Alexa Fluor 555 Reactive Dye (#A32756; Thermo Fisher Scientific) as recommended in the manual of the Amino Allyl MessageAmp™ II Kit (twofold downscaled reaction volumes).

cRNA fragmentation, hybridisation and washing steps were carried- out as recommended in the 'One-Color Microarray-Based Gene Expression Analysis Protocol V5.7', 750ng of each fluorescently labelled cRNA population were used for hybridization. Slides were scanned using the Agilent Micro Array Scanner G2565CA (pixel resolution 3 μm, bit depth 20). Data extraction was performed with the 'Feature Extraction Software V10.7.3.1' by use of the extraction protocol file 'GE1_107_Sep09.xml'. Measurements of on-chip replicates were averaged using the geometric mean of processed intensity values of the green channel, 'gProcessedSignal' (gPS) to retrieve one resulting value per unique non-control probe. Single Features were excluded from averaging, if they i) were manually flagged, ii) were identified as Outliers by the Feature Extraction Software, iii) lay outside the interval of '1.42 x interquartile range' regarding the normalized gPS distribution of the respective on-chip replicate population, or, iv) showed a coefficient of variation of pixel intensities per Feature that exceeded 0.5. Averaged gPS values were normalized by global linear scaling. A lower intensity threshold (surrogate value) was defined based on intensity distribution of negative control features and

fixed at 15 (aorta) or 10 (kidney) normalized gPS units.

Tissue preparation for single cell RNA sequencing

Briefly, kidneys from three male C57Bl/6 control mice (no surgery) and three male C57Bl/6 mice seven days after 27min unilateral renal IR were digested as described.[34,35] Tissues were pooled for each condition. Following red blood cell lysis and dead cell removal using a Miltenyi Dead Cell Removal Kit according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany) 10,000 cells per sample were subjected to single cell mRNA-Seq analysis (Chromium Single Cell 3 Reagent Kits v3 User Guide, Document Number CG000183, Rev A; 10x Genomics). Equimolar amounts of libraries were pooled, denatured with NaOH, and finally diluted to 1.8 pM according to the Denature and Dilute Libraries Guide (Document # 15048776 v02; Illumina). 1.3ml of the denatured pool was sequenced on an Illumina NextSeq 550 sequencer using one third of a High Output Flowcell for 75 cycles per sample (#20024906; Illumina). The proprietary 10x Genomics CellRanger pipeline (v3.0.2) was employed with default parameters.

Bioinformatic analysis of single cell sequencing data

CellRanger was used to build a "pre-mRNA" reference package from reference genome provided by 10x Genomics (Mouse reference dataset 3.0.0; November 19, 2018; mm 10) as described in https://support.10xgenomics.com/single- cell- gene- expression/ software/pipelines/latest/advanced/references. Read data were then aligned to the "premRNA" reference package with CellRanger using the aligner STAR to count aligned reads per gene and calculate clustering and summary statistics. Seurat was used to generate and visualize clusters using the standard Seurat workflow: (1) reads were filtered for based on genes and reads per cell as well as mtRNA abundance. (2) Dimensionality reduction with PCA, followed

by network-based clustering. (3) Visualization of clusters was done using T-SNE. (4) Cluster specific marker genes were generated using the fisher statistic.

Clusters were labeled with cell types by comparing the cluster specific marker genes with previously defined cell type marker genes. Cell types were matched to clusters using the Mann Whitney test between the ranking of the cluster marker genes which were part of a given cell type annotation list and those that were not. In the preprocessing cells with less than 200 or more than 8000 expressed genes were removed. Further cells with more than 15% mt-genes were removed. The data was normalized, excluding highly expressed genes and log transformed. The doublets were removed using Scrublet. The data was clustered using Louvain (neighbors=5, PCAs=10) and visualized with UMAP.

Flow cytometry

The following antibodies were used: anti-CD115 (AFS98), anti-MHCII (M5/114.15.2), anti-CD11b (M1/70), anti-CD11c (N418), anti-TCRαβ (H57-597), anti-Gr1 (R B6-8C5, anti-CD19 (6D5), anti-CD45 (30-F11), anti-CD45.1(A20), anti-CD45.2(104) (all biolegend, San Diego, CA). Yellow LIVE/DEAD® Fixable Dead Cell Stain Kit (Invitrogen, Eugene, OR) was used according to the manufacturer's instructions. Flow cytometry analysis was performed on a Becton-Dickinson FACSCanto and LSRII (Franklin Lakes, NJ) and on an ID7000 Spectral Cell Analyzer (Sony Biotechnologies, San Jose, CA). Data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

Suppl. tables

Suppl. table 1: Characteristics of LDLr$^{-/-}$ mice after 10 weeks of a high fat diet

	ctrl.	IR	p value
Body (g)	33.3±2.0 (11)	33.3±1.7 (12)	>0.99
Spleen (mg)	133.9±10.3 (11)	120.8±7.4 (12)	0.32
Rel. spleen weight (%)	0.4±0.04 (11)	0.4±0.03 (12)	0.46
Contralateral kidney (mg)	205.7±8.9 (11)	260.2±10.3 (12)	*** <0.001
Rel. cont. kidney weight (%)	0.6±0.04 (11)	0.8±0.05 (12)	* 0.02
IR (sham) kidney (mg)	198.1±3.9 (11)	76.1±7.5 (12)	*** <0.001
Rel. IR (sham) kidney weight (%)	0.6±0.03 (11)	0.2±0.03 (12)	*** <0.001
Leukocytes (10^3/µl)	6.0±0.5 (11)	7.2±1.2 (12)	0.35
Monocytes (10^3/µl)	0.5±0.04 (11)	0.6±0.08 (12)	0.38
Lymphocytes (10^3/µl)	2.3±0.2 (11)	2.7±0.4 (12)	0.37
Granulocytes (10^3/µl)	3.2±0.4 (11)	3.9±0.8 (12)	0.42
Thrombocytes (10^3/µl)	834.8±57.4 (11)	777.3±68.5 (12)	0.53
Erythrocytes (10^6/µl)	10.6±0.5 (11)	11.1±0.5 (12)	0.49
Urea (mmol/l)	9.2±0.9 (7)	13.3±1.2 (9)	* 0.02
Creatinine (µmol/l)	24.0±3.8 (7)	29.8±4.1 (10)	0.31
Phosphorous (mmol/l)	2.5±0.3 (9)	2.6±0.1 (12)	0.69
Calcium (mmol/l)	2.7±0.07 (8)	2.8±0.03 (10)	0.24
Cholesterol (mmol/l)	51.5±4.5 (11)	52.7±3.5 (12)	0.84
Triglycerides (mmol/l)	5.7±0.9 (11)	4.5±0.4 (12)	0.24

Values are mean ± SEM (n). P values are given for unpaired t test after Welch's correction. Ctrl: mice after control surgery; IR: mice after unilateral ischemia reperfusion injury.

Suppl. table 2: Characteristics of LDLr$^{-/-}$ mice after 3 weeks of a high fat diet

	Ctrl.	**IR**	**P value**
Body (g)	25.9±0.4 (7)	23.6±0.6 (9)	**0.008
Spleen (mg)	96.7±7.7 (7)	105.2±6.1 (9)	0.40
Rel. spleen weight (%)	0.37±0.03 (7)	0.45±0.02 (9)	0.05
Contralateral kidney (mg)	176.9±9.6 (7)	211.3±6.1 (9)	*0.01
Rel. cont. kidney weight (%)	0.7±0.03 (7)	0.9±0.05 (9)	** 0.005
IR (sham) kidney (mg)	171.3±6.9 (7)	72.4±4.4 (9)	*** <0.001
Rel. IR (sham) kidney weight (%)	0.7±0.02 (7)	0.3±0.02 (9)	*** <0.001
Leukocytes (10^3/µl)	6.4±0.9 (7)	6.6±0.7 (9)	0.85
Monocytes (10^3/µl)	0.5±0.07 (7)	0.6±0.06 (9)	0.62
Lymphocytes (10^3/µl)	2.7±0.4 (7)	3.1±0.4 (9)	0.47
Granulocytes (10^3/µl)	3.2±0.6 (7)	2.9±0.3 (9)	0.76
Thrombocytes (10^3/µl)	814±58 (7)	697±76 (9)	0.24
Erythrocytes (10^6/µl)	10.6±0.2 (7)	10.6±0.3 (9)	0.84
Urea (mmol/l)	7.9±0.6 (6)	11.1±0.8 (9)	**0.01
Creatinine (µmol/l)	22.2±2.3 (6)	28.5±5.8 (8)	0.34
Phosphorous (mmol/l)	2.9±0.2 (7)	3.0±0.2 (9)	0.73
Calcium (mmol/l)	2.4±0.44 (4)	2.8±0.08 (8)	0.40
Cholesterol (mmol/l)	32.3±3.3 (6)	38.2±2.3 (9)	0.18
Triglycerides (mmol/l)	5.3±0.3 (7)	4.5±0.5 (9)	0.22

Values are means ± SEM (n). P values are given for unpaired t test with Welch's correction. Ctrl: mice after control surgery; IR: mice after unilateral ischemic reperfusion injury.

Suppl. table 3: LDLr$^{-/-}$ mice after reconstitution with wildtype or Ccr2$^{-/-}$ bone marrow followed by renal ischemia reperfusion or control surgery and 10 weeks of a high fat diet

	wildtype		***Ccr2*$^{-/-}$**		**ctrl. vs. IR**		**wt vs. *Ccr2*$^{-/-}$**	
	ctrl.	**IR**	**ctrl.**	**IR**	**wt**	***Ccr2*$^{-/-}$**	**ctrl.**	**IR**
Body (g)	25.4±0.4 (11)	24.3±0.5 (13)	24.6±0.3 (8)	24.1±0.4 (9)	ANOVA not significant			
Spleen (mg)	118.9±9 (11)	111.4±8 (13)	113.3±10 (8)	108.8±10 (9)	ANOVA not significant			
Rel. spleen weight (%)	0.4±0.03 (11)	0.5±0.04 (13)	0.5±0.04 (8)	0.5±0.04 (9)	ANOVA not significant			
Contralateral kidney (mg)	163.1±8 (11)	207.7±11 (13)	155.9±5 (8)	198.6±6 (9)	*0.01	***<0.001	0.92	0.91
Rel. cont. kidney weight (%)	0.6±0.03 (11)	0.9±0.04 (13)	0.6±0.02 (8)	0.8±0.02 (9)	**0.002	***<0.001	>0.99	0.92
IR (sham) kidney (mg)	166.9±7 (11)	34.3±3 (13)	147.0±4 (8)	27.5±2 (9)	***<0.001	***<0.001	0.10	0.20
Rel. IR (sham) kidney weight (%)	0.7±0.03 (11)	0.1±0.01 (13)	0.6±0.01 (8)	0.1±0.01 (9)	***<0.001	***<0.001	0.17	0.27
Blood								
Leukocytes (10^3/µl)	8.8±1.2 (11)	10.2±1.0 (13)	8.7±0.8 (8)	11.3±1.3 (9)	ANOVA not significant			
Monocytes (cells/µl)	853±139 (11)	801±95 (11)	270±43 (7)	396±59 (6)	>0-99	0.36	**0.007	*0.009
$GR1^{HIGH}$ Monocytes (cells/µl)	317±55 (11)	344±56 (11)	44±18 (7)	55±18 (6)	>0.99	0.98	**0.002	**0.001
$GR1^{LOW}$ Monocytes (cells/µl)	536±106 (11)	457±64 (11)	227±35 (7)	340±55 (6)	ANOVA not significant			
B lymphocytes (cells/µl)	3641±887 (11)	3991±466 (11)	2818±523 (7)	4669±978 (6)	ANOVA not significant			
T lymphocytes (cells/µl)	1665±215 (11)	2055±236 (11)	1248±121 (7)	1635±189 (6)	0.64	0.35	0.53	0.36
Neutrophils (cells/µl)	2285±248 (11)	2597±477 (11)	3156±822 (7)	4028±1367 (6)	ANOVA not significant			
Thrombocytes (10^3/µl)	641±46 (11)	716±47 (13)	882±80 (8)	886±132 (9)	ANOVA not significant			
Erythrocytes (10^6/µl)	10.3±0.6 (11)	9.7±0.3 (13)	9.7±0.6 (8)	8.9±0.4 (9)	ANOVA not significant			
Urea (mmol/l)	7.4±0.5 (7)	11.9±1.0 (7)	7.4±0.6 (6)	11.7±0.6 (8)	*0.01	**0.001	>0.99	>0.99
Creatinine (µmol/l)	20.7±2.2 (11)	25.7±1.7 (13)	23.7±2.9 (8)	29.7±2.6 (8)	ANOVA not significant			
Phosphorous (mmol/l)	2.8±0.1 (11)	2.9±0.1 (11)	2.7±0.2 (8)	2.5±0.1 (8)	ANOVA not significant			
Calcium (mmol/l)	2.7±0.03 (11)	2.7±0.05 (11)	2.5±0.18 (7)	2.7±0.04 (8)	ANOVA not significant			
Cholesterol (mmol/l)	37.7±4.9 (11)	37.3±2.0 (13)	35.4±5.0 (8)	33.6±2.7 (9)	ANOVA not significant			
Triglycerides (mmol/l)	7.9±1.3 (11)	8.2±0.7 (13)	6.3±1.1 (8)	6.8±1.2 (9)	ANOVA not significant			

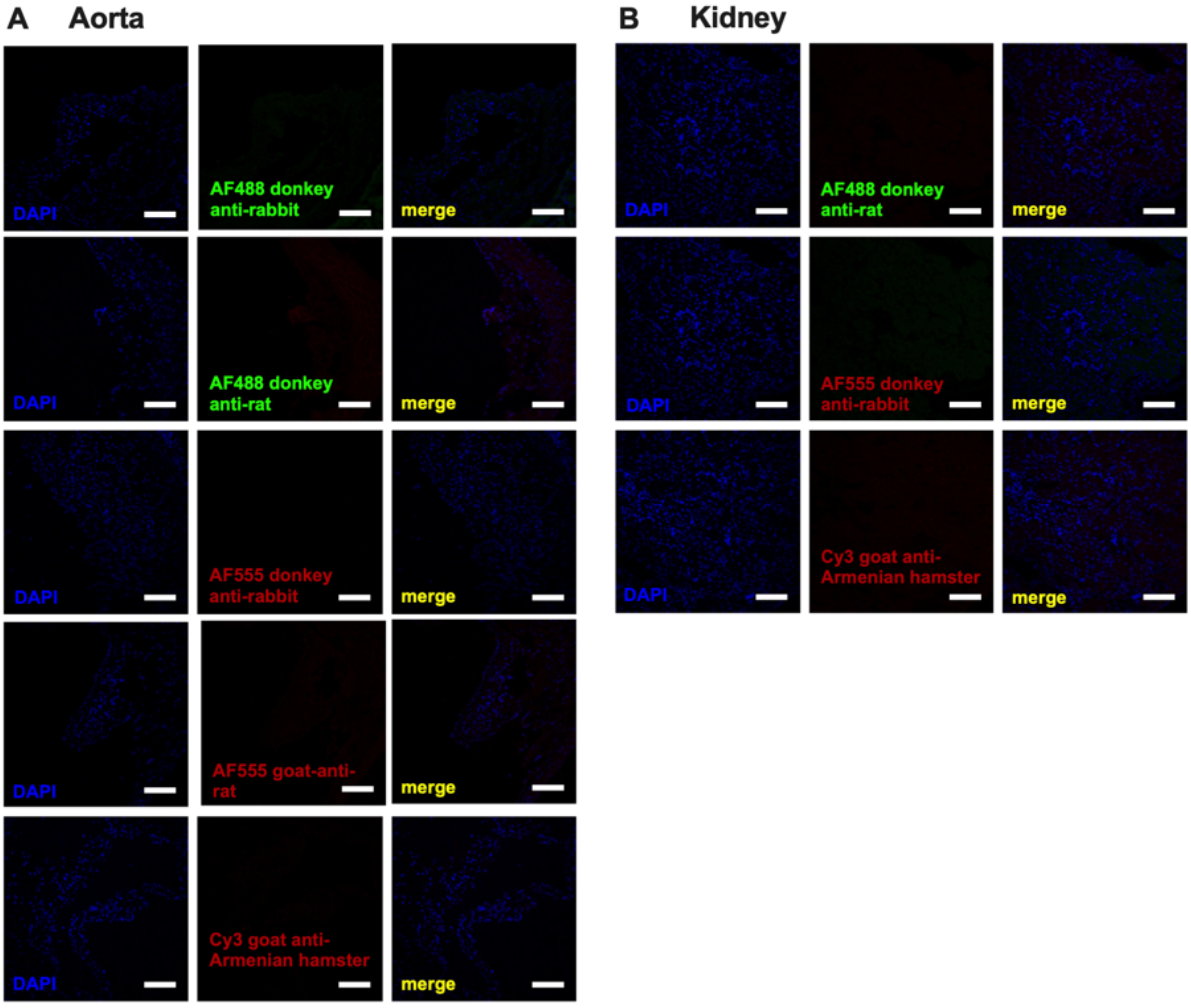
Values are mean ± SEM (n). P values are given for Dunnett's test if ANOVA was significant.
Ctrl: mice after control surgery; IR: mice after unilateral ischemia reperfusion injury.

Suppl. table 4: LDLr$^{-/-}$ mice after reconstitution with wildtype or Ccr2$^{-/-}$ bone marrow followed by renal ischemia reperfusion or control surgery and 3 weeks of a high fat diet

	wildtype		***Ccr2*$^{-/-}$**		**ctrl. vs. IR**		**wt vs. *Ccr2*$^{-/-}$**	
	ctrl.	**IR**	**ctrl.**	**IR**	**wt**	***Ccr2*$^{-/-}$**	**ctrl.**	**IR**
Body (g)	23.6±0.7 (5)	22.4±0.7 (5)	23.6±0.5 (4)	22.4±0.5 (7)	ANOVA not significant			
Spleen (mg)	112.0±17 (5)	95.9±7 (5)	119.3±10 (4)	117.4±16 (7)	ANOVA not significant			
Rel. spleen weight (%)	0.5±0.1 (5)	0.4±0.03 (5)	0.5±0.04 (4)	0.5±0.1 (7)	ANOVA not significant			
Contralateral kidney (mg)	154±12 (5)	172±10 (5)	160±13 (4)	184±9 (7)	ANOVA not significant			
Rel. cont. kidney weight (%)	0.7±0.05 (5)	0.8±0.03 (5)	0.7±0.04 (4)	0.8±0.03 (7)	ANOVA not significant			
IR (sham) kidney (mg)	152±11 (5)	48±2 (5)	154±14 (4)	51±2 (4)	**0.002	*0.02	>0.99	0.60
Rel. IR (sham) kidney weight (%)	0.6±0.04 (5)	0.2±0.01 (5)	0.6±0.05 (4)	0.2±0.01 (7)	**0.001	*0.01	>0.99	0.62
Blood								
Leukocytes (10^3/µl)	9.6±1.5 (8)	6.4±0.5 (7)	10.5±1.7 (8)	8.1±0.9 (14)	ANOVA not significant			
Monocytes (cells/µl)	1119±238 (8)	493±36 (7)	343±53 (8)	252±28 (14)	0.12	0.47	*0.05	***<0.001
GR1^{HIGH} Monocytes (cells/µl)	549±122 (8)	228±17 (7)	71±23 (8)	51±8 (14)	0.12	0.86	*0.02	***<0.001
GR1^{LOW} Monocytes (cells/µl)	571±155 (8)	277±26 (7)	272±39 (8)	201±24 (14)	ANOVA not significant			
B lymphocytes (cells/µl)	3705±629 (8)	2537±304 (7)	4229±1150 (8)	4248.2±665 (14)	ANOVA not significant			
T lymphocytes (cells/µl)	1491±230 (8)	1134±70 (7)	1167±188 (8)	976±81 (14)	ANOVA not significant			
Neutrophils (cells/µl)	2953±479 (8)	1936±482 (7)	4226±1120 (8)	2260±292 (14)	ANOVA not significant			
Thrombocytes (10^3/µl)	648±59 (8)	656±34 (7)	838±121 (8)	786±46 (14)	ANOVA not significant			
Erythrocytes (10^6/µl)	11.1±0.2 (8)	10.9±0.2 (7)	10.4±0.1 (8)	10.4±0.3 (14)	0.94	>0.99	0.06	0.41
Urea (mmol/l)	8.3±0.6 (5)	13.5±0.8 (5)	8.0±1.0 (4)	12.3±0.8 (6)	**0.007	*0.04	>0.99	0.75
Creatinine (µmol/l)	22.7±1.7 (5)	29.2±1.8 (5)	22.2±2.1 (4)	30.1±1.0 (7)	0.1	0.08	>0.99	0.98
Phosphorous (mmol/l)	3.1±0.2 (5)	2.8±0.3 (5)	2.8±0.2 (4)	2.7±0.2 (7)	ANOVA not significant			
Calcium (mmol/l)	2.6±0.1 (5)	2.8±0.06 (5)	2.6±0.1 (4)	2.8±0.04 (5)	ANOVA not significant			
Cholesterol (mmol/l)	28.8±4.2 (5)	34.1±3.0 (5)	33.3±6.2 (4)	25.3±0.9 (7)	ANOVA not significant			
Triglycerides (mmol/l)	4.5±0.9 (4)	5.6±0.7 (5)	4.2±0.7 (4)	4.0±0.4 (7)	ANOVA not significant			

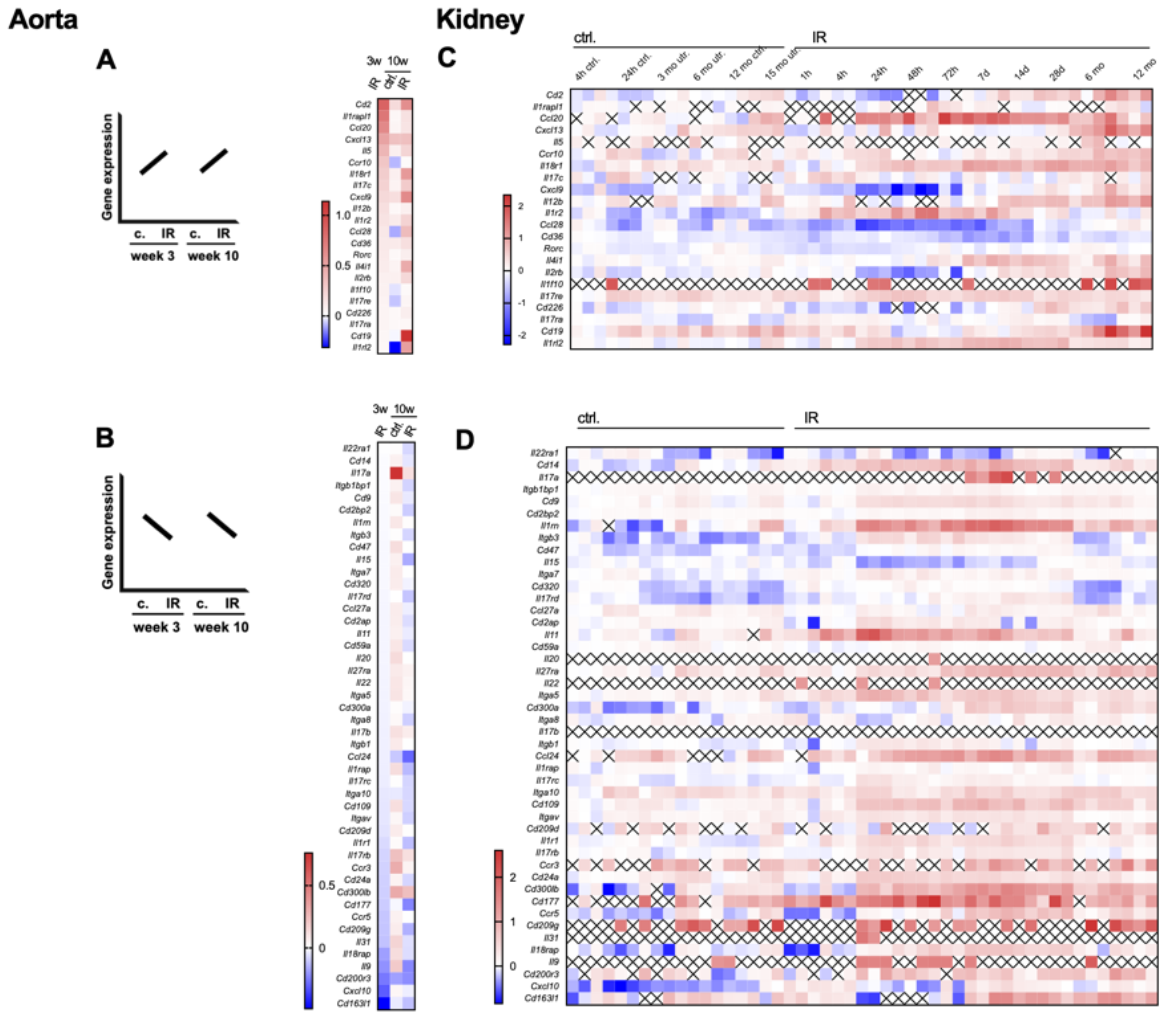
Values are mean ± SEM (n). P values are given for Dunnett's test if ANOVA was significant.
Ctrl: mice after control surgery; IR: mice after unilateral ischemia reperfusion injury.

Supplemental figures and figure legends:



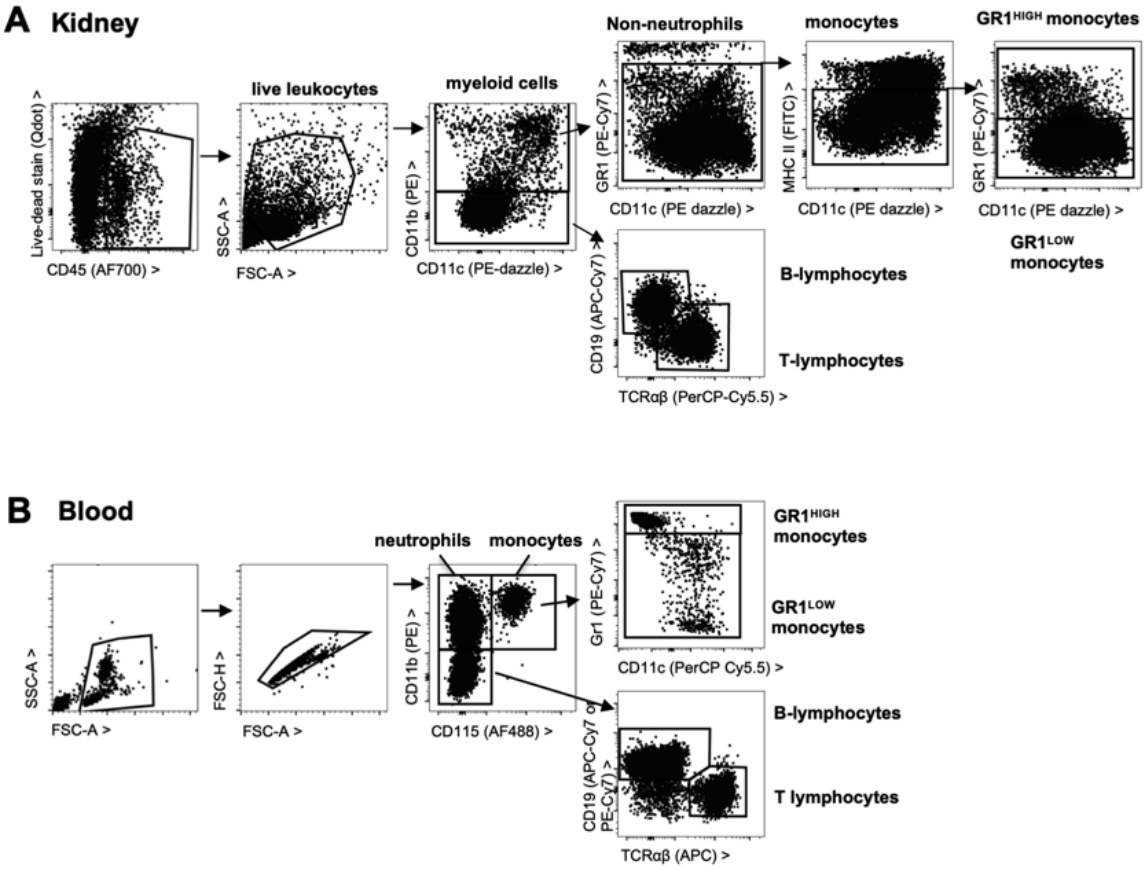
Suppl. figure 1: Immunostaining specificity controls

(A,B) Confocal images of aortic root (A) and kidney (B) sections imaged as in figures 1, 4 and 6 but stained with omission of the primary antibodies. Secondary antibodies were used as indicated in methods as follows: AF488 donkey anti-rat and AF555 goat anti-rat for F4/80, AF488 donkey anti-rabbit and AF555 donkey anti-rabbit for CD3, AF488 donkey anti-rat for CD11b, Cy3-goat anti-Armenian hamster for CD11c (blue: DAPI, size bars=100µm).



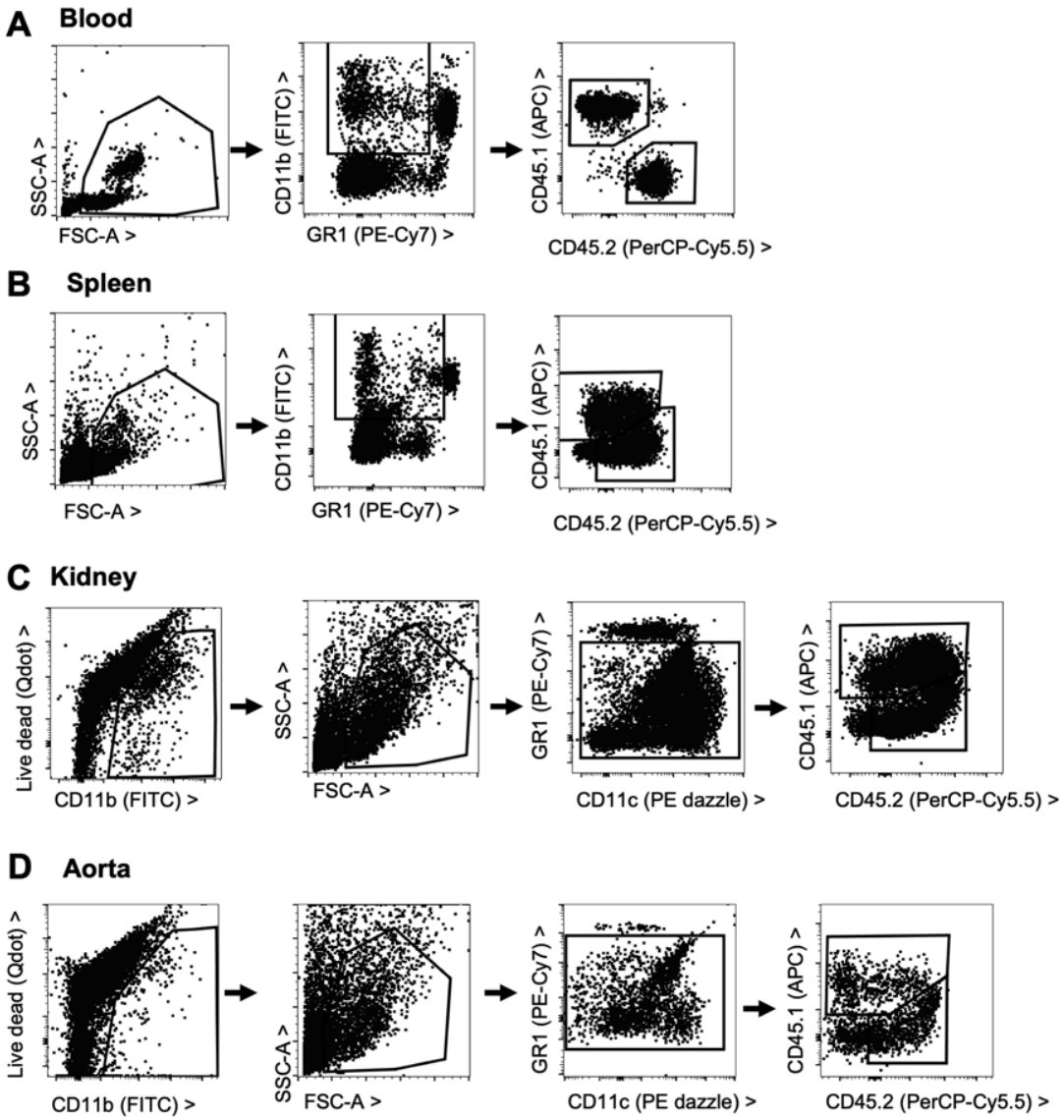
Suppl. figure 2: Parallels in aortic and renal post-ischemic inflammatory gene expression

(A,B) Differentially expressed immune genes in atherosclerotic aortas from mice after renal IR and controls were grouped in genes upregulated upwards (A) or downwards (B) 1.5fold or more in both early and late atherosclerosis as detailed in methods. **(C,D)** Regulation of these gene sets in the post-ischemic murine kidney (n=3-4/timepoint, expression relative to baseline, x=not detected in the dataset[25]).



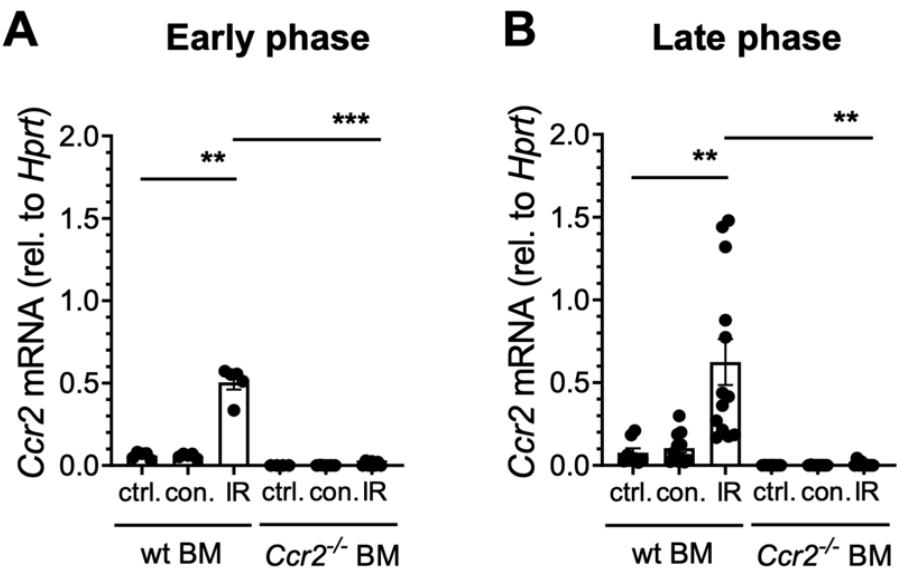
Suppl. figure 3: Flow cytometric gating strategies

(A,B) Gating strategies of renal (A) and blood (B) leukocytes are shown.



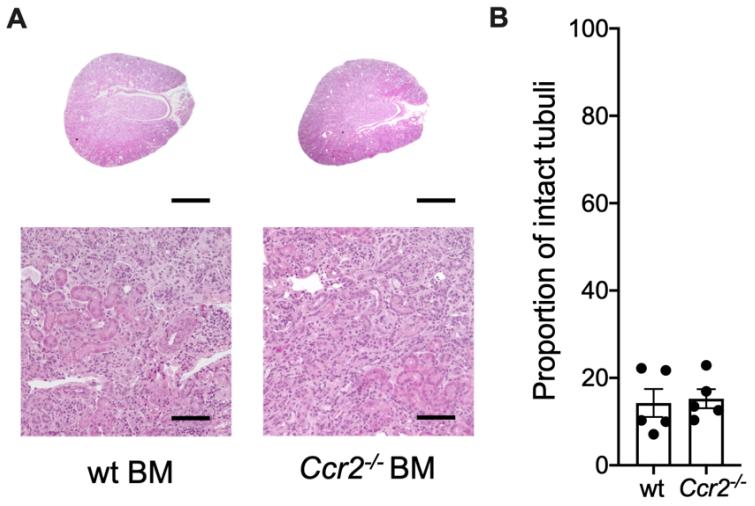
Suppl. figure 4: Flow cytometric gating strategies for assessment of mixed bone marrow chimeras

(A-D) Gating strategies of blood (A), spleen (B), kidney (C) and aortic (D) leukocyte for $CD11b^{+}$ myeloid cells and CD45.1 and CD45.2 syngenic markers are shown.



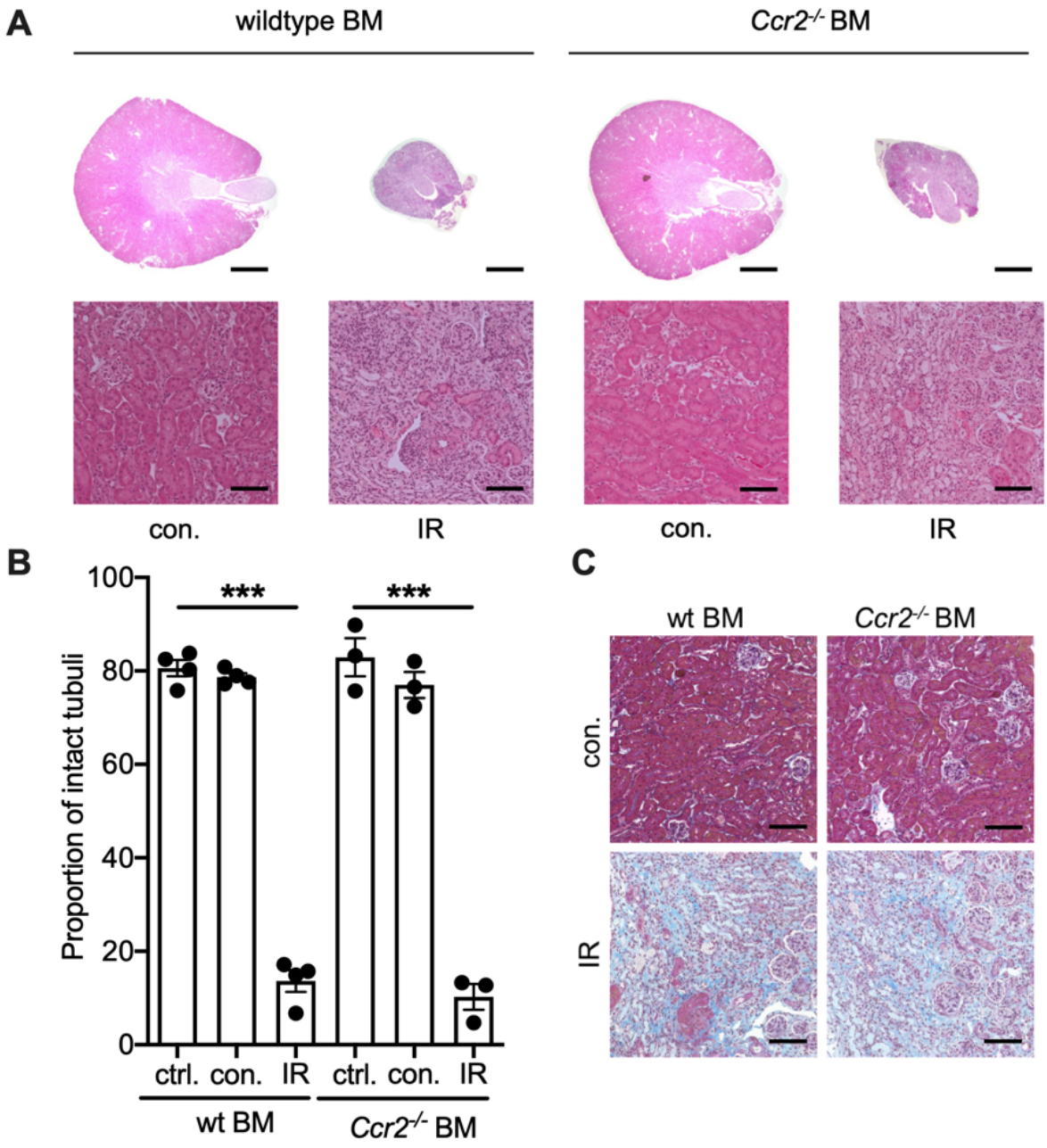
Suppl. figure 5: Renal Ccr2 expression after ischemia reperfusion injury

(A,B) Renal *Ccr2* expression was assessed by qPCR in *LDLr*$^{-/-}$ mice after IR or control surgery and after reconstitution with wildtype or *Ccr2*$^{-/-}$ bone marrow as depicted in figure 5A after three (A) and ten weeks (B) of atherosclerosis induction (A: n=4-7 mice per group from 6 indep. exp., B: n=7-13 mice per group from 10 indep. exp., Dunnett's after ANOVA).



Suppl. figure 6: Histologic outcome in LDLr-/- mice in the absence and presence of myeloid CCR2

(A,B) Renal histology after IR was assessed in *LDLr*$^{-/-}$ mice reconstituted with wildtype or *Ccr2*$^{-/-}$ bone marrow after three weeks of atherosclerosis induction (A, Representative HE-stained kidney sections (size bars= 1mm and 100µm, B: proportion of intact tubuli in cortex and outer stripe of outer medulla, n=5 mice per group, n= 5 indep. exp., t-test).



Suppl. figure 7: Similar histologic outcome after IR in the absence and presence of myeloid CCR2

(A-C) Renal histology was assessed in *LDLr*$^{-/-}$ mice after IR or control surgery and after reconstitution with wildtype or *Ccr2*$^{-/-}$ bone marrow as depicted in figure 5A after ten weeks of atherosclerosis induction (A, Representative HE-stained kidney sections (size bars= 1mm and 100µm, B: proportion of intact tubuli in cortex and outer stripe of outer medulla, n=3–4 mice per group, n= 2 indep. exp., Dunnett's after ANOVA). **(C)** Fibrosis was assessed by Masson Trichrome-staining (representative. sections, size bars= 100µm).