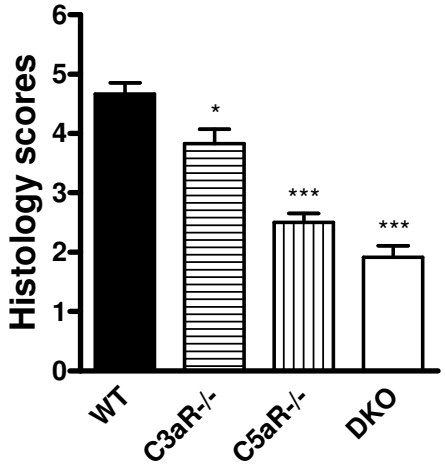
Supplemental figure 1

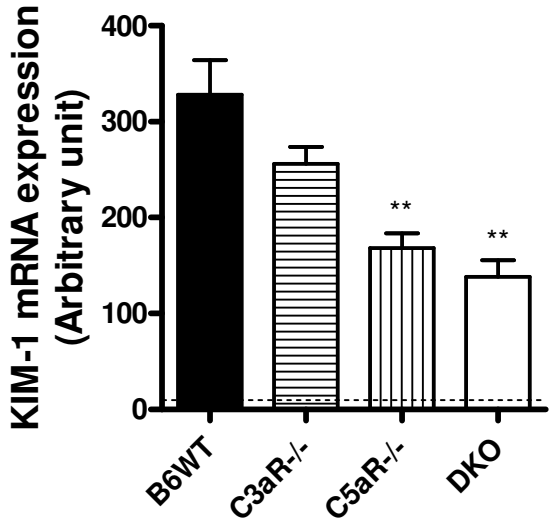
Histopathology of kidneys after 48h reperfusion



Renal IR injury was induced in four groups of mice: WT (n=12), C3aR-/- (n=6), C5aR-/- (n=6) and DKO (n=12 per group). Kidney tissues were collected at 48h after reperfusion. Tissue injury were analyzed in PAS stained sections by histological scores. Data were shown as mean ± SEM (n=4 per group, 3 fields of each kidney) and analyzed by Mann-Whitney test. * $P < 0.05$, *** $P < 0.0001$, comparing WT and C3aR-/- or C5aR-/- or DKO mice.

Supplemental figure 2

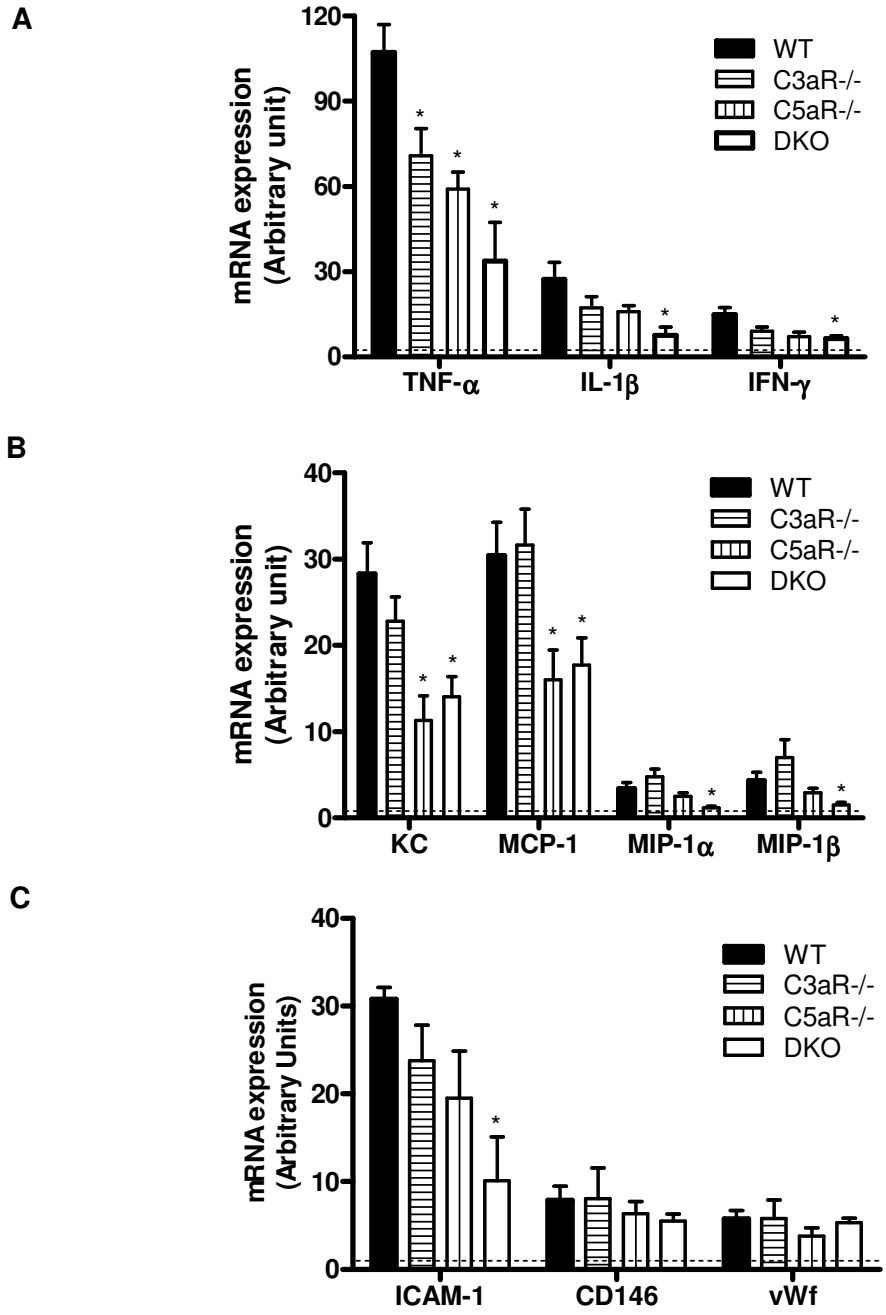
Renal expression of KIM-1 is reduced in C3aR-/-, C5aR-/- and DKO mice, compared with WT mice



Renal IR injury was induced in four groups of mice: WT, C3aR-/- , C5aR-/- and DKO (n=6 per group). kidney tissues were collected at 48h after reperfusion. KIM-1 expression in postischemic kidneys was analysed by quantitative real time RT-PCR. The dotted line indicates KIM-1 gene expression level in normal kidney tissues. Data were shown as mean ± SEM and analyzed by Mann-Whitney test, ** $P < 0.005$, comparing WT and C3aR-/- or C5aR-/- or DKO mice.

Supplemental figure 3

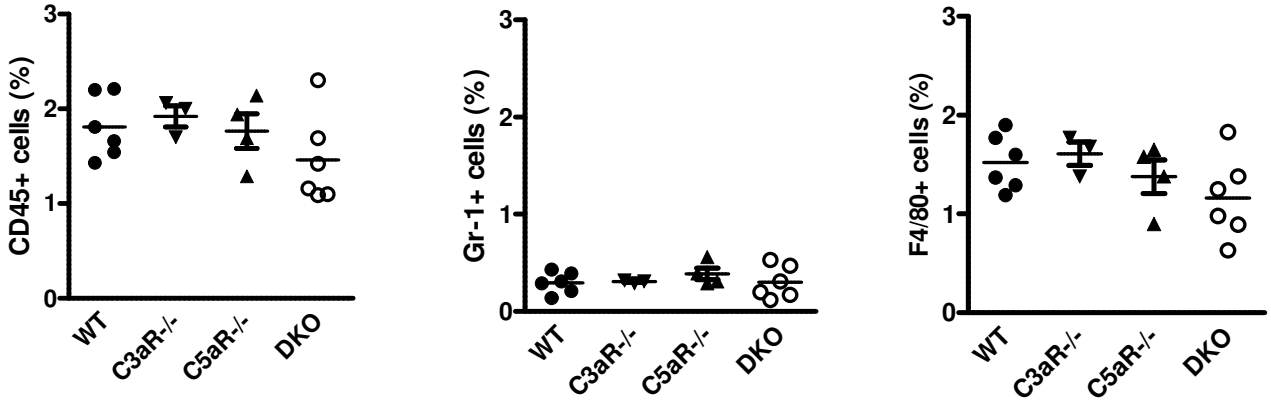
Deficiency of C3aR/C5aR reduces renal production of inflammatory mediators following renal IR



Renal IR injury was induced in WT, C3aR-/-, C5aR-/- and DKO mice (n=5 per group). Kidney tissues were harvested at 48h after reperfusion and used for quantitative real time RT-PCR analysis. A. Pro-inflammatory cytokines (TNF-α, IL-1β and IFN-γ). B. Chemokines (KC, MIP-1α, MIP-1β and MCP-1). C. Adhesion molecules and endothelial activation/injury markers (ICAM-1, CD146 and vWF). The dotted lines in A.B.C indicate the gene expression levels in normal kidney tissue. Data were shown as mean ± SEM and analyzed by Mann-Whitney test, * $P < 0.05$, comparing WT and C3aR-/- or C5aR-/- or DKO mice.

Supplemental figure 4

Proportions of CD45$^+$, Gr-1$^+$ or F4/80$^+$ cells in kidneys from naïve WT, C3aR-/-, C5aR-/- and DKO mice



Renal single cell suspensions were prepared from naïve WT, C3aR-/-, C5aR-/- and DKO mice (n=6 per group). Flow cytometric analysis of CD45$^+$, Gr-1$^+$ and F4/80$^+$ cells was performed in the cell suspensions prepared from individual kidneys.

Supplemental table

PCR primer sequences and product sizes

Primers	Oligonucleotide Sequence (5' → 3')	Product Size (bp)	Gene bank code
18S-1 18S-2	ATCCCTGAGAAGTTCCAGCA CCTCTTGGTGAGGTCGATGT	153	NM_011296.1
C5aR-1 C5aR-2	CAGGACATGGACCCCATAGAT ACCAGGAACACCACCGAGTAG	155	NM_007577.2
C3aR-1 C3aR-2	GATTCCATCTCAGTGTGCTTGAC GTGTCCTTGGAGAATCAGGTGAG	290	NM_009779.1
TNFα-1 TNFα-2	TGAGCACAGAAAGCATGATCC GCCATTTGGGAACTTCTCATC	200	ENSMUSG00000024401
IL-1β-1 IL-1β-2	GCTCTCCACCTCAATGGACA TTGGGATCCACACTCTCCAG	182	ENSMUSG00000027398
IFNγ-1 IFNγ-2	ACT GGC AAA AGG ATG GTG AC TGA GCT CAT TGA ATG CTT GG	237	NM_008337.3
KC-1 KC-2	CTT GAA GGT GTT GCC CTC AG ACA GGT GCC ATC AGA GCA GT	181	ENSMUST00000031327
MCP-1-1 MCP-1-2	GGCTCAGCCAGATGCAGTTA ATTTGGTTCCGATCCAGGTT	219	ENSMUSG00000035385
MIP-1α-1 MIP-1α-2	CACTGCCCTTGCTGTTCTTC GGCATTCAGTTCCAGGTCAG	262	ENSMUSG00000000982
MIP-1β-1 MIP-1β-2	CCCAGCTCTGTGCAAACCTA TCTGCCTCTTTTGGTCAGGA	248	ENSMUSG00000018930
ICAM-1-1 ICAM-1-2	AGAGGTGACTGAGGAGTTCGAC AGAAGCTTCGTTTGTGATCCTC	284	NM_010493.2
CD146-1 CD146-2	AGCCCCAGAGGAACCAACTA AGGCGTGCACTCAGAACACT	234	ENSMUST00000098852
vWf-1 vWf-2	CTTTGGGGACGACTTCATCA TAGGGCATGGAGATGCTTTG	228	ENSMUSG00000001930
KIM-1-1 KIM-1-2	AAGCCGCAGAAAAACCCTAC TTGTCTTCAGCTCGGGAATG	206	ENSMUSG00000040405

* Primer-1 is identical to the coding strand; primer-2 is complementary to the coding strand. All primers were designed such that there are intronic sequences between the primer 1 and primer 2.