Vascular-resident CD169-positive Monocytes and Macrophages Control Neutrophil Accumulation in the Kidney with Ischemia-reperfusion Injury

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Supplemental discussion

Time course of gene recombination by Cre recombinase

In CD169-Cre-YFP mice, YFP^+ cells may not correspond to CD169-expressing cells because Cre recombinase remains active even after the cells cease to express CD169. Thus, YFP-positive cells may not necessarily express CD169. On the other hand, activation of CD169 gene promoter does not instantly initiate YFP expression. When ES cells expressing tamoxifen-inducible modified estrogen receptor were cultured *in vitro* in the presence of tamoxifen, fluorescence intensity continued to increase up to day 4 [1]. Hori et al generated mice that express GFP-Cre fusion protein under the control of Foxp3 promoter and crossed them with ROSA26-red fluorescent protein (RFP) mice. In those mice, $Foxp3^+$, $CD4^+$ thymocytes contained many $RFP^{-\text{ or lo}}$ cells until 4 days after the birth, indicating the presence of newly developed $Foxp3^+$ cells that have initiated Foxp3 transcription but have not fully accumulated RFP [2]. Compatible with the reported kinetics, M-CSF-cultured BM cells express YFP more than 3 days after CD169 expression (**Fig. S3**). Disappearance of monocytes in CD169-DTR mice was more profound than the frequency of YFP-positive cells in the peripheral blood. We argue that the gap results from a delayed gene recombination in peripheral blood $CD169^+$ monocytes. Due to the rapid turnover of peripheral blood monocytes, they may differentiate into tissue macrophages before they express YFP in blood stream.

The interaction of perivascular kidney CD169 macrophages with vascular endothelial cells

Our results strongly suggest that some CD169 monocytes attach to vascular endothelial cells from the luminal side, whereas perivascular kidney CD169 macrophages interact with vascular endothelial cells from the side of basal lamina. Although we have not determined

the details of interaction between perivascular macrophages and capillary endothelial cells, we speculate that the mechanism of interaction might be similar to that of interaction between pericytes and capillary endothelial cells. The capillary wall consists of vascular endothelial cells, the basal lamina, and pericytes. Pericytes are located in the perivascular region, and have been recently reported to interact closely with endothelial cells and to regulate the functions of these cells[3, 4]. From these findings, it is possible for perivascular macrophages to interact with vascular endothelial cells, and to regulate these functions.

References

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3. Armulik A, Genové G, Betsholtz C: Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell*, 21: 193-215, 2011.

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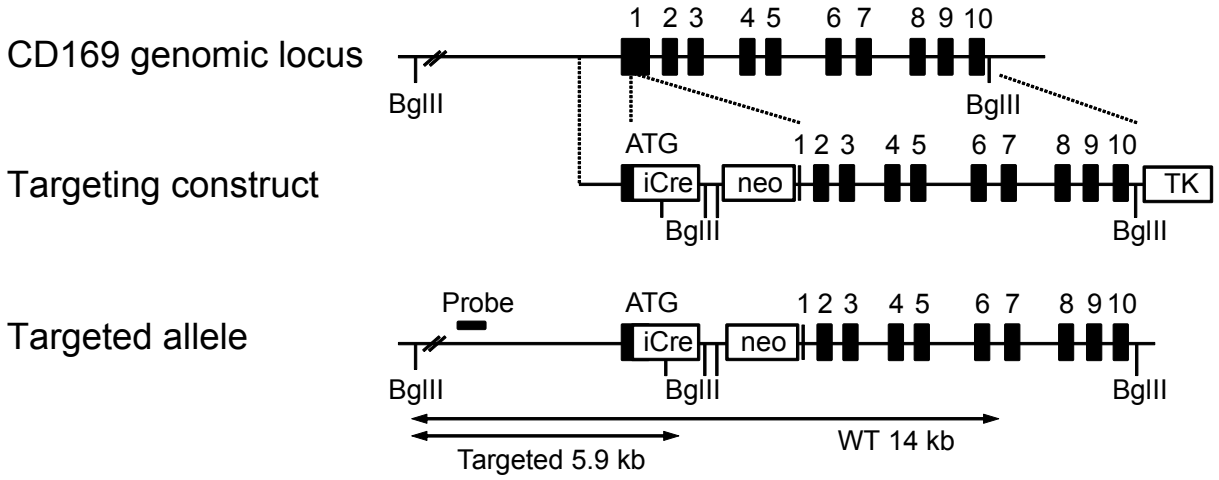
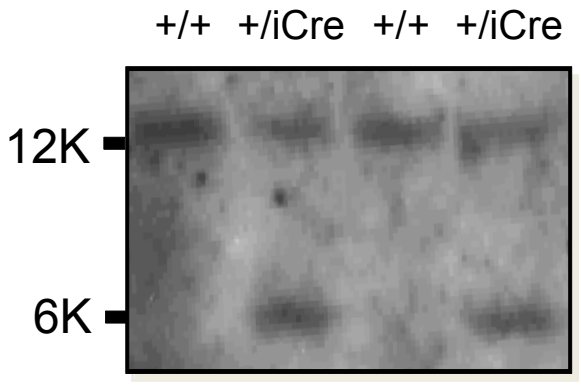
Karasawa et al. Fig.S1.

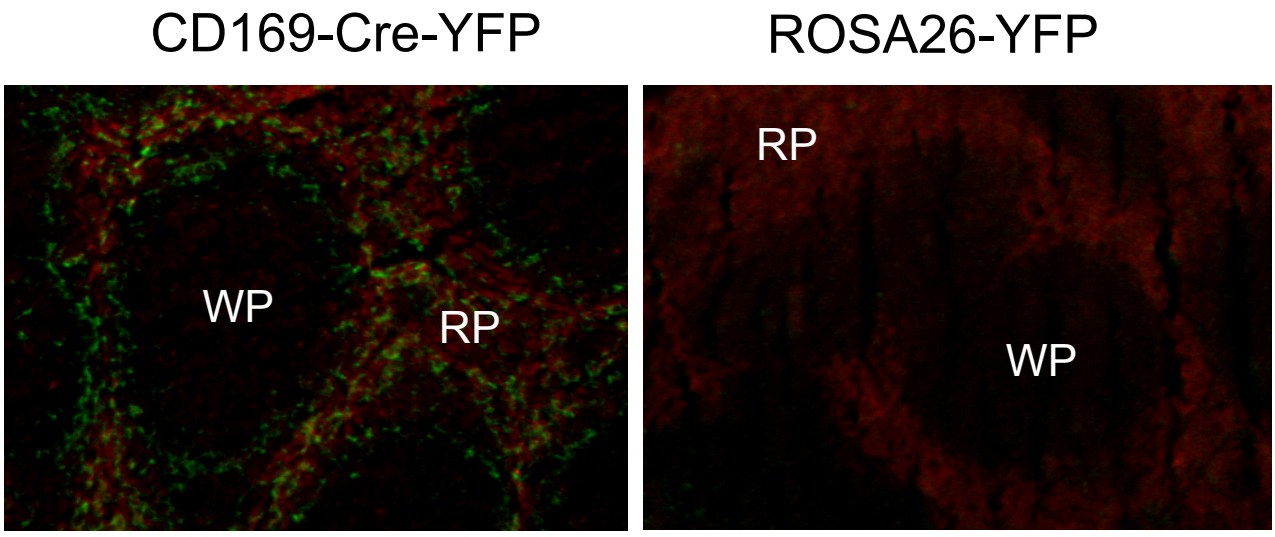




Supplementary Fig S1 . CD169-Cre-YFP mice

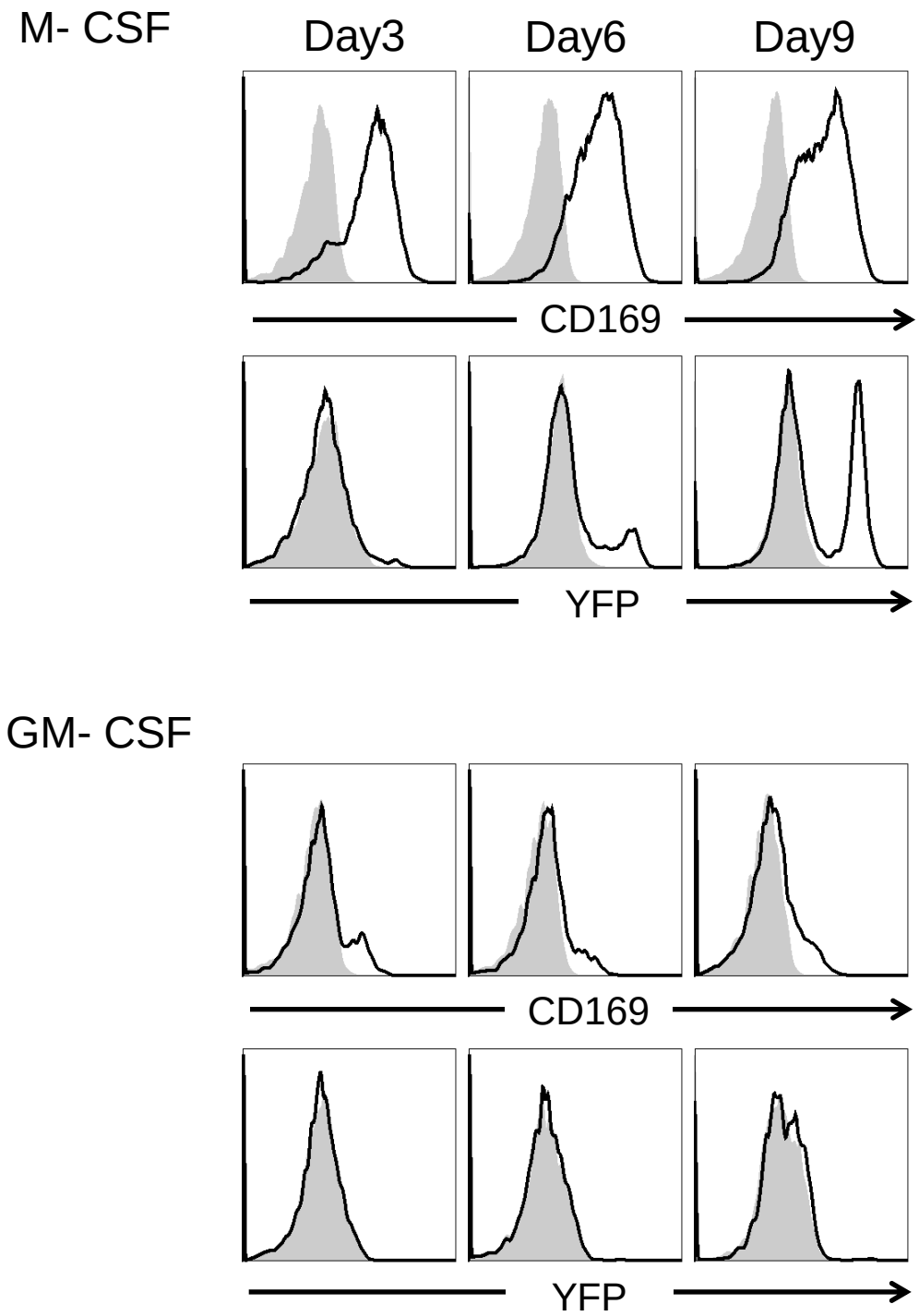
Schematic diagram of the CD169-Cre targeting construct. Exons of the CD169 gene (*siglec1*) are indicated by solid boxes. The improved Cre recombinase cDNA (iCre), the neomycin resistant gene (neo), and the HSV-thymidine kinase (TK) gene are indicated by open boxes. The probe used for Southern blot analysis is indicated by a solid line together with the predicted hybridization fragments. Genomic Southern blot analysis of WT (+/+) and CD169-Cre (+/iCre) mice was carried out using Bgl II digest.

Karasawa et al. Fig.S2.



Supplementary Fig S2. Immunohistochemical analysis of the spleen of CD169-Cre-YFP mice Cryosections of spleens from CD169-Cre-YFP mice (left) or ROSA26-YFP mice (right) were stained for YFP (Green) and F4/80 (red). WP: white pulp, RP: red pulp. Original magnification, x 40.

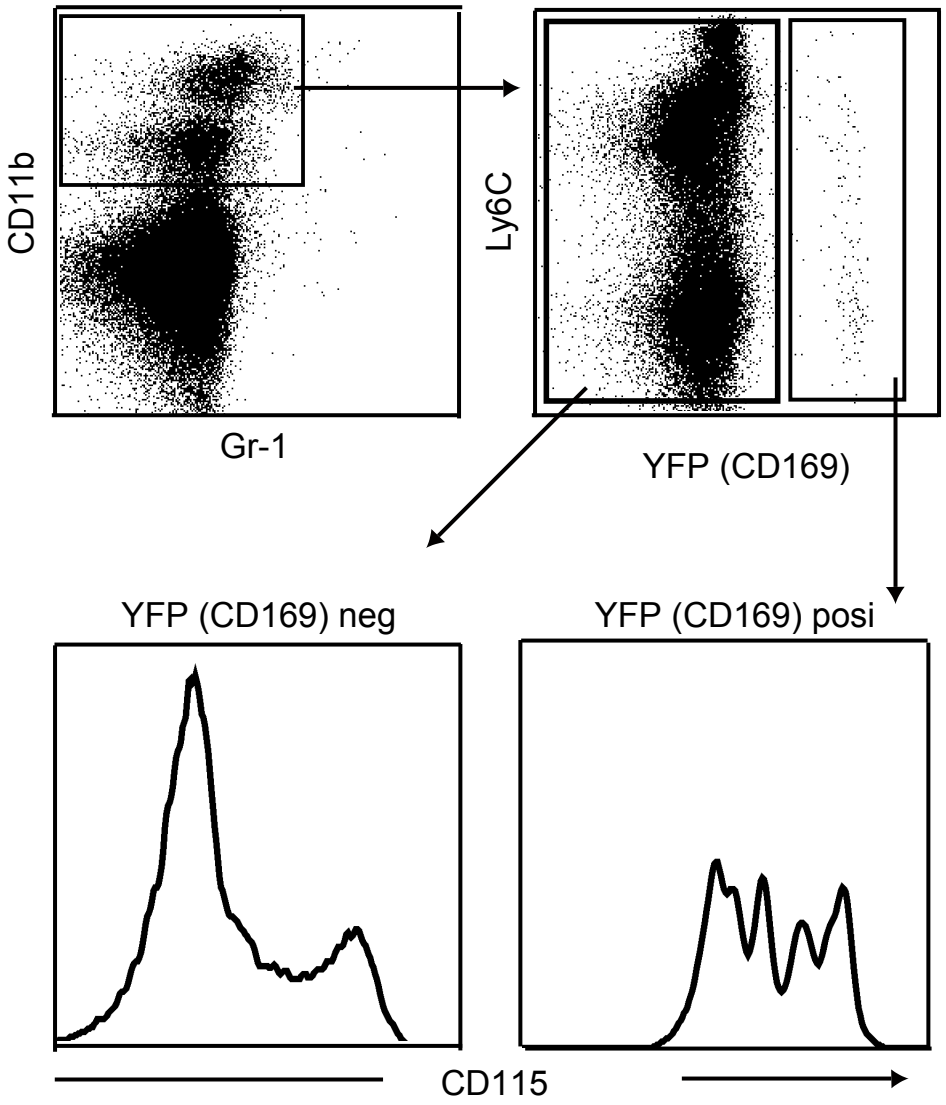
Karasawa et al. figure.S3.



Supplementary Fig S3. YFP expression in bone marrow-derived macrophages and dendritic cells from CD169-Cre-YFP mice

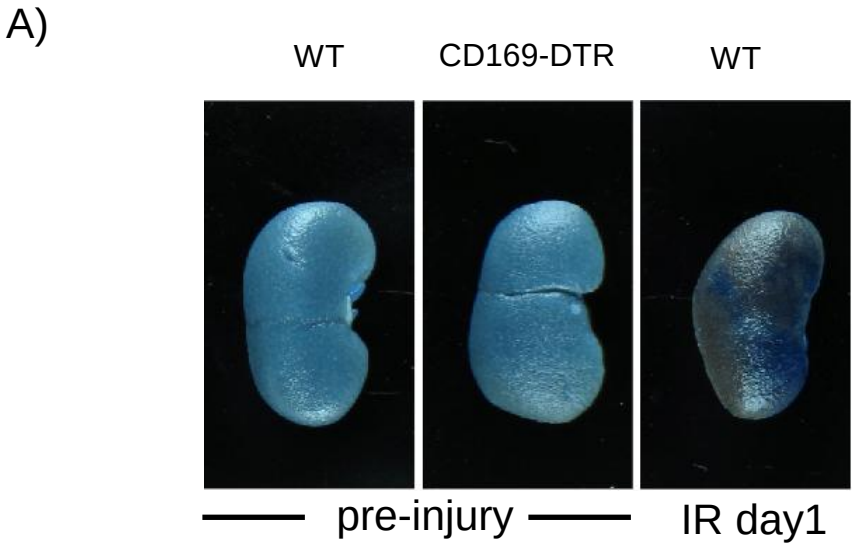
Bone marrow cells from CD169-Cre-YFP mice were cultured either in DMEM/10% FCS supplemented with M-CSF (CMG 14-12 culture medium) or RPMI/10% FCS supplemented with GM-CSF (MGM-5 culture medium). YFP expression levels in $CD11b^+$, $F4/80^+$ fraction (M-CSF) or $CD11b^+$, $CD11c^+$ fraction (GM-CSF) were detected by a flowcytometer every 3 days up to 9 days after the culture. CD169 expression levels of those cells were detected with anti-CD169 antibody (clone M7). Shadow indicates unstained control for CD169 or YFP intensity of cells prepared from ROSA26-YFP mice. Data is representative of 2 independent experiments.

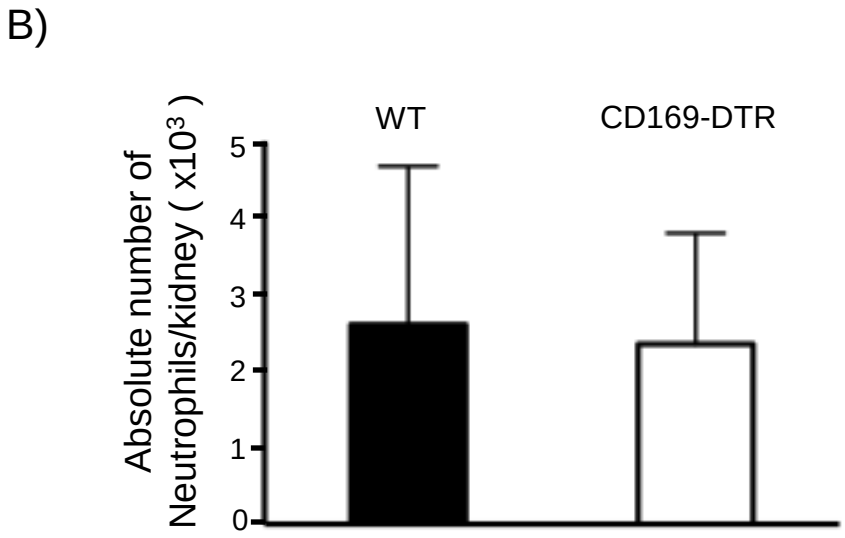
Karasawa et al. Fig.S4.



Supplementary Fig S4. YFP-positive cells in peripheral blood express CD115
Peripheral blood was obtained from CD169-Cre-YFP mice treated with anti-Gr-1 (Clone RB6-8C5) antibody. RBCs were lysed and residual leukocytes were stained for CD11b, Gr-1, Ly6C, and CD115, and analyzed with a flowcytometer. Data is representative of 3 independent experiments.

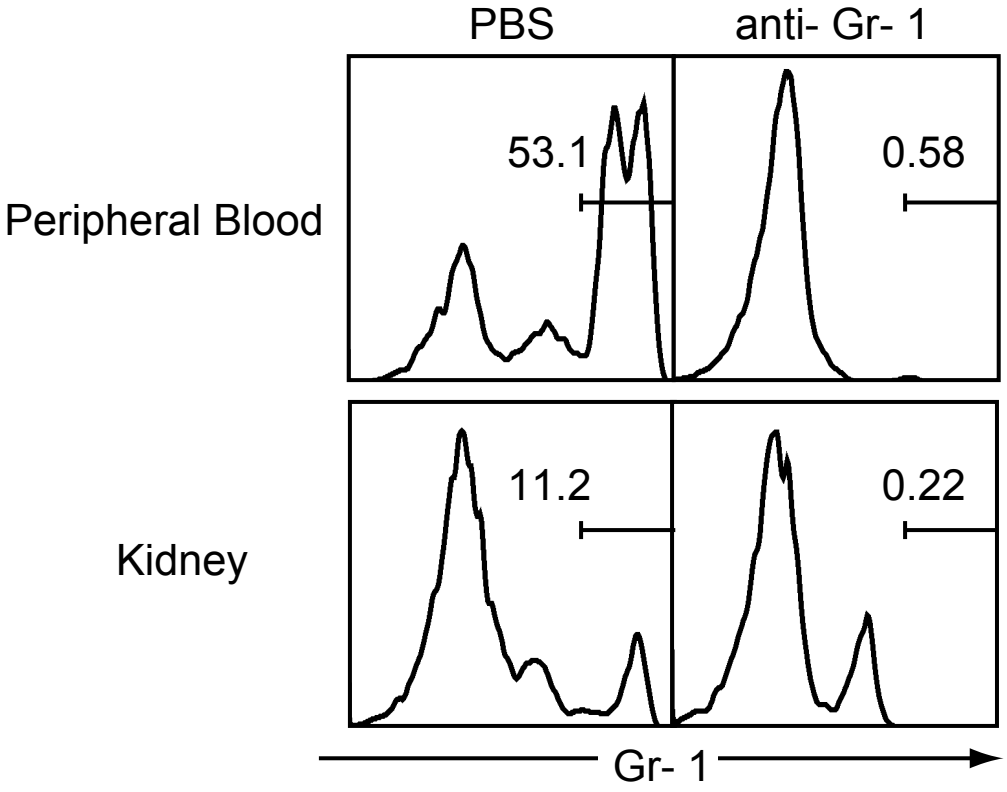
Karasawa et al. figure.S5.





Supplementary Fig S5. A) Vascular permeability assay. Left and middle: DT was injected intraperitoneally into WT or CD169-DTR mice. Thirty-six hours later, Evans blue (200μl of 25mg/dl) was injected intravenously into WT or CD169-DTR mice. **Right:** DT was injected intraperitoneally into WT mice. Thirty-six hours later, unilateral IR was performed, followed by the i.v. injection of Evans blue. **B) The numbers of neutrophils in kidneys of WT and CD169-DTR mice in the pre-injury state.** DT was injected intraperitoneally into WT or CD169-DTR mice. Thirty-six hours later, absolute nembers of $CD11b^{+}$, $F4/80^{lo}$, $Gr\text{-}1^{high}$ neutrophils were quantitated by a flowcytometer.

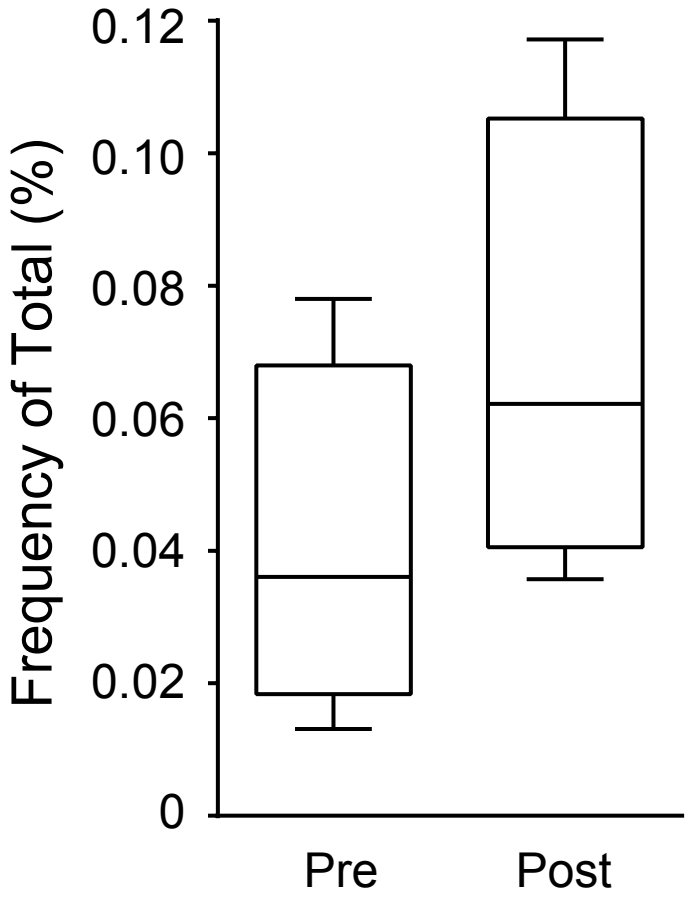
Karasawa et al. Fig.S6.



Supplementary Fig S6. Injection of anti-Gr-1 antibody selectively depletes neutrophils

One hundred μg of anti-Gr-1 (Clone RB6-8C5) antibody was injected intraperitoneally into WT mice. One day after the antibody injection, blood was collected and kidneys were resected, stained with anti-CD11b and anti-Gr-1 antibodies, and analyzed with a flowcytometer.

Karasawa et al. Fig.S7.



Supplementary Fig S7.

CD169^{+} monocytes adhere to vascular surface in a CD11b-dependent manner.

Anti-Gr-1 antibody was injected into CD169-Cre-YFP mice in order to deplete neutrophils. Fifteen hours after the anti-Gr-1 injection, 100 μg of anti-CD11b antibody (clone 5C6) was injected intravenously into the mice. The frequency of YFP^{+} cells in Gr-1^{-}, CD11b^{+}, CD115^{+} fraction of peripheral blood monocytes was quantitated by a flowcytometer 3h before and 1 h after anti-CD11b antibody injection. Each symbol represents data from an individual animal. n = 4 mice/ group. Lines indicate the minimum, average +/- SD, and maximum values of each group.

Karasawa et al. Table.S1.

Sequences of quantitative RT-PCR primers

Genes	Sequence (5' →3')	Position	Length (bp)
icam1 (NM_010493.2)			
Fwd	ATCTCAGGCCGCAAGGG	653-669	66
Rev	CGAAAGTCCGGAGGCTCC	701-718	
icam2 (NM_010494.1)			
Fwd	TATGAGCCGATGCAGGACAAC	800-820	108
Rev	GTGCTGGCCAAAGATAAAGCATAG	884-907	
tnf (NM_013693.2)			
Fwd	ACCCTCACACTCAGATCATC	385-404	188
Rev	GAGTAGACAAGGTACAACCC	553-572	
il1β (NM_008361.3)			
Fwd	GGATGAGGACATGAGCACCT	323-342	174
Rev	AGCTCATATGGGTCCGACAG	477-496	
cxcl1 (NM_008176.3)			
Fwd	ACTGCACCCAAACCGAAGTC	239-258	114
Rev	TGGGGACACCTTTTAGCATCTT	331-352	
cxcl2 (NM_009140.2)			
Fwd	ACAGAAGTCATAGCCACTCTC	239-259	103
Rev	CCTTGCCTTTGTTCAGTATC	322-341	
ccl2 (NM_011333.3)			
Fwd	TGTTGGCTCAGCCAGATGCA	147-166	82
Rev	AGCCTACTCATTGGGATCATCTTG	205-228	

The positions of the primers are annotated according to the sequences derived from GenBank (accession numbers are given in parenthesis). Fwd, forward; Rev, reverse.