SUPPLEMENTAL MATERIAL

Yamanishi et al., http://www.jem.org/cgi/content/full/jem.20090581/DC1

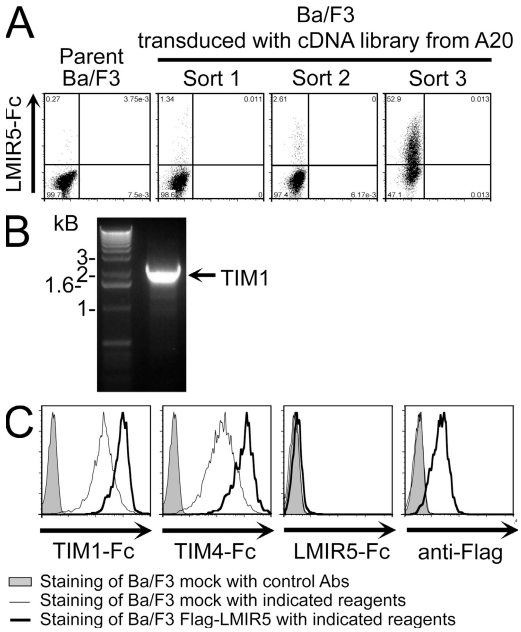

Figure S1. Identification of TIM1 as the ligand for LMIR5 by retrovirus-mediated expression cloning. (A) After a cDNA library from A20 cells was retrovirally transfected into Ba/F3 cells, the transduced Ba/F3 cells stained with LMIR5-Fc were sorted by a fluorescence-activated cell sorter, expanded in culture, and sorted again. This cycle of sorting and expansion was repeated three times. (B) Using single-cell clones stained with LMIR5-Fc, the genes derived from the cDNA library were amplified by PCR and sequenced. TIM1 cDNA, including a full-length TIM1 coding region, was isolated from all three clones. (C) TIM1-Fc or TIM4-Fc bound LMIR5-transduced Ba/F3 cells more strongly than mock-transduced Ba/F3 cells. Ba/F3 cells transduced with Flag-tagged LMIR5 or mock were stained with TIM1-Fc, TIM4-Fc, LMIR5-Fc, or anti-Flag antibody. The control histogram is represented by staining of mock-transduced Ba/F3 cells with control antibodies. All the data are representative of three independent experiments.

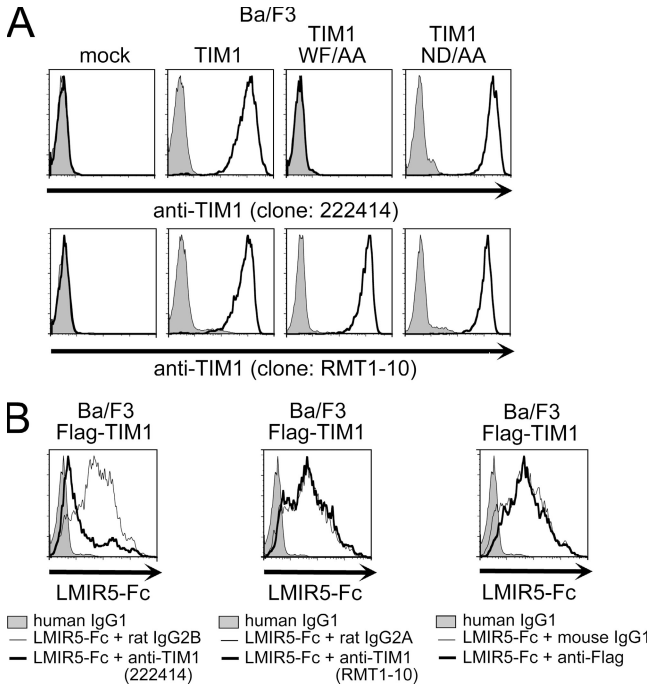

Figure S2. Differential blocking effects of anti-TIM1 antibodies on the TIM1–LMIR5 interaction. (A) Ba/F3 cells transduced with either TIM1, TIM1 (WF/AA), TIM1 (ND/AA), or mock were stained with anti-TIM1 antibody (222414) or anti-TIM1 antibody (RMT1-10; continuous line). The control histogram was represented by staining with control antibodies (shaded). (B) Ba/F3 cells transduced with Flag-tagged TIM1 were preincubated with 100 μg/ml anti-TIM1 antibody (222414), anti-TIM1 antibody (RMT1-10), anti-Flag antibody, or control antibody before staining with LMIR5-Fc. All data are representative of three independent experiments.

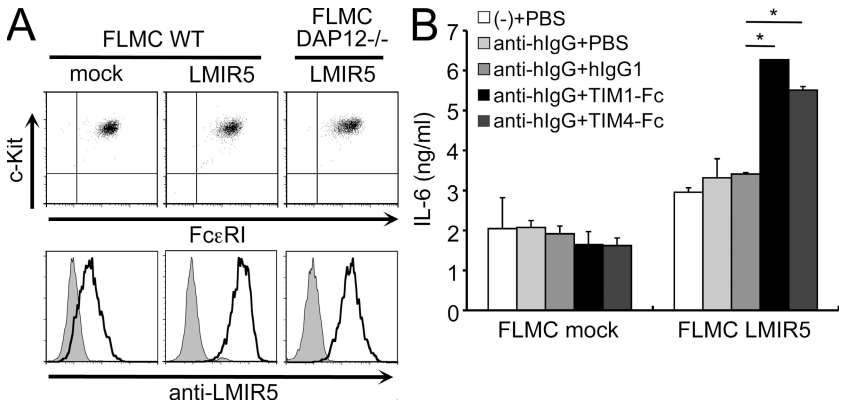

Figure S3. TIM4–Fc as well as TIM1–Fc induced LMIR5–mediated activation of mast cells. (A) Surface expression levels of LMIR5 in WT or DAP12-deficient FLMCs transduced with LMIR5 or mock. Surface expression levels of LMIR5 (bottom) as well as FcεRI and c-kit (top) were examined in FLMCs transduced with LMIR5 or mock, or DAP12-deficient FLMCs transduced with LMIR5. Data are representative of three independent experiments. (B) FLMCs transduced with LMIR5 or mock were stimulated with TIM1-Fc, TIM4-Fc, or hIgG1. All data points correspond to the means ± SD of triplicate samples. Data are representative of three independent experiments. *, $P < 0.05$.

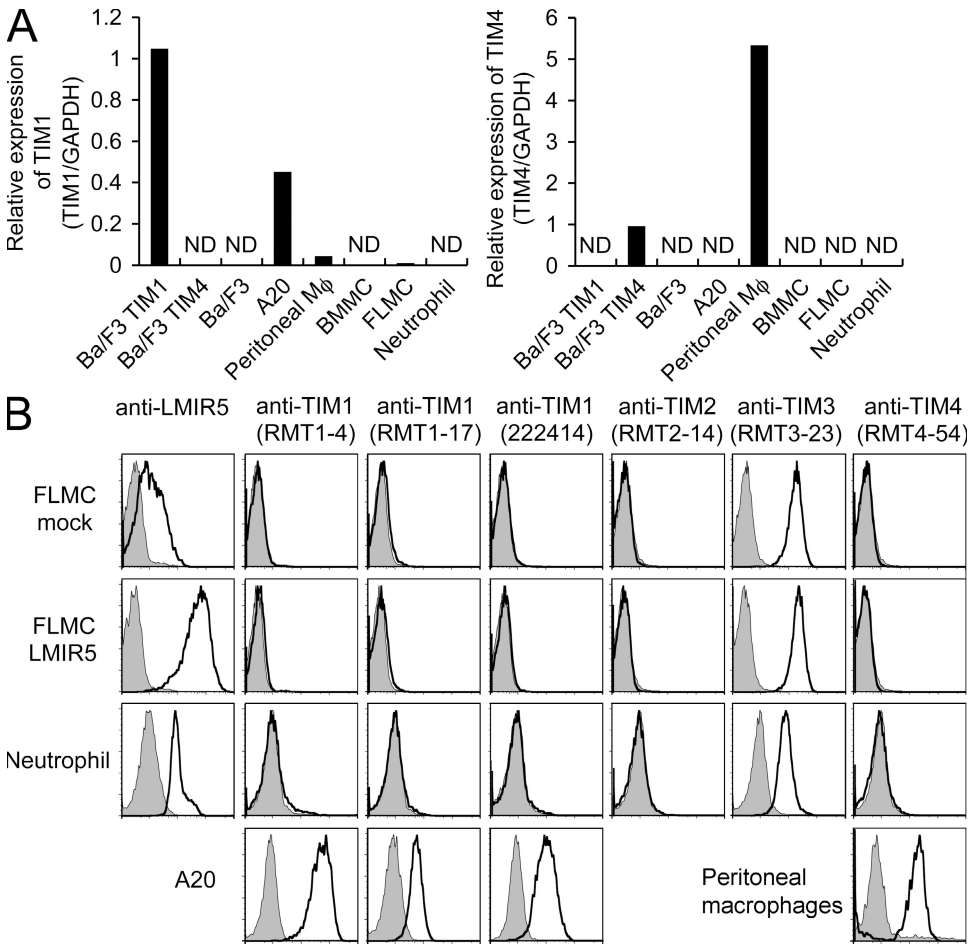

Figure S4. No detectable expression levels of TIM1 and TIM4 in BMMCs, FLMCs, or neutrophils. (A) Relative gene expression levels of TIM1 and TIM4 among Ba/F3 cells transduced with TIM1, TIM4, or mock, A20 cells, peritoneal macrophages, BMMCs, FLMCs, or neutrophils were estimated by using real-time PCR. One representative out of three independent experiments is shown. (B) Surface expression levels of LMIR5, TIM1, TIM2, TIM3, or TIM4 (continuous line histograms) in FLMCs transduced with LMIR5 or mock, neutrophils, A20 cells, or peritoneal macrophages were examined by staining with the indicated antibodies. Staining with control antibodies is shown (shaded histograms). ND, not detected.

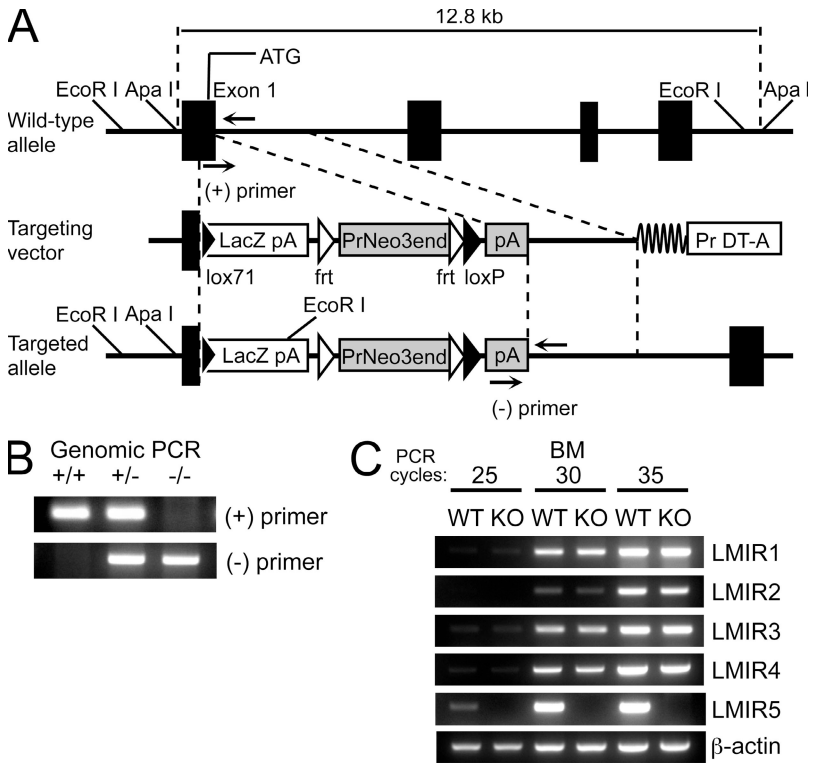

Figure S5. Gene targeting of *LMIR5*. (A) Schematic representation of the WT *LMIR5* allele, targeted allele, and targeting vector. Black boxes indicate exons. PrDT-A is the diphtheria toxin A fragment gene directed by the MC1 promoter for the negative selection of homologous recombinants, and the zig-zag line indicates the vector-derived sequences. PrNeo3end is the neomycin-resistant gene directed by the PGK gene promoter; it was flanked by frt sequences and conjugated to the lacZ gene with the rabbit β-globin polyadenylation signal. These are further flanked by mutant lox71 and WT loxP sequences at the 5′ and 3′ ends, respectively, and combined to the PGK polyadenylation signal (http://www.cdb.riken.jp/arg/cassette.html). (B) Genomic PCR analysis of the LMIR5-targeted allele. Genomic DNA isolated from +/+, +/−, and −/− mice were amplified with primer pairs for the WT allele (+) or the targeted allele (−), as depicted by the arrows in A. (C) Gene expression levels of LMIR family members in BM derived from WT and LMIR5$^{-/-}$ mice. The gene expression levels of LMIR1/2/3/4/5 or β-actin were determined by RT-PCR with 25, 30, and 35 cycles of amplification. Data are representative of three independent experiments.

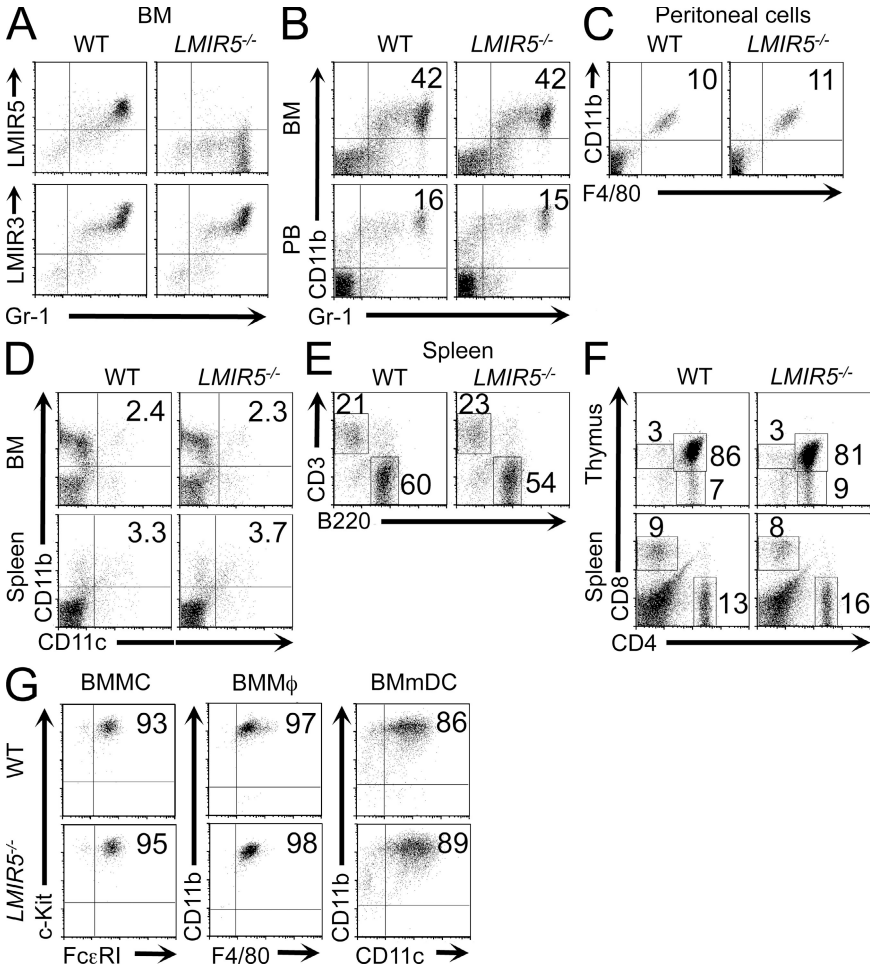

Figure S6. Normal development of myeloid cells and lymphocytes in LMIR5$^{-/-}$ mice. (A) Surface expression of LMIR5 and LMIR3 in BM cells derived from WT and LMIR5$^{-/-}$ mice. Cells were stained with anti–Gr-1 antibody plus anti-LMIR5 antibody or anti-LMIR3 antibody. FSC^{high}SSC^{high} populations representing the myeloid lineage were gated. (B–D) BM cells (B and D, top), peripheral blood cells (B, bottom), peritoneal cells (C), and spleen cells (D, bottom) derived from WT or LMIR5$^{-/-}$ mice were stained with the indicated antibodies to examine the development of myeloid cells, including neutrophils, macrophages/monocytes, and dendritic cells. (E and F) Spleen cells (E and F, bottom) and thymus cells (F, top) were stained with the indicated antibodies to examine the development of lymphocytes. (G) BMMCs, BM-derived macrophages (BMMϕ), and BM-derived myeloid dendritic cells (BMmDC) derived from WT or LMIR5$^{-/-}$ mice were stained with the indicated antibodies. The percentages of cells in the regions are indicated. Results are representative of three different experiments using 8-wk-old WT and LMIR5$^{-/-}$ mice.

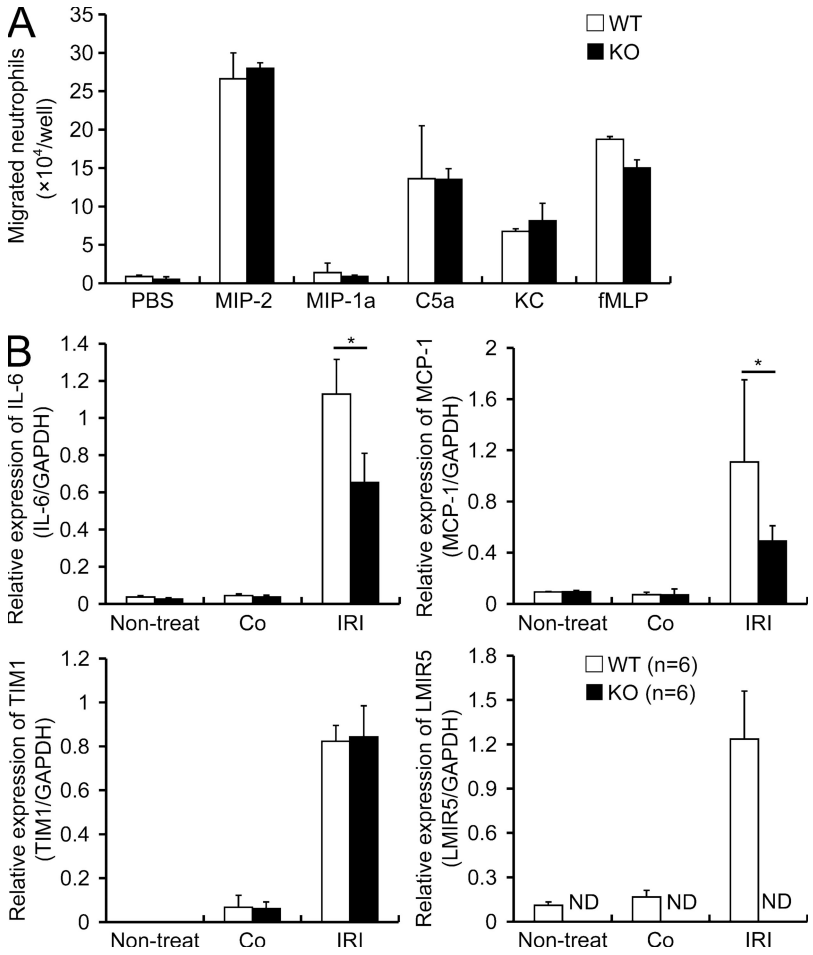

Figure S7. Relative gene expression levels of IL-6, MCP-1, TIM1, or LMIR5 in the postischemic kidney from WT or LMIR5$^{-/-}$ mice. (A) The migratory activity of LMIR5-deficient neutrophils is comparable to that of WT neutrophils. BM neutrophils (10^6 cells/upper well) migrated through the 3-µm polycarbonate filters into the lower wells containing 50 ng/ml MIP-2, 100 ng/ml MIP-1α, 50 ng/ml C5a, 50 ng/ml KC, or 10 µM fMLP. Neutrophils migrated into the lower wells for 90 min were counted. All data points correspond to the means ± SD of triplicate samples. Data are representative of three independent experiments. (B) Relative gene expression levels of IL-6, MCP-1, TIM1, or LMIR5 (real-time PCR) in contralateral (Co) or IRI kidneys from WT or LMIR5$^{-/-}$ mice at 24 h after surgery or in nontreated kidneys from WT mice. Data are means ± SD (n = 6 mice in each group). Statistically significant differences are shown. *, $P < 0.05$. Three independent experiments were performed. ND, not detected.

Table S1. Gene-specific primers used in this study

Primer name	Sequence	Used for
LMIR5 forward	5′-TTACCATGGAGATGCTCAGG-3′	Real-time PCR
LMIR5 reverse	5′-GGTCCCACGGTCAGTTCCGAA-3′	Real-time PCR
TIM1 forward	5′-TGGTTGCCTTCCGTGTCTCT-3′	Real-time PCR
TIM1 reverse	5′-TCAGCTCGGGAATGCACAA-3′	Real-time PCR
TIM4 forward	5′-GGCTCCTTCTCACAAGAAACCACA-3′	Real-time PCR
TIM4 reverse	5′-TCAGCTGTGAAGTGGATGGGAGA-3′	Real-time PCR
IL-6 forward	5′-GCCAGAGTCCTTCAGAGAGATACA-3′	Real-time PCR
IL-6 reverse	5′-CTTGGTCCTTAGCCACTCCTTC-3′	Real-time PCR
MCP-1 forward	5′-TTAACGCCCCACTCACCTGCTG-3′	Real-time PCR
MCP-1 reverse	5′-GCTTCTTTGGGACACCTGCTGC-3′	Real-time PCR
GAPDH forward	5′-ATGTGTCCGTCGTGGATCTGA-3′	Real-time PCR
GAPDH reverse	5′-TTGAAGTCGCAGGAGACAACC-3′	Real-time PCR
LMIR1 RT-PCR forward	5′-CAAGTCAGGTAGAAGTGGTGG-3′	RT-PCR
LMIR1 RT-PCR reverse	5′-AGGCTAAGAGGAGAGAGCCAG-3′	RT-PCR
LMIR2 RT-PCR forward	5′-GAGCCTTGAGAGTGGTAGAGAT-3′	RT-PCR
LMIR2 RT-PCR reverse	5′-AGGAGCTGTGTTTAGGGACAG-3′	RT-PCR
LMIR3 RT-PCR forward	5′-GCCTCGCTCTTTGCTTGG-3′	RT-PCR
LMIR3 RT-PCR reverse	5′-GTCAGAGCGGCATATGAAACC-3′	RT-PCR
LMIR4 RT-PCR forward	5′-CTGAGATTGCAAGCATACACG-3′	RT-PCR
LMIR4 RT-PCR reverse	5′-GATTCCTGCAGTTGACCTCC-3′	RT-PCR
LMIR5 RT-PCR forward	Same as LMIR5 forward	RT-PCR
LMIR5 RT-PCR reverse	5′-TCGCTACAGAGAGTGTGTCTCC-3′	RT-PCR
β-Actin RT-PCR forward	5′-CATCACTATTGGCAACGAGC-3′	RT-PCR
β-Actin RT-PCR reverse	5′-ACGCAGCTCAGTAACAGTCC-3′	RT-PCR