Antibodies, reagents, and cells
PBS-liposomes and Clodronate-liposomes were prepared as published. Alum hydroxide gel, bovine serum albumin (BSA), albumin from chicken egg grade V (ova), DNP<sub>30</sub>-coupled human serum albumin (DNP<sub>30</sub>-HSA), rabbit Glucose-6-phosphate Isomerase (GPI), gadolinium(III)-chloride (GdCl<sub>3</sub>), Pluronic F-127, complete and incomplete Freund’s adjuvant (CFA, IFA), β-hexosaminidase substrate, anti-FLAG mAb, anti-ovalbumin mIgG1 (OVA-14), anti-ova rabbit serum and purified human serum IgG were obtained from Sigma-Aldrich. Anti-mouse CD11b, CD11c, CD3, CD19, Gr1, SiglecF, NK1.1, c-kit, anti-IgE and anti-human FcγRI, FcγRII, FcγRIII, CD3, CD19, CD56, CD24, CD14, CDw125 mAbs were purchased from BD Biosciences (San Diego, CA); isotype controls from BD and Clinisciences (Montrouge, France); anti-mouse DX5, anti-mouse FceRI and anti-human FcεRI mAbs from eBioscience (San Diego, CA); anti-mouse FcγRI (290322), anti-mouse FcγRIIIA (275003) and anti-human CCR3 from R&D systems; anti-human FcγRIIA mAb (IV.3) from StemCell Technologies. The hybridoma producing mAbs anti-mFcγRIV (9E9) was provided by J.V. Ravetch (Rockefeller University, NY, USA), anti-Gr1 (RB6-8C5) by R. Coffman (DNAX, Palo Alto, CA), anti-human FcγRIIA (IV.3) by C.L. Anderson (Heart & Lung Research Institute, Columbus, OH, USA), anti-Ly-6G (NIMP-R14) by C. Leclerc (Institut Pasteur, Paris, France), IgE anti-DNP (2682I) by C.A. Molinaro (Scripps Clinic and Research Foundation, La Jolla, CA, USA). Purified mAbs mouse anti-hFcγRIIB/C (GB3) were provided by U. Jacob (SuppreMol, Martinsried/München, Germany), anti-CD200R3 (Ba103) by H. Karasuyama (JST, CREST, Tokyo Medical and Dental University Graduate School, Tokyo, Japan), anti-CCR3 by J.J. Lee (Mayo Clinic, AZ, USA).

Rabbit antiserum against the intracellular portion of human FcγRIIA was described before. ELISA kit for MPO was obtained from HyCult Biotech, for MIP2 from R&D Systems, for LTC<sub>4</sub> and histamine from Neogen and for PDG<sub>2</sub> from Cayman Chemical. CHO-K1 cells stably transfected with FLAG-tagged mouse FcγRs<sup>25</sup> or human FLAG-tagged FcγRs<sup>18</sup> were cultured as described.

Flow cytometry analysis
Human blood from healthy donors or murine blood from FcγRIIA transgenic mice was incubated with IV.3-FITC or isotype control (mlgG2b-FITC). Human blood cell populations were discriminated based on FSC/SSC properties and staining of CD3 (T cells), CD19 (B cells), CD14 (monocytes), CD56 (NK cells), FceRI/CD203c (basophils), CD24 (neutrophils) and CCR3/CDw125 (esoinophils). Mouse blood cell populations were discriminated based on FSC/SSC properties and staining of CD3 (T cells), B220 (B cells), CD11b/CD115 (monocytes), NK1.1/DX5 (NK cells), IgE/DX5 (basophils), CD11b/Gr1<sup>high</sup> (neutrophils) and Gr1<sup>int</sup>/SiglecF (esoinophils). Macrophages in the peritoneum were identified as CD11b<sup>+</sup>/Gr1<sup>neg</sup> cells, mast cells as IgE<sup>+</sup>c-kit<sup>+</sup> expressing cells. Cells in BAL were stained with CD11c/Gr1 and enumerated using Counting beads (Caltag). Expression of different Flag-tagged FcRs in CHO-K1 cells was compared using anti-FLAG antibody.

Immune complex binding: CHO-K1 cells were incubated with preformed ICs made of 0.5 µg/mL GPI and 1 µg/mL anti-GPI polyclonal antibodies purified from KRN serum, or of 1 µg/ml ova and rabbit anti-ova antiserum (1/500 dilution), for 1 hour at 4°C. PCMC were incubated with preformed ICs made of 20 µg/ml GPI and KRN serum (1/100 dilution) or of 100 µg/ml ovalbumin and 100 µg/ml mAb OVA-14, for 30 min at 4°C. Bound ICs were detected using F(ab')<sub>2</sub> GAM-F(ab')<sub>2</sub>-PE or DAM-FITC.
Flow cytometric analysis of Ca2+ mobilization: 1 × 10⁶ PCMC/ml were preloaded with 50 µg Fluo-3 AM (Molecular Probes, Eugene, OR) in 0.2% Pluronic F-127 for 30 min and [Ca²⁺]i was monitored by flow cytometry.

In vitro cell activation

PCMC degranulation and mediator production
PCMC were generated as described²² and challenged with preformed ICs made of 50 µg/ml GPI either with 1/100 dilution of anti-GPI antiserum or with Protein G-purified IgG anti-GPI (100 µg/ml), or ICs made of 100 µg/ml mAb OVA-14 and 100 µg/ml ova, at 37°C. As a positive control, mast cells were sensitized with IgE anti-DNP 2682I and challenged with 0,01 µg/ml DNP-HSA. β-hexosaminidase was assayed after 15 min stimulation, and histamine, LTC₄ and PDG₂ after 30 min stimulation.

Human skin-derived mast cells
Mast cells were obtained from human skin as described²³ and resuspended in serum-free X-VIVO 15 medium containing 100 ng/ml of rhSCF (gift of Amgen Inc.). 10⁶/ml 6-16 week-old mature mast cells were incubated at 37°C for 30 min with anti-hFcεRI mAb 22E7 (1 µg/ml) or with a mix of IV.3 mAb (1 µg/ml) and F(ab’)2 goat-anti-mouse IgG Fcγ–specific (2 µg/ml) to cross-link FcεRI and FcγRIIA, respectively. β-hexosaminidase release was determined as described.²⁴

Activation of primary mouse peritoneal mast cells
Mouse peritoneal cells were stimulated ex vivo with preformed IC or 10 µg/ml anti-IgE (R35-72, BD Pharmingen) for 15 min at 37°C, followed by incubation with toluidine blue at 0°C. Degranulation was assessed morphologically under the microscope on at least 200 cells per experimental point.

Western blot analysis
Immediately after stimulation with indicated reagents, PCMC were lysed in 10 mM Tris (pH 7.2) 1% SDS lysis buffer at 95°C. 20 µg of proteins (quantified by Dc protein assay, Bio-Rad) were separated by electrophoresis, transferred on PDVF membranes. Membranes were incubated with anti-pERK1/2 (Thr202/Tyr204), anti-pSyk (Tyr525/526), anti-pAkt (S473), anti-pIκB (Ser32), anti-actin (I-19) mAbs (Cell Signaling), anti-pSHIP (Stemcell Technologies), anti-pLat (Tyr191) (Millipore), anti-pPLCγ₁ (Tyr783) (Santa Cruz) or rabbit serum anti-hFcγRIIA intracellular domain, followed by incubations with HRP-labeled secondary antibodies (Santa Cruz), detected using enhanced chemoluminescence (Amersham Biosciences).

Human monocyte and neutrophil activation
Blood samples from normal human donors were fractionated by density centrifugation on Lympholyte-poly (Cedarlane Labs). Monocytes were purified from PBMCs, and neutrophils from granulocytes, using anti-CD14 or anti-CD15 magnetic beads (Miltenyi), respectively. 0.5-1 × 10⁶ purified cells were incubated or not with HA-human IgG or -IV.3 mAb (100 µg/ml) for 20 min at 37°C in HBSS buffer supplemented with 1.26mM CaCl₂ and 0.9mM MgCl₂. A small fraction of the cells was used to assess cell activation using CD62L expression by flow cytometry; the major fraction of the cells was centrifuged and used for lipid mediator measurement.
**Lipid mediator measurement:** Culture supernatants were mixed 1:10 v/v in methanol, or cell pellets resuspended in methanol, and centrifuged at 15,000rpm for 10min. Resulting supernatants were frozen at -20°C. Lipid mediators were quantified as described. After adding deuterized PAF and LTB₄ (PAF-d4 and LTB₄-d4, Cayman chemical, Ann Arbor, MI) as internal standards, lipid mediators were extracted with Oasis HLB solid phase extraction cartridge (1cc/10mg, Waters, Milford, MA). PAF, LTB₄ and LTC₄ were quantified using liquid chromatography-tandem mass spectrometry on a TSQ Quantum Ultra mass spectrometer (Thermo Fischer Scientific, Waltham, MA) that was operated in negative electrospray ionization. Selected reaction monitoring (SRM) mode was used to monitor 568.5 to 59 m/z transitions for PAF, 572.5 to 59 m/z transitions for PAF-d4, 335 to 195 m/z transitions for LTB₄, and 624 to 272 m/z transitions for LTC₄. Commercial PAF, LTB₄ and LTC₄ (Cayman chemical) mixed with PAF-d4 and LTB₄-d4 were used to draw calibration curves, and data were processed using the Xcalibur 2.0 software (Thermo Fischer Scientific). PAF, LTB₄ and LTC₄ values less than 4pg should be considered under the lower quantification limit (represented by a dashed line in Fig.4).

**Lung histology**
3µm sections of paraffin-embedded mouse lungs or human lung biopsies were stained for FcyRIIA using rabbit antiserum against the intracellular portion of human FcyRIIA and Histofine (simple stain mouse MAX PO, Nichirei Bioscience Inc.) followed by revelation with AEC (Sigma), and counterstained with Hematoxylin (Merck).

**Statistical analyses**
Data was analyzed using one-way ANOVA with Bonferroni post-test (Fig. 2A,C-D, and 2E-F lower panels, Fig. 3, 4, 6H, 7H-I), two-way ANOVA with Bonferroni post-test (Fig. 1G, 2E and F upper panels, Fig. 5A-C, 6E-F, 7K-L, S3A-B), Mantel Cox test for all Survival curves, or Student’s t-test (all other data).
Figure S1

(A–B) Indicated mice were immunized with BSA in alum (A) or in Freund’s adjuvant (B) and challenged with BSA. Central body temperatures and survival rates were monitored. ASA in wt (A, n=6; B, n=5) and FcRγ<sup>-/-</sup> mice (A, n=7; B, n=7). (C) Representative histogram plots of human FcR expression on human blood basophils (gated on FcεRI<sup>+</sup>/CD203c<sup>+</sup> cells).
Figure S2
(A) Indicated mice were injected with preformed polyclonal IgG-IC (mouse anti-GPI antiserum plus GPI), and central temperatures were monitored (n=3). (B–E) Representative density plots of mouse blood stained with indicated antibodies upon cell depletion as used in experiments represented in Fig. 3. (B,C) Depletion of mouse neutrophils following injection of anti-Gr1 (B) or anti-Ly6G (C) mAbs. (D) Depletion of monocytes by injection of toxic liposomes. PBS liposomes were used as controls. (E) Depletion of basophils following anti-CD200R3 injection. (B,C,E; left panels) Matched isotype controls are shown for each specific mAb injection. (A) Data are represented as mean ± SEM. (A–E) Data are representative from at least two independent experiments.
Figure S3

(A–E) Mice were injected intradermally with indicated reagents and i.v. with Evan’s blue. (A,B) PCA in 5KO (open symbols: A, n=3; B, n=3), 5KOIIA (black symbols: A, n=4; B n=3). Quantification of Evan’s blue extracted from skin tissue are represented (left panel). Data are represented as single measured points, and mean ± SEM and are representative of at least 2 independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001. Representative photographs of the four injections sites on the skin (middle panel) and the corresponding doses of reagents (right panel) are indicated. (C–E) Representative photographs of the four injections sites on the skin (left panel) and the corresponding doses of reagents (right panel) corresponding to the PCA reactions from Fig. 5A (C), Fig. 5B (D) and Fig. 5C (E).