

Supplementary Materials:

NASA study group

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*Deceased

Figure S1

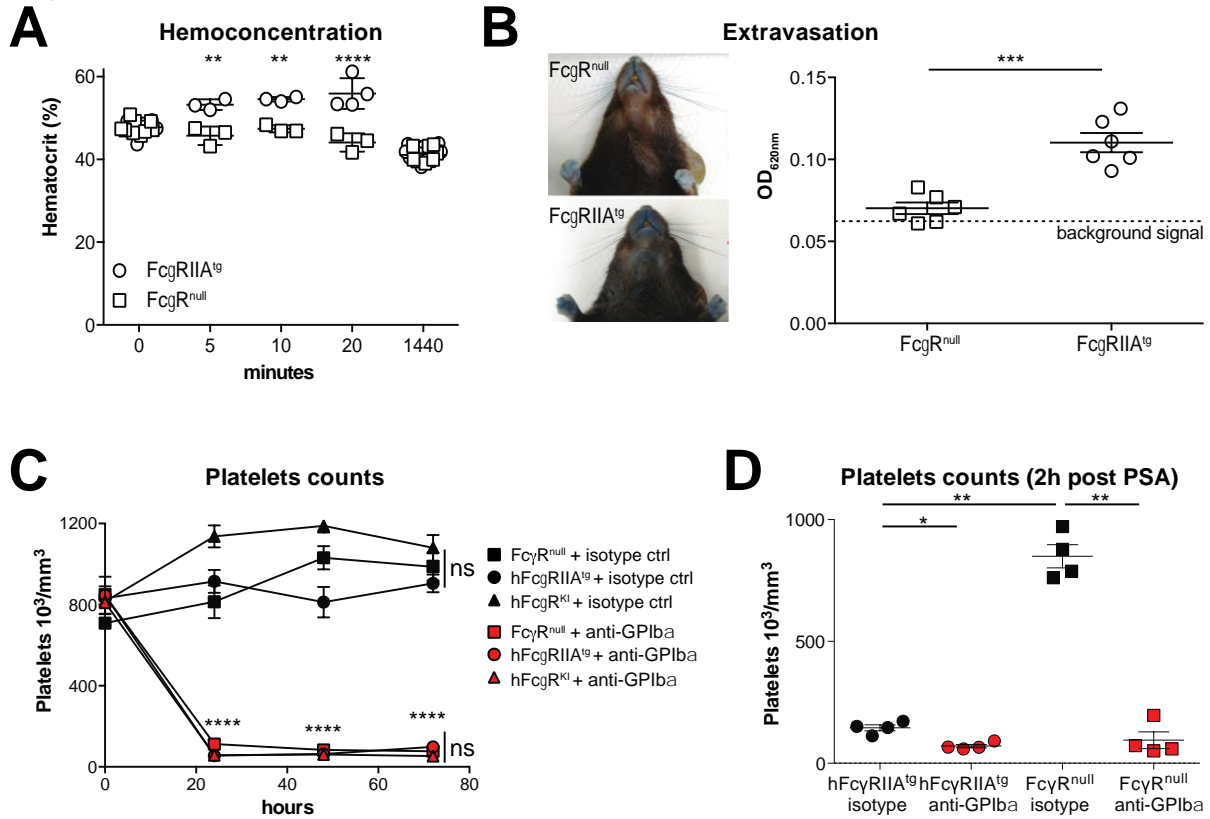


Figure S1. Clinical signs of HA-hIgG anaphylaxis and platelet depletion using anti-GPIIb α antibodies.

(A) Change in hematocrit during HA-hIgG-anaphylaxis in hFcγRIIA^{tg} (circles, n=4) and FcγR^{null} (squares, n=4) mice. (B) Augmented vascular permeability during HA-hIgG-anaphylaxis in hFcγRIIA^{tg} but not FcγR^{null} mice revealed by extravasation of Evans Blue 20 minutes after anaphylaxis induction; Left: Exemplary photograph of each genotype; Right: Quantification of extracted dye from front paws at OD_{620nm}. (C) Efficacy and kinetics of platelet depletion using anti-GPIIb α antibodies (red symbols) or isotype control (black symbols) in FcγR^{null} (squares, n=4), hFcγRIIA^{tg} (circles, n=3) and hFcγR^{KI} (triangles, n=4) mice over time. (D) Platelet counts 2 hours after HA-hIgG injection into hFcγRIIA^{tg} (circles) and FcγR^{null} (squares) mice, pretreated with either anti-GPIIb α antibodies (red symbols) or isotype control (black symbols), n=4. Significant differences between experimental groups are indicated. *p<0.05, **p<0.005, ****p<0.0001.

Figure S2

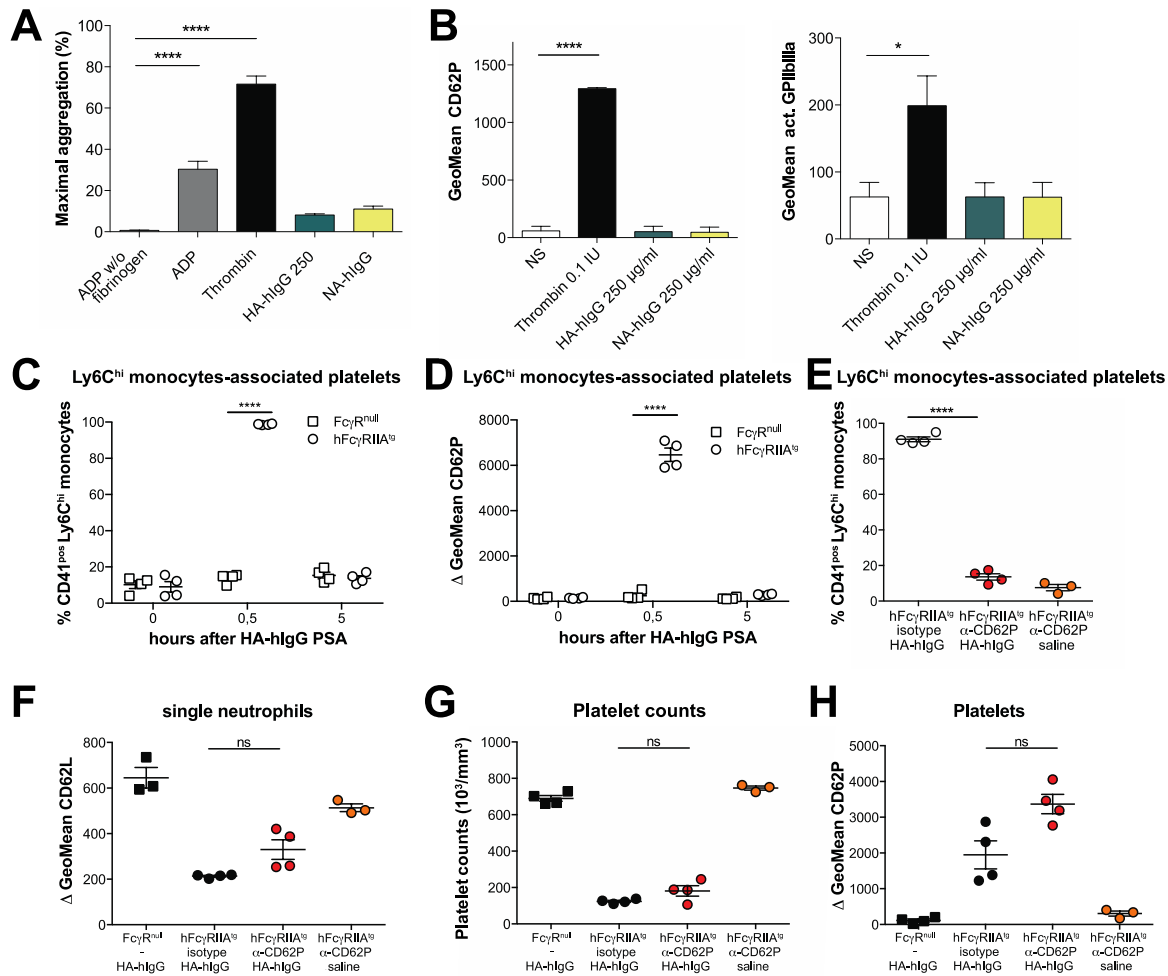


Figure S2. Platelets associate with Ly6C^{hi}-monocytes during IgG anaphylaxis and require hFc γ RIIA expression for activation. (A) Platelets require hFc γ RIIA expression for activation. Maximal aggregation (%) of platelets compared to baseline and (B) expression of platelet activation markers CD62P (left) and activated α IIB β 3 integrin (right) following *in vitro* incubation of washed mouse platelets from Fc γ R^{null} mice with indicated stimuli. (C-E) hFc γ RIIA^{tg} (open circles, n=4) and Fc γ R^{null} (open squares, n=4) mice were challenged or not with HA-hlgG. (C) Percentage of Ly6C^{hi} monocytes associated to CD41⁺-platelets within the Ly6C^{hi} monocyte population. (D) Δ GeoMean CD62P expression (GeoMean CD62P – GeoMean isotype staining) within platelet-Ly6C^{hi} monocyte aggregates. (E) Percentage of Ly6C^{hi} monocytes associated to CD41⁺-platelets within the Ly6C^{hi} monocyte population 30 min following injection of HA-hlgG or saline in hFc γ RIIA^{tg} mice pretreated with anti-CD62P mAb or an isotype control (n=4). (F-H) hFc γ RIIA^{tg} mice pre-treated with anti-CD62P mAb (red circles, n=4) or isotype

control (black circles, n=4) and FcγR^{null} mice (black squares, n=4) were challenged with HA-hIgG; a group of hFcγRIIA^{tg} mice treated with anti-CD62P mAb (orange circles, n=3) were injected with saline. **(F)** ΔGeoMean CD62L expression (GeoMean CD62L – GeoMean isotype staining) on single neutrophils, **(G)** platelet counts in indicated mice and **(H)** ΔGeoMean CD62P expression (GeoMean CD62P – GeoMean isotype staining) on single platelets was determined. **(A/B)** Data are pooled from three independent experiments. **(C-H)** Data are representative of at least two independent experiments. *p<0.05, ****p<0.0001.

Figure S3

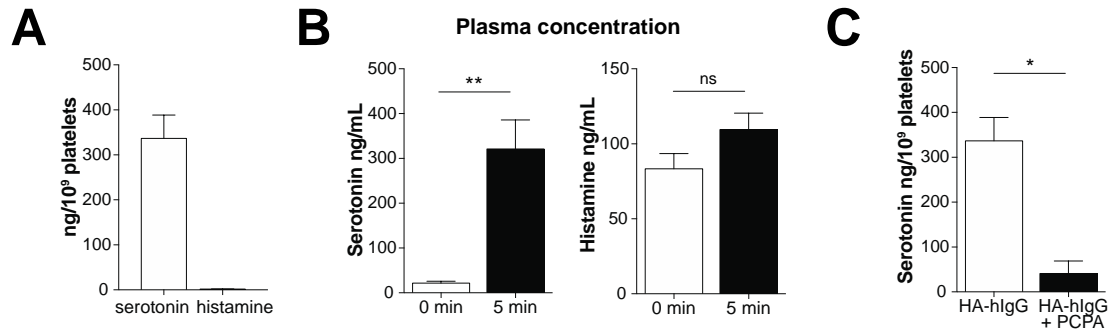


Figure S3. Serotonin but not histamine concentration is augmented during HA-hIgG anaphylaxis. (A) Serotonin and histamine content in releasate from washed hFcγRIIA-expressing platelets stimulated with HA-hIgG, n=3. **(B)** Serotonin and histamine concentration in the plasma of hFcγRIIA^{tg}-WT mice before and 5 minutes after injection of HA-hIgG, n≥4. **(C)** Serotonin content in releasate from HA-hIgG-activated washed platelets from hFcγRIIA^{tg} mice treated or not with PCPA, n≥2. Significant differences between experimental groups are indicated. *p<0.05, **p<0.01.

Figure S4

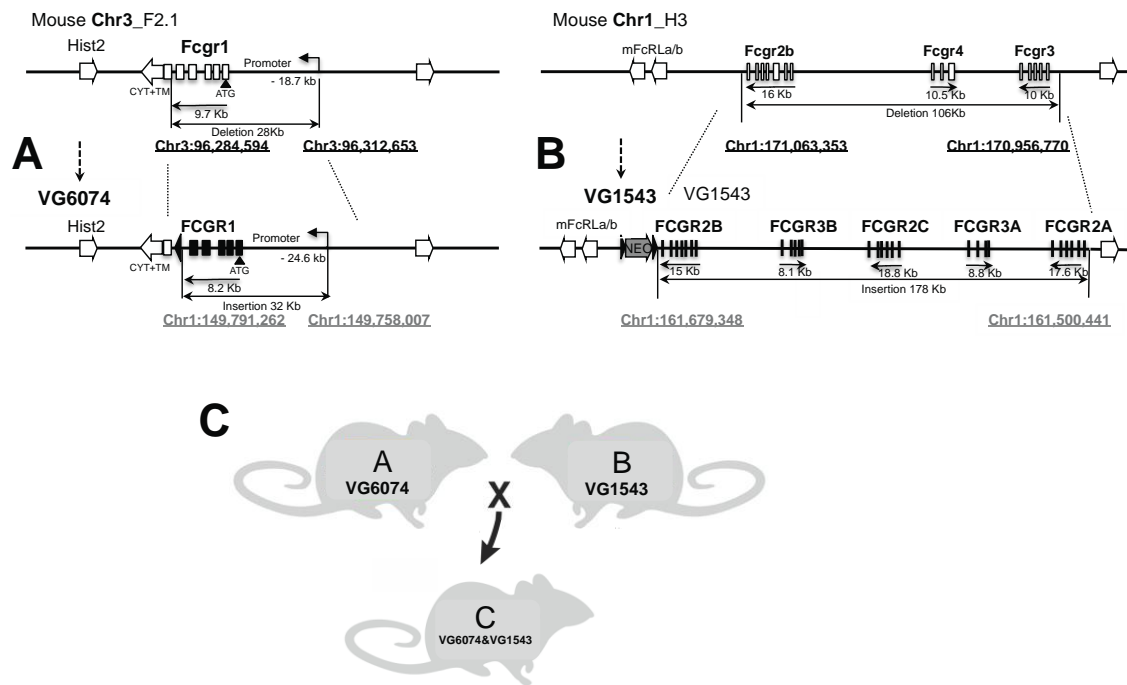


Figure S4. Generation of hFc γ R^{KI} mice. Humanization of the mouse high- and low-affinity receptors loci. Representations are not drawn to scale. Coordinates are based on mouse (GRCm38.p4) and human (GRCh38.p7) genomic information: mouse genes are in empty rectangles, genomic coordinates are in black; human genes are in solid rectangles, genomic coordinates are in grey. Black triangles represent LoxP sites. **(A)** Humanization of the promoter and the ectodomain of mouse Fcgr1 with human FCGR1 promoter and ectodomain. **(B)** Humanization of mouse Fcgr2b, Fcgr4 and Fcgr3 genes (encoding mFc γ RIIB, mFc γ RIV and mFc γ RIII) with human FCGR3A, FCGR2A, FCGR2C, FCGR2B and FCGR3B encoding hFc γ RIIIA(V158), hFc γ RIIA(H131), hFc γ RIIC(stop13), hFc γ RIIB(I232) and hFc γ RIIB(NA2), with parenthesis indicating the polymorphic variant expressed (24). **(C)** Breed VG6074 (mouse A) and VG1543 (mouse B) to get hFc γ R^{KI} mice (mouse C).

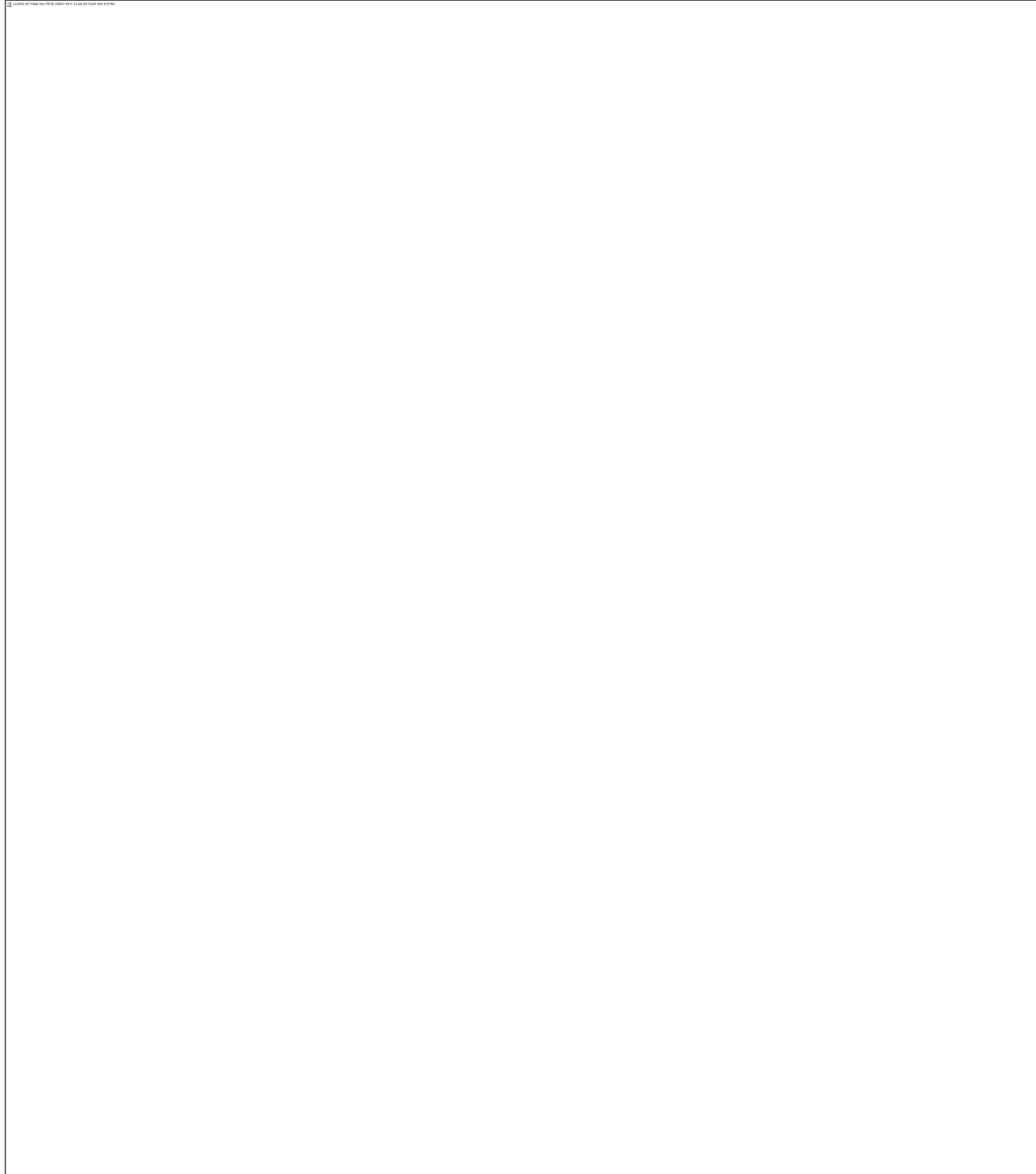


Figure S5. Expression of hFcγRs in blood of humans or transgenic mice. hFcγRIIA expression compared to isotype control (grey) in myeloid and lymphoid compartments of (A) human blood leukocytes from blood bank donors (red), (B) FcγR^{null} (green), hFcγRIIA^{tg} (blue) or hFcγR^{KI} (purple) peripheral mouse blood. Three individuals are represented per group. (right column) The merged histograms from one representative mouse of each genotype of FcγR^{null} (green), hFcγRIIA^{tg} (blue) and hFcγR^{KI} (purple) illustrates variation of hFcγRIIA expression between the “simple” transgenic hFcγRIIA^{tg} mouse model and the “complex” hFcγR^{KI} knock-in mouse model that also expresses hFcγRI, hFcγRIIB, hFcγRIIIA and hFcγRIIIB (24). (C) Representative histograms showing expression of hFcγRI (top), hFcγRIIB (middle) and hFcγRIII (bottom) on indicated blood cell populations from FcγR^{null} (grey), and hFcγR^{KI} (purple) mice. (D) Representative histograms showing expression of indicated human and mouse FcγRs on platelets from WT (black), FcγR^{null} (green), and hFcγRIIA^{IIA} (blue) and hFcγR^{KI} (purple) mice.

Figure S6

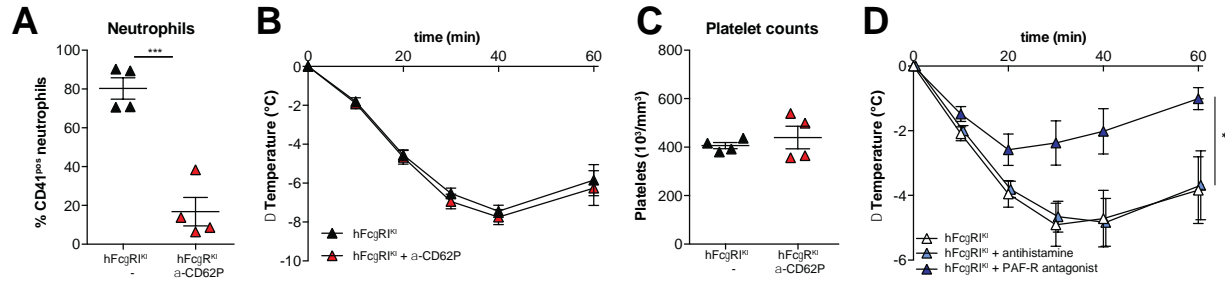


Fig. S6. Formation of platelet/neutrophil aggregates is dispensable for HA-hIgG anaphylaxis in hFcγR^{KI} mice. (A-C) hFcγR^{KI} mice pre-treated with anti-CD62P mAb (red triangles, n=4) or not (black triangles, n=4) were challenged with HA-hIgG. (A) Percentage of neutrophils associated to CD41⁺-platelets within the neutrophil population, (B) changes in body temperature and (C) platelet counts in indicated mice were determined. (A-C) Data are representative of at least two independent experiments. (D) Anaphylaxis was induced in hFcγR^{KI} mice following i.v. injection of 1 mg of HA-hIgG, and central temperatures were monitored: indicated mice were pretreated with ABT-491 (PAF-R antagonist; n=10), the H1-receptor antagonist cetirizine (antihistamine; n=9) or vehicle (NaCl, n=11). Data are represented as means ± SEMs and are pooled from three independent experiments. *p<0.1, ***p<0.001.

Figure S7

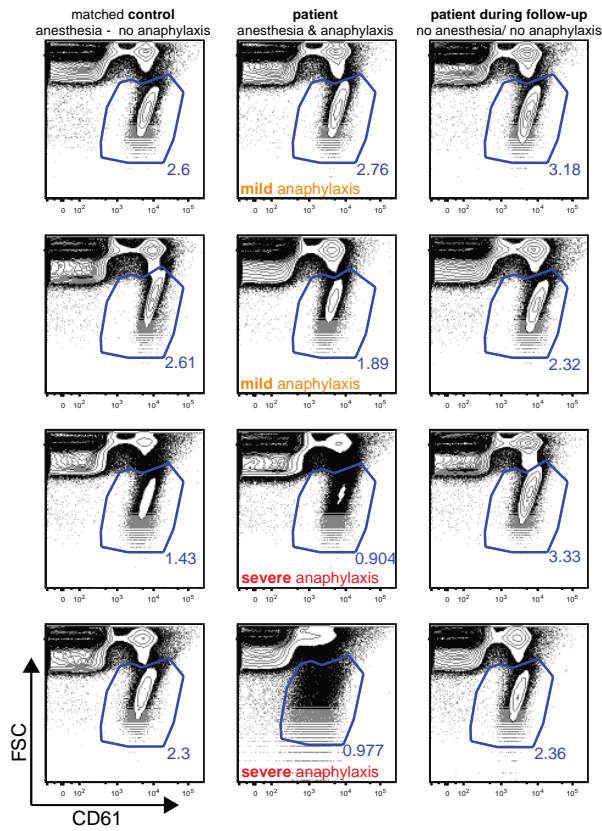


Figure S7: Platelet gating in NASA study. Examples of the gating strategy used to identify single CD61⁺ platelets in whole blood of patients during anesthesia-related anaphylaxis (middle), 6-8 weeks later (right, follow-up) and their matched controls, that were anesthetized but did not experience anaphylaxis (left). Two examples of patients with mild or severe anaphylaxis are represented. Percentages of single CD61⁺ platelets among total blood cells are indicated.

Figure S8

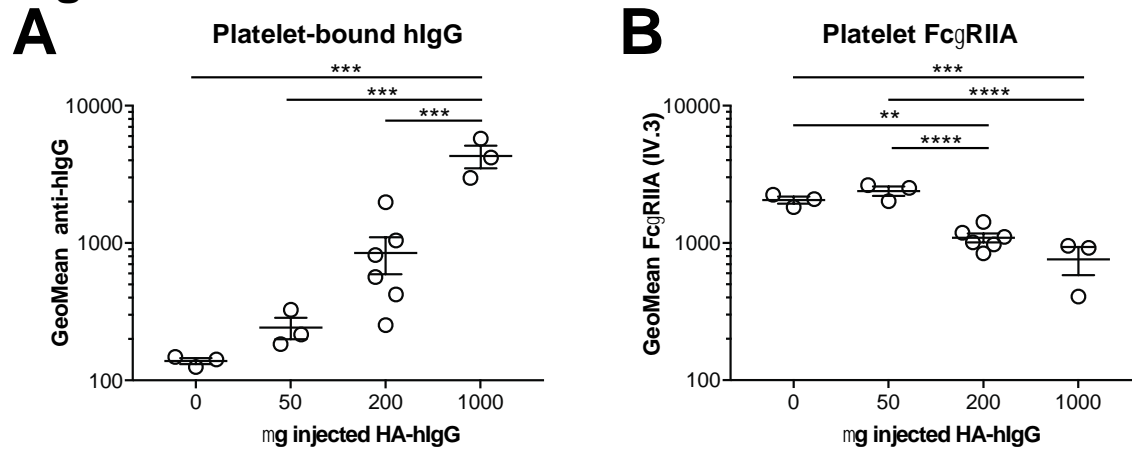


Figure S8: Platelet-bound hIgG and hFcγRIIA expression on platelets *ex vivo* following HA-hIgG injection into hFcγRIIA^{tg} mice. Detection of hIgG (A) and hFcγRIIA (B) on the surface of platelets from hFcγRIIA^{tg} mice *ex vivo* 15 minutes after injection with indicated doses of HA-hIgG. (A/B) Data are representative of at least two independent experiments. **p<0.01, ***p<0.001, ****p<0.0001.