Supporting Information

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SI Materials and Methods

Generation of FcRα$^{-/-}$ Mice. The *FcRα*$^{-/-}$ mouse was designed to contain a 95-kb deletion spanning the *Fcgr2b*, *Fcgr3*, and *Fcgr4* loci on chromosome 1. The *Fcgr3* and *Fcgr2b* genes are 5′ and 3′ of the *Fcgr4* gene, respectively. A first *LoxP* element was introduced into the *Fcgr2b* gene and a second LoxP element into the *Fcgr3* gene to delete the intervening genomic DNA by Cre recombination. This strategy relies upon sequential gene-targeted events into the same chromosomal arm of white C57BL/6 ES cells (Rockefeller University Gene Targeting Facility).

Conditional knockout targeting vector for FcgR2. The conditional targeting construct was generated by subcloning genomic pieces of the *Fcgr2* gene into an ES cell targeting vector. For the first homologous arm of the conditional knockout targeting construct, an 8.5-kb DNA fragment containing the exons coding for the S2, EC1, EC2, and TM domains of *Fcgr2* was generated by PCR amplification (Expand Long Template PCR; Roche) of C57BL/6 genomic DNA and cloned into Cla1/NotI sites of the targeting vector (pEasyFlox). A *LoxP-Neo-LoxP* cassette was inserted after this 8.85 kb fragment into the NotI/SalI multiple cloning site of pEasyFlox. The location of this cassette with respect to the gene placed it 1300 bp downstream of the TM exon (exon 5) in intron 5. The second homologous arm of the construct, a 4.3 kb DNA fragment containing the exons coding for the 3 intracellular domains (IC1, IC2, and IC3) was generated by PCR amplification and cloned into the SalI/XhoI multiple cloning site of pEasyFlox. The third *LoxP* site was inserted into a HindIII site that was 134 bp upstream to the EC1 exon.

Conditional knockout targeting vector of FcgR3. The conditional targeting construct was generated using a recombineering method (1) to capture a 12 kb fragment from a murine BAC clone containing the *Fcgr3* gene made from C57BL/6 genomic DNA into pBluescript SK+ (Agilent). Engineered into this construct are two unique restriction sites (QuikChange II Site-Directed Mutagenesis; Agilent) that allow for the insertion of a *LoxP-Neo-LoxP* cassette 5′ to the EC2 exon and a third *LoxP* site 3′ to the transmembrane/intracellular (TM/IC) exon. The gene targeting sequence was removed from pBLuescript SK+ and placed into the pDTKA vector, which contains positive and negative selection markers.

Embryonic stem cell targeting. Transfection, selection, and screening were performed by the Rockefeller University Gene Targeting Facility. Clones containing the targeted *Fcgr2b* allele were identified by Southern blot analysis of EcoRV-digested genomic DNA with a probe that hybridized outside of the targeting vector. A hybridized 13.7 kb band identified the wild-type *Fcgr2b* allele and a 10.1-kb band indicated the presence of an EcoRV site in the *Neo* cassette that identified the mutant *Fcgr2b* allele (Fig. S5). Several clones were selected for microinjection into C57BL/6 embryos, and chimeric male offspring were bred to C57BL/6 females. Litters resulting in offspring with white coat color were screened by PCR and Southern analysis to identify successful germline transmission. After identifying a white ES cell clone (R2B6-231) that could stably transmit the conditionally targeted allele after microinjection into mouse blastocysts, ES clone R2B6-231 was transiently transfected along with a Cre-expressing plasmid, and clones with the *Neomycin* resistance gene removed were selected. Removal of this cassette was required to proceed with the secondary transfection into this ES line, creating a *cis* integration with the *Fcgr3* targeting construct into same arm of the chromosome that had already been targeted for the mutated *Fcgr2b* allele (Fig. 1*A*). The targeting vector was introduced into clone R2B6-231-cre39F ES cells by electroporation, and clones were selected under neomycin resistance. Clones were then picked into 96-well plates, replica plated, and transiently transfected with a Cre-expressing plasmid. DNA purified from ES cell clones was screened by PCR using a primer from the *Fcgr2b* gene and a primer from the *Fcgr3* gene. Clones in which the lox sites were successfully integrated into the desired loci resulted in the ability to generate a PCR product that verified the intramolecular deletion of the α-chain locus on chromosome 1. Properly integrated ES cell clones were injected into blastocysts to generate chimeric mice, which were subsequently bred to C57BL/6 mice to confirm germline transmission. Those mice that transmitted were crossed to mice expressing Cre recombinase under the control of the cytomegalovirus immediate early enhancer-chicken β-actin hybrid (CAG) promoter for the intramolecular deletion of the α-chain locus in the mouse. Southern blotting analysis confirmed the FcRα deletion (Fig. S6) These FcRα$^{-/-}$ were crossed to *FcgR1*$^{-/-}$ mice (2) that were provided by P. Mark Hogarth (Burnet Institute, Melbourne, Australia). Mice lacking both the α-chain locus and FcγRI were mated to each other to establish homozygous mutations, and resulting progeny were termed FcRα null mice.

Generation of huFcγRIIB Transgenic Mice. Transgenic mice carrying the human transgene for FCGR2^{I232} were made using a genomic DNA library isolated from human blood (BAC clone RPCI-11-474I16, CHORI). The BAC clone was linearized with the Not I restriction enzyme and isolated using a pulse field gel electrophoresis system (Bio-Rad). The purified genomic fragment was purified and microinjected into the pronuclei of fertilized oocytes from C57BL/6 mice. Transgenic founders were mated with C57BL/6 mice and were maintained on this genetic background.

Generation of FcγR Humanized Mice. Mice expressing either human FcγRIIIA^{F158} and FcγRIIIB were previously generated and characterized (3). Mice transgenically expressing human FcγRI (4) and FcγRIIA^{R131} (5) were obtained from J. G. J. van de Winkel (Utrecht University, Utrecht, The Netherlands) and the Jackson Laboratories, respectively. Transgenic mice expressing huFcγRI, huFcγRIIA^{R131}, huFcγRIIB^{I332}, huFcγRIIIA^{F158}, and huFcγRIIIB were individually mated together to create a mouse containing the full repertoire of human FcγRs. This mouse was then mated with the FcRα null mouse to establish the FcγR humanized mouse.

SPR Experiments. All experiments were performed with a Biacore T100 SPR system (Biacore, GE Healthcare) at 25 °C in HBS-EP+ buffer [10 mM Hepes (pH 7.4), 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20]. Soluble ectodomains of human FcγR were obtained by transient transfection of 293T cells and purified using Hig-Tag isolation and pull-down dynabeads (Invitrogen). Purity (assessed by SDS/PAGE/Coomassie staining) was estimated to be >90%. Soluble FcγR at 20 μg/mL were immobilized on CM5 chips by amine coupling at pH 4.5 (except for FcγRIIb: pH 5.0), resulting in a density of 2,000 response units. Recombinant IgG were injected through flow cells at six different concentrations (1,000 to 31.25 nM; 1:2 successive dilutions) at a flow rate of 50 μL/min with 2-min associations and 3.5-min dissociations. After each assay cycle, the sensor surface was regenerated with a 30-second injection of 25 mM NaOH at a flow rate of 30 μL/min. Background binding to blank immobilized flow cells was subtracted and affinity constants were calculated using BIAcore T100 Evaluation software using the 1:1 Langmuir binding model.

1. Liu P, Jenkins NA, Copeland NG (2003) A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Res* 13:476–484.
2. Barnes N, et al. (2002) FcgammaRI-deficient mice show multiple alterations to inflammatory and immune responses. *Immunity* 16:379–389.
3. Li M, Wirthmueller U, Ravetch JV (1996) Reconstitution of human Fc gamma RIII cell type specificity in transgenic mice. *J Exp Med* 183:1259–1263.
4. Heijnen IA, et al. (1996) Antigen targeting to myeloid-specific human Fc gamma RI/CD64 triggers enhanced antibody responses in transgenic mice. *J Clin Invest* 97:331–338.
5. McKenzie SE, et al. (1999) The role of the human Fc receptor Fc gamma RIIA in the immune clearance of platelets: A transgenic mouse model. *J Immunol* 162:4311–4318.
6. Richards JO, et al. (2008) Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. *Mol Cancer Ther* 7:2517–2527.

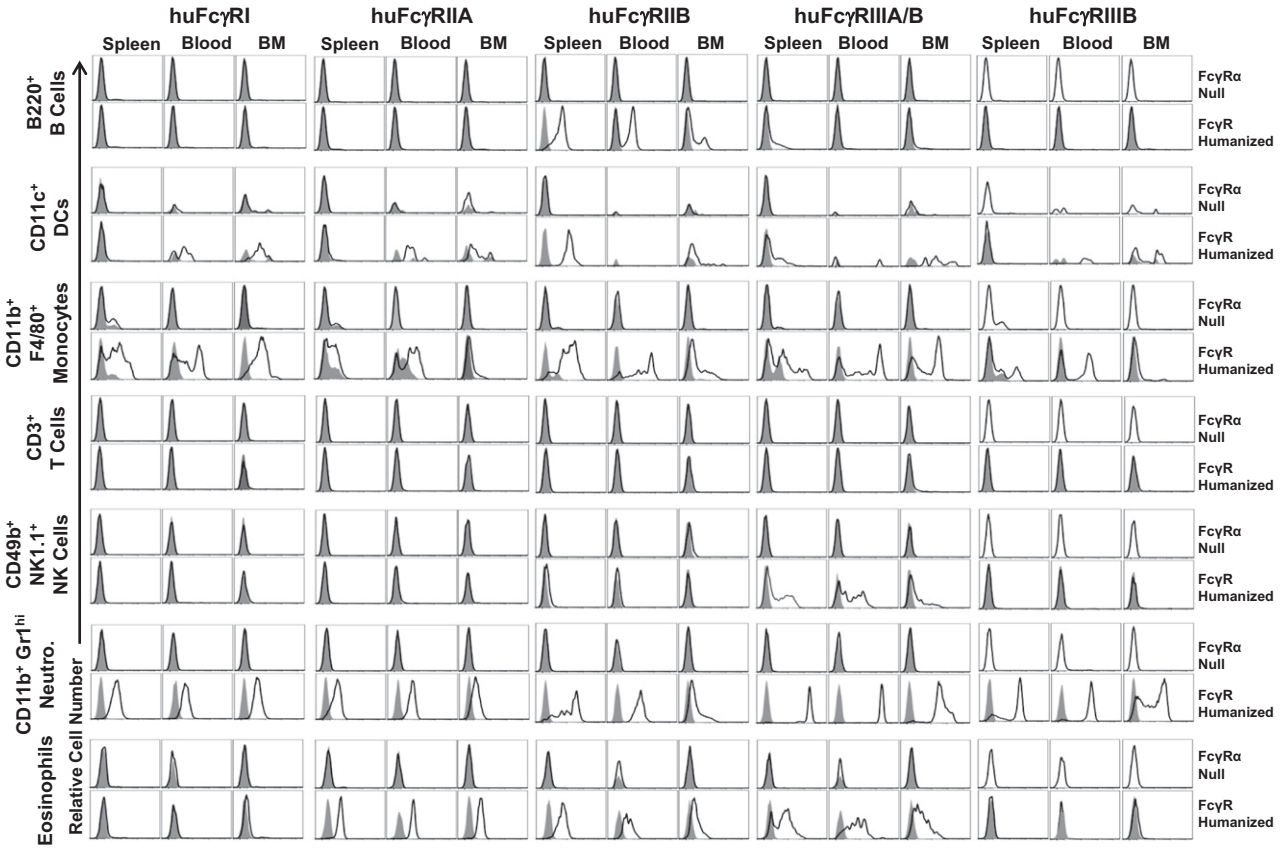

Fig. S1. HuFcγR expression in FcγR humanized mice. The indicated cell types from the indicated tissues were analyzed for huFcγR expression (heavy lines) by immunofluorescence staining and flow cytometric analysis. Shaded histograms show background staining by isotype control mAbs, except for the FcγRIIIB histograms, where shaded histograms show FcγRIIIB staining on cells from FcRα null mice.

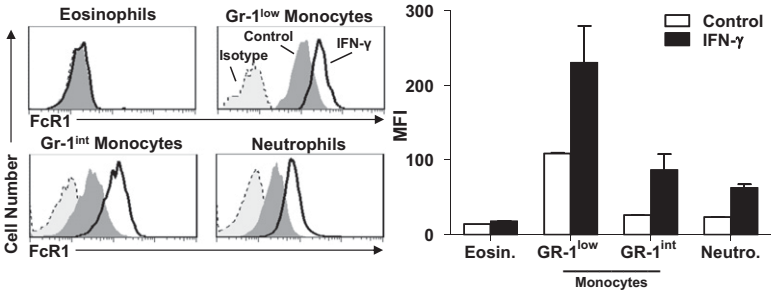

Fig. S2. HuFcγRI expression by innate cells is induced by IFN-γ in vitro. Whole blood was harvested from FcγR humanized mice and treated with IFN-γ for 60 min. FcγRI expression by eosinophils, $CD11b^{+}Gr1^{low}$ monocytes, $CD11b^{+}Gr1^{int}$ monocytes, and $CD11b^{+}Gr1^{hi}$ neutrophils was assessed by immunofluorescence staining with flow cytometric analysis. Representative flow cytometric histograms showing FcγRI expression by control-treated (shaded line) or IFN-γ-treated (heavy line) cells is shown. Dashed line indicated background staining by isotype control mAb. Values in the graph represent mean (± SEM) mean fluorescence intensities (MFI) of FcγRI expression by the indicated cell types.

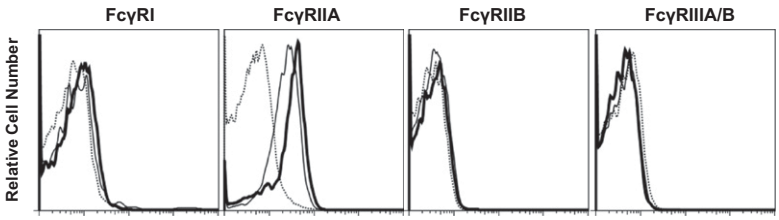

Fig. S3. Platelets in FcγR humanized mice express huFcγRIIA. Platelets were purified from the peripheral blood of a human donor (thin line), FcγR humanized mice (heavy line), and FcRα null mice (dotted line) and analyzed for huFcγR expression by immunofluorescence staining with flow cytometric analysis. Representative flow cytometric histograms for huFcγRIIA staining are shown.

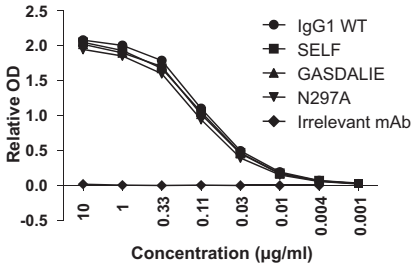

Fig. S4. Point mutations in the huIgG1 Fc domain do not affect the affinity of mAbs for Ag. TA99 mAbs with the indicated Fc mutations were diluted and used in an ELISA with gp75-coated ELISA plates. Values represent the mean relative OD values from duplicate ELISA measurements.

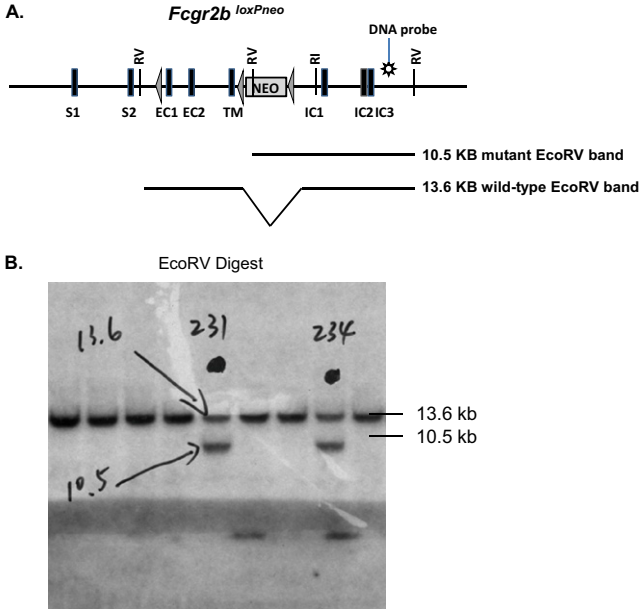

Fig. S5. Targeting of *Fcgr2b* and Southern Blot analysis of targeted White ES cell clones. (*A*) To generate a *loxPneo* allele for *Fcgr2b* using standard gene targeting techniques in ES cells, the *loxP neo* cassette was inserted between the transmembrane domain (TM) and the first intracellular domain. A third *loxP* site was inserted 5′ to the first extracellular domain to complete the conditional targeting construct. Black boxes represent the coding regions of the *Fcgr2b* gene, and gray triangles represent the *loxP* sites. RI, *EcoRI*; RV, *EcoRV*. Expected sizes of *EcoRV*-digested genomic DNA for the wild-type and targeted alleles are shown. (*B*) Verification of the conditional targeted *Fcgr2b* allele was completed by Southern blot analysis of *EcoRV*-digested ES cell genomic DNA probed with a DNA probe (star) inside of the gene but outside of the targeting construct. A 13.6-kb band represents the wild-type *Fcgr2b* allele, and a 10.5-kb band identifies the correctly targeted *Fcgr2b* allele.

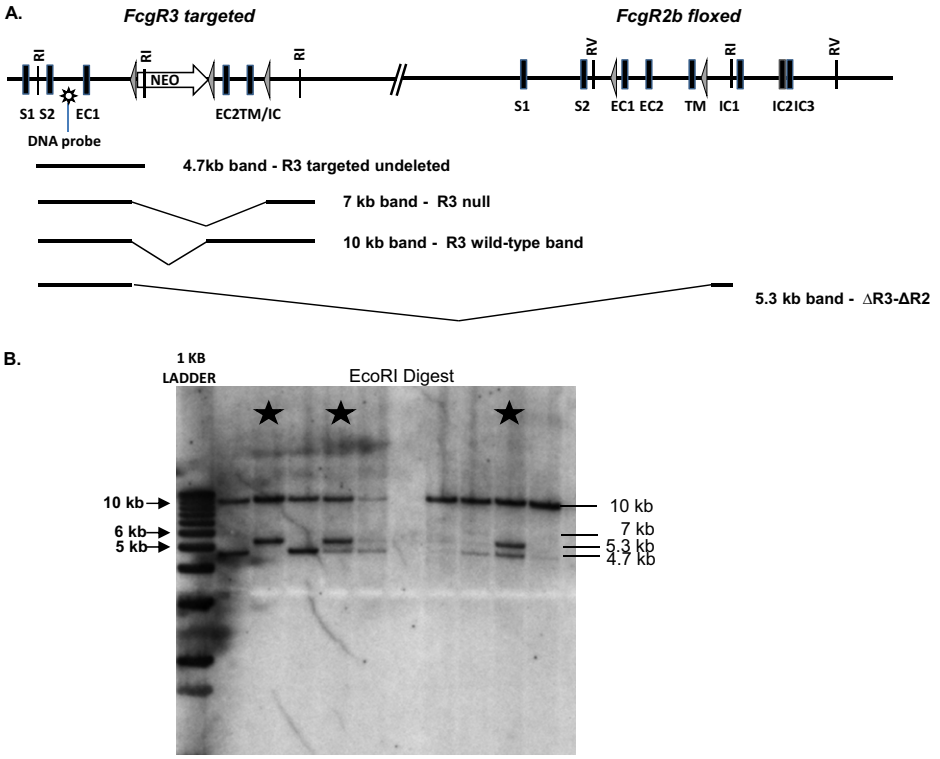

Fig. S6. Sequential Targeting of *Fcgr2b*^{floxed} ES Cell Clone with the *Fcgr3* targeting construct and Southern blot analysis of FcRα chain Deletion. (*A*) To generate a *loxPneo* allele for *Fcgr3* using standard gene targeting techniques in ES cells, the *loxP neo* cassette was inserted between the extracellular domains (EC1 & EC2). A third *loxP* site was inserted 3′ to the transmembrane/intracellular domain (TM/IC) to complete the conditional targeting construct. Black boxes represent the coding regions of the *Fcgr3* gene, and gray triangles represent the *loxP* sites. RI, *EcoRI*; RV, *EcoRV*. Expected sizes of *EcoRI*-digested genomic DNA for the wild-type and targeted alleles in the α-chain locus after expression of Cre recombinase are shown. (*B*) Verification of the conditionally targeted *Fcgr3* and *Fcgr2b* alleles and complete excision of the α-chain locus was completed by Southern blot analysis of *EcoRI*-digested genomic DNA probed with a DNA probe inside of the *Fcgr3* gene but outside of the targeting construct. A 10-kb band represents the wild-type *Fcgr3* allele, and a 5.3-kb band identifies the correctly targeted *Fcgr3* allele and subsequently deleted α chain locus (lanes labeled with stars). A 4.7-kb and a 7-kb band represent other possible recombination events that were not desired but expected.

Table S1. huFcγR expression pattern comparison between human blood and mouse spleen

	Human blood					FcγR humanized mouse spleen				
Cell type	FcγRI	FcγRIIA	FcγRIIB	FcγRIIIA	FcγRIIIB	FcγRI	FcγRIIA	FcγRIIB	FcγRIIIA	FcγRIIIB
B cell	—	—	+	—	—	—	—	+	—	—
Mono	+	+	+	+	—	+	+	+	+	—
Neutro	+	+	+	—	+	+	+	+	—	+
DC*	—	+	+	—	—	—	+	+	—	—
NK Cell	—	—	—	+	—	—	—	—	+	—
T Cell	—	—	—	—	—	—	—	—	—	—

Mono, monocyte; Neutro, neutrophil.
*Monocyte-derived DCs from human blood and bone marrow-derived DCs from mice.

Table S2. Affinities and A/I ratios of human IgG1 mutant Fc for human FcγRs

	Human soluble mAb (K_D in M)											
Human IgG1 Fc	FcγRIIA^{R131}	Fold	FcγRIIA^{H131}	Fold	FcγRIIB	Fold	FcγRIIIA^{V158}	Fold	FcγRIIIA^{F158}	Fold	A/I*	A/I†
WT IgG1	1.55×10^{-6}	1.0	1.38×10^{-6}	1.0	2.52×10^{-6}	1.0	4.32×10^{-7}	1.0	2.95×10^{-6}	1.0	0.9	1.6
N297A	n.d.b.		n.d.b.		n.d.b.		n.d.b.		n.d.b.		N/A	N/A
S267E (SE)	9.80×10^{-8}	15.8	1.48×10^{-6}	0.93	8.33×10^{-8}	30.2	5.92×10^{-6}	0.07	n.d.b.		0	0.8
S267E/L328F (SELF)	1.0×10^{-8}	147	3.70×10^{-6}	0.37	1.52×10^{-8}	166	n.d.b.		n.d.b.		0	1.5
S239D/I332E (SDIE)‡	1.2×10^{-7}	7.6	2.3×10^{-7}	3.7	1.7×10^{-7}	14	1.6×10^{-8}	18	3.0×10^{-8}	33	5.7	1.4
G236A/S239D/A330L/I332E (GASDALIE)	6.32×10^{-8}	24.5	8.16×10^{-8}	16.9	7.36×10^{-7}	3.42	1.24×10^{-7}	3.49	1.00×10^{-7}	29.5	7.4	11.6

Binding constants were obtained by SPR analysis with immobilized FcγRs and soluble mAbs. A/I, activating to inhibitory receptor binding ratio; N/A, not applicable; n.d.b., no detectable binding.

*A/I ratios for FcγRIIIA^{F158}.

†A/I ratios for FcγRIIA^{R131}.

$^{\ddagger}K_D$ values and fold changes compared with WT IgG1 for the SDIE mutant are from ref. 6. A/I ratios were calculated based on these data.