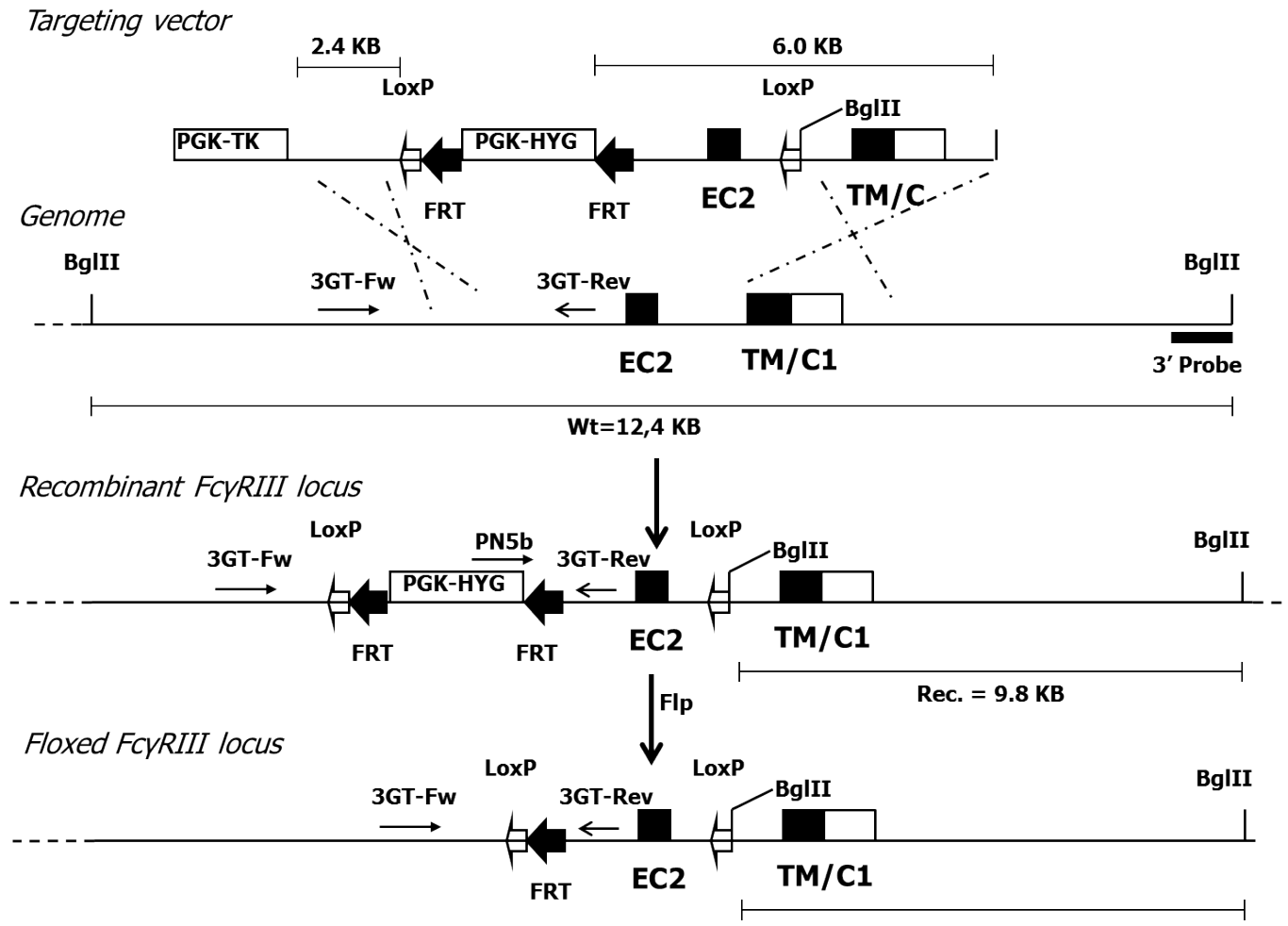
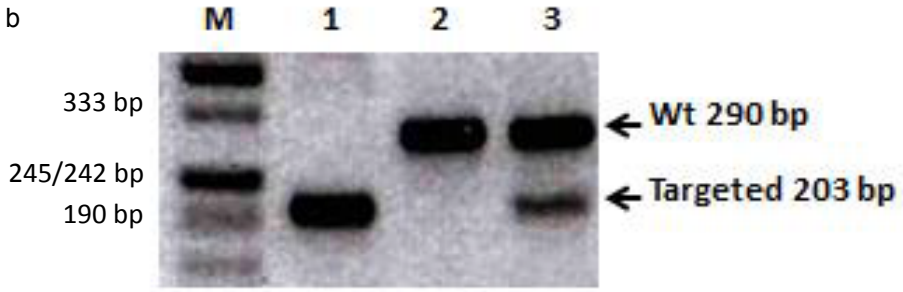
Supplementary figure 1





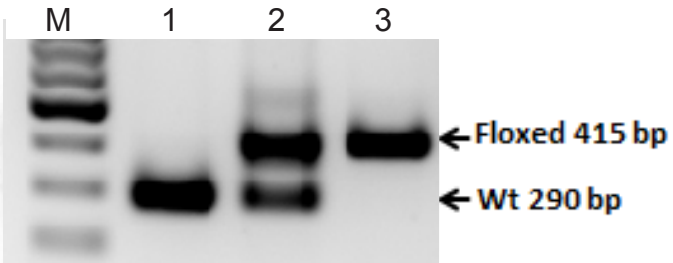


Fig. S1 Generation of a FcγRIII conditional KO mouse model.
A targeting vector was constructed based on a 8.4 kb genomic fragment containing exons 4, encoding extracellular domain 2 (EC2), and exon 5, encoding transmembrane and cytoplasmic domain (TM/C) of the FcγRIII gene, from BAC clone RPCI23-87B18 of the RPCI 23 female (C57Bl/6J) mouse BAC genomic library (BACPAC Resources Center, Children's Hospital Oakland Research Institute, Oakland, California). A LoxP site downstream of the EC2 exon as well as a LoxP-FRT-Hygro-FRT cassette upstream of the EC2 exon was inserted. Gene targeting was performed in C57Bl/6-derived ES cells (Bruce4). Clones in which homologous recombination occurred were identified by Southern blotting and subsequently injected in C57Bl/6 blastocysts. The obtained chimeras were crossed with C57Bl/6J mice and the F1 offspring positive for the FcγRIII targeted allele was crossed with a C57BL/6 Flp deleter strain resulting in mice with a floxed FcγRIII allele. Flp-mediated recombination was analyzed with PCR.a. From top to bottom schematic representation of the FcγRIII targeting vector, the relevant part of the WT mouse FcγRIII genomic locus, the targeted recombinant FcγRIII allele and the floxed FcγRIII allele after removal of the PGK-Hyg selection marker gene by Flp recombinase. The FcγRIII exon 4 and 5 (black boxes) are marked in accordance to the functional domains they encode: EC2, the extracellular immunoglobulin-like domain 2; TM/C, transmembrane-cytoplasmic tail region. Coding parts are depicted as closed boxes, non-coding parts as open boxes. Indicated are BglII restriction sites and location of a 3'probe used for the identification of the recombinant locus by Southern blot analysis (data not shown). Primers for PCR based genotyping are depicted as small arrows. b. PCR analysis of genomic DNA from tail biopsies. Top panel: chimeric mouse with targeted FcγRIII allele. Use of primer pair PN5b/3GT-Rev resulted in the amplification of a 203 bp fragment of the recombinant FcγRIII allele only (lane 1). Use of primer pair 3GT-Fw/3GT-Rev resulted in the amplification of a 290 bp fragment of the Wt FcγRIII allele only (lane 2). Lane 3 shows the PCR fragments when all three PCR primers were used. M: mol.weight marker Bottom panel: Use of primer pair 3GT-Fw/3GT-Rev resulted in the amplification of a 415 bp fragment of the floxed FcγRIII allele after Flp recombination. M: 100bp ladder. Primer sequences: 3GT-Fw: GAGGGCATCCGATTTCATTA; 3GT-Rev: GCTGTAGCTATCTCTCAGCAGAA; PN5b: CTAAAGCGCATGCTCCAGACT