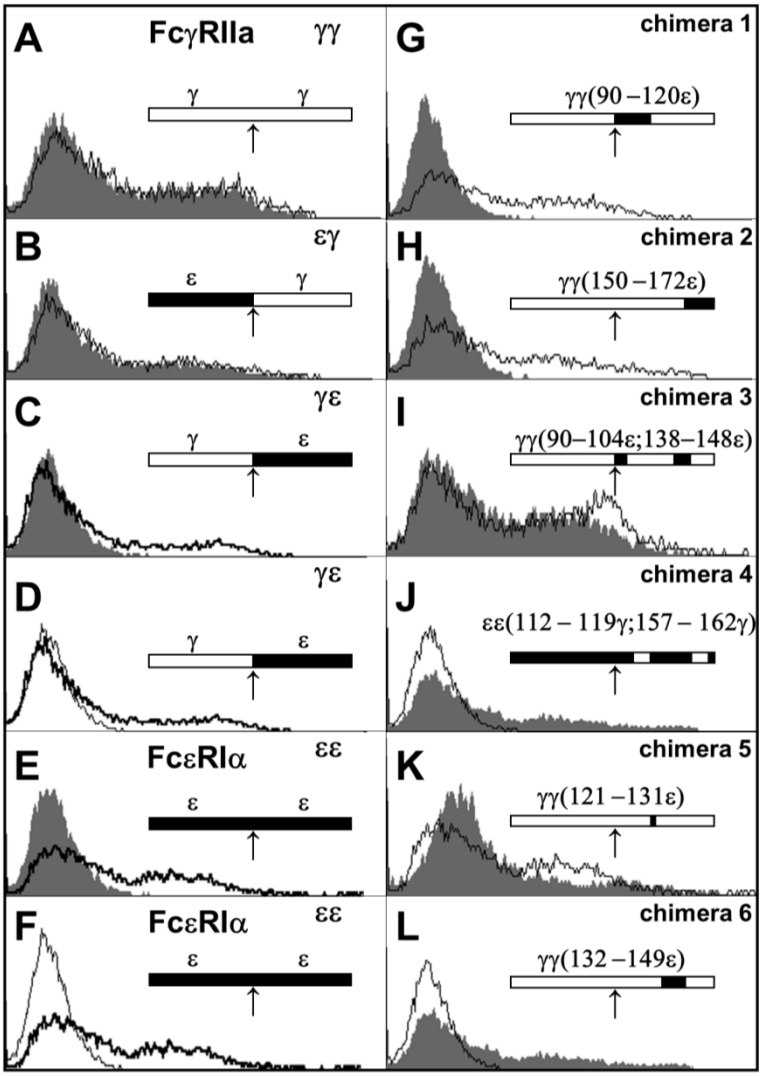
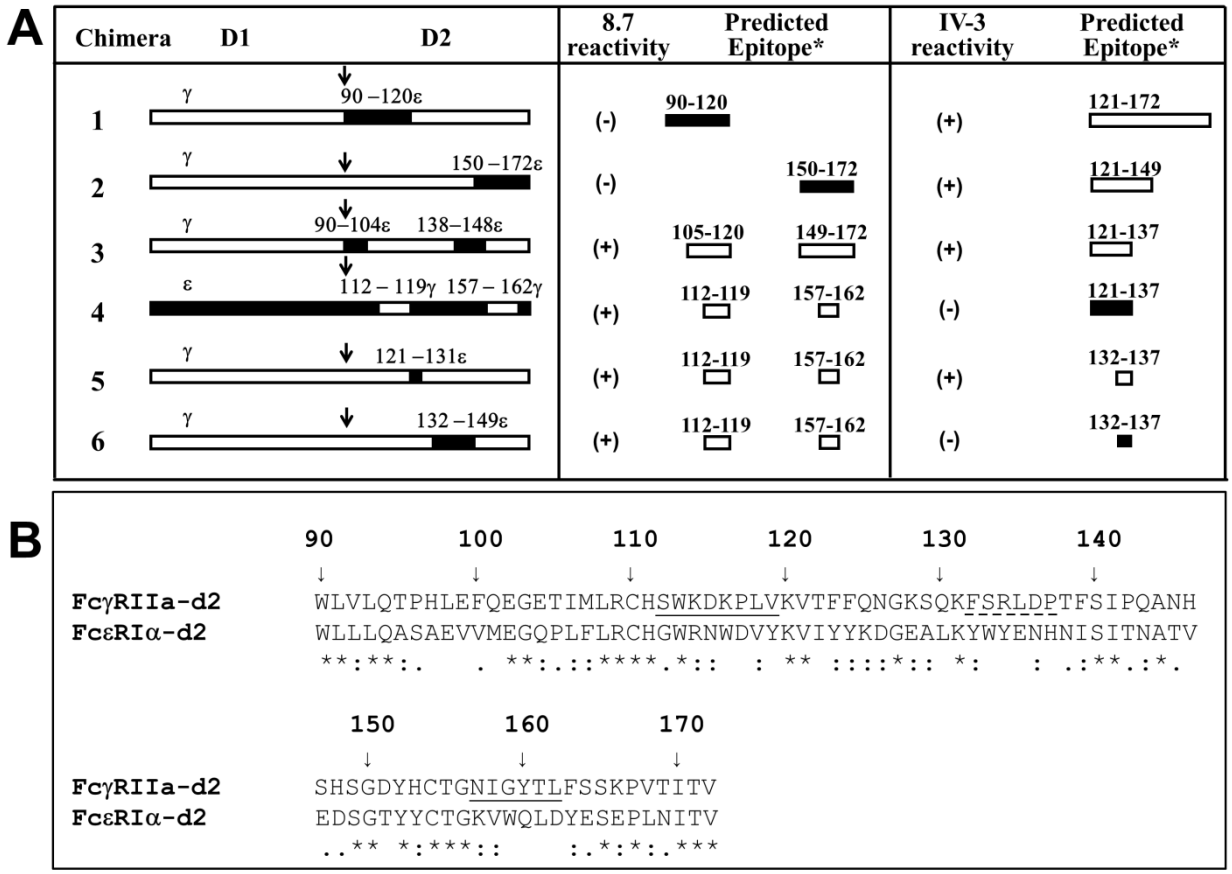
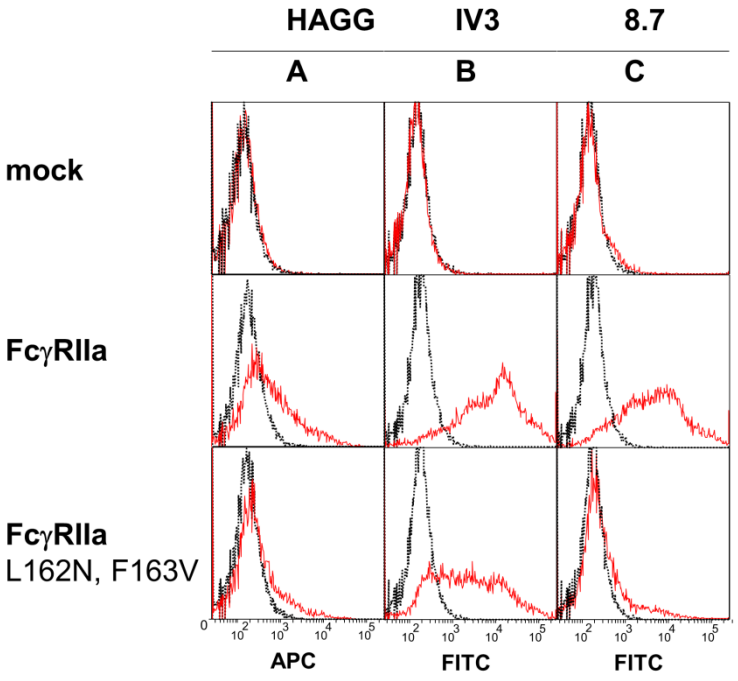


Supplemental Fig. 1. FACS analysis of binding of mAb 8.7 and IV-3 to FcγRIIa/FcεRI chimeric receptors. The chimeric receptors are shown schematically as bars with regions originating from FcγRIIa represented in white "γ" and from FcεRI in black "ε". The arrow (↑) indicates the junction between ectodomain 1 (D1) and ectodomain 2 (D2). Thus; γγ = FcγRIIa, εε = FcεRI, γε = FcγRIIa-D1/FcεRI-D2, εγ = FcεRI-D1/FcγRIIa-D2, γγ(90–120ε) = FcγRIIa with residues 90-120 exchanged with the equivalent residues of FcεRI. COS-7 cells were transiently transfected with receptor constructs and antibody binding was determined by flow cytometry. Cells were incubated with 1μg IV.3 Fab or 1μg 8.7 F(ab)'$_2$, mAbs for 30 minutes on ice. Unbound antibodies were removed by washing in 5 ml of PBS containing 0.5% BSA. Cells were resuspended in 50μls of FITC-conjugated anti-mouse IgG (Silenus, Melbourne, Australia) for 30 minutes on ice in the dark, washed in PBS BSA and analysed on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA). Binding of the 8.7 mAb is shown as filled histograms and that of IV-3 mAb as unfilled thin black line. The binding of mAb X52-47-5.4 is shown as thick black line, unfilled histogram in panels C, D, E, F) This antibody detects an epitope in the second domain of FcεRI and was used as a positive control for the detection of the domain exchange chimera γε.



Supplemental Fig. 2. Definition of mAb 8.7 and IV-3 epitopes using FcγRIIa/FcεRI chimeric receptors. **A.** The nomenclature for chimeric receptors are shown schematically as bars with regions originating from FcγRIIa represented in white "γ" and from FcεRI in black "ε". D1 = ectodomain 1; D2 = ectodomain 2 as in supplementary Fig. 1. Antibody binding determined by flow cytometry to be equivalent to binding to wild type receptor is scored (+) and the absence of mAb binding is scored (-). *Sequence segments associated with the retention of binding (white boxes) and loss of binding (filled boxes) are shown in the right hand panels for the predicted epitopes of 8.7 and IV-3. B. Sequence alignment of the second ectodomains of FcγRIIa and FcεRI. The numbering refers to FcγRIIa. The two elements of the mAb 8.7 epitope determined by chimeric receptor analysis are shown solid underlined. Likewise the segment required for mAb IV-3 binding is shown with dashed underline. Sequence identity is indicated by "*", highly homologous residues by ":" and similar residues by ".".



Supplemental Fig. 3. Binding of mAbs to the gain of glycosylation mutant of FcγRIIa (L162N, F163V) examined by flow cytometry. CHOP cells transiently transfected with pcDNA3 alone (top row, mock), WT FcγRIIa (middle row) or the mutant (L162N, F163V) receptor were reacted with a model immune complex (HAGG) or the indicated mAbs and binding determined flow cytometry after labelling with APC-anti human IgG or FITC-anti mouse IgG.