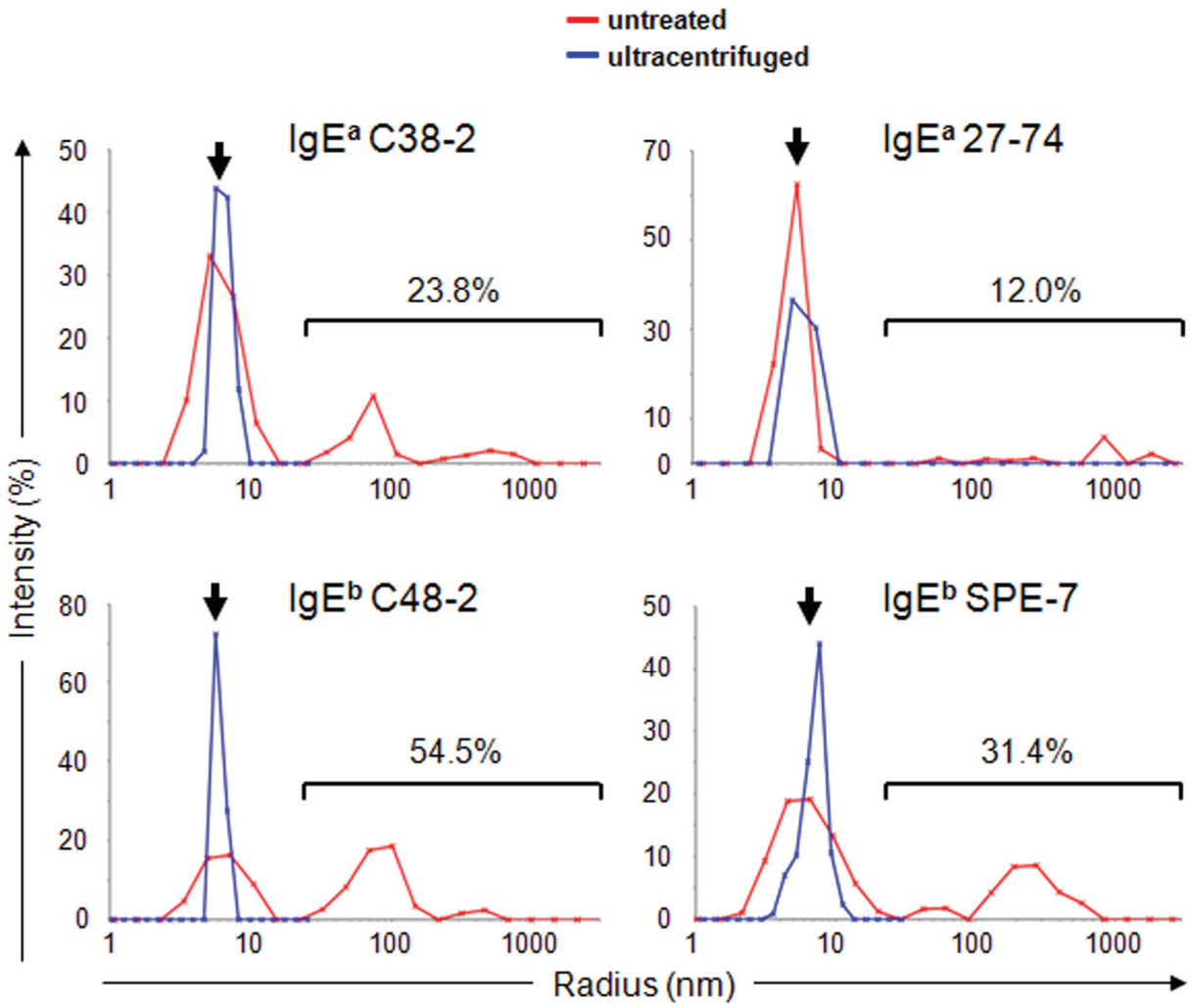
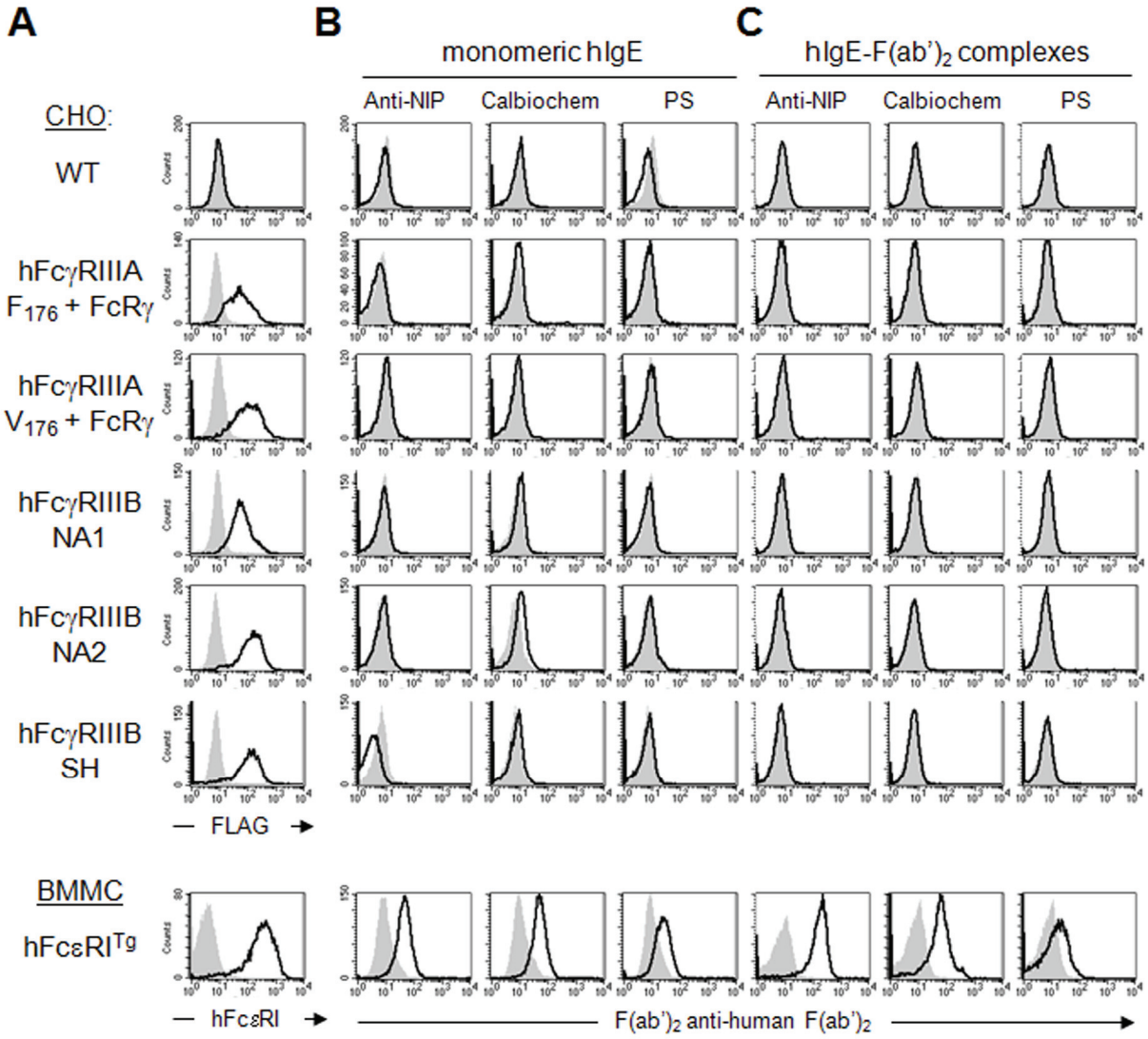
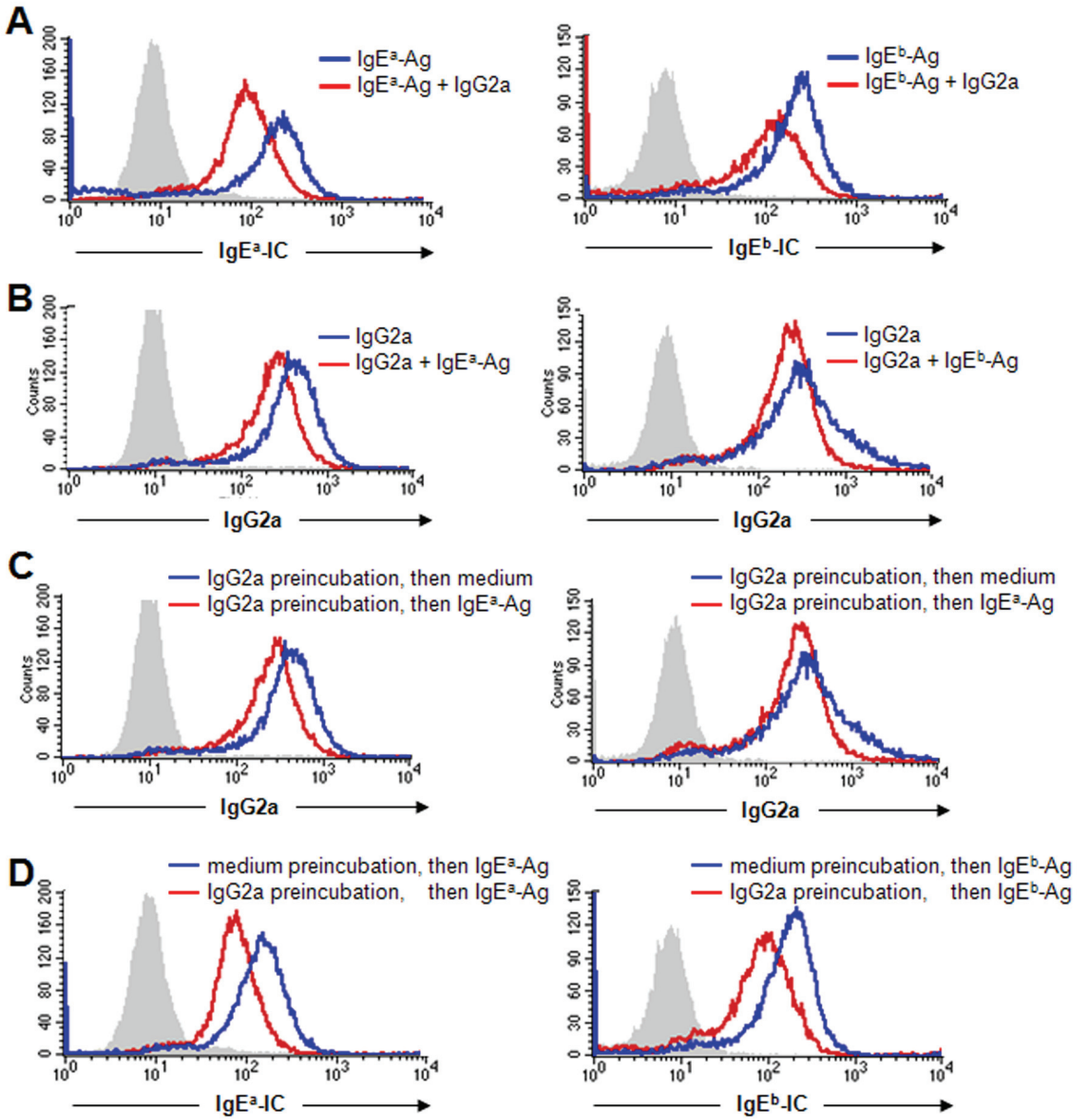


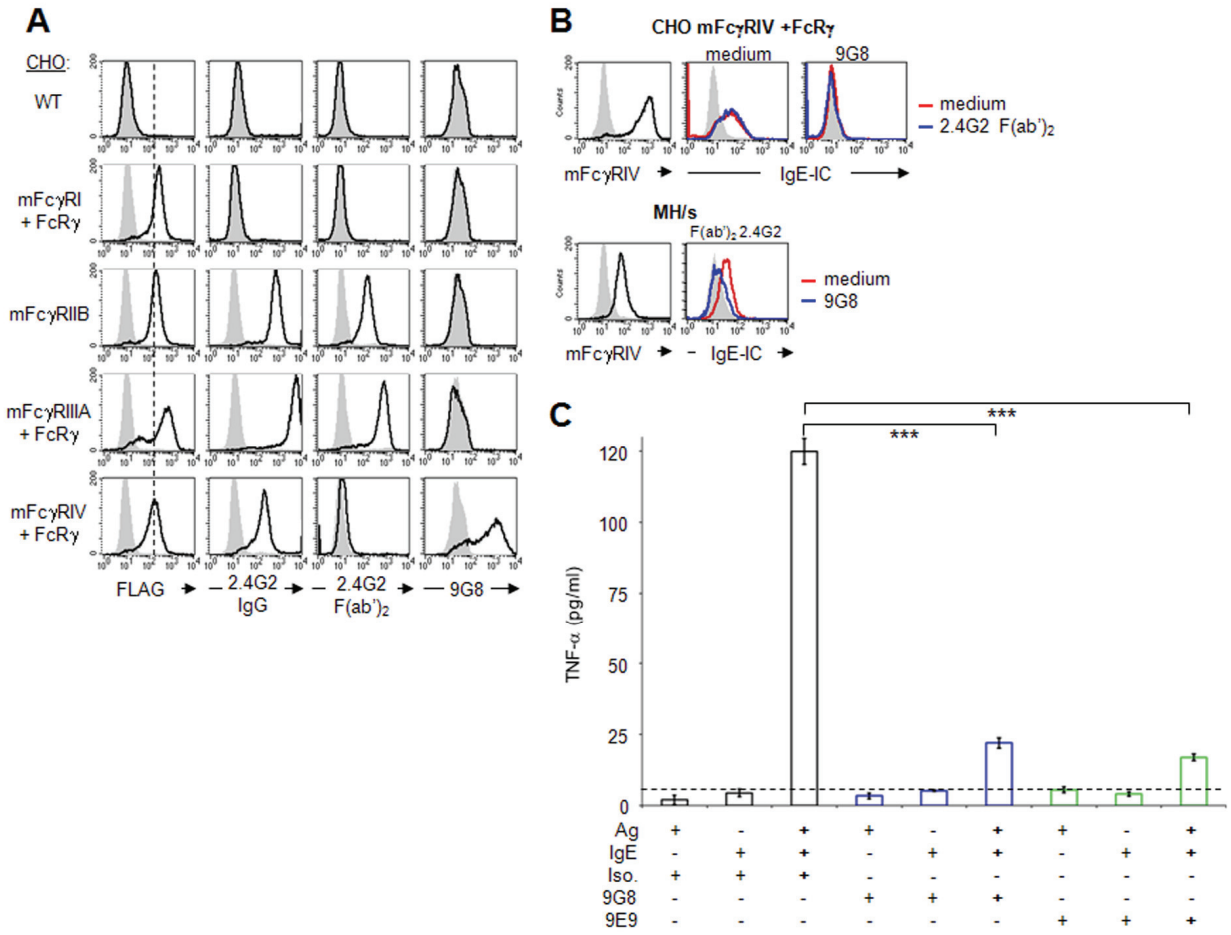
Supplemental Figure 1. Regulation histograms of IgE[a] (C38-2[a] and 27-74[a]) and IgE[b] (C48-2[b] and SPE-7[b]) indicating a homogeneous monomeric species only when ultracentrifuged. IgE solutions at 300 μg/ml were analyzed by Dynamic Light Scattering at 833 nm. Arrows indicate monomeric IgEs. The relative proportion of signal due to aggregates in commercial IgE solutions are indicated in the figure.



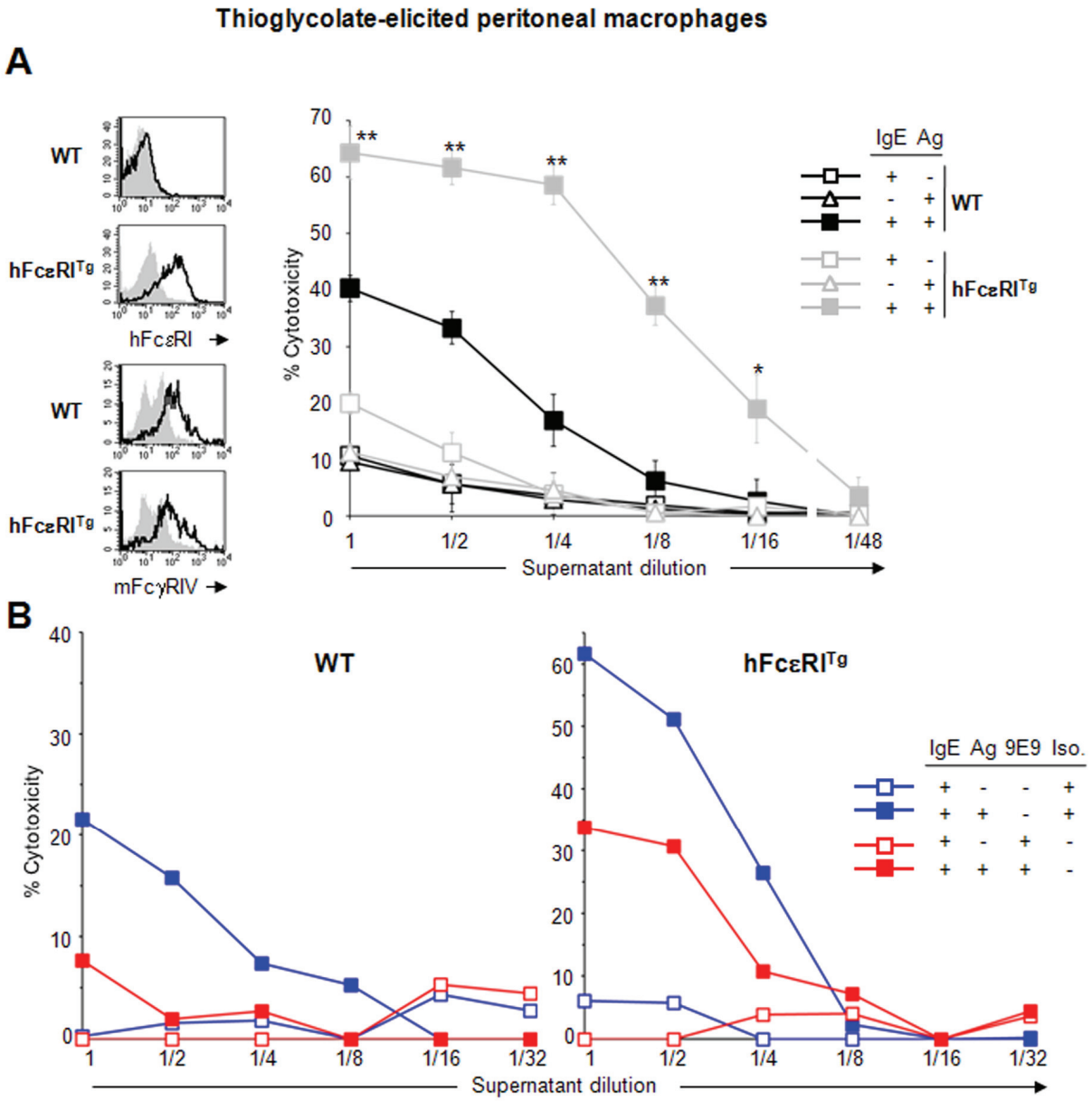
Supplemental Figure 2. hFcγRIIIA and hFcγRIIIB do not bind human IgE. (**A**) Histograms show the binding of anti-FLAG mAb to FLAG-tagged FcγR on CHO transfectants and the binding of anti-hFcεRI mAb to hFcεRI^{Tg}-BMMC. Solid gray histograms show the binding of isotype control. (**B, C**) Histograms show the binding of 30μg/ml 100,000g-ultracentrifuged monomeric human IgE (B) or 30μg/ml human IgE in complex with 16.7μg/ml PE-F(ab')$_2$ anti-human F(ab')$_2$ (**C**) to FcγR$^+$-CHO and to BMMC. Solid gray histograms show the binding of PE-F(ab')$_2$ anti-human F(ab')$_2$. Identical data was obtained in two independent experiments.



Supplemental Figure 3. IgE IC compete with IgG2a for mFcγRIV binding and displace mFcγRIV-bound IgG2a. (**A, B**) mFcγRIV$^+$-CHO were incubated with a mixture of 30μg/ml IgG2a and IgE^a IC (C38-2^a, left panel) or IgE^b IC (C48-2^b, right panel). Histograms represent the binding of IgE IC (**A**) or IgG2a (**B**). (**C, D**) mFcγRIV$^+$-CHO were preincubated with 30μg/ml of IgG2a, followed by an incubation with IgE^a IC (C38-2^a, left panel) or IgE^b IC (C48-2^b, right panel). Histograms represent the binding of IgG2a (**C**) or IgE IC (**D**). Solid grey histograms show the background binding of fluorescent reagents alone.



Supplemental Figure 4. mAbs 9G8 and 9E9, but not F(ab')$_2$ fragments of mAb 2.4G2, bind to mFcγRIV and block the binding of IgE IC. (**A**) Histograms show the binding of anti-FLAG mAb, of intact 2.4G2 IgG, of 2.4G2 F(ab')$_2$ fragments (2.5µg/ml) and of 9G8 mAb to FcγR$^+$-CHO. Binding of 2.4G2 was revealed by anti-rat IgG staining. Binding of 9G8 was revealed by F(ab')$_2$ anti-Hamster IgG staining. Solid gray histograms show the background binding of fluorescent reagents alone. (**B**) Histograms show the binding of 9G8 or isotype control (solid gray histograms) to mFcγRIV$^+$-CHO transfectants or MH-S cells. The same cells were pre-incubated with 10µg/ml 2.4G2 F(ab')$_2$ or without, in the presence of 10µg/ml 9G8 or without, as indicated. IgE^b IC (C48-2^b) binding was revealed by neutravidin staining. Solid gray histograms represent the binding of antigen alone. (**C**) MH-S cells were pre-incubated with 10µg/ml 9G8, 9E9 or Hamster IgG (Iso.) and assayed for TNF-α secretion following incubation on IgE-, Ag- or IgE^b IC (C48-2^b) by ELISA. All reagents were treated with Polymyxin B. Mean ± SD of triplicates are represented. Significant differences between cells triggered by IgE+Ag are indicated (***, $p < 0.0001$; Student's *t* test).



Supplemental Figure 5. Both mFcγRIV and hFcεRI engagement by IgE IC induces TNF-α secretion by peritoneal macrophages. (A) Histograms show the binding of anti-hFcεRI or 9G8 mAb (black line) or isotype controls (solid gray) to thioglycolate-elicited peritoneal macrophages from indicated mice. The same cells were assayed for TNF-α secretion following incubation on IgE-, Ag- or IgE^{b} IC (C48-2^{b})-coated wells. Mean ± SD of triplicates in the TNF-α bioassay are represented. Significant differences between cells from w.t. or hFcεRI^{Tg} mice triggered by IgE+Ag are indicated (**, $p < 0.001$; *, $p < 0.01$; Student's *t* test). Data are representative of two independent experiments. (B) Indicated mice were injected intravenously with 9E9 or irrelevant hamster IgG (Iso.) 1 day before recovery of thioglycollate-elicited macrophages. The cells were assayed for TNF-α secretion following incubation on IgE-, Ag- or IgE^{b} IC (C48-2^{b})-coated wells. All reagents were treated with Polymyxin B. Curves represent the percentage of cytotoxicity as a function of supernatant dilution.