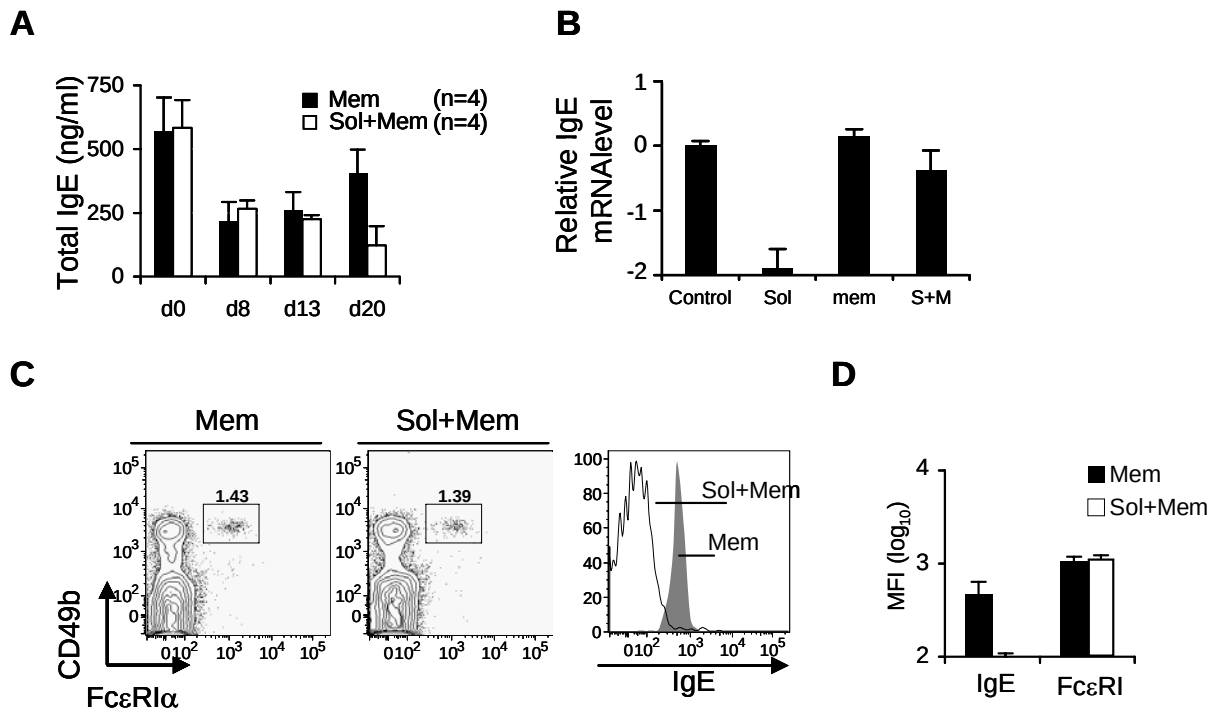
Light chain
DIVLTQSPAL AVSLGQRATI SCRASQSVSI SRYTLMHWYQ KKPGQRPKVL
                                          Q   P L
IYRASNLASG IPARFSGSGS GTDFTLTINP VQADDIATYY CHQSRESPPT
            Linker   Heavy chain
FGGGTKLELK RGGGGSGGGG SGGGGSQVTL KESGPGILQP SQTLSLTCSF

SGFLLSASSV RVAWIRQPSG KGLEWLATIG WEDVKHYNPS LKSRLTISKD

TSNNQVFLKI SSVDTADTGT YYCAHSSFDQ GTYFDYWGQG VMVTVSS

Supplementary Figure 1. Development of recombinant R1E4.

Recombinant R1E4 plasmid scFv amino acid sequence. From R1E4 hybridoma cells, Ig heavy and light chain variable region sequences was determined. Light and heavy chain sequences were fused with (Gly4Ser)3 linker. To decrease the immunogenicity, 3 amino acids were replaced (to the amino acids shown below).



Supplementary Figure 2. Analysis of the effects of plasmids encoding membrane-bound or secreted forms of R1E4 anti-IgE on markers of IgE expression.

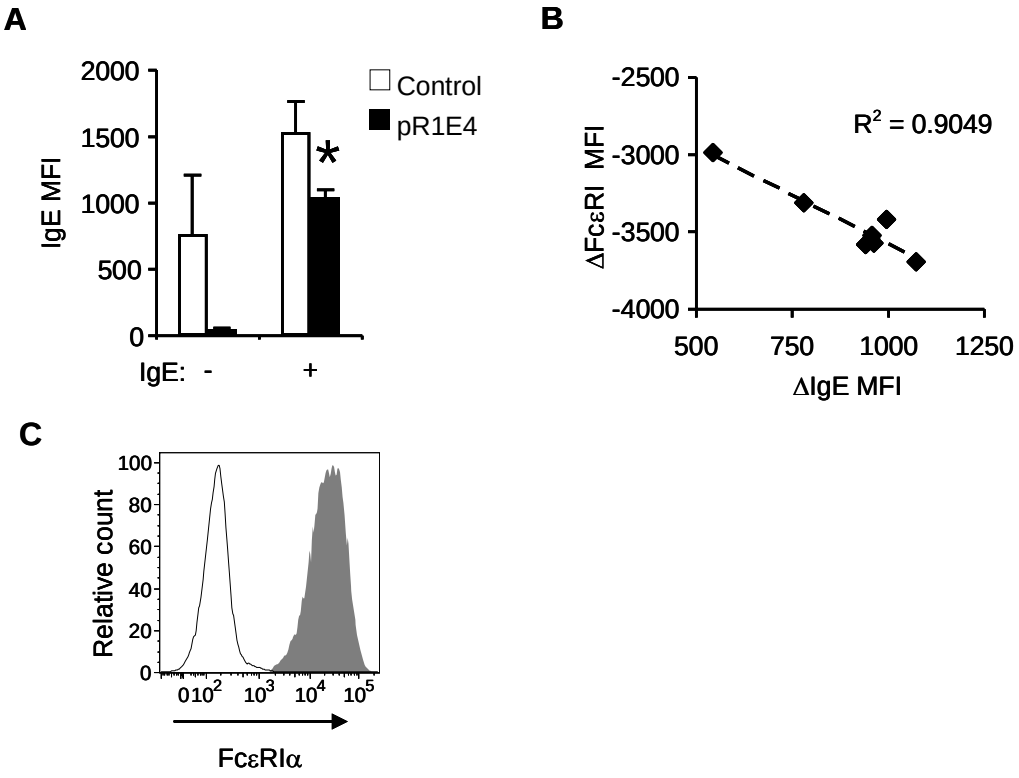
Each mouse was injected with 30 μg of the indicated plasmids encoding the membrane form of recombinant R1E4 (Mem), the soluble form (Sol), both (Sol+Mem), or a control plasmid lacking scFv insert (Control). Each treatment group consisted of 4 mice.

(**A**) Total serum IgE levels measured on the indicated days post plasmid injection.

(**B**) qPCR analysis of the levels of IgE H-chain mRNA in spleens of mice injected with the indicated plasmids 13 days previously.

(**C**) Representative comparison of IgE and FcεRI levels on splenic basophils of the indicated mice 13 days post plasmid injection.

(**D**) Mean fluorescence intensity (MFI) of staining for (in vivo bound) IgE or FcεRI on splenic basophils from the indicated mice at day 13 post treatment.



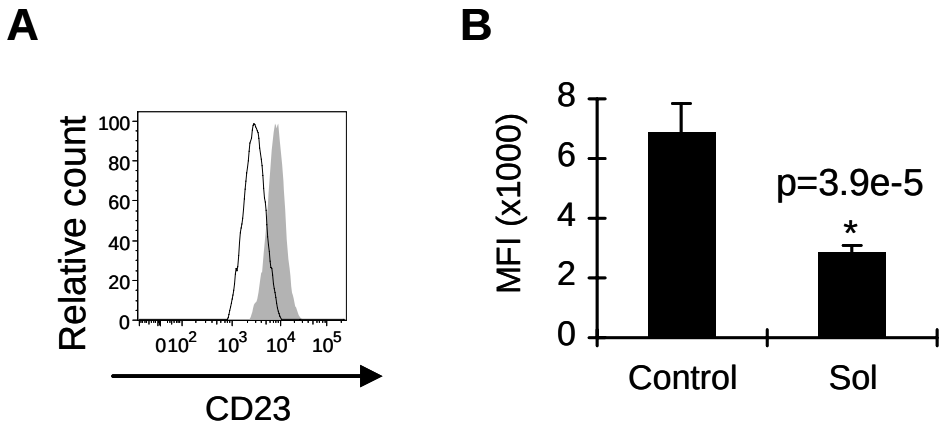
Supplementary Figure 3. Lower FcεR1 expression level on basophils in pR1E4-treated mice and properties of anti-FcεRIα antibody MAR-1 .

Two month old BALB/c mice were treated with pR1E4 or control plasmid and analyzed 13 days later. In some experiments, harvested bone marrow cells were incubated with free IgE to saturate free FcεRI as shown in materials and methods. Control (n=3) or pR1E4 (n=4) plasmid treated mouse bone marrow cells were incubated with purified IgE at 10μg/ml for 30 minutes on ice and bound IgE assessed. The difference of surface IgE and FcεRI MFI on basophils between non-saturated and IgE-saturated cells was calculated.

(**A**) Comparison of surface IgE level on bone marrow basophils with (+) or without (-) exogenous IgE addition.

(**B**) Surface FcεRI and IgE level change on bone marrow basophils caused by exogenous IgE addition was quantitated. In the plot shown, each data point represents the value measured in an individual mouse.

(**C**) Characteristics of anti-FcεRIα antibody MAR-1. Bone marrow derived mast cells expanded in WEHI3 cell culture supernatant (with IL3 bioactivity) were stained with anti-FcεRIα antibody after incubation with IgE (10 μg/ml) (black line). Histogram marked by gray shadow shows binding by MAR-1 in the absence of IgE.



Supplementary Figure 4. CD23 down-regulation on B cells of pR1E4-treated mice.

(**A**) CD23 expression level was compared between Ova/alum immunized mice receiving either pR1E4 (black line) or control plasmid (gray fill).

(**B**) Analysis of CD23 staining in each group. Note: staining was carried out in the presence of 1 mM EDTA to strip bound IgE.