Supporting Information

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Fig. S1. Serum IgE levels increase in IL-18-injected mice but not in PBS-injected controls. Total IgE levels are shown in mice injected with PBS or IL-18 for 12 d. Mean and SEM from six mice per group [preimmune (PI) and indicated time points] are plotted.



Fig. 52. Expression of the IL-18R in the spleen. (*A*) Immunofluorescence of the spleen for expression of the IL-18R (red/pink) on T cells (CD4⁺) and macrophages (F4/80⁺) in untreated WT mice. (*B* and *C*) FACS analysis of IL-18R expression on splenic (*B*) T cells and invariant NKT cells and (*C*) mature B-cell subsets. Histograms of IL-18R expression on the indicated cell types (shaded area shows isotype control) and FACS plots show the gating strategy for (*B*) T cells and invariant NKT cells on B220⁻ splenocytes and (*C*) B cells, FoBs, and MZBs on CD19⁺ splenocytes.



Fig. S3. Gating strategy for splenic B-cell populations and quantification of immature B-cell subsets in IL-18–injected mice. (*A*) Representative FACS plots of the spleen [preimmune (PI) and day 6] showing FoBs and MZBs gated on B220⁺ splenocytes as well as the B1a subset gated on TCR- β^- splenocytes. (*B*) Representative FACS plots of the spleens of untreated mice showing the gating strategy for the immature B-cell subsets, transitional type 1 (T1) gated on CD23⁻ B cells and transitional type 2 (T2) as well as transitional type 2-MZB precursor (T2-MZP) gated on CD23⁺ B cells. Quantification of the T1, T2, and T2-MZP subsets in IL-18–injected mice is shown. Mean and SEM from three to five mice per group (PI and day 6) are plotted. **P* < 0.05 by comparing untreated and IL-18–injected mice with a Mann–Whitney test.



Fig. S4. Gating strategy for MZBs in the spleens of CD19^{-/-} mice injected with IL-18 or BAFF. (A and B) Representative FACS plots of the MZB gating on B220⁺ splenocytes in (A) IL-18–injected mice [preimmune (PI) and day 10] and (B) BAFF- or PBS-injected mice (day 6).



Fig. S5. Reconstitution of the MZB population in the NKT cell-deficient $CD1d1^{-/-}$ mice restores the IL-18–induced IgE response. (A) Serum levels of total IgE in $CD1d1^{-/-}$ (N6, generation 6) and WT (C57BL/6) control mice injected with IL-18. Mean and SEM from 5 mice per group are shown. (*B*) FACS analysis of the mature splenic B-cell population in naive WT, $CD1d^{-/-}$, and $CD1d1^{-/-}$ (N6) mice. Mean and SEM from 10–12 mice per group are shown as well as representative FACS plots showing the MZB and FoB gating on B220⁺ splenocytes. (C) FACS analysis of the MZB population in naive WT and congenic $CD1d1^{-/-}$ (N12, generation 12) mice. Mean and SEM from 3–5 mice per group are shown. (*D* and *E*) $CD1d1^{-/-}$ (N6) mice after injections of IL-18. Mean and SEM from 5 mice per group are shown of the CD1d1^{-/-} (N6) mice are lethally irradiated and reconstituted with $CD1d^{-/-}$ BM. (*D*) Serum levels of total IgE in BM reconstituted $CD1d^{-/-}$ mice and $CD1d^{-/-}$ (N6) mice after injections of IL-18. Mean and SEM from 5 mice per group are shown. (*B*) FACS analysis of the MZB population in naive WT and congenic $CD1d1^{-/-}$ (N12, generation 12) mice. Mean and SEM from 3–5 mice per group are shown. (*D*) and *E*) $CD1d^{-/-}$ (N6) mice were lethally irradiated and reconstituted with $CD1d^{-/-}$ BM. (*D*) Serum levels of total IgE in BM reconstituted $CD1d^{-/-}$ mice and $CD1d^{-/-}$ (N6) mice after injections of IL-18. Mean and SEM from 5 mice per group (PI and indicated time points) are shown. (*E*) Representative FACS plots of the MZB population (gated on B220⁺ splenocytes) in $CD1d1^{-/-}$ (N6) mice before and after BM transfer. $CD1d^{-/-}$ mice are shown for comparison. **P* < 0.05 and ****P* < 0.001 by comparing the $CD1d1^{-/-}$ mice to the WT mice in *A*–*C* or to the $CD1d^{-/-}$ mice in *D* with a Mann–Whitney test.



Fig. S6. Production of Th1- and Th2-type cytokines in the spleens of IL-18–injected mice and IL-18–induced IgE response in IFN_YR-deficient mice. (*A* and *B*) Expression of mRNA for (*A*) *ifn_Y* and (*B*) *il4* in the spleens of IL-18–injected mice measured with quantitative PCR. Mean and individual mice [preimmune (PI) and indicated time points] are plotted. Relative expression of *ifn_Y* and *il4* was normalized to that of *gapdh* and the ΔCt values from IL-18–injected mice were compared with the mean ΔCt value of the PI mice. (*C*) IFN- γ produced in anti-CD3–stimulated ex vivo splenocyte cultures from IL-18–injected mice. Mean and SEM from 5–6 mice per group (PI and day 6) are plotted. (*D*) Serum levels of IgE in IFN_YR^{-/-} and WT (C57BL/6) mice injected with IL-18. Mean and SEM from 10 mice per group (PI and indicated time points) are plotted. **P* < 0.05 by comparing IL-18–injected mice to the PI group (*C*) or the IFN γ R^{-/-} mice to the WT group (*D*) with a Mann–Whitney test.