Eosinophils are required

for the maintenance of plasma cells in the bone marrow

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Supporting online material

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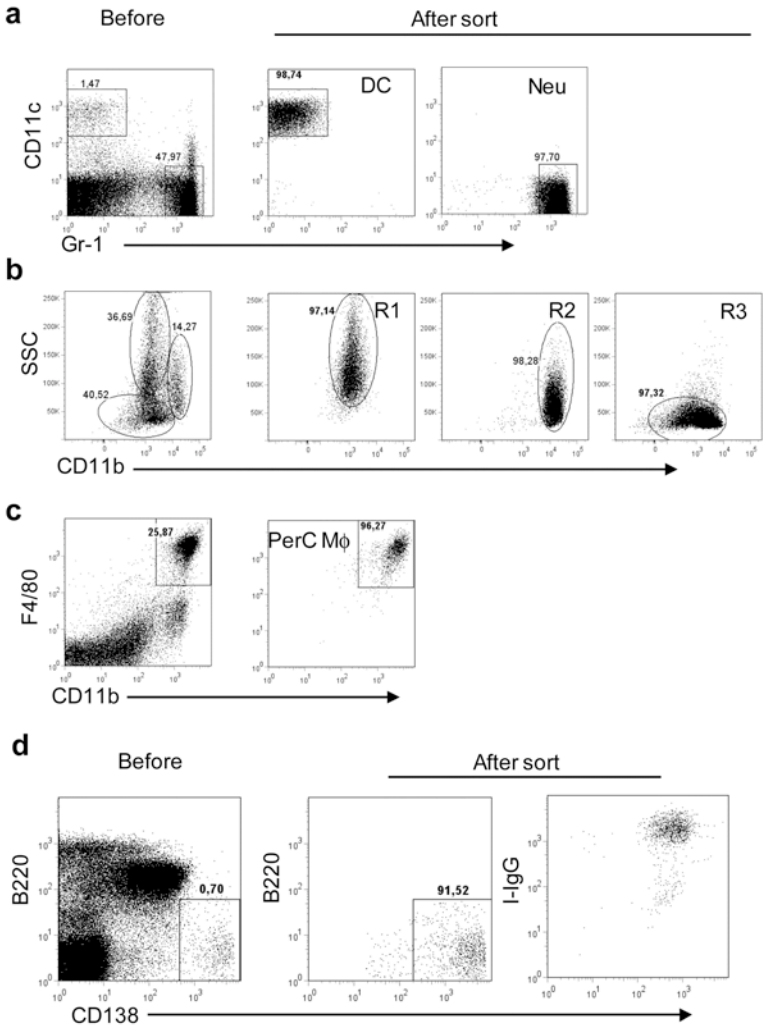
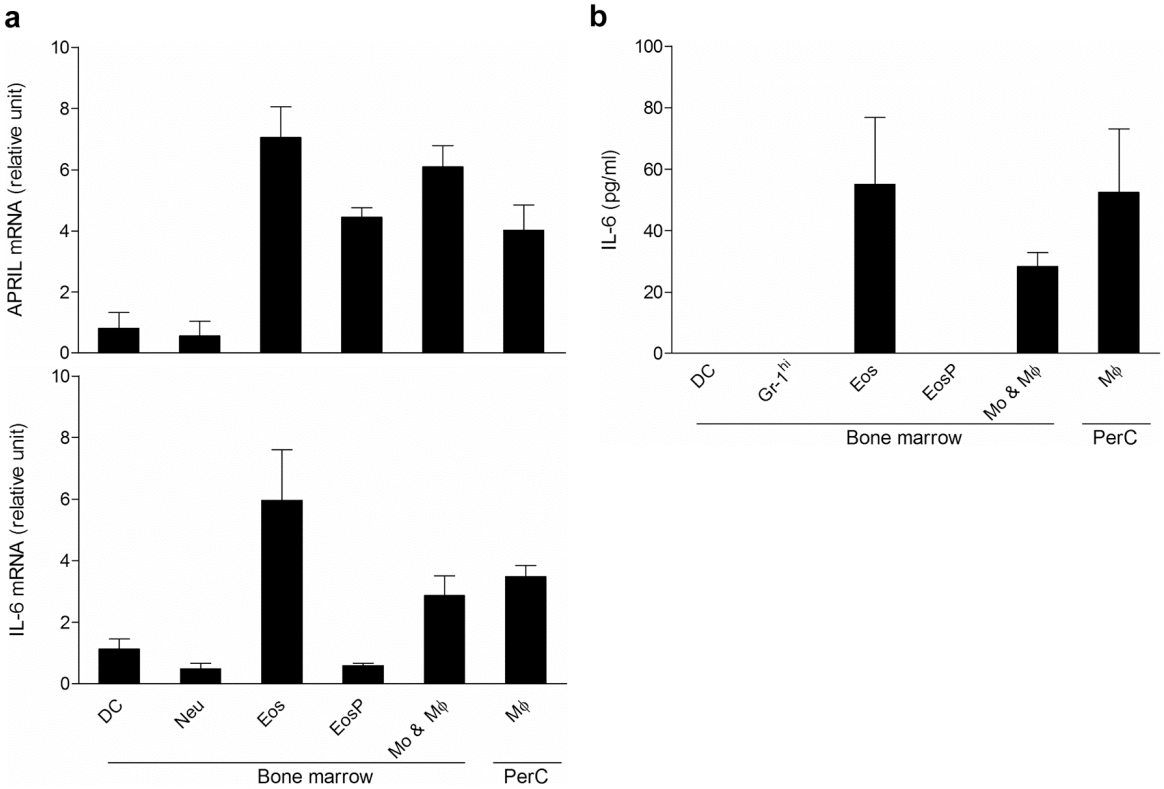
Supplementary Figure 1



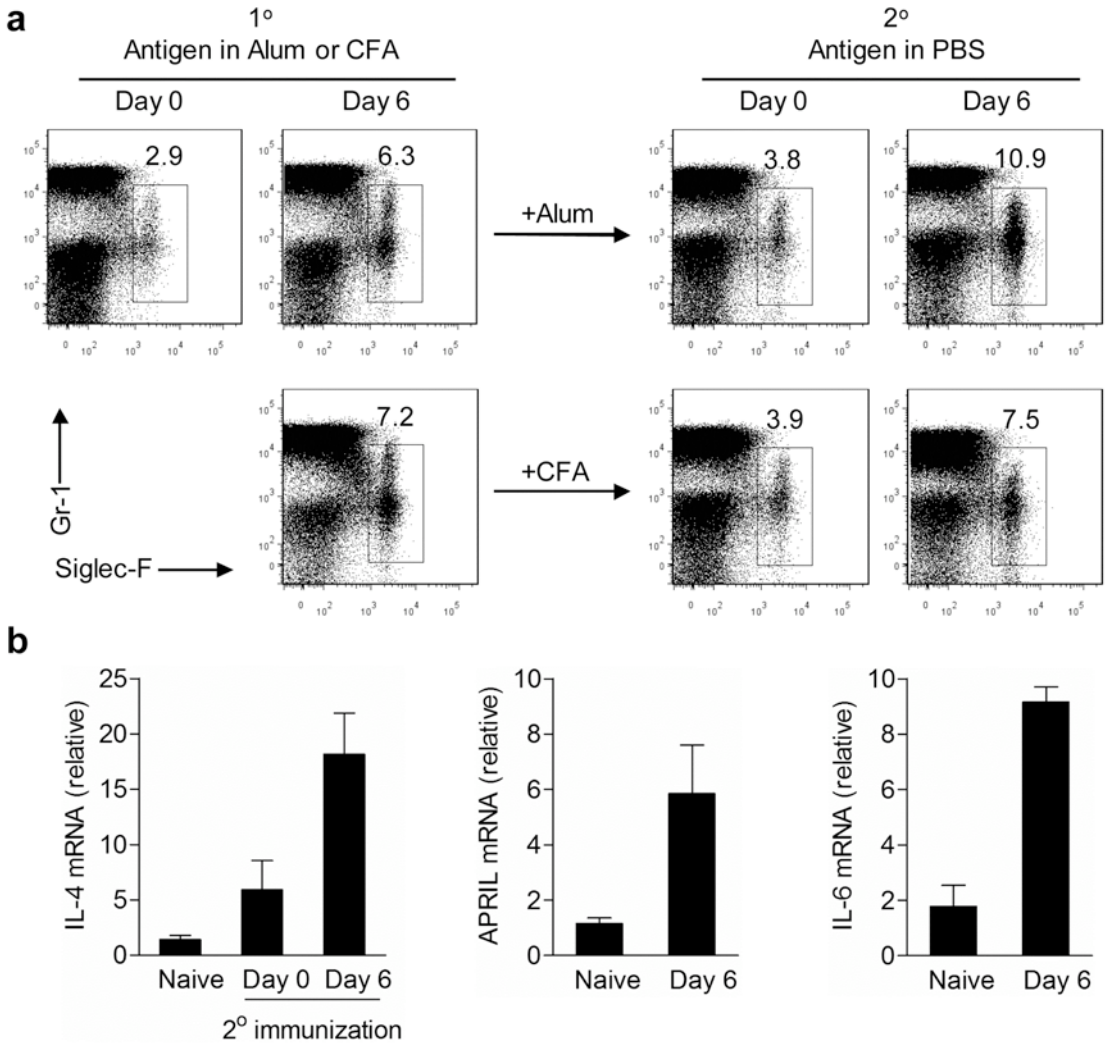
Sorting BM cell subsets. (**a**) To isolate dentritic cells (DC), BM cells were stained with anti-CD11c-PE, incubated with anti-PE microbeads, enriched by MACS and sorted by FACS. The negative fraction was stained with anti-Gr-1 and anti-CD11b and neutrophils (Neu) sorted. (**b**) To isolate R1 (eosinophils), R2 (immature eosinophils) and R3 (Mo/Mϕ) cells, BM suspension was depleted of B and T cells by MACS and cells stained with anti-CD11c/FcεRIα, anti-Gr-1, anti-F4/80 and anti-CD11b. DC, basophils/mast cells and Gr-1^{hi} cells were excluded by gating. The R1, R2 and R3 cell subsets were sorted based on SSC and on their expression levels of CD11b. (**c**) Peritoneal cavity cells were double stained with F4/80 and CD11b specific antibodies and Mϕ (PerC-Mϕ) enriched. (**d**) Plasma cells were sorted and purity controlled by staining with anti-B220 or intracellular IgG.

Supplementary Figure 2



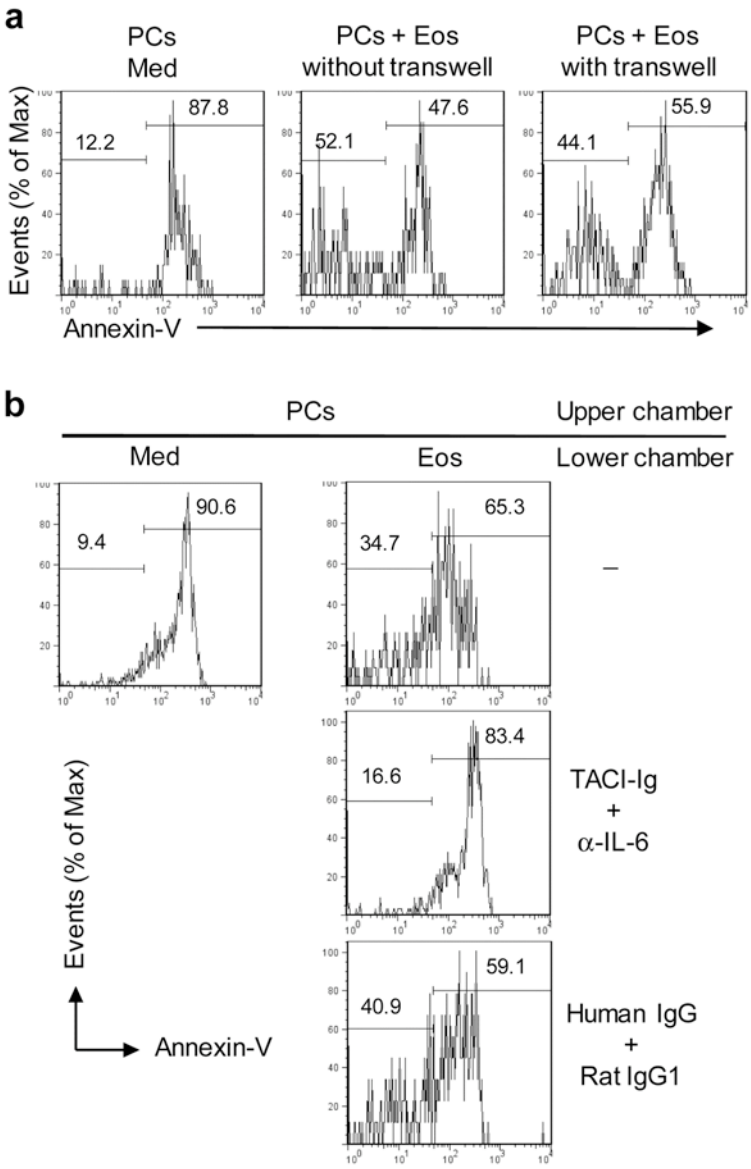
Ex vivo **cytokine expression in BM cell subsets.** (**a**) The different BM cell subsets were sorted, mRNA prepared and the level of APRIL and IL-6 mRNA compared by real-time PCR. The expression levels were normalized to that of ß-actin. (**b**) Sorted BM cell subsets were cultured for 24h and constitutive IL-6 expression determined. The results from 3 independent experiments are shown. All error bars are s.d.

Supplementary Figure 3



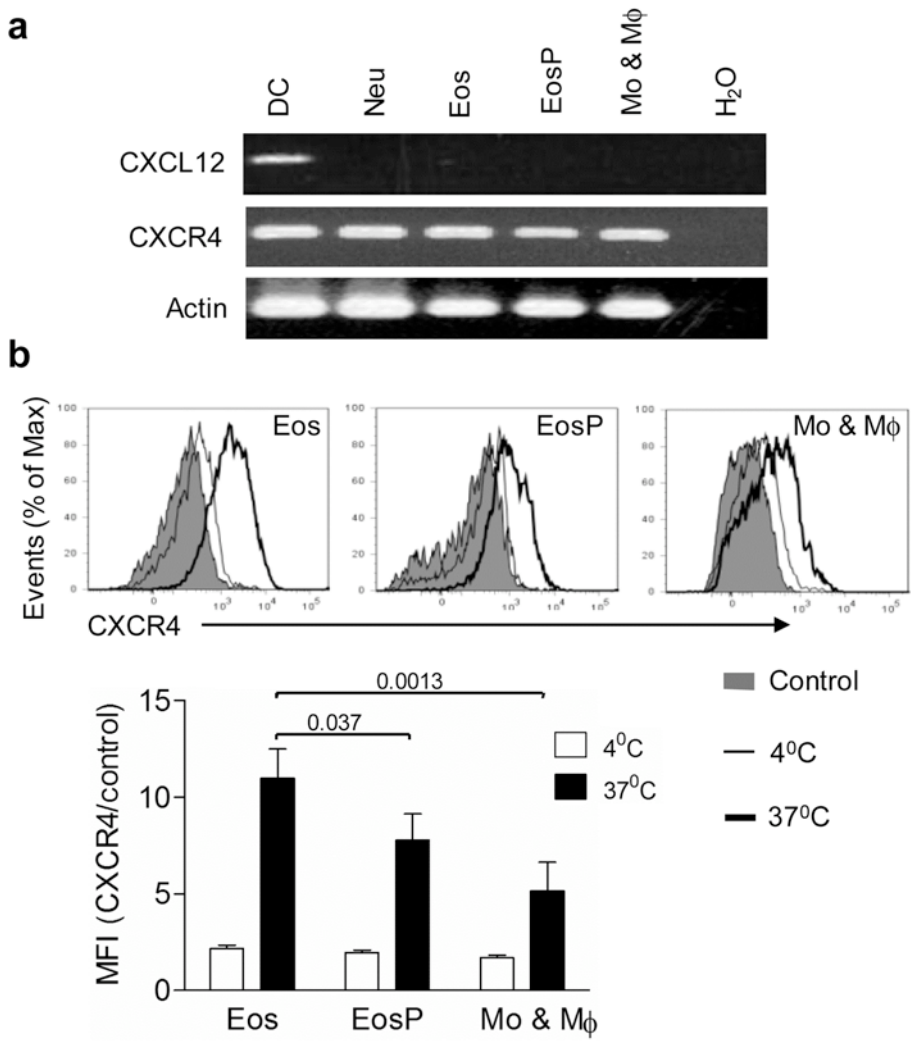
Activation of BM eosinophils *in vivo*. (**a**) BALB/c mice were immunized i.p. with Alum-precipitated or CFA-emusified phOx-CSA (1° immunization) and 8 weeks later boosted i.v. with soluble antigen (2° immunization). At indicated time points BM cells were stained with antibodies specific for Gr-1 and Siglec-F. The percentage of eosinophils is shown as dot plots. (**b**) BALB/c mice were immunized i.p. with CFA-emusified phOx-CSA and 8 weeks later boosted i.v. with soluble antigen. Eosinophils were sorted from the BM of naive BALB/c and at days 0 and 6 of secondary immunization. The relative amount of IL-4, APRIL and IL-6 mRNA was determined. 4 mice per group were analyzed. All error bars are s.d.

Supplementary Figure 4



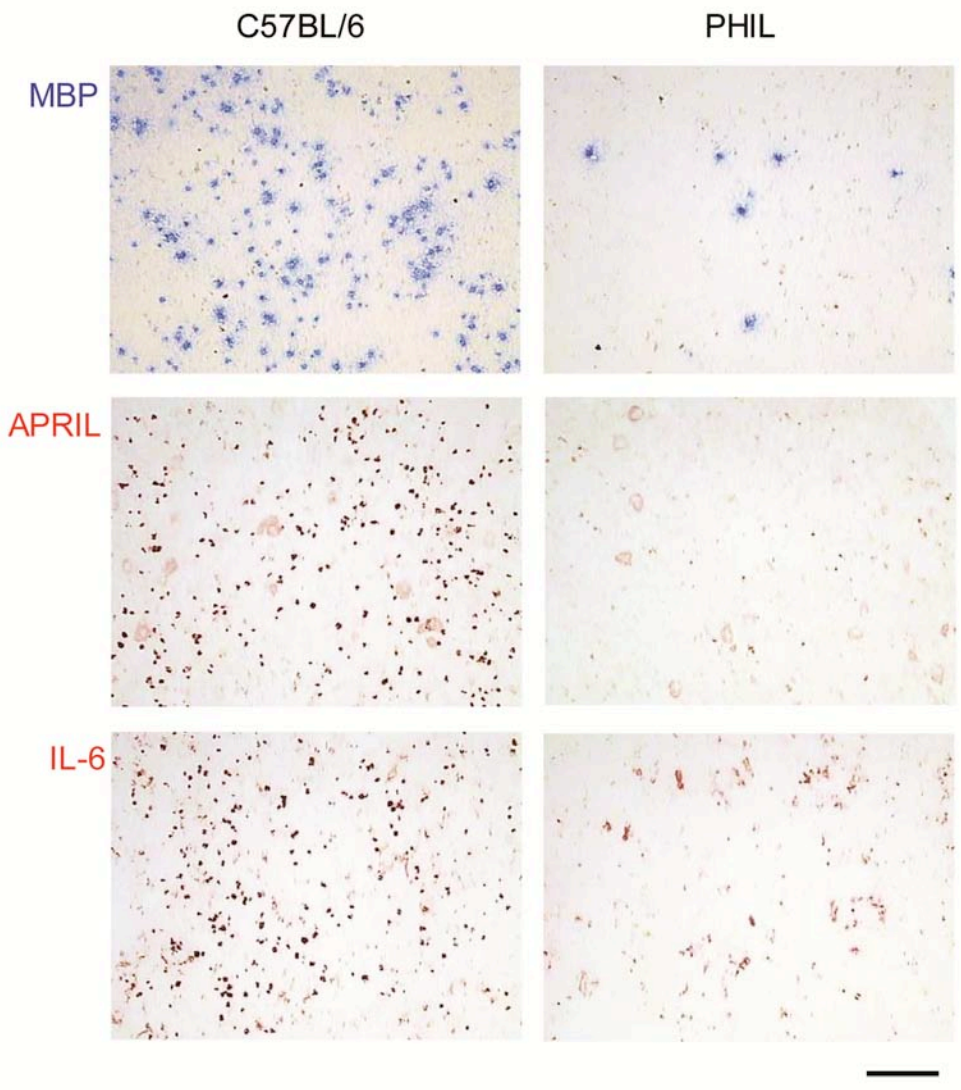
Eosinophils support plasma cell survival through secretion of soluble factors. (**a**) 10^4 BM plasma cells were cultured with medium (Med) or eosinophils (Eos, ratio 1:1) in transwells with or without inserts. (**b**) Inhibitors were added as indicated. Human IgG and rat IgG1 were used as isotype controls. After 48h culture cells were stained with anti-CD138 and Annexin-V and analyzed by flow cytometry. The percentage of apoptotic and Annexin-V^{neg} surviving cells was determined. A representative result from 2 similar experiments is shown.

Supplementary Figure 5



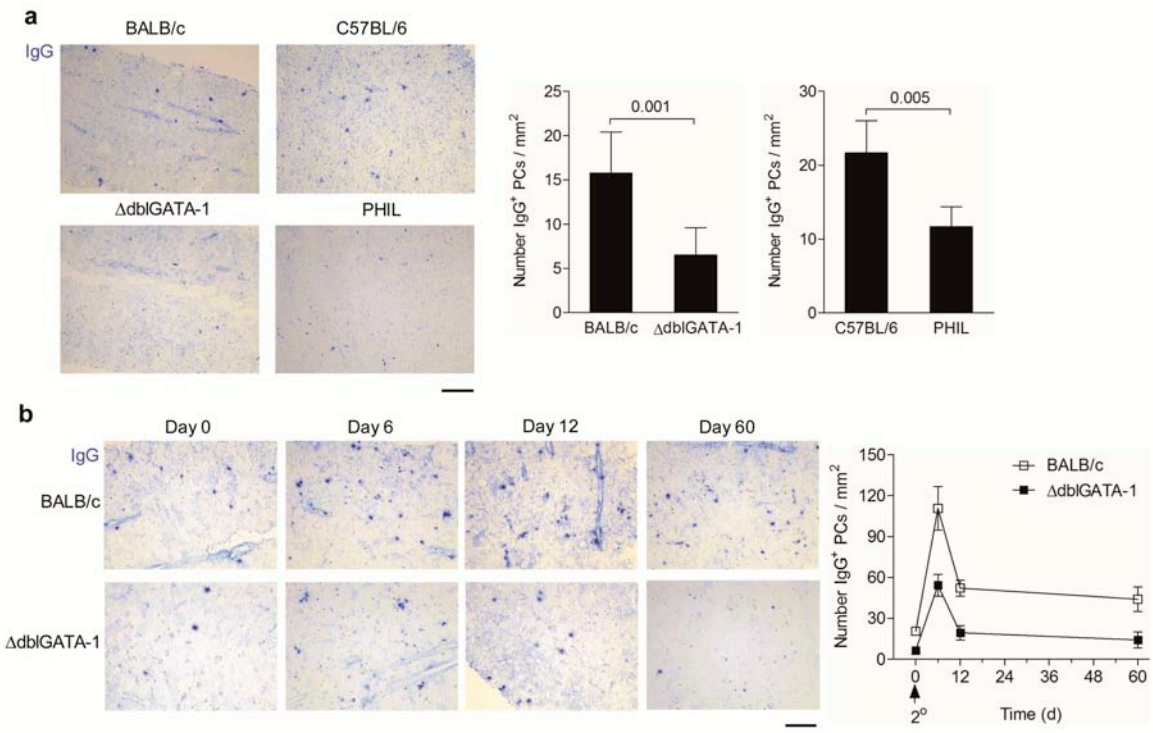
BM eosinophils do not produce CXCL12, but express CXCR4. (**a**) BM cell subsets were sorted and CXCL12 and CXCR4 transcripts determined by RT-PCR. (**b**) CXCR4 surface expression for eosinophils (Eos), immature eosinophils (EosP) and Mo & Mϕ was analyzed by flow cytometry. Histograms show overlays of isotype control (grey), surface expression at 4°C (normal line) or after incubation at 37°C (heavy line). MFI for CXCR4 expression was determined at 4° ( ) and 37° ( ). P values are indicated and error bars indicate s.d.

Supplementary Figure 6



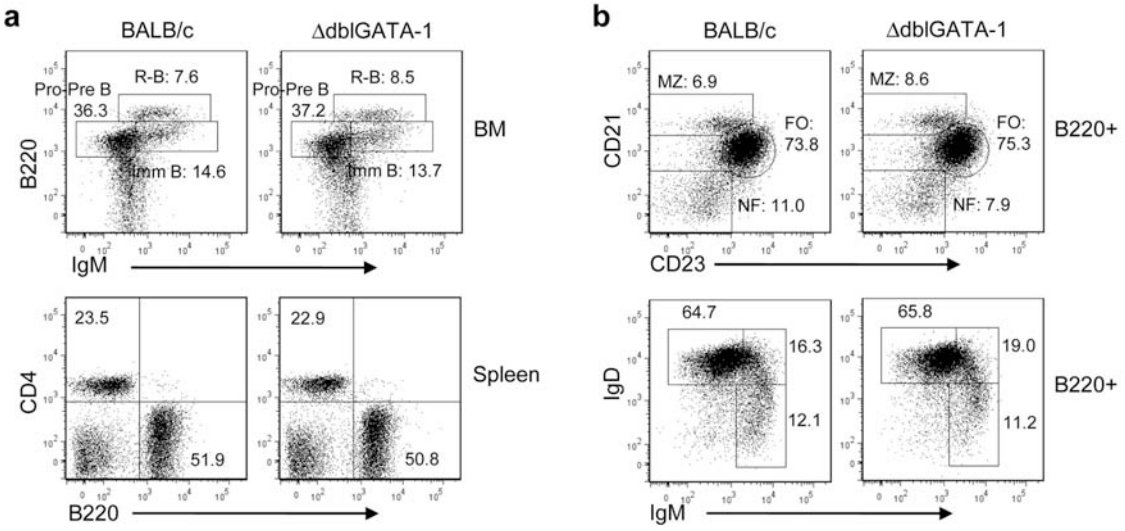
The level of APRIL and IL-6 expression is reduced in the BM of PHIL mice. BM tissue sections of C57BL/6 and PHIL mice were immunohistochemically stained with antibodies specific for MBP, APRIL and IL-6. Scale bar 150 μm.

Supplementary Figure 7



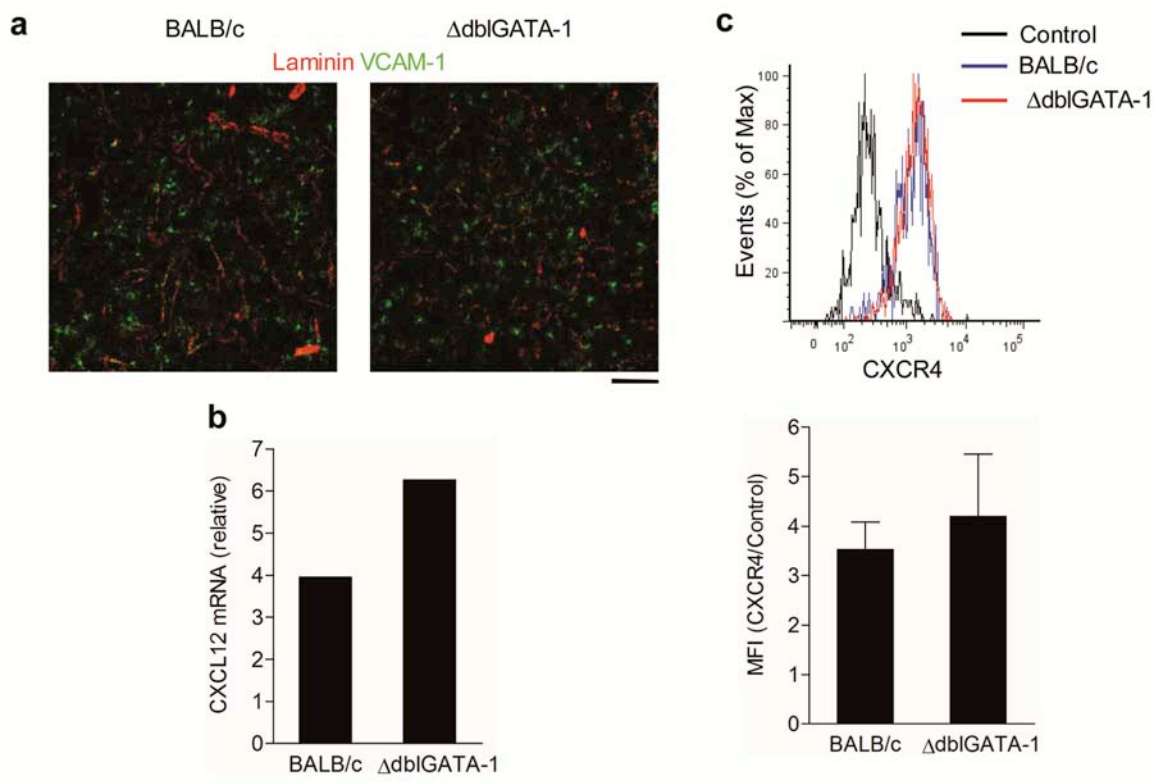
Plasma cell accumulation in the BM is impaired in eosinophil-deficient mice. BM sections were prepared from (**a**) naive BALB/c (n=4), C57BL/6 (n=2), dblGATA-1 (n=4) and PHIL (n=2) mice and (**b**) from BALB/c ( ) and dblGATA-1 ( ) mice at different time points after secondary immunization. Sections were stained with anti-mouse IgG and the number of plasma cells counted. For secondary immunization 3 animals per group were analyzed at each time point, data summarized and presented as graph. Scale bar (**a**) 300 μm and (**b**) 150 μm. P values are shown and error bars indicate s.d.

Supplementary Figure 8



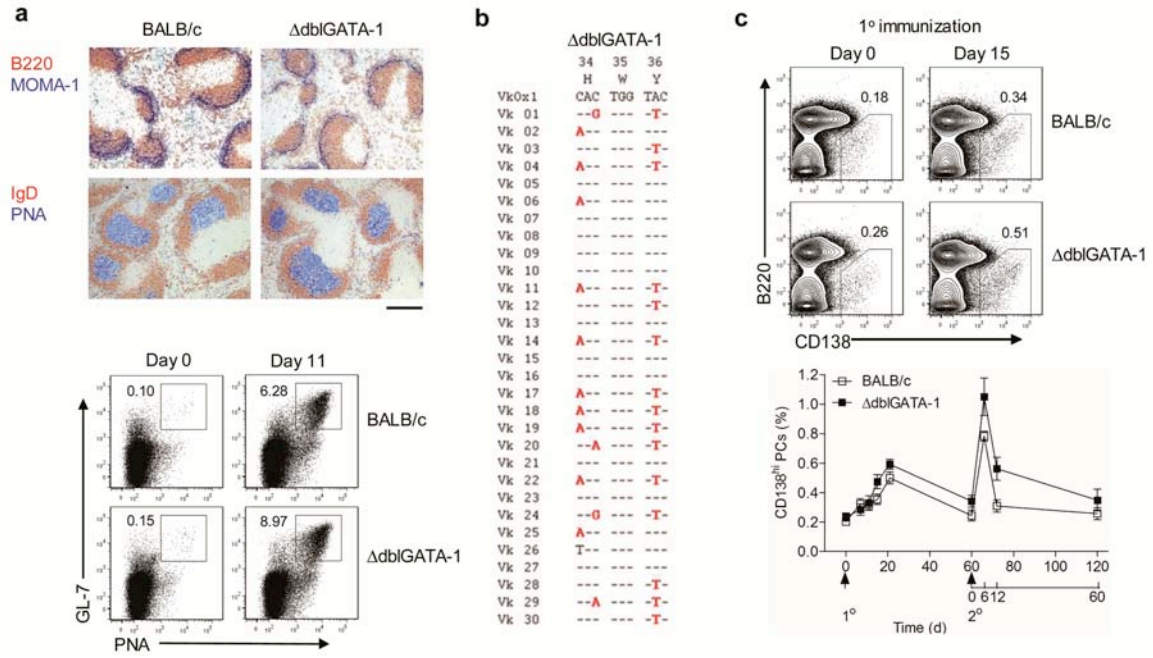
B cell development in dblGATA-1 mice is normal. (**a**) BM and spleen cell suspensions were stained with anti-B220 and IgM or CD4, respectively. Gating on living lymphocytes showed that the percentage of pro-pre, immature (Imm), re-circulating (R) B cell subsets in BM of dblGATA-1 mice is normal. In the spleen the ratio of CD4 T cells and B cells are normal. (**b**) Splenocytes were stained with for B220, CD21, CD23, IgM and IgD. Gating on B220 showed normal development of newly formed (NF) T1 and T2, marginal zone (MZ) and follicular (FO) B cells. Representative data from one of 4 animals are shown.

Supplementary Figure 9



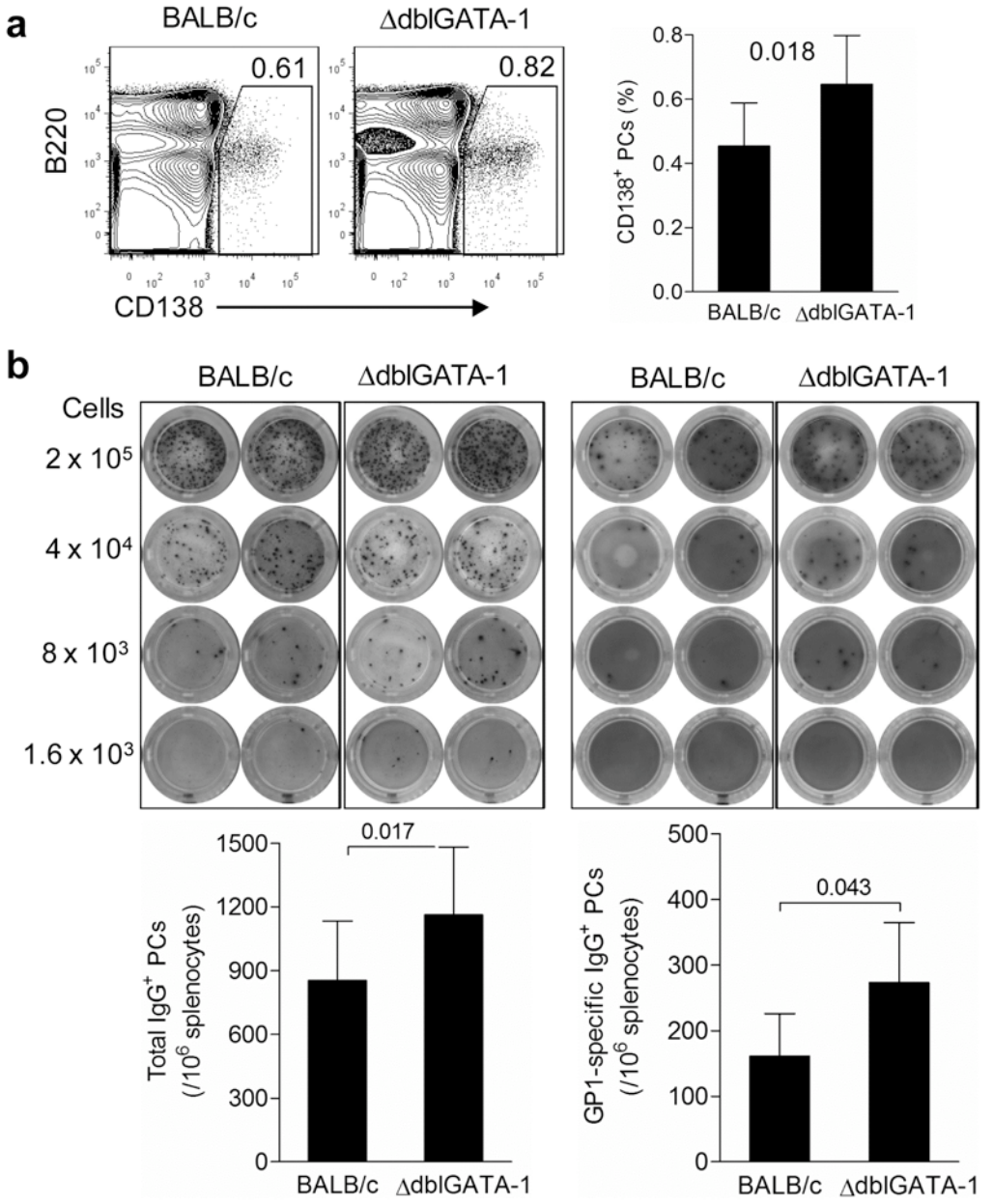
BM architecture and CXCR4 chemokine receptor expression are normal in dblGATA-1 mice. (**a**) BM sections of BALB/c and dblGATA-1 mice were stained with anti-Laminin (red) and anti-VCAM-1 (green). Scale bar 75 μm. (**b**) The expression level of BM CXCL12 mRNA was compared by real-time PCR. The expression levels were normalized to that of ß-actin, mean values are shown. Representative data for one of 4 animals are shown. (**c**) A histogram shows overlays of CXCR4 expression on the surface of splenic $CD138^+$ plasma cells from BALB/c (blue), dblGATA-1 (red) and isotype control (black) at day 6 of secondary immunization. Ratio of MFI of CXCR4 expression on plasma cells to the MFI of the isotype control is shown. 4 animals per group were analyzed. All error bars are s.d.

Supplementary Figure 10



Normal initial immune response in dblGATA-1 mice. BALB/c and dblGATA-1 mice were immunized i.p. with phOx-CSA in alum. (**a**) Staining of splenic tissue sections with B220 and MOMA-1 or anti-IgD and PNA shows normal lymphoid structures and GC development. Scale bar 300 μm. Splenocytes were stained with anti-B220, GL-7, CD138 and PNA. Frequency of germinal center B cells is indicated. (**b**) 15 days after immunization PNA^{hi} B cells from dblGATA-1 were sorted and Vk-Ox1 V-region genes amplified and sequenced. The key mutations conferring high affinity are highlighted. (**c**) The percentage of $CD138^{+}$ plasma cells was determined in the spleen of BALB/c and dblGATA-1 at day 0 and day 15 after primary immunization (contour plots). The percentage of $CD138^{+}$ plasma cells in spleen during primary and secondary immunization was summerized from 4 animals per each group (graph). All error bars are s.d.

Supplementary Figure 11



Eosinophil-deficiency affects numbers of plasma cells in spleen of LCMV infected mice. BALB/c and dblGATA-1 mice were infected with LCMV and 7 months later the response analyzed. (**a**) The frequency of plasma cells in spleen was determined by flow cytometry. (**b**) Serial dilutions of splenocytes were analyzed by ELISPOT assays to determine the number of total (left panels) or GP1 (right panels) specific IgG plasma cells. Data from 6 animals for each group are summarized (graphs). P values are included, error bars display as s.d.

Supplementary Table 1: List of PCR primer sequences

Gene	5´ Primer sequence	3´ Primer sequence
CXCL12	5´-ggacgccaaggtcgtcgccgtgctg	5´-ctccaggtactcttggatccac
CXCR4	5´-gccatggaaccgatcagtgtgag	5´-ccatgaccaggatcaccaatcc
APRIL	5´-gcaaccagtacttaggcgtgg	5´-aggcacggtcaggatcagaag
IL-6	5´-ccacttcacaagtcggaggct	5´-ggtactccagaagaccagagg
ß-actin	5´-gacaggatgcagaaggagatcact	5´-tgatccacatctgctggaggt