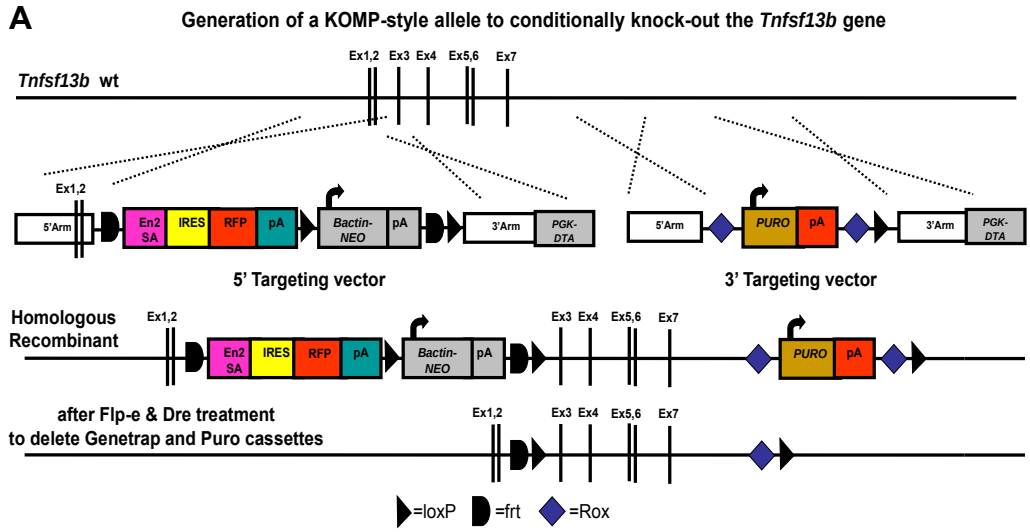
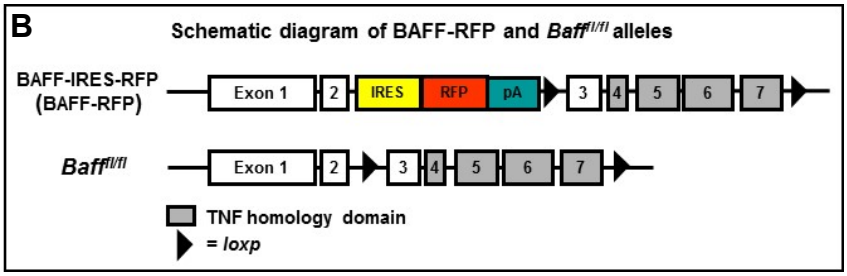
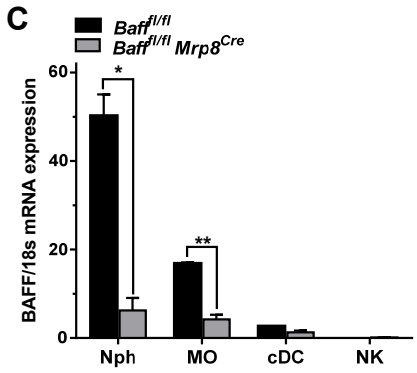
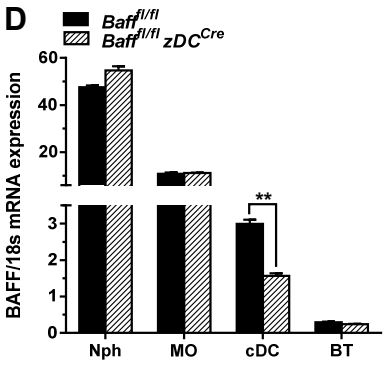
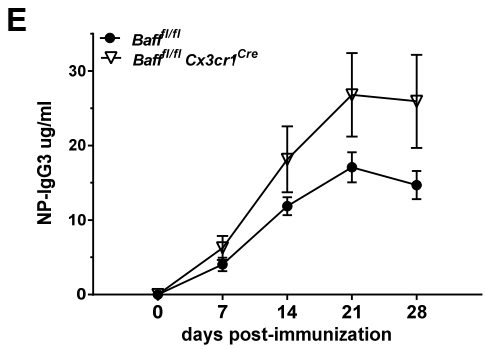
Supplemental Fig. S1









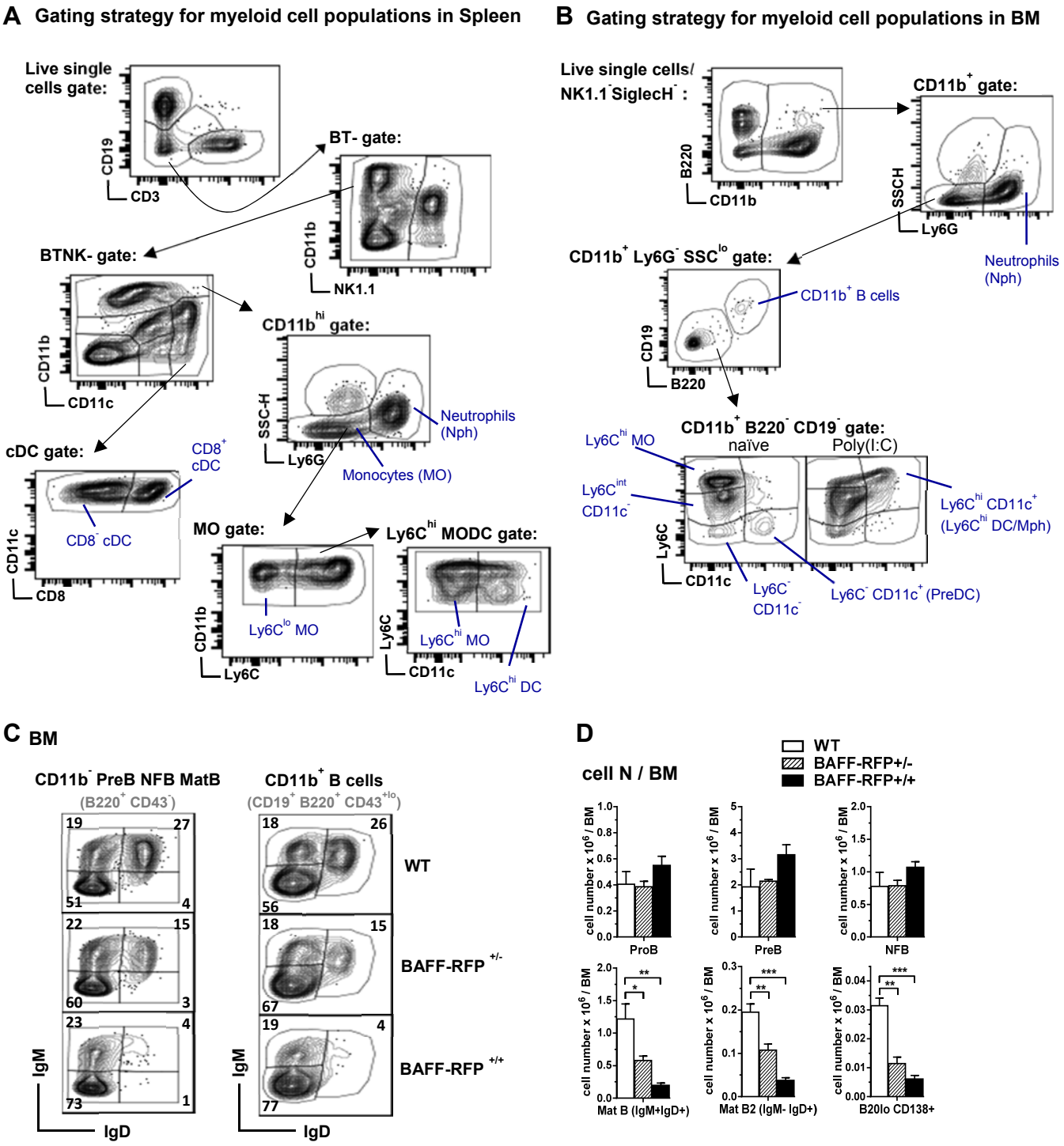


Supplemental Figure S1. *Tnfsf13b* (BAFF) reporter mice expressing RFP, *Baff*$^{fl/fl}$ mice, and verification of conditional KO mice. A. Schematic of the two-construct approach used to generate a KOMP-style allele to conditionally knock-out the *Tnfsf13b/Baff* gene. The *Tnfsf13b*-5'construct included a Genetrap Cassette expressing the TagRFPT) red fluorescence protein (RFP), under the control of an internal ribosome entry site (IRES) and flanked by frt sites for selective deletion, a loxP site and arms of homology of ~5kb each. The *Tnfsf13b*-5'construct was inserted between exons 2 and 3 of *Tnfsf13b* gene. Neomycin (*Neo*) and Diphtheria Toxin A (DTA) elements for positive and negative selection are indicated. The *Tnfsf13b*-3'construct was inserted after the last exon 7 in the intergenic region, and included the other loxP site, arms of homology (~5kb each), a Puromycin (*Puro*) and a DTA element. The *Tnfsf13b*-3'construct was electroporated first into ES cells, and a positive clone electroporated with the *Tnfsf13b*-5'construct. Homologous recombination of the two constructs generated the heterozygous BAFF-IRES-RFP (BAFF-RFP) reporter mice where *Tnfsf13b* is functionally knocked-out and the reporter expresses the IRES-RFP under *Tnfsf13b* promoter and the loxP sites between exon 3 and 7. The germline BAFF-RFP+/- mice were bred with B6N(B6J)-Tg(CAG-Flpo) mice to excise the Genetrap Cassette and *Neo*, and then with B6;129-Tg(CAG-dre) mice to remove *Puro*. The Flpo and Dre recombinases were bred out to generate *Baff*$^{fl/fl}$ mice expressing wild type *Baff* with floxed exons 3-7. **B**, Schematic diagram of BAFF-IRES-RFP (BAFF-RFP) and *Baff* $^{fl/fl}$ alleles. **C**, **D**, Splenocytes harvested from *Baff*$^{fl/fl}$ (**C**, **D**), *Baff*$^{fl/fl}$ *Mrp8*^{Cre} mice (**C**), or *Baff*$^{fl/fl}$ *zDC*^{Cre} mice (**D**), enriched with CD11b^{+} CD11c^{+} magnetic bead positive selection, sorted on a FACS Aria cell sorter and assessed for *Baff* mRNA by qPCR. **C**, **D**, Data show means ± SEM of relative *Baff* mRNA from one experiment pooling cells from six mice/group (**C**), or from one representative of two experiments pooling cells from six-seven mice/group (**D**). Selective depletion of BAFF in Nphs from *Baff*$^{fl/fl}$ *Mrp8*^{Cre} mice was confirmed with sorted BM cells. **C**, **D**, Sorted cells from *Mrp8*^{Cre} mice and *zDC*^{Cre} mice showed similar *Baff* mRNA levels as *Baff*$^{fl/fl}$ mice (not shown). **C**, **D**, Statistics were determined with two-tailed unpaired Student's *t-test*, * $p<0.05$, ** $p<0.01$. **E,** *Baff* $^{fl/fl}$ mice crossed with *Cx3cr1*^{Cre} mice (1) to generate *Baff*$^{fl/fl}$ *Cx3cr1*^{Cre} mice. *Baff* mRNA in sorted splenic MOs from *Baff*$^{fl/fl}$ *Cx3cr1*^{Cre} mice was lower than in control MOs, confirming that BAFF was

knocked down just in the appropriate subset. *Baff* $^{fl/fl}$ mice and *Baff*$^{fl/fl}$ *Cx3cr1*^{Cre} mice were immunized i.p. with 20 μg of NP-Ficoll, bled at the indicated time points, NP-specific IgG3 was analyzed by ELISA. Data show means ± SEM of NP-IgG3 and are from one experiment; *Baff*$^{fl/fl}$, N=8; *Baff*$^{fl/fl}$ *Cx3cr1*^{Cre}, N=6. Statistics were performed with 2-way ANOVA with Tukey's multiple comparison test.

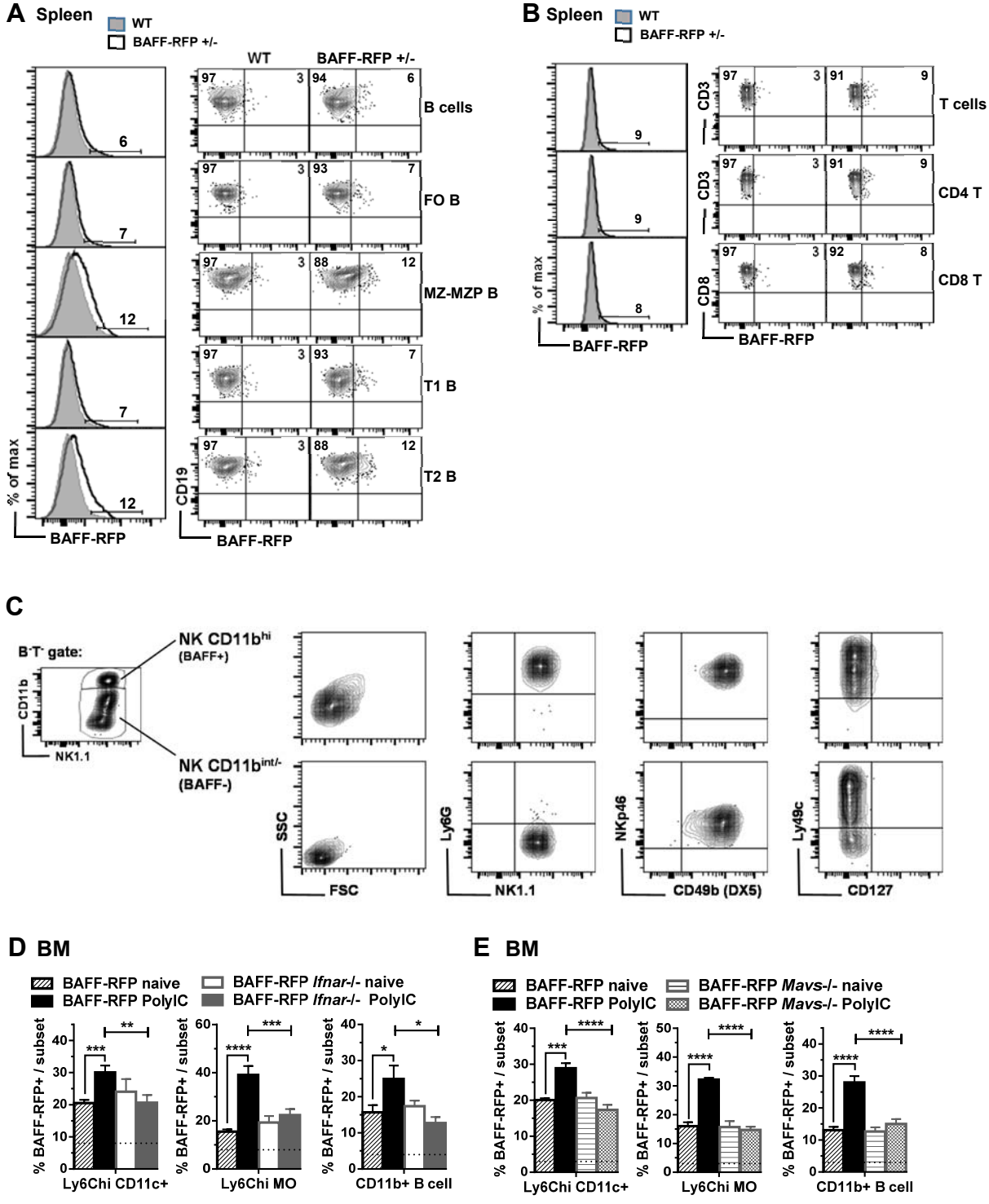
1. Yona, S., K.-W. Kim, Y. Wolf, A. Mildner, D. Varol, M. Breker, D. Strauss-Ayali, S. Viukov, M. Guilliams, A. Misharin, D. A. Hume, H. Perlman, B. Malissen, E. Zelzer, and S. Jung. 2013. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. *Immunity* 38: 79–91.

Supplemental Fig. S2



Supplemental Figure S2. Analyses of myeloid cells and B cells in spleens and BMs from WT, BAFF-RFP$^{+/-}$ and BAFF-RFP$^{+/+}$ mice. Gating strategy to identify myeloid cell populations in WT splenic (**A**) and BM cells (**B**). Debris, doublets and nonviable cells were excluded. **A,** splenic **B cells** (CD19^{+}CD3^{-}) and **T cells** (CD19^{-}CD3^{+}) were defined first; in non-B cells and non-T cells (CD19^{-}CD3^{-} gate) populations were defined as follows: **NK cells**, NK1.1^{+}CD11b$^{+/-}$; neutrophils (**Nphs**), CD11b^{hi}Ly6G^{hi}Ly6C^{int}SSC^{int}NK1.1^{-}; Ly6C^{hi} monocytes (**Ly6C^{hi} MOs**), CD11b^{hi}Ly6C^{hi}CD11c^{-}SSC^{-}Ly6G^{-}NK1.1^{-}; Ly6C^{hi} dendritic cells (**Ly6C^{hi} DCs**), CD11b^{hi}Ly6C^{hi}CD11c^{hi} SSC^{-}Ly6G^{-}NK1.1^{-}; **Ly6C^{lo} MOs**, CD11b^{int}CD11c^{-}Ly6C^{lo}SSC^{-}Ly6G^{-}NK1.1^{-}; plasmacytoid DCs (pDCs), SiglecH^{+}CD11b^{-}CD11c^{lo}B220^{+}Ly6G^{-}NK1.1^{-}; **CD8^{+} cDCs**, CD11c^{hi}CD8^{+}B220^{-}Ly6G^{-}NK1.1^{-}; **CD8^{-} cDCs**, CD11c^{hi}CD8^{-}B220^{-}Ly6G^{-}NK1.1^{-}. **B**, For the CD11b^{+}B220^{-}CD19^{-} gate, BM cells are shown from mice treated or not for 24 h with Poly(I:C). BM NK cells (NK1.1^{+}SiglecH^{-}) and pDCs (NK1.1^{-}SiglecH^{+}) were defined and then gated out. In the NK1.1^{-}SiglecH^{-} gate CD11b^{+} gated myeloid populations were defined as follows: neutrophils (**Nphs**), CD11b^{+}Ly6G^{hi}Ly6C^{int}SSC^{int}; **CD11b^{+} B cells**, B220^{+}CD19^{+}CD11b^{+}Ly6G^{-}SSC^{-}. In the **CD11b^{+}**B220^{-}CD19^{-}Ly6G^{-}SSC^{-} gate we identified 5 populations of myeloid cells and precursors defined as follows (See also **Table S1**): **CD11b^{+} myeloid precursors**, Ly6C^{-}CD11c^{-}CD115^{-}CX3CR1^{-}CCR2^{-}MHCII^{-}; **Nph precursors**, Ly6C^{int}CD11c^{-}CD115^{-}CX3CR1^{-}CCR2^{-}MHCII^{-}; **Ly6C^{hi} MOs**, CD11c^{-}CD115^{+}CX3CR1^{+}CCR2^{hi} MHCII^{-}; **preDCs**, CD11c^{+}Ly6C^{-}CD115^{+} CX3CR1^{hi} CCR2^{+} MHCII^{+}. After treatment with Poly(I:C) a BM Ly6C^{hi} DC/Mph precursor (CD11c^{+}CD115^{hi}CX3CR1^{hi}CCR2^{hi} MHCII^{+}) was substantially upregulated. In the NK1.1^{-}SiglecH^{-}CD11b^{-} gate, B cell precursors were defined as described in Methods. **C**, **D**, B cell populations from naïve WT mice, BAFF-RFP$^{+/-}$ mice and BAFF-RFP$^{+/+}$ mice. **C,** representative plots of B220^{+} CD43^{-} (PreB NFB MatB) cells include preB cells, NF B cells, and mature B cells (*left panels*), and CD11b^{+} B cells (*right panels*). **D** graphs show means ± SEM of cell numbers. Data are from one of two independent experiments performed with three mice per group. Statistics were performed by one-way ANOVA corrected with Holm-Sidak method for multiple comparisons; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplemental Fig. S3



Supplemental Figure S3. BAFF-RFP in B cell and and T cell subsets, BAFF^{+} CD11b^{hi} NK cell phenotypes and requirement for MAVS for BAFF up-regulation in BM myeloid and B cell subsets by Poly(I:C). A and **B**, BAFF-RFP expression detected by flow cytometry in splenic B cell subsets (**A**) and T cell subsets (**B**) from naïve BAFF-RFP$^{+/-}$ mice. RFP background control in WT (C57BL/6) mice is also shown. **A** and **B**, BAFF-RFP levels are shown as histograms on the left, BAFF-RFP$^{+/-}$ mice (*black empty histograms*), WT mice (*grey filled histograms*); and on the right as dot plots. Data show one representative of more than three experiments. For gating strategy of B cell subsets and T cell subsets see Methods. **C.** Phenotype of NK CD11b$^{int/-}$ cells and NK CD11b^{hi} cells upregulated in spleen after Poly(I:C) i.p. injection. NK cell subset phenotypes: CD11b$^{int/-}$ NK cells, NK1.1^{+}Ly6G^{-}CD49b^{+}NKp46^{lo}Ly-49C$^{+/-}$CD127^{-}; CD11b^{hi} NK cells, NK1.1^{+}Ly6G^{hi}CD49b^{+}NKp46^{hi}Ly-49C^{hi}CD127^{-}. **D** and **E**, BAFF-RFP mice and BAFF-RFP *Ifnar*$^{-/-}$ mice (**D**) and BAFF-RFP *Mavs*$^{-/-}$ mice (**E**) were left untreated or treated i.p. with 200μg/mouse Poly(I:C) for 24 hrs. **D** and **E**, bar graphs show means ± SEM of % of BAFF-RFP^{+} BM cells and summarize data from two independent experiments (N=6-7). Dotted lines show RFP^{+} background in WT and *Ifnar*$^{-/-}$ (**D**) or *Mavs*$^{-/-}$ (**E**) mice that were included as controls in each experiment. Statistics were performed by one-way ANOVA corrected with Holm-Sidak method for multiple comparisons; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplemental Table S1. Phenotype of myeloid precursors in BM

Cell Phenotype	Cell Name	Cell marker					
		CD115	CX3CR1	CCR2	Ly6G	MHCII	BAFF-RFP
$Ly6C^{-}$ $CD11c^{-}$	CD11b+ myeloid precursor	-	-	-	-	-	++
$Ly6C^{int}$ $CD11c^{-}$	Nph prec	-	-	-	+	-	++
$Ly6C^{int}$ $CD11c^{-}$	Nph	-	-	-	+++	-	+++
$Ly6C^{hi}$ $CD11c^{-}$	$Ly6C^{hi}$ MO	++	++	++++	-	-	+/-
$Ly6C^{hi}$ $CD11c^{+}$	$Ly6C^{hi}$ DC/Mph	+++	+++	+++	+	+	++
$Ly6C^{-}$ $CD11c^{+}$	preDC	++	+++	+	-	+	+

+ and – indicates MFI levels of cell markers