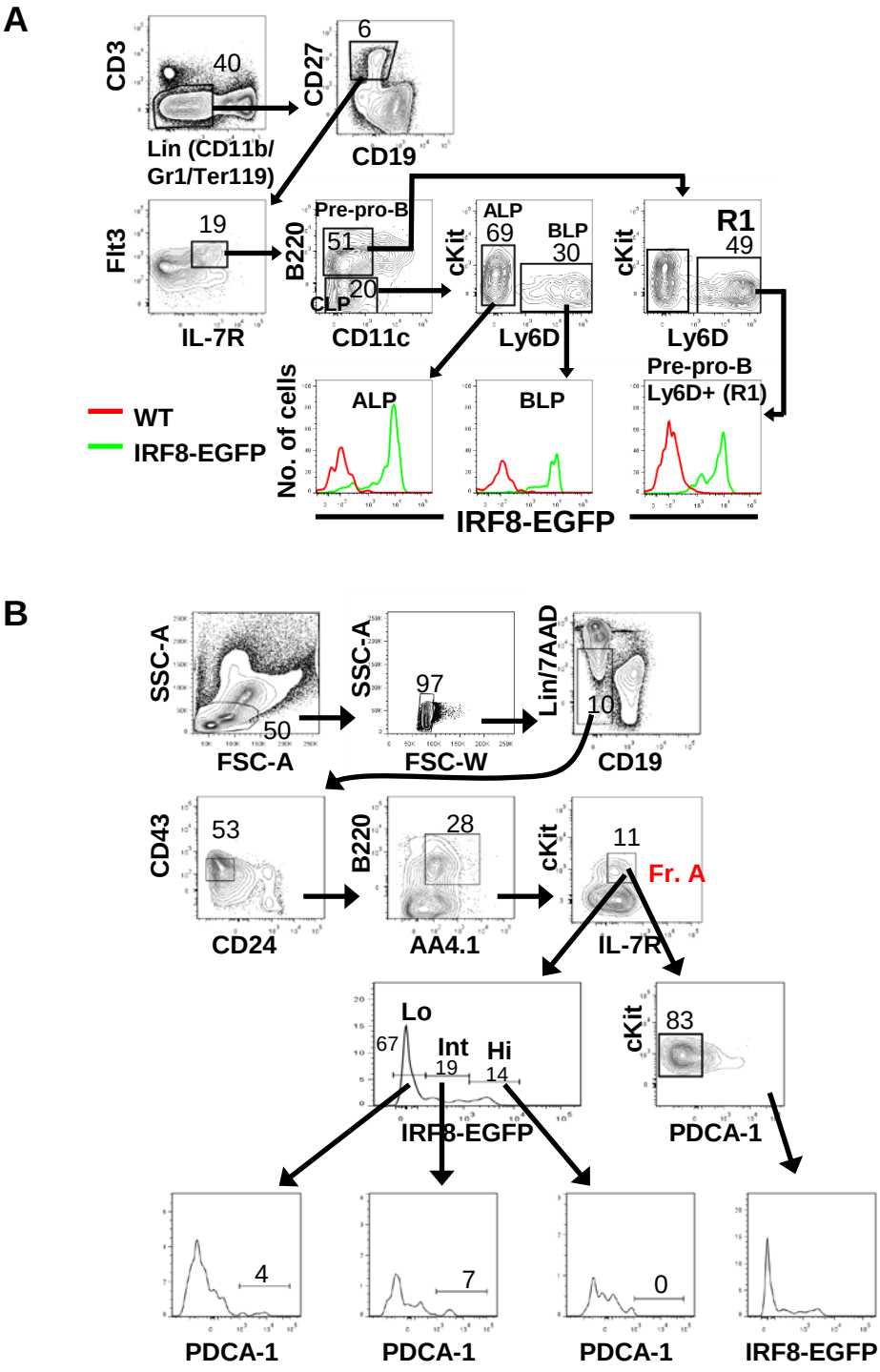
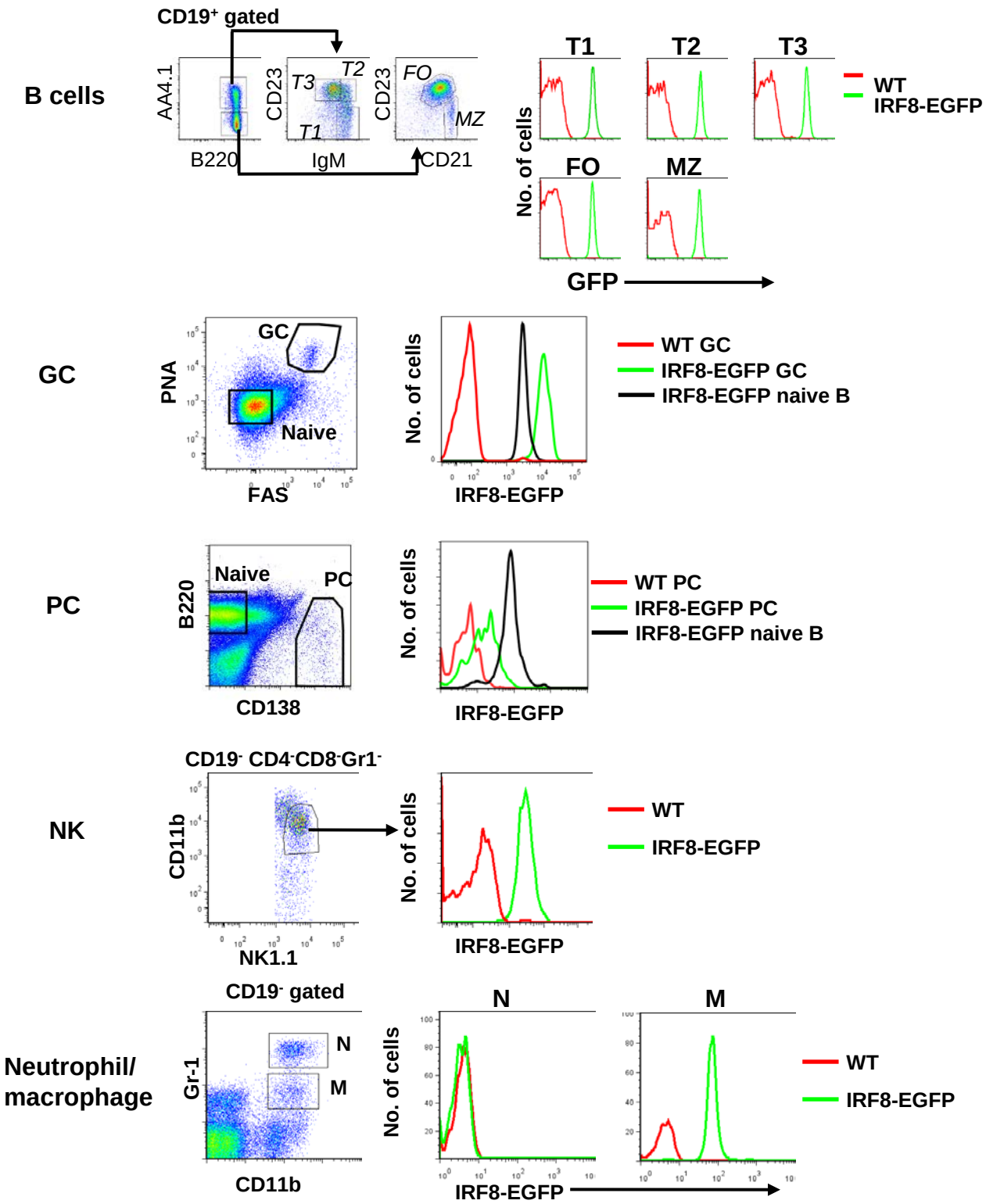
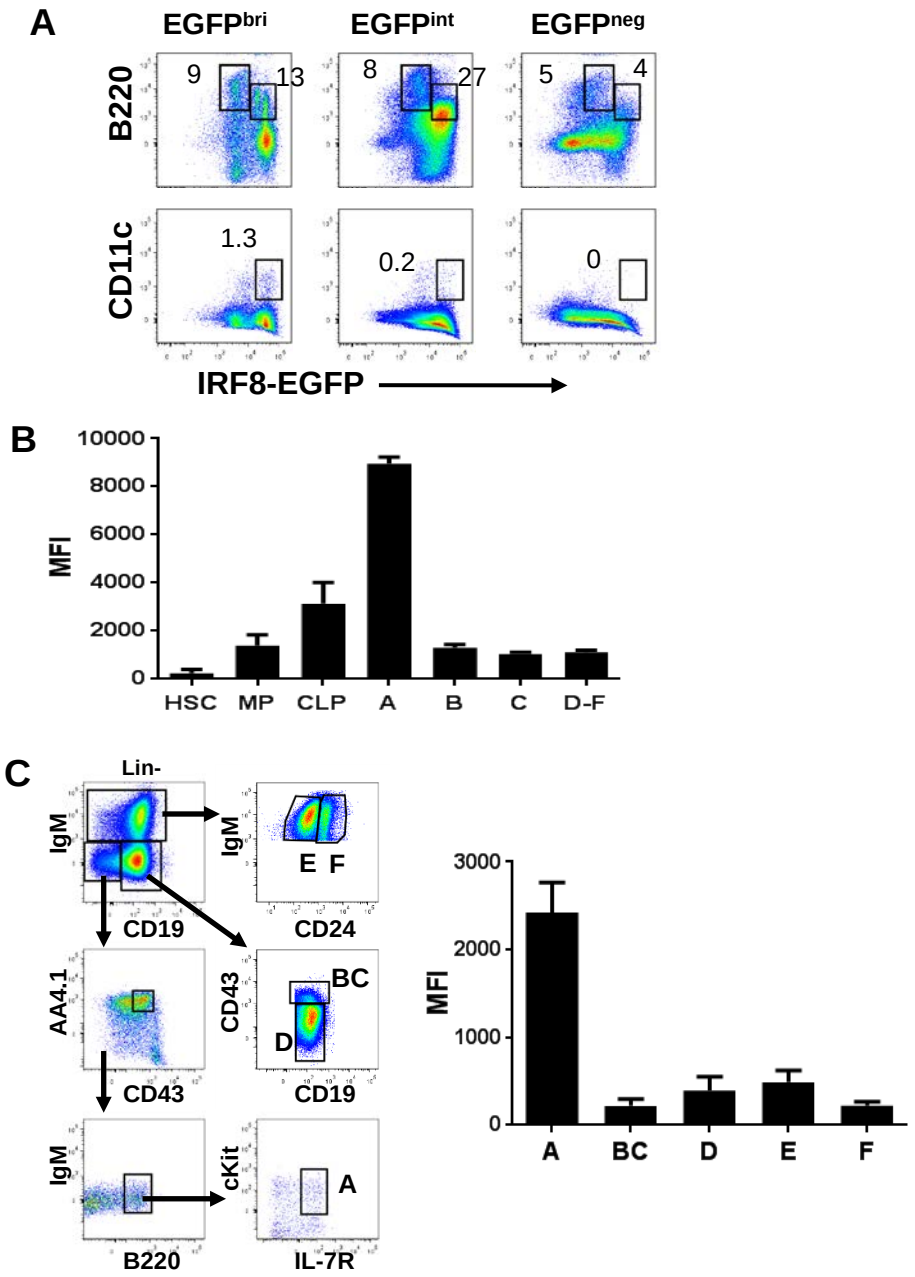


Supplemental Fig. 1. Analysis of early B cell populations using the Weissman nomenclature (A) or the modified Hardy nomenclature (B). The BM cells were stained and analyzed by FACS. The numbers are percentages of cells falling in each gate. The lineage panel (Lin) contains anti-CD3, CD11b, Gr-1, Ly6C, DX5, Ter119 Abs. The overlays are expression of IRF8-EGFP over WT controls. Data are representative of three independent experiments.



Supplemental Fig. 2. IRF8-EGFP expression in splenic B cell subsets, GCs, PCs, NK and neutrophils/macrophages. Splenocytes of WT and IRF8-EGFP mice were stained and analyzed by flow cytometry. The dot plot showed gating schemes used to define specific populations. The overlays (right panels) are expression of IRF8-EGFP over WT controls. Data are representative of more than five independent experiments. Note that GC and PC analysis was done with mice immunized with NP-KLH/alum for 8 days.



Supplemental Fig. 3. Evaluation of IRF8-EGFP (A and B) or IRF8 (C) expression in early B cells. (A) IRF8-EGFPl$^{o/-}$ pre-pro-B cells become IRF8-EGFP^{+} cells in vitro. BM pre-pro-B cells were sort-purified for EGFP$^{lo/-}$, EGFP^{int} and EGFP^{bri} as shown in Fig. 5A and were cultured with IL-7, Flt3L, SCF, and IL-15 for 5 days. The cells were then analyzed by flow cytometry. The numbers are percentages of cells falling in each gate. Data are representative of three independent experiments with similar results. (B) BM B cell subsets were defined as depicted in Fig. 2 and Fig. 4. The HSC included both LT- and ST-HSCs. The MP included GMPs, CMPs and MEPs. MFI are absolute values excluding background values obtained from a wild-type control mouse. (C) FACS analysis of intracellular IRF8 protein expression in B6 BM B cells. BM cells were pre-purified with biotin-labeled antibodies against Gr-1, CD11b, Ter119, CD3, Ly6C and Dyna beads. The cells were then stained with antibodies as indicated, followed by fixation, permeabilization and intracellular staining with an anti-IRF8 Ab (C-19x, Santa Cruz) and an anti-goat secondary antibody. Left panel, gating schemes used for defining each B cell subsets. Right panel, MFI are absolute values excluding background which was determined from an IRF8$^{-/-}$ sample. Error bars are of 3 mice. Data represent four independent experiments.

Supplemental Table 1. Antibodies for FACS analysis

Antibodies	Clone	Fluorochrome	Source
CD3	145-2C11	PE.Cy7, biotin	Biolegend
CD4	GK1.5	FITC, PE, Pacific blue (PB), Percp.Cy5.5	Biolegend
CD5	53-7.3	PE	eBioscience
CD8	53-6.7	FITC, PE	BD Biosciences
CD11b	M1/70	Alexa Fluor 700, 647	Biolegend
CD11c	HL3	PE, PE.Cy7	BD Biosciences
CD19	ID3	FITC, PE, APC, Percp.Cy5.5	BD Biosciences
CD21	4E3	FITC, PE	eBioscience
CD23	B3B4	PE, biotin	Biolegend
CD24	M1/69	FITC, PE	BD Biosciences
CD25	PC61.5	PE	eBioscience
CD27	LG.3A10	PE, APC	BD Biosciences, Biolegend
CD16/CD32	2.4G2	PE	BD Biosciences
CD34	RAM34	PB	eBioscience
B220	RA3-6B2	FITC, PE, APC, PB, APC.Cy7, Alexa Fluor 700, biotin, Qdot655	BD Biosciences or invitrogen
CD43	S7	PE	BD Biosciences
CD44	IM7	FITC, Alexa Fluor 700	BD Biosciences
CD71	R17217	PE	eBioscience
CD115	AFS98	APC	Biolegend
CD117 (cKit)	2B8	APC, APC.Cy7	BD Biosciences
CD138	281-2	PE	Biolegend
Gr-1	RB6-8C5	PE.Cy7, PB, APC.Cy7, biotin	BD Biosciences
MHC II	AF6-120.1	Biotin	BD Biosciences
Siglec H	551	PB	Biolegend
pDCA	eBio927	FITC, Percp-eFluor 710	eBioscience
Ter119	Ter-119	Biotin	BD Biosciences
IL-7R	A7R34	PE	eBioscience
Ly6D	49-H4	APC	BD Biosciences (self-labeled)
CD135(Flt3)	A2F10	PE.Cy5, BV421	eBioscience, Biolegend
DX5	DX5	PE.Cy7	eBioscience
GL7	GL-7	FITC	BD Biosciences
PNA	-	Biotin	Sigma-Aldrich
Sca-1	E13-161.7 or D7	FITC, Alexa Fluor 700	BD Biosciences or eBioscience
AA4.1	AA4.1	PE	BD Biosciences
BP-1	6C3	Biotin	BD Biosciences
IgM	II/41	APC	BD Biosciences
NK1.1	PK136	Percp.Cy5.5, biotin	BD Biosciences
FAS (CD95)	Jo2	PE	BD Biosciences
CD4	RM4-5	Percp.Cy5.5	eBioscience
Ly6C	AL-21	Biotin	BD Biosciences
CD40	3/23	biotin	BD Biosciences
CD86	GL1	PE	BD Biosciences
CD150	TC15-12F12.2	APC	Biolegend