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Supplemental information

EAF2 deficiency attenuates autoimmune disease in *Fas*^{lpr} mice by modulating B cell activation and apoptosis

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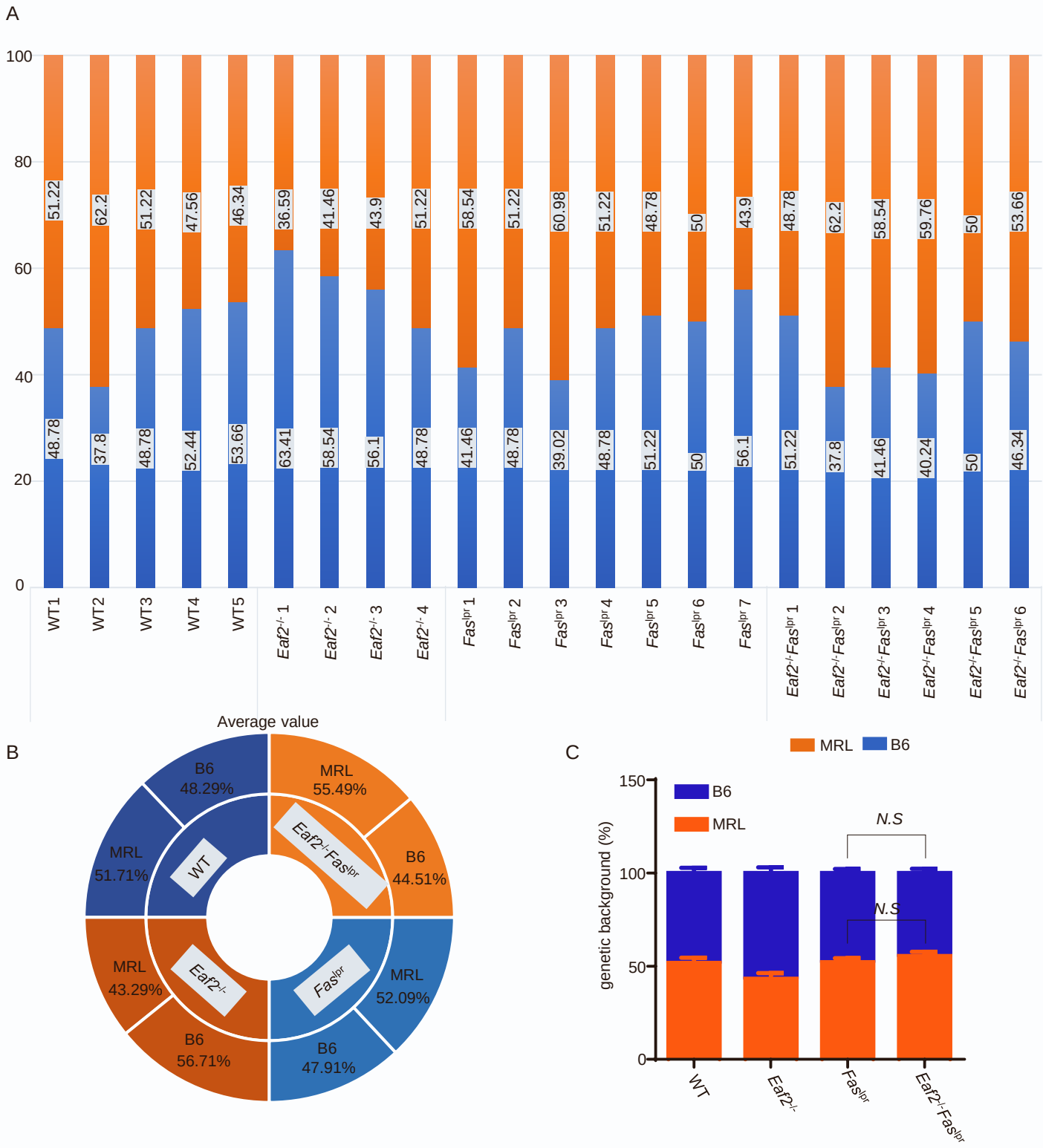
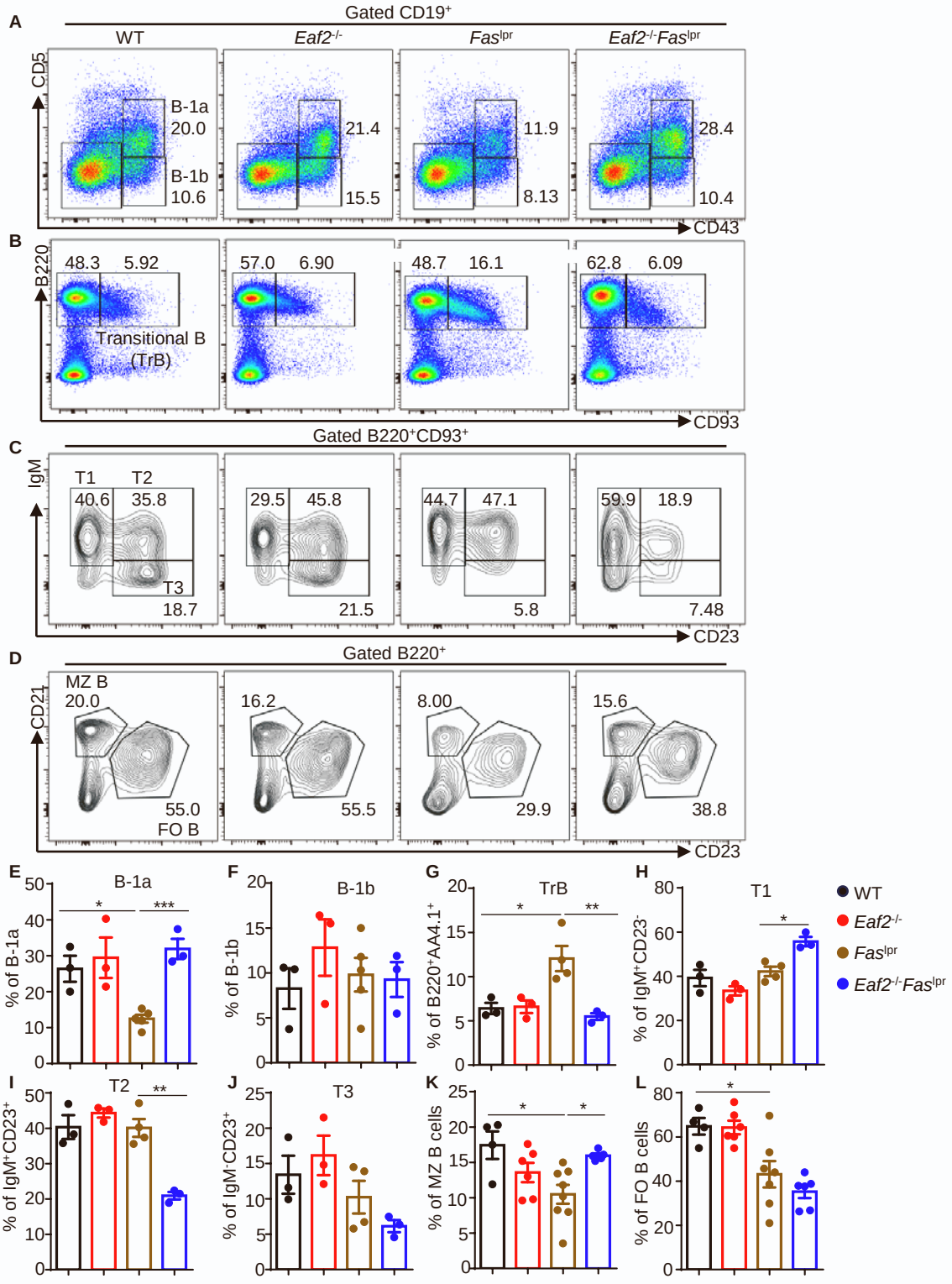
Supplemental Figure 1



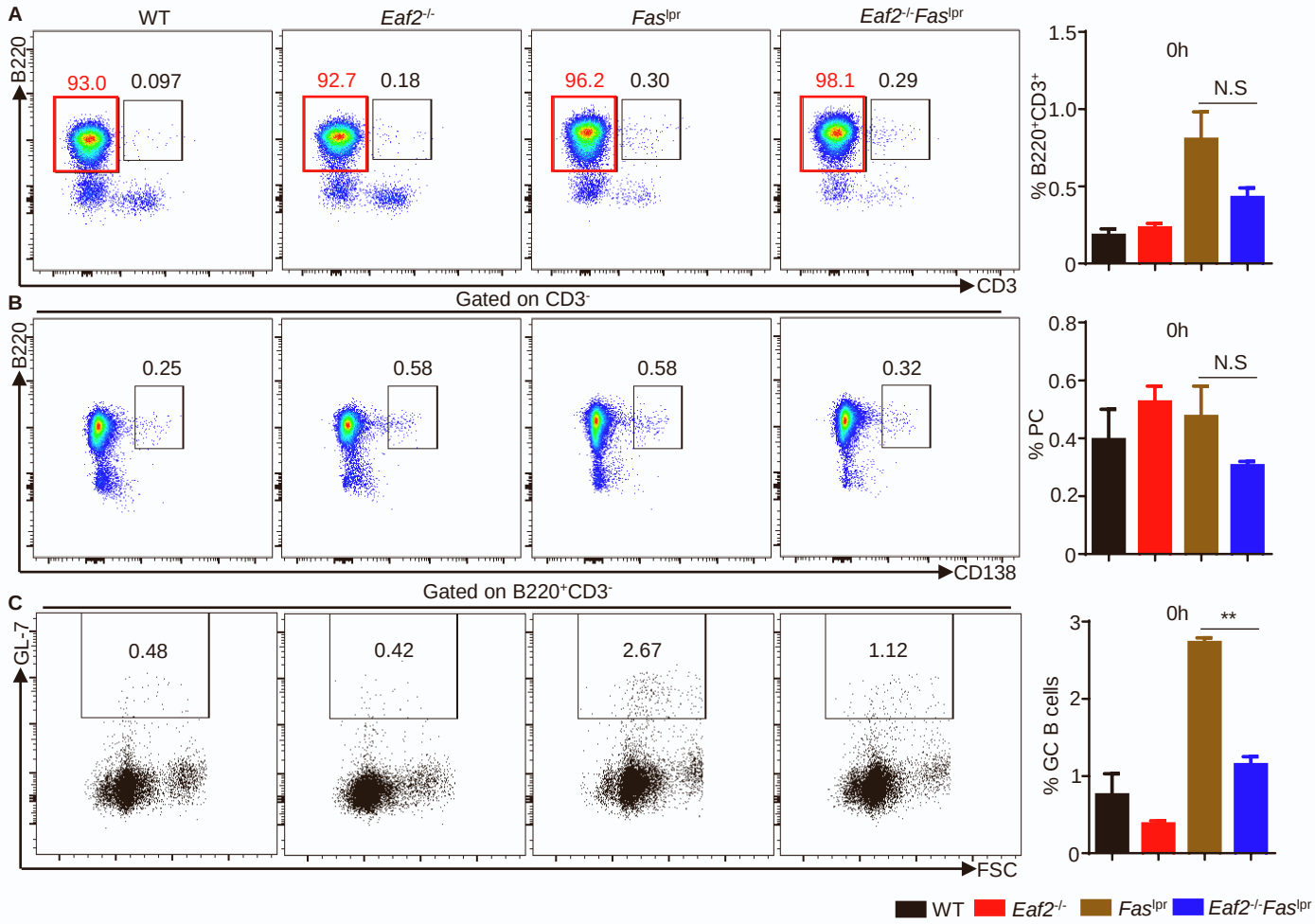
Supplemental Figure 1. Genetic backgrounds of WT, *Eaf2*$^{-/-}$, *Fas*^{lpr}, *Eaf2*$^{-/-}$*Fas*^{lpr} female mice. Related to Figure 1. The backgrounds were determined using genomic single nucleotide polymorphism (SNP) testing (n ≥ 3). (A) Percentage of B6 and MRL genetic backgrounds in each mouse. (B) Average genetic background values for each genotype. (C) Statistical analysis of B6 and MRL genetic backgrounds across groups.

Supplemental Figure 2



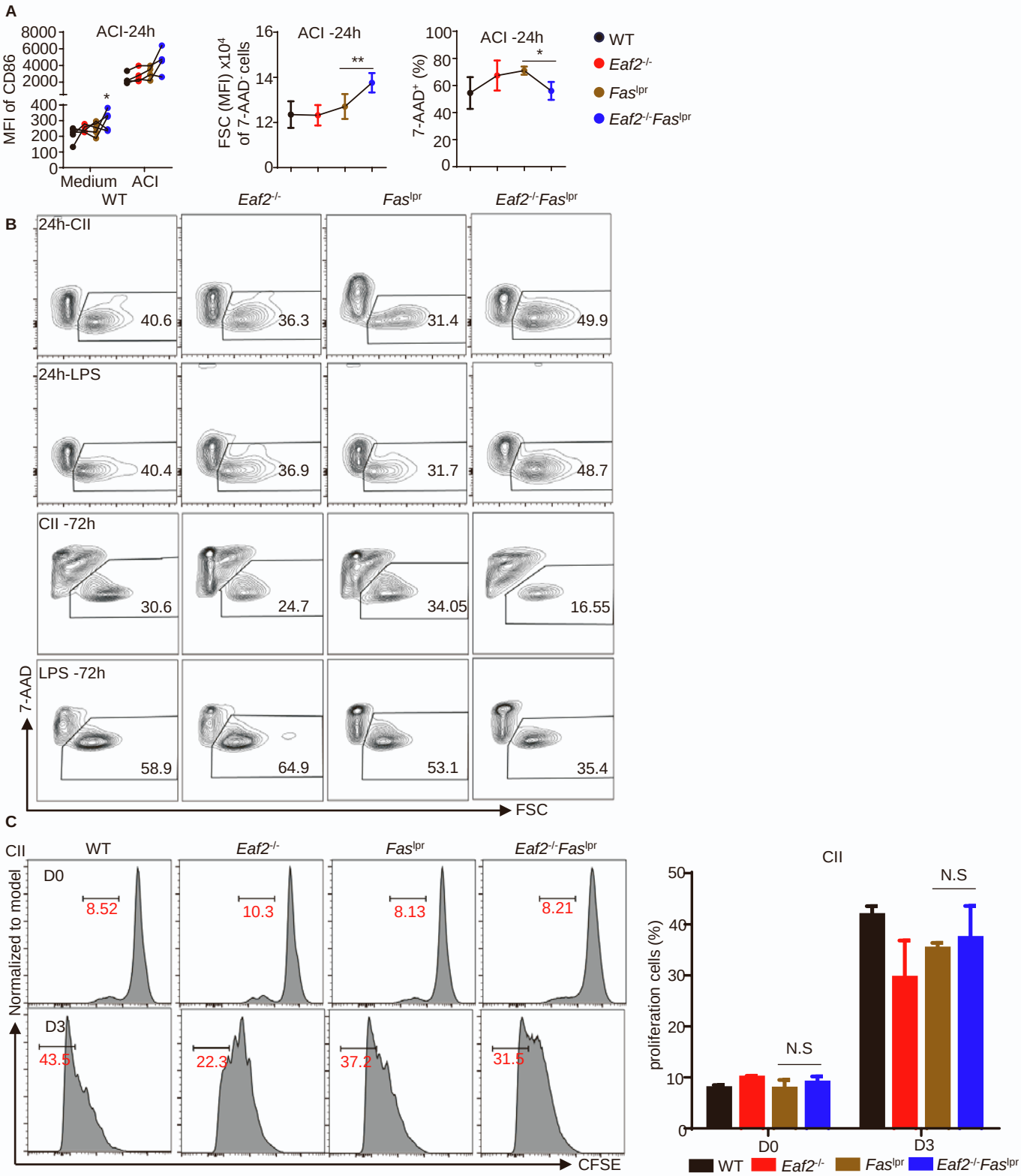
Supplemental Figure 2. Impact of EAF2 deficiency on B cell development and maturation in *Fas*^{lpr} mice. Related to Figure 3. Spleen cells from WT, *Eaf2*$^{-/-}$, *Fas*^{lpr}, *Eaf2*$^{-/-}$*Fas*^{lpr} female mice (aged 7-12 weeks, n ≥ 3) were stained and analyzed by flow cytometry for various B cell subsets. Representative flow cytometry plots show (A) B-1a ($CD19^+CD43^+CD5^+$), B-1b ($CD19^+CD43^+CD5^-$) and B-2 ($CD19^+CD43^-CD5^-$) cells; (B) Total transitional B ($CD19^+B220^+CD93^+$) cells; (C) T1 ($B220^+CD93^+IgM^+CD23^-$), T2 ($B220^+CD93^+IgM^+CD23^+$), and T3 ($B220^+CD93^+IgM^-CD23^+$) subsets; (D) MZ B ($B220^+CD21^{hi}CD23^{lo}$) and FO B ($B220^+CD21^+CD23^+$) cells. (E-L) Mean ± SEM of various B cell subsets from at least three independent experiments. Statistical significance was assessed by one-way ANOVA. *p < 0.05; **p < 0.01; *** p < 0.001.

Supplemental Figure 3



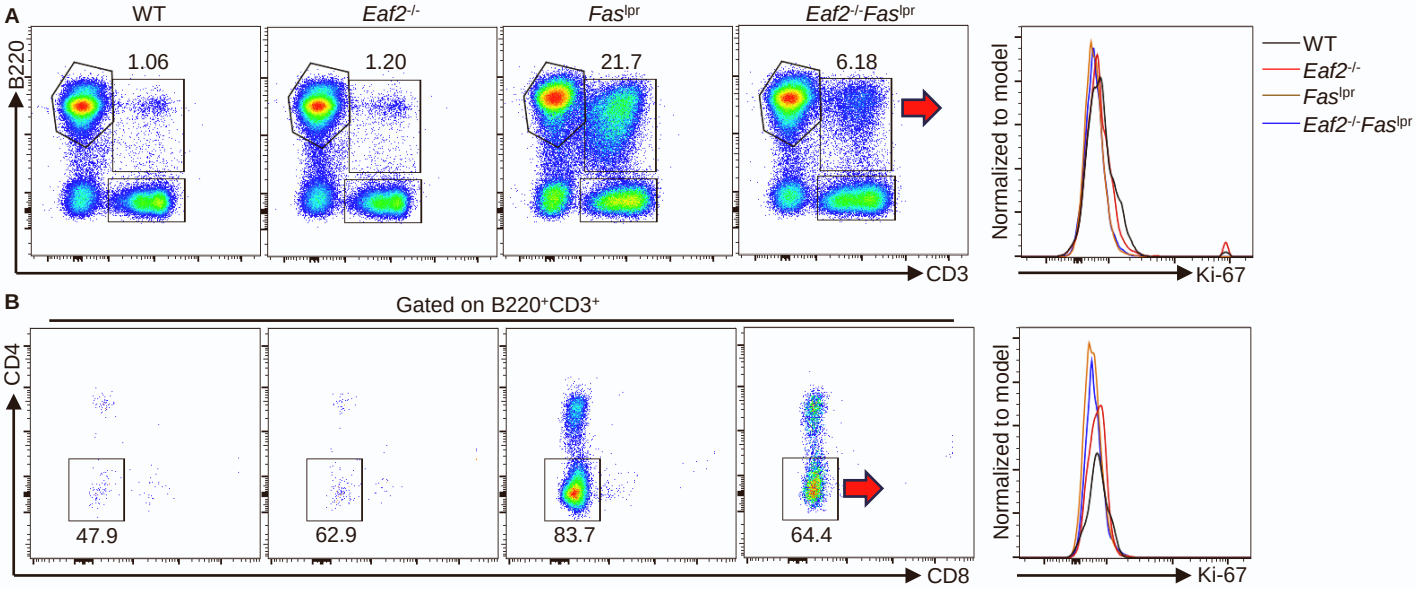
Supplemental Figure 3. Phenotype of purified B cells. Related to Figure 4. B cells from WT, *Eaf2*$^{-/-}$, *Fas*^{lpr}, *Eaf2*$^{-/-}$*Fas*^{lpr} female mice (7-12 weeks of age, n ≥ 3) were analyzed by flow cytometry for their (A) B220 and CD3, (B) B220 and CD138, and (C) FSC and GL-7 expression.

Supplemental Figure 4

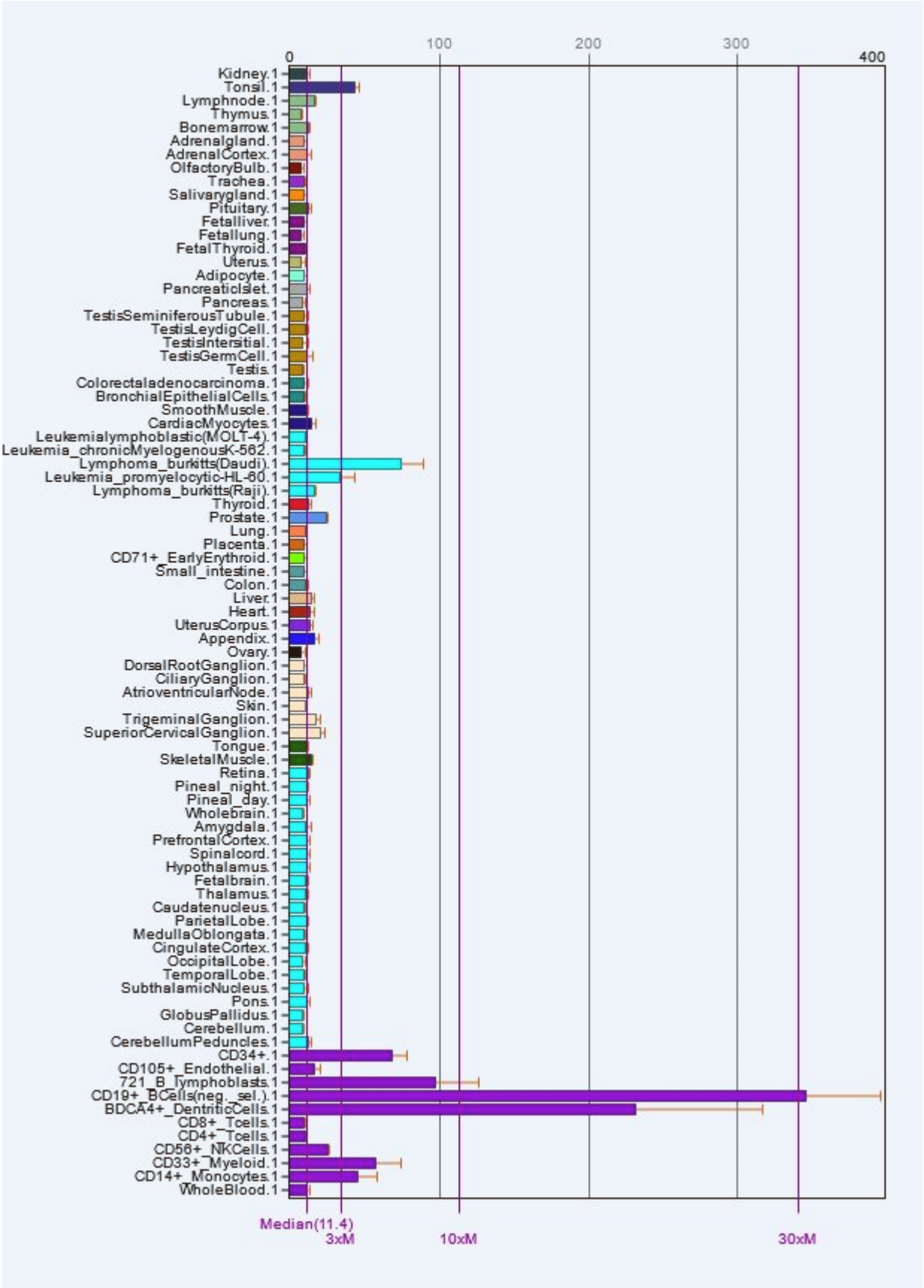


Supplemental Figure 4. B cell activation, death and proliferation analysis. Related to Figure 4. (A) B cells from WT, *Eaf2*$^{-/-}$, *Fas*^{lpr}, *Eaf2*$^{-/-}$*Fas*^{lpr} female mice (7-12 weeks of age, n ≥ 3) were stimulated with ACI for 24 h and analyzed for CD86 expression, FSC, and cell viability. (B) Representative flow cytometry of 7-AAD staining after CD40L+IL4+IL21 and LPS stimulation for 24 h and 72 h. (C) Purified spleen B cells were labeled with CFSE and stimulated with CII for 3 days. CFSE dilution was analyzed by flow cytometry. Left, representative flow cytometry profiles; right, summary of three independent experiments.

Supplemental Figure 5



Supplemental Figure 5. Ki-67 expression in the abnormal B220$^+$CD3$^+$ T and B220$^+$CD3$^+$CD4$^-$CD8$^-$ (DN) T cells. Related to Figure 5. Spleen cells from WT, *Eaf2*$^{-/-}$, *Fas*^{lpr}, *Eaf2*$^{-/-}$*Fas*^{lpr} female mice (7-12 weeks of age, n ≥ 3) were stained with anti-Ki-67 and analyzed for Ki-67 expression in gated B220$^+$CD3$^+$ T (upper panels) and B220$^+$CD3$^+$CD4$^-$CD8$^-$ (DN) T cells (lower panels). Left, gating strategy; right, overlay of Ki-67 expression.



Supplemental Figure 6. Expression of human *EAF2* in B cells and Burkitt's lymphomas. Related to Figure 6. Data sourced from BioGPS (EAF2 (ELL associated factor 2) | Gene Report | BioGPS).