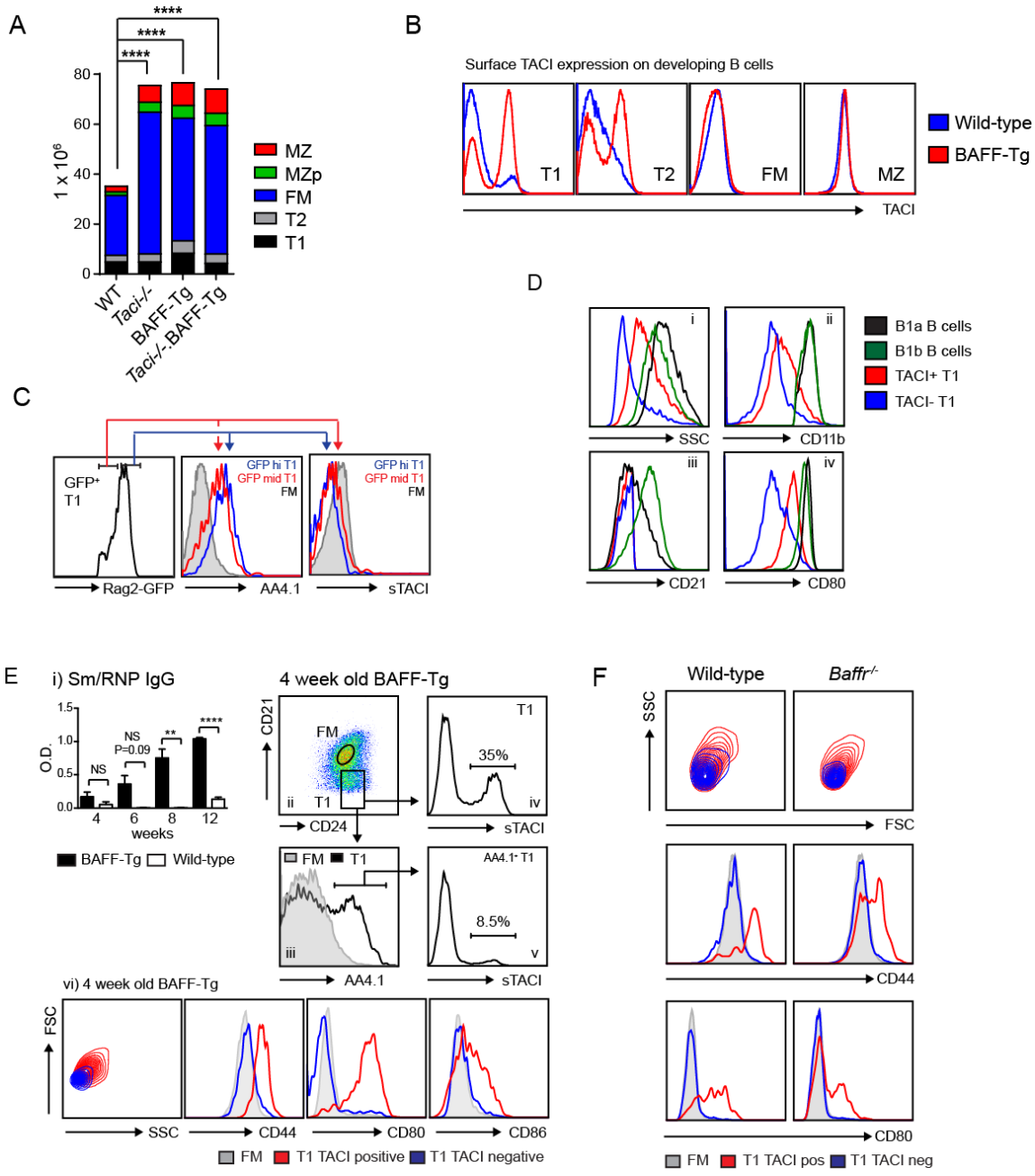
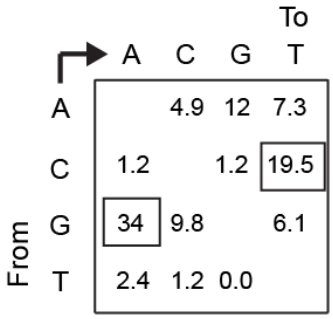
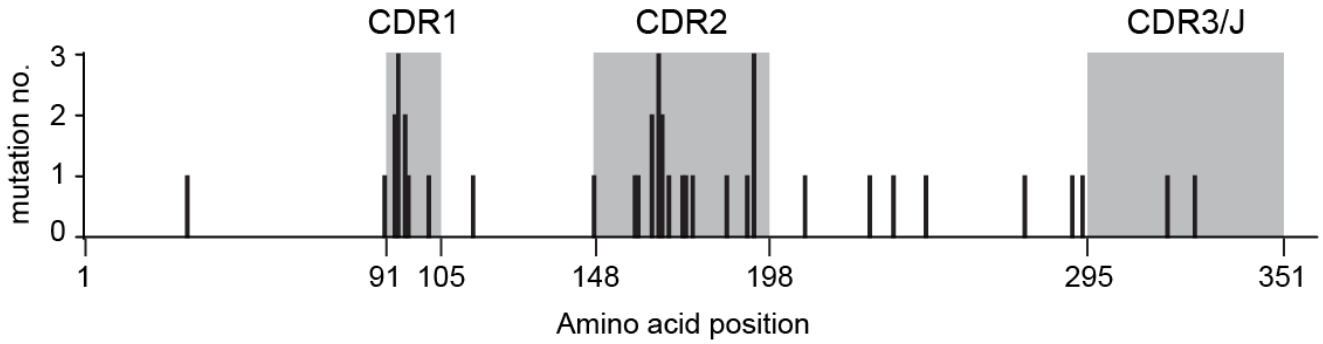


Supplemental Figure 1: The TACI$^+$ transitional subset in BAFF-Tg mice are derived from transitional B cells
(A) Total splenic B cells and B cell developmental subsets in WT, *Taci*$^{-/-}$, BAFF-Tg and *Taci*$^{-/-}$.BAFF-Tg mice. ****, P<0.0001, by one-way ANOVA, followed by Tukey's multiple comparison test. (B) sTACI on splenic B cell subsets from wild-type (blue) and BAFF-Tg (red) mice. (C) AA4.1 is downregulated on a subset of recent BM emigrant T1 B cells. GFP$^+$ T1 B cells from Rag2-GFP$^+$ mouse were subdivided into GFP^{hi} and GFP^{mid} subsets (such that GFP^{mid} T1 B cells have undergone ~50% GFP dilution). Left panel: Rag2-GFP expression (gated on GFP$^+$ T1 B cells) showing GFP^{mid} and GFP^{hi} gating strategy. Overlaid histograms showing AA4.1 (middle panel) and sTACI (right panel) expression in Rag2-GFP^{mid} T1 (red), Rag2-GFP^{hi} T1 (blue) and FM (grey) B cells. Notably, AA4.1 staining was lower and sTACI expression higher on GFP^{mid} T1 relative to GFP^{hi} T1 B cells, consistent with reciprocal regulation of AA4.1 and sTACI within the transitional compartment. (D) TACI$^+$ T1 B cells from BAFF-Tg mice are not peritoneal B1 B cells. (i-iv) Overlaid histograms showing surface phenotype of BAFF-Tg TACI$^+$ (red) and TACI$^-$ (blue) T1 B cells compared with BAFF-Tg peritoneal B1a (black) and B1b (green) B cells. (i) While TACI$^+$ T1 are larger than TACI$^-$ T1, the TACI$^+$ subset is smaller than both B1a and B1b B cells. (ii) T1 B cells lack surface expression of CD11b, a marker of peritoneal B1 B cells (1). (iii) CD21 expression is higher on B1b cells (hypothesized to contribute to BAFF-Tg autoimmunity (2)) relative to BAFF-Tg TACI$^+$ and TACI$^-$ T1 B cells. (iv) Although CD80 is upregulated on activated TACI$^+$ T1 B cells from BAFF-Tg mice, surface CD80 expression is significantly higher on peritoneal B1 B cells. (i-iv) Peritoneal B1 cells were gated as CD19^{hi} IgM^{hi} CD11b(Mac-1)$^+$ and further subdivided as CD5^{pos} B1a and CD5^{neg} B1b. (E) BAFF-Tg TACI$^+$ T1 B cells develop prior to systemic autoimmunity. (i) Serum anti-Sm/RNP IgG autoantibodies in WT (white) and BAFF-Tg (black) mice at indicated ages. Error bars, SEM; **, P<0.01; ****, P<0.0001, by two-tailed Student's t-test. (ii) T1 and FM gates in 4-week-old BAFF-Tg mouse. (iii) AA4.1 expression on BAFF-Tg FM and T1 B cells; gate indicates AA4.1$^+$ cells. (iv, v) Representative histograms of sTACI expression on BAFF-Tg CD21^{lo}CD24^{hi} T1 B cells (iv); as well as AA4.1$^+$ T1 B cells (v). Number indicates % within gate. Although AA4.1 expression was decreased on T1 B cells from young BAFF-Tg mice, we still noted a distinct subpopulation of TACI$^+$ B cells when gated on AA4.1$^+$ T1 B cells (in keeping with a transitional origin for this novel subset). (vi) Cell size (by forward (FSC) and side (SSC) scatter); and surface activation markers in BAFF-Tg FM (grey), TACI$^-$ (blue) and TACI$^+$ (red) T1 B cells demonstrating identical surface phenotype of TACI$^+$ T1 B cells in BAFF-Tg mice prior to the onset of autoimmunity. (F) TACI$^+$ T1 B cells from WT and *Baffr*$^{-/-}$ mice exhibit activated surface phenotype. Cell size (by forward (FSC) and side (SSC) scatter; upper); and representative histograms of CD44 (middle), CD80 (lower) in WT (left) and *Baffr*$^{-/-}$ (right) TACI$^+$ T1 (red), *Baffr*$^{-/-}$ TACI$^-$ T1 (blue) and WT FM (grey) B cells.

A Nucleotide substitution pattern in mutated GFP^{lo} clones



B Location of heavy chain mutations



Supplemental Figure 2: Immunoglobulin mutations in cycling BAFF-Tg transitional cells exhibit characteristics of AID-mediated somatic hypermutation (SHM). (A) Summary of nucleotide substitution patterns in mutated sequences from cloned GFP$^-$ BCRs sorted from Rag2-GFP.BAFF-Tg mice (numbers indicate percentage of each specific type of substitution among cloned BCRs exhibiting evidence for SHM), demonstrating bias for G to A and C to T transitions (3). (B) Location of heavy chain mutations in cloned GFP$^-$ BCRs from sorted T1/T2 Rag2-GFP.BAFF-Tg transitional B cells. Mutations are targeted to complementary determining regions (CDR1-2), consistent with AID-dependent somatic hypermutation (4). CDRs shaded in grey.

Supplemental References:

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