Supplemental material

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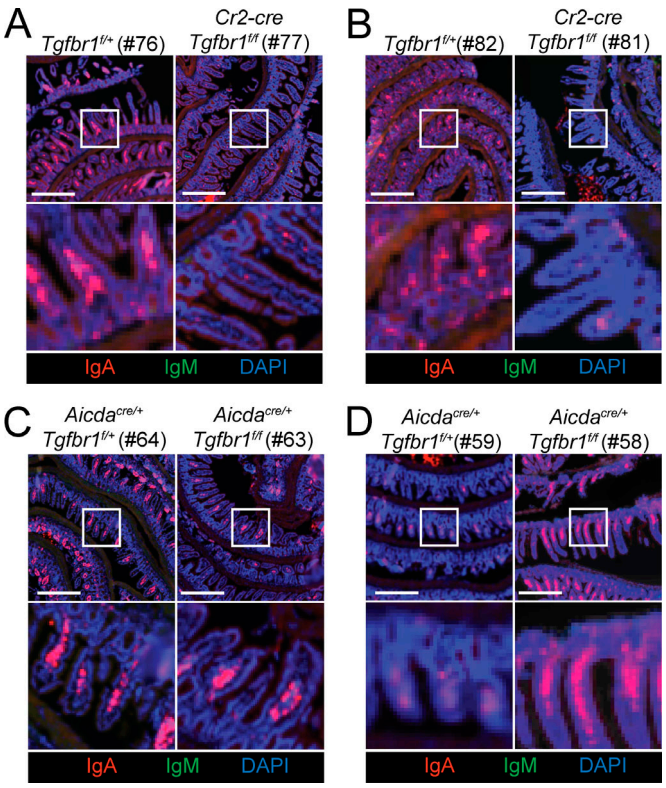

Figure S1. **Induction of IgA⁺ plasma cells in small intestine lamina propria is not dependent on TGFβ signaling PP GCs. (A–D)** Additional examples of immunofluorescence of sections of small intestine from *Tgfbr1*$^{f/+}$ or *Cr2-cre Tgfbr1*$^{f/f}$ animals (A and B) or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/+}$ or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/f}$ animals (C and D) stained as in Fig. 2. Scale bars: 500 µm in low-power images and 125 µm in insets.

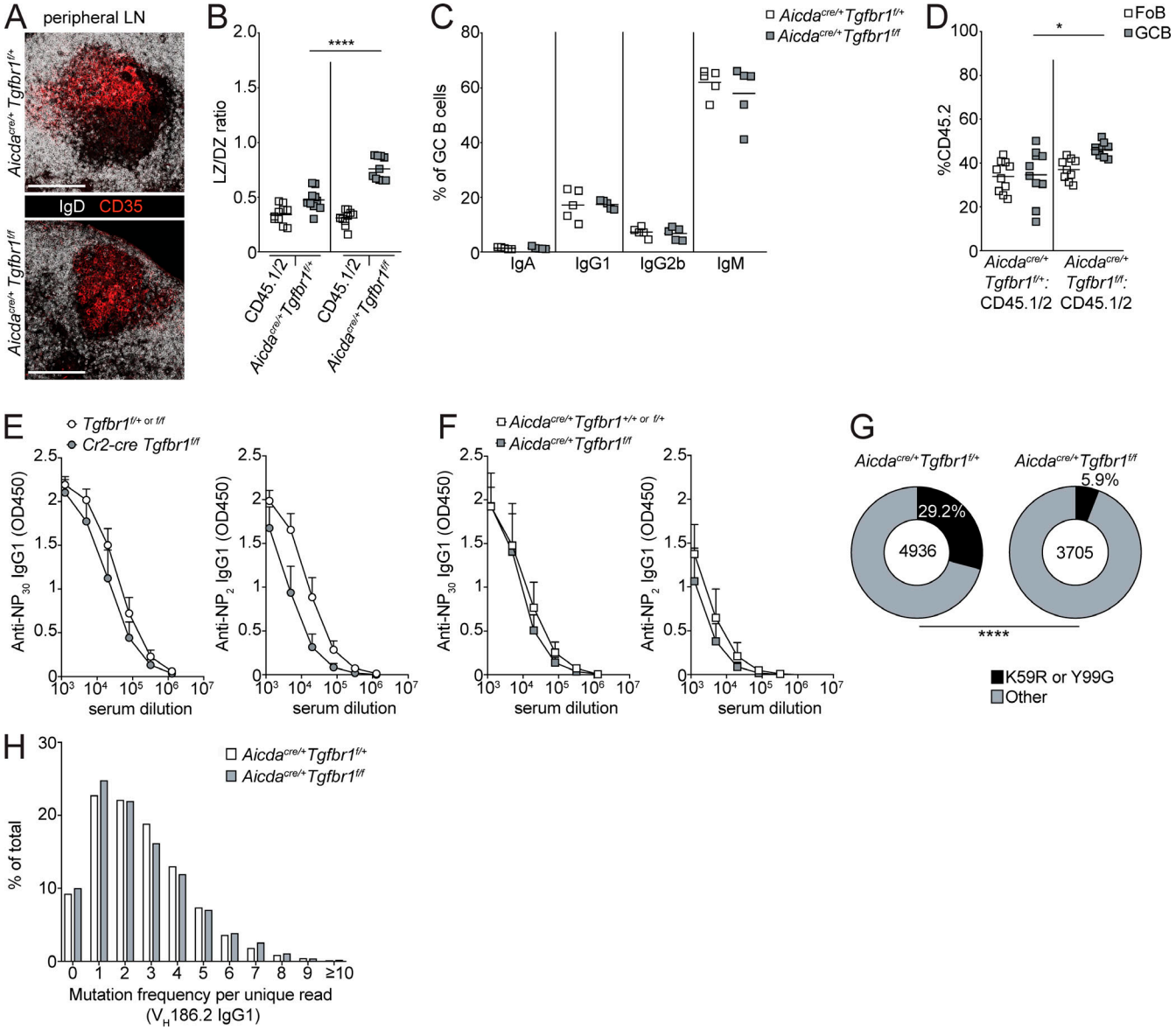

Figure S2. **TGFβ signaling promotes the LZ to DZ transition and antibody affinity maturation in nonmucosal GCs. (A)** Confocal microscopy of pLNs from NP-CGG–immunized *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/+}$ or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/f}$ animals stained for IgD (white) and CD35 (red). Scale bars: 150 µm. Data are representative of at least three GCs of each type from three independent experiments. **(B–D)** Ratio of LZ to DZ GCBs (D); percentages of CD45.2$^+$ GCBs staining positive for IgA, IgG1, IgG2b, or IgM (E); or frequency of FoBs or GCBs derived from CD45.2 bone marrow (F) in NP-CGG–immunized pLNs from mixed bone marrow chimeras generated with a mixture of 70% WT (CD45.1/2) and 30% CD45.2 bone marrow that was *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/+}$ or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/f}$ assessed by FACS. Data are from one (C) or two (B and D) independent experiments with five mice per group. **(E and F)** ELISA of total IgG1 binding to NP (NP_{30}) or high-affinity IgG1 binding to NP (NP_2) in serum of littermate control or *Cr2-cre Tgfbr1*$^{f/f}$ (E) or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/+}$ or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/f}$ (F) animals that had been immunized 14 d prior with NP-CGG in alum. Data are means + SD. Data in E are from one experiment representative of two with five mice per group. Data in F are from one experiment representative of three independent experiments with seven and eight mice per group, respectively. **(G and H)** Frequency of K59R or Y99G mutations (G) or overall mutation frequency (H) in unique V_H186.2 IgG1 sequences with two or more reads from heavy-chain repertoire sequencing of pLN GCBs from NP-CGG–immunized *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/+}$ or *Aicda*$^{cre/+}$ *Tgfbr1*$^{f/f}$ animals. Data are pooled from three mice per group from one experiment. *, P < 0.05; ****, P < 0.0001; unpaired two-tailed Student's *t* test for data in A–F. ****, P < 0.0001; χ^2 test for G.

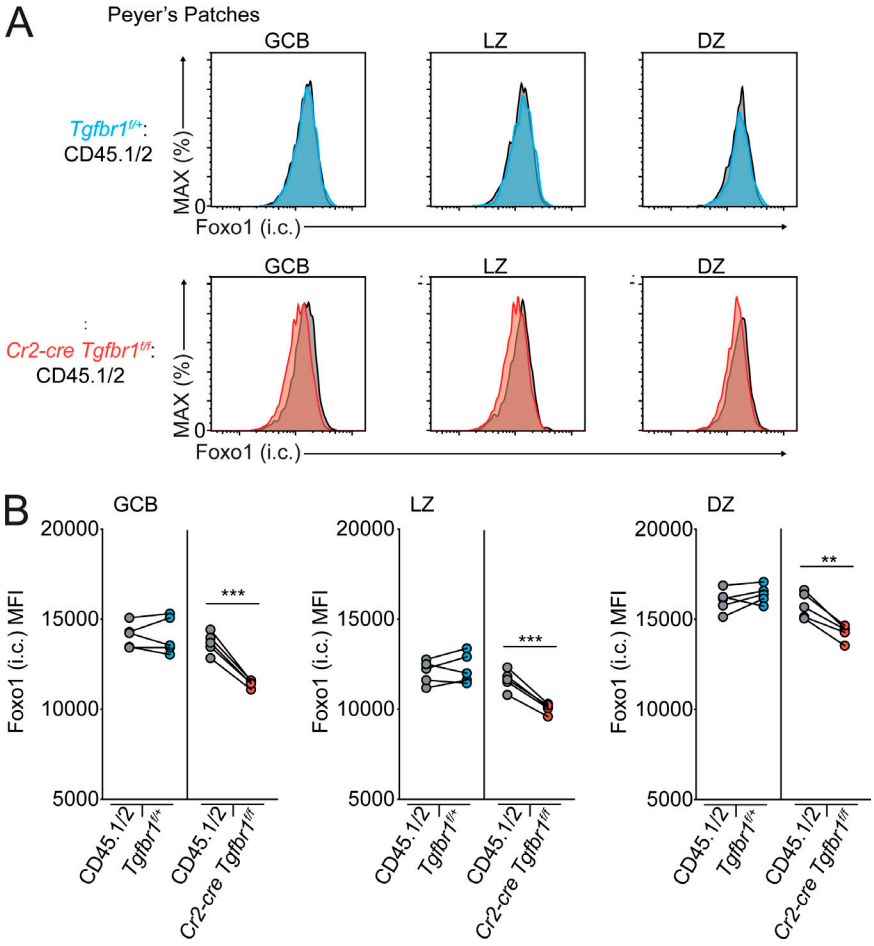

Figure S3. **TGFβ signaling promotes the transition from the LZ to DZ via Foxo1. (A and B)** Intracellular FACS of Foxo1 in total (left), LZ (middle), and DZ (right) GCBs from PPs of mixed chimeras generated with 30% CD45.2 bone marrow *Tgfbr1*$^{f/+}$ (blue) or *Cr2-cre Tgfbr1*$^{f/f}$ (red) and 70% WT CD45.1/2 (gray) bone marrow. Representative histograms are shown in A, and mean fluorescence intensity is shown in B. Data in B are from five mice of each type from one experiment representative of two independent experiments. **, $P < 0.01$; ***, $P < 0.001$; paired two-tailed Student's *t* test. MAX, maximum.