Supplemental material

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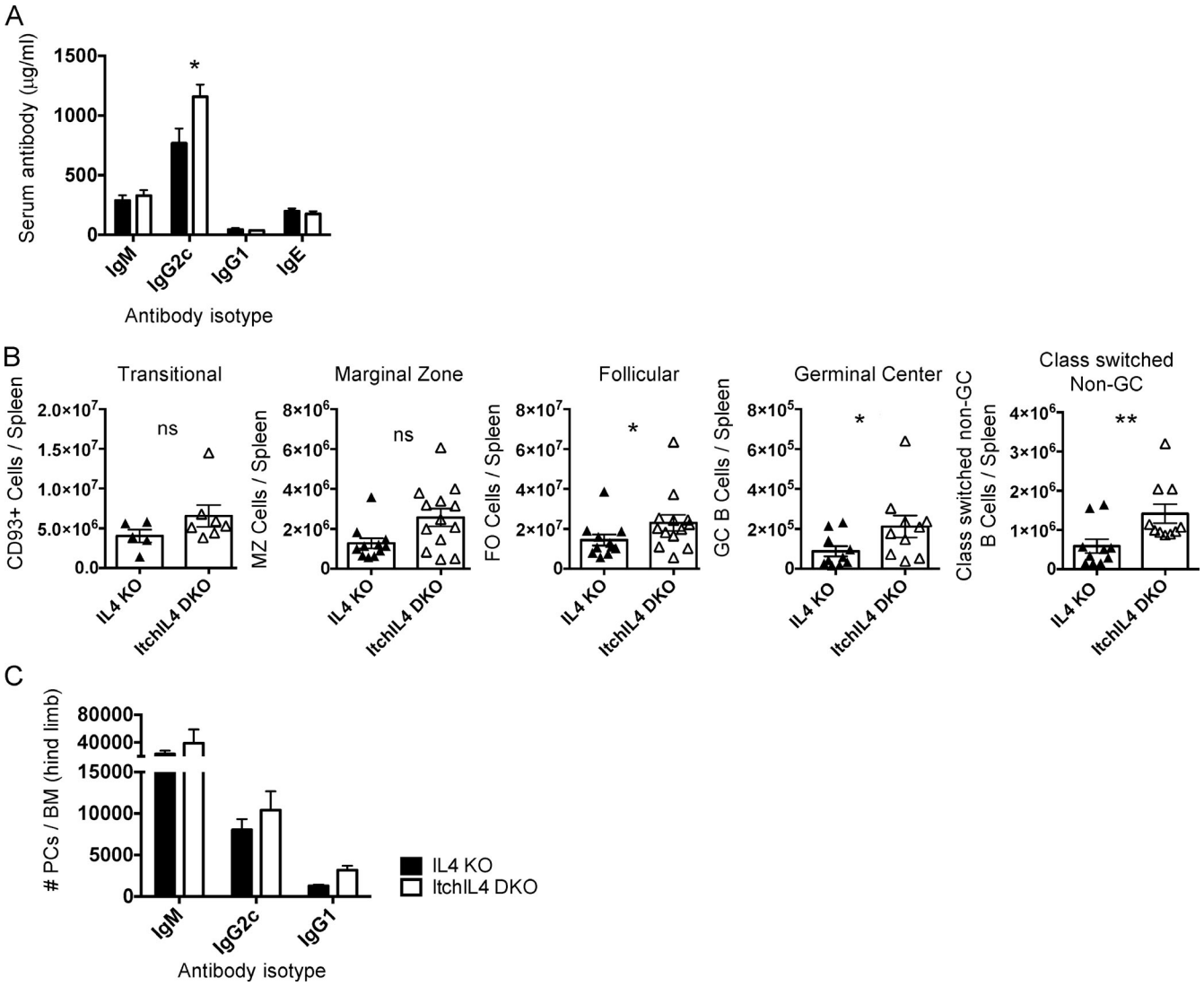

Figure S1. **Itch limits antibody and activated B cells in the absence of IL-4. (A)** Quantity of total antibody isotypes in serum of IL4 KO and Itch/IL4 DKO mice was determined by ELISA (mice between 8–12 wk old, for IgM $n = 6$ or 7, for IgG1 $n = 12$ or 13, for IgG1 $n = 4$, multiple t tests, Holm–Sidak correction). **(B)** Pre-immune and activated B cell subsets in spleen were quantified by flow cytometry. Cells were gated on live singlets, and then transitional B cells were $CD19^+CD93^+$, FO B cells were CD19⁺CD93-CD23⁺, and marginal zone B cells were $CD19^+CD23^{lo/-}CD21^+$. For GC and class-switched non-GC B cells, cells were first gated on $IgD^-CD4^-CD8^-F480^-GR1^-CD19^+IgM^-$, and then GC B cells were $GL7^+CD38^-$, and class-switched non-GC B cells were $GL7^-$ ($n = 8$–10, compiled from three independent experiments, Mann–Whitney test). **(C)** BM IgM, IgG1, and IgG2c-secreting PCs were enumerated by ELISPOT ($n = 3$, two-way ANOVA with Sidak post-test, IL4 KO mice were on B6.SJL background [CD45.1]). Error bars indicate SEM. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

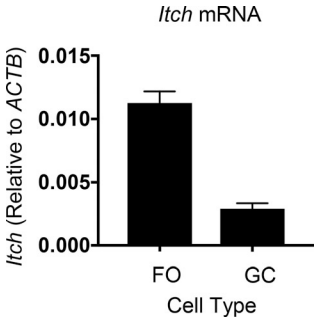

Figure S2. **Itch is expressed in naive and GC B cells.** RNA was extracted from purified splenic FO and GC B cells directly ex vivo. Reverse transcription and real-time PCR for *Itch* and β actin transcripts were performed (n = 3, two independent experiments; error bars indicate SEM; WT mice were C57Bl/6 [CD45.2]).

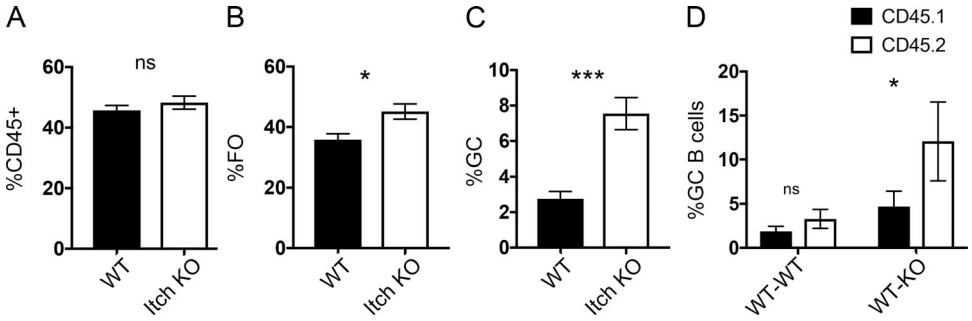

Figure S3. **Itch limits GC B cells in a B cell intrinsic manner. (A–C)** Mixed chimeras from Fig. 2 are analyzed in a different way. **(A)** Gated on live singlets, then the percent CD45.1$^+$ or CD45.2$^+$ is shown. **(B)** Gated on CD45.1$^+$ or CD45.2$^+$, then the percent IgD$^+$ cells is shown. **(C)** Cells were gated on CD45.1$^+$IgD$^-$ or CD45.2$^+$IgD$^-$, then the percent GC B cells is shown (n = 6, two independent experiments, unpaired t test). **(D)** Mixed chimeras were generated by injecting mixtures of equal numbers of T cell–depleted BM cells from WT (B6.SJL CD45.1) and WT (C57Bl/6 CD45.2) mice or WT (B6.SJL CD45.1) and Itch KO (CD45.2) mice into sublethally irradiated (400 rad) Rag KO mice. After 8 wk, spleens were harvested, and the cells were gated on CD45.1$^+$CD19$^+$ or CD45.2$^+$CD19$^+$, and the percent GC B cells is shown (n = 4 or 5, two independent experiments, two-way ANOVA with Sidak post-test). Error bars indicate SEM. *, P < 0.05; ***, P < 0.001; ns, not significant.

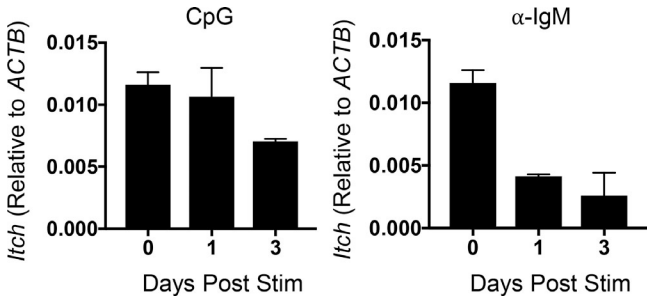

Figure S4. **Itch expression in B cells after in vitro stimulation.** FO B cells were purified from Itch-sufficient IL4 KO mice and cultured for 3 d with or without CpG or anti-IgM stimulation. RNA was extracted, cDNA was made by reverse transcription, and real-time PCR for *Itch* and *actb* transcripts was performed (for CpG, n = 3 or 4, two independent experiments, and for IgM, n = 2, one experiment. Error bars indicate SEM. IL4 KO mice were B6.SJL [CD45.1]). Post stim, post stimulation.

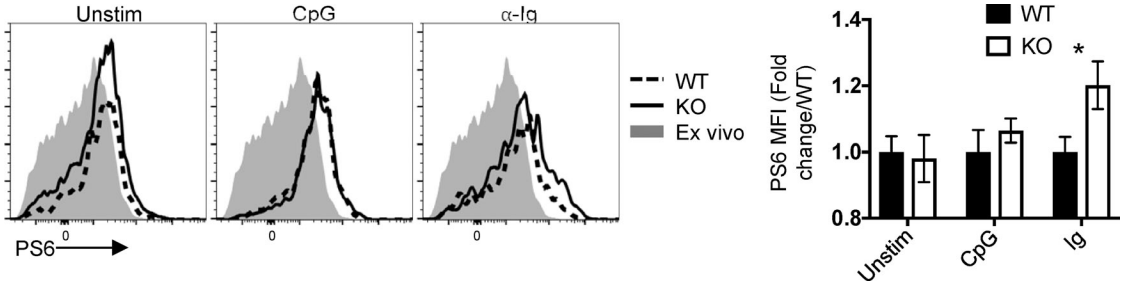

Figure S5. **Itch limits mTORC1 activity in spontaneous GC B cells.** Total B cells were isolated from WT or Itch KO spleens using negative selection. Cells were cultured in B cell media for 1 h, and were stimulated with 1 µM CpG or 10 µM anti-Ig. Cells were stained for GC markers and P-S6, then analyzed by flow cytometry. GC B cells were gated on $CD19^+IgD^-IgM^-GL7^+CD38^-$, and MFI of P-S6 was determined. Fold change indicates MFI/average MFI of WT for each independent experiment (n = 6, three independent experiments, multiple t tests; error bars indicate SEM). *, $P < 0.05$. Unsim, unstimulated.