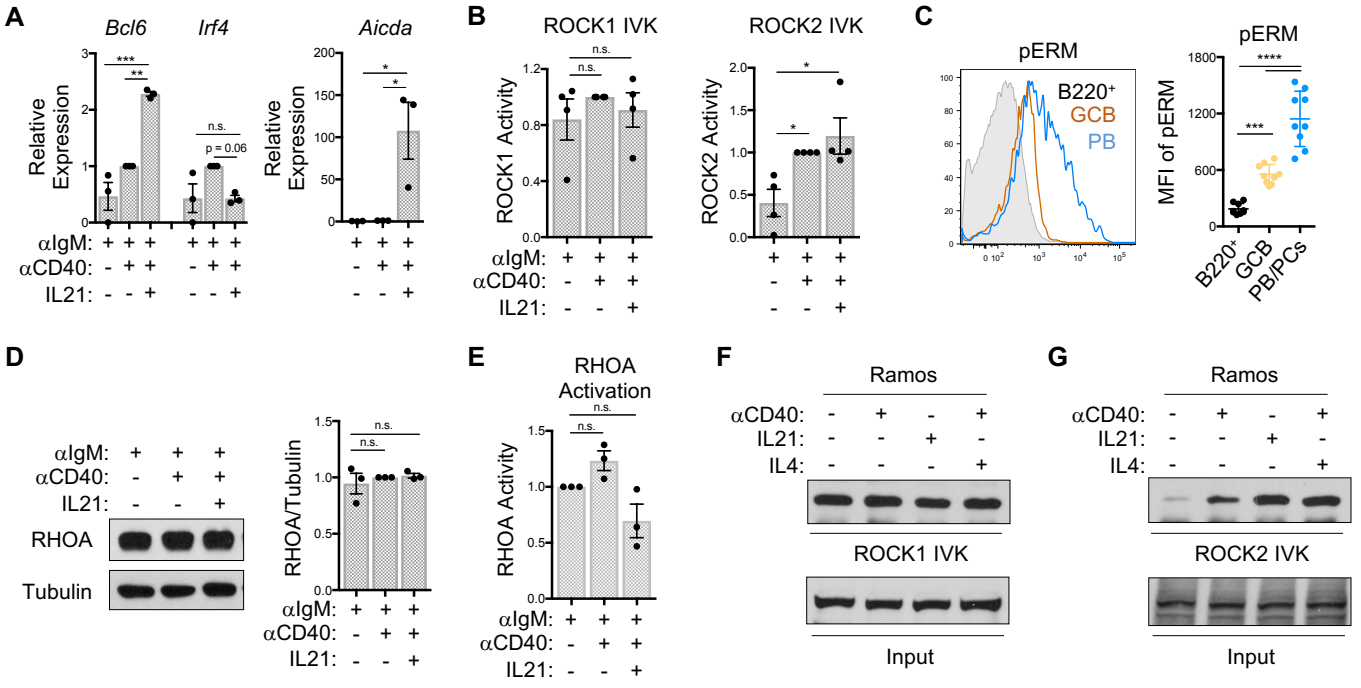
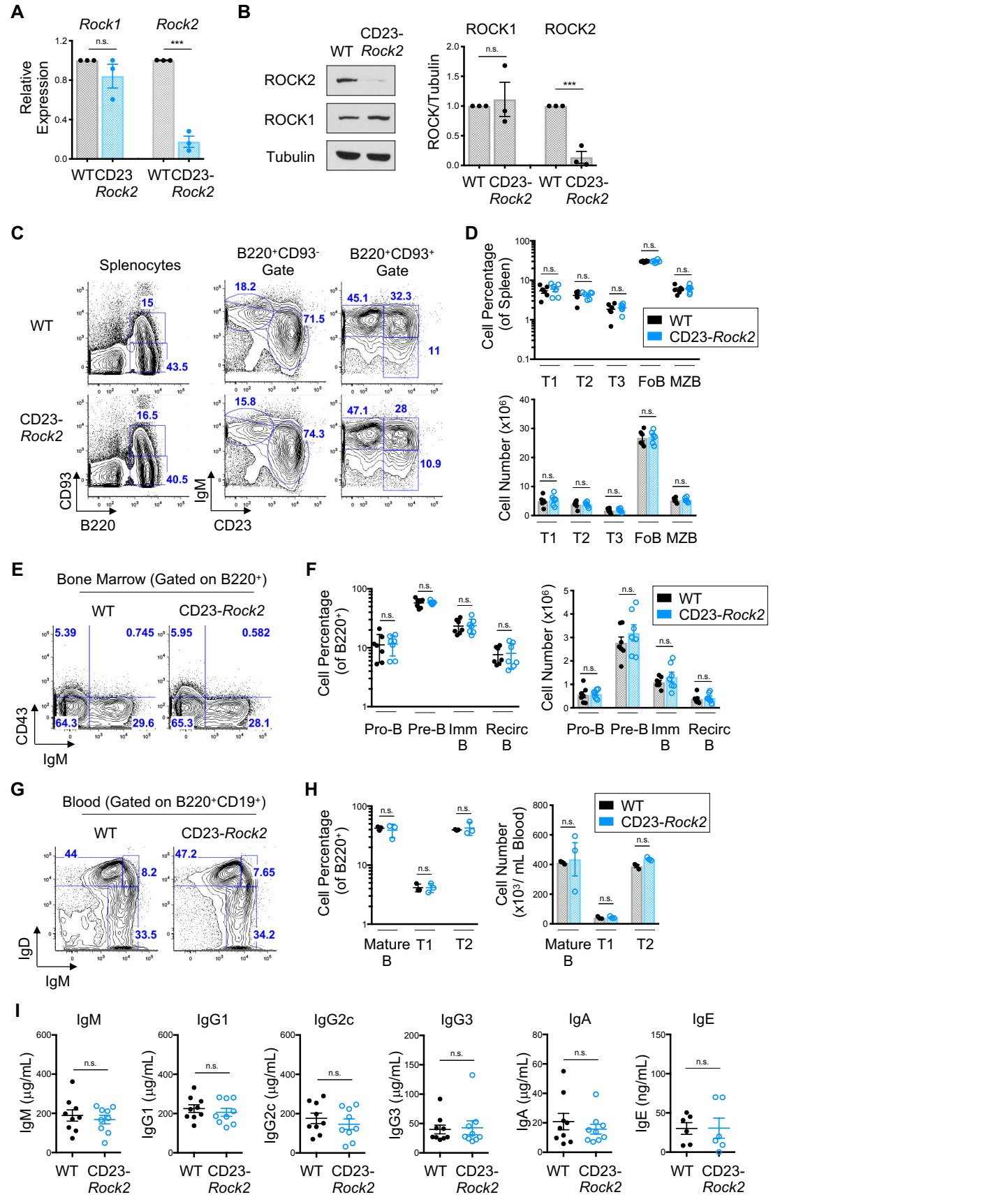
Supplementary Figure 1, related to Figure 1.



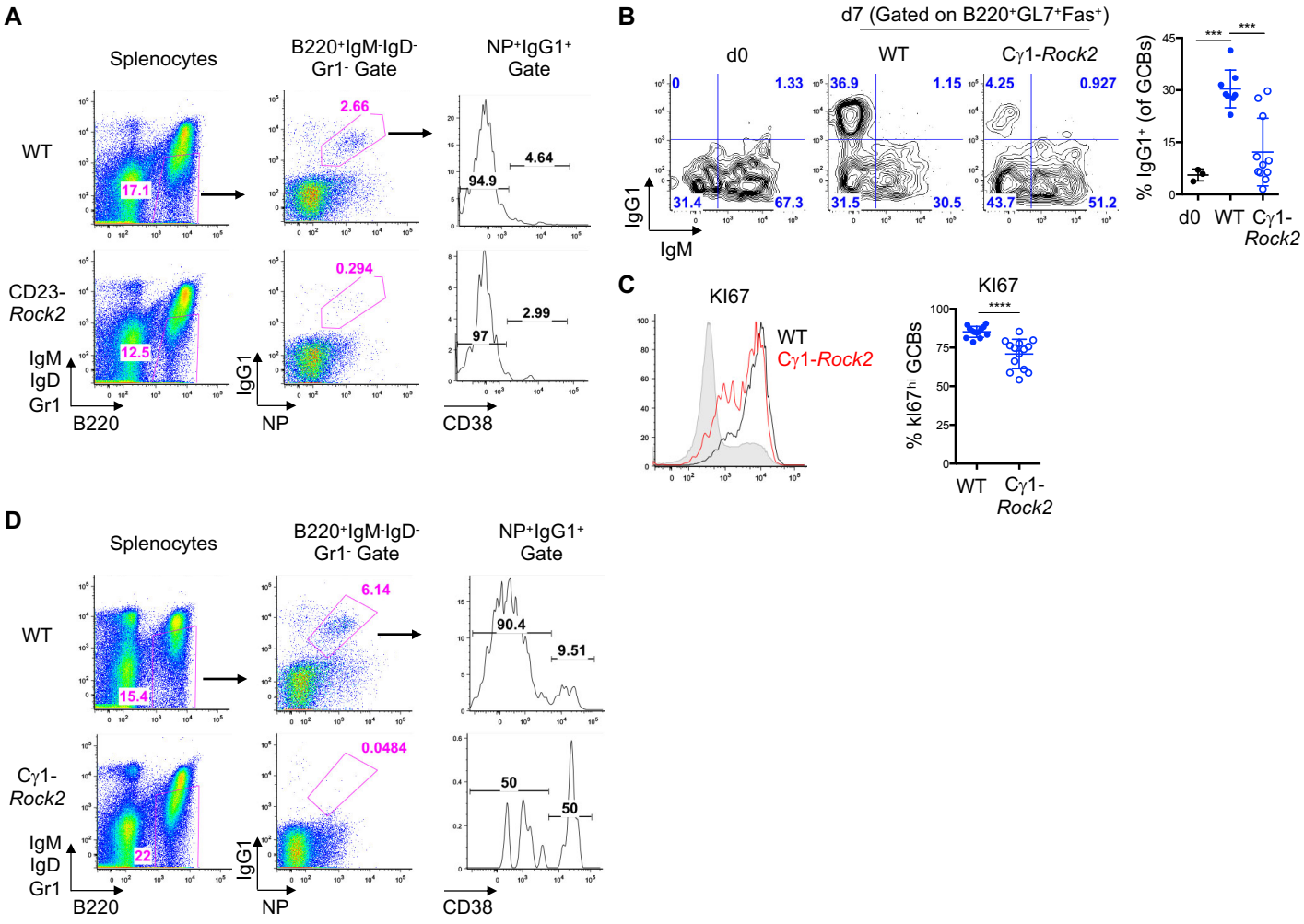
Supplementary Figure 1, related to Figure 1. CD23$^+$ B cells were purified from C57BL/6 mice and cultured for 3d as in Figure 1A-B. (A) RT-qPCR analysis of *Bcl6*, *Irf4*, and *Aicda* relative to *Ppia* expression. Data is pooled from 3 independent experiments (mean +/- SEM; *n*=3; *p*-value by 1-way ANOVA followed by Dunnett's test for multiple comparisons). (B) Quantifications of ROCK1 and ROCK2 IVKs from Figure 1A-B plotted as the ratio of pMYPT1 to total input protein (mean +/- SEM; *n*=3; *p*-value by 1-way ANOVA followed by Dunnett's test for multiple comparisons). (C) Representative histogram and pooled quantifications of the expression of phosphorylated ERM (pERM) proteins in GC B cells, plasmblasts/plasma cells (PB/PCs), and B220$^+$ cells from *Rock2$^{flox/flox}$* control mice that were immunized with NP-CGG for 7d. Data is pooled from 3 independent experiments (mean +/- SEM; *n*=9; p-value by 1-way ANOVA followed by Tukey's test for multiple comparisons). (D) Representative immunoblot analysis of RhoA and housekeeping protein Tubulin from extracts of CD23$^+$ cultures. Quantifications *(right)* show the ratio of RhoA to Tubulin protein expression (mean +/- SEM; *n=3; p*-value by 1-way ANOVA followed by Dunnett's test for multiple comparisons). (E) Representative RhoA G-LISA activity assay from CD23$^+$ cultures. Pooled data from 3 independent experiments (mean +/- SEM; *n*=3; p-value by 1-way ANOVA followed by Dunnett's test for multiple comparisons). (F-G) Ramos cells were either left unstimulated or stimulated for 6hr with αCD40 (1μg/mL) and/or IL-21 (100ng/mL) or IL-4 (50ng/mL). ROCK1 *(F)* and ROCK2 *(G)* kinase activity assays were performed as in Figure 1C-D. Data representative of 2 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 2, related to Figure 2.



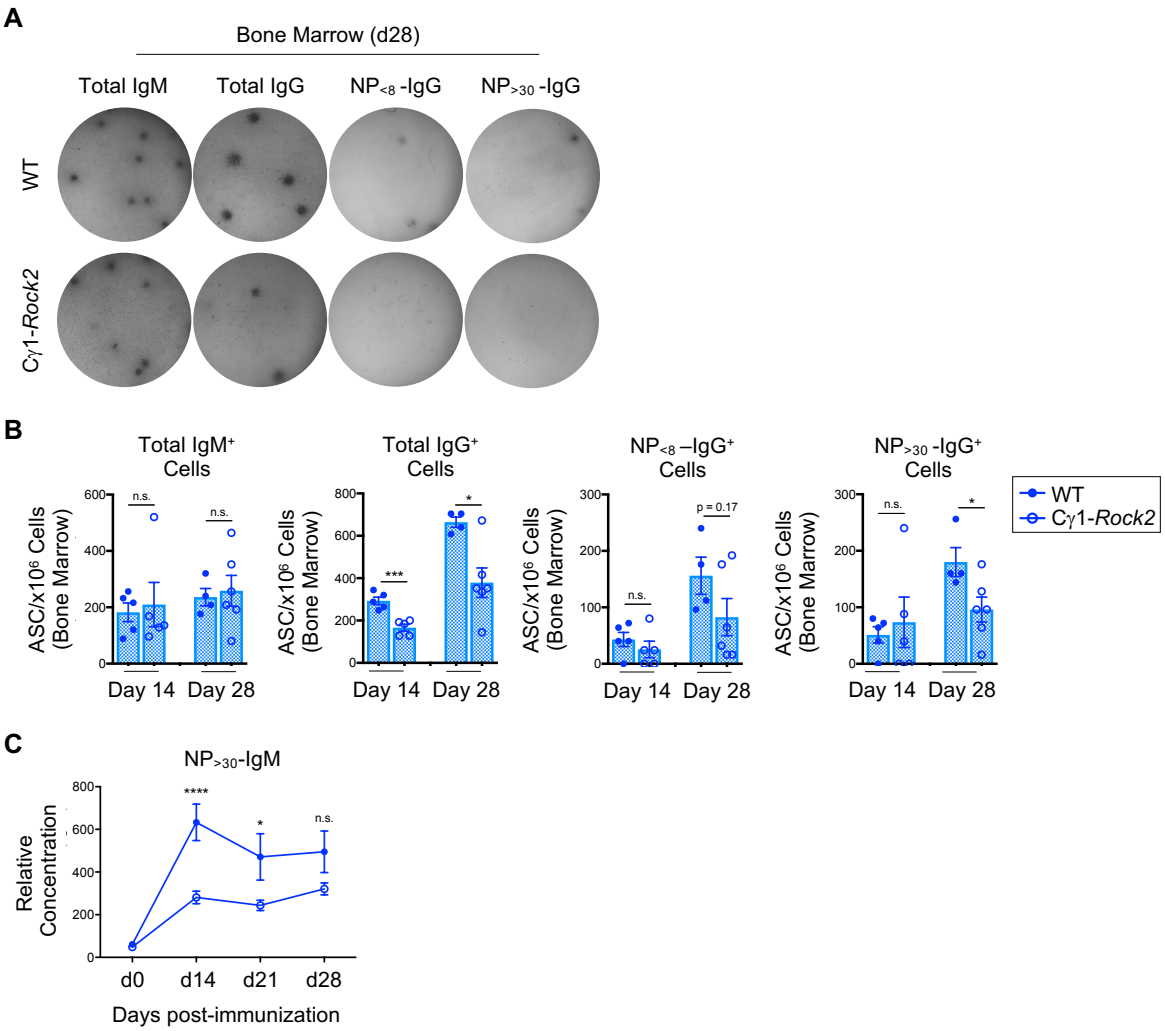
Supplementary Figure 2, related to Figure 2. (A) Pooled RT-qPCR analysis of *Rock1* and *Rock2* expression in $CD23^+$ purified B cells from WT or CD23-*Rock2* mice. Data shows mean +/- SEM; *n*=3; p-value by unpaired two-tailed t-test. (B) Representative immunoblot and pooled quantifications of ROCK1 and ROCK2 protein from $CD23^+$ purified B cells from WT or CD23-*Rock2* mice. Quantification shows ratio of ROCK protein to Tubulin (mean +/- SEM; *n=3; p*-value by unpaired two-tailed t-test. (C-D) Representative FACS plots *(C)* and pooled quantifications *(D)* of transitional B cells (T1: $B220^+CD93^+IgM^+CD23lo$; T2: $B220^+CD93^+IgM^+CD23hi$; T3: $B220^+CD93^+IgM^-CD23^{hi}$), follicular B cells (FoB: $B220^+CD93^-CD23^{hi}$), and marginal zone B cells (MZB: $B220^+CD93^-IgM^+CD23^{lo}$) from the spleens of 6-10wk old WT or CD23-*Rock2* mice. Data pooled from 2 independent experiments (mean +/- SEM; *n=6; p*-value by unpaired two-tailed t-test. (E-F) Representative FACS plots *(E)* and pooled quantifications *(F)* of pro-B cells (pro-B: $B220^+CD43^+IgM^-$), pre-B cells (pre-B: $B220^+CD43^-IgM^-$), immature B cells (ImmB: $CD43^-IgM^+B220^{lo}$), and recirculating B cells (RecircB: $CD43^-IgM^+B220^{hi}$) from the bone marrow of 6-10wk old WT or CD23-*Rock2* mice. Data pooled from 3 independent experiments (mean +/- SEM; *n=7; p*-value by unpaired two-tailed t-test. (G-H) Representative FACS plots *(G)* and pooled quantifications *(H)* of mature B cells (Mature B: $B220^+CD19^+IgD^{hi}IgM^{mid/lo}$) and transitional B cells (T1: $B220^+CD19^+IgD^-IgM^{hi}$; T2: $B220^+CD19^+IgD^{hi}IgM^{hi}$) from the blood of 6-10wk old WT or CD23-*Rock2* mice. Data shows mean +/- SEM; *n=3;* p-value by unpaired two-tailed t-test. (I) Pooled ELISA analysis of total Ig isotypes from 6-10wk old WT or CD23-*Rock2* mice. Data pooled from 5 independent experiments (mean +/- SEM; *n=9; p*-value by unpaired two-tailed t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary Figure 3, related to Figure 2.



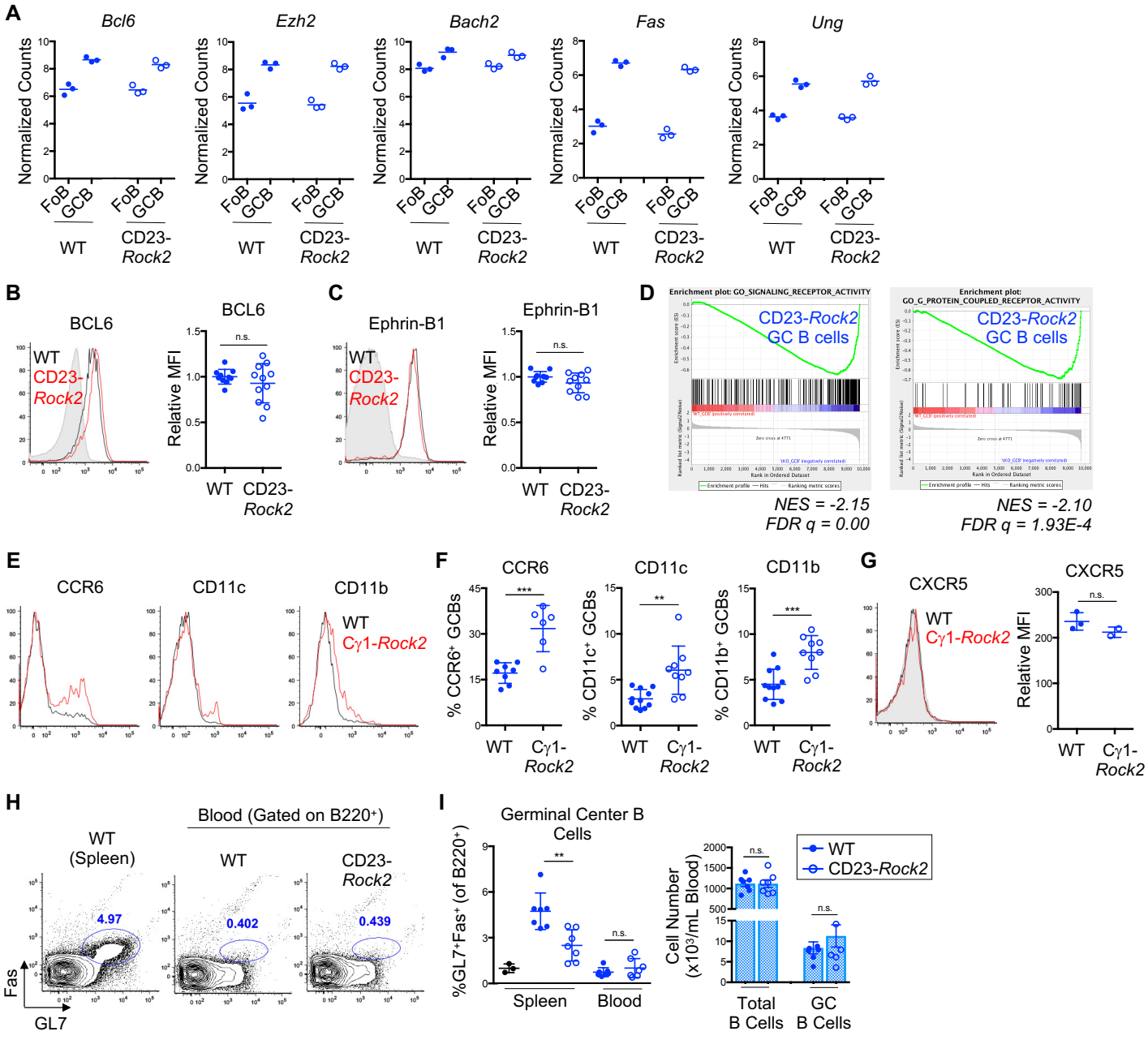
Supplementary Figure 3, related to Figure 2. (A) WT and CD23-*Rock2* mice were immunized with 100μg NP-CGG for 7-10d. (A) Representative FACS plots of NP-specific B cells ($B220^+IgM^-IgD^-Gr1^-NP^+IgG1^+$). Data pooled from at least 2 independent experiments per timepoint (*n*>6). (B-D) WT and Cγ1-*Rock2* mice were immunized with 100μg NP-CGG for 7-10d. (B) Representative FACS plots of $IgG1^+$ GC B cells from WT or Cγ1-*Rock2* mice at d7 after immunization. (C) Representative FACS histograms and pooled quantifications of Ki67 expression in WT or Cγ1-*Rock2* mice at d7 after immunization. (D) Representative FACS plots of NP-specific B cells. Data pooled from at least 2 independent experiments per timepoint (*n*>6). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 4, related to Figure 3.



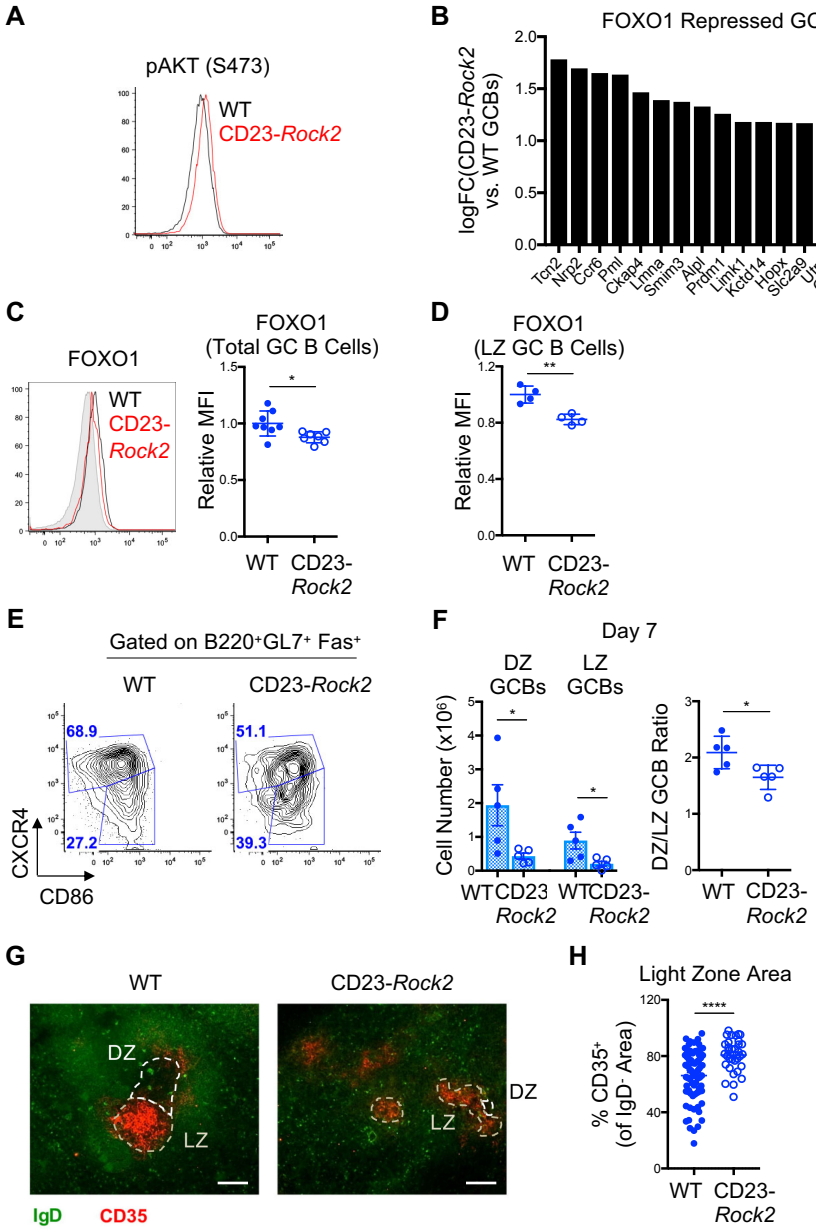
Supplementary Figure 4, related to Figure 3. WT or Cγ1-*Rock2* mice were immunized with 100μg NP-CGG for 7-28d. (A-B) Representative ELISPOT images *(A)* and pooled quantifications *(B)* of total and NP-specific antibody-secreting cells (ASCs) in bone marrow from the indicated mice at d28 after immunization. Data pooled from 2 independent experiments per timepoint (mean +/- SEM; *n*>=4; p-value by unpaired two-tailed t-test). (C) Pooled ELISA analysis of NP-specific IgM from the serum of indicated mice at 0-28d after immunization. Data from 2 independent experiments (mean +/- SEM; *n*=8; p-value by 2-way ANOVA followed by Sidak's test for multiple comparisons). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 5, related to Figure 4.



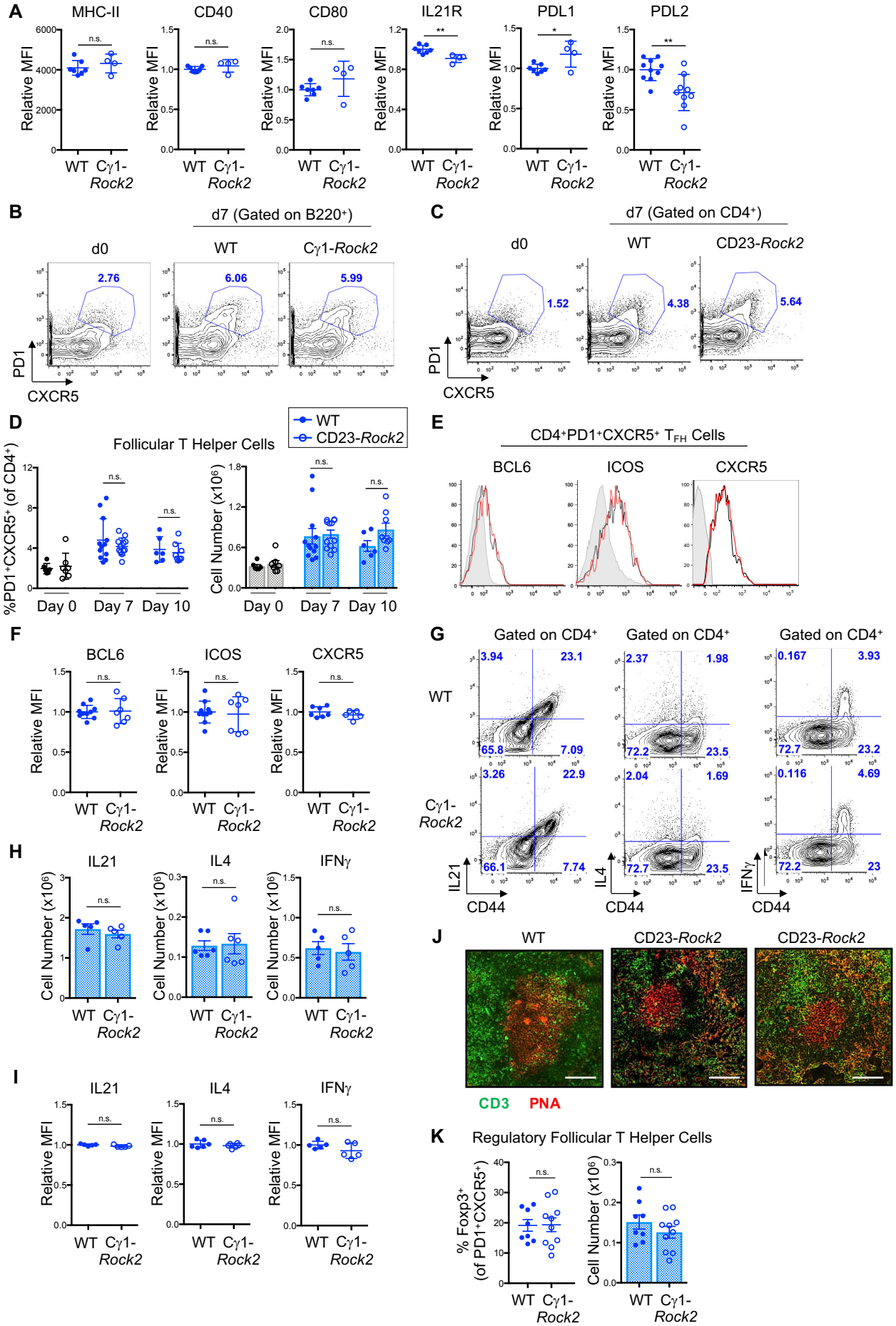
Supplementary Figure 5, related to Figure 4. (A) Plots showing the normalized log-transformed counts per millions for the indicated genes from the RNA-seq analysis in Figure 4. (B-C) Representative FACS plots and pooled quantifications of BCL6 *(B)* and Ephrin-B1 *(C)* expression in GC B cells ($B220^{+}Fas^{+}GL7^{+}$) from WT or CD23-*Rock2* mice 7d after immunization. Data from 3 independent experiments (mean +/- SEM; *n*>9; p-value by unpaired two-tailed t-test). (D) GSEA plots showing enrichment of the indicated gene sets in CD23-*Rock2* GC B cells as compared to WT GC B cells. (E-F) Representative FACS plots *(E)* and pooled quantifications *(F)* of the indicated markers on GC B cells from WT or Cγ1-*Rock2* mice 7d after immunization. Data from at least 2 independent experiments (mean +/- SEM; *n>6;* p-value by unpaired two-tailed t-test). (G) Representative histogram and pooled quantification of CXCR5 expression on the surface of WT *(black)* and Cγ1-*Rock2 (red)* GC B cells ($B220^{+}GL7^{+}CD38^{lo}$). CXCR5 expression on non-GC B cells from WT mice *(shaded gray)* are plotted as a control (mean +/- SEM; *n*>2; p-value by unpaired two-tailed t-test). (H-I) Representative FACS plots *(H)* and pooled quantifications *(I)* of the frequency and total numbers of GC B cells in the blood from WT and CD23-*Rock2* mice at d7 after immunization. Data from 2 independent experiments (mean +/- SEM; *n*>6; p-value by unpaired two-tailed t-test). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary Figure 6, related to Figure 5.



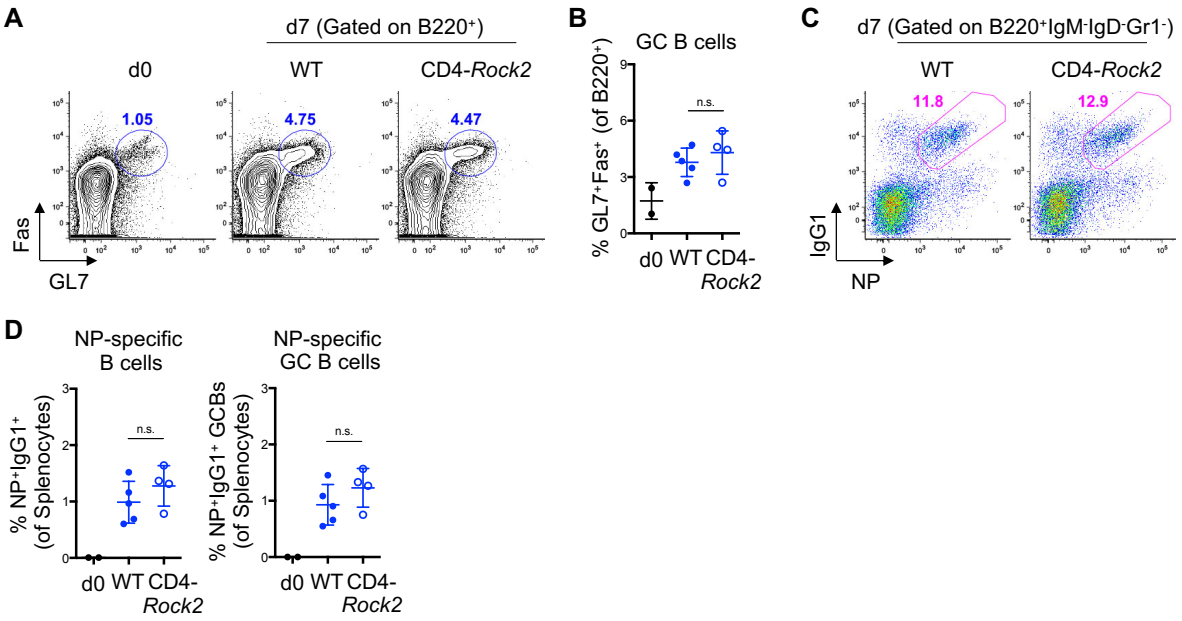
Supplementary Figure 6, related to Figure 5. (A) Representative histogram of AKT phosphorylation at S473 in CD23$^+$ B cells stimulated as in Figure 5B. (B) Plot showing the logFC values of the top 20 genes enriching the FOXO1_Repressed_GCB Geneset in CD23-*Rock2* GC B cells from Figure 5D. (C-D) Representative histogram and pooled quantifications of FOXO1 protein levels in total GC B cells *(C)* and in LZ GC B cells *(D)* from WT or CD23-*Rock2* mice at d7 after immunization. (E-F) Representative FACS plots *(E)* and pooled quantifications *(F)* of dark zone GC B cells (DZ GCBs; CXCR4^{hi}CD86^{lo}) and light zone GC B cells (LZ GCBs; CXCR4^{lo}CD86^{hi}) from WT or CD23-*Rock2* mice at d7 after immunization. Data from 2 independent experiments (mean +/- SEM; *n*=5; p-value by unpaired two-tailed t-test). (G) Representative IF images showing IgD *(green)* and CD35 *(red)* expression in splenic sections from WT and CD23-*Rock2* mice at d7 after immunization. Data representative of 2 independent experiments. Scale bars show 100μm. (H) Quantification of %CD35$^+$ area within total IgD$^-$ GC area from Supplementary Figure 6H (mean +/- SEM; *n*>33 GCs across 3 mice per genotype; p-value by Mann-Whitney test). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 7, related to Figure 6.



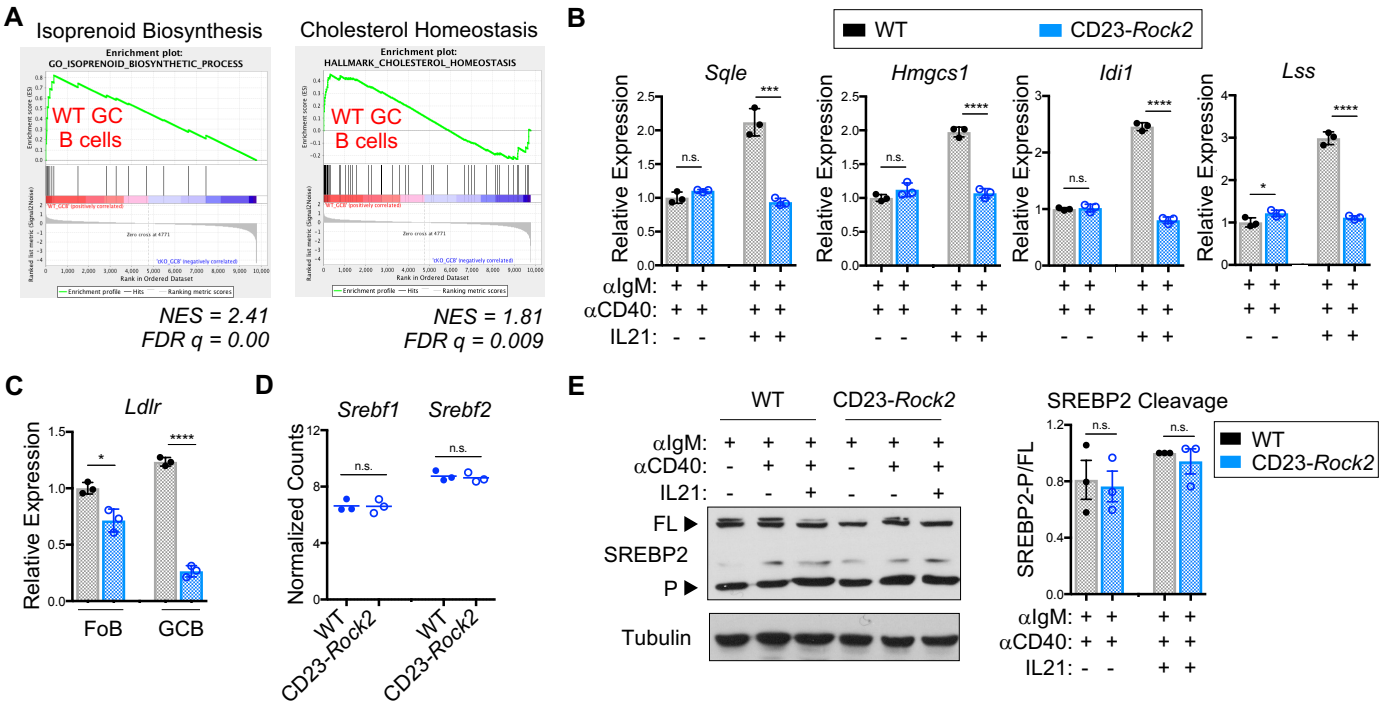
Supplementary Figure 7, related to Figure 6. (A) Quantifications of MHC-II, CD40, CD80, IL21R, PD-L1, and PD-L2 on the surface of GC B cells from WT or Cγ1-*Rock2* mice at d7 after immunization, as in Figure 6A. Data from 2 independent experiments (mean +/- SEM; *n*>4; p-value by unpaired two-tailed t-test). (B-D) Representative FACS plots *(B-C)* and pooled quantifications *(D)* of the frequency and numbers of T_{FH} cells ($CD4^+CXCR5^+PD1^+$) from the indicated mice 7-10d after immunization. Data from at least 2 independent experiments per timepoint (mean +/- SEM; *n*>6; p-value by unpaired two-tailed t-test). (E-F) Representative histograms *(E)* and pooled quantifications *(F)* of BCL6, ICOS, and CXCR5 expression on T_{FH} cells from WT or Cγ1-*Rock2* mice at d7 after immunization. WT non-T_{FH} $CD4^+$ cells *(shaded grey)* are shown as a control. Data from at least 2 independent experiments (mean +/- SEM; *n*>5; p-value by unpaired two-tailed t-test). (G-I) Splenocytes from d10 immunized mice with PMA and Ionomycin for 4hr in the presence of BrefeldinA. (G) Representative FACS plots of IL-21, IL-4, and IFNγ production by $CD4^+$ T cells. (H-I) Plots showing the total cell numbers of cytokine-producing T cells *(H)* and the normalized MFI of IL-21, IL-4, and IFNγ expression from the cytokine-producing $CD44^+$ T cells *(I)* from Supplementary Figure 7G. Data from 2 independent experiments (mean +/- SEM; *n*>5; p-value by unpaired two-tailed t-test). (J) Representative IF images showing CD3 *(green)* and PNA *(red)* expression in splenic sections from WT and CD23-*Rock2* mice 7d after immunization. Data representative of 2 independent experiments. Scale bar shows 100μm. (K) Representative FACS plots *(left)* and pooled quantifications *(right)* of the frequencies and cell numbers of T_{FR} cells ($CD4^+CXCR5^+PD1^+FOXP3^+$) from the indicated mice 7d after immunization. Data from 2 independent experiments (mean +/- SEM; *n*>8; p-value by unpaired two-tailed t-test). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 8, related to Figure 6.



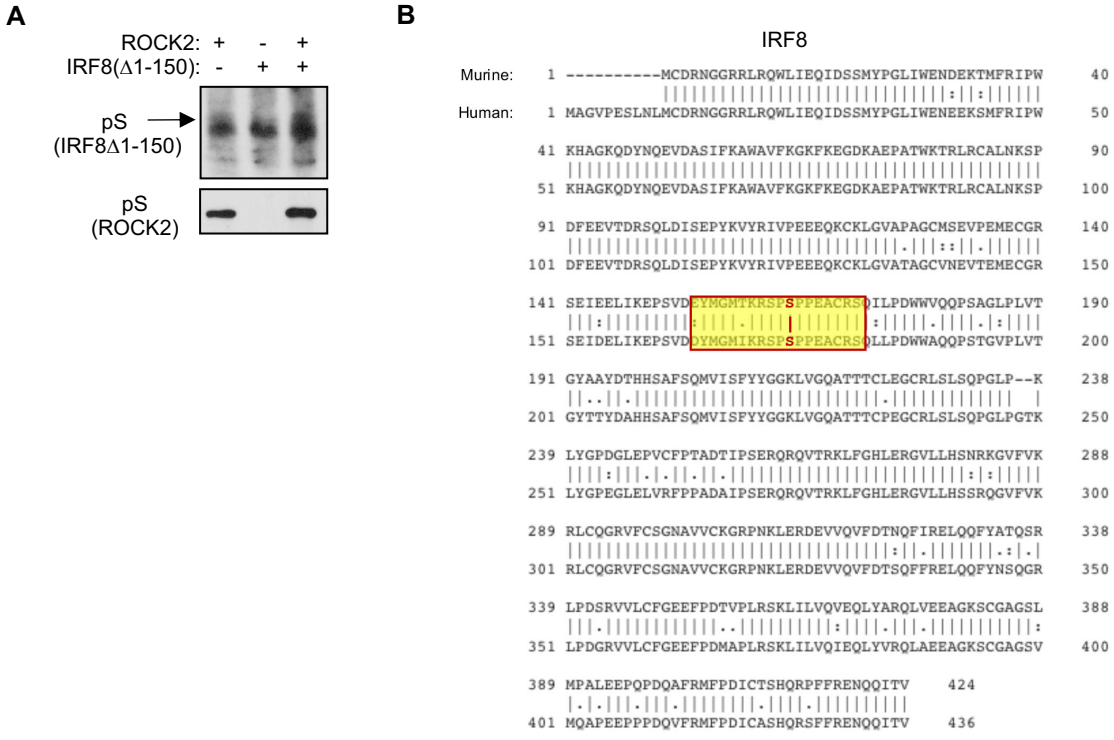
Supplementary Figure 8, related to Figure 6. WT and CD4-*Rock2* mice were immunized with 100μg NP-CGG for 7-28d. (A-D) Representative FACS plots and quantifications of GC B cells *(A-B)* and NP-reactive B cells *(C-D)* from CD4-*Rock2* or WT mice immunized for 7d with NP-CGG. Data representative of 2 independent experiments (mean +/- SEM; *n*>5; p-value by 1-way ANOVA followed by Tukey's test for multiple comparisons). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 9, related to Figure 7.



Supplementary Figure 9, related to Figure 7. (A) GSEA plots showing the downregulation of GO_Isoprenoid_Biosynthetic_Process and HALLMARK_Cholesterol_ Homeostasis gene sets in CD23-*Rock2* GC B cells. (B) Representative RT-qPCR analysis of indicated genes from CD23$^+$ cultures. Data shows technical triplicates and is representative of 3 independent experiments (mean +/- SD; *n*=3; p-value by unpaired two-tailed t-test). (C) Representative RT-qPCR of *Ldlr* expression in sorted GC B cells from WT and CD23-*Rock2* mice. Data show technical triplicates and is representative of 3 independent experiments (mean +/- SD; *n*=3; p-value by unpaired two-tailed t-test). (D) Plot showing the normalized counts of *Srebf1* and *Srebf2* in sorted GC B cells from WT and CD23-*Rock2* mice as assessed by RNA-seq analysis. (E) Representative immunoblot and pooled quantifications of full-length SREBP2 (FL) and processed SREBP2 (P) protein from CD23$^+$ cultures. Quantifications calculated as the ratio of P/FL SREBP2. Data from 3 independent experiments (mean +/- SEM; *n*=3; p-value by unpaired two-tailed t-test). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

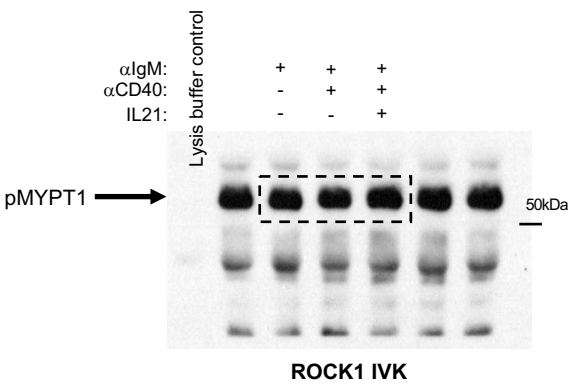
Supplementary Figure 10, related to Figure 8.



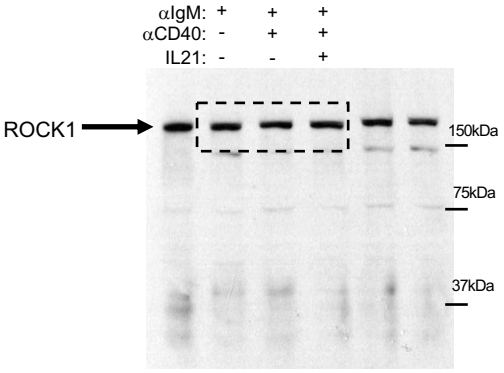
Supplementary Figure 10, related to Figure 8. (A) IRF8 was immunoprecipitated from 293T cells over-expressing mutant IRF8 protein lacking a DNA-binding domain (IRF8Δ1-150) and incubated with constitutively active ROCK2. Detection of phosphorylated IRF8 was performed by immunoblot with an anti-phosphorylated serine antibody. (B) Schematic showing the similarity between the human and mouse IRF8 protein sequence. The ROCK consensus site is highlighted in yellow. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Full Uncropped Gels.

Full Uncropped Gels for Figure 1A



ROCK1 IVK



Inputs

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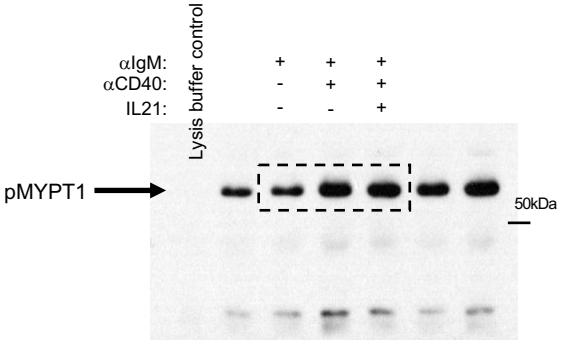
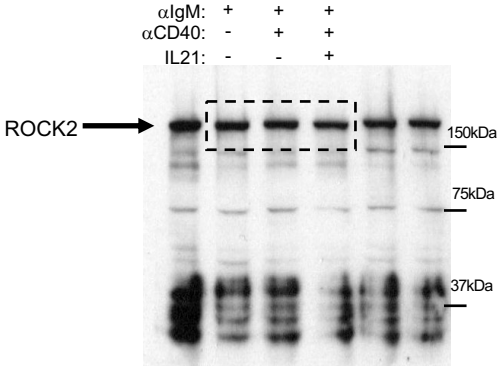


ROCK2 IVK



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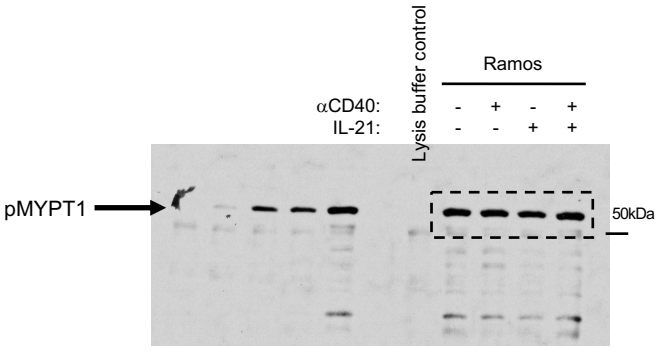
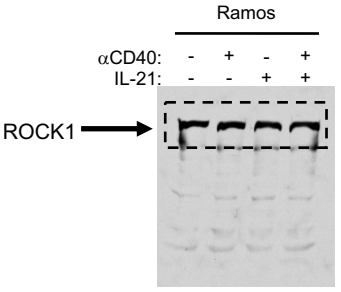


ROCK1 IVK



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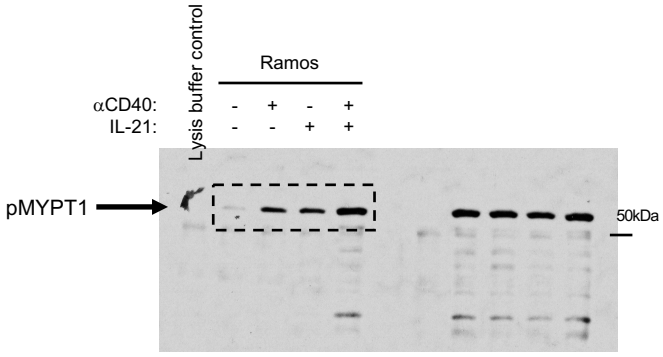
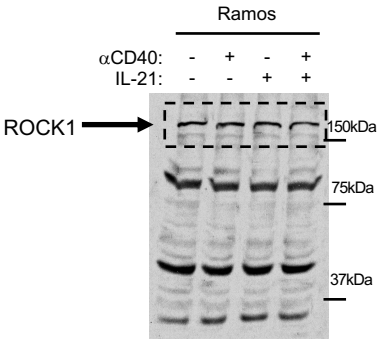


ROCK2 IVK



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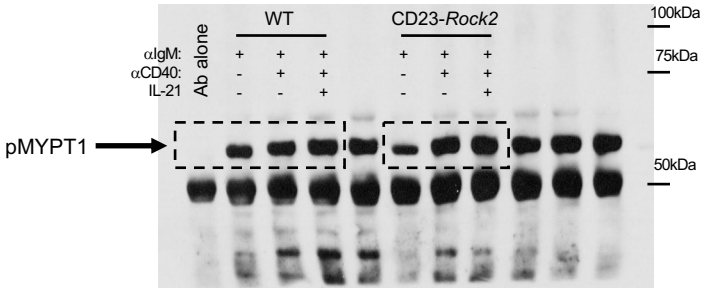
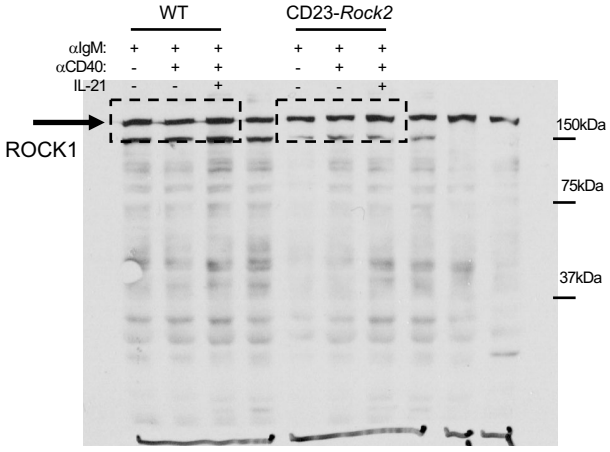


ROCK1 IVK



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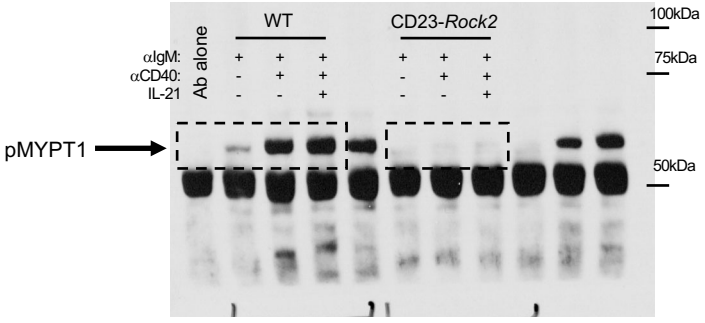
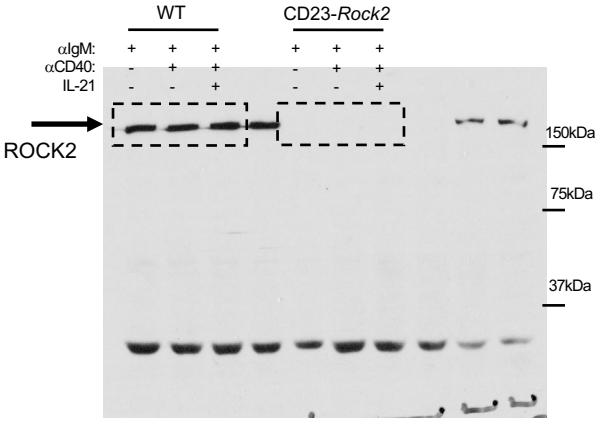


ROCK2 IVK



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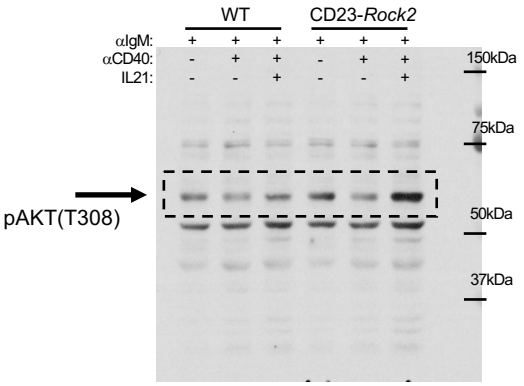

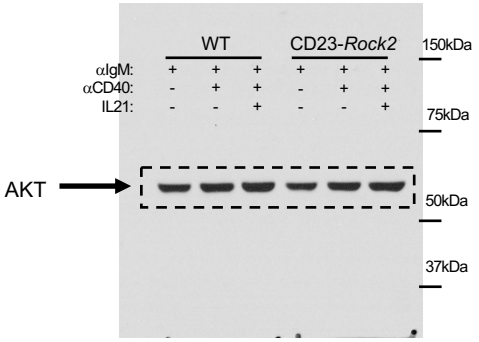

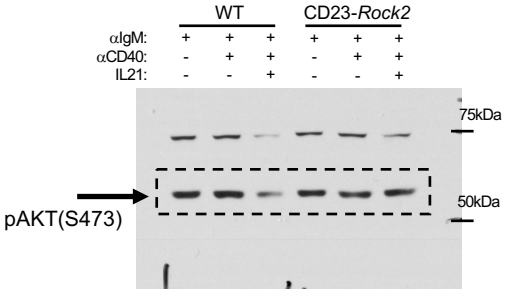

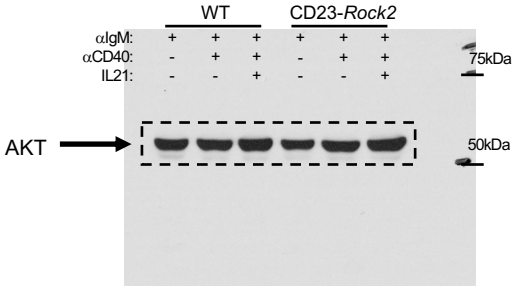

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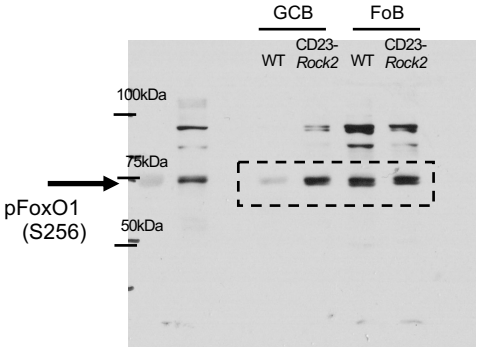

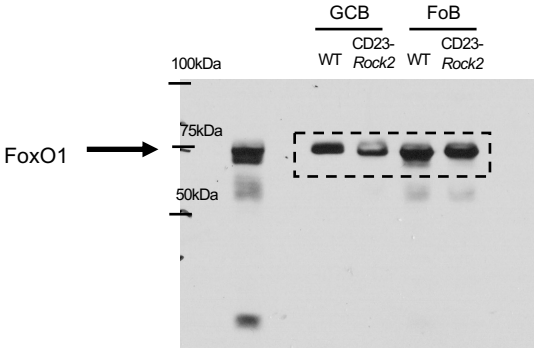

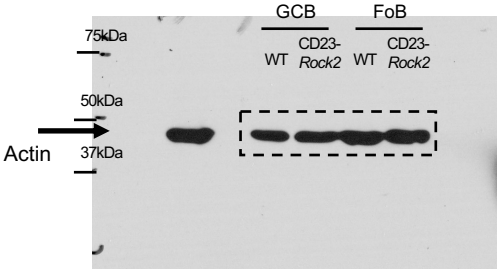

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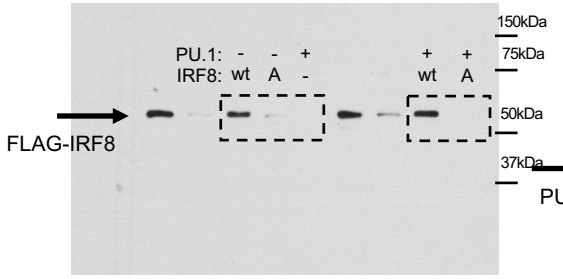

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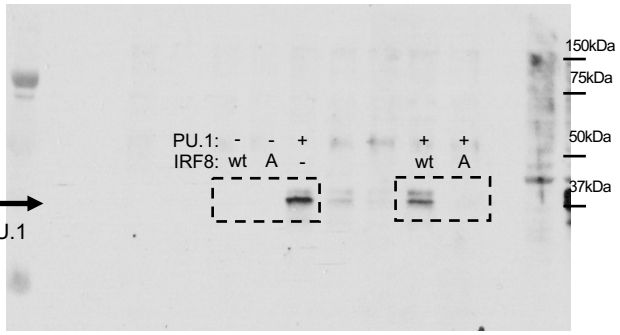

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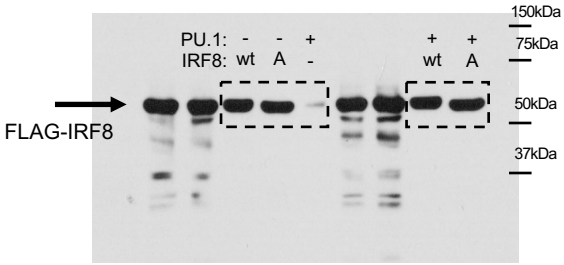

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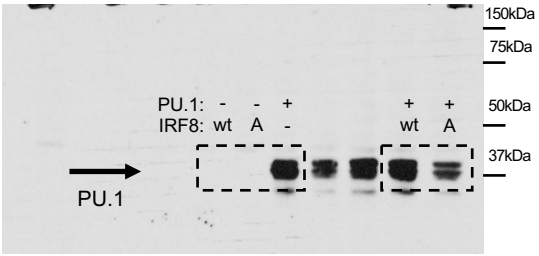

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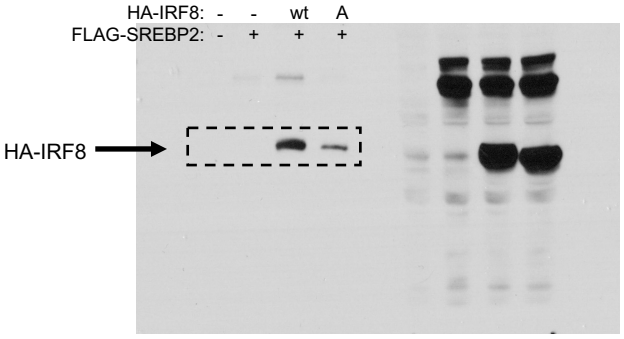
Full Uncropped Gels for Figure 8E



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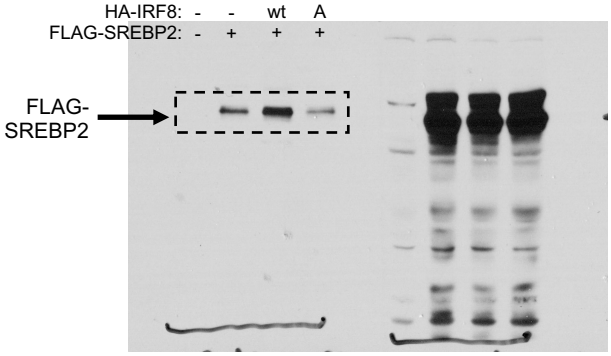

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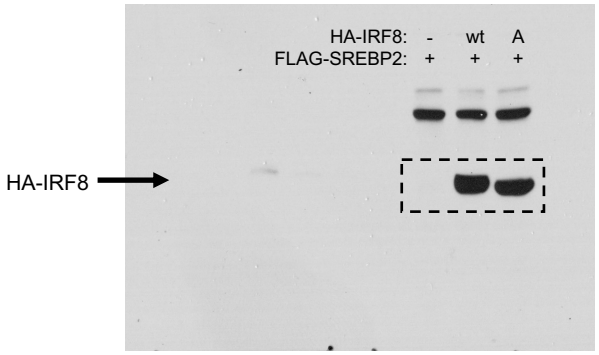

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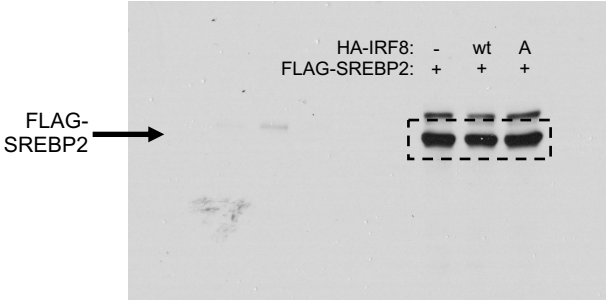

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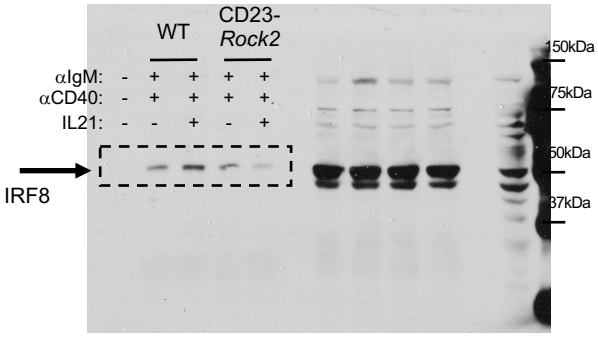
Full Uncropped Gels.

Full Uncropped Gels for Figure 8F



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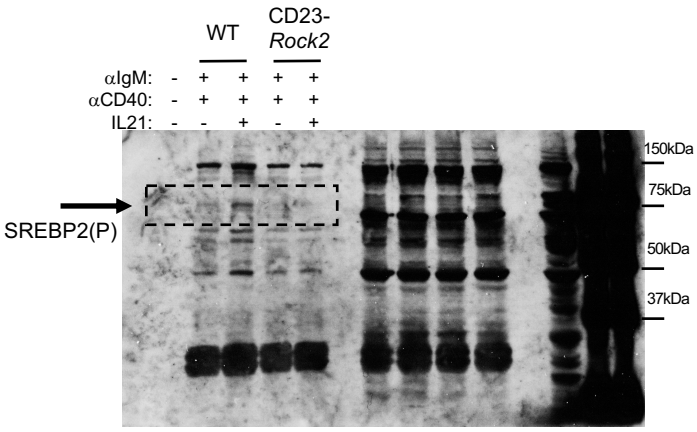

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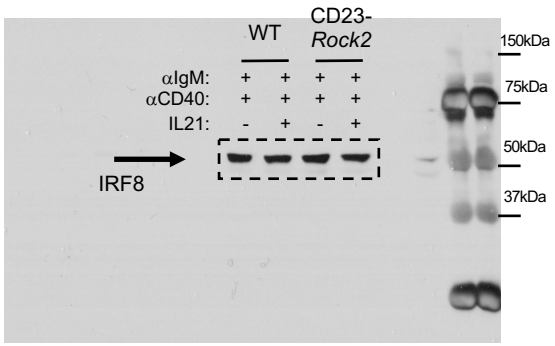

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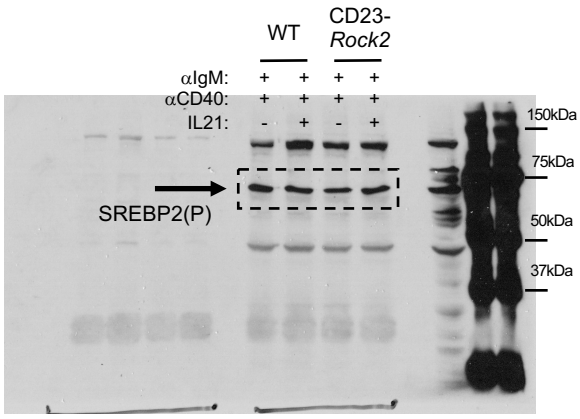

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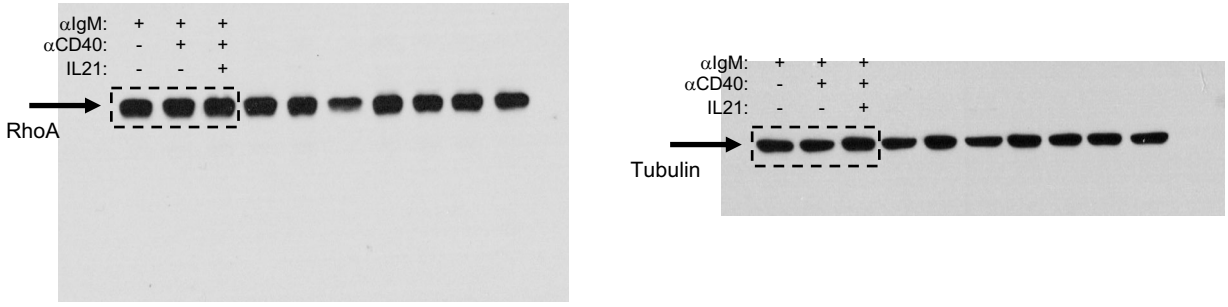
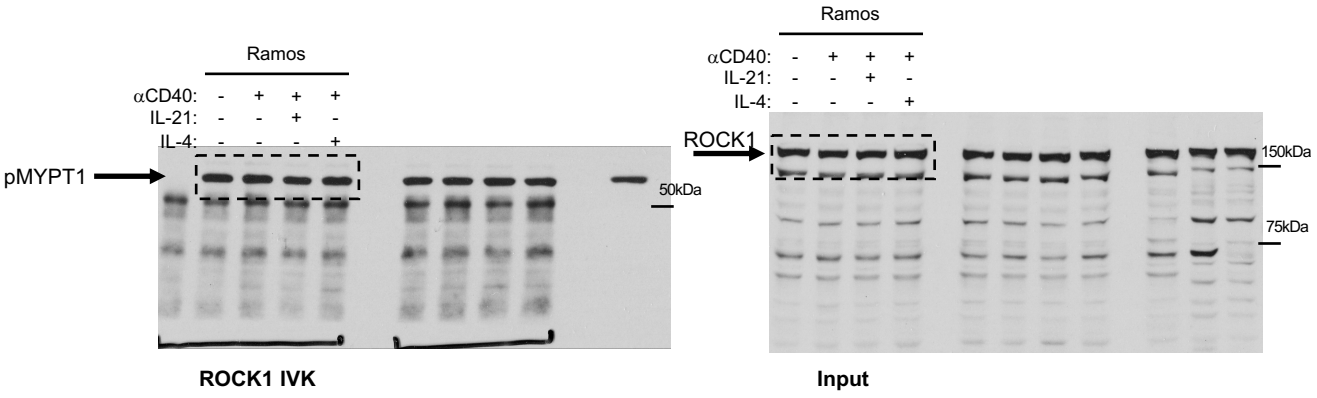
Full Uncropped Gels.

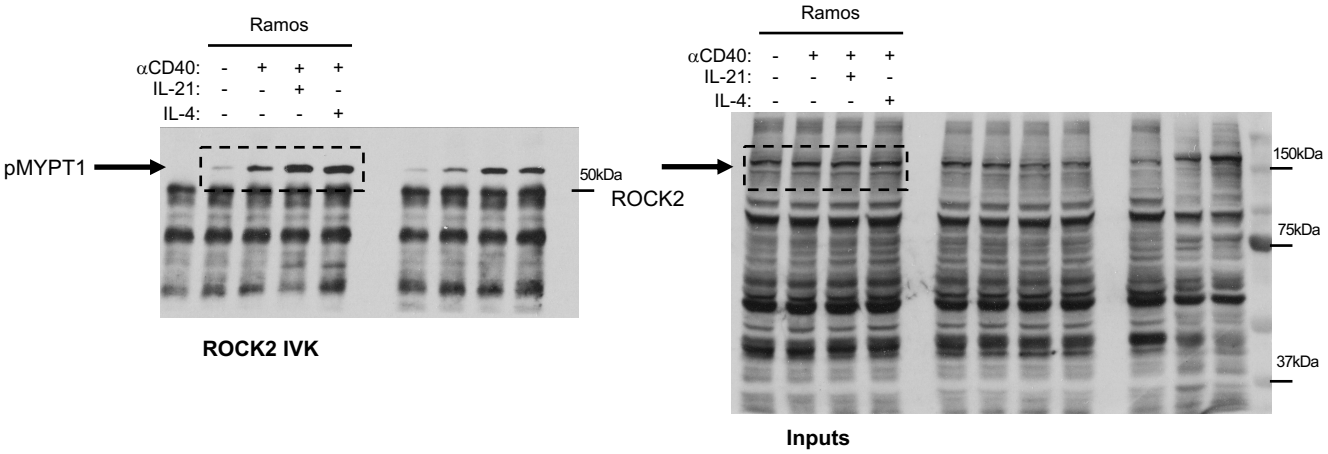
Full Uncropped Gels for Supplementary Figure 1D



Full Uncropped Gels for Supplementary Figure 1F

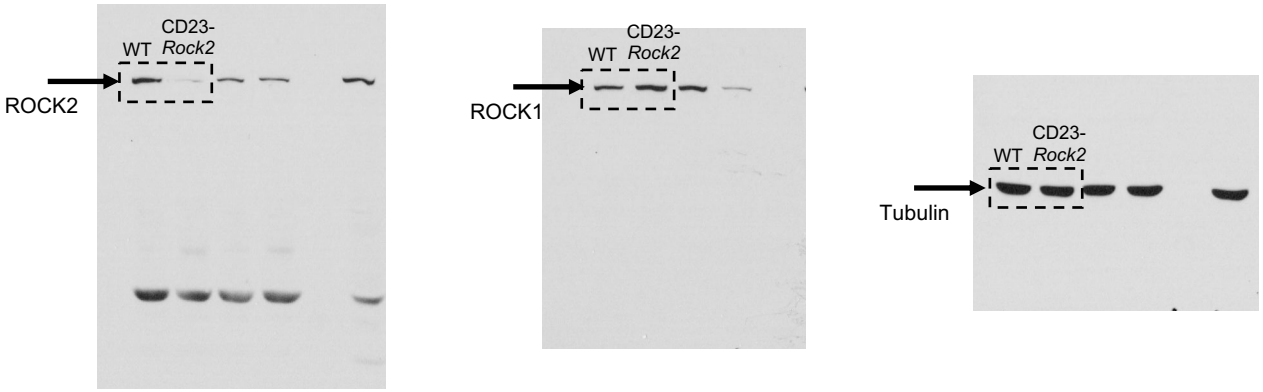


Full Uncropped Gels for Supplementary Figure 1G

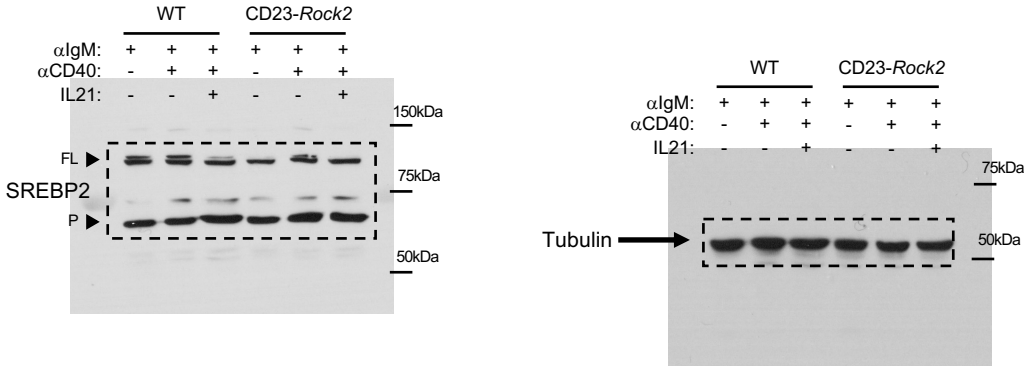


Full Uncropped Gels.

Full Uncropped Gels for Supplementary Figure 2B



Full Uncropped Gels for Supplementary Figure 7E



Full Uncropped Gels for Supplementary Figure 9D