SUPPLEMENTAL INFORMATION

Toll-like receptor mediated inflammation directs B cells towards protective antiviral extrafollicular responses

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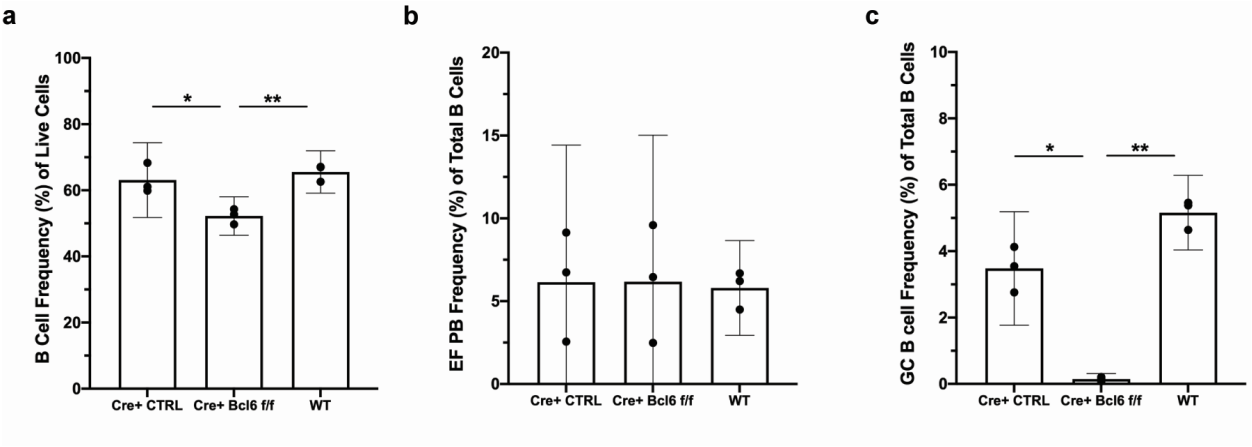
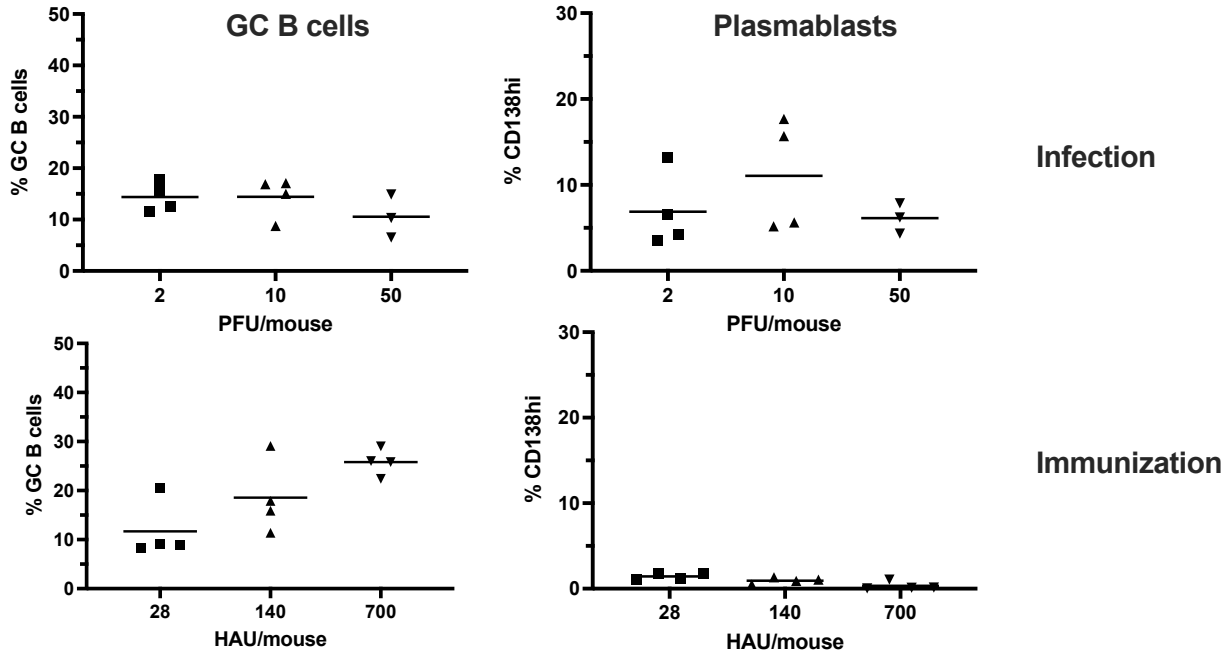
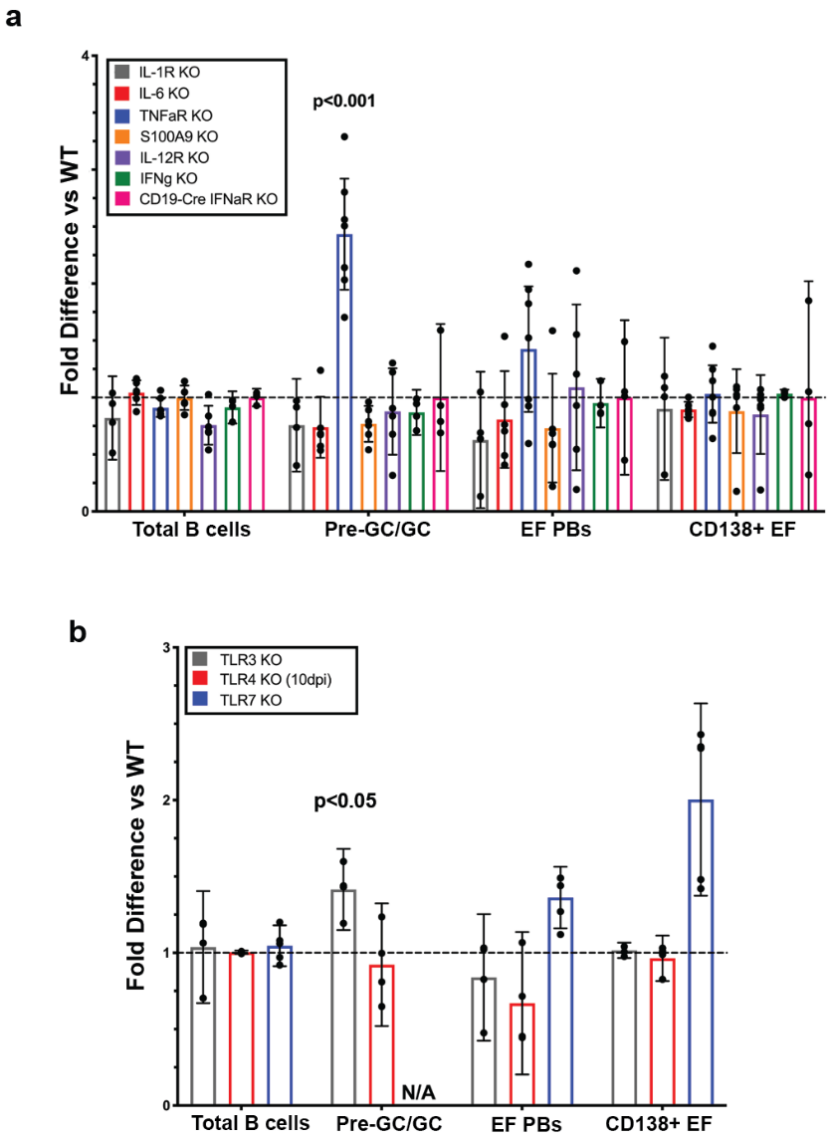
Supplemental Figures 1-12



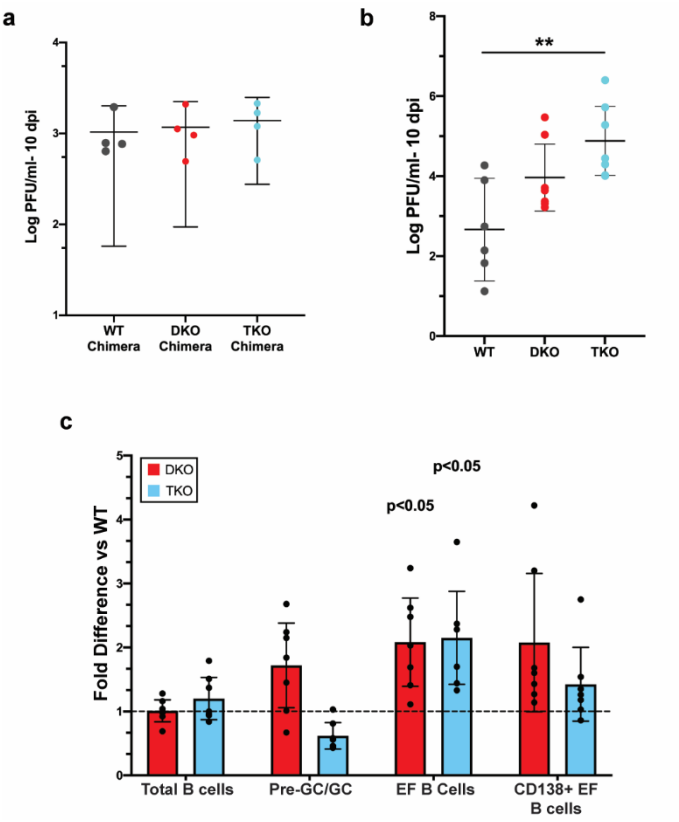
Supplemental Figure 1. EFR are not generated through GC reactions WT, Mb-1 Cre+ control, and Mb-1 Cre+ Bcl6 f/f mice were infected with 10 PFU A/PR8 and medLNs were analyzed at 10 dpi. **(a)** B cell frequency of total cells. **(b)** EF PB frequency of total B cells. **(c)** GC B cell frequency of total B cells. Error bars represent 95% CI, p-values determined by unpaired Student's t-test with Welch's correction. *: $p<0.05$, **: $p<0.01$.



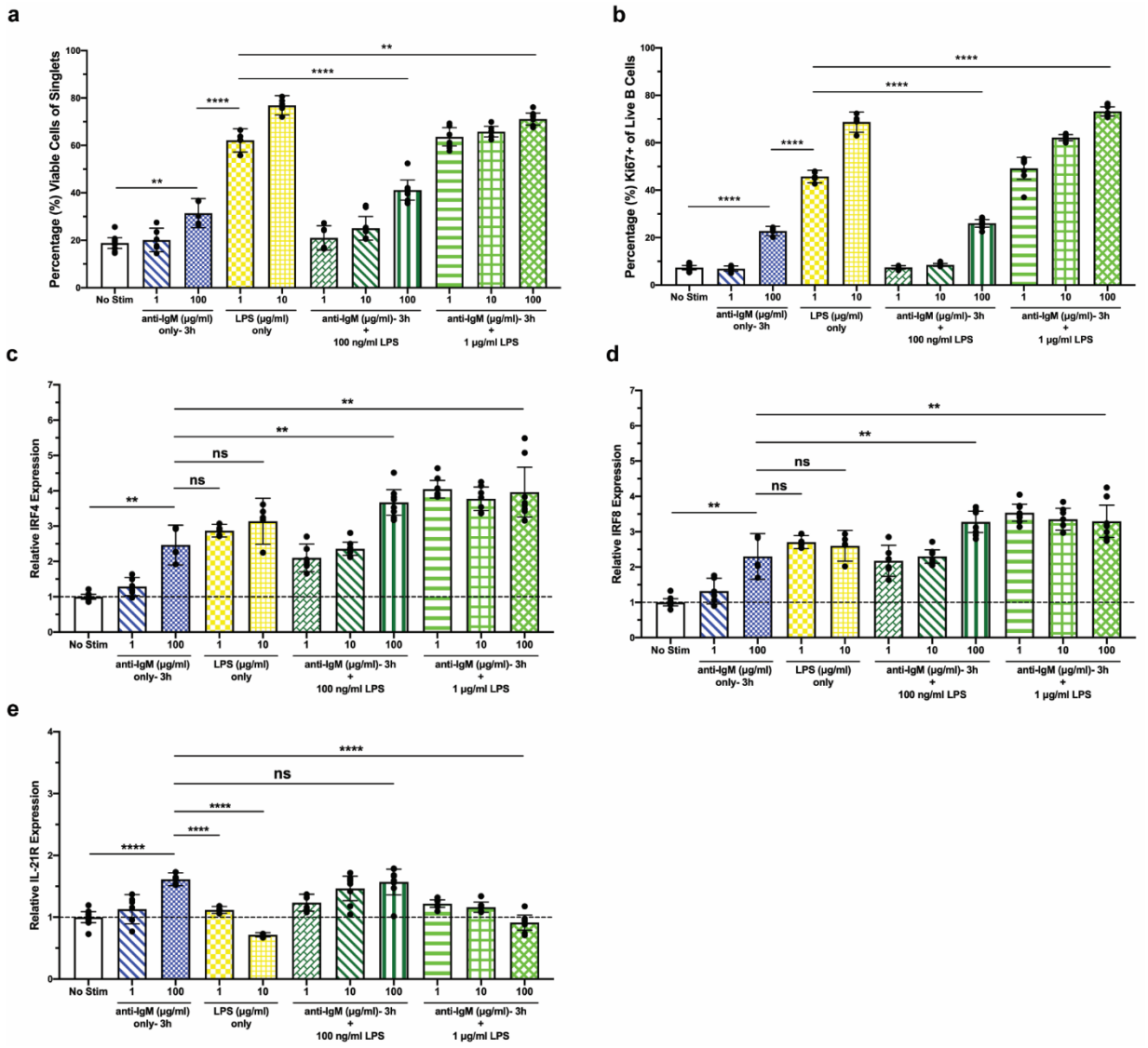
Supplemental Figure 2. Antigen-dose increases germinal centers but not plasmablast responses. Scatter plots indicate frequencies of B220+ CD38+ CD24hi germinal center B cells (left) and B220lo CD138hi plasmablasts in draining lymph nodes of BALB/c mice (n=4 per group) either infected intranasally with indicated PFU influenza A/PR8 (top) or immunized s.c. with indicated HAU sucrose-gradient purified influenza A/PR8 virion emulsified in Complete Freund's adjuvant. Analysis was done on day 7 after infection/immunization. All data are frequencies of cells after gating for live, lymphocytes.



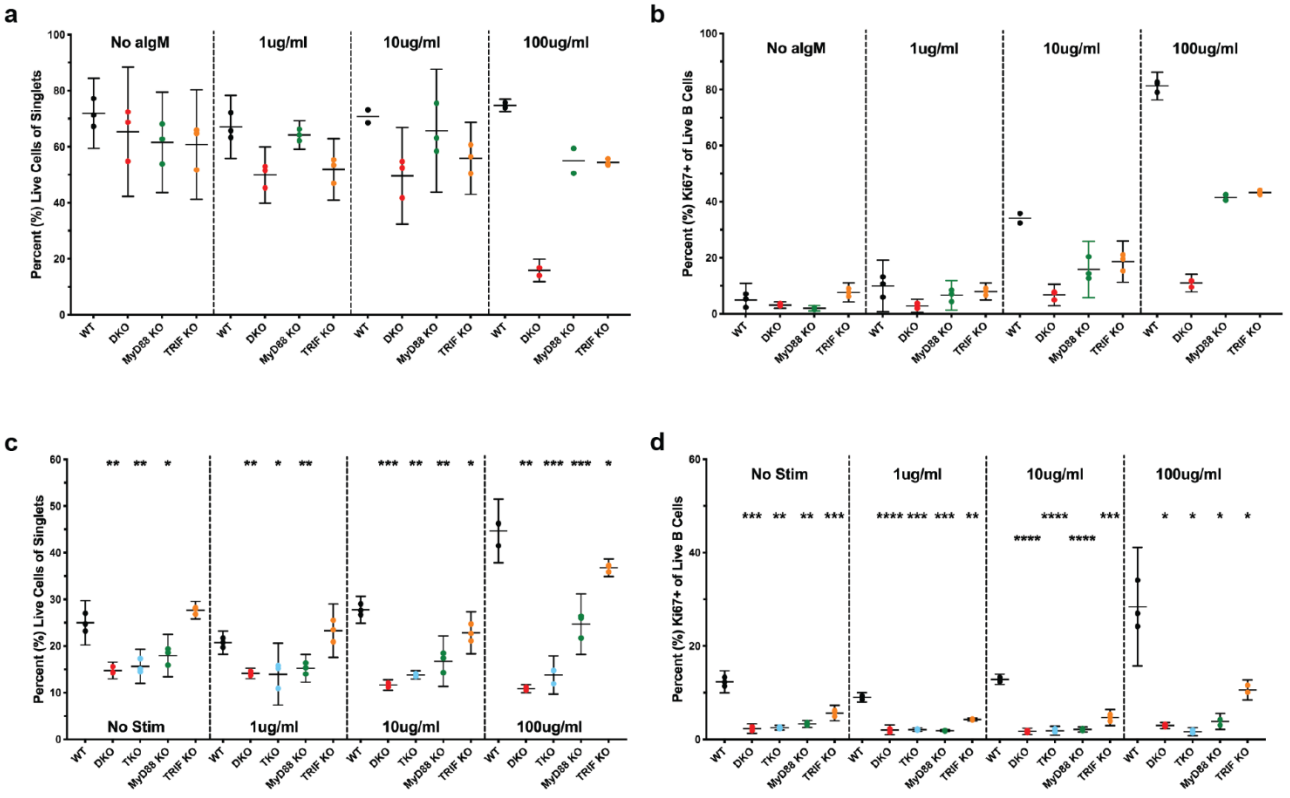
Supplemental Figure 3. Lack of single cytokine or TLR pathway does not affect EFR to influenza. Knockout and WT mice were infected with 10 PFU A/PR8 and medLNs were collected at 7 days post-infection (dpi). Shown are fold-differences in major B cell subsets in C57BL/6 WT and indicated gene-targeted mice (**a**), as well as **(b)** single TLR-knockouts. Error bars represent 95% CI, p-values determined by unpaired Student's t-test with Welch's correction. P-values indicated on charts.



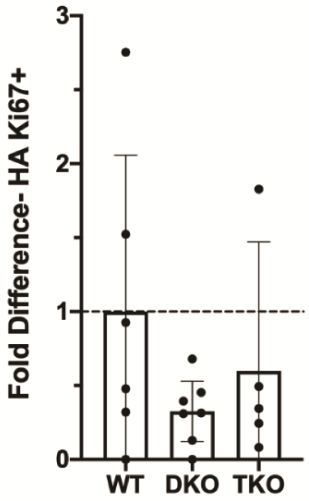
Supplemental Figure 4. **Passive protection with TKO serum and late-stage viral loads and EF responses.** (**a,b**) Viral loads as analyzed by viral qRt-PCR on lung homogenates at 10 dpi of B cell-specific chimera (a) and global knockout (b) mice. **c)** Fold-differences of B cell subsets in DKO, TKO and WT mice at 7 dpi. Graphs are representative of two experiments (n>4). Error bars represent 95% CI. Statistical significance determined by one-way ANOVA and unpaired Student's t-test with Welch's correction. *: p<0.05 (or shown) **: p<0.01 ***: p<0.001, ****: p<0.0001. *****" p>1E-5.



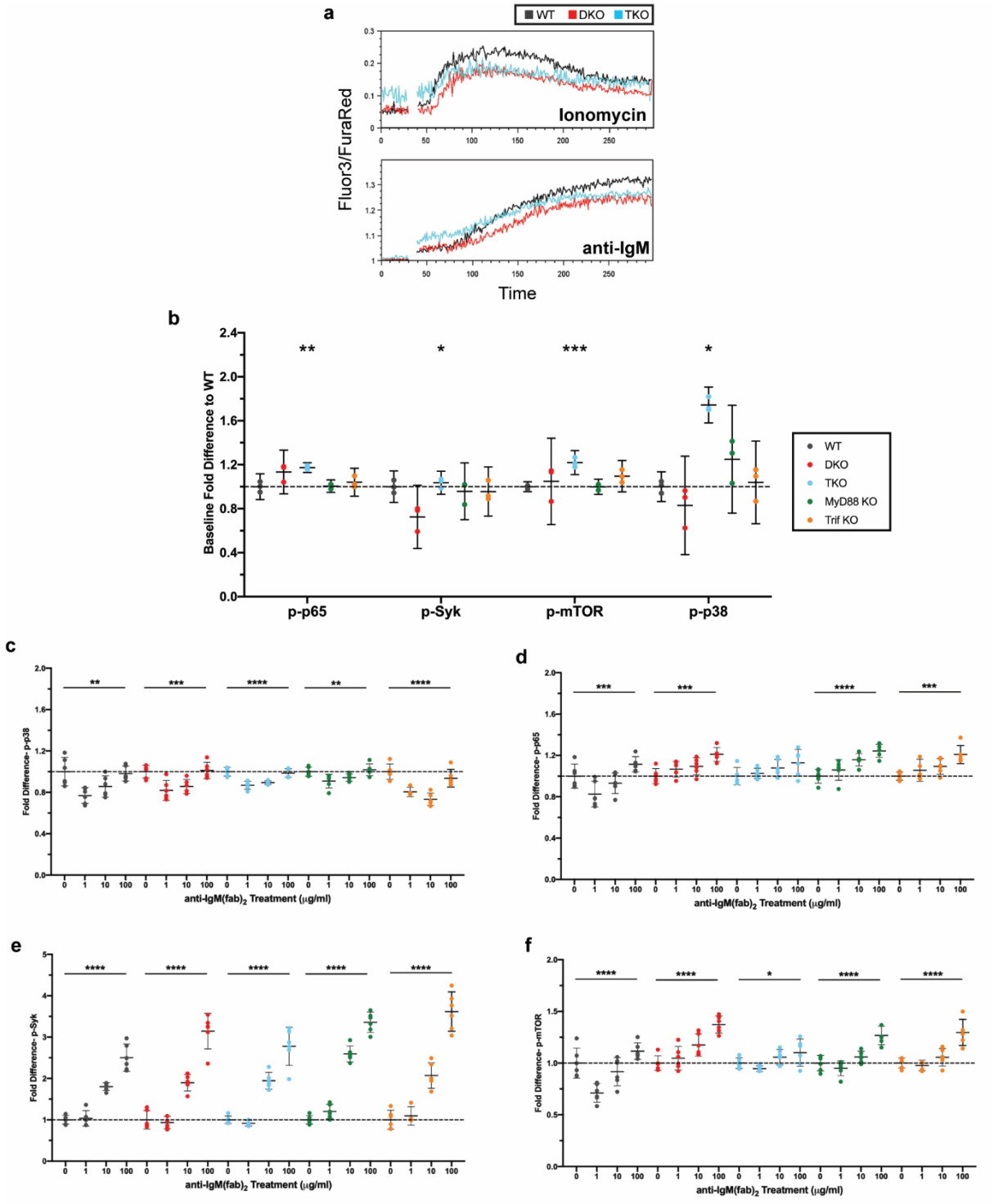
Supplemental Figure 5. BCR and TLR4 stimulation have additive effects on cell viability, proliferation, and activation phenotype. Pooled and enriched splenic and lymph node WT B cells were cultured with indicated graded concentrations anti-IgM 3h pulsed or graded, sustained concentrations of LPS and assessed for **(a**) cell viability, **(b**) proliferation, (**c**) IRF4 (**d**) IRF8, and (**e**) IL-21R expression. Graphs are representative of two experiments (n=4). Error bars represent 95% CI. Statistical significance determined by one-way ANOVA and unpaired Student's t-test with Welch's correction. *: p<0.05 **: p<0.01 ***: p<0.001, ****: p<0.0001.



Supplemental Figure 6. MyD88 and TRIF non-redundantly regulate B cell viability and proliferation in response to BCR stimulus. Pooled splenic/LN WT, DKO, MyD88 KO, and TRIF KO B cells were negatively enriched (>98%) and pulsed with graded anti-IgM for 3 hours, then stimulated with CD40L and BAFF for 48 hours and assessed for **(a)** viability and **(b)** proliferation. **(c,d)** Cells as in **(a)** were pulsed with graded doses anti-IgM for 3 hours followed by incubation in complete medium only for 48h, then assessed for (**c**) cell viability and (**d**) proliferation. Error bars represent 95% CI. Statistical significance determined by one-way ANOVA and unpaired Student's t-test with Welch's correction. *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$, ****: $p<0.0001$.



Supplemental Figure 7. Antigen-specific B cells have greater proliferative capacity than TLR-signaling deficient B cells after influenza infection. Relative fold-change in HA-specific, Ki67+ B cells compared to WT controls in medLN of mice at 5 dpi with influenza A/PR8. Graph is representative of two experiments (n>/=5). Error bars represent 95% CI.



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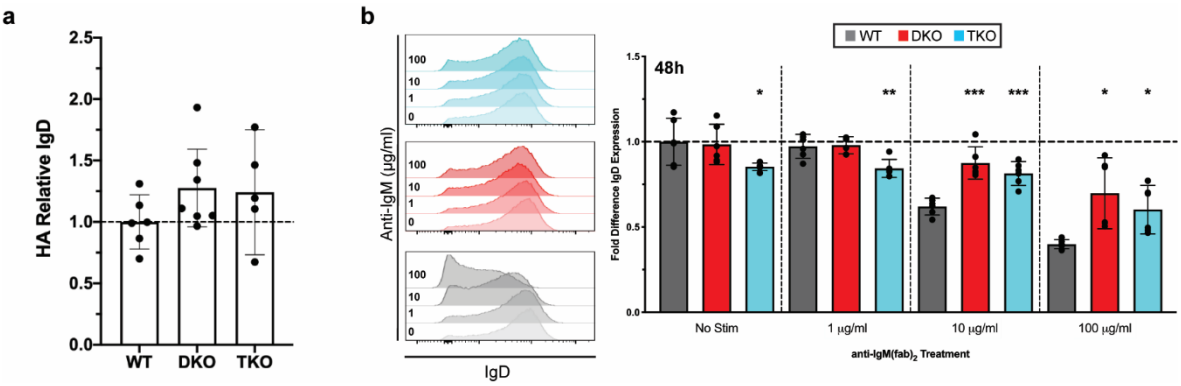
79 **Supplemental Figure 8. TLR-signaling deficient B cells have nominal immediate**

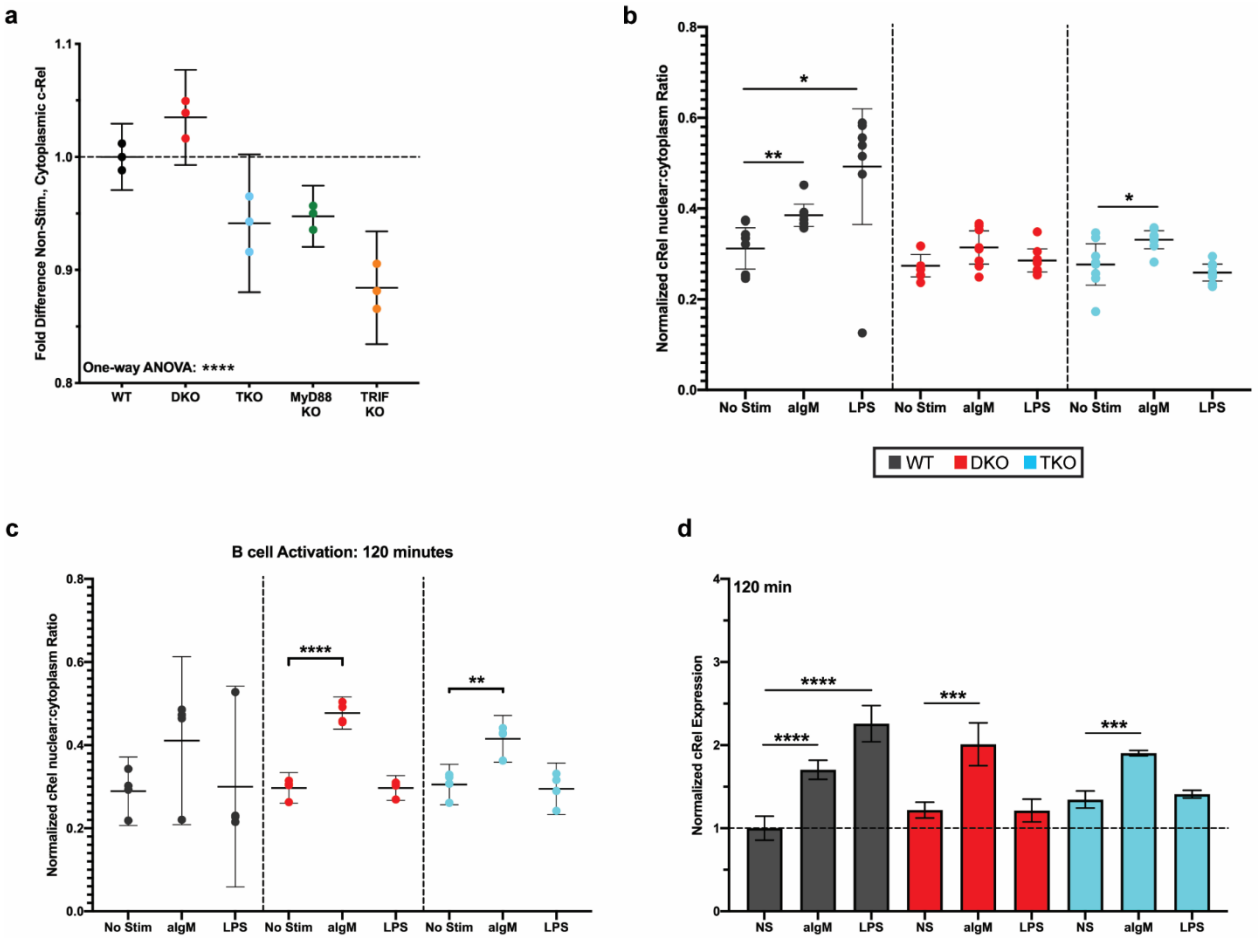
80 **activation and functional downstream effector protein activation**. (**a**) Calcium flux of BCR-

81 independent (iono) and BCR-mediated, anti-IgM(Fab)$_2$ activation in purified WT, DKO, and TKO

B cells. (**b,c**) Pooled splenic/LN WT, DKO, TKO, MyD88 KO, and TRIF KO B cells were negatively enriched (>98%) and stimulated with graded indicated doses anti-IgM$(Fab)_2$ for 30 minutes, then assessed for phosphorylated, intracellular proteins. (**b**) Baseline fold-difference in phosphorylation between WT and knockout B cells. (**c**) Fold-changes in protein phosphorylation of target proteins relative to non-stimulated conditions for each strain. Graphs are representative of two experiments (n=6) except for (b) (n=3). Error bars represent 95% CI. Statistical significance determined by one-way ANOVA only. *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$, ****: $p<0.0001$.



Supplemental Figure 9. Early HA-specific B cells in DKO and TKO BMCs have an anergic phenotype early during influenza infection. (**a,b**) Relative expression of IgD *in vivo* at 5 dpi in HA-specific B cells from bone marrow chimeras (a) and *in vitro* after 48h anti-IgM pulse plus CD40L/BAFF treatment (b). Graphs are representative of two experiments (n>/=5). Error bars represent 95% CI. Statistical significance determined by one-way ANOVA only. *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$.



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107 **Supplemental Figure 10. Immediate activation of c-Rel after BCR stimulation is defective**

108 **in TLR-signaling deficient B cells**. **(a)** (**b-c**) Shown are the ratios of nuclear vs cytoplasmic c-

109 Rel in WT, DKO, and TKO B cells as determined by ELISA on nuclear versus cytoplasmic

110 protein extracts at 60 minutes **(b)** and 120 minutes **(c)** after anti-IgM or LPS stimulation. (**d**)

111 Total c-Rel expression after 120-minute anti-IgM or LPS stimulation as assessed by flow

112 cytometry. Error bars represent 95% CI. Statistical significance determined by one-way ANOVA

113 (a, intra-strain comparisons in b-d) and unpaired Student's t-test with Welch's correction (b-d). *:

114 $p<0.05$ **: $p<0.01$ ***: $p<0.001$, ****: $p<0.0001$.

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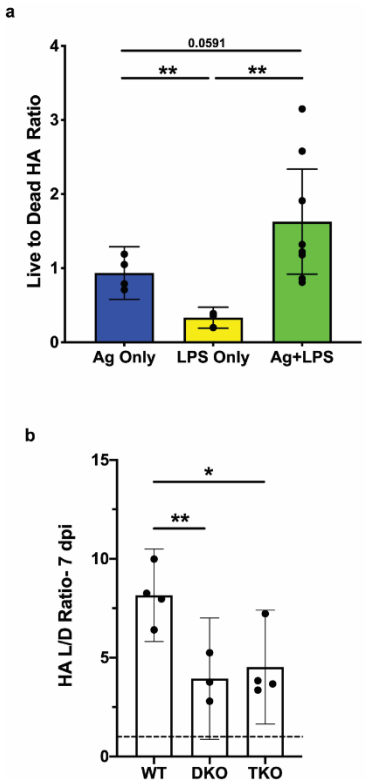
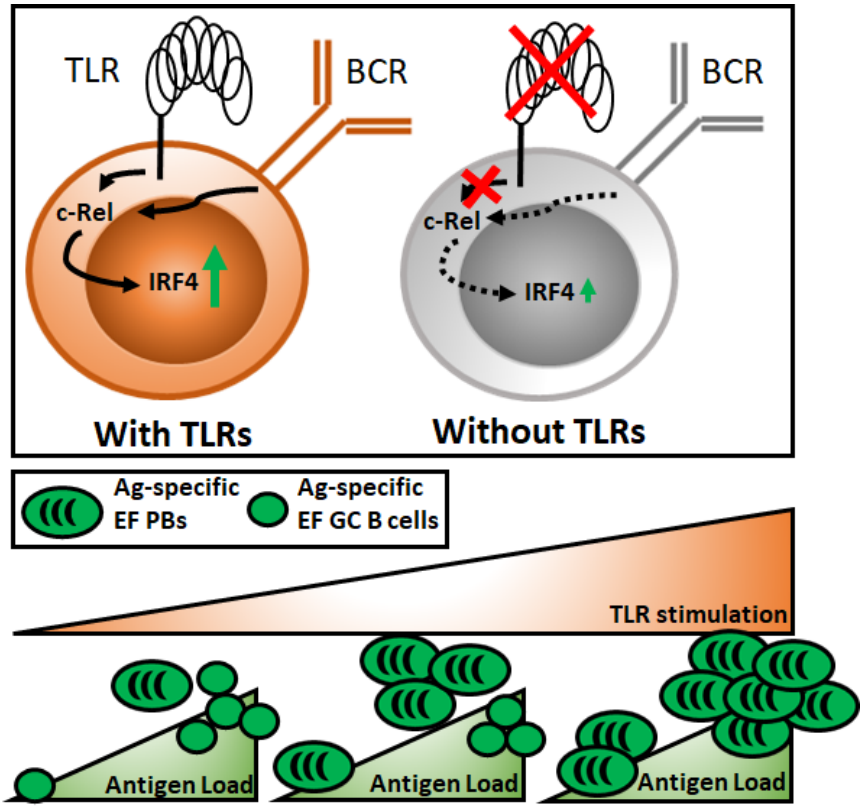


Supplemental Figure 11. HA-specific B cell survival is modulated by TLR signals. (a) Mice were immunized s.c. with or without influenza in alum and with or without LPS, then boosted with either LPS or PBS on days specified, followed by analysis of draining LN. The ratio of live versus dead HA-specific B cell populations were assessed for each treatment. (**b)** Analysis of live versus dead HA-specific B cells as in (a) but from infected mice, 7 dpi. Graphs are representative of two experiments (n>/=3). Error bars represent 95% CI, statistical significance determined by one-way ANOVA ($p<0.01$ for both a,b) and unpaired Student's t-test with Welch's correction (d,e). *: $p<0.05$ **: $p<0.01$.



Supplemental Figure 12. Model of TLR-mediated induction of B cell differentiation and EFRs. (Top) With a functional TLR axis either through MyD88/TRIF or TLR2/4/unc93b, BCR stimulation leads to efficient c-Rel nuclear localization and sustained upregulation of IRF4. Without a functional TLR axis, BCR signaling leads to delayed c-Rel localization, lack of long-term c-Rel expression, and a lack of sustained IRF4 induction and upregulation. (Bottom) During infection or immunization, TLR agonism or an equivalent aggregate of inflammatory stimuli polarizes antigen-specific B cells towards EFR and away from the GC response when antigen is limiting or in excess, resulting in early and protective antigen-specific antibody generation.