## **Appendix Figure Legends**

#### Appendix Figure S1. BKO mouse B cells are hyperactive and hyperproliferative

(A) MFI of MHC II and CD86 activation biomarkers for CD19+ B cells in lymph nodes of WT (n=6) and BKO (n=9) mice. Mean  $\pm$  SD; \*\*p=0.0022 and \*p=0.03 two-tailed, unpaired Student's t test, respectively

(B) MFI of CD69 for CD19+ LKB1+YFP- and LKB1-YFP+ B cells from BKO-YFP mice (n=4). Mean ± SD

(C) Flow cytometry of in vitro cell division over 3 days using Celltracer dye dilution for anti-CD40 Ab plus IL-4 stimulated splenic B cells from WT-YFP and BKO-YFP mice. Plots shown are representative of 3 independent experiments.

(D) Flow cytometry of in vitro cell division over 3 days using Celltracer dye dilution for anti-CD40 Ab plus IL-4 stimulated splenic B cells BKO-YFP mice. (Right panel) The percentage of live LKB1-YFP+ and LKB1+YFP- B cells in each generation is graphed. Mean ± SD for three independent experiments, \*\*\*p=0.004 and 0.004 two-tailed, unpaired Student's t test, respectively

(E) (Left panel) Percentage of harvested splenic B cells from WT-YFP and BKO-YFP mice that incorporate BrdU over 2 days and (Right panel) percentage of LKB1+YFP- and LKB1-YFP+ B cells from BKO-YFP mice that incorporate BrdU over 2 days. Mean  $\pm$  SD from three independent experiments; \*\*p=0.007 two-tailed, unpaired Student's t test

(F) Flow cytometry for the expression of CD44 on CD4+ T cells in the lymph nodes of WT (n=5) and BKO (n=5) mice. Mean  $\pm$  SD; \*\*p=0.008 by Mann-Whitney U test

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### Appendix Figure S2. BKO B cells secrete inflammatory cytokines

(A) Cytokine and chemokine array surveyed with conditioned media from unstimulated CD43 depleted B cells from WT and BKO spleens. Mean values are plotted for proteins from two WT and two BKO mice that were increased 1.4-fold or more in BKO versus WT conditioned media (B) Cytokine profiles of CD43 depleted naïve WT-YFP and naïve BKO-YFP B cells were compared with equivalent numbers of WT B cells stimulated with LPS for 24 hours and BMDM stimulated with LPS for 4 hours by ELISA for RANTES, Mip-1 $\alpha$ , MIP-1 $\beta$ , IP-10, and IL-6. Corrected absorbance is shown as mean  $\pm$  SD (n=3); \*\*\*\*p=0.0001, 1.9E-07, 2.2E-06, 1.5E-05, 7.6E-05, \*\*p=0.0054, 0.0091, and \*p=0.034 by two-tailed, unpaired Student's t test, respectively

### Appendix Figure S3. LKB1 regulates IL-6 production through NF-κB signaling

- (A) Western blot analysis of signaling through ERK1/2 in WT and LKB1- MEFs
- (B) Western blot analysis of signaling through p38 in WT and LKB1- MEFs
- (C) Western blot analysis of signaling through JNK in WT and LKB1- MEFs
- (D) Western blot analysis of signaling to CREB transcription factor in WT and LKB1- MEFs
- (E) Western blot analysis of JunB expression in WT and LKB1– MEFs

(F) qRT-PCR analysis of *II6* expression, relative to *36b4* expression, from LKB1– MEFs treated with DMSO or an escalating concentration of the NF- $\kappa$ B inhibitor JSH-23. Mean ± SEM

# Walsh, et al., Appendix Figure S1



Walsh, et al., Appendix Figure S2







Α

# Walsh, et al., Appendix Figure S3



DMSO 50

рΜ

500

pМ

5

nM

50

nΜ

500

nM

5

μΜ

50

μМ