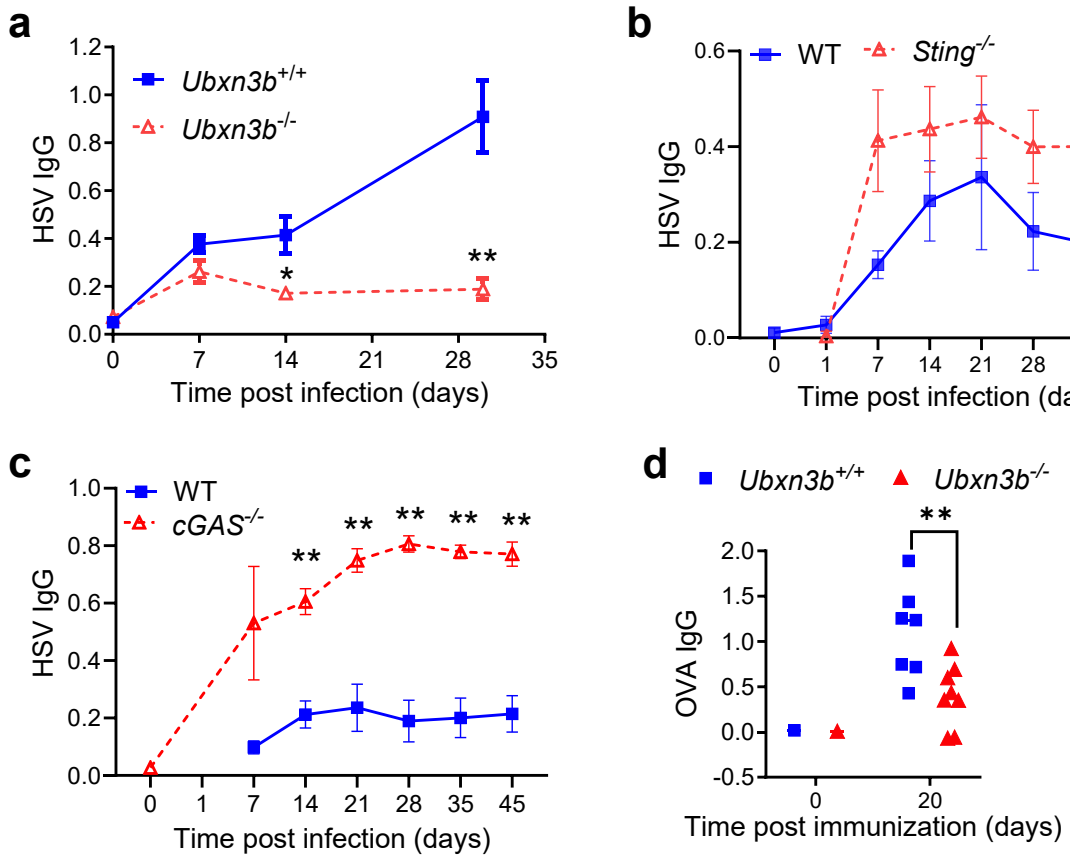
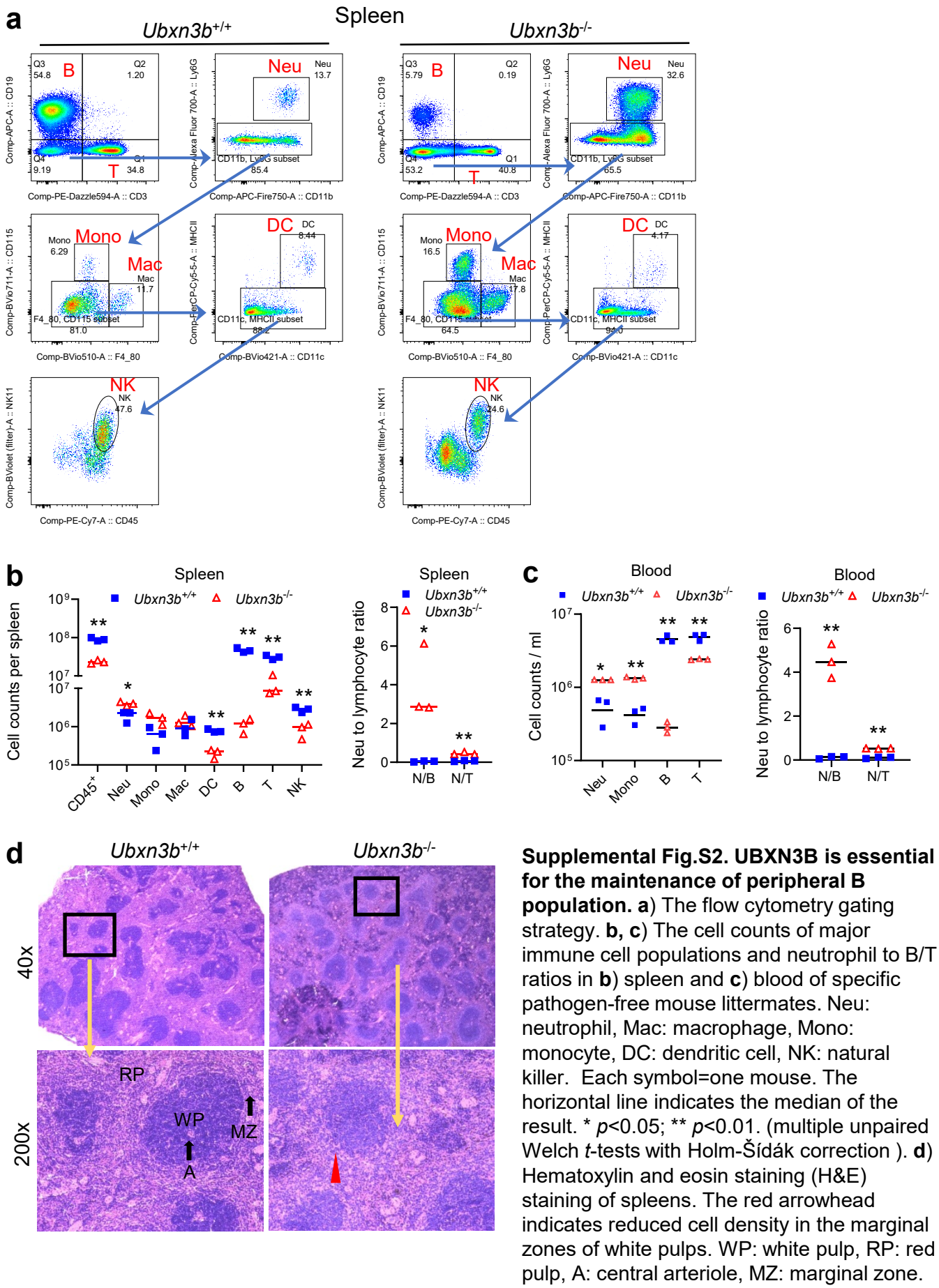
UBXN3B Is Crucial for B Lymphopoiesis

Tingting Geng et al.

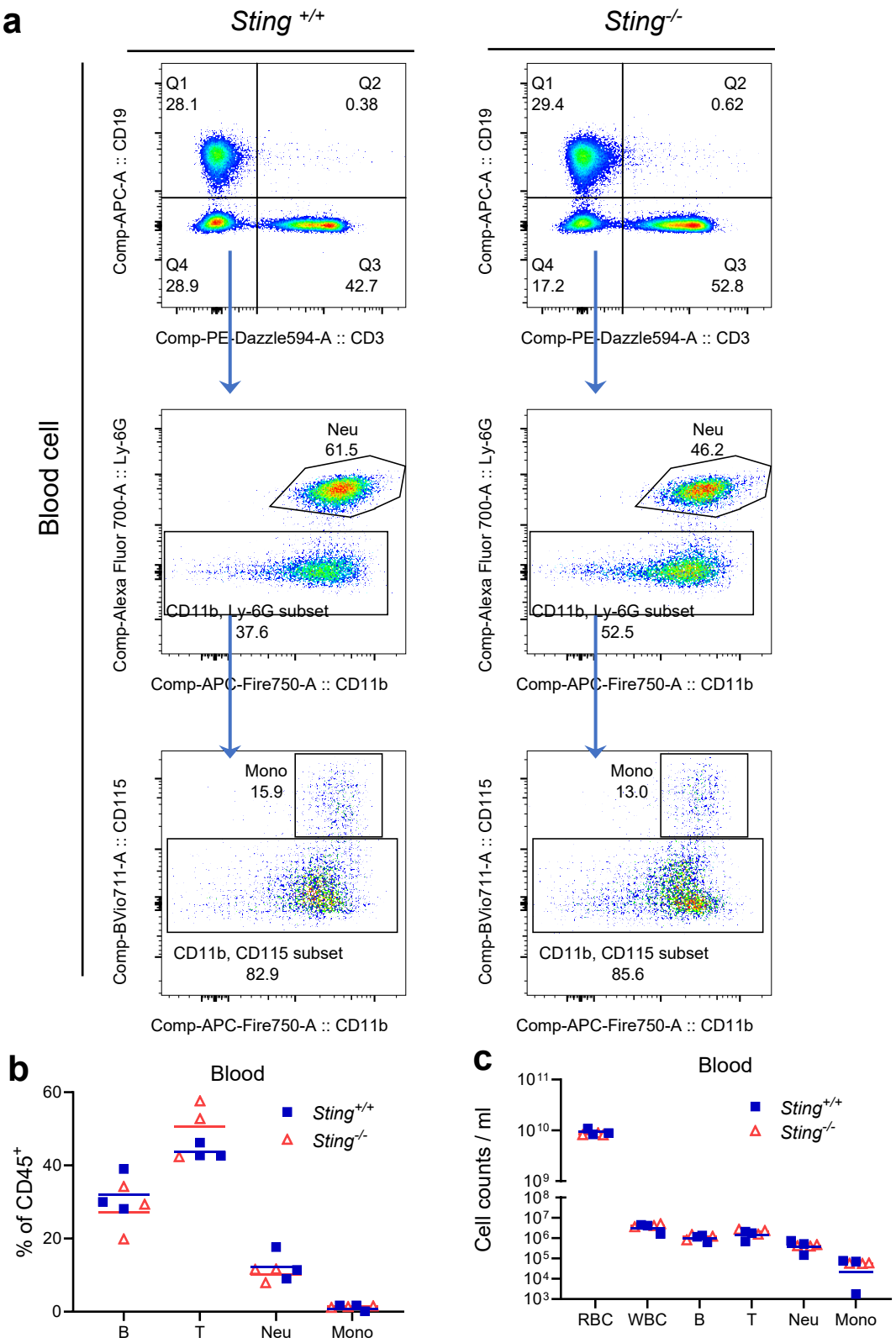
This file contains 15 supplemental figures and legends.



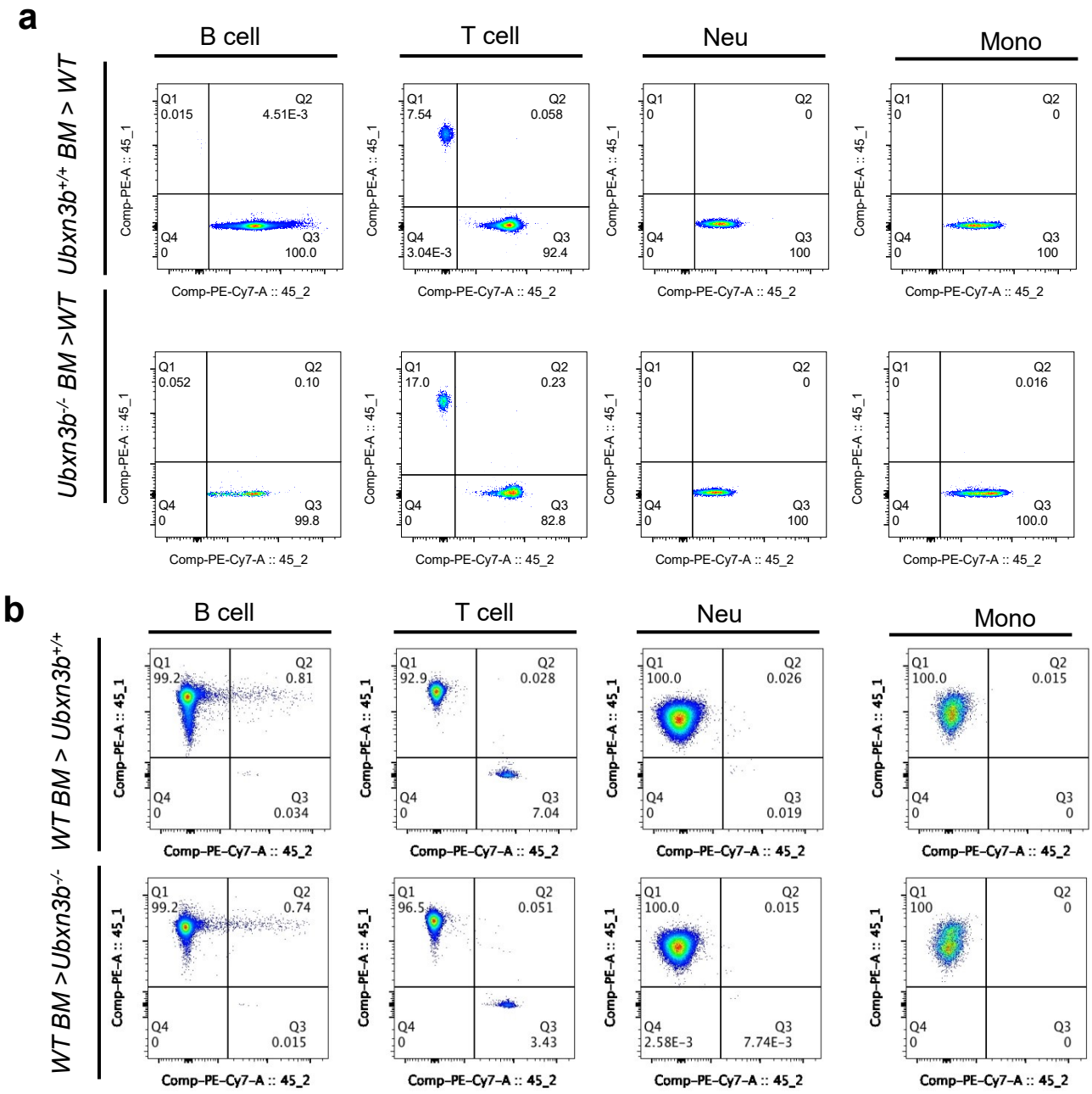
Supplemental Fig.S1. UBXN3B is critical for the IgG response to viral infection and immunization. a-c) Age and sex-matched mice were infected with $1x10^4$ plaque forming units (PFU) of HSV-1 virions intraperitoneally, or **d**) immunized with 10 µg of chicken ovalbumin (OVA) twice. The serum HSV-1-or OVA-specific IgG were quantified by ELISA and presented as optical density $O.D.450_{nm}$. In **a-c**), data point: mean ± S.E.M, N=6 for *Ubxn3b*$^{+/+}$ and *Ubxn3b*$^{-/-}$ respectively, N= 6 for WT and 5 for *Sting*$^{-/-}$ respectively, N=6 mice for WT and 3 for *cGas*$^{-/-}$ respectively. **p*<0.05, ***p*<0.01, (repeated measures two-way ANOVA with Bonferroni's multiple comparisons test). In **d**), each symbol=one animal, N=7 mice for days 24 p.i . ** *p*<0.05, two tailed unpaired *t*-test.



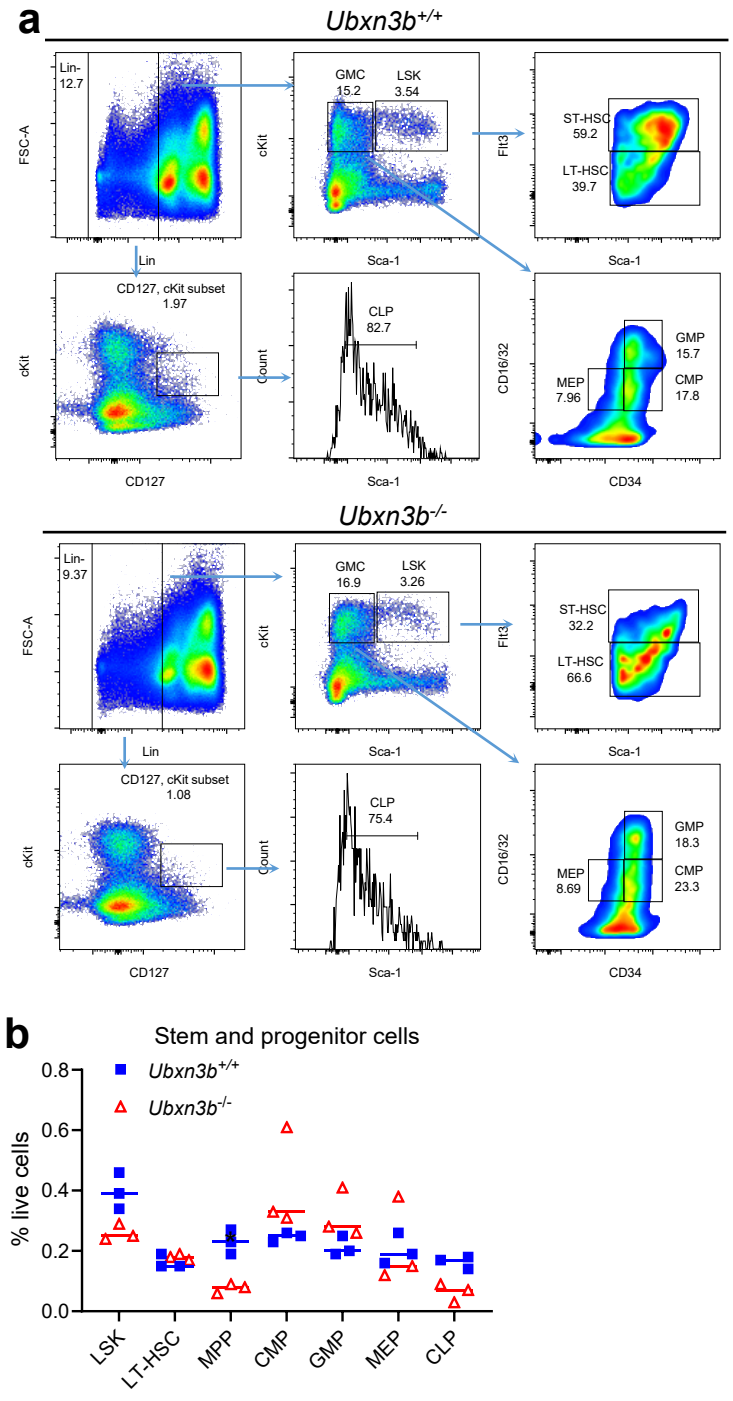
Supplemental Fig.S2. UBXN3B is essential for the maintenance of peripheral B population. a) The flow cytometry gating strategy. **b, c**) The cell counts of major immune cell populations and neutrophil to B/T ratios in **b**) spleen and **c**) blood of specific pathogen-free mouse littermates. Neu: neutrophil, Mac: macrophage, Mono: monocyte, DC: dendritic cell, NK: natural killer. Each symbol=one mouse. The horizontal line indicates the median of the result. * p<0.05; ** p<0.01. (multiple unpaired Welch t-tests with Holm-Šídák correction). **d**) Hematoxylin and eosin staining (H&E) staining of spleens. The red arrowhead indicates reduced cell density in the marginal zones of white pulps. WP: white pulp, RP: red pulp, A: central arteriole, MZ: marginal zone.



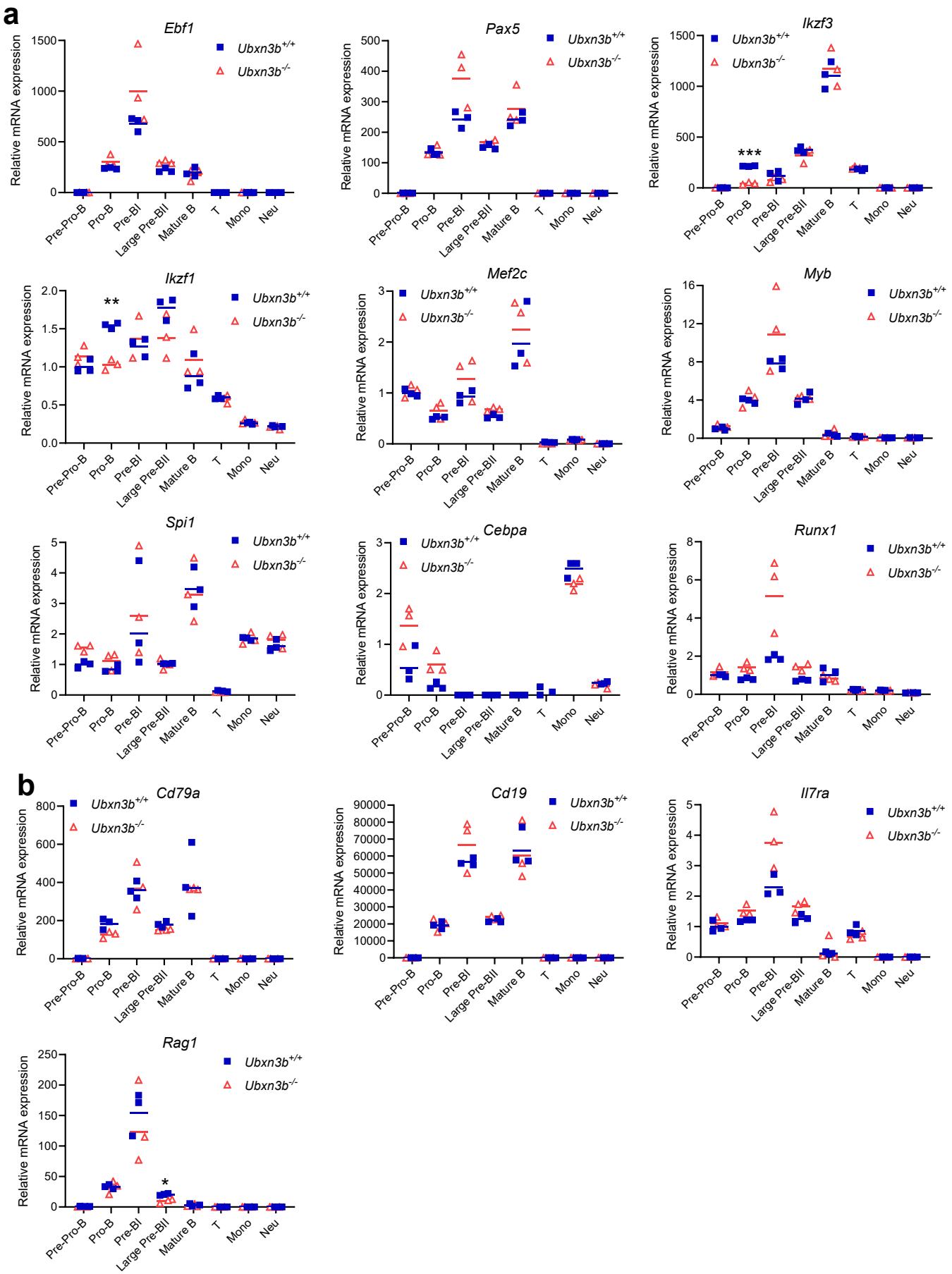
Supplemental Fig.S3. STING is dispensable for steady-state hematopoietic homeostasis. **a**) The flow cytometry gating strategy, **b**) The percentage and **c**) counts of major blood cell types in *Sting*$^{+/+}$ and *Sting*$^{-/-}$ littermates, quantitated by flow cytometry. Each symbol=one animal. N=3 mice/genotype. The horizontal line indicate the mean of the results. RBC: red blood cell, WBC: white blood cell, CD19^{+}: B cell, CD3^{+} : T cell, Neu: neutrophil, Mono: monocyte. Multiple Unpaired Welch *t*-test was use for b) and c) with no significant difference.



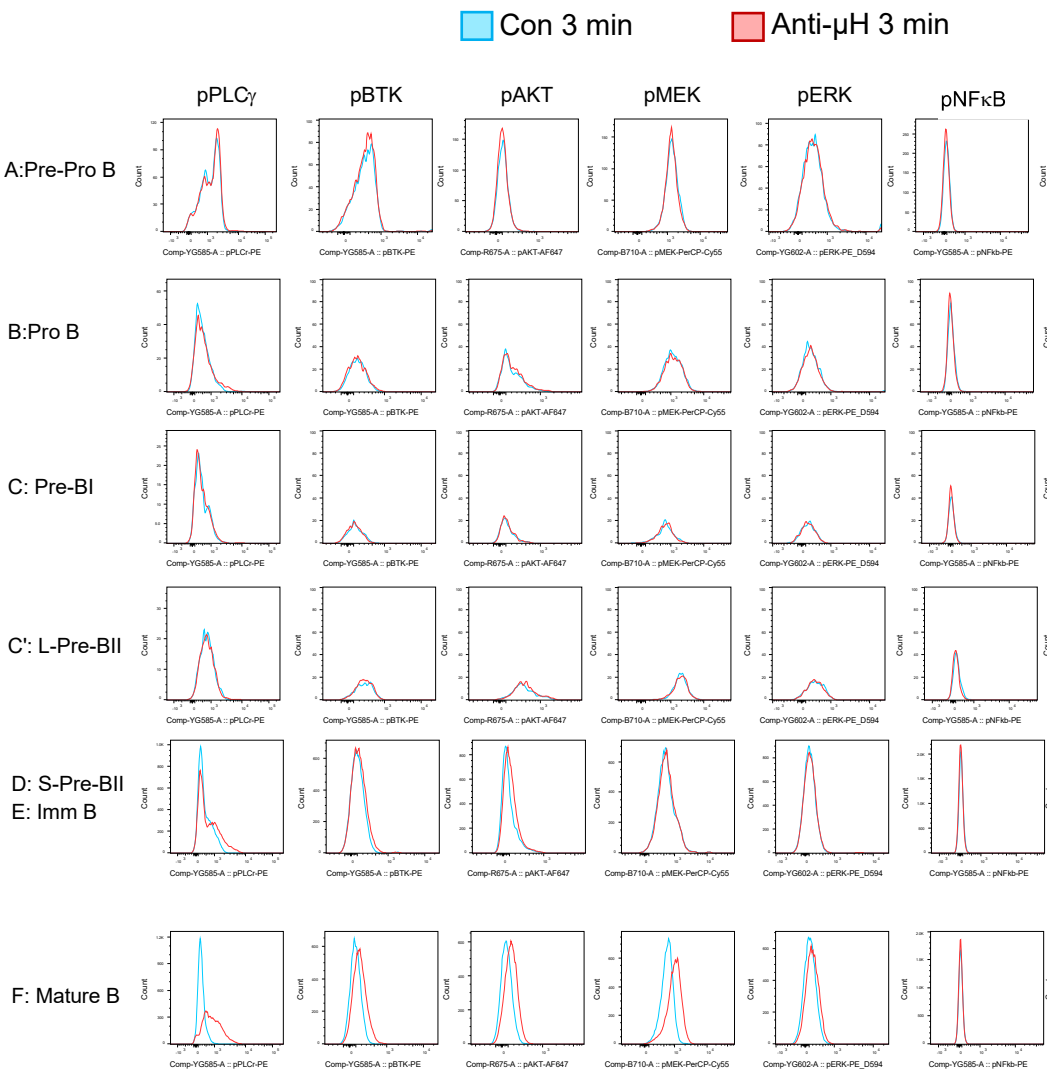
Supplemental Fig.S4. The percentage of blood CD45.1 and CD45.2 cells 1.5 month after bone marrow transplantation (BMT). a) Irradiated wild type (WT, CD45.1) mice were transplanted with *Cre*$^{+}$*Ubxn3b*$^{f/f}$ (CD45.2) bone marrow . The mice were then treated with tamoxifen (TMX) to delete Ubxn3b (designated *Ubxn3b*$^{-/-}$ BM–WT) or not (designated *Ubxn3b*$^{+/+}$ BM–WT) in hematopoietic cells. Over 99% B/Neu/Mono are CD45.2^{+}, over 82% T cell are CD45.2^{+}, indicating successful irradiation and reconstitution. **b**) Irradiated *Cre*$^{+}$*Ubxn3b*$^{f/f}$ and *Ubxn3b*$^{f/f}$ (CD45.2) mice were transplanted with wild type (WT, CD45.1) bone marrow . The mice were then treated with tamoxifen (TMX) to delete Ubxn3b (designated WT BM–*Ubxn3b*$^{-/-}$) or not (designated WT BM–*Ubxn3b*$^{+/+}$) in non-hematopoietic cells. Over 99% B/Neu/Mono are CD45.1^{+}, over 92% T cell are CD45.1^{+}, indicating successful irradiation and reconstitution. Neu: neutrophil, Mono: monocyte.



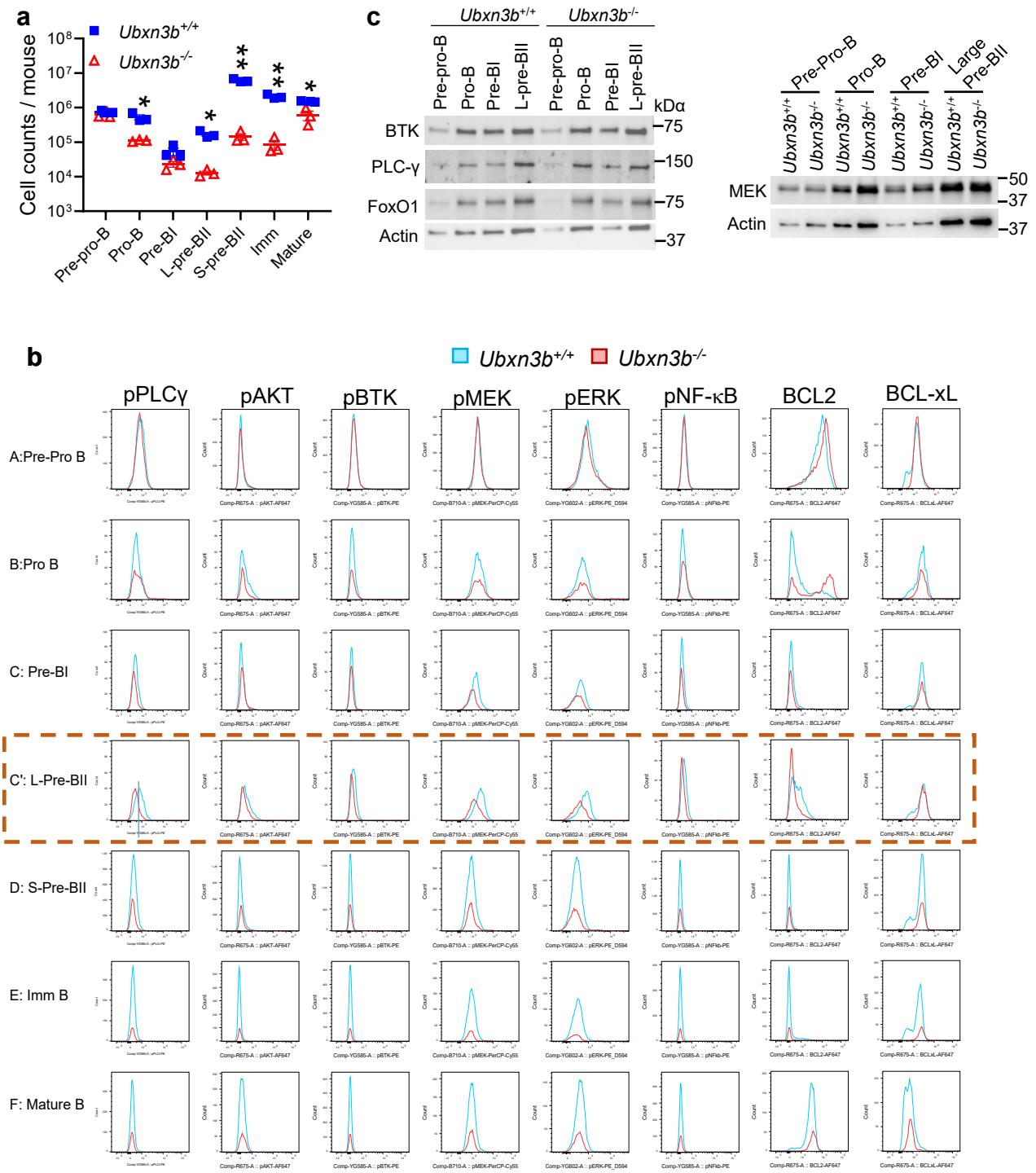
Supplemental Fig.S5. Bone marrow stem cells and CLPs are modestly reduced in *Ubxn3b*$^{-/-}$ mice. **a**) The flow cytometry gating strategy for hematopoietic stem cells (HSC, Lin$^-$, Sca1$^+$ Kit$^+$) and lineage progenitors in the bone marrows of *Ubxn3b*$^{+/+}$ and *Ubxn3b*$^{-/-}$ littermates. **b**) The percentage of each cell type. Each symbol=one animal. N=3 mice/genotype. The horizontal line indicate the mean of the results. LT-HSC: long-term HSC, MPP: multipotent progenitor, CLP: common lymphoid progenitor, CMP: common myeloid progenitor, GMP: granulocyte-macrophage progenitor, MEP: megakaryocyte-erythrocyte progenitors.



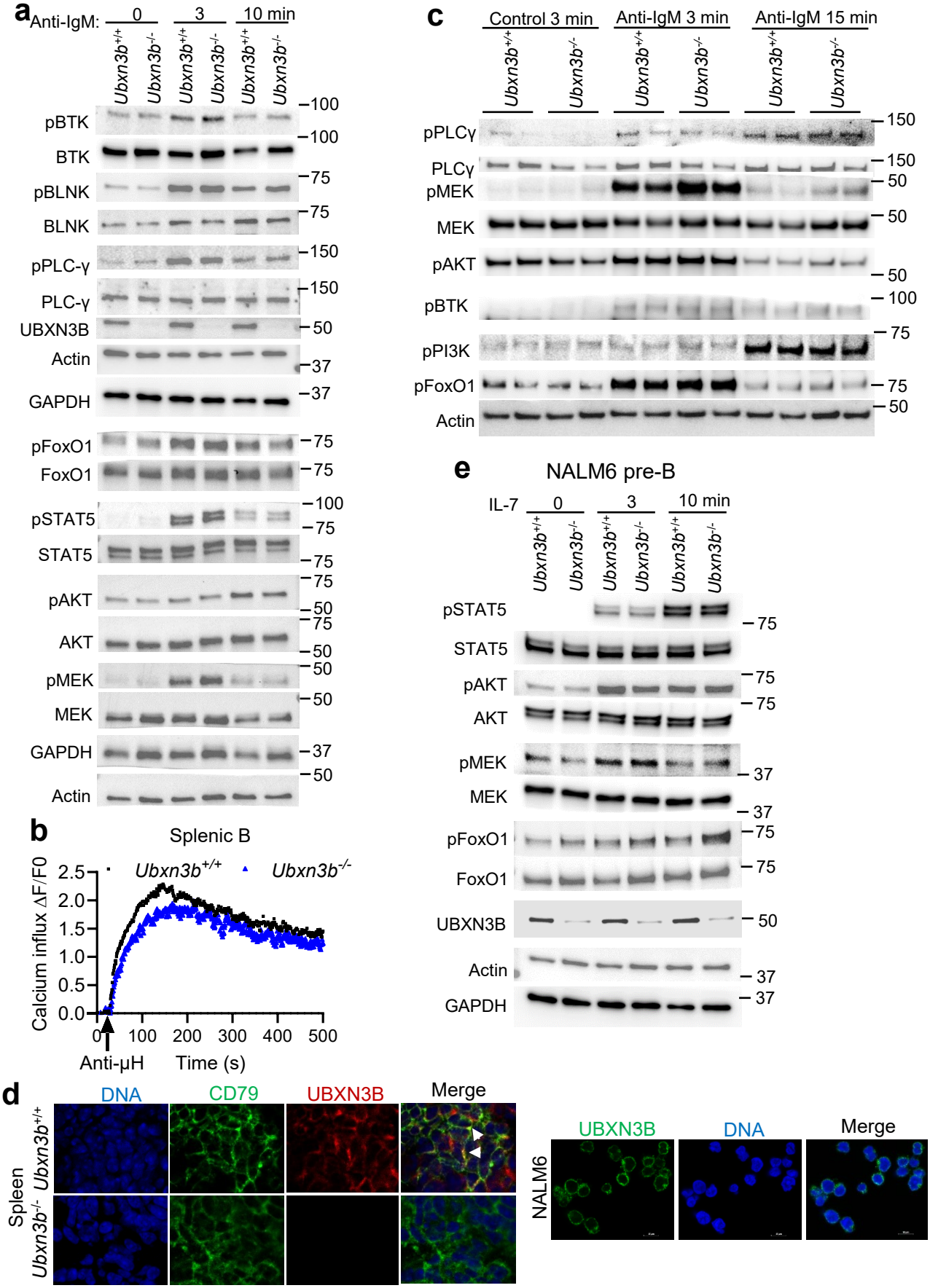
Supplemental Fig.S6. qRT-PCR quantification of transcription factors and surface markers in steady-state *Ubxn3b*$^{+/+}$ and *Ubxn3b*$^{-/-}$ littermates. The bone marrow cell compartments were sorted by flow cytometry and the mRNA levels of **a**) transcription factors and **b**) cell surface markers and *Rag1* were quantitated by qRT-PCR. Each symbol=one animal. N=3 mice/genotype. The horizontal line indicates the mean of the results. ** p<0.01 (multiple unpaired Welch t-tests with Holm-Šídák correction).



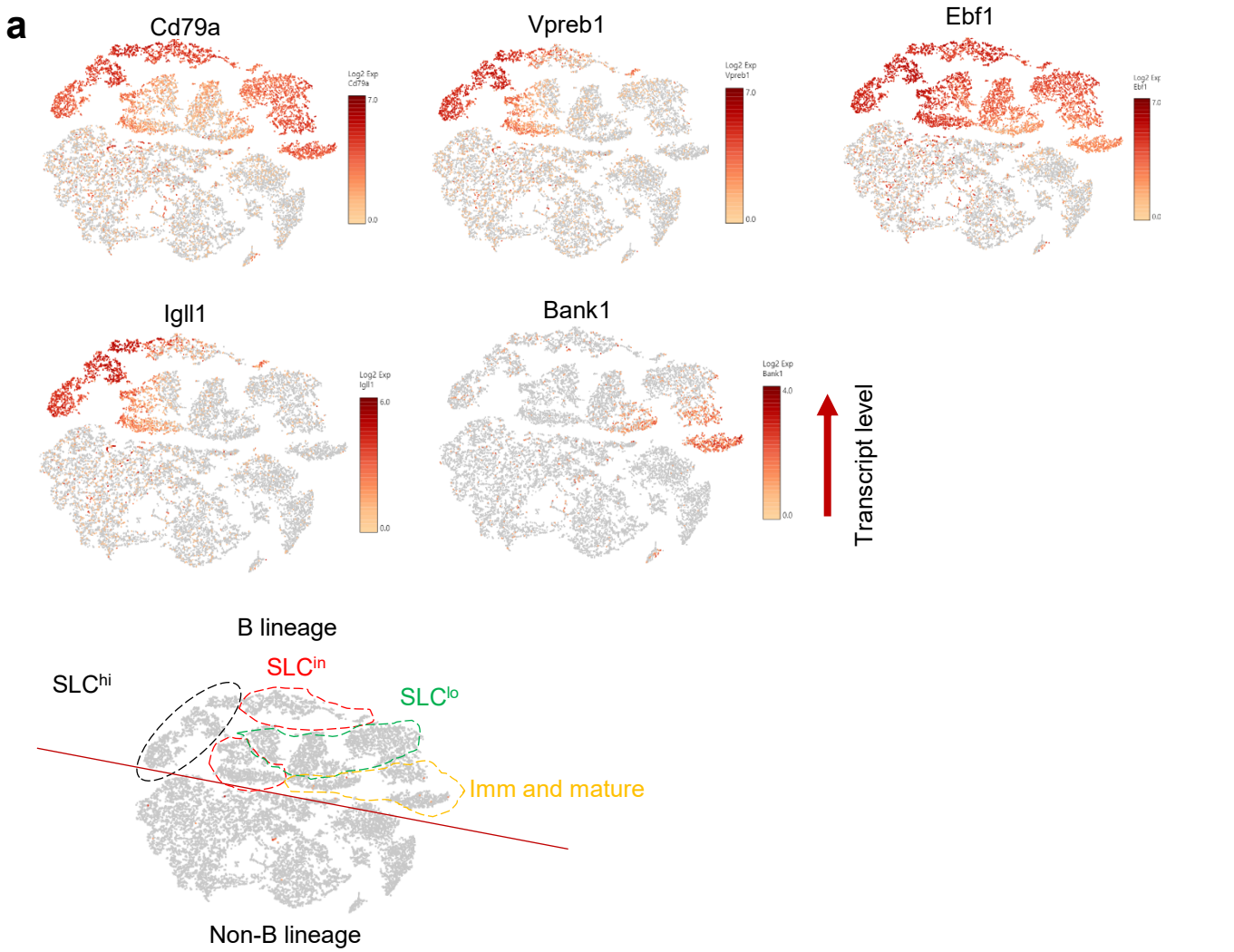
Supplemental Fig.S7. Cross-linking of BCR by an anti-μH antibody stimulates BCR signaling robustly only in mature B cells. C57/BL6 mouse bone marrow B cells were stimulated with an anti-μH antibody or isotype (control) for 3 min, immunostained for the indicated proteins and quantified by flow cytometry. Shown are the histograms of indicated proteins. Y-axis: cell count, X-axis: fluorescence intensity. P denotes phosphorylation.

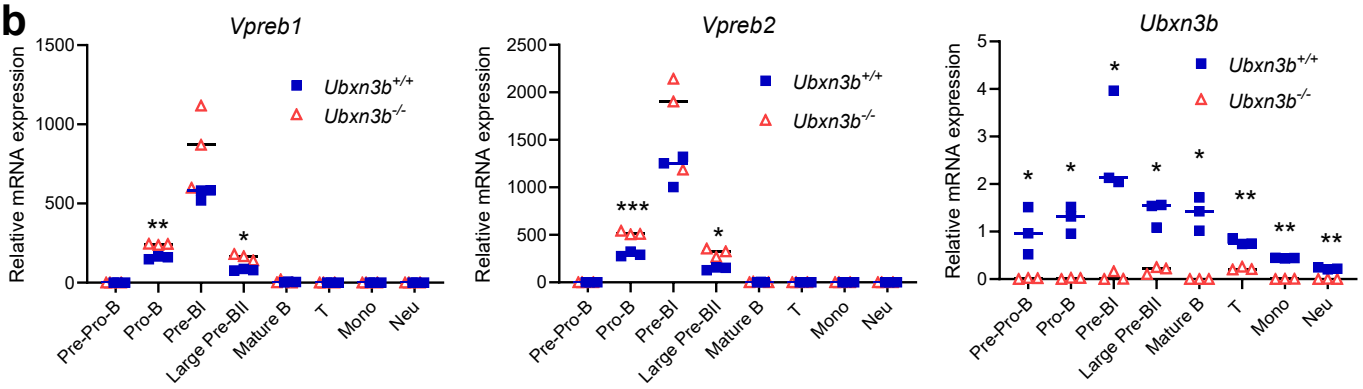


Supplemental Fig.S8. Constitutive pre-BCR signaling is impaired modestly in pre-BI and significantly in large pre-BII. ERT2-Cre *Ubxn3b*$^{flox/flox}$ mice were treated with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3b*$^{+/+}$) every two days for 8 days. At 5 days after the last dose of TMX, bone marrow B cells were collected for the quantification of **a**) bone marrow B fractions, **b**) indicated phosphorylated proteins by flow cytometry, and **c**) total proteins by immunoblotting. Each symbol=one animal. N=3 mice/genotype. In **a**), the horizontal line indicates the mean of the results. * p<0.05, ** p<0.01 (multiple unpaired Welch t-tests with Holm-Šídák correction). In **b**), the histograms represent one out of 3 mice. In **c**), the red arrow points to BLNK, the green stars indicate non-specific bands. L: large, S: small. Imm: immature. X-axis: fluorescence intensity, Y-axis: cell count.

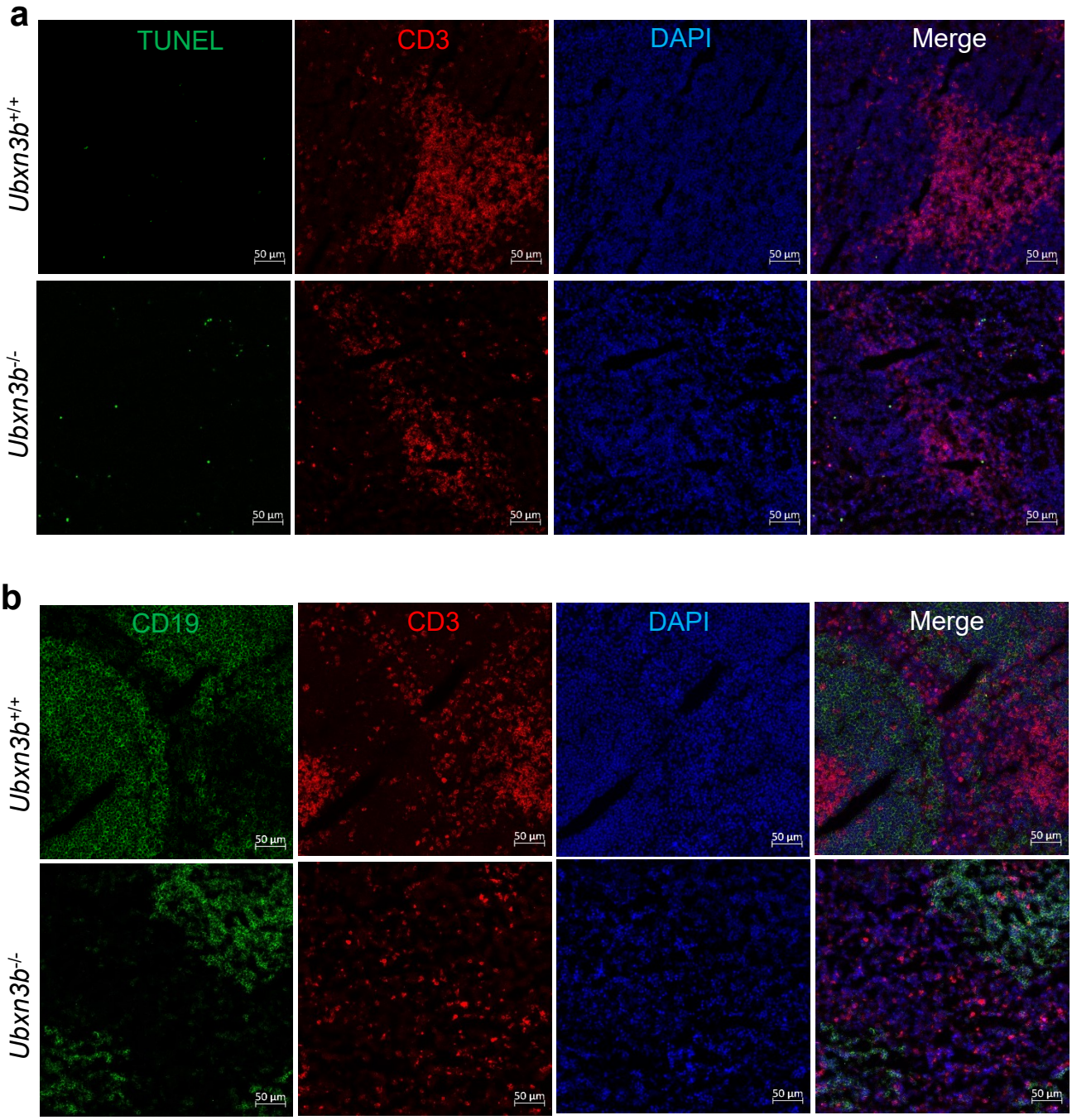


Supplemental Fig.S9. UBXN3B is dispensable for mature BCR and IL-7R signaling. a, b) ERT-Cre^{+} *Ubxn3b*$^{flox/flox}$ splenocytes were treated *ex vivo* with 4-OH tamoxifen to induce *Ubxn3b* deletion or with solvent, dimethyl sulfoxide. B cells were then purified and stimulated with an anti-human IgM antibody. **a**) The immunoblots of phosphorylated (p) and total proteins. **b**) The ratio of ΔF (the difference of calcium load between any a given time after anti-IgM μH treatment and time point zero F0) to F0. **c, d**) Cre^{+} Ubxn3b$^{f/f}$ mice were injected with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3b*$^{+/+}$) every two days for 8 days. Splenic B cells were purified and stimulated at 5 days after the last dose of TMX. **c**) The immunoblots of indicated proteins from two mice per genotype. **d**) Immunofluorescence staining. **e**) The immunoblots of indicated proteins in NALM6 cells treated with a recombinant human IL-7 protein.

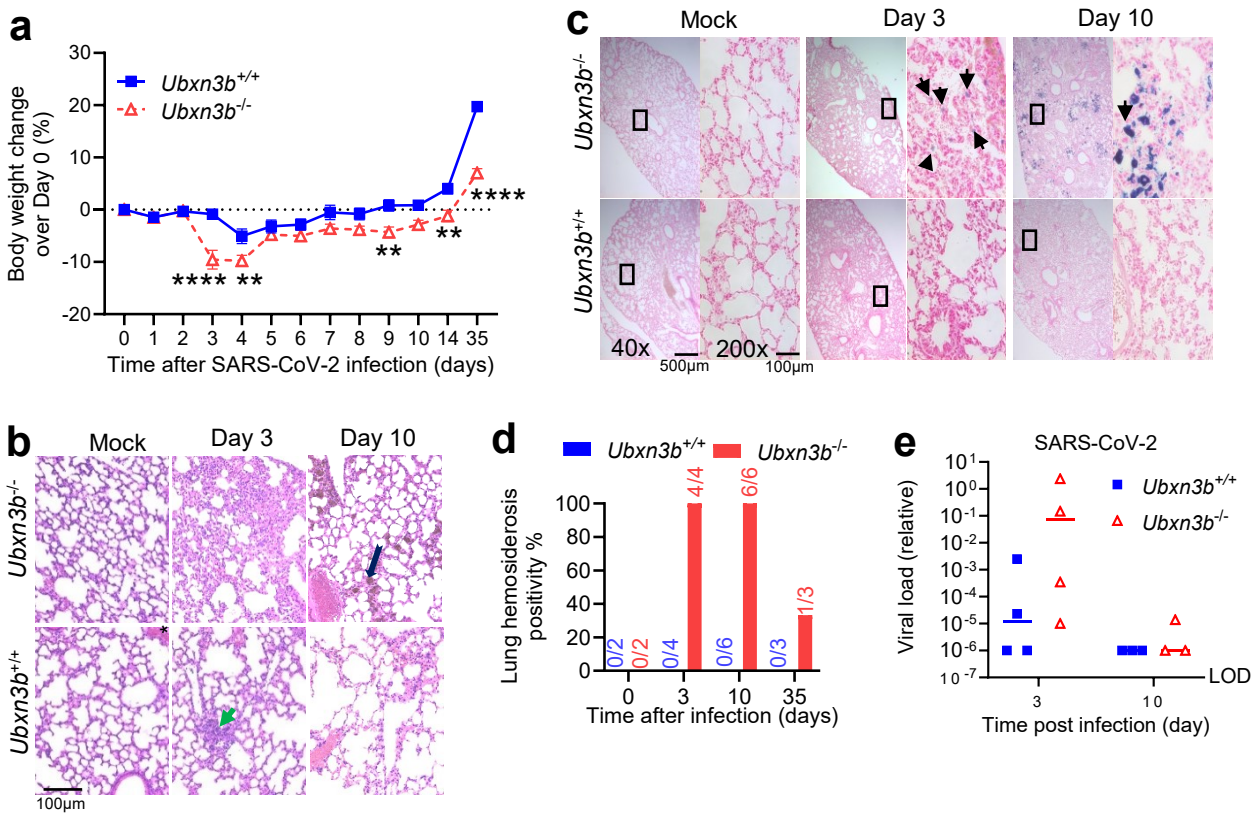




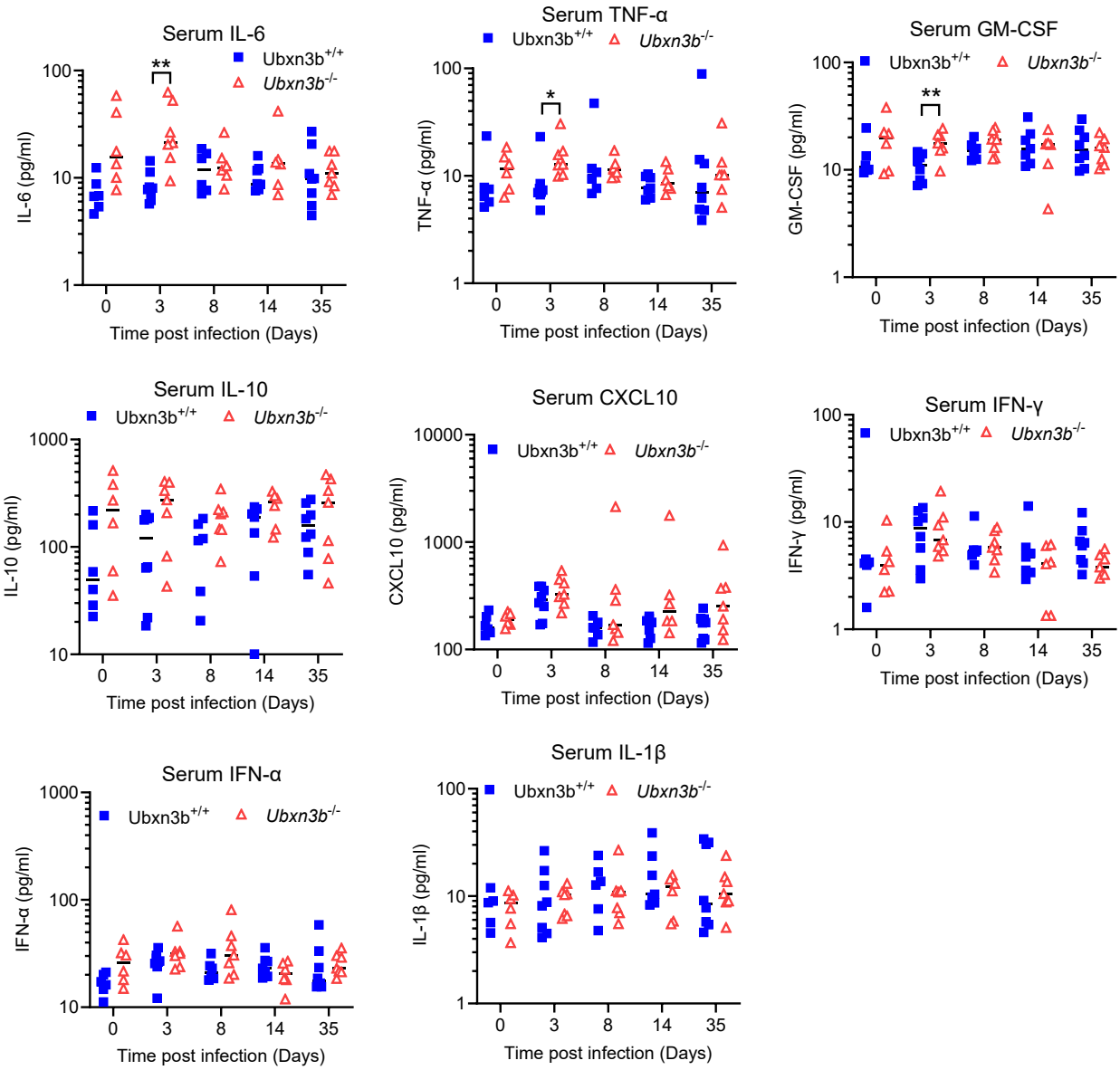
Supplemental Fig.S10. Clustering of the SLC^{hi} B fraction. Bone marrow B cells were sorted by flow cytometry and transcriptomes were analyzed by scRNA-seq. **a**) The t-distributed stochastic neighbor embedding (t-SNE) of surrogate light chain (SLC) genes, *Vpreb1* and *Igll1*, B cell specific surface marker *Cd79a* and transcription factor *Ebf1* expression. *Bank1* is expressed in immature and mature B. Hi: high, lo: low, in: intermediate SLC expression. **b**) Quantification of the mRNA expression of *Vpreb* and *Ubxn3b* by qRT-PCR and normalized to a house keeping gene, *Actb*. Each symbol=one animal. N=3 mice/genotype. The horizontal line indicates the mean of the results. *, p<0.05; **, p<0.01, ***, p<0.001 (multiple unpaired Welch t-tests with Holm-Šídák correction).



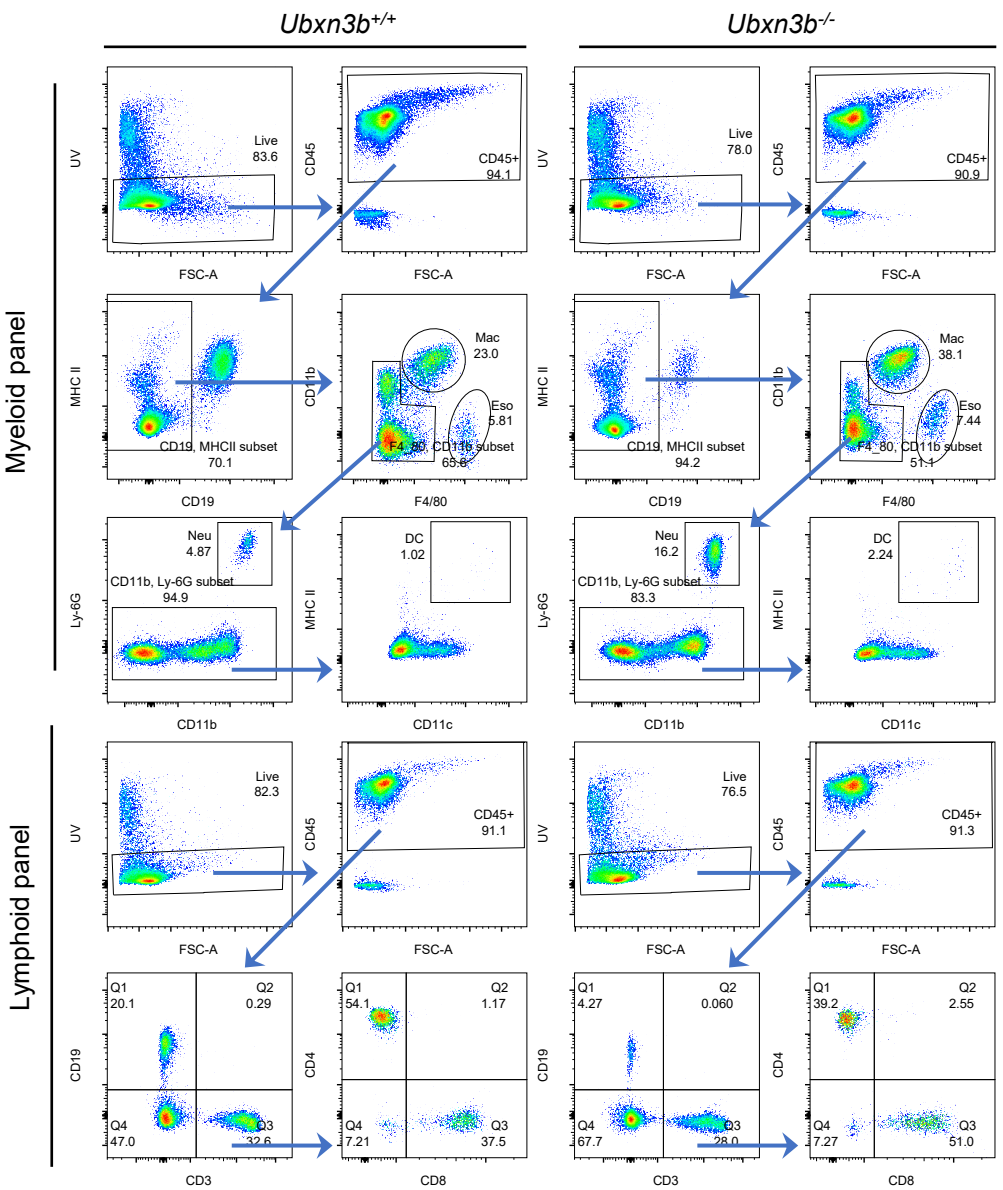
Supplemental Fig.S11. UBXN3B is dispensable for peripheral T cell survival. ERT2-Cre Ubxn3b$^{flox/flox}$ mice were treated with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3b*$^{+/+}$) every two days for 8 days. Shown is the immunofluorescence staining for **a**) apoptotic (TUNEL) and T cells (CD3), **b**) B (CD19) and T cells in the spleen from the at 7 days after the last dose of tamoxifen). DAPI is a counterstain for DNA. Objective: 20x.



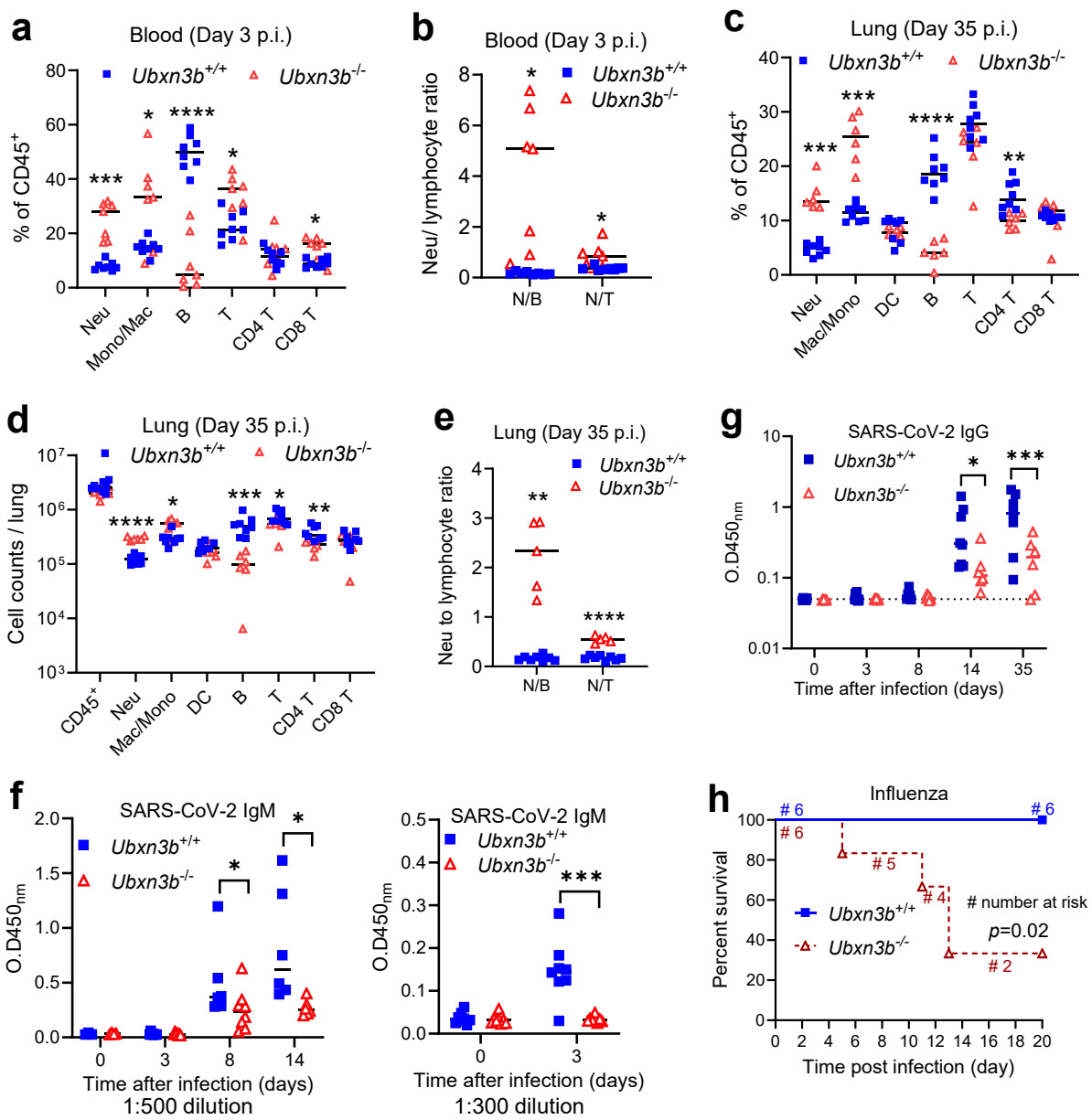
Supplemental Fig.S12. UBXN3B is essential for controlling SARS-CoV-2 pathogenesis. ERT2-Cre^{+} *Ubxn3b*$^{flox/flox}$ mice were treated with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3*$^{+/+}$) every two days for 7 days. Three weeks after the first treatment, sex- and age-matched littermates were sensitized with Ad5-hACE2 intranasally for 5 days, then administered 2x10^{5} plaque forming units (PFU) of SARS-CoV-2 intranasally. **a**) The percent changes in the body mass of *Ubxn3b*$^{+/+}$ and *Ubxn3b*$^{-/-}$ littermates over their baseline (day 0). Data point: mean ± SEM, N=6 mice/genotype. **b**) Representative micrographs of hematoxylin and eosin staining (H&E) of lung sections from mock or SARS-CoV-2-infected mice at 3- and 10-days post infection (*p.i.*). The green arrow points to a cluster of immune infiltrates. The blue arrow indicates a cluster of brownish cells of hemosiderosis. Magnification 400 x. **c**) Iron-staining (blue) of lung sections from **b**). N=2 (mock), 4 (Day 3), 7 (Day 10), 3 (Day 35) mice per genotype. **d**) The positivity (%) of iron-positive mice from **c**). **e**) Quantitative RT-PCR (qPCR) quantification of SARS-CoV-2 loads in the lung at days 3 and 10 post infection (p.i). N=4 *Ubxn3b*$^{+/+}$ and 3-4 *Ubxn3b*$^{-/-}$ mice. Each symbol=one mouse. The horizontal line indicates the median of the results. ***p*<0.01, *****p*<0.0001
(repeated measures two-way ANOVA with Bonferroni's multiple comparisons test for **a**).



Supplemental Fig.S13. UBXN3B is essential for controlling SARS-CoV-2 pathogenesis. ERT2-Cre^{+} *Ubxn3b*$^{flox/flox}$ mice were treated with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3*$^{+/+}$) every two days for 7 days. Three weeks after the first treatment, sex-and-age matched littermates were administered 2x10^{5} plaque forming units (PFU) of SARS-CoV-2 intranasally. Shown is quantification of serum cytokines by a multiplex ELISA. Each symbol= one mouse, N=8 *Ubxn3b*$^{+/+}$ and 7 *Ubxn3b*$^{-/-}$ mice. The small horizontal line: the median of the result. *, p<0.05; **, p<0.01 (unpaired multiple Mann-Whitney tests with Holm-Šídák correction) between *Ubxn3b*$^{+/+}$ and *Ubxn3b*$^{-/-}$ littermates at each time point.



Supplementary Fig.S14. UBXN3B is required for immune cell homeostasis in the lung during SARS-CoV-2 infection. ERT2-Cre^{+} *Ubxn3b*$^{flox/flox}$ mice were treated with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3*$^{+/+}$) every two days for 7 days. Three weeks after the first treatment, sex-and-age matched mice were sensitized with Ad5-hACE2 intranasally for 5 days then administered 2x10^{5} plaque forming units (PFU) of SARS-CoV-2 intranasally. Shown are the flow cytometry gating strategy for various immune cell compartments and their percentages in the lung at 35 days after infection.



Supplemental Fig.S15. UBXN3B is essential for controlling SARS-CoV-2 and influenza pathogenesis. ERT2-Cre^{+} *Ubxn3b*$^{flox/flox}$ mice were treated with tamoxifen (TMX) (*Ubxn3b*$^{-/-}$) or corn oil (*Ubxn3*$^{+/+}$) every two days for 7 days. **a-g**) Three weeks after the first treatment, sex- and age-matched littermates were sensitized with Ad5-hACE2 intranasally for 5 days, then administered 2x10^{5} plaque forming units (PFU) of SARS-CoV-2 intranasally. **a**) The percentage (relative to CD45^{+} cells) and **b**) neutrophil-to-T/B cell ratio (N/B, N/T) at 3 days *p.i.* The **c**) percentage, **d**) cell counts of various immune cell compartments, and **e**) the neutrophil-to-B/T ratios, in the lungs at 35 days *p.i.* The concentrations of serum **f**) IgM and **g**) IgG against SARS-CoV-2 Spike as presented as optical density (OD) at λ_{450nm}. In **a, b**) N=8 *Ubxn3b*$^{+/+}$ and 7 *Ubxn3b*$^{-/-}$. In **c-e**) N=8 *Ubxn3b*$^{+/+}$ and 6 *Ubxn3b*$^{-/-}$. In **f**) N for Day 0=3/3, Day 3=8/7, Day 8=6/7, Day 14=6/6 *Ubxn3b*$^{+/+}$ / *Ubxn3b*$^{-/-}$ mice respectively. In **g**) N for Day 0=6/6, Day 3=8/7, Day 8=6/7, Day 14=8/6, Day 35=7/6. Neu: neutrophil, Mac/Mono: macrophage/monocyte, DC: dendritic cell. Each symbol=one mouse. The horizontal line indicates the median of the results. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 [unpaired multiple Welch *t*-test with Holm-Šídák correction for **a-e**), mixed effect model with Bonferroni's multiple comparisons test for **f** and **g**) respectively]. **h**) After the tamoxifen/oil treatment, sex- and age-matched littermates were infected with 350 $CCID_{50}$ (cell culture infectious dose 50% assay) influenza A PR/8/34 H1N1 intranasally. Mice were monitored daily from Day 1 through 20 post infection (*p.i.*). Shown are the survival curves with the subject number at risk. N=6 mice per genotype, p=0.02 (Log-Rank test).