Supplemental material

Myeloid Loss of *Beclin 1* Promotes PD-L1^{hi} Precursor B Cell Lymphoma Development

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Supplemental Figure 1-6.

Supplemental Table 1-7.



Analysis of immune cell populations in WT and *Becn1*^{ΔM} mice. Related to Figure 1. (A) Immunoblot analysis (IB) on dendritic cells (DC), peritoneal macrophages (pMAC), T cells, B cells, and neutrophils (Neu) from WT and Becn1^{ΔM} mice for Becn1 expression (n=3). (B) Total splenocytes number in 6-to-8-week-old WT and Becn1^{ΔM} mice. (C) Giemsa staining of neutrophils morphology in peripheral blood (PB) smear and Immunohistochemical analysis of spleen (SP) stained with anti-Ly6G antibody in WT and Becn1^{Δ M} mice (n=3). Scale bars, 500 μ m. (**D**) FACS analysis of CD11b⁻ gated B220⁺CD11c⁺ pDC (Plasmacytoid dendritic cell) in the spleen of WT and *Becn1*^{ΔM} mice (n=3). (E) Intracellular staining and FACS analysis of different subsets of CD4⁺ T cells in the presence of PMA and ionomycin isolated from the mixture of Spleen and LNs of 10week-old WT or *Becn1*^{ΔM} mice (n=3). (F) Intracellular staining and FACS analysis of IFNy⁺CD8⁺T cells in the presence of PMA and ionomycin isolated from a mixture of Spleen and LNs of 10-week-old WT or Becn1^{ΔM} mice (n=3). (G) Apoptosis was assayed with Annexin V/7-AAD apoptosis staining and FACS analysis of spleen CD3⁺ T cells, B220⁺ B cells. CD11b⁺F4/80⁺ macrophages and CD11b⁺Gr-1⁺neutrophils (n=3). (H) Diagram of mixed BM chimera generation (upper). WT or Becn1^{ΔM} (CD45.2⁺) BM cells were mixed at 1:1 ratio with helper WT (CD45.1⁺) BM cells and transplanted into lethally irradiated WT (CD45.1⁺) hosts. Resulting chimeric mice were analyzed 12 weeks posttransplantation (n=4). Percentage of CD45.1⁺ and CD45.2⁺ cells in mice reconstituted with a mixed WT BM (WT/WT \rightarrow WT) or a mixed of WT and *Becn1*^{ΔM} BM cells ($\Delta M/WT$ \rightarrow WT) (bottom). (I) Flow cytometric (left) and statistical (right) analysis of neutrophils in spleen of mixed BM chimeric mice with anti-Gr-1 and anti-CD11b antibodies. Cell

populations are gated and numbers indicate the percentage of cells in the gate. Cells were first gated based on of CD45.1 or CD45.2 expression (n=4). Data are presented as boxplots whiskers span minimum and maximum with individual data points, line represents median with error bars show the mean \pm SEM (**B**, **H**, **I**). Statistical between groups calculated using Students' unpaired t-test with significance indicated (**P* <0.05, ***P* <0.01, NS, not significant).



Becn1 deficiency skews BMDM differentiation to M1. Related to Figure 2. (A and B) WT or Becn1-deficient neutrophils (A) or peritoneal macrophages (pMAC) (B) were treated with TLR2 agonist Pam3CSK4 for the indicated time points, followed by immunoblot analysis with indicated antibody (n=3). (C) gRT-PCR for analysis of mRNA levels of Scarb1 in pMAC and neutrophils isolated from WT or *Becn1*^{ΔM} mice with or without LPS treatment (n=4). (D) gRT-PCR of Becn1 mRNA from BMDM cultured for 24hr in the presence of LPS/IFN-y (M1) or IL-4/IL-10 (M2) for macrophage differentiation (n=4). (E) gRT-PCR of M1 genes expression in the presence of LPS (upper panel) and M2 genes expression in the presence of IL-4 (lower panel) at indicated time points (n=4). (F) WT and *Becn1*-deficient neutrophils (upper, Neu) or peritoneal macrophages (bottom, pMAC) were stimulated by LPS for 3 h. ROS production was measured by staining cells with CM-H₂DCFDA for 30 min followed by flow cytometry. (G) FACS analysis of the percentage of macrophages treated with PBS- or clodronate-containing liposomes or neutrophils treated with IgG control or anti-Ly6G antibodies. (H) KEGG pathway enrichment of highly differentially expressed genes in LPS-treated neutrophils from Becn1^{△M} mice compared with WT control. (I) ELISA measurement of IL-17A and IL-21 production in Neu or pMAC from WT and Becn1^{Δ M} mice (n=3) after 100 ng/mL LPS treatment for indicated time points. Data are presented as boxplots whiskers span minimum and maximum with individual data points, line represents median with error bars show the mean ± SEM (C, D). Statistical between groups calculated using one-way ANOVA with Dunnett's multiple comparison test (C, D) or Students' unpaired t-test (E, I) with significance indicated (**P < 0.01, NS, not significant).



Becn1^{Δ M} tumor-bearing mice exhibit increased infiltrates of immature B cells in different tissues. Related to Figure 5. (A) Hematoxylin and eosin (H&E) staining in the section from lung, thymus, heart, and intestine isolated from WT or *Becn1^{\DeltaM}* tumorbearing mice (n=3). Scale bars, 500 µm. The squared area was enlarged from original magnification, x10, x40, x100. (B) Immunohistochemical analysis of paraffin-embedded heart, kidney, and liver tissue sections from *Becn1^{\DeltaM}* tumor-bearing mice with anti-B220 antibody (n=3). Scale bars, 50 µm. (C) Immunohistochemical analysis of paraffinembedded lung sections from WT and *Becn1^{\DeltaM}* tumor-bearing mice with anti-Ly6G (neutrophils), anti-B220 (B cells), anti-Mac-3 (monocyte), anti-Mac-1 (macrophage) (n=3). Scale bars, 200 µm. (**D**) Immunofluorescence of tumor from *Becn1*^{Δ M} mice stained with the anti-CD3 (pink), anti-Ly6G (yellow), anti-macrophage (cyan) and anti-B220 (green) (n=3). (**E**) Representative flow cytometry plot of gated total B220⁺ cells analyzed for recirculating (IgM⁺IgD^{hi}), transitional (IgM⁺IgD^{lo}) and immature B (IgM⁺IgD⁻) populations in BM, SP, LN isolated from WT and *Becn1*^{Δ M} tumor-bearing mice (n=3). (**F**) Giemsa staining of BM cytospin from WT and *Becn1*^{Δ M} mice (upper) and H&E staining of the bone section in WT and *Becn1*^{Δ M} mice (n=3). Scale bars, 100 µm.



Tumor transcriptome analysis shows upregulation of inflammation and decreased tight-junction. Related to Figure 6

(A) IF and FACS analysis of cell populations in the tumor. White line circled tumor area. Original magnification, x40. Scale bars, 50 µm. (B) Log2 ratio of mRNA variations in the tumor from $Becn1^{\Delta M}$ mice compared to WT controls by RNA-seq. (C) Scatter plot comparing global gene expression profiles between LNs from *Becn1*^{ΔM} mice or WT controls. (D) Gene ontology (GO) enrichment analysis. Enrichments for biological process ontology is shown. (E) KEGG pathway enrichment of highly differentially expressed genes in the tumor from $Becn1^{\Delta M}$ mice compared with WT control. (F) qRT-PCR for analysis of the mRNA levels of the indicated genes in WT LNs, Becn1^{ΔM} LNs (no tumor), and Becn1^{Δ M} lymphomas (n=4). (G) gRT-PCR confirmation of junction molecules Cldn1, Cldn2, Cldn3 and Tjp1 in the tumor isolated from *Becn1*^{ΔM} tumor-bearing mice compared to WT control. (H) FACS analysis of Mo-MDSC (Ly6ChiLy6G) and PMN-MDSC (Ly6C^{lo}Ly6G⁺) in the tumor from *Becn1*^{ΔM} tumor-bearing mice compared to WT controls. (I) T cell proliferation in neutrophils isolated from wildtype or $Becn1^{\Delta M}$ mice with (T) or without tumor (N). Proliferation was determined by incorporation of [³H] thymidine. CPM, counts per min. Data are presented as boxplots whiskers span minimum and maximum, line represents median with error bars show the mean ± SEM, n=4 (F, G, I). Statistical between groups calculated using one-way ANOVA with Dunnett's multiple comparison test with significance indicated (*P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant).



Supplemental Figure 5

Myeloid Becn 1 regulates PD-L1 expression in B cell through JAK/STAT and ERK signaling. Related to Figure 7. (A) Immunoblot analysis of phosphorylation of STAT1, STAT3, STAT5 (IL-21 downstream), AKT and FOXO1 (Pten downstream) as well as total STAT3, MCL-1 (a downstream target of STAT5) and non-canonical p-NF- κ B p52 (BAFF downstream) expression in B cells directly isolated and lysed from WT and *Becn1*^{ΔM} tumor-bearing mice. (B) FACS analysis of apoptotic B cells from WT and *Becn1*^{ΔM} tumor-bearing (T) mice by Pl/Annexin V staining, n=4. Early apoptosis, Pl-AnnexinV⁺; late

apoptosis, PI*AnnexinV*. (**C**) IB of WT B cells co-cultured with WT or tumor-associated neutrophils or pMAC (n=2). (**D**) qRT-PCR for analysis of mRNA levels of Cxcl9 and Cxcl10 in B cells co-cultured with WT or *Becn1*-deficient (KO) Neu (n=4). (**E** and **F**) IB of WT B cells co-cultured with tumor-associated neutrophils in the presence of indicated antibody (E) or inhibitors (F). PD-L1 levels were quantified by densitometric scanning of blots and normalized to β -actin. (**G**) qRT-PCR for analysis of indicated genes after Neu/B co-culture in the presence of indicated inhibitors (n=4). (**H**) qRT-PCR for analysis of mRNA levels of Cmtm4 and Cmtm6 in B cells co-cultured with WT or *Becn1*-deficient (KO) Neu (n=4). Data are presented as boxplots whiskers span minimum and maximum, line represents median with error bars show the mean ± SEM, n=4 (**B**, **D**, **G**, **H**). Statistical between groups calculated using one-way ANOVA with Dunnett's multiple comparison test (**D**, **G**, **H**) or using Students' unpaired t-test (**B**) with significance indicated (**P* <0.05, ***P* <0.01, NS, not significant).



IL-21 secreted by *Becn1*-deficient neutrophils is essential for the immunosuppressive function of B cell lymphoma. Related to Figure 7.

(A) Tumor formation in *Becn1^{ΔM}* mice 2 months after stimulation (n=4, i.p.10 µg/mouse per week). Representative imaging of LNs from mice injected with PBS or IL-21 (n=3). (B) FACS and IB of PD-L1 in CD19⁺ B cells from mice injected with IL-21. (C) IHC staining of tumor with CD8 antibody from *Becn1*^{△M} tumor-bearing mice injected with IgG control, PD-L1 or IL-21R antibody, compared to WT control. Statistical analysis of infiltrated CD8⁺ T cells per field was shown (n=4). (**D**) FACS analysis of CD8⁺IFN-y⁺ and CD8⁺Granzyme B⁺T cells from *Becn1*^{Δ M} (T) mice injected with IgG control, PD-L1 or IL-21R antibody (n=4). (E) gRT-PCR and ELISA assay for the analysis of the expression of B cell helper factors (IL-12b, CD40L, IL-1β, IL-21, BAFF) in neutrophils from WT, *Becn1*^{ΔM} tumor-bearing mice, and $Becn1^{\Delta M}$: Mapk14^{\Delta M} mice (n=10). (F) B cells isolated from WT, $Becn1^{\Delta M}$ tumorbearing mice, and Becn1^{ΔM}: Mapk14^{ΔM} mice were purified and lysed for immunoblot analysis with indicated antibody. (G) FACS and statistical analysis of BM and LN B220⁺ cells from WT, Becn1^{ΔM}, and Becn1^{ΔM}:Mapk14^{ΔM} mice. (H) Kaplan-Meier curves for tumor-free animals were calculated based on the tumor latency of the WT (n=10, black), Becn1^{ΔM} mice (n=10, red) and Becn1^{ΔM}:Mapk14^{ΔM} (n=10, blue) mice. (I) Human Becn1 gene expression profile (GEP) in patients with pre-B cell acute lymphoblastic leukemia (pre-B ALL, without mutations or gene rearrangements) or various non-Hodgkin's B cell (NHB) lymphoma compared to reactive LN controls. The boxes show the median±1 guartile, with whiskers extending to the most extreme data point within 1.5 interguartile ranges from the box boundaries. (J) Correlation of IL-21, PD-L1 and neutrophil marker

(CD177) in B-ALL dataset (TARGET). Data are presented as dot plots or boxplots whiskers span minimum and maximum, line represents median with error bars show the mean \pm SEM (**C**, **D**, **E**, **I**). Statistical between groups calculated using one-way ANOVA with Dunnett's multiple comparison test (**C**, **D**, **E**, **I**) and Mantel-Cox log-rank test (**H**) with significance indicated (**P* <0.05, ***P* <0.01).