

Figure S1: Dlg1 is not essential for BMDC development.

(A) Representative flow cytometric graphs and quantification of BMDCs from bone marrow cell culture. Bone marrow cells (BMCs) (4×10^5 cells per well) from WT (Cd11c-Cre-GFP) and KO (*Dlg1^{fl/fl}*Cd11c-Cre-GFP) mice were cultured in the presence of GM-CSF (200 ng/mL) and IL-4 (10 ng/mL) for 7 days to generate BMDCs. Data are pooled from six mice in each group. (B-C) BMCs from WT (Cd11c-Cre-GFP) and KO (*Dlg1^{fl/fl}*Cd11c-Cre-GFP) mice were cultured in Flt3L (200 ng/mL) for 7 days. "+GM-CSF" means adding GM-CSF (20 ng/mL) during last 2 days of culture. "-GM-CSF" means no GM-CSF during last 2 days. (C) Cells were gated from CD11c⁺ Siglec H⁺ population in (B). Data were pooled from six mice in each group.

Figure S2

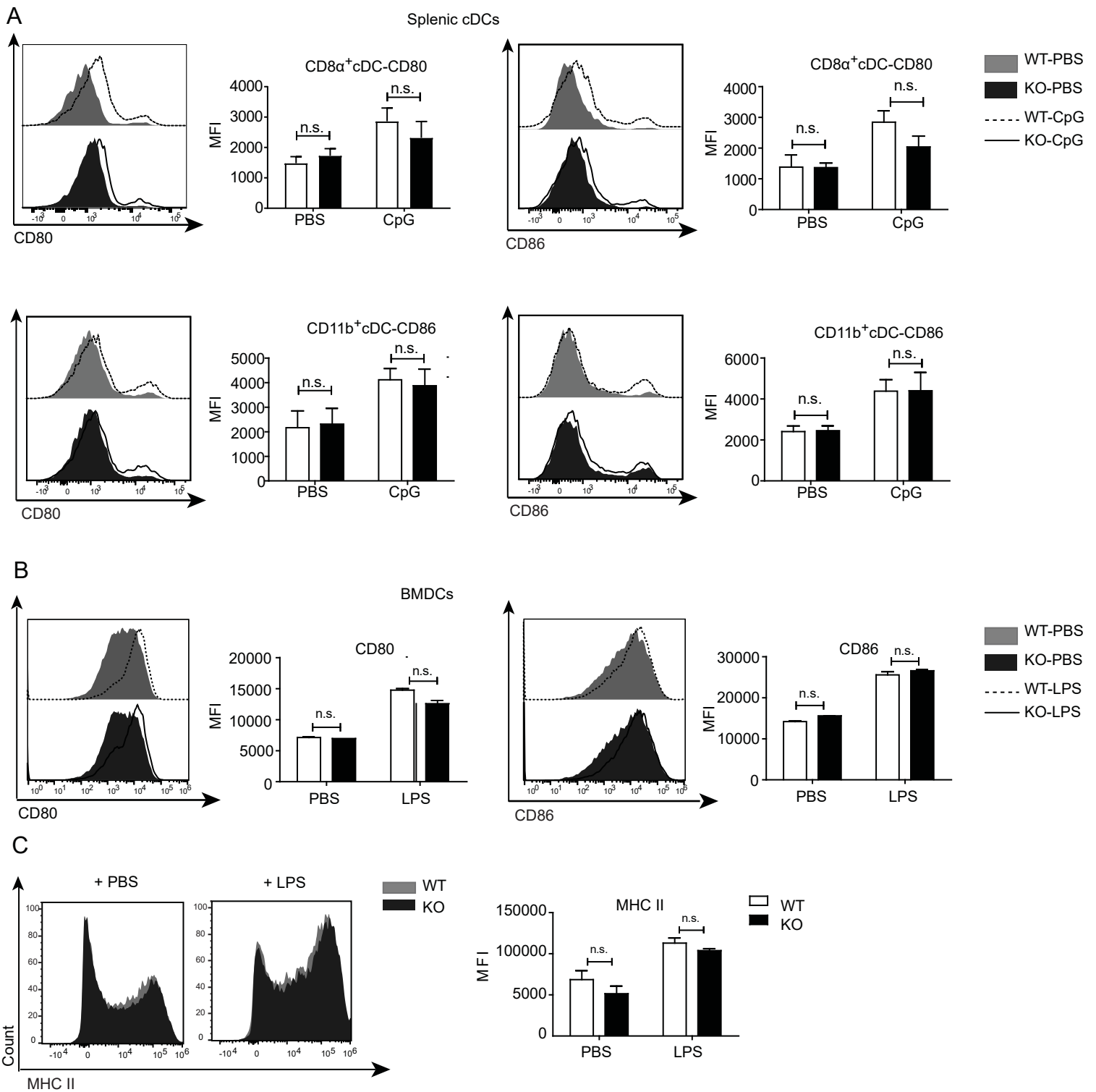


Figure S2: Dlg1 is not essential for DC maturation.

(A) Representative histogram and median fluorescence intensity (MFI) quantification of CD80 and CD86 on splenic cDCs stimulated with CpG ODN (20 nM) or PBS as control. Data were pooled from three mice with assayed triplicated wells. (B-C) Representative histogram and quantification of CD80, CD86, and MHC II on stimulated BMDCs. BMDCs were stimulated with LPS (100 ng/mL) overnight and gated on CD11c $^+$ BMDCs for analysis. Data were pooled from three mice with assayed triplicated wells.

Figure S3

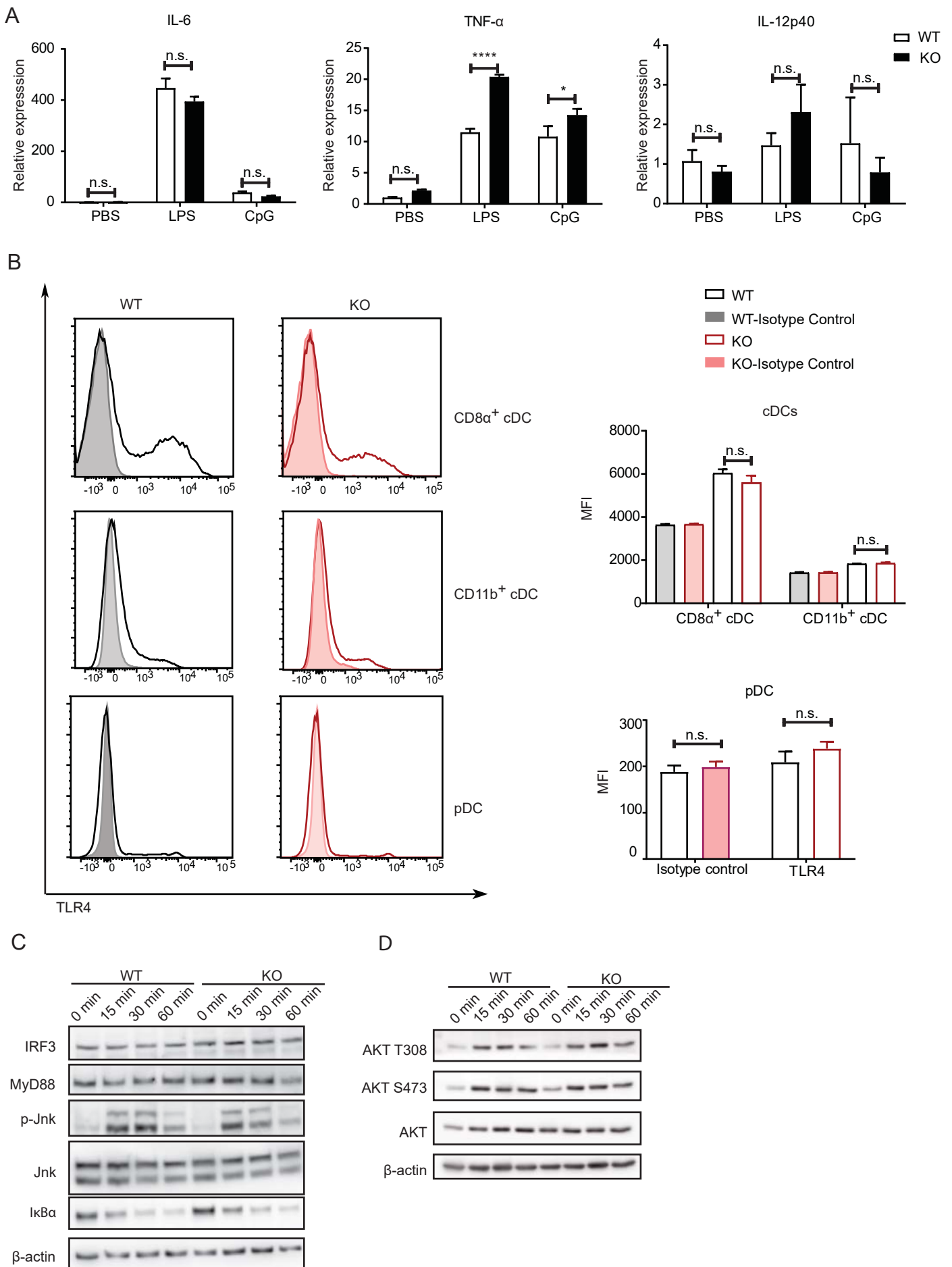


Figure S3. Dlg1 does not alter TLR Signaling pathway.

(A) IL-6, TNF- α , and IL-12p40 mRNA relative expression of CD11c⁺BMDCs induced with LPS (100 ng/mL) or CpG ODN (0.3 μ M) for 6 hrs. Data were pooled from three independent experiments (one mouse in each group and assayed in triplicate wells) (Two-way ANOVA analyses). **(B)** TLR4 expression level in DCs. Splenic cells from WT (Cd11c-Cre-GFP) and KO (*Dlg1^{fl/fl}*/Cd11c-Cre-GFP) mice were enriched and subjected to TLR4 expression analysis by flow cytometry. Representative histogram (left) and quantification data (right) are shown. Data are representative from two independent experiments (Two-way ANOVA analyses, n=4 mice in each group). **(C-D)** Signaling induced by LPS in BMDCs. CD11c⁺ BMDCs were purified and treated with LPS (100 ng/mL) for indicated time. Whole cell lysate were subjected to Western blot for detection of IRF3, MyD88, p-Jnk, Jnk, I κ B α , p-AKT(T308), p-AKT(S473), and AKT. β -actin was presented as the loading control. Data are representative of at least two independent experiments.

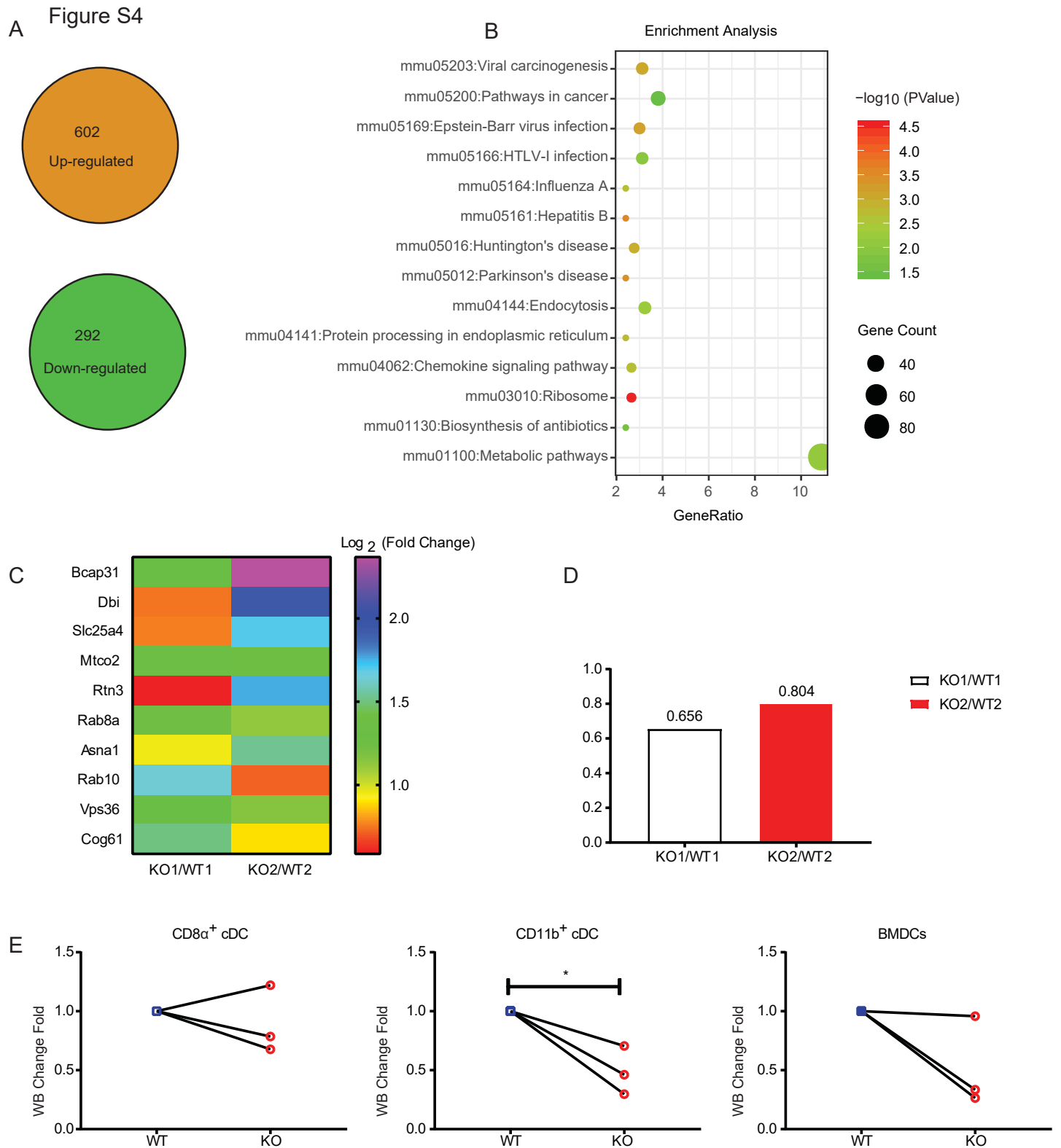


Figure S4. Total protein mass spectrometry (MS) analyses.

(A) Total number of proteins revealing up-regulation or down-regulation in Dlg1-KO CD11b $^+$ cDCs. (B) Top 10 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway categories of both up-regulated and down-regulated proteins from Dlg1-KO CD11b $^+$ cDCs. Plot colors represent the statistical significance and GeneRatio (%) represent the proportion of genes in total genes. Plot sizes represent Gene Count. Data were analyzed by R language. (C) Top 10 up-regulated proteins in transport biological process group. (D) Kcna2 quantitative proteomics analysis data from protein mass spectrometry. Each column indicated ratio of one group (KO/WT). (E) Quantification for anti-pan Kv β potassium subunit western blot data in Figure 6 (C-D) (Image J). Each blue plot and red plot represents one independent experiment. Bands were quantified by Image J and normalized by Kv β / β -actin ratio in each group. Then WT groups were normalized to "1.0".