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Supplemental Information

Cholesterol Accumulation in Dendritic Cells Links the Inflammasome to Acquired Immunity

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Supplemental Figures

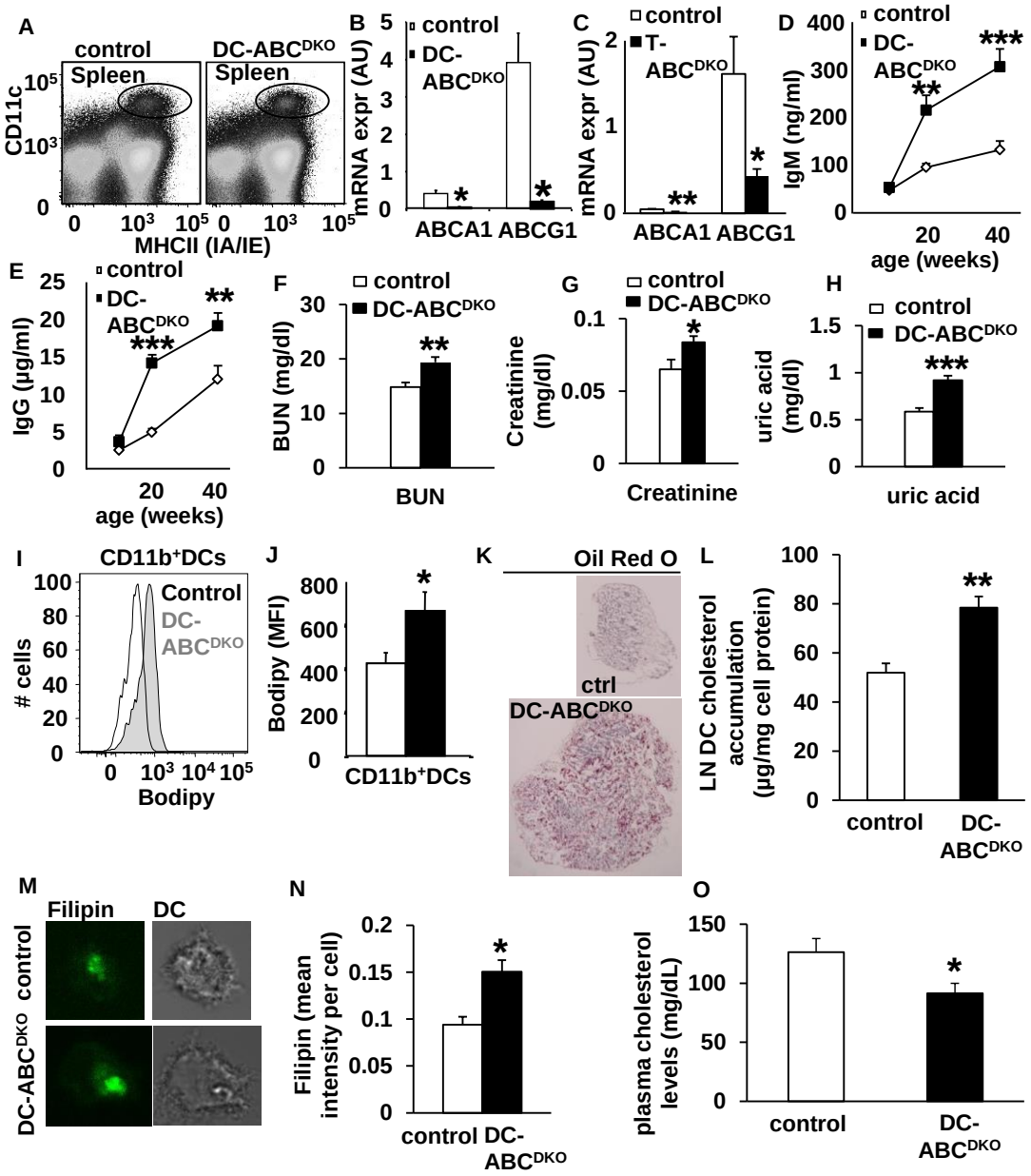

Figure S1, related to Figure 2. DC-ABC^{DKO} and T-ABC^{DKO} mice show >90% and >80% decreased ABCA1 and ABCG1 mRNA expression in dendritic cells (DCs) and T-cells, respectively and DC-*Abca1/g1* deficiency induces lipid accumulation in DCs. (**A**) Gating strategy for isolation of splenic CD45^{+}CD115^{-}CD11c^{+}MHCII^{+} DCs in control and DC-ABC^{DKO} mice. (**B**) Splenic DCs were sorted by flow cytometry and the *Abca1* and *Abcg1* mRNA expression levels were determined and corrected for the housekeeping gene m36B4. n=6. (**C**)

Blood $CD4^+$ T-cells were sorted by flow cytometry and the *Abca1* and *Abcg1* mRNA expression levels were determined and corrected for the housekeeping gene m36B4. n=4. (**D-E**) Increased plasma levels of IgM (**D**) and IgG (**E**) in DC-ABC^{DKO} mice. n=6-10. (**F-H**) Increased plasma levels of blood urea nitrogen (BUN) (**F**), creatinine (**G**), and uric acid (**H**) in DC-ABC^{DKO} mice. n=10. (**I-J**) Increased lipid accumulation in splenic DC-ABCDKO $CD11b^+$ DCs assessed by BODIPY staining. n=6. (**K**) Inguinal LNs were embedded in OCT and frozen sections were made and stained for Oil Red O. Lipid accumulation in DC-ABC^{DKO}, but not in control LNs. All sections (n=6) studied showed Oil Red O staining in DC-ABC^{DKO} but not in control LNs. Representative pictures are shown. (**L**) DC-*Abca1/g1* deficiency enhances cholesterol accumulation in DCs. $CD11c^+$ cells were isolated from the lymph nodes of control and DC-ABC^{DKO} mice using CD11c positive beads, lipids were extracted, and cholesterol was assessed using an enzymatic assay and corrected for cell protein. n=6. (**M-N**) Bone marrow cells were differentiated into DCs by treatment with GM-CSF and were stained with filipin. Representative pictures of n=5 fields are shown (**M**) and filipin staining was quantified (**N**). (**O**) Plasma cholesterol levels in 20 week old control and DC-ABC^{DKO} mice fed a chow diet. n=10. Data in **B**, **C-H**, **J**, **L**, **N**, and **O** are presented as mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, by t-test.

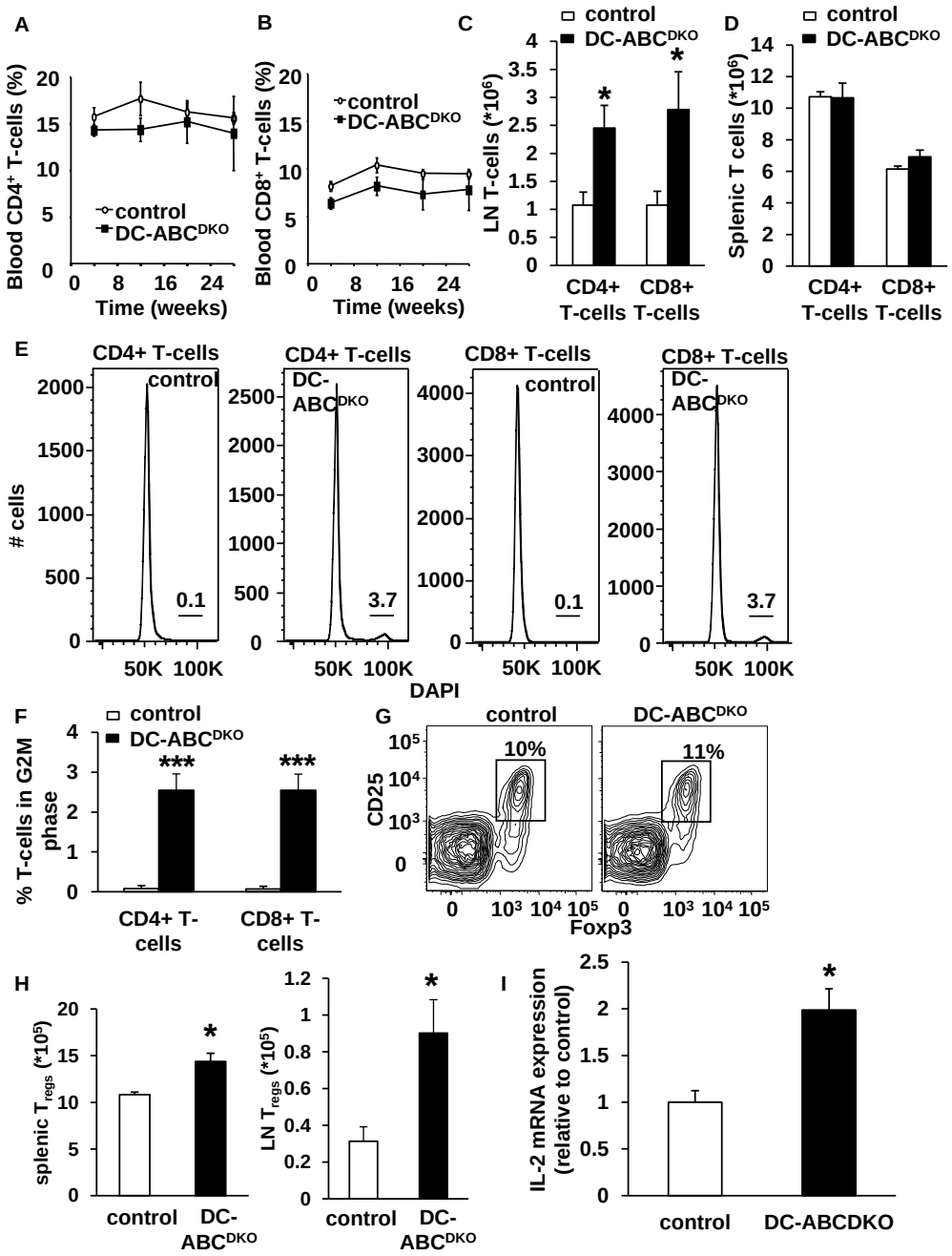

Figure S2, related to Figure 3. Effect of DC *Abca1/g1* deficiency on CD4$^+$ and CD8$^+$ T-cell numbers, T-cell proliferation, and T_{regs}. CD4$^+$ and CD8$^+$ T-cell percentage was assessed in blood (**A**-**B**), and CD4$^+$ and CD8$^+$ T-cell numbers in inguinal lymph nodes (**C**) and spleen (**D**) n=5-8. All data in (**A**-**D**) were measured concurrently with T-cell activation measurements. (**E** and **F**) CD4$^+$ and CD8$^+$ T-cell proliferation was assessed using DAPI in lymph nodes and

calculated in Flow Jo using the Watson model. (**E**) T-cell proliferation graphs. Percentages show cells in the G2M phase. (**F**) Quantification of T-cells in the G2M phase. n=5. (**G**) and (**H**) DC-ABC^{DKO} mice show increased T_{regs} in spleen and lymph nodes. (**G**) Gating strategy. (**H**) Splenic T_{reg} and lymph node T_{reg} numbers. n=7. (**I**) DC-ABC^{DKO} increases IL-2 mRNA in CD4^{+} T-cells. CD4^{+} T-cells were sorted from LNs using flow cytometry, RNA extracted, and IL-2 mRNA levels assessed. Data in **A-D**, **F**, **H**, and **I** are presented as mean ± SEM. **P*<0.05, ****P*<0.001, by t-test.

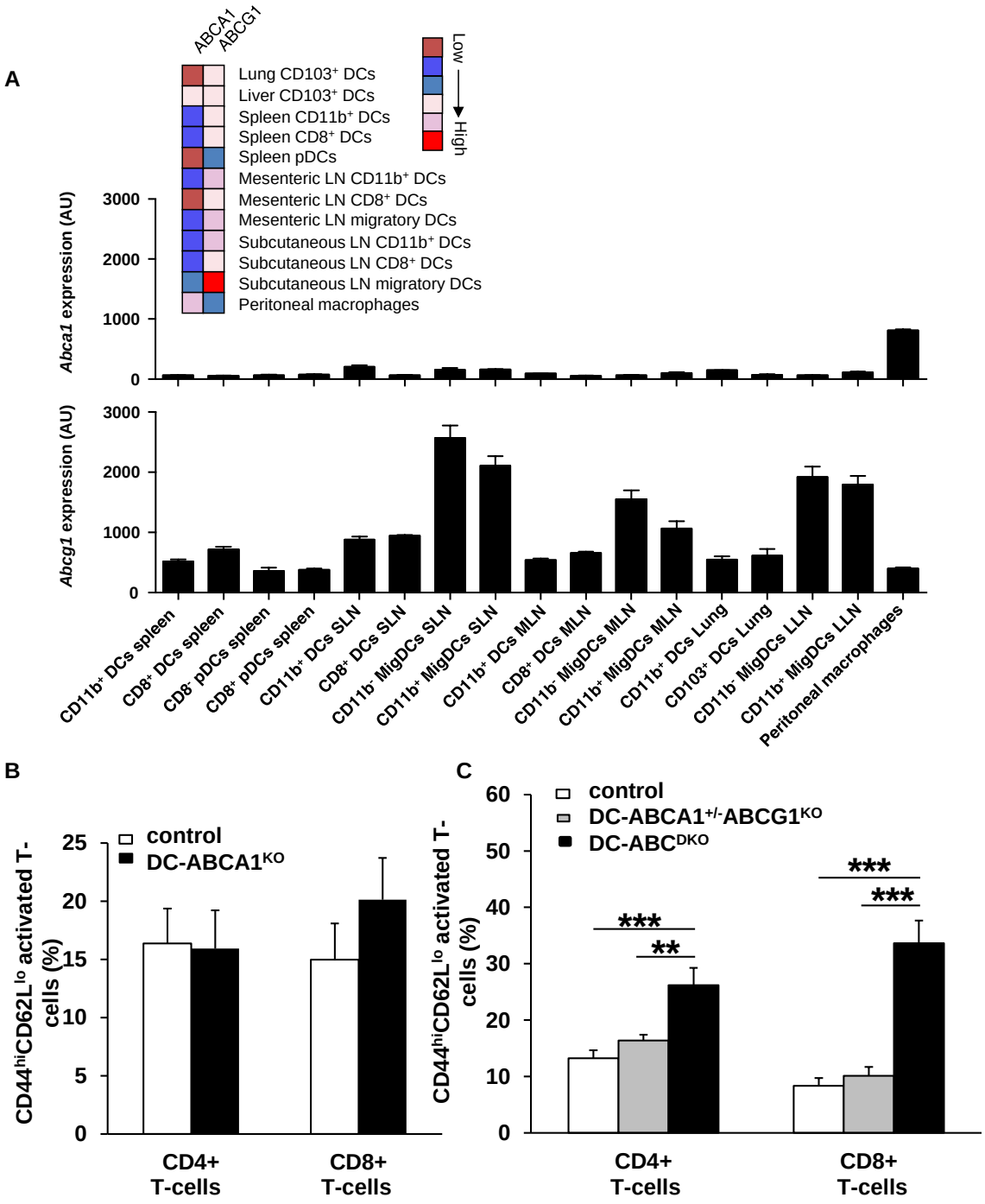

Figure S3, related to Figure 3. ABCG1 is highly expressed in several DC subtypes but only combined deficiency of ABCA1 and ABCG1 induces T-cell activation. (**A**) Expression of *Abca1* and *Abcg1* in several DC subtypes and peritoneal macrophages as assessed by microarray. Top: heatmap. Bottom: quantification of *Abca1* and *Abcg1* expression. These data were obtained

as part of the Immunological Genome Project. (**B-C**) Combined deficiency of ABCA1 and ABCG1 in DCs is required for the T-cell activation phenotype. (**B**) $CD44^{hi}CD62L^{lo}$ activated $CD4^{+}$ and $CD8^{+}$ T-cells in the blood of control and DC-$ABCA1^{KO}$ mice. n=5. (**C**) $CD44^{hi}CD62L^{lo}$ activated $CD4^{+}$ and $CD8^{+}$ T-cells in the blood of control, DC-$ABCA1^{+/-}$ $ABCG1^{KO}$ and DC-ABC^{DKO} mice. n=5. **B** and **C**, $^{**}P<0.01$, $^{***}P<0.001$, by one-way ANOVA with Bonferroni post-test.

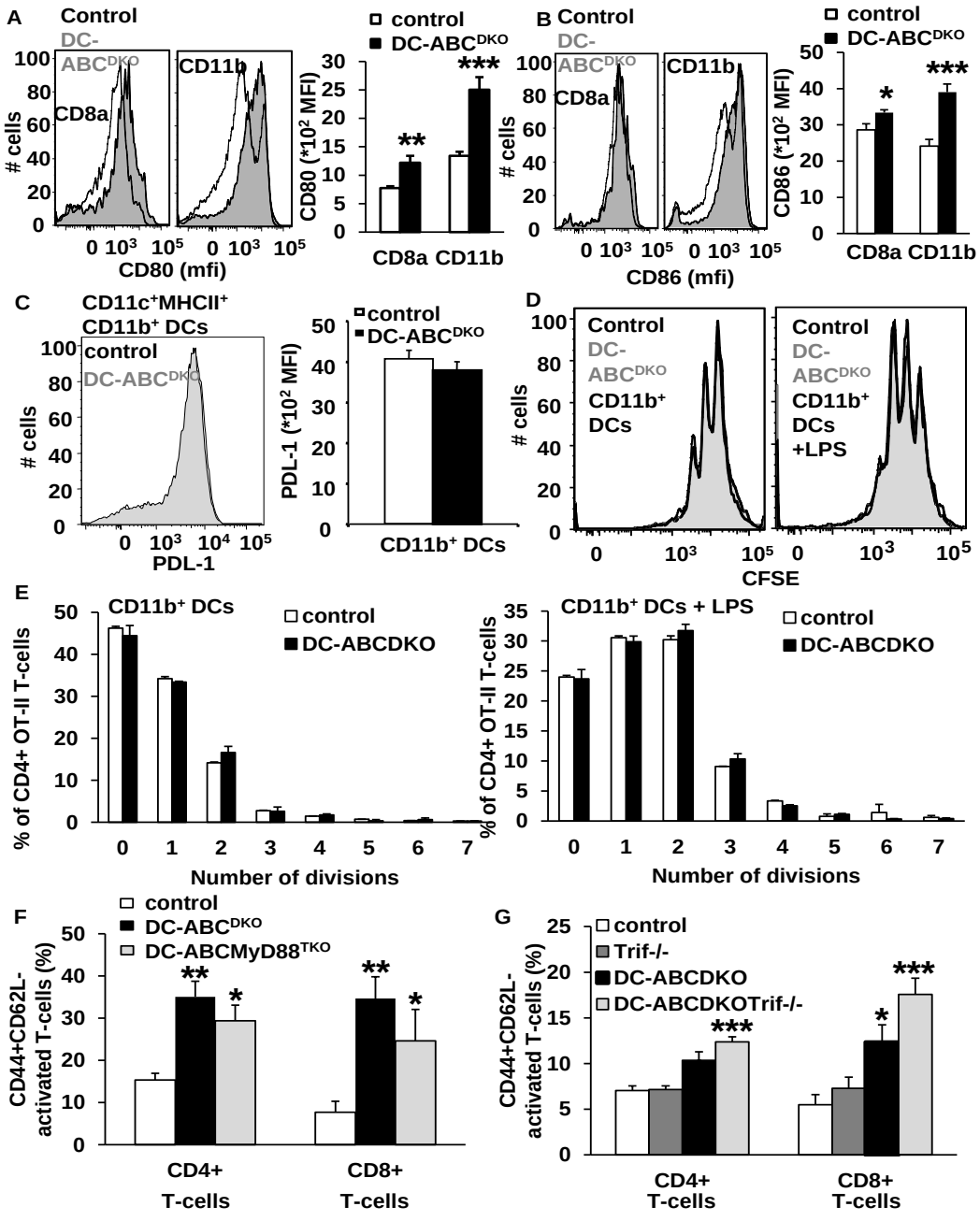

Figure S4, related to Figure 4. DC-*Abca1/g1* deficiency enhances CD80/86 expression, but does not affect PDL-1 surface expression in CD11b^{+} DCs and antigen presentation by CD11b^{+} DCs *in vitro*, and T-cell activation in DC-ABC^{DKO} mice is not due to enhanced MyD88 or Trif signaling. (**A-C**) CD80 (**A**) and CD86 (**B**) surface levels on splenic CD11c^{+}MHC-II^{+}CD8a^{+} and CD11c^{+}MHC-II^{+}CD11b^{+} DCs and PDL-1 (**C**) surface levels on

splenic $CD11c^+MHC\text{-}II^+CD11b^+$ DCs determined by flow cytometry. Representative examples and quantification. n=4-6. *P<0.05, **P<0.01, ***P<0.001, by t-test. (**D**-**E**) Bone marrow cells were stimulated with GM-CSF for 8 days to generate $CD11b^+$ DCs, incubated with or without LPS for 24 h, and then incubated with OT-II peptide in the presence of CFSE labeled $CD4^+$OTII T-cells (ratio DC:T-cells 1:5). CFSE dilution in $CD4^+$ T-cells was assessed at 72 h after co-incubation. Representative CFSE dilutions are shown (**D**) and quantified using Flow Jo software (**E**). n=4. Mice were fed chow diet for 16-20 weeks (**F** and **G**) and activated $CD44^+CD62L^-$ $CD4^+$ and $CD44^+CD62L^-$ $CD8^+$ T-cells in blood were measured using flow cytometry. n=6. The experiment was done twice at different timepoints. Data are presented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, compared to control, by one way ANOVA and Bonferroni post-test.

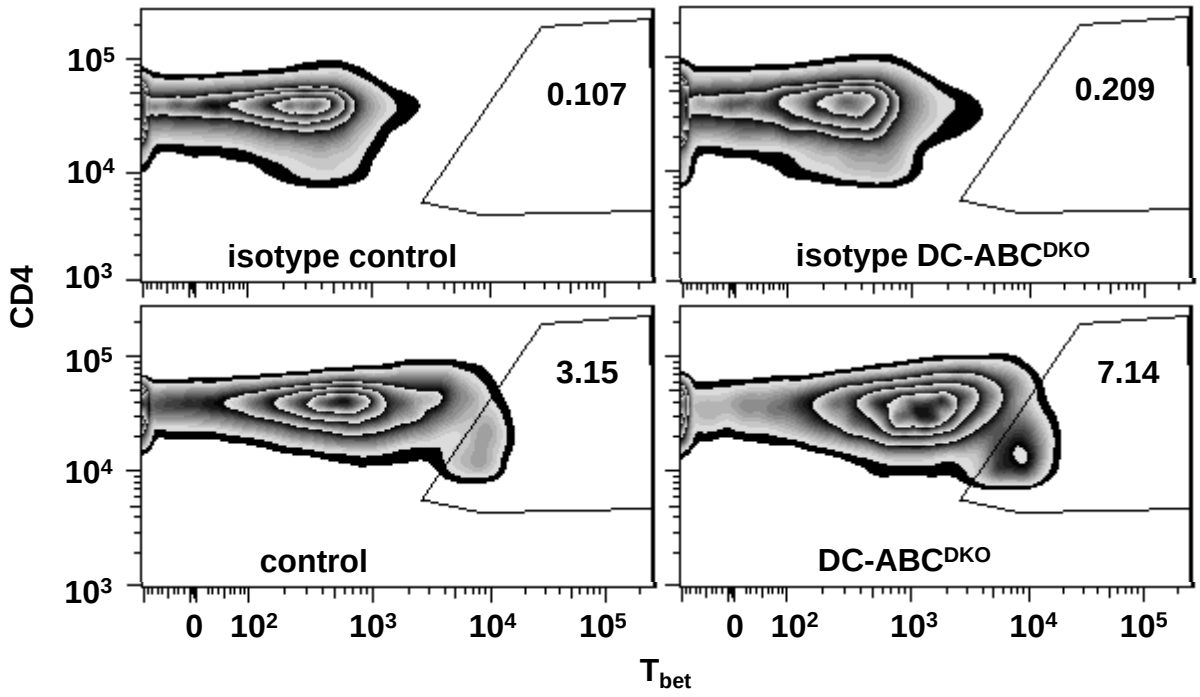

Figure S5, related to Figure 5. Splenic cell homogenates were stained with an antibody to CD4, fixed and permeabilized, and stained for T_{bet} or its isotype control. Representative FACS plots are shown.

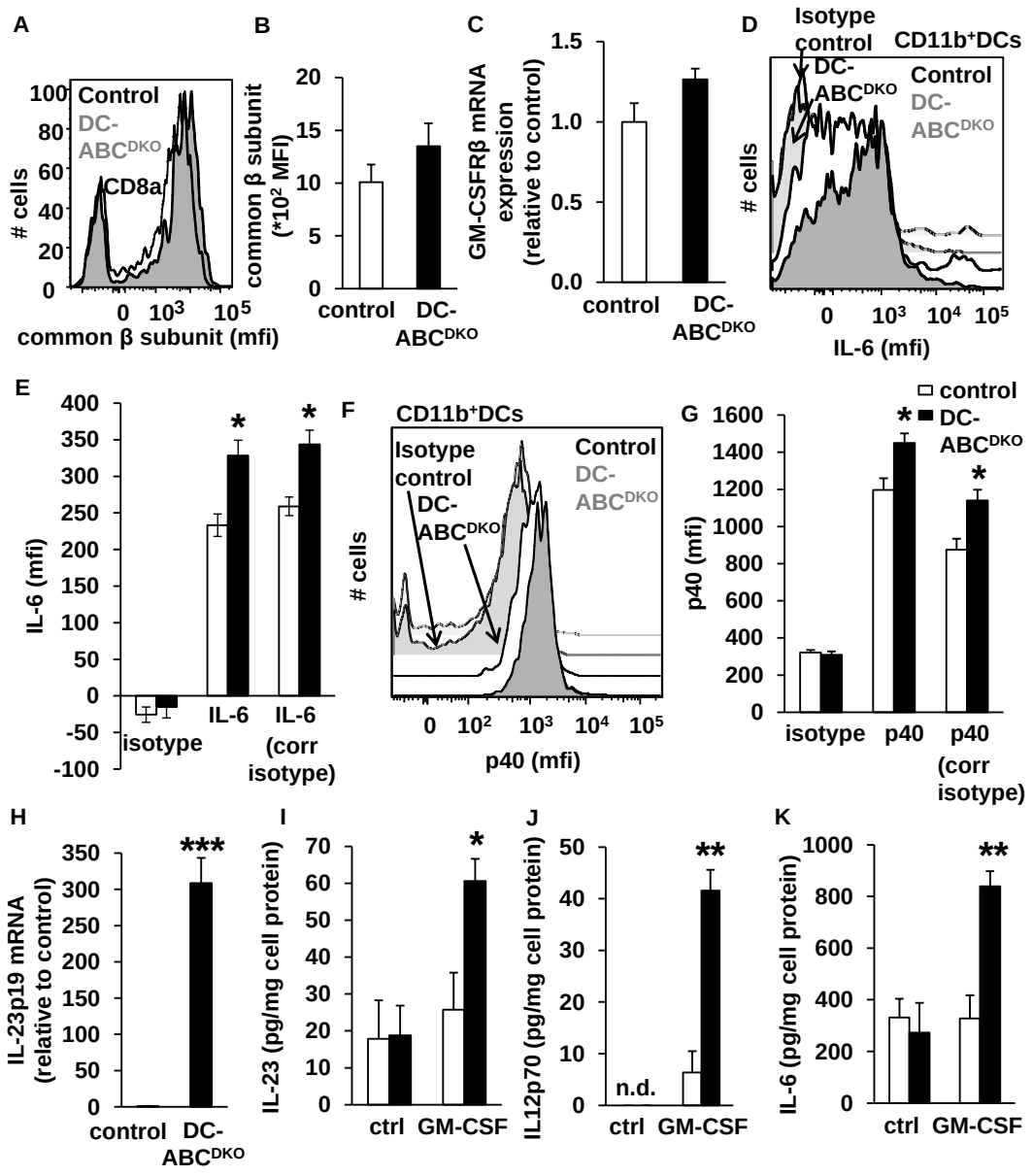

Figure S6 related to Figure 7. DC *Abca1/g1* deficiency does not affect GM-CSFRβ surface on CD8a$^+$ DCs or GM-CSFRβ mRNA expression in CD11b$^+$ DCs, but enhances intracellular cytokines in CD11b$^+$ DCs, and secretion of interleukins induced by GM-CSF in CD11c$^+$ DCs. Spleens were isolated from 20 week old mice. Spleens were isolated (**A-B**), stained for DC markers in combination with the indicated antibodies, and analyzed by flow cytometry. (**A-B**) DC-*Abca1/g1* deficiency did not affect the surface level of the common β subunit of the GM-CSF receptor on splenic CD8a$^+$ DCs. n=6. (**C**) CD11c$^+$MHCII$^+$CD11b$^+$ DCs were sorted using flow cytometry, RNA was extracted and GM-CSFRβ mRNA levels assessed. n=4. (**D-G**) Intracellular staining of IL-6 (**D**, **E**), and p40 (**F**, **G**) in CD11c$^+$MHCII$^+$CD11b$^+$ DCs.

FACS plots (**D**, **F**) and quantifications (**E**, **G**) are shown. n=4. (**H**) IL23p19 mRNA levels in $CD11c^{+}MHCII^{+}CD11b^{+}$ DCs as sorted in (**C**). n=4. (**I-K**) $CD11c^{+}$ cells were sorted from the spleen of 20 week old control and $DC\text{-}ABC^{DKO}$ mice using CD11c positive beads and then incubated with or without GM-CSF (20 ng/ml) for 24 h. IL-23 (**I**), IL-12p70 (**J**), and IL-6 (**K**) secretion were assessed and corrected for cell protein. n=4. Data in **B**, **C**, **E**, **G-K** are presented as mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, by t-test.

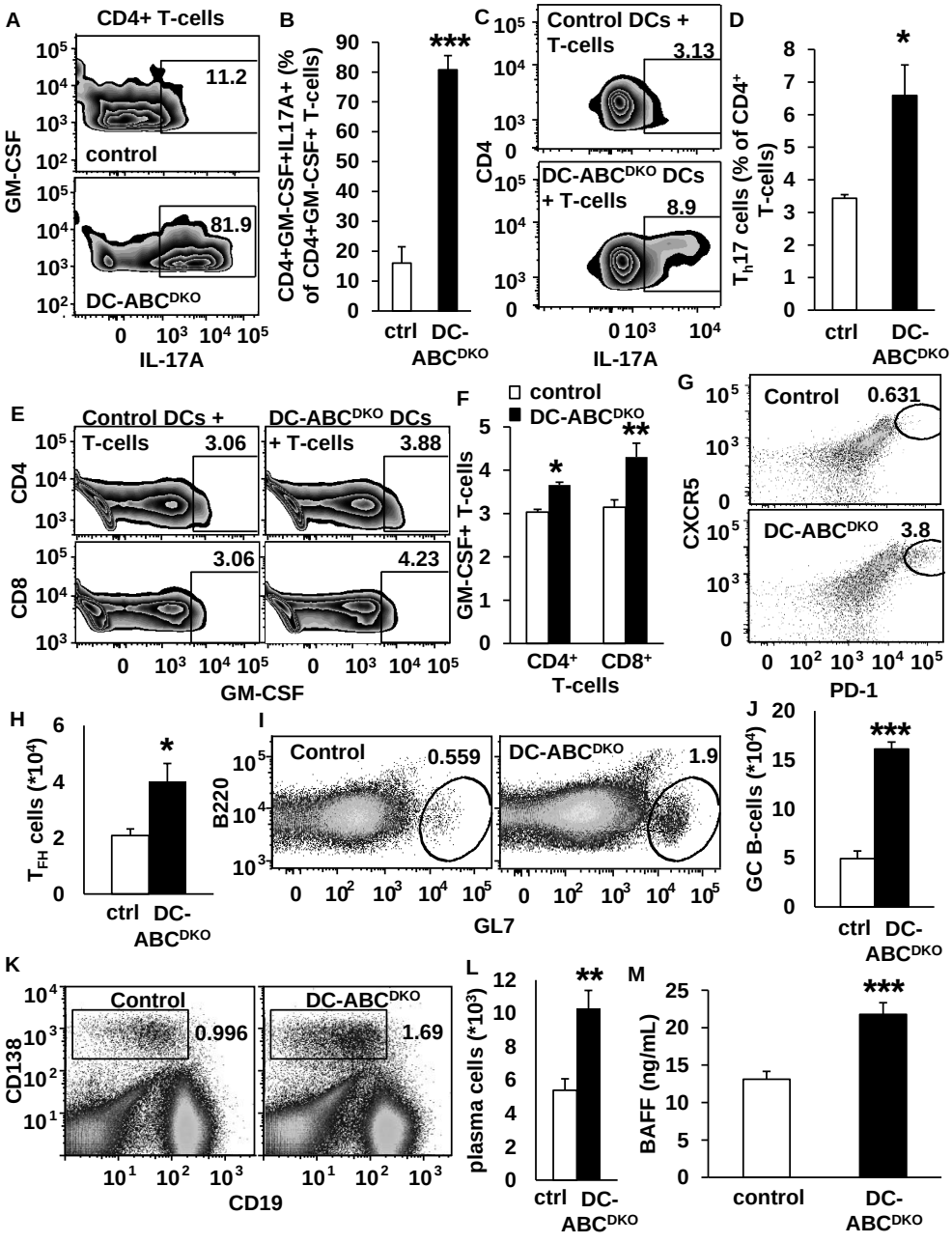

Figure S7, related to Figure 7. DC-*Abca1/g1* deficiency leads to expansion of several T-cell subsets. Spleens were isolated from 20 week old mice. (**A-B**) In DC-ABC^{DKO} mice, GM-CSF^{+} T-cells are highly positive for IL17A. Splenic cell homogenates were stained with antibodies to CD4, fixed and permeabilized, stained for GM-CSF and IL17A, and IL17A expression was

assessed in GM-CSF$^+$ T-cells. FACS plots (**A**), and quantification (**B**). n=4. (**C-F**) *Abca1/g1* deficiency in DCs stimulates differentiation of T-cells towards T_h17 cells (**C-D**) and GM-CSF$^+$ T-cells (**E-F**) in co-incubation experiments of DCs with naïve T-cells. CD11b$^+$ DCs were isolated from splenic homogenates of control and DC-ABC^{DKO} mice using CD11b$^+$ beads and co-incubated with splenic T-cells of 8 week old wild-type mice. Cells were co-cultured in a DC:T-cell ratio of 1:5 for 5 days and subsequently stained with antibodies to CD4, fixed and permeabilized, stained for IL17A, and T_h17-cells were assessed (**C-D**). n=4. Alternatively, after co-incubation, cells were stained with antibodies to CD4 and CD8, fixed and permeabilized, stained for GM-CSF and GM-CSF$^+$ T-cells were assessed (**E-F**). n=4. (**G-L**) Splenic T- and B-cell subsets were analyzed by flow cytometry. T follicular helper (T_{FH}) cells were identified as TCRβ$^+$CD4$^+$CXCR5$^+$PD1$^+$ (**G-H**). Germinal center B-cells were identified as B220$^+$GL7$^+$ (**I-J**). Plasma cells were identified as CD19$^-$CD138$^+$ (**K-L**). n=6. (**M**) DC-*Abca1/g1* deficiency increased plasma BAFF levels. n=10. Data in **B**, **D**, **F**, **H,** **J**, **L**, and **M** are presented as mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, by t-test.