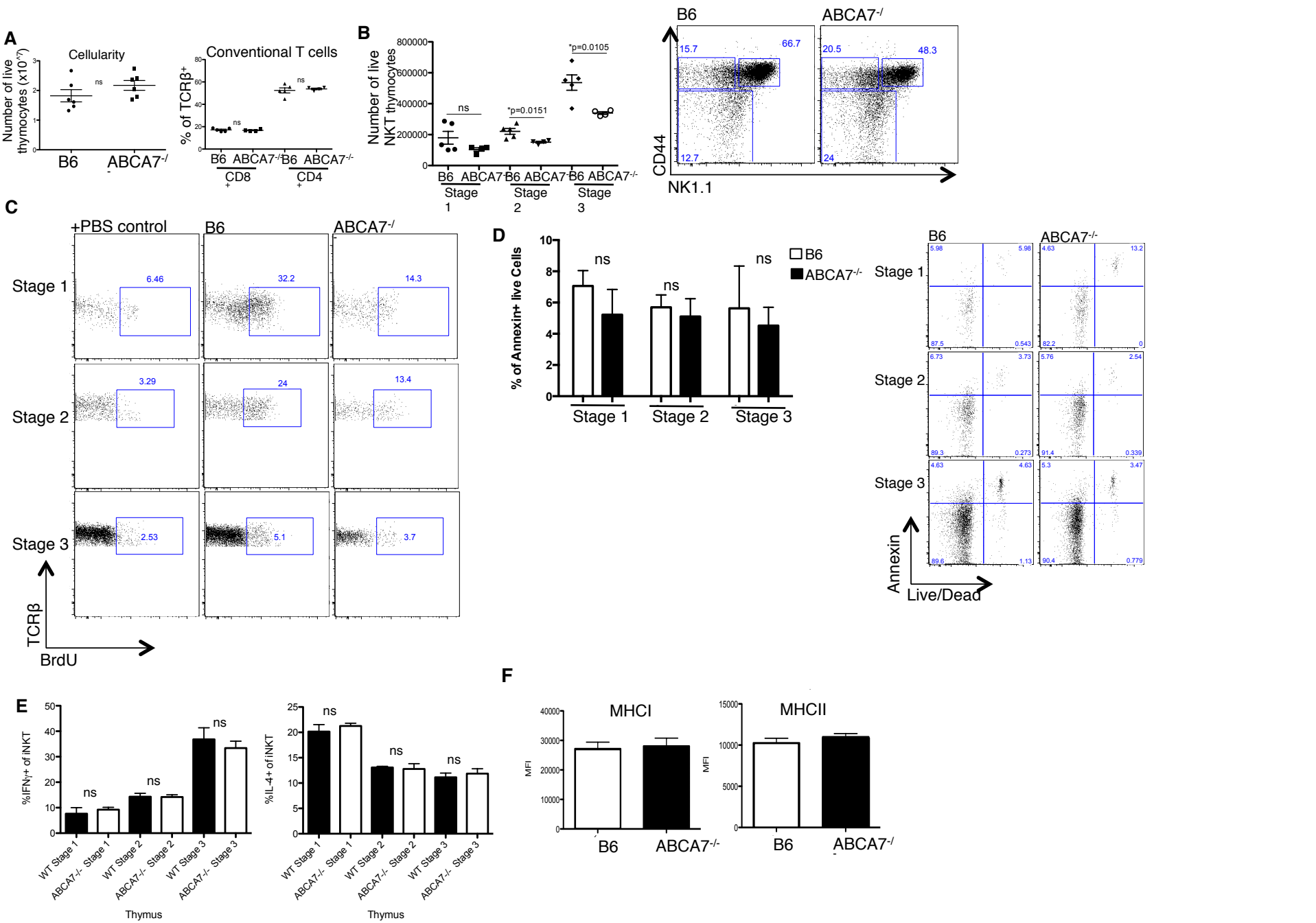Supplemental Information for:

ATP Binding Cassette Transporter ABCA7 Regulates NKT Cell Development and Function by Controlling CD1d Expression and Lipid Raft Content

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A

Cellularity

Conventional T cells

B

B6   ABCA7⁻/⁻

*p=0.0105

*p=0.0151

ns

CD44 / NK1.1

15.7   66.7   20.5   48.3
12.7   24

C

+PBS control   B6   ABCA7⁻/⁻

Stage 1   6.46   32.2   14.3

Stage 2   3.29   24   13.4

Stage 3   2.53   5.1   3.7

TCRβ / BrdU

D

% of Annexin+ live Cells

B6   ABCA7⁻/⁻

B6   ABCA7⁻/⁻

Stage 1   5.98 5.98   4.63 13.2
87.5 0.543   82.2 0

Stage 2   6.73 3.73   5.76 2.54
89.3 0.273   91.4 0.339

Stage 3   4.63 4.63   5.3 3.47
89.6 1.13   90.4 0.779

Annexin / Live/Dead

E

%IFNγ+ of iNKT

%IL-4+ of iNKT

Thymus

Thymus

F

MHCI

MHCII

B6   ABCA7⁻/⁻

B6   ABCA7⁻/⁻

Supplemental Figure 1

A) Total number of live thymocytes and total number of live TCRb$^+$CD4$^+$ and TCRb$^+$CD8$^+$ T cells in the thymus of WT (B6) and *Abca7-/-* mice. B) Number of thymic NKT cell stages (Stage 1 CD44^{lo}NK1.1$^-$, Stage 2 CD44^{hi}NK1.1$^-$, Stage 3 CD44^{hi}NK1.1$^+$) in B6 versus *Abca7-/-* mice C) BrdU$^+$ NKT cells at Stage 1-3 of development D) Frequency of Annexin-V$^+$, Live cells of thymic NKT cell stages (Stage 1 CD44^{lo}NK1.1$^-$, Stage 2 CD44^{hi}NK1.1$^-$, Stage 3 CD44^{hi}NK1.1$^+$) in B6 versus *Abca7-/-* mice E) NKT cells from the thymus stimulated in vitro with anti-CD3 and anti-CD28 antibodies for 72 hours. Intracellular staining for IFNg and IL4 in NKT cells at Stage 1-3 of thymic development. F)MFI of MHCI and MHCII in thymocytes isolated from B6 and *Abca7-/-* mice. Data are representative of at least two separate experiments with at least three age and sex matched mice per group. P value, unpaired, Student's two-tailed t-test. *p<0.05. ns: not significant, p>0.05.
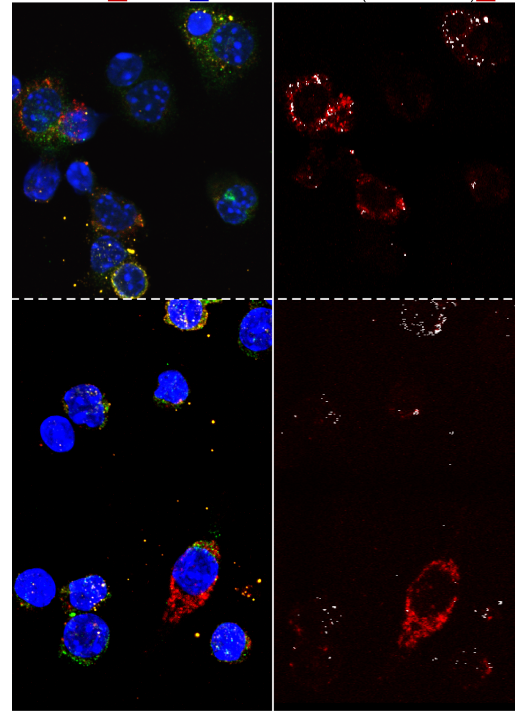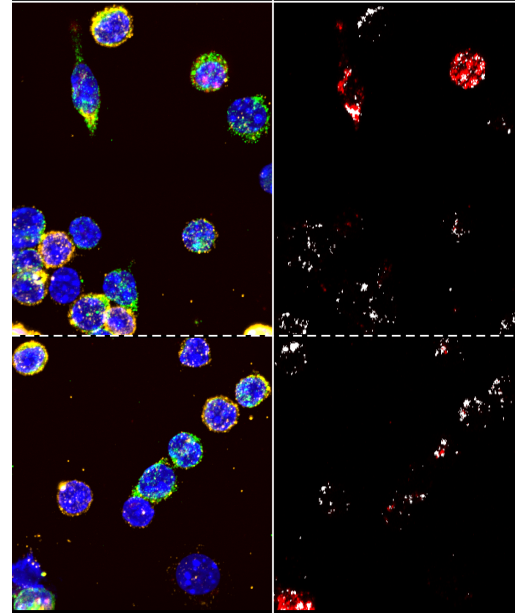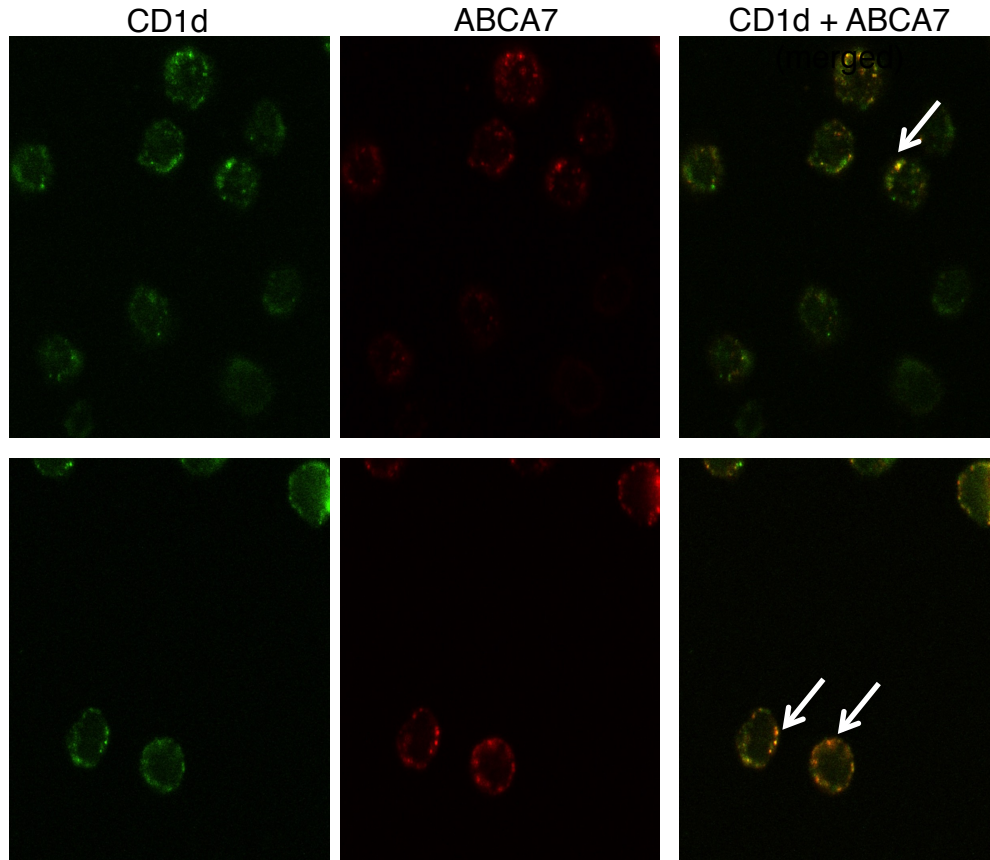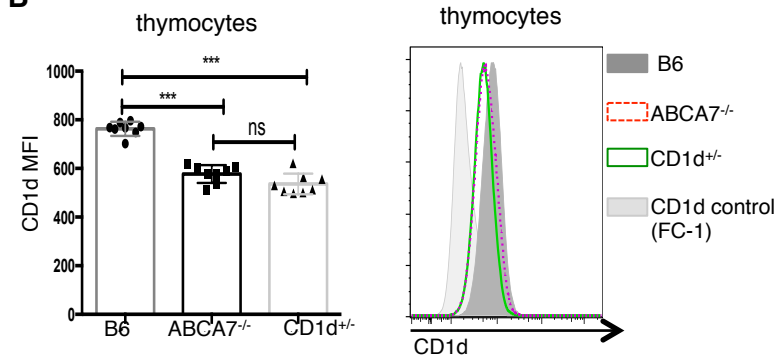
Supplemental Figure 2. **Colocalization of CD1d and LAMP-1.**
Additional images showing colocalization by confocal microscopy of CD1d, Caveolin-1, and LAMP-1 on peritoneal macrophages harvested from WT and *Abca7*^{-/-} mice (representative images). CD1d is shown in orange, caveolin in green, LAMP-1 in red, and Hoechst in blue. Left panels show all 4 markers; right panel shows colocalization of CD1d and LAMP-1.

Supplemental Figure 3. A) Confocal microscopy of CD1d and ABCA7 co-localization on DP thymocytes isolated from WT (B6) mice. Top and bottom panels show two different images. White arrows point to some areas of colocalization. B) CD1d MFI on B6, *Abca7-/-*, and *CD1d+/-* thymocytes. Data are representative of at least two separate experiments with at least three age and sex matched mice per group. P value, unpaired, two-tailed t-test. ***p<0.0005. ns: not significant, p>0.05