ABCG1 regulates pulmonary surfactant metabolism in mice and men

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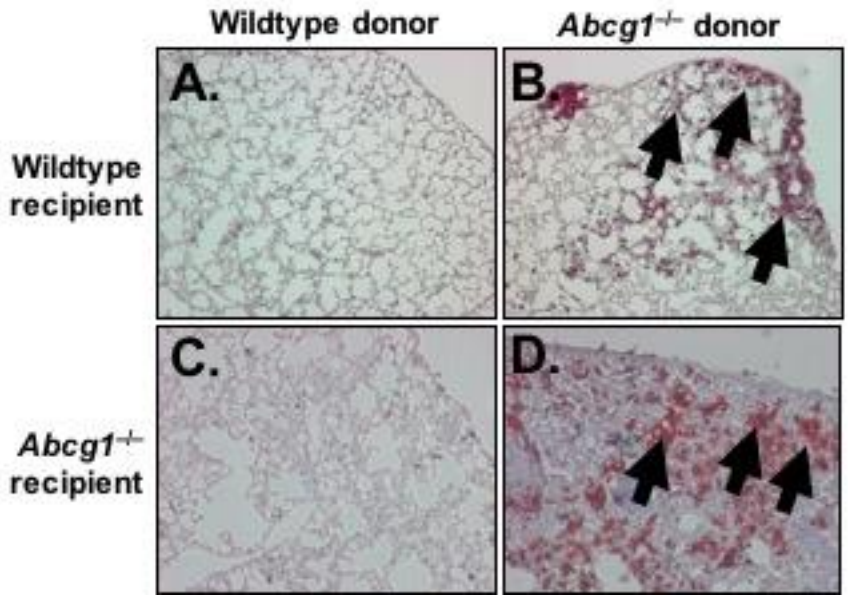
Running Title: Dissecting the Pulmonary Lipidosis of *Abcg1*$^{-/-}$ Mice

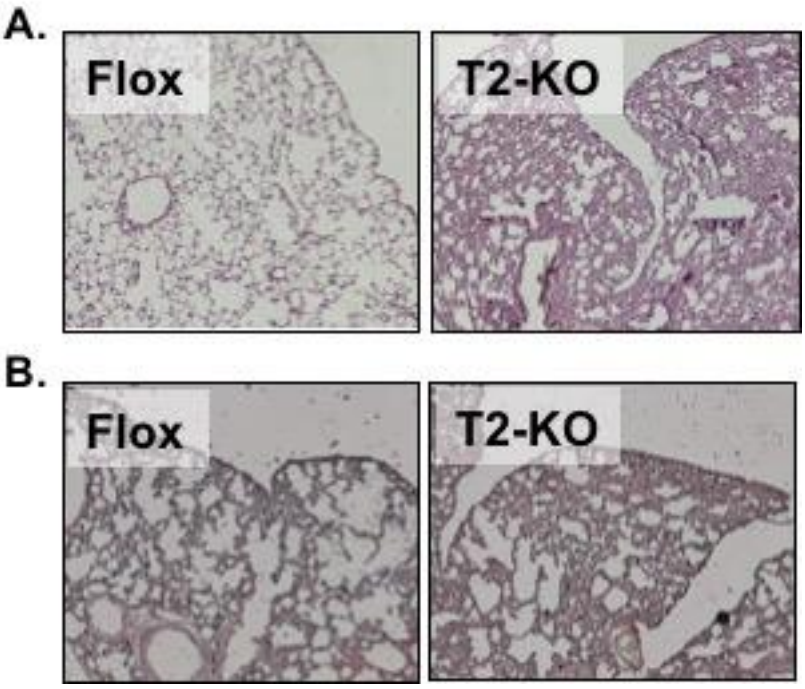
Supplemental Data

Supplemental Table S1. Characteristics of PAP patients.

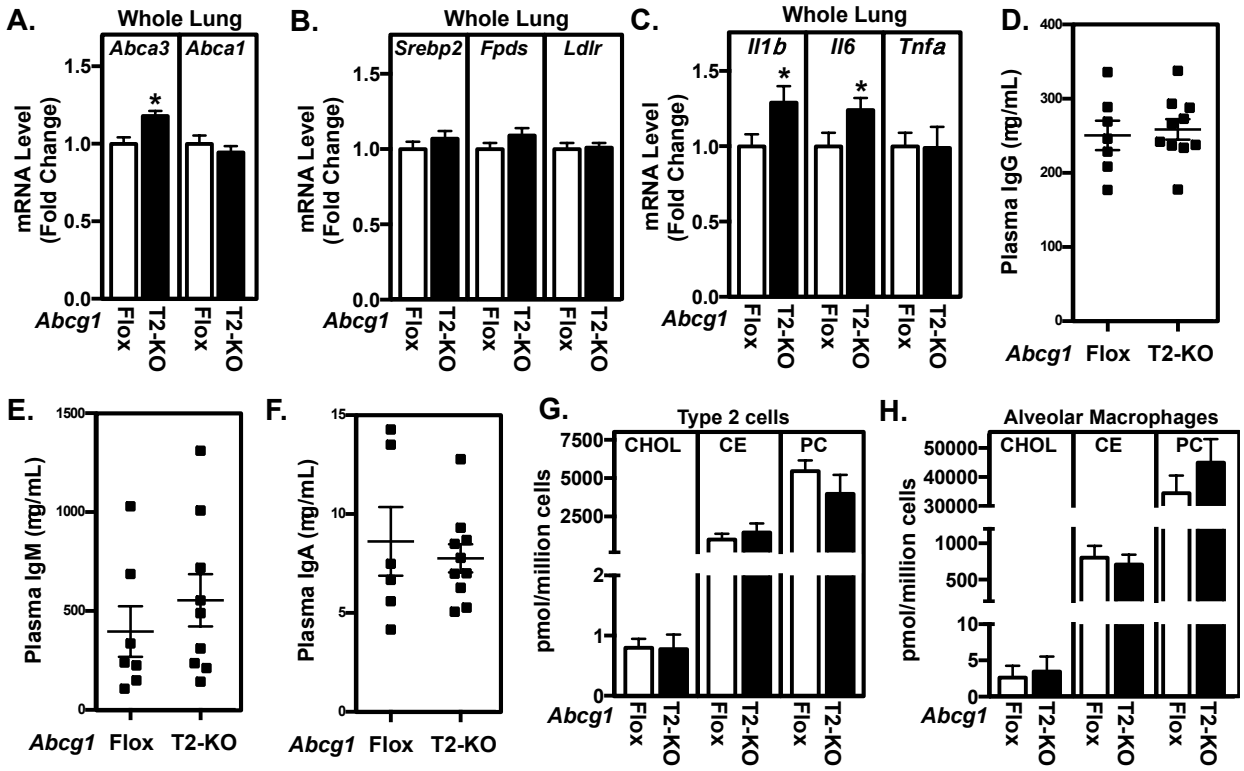
Patient Characteristics	**Cohort**
Number of subjects	7
Males	6
Females	1
White	4
African-American	1
Hispanic	2
Median Age (yr) Male Female	 46.5 50



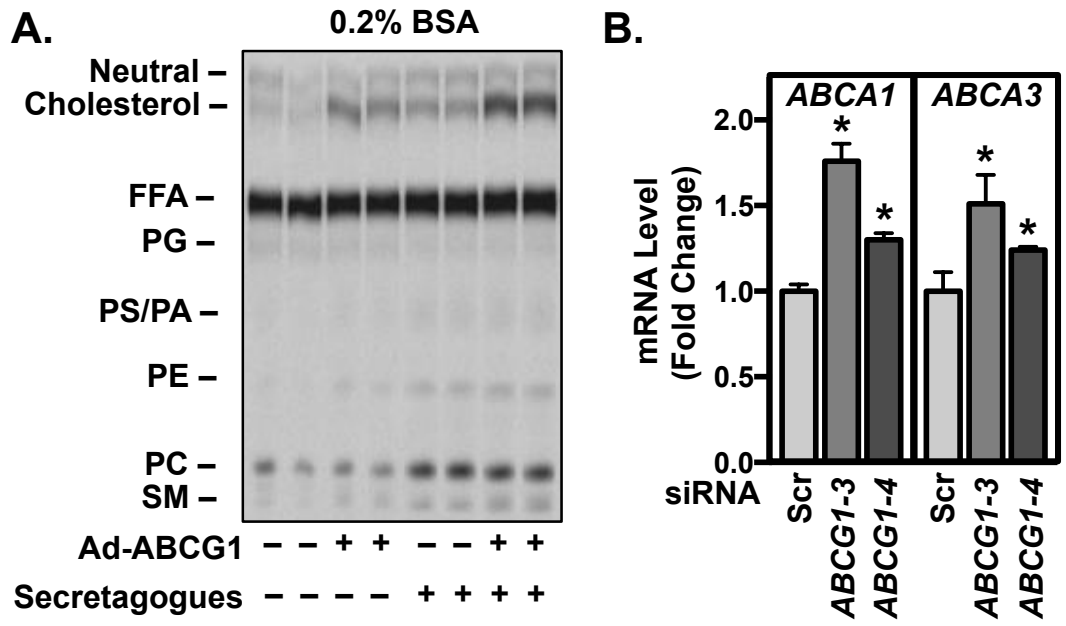
Supplemental Figure S1. (A-D) Frozen lung sections (10 μM) from mice treated as in Figure 1 were stained with Oil red O to identify neutral lipids. Arrows indicate positively stained areas. **(A)** *Abcg1*$^{+/+}$ donor bone marrow (BM) → *Abcg1*$^{+/+}$ recipient mice. **(B)** *Abcg1*$^{-/-}$ donor bone marrow (BM) → *Abcg1*$^{+/+}$ recipient mice. **(C)** *Abcg1*$^{+/+}$ donor bone marrow (BM) → *Abcg1*$^{-/-}$ recipient mice. **(D)** *Abcg1*$^{-/-}$ donor bone marrow (BM) → *Abcg1*$^{-/-}$ recipient mice.



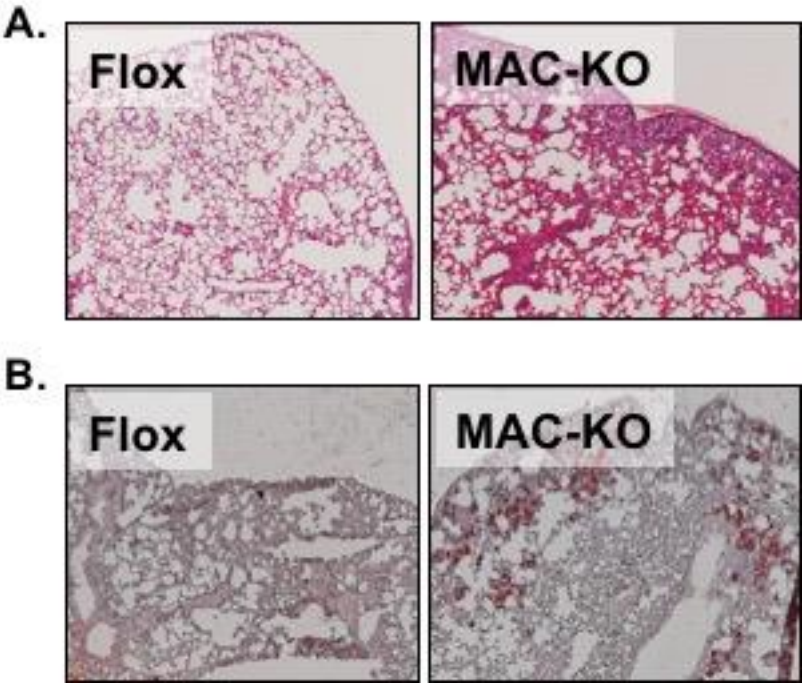
Supplemental Figure S2. Frozen lung sections (10 μM) from *Abcg1*$^{fl/fl}$ (Flox) and *Abcg1*$^{T2\text{-}KO}$ (T2-KO) mice were stained with hematoxylin and eosin **(A)** or Oil red O **(B)**.



Supplemental Figure S3. (A-C) Whole lung mRNA expression of **(A)** *Abca1* and *Abca3*, **(B)** *Srebp-2*, *Fdps*, and *Ldlr*, and **(C)** *Il1β*, *Il6*, and *Tnfα* in *Abcg1*$^{fl/fl}$ (Flox) and *Abcg1*$^{T2\text{-}KO}$ (T2-KO) mice. Gene expression was normalized to 36B4 and presented as fold change. Data are expressed as mean mRNA level ±SEM (n = 4-6 mice/genotype). **(D-F)** Plasma was diluted 1:250-1:1000 and tested for binding to IgG **(D)**, IgM **(E)** and IgA **(F)**. HRP-conjugated antibodies were used for detection. Data are presented as mean antibody titer (ng/mL) ±SEM (n = 3-6 mice/genotype). **(G-H)** Cholesterol, cholesteryl ester, phosphatidylcholine and their derivatives were quantified by ESI-MS/MS in T2 cells **(G)** and alveolar macrophages **(H)** from *Abcg1*$^{fl/fl}$ and *Abcg1*$^{T2\text{-}KO}$ mice. Data are presented as mean lipid level (pmol/million cells) ±SEM (n = 3-6 mice/genotype). Significance was measured by Student's t test. * $p < 0.05$.



Supplemental Figure S4. (A) A549 T2 cells were infected as in Figure 4. Cells were pulse labeled with ^{14}C-acetate for 4 h, followed by a 2 h chase in media containing 0.2% BSA in the presence or absence of a secretagogue cocktail (100 μM ATP, 0.1 μM phorbol-12-myristate-13-acetate, 20 μM terbutaline). Total secreted lipids were extracted from the media and separated by thin layer chromatography to determine the levels of phospholipids. **(B)** Increased *ABCA3* and *ABCA1* expression in A549 cells treated with *ABCG1* siRNA. Data are presented as mean ±SEM (n = 6 replicates/condition). Significance was measured by one-way ANOVA followed by Bonferroni correction. * $p < 0.05$.



Supplemental Figure S5. Frozen lung sections (10 μM) from *Abcg1*$^{fl/fl}$ (Flox) and *Abcg1*$^{MAC\text{-}KO}$ (MAC-KO) mice were stained with hematoxylin and eosin **(A)** or Oil red O **(B)**.