Supplemental Figures

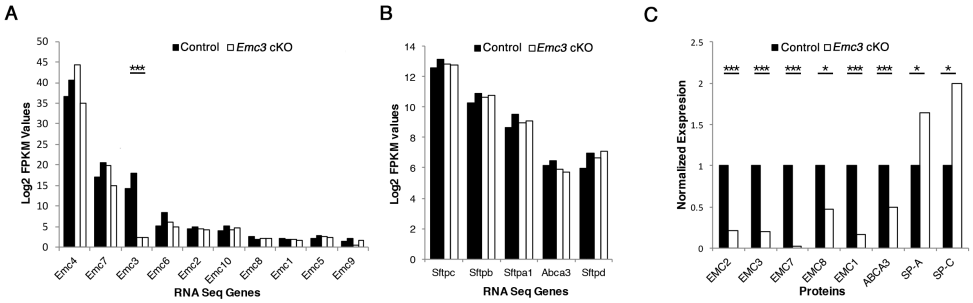

Figure S1. RNA sequencing and proteomic data of selected genes in E18.5 control and *Emc3* cKO EpCAM+ lung cells.

(A) RNA sequencing data of *Emc* genes were obtained in E18.5 control and *Emc3* cKO EpCAM+ lung cells. *Emc3* mRNA was decreased without changes in RNA transcripts encoding other EMC subunits in *Emc3* cKO mice.

(B) RNA sequencing data related to surfactant associated genes in E18.5 control and *Emc3* cKO EpCAM+ lung cells. No significant changes in Abca3 or surfactant associated protein mRNAs were observed in *Emc3* cKO mice.

(C) Proteomic analysis on E18.5 control and *Emc3* cKO EpCAM+ lung cells. EMC proteins and ABCA3 were decreased and surfactant proteins SP-A and SP-C were increased in EpCAM+ cells of *Emc3* cKO lung.

For RNA sequencing, EpCAM+ lung cells from two *Emc3* cKO and two control samples were analyzed. For proteomics, four samples of each genotype were analyzed. *P<0.05, ***P<0.001.

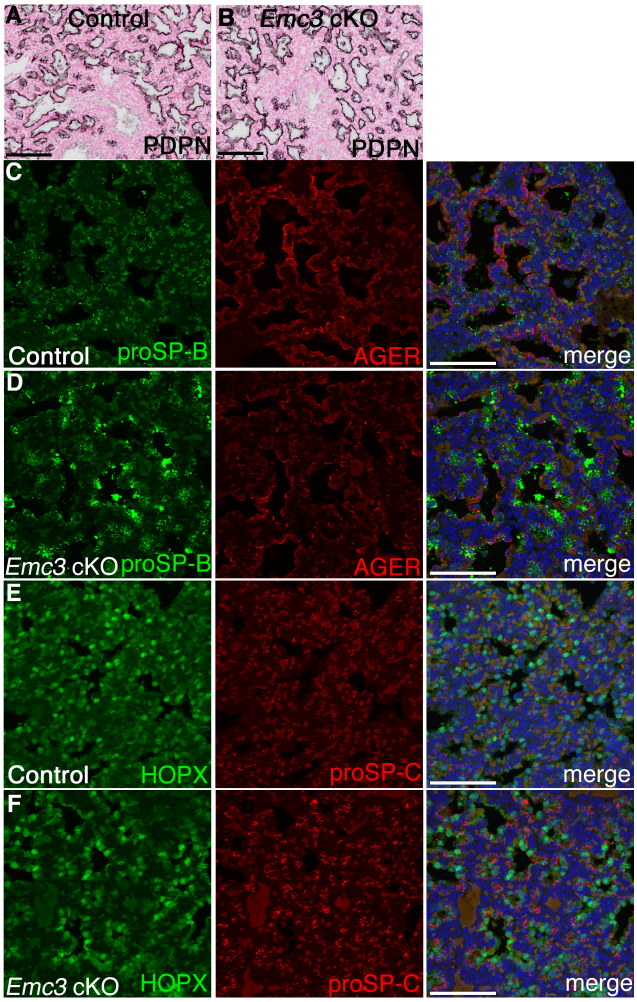

Figure S2. *Emc3* deletion in the epithelial cells does not disrupt AT1 cell differentiation.

(A and B) Immunohistochemical staining for Podoplanin (PDPN) on lung sections of control and *Emc3* cKO embryos at E18.5.

(C-F) Immunofluorescence staining for AGER and HOPX on lung sections of control and *Emc3* cKO embryos at E18.5.

All AT1 cell markers, PDPN, AGER or HOPX demonstrate normal patterns of staining after deletion of *Emc3*. Scale bars: 100 μm.

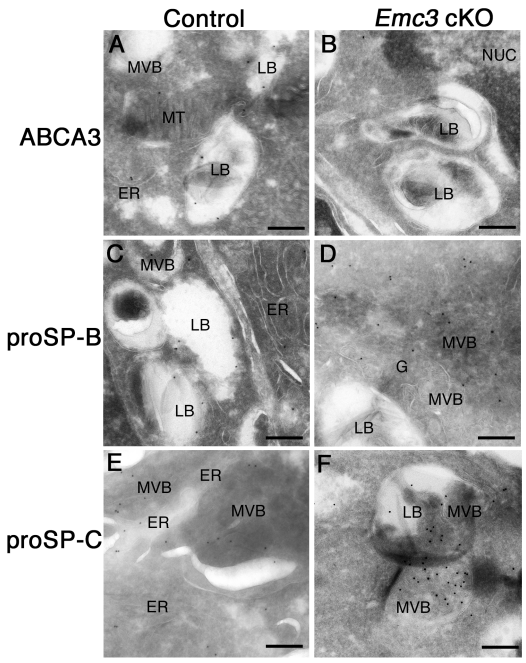

Figure S3. Immunogold labeling of ABCA3, proSP-B, and proSP-C in E18.5 control and *Emc3* cKO embryos.

In control AT2 cells, ABCA3 labeling was present in limiting membranes of lamellar bodies; proSP-B and proSP-C were normally processed and did not accumulate intracellularly. In *Emc3* cKO AT2 cells, ABCA3 staining was markedly decreased; both proSP-B and proSP-C accumulated in the multivesicular bodies. ER: endoplasmic reticulum; G: Golgi apparatus; LB: lamellar body; MT: mitochondria; MVB: multivesicular body; NUC: nucleus. Scale bars: 250 nm.

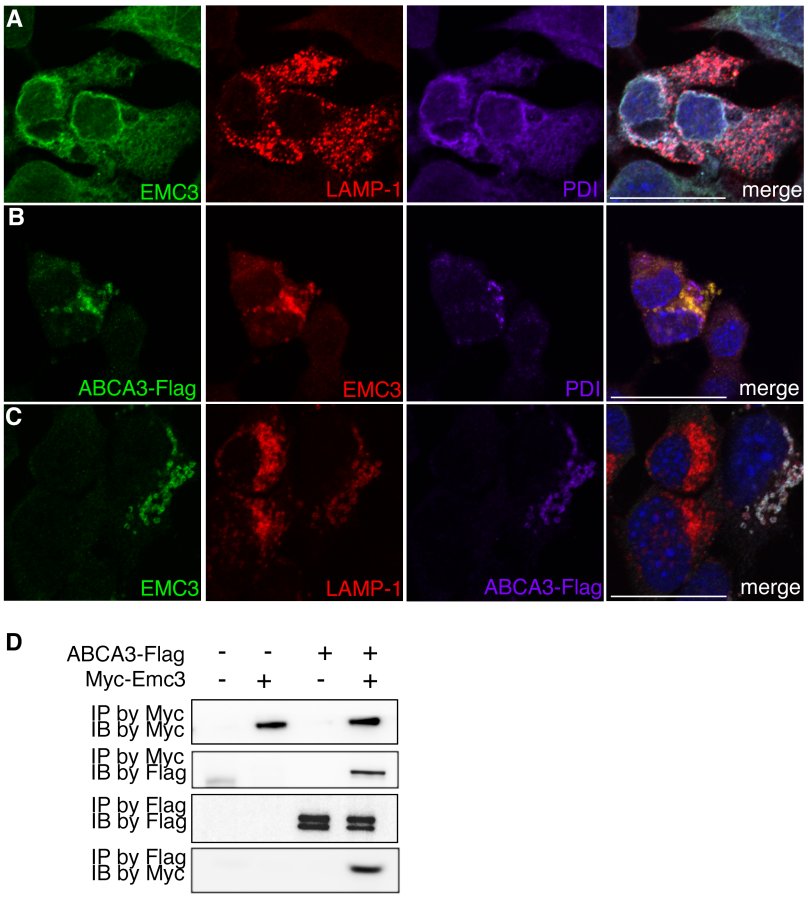

Figure S4. Interaction between EMC3 and ABCA3 in MLE-15 cells.

(A) Staining of MLE-15 cells with EMC3 antibody shows colocalization of endogenous EMC3 with ER marker, PDI. Scale bar: 25μm.

(B and C) MLE-15 cells were transfected with ABCA3-Flag alone and immunostained 48 hours later. ABCA3-Flag expression increased endogenous EMC3 levels and recruited EMC3 to LAMP-1 positive vesicles. Scale bars: 25μm.

(D) Equal numbers of MLE-15 cells were transfected with control vectors or Myc-Emc3 cDNA alone or ABCA3-Flag alone or both together. Cells were lysed 48 hours later and immuno-precipitated by beads conjugated with Myc antibody or Flag antibody. Samples were eluted and processed for immunoblotting. Three independent experiments were performed and representative results are shown. Antibodies used for immunoblotting are indicated on the left of the panels.

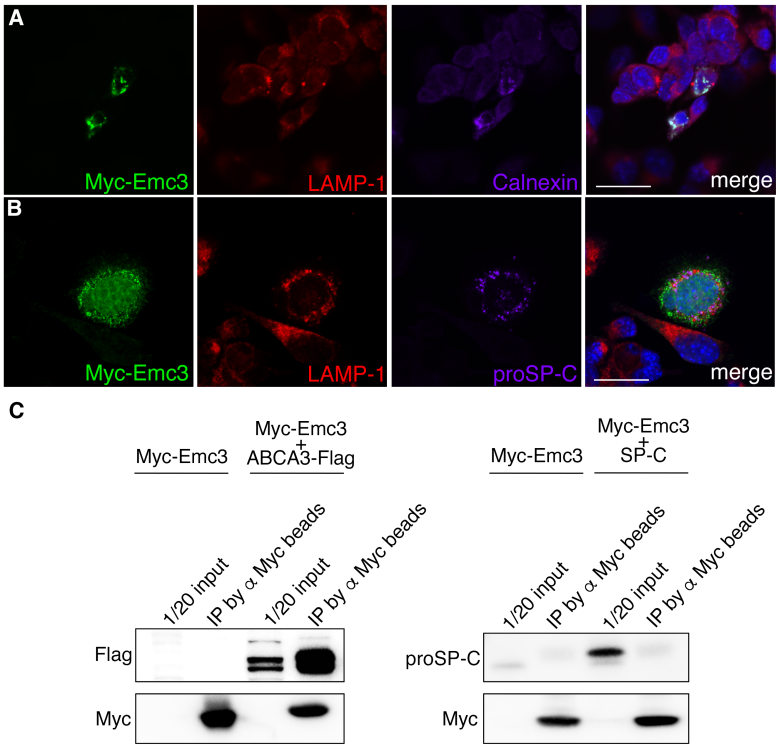

Figure S5. Lack of EMC3-ProSP-C interaction in MLE-15 cells.

(A) MLE-15 cells were transfected with Myc-Emc3 and immunostained 48 hours later. Scale bar: 25μm.

(B) MLE-15 cells were co-transfected with *SFTPC* cDNA and Myc-Emc3 and immunostained 48 hours later. Scale bar: 25μm.

(C) Equal numbers of MLE-15 cells were transfected with Myc-Emc3 cDNA alone or together with cDNAs expressing *Abca3-Flag* or *SFTPC*. Cells were lysed 48 hours later and immuno-precipitated by beads conjugated with Myc antibody. Samples were eluted and processed for immunoblotting. Three independent experiments were performed and representative results are shown. Antibodies used for immunoblotting are indicated on the left of the panels.

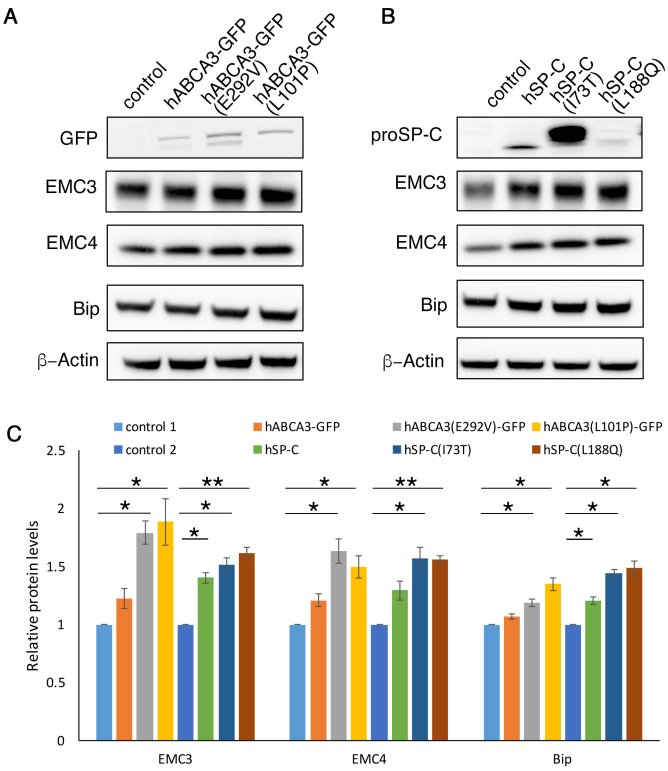

Figure S6. Expression of human ABCA3 and SP-C variant proteins induced Bip, EMC3 and EMC4.

(A and B) MLE-15 cells were transfected with equal amount of empty vector or constructs encoding different ABCA3 (A) or SP-C (B) variants. Cells were harvested 60 hours after transfection. Lysates were subjected to immunoblotting using indicated antibodies.

(C) Quantification of western blots shown in (A) and (B) from three independent experiments. Means and ± SEM; *P<0.05, **P<0.01 using unpaired, 2-tailed Student's t test.

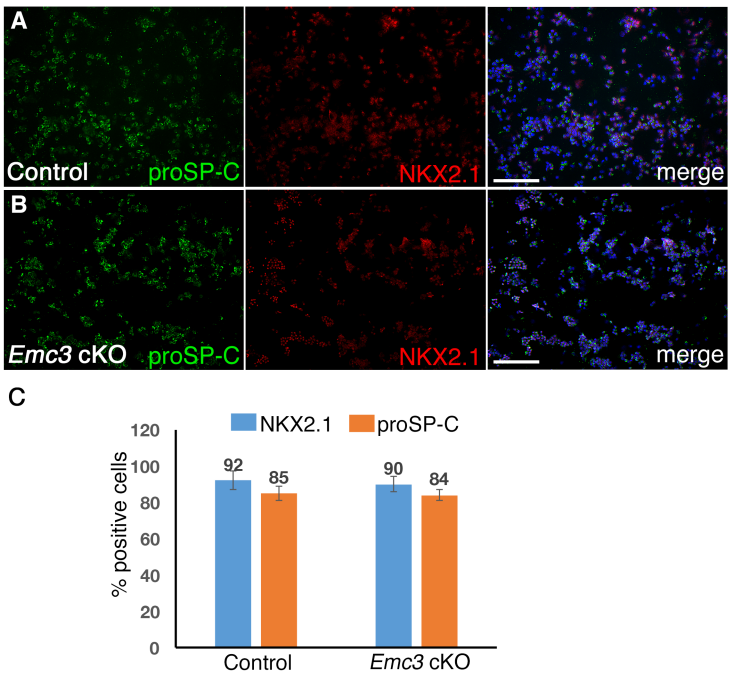

Figure S7. Enrichment of AT2 cells after EpCAM sorting.

EpCAM+ epithelial cells were isolated from control and *Emc3* cKO fetal lungs (E18.5) using magnetic beads.

(A and B) Immunostaining of cytospins of sorted cells with NKX2.1/proSP-C and with DAPI. Representative immunofluorescence images are shown. Scale bars, 100 μm.

(C) Quantification of the ratios of epithelial cells (NKX2.1+) and AT2 cells (proSP-C+) in DAPI+ cells from the staining shown in (A) and (B). Means ± SEM are shown. n=4/group.

Supplemental Tables

Genes	Fold change in *Abca3* cKO	Fold change in *Emc3* cKO	Correlation
Aqp3	-7.09	3.15	Opposite
Sftpa1	-3.95	1.11	Opposite
Gjb6	-3.64	-2.30	Same
Aqp5	-3.20	-1.21	Same
Lipg	-2.45	1.27	Opposite
Pnliprp1	-2.16	-26.67	Same
Lpcat1	-2.11	1.01	Opposite
Rab27a	-1.90	-1.23	Same
Pnpla2	-1.59	-1.23	Same
Sc5d	1.71	1.30	Same

Table S1. Comparison of differentially expressed genes in *Abca3* cKO and *Emc3* cKO lungs.

Changes in mRNAs in whole lung from E18.5 ABCA3 gene deleted embryos (*SP-C-rtTA*$^{tg/wt}$*;TetO-Cre*$^{tg/wt}$*;Abca3*$^{flox/flox}$ deleted doxycycline administration to the dam from E6.5 to E14.5) (1) were compared with whole lung RNA sequencing data. Transcriptional changes in E18.5 *Emc3* cKO lungs were assessed by RNA sequencing of whole lung mRNA.

A

Lipid enzymes	P value	Fold change
BDH2	0.0025	-10.36
GDPD2	0.0048	-7.59
MGST2	0.0043	-7.56
CES1D	0.0015	-5.80
SCD1	0.0033	-5.13
LPL	0.0044	-4.78
ACSS2	0.0150	-4.40
ACOXL	0.0101	-4.31
ACSS1	0.0134	-3.83
ISYNA1	0.0173	-3.77
TAMM41	0.0485	-3.75
MCEE	0.0352	-3.47
PAFAH2	0.0296	-3.24
ACSL1	0.0392	-3.04
GPX1	0.0487	-2.87
SCD2	0.0033*	-2.28

Other lipid genes	P value	Fold change
PON1	0.0049	-26.72
ABCA1	0.00003	-11.20
FABP12	0.0003	-8.58
APOC1	0.0024	-7.81
APOE	0.0475	-5.54
FABP5	0.0041	-4.81
TECRL	0.0087	-4.34
SCP2	0.0096	-4.05
MID1IP1	0.0131	-3.88
PDP2	0.0190	-3.79
CERS4	0.0235	-3.46
INSIG1	0.0372	-3.19
FDX1	0.0470	-3.10
NR2F2	0.0408	-3.08
SLC16A1	0.0367	-3.05
NSMAF	0.0432	-2.95

UPR Genes	P value	Fold change
CREB3L1	0.0194*	-2.08
ATF4	0.0178*	1.54
DDIT3	0.0084*	1.70
BHLHA15	0.0030*	1.72
HSPA5	0.0036*	1.77
MAP3K5	0.0430*	1.86
TRIB3	0.0377*	1.88
PDIA5	0.0041*	2.00
DNAJB9	0.0376*	2.15
KDELR3	0.0057*	2.16
ASNS	0.0341*	2.25
ATF3	0.0190	4.20
THBS1	0.0027	5.40

* P value from Altanalyze analysis

B

Lipid enzymes	P value	Fold change
SOAT1	0.0009	-5.21
MCCC1	0.0019	-2.31
GALC	0.0071	-2.11
GDE1	0.0002	-2.02
CES1D	0.0008	-1.95
PCCA	0.0018	-1.82
CAT	0.0003	-1.81
IVD	0.0011	-1.64
MCCC2	0.0012	-1.58
PCCB	0.0068	-1.56
NAGA	0.0194	-1.48
PIGU	0.0077	-1.46
NSDHL	0.0486	-1.41
ACLY	0.0013	-1.38
HMGCS1	0.0248	-1.38
ECI2	0.0081	-1.38
ASAH1	0.0010	-1.37
ACAA2	0.0029	-1.36
ACSL1	0.0360	-1.34
HACD3	0.0107	-1.33
MUT	0.0014	-1.33
ADH5	0.0171	-1.32
ACOT2	0.0018	-1.31
ECHS1	0.0104	-1.31
ACAT2	0.0340	-1.30
ECI1	0.0083	-1.30
GPX1	0.0097	-1.28
ACSL5	0.0019	-1.26
ACAT1	0.0046	-1.25
PRDX6	0.0082	-1.24
GCDH	0.0165	-1.23
ACACA	0.0471	-1.22
ATG7	0.0483	-1.22
CPT2	0.0033	-1.20
ANXA1	0.0019	1.43
GDPD2	0.0000	†Absent
SCD1	0.0451	†Absent
MCEE	0.0000	†Absent

Other lipid proteins	P value	Fold change
GOLM1	0.0237	-2.90
FABP5	0.0024	-2.62
NPC2	0.0136	-2.60
SCPEP1	0.0001	-2.05
ABCA3	0.0004	-2.03
SFTPB	0.0011	-2.02
ATP1A1	0.0000	-1.92
ABCD3	0.0016	-1.77
SCP2	0.0019	-1.68
SORL1	0.0075	-1.68
ERLIN1	0.0031	-1.58
PC	0.0029	-1.39
STOML2	0.0377	-1.26
PON1	0.0494	†Absent
SLC44A1	0.0000	†Absent
AGK	0.0000	†Absent
EIF6	0.0411	1.24
LAMTOR1	0.0024	1.26
AKT1	0.0130	1.34
PSAP	0.0108	1.73
C3	0.0373	1.90

UPR proteins	P value	Fold change
ASNA1	0.0060	-1.65
ATP6V0D1	0.0478	-1.59
SEC61A1	0.0044	-1.36
UBA5	0.0393	1.21
SEC31A	0.0082	1.21
GFPT1	0.0075	1.23
FAM129A	0.0018	1.26
LMNA	0.0065	1.27
TMEM33	0.0271	1.33
PDIA5	0.0162	1.33
ERP44	0.0369	1.38
MANF	0.0042	1.43
GOSR2	0.0380	2.48
THBS1	0.0423	Induced in KO only

Cathepsins	P value	Fold change
CTSZ	0.0012	-3.71
CTSC	0.0004	-3.00
CTSB	0.0039	-1.88
CTSH	0.0036	-1.58
*NAPSA	*0.0650*	*-1.28*

*P value >0.05

†Absent: not detectable in the KO but present in the control

Table S2. Changes of mRNAs (A) and proteins (B) in EpCAM+ cells sorted from *Emc3* cKO fetal lungs (E18.5). Results were obtained by RNA sequencing and proteomic analysis from control and *Emc3* cKO AT2 cells as described in Methods.

Phosphatidylcholine Species	Fold change
PC(O-38:5)	1.858
PC(15:0/16:0)	1.506
PC(16:0/22:4)	1.263
PC(16:0/20:3)	-1.252
PC(16:0/16:1)	-1.253
PC(18:0/18:1)	-1.285
PC(15:0/22:6)	-1.339
PC(20:3/20:4)	-1.366
PC(14:0/22:6)	-1.378
PC(20:4/20:4);PC(18:2/22:6)	-1.474
PC(22:6/22:6)	-1.561
PC(18:1/20:1)	-1.579
PC(20:4/22:6)	-1.636
PC(16:0/20:5)	-1.644
PC(18:2/22:5)	-1.657
PC(14:0/16:1)	-1.934
PC(16:1/20:4)	-1.945
PC(16:1/22:6)	-2.053
PC(16:1/0:0)	-2.069
PC(16:1/18:2)	-2.248
PC(16:1/16:1)	-2.764
PC(16:1/20:5)	-3.296

Phosphatidylglycerol Species	Fold change
PG(14:0/15:0)	-1.374
PG(16:0/16:0)	-1.376
PG(16:0/22:4)_B	-1.455
PG(14:0/16:0)	-1.480
PG(18:1/18:2)_A	-1.486
PG(16:1/20:4)_A	-1.579
PG(22:6/0:0)	-1.607
PG(16:1/0:0)	-1.666
PG(14:0/18:1);PG(16:0/16:1)	-1.785
PG(16:0/18:1)_A	-1.843
PG(16:1/18:2)_A	-1.850
PG(16:0/22:6)_B	-1.916
PG(18:1/20:4)_B	-1.956
PG(18:1/18:1)	-2.104
PG(18:1/22:6)_B	-2.109
PG(16:2/16:0)	-2.157
PG(18:1/22:6)_C	-2.266
PG(14:0/14:0)	-2.370
PG(16:1/18:3)_B	-2.400
PG(18:1/0:0)_D	-2.446
PG(18:1/18:2)_B	-2.529
PG(15:0/16:1)	-2.533
PG(16:0/20:5)	-2.557
PG(16:1/18:1)_B	-2.752
PG(18:2/22:6)_B	-2.903
PG(14:1/16:0);PG(14:0/16:1)	-3.153
PG(16:0/18:3)_A	-3.356
PG(17:1/18:2)	-3.446
PG(16:0/18:2)_A	-3.481
PG(16:3/16:0)	-3.847
PG(16:1/22:6)_B	-4.043
PG(16:1/18:2)_B	-4.516
PG(16:1/20:4)_B	-4.578
PG(16:0/18:3)_B	-4.918
PG(16:1/17:1)	-5.484
PG(16:1/16:1)	-5.854
PG(16:1/20:5)	-6.608

Triglyceride Species	Fold change
TG(16:0/16:0/16:0)	3.396
TG(18:0/18:1/20:4)	2.431
TG(16:0/16:0/18:1)	2.309
TG(14:0/14:0/16:0)	2.271
TG(16:0/16:0/22:6)	2.262
TG(18:1/18:1/22:3)	2.243
TG(49:1)	2.208
TG(54:5)	2.197
TG(18:1/22:4/22:4)	2.130
TG(18:1/22:1/22:6)	2.068
TG(16:0/16:0/16:1);TG(14:0/16:0/18:1)	2.060
TG(18:1/18:1/20:4);TG(16:0/18:1/22:5)	2.041
TG(18:1/20:1/22:3)	2.032
TG(16:0/18:1/18:1)	1.942
TG(18:2/18:2/20:4)	1.932
TG(54:1)	1.928
TG(16:0/16:0/18:0)	1.906
TG(58:4)	1.898
TG(51:2)	1.880
TG(16:0/18:1/22:6)	1.874
TG(53:2)	1.866
TG(52:3)	1.866
TG(18:0/20:4/22:6)	1.830
TG(16:0/16:0/18:2);TG(16:0/16:1/18:1)	1.809
TG(14:0/22:6/22:6)	1.804
TG(49:2)	1.776
TG(16:0/18:1/24:0)	1.760
TG(18:2/20:4/20:4);TG(16:0/20:4/22:6)	1.751
TG(18:1/22:4/22:6);TG(18:1/22:4/22:6)	1.723
TG(14:0/16:0/16:1)	1.698
TG(56:1)	1.691
TG(18:2/20:4/22:6)	1.684
TG(20:4/20:4/22:6)	1.683
TG(52:5)	1.592
TG(18:1/18:1/22:1);TG(18:1/20:1/20:1)	1.591
TG(14:0/20:4/22:6)	1.545
TG(50:3)	1.538
TG(18:1/22:6/22:6)	1.433

Table S3. Changes in the concentrations of lipid species in EpCAM+ cells sorted from *Emc3* cKO fetal lungs (E18.5).

Results were obtained by lipidomics analysis as described in Methods. Fold changes of significantly changed lipid species are shown, $P < 0.05$ using two-tailed, homoscedastic t-tests. n = 4/group.

Reference

1. Besnard V, Matsuzaki Y, Clark J, Xu Y, Wert SE, Ikegami M, Stahlman MT, Weaver TE, Hunt AN, Postle AD, et al. Conditional deletion of Abca3 in alveolar type II cells alters surfactant homeostasis in newborn and adult mice. *Am J Physiol Lung Cell Mol Physiol.* 2010;298(5):L646-59.