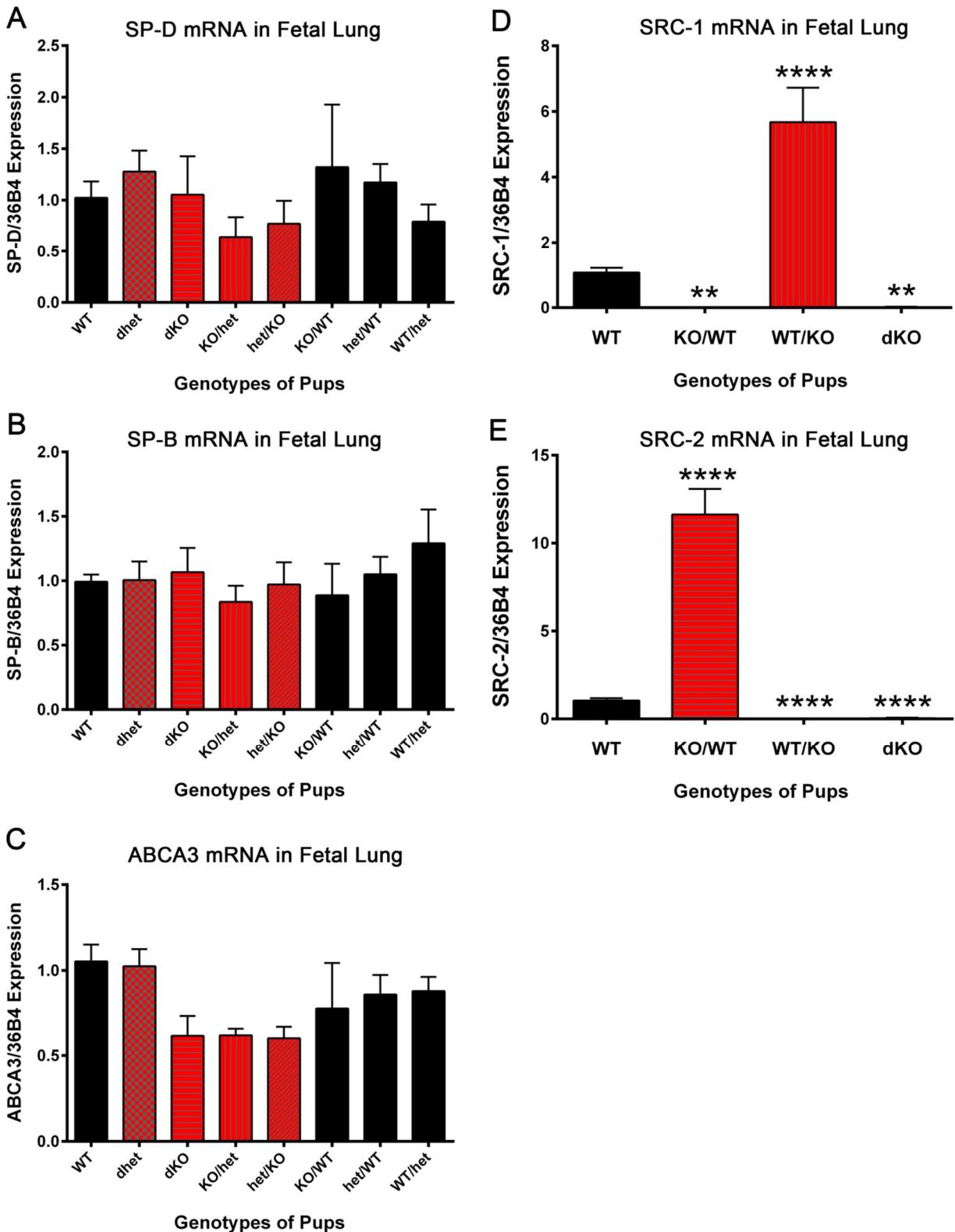


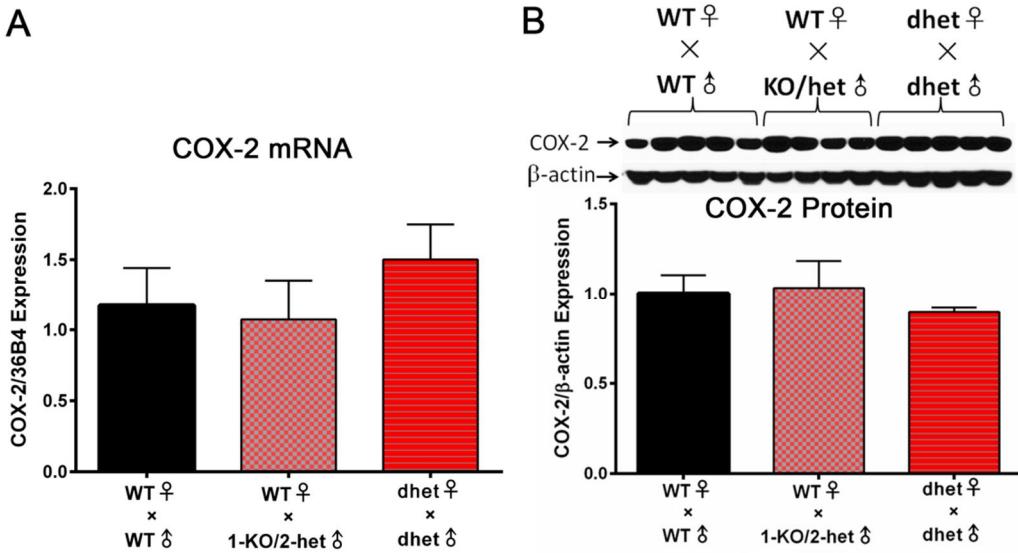
SUPPLEMENTAL FIGURES



Supplemental Figure 1. Expression of SP-B, SP-D, the lipid transporter ABCA3, as well as SRC-1 and SRC-2, in *SRC-1/-2* deficient fetal lungs. Related to Figure 1.

- (A) SP-D mRNA levels in lung tissues of WT (n = 3), *SRC-1/-2 dhet* (*dhet*; n = 14), *SRC-1/-2 dKO* (*dKO*; n = 4), *SRC-1 KO/SRC-2 het* (*KO/het*; n = 6), *SRC-1 het/SRC-2 KO* (*het/KO*; n = 3), *SRC-1 KO/SRC-2 WT* (*KO/WT*; n = 3), *SRC-1 het/SRC-2 WT* (*het/WT*; n = 13), *SRC-1 WT/SRC-2 het* (*WT/het*; n = 4) fetuses at 18.5 dpc from matings of *SRC-1/-2 dhet* ♀ bred to *SRC-1/-2 dhet* ♂.
- (B) SP-B mRNA levels in lung tissues of WT (n = 6), *dhet* (n = 13), *dKO* (n = 3), *KO/het* (n = 5), *het/KO* (n = 3), *KO/WT* (n = 3), *het/WT* (n = 13), *WT/het* (n = 4) fetuses at 18.5 dpc.
- (C) ABCA3 mRNA levels in lung tissues of WT (n = 4), *dhet* (n = 15), *dKO* (n = 3), *KO/het* (n = 6), *het/KO* (n = 3), *KO/WT* (n = 3), *het/WT* (n = 13), *WT/het* (n = 4) fetuses at 18.5 dpc.
- (D) SRC-1 mRNA levels in lung tissues of WT (n = 7), *KO/WT* (n = 6), *WT/KO* (n = 4), *dKO* (n = 6) fetuses at 18.5 dpc.
- (E) SRC-2 mRNA levels in lung tissues of WT (n = 7), *KO/WT* (n = 6), *WT/KO* (n = 4), *dKO* (n = 6) fetuses at 18.5 dpc.

Data shown are mean ± SEM. **P<0.01, ****P<0.0001 compared to WT (ANOVA).

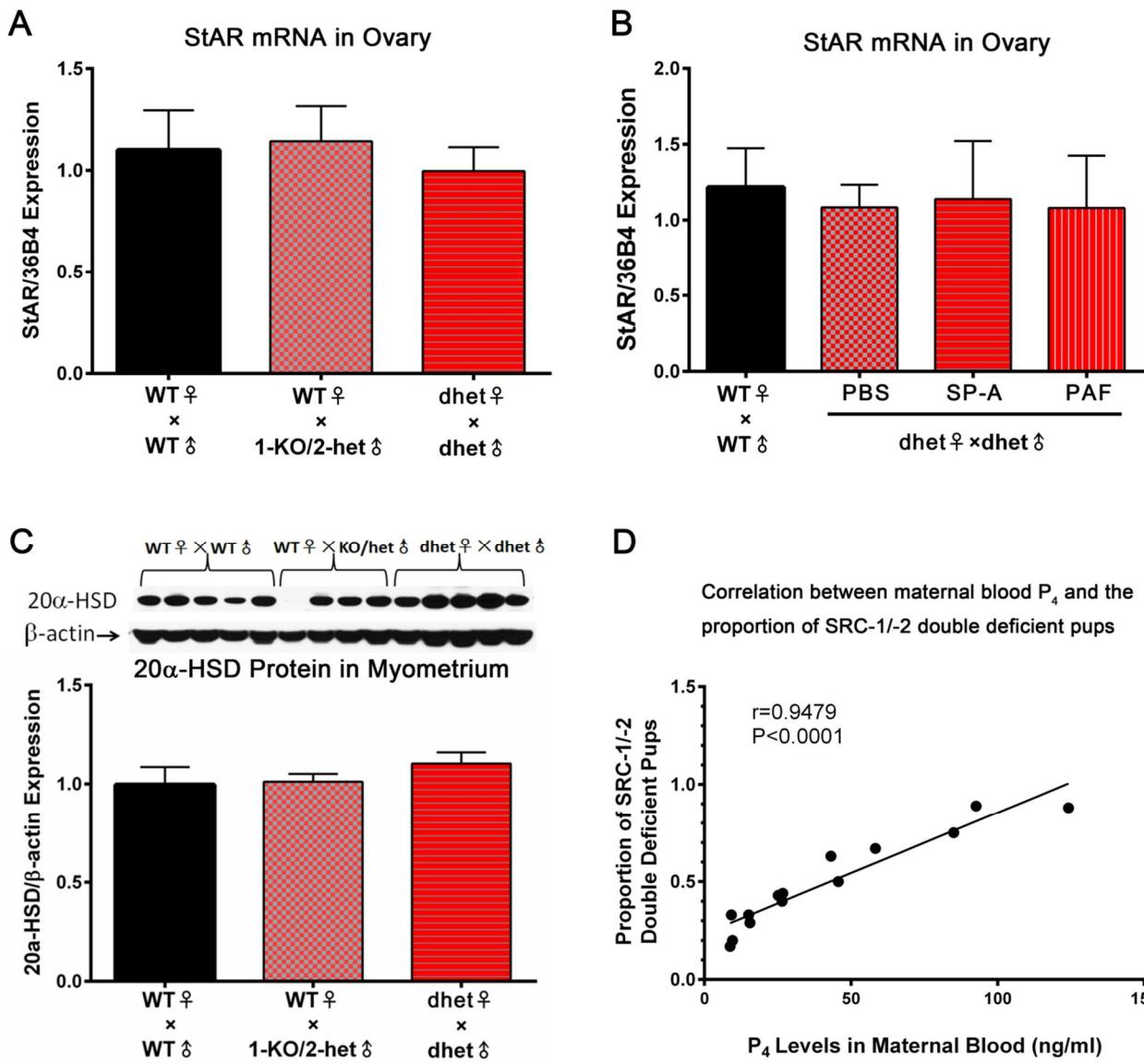


Supplemental Figure 2. COX-2 expression is not altered in myometrium of *SRC-1/-2* deficient pregnant mice. Related to Figure 2.

(A) COX-2 mRNA levels in the myometrium of 18.5 dpc female mice from indicated crosses. WT ♀ × WT ♂ (n=12), WT ♀ × *SRC-1* *KO/SRC-2* *het* (1-KO/2-het) ♂ (n = 8), and *SRC-1/-2* *dhet* (dhet) ♀ × *dhet* ♂ (n = 12).

(B) Upper panel is a representative immunoblot of COX-2 protein in the myometrium of 18.5 dpc female mice from indicated crosses. In the lower panel are combined data from densitometric scans of immunoblots for COX-2 plotted for WT ♀ × WT ♂ (n=5), WT ♀ × 1-KO/2-het ♂ (n=4) and dhet ♀ × dhet ♂ (n=5). The same representative immunoblot of β-actin was also used for the normalization of CX43 (Fig. 2D), AKR1B3 (Fig. 3B), PDGH (Fig. 3D) and 20α-HSD (Supplemental Figure 3C), since these were probed using the same blot.

Data shown in bar graphs are mean ± SEM (ANOVA).



Supplemental Figure 3. Expression of genes involved in progesterone synthesis and metabolism in ovary and myometrium, effects of SP-A or PAF rescue on StAR expression in ovary, as well as circulating P₄ levels in *SRC-1/-2 dhet* ♀ bred to *SRC-1/-2 dhet* ♂, plotted according the proportion of *SRC-1/SRC-2 double-deficient fetuses*. Related to Figure 3 and Figure 7.

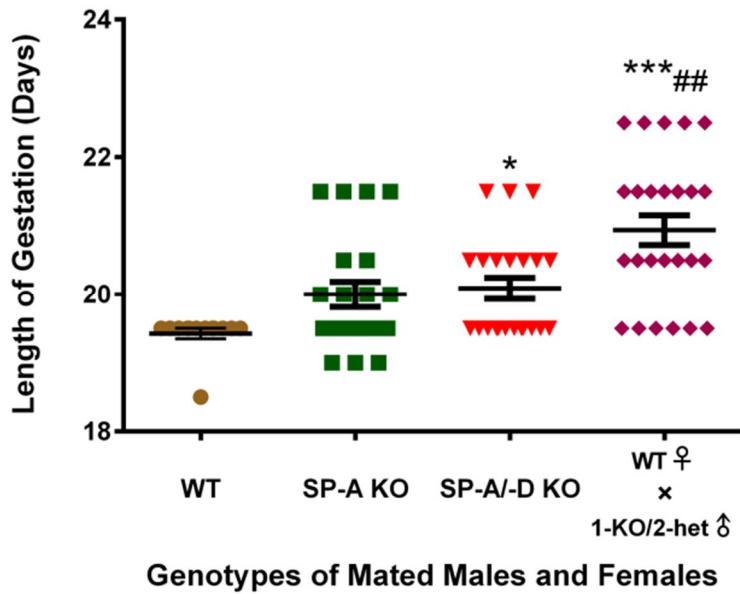
(A) StAR mRNA in 18.5 dpc ovaries from indicated crosses. WT ♀ × WT ♂ (n=6), WT ♀ × 1-KO/2-het ♂ (n=6), and dhet ♀ × dhet ♂ (n=16).

(B) StAR mRNA in 18.5 dpc ovaries from WT or *SRC-1/-2 dhet* ♀ bred to *SRC-1/-2 dhet* ♂ intra-amniotically injected with PBS (50 µl/sac), SP-A (3.0 µg/sac) or PAF (0.25 ng/sac) at 17.5 dpc, with tissues harvested one day later (18.5 dpc). n=6 for each group.

(C) 20 α -HSD protein expression in myometrium of indicated crosses at 18.5 dpc. WT ♀ × WT ♂ (n=5), WT ♀ × *I-KO/2-het* ♂ (n=4) and *dhet* ♀ × *dhet* ♂ (n=5).

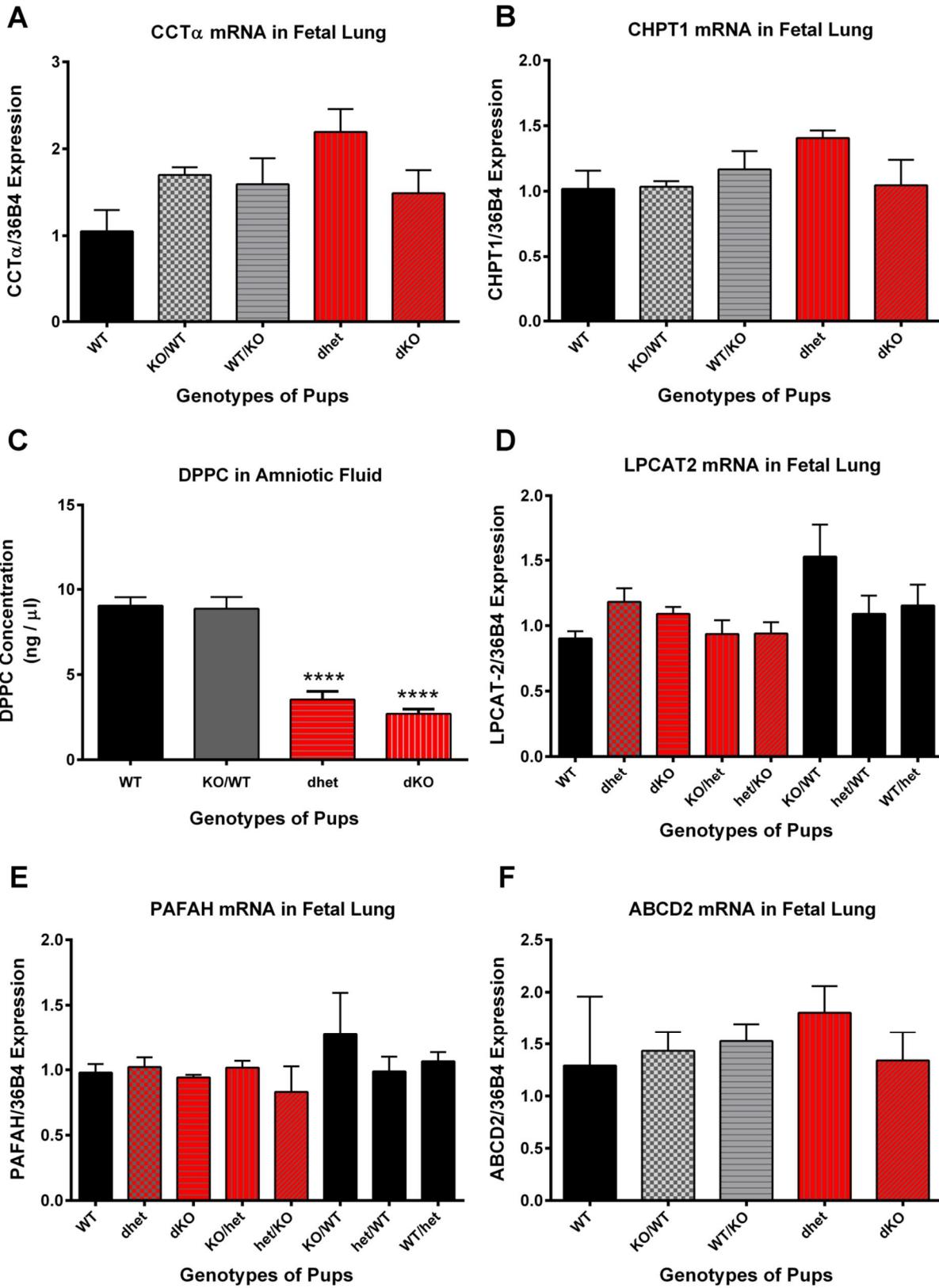
(D) Correlation between maternal P₄ levels and the proportion of *SRC-1/SRC-2* double-deficient fetuses (n=14).

Data shown are mean ± SEM (ANOVA).



Supplemental Figure 4. Delay in labor of WT ♀ bred to SRC-1 KO/SRC-2 het ♂ is more pronounced than that of SP-A KO ♀ crossed with SP-A KO ♂ and SP-A/D dKO ♀ crossed with SP-A/D dKO ♂ (gestation timing in SP-A and SP-A/D dKO mice is from Reference #15 in manuscript). Related to Figure 1.

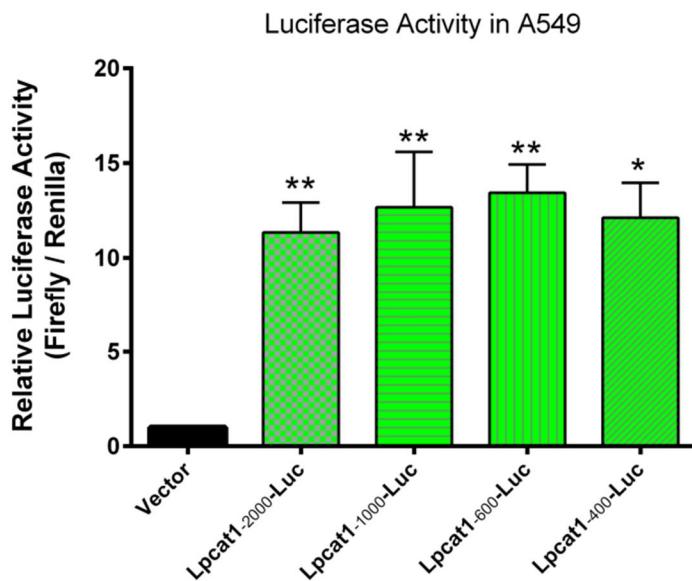
*P<0.05, ***P<0.001, compared to WT crosses; ## P<0.01 compared to SP-A/-D KO crosses (ANOVA).



Supplemental Figure 5. Expression of genes encoding enzymes involved in surfactant glycerophospholipid synthesis and secretion in fetal lungs, and amniotic fluid DPPC levels of 18.5 dpc SRC-1/-2 deficient mice. Related to Figure 4.

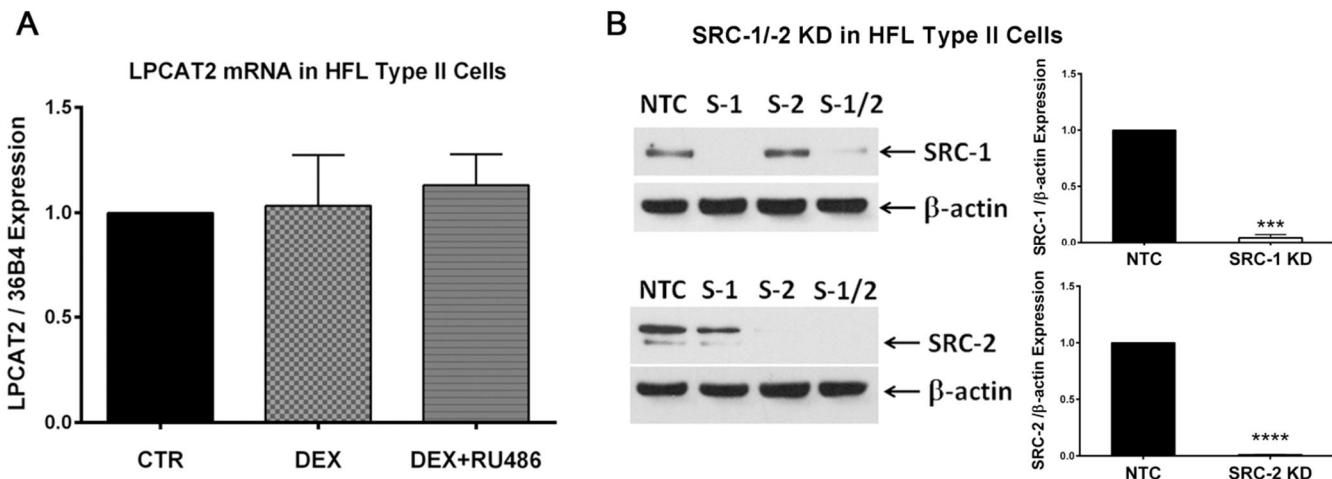
- (A) Choline phosphate cytidylyltransferase (CCT α) mRNA expression in fetal lungs according to genotype. WT (n=3), *KO*/WT (n=5), WT/*KO* (n=4), *dhet* (n=3) and *dKO* (n=8).
- (B) Choline phosphotransferase 1 (CHPT1) mRNA expression in lungs of 18.5 dpc WT and SRC-1/-2 deficient fetal mice. WT (n=3), *KO*/WT (n=5), WT/*KO* (n=4), *dhet* (n=3) and *dKO* (n=8).
- (C) DPPC levels in amniotic fluid of 18.5 dpc WT (n=13), *KO*/WT (n=7), *dhet* (n=12) and *dKO* (n=6) fetuses.
- (D) Lysophosphatidylcholine acytransferase 2 (LPCAT2) mRNA expression in lungs of 18.5 dpc WT (n=5), *dhet* (n=14), *dKO* (n=3), *KO/het* (n=5), *het/KO* (n=3), *KO*/WT (n=3), *het*/WT (n=13), WT/*het* (n=4) fetal mice.
- (E) mRNA expression of the PAF degrading enzyme, platelet-activating factor acetylhydrolase (PAFAH), in fetal lungs of 18.5 dpc WT (n=5), *dhet* (n=14), *dKO* (n=3), *KO/het* (n=5), *het/KO* (n=3), *KO*/WT (n=3), *het*/WT (n=13), WT/*het* (n=4) fetal mice.
- (F) mRNA expression of ABCD2, a transporter of very long chain acyl-CoAs in 18.5 dpc fetal lungs, according to genotype. WT (n=3), *KO*/WT (n=5), WT/*KO* (n=4), *dhet* (n=3) and *dKO* (n=8).

Data represent mean \pm SEM. ***P<0.0001 (ANOVA).



Supplemental Figure 6. Luciferase activities of all *Lpcat1* promoter constructs were comparably increased above that of empty vector control. Related to Figure 5.

Luciferase assays of different reporter constructs without treatment. (n=4 for each genotype). Mean ± SEM. *P<0.05, **P<0.01 (ANOVA).



Supplemental Figure 7. LPCAT2 in human fetal lung (HFL) type II cells is unaffected by glucocorticoids. Recombinant lentiviruses containing shRNA targeting SRC-1 and SRC-2 significantly decrease expression of SRC-1 and SRC-2 in HFL type II cells. Related to Figure 5.

(A) LPCAT2 expression in HFL type II cells treated with Dex \pm RU486. (n=4).

(B) Representative immunoblot of SRC-1 and SRC-2 proteins in HFL type II cells infected with lentiviruses expressing SRC-1 and SRC-2 shRNAs. The efficacies of knockdown for SRC-1 and SRC-2 are $95.8 \pm 3.0\%$ and $98.1 \pm 0.6\%$, respectively. NTC, non-targeting control; S-1, cells infected with lentivirus expressing SRC-1 shRNA; S-2, cells infected with lentivirus expressing SRC-2 shRNA; S-1/2, cells infected with both lentiviruses. Quantifications of SRC-1 and SRC-2 protein levels from 3 independent experiments are shown in far right panel.

Data represent mean \pm SEM. ***P<0.001, ****P<0.0001 (ANOVA).

SUPPLEMENTAL TABLES

Supplemental Table 1. Gestation timing, litter size and proportion of deficient pups of females with indicated genotypes bred to males with indicated genotypes from first pregnancies. Related to Figure 1.

Crosses	Gestation length (dpc)	Litter Size/Viability (total / survival)	Proportion of Deficient Pups
WT♀×WT♂	19.42 ± 0.08 (n=13)	7.08 ± 0.42/ 96.53 ± 2.40% (n=12)	
1	19.5	8/8	-
2	19.5	8/6	-
3	19.5	7/7	-
4	19.5	6/6	-
5	19.5	8/8	-
6	19.5	5/5	-
7	19.5	7/7	-
8	19.5	8/8	-
9	19.5	5/5	-
10	19.5	6/5	-
11	19.5	7/7	-
12	19.5	10/10	-
13	18.5		-
<i>SRC-1^{+/+}/SRC-2^{+/+} ♀</i> × <i>SRC-1^{+/+}/SRC-2^{+/+} ♂</i>	20.89 ± 0.13 (n=49)****	6.83 ± 0.73 / 90.68 ± 2.71% (n=39)	-
1	19.5	10/9	0.429
2	20.5	7/7	0.429
3	19.5	7/7	0.200
4	19.5	9/9	0.333
5	19.5	7/7	0.143
6	20.5	7/6	0.800
7	20.5	7/7	0.571
8	20.5	2/2	0.500
9	20.5	8/8	0.625
10	20.5	7/6	0.429
11	20.5	5/5	0.710
12	20.5	6/6	0.833
13	20.5	7/7	0.571
14	21.5	5/2	1.000
15	22.5	all pups eaten	N/A
16	20.5	6/6	0.500
17	21.5	8/8	0.750
18	20.5	7/7	0.500
19	20.5	7/6	0.429
20	22.5	8/3	0.750
21	21.5	5/5	0.600
22	22.5	7/5	0.857
23	21.5	7/7	0.571
24	21.5	9/6	0.667

25	21.5	2/2	0.500
26	19.5	6/6	0.333
27	20.5	6/3	0.500
28	21.5	8/8	0.750
29	20.5	7/6	0.571
30	22.5	8/8	0.875
31	21.5	7/7	0.714
32	21.5	9/8	0.667
33	22.5	8/8	0.875
34	21.5	7/7	0.714
35	20.5	5/5	0.400
36	22.5	7/5	1.000
37	22.5	6/6	0.833
38	21.5	9/7	0.667
39	22.5	8/8	0.750
40	19.5	7/7	0.286
41	20.5	ND	ND
42	20.5	ND	ND
43	20.5	ND	ND
44	19.5	ND	ND
45	20.5	ND	ND
46	20.5	ND	ND
47	19.5	ND	ND
48	20.5	ND	ND
49	20.5	ND	ND

WT[♀]×SRC-I^{+/−}/SRC-2^{+/−}♂ **20.94 ± 0.22 (n=25)****** **6.41 ± 0.41 / 90.28 ± 8.32% (n=12)**

1	19.5	6/5	ND
2	20.5	5/5	ND
3	21.5	4/4	ND
4	20.5	7/7	ND
5	20.5	6/6	ND
6	21.5	4/0	ND
7	22.5	9/9	ND
8	20.5	5/5	ND
9	21.5	8/8	ND
10	21.5	6/6	ND
11	22.5	7/6	ND
12	20.5	7/7	ND
13	19.5	ND	ND
14	19.5	ND	ND
15	21.5	ND	ND
16	21.5	ND	ND
17	20.5	ND	ND
18	22.5	ND	ND
19	19.5	ND	ND
20	22.5	ND	ND
21	21.5	ND	ND
22	19.5	ND	ND
23	20.5	ND	ND
24	22.5	ND	ND
25	19.5	ND	ND

<i>SRC-1</i> ^{-/-} /WT	21.06 ± 0.18 (n=9)****	6.78 ± 0.40 / 93.14±3.50% (n=9)	
1	20.5	6/5	ND
2	19.5	6/6	ND
3	21.5	7/7	ND
4	20.5	8/6	ND
5	20.5	5/4	ND
6	20.5	6/6	ND
7	22.5	7/7	ND
8	21.5	7/7	ND
9	22.5	9/9	ND
<i>SRC-1</i> ^{-/-} × <i>SRC-1</i> ^{-/-}	19.44 ± 0.07 (n=16)	7.44 ± 0.44 / 95.64±1.85% (n=16)	
1	19.5	10/9	1.0
2	19.5	7/7	1.0
3	19.5	8/7	1.0
4	19.5	10/10	1.0
5	19.5	6/6	1.0
6	19.5	6/6	1.0
7	19.5	9/9	1.0
8	19.5	5/5	1.0
9	19.5	8/6	1.0
10	19.5	9/8	1.0
11	19.5	7/7	1.0
12	19.5	6/6	1.0
13	18.5	8/8	1.0
14	19.5	9/8	1.0
15	19.5	7/7	1.0
16	19.5	4/4	1.0
<i>SRC-2</i> ^{+/-} × <i>SRC-2</i> ^{+/-}	19.50 ± 0.0 (n=10)	7.3 ± 0.47 / 91.21±4.52% (n=10)	
1	19.5	7/6	ND
2	19.5	7/7	ND
3	19.5	8/7	ND
4	19.5	7/7	ND
5	19.5	6/6	ND
6	19.5	9/5	ND
7	19.5	10/10	ND
8	19.5	6/5	ND
9	19.5	8/8	ND
10	19.5	5/5	ND

**** $P<0.0001$

ND – Not determined

Supplemental Table 2. Gestation length in females of different genotypes bred to males of different genotypes from second and subsequent pregnancies.

Crosses	Gestation length (dpc)
WT♀ × WT♂	19.50 ± 0 (n=4)
1	19.5
2	19.5
3	19.5
4	19.5
<i>SRC-1^{+/+}/SRC-2^{+/+}♀</i> × <i>SRC-1^{+/+}/SRC-2^{+/+}♂</i>	20.5 ± 0.31 (n=5)
1	20.5
2	19.5
3	20.5
4	20.5
5	21.5
WT♀ × SRC-1^{-/-}/SRC-2^{+/+}♂	21.1± 0.50 (n=5)
1	21.5
2	19.5
3	21.5
4	20.5
5	22.5

Supplemental Table 3. Primer sequences used for quantitative PCR and ChIP-qPCR analysis.

Related to Figures 1-7.

Gene	Primer Sequence
mouse (m)36B4	Forward: CACTGGTCTAGGACCCGAGAAG Reverse: GGTGCCTCTGGAGATTTCG
mSP-A	Forward: TCCAGGGTTCCAGCTTACCT Reverse: GACAGCATGGATCCTTGCAAG
mSP-B	Forward: GTGCACAAGGCCCTCAATT Reverse: TGGCACAGGTCAATTAGCTCC
mSP-D	Forward: GGACTCAAGGGGGACAGAG Reverse: AGCTTCTGATAGTGGGAGAAGG
mABCA3	Forward: CCCTACCAGTGGACATGCTT Reverse: CAGGCTGAGGATGTGTAGCA
mCXN43 (GJA1)	Forward: TCCAAGGAGTTCCACCACTT Reverse: TGGAGTAGGCTTGGACCTTG
mOXTR	Forward: TTCTTCGTGCAGATGTGGAG Reverse: TGTAGATCCATGGGTTGCAG
mAKR1B3	Forward: GAACTCAACAACGGCACCAA Reverse: GAAGAGATCCTGCCGCTTCA
mPGDH	Forward: CAACAATGCAGGCGTGAACA Reverse: GCGTGTGAATCCGATGTGC
mCOX-2	Forward: CAGCCAGGCAGCAAATCC Reverse: ACATTCCCCACGGTTTGAC
mStAR	Forward: TCGCTACGTTCAAGCTGTGT Reverse: ACGTCGAACTTGACCCATCC
m20 α -HSD	Forward: GGAGGCCATGGAGAAGTGT Reverse: ATGGCATTCTACCTGGTTGC
mLPCAT1	Forward: GGTGCTACGCTACCCAAACA Reverse: TGGCATACAGGGCAGGATT
mLPCAT2	Forward: GCATGGCCATTGCTGTCAT Reverse: CACGCGCCAGAAATGTCAA
mPAFAH	Forward: AAGACCCTCCGTGTATGGGA Reverse: GTAACCACATAGGGTGCCGT
mCCT α	Forward: GAAGCAGGAGGCTACTGTGA Reverse: CCGTAAACCAACTGCGAAC
mCHPT1	Forward: TACTGCCCTACAGTCACCGA Reverse: AGCGCCGATGCCATAAATA

mABCD2	Forward: AAATTGGTTCGCTGGTGGC Reverse: CCACAGCTGCTCCACACATA
mSRC1	Forward: GCTCCCCAGTTGGCATTACT Reverse: CTGTGAGCTCATCTGCCGAA
mSRC2	Forward: AGCGAGCCCCAAGAAGAAAG Reverse: TGTCTGGGAAAAGCTGTGGT
human (h) 36B4	Forward: TGCATCAGTACCCCATTCTATCA Reverse: AAGGTGTAATCCGTCTCCACAGA
hSRC-1	Forward: TCTGAGGGGCTTAGAAATTAACA Reverse: TGATGTTTCCAGAGATGGCT
hSRC-2	Forward: CTGACGGCGTGACCGAC Reverse: TTTCTCCCATCCCACTCATC
hLPCAT1	Forward: CCATGAGGCTGCGGGGAT Reverse: GGGAAAGAGCGTCAGTGTCA
hLPCAT2	Forward: ACCAGGAGCCTTCATTCCAG Reverse: TCATCATTGGTACTTGAACGGC
LPCAT1-promoter	
GRE1/2	Forward: TGTGGACTTGGGTCAACACC Reverse: TGGGCCAGGATTCTGTAGC
GRE3	Forward: CAGTGGAATCTAGGTACAGGAC Reverse: GAGTGCTGGGATTAAAGGCG
GRE4	Forward: TTTCTGAGTTCGAGGCCAGC Reverse: ACAGGGTTCTCTGTTAGCCC
GRE5	Forward: CAGAGAAACCTGTCTGAAAAA Reverse: TCCTCTGGCTAAGAAGGCT
