Supplemental Materials

Molecular Biology of the Cell

Gijs et al.

Figure S1

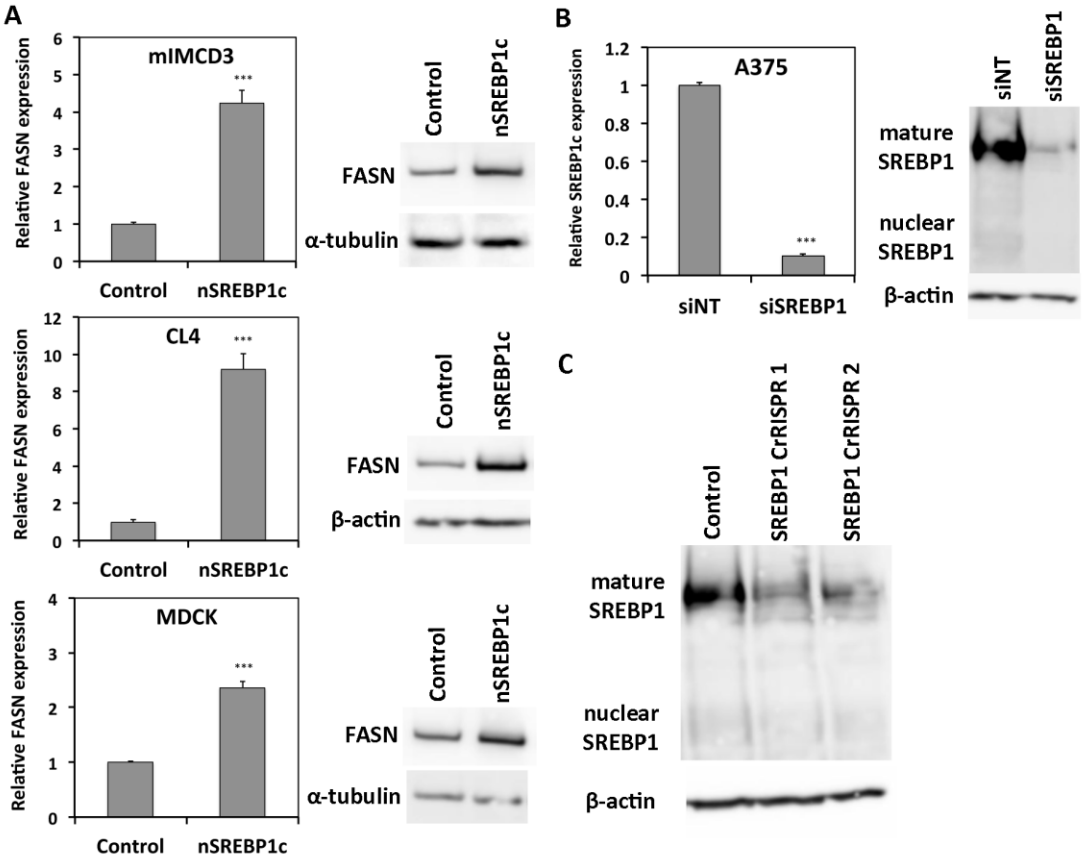

Figure S1: Modulation of SREBP1c expression.
A. Efficient upregulation of FASN upon SREBP1c transduction. QRT-PCR (left panel) and western blot (right panel) analysis of FASN in mIMCD3, CL4 and MDCK cells after infection with adenovirus encoding nSREBP1c versus control vector. **B.** Efficient siRNA-mediated knockdown of SREBP1. QRT-PCR (left panel) and western blot (right panel) analysis of SREBP1 in A375 cells after transfection with SREBP1-targeting siRNA (siSREBP1) or non-targeting siRNA (siNT). **C.** Efficient CRISPR/Cas9-mediated knockdown of SREBP1. Western blot analysis of SREBP1 in A375 cells transfected with SREBP1-targeting CRISPR/Cas9 plasmids or in control cells.
Error bars, SEM; *** is P-value < 0.0005 using student's t-test. α-tubulin or β-actin is used as loading control for western blotting.

Figure S2

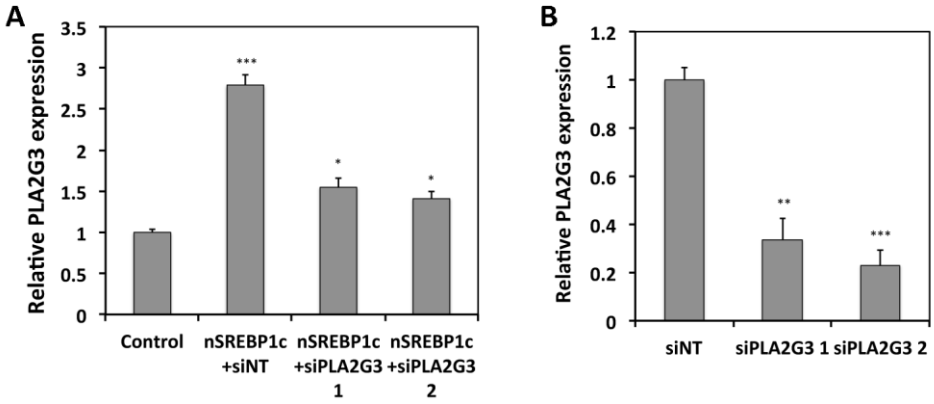

Figure S2: Modulation of PLA2G3 expression.
A. Efficient attenuation of PLA2G3 in nSREBP1c-transduced cells. QRT-PCR analysis of mIMCD3 cells 48h after transfection with nSREBP1c or control adenovirus in combination with siRNAs targeting PLA2G3 (siPLA2G3) or non-targeting siRNA (siNT).**B.** Efficient knockdown of PLA2G3. QRT-PCR analysis of A375 cells 48h after transfection with siPLA2G3s or siNT.
N = 3; Error bars, SEM; * is P-value < 0.05, ** is P-value< 0.005 and *** is P-value < 0.0005 using student's t-test.

Figure S3

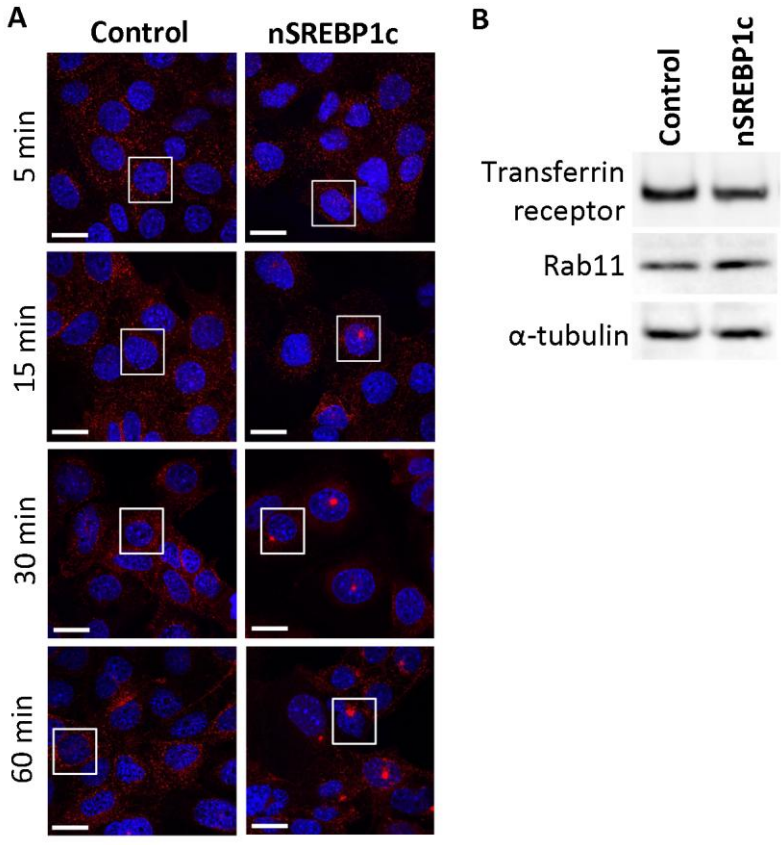

Figure S3: SREBP1c impairs vesicle recycling, but not transferrin receptor or Rab11 expression.
A. SREBP1c-overexpressing cells accumulate transferrin in a perinuclear region. mIMCD3 cells were infected with nSREBP1c or control adenovirus. Cells were fixed after 5, 15, 30 and 60 min of Alexa-Fluor 564 conjugated transferrin uptake (red) and stained with DAPI (blue) (N = 3; Bar, 20 μM). White square: representative regions shown in Figure 5A. **B.**nSREBP1c has no effect on transferrin receptor and rab11 expression. Western blot analysis of transferrin receptor, rab11 and α-tubulin in mIMCD3 cells 72h after infection with nSREBP1c or control adenovirus.

Figure S4

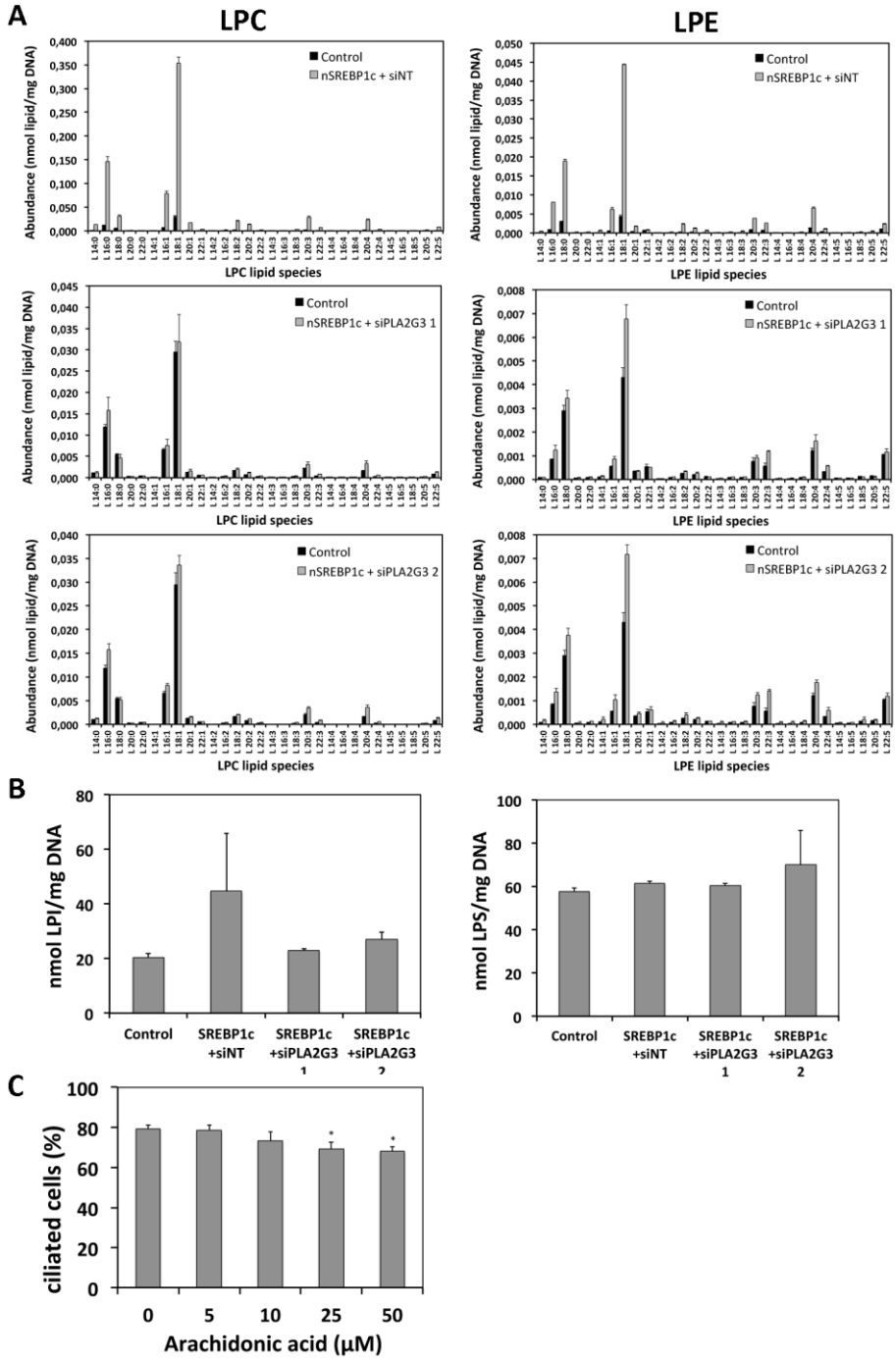

Fig. S4: Lysophospholipid analysis and effect of arachidonic acid treatment on ciliogenesis.

A, B. Effect of nSREBP1c-induced PLA2G3 expression on lysophospholipid levels.mIMCD3 cells were infected with control or nSREBP1c adenovirus in combination with PLA2G3 targeting siRNA (siPLA2G3) or non-targeting siRNA (siNT). Lysophospholipids (**A:** LPC, LPE and **B:** LPI, LPS) were measured by ESI-MS/MS after 72h.**C.** Minor effects of arachidonic acid treatment on ciliogenesis. mIMCD3 cells were treated with different concentrations of arachidonic acid during 72h and were stained for acetylated α-tubulin and DAPI. For

quantification, 650 – 750 cells were scored (Error bars, SEM; * is P-value < 0.05 and ** is P-value < 0.005 using student's t-test).

Table S1

siRNA	sequence
mouse PLA2G3 siRNA 1	CGAAACUACCGAUUCCACA
mouse PLA2G3 siRNA 2	GCACCAGCGAGAGAAGAGA
human PLA2G3 siRNA 1	GACACCAGCUCCAGGAUAA
human PLA2G3 siRNA 2	GGACUGGCCCUGAUCCAUGA
human SREBP1 siRNA	CGGAGAAGCUGCCUAUCAAUU
primer	**sequence**
mouse FASN Fw	AGAGATCCCGAGACGCTTCT
mouse FASN Rev	AGCCGGTTGGCCATCATT
mouse CDKN2B Fw	CGCCCAATCCAGGTCATGAT
mouse CDKN2B Rev	AGTTGGGTTCTGCTCCGTG
mouse MX2 Fw	CGAGAATTGCCAGGGTTTGT
mouse MX2 Rev	GGCAGCTCGTACAATTTCAGT
mouse PLA2G3 Fw	GGCTGAGGCCACCTCATATACTTC
mouse PLA2G3 Rev	TCCTTTGCCCTCAGCACAGTCAAG
Pig FASN Fw	TTGTGGACGGAGGCATCAAC
Pig FASN Rev	GCATCAGAACTGCTCACACC
pig CDKN2B Fw	GGAGACCGTGCGTCAACTTC
pig CDKN2B Rev	CTGCCCATCATCATGACCTGGAT
pig MX2 Fw	GGAAATACGCAAAGCCCAGG
pig MX2 Rev	TGATGCCGGGAAGATCGATG
Pig PLA2G3 Fw	AGCCTTCTTCAACGTGCTGG
Pig PLA2G3 Rev	CATACCTTCTACACCCGCCC
dog FASN Fw	CACGATGATCCCCTGCTC
dog FASN Rev	GTGCATCCTGAGACTGGTCA
dog CDKN2B Fw	CTTCCTGGACACGCTGGT
dog CDKN2B Rev	CTCCTCAGCCAGGTCCAC
dog MX2 Fw	ATCGGAGTGCAGATCAAGGC
dog MX2 Rev	AATGTCCACGTTGCAGGG
dog PLA2G3 Fw	GGCCTCCATCAAACCTGATA
dog PLA2G3 Rev	CCGATCTGCTGTTCACACTG
human SREBP1c Fw	GCCATGGATTGCACTTT
human SREBP1c Rev	CAAGAGAGGAGCTCAATG
human PLA2G3 Fw	TCCACTGGACTGTGTGGAAG
human PLA2G3 Rev	GTGCCTTTATCCTGGAGCTG
18S Fw	CGCCGCTAGAGGTGAAATTC
18S Rev	TTGGCAAATGCTTTCGCTC
PLA2G3 deletion SRE1 Fw	TACAAGCCCAAGATACTCCAGCCTCTTTCCTCCTCCTCCCGCAGC
PLA2G3 deletion SRE1 Rev	GCTGCGGGAGGAGGAGGAAAGAGGCTGGAGTATCTTGGGCTTGTA
PLA2G3 mutation SRE1 Fw	CGGTTACAAGCCCAAGATCACCGATTACTCCAGCCTCTTTCC
PLA2G3 mutation SRE1 Rev	GGAAAGAGGCTGGAGTAATCGGTGATCTTGGGCTTGTAACCG
dog PLA2G3 ChIP Fw	TGCCTCATCATGCCAGCGA
dog PLA2G3 ChIP Rev	GCTCATGCCCCAAATAAAACTCCAA
dog FASN ChIP Fw	ACCCTGACGCGACTGAACTC
dog FASN ChIP Rev	CATAGGTGGGGATGCTGAGC
mouse PLA2G3 ChIP Fw	TGCATCCCTCAGCCAATCAG
mouse PLA2G3 ChIP Rev	GGGTGTCAGGGCCAATGAAT
mouse FASN ChIP Fw	GTGGCAAAGGTACGTACGTGT
mouse FASN ChIP Rev	CCCTATAGTGGCAAACAGGTCA

Table S1:Nucleotide sequences of oligonucleotides and siRNAs used in this study.