Supporting Information

Kamphorst et al. 10.1073/pnas.1307237110

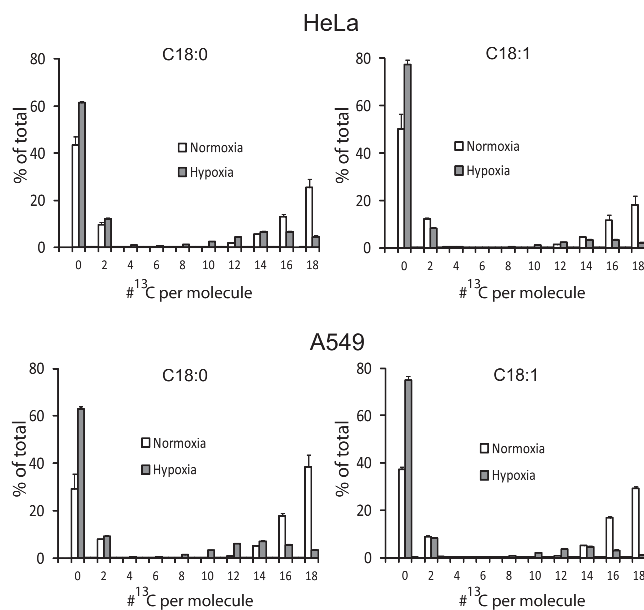

Fig. S1. Fatty acid labeling in normoxia and hypoxia. Labeling patterns of stearate (C18:0) and oleate (C18:1) from saponified cellular lipid extracts from cells grown in [U-^{13}C]glucose and [U-^{13}C]glutamine, in normoxia and hypoxia (1% O_2 for HeLa, and 0.5% for A549) for 72 h. All data are means ± SD of n = 3.

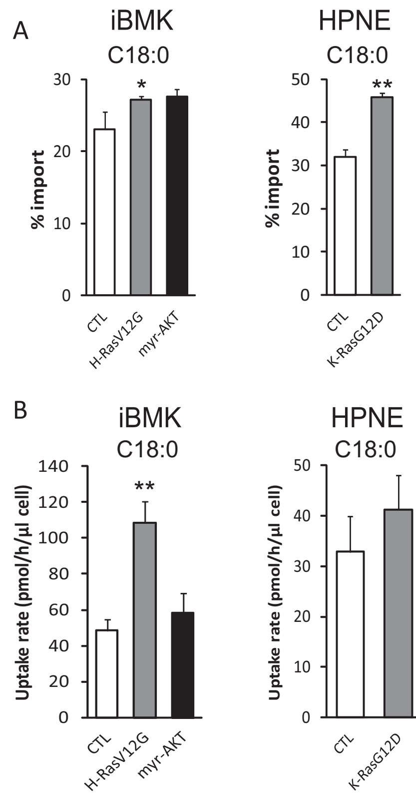

Fig. S2. Effect of oncogenic Ras on de novo synthesis versus import of stearate (C18:0). (*A*) Percentage import of C18:0, as measured by fatty acid labeling (72 h) from [U-^{13}C]glucose and [U-^{13}C]glutamine, in immortalized baby mouse kidney (iBMK) isogenic cell lines engineered to express myrAkt or H-Ras^{V12G} versus vector control (CTL), and human pancreatic nestin-expressing (HPNE) cells with oncogenic K-Ras^{G12D} versus vector control (CTL). (*B*) Uptake rates of C18:0, based on measurements of saponified lipids from fresh and spent medium (10% serum; 72 h of incubation). All data are means ± SD of $n \geq 3$. *$P < 0.05$; **$P < 0.01$ (two-tailed *t* test).

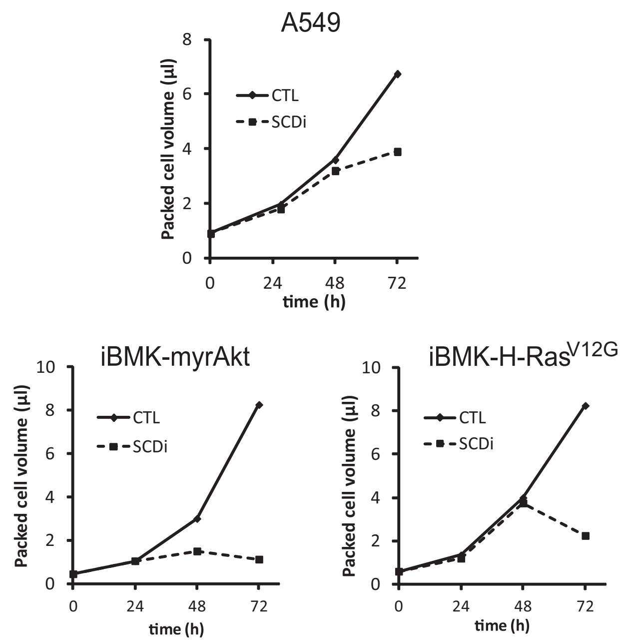

Fig. S3. Effect of stearoyl-CoA desaturase (SCD)1 inhibition on cell growth in 6-cm tissue culture dishes. Onset of growth inhibition, upon treatment with 200 nM CAY10566, occurs earlier in the tissue culture dishes than when using the xCELLigence system where growth is on 96-well culture plates, because the cells grow faster and therefore more rapidly consume medium lipids on the 6-cm dishes. (Mean, $n = 2$.)

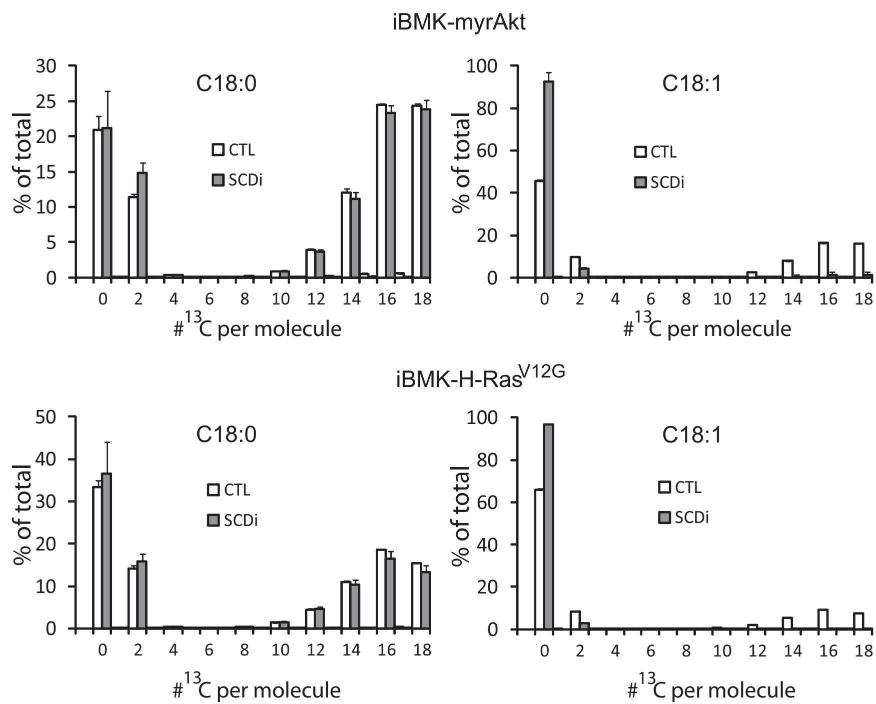

Fig. S4. CAY10566 at a concentration of 200 nM is sufficient to fully block C18:1 labeling. Cells were treated with 200 nM CAY10566 [SCD1 inhibitor, SCDi] or vehicle control (CTL) while cultured in medium with [U-^{13}C]glucose and [U-^{13}C]glutamine for 48 h. All data are means ± SD of $n \geq 3$.

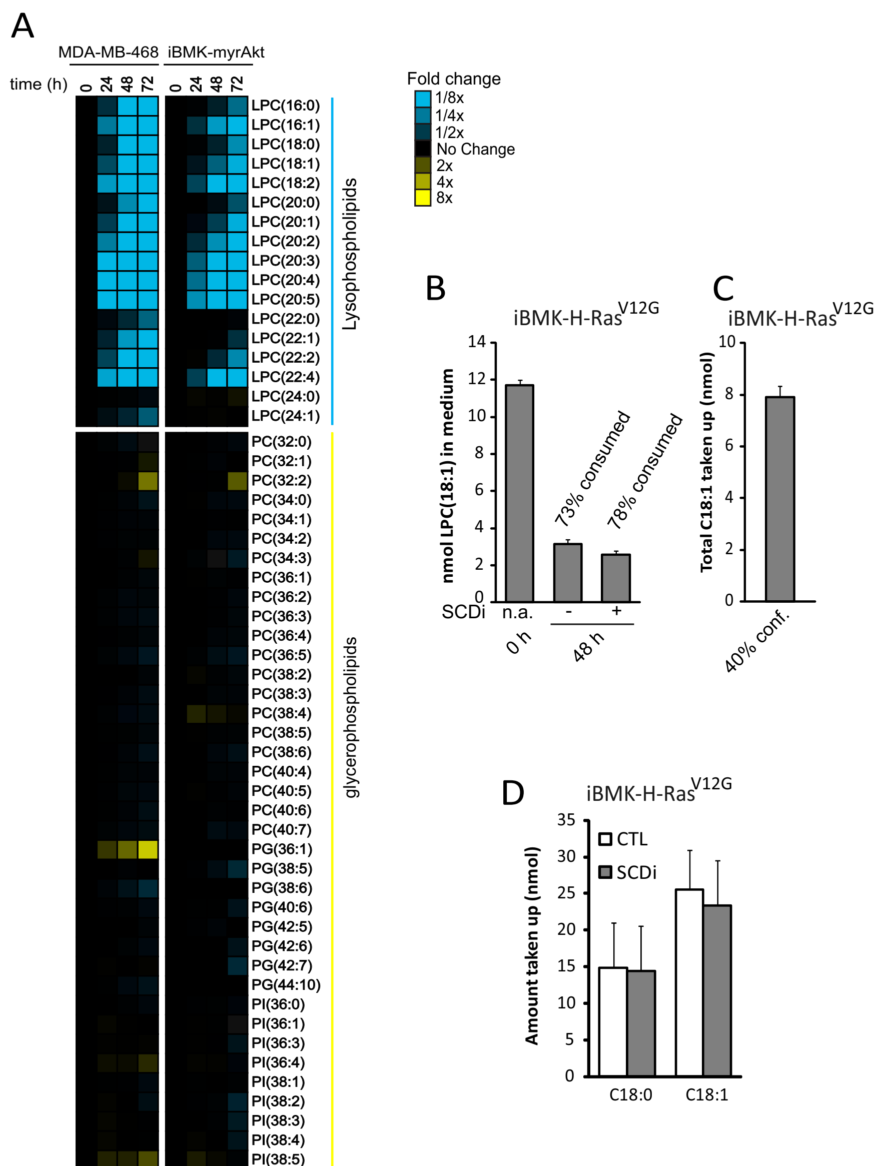

Fig. S5. Lysophospholipid consumption by cancer cells. (*A*) Fold changes in medium phospholipids during growth of MDA-MB-468 and iBMK-myrAkt cells (relative to fresh medium with 10% serum). (*B*) Consumption (in nanomoles) of lysophosphatidylcholine (LPC)(18:1) from medium by iBMK–H-Ras^{V12G} cells treated with 200 nM CAY10566 (SCD1 inhibitor, SCDi) or vehicle control (CTL), for 48 h. (*C*) Total C18:1 import (based on uptake rates from Fig. 3*C*) for cells cultured to ~40% confluence. (*D*) Effect of SCDi inhibition on total C18:1 uptake, based on measurements of saponified lipids from fresh and spent medium (10% serum; 48 h of incubation). Cells were seeded at higher density than usual to facilitate optimal determination of fatty acid consumption. All data are means ± SD of $n \geq 3$.

Table S1. Total and free (nonesterified) fatty acid concentrations in medium (10% serum) and total fatty acid concentrations in A549 and iBMK–H-RAS^{V12G} cells

Fatty acid	Medium total fatty acids, μM	Medium free fatty acids, μM	A549 total fatty acids, nmol/μL cells	iBMK–H-Ras^{V12G} total fatty acids, nmol/μL cells
C16:0	22.3 ± 1.5	0.85 ± 0.08	8.7 ± 0.5	8.1 ± 0.6
C16:1	5.1 ± 0.5	NA	3.3 ± 0.2	2.2 ± 0.6
C18:0	12.2 ± 0.3	0.25 ± 0.08	2.8 ± 0.3	4.4 ± 0.08
C18:1	20.9 ± 0.9	1.1 ± 0.04	10.5 ± 0.3	8.1 ± 0.2

Medium free fatty acids constitute less than 5% of total medium fatty acids.