Supplemental Figure Legends

Supplemental Figure 1. Inhibition of lipogenic transcriptional program in various cancer cells. (A) Lipogenic gene expression determined by q-PCR in T98G (GBM), SUM159 (breast cancer) and CWR (prostate cancer) cells treated with 25μM 25-hydroxycholesterol for 24 h cultured in media and 1% serum. (B,C) Lipogenic gene expression determined by q-PCR in T98G (GBM), SUM159 (breast cancer) and CWR (prostate cancer) cells treated with 10μM fatostatin (10μM) or compound 24 (10μM or 25μM) for 24 h cultured in media and 1% serum. (D) Growth curve for wild-type U87 cells cultured in media and 1% serum. In addition indicated cultures were treated with vehicle or 10μM fatostatin. (E) Growth curve performed as described above with the exception that wild-type U251 cells were treated with vehicle or 10μM Compound 24. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Supplemental Figure 2. The lipogenic program and growth of glioma cells is SCAP sensitive. (A) Western blots of U87 or U251 lysates collected after treatment with indicated dose of Compound 24 for 24 h. Cells were cultured in complete media and 1% serum for 24 h. (B) Western blots of lysates from U87 shControl, shSREBP1 or shSCAP cells cultured in complete media and 1% serum for 24 h. (C) Lipogenic gene expression determined by q-PCR in stable U87 control and SREBP2 knockdown lines cultured in 1% serum for 24 h. (D) Lipogenic gene expression determined by q-PCR in stable U87 control and SCAP knockdown cells lines cultured in complete media and 1% serum for 24 h. (E) Gene expression of glycolysis/hypoxia metabolism genes determined by q-PCR U87 in shControl, shSREBP1, shSREBP2 or shSCAP cells indicating no difference. (F) Gene expression of lipogenic genes determined by q-PCR in U87 shControl, or shSCAP cells treated with 25μM compound 24 for 24 h.

Supplemental Figure 3. Cholesterol content is unchanged in SREBP loss-of-function cells. (A) Growth assay of U251 glioma cells stably expressing shSREBP1, shSREBP2, or shSCAP cultured in compete media and 1% serum. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 4. Cholesterol content is unchanged in SREBP loss-of-function cells. (A) SREBP and lipogenic gene expression determined by q-PCR in wild-type U87 cultured in complete media and 10% or 1% serum for 24 h. In addition, some cultures were treated with 10μM fatostatin. (B) Total cellular cholesterol from control and SREBP knockdown cells. Lipid fraction was extracted and cholesterol quantitatively measured by colorimetric assay. Cholesterol content is expressed normalized to cell number. (C) Percentage of *de novo* synthesized cholesterol in the total pool of cellular cholesterol from WT U87 cells cultured in 50% mixture of U-^{13}C-glucose and 10% or 1% serum as indicated over a 48 h labeling period. (D) Schematic for determination of cellular cholesterol/fatty acid from *de novo* biosynthesis using isotopic enrichment analysis in cultures treated with SCAP antagonist compound 24 for 48 h. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 5. Loss of SREBP signaling results in the uncoupling of saturated fatty acid biosynthesis from desaturase activity. (A) The total amount of indicated long chain fatty acids/cell from control and SCAP knockdown U251 cells cultured in complete media and 1% serum for 48 h. (B) The normalized ratios of the indicated saturated to monounsaturated fatty acids from U251 cells. (C) Percentage of indicated fatty acid synthesized by U251control or SCAP knockdown cells cultured in complete media with a 50% mixture of U-^{13}C-glucose and 1% serum over a 48 h labeling period. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 6. Expression of mature SREBP1 alters the ratio of saturated to monounsaturated fatty acids. (A) Lipogenic gene expression determined by q-PCR in U87 stably expressing mature SREBP1 (mSREBP1a) and SREBP2 (mSREBP2). (B) Relative amount of indicated fatty acid from U87 cells stably expressing mSREBP1, mSREBP2 or vector control. Each analyte is normalized to control cells. (C) Lipogenic gene expression determined by qPCR from control, SREBP1 or SCAP knockdown U87 xenografts harvested on day 19 post-implantation. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 7. Knockdown of SCD1 does not alter cholesterol homeostasis. (A) ROS levels from shSREBP1, shSCAP or shControl U87 cells cultured in complete media and 1% serum treated with 5mM N-acteylcysteine for 48 h. (B) Growth curve of shSREBP1, shSCAP or shControl U87 cells cultured in complete media and 1% serum treated with 5mM N-acteylcysteine for 48 h. (C) Lipogenic gene expression determined by qPCR in stable U87 control and SCD1 knockdown lines. (D) The total amount of cholesterol from shControl and SCD1 knockdown U87 cells cultured in complete media and 1% serum for 48 h. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 8. Graphical representation of method used to determine the relative contribution of *de novo* cholesterol and long chain fatty acids synthesis to the total cellular pool. The "Cellular Lipid Pool" distribution is modeled as a weighted mixture of two independent distributions: the "*De Novo* Synthesized Lipid" distribution and the "Non-Synthesized Lipid" distribution. The weighting factor, s, represents the percent of the total

cellular lipid pool that has been synthesized since addition of label glucose. Lipids are synthesized using random draws from the cytoplasmic AcCoA pool. The cytoplasmic AcCoA pool distribution is also modeled as a weighted mixture of two independent distributions: the "Label Glucose" distribution and the "Natural Glucose and Other Carbon Sources" distribution. The weighting factor, p, represents the percent of the total cytoplasmic AcCoA pool that is derived from label glucose. "e" and "q" represent the enrichments of 13C in the label glucose and natural glucose and other carbon sources, respectively. See method for additional detail and (21, 22).

Supplemental Table 1. Gene set enrichment analysis on the ten most statistically significant down-regulated metabolic pathways in SCAP loss-of-function cells.