**Ricoult, *et al.***

## Supplemental Figures

**Figure S1.** Effects of mTOR inhibitors and SREBP1/2 knockdowns on the induction of *SREBF1, SREBF2*, and *ACACA* transcription by oncogenic PI3K and K-Ras. (**a**) Oncogene and mTORC1-dependent regulation of *SREBF1, SREBF2*, and *ACACA* gene expression. RNA was isolated from MCF10a cells (from **2b**) stably expressing empty vector, PIK3CA$^{H1047R}$,or K-Ras$^{G12V}$ serum starved for 16 h in the presence of vehicle, rapamycin (20 nM), or Torin1 (250 nM) for analysis by qRT-PCR, n=2. (**b**) Effect of SREBP knockdown on *SREBF1, SREBF2*, and *ACACA* gene expression. MCF10a cells from **2c** were transfected with siRNAs targeting SREBP1, SREBP2, or both. RNA was isolated from cells 72 h post-transfection following 20 h starvation, n=3. Representative data are shown as mean ± s.e.m. relative to vector-expressing cells. # P-value < 0.05 compared to vector-expressing cells; * P-value < 0.05 compared to vehicle-treated cells expressing the same oncogene.

**Figure S2.** mTOR inhibitors decreases the expression of SREBP and its target genes in breast cancer cells. (**a**) mTORC1-dependent *SREBF1* and *SREBF2* expression in breast cancer cells. qRT-PCR analysis of RNA isolated from cells (from **3b**) serum starved for 18 h in the presence of vehicle, rapamycin (20 nM), or Torin1 (250 nM). Data are shown as mean ± s.e.m. relative to vehicle-treated cells. * P-value < 0.05 compared to vehicle-treated cells. (**b**) Immunoblots demonstrating the specificity of SREBP1 and SREBP2 antibodies. MDA-MB-468, MDA-MB-453, and Hs578T cells were transfected with siRNAs targeting SREBP1 and SREBP2 for 72 h. Cytosolic and nuclear fractions

were collected after 18 h serum starvation to detect the cytosolic precursor (P) and the nuclear mature (M) forms of SREBP1 and 2, and the C-terminal cleaved form of SREBP2 (C). (**c**) Effect of long-term mTOR inhibition on FASN and SCD levels. MDA-MB-453 cells were serum starved for 96 h and rapamycin (20 nM) or Torin1 (250 nM) was added in 24 h increments.

**Figure S3.** Effect of SREBP1 or SREBP2 knockdown on expression of *SREBF1*, *SREBF2*, and *SCD* in breast cancer cells. Breast cancer cells were transfected with siRNAs targeting SREBP1, 2 or both. RNA was extracted for qRT-PCR analysis 72 h after transfection, following 18 h serum starvation. Representative data are shown as mean ± s.e.m. relative to vehicle-treated cells, n=2. * P-value < 0.05 compared to cells with control siRNAs.

**Figure S4.** Effects of SREBP1 and SREBP2 depletion on the proliferation of breast cancer cells in full, lipid-rich serum. MDA-MB-468, MDA-MB-453, and Hs578T cells were transfected with siRNAs targeting SREBP1, SREBP2, or both. Cells cultured in full serum were counted every 24 h, starting 24 h post-transfection (t = 0 h). Data are shown as mean ± s.e.m., n=3. * P-value < 0.05 compared to cells with control siRNAs at that time point.

**Figure S5.** Validation of antibodies for use in tissue array dot blots. MDA-MB-468 cells were serum starved for 20 h in the presence of vehicle or Torin1 (250 nM). Dilutions of protein extract from these cells were spotted onto nitrocellulose membranes and immunoblotted for the indicated proteins.

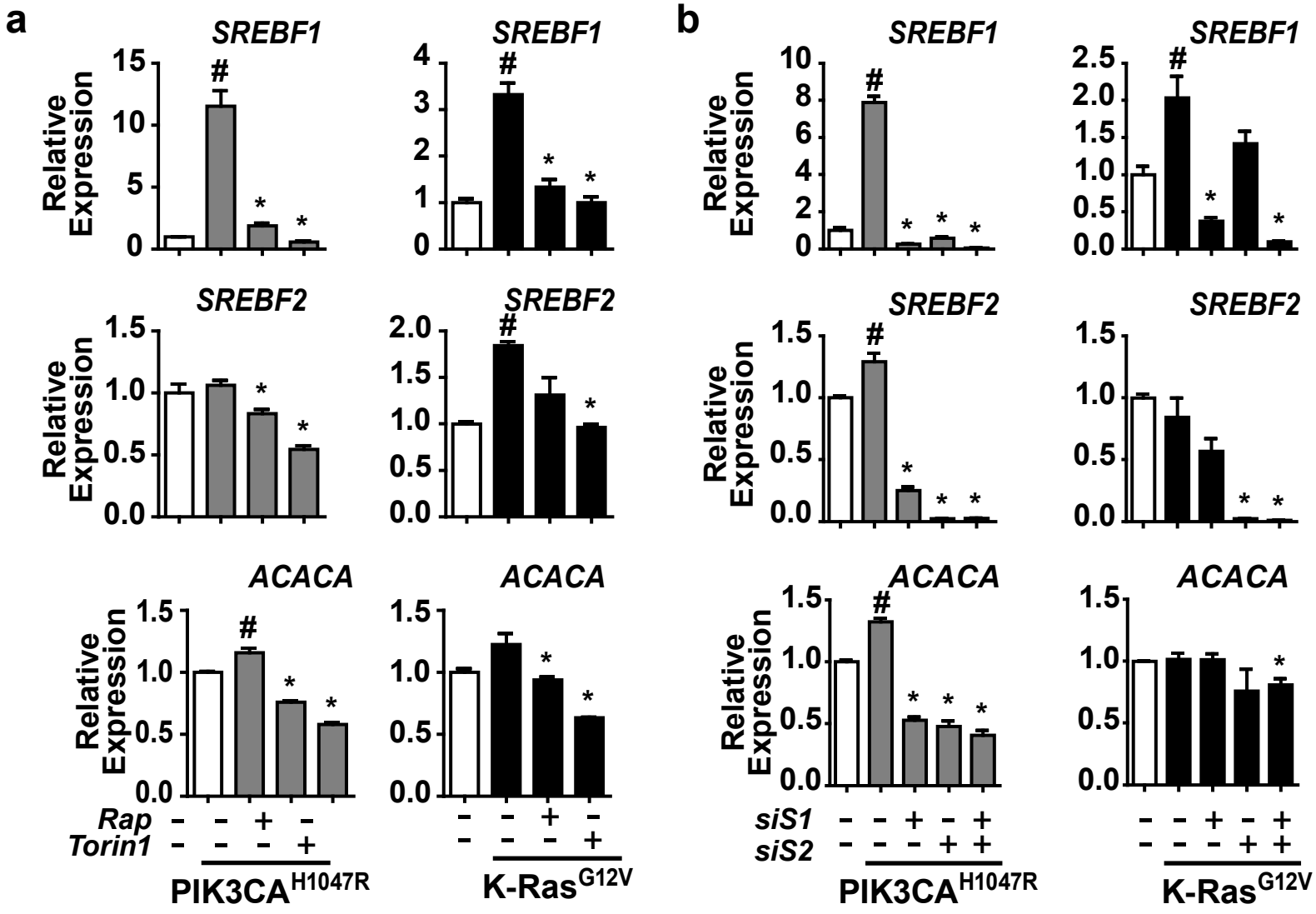
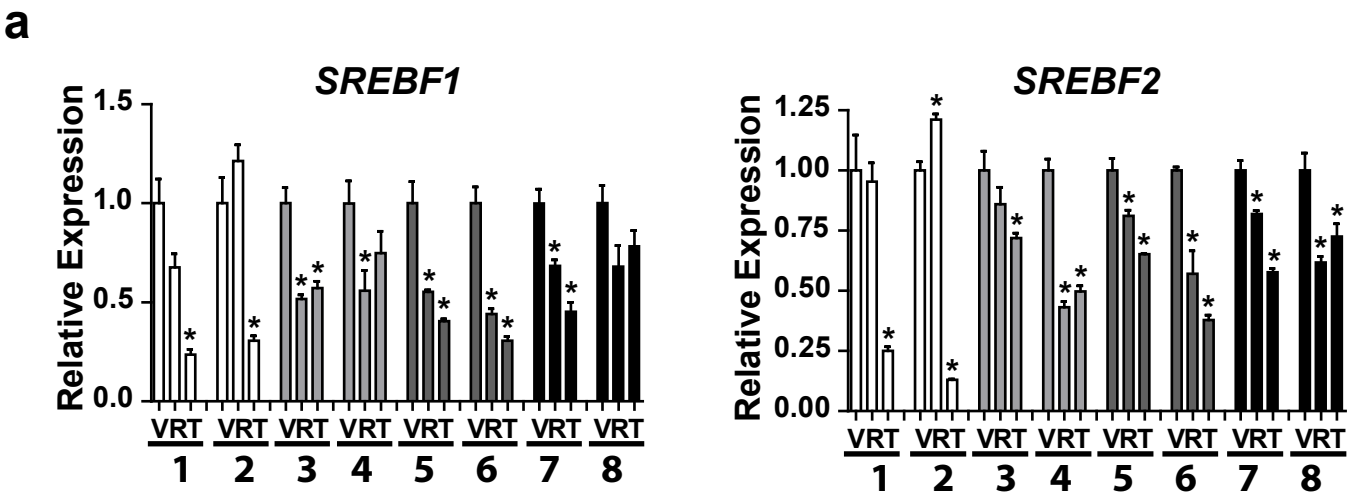
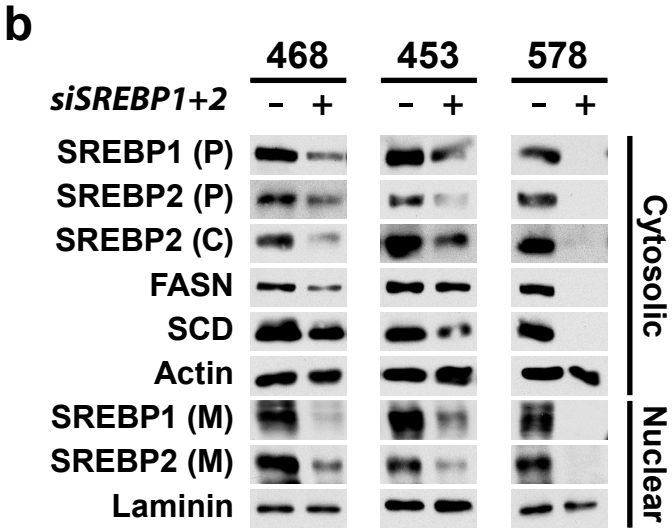

a
b
SREBF1
SREBF2
ACACA
Relative Expression
Rap
Torin1
siS1
siS2
PIK3CA H1047R
K-Ras G12V

Figure S1


a
SREBF1
Relative Expression
1.5
1.0
0.5
0.0
VRT
1
2
3
4
5
6
7
8
SREBF2
Relative Expression
1.25
1.00
0.75
0.50
0.25
0.00


b
468
453
578
siSREBP1+2
- + - + - +
SREBP1 (P)
SREBP2 (P)
SREBP2 (C)
FASN
SCD
Actin
SREBP1 (M)
SREBP2 (M)
Laminin
Cytosolic
Nuclear

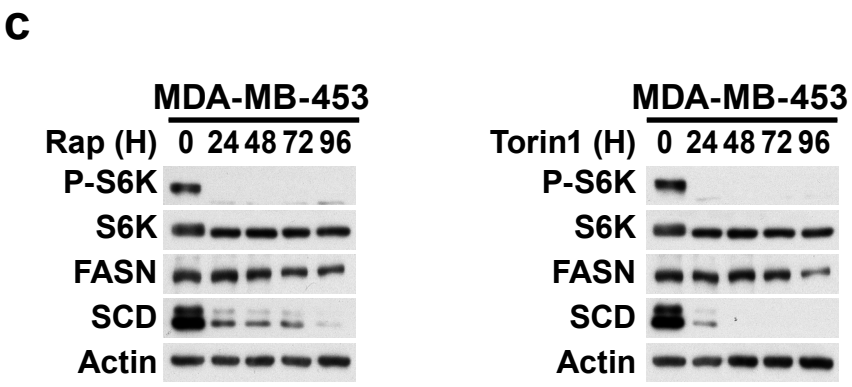

c
MDA-MB-453
Rap (H) 0 24 48 72 96
P-S6K
S6K
FASN
SCD
Actin
MDA-MB-453
Torin1 (H) 0 24 48 72 96
P-S6K
S6K
FASN
SCD
Actin

Figure S2

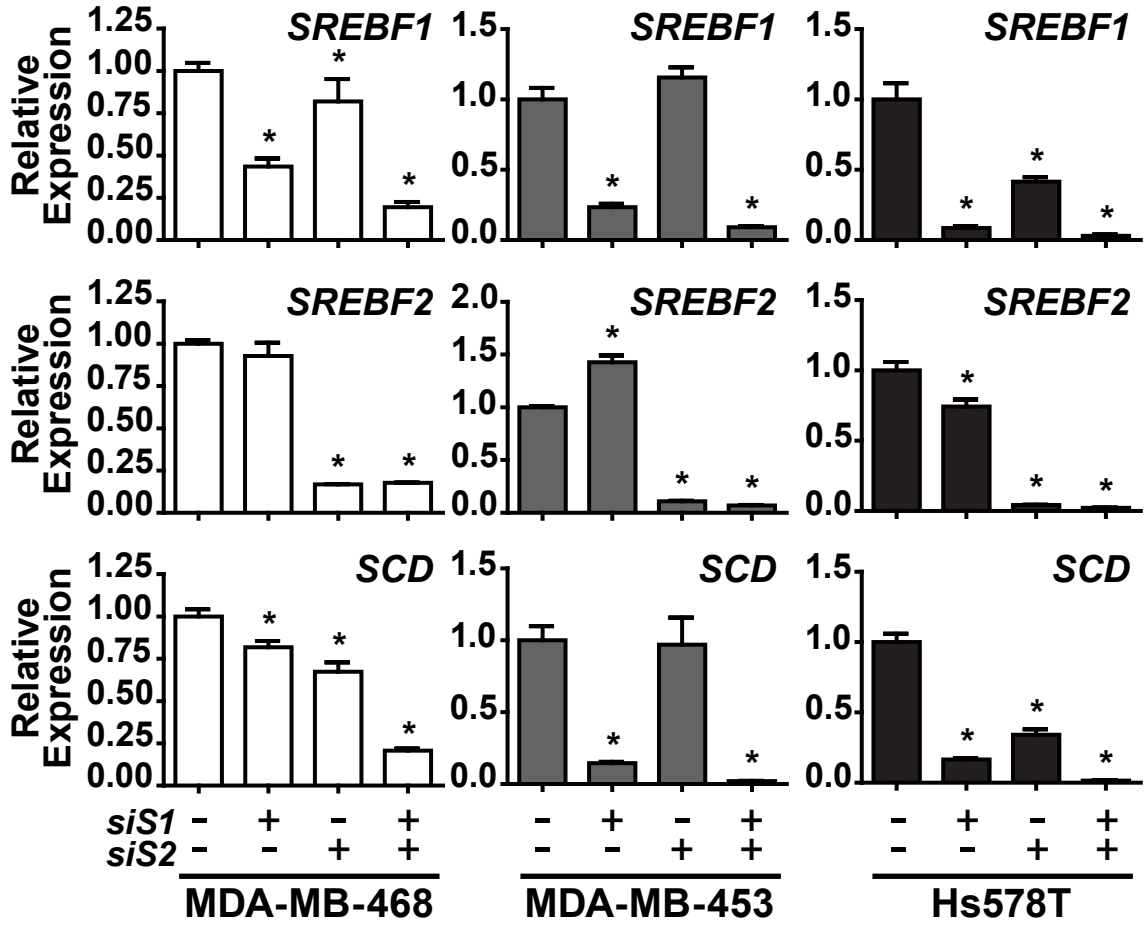

SREBF1
SREBF2
SCD
Relative Expression
siS1
siS2
MDA-MB-468
MDA-MB-453
Hs578T

**Figure S3**

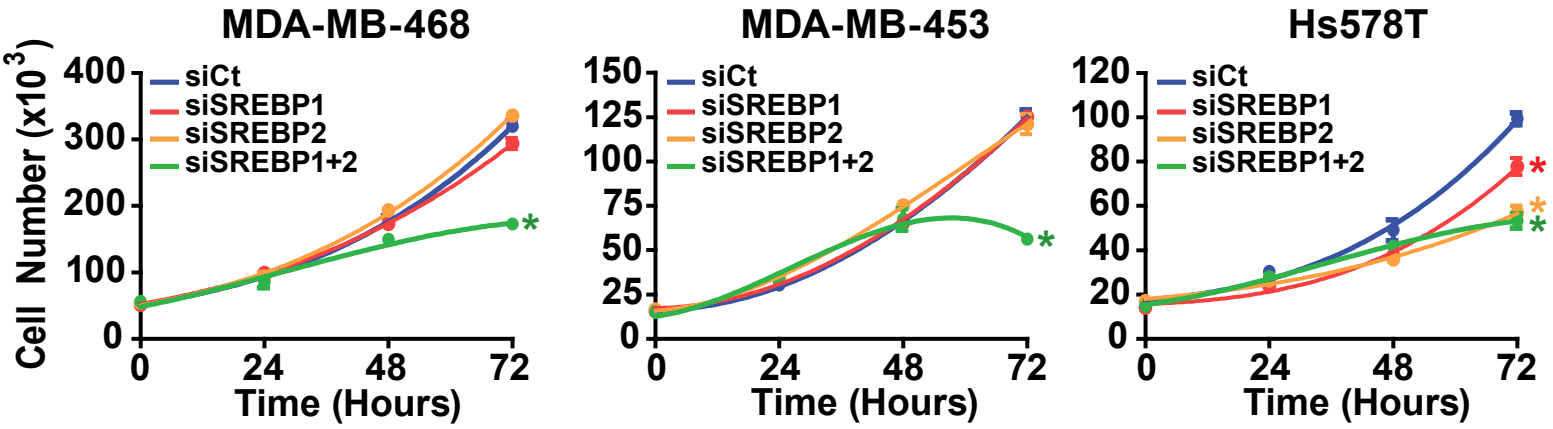

MDA-MB-468
MDA-MB-453
Hs578T
Cell Number (x10^3)
siCt
siSREBP1
siSREBP2
siSREBP1+2
Time (Hours)
0
24
48
72
400
300
200
100
150
125
75
50
25
120
80
60
40
20
*

**Figure S4**

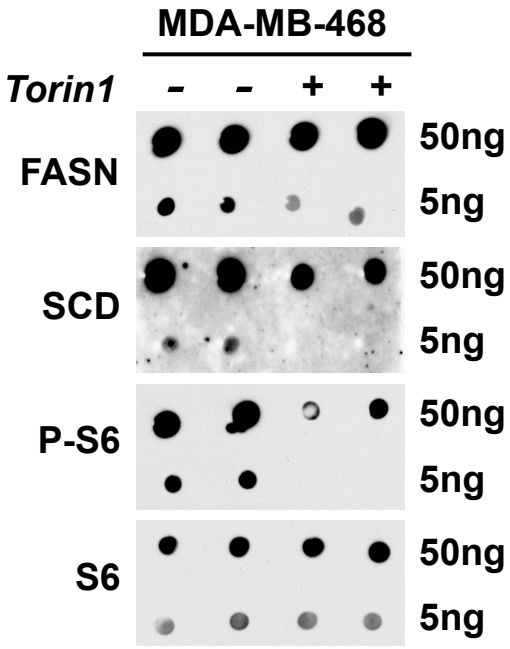

MDA-MB-468
Torin1
- - + +
FASN
SCD
P-S6
S6
50ng
5ng
50ng
5ng
50ng
5ng
50ng
5ng

**Figure S5**