Supplemental Methods

Acyl-CoA analysis by LC-MS/MS

The prepared samples were examined by LC-MS/MS. A Luna® C18(2) 100 Å LC column (100 × 2 mm, 3 μm) coupled with a Phenomenex SecurityGuard C-18 guard column (4.0 mm × 2.0 mm) was used to separate the analytes. The column temperature was maintained at 32 °C. The mobile phase A was 10 mM ammonium acetate (pH 6.8) in water, and mobile phase B was acetonitrile. The injection volume was 30 μL. Analytes were separated using a gradient method, with a 0.2 mL/min flow rate, (time/minute, % mobile phase B): (0, 20), (15, 100), (15.01, 100), (22.5, 100), (22.51, 20), and the analysis time was 30 min. The LC system was interfaced by a six-port divert valve to the mass spectrometer, introducing eluents from 1.0 to 22.5 min to the ion source. The autosampler injection needle was washed with methanol after each injection. Samples were analyzed by the mass spectrometer in positive ion ESI mode. MS parameters were optimized by direct infusion of acyl-CoAs dissolved in methanol (each at 10 μg/mL) at 30 μL/min into the instrument. The capillary voltage was 3.00 kV, the cone voltage was 40 V, the extractor voltage was 0 and RF lens voltage was 3.0 V. Nitrogen was used as the desolvation gas at a flow rate of 500 L/h. The desolvation temperature was 500 °C and the source temperature was 120 °C. Argon was used as the collision gas; the collision cell pressure was 3.5×10^{-3} mbar and the collision energy was 40 eV. A multiple reaction monitoring (MRM) function was applied for simultaneous detection of analytes. The ion transitions monitored were 1006 → 499 for palmitoyl-CoA, 992 → 485 for pentadecanoyl-CoA, 978 → 471 for myristoyl-CoA, 950 → 443 for lauroyl-CoA and 922 → 415 for decanoyl-CoA.

To analyze the amount of acyl-CoA in the samples, peak areas for analytes and the internal standard were automatically calculated using Masslynx 4.0 software. The response ratios

between analytes and the internal standard (pentadecanoyl-CoA) were calculated to minimize systemic and random errors present in the experiment. To compare among samples potentially with different cell numbers, the response ratio was normalized to per 1 mg cell protein (about 3.6 $\times 10^6$ cells); to compare among cell groups with different treatment conditions, data were further normalized to the control group. The control group represents 1, and the fold change of the treatment was calculated based on the control group.

Supplemental figure legends

Figure S1. The endogenous expression levels of Src kinase and other proteins. (A-B) Endogenous expression levels of relevant proteins in human prostate cancer cell lines. Prostate cancer cell lines (LNCaP, 22Rv1, DU145, PC-3, VCaP), colon cancer cell lines (HT-29 and HCT116), and NIH3T3 cell line were obtained from ATCC. Cells were grown with serum (10% FBS) and analyzed for the indicated proteins by immunoblotting. The PC-3 prostate cancer cell line had the highest expression levels of pSrc among the tested prostate cancer cell lines (A) and the two colon cancer cell lines, HT-29 and HCT116 (B). The data represent three independent experiments in panel A and panel B.

Figure S2. Knock down of Src kinase in PC-3 cells. (A) Diagram of the lentiviral vector expressing the shRNA targeting Src kinase. **(B)** Four lentiviral vectors carrying shRNA targeting human Src kinase were created. PC-3 cells were transduced with Mock (no vector), shRNA-Control (shCon) or shSrc by lentiviral infection. The efficiency of knockdown was evaluated by immunoblotting. shRNASrc #3 had the best efficiency in knocking down Src expression and was therefore used for Figure 2. The shRNA-Src has been confirmed either *in vitro* and *in vivo* experiments for more than 3 times.

Figure S3. Expression levels of Ki67, CD34, and inflammation markers in xenograft tumors. (A-D) IHC staining of Ki67 (A), CD34 (B), IL-1β (C), and TNF-α (D) in PC-3 xenografts expressing shRNA-Control or shRNA-Src under 10% or 45% fat diet (scale bar, 200 μm).

Figure S4. Body weight, major organs or tissues, and calorie intake from the diets of host SCID mice carrying Src(Y529F) induced tumors. (A) The calorie intake from protein, carbohydrate, and fat in 10 and 60% fat diets (n=16 per group). **(B)** The change of mouse body

weight before and after 8 weeks on the different diets. Body weight is represented as mean ± SEM (n=6 per group). **(C)** Weight of liver, white adipose tissue (WAT), kidney, heart, and lung of host mice on 10 and 60% fat diets after 8 weeks. **(D)** Representative images from H&E staining of white adipose tissue and hepatocytes after 8 weeks. Host mice on the HFD weighed more than those on the LFD after 8 weeks, and showed increases in the weight of liver and white adipose tissue with no change of kidney, heart or lung weight. The size of adipocytes and the accumulation of ectopic fat in hepatocytes increased in the 60% fat diet group (n=8 per diet) (Scale bar, 100 μm). N.S., not significant. # and ### represent $p<0.05$ and $p<0.005$, respectively.

Figure S5. Expression levels of pSrc and pErk in regenerated prostate tumors. The expression levels of pSrc(Y416) (A) and pErk (B) in regenerated tissues of Src(Y529F) or Src(Y529F/G2A) under 10, 45, and 60% fat diets with vehicle or dasatinib treatment shown in Figure 4E-F were analyzed by the computer software ImageJ (three independent experiments).

Figure S6. Biosynthesis of acyl-CoAs from exogenous fatty acids. 293T cells expressing doxycycline inducible Src(Y529F) were treated with myristic (MA, C14:0) or palmitic acid (PA, C16:0) for 24, 48, and 72 h. The levels of myristoyl-CoA were analyzed by LC-MS/MS (three repeats per group). # and ## represent statistical significance of $p<0.05$ and $p<0.01$, respectively.

Figure S7. The incorporation of exogenous fatty acids with Src kinase. (A-C) SYF1+Src(WT) cells were cultured with 0, 20, 60, 120, 240, or 480 μM myristic acid (C14:0, MA) (A), lauric acid (C12:0, LA) (B), or palmitic acid (C16:0, PA) (C) together with 20 μM of myristic acid-azide. Myristoylated proteins were detected by the click chemistry reaction. The data represent three independent experiments.

Figure S8. Doxycycline (Dox) regulates the expression levels of Src kinase and downstream MAPK signaling. 293T cells carrying the tetracycline transcriptional activator were transduced

with Dox inducible Src(Y529F) by lentiviral infection. Cells were treated with (**A)** increasing concentrations of Dox for 24 h, or (**B)** with 500 ng/ml Dox for various times. The expression levels of total Src, pSrc(Y416), total Erk, or pErk1/2 were determined by immunoblotting. The expression levels of Src, pSrc(Y416), and its downstream MAPK signaling were dependent on the Dox dosage and the stimulation time.

Figure S9. Expression levels of Src kinase in different cellular fractions of 3T3 cells. 3T3 cells were transduced with NMT1 by lentiviral infection. SYF1 (negative control of Src expression), 3T3, and 3T3 over-expressing NMT1 (3T3/NMT1) cells were grown in DMEM medium. Protein lysates were harvested and were further fractionated into cytosol (Cyt) and membrane fraction (Mem). The levels of total Src, caveolin-1, and GAPDH were determined by immunoblotting. Caveolin-1 and GAPDH were used as a marker for the membrane and the cytosol fraction, respectively. The band at the lower molecule weight than Src kinase (Lane 4) is likely a Src family member being detected by the Src antibody non-specifically (three independent experiments).

Table S1. Sucrose-matched rodent diets with 10, 45, 60% fat

Diets *	**10% fat diet (kcal)**	**45% fat diet (kcal)**	**60% fat diet (kcal)**
Protein (%)	20	20	20
Carbohydrate (%)**	70	35	20
Fat (%)***	10	45	60
Total	100	100	100

* The total amount of calories is the same in all three diets.

**: Corn starch is the major dietary component to match the calorie count in the three different diets.

***: Lard and soybean oil are the main components of the fat. Soybean is the same in all diets, and the alteration of the amount of lard is the major component contributing to the difference in fat.

Table S2. Typical fatty acid composition of fats in rodent diets

Diets	**10% fat diet (g)**	**45% fat diet (g)**	**60% fat diet (g)**
Capric (C10)	0	0.1	0.1
Lauric (C12)	0	0.2	0.2
Myristic (C14)	0.2	2.0	2.8
C15	0	0.1	0.2
Palmitic (C16)	6.5	36.9	49.9
Palmitoleic (C16:1)	0.3	2.4	3.4
C17	0.1	0.7	0.9
Stearic (C18)	3.1	19.8	26.9
Oleic (C18:1)	12.6	64.4	86.6
Linoleic (C18:2)*	18.3	56.7	73.1
Linolenic (C18:3)**	2.2	4.3	5.2
Arachidic (C20)	0	0.3	0.4
C20:1	0.1	1.1	1.5
C20:2	0.2	1.4	2.0
C20:3	0	0.2	0.3
Arachidonic (C20:4)*	0.1	0.5	0.7
Docosapentaenoic (C22:5)	0	0.2	0.2
Total (g)	43.7	191.3	254.5
Omega-6/Omega-3	8.3	13.3	14.2
PUFA/SFA	2.1	1.1	1.0

*: Omega-6 fatty acids
**: Omega-3 fatty acids

Table S3. Primer sequences used for creating Src mutants and shRNA-Src

Gene	Direction	Sequence (5'-3')
F-G2A	**Forward**	CACTCTAGAAGGACCATGGCCAGCAACAAGAGCAAGCCC AAG
R-G2A	**Reverse**	CACTCAATGAATTCCACAGTCC
Src shRNA 1	**Forward**	TCCCGGAGGCTTCAACTCCTCGGACACCGTCACTTCAAGAGAGTGACGGT GTCCGAGGAGTTGAAGCCTCC
	Reverse	AAAAGGAGGCTTCAACTCCTCGGACACCGTCACTCTCTTGAAGTGACGGT GTCCGAGGAGTTGAAGCCTCC
Src shRNA 2	**Forward**	TCCCAAGAAAGGCGAGCGGCTCCAGATTGTCAATTCAAGAGATTGACAAT CTGGAGCCGCTCGCCTTTCTT
	Reverse	AAAAAAGAAAGGCGAGCGGCTCCAGATTGTCAATCTCTTGAATTGACAAT CTGGAGCCGCTCGCCTTTCTT
Src shRNA 3	**Forward**	TCCCGCAGTTGTATGCTGTGGTTTCAGAGGAGCTTCAAGAGAGCTCCTCTG AAACCACAGCATACAACTGC
	Reverse	AAAAGCAGTTGTATGCTGTGGTTTCAGAGGAGCTCTCTTGAAGCTCCTCTG AAACCACAGCATACAACTGC
Src shRNA 4	**Forward**	TCCCCTGGAGGCAATCAAGCAGACATAGAAGAGTTCAAGAGACTCTTCTA TGTCTGCTTGATTGCCTCCAG
	Reverse	AAAACTGGAGGCAATCAAGCAGACATAGAAGAGTCTCTTGAACTCTTCTA TGTCTGCTTGATTGCCTCCAG

Figure S1

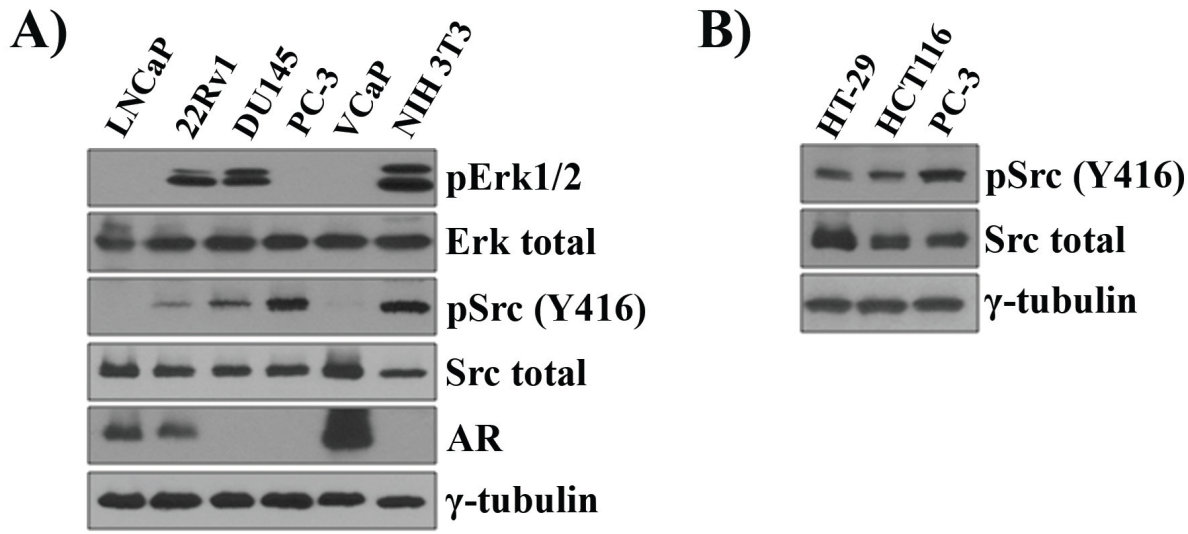

A)
LNCaP
22Rv1
DU145
PC-3
VCaP
NIH 3T3
pErk1/2
Erk total
pSrc (Y416)
Src total
AR
γ-tubulin
B)
HT-29
HCT116
PC-3
pSrc (Y416)
Src total
γ-tubulin

Figure S2

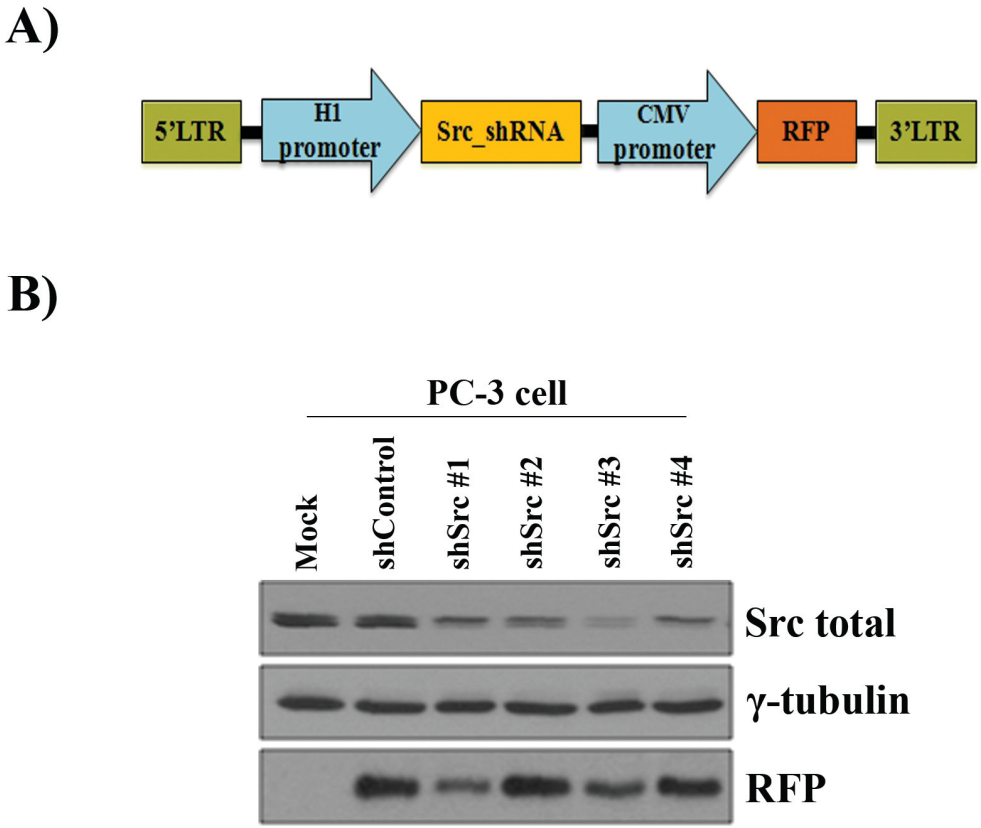

A)
5'LTR
H1 promoter
Src_shRNA
CMV promoter
RFP
3'LTR
B)
PC-3 cell
Mock
shControl
shSrc #1
shSrc #2
shSrc #3
shSrc #4
Src total
γ-tubulin
RFP

Figure S3

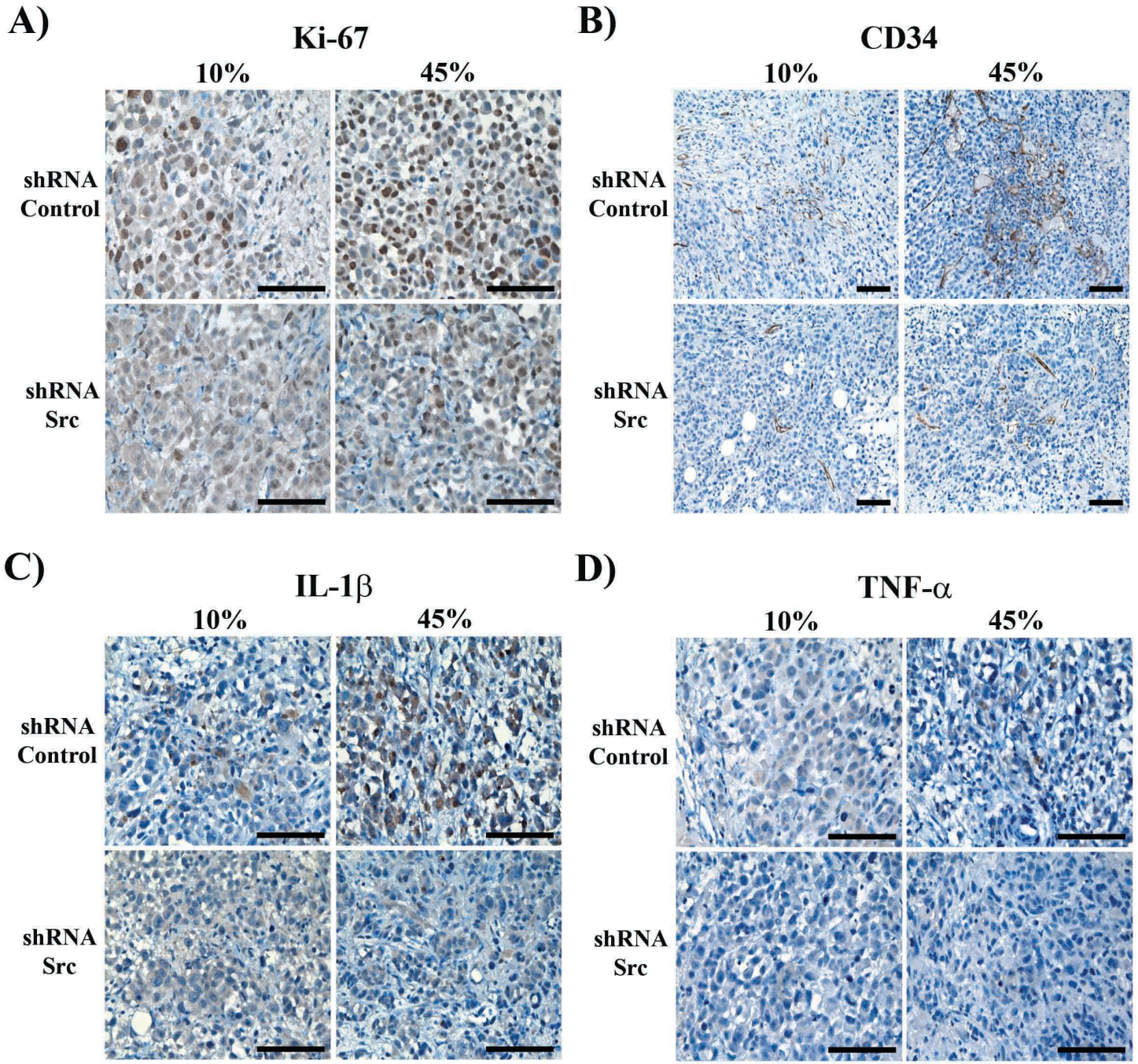

A)
Ki-67
10%
45%
shRNA Control
shRNA Src
B)
CD34
10%
45%
shRNA Control
shRNA Src
C)
IL-1β
10%
45%
shRNA Control
shRNA Src
D)
TNF-α
10%
45%
shRNA Control
shRNA Src

Figure S4

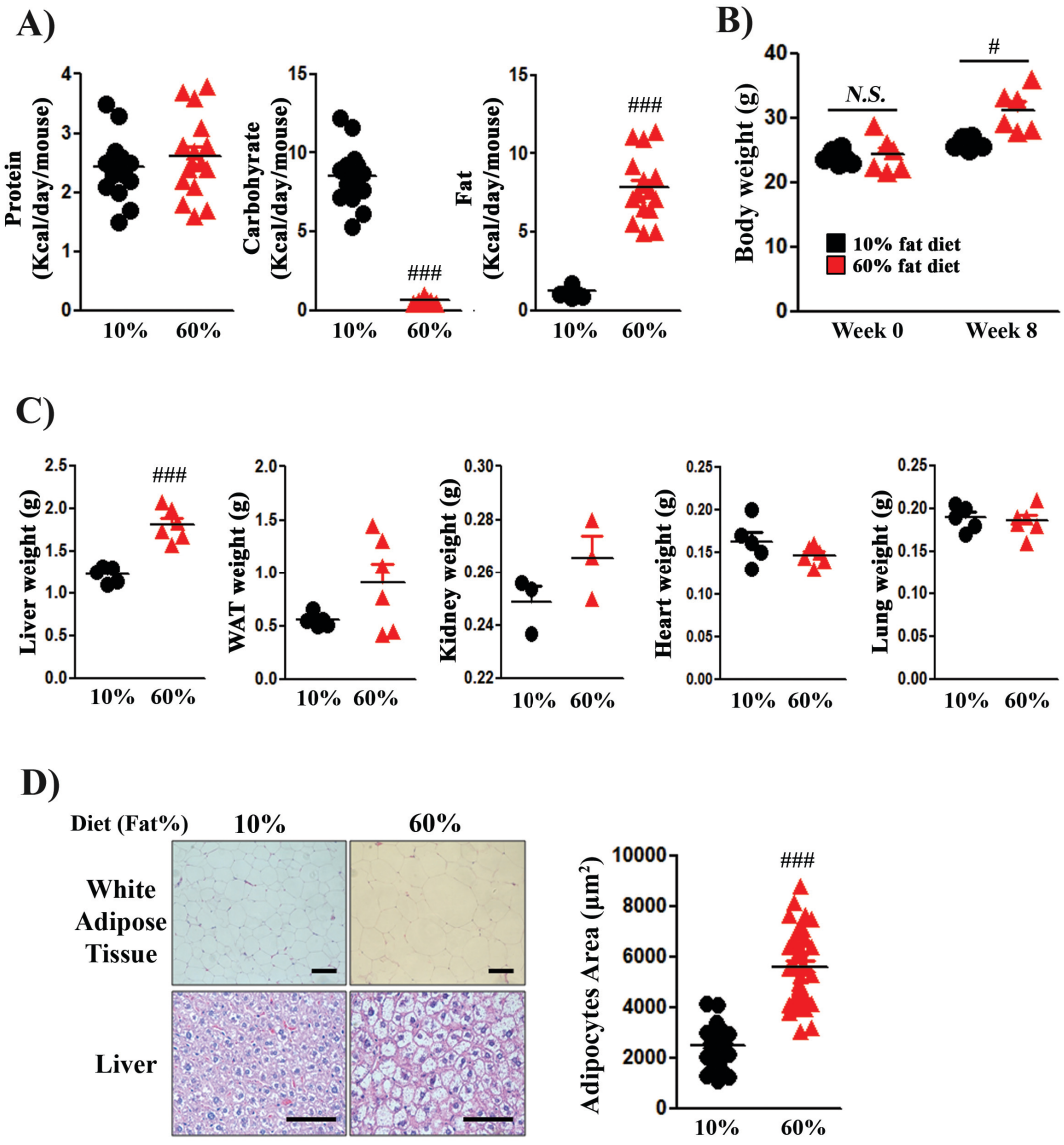

A)
Protein (Kcal/day/mouse)
Carbohyrate (Kcal/day/mouse)
Fat (Kcal/day/mouse)
###
10%
60%
B)
Body weight (g)
N.S.
#
10% fat diet
60% fat diet
Week 0
Week 8
C)
Liver weight (g)
WAT weight (g)
Kidney weight (g)
Heart weight (g)
Lung weight (g)
D)
Diet (Fat%)
White Adipose Tissue
Liver
Adipocytes Area (μm²)

Figure S5

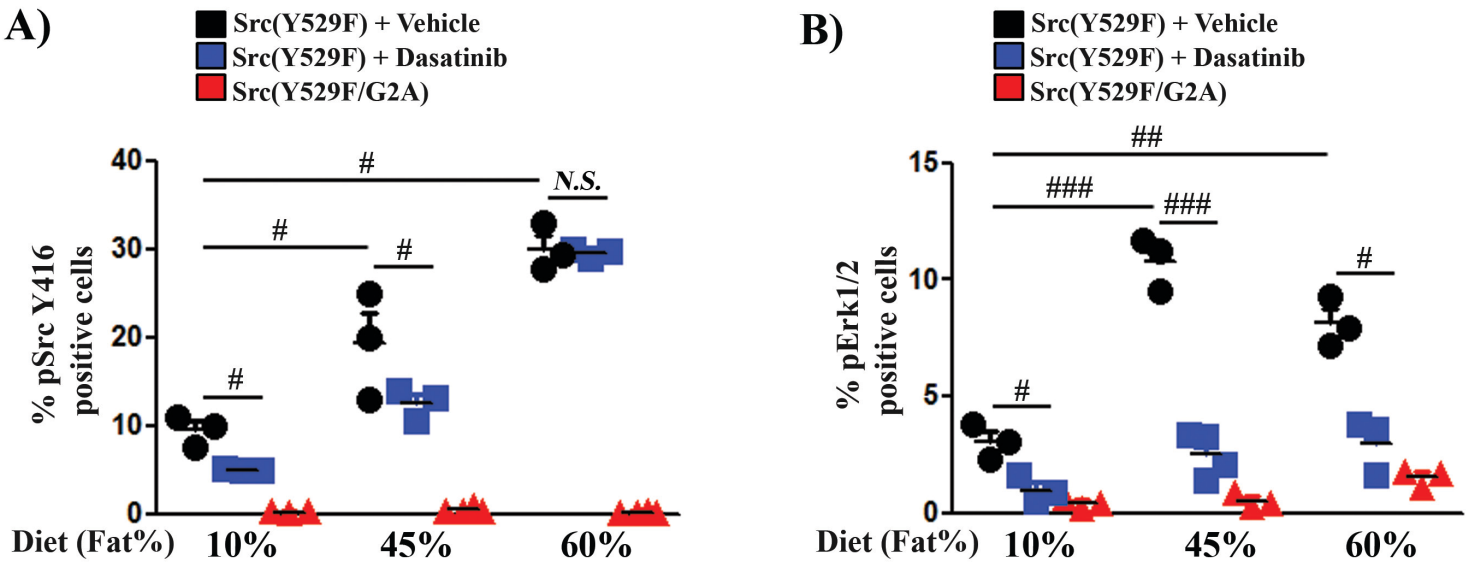

A)
Src(Y529F) + Vehicle
Src(Y529F) + Dasatinib
Src(Y529F/G2A)
% pSrc Y416 positive cells
40
30
20
10
0
#
#
#
N.S.
Diet (Fat%)
10%
45%
60%
B)
Src(Y529F) + Vehicle
Src(Y529F) + Dasatinib
Src(Y529F/G2A)
% pErk1/2 positive cells
15
10
5
0
##
###
###
#
#
Diet (Fat%)
10%
45%
60%

Figure S6

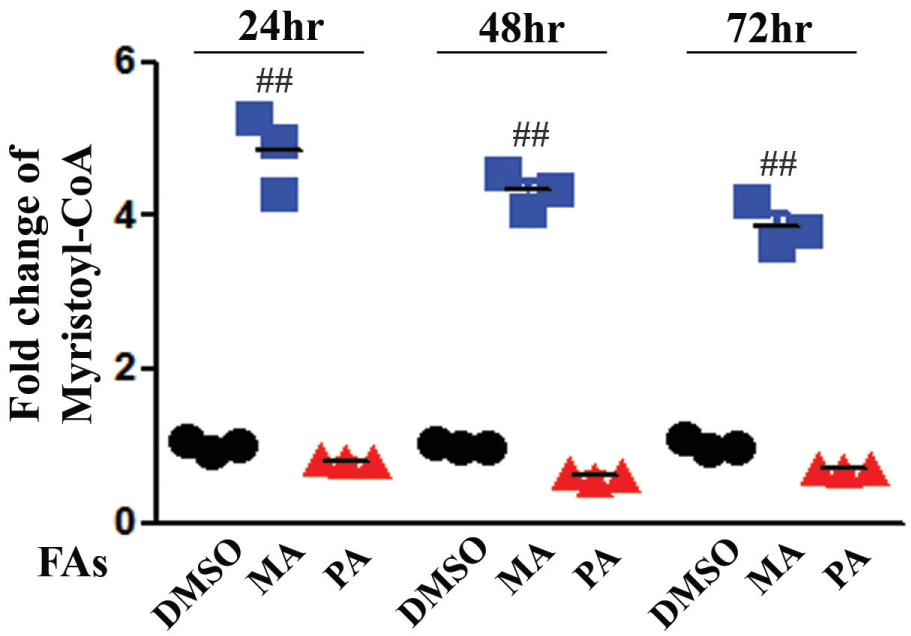

24hr
48hr
72hr
##
##
##
Fold change of Myristoyl-CoA
6
4
2
0
FAs
DMSO
MA
PA
DMSO
MA
PA
DMSO
MA
PA

Figure S7

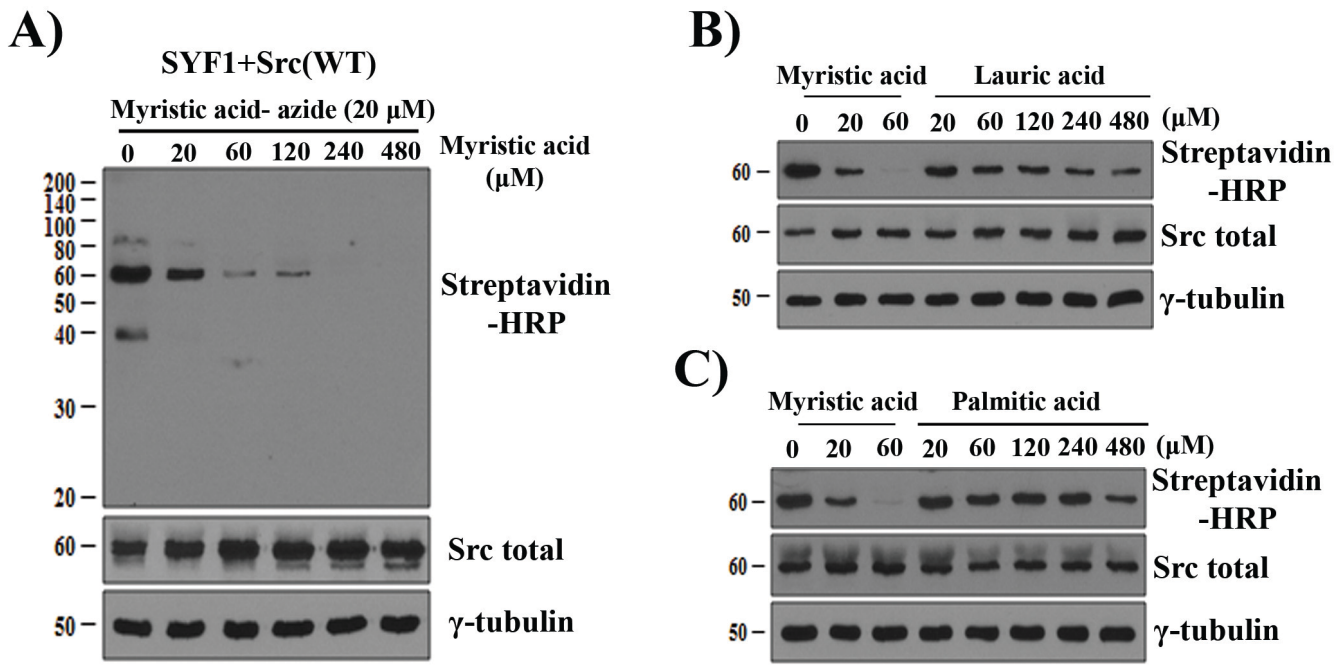

A)
SYF1+Src(WT)
Myristic acid- azide (20 μM)
0 20 60 120 240 480
Myristic acid (μM)
200
140
100
80
60
50
40
30
20
Streptavidin -HRP
60
Src total
50
γ-tubulin
B)
Myristic acid
Lauric acid
0 20 60 20 60 120 240 480 (μM)
60
Streptavidin -HRP
60
Src total
50
γ-tubulin
C)
Myristic acid
Palmitic acid
0 20 60 20 60 120 240 480 (μM)
60
Streptavidin -HRP
60
Src total
50
γ-tubulin

Figure S8

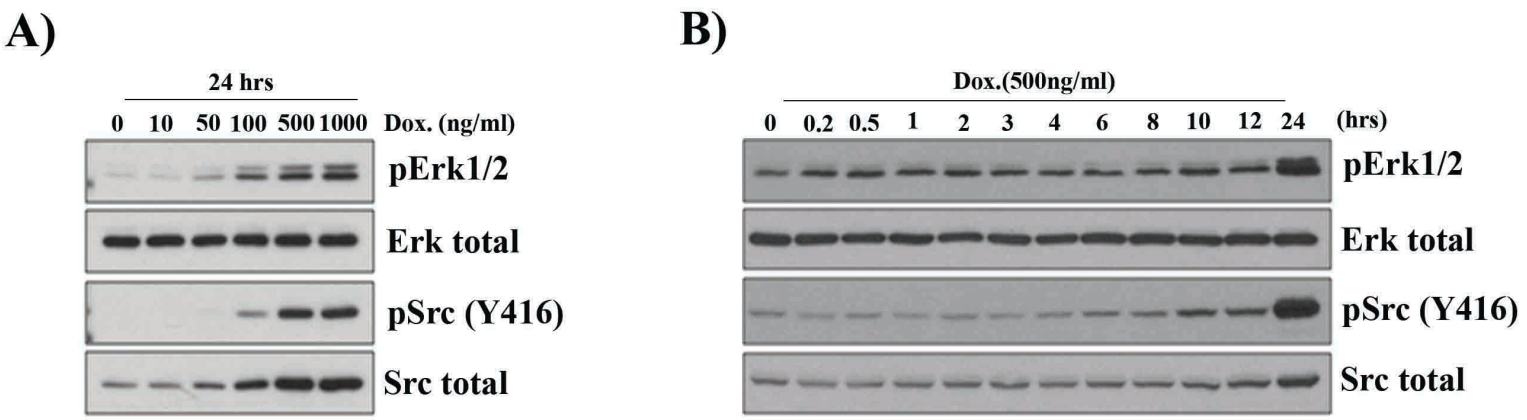
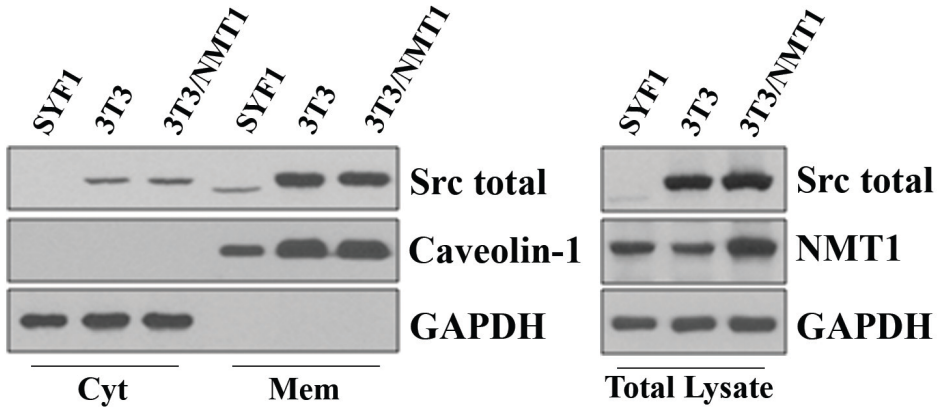

A)
24 hrs
0 10 50 100 500 1000 Dox. (ng/ml)
pErk1/2
Erk total
pSrc (Y416)
Src total
B)
Dox.(500ng/ml)
0 0.2 0.5 1 2 3 4 6 8 10 12 24 (hrs)
pErk1/2
Erk total
pSrc (Y416)
Src total

Figure S9


SYF1
3T3
3T3/NMT1
SYF1
3T3
3T3/NMT1
SYF1
3T3
3T3/NMT1
Src total
Caveolin-1
GAPDH
Src total
NMT1
GAPDH
Cyt
Mem
Total Lysate