

Supplementary Information

Fatostatin Displays High Anti-Tumor Activity in Prostate Cancer by Blocking SREBP-Regulated Metabolic Pathways and Androgen Receptor Signaling

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Supplementary Methods

Supplementary Figure Legends

Supplementary Table S1

Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure S3

Supplementary Methods

Immunofluorescence staining

Prostate cancer cells treated with vehicle or fatostatin (10 $\mu\text{mol/L}$) were fixed with 10% formalin and permeabilized with 0.1% Triton X-100 in PBS. After washing with PBS, cells were blocked with 1% BSA for 1 hours. Anti-SREBP-1 (Santa Cruz Technology) and SREBP-2 (Abcam) and AR (Milipore) primary antibodies were added and incubated for overnight at 4 °C. After washing twice in PBS, secondary anti-rabbit IgG-R or IgG-FITC (sc-2095 and sc-2090, Santa Cruz Technology) was used and incubated for 1 hour. For the examination of nuclear morphology, cells were stained with DAPI solution (1 $\mu\text{g/mL}$) for 10 minutes. Fluorescence images were visualized by fluorescence microscopy (Nikon Eclipse Ti)(1).

Supplementary References

1. Walker AK, Yang F, Jiang K, Ji JY, Watts JL, Purushotham A, et al. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev.* 2010;24:1403-17.

Supplementary Figure Legends

Supplementary Figure S1. Chemical structure of fatostatin

Supplementary Figure S2. Fatostatin blocks nuclear abundance of SREBP-1 and SREBP-2 in LNCaP and C4-2B cells. Cells were treated with vehicle or fatostatin for 24 hours and then stained with antibodies against SREBP-1 or SREBP-2 along with DAPI, and were visualized by immunofluorescence. Representative images are shown. Scale bar = 50 μm .

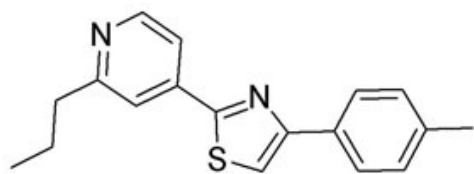
Supplementary Figure S3. Fatostatin inhibits the transcriptional activity of SREBPs determined by the SREs (sterol regulatory elements)-luciferase (Luc) reporter assay. Fatostatin significantly decreased the SRE-Luc activity in the presence of SREBP-1 (A) or SREBP-2 (B) expression vector but didn't affect the SRE-Luc activity in the presence of dominant negative forms (A-SREBP1 or A-SREBP2). The relative luciferase activity was normalized by β -galactosidase activity and assigned as 100% in the presence of SREBP-1 or -2 expression vector. The data were shown as mean \pm SD, *, $P < 0.05$ and **, $P < 0.01$.

Supplementary Figure S4. Fatostatin blocks AR nuclear translocation in LNCaP and C4-2B cells. Cells were exposed to vehicle or fatostatin (10 $\mu\text{mol/L}$) for 12 hours, stained with anti-AR antibody and visualized by immunofluorescence. Representative images are shown. Scale bar = 20 μm .

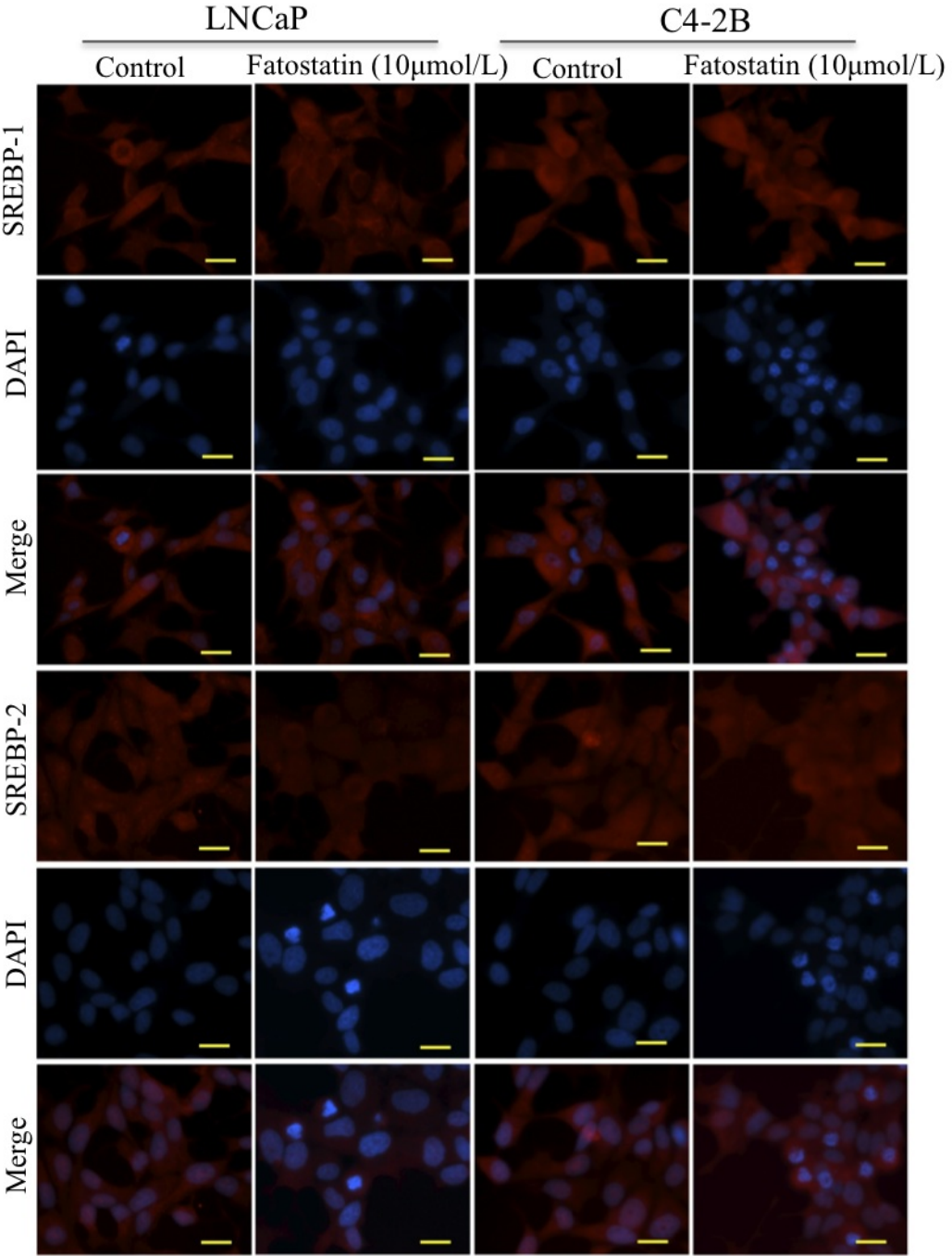
Supplementary Table S1. The Sequences of primers used for qRT-PCR

Gene	Sequence (5' to 3')
ACL	F: TGTAACAGAGCCAGGAACCC R: CTGTACCCCAGTGGCTGTTT
FASN	F: CGGTACGCGACGGCTGCCTG R: GCTGCTCCACGAACTCAAACACCG
SCD-1	F: CACTTGGGAGCCCTGTATGG R: AGCCGAGCTTTGTAAGAGCG
HMGCS1	F: GAGGGCTTCGTGGGACACATA R: GCCACTGGGATGGATCTTT
HMGCR	F: GTCATTCCAGCCAAGGTTGT
	R: GGGACCACTTGCTTCCATTA
MVK	F: CCTTGTGGCTGGCGTCAGAAA R: CGAGGGCATTTCAGATGGTGCT
MVD	F: ACCACGGGGACACCAAGGT R: CCACACAGCAGCCACAAACTC
INSIG1	F: GGACGACAGTTAGCTATGGGTGTT R: GAGTCATTTGTACAGTCAGCCCGA
SCAP	F: TATCTCGGGCCTTCTACAACCA R: ACACAACTCCTCCAAGCTCCTG
β -actin	F: CAAGGCCAACCGCGAGAAGATGAC R: GCCAGAGGCGTACAGGGATAGCACA

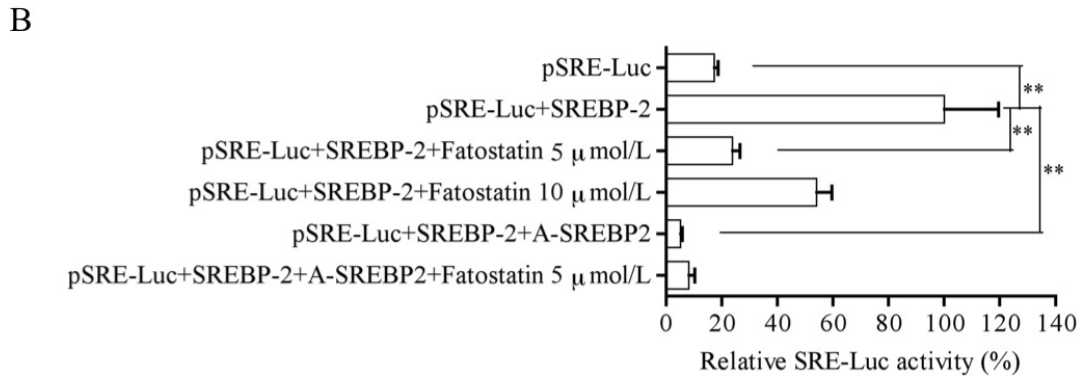
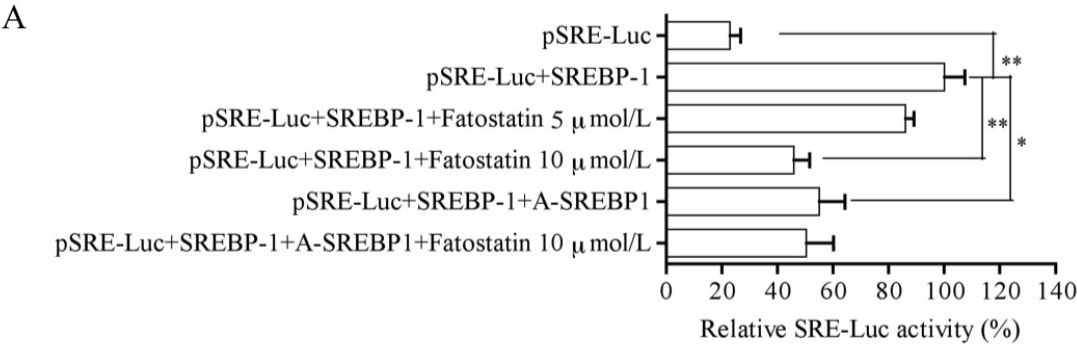
Supplementary Figure S1



Supplementary Figure S2.



Supplementary Figure S3



Supplementary Figure S4

