Supplemental Figure Legends

Supplemental Fig. 1S. Effects of autophagy inhibitors on prostate cancer cell viability. A, LNCaP or VCaP cells were treated for 72 hours with vehicle or the synthetic androgen R1881 in combination with vehicle or increasing concentrations of various inhibitors of autophagy (chloroquine: 10, 40 μM; 3-MA: 1, 10 mM; bafilomycin A1: 1, 10, 100 nM). Cell viability was then assayed using a resazurin-based CellTiter-Blue Cell Viability Assay. Each sample was performed in triplicate and results from a representative experiment are shown. Results are expressed as mean relative cell number + SE (n = 2). *, significant changes from vehicle (no R1881)-treated cells. $^{\#}$, significant changes from vehicle (no autophagy inhibitor)-treated cells. B, qPCR analysis of Atg7 mRNA levels in LNCaP cells following 72 hour siRNA transfection to demonstrate knockdown efficiency. Results are expressed as normalized fold Atg7 mRNA levels + SE (n = 2). *, significant changes from mock-transfected cells. C, LNCaP cells were transfected with mock, a control siRNA or siRNAs targeting 3 separate regions of the core autophagy molecule ATG7 and then treated for 72 hours ± R1881. Cell viability was then quantitated as in A. Each sample was performed in triplicate and results from a representative experiment are shown. Results are expressed as mean relative cell number + SE (n = 2). *, significant changes from vehicle (no R1881)-treated cells. $^{\#}$, significant changes from mock-transfected cells.

Supplemental Fig. 2S. Androgens increase autophagic flux. A, VCaP cells stably expressing an eGFP-LC3B fusion were treated for 3 days with vehicle or 10 nM R1881. Cells were then fixed and stained with LysoTracker to identify acidic organelles. eGFP-LC3B and LysoTracker colocalization was determined using immunofluorescence deconvolution microscopy. Scale bars, 10 μm. Quantification of colocalization (Fig. 3A, *right*) was done using ImageJ software. B, LNCaPs were transfected with an mCherry-GFP-LC3B construct and treated for 3 days with vehicle, DHT (10 nM) or R1881 (10 nM) to assess autophagic flux. Cells were then fixed and visualized using an immunofluorescence confocal

microscope. Scale bars, 10 μm. C, LNCaP cells were treated for 3 days ± 40 μM chloroquine (lysosomal inhibitor) and ± 10 nM R1881. Cells were then subjected to Western blot analysis as in Fig. 2.

Supplemental Fig. 3S. Effect of ATG7 knockdown on cell cycle regulatory proteins. LNCaP cells were siRNA transfected and treated as in Supplemental Figure 1SC. Cell lysates were then subjected to Western blot analysis to determine cyclin D1, cyclin D3, p21 Waf1/Cip1, p27 Kip1, CDK4, p15 INK4B, p18 INK4C, LC3BI/II and GAPDH (loading control) protein levels. Representative blots are shown (n = 2).

Supplemental Fig. 4S. Androgens promote intracellular neutral lipid accumulation through an AR-dependent mechanism. A, prostate cancer cells were treated for 3 days with vehicle or 10 nM R1881. Intracellular triglyceride (TG) levels were then determined using a fluorescent Nile Red-based stain (AdipoRed) and normalized to cell numbers that were determined using duplicate plates that were instead subjected to the fluorescent DNA-binding dye described in Fig. 1. Each sample was performed in triplicate and results from a representative experiment are shown. Results are expressed as TG levels normalized to cell numbers + SE (n = 2). *, significant changes from vehicle-treated cells. B, LNCaP cells were transfected with siRNAs targeting either a scrambled sequence (siControl) or AR and then treated for 3 days with vehicle or 10 nM R1881. Intracellular triglyceride (TG) levels were then determined as described in A. Each sample was performed in triplicate and results from a representative experiment are shown. Results are expressed as TG levels normalized to cell numbers + SE (n = 2). *, significant changes from vehicle-treated cells.

Supplemental Fig. 5S. Androgen-mediated autophagy is associated with lipogenesis. VCaP cells stably expressing an eGFP-LC3B fusion were treated for 3 days with vehicle, DHT (10 nM) or R1881 (10 nM). Cells were then fixed and stained with LipidHTX (neutral lipid depots) and DAPI (nucleus). eGFP-LC3B,

LipidHTX and DAPI colocalization was then visualized using immunofluorescence deconvolution microscopy. Scale bars, 20 μm.

Supplemental Fig. 6S. Western blot controls for Figs. 4D and E. Inhibition of ATG7 blocks entry into autophagy and thus decreases in LC3BII levels are seen. Chloroquine is a lysosomal inhibitor and therefore blocks autophagic flux including the breakdown of LC3B. Hence, chloroquine treatment leads to elevated LC3BII levels.

Supplemental Fig. 7S. Knockdown of ATG7 does not affect AR-mediated transcription. A and B, LNCaP cells were transfected and treated as described in Fig. 4D. C and D, LNCaP ATG7 shRNA-inducible stable cells were treated for 2 days ± 800 ng/ml doxycycline (Dox) followed by 72 hour treatment with or without 10 nM R1881. A-D, after treatment, cells were lysed, and RNA was isolated and reverse transcribed. The expression of the classical AR target genes *FKBP51* (A and C) and *PSA* (B and D) was assessed using qPCR. A and B, Results are expressed as normalized fold induction over vehicle-treated and mock-transfected cells + SE (n = 2). C and D, Results are expressed as normalized fold induction over double vehicle-treated cells + SE (n = 2).

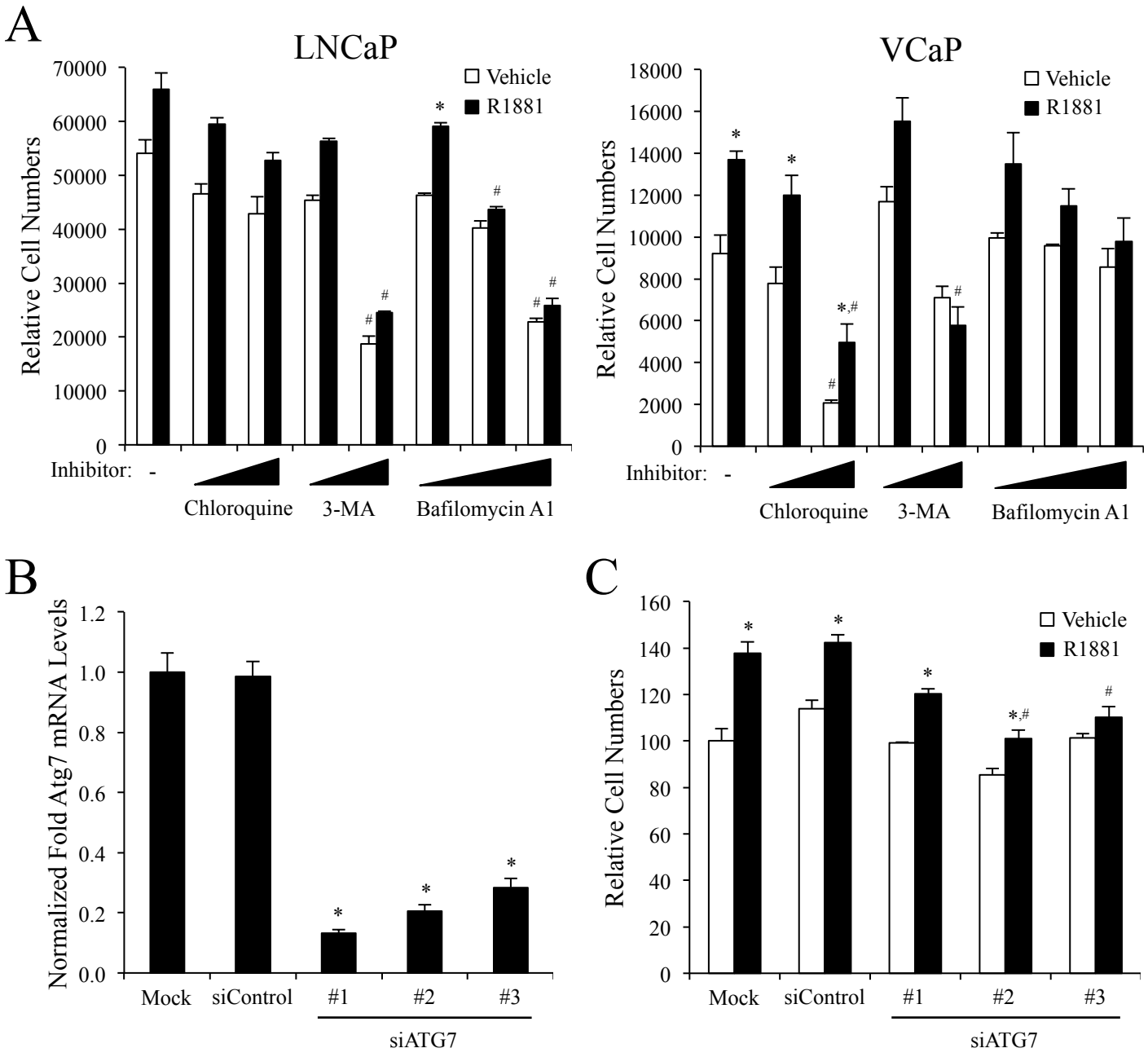
Supplemental Fig. 8S. Inhibition of autophagy does not affect androgen-mediated lipid metabolism enzyme levels. A, LNCaP ATG7 shRNA-inducible stable cells were treated for 2 days ± 800 ng/ml doxycycline (Dox) followed by 72 hour treatment with or without 10 nM R1881. After treatment, cells were lysed, and RNA was isolated and reverse transcribed. FAS mRNA levels were assessed using qPCR. Results are expressed as normalized fold induction over double vehicle-treated cells + SE (n = 2). B, LNCaP cells were treated and subjected to Western blot analysis as described in Fig. 3C. Here, cell lysates were also assessed for FAS protein levels. C, LNCaP cells were siRNA transfected and treated as in Supplemental Fig. 3S. Cell lysates were then subjected to Western blot analysis to determine acetyl-

CoA carboxylase (ACC), ATP-citrate lyase (ACL), acetyl-CoA synthetase (AceCS1), long-chain acyl-CoA synthetase (ACSL), lipin1, LC3BI/II and GAPDH (loading control) protein levels. Representative blots are shown (n = 2).

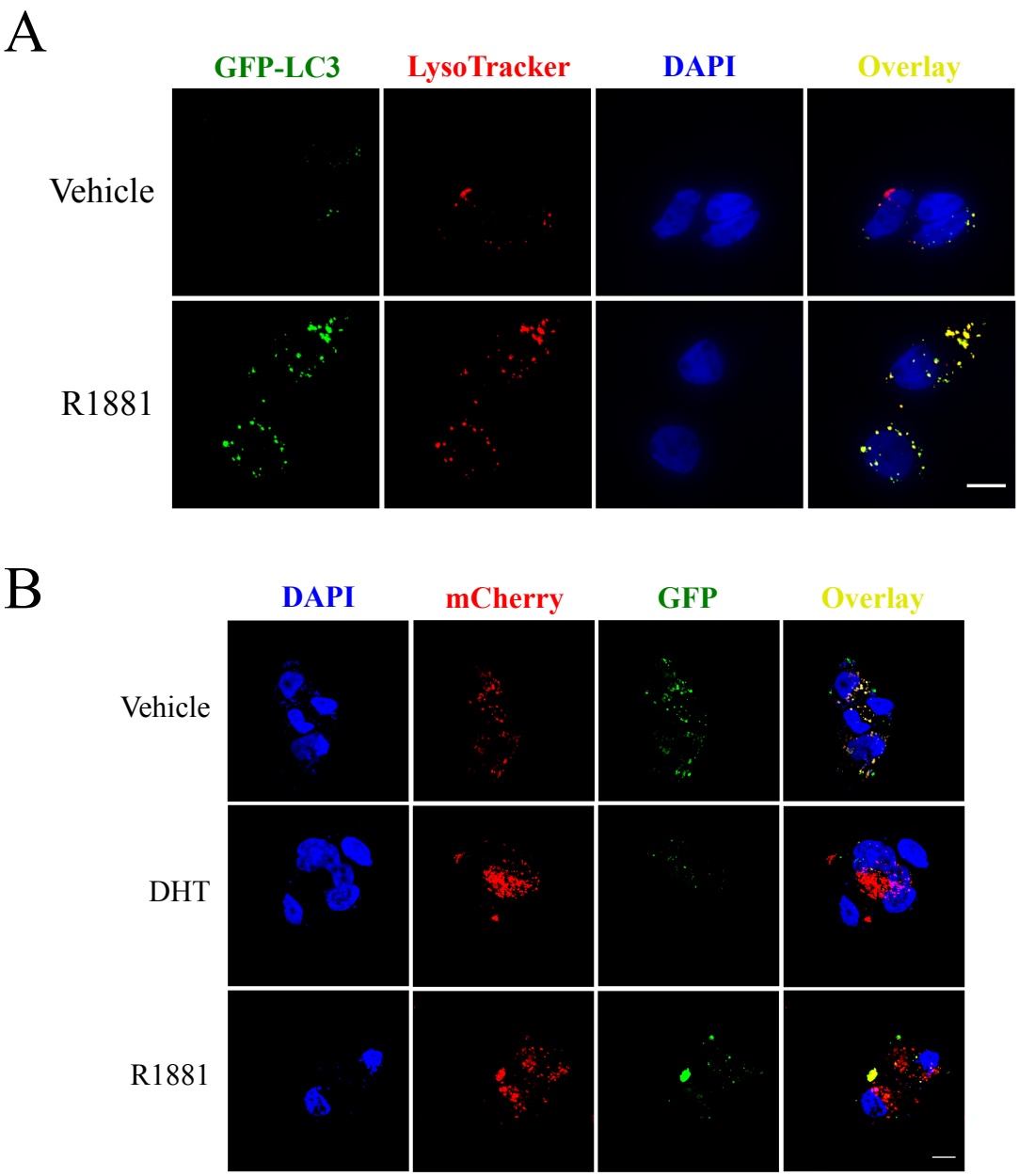
Supplemental Fig. 9S. Androgens increase intracellular ROS levels in LNCaP and VCaP cells. LNCaP or VCaP cells were treated for 3 days with vehicle, DHT (10 nM) or R1881 (10 nM). Intracellular ROS levels were then quantified using CM-H2DCFDA FACS analysis.

Supplemental Fig. 10S. Antioxidants block androgen-mediated autophagy. LNCaP cells were cotreated for 3 days ± 5 mM NAC and ± 10 nM R1881. Cells were then subjected to Western blot analysis for LC3B and GAPDH (loading control).

Supplemental Figure 1S



Supplemental Figure 2S



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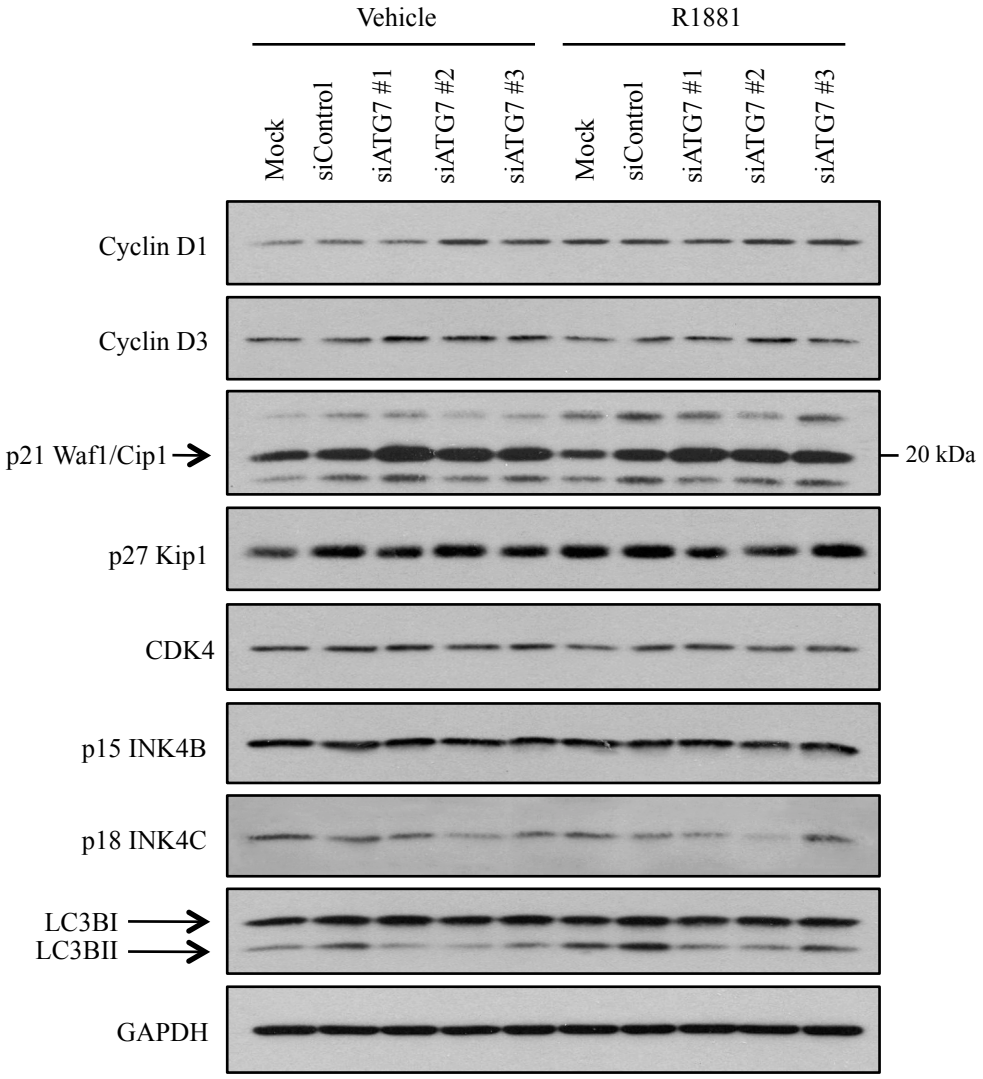
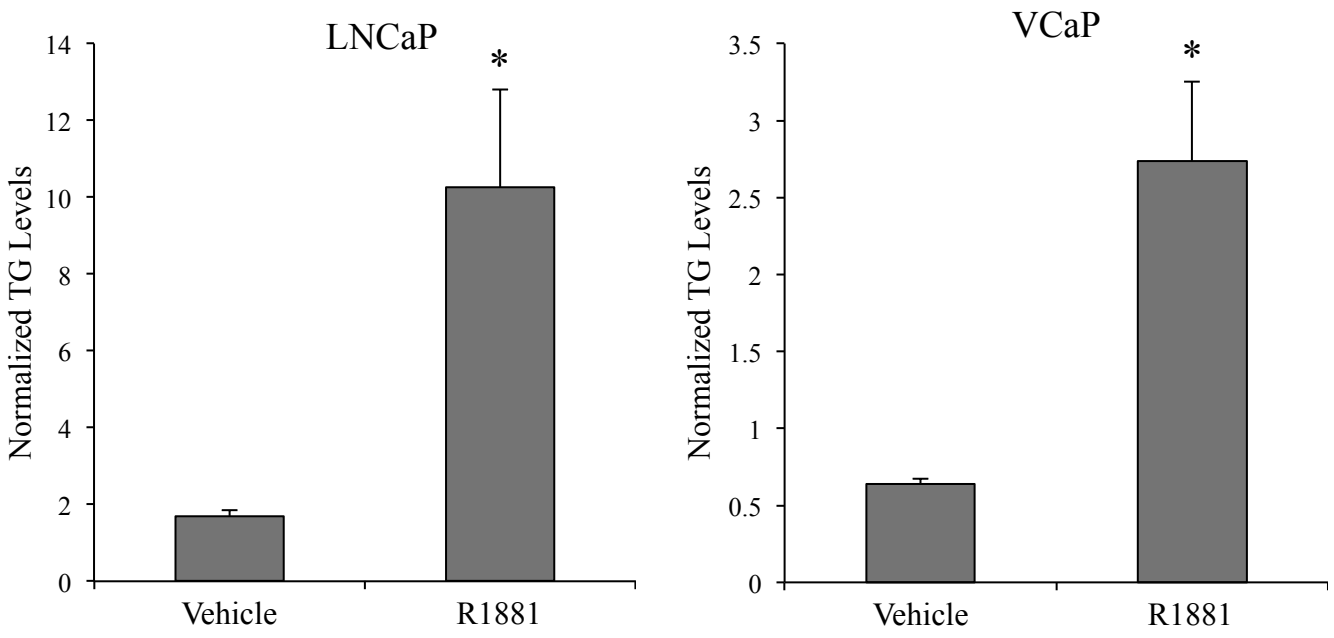


Supplemental Figure 4S

A



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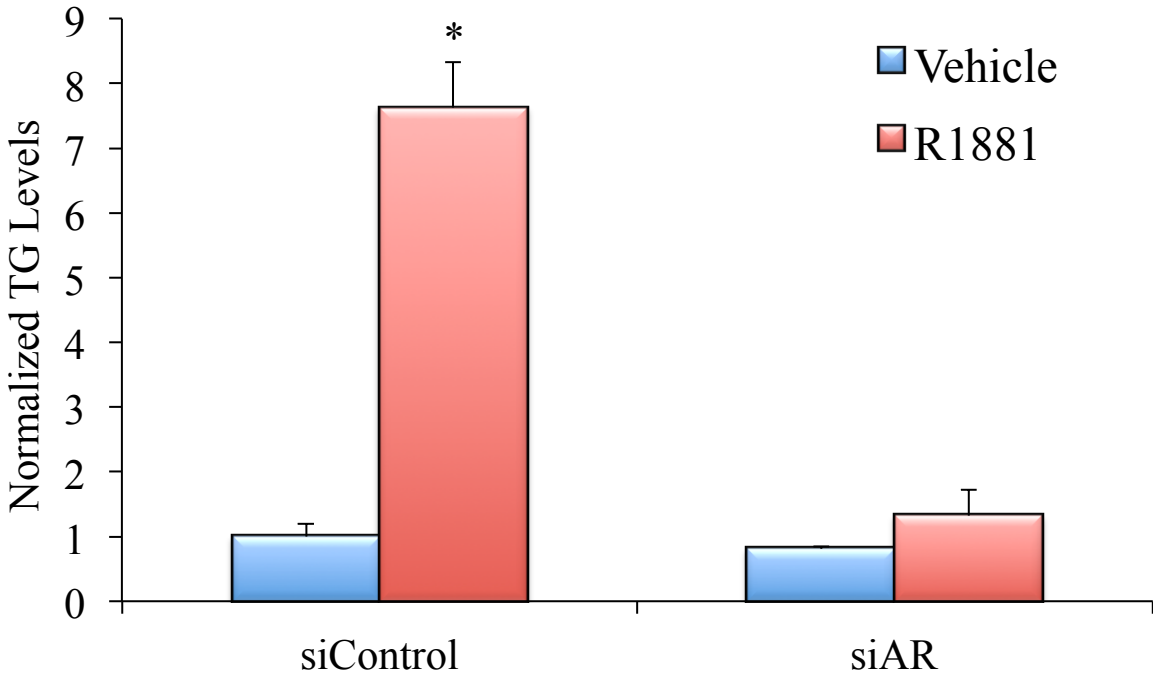

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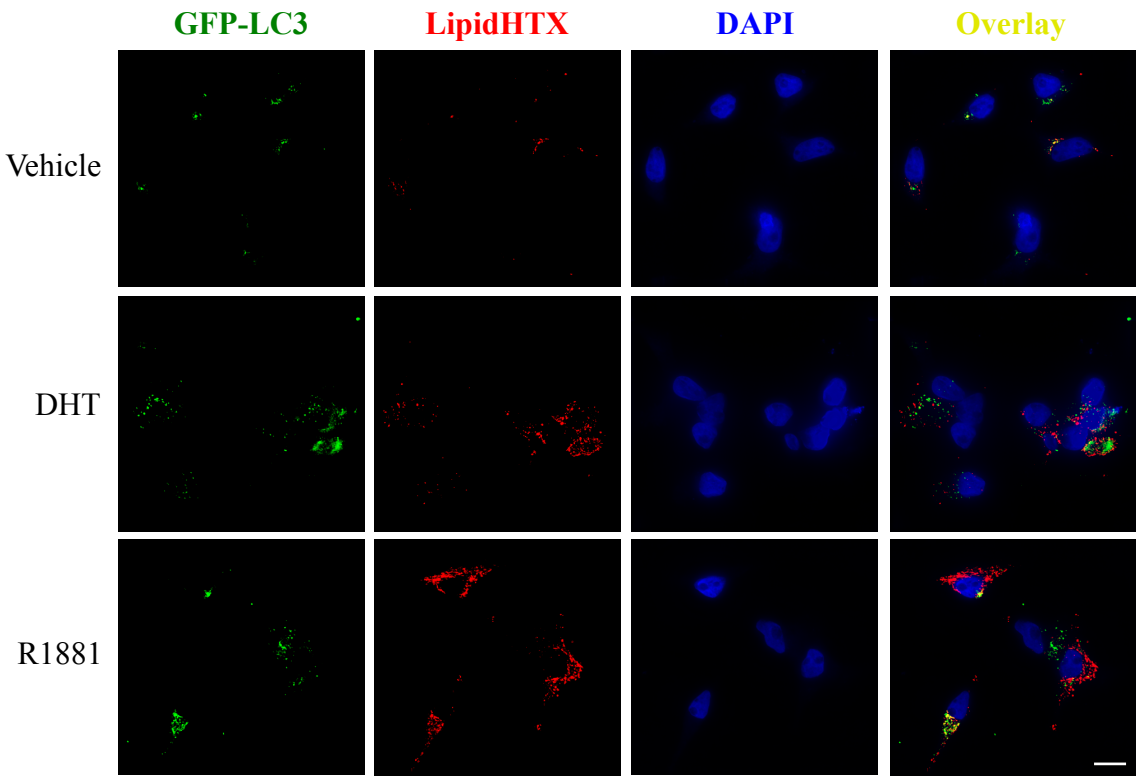
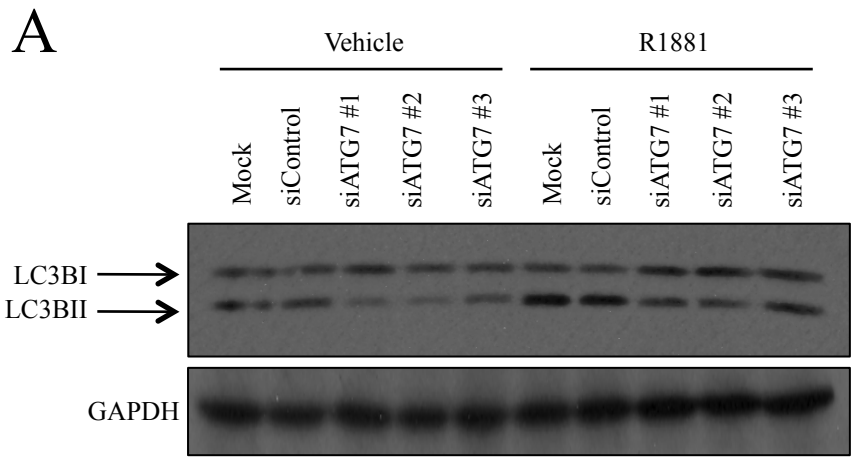
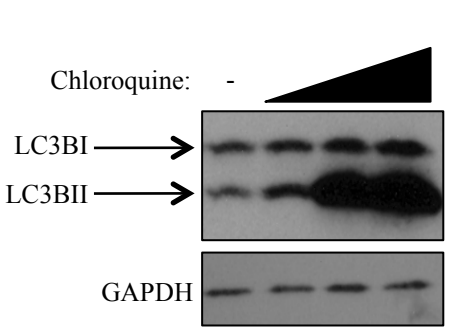
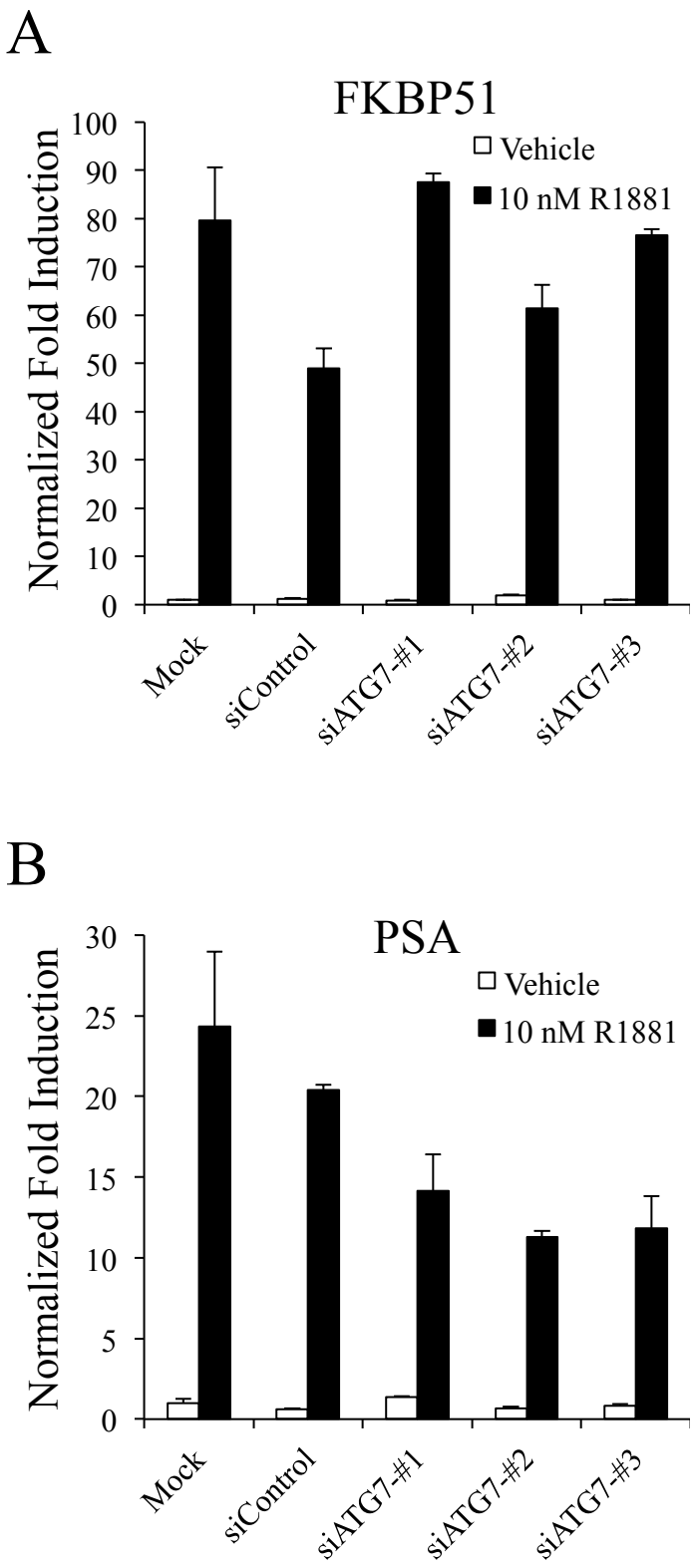


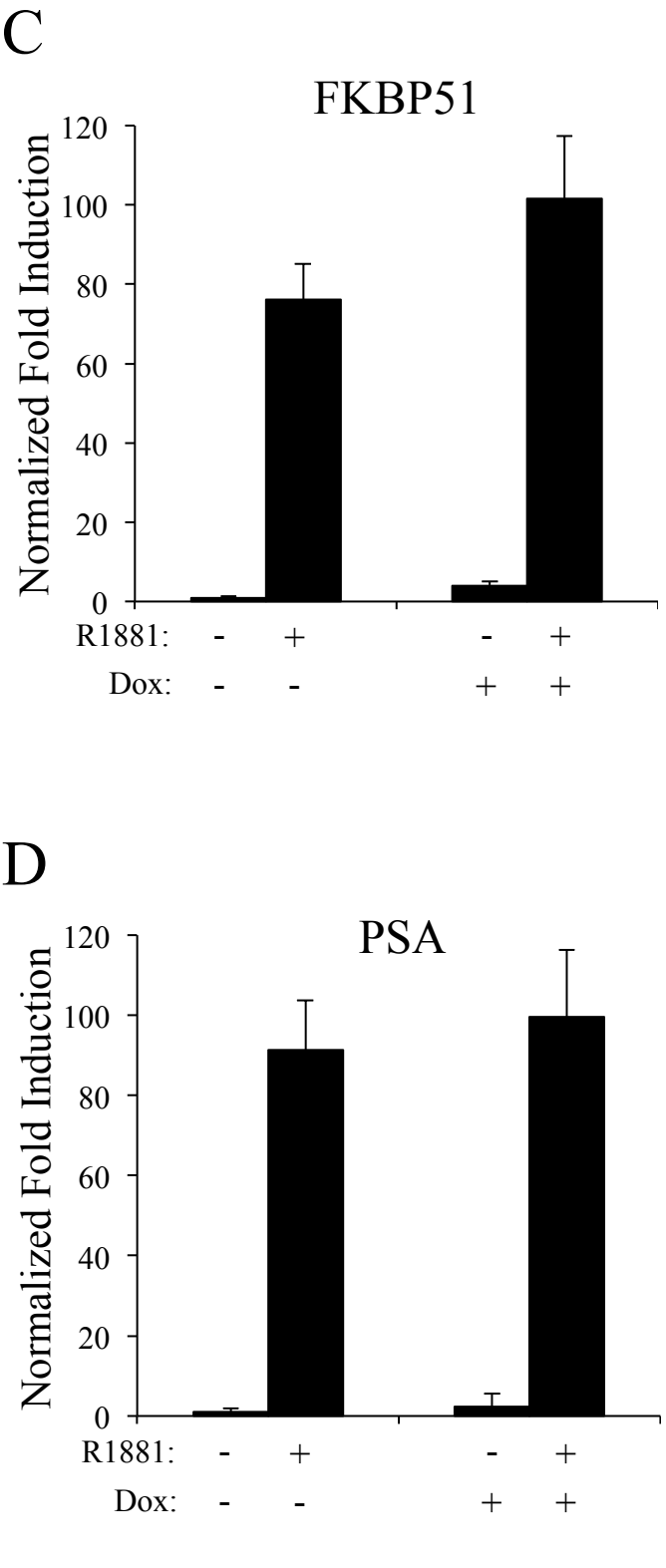
Supplemental Figure 6S



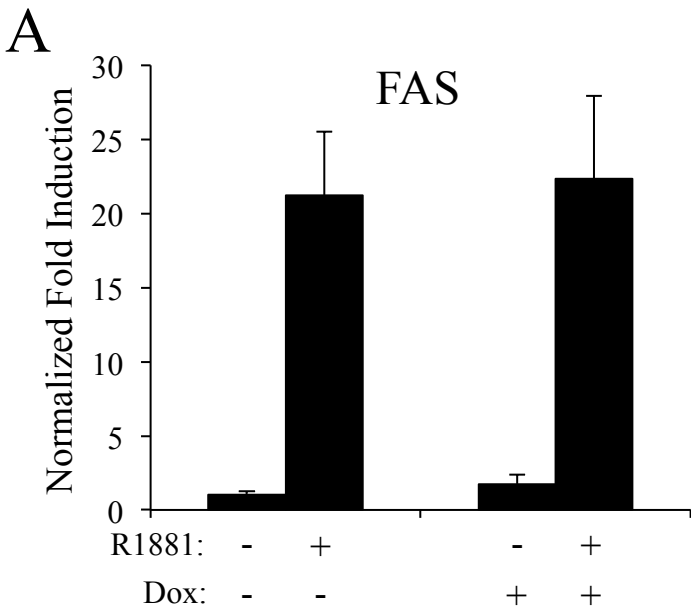


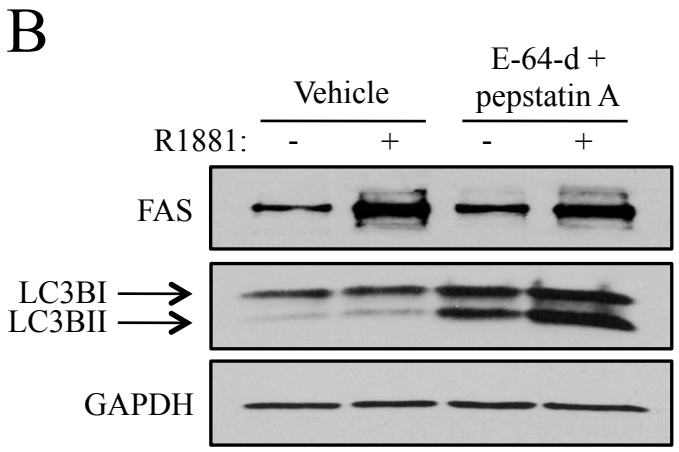
Supplemental Figure 7S

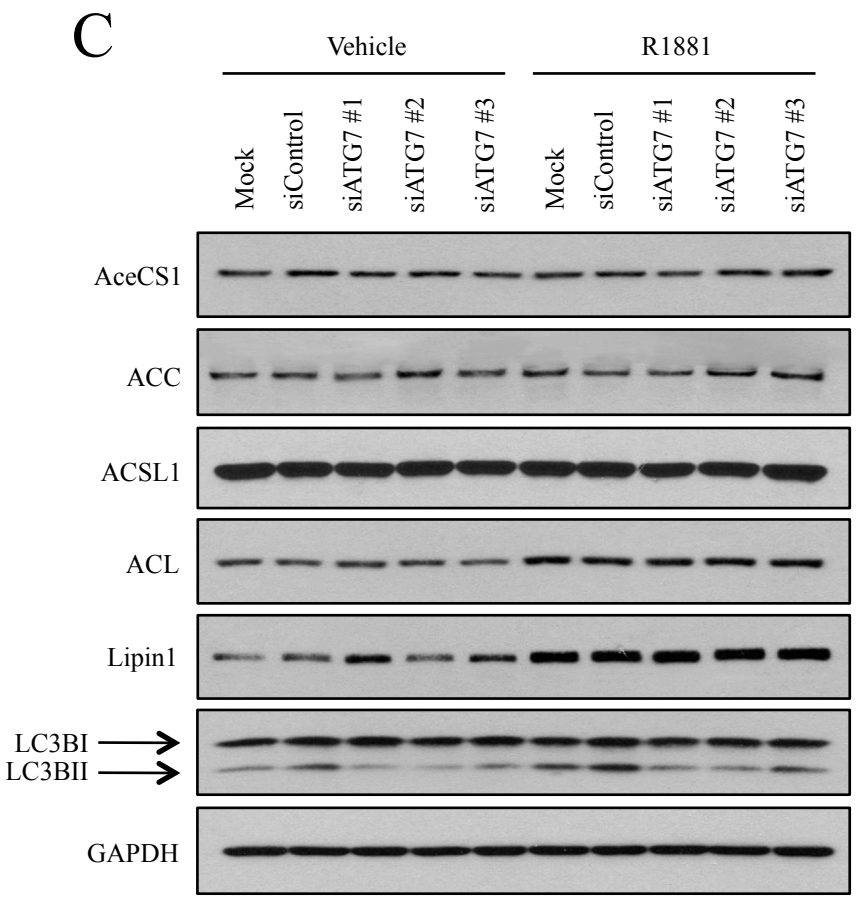




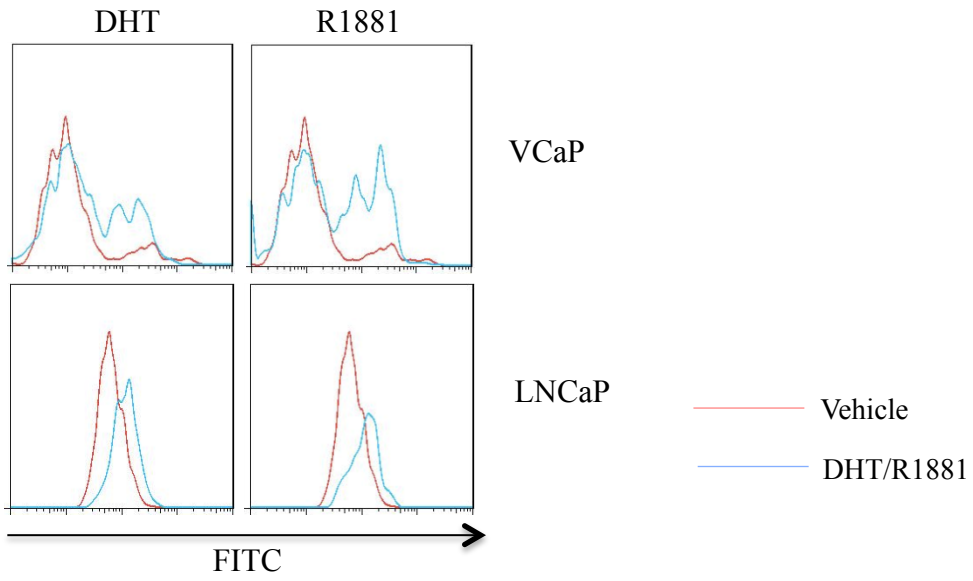
Supplemental Figure 8S



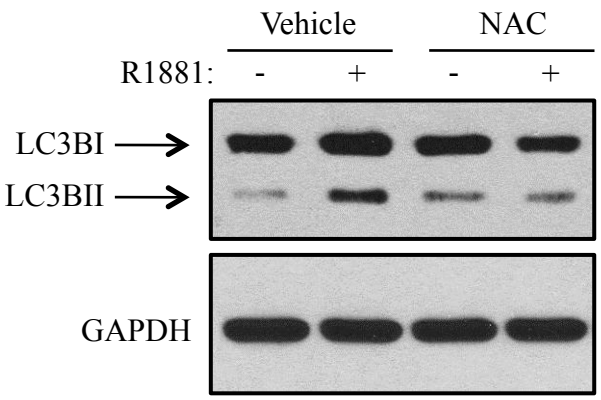




Supplemental Figure 9S



Supplemental Figure 10S



Supplementary Table 1. RNAi sequences used in these studies	
RNAi	***Sequence***
siRNA sequences	
ATG7 #1	5'-GCCGUCAUUGCUGCAAGCAAGAGAA-3'
ATG7 #2	5'-UCUUCGAAGUGAAGCUUCCAGAAAU-3'
ATG7 #3	5'-CCAAUCCUGUGAGGCAGCCUCUCUA-3'
FAS #1	5'-ACUUCCUCUGGGAUGUGCCUUCCAA-3'
FAS #2	5'-CCUCCCAGGACAGCCUGCUAGGUAU-3'
FAS #3	5'-CAGCCGCCGUGGACCUGAUCAUCAA-3'
shRNA sequence	
ATG7	5'-CCAGCATCATCTTCGAAGTGAA-3'