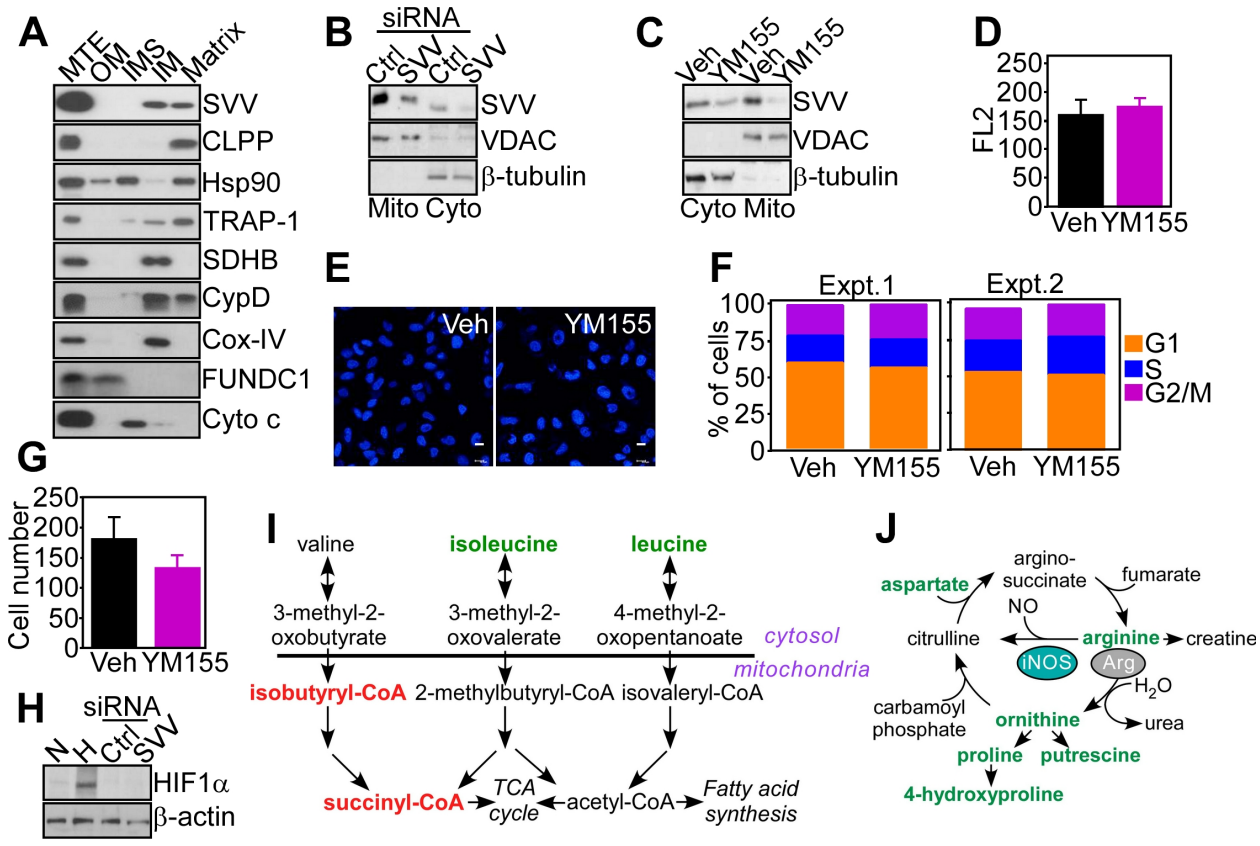


Figure S1. Mitochondrial survivin. (**A**) Mitochondrial extracts (MTE) isolated from PC3 cells were separated in outer membrane (OM), inter-membrane space (IMS), inner membrane (IM) and matrix-containing fractions, and analyzed by Western blotting. Blots are representative of two independent experiments. (**B, C**) PC3 cells were transfected with control non-targeting siRNA (Ctrl) or siRNA directed against survivin (SVV) (**B**), or treated with vehicle (Veh) or the small molecule survivin suppressant YM155 (10 nM) (**C**), and cytosol (Cyto) or mitochondrial (Mito) extracts were analyzed by Western blotting. Blots are representative of two independent experiments for (B) and (C). (**D**) PC3 cells were treated with vehicle (Veh) or 10 nM YM155 and analyzed for mitochondrial membrane potential by TMRE labeling and flow cytometry. Graph shows means±SD from three independent experiments. (**E**) PC3 cells treated as in (**D**) were stained with DAPI and analyzed for nuclear morphology by fluorescence microscopy.

Images are representative of two independent experiments. Scale bar, 10 μm. (**F**) Treated PC3 cells were analyzed for changes in cell cycle transitions by propidium iodide staining and flow cytometry. The percentage of cells in the various cell cycle phases is indicated. Two independent experiments (Expt) are shown. (**G**) Treated PC3 cells were analyzed for cell proliferation after 48 h by direct cell counting. Graph shows means±SD from three independent experiments. (**H**) PC3 cells were maintained under normoxic (N) or hypoxic (H) conditions, transfected with control siRNA (Ctrl) or siRNA directed against survivin (SVV) and analyzed by Western blotting. Two independent experiments. (**I, J**) PC3 cells transfected with control siRNA or survivin-directed siRNA were analyzed after 48 h in a global metabolomics screen (n=5 biological replicates). Changes in metabolite concentrations implicated in branched chain amino acids metabolism (**I**) or arginine metabolism (**J**) are shown. Red, increased concentrations; green, increased concentrations. Only significant changes ($p<0.05$) are shown, except for proline levels that trended downwards without reaching statistical significance.

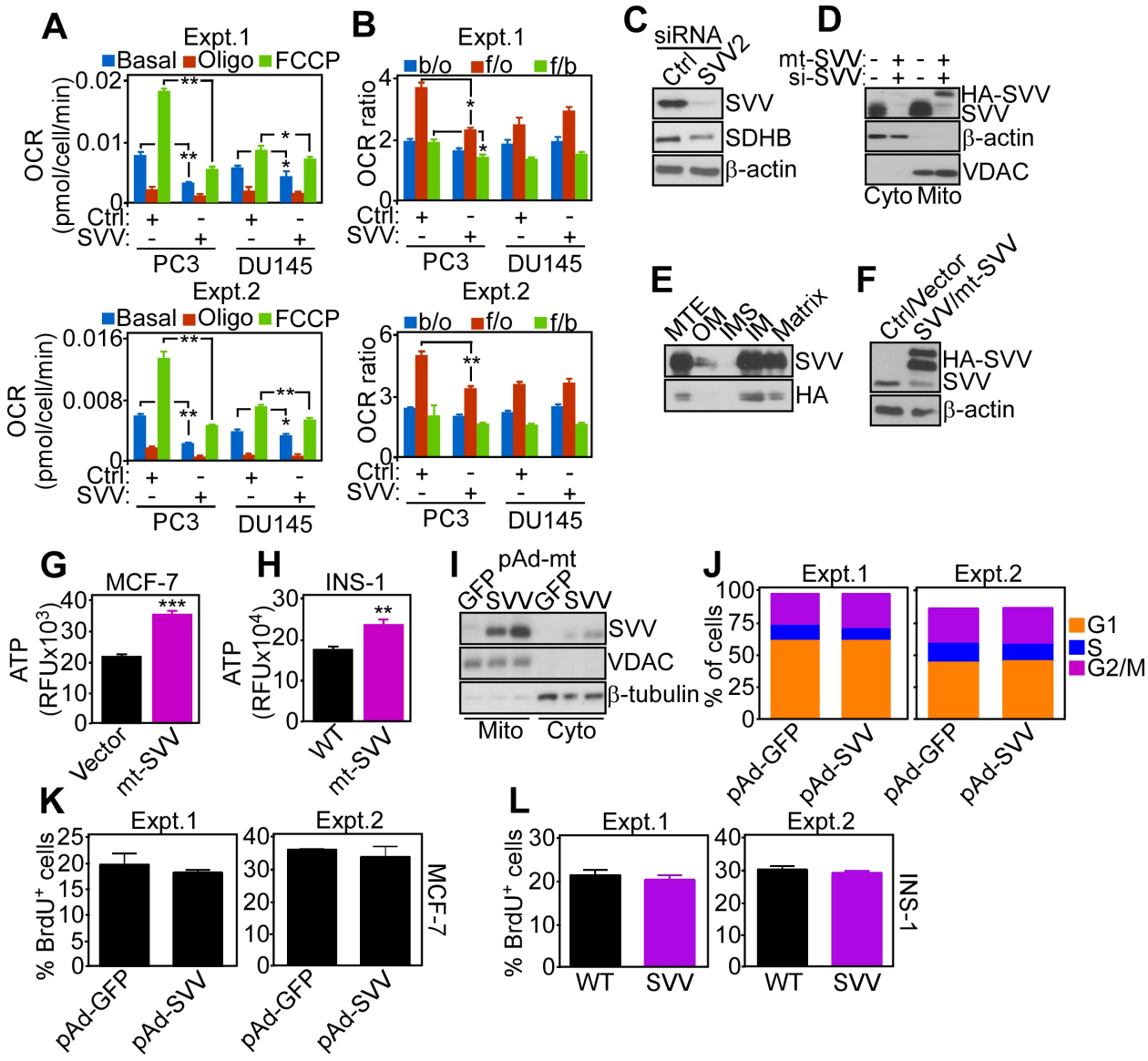


Figure S2. Survivin regulation of mitochondrial bioenergetics. (**A**) PC3 or DU145 cells transfected with control non-targeting siRNA (Ctrl) or survivin (SVV)-directed siRNA were analyzed for oxygen consumption rates (OCR) normalized by the cell number. Extra-mitochondrial respiration after addition of antimycin (2 μM) was subtracted as background. Oligo, oligomycin. Graphs show means±SEM of replicates from two independent experiments (Expt). *, $p<0.05$; **, $p<0.01$. (**B**) siRNA-transfected PC3 or DU145 cells were quantified for OCR ratio between: b/o, basal condition (before any addition) and after oligomycin addition; f/o,

after FCCP and oligomycin addition; f/b, after FCCP addition and basal condition. Mean±SEM. *,p<0.01.Graphs show means±SEM of replicates from two independent experiments (Expt).. (**C**) PC3 cells were transfected with a second, independent siRNA sequence targeting survivin (SVV2) and analyzed by Western blotting. Blots are representative of two independent experiments. (**D**) PC3 cells were transfected with survivin-directed siRNA (si-SVV) with or without mitochondrial-targeted HA-SVV cDNA (mt-SVV) and cytosolic (Cyto) or mitochondrial (Mito) fractions were analyzed by Western blotting. Blots are representative of two independent experiments. (**E**) Submitochondrial fractions isolated from PC3 cells transfected with mt-SVV were analyzed by Western blotting. MTE, mitochondrial extracts, OM, outer membrane; IMS, inter-membrane space; IM, inner membrane. Antibodies to survivin (SVV) or HA were used. Blots are representative of two independent experiments. (**F**) PC3 cells were silenced for endogenous survivin by siRNA, transfected with vector or mt-SVV cDNA, and analyzed by Western blotting. The position of endogenous (SVV) or over-expressed mt-SVV (HA-SVV) is indicated. Blots are representative of two independent experiments. (**G, H**) MCF-7 (**G**) or rat insulinoma INS-1 cells (**H**) were transfected with mt-SVV cDNA, and analyzed for ATP production. RFU, relative fluorescence units. Graphs in (G) and (H) show means±SEM from three independent experiments. **, p=0.007; ***, p<0.0001. (**I**) MCF-7 cells were transduced with adenovirus encoding mitochondrial-targeted GFP (pAd-mt-GFP) or mitochondrial-targeted survivin (pAd-mt-SVV), and cytosolic (Cyto) or mitochondrial (Mito) fractions were analyzed by Western blotting. VDAC is a mitochondrial marker. Blots are representative of two independent experiments. (**J, K**) MCF-7 cells transduced with the indicated mitochondrial-targeted pAd constructs were analyzed for cell cycle progression by propidium iodide (PI) staining (**J**) or BrdU incorporation and flow cytometry (**K**)). The percentage of cells

in each cell cycle phase (J) or BrdU-positive cells (K) is indicated. Two independent experiments (Expt.) are shown. (**L**), Wild type INS-1 cells or INS-1 cells expressing mitochondrial survivin (SVV) were analyzed by BrdU incorporation and flow cytometry. The percentage of BrdU-positive cells is indicated. Two independent experiments (Expt.) are shown..

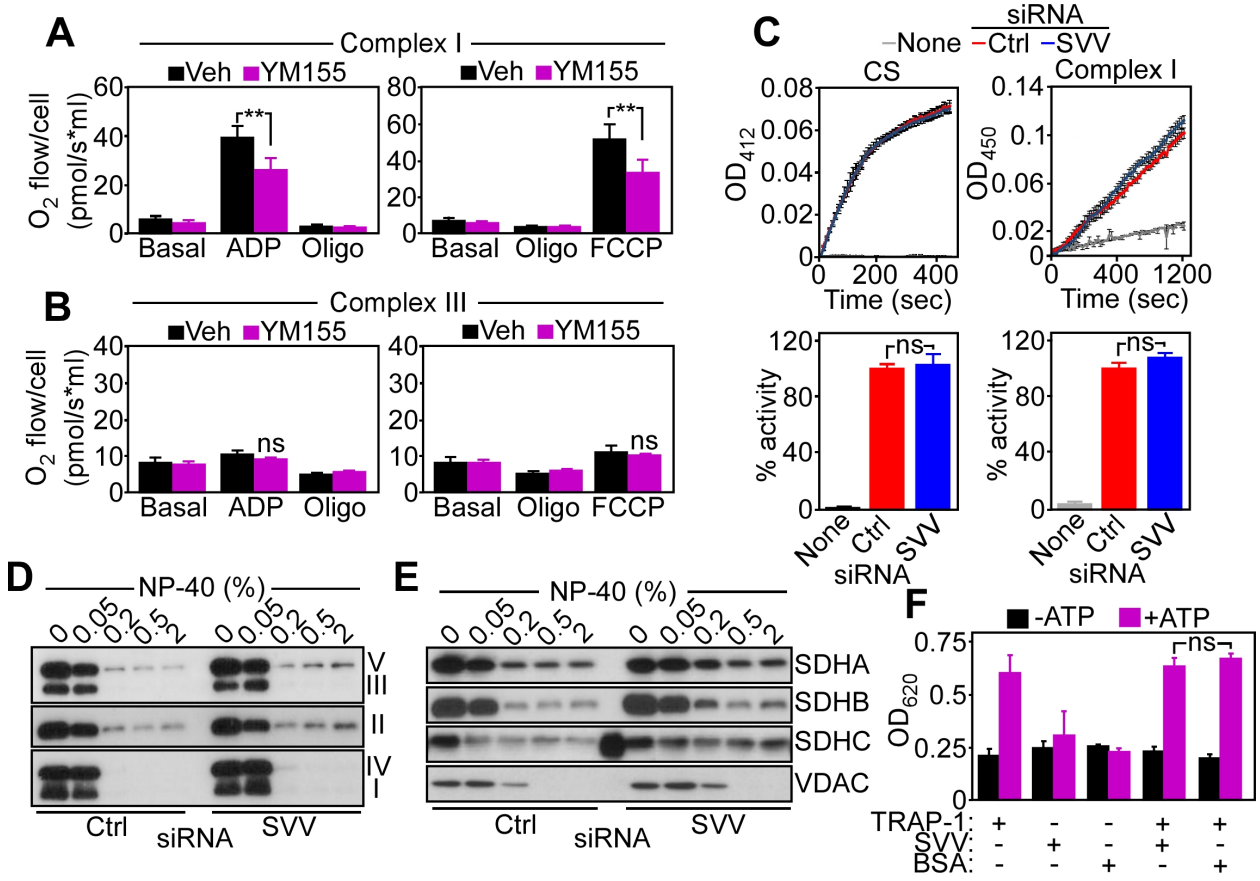


Figure S3. Modulation of oxidative phosphorylation by survivin. (**A, B**) PC3 cells were treated with vehicle (Veh) or 10 nM YM155, permeabilized with digitonin and analyzed for Complex I activity in the presence of 10 mM glutamate and 2 mM malate (**A**) or Complex III activity in the presence of 5 mM malonic acid and 10 mM glycerol phosphate (**B**) using a 2-chamber high-resolution respirometer. Graph shows means±SEM from three independent experiments. **, p<0.01; ns, not significant. (**C**) PC3 cells were transfected with control non-targeting siRNA (Ctrl) or survivin-directed (SVV) siRNA, and analyzed for citrate synthase (CS) or Complex I activity. *Bottom*, quantification of individual activity. Graph shows means±SEM from three independent experiments. ns, not significant, p=0.78-0.86. (**D, E**) PC3 cells were transfected with control non-targeting siRNA (Ctrl) or survivin-directed (SVV) siRNA and

detergent (NP-40)-insoluble material was analyzed for changes in expression of oxidative phosphorylation subunits (**D**) or Complex II subunits (**E**) by Western blotting. The extra band in the SDHC blot in (**E**) corresponds to non-specific reactivity with a molecular weight marker. Blots are representative of two independent experiments for (D) and (E). (**F**) Recombinant TRAP-1 (1 μM) was incubated with recombinant survivin (SVV) (1 μM) or BSA (2 μM) in the presence or absence of ATP (100 μM) for 16 h, and ATP hydrolysis was quantified by absorbance at OD_{620}. Graph shows means±SD from three experiments. ns, not significant, p=0.12.

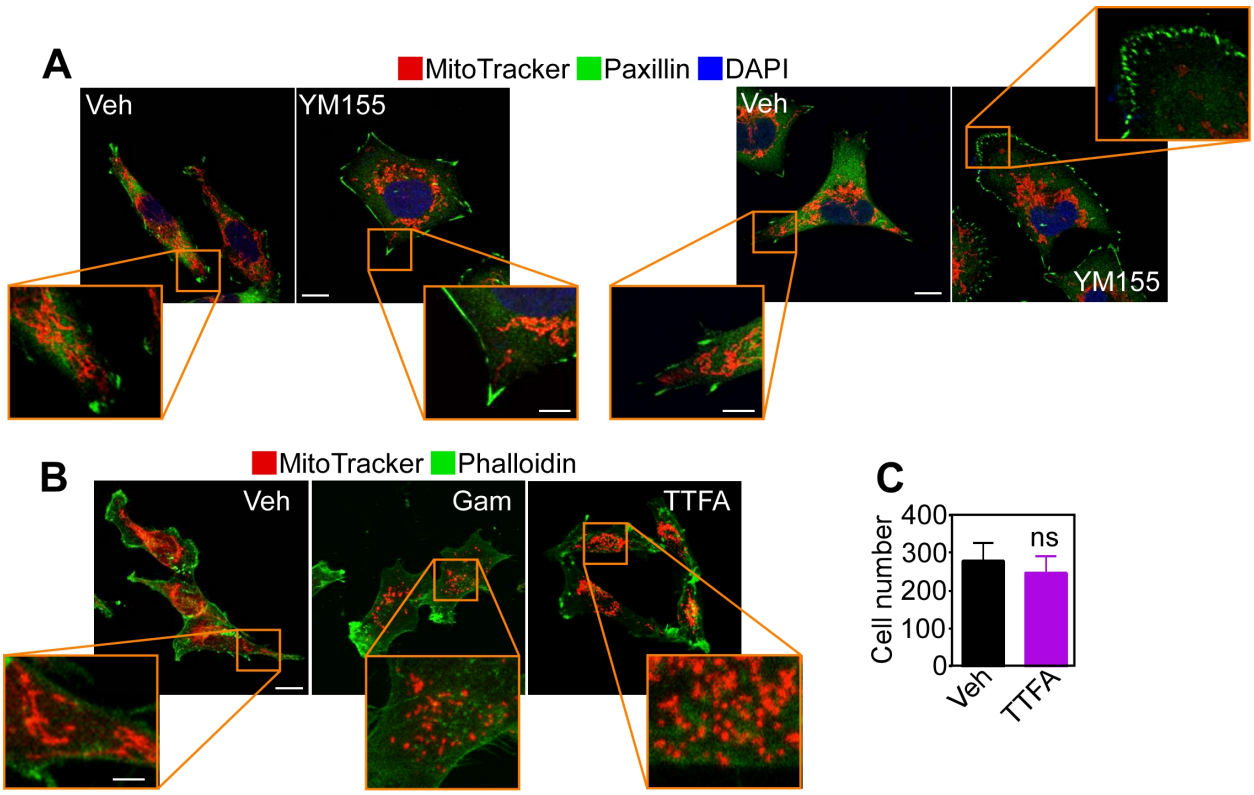
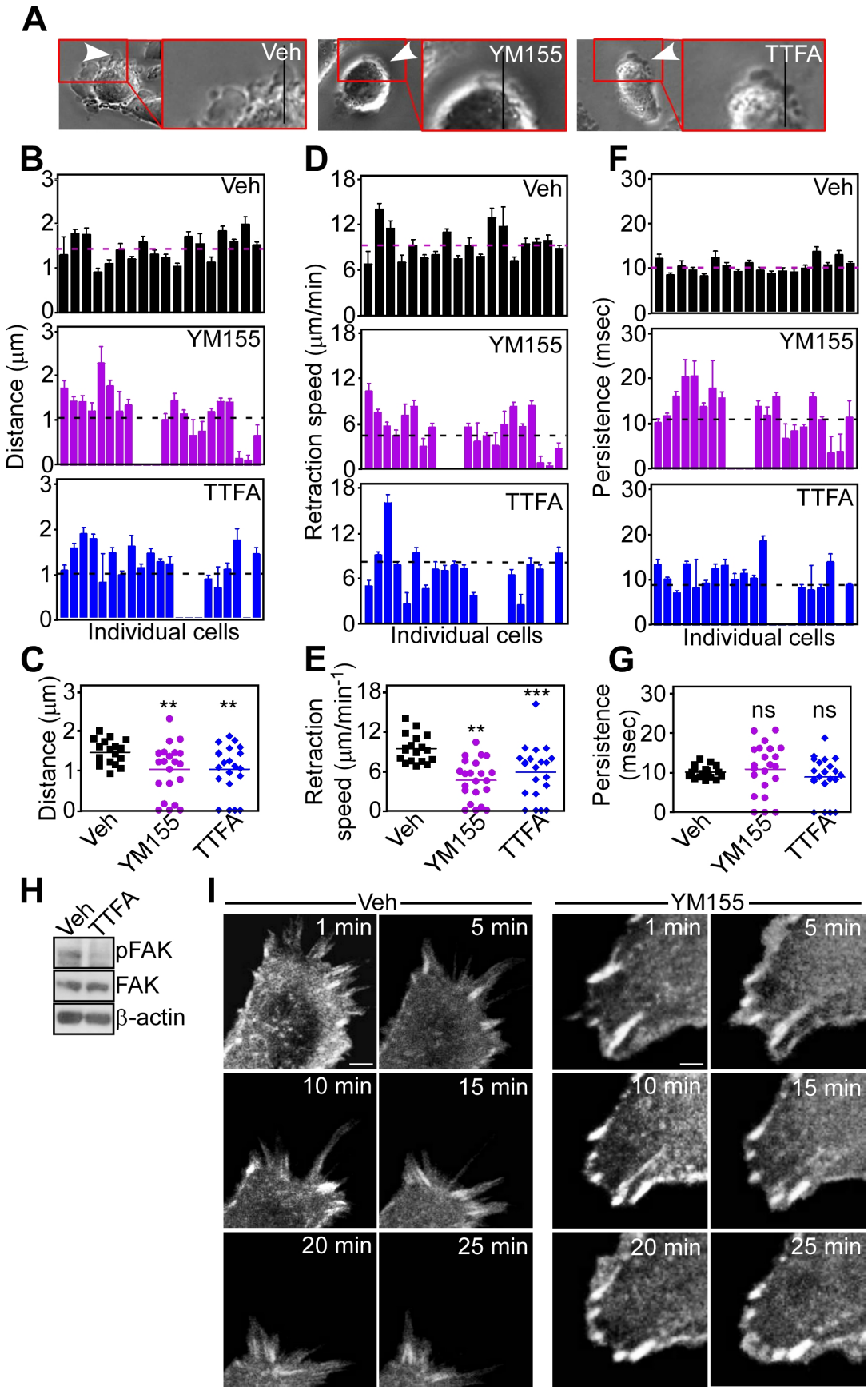


Figure S4. Survivin regulation of mitochondrial trafficking. (**A**) Treated PC3 cells were labeled for mitochondria (MitoTracker, red), focal adhesion (FA) complexes (Paxillin, green) and nuclei (DAPI, blue), and analyzed by confocal fluorescence microscopy. Two independent fields per condition are shown. Imaged in two independent experiments. Scale bar, 10 μm; insets, 5 μm. (**B**) PC3 cells were treated with vehicle (Veh), CCCP (12.5 μM), Gamitrinib (Gam, 5 μM) or TTFA (200 μM) for 16 h, stained for mitochondria (MitoTracker, red) and actin (phalloidin, green), and analyzed by fluorescence microscopy. 45 cells/treatment condition imaged in two independent experiments. Scale bar, 10 μm; insets, 5 μm. (**C**) PC3 cells treated with vehicle (Veh) or TTFA (200 μM) were analyzed for cell proliferation by direct cell counting. Data are the mean±SEM of three independent experiments.


A
Veh
YM155
TTFA
B
Distance (μm)
Individual cells
D
Retraction speed (μm/min)
Individual cells
F
Persistence (msec)
Individual cells
C
Distance (μm)
**
**
Veh
YM155
TTFA
E
Retraction speed (μm/min-1)
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G
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H
Veh
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pFAK
FAK
β-actin
I
Veh
YM155
1 min
5 min
10 min
15 min
20 min
25 min

Figure S5. Regulation of membrane dynamics by survivin. (**A**) Time-lapse videomicroscopy of PC3 cells treated with vehicle (veh), YM155 (10 nM) or TTFA (200 μM) at time T_0. Arrow, region analyzed. Inset, one SACED line from which stroboscopic images were digitalized is shown (black line=16 μm). (**B-G**) PC3 cells were treated with vehicle (Veh), YM155 (10 nM) or TTFA (200 μM) and analyzed for membrane ruffling dynamics by real-time stroboscopic microscopy. Quantification of ruffle distance traveled (**B, C**), ruffle retraction speed (**D, E**), and time of ruffle persistence (**F, G**) is shown. For panels (**B, D, F**), each bar corresponds to an individual cell. Broken lines, average values. For panels (**C, E, G**) the individual values are as follows: ruffle distance (**C**), Veh, 1.45±0.07 (n=18); YM155, 1.026±0.13 (n=22), p=0.009; TTFA, 1.034±0.13 (n=21), p=0.008; ruffle retraction speed (**E**), Veh, 9.27±0.49 (n=18); YM155, 4.57±0.65 (n=22), p<0.0001; TTFA, 5.78±0.87 (n=21), p=0.0015; time of ruffle persistence (**G**), Veh, 10.1±0.37 (n=18); YM155, 10.9±1.35 (n=22), p=0.61; TTFA, 8.92±1.13 (n=21), p=0.34. The indicated number of cells as above for each treatment condition were imaged in two independent experiments. (**H**) PC3 cells treated with vehicle (Veh) or TTFA (200 μM) were analyzed by Western blotting. p, phosphorylated. Two independent experiments. (**I**) PC3 cells were transfected with GFP-α-actinin 1, treated with vehicle (Veh) (*left*) or 10 nM YM155 (*right*), and analyzed for FA complex dynamics by time-lapse videomicroscopy. Representative images at the indicated time intervals are shown per each condition tested. Cells were imaged in two independent experiments.

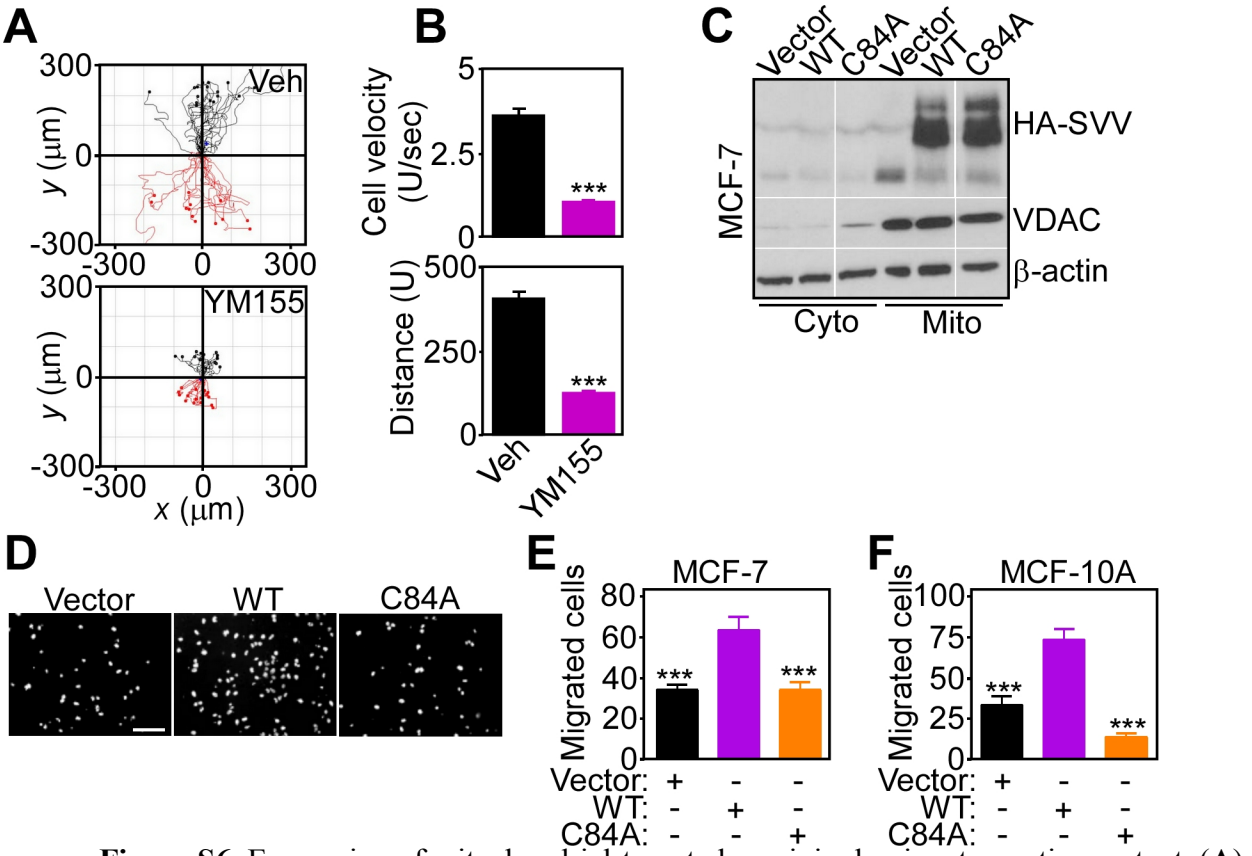

Figure S6. Expression of mitochondrial-targeted survivin dominant-negative mutant. (**A**) PC3 cells were treated with vehicle (Veh) or 10 nM YM155 and analyzed by time-lapse videomicroscopy in a wound closure assay for 24 h. Each tracing corresponds to an individual cell. (**B**) Migration velocity (top) or total distance traveled (bottom) of the cells in (A) was measured. 30 cells/treatment condition imaged in two independent experiments. (**C**) MCF-7 cells were transfected with vector, wild type (WT) mt-SVV or mitochondrial-targeted survivin Cys84Ala (C84A) dominant negative mutant, and isolated cytosol (Cyto) or mitochondrial (Mito) extracts were analyzed by Western blotting. TCE, total cell extracts. Blots are representative of two independent experiments. (**D**) MCF-7 cells transfected as in (**C**) were analyzed for Matrigel invasion. Images are representative of two independent experiments. Scale bar, 200 μm.(**E, F**) MCF-7 (**E**) or MCF-10A (**F**) cells transfected as in (**C**) were quantified for

migration across Transwell membranes under the various conditions tested. Data are presented as mean±SEM of three independent experiments. ***, p=0.0001-0.0005..

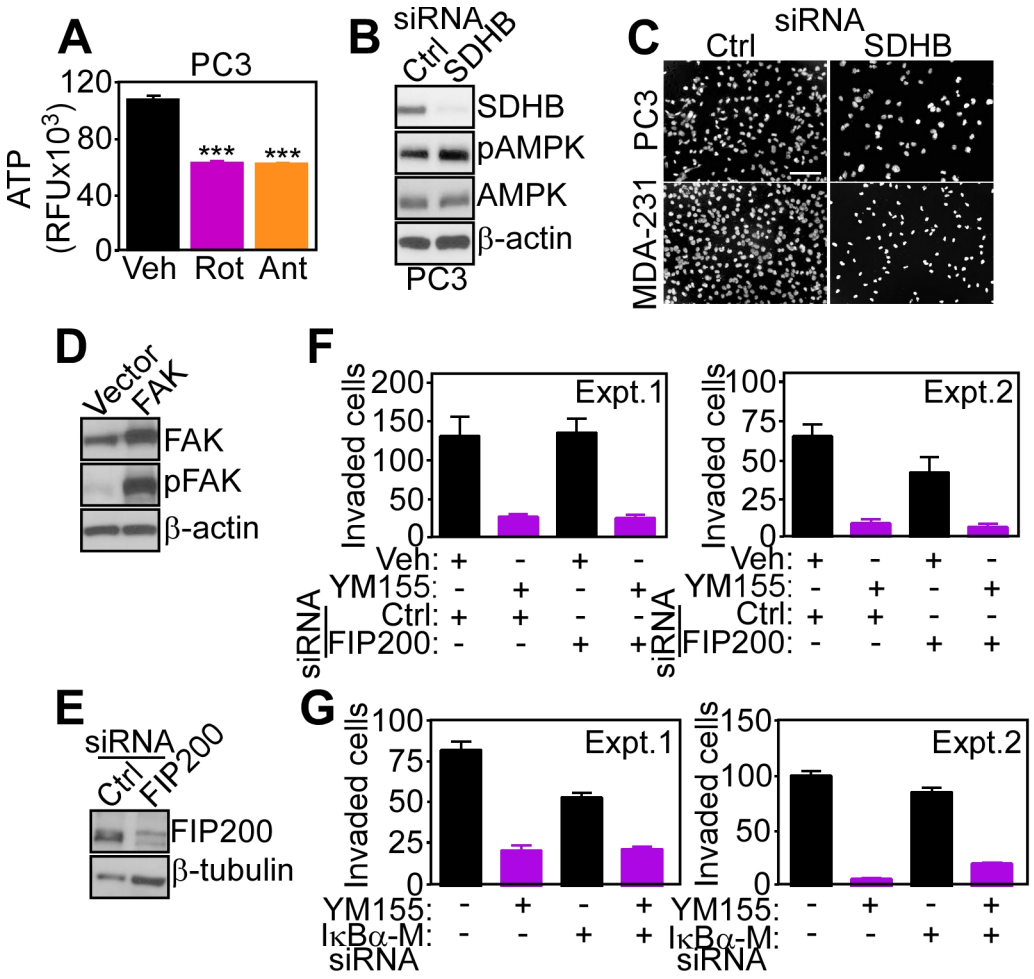

Figure S7. Mitochondrial survivin-complex II regulation of tumor cell motility. (**A**) PC3 cells treated with vehicle (Veh), 4 μM Rotenone (Rot; Complex I inhibitor) or 30 μM Antimycin A (Ant; Complex III inhibitor) for 16 h were analyzed for changes in ATP production. Graph shows means±SEM from three independent experiments. ***, $p<0.0001$. (**B**) PC3 cells were transfected with control (Ctrl) or siRNA directed against SDHB and analyzed by Western blotting. Blots are representative of two independent experiments. (**C**) PC3 or MDA-MB231 cells were transfected as in (**B**) and analyzed for Matrigel invasion. Images are representative of two independent experiments. Scale bar, 200 μm. (**D**) PC3 cells were transfected with vector or FAK cDNA and analyzed by Western blotting. p, phosphorylated. Blots are representative of two

independent experiments. (**E, F**) PC3 cells were transfected with control non-targeting siRNA (Ctrl) or FIP200-directed siRNA and analyzed by Western blotting (**E**) or Matrigel invasion in the presence of vehicle (Veh) or 10 nM YM155 (**F**). Mean±SEM. ***, $p<0.0001$. Blots in (E) are representative of two independent experiments. Two independent experiments (Expt.) are shown for the data in (**F**). (**G**) PC3 cells transfected with vector or IκBα super-repressor mutant cDNA were treated with vehicle (Veh) or 10 nM YM155 and analyzed for Matrigel invasion. Mean±SEM. ns, not significant. Two independent experiments (Expt) are shown.