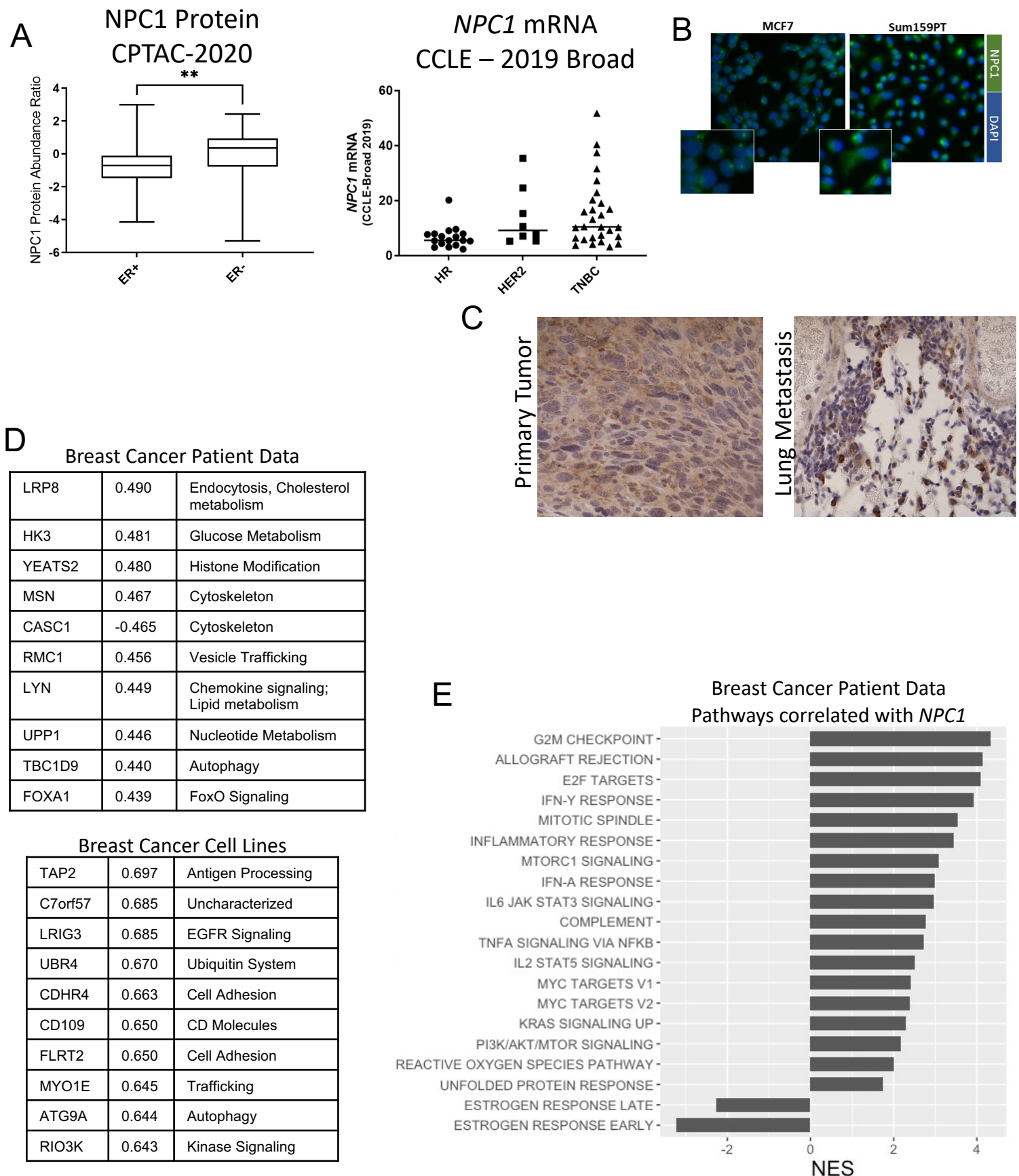


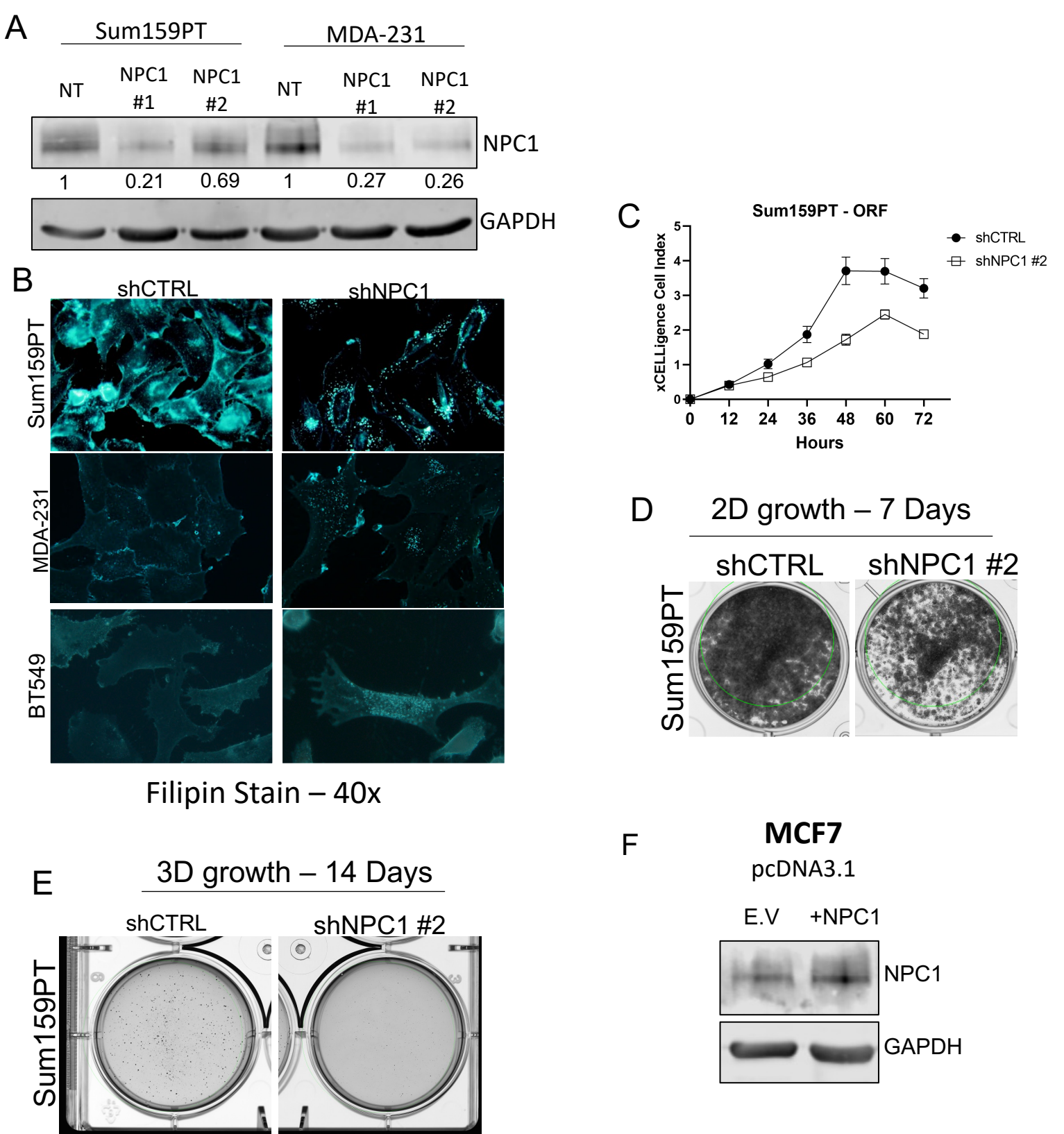
Supplemental Figure S1

A) Metabolic Pathways enriched in previously published gene array (miR-200c +/- for 72hr in BT549 cells. GSEA analysis using Hallmarks gene set. **B)** GSEA maps for Cholesterol Homeostasis and Adipogenesis genes. **C)** Top hits within cholesterol homeostasis/adipogenesis gene sets (Fold change miR-200c/SCR, P-value student's T Test) **D)** quantification (imageJ) of western blot from figure 1F. **E)** qPCR of *LDLR* expression in TNBC cell lines, 96 hours post miR-200c transfection. **F)** Western of *LDLR* protein expression, 96 hours post miR-200c transfection.

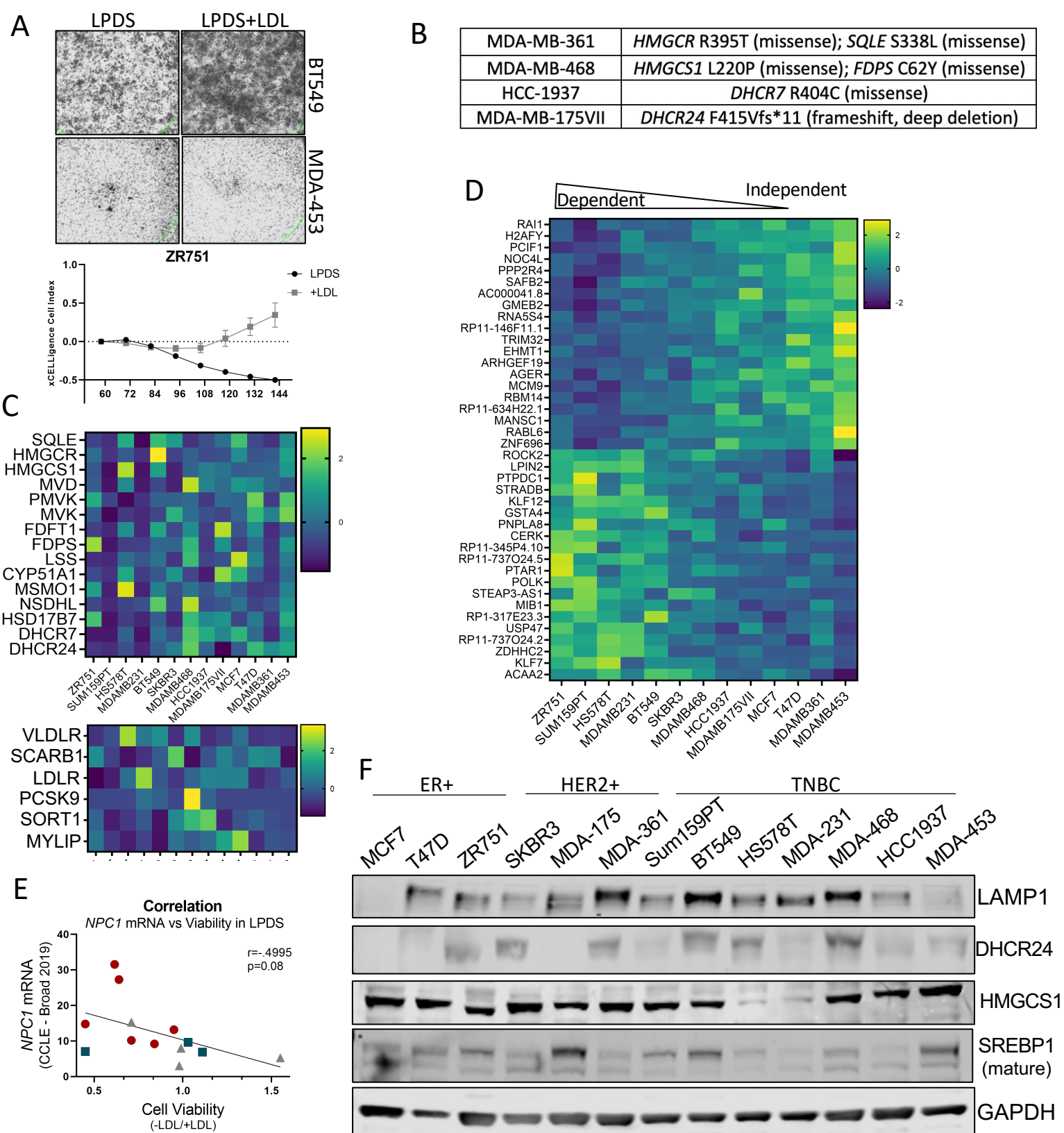


Supplemental Figure S2

A) NPC1 in publicly available datasets: Protein abundance in Breast Cancer-Patient Primary Tumors (Right) and Cancer Cell Line Encyclopedia – Breast Cancer mRNA expression of *NPC1* (Left), accessed through cbiportal. **B)** Immunocytochemistry of NPC1 (green) and DAPI (blue) in MCF7 (ER+) and Sum159PT (TNBC) cells, 10x. **C)** IHC of NPC1 in MMTV-driven mammary tumor (top) and lung metastasis (bottom). **D)** Genes most highly correlated with *NPC1* in 2016 METABRIC patient dataset (left) and CCLE-Breast Cancer cell line database (right). **E)** Pathway analysis of *NPC1* correlation with genome-wide data from 2016 METABRIC patient breast cancers. Analyzed with GSEA – Pre-ranked, Hallmarks gene sets.

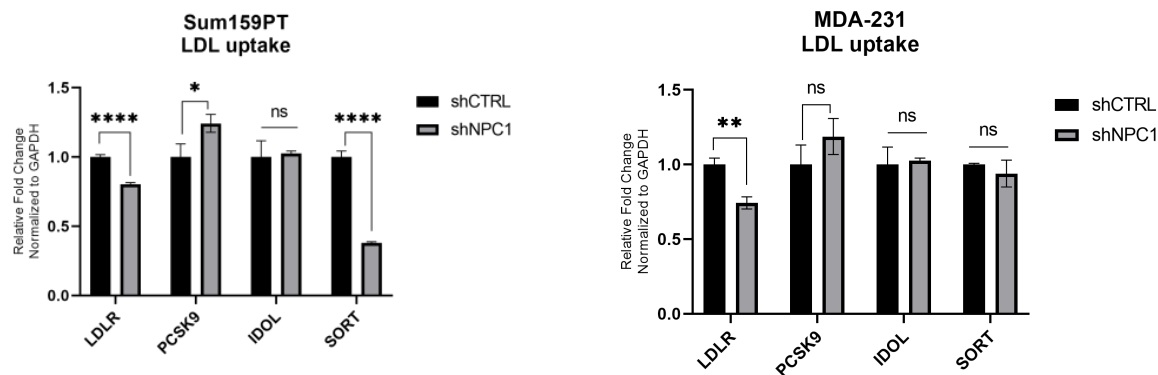


Supplemental Figure S3. A) Western blot confirming NPC1 knockdown by two unique shRNAs. **B)** Filipin (neutral cholesterol staining) of stable shNPC1 knockdown versus empty vector cell lines. **C)** xCELLigence growth assay of Sum159PT “shRNA #2”, shNPC/empty vector over 72 hours. **D)** Representative images of confluency of Sum159PT “shRNA #2” cells after 7 days in 2D culture. 12 well dish with initial seeding density of 3,000 cells/well. **E)** Representative images of colony formation of Sum159PT “shRNA #2” in soft agar (0.5% bottom layer, 0.3% top layer) over 14 days. **F)** Confirmation of NPC1 overexpression in MCF7 cells used for soft agar and invasion assay, figure 2F-2G.

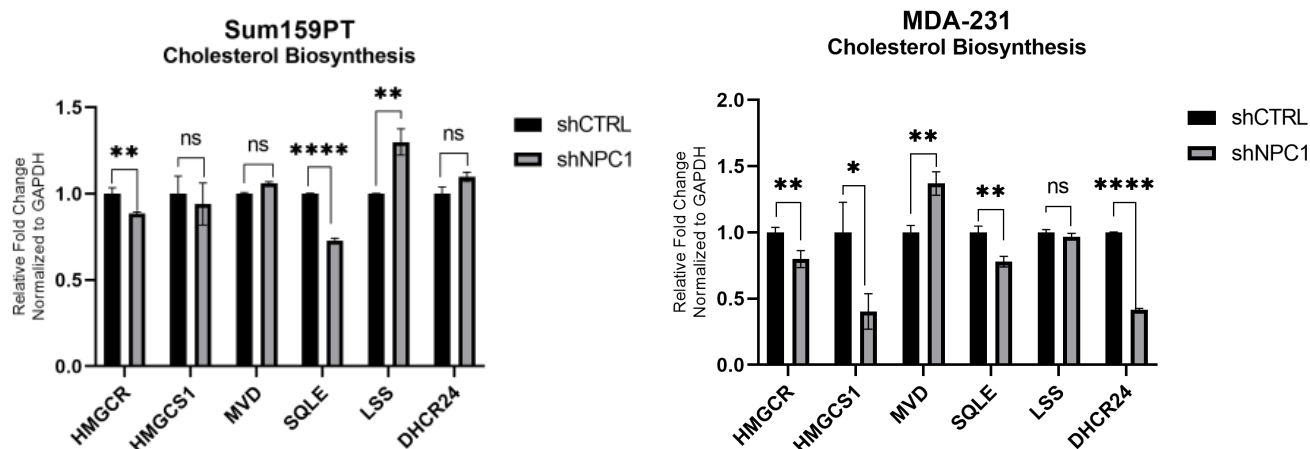


Supplemental Figure S4. A) Top: growth in LPDS \pm LDL of MDA-453 (cholesterol independent) and BT549 (cholesterol auxotroph). Bottom: Growth over time in ZR75-1 (cholesterol auxotroph) days 2-7, when seeded at equal cell number following 48 hour lipoprotein starvation. **B)** Mutations in cholesterol biosynthesis genes, data obtained from CCLE accessed via cbiportal. **C)** Cholesterol biosynthesis (top) and uptake (bottom) genes in panel of breast cancer cell lines, CCLE via cbiportal. **D)** Top genes correlated with cholesterol auxotrophy, CCLE accessed via cbiportal. **E)** Correlation of *NPC1* mRNA versus cholesterol auxotrophy; mRNA expression obtained from CCLE/cbiportal and compared to viability from figure 3A. **F)** Western of key cholesterol metabolism enzymes in breast cancer cell line panel.

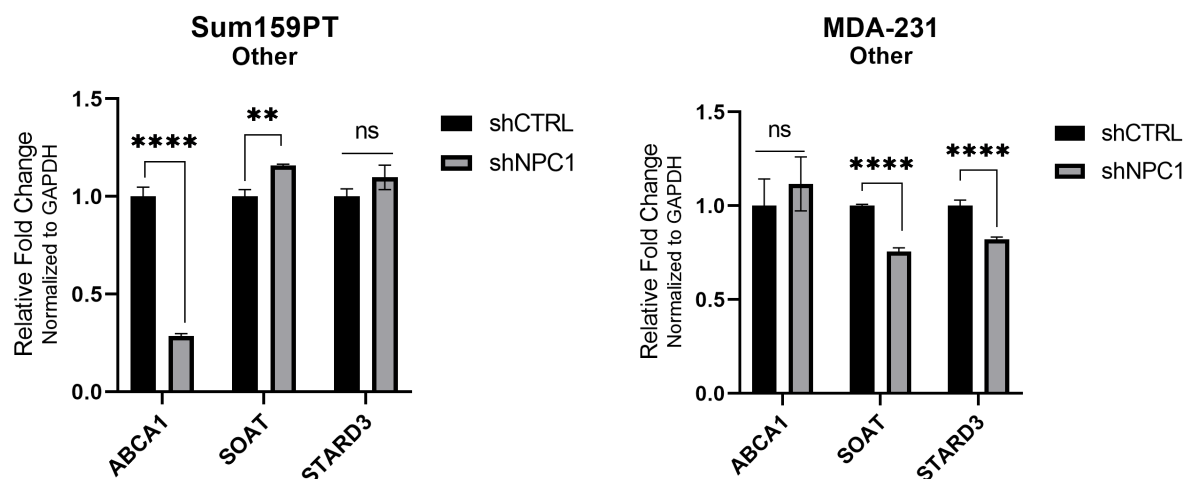
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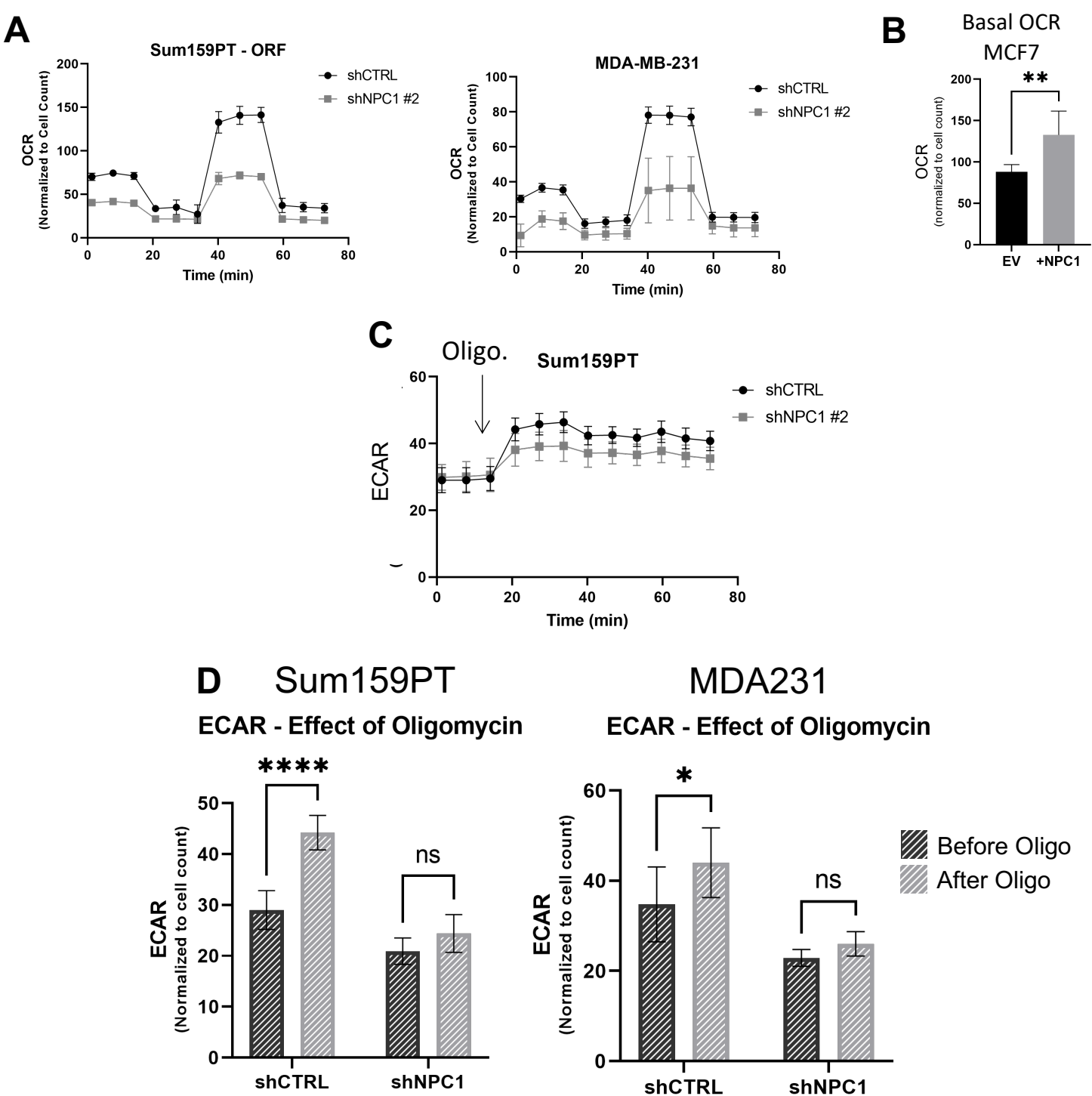
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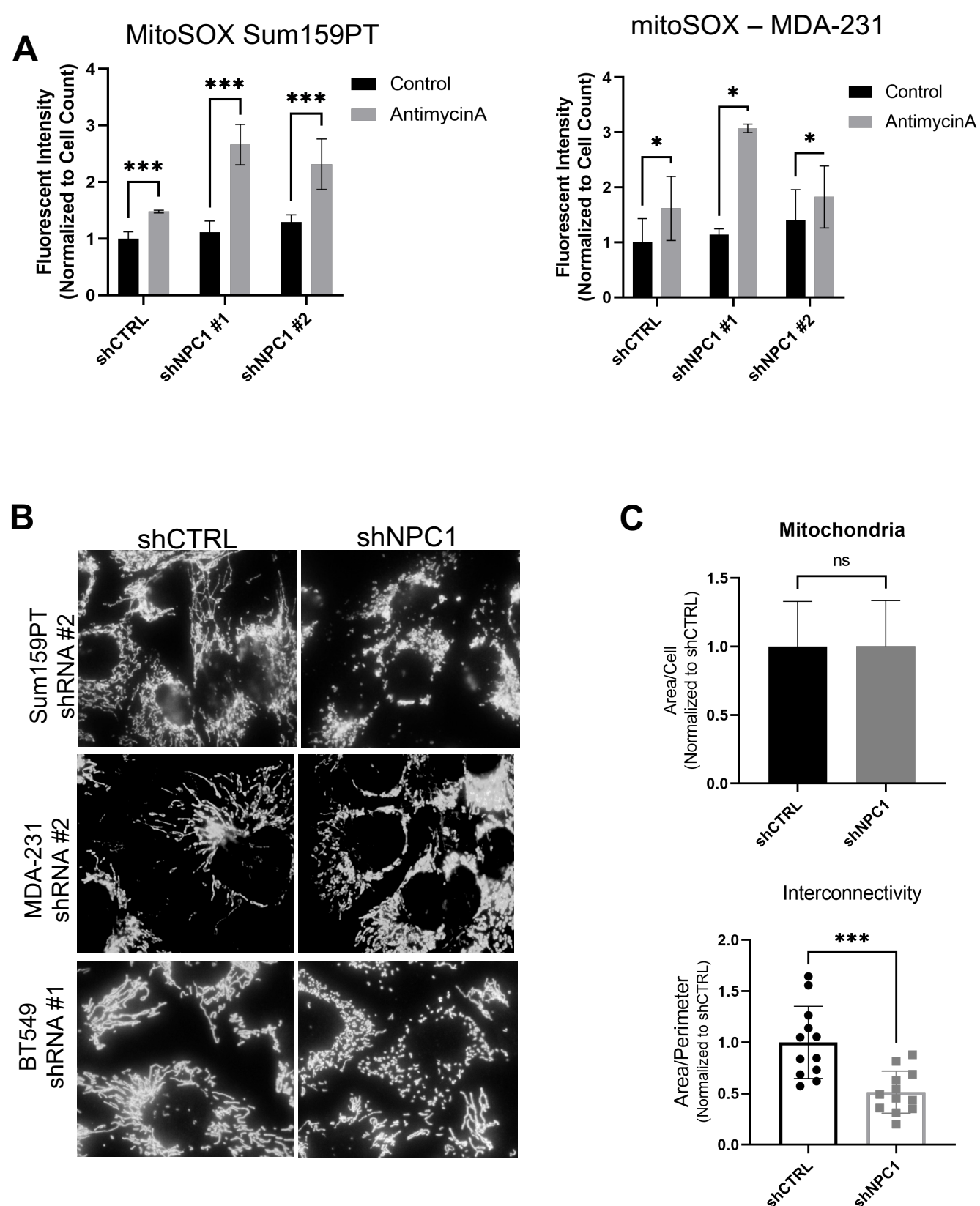
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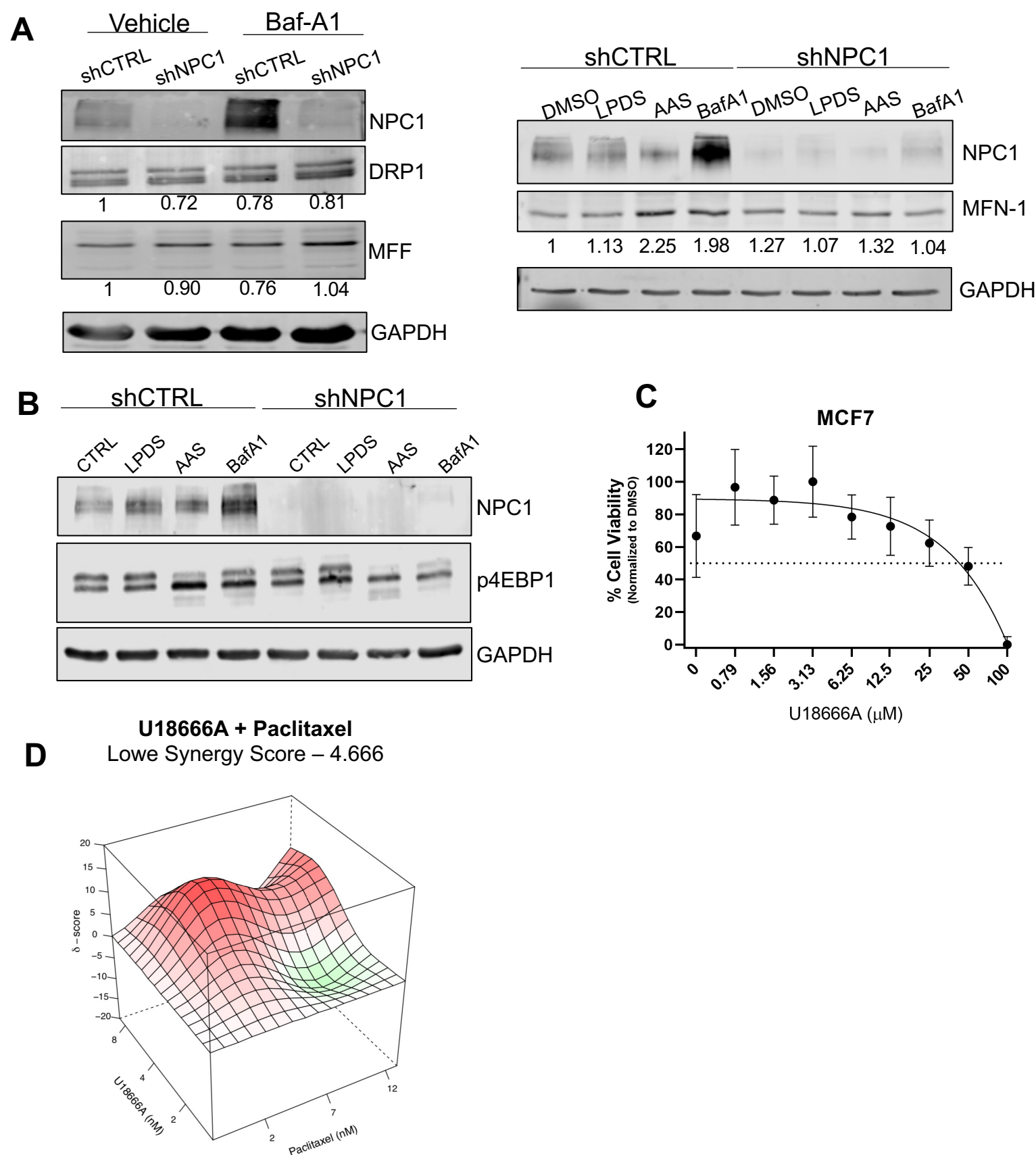
Supplemental Figure S5. qPCR for shRNA#1 cell lines, **A)** genes reportedly involved in cholesterol uptake and LDLR regulation. **B)** Cholesterol biosynthesis genes. **C)** misc. cholesterol-related genes, *ABCA1* (cholesterol efflux), *SOAT1* (cholesterol esterification), and *STARD3* (lysosome-to-mitochondrial cholesterol transporter).



Supplemental Figure S6. **A)** Seahorse mitochondrial stress test in shRNA#2 cell lines. **B)** Quantification of basal respiration (OCR) in MCF7 cells. **C)** ECAR changes in Sum159PT cells, with arrow indicating addition of Oligomycin. **D)** ECAR before and after addition of Oligomycin in Sum159PT and MDA-231 cells, demonstrating glycolytic capacity.



Supplemental Figure S7. **A)** Effect of Antimycin A (used as a positive control) on mitoSOX staining in shCTRL vs. shNPC1 cell lines. **B)** Representative images of mitochondria morphology using additional cell lines/shRNA constructs. **C)** Mitochondrial number (top) and mitochondrial interconnectivity (bottom), based on quantification of confocal microscopy by imagej.



Supplemental Figure S8. A) Western blot of various fission/fusion proteins in NPC1^{+/−} cells under various metabolic and lysosome stressors (24 hour treatment. BafA1 5nM). **B)** Western blot of p4EBP1 branch of mTOR signaling under metabolic and lysosome stress. **C)** Dose Response curve of U18666A in MCF7 cells, viability relative to DMSO control over 48 hours, analyzed with Crystal Violet. **D)** Low synergy score of U18666A (μ M) and Paclitaxel (nM) in BT549 at 48 hours. Calculated using SynergyFinder 2.0.