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| Authors | Lyons, Amy;Coleman, Michael;Riis, Sarah;Favre, Cedric;O'Flanagan, Ciara;Zhdanov, Alexander V.;Papkovsky, Dmitri B.;Hursting, Stephen D.;O'Connor, Rosemary |
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**Lyons et al., Supplemantary Material**

**Table I**

**Sequences of oligonucleotide primers used for RT-PCR**

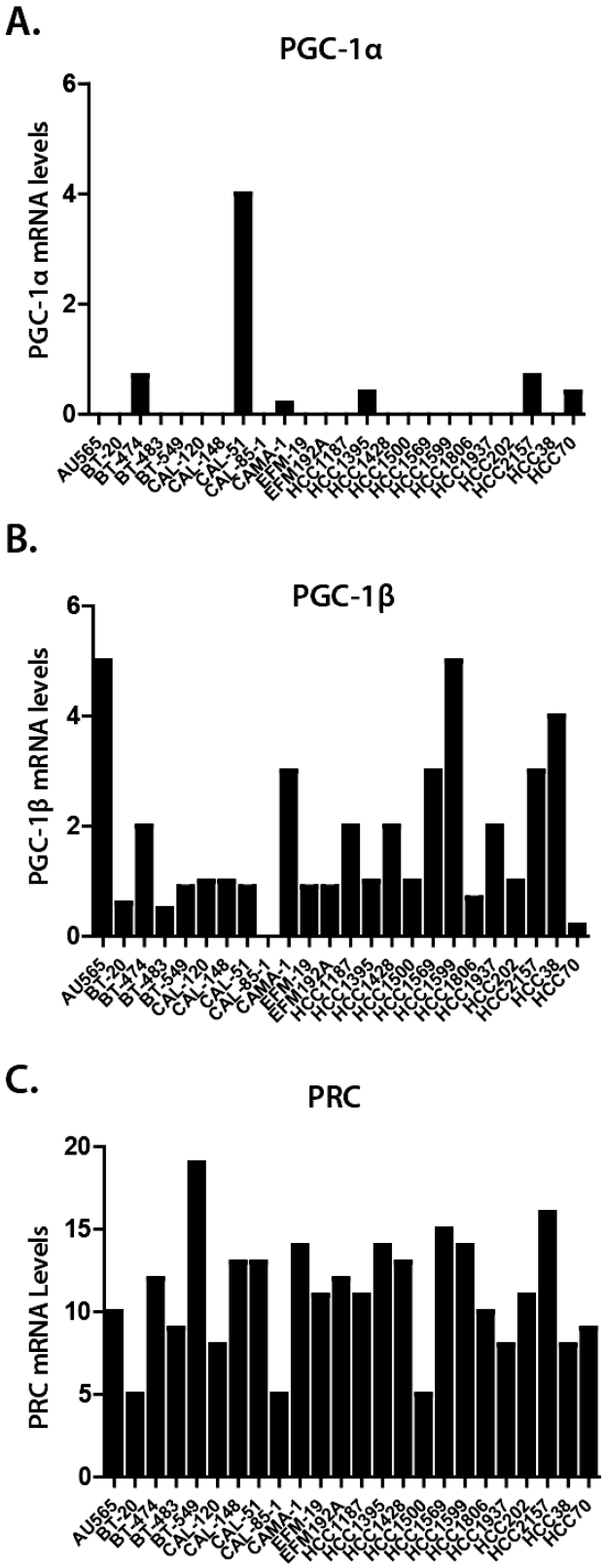
| Primer Set | Sequences |
|---|---|
| PGC-1β | F: 5'-GCTCAAGCTCTGGCTCTTCA-3'<br>R: 5'-ATGCTTGGCGTTCTGTCTGA-3' |
| PRC | F: 5'-CAAGCAGAAACAGAAGAGAGAAG-3'<br>R: 5'-GGTGGGATGACAAGACAAGG-3' |
| PGC-1α | F 5'-CAAGCCAAACCAACAACTTTATCTCT-3'<br>R 5'-CACACTTAAGGTGCGTTCAATAGTC-3' |
| NRF-2α | F 5'-AACAAGAACGCCTTGGGATAC-3'<br>R 5'-GTGAGGTCTATATCGGTCATGCT-3' |
| MFN1 | F 5'-TGTTTTGGTCGCAAACTCTG-3'<br>R 5'-CTGTCTGCGTACGTCTTCCA-3' |
| PINK1 | 5' AGACGCTTGCAGGGCTTTC-3'<br>5' GGCAATGTAGGCATGGTGG-3' |
| BNIP3 | F 5'-CGTTCCAGCCTCGGTTTCTA-3'<br>R 5'-ATCTTGTGGTGTCTGCGAGC-3' |
| BNIP3L | F 5'-AATGTCGTCCCACCTAGTCG-3'<br>R 5'-TAGCTCCACCCAGGAACTGT-3' |
| Mouse PGC-1α | F 5'-AAGCACTTCGGTCATCCCTG-3'<br>R 5'-TGAGTCTCGACACGGAGAGT-3' |
| Mouse PGC-1β | F 5'-GACTTGCCAGAGCTTGACCT-3'<br>R 5'-GAAGAGCTCGGAGTCATCGG-3' |
| Mouse PRC | F 5'-ATCCTAAGGTCGTCAGCCCT-3'<br>R 5'-CTCTGGGAGACTAGGCCACT-3' |
| PNC1 | F 5'-GCTCTGCAGCT TTTATCACAAATTC-3'<br>R 5'-AACGTAACGAGCACACTGGAGTG-3' |
| ADP/ATP trans | F 5'-CCTCCTCCACACACACACAC-3'<br>R 5'-GTACTCGGCTGTGCAAAACA-3' |
| Aralar | F 5'-CGAGACATTCCCTTCTCTGC-3'<br>R 5'-GTCCCTTTCCAAAATGCTGA-3' |

| | |
|---|---|
| COX1 | F 5'-CTCCTACTCCTGCTCGCATC-3'<br>R 5'-GGGTGACCGAAAAATCAGAA-3' |
| Cyto B | F 5'-ATGGCTGAATCATCCGCTAC-3'<br>R 5'-GTGTGAGGGTGGGACTGTCT-3' |
| NFE2L2 | F 5'-CTACTCCCAGGTTGCCCACA-3'<br>R 5'-CGACTCATGGTCATCTACAAATGG-3' |
| NRF-1 | F 5'-CGCTCTGAGAACTTCATGGAGGAACAC-3'<br>R 5'-GCCACATGGACCTGCTGCACT T-3' |

**Supplementary Table I**

List of all oligonucleotide primer sequences used for PCR in the manuscript.

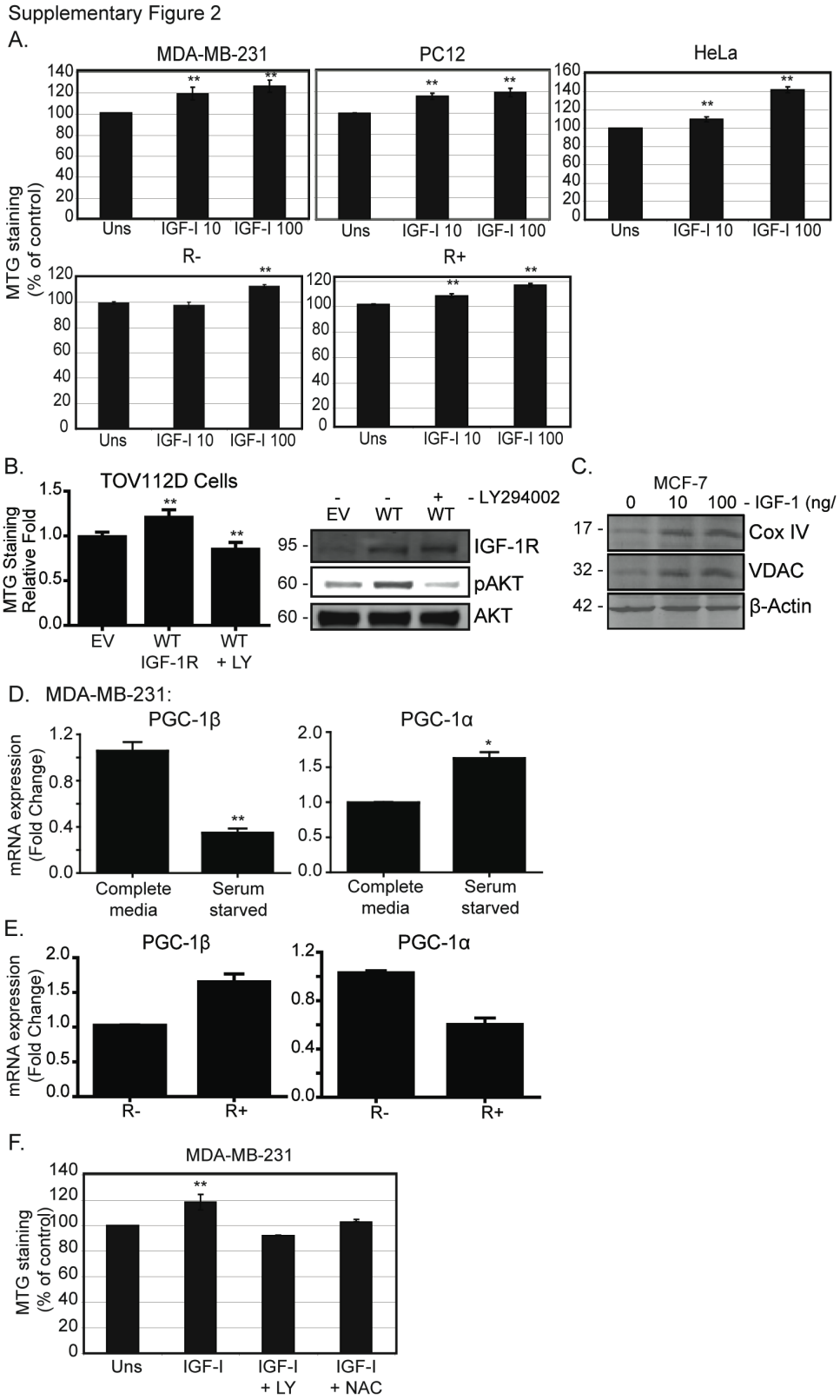
Supplemantary Figure 1




**Supplementary Figure 1:**

**PGC-1α, PGC-1β and PRC expression in breast cancer cell lines**

Publically available RNA-Seq data for human cancer cell lines from the Cancer Cell Line Encyclopaedia was used to plot the relative expression level of PGC-1α, PGC-1β and PRC in a panel of breast cell lines.

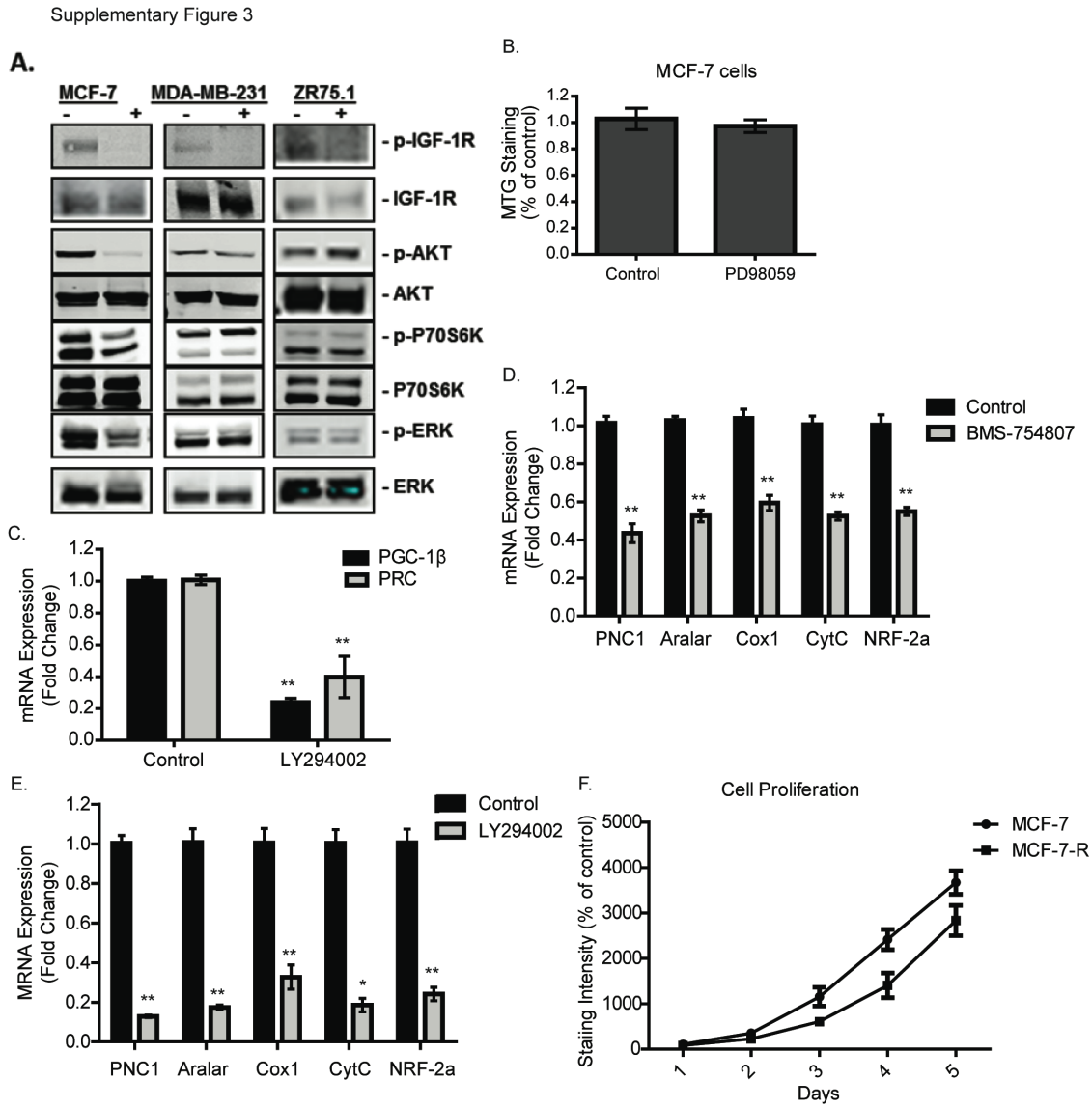


**Supplementary Figure 2:**

**IGF-1 induction of mitochndrial mass and expression of PGC1 isoforms in different cell lines.**

**A.** Mitochondrial Mass in Hela, PC12, MDA-MB-231 cells, R- and R+ cells. Cells were serum-starved for 4 hours and stimulated with 10ng/ml or 100ng/ml IGF-1 for a further 20 hours. Mitochondrial mass was measured by FACS using the MitoTracker Green probe.

**B.** Mitochondrial Mass in TOV112D cells. A stable pool of TOV112D cells overexpressing IGF-1R was generated by transfection with pcDNA3-EV or pcDNA3-IGF-1R and selection geneticin. Mitochondrial mass was measured by flow cytometry using the MitoTracker Green probe. Data are presented as fold difference of control (EV cells) set as 1. Student's t-test *$p<0.05$, **$p<0.01$. The western blot shows expression of IGF-1R in TOV112D cells and that IGF-1 induces phosphorylation of Akt (probed with anti-IGF-1R, and anti-phospho-AKT and anti-AKT antibodies). **C.** Western blot analysis was used to determine expression of CoxIV and VDAC in MCF-7 cells stimulated with IGF-1 for 24 h. **D**. QPCR showing expression levels of PGC-1β and PRC in MDA-MB-231 cells that were either cultured in complete medium or seru-starved for 24 hours, and in R- and R+ cells cultured in complete medium. The expression level of each gene was normalized to the housekeeping gene, UBC, and the levels are shown as fold-change of the control set at 1. Data are presented from three independent experiments as mean ± Standard Error of the Mean (SEM). Statistical Analysis for each experiment was performed using the Student's t-test (*$P<0.05$, **$P<0.01$). (E) Mitochondrial mass in MDA-MB-231 cells in the presence of the PI-3K inhibitor LY294002 (LY) or N Acetyl cysteine (NAC). Cells were serum starved for 4 hours and stimulated with 10ng/ml of IGF-I in presence or absence of inhibitors for 18 hours. Mitochondrial mass was then measured by flow cytometry using Mitotracker Green. The data are presented as the percentage of fluorescence unstimulated cells and as the mean and standard deviation from three separate experiments (Student T-Test ** $P<0.01$).

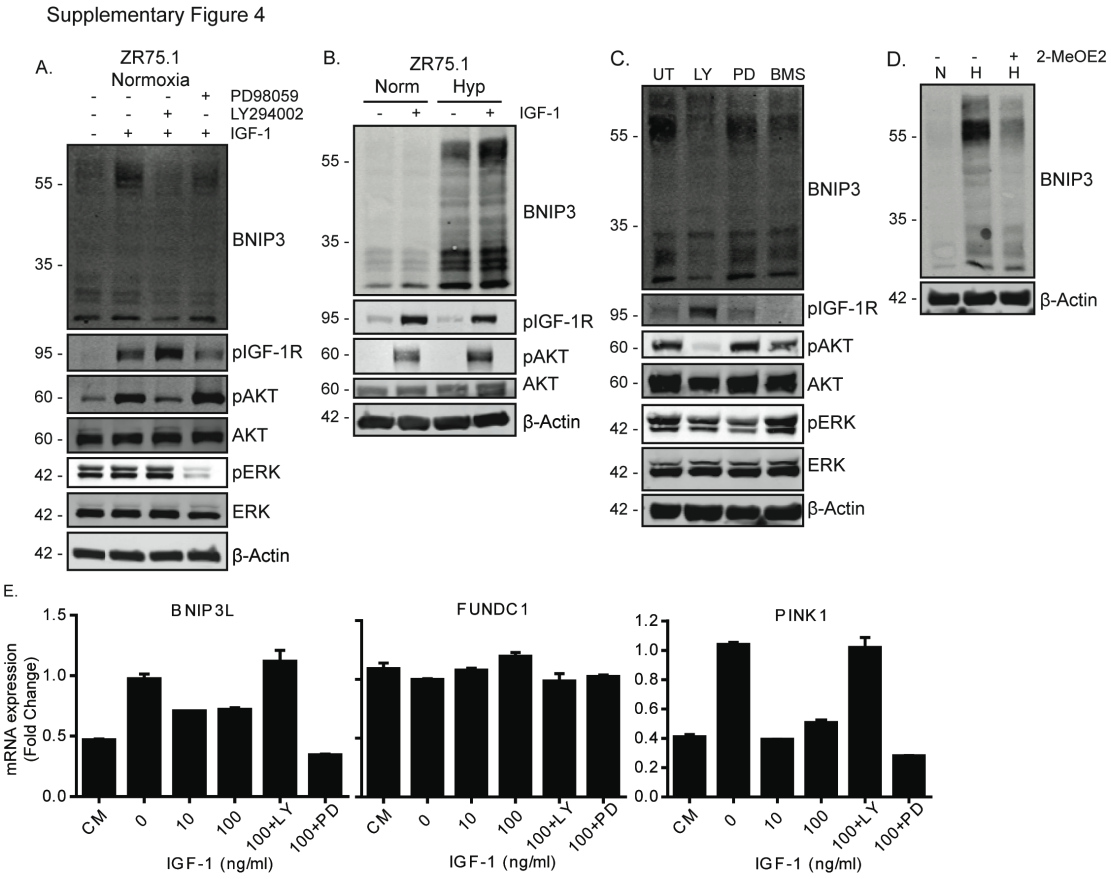



**Supplementary Figure 3:**

**Inhibition of IGF-1R/PI-3K signalling results in reduced mitochondrial biogenesis**

**A.** Western Blot showing IGF-1R/PI-3K activity in MCF-7, MDA-MB-231, and ZR75.1 cells following treatment with BMS-754807. Cells were maintained in complete media and were treated with 500nM BMS-754807 for 24 hours. Cells were lysed and prepared for immunoblotting with anti-phospho-IGF-1R, anti-IGF-1R, anti-phospho-AKT, anti-AKT, anti-phospho-P70S6K, anti-P70S6K, anti-phospho-ERK and anti-ERK antibodies. **B.** Mitochondrial mass in MCF-7 cells following MAPK inhibition. MCF-7 cells were treated with PD98059 for 24 hours and mitochondrial mass was determined by FACS analysis using the MitoTracker Green probe. **C.** QPCR showing PGC-1β and PRC expression in MCF-7 cells following treatment with LY294002. Cells maintained in complete media were treated with 20μM

LY294002 for 24 hours after which they were harvested for QPCR. PGC-1β and PRC levels were normalized to the housekeeping gene, UBC, and the levels are shown as fold-change of the control set at 1. **D.** QPCR showing mitochondrial gene expression in MCF-7 cells following treatment with BMS-754807. Cells maintained in complete media were treated with BMS-754807 for 24 hours after which they were harvested for QPCR. Expression levels of each gene was normalized to the housekeeping gene, UBC, and the levels are shown as fold-change of the control set at 1. **E.** QPCR showing mitochondrial gene expression in MCF-7 cells following treatment with LY294002. Cells maintained in complete media were treated with LY294002 for 24 hours after which they were harvested for QPCR. Expression levels of each gene was normalized to the housekeeping gene, UBC, and the levels are shown as fold-change of the control set at 1 **F.** Growth rates of MCF-7 parental and MCF-7-R cells. Cells were initially plated at a density of $2.5x10^4$/well of a 24-well plate and cell number was assessed every 24 hours by crystal violet staining. MCF-7-R cells were cultured in the presence of 500nM BMS-754807. Statistical Analysis for each experiment was performed using the Student's t-test (*P<0.05, **P<0.01).

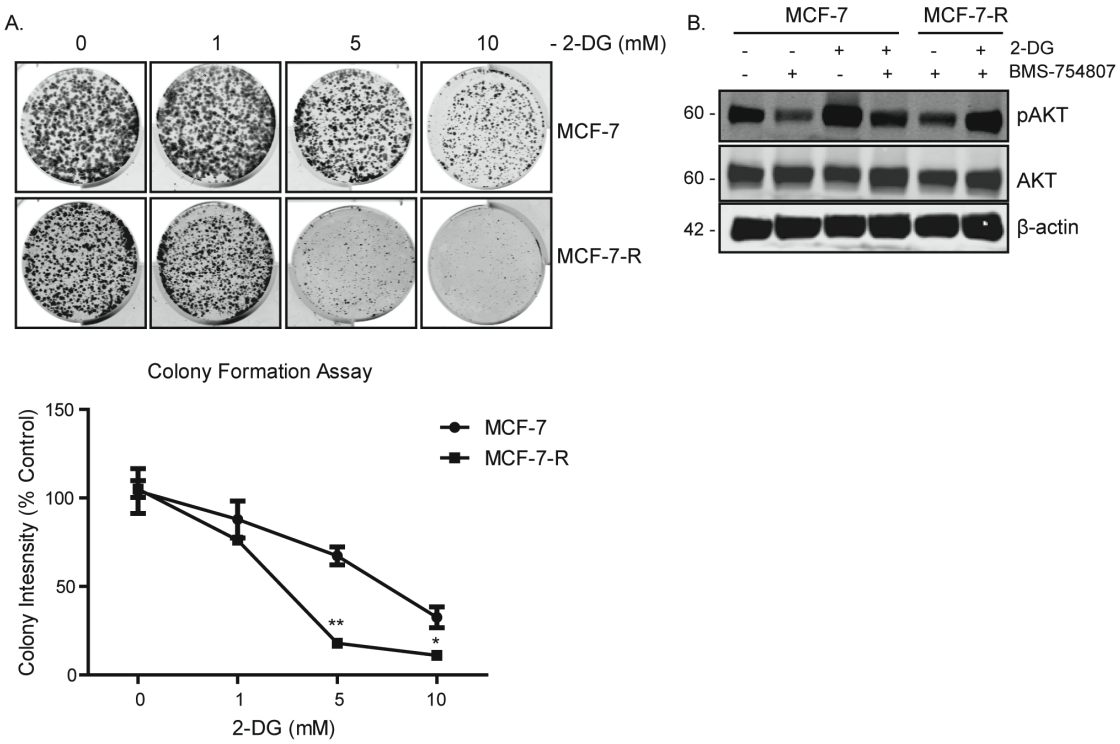



**Supplementary Figure 4:**

**IGF-1-regulated expression of mitophagy regulatora BNIP3 FUNDC1 and PINK1.**

**A.** Western blot showing BNIP3 expression in ZR75.1 cells following stimulation with IGF-1. Cells were serum starved for 4 hours and stimulated with 100ng/ml IGF-1 for 20 hours in the presence or absence of LY294002 or PD98059. Cells were then lysed and harvested for western blot analysis. Blots were probed with anti-BNIP3, anti-phospho-IGF-1R, anti-phospho-AKT, anti-AKT, anti-phospho-ERK, anti-ERK and anti-β-Actin antibodies. **B.** ZR75.1 cells were serum starved for 4 hours and stimulated with 100ng/ml IGF-1 for 20 hours under either normoxic or hypoxic (1% $O_2$) conditions. Cells were then lysed and harvested for western blot analysis. Blots were probed with anti-BNIP3, anti-phospho-IGF-1R, anti-phospho-AKT, anti-AKT, and anti-β-Actin antibodies. **C.** MCF-7 cells were incubated with 20μM LY294002, 20μM PD98059 or 500nM BMS-754807 for 24 hours. Cells were then lysed and cell lysates were harvested for western blot analysis. Blots were probed with anti-BNIP3, anti-phospho-IGF-1R, anti-phospho-AKT, anti-AKT, anti-phospho-ERK and anti-ERK and anti-β-Actin antibodies. **D.** MCF-7 cells were placed in normoxia or hypoxia for 20 hours. Hypoxic cells were cultured in the presence or absence of 2MeOE2. Cells were then lysed and cell lysates were harvested for western blot analysis. Blots were probed with anti-BNIP3 and anti-β-Actin antibodies. **E.** QPCR showing levels of BNIP3L, PINK1, and FUNDC1 following stimulation with IGF-1. MCF-7 cells were cultured in complete media or serum starved for 4 hours and stimulated with 10ng/ml or 100ng/ml IGF-1 for 24 hours in the presence or absence of LY294002 or PD98059. Levels of each gene was normalized to the housekeeping gene, UBC, and the levels are shown as fold-change of the control set at 1 Data is included from three separate experiments.

Supplementary Figure 5




**Supplementary Figure 5:**

**MCF-7-R cells display increased sensitivity to glycolysis inhibition compared to parental cells.**

**A.** MCF-7 and MCF-7-R cells were seeded into colony formation assays and incubated with concentrations of 2-DG (1mm-10mM) and allowed to grow for 14 days. The drug was replenished every 4 days. Cells were fixed and stained with crystal violet. Odyssey Infrared Imaging system was used to image the assay. Data presented on the graph are the average +/- SEM of triplicate wells of two individual experiments. B. Representative immunoblot of MCF-7 parental and MCF-7-R cells treated with BMS-754807, 2-DG, or both in combination. Cells were treated with 500nM BMS-754807 or 25mM 2-DG for 24 hours in complete culture media before being harvested for western blot analysis. The blot was probed anti-phospho-AKT, anti-AKT, and anti-β-Actin antibody.