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Supplemental Material to:

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Glucose promotes breast cancer aggression and reduces metformin efficacy

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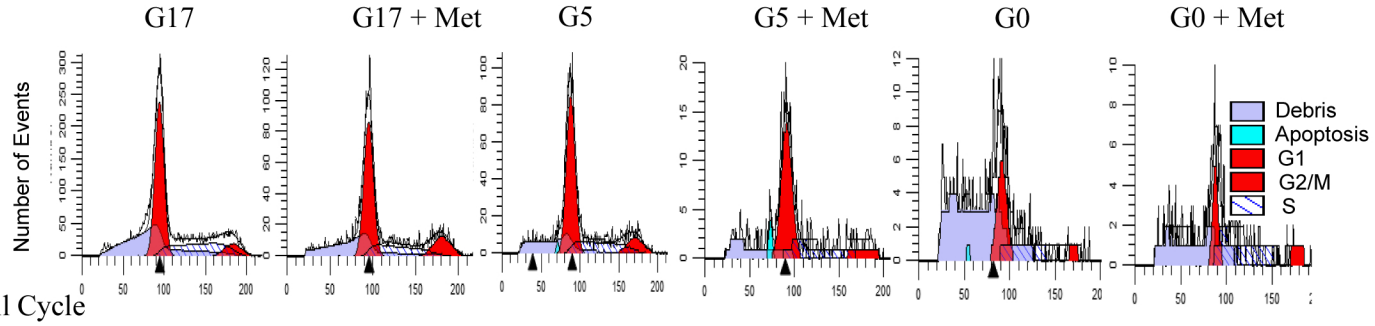
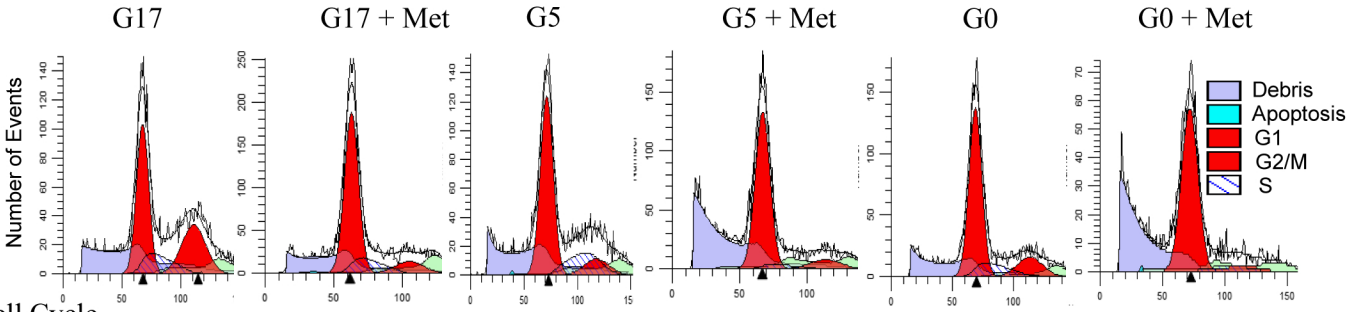
Supplementary 1

A. MCF7



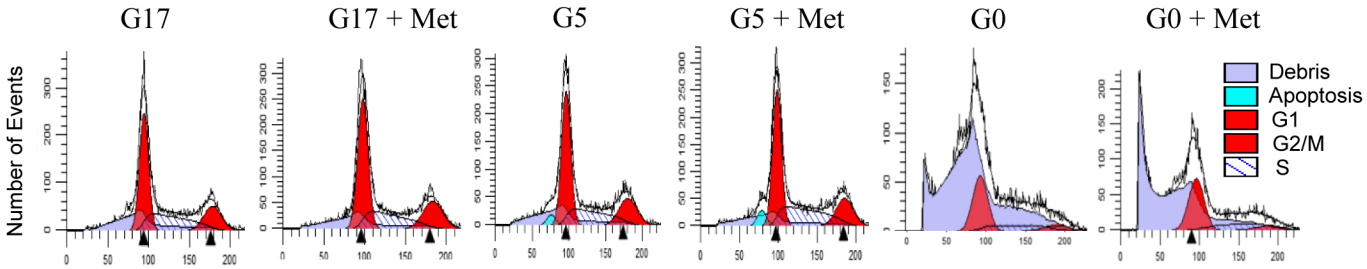
Cell Cycle	G17	G17 + Met	G5	G5 + Met	G0	G0 + Met
Apoptosis	0.00%	0.00%	1.52%	4.93%	2.69%	5.74%
G1	55.54%	61.58%	61.55%	58.77%	54.41%	29.49%
S	26.11%	24.12%	26.22%	28.76%	35.50%	58.81%
G2/M	18.34%	14.30%	12.23%	12.48%	10.10%	11.70%

B. SK-BR-3



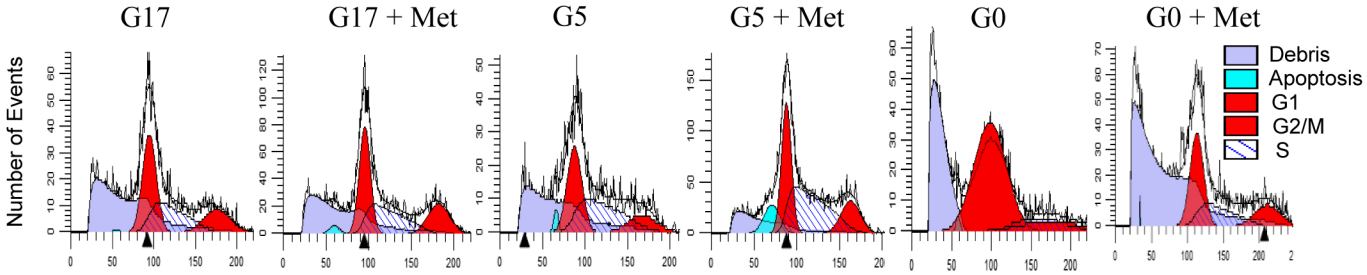
Cell Cycle	G17	G17 + Met	G5	G5 + Met	G0	G0 + Met
Apoptosis	0.00%	0.00%	0.00%	0.76%	0.00%	0.43%
G1	57.39%	81.46%	63.76%	88.76%	69.98%	86.70%
S	19.77%	18.54%	28.29%	9.35%	14.13%	7.03%
G2/M	22.85%	0.00%	7.95%	1.89%	15.89%	6.27%

C. MDA-MB-231



Cell Cycle						
Apoptosis	0.00%	0.00%	3.90%	5.56%	0.00%	0.00%
G1	48.38%	51.83%	52.29%	48.24%	58.05%	60.87%
S	31.77%	26.70%	27.11%	33.00%	28.17%	28.25%
G2/M	19.80%	21.48%	20.90%	18.76%	13.79%	10.88%

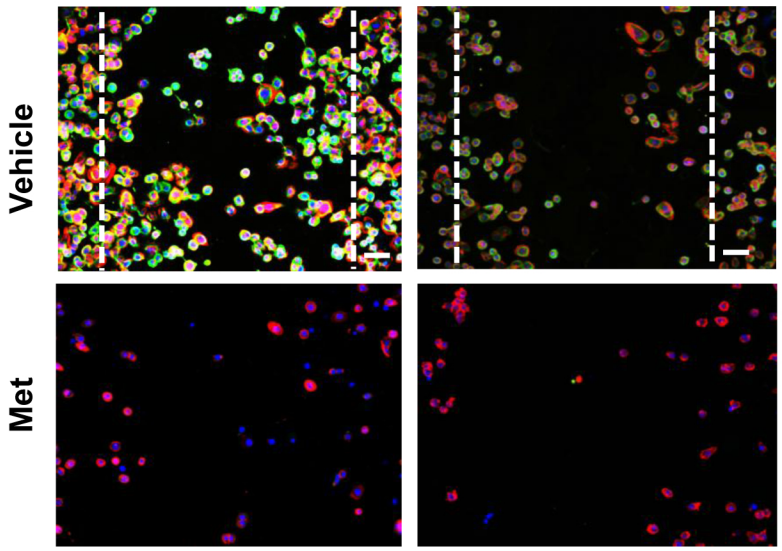
D. BT-549



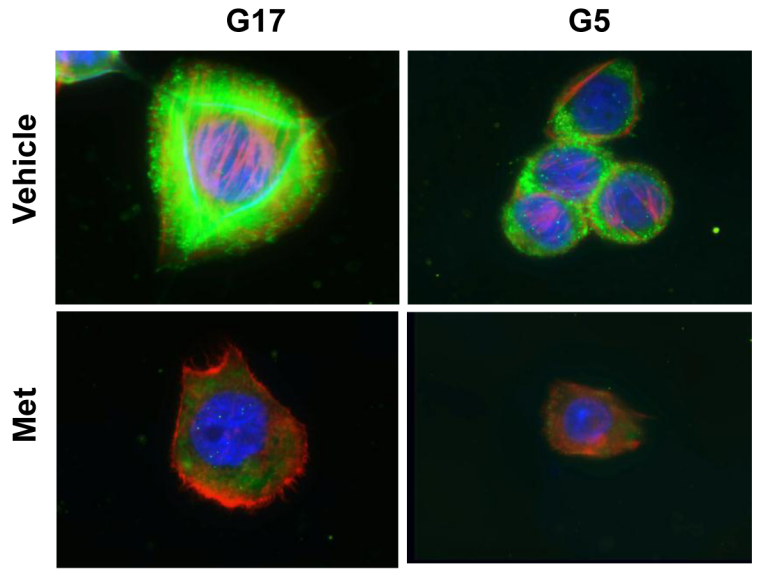
Cell Cycle						
Apoptosis	0.66%	2.85%	3.36%	11.05%	1.26%	0.76%
G1	45.56%	41.663%	39.38%	35.55%	72.83%	48.40%
S	33.68%	35.74%	44.71%	47.17%	13.99%	30.19%
G2/M	20.76%	22.60%	15.19%	17.29%	13.18%	21.41%

Supplementary 2

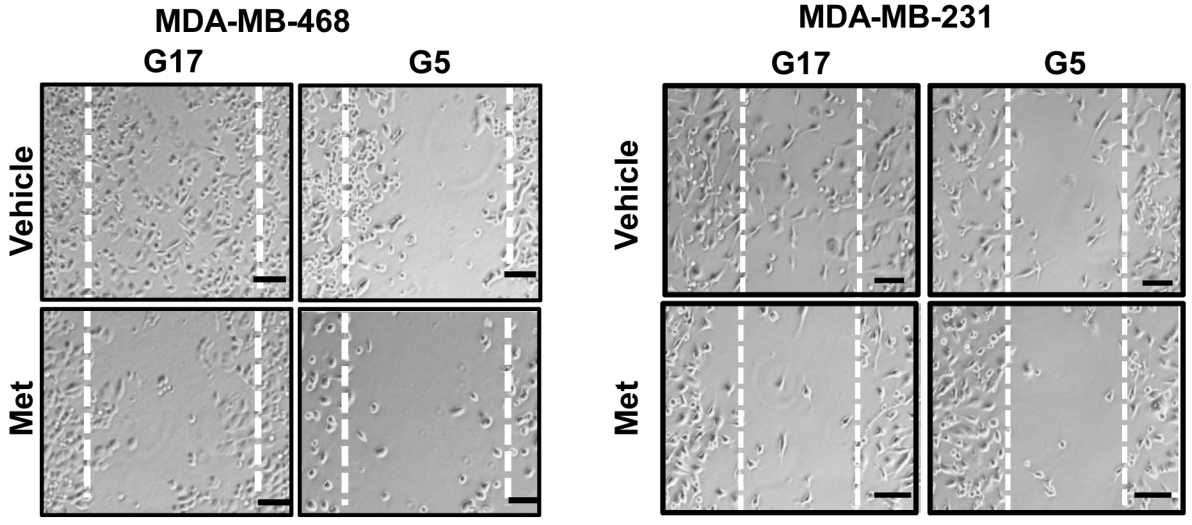
A. MDA-MB-468



B.

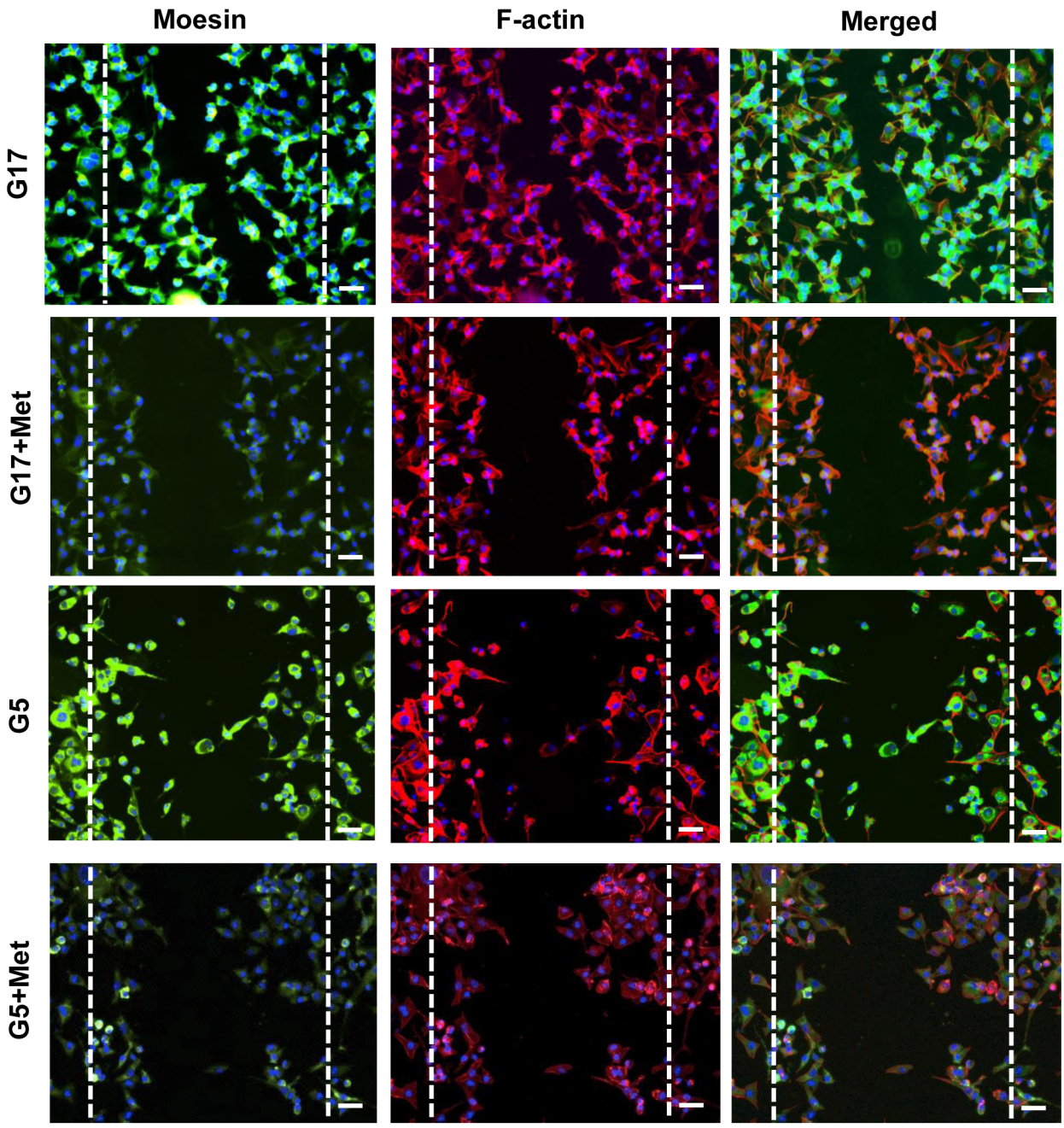


C.



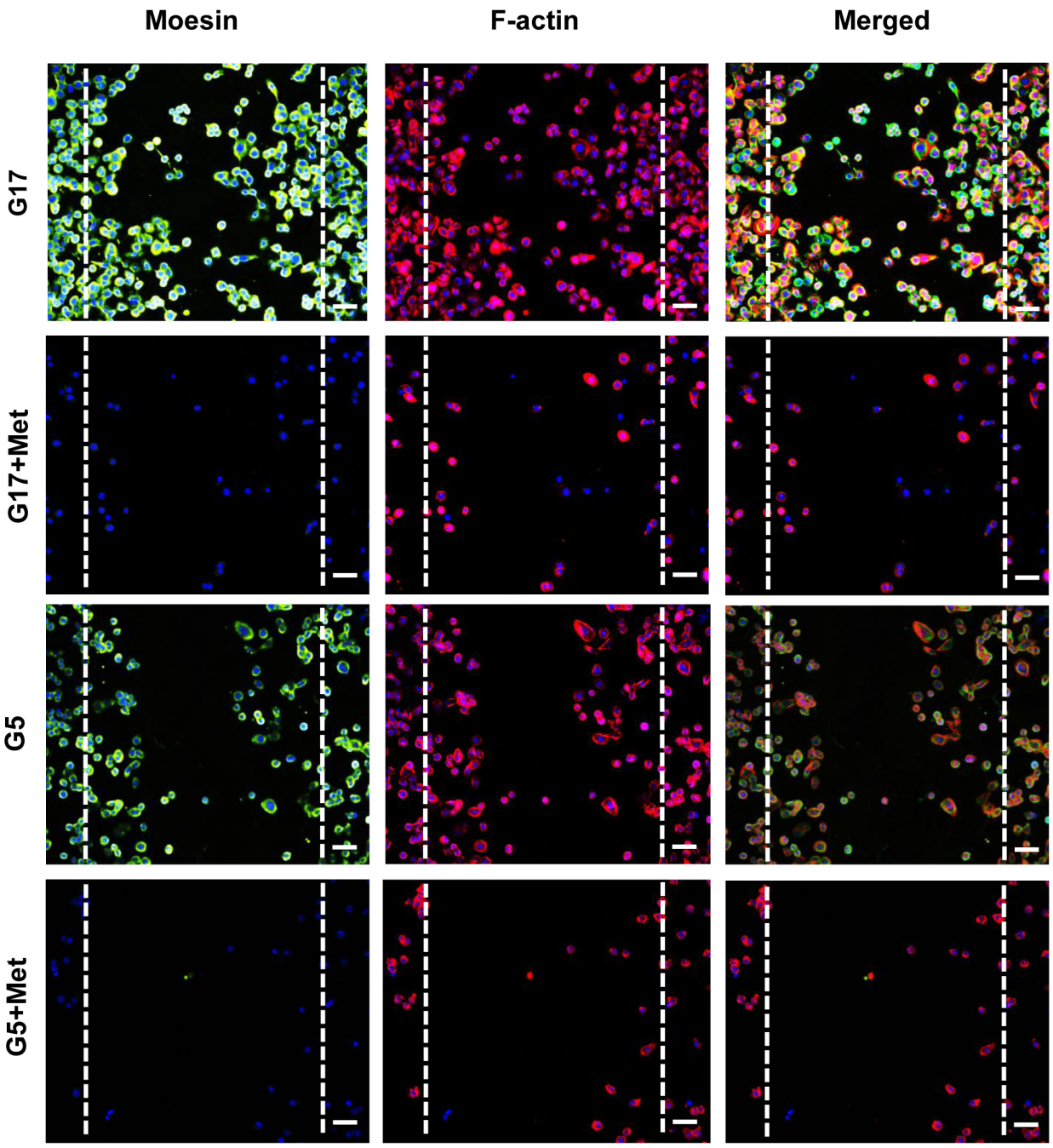
Supplementary 3

A. MDA-MB-231



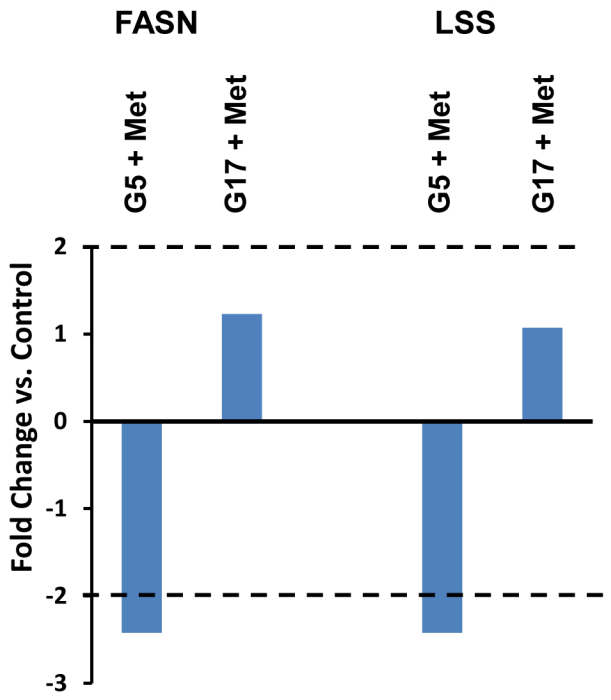
Supplementary 3

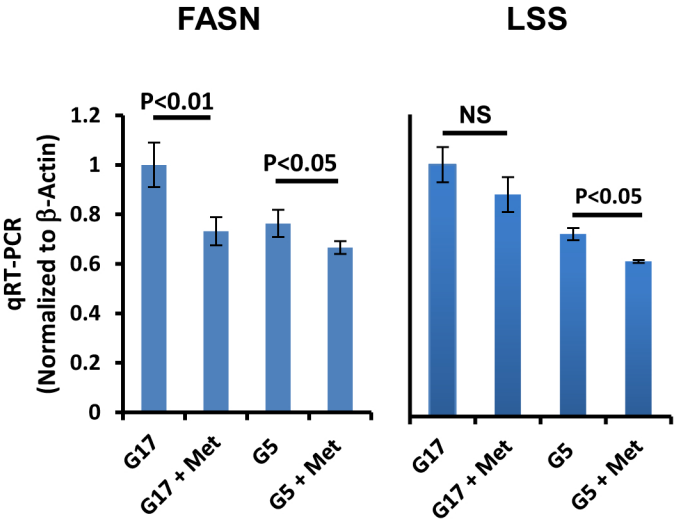
B. MDA-MB-468



Supplementary 4

A. **Selected Genes Differentially Regulated by Metformin in MDA-231**





Supplemental Figures 1. Reduction of Glucose levels enhances metformin-mediated apoptosis in breast cancer cells lines. Breast cancer cell lines MCF-7 **(A)**, SK-BR-3 **(B)**, MDA-MB-231 **(C)**, and BT-549 **(D)** were treated as described in Fig. 1 and cell cycle analysis was performed by flow cytometry for the percentage of cells in apoptosis, G1, S and G2/M. Mod Fit analysis is representative of three independent experiments.

Supplemental Figure 2. Metformin inhibits cell motility and wound closure in MDA-MB-468 cells. A. MDA-MB-468 cell were processed as described in Fig.4. Cover slips were stained using immol/Lunofluorescence (IF) for expression of moesin (green), F-actin (red) and DAPI (blue) and imaged at 10x, bar is representative of 40 μM. **C.** MDA-MB-468 cells treated as described above were imaged at 100x on a Nikon microscope. Bar is representative of 20 μM. C. MDA-MB-468 (left) and MDA-MB-231 (right) cells were treated as described above and relative wound closure was monitored in phase contrast imaging using a Nikon microscope at 10x. Images are representative of three independent experiments, dotted line is indicative of scratch margins at time zero.

Supplemental Figure 3. Metformin inhibits wound closure in MDA-MB-231 and MDA-MB-468 cells. MDA-MB-231 **(A)** or MDA-MB-468 **(B)** cell were processed as described in Figure 4 and Supplementary Fig. 2 by IF. Double labeled images of moesin (green)/DAPI (blue), F-actin (red)/DAPI (blue) or triple-labeled moesin (green)/F-actin (red)/ DAPI (blue) are representative of three independent experiments. Dotted lines indicates scratch margins at time zero, bar is representative of 40 μM.

Supplemental Figure 4. Microarray analysis of MDA-MB-231 cells with normal (5 mmol/L) or high (17 mmol/L) glucose in the presence or absence of metformin. **A.** MDA-MB-231 cells were treated with 10 mmol/Lol/L metformin in either 5 mmol/L or 17 mmol/L glucose medium for 24 h and analyzed on the Affymetrix Human Gene 1.0 ST array platform. Selected genes differentially regulated as determined by Affymetrix gene array are shown. Columns are representative of fold change relative to controls, and are averages of biological triplicate determinants ± SE. **B.** Quantitative RT-PCR was performed on MDA-MB-231 treated as indicated (Fig.6) normalized to β-actin control. Columns are representative of triplicate determinants ± SE.