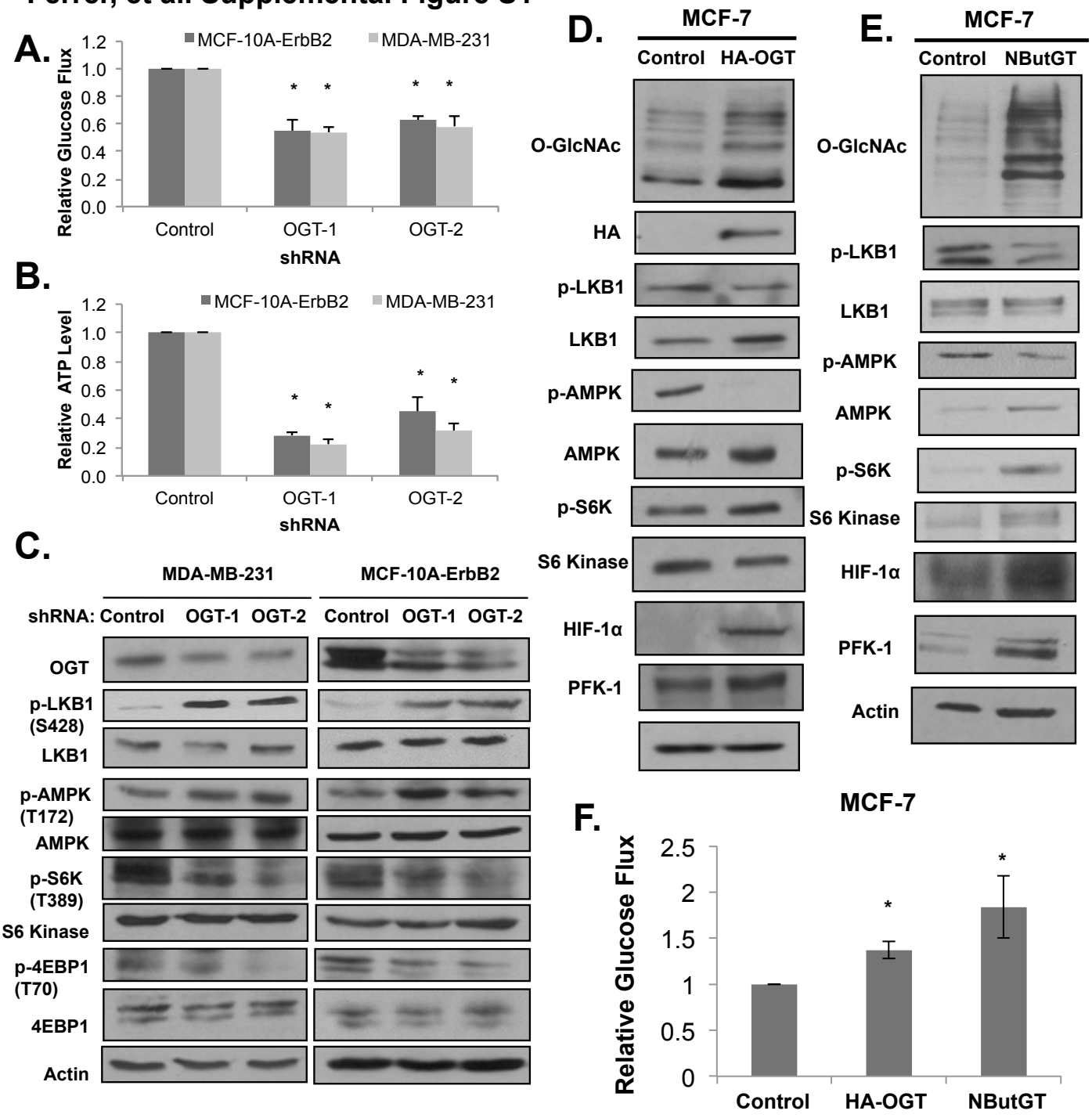
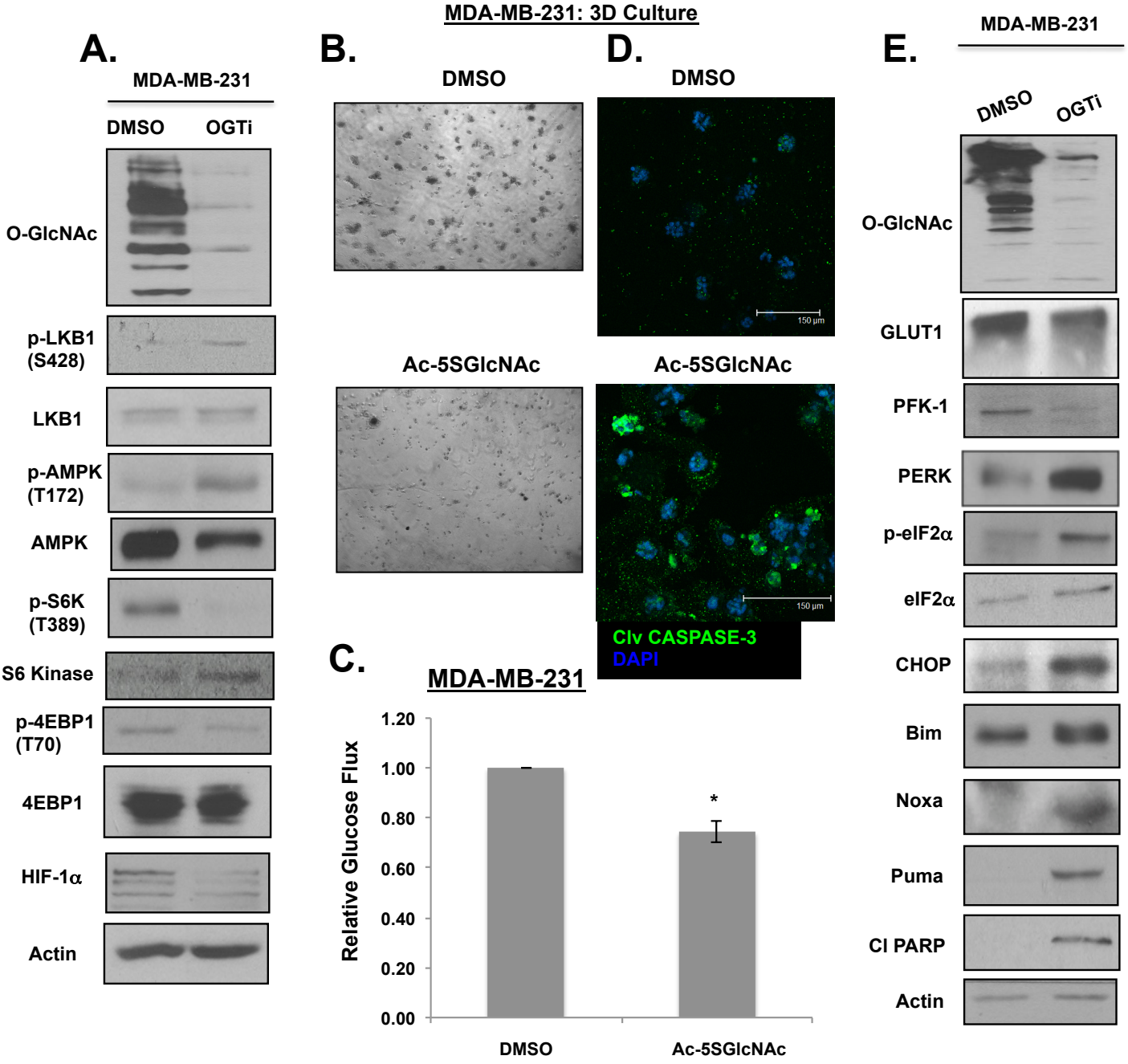
Ferrer, et al. Supplemental Figure S1



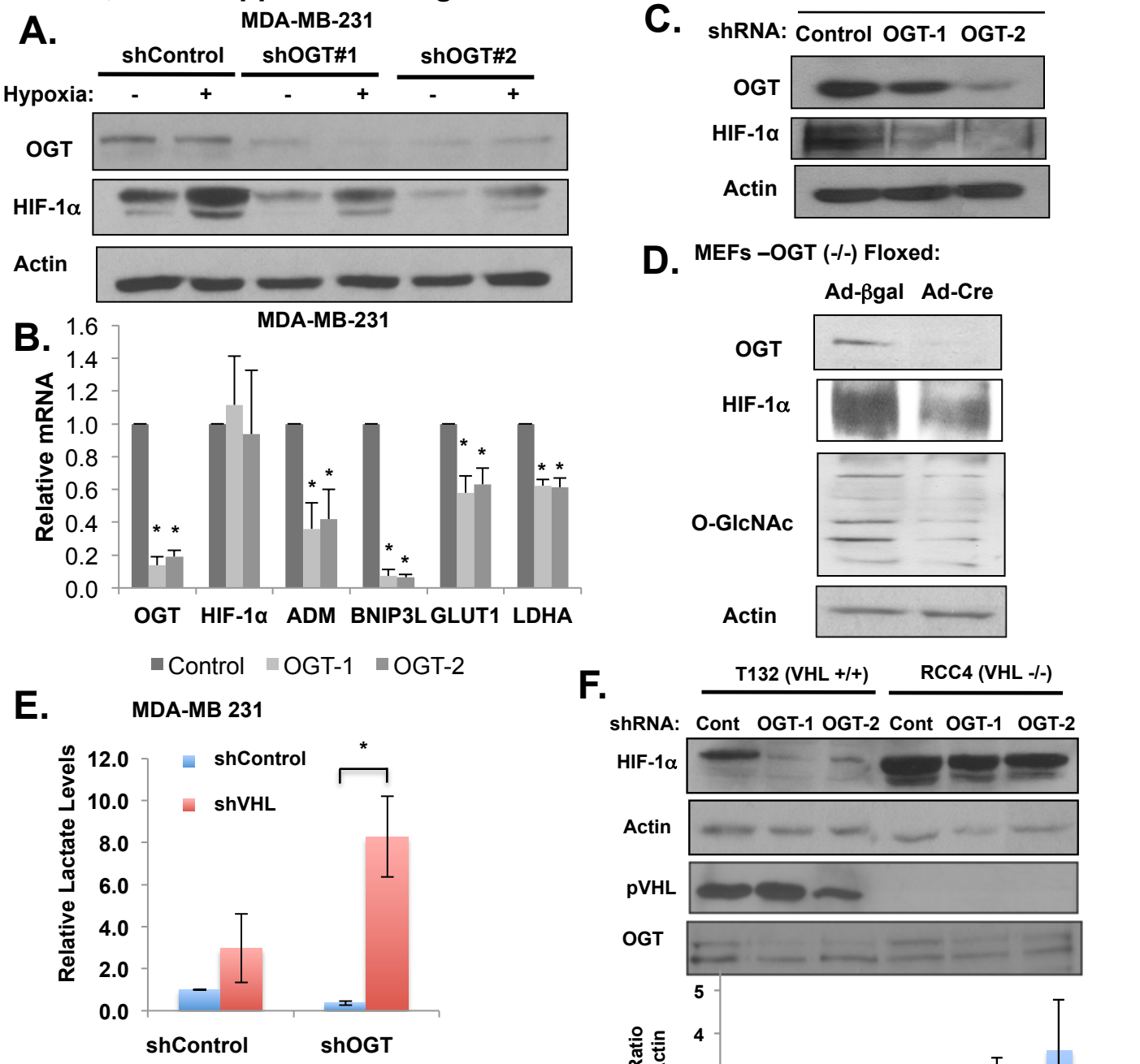
Supplemental Figure 1. OGT/O-GlcNAc regulates glycolytic flux and metabolic signaling in breast cancer cells. MDA-MB-231 and MCF-10A-ErbB2 cells stably expressing control, OGT-1 or OGT-2 shRNA pLKO.1, and glucose flux (A) and ATP levels (B) were measured and normalized to control shRNA treated cells. (C) MDA-MB-231 and MCF-10A -ErbB2 cells were infected with control, OGT-1 or OGT-2 shRNA, and cell lysates from were used for immunoblot analysis with indicated antibodies. (D) MCF-7 cells stably overexpressing control or HA-OGT. (E) MCF-7 cells were treated with control or 100 μM O-GlcNAcase inhibitor NButGT for 48 hours. Protein lysates were collected for immunoblot analysis with indicated antibodies. (F) Glucose uptake levels were measured in cells in D and E. Changes in were normalized to control treated cells. Data are presented as an average from three or more independent experiments. *p-value <0.05. Related to Figure 1.

Ferrer, et al. Supplemental Figure S2



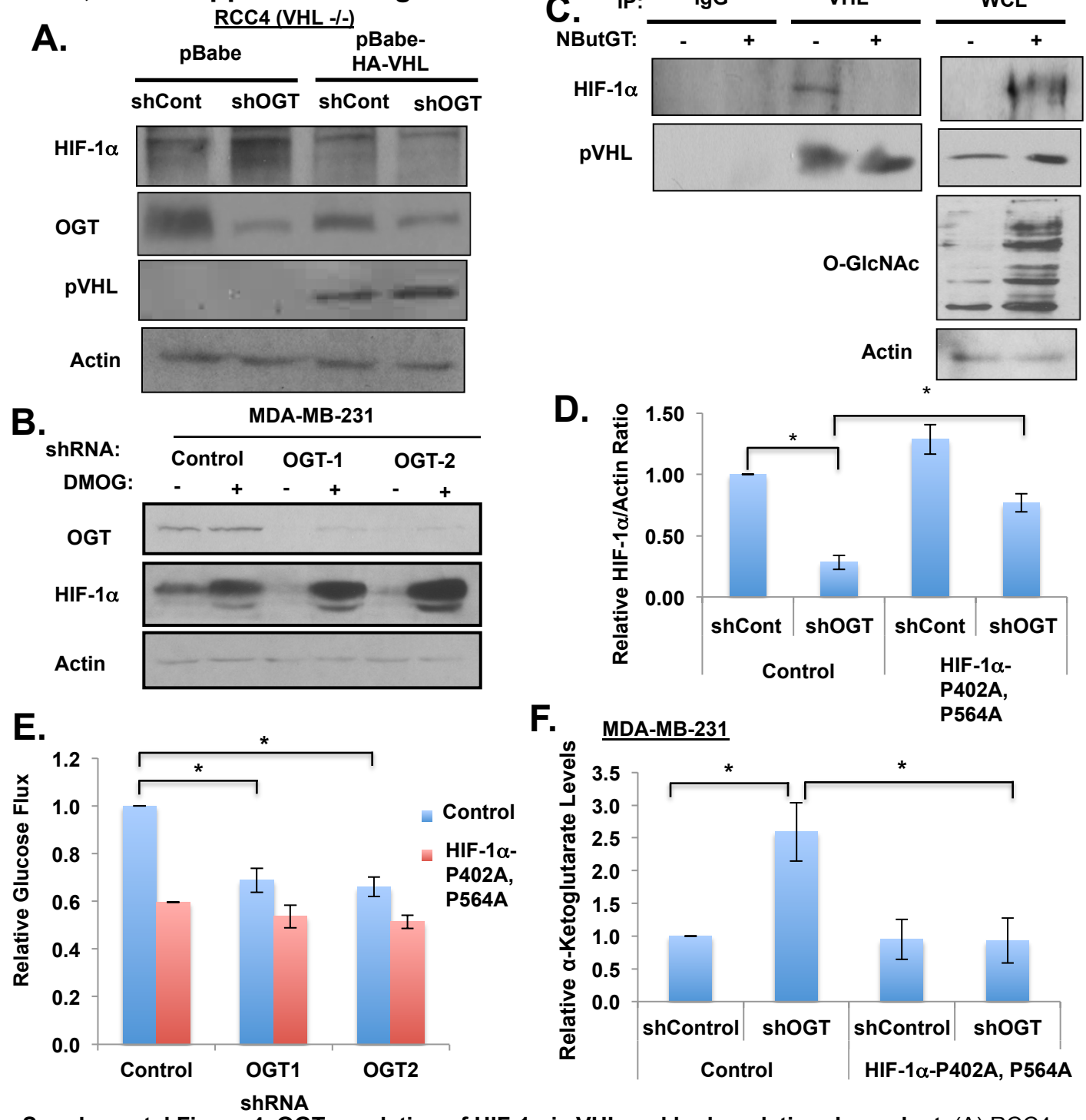
Supplemental Figure 2 . Pharmacological inhibition of OGT regulates LKB1-AMPK- mTOR signaling, glycolytic metabolism, apoptosis and ER stress in breast cancer cells. (A) MDA-MB-231 cells were treated with control or 100 μM Ac-5SGlcNAc (OGTi) for 24 h. Cells were lysed and proteins were analyzed by immunobloting with indicated antibodies. (B) MDA-MB-231 cells were placed in 3D culture and treated with 100 μM Ac-5SGlcNAc (OGTi) or control. At day 6, phase images of the acini were acquired. (C) MDA-MB-231 cells were treated with DMSO or 100 μM OGT inhibitor, Ac-5SGlcNAc for 24 hours. Changes in glucose uptake were measured and normalized to DMSO treated cells. Data are presented as an average from three or more independent experiments. (D). Cells as in B at day 6, cells were fixed and stained with indicated antibodies and representative images were taken with confocal microscope. (E). MDA-MB-231 cells as in A were lysed and proteins were were analyzed by immunobloting with indicated antibodies. * represents p-value <0.05. Related to Figure 1 and 4.

Ferrer, et al. Supplemental Figure S3



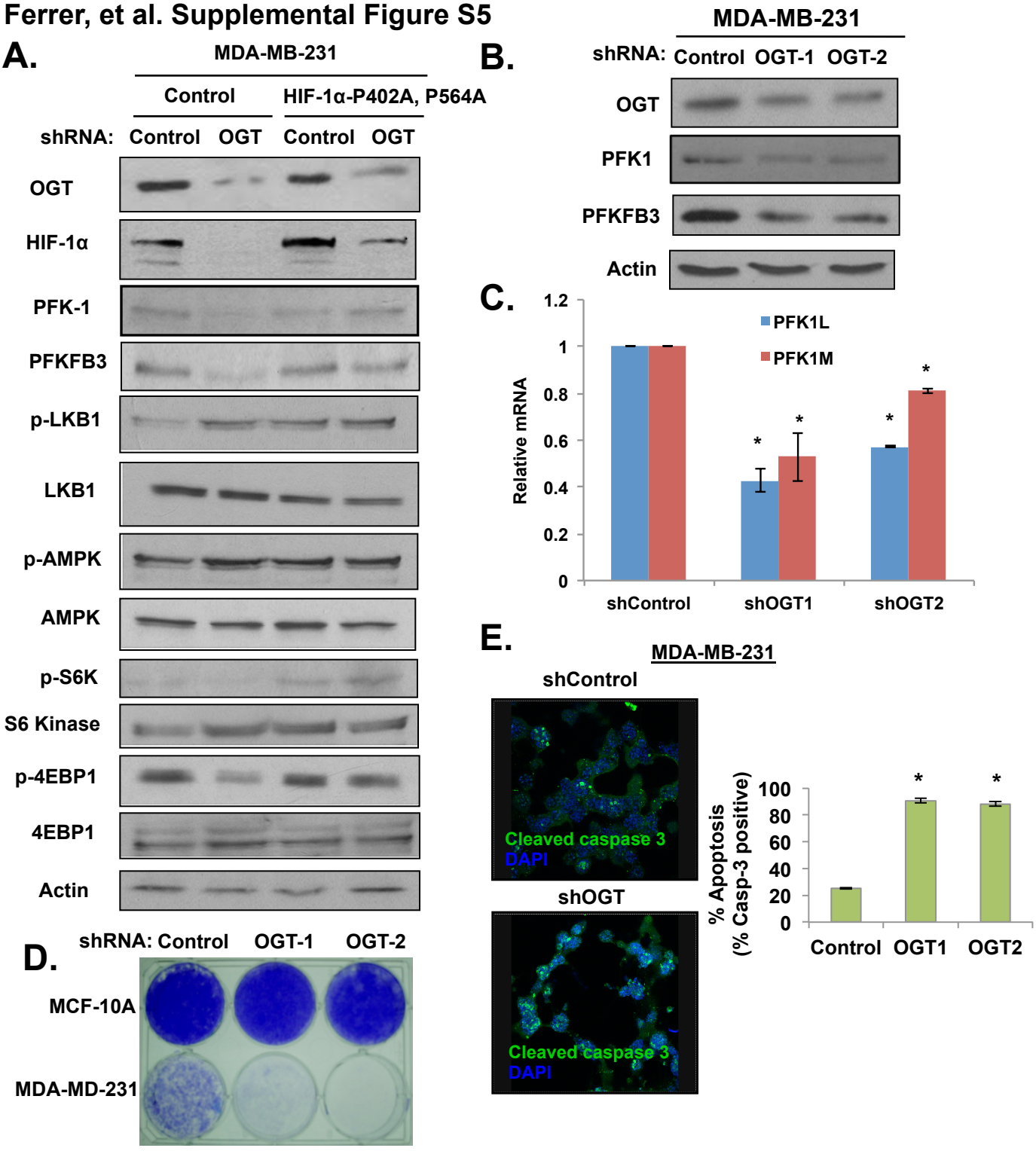
Supplemental Figure 3. Reducing OGT levels alters HIF-1α protein levels, target gene expression in breast cancer cells, immortalized mouse fibroblasts and is dependent on pVHL. (A) MDA-MB-231 cell lysates from control, OGT-1, or OGT-2 shRNA after exposure to normoxia (−) or hypoxia (1% O_2) (+) for 6 hours were collected and analyzed by immunobloting. (B) RNA collected from MDA-MB-231 cells with control, OGT-1 or OGT-2 shRNA and expression levels of HIF-1α targets were measured via RT-PCR and normalized to control shRNA treated cells. Data are presented as an average from three or more independent experiments. (C) Lysates from MCF-7 cells expressing control, OGT-1 or OGT-2 shRNA were analyzed by immunobloting. (D). Mouse embryonic fibroblasts containing OGT floxed allele were infected with adenovirus expressing either control β-galactosidase or Cre-recombinase for 24 hours, followed by incubation in 1% O_2 for 6 hours. Protein lysates were collected for immunoblot analysis with indicated antibodies. (E) MDA-MB-231 cells expressing control or VHL shRNA were infected with control or OGT-2 shRNA. Lactate levels were measured and normalized to control shRNA treated cells. (F) Cell lysates from T132 (VHL +/+) and RCC4 (VHL-/-) renal carcinoma cells expressing control, OGT-1, or OGT-2 shRNA and were analyzed by immunobloting. Data are quantified (n=3). * p-value<0.05. Related to Figure 2.

Ferrer, et al. Supplemental Figure S4



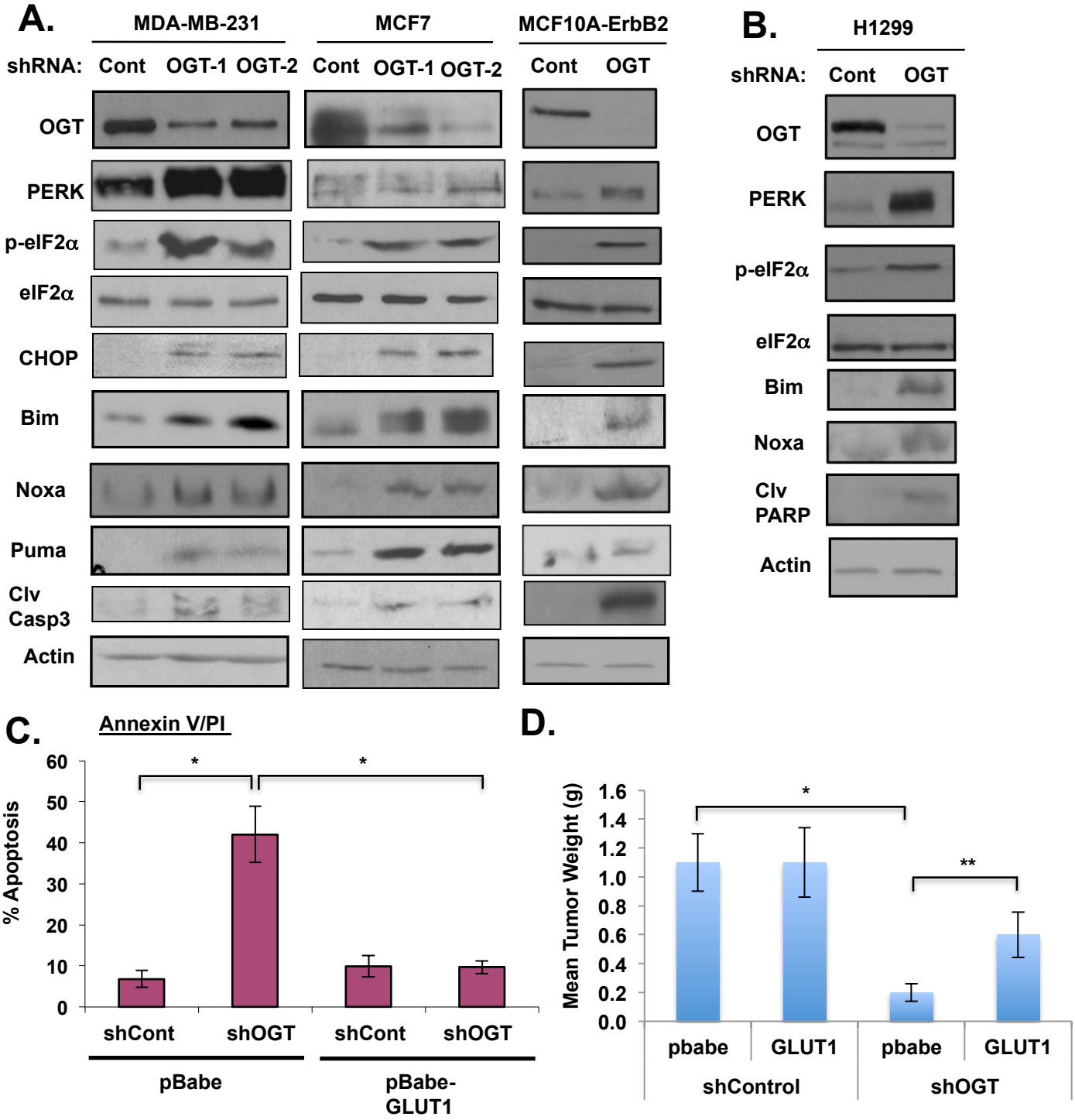
Supplemental Figure 4. OGT regulation of HIF-1α is VHL and hydroxylation dependent. (A) RCC4 (VHL-/-) cells stably expressing pBabe or pBabe-HA-VHL and infected with control or OGT-2 shRNA and immunoblot analysis was performed with indicated antibodies. (B) MDA-MB-231 cells stably expressing control or OGT shRNA were treated with DMSO or 1 mM DMOG for 4 hours and immunoblot analysis was performed with indicted antibodies. (C) MDA-MB-231 cells were treated with control (DMSO) or 100 μM NButGT (OGAi) for 24 hours and immunoprecipitation was performed with indicated antibodies. (D) MDA-MB-231 cells overexpressing control or HIF-1α-P402A, P546A and either infected with control or OGT-1 shRNA. HIF-1a levels were quantified relative to actin from immunoblot analysis (n=3). MDA-MB-231 cells overexpressing control or HIF-1α-P402A-P546A and either infected with control or OGT-1 or OGT-1 shRNA were measured for changes in glucose flux (E) or α-ketoglutarate levels (F). All levels were measured and normalized to control shRNA treated cells. * p-value<0.05. Related to Figure 2 and 3.

Ferrer, et al. Supplemental Figure S5



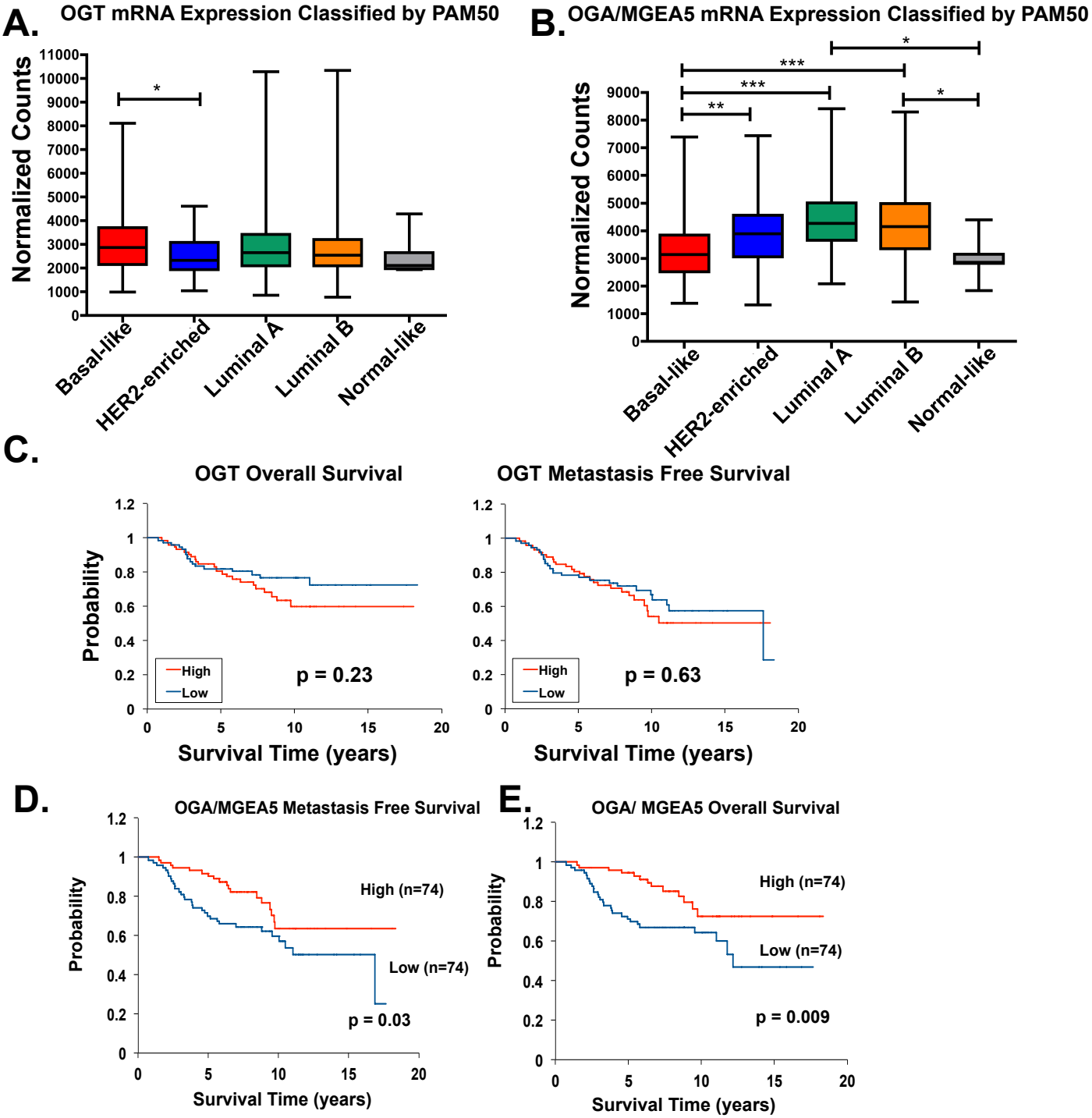
Supplemental Figure 5. OGT Regulation of metabolic signaling is dependent on HIF-1α hydroxylation in breast cancer cells. (A) MDA-MB-231 cells expressing control or HIF-1α-P402A, P546A and either infected with control or OGT-1 shRNA. Protein lysates were collected for immunoblot analysis. (B) Immunoblot analysis from lysates of MDA-MB-231 cells expressing control, OGT-1 or OGT-2 shRNA. (C) RNA was isolated from MDA-MB-231 cells expressing control, OGT-1 or OGT-2 shRNA and levels of PFK-L and PFK-M isoforms were measured via RT-PCR and normalized to control shRNA treated cells. (D) MCF-10A and MDA-MB-231 cells expressing control, OGT-1 or OGT-2 shRNA were stained with crystal violet 8 days post-infection.(E) MDA-MB-231 cells expressing control, OGT-1 or OGT-2 shRNA were placed in 3D culture. At day 6, cells were fixed and stained with indicated antibodies and representative images were taken using confocal microscope. Caspase-3 positive structures were quantified (n=3). * p-value<0.05. Related to Figure 3.

Ferrer, et al. Supplemental Figure S6



Supplemental Figure 6. Targeting OGT induces ER stress, BH3-only proteins and phenotypes can be reversed with GLUT1 overexpression. (A) MDA-MB-231, MCF-10A-ErbB2, and MCF-7 cells expressing control, OGT-1 or OGT-2 shRNA, and protein lysates were collected for immunoblot analysis with indicated antibodies. (B) H1299 cells were treated as in (A). (C) MDA-MB-231 cells stably infected with control or GLUT1 were analyzed for apoptosis after lentiviral infection with control or OGT shRNA. Cells were collected, stained with Annexin V/PI and analyzed by flow cytometry (*p <0.05). (D) Mean tumor weights (g) were taken *ex vivo* of MDA-MB-231-control or MDA-MB-231-GLUT1 expressing control shRNA and OGT-1 shRNA cells 8 weeks after injection (n=13) (*P-value<0.01, ** P-value<0.05).
Related to Figure 4 and 6.

Ferrer, et al. Supplemental Figure S7



Supplemental Figure 7. OGT and OGA Expression in Human Breast Cancer. The TCGA breast cancer dataset was mined to correlate normalized mRNA expression of OGT (A) or OGA/MGEA5 (B) among breast cancer subtypes according to PAM50 classification guidelines. Significant comparisons among subtypes are indicated with an asterisk (*, **, *** represent a *p*-value of <0.05, <0.01 or <0.001, respectively, by ANOVA with Bonferroni correction). (C), (D) and (E) The relationship between individual gene expression, overall survival and metastasis-free survival was analyzed using the van't Veer dataset. No significant correlation was observed for high OGT expression with decreased overall or metastasis-free survival. (D) Significant correlation was observed for low OGA/MGEA5 expression with decreased metastasis-free survival and overall survival (E) when expression was stratified by high (top 75%) versus low expression (bottom 25%), (p <0.05, Cox log-rank test). Related to Figure 7.