SUPPLEMENTARY METHODS

Glycolysis stimulation assay

MCF-7 and MCF-10A cells were seeded in Seahorse Bioscience V7 tissue culture plates. Following overnight incubation, cells were washed and changed to assay media lacking glucose and sodium bicarbonate [DMEM (Cell-gro)], 4 mM L-Glutamine, 1 mM pyruvate). Five baseline measurements of ECAR were then made. All five measurements from each well were averaged to give the 100% starting value. Glucose was then injected to either 5.5, or 17.5 mM. Five further measurements of ECAR were then made before injection of 1 μg/ml oligomycin. ECAR was again measured five times, and then 2-DG was injected to a final concentration of 25 mM in all wells.

In vitro luciferase assay

MDA-MB-231-luc cells were seeded at 2×10^4 per well in 96-well plate. Following an overnight incubation, cells were treated with 2-DG in the presence and absence of 1 μM Mito-CP for 6 h. The substrate luciferin was added to the wells to a final concentration of 150 μg/ml and incubated for 2 min. The microplates were imaged in an IVIS Imaging System to determine the total luciferase activity of the cells according to the manufacturer's instructions (Caliper Life Sciences).

Cell viability assay

MCF-7 and MCF-10A cells seeded at 2×10^4 per well in 96-well plates were treated with 2-DG in the presence and absence of 1 μM MTDs for 6 h or 24 h. The cell

viabilities were monitored by staining with PrestoBlue™ Cell Viability Reagent per the manufacturer's instructions (Invitrogen) and the fluorescence intensities were acquired using a plate reader (Beckman Coulter DTX-880; Beckman-Coulter) equipped with 535 nm excitation and 595 nm emission filters.

SUPPLEMENTARY TABLE 1.

Effects of 2-DG in the presence and absence of Mito-CP on body weight and tissue weight in xenograft mouse models

	Body weight (g)	Kidney (mg)	Liver (mg)	Heart (mg)
Control	21.7 ± 0.4	148.6 ± 4.3	1163.8 ± 50.2	136.1 ± 6.2
Mito-CP (40 mg/kg)	20.5 ± 0.5	151.0 ± 5.2	1161.4 ± 51.1	132.7 ± 7.4
2-DG (1 g/kg)	22.2 ± 0.5	168.3 ± 8.6	1282.4 ± 43.9	141.7 ± 7.3
Mito-CP+2-DG	19.8 ± 0.3	151.1 ± 4.4	1117.1 ± 37.5	156.6 ± 6.8

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Glycolytic capacity is higher in MCF-10A cells than MCF-7 cells. (**A**) MCF-7 and MCF-10A cells were cultured in media containing the glucose concentrations indicated. After establishing baseline ECAR, oligomycin was injected to the final indicated concentration. Five further measurements of ECAR were taken, and the final stimulated ECAR was plotted as a function of oligomycin concentration. Data shown are the mean ± sem. n≥3 per group. (**B**) MCF-7 and MCF-10A cells were assayed in DMEM lacking glucose. Five baseline ECAR values were averaged to yield the starting ECAR. Glucose was then injected to either 5.5 or 17 mM and stimulation of ECAR was monitored. Oligomycin was then added to 1 µg/ml in all wells, and ECAR stimulation was again assessed. Lastly, 2-DG was injected to a final concentration of 25 mM. (**C**) Stimulation of ECAR by oligomycin was calculated from the data in Panel B as the percent increase over values measured in the presence of glucose. Data shown are the mean ± SEM. n=5, p≤0.05 as determined by two-tailed unpaired t-test.

Supplementary Figure 2. The effects of 2-DG and CP, Me-TPP$^+$, or Dec-TPP$^+$ on intracellular ATP levels. To confirm that the decrease in ATP levels seen in Figure 3 was induced by MTD, multiple controls were performed. MCF-7 and MCF-10A cells seeded in 96-well plates were treated continuously with 2-DG in the presence and absence of 1 µM of non-targeted carboxy-proxyl (CP, as a control for Mito-CP) (**A**), Me-TPP$^+$ (**B**), or Dec-TPP$^+$ (**C**) as controls for the targeting moiety for 6 h. Data shown are the means ± SEM, n=4.

Supplementary Figure 3. The effects of 2-DG, MTDs and the relevant control compounds on intracellular ATP levels in MDA-MB-231 cells. MDA-MB-231 cells seeded in 96-well plates were treated continuously with 2-DG in the presence and absence of 1 μM of the indicated MTDs (**A**) or relevant controls (**B**) for 6 h. Data are represented as a percentage of control (non-treated) cells after normalization to total protein for each well. The calculated absolute values of ATP (nmol ATP/μg protein) for MDA-MB-231 control cells were 27.6±0.9. Data shown are the means ± SEM, n=4.

Supplementary Figure 4. The effects of 2-DG in the presence and absence of MTDs on cell viabilities in MCF-7 and MCF-10A cells. Cell viabilities were monitored by staining with PrestoBlue™ Cell Viability Reagent (Invitrogen) per the manufacturer's instructions, after incubation for 6 h (**A, C**) or 24 h (**B, D**) with the compounds tested.

Supplementary Figure 5. The effects of 2-DG in the presence and absence of Mito-CP on the extent of cell death in MCF-7 and MCF-10A cells under same glucose level. MCF-7 cells cultured in media containing 17.5 mM glucose same as of MCF-10A cells were treated with 2-DG in the presence and absence of 1 μM Mito-CP for 24 h, and cell death was monitored by staining with Sytox Green. Data are represented as a percentage of dead cells after normalization to total cell number for each group. Data shown are the means ± SEM, n=5.

Supplementary Figure 6. Cytotoxicity of Mito-CP is not due to the untargeted CP or the TPP^+ targeting moiety. To confirm that cell death induced by Mito-CP

was not due to the nitroxide alone (untargeted compound) or the targeting moiety itself, cells were treated with CP or Me-TPP^+ in combination with 2-DG. MCF-7 (**A**) and MCF-10A (**B**) cells seeded in 96-well plates were treated with 2-DG in the presence and absence of 1 μM MTDs for 24 h, and cytotoxicity was monitored by staining with Sytox Green as described in Figure 4.

Supplementary Figure 7. Schematic representation of the bioenergetic function assay. To determine the mitochondrial and glycolytic function of MCF-7 and MCF-10A cells in response to Mito-CP, Mito-Q, and 2-DG, we used the bioenergetic function assay previously described with several modifications (ref. 30). After seeding and treatment, MCF-7 cells and MCF-10A cells were then washed with complete media (MEM-α for MCF-7 and DMEM/F12 for MCF-10A) and either assayed immediately, or returned to a 37°C incubator for 36 or 60 h. The relative time of treatment and post-treatment incubation that corresponds to the appropriate figures is also given.

Supplementary Figure 8. The effects of 2-DG and MTDs on OCR and ECAR in MCF-7 and MCF-10A cells immediately after a 6 h treatments. (A-D) OCR and ECAR were monitored as described in Figure 6 with the following modifications. MCF-7 cells and MCF-10A cells (30,000 cells/well) seeded in V7 culture plates were treated with the indicated compounds for 6 h. (**E**) *, $P \leq 0.01$ (n = 5) comparing MCF-7 with MCF-10A under the same treatment conditions.

Supplementary Figure 9. The effects of 2-DG and MTDs on OCR and ECAR in MCF-7 and MCF-10A cells measured after 60 h incubation following washout of treatments. (A-D) OCR and ECAR were monitored as described in Figure 6 with the following modifications. MCF-7 cells and MCF-10A cells (15,000 cells/well) seeded in V7 culture plates were treated with the indicated compounds for 6 h. The cells were then washed with complete media (MEMα for MCF-7 and DMEM/F12 for MCF-10A) and returned to a 37°C incubator for 60 h. (**E**) *, $P \leq 0.01$ (n = 5) comparing MCF-7 with MCF-10A under the same treatment conditions.

Supplementary Figure 10. Hematoxylin and eosin (H&E) staining. Representative images of tissue collected from Control, Mito-CP-treated, 2-DG-treated, and Mito-CP/2-DG-treated mice. Shown are examples of portal vein and hepatic duct (**A**), central vein (**B**), glomeruli (**C**), renal corticomedullary junction (**D**), and ventricle (**E**).

Supplementary Figure 11. The effects of 2-DG and Mito-CP on luciferase activity in MDA-MB-231-luc cells. MDA-MB-231-luc cells seeded in 96-well plates were treated continuously with 2-DG in the presence and absence of 1 μM of Mito-CP for 6 h. The microplates were imaged in IVIS-100 Imaging System. Shown are plate layout (**A**) and luminescence intensity image (**B**).