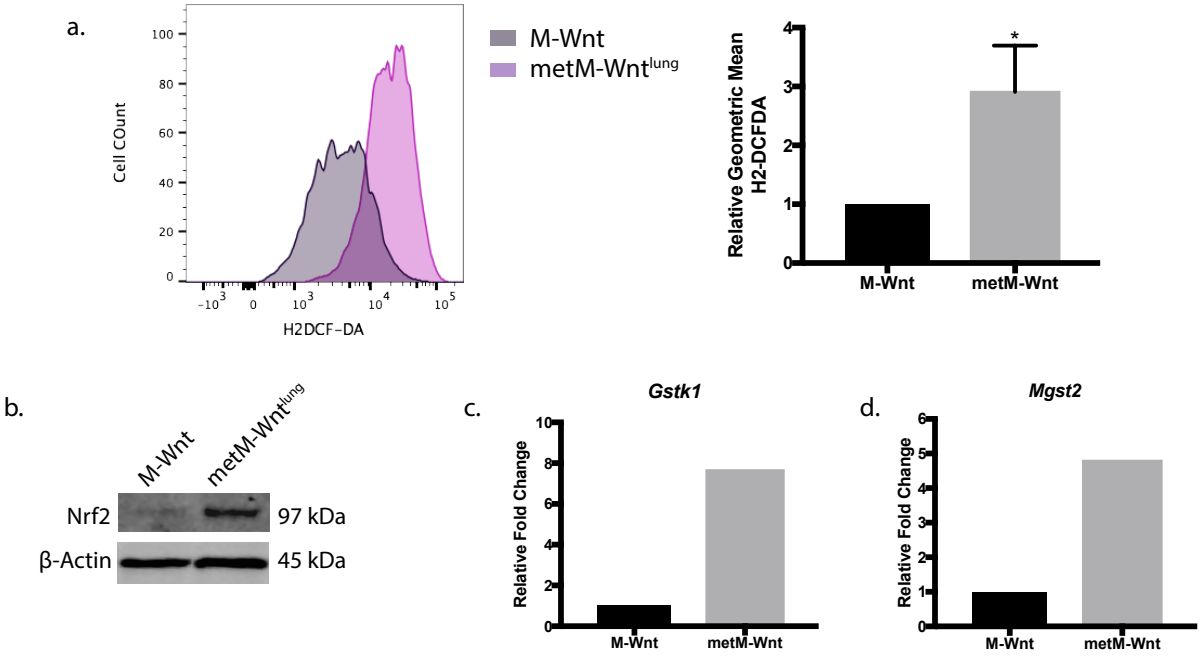

Figure 1. metM-Wnt^{lung} cells exhibited higher basal oxidative stress compared to M-Wnt cells. (a) Endogenous ROS were stained using a H2-DCFDA fluorescent probe. The DCF fluorescence was detected using FL-1 channel at 520 nm. Cellular ROS level was higher in metM-Wnt^{lung} relative to M-Wnt, as measured using geometric mean of FL-1 signals ($n = 3$, * $p < 0.05$). (b) Redox defense signal Nrf2, determined by immunoblotting, was higher in metM-Wnt^{lung} cells compared to M-Wnt. (c)(d) Microarray analysis showed metM-Wnt^{ung} cells exhibited higher glutathione utilization enzymes mRNA expression ($q < 0.001$).

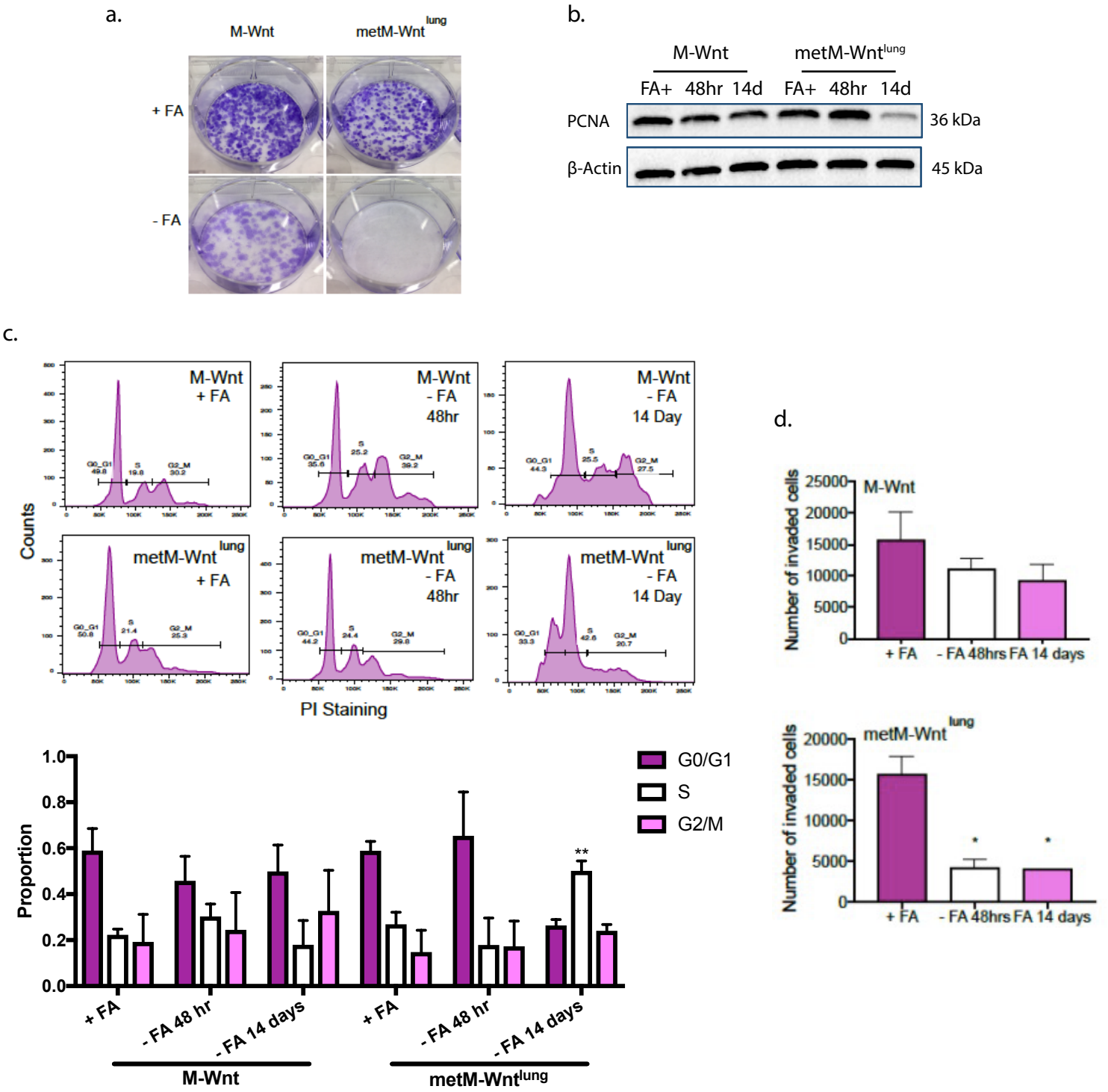

Figure 2. Differential effects of folate depletion on cell growth and invasion in M-Wnt and metM-Wnt^{lung} cells. (a) M-Wnt and metM-Wnt^{lung} cells (1,000) were seeded into each well of a 6-well plate in complete media. Media was changed to either complete media (+ FA) and media lacking folic acid (- FA). The colonies were allowed to form for 21 days. (b) Cell proliferation marker PCNA expression was determined by immuno-blotting. (c) Cells were lysed with 0.1% NP-40 and DNA was stained with 50 µg/mL propidium iodide (PI). metM-Wnt^{lung} cells showed a significant increase percentage in S phase following 14-day folate depletion (n = 3, ** $p < 0.01$). (d) *In vitro* invasion assay using matrigel showed significant decrease of cell invasion in metM-Wnt^{lung} cells, but not M-Wnt cells (n = 3, * $p < 0.05$).

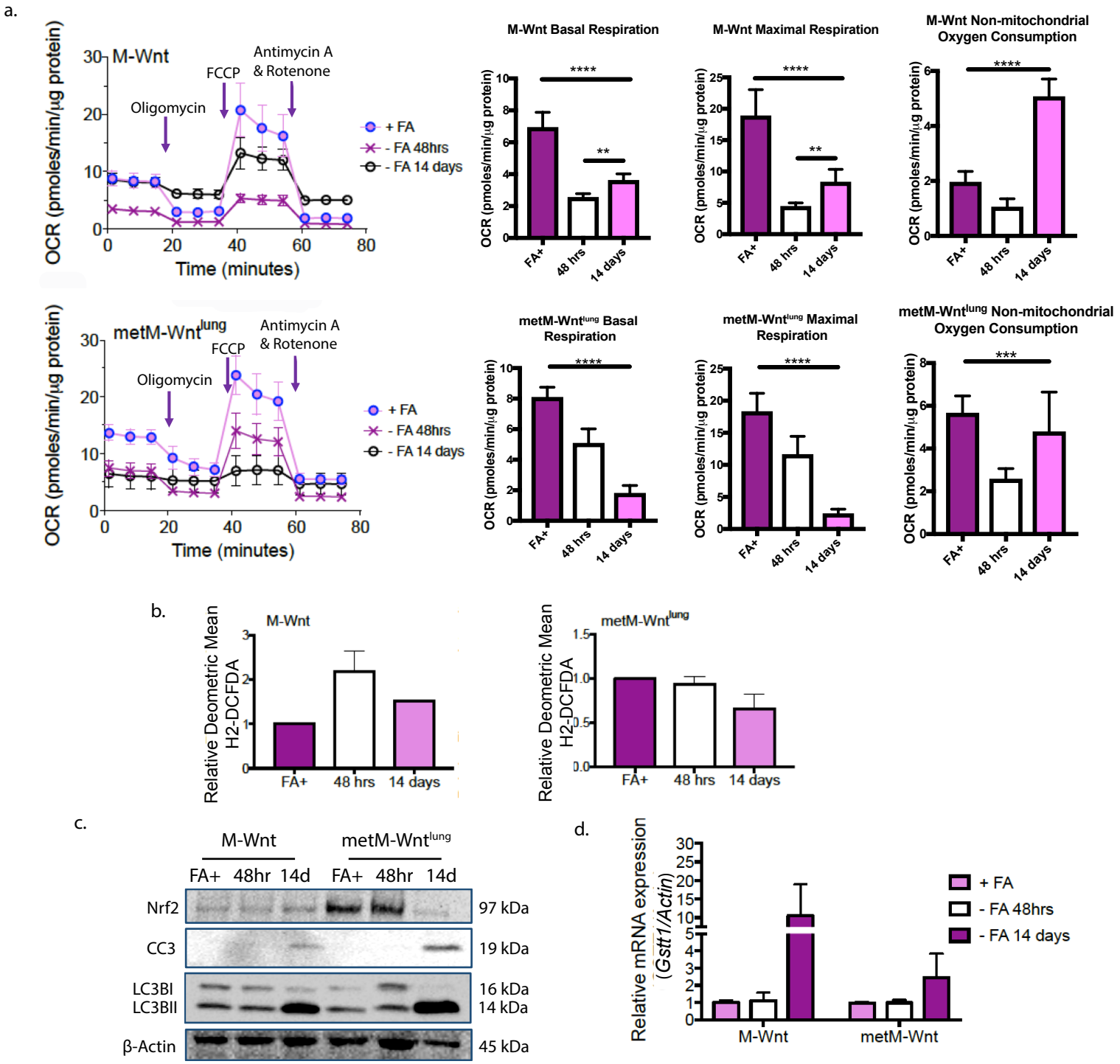

Figure 3. Cellular metabolic reprogramming that underlied differential response of folate depletion in M-Wnt and metM-Wnt^{lung} cells. (a) Mitochondrial stress tests on M-Wnt and metM-Wnt^{lung} cells were performed by sequential injections of oligomycin (1.0 µM), FCCP (1.0 µM), and rotene/antimycin A (0.5 µ M). Cellular oxidative phosphorylation was measured by oxygen consumption rates (OCR). Data were normalized by the protein amount (n = 8; **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$). (b) Endogenous ROS of cells grown in control media (FA+) and media lacking folic acid for 48-hour and 14-day were stained using a H2-DCFDA fluorescent probe. The DCF fluorescence was detected using FL-1 channel at 520 nm (n = 3). (c) Redox defense signal in cells grown with or without folic acid was determined by Nrf2 expression using immunoblotting. Autophagic flux was indicated by LC3B lipidation. Apoptosis signals were indicated by cleaved caspase 3 (CC3) expression. (d) qRT-PCR showed upregulation of *Gstt1* in M-Wnt cells, but not metM-Wnt^{lung} cells, under long-term folate depletion. mRNA levels were normalized by *β-actin* expression (n = 3).

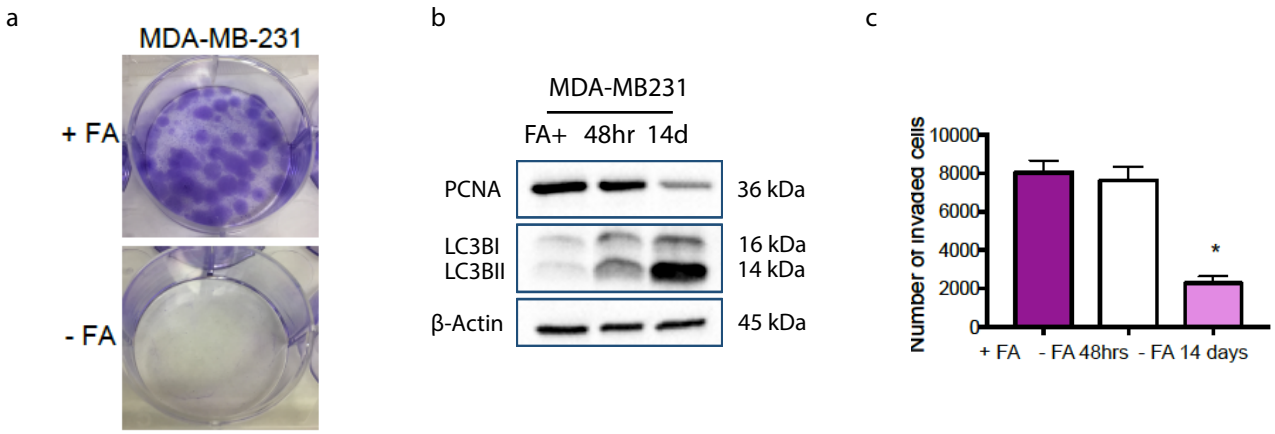

Figure 4. Folate depletion impaired growth and invasion in human metastatic TNBC cell line MDA-MB-231. (a) MDA-MB-231 cells (1,000) were seeded into each well of a 6-well plate in complete media. Media was changed to either complete media (+ FA) and media lacking folic acid (- FA). The colonies were allowed to form for 21 days. (b) In MDA-MB-231 grown in media with or without folic acid, cell proliferation marker PCNA and LC3B lipidation were determined by immunoblotting. (c) *In vitro* invasion assay using matrigel showed significant decrease of cell invasion in MDA-MB231 ($n = 3$, * $p < 0.05$).