Supplementary figure legends

Figure S1. Antifolate growth inhibition assay of HeLa cells under DMOG treatment. Growth inhibition assay was performed by exposure of HeLa cells to 1mM DMOG for 16h following which increasing concentrations of various cytotoxic drugs with or without 0.5mM DMOG was administered for additional 72h and cell survival was determined using the XTT assay. Growth inhibition was assessed using a lipophilic antifolate (A) and hydrophilic antifolates (B-C). Results are normalized to the drug-free control for each treatment and are the means of at least three independent experiments performed in triplicates ± SD.

Figure S2. Antifolate resistance under DMOG treatment is a widespread phenomenon in various carcinoma cell lines. Human carcinoma cell lines of different cell lineage were exposed to 1μM PMX in the presence or absence of 0.5mM DMOG for 72h, after which cell survival was determined by viable cell counting using Trypan blue. Viable cell counts were normalized to PMX-free controls. Results are the means of at least three independent experiments ± SD. *P-value refers to all of the examined tumor cell lines.

Figure S3. The effect of hypoxic conditions on PMX-induced PNKP S114 phosphorylation. HeLa cells were exposed to 1μM PMX under normoxia for 24h or 48h (lanes 2 and 3), 0.5mM DMOG for 48h with and without co-exposure to 1μM PMX (lanes 4 and 6), hypoxia for 48h with and without co-exposure to 1μM PMX (lanes 5 and 7) and the DNA damaging agent VP16 (40μM for 20min, lane 8). Nuclear protein extracts were subjected to Western blotting using a PNKP pS114 antibody. Equal loading was verified by reprobing with a β-actin antibody.

Figure S4. HeLa cells are arrested and viable under DMOG treatment. HeLa cells were labeled with the viable cell dye CFSE as described in the supplemental methods. CFSE content

was determined by flow cytometry after 88h normoxia, 88h DMOG and 72h after DMOG removal. The x-axis represents the intensity of green fluorescence, with the progression of cell division moving right to left owing to dilution of intracellular fluorescein. The y-axis represents the number of events. The displayed experiment is a representative of three similar independent experiments.

Figure S5. Growth inhibition assay of cytarabine under DMOG treatment. Growth inhibition assay was performed by exposure of HeLa cells to 1mM DMOG for 16h following which increasing concentrations of cytarabine in the presence or absence of 0.5mM DMOG were administered for additional 72h. Cell survival was determined using the XTT assay. Results are normalized to the drug-free control for each treatment; results depicted are the means of three independent experiments performed in triplicates ± SD.