Figure S1. Effects of TMZ and Bru on relative cell number and EGFR phosphorylation in U138MG cells under normoxic and hypoxic conditions. U138MG cells were treated with TMZ (0-300 μM) in the presence or absence of Bru (30 nM) for 72 h, and relative cell number was assessed by DAPI-based cell counting. TMZ dose-response curves were generated under (A) normoxic and (B) hypoxic conditions. Data are presented as percentages of control within each treatment condition (set to 100%). TMZ alone and TMZ plus Bru at matched TMZ concentrations were compared using unpaired two-tailed Student's t-test. (C) Representative western blots and (D) quantification of the p-EGFR/EGFR ratio under normoxic and hypoxic conditions. Bru, brusatol; TMZ, temozolomide; EGFR, epidermal growth factor receptor; p-, phosphorylated.

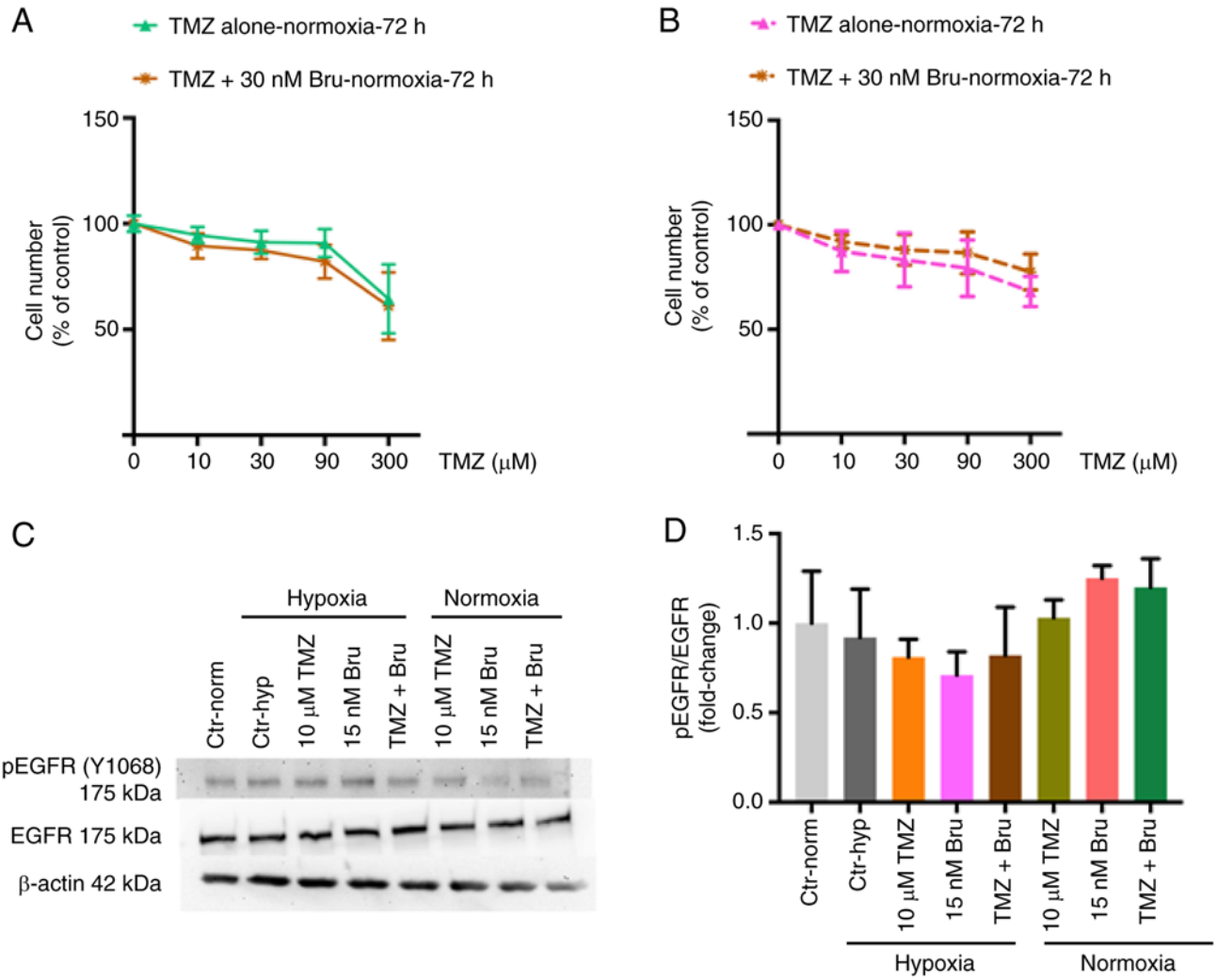

Figure S2. Hypoxia induces Nrf2 target-gene expression in U87MG cells. U87MG cells were exposed to hypoxia (1% O_2) for the indicated durations. The mRNA expression levels of the Nrf2 target genes GSTP1 and NQO1 were determined by reverse transcription-quantitative PCR. Data are presented as the mean ± SD from at least three independent experiments (n≥3). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test using normoxic cells as the reference group. *P<0.05, **P<0.01 and ***P<0.001 vs. normoxic cells. GSTP1, glutathione S-transferase pi 1; NQO1, NAD(P)H quinone oxidoreductase 1.

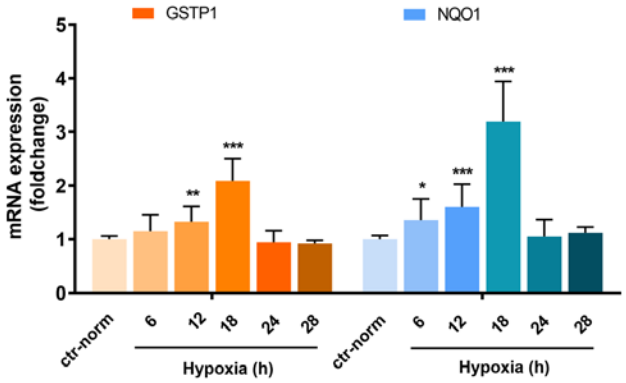

Figure S3. Effects of TMZ and Bru on gene expression in U87MG cells under normoxic conditions. U87MG cells were treated with TMZ (10 μM), Bru (15 nM), or their combination for 18 h under normoxia. Relative mRNA expression levels of the indicated genes were determined by reverse transcription-quantitative PCR and visualized as a heat map. Gene expression is shown relative to the untreated control, with blue and red indicating lower and higher expression, respectively. TMZ, temozolomide; Bru, brusatol.

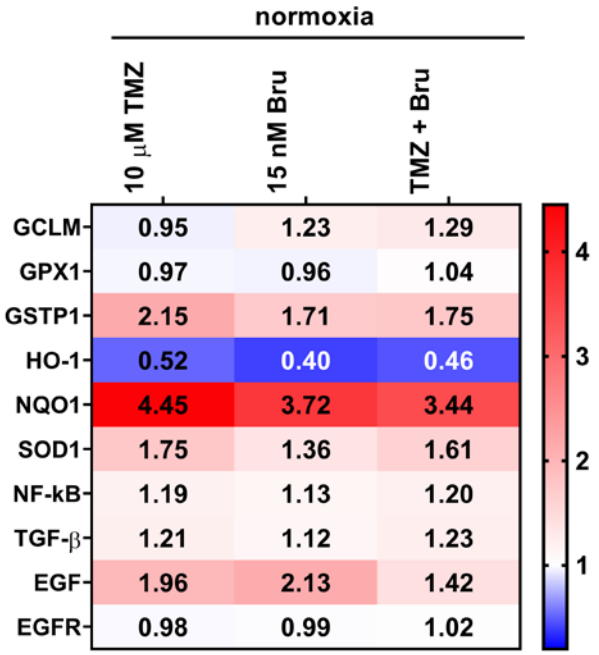

Figure S4. Effects of TMZ and Bru on PGC-1α subcellular localization in U87MG cells under normoxic conditions. U87MG cells were treated with TMZ (10 μM), Bru (15 nM), or their combination for 24 h under normoxia. (A) Representative immunofluorescence images show PGC-1α (red) and DAPI-counterstained nuclei (blue); magnification, x40 objective. Quantitative analysis includes (B) the nuclear-to-cytoplasmic fluorescence-intensity ratio and (C) nuclear, (D) cytoplasmic, and (E) whole-cell mean PGC-1α fluorescence intensity. Data are presented as the mean ± SD from at least three independent experiments. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple-comparison test vs. the normoxic control *P<0.05, **P<0.01 and ***P<0.001. TMZ, temozolomide; Bru, brusatol; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; ctr-norm, normoxic control.

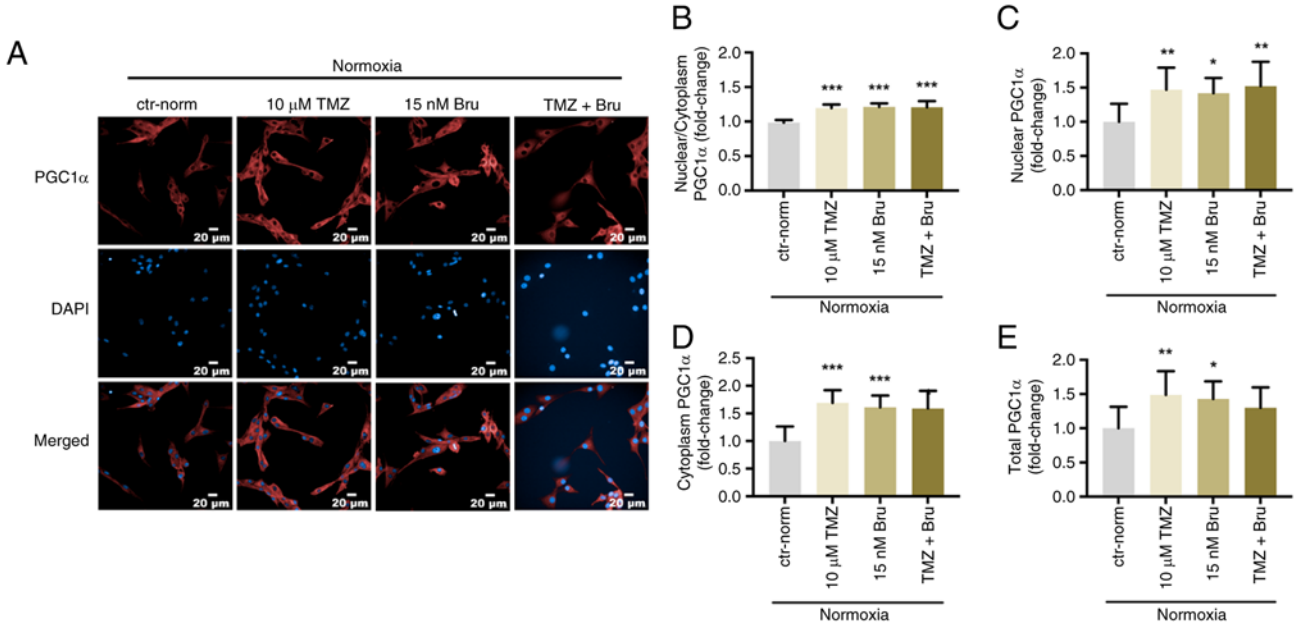

Figure S5. Effects of TMZ and brusatol on PGC-1α and downstream mitochondrial gene expression in U87MG cells under normoxic conditions. U87MG cells were treated with TMZ (10 μM), brusatol (15 nM), or their combination for 24 h under normoxia. The mRNA expression levels of (A) PGC-1α, (B) SIRT3, (C) CYCS, and (D) ATP5F1B were determined by qPCR. Data are presented as the mean ± SD from at least three independent experiments. Statistical significance was analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test vs. the normoxic control. ***P<0.001. TMZ, temozolomide; Bru, brusatol; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; SIRT3, sirtuin 3; CYCS, cytochrome c; ATP5F1B, ATP synthase F1 subunit β; qPCR, quantitative polymerase chain reaction.

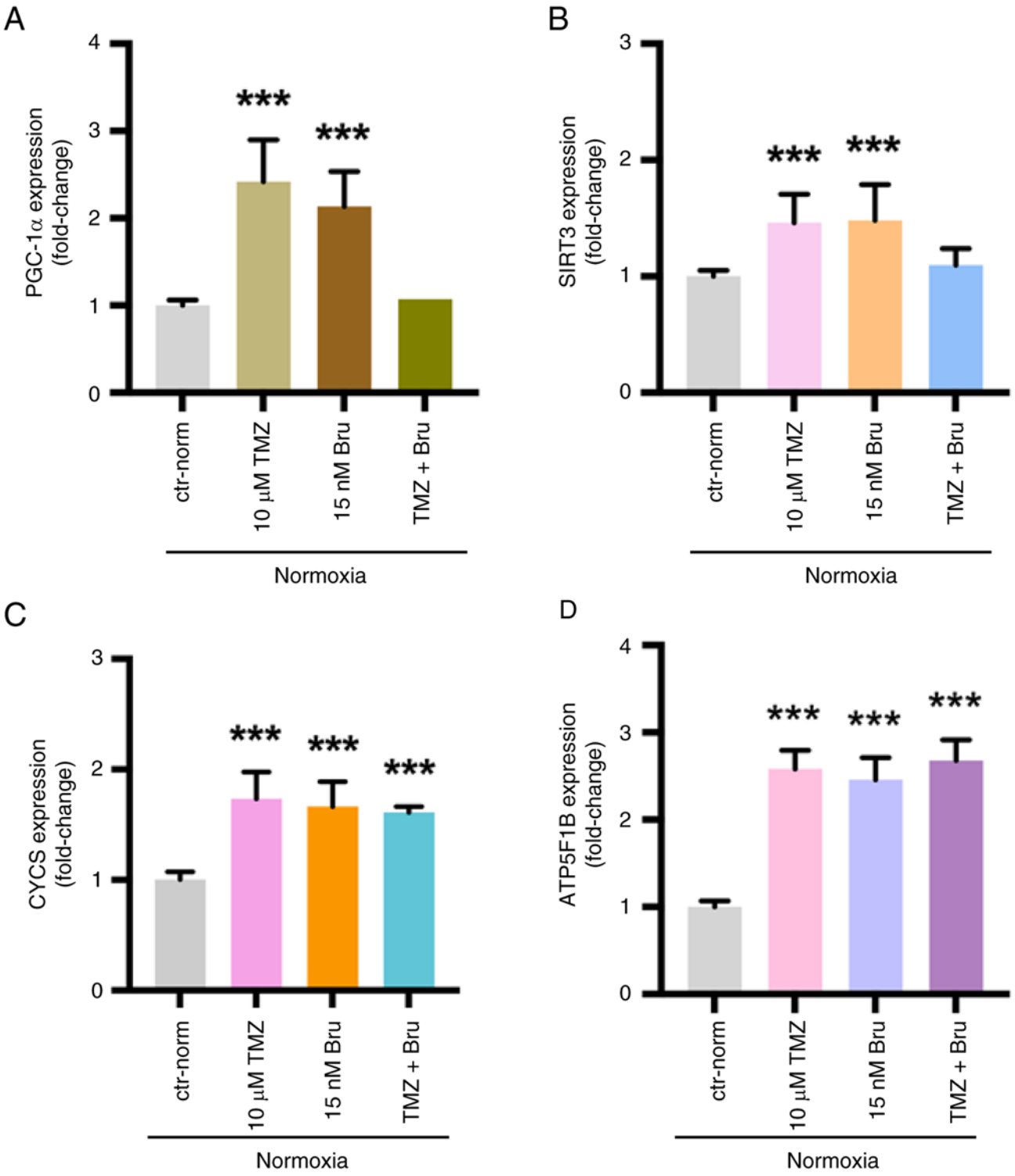

Figure S6. Effects of TMZ and Bru on oxidative DNA damage in U87MG cells under normoxic conditions. U87MG cells were treated with TMZ (10 μM), Bru (15 nM), or their combination for 24 h under normoxia. Oxidative DNA damage was assessed by immunofluorescence staining of 8-OHdG. (A) Representative fluorescence images (magnification, x40 objective) and (B) quantification of whole-cell mean 8-OHdG fluorescence intensity. Data are presented as the mean ± SD from at least three independent experiments. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple-comparison test vs. the normoxic control. *P<0.05 and **P<0.01. TMZ, temozolomide; Bru, brusatol; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.