Figure S1. Translocation of HMGB1 in glioma cells following oxidative stress. U87-MG glioma cells in normal culture medium (A) or treated with H_2O_2 (B) were labeled with the mitochondrial probe Mitotracker Red, followed by immunostaining for HMGB1. The boxed area of a cell representative of the staining is enlarged and displayed in the left upper corner. Nuclei were stained with 4'6-diamidino-2-phenylindole. It should be considered that H_2O_2 treatment increased the cytoplasmic translocation of HMGB1 and its localization on mitochondria. Scale bars, 5 μm, and for the enlarged images, 2.5 μm. HMGB1, high mobility group box 1.

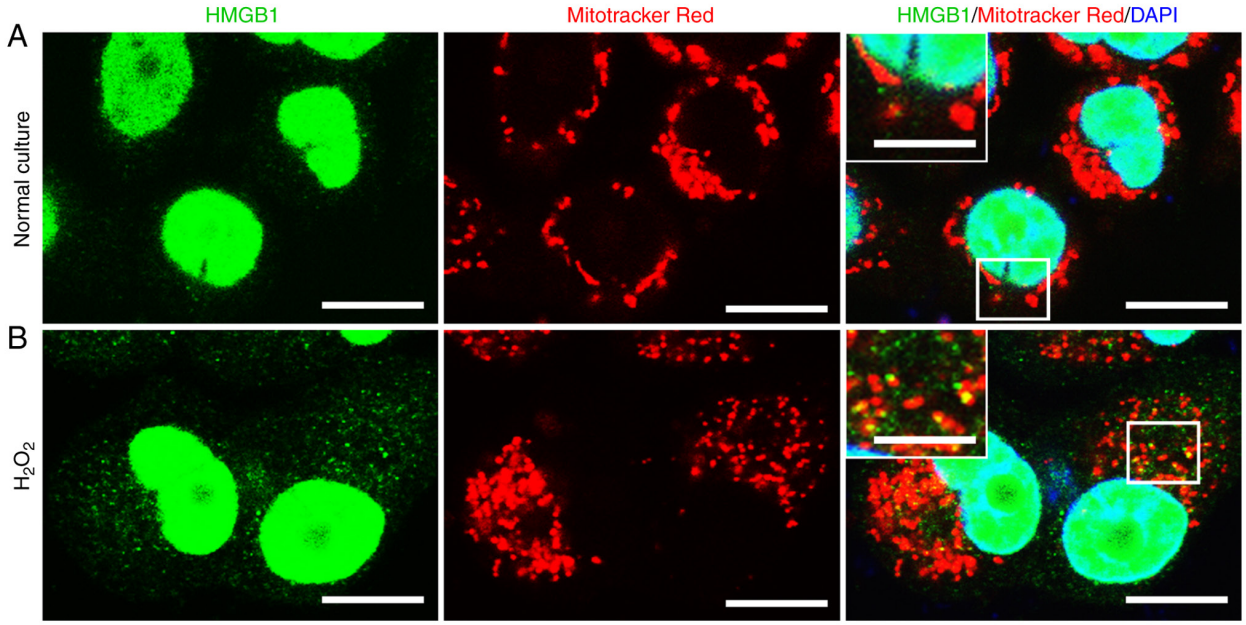

Figure S2. MAMs were increased in glioma tissues and cells. (A) MAMs (overlap between the ER and mitochondria) in HA1800 astrocytes and three glioma cell lines. ER and mitochondria were labeled with CANX and Mitotracker Red, respectively. (B) Manders' overlap coefficient between the ER and mitochondria in astrocytes and three glioma cell lines (n=3). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post-hoc test; $^{**}P<0.01$. (C) MAMs in normal brain tissues and glioma tissues. ER and mitochondria were labeled with CANX and ATP5A, respectively. Cells marked with asterisks are amplified in the boxed images. (D) Manders' overlap coefficient between the ER and mitochondria in normal brains (n=3) and glioma tissues (n=6). An unpaired Student's t-test was used to evaluate statistical significance. $^{*}P<0.05$. ns, not significant. Nuclei were stained with 4'6-diamidino-2-phenylindole. Scale bars, 5 μm, 1 μm in the boxed images. (E) Transmission electron microscopy of a case of GBM (male, 66 years). The mitochondria (M) with partial or total cristolysis were in close association with ER profiles establishing MAMs (indicated by arrows). Scale bars, 500 nm. MAMs, mitochondria-associated endoplasmic reticulum membranes; ER, endoplasmic reticulum; CANX, calnexin; ATP5A, ATP synthase F1 subunit alpha; GBM, glioblastoma multiforme.

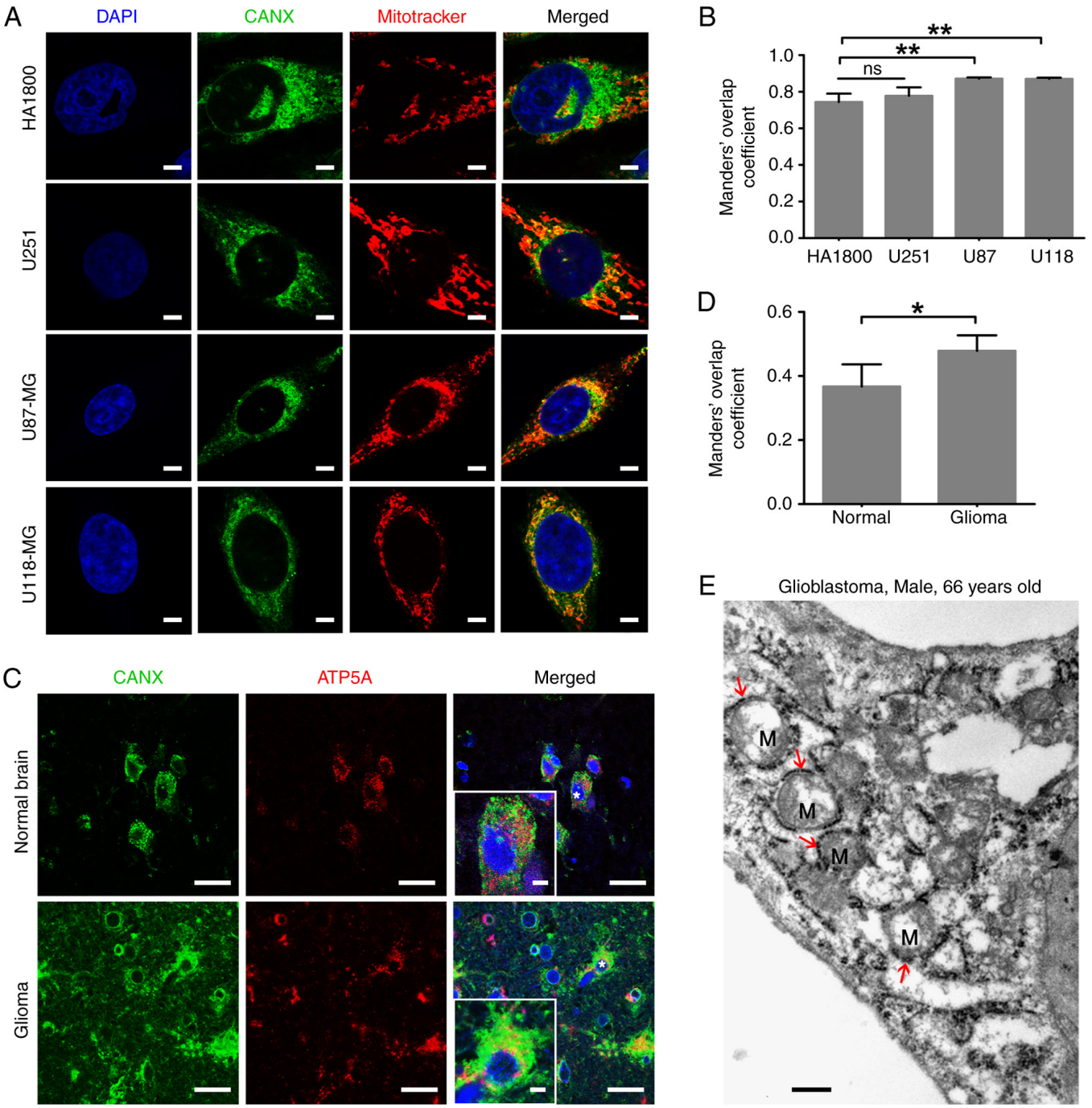

Figure S3. HMGB1 localized at MAMs in glioma cells. HBSS-treated U87-MG cells were co-stained for HMGB1, CANX and Mitotracker Red. White arrows indicate the localization of HMGB1 in MAMs. The 'inset' image is an enlarged image of the boxed region in the merged image. Scale bars, 5 μm in the lower magnified image and 2 μm in the higher magnified images. HMGB1, high mobility group box 1; MAMs, mitochondria-associated endoplasmic reticulum membranes; HBSS, Hank's balanced salt solution; CANX, calnexin.

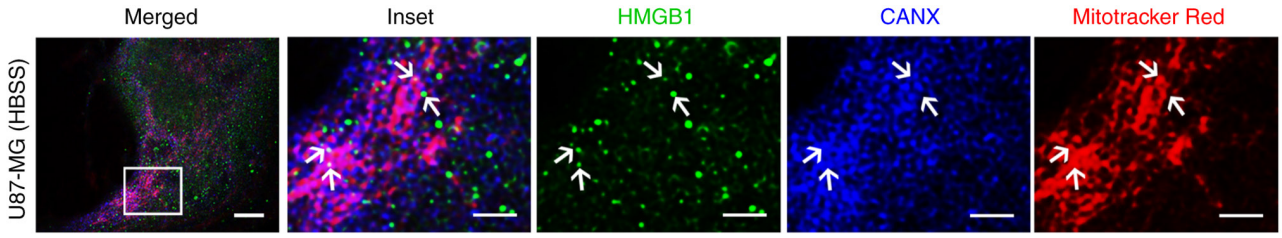

Figure S4. Overexpression of HMGB1 in U87-MG cells. Both the (A) immunocytochemistry images and (B) western blots indicated that HMGB1 was successfully overexpressed by the pHMGB1-EGFP-C1 transfection. p-EGFP-C1 was used as the transfection control. Scale bars, 50 μm; Con: control vector; OE, overexpression vector; HMGB1, high mobility group box 1; Exo-HMGB1, exogenous HMGB1; Endo-HMGB1, endogenous HMGB1. GAPDH was used as the loading control.

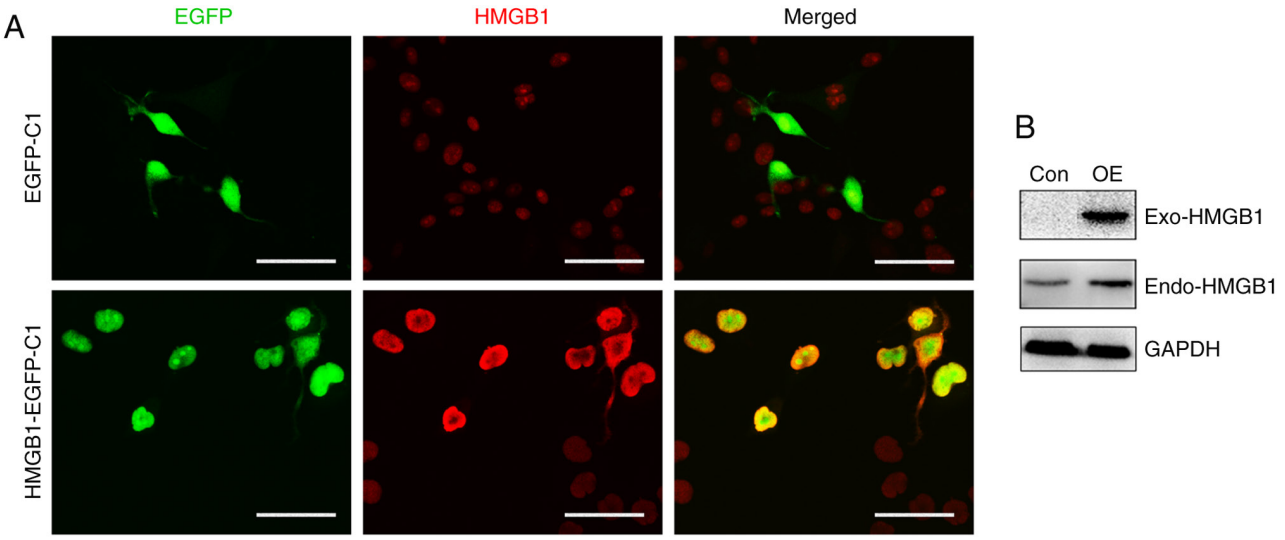

Figure S5. Starvation with HBSS induces autophagy in U251 glioma cells. (A) U251 glioma cells were treated with HBSS for 3 h, followed by immunoblotting for LC3B and P62 (n=3). (B) Statistical analysis of the ratio of LC3B-II to LC3B-I and p62 to β-actin in (A) (n=3). Following HBSS treatment, the expression of the autophagic marker, LC3B-II, was significantly increased, whereas that of the substrate of autophagy, p62, was reduced. Statistical significance was evaluated using an unpaired Student's t-test. $^{*}P<0.05$ and $^{**}P<0.01$. HBSS, Hank's balanced salt solution; LC3B, microtubule-associated proteins 1A/1B light chain 3B; P62, sequestosome 1; HBSS, Hank's balanced salt solution.