

Supplementary data

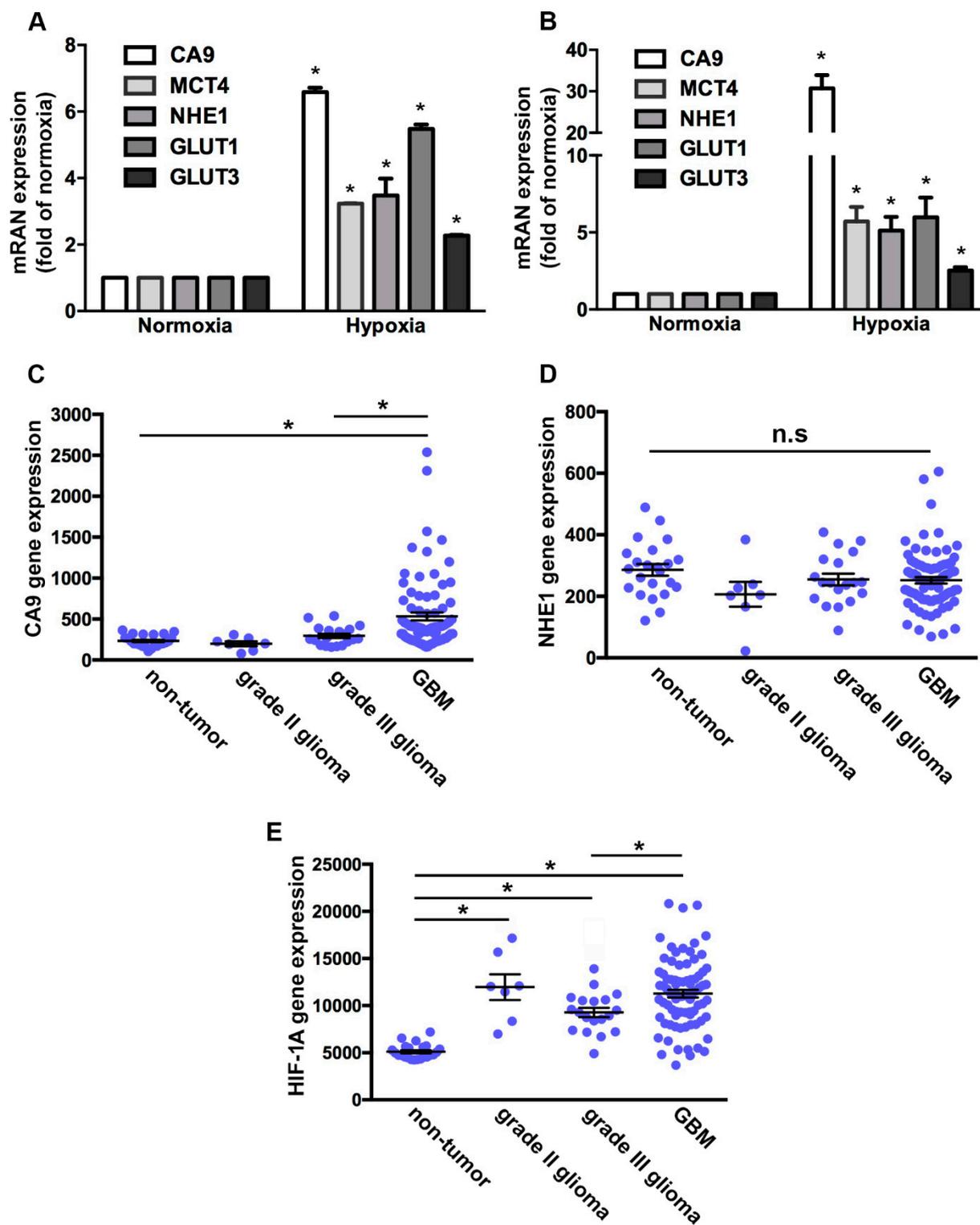


Figure S1. Hypoxia induces CAIX and pH-regulating proteins expression in GBM. U87 were exposed to hypoxic conditions (1% O₂) for 6 h (A) or 24 h (B), mRNA expressions were analyzed by qPCR for indicated proteins. **p* < 0.05 compared with the normoxia group. One-way ANOVA with a post-hoc Bonferroni test was used to examine the significance of the mean. Quantitative data are presented as mean ± s.e.m. (representative of *n* = 3). Messenger RNA levels of CAIX (C), NHE (D), and HIF-1A (E) in patients' specimens from

human glioma microarray datasets GSE4290. * $p < 0.05$ compared with the non-tumor or grade III glioma. ns: not significant. One-way ANOVA with a post-hoc Bonferroni test was used to examine the significance of the mean.

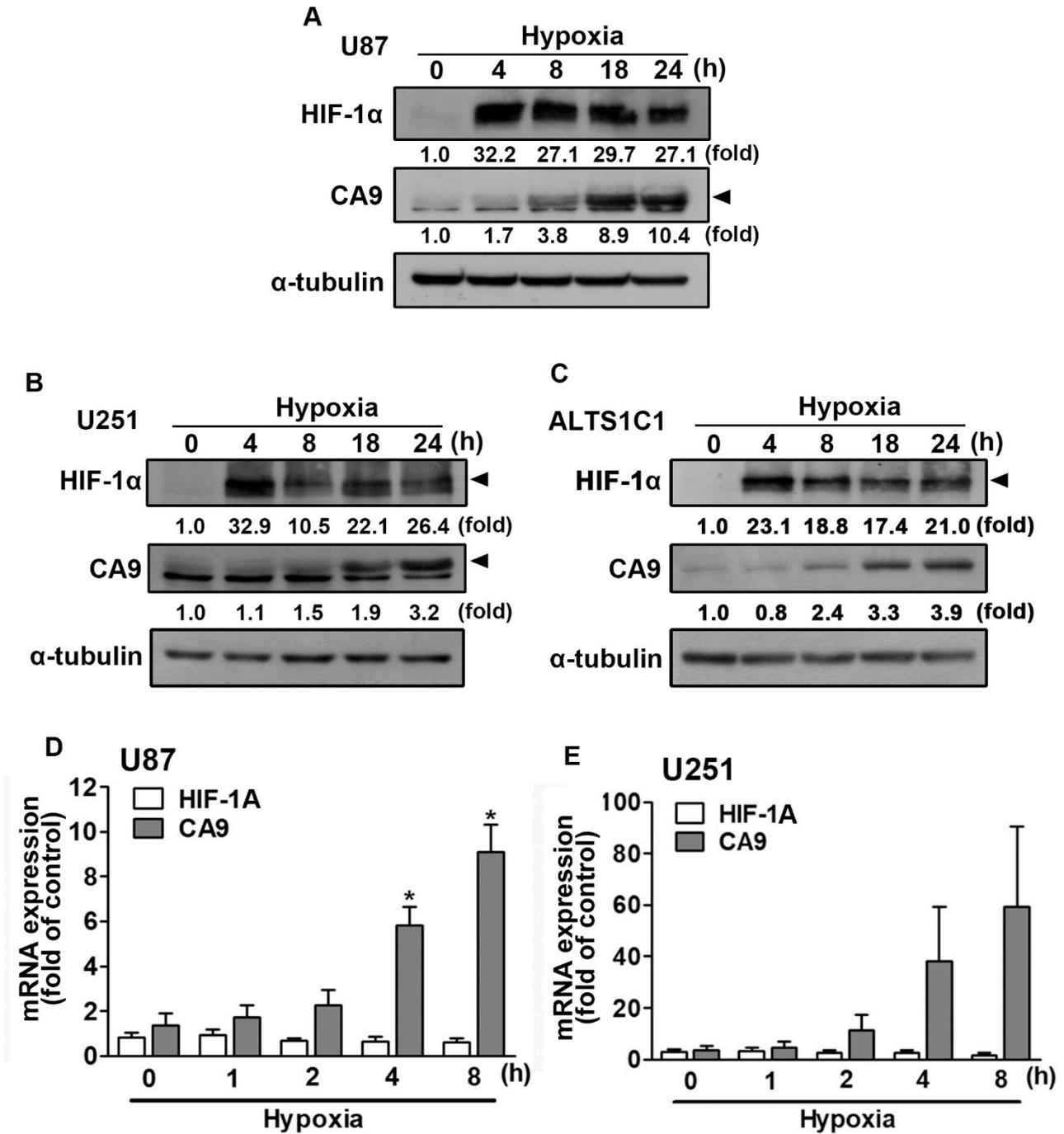


Figure S2. Hypoxia induces HIF-1 α and CAIX expression in GBM. U87 (A), U251 (B), and ALTS1C1 (C) were exposed to hypoxic conditions (1% O₂) for indicated time periods (4, 8, 16, or 24 h). HIF-1 α and CAIX protein expression were determined by western blotting using whole cell lysates. U87 (D) and U251 (E) were exposed to hypoxia condition (1% O₂) for indicated time periods (1, 2, 4, or 8 h), and *HIF-1A* and *CAIX* mRNA expression were determined using qPCR. Each time points was compared with the same time periods of normoxic conditions. * $p < 0.05$ compared with the control group. Quantitative data are presented as mean \pm s.e.m. (representative of $n = 3$).

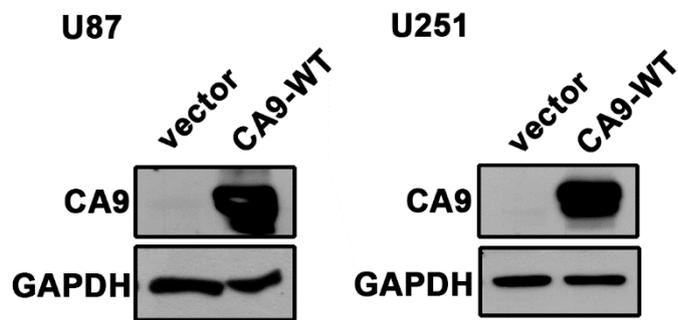


Figure S3. Overexpression of CAIX in human GBM cells. U87 and U251 cells were transfected with empty vector or wild-type plasmid of CAIX for 24 h and subsequently exposed to hypoxic conditions (1 % O₂) for another 24 h. CAIX expression was determined by western blotting using whole cell lysates.

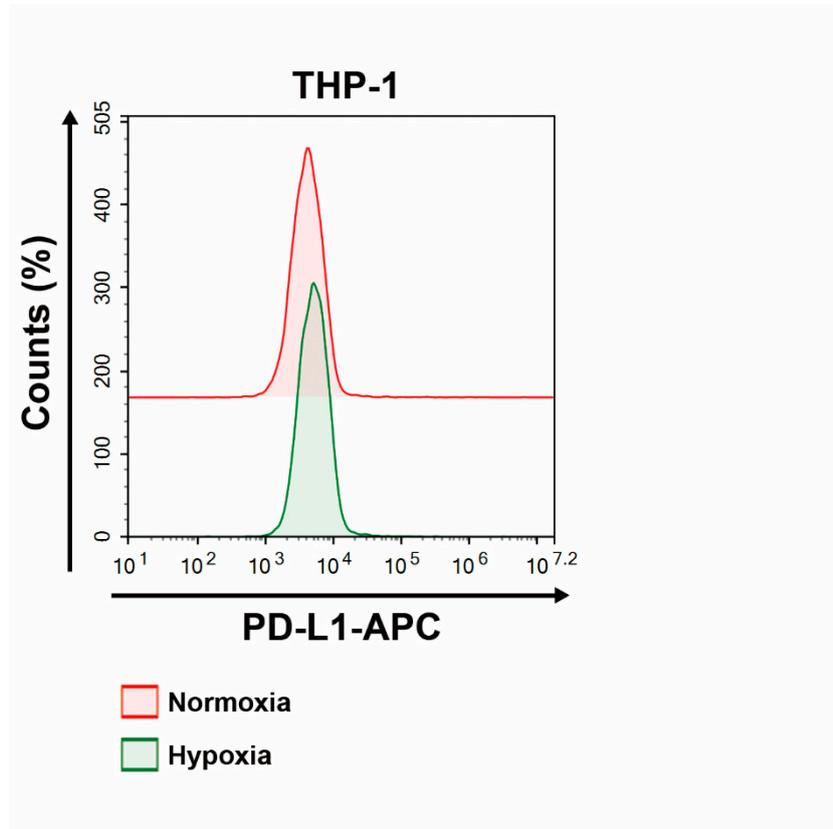


Figure S4. Expression of PD-L1 on THP-1 monocytes under hypoxic conditions. THP-1 monocytes were incubated under hypoxic or normoxic conditions for 48 h. The levels of programmed death ligand 1 (PD-L1) were determined by flow cytometry.