**Supplementary information**

# Hypoxia-induced ROS aggravate tumor progression through HIF-1α-SERPINE1 signaling in glioblastoma

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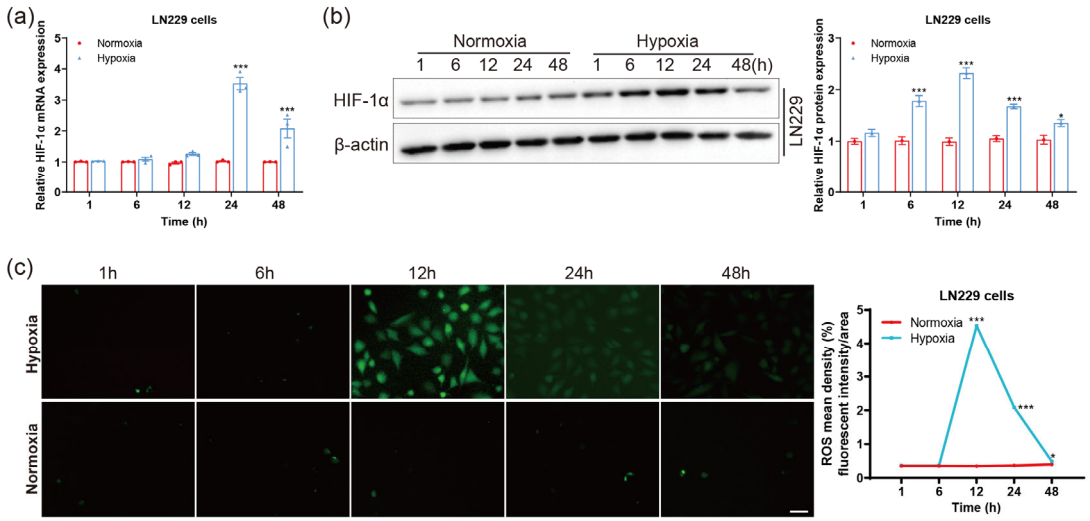


**Fig. S1 Hypoxia promotes HIF-1α expression and ROS production in LN229 cells. (a) q-PCR and (b) western blotting were performed to detect HIF-1α mRNA and protein expression under normoxic or hypoxic conditions for 1, 6, 12, 24, and 48 h. (c) ROS levels were assessed by fluorescence at indicated time intervals after normoxic or hypoxic exposure. Scale bar: 100 μm. ($^{*}$ *P*<0.05; $^{***}$ *P*<0.001).**

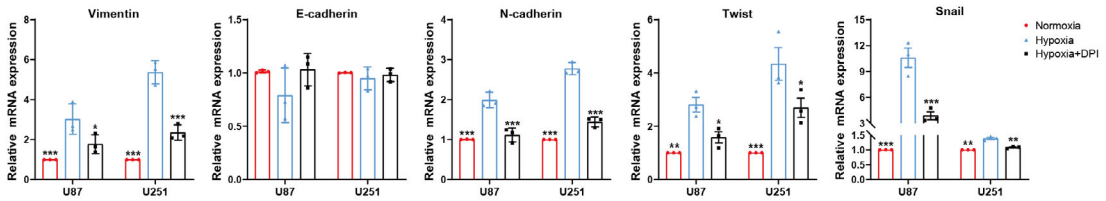


**Fig. S2 Hypoxia contributes to EMT. The mRNA expression of EMT-related proteins was examined by q-PCR in U87 and U251 cells. Cells were treated with or without 10 μmol/L DPI for 4 h, and then subjected to hypoxia for 24 h. The normoxia group was used as negative control. ($^{*}$ *P*<0.05; $^{**}$ *P*<0.01; $^{***}$ *P*<0.001).**

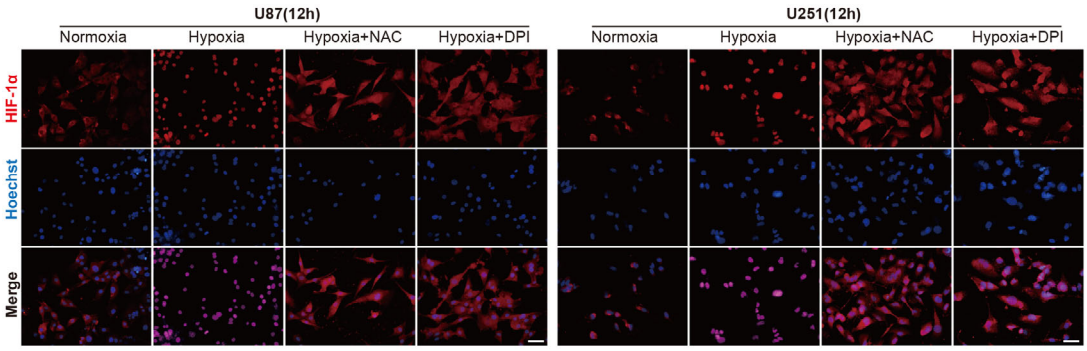


**Fig. S3 ROS are involved in HIF-1α nuclear accumulation induced by hypoxia. Immunostaining was performed to determine the localization of HIF-1α in U87 and U251 cells. Cells were incubated in NAC (5 mmol/L) or DPI (10 μmol/L) for 4 h and then in hypoxic environments for 12 h. Scale bar: 25 μm.**

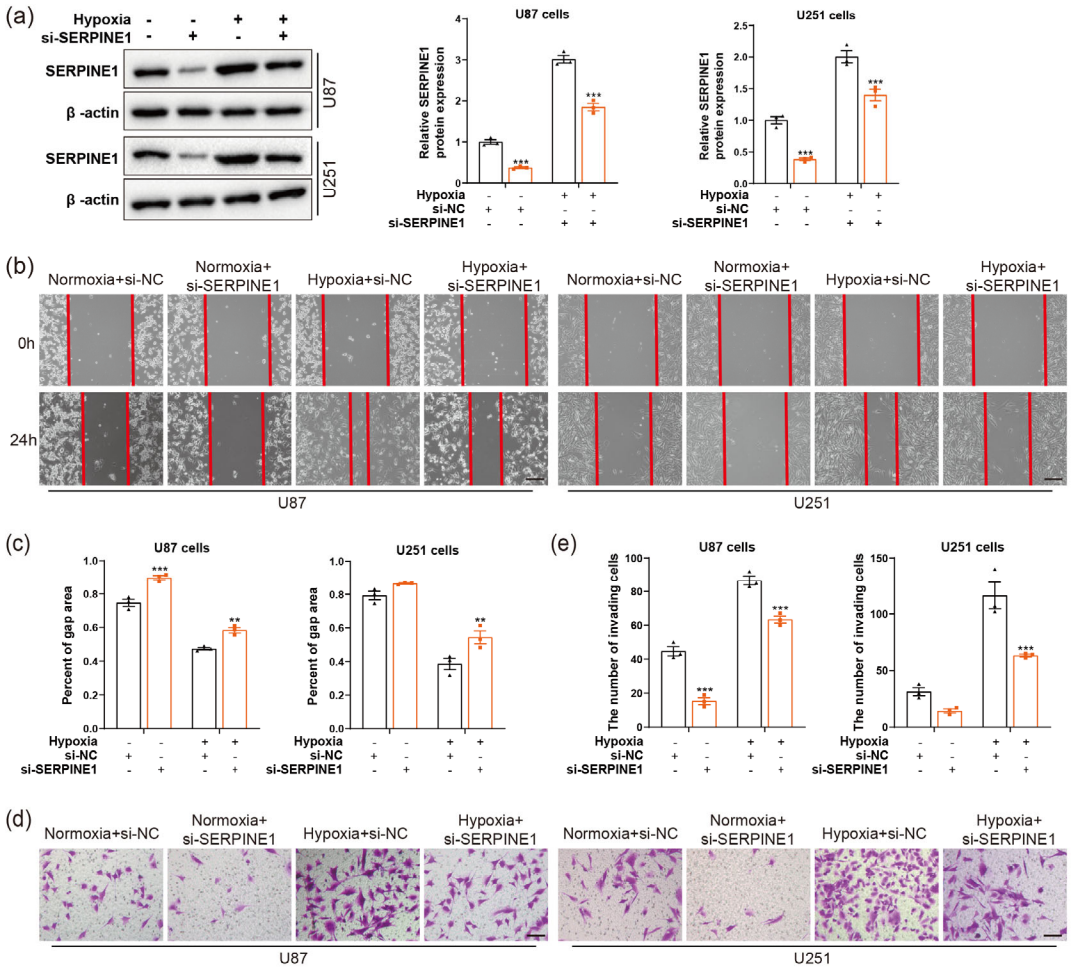


**Fig. S4 SERPINE1 knockdown inhibits cell migration and invasion enhanced by hypoxia in glioblastoma cells. (a) The expression of SERPINE1 was measured by western blotting in normoxia or hypoxia when SERPINE1 was knockdowned in U87 and U251 cells. (b, c) Scratch wound assay was used to analyze the effect of SERPINE1 knockdown on cell migration under normoxic or hypoxic conditions. Scale bar: 200 μm. (d, e) Transwell matrigel assay was conducted to assess the effect of SERPINE1 knockdown on cell invasion under normoxic or hypoxic environments. Scale bar: 100 μm ($^{**}$ *P*<0.01; $^{***}$ *P*<0.001).**

**Table S1 Primer sequences for qPCR**

| Gene | Forward sequence | Reverse sequence |
|---|---|---|
| HIF-1α | 5'-TAAAGGAATTTCAATATTTGATGGG-3' | 5'-AAAGGGTAAAGAACAAAACACACAG-3' |
| SERPINE1 | 5'-GAGACCAAGAGCCTCTCCAC-3' | 5'-GGTTCCATCACTTGGCCCAT-3' |
| ZEB1 | 5'-GTGGCGGTAGATGGTAAT-3' | 5'-GGAAGACTGATGGCTGAA-3' |
| Twist | 5'-GTCCGCAG-TCTTACGAGGAG-3' | 5'-CCAGCTTGAGGGTCTGAATC-3' |
| E-cadherin | 5'-TTGCTACTGGAACAGGGACAC-3' | 5'-CCCGTGTGTTAG-TTCTGCTGT-3' |
| N-cadherin | 5'-TTATCCTTGTGCTGATGTTTGTG-3' | 5'-TCTTCTTCTCCTCCACCTTCTTC-3' |
| Vimentin | 5'-GAGAACTTTGCCGTTGAAGC-3' | 5'-TCCAGCAGCTTCCTGTAG-3' |
| Snail | 5'-CTTCCAGCAGCCCTACGA-3' | 5'-AGCCTTTCCCACTGTCCTC-3' |
| β-actin | 5'-CCCCGCGAGCACAGAG-3' | 5'-TCATCATCCATGGTGAGCTGG-3' |

**Table S2 Antibody information**

| Antibody name | Application in this work | Catalog number | Company |
|---|---|---|---|
| HIF-1α | Western blotting and IF | AF1009 | AFFINITY |
| SERPINE1 | Western blotting | 66261-1-Ig | Proteintech |
| β-catenin | Western blotting | 51067-2-AP | Proteintech |
| E-cadherin | Western blotting | 20874-1-AP | Proteintech |
| N-cadherin | Western blotting | 22018-1-AP | Proteintech |
| Vimentin | Western blotting | 10366-1-AP | Proteintech |
| LaminA/C | Western blotting | BA1227 | Boster |
| β-actin | Western blotting | GB11001 | Servicebio |

**Table S3 siRNA oligo sequences**

| siRNA name | Sense | Antisense |
|---|---|---|
| si-SERPINE1-NC | UUCUCCGAACGUGUCACGUTT | ACGUGACACGUUCGGAGAATT |
| si-SERPINE1-#1 | CCCUCCGGCAUCUGUACAATT | UUGUACAGAUGCCGGAGGGTT |
| si-SERPINE1-#2 | CCGGAGCACGGUCAAGCAATT | UUGCUUGACCGUGCUCCGGTT |
| si-SERPINE1-#3 | GCCACUGGAAAGGCAACAUTT | AUGUUGCCUUUCCAGUGGCTT |