

Figure S1. P1 glioma cells were treated with MitoBloCK-10 (MB-10, for 25 μ M) or the vehicle control ("Veh", 0.1% DMSO), and cells were further cultivated for designated hours; Cell viability, proliferation, migration and invasion were examined by CCK-8 (**A**), nuclear EdU staining (**B**), Transwell (**C**) and "Matrigel Transwell" (**D**) assays, respectively. Mitochondrial depolarization (by measuring JC-1 green monomers, **E**), CellROX intensity OD (**F**) and cell apoptosis (TUNEL assays, **G**) were tested as well. The data were presented as mean \pm standard deviation (SD). * $P < 0.05 \ vs.$ "Veh". Experiments were repeated five times with similar results obtained. Scale bar = 100 μ m.

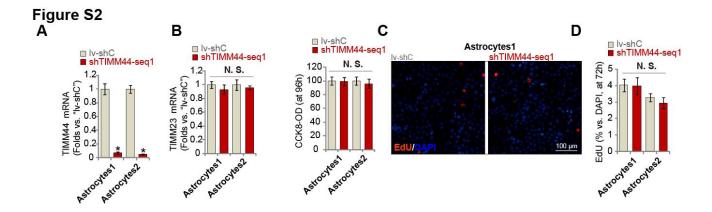


Figure S2. Expression of listed mRNAs in the primary human astrocytes ("Astrocytes1/2", derived from two patients) with the TIMM44 shRNA ("shTIMM44-seq1") or the lentiviral scramble control shRNA ("lv-shC") was shown (**A** and **B**). Astrocytes were further cultivated for designated hours, and cell viability (**C**) and proliferation (**D**) were tested. The data were presented as mean \pm standard deviation (SD). * $P < 0.05 \ vs.$ "lv-shC". "N.S." stands for non-statistical difference (P > 0.05). The *in vitro* experiments were repeated five times with similar results obtained. Scale bar = 100 µm.

Figure S3 □ Iv-shC■ shTIMM44-seq1 Α P1 glioma cells Ε С □ lv-shC **]■** shTIMM44-seq1 (Folds vs. "Pare" at 48h) ■PBS ■ATP ■NAC Mito-complex I activity 1 0.6 CellROX OD, at 48h) CCK8-OD (at 96h) 0.8 0.4 0.8 0.6 0.4 0.2 0.4 0.2 0 0 A172 U251 P2 Р3 P2 P3 A172 U251 lv-shC shTIMM44-seq1 Pirmary glioma cells Pirmary glioma cells □ Iv-shC ■ shTIMM44-seq1 В D □ Iv-shC TUNEL (% vs. DAPI, at 96h) (Folds vs. "Iv-shC" at 48h) shTIMM4 P1 glioma cells 40-1 Trypan blue%, at 96h PBS 8.0 ATP contents ATP NAC 30-0.6 # * I 20-0.4 0.2 10-

P2

0

lv-shC shTIMM44-seq1

P3

Pirmary glioma cells

A172

U251

P2

Р3

Pirmary glioma cells

A172 U251

Figure S3. P1 glioma cells with the TIMM44 shRNA (shTIMM44-seq1) or the lentiviral scramble control shRNA ("lv-shC") were treated with ATP (2 mM), N-Acetyl-L-cysteine (NAC, 400 µ M) or PBS for 96h, cell viability (A) and death (B) were examined by CCK-8 and Trypan blue staining assays, respectively. The primary glioma cells ("P2" and "P3") or established lines (A172 and U251) with the TIMM44 shRNA (shTIMM44-seq1) or the lentiviral scramble control shRNA ("lv-shC") were cultivated for designated hours, the relative mitochondrial complex I activity (C), cellular ATP contents (D), CellROX intensity OD (E) and nuclear TUNEL ratio (F) were tested. The data were presented as mean \pm standard deviation (SD). * P < 0.05 vs. "lv-shC". # P < 0.05. "PBS" (A and B). The in vitro experiments were repeated five times with similar results obtained.

Figure S4

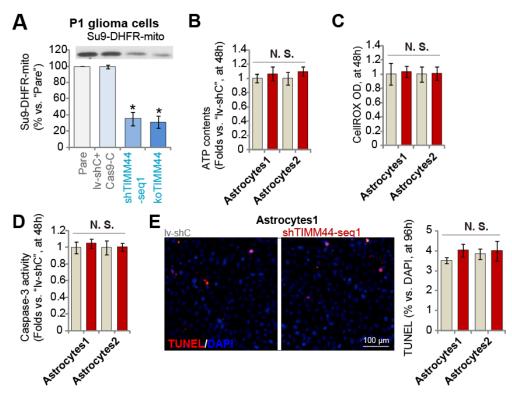


Figure S4. The isolated mitochondria (200 μg/mL) of P1 glioma cells with applied genetic modifications was incubated with [35 S]-methionine and cysteine-labeled precursor Su9-DHFR protein, and mitochondria protein import was stopped by adding trypsin after 15 min. The mitochondrial Su9-DHFR protein ("Su9-DHFR-mito") was tested by SDS-PAGE and autoradiography (**A**), the relative level of Su9-DHFR-mito was quantified from five repeats (**A**). The primary human astrocytes ("Astrocytes1/2", derived from two patients) with the TIMM44 shRNA ("shTIMM44-seq1") or the lentiviral scramble control shRNA ("lv-shC") were cultivated for designated hours, cellular ATP contents (**B**), CellROX intensity OD (**C**), Caspase-3 activity (**D**) and cell apoptosis (**E**) were tested. The data were presented as mean \pm standard deviation (SD). * P < 0.05 vs. "Pare". "N. S." stands for non-statistical difference (P > 0.05). The *in vitro* experiments were repeated five times with similar results obtained. Scale bar = 100 μm.

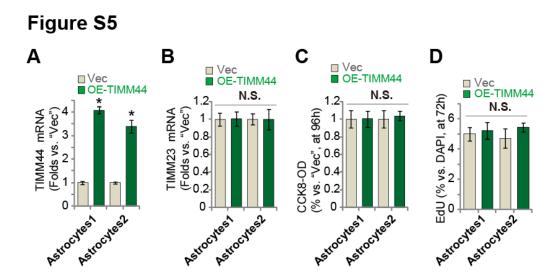


Figure S5. Expression of listed mRNAs in the primary human astrocytes ("Astrocytes1/2", derived from two patients) with the lentiviral TIMM44-expressing vector ("OE-TIMM44") or the empty vector ("Vec") was shown (**A** and **B**). Astrocytes were further cultivated for designated hours, and cell viability (**C**) and EdU-positive nuclei ratio (**D**) were tested. The data were presented as mean \pm standard deviation (SD). * P < 0.05 vs. "Vec". "N.S." stands for non-statistical difference (P > 0.05). The *in vitro* experiments were repeated five times with similar results obtained.

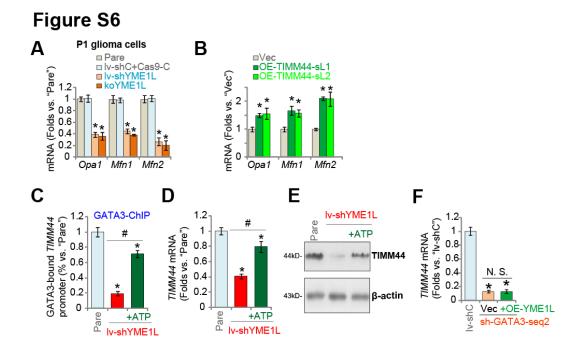


Figure S6. P1 primary human glioma cells, stably expressing the YME1L shRNA (lv-shYME1L), the lenti-CRSIPR/Cas9-YME1L-KO-puro construct ("koYME1L"), the lentiviral scramble shRNA plus the CRSIPR/Cas9 empty vector ("lv-shC+Cas9-C"), the YME1L-expressing construct ("OE-YME1L-sL1 or OE-YME1L-sL2", two stable selections) or the empty vector ("Vec"), were established, expression of listed mRNAs was shown (A and B). P1 primary human glioma cells, stably expressing the YME1L shRNA (lv-shYME1L) were treated with or without ATP (2 mM) for 24h, chromosome IP (ChIP) showed the relative levels of the proposed *TIMM44* promoter binding to GATA3 protein in the nuclei (C); Expression of *TIMM44* mRNA (D) and listed proteins (E) was tested. The sh-GATA3-seq2-expressing P1 glioma cells were further transduced with or without the lentiviral YME1L-expressing construct ("+OE-YME1L"), stable cells were formed by selection, and expression of *TIMM44* mRNAwas tested (F). The data were presented as mean ± standard deviation (SD). * P < 0.05 vs. "Pare"/"Vec"/"Iv-shC". * P < 0.05. "N.S." stands for non-statistical difference (P > 0.05). The experiments were repeated five times with similar results obtained.