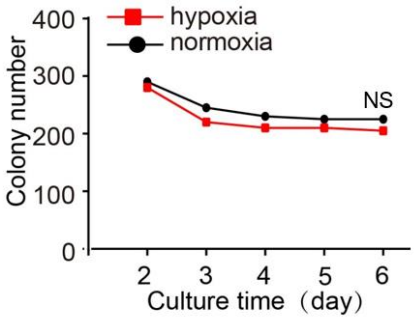
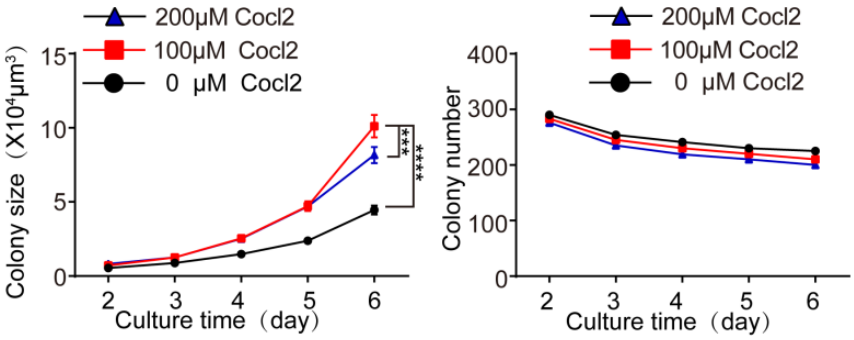
Supplemental Figure S1



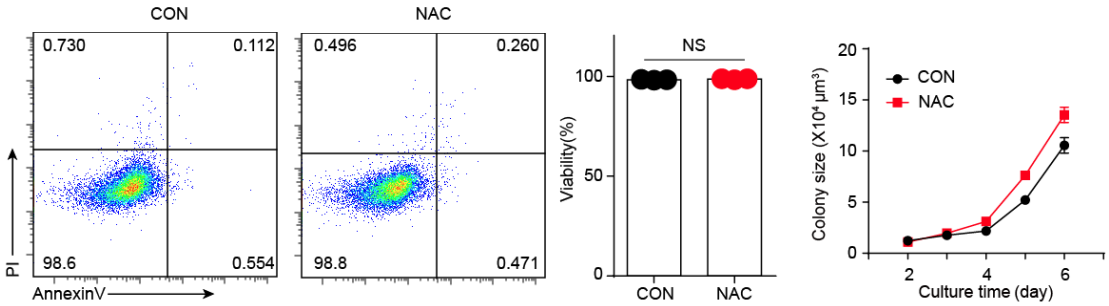
Supplemental Figure S1, related to Figure 2. MCF-7 cells were seeded in soft 3D fibrin gels under 21% O_2 (normoxia) or 1% O_2 (hypoxia) for 6 days, the colony number was calculated. NS, no significance (Student's t-test).

Supplemental Figure S2



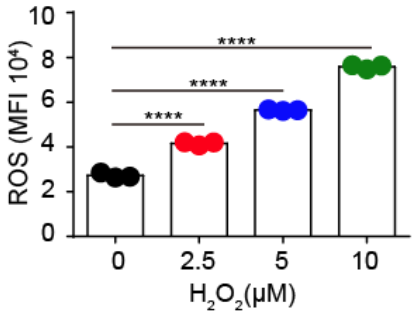
Supplemental figure S2, related to Figure 2. MCF-7 TRCs were cultured in different concentration of $CoCl_2$ induced hypoxia, the colony size and number were calculated. Data shown are representative of three independent experiments and error bars represent mean ± SEM. ***p< 0.001, ****p< 0.0001 (Student's t-test).

Supplemental figure S3



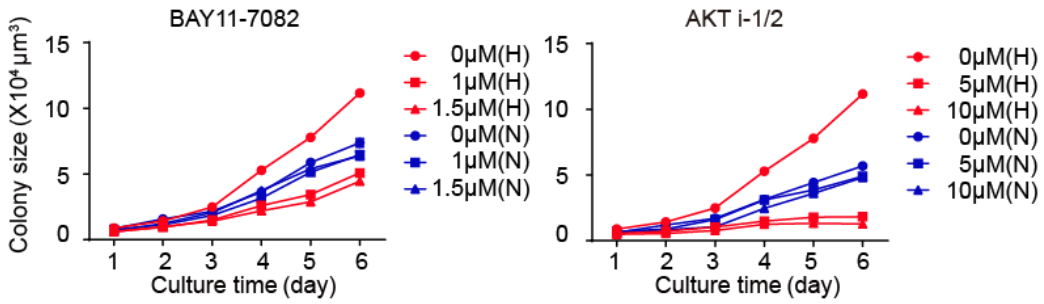
Supplemental figure S3, related to figure 3. MCF-7 cells were cultured in 90 Pa soft 3D fibrin gels under normoxic conditions in the presence of 20 mM NAC for 5 days，cells were collected and the apoptosis was analyzed by PI and AnnexinV staining as described in methods, and the colony size was also analyzed.

Supplemental figure S4



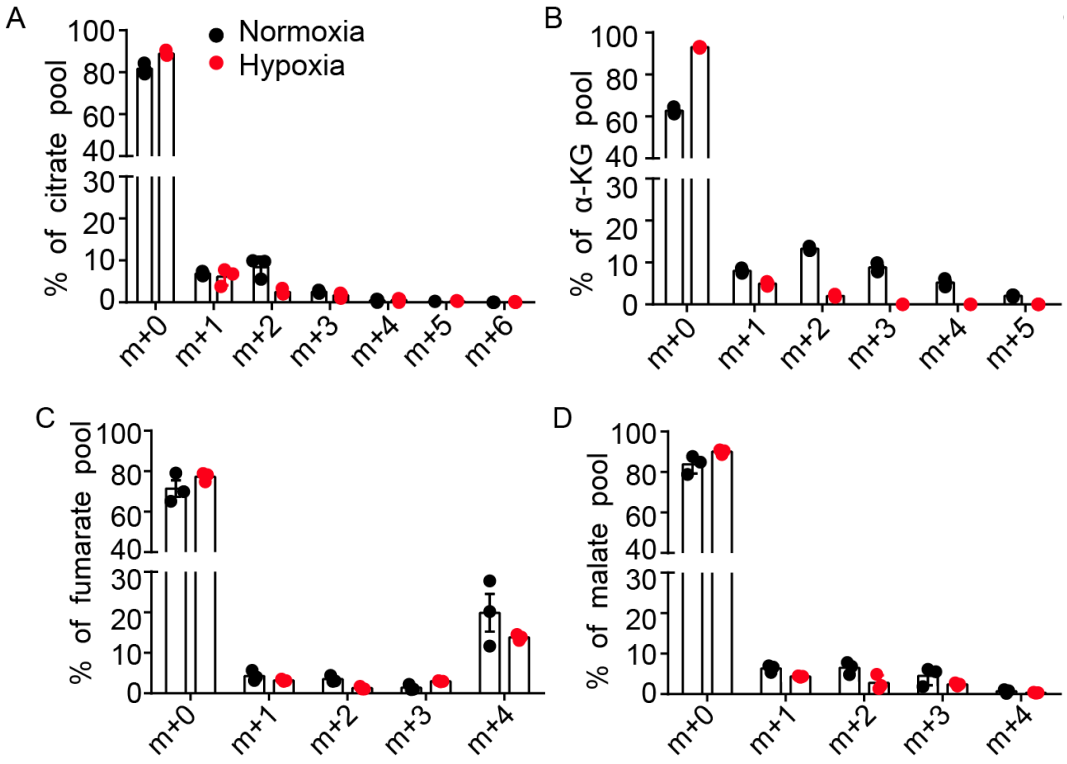
Supplemental figure S4, related to Figure 3. MCF-7 TRCs were treated with H_2O_2 (2.5 μM, 5.0 μM or 10 μM) under normoxic condition for 24 hours, then ROS levels were detected by flow cytometry. Data shown are representative of three independent experiments and error bars represent mean ± SEM. ****$p < 0.0001$ (Student's t-test).

Supplemental Figure S5



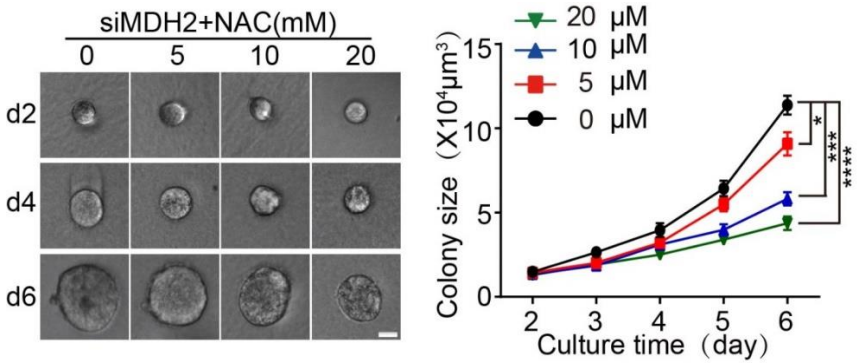
Supplemental Figure S5, related to Figure 3. MCF-7 TRCs were treated with BAY11-7082 or AKTi-1/2 in different dose under normoxic (N) or hypoxic (H) condition, the colony size was calculated.

Supplemental Figure S6



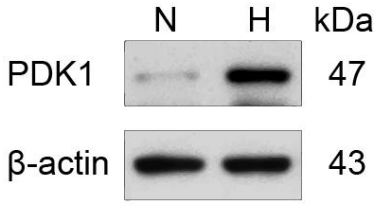
Supplemental Figure S6, related to Figure 4. MCF-7 TRCs were cultured in normoxic (21% O_2) or hypoxia (1% O_2) conditions. On day 4 of culture, cells were washed, then cultured with [U6]-^{13}C glucose for 24 hours. Cells were washed twice in saline and lysed in extraction solvent (80% methanol/20% water) for 30 min at -80℃. After centrifugation at 13,000 g, 10 min at 4℃, supernatant extracts for analysis of the mass isotopomer distribution of citrate (A), a-KG (B), fumarate(C) and malate (D)were analyzed by LC/MS/MS.

Supplemental Figure S7



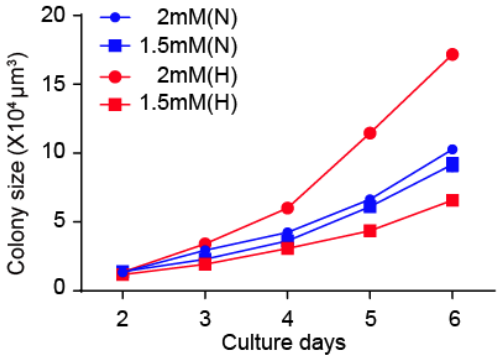
Supplemental Figure S7, related to Figure 4. MCF-7 TRCs were transfected with scramble or MDH2 siRNA，at the same time，added different concentration NAC for the indicated days. The colony size is presented photographically and graphically. Data shown are representative of three independent experiments and error bars represent mean ± SEM. Bar 20μm. *p< 0.05, ***p< 0.001, ****p< 0.0001 (Student's t-test).

Supplemental Figure S8



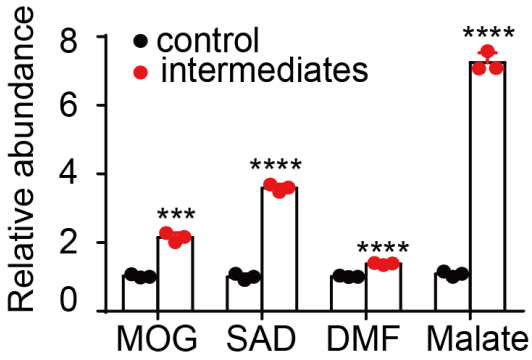
Supplemental Figure S8, related to figure 5. MCF-7 cells were seeded in 90 Pa soft 3D fibrin gels under normoxic and hypoxic conditions for 5 days, then cells were collected and PDK1 expression was detected by western blotting.

Supplemental Figure S9



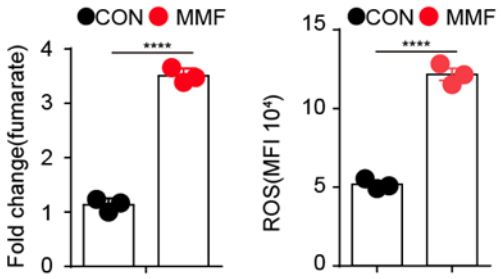
Supplementary Fig. S9, related to figure 5. MCF-7 TRCs were cultured in normoxia (N, 21%O_2) or hypoxia (H, 1%O_2) in the different doses of glutamine. The colony size was analyzed from day 2 to 6. Data shown are representative of three independent experiments and error bars represent mean ± SEM.

Supplemental Figure S10



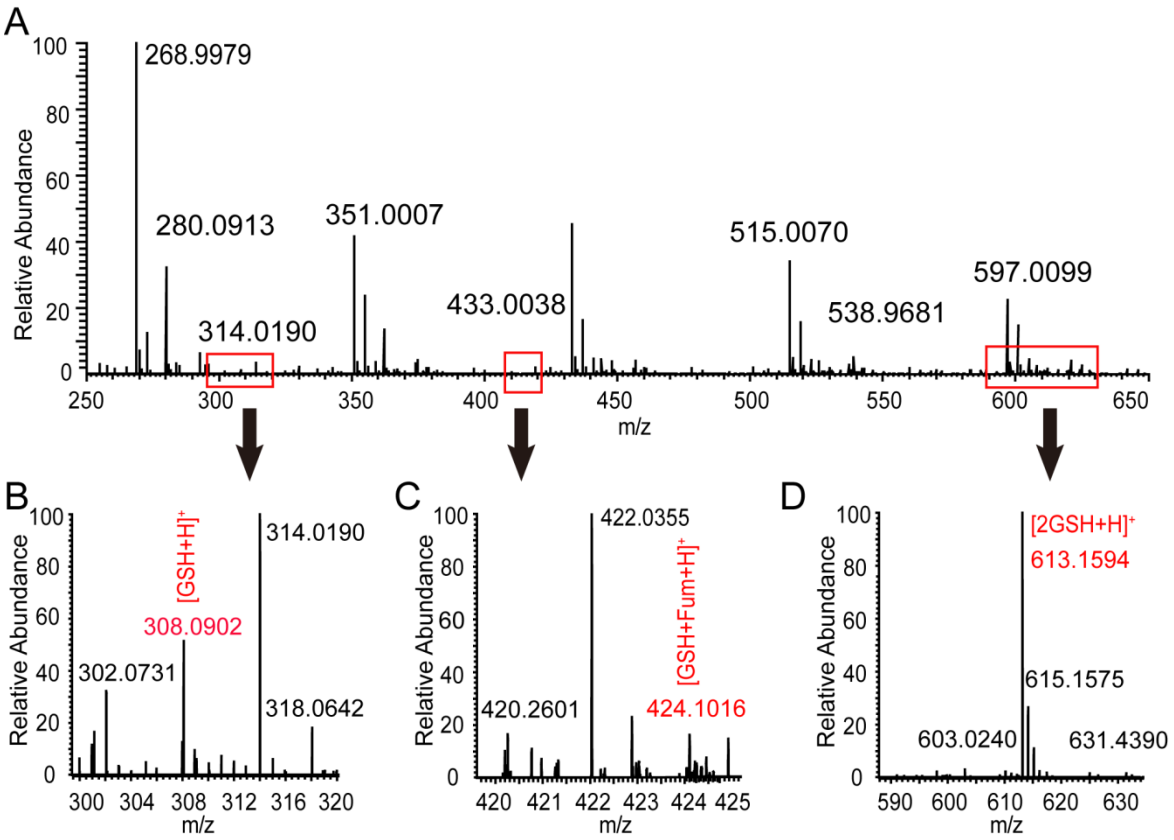
Supplementary Figure S10, related to Figure 5. MCF-7 cells were seeded in 90 Pa soft 3D fibrin gels for 5 days in hypoxia, and then were treated with MOG (5mM), SAD (5mM), DMF (100μM) and malate (5mM). 24 hours later, LC-MS were performed for citrate, α-KG, succinate, fumarate and malate detection respectively.

Supplemental Figure S11



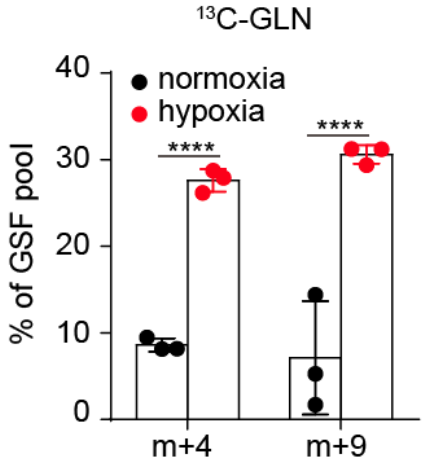
Supplemental Figure S11, related to Figure 5. MCF-7 cells were seeded in 90 Pa soft 3D fibrin gels in hypoxia and treated with MMF on day 5 for another 24 hours, fumarate was analyzed by LC-MS and ROS levels were determined by flow cytometry.

Supplemental Figure S12



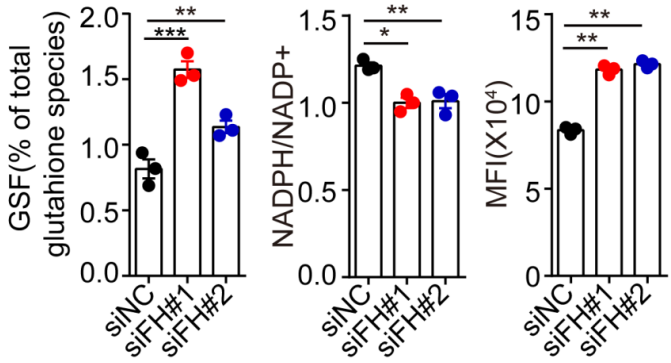
Supplemental Figure S12, related to Figure 5. MCF-7 TRCs were cultured in normoxia (21%O_2) or hypoxia (1%O_2). 5 days later, Cells were washed twice in saline and lysed in extraction solvent (80% methanol/20% water) for 30 min at -80℃. After centrifugation at 13,000 g, 10 min at 4℃, supernatant extracts were analyzed by LC/MS/MS. Representative MRM chromatograms of GSH (B)、GSF(C) and GSSG(D)

Supplemental Figure S13



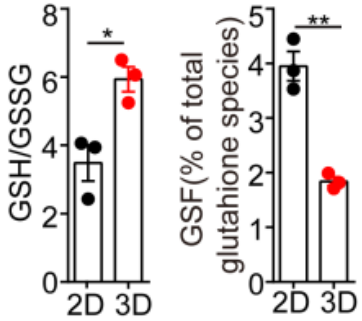
Supplemental Figure S13, related to Figure 5. MCF-7 cells were cultured in 90 Pa soft 3D fibrin gels for 4 days under normoxic or hypoxic conditions, and then incubated with ^{13}C glutamine (GLU) for another 24 hours; LC-MS were performed for glutamine derived GSF.

Supplemental Figure S14



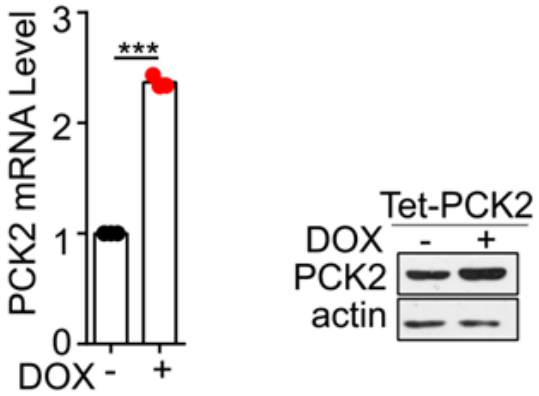
Supplemental Figure S14, related to Figure 5. Hypoxia MCF-7 TRC were transfected with scramble or FH siRNA, cultured in soft 3D fibrin gels for 3 days, Quantification of LC/MS/MS analysis of succinic GSH. Data shown are representative of three independent experiments and error bars represent mean ± SEM.*p< 0.05, **p< 0.01, ***p< 0.001 (Student's t-test).

Supplemental Figure S15



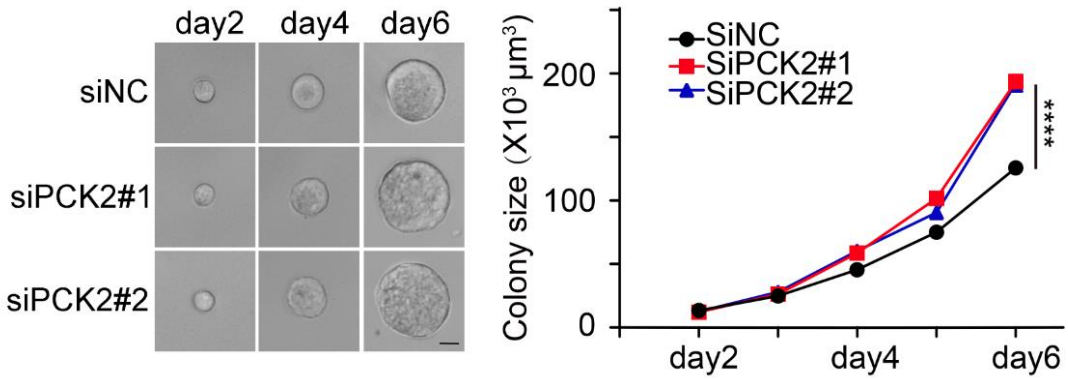
Supplemental Figure S15, related to Figure 5. MCF-7 cells were seeded in rigid dish and soft 3D fibrin gels, then cultured them under hypoxic conditions, respectively. Five days later, cells were collected and lysed in extraction solvent (80% methanol/20% water), and then GSF, GSH/GSSG Ratio were analyzed by LC-MS. Data shown are representative of three independent experiments and error bars represent mean ± SEM.*$p < 0.05$, **$p < 0.01$ (Student's t-test).

Supplemental Figure S16



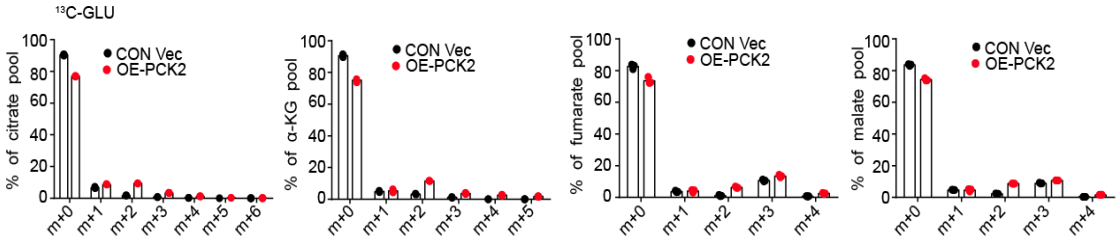
Supplemental Figure S16, related to Figure 6. The Tet-PCK2 MCF-7 cells was administered with or without 2 μg/ml doxycycline for 2 days and then was analyzed by western blotting for PCK2 expression. Data shown are representative of three independent experiments and error bars represent mean ± SEM. ***p< 0.001 (Student's t-test).

Supplemental Figure S17



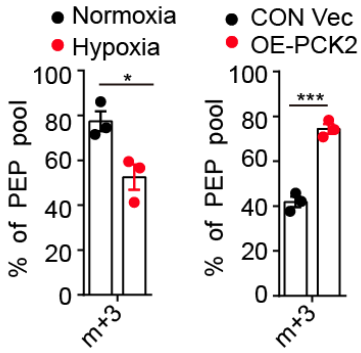
Supplementary Fig. S17, related to Fig. 6. MCF-7 cells were transfected with scramble or PCK2 siRNA, and then cultured in soft 3D fibrin gels for 6 days in normoxia. The colony size is presented photographically and graphically. Data shown are representative of three independent experiments and error bars represent mean ± SEM. Bar 20µm. ****p< 0.0001 (Student's t-test).

Supplemental Figure S18



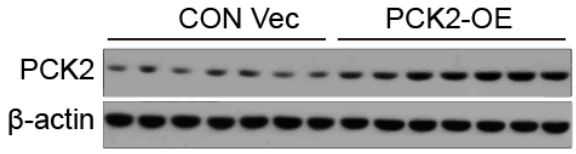
Supplemental Figure S18, related to Figure 6. Tet-PCK2 MCF-7 cells were cultured in 90 Pa soft 3D fibrin gels with or without doxycycline in hypoxia for 4 days under hypoxic conditions, and then incubated with ^{13}C glucose (GLU) for another 24 hours; LC-MS were performed for full mass isotopologue distribution of citrate, α-KG, fumarate and malate.

Supplemental Figure S19



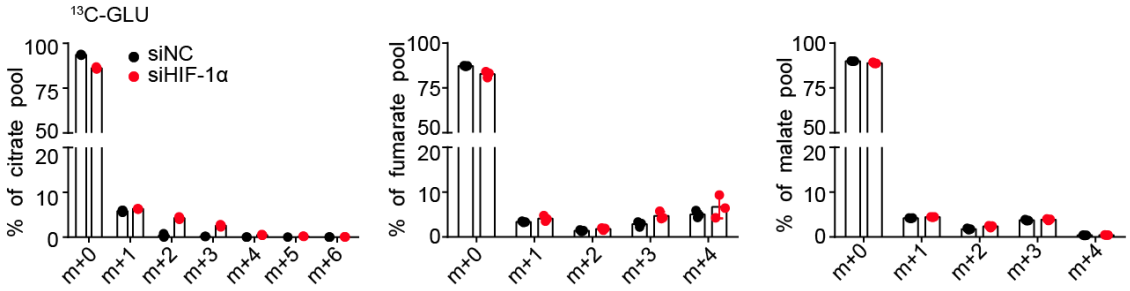
Supplemental Figure S19, related to Figure 6. MCF-7 cells were cultured in 90 Pa soft 3D fibrin gels under normoxic or hypoxic conditions (left) or Tet-PCK2 MCF-7 cells were cultured in 90 Pa soft 3D fibrin gels with or without doxycycline under hypoxic conditions for 4 days (right), and then incubated with ^{13}C glutamine (GLN) for another 24 hours; LC-MS were performed for m+3 isotopologue distribution of PEP.

Supplemental Figure S20



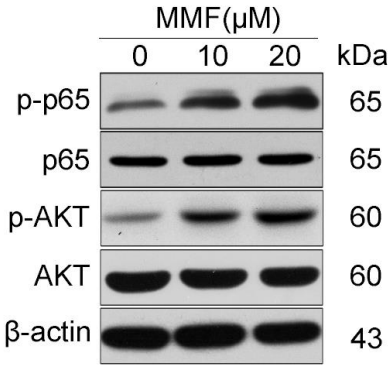
Supplemental Figure S20, related to Figure 6. Mice was sacrificed and tumor tissue were selected from control vector or PCK2-overexpressing group, protein was extracted and PCK2 were detected by western blotting.

Supplemental Figure S21



Supplemental Figure S21, related to figure 7. MCF-7 cells were transfected with HIF-1α siRNA and then cultured in 90 Pa soft 3D fibrin gels for 4 days under hypoxic conditions, and then incubated with ^{13}C glucose (GLU) for another 24 hours; LC-MS were performed for full mass isotopologue distribution of citrate, fumarate and malate.

Supplemental Figure S22



Supplemental Figure S22, related to Figure 7. MCF-7 TRCs were treated with MMF (10, 20 μM) under normoxic condition for 24 hours, then proteins were collected and NF-κB (p65) and Akt phosphorylation were detected by western blotting.

Supplemental Table 1, siRNAs sequences were shown as follows.

MDH2	siRNA1 #:GTGGAATGTTCCTTCGTTA
	siRNA2 #:GAGTCAACGTCCCTGTCAT
IDH3G	siRNA1 #:GCAAGAGTATCGCCAATAA
	siRNA2 #:GTGTACCAGTGGACTTTGA
FH	siRNA1 #:GATCTACGATGAACTTTAA
	siRNA2 #:GGATGCTGTTCCACTTACT