# Supplementary Materials: Blockade LAT1 Mediates Methionine Metabolism to Overcome Oxaliplatin Resistance under Hypoxia in Renal Cell Carcinoma

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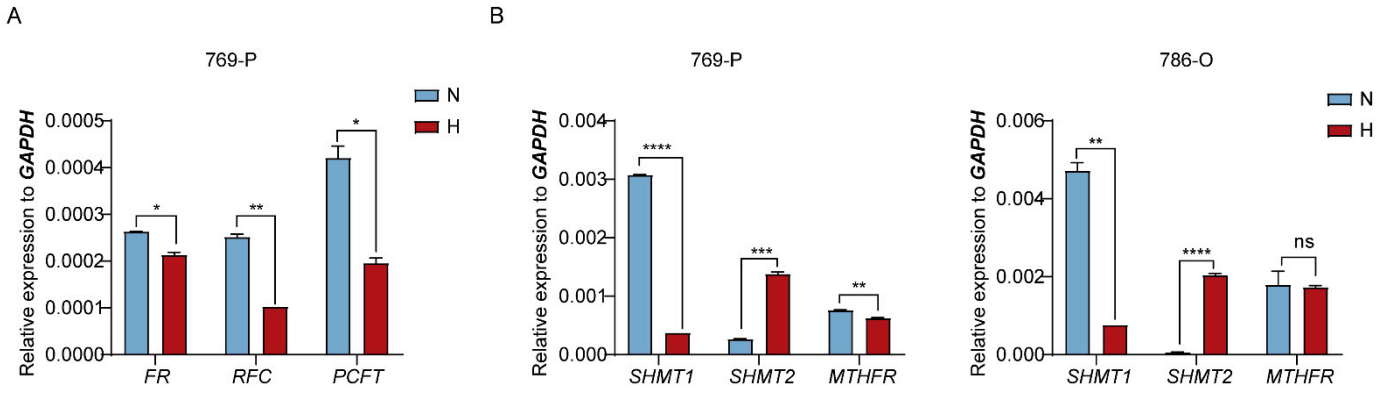


**Figure S1.** The effect of hypoxia on folate metabolism. (**A**) The mRNA expression of folate transporters (*RFC*, *FR*, and *PCFT*) in 769-P cells exposed in hypoxic and normoxic conditions for 72 h, respectively. RFC: Reduced folate carrier; PCFT: Proton-coupled folate transporter; FR: Folate receptor. (**B**) The mRNA expression of enzymes (*SHMT1*, *SHMT2*, and *MTFHR*) of folate cycle in 786-O and 769-P cells cultured under normoxia and hypoxia for 72 h, respectively. N, normoxia; H, hypoxia. *GAPDH* was used as the normalizing gene. Data are the mean ± SEM for biological triplicates. Student's *t* test (two-tailed) was used. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. ns: no significance.

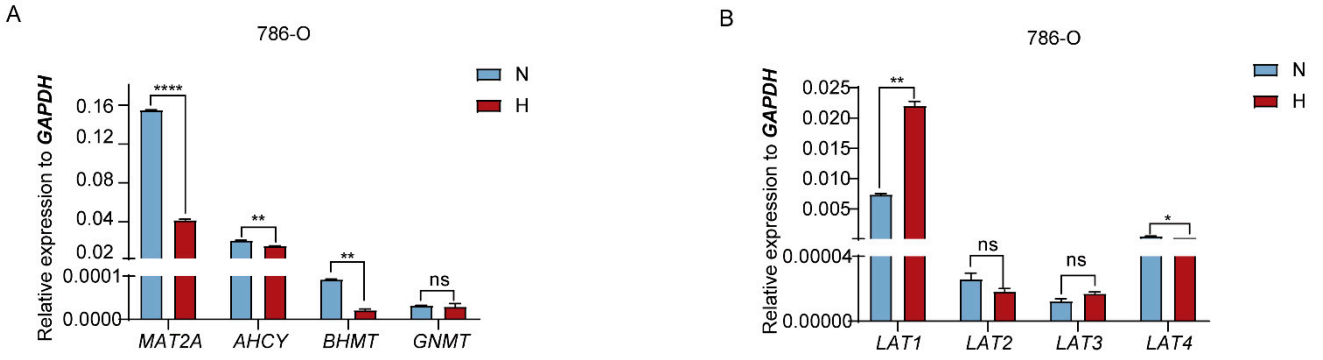


**Figure S2.** Expression level of enzymes and transporters of methionine cycle in RCC cells under hypoxia. (**A**) The mRNA expression of pivotal enzymes (*MAT2A*, *AHCY*, *BHMT*, and *GNMT*) of methionine cycle in 786-O cells cultured under normoxia and hypoxia for 72 h, respectively. MAT2A: Methionine adenosyltransferase 2A; GNMT: Glycine N-methyltransferase; AHCY: Adenosylhomocysteinase; BHMT: Betaine-homocysteine S-methyltransferase. (**B**) The mRNA expression of methionine uptake transporters (*LAT1*, *LAT2*, *LAT3*, and *LAT4*) in 786-O cells cultured under normoxia and hypoxia for 72 h, respectively. LAT: L-type amino acid transporter. N, normoxia; H, hypoxia. *GAPDH* was used as the normalizing gene. Data are the mean ± SEM for biological triplicates. Student's *t* test (two-tailed) was used. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. ns: no significance.

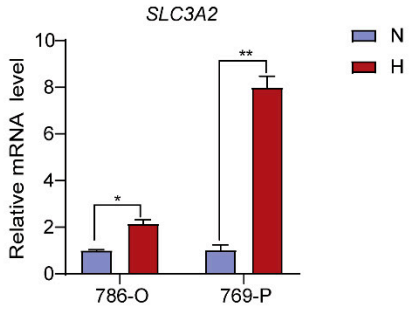


**Figure S3.** The mRNA level of *SLC3A2* in 786-O and 769-P cells cultured under normoxia and hypoxia for 72 h, respectively. N, normoxia; H, hypoxia. *GAPDH* was used as the normalizing gene. Data are the mean ± SEM for biological triplicates. Student's *t* test (two-tailed) was used. * $p < 0.05$; ** $p < 0.01$.

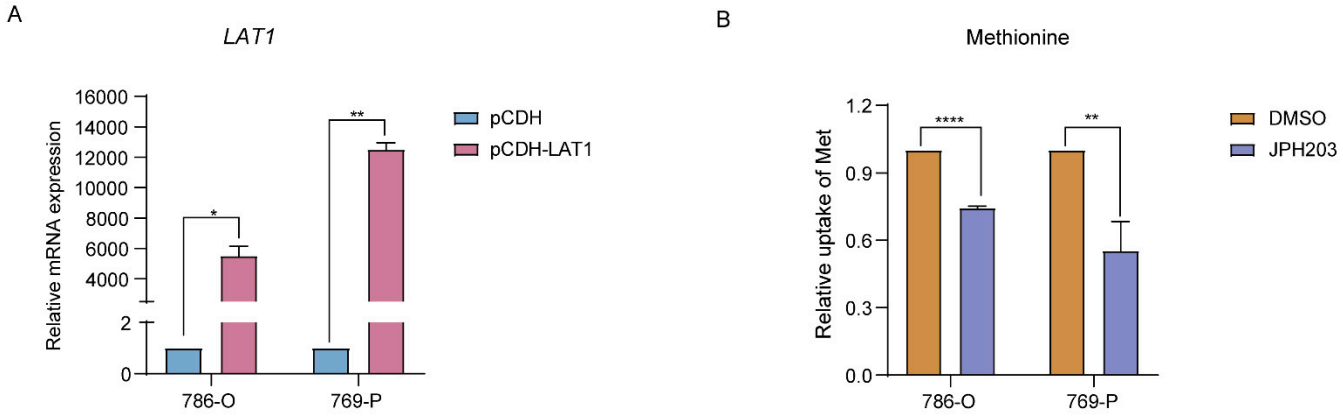


**Figure S4.** Efficiency of overexpression and inhibition of LAT1 in RCC cells. (**A**) The mRNA expression of *LAT1* in 769-P and 786-O cells with LAT1 overexpression (versus vector). *GAPDH* was used as the normalizing gene. (**B**) Relative methionine uptake was examined using LC-MS in 769-P and 786-O cells treated with 10 μmol/L JPH203 (versus DMSO) for 72 h. Met: methionine. Data are the mean ± SEM for biological triplicates. Student's *t* test (two-tailed) was used. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.

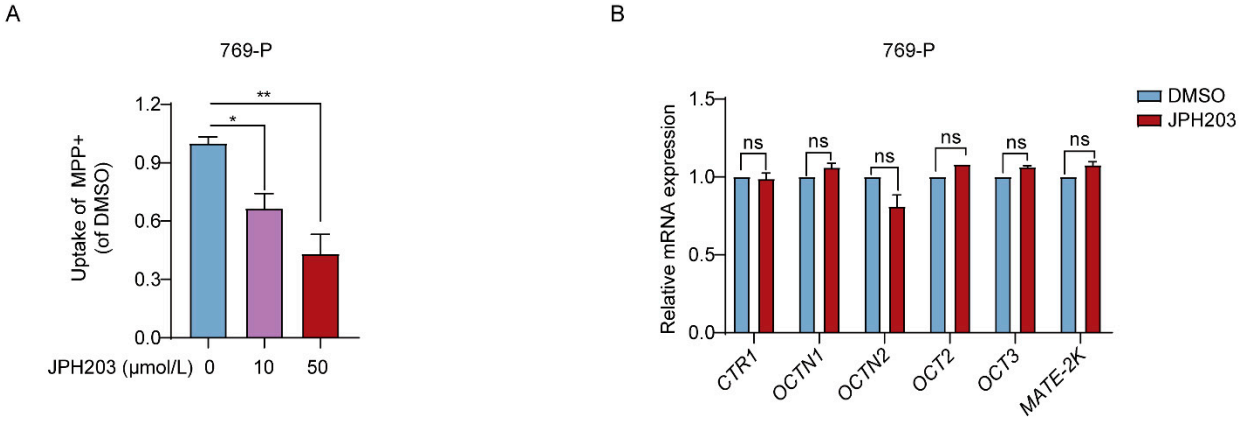


**Figure S5.** The expression and uptake capacity of transporter of oxaliplatin in RCC cells after being treated with JPH203 (**A**) The uptake of $MPP^+$ (1-methyl-4-phenylpyridinium) was examined in 769-P cells after being treated with JPH203 (0, 10, 50 μmol/L). (**B**) The mRNA expression of organic cation/carnitine transporters (*OCTN1* and *OCTN2*), organic cation transporters (*OCT2* and *OCT3*), high affinity copper uptake protein (*CTR1*), and multidrug and toxin extrusion protein (*MATE-2K*) in 769-P cells after being treated with 10 μmol/L JPH203. *GPADH* was used as the normalizing gene.

Data are the mean ± SEM for biological triplicates. Student's *t* test (two-tailed) was used. * $p < 0.05$; ** $p < 0.01$. ns: no significance.

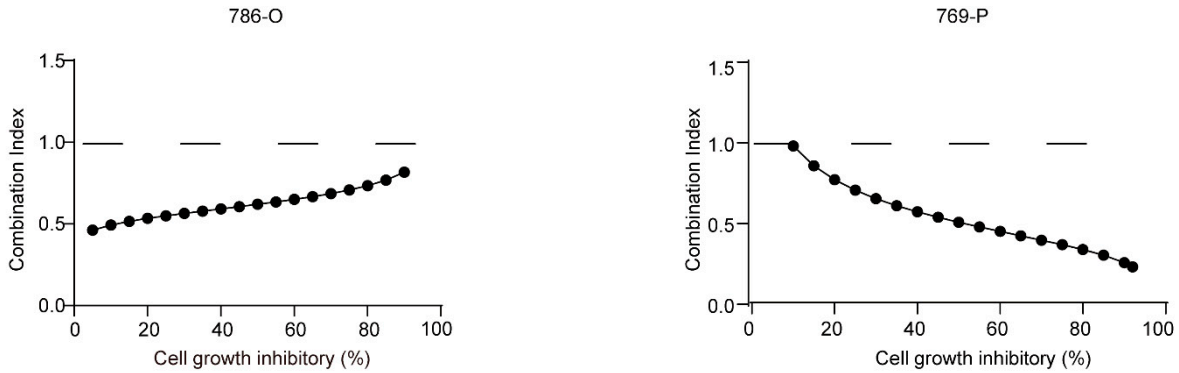


**Figure S6.** Combination index–fraction affected plots of JPH203 and oxaliplatin combinations in 786-O and 769-P cells. Combination index (CI) < 1 represents synergism.

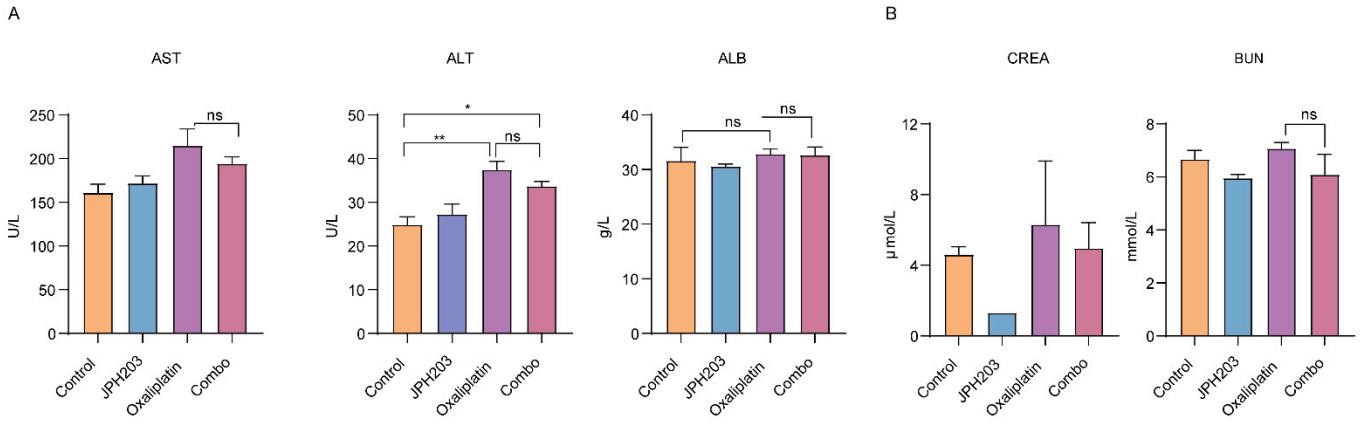


**Figure S7.** The biocompatibility of combination strategy. (**A**) The amount of AST (Aspartate aminotransferase), ALT (Alanine transaminase), and ALB (Albumin) in plasma of mice bearing different drug treatment. (**B**) The amount of CREA (Creatinine) and BUN (Blood urea nitrogen) in plasma of mice bearing different drug treatment. Combo: JPH203 in combination with oxaliplatin. Data are the mean ± SEM for biological triplicates. One-way ANOVA analysis was used. * $p < 0.05$; ** $p < 0.01$; ns, no significance.

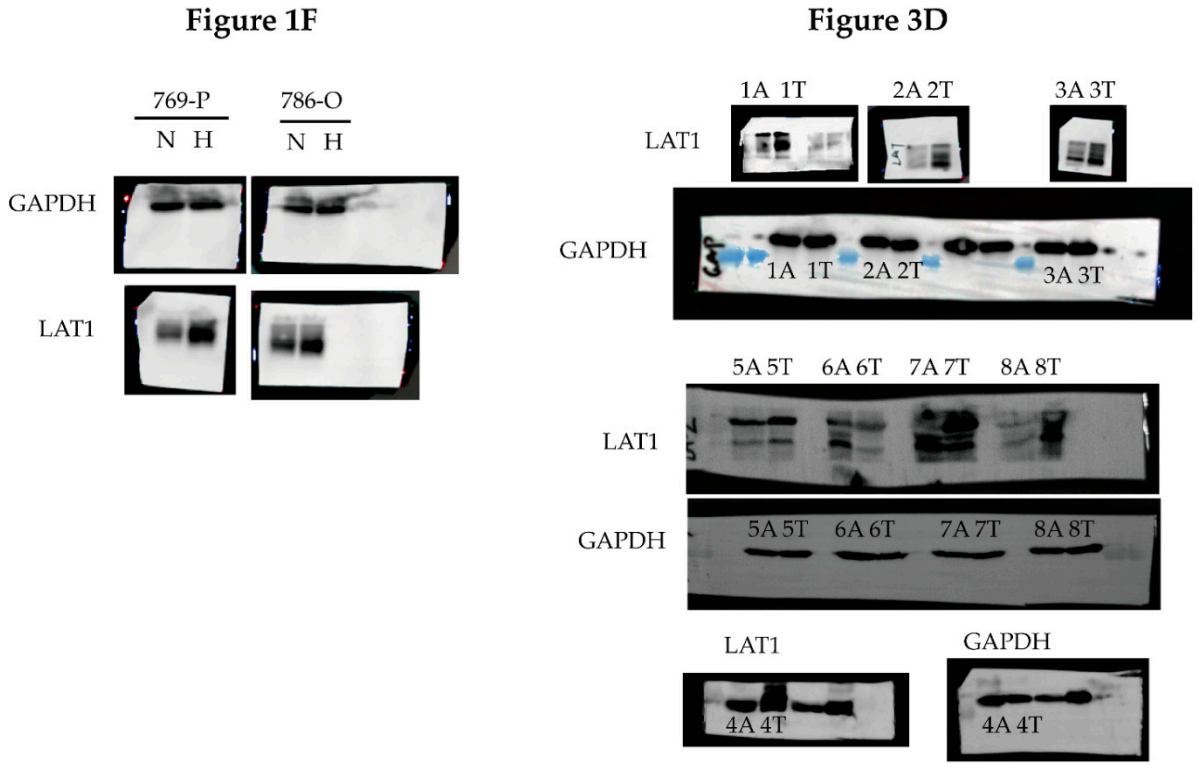


**Figure S8.** Original western blots of Figure 1F and Figure 3D.

**Table S1.** Primers used in this study.

| Gene | Forward (5′ to 3′) | Reverse (5′ to 3′) |
|---|---|---|
| *GAPDH* | AGGTGAAGGTCGGAGTCA | GGTCATTGATGGCAACAA |
| *LAT1* | GCATCGGCTTCACCATCATC | ACCACCTGCATGAGCTTCTGAC |
| *PCFT* | AGAGCTGGACAATGGATCGGT | GCCTTGCTGATAGCCATGACTC |
| *RFC* | GGGCTTTGTTGCTGGAAG | GGCAGAAAGGATTTGTCTCAAG |
| *SHMT1* | CGCAGAGTGCACCTTCCTGA | CACTGGTTCGAAGCTGCCTA |
| *SHMT2* | CTGCAGGAAGACCCTCTTGT | CGGAGTAGGGCTGGACATTG |
| *AHCY* | TAGCAGGCTATGGTGATGTGG | ATGGGGTCAATCTCGGTGATG |
| *MTHFR* | CCGCCGTGAACTACTGTGG | AGATGGCCCGTGATCTCCTC |
| *MAT2A* | ACCAGAAAGTGGTTCGTGAAG | CAAGGCTACCAGCACGTTACA |
| *GNMT* | GTATATCGGAGAGACACCCGCAG | CACTCTGGTCCCCTTTGCAG |
| *BHMT* | TGCTGGAGAGATTGTGATTGGA | CTTGTCTTCACTCGCATAGAAGG |
| *FR* | TTAGCCTGGCCCTAATGCT | GCAGGGATTTCCAGGTATCA |

**Table S2.** Tissue specimen information.

| Number | Gender | Age | Subtype | TNM stage |
|---|---|---|---|---|
| 1 | Male | 37 | Clear cell renal cell carcinoma | T1bN0M1 |
| 2 | Male | 70 | Papillary cell renal cell carcinoma | T1bN0M0 |
| 3 | Male | 71 | Clear cell renal cell carcinoma | T4aN2M0 |
| 4 | Female | 51 | Clear cell renal cell carcinoma | T2aN0M0 |
| 5 | Female | 43 | Clear cell renal cell carcinoma | T1aN0M0 |
| 6 | Male | 58 | Clear cell renal cell carcinoma | T1bN0M0 |
| 7 | Male | 50 | Clear cell renal cell carcinoma | T3N0M0 |
| 8 | Male | 52 | Clear cell renal cell carcinoma | T4N1M0 |