**Supplementary Fig. 1 Quality control plots for the genome-wide CRISPR-Cas9 screen.**

**a, b** Correlation plot between replicates for SNU398 (**a**) and HepG2 (**b**) cells.**c, d** Distribution of the negative and positive controls for SNU398 (**c**) and HepG2 (**d**) cells. The F-measure in the title of the distribution plots is a metric for how well the two distributions are separated. The higher the value, the better the separation. The maximum value is 1 and the lowest value is 0. ‘n’, non-targeting control sgRNAs (blue); ‘p’, sgRNAs targeting essential genes (red); ‘x’, sgRNAs targeting 19114 human genes (grey). T0, SNU398 or HepG2 cells after puromycin selection; Tu, SNU398 or HepG2 cells cultured for 14 days.

**Supplementary Fig. 2 Role of mitochondrial translation in liver cancer cells.**

**a, b** Enriched Gene Ontology (GO) terms in cellular component (CC) (**a**) and biological process (BP) (**b**) for 455 common hits identified in the screen of SNU398 and HepG2 cells. Mitochondria-related pathways are marked by red dots. (**c**) Gene set variation analysis (GSVA) scores of mitochondrial translation were evaluated in 50 pairs of tumour (T) and corresponding non-tumour (N) tissues of LIHC. n=50. Values are shown as mean ± SEM. *P* values are calculated by two-tailed paired t test.

**Supplementary Fig. 3 Knockdown of mitochondrial translation-related genes suppresses cell proliferation and reduces protein levels of genes coded by mitochondrial DNA.**

**a, b** Cell growth assays measured by CCK8 for SNU398 (**a**) and HepG2 (**b**) cells transfected with shRNAs of *GFM1*, *MRPL4*, or *MRPS23*. **c, d** Western blot analyses for subunits of respiratory complex (CIII-CV) in SNU398 (**c**) and HepG2 (**d**) cells transfected with shRNAs of *GFM1*, *MRPL4*, or *MRPS23*. Values are shown as mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 4 Long-term cell growth assays for tigecycline in liver cancer cell lines.**

Long-term cell growth assays for tigecycline in liver cancer cell lines. Growth inhibition (%) was counted by image J and displayed with a heat map.

**Supplementary Fig. 5 MEK inhibitors act as synergistic inhibitors in tigecycline-insensitive cells.**

**a, b** Short-term cell growth assays for tigecycline, trametinib (**a**), cobimetinib (**b**), and combination of tigecycline and trametinib or cobimetinib in MHCC97H, PLC/PRF/5, and Li7 cells. Compounds were added into cells at the indicated concentration for three days before CCK8 test. **c, d** Incucyte cell proliferation and long-term cell growth assays showed the synergistic effects of drug combination of tigecycline and trametinib (**c**) or cobimetinib (**d**) in Li7 cells. Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 6 Oxygen consumption rate and extracellular acidification rate with treatment of tigecycline in tigecycline-sensitive and -insensitive cells.**

Tigecycline was added into tigecycline-sensitive and -insensitive cells at the indicated concentration for three days before the Seahorse glycolysis and mitochondrial stress tests. The tigecycline-sensitive cells were SNU398, HepG2, and Huh6, while tigecycline-insensitive cell lines were MHCC97H, PLC/PRF/5, and Li7. **a** Oxygen consumption rate (OCR) of the tigecycline-sensitive cells with treatment of tigecycline. **b** OCR of the tigecycline-insensitive cells with treatment of tigecycline. **c**Extracellular acidification rate (ECAR) of the tigecycline-sensitive cells with treatment of tigecycline. **d** ECAR of the tigecycline-insensitive cells with treatment of tigecycline. Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 7 Combined regimen of tigecycline and trametinib inhibits both oxidative phosphorylation and glycolysis.**

Tigecycline and trametinb were added into the tigecycline-insensitive cells at the indicated concentration for three days before the Seahorse glycolysis and mitochondrial stress test. **a** Oxygen consumption rate (OCR) of Li7 cells with treatment of tigecycline and trametinib. **b** Extracellular acidification rate (ECAR) of Li7 cells with treatment of tigecycline and trametinib. **c-e** Maximal respiration (**c**), ATP production (**d**), and glycolytic capacity (**e**) of MHCC97H, PLC/PRF/5, and Li7 cells with treatment of tigecycline and trametinib. Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 8 RNA-sequencing analyses for tigecycline-insensitive cells****.**

**a** Volcano plots of differentially expressed genes (DEGs) of the tigecycline group versus control group in MHCC97H and PLC/PRF/5 cells. Log2(Fold change) >1 or log2(Fold change) <-1, *P* value <0.05. **b** Top downregulated KEGG pathways on the tigecycline group versus control group for MHCC97H and PLC/PRF/5 cells. **c** qRT-PCR measured mRNA levels of one carbon pool by folate pathway-related enzymes in MHCC97H and PLC/PRF/5 cells with treatment of tigecycline.

**Supplementary Fig. 9 AREG and EREG activate EGFR-ERK1/2-MYC cascade in tigecycline-insensitive cells with treatment of tigecycline.**

**a** qRT-PCR measured mRNA levels of AREG and EREG of MHCC97H and PLC/PRF/5 cells. **b, c** ELISA measured [secretion](file:///D:/LenovoSoftstore/Install/wangyiyoudaocidian/8.10.3.0/resultui/html/index.html#/javascript:;) levels of AREG (**b**) and EREG (**c**) in supernatants of PLC/PRF/5 cells that were treated for three days with DMSO or 10μM tigecycline. **d** Western blot analyses of MAPK cascade in PLC/PRF/5 cells treated with tigecycline (10 μM), gefitinib (2 μM) or their combination for three days. **e** Long-term cell growth assays for the combined regimen of tigecycline and gefitinib. **f** Biochemical responses of PLC/PRF/5 cells with treatment of tigecycline (10 μM), trametinib (0.5 μM) or their combination were recorded by Western blot analysis.

**Supplementary Fig. 10 Differences in feedback activation of MAPK-ERK1/2 cascade in tigecycline-sensitive and -insensitive cells with treatment of tigecycline.**

**a, b**Tigecycline-sensitive (**a**) and -insensitive (**b**) cells were treated with 20 μM tigecycline for 0, 6, 12, 24, 48, and 72 hours. Western blot analyses for levels of p-ERK1/2 and ERK1/2. SNU398 and HepG2 are tigecycline-sensitive cells, and MHCC97H and PLC/PRF/5 are tigecycline-insensitive cells.

**Supplementary Fig. 11 Knockdown of *MYC* decreased oxidative phosphorylation and glycolysis in tigecycline-insensitive cells with treatment of tigecycline.**

**a** Western blot analyses of MYC, SHMT2, HK2, PKM2 and LDHA in PLC/PRF/5 cells with knockdown of *MYC*. **b** Maximal respiration, ATP production, and glycolytic capacity were measured after knockdown of *MYC* in MHCC97H cells treated with tigecycline. **c** Oxygen consumption rate (OCR), basal OCR, maximal respiration, and ATP production of PLC/PRF/5 cells with knockdown of *MYC* and treatment of tigecycline. **d** Extracellular acidification rate (ECAR), glycolysis, and glycolytic capacity of PLC/PRF/5 cells with knockdown of *MYC* and treatment of tigecycline. Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 12 Knockdown of *HK2* decreased oxidative phosphorylation and glycolysis in tigecycline-insensitive cells with treatment of tigecycline.**

**a** Western blot analyses for HK2 in MHCC97H and PLC/PRF/5 cells with knockdown of *HK2*. **b** Oxygen consumption rate (OCR), basal OCR, maximal respiration, and ATP production of MHCC97H and PLC/PRF/5 cells. **c** Extracellular acidification rate (ECAR), glycolysis, and glycolytic capacity of MHCC97H and PLC/PRF/5 cells. **d** Long-term cell growth assays for MHCC97H and PLC/PRF/5 cells treated with tigecycline and 2-Deoxy-D-glucose (2-DG). Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 13 Knockdown of *SHMT2* decreased oxidative phosphorylation in tigecycline-insensitive cells with treatment of tigecycline.**

**a** Western blot analyses of SHMT2 and oxidative phosphorylation-related genes for MHCC97H and PLC/PRF/5 cells that were transfected with *SHMT2* shRNAs and treated with tigecycline. **b** Oxygen consumption rate (OCR), basal OCR, maximal respiration, and ATP production of MHCC97H and PLC/PRF/5 cells.

**Supplementary Fig. 14 IHC staining of MHCC97H subcutaneous tumour tissues.**

**a** Body weight of mice for each group. **b** Tumour weight. **c** Representative staining for Ki67, p-ERK1/2, MTCO2, ATP6, and CYTB are shown. **d** H scores of IHC staining. n=6. Scale bar=100μM. Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Fig. 15 Blocking EGFR-ERK1/2-MYC cascade sensitizes subcutaneous tumour to tigecycline in immune-competent mice.**

**a** Body weight of mice for each group. **b** IHC analyses of F4/80+ (a marker of macrophages) cells. Each group counted 12 high magnification fields. Data are mean ± SEM. Unpaired two-sided t-test.

**Supplementary Table 1. List of 26 genes related to mitochondrial translation.**

**Supplementary Table 2. Univariate and multivariate analyses for the protein levels of GFM1 and clinicopathological features associated with overall survival and recurrence in 243 patients with HCC.**

**Supplementary Table 3. Univariate and multivariate analyses for the protein levels of MRPS23 and clinicopathological features associated with overall survival and recurrence in 243 patients with HCC.**

**Supplementary Table 4. Univariate and multivariate analyses for the protein levels of MRPL4 and clinicopathological features associated with overall survival and recurrence in 243 patients with HCC.**

**Supplementary Table 5. List of differentially expressed genes (DEGs) of the tigecycline group versus control group in MHCC97H cells.**

**Supplementary Table 6. List of differentially expressed genes (DEGs) of the tigecycline group versus control group in PLC/PRF/5 cells.**