

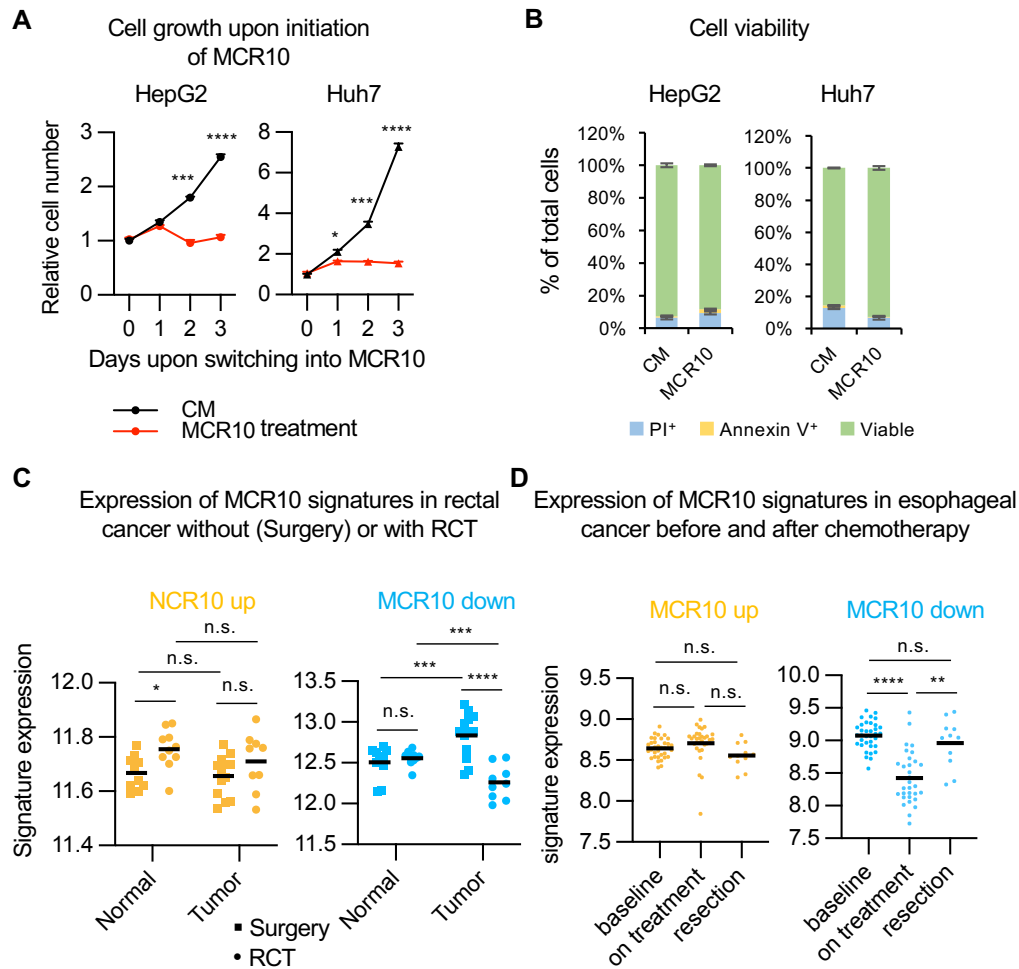


Figure S1. Phenotypic characterization of MCR10 persisters in HCC

(A) MCR10 medium induces growth arrest of liver cancer cells. Parental HepG2 and Huh7 cultured in complete medium (CM) were continued growth in CM or switched into MCR10 medium, live cell number was monitored daily for three days ($n=4$ replicates/group, multiple 2-tailed unpaired Student's t-test, $***p<0.001$, $****p<0.0001$). (B) MCR10 medium does not increase cell death of liver cancer cells. HepG2 and Huh7 cultured in CM or MCR10 medium for 48 hours were analyzed by FACS after Annexin/PI staining (Invitrogen V13241). (C) Expression of MCR10 signatures in rectal cancer without (Surgery) or with radio-chemotherapy (RCT). A publicly available RNA-seq dataset of resectable rectal cancer patients with or without RCT 28 was analyzed for the expression of MCR10 up signatures and down signatures ($n=10$, 10, 13, and 9 samples, respectively; 2-way ANOVA adjusted for FDR, $*q<0.05$, $***q<0.001$, $****q<0.0001$, n.s., not significant). (D) Expression of MCR10 signatures in esophageal cancer before and after chemotherapy. A publicly available RNA-seq dataset of resectable esophageal adenocarcinoma on combined neoadjuvant chemoradiotherapy and atezolizumab treatment 29 was analyzed for the expression of MCR10 up signatures and down signatures ($n=35$, 31, and 11 patient samples, respectively; Brown-Forsythe and Welch ANOVA test adjusted for FDR, $**q<0.01$, $****q<0.0001$, n.s., not significant).

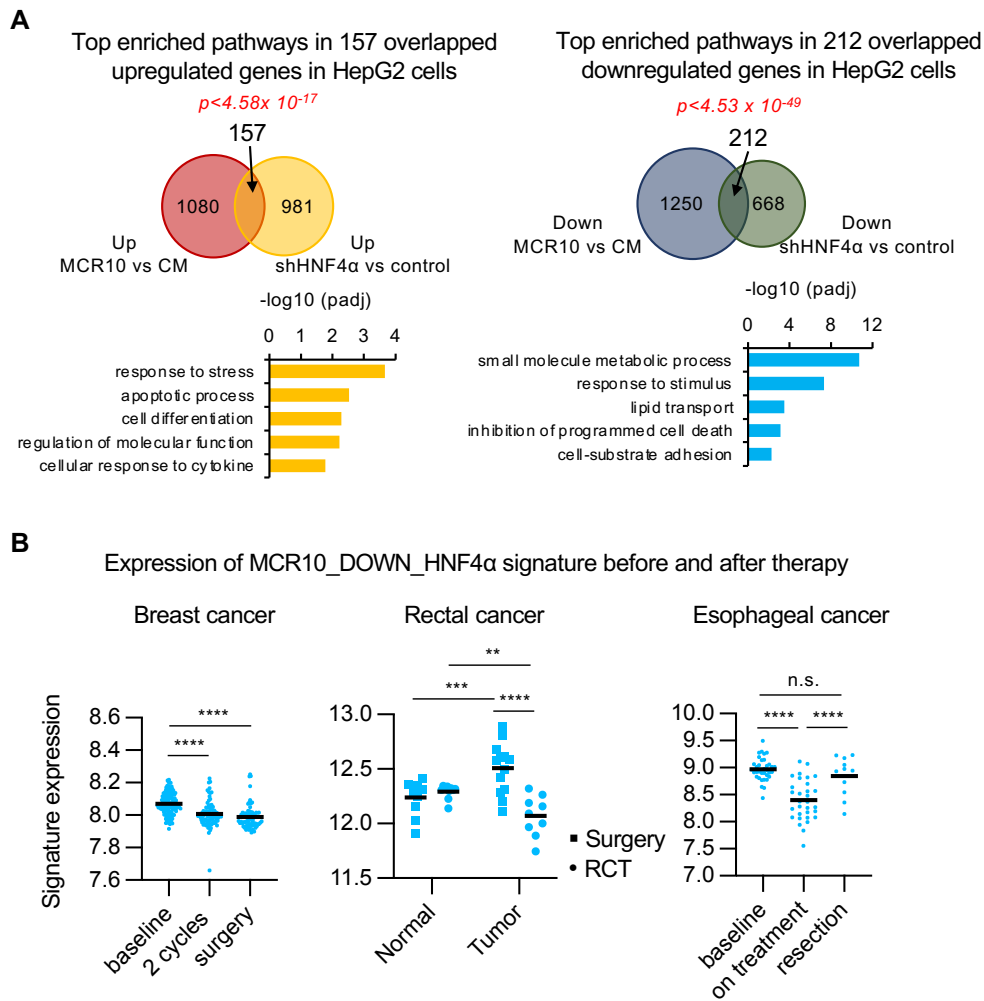


Figure S2. MCR10 persists share molecular similarities with HNF4 α knockdown cells

(A) The pathways of MCR10 persisters share similarity with HNF4 α KD cells. P values were calculated using an exact hypergeometric probability test, with 17,664 total protein-coding genes in HepG2 and Huh7 cells as the background. (B) The expression of MCR10_DOWN_HNF4 α signature is significantly reduced in residual tumors after treatment. The expression of 218 HNF4 α target genes in 556 MCR10_DOWN signature was analyzed as described in Methods. Breast cancer (n=122, 82, and 71 patient samples, respectively; Brown-Forsythe and Welch ANOVA test after adjusted by FDR, **** $q < 0.0001$). Rectal cancer (n=10, 10, 13, and 9 samples, respectively; 2-way ANOVA after adjusted by FDR, ** $q < 0.01$, *** $q < 0.001$, **** $q < 0.0001$, n.s., not significant). Esophageal cancer (n=35, 31, and 11 patient samples, respectively; Brown-Forsythe and Welch ANOVA test after adjusted by FDR, **** $q < 0.0001$, n.s., not significant).

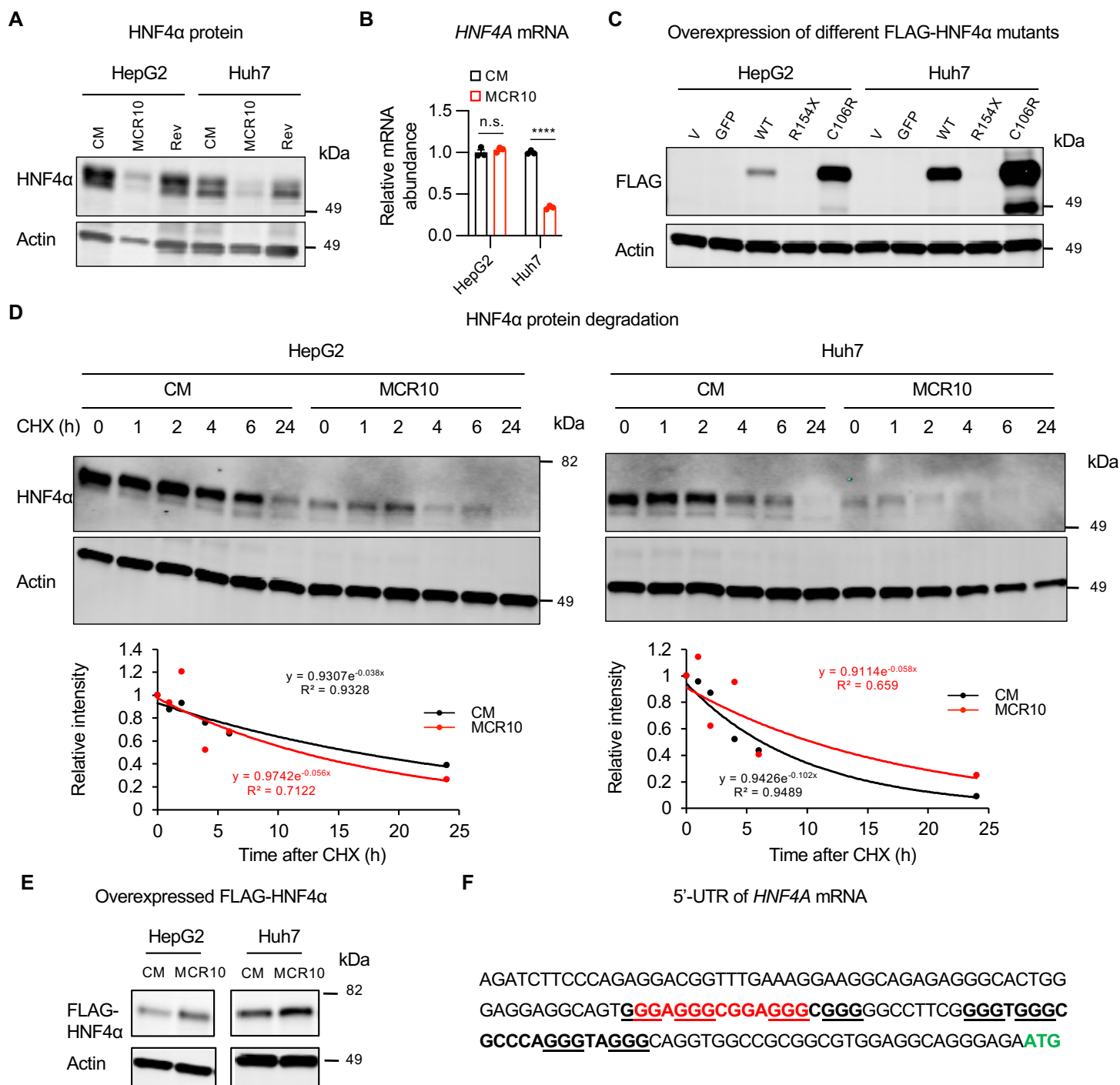


Figure S3. Methionine restriction reduces HNF4α protein

(A) The reversible changes of HNF4α protein. MCR10 persisters were either cultured in MCR10 medium for an additional 6 days (MCR10) or switched back to complete medium for 6 days (Rev). (B) mRNA levels of *HNF4A* in parental cells cultured in CM or MCR10 persisters (n=3 replicates/group, 2-tailed unpaired Student's t-test, ****p<0.0001, n.s., not significant). (C) The protein levels of different HNF4α mutants in Figure 2G. (D) HNF4α protein in parental cells cultured in CM or MCR10 persisters. Parental cells and MCR10 persisters were treated with 100 μg/ml cycloheximide for indicated times. (E) HNF4α protein expressed from a construct without endogenous 5'-UTR is not sensitive to MCR10 induced reduction. (F) 5'-UTR of *HNF4A* mRNA contains highly conserved rG4-forming sequences (underlined). Green, start codon; Red, predicted SRSF1 binding sites.

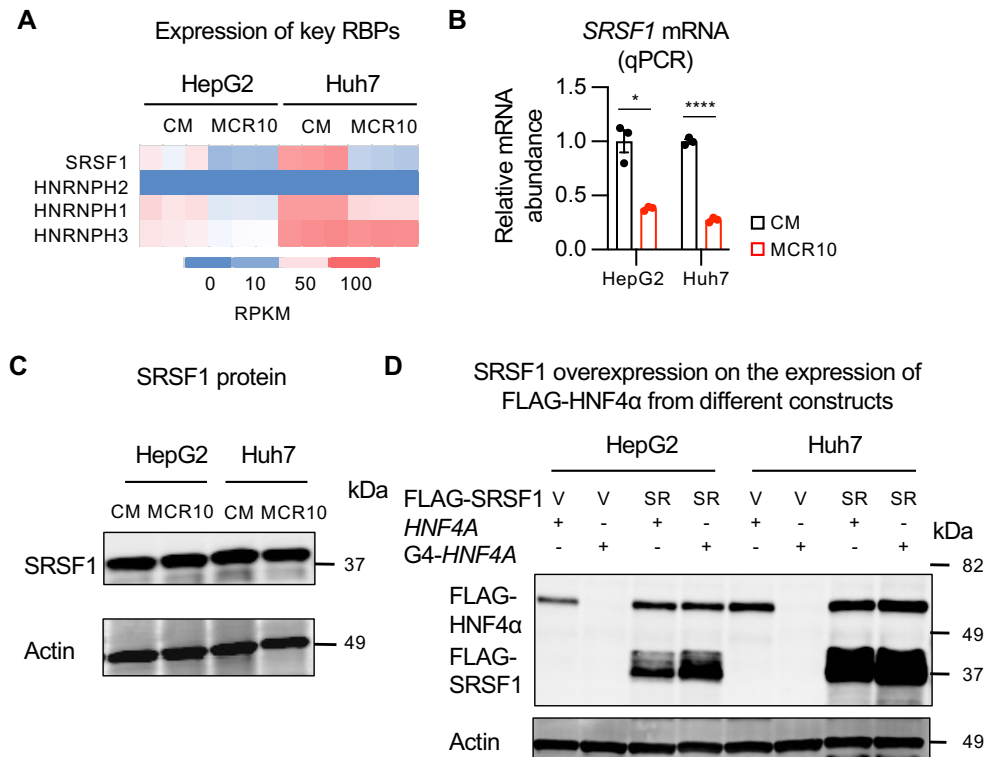


Figure S4. Methionine restriction reduces HNF4α protein through SRSF1.

(A) Expression of key RBPs in parental cells or MCR10 persisters. The mRNA levels of indicated RBPs were analyzed by RNA-seq as described in Methods. (B) *SRSF1* mRNA abundance is decreased in MCR10 persisters as analyzed by qPCR (n=3 replicates/group, 2-tailed unpaired Student's t-test, *p<0.05, ****p<0.0001). (C) The level of SRSF1 protein is not changed in MCR10 persisters. (D) Overexpression of SRSF1 restores the expression of HNF4α protein from an rG4 containing construct.

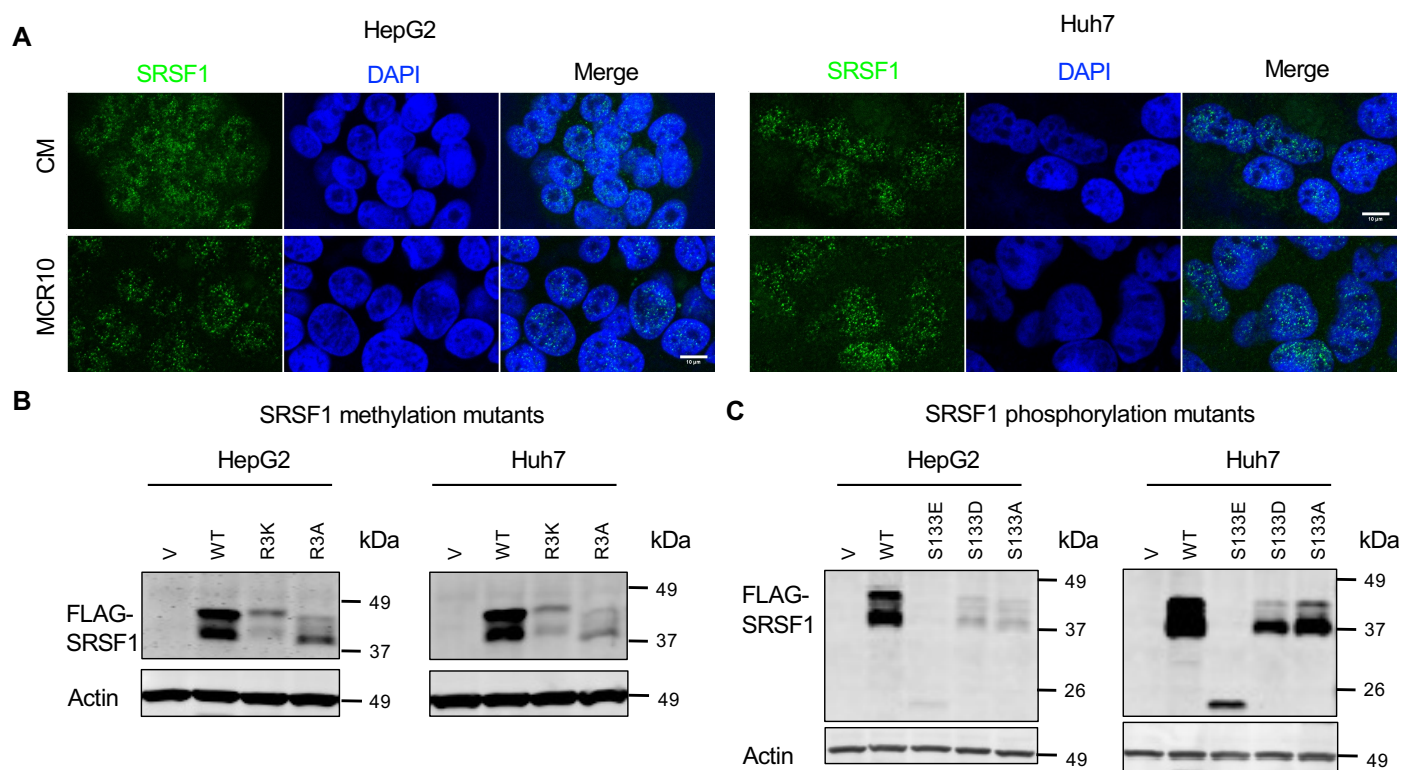


Figure S5. Phosphorylation of SRSF1 by AMPK impairs HNF4 α translation.

(A) The subcellular localization of SRSF1 is not altered in MCR10 persisters. (B) Protein levels of WT and methylation mutant SRSF1 in parental HepG2 and Huh7 cells. (C) Protein levels of WT and S133 mutant SRSF1 in parental HepG2 and Huh7 cells.