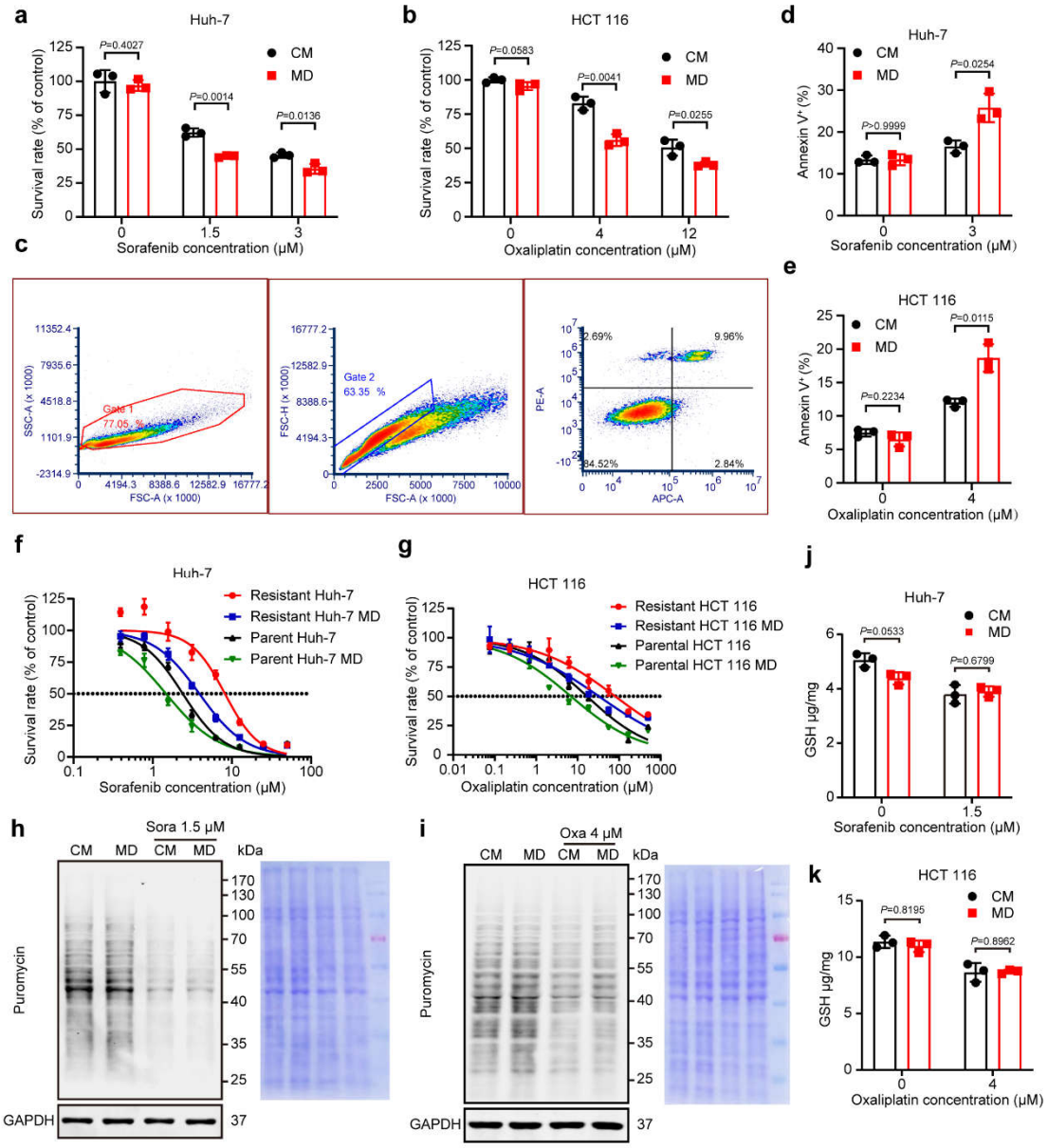
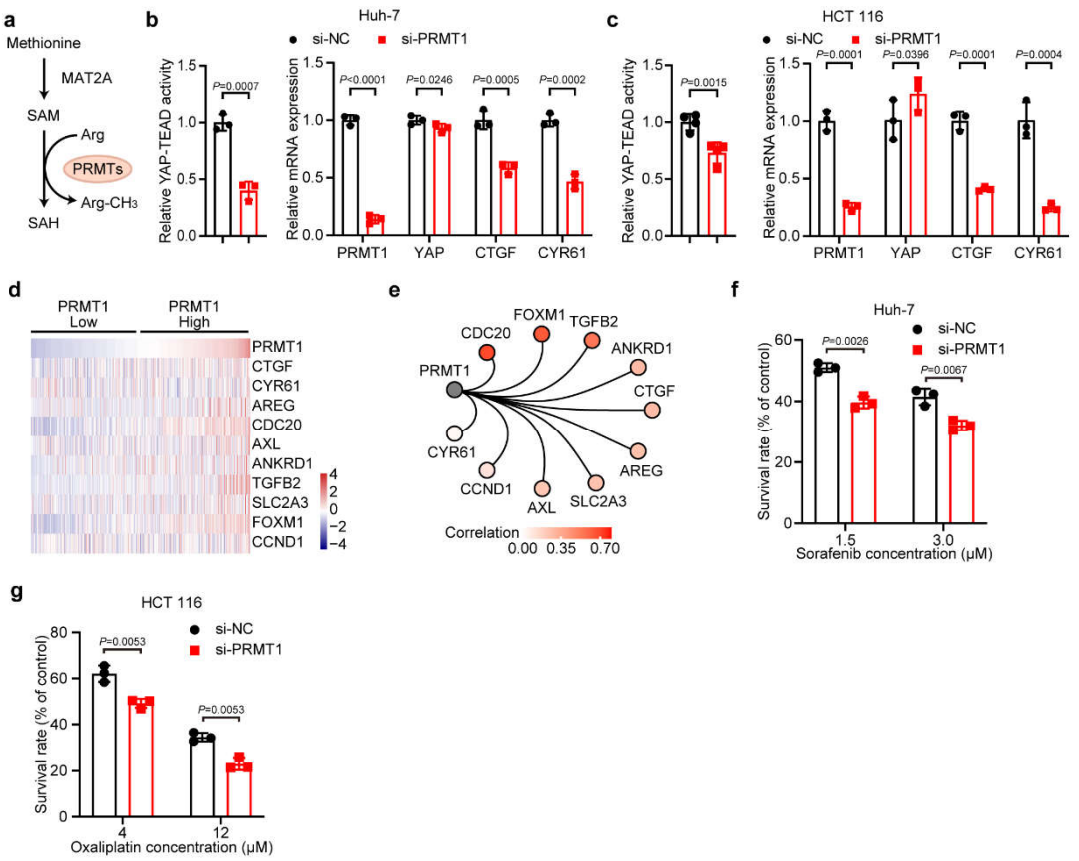
Supplementary figure



Supplementary Fig. 1 Methionine confers potent drug resistance to HCC and CRC cells.

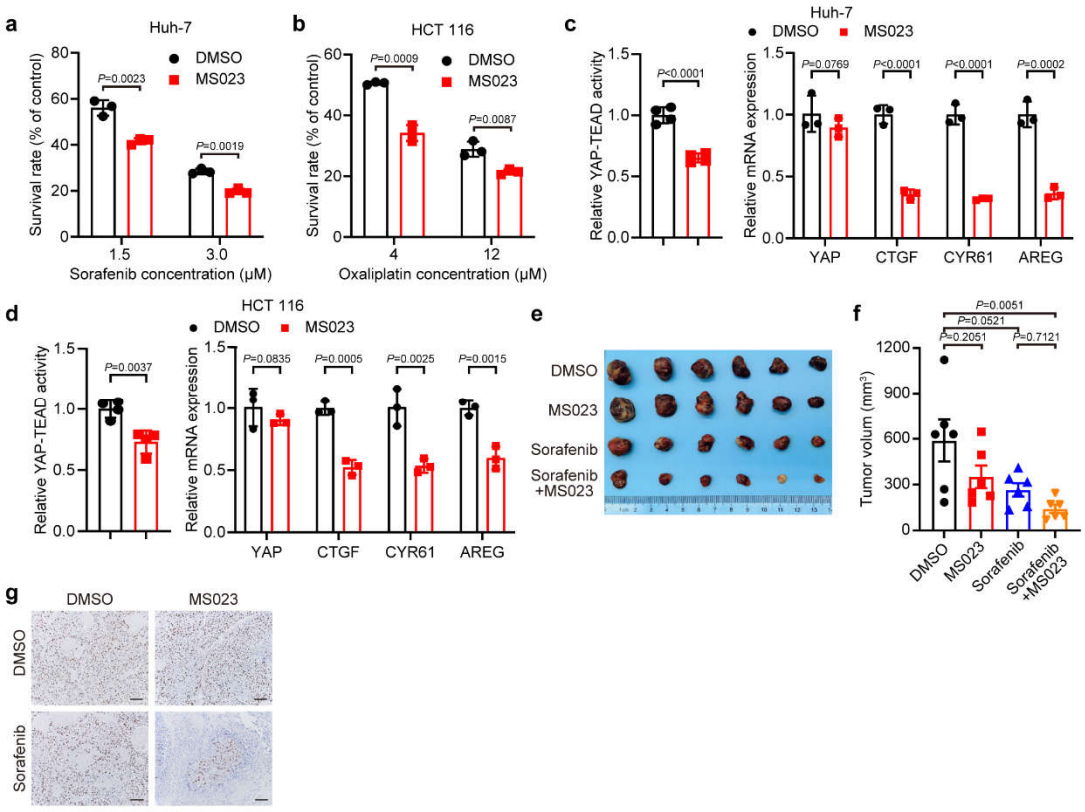
a The cell viability in Huh-7 cells treated with indicated concentration of sorafenib and cultured in methionine-depleted medium (MD) for 24 hours. **b** The cell viability in HCT 116 cells treated with indicated concentration of oxaliplatin and cultured in MD for 48 hours. **c** The gating strategy for flow cytometry assay. The rate of Annexin V positive cells represent apoptosis index. **d** The apoptosis in Huh-7 cells treated with indicated concentration of sorafenib and cultured in MD for 48 hours. **e** The apoptosis in HCT 116 cells treated with indicated concentration of oxaliplatin and cultured in MD for 72 hours. **f, g** The IC50 of

indicated Huh-7 cells cultured in MD for 24 hours (**f**) and indicated HCT 116 cells cultured in MD for 48 hours (**g**). **h, i** immunoblot (left) and Coomassie blue staining (right) of protein synthesis in Huh-7 cells cultured in MD and treated with sorafenib (1.5 μM) for 24 hours (**h**) and HCT 116 cells cultured in MD and treated with oxaliplatin (4 μM) for 48 hours (**i**). Puromycin (1 mg/mL, 1:1000) was added 1 hour before harvesting cell lysate. **j, k** The GSH concentration in Huh-7 cells cultured in MD and treated with sorafenib (**j**) and HCT 116 cells cultured in MD and treated with oxaliplatin (**k**) for 48 hours. Statistical analyses were performed using two-sided unpaired Student's t-test (**a, b, d, e, j, k**). Data were presented as mean ± SD. $n = 3$ (**a, b, d-g, j, k**) biologically independent samples. The data presented in (**h, i**) are representative of two independent experiments. Source data are provided as a Source Data file.



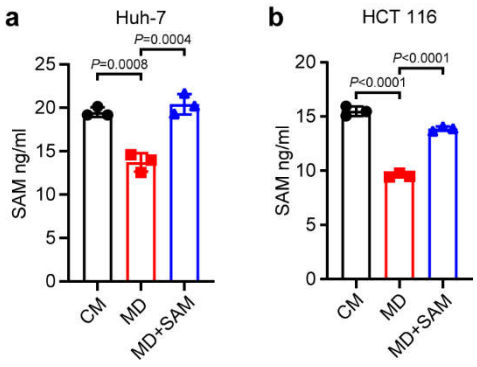
Supplementary Fig. 2 PRMT1 promotes drug resistance and YAP–TEAD activity.

a Schematic of methionine metabolites and arginine methylation process. SAM: S-adenosyl methionine. SAH: S-adenosyl homocysteine. **b, c** The YAP-TEAD activity (left panel) and the transcriptional expression of YAP and YAP target genes (right panel) in Huh-7 cells (**b**) and HCT 116 cells (**c**) transfected with si-PRMT1 for 48 hours. **d** Heatmap from TCGA dataset depicts differential expression of YAP target genes in HCC patients with high and low expression of PRMT1. **e** The correlation network map shows the positive correlations of PRMT1 with the indicated YAP target genes in HCC patients. **f** The cell viability in Huh-7 cells treated with indicated concentration of sorafenib and transfected with si-PRMT1 for 24 hours. **g** The cell viability in HCT 116 cells treated with indicated concentration of oxaliplatin and transfected with si-PRMT1 for 48 hours. Statistical analyses were performed using two-sided unpaired Student's t-test (**b, c, f, g**) or two-tailed Spearman's correlation test (**e**). Data were presented as mean ± SD. n = 3 (**b, c (right panel), f, g**) or 4 (**c (left panel)**) biologically independent samples. Source data are provided as a Source Data file.



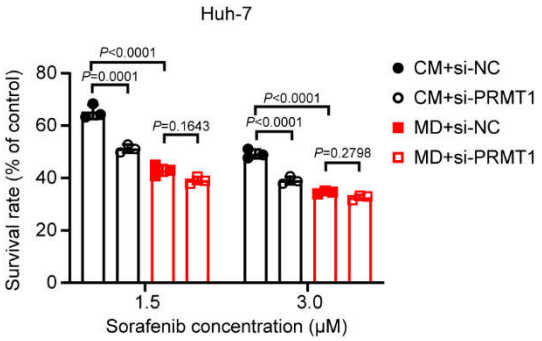
Supplementary Fig. 3 MS023 sensitizes HCC to sorafenib treatment.

a The cell viability in Huh-7 cells treated with indicated concentration of sorafenib and MS023 (200 nM) for 24 hours. **b** The cell viability in HCT 116 cells treated with indicated concentration of oxaliplatin and (600 nM) for 48 hours. **c** The YAP-TEAD activity (left panel) and the transcriptional expression of YAP and YAP target genes (right panel) in Huh-7 cells treated with MS023 (200 nM) for 48 hours. **d** The YAP-TEAD activity (left panel) and the transcriptional expression of YAP and YAP target genes (right panel) in HCT 116 cells treated with MS023 (600 nM) for 48 hours. **e** Representative image of tumor nodules in mice treated with MS023 and/or sorafenib. n = 6 per group. **f** The analysis of tumor volume in mice described in Supplementary Fig. 2e. **g** Representative IHC image of ki-67 in mice described in Supplementary Fig. 2e. Scale bar = 50 μm. Statistical analyses were performed using two-sided unpaired Student's t-test (**a-d**) and one-way ANOVA with multiple comparisons (**f**). Data were presented as mean ± SD. *n* = 3 (**a, b, c (right panel), d (right panel)**), 4 (**c (left panel), d (left panel)**) or 6 (**f**) biologically independent samples. Source data are provided as a Source Data file.

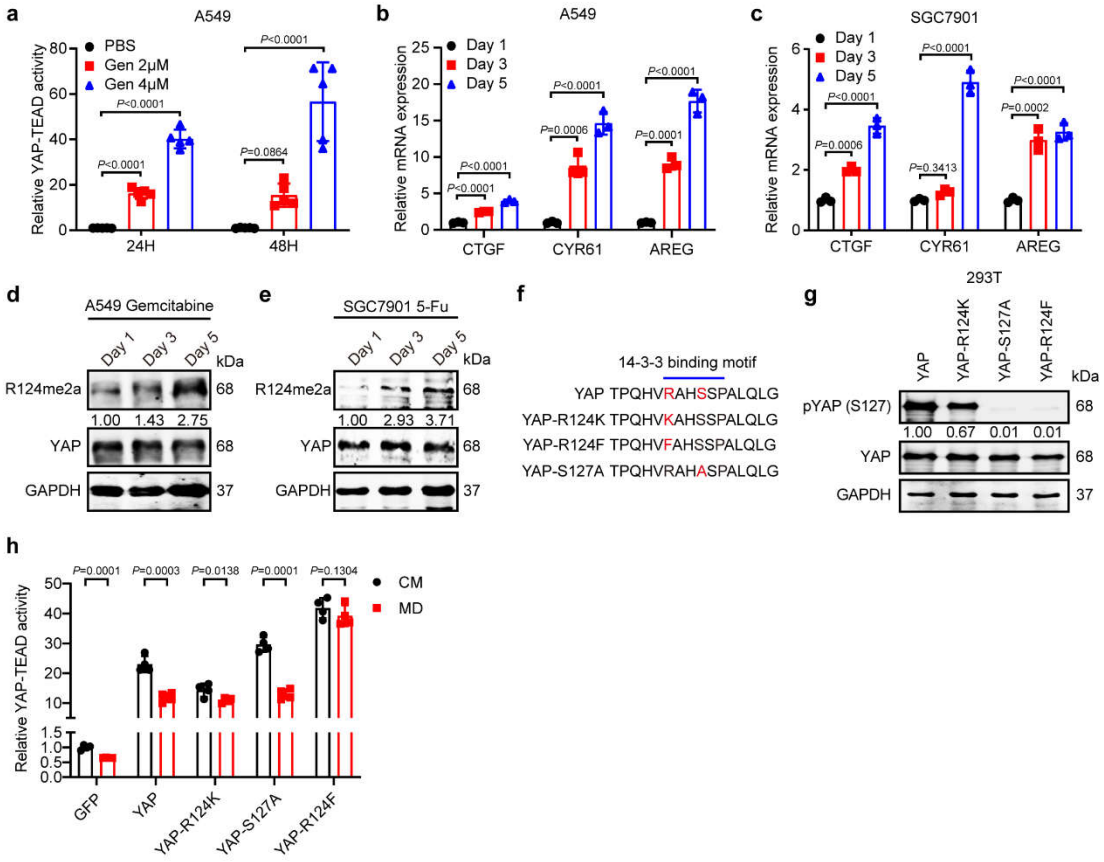


Supplementary Fig. 4 The SAM concentration in Huh-7 cells and HCT 116 cells.

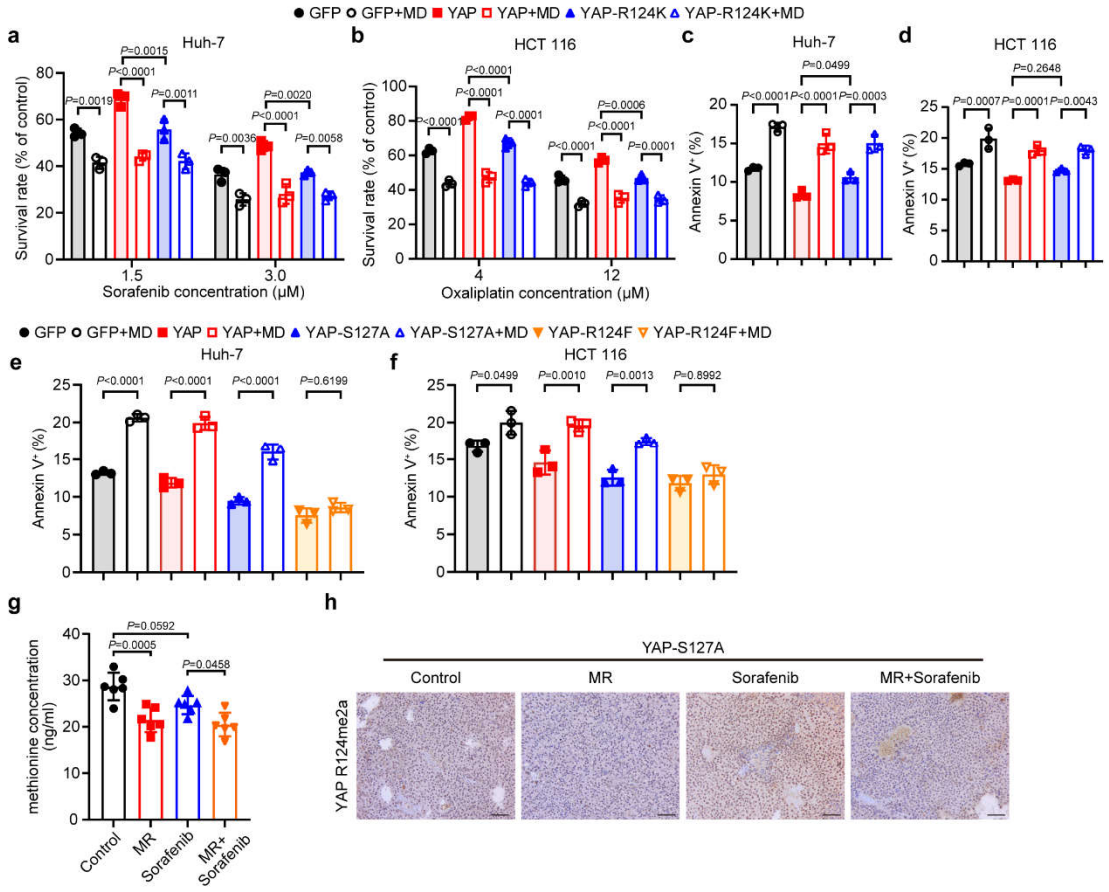
a, b The SAM concentration in Huh-7 cells (**a**) and HCT 116 cells (**b**) detected by ELISA. Cells were cultured in MD for 24 hours and then supplemented with SAM (50 μM) for another 24 hours. Statistical analyses were performed using one-way ANOVA with multiple comparisons (**a, b**). Data were presented as mean ± SD. $n = 3$ (**a, b**) biologically independent samples. Source data are provided as a Source Data file.



Supplementary Fig. 5 The cell viability of Huh-7 cells transfected with si-PRMT1 and treated with methionine deprivation and sorafenib for 48 hours. Statistical analyses were performed using one-way ANOVA with multiple comparisons. Data were presented as mean ± SD. n = 3 biologically independent samples. Source data are provided as a Source Data file.

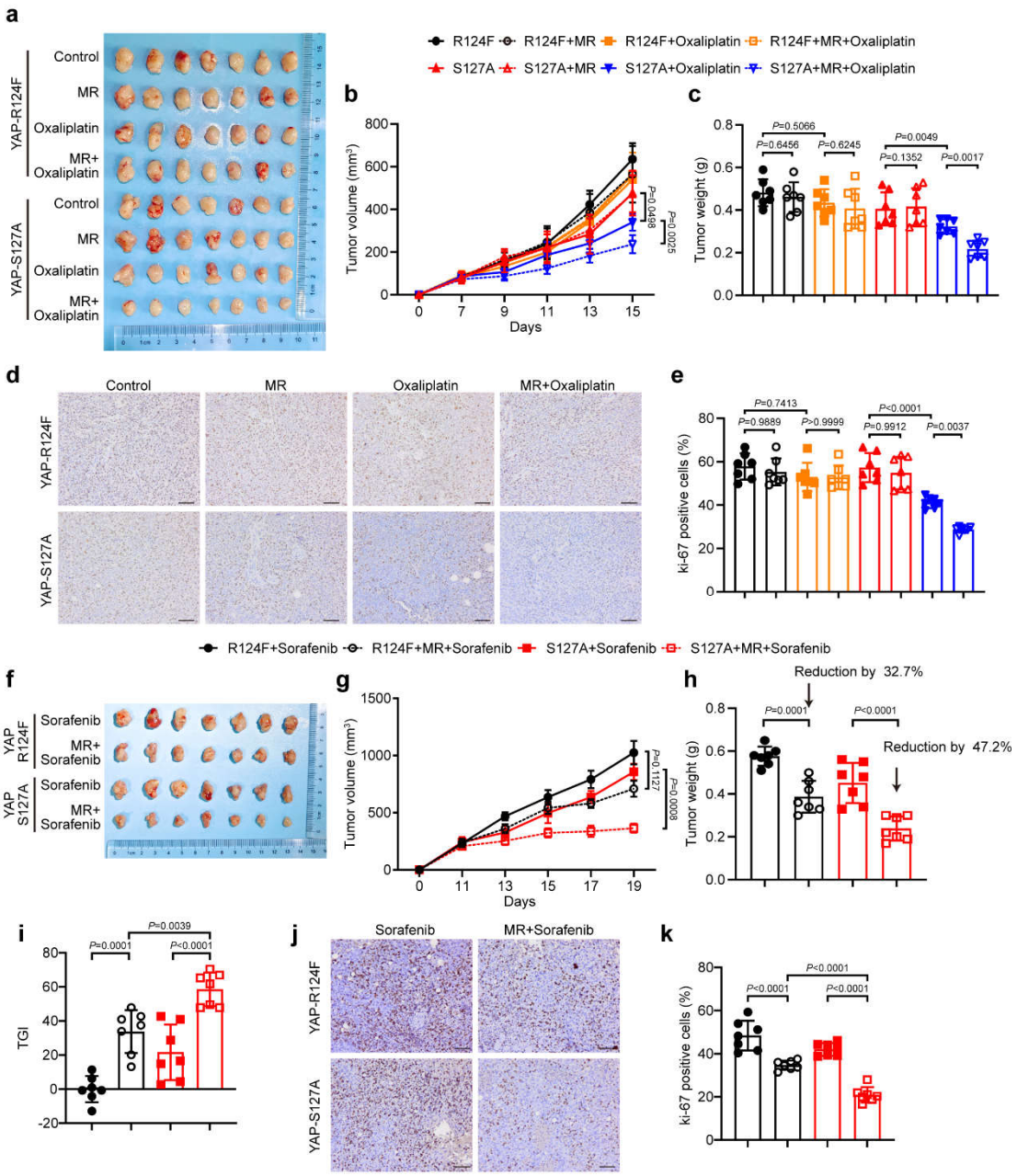


Supplementary Fig. 6 R124 asymmetric dimethylation of YAP promotes YAP-related drug resistance. a The relative YAP-TEAD activity in A549 cells treated with indicated concentration of gemcitabine. **b, c** The relative expression of YAP target genes in A549 cells treated with gemcitabine (2 μM) (**b**) and SGC7901 cells treated with 5-Fu (5 μM) (**c**) for 72 hours. **d, e** The level of R124 methylation of YAP in A549 cells treated with gemcitabine (2 μM) (**d**) and SGC7901 cells treated with and 5-Fu (5 μM) (**e**) for 72 hours. The data represents the relative ratio of YAP R124me2a to YAP. **f** The consensus sequence of human YAP around residues R124 and S127 and the mutant sequence of YAP-R124K, YAP-S127A, YAP-R124F. **g** The level of YAP phosphorylation in 293T cells infected with YAP or YAP mutation lentivirus for 48 hours. The data represents the relative ratio of pYAP (S127) to YAP. **h** The YAP-TEAD activity in Huh-7 cells infected with YAP or YAP mutation lentivirus and cultured in MD for 96 hours. Statistical analyses were performed using one-way ANOVA with multiple comparisons (**a-c, h**). Data were presented as mean ± SD. *n* = 5 (**a**), 3 (**b, c**) or 4 (**h**) biologically independent samples. The data presented in (**d, e, g**) are representative of three independent experiments. Source data are provided as a Source Data file.



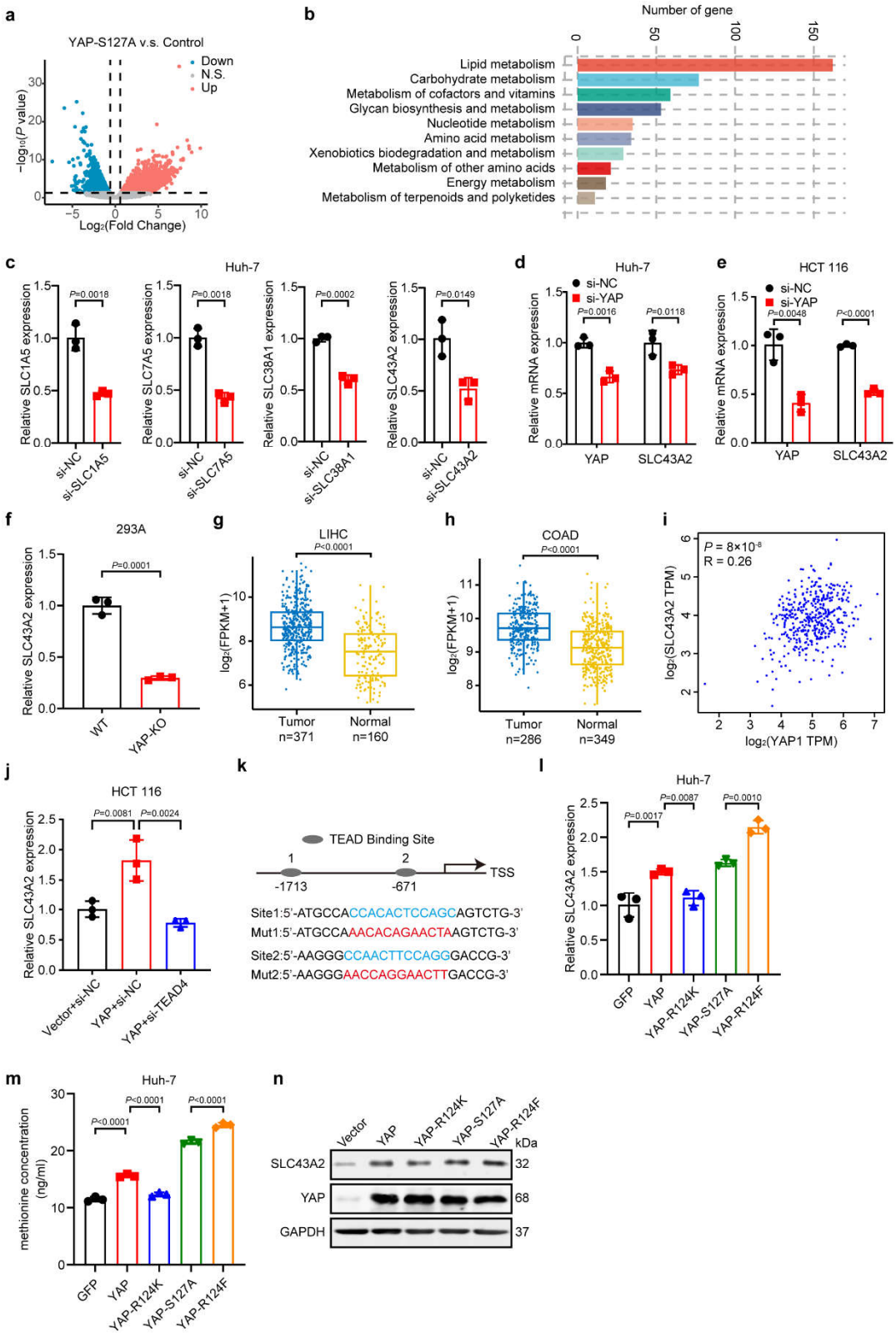
Supplementary Fig. 7 Methionine deficiency enhances drug efficacy by reducing YAP R124me2a. a The cell viability of YAP-, or YAP-R124K-overexpressed Huh-7 cells cultured in MD and treated with sorafenib for 24 hours. **b** The cell viability of YAP-, or YAP-R124K-overexpressed HCT 116 cells cultured in MD and treated with oxaliplatin for 48 hours. **c** The apoptosis of YAP-, or YAP-R124K-overexpressed Huh-7 cells cultured in MD and treated with sorafenib for 48 hours. **d** The apoptosis of YAP-, or YAP-R124K-overexpressed HCT 116 cells cultured in MD and treated with oxaliplatin for 72 hours. **e** The apoptosis of YAP-, YAP-S127A-, or YAP-R124F-overexpressed Huh-7 cells cultured in MD and treated with sorafenib for 48 hours. **f** The apoptosis of YAP-, YAP-S127A-, or YAP-R124F-overexpressed HCT 116 cells cultured in MD and treated with oxaliplatin for 72 hours. **g** The plasma methionine concentration in subcutaneous tumor model mice in Fig. 3c. n = 6 per group. **h** Representative IHC image of YAP R124me2a in tumors from YAP-S127A-overexpressed tumors treated with different regimens, Scale bar = 50 μm. Statistical analyses were performed using one-way ANOVA with multiple comparisons

(**a-g**). Data were presented as mean ± SD. n = 3 (**a-f**) or 6 (**g**) biologically independent samples. Source data are provided as a Source Data file.



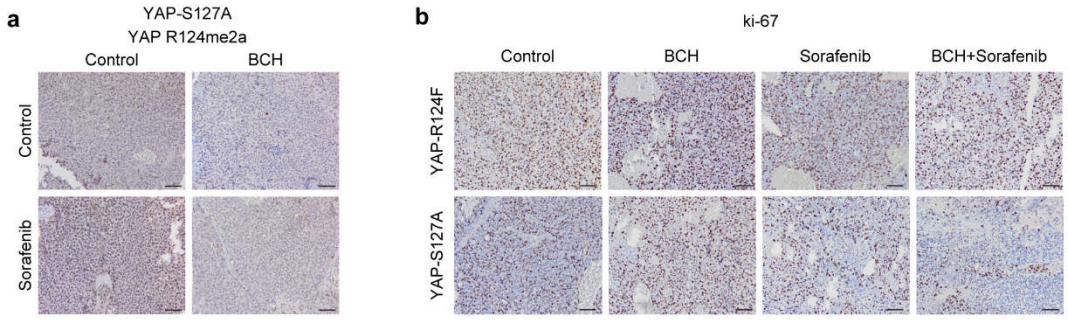
Supplementary Fig. 8 Dietary methionine restriction enhances drug efficacy by reducing YAP R124me2a *in vivo*. a Representative image of tumor nodules in different conditions. *Balb/c* nude mice were injected with HCT 116 cells overexpressing YAP-S127A or YAP-R124F and administrated with methionine-restricted diet and/ or oxaliplatin. **b, c** The statistical analysis of tumor volume (**b**) and tumor weight (**c**) in different groups described in supplementary Fig. 8a. **d, e** Representative images (**d**) and quantitation (**e**) of ki-67 staining in tumors from different groups were shown. scale bar = 50 μm. **f** Representative image of tumor nodules in different conditions. *C57BL* mice were injected with Hepa1-6 cells overexpressing YAP-S127A or YAP-R124F and administrated with methionine-restricted diet

and/ or sorafenib. **g-i** The statistical analysis of tumor volume (**g**), tumor weight (**h**) and TGI (**i**) in different groups described in supplementary Fig. 8f. The data in (**h)** represented the ratio of average tumor weight in YAP-R124F (or YAP-S127A) +MR+Sorafenib group to average tumor weight in YAP-R124F (or YAP-S127A)+Sorafenib group. **j, k** Representative images (**j**) and quantitation (**k**) of ki-67 staining in tumors from different groups were shown. scale bar = 50 μm. Statistical analyses were performed using one-way ANOVA with multiple comparisons (**c, e, h, i, k**) or two-way ANOVA with multiple comparisons (**b, g**). Data were presented as mean ± SEM for **b, g** and mean ± SD for **c, e, h, i, k**. $n = 7$ (**b, c, e, g, h, i, k**) biologically independent samples. Source data are provided as a Source Data file.

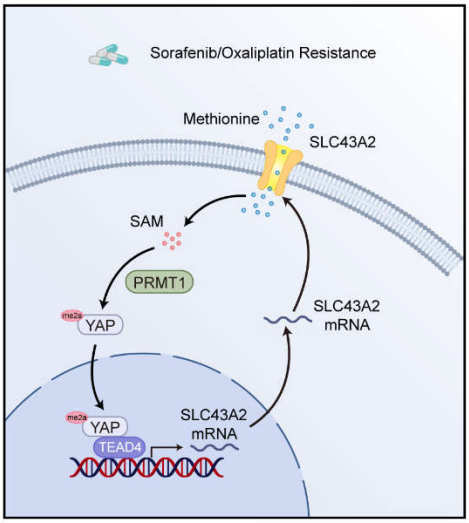


Supplementary Fig. 9 YAP promotes methionine uptake by upregulating SLC43A2 expression. a Volcano plot of the $\log_{10}$ (*P* value) against the $\log_2$ (Fold Change) of gene transcripts. Blue and red dots indicate significantly decreased and increased genes in YAP-S127A-overexpressed hepatocytes, respectively. **b** KEGG analysis of metabolism

pathway enrichment in YAP-S127A-overexpressed hepatocytes. **c** The knockdown efficiency of methionine transporters in Huh-7 cells. **d, e** The relative mRNA expression of SLC43A2 in Huh-7 cells (**d**) and HCT 116 cells (**e**) transfected with si-YAP for 72 hours. **f** The relative mRNA expression of SLC43A2 in YAP-knockout 293A cells. **g, h** The SLC43A2 mRNA levels in tumors and normal tissues from patients with LIHC (**g**) and COAD (**h**) in the TCGA dataset. **i** Spearman correlation between YAP and SLC43A2 in tumors and matched normal tissues from patients with COAD or READ in the TCGA dataset. **j** The relative mRNA expression of SLC43A2 in HCT 116 cells transfected with YAP and/or si-TEAD4 for 72 hours. **k** Predicted sequence of TEAD binding sites in SLC43A2 promoter and the parallel mutant sequences. **l, m** The relative transcriptional expression of SLC43A2 (**l**) and intracellular methionine concentration (**m**) in Huh-7 cells infected with YAP or YAP mutation lentivirus for 96 hours. **n** The protein level of SLC43A2 in Huh-7 cells infected with YAP or YAP mutation lentivirus for 96 hours. Statistical analyses were performed using two-sided unpaired Student's t-test (**c-f**), Wi Wilcoxon test (**g, h**), one-way ANOVA with multiple comparisons (**j, l, m**) or two-tailed Spearman's correlation test (**i**). Data were presented as mean ± SD. n = 3 (**c-f, j, l, m**) biologically independent samples. The data presented in (**n**) are representative of three independent experiments. Source data are provided as a Source Data file.



Supplementary Fig. 10 Disrupting the positive feedback between YAP R124 methylation and SLC43A2 improves drug sensitivity. a Representative IHC image of YAP R124me2a in YAP-S127A-overexpressed tumors treated with different regimens. Scale bar = 50 μm. **b** Representative image of ki-67 staining in tumors from different groups were shown. Scale bar = 50 μm.



Supplementary Fig. 11 Working model of the proposed mechanism in this study.

Methionine enhances resistance to sorafenib and oxaliplatin by elevating PRMT1-catalyzed methylation of YAP R124. Furthermore, YAP activates the expression of SLC43A2, the major methionine transporter, to sustains high methionine levels in cancer cells by interacting with TEAD4. We establish the positive feedback between YAP R124 methylation and methionine transporter SLC43A2 during drug resistance.

Supplementary Table 1: The oligonucleotides used in this paper		
si-NC	UUCUCCGAACGUGUCACGU	
si-PRMT1	GAGUUCACACGCUGCCACA	
si-YAP	GGAAGGAGAUGGAAUGAACAUAG AA	
si-SLC1A5	GUACCGUCCUCAAUGUAGA	
si-SLC7A5	CUCUUUGCCUAUGGAGGAU	
si-SLC38A1	CAAUUUACAGUGAGCUUAA	
si-SLC43A2	CCUUCAUCCUGCACACAAU	
si-TEAD4	GGGCAGACCUCAACACCAA	
Human GAPDH qPCR	F: TTGATTTTGGAGGGATCTCG	R: GAGTCAACGGATTTGGTCGT
Human YAP qPCR	F: CAGCAACTGCAGATGGAGAA	R: TGGATTTTGAGTCCCACCAT
Human CTGF qPCR	F: GTAATGGCAGGCACAGGTCT	R: CCGTACTCCCAAAATCTCCA
Human CYR61 qPCR	F: GGTCAAAGTTACCGGGCAGT	R: GGAGGCATCGAATCCCAGC
Human AREG qPCR	F: GCTGTCGCTCTTGATACTCG	R: ACGCTTCCCAGAGTAGGTGT
Human SLC1A5 qPCR	F:TCATGTGGTACGCCCCTGT	R:GCGGGCAAAGAGTAAACCCA
Human SLC7A5 qPCR	F:CCGTGAACTGCTACAGCGT	R:CTTCCCGATCTGGACGAAGC
Human SLC38A1 qPCR	F:AACCTCCTTAGGCATGTCTGT	R:GCAAAGGCGAGTCCCAAAAT
Human SLC43A2 qPCR	F:TGGGTATCGTCATGGACAAGT	R:CGGAGAGAGCGTTTGGTTTAC
Human PRMT1 qPCR	F:CAGGCGGAAAGCAGTGAGAA	R:TGGAGTTGCGGTAAGTGAGG
Human TEAD4 qPCR	F:GGACACTACTCTTACCGCATCC	R:TCAAAGACATAGGCAATGCAC A
SLC43A2 CHIP-qPCR-1	F:GTCATGCCTGTAATCCCT	R:AGCCTCCCAAGTAGCTG
SLC43A2 CHIP-qPCR-2	F:GGAGCACCCAGATGACGA	R:GCTGGTCTGTCCCGTCCT
CYR61 CHIP-qPCR	F:CTCGCCCCTCACGACCCTCC	R:GCCTCCCCTGGCTCCATTGC

Supplementary Table 2: The antibodies used in this paper			
Antibodies	Source	Identifier	Dilution
Western blot antibodies:			
Mouse anti-YAP	Cell Signaling Technology	CAT#12395	1:1,000
Rabbit anti-YAP R124me2a	Chinapeptides	N/A	1:400
Rabbit anti-Phospho-YAP (Ser127)	Cell Signaling Technology	CAT#13008	1:1,000
Rabbit anti-SLC43A2	Thermo Fisher Scientific	CAT#PA5-54451	1:1,000
Rabbit anti-PRMT1	Cell Signaling Technology	CAT#2449	1:1,000
Rabbit anti-puromycin	ABclonal	CAT#A21205	1:3,000
Rabbit anti- phospho-LATS	ABclonal	CAT#AP1517	1:1,000
Rabbit anti-LATS1	Cell Signaling Technology	CAT #3477	1:1,000
Rabbit anti- LATS2	ABclonal	CAT #A16249	1:1,000
Rabbit anti-GAPDH	Cell Signaling Technology	CAT#97166	1:5,000
800CW Goat anti-Rabbit IgG Secondary Antibody	LI-COR Biosciences	CAT#926-32210	1:3,000
680CW Goat anti-Mouse IgG Secondary Antibody	LI-COR Biosciences	CAT#926-68071	1:7,000
IHC antibodies:			
Mouse anti-ki-67	Servicebio	CAT#GB121141-100	1:3,000
Rabbit anti-YAP R124me2a	Chinapeptides	N/A	1:400
Rabbit anti-SLC43A2	Thermo Fisher Scientific	CAT#PA5-54451	1:50
ChIP-qPCR antibodies:			
Rabbit anti-YAP	CST	CAT#14074	1:50
Mouse anti-TEAD4	Abcam	CAT#AB58310	1:50