Supplementary Figures

Targeting METTL3 enhances the chemosensitivity of non-small cell lung cancer cells by decreasing ABCC2 expression in an m^{6}A-YTHDF1-dependent manner

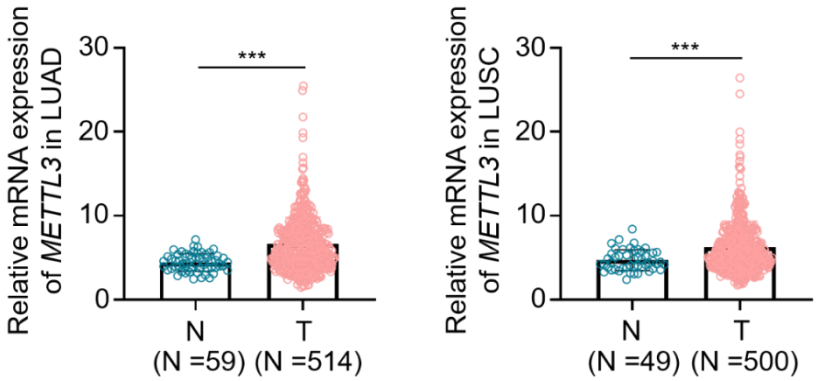

Figure S1. *METTL3* mRNA expression in lung cancers

The mRNA expression of METTL3 was analyzed in LUADs and LUSCs and noncancerous lung tissues (the data from the TCGA database). N: noncancerous lung tissue, T: tumor tissue. Data were presented as mean ± SD. ***, $P < 0.001$.

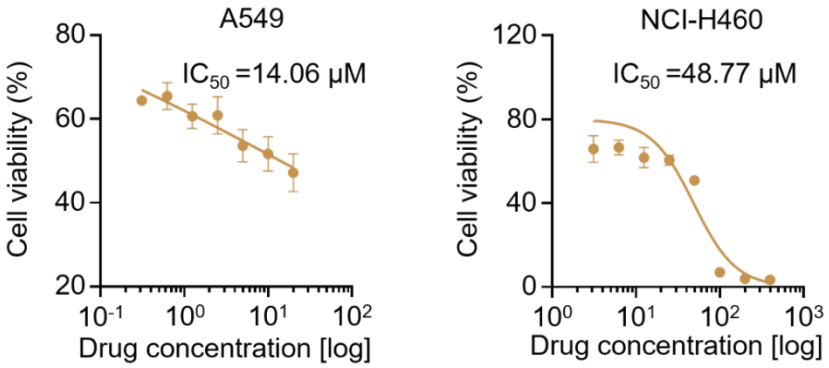

Figure S2. The IC_{50} values of STM2457 in NSCLC cells.

A549 and NCI-H460 cells were treated with indicated concentrations of STM2457 for 48 h. MTT assay was then performed to evaluated cell viability, and Reed-Muench method was used to calculated the IC_{50} values. The data were presented as the mean ± SD.

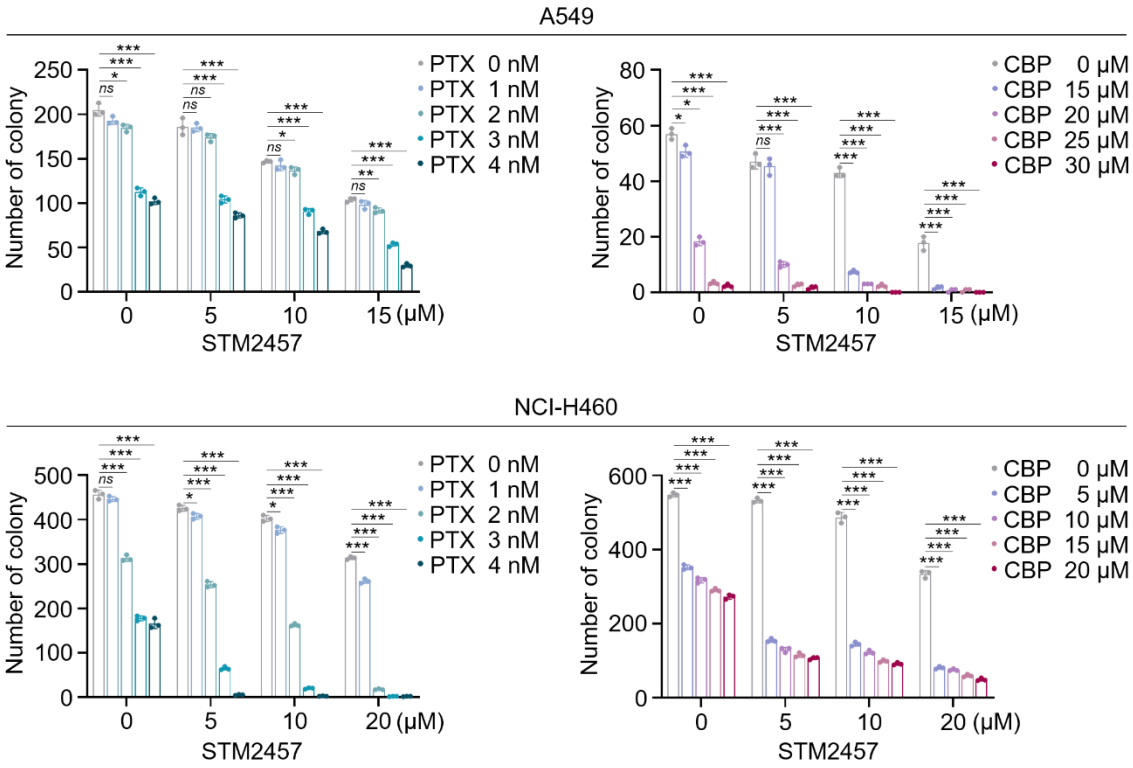

Figure S3. The statistical analysis of clones

A549 and NCI-H460 cells were treated with indicated PTX or CBP individually or in combination with different dose of STM2457, and their effect on cell colony formation ability was then evaluated. The number of clones was statistically analyzed. The data were presented as the mean ± SD. *, P <0.05; **, P <0.01; ***, P <0.001; *ns*, no significance.

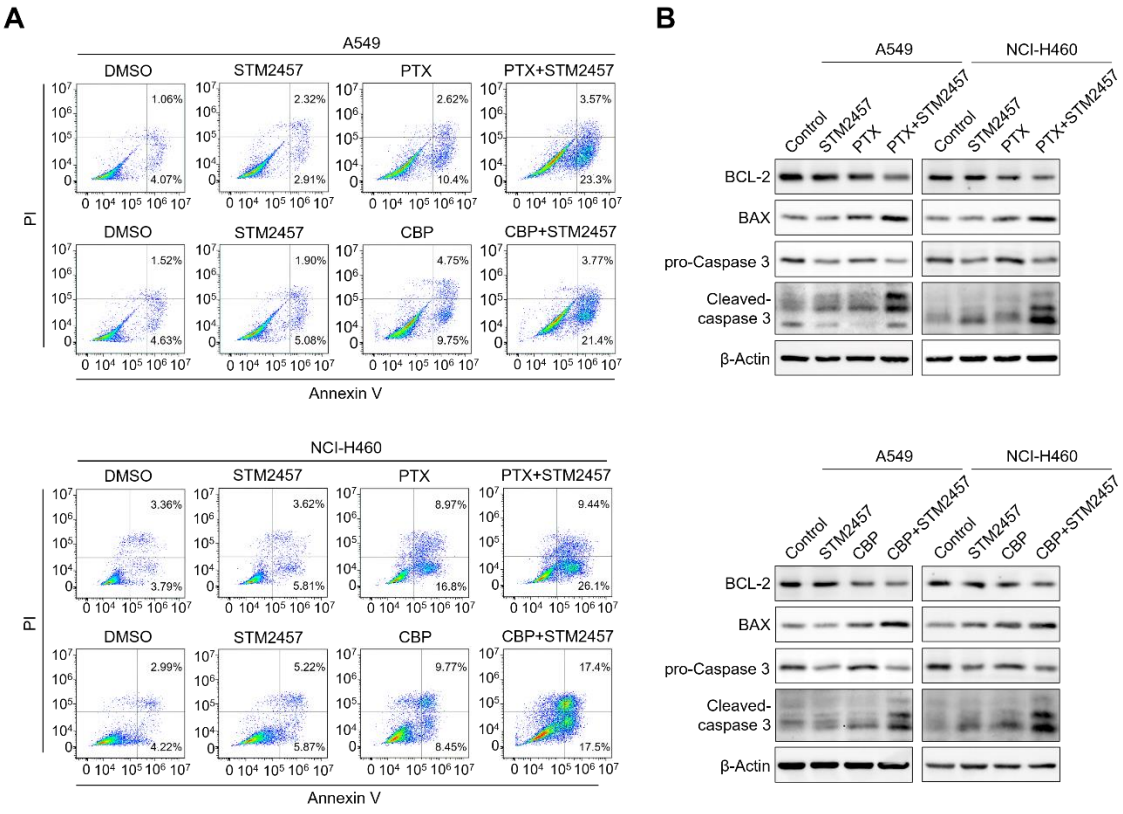

Figure S4. The effect of STM2457, individually or in combination with PTX or CBP, on the apoptosis of NSCLC cells

A549 and NCI-H460 cells were treated with 5 μM or 20 μM STM2457 individually or in combination with 5 nM PTX or 30 μM CBP for 48 h. (**A**) Cell apoptosis was then assessed by flow cytometry. (**B**) The levels of apoptosis-related markers were detected by western blotting analysis. β-Actin was used as a loading control.

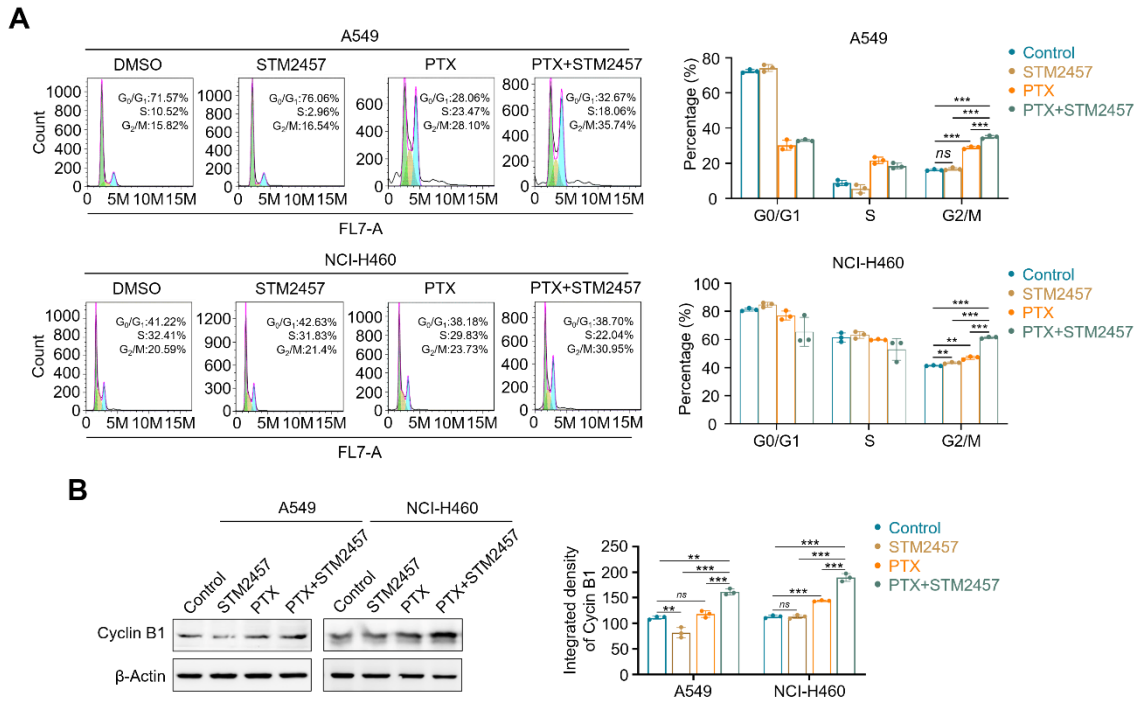

Figure S5. The effect of STM2457, individually or in combination with PTX or CBP, on cell cycle distribution of NSCLC cells

(A) A549 and NCI-H460 cells were treated with 5 μM or 20 μM STM2457 individually or in combination with 5 nM PTX or 30 μM CBP. After a 24-h treatment, cell cycle was assessed by flow cytometry. Representative flow cytometric histograms were shown in the left panel, and the percentage of each cell cycle phase was indicated in the right panel. **(B)** The levels of cyclin B1 were detected by western blotting analysis and integrated density was analyzed statistically. The representative images of cyclin B1 were shown in the left panels, and statistical analysis of integrated density of cyclin B1 was shown in the right panel. β-Actin was used as a loading control. Data were presented as mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; *ns*, no significance.

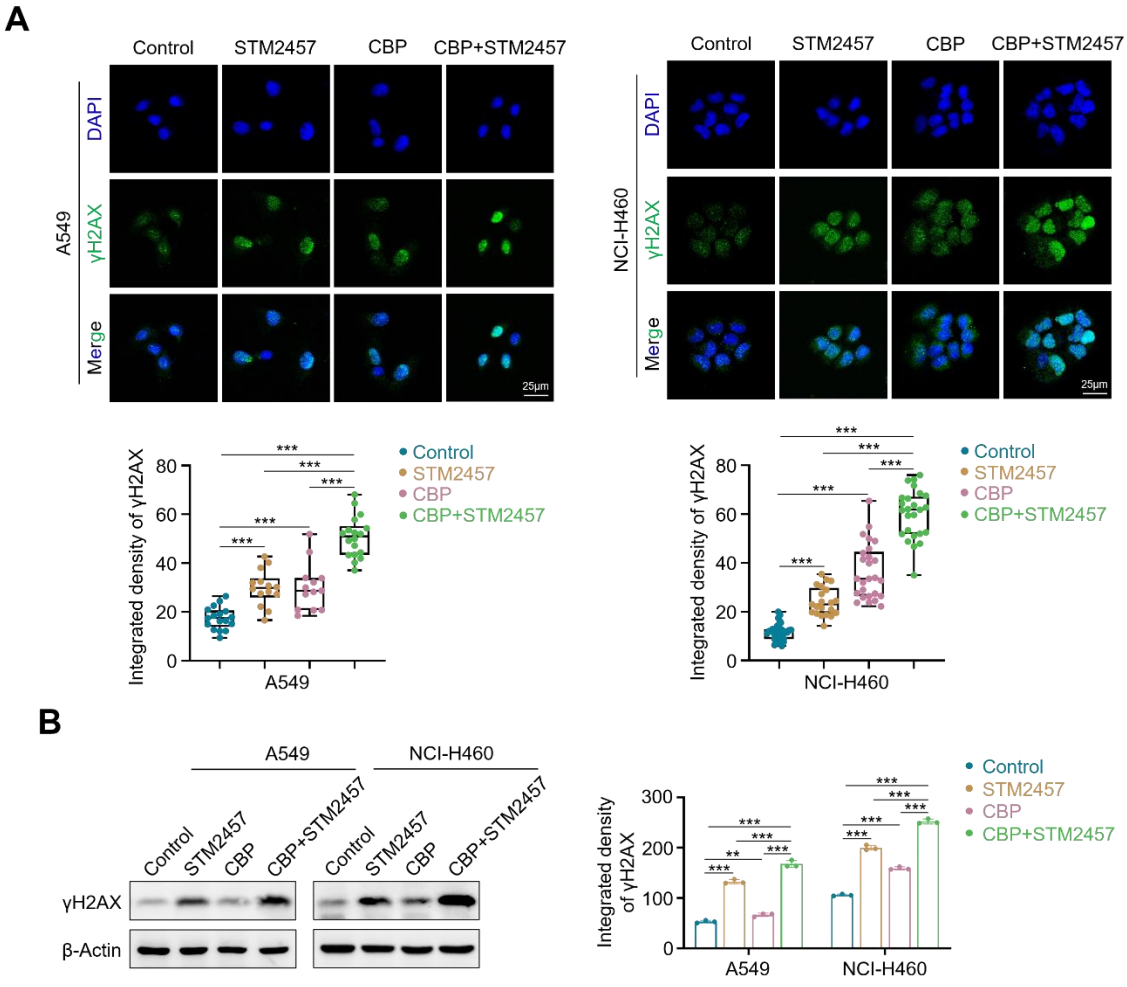

Figure S6. The effect of STM2457, individually or in combination with PTX or CBP, on DNA damage of NSCLC cells

(A) A549 and NCI-H460 cells were treated with 5 μM or 20 μM STM2457 individually or in combination with 5 nM PTX or 30 μM CBP. After a 48-h treatment, γH2AX foci was detected by immunofluorescence assay. The representative immunofluorescence images of γH2AX were shown in the upper panels, and statistical analysis of integrated density of γH2AX was shown in the lower panels. Blue color indicates the staining of nuclei, and

green color indicates the staining of γH2AX. Scale bars, 25 μm. **(B)** A549 and NCI-H460 cells were treated with the same conditions as above for 48 h. The protein expression of γH2AX was detected by western blotting analysis. The representative images of γH2AX were shown in the left panel, and statistical analysis of integrated density of γH2AX was shown in the right panel. β-Actin was used as a loading control.

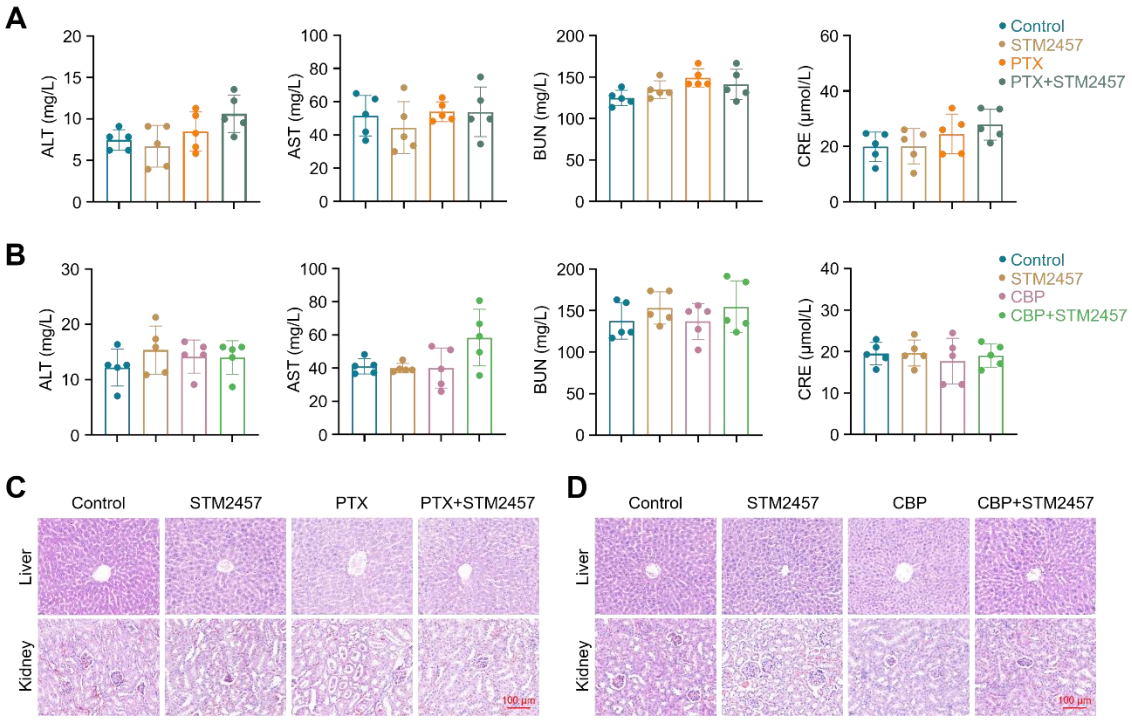

Figure S7. Safety evaluation of drugs in nude mice

(A-B) Tumor-bearing nude mice were grouped randomly (n=5/group) and treated with STM2457 (30 mg/kg, once a day) and PTX (3 ng/kg, once every two days) or CBP (30 mg/kg, once every two days), individually or in combination, for 2 weeks. The levels of alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and serum creatinine (CRE) were detected by corresponding kits. **(C-D)** Representative

H&E staining of kidney and liver sections from the indicated groups. Scale bar, 100 μm. Data were presented as mean ± SD.

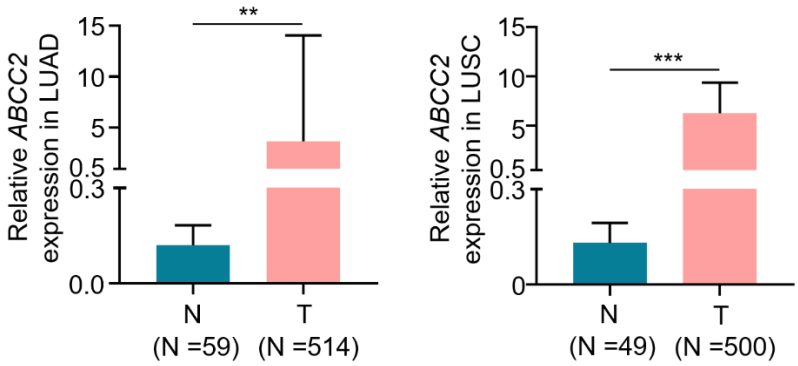

Figure S8. *ABCC2* mRNA expression in lung cancers

ABCC2 mRNA expression was analyzed in LUADs and LUSCs in comparison with unpaired noncancerous lung tissues (the data from the TCGA database). N: noncancerous lung tissue, T: tumor tissue. Data were presented as mean ± SD. **, $P < 0.01$; ***, $P < 0.001$.

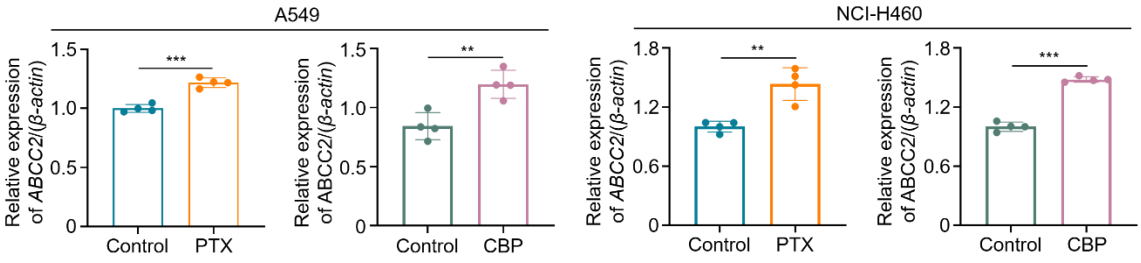

Figure S9. The effect of PTX or CBP on mRNA expression of ABCC2

A549 cells were treated 3 nM PTX or 20 μM CBP and NCI-H460 cells were treated 5 nM PTX or 30 μM CBP for 24 h, the mRNA expression of *ABCC2* was measured by qRT-PCR

assay. *β-actin* was used as the normalization control. Data were presented as mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

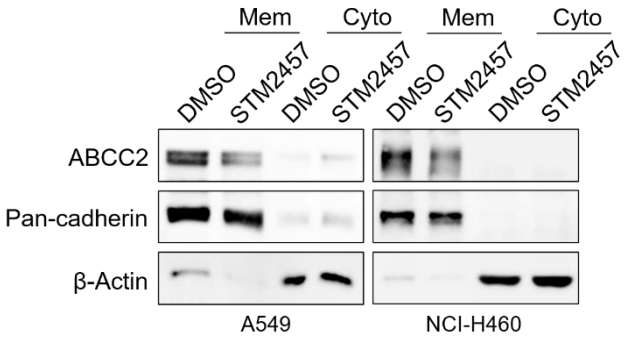

Figure S10. The protein expression of ABCC2 in cytomembrane and cytosol

A549 and NCI-H460 cells were treated with 5 μM or 20 μM STM2457, respectively. The proteins in membrane and cytosol were extracted separately, and western blotting analysis was then performed to detect the production of ABCC2 in cytomembrane and cytosol. β-Actin was used as a loading control of cytoplasm proteins, and pan-cadherin was used a loading control of cytomembrane proteins. Mem, Membrane; Cyto, Cytosol.

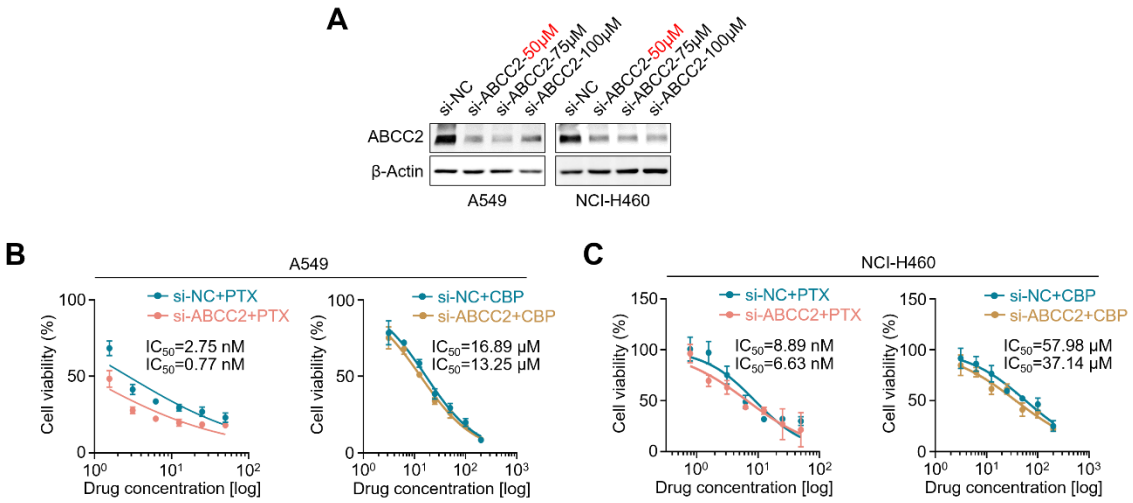

Figure S11. The regulatory effect of ABCC2 on the sensitivity of PTX and CBP

(A) A549 and NCI-H460 cells were treated with different doses of siRNA targeting ABCC2, and the protein levels of ABCC2 were measured by western blotting analysis. β-Actin was used as a loading control. (**B**-**C**) ABCC2-knockdown A549 and NCI-H460 cells and their control cells were treated with the indicated doses of PTX or CBP for 48 h. MTT assay was then performed to evaluated cell viability, and Reed-Muench method was used to calculated the IC_{50} values. The data were presented as the mean ± SD.

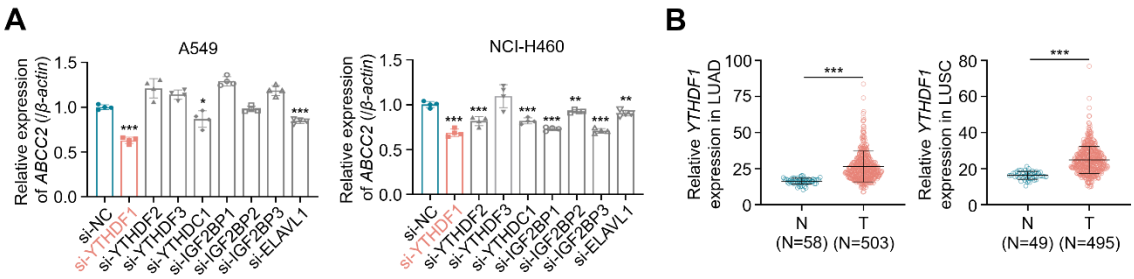

Figure S12. The regulatory effect of YTHDF1 on mRNA expression of *ABCC2*

(A) The indicated m^6A reader proteins were knocked down in A549 and NCI-H460 cells,

and the mRNA expression of *ABCC2* was measured by qRT-PCR. *β-actin* was used as a normalization control. **(B)** *YTHDF1* mRNA expression was analyzed in LUAD and LUSC tissues in comparison with unpaired normal lung tissues (the data from the TCGA database). N: noncancerous lung tissue, T: tumor tissue. Data were presented as mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplementary Tables

Supplementary Table S1: Sequence of siRNAs

siRNAs	Sequence (5’-3’)
si-NC	5'-UUCUCCGAACGUGUCACGUTT-3’
si-METTL3#1	5'-CAAGTATGTTCACTATGAA-3’
si-METTL3#2	5'-GACTGCTCTTTCCTTAATA-3’
si-YTHDF1#1	5'-ACGGCAGAGTCGAAACAAA-3’
si-YTHDF1#2	5′-CCTCCACCCATAAAGCATA-3′
si-YTHDF2	5′-AAGGACGTTCCCAATAGCCAA-3′
si-YTHDF3	5′-AGAUGGUGUAUUUAGUCAACC-3′
si-YTHDC1	5’-GAAGUGGAUAGACGUGCAATT-3’
si-IGF2BP1	5’-GGCCCAUAAUAACUUUGUATT-3’
si-IGF2BP2	5’-CATGCCGCATGATTCTTGA-3’
si-IGF2BP3	5’-GCAGGAAUUGACGCUGUAUTT-3’
si-ELAVL1	5′-AAGAGGCAAUUACCAGUUUCA-3′
si-ABCC2	5′-CCATAGCTTCATTCCTGAGTA-3′

Supplementary Table S2: The primers of qRT-PCR used in this study

Genes	Primer sequences
β-actin	Forward primer (5’-3’), CCTTGCACATGCCGGAG;
	Reverse primer (3’-5’), GCACAGAGCCTCGCCTT.
METTL3	Forward primer (5’-3’), CAAGCTGCACTTCAGACGAA;
	Reverse primer (3’-5’), GCTTGGCGTGTGGTCTTT.
ABCC2	Forward primer (5’-3’), CCCTGCTGTTCGATATACCAATC;
	Reverse primer (3’-5’), TCGAGAGAATCCAGAATAGGGAC.
18S rRNA	Forward primer (5’-3’), CAGCCACCCGAGATTGAGCA;
	Reverse primer (3’-5’), TAGTAGCGACGGGCGGTGTG.
YTHDF1	Forward primer (5’-3’), ACCTGTCCAGCTATTACCCG;
	Reverse primer (3’-5’), TGGTGAGGTATGGAATCGGAG.

YTHDF2	Forward primer (5’-3’), TAGCCAACTGCGACACATTC;
	Reverse primer (3’-5’), CACGACCTTGACGTTCCTTT.
YTHDF3	Forward primer (5’-3’), GGTGTATTTAGTCAACCTGGGG;
	Reverse primer (3’-5’), AAGAGAACTAGGTGGATAGCCAT.
YTHDC1	Forward primer (5’-3’), AAGGAGGGCCAAATCTCCTA;
	Reverse primer (3’-5’), CAGTGTTGTTCCCTTGCTCA.
IGF2BP1	Forward primer (5’-3’), AGAATGATGTGGCTGCCATGA;
	Reverse primer (3’-5’), AAAGGAGCTATAGGGAGCAGC.
IGF2BP2	Forward primer (5’-3’), AGCTAAGCGGGCATCAGTTTG;
	Reverse primer (3’-5’), CCGCAGCGGGAAATCAATCT.
IGF2BP3	Forward primer (5’-3’), TATATCGGAAACCTCAGCGAGA;
	Reverse primer (3’-5’), GGACCGAGTGCTCAACTTCT.
ELAVL1	Forward primer (5’-3’), TGTTCTCTCGGTTTGGGCGGAT;
	Reverse primer (3’-5’), TCTTCTGCCTCCGACCGTTTGT.
ABCA1	Forward primer (5’-3’), GCAAGGCTACCAATTACATTTG;
	Reverse primer (3’-5’), GGTCAGAAACATCACCTCCTG.
ABCA2	Forward primer (5’-3’), GCCCAGGTCTGGCTCAACATCTC;
	Reverse primer (3’-5’), CTCACCTTGGACATGAACTGGAT.
ABCA3	Forward primer (5’-3’), CTTGACAGTCGCAGAGCACCTT;
	Reverse primer (3’-5’), CTCCGTGAGTTCCACTTGTCCT.
ABCA8	Forward primer (5’-3’), GGCCCTTTTCTTGGCACTTG;
	Reverse primer (3’-5’), CAGGCCGGTGAGGAAAGATT.
ABCB1	Forward primer (5’-3’), TGCTCAGACAGGATGTGAGTTG;
	Reverse primer (3’-5’), AATTACAGCAAGCCTGGAACC.
ABCB4	Forward primer (5’-3’), GCATCAGCAGCAAACAAAAA;
	Reverse primer (3’-5’), GCAGCGACAAGGAAAAGTTC.
ABCB5	Forward primer (5’-3’), TTTCTCCGCCAGCATTCCAT;
	Reverse primer (3’-5’), GCTGAGGAATCCACCCAATCT.
ABCB8	Forward primer (5’-3’), CTGTCAGGTACTCTGATGGCT;
	Reverse primer (3’-5’), TCCATCTGGGAGCTAGGGG.
ABCB11	Forward primer (5’-3’), ACTAGATGAAGCCACTTCTGCCTTA;

	Reverse primer (3’-5’), TGCACCGTCTTTTCACTTTCTGT.
ABCC1	Forward primer (5’-3’), CTCTATCTCTCCCGACATGACC;
	Reverse primer (3’-5’), AGCAGACGATCCACAGCAAAA.
ABCC3	Forward primer (5’-3’), AGCTCGGCTCCAAGTTCTG;
	Reverse primer (3’-5’), GACCCACAGGTAGATGCAGG.
ABCC4	Forward primer (5’-3’), AGCTGAGAATGACGCACAGAA;
	Reverse primer (3’-5’), ATATGGGCTGGATTACTTTGGC.
ABCC5	Forward primer (5’-3’), AGTCCTGGGTATAGAAGTGTGAG;
	Reverse primer (3’-5’), ATTCCAACGGTCGAGTTCTCC.
ABCC6	Forward primer (5’-3’), CTGGACGAGGCTACTGCTG;
	Reverse primer (3’-5’), TTGTCCATGACCAGAACCC.
ABCC10	Forward primer (5’-3’), CGGCCTGCTCTATGCTCTG;
	Reverse primer (3’-5’), CCCCGTGCCTGAAGTGTTA.
ABCC11	Forward primer (5’-3’), CCTACTTCATTATTGGATACACTGC;
	Reverse primer (3’-5’), CTTGTCATGAATACCGCCAG.
ABCE1	Forward primer (5’-3’), CACAGGTTGCCTATCCCTCG;
	Reverse primer (3’-5’), AAATAAGTCAAAATCTCCTGCCAGTCAG.
ABCG1	Forward primer (5’-3’), GTCTGAACTGCCCAACCTACCAC;
	Reverse primer (3’-5’), CCGACTGTTCTGATCACCGTACTC.
ABCG2	Forward primer (5’-3’), CAGGTGGAGGCAAATCTTCGT;
	Reverse primer (3’-5’), ACCCTGTTAATCCGTTCGTTTT.

Supplementary Table S3. The antibodies used in this study

Antibodies	Catalog#	Source
anti-METTL3	ab195352	Abcam
anti-ABCC2	ab172630	Abcam
anti-m^6A	ab151230	Abcam
anti-Ki67	550609	BD Pharmingen
anti-YTHDF1	R27444	zenbio
anti-cyclin B1	R23304	zenbio
anti- Goat Anti-Rabbit IgG H&L (AF488)	550037	zenbio

anti-γH2AX (phospho S139)	3924583	Merck
anti-BCL-2	12789-1-AP	proteintech
anti-β-Actin	sc-1616	Santa Cruz
anti-pan-cadherin	R25274	zenbio
anti-BAX	380709	zenbio
anti-caspase 3	sc-56053	Santa Cruz

Supplementary Table S4. The primers used for MeRIP-qPCR

Site	**Primer sequences**
ABCC2-m^{6}A	Forward primer (5'-3'), TGAAGGAAGACGAAGAAC; Reverse primer (3'-5'), CTTCACCTTTCCAGTTTCTATG.