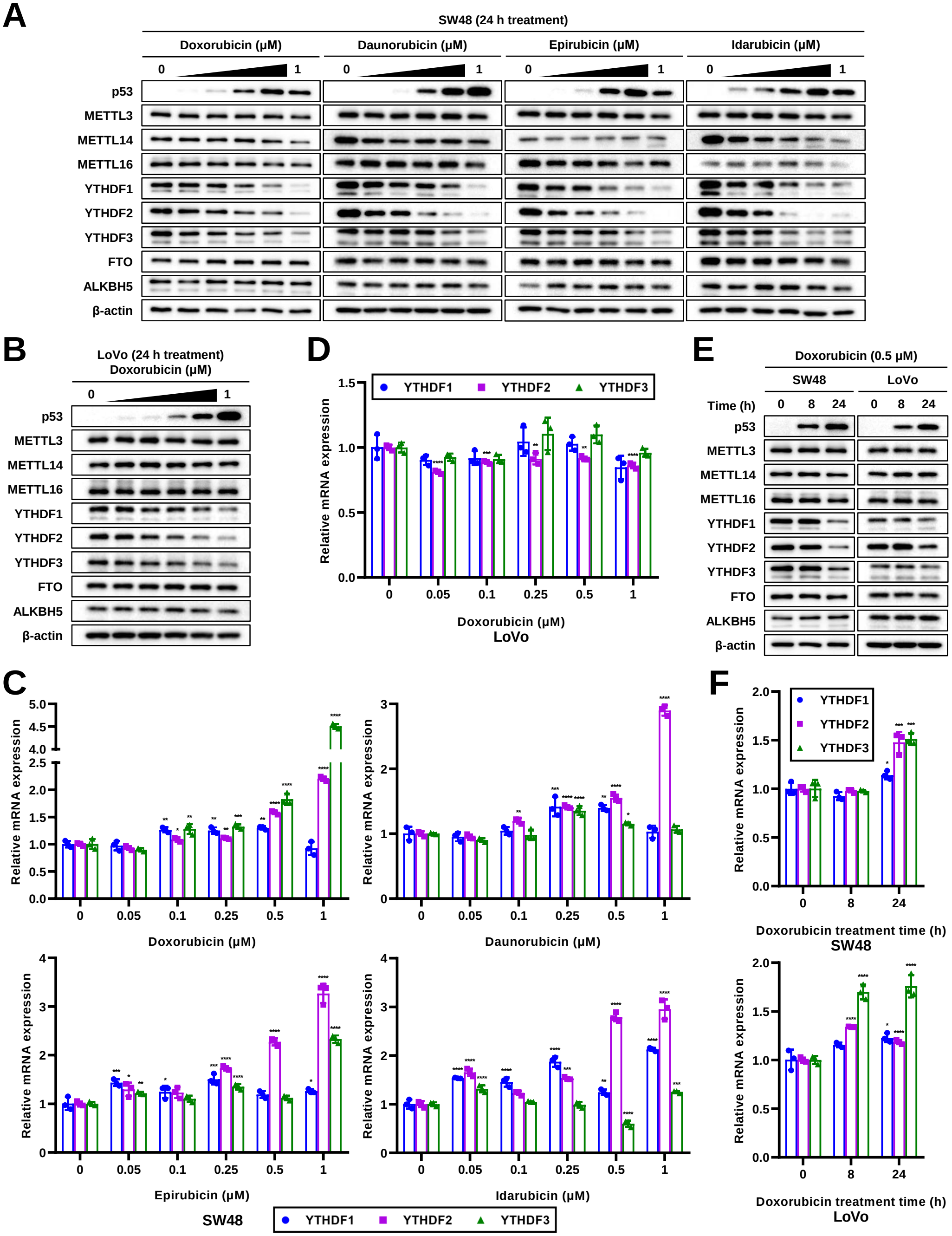
Supplementary Fig. 1



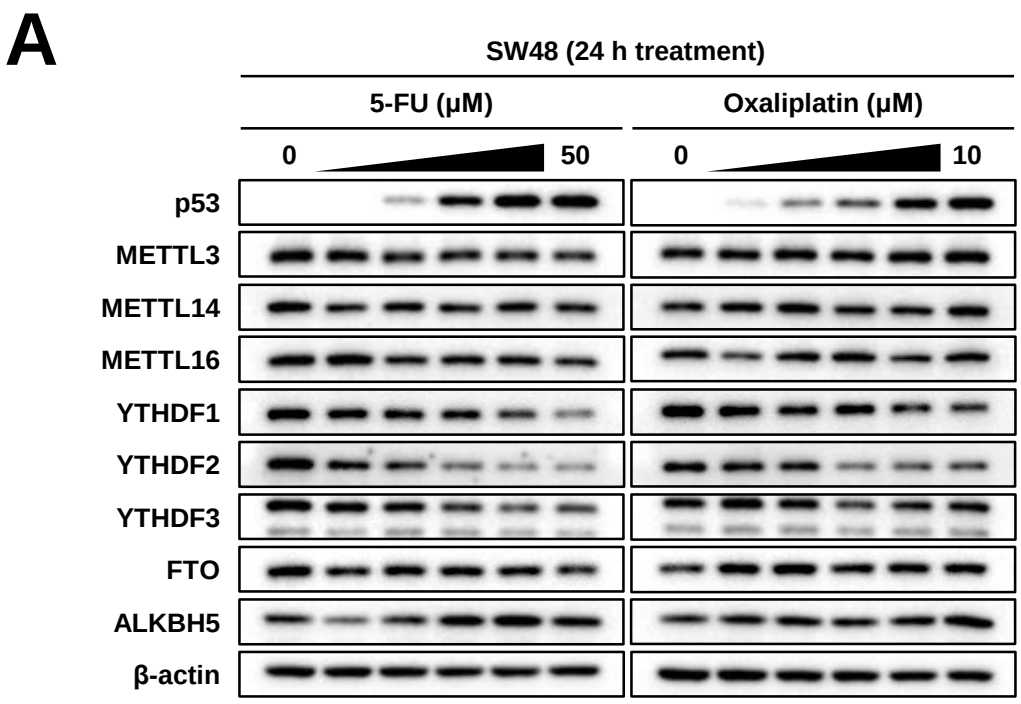
Supplementary Fig. 1 Validation of anthracycline-induced downregulation of YTHDF family proteins in additional cell lines.

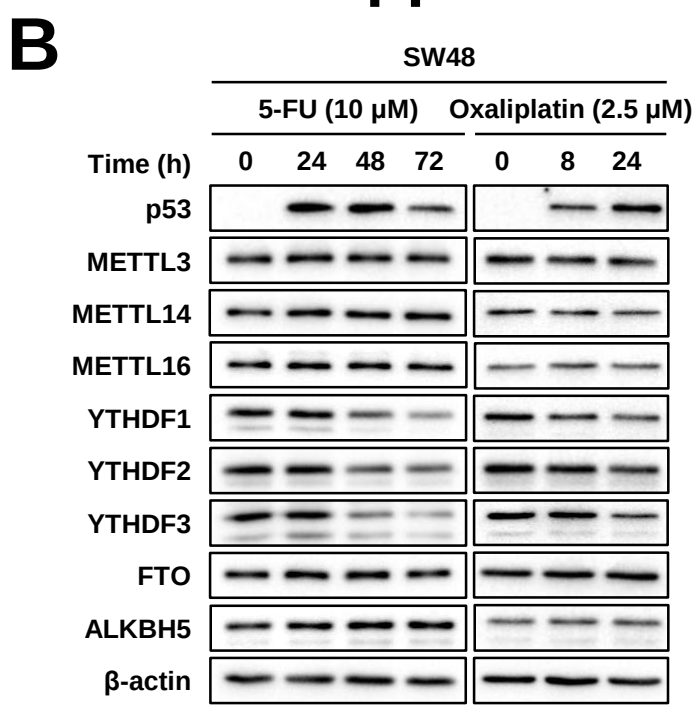
A-**D** Western blot (**A**, **B**) and quantitative real-time PCR (**C**, **D**) in SW48 and LoVo cells following anthracycline treatment. SW48 cells were treated with doxorubicin, daunorubicin, epirubicin, or idarubicin (0, 0.05, 0.1, 0.25, 0.5, and 1 μM) (**A**, **C**), whereas LoVo cells were treated with doxorubicin (0, 0.05, 0.1, 0.25, 0.5, and 1 μM) (**B**, **D**) for 24 h.

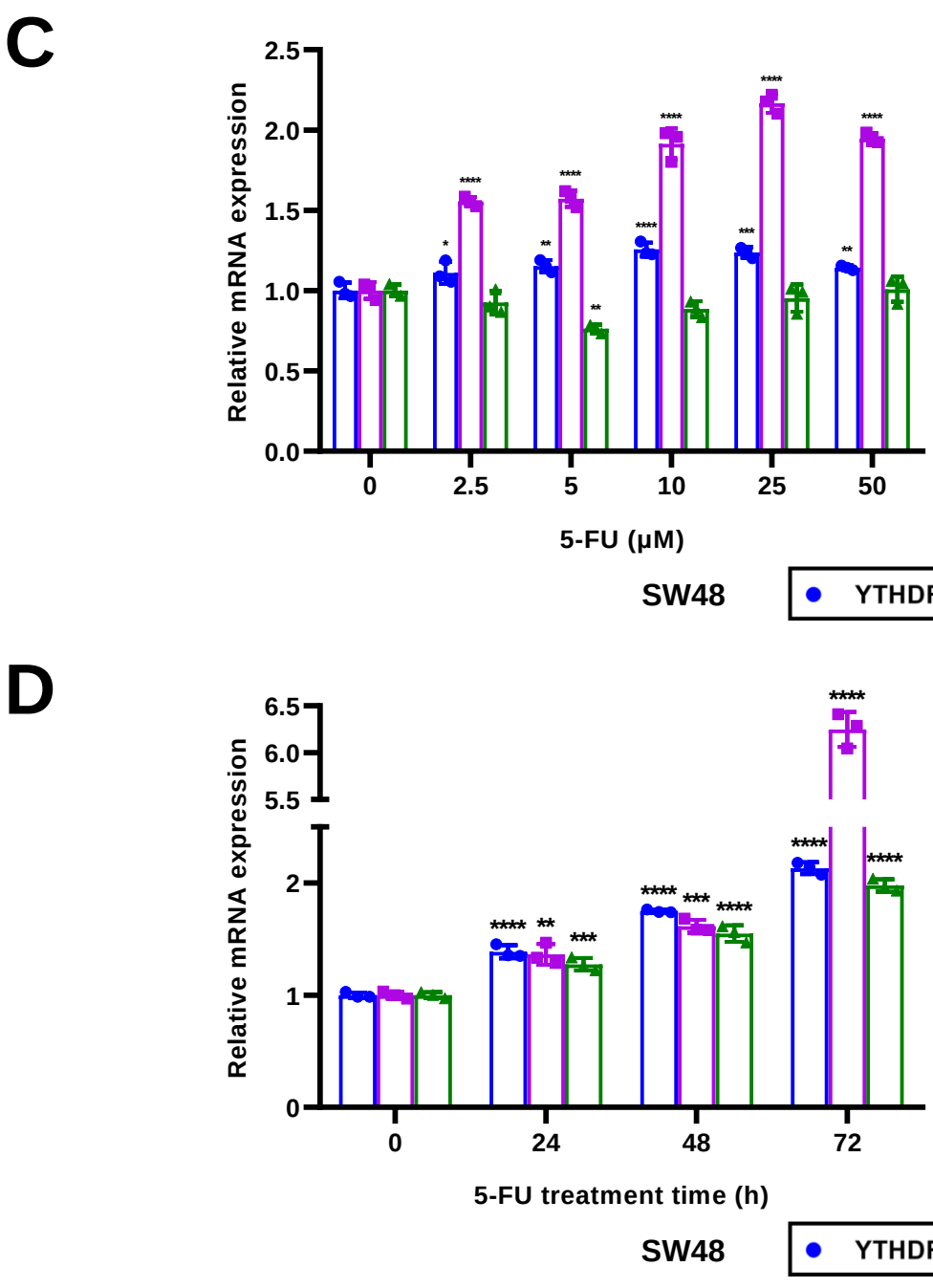
E, **F** Western blot (**E**) and quantitative real-time PCR (**F**) in SW48 and LoVo cells following doxorubicin treatment. SW48 and LoVo cells were treated with 0.5 μM doxorubicin for 0, 8, and 24 h.

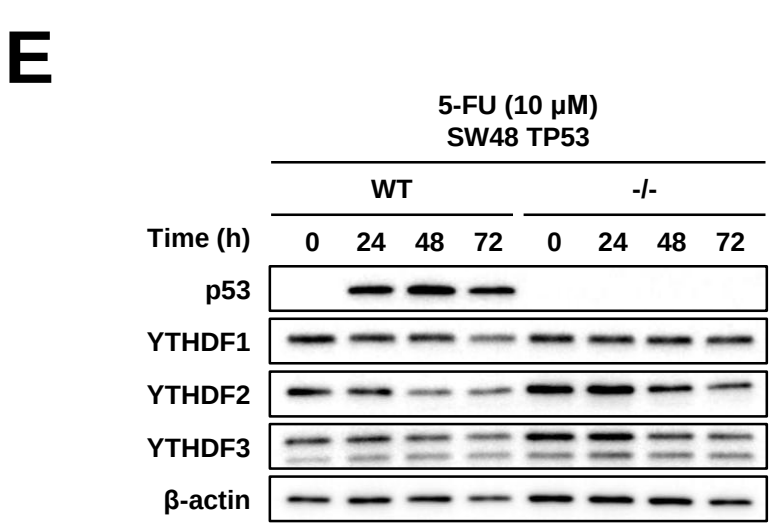
Data are presented as mean ± SD from experiments performed in triplicate. Differences among groups were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test (**C**, **D**, **F**).

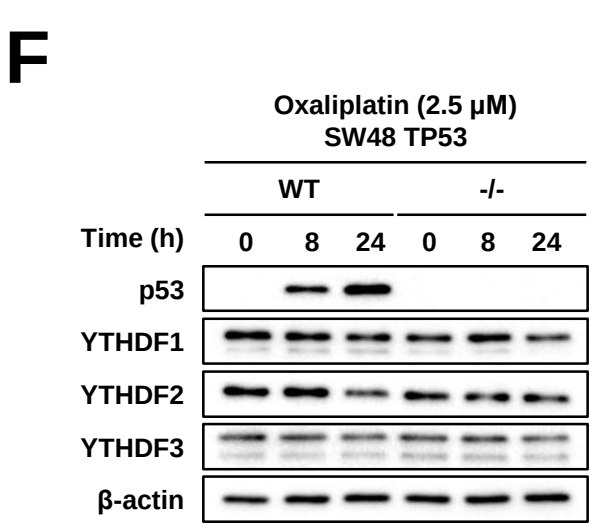
Supplementary Fig. 2









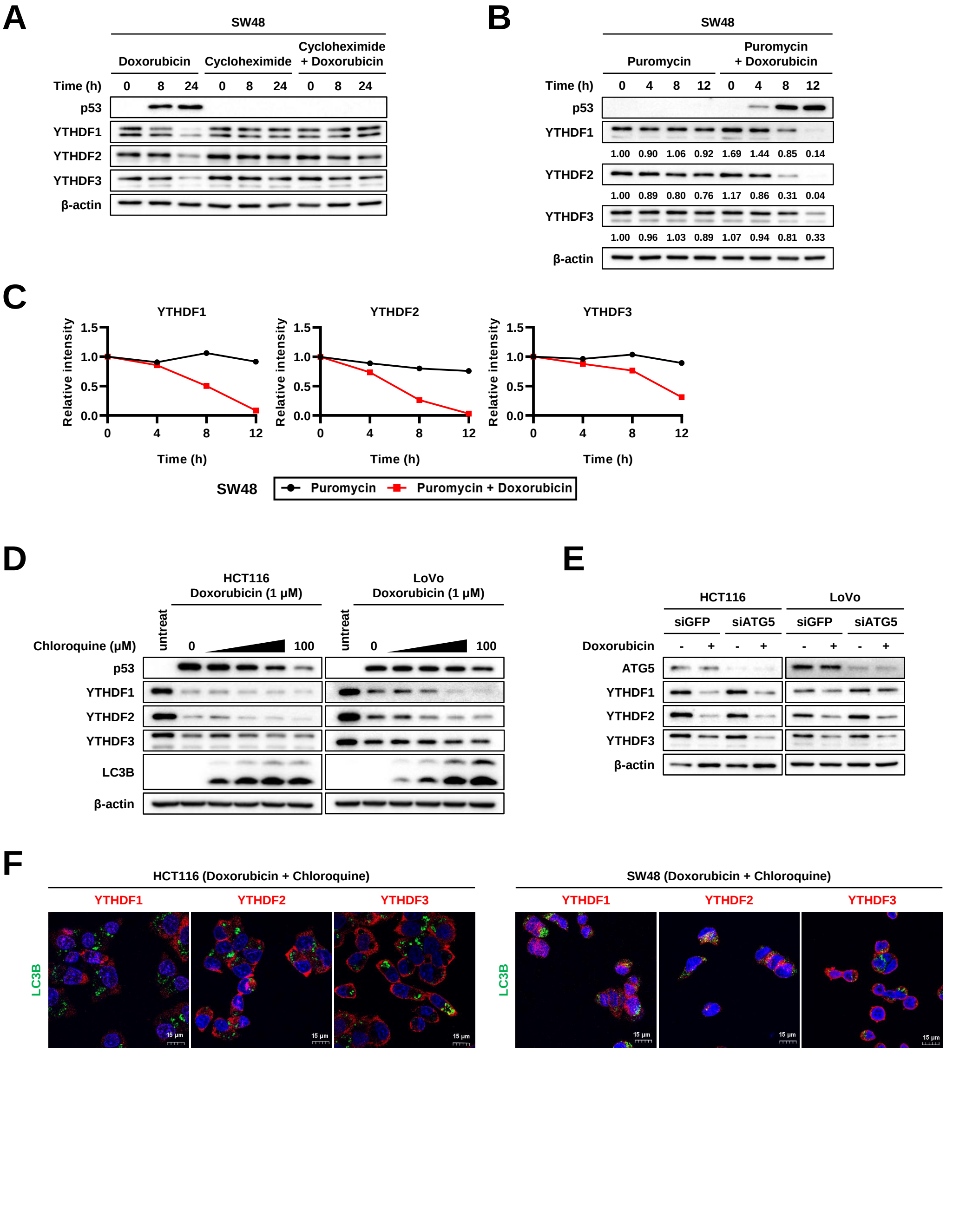


Supplementary Fig. 2 Effects of 5-FU and oxaliplatin on YTHDF1, YTHDF2, and YTHDF3 expression in SW48 cells and TP53 isogenic models.

A-**D** Western blot (**A**, **B**) and quantitative real-time PCR (**C**, **D**) in SW48 cells following 5-FU or oxaliplatin treatment. SW48 cells were treated with 5-FU (0, 2.5, 5, 10, 25, and 50 μM) or oxaliplatin (0, 0.5, 1, 2.5, 5, and 10 μM) for 24 h (**A**, **C**), or with 10 μM 5-FU for 0, 24, 48, and 72 h or 2.5 μM oxaliplatin for 0, 8, and 24 h (**B**, **D**). Data are presented as mean ± SD from experiments performed in triplicate. Differences among groups were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test (**C**, **D**).

E, **F** Western blot in SW48 isogenic cell lines with TP53 wild-type (WT) or TP53 knockout (-/-) following 5-FU or oxaliplatin treatment. SW48 isogenic cell lines were treated with 10 μM 5-FU for 0, 24, 48, and 72 h (**E**) or 2.5 μM oxaliplatin for 0, 8, and 24 h (**F**).

Supplementary Fig. 3



Supplementary Fig. 3 Cell line-dependent contribution of autophagy to doxorubicin-induced degradation of YTHDF family proteins.

A Western blot in SW48 cells following a protein stability assay. SW48 cells were treated with 200 μg/ml cycloheximide and/or 1μM doxorubicin for 0, 8, and 24 h.

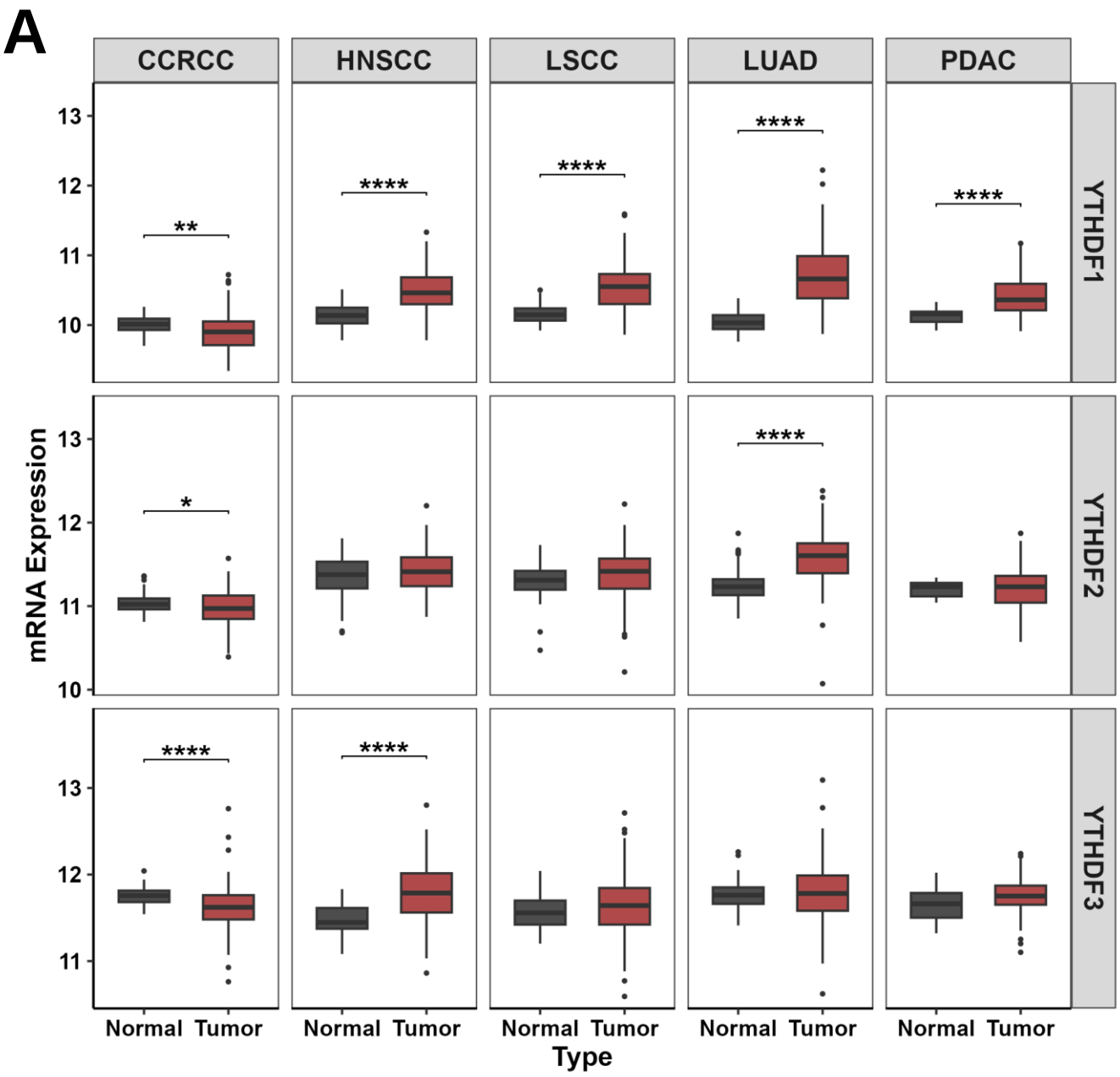
B, **C** Western blot (**B**) and densitometric quantification (**C**) in SW48 cells following a protein stability assay. SW48 cells were treated with 1 μg/ml puromycin in the presence or absence of 0.5 μM doxorubicin for 0, 4, 8, and 12 h. Band intensities were normalized to β-actin and expressed relative to the 0 h time point.

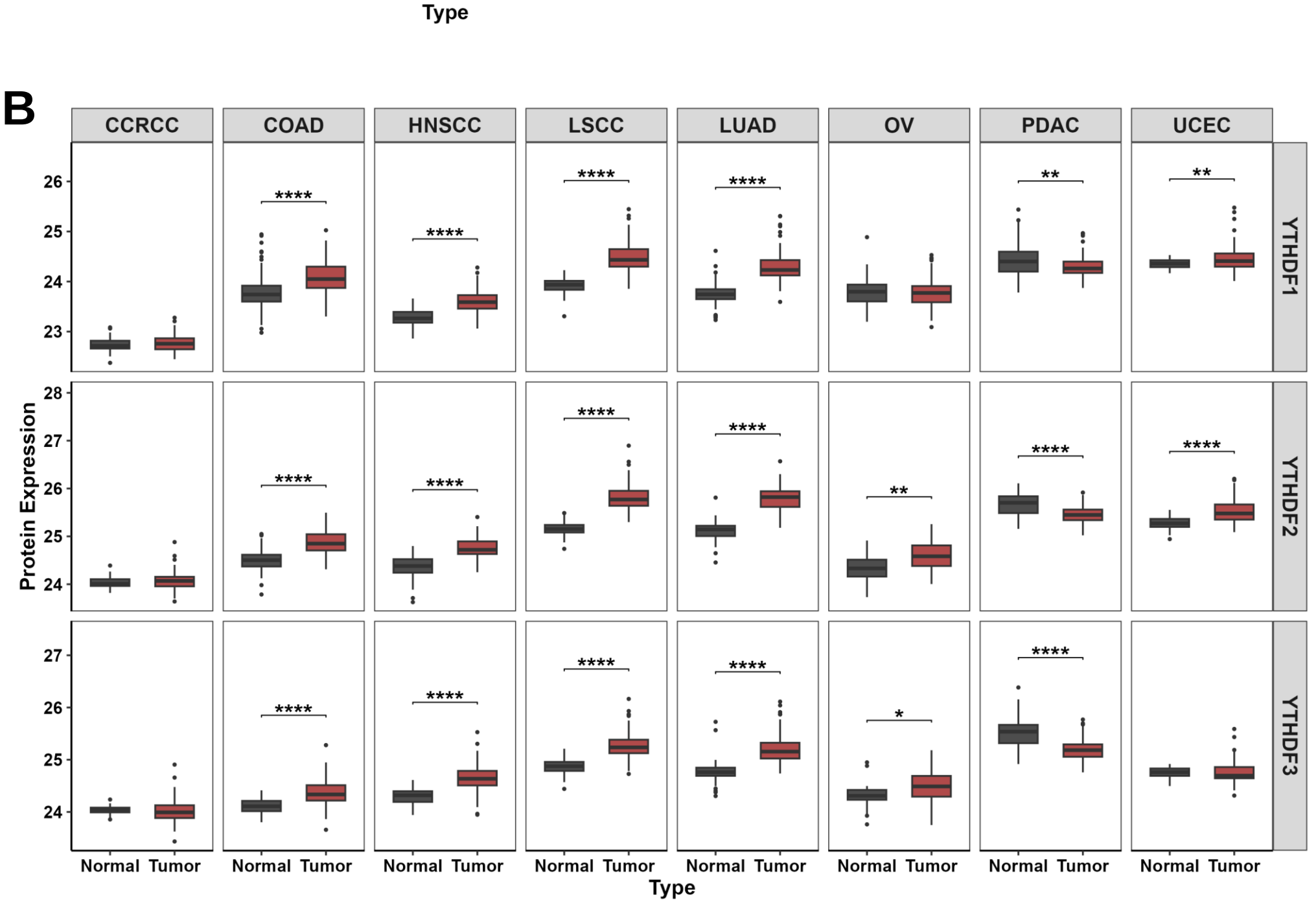
D Western blot in HCT116 and LoVo cells following doxorubicin and chloroquine treatment. HCT116 cells were treated with 1 μM doxorubicin in the presence of chloroquine (0, 25, 50, 75, and 100 μM), whereas LoVo cells were treated with 1 μM doxorubicin in the presence of chloroquine (0, 10, 20, 50, and 100 μM) for 24 h.

E Western blot in HCT116 and LoVo cells following siRNA transfection and doxorubicin treatment. HCT116 and LoVo cells were transfected with control siRNA (siGFP) or siATG5 for 24 h and subsequently treated with 1 μM doxorubicin for 24 h.

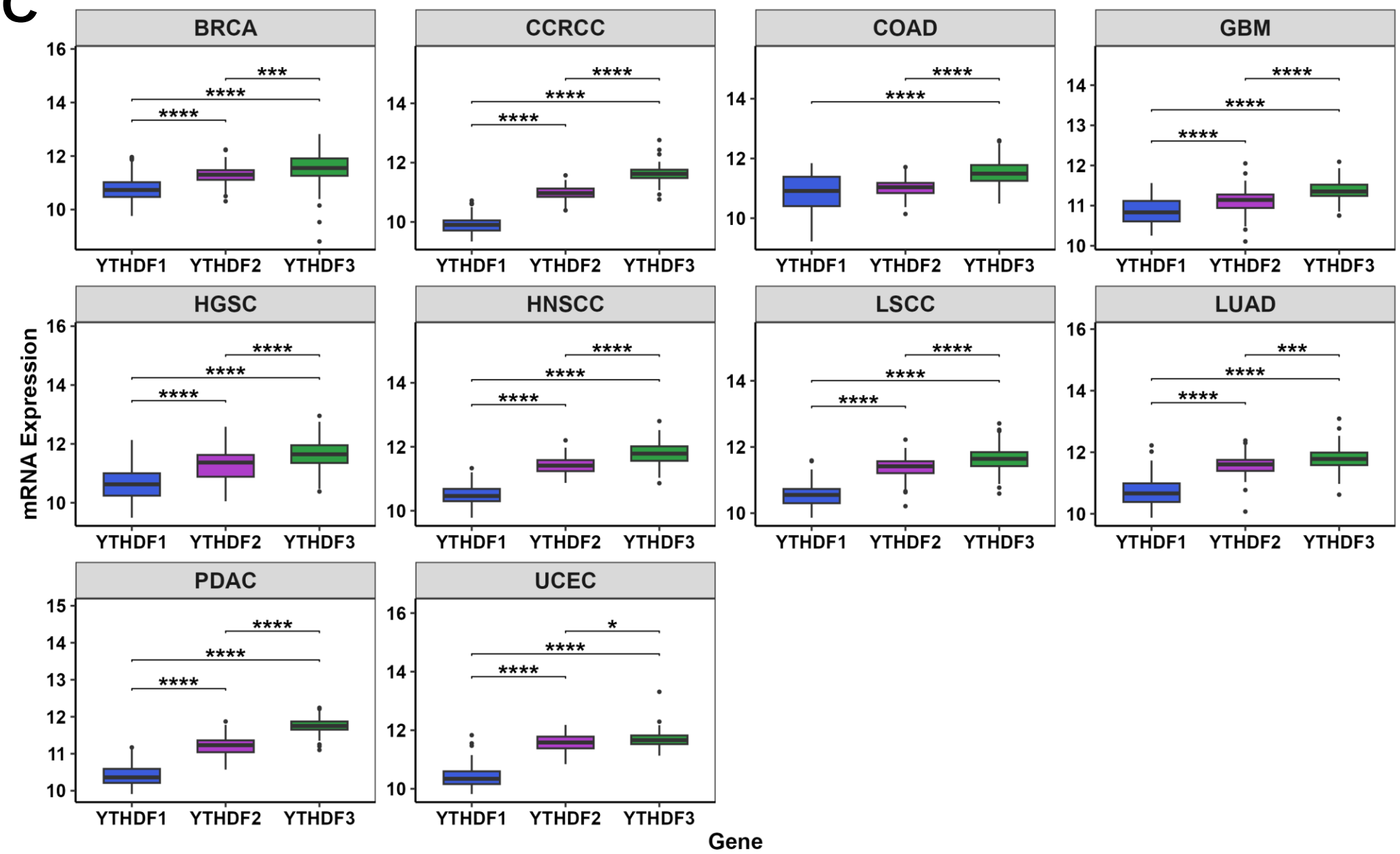
F Confocal immunofluorescence images of YTHDF1, YTHDF2, and YTHDF3 proteins colocalization with LC3B in HCT116 and SW48 cells following doxorubicin and chloroquine treatment. HCT116 cells were treated with 1 μM doxorubicin and 100 μM chloroquine, whereas SW48 cells were treated with 0.5 μM doxorubicin and 20 μM chloroquine for 24 h. Cells were stained for YTHDF1, YTHDF2, or YTHDF3 (red) and LC3B (green), with nuclei counterstained using DAPI (blue). Representative confocal images are shown.

Supplementary Fig. 4

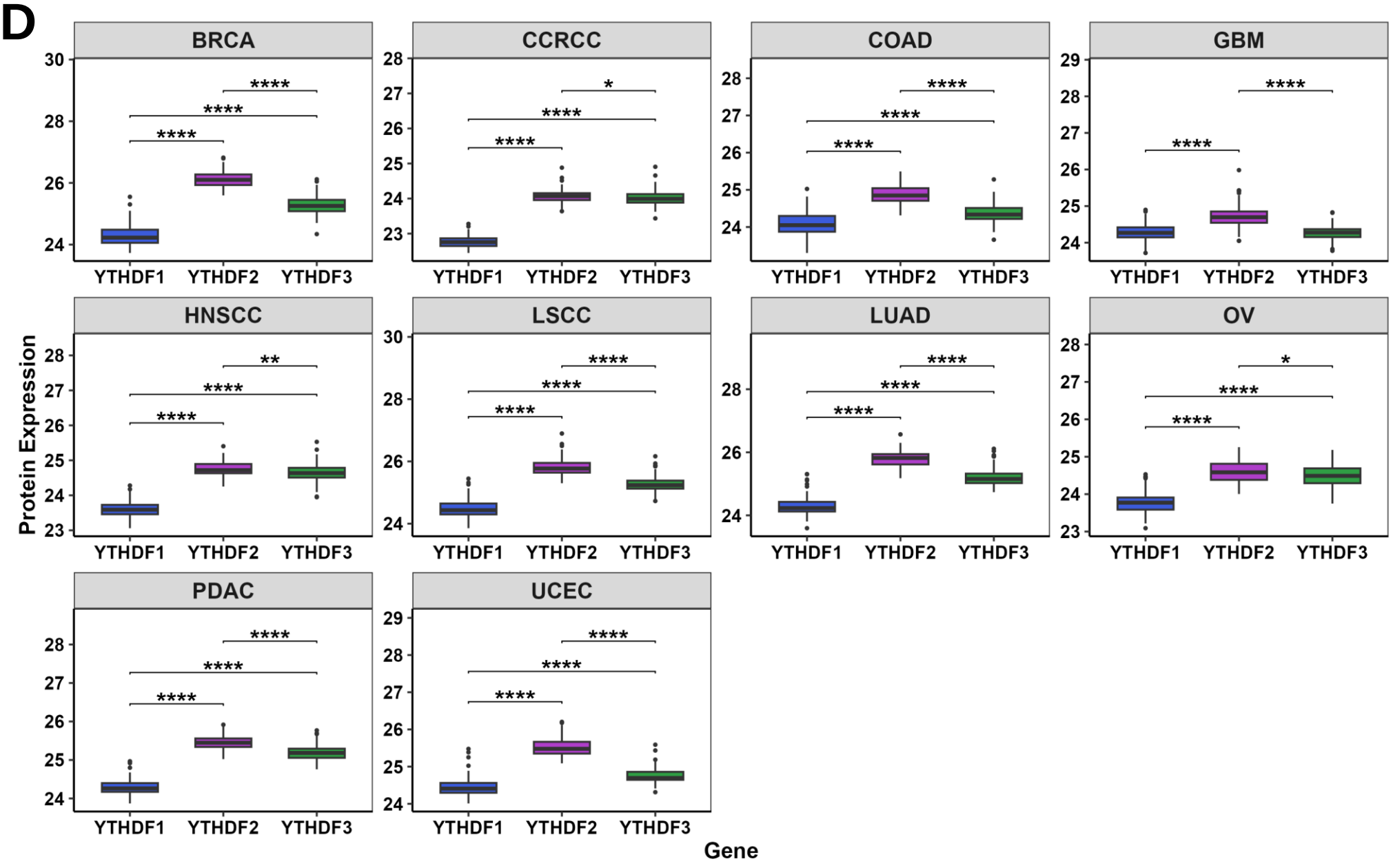




C


BRCA
CCRCC
COAD
GBM
HGSC
HNSCC
LSCC
LUAD
PDAC
UCEC
mRNA Expression
YTHDF1
YTHDF2
YTHDF3
Gene

D


BRCA
CCRCC
COAD
GBM
HNSCC
LSCC
LUAD
OV
PDAC
UCEC
Protein Expression
YTHDF1
YTHDF2
YTHDF3
Gene

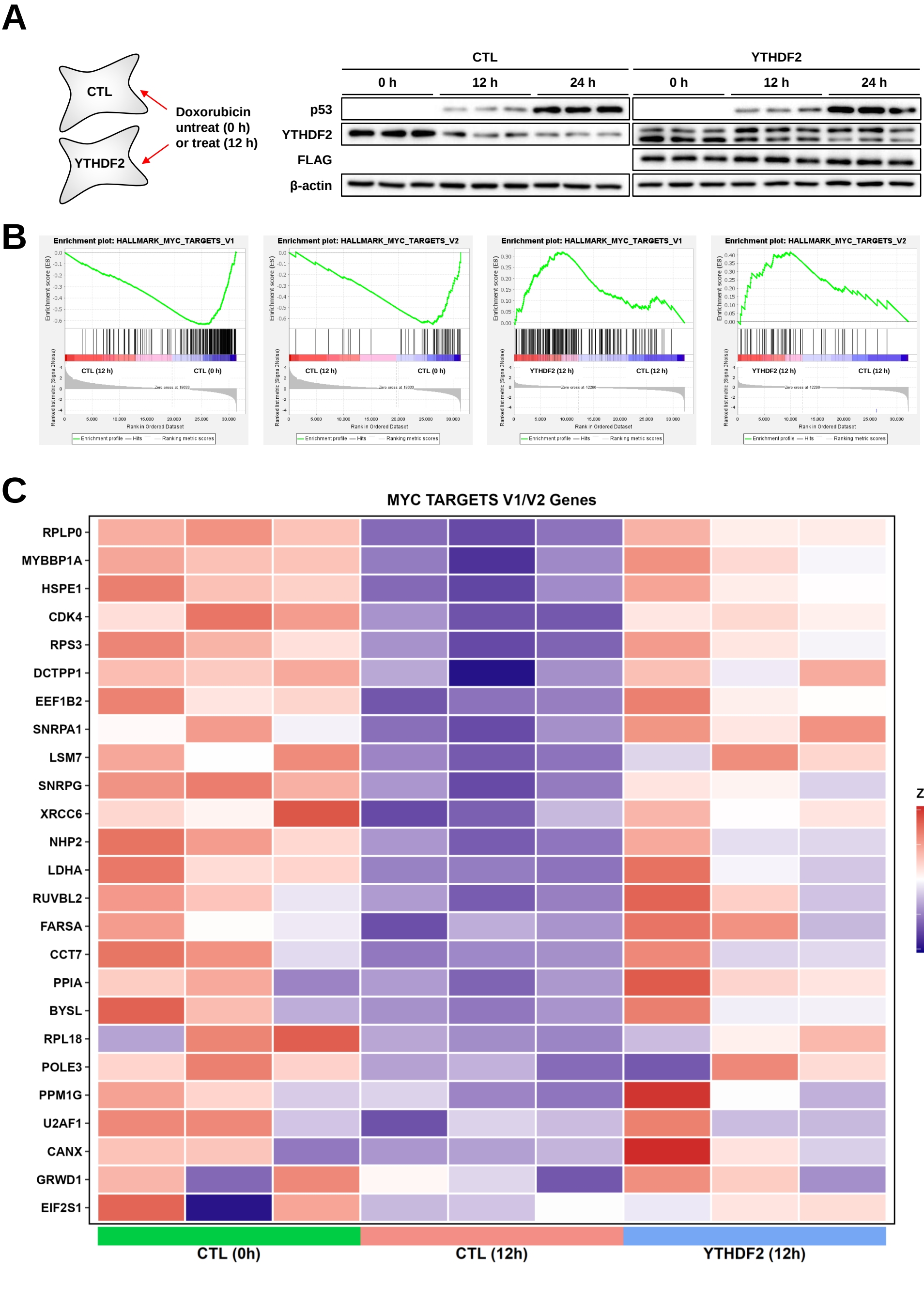
Supplementary Fig. 4 Pan-cancer expression profiles of YTHDF1, YTHDF2, and YTHDF3 in CPTAC datasets.

A, **B** Comparison of YTHDF1, YTHDF2, and YTHDF3 mRNA (**A**) and protein (**B**) expression levels between normal and tumor tissues across multiple cancer types in the CPTAC datasets.

C, **D** Comparison of YTHDF1, YTHDF2, and YTHDF3 mRNA (**C**) and protein (**D**) expression levels across multiple cancer types in the CPTAC datasets.

Boxplots represent the distribution of mRNA (**A**, **C**) or protein (**B**, **D**) expression levels, with the center line indicating the median and the box boundaries representing the interquartile range. Differences between normal and tumor tissues were analyzed using a two-tailed unpaired Welch's t-test (**A**, **B**) and differences among groups were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test (**C**, **D**).

Supplementary Fig. 5



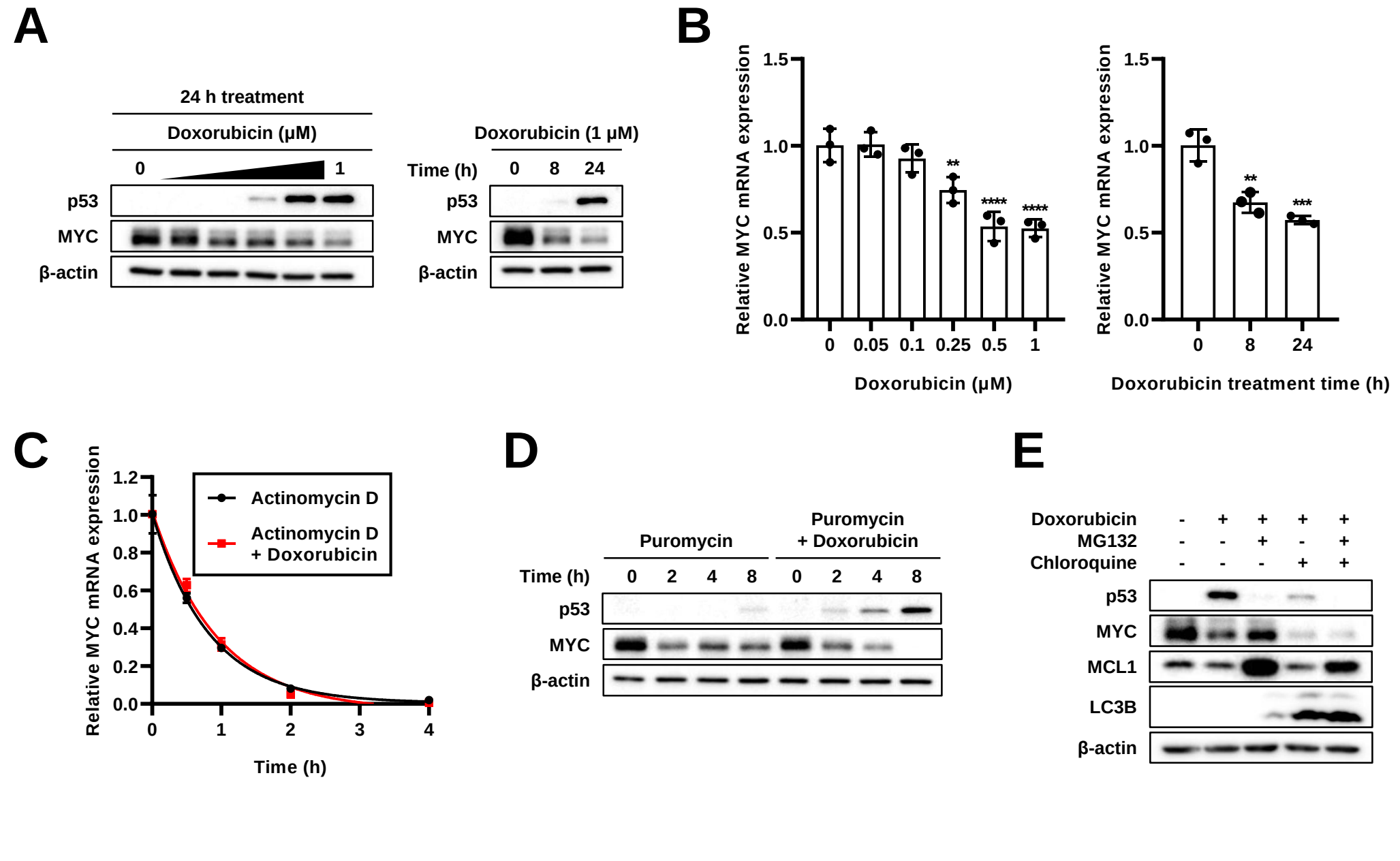
Supplementary Fig. 5 RNA-sequencing analyses of YTHDF2-regulated MYC signaling pathway.

A Experimental design and validation of samples used for RNA-sequencing. HCT116 cells were transfected with control (CTL) or YTHDF2 expression vectors and subsequently treated with 1 μM doxorubicin for 0, 12, and 24 h. RNA-sequencing was performed using samples collected at 0 and 12 h (left). Western blot of YTHDF2 and p53 proteins was performed to confirm successful YTHDF2 overexpression and doxorubicin activity, respectively (right).

B Gene Set Enrichment Analysis (GSEA) enrichment plots of MYC Targets V1 and MYC Targets V2 gene sets based on RNA-sequencing data from HCT116 cells. The left two panels compare CTL (12 h) and CTL (0 h), whereas the right two panels compare YHTDF2 (12 h) and CTL (12 h).

C Heatmap of representative genes from the MYC Targets V1 and MYC Targets V2 gene sets based on RNA-sequencing data from HCT116 cells. Gene expression levels in CTL (0 h), CTL (12 h), and YTHDF2 (12 h) samples were quantified as transcripts per million (TPM) and normalized by Z-score transformation across samples. Colors indicate relative expression levels, with red and blue representing higher and lower expression, respectively.

Supplementary Fig. 6



Supplementary Fig. 6 Assessment of MYC mRNA and protein stability following doxorubicin treatment.

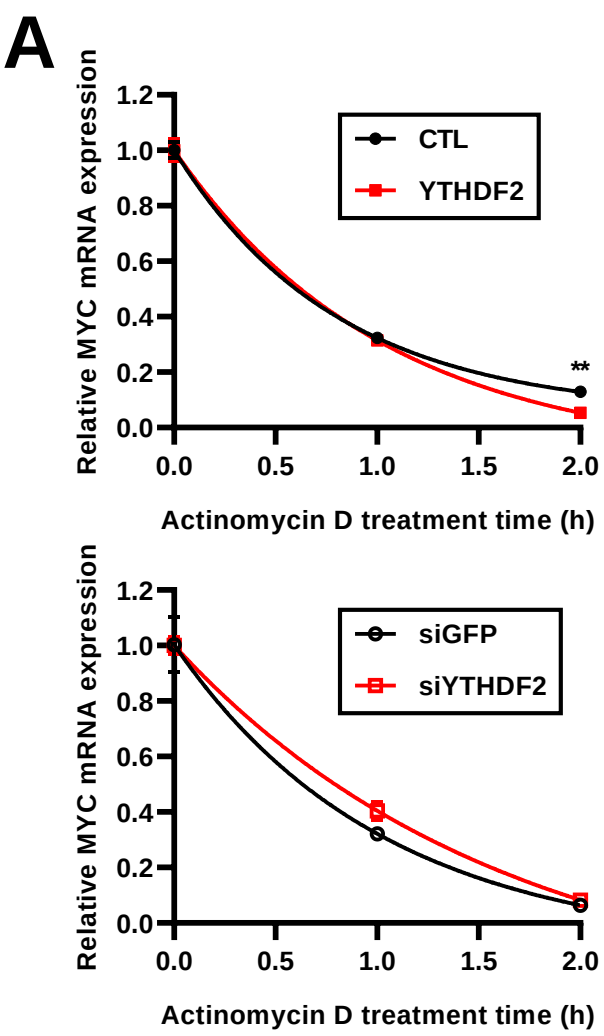
A, **B** Western blot (**A**) and quantitative real-time PCR (**B**) in HCT116 cells following doxorubicin treatment. HCT116 cells were treated with doxorubicin (0, 0.05, 0.1, 0.25, 0.5, and 1 μM) for 24 h (**A**, **B**; left) and 1 μM doxorubicin for 0, 8, and 24 h (**A**, **B**; right). Data are presented as mean ± SD from experiments performed in triplicate. Differences among groups were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test (**B**).

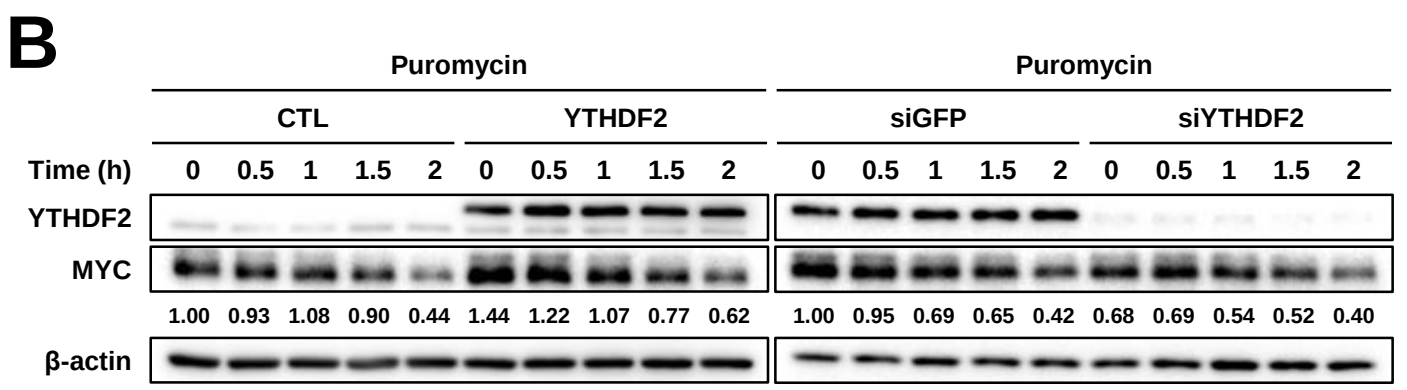
C Quantitative real-time PCR in HCT116 cells following a mRNA stability assay. HCT116 cells were treated with 5 μg/ml actinomycin D in the presence or absence of 1 μM doxorubicin for 0, 0.5, 1, 2, and 4 h. Data are presented as mean ± SD from experiments performed in triplicate. mRNA decay curves were fitted by nonlinear regression using a one phase decay model and differences between groups were analyzed using two-way ANOVA followed by Sidak's multiple comparisons test.

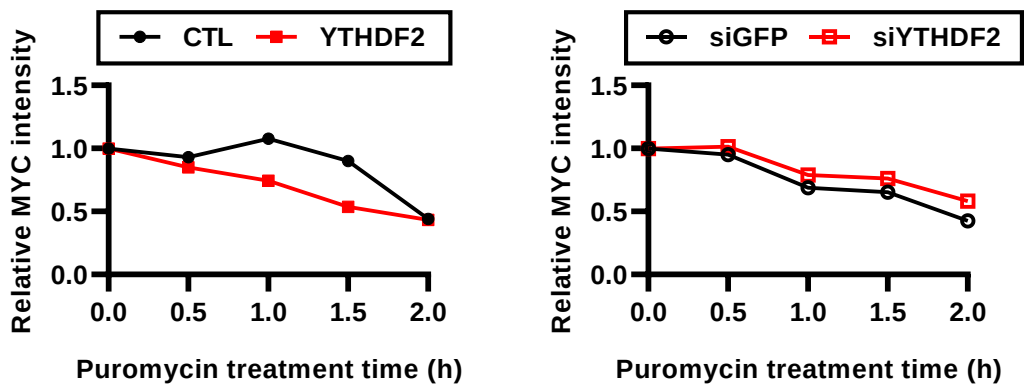
D Western blot in HCT116 cells following a protein stability assay. HCT116 cells were treated with 1 μg/ml puromycin in the presence or absence of 1 μM doxorubicin for 0, 2, 4, and 8 h.

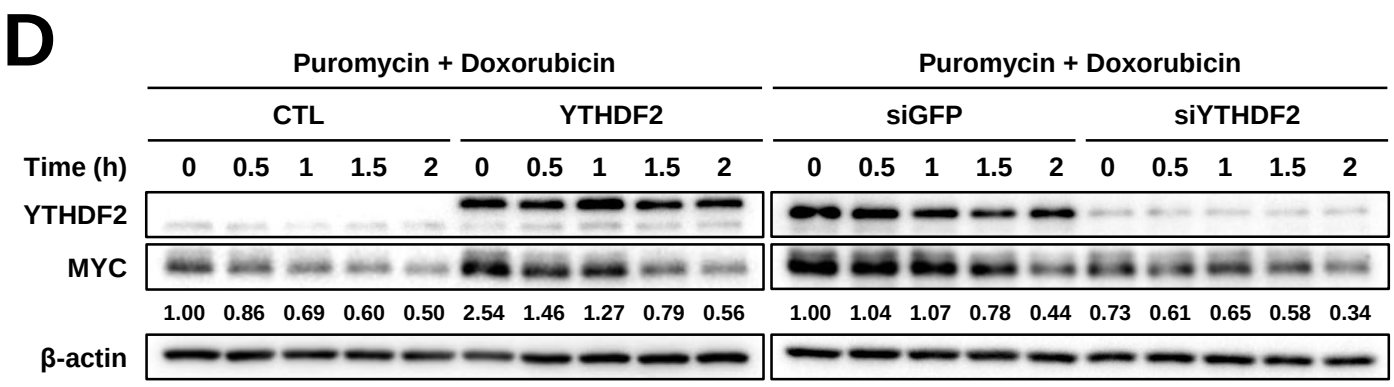
E Western blot in HCT116 cells following treatment with doxorubicin and protein degradation pathway inhibitors. HCT116 cells were treated with 1 μM doxorubicin, 1 μM MG132, and 100 μM chloroquine. Drugs were administered in the indicated combination for 24 h.

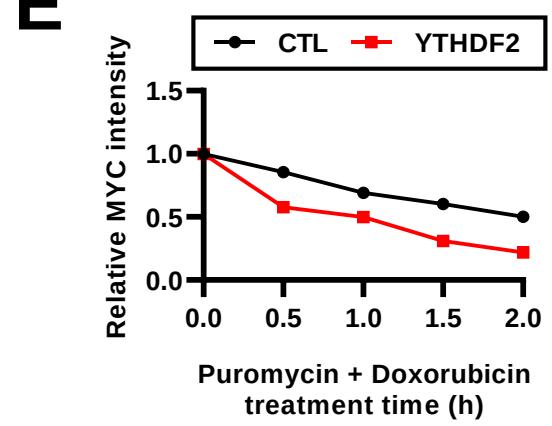
Supplementary Fig. 7

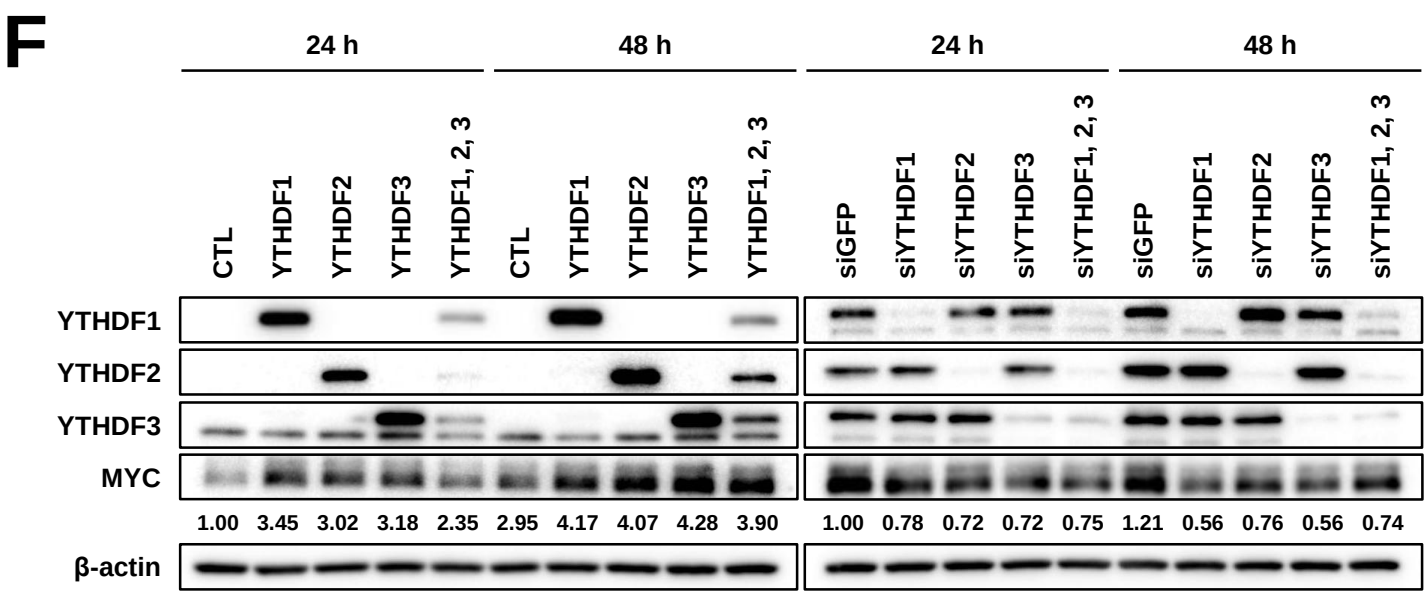


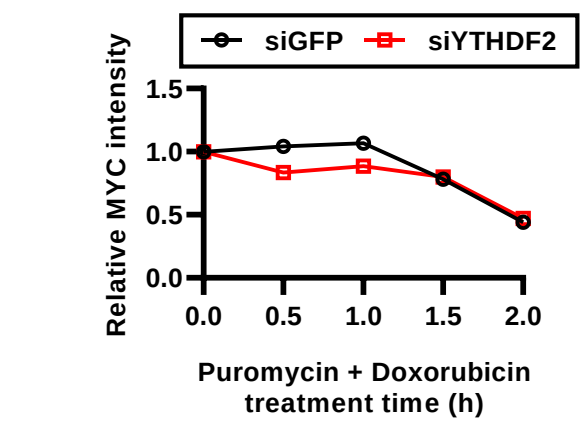


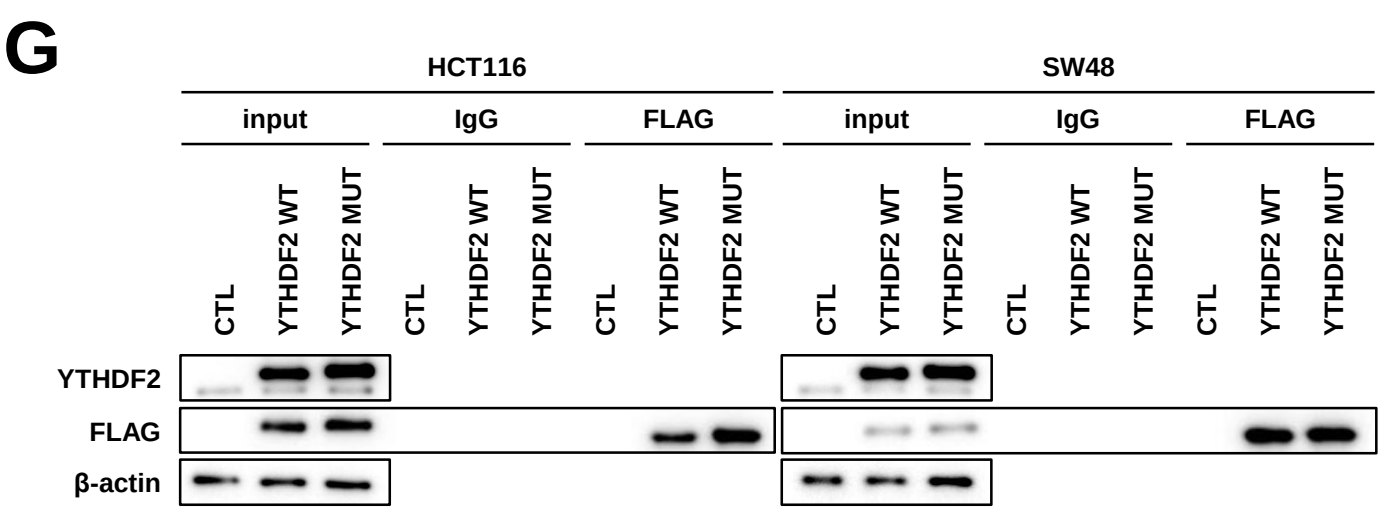












Supplementary Fig. 7 MYC stability analyses and validation of YTHDF2-MYC interaction.

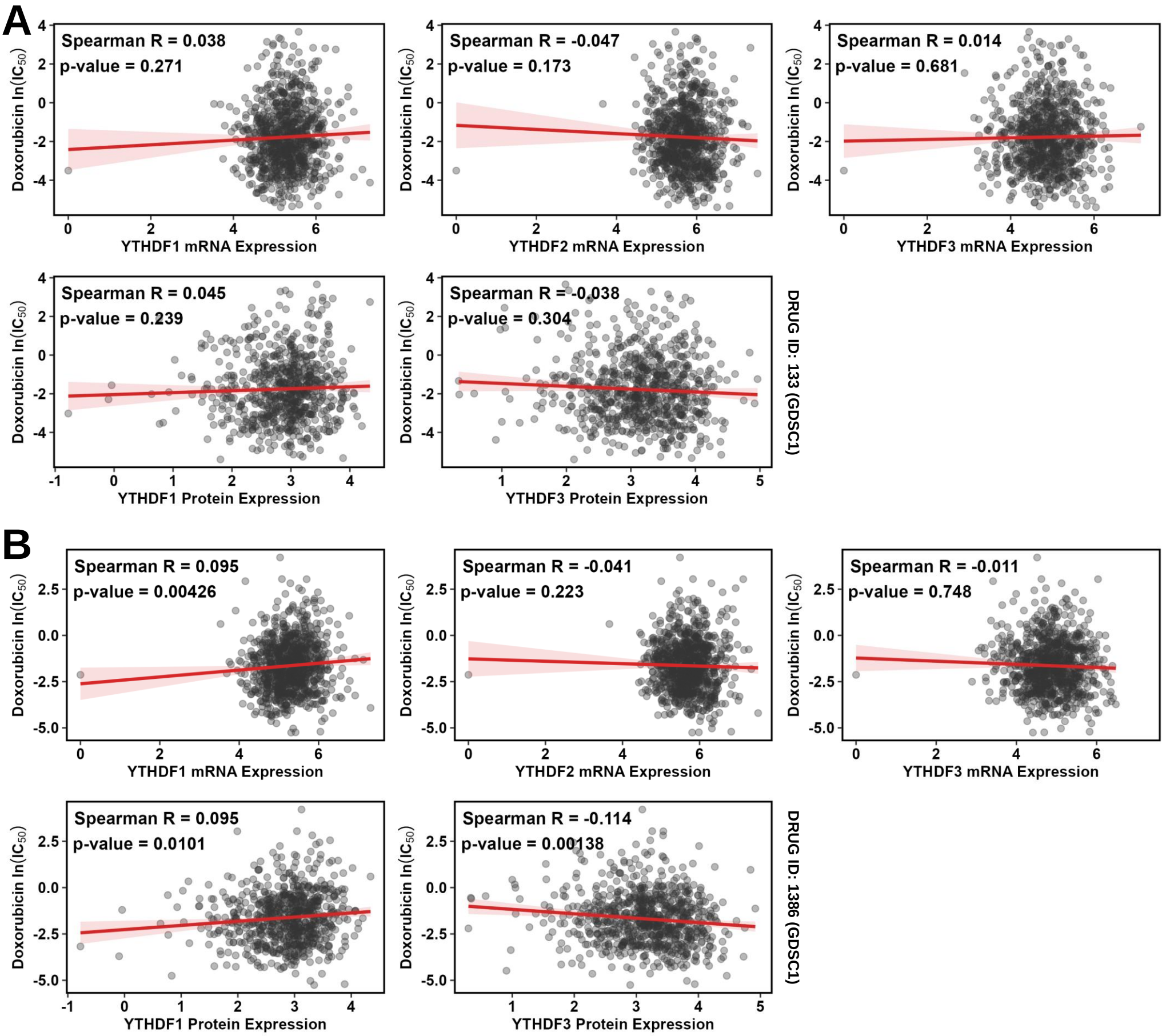
A Quantitative real-time PCR in HCT116 cells following a mRNA stability assay. HCT116 cells were transfected with indicated plasmid vectors or siRNAs for 24 h and subsequently treated with 5 μg/ml actinomycin D for 0, 1, and 2 h. Data are presented as mean ± SD from experiments performed in triplicate. mRNA decay curves were fitted by nonlinear regression using a one phase decay model. Differences between groups were analyzed using two-way ANOVA followed by Sidak's multiple comparisons test.

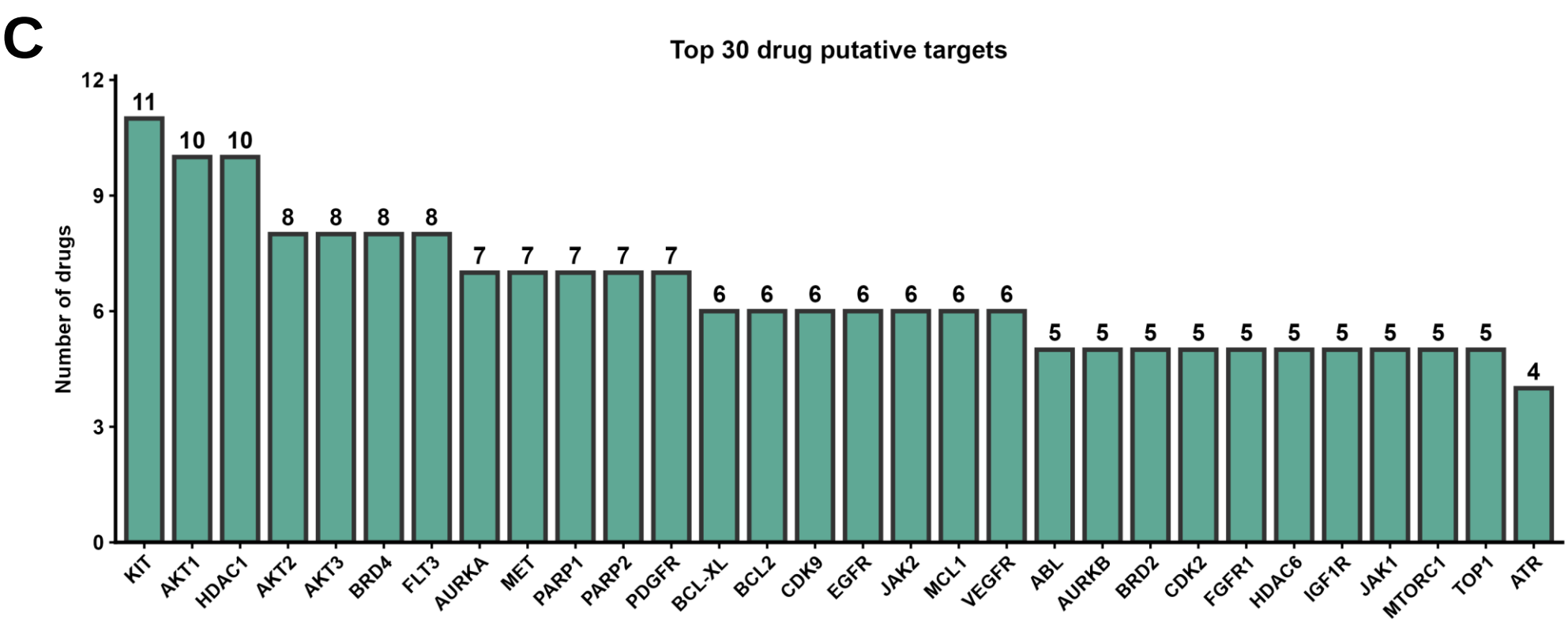
B-**E** Western blot (**B**, **D**) and densitometric quantification (**C**, **E**) in HCT116 cells following a protein stability assay. HCT116 cells were transfected with indicated plasmid vectors or siRNAs for 24 h and subsequently treated with 1 μg/ml puromycin in the absence (**B**, **C**) or presence (**D**, **E**) of 1 μM doxorubicin for 0, 0.5, 1, 1.5, and 2 h. Band intensities were normalized to β-actin and expressed relative to the 0 h time point.

F Western blot in HCT116 cells following transfection with indicated plasmid vectors or siRNAs for 24 and 48 h.

G Western blot validation of immunoprecipitation efficiency for RIP-qPCR. HCT116 and SW48 cells were transfected with control (CTL), YTHDF2 wild-type (WT), or m6A-binding-deficient YTHDF2 mutant (MUT) expression vectors for 24 h. Input, IgG, and FLAG immunoprecipitates were analyzed by western blot to verify specific enrichment of FLAG-YTHDF2 proteins.

Supplementary Fig. 8





Supplementary Fig. 8 Pharmacogenomic analyses associated with YTHDF1, YTHDF2, and YTHDF3 expression and MYC signaling.

A, **B** Scatter plots showing the correlations between YTHDF1 mRNA expression (upper left), YTHDF2 mRNA expression (upper middle), YTHDF3 mRNA expression (upper right), YTHDF1 protein expression (lower left) or YTHDF3 protein expression (lower middle) and doxorubicin response [ln(IC_{50})] across cancer cell lines from the GDSC database. Analyses were performed using DRUG ID: 133 (GDSC1) (**A**) and DRUG ID: 1386 (GDSC1) (**B**). Each point represents an individual cell line, and the red line indicates the fitted linear regression. Correlation was analyzed using Spearman's rank correlation analysis.

C Bar graph showing the top 30 drug putative targets among 441 unique drugs identified from the 527 overlapping drug IDs that were significantly negatively correlated with both YTHDF2 protein and MYC mRNA expression. Putative targets were ranked according to the number of associated drugs. The x-axis represents the putative targets and y-axis indicates the number of drugs associated with each target.

Supplementary Table. 1 Antibody information

Antibody	Company	Catalog #	Usage
β-actin	Sigma-Aldrich	A5441	Western blot
p53	Cell Signaling Technology	2524	Western blot
METTL3	Cell Signaling Technology	86132	Western blot
METTL14	Cell Signaling Technology	51104	Western blot
METTL16	Cell Signaling Technology	17676	Western blot
YTHDF1	Cell Signaling Technology	86463	Western blot
YTHDF1	Cell Signaling Technology	57530	Immunofluorescence
YTHDF2	Cell Signaling Technology	80014	Western blot
YTHDF2	Cell Signaling Technology	71283	Immunofluorescence
YTHDF3	Proteintech	25537-1-AP	Western blot, Immunofluorescence
FTO	Cell Signaling Technology	45980	Western blot
ALKBH5	Proteintech	16837-1-AP	Western blot
p21	Cell Signaling Technology	2947	Western blot
MCL1	Cell Signaling Technology	5453	Western blot
LC3B	Cell Signaling Technology	3868	Western blot
LC3B	Cell Signaling Technology	83506	Immunofluorescence
ATG5	Cell Signaling Technology	8540	Western blot
Ubiquitin	Santa Cruz	sc-8017	Western blot
cleaved caspase-3	Cell Signaling Technology	9661	Western blot
MYC	Cell Signaling Technology	5605	Western blot
PARP	Cell Signaling Technology	9542	Western blot
FLAG	Sigma-Aldrich	F1804	Western blot, RIP-qPCR
m6A	Synaptic Systems	202 003	MeRIP-qPCR
normal mouse IgG	Sigma-Aldrich	CS200621	RIP-qPCR
Puromycin	Sigma-Aldrich	MABE343	Western blot

Supplementary Table. 2 qRT-PCR primer sequence

Gene	Forward	Reverse
GAPDH	5'-GAAGGTGAAGGTCGGAGT-3'	5'-GAAGATGGTGATGGGATTTC-3'
YTHDF1	5'-CAAGCACACAACCTCCATCTTCG-3'	5'-GTAAGAAACTGGTTCGCCCTCAT-3'
YTHDF2	5'-CCTTAGGTGGAGCCATGATTG-3'	5'-TCTGTGCTACCCAACTTCAGT-3'
YTHDF3	5'-TGACAACAAACCGGTTACCA-3'	5'-TGTTTCTATTTCTCTCCCTACGC-3'
MYC	5'-CAGCTGCTTAGACGCTGGATT-3'	5'-GTAGAAATACGGCTGCACCGA-3'
MYC site #1	5'-CACATCAGCACAACTACGCA-3'	5'-GGTGCATTTTCGGTTGTTGC-3'
MYC site #2	5'-AGCATACATCCTGTCCGTCC-3'	5'-TCCTTACTTTTCCTTACGCACA-3'
MYC site #3	5'-AATGTCCTGAGCAATCACCT-3'	5'-AAAGATGGTAAGCATAAAAAAG-3'