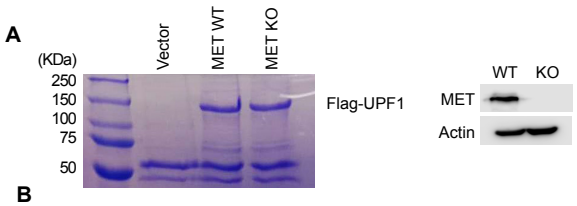
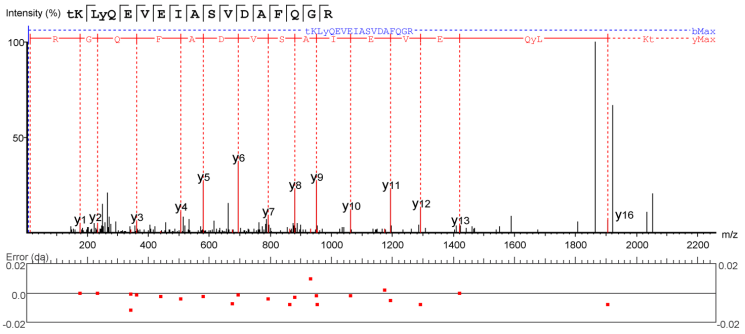
Supplementary Figure S8



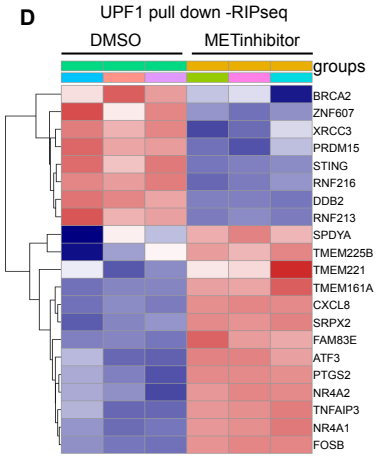
B

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	182.02	164.01	164.99	91.51	T(+79.97)					18
2	310.12	292.11	293.09	155.56	K	2032.97	2014.96	2015.95	1016.99	17
3	423.20	405.19	406.17	212.10	L	1904.89	1886.87	1887.85	952.95	16
4	666.23	648.22	649.20	333.62	Y(+79.97)	1791.79	1773.78	1774.77	896.40	15
5	794.29	776.28	777.26	397.64	Q	1548.77	1530.75	1531.74	774.88	14
6	923.33	905.32	906.30	462.17	E	1420.71	1402.70	1403.68	710.85	13
7	1022.40	1004.39	1005.37	511.70	V	1291.67	1273.65	1274.64	646.33	12
8	1151.44	1133.43	1134.42	576.22	E	1192.60	1174.58	1175.57	596.80	11
9	1264.53	1246.52	1247.50	632.76	I	1063.55	1045.54	1046.53	532.28	10
10	1335.56	1317.55	1318.54	668.28	A	950.47	932.45	933.44	475.73	9
11	1422.60	1404.59	1405.57	711.80	S	879.44	861.42	862.41	440.22	8
12	1521.66	1503.65	1504.64	761.33	V	792.40	774.39	775.37	396.70	7
13	1636.69	1618.68	1619.66	818.85	D	693.33	675.32	676.31	347.17	6
14	1707.73	1689.72	1690.70	854.36	A	578.31	560.29	561.28	289.65	5
15	1854.80	1836.79	1837.77	927.90	F	507.27	489.26	490.24	254.13	4
16	1982.86	1964.84	1965.83	991.93	Q	360.20	342.20	343.17	180.60	3
17	2039.88	2021.87	2022.85	1020.44	G	232.14	214.13	215.11	116.57	2
18					R	175.12	157.11	158.09	88.06	1

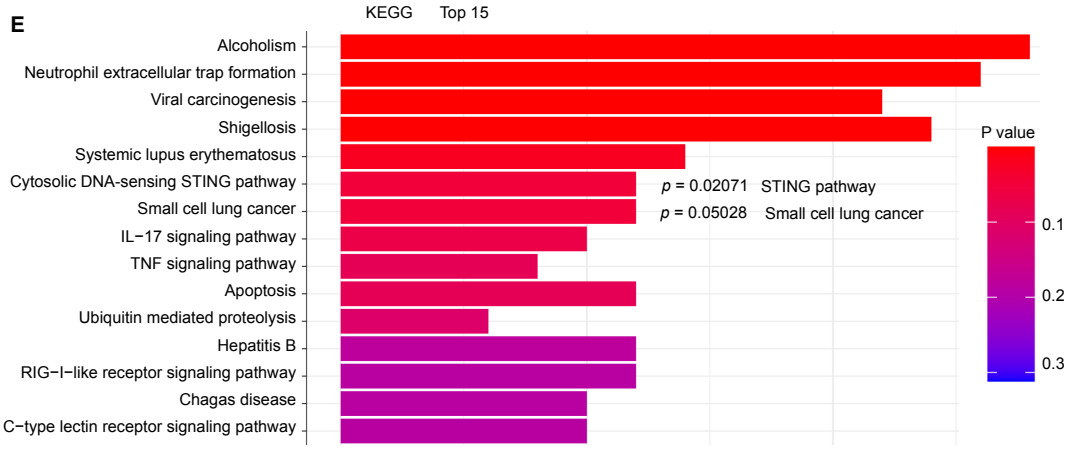
C



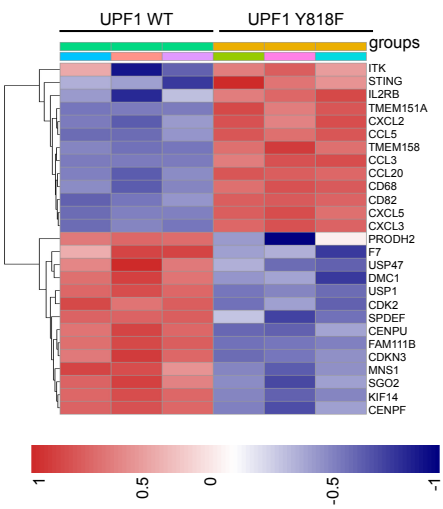
D



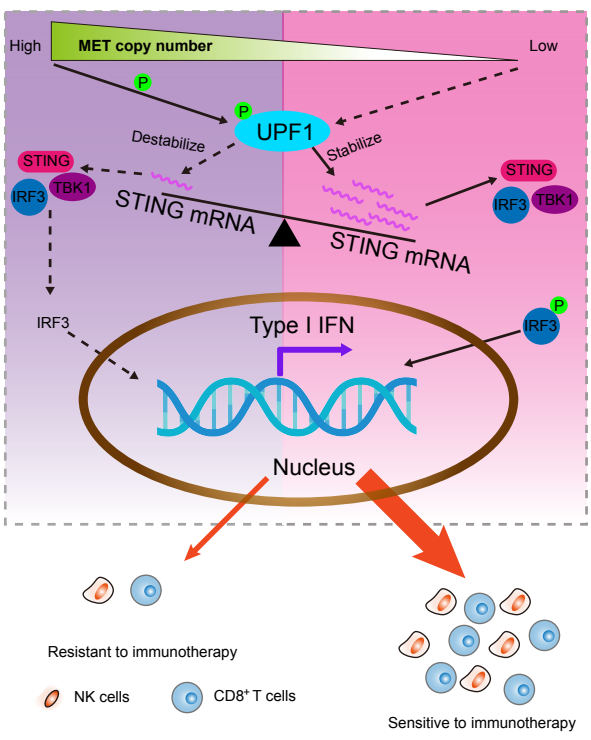
E



F



G



Supplementary Figure S8. UPF1 can be phosphorylated at tyrosine residues in a MET kinase-dependent manner

A, Flag-tagged UPF1 protein was expressed in A549 MET WT and MET KO cells and purified. Coomassie Brilliant Blue (CBB) staining of immunoprecipitation products was resolved on a 4–12% NuPAGE gel. Mass spectrometry was performed to map potential tyrosine phosphorylation sites on UPF1.

B-C, Mass spectrometry was performed to map potential acetylation sites on UPF1. The MS/MS spectrum unambiguously identifies Y818 as the phosphorylated amino acid within the peptide.

D, RIP was performed in MET inhibitor (tivantinib 100 nM for 48h) or DMSO treated H1993 cells using UPF1 antibody, followed by RNA-seq on the extracted RNAs. Heatmap of genes enriched by UPF1 through RIP-seq is shown (three independent replicates).

E, Gene Ontology (GO) analysis of RIP-seq that interacted with anti-UPF1 that were significantly enriched in multiple important biological pathways, including STING and immune-related pathways. The data are shown as *p* value.

F, UPF1 WT or Y818 mutant was expressed in MET amplification cell H1993 with endogenous UPF1 knocked down. RNA-seq was performed on the extracted RNAs. Heatmap of UPF1Y818F-related genes is shown (three independent replicates).

G, Working model: oncogenic MET signaling induces phosphorylation of UPF1 and regulates STING mRNA stability via UPF1. MET mediates immune evasion and modulates the efficiency of anti-PD1/PDL1 immunotherapy via STING signaling.