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Supplemental information

Overcoming MET-mediated resistance in oncogene-driven NSCLC

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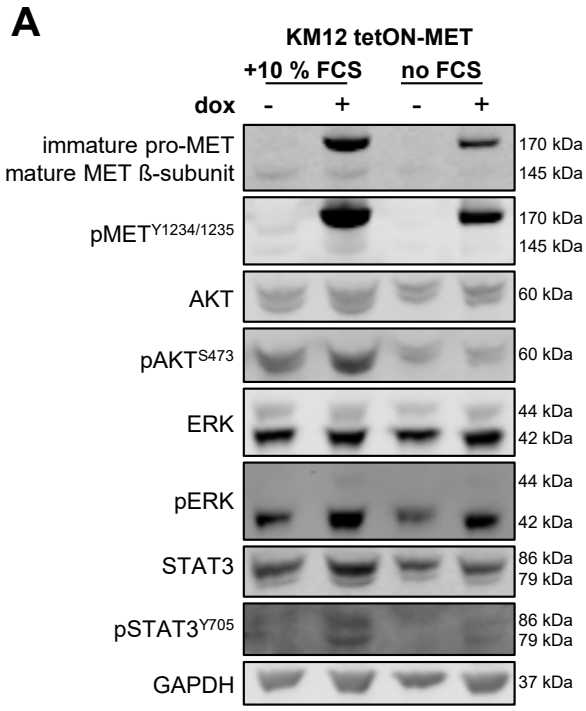
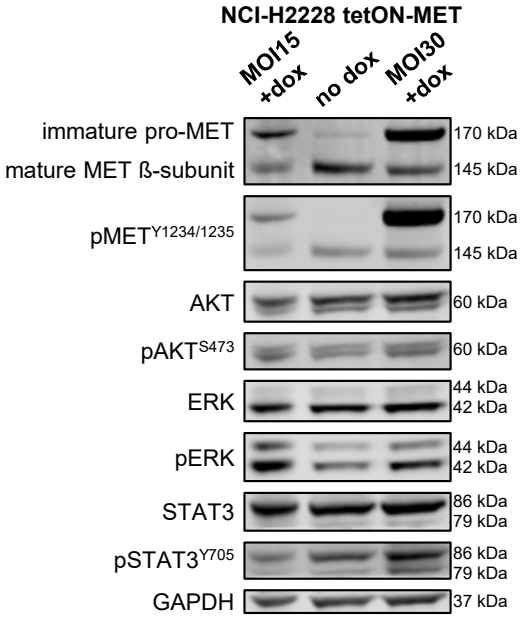
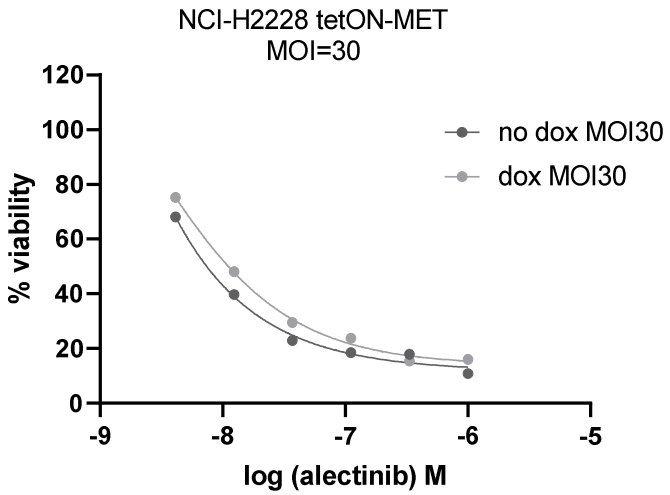




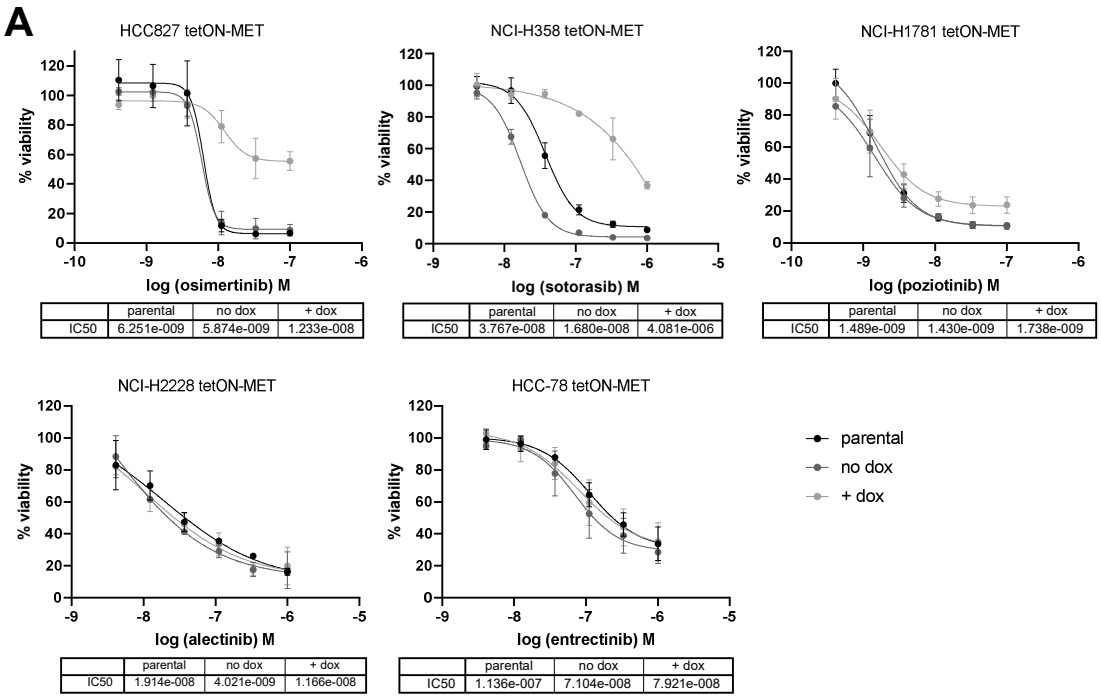


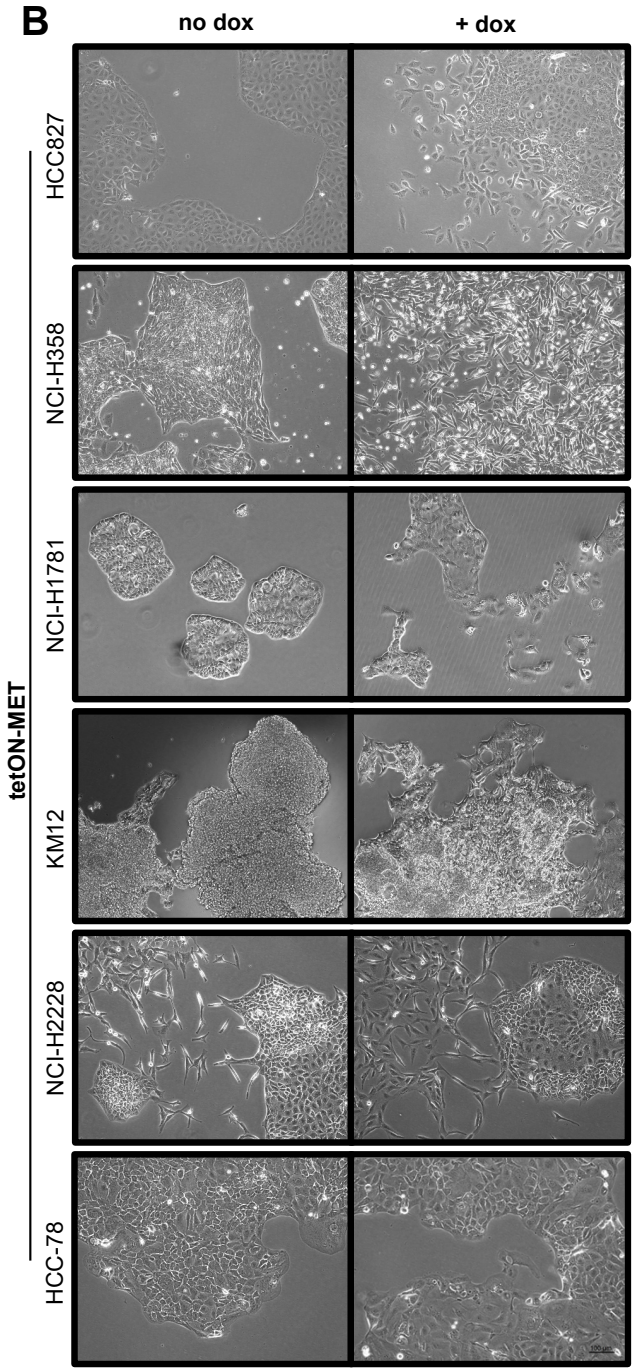
	no dox MOI30	dox MOI30
IC50	~ 1.840e-012	2.102e-009

Supplementary Figure S1: Further investigation of KM12 tetON-MET and NCI-H2228 tetON-MET cells, related to Figure 2.

A, KM12 tetON-MET cells were treated with/ without doxycycline and with/ without 10% FCS for 48 hours, before lysis, and Western blot analysis was performed with the indicated antibodies.

B, Western blot analysis of NCI-H2228 tetON-MET cells transduced with multiplicity of infection (MOI) = 15 compared to MOI = 30 and cell viability analysis of NCI-H2228 tetON-MET cells transduced with MOI = 30.



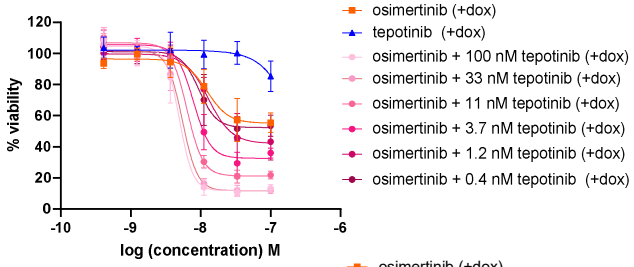


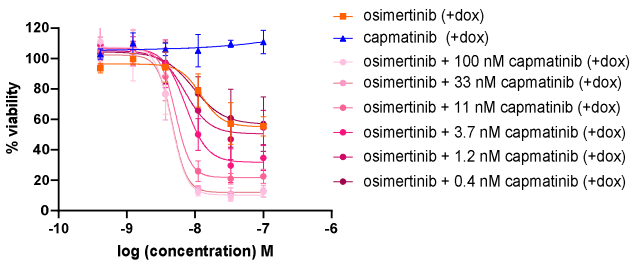
Supplementary Figure S2: Further investigation of the tetON MET cell lines, related to Figure 2.

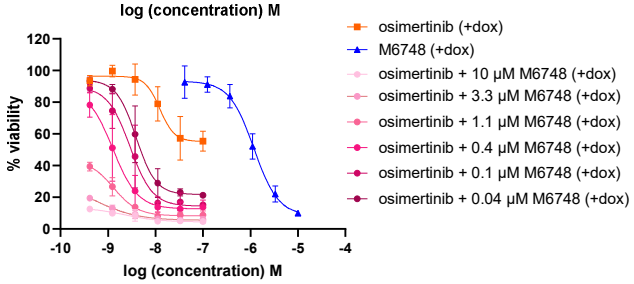
A, The indicated cells were cultured with doxycycline for 24 hours before they were treated with the respective targeted therapies. Cellular viability was measured six days from treatment onset. Data are represented as mean ± SD (n = 3).

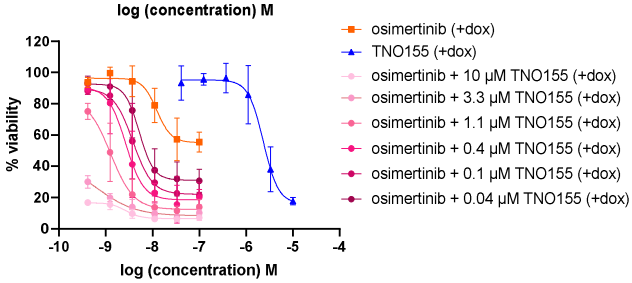
B, Brightfield microscopy images of the tetON-MET cells used in this study after 14 days of doxycycline treatment.

HCC827 tetON-MET (*EGFR*^{ex19del})

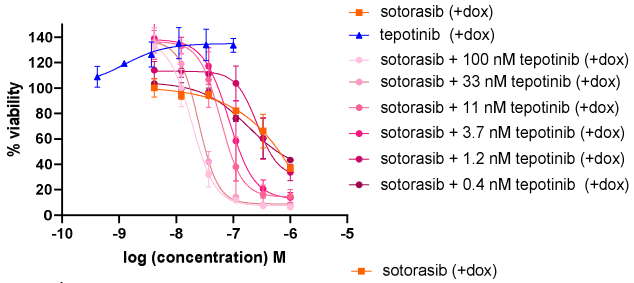


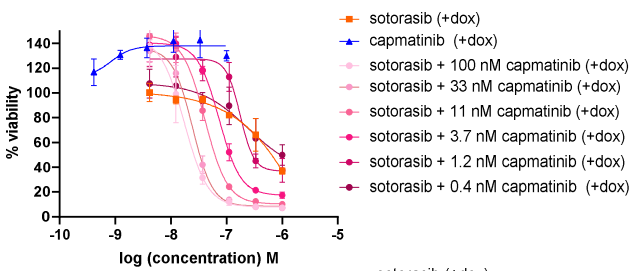


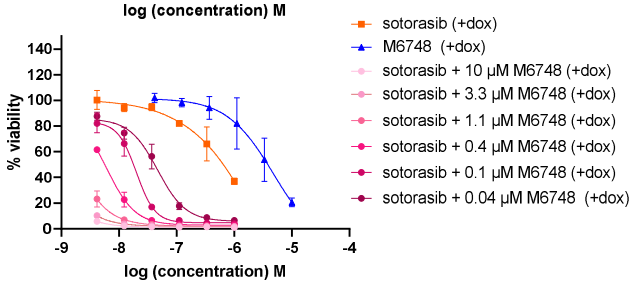


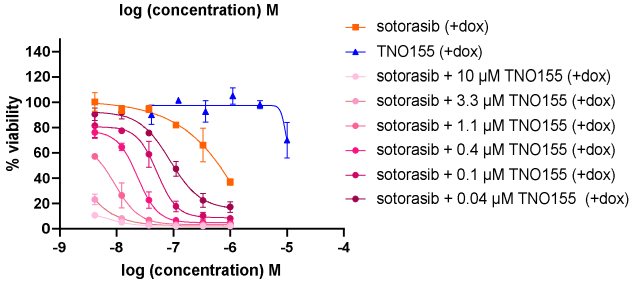


NCI-H358 tetON-MET (*KRAS*^{G12C})

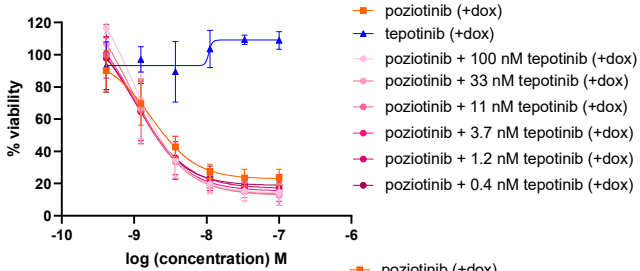


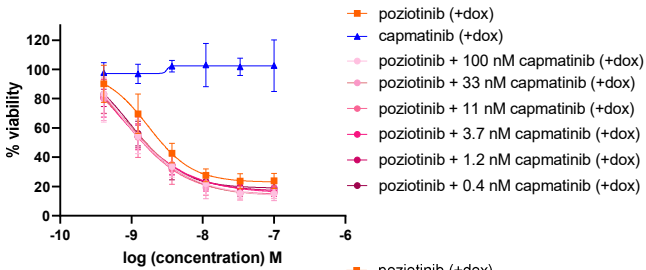


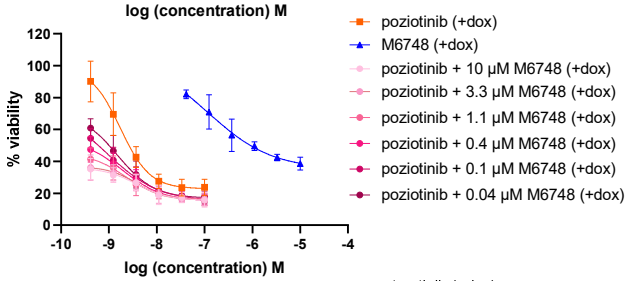


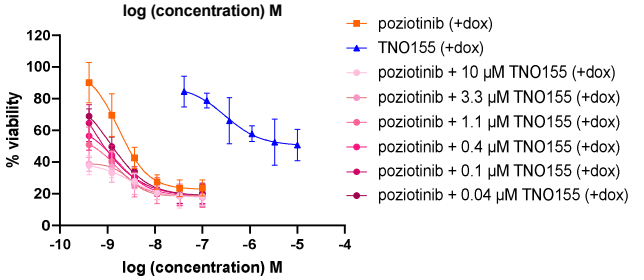


NCI-H1781 tetON-MET (*HER2*^{ex20ins})

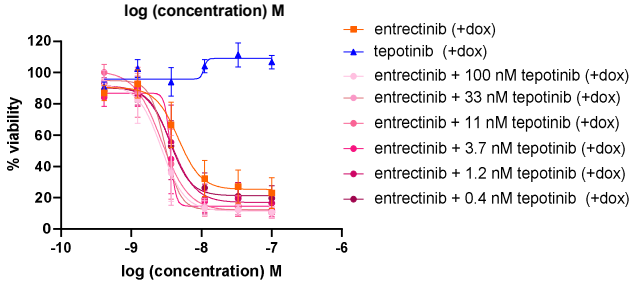


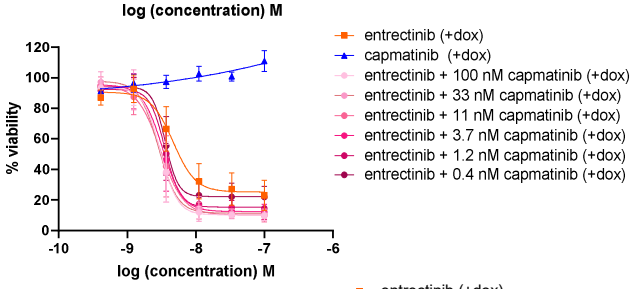


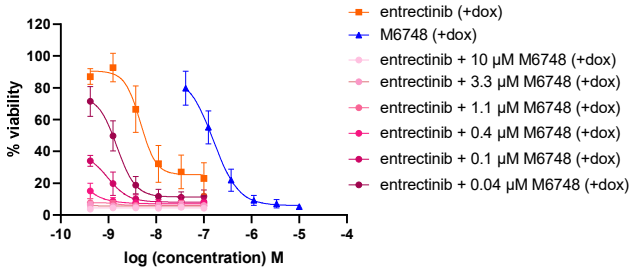


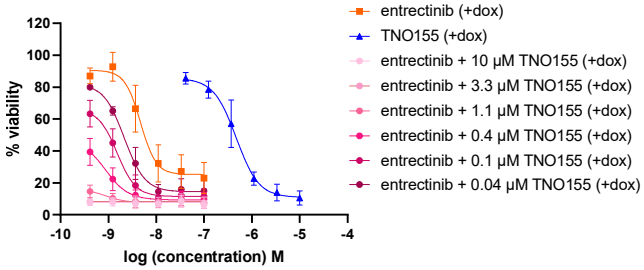


KM12 tetON-MET (*TPM3-NTRK1*)

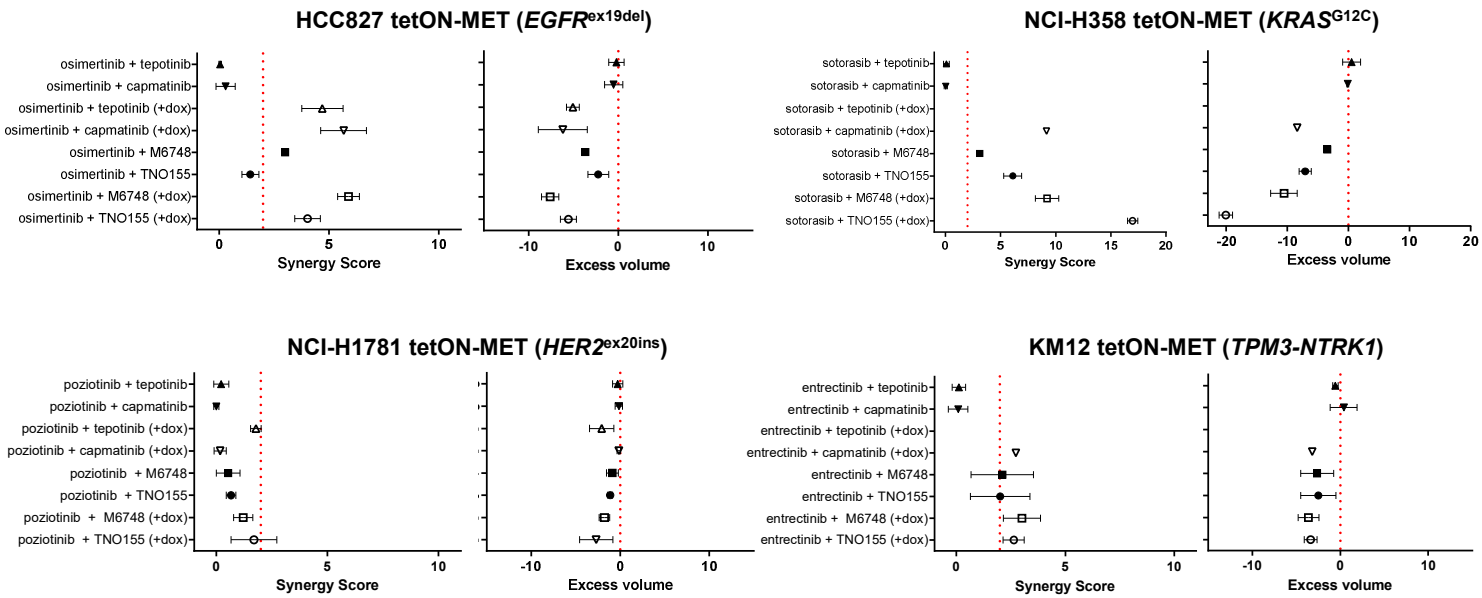






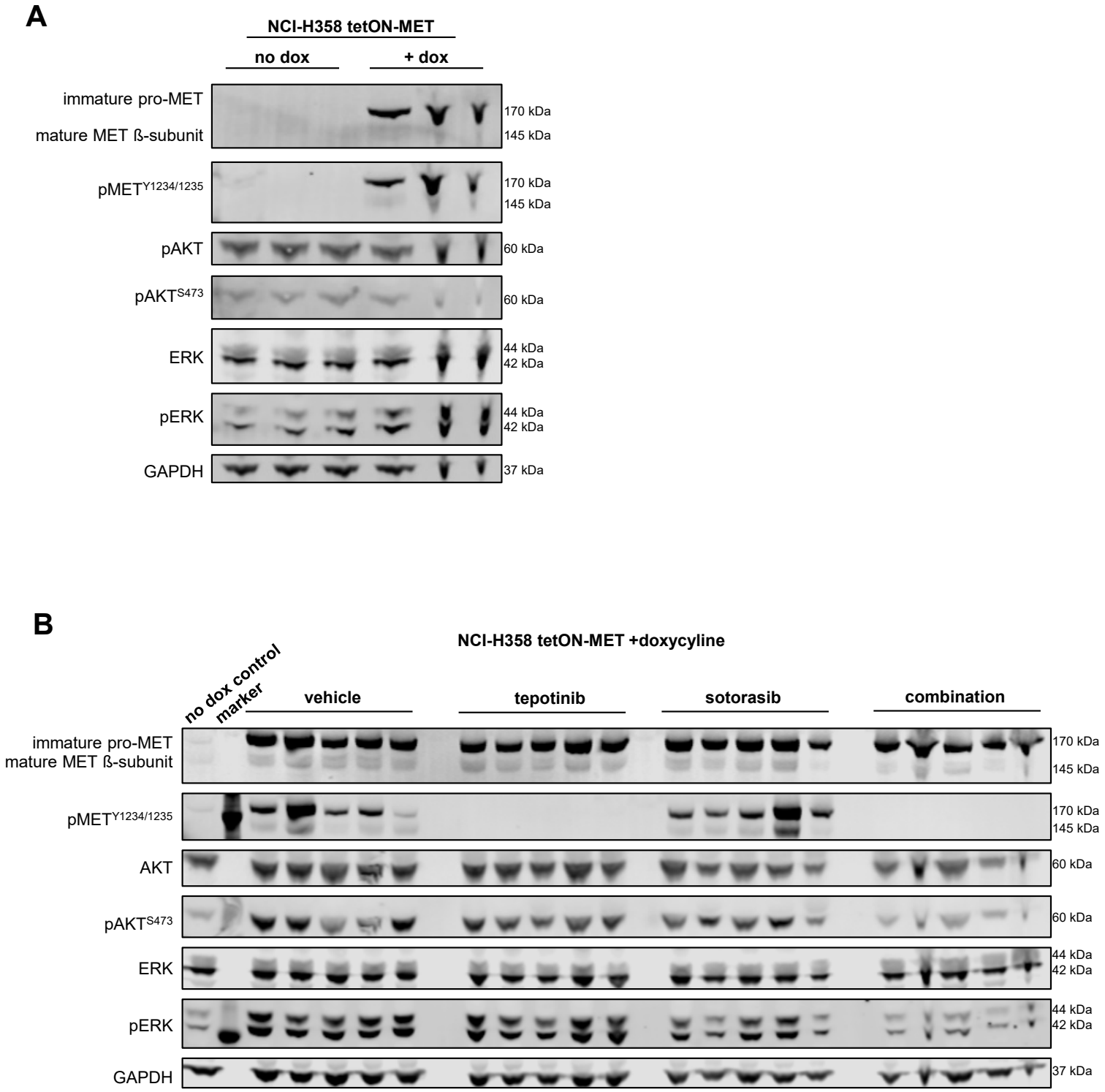


Supplementary Figure S3: Cell viability analyses of tetON-MET cells upon combination treatments, related to Figure 4.
The antiproliferative effects of the targeted therapies combined with tepotinib/ capmatinib or M6748/ TNO155 were assessed in the respective tetON-MET cell lines using combination dose matrices in 6-day viability assays. Graphs visualizing the mean ± SD (n = 3) are shown.



Supplementary Figure S4: Synergy analyses of targeted therapies and MET or SHP2 inhibitors in tetON-MET cells, related to Figure 4.

The antiproliferative effects of the targeted therapies combined with tepotinib/ capmatinib or M6748/ TNO155 were assessed in the respective tetON-MET cell lines by combination dose matrices in 6-day viability assays. The mean Synergy Scores and excess volumes of three independent experiments are shown. Synergism: Synergy Score > 2, Excess volume < 0.



Supplementary Figure S5, Western blot analyses of NCI-H358 tetON-MET xenograft tumors, related to Figure 5.

A, Overexpression of MET in NCI-H358 tetON-MET tumor lysates of mice fed with a doxycycline-containing diet. **B**, Tumor lysates of mice with NCI-H358 tetON-MET tumors fed with a doxycycline-containing diet and treated with tepotinib, sotorasib or the combination of both were analyzed by Western blot after treatment termination.

Supplementary Table S1: Table listing all liquid (blood, cerebrospinal fluid, pleural effusion) biopsies (LBx) and tumor or cytological biopsies (TBx) collected from 28 patients with NSCLC who had disease progression on ALK (n=15), ROS1 (n=8) or RET (n=5) TKIs, related to Figure 1.

Unless otherwise indicated, the TKI was the 1st line treatment.
Patients were considered *METamp* positive by FISH if they showed: (i) a *MET*/CEP7 ratio (r) ≥ 2 and (ii) *MET* gene copy number (GCN) per cell ≥ 6. Patients were considered MET/pMET immunohistochemistry (IHC) positive if intense membrane staining (3+) was observed in ≥ 50% of the tumor cells.

	Patient No.	Sex Age [1] Stage [1]	Type of rearrangement	Type of biopsy	Date of biopsy	Progression on	PFS (months)	NGS [2]	nCounter fusions	nCounter *MET* mRNA [3]	*MET* r & GCN by FISH [3]	MET IHC [3]	pMET IHC [3]
LBx only	P1	unknown unknown unknown	*KIF5B-RET* (ex15-ex12)	blood	13/09/2019	alectinib	10	ND	*KIF5B-RET* (ex15-ex12)				
	P2	Female unknown unknown	*CCDC6-RET (ex1-ex12)*	blood	18/02/2020	alectinib	2	ND	ND				
	P3	unknown unknown unknown	*KIF5B-RET* (ex15-ex12)	blood	21/02/2020	alectinib	3	ND	*KIF5B-RET* (ex15-ex12)				
	P4	Female 67 IV	*ALK*, variant unknown	blood	04/03/2020	ceritinib/ brigatinib	unknown	ND	NA				
	P5	Female 65 IV	*ALK*v3	blood	08/05/2020	alectinib	5	ND	ND				
	P6	Female 58 II	*ALK*v3	blood	30/09/2020	alectinib	19	ND	ND				
				blood	28/01/2021	lorlatinib (2nd line)	4	ND	NA				
				blood	05/10/2021	pemetrexed + lorlatinib (3rd line)	9	*MET*amp	ND				
	P7	unknown unknown unknown	*KIF5B-RET* (ex15-ex12)	blood	02/12/2020	alectinib	17	ND	ND				
	P8	Female 59 unknown	*ALKv3a* (E6a/A20)	pleural effusion	13/08/2021	alectinib	11	ND	*ALKv3*				
				blood	26/10/2021	alectinib	11	*ALK*: p.I1171N	ND				
	P9	Female 59 unknown	*RET*	blood	06/10/2021	selpercatinib	17	ND	ND				
	P10	Female unknown unknown	*ALK*v1	blood	20/10/2020	lorlatinib	48	ND	*ALKv1*				

LBx only	P11	Male unknown unknown	*PRKAR1A-ALK*	blood	29/11/2021	alectinib	unknown	*ALK*: p.L1196M	ND				
	P12	Female unknown unknown	*ROS1*, variant unknown	blood	01/12/2021	crizotinib	36	ND	NA				
	P13	Female unknown IV	*SLC34A2-ROS1*	blood	03/12/2021	ceritinib	3	ND	*SCL34A2-ROS1*				
	P14	Male 47 unknown	*ALK*, variant unknown	blood	20/12/2021	alectinib	unknown	ND	ND				
	P15	Female unknown unknown	*CD74-ROS1*	blood	19/01/2022	crizotinib	unknown	ND	ND				
	P16	Male 62 unknown	*ALK*, variant unknown	blood	24/01/2022	crizotinib	unknown	ND	ND				
	P17	Female 36 unknown	*CD74-ROS1*	blood	28/01/2022	lorlatinib	unknown	ND	*CD74-ROS1*				
	P18	Male 71 IV	*ROS1*, variant unknown	blood	31/01/2022	lorlatinib	4	ND	ND				
	P19	Male 45 IV	*ALK*, variant unknown	blood	19/04/2022	alectinib	unknown	ND	ND				
	P20	Female unknown IV	*ALK*, variant unknown	blood	06/05/2022	lorlatinib	64	ND	ND				
TBx only	P21	Male 56 IV	CD74-ROS1	tissue	12/04/2022	crizotinib	35	*	*	*	*	NEG (0)	*
LBx and TBx	P22	Male unknown IV	*ALK*v1	blood	16/03/2018	alectinib	10	ND	ND				
				tissue	07/05/2018	alectinib	12	ND	*ALK*v1	7	NEG (r=0.3; GCN=2.2)	NEG (80% 2+)	NEG (0)
	P23	Male unknown unknown	*ALK*v5	blood	18/07/2018	alectinib	9	*ALK:* p.G1269A	*ALK*v5				
				tissue	06/08/2018	alectinib	10	*ALK:* p.G1269A	*ALK*v5	35	NEG (r=1.3, GCN=4.1)	NEG (60% 2+)	NEG (0)

LBx and TBx	P24	Male 56 IV	*ALK*v1	blood	15/02/2019	lorlatinib	2	ND	NA				
				tissue	25/02/2019	lorlatinib	2	*MET*amp	*ALK*v1	105	POS (r>5, GCN>15)	POS (95% 3+)	POS (100% 3+)
	P25	Female 48 IV	*ALK*v5	tissue	10/03/2019	alectinib	23	*ALK:* p.G1202R	*ALK*v5	31	NEG (r=1.0; GCN=2.8)	NEG (60% 2+)	NEG (5% 3+)
				blood	16/08/2019	lorlatinib (2nd line)	5	ND	NA				
				cerebrospinal fluid	16/08/2019	lorlatinib (2nd line)	5	ND	NA				
	P26	Female 64 IV	*CD74-ROS1*	blood	15/06/2020	crizotinib	6	ND	ND				
				tissue	28/10/2020	crizotinib	10	ND	*CD74-ROS1*	99	NEG (r=1.2; GCN=2.8)	POS (80% 3+)	NEG (0) [4]
	P27	Male 57 IV	*ALK*v1	blood	08/10/2020	brigatinib	2	ND	NA				
				cerebrospinal fluid	08/10/2020	brigatinib	2	ND	*ALKv1*				
				cerebrospinal fluid	26/02/2021	lorlatinib (2nd line)	4	ND	*ALKv1*				
				pleural effusion	29/04/2021	lorlatinib (2nd line)	6	*MET*amp	NA				
				cytological	29/04/2021	lorlatinib (2nd line)	6	*MET*amp	*ALK*v1				
	P28	Female unknown unknown	*CD74-ROS1*	pleural effusion	08/10/2021	lorlatinib	9	*ROS1:* p.G2026R	ND				
				cytological	08/10/2021	lorlatinib	9	*ROS1:* p.G2026R	*CD74-ROS1*				

[1] Age and stage are reported at the time of diagnosis.
[2] NGS analysis covers mutations and copy number variations (CNVs) in 30 genes frequently altered in lung cancer (*EGFR, BRAF, MET, ERBB2, ALK, ROS1, RET, PIK3CA, KRAS, NRAS, KIT, PDGFRA, TP53, STK11, KEAP1, ARID1A, FAT1, NFE2L2, SETD2, POLE, POLD1, IDH1, IDH2, ERBB4, FGFR1, FGFR2, FGFR3, MYC, CDK4, CDK6*). ND means that no mutations or CNVs in the 30 genes were detected.
[3] nCounter *MET* mRNA, *MET* r & GCN by FISH, MET IHC and pMET IHC were only analyzed in tissue samples.
[4] The negative pMET result of the tissue biopsy of P26 might be an artifact due to inaccurate tissue fixation. A primary cell culture established from P26 at progression showed positive pMET IHC.
* Insufficient material
ND: Not detected
NA: Not analyzed

Supplementary Table S2: Table listing the probe set for nCounter hybridization, related to Figure 1 and Supplementary Table S1.

Assay Type	Target Variant	Accession Number	Target Sequence
Fusion	*EML4-ALK__E13:A20*	PFUS_001.1:1	ATATGGAGCAAAACTACTGTAGAGCCCACACCTGGGAAAGGACCTAAAGTGTAC CGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*EML4-ALK__E20:A20*	PFUS_002.1:1	GACAACAAGTATATAATGTCTAACTCGGGAGACTATGAAATATTGTACTTGTACCG CCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*EML4-ALK__E6:A20*	PFUS_003.1:1	AAAGTTACCAAAACTGCAGACAAGCATAAAGATGTCATCATCAACCAAGTGTACC GCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*EML4-ALK__E2:A20*	PFUS_006.1:1	ATCTCTGAAGATCATGTGGCCTCAGTGAAAAAATCAGTCTCAAGTAAAGTGTACC GCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*EML4-ALK__E18:A20*	PFUS_008.1:1	ATCCACACAGACGGGAATGAACAGCTCTCTGTGATGCGCTACTCAATAGTGTACC GCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*TFG-ALK__T5:A20*	PFUS_016.1:1	CAGCAGCCACCATATACAGGAGCTCAGACTCAAGCAGGTCAGATTGAAGTGTAC CGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*KIF5B-ALK__K17:A20*	PFUS_031.1:0	TTGGAGGAATCTGTCGATGCCCTCAGTGAAGAACTAGTCCAGCTTCGAGCACAA GTGTACCGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGC
Fusion	*EZR-ROS1_E10:R34*	PFUS_032.1:2	AAGGAGGAGTTGATGCTGCGGCTGCAGGACTATGAGGAGAAGACAAAGAAGGC AGAGAGAGATGATTTTTGGATACCAGAAACAAGTTTCATACTTACTA
Fusion	*GOPC-ROS1_G4:R36*	PFUS_023.1:0	CCCTGGTGCTAGTTGCAAAGACACAAGTGGGGAAATCAAAGTATTACAAGTCTG GCATAGAAGATTAAAGAATCAAAAAAGTGCCAAGGAAGGGGTGACA
Fusion	*TPM3-ROS1_T8:R35*	PFUS_035.1:21	AGTTTGCTGAGAGATCGGTAGCCAAGCTGGAAAAGACAATTGATGACCTGGAAG TCTGGCATAGAAGATTAAAGAATCAAAAAAGTGCCAAGGAAGGGGT
Fusion	*LRIG3-ROS1 _L16:R35*	PFUS_027.1:32	AGTTTGTCACATCTTCAGGTGCTGGATTTTTCTTACCACAACATGACAGTAGTGTC TGGCATAGAAGATTAAAGAATCAAAAAAGTGCCAAGGAAGGGGT
Fusion	*GOPC-ROS1_G7:R35*	PFUS_022.1:0	TATGGGGCGAGACTAGCTGCCAAGTACTTGGATAAGGAACTGGCAGGAAGTACT CTTCCAACCCAAGAGGAGATTGAAAATCTTCCTGCCTTCCCTCGGG
Fusion	*SLC34A2-ROS1_S13del2046:R32*	PFUS_034.1:23	AAGGCTCCTGAGACCTTTGATAACATAACCATTAGCAGAGAGGCTCAGGCTGGA GTCCCAAATAAACCAGGCATTCCCAAATTACTAGAAGGGAGTAAA

Fusion	*CD74-ROS1_C6:R32*	PFUS_030.1:23	AATGAGCAGGCACTCCTTGGAGCAAAAGCCCACTGACGCTCCACCGAAAGCTGG AGTCCCAAATAAACCAGGCATTCCCAAATTACTAGAAGGGAGTAAA
Fusion	*SDC4-ROS1_S2:R32*	PFUS_024.1:35	GCCCGGGCAGGAATCTGATGACTTTGAGCTGTCTGGCTCTGGAGATCTGGCTGG AGTCCCAAATAAACCAGGCATTCCCAAATTACTAGAAGGGAGTAAA
Fusion	*SDC4-ROS1_S4:R34*	PFUS_033.1:7	GGTGTCAATGTCCAGCACTGTGCAGGGCAGCAACATCTTTGAGAGAACGGAGGT CCTGGCAGATGATTTTTGGATACCAGAAACAAGTTTCATACTTACT
Fusion	*SLC34A2_S4:ROS1-Common*	PFUS_020.1:5	GTGTGCTCCCTGGATATTCTTAGTAGCGCCTTCCAGCTGGTTGGAGCTGGAGTC CCAAATAAACCAGGCATTCCCAAATTACTAGAAGGGAGTAA
Fusion	*KIF5B-RET_K16:R12*	PFUS_025.1:7	AAGAAAATGAAAAGGAGTTAGCAGCATGTCAGCTTCGTATCTCTCAAGAGGATCC AAAGTGGGAATTCCCTCGGAAGAACTTGGTTCTTGGAAAAACTCT
Fusion	*KIF5B-RET_K22:R12*	PFUS_026.1:12	ACCTGCGCAAACTCTTTGTTCAGGACCTGGCTACAAGAGTTAAAAAGGAGGATCC AAAGTGGGAATTCCCTCGGAAGAACTTGGTTCTTGGAAAAACTCT
Fusion	*KIF5B-RET_K23:R12*	PFUS_029.1:51	CCTTTCTTGAAAATAATCTTGAACAGCTCACTAAAGTGCACAAACAGGAGGATCC AAAGTGGGAATTCCCTCGGAAGAACTTGGTTCTTGGAAAAACTCT
Fusion	*CCDC6-RET_C1:R12*	PFUS_039.1:10	GGAGGAGAACCGCGACCTGCGCAAAGCCAGCGTGACCATCGAGGATCCAAAGT GGGAATTCCCTCGGAAGAACTTGGTTCTTGGAAAAAC
Fusion	*KIF5B_K24-Common*	PFUS_013.1:1	GCAGTCAGGTCAAAGAATATGGCCAGAAGAGGGCATTCTGCACAGATTGTGTAC CGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAG
Fusion	*KIF5B_K15-Common*	PFUS_028.1:40	AAGACCTTGCAGAAATAGGAATTGCTGTGGGAAATAATGATGTAAAGGAGGATCC AAAGTGGGAATTCCCTCGGAAGAACTTGGTTCTTGGAAAAACTCT
METΔex14	*MET*	RCC_AS01_065.1:27_T053	TCCTGTGGCTGAAAAAGAGAAAGCAAATTAAAGATCAGTTTCCTAATTCATCTCAG AACGGTTCATGCCGACAAGTGCAGTATCCTCTGACAG
MET wt	*MET*	RCC_AS01_066.2:1_T052	CCTGTGGCTGAAAAAGAGAAAGCAAATTAAAGATCTGGGCAGTGAATTAGTTCGC TACGATGCAAGAGTACACACTCCTCATTTGGATAGGC
Endogenous	*GAPDH*	NM_002046.3:35_T001	TCCTCCTGTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGCCAGCCGAGCCACA TCGCTCAGACACCATGGGGAAGGTGAAGGTCGGAGTCAACGGATTT
Endogenous	*MRPL19*	NM_014763.3:364_T003	GGAAGTATTCTTCGTGTTACTACAGCTGACCCATATGCCAGTGGAAAAATCAGCC AGTTTCTGGGGATTTGCATTCAGAGATCAGGAAGAGGACTTGGAG
Endogenous	*PSMC4*	NM_006503.2:250_T004	TTTCTCCATGCCCAGGAGGAGGTGAAGCGAATCCAAAGCATCCCGCTGGTCATC GGACAATTTCTGGAGGCTGTGGATCAGAATACAGCCATCGTGGGCT