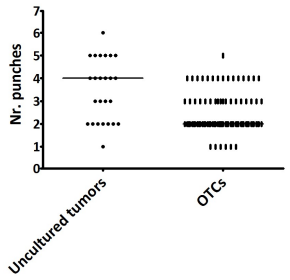
A

Site	N	(%)
Oral cavity	11	45.83
Oropharynx	9	37.5
Larynx	4	16.67
T-stage		
1	1	4.17
2	12	50
3	3	12.5
4	8	33.33
N-stage		
0	9	37.5
1	1	4.17
2	13	54.16
3	1	4.17
Overall stage		
I	0	0
II	8	33.33
III	1	4.17
IV	15	62.50

B



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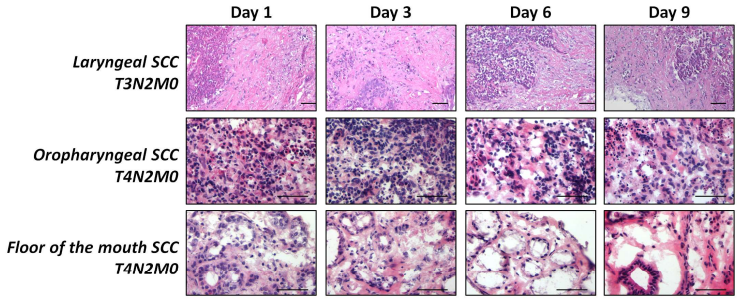

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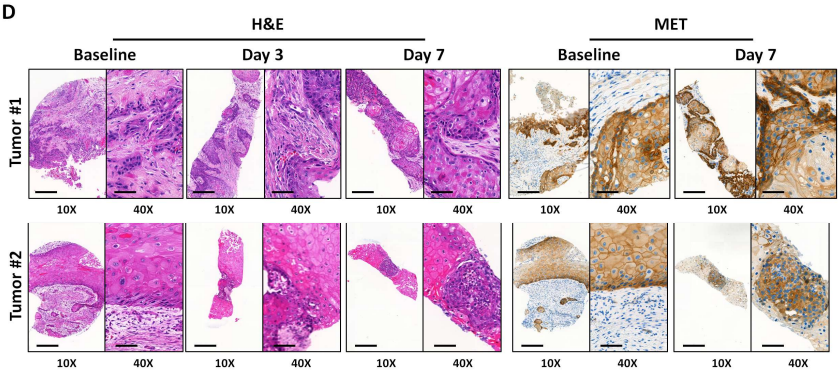

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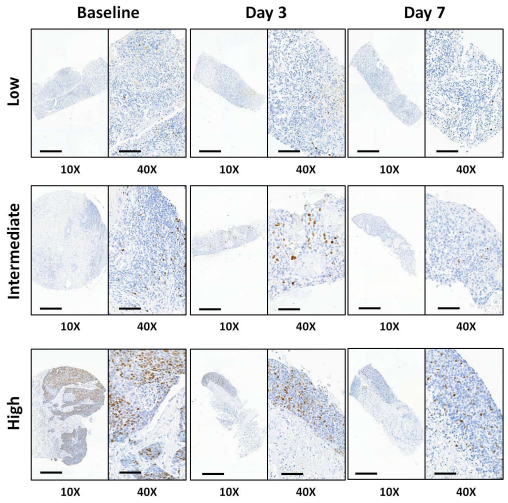

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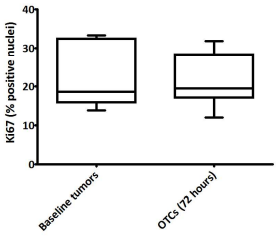
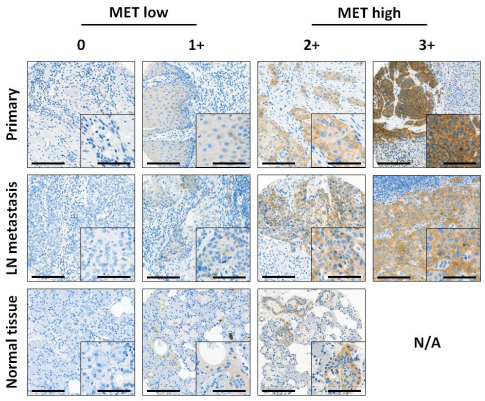


Suppl. Fig. 1. Establishment of organotypic tissue cultures (OTCs) from freshly-resected HNSCCs. A, Tumor-related features of included samples (n=24=). B, Number of punches from uncultured tumors and OTCs included in the final TMA. C, Evaluation of morphology (H&E) upon culture in tumors of different anatomic subsites (larynx, oropharynx and oral cavity); morphology was preserved up to 3 days of culture and variably thereafter. D, Levels of MET expression were determined by immunohistochemistry at baseline and after 7 days, without significant changes in expression. E, Assessment of proliferation (Ki67 labeling index) at baseline, day 3 and day 7 in tumors with low, intermediate and high proliferation rates. No significant changes in Ki67 immunoreactivity were seen in any group after 3 days, whereas after 7 days there was a tendency for loss of cell proliferation in some tumors. F, Baseline tumors and OTCs after 3 days of culture did not reveal significant differences in Ki67 expression ($p=0.4275$, paired t-test).

A



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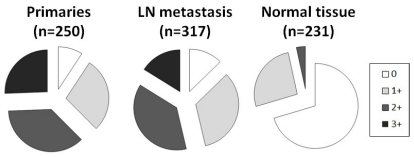

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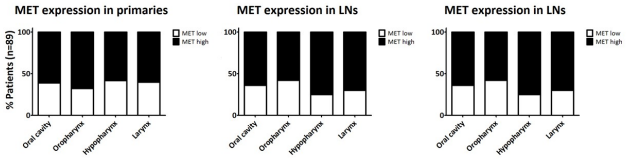

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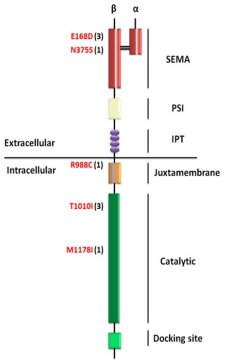

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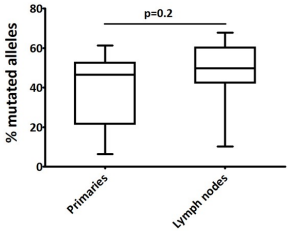
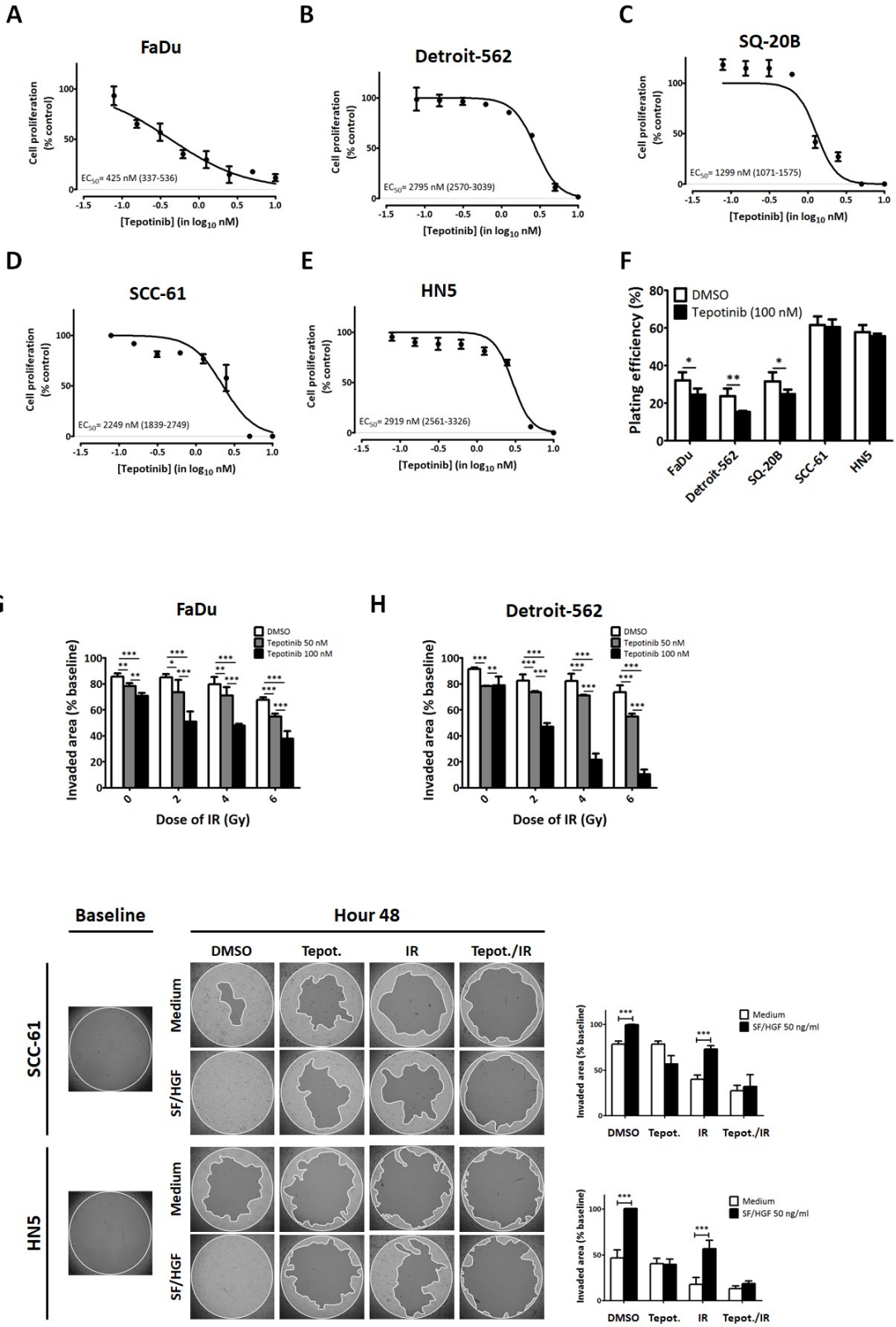


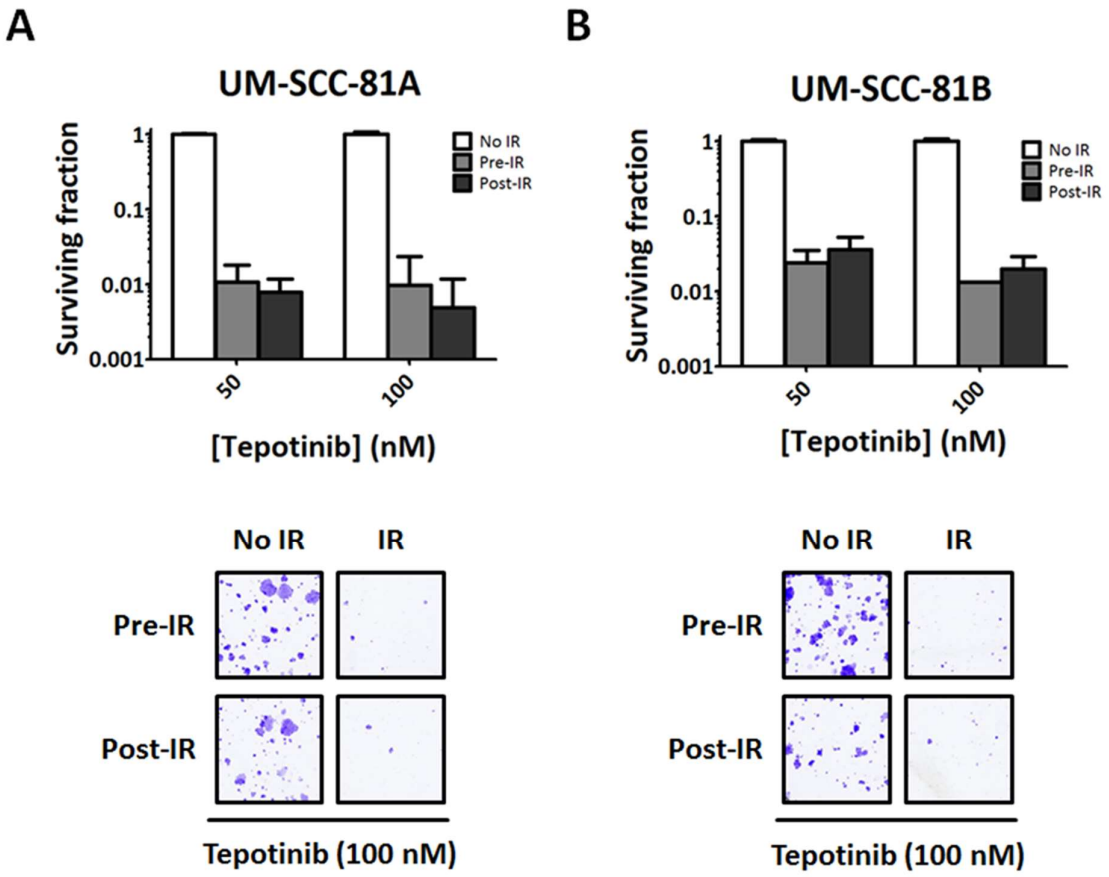
F

Characteristic		N	%
Gender	*Male*	164	73.54
	Female	59	26.46
Age	*Aver ± SD (range)*	60.06± 10.31 (20.07-88.08)	
Location primary tumor	*Oral cavity*	69	30.94
	Oropharynx	109	48.88
	Hypopharynx	23	10.31
	Larynx	16	7.17
	Multiple locations within HN	6	2.69
Stage primary tumor	*Stage III*	29	13.00
	Stage IVA	183	82.06
	Stage IVB	11	4.93
T-stage	*T1*	28	12.56
	T2	90	40.36
	T3	56	25.11
	T4	49	21.97
N-stage	*N0*	0	0.00
	N1	39	17.49
	N2	173	77.58
	N3	11	4.93

Suppl. Fig. 2. MET expression and mutations in HNSCC. A, Representative images of immunohistochemical staining intensities in primary tumors, lymph node metastases (LNMs) and normal salivary gland tissue (larger image 20X magnification, size bar 100 µm; smaller image 40X magnification, size bar 50 µm). B, Frequencies of MET expression immunohistochemical intensities. High MET expression was found in 62.40% of primaries, 53.63% of LNs and only 3.39% of normal tissue. No normal tissue was scored as 3+. C, MET expression in primaries and LNMs according to HNSCC anatomical subsites. D, Schematic representation of the MET receptor displaying mutations identified in our screening approach. E, Relative abundance of mutated alleles in primaries vs. lymph node metastases. F, Characteristics of patients whose tissues were used for mutational screening. G, Individual details of patients with MET-mutated HNSCC.


A
FaDu
Cell proliferation (% control)
EC50= 425 nM (337-536)
[Tepotinib] (in log10 nM)
B
Detroit-562
EC50= 2795 nM (2570-3039)
C
SQ-20B
EC50= 1299 nM (1071-1575)
D
SCC-61
EC50= 2249 nM (1839-2749)
E
HN5
EC50= 2919 nM (2561-3326)
F
DMSO
Tepotinib (100 nM)
Plating efficiency (%)
FaDu
Detroit-562
SQ-20B
SCC-61
HN5
G
FaDu
DMSO
Tepotinib 50 nM
Tepotinib 100 nM
Invaded area (% baseline)
Dose of IR (Gy)
H
Detroit-562
I
Baseline
Hour 48
DMSO
Tepot.
IR
Tepot./IR
SCC-61
HN5
Medium
SF/HGF
SF/HGF 50 ng/ml

Suppl. Fig. 3. Effects of tepotinib *in vitro*. EC50 value determination and effect of tepotinib on plating efficiency *in vitro*. A-E, EC_{50} values were determined by exposing cells to increasing concentrations of tepotinib (0-10 μM) for 72 hours. Cell proliferation was evaluated with a resazurin reduction based assay after 72 hours. F, For evaluation of plating efficiency, cells were seeded onto 6-well plates and treated with tepotinib (100 nM) or DMSO for 8-12 days. After this period, colonies of >50 cells were then quantified and plating efficiency was calculated using the formula "colonies/plated cells". G-H, Cell migration in FaDu and Detroit-562 cells. Cells were plated and left to attach. Subsequently, cells were treated with tepotinib (50 nM and 100 nM), and irradiated after 6 hours. Invaded area was determined after 48 hours. I, SF/HGF induced cell migration in cells with low basal MET expression. Cells were plated and left to attach. Serum-starvation took place for 12 hours and cells were then stimulated with exogenous SF/HGF 50 ng/ml for 6 supplementary hours. Thereupon, tepotinib (100 nM) or DMSO was added in complete medium (without removing SF/HGF), and 6 hours later IR (6 Gy) was delivered. Cells migration was followed for 48 hours. P-values: *<0.05, **<0.01, ***<0.001.

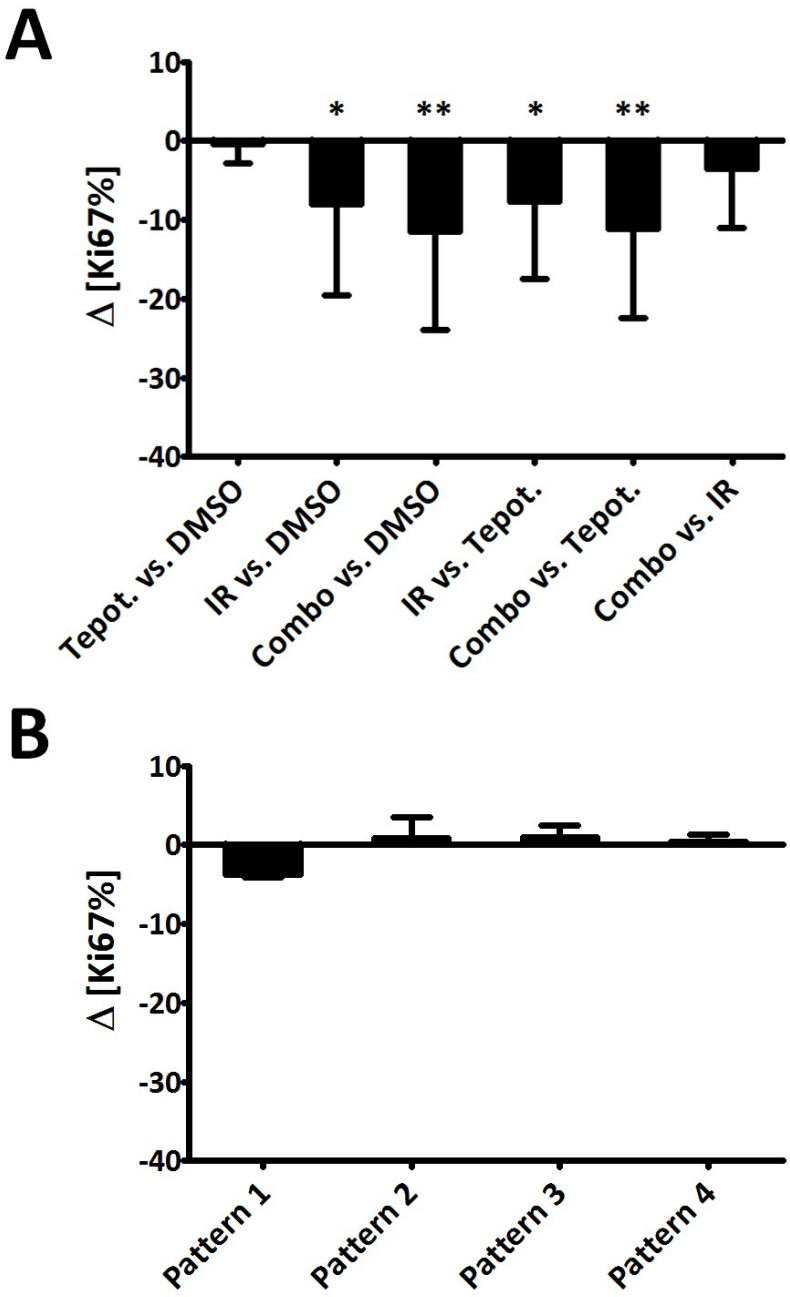


C

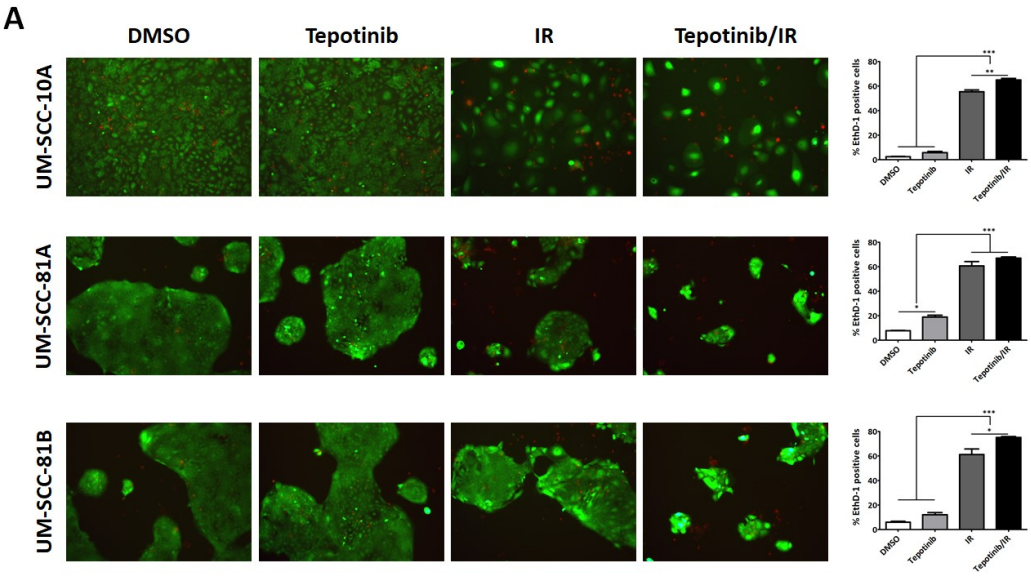
Cell line	RER (doses of tepotinib)		
	0 vs 50	***0 vs 100***	***50 vs 100***
FaDu	0.978±0.007	0.979±0.002	1.000±0.010
Detroit-562	***1.084±0.000***	***1.259±0.010***	***1.161±0.009***
UM-SCC-10A	***1.138±0.003***	***1.179±0.011***	***1.037±0.007***
UM-SCC-10B	***1.232±0.007***	***1.190±0.016***	0.955±0.007
HN5	***1.156±0.037***	***1.201±0.027***	1.040±0.057
SCC-61	***1.143±0.084***	***1.182±0.055***	1.035±0.027
UM-SCC-81A	***1.082±0.011***	***1.206±0.024***	***1.115±0.034***
UM-SCC-81B	***1.095±0.012***	***1.453±0.089***	***1.326±0.066***
SQ-20B	***1.192±0.079***	**1.602±0.313**	1.336±0.172

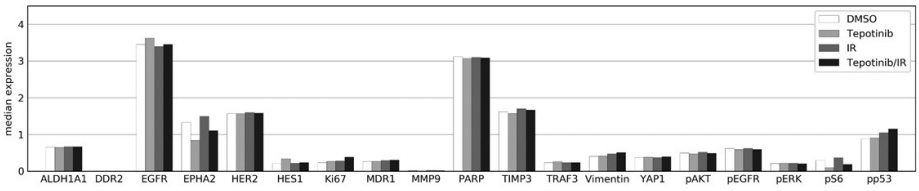
Suppl. Fig. 4. Effects of pre- and post-irradiation tepotinib treatment. Administration of

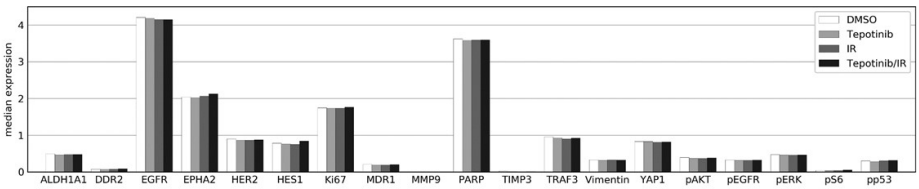
tepotinib (50 and 100 nM) 16 hours prior or after IR (6 Gy) did not significantly alter final results. Surviving fraction normalized to plating efficiency and corrected for drug toxicity (upper panels) and representative images for UM-SCC-81A (A) and UM-SCC-81B (B). C, Radiosensitization was determined by calculating radiation enhancement ratios (RER). RERs significantly superior to 1 according to one-sample t tests (shown in bold characters in italics) indicate radiosensitization.

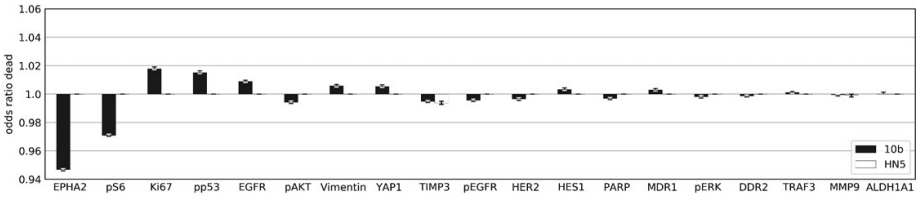


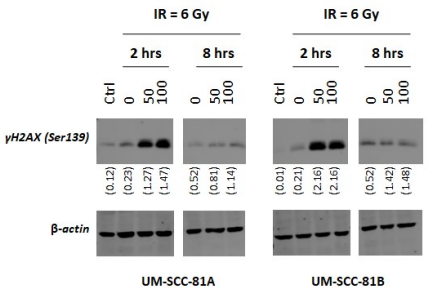
Suppl. Fig. 5. Responses to MET inhibition and IR in OTCs. A, Comparisons of responses (expressed in variation of percentage of Ki67 positive nuclei, Δ[Ki67%]). P-values: *<0.05, **<0.01; calculated with one-sample t test). B, Single MET inhibition (tepotinib 1 µM) did not lead to significant reduction of Ki67 expression in any of the identified patterns.


A
DMSO
Tepotinib
IR
Tepotinib/IR
UM-SCC-10A
UM-SCC-81A
UM-SCC-81B
% EthD-1 positive cells
DMSO
Tepotinib
IR
Tepotinib/IR


B
UM-SCC-10B
median expression
DMSO
Tepotinib
IR
Tepotinib/IR
ALDH1A1 DDR2 EGFR EPHA2 HER2 HES1 Ki67 MDR1 MMP9 PARP TIMP3 TRAF3 Vimentin YAP1 pAKT pEGFR pERK pS6 pp53


C
HN5
median expression
DMSO
Tepotinib
IR
Tepotinib/IR
ALDH1A1 DDR2 EGFR EPHA2 HER2 HES1 Ki67 MDR1 MMP9 PARP TIMP3 TRAF3 Vimentin YAP1 pAKT pEGFR pERK pS6 pp53


D
odds ratio dead
10b
HN5
EPHA2 pS6 Ki67 pp53 EGFR pAKT Vimentin YAP1 TIMP3 pEGFR HER2 HES1 PARP MDR1 pERK DDR2 TRAF3 MMP9 ALDH1A1


E
IR = 6 Gy
IR = 6 Gy
2 hrs
8 hrs
2 hrs
8 hrs
Ctrl
0
50
100
0
50
100
Ctrl
0
50
100
0
50
100
γH2AX (Ser139)
(0.12) (0.23) (1.27) (1.47)
(0.52) (0.81) (1.14)
(0.01) (0.21) (2.16) (2.16)
(0.52) (1.42) (1.48)
β-actin
UM-SCC-81A
UM-SCC-81B

Suppl. Fig. 6. Mechanisms of radiosensitization *in vitro*. A, Live-dead assays were performed as described in the legend of Fig. 5, in 3 cell lines sensitized to IR by MET inhibition. P-values: *<0.05, **<0.01, ***<0.001. CyTOF analysis of expression of 19 proteins in cell lines treated with either DMSO or tepotinib for 16 hours prior to IR. Panels show the median arcsinh-transformed expression of the proteins upon each treatment condition in UM-SCC-10B (Panel B) and HN5 (Panel C). The 95% confidence intervals are too small to be shown due to the large number of events analyzed (around 100,000 per cell line and treatment condition). D, Logistic regression analysis of all 19 included proteins. E, UM-SCC-10A, UM-SCC-81A, and UM-SCC-81B were exposed to DMSO or tepotinib (100 nM) 16 hours prior to IR. Cells were lysed 2 and 8 hours after IR and γH2AX levels assessed by immunoblot.