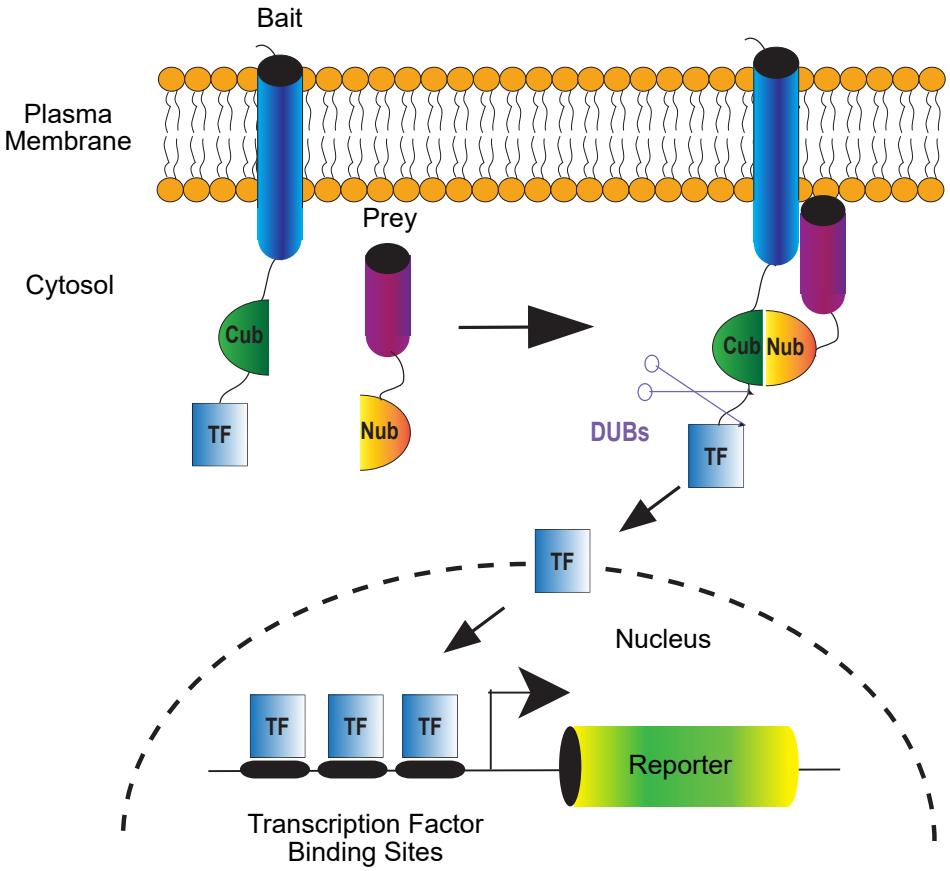
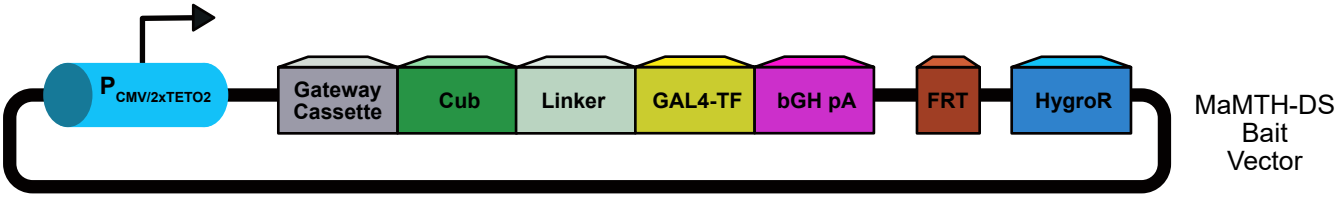


Supplementary Figure 1. Principles of MaMTH. Integral membrane 'bait', fused to the C-terminus of ubiquitin (Cub) and an artificial transcription factor (TF), is expressed alongside cytosolic or membrane-bound 'prey' fused to the N-terminus of ubiquitin (Nub) in cells carrying a luciferase reporter. Interaction of bait and prey leads to ubiquitin reconstitution, proteolytic cleavage by deubiquitinating enzymes (DUBs) and subsequent release of the TF, which then enters the nucleus of the cell and activates the reporter system. In MaMTH-DS, small molecules which disrupt interaction of bait and prey can be identified by loss of luciferase activity.

a



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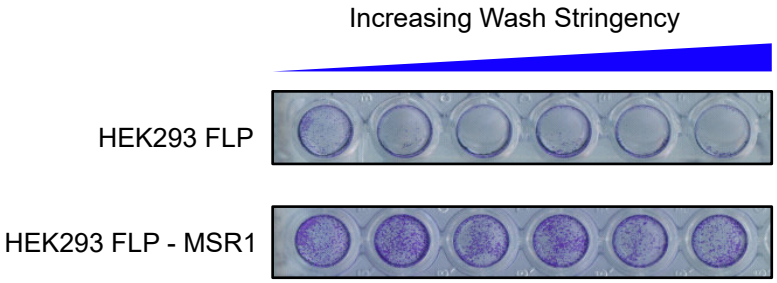

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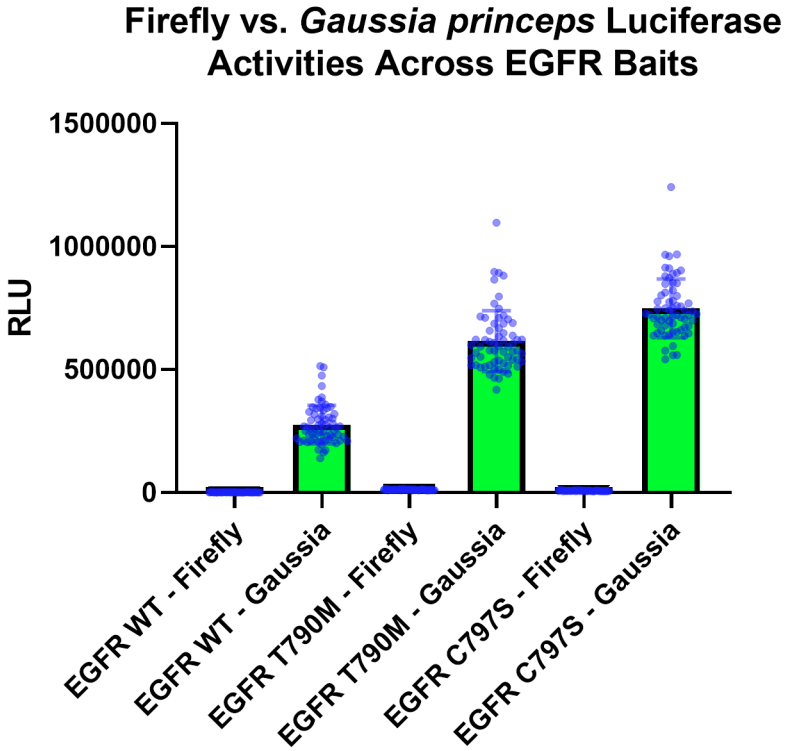
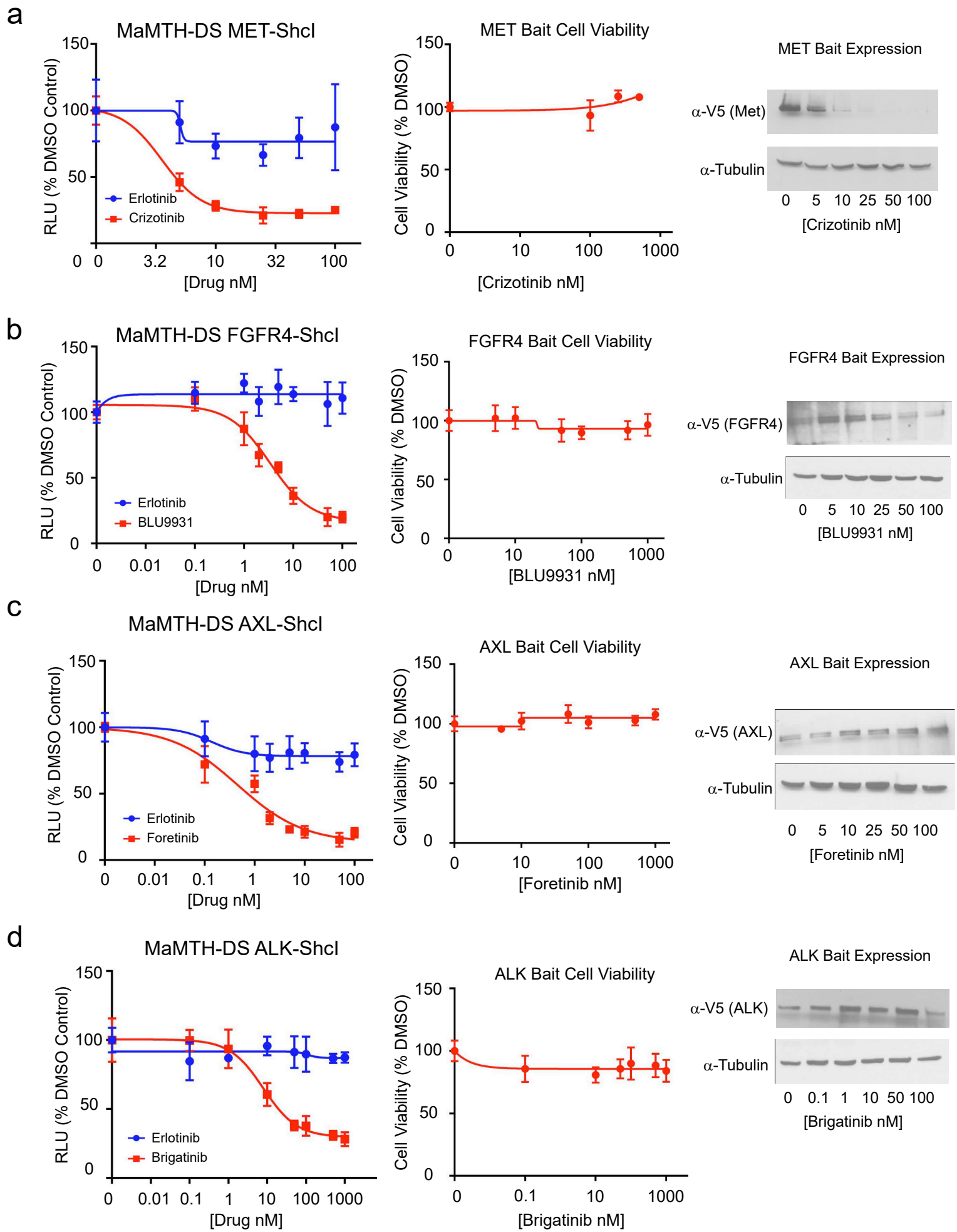
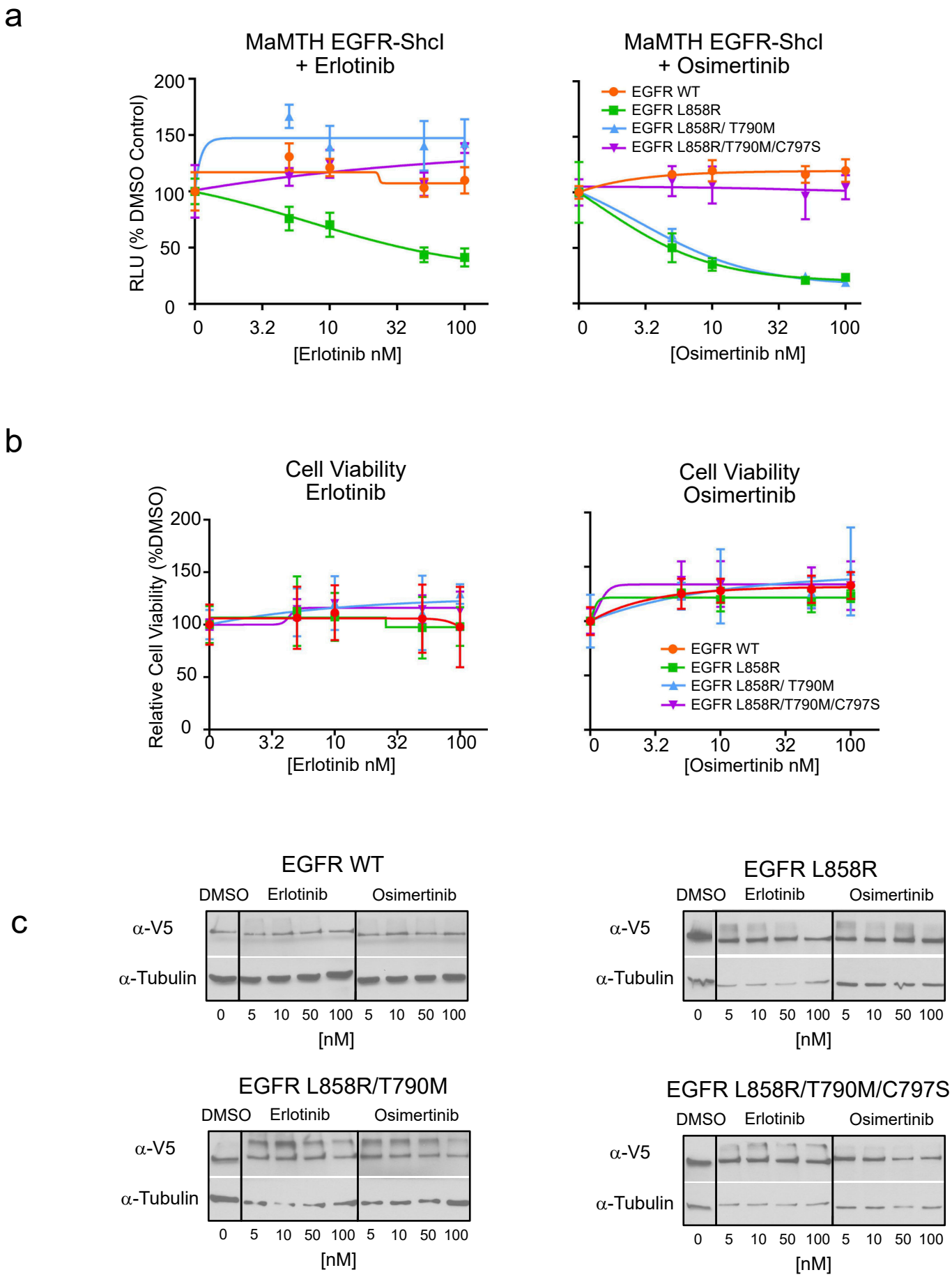


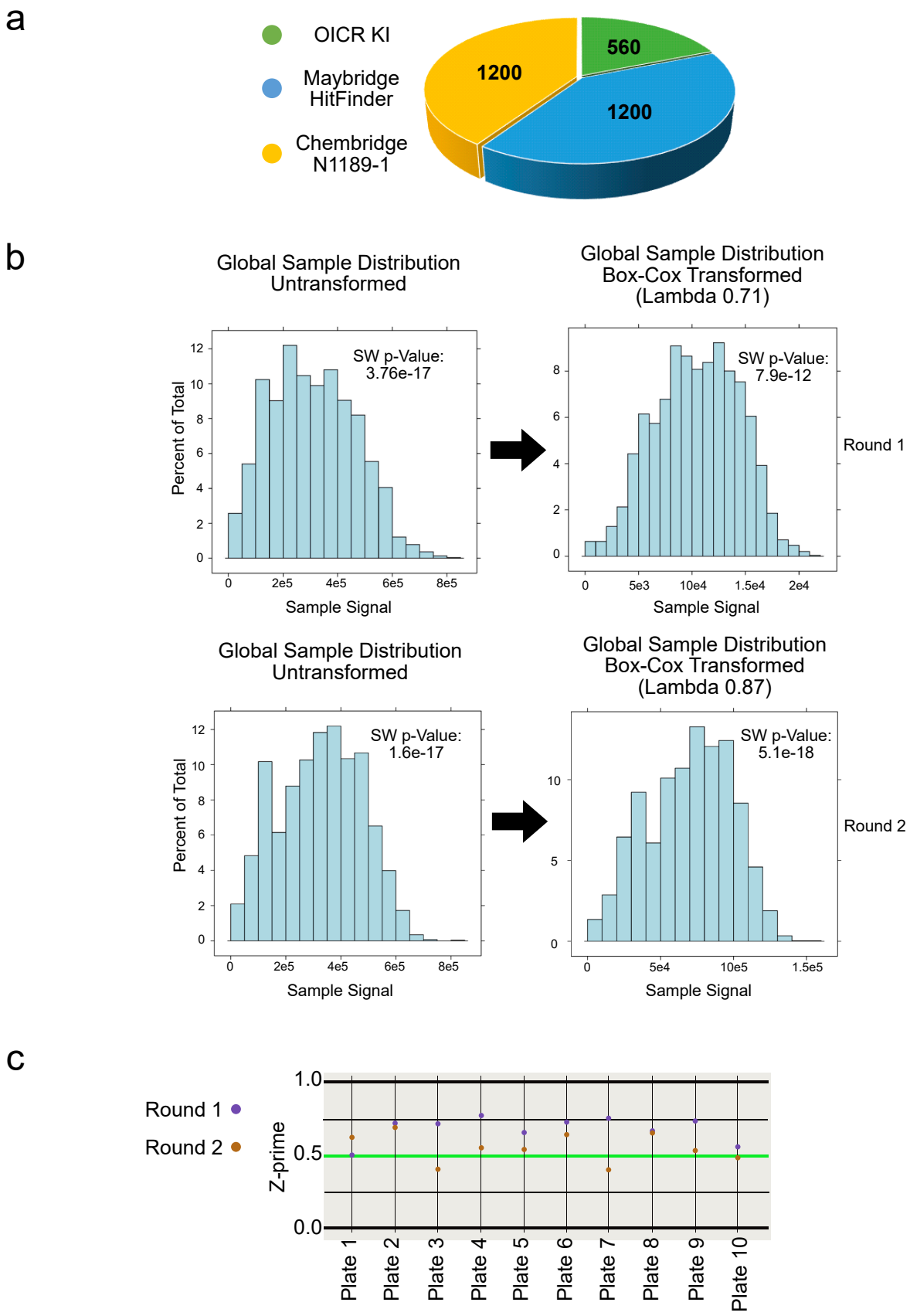
Supplementary Figure 2. Overview of key modifications employed in the MaMTH-DS platform. (a) Schematic diagram of Flp-In TREx and Gateway compatible MaMTH-DS bait vector. (b) Methylene blue rinse test demonstrating enhanced adherence of Flp-compatible HEK293 cells carrying randomly integrated macrophage scavenger receptor 1 (MSR1, bottom panel) vs Flp-compatible HEK293 WT (top panel). Experiments were carried out four times with consistent results obtained each time. (c) Comparison of Firefly vs *Gaussia princeps* luciferase activity across EGFR WT and mutants in MaMTH assays performed in a 384-well format. EGFR T790M = EGFR L858R/T790M, EGFR C797S = EGFR L858R/T790M/C797S. Values are the average of 48 or 64 independent measurements (for Firefly and Gaussia luciferases, respectively) with error bars showing standard deviation.



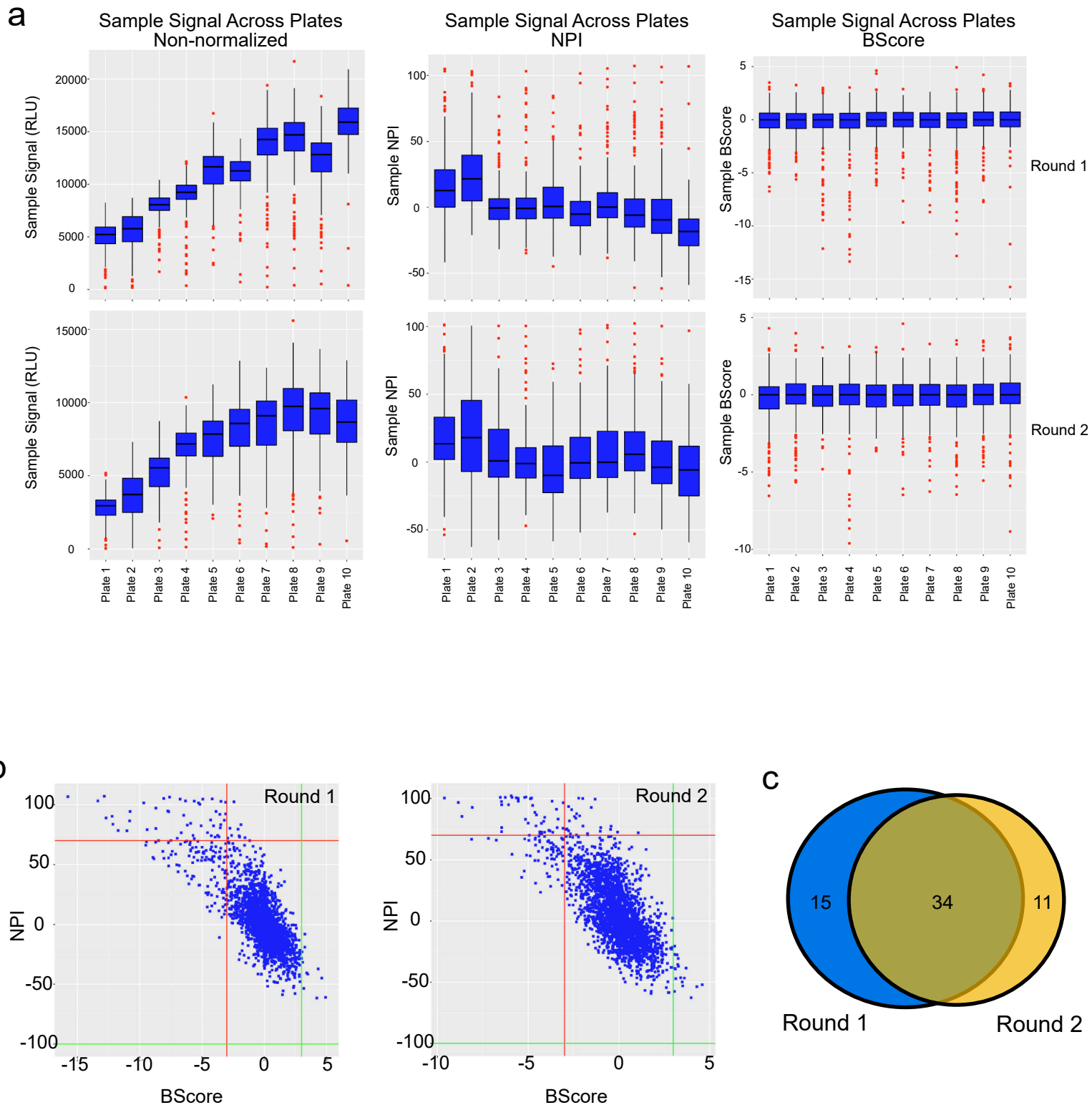
Supplementary Figure 3. Effect of TKI therapeutics on MaMTH-DS RTK baits. Left panels show the effect of the indicated compounds on MaMTH-DS activity in reporter cells stably expressing RTK bait in the presence of transiently transfected Shc1 prey. Middle panels show the effects of the indicated compounds on the viability of reporter cells expressing RTK bait. Right panels show the effect of indicated compounds on the expression of RTK bait in reporter cells. (a) MET receptor with Crizotinib. (b) FGFR4 receptor with BLU9931. (c) AXL receptor with Foretinib. (d) ALK receptor with Brigatinib. All plotted values are the average of three independent measurements, with error bars showing standard deviation. See Supplementary Fig 29 for source blot images.



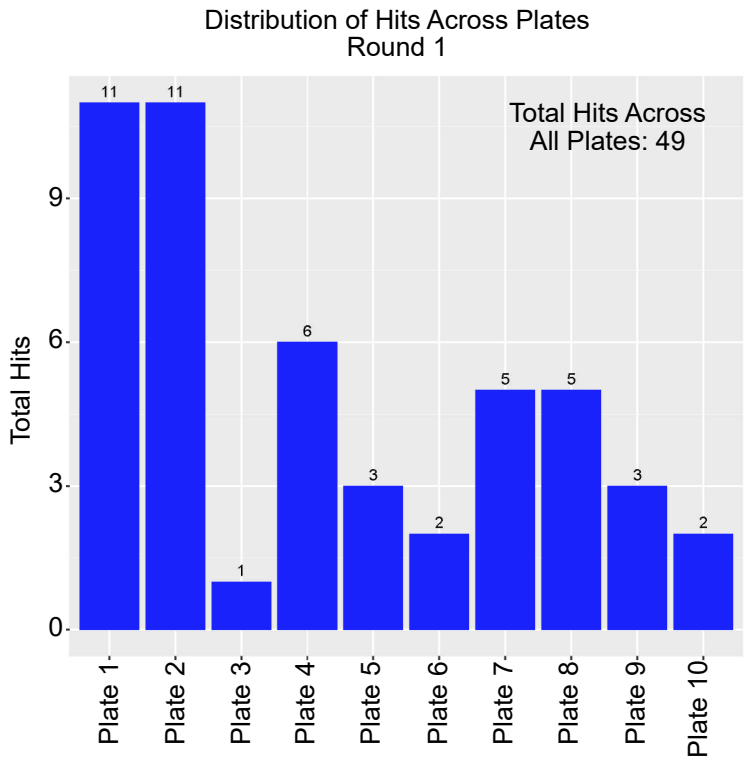
Supplementary Figure 4. Effect of TKI therapeutics on MaMTH reporter cells stably expressing EGFR WT and mutant baits. (**a**) Effect of Erlotinib and Osimertinib on EGFR WT and mutants measured using MaMTH-DS. (**b**) Effect of reported TKI therapeutics on MaMTH EGFR bait reporter cell viability. (**c**) Effect of reported TKI therapeutics on EGFR bait expression. Baits were probed with α-V5 anitbody. Tubulin levels were measured as a loading control. For graphs, all plotted values are the average of three independent measurements, with error bars showing standard deviation. For blots, results are representative of at least two independent experiments. See Supplementary Fig 29 for source blot images.

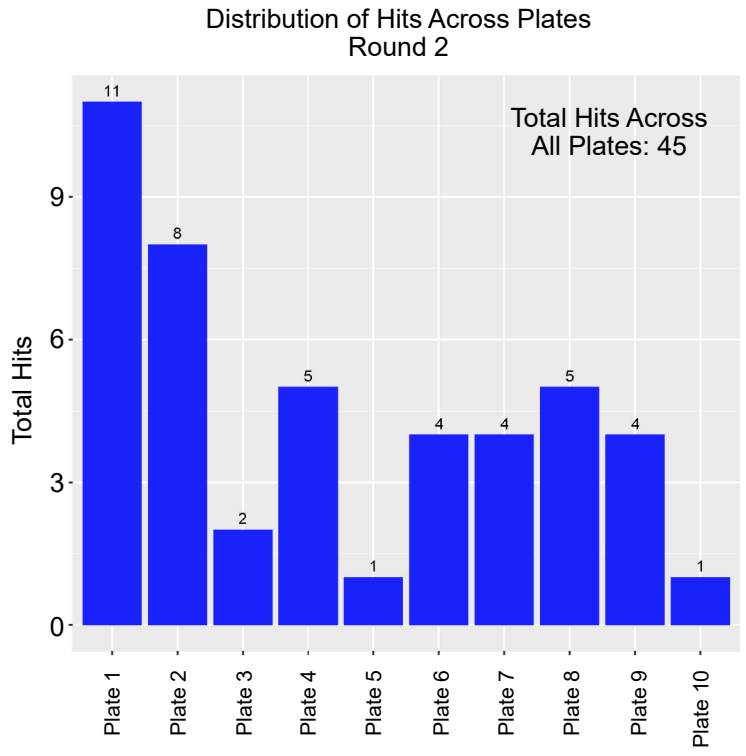


Supplementary Figure 5. MaMTH-DS pilot screen compound overview and initial data analysis. (a) Source and number of small molecules used in our pilot screening library. Library included compounds from the Maybridge HitFinder TM, Chembridge N1189-1 and Ontario Institute for Cancer Research (OICR) kinase inhibitor collections. (b) Sample distributions (n=2960) of untransformed and Box-Cox power transformed data (using a value of Lambda = 0.71 and 0.87 for screening rounds 1 and 2, respectively). P-Values from Shapiro-Wilk's normality test of data are shown in inset. (c) Z' values across all ten plates used in each round of screening.



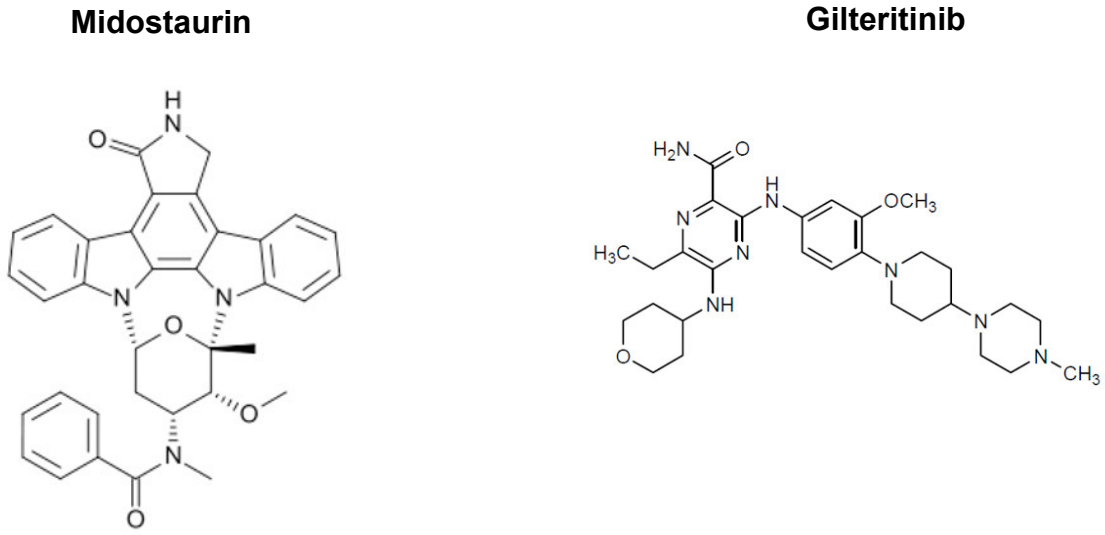
Supplementary Figure 6. MaMTH-DS pilot screen data normalization and hit identification. (a) Box and whisker plots showing distribution of sample values across plates for non-normalized, NPI-normalized and BScore-normalized data. Medians are indicated by thick black lines. Filled blue boxes encompass the 25th to 75th percentiles. Whiskers extend to the largest and smallest values not greater than 1.5 times the IQR. Outlying points beyond the whiskers are shown individually in red. (b) Scatterplot of NPI vs. BScore for all samples in screens. Horizontal red and green lines correspond to NPI values of 70% and -100%, respectively. Vertical red and green lines correspond to BScore values of -3 and +3, respectively. All values in the upper left quadrants were scored as hits. (c) Total hits and overlap for screening Rounds 1 and 2.





Supplementary Figure 7. MaMTH-DS hits from screen of EGFR L858R/T790M/C797S in the presence of Shc1. Hit distributions across plates for Rounds 1 and 2 of screening are shown, with the total number of hits per screen labelled inset. Distribution of hits is relatively even across all plates, with a slightly greater hit rate in the OICR Kinase Inhibitor Collection (Plates 1 and 2), consistent with the expected activity of these compounds towards RTKs such as EGFR.

a



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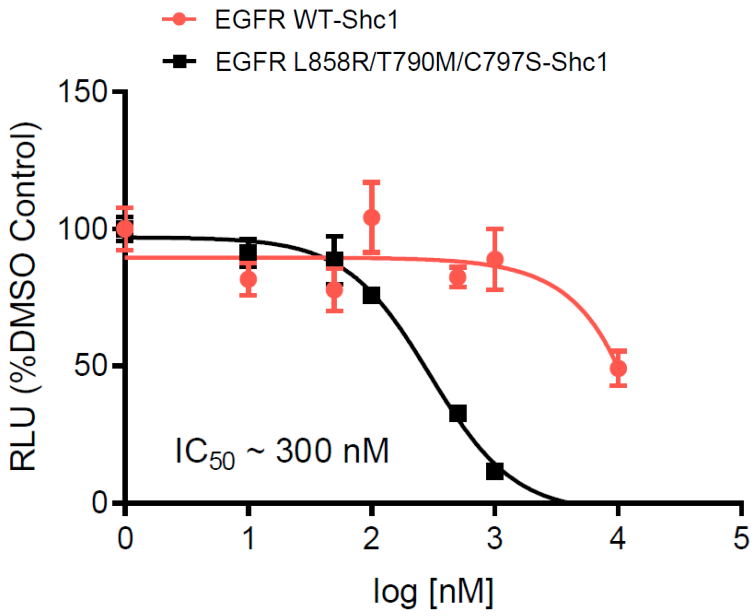
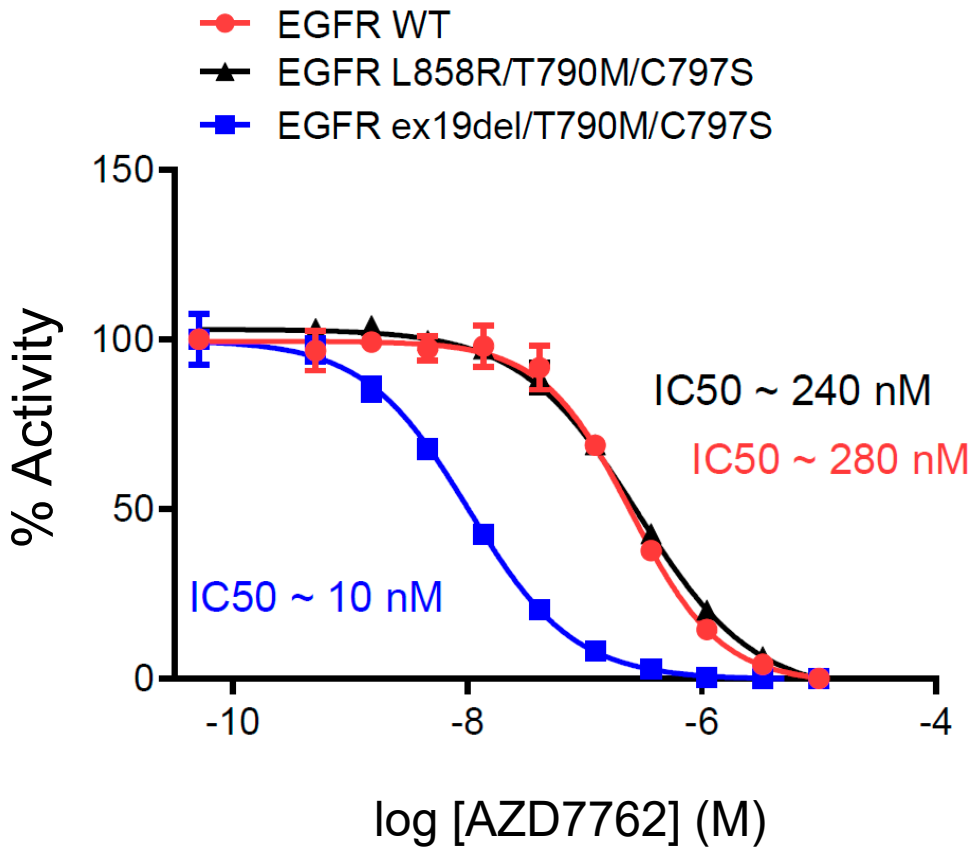
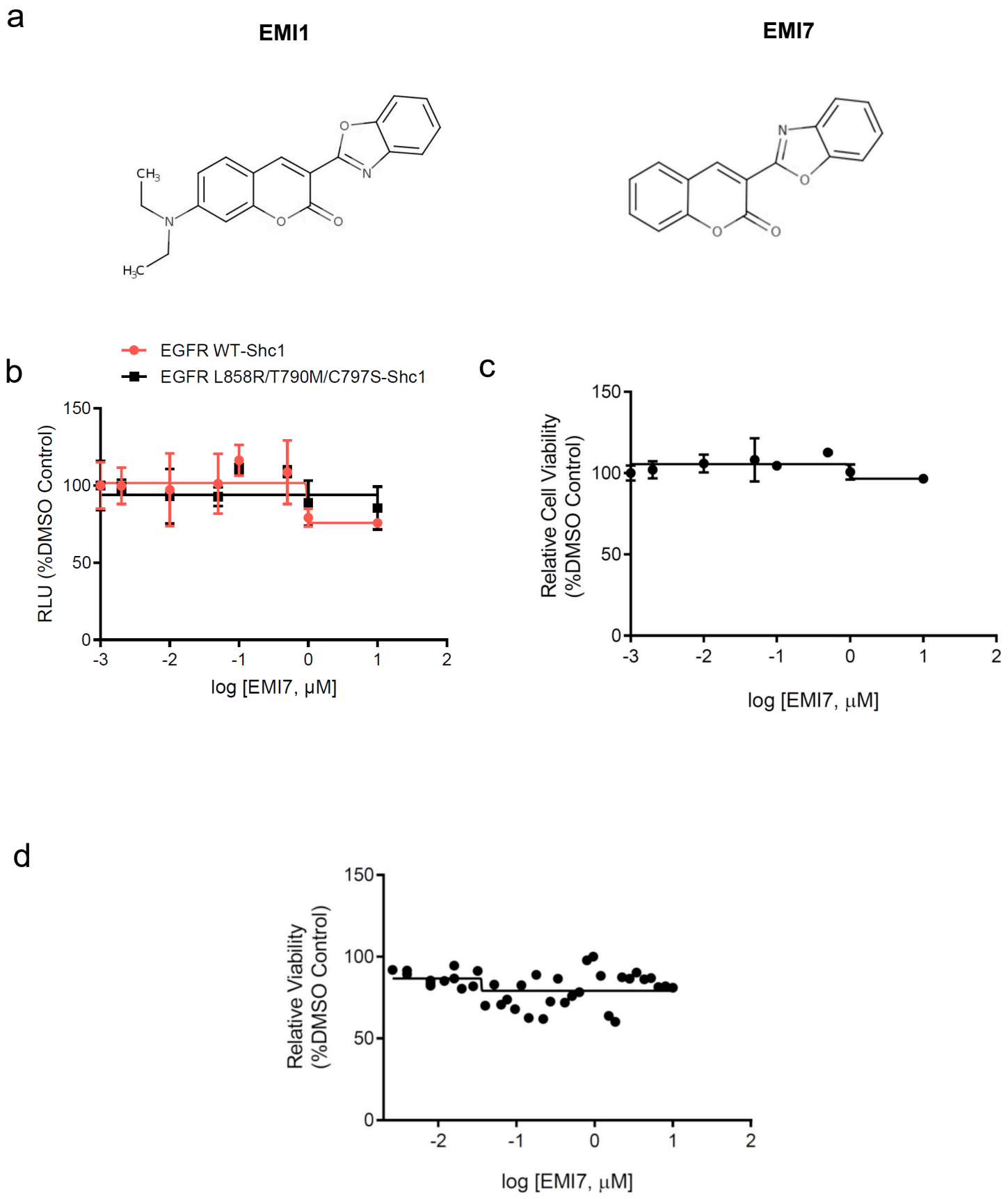


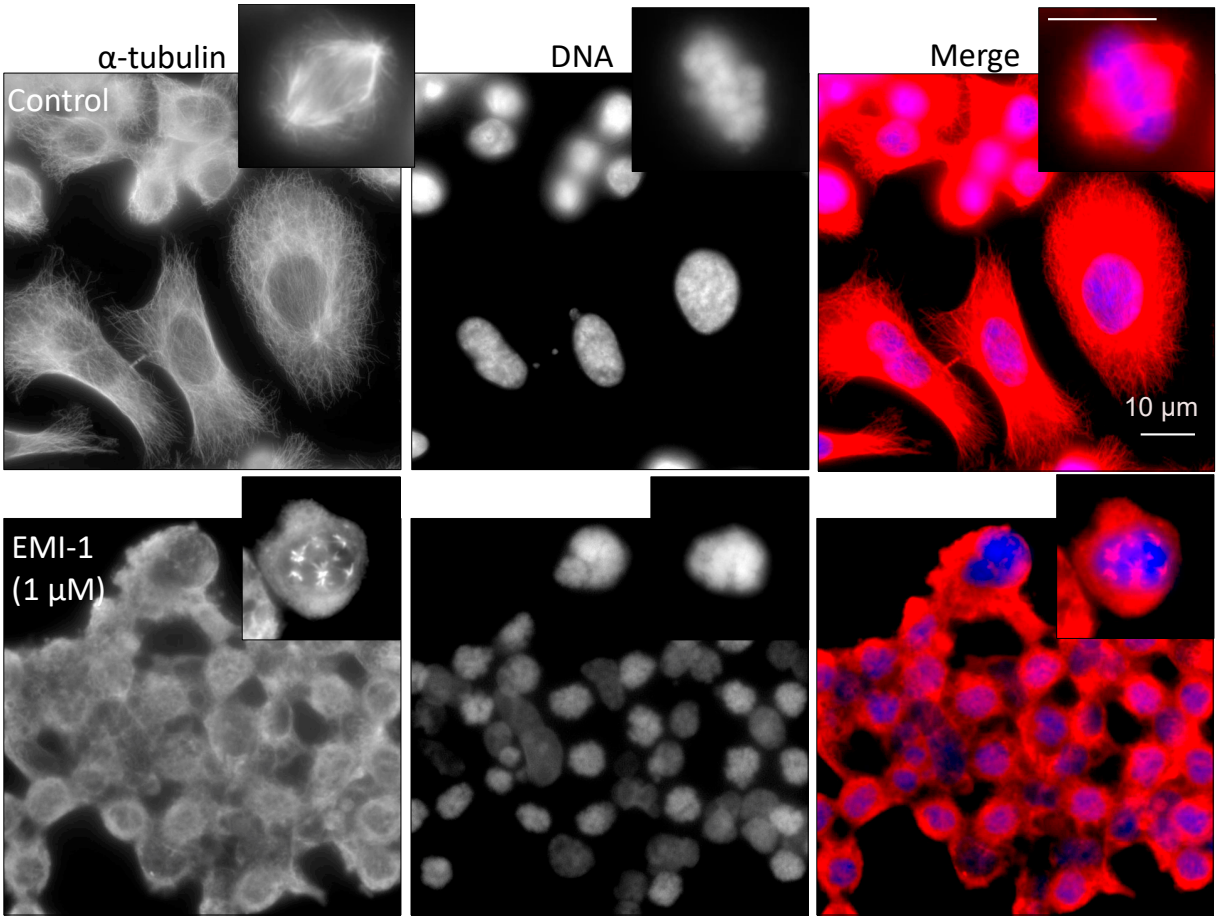
Supplementary Figure 8. Comparison of Midostaurin and Gilteritinib structures and analysis of Gilteritinb activity in MaMTH-DS. (a) Chemical structures of Midostaurin and Gilteritinib. (b) MaMTH-DS analysis of Gilteritinib demonstrating that it is a potent, mutant-specific and dose-responsive inhibitor of the interaction of EGFR L858R/T790M/C797S triple mutant with Shc1. Results are shown as the average ± SD for three independent experiments.



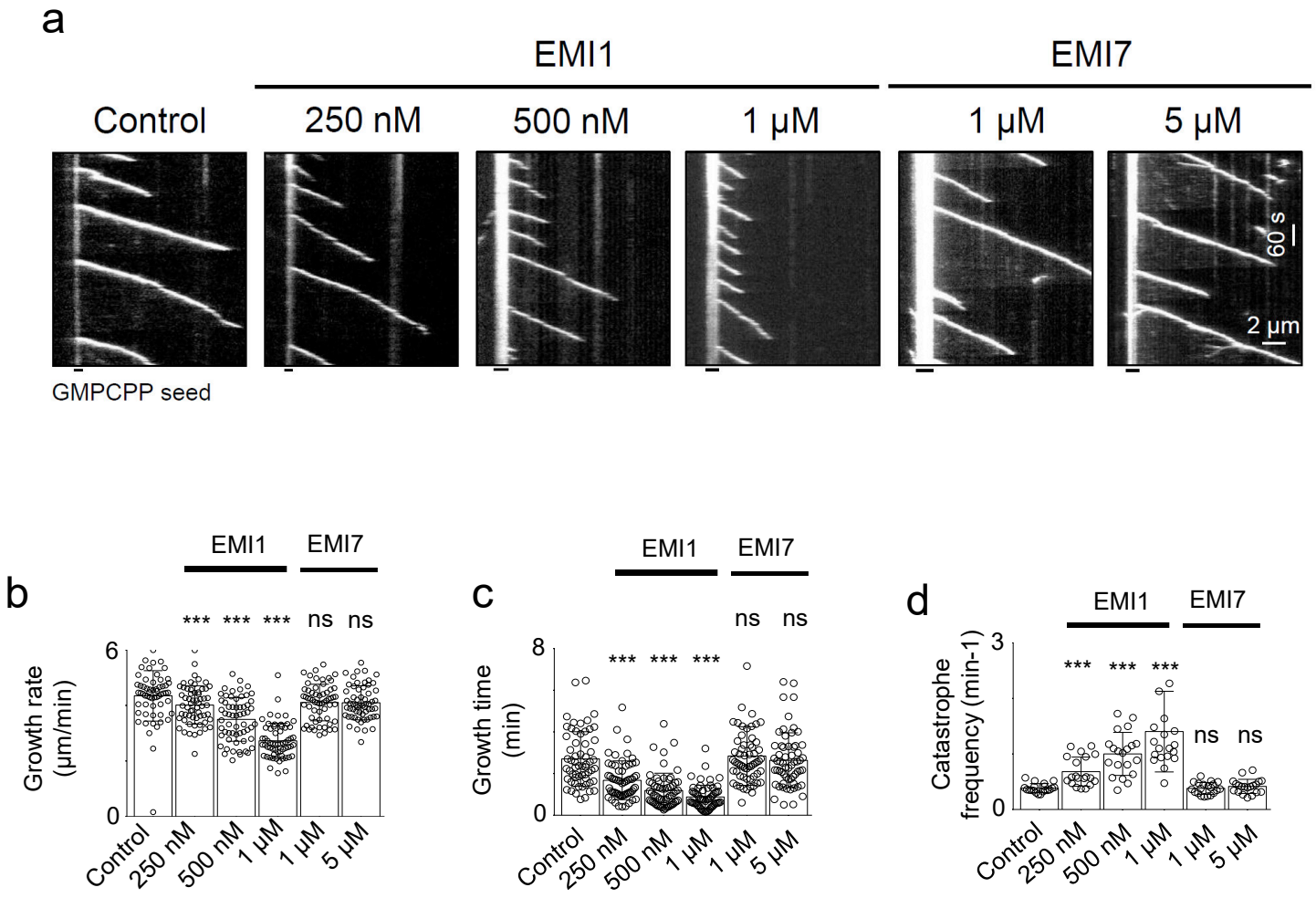
Supplementary Figure 9. Inhibition of recombinant EGFR WT and triple mutant kinase domain activity by AZD7762 measured using *in vitro* kinase assay. Results are shown as the average ± SD for two independent experiments.



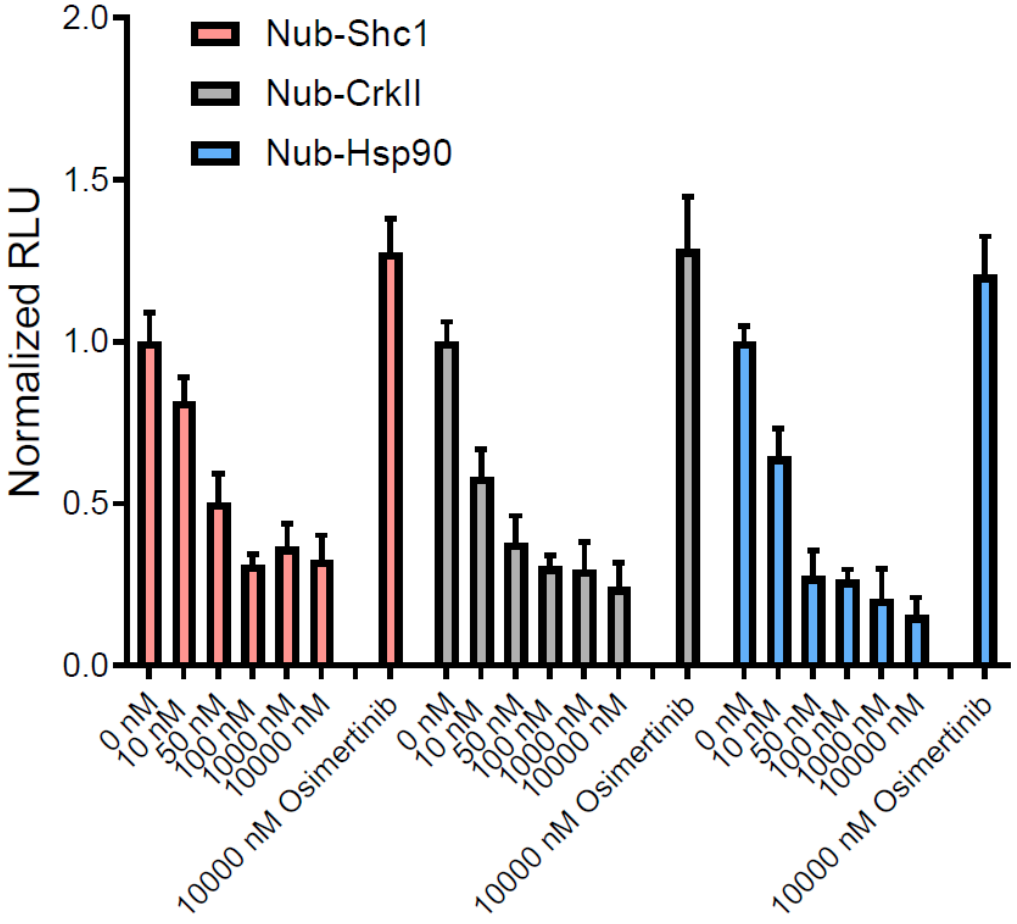
Supplementary Figure 10. Characterization of EMI7 control compound. (a) Chemical structures of EMI1 and EMI7 analog. (**b**) MaMTH-DS analysis of EMI7 demonstrating that it has no effect on the interaction of EGFR L858R/T790M/C797S triple mutant with Shc1. Results are shown as the average ± SD for three independent experiments. (**c**) Cell viability assay showing that EMI7 does not affect the growth of PC9 EGFR ex19del/T790M/C797S triple mutant cells. Results are shown as the average ± SD for three independent experiments. (**d**) Viability assay showing that EMI7 does not affect the growth of PC9 EGFR ex19del/T790M/C797S organoids. Results are shown as single 36-point dose response experiments.



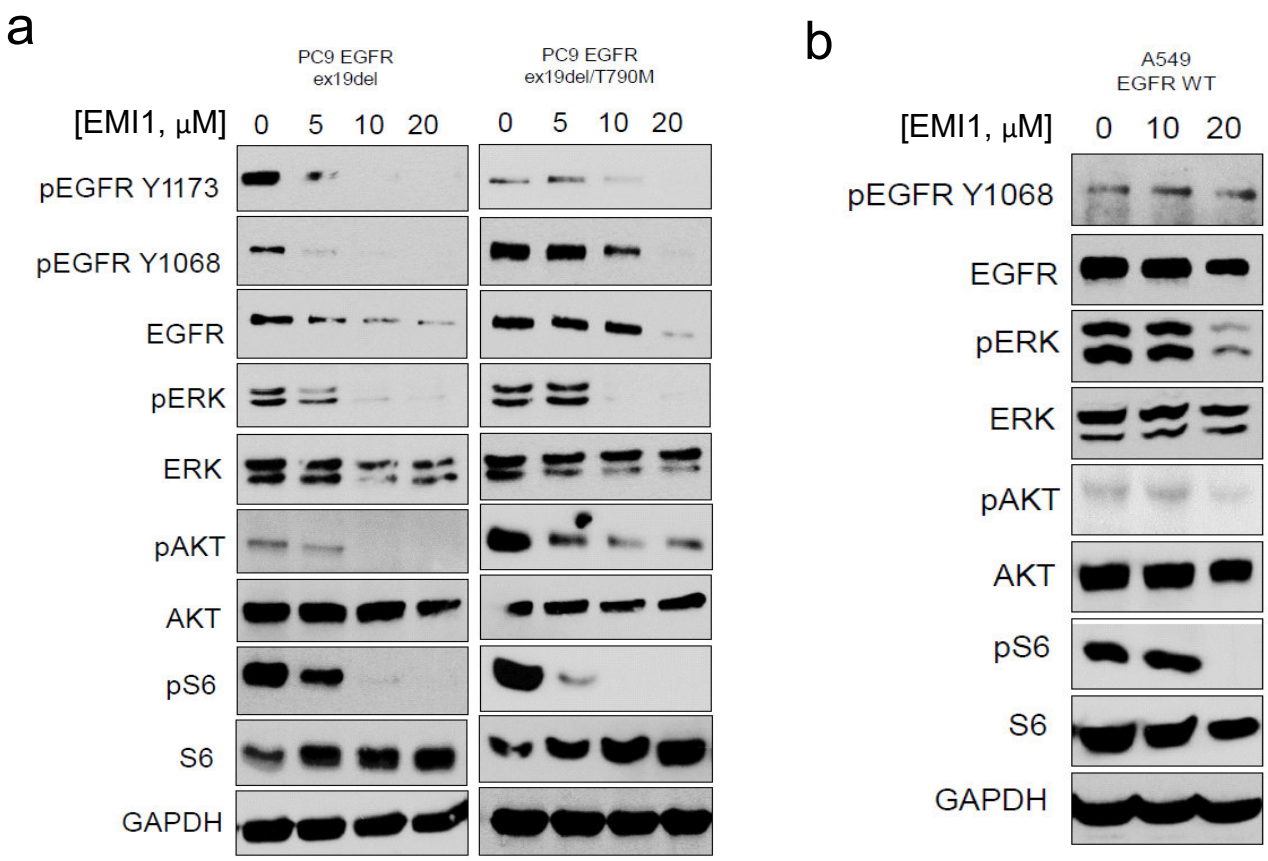
Supplementary Figure 11. Assessing the effect of EMI1 on microtubule dynamics in PC9 EGFR ex19del/T790M/C797S cells. EMI1 depolymerized interphase microtubules and perturbed spindle formation (inset). PC9 cells were incubated without (Control, upper panel) or with 1 µM EMI1 (lower panel) for 20 hours. Scale bar is 10 µm. Results are representative of at least two independent experiments



Supplementary Figure 12. Assessing the effect of EMI1 and EMI7 on microtubule dynamics *in vitro*. (a) Representative kymographs showing the effect of EMI1 and EMI7 on microtubule polymerization from GMPCPP stabilized microtubule seeds in the presence of plus-end binding protein EB3. (b) Quantification of microtubule growth rate. n = 60 in each case. (c) Quantification of microtubule growth time. n = 60 in each case. (d) Quantification of microtubule catastrophe frequency. Catastrophe frequencies were quantified as an inverted average growth time per microtubule. n = 20 in each case. EMI1 reduces microtubule growth and increases catastrophe frequency, while EMI7 does not. 2 independent sets of experiments. Results are shown as the average ± SD. P-value was calculated using the Mann-Whitney test, comparing treatment to DMSO control. ***P value <0.0001. ns, not significant.

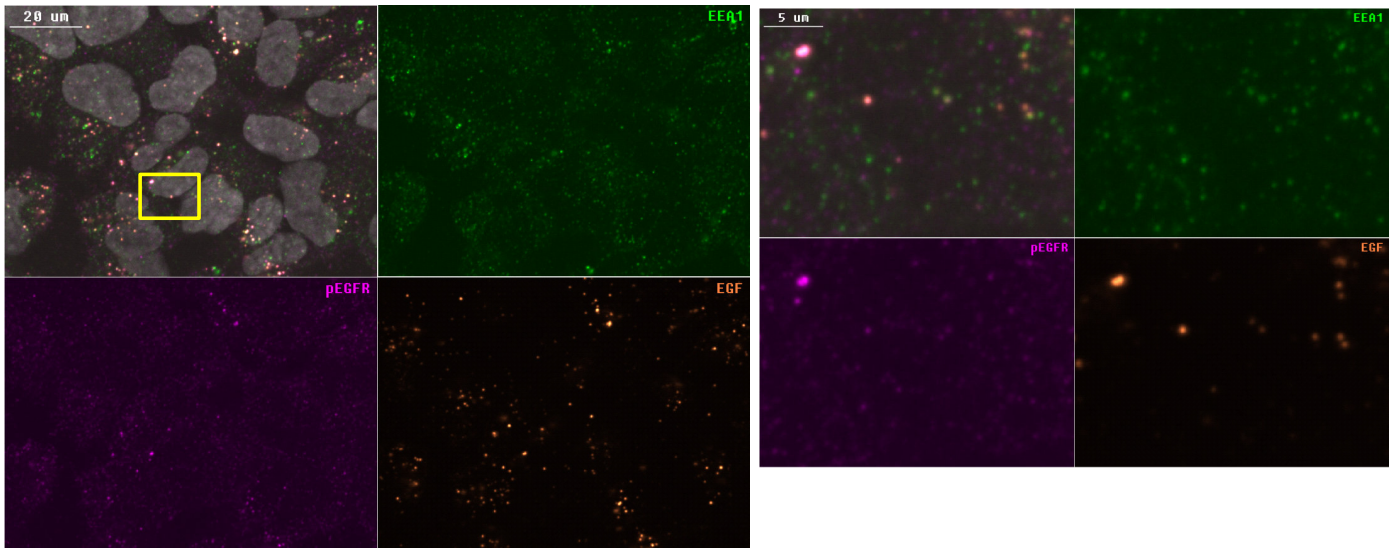


Supplementary Figure 13. MaMTH-DS assays showing that compound EMI1 is not a specific protein-protein interaction inhibitor. EMI1 at various concentrations (as indicated) reduces the interaction of EGFR L858R/T790M/C797S with Shc1, CrkII and Hsp90 in a dose-dependent manner. Osimertinib negative control, which does not target the triple mutant, has no effect at even high concentration. Data was normalized to DMSO control and shown as the average ± SD for three independent experiments.



Supplementary Figure 14. EMI1 induces mutant-EGFR degradation and impairs its signalling in NSCLC cells. (a) Effect of EMI1 on EGFR activation and downstream signalling in PC9 EGFR ex19del and PC9 EGFR ex19del/T790M cells. (c) Effect of EMI1 on EGFR activation and downstream signalling in A549 EGFR WT cells. Results are representative of at least two independent experiments. See Supplementary Fig 30 and 31 for source blot images.

DMSO



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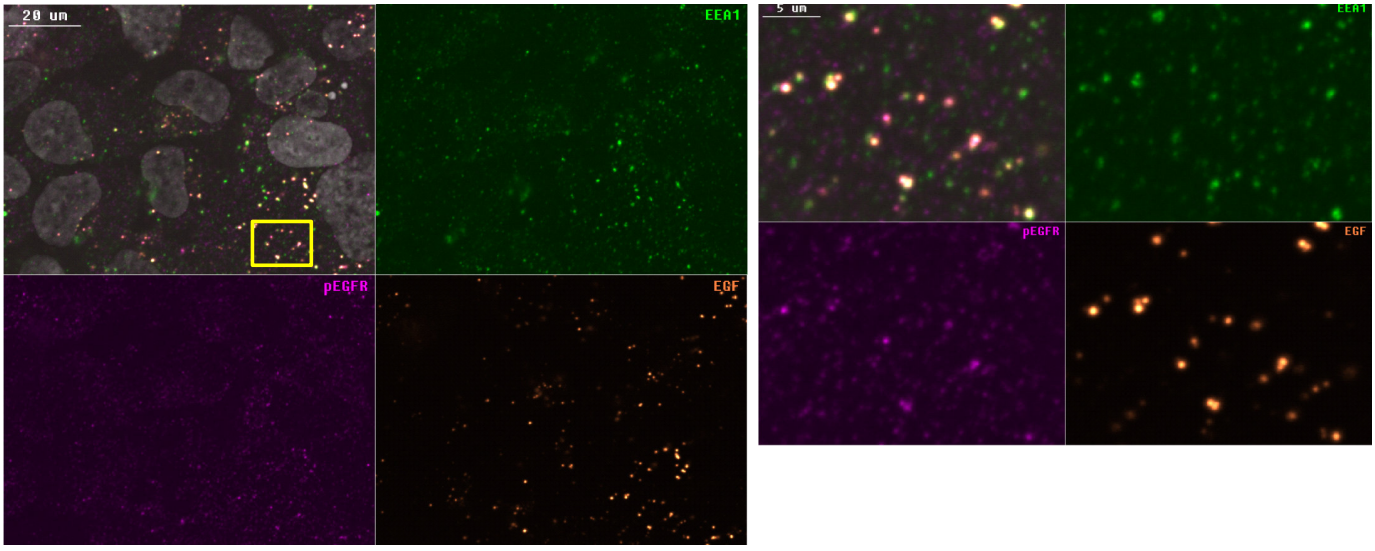
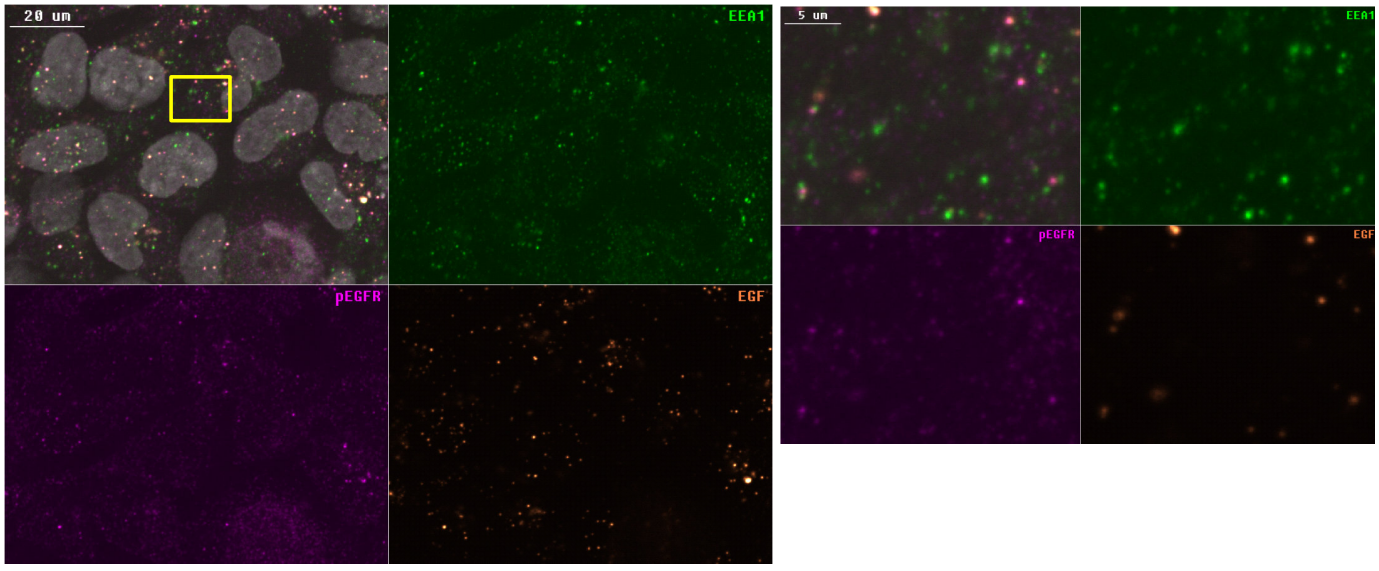
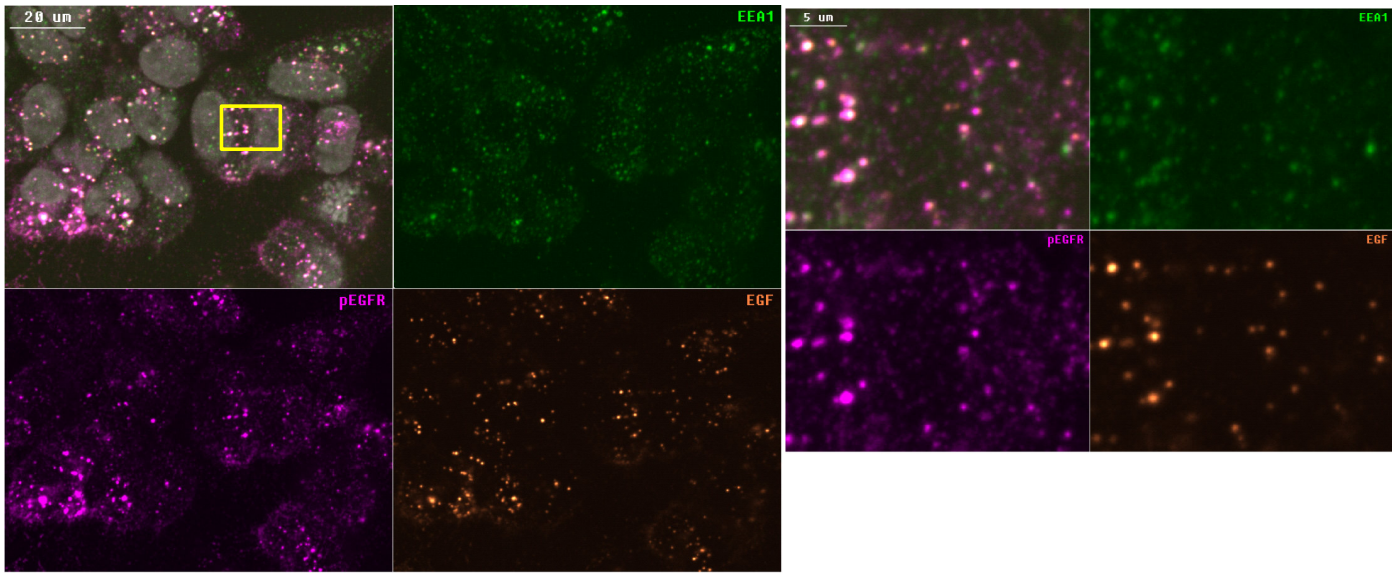


Midostaurin



Supplementary Figure 15. Investigating effect of EMI1 and Midostaurin on activated EGFR WT endosomal trafficking. Images in zoom show higher magnification (scale bar is 5 μm) of respresentative vesicular signals. Results are representative of at least two independent experiments.

DMSO



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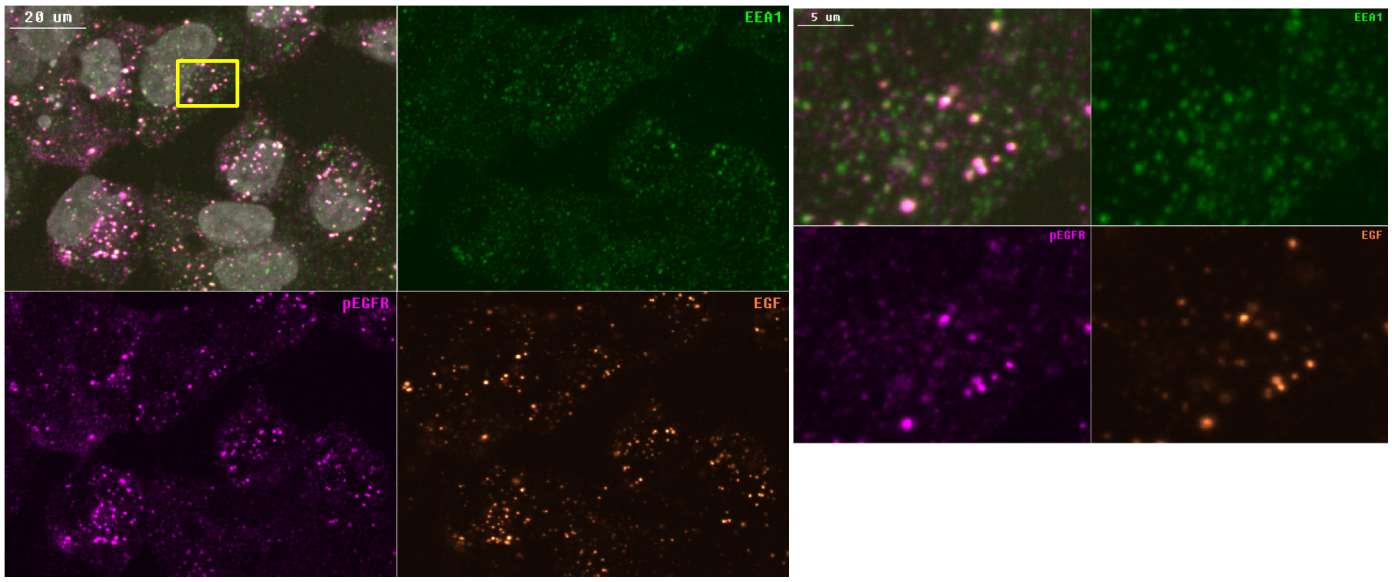

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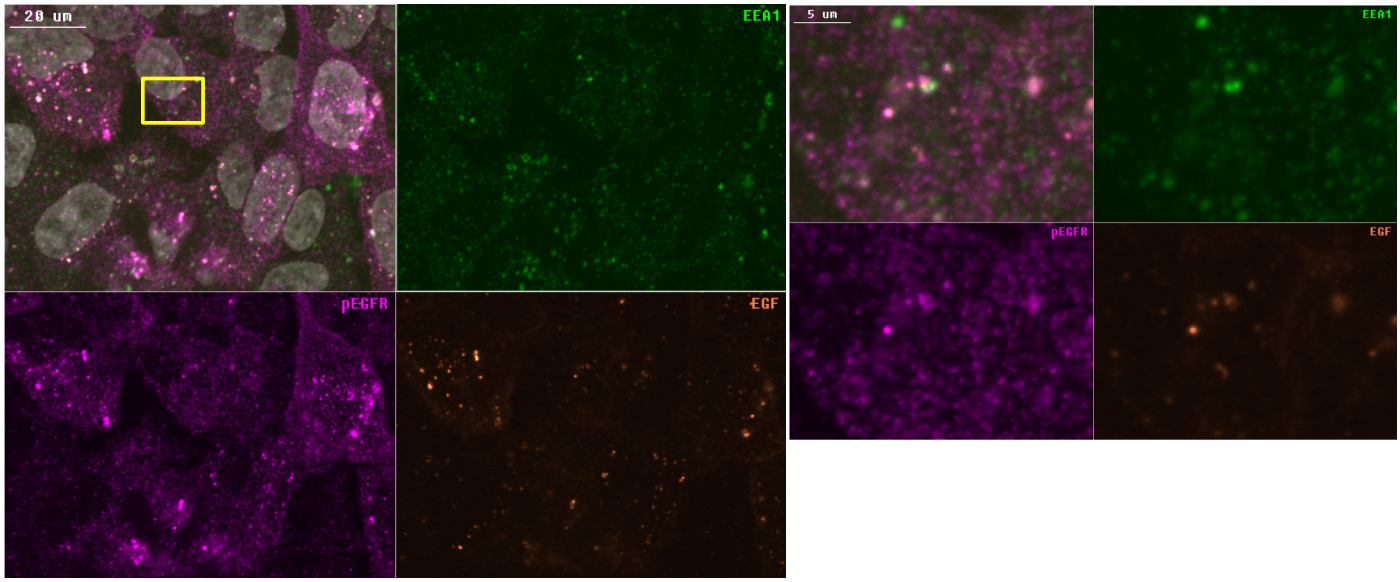
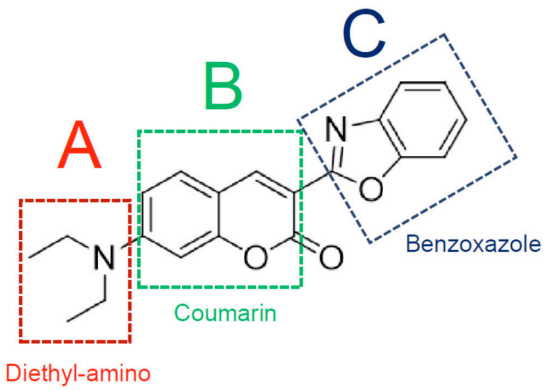
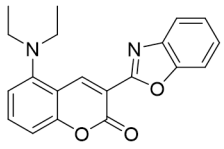


Supplementary Figure 16. Investigating effect of EMI1 and Midostaurin on activated EGFR L858R/T790M/C797S endosomal trafficking. Images in zoom show higher magnification (scale bar is 5 µm) of respresentative vesicular signals. Results are representative of at least two independent experiments.





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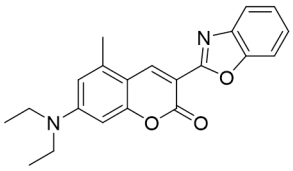

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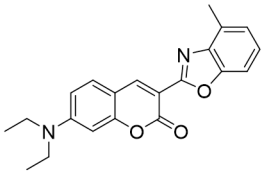

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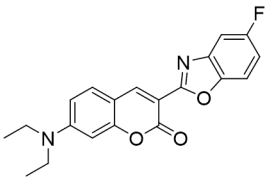

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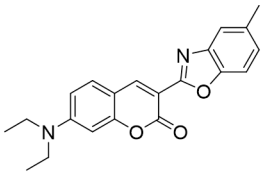

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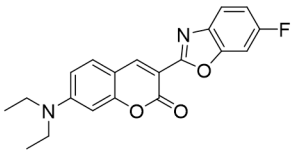

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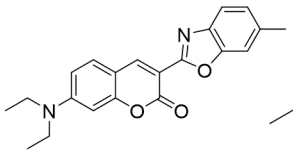

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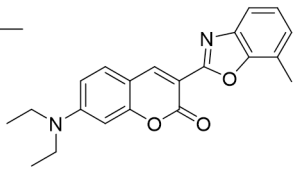

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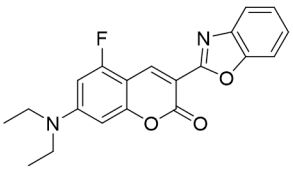

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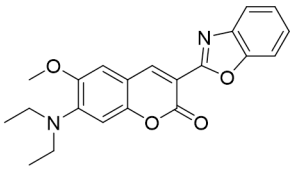

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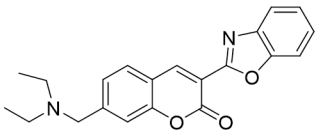

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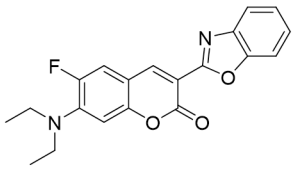

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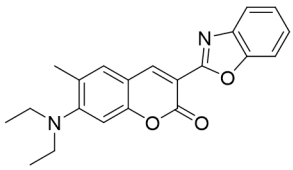

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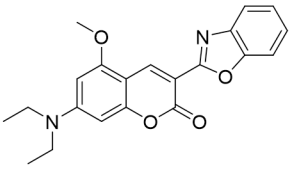

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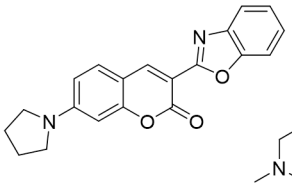

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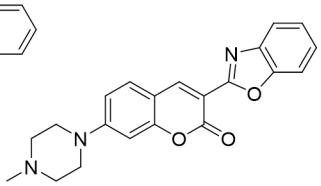
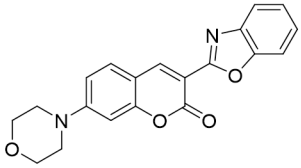
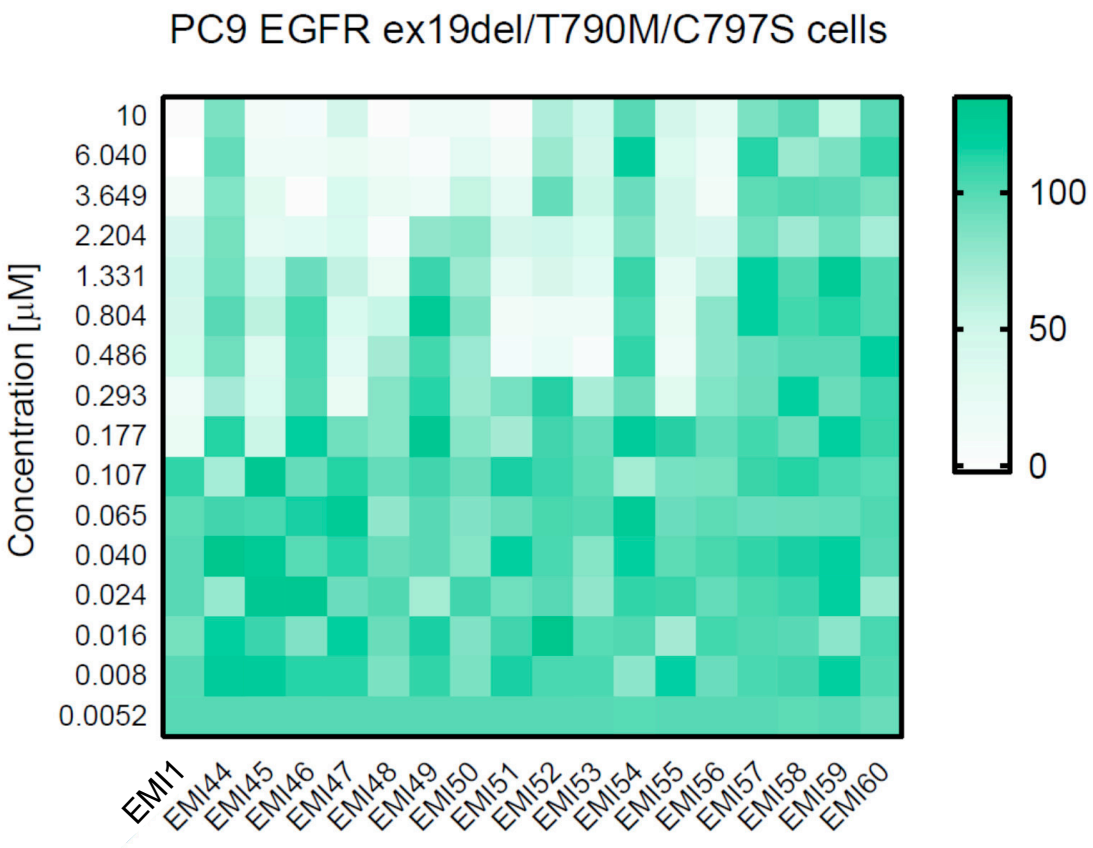


EMI59

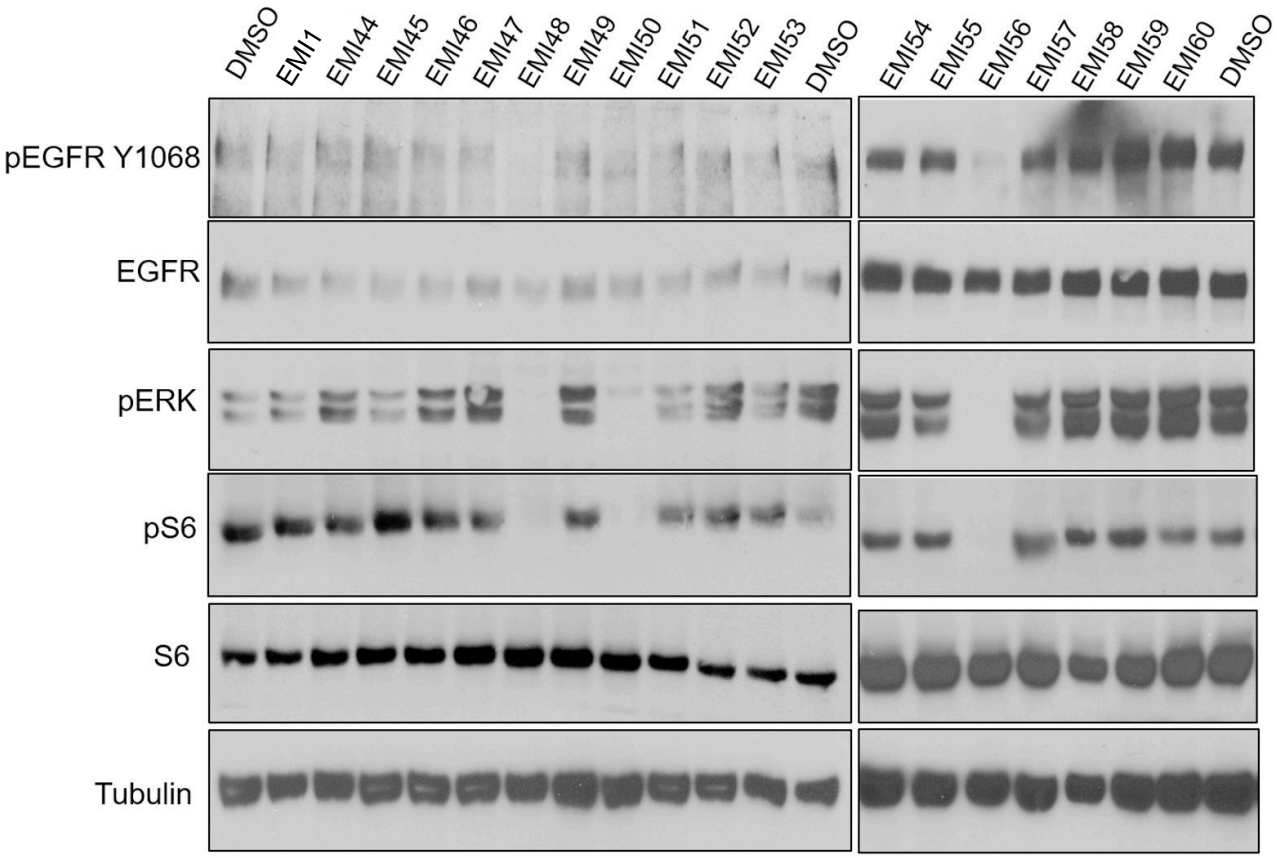


EMI60

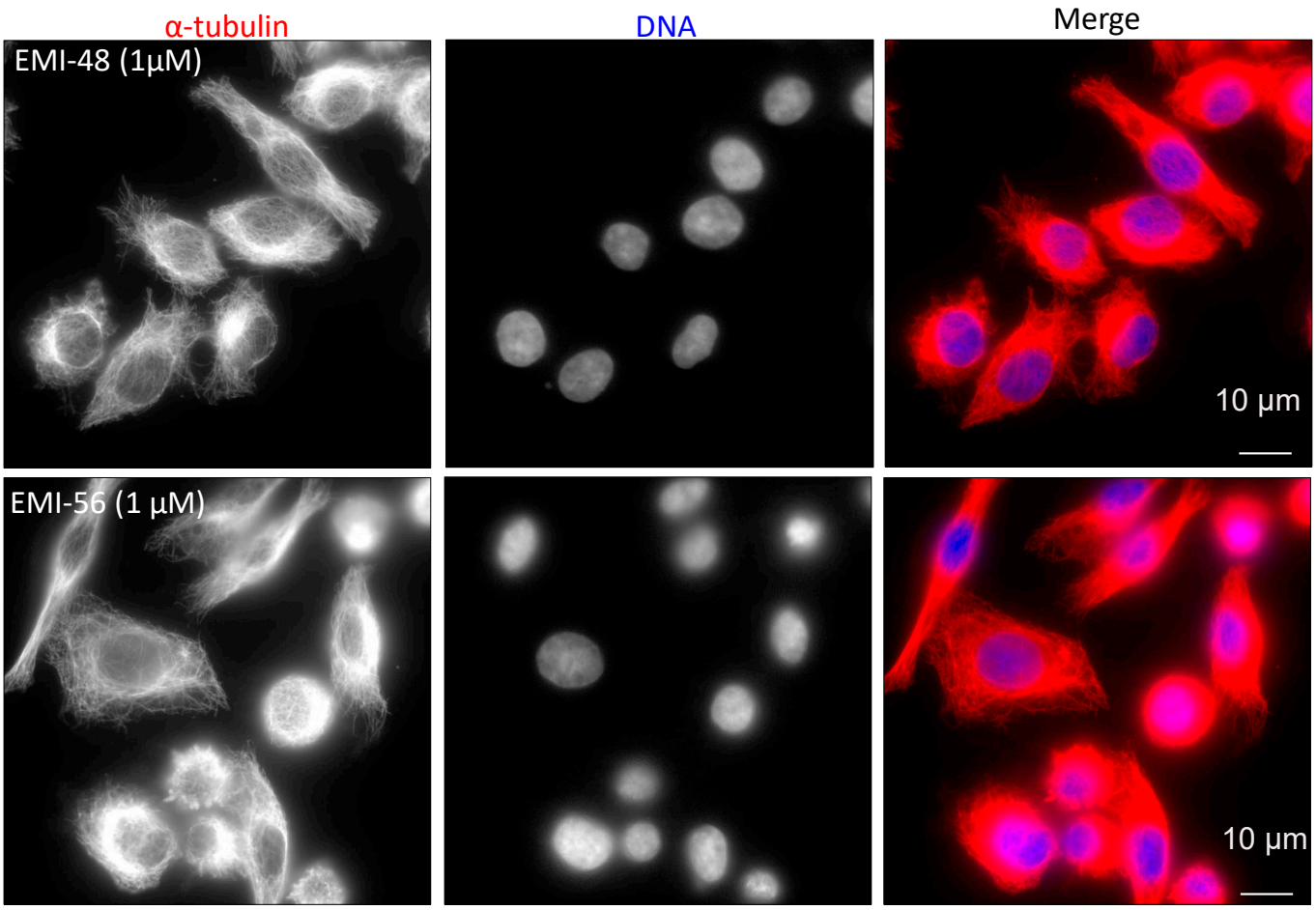
Supplementary Figure 17. Structures of 17 analogs of EMI1 generated using medicinal chemistry. Further information on medicinal chemistry synthesis schemes can be found in Supplementary Note 2. 1H NMR, 13C NMR and HRMS spectra are provided for EMI48 and EMI56 in Supplementary Note 2.



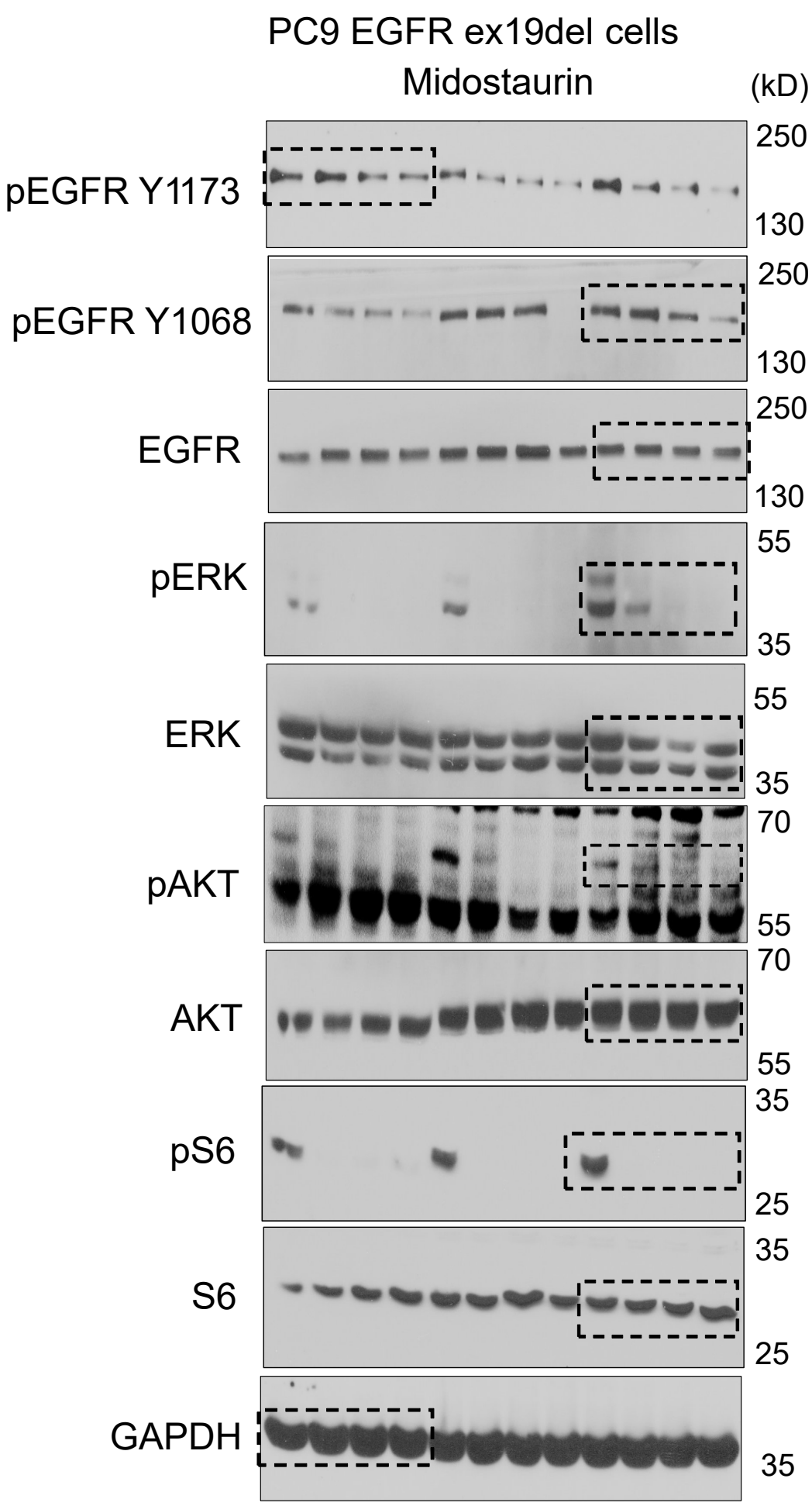
Supplementary Figure 18. Effect of EMI1 derivatives on viability of PC9 EGFR ex19del/T790M/C797S cells. Results are shown as single 16-point dose response experiments



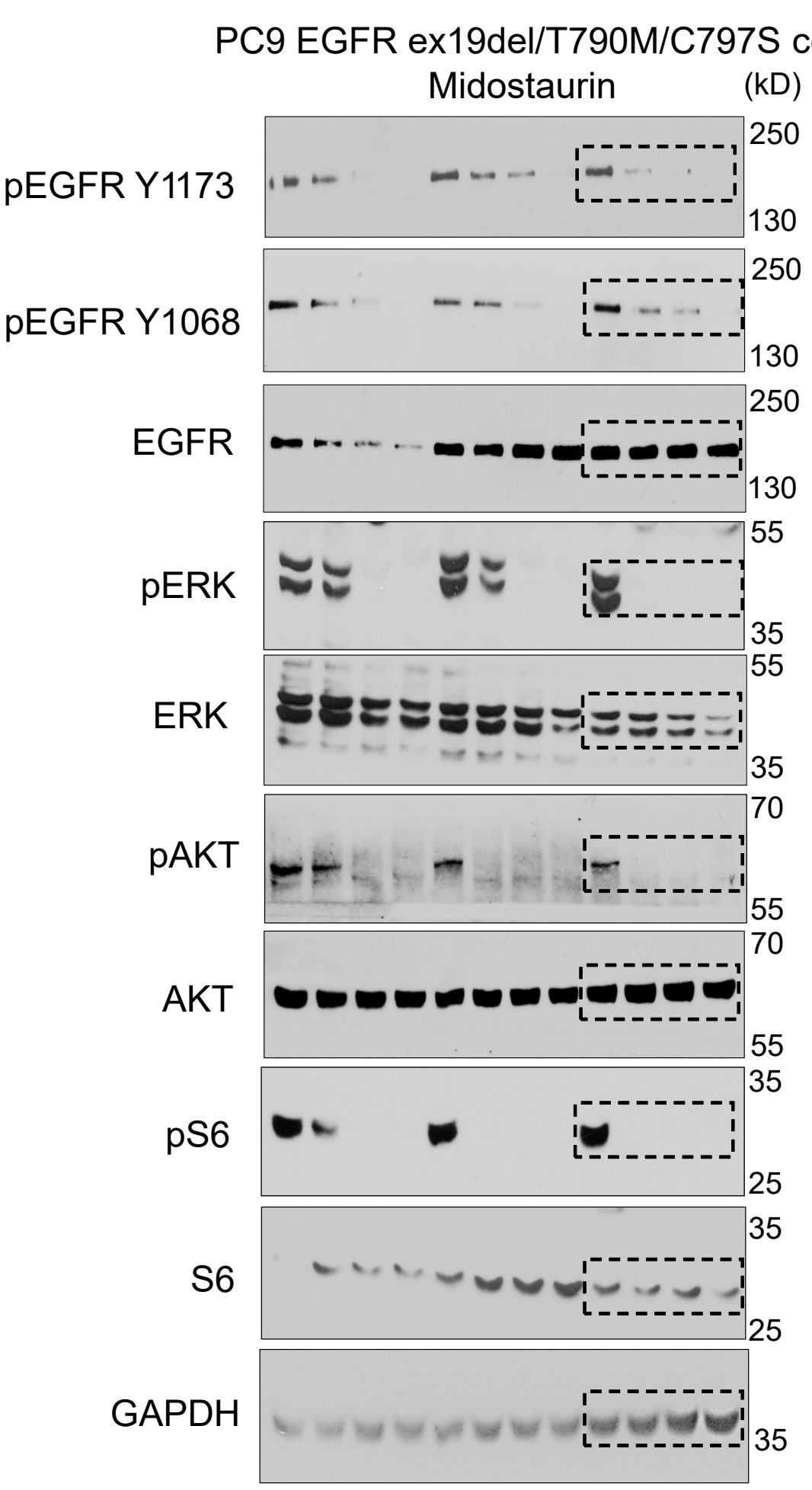
Supplementary Figure 19. Effect of EMI1 derivatives on EGFR activation and downstream signalling in PC9 EGFR ex19del/T790M/C797S cells. Results are representative of at least two independent experiments. See Supplementary Fig 32 for source blot images.



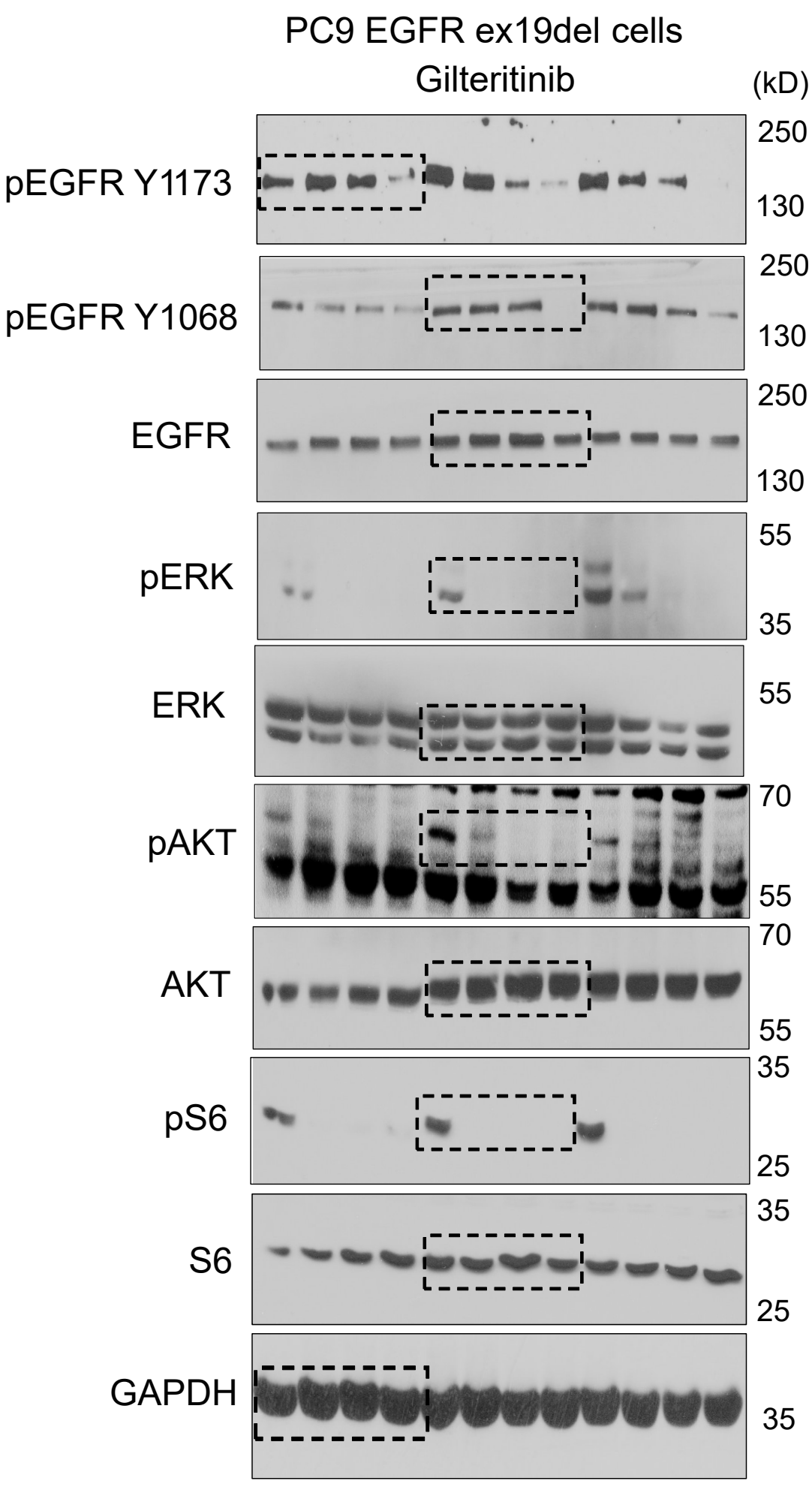
Supplementary Figure 20. Assessing the effect of EMI48 and EMI56 on microtubule dynamics in PC9 EGFR ex19del/T790M/C797S cells. Both analogs do not affect interphase microtubules and don't perturb spindle formation. PC9 cells were incubated with 1 μM EMI48 (upper panel) or EMI56 (lower panel) for 20 hours. Scale bar is 10 μm. Results are representative of at least two independent experiments.



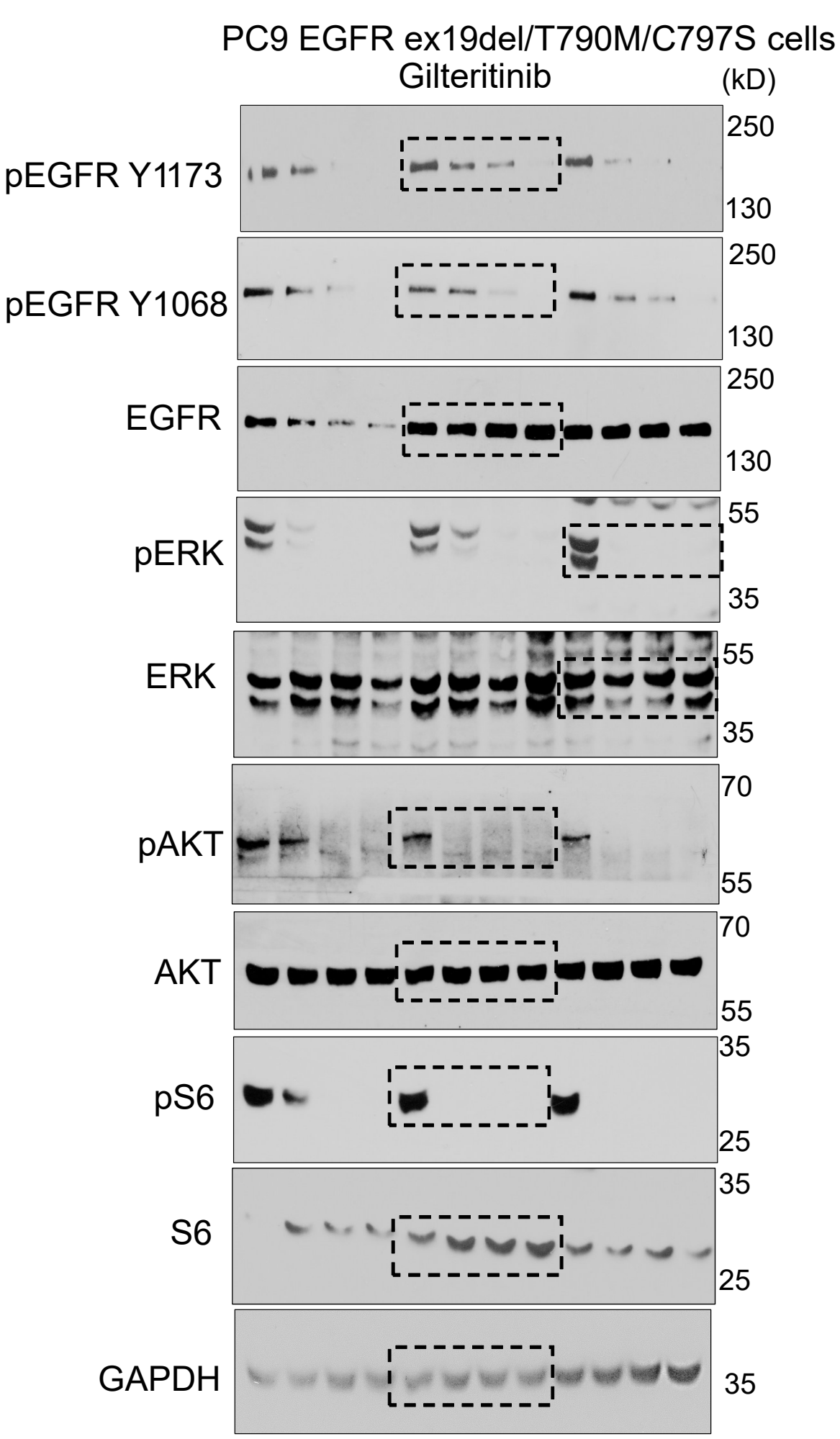
Supplementary Figure 21. Source blot images for Figure 2c Midostaurin treatment of PC9 ex19del cells.



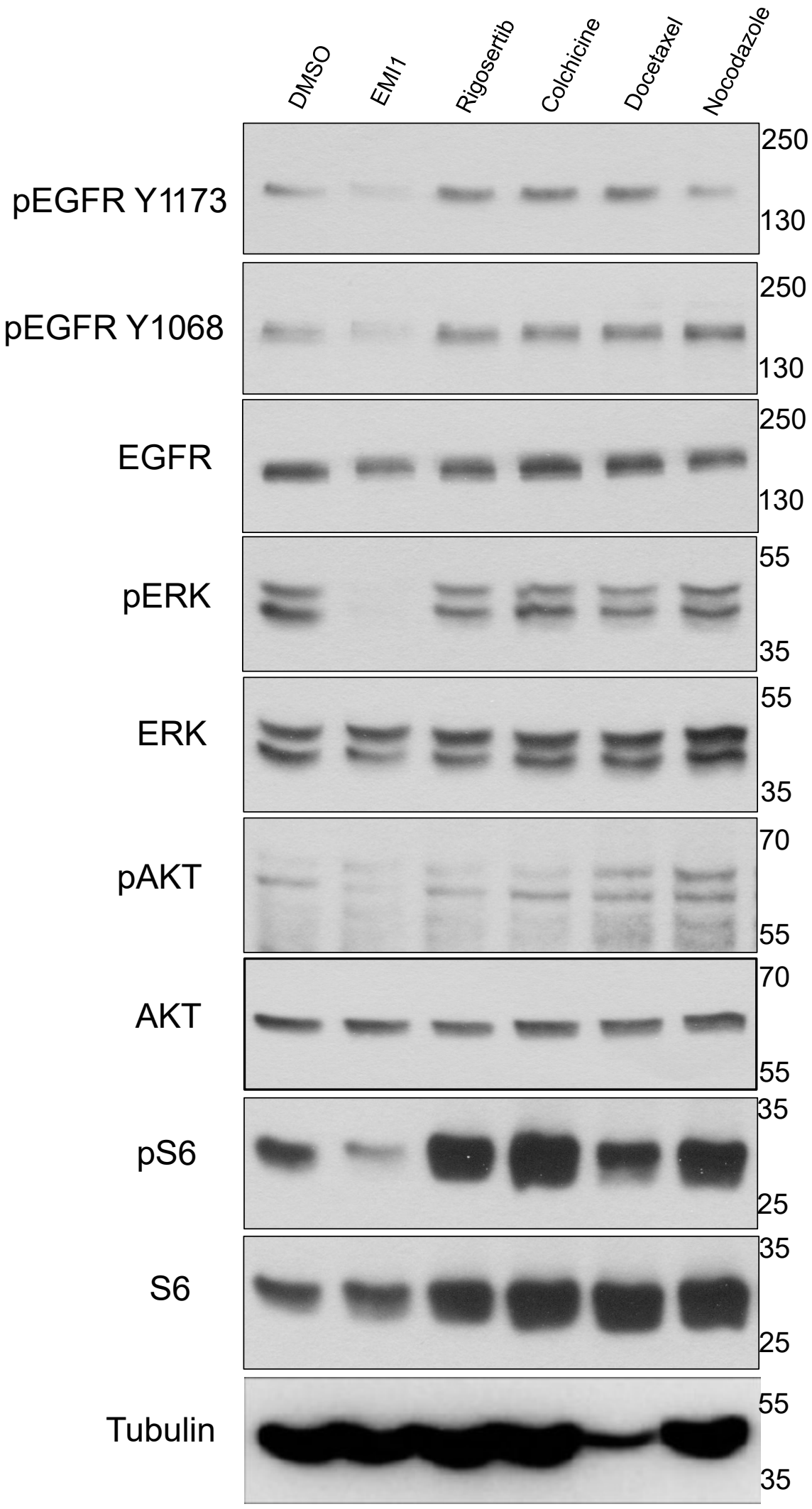
Supplementary Figure 22. Source blot images for Figure 2c Midostaurin treatment of PC9 ex19del/T790M/C797S cells.



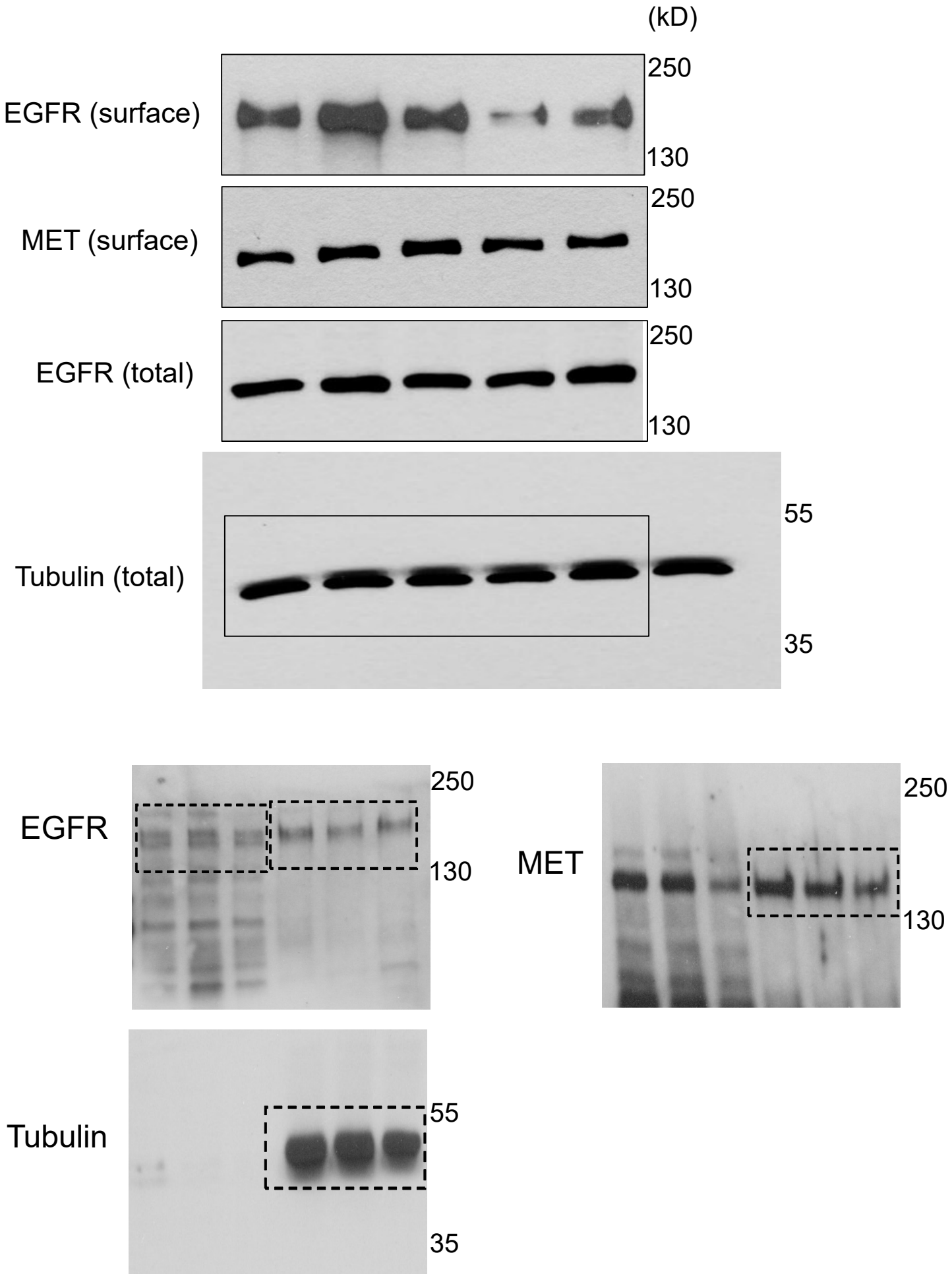
Supplementary Figure 23. Source blot images for Figure 2c Gilteritinib treatment of PC9 ex19del cells.



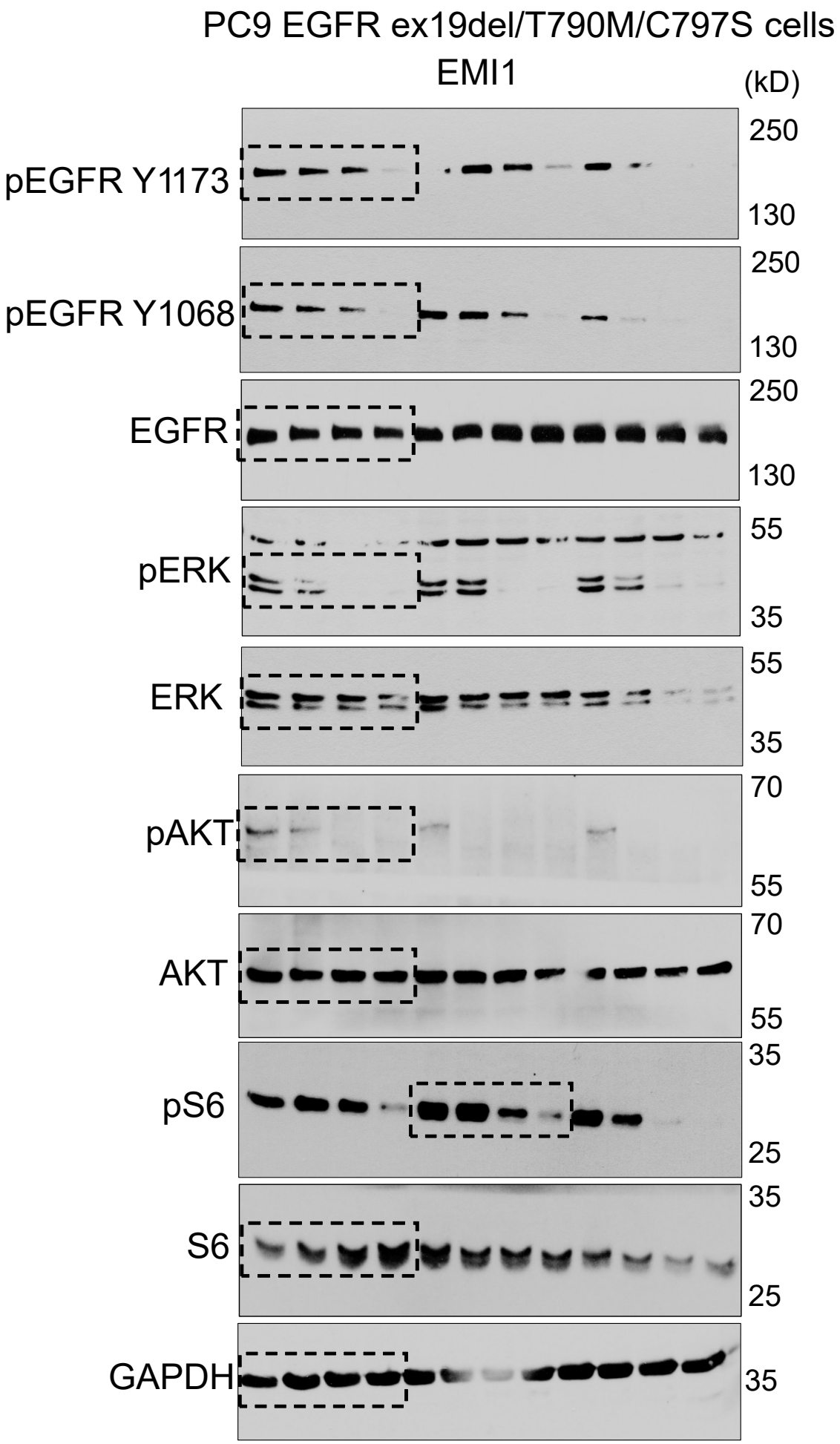
Supplementary Figure 24. Source blot images for Figure 2c gilteritinib treatment of PC9 ex19del/T790M/C797S cells.



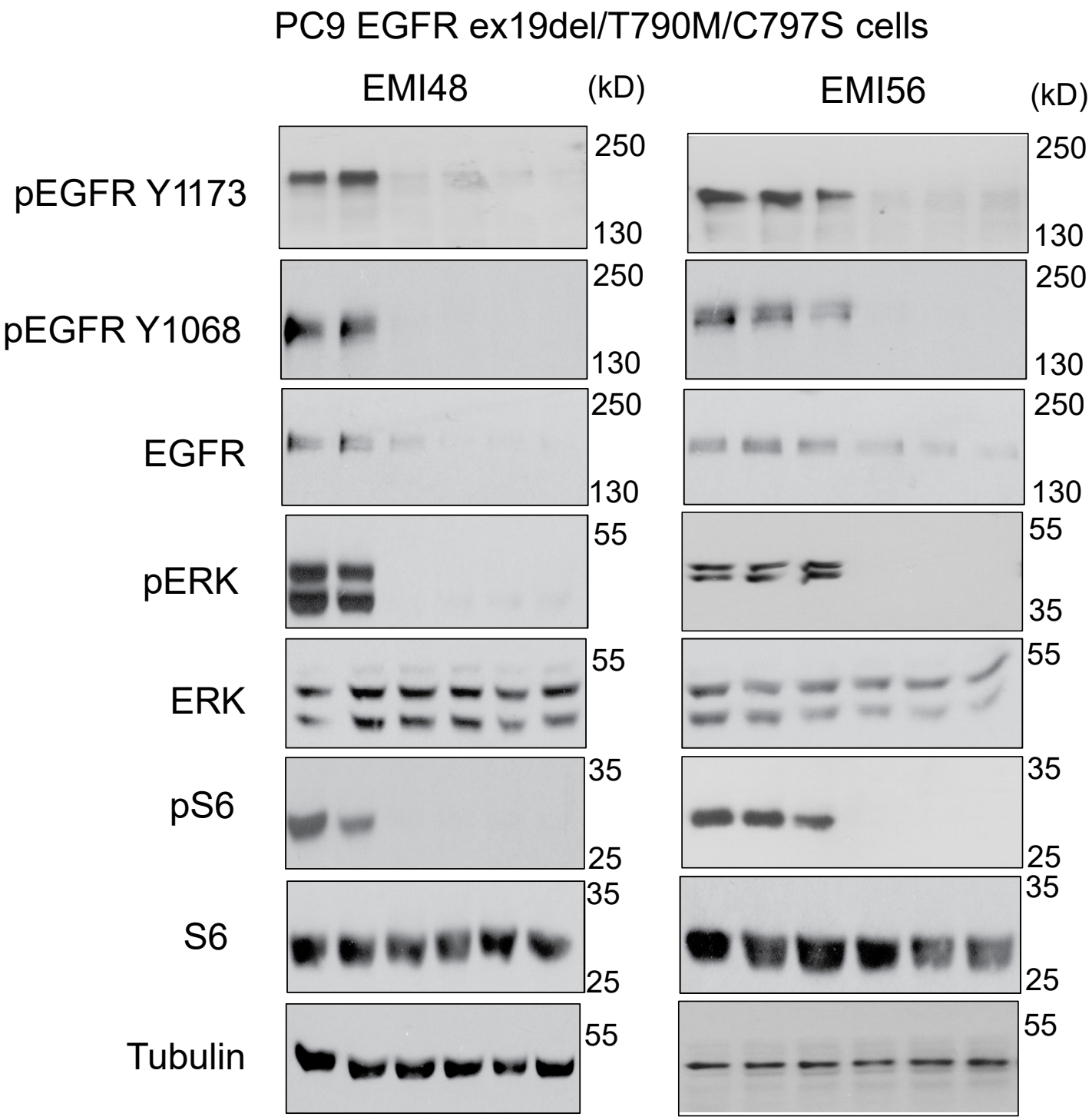
Supplementary Figure 25. Source blot images for Figure 3g.



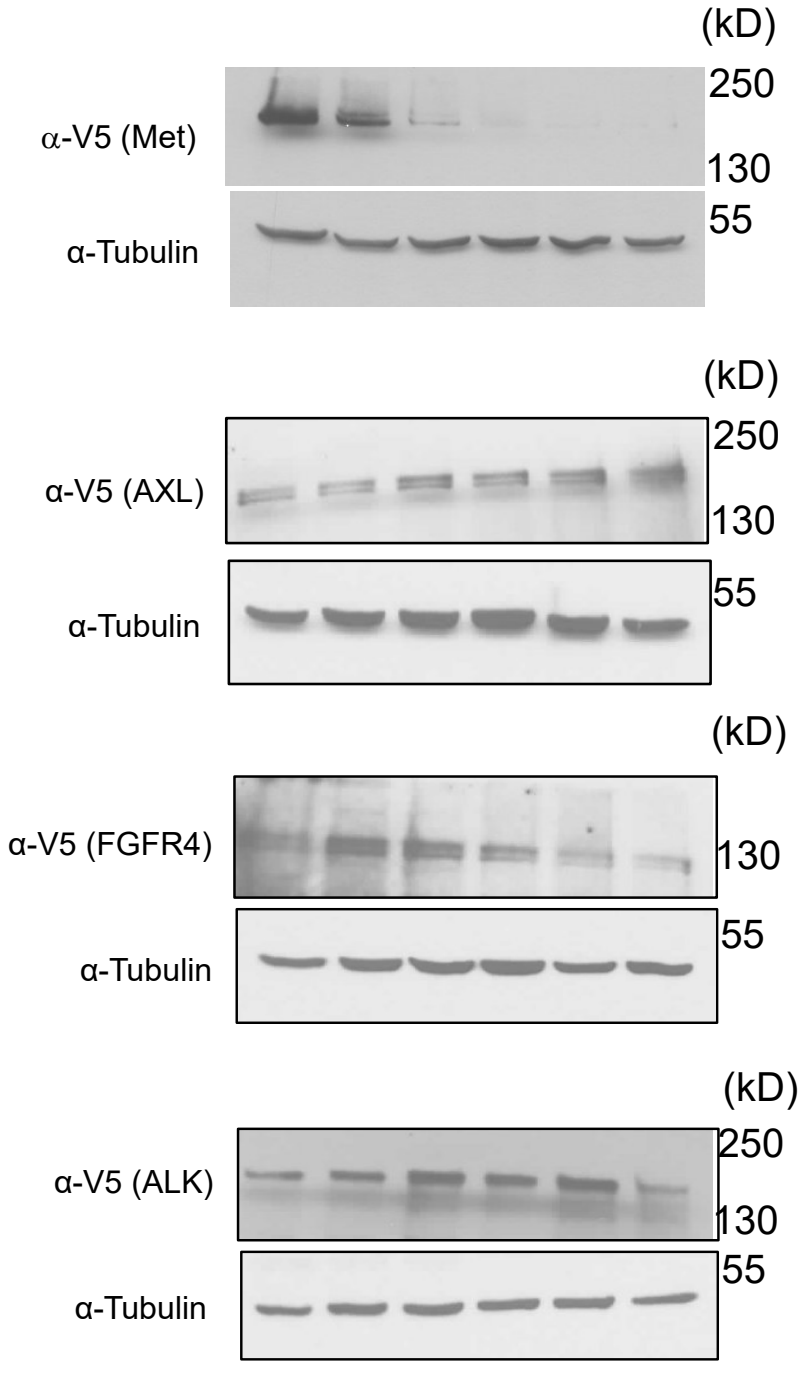
Supplementary Figure 26. Source blot images for Figure 4c and 4d.



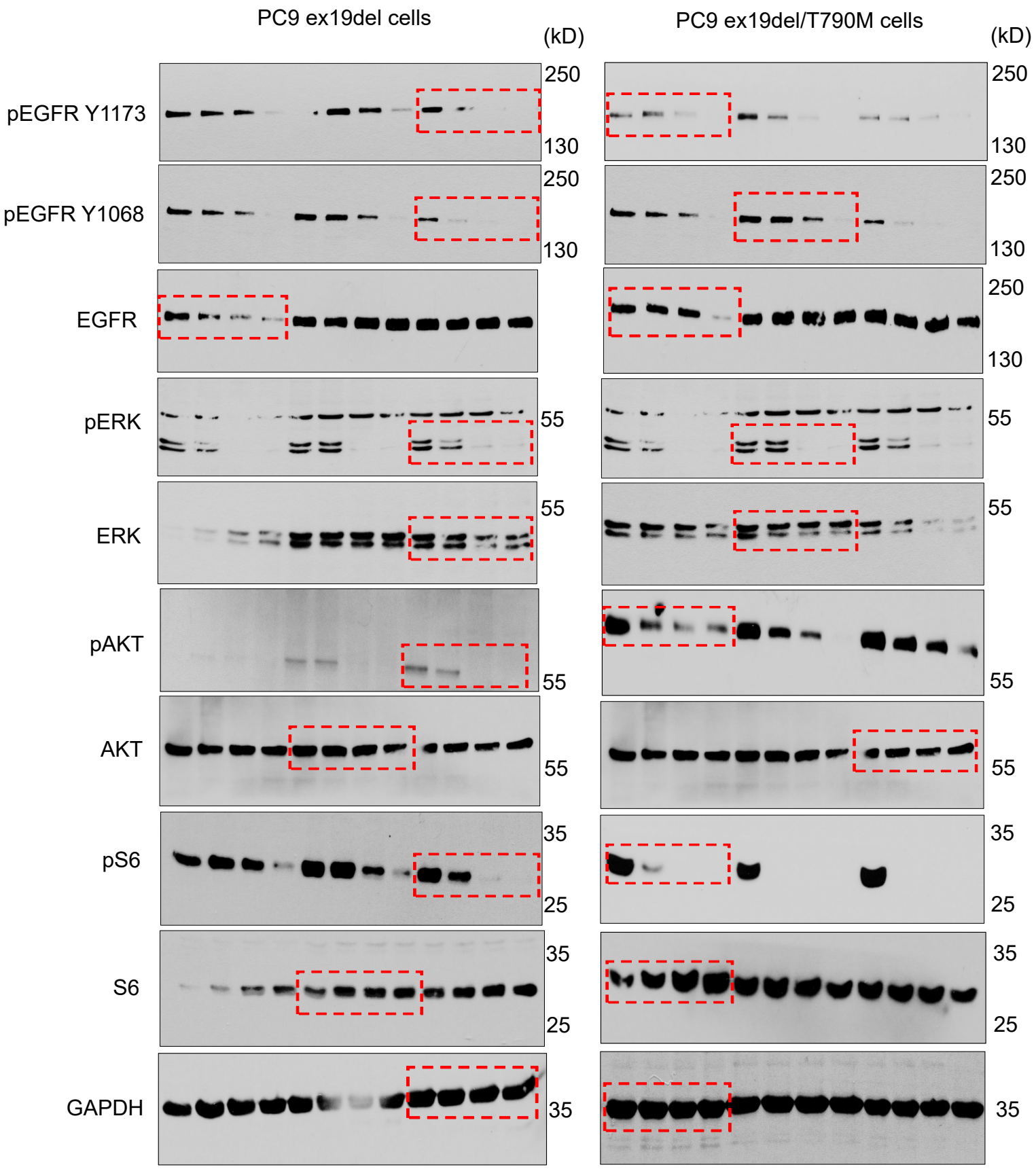
Supplementary Figure 27. Source blot images for Figure 5a..



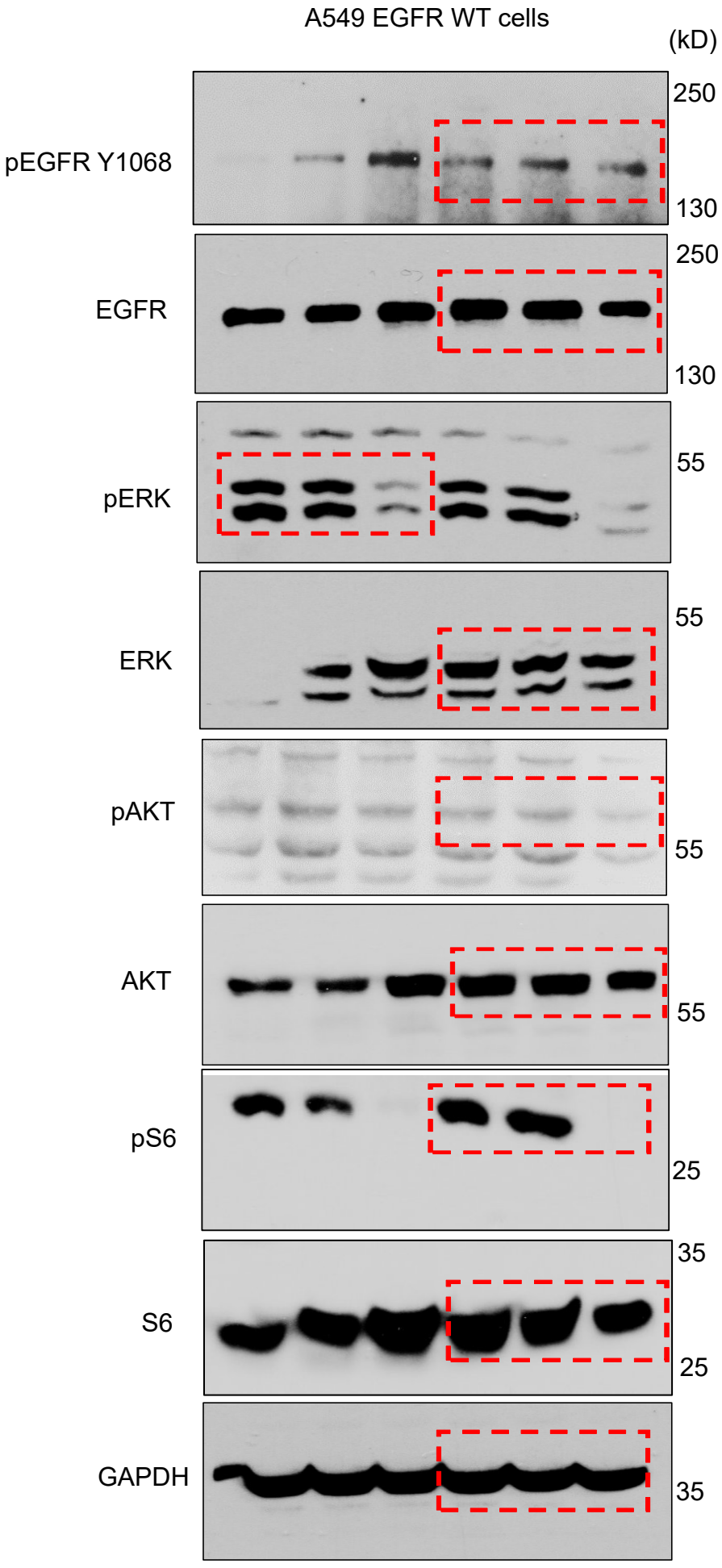
Supplementary Figure 28. Source blot images for Figure 5b and 5c.



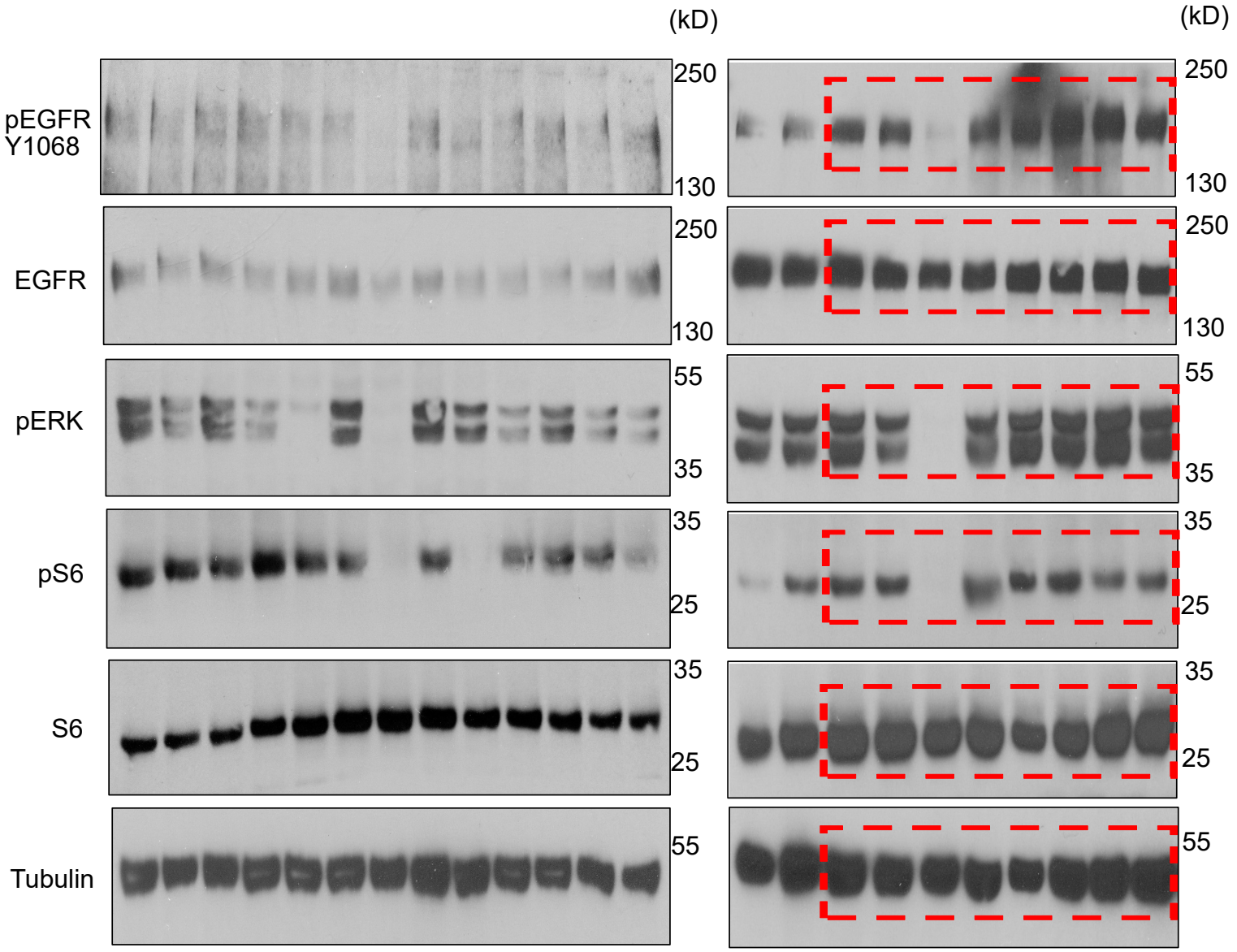
Supplementary Figure 29. Source blot images for Supplementary Figure 3 and 4..



Supplementary Figure 30. Source blot images for Supplementary Figure 14a.



Supplementary Figure 31. Source blot images for Supplementary Figure 14b.



Supplementary Figure 32. Source blot images for Supplementary Figure 19.

Supplementary Table 1. Specificity and reproduciblity testing of MaMTH-DS hits, identified in both rounds of screening, against EGFR WT and EGFR L858R/T790M/C797S baits in the presence of ShcI. Samples were run in triplicate (n=3).

		WT						C797S									
Compound.Source	Compound.ID	Luciferase Activity1.Percent	Luciferase Activity2.Percent	Luciferase Activity3.Percent	Average.Percent	Std.Dev.Percent	%CV.Percent	Luciferase Activity1.Percent	Luciferase Activity2.Percent	Luciferase Activity3.Percent	Average.Percent	Std.Dev.Percent	%CV.Percent	WT>50% Inhibition	C797S>50% Inhibition	p-Value	Fold.WT.C797S
OICR TKI Collection	PKC-412, CGP412	96.7	141.4	149.8	129.3	28.5	22.1	7.9	13.1	10.4	10.5	2.6	24.9	NO	YES	0.017991867	12.3
Chembridge Diverset	5213777	118	70.3	127.5	105.3	30.6	29.1	31.9	15.3	36.1	27.8	11	39.7	NO	YES	0.036213511	3.8
OICR TKI Collection	(5Z)-7-Oxozeaeno	85.8	70.9	83.9	80.2	8.1	10.1	17.7	17.6	19.7	18.3	1.2	6.6	NO	YES	0.004903998	4.4
OICR TKI Collection	CGP-74514A hydr	57.8	58.2	56.4	57.5	0.9	1.6	16.5	16.7	15.8	16.3	0.5	2.9	NO	YES	7.30E-06	3.5
OICR TKI Collection	AZD 7762, AZD-77	47.1	61.7	60.4	56.4	8.1	14.3	17.3	15.4	14.7	15.8	1.4	8.6	NO	YES	0.011228412	3.6
Maybridge HitFinder	BR00086SC	24.4	25.2	25.2	24.9	0.5	1.9	5.7	6.4	6.4	6.2	0.4	6.9	YES	YES	9.30E-07	4
Maybridge HitFinder	SPB01851SC	18.4	30	25.2	24.5	5.9	23.8	5.6	7.5	4.2	5.8	1.6	28.1	YES	YES	0.024156361	4.3
Maybridge HitFinder	SEW06379SC	21.8	22.4	25.7	23.3	2.1	8.9	14	18.9	18.3	17.1	2.6	15.5	YES	YES	0.035165788	1.4
Chembridge Diverset	5304079	24.4	18.4	21.6	21.5	3	13.9	13.9	13.8	7.4	11.7	3.7	31.8	YES	YES	0.025512837	1.8
OICR TKI Collection	Hesperadin	12.9	25	21.9	19.9	6.3	31.7	2.4	7	3.9	4.5	2.3	52.6	YES	YES	0.038481202	4.5
Maybridge HitFinder	BTB08928SC	15.3	18.7	17.8	17.3	1.8	10.4	4.8	5.9	5.3	5.3	0.5	9.9	YES	YES	0.004433972	3.2
Chembridge Diverset	5106405	12.5	18.3	16.8	15.8	3	18.8	1.9	4	2.3	2.7	1.1	41.6	YES	YES	0.009345886	5.8
Maybridge HitFinder	S14919SC	16.7	11.7	12.8	13.7	2.7	19.4	2.7	1.7	4.3	2.9	1.3	44.9	YES	YES	0.008764106	4.8
OICR TKI Collection	IRAK4 Inhibitor	14.3	11.3	9	11.5	2.7	23.3	4.9	4.1	4.9	4.6	0.5	9.8	YES	YES	0.043699193	2.5
OICR TKI Collection	PF-3758309, PF-0	6.7	9.8	6.4	7.6	1.9	24.4	1	0.7	1.1	0.9	0.2	25.2	YES	YES	0.023346445	8.1
Chembridge Diverset	5357830	8.5	5.4	4.6	6.2	2	33.1	1.2	0.8	0.9	0.9	0.2	22	YES	YES	0.046079898	6.6
Chembridge Diverset	5274945	4.6	4.7	3.8	4.4	0.5	12	0.4	0.2	0.5	0.4	0.1	33.5	YES	YES	0.004005023	12
Maybridge HitFinder	KM08160SC	5.4	4	2.8	4.1	1.3	32	1	0.4	0.4	0.6	0.3	54	YES	YES	0.037344862	6.6
OICR TKI Collection	Staurosporine	2.7	1.9	2.2	2.3	0.4	19.6	0.8	0.2	0.4	0.5	0.3	71.5	YES	YES	0.006113406	5
Chembridge Diverset	5238658	1.1	0.7	0.9	0.9	0.2	26.7	0.3	0.1	0.2	0.2	0.1	56.1	YES	YES	0.022392801	4.4
OICR TKI Collection	Compound 71	3.7	9	6.8	6.5	2.7	40.9	1	0.2	0.4	0.5	0.4	74.7	YES	YES	0.057112926	12.4
OICR TKI Collection	PIK-75 hydrochlor	4.5	8.3	3.7	5.5	2.5	45.2	0.6	0.3	0.1	0.3	0.2	65.6	YES	YES	0.068205877	16.3
OICR TKI Collection	A-443654, A-654	12	5.6	4.3	7.3	4.1	56.7	0.3	0.3	0.2	0.3	0.1	25.9	YES	YES	0.098676818	26.6
Maybridge HitFinder	CD03862SC	6.4	2.9	6.2	5.2	2	38.5	2.4	1.5	3.2	2.4	0.8	35.3	YES	YES	0.120873261	2.2
Chembridge Diverset	5109882	17.2	14	13.1	14.8	2.2	14.6	12.5	10.7	12.4	11.9	1	8.5	YES	YES	0.1303195	1.2
Maybridge HitFinder	JA00113SC	2.3	1.9	6	3.4	2.3	66.5	0.5	0.3	0.3	0.4	0.1	32.1	YES	YES	0.146340884	8.9
OICR TKI Collection	Alvocidib, HMR-12	2.7	2.2	8.4	4.4	3.4	77.9	0.3	0.2	0.5	0.3	0.2	55.4	YES	YES	0.175813511	13.1
OICR TKI Collection	Dinaciclib, MK-79	4.2	13	2.2	6.5	5.7	88.4	0.3	0.3	0.3	0.3	0	7.5	YES	YES	0.203901998	20
Chembridge Diverset	5792598	79	96	77.7	84.2	10.2	12.2	81.5	138.6	124.5	114.8	29.8	25.9	NO	NO	0.209695268	0.7
Chembridge Diverset	5333931	43.6	47.2	46.1	45.6	1.8	3.9	45.9	38.6	41.5	42	3.7	8.7	YES	YES	0.221411804	1.1
OICR TKI Collection	PHA-767491 hydr	11.5	12	10.9	11.5	0.5	4.6	10.8	16.7	16.1	14.5	3.2	22.3	YES	YES	0.239198175	0.8
OICR TKI Collection	LY-2606368	23.67	38.64	37.98	33.43	8.46	25.3	38.67	39.01	37.03	38.24	1.06	2.77	YES	YES	0.429149913	0.9
Maybridge HitFinder	SEW02515SC	8.5	5	6.4	6.7	1.8	26.5	7.4	5.2	6.6	6.4	1.1	17.4	YES	YES	0.830386676	1
Chembridge Diverset	5270140	14	3.8	7	8.3	5.2	62.7	9.4	4.3	12.2	8.6	4	46.5	YES	YES	0.931181228	1

Supplementary Table 2. Inhibition of oncogenic EGFR mutants by Midostaurin and Gilteritinib

Enzyme (IC_{50}, nM)	Midostaurin	Gilteritinib
EGFR WT	> 10,000	450
EGFR L858R	545.6	358.5
EGFR ex19del	616.5	257.7
EGFR L858R/T790M	15.2	179.1
EGFR ex19del/T790M	33.6	92.2
EGFR L858R/T790M/C797S	29.1	58.9
EGFR ex19del/T790M/C797S	5.2	2.45
IC50: 50% inhibition concentration. The IC50 was calculated from data collected at concentrations ranging from 0.05 nM to 10 μM (n = 2).		

Supplementary Datasets

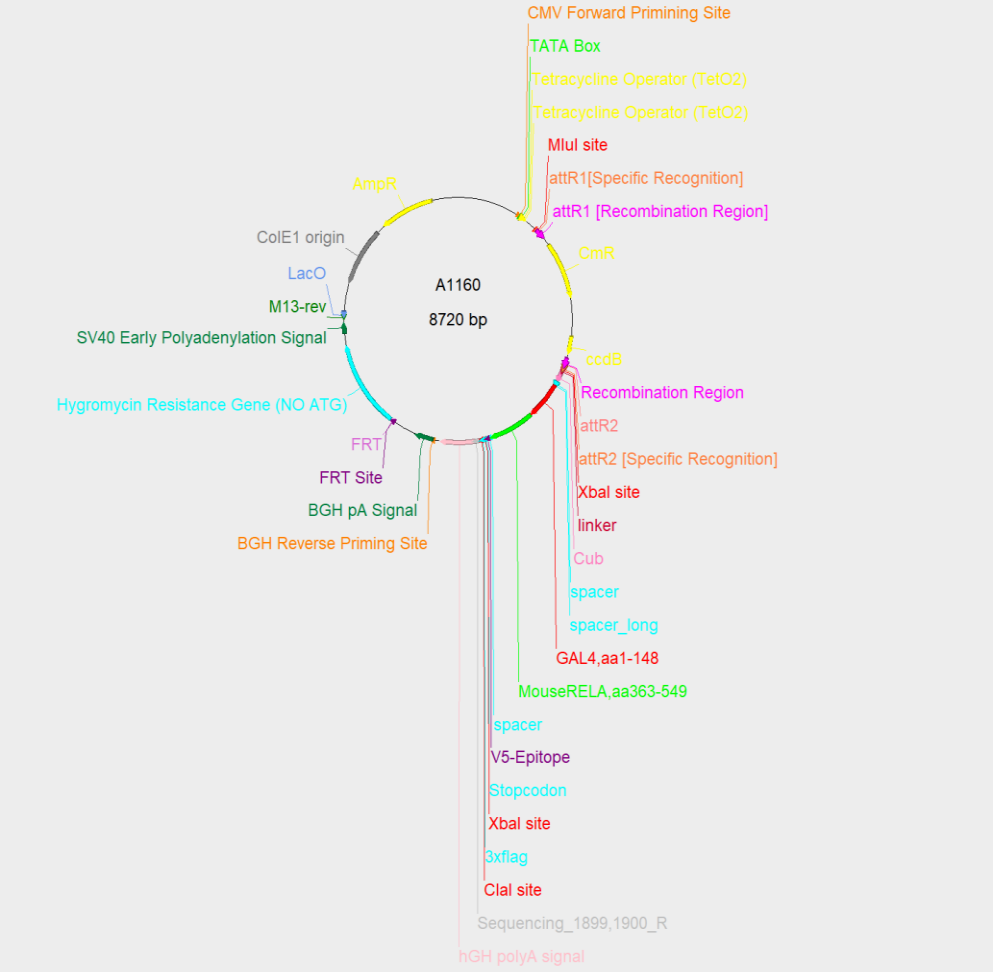
Supplementary Dataset 1: Collection of compounds used in MaMTH-DS pilot screening.

Supplementary Dataset 2: Results of MaMTH-DS screening of EGFR L858R/T790M/C797S in the presence of ShcI (Round 1). A total of 2960 compounds were tested in singlicate (n=1). DMSO and AZD9291 controls were tested at n=16.

Supplementary Dataset 3: Results of MaMTH-DS screening of EGFR L858R/T790M/C797S in the presence of ShcI (Round 2). A total of 2960 compounds were tested in singlicate (n=1). DMSO and AZD9291 controls were tested at n=16.

Supplementary Note 1. MaMTH-DS bait and prey vector sequences and maps

A1160 MaMTH ‘bait’ vector map and sequence



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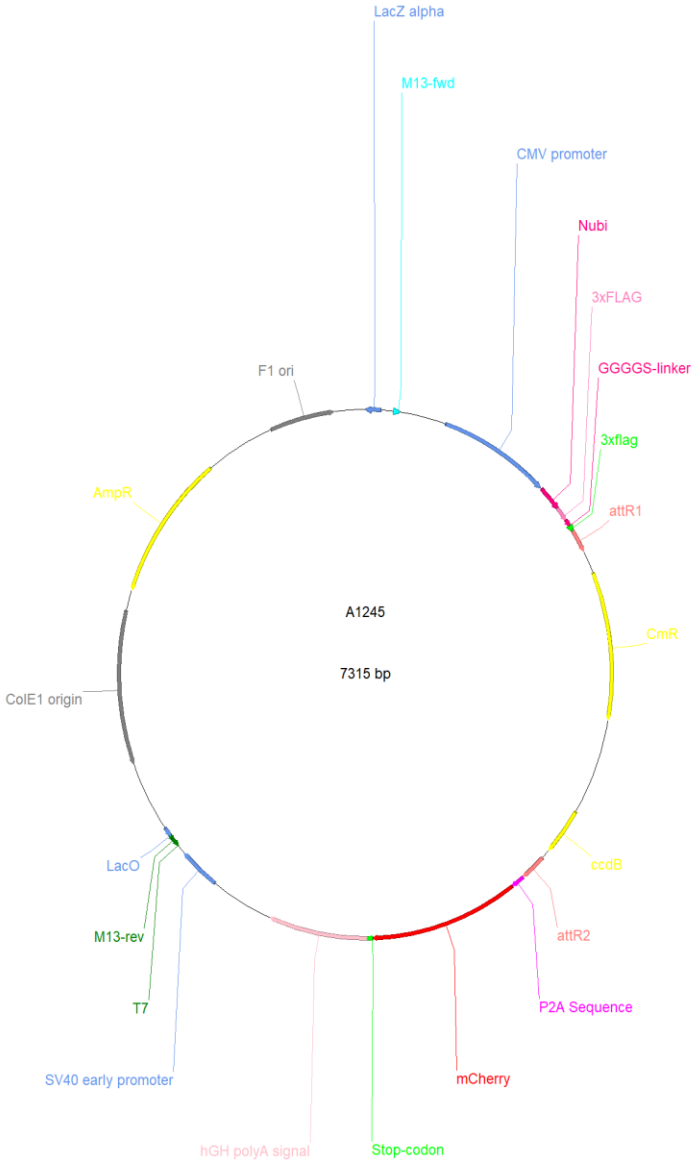
A1245 MaMTH ‘prey’ vector map and sequence



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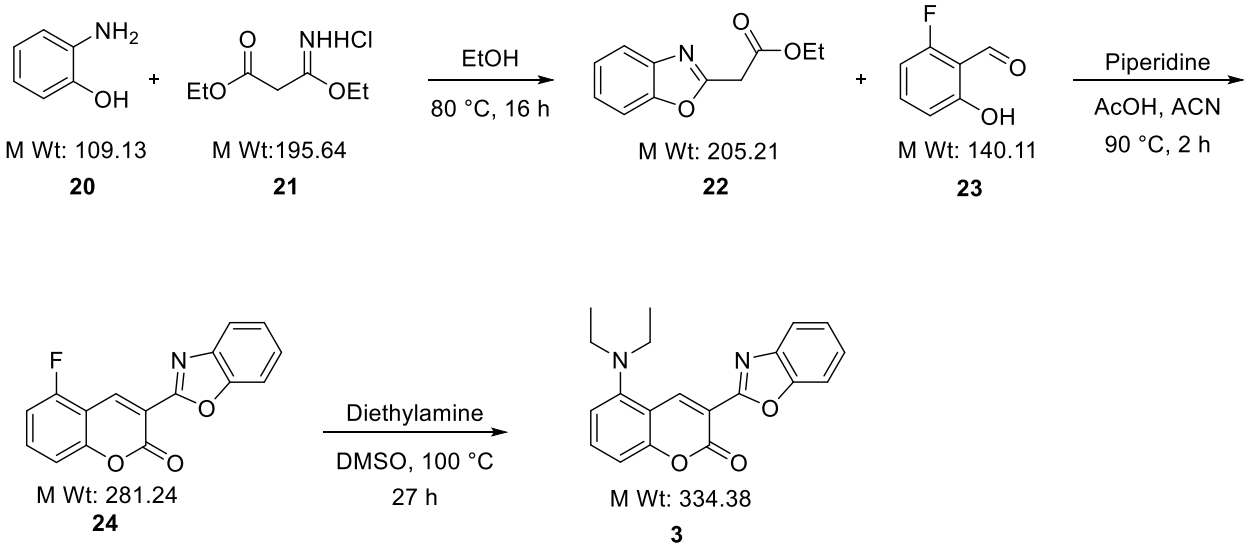
Supplementary Note 2: Synthetic procedures

EMI1 (1) was purchased from InterBioScreen Ltd., with a purity greater than 95%.

EMI7 (2) was purchased from Life Chemicals Inc., with a purity greater than 99%.

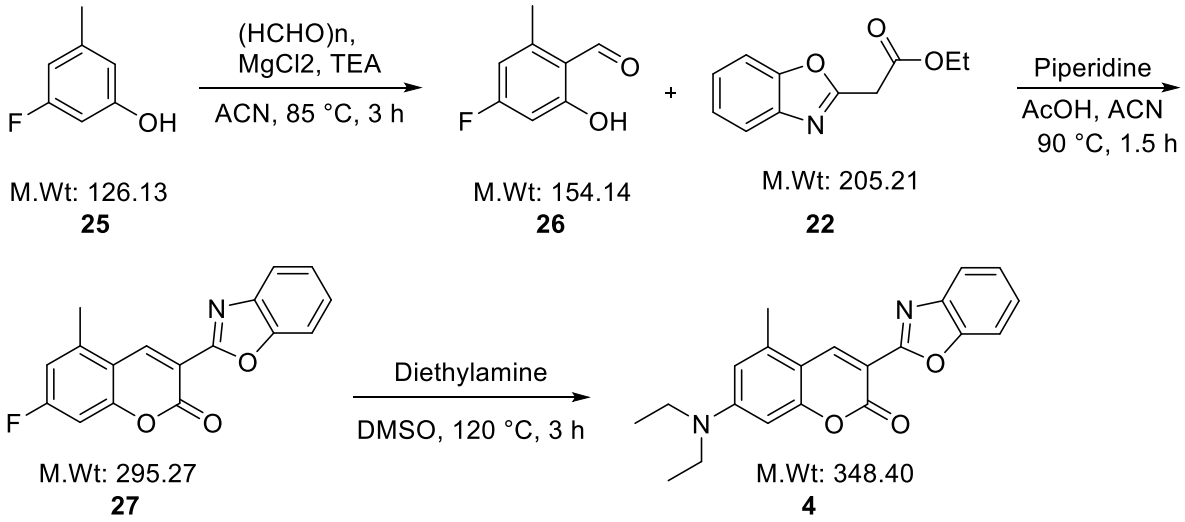
Synthesis of EMI44 (3):



M Wt: 109.13 **20** + M Wt:195.64 **21** → M Wt: 205.21 **22** + M Wt: 140.11 **23**

M Wt: 281.24 **24** → M Wt: 334.38 **3**

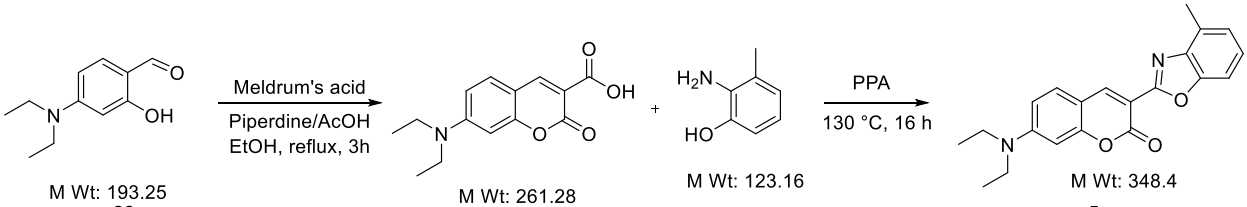
Synthesis of EMI45 (4):



M.Wt: 126.13 **25** → M.Wt: 154.14 **26** + M.Wt: 205.21 **22**

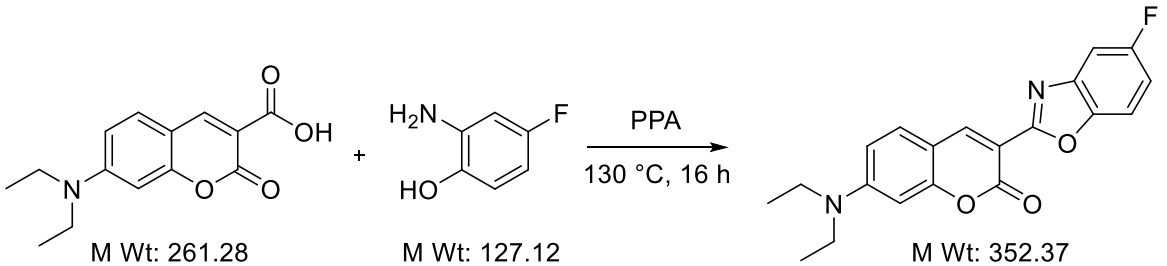
M.Wt: 295.27 **27** → M.Wt: 348.40 **4**

Synthesis of EMI46 (5):



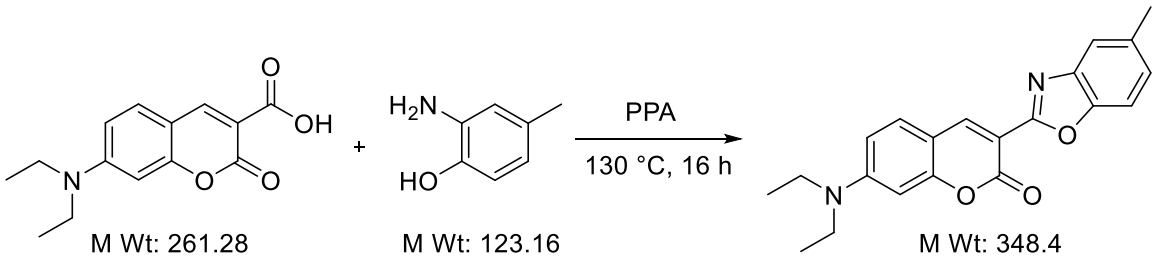
M Wt: 193.25 **28** | M Wt: 261.28 **29** | M Wt: 123.16 **30** | M Wt: 348.4 **5**

Synthesis of EMI47 (6):



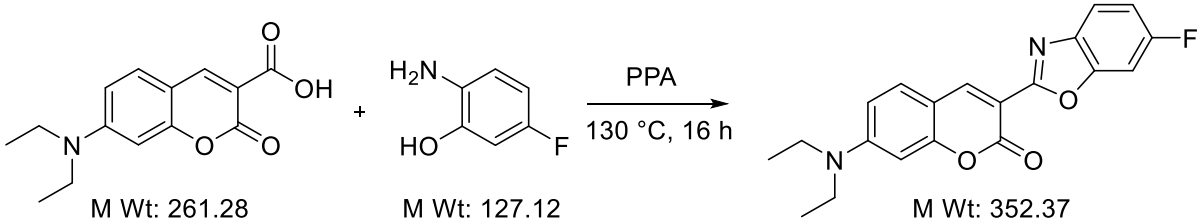
M Wt: 261.28 **29** | M Wt: 127.12 **31** | M Wt: 352.37 **6**

Synthesis of EMI48 (7):



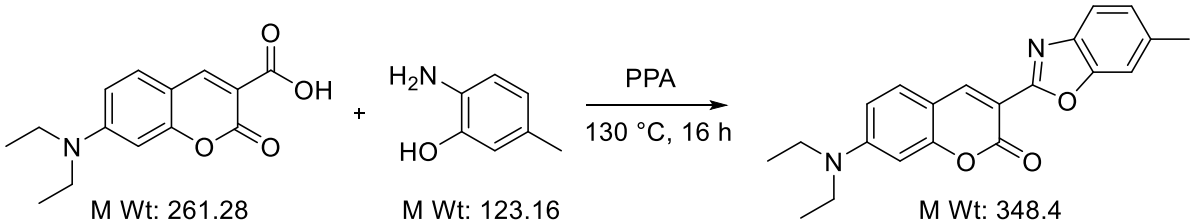
M Wt: 261.28 **29** | M Wt: 123.16 **32** | M Wt: 348.4 **7**

Synthesis of EMI49 (8):



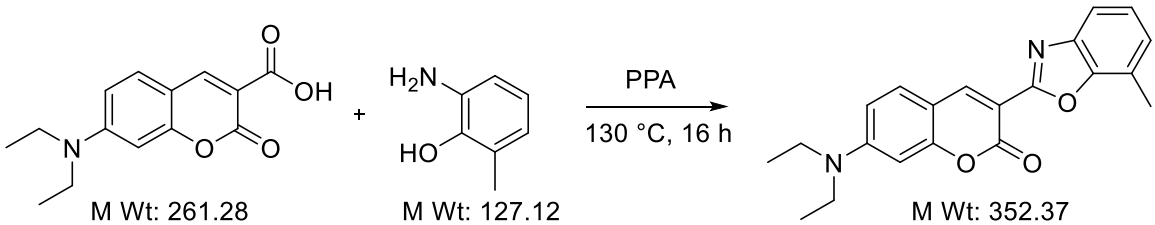
M Wt: 261.28 **29** | M Wt: 127.12 **33** | M Wt: 352.37 **8**

Synthesis of EMI50 (9):



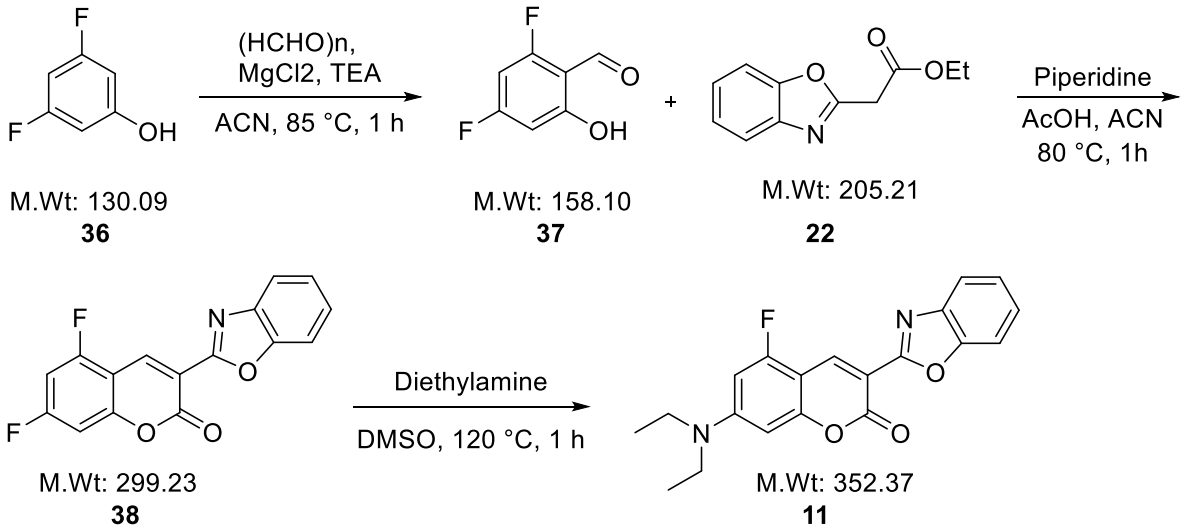
M Wt: 261.28 **29** + M Wt: 123.16 **34** → M Wt: 348.4 **9**

Synthesis of EMI51 (10):



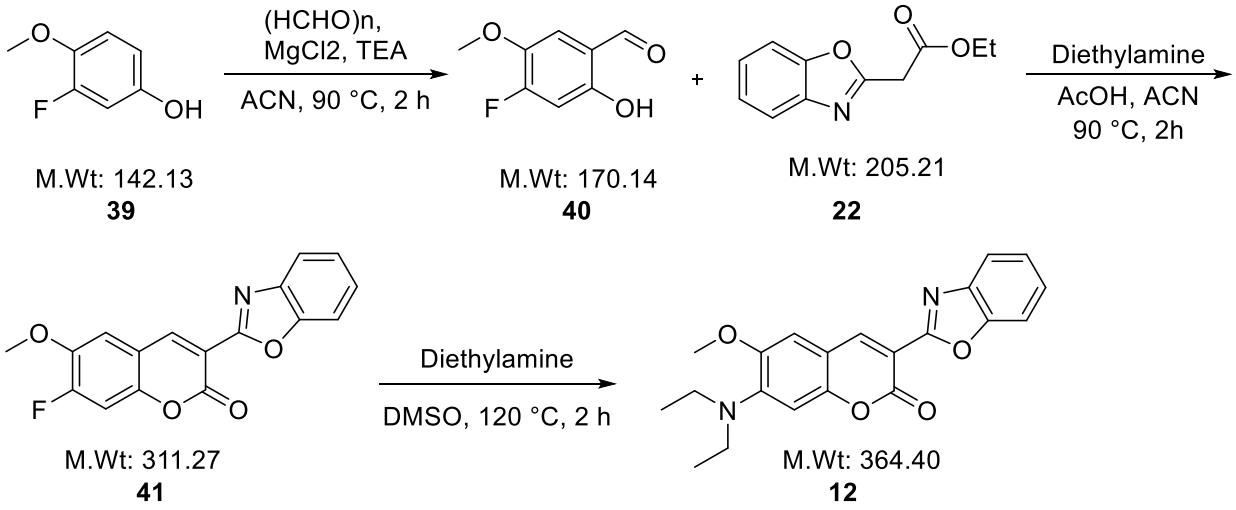
M Wt: 261.28 **29** + M Wt: 127.12 **35** → M Wt: 352.37 **10**

Synthesis of EMI52 (11):

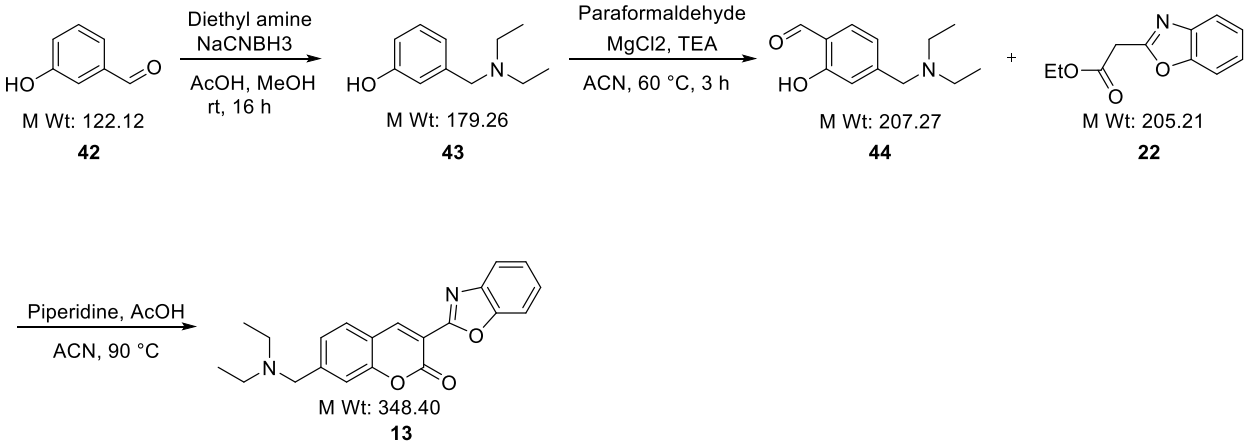


M.Wt: 130.09 **36** → M.Wt: 158.10 **37** + M.Wt: 205.21 **22** → M.Wt: 299.23 **38** → M.Wt: 352.37 **11**

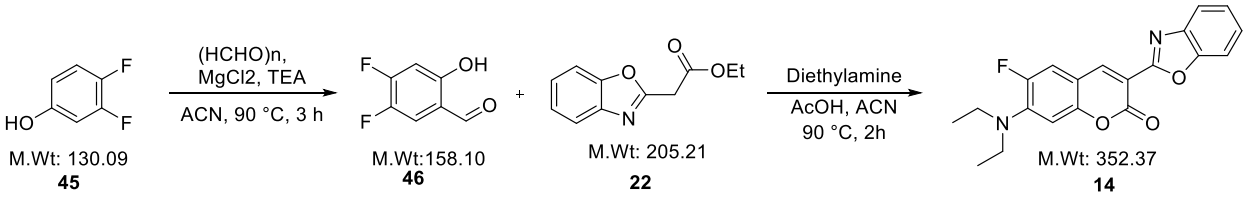
Synthesis of EMI53 (12):



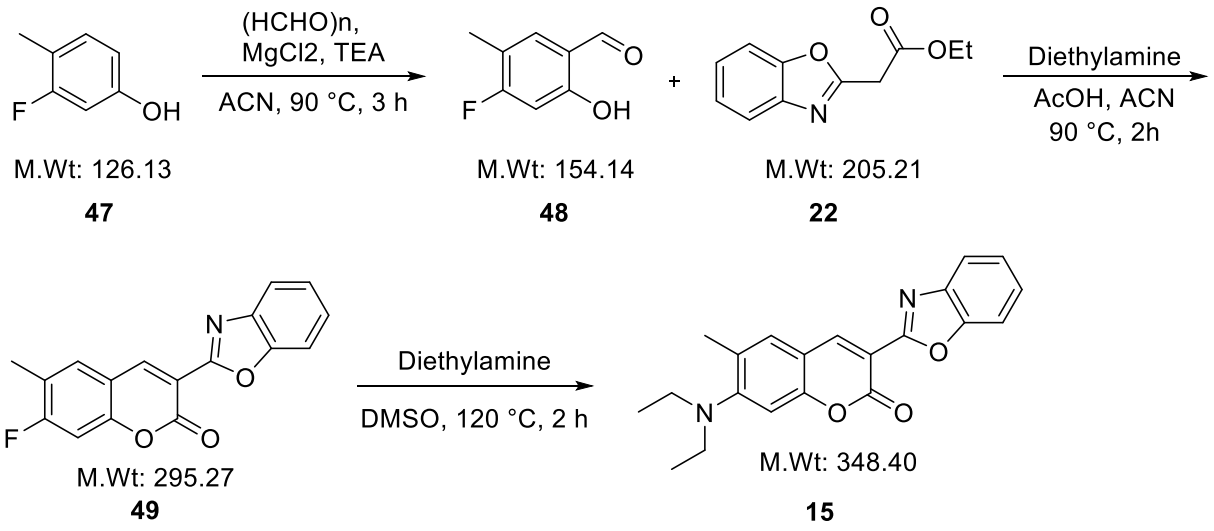
Synthesis of EMI54 (13):



Synthesis of EMI55 (14):



Synthesis of EMI56 (15):



M.Wt: 126.13 **47**

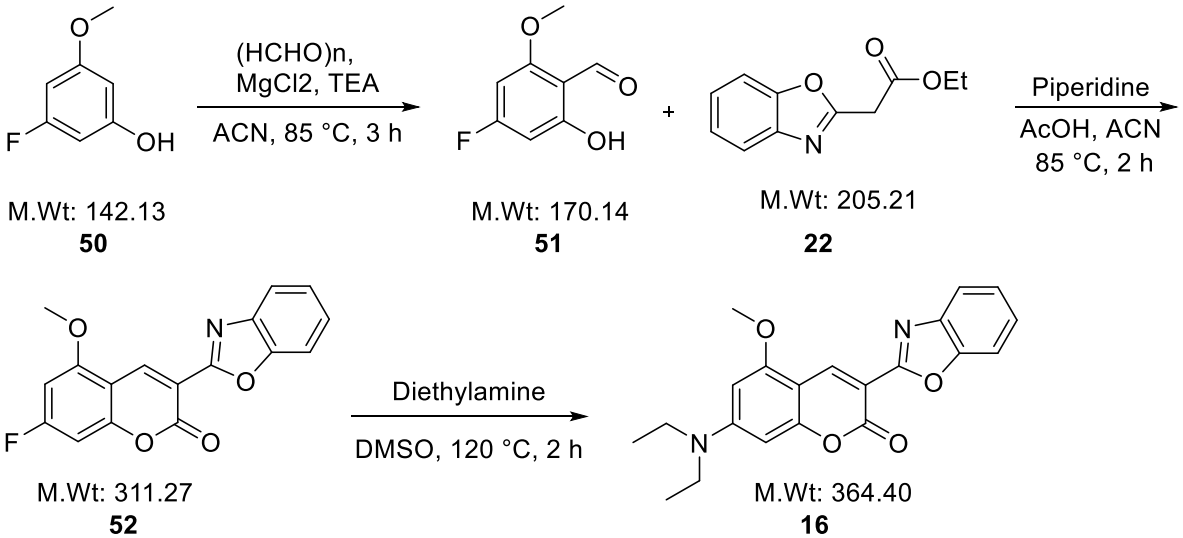
M.Wt: 154.14 **48**

M.Wt: 205.21 **22**

M.Wt: 295.27 **49**

M.Wt: 348.40 **15**

Synthesis of EMI57 (16):



M.Wt: 142.13 **50**

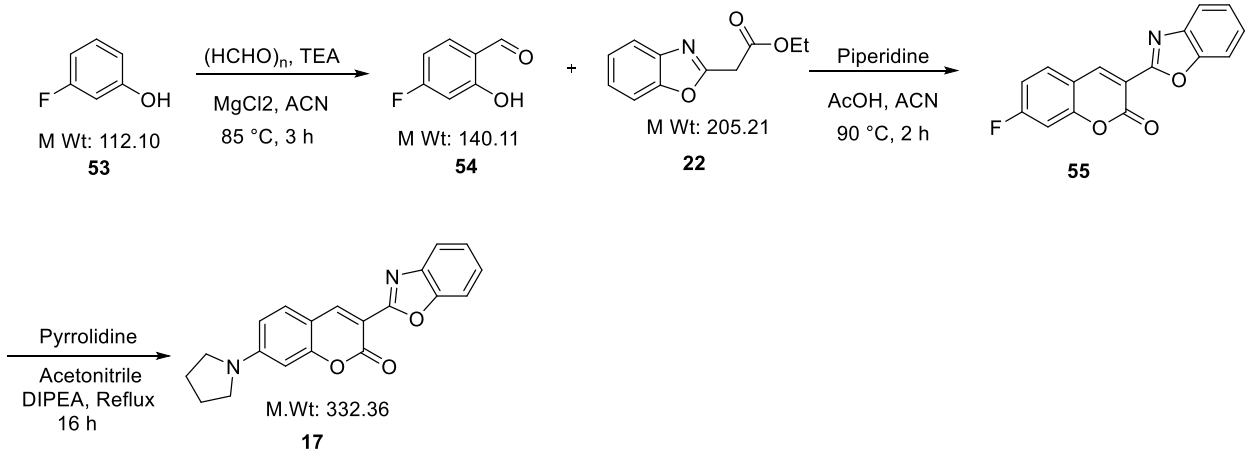
M.Wt: 170.14 **51**

M.Wt: 205.21 **22**

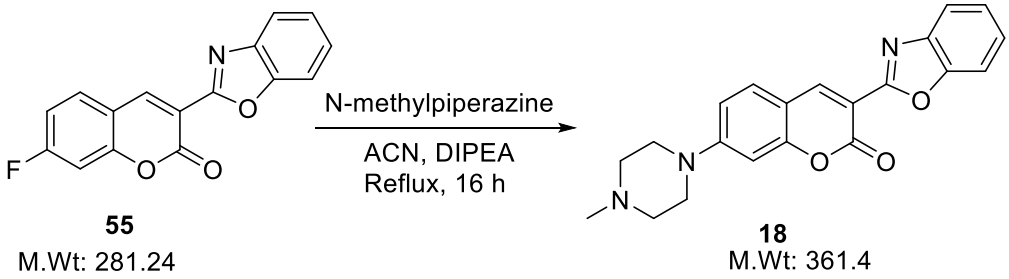
M.Wt: 311.27 **52**

M.Wt: 364.40 **16**

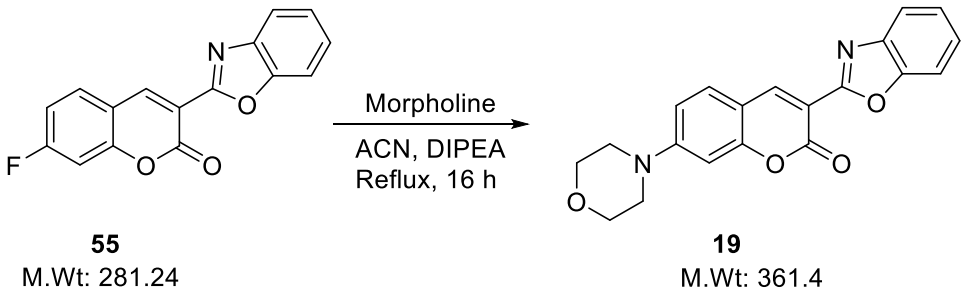
Synthesis of EMI58 (17):



Synthesis of EMI59 (18):



Synthesis of EMI60 (19):



Preparation of EMI48 (7):

To a stirred solution of **29** (200 mg, 0.766 mmol) polyphosphoric acid (10 g) was added followed by the addition of **32** (189 mg, 1.532 mmol) at room temperature and the resulting reaction mixture was stirred at 130 °C for 16 h. The progress of the reaction was monitored by TLC (5% methanol in chloroform, 0.5 R_f, U.V active) indicating completion of the reaction. The reaction mixture was then cooled to room temperature and poured in ice cold water and extracted with ethyl acetate. The organic layer was washed with cold water twice and saturated with sodium chloride solution. The organic layer was then dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield 300 mg of crude compound. The crude compound was purified by silica gel column chromatography by eluting with 1% methanol in chloroform afforded 110 mg (41%) of 7-(diethylamino)-3-(5-methylbenzo-[d]oxazol-2-yl)-2H-chromen-2-one **(7)** as a yellow color solid with a purity of 97.8% by HPLC, confirmed by mass and NMR. ^{1}H NMR (400 MHz, CDCl3) δ 8.60 (s, 1H), 7.57 (s, 1H), 7.45 (d, *J*=6.4 Hz, 1H), 7.41 (d, *J*= 7.2 Hz, 1H), 7.13 (d, *J*=6.4 Hz, 1H), 6.64 (dd, *J*=7.2 & 2.0 Hz, 1H), 6.54 (d, *J*=2.0 Hz, 1H), 3.46 (q, *J*=5.6 Hz, 4H), 2.47 (s, 3H), 1.24 (t, *J*=5.6 Hz, 6H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 160.0, 157.4, 156.9, 152.4, 148.1, 146.0, 141.6, 134.0, 131.3, 126.0, 119.1, 109.9, 109.9, 107.6, 104.9, 96.1, 44.4, 21.0, 12.3. HRMS (*m/z*): [M+H] calcd for C21H20N2O3, 348.147; found, 349.1545

Preparation of EMI56 (15):

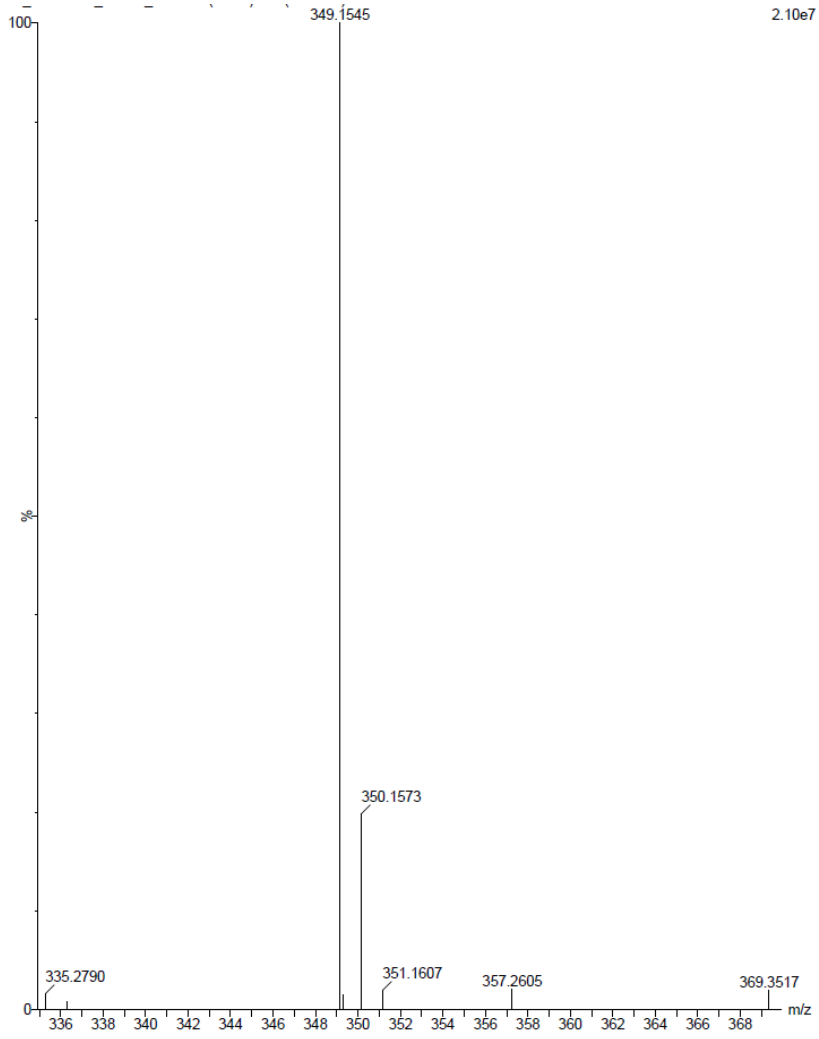
Step 1: Preparation of compound 48: To a solution of **47** (1 g, 7.930 mmol) in dry acetonitrile (37.5 mL) was added to magnesium chloride (5.59 g, 57.89 mmol), dry TEA (4.2 mL, 29.73 mmol), paraformaldehyde (1.73 g, 57.89 mmol) at room temperature and the resulting reaction mixture was heated to 90 °C, and stirred for 3 h. The progress of the reaction was monitored by TLC (10% ethyl acetate in hexane, 0.6 R_f, U.V and KMnO4 active) indicating the completion of the reaction. The reaction mixture was cooled to room temperature, acidified with 5% HCl and extracted with ethyl acetate. The organic layer was then washed with cold water, saturated sodium chloride solution, and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford 1 g (82%) of 4-fluoro-2-hydroxy-5-methyl-benzaldehyde **(48)** as a brown color solid. It was used for the next step without further purification.

Step 2: Preparation of compound 49: To a solution of **48** (600 mg, 3.894 mmol) in acetonitrile (12 mL) **22** (800 mg, 3.894 mmol) was added followed by the addition of diethyl amine (1.42 g, 19.48 mmol), acetic acid (16 mg, 0.257 mmol) at room temperature. Then the resulting reaction mixture was heated to 90 °C and stirred for 2 h. The progress of the reaction was monitored by TLC (30% ethyl acetate in hexane, 0.5 R_f, U.V active) indicates the completion of the reaction. The reaction mixture was then cooled to 0 °C and the solid was filtered, washed with cold acetonitrile and dried under vacuum

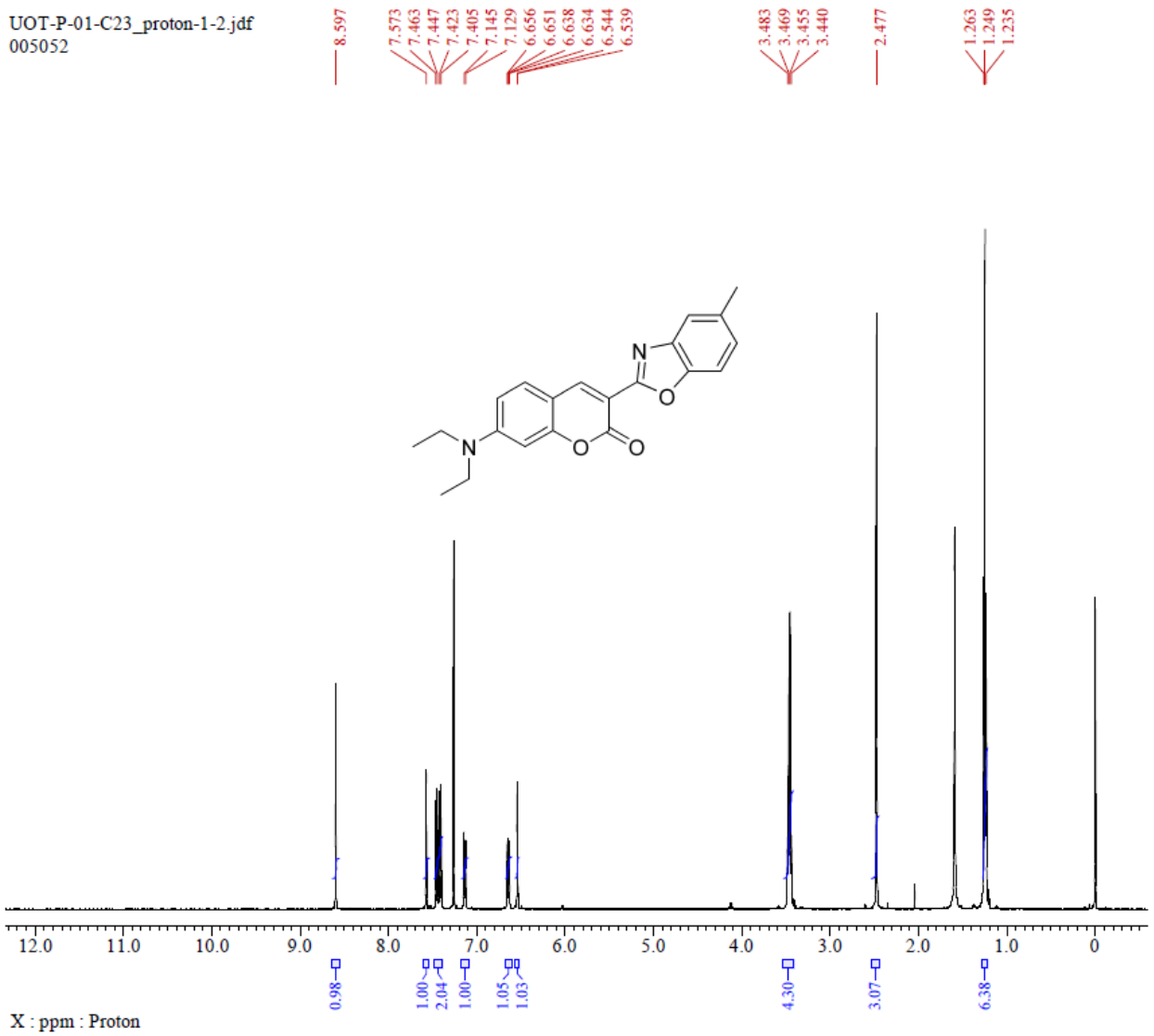
to afford 500 mg of crude 3-(benzo[d]oxazol-2-yl)-7-fluoro-6-methyl-2H-chromen-2-one **(49)** as a yellow color solid, and it was used for next step without further purification.

Compound EMI56 (15): To a solution of **49** (600 mg, 2.032 mmol) in dry dimethyl sulfoxide (12 mL) diethyl amine (1.5 g, 20.32 mmol) was added at room temperature. The resulting reaction mixture was stirred at 120 °C for 2 h. The progress of the reaction was monitored by TLC (5% ethyl acetate in chloroform, 0.4 R_f, U.V active) indicating the completion of the reaction. The reaction mixture was then cooled to room temperature and cold water was added, extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford 500 mg of crude compound and it was purified by column chromatography by eluting with chloroform afforded 100 mg (14%) of 3-(benzo[d]oxazol-2-yl)-7-(diethylamino)-6-methyl-2H-chromen-2-one (**15**) as a yellow color solid. ^{1}H NMR (400 MHz, CDCl3) δ 8.66 (s, 1H), 7.83-7.81 (m, 1H), 7.62-7.60 (m, 1H), 7.37-7.35 (m, 3H), 6.93 (s, 1H), 3.20 (q, *J*=7.6 Hz, 4H), 2.34 (s, 3H), 1.12 (t, *J*=7.2 Hz, 6H). ^{13}C NMR (DMSO-d6, 126 MHz) δ 160.0, 157.4, 156.9, 152.4, 148.1, 146.0, 141.6, 134.0, 131.3, 126.0, 119.1, 109.9, 109.9, 107.6, 104.9, 96.1, 44.4, 40.1, 21.0, 12.3. HRMS (*m/z*): [M+H] calcd for C21H20N2O3, 348.147; found, 349.1548

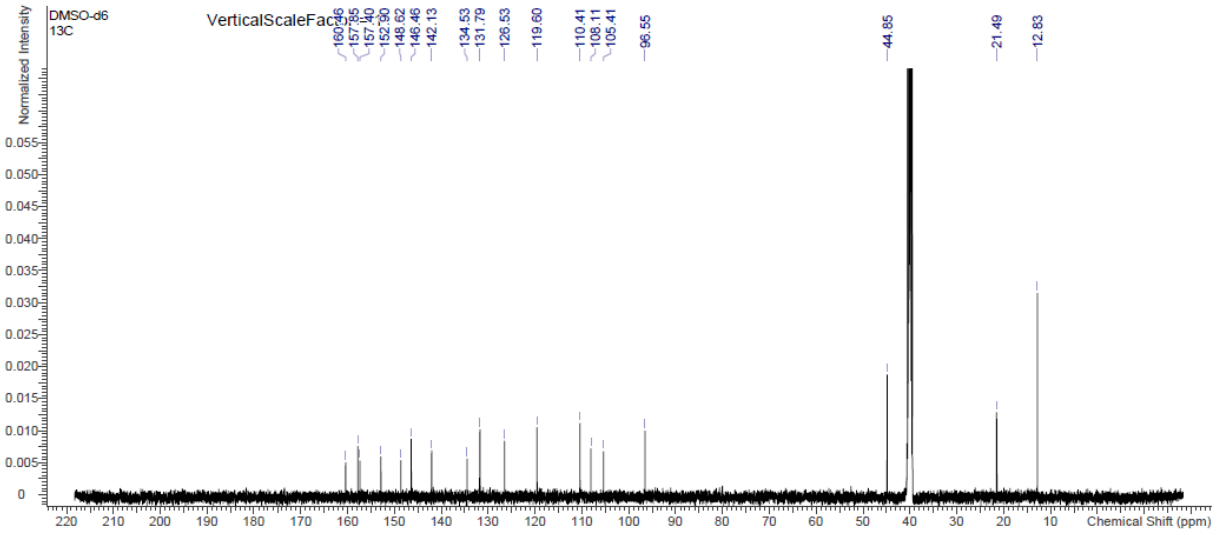
HRMS spectrum of EMI48



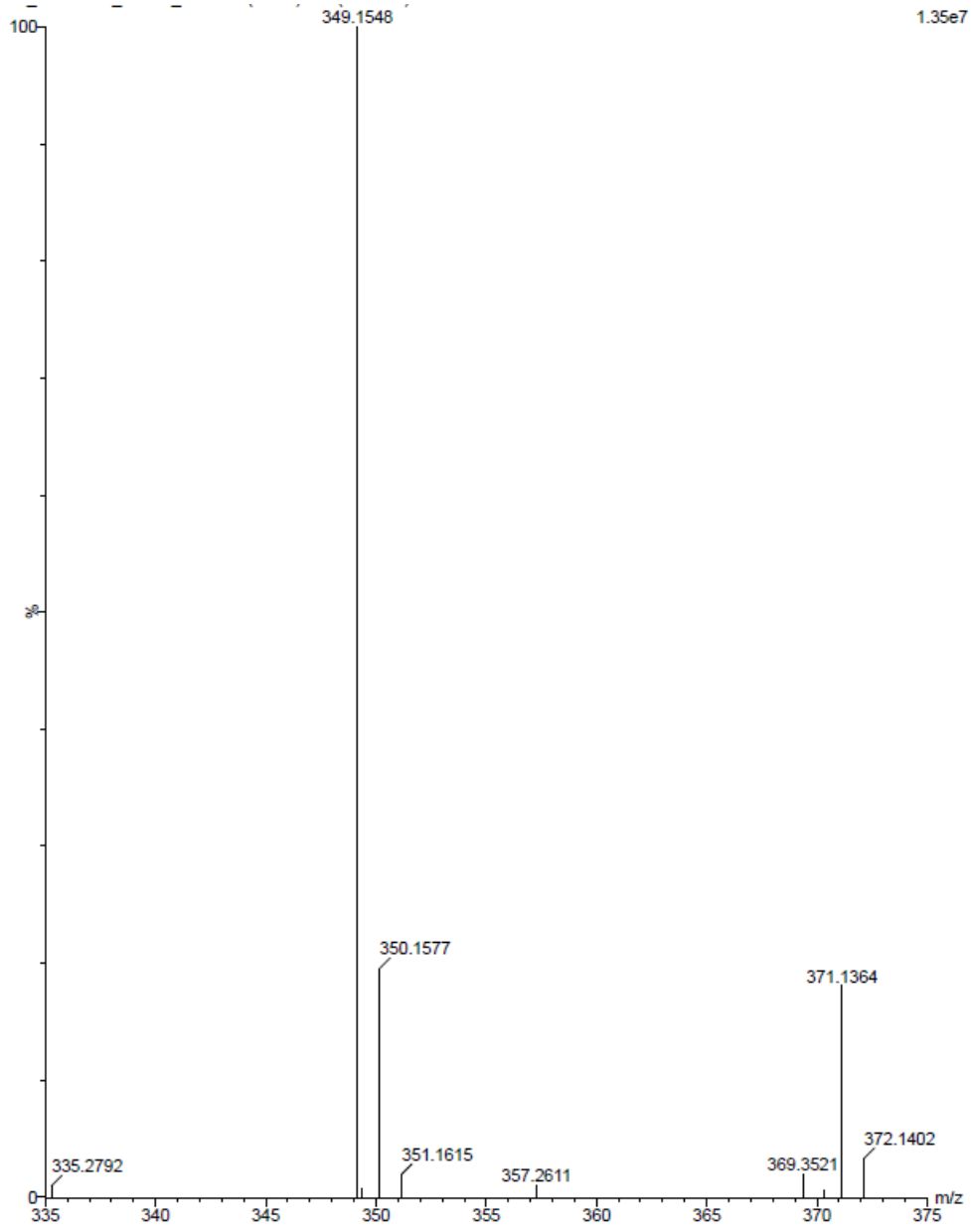
^{1}H NMR spectrum of EMI48



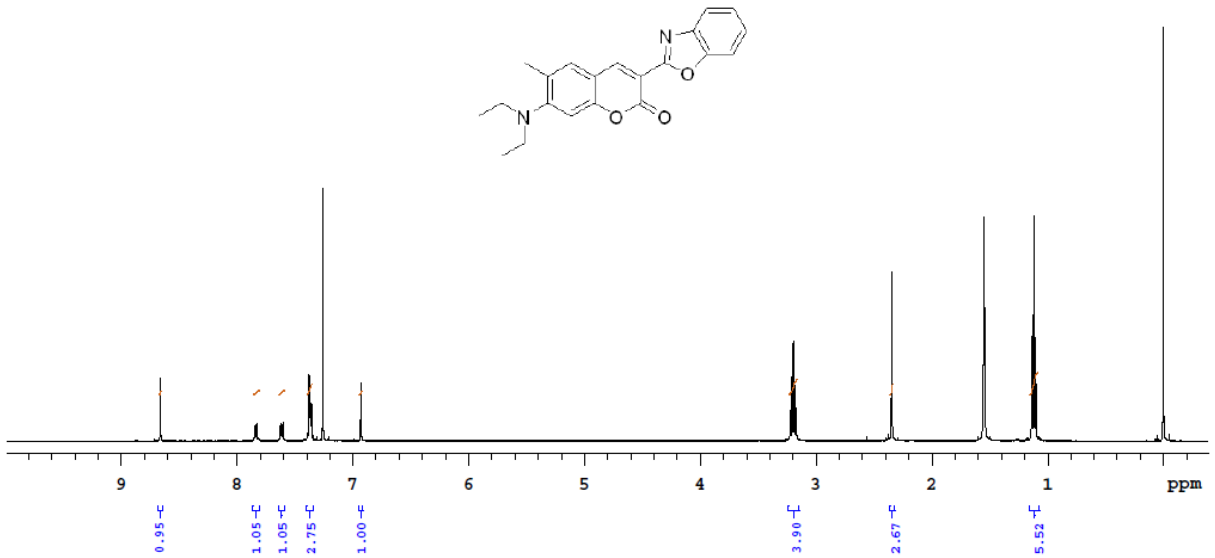
^{13}C NMR spectrum for EMI48



HRMS spectrum of EMI56



^{1}H NMR spectrum of EMI56



^{13}C NMR spectrum for EMI56