

**Supplemental table 1: Paired biopsies in 38 patients with site of biopsy and EGFR mutations identified**

Patient	Site for MSK-IMPACT	EGFR mutation	Same site Y/N
1	Right side Pleura	L747_P753delinsS	Y
	Right side Pleura	T790M+L747_P753delinsS	
2	Left lower lobe lung	E746_A750del	Y
	Left lower lobe lung	T790M+E746_A750del	
3	Left Lung	L858R	N
	Sacrum	L858R	
4	Hilar Lymph node	L858R	N
	Liver	T790M+L858R	
5	Right lower lobe Lung	L858R	Y
	Right lower lobe Lung	L858R	
6	Segment 6 Liver	L858R+V689M	N
	Segment 6 Liver	L858R+V689M	
	Left lateral segment Liver	L858R+V689M	
7	Supraclavicular Lymph node (Left)	L858R+D761Y	Y
	Supraclavicular Lymph node (Left)	T790M+L858R+D761Y	
8	Right lower lobe lung	L858R+E709K	Y
	Right lower lobe lung	L858R	
9	Pleura	E746_A750del	N
	Lingula	T790M+E746_A750del	
10	Brain	L747_T751del	N
	Paratracheal Lymph node	L747_T751del	
11	Left upper lobe Lung	L858R+T854S	N
	Mediastinal Lymph node	T790M+L858R+T854S	
12	Brain	E746_A750del	N
	Left lower lobe lung	T790M+E746_A750del	
13	Sacrum	E746_A750del	N
	Right upper lobe Lung	T790M+E746_A750del	
14	Paratracheal Lymph node	E746_A750del	

	Right upper lobe Lung	E746_A750del	N
15	Subcarinal Lymph node	E709_T710delinsD	N
	Left upper lobe Lung	E709_T710delinsD	
16	Left Pleura	E746_A750del	N
	Left upper lobe Lung	T790M+E746_A750del	
17	Paratracheal Lymph node	L858R+D1014V	N
	Supraclavicular Lymph node (Left)	L858R+D1014V	
18	Brain	E746_A750del	N
	Right lower lobe Lung	T790M+E746_A750del	
19	Left upper lobe Lung	L858R+E709K	N
	Pericardium	L858R+E709K	
20	Left lower lobe Lung	E709_T710delinsD	N
	Right upper lobe Lung	E709_T710delinsD	
21	Subcarinal Lymph node	L858R	N
	Liver	L858R	
22	Right Pleura	EGFR-KDD	N
	Right lower lobe Lung	EGFR-KDD KRAS	
23	Right upper lobe Lung	T790M+L858R	N
	Pleural fluid	L858R	
24	Paratracheal Lymph node	EGFR-KDD	N
	Rib (bone)	EGFR-KDD	
25	Right upper lobe Lung	E746_A750del	N
	Right bronchus intermedius	E746_A750del	
26	T11 bone	E746_A750del	N
	Right upper lobe Lung	E746_A750del	
27	Right lower lobe Lung	E746_A750del	N
	Pleural fluid	E746_A750del	
28	Right Paratracheal Lymph node	L858R	N
	Right Axillary Lymph node	T790M+L858R	
29	Level 11R Lymph Node	E746_A750del	N
	Left upper lobe Lung	T790M+E746_A750del	
30	Right ischiorectal mass	E746_A750del	N
	Left upper lobe Lung	E746_A750del	

31	Pleural fluid	E746_A750del	N
	Omentum	T790M+E746_A750del	
32	Left Pleura	E709_T710delinsD	N
	Right Pleural Fluid	E709_T710delinsD	
33	Right Pleura	L858R	N
	Right upper lobe Lung	L858R	
34	Brain	K745_E746insVPAIK	N
	Right upper lobe Lung	K745_E746insVPAIK	
35	L1 epidural tumor	L858R	N
	Right Iliac Bone	L858R	
36	Right lower lobe Lung	L747_P753delinsS+A755G	N
	Left Proximal Femur	L747_P753delinsS+A755G	
37	Right Paratracheal Lymph node	E746_T751delinsA	N
	Right Supraclavicular Lymph node	T790M+E746_T751delinsA	
38	Left upper lobe Lung	T790M+E746_A750del	N
	Liver	E746_A750del	

Supplementary Table 2: Alterations detected in 38 patients that had paired samples assessed by IMPACT prior and upon resistance to EGFR-TKI therapy

Pt	Baseline			Acquired				
	EGFR	Concurrent		T790M	Gained		Lost	
1	Ex19del	Mtn	L747_P753delinsS, TP53 E298*, PDGFRA R804Q, SETD2 T1641Kfs*27	Positive	Mtn	T790M	Mtn	
		Amp	EGFR (11.6 vs 11.5), MYC (2.4 vs 2.2), RECLQL4 (2.1 vs 2.1), RB1 del (-6.6 vs -3.3)		Amp	ERBB2 (2.7), SDHA (2.1), TERT (2.1), IL7R (2.1), RICTOR (2.1), RAD21 (2.2)	Amp	
		Del			Del		Del	
2	L858R	Mtn	TP53 A159P, SMAD2 P221S, L858R	Positive	Mtn	T790M	Mtn	
		Amp	FGFR1 (2.0), NKX2-1 (2.3), FOXA1 (2.3), CCNE1 (3.3), AKT2 (4.7)		Amp		Amp	FGFR, NKX2-1, FOXA1, CCNE1, AKT2
		Del			Del		Del	
3	V689M L858R	Mtn	L858R, TP53 P36fs, FGFR3 R399C, AR G578*, ATRX S784F, BARD1 N73S, EPHA3 F152S, SMARCA4 P1277L		Mtn	IDH1 R132G	Mtn	ATRX S784F, BARD1 N73S, EPHA3 F152S SMARCA4 P1277L
		Amp			Amp	YES1 (2.2)	Amp	
		Del			Del		Del	
4	L858R D761Y	Mtn	L858R, D761Y, PTEN R233*, CTNNB1 S33F	Positive	Mtn	T790M	Mtn	
		Amp	AURKA (2.9 v 1.8), TERT (2.2 v 2.0), GNAS (2.9 v 1.8), SDHA (2.9 v 2.0), PTPRT (2.9 v 2.1), MCL1 (2.3), RIT1 (2.3), DIS3 (2.2)		Amp		Amp	MCL1, RIT1, DIS3
		Del			Del		Del	
5	L858R E709K	Mtn	L858R, E709K, TP53 M273I, CARD11 E1080Q, MSH2 F58L		Mtn	ATR E1986K, NOTCH2 L395V, RAD50 E620K, MST1R G886V	Mtn	EGFR E709K, MSH2
		Amp	CCNE1 (1.9 vs 5.4), EGFR (1.6 vs 2.8)		Amp	ERBB2 (22.7), AKT2 (3.2), CDK12 (3.7), IKZF1 (2.8), CEBPA (3.2)	Amp	
		Del			Del		Del	
6	L858R T854S	Mtn	L858R +T854S, TP53 ex7 splice, PBMR1 N110fs, PTEN L247fs, NTRK3 R306H, RB1 V654M	Positive	Mtn	T790M, SMARCA4 D1114H, HLA-A E197fs	Mtn	RB1 V654M
		Amp			Amp		Amp	
		Del			Del		Del	
7	Ex19del	Mtn	E746_A750del, TP53 C275W, RB1, TET1 Q638P, GNAS K25N, STAG2 M498I, GNA11 R210L	Positive	Mtn	T790M	Mtn	GNAS K25N, GNA11 R210L
		Amp	EGFR (2.2), RAC1 (2.4), PMS2 (2.5), FOXA1 (3.2), NKX2-1 (3.2)		Amp		Amp	PMS2, RAC1, EGFR
		Del	PTEN (-8,4)		Del		Del	PTEN
8	L858R + D1014V	Mtn	L858R+D1014V, TP53 R280I, JAK2 S1035L, ATRX D1287E, B2M M1T, EPHA5 G838R, TSC2 S1130C,PALB2 P358L, ARAF E98*,CRLF2 D87N, RAD21 L8V		Mtn		Mtn	B2M M1T, EPHA5 G838R, TSC2 S1130C,PALB2 P358L, ARAF E98*,CRLF2 D87N, RAD21 L8V
		Amp			Amp		Amp	
		Del			Del		Del	
9	L858R	Mtn	L858R, TP53 C238R, STK11 (Q100*), TGFBR2 R485H, E2F3 Q302E, NEGR1 T117A, IGF1R E1162Q		Mtn	STK11(S69*), MDM4 Q9H	Mtn	

		Amp	TERT (2.7 v 3.6), SDHA (2.7 v 3.6), EGFR (5.0)		Amp	MDM4 (2.1), RIT1 (2.7)	Amp	EGFR
		Del			Del	KEAP1 Del	Del	
10	Ex19del	Mtn	E746_A750del, TP53 F328fs, <i>NF1 Q2723R</i> , <i>PTPRS A1115T</i> , <i>SMO R512L</i> , <i>CRKL V281I</i>		Mtn	<i>NOTCH2 R1931C</i>	Mtn	
		Amp			Amp		Amp	
		Del			Del		Del	
11	Ex19del	Mtn	E746_A750del, PIK3CA E545K, TP53 P177_C182del, <i>SETD2T2316A</i>		Mtn		Mtn	<i>EPHB1 E144K</i>
		Amp	EGFR (1.9 v 1.3)		Amp	MET (1.7)	Amp	
		Del			Del		Del	
12	Ex19del	Mtn	E746_AT750del, TP53 R248Q	Positive	Mtn	<i>T790M</i> , <i>CREBBP V^722E</i> , <i>MDC1 E952Q</i> , <i>PTPN11 M355I</i> , <i>FAM46C A232T</i>	Mtn	
		Amp			Amp	EGFR (3.3)	Amp	
		Del			Del		Del	
13	Ex19del	Mtn	E746_A750del	Positive	Mtn	T790M	Mtn	
		Amp	CDK4 (4.9 v 3.4), MDM2 (5.0 v 3.4)		Amp		Amp	
		Del			Del		Del	
14	Ex19del	Mtn	E746_A750del, TP53 V272_R273insL, PIK3CA N345K, <i>JAK3 S1000*</i>	Positive	Mtn	T790M	Mtn	
		Amp	GNAS (1.8)		Amp		Amp	GNAS (1.8)
		Del			Del		Del	
15	L858R	Mtn	L858R, TP53 H179L, H214R		Mtn	MET S22N	Mtn	
		Amp	EGFR (1.7)		Amp		Amp	EGFR (1.7)
		Del			Del		Del	
16	Ex19del	Mtn	<b>TP53 R342P, RB1 Ex12 splicing variant, E746_A750del, BCOR E1096K, MDC1 D221N, NFE2L2 D321N</b>	Positive	Mtn	<b>T790M, PIK3CA E545K, IKZF1 R511Q (Small cell transformation)</b>	Mtn	<b>BCOR,MDC, NFE2L2 PIK3CA M1043I</b>
		Amp			Amp		Amp	
		Del	<b>PTPRT (-2.2),TOP1 (-2.2), SRC (-2.2),NCOA3 (-2.2)</b>		Del		Del	<b>NCOA3</b>
17	Ex19del	Mtn	L747_T751del, TP53 V173*, <i>SMARCA4 G243_P244dup</i> , <i>HGFY259C</i>		Mtn	TP53 G334W, AGK-BRAF fusion	Mtn	
		Amp	FOXA1 (5.0 v 2.1), EGFR (10.2 v 2.3), NKX2-1 (5.0 v 2.1)		Amp		Amp	
		Del	CDKN2A (-3.1) KDM5C (-2.1), CDKN2B (-3.1)		Del		Del	CDKN2A, KDM5C, CDKN2B
18	Ex19del	Mtn	E746_A750del, TP53 R248G, RB1 L337fs	Positive	Mtn	<i>T790M</i> , <i>TGFBR1 A24_A26del</i> , <i>CDKN1A T97I</i> , <i>NEGR1 S331F</i>	Mtn	
		Amp	SDHA (2.4)		Amp	EGFR (2.2)	Amp	SDHA
		Del			Del		Del	
19	Ex18del	Mtn	E709_T710delinsD, NTRK3 Y376C, PTPRT V1085I		Mtn	PIK3CA E542K, <i>KMT2C Y3879C</i> , <i>ATRX G630V</i>	Mtn	<i>PTPRT</i>
		Amp			Amp	EGFR (2.3)	Amp	
		Del			Del		Del	
20	Ex18del	Mtn	E709_T710delinsD, PIK3CA E545K, ARID2 ex3 splicing variant, RBM10 E280*, <i>MED12 Ex37 splice</i> , <i>AMER1 E283*</i> , <i>CSF1R Q764P</i> , <i>KMT2C</i>		Mtn		Mtn	<i>KMT2C</i>

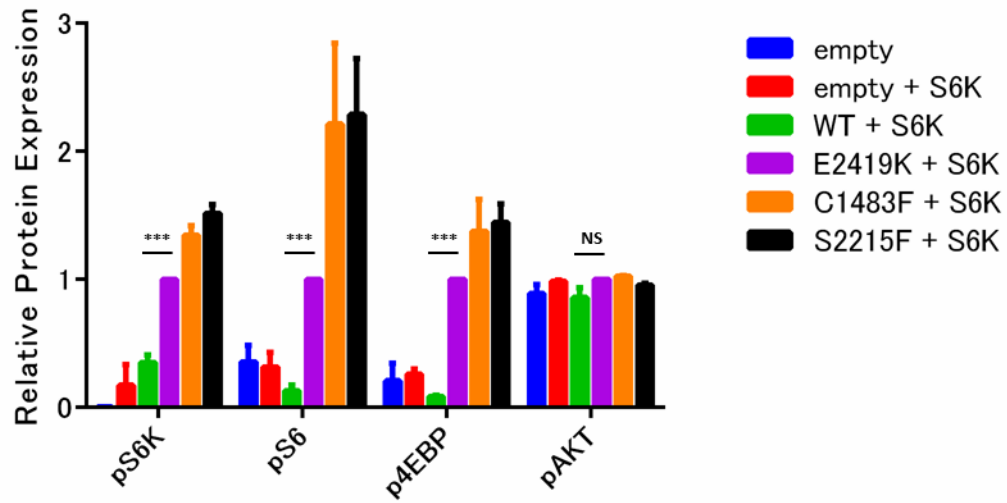
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		Amp	EGFR (V 4.2), RAC1 (2.1), ETV1 (2.0), PMS2 (2.2)		Amp	IKZF1 (2.2)	Amp	RAC1, ETV1, PMS2
		Del	CDKN2A (-2.8), CDKN2B (-2.8)		Del		Del	
21	EGFR-KDD	Mtn	EGFR-KDD, CTNNB1 T41A		Mtn	KRAS G12A, SMARCB1 R377C, RICTOR E372*, MAX V50_P51del	Mtn	
		Amp			Amp		Amp	
		Del			Del		Del	
22	EGFR-KDD	Mtn	EGFR-KDD, TP53 D49V, PLK2 I445M		Mtn	NF2 V81L	Mtn	
		Amp	EGFR (1.5 vs 2.8)		Amp		Amp	
		Del			Del		Del	
23	Ex19del	Mtn	E746_A750del, APC Q1477*, PIK3R1 D464_Y464del	Positive	Mtn	T790M, KDM5C I10006V	Mtn	
		Amp			Amp		Amp	
		Del	CDKN2A (-3.9 v -2.7), TP53 del, ETV6 (-4.3 v -3.2)		Del		Del	
24	Ex19del	Mtn	E746_A750del, TP53 E180K, SMARCA4 K1044N, JAK1 D388N	Positive	Mtn	T790M	Mtn	
		Amp			Amp	EGFR (1.6)	Amp	
		Del			Del		Del	
25	Ex18del	Mtn	E709_T710delinsD, TP53 P152L, SMAD2 W437_I438insR		Mtn	STAT5A R778C	Mtn	SMAD2
		Amp			Amp	CCNE1 (2.8), NKX2-1 (4.7), ETV1 (2.7), EGFR (2.9), RAC1 (3.4), AKT1 (4.2), PMS2 (3.4), CARD11 (2.4), IKZF1 (2.2), INHBA (2.2), MAX (5.2), DICER1 (5.1)	Amp	
		Del			Del	CDKN2A (-6.6), CDKN2B (-14.3), NKX3-1 (-3.0) EPHA7 (-2.0), BRCA2 (-2.9), FOXO1 (-2.9), ERCC5 (-2.8), IRS2 (-3.1), SOX17 (-2.9)	Del	
26	L858R	Mtn	L858R, PIK3CA C420R, TP53 (G266R), RB1 Y173fs, RHEB V98A		Mtn	TP53(E56*), ARID1A E1032K	Mtn	TP53(G266R), RB1, RHEB
		Amp	FOXA1 (3.9 v 2.4), NKX2-1 (3.9 v 2.4), PTPRT (3.0 v 1.7), ERBB2 (2.4 v), MDM2 (3.1), RICTOR (4.0), IL7R (2.3), MYCL1 (2.6)		Amp		Amp	ERBB2 (2.4 v), MDM2 (3.1), RICTOR (4.0), IL7R (2.3), MYCL1 (2.6)
		Del			Del		Del	
27	L858R	Mtn	L858R, DOTL1 I232N, YAP1 F385L, MAPK3 R96H		Mtn		Mtn	MAPK3 R96H
		Amp	EGFR (2.2 v 3.1), FGFR4 (2.0 v 2.9), NSD1 (2.0 v 2.9), NPM1 (2.0 v 2.9)		Amp	ETV1 92.1), RAC1 (2.5), PMS2 (2.6), CARD11 (3.2), RBM10 (2.7), ARAF (2.0), KDM6A (2.2), Gain: CSF1R (1.8), PDGFRB (1.8)	Amp	
		Del			Del	PIK3R1 loss (-1.8)	Del	
28	L858R E709K	Mtn	L858R+E709K		Mtn	TP53 M246K		
		Amp			Amp			
		Del			Del			
29	L858R	Mtn	L858R, TP53 M246V, RUNX1 L129_T131del	Positive	Mtn	T790M, APC S1100fs, BRIP1 I983fs, FBXW7 C573Y	Mtn	

		Amp			Amp		Amp	
		Del			Del		Del	
30	Ex19del	Mtn	E746_A750del CTNNB1 S37C		Mtn	PIK3CA E545K, SETD2 C667Lfs, GRIN2A E1176K, PTPRS E957K, <u>FGFR3-TACC3 fusion</u> , CDKN1A R84Q, SESN1 rearrangement	Mtn	CTNNB1 S37C
		Amp	ERBB2 (2.3 v 2.0), MDM2 (2.6 v 2.4), CDK12 (2.3 v 2.0), TERT (2.6 v 3.2) RARA (2.3)		Amp		Amp	RARA (2.3)
		Del			Del	CDKN2A (-1.6)	Del	CXCR4
31	Ex19del	Mtn	E746_A750del, PIK3CA E545K, TP53 R196*, SF3B1 K700E, PTPRD M1588I, BCL6 E311D, NSD1 D1419H, FLT3 E800Q, MDM2 P320A, MDM4 I166L		Mtn		Mtn	PIK3CA E545K, TP53 R196*, SF3B1 K700E, PTPRD M1588I, BCL6 E311D, NSD1 D1419H, FLT3 E800Q, MDM2 P320A, MDM4 I166L
		Amp	PAX5 (2.2)		Amp		Amp	PAX5
		Del	CDKN2A (-1.8), CDKN2B (-2.0)		Del		Del	CDKN2A, CDKN2B
32	L858R	Mtn	L858R, TP53 R282W, APC D962fs, MTOR P568S, FLT1 I464V, PDGFRA V859M	Positive	Mtn	T790M	Mtn	MTOR, FLT1, PDGFRA
		Amp	TERT (2.2), FOXA1 (3.4), NKX2-1 (3.4), TSHR (2.3), NFKBIA (3.4)		Amp	EGFR (3.0), CCNE1 (4.0), AKT2 (4.7), CEBPA (3.3), FGFR4 (3.0), IKZF1 (3.0), NSD1 (3.0), INHBA (3.0)	Amp	TERT (2.2), FOXA1 (3.4), NKX2-1 (3.4), TSHR (2.3), NFKBIA (3.4)
		Del			Del		Del	
33	Ex19INS	Mtn	I740_P741insSRYYQG, TP53 R248G, ROS1 G367V		Mtn	SMYD3 A311V	Mtn	
		Amp	EGFR (9.9 v 1.7), MYC (2.4)		Amp		Amp	MYC
		Del	CDKN2A (-4.7) CDKN2B (-4.7)		Del		Del	CDKN2A
34	Ex19del	Mtn	L747_P753delinsS +A755G, TP53 S269fs, BAP1 P522fs, MED12 R1170G, MDM4 E243V		Mtn	NOTCH3 M458V, CARD11 R869W	Mtn	
		Amp	EGFR (2.2 v 2.1), FOXA1 (1.9), NKX2-1 (1.9), NFKBIA (1.9)		Amp	MET (3.7), MYC (2.2), BRAF (1.8)	Amp	NKX2-1, FOXA1, NFKBIA
		Del			Del	CDKN2A (-1.7)	Del	
35	Ex19del	Mtn	E746_T751delinsA	Positive	Mtn	T790M, APC Q1444*, FAT1 E2052K, ERRF1 K409*	Mtn	
		Amp			Amp		Amp	
		Del			Del		Del	
36	Ex19del	Mtn	E746_A750del, TP53 R337L, ATR D2331Y	Positive	Mtn	T790M, KMT2C D2690N, RAD51B L209V	Mtn	TP53, ATR
		Amp			Amp		Amp	
		Del			Del		Del	
37	L858R	Mtn	L858R, TP53 C238Y, ARID1B S1246*, GRIN2A T888M, PTPRD E698Q, BRCA2 M1149I, BCOR S1180F, CARD11 ex8-9 splice variant, TBX3, RET, PIK3CB, TAP1, FAT1		Mtn	ERBB3 S781L, AKT1 L213V, FUBP1 G395A, RTEL1 ex7 splice, PBRM1 Ex28 splice, CARD11 ex8-9 splice, ATRX Q1632K, DROSHA R1075H, MSH6 E899V, FUBP1 G395A, RTEL1 ex7 splice	Mtn	TAP1, CARD11 ex8-9 splicing variant, TBX3, PIK3CB, RET, PIK3CB, FAT1,
		Amp	CDK4 (3.3 v 3.0), CCND1 (2.8 v 3.3), EGFR (5.8)		Amp	MET (6.8)	Amp	PRKD1 (2.4), FOXA1 (1.8)

			v 6.8), NKX2-1 (1.8 v 2.4), RICTOR (1.9 v 2.1), FGF4 (2.8 v 3.3), FGF19 (2.8 v 3.3), FGF3 ( 2.8v 3.3), NFKBIA (1.8 v 2.4), PRKD1 (2.4), FOXA1 (1.8)				
		Del			Del		Del
38	L858R	Mtn	L858R, APC K581fs,RBM10 H842R		Mtn	MTOR E2419K, NUP93 E14K	Mtn
		Amp			Amp		Amp
		Del			Del		Del

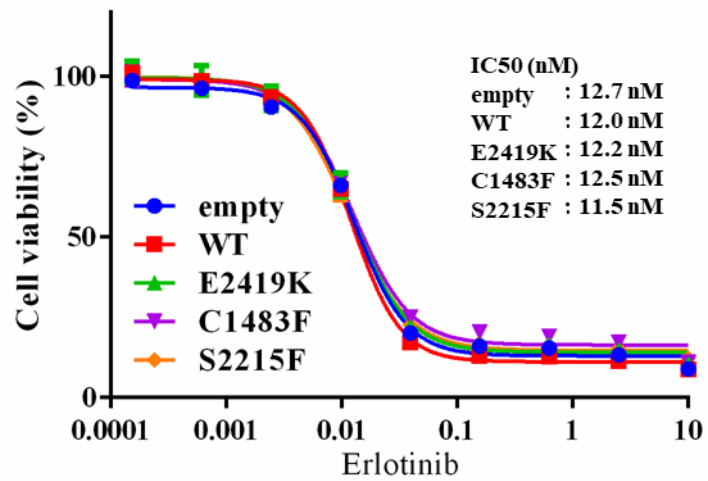


**Supplemental Figure 1: Relative expression of phosphoryrated proteins in transient cell line model.** 293T cells were transiently transfected with pcDNA3 Flag-mTOR (WT, E2419K, S1483F, S2215F), vector control, or HA-S6K1. Thirty-six hours after the transfection, cells were serum starved overnight and subsequently nutrition starved in PBS for 1 hour. Lysates were subjected to immunoblotting. Band intensities were quantified using ImageJ software, and data are representative of two independent experiments (mean  $\pm$  SE). \*\*\* $p < 0.001$ , compared to the respective WT+S6K group.



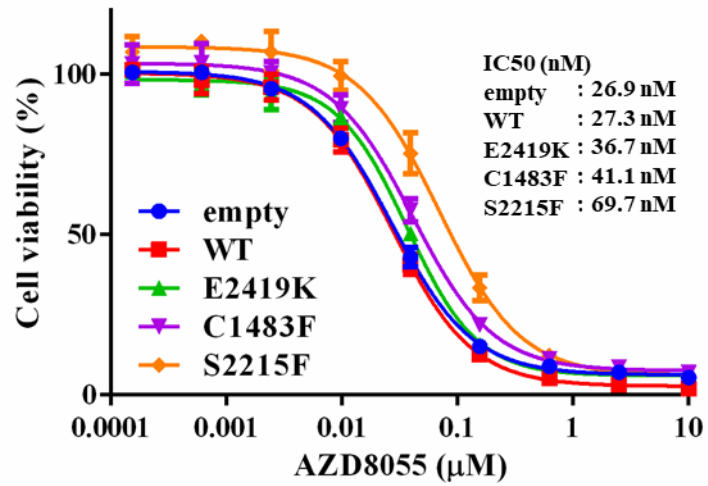
**Supplemental Figure 2: Cell growth and viability of isogenic PC9-mTOR cell line model with erlotinib treatment.**

PC9-mTOR cell lines were treated with erlotinib for 96 hours. Each experiment was assayed in six-replicate determinations and data are representative of three independent experiments (mean  $\pm$  SE).



**Supplemental Figure 3: Cell growth and viability of isogenic PC9-mTOR cell line model with AZD8055 treatment.**

PC9-mTOR cell lines were treated with AZD8055 for 96 hours. Each experiment was assayed in six-replicate determinations and data are representative of three independent experiments (mean  $\pm$  SE).



#### Supplemental Figure 4: Changes of body weight

PC9-*mTOR* E2419K cells were implanted subcutaneously into the flank of athymic nude mice. Once tumors reached approximately 100 mm<sup>3</sup>, mice were treated with vehicle, 25 mg/kg erlotinib, 20 mg/kg AZD8055, or a combination of 25 mg/kg erlotinib and 20 mg/kg AZD8055 daily. Body weight was determined on the indicated days after the onset of treatment. Data represent mean  $\pm$  SE (n = 5).

