



Supplementary Materials: Overactivation of Akt Contributes to MEK Inhibitor Primary and Acquired Resistance in Colorectal Cancer Cells

		Cell lines								
	Mutation	Caco-2	Colo-205							
	p.G12D	_	_	_	_	_				
	p.G12V	_	_	_	_	—				
	p.G13D	_	_	_	_	_				
	p.G12C	_	—	_	_	_				
	p.G12A	_	_	_	_	_				
	p.G12S	_		_		_				
	p.G12R	_	_	_	_	_				
	p.G13C	_	_	_	_	_				
	p.Q61H	_	_	_	_	_				
KDV6	p.G13S	_	_	_	_	_				
INAS	p.Q61L	_	_	_	_	_				
	p.Q61H	_	_	_	_	_				
	p.A146T	_	_	_	_	_				
	p.G13R	_	_	_	_	_				
	p.Q61R	_	_	_	_	_				
	p.G12F	_	_	_	_	_				
	p.G13A	_	_	_	_	_				
	p.Q61K	_	_	_	_	_				
	p.G13D	_	+	_	+	_				
	p.G13V	_	_	_	_	_				

Figure S1. mutation analysis of KRASusing the Somatic Mutation PCR arrayin Caco-2, DLD-1, HT-29, LoVo, and Colo-205 cells.

		Cell lines								
	Mutation	Caco-2	DLD-1	HT-29	LoVo	Colo-205				
	p.V600E	_	_	+	_	+				
	p.V600K	—	—	_	—	_				
	p.K601E	_	_	_	_	_				
	p.V600R	_	_	_	_	_				
	p.V600M	_	_	_	_	—				
	p.V600A	_		_		_				
	p.V600E	_		_		_				
	p.G469A	_	_	_	_	_				
BBAE	p.V600D	_	_	_		_				
	p.V600G	_		_		_				
	p.G469V	_	_	_	_	_				
	p.G466V	_	_	_	_	_				
	p.L597V	_		_		_				
	p.L597S	_	_	_	_	_				
	p.L597Q	_		_	_	_				
	p.L597R	_		_		_				
	p.G464E	_	_	_	_	_				
	p.G469E	_		_		_				
	p.Y472S	_	_	_	_	_				
	p.G464V	_	_	_	_	_				

Figure S2. mutation analysis of BRAFusing the Somatic Mutation PCR arrayin Caco-2, DLD-1, HT-29, LoVo, and Colo-205 cells.

		Cell lines							
	Mutation	Caco-2	DLD-1	HT-29	LoVo	Colo-205			
	p.H1047R	-	_	-	_	-			
	p.E545K	-	+	-	_	-			
	p.E542K	_	_	_	_	_			
	p.H1047L	_	_	_	_	-			
	p.E545A	_	_	_	_	_			
	p.E545G	_	_	_	_	-			
	p.Q546K	-	_	-	_	-			
	p.H1047Y	_	_	_	_	_			
	p.N345K	_	_	_	_	_			
	p.M1043I	-	_	_	_	-			
	p.T1025T	_	_	_	_	_			
	p.T1025A	_	_	_	_	_			
	p.Y1021C	_	_	_	_	_			
	p.P539R	-	_	_	_	_			
	p.Q546R	-	_	_	_	_			
	p.G1049R	_	_	_	_	_			
	p.N1068fs*4	_	_	_	_	_			
	p.M1043V	_	_	_	_	-			
	p.G1049S	_	_	_	_	-			
	p.Q546E	_	_	_	_	_			
	p.E545Q	_	_	_	_	_			
	p.E545D	_	_	_	_	_			
PIK3CA	p.E542V	-	_	_	_	-			
	p.Q546P	-	_	_	_	-			
	p.K111E	_	_	_	_	-			
	p.E545D	-	_	_	_	-			
	p.E542Q	-	_	_	_	-			
	p.T1052K	_	_	_	_	_			
	p.R93W	_	_	_	_	_			
	p.R108H	_	_	_	_	-			
	p.E453K	_	_	_	_	_			
	p.K111N	-	_	_	_	-			
	p.G1007R	_	_	_	_	_			
	p.Q546L	-	_	_	_	-			
	p.H1065L	-	_	_	_	_			
	p.G106V	-	_	_	_	-			
	p.G1049A	_	_	_	_	-			
	p.A1066V	-	_	_	_	_			
	p.T1025N	_	_	_	_	_			
	p.S1015F	-	_	_	_	-			
	p.E522K	-	_	_	_	-			
	p.L1001I	_	_	_	_	_			
	p.G1050D	-	_	_	_	-			
	p.I1062V	-	-	-	_	-			

HT-29: Previously reported p.P449Tmutations not covered by our assays.(Oncogenesis. 2013 Sep 16;2:e71.)

Figure S3. mutation analysis of PIK3CAusing the Somatic Mutation PCR arrayin Caco-2, DLD-1, HT-29, LoVo, and Colo-205 cells.



Figure S4. of trametinib and PD0325901 on cell viability in DLD-1, HT-29, LoVo, and Colo-205 cells, evaluated by MuseTM Count and Viability kit.Cell viability of trametinib-and PD0325901-treated DLD-1, HT-29, LoVo, and Colo-205 cells as measured by the MuseTM Count and Viability kit(the dual DNA intercalating fluorescent dyes kit). These cells were administrated with indicated concentrations of trametinib and PD0325901 for 1, 3, or 5 days. The results showed the 5 independent experiments. *p < 0.05vs. controls (Shapiro-Wilk test and Kruskal-Wallis test followed by Steel test).

Cell line	IC ₅₀ (nM)						
Ven inte	PD0325901	Trametinib					
LoVo	11.507	3.404					
LoVo/PR	10314.327	5949.128					
IC ₅₀ -fold increase	896.352	1747.687					
LoVo/TR	21522.609	19755.481					
IC ₅₀ -fold increase	1870.392	5803.608					
Colo-205	9.138	8.878					
Colo-205/PR	4176.477	1846.866					
IC ₅₀ -fold increase	457.045	208.027					

Figure S5. Of various MEK inhibitors for LoVo, LoVo/PR, LoVo/TR, Colo-205, and Colo205/PRcells.Cell viability was analyzed by Trypan blue dye exclusion assay. The IC50s were calculated by fitting the data to a logistic curve.

Western Blots

Figure 1B



Figure 2

DLD-1		Tr	ametinib	(nM)			LoVo				Trameti	inib (n	M)		
	0	50	100	500	1000		2010					-	40	50	
46 kDa	-	-	-			Phospho-ERK1/2		46 kDa	_	1		5	10	50	Phospho-FRK1/2
30 KDa	-	1000				•		30 kDa							Thospho-Entri2
40 kDa	-		-			ERK1/2		46 kDa		-			STATE 1		ERK1/2
00 kDa								30 kDa	-			200	Contraction of		ERRITZ
58 kDa		_				Phospho-Akt		80 kDa		10000		Sec.			Phospho-Akt
50 kDa								58 kDa	Contractor of	10 - 614		Column .	Association of the	and the second s	
58 kDa —						Akt		80 kDa	- 22		18. A.S.	2.3	2223	12.2.4	Akt
80 kDa —						Phospho-NE-rB		58 KDa 80 kDa		_					
58 kDa —			-			r noopno ni ko		58 kDa	1000						Phospho-NF-KB
80 kDa —			22/22					80 kDa							
58 kDa —						NF-KD		58 kDa	_				_		NF-KD
46 kDa —			-			R-actin		46 kDa							6
30 kDa			-	_		-acon		30 kDa	-				1992		p-actin
Phospho-ERK		1 0.251232	0.12315	0.08745	6 0.074565	5	P	hospho-ERK		1 0.21	2354 0.18	81353	0.074465	0.065548	
ERK		1 1		1	1 1		E	RK		1	1	1	1	1	
Phospho-Akt		1 1.954665	2.45156	2.54646	9 2.64648		P	hospho-Akt		1 1.02	1314 0.95	54565	0.984547	1.051355	
AKt Dheepha NE vD		1 1 5 4 6 5 4 7	0 405 40	1	1 2 5 46 480		A	kt		1	1	1	1	1	
ME vB		1 1.040047	2.12040	1 2.04004	1 3.340405	,	P	hospho-NF-KB		1 0.98	4565 1.03	32135	1.084546	0.984655	
B-actin		1 1		1 1	1 -	-	N	F-KB	-	1	1	1	1	1	
puoun					-	1	p.	-acun							
HT-29		Tr	ametinik	(nM)			Colo-205	i			Trame	etinib ((nM)		
	0	50	100	500	1000) .	1	5	10	50	
46 kDa			100	000	1000	Phospho-ERK1/2		46 kDa	-			_			Phoenho ERK1/2
30 kDa	10000000		Call Second			Thospho-Entrinz		30 kDa	-						Filospilo-EKK l/2
46 kDa —					-	ERK1/2		46 kDa	_	-	_	-		-	ERK1/2
30 kDa			2.2.2.2					30 kDa	_						
80 kDa				113518.7		Dheenhe Ald		80 kDa	-		1. S. 17. S.	1000	-1225	110,10	Phospho-Akt
58 kDa —						Phospho-Akt		58 kDa	_						
80 kDa —	12000	Sugardin .			and the second second	Akt		80 kDa	-						Akt
58 kDa —		- And - Colored States	-	-	and the second se			58 KDa 80 kDa							-
80 kDa —			Sec.	Section 1		Phoenho NE vP		58 kDa	000	100000	1993.89	1000	and the second	and the second	Phospho-NF-KB
58 kDa —		-				ноэрно-м-кв		80 kDa	_		1000 CT				1
80 kDa —						NF-rB		58 kDa		1000	1000		Section 2		NF-KB
58 kDa —	_	_						46 kDa			_				
46 kDa 🗕	-		_			R-actin		30 kDa	_						β-actin
30 kDa	-					placan	Ph	ospho-ERK		1 0.54	6547 0.49	98465	0.312135	0.105465	ō
Phospho-ERK	1	1 0.512132	0.24123	5 0.125465	0.084568		EF	RK		1	1	1	1	1	
ERK	1	1 1		1 1	1		Ph	ospho-Akt		1 1.05	4562 1.06	64565	0.983455	0.954686	Ď
Phospho-Akt	1	1 1.234546	2.12313	5 2.245647	2.545465		Ak	t		1	1	1	1	1	
Akt	1	1 1	4.0450	1 1	1		Ph	ospno-NF-KB		1 1.05	4565 1.06	65467	1.081314	0.984547	
Phospho-NF-KB		1 1.084655	1.64564	1 2.546547	3.123121		NF 0.4	-KD	+	1	1	1	1	1	-
B-actin		1 1		1 1	1		p-6	ac an I	_	1	1	1	1		u
				1. II.											

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Figure 3











Figure 4B



Figure 6C





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