

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

	Mutation	Cell lines				
		Caco-2	DLD-1	HT-29	LoVo	Colo-205
KRAS	p.G12D	–	–	–	–	–
	p.G12V	–	–	–	–	–
	p.G13D	–	–	–	–	–
	p.G12C	–	–	–	–	–
	p.G12A	–	–	–	–	–
	p.G12S	–	–	–	–	–
	p.G12R	–	–	–	–	–
	p.G13C	–	–	–	–	–
	p.Q61H	–	–	–	–	–
	p.G13S	–	–	–	–	–
	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 KRAS using the Somatic Mutation PCR array in Caco-2, DLD-1, HT-29, LoVo, and Colo-205 cells.

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

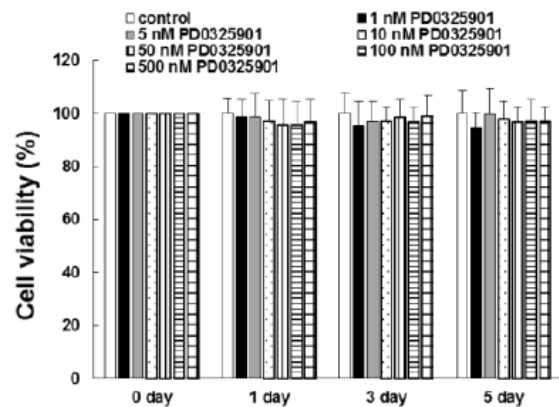
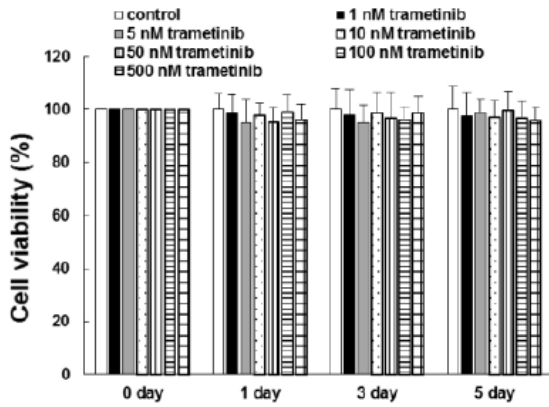
Figure S2. mutation analysis of BRAF using the Somatic Mutation PCR array in Caco-2, DLD-1, HT-29, LoVo, and Colo-205 cells.

	Mutation	Cell lines				
		Caco-2	DLD-1	HT-29	LoVo	Colo-205
PIK3CA	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	-	-	-	-	-
	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	-	-	-	-	-	

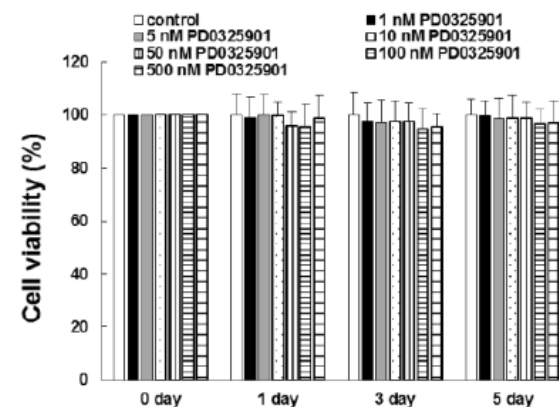
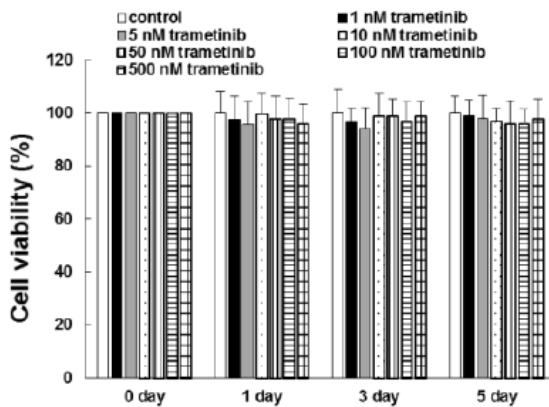
HT-29: Previously reported p.P449T mutations not covered by our assays. (Oncogenesis. 2013 Sep 16;2:e71.)

Figure S3. mutation analysis of PIK3CA using the Somatic Mutation PCR array in Caco-2, DLD-1, HT-29, LoVo, and Colo-205 cells.

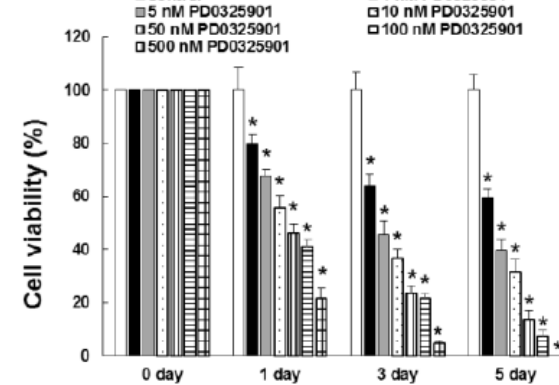
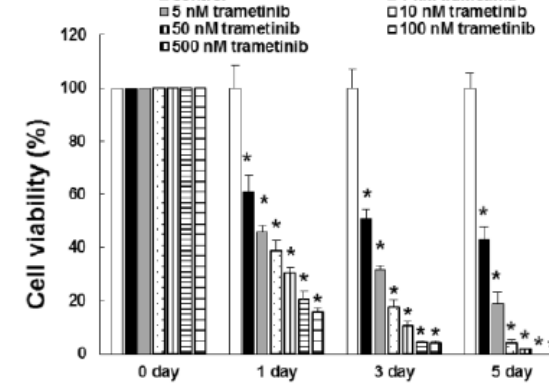
DLD-1



HT-29



LoVo



Colo-205

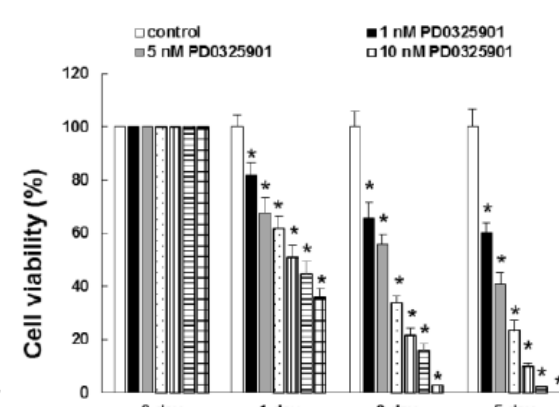
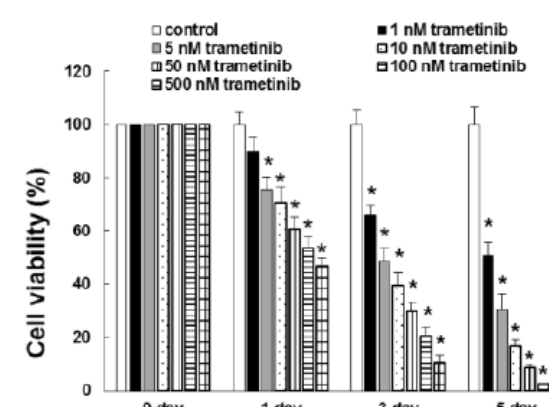


Figure S4. of trametinib and PD0325901 on cell viability in DLD-1, HT-29, LoVo, and Colo-205 cells, evaluated by Muse™ Count and Viability kit. Cell viability of trametinib-and PD0325901-treated DLD-1, HT-29, LoVo, and Colo-205 cells as measured by the Muse™ Count and Viability kit (the dual DNA intercalating 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. * $p < 0.05$ vs. controls (Shapiro-Wilk test and Kruskal-Wallis test followed by Steel test).

Cell line	IC ₅₀ (nM)	
	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/PR cells. 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

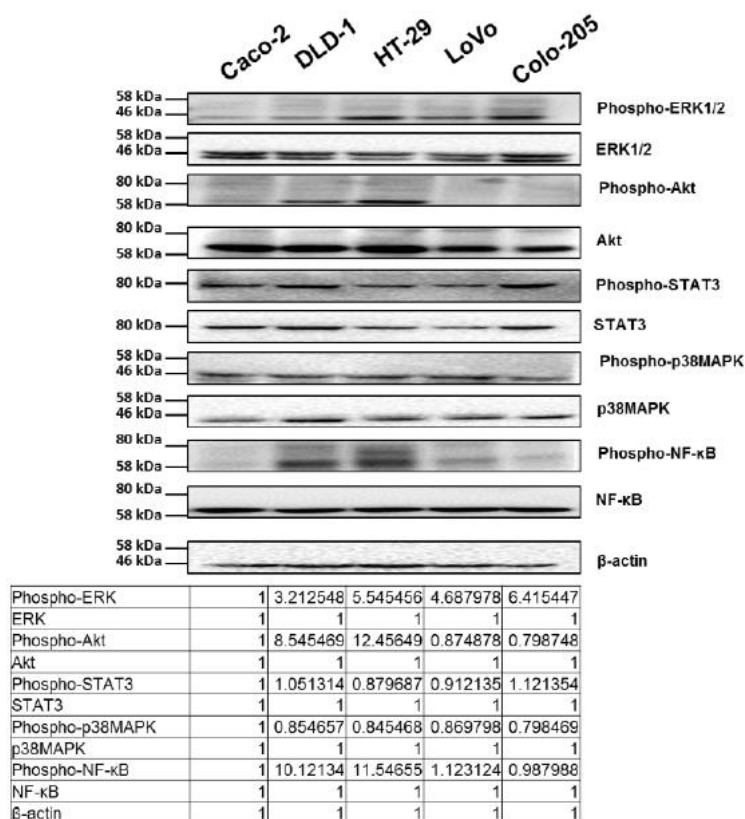
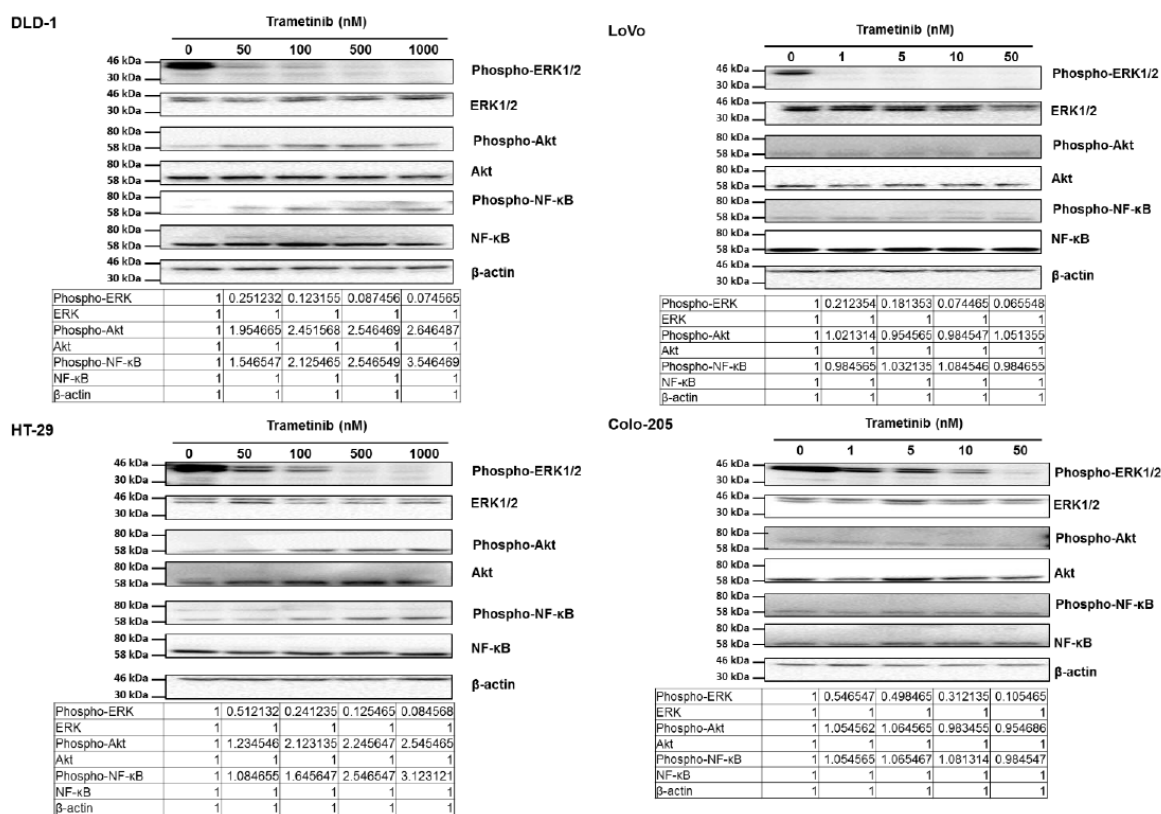


Figure 2



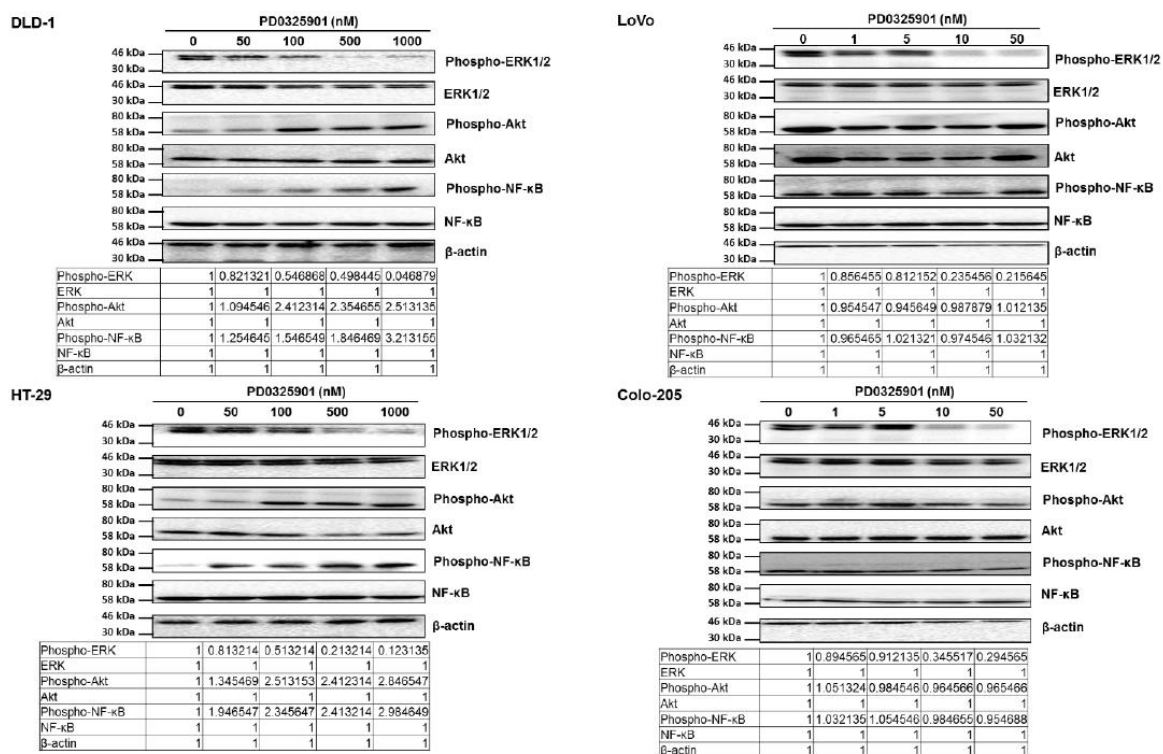


Figure 3

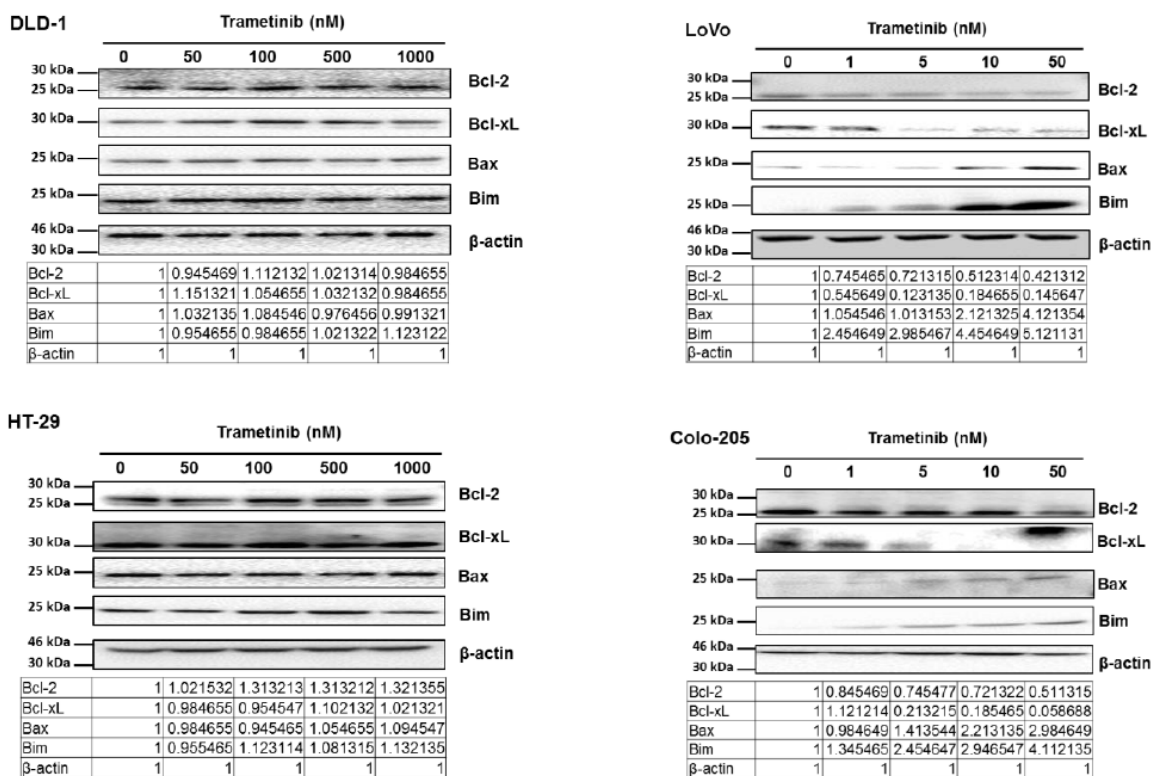
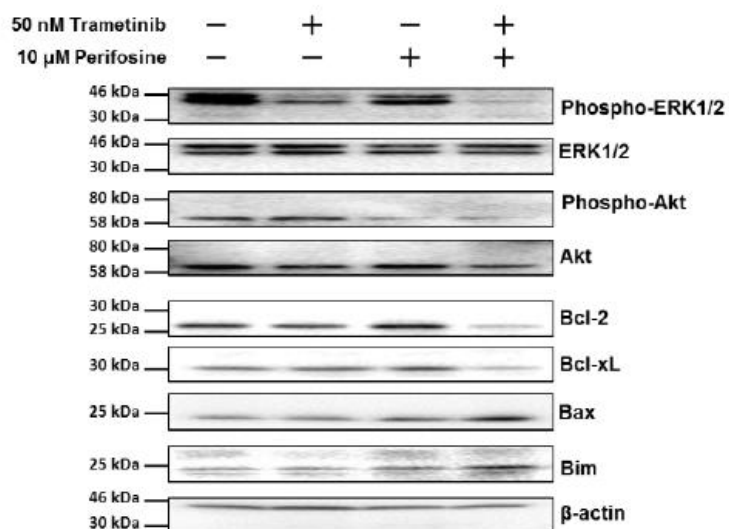
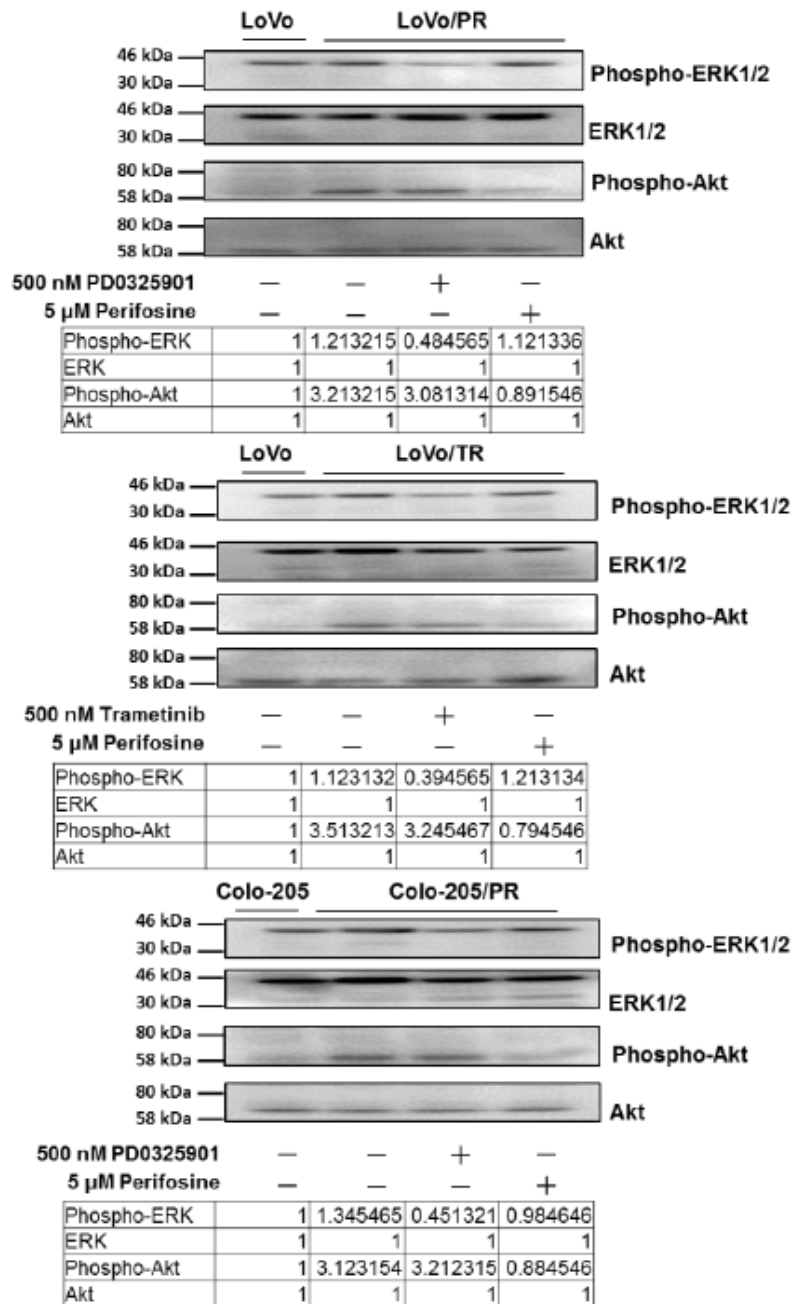


Figure 4B



Phospho-ERK	1	0.405647	0.984547	0.231343
ERK	1	1	1	1
Phospho-Akt	1	0.894565	0.123155	0.105469
Akt	1	1	1	1
Bcl-2	1	0.894655	0.945647	0.423132
Bcl-xL	1	0.945649	0.813213	0.184565
Bax	1	1.051355	1.554658	4.213132
Bim	1	1.032313	1.456156	3.546568
β-actin	1	1	1	1

Figure 6C



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