Dual specificity protein phosphatase DUSP4 regulates response to MEK inhibition in *BRAF* wild-type melanoma

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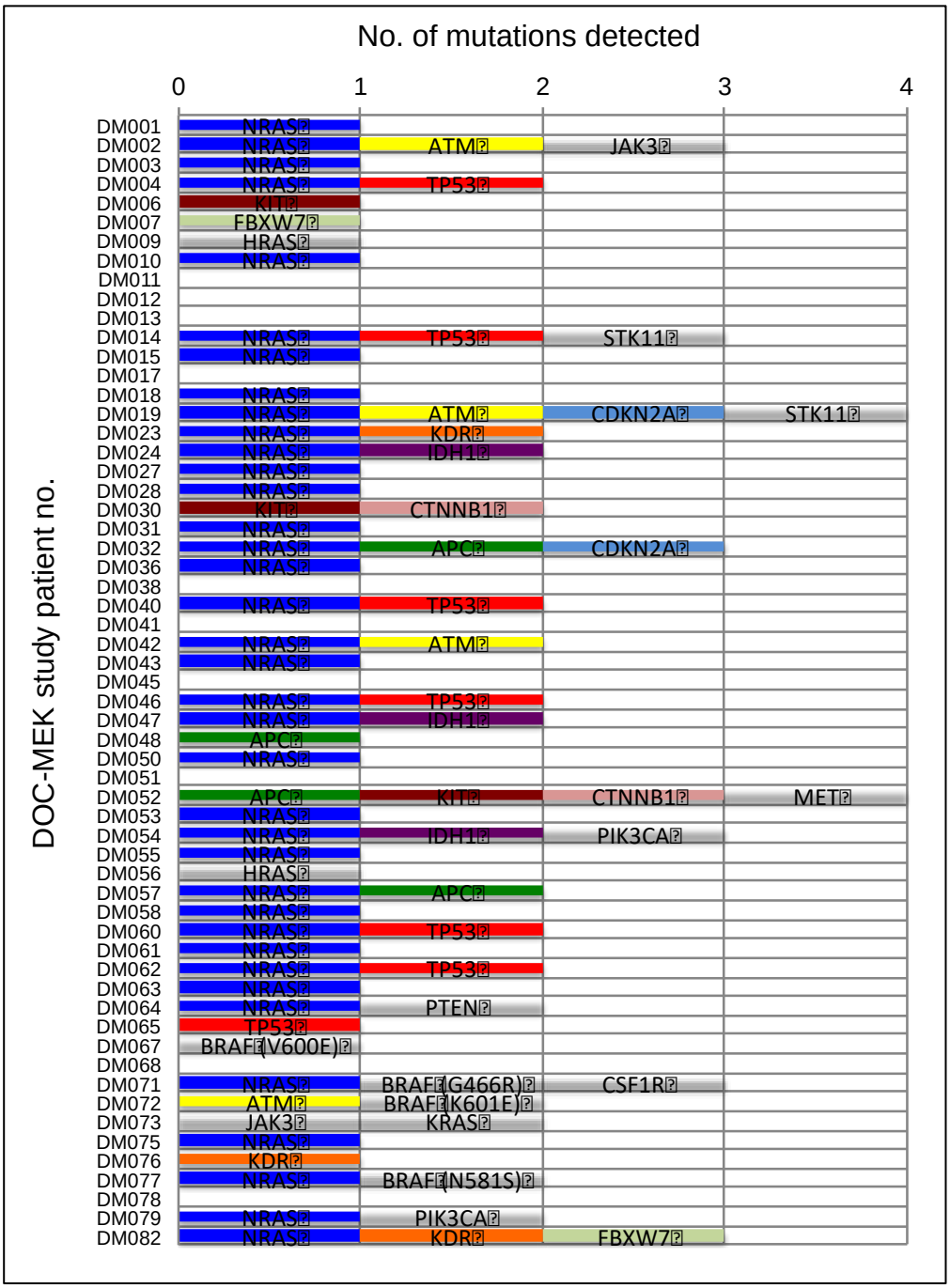
Supplementary Data

SupplementaryTables

Genes validated for diagnostic use	Genes presented on a research basis			
BRAF	ABL1	ERBB2	HRAS	NPM1
EGFR	AKT1	ERBB4	IDH1	PTPN11
KIT	ALK	FBXW7	JAK2	RB1
KRAS	APC	FGFR1	JAK3	RET
NRAS	ATM	FGFR2	KDR	SMAD4
PDGFRA	CDH1	FGFR3	MET	SMARCB1
PIK3CA	CDKN2A	FLT3	MLH1	SMO
PTEN	CSF1R	GNAS	MPL	SRC
TP53	CTNNB1	HNF1A	NOTCH1	STK11
				VHL

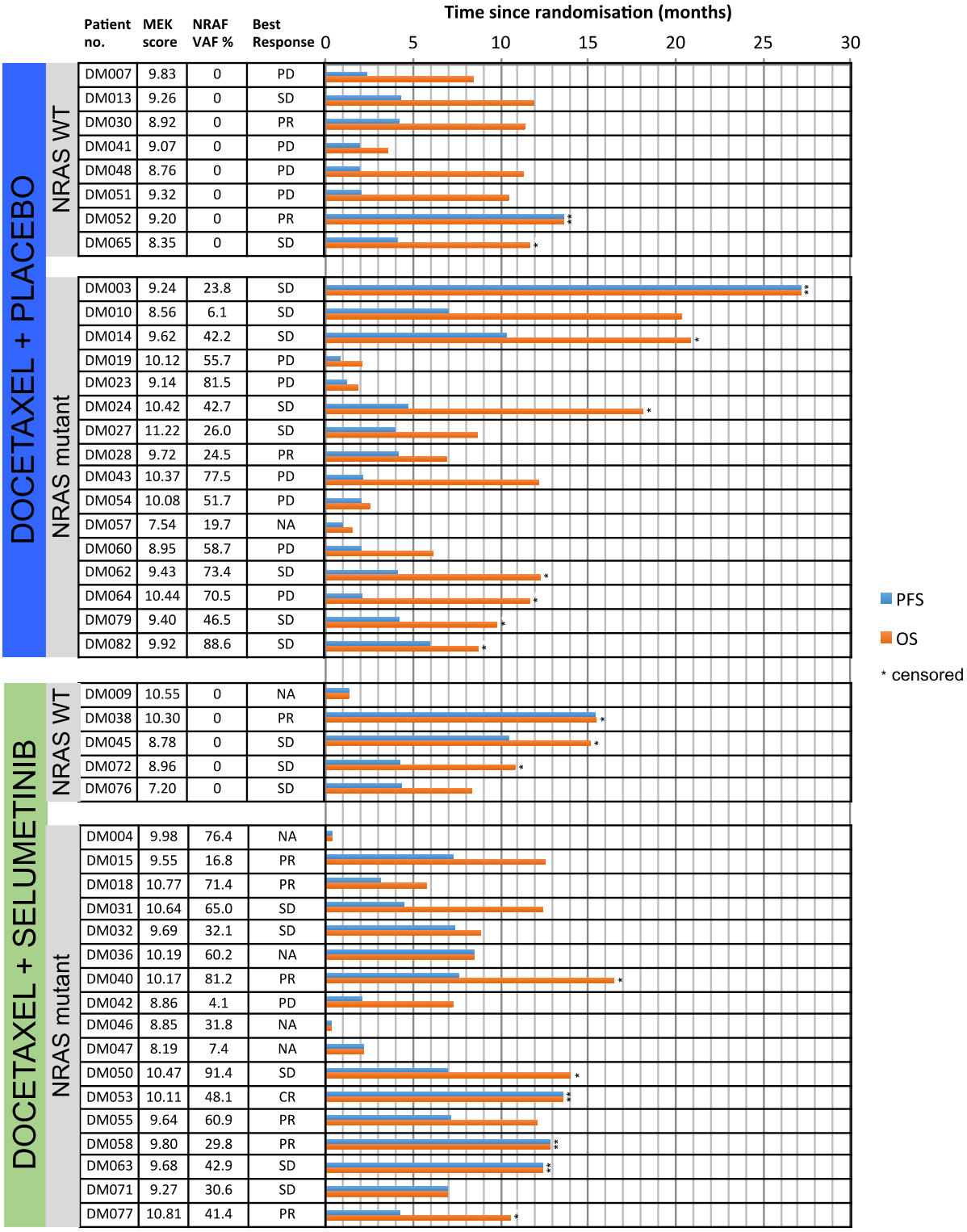
Supplementary Table 1: Genes in 46 cancer gene panel. Hotspot mutation detection was performed using IonTorrent Personal Genome Machine. Assays for mutations in nine of these genes have been validated for clinical use and the remaining assays are available for research use.



Supplementary Table 2: Number and type of mutations detected per case: Distribution of mutations detected in samples from 59 patients tested using the 46 gene cancer panel.

No. of mutations	0	1	≥ 2
No. of cases	9	24	24
Median PFS (months, PP population)	4.27	4.39	4.14
Median OS (months, PP population)	10.43	11.65	9.30

Supplementary Table 3: Summary of mutations detected per sample and patient outcome. PFS, progression free survival; OS, overall survival; PP, per protocol. There were no significant differences in PFS or OS between patients whose melanomas contained 0, 1 or ≥2 mutations ($p>0.05$ by one-way ANOVA).



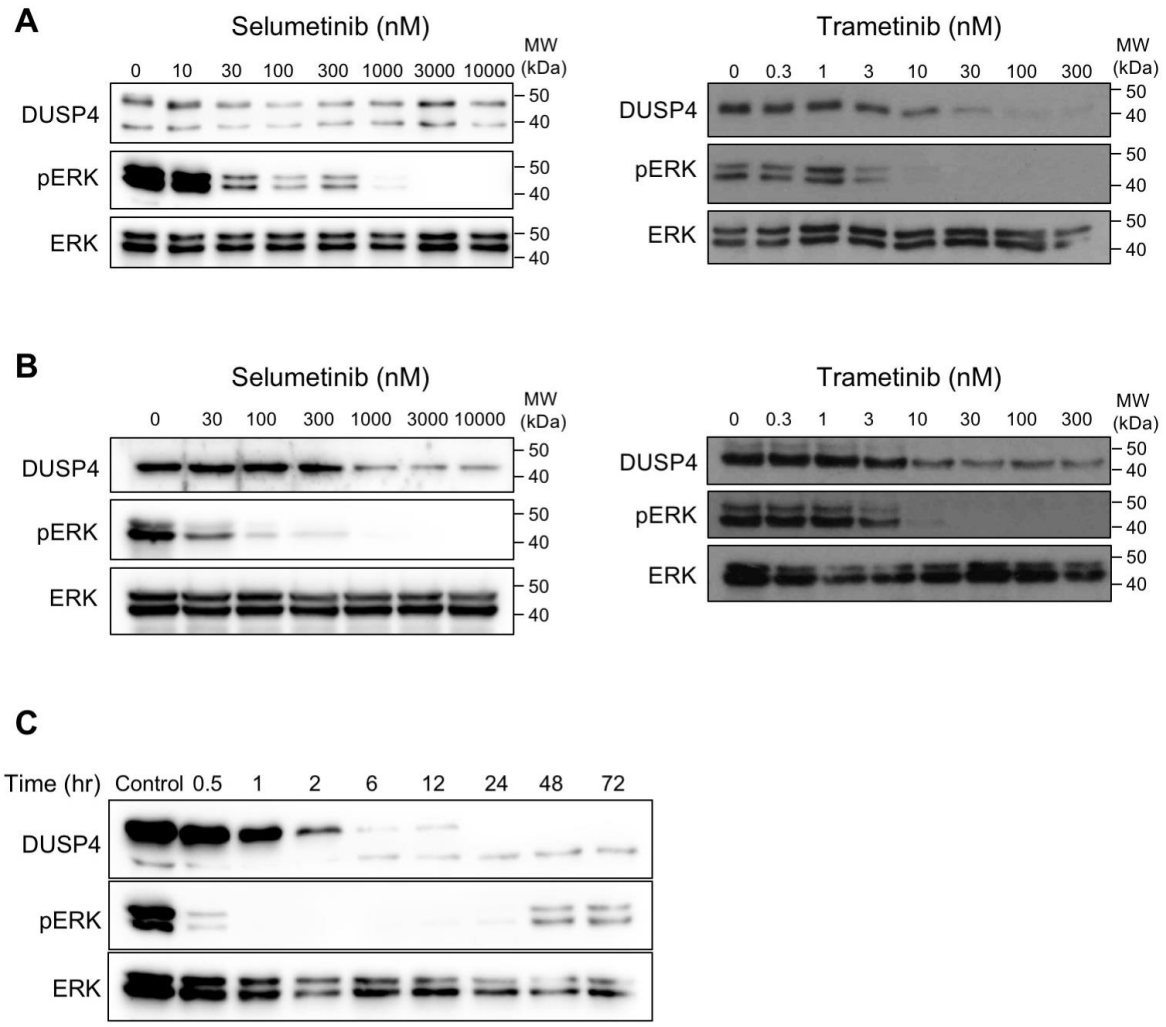
Patient no.	MEK score	NRAF VAF %	Best Response
DM007	9.83	0	PD
DM013	9.26	0	SD
DM030	8.92	0	PR
DM041	9.07	0	PD
DM048	8.76	0	PD
DM051	9.32	0	PD
DM052	9.20	0	PR
DM065	8.35	0	SD
DM003	9.24	23.8	SD
DM010	8.56	6.1	SD
DM014	9.62	42.2	SD
DM019	10.12	55.7	PD
DM023	9.14	81.5	PD
DM024	10.42	42.7	SD
DM027	11.22	26.0	SD
DM028	9.72	24.5	PR
DM043	10.37	77.5	PD
DM054	10.08	51.7	PD
DM057	7.54	19.7	NA
DM060	8.95	58.7	PD
DM062	9.43	73.4	SD
DM064	10.44	70.5	PD
DM079	9.40	46.5	SD
DM082	9.92	88.6	SD
DM009	10.55	0	NA
DM038	10.30	0	PR
DM045	8.78	0	SD
DM072	8.96	0	SD
DM076	7.20	0	SD
DM004	9.98	76.4	NA
DM015	9.55	16.8	PR
DM018	10.77	71.4	PR
DM031	10.64	65.0	SD
DM032	9.69	32.1	SD
DM036	10.19	60.2	NA
DM040	10.17	81.2	PR
DM042	8.86	4.1	PD
DM046	8.85	31.8	NA
DM047	8.19	7.4	NA
DM050	10.47	91.4	SD
DM053	10.11	48.1	CR
DM055	9.64	60.9	PR
DM058	9.80	29.8	PR
DM063	9.68	42.9	SD
DM071	9.27	30.6	SD
DM077	10.81	41.4	PR

Supplementary Table 4: Summary of gene expression data correlated with clinical outcome. *NRAS* status was determined by NGS using the 46 gene cancer panel. WT, wild-type; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; NA, not available; c, censored.

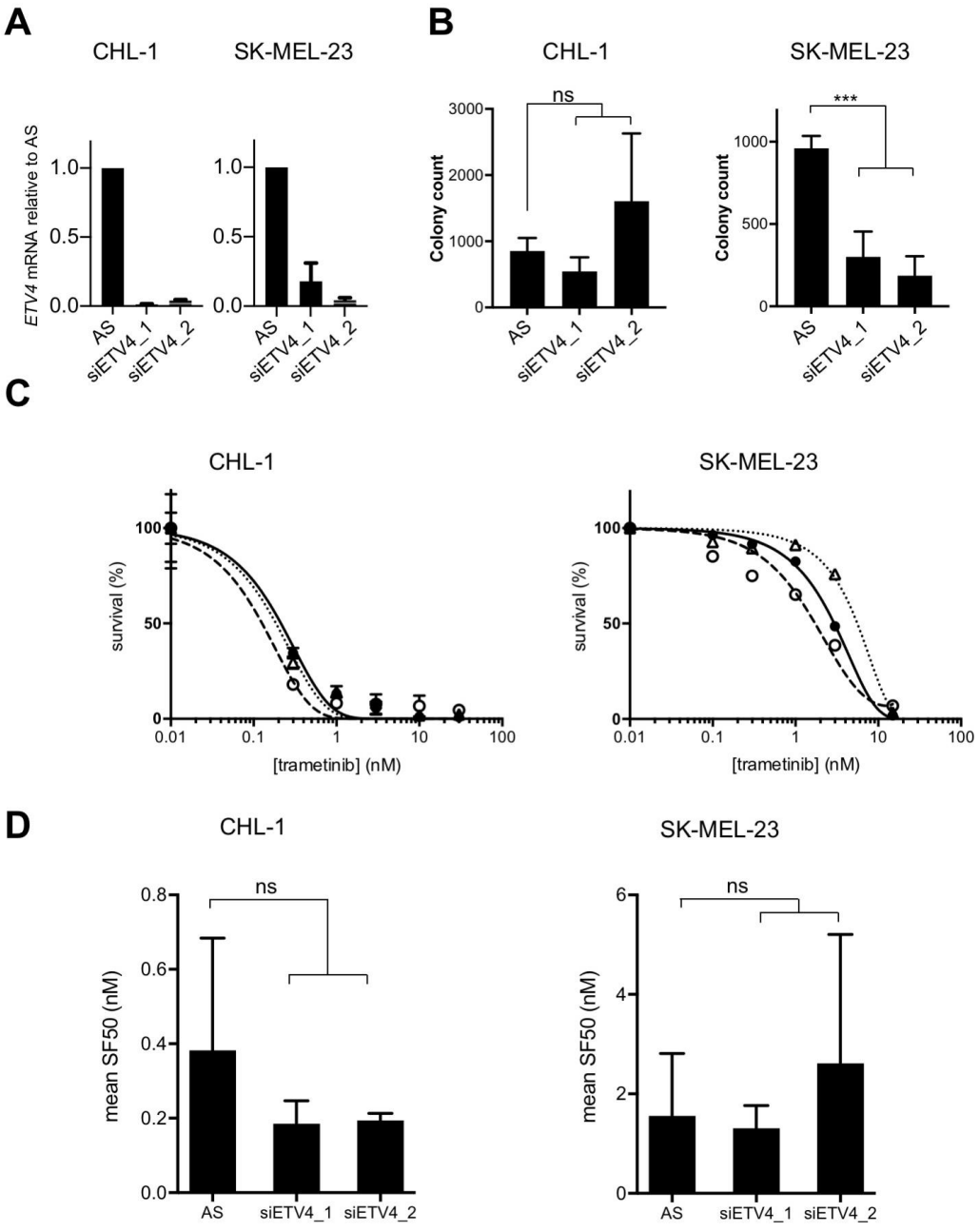
Trial no.	Normalised values					
	DUSP4	**DUSP6**	**ETV4**	**ETV5**	**PHLDA1**	**SPRY2**
PR/CR						
DM028	9.887194	10.955445	8.085209	8.990286	10.997901	9.386733
DM030	8.934151	8.182466	8.340894	10.365277	10.358002	7.321323
DM052	10.009136	7.743555	7.967867	11.215295	9.771230	8.513439
Mean	**9.610160**	**8.960489**	**8.131323**	**10.190286**	**10.375711**	**8.407165**
PD						
DM007	9.495310	10.402783	9.047305	9.785372	11.260327	8.960709
DM019	10.720741	10.895120	8.515907	9.836377	10.748692	9.988248
DM028	8.939076	9.317774	7.439848	9.508937	11.026119	8.633325
DM029	8.873473	10.139463	7.626767	10.602202	11.011783	7.857903
DM041	10.544330	7.109423	7.730938	10.536587	10.183001	8.332260
DM043	10.937361	11.041192	9.230746	10.540165	11.073405	9.404785
DM048	6.692641	9.119352	9.004512	9.591143	9.186803	8.991086
DM051	8.481253	9.441539	9.363928	10.993551	10.513146	7.128626
DM054	10.112275	10.847430	6.951539	11.284806	10.981491	10.297288
DM060	10.225808	8.197017	6.634277	9.441036	10.712816	8.513294
DM064	11.035885	10.770720	9.123161	10.884652	11.007795	9.842820
Mean	**9.641650**	**9.752892**	**8.242630**	**10.273166**	**10.700489**	**8.904577**
2 tailed p value	**0.969166**	**0.385887**	**0.854082**	**0.869125**	**0.414625**	**0.444763**

Supplementary Table 5: Gene expression data for each gene in the MEK 6 gene score in the Docetaxel plus placebo group. Shaded rows: patients with PR/CR to docetaxel plus placebo; unshaded: patients with PD at first assessment.

Supplementary Figures



Supplementary Figure 1. Concentration and time -dependent effect of MEK inhibition on ERK phosphorylation and DUSP4 expression in BRAF wild-type melanoma cells. A, CHL-1 cells were treated with increasing concentrations of: left, selumetinib (n=1); right, trametinib (n=3, representative result is shown) for 1 hr before analysis by Western blot. B, SK-MEL-23 cells were treated with increasing concentrations of: left, selumetinib (n=1); right, trametinib (n=2, representative result is shown) and analysed as A. C, SK-MEL-23 cells were treated with 10nM trametinib for the indicated times. Control cells were treated with solvent and harvested at 24hr (n=1).



Supplementary Figure 2: ETV4 depletion does not influence sensitivity to MEK inhibition in BRAF wild-type melanoma cells. A, Quantification of *ETV4* expression determined by qRT-PCR in: left, CHL-1 and right, SK-MEL-23 cells transfected with control Allstars siRNA (AS) and 2 siRNAs against ETV4 (siETV4_1 and siETV4_2). Data are mean ± SEM from triplicate readings from 3 experiments. B, effect of ETV4 depletion by siETV4_1 and _2 on colony count for: left, CHL-1 and right, SKMEL-23 cells. In each case 3000 cells were originally seeded. C, compared with control Allstars siRNA transfectants (closed circles), ETV4 depletion using siETV4_1 (dashed line, open circles) and _2 (dotted line, open triangles) did not influence cell survival in response to trametinib in: left, CHL-1 and right, SK-MEL-23 cells. Values represent mean ± SD % survival in triplicate dishes for each condition. D, Summary of effect of ETV4 depletion on SF50 values for trametinib in: left, CHL-1 and right, SK-MEL-23 cells. Values represent mean ± SEM of triplicate values from three independent experiments for both cell lines.