Supplementary Data

Table 1 Details of the ATP-based cell viability assay

Cell lines	Culture media	Cells plated/well	Doubling time (hr)
MEF $KRAS^{G12D}$	DMEM+10%FBS+1mM Sodium Pyruvate (Thermo Fisher Scientific, 11965)	1500(384-well)/ 300 (1536-well)	26
MEF $HRAS^{WT}$	DMEM+10%FBS+1mM Sodium Pyruvate	1500(384-well)/ 300 (1536-well)	27
MEF NRAS	DMEM+10%FBS+1mM Sodium Pyruvate	1500 (384-well)	40
SW620	DMEM+10%FBS+1mM Sodium Pyruvate	1500 (384-well)	30
LS513	RPMI-1640+10%FBS+2mM L-Glutamine (Thermo Fisher Scientific, 31870)	1000 (384-well)	54
Colo320	RPMI-1640+10%FBS+2mM L-Glutamine	1000 (384-well)	27

The doubling time was calculated as $[T \times (\ln 2)]/[\ln(C_{T0}/C_{Te})]$, where T = assay time duration, C_{T0} = cell number at time = 0, C_{Te} = cell number at the end of the experiment.

Table 2 Kinases inhibited by selected compounds

Series	Cluster 44	Cluster 06	Cluster 23	Cluster 04		Natural product
Cluster size	300	152	200	85 (sub-group 1)	35 (sub-group 2)	5
Best compound	44-1	6-1	23-1	4-1	4-2	NP-1
Human kinases inhibited	TRKC	CDK9/cyclin T1	CDK9/cyclinT1	CDK9/cyclinT1	CDK9/cyclin T1	No kinases
(> 50% inhibition @0.1μM)	MKK6	CaMKII delta	AAK1	AMPKα2	CDKL3	
		CDK5/p25	ARK5	CaMKIIδ		
		CLK2	AURORA-B	CDK1/cyclinB		
		DYRK2	AURORA-C	CDK5/p25		
		FLT3	BIKe	CLK2		
		LRRK2	CaMKIIγ	FLT3		
		MELK	CaMKK2	GSK3α		
		p70S6K	CDK7/cyclinH/MAT1	GSK3β		
		PIM-1	CDKL2	IKKε		
		PKA	CDKL3	KDR		
		PKB alpha	CDKL4	LYN		
		PKCθ	CK2α2	LRRK2		
		PKCζ	CLK1	PKA		
		RSK1	CLK2			
			CLK4			
			DDR1			
			DRAK1			
			DYRK1A			
			DYRK1B			
			FLT1			
			FLT3			
			GCK			
			HCK activated			
			HIPK1			
			HIPK2			
			HIPK3			
			IRAK1			
			IRAK4			
			LCK			
			MAP4K3			
			MAP4K4			
			MELK			
			MYLK2			
			PhKγ1			
			PhKγ2			
			RIPK2			
			TAF1L			
			TAK1			
			TIE2			
			TRB2			

Table 3 Cell-based and biochemical assay data of selected compounds

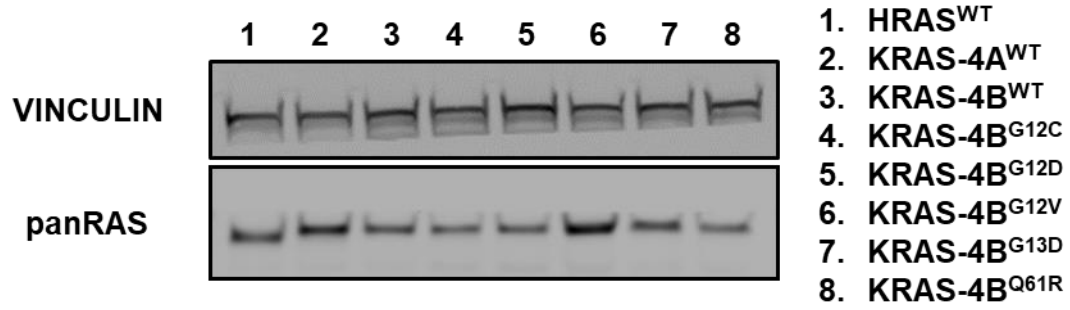
	Series	Cluster 44	Cluster 06	Cluster 23	Cluster 04		Natural product	Positive control
	Best compound	**44-1**	**6-1**	**23-1**	**4-1**	**4-2**	**NP-1**	
Cell-based assays	pEGRF HTRF*	ND	146.02%	ND	ND	ND	ND	AG1478 (28.4%)
	pERK HTRF	NA	ND	1.80E-08	ND	>1.22E-05	NA	Trametinib (IC50=37.3nM)
	pAKT HTRF	5.12E-06	ND	ND	ND	>1.22E-05	NA	Proprietory (IC50=759nM)
Biochemical assays	BRAF V600E	NA	3.51E-06	2.06E-06	9.37E-07	>3.03E-05	ND	Vemurafenib (IC50=749nM)
	MEK1	NA	1.49E-05	>3.03E-05	3.69E-06	>3.03E-05	ND	Proprietory (IC50=54.5nM)
	ERK2	NA	>2.99E-05	>3.03E-05	3.15E-06	>3.03E-05	ND	Proprietory (IC50=224nM)
	PI3K alpha	NA	NA	>1.82E-05	>1.82E-05	NA	ND	Wortmanin (IC50=165nM)
	AKT	NA	1.60E-07	>3.03E-05	2.51E-06	>3.03E-05	ND	Staurosporine (IC50=857nM)
	mTOR	NA	NA	NA	>1.82E-05	>1.82E-05	ND	PI-103 (IC50=118nM)

NA: no activity
ND: not determined

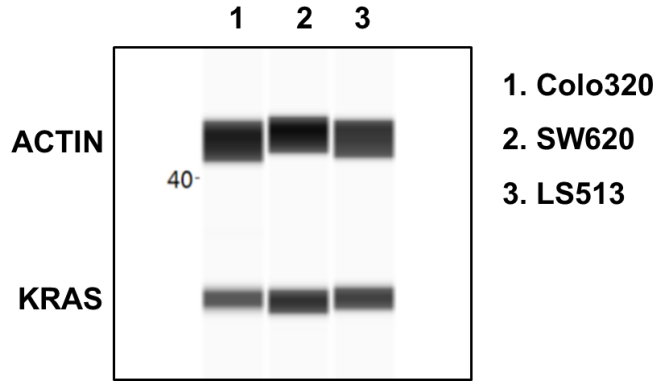
*pEGFR HTRF: Compounds (single dose of 5μM) were tested in BxPC-3 cells in the pEGFR HTRF assay. Data were presented as relative to the DMSO-treated samples. 1.25μM AG1478 was used as the positive control.

Figure 1 RAS levels in MEFs and colon cancer cell lines

A



B

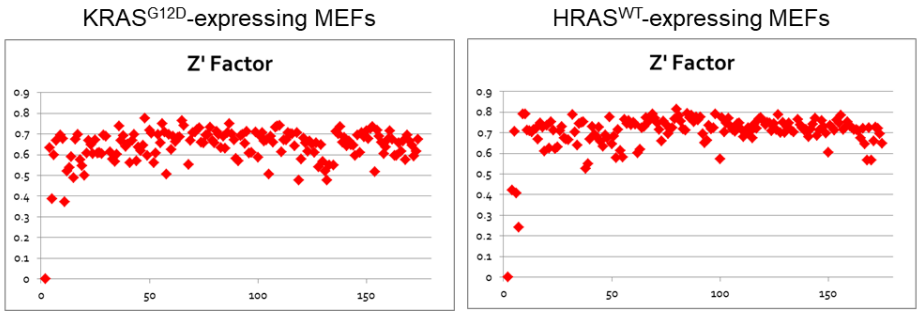


(A) The RAS levels of MEF cell lines were determined by western blots, with antibodies against RAS (Thermo Fisher Scientific, 1862335) and Vinculin (Cell Signaling Technology, 13801).

(B) The RAS levels of colon cancer cell lines were determined with the Sally Sue apparatus (Protein Simple), using antibodies against KRAS (Sigma, WH0003845M1) and Actin (Cell Signaling Technology, 4967).

Figure 2 Selection criteria and statistics of the Phase 1 high throughput screen

A



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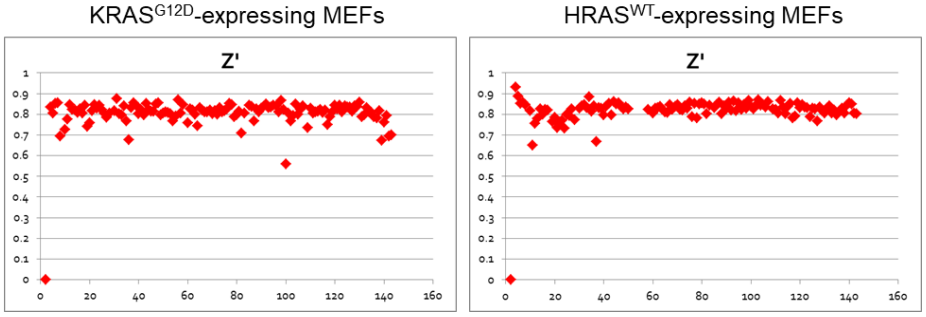

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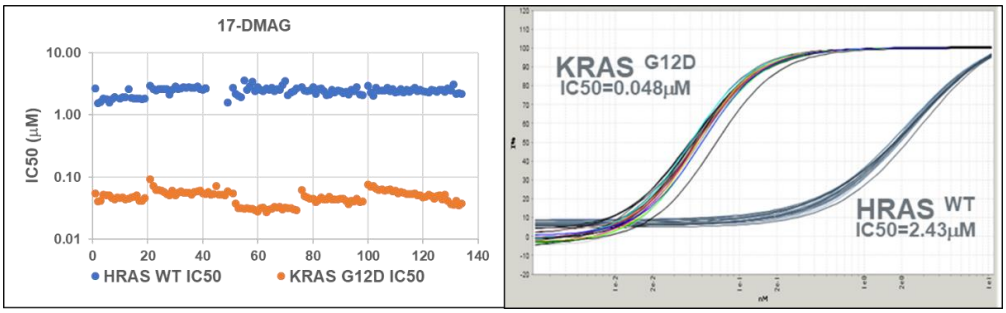
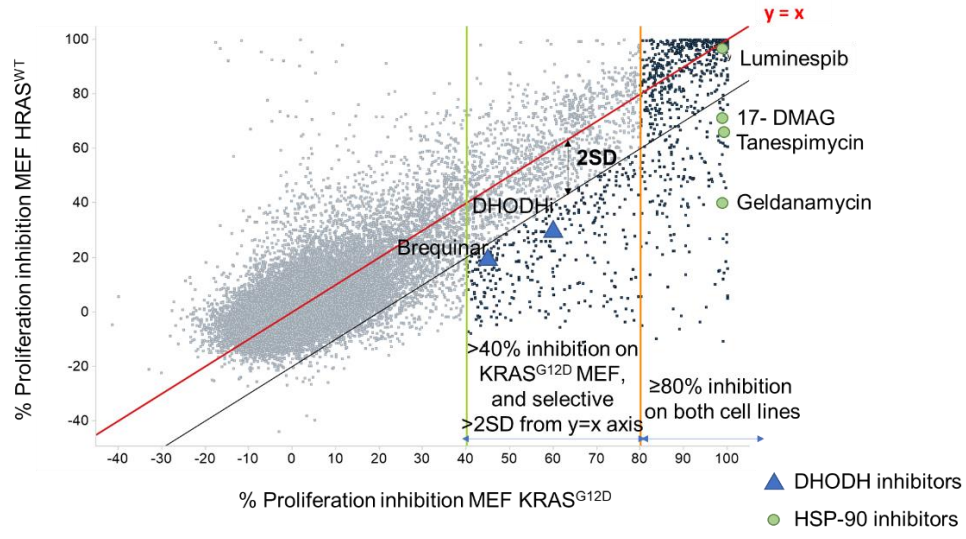


D

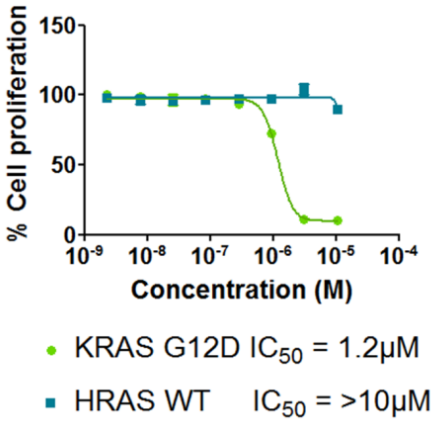


(A, B) The Z’ factor of the CellTiter-Glo® assay of each assay plate (A: 1536-well plates; B: 384-well plates) was determined, and data from some of the assay plates were shown.

(C) The dose-response of a reference compound, 17-DMAG, was determined in each assay plate. Data from some of the assay plates were shown.

(D) Selection metric of the primary screen with $KRAS^{G12D}$-expressing and $HRAS^{WT}$-expressing MEFs, with targets identified by previous screens (DHODH and HSP-90). For clarity only 40,000 compounds were shown.

Figure 3 KRAS^{G12D}-expressing MEFs were more sensitive than HRAS^{WT}-expressing MEFs to SNS032



MEFs were treated for 72 hours with SNS032 and examined with CellTiter-Glo® assay. IC50 values were determined by dose-response curves generated from two independent experiments. Data were presented as the percentage to the DMSO-treated samples.