An integrative pharmacogenomics analysis identifies therapeutic targets in KRAS-mutant lung cancer

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Abstract: Background
KRAS mutations are the most frequent oncogenic aberration in lung adenocarcinoma. Due to differences in protein structure and GTPase activity, KRAS mutant isoforms shape tumor biology and therefore may influence the treatment response in non-small-cell lung cancer. This heterogeneity challenges the development of effective targeted therapies for KRAS-driven lung cancer.
Methods
Here, we systematically investigated MEK/ERK inhibitors sensitivity for different KRAS mutant isoforms. Then we developed an integrative pharmacogenomics analysis to identify potential targets in lung cancer with KRAS(G12C) mutation, the most frequent aberration in patients with primary or metastatic KRAS mutant non-small cell lung cancer. We further validated our prediction by siRNA-mediated gene knockdown and TOPFlash reporter assay.
Findings
Our computational analysis identifies casein kinase 2A1 (CSNK2A1) as a mediator of MEK inhibitor resistance in KRAS(G12C) mutant cells which is not observed in cells with non-KRAS(G12C) mutations and in those harboring other oncogenic drivers as e.g. activating mutations in EGFR, BRAF, or NRAS. Knockdown of CSNK2A1 reduces proliferation, inhibits Wnt/β-catenin signalling and increases the anti-proliferative effect of selumetinib in KRAS(G12C) mutant lung cancer cells.
Interpretation
Our study suggested that accurate patients stratification will be necessary in order to observe significant benefit upon CK2 inhibition - alone or in combination - in a subset of patients with a favorable intratumoral genetic context. We provide a promising approach towards developing precision treatments for various subtypes of KRAS mutant lung cancer.
Fund
This work was supported by grants from National Natural Science Foundation of China (31571363, 31771469, and 81573023 to HW) the National key research and development program of China (2017YFC0908500 to HW), the

Lung Cancer Research Foundation (to CA) and a Mildred-Scheel postdoctoral fellowship from the German Cancer Aid Foundation (70111755 to JK).

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Research in context

Evidence before this study

In NSCLC, different KRAS mutations have been identified according to the amino acid substitution which can affect drug sensitivity and tumor biology.

Added value of this study

We interrogated the publicly available pharmacogenomics dataset CGP to systematically unravel that cancer cells with different KRAS mutant isoforms differ in their drug sensitivities to MEK/ERK inhibitors. We further developed a computational pipeline to systematically identify novel therapeutic targets for KRAS(G12C) mutation, the most dominant KRAS mutation in lung cancer.

Implications of all the available evidence

Predicting novel therapeutic targets by considering the mutational heterogeneity of cancer histotypes will help to guide therapeutic decision-making and improve treatment outcomes. Our pipeline can potentially be extended to other mutant KRAS isoforms given that a large enough sample size is available for statistical analysis.

Introduction

The Kirsten rat sarcoma oncogene (KRAS) encodes for a small GTPase that couples growth factor signalling to the MAPK signalling cascade. Despite being an oncogene with a prevalence of 30% in non-small cell lung cancer (NSCLC), the development of KRAS targeted therapies has been largely unsuccessful in the past. This is mainly due to the higher affinity of RAS for GTP [1,2]. Very recently, the pharmacokinetic and pharmacodynamic improvement of direct G12C inhibitors has raised great excitement [3], leading to two clinical studies that are currently on-going (https://clinicaltrials.gov/ct2/results?cond=G12C&term=&cntry=&state=&city=&dist=). As an alternative, inhibitors targeting kinases downstream of KRAS, such as BRAF and MEK, have been developed which showed promising activity in metastatic melanoma but were less active in KRAS mutant NSCLC. Furthermore, drug efficacy is limited by the development of acquired resistance or KRAS copy number variations [4-12]. Hence, there is still an unmet need to develop more efficacious targeted treatment strategies for KRAS mutant lung cancer.

In NSCLC, different KRAS mutations have been identified according to the amino acid substitution which can affect drug sensitivity and tumor biology [13,14]. The heterogenous behaviour of different KRAS mutations is due to differences in protein structure and GTPase activity [15-17] which needs to be considered when investigating potential targets for KRAS mutant lung cancer.

Here, we perform a pan-cancer analysis to systematically investigate differences in treatment response to MEK inhibitors due to different KRAS mutational subtypes. An integrative pharmacogenomics analysis pipeline is then developed to identify potential targets in lung cancer with KRAS(G12C) mutation, the most frequent mutation (>40%) in patients with primary or metastatic KRAS mutant non-small cell lung cancer (NSCLC) [17]. The most promising target predicted by this pipeline is casein kinase 2A1 (CSNK2A1) which encodes for the casein kinase 2 subunit alpha (CK2 alpha), a serine/threonine protein kinase that phosphorylates acidic proteins such as casein. Although there is strong evidence that CK2 plays a role in the pathogenesis of cancer [18-20] and several CK2 inhibitors have entered clinical trials, the role of CSNK2A1 as a therapeutic target in KRAS mutant lung cancer remains unknown to date. Our study links

CSNK2A1 to Wnt/β-catenin signaling and explores its potential as therapeutic target for treating KRAS mutant lung cancer.

Materials & Methods

Key Resources Table

KRAS(G12C) mutant cell lines for integrative pharmacogenomics analysis

Cell line	KRAS	Tissue	TP53
LU-65	G12C	NSCLC_large cell	E11Q
NCI-H2030	G12C	NSCLC_adenocarcinoma	Q16L
NCI-H2122	G12C	NSCLC_adenocarcinoma	C176F,Q16L
LU-99A	G12C	NSCLC_large cell	wt
NCI-H1792	G12C	NSCLC_adenocarcinoma	ess_splice
HCC-44	G12C	NSCLC_adenocarcinoma	p.R175L,S94*
NCI-H23	G12C	NSCLC_adenocarcinoma	M246I
NCI-H2291	G12C;G12V	NSCLC_adenocarcinoma	G154V
NCI-H358	G12C	NSCLC_adenocarcinoma	wt
SW1573	G12C	NSCLC_adenocarcinoma	wt
IA-LM	G12C	NSCLC_large cell	Q192*
HOP-62	G12C	NSCLC_adenocarcinoma	ess_splice

KRAS mutant cell lines for assays in vitro

Cell line	KRAS	Tissue	TP53
Calu1	G12C	NSCLC_ adenocarcinoma	wt
H2030	G12C	NSCLC_adenocarcinoma	Q16L
A549	G12S	NSCLC_adenocarcinoma	wt

H2009	G12A	NSCLC_adenocarcinoma	R273L

Pharmacogenomics analysis to identify potential targets in KRAS(G12C) mutant lung cancer

The Cancer Genome Project (CGP) at the Wellcome Trust Sanger Institute resulted in a large-scale, high-throughput pharmacogenomic dataset for 1001 human cancer cell lines, including the mutation status of 19,100 genes, genome-wide DNA copy number variation (CNV) status, mRNA expression profiling of 17,419 genes, and pharmacological profiling for 267 anti-cancer drugs. The drug response is represented by the natural logarithm of the IC50 value, which corresponds to the half maximal inhibitory concentration of an anti-cancer drug. In this dataset, there are five MEK inhibitors including PD-0325901, selumetinib, CI-1040, trametinib, and refamtinib, and two ERK inhibitors including FR-180204 and VX-11e. Among 137 cancer cells harboring KRAS mutations, 35 cells are derived from lung cancer.

We developed a computational pipeline to identify novel therapeutic targets for KRAS mutant lung cancer (Fig. 1). First, we started the analysis with CGP dataset. With respect to tumor heterogeneity generated from the different KRAS mutation isoforms, here, we only focused on lung cancer cell lines with KRAS(G12C) mutation, the most frequent among KRAS mutation isoforms in lung cancer. In total, we included 12 cell lines into our analyses. MAPK signalling inhibitors, 5 types of MEK inhibitors including PD-0325901, selumetinib, CI-1040, trametinib, and refamtinib, and 2 types of ERK inhibitors including FR-180204 and VX-11e were included in this analysis. The expression of 17,420 genes was used to individually calculate their correlation with drug sensitivity. As expected, the high expression of some genes was significantly correlated with the decreased drug sensitivity of MEK inhibitors or ERK inhibitors. Of these, the genes with association with more than two MAPK signalling inhibitions were considered as the potential targets. Second, they were upregulated in KRAS(G12C) mutant Lung adenocarcinoma (LUAD) patients in comparison with normal samples in TCGA database. Optionally, the high expression of the genes has a poorer clinical prognosis. Third, we further filter the genes with selection criteria that required the genes to be part of cancer core pathways and to be known drug targets. Finally, by integrative analysis of the above-mentioned criteria, we identified potential targets for KRAS mutant lung cancer.

TCGA data analysis

The RNA-seq and clinical data of LUAD patients were downloaded from TCGA cBioProtal (http://www.cbioportal.org/index.do). The abundance of each gene was quantified as RSEM value, which was evaluated by a statistical method RSEM (RNA-Seq by Expectation Maximization). RSEM uses a generative model of RNA-seq reads and the EM algorithm, taking read mapping uncertainty into account and achieving the most accurate abundance estimates [21]. The statistical analysis of differentially expressed genes between cancer and normal samples was performed using DESeq2 [22]. LUAD patients were divided into high and low expressing group, based on the median value of gene expression across the patients. Kaplan-Meier test was used to compare the overall survival and cancer relapse between two groups.

Cell lines

The human lung cancer cell lines A549, H2030, H2009 and Calu1 were purchased from ATCC and grown at 37°C in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 µg/ml penicillin and 100 units/ml streptomycin (complete medium). The cell lines were authenticated using the Promega GenePrint 10 System at the RTSF Genomics Core at Michigan State University. All cell lines used in the study tested negative for Mycoplasma as determined by the Mycoplasma Plus PCR Primer Set (Agilent).

Assessment of cellular proliferation

Cells (1×10^3) were seeded in 96-well plates in 100 µl RPMI media supplemented with 10% FBS and penicillin/streptomycin. The following day, plates were incubated in the IncuCyte ZOOM™ (Essen BioScience) for real-time imaging, with three fields imaged per well under 10x magnification every two hours for a total of 120 hours. Data were analyzed using the IncuCyte Confluence version 1.5 software (Essen BioScience), which quantifies cell surface area coverage as confluence values. IncuCyte experiments were performed in triplicate. A representative growth curve is shown for each condition.

Western blot analysis

Cells from *in vitro* culture were lysed in RIPA lysis buffer (#89900 Thermo Fisher) supplemented with protease and phosphatase inhibitor cocktail tablets (Roche). The antibodies used for western blotting

included those against: HSP90 (H114) (Santa Cruz Biotech Cat#sc-7947), phosphorylated Akt (Ser473) (Cell Signaling Cat#4060), Akt (Cell Signaling Cat#9272), phosphorylated ERK1/2 (Cell Signaling Cat#4370), ERK1/2 (Cell Signaling Cat#4695), phosphorylated MEK (Cell Signaling Cat#9154), MEK (Cell Signaling Cat#8727), phosphorylated S6 (Ser235/236) (Cell Signaling Cat#4858), S6 ribosomal protein (Cell Signaling Cat#2217), β-catenin (Cell Signaling Cat#8480), p27 (Cell Signaling Cat#3688), cMyc (Cell Signaling Cat#2276), anti-rabbit IgG, HRP-linked secondary antibody Cell Signaling (Cat#7074P2), ECL Sheep anti-Mouse IgG, HRP-linked secondary antibody (GE Healthcare Cat#NA931V), ECL Donkey anti-Rabbit IgG, HRP-linked secondary antibody (GE Healthcare Cat#NA934V). Western blotting showed in the manuscript are representative of at least three independent experiments.

SiRNA-mediated gene knockdown

Cells ($1{,}5x10^6$) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, media was replaced by antibiotic free full media and the mixture of siRNA (scrambled, CSNK2A1, Dharmacon) at a final concentration of 20nM together with DharmaFECT 1 was added after allowing 30min of complex formation in serum-free media. Knockdown efficacy was assessed by Western blot and qRT-PCR after 48hrs of transfection. For treatment experiments cells were harvested and re-seeded after 48hrs of siRNA treatment and treated with selumetinib for another 24 to 96hrs.

TOPFlash reporter assay

Cells ($1{,}5x10^6$) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, cells were transiently transfected with 1μg of M50 Super 8x TOPFlash reporter plasmid, 100ng of a pRL Renilla Luciferase control reporter plasmid (Promega) and FuGENE HD (Promega). M50 Super 8x TOPFlash was a gift from Randall Moon (Addgene plasmid #12456). After 24hrs, cells were washed with PBS and full media was added for another 24hrs without or with Mek inhibitor (selumetinib, 1μm). Luciferase activity was measured with the Dual Luciferase reporter assay (Promega).

Results

Cancer cells with different KRAS mutant isoforms differ in their drug sensitivities to MEK/ERK inhibitors

We first interrogated the publicly available pharmacogenomics dataset CGP, which includes mutational and pharmacological profiles of >1000 human cancer cell lines treated with 265 anti-cancer drugs [23]. Drug sensitivities are represented by the natural logarithm of the drug's IC50 value. To investigate MEK/ERK inhibitors sensitivity for different KRAS mutant isoforms, we grouped all cancer cells based on their KRAS mutation status, and then used the Kruskal-Wallis H-test to compare drug sensitivities between multiple groups and the t-test to compare drug sensitivities between two groups.

KRAS mutant cancer cell lines were divided into 12 groups, respectively, with A146T, G12A, G12C, G12D, G12R, G12S, G12V, G13C, G13D, K117N, Q61H, or Q61L mutations. We found that MEK/ERK inhibitors drug sensitivities vary in cell lines with different KRAS mutations, including CI-1040, refametinib (RDEA119), PD-0325901, selumetinib, trametinib, and VX-11e (Fig. 2). Cells lines with G12R mutation were in general more sensitive to MEK inhibitors in comparison with other types of KRAS mutations (Fig. 2a-f). To address the question if also the tissue of origin influences response to MAPK pathway inhibition, we furthermore investigated the effect of different KRAS mutations on drug sensitivities in the two major cancer histotypes of lung and pancreatic cancer. Differences in MEK/ERK inhibitors sensitivities across the different types of KRAS mutations were observed in both cancer types, being pancreatic cancer cells with G12R mutation (Additional file 1: Figure S1a-c) and lung cancer cells with G12A mutation (Additional file 1: Figure S1d-e) most sensitive to MEK inhibition, respectively.

We surveyed the datasets of primary LUAD patients from TCGA and metastatic LUAD patients from MSK-IMPACT to investigate the prevalence of different KRAS mutational isoforms (Fig. 3). 75 (33%) of patients with primary LUAD and 241 (27%) of patients with metastatic LUAD patients harbor KRAS mutations, respectively. In total, we observed ten different types of KRAS mutations, including G12C, G12D, G12A, G12F, G12R, G12S, G12V, G12Y, Q61L, D33E, in the primary LUAD TCGA dataset (Additional file 2: Table S1). Whereas KRAS(G12C) is the dominant mutation in patients with primary KRAS mutant NSCLC (48.00%, Fig. 3a), patients with metastatic LUAD exhibit a more complex pattern

of KRAS mutations. Among 19 types of KRAS mutations, 11 types (A146T, A146V, A59T, AG59GV, G13C, G13D, G13E, G13R, G13V, Q61R, and T58I) are exclusively observed in patients with metastatic LUAD but not in primary tumors (Fig. 3b, Additional file3: Table S2). In metastatic LUAD patients, the KRAS(G12C) mutation is also the most prevalent one (42.74%), followed by G12V (15.35%) and G12D (15.35%) mutations.

Pharmacogenomics analysis to identify potential targets in lung cancer harboring KRAS(G12C) mutations

Our above-mentioned analysis of the CGP dataset suggests that cancer cell lines with different KRAS mutations exhibit different sensitivities to MAPK pathway inhibition. In the present analysis, we focused on lung cancer cell lines with KRAS(G12C) mutation, the most dominant KRAS mutation in lung cancer. In our analysis, a total of 12 cell lines were included (see Methods). We developed a computational pipeline to identify novel therapeutic targets for KRAS mutant lung cancer (Fig. 1). In a first step of this pipeline, 1212 genes with association with more than two inhibitors of MAPK signalling were considered as potential targets. In a second step, using the TCGA database, we selected 494 genes which are upregulated in LUAD patients and are associated with poor survival. Finally, we narrowed down the number of genes by requiring them to be part of core cancer pathways as well as to be known drug targets. This algorithm finally led to the identification of 14 potential therapeutic targets for KRAS mutant lung cancer (Fig. 1d), including AARS2, ALKBH2, CARS, CDK8, COMP, CSNK2A1, DARS, EPRS, HDAC1, IARS2, MAPK8, PARS2, RPL8 and YARS (Additional file 4: Table S3).

Among the 14 candidate genes identified by our pharmacogenomics analysis, CSNK2A1 was ranked as the most promising gene. CSNK2A1 encodes for a protein which is a component of the highly conserved serine/threonine protein kinase CK2 alpha. CK2 alpha itself is part of various pathways relevant for cancer cell biology among them Wnt (Fig. 4a) and NF-kappa B signaling [20]. This is especially relevant as there is an increased interest for CSNK2 as a therapeutic target in ongoing clinical trials [24]. The association of CSNK2A1 expression and reduced MEK/ERK inhibitors sensitivity was repeatedly observed for 4 different MEK inhibitors, including 2 replicate datasets for refametinib and selumetinib (Fig. 4b). Importantly,

LUAD patients or LUAD patients with KRAS(G12C) mutation showed an increased expression of CSNK2A1 in comparison with normal lung tissue (Fig. 4c, *p=1.35e-18*). Moreover, LUAD patients with high CSNK2A1 expression had a trend towards poorer overall survival (Additional file 5: Figure S2).

Correlation of CSNK2A1 levels and MEK inhibitor resistance is neither observed in non-KRAS(G12C) mutant lung and pancreatic cancer cells, nor in lung cancer with EGFR, BRAF or NRAS mutations

We next investigated if the correlation between CSNK2A1 expression and MEK inhibitor resistance can also be observed in non-KRAS(G12C) mutant cancer cells. As KRAS(G12V) mutations represent the second most frequent mutation in LUAD, nine KRAS(G12V) mutant lung cancer cell lines from CGP were included in the statistical analysis. No correlation was found between CSNK2A1 expression and drug sensitivity to 7 MEK inhibitors in KRAS(G12V) mutant lung cancer cells (Fig. 5a). Due to the limited sample size for other non-KRAS(G12C) mutations, we pooled the remaining lung cancer cells with non-KRAS(G12C) mutations, for which no positive correlation between CSNK2A1 expression and drug sensitivity was observed (Fig. 5b). As KRAS(G12V) and KRAS(G12D) mutations occur more frequently in pancreatic cancer, we therefore also investigated the correlation of CSNK2A1 levels and MEK inhibitor resistance in KRAS(G12V) or KRAS(G12D) mutant pancreas cancer cells. This analysis also showed no correlation between CSNK2A1 expression and drug sensitivity (Fig. 5c,d).

We furthermore investigated if there is any correlation between CSNK2A1 expression and MEK inhibitor resistance in lung cancer cell lines with other oncogenic mutations affecting the MAPK signaling pathway as for example BRAF, EGFR, or NRAS. However, our analysis showed no correlation between CSNK2A1 expression and MEK inhibitor resistance for BRAF- (Fig. 6a), EGFR- (Fig. 6b), or NRAS-mutant (Fig. 6c) lung cancer cells. Cell lines we used for the analysis were in Additional file 6: Table S4.

CSNK2A1 knockdown reduces proliferation and Wnt/β-catenin reporter activity in KRAS(G12C) mutant lung cancer cells, and increases the anti-proliferative effect of selumetinib

We selected two KRAS(G12C) mutant lung cancer cell lines (Calu1 and H2030) and two non-KRAS(G12C) mutant cell lines (A549 and H2009) to investigate the effect of siRNA-mediated CSNK2A1 knockdown on cell proliferation and efficacy of MAPK pathway inhibition with 1μM of selumetinib. Knockdown of CSNK2A1 (Fig. 7a) alone significantly decreased proliferation of KRAS(G12C) mutant cells Calu1 (Fig. 7b) and H2030 (Fig. 7c) and increased the anti-proliferative activity of simultaneous MEK inhibition in Calu1 cells (Fig. 7b). These effects were not observed in non-KRAS(G12C) mutant lung cancer cell lines (Fig. 7d-f).

As casein kinases have previously been connected to the drug resistance mediating Wnt/β-catenin pathway [25,26], we therefore investigated if CSNK2A1 influences Wnt/β-catenin signaling in KRAS(G12C) mutant lung cancer. We used gene expression profiles of LUAD patients (TCGA) and cancer cell lines (CCLE) [27] and categorized samples into CSNK2A1 high and low expressing groups. Deseq2 [28] was applied to call differentially expressed genes between the two groups. Gene set enrichment analysis (GSEA) [29] was further employed to determine the pathways enriched by a pre-ranked list of all genes, which were sorted by the statistical significance of differential expression defined by the Deseq2 analysis. GSEA showed that the Wnt signaling pathway was significantly enriched in the CSNK2A1 high expressing group in CCLE (Fig. 8a, *p=0.008*) and TCGA (Fig. 8b, *p=0.014*).

To support our computational findings, we also investigated the differences in Wnt/β-catenin signaling parameters between KRAS(G12C) and KRAS(non-G12C) cell lines *in vitro*. After 24hrs of selumetinib treatment, accumulation of the cell cycle inhibitor p27 upon CSNK2A1 knockdown was relatively increased in KRAS(G12C) mutant Calu1 and H2030 cells compared to KRAS(non-G12C) A549 and H2009 cells (Fig. 8c). This suggests that Calu1 and H2030 cells are more dependent on CSNK2A1 to overcome MEK inhibitor induced growth arrest. This is also in agreement with the stronger anti-proliferative effect of CSNK2A1 knockdown itself in cells with KRAS(G12C) mutation (Additional file 7: Figure S3, Fig. 7b,e). Furthermore, transient transfection of the Wnt/TCF reporter plasmid 8xTOPFlash showed stronger reduction of reporter activity in Calu1 cells with KRAS(G12C) mutation than in

KRAS(G12S) mutant A549 cells upon CSNK2A1 knockdown and upon simultaneous treatment with selumetinib (Fig. 8d).

Discussion

In this study, we used pharmacogenomics data to systematically unravel the heterogeneity of responses to MAPK pathway inhibition due to different KRAS mutation isoforms. Subsequently, we developed a pharmacogenomics analysis pipeline to identify novel targets for the subgroup of KRAS(G12C) mutant lung cancer. Our computational pipeline identified a correlation between CSNK2A1 expression and MEK inhibitor resistance in KRAS(G12C) mutant cells, a finding that was exclusively observed in this mutational subset of lung cancer cells, but not in KRAS(non-G12C) mutant cells and neither in tumor cells harboring other oncogenic mutations as e.g. EGFR, BRAF, or NRAS. This suggests that the correlation between CSNK2A1 expression and MEK inhibitor resistance may depend on the context of KRAS(G12C) mutation in lung cancer. A pan-cancer analysis of the TCGA dataset showed that CSNK2A1 is upregulated in a wide range of cancers (Additional file 8: Figure S4) and that pancreatic cancer patients with high intratumoral CSNK2A1 expression have a worse overall and relapse-free survival (Additional file 9: Figure S5).

CSNK2A1 encodes for a protein that is a component of the highly conserved serine/threonine protein kinase CK2 alpha. Previous studies have identified CSNK2 as an oncogene when overexpressed in mice, playing a key role in the pathogenesis of cancer, including breast, lung, colon, and prostate cancer, as well as hematologic malignancies [19,20,30]. Moreover, a cancer context-dependent effect of CK2 on signaling pathways such as Wnt signaling [20], JAK/STAT [31], NF-κB [20], and PTEN/PI3K/Akt-PKB [32,33] has been described in the past. In the Wnt pathway, CK2 acts by phosphorylating and stabilizing Dvl and β-catenin and promotes T-cell factor/lymphoid enhancer-binding factor (TCF) DNA binding in the nucleus (Fig. 4a). Based of these observations, CK2 has recently arisen as a promising candidate for targeted therapy, with two CK2 inhibitors in ongoing clinical trials (https://clinicaltrials.gov/ct2/results?cond=&term=CK2&cntry=&state=&city=&dist=). Our results suggest that accurate patients stratification will likely be necessary in order to observe significant benefit upon CK2

inhibition - alone or in combination - in a subset of patients with a favorable intratumoral genetic context.

In agreement with our computational results, CSNK2A1 knockdown significantly reduced the proliferation of KRAS(G12C) mutant lung cancer cells, an effect that was not observed in cells with non-G12C KRAS mutations (Fig. 7). This identifies CSNK2A1 as an interesting target *per se* in KRAS(G12C) mutant NSCLC cell lines. Furthermore, simultaneous CSNK2A1 knockdown and MEK inhibition with selumetinib increased the anti-proliferative effect of selumetinib in KRAS(G12C) mutant but not in KRAS(non-G12C) mutant cells. Mechanistically, our analysis including transient 8xTOPFlash reporter transfection shows that CSNK2A1 mediates TCF transcriptional activity in KRAS(G12C) mutant lung cancer (Fig. 8d). The importance of CSKN2A1 for Wnt signaling is also supported by our gene-set enrichment analysis (GSEA) of the CCLE and TCGA datasets which show significant enrichment for the Wnt signaling pathway (Fig. 8a, b).

For a given oncogene, different types of nonsynonymous or indel variants impact differently on the biological function of the respective protein. This results in different oncogenic activities in cancer cells with different oncogenic mutations and challenges the development of effective targeted therapies. Therefore, predicting novel therapeutic targets by considering the mutational heterogeneity of cancer histotypes will help to guide therapeutic decision-making and improve treatment outcomes. Although the pharmacogenomics approach suggested by us here was applied to KRAS(G12C) mutant lung cancer cells, this pipeline can potentially be extended to other mutant KRAS isoforms given that a large enough sample size is available for statistical analysis.

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to HW), the Lung Cancer Research Foundation (to CA) and a Mildred-Scheel postdoctoral fellowship from the German Cancer Aid Foundation (70111755 to JK).

Declarations of interests

The authors declare no competing financial interests.

Authors' contributions

HW, JK and CA conceived the hypothesis. HW and QL designed and performed the data analysis. YX, ZC, JZ, XC, and YD collected and preprocessed the data. JK and CA performed experimental validation. HW, JK and CA wrote the manuscript. PJ provided the resources of experimental validation and helpful suggestions on the validation.

Reference

1. Baines AT, Xu D, Der CJ. Inhibition of Ras for cancer treatment: the search continues. *Future Med Chem* 2011; **3**(14): 1787-808.

2. Young A, Lyons J, Miller AL, Phan VT, Alarcon IR, McCormick F. Ras signaling and therapies. *Adv Cancer Res* 2009; **102**: 1-17.

3. Janes MR, Zhang J, Li LS, et al. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell* 2018; **172**(3): 578-89 e17.

4. Sun C, Hobor S, Bertotti A, et al. Intrinsic resistance to MEK inhibition in KRAS mutant lung and colon cancer through transcriptional induction of ERBB3. *Cell Rep* 2014; **7**(1): 86-93.

5. Lito P, Saborowski A, Yue J, et al. Disruption of CRAF-mediated MEK activation is required for effective MEK inhibition in KRAS mutant tumors. *Cancer Cell* 2014; **25**(5): 697-710.

6. Lito P, Rosen N, Solit DB. Tumor adaptation and resistance to RAF inhibitors. *Nat Med* 2013; **19**(11): 1401-9.

7. Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 2010; **464**(7287): 427-30.

8. Montagut C, Sharma SV, Shioda T, et al. Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. *Cancer Res* 2008; **68**(12): 4853-61.

9. Johannessen CM, Boehm JS, Kim SY, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* 2010; **468**(7326): 968-72.

10. Marusiak AA, Edwards ZC, Hugo W, et al. Mixed lineage kinases activate MEK independently of RAF to mediate resistance to RAF inhibitors. *Nat Commun* 2014; **5**: 3901.

11. Burgess MR, Hwang E, Mroue R, et al. KRAS Allelic Imbalance Enhances Fitness and Modulates MAP Kinase Dependence in Cancer. *Cell* 2017; **168**(5): 817-29 e15.

12. Ambrogio C, Kohler J, Zhou ZW, et al. KRAS Dimerization Impacts MEK Inhibitor Sensitivity and Oncogenic Activity of Mutant KRAS. *Cell* 2018; **172**(4): 857-68 e15.

13. Garassino MC, Marabese M, Rusconi P, et al. Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer. *Ann Oncol* 2011; **22**(1): 235-7.

14. Nadal E, Chen G, Prensner JR, et al. KRAS-G12C mutation is associated with poor outcome in surgically resected lung adenocarcinoma. *J Thorac Oncol* 2014; **9**(10): 1513-22.

15. Lu S, Jang H, Nussinov R, Zhang J. The Structural Basis of Oncogenic Mutations G12, G13 and Q61 in Small GTPase K-Ras4B. *Sci Rep* 2016; **6**: 21949.

16. Smith MJ, Neel BG, Ikura M. NMR-based functional profiling of RASopathies and oncogenic RAS mutations. *Proc Natl Acad Sci U S A* 2013; **110**(12): 4574-9.

17. Haigis KM. KRAS Alleles: The Devil Is in the Detail. *Trends Cancer* 2017; **3**(10): 686-97.

18. Ruzzene M, Pinna LA. Addiction to protein kinase CK2: a common denominator of diverse cancer cells? *Biochim Biophys Acta* 2010; **1804**(3): 499-504.

19. Seldin DC, Landesman-Bollag E, Farago M, Currier N, Lou D, Dominguez I. CK2 as a positive regulator of Wnt signalling and tumourigenesis. *Mol Cell Biochem* 2005; **274**(1-2): 63-7.

20. Dominguez I, Sonenshein GE, Seldin DC. Protein kinase CK2 in health and disease: CK2 and its role in Wnt and NF-kappaB signaling: linking development and cancer. *Cell Mol Life Sci* 2009; **66**(11-12): 1850-7.

21. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011; **12**: 323.

22. Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biology* 2010; **11**(10): R106.

23. Garnett MJ, Edelman EJ, Heidorn SJ, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* 2012; **483**(7391): 570-5.

24. Rabalski AJ, Gyenis L, Litchfield DW. Molecular Pathways: Emergence of Protein Kinase CK2 (CSNK2) as a Potential Target to Inhibit Survival and DNA Damage Response and Repair Pathways in Cancer Cells. *Clin Cancer Res* 2016; **22**(12): 2840-7.

25. Ponce DP, Yefi R, Cabello P, et al. CK2 functionally interacts with AKT/PKB to promote the beta-catenin-dependent expression of survivin and enhance cell survival. *Mol Cell Biochem* 2011; **356**(1-2): 127-32.

26. Yefi R, Ponce DP, Niechi I, et al. Protein kinase CK2 promotes cancer cell viability via up-regulation of cyclooxygenase-2 expression and enhanced prostaglandin E2 production. *J Cell Biochem* 2011; **112**(11): 3167-75.

27. Barretina J, Caponigro G, Stransky N, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 2012; **483**(7391): 603-7.

28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; **15**(12): 550.

29. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005; **102**(43): 15545-50.

30. Zhang HX, Jiang SS, Zhang XF, et al. Protein kinase CK2alpha catalytic subunit is overexpressed and serves as an unfavorable prognostic marker in primary hepatocellular carcinoma. *Oncotarget* 2015; **6**(33): 34800-17.

31. Zheng Y, Qin H, Frank SJ, et al. A CK2-dependent mechanism for activation of the JAK-STAT signaling pathway. *Blood* 2011; **118**(1): 156-66.

32. Di Maira G, Salvi M, Arrigoni G, et al. Protein kinase CK2 phosphorylates and upregulates Akt/PKB. *Cell Death Differ* 2005; **12**(6): 668-77.

33. Park JH, Kim JJ, Bae YS. Involvement of PI3K-AKT-mTOR pathway in protein kinase CKII inhibition-mediated senescence in human colon cancer cells. *Biochem Biophys Res Commun* 2013; **433**(4): 420-5.

Figures and Tables Legend

Figure 1. A pharmacogenomics analysis identifies the potential therapeutic targets in KRAS-mutant lung cancer cells. A, pharmacogenomics data and analysis for identifying genes whose high expression associates with decreased drug sensitivity. B, TCGA data analysis for evaluating the expression of genes in lung adenocarcinoma (LUAD) patients as well as their correlation with clinical prognosis. C, Cancer core pathway and DrugBank information for further investigating the biological relevance of candidate genes. D, Overall integrative analysis for determining the potential therapeutic targets.

Figure 2. Pan-cancer analysis of drug sensitivities to MEK/ERK inhibitors, including CI-1040, refametinib, PD-0325901, selumetinib, trametinib, and VX-11e, in cancer cells with different types of KRAS mutations. The p value of the multiple-groups comparison is indicated. A symbol * denotes the pairwise comparison with p value smaller than 0.05.

Figure 3. **Frequencies of different KRAS mutations in the primary LUAD patients in TCGA dataset and the metastatic LUAD patients in MSK-IMPACT dataset.** KRAS(G12C) is the most common mutation (>40%) across LUAD patients.

Figure 4. A pharmacogenomics analysis identifies CSNK2A1 as a potential therapeutic target in KRAS-mutant lung cancer cells. A, The protein encoded by CSNK2A1 is a serine/threonine protein kinase, which is involved in various cellular processes, including the Wnt signalling pathway. B, High expression of CSNK2A1 is associated with decreased drug sensitivity to MEK inhibitors refametinib, selumetinib, CI-1040, and trametinib. C, CSNK2A1 expression is significantly higher in LUAD compared to normal lung tissue.

Figure 5. (A) Expression of CSNK2A1 in lung cancer cell lines with KRAS(G12V) mutation, the second most frequent mutation in lung cancer, is not correlated with drug sensitivity to MEK inhibitors. (B) Expression of CSNK2A1 in other non-G12C mutant lung cancer cell lines, is not positively correlated with drug sensitivity to MEK inhibitors. (C) Expression of CSNK2A1 in pancreatic cancer cell lines with KRAS(G12V) mutation, the most frequent mutation in pancreatic cancer, is not correlated with drug sensitivity to MEK inhibitors. (D) Expression of CSNK2A1 in pancreatic cancer cell lines with KRAS(G12D) mutation, the second most frequent mutation in pancreatic cancer, is not correlated with drug sensitivity to MEK inhibitors.

Figure 6. Expression of CSNK2A1 is not correlated with MEK inhibitor sensitivity in lung cancer cell lines with BRAF (A), EGFR (B) or NRAS (C) mutations.

Figure 7. CSNK2A1 Knockdown reduces the proliferation of KRAS(G12C) mutant lung cancer cells and increases the anti-proliferative effect of the MEK inhibitor selumetinib. (A) Western blot analysis of CSNK2A1 protein in KRAS(G12C) mutant cell lines Calu1 and H2030 transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1 μm) treatment. Growth rates of Calu1 (B) and H2030 (C) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA (red and grey) without or with simultaneous selumetinib (1 μm) treatment. Phase-contract images are shown at the bottom. (D) Western blot analysis of CSNK2A1 protein in KRAS(G12S) mutant cell line A549 and in KRAS(G12A) mutant cell lines H2009 transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1 μm) treatment. Growth rates of A549 (E) and H2009 (F) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA (red and grey) without or with simultaneous selumetinib (1 μm) treatment. Phase-contract images are shown at the bottom.

Figure 8. CSNK2A1 knockdown inhibits the activation of Wnt/β-catenin signaling in KRAS(G12C) mutant lung cancer cells. GSEA analysis based on CCLE lung cancer cells (A) and TCGA LUAD patients (B) with KRAS mutation shows that Wnt signaling pathway is enriched in tumors with high CSNK2A1 expression. (C) Western blot analysis shows increased p27 induction upon selumetinib

treatment in siCSNK2A1 treated Calu1 and H2030 compared to A549 and H2209. (D) Reporter activity of Wnt/TCF reporter assay in Calu1 KRAS(G12C) and A549 KRAS(G12S). Student t-test are performed, with a symbol *, **, and **** respectively representing the comparison with p value smaller than 0.05, 0.01 and 0.0001.

Additional file1: Figure S1. Pan-cancer analysis investigates the drug sensitivity to MEK/ERK inhibitors, including CI-1040, refametinib, PD-0325901, selumetinib, trametinib, and VX-11e, of the different types of KRAS mutations in pancreatic cancer and lung cancer cell lines. The p value of the multiple-groups comparison is given. A symbol * denotes the pairwise comparison with p value smaller than 0.05.

Additional file2: Table S1. The prevalence of different KRAS mutational isoforms in primary LUAD patients

Additional file3: Table S2. The prevalence of different KRAS mutational isoforms in metastatic LUAD patients

Additional file4: Table S3. Fourteen potential therapeutic targets for KRAS mutant lung cancer.

Additional file5: Figure S2. LUAD patients with higher expression of CSNK2A1 tend to have shorter overall survival.

Additional file6: Table S4. Information of cell lines with KRAS(G12C) mutation in lung cancer, with KRAS(G12V) mutation in lung cancer, with KRAS(non-G12C) mutation in lung cancer, with KRAS(G12V) mutation in pancreatic cancer, with KRAS(G12D) mutation in pancreatic cancer, with BRAF mutation in lung cancer, with EGFR mutation in lung cancer, and with NRAS mutation in lung cancer.

Additional file7: Figure S3. Growth rates of Calu1 KRAS(G12C) (a) and A549 KRAS(G12S) (b) transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1umettreatment. Student t-test are performed, with a symbol *, ****, and n.s. respectively representing the comparison with p value smaller than 0.05, 0.0001 and greater than 0.05.

Additional file8: Figure S4. Expression of CSNK2A1 is upregulated in a wide range of cancers.

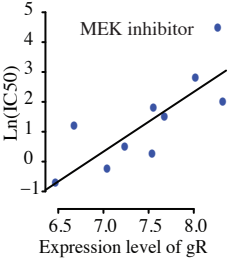
Additional file9: Figure S5. Pancreatic cancer patients with higher expression of CSNK2A1 significantly have poorer survival.

Figure1

Click here to download Figure: Fig1.analysis pipeline.pdf

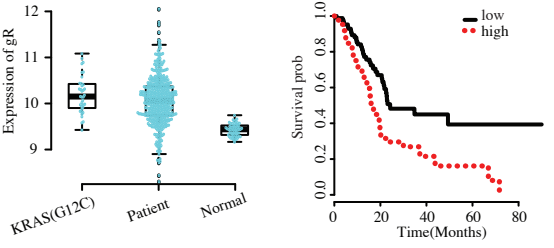
a

Pharmacogenomics data (CGP dataset)
* 12 KRAS(G12C) mutant lung cell lines
* 6 MEK inhibitors, 2 ERK inhibitors
* genome-wide gene expression



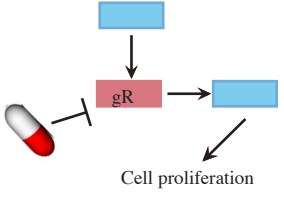
b

TCGA data (LUAD patients)
* 36 KRAS(G12C) mutant patients
* 576 samples (517 patients vs. 59 normal)
* genome-wide gene expression
* clinical prognosis



c

Cancer core pathway
* KEGG
* REACTOME
* BIOCARTA et al.
DrugBank



d

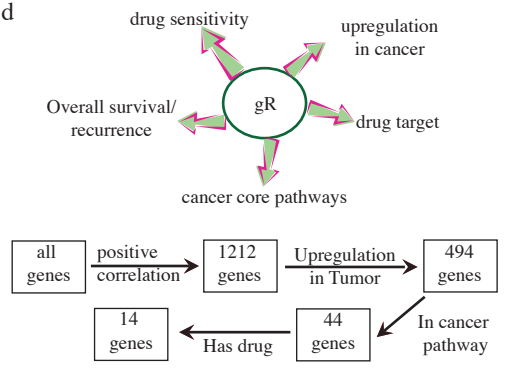


Figure2

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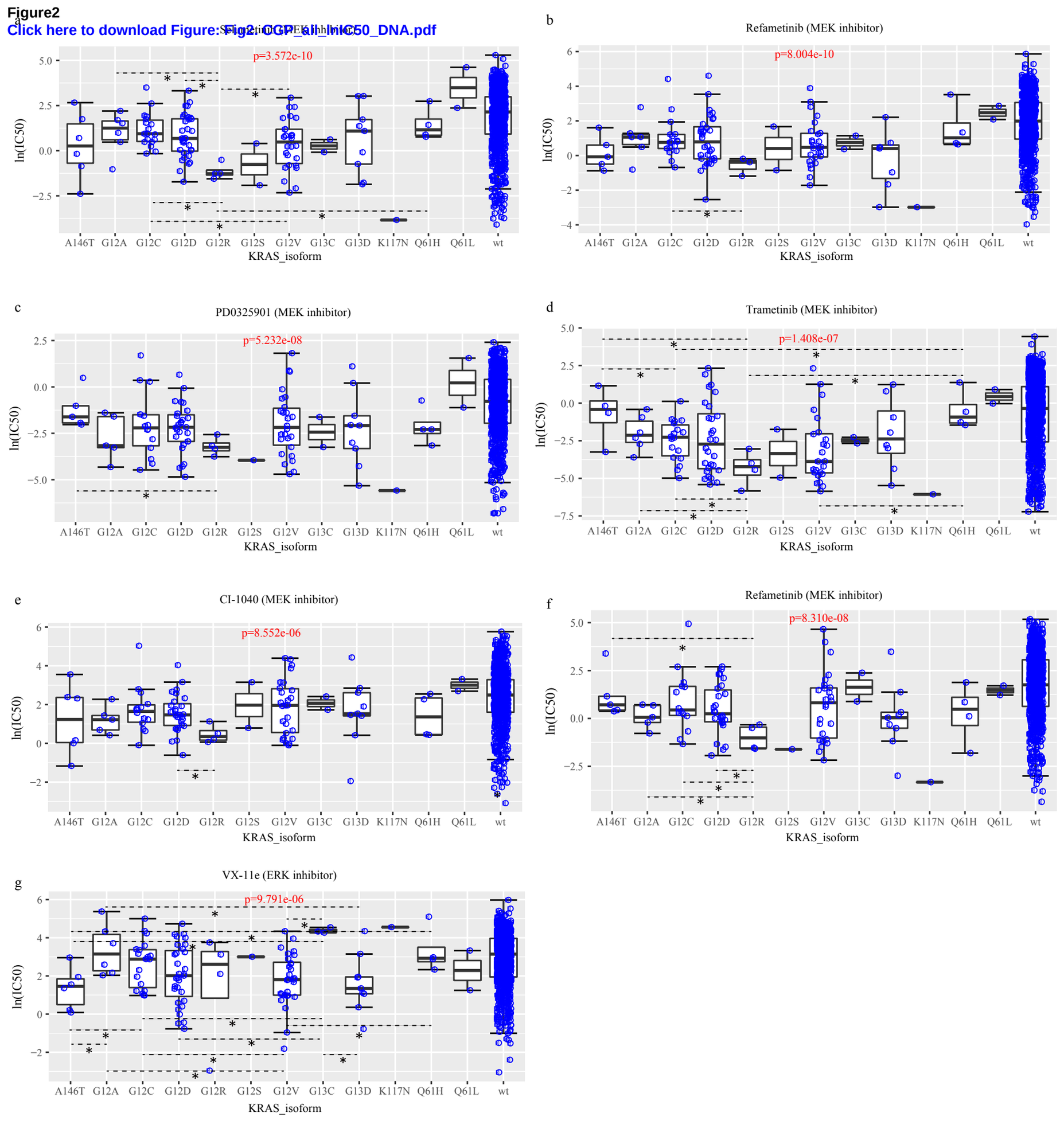

Figure3

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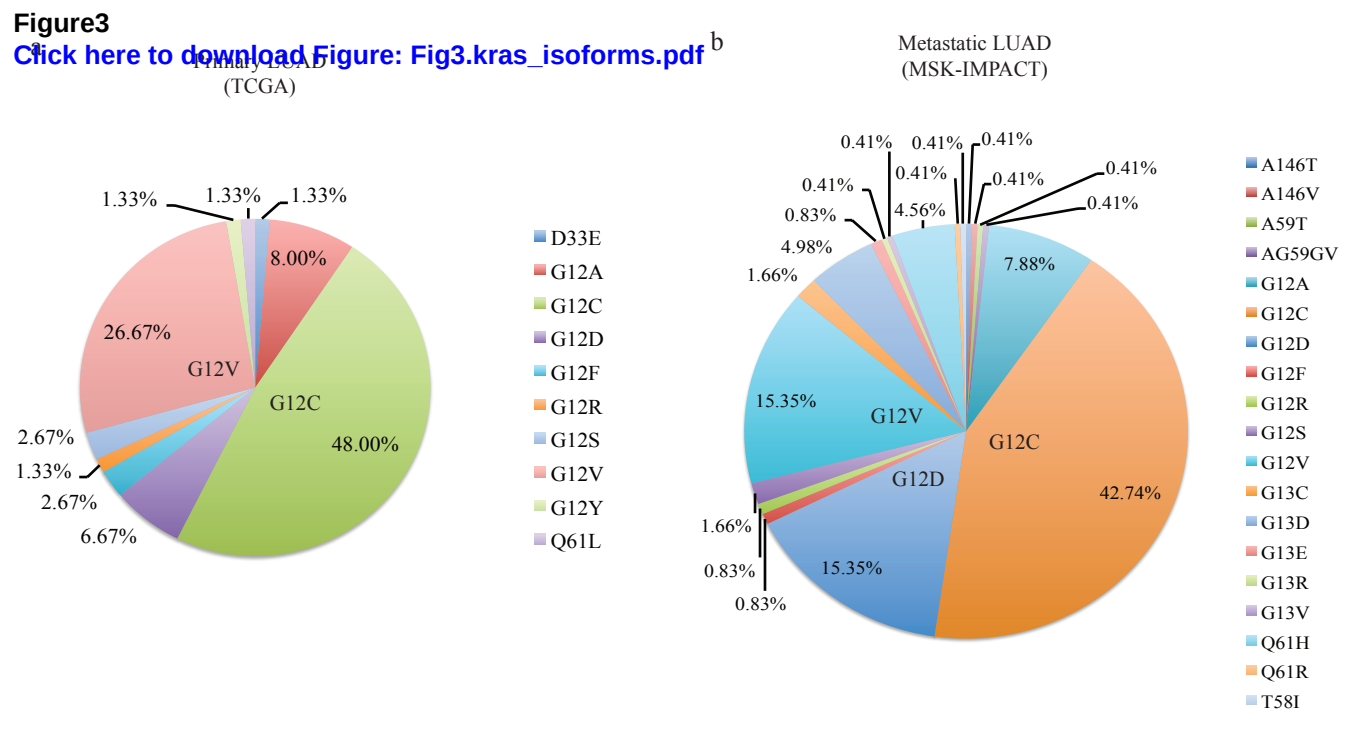

Figure4

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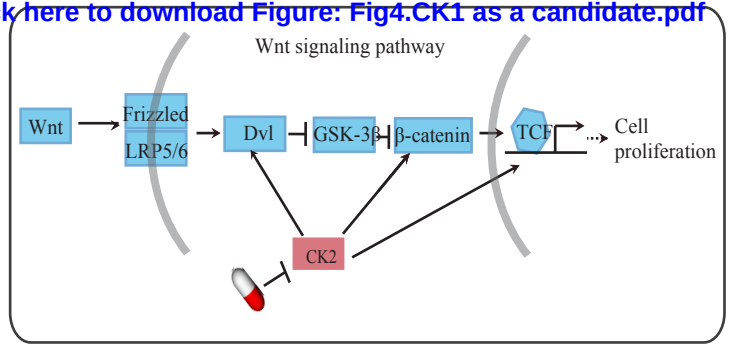

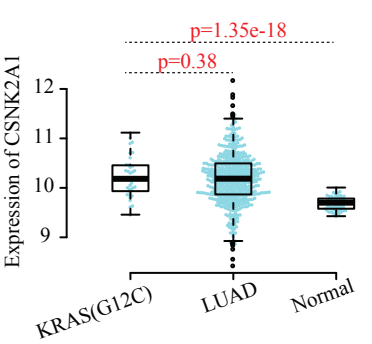

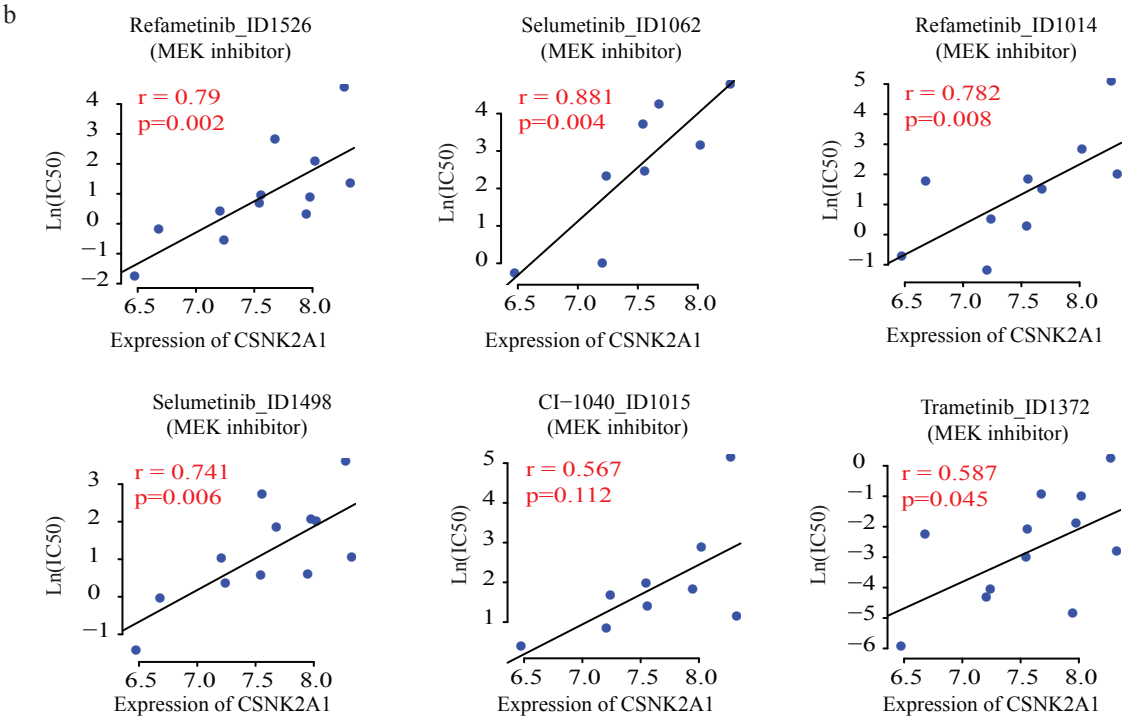

Figure5

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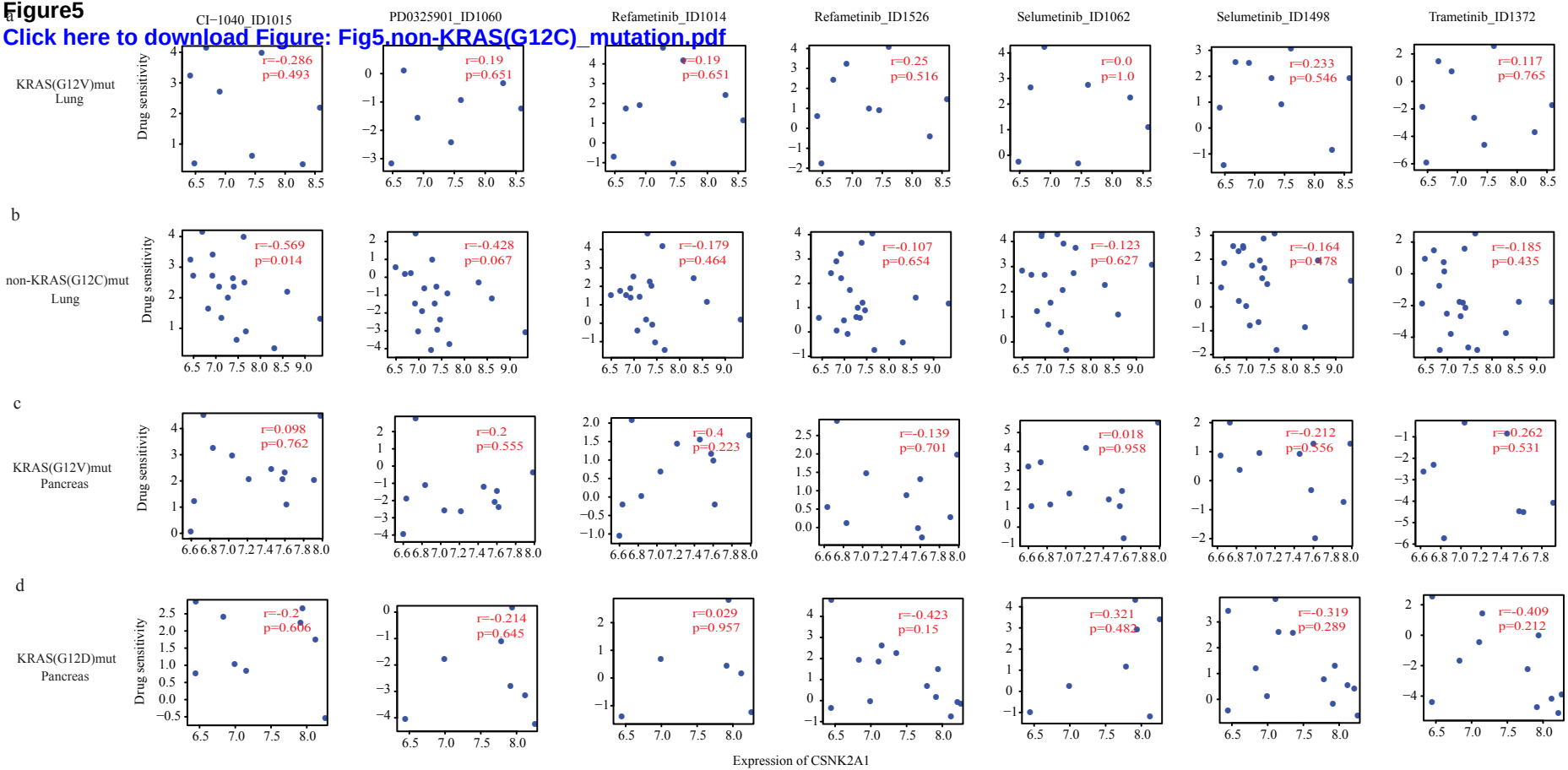

Figure6

Click here to download Figure: Fig6. BRAF_EGFR_NRAS_mutation.pdf

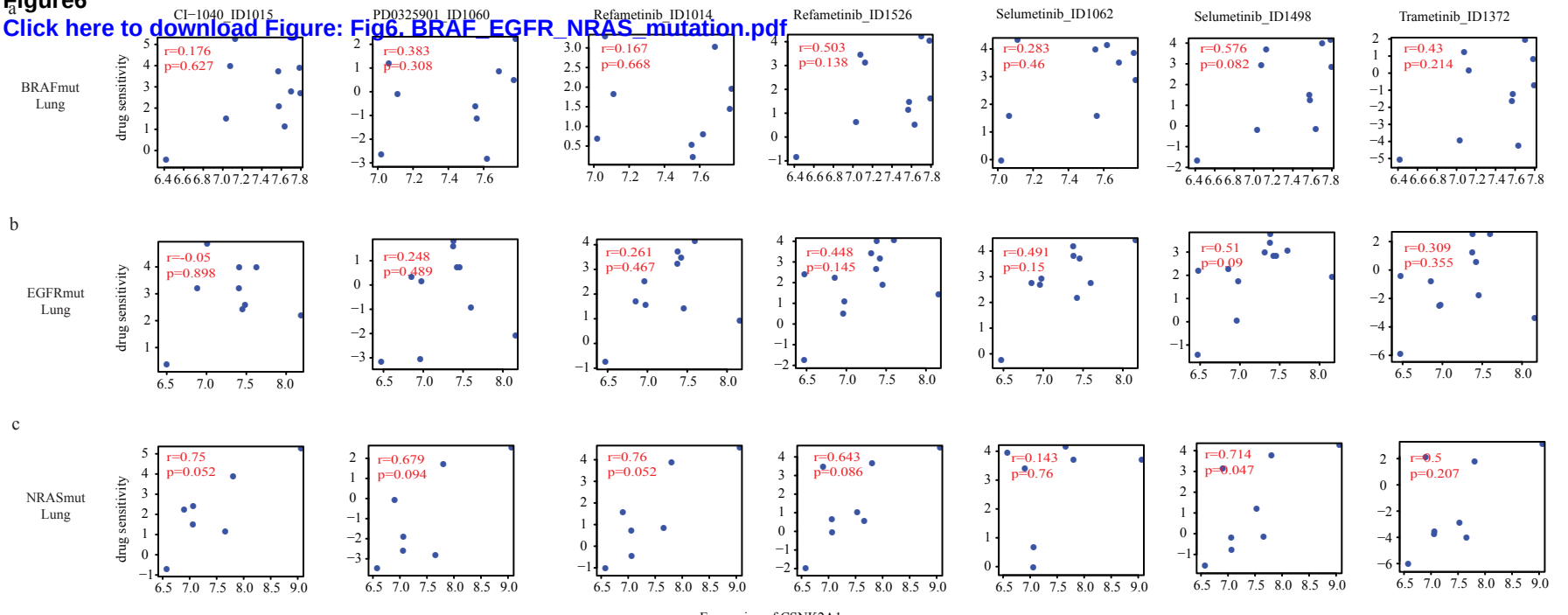

Figure7

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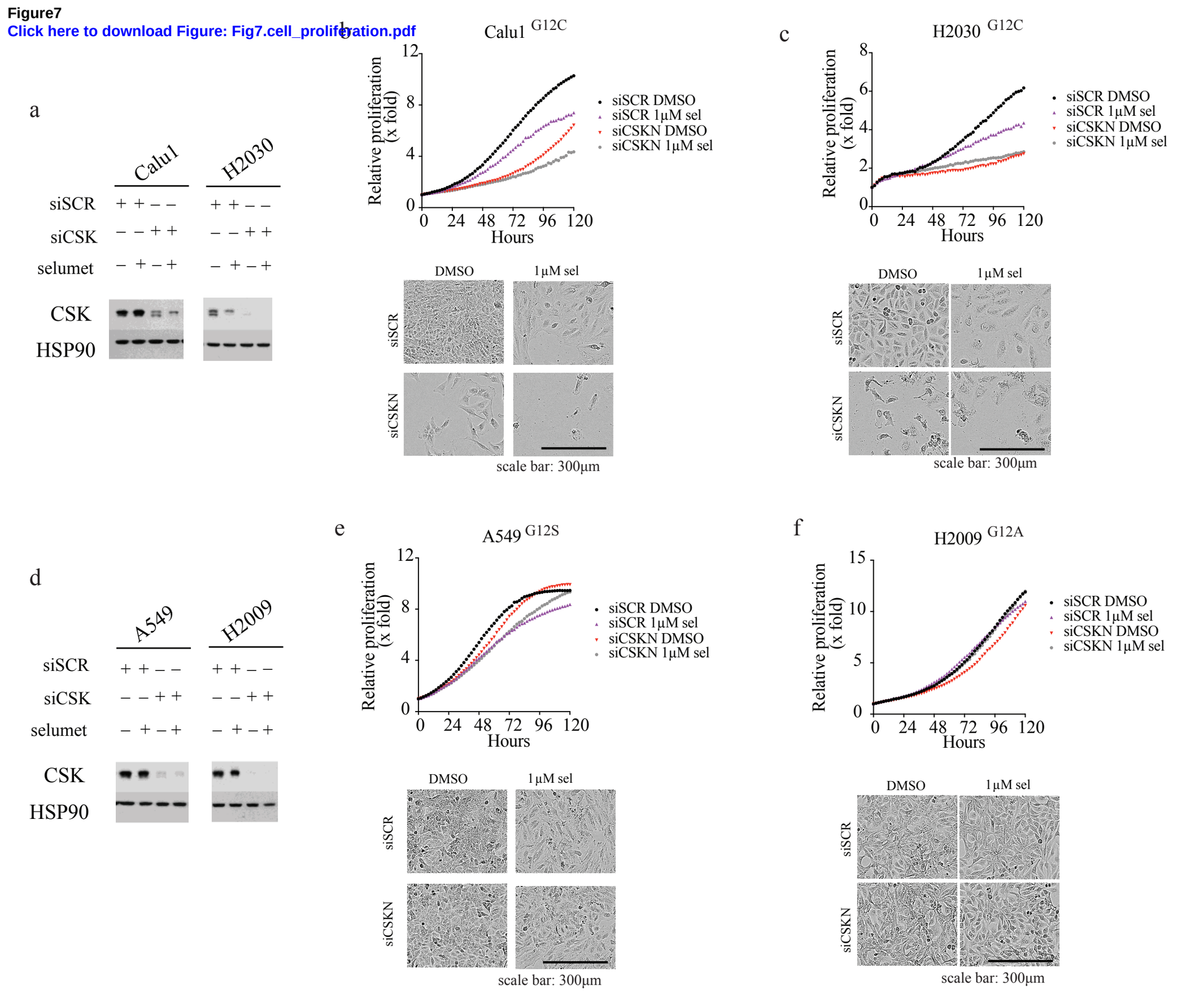

Figure8

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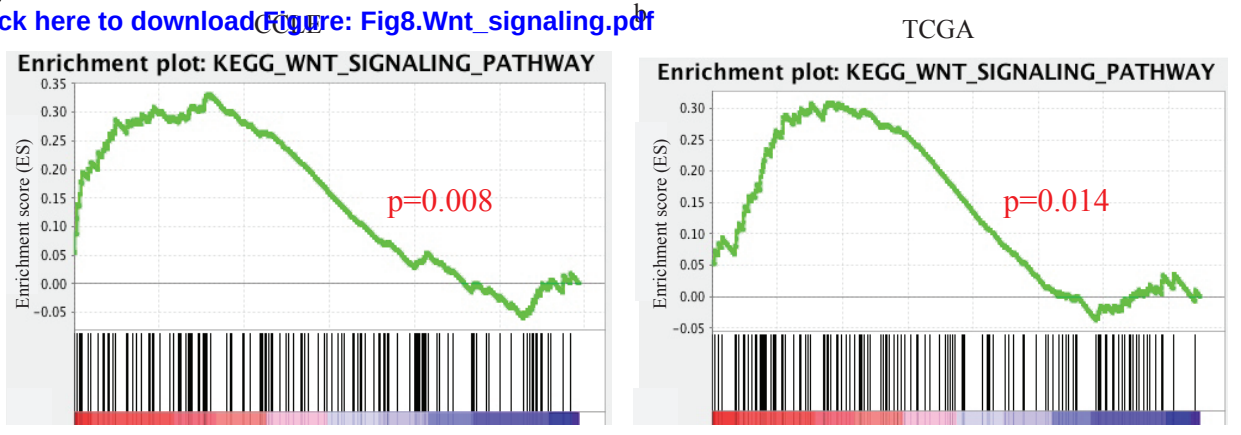

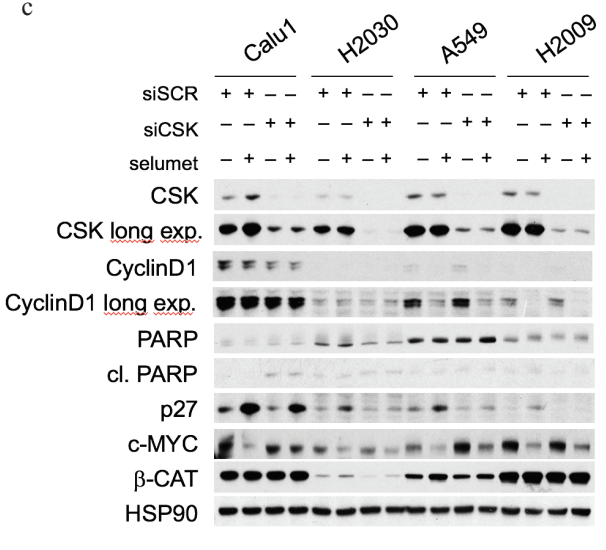

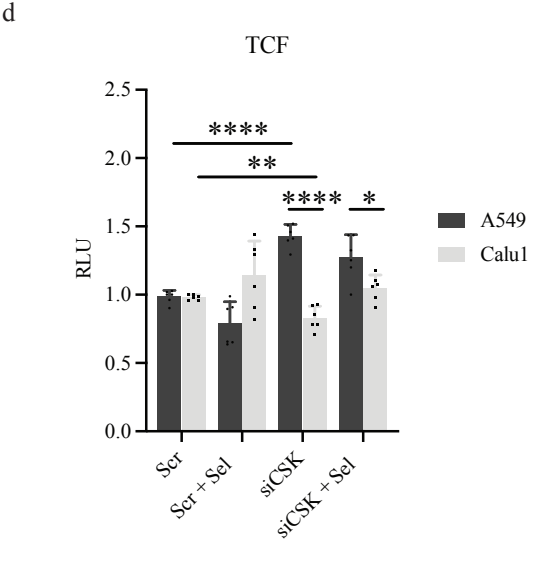

Supplementary Figure S1

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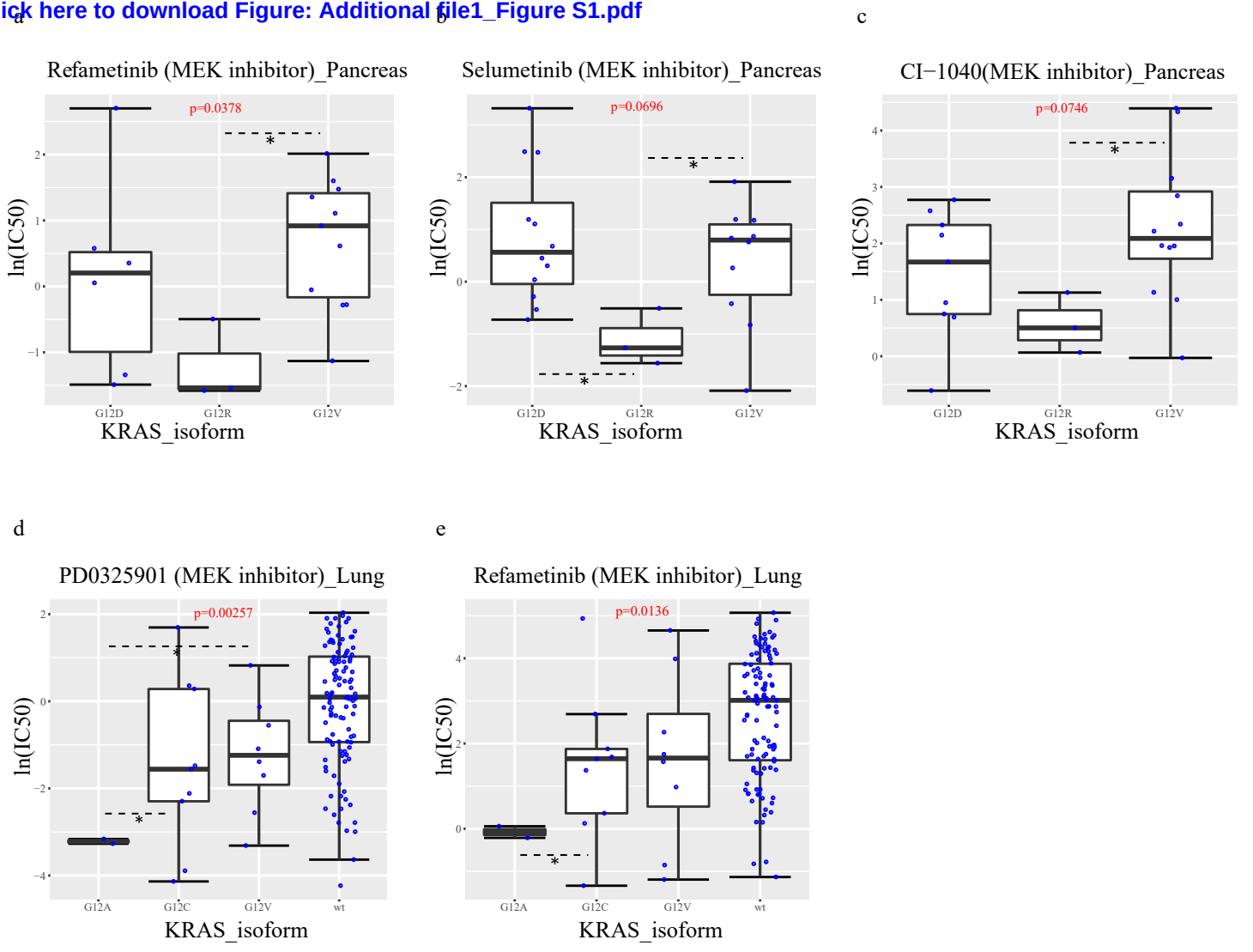

Supplementary Figure S2

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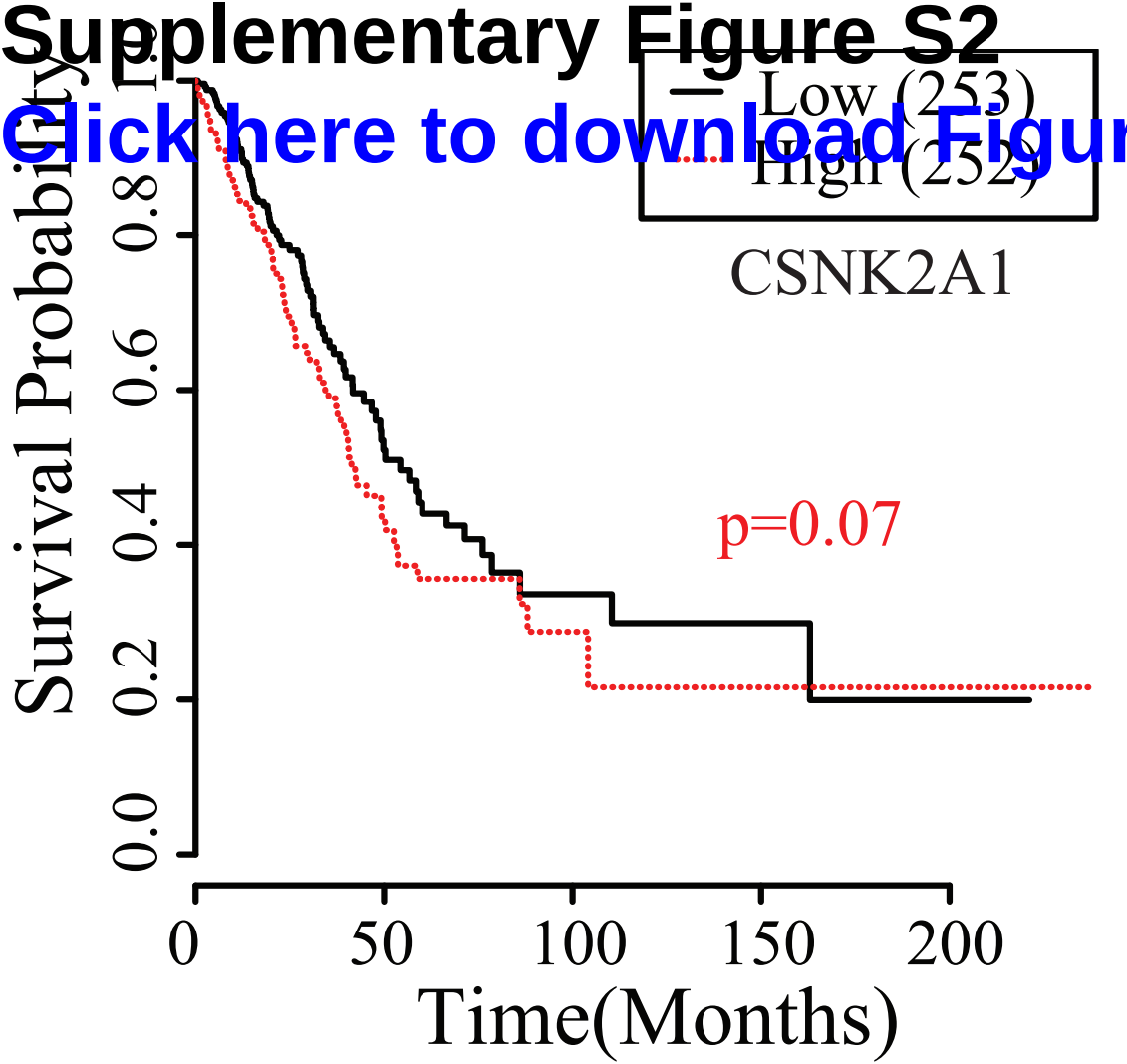

Supplementary Figure S3

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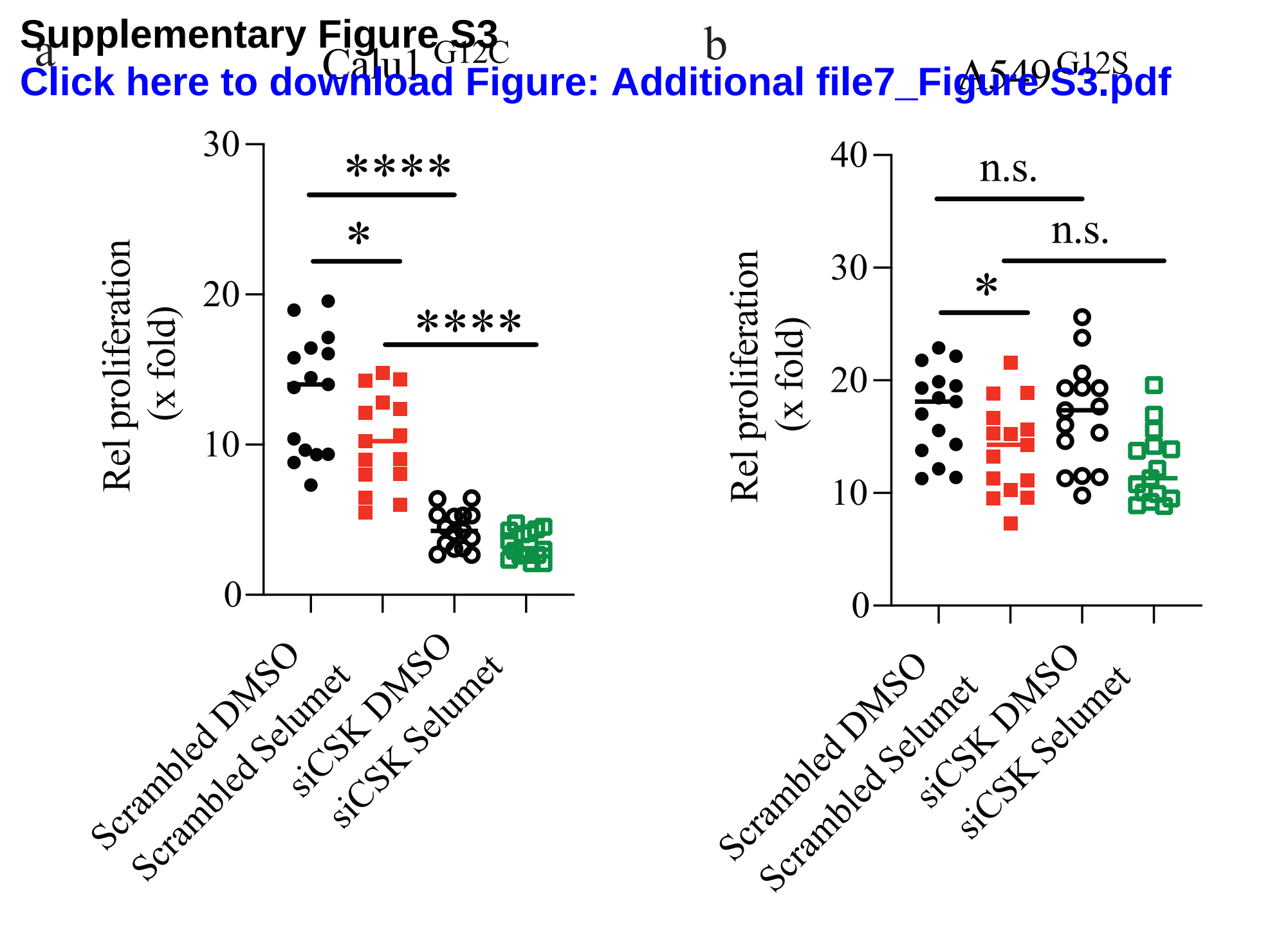

Supplementary Figure S4

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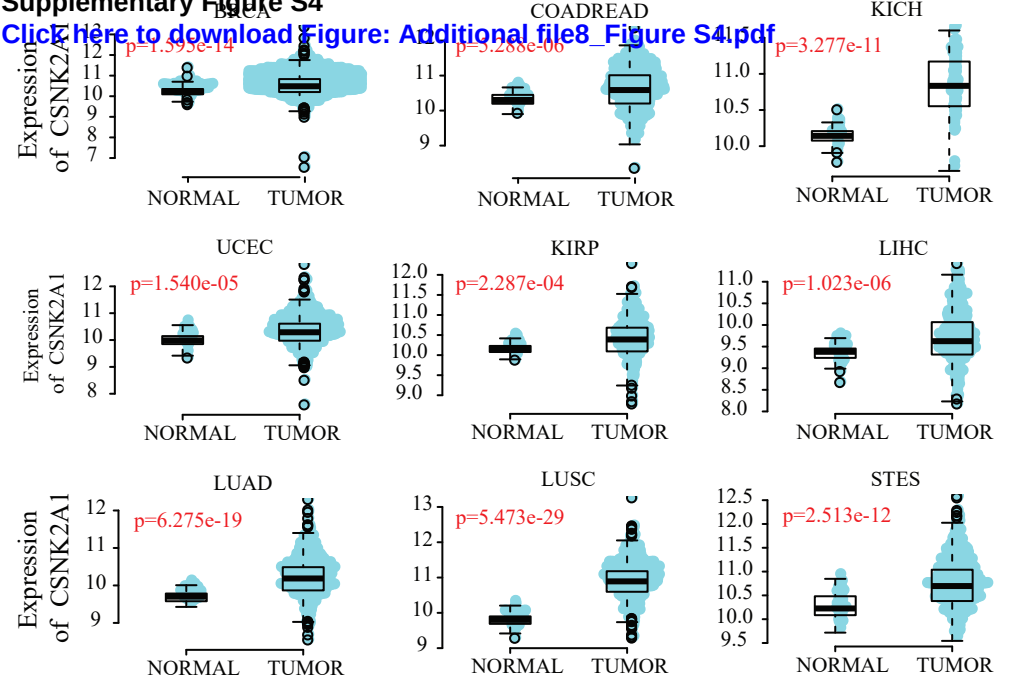

Supplementary Figure S5

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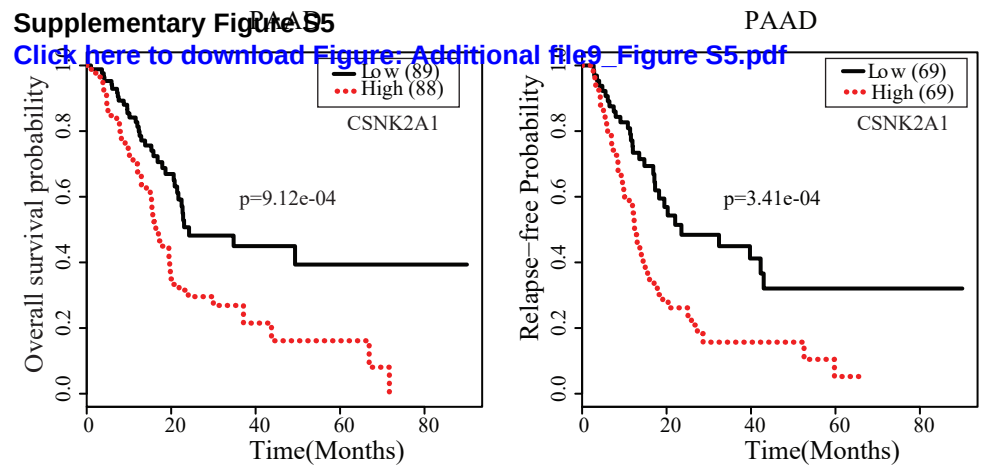

Supplementary Table S1

Table S1. The prevalence of different KRAS mutational isoforms in primary LUAD patients

KRAS mutation	# of patients
D33E	1
G12A	6
G12C	36
G12D	5
G12F	2
G12R	1
G12S	2
G12V	20
G12Y	1
Q61L	1
total	75

Supplementary Table S2

Table S2. The prevalence of different KRAS mutational isoforms in metastatic

KRAS mutation	# of patients
A146T	1
A146V	1
A59T	1
AG59GV	1
G12A	19
G12C	103
G12D	37
G12F	2
G12R	2
G12S	4
G12V	37
G13C	4
G13D	12
G13E	2
G13R	1
G13V	1
Q61H	11
Q61R	1
T58I	1
total	241

Supplementary Table S3

drugId	drugName	target_gene	target_pathwa	gene	cells	Spearman	pval	adjPval
1372	Trametinib	MEK1, MEK2	ERK MAPK si	AARS2	KRASG12C	0.657	0.02	0.397945292
1526	RDEA119	MEK1, MEK2	ERK MAPK si	AARS2	KRASG12C	0.615	0.033	0.533334017
1060	PD-0325901	MEK1, MEK2	ERK MAPK si	ALKBH2	KRASG12C	0.915	0	0
1014	RDEA119	MEK1, MEK2	ERK MAPK si	ALKBH2	KRASG12C	0.721	0.019	0.387727888
1498	selumetinib	MEK1, MEK2	ERK MAPK si	CARS	KRASG12C	0.811	0.001	0.031604522
1526	RDEA119	MEK1, MEK2	ERK MAPK si	CARS	KRASG12C	0.79	0.002	0.061137987
1372	Trametinib	MEK1, MEK2	ERK MAPK si	CARS	KRASG12C	0.748	0.005	0.138871421
1062	selumetinib	MEK1, MEK2	ERK MAPK si	CARS	KRASG12C	0.81	0.015	0.330350852
1014	RDEA119	MEK1, MEK2	ERK MAPK si	CARS	KRASG12C	0.673	0.033	0.533334017
1498	selumetinib	MEK1, MEK2	ERK MAPK si	CDK8	KRASG12C	0.769	0.003	0.088607594
1526	RDEA119	MEK1, MEK2	ERK MAPK si	CDK8	KRASG12C	0.601	0.039	0.584552683
1015	CI-1040	MEK1, MEK2	ERK MAPK si	COMP	KRASG12C	0.817	0.007	0.184018247
1498	selumetinib	MEK1, MEK2	ERK MAPK si	COMP	KRASG12C	0.692	0.013	0.299435549
1526	RDEA119	MEK1, MEK2	ERK MAPK si	CSNK2A1	KRASG12C	0.79	0.002	0.061137987
1062	selumetinib	MEK1, MEK2	ERK MAPK si	CSNK2A1	KRASG12C	0.881	0.004	0.115864071
1498	selumetinib	MEK1, MEK2	ERK MAPK si	CSNK2A1	KRASG12C	0.741	0.006	0.163150558
1014	RDEA119	MEK1, MEK2	ERK MAPK si	CSNK2A1	KRASG12C	0.782	0.008	0.205302231
1372	Trametinib	MEK1, MEK2	ERK MAPK si	CSNK2A1	KRASG12C	0.587	0.045	0.624014981
1060	PD-0325901	MEK1, MEK2	ERK MAPK si	DARS	KRASG12C	0.697	0.025	0.461067753
1014	RDEA119	MEK1, MEK2	ERK MAPK si	DARS	KRASG12C	0.697	0.025	0.461067753
1498	selumetinib	MEK1, MEK2	ERK MAPK si	EPRS	KRASG12C	0.881	0	0
1526	RDEA119	MEK1, MEK2	ERK MAPK si	EPRS	KRASG12C	0.811	0.001	0.031604522
1014	RDEA119	MEK1, MEK2	ERK MAPK si	EPRS	KRASG12C	0.818	0.004	0.115864071
1372	Trametinib	MEK1, MEK2	ERK MAPK si	EPRS	KRASG12C	0.692	0.013	0.299435549
263	FR-180204	ERK	ERK MAPK si	HDAC1	KRASG12C	0.72	0.008	0.205302231
262	VX-11e	ERK	ERK MAPK si	HDAC1	KRASG12C	0.615	0.033	0.533334017
1060	PD-0325901	MEK1, MEK2	ERK MAPK si	IARS2	KRASG12C	0.818	0.004	0.115864071
1014	RDEA119	MEK1, MEK2	ERK MAPK si	IARS2	KRASG12C	0.721	0.019	0.387727888
262	VX-11e	ERK	ERK MAPK si	MAPK8	KRASG12C	0.762	0.004	0.115864071
263	FR-180204	ERK	ERK MAPK si	MAPK8	KRASG12C	0.699	0.011	0.261266107
1526	RDEA119	MEK1, MEK2	ERK MAPK si	PARS2	KRASG12C	0.72	0.008	0.205302231
1498	selumetinib	MEK1, MEK2	ERK MAPK si	PARS2	KRASG12C	0.587	0.045	0.624014981
1526	RDEA119	MEK1, MEK2	ERK MAPK si	RPL8	KRASG12C	0.72	0.008	0.205302231
1498	selumetinib	MEK1, MEK2	ERK MAPK si	RPL8	KRASG12C	0.636	0.026	0.467907815
1372	Trametinib	MEK1, MEK2	ERK MAPK si	RPL8	KRASG12C	0.608	0.036	0.56542678
1372	Trametinib	MEK1, MEK2	ERK MAPK si	YARS	KRASG12C	0.678	0.015	0.330350852
1498	selumetinib	MEK1, MEK2	ERK MAPK si	YARS	KRASG12C	0.671	0.017	0.361519728
1014	RDEA119	MEK1, MEK2	ERK MAPK si	YARS	KRASG12C	0.636	0.048	0.637656047

Supplementary Table S4_1

Table S4-1. Information of cell lines with KRAS(G12V) mutation in lung cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H727	724855_lung	KRAS	(missense:c.35G>T:p.G12V)	(Missense:c.49
NCI-H2444	1298356_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.70
LCLC-97TM1	946361_lung	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.1
NCI-H2291	724874_lung	KRAS	(missense:c.35G>T:p.G12V)(missense:c.	(missense:c.46
SHP-77	724872_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.52
COLO-668	910692_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.10
SW900	724879_lung	KRAS	(missense:c.35G>T:p.G12V)	(nonsense:c.49
NCI-H441	908460_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.47
COR-L23	687780_lung	KRAS	(missense:c.35G>T:p.G12V)	wt

Table S4-2. Information of cell lines with KRAS(non-G12C) mutation in lung cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1355	724866_lung	KRAS	(missense:c.37G>T:p.G13C)	(missense:c.85
Calu-6	724859_lung	KRAS	(missense:c.181C>A:p.Q61K)	(nonsense:c.58
NCI-H727	724855_lung	KRAS	(missense:c.35G>T:p.G12V)	(Missense:c.49
NCI-H1944	1240185_lung	KRAS	(missense:c.38G>A:p.G13D)	wt
SK-LU-1	909721_lung	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.57
NCI-H650	722066_lung	KRAS	(missense:c.182A>T:p.Q61L)	(missense:c.49
A427	910851_lung	KRAS	(missense:c.35G>A:p.G12D)	wt
EMC-BAC-2	1503370_lung	KRAS	(missense:c.35G>C:p.G12A)	wt
NCI-H2444	1298356_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.70
NCI-H1573	908472_lung	KRAS	(missense:c.35G>C:p.G12A)	(missense:c.74
NCI-H647	1240191_lung	KRAS	(missense:c.38G>A:p.G13D)	(ess_splice:c.7
LCLC-97TM1	946361_lung	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.1
SHP-77	724872_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.52
NCI-H460	905943_lung	KRAS	(missense:c.183A>T:p.Q61H)	wt
A549	905949_lung	KRAS	(missense:c.34G>A:p.G12S)	wt
COLO-668	910692_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.10
NCI-H2009	724873_lung	KRAS	(missense:c.35G>C:p.G12A)	(missense:c.81
SW900	724879_lung	KRAS	(missense:c.35G>T:p.G12V)	(nonsense:c.49
NCI-H1734	722058_lung	KRAS	(missense:c.37G>T:p.G13C)	(missense:c.81
NCI-H2347	687820_lung	KRAS	(missense:c.57G>T:p.L19F)	wt
NCI-H1155	908467_lung	KRAS	(missense:c.183A>T:p.Q61H)	(missense:c.81
NCI-H441	908460_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.47
COR-L23	687780_lung	KRAS	(missense:c.35G>T:p.G12V)	wt

Supplementary Table S4_2

Table S4-3. Information of cell lines with KRAS(G12V) mutation in pancreatic cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
DAN-G	1290797_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(ess_splice:c.9
QGP-1	1298534_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.2
PANC-03-27	925346_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(ess_splice:c.3
KP-3	1298219_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.4
MZ1-PC	753595_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.6
CFPAC-1	906821_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.72
PA-TU-8902	1298526_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.52
CAPAN-1	753624_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.47
Capan-2	910915_pancreas	KRAS	(missense:c.35G>T:p.G12V)	wt
YAPC	909904_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.53
HuP-T4	907286_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.76
PA-TU-8988T	1240201_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.84

Table S4-4. Information of cell lines with KRAS(G12D) mutation in pancreatic cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
HPAF-II	724869_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.45
PANC-02-03	1298475_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.74
PANC-04-03	1298476_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.73
PANC-08-13	925347_pancreas	KRAS	(missense:c.35G>A:p.G12D)	wt
PL4	1298533_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.79
KP-1N	1298216_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.81
HPAC	1298136_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.55
SW1990	910907_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(inframe:c.572_
PANC-10-05	925348_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.76
AsPC-1	910702_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(frameshift:c.4
SUIT-2	1240219_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.81
SU8686	1240218_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.10

Table S4-5. Information of cell lines with BRAF mutation in lung cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1651	910900_lung	BRAF	(missense:c.1125A>T:p.E375D)	(missense:c.52
NCI-H1395	684681_lung	BRAF	(missense:c.1406G>C:p.G469A)	wt
NCI-H2227	688018_lung	BRAF	(missense:c.958G>T:p.A320S)	(ess_splice:c.7
SW1271	1299062_lung	BRAF	(missense:c.2048G>A:p.S683N)	(missense:c.83
IST-SL2	753565_lung	BRAF	(ess_splice:c.608+1G>T:p.?)	(nonsense:c.88
NCI-H1666	908473_lung	BRAF	(missense:c.1397G>T:p.G466V)	wt
NCI-H1755	908475_lung	BRAF	(missense:c.1406G>C:p.G469A)	(missense:c.72
NCI-H2087	724834_lung	BRAF	(missense:c.1789C>G:p.L597V)	(missense:c.46
NCI-H2405	687821_lung	BRAF	(inframe:c.1454_146916>A:p.L485_P49	(missense:c.81
CAL-12T	753540_lung	BRAF	(missense:c.1397G>T:p.G466V)	(missense:c.40

Supplementary Table S4_3

Table S4-6. Information of cell lines with EGFR mutation in lung cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1355	724866_lung	EGFR	(missense:c.3477G>C:p.Q1159H)	(missense:c.85
NCI-H1975	924244_lung	EGFR	(missense:c.2369C>T:p.T790M)(missens	(missense:c.81
MS-1	753594_lung	EGFR	(missense:c.771A>T:p.E257D)	(missense:c.73
NCI-H1650	687800_lung	EGFR	(inframe:c.2235_2249del15:p.E746_A75	(ess_splice:c.67
PC-14	753608_lung	EGFR	(inframe:c.2235_2249del15:p.E746_A75	(missense:c.74
HCC-827	1240146_lung	EGFR	(inframe:c.2236_2250del15:p.E746_A75	(inframe:c.652_
SHP-77	724872_lung	EGFR	(missense:c.2102A>G:p.Q701R)	(missense:c.52
NCI-H2291	724874_lung	EGFR	(missense:c.1774G>T:p.V592F)	(missense:c.46
H3255	1247873_lung	EGFR	(missense:c.2573T>G:p.L858R)	(ess_splice:c.5
NCI-H1793	908463_lung	EGFR	(missense:c.932G>T:p.C311F)	(missense:c.81
NCI-H1568	1298348_lung	EGFR	(missense:c.3379G>A:p.D1127N)	(missense:c.53
PC-3 [JPC-3]	1240202_lung	EGFR	(inframe:c.2236_2244delGAATTAAGA	(missense:c.84

Table S4-7. Information of cell lines with NRAS mutation in lung cancer

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1048	687995_lung	NRAS	(frameshift:c.59_60delCA:p.T20fs*11)	(missense:c.81
COR-L279	910937_lung	NRAS	(missense:c.14A>G:p.K5R)	(ess_splice:c.3
SW1271	1299062_lung	NRAS	(missense:c.182A>G:p.Q61R)	(missense:c.83
NCI-H1299	724831_lung	NRAS	(missense:c.181C>A:p.Q61K)	(ess_splice:c.1_
NCI-H2087	724834_lung	NRAS	(missense:c.181C>A:p.Q61K)	(missense:c.46
NCI-H2135	1298352_lung	NRAS	(missense:c.181C>A:p.Q61K)	(missense:c.83
HCC-15	1240143_lung	NRAS	(missense:c.557G>T:p.C186F)(missense	(missense:c.77
NCI-H2347	687820_lung	NRAS	(missense:c.182A>G:p.Q61R)	wt