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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).

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

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28

29 **Abstract**

30 **Background**

31 KRAS mutations are the most frequent oncogenic aberration in lung adenocarcinoma. Due to differences in
32 protein structure and GTPase activity, KRAS mutant isoforms shape tumor biology and therefore may
33 influence the treatment response in non-small-cell lung cancer. This heterogeneity challenges the
34 development of effective targeted therapies for KRAS-driven lung cancer.

35 **Methods**

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37 Then we developed an integrative pharmacogenomics analysis to identify potential targets in lung cancer
38 with KRAS(G12C) mutation, the most frequent aberration in patients with primary or metastatic KRAS
39 mutant non-small cell lung cancer. We further validated our prediction by siRNA-mediated gene
40 knockdown and TOPFlash reporter assay.

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42 Our computational analysis identifies casein kinase 2A1 (CSNK2A1) as a mediator of MEK inhibitor
43 resistance in KRAS(G12C) mutant cells which is not observed in cells with non-KRAS(G12C) mutations
44 and in those harboring other oncogenic drivers as e.g. activating mutations in EGFR, BRAF, or NRAS.
45 Knockdown of CSNK2A1 reduces proliferation, inhibits Wnt/ β -catenin signalling and increases the anti-
46 proliferative effect of selumetinib in KRAS(G12C) mutant lung cancer cells.

47 **Interpretation**

48 Our study suggested that accurate patients stratification will be necessary in order to observe significant
49 benefit upon CK2 inhibition - alone or in combination - in a subset of patients with a favorable intratumoral
50 genetic context. We provide a promising approach towards developing precision treatments for various
51 subtypes of KRAS mutant lung cancer.

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56 postdoctoral fellowship from the German Cancer Aid Foundation (70111755 to JK).

57 **Keywords:** Pharmacogenomic profiles, KRAS mutations, Lung adenocarcinoma, CSNK2A1

58

59 **Research in context**

60 **Evidence before this study**

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

63 **Added value of this study**

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

68 **Implications of all the available evidence**

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

73

74

75

76

77 **Introduction**

78 The Kirsten rat sarcoma oncogene (KRAS) encodes for a small GTPase that couples growth factor
79 signalling to the MAPK signalling cascade. Despite being an oncogene with a prevalence of 30% in non-
80 small cell lung cancer (NSCLC), the development of KRAS targeted therapies has been largely
81 unsuccessful in the past. This is mainly due to the higher affinity of RAS for GTP^{1,2}. Very recently, the
82 pharmacokinetic and pharmacodynamic improvement of direct G12C inhibitors has raised great excitement
83³, leading to two clinical studies that are currently on-going
84 (<https://clinicaltrials.gov/ct2/results?cond=G12C&term=&cntry=&state=&city=&dist=>). As an alternative,
85 inhibitors targeting kinases downstream of KRAS, such as BRAF and MEK, have been developed which
86 showed promising activity in metastatic melanoma but were less active in KRAS mutant NSCLC.
87 Furthermore, drug efficacy is limited by the development of acquired resistance or KRAS copy number
88 variations⁴⁻¹². Hence, there is still an unmet need to develop more efficacious targeted treatment strategies
89 for KRAS mutant lung cancer.

90

91 In NSCLC, different KRAS mutations have been identified according to the amino acid substitution which
92 can affect drug sensitivity and tumor biology^{13,14}. The heterogenous behaviour of different KRAS
93 mutations is due to differences in protein structure and GTPase activity¹⁵⁻¹⁷ which needs to be considered
94 when investigating potential targets for KRAS mutant lung cancer.

95

96 Here, we perform a pan-cancer analysis to systematically investigate differences in treatment response to
97 MEK inhibitors due to different KRAS mutational subtypes. An integrative pharmacogenomics analysis
98 pipeline is then developed to identify potential targets in lung cancer with KRAS(G12C) mutation, the most
99 frequent mutation (>40%) in patients with primary or metastatic KRAS mutant non-small cell lung cancer
100 (NSCLC)¹⁷. The most promising target predicted by this pipeline is casein kinase 2A1 (CSNK2A1) which
101 encodes for the casein kinase 2 subunit alpha (CK2 alpha), a serine/threonine protein kinase that
102 phosphorylates acidic proteins such as casein. Although there is strong evidence that CK2 plays a role in
103 the pathogenesis of cancer¹⁸⁻²⁰ and several CK2 inhibitors have entered clinical trials, the role of
104 CSNK2A1 as a therapeutic target in KRAS mutant lung cancer remains unknown to date. Our study links

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

107

108 **Materials & Methods**

109 **Key Resources Table**

110 **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 |

111

112 **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 |

113

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

115 The Cancer Genome Project (CGP) at the Wellcome Trust Sanger Institute resulted in a large-scale, high-
116 throughput pharmacogenomic dataset for 1001 human cancer cell lines, including the mutation status of
117 19,100 genes, genome-wide DNA copy number variation (CNV) status, mRNA expression profiling of
118 17,419 genes, and pharmacological profiling for 267 anti-cancer drugs. The drug response is represented by
119 the natural logarithm of the IC50 value, which corresponds to the half maximal inhibitory concentration of
120 an anti-cancer drug. In this dataset, there are five MEK inhibitors including PD-0325901, selumetinib, CI-
121 1040, trametinib, and refametinib, and two ERK inhibitors including FR-180204 and VX-11e. Among 137
122 cancer cells harboring KRAS mutations, 35 cells are derived from lung cancer.

123

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

139

140 **TCGA data analysis**

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

149

150 **Cell lines**

151 The human lung cancer cell lines A549, H2030, H2009 and Calu1 were purchased from ATCC and grown
152 at 37°C in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 μg/ml penicillin and 100
153 units/ml streptomycin (complete medium). The cell lines were authenticated using the Promega GenePrint
154 10 System at the RTSF Genomics Core at Michigan State University. All cell lines used in the study tested
155 negative for Mycoplasma as determined by the Mycoplasma Plus PCR Primer Set (Agilent).

156

157 **Assessment of cellular proliferation**

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

164

165 **Western blot analysis**

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

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

178

179 **SiRNA-mediated gene knockdown**

180 Cells (1.5×10^6) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, media was
181 replaced by antibiotic free full media and the mixture of siRNA (scrambled, CSNK2A1, Dharmacon) at a
182 final concentration of 20nM together with DharmaFECT 1 was added after allowing 30min of complex
183 formation in serum-free media. Knockdown efficacy was assessed by Western blot and qRT-PCR after
184 48hrs of transfection. For treatment experiments cells were harvested and re-seeded after 48hrs of siRNA
185 treatment and treated with selumetinib for another 24 to 96hrs.

186

187 **TOPFlash reporter assay**

188 Cells (1.5×10^6) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, cells were
189 transiently transfected with 1 μ g of M50 Super 8x TOPFlash reporter plasmid, 100ng of a pRL Renilla
190 Luciferase control reporter plasmid (Promega) and FuGENE HD (Promega). M50 Super 8x TOPFlash was
191 a gift from Randall Moon (Addgene plasmid #12456). After 24hrs, cells were washed with PBS and full
192 media was added for another 24hrs without or with Mek inhibitor (selumetinib, 1 μ m). Luciferase activity
193 was measured with the Dual Luciferase reporter assay (Promega).

194

195 **Results**

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

198 We first interrogated the publicly available pharmacogenomics dataset CGP, which includes mutational and
199 pharmacological profiles of >1000 human cancer cell lines treated with 265 anti-cancer drugs²³. Drug
200 sensitivities are represented by the natural logarithm of the drug's IC50 value. To investigate MEK/ERK
201 inhibitors sensitivity for different KRAS mutant isoforms, we grouped all cancer cells based on their KRAS
202 mutation status, and then used the Kruskal-Wallis H-test to compare drug sensitivities between multiple
203 groups and the t-test to compare drug sensitivities between two groups.

204

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

216

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

224 of KRAS mutations. Among 19 types of KRAS mutations, 11 types (A146T, A146V, A59T, AG59GV,
225 G13C, G13D, G13E, G13R, G13V, Q61R, and T58I) are exclusively observed in patients with metastatic
226 LUAD but not in primary tumors (Fig. 3b, Additional file3: Table S2). In metastatic LUAD patients, the
227 KRAS(G12C) mutation is also the most prevalent one (42.74%), followed by G12V (15.35%) and G12D
228 (15.35%) mutations.

229

230 **Pharmacogenomics analysis to identify potential targets in lung cancer harboring KRAS(G12C)** 231 **mutations**

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

244

245 Among the 14 candidate genes identified by our pharmacogenomics analysis, CSNK2A1 was ranked as the
246 most promising gene. CSNK2A1 encodes for a protein which is a component of the highly conserved
247 serine/threonine protein kinase CK2 alpha. CK2 alpha itself is part of various pathways relevant for cancer
248 cell biology among them Wnt (Fig. 4a) and NF-kappa B signaling²⁰. This is especially relevant as there is
249 an increased interest for CSNK2 as a therapeutic target in ongoing clinical trials²⁴. The association of
250 CSNK2A1 expression and reduced MEK/ERK inhibitors sensitivity was repeatedly observed for 4 different
251 MEK inhibitors, including 2 replicate datasets for refametinib and selumetinib (Fig. 4b). Importantly,

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

255

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

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

270

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

276

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

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

286

287 As casein kinases have previously been connected to the drug resistance mediating Wnt/ β -catenin pathway
288 ^{25,26}, we therefore investigated if CSNK2A1 influences Wnt/ β -catenin signaling in KRAS(G12C) mutant
289 lung cancer. We used gene expression profiles of LUAD patients (TCGA) and cancer cell lines (CCLE) ²⁷
290 and categorized samples into CSNK2A1 high and low expressing groups. Deseq2 ²⁸ was applied to call
291 differentially expressed genes between the two groups. Gene set enrichment analysis (GSEA) ²⁹ was further
292 employed to determine the pathways enriched by a pre-ranked list of all genes, which were sorted by the
293 statistical significance of differential expression defined by the Deseq2 analysis. GSEA showed that the
294 Wnt signaling pathway was significantly enriched in the CSNK2A1 high expressing group in CCLE (Fig.
295 8a, $p=0.008$) and TCGA (Fig. 8b, $p=0.014$).

296

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

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

308

309 **Discussion**

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

322

323 CSNK2A1 encodes for a protein that is a component of the highly conserved serine/threonine protein
324 kinase CK2 alpha. Previous studies have identified CSNK2 as an oncogene when overexpressed in mice,
325 playing a key role in the pathogenesis of cancer, including breast, lung, colon, and prostate cancer, as well
326 as hematologic malignancies^{19,20,30}. Moreover, a cancer context-dependent effect of CK2 on signaling
327 pathways such as Wnt signaling²⁰, JAK/STAT³¹, NF- κ B²⁰, and PTEN/PI3K/Akt-PKB^{32,33} has been
328 described in the past. In the Wnt pathway, CK2 acts by phosphorylating and stabilizing Dvl and β -catenin
329 and promotes T-cell factor/lymphoid enhancer-binding factor (TCF) DNA binding in the nucleus (Fig. 4a).
330 Based of these observations, CK2 has recently arisen as a promising candidate for targeted therapy, with
331 two CK2 inhibitors in ongoing clinical trials
332 (<https://clinicaltrials.gov/ct2/results?cond=&term=CK2&cntry=&state=&city=&dist=>). Our results suggest
333 that accurate patients stratification will likely be necessary in order to observe significant benefit upon CK2

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

335

336 In agreement with our computational results, CSNK2A1 knockdown significantly reduced the proliferation
337 of KRAS(G12C) mutant lung cancer cells, an effect that was not observed in cells with non-G12C KRAS
338 mutations (Fig. 7). This identifies CSNK2A1 as an interesting target *per se* in KRAS(G12C) mutant
339 NSCLC cell lines. Furthermore, simultaneous CSNK2A1 knockdown and MEK inhibition with selumetinib
340 increased the anti-proliferative effect of selumetinib in KRAS(G12C) mutant but not in KRAS(non-G12C)
341 mutant cells. Mechanistically, our analysis including transient 8xTOPFlash reporter transfection shows that
342 CSNK2A1 mediates TCF transcriptional activity in KRAS(G12C) mutant lung cancer (Fig. 8d). The
343 importance of CSNK2A1 for Wnt signaling is also supported by our gene-set enrichment analysis (GSEA)
344 of the CCLE and TCGA datasets which show significant enrichment for the Wnt signaling pathway (Fig.
345 8a, b).

346

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

355

356 **Acknowledgements**

357 Not applicable

358

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361 31771469, and 81573023 to HW), the National key research and development program (2017YFC0908500

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

364

365 **Declarations of interests**

366 The authors declare no competing financial interests.

367

368 **Authors' contributions**

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

373

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447

448 **Figures and Tables Legend**

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

455

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

460

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

464

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

471

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

480

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

483

484 **Figure 7. CSNK2A1 Knockdown reduces the proliferation of KRAS(G12C) mutant lung cancer cells**

485 **and increases the anti-proliferative effect of the MEK inhibitor selumetinib.** (A) Western blot analysis

486 of CSNK2A1 protein in KRAS(G12C) mutant cell lines Calu1 and H2030 transfected with scrambled or

487 CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1 μ m) treatment. Growth rates of

488 Calu1 (B) and H2030 (C) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA

489 (red and grey) without or with simultaneous selumetinib (1 μ m) treatment. Phase-contrast images are

490 shown at the bottom. (D) Western blot analysis of CSNK2A1 protein in KRAS(G12S) mutant cell line

491 A549 and in KRAS(G12A) mutant cell lines H2009 transfected with scrambled or CSNK2A1 targeting

492 siRNA without or with simultaneous selumetinib (1 μ m) treatment. Growth rates of A549 (E) and H2009

493 (F) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA (red and grey) without or

494 with simultaneous selumetinib (1 μ m) treatment. Phase-contrast images are shown at the bottom.

495

496 **Figure 8. CSNK2A1 knockdown inhibits the activation of Wnt/ β -catenin signaling in KRAS(G12C)**

497 **mutant lung cancer cells.** GSEA analysis based on CCLE lung cancer cells (A) and TCGA LUAD

498 patients (B) with KRAS mutation shows that Wnt signaling pathway is enriched in tumors with high

499 CSNK2A1 expression. (C) Western blot analysis shows increased p27 induction upon selumetinib

500 treatment in siCSNK2A1 treated Calu1 and H2030 compared to A549 and H2209. (D) Reporter activity of
501 Wnt/TCF reporter assay in Calu1 KRAS(G12C) and A549 KRAS(G12S). Student t-test are performed,
502 with a symbol *, **, and **** respectively representing the comparison with p value smaller than 0.05,
503 0.01 and 0.0001.

504

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

509

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

512

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

515

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

517

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

520

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

526

527 **Additional file7: Figure S3.** Growth rates of Calu1 KRAS(G12C) (a) and A549 KRAS(G12S) (b)
528 transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib
529 (1umettreatment. Student t-test are performed, with a symbol *, ****, and n.s. respectively representing the
530 comparison with p value smaller than 0.05, 0.0001 and greater than 0.05.

531

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

533

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

536

537

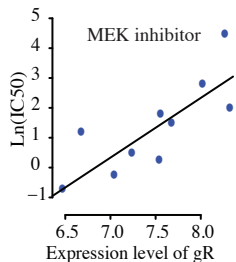
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Figure1

[Click here to download Figure: Fig1.analysis pipeline.pdf](#)

Pharmacogenomics data (CGP dataset)

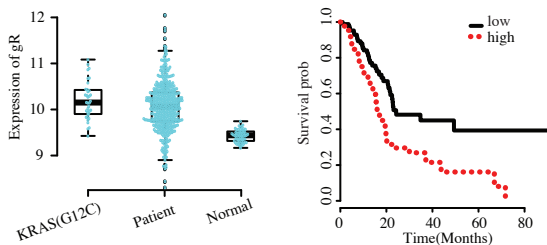
- * 12 KRAS(G12C) mutant lung cell lines
- * 6 MEK inhibitors, 2 ERK inhibitors
- * genome-wide gene expression



b

TCGA data (LUAD patients)

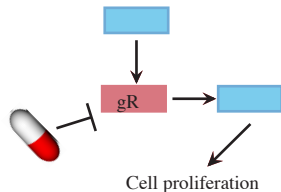
- * 36 KRAS(G12C) mutant patients
- * 576 samples (517 patients vs. 59 normal)
- * genome-wide gene expression
- * clinical prognosis



c

Cancer core pathway

- * KEGG
- * REACTOME
- * BIOCARTA et al.
- DrugBank



d

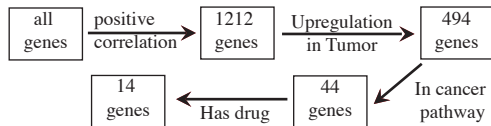
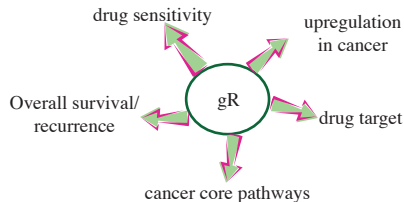


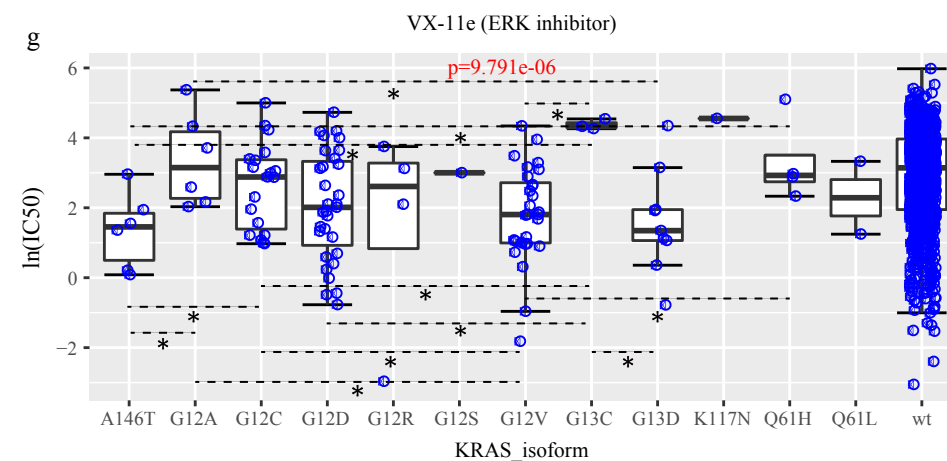
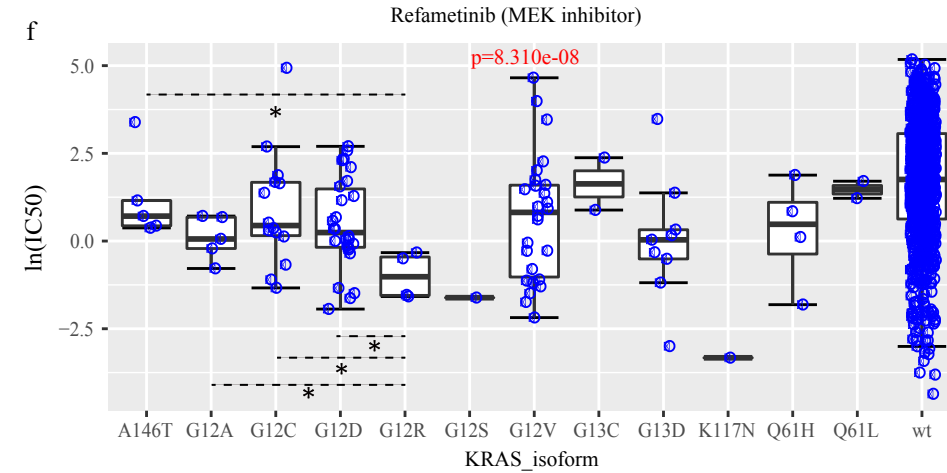
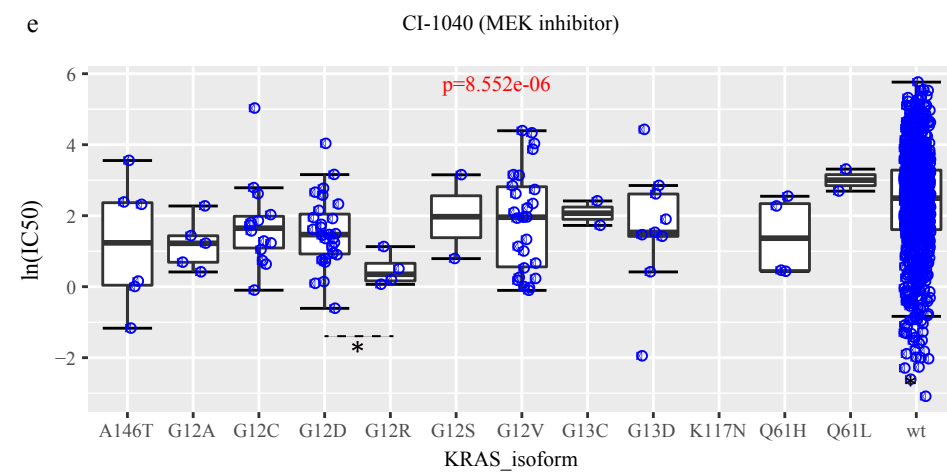
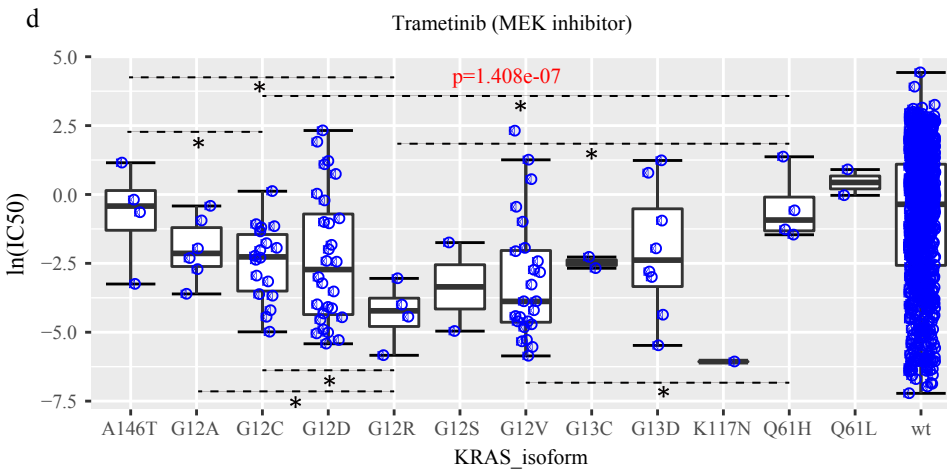
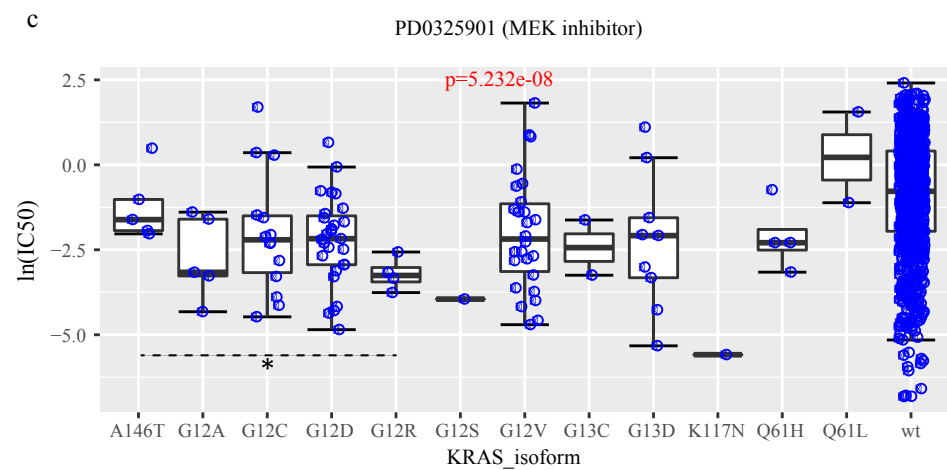
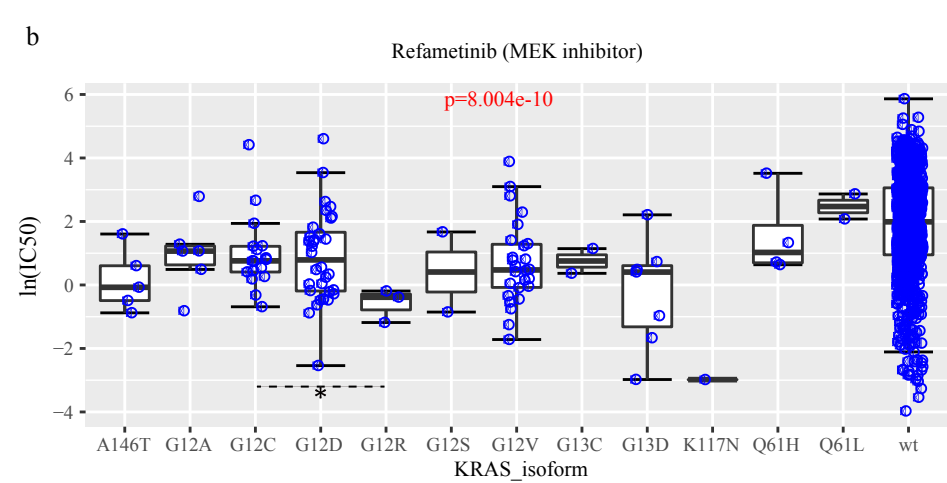
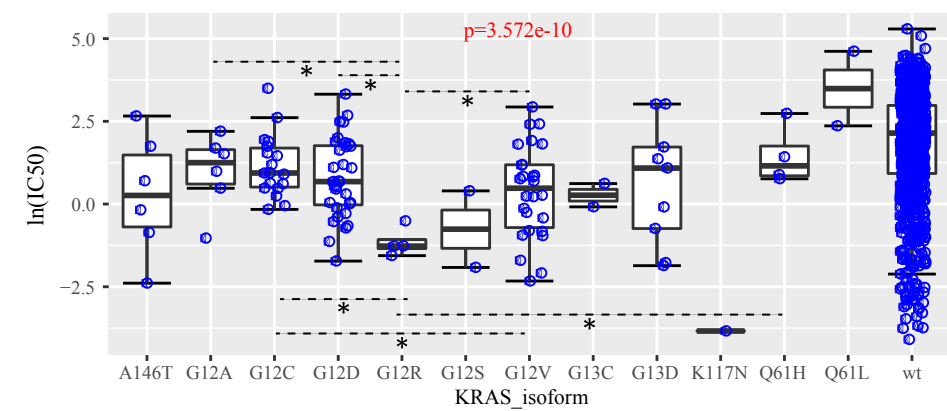
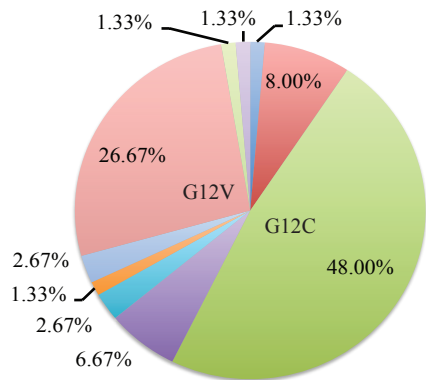
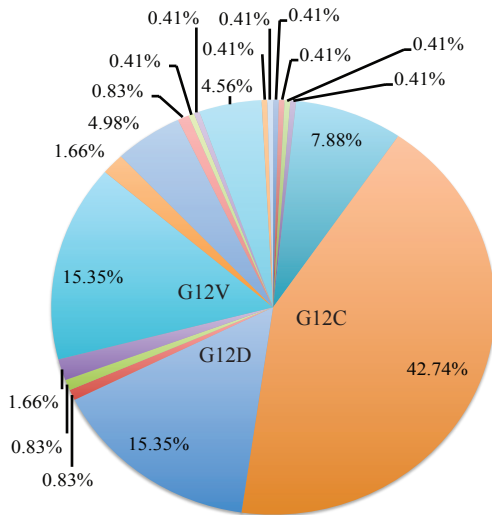
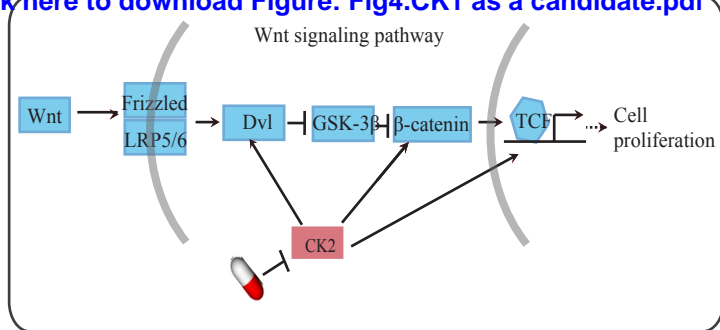
Figure 2[Click here to download Figure: Fig2_CGP_all_InIC50_DNA.pdf](#)

Figure3[Click here to download Figure: Fig3.kras_isoforms.pdf](#)**b**Primary LUAD
(TCGA)Metastatic LUAD
(MSK-IMPACT)

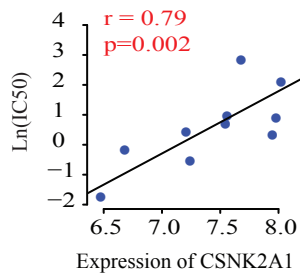
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G12C
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G12F
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G12S
G12V
G12Y
Q61L



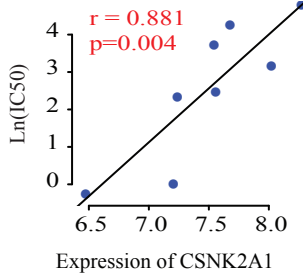
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T58I

Figure 4[Click here to download Figure: Fig4.CK1 as a candidate.pdf](#)**b**

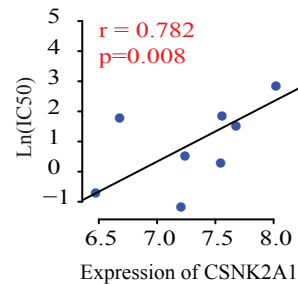
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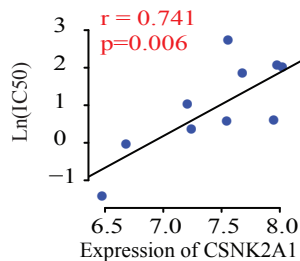
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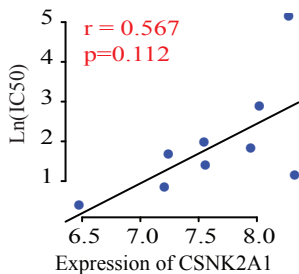
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Selumetinib_ID1498
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CI-1040_ID1015
(MEK inhibitor)



Trametinib_ID1372
(MEK inhibitor)

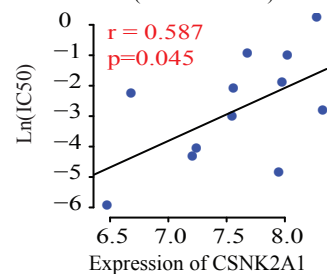
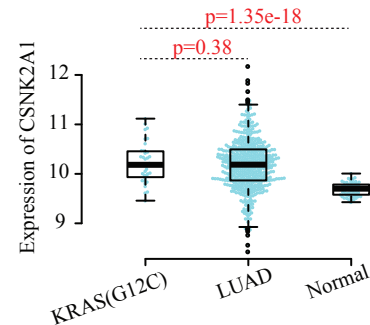
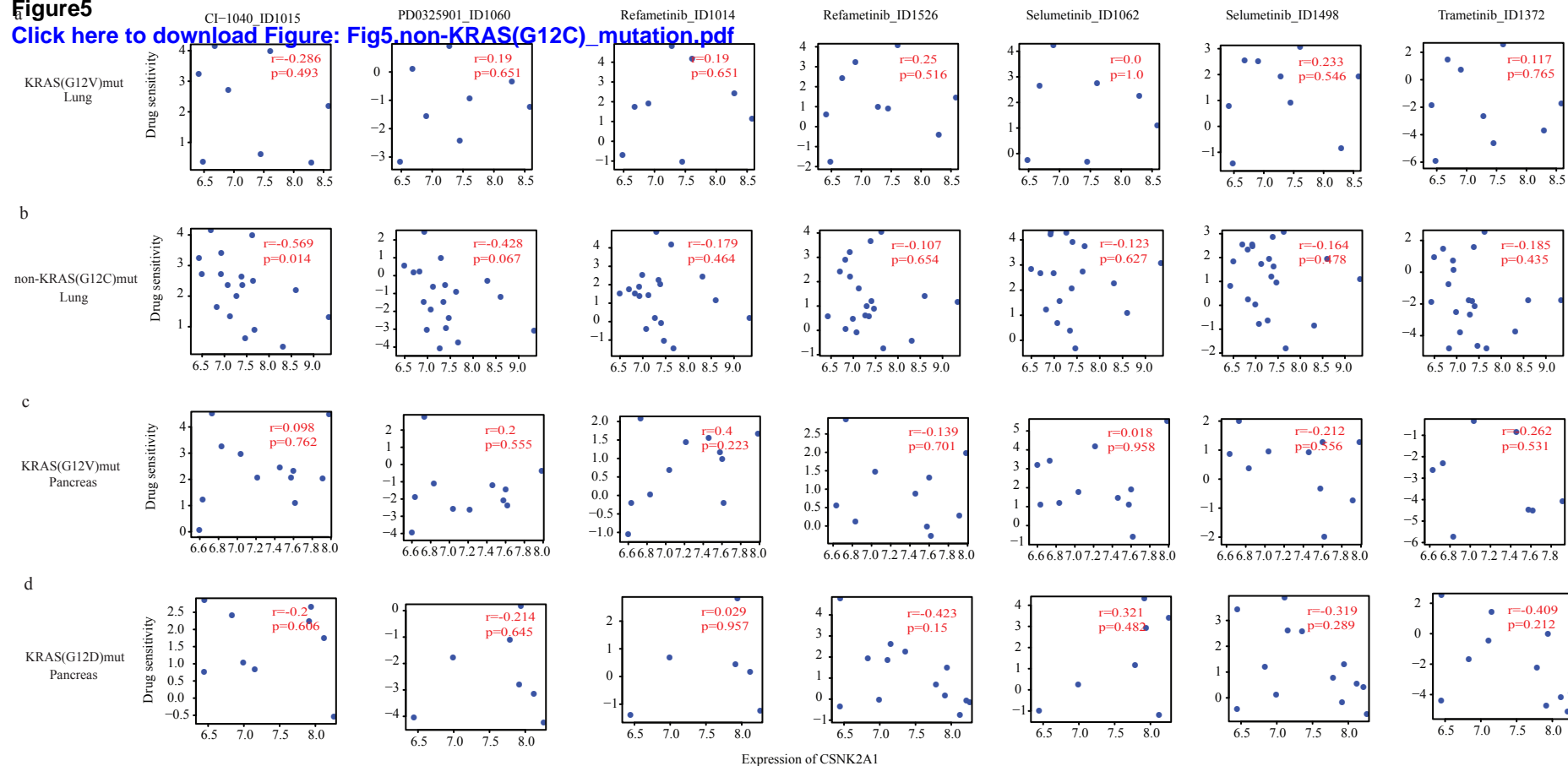
**c**

Figure 5[Click here to download Figure: Fig5 non-KRAS\(G12C\) mutation.pdf](#)

Expression of CSNK2A1

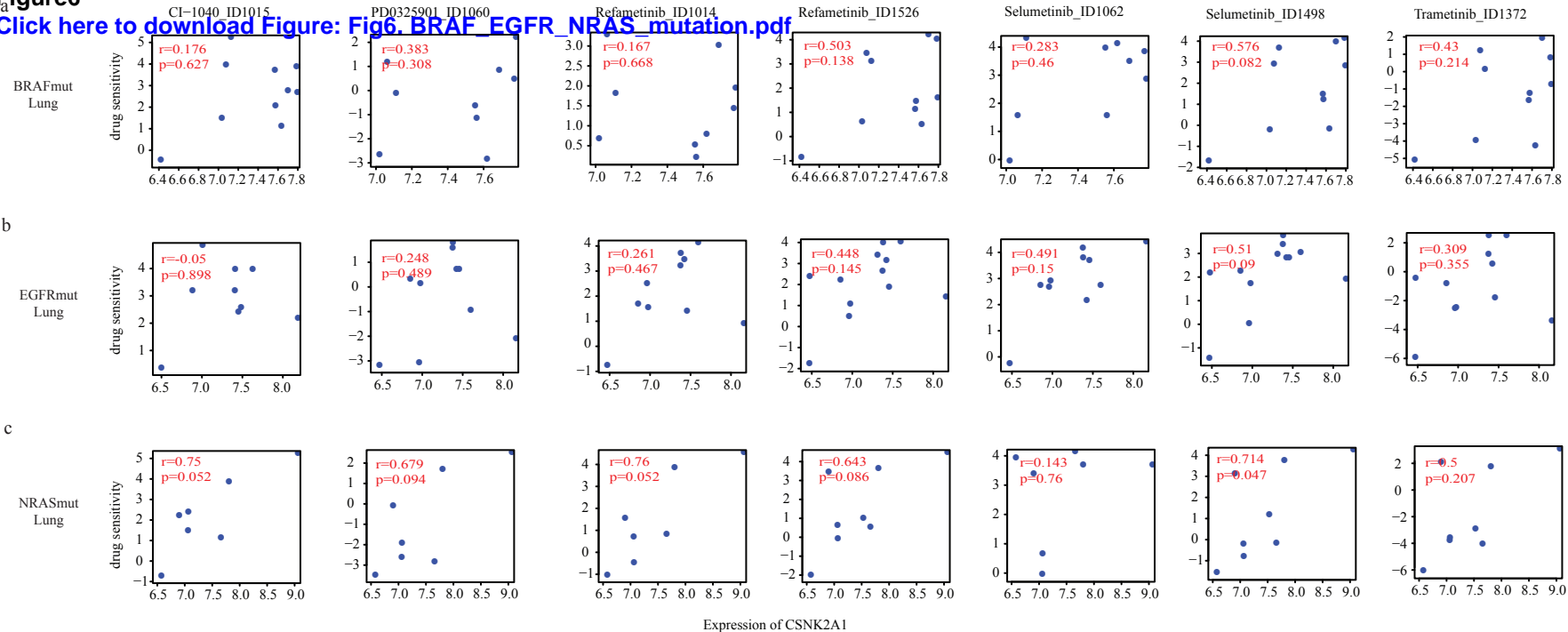
Figure 6[Click here to download Figure: Fig6. BRAF_EGFR_NRAS_mutation.pdf](#)

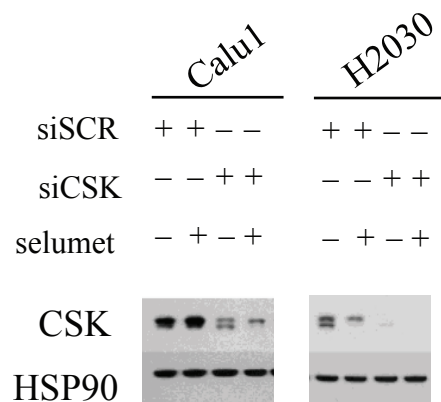
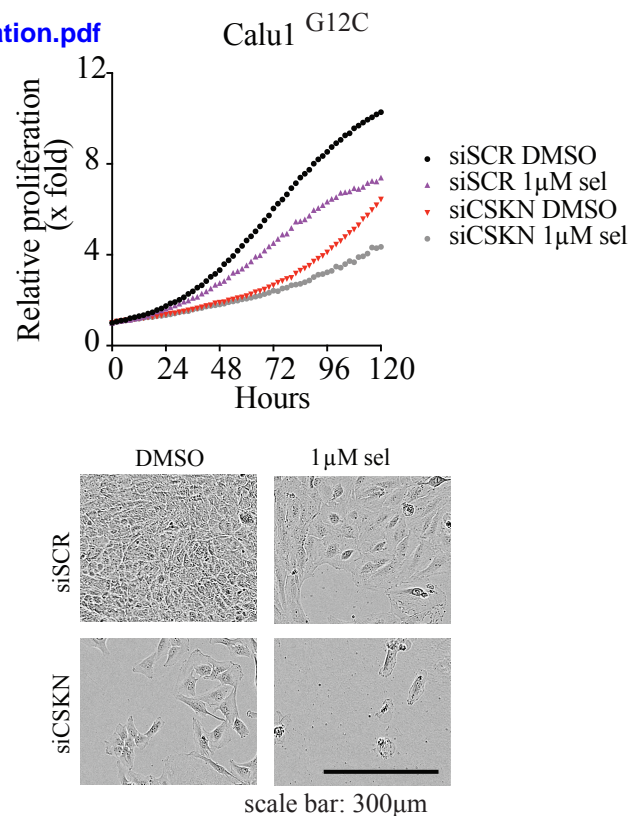
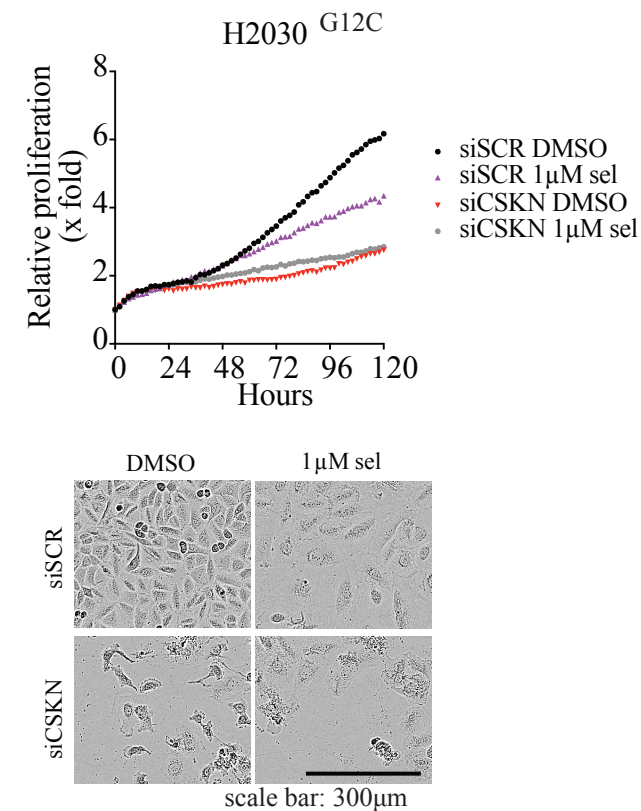
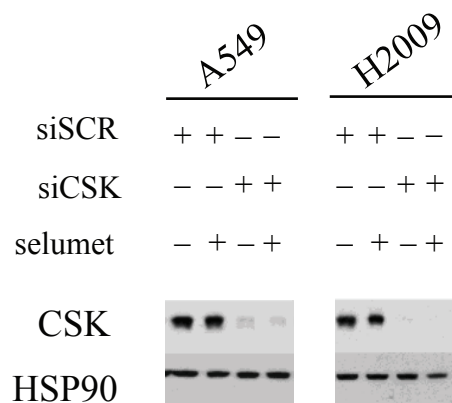
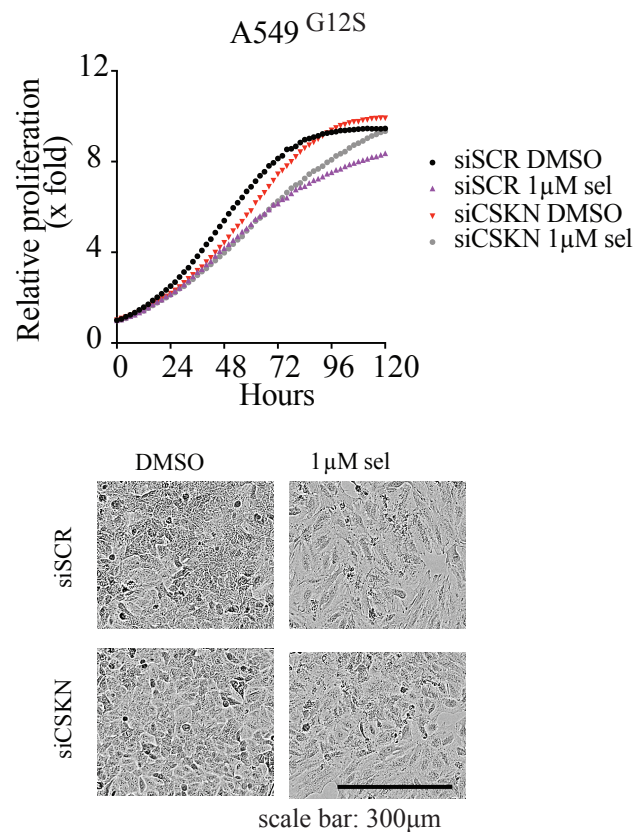
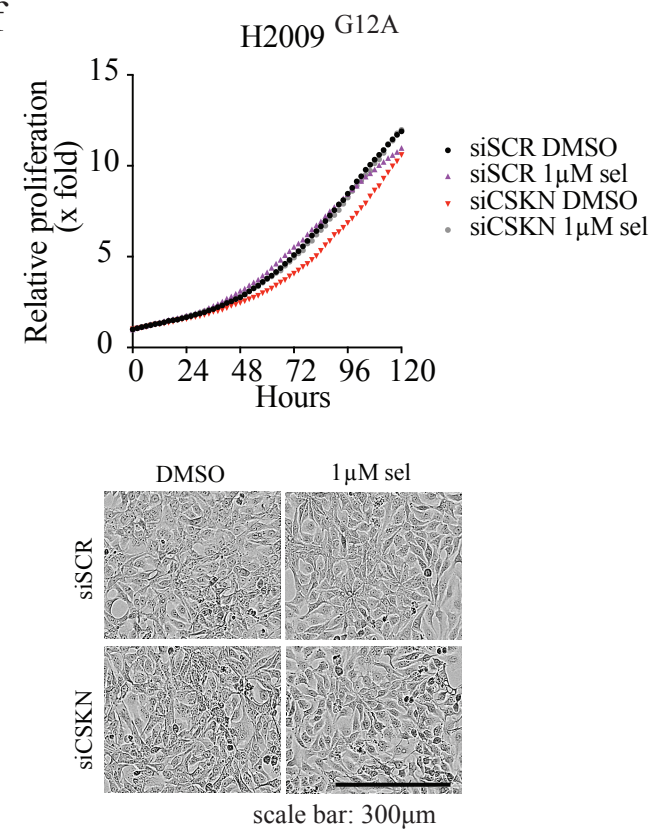
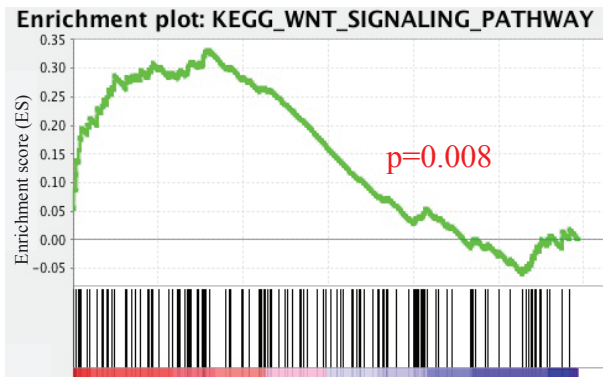
Figure7[Click here to download Figure: Fig7.cell_proliferation.pdf](#)**a****b****c****d****e****f**

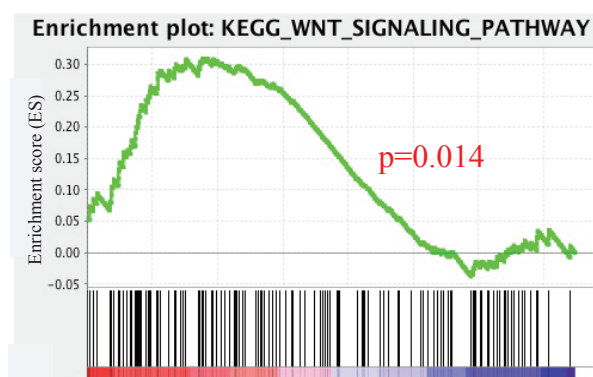
Figure8

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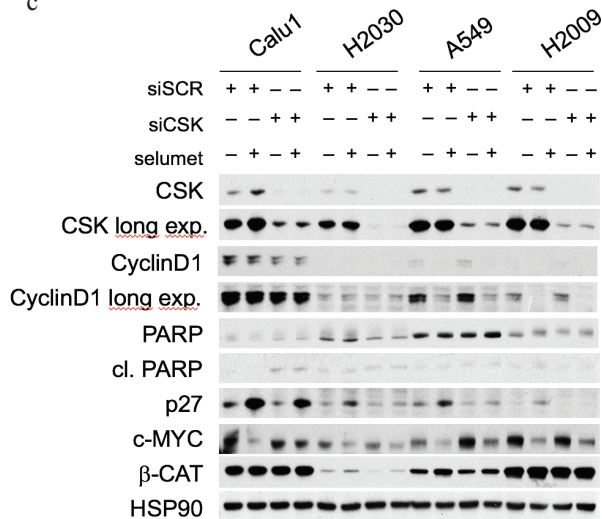
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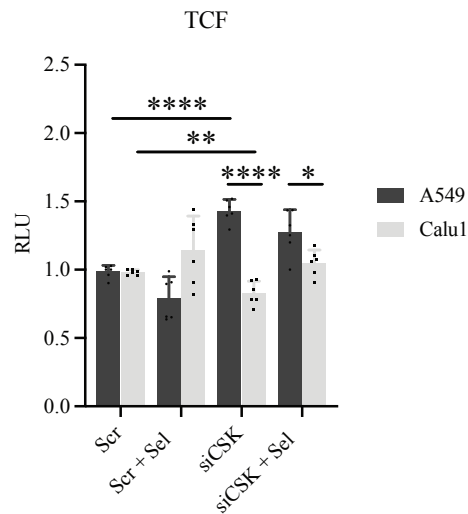
TCGA



c



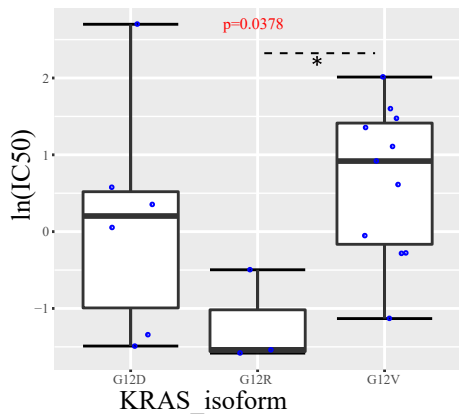
d



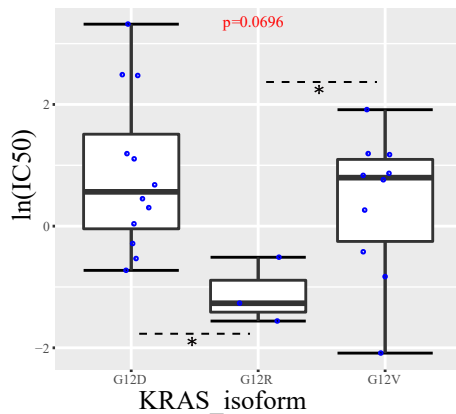
Supplementary Figure S1

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a Refametinib (MEK inhibitor)_Pancreas

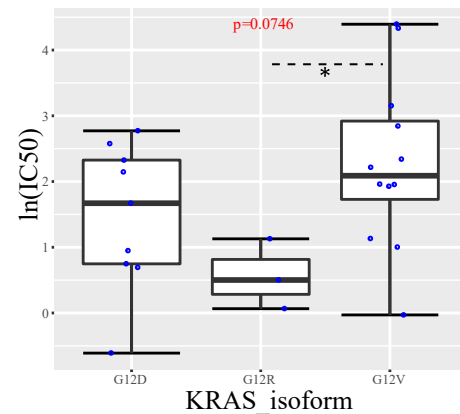


b Selumetinib (MEK inhibitor)_Pancreas



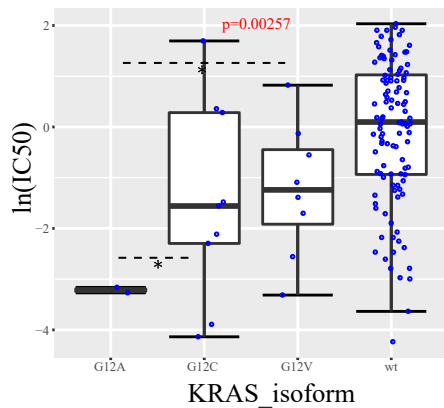
c

CI-1040(MEK inhibitor)_Pancreas



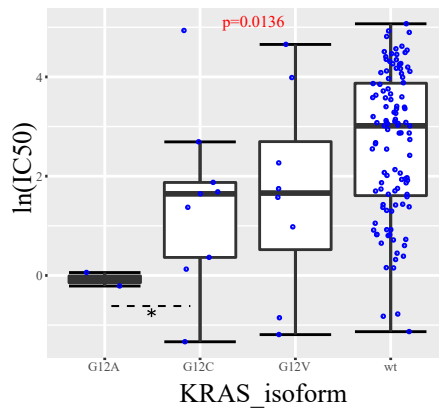
d

PD0325901 (MEK inhibitor)_Lung



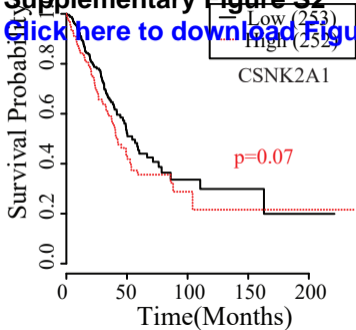
e

Refametinib (MEK inhibitor)_Lung



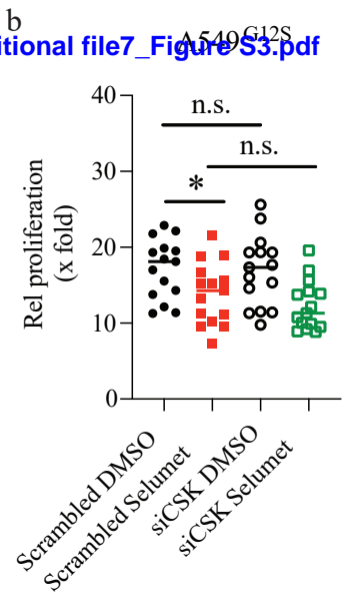
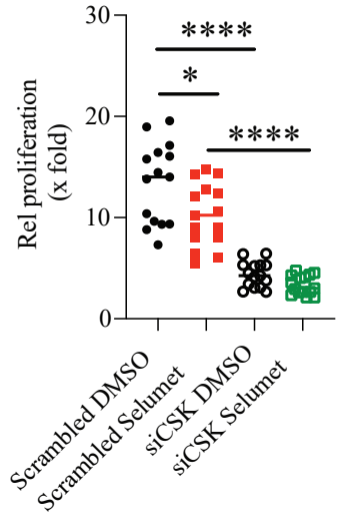
Supplementary Figure S2

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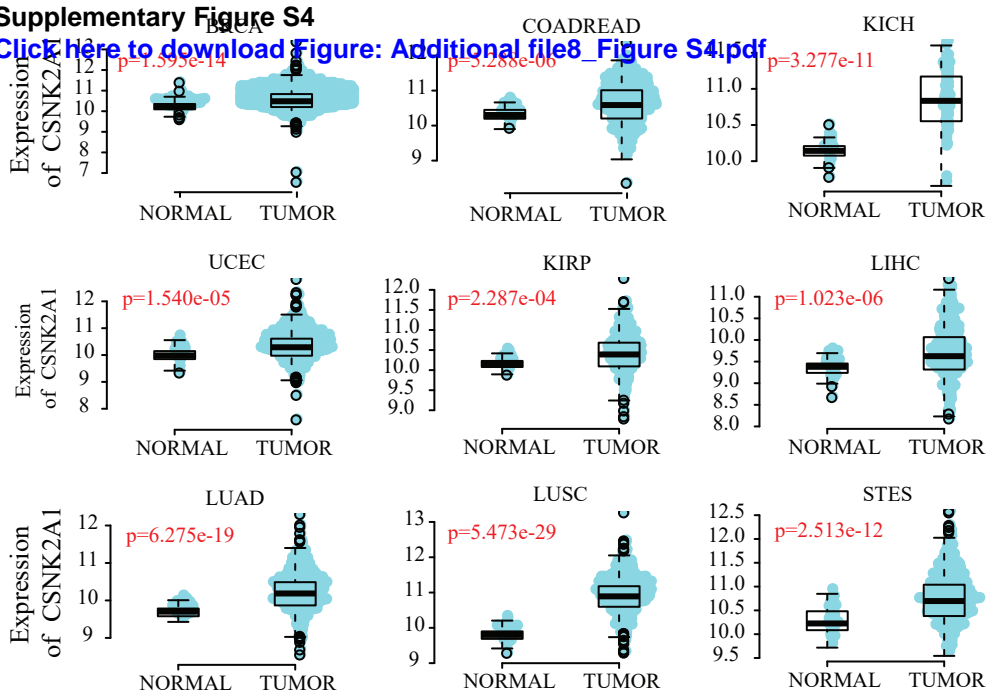
Supplementary Figure S3

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Supplementary Figure S4

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Supplementary Figure S5

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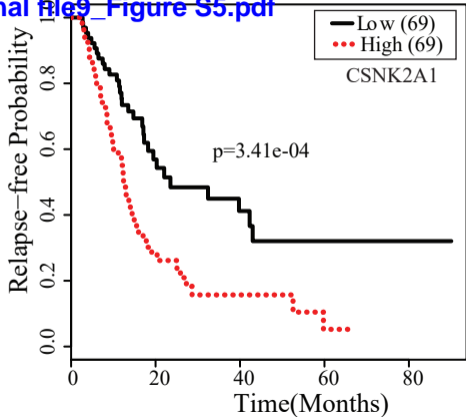
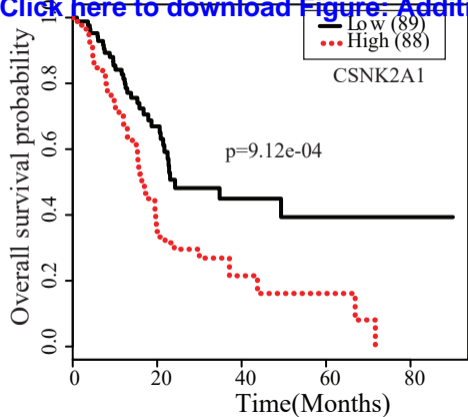


Table S1. The prevalence of different KRAS mutational isoforms in primary LUAD patients

| 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 |

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_pathway_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 |

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.10 |
| 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.78 |
| LCLC-97TM1 | 946361_lung | KRAS | (missense:c.35G>T;p.G12V) | (frameshift:c.10 |
| 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 |

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 |
| MZI-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 |

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)(missense:c.81 | (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.6 |
| 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.56 |
| 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 |