**Supplementary Information Appendix for**

# TUMOR REGRESSION AND RESISTANCE MECHANISMS UPON CDK4 AND RAF1 INACTIVATION IN KRAS/P53 MUTANT LUNG ADENOCARCINOMAS

Laura Esteban-Burgos, Haiyun Wang, Patricia Nieto, Jie Zheng, Carmen Blanco-Aparicio, Carmen Varela, Gonzalo Gómez-López, Fernando Fernández-García, Manuel Sanclemente, Carmen Guerra, Matthias Drosten, Javier Galán, Eduardo Caleiras, Jorge Martínez-Torrecuadrada, Lluis Fajas, Sheng-Bin Peng, David Santamaría, MonicaMusteanu and Mariano Barbacid

Correspondence: mmusteanu@cnio.es and mbarbacid@cnio.es

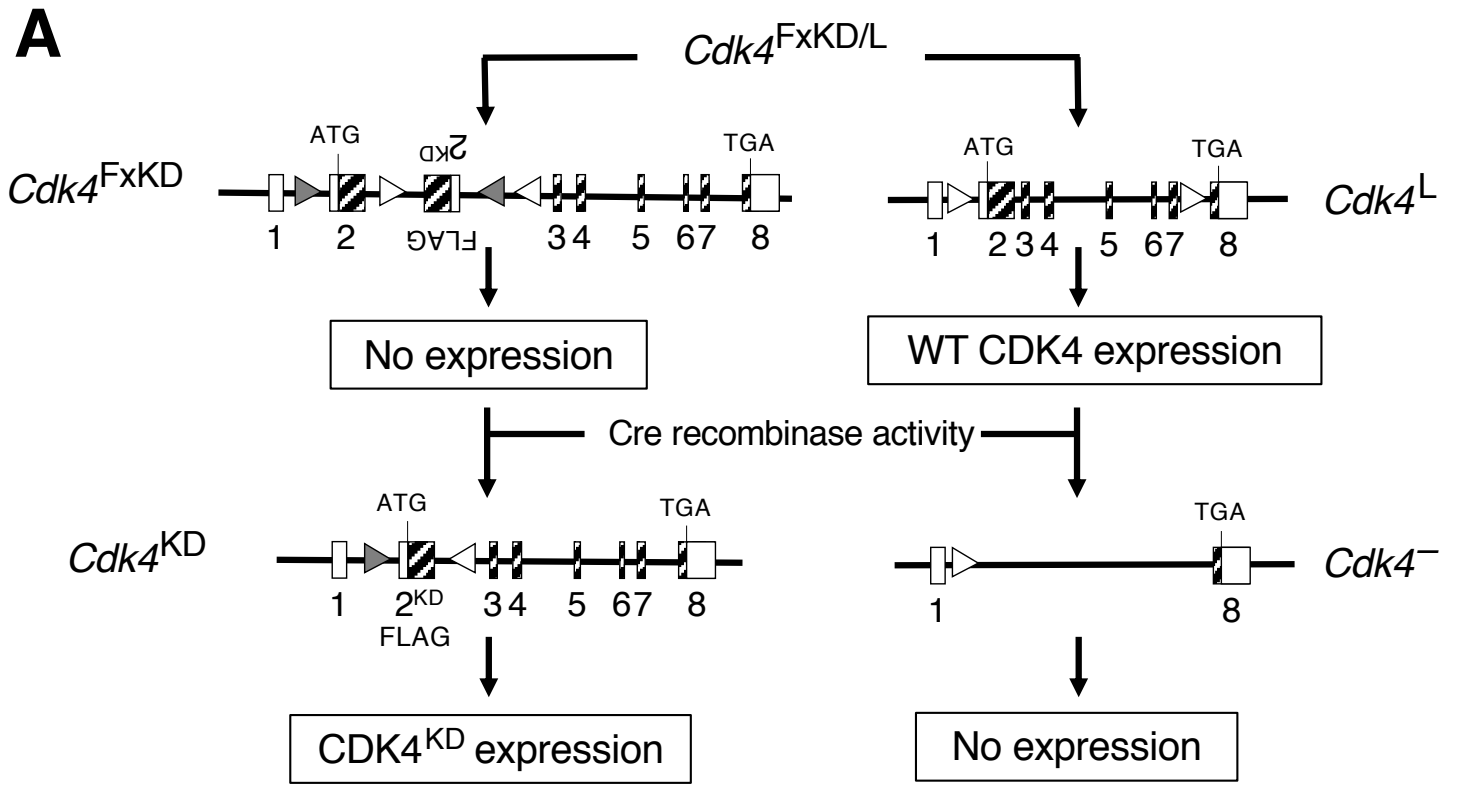
**This PDF file includes:**

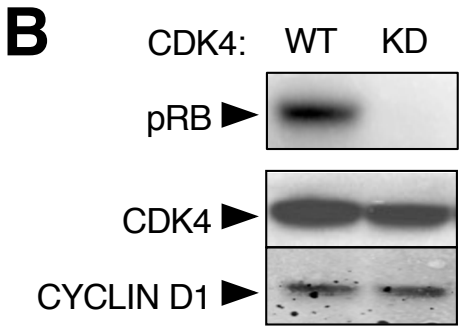
## SI Appendix Materials and Methods

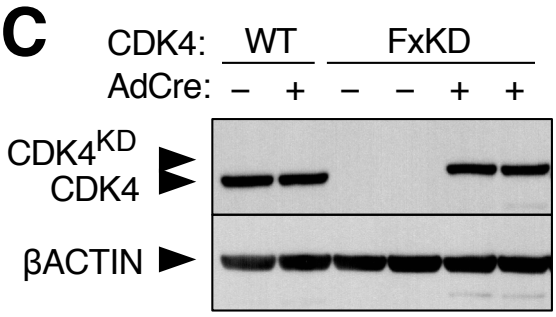
**Tumor genotyping.** Semiquantitative genotyping was performed using nested PCR. The first PCR contains each locus-specific primer needed to detect the ratio between unrecombined (FxKD/L) versus recombined (KD/–) alleles. For the second real time PCR (RT-PCR), the first PCR products were diluted 1:20 in water and used as a 5x stock. The RT-PCR was prepared with fluorescent reporter-quencher custom probes indicated by an asterisk (see below). All reactions were performed in triplicate. Results were analyzed with the CFX Maestro Software (BioRad). Firstly, thresholds are separately set for each fluorophore channel lying above their respective general noise but still within the curves' linear range and subsequently taken to the same consensus value within their ranges. The cycle number where its amplification curves cross the common threshold (Cq as determined in "single threshold" mode) is taken, and used to estimate allele frequencies. Cq values for *Cdk4*$^{FxKD/L}$ and *Cdk4*$^{KD/–}$ alleles are averaged among sample replicates, and these two averages are subtracted from one another. The absolute value of this resulting delta Cq is used to estimate the corresponding fold differences between the unrecombined and recombined alleles abundance in the sample. Oligonucleotides (Integrated DNA Technologies Inc.) used for sample genotyping were:

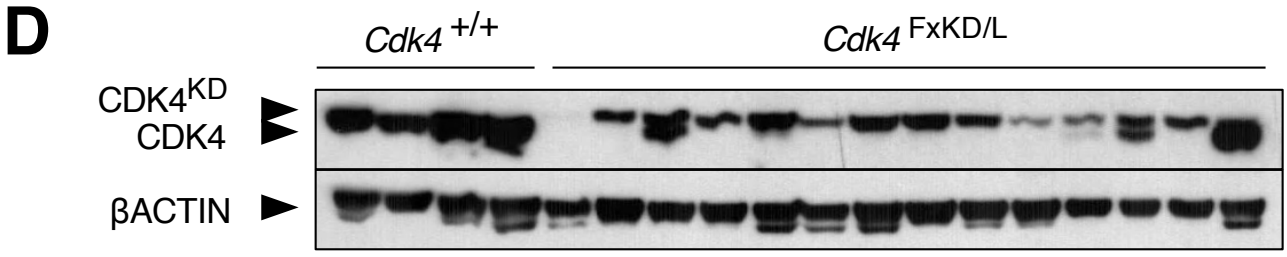
| ***Cdk4*$^{KD}$** | | |
|---|---|---|
| GGTGGAGAGGACAATAGGAC | KD | Fw primer |
| AAACGCTAGTGAGCTCGA | FxKD | Fw primer |
| GAACAAATGATCACCAGCTAGTC | FxKD, KD | Rev primer |
| TTGAACATCCCAATGTTGTACG | KD | Fw primer(*) |
| GGAACTCCTGCACAAGGT | FxKD | Fw primer(*) |
| CAGACATCCATCAGCCTGA | FxKD, KD | Rev primer(*) |
| Fam-CGGATCCATCGACCCATAACTT-IowaBlack | FxKD | probe |
| Cy5/Fam-CGTTGAGGATCTTCTAGAGCTTATAA-IowaBlack | KD | probe |

| ***Cdk4$^{L}$*** | | |
|---|---|---|
| GCCATCTCTCCAGTCCTGTA | L | Fw primer |
| GCAGTCTCATCCAGGATCG | – | Fw primer |
| ACTCTGTCAGCGCTGTATTAC | – | Rev primer |
| GTTATATTATGTACCGAAGTTCCTATACT | L | Rev primer |
| TGCTCTTAGCTGCTGAGC | L | Fw primer(*) |
| GAGCGTAAGGTGAGTGCA | – | Fw primer(*) |
| GCCTTCCATCTCATTGGAGAC | L, – | Rv primer(*) |
| Fam-ATCGAATTCCGAAGTTCCTATTCTC-IowaBlack | L | probe |
| Cy5/Fam-CCGCATTCTGGTACCAGGGC-IowaBlack | – | probe |
| ***Raf1$^{L}$*** | | |
| AGACATCCAGAGACAGGCA | L | Fw primer |
| CTTGGATCCACTAGTTCTAGAGC | – | Fw primer |
| CAGCAGTTAGGTAAGCAGGC | L, – | Rev primer |
| CTGATTGCCCAACTGCCATAA | L | Fw primer(*) |
| CACGATGCATGTAACCTGTGT | – | Fw primer(*) |
| ACTGATCTGGAGCACAGCAAT | L, – | Rev primer(*) |
| Fam-AGCTCTGCAGATAACTTCGT-IowaBlack | L | probe |
| Cy5/Fam-TAGACTCGAGGAATTCCGATCATA-IowaBlack | – | probe |


A
Cdk4FxKD/L
Cdk4FxKD
ATG
2KD
TGA
1 2 FLAG 3 4 5 67 8
ATG
TGA
1 2 3 4 5 67 8
Cdk4L
No expression
WT CDK4 expression
Cre recombinase activity
Cdk4KD
ATG
TGA
1 2KD FLAG 3 4 5 67 8
TGA
1 8
Cdk4−
CDK4KD expression
No expression


B
CDK4: WT KD
pRB
CDK4
CYCLIN D1


C
CDK4: WT FxKD
AdCre: – + – – + +
CDK4KD
CDK4
βACTIN


D
Cdk4 +/+
Cdk4 FxKD/L
CDK4KD
CDK4
βACTIN

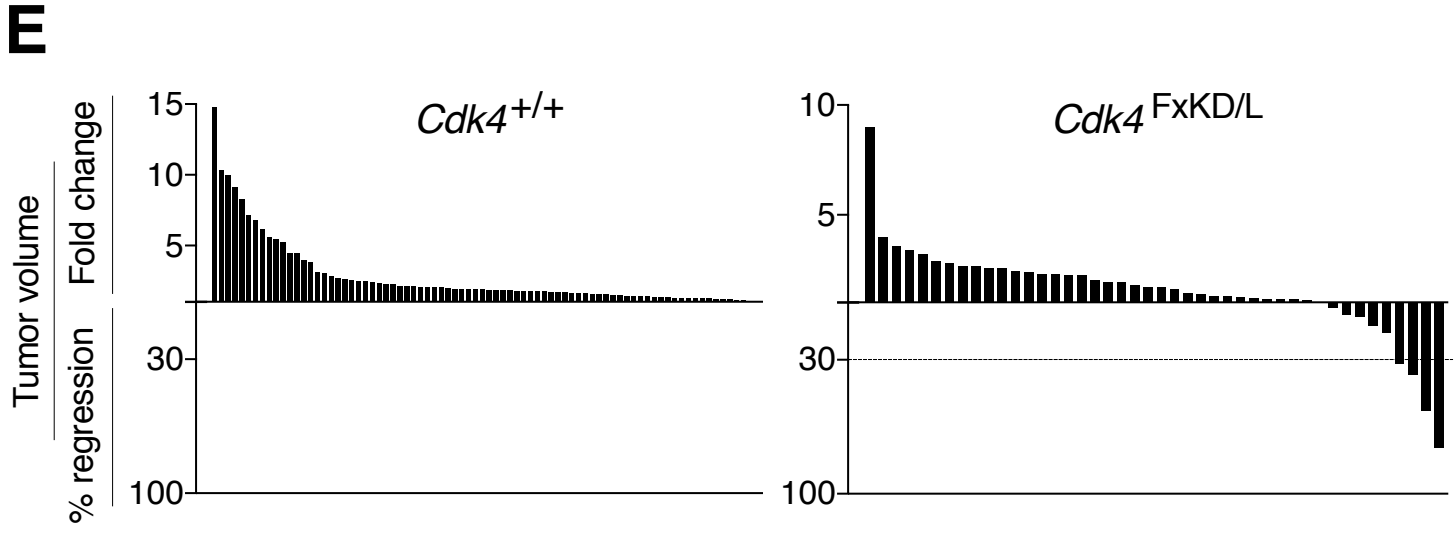
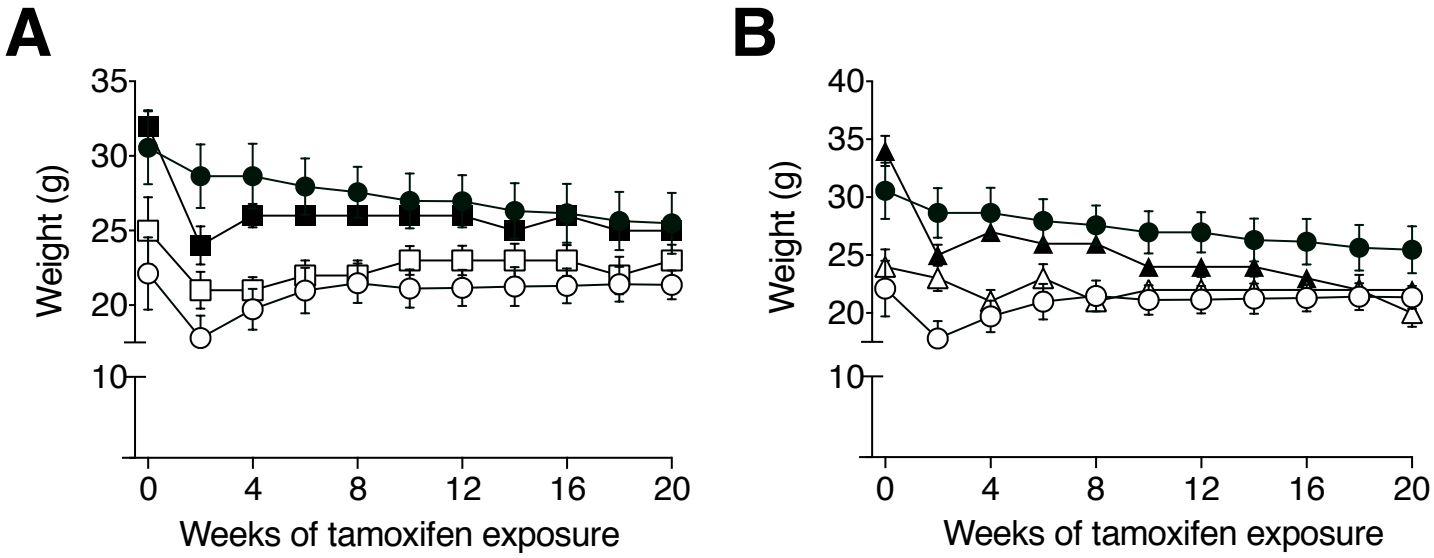
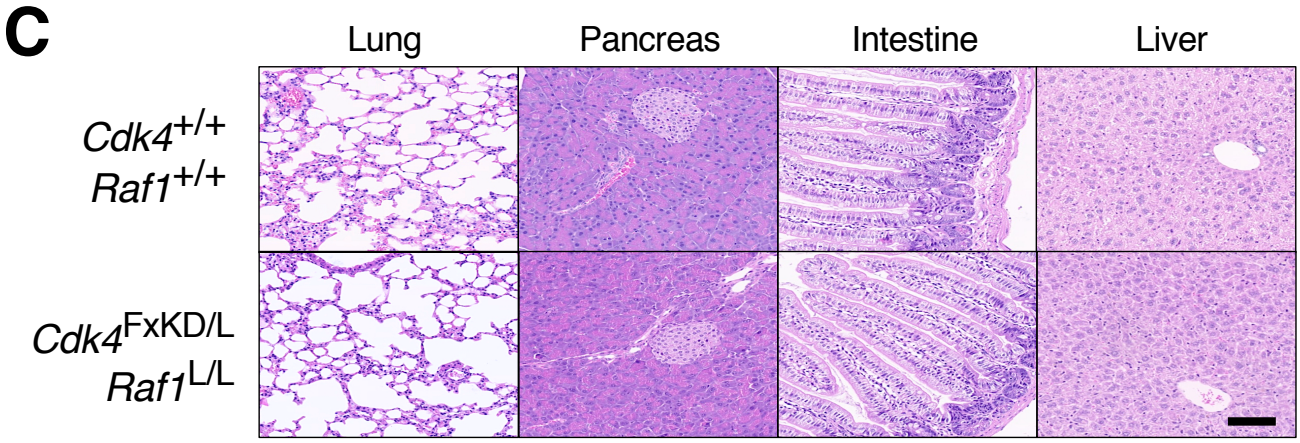

E
Tumor volume
Fold change
% regression
Cdk4+/+
15
10
5
30
100
Cdk4 FxKD/L
10
5
30
100

**Fig. S1. Expression and anti-tumor effect of a CDK4$^{KD}$ kinase dead isoform**. **(A)** Schematic representation of the conditional *Cdk4*$^{FxKD}$ and *Cdk4*$^{L}$ alleles and the resulting *Cdk4*$^{KD}$ and *Cdk4*$^{-}$ alleles generated upon Cre-mediated recombination. Expression of wild type CDK4 or kinase dead CDK4$^{KD}$ proteins by the corresponding alleles is indicated. **(B)** Autoradiography of incorporated [$^{32}$P]-ATP to recombinant RB protein to determine the kinase activity associated with wild type CDK4 and mutant CDK4$^{KD}$ recombinant proteins incubated with mouse CYCLIN D1 expressed in a baculovirus system as determined by Western blot analysis. Migration of the above proteins is indicated by arrowheads. **(C)** Western blot analysis of CDK4 and CDK4$^{KD}$ expression in whole cell extracts obtained from *Cdk4*$^{+/+}$ and *Cdk4*$^{FxKD/FxKD}$ mouse embryonic fibroblasts before and after exposure to Adeno Cre (AdCre) particles. βACTIN was used as loading control. Migration of the above proteins is indicated by arrowheads. **(D)** Western blot analysis of CDK4 and CDK4$^{KD}$ expression in tumor lysates from *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$ mice harboring *Cdk4*$^{+/+}$ or *Cdk4*$^{FxKD/L}$ alleles after 9 wk of TX exposure. βACTIN was used as loading control. **(E)** Waterfall plots representing the increase in tumor volume (fold change) and the percentage of tumor regression as determined by CT scans performed at the beginning and at the end of the trial of individual lung tumors present in *Kras*$^{+/FSFG12V}$;h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{+/+}$ (n=25mice/85tumors) and *Kras*$^{+/FSFG12V}$;h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{FxKD/L}$ (n=19 mice/44 tumors) mice exposed to TX for 9 wk.


A
Weight (g)
35
30
25
20
10
0
4
8
12
16
20
Weeks of tamoxifen exposure
B
Weight (g)
40
35
30
25
20
10
0
4
8
12
16
20
Weeks of tamoxifen exposure


C
Lung
Pancreas
Intestine
Liver
Cdk4+/+
Raf1+/+
Cdk4FxKD/L
Raf1L/L

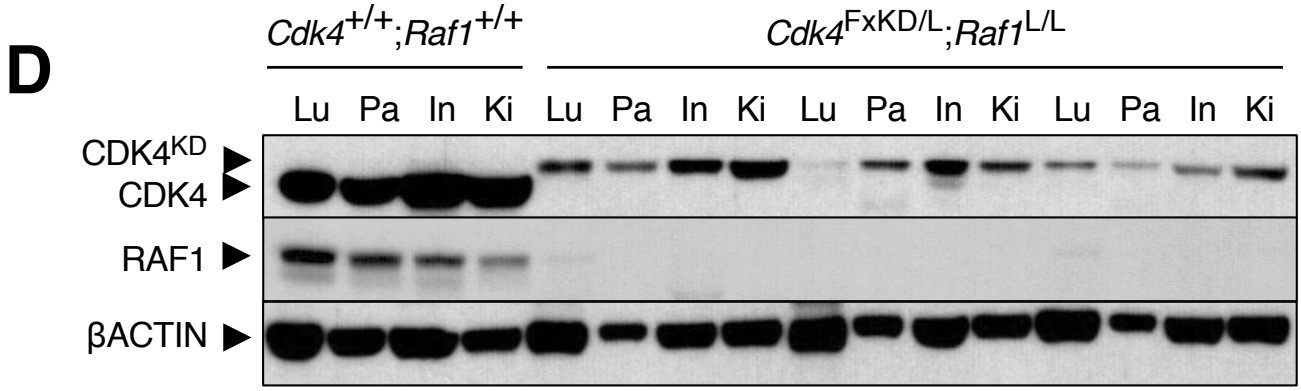

D
Cdk4+/+;Raf1+/+
Cdk4FxKD/L;Raf1L/L
Lu Pa In Ki Lu Pa In Ki Lu Pa In Ki Lu Pa In Ki
CDK4KD
CDK4
RAF1
βACTIN

**Fig. S2: Limited toxicities upon concomitant CDK4 and RAF1 inactivation in adult mice.** **(A)** Body weight change in grams (g) of non-tumor-bearing male (solid) and female (open) mice exposed to TX for 20 wk. h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ (circles) and h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{FxKD/L}$;*Raf1*$^{+/+}$ (squares) mice. n=5 mice/group. Error bars indicate mean ± SEM. **(B)** Body weight change in grams (g) of non-tumor-bearing male (solid) and female (open) mice exposed to TX for 20 wk. h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{+/+}$*;Raf1*$^{L/L}$ (circles) and h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{FxKD/L}$*;Raf1*$^{L/L}$ (triangles) mice. n=5 mice/group. Error bars indicate mean ± SEM. **(C)** Representative images of H&E stained histological sections of paraffin embedded organs of h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ and h*UBC-CreERT2*$^{+/T}$*;Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ mice sacrificed after 20 wk of continuous exposure to TX. (Scale bar, 0.1 mm.). **(D)** Western blot analysis of CDK4, CDK4$^{KD}$ and RAF1 expression levels in tissue lysates obtained from the indicated organs isolated from h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ and h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ mice after 9 wk of TX exposure. βACTIN was used as loading control. Migration of the above proteins is indicated by arrowheads. Lu: lung, Pa: pancreas, In: intestine, Ki: kidney.

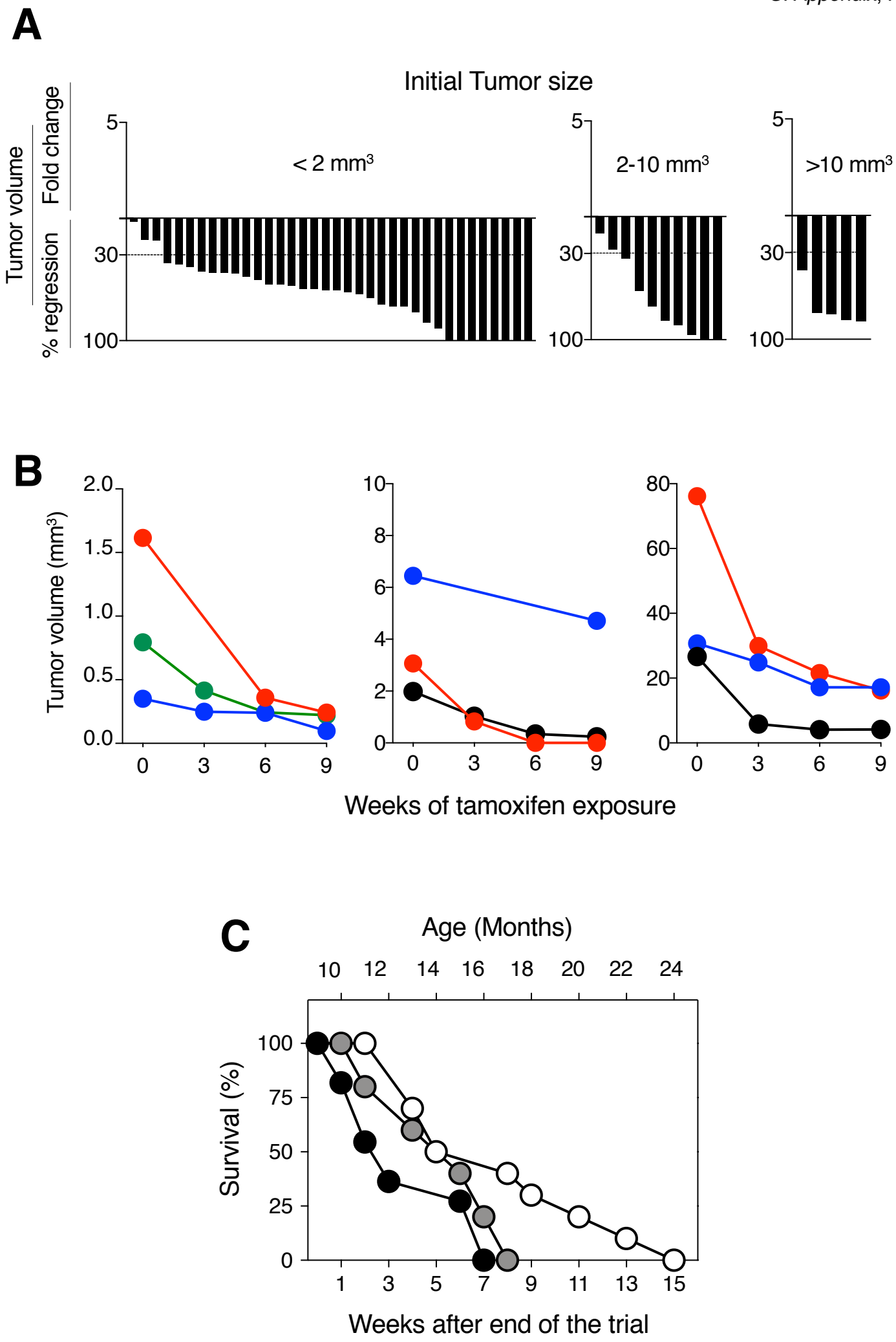

A
Initial Tumor size
Tumor volume
Fold change
% regression
5
30
100
< 2 mm3
2-10 mm3
>10 mm3
B
Tumor volume (mm3)
2.0
1.5
1.0
0.5
0.0
10
8
6
4
2
0
80
60
40
20
0
0
3
6
9
Weeks of tamoxifen exposure
C
Age (Months)
10
12
14
16
18
20
22
24
Survival (%)
100
75
50
25
0
1
3
5
7
9
11
13
15
Weeks after end of the trial

**Fig. S3: Tumor regression upon CDK4/RAF1 inactivation is independent of their initial size. (A)** Waterfall plot representing the increase in tumor volume (fold change) and the percentage of tumor regression as determined by CT scans performed at the beginning and at the end of the trial of individual lung tumors present in *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ (n=19 mice/51tumors) exposed to TX for 9 wk and classified according to their initial tumor volume of less than 2 $mm^3$, 2-10 $mm^3$ or more than 10 $mm^3$. **(B)** Tumor volume monitoring during the 9 wk-long trial of 3 representative tumors from each of the three groups indicated in (**A)**. Each color represents tumors from the same mouse. (**C**) Survival of *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ (solid circles; n=11), *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$;*Raf1*$^{L/L}$ (gray circles; n=6) and *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ (open circles; n=10) mice after the 9 wk-long trial. The upper time scale indicates the age of the mice.

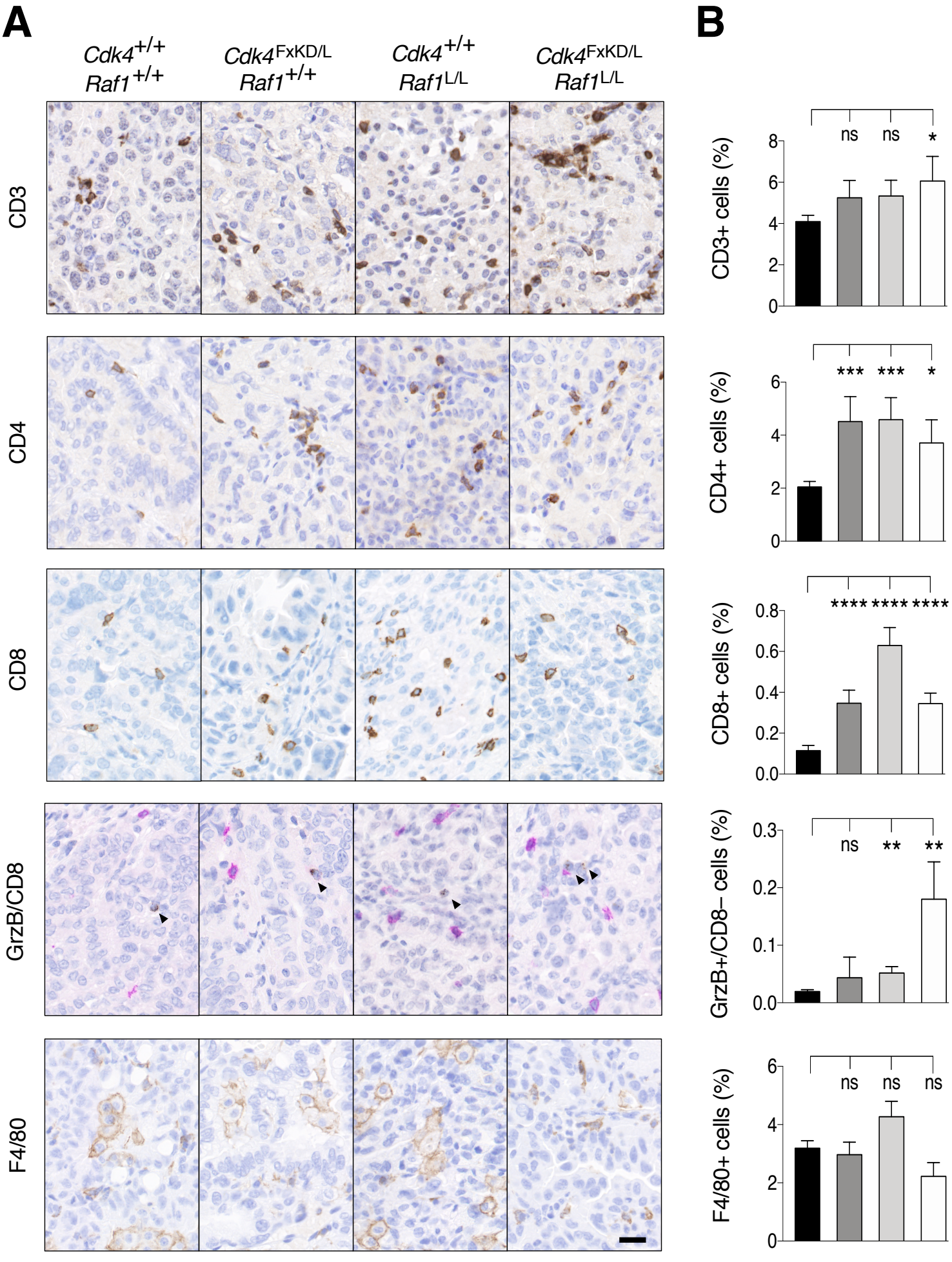

A
Cdk4+/+ Raf1+/+
Cdk4FxKD/L Raf1+/+
Cdk4+/+ Raf1L/L
Cdk4FxKD/L Raf1L/L
CD3
CD4
CD8
GrzB/CD8
F4/80
B
CD3+ cells (%)
ns ns *
CD4+ cells (%)
*** *** *
CD8+ cells (%)
**** **** ****
GrzB+/CD8– cells (%)
ns ** **
F4/80+ cells (%)
ns ns ns

**Fig. S4. Immune cell infiltration in tumors upon combined CDK4 and RAF1 inactivation.** (**A**) Immuno-staining of representative paraffin embedded sections of tumors from *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$ mice harboring *Cdk4*$^{+/+}$;*Raf1*$^{+/+}$, *Cdk4*$^{FxKD/L}$;*Raf1*$^{+/+}$, *Cdk4*$^{+/+}$;*Raf1*$^{L/L}$ and *Cdk4*$^{FxKD/L}$*;Raf1*$^{L/L}$ alleles after 9 wk of TX exposure with antibodies against CD3; CD4; CD8; GranzymeB/CD8 and F480. Scale bar: 0.02 mm. (**B**) Quantification of the results depicted in (**A**). Tumors from *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$ mice harboring *Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ (solid bars) (n=3/28tumors), *Cdk4*$^{FxKD/L}$;*Raf1*$^{+/+}$ (dark gray bars) (n=3/28tumors), *Cdk4*$^{+/+}$;*Raf1*$^{L/L}$ (light gray bars) (n=3/23tumors) and *Cdk4*$^{FxKD/L}$*;Raf1*$^{L/L}$ (open bars) (n=8/28tumors) alleles after 9 wk of TX exposure. *P* values were calculated using the unpaired Student´s *t* test. ****$P$ <0.0001, **$P$ < 0.01, and *$P$ < 0.05, ns, not significant. (Scale bar, 0.02 mm.)

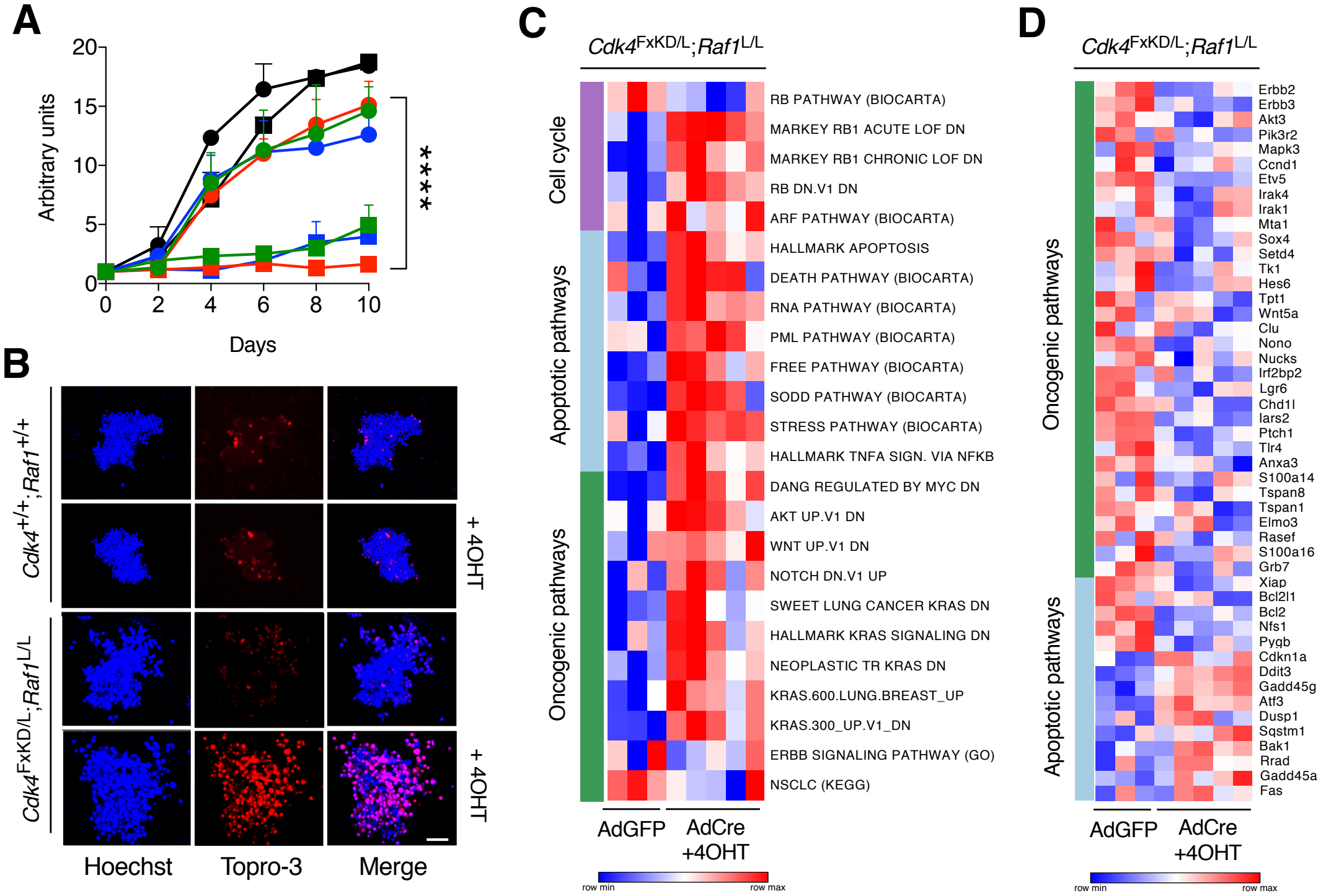
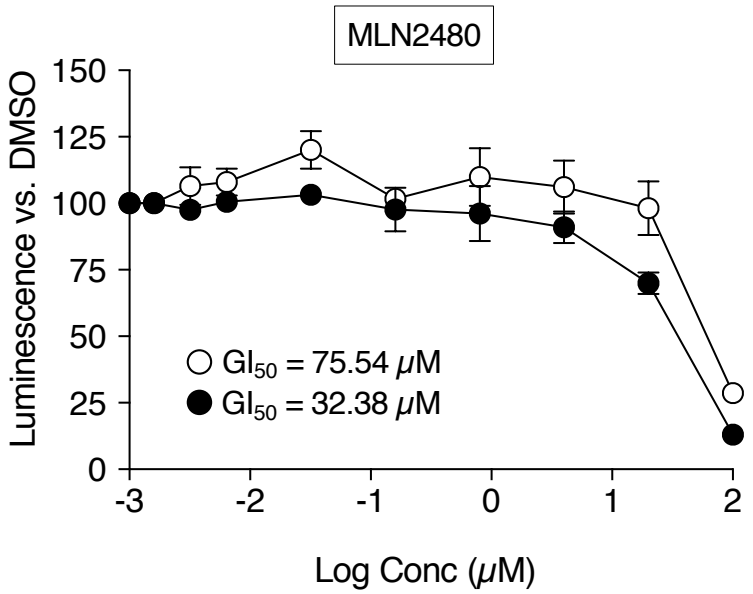
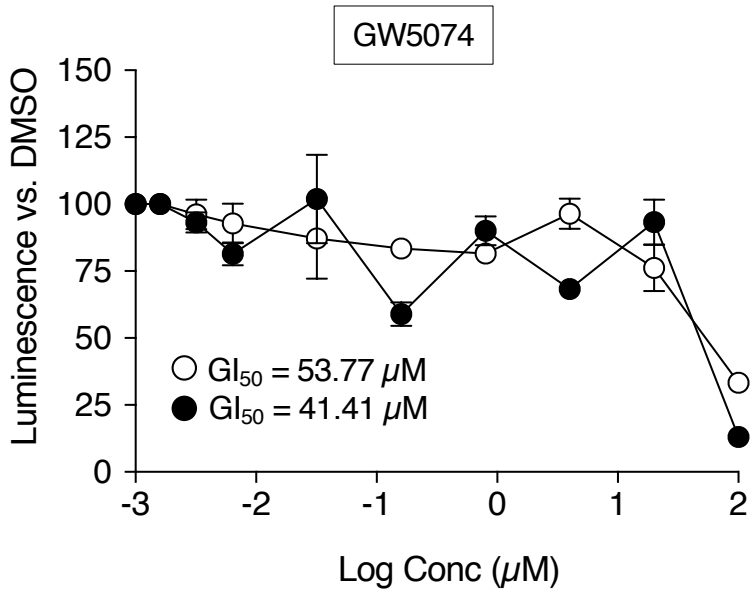
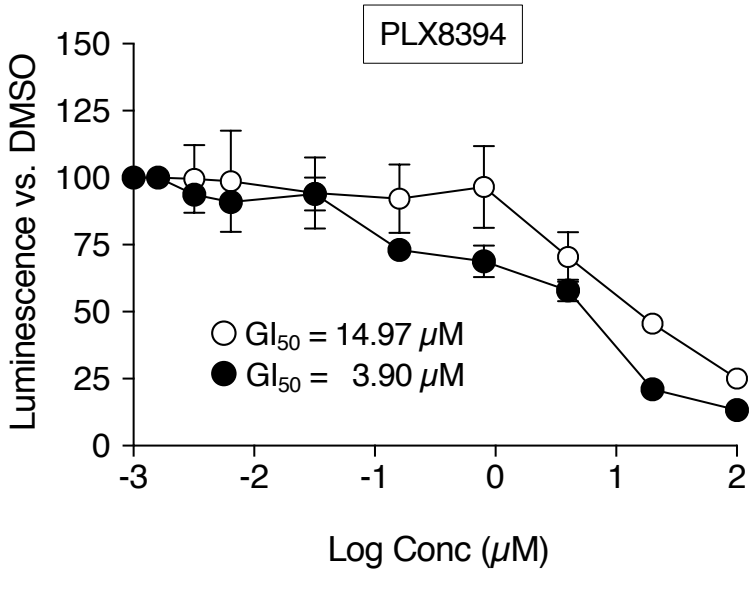

A
Arbitrary units
20
15
10
5
0
0
2
4
6
8
10
Days
****
B
Cdk4+/+;Raf1+/+
Cdk4FxKD/L;Raf1L/L
+ 4OHT
+ 4OHT
Hoechst
Topro-3
Merge
C
Cdk4FxKD/L;Raf1L/L
Cell cycle
Apoptotic pathways
Oncogenic pathways
RB PATHWAY (BIOCARTA)
MARKEY RB1 ACUTE LOF DN
MARKEY RB1 CHRONIC LOF DN
RB DN.V1 DN
ARF PATHWAY (BIOCARTA)
HALLMARK APOPTOSIS
DEATH PATHWAY (BIOCARTA)
RNA PATHWAY (BIOCARTA)
PML PATHWAY (BIOCARTA)
FREE PATHWAY (BIOCARTA)
SODD PATHWAY (BIOCARTA)
STRESS PATHWAY (BIOCARTA)
HALLMARK TNFA SIGN. VIA NFKB
DANG REGULATED BY MYC DN
AKT UP.V1 DN
WNT UP.V1 DN
NOTCH DN.V1 UP
SWEET LUNG CANCER KRAS DN
HALLMARK KRAS SIGNALING DN
NEOPLASTIC TR KRAS DN
KRAS.600.LUNG.BREAST_UP
KRAS.300_UP.V1_DN
ERBB SIGNALING PATHWAY (GO)
NSCLC (KEGG)
AdGFP
AdCre +4OHT
row min
row max
D
Cdk4FxKD/L;Raf1L/L
Oncogenic pathways
Apoptotic pathways
Erbb2
Erbb3
Akt3
Pik3r2
Mapk3
Ccnd1
Etv5
Irak4
Irak1
Mta1
Sox4
Setd4
Tk1
Hes6
Tpt1
Wnt5a
Clu
Nono
Nucks
Irf2bp2
Lgr6
Chd1l
Iars2
Ptch1
Tlr4
Anxa3
S100a14
Tspan8
Tspan1
Elmo3
Rasef
S100a16
Grb7
Xiap
Bcl2l1
Bcl2
Nfs1
Pygb
Cdkn1a
Ddit3
Gadd45g
Atf3
Dusp1
Sqstm1
Bak1
Rrad
Gadd45a
Fas
AdGFP
AdCre +4OHT
row min
row max

**Fig. S5. Concomitant inactivation of CDK4 and RAF1 ablation halts proliferation of lung tumor cells. (A)** Proliferation of *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$ tumor cell lines harboring *Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ (black), *Cdk4*$^{FxKD/L}$;*Raf1*$^{+/+}$ (blue), *Cdk4*$^{+/+}$;*Raf1*$^{L/L}$ (green) and *Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ (red) alleles infected with AdGFP (circles) or with AdCre followed by exposure to 4OHT (squares) to induce recombination of the conditional alleles. Error bars indicate mean ± SEM. *P* values were calculated using the unpaired Student´s *t* test. ****$P$ <0.0001. **(B)** Representative images of TO-PRO-3 and Hoechst stained 3D spheroids derived from *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$ lung tumor cells harboring *Cdk4*$^{+/+}$;*Raf1*$^{+/+}$ or *Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ alleles either untreated or exposed to 4OHT. (Scale bar, 0.1 mm.). **(C)** Heatmap of ssGSEA normalized enrichment scores (NES) for gene sets related to cell cycle (purple), apoptosis (blue), and oncogenic (green) pathways. Columns represent individual *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ lung tumor cell lines infected with AdGFP or with AdCre followed by exposure to 4OHT. (**D)** Heatmap of 48 differentially expressed genes related to apoptosis (blue) and oncogenic (green) pathways. Columns represent the same *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$;h*UBC-CreERT2*$^{+/T}$;*Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ cell lines used in (**B)** infected with AdGFP or with AdCre followed by exposure to 4OHT. Adjusted *P* value < 0.05.


MLN2480
Luminescence vs. DMSO
150
125
100
75
50
25
0
-3
-2
-1
0
1
2
Log Conc (μM)
GI50 = 75.54 μM
GI50 = 32.38 μM


GW5074
Luminescence vs. DMSO
150
125
100
75
50
25
0
-3
-2
-1
0
1
2
Log Conc (μM)
GI50 = 53.77 μM
GI50 = 41.41 μM


PLX8394
Luminescence vs. DMSO
150
125
100
75
50
25
0
-3
-2
-1
0
1
2
Log Conc (μM)
GI50 = 14.97 μM
GI50 = 3.90 μM

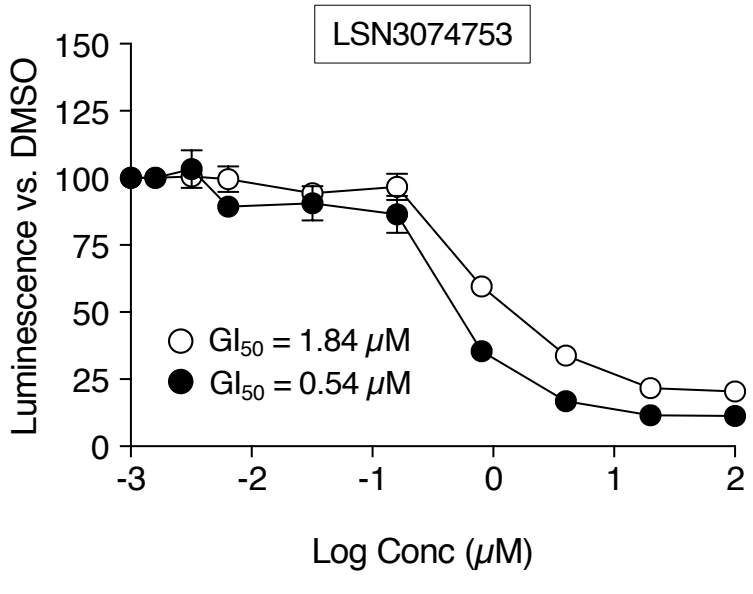

LSN3074753
Luminescence vs. DMSO
150
125
100
75
50
25
0
-3
-2
-1
0
1
2
Log Conc (μM)
GI50 = 1.84 μM
GI50 = 0.54 μM

**Fig. S6. Effect of panRAF inhibitors in PDX derived lung tumor cell lines.** Cell viability assays in PDX-derived cell lines including PDX dc-1 (solid circles) and PDX dc-2 (open circles) treated with 4 different panRAF inhibitors, MLN2480, GW5074, PLX8394 and LSN3074753 for 72h. The GI50 calculated for each cell line is indicated in the figure.

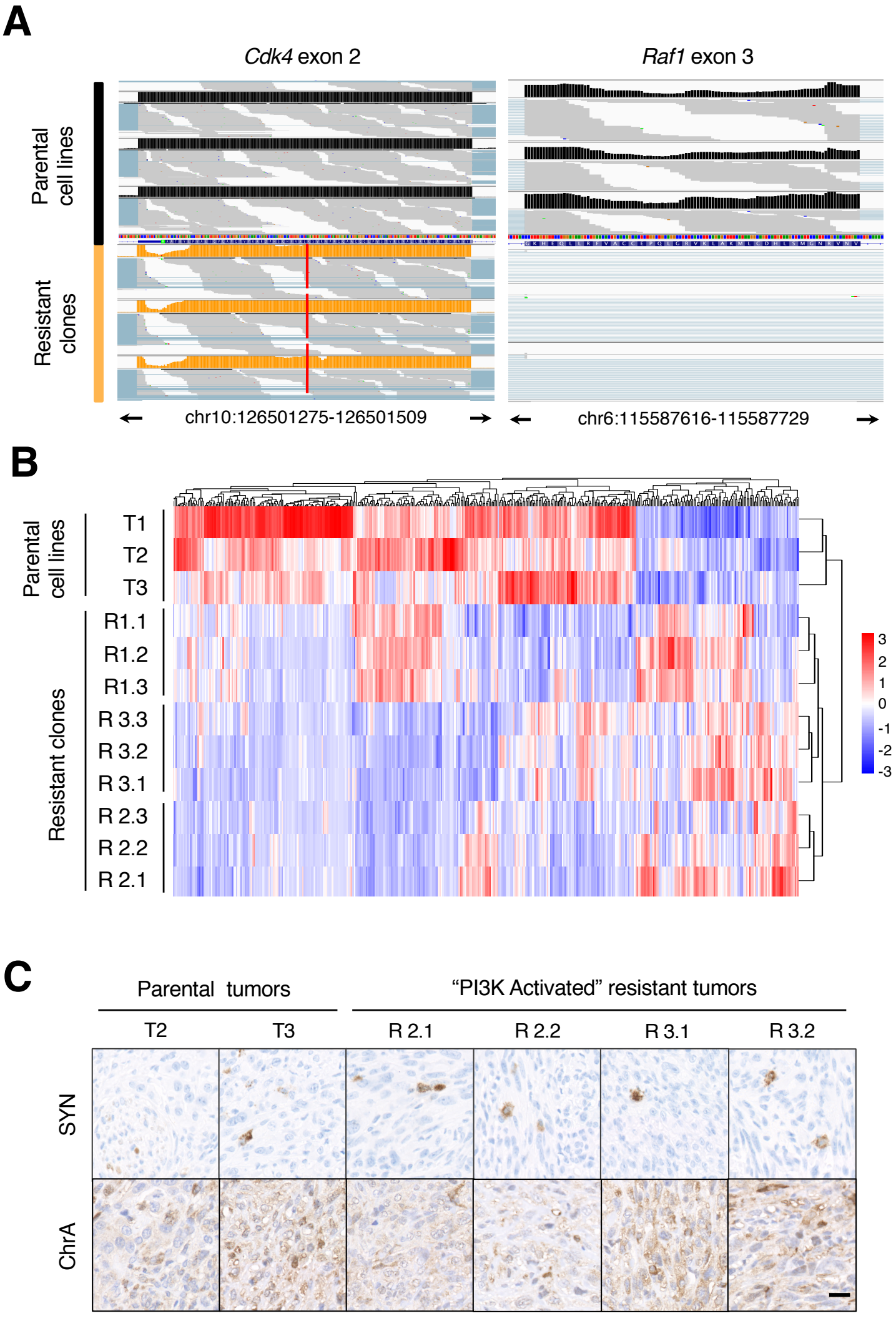

A
Cdk4 exon 2
Raf1 exon 3
Parental cell lines
Resistant clones
chr10:126501275-126501509
chr6:115587616-115587729
B
Parental cell lines
T1
T2
T3
Resistant clones
R1.1
R1.2
R1.3
R 3.3
R 3.2
R 3.1
R 2.3
R 2.2
R 2.1
3
2
1
0
-1
-2
-3
C
Parental tumors
"PI3K Activated" resistant tumors
T2
T3
R 2.1
R 2.2
R 3.1
R 3.2
SYN
ChrA

**Fig. S7. Transcriptional and phenotypic characterization of CDK4/RAF1 resistant clones.**

**(A)** Short-reads alignments of *Cdk4* exon 2 and *Raf1* exon 3 sequences from RNAseq data mapped to the genome reference (Genome assembly MGSCv37, mm9) in parental cell lines before CDK4 inactivation and RAF1 ablation (solid bars) and in CDK4/RAF1 resistant clones (orange bars). The red lines in *Cdk4* exon 2 show the AAG to ATG mutation responsible for the K35M mutation present in the $CDK4^{KD}$ kinase dead isoform. The lack of sequences in *Raf1* exon 3 reflect the Cre-mediated recombination in the $Raf1^{L}$ alleles responsible for the ablation of RAF1 expression. Three samples for each genotype are shown for illustrative purposes. **(B)** Heatmap representing color-coded expression levels of differentially expressed genes in CDK4/RAF1 resistant clones (R1.1, R1.2, R1.3, R2.1 R2.2, R2.3, R3.1, R3.2 and R3.3) and their respective parental cell lines (T1, T2 and T3) before CDK4 and RAF1 inactivation. **(C)** Immuno-staining with antibodies against synaptophysin (SYN) and chromogranin A (ChrA) in paraffin embedded sections of tumors generated from subcutaneously implanted parental (T) cells or CDK4/RAF1 "PI3K Activated" resistant clones (R) in immunodeficient mice. (Scale bar, 0.02 mm.)

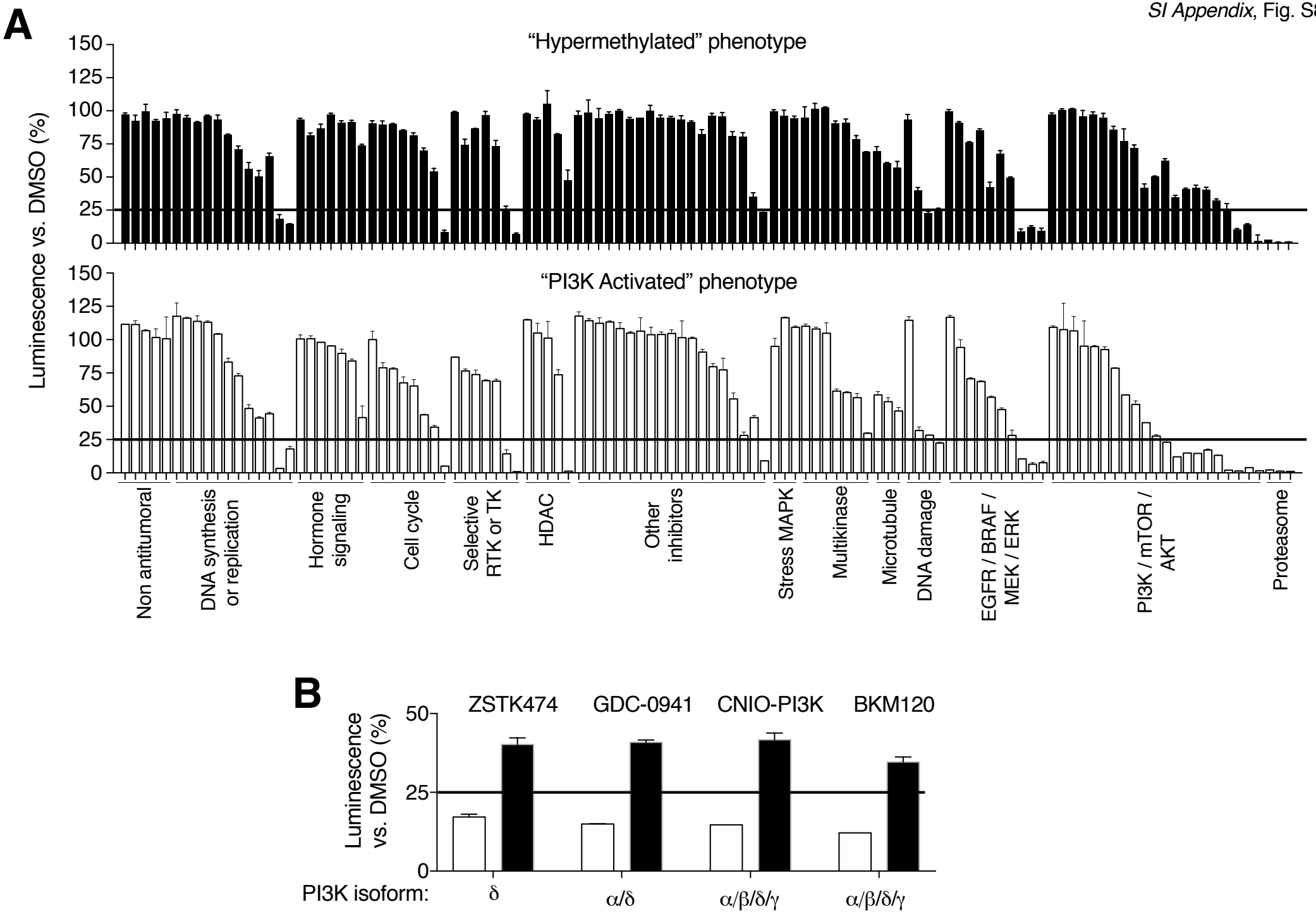

A
"Hypermethylated" phenotype
"PI3K Activated" phenotype
Luminescence vs. DMSO (%)
150
125
100
75
50
25
0
Non antitumoral
DNA synthesis or replication
Hormone signaling
Cell cycle
Selective RTK or TK
HDAC
Other inhibitors
Stress MAPK
Multikinase
Microtubule
DNA damage
EGFR / BRAF / MEK / ERK
PI3K / mTOR / AKT
Proteasome
B
ZSTK474
GDC-0941
CNIO-PI3K
BKM120
Luminescence vs. DMSO (%)
50
25
0
PI3K isoform:
δ
α/δ
α/β/δ/γ
α/β/δ/γ

**Fig. S8. Search for additional drug vulnerabilities in CDK4/RAF1 resistant cells. (A)** Cell viability assay of R1 (solid bars) and R2 and R3 (open bars) resistant clones after screening with a library of 114 compounds at a unique 5 μM dose during 72 hours. The solid horizontal lines represent the cut-off for 25% inhibition (**B)** Compounds that achieved >75% cell growth inhibition for R2 and R3 resistant clones having a "PI3K Activated" phenotype (open bars) compared to R1 resistant clones having a "hypermethylated" phenotype (solid bars). The designation and PI3K isoform specificity of each compound is indicated. The solid horizontal line represents the 25% inhibition cut-off. Error bars indicate mean ± SEM.

**Table S1**. Number of tumors and therapeutic responses observed upon inactivation of CDK4 alone or in combination with RAF1 ablation after 9 weeks of TX exposure. Stable Disease (SD), Partial Response (PR), Complete Response (CR) and Progressive Disease (PD).

| Tumor number and therapeutic response | *Kras*$^{+/FSFG12V}$; h*UBC-CreERT2*$^{+/T}$; *Cdk4*$^{+/+}$ | | *Kras*$^{+/FSFG12V}$; h*UBC-CreERT2*$^{+/T}$; *Cdk4*$^{FxKD/L}$ | | *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$; h*UBC-CreERT2*$^{+/T}$; *Cdk4*$^{+/+}$ | | *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$; h*UBC-CreERT2*$^{+/T}$; *Cdk4*$^{FxKD/L}$ | |
|---|---|---|---|---|---|---|---|---|
| | Number of tumors | | Number of tumors | | Number of tumors | | Number of tumors | |
| Initial tumor number | 105 | | 51 | | 70 | | 31 | |
| Final tumor number | 85 | | 44 | | 45 | | 27 | |
| SD (<30%) | 0 | 0% | 5 | 11% | 0 | 0% | 1 | 4% |
| PR (>30%) | 0 | 0% | 4 | 9% | 0 | 0% | 2 | 7% |
| CR | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% |
| PD | 85 | 100% | 35 | 80% | 45 | 100% | 24 | 89% |

| Tumor number and therapeutic response | *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$; h*UBC-CreERT2*$^{+/T}$; *Cdk4*$^{+/+}$;*Raf1*$^{L/L}$ | | *Kras*$^{+/FSFG12V}$;*Trp53*$^{F/F}$; h*UBC-CreERT2*$^{+/T}$; *Cdk4*$^{FxKD/L}$;*Raf1*$^{L/L}$ | |
|---|---|---|---|---|
| | Number of tumors | | Number of tumors | |
| Initial tumor number | 66 | | 56 | |
| Final tumor number | 62 | | 51 | |
| SD (<30%) | 7 | 11% | 5 | 10% |
| PR (>30%) | 34 | 55% | 34 | 66% |
| CR | 6 | 10% | 12 | 24% |
| PD | 15 | 24% | 0 | 0% |

**Table S2**. Percentage of allele excision in residual lesions as determined by laser capture microdissection

| | Percentage of allele recombination | | |
|---|---|---|---|
| Tumor | *Cdk4*$^{L}$ | *Cdk4*$^{FxKD}$ | *Raf1*$^{L}$ |
| T1 | 77% | 94% | 100% |
| T2 | 99% | 98% | 85% |
| T3 | 100% | 94% | 100% |
| T4 | 98% | 98% | 95% |
| T5 | 100% | 85% | 100% |
| T6 | 100% | 80% | 98% |
| T7 | 100% | 100% | 100% |
| T8 | 100% | 35% | 99% |
| T9 | 79% | 56% | 100% |
| T10 | 83% | 36% | 84% |
| T11 | 100% | 55% | 98% |
| T12 | 95% | 27% | 98% |
| T13 | 99% | 55% | 99% |
| T14 | 100% | 60% | 99% |

**Table S3**. Compounds used in the drug screening assay described in *SI Appendix*, Fig. S8

| | Drug Name |
|---|---|
| Non antitumoral | EFLORNITHINE |
| | SURAMIN |
| | METFORMIN |
| | DISULFIRAM |
| | TEMPOL |
| DNA synthesis or replication | OXALIPLATIN |
| | TEMOZOLOMIDE |
| | IRINOTECAN |
| | CYCLOPHOSPHAMIDE |
| | CISPLATINUM |
| | LOMUSTINE |
| | 5-FLUORACIL |
| | MITOMYCIN C |
| | SN-38 |
| | ETOPOSIDE VP16-213 |
| | DOXORUBICINE |
| | GEMCITABINE |
| Hormone signalling | LETROZOLE |
| | MIFEPRISTONE |
| | GENISTEIN |
| | ABIRATERONE |
| | KETOCONAZOLE |
| | FULVESTRANT |
| | TAMOXIFEN |
| Cell cycle | ROSCOVITINE |
| | SNS-314 MESYLATE |
| | TOZASERTIB |
| | ALISERTIB |
| | INH CDK4/6 |
| | AT7519 |
| | GSK461364 |
| | FLAVOPIRIDOL |
| Selective RTK or TK | NVP-BGJ398 |
| | QUIZARTINIB |
| | LY2801653 |
| | OSI-906 |
| | BAY 61-3606 |
| | TX-1123 |
| | CRIZOTINIB |

| | Drug Name |
|---|---|
| HDAC | VALPROIC ACID |
| | RICOLINOSTAT |
| | PHENYLBUTYRATE |
| | VORINOSTAT |
| | PANOBINOSTAT |
| Other inhibitors | AICAR |
| | CNIO-PIM |
| | ZILEUTON |
| | FINASTERIDE |
| | PX-478 |
| | EX-527 |
| | SILMITASERTIB |
| | VISMODEGIB, |
| | GALUNISERTIB |
| | SEMAGACESTAT |
| | S-RUXOLITINIB |
| | LY-411575 |
| | SB 505124 |
| | BAY 87-2243 |
| | BARDOXOLONE |
| | ELESCLOMOL |
| | S7289 |
| | PEMETREXE |
| | GELDANAMYCIN |
| Stress MAPK | TANZISERTIB |
| | SB 203580 |
| | DORAMAPIMOD |
| Multikinase | LINIFANIB |
| | IMATINIB |
| | SORAFENIB |
| | PAZOPANIB |
| | CUDC-101 |
| | DOVITINIB |
| | DASATINIB |
| Micro-tubule | DOCETAXEL |
| | PACLITAXEL |
| | VINCRISTINE |

| | Drug Name |
|---|---|
| DNA damage | OLAPARIB |
| | KU-57788 |
| | AZ20 |
| | CNIO-ATR |
| EGFR/BRAF/ MEK/ERK | VEMURAFENIB |
| | DABRAFENIB |
| | ERLOTINIB |
| | GEFITINIB |
| | LAPATINIB |
| | GDC-0994 |
| | SELUMETINIB |
| | PD-0325901 |
| | TRAMETINIB |
| | SCH772984 |
| PI3K/mTOR/AKT | PERIFOSINE |
| | GSK2636771 |
| | PF 4708671 |
| | PILARALISIB |
| | AZD5363 |
| | TGX-221 |
| | IDELALISIB |
| | RAPAMYCIN |
| | BYL-719 |
| | MK-2206 |
| | KU-0063794 |
| | GEDATOLISIB |
| | BKM120 |
| | GDC-0941 |
| | PI3K-CNIO |
| | ZSTK474 |
| | BEZ-235 |
| | GSK2126458 |
| | CNIO-PI3K-2 |
| | CNIO-PI3K-3 |
| | CUDC-907 |
| Protea-some | IXAZOMIB |
| | BORTEZOMIB |
| | CARFILZOMIB |