Figure S1: (A) Pie graph showing the proportion of different KRAS mutant alleles present in the cohort of cell lines employed. Heatmap showing the relative expression of genes that denote the quasi-mesenchymal and classical subtypes of pancreatic cancer. The models fall into either the classical or quasi-mesenchymal subtypes as indicated in the table. (B) The indicated pancreatic cancer cell lines expressing H2B-GFP were treated with 100 nM palbociclib (PD) and proliferation was determined by live cell imaging over the indicated time course. Representative images and quantification as a function of time is shown. The 7310 model lacks RB and is completely unresponsive to palbociclib (PD). (C) The MCF7 breast cancer cell line expressing H2B-GFP was treated with 100 nM palbociclib (PD) and proliferation was determined by live cell imaging over the indicated time course. (D) The indicated cell lines were treated with increasing doses of palbociclib (PD) and protein levels were determined by immunoblotting. (E) The 3226 cell line was treated with palbociclib (PD) for the indicated time-course and the indicated proteins were detected by immunoblotting.

Figure S2: (A) The indicated cell lines were treated with abemaciclib for 48 hours and the levels of the indicated proteins was determined by immunoblotting. (B) The RB-deficient 7310 cell line was treated with palbociclib (PD) for 48 hours and the levels of the indicated proteins was determined by immunoblotting. (C) The indicated gene levels were determined by RNA sequencing from the cells treated with palbociclib. (D) Stability of cyclin D1 and cyclin E1 was determined after cycloheximide (CHX) treatment for the indicated times in the presence and absence of PD0332991 (200 nM) pre-treatment. Protein expression was analyzed by immunoblotting. (E) The indicated cell lines were treated with palbociclib (PD) (200 nM) for 48 hours and the localization of cyclin D1 (upper row) and cyclin E1(lower row) was determined by immunofluorescent microscopy. Representative confocal images are shown. (F) Gene expression

analysis from cells treated with either palbociclib (PD), the combination with TAK228 or trametinib (at 100 nM) is shown. ETV4 and DUSP6 are established MEK regulated genes, while ACAT2 and ALDH3A1 are regulated by MTOR. The graph shows data from 4 independent cell lines that were sequenced in triplicates (**p < 0.01, ***p < 0.001 as determined by t test). Immunoblot analysis to determine the effect of palbociclib (100 nM) +/- TAK228 (100 nM) on mitogenic signaling pathways following 48 hours exposure.

Figure S3: (A) Cell lysates from cell treated with DMSO or 200 nM palbociclib (PD) were subjected to immunoprecipitation with IgG, anti-p21, or anti-p27. Resultant immunoprecipitates were resolved and the indicated proteins detected by immunoblotting. The input serves as the control for relative protein abundance. (B) BrdU incorporation assay and immunoblot analysis for the 226 cell line transfected with CDK2 and non-target (NT) RNAi in the presence and absence of palbociclib (PD). The mean and SD are shown (***p < 0.001 as determined by t test). (C) BrdU incorporation assay and immunoblot analysis for the indicated cell lines transfected with cyclin E1 and non-target (NT) RNAi in the presence and absence of palbociclib (PD). The mean and SD are shown (**p < 0.01 as determined by t test). (D) BrdU incorporation assay and immunoblot analysis for the indicated cell lines transfected with cyclin D1 and non-target (NT) RNAi in the presence and absence of palbociclib (PD). The mean and SD are shown (**p < 0.01, **p<0.001 as determined by t test). (E) Immunoblot analysis for the indicated phosphorylation sites on CDK4 and CDK2.

Figure S4: (A) The indicated PDX models were randomized when tumor volume reached ~200 mm^3 for treatment with vehicle or palbociclib. Tumors were treated for 21 days and tumor volume was monitored every 48 hours. Data shows the mean and standard error of the mean. Greater than n=5 tumors mice were treated for each

condition. For all PDX models, except 827 PDX, the effect of palbociclib was significant for reducing tumor size ($p<0.01$). In the 827 PDX model there was rapid progression during the first 9 days of treatment. (B) Correlation between the cell cycle response observed in cell lines as determined by the AUC of BrdU incorporation inhibition versus the tumor volume at 20 days of treatment. (C) Association between the response to palbociclib and cyclin D1 and cyclin E1 base line gene expression levels, the established transcriptional subtypes of pancreatic cancer, and canonical mutations in PDX models. (D) Analysis of the relationship between the indicated gene expression levels (x-axis) and therapeutic response in PDX models. Neither KRAS nor RB1 are significantly associated with response $p>0.05$. The expression of FLG and SMAD9 genes are associated with response $p<0.01$.

Figure S5: (A) Heatmap of genes that correlated with response to palbociclib in PDX models. DNA replication represents one of the networks wherein the level of transcriptional repression is associated with response. (B) RNA sequencing from tumors that become resistant to palbociclib during the course of treatment compared against sensitive modes. The log fold change relative to the vehicle control is plotted. The genes that were selectively repressed in the sensitive models are shown in red. The top gene ontologies of those genes are shown.

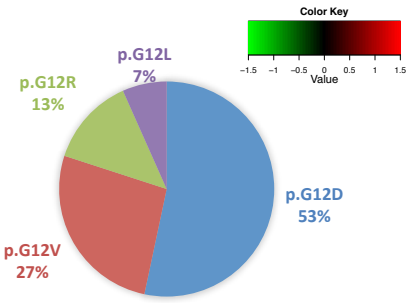
Figure S6: (A) Immunoblot for the levels of CDK2 in cells treated with the indicated agents. (B) Isobologram analysis showing dose response relationship with both palbociclib (PD) and TAK228. Heatmaps depict relative level of BrdU incorporation at 48 hours post treatment. Blue shading is associated with response, inset numbers show the fractional BrdU incorporation relative to DMSO control. (C) Live cell imaging of cells treated with DMSO, palbocicib (100 nM), TAK228 (100 nM), or the combination in

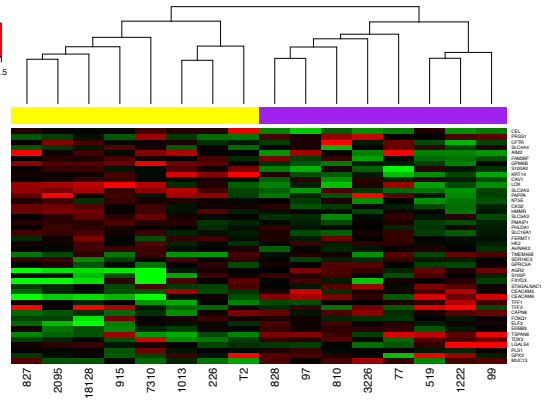
the RB deficient 7310 cell line (D) The mouse weight from a representative PDX cohort treated with palbociclib (PD) and the combination of palbociclib and TAK 228 (PD+TAK).

Figure S7: (A) Representative images of H&E and immunohistochemical staining of the indicated PDX models treated with palbociclib (PD) or the combination with TAK228. (B) Valcono plot shows genes selectively repressed by PD+TAK (red) vs. PD and PD+TAK (blue). Gene ontology terms for each gene set. (C) Heatmap of gene set variance analysis terms that are selectively repressed by the combination of palbociclib with TAK228 (PD+TAK).

Fig S1

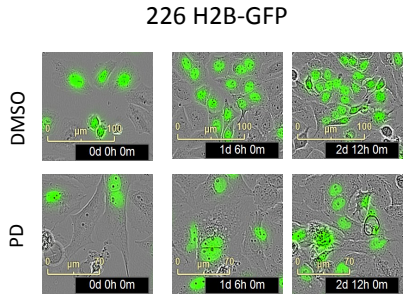
A.

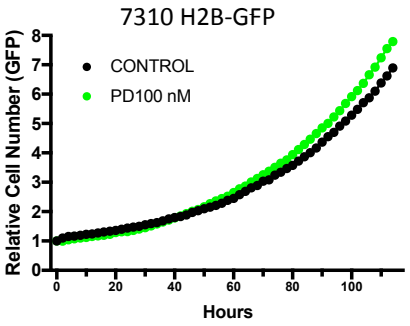


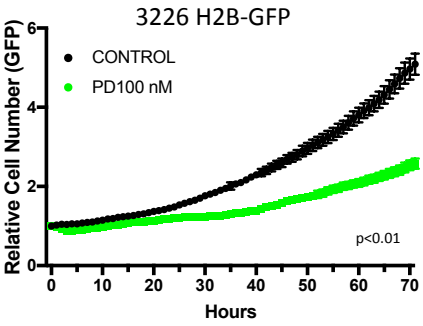


Model	Collisson Subtype
828	Classical
915	Quasi-mesenchymal
519	Classical
77	Quasi-mesenchymal
97	Classical
18128	Quasi-mesenchymal
2095	Quasi-mesenchymal
1222	Classical
810	Classical
1013	Quasi-mesenchymal
827	Quasi-mesenchymal
3226	Quasi-mesenchymal
226	Quasi-mesenchymal
T2	Classical
99	Classical
7310	Quasi-mesenchymal

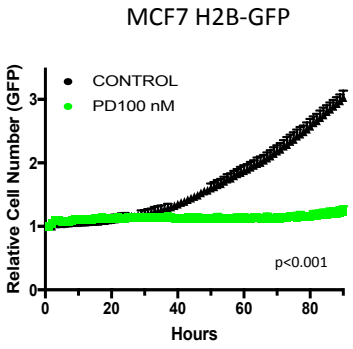
B.



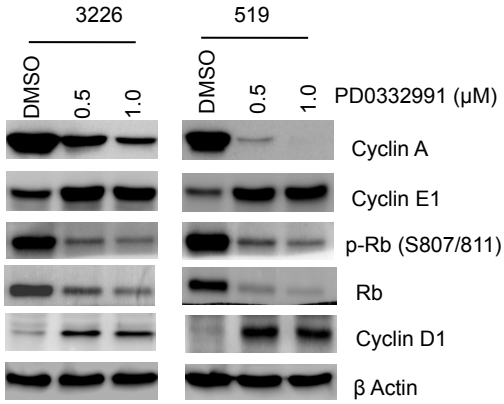




C.



D.



E.

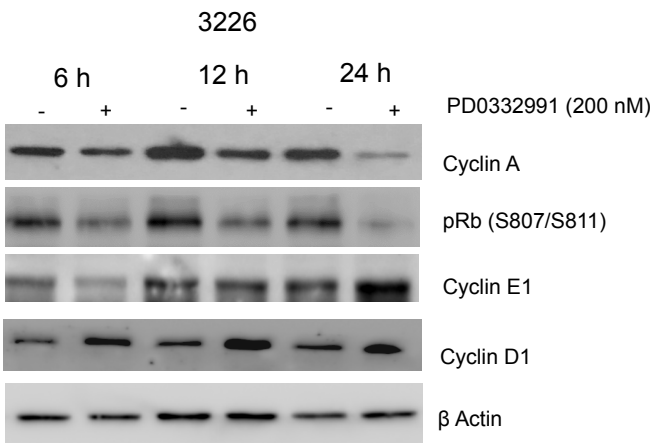


Fig S2

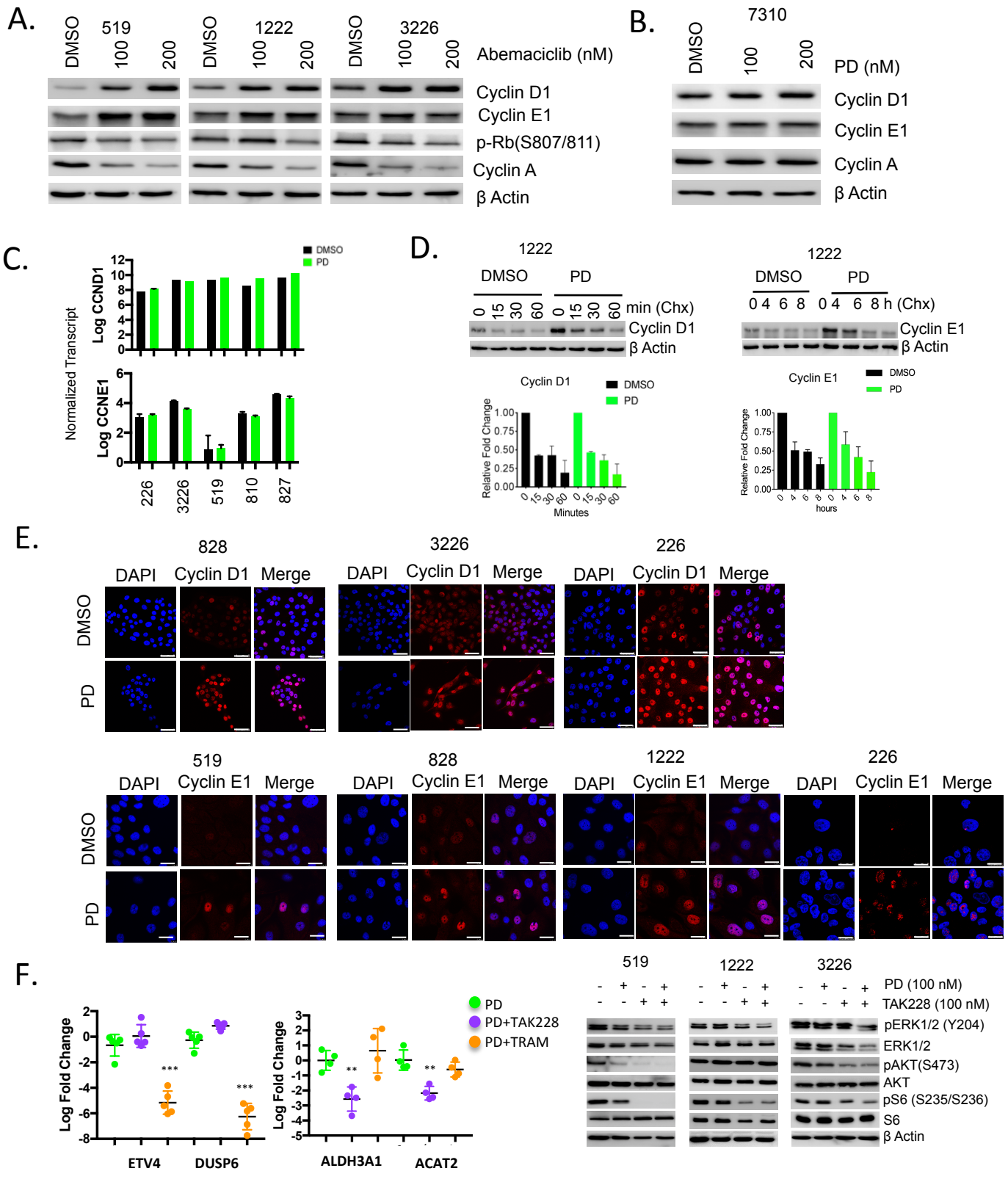

A.
519
1222
3226
DMSO
100
200
Abemaciclib (nM)
Cyclin D1
Cyclin E1
p-Rb(S807/811)
Cyclin A
β Actin
B.
7310
PD (nM)
C.
DMSO
PD
Normalized Transcript
Log CCND1
Log CCNE1
226
3226
519
810
827
D.
1222
min (Chx)
h (Chx)
Relative Fold Change
Minutes
hours
E.
828
DAPI
Cyclin D1
Merge
Cyclin E1
F.
PD
PD+TAK228
PD+TRAM
Log Fold Change
ETV4
DUSP6
ALDH3A1
ACAT2
PD (100 nM)
TAK228 (100 nM)
pERK1/2 (Y204)
ERK1/2
pAKT(S473)
AKT
pS6 (S235/S236)
S6

Fig S3

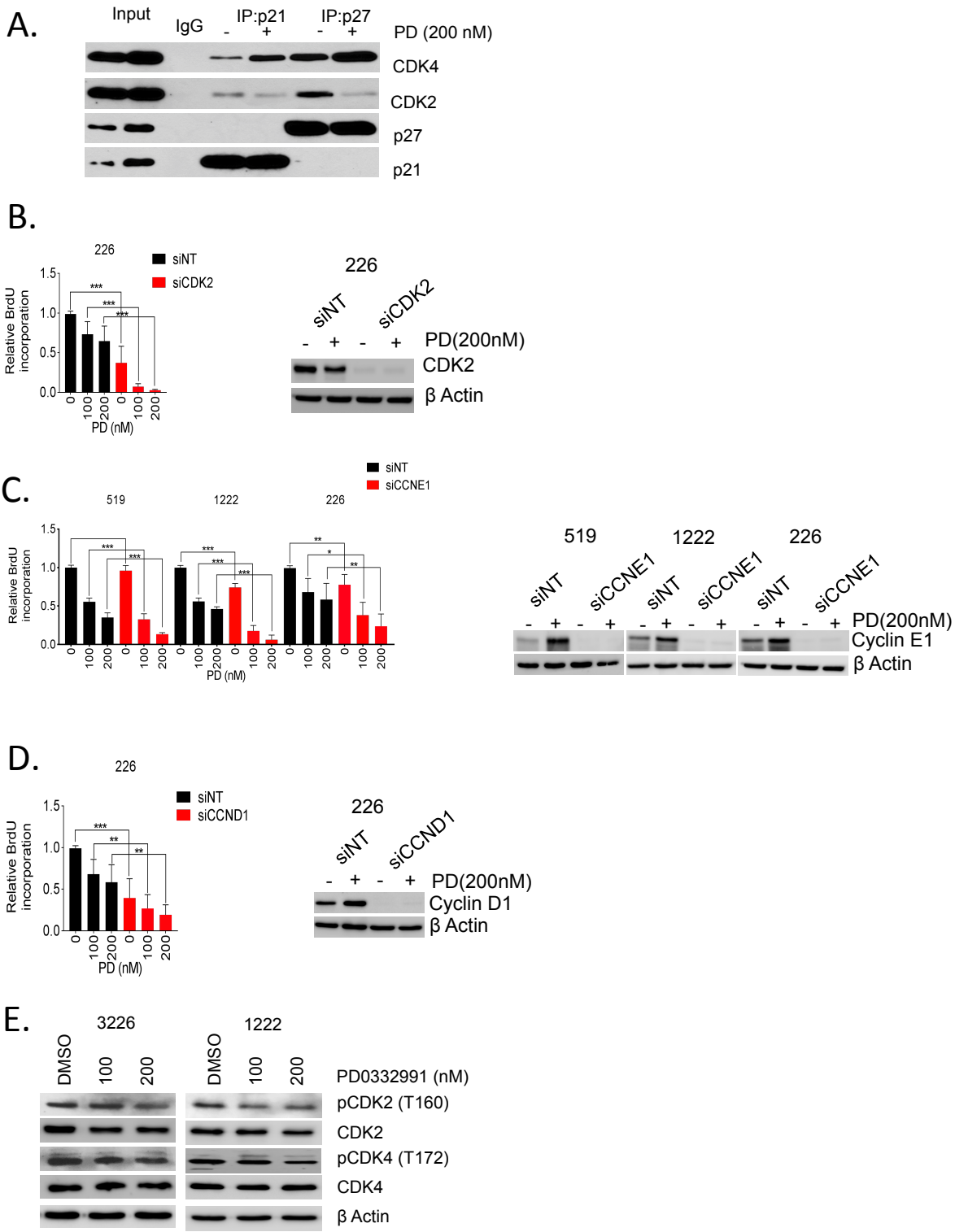
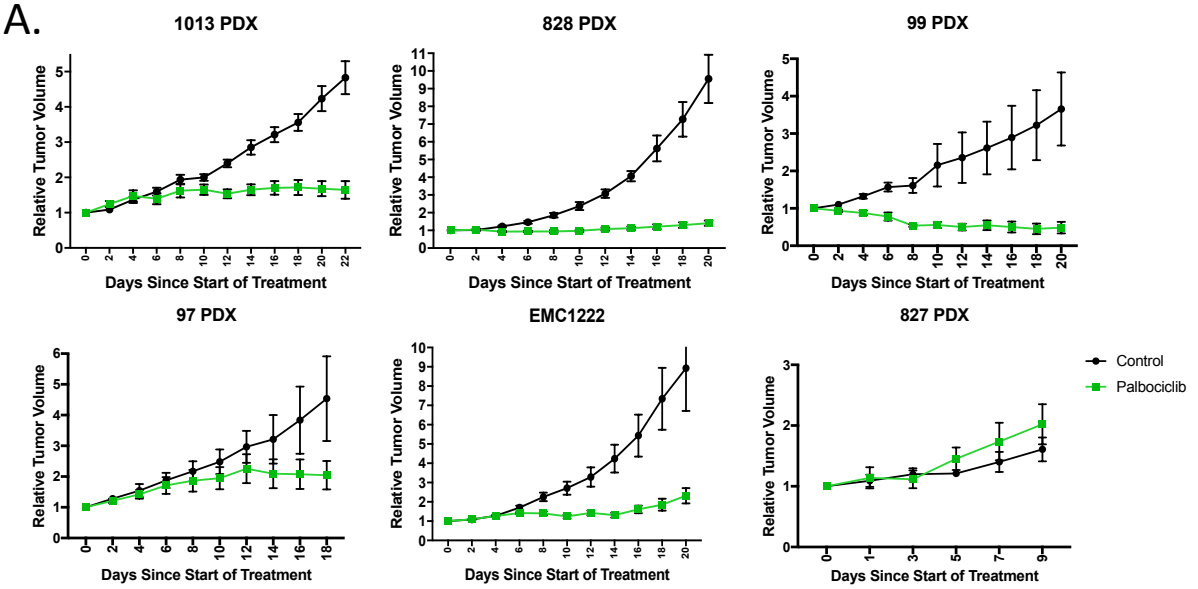
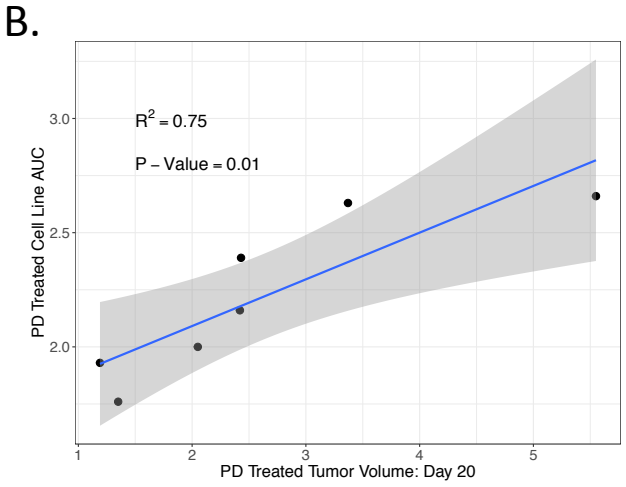
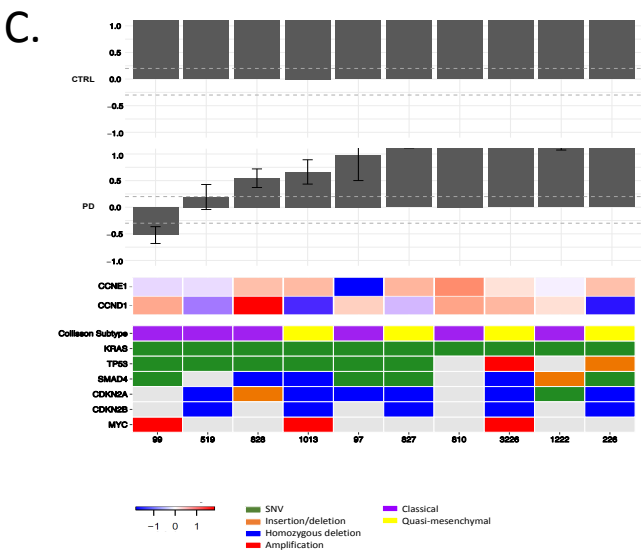

A.
Input
IgG
IP:p21
IP:p27
PD (200 nM)
CDK4
CDK2
p27
p21
B.
226
siNT
siCDK2
Relative BrdU incorporation
PD (nM)
226
siNT
siCDK2
PD(200nM)
CDK2
β Actin
C.
siNT
siCCNE1
519
1222
226
Relative BrdU incorporation
PD (nM)
519
1222
226
siNT
siCCNE1
PD(200nM)
Cyclin E1
β Actin
D.
226
siNT
siCCND1
Relative BrdU incorporation
PD (nM)
226
siNT
siCCND1
PD(200nM)
Cyclin D1
β Actin
E.
3226
1222
DMSO
100
200
PD0332991 (nM)
pCDK2 (T160)
CDK2
pCDK4 (T172)
CDK4
β Actin

Fig S4


A.
1013 PDX
828 PDX
99 PDX
97 PDX
EMC1222
827 PDX
Relative Tumor Volume
Days Since Start of Treatment
Control
Palbociclib


B.
R2 = 0.75
P – Value = 0.01
PD Treated Cell Line AUC
PD Treated Tumor Volume: Day 20


C.
CTRL
PD
CCNE1
CCND1
Collisson Subtype
KRAS
TP53
SMAD4
CDKN2A
CDKN2B
MYC
99
519
828
1013
97
827
510
3226
1222
226
SNV
Insertion/deletion
Homozygous deletion
Amplification
Classical
Quasi-mesenchymal

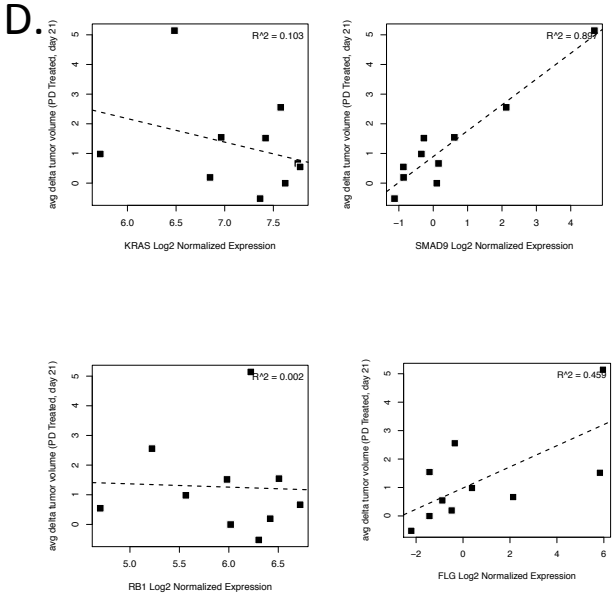
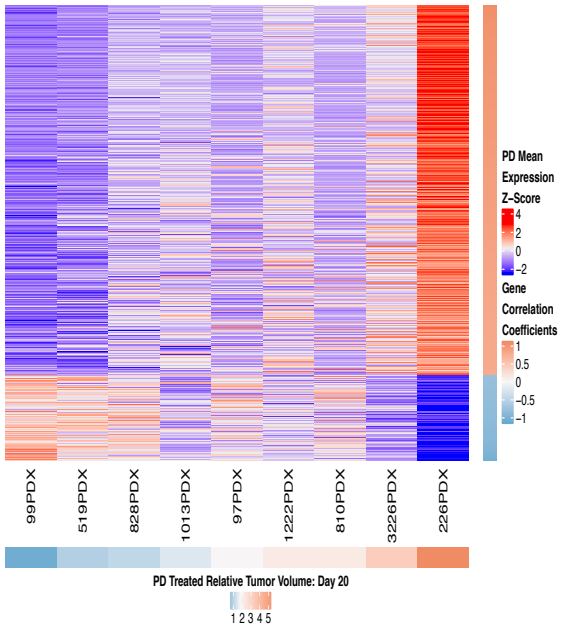
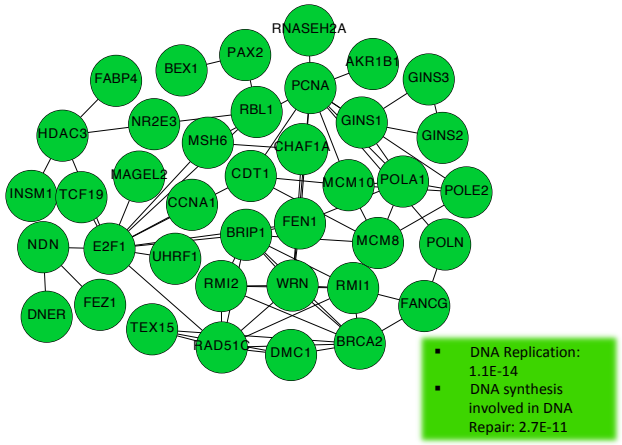

D.
R^2 = 0.103
KRAS Log2 Normalized Expression
SMAD9 Log2 Normalized Expression
R^2 = 0.002
RB1 Log2 Normalized Expression
FLG Log2 Normalized Expression
avg delta tumor volume (PD Treated, day 21)

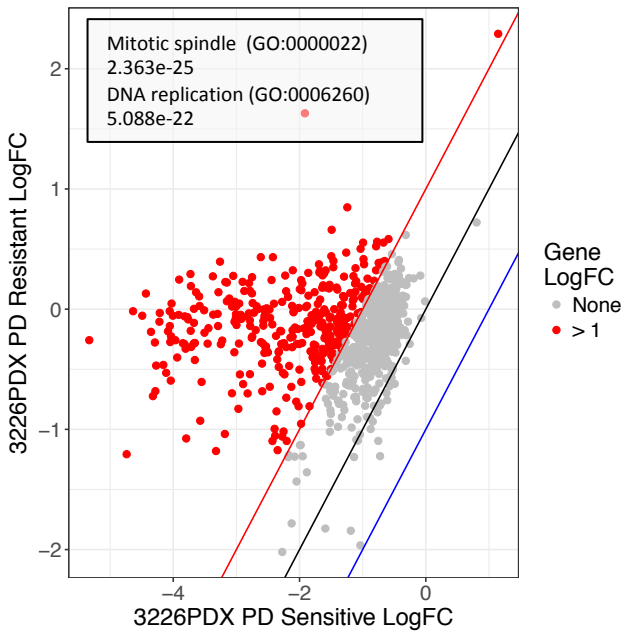
Fig S5

A.


PD Mean Expression Z-Score
4
2
0
−2
Gene Correlation Coefficients
1
0.5
0
−0.5
−1
99PDX
519PDX
828PDX
1013PDX
97PDX
1222PDX
810PDX
3226PDX
226PDX
PD Treated Relative Tumor Volume: Day 20
1 2 3 4 5


RNASEH2A
PAX2
AKR1B1
FABP4
BEX1
PCNA
GINS3
RBL1
NR2E3
GINS1
HDAC3
MSH6
GINS2
CHAF1A
MAGEL2
CDT1
INSM1
TCF19
MCM10
POLA1
POLE2
CCNA1
FEN1
BRIP1
NDN
E2F1
MCM8
POLN
UHRF1
RMI2
WRN
RMI1
FANCG
DNER
FEZ1
TEX15
RAD51C
DMC1
BRCA2
DNA Replication: 1.1E-14
DNA synthesis involved in DNA Repair: 2.7E-11

B.


Mitotic spindle (GO:0000022)
2.363e-25
DNA replication (GO:0006260)
5.088e-22
3226PDX PD Resistant LogFC
3226PDX PD Sensitive LogFC
Gene LogFC
None
> 1

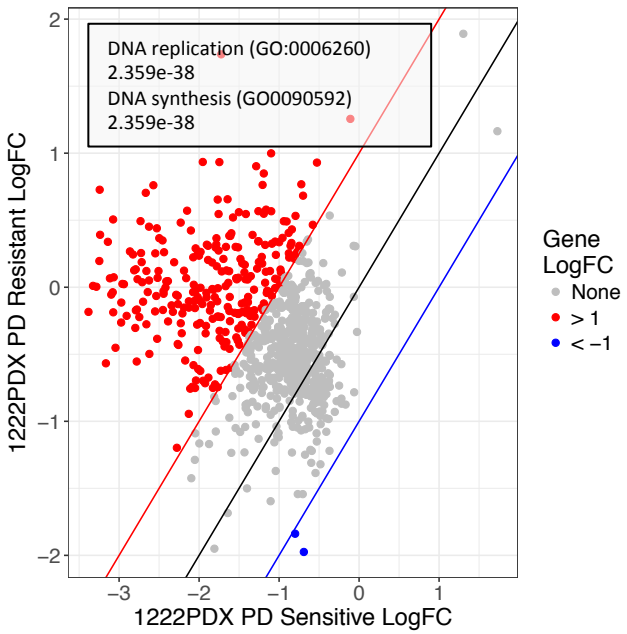
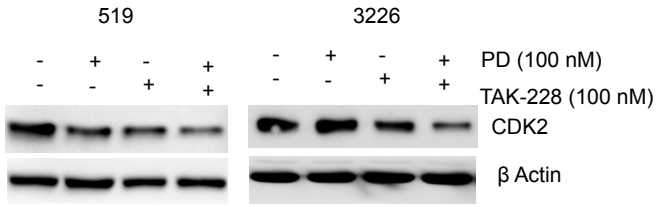
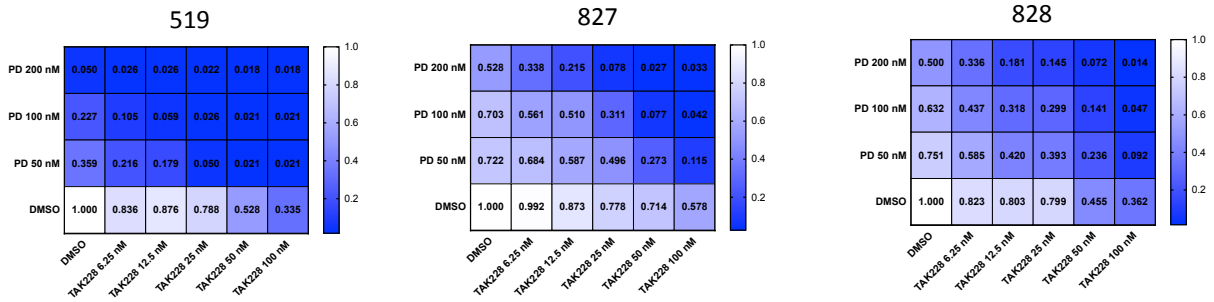

DNA replication (GO:0006260)
2.359e-38
DNA synthesis (GO0090592)
2.359e-38
1222PDX PD Resistant LogFC
1222PDX PD Sensitive LogFC
Gene LogFC
None
> 1
< −1

Fig S6

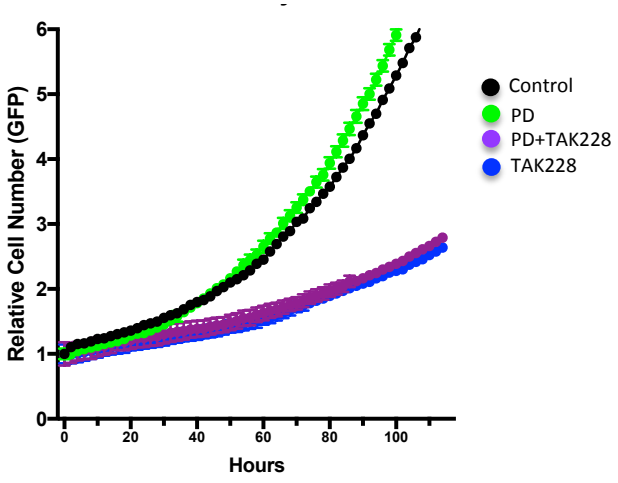
A.


519
3226
PD (100 nM)
TAK-228 (100 nM)
CDK2
β Actin

B.


519
827
828
PD 200 nM
PD 100 nM
PD 50 nM
DMSO
TAK228 6.25 nM
TAK228 12.5 nM
TAK228 25 nM
TAK228 50 nM
TAK228 100 nM

C.


Relative Cell Number (GFP)
Hours
Control
PD
PD+TAK228
TAK228

D.

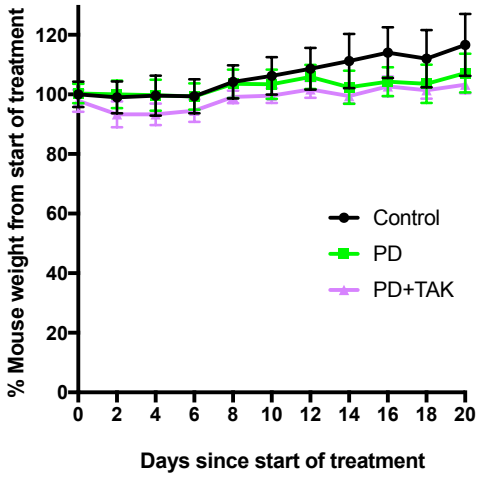
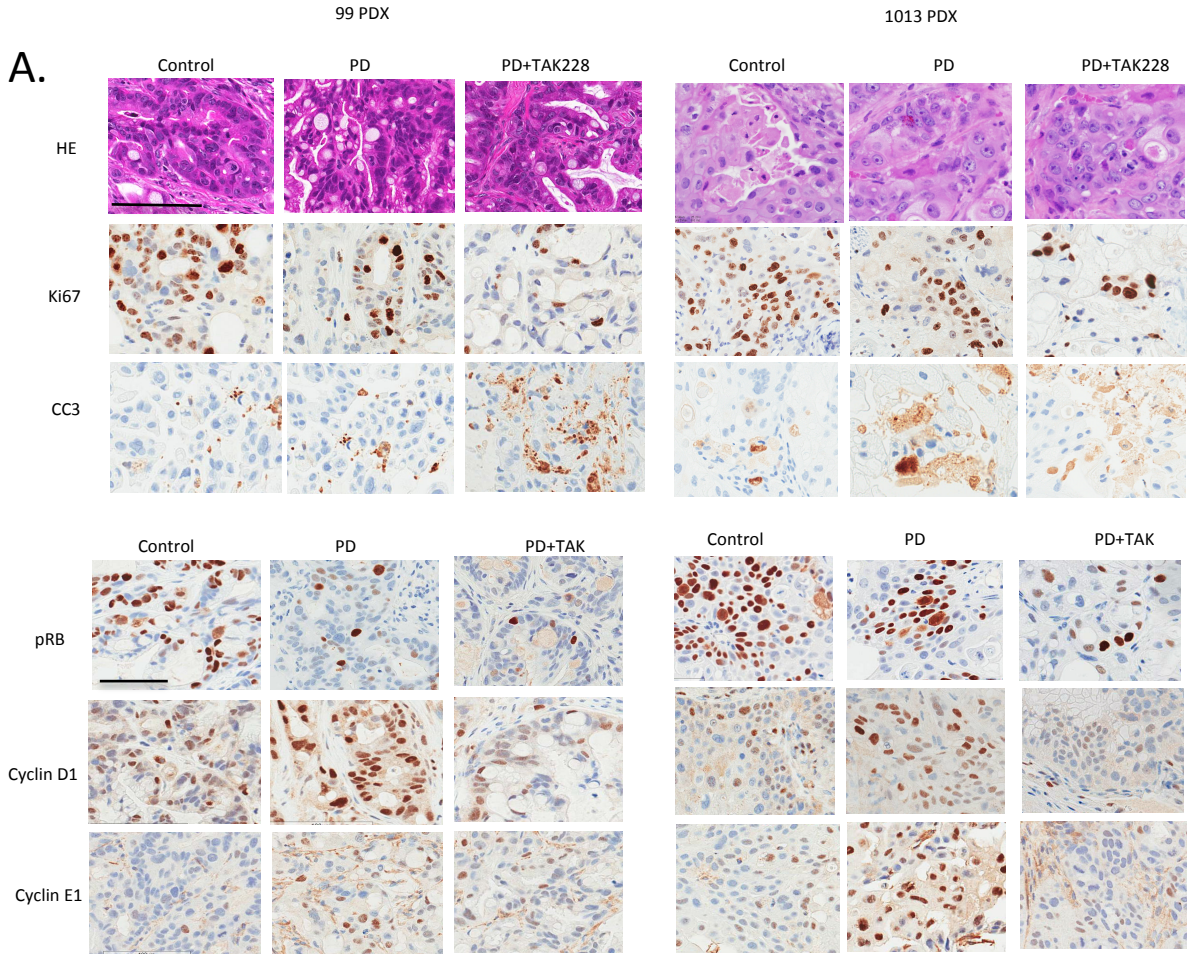
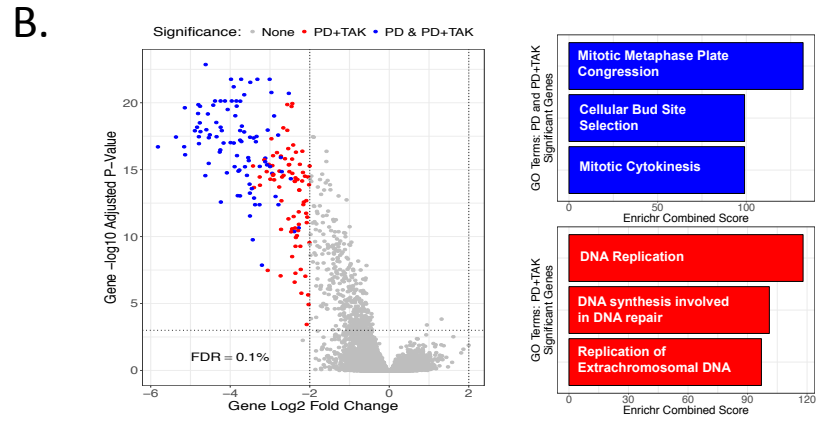
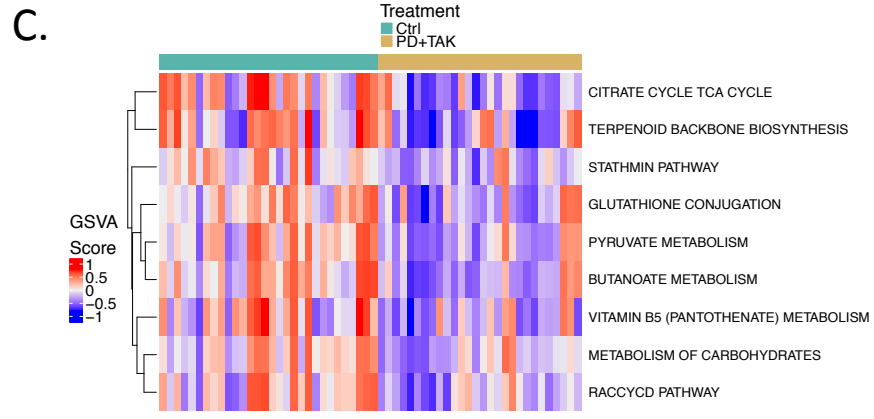

% Mouse weight from start of treatment
Days since start of treatment
Control
PD
PD+TAK

Fig S7


A.
99 PDX
1013 PDX
Control
PD
PD+TAK228
HE
Ki67
CC3
PD+TAK
pRB
Cyclin D1
Cyclin E1


B.
Significance: None PD+TAK PD & PD+TAK
Gene −log10 Adjusted P-Value
Gene Log2 Fold Change
FDR = 0.1%
GO Terms: PD and PD+TAK Significant Genes
Mitotic Metaphase Plate Congression
Cellular Bud Site Selection
Mitotic Cytokinesis
Enrichr Combined Score
GO Terms: PD+TAK Significant Genes
DNA Replication
DNA synthesis involved in DNA repair
Replication of Extrachromosomal DNA
Enrichr Combined Score


C.
Treatment
Ctrl
PD+TAK
GSVA Score
CITRATE CYCLE TCA CYCLE
TERPENOID BACKBONE BIOSYNTHESIS
STATHMIN PATHWAY
GLUTATHIONE CONJUGATION
PYRUVATE METABOLISM
BUTANOATE METABOLISM
VITAMIN B5 (PANTOTHENATE) METABOLISM
METABOLISM OF CARBOHYDRATES
RACCYCD PATHWAY