Supplementary Figure 1: Palbociclib sensitive cell lines are predominately CDK2^{low} and palbociclib resistant cell lines are predominately CDK2^{high} post-mitosis.

A and **B.** Single cell CDK2 activity traces of palbociclib sensitive MDA-MB-453 (LAR TNBC) and MCF7 (estrogen receptor positive) cell lines that exit mitosis with low CDK2 activity levels. Dotted line: 2 hours time point.

C and **D.** Single cell CDK2 activity traces of palbociclib resistant HCC1143 (basal-like TNBC) and CAL51 (MES TNBC) cell lines that exit mitosis with a CDK2^{high} population with rapid increase in CDK2 activity and shorter cell cycle.

E and **F.** Single cell CDK2 activity post-mitosis in SUM149 cells exposed to palbociclib (500nmol) for **E.** 24 hours and, **F.** chronically exposed for 14 days with regular change in drug and media (every 3 days).

Supplementary Figure 2: Cyclin E1 dysregulation in TNBC.

A. Metabric dataset analysis comparing the expression of the indicated genes in LAR (n=35) versus basal-like TNBC tumours (n=97). *CCNE1* (cyclin E1), *CDK2*, *CDKN1A* (p21), *CDKN1B* (p27), *CCND1* (cyclin D1) and *RB1* (retinoblastoma protein 1). *CDKN1B, CCND1* and *RB1* are not statistically significant. Error bars = mean expression and SD.

B. TCGA data set analysis of gene expression *(bottom)*, copy number aberration *(middle)* and mutational status *(top-orange)* in 75 TNBC tumours, classified according to TNBC subgroups (13 LAR, 8 MSL, 24 MES and 30 basal-like), for the indicated genes.

C. Correlation for *CCNE1* mRNA and cyclin E1 protein levels in breast cancer tumours *(black)*, with TNBC highlighted *(red)* from TCGA database.

D. Correlation for *CCNE1* mRNA and cyclin E1 protein levels in TNBC breast cancer tumours according to TNBC subtypes from TCGA database: LAR *(green)*, MSL *(light blue)*, M *(dark blue)*, basal-1 *(black)* and basal-2 *(red)*.

E. Nuclear cyclin E1 protein expression in single cells shown for palbociclib sensitive MDAMB453 *vs.* Palbociclib resistant SUM149 cell lines (p=0.0015 Student T test), 1-3 hours after mitoses, using immunofluorescent staining. Mean and SD (error bars).

F. Single cell live cell traces of nuclear intensity of a PCNA sensor post-mitosis in single SUM149 cells[2]. ***Red line:*** time point of S phase entry - increased PCNA intensity and appearance of nucleoli. Median time to S phase entry for SUM149 cells was 6 hours and range 2.83 hours.

G. CDK2 activity 2 hours post-mitosis for SUM149 cells shown in **main figure 4F** transfected with *siCON1 or siCCNE1*. Mean and SD (error bars).

H. Western blot of CAL51 cells transfected with indicated siRNA and blotted for cyclin E1 protein and actin.

I. Relative BrdU incorporation in SUM149 cells transfected 72 hours earlier with *siCON1* or *siCCNE1* pool, treated with or without palbociclib (500nmol). *siUBB* (Ubiquitin B) shown as positive toxicity control. P value Student's T-test.

Supplementary Figure 3: CDK2^{high} and CDK2^{low} cell subpopulation arise from single cell.

A. Left: Single cell FACS sorted and plated per well (cell line CAL51). After 4 weeks, the developed colonies were transfected with CDK2L sensor and imaged. **Right:** Relative change in CDK2 activity for each transfected cells in the indicated wells: Wells A – D.

B and **C.** Long term stability of CDK2 activity shown by correlating CDK2 activity at 2 hours between randomly paired sister cells in individual clones from **B.** CAL51 cell line (p=0.41 Spearman's correlation coefficient) and **C.** SUM149 cells (p=0.55 Spearman's correlation coefficient).

Supplementary Figure 4: PI3 kinase / mTOR (vistusertib; AZD2014) combinations with palbociclib in TNBC

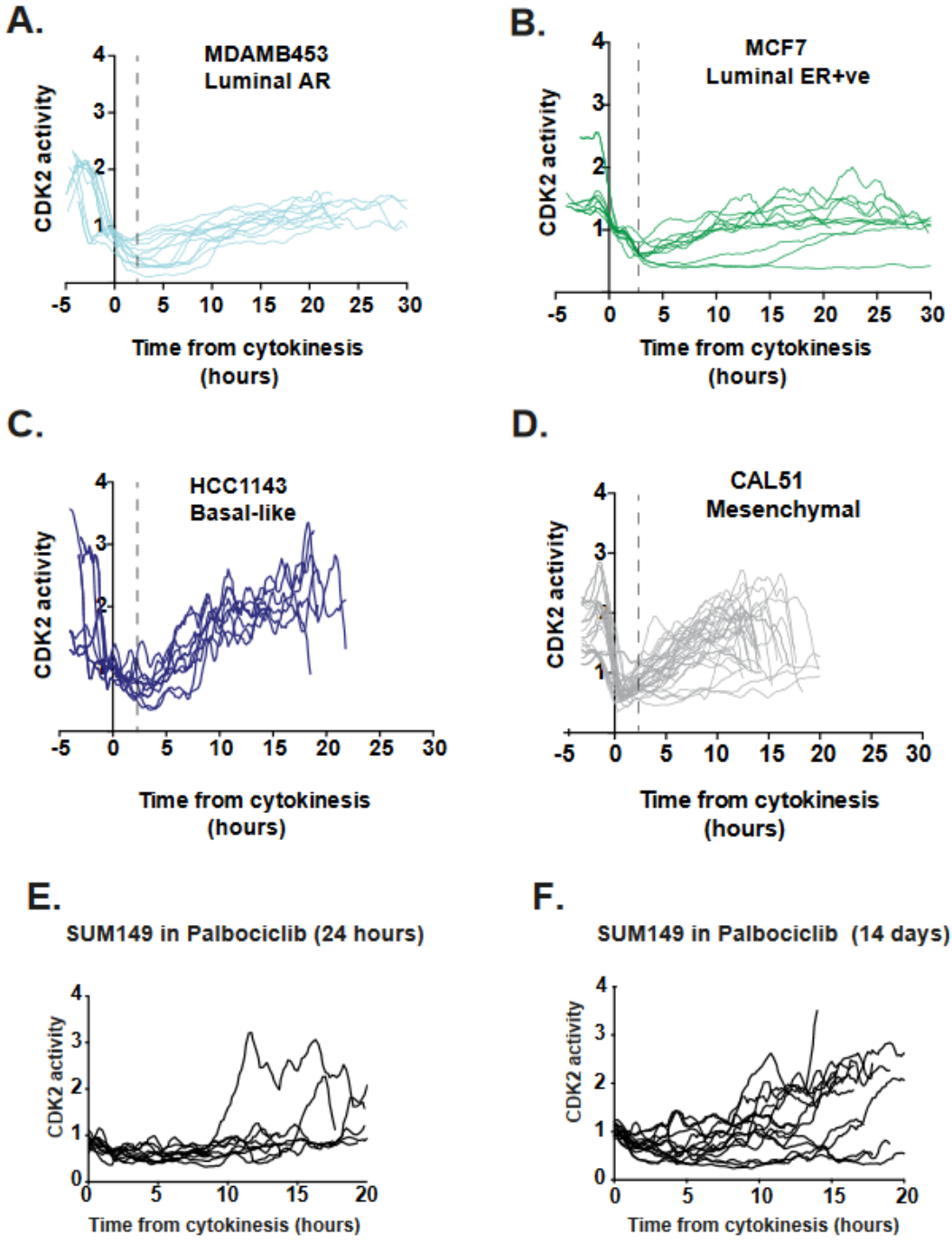
A. Synergy heat maps from clonogenic assays in TNBC cell lines treated with palbociclib and/or AZD2014 (dual mTORC1 and mTORC2 inhibitor) at increasing concentrations. 1 small square = 1 well of 6 well plate; Red on heat map= no colony formation.

B. Relative BrdU incorporation in SUM159 cells (MSL) exposed to the indicated compounds alone or in combination for 24 or 72 hours**.**

C. Immunohistochemistry sections of tumours from MDAMD453 mouse xenografts stained for phospho S6 ribosomal protein (pS6RP) and cleaved caspase-3 treated under indicated conditions: Vehicle, Taselisib, Palbociclib and Taselisib/Palbociclib combination.

D. CDK2 activity pre-cytokinesis in *PIK3CA* mutant CAL51 cells treated taselisib 100nmol. Proliferative – cells that post-mitosis re-enter the cell cycle within 10 hours (CDK2 activity >1.0 at 10 hours) *versus* Quiescent – cells that post-mitosis do not re-enter the cell cycle within 10 hours (CDK2 activity <1.0 at 10 hours)..

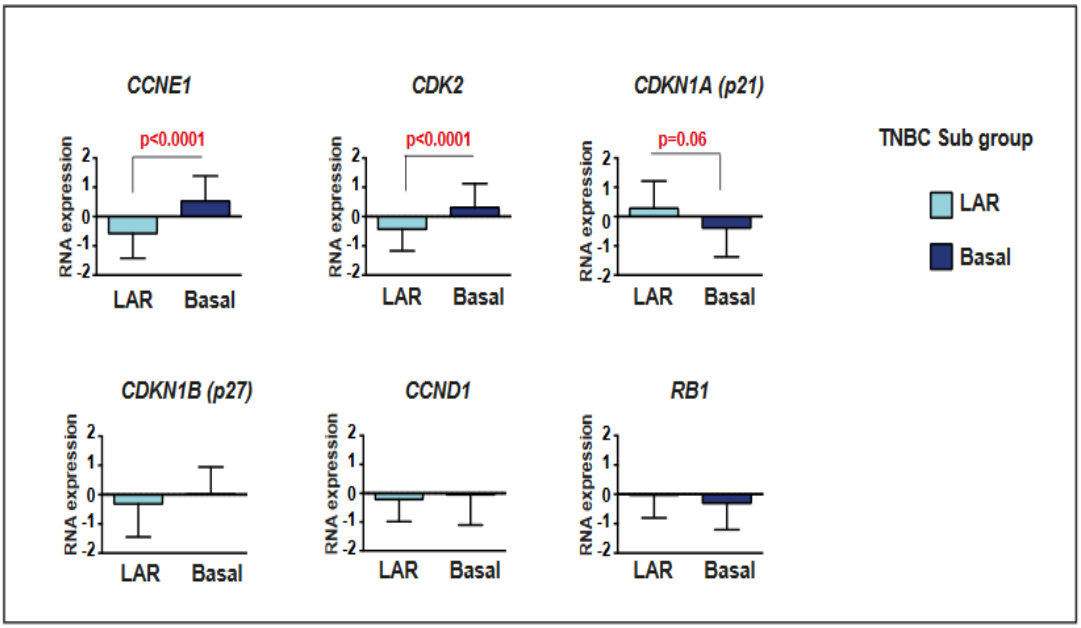
Supplementary Figure 1



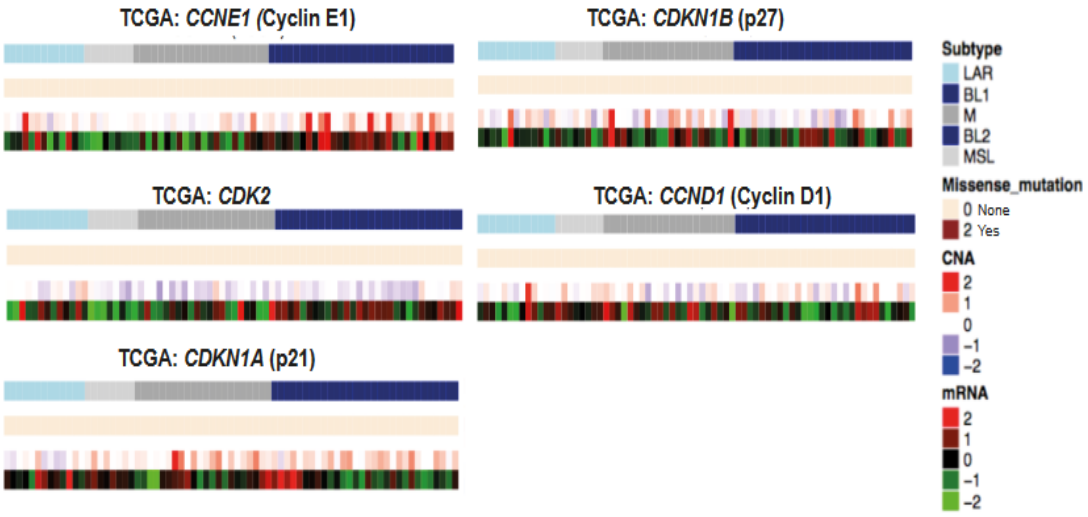
Supplementary Figure 2

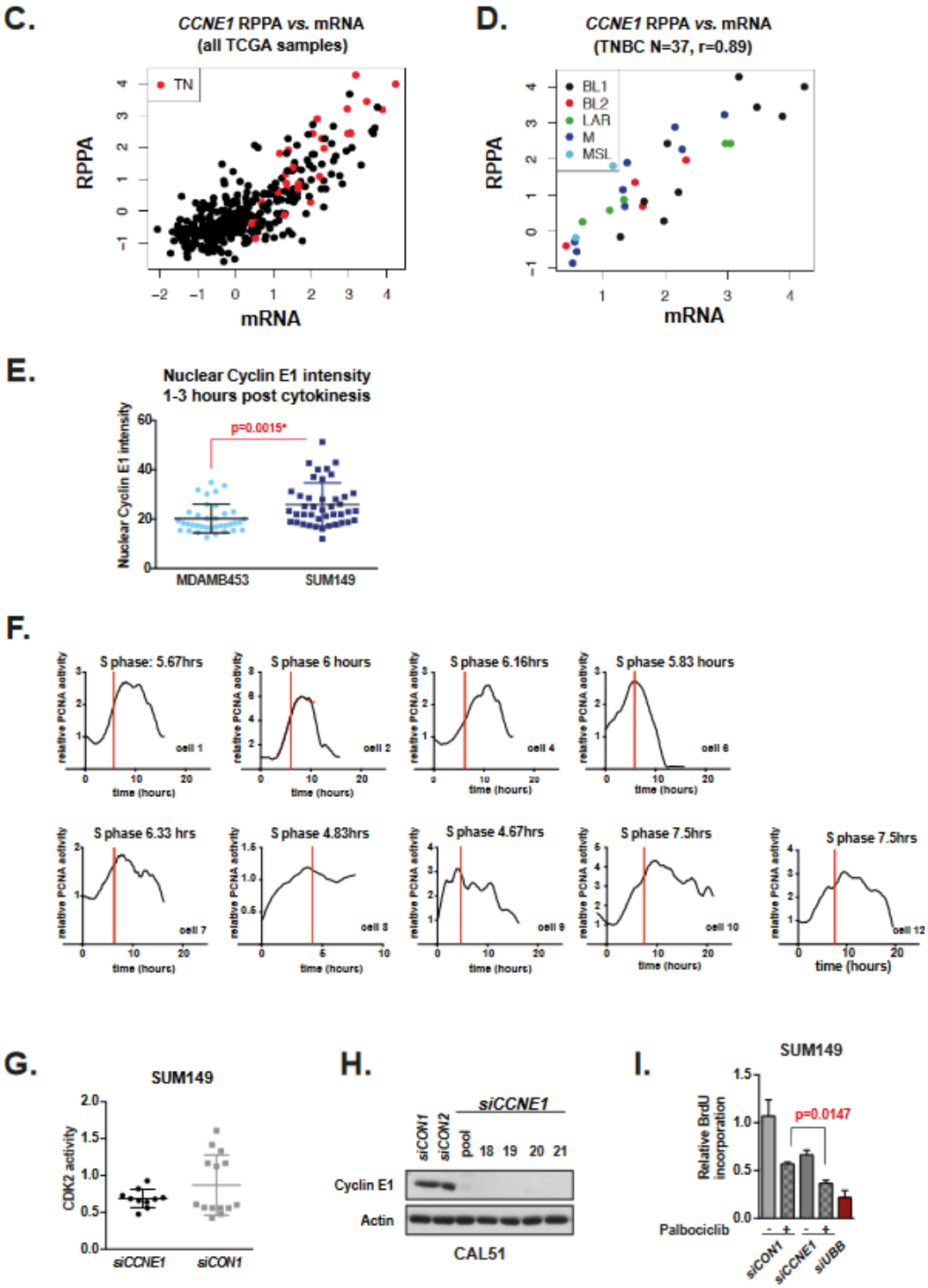
A.

TNBC Gene expression (Metabric dataset)



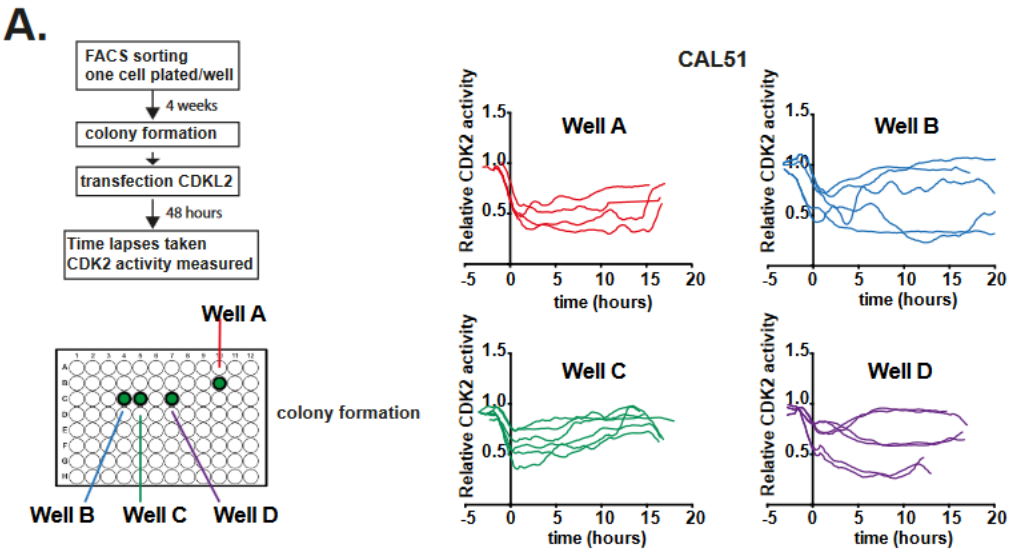
B.

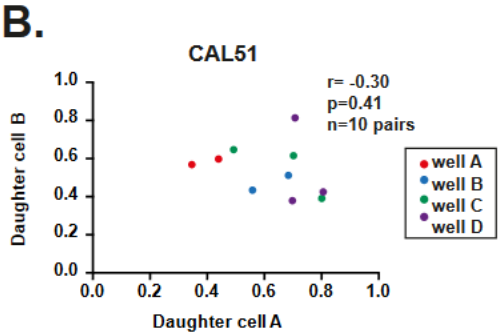


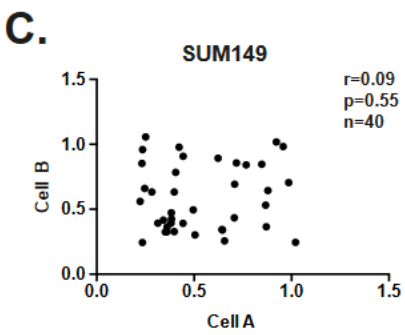


Supplementary Figure 2

Supplementary Figure 3

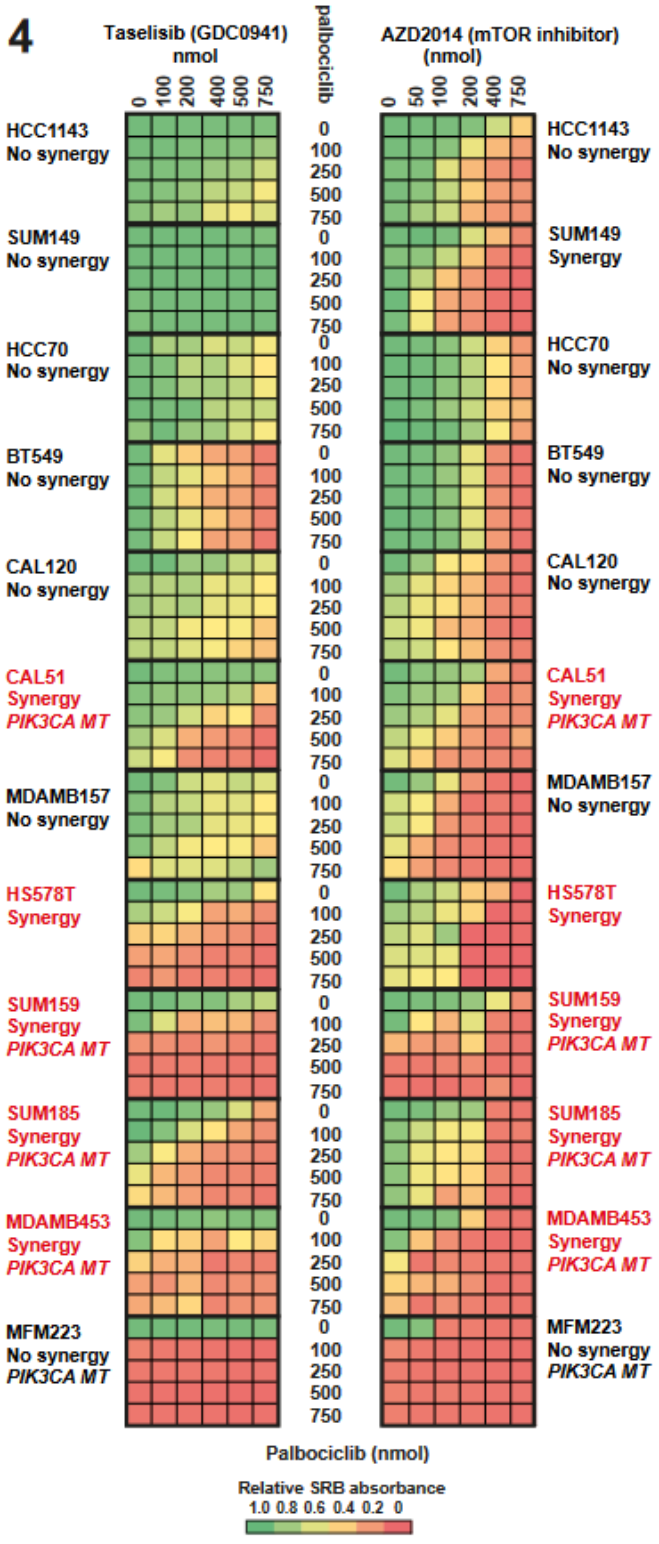






Suppl. Figure 4

A.



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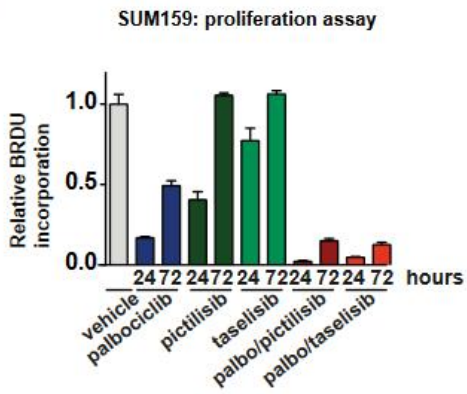
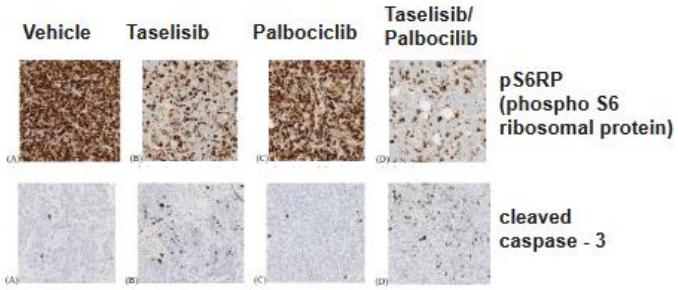
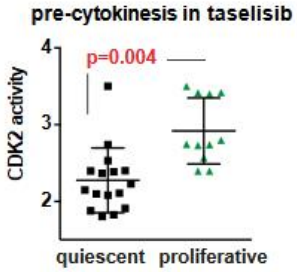

SUM159: proliferation assay
Relative BRDU incorporation
1.0
0.5
0.0
24 72 24 72 24 72 24 72 24 72 hours
vehicle
palbociclib
pictilisib
taselisib
palbo/pictilisib
palbo/taselisib

C.


MDAMB453 mouse xenograft model
post treatment: 4 hours
Vehicle
Taselisib
Palbociclib
Taselisib/
Palbocilib
pS6RP
(phospho S6
ribosomal protein)
cleaved
caspase - 3

D.


pre-cytokinesis in taselisib
p=0.004
CDK2 activity
4
3
2
quiescent
proliferative

Supplementary Table 1: Cell cycle lengths (hours) according to CDK2 status ($CDK2^{high}$ *vs.* $CDK2^{low}$)

Cell line	CDK2 status	n	%	Mean ± SD (hours)	Median (IQR) (hours)
MFM223 LAR	Low	13	65	>72*[1]	>72*
	High	7	35	37.03 ± 7.995	38.30 (34.40 - 43.10)
MDAMB453 LAR	Low	7	50	>50*	>50*
	High	7	50	34.1 ± 8.811	33.00 (26.00-40.00)
CAL51 MES	Low	7	19	33.46 ± 8.153	31.30 (15.30-20.20)
	High	29	81	18.06 ± 3.576	17.20 (15.30-20.20)
SUM149 Basal-like	Low	8	30	39.54 ± 4.454	41.00 (37.33-42.35)
	High	19	70	25.48 ± 4.454	26.20 (20.80-30.00)
HCC1143 Basal-like	Low	2	18	41.80 ± 0.2828	41.80 (41.60-42.00)
	High	11	82	32.92 ± 5.695	34.00 (30.30-36.00)

* Cell cycle length (cytokinesis→next cytokinesis) not captured due to duration of time-lapse imaging experiment.
For example, >72hrs = cytokinesis to end of time-lapse experiment was 72 hours in the absence of further mitotic event.

Supplementary Table 2

TNBC subtype	Cell line	RB1 status	Mutations in cancer genes (Verified or previously reported)
Basal-like	HCC1143	WT	*TP53; NUP98; PLAG1; PTPRC; GRIN2A RANBP17; PML*
	SUM149	WT	*BRCA1*; CDKN2A*; FBXW7**
	HCC70	WT	*TP53; PTEN; APOBEC3B*
Mesenchymal	BT-549	Mutant	*RB1; TP53; PTEN; PTPRT; RNF213; CYLD; MUTYH; PCM1*
	CAL-51	WT	PIK3CA; PTEN; CCND1; ARID1A; JAK1; MAP2K4; BCORL1; NFATC2; NSD1; MN1; ZFHX3; RAC1; ARID1B; KMT2A; EXT1; MYH9; ALK; MECOM; NUMA1; RPL22 *FBXW7**, CCNE1***
	CAL-120	WT	*TP53; KDM6A; LRP1B; FANCG*
Mesenchymal stem-like	HS-578-T	WT	*TP53; HRAS; PIK3R1; GMPS; NF1*
	SUM159	WT	*TP53; PIK3CA*; HRAS**
	MDA-MB-157	WT	*TP53; MSH6; NF1*
Luminal Androgen Receptor	MFM223	WT	*TP53; PIK3CA; ARID1A; ALK; FLT3*
	MDA-MB-453	WT	*PIK3CA; PTEN; AR; LRP1B; NUP98; CDH1; THRAP3; WHSC1L1; FANCE; SUZ12*
	SUM185	WT	*PIK3CA**

Mutations (verified) in cancer genes obtained from CELL LINE PROJECT
**** CELL LINE PROJECT-unverified but related to cell cycle**
***COSMIC (unverified, but previously reported)**