# Table S1 Antibodies used in this study

Antigen	Antibody
Aurora	Cell Signalling Technology #14475
Phospho-Aurora A T288	Cell Signalling Technology #3079
BCKDK	Bethyl laboratories A303- 790A-M
mTORC	Cell Signalling Technology, #2972
Мус	Santa-Cruz, SC-40
Histone H3	Cell Signalling Technology #4499
PARP	Cell Signalling Technology #9542

# Table S2: Activity of DCBC and CMVA in cell growth assays.

The potency of CMVA and DCBC as single agents in cell growth assays. The potency of CMVA and DCBC as single agents in cell growth assays was determined in the indicate cell lines. Results are expressed as the  $IC_{50}$ (mean±S.D,n=3). ">200" indicates the compounds were insufficiently potent for a reliable  $IC_{50}$  determination so the  $IC_{50}$  is reported as being greater than 200 µM.

Cell line	IC50 (μM)		
	CMVA	DCBC	
OVCAR-4	>200	>200	
COV362	>200	>200	
COV318	>200	>200	
FUOV-1	>200	>200	
MCF-7	180 ± 90	120 ± 40	
MDA-MB-468	>200	120 ± 20	
MDA-MB-231	>200	>200	





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#### **Supplementary figure 1**

Quantification of BCKDK and BCKDH expression. The expression of the BCKDK and BCKDH in the indicated ovarian (A) and breast (B) cancer cell lines was analysed by immunoblotting. The resulting chemiluminescent signal was quantified and normalized to GAPDH in each experiment. The results of several experiments (mean  $\pm$  S.D n= 3) normalized to the expression in HOE (normal human ovarian epithelial cells) or MCF-7 cells respectively.







**Supplementary figure 2**. Repression of BCKDK expression by siRNA. The expression of BCKDK was determined by western blotting of protein lysates obtained from OVCAR-4, MCF-7 or MDA-MB-468 cell lines 48 hours after transfection with the indicated BCKDK siRNA or non-targetting siRNA (NT-1). The results are representative of three experiments. Note that siRNA #1 was replaced with #17 following the supplier's recommendation and that differences in efficiency of knockdown may represent differences in transfection efficiencies between different cell lines. A. repression of expression in MDA-MB-468 cells. **B**. Quantification of expression.



**Supplementary figure 3** The effect of paclitaxel (30 nM, 72 hours) was measured using spheroids formed from either HAP-1 or BCKDK<sup>-/-</sup> cells. Relative viable cell number was assessed by measurement of intracellular ATP and are expressed as a fraction of paclitaxel in HAP-1 cells. The results (mean ± S.D, n=6) are significantly different where indicated (\*\*\*,  $P < 1x \ 10^{-6}$ , paired *t*-test).

# S4 Experimental design for cell synchronization studies and treatment with BCKDKi



В





**Supplementary figure 4**. **A**. The indicated cells were synchronized with nocodazole (1 µg/ml, 24 h), washed and lysed after the indicated times. Aurora phosphorylation was detected by immunoblotting. **B**. Scheme showing the experimental use of nocodazole-induced arrest and BCKDK inhibitors. This scheme was adopted because it allowed both the cells to be synchronized but at the same time allow sufficient time for the BCKDK it o drive BCAA metabolism. Different schemes were used with OVCAR-4 and MCF-7 because of the slower growth of the former cells.

# S5 Quantification of immunoblotting to assess the effect of BCKDKi on mTORC and Aurora phosphorylation



Supplementary Fig 5 Quantification of the effects of BCKDK inhibitors on phosphorylation of mTORC and Aurora. OVCAR-4 or MCF-7 cells were synchronized with nocodazole, then treated with 100  $\mu$ M CMVA and DCBC for 72h. A second set of cells were supplemented with 1mM BCAAs for 30 min before cell collection. Following immunoblotting, the intensity of the signal was quantified and normalized to the corresponding bands for GAPDH. These results are from three independent experiments and are presented as means ± SD.



Supplementary Figure S6 Quantification of the effects of BCKDKi on Myc expression. OVCAR-4 OR MCF-7 cells were synchronized with nocodazole as described in the methods, treated with DMSO or 100  $\mu$ M CMVA or DCBC for 72h, lysed and analysed by immunoblotting as shown. The results were quantified and normalized to Histone H3 expression.

S7 Effect of BCKDKi and paclitaxel in MDA-MB-468 cells



#### **MDA-MB-468**



Supplementary Fig S7. Effect of BCKDK inhibitors on M-phase arrest induced by paclitaxel. A, MDA-MB-4868 cells were exposed to the indicated combinations of paclitaxel (9.5 nM), CMVA (100 µM) or DCBC (100 µM) and monitored by time-lapse microscopy. The time at which the cells rounded and their subsequent behaviour was classified (mean ± S.D., n=3 total of 100 cells counted for each condition) as "reversed cytokinesis" (cells started to divide, but then aborted division), "rounded then flattened" (cells did not divide but instead reverted to a flat morphology) or "rounded, flattened then re-rounded (as the previous group, but subsequently rounded again) or "remained rounded" (the cells remained rounded until the end of the recording period). Some cells fragmented, consistent with apoptosis and this is here recorded as "lysis" due to the lack of additional confirmatory evidence. Cell fate maps are shown for each cell in Fig S13-18. B. The time at which cells rounded was noted and time to flatten again determined and the interval this encompassed taken to represent the time required to complete mitosis. Cells treated with paclitaxel remained rounded throughout the 24h duration of the video and hence are labelled ">24 h".

#### MCF7 cell fate Treatment: DMSO

- Flat
- Round

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MCF7 cell fate Treatment: CMVA

- Round
- Divided into two cells
- Reversed cytokinesis

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# MCF7 cell fate Treatment: DCBC

- Flat
- Round
- Divided into two cells
- Reversed cytokinesis



Cell number

# MCF7 cell fate Treatment: Paclitaxel

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Divided into two cells

Reversed cytokinesis

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MCF7 cell fate Treatment: Paclitaxel & CMVA

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- Round
- Divided into two cells
- Reversed cytokinesis

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MCF7 cell fate **Treatment: Paclitaxel & DCBC** 

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- Divided into two cells
- Reversed cytokinesis

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- Divided into two cells
- Cell lysis
- Reversed cytokinesis

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-1					9 24



Flat

Round

Cell lysis

Reversed cytokinesis

07         86         85         84         83         82         81         80         79         78         77         76         75         74         72		
71       70       69       68       67       66       65       64       63       64       63       64       63       64       63       64       65       64       65       64       65       64       65       64       65       64       65       64       65       64       65       66       58       57		
56       55       54       53       52       51       50       49       48       47       46       45       43       42		
41 40 39 38 37 36 35 34 33 32 31 30 29 28 27		
26       25       24       23       22       21       20       19       18       17       16       15       14       13       12		
11 10 9		

Flat

Round

Cell lysis

Reversed cytokinesis



MDA-MB-468 cell fate Treatment: Paclitaxel & CMVA

FlatRound

Cell lysis

Reversed cytokinesis



MDA-MB-468 cell fate Treatment: DCBC & paclitaxel

FlatRound

Cell lysis

Reversed cytokinesis





DMSO

DCBC

# S20: All MDA-MB-468 fate maps





DCBC

Supplementary Fig 8-21. Cell fate maps for cells exposed to combinations of paclitaxel and BCKDKi. MCF-7 (S7-12) or MDA-MB-468 (S13-18) cells were exposed to the indicated combinations of paclitaxel (9 nM), CMVA (100  $\mu$ M) or DCBC (100  $\mu$ M) and monitored by time-lapse microscopy. The time at which the cells rounded and their subsequent behaviour was recorded. Figure 20 (MCF-7) and figure 21 (MDA-MB-468) show a composite of the previous figures, to facilitate comparison.



# Supplementary figure 22 Cell cycle analysis of cells treated with the drug combination.

MCF-7 or MDA-MA-468 cells were exposed to DMSO, paclitaxel (9.5 nM) and/or CMVA (100  $\mu$ M) or DCBC (100  $\mu$ M). After 48 hours, the cells were collected, stained with propidium iodide and analysed by flow cytometry (mean ± S.D. n = 3). Inclusion of the BCKDK inhibitors with paclitaxel did not significantly increase the number of cells with greater than 4N content compared to cells exposed to paclitaxel alone.

#### Supplementary videos 1-12.

The videos show MCF7 (videos 1-5) or MDA-MB-468 cells (videos 6-12) treated with either paclitaxel alone (video 1 & 6) or paclitaxel and DCBC (remaining videos). The videos exemplify different types of cell behaviour after exposure to the drugs including mitotic arrest after exposure to paclitaxel (videos 1 and 6), mitosis (videos 3 and 8), cells rounding then re-flattening without dividing (videos 4 and 9,10), starting cytokinesis then reverting to a single cell ("reversing cytokinesis", video 5 and 11) and cell fragmenting ("lysis", video 12). One second of the video represents 8.5 minutes of real time.