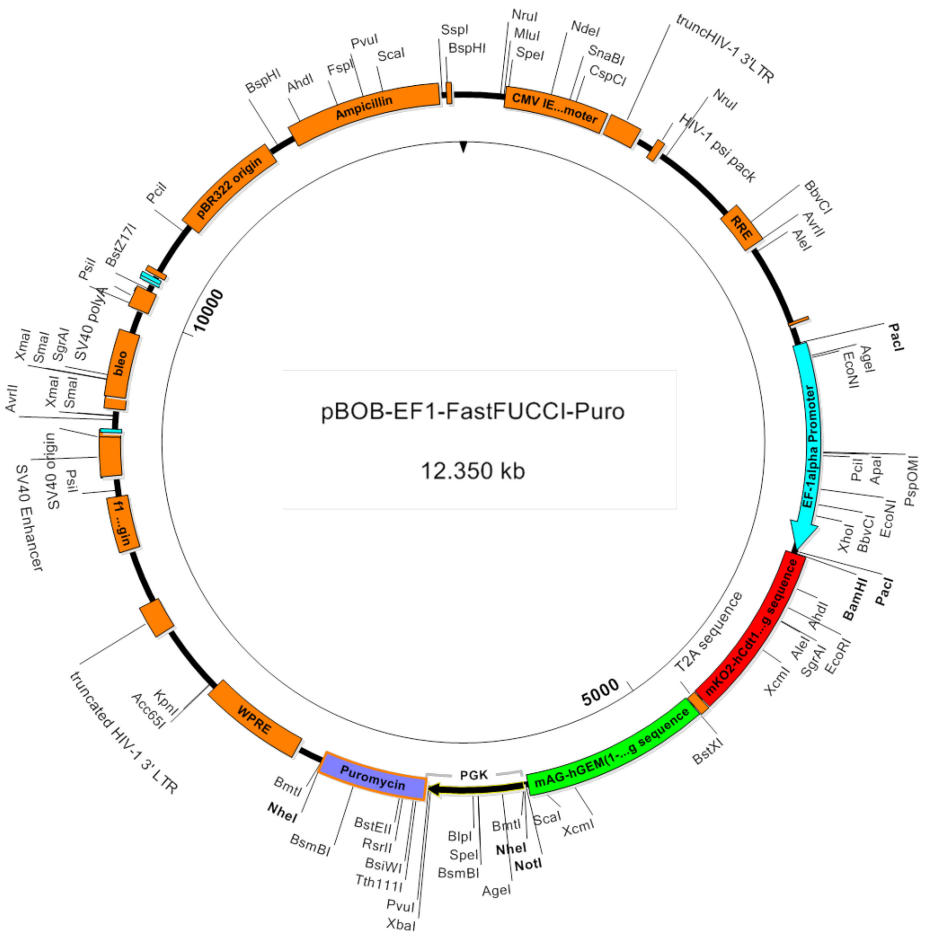

Figure S1, related to Figure 1. pBOB-EF1-FastFUCCI-Puro construct
Full map of the pBOB-EF1-FastFUCCI-Puro construct. See Supplementary Table S1 for all single and two-cutter 6+ nucleotide restriction enzymes with respective positions and actual nucleotide numbers.

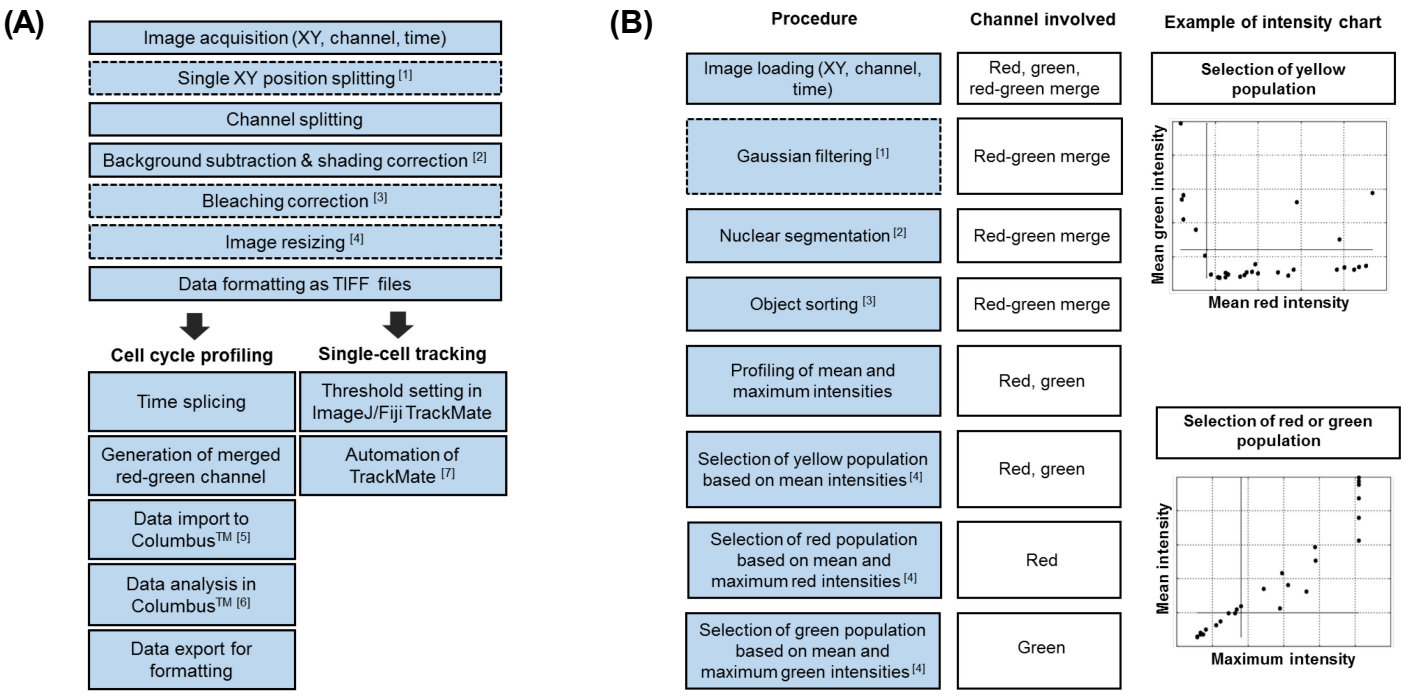

Figure S2, related to Figure 2. FastFUCCI imaging assay

(A) Description of the FastFUCCI imaging pipeline. [1] XY position splitting is required if imaging software generates one single merged file for the entire video acquisition. [2] Image processing should be tested on several videos for quality control prior to automation. [3] This step would be useful if there is exhibition of auto-fluorescence. Exponential fit in the ImageJ/Fiji plug-in "Bleaching correction" is recommended. [4] Image resizing is optional to expedite the analysis process but it should only be applied if it does not affect segmentation. [5] Data import requires a switch from "Project" to "Screen" mode within the Columbus™ software to allow for batch mode analysis. [6] Analysis protocol is in (B). [7] Automation is achieved by the AutoClick Robot software (Java code), with a GUI to schedule click events. Red and green channels could be analysed separately if selected thresholds preclude batch analysis. Data export is included in the automation. (B) Protocol for Columbus™ analysis. [1] Gaussian filtering could be performed to improve contour definition for nuclear segmentation. [2-4] Thresholds for segmentation, nuclear size and red/green intensities are user-defined prior to batch analysis. [3] Objects near the periphery of field of view, as well as abnormally small and large objects are discarded during sorting. [4] Once an object is classified, it would be excluded from subsequent selection.

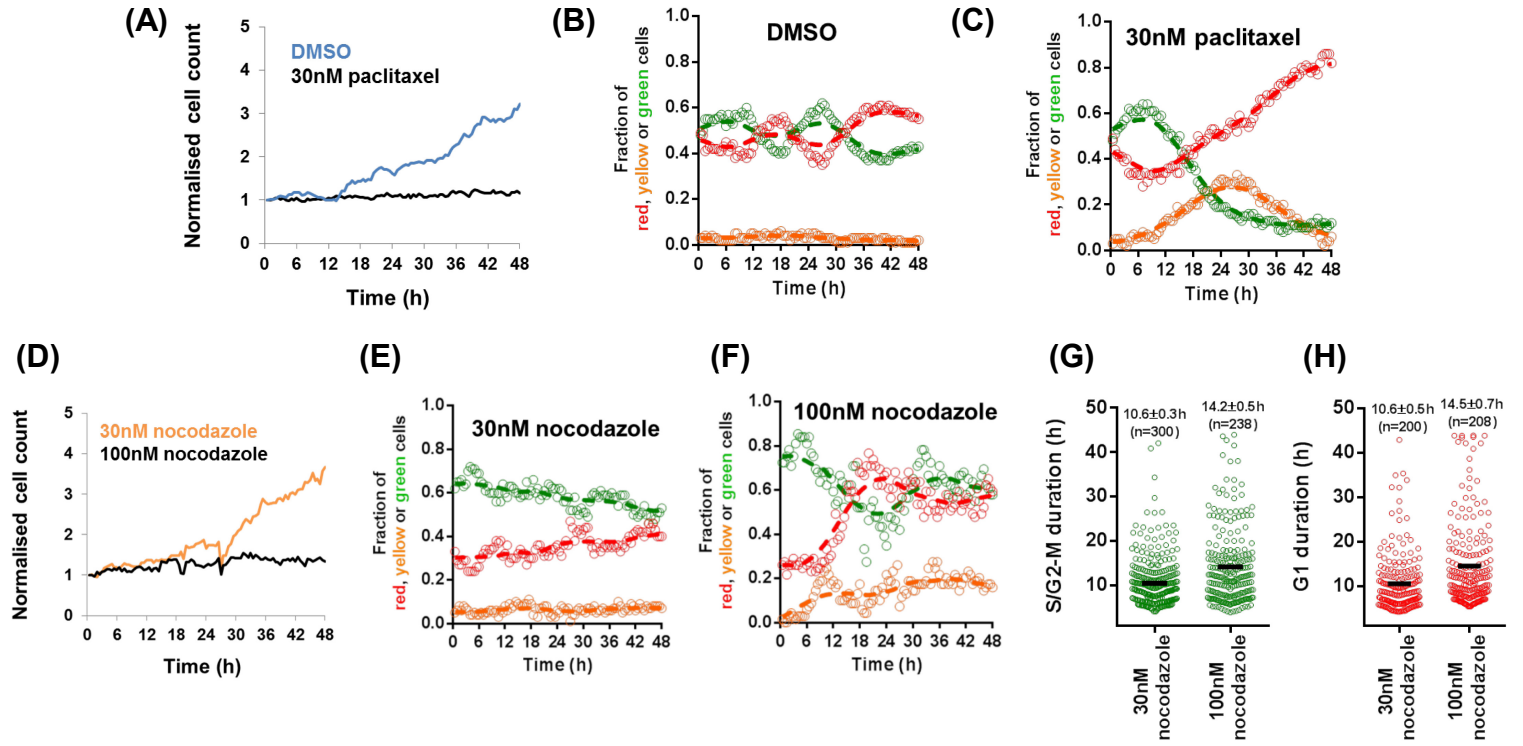

Figure S3, related to Figure 3. Real-time quantification of cell cycle modulation
(A-C) Real-time monitoring of FastFUCCI-expressing Panc-1 cells treated as indicated. Data shown were from five fields of view per time-point, with a mean total of 15,684 cells per condition scored. (D-F) Real-time monitoring of FastFUCCI-expressing MIA PaCa-2 cells treated as indicated. Data shown were from three fields of view per time-point, with a mean total of 11,226 cells per condition scored. (G) S/G2-M and (H) G1 duration of individual FastFUCCI-expressing MIA PaCa-2 cells treated as indicated. Duration is reported as mean ± SEM, n=number of cells, collected from imaging for 72 hours after drug addition. (D-H) Corresponding data for DMSO control are in Figure 3.

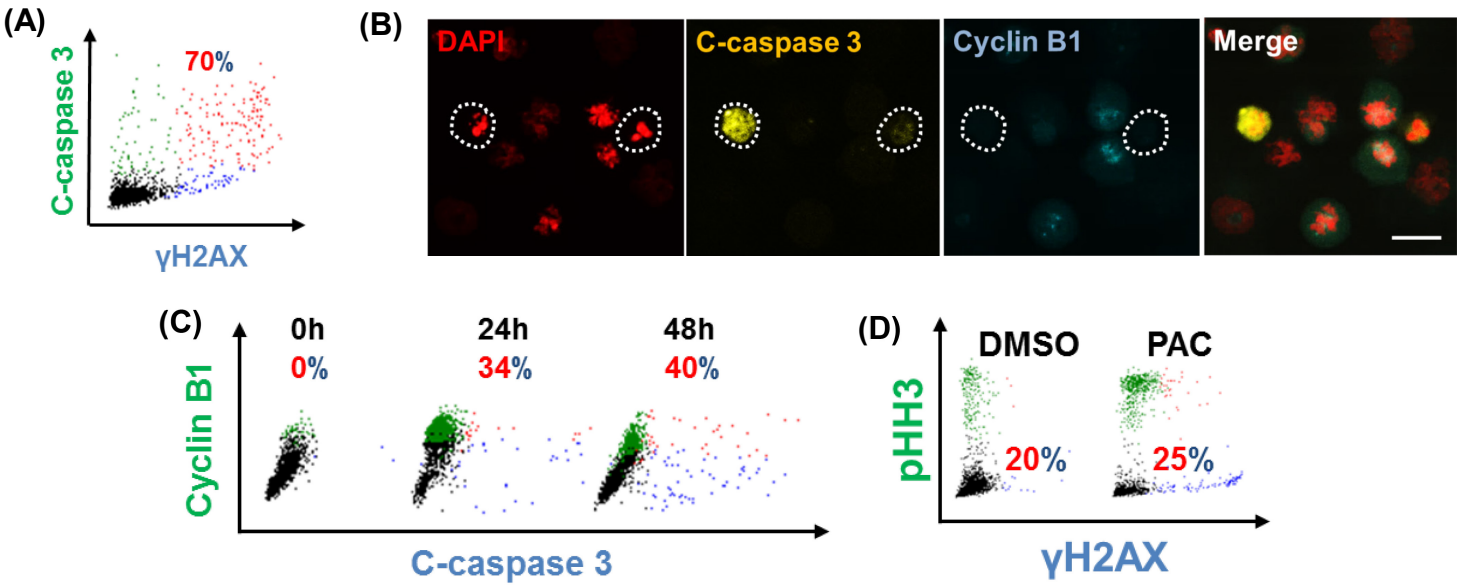

Figure S4, related to Figure 5. Correlation of paclitaxel-induced cell death and mitosis
(A) Quantification of parental MIA PaCa-2 cells treated with 30 nM paclitaxel for 24 hours. Each dot represents a single cell: black dot denotes negative cell, blue dot denotes γH2AX-positive cell, green dot denotes c-caspase 3-positive cell, red dot denotes γH2AX/c-caspase 3 double positive cell. Number denotes percentage of c-caspase 3-positive cells that were positive for γH2AX. (B) Immunofluorescence of parental Panc-1 cells treated with 30 nM paclitaxel for 24 hours. Scale bar, 25 µm. (C) Quantification of parental Panc-1 cells treated with 30 nM paclitaxel over the course of 48 hours. Each dot represents a single cell: black dot denotes negative cell, blue dot denotes c-caspase 3-positive cell, green dot denotes cyclin B1-positive cell, red dot denotes c-caspase 3/cyclin B1 double positive cell. Number denotes percentage of c-caspase 3-positive cells that were positive for cyclin B1. (D) Quantification of parental Panc-1 cells treated with vehicle or 30 nM paclitaxel for 48 hours. Each dot represents a single cell: black dot denotes negative cell, blue dot denotes γH2AX-positive cell, green dot denotes pHH3-positive cell, red dot denotes γH2AX/pHH3 double positive cell. Number denotes percentage of γH2AX-positive cells that were positive for pHH3. (A, C, D) A total of >2000 single cells per sample were scored.

Table S1, related to Figure 1. All single and two-cutter 6+ nucleotide restriction enzymes with respective positions and actual nucleotide numbers

Name	Frequency	Position
Acc65I	1	7761 (12350)
AgeI	2	2607 (3352), 5959 (8998)
AhdI	2	3904 (7524), 11428 (4826)
AleI	2	1954 (2146), 4100 (10204)
ApaI	1	3146 (12350)
AvrII	2	1911 (7480), 9391 (4870)
BamHI	1	3722 (12350)
BbvCI	2	1801 (1630), 3431 (10720)
BlpI	1	6122 (12350)
BmtI	2	5834 (1162), 6996 (11188)
BsiWI	1	6448 (12350)
BsmBI	2	6094 (690), 6784 (11660)
BspHI	2	11255 (1008), 12263 (11342)
BstEII	1	6526 (12350)
BstXI	1	4771 (12350)
BstZ17I	1	10156 (12350)
CspCI	1	628 (12350)
EcoNI	2	2618 (728), 3346 (11622)
EcoRI	1	4016 (12350)
FspI	1	11650 (12350)
KpnI	1	7765 (12350)
MluI	1	230 (12350)
NdeI	1	486 (12350)
NheI	2	5830 (1162), 6992 (11188)
NotI	1	5823 (12350)
NruI	2	210 (1000), 1210 (11350)
PacI	2	2520 (1192), 3712 (11158)
PciI	2	3162 (7373), 10535 (4977)
PsiI	2	8992 (1051), 10043 (11299)
PspOMI	1	3142 (12350)
PvuI	2	6372 (5426), 11798 (6924)
RsrII	1	6508 (12350)
ScaI	2	5771 (6137), 11908 (6213)
SgrAI	2	4097 (5537), 9634 (6813)
SmaI	2	9414 (196), 9610 (12154)
SnaBI	1	592 (12350)
SpeI	2	251 (5843), 6094 (6507)
SspI	1	12232 (12350)
Tth111I	1	6434 (12350)
XbaI	1	6360 (12350)
XcmI	2	4282 (1302), 5584 (11048)
XhoI	1	3494 (12350)
XmaI	2	9412 (196), 9608 (12154)