Figure S1

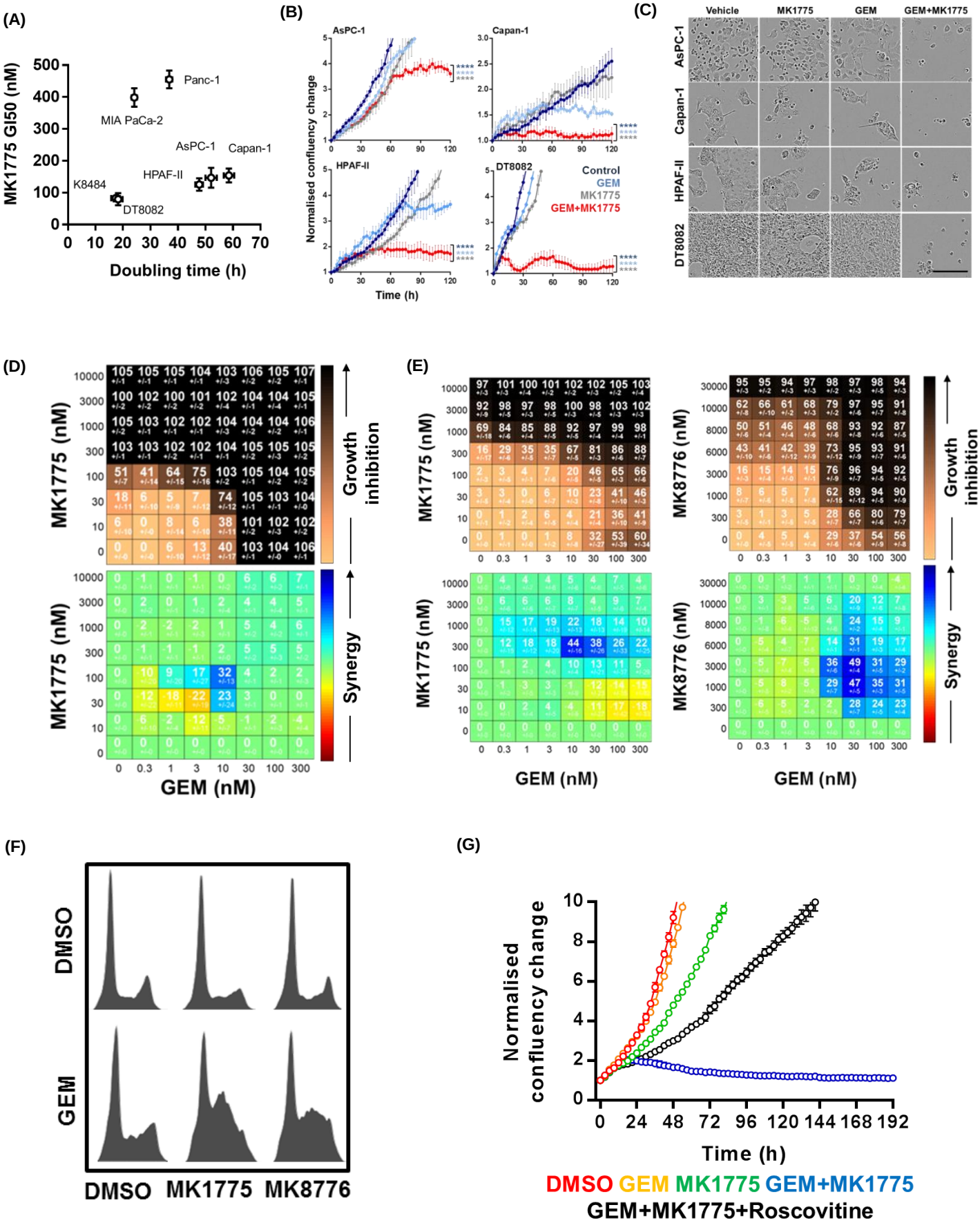
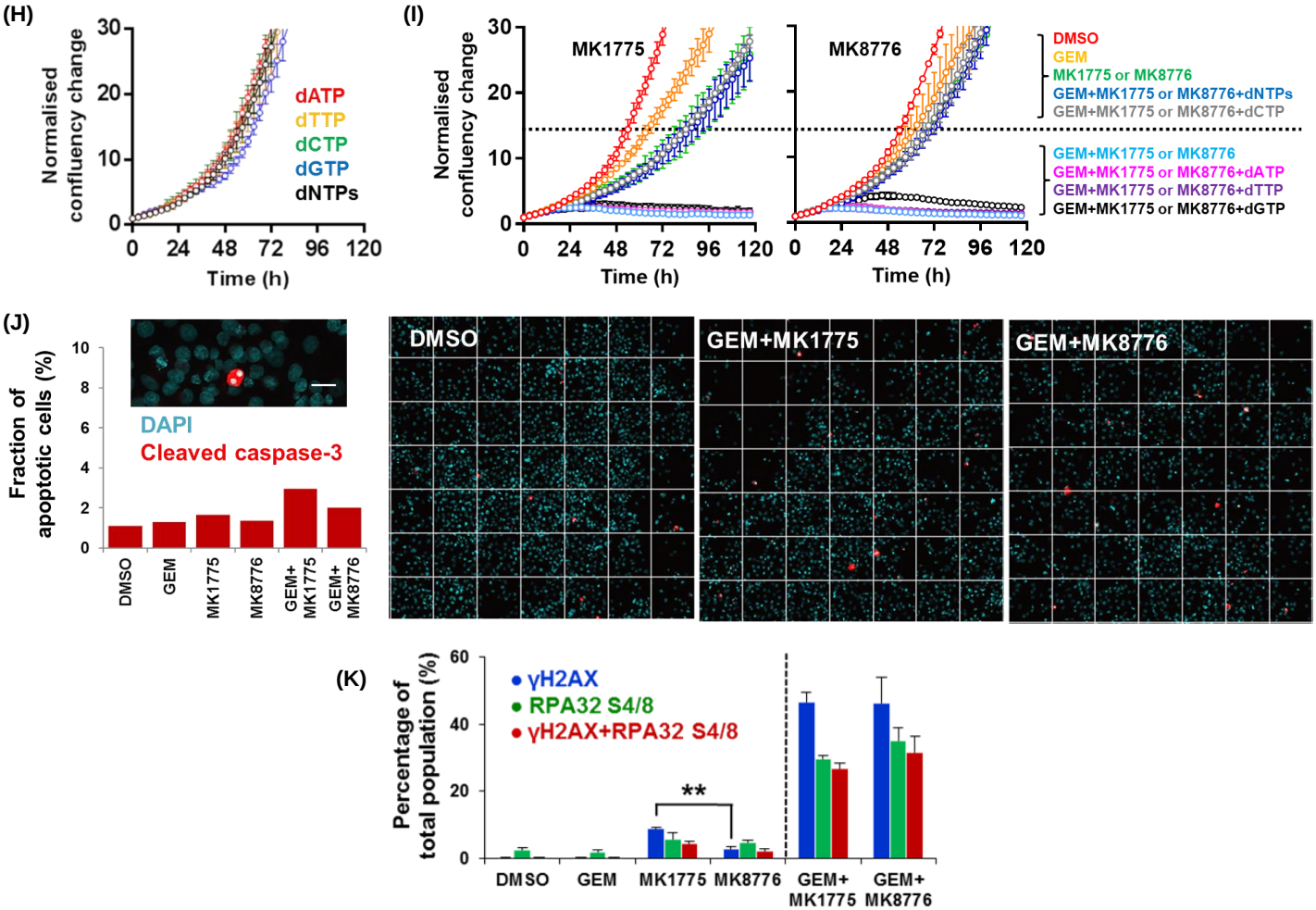

(A)
MK1775 GI50 (nM)
Doubling time (h)
Panc-1
MIA PaCa-2
AsPC-1
Capan-1
HPAF-II
K8484
DT8082
(B)
AsPC-1
Capan-1
HPAF-II
DT8082
Normalised confluency change
Time (h)
Control
GEM
MK1775
GEM+MK1775
(C)
Vehicle
MK1775
GEM
GEM+MK1775
AsPC-1
Capan-1
HPAF-II
DT8082
(D)
MK1775 (nM)
GEM (nM)
Growth inhibition
Synergy
(E)
MK1775 (nM)
MK8776 (nM)
GEM (nM)
Growth inhibition
Synergy
(F)
DMSO
GEM
DMSO
MK1775
MK8776
(G)
Normalised confluency change
Time (h)
DMSO
GEM
MK1775
GEM+MK1775
GEM+MK1775+Roscovitine

Figure S1



(A) Sensitivity assay. Cells were treated with a range of MK1775 concentrations for 5 days. Doubling time was derived from exponential phase. Data are represented as mean ± SEM, n=3.

(B) Growth kinetic assay. 10 nM GEM and 100 nM MK1775 were used in AsPC-1, Capan-1 and DT8082; 30 nM GEM and 100 nM MK1775 were used in HPAF-II. Data are represented as mean ± SEM, n=3. A two-way ANOVA analysis was performed, ****p≤0.0001.

(C) Representative images of cells treated as in Fig. S1B after 120 hours. Scale bar, 200 µm.

(D) Combination assay. K8484 cells were treated as indicated for 72 hours. Data were analysed with the Bliss synergy model.

(E) Combination assay. Panc-1 cells were treated as indicated for 72 hours. Data were analysed with the Bliss synergy model.

(F) Cell cycle profile of MIA PaCa-2 cells treated as indicated for 24 hours (10 nM GEM, 300 nM MK1775, 1 µM MK8776).

(G) Cell proliferation of MIA PaCa-2 cells treated as indicated. Concentrations used were as follows: 10 nM GEM, 300 nM MK1775, 10 µM roscovitine. Data are represented as mean ± SEM, n=3.

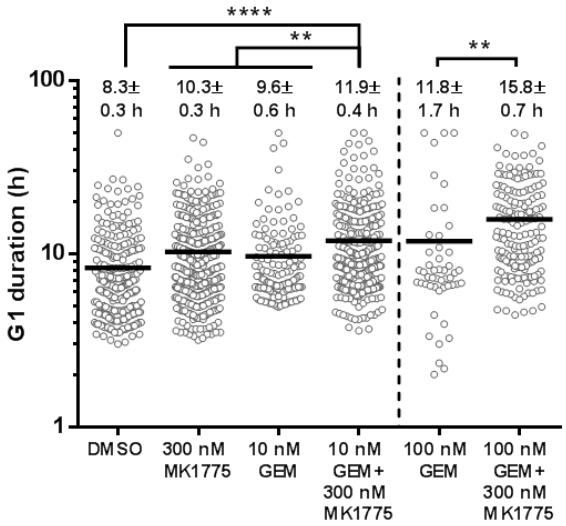
(H-I) Cell proliferation of MIA PaCa-2 cells treated as indicated. Concentrations used were 10 µM dNTPs (Invitrogen), 10 nM GEM, 300 nM MK1775, 1 µM MK8776. Data are represented as mean ± SEM, n=3.

(J) Quantitative immunofluorescence on MIA PaCa-2 cells treated as indicated for 24 hours (10 nM GEM, 300 nM MK1775, 1 µM MK8776). Cleaved caspase-3 was used as a marker of apoptosis. At least 4000 cells were analysed per condition. Grid, 200X200 µm. Scale bar, 30 µm.

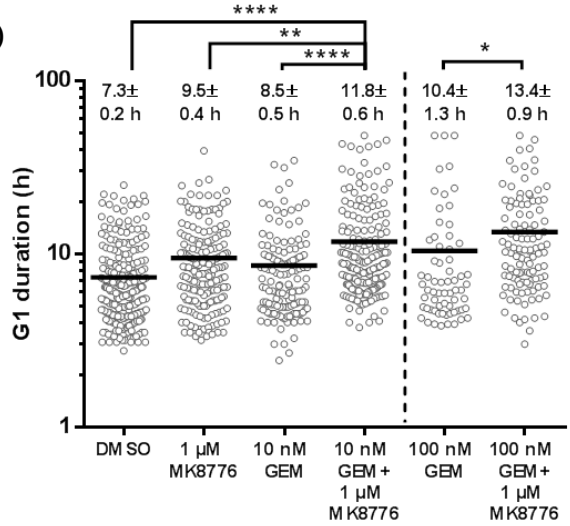
(K) Quantification for Fig. 1C. Data are represented as mean ± SEM, n=3. A two-tailed t-test was performed, **p≤0.01.

Figure S2

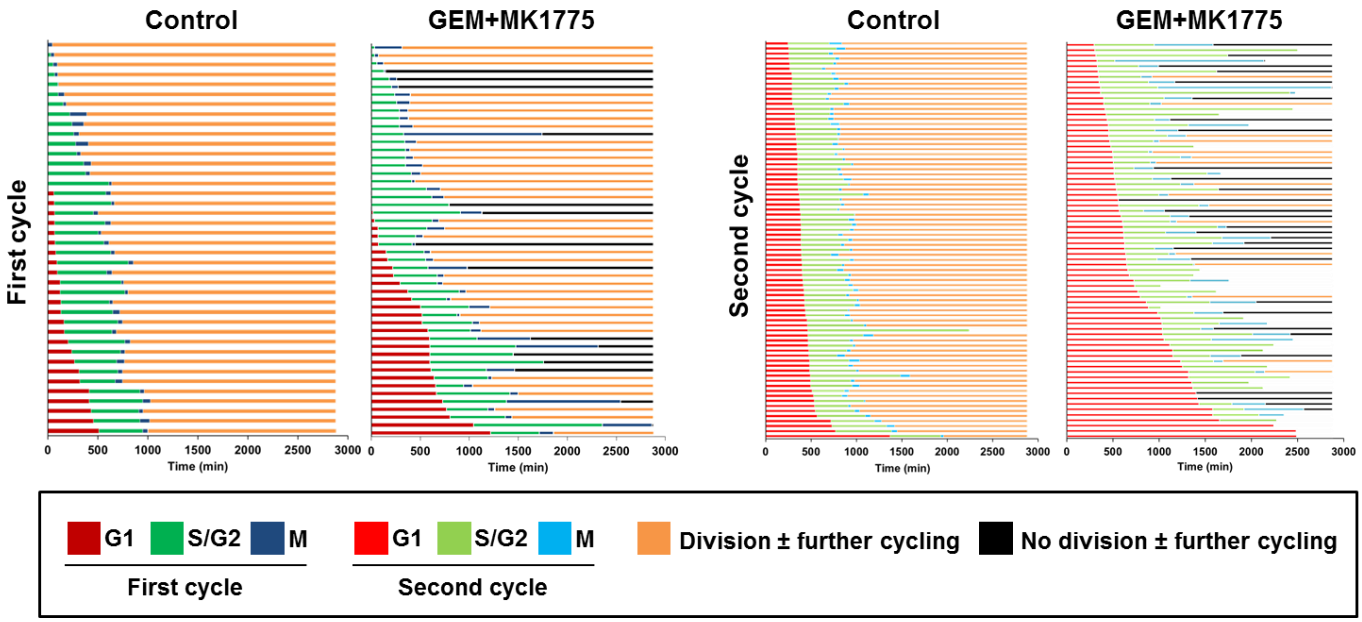
(A)



(B)



(C)



(D)

First generation	Second generation: Cell fate	Division	Cytokinesis failure	Cell cycle arrest G1	Cell cycle arrest S/G2	Cell cycle arrest M	Cell death G1	Cell death S/G2	Cell death M
	Division	16	18	3	15	7	0	2	4
	Cytokinesis failure	0	3	2	1	0	1	0	0

(E)

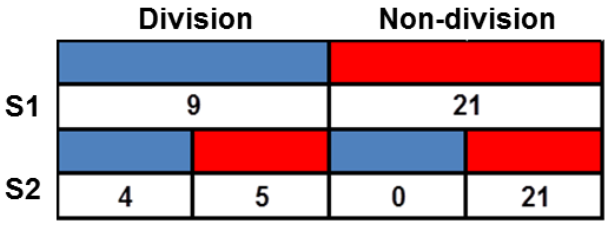


Figure S2

(A-B) G1 duration of MIA PaCa-2 FastFUCCI cells treated as indicated. Except for 100 nM GEM samples where 66 (A) and 49 (B) cells were analysed, at least 100 cells were analysed in all other conditions. Data are represented as mean ± SEM. A one-way ANOVA analysis was performed, *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

(C) Individual fate profiles of MIA PaCa-2 FastFUCCI cells treated in Fig. 2C. Red, green and blue bars denote the duration of the specified phase of each cell. Profiles are ranked first according to G1 duration (red bar), if not to S/G2 duration (green bar). Orange and black bars denote the outcome of the observed mitosis without further temporal reference. Truncation denotes the end of experiment.

(D) Cross-generation fate analysis of cells treated in Fig. 2D. The fates of first-generation cells and the corresponding second-generation cells were tracked in parallel and grouped accordingly. The number of second-generation cells arising from the first-generation cells for each corresponding fate is indicated. First-generation cells that died without exiting mitosis (i.e. no second-generation cells) were excluded from the analysis.

(E) Sister cell fates. 30 pairs of daughter cells arising from the same mother cells treated in Fig. 2D were followed over time and grouped according to their eventual fate.

Figure S3

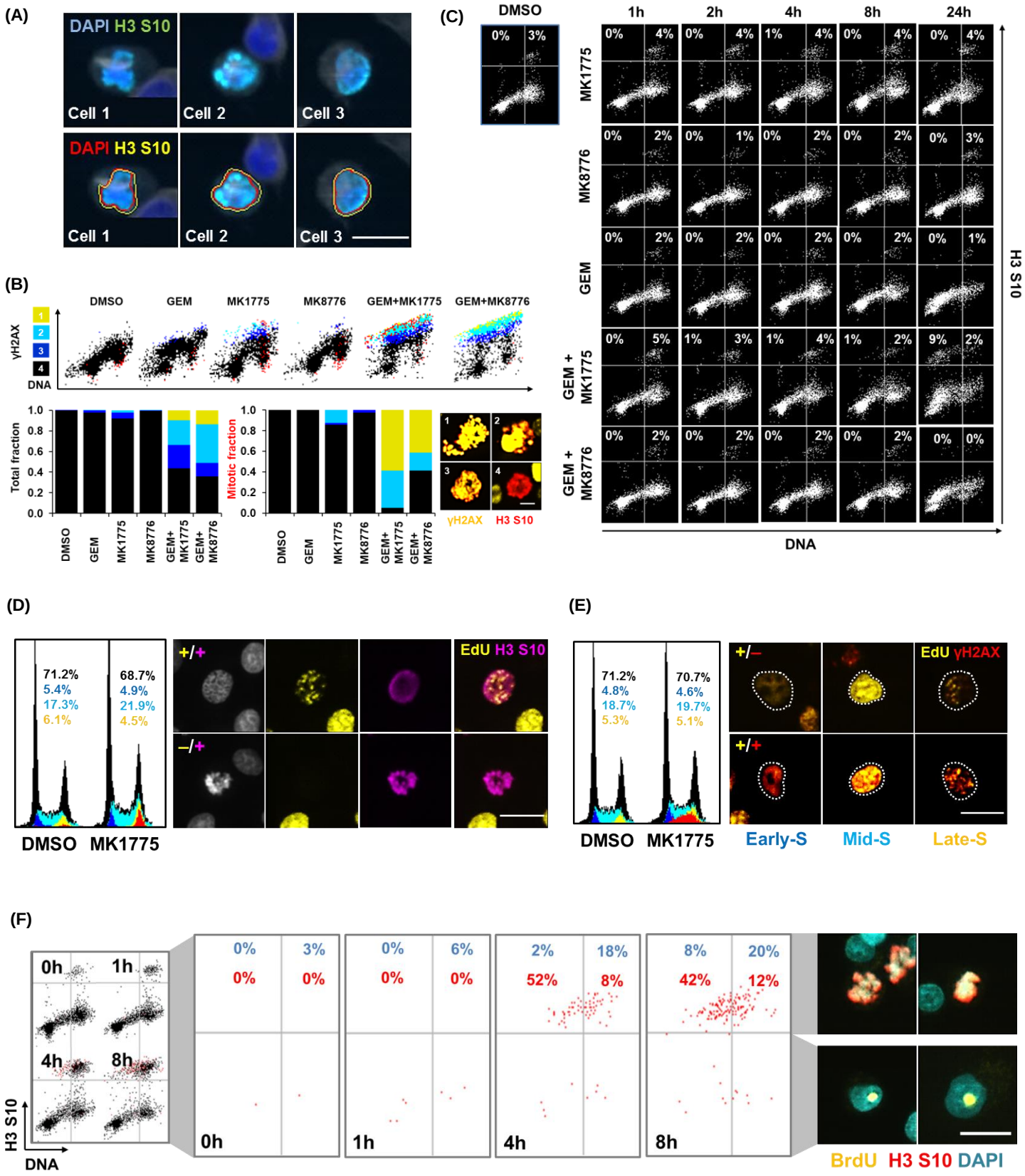
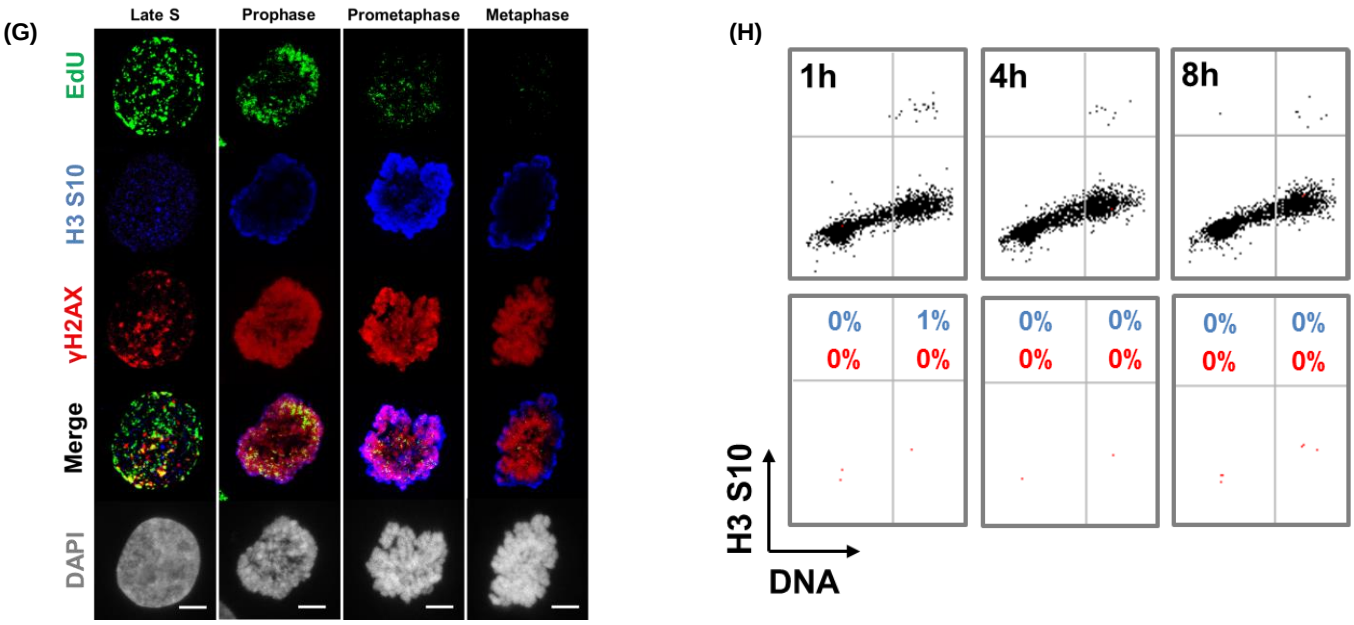

(A)
DAPI H3 S10
Cell 1
Cell 2
Cell 3
DAPI H3 S10
Cell 1
Cell 2
Cell 3
(B)
DMSO
GEM
MK1775
MK8776
GEM+MK1775
GEM+MK8776
γH2AX
DNA
Total fraction
Mitotic fraction
γH2AX
H3 S10
(C)
DMSO
1h
2h
4h
8h
24h
MK1775
MK8776
GEM
GEM + MK1775
GEM + MK8776
H3 S10
DNA
(D)
71.2%
5.4%
17.3%
6.1%
68.7%
4.9%
21.9%
4.5%
+/+
–/+
EdU H3 S10
DMSO
MK1775
(E)
71.2%
4.8%
18.7%
5.3%
70.7%
4.6%
19.7%
5.1%
+/–
+/+
EdU γH2AX
DMSO
MK1775
Early-S
Mid-S
Late-S
(F)
0h
1h
4h
8h
H3 S10
DNA
0%
3%
0%
0%
0%
6%
0%
0%
2%
18%
52%
8%
8%
20%
42%
12%
0h
1h
4h
8h
BrdU
H3 S10
DAPI

Figure S3



(A) Quantification of DNA content. Each mitotic cell detected by H3 S10 staining was scored for its DNA content as measured by DAPI. Scale bar, 25 µm.

(B) Differential analysis of DNA damage in Panc-1 cells treated for 24 hours. Each dot represents a single cell, colour-coded (black, blue, cyan, yellow) according to degree of damage. Red dot denotes H3 S10-positive (mitotic) cells. Bottom panel shows the fractions of total (left) and mitotic (middle) populations in each damage classification, with examples (right). Scale bar, 10 µm.

(C) 2D scatter diagrams for Panc-1 cells treated over the course of 24 hours (30 nM GEM, 300 nM MK1775, 1 µM MK8776). Percentage of cells in the two upper quadrants is shown.

(D) Cell cycle profile of EdU-pulsed Panc-1 cells treated with DMSO or 3 µM MK1775 in Fig. 3C. Black denotes non-S subset; blue denotes early-S subset; cyan denotes mid-S subset; yellow denotes late-S subset; red denotes mitotic subset. Images depict examples of replicating (top panel) and non-replicating (bottom panel) mitotic cells. Scale bar, 25 µm.

(E) Cell cycle profile of EdU-pulsed Panc-1 cells treated with DMSO or 3 µM MK1775 in Fig. 3D. Black denotes non-S subset; blue denotes early-S subset; cyan denotes mid-S subset; yellow denotes late-S subset; red denotes γH2AX-positive subset. Images depict examples of γH2AX-negative (top panel) and γH2AX-positive (bottom panel) cells in three stages of S phase. Scale bar, 25 µm.

(F) 2D scatter diagrams for Panc-1 treated in Fig. 3E-F. The profile for the whole cell population and the position of native BrdU-positive cells (red dots) are shown on the far left. Percentage of mitotic (H3 S10-positive) cells in the two upper quadrants is shown in blue, percentage of native BrdU-positive mitotic cells (out of the respective mitotic fractions) in red. Examples of mitotic and non-mitotic native BrdU-positive cells are shown on the far right. Scale bar, 25 µm.

(G) Immunofluorescence of premature mitotic Panc-1 cells treated with 3 µM MK1775 for 4 hours followed by 10 µM EdU for 45 minutes. Scale bar, 5 µm.

(H) 2D scatter diagrams for Panc-1 grown with 10 µM BrdU for 48 hours, treated with 30 µM MK8776 over 8 hours. Top row shows the profile for the whole cell population. Bottom row shows the position of the few native BrdU-positive cells (red dots). Percentage of mitotic (H3 S10-positive) cells in the two upper quadrants is shown in blue, percentage of native BrdU-positive mitotic cells (out of the respective mitotic fractions) in red.

Figure S4

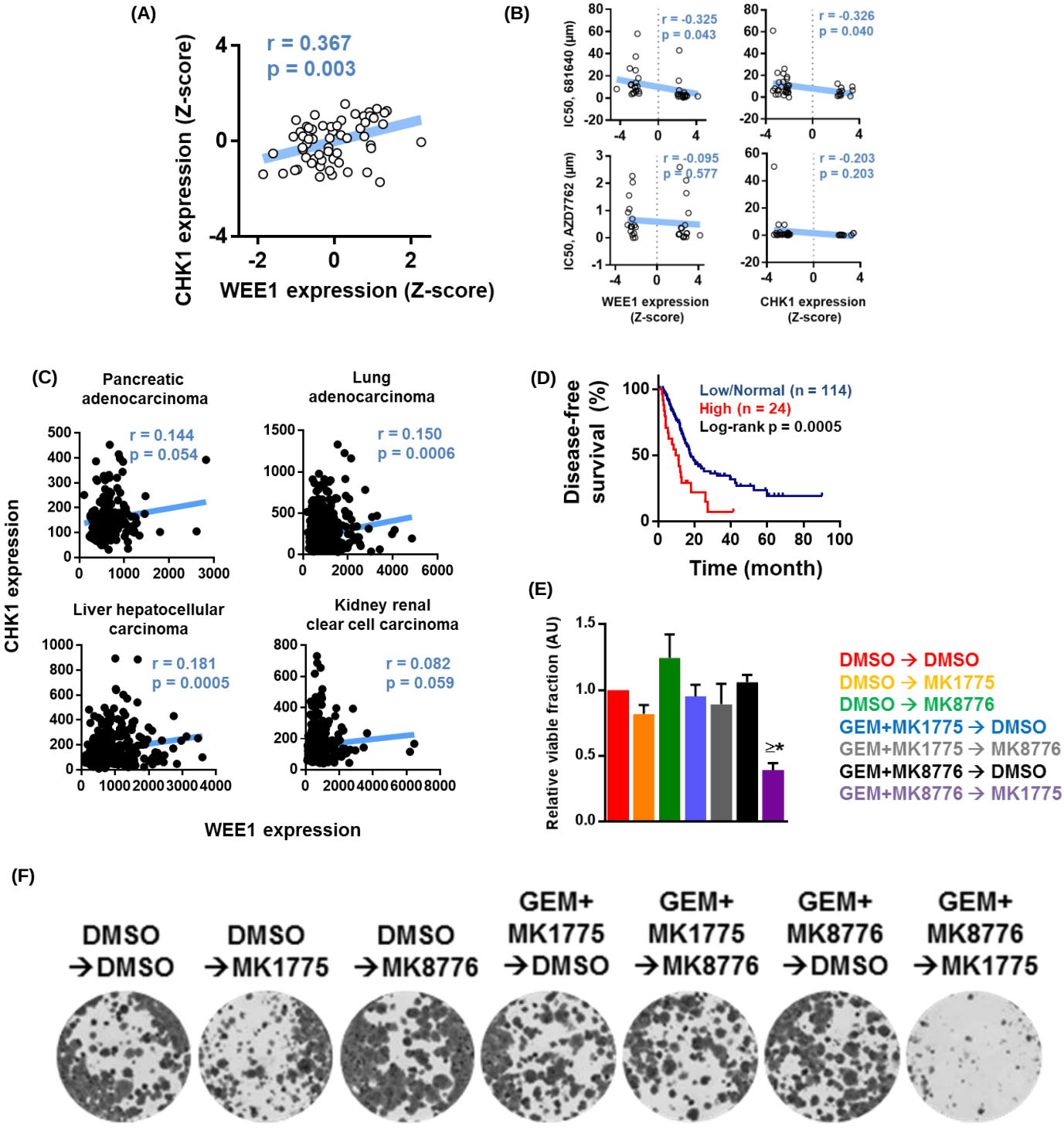

(A)
r = 0.367
p = 0.003
CHK1 expression (Z-score)
WEE1 expression (Z-score)
(B)
IC50, 681640 (μm)
IC50, AZD7762 (μm)
r = -0.325
p = 0.043
r = -0.326
p = 0.040
r = -0.095
p = 0.577
r = -0.203
p = 0.203
WEE1 expression (Z-score)
CHK1 expression (Z-score)
(C)
Pancreatic adenocarcinoma
r = 0.144
p = 0.054
Lung adenocarcinoma
r = 0.150
p = 0.0006
Liver hepatocellular carcinoma
r = 0.181
p = 0.0005
Kidney renal clear cell carcinoma
r = 0.082
p = 0.059
CHK1 expression
WEE1 expression
(D)
Disease-free survival (%)
Low/Normal (n = 114)
High (n = 24)
Log-rank p = 0.0005
Time (month)
(E)
Relative viable fraction (AU)
≥*
DMSO → DMSO
DMSO → MK1775
DMSO → MK8776
GEM+MK1775 → DMSO
GEM+MK1775 → MK8776
GEM+MK8776 → DMSO
GEM+MK8776 → MK1775
(F)
DMSO →DMSO
DMSO →MK1775
DMSO →MK8776
GEM+ MK1775 →DMSO
GEM+ MK1775 →MK8776
GEM+ MK8776 →DMSO
GEM+ MK8776 →MK1775

Figure S4

(A) Correlative analysis between WEE1 and CHK1 mRNA expression in 60 tumour cell lines from the NCI-60 cell line project. Pearson correlation coefficient r and p values are indicated.
(B) Correlative analysis between WEE1 or CHK1 gene expression in tumour cell lines and sensitivity to either WEE1/CHK1i 681640 or CHK1/2i AZD7762. Data were sourced from the Catalogue of Somatic Mutations in Cancer. Pearson correlation coefficient r and p values are indicated.
(C) Correlative analysis between WEE1 and CHK1 mRNA expression in indicated primary tumour samples. Pearson correlation coefficient r and p values are indicated. Data were sourced from the TCGA Research Network.
(D) Kaplan-Meier analysis of RNASeq V2 data on WEE1 or CHK1 expression and patient disease-free survival in pancreatic adenocarcinoma. Data were sourced from the TCGA Research Network.
(E) Clonogenic assay. Panc-1 cells were treated with the first condition for 24 hours, after which the medium was replaced with the second condition for 10 days. Data are represented as mean ± SEM, n=3. A one-way ANOVA analysis was performed, *$p \leq 0.05$.
(F) Representative images of clonogenic assays in Fig. S4E.

Figure S5

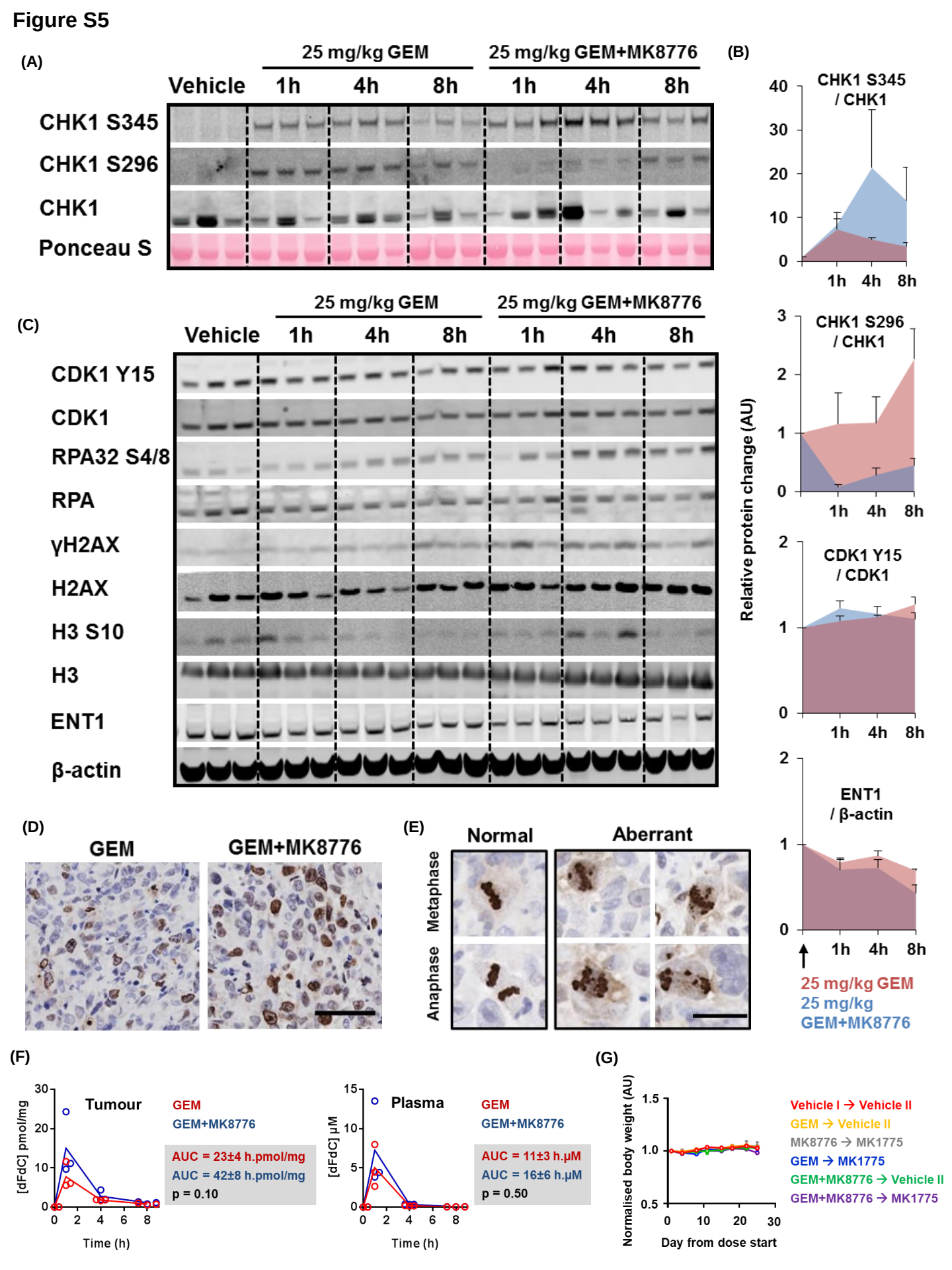

(A)
25 mg/kg GEM
25 mg/kg GEM+MK8776
Vehicle
1h
4h
8h
1h
4h
8h
CHK1 S345
CHK1 S296
CHK1
Ponceau S
(B)
CHK1 S345 / CHK1
CHK1 S296 / CHK1
CDK1 Y15 / CDK1
ENT1 / β-actin
Relative protein change (AU)
(C)
CDK1 Y15
CDK1
RPA32 S4/8
RPA
γH2AX
H2AX
H3 S10
H3
ENT1
β-actin
25 mg/kg GEM
25 mg/kg GEM+MK8776
(D)
GEM
GEM+MK8776
(E)
Normal
Aberrant
Metaphase
Anaphase
(F)
Tumour
GEM
GEM+MK8776
AUC = 23±4 h.pmol/mg
AUC = 42±8 h.pmol/mg
p = 0.10
[dFdC] pmol/mg
Time (h)
Plasma
AUC = 11±3 h.μM
AUC = 16±6 h.μM
p = 0.50
[dFdC] μM
(G)
Normalised body weight (AU)
Day from dose start
Vehicle I → Vehicle II
GEM → Vehicle II
MK8776 → MK1775
GEM → MK1775
GEM+MK8776 → Vehicle II
GEM+MK8776 → MK1775

Figure S5

(A-C) Immunoblotting of tumour samples from MIA PaCa-2 xenografts treated and harvested as indicated. The graphs in (B) show the densitometric analysis of the immunoblots. Data are represented as mean ± SEM, n=3.

(D) Examples of γH2AX immunohistochemistry quantified in Fig. 5B. Images were from MIA PaCa-2 tumour sample treated with 25 mg/kg GEM or 25 mg/kg GEM+MK8776 for 8 hours. Scale bar, 50 µm.

(E) Examples of aberrant mitotic cells in MIA PaCa-2 tumour sample treated with 25 mg/kg GEM+MK8776. Scale bar, 25 µm.

(F) Pharmacokinetic profiles of GEM. Tumour and plasma samples from MIA PaCa-2 xenografts treated with either 25 mg/kg GEM or 25 mg/kg GEM+MK8776 were analysed for the native metabolite of GEM (dFdC) at specified time-points. Area under the curve (AUC) and p values are indicated.

(G) Normalised body weight of MIA PaCa-2 xenografts in Fig. 5E. Data are represented as mean ± SEM, n=3.