Electronic Supplementary Material (ESI) for Analyst.

Identifying fates of cancer cells exposed to mitotic inhibitors by quantitative phase imaging

Dian Huang, Irena J. Roy, Graeme F. Murray, Jason Reed, Thomas A. Zangle, and Michael A. Teitell

SUPPLEMENTARY FIGURES 1 – 6

SUPPLEMENTARY TABLES 1 and 2

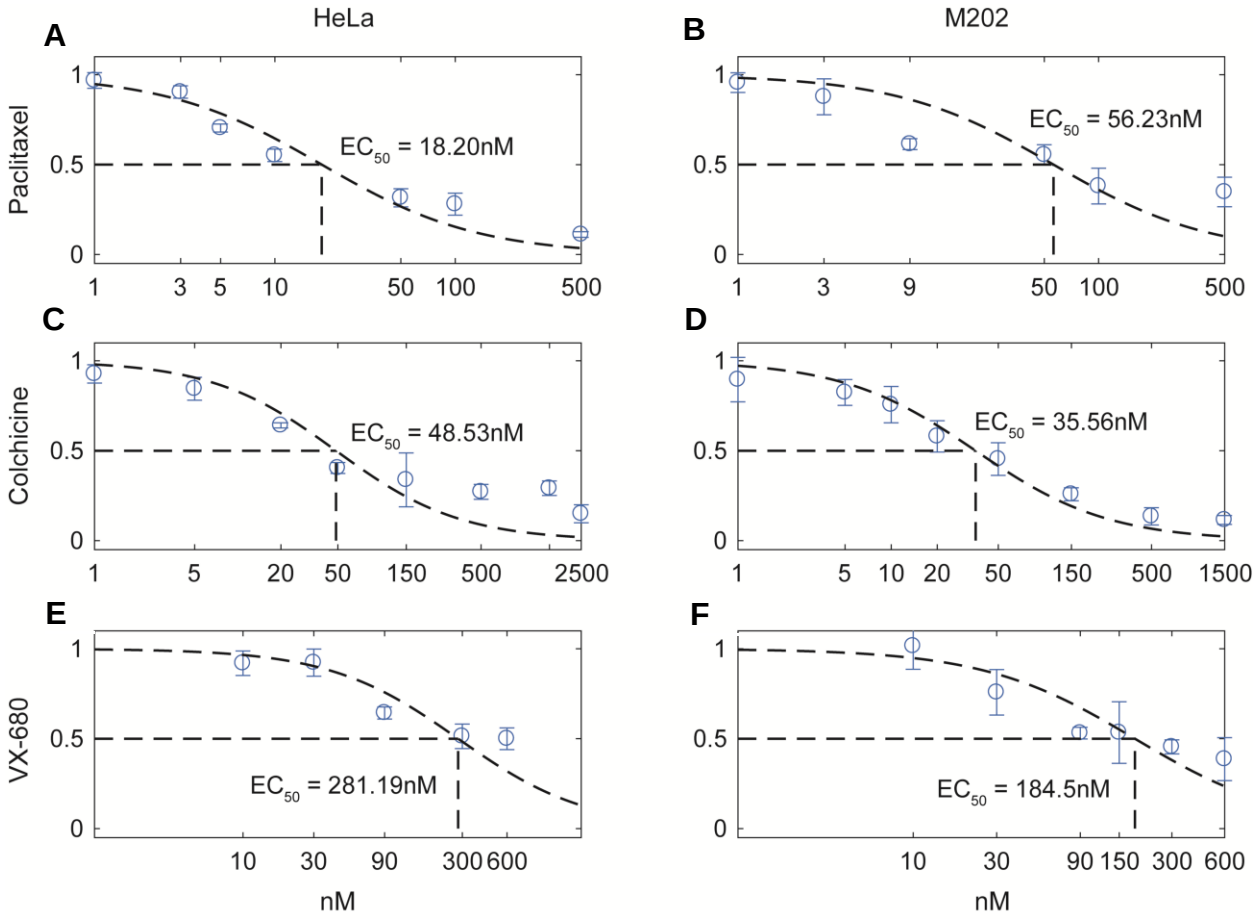

Figure S1 - EC_{50} Values from Live Cell Counting After 24-hour Drug Treatments. Data points indicate average cell counts across three technical replicates. Error bars indicate standard deviation of the three technical replicates. (A) Live cell counting assay for HeLa cells after 24 hours of paclitaxel treatment. (B) Live cell counting assay for M202 cells after 24 hours of paclitaxel treatment. (C) Live cell counting assay for HeLa cells after 24 hours of colchicine treatment. (D) Live cell counting assay for M202 cells after 24 hours of colchicine treatment. (E) Live cell counting assay for HeLa cells after 24 hours of VX-680 treatment. (F) Live cell counting assay for M202 cells after 24 hours of VX-680 treatment.

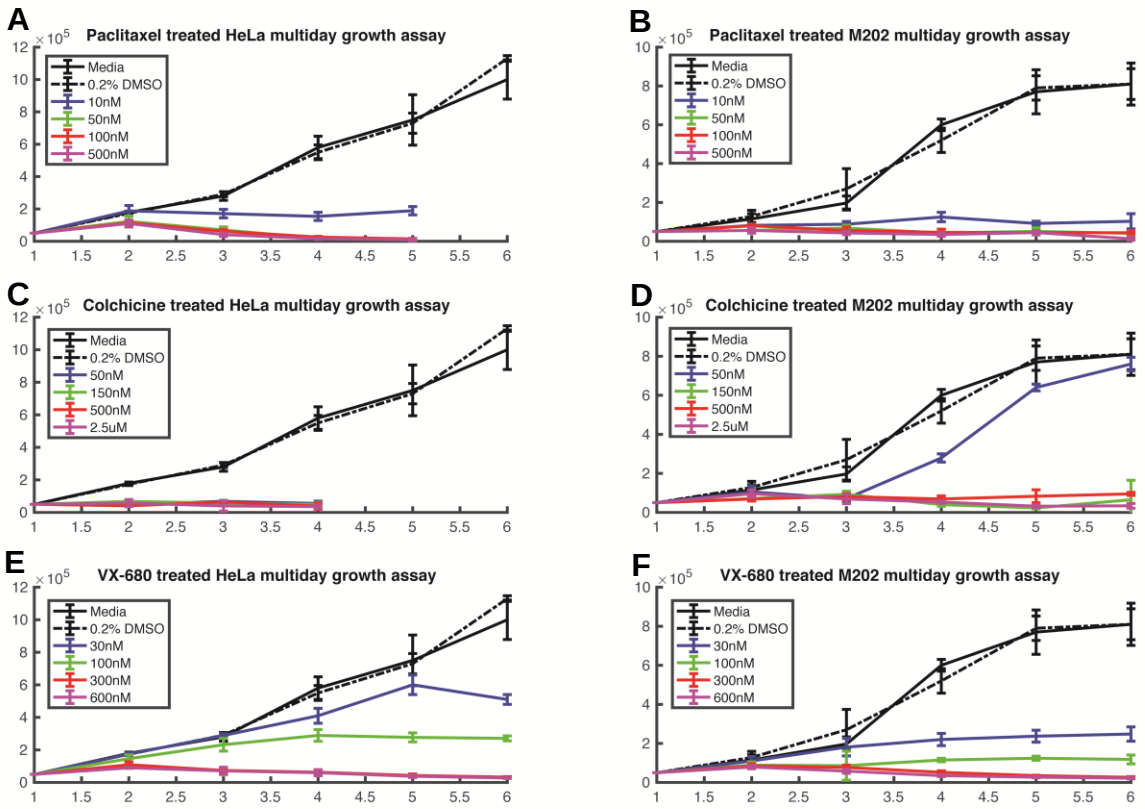

Figure S2 - Multi-day Cell Counting Assays. Data points indicate average cell counts across three technical replicates. Error bars indicate standard deviation of the three technical replicates. (A) Multi-day cell counting assay for HeLa cells under paclitaxel treatment. (B) Multi-day cell counting assay for M202 cells under paclitaxel treatment. (C) Multi-day cell counting assay for HeLa cells under colchicine treatment. (D) Multi-day cell counting assay for M202 cells under colchicine treatment. (E) Multi-day cell counting assay for HeLa cells under VX-680 treatment. (F) Multi-day cell counting assay for M202 cells under VX-680 treatment.

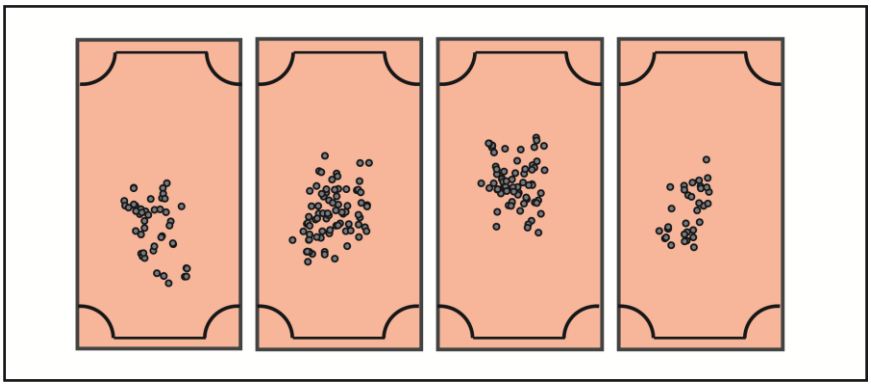

Figure S3 - Single cell location distribution within imaging wells. Each dot represents the location of an individual cell (n = 229) that was tracked in this example experiment. The layout and structure of an ibidi 4-well $^{Ph+}$ μ-slide is also shown. Due to image distortion and aberrations in the optical path that occur near the edges and corners of each well, random locations were selected within the central region of each well where imaging conditions are optimal for QPI.

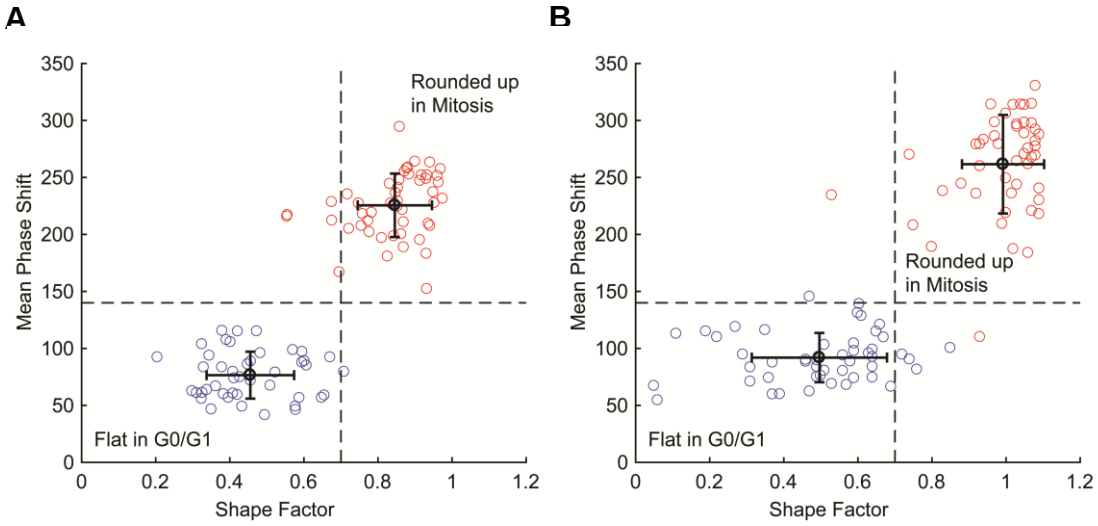

Figure S4 - Mean phase-shift and shape factor thresholds. (A) Mean phase-shift and shape factor values for flat and mitotic HeLa cells (*n* = 93). Thresholds set at 140 for mean phase- shift and 0.7 for shape factor. (B) Mean phase-shift and shape factor values for flat and mitotic M202 cells (*n* = 96). Same thresholds also apply to these cells.

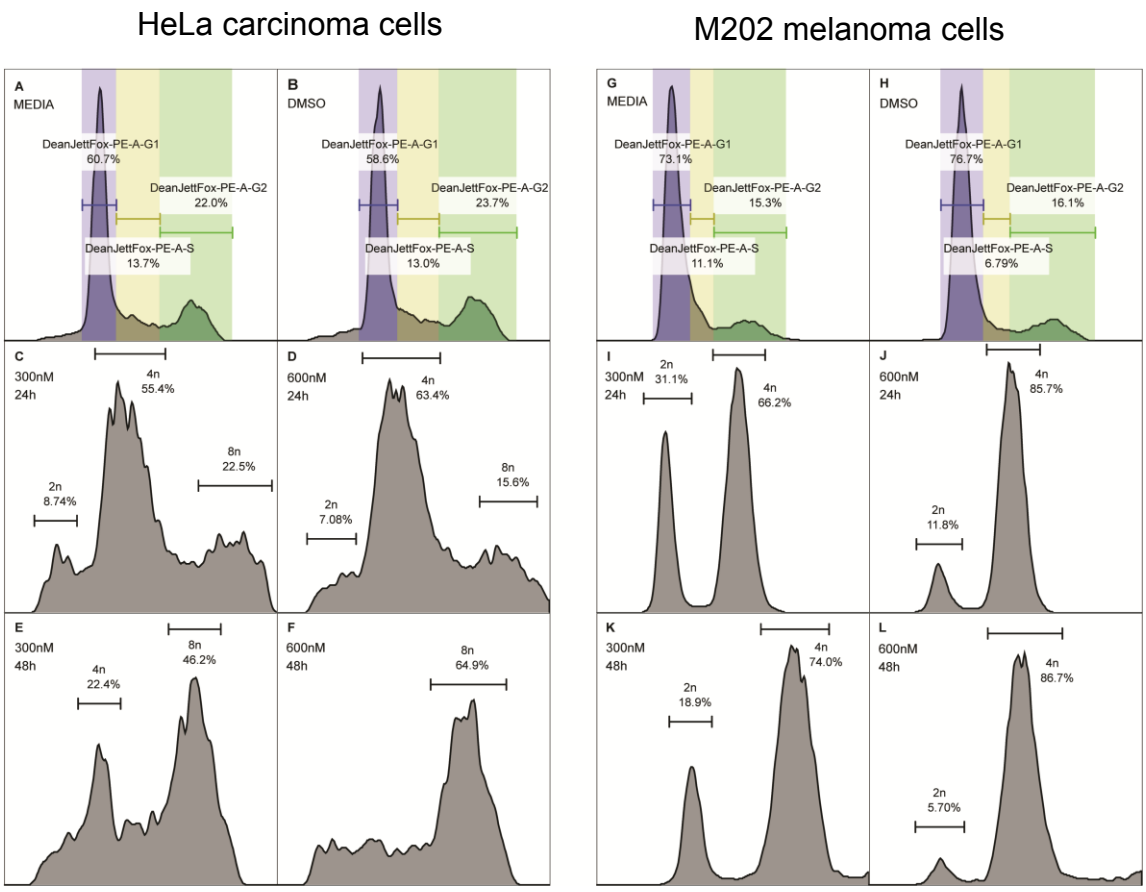

Figure S5 - Flow cytometry analysis of QPI-defined endocycling cells. (A) DNA content profile of HeLa cells in media control condition. (B) DNA content profile of HeLa cells in DMSO (0.1%) control condition. (C) DNA content profile of HeLa cells in 300nM of VX-680, 24 h. (D) DNA content profile of HeLa cells in 600nM of VX-680, 24 h. (E) DNA content profile of HeLa cells in 300nM of VX-680, 48 h. (F) DNA content profile of HeLa cells in 300nM of VX-680, 24 h. (G) DNA content profile of M202 cells in media control condition. (H) DNA content profile of M202 cells in DMSO (0.1%) control condition. (I) DNA content profile of M202 cells in 300nM of VX-680, 24 h. (J) DNA content profile of M202 cells in 600nM of VX-680, 24 h. (K) DNA content profile of M202 cells in 300nM of VX-680, 48 h. (L) DNA content profile of M202 cells in 300nM of VX-680, 24 h.

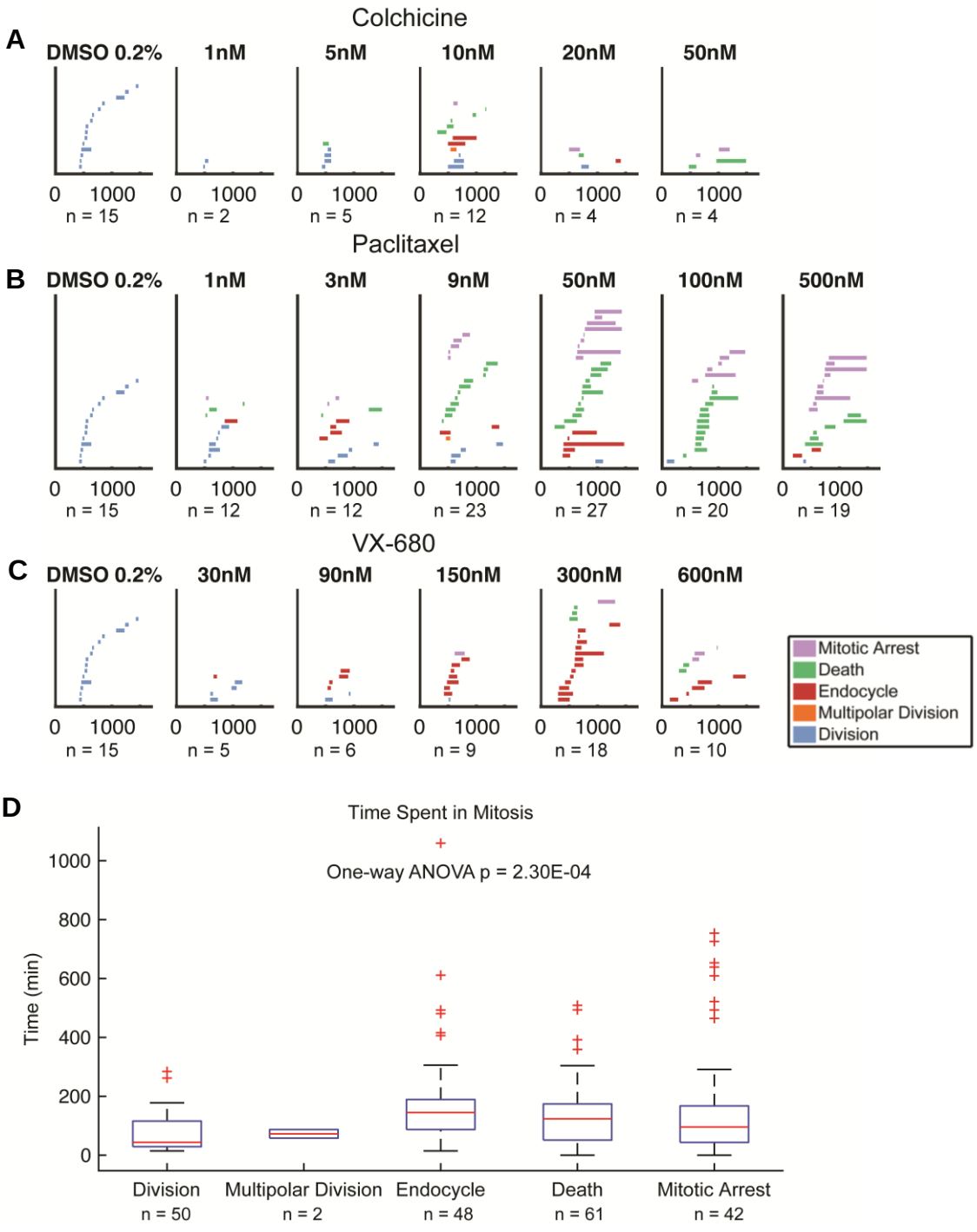
A
Colchicine
DMSO 0.2%
1nM
5nM
10nM
20nM
50nM
0 1000
n = 15
n = 2
n = 5
n = 12
n = 4
n = 4
B
Paclitaxel
DMSO 0.2%
1nM
3nM
9nM
50nM
100nM
500nM
0 1000
n = 15
n = 12
n = 12
n = 23
n = 27
n = 20
n = 19
C
VX-680
DMSO 0.2%
30nM
90nM
150nM
300nM
600nM
0 1000
n = 15
n = 5
n = 6
n = 9
n = 18
n = 10
Mitotic Arrest
Death
Endocycle
Multipolar Division
Division
D
Time Spent in Mitosis
One-way ANOVA p = 2.30E-04
Time (min)
1000
800
600
400
200
0
Division
n = 50
Multipolar Division
n = 2
Endocycle
n = 48
Death
n = 61
Mitotic Arrest
n = 42

Figure S6 - M202 cells division time visualization. (A) Time spent in mitosis for M202 single cells with colchicine exposure. The length of each color bar indicates the amount of time spent in mitosis for that individual cell. The color of the bar corresponds to its cell fate outcome. (B) Time spent in mitosis for M202 single cells with palitaxel exposure. (C) Time spent in mitosis for M202 single cells with VX-680 exposure. (D) Box plot of time spent in mitosis for five cell fate outcomes. One-way ANOVA with unbalanced sample groups performed in MatLab provide a *p*-value of 2.30E-4.

Data Group	EC_{50} (nM)	95% Confidence Bounds (nM)	R-square	ΔEC_{50} (nM)	KS Test p-value
HeLa_Colchicine_Division_Rate	$EC_{50,Growth}$ = 6.53	(2.67, 15.96)	0.9791	2.25	0.00075491
HeLa_Colchicine_Death/Arrest_Rate	$EC_{50,Death}$ = 8.78	(2.53, 30.48)	0.9492		
HeLa_Paclitaxel_Division_Rate	$EC_{50,Growth}$ = 7.32	(4.33, 12.39)	0.9784	8.31	0.0369
HeLa_Paclitaxel_Death/Arrest_Rate	$EC_{50,Death}$ = 15.63	(7.17, 34.12)	0.9239		
HeLa_VX-680_Division_Rate	$EC_{50,Growth}$ = 8.37	(1.92, 36.31)	0.9855	927.04	2.9256E-30
HeLa_VX-680_Death/Arrest_Rate	$EC_{50,Death}$ = 935.41	(695.02, 1256.03)	0.961		
M202_Colchicine_Division_Rate	$EC_{50,Growth}$ = 7.4	(2.59, 21.18)	0.854	5.34	0.0129
M202_Colchicine_Death/Arrest_Rate	$EC_{50,Death}$ = 12.74	(5.50, 29.51)	0.8791		
M202_Paclitaxel_Division_Rate	$EC_{50,Growth}$ = 1.51	(1.20, 1.90)	0.9788	2.96	0.0524
M202_Paclitaxel_Death/Arrest_Rate	$EC_{50,Death}$ = 4.47	(1.96, 10.19)	0.7976		
M202_VX-680_Division_Rate	$EC_{50,Growth}$ = 46.77	(17.86, 122.18)	0.8327	808.30	2.5882E-16
M202_VX-680_Death/Arrest_Rate	$EC_{50,Death}$ = 855.07	(480.84, 1524.05)	0.8799		

Table S1 - Drug response curve fitting, EC50 values and Kolmogorov-Smirnov test *p*-values.

Table Analyzed	HeLa (Colchicine)	HeLa (Paclitaxel)	HeLa (VX-680)	M202 (Colchicine)	M202 (Paclitaxel)	M202 (VX-680)
Chi-square	607.7	302	381.9	543.5	502	517
df	20	16	9	20	24	15
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
p-value summary	****	****	****	****	****	****
One- or two-tailed	NA	NA	NA	NA	NA	NA
Statistically significant? (alpha<0.05)	Yes	Yes	Yes	Yes	Yes	Yes
Number of rows (Concs.)	6	5	4	6	7	6
Number of columns (Fates)	5	5	4	5	5	4

Table S2 - Chi-square test results for cell fate distribution (Prism 6, Graphpad).