Profiling and targeting of cellular bioenergetics: Inhibition of pancreatic cancer cell proliferation

SUPPLEMENTAL INFORMATION

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Suppl. Table 1. The basal intracellular ATP levels measured in pancreatic cancer cells across all experimental plates. Intracellular ATP levels were measured using a luciferase-based assay. Data are represented as the calculated absolute values of ATP level after normalization to protein content (nmol ATP/mg protein). Data shown are the means ± SD, n represents the number of independent experiments over *ca.* 6 months period.

	n	ATP (nmol ATP/mg protein)
AsPC-1	38	35.6 ± 6.9
MiaPaCa-2	49	35.0 ± 5.7
PANC-1	35	38.4 ± 5.2
HPAF-II	38	27.9 ± 3.8
Capan-1	34	26.2 ± 3.8
Capan-2	43	30.9 ± 5.5

Suppl. Table 2. The effect 2-DG on intracellular ATP levels in MiaPaCa-2. Cells were treated with 2-DG (0.1-30 mM) for indicated periods of time. Intracellular ATP levels were measured using a luciferase-based assay. Data are represented as a percentage of control (non-treated) cells after normalization to total cellular protein for each well. Data shown are the means ± SD, n=4.

2-DG (mM)	*Time of exposure*														
	2 h			**4 h**			**6 h**			**12 h**			**24 h**		
0	100.0	±	3.6	100.0	±	7.3	100.0	±	4.7	100.0	±	2.3	100.0	±	2.1
0.1	97.8	±	1.7	96.2	±	8.1	93.4	±	1.6	86.2	±	2.7	87.7	±	1.8
0.3	89.3	±	2.0	87.4	±	5.3	85.8	±	2.0	77.6	±	2.0	78.4	±	1.9
1	85.1	±	2.0	81.6	±	4.8	81.7	±	2.7	73.7	±	0.7	73.1	±	2.8
3	74.1	±	3.3	70.6	±	1.9	69.2	±	1.8	61.3	±	1.8	53.4	±	4.8
5	66.4	±	1.0	62.6	±	2.0	62.7	±	2.4	58.0	±	3.4	52.6	±	1.6
10	55.5	±	2.1	52.3	±	0.7	53.0	±	2.3	50.7	±	2.3	45.0	±	1.6
30	36.5	±	0.6	34.6	±	1.1	34.9	±	0.8	33.9	±	0.4	28.8	±	0.8

Suppl. Table 3. The effect 2-DG on intracellular ATP levels in Capan-2. Cells were treated with 2-DG (0.1-30 mM) for indicated periods of time. Intracellular ATP levels were measured using a luciferase-based assay. Data are represented as a percentage of control (non-treated) cells after normalization to total cellular protein for each well. Data shown are the means ± SD, n=4.

2-DG (mM)	*Time of exposure*														
	2 h			**4 h**			**6 h**			**12 h**			**24 h**		
0	100.0	±	4.4	100.0	±	2.0	100.0	±	5.6	100.0	±	13.3	100.0	±	6.2
0.1	96.1	±	5.9	98.3	±	4.5	95.3	±	6.8	103.8	±	8.7	90.6	±	13.6
0.3	97.4	±	4.2	95.0	±	5.5	91.5	±	11.1	97.5	±	4.2	95.8	±	3.6
1	95.2	±	1.7	96.7	±	2.7	96.5	±	16.7	94.7	±	2.4	96.1	±	5.0
3	96.7	±	5.2	101.6	±	1.5	113.8	±	2.7	109.9	±	10.0	100.4	±	23.7
5	102.5	±	5.1	90.6	±	2.7	105.1	±	5.6	111.9	±	7.3	109.1	±	4.8
10	104.0	±	3.8	89.6	±	8.8	95.8	±	3.4	108.2	±	2.4	103.6	±	1.7
30	86.9	±	2.7	83.5	±	4.1	85.2	±	3.8	86.6	±	2.2	86.1	±	4.6

Suppl. Table 4. The effect 2-DG on intracellular ATP levels in pancreatic cancer cells. Cells were treated with 2-DG (1-10 mM) for indicated periods of time. Intracellular ATP levels were measured using a luciferase-based assay. Data are represented as a percentage of control (non-treated) cells after normalization to total cellular protein for each well. Data shown are the means ± SD, n=4.

2-DG (mM)		AsPC-1	PANC-1	HPAF-II	Capan-1
3 h	**0**	100.0 ± 4.6	100.0 ± 5.0	100.0 ± 3.2	100.0 ± 6.3
	1	86.6 ± 2.2	88.4 ± 1.7	85.2 ± 1.2	91.9 ± 3.5
	3	80.3 ± 4.1	85.6 ± 1.7	82.0 ± 1.8	84.8 ± 4.2
	10	56.8 ± 3.0	77.2 ± 3.0	67.5 ± 0.7	72.5 ± 3.5
6 h	**0**	100.0 ± 4.6	100.0 ± 2.3	100.0 ± 3.6	100.0 ± 11.9
	1	79.4 ± 4.4	86.1 ± 4.8	91.3 ± 6.1	93.8 ± 6.0
	3	76.5 ± 2.9	83.4 ± 4.4	89.5 ± 4.5	92.1 ± 6.0
	10	58.1 ± 2.8	81.3 ± 2.1	78.1 ± 2.6	77.2 ± 5.5
8 h	**0**	100.0 ± 4.4	100.0 ± 6.6	100.0 ± 4.8	100.0 ± 7.1
	1	79.3 ± 2.5	90.1 ± 2.9	89.6 ± 4.6	89.4 ± 5.5
	3	77.7 ± 4.1	86.7 ± 5.9	89.9 ± 8.1	89.5 ± 4.4
	10	63.1 ± 2.4	83.9 ± 3.2	83.5 ± 3.1	78.2 ± 3.6
12 h	**0**	100.0 ± 3.0	100.0 ± 2.5	100.0 ± 5.9	100.0 ± 7.0
	1	71.5 ± 3.9	80.2 ± 6.0	83.8 ± 4.8	91.8 ± 7.8
	3	64.1 ± 3.4	74.8 ± 3.0	79.8 ± 5.6	89.7 ± 6.2
	10	53.3 ± 3.2	65.0 ± 5.6	76.0 ± 3.2	77.8 ± 3.2
24 h	**0**	100.0 ± 5.0	100.0 ± 3.8	100.0 ± 5.8	100.0 ± 4.7
	1	61.4 ± 2.0	76.0 ± 4.5	91.8 ± 10.2	87.8 ± 14.1
	3	49.4 ± 1.6	70.2 ± 2.7	101.7 ± 5.0	93.6 ± 12.7
	10	44.8 ± 1.9	58.7 ± 2.4	86.0 ± 7.9	85.8 ± 2.7

Supplemental Figure Legends

Suppl. Figure 1. (**A**) Kinetics of signal appearance of NADH, as monitored at 340 nm in enzyme-based glucose assay. Assay vehicle was DMEM with 10% FBS. Right panel shows the representative calibration curve for glucose, constructed from the data collected 60 min (as indicated by the dashed line) after starting the reaction. (**B**) Effect of 2-DG on the detection of glucose under conditions same as in (**A**) but in the presence of different assay media and 2-DG concentrations, as indicated in the attached table.

Suppl. Figure 2. Changes in intracellular ATP levels of pancreatic cancer cells treated with 2-DG (1, 3 and 10 mM). Experimental conditions are similar to those described in Figure 1B. Graphs show a three dimensional representation of both treatment time and concentration effect of 2-DG on intracellular ATP.

Suppl. Figure 3. Relationship between the extent of ATP depletion, inhibition of colony formation and basal glucose consumption rates in pancreatic cancer cells treated with 2-DG. Experimental conditions are similar to those in Figure 3 except that basal PPR values are replaced by glucose consumption rates.

Suppl. Figure 4. Effect of metformin on mitochondrial respiration in pancreatic cancer cells. OCR was monitored in real time with Seahorse analyzer and arrows indicate the time point of metformin injection. 100% corresponds to the OCR value recorded immediately before injection of metformin. The arrows indicate the time point of metformin injection.

Suppl. Figure 5. (*Top*) The effect of 2-DG on hexokinase activity in enzyme-based assay measured in a 96-well plate format. (*Bottom*) The effect of metformin on hexokinase

activity. Hexokinase activity was measured with the glucose detection kit, as described in the method section. Assay vehicle was DMEM without glucose and FBS. O.D. corresponds to optical density (absorbance) at 340 nm due to NADH produced.

Suppl. Figure 6. Synergistic depletion of ATP by 2-DG and metformin in MiaPaCa-2 cells. MiaPaCa-2 cells were treated with 2-DG (0.3-3 mM) or metformin (0.3-30 mM) independently and together for 6 h (A) or 24 h (C) and intracellular ATP levels were determined, normalized to total cellular protein amount and expressed as percentage of untreated cells. A three-dimensional representation showing the concentration dependent effects of 2-DG or metformin alone and together on intracellular ATP levels in MiaPaCa-2 cells. The combination index-fraction affected (CI-Fa) plots are shown (B,D). Fraction affected parameter is used as a measure of the drug(s) efficiency, with a value of zero indicating the lack of effect on intracellular ATP and the value of 1 indicating total depletion of intracellular ATP.

Suppl. Figure 7. Effects of 2-DG on cell proliferation in MiaPaCa-2 and Capan-2 cells. Cell proliferation was monitored in real-time with the continuous presence of indicated treatments until the end of each experiment. Changes in cell confluence are used as a surrogate marker of cell proliferation. Data shown are the mean ± SD. n=6.

Suppl. Figure 8. A label-free confluence assay by IncuCyte™ Live-Cell Imaging Systems. MiaPaCa-2 cells were seeded at 1,000 cells/well overnight in a 96-well plate. The cells were photographed and confluence calculated every 2 h by IncuCyte 2011A software. The IncuCyte Analyzer provides real-time updates on cell confluence, based on segmentation of high definition-phase contrast images. (***Left***) Representative original high definition-phase contrast images derived from IncuCyte and calculated cell

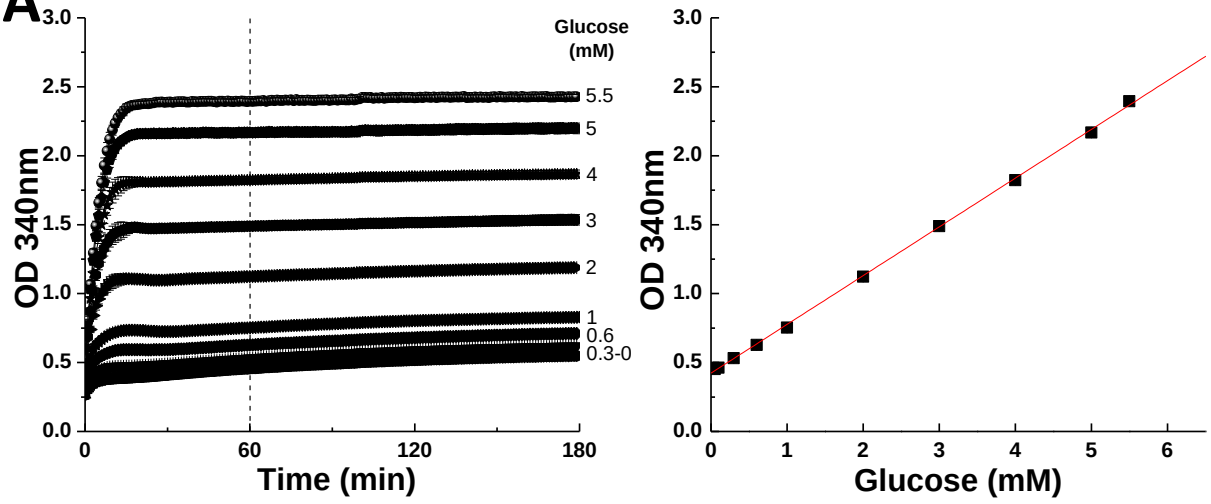
confluence values (*inserts*). (***Right***) Same images processed by Image J 1.47v software in order to show the segmentation of HD-phase contrast images (*segmentation mask illustrated in black*).

Suppl. Figure 9. Inhibition of cell proliferation by 2-DG combined with gemcitabine or doxorubicin. Effects of 2-DG and gemcitabine (**A**) or doxorubicin (**B**) alone and together, on cell proliferation. MiaPaCa-2 cells were treated with 2-DG (0.5 mM) or gemcitabine (Gem, 10 nM) or doxorubicin (Dox, 10 nM) alone and together. Cell proliferation was monitored in real-time with the continuous presence of indicated treatments until the end of each experiment. The changes in cell confluence are used as a surrogate marker of cell proliferation. Data shown are the mean ± SD (n=4).

Suppl. Figure 10. Synergistic depletion of ATP by 2-DG and celecoxib in Capan-2 cells. (A) The relationship between the glycolytic rates (as indicated by PPR) and COX-2 protein expression (taken from Deer EL et al., *Pancreas* 39: 425-435, 2010). (B) A three-dimensional representation showing the concentration dependent effects of 2-DG or celecoxib alone and together on cellular ATP levels in Capan-2 cells. Capan-2 cells were treated with 2-DG (0.3-10 mM) or celecoxib (30-100 μM) independently and together for 24 h (B) and total cellular ATP levels were determined and expressed as percentage of untreated cells. (C) The combination index-fraction affected (CI-Fa) plots is shown. Fraction affected parameter is used as a measure of the drug(s) efficiency, with a value of zero indicating the lack of effect on intracellular ATP and the value of 1 indicating complete depletion of intracellular ATP.

Suppl. Figure 1

A



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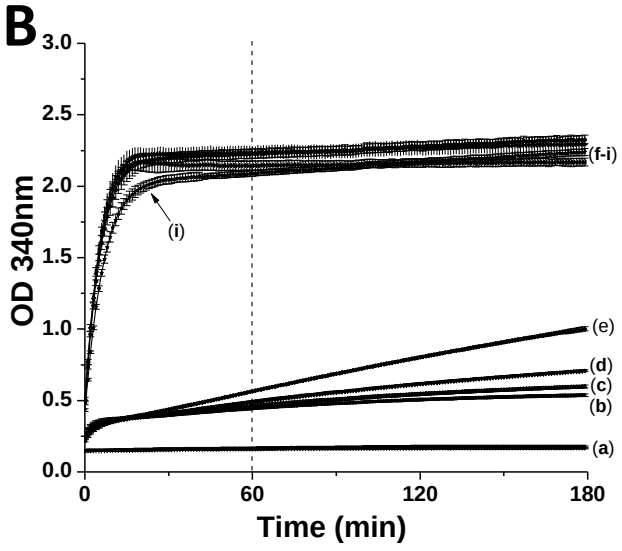
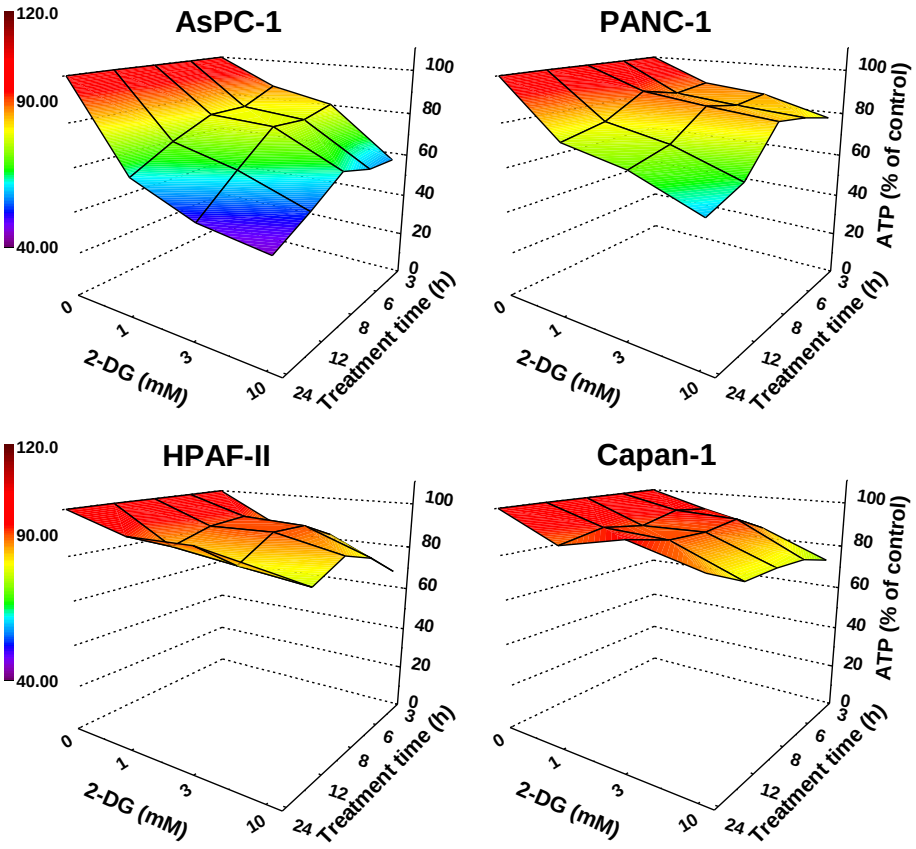


	FBS	Glucose	2-DG (mM)
(a)	–	–	–
(b)	+	–	–
(c)	+	–	1
(d)	+	–	3
(e)	+	–	10
(f)	+	+	–
(g)	+	+	1
(h)	+	+	3
(i)	+	+	10

Suppl. Figure 2



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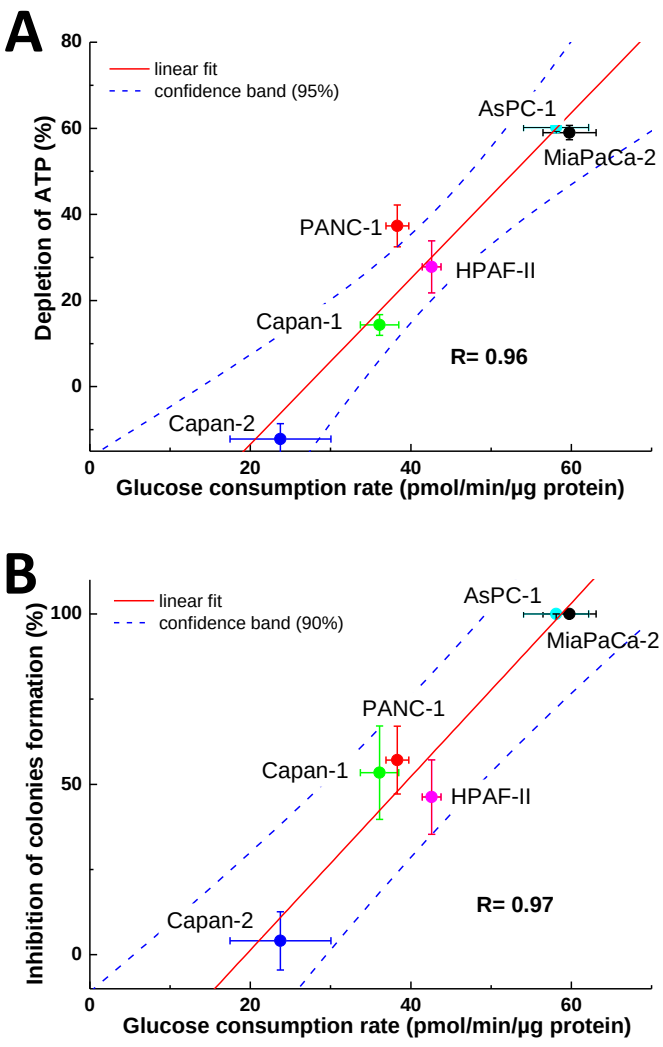

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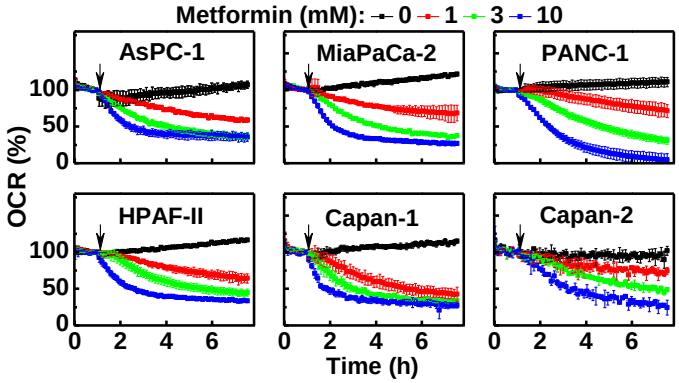

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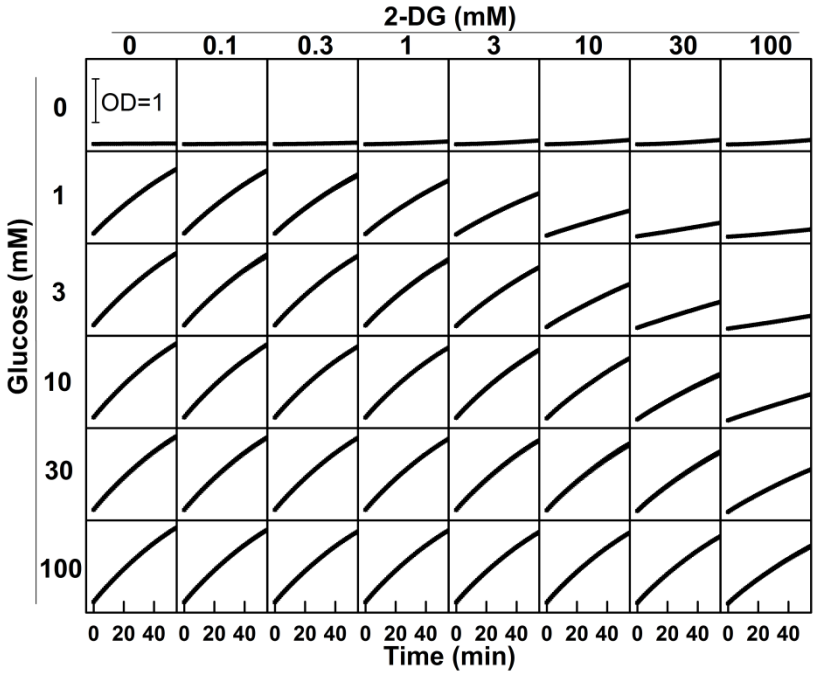
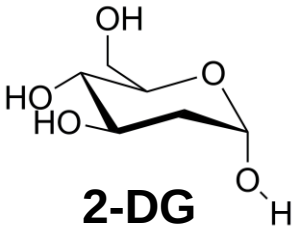
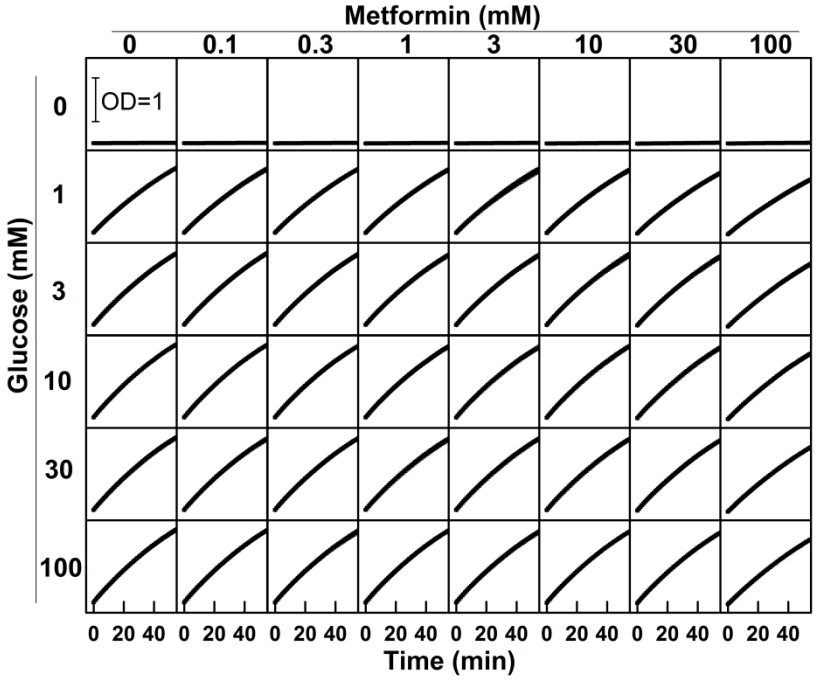
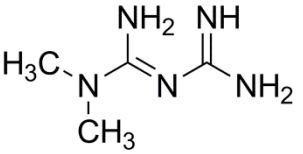




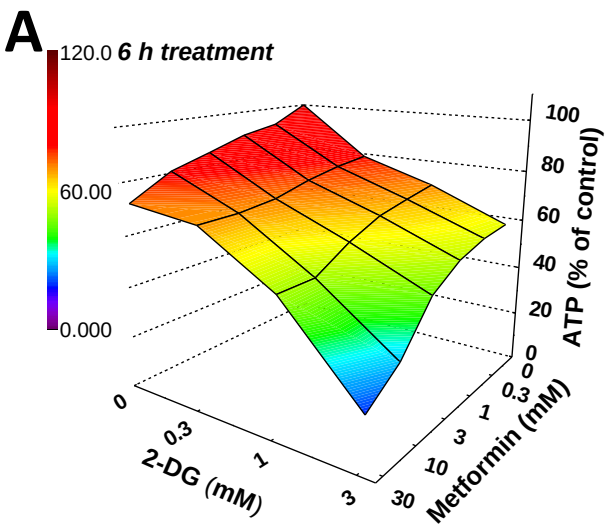
2-DG

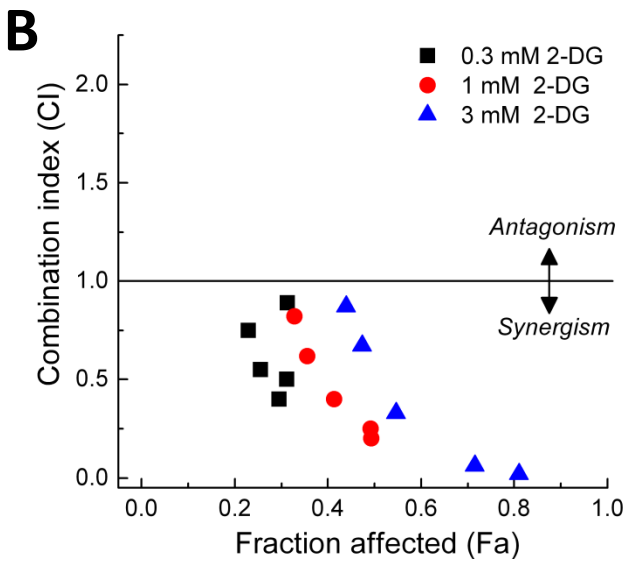


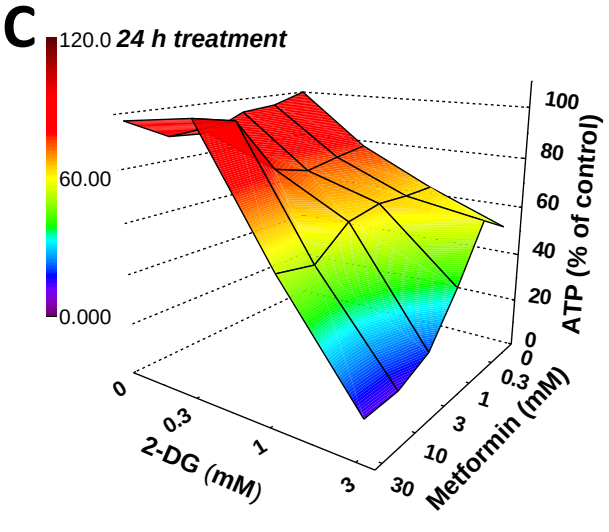


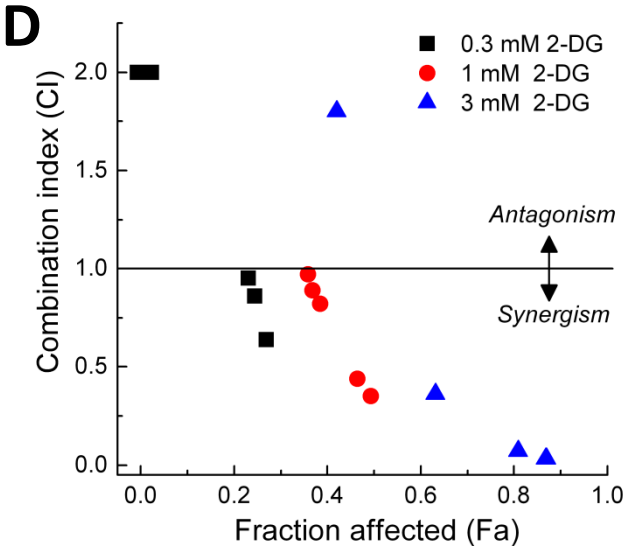
Metformin

Suppl. Figure 6









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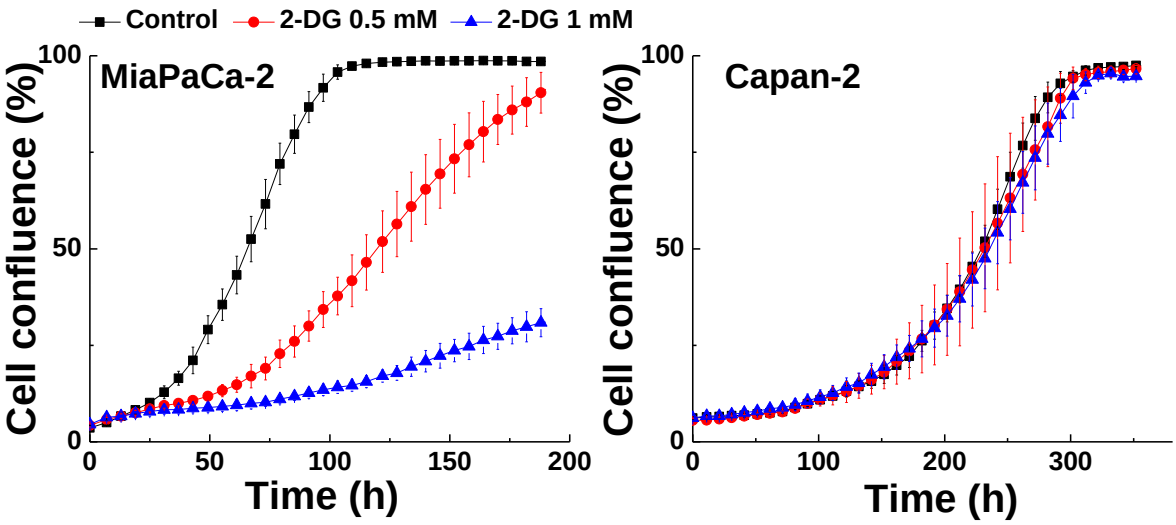

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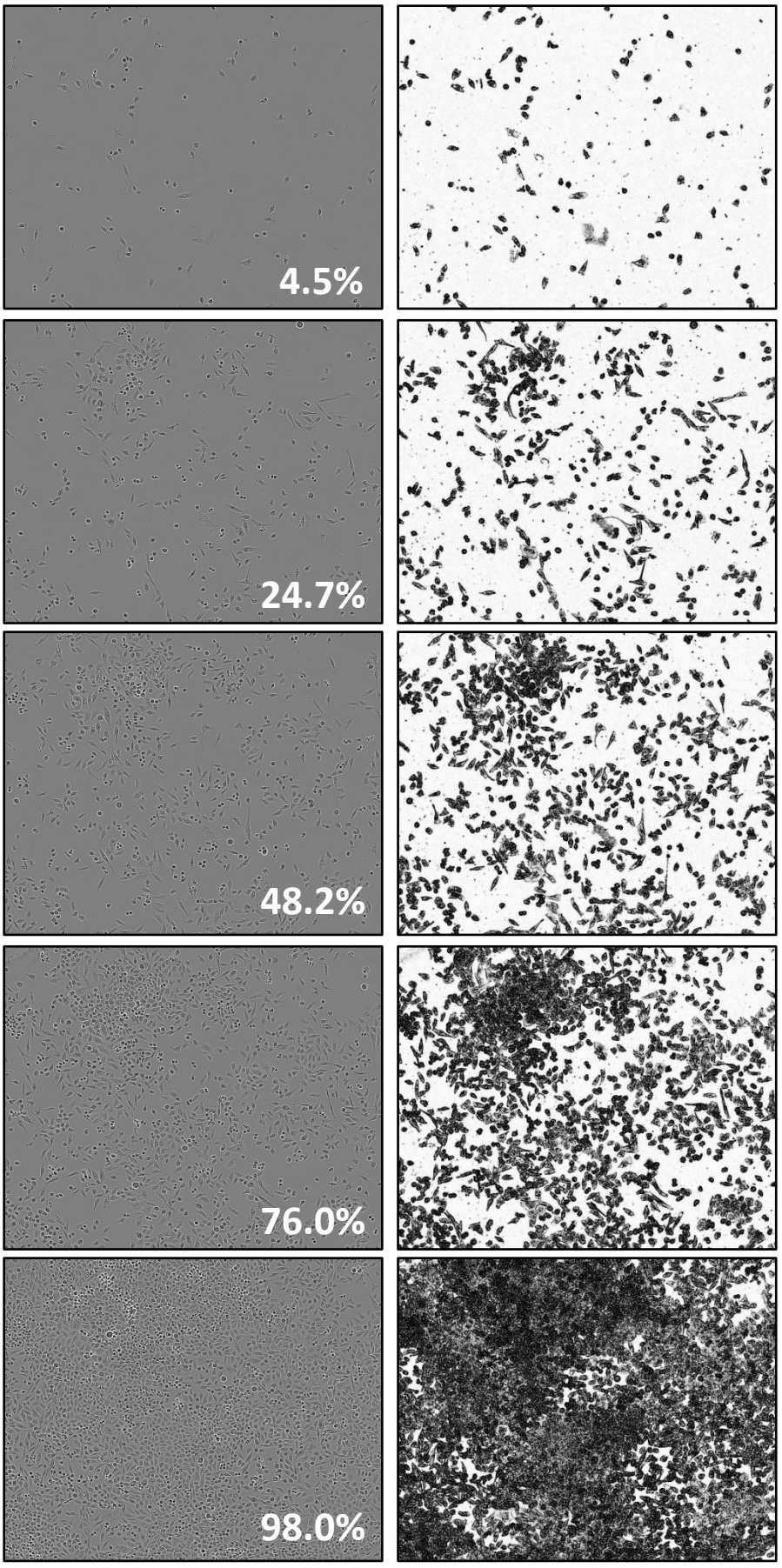

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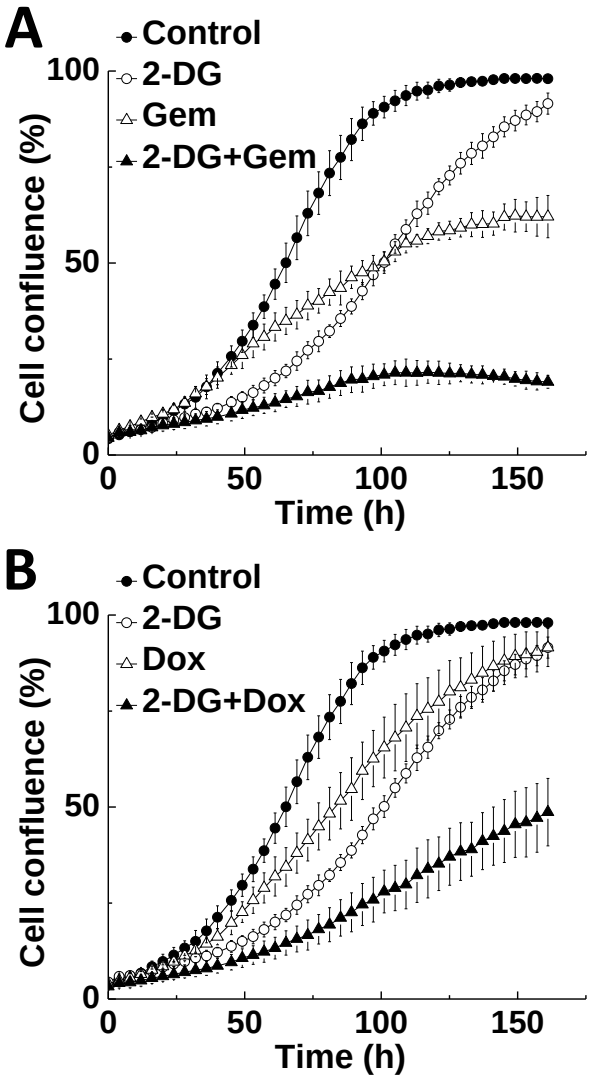

Suppl. Figure 10