

Supplementary Materials

Hyperthermia Enhances Efficacy of Chemotherapeutic Agents in Pancreatic Cancer Cell Lines

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Supplementary Tables

Supplementary Table S1. Serial dilution of chemotherapeutics applied in pancreatic cancer cell lines.

| | | | | | | | | | | |
|----------------|--------|---------|---------|--------|---------|---------|----------|----------|-----------|---------|
| 5-Fluorouracil | 200 µM | 66.6 µM | 22.2 µM | 7.4 µM | 2.47 µM | 0.82 µM | 0.27 µM | 0.091 µM | 0.030 µM | Vehicle |
| Gemcitabine | 200 µM | 50 µM | 12.5 µM | 3.1 µM | 0.78 µM | 0.19 µM | 0.049 µM | 0.012 µM | 0.0030 µM | Vehicle |
| Cisplatin | 200 µM | 66.6 µM | 22.2 µM | 7.4 µM | 2.47 µM | 0.82 µM | 0.27 µM | 0.091 µM | 0.030 µM | Vehicle |

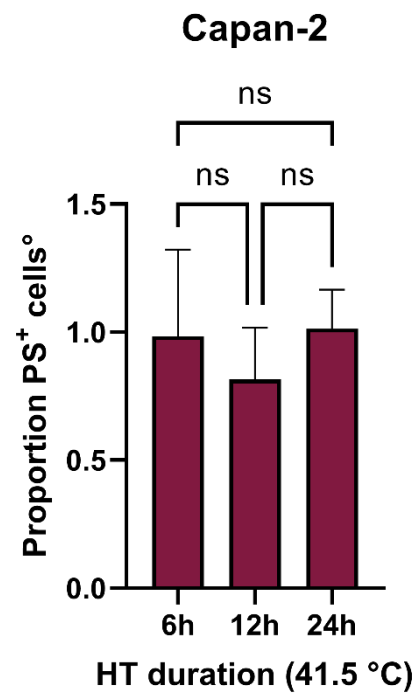
Supplementary Table S2. IC_{50,drug}, IT_{50,HT} and combination doses between hyperthermia and 5-fluorouracil, gemcitabine, and cisplatin. (6-, 12-, and 24 hours).

| | | | BxPC-3 | | | PANC-1 | | | Capan-1 | | | Capan-2 | | | MIA-PaCa-2 | | |
|----------------|------|------------------|-----------------------------|--------------------------------------|-------------------------------------|-----------------------------|--------------------------------------|-------------------------------------|-----------------------------|--------------------------------------|-------------------------------------|-----------------------------|--------------------------------------|-------------------------------------|-----------------------------|--------------------------------------|-------------------------------------|
| Drug | Time | T_{HT} (°C) | IT _{50,HT} (°C) | IC _{50,drug} (μM) | C _{50,drug} (μM) | IT _{50,HT} (°C) | IC _{50,drug} (μM) | C _{50,drug} (μM) | IT _{50,HT} (°C) | IC _{50,drug} (μM) | C _{50,drug} (μM) | IT _{50,HT} (°C) | IC _{50,drug} (μM) | C _{50,drug} (μM) | IT _{50,HT} (°C) | IC _{50,drug} (μM) | C _{50,drug} (μM) |
| 5-Fluorouracil | 6 h | 40.5 | 44.6 | 4.14 | 4.24 | 48 | 5.32 | 4.09 | 42.6 | 0.31 | 0.25 | 46.1 | 57.76 | 78.22 | 44.6 | 6.70 | 7.50 |
| | | 41 | | | 4.38 | | | 5.91 | | | 0.32 | | | 96.02 | | | 7.31 |
| | | 41.5 | | | 5.04 | | | 25.99 | | | 0.14 | | | 47.49 | | | 7.16 |
| | 12 h | 40.5 | 43.5 | 4.38 | 1.25 | 46.4 | 7.69 | 6.39 | 41.2 | 0.35 | 0.13 | 45.1 | 60.83 | 79.21 | 43.4 | 7.06 | 5.26 |
| | | 41 | | | 0.90 | | | 7.23 | | | 0.05 | | | 74.52 | | | 5.04 |
| | | 41.5 | | | 1.03 | | | 2.98 | | | 0.0004 | | | 39.44 | | | 3.17 |
| | 24 h | 40.5 | 41 | 5.04 | 0.90 | 41.5 | 9.11 | 1.63 | 41 | 0.26 | 0.018 | 41.9 | 41.36 | 13.57 | 41.45 | 5.9 | 4.70 |
| | | 41 | | | 0.04 | | | 0.12 | | | 0.0036 | | | 6.03 | | | 2.37 |
| | | 41.5 | | | 0.00015 | | | 0.13 | | | 0.0002 | | | 1.18 | | | 0.03 |
| Gemcitabine | 6 h | 40.5 | 44.6 | 0.21 | 0.30 | 48 | 76.84 | 150.70 | 42.6 | 0.14 | 0.12 | 46.1 | 2.32 | 2.75 | 44.6 | 0.15 | 0.16 |
| | | 41 | | | 0.29 | | | 56.14 | | | 0.11 | | | 2.27 | | | 0.14 |
| | | 41.5 | | | 0.27 | | | - | | | 0.06 | | | 2.07 | | | 0.17 |
| | 12 h | 40.5 | 43.5 | 0.08 | 0.06 | 46.4 | 25.02 | - | 41.2 | 0.07 | 0.02 | 45.1 | 3.06 | 5.40 | 43.4 | 0.13 | 0.10 |
| | | 41 | | | 0.024 | | | 17.57 | | | 0.009 | | | 5.44 | | | 0.09 |
| | | 41.5 | | | 0.022 | | | 5.57 | | | 0.00005 | | | 3.93 | | | 0.08 |
| | 24 h | 40.5 | 41 | 0.06 | 0.01 | 41.5 | 19.34 | 0.20 | 41 | 0.06 | 0.0055 | 41.9 | 0.40 | 0.28 | 41.45 | 0.12 | 0.10 |
| | | 41 | | | 0.0016 | | | 0.05 | | | 0.0013 | | | 0.08 | | | 0.06 |
| | | 41.5 | | | 0.00006 | | | - | | | 0.0002 | | | 0.015 | | | 0.0008 |
| Cisplatin | 6 h | 40.5 | 44.6 | 2.39 | 1.77 | 48 | 5.14 | 7.31 | 42.6 | 0.32 | 0.33 | 46.1 | 13.55 | 10.52 | 44.6 | 6.30 | 6.43 |
| | | 41 | | | 1.75 | | | 7.63 | | | 0.34 | | | 9.76 | | | 6.40 |
| | | 41.5 | | | 1.69 | | | 5.32 | | | 0.18 | | | 11.58 | | | 5.54 |
| | 12 h | 40.5 | 43.5 | 1.56 | 0.67 | 46.4 | 5.27 | 4.07 | 41.2 | 0.28 | 0.21 | 45.1 | 0.28 | 0.21 | 43.4 | 4.67 | 4.35 |
| | | 41 | | | 0.57 | | | 3.25 | | | 0.07 | | | 0.07 | | | 3.56 |
| | | 41.5 | | | 0.36 | | | 2.18 | | | 0.0018 | | | 0.0019 | | | 2.17 |
| | 24 h | 40.5 | 41 | 1.40 | 0.12 | 41.5 | 12.11 | 1.03 | 41 | 0.27 | 0.017 | 41.9 | 0.27 | 0.017 | 41.45 | 5.59 | 2.72 |
| | | 41 | | | 0.0016 | | | 0.06 | | | 0.005 | | | 0.0053 | | | 0.33 |
| | | 41.5 | | | 0.00012 | | | 0.014 | | | 0.0007 | | | 0.0007 | | | 0.0007 |

Supplementary Table S3. IC_{50, ratio, drug} and combination index (CI) values of chemotherapy combined with hyperthermia treatment. CI) lower than 0.9 indicates synergism, between 0.9 and 1.1, additivity and higher than 1.1 indicates antagonism.

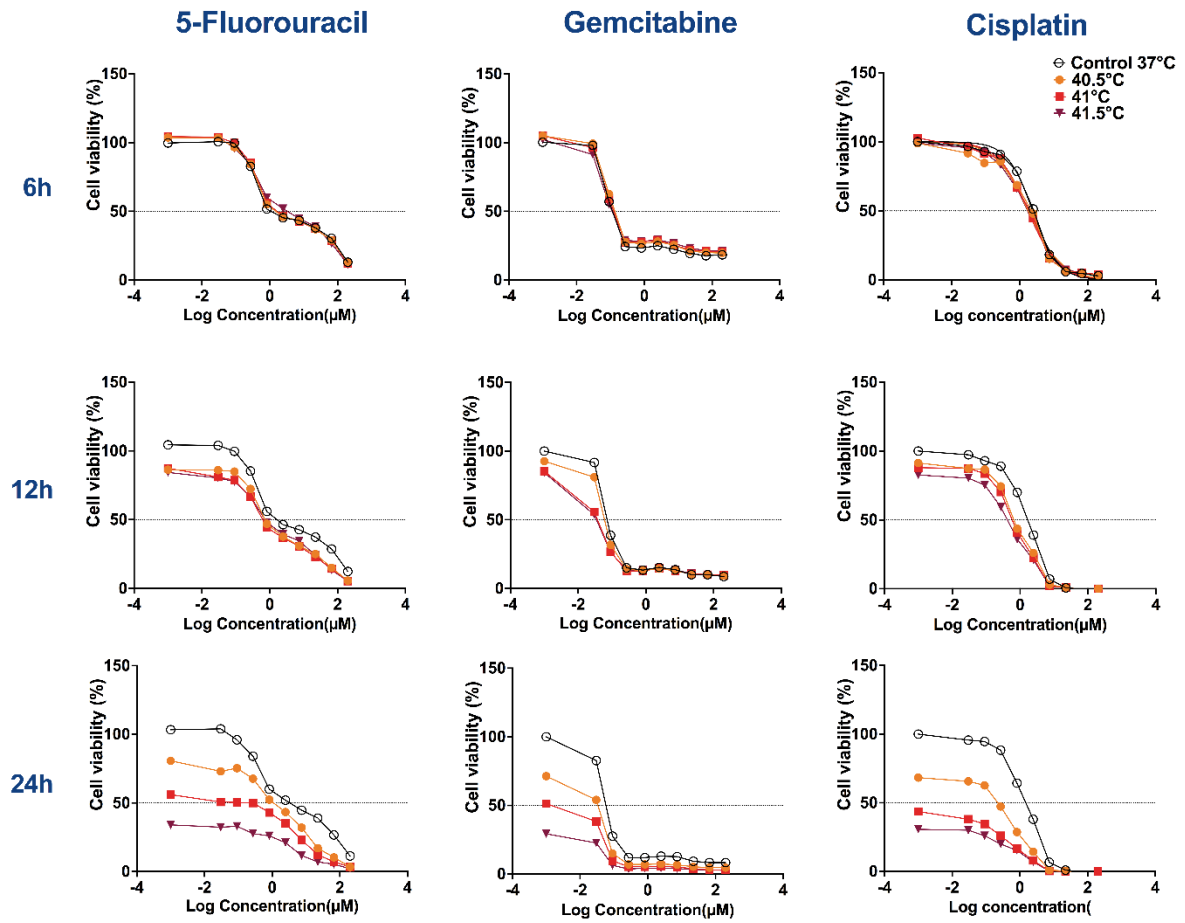
| | | BxPC-3 | | | PANC-1 | | | Capan-1 | | | Capan-2 | | | MIA-PaCa-2 | | |
|----------------|---------------|-----------------------------|------|-----------|-----------------------------|------|-----------|-----------------------------|------|-----------|-----------------------------|------|-----------|-----------------------------|------|-----------|
| Drug | Thermal dose | IC50 _{ratio, drug} | CI | Type | IC50 _{ratio, drug} | CI | Type | IC50 _{ratio, drug} | CI | Type | IC50 _{ratio, drug} | CI | Type | IC50 _{ratio, drug} | CI | Type |
| 5-Fluorouracil | 6h at 40.5°C | 1,02 | 1,49 | Antagonic | 0,77 | 1,09 | Additive | 0,80 | 1,43 | Antagonic | 1,35 | 1,74 | Antagonic | 1,12 | 1,58 | Antagonic |
| | 6h at 41°C | 1,06 | 1,58 | Antagonic | 1,11 | 1,47 | Antagonic | 1,05 | 1,76 | Antagonic | 1,66 | 2,1 | Antagonic | 1,09 | 1,62 | Antagonic |
| | 6h at 41.5°C | 1,22 | 1,81 | Antagonic | 4,88 | 5,29 | Antagonic | 0,44 | 1,25 | Antagonic | 0,82 | 1,32 | Antagonic | 1,07 | 1,66 | Antagonic |
| | 12h at 40.5°C | 0,29 | 0,83 | Synergic | 0,83 | 1,2 | Antagonic | 0,37 | 1,21 | Antagonic | 1,30 | 1,73 | Antagonic | 0,75 | 1,29 | Antagonic |
| | 12h at 41°C | 0,20 | 0,82 | Synergic | 0,94 | 1,37 | Antagonic | 0,15 | 1,1 | Additive | 1,23 | 1,72 | Antagonic | 0,71 | 1,34 | Antagonic |
| | 12h at 41.5°C | 0,24 | 0,93 | Additive | 0,39 | 0,87 | Synergic | 0,001 | 1,07 | Additive | 0,65 | 1,2 | Antagonic | 0,45 | 1,15 | Antagonic |
| | 24h at 40.5°C | 0,18 | 1,05 | Additive | 0,18 | 0,96 | Additive | 0,07 | 0,94 | Additive | 0,33 | 1,04 | Additive | 0,80 | 1,58 | Antagonic |
| | 24h at 41°C | 0,008 | 1,01 | Additive | 0,01 | 0,9 | Additive | 0,01 | 1,01 | Additive | 0,15 | 0,96 | Additive | 0,40 | 1,3 | Antagonic |
| | 24h at 41.5°C | 2,95E-05 | 1,13 | Antagonic | 0,01 | 1,01 | Additive | 0,0007 | 1,13 | Antagonic | 0,03 | 0,95 | Additive | 0,006 | 1,02 | Additive |
| Gemcitabine | 6h at 40.5°C | 1,44 | 1,9 | Antagonic | 1,96 | 2,28 | Antagonic | 0,88 | 1,5 | Antagonic | 1,18 | 1,57 | Antagonic | 1,07 | 1,53 | Antagonic |
| | 6h at 41°C | 1,40 | 1,93 | Antagonic | 0,73 | 1,09 | Additive | 0,80 | 1,52 | Antagonic | 0,98 | 1,42 | Antagonic | 0,95 | 1,47 | Antagonic |
| | 6h at 41.5°C | 1,30 | 1,88 | Antagonic | - | - | - | 0,42 | 1,22 | Antagonic | 0,89 | 1,39 | Antagonic | 1,15 | 1,74 | Antagonic |
| | 12h at 40.5°C | 0,77 | 1,31 | Antagonic | - | - | - | 0,30 | 1,13 | Antagonic | 1,76 | 2,2 | Antagonic | 0,81 | 1,36 | Antagonic |
| | 12h at 41°C | 0,30 | 0,91 | Additive | 0,70 | 1,13 | Antagonic | 0,13 | 1,09 | Additive | 1,78 | 2,27 | Antagonic | 0,74 | 1,37 | Antagonic |
| | 12h at 41.5°C | 0,27 | 0,96 | Additive | 0,22 | 0,7 | Synergic | 0,0008 | 1,07 | Additive | 1,28 | 1,84 | Antagonic | 0,60 | 1,31 | Antagonic |
| | 24h at 40.5°C | 0,17 | 1,05 | Additive | 0,01 | 0,79 | Synergic | 0,085 | 0,96 | Additive | 0,69 | 1,4 | Antagonic | 0,82 | 1,6 | Antagonic |
| | 24h at 41°C | 0,026 | 1,03 | Additive | 0,002 | 0,89 | Synergic | 0,02 | 1,02 | Additive | 0,19 | 1,01 | Additive | 0,52 | 1,41 | Antagonic |
| | 24h at 41.5°C | 0,001 | 1,13 | Antagonic | - | - | - | 0,003 | 1,13 | Additive | 0,04 | 0,95 | Additive | 0,007 | 1,02 | Additive |
| Cisplatin | 6h at 40.5°C | 0,74 | 1,2 | Antagonic | 1,4 | 1,74 | Antagonic | 1,00 | 1,63 | Antagonic | 0,78 | 1,16 | Antagonic | 1,02 | 1,48 | Antagonic |
| | 6h at 41°C | 0,73 | 1,26 | Antagonic | 1,48 | 1,85 | Antagonic | 1,04 | 1,76 | Antagonic | 0,72 | 1,16 | Antagonic | 1,01 | 1,54 | Antagonic |
| | 6h at 41.5°C | 0,70 | 1,3 | Antagonic | 1,04 | 1,44 | Antagonic | 0,56 | 1,37 | Antagonic | 0,85 | 1,35 | Antagonic | 0,88 | 1,47 | Antagonic |
| | 12h at 40.5°C | 0,43 | 0,97 | Additive | 0,77 | 1,14 | Antagonic | 0,75 | 1,58 | Antagonic | 0,75 | 1,18 | Antagonic | 0,93 | 1,48 | Antagonic |
| | 12h at 41°C | 0,36 | 0,98 | Additive | 0,62 | 1,04 | Additive | 0,26 | 1,21 | Antagonic | 0,26 | 0,76 | Synergic | 0,76 | 1,39 | Antagonic |
| | 12h at 41.5°C | 0,23 | 0,92 | Additive | 0,41 | 0,89 | Synergic | 0,007 | 1,08 | Additive | 0,007 | 0,56 | Synergic | 0,47 | 1,17 | Antagonic |
| | 24h at 40.5°C | 0,08 | 0,96 | Additive | 0,08 | 0,86 | Synergic | 0,06 | 0,94 | Additive | 0,06 | 0,78 | Synergic | 0,49 | 1,27 | Antagonic |
| | 24h at 41°C | 0,001 | 1 | Additive | 0,005 | 0,89 | Synergic | 0,02 | 1,02 | Additive | 0,02 | 0,84 | Synergic | 0,06 | 0,96 | Additive |
| | 24h at 41.5°C | 8,50E-05 | 1,13 | Antagonic | 0,001 | 1 | Additive | 0,003 | 1,13 | Antagonic | 0,003 | 0,92 | Additive | 0,0001 | 1,01 | Additive |

Supplementary Figures



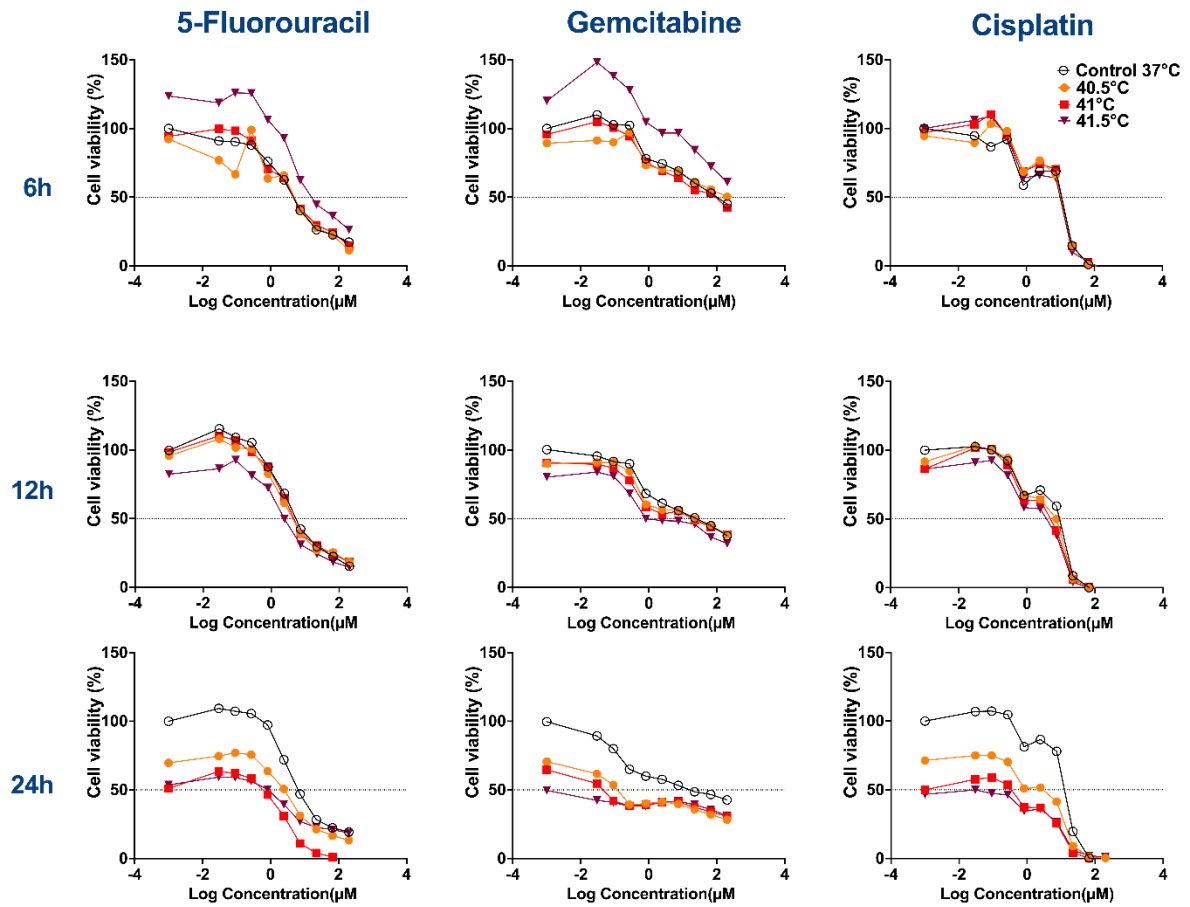
Supplementary Figure S1: Expression of phosphatidylserine (PS) on the outer membrane of Capan-2. No significant differences in PS positivity were observed.

BxPC-3



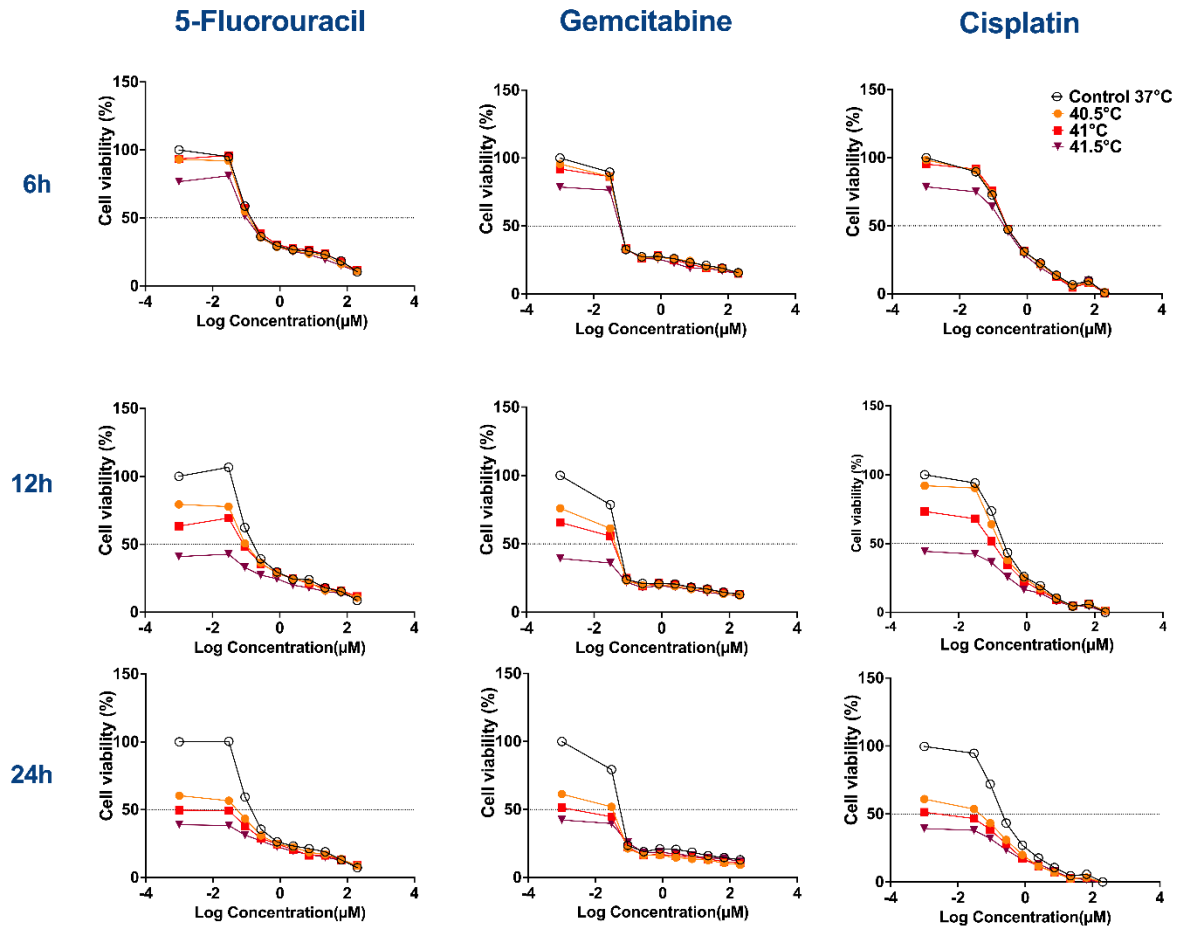
Supplementary Figure S2: Dose curve response of BxPC-3 to 5-fluorouracil, gemcitabine and cisplatin. These data represent triplicates.

PANC-1



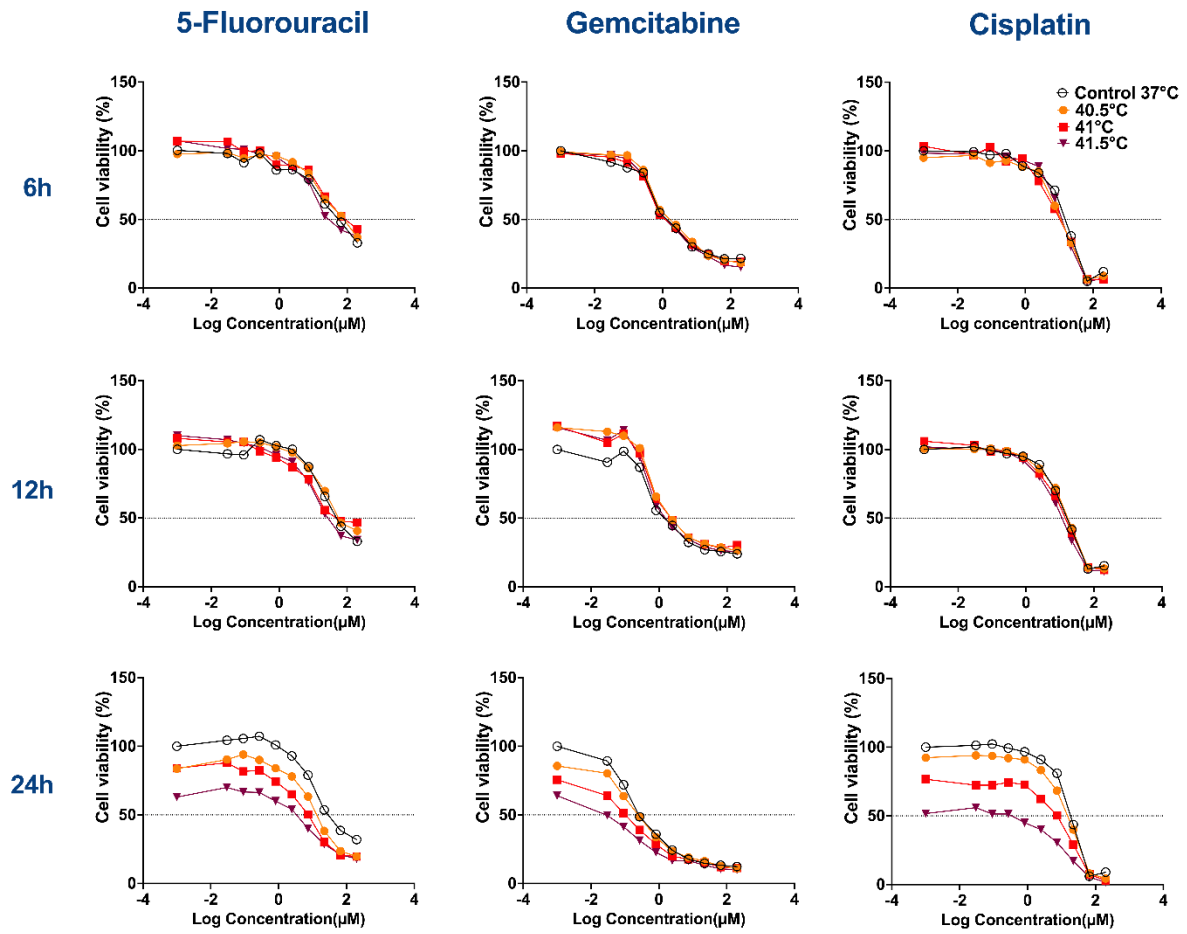
Supplementary Figure S3: Dose curve response of PANC-1 to 5-fluorouracil, gemcitabine and cisplatin. These data represent triplicates.

Capan-1



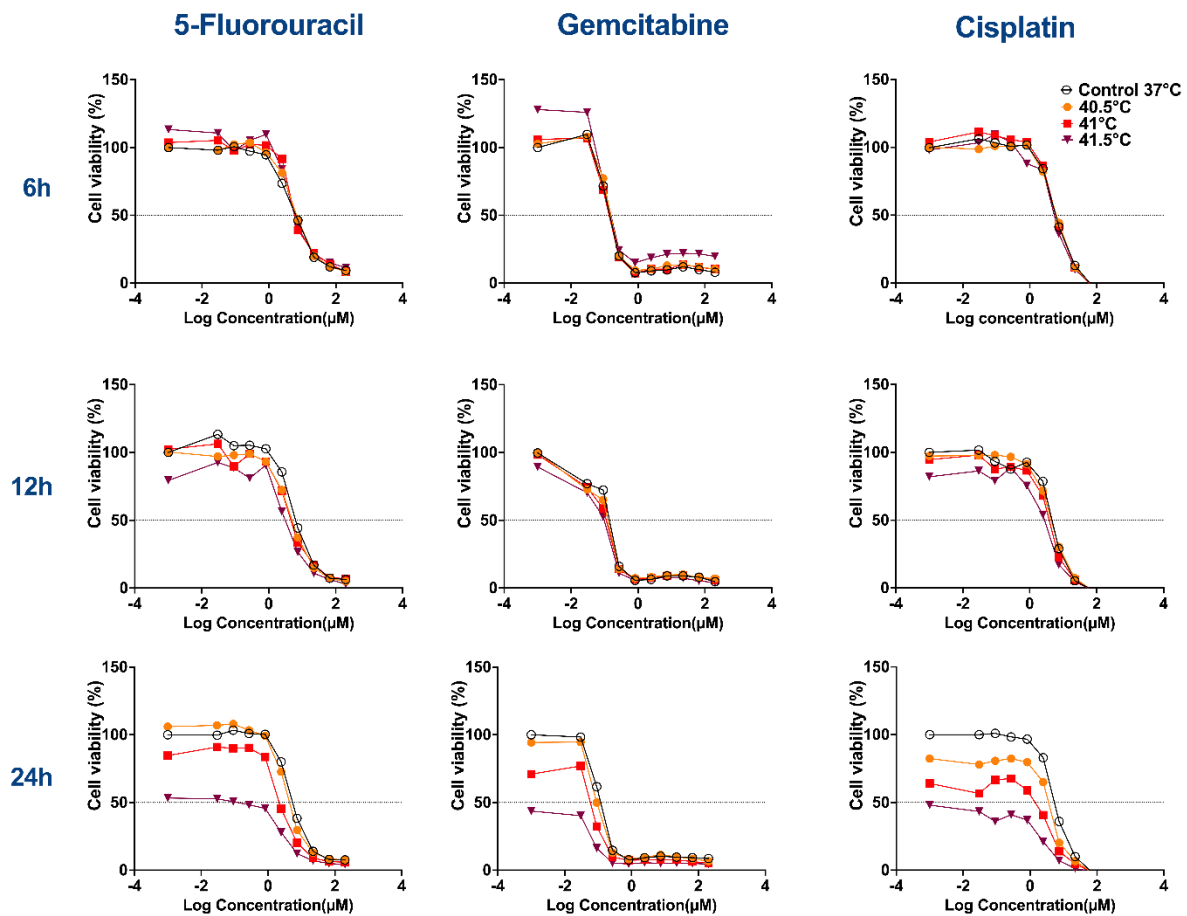
Supplementary Figure S4: Dose curve response of Capan-1 to 5-fluorouracil, gemcitabine and cisplatin. These data represent triplicates.

Capan-2



Supplementary Figure S5: Dose curve response of Capan-2 to 5-fluorouracil, gemcitabine and cisplatin. These data represent triplicates.

MIA PaCa-2



Supplementary Figure S6: Dose curve response of MIA PaCa-2 to 5-fluorouracil, gemcitabine and cisplatin. These data represent triplicates.