Supporting Information

Single Cell Chemical Proteomics (SCCP) Interrogates the Timing and Heterogeneity of Cancer Cell Commitment to Death

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Supplementary figures

Figure S1. A) Summary table with the number of peptides and proteins identified in the raw data before filtering and after applying the data analysis workflow. B) Schematic representation of the bioinformatic workflow applied to the data acquired with single cells. C) As an example, PCA of eight TMT-sets are depicted before and after the batch correction.

|          | Before filtering | After filtering |
|----------|------------------|-----------------|
|          | Peptide          | Protein         | Peptide          | Protein         |
| MTX_3h   | 17757            | 2325            | 12910            | 1931            |
| MTX_6h   | 13731            | 1988            | 9767             | 1625            |
| MTX_12h  | 20415            | 2481            | 19405            | 1585            |
| MTX_24h  | 17874            | 2240            | 17074            | 1627            |
| MTX_48h  | 19664            | 2397            | 11692            | 1738            |
| CPT_12h  | 18055            | 2188            | 9945             | 1487            |
| CPT_24h  | 16099            | 1958            | 10802            | 1570            |
| CPT_48h  | 17710            | 2244            | 10940            | 1662            |
| TDX_12h  | 17744            | 2169            | 12808            | 1786            |
| TDX_24h  | 15790            | 2037            | 15212            | 1746            |
| TDX_48h  | 16464            | 2132            | 12076            | 1833            |

A

|          | Before filtering | After filtering |
|----------|------------------|-----------------|
|          | Peptide          | Protein         | Peptide          | Protein         |
| MTX_3h   | 17757            | 2325            | 12910            | 1931            |
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| TDX_24h  | 15790            | 2037            | 15212            | 1746            |
| TDX_48h  | 16464            | 2132            | 12076            | 1833            |

B

Raw files → Pd → CSV → Peptide level (unique peptides) → Filter RIA (>10%) → Normalization (median) → Log2 transformation → Peptide → Proteins (median intensity) → Protein normalization (median) → Imputation (Normal distribution, kNN) → Batch correction
Before batch correction

After batch correction
Figure S2. Cell viability diagrams for three drugs (methotrexate, MTX; camptothecin, CPT; tomudex, TDX) used with A549 cells.
**Figure S3.** FACS diagrams with gating conditions for isolating individual DMSO-treated (A) and MTX-treated (B) A549 cells after 48 h.

**Figure S4.** PCA plots of the proteomics results on MTX treated single cell presented in Figure 2, coloring the cells after the batches.
**Figure S5.** Same as in Figure S4, coloring the cells after the TMT channels.
Figure S6. Clustering of the proteomes of attached treated single cells provides two groups of cells, G1 and G2.
Figure S7. Same as in Figure S4, coloring the treated cells after the G1 and G2 subpopulations (depicted in red and blue colors, respectively; while untreated cells are shown as empty circles).
**Figure S8.** Volcano plots of data presented in Figures 2 and 4, with proteins re-colored according to their relative abundance in the sample (low to high proteins abundance = red-yellow-blue).
Figure S9. Proteins ranked after their abundances in data sets shown in Figures 2 and 4. The significantly regulated proteins are depicted in red color.
Figure S10. A) PCA plot of all single cell proteomics data acquired with MTX-treated and control cells. The cells were color-coded according to their treatment time and treatment status (see legend). B) The histogram of cells’ first principal component (t[1]) for different treatment times.
**Figure S11.** Pathway analysis of 179 proteins with significantly different abundances in G1 versus G2 subpopulations at 12 h past MTX treatment revealed that they preferentially belong to metabolic pathways and carbon metabolism (enriched in G2), as well as ribosome- and proteasome-related pathways (enriched in G1).