Supporting Information

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

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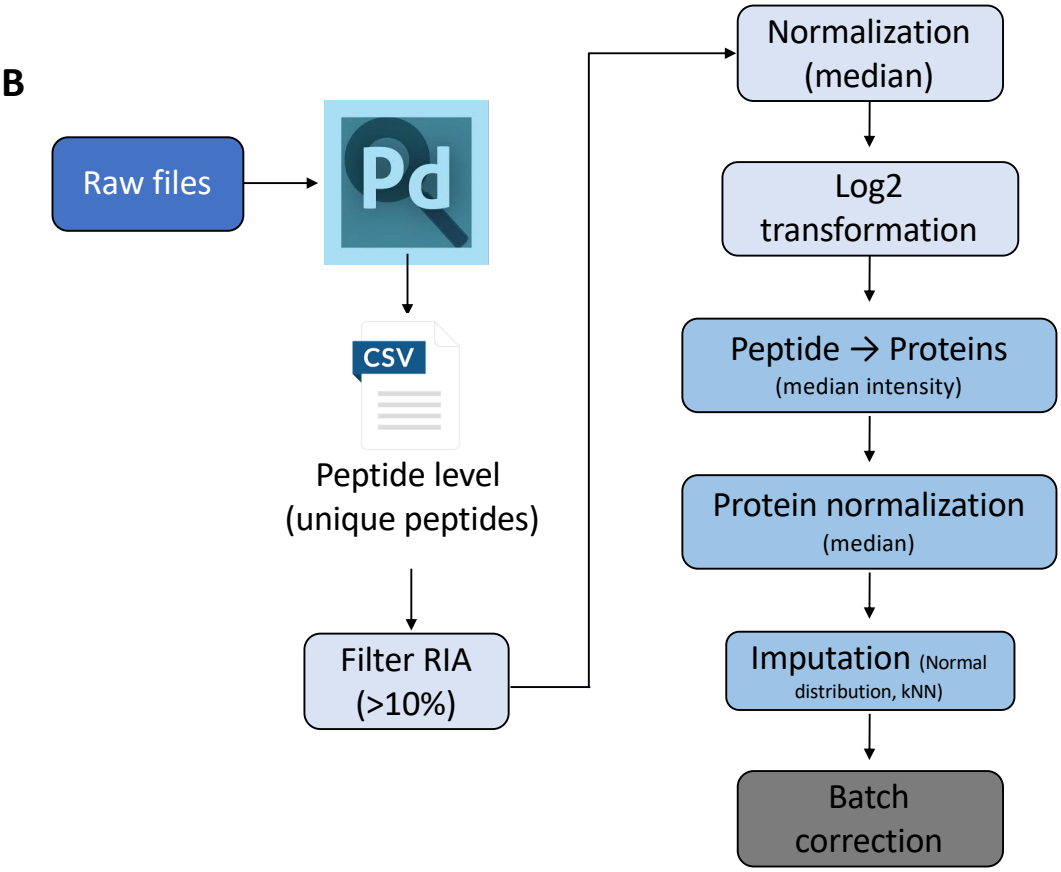
Supplementary figures

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.

A

	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

B



C

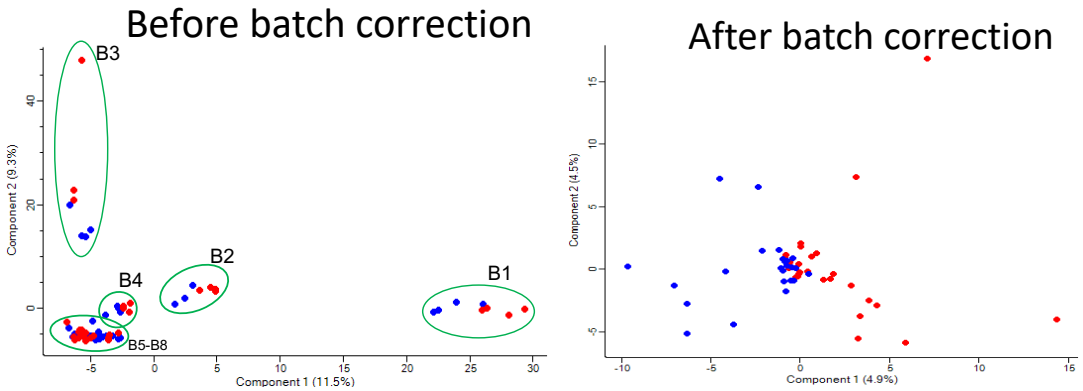
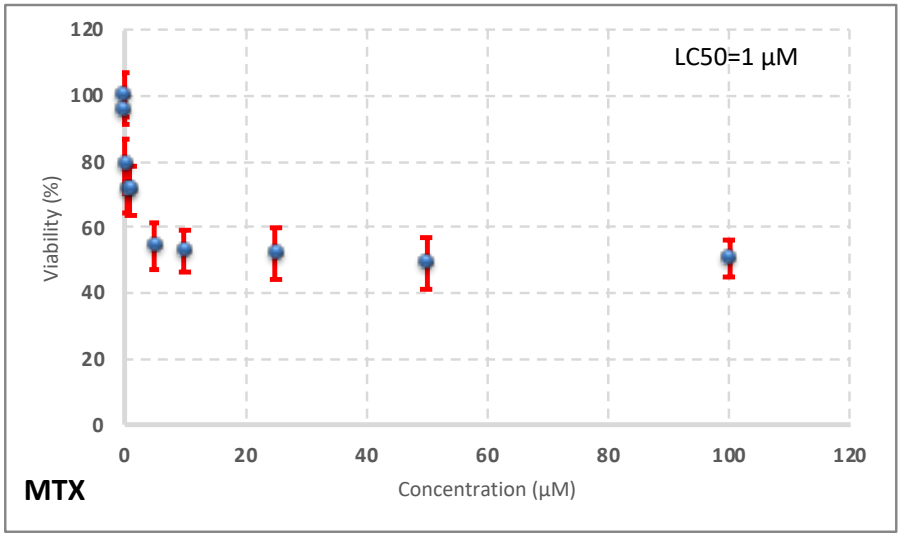
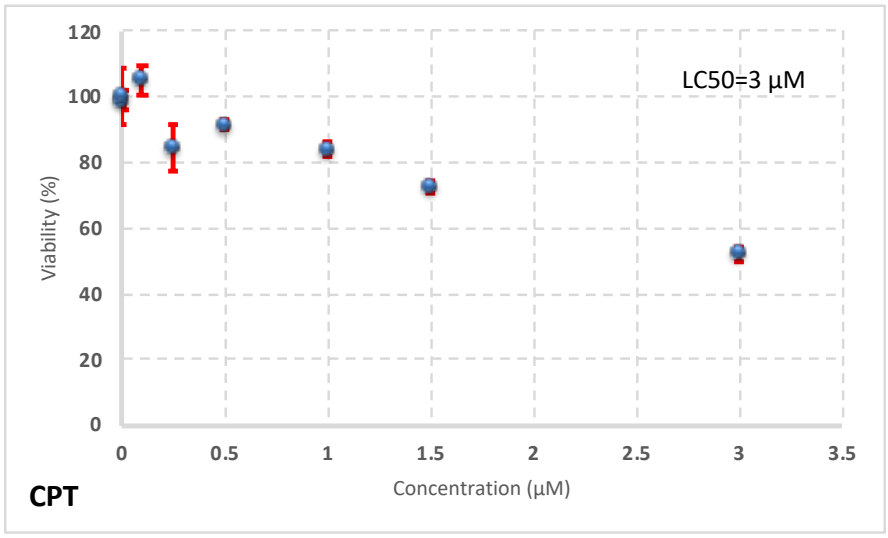

Before batch correction
After batch correction
B3
B2
B4
B1
B5-B8
Component 2 (9.3%)
Component 1 (11.5%)
Component 2 (4.5%)
Component 1 (4.9%)

Figure S2. Cell viability diagrams for three drugs (methotrexate, MTX; camptothecin, CPT; tomudex, TDX) used with A549 cells.





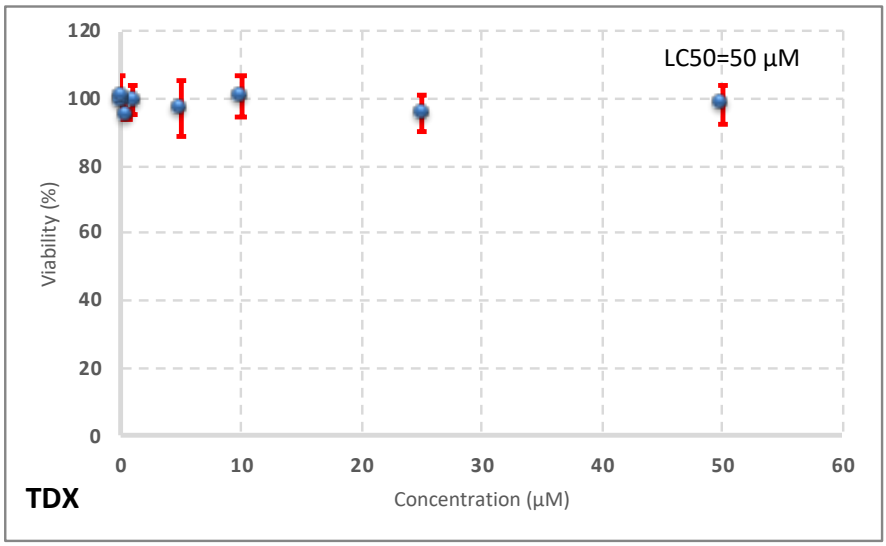


Figure S3. FACS diagrams with gating conditions for isolating individual DMSO-treated (A) and MTX-treated (B) A549 cells after 48 h.

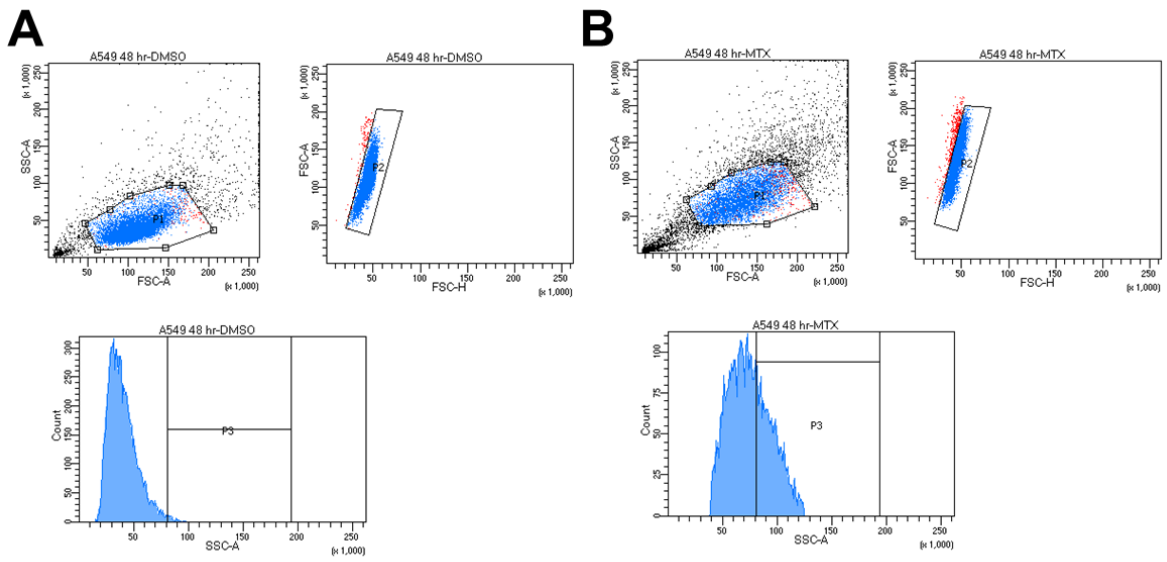
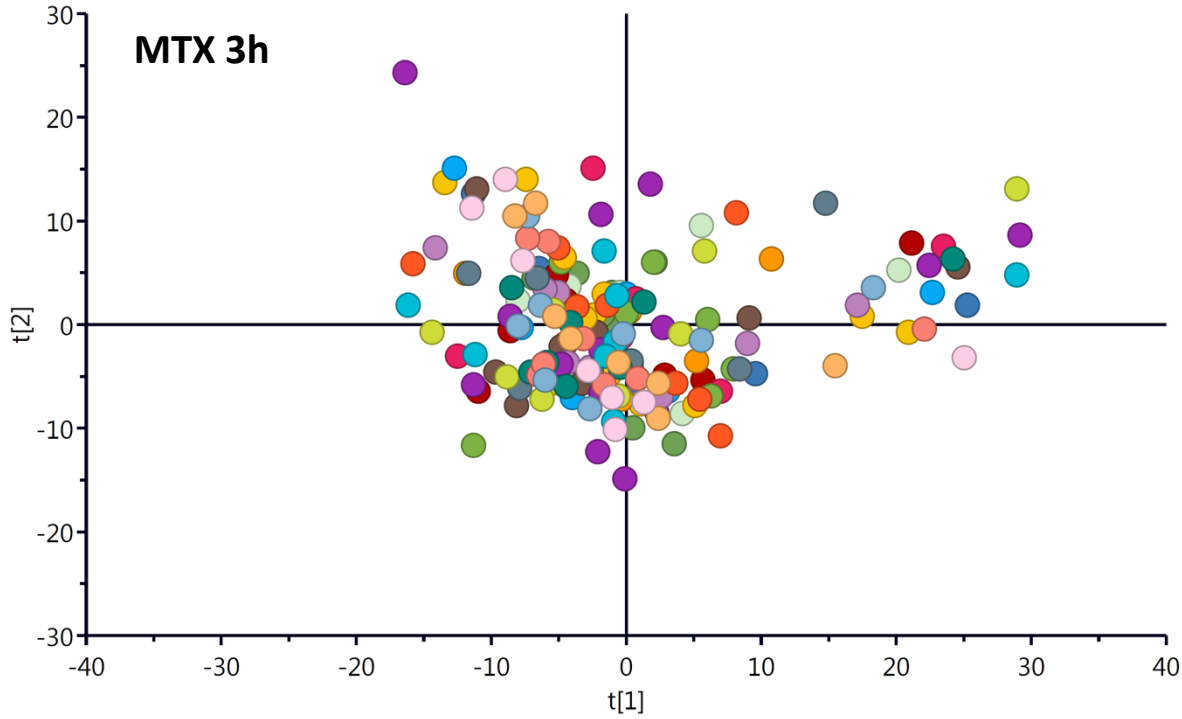
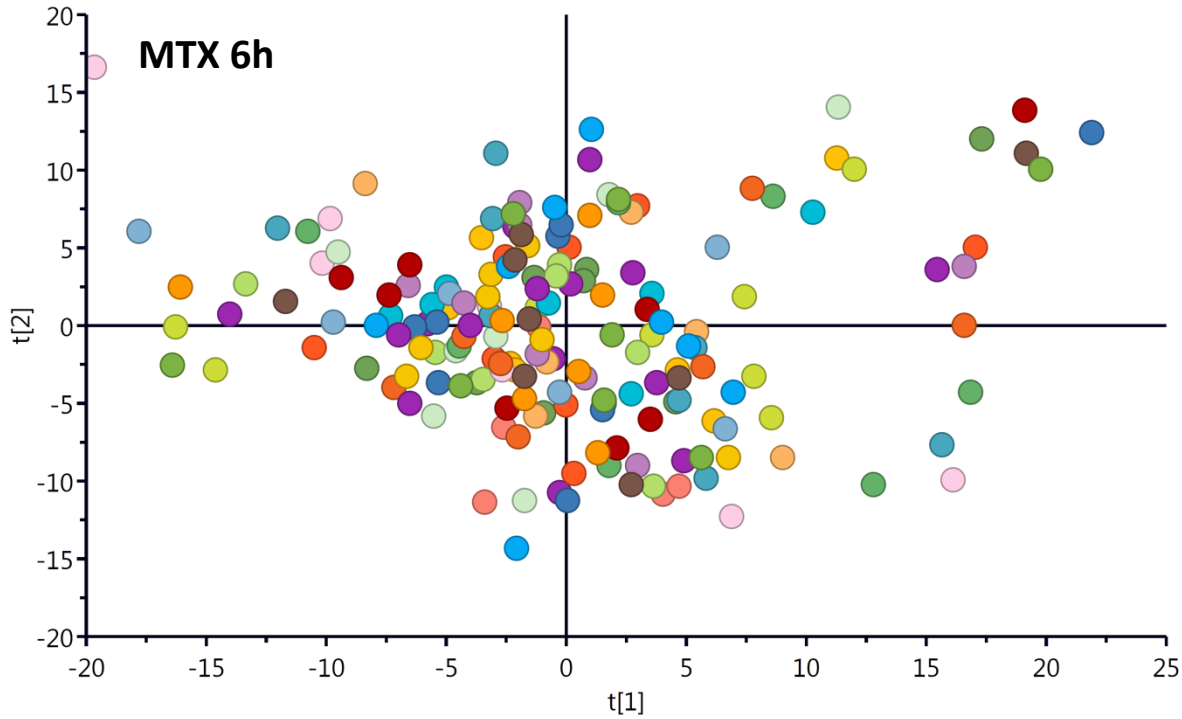
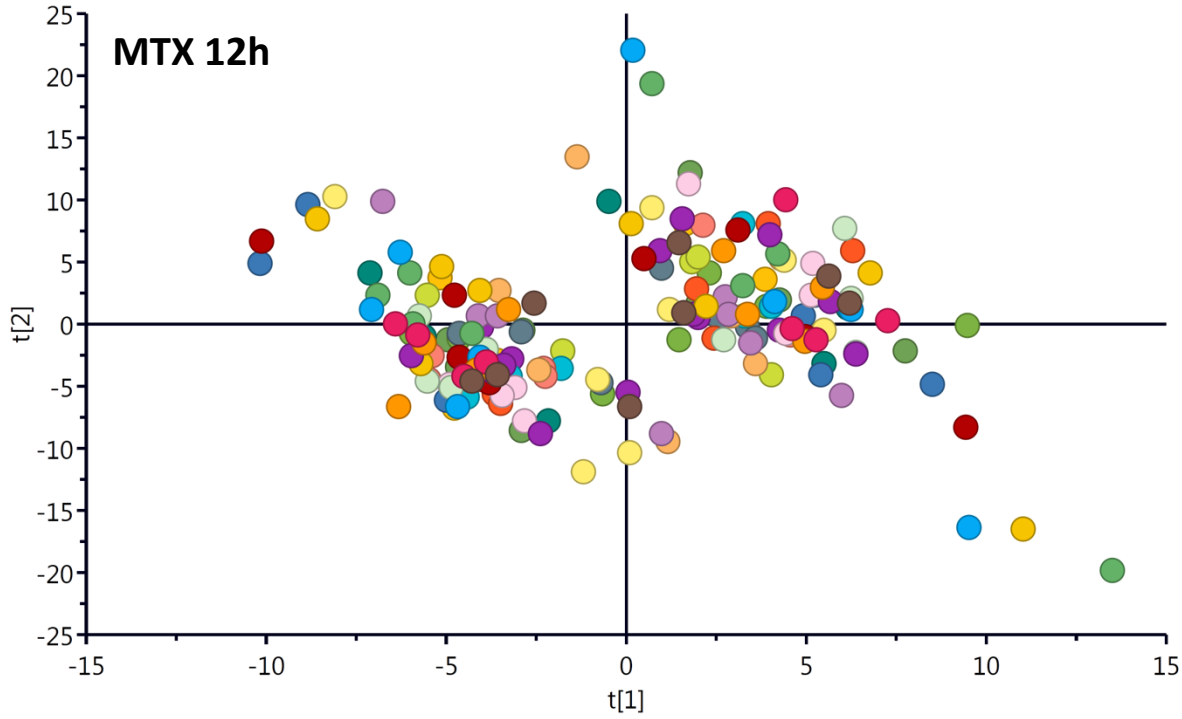
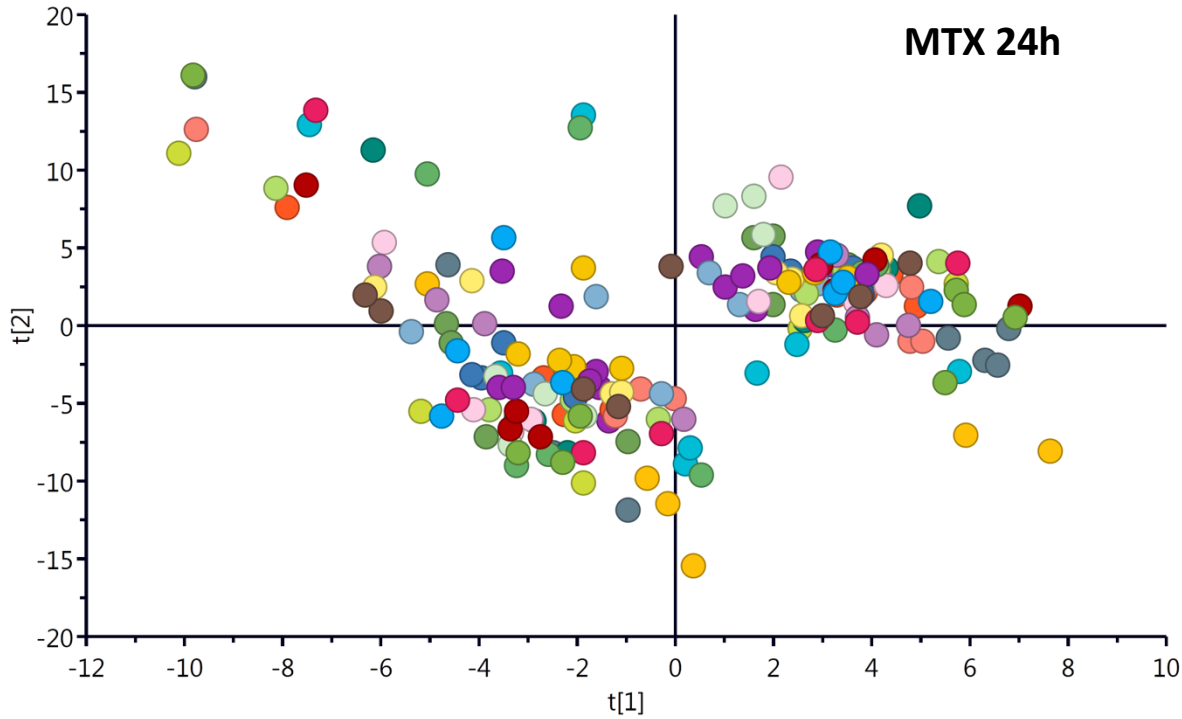


Figure S4. PCA plots of the proteomics results on MTX treated single cell presented in *Figure 2*, coloring the cells after the batches.




MTX 6h
t[2]
t[1]
20
15
10
5
0
-5
-10
-15
-20
-20
-15
-10
-5
0
5
10
15
20
25


MTX 12h
t[2]
t[1]
25
20
15
10
5
0
-5
-10
-15
-20
-25
-15
-10
-5
0
5
10
15


MTX 24h
t[2]
t[1]
20
15
10
5
0
-5
-10
-15
-20
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10

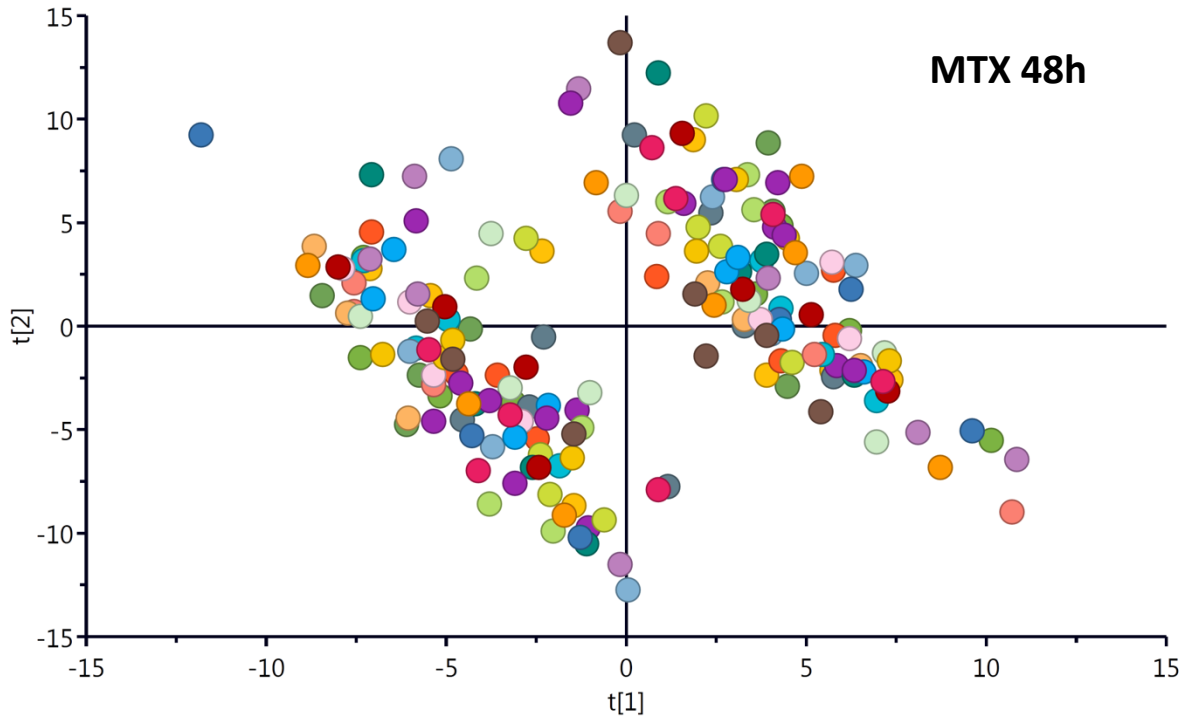
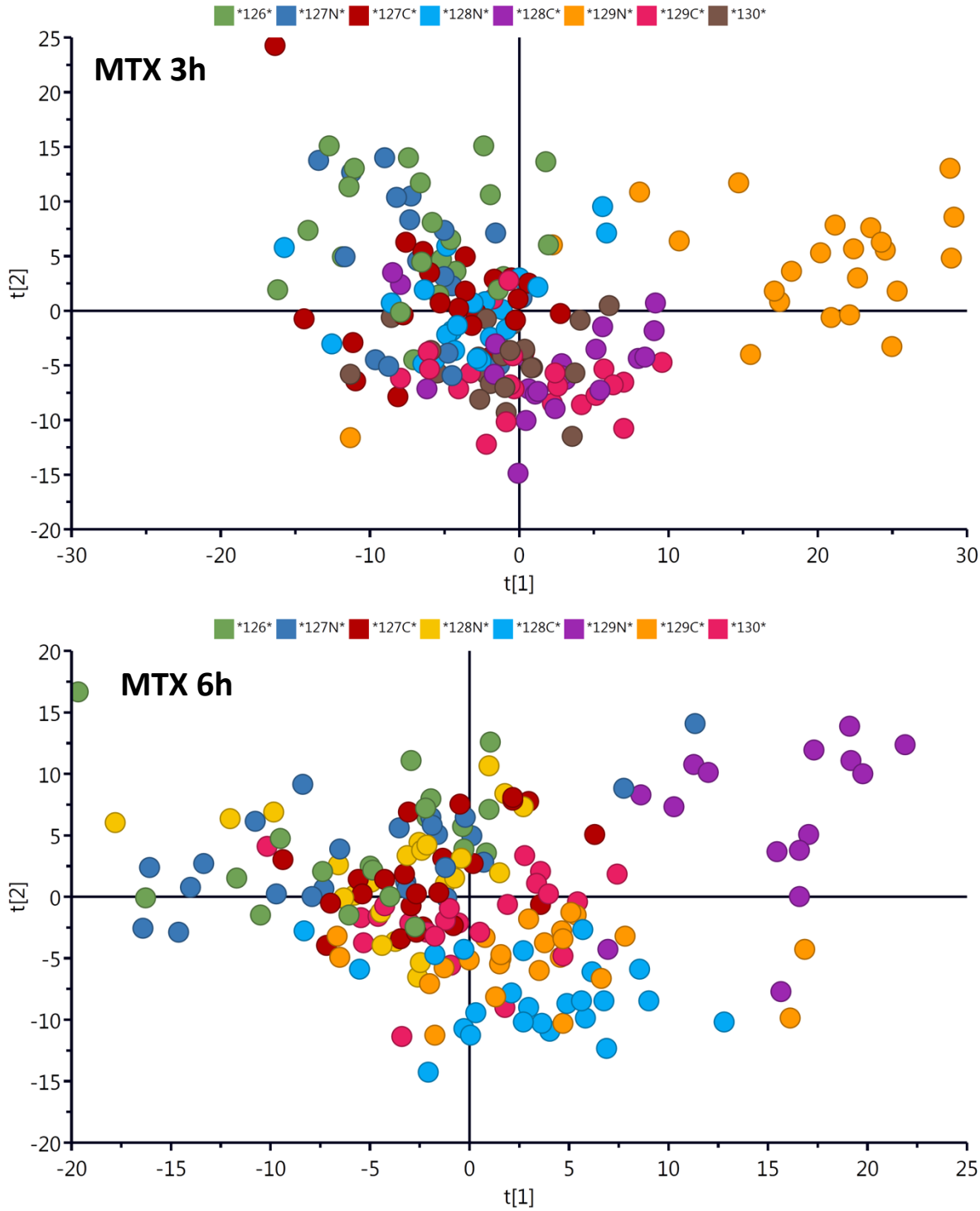
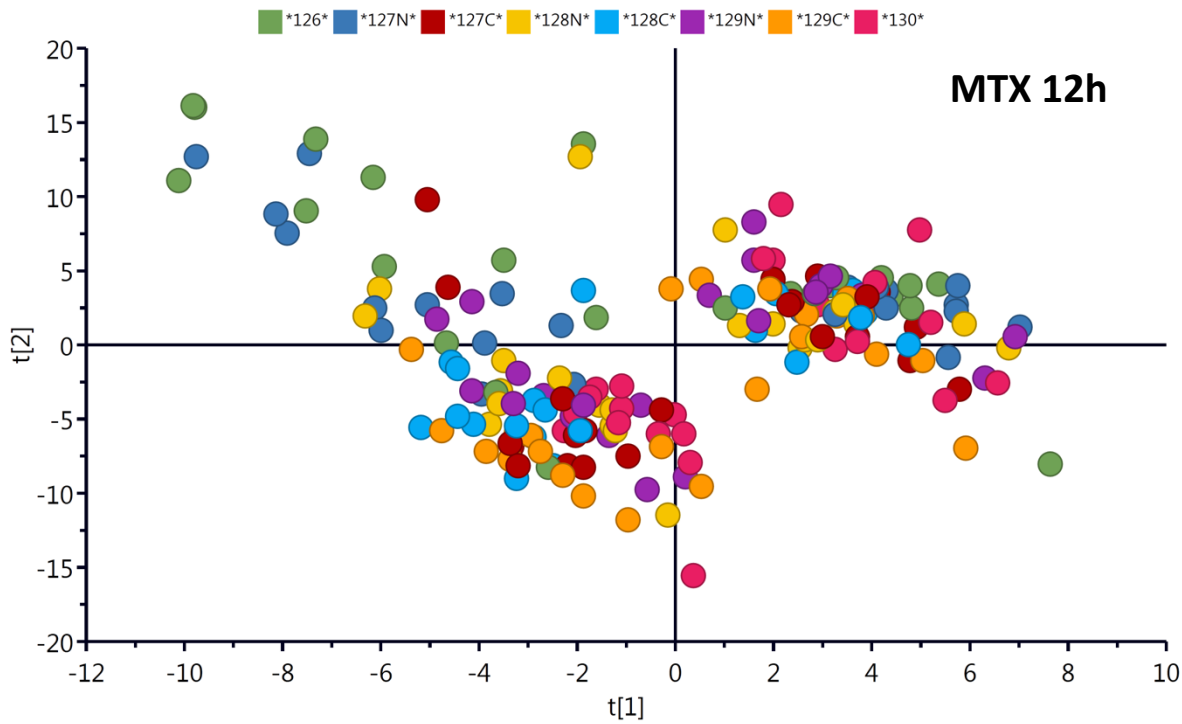
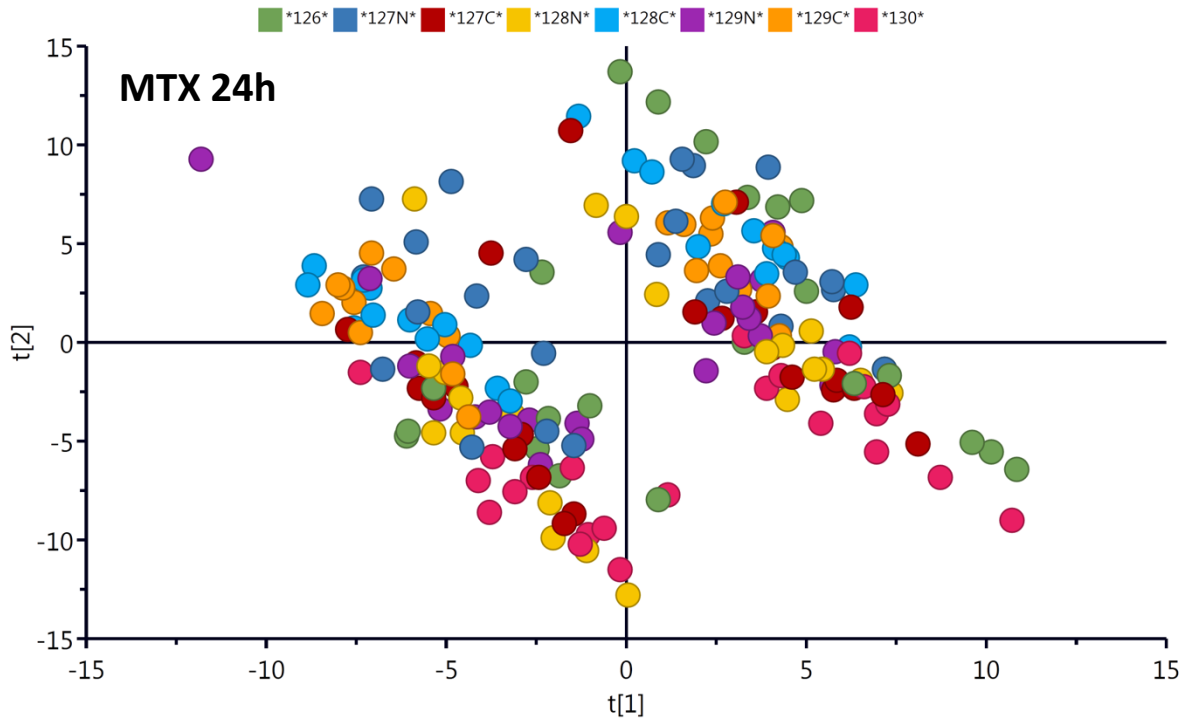

MTX 48h
t[2]
t[1]
15
10
5
0
-5
-10
-15
-15
-10
-5
0
5
10
15

Figure S5. Same as in Figure S4, coloring the cells after the TMT channels.




126 *127N* *127C* *128N* *128C* *129N* *129C* *130*
MTX 12h
t[2]
t[1]


126 *127N* *127C* *128N* *128C* *129N* *129C* *130*
MTX 24h
t[2]
t[1]

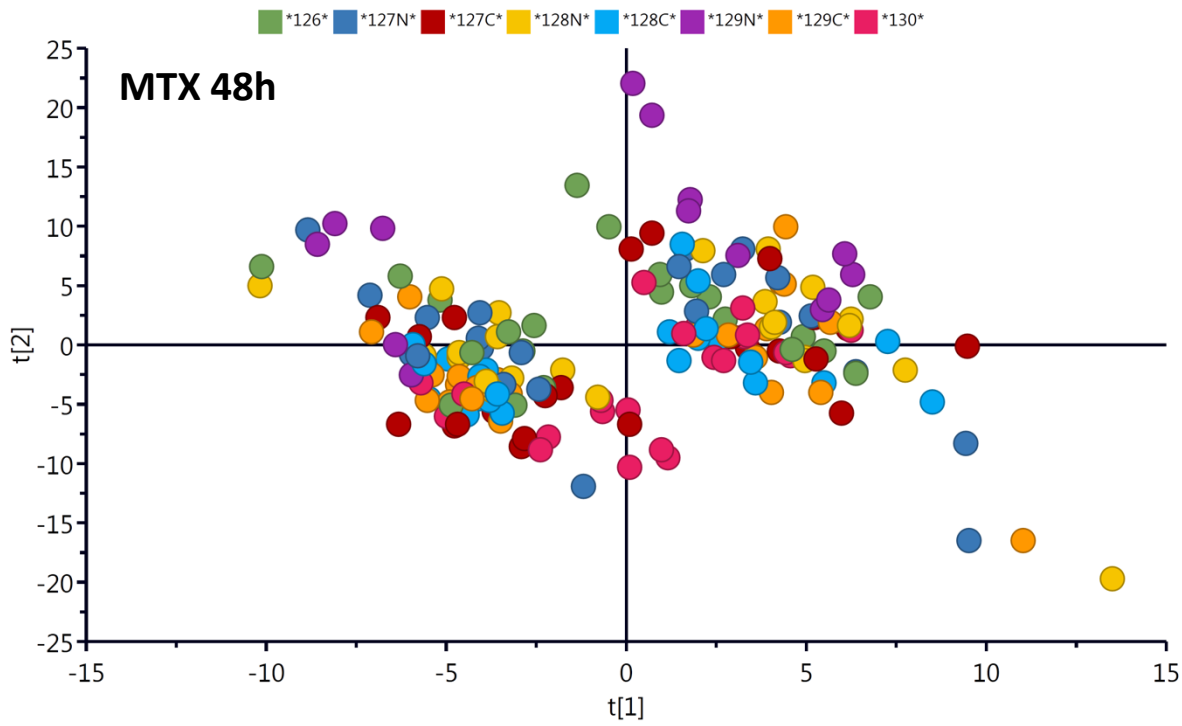
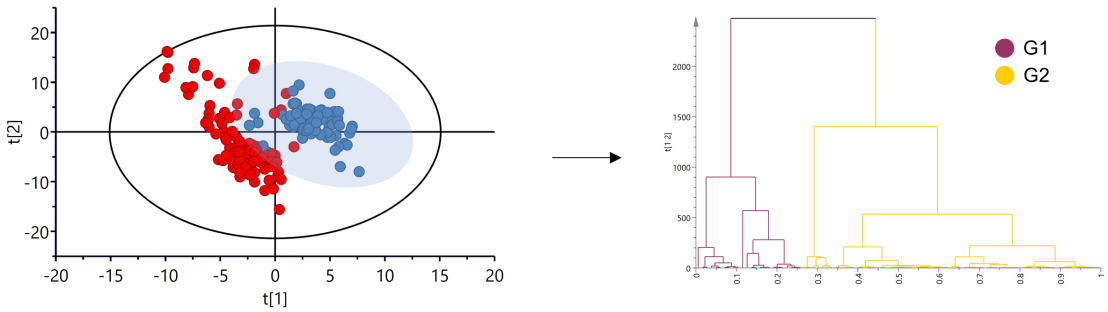


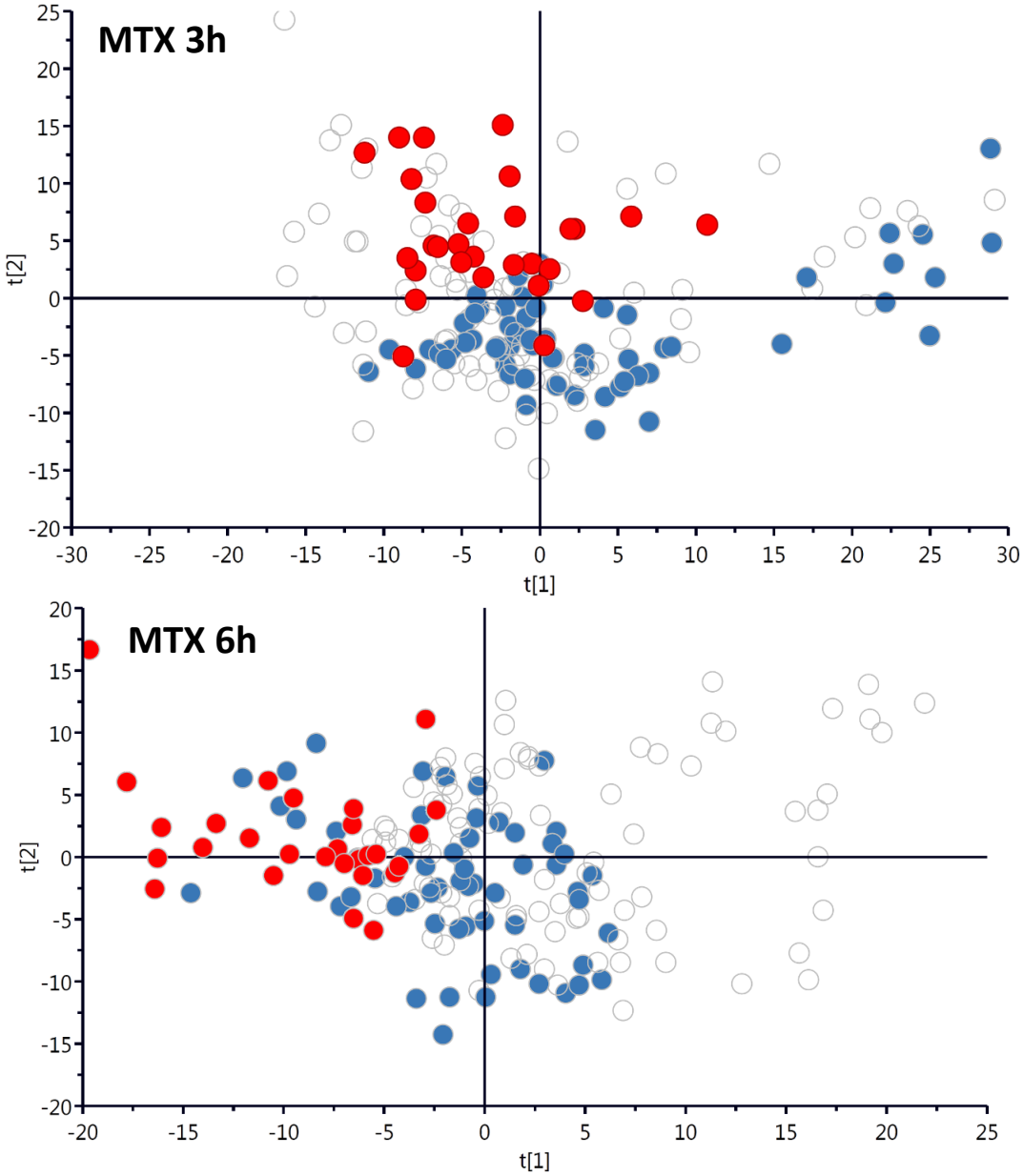
Figure S6. Clustering of the proteomes of attached treated single cells provides two groups of cells, G1 and G2.

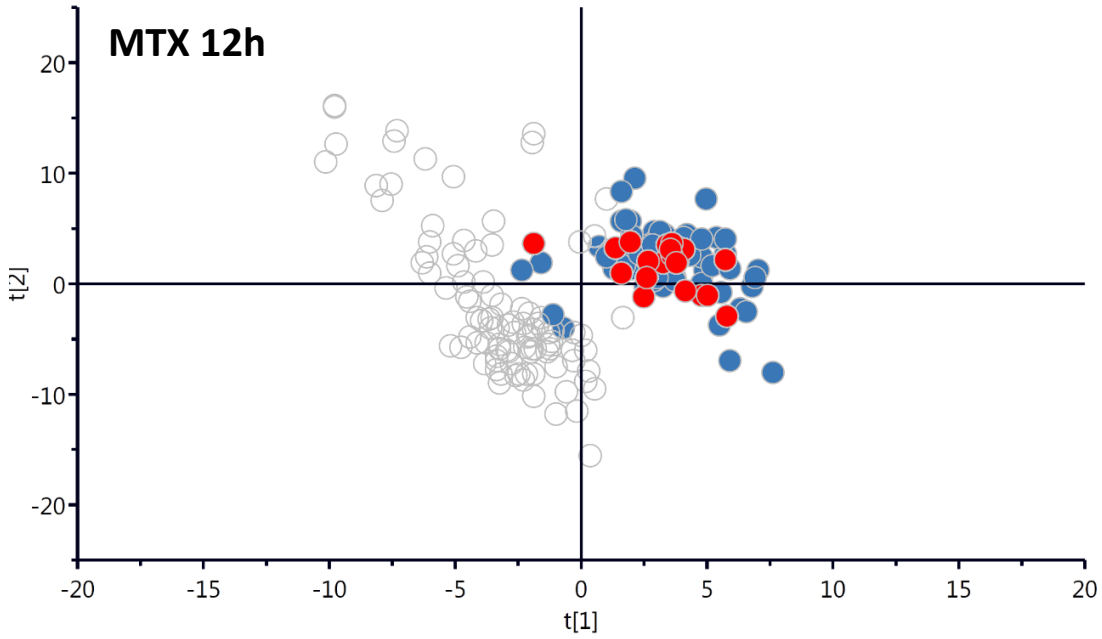


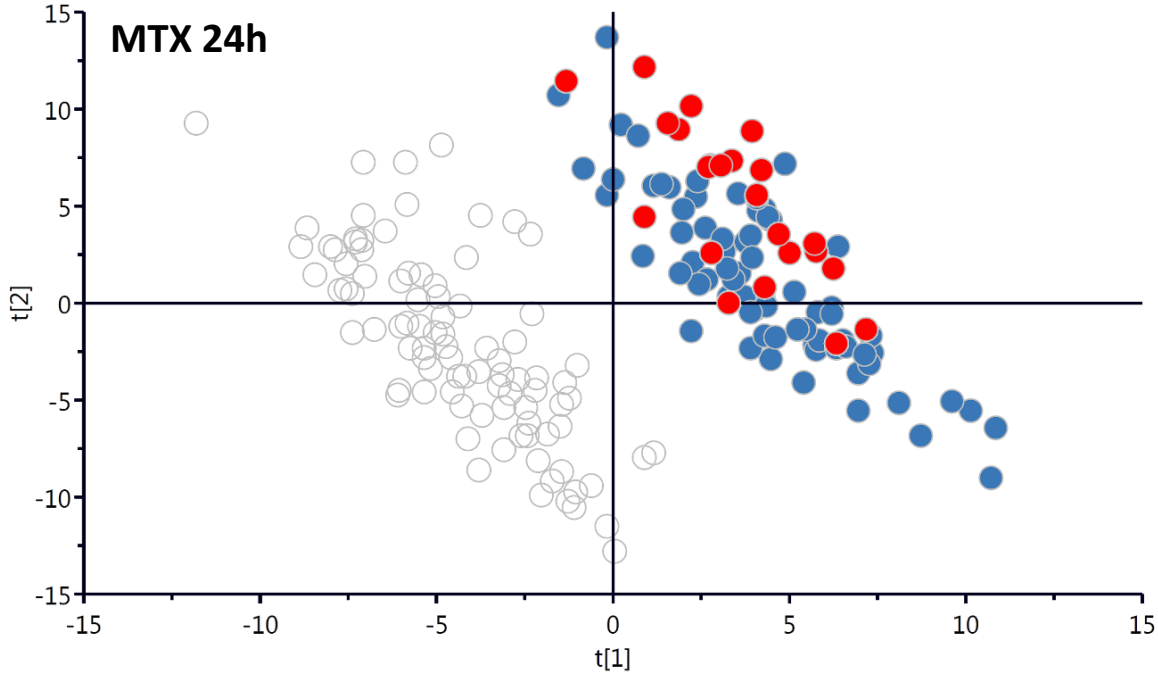
MTX, 12h PCA

Dendrogram (two main groups)

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).




MTX 12h
20
10
0
-10
-20
t[2]
-20
-15
-10
-5
0
5
10
15
20
t[1]


MTX 24h
15
10
5
0
-5
-10
-15
t[2]
-15
-10
-5
0
5
10
15
t[1]

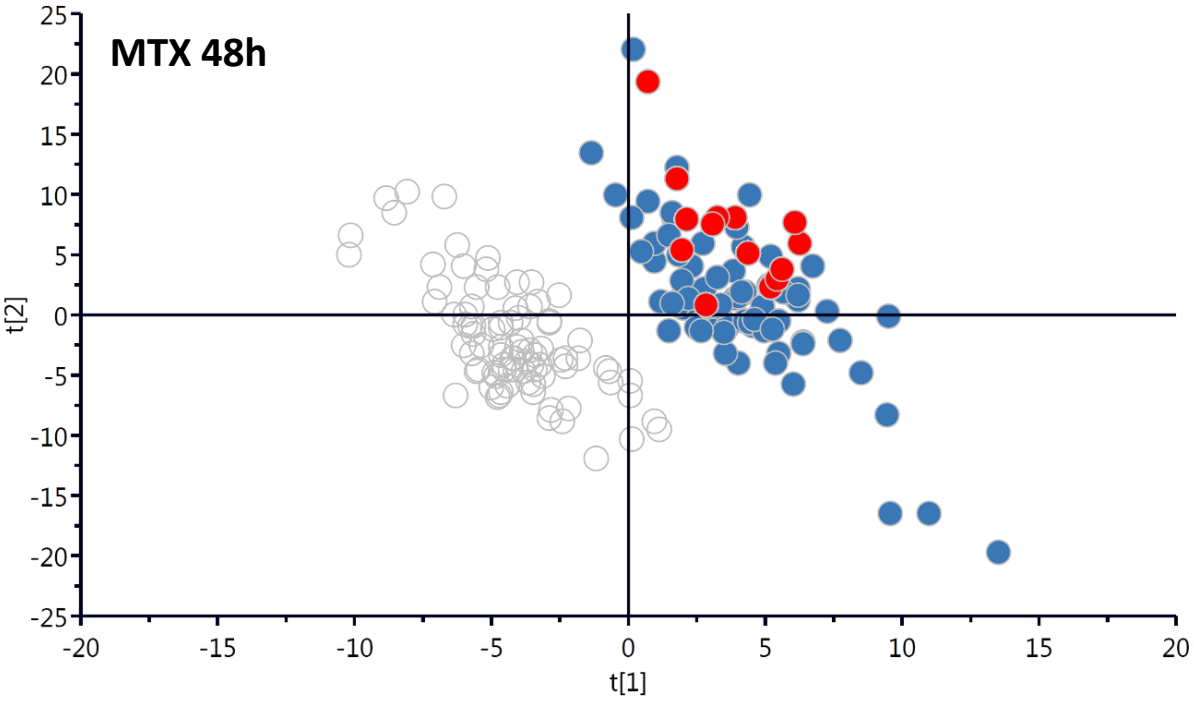
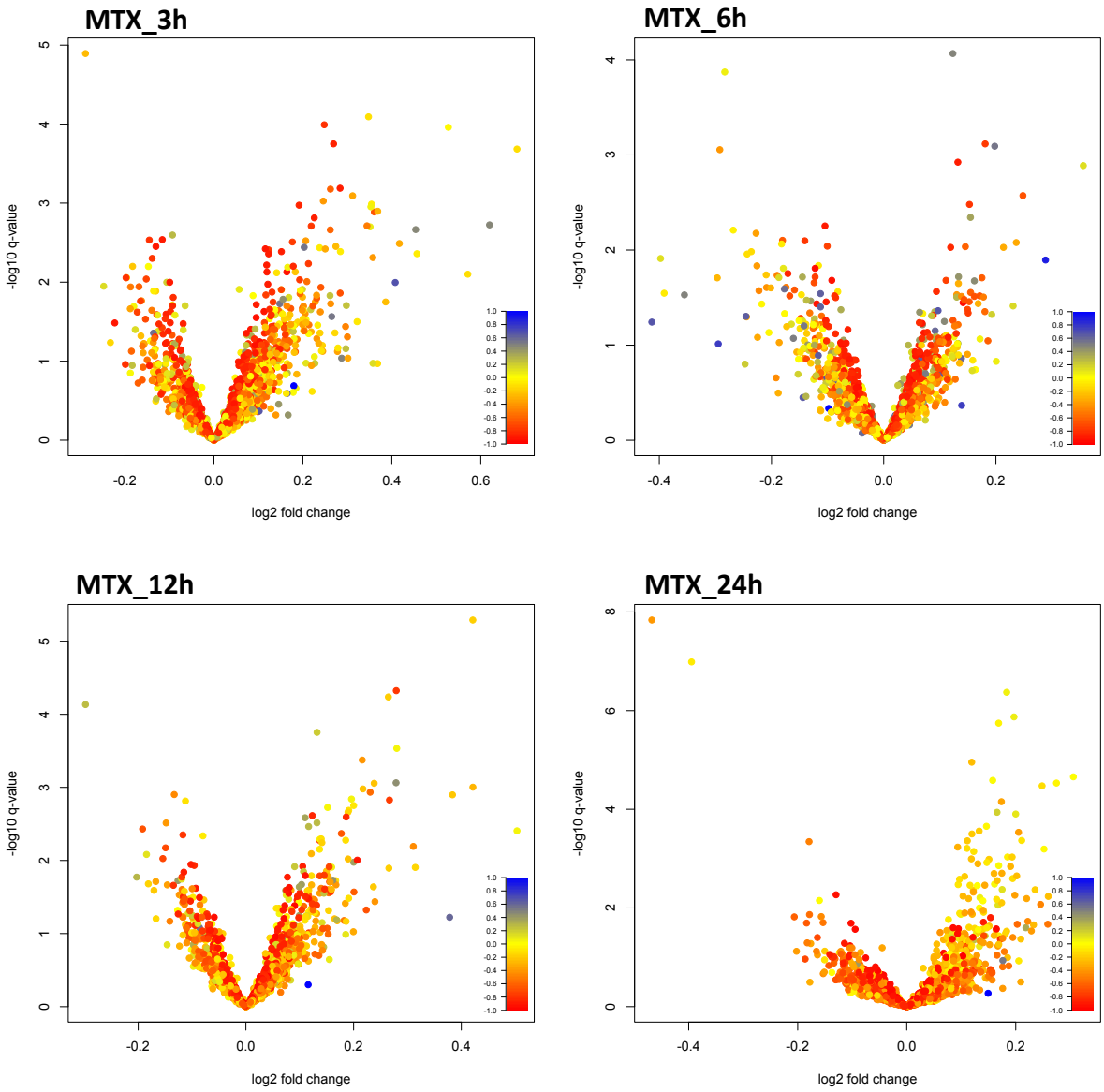
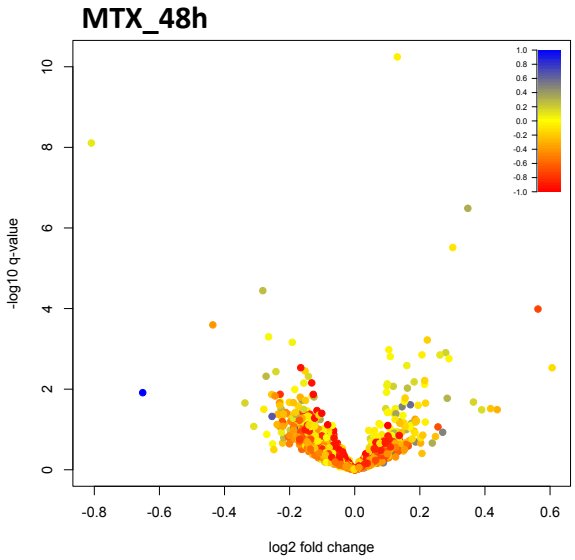
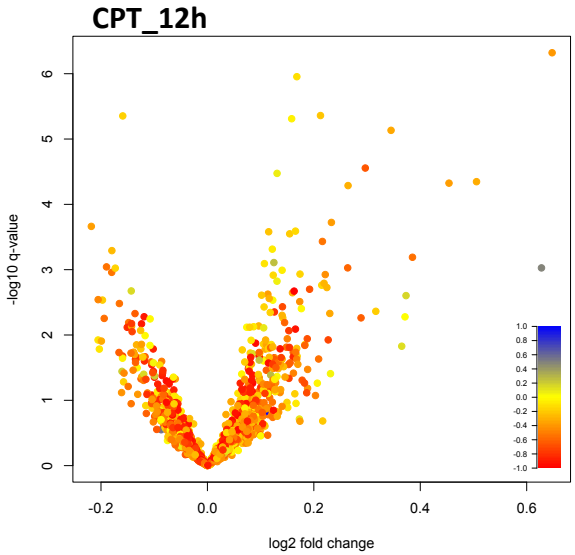
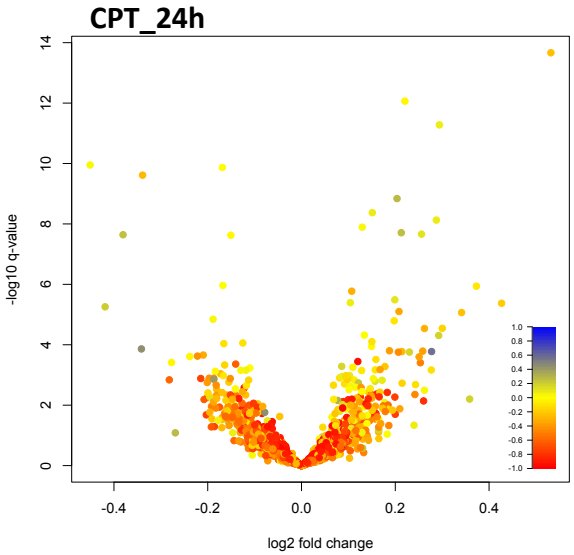

MTX 48h
t[2]
t[1]
25
20
15
10
5
0
-5
-10
-15
-20
-25
-20
-15
-10
-5
0
5
10
15
20

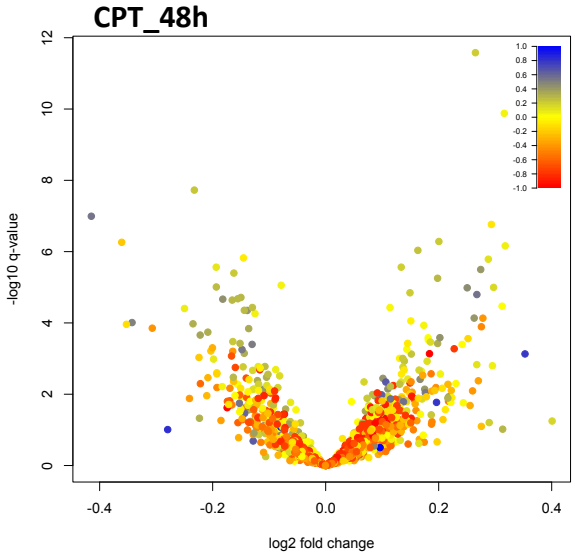
Figure S8. Volcano plots of data presented in *Figures 2* and *4*, with proteins re-colored according to their relative abundance in the sample (low to high proteins abundance = red-yellow-blue).

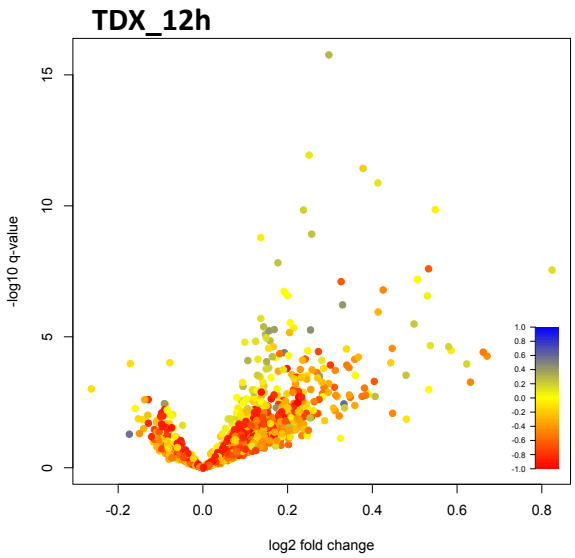


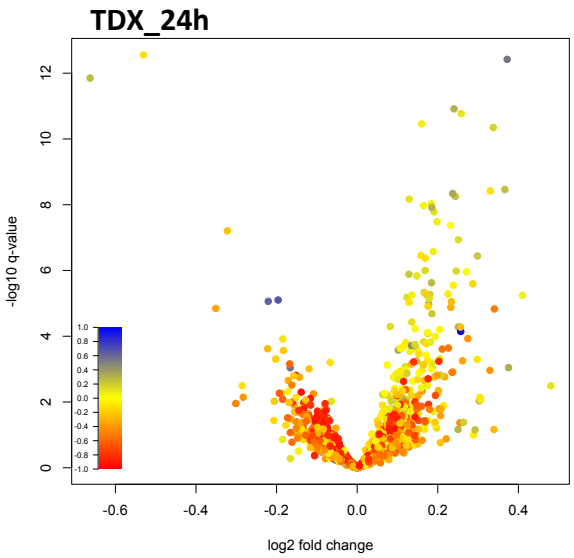

MTX_48h
-log10 q-value
log2 fold change


CPT_12h
-log10 q-value
log2 fold change


CPT_24h
-log10 q-value
log2 fold change


CPT_48h
-log10 q-value
log2 fold change


TDX_12h
-log10 q-value
log2 fold change


TDX_24h
-log10 q-value
log2 fold change

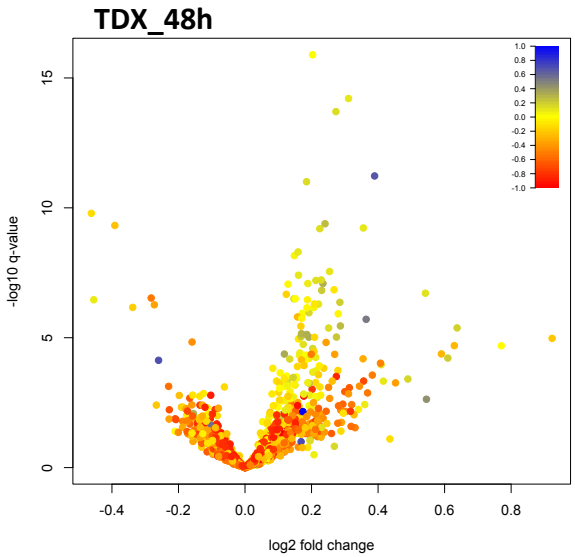
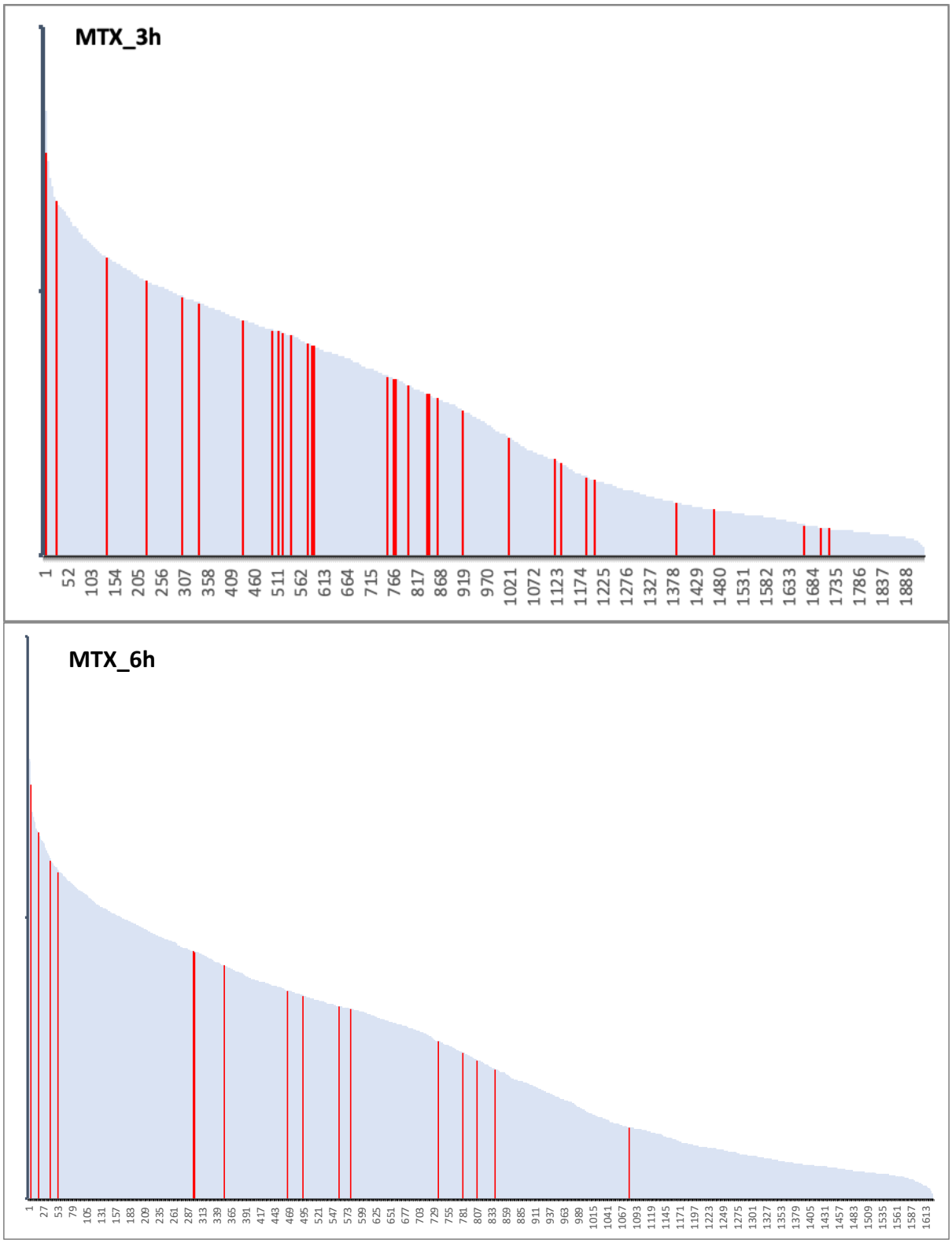
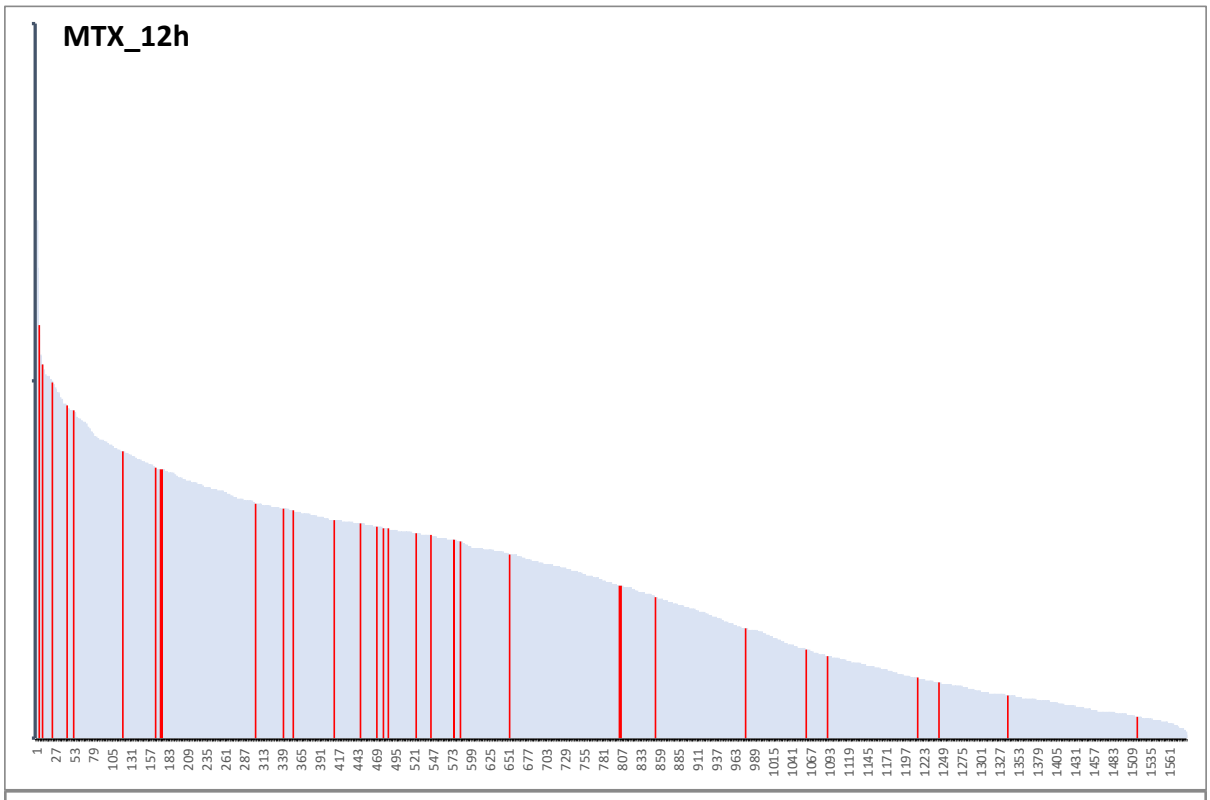
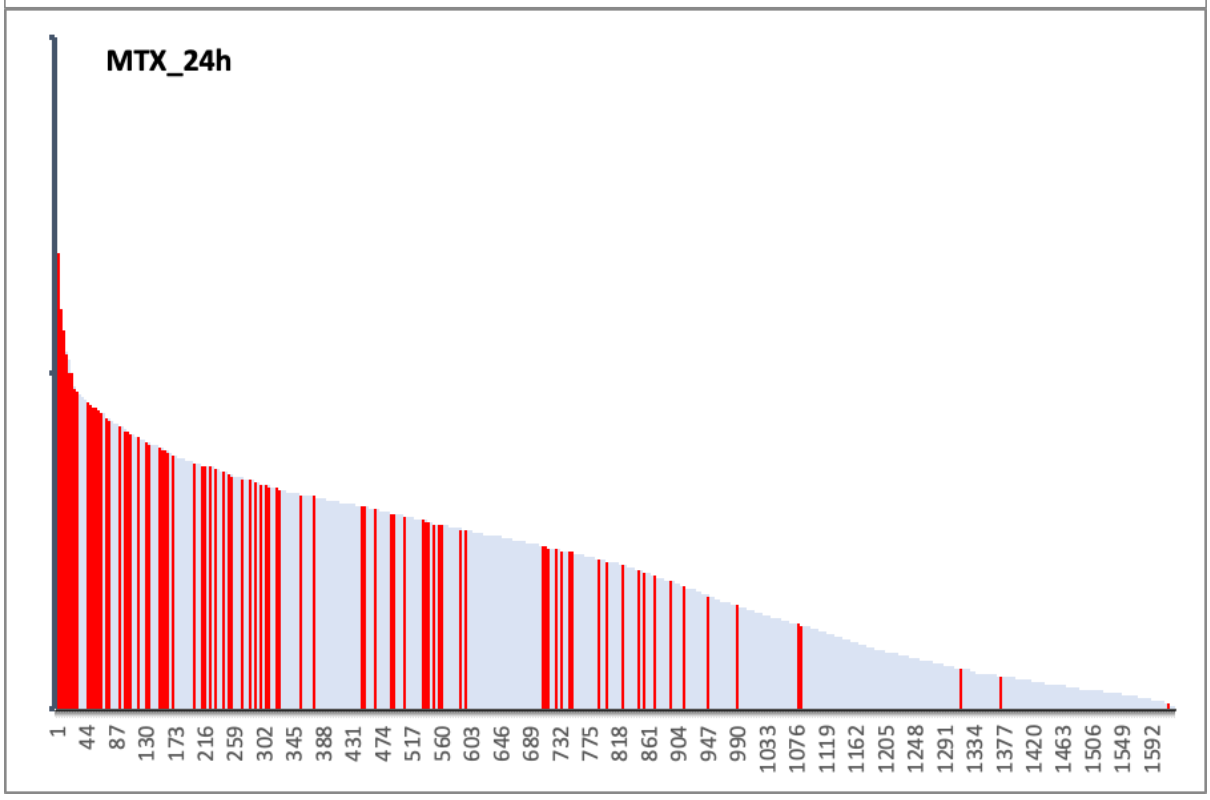
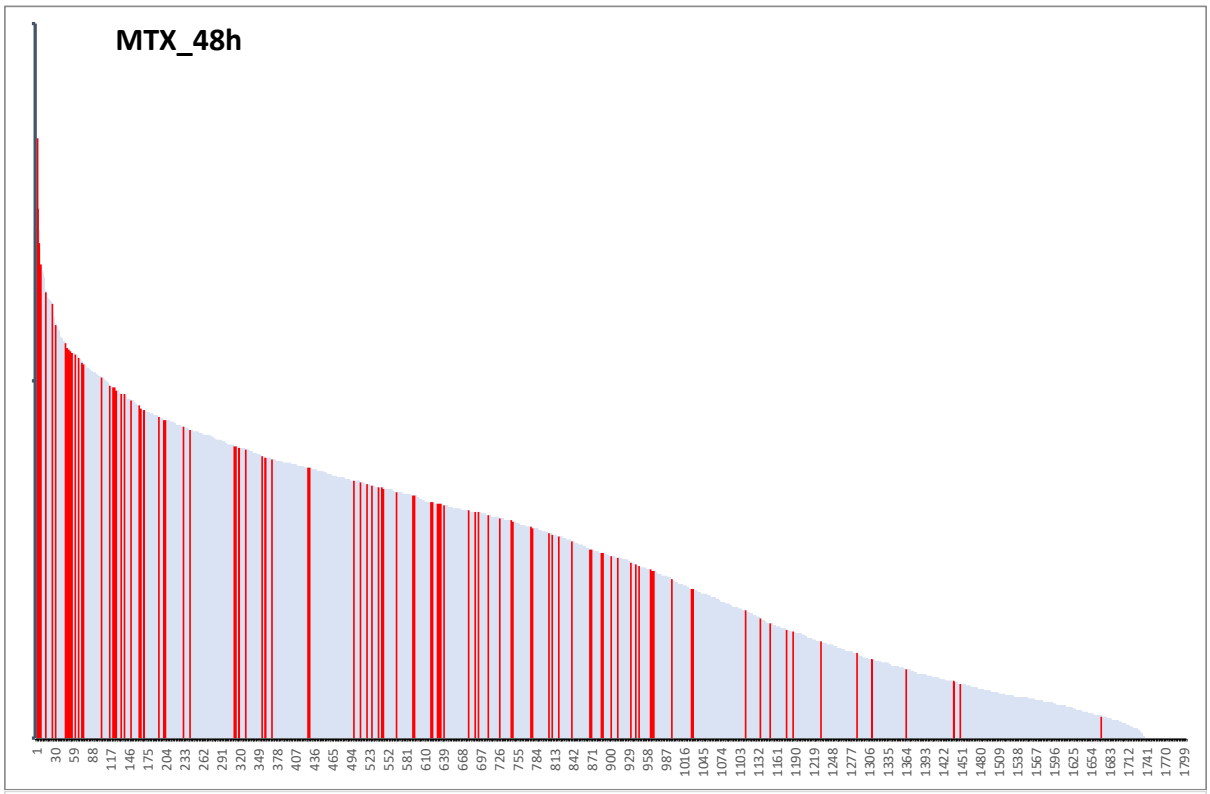

TDX_48h
-log10 q-value
log2 fold change

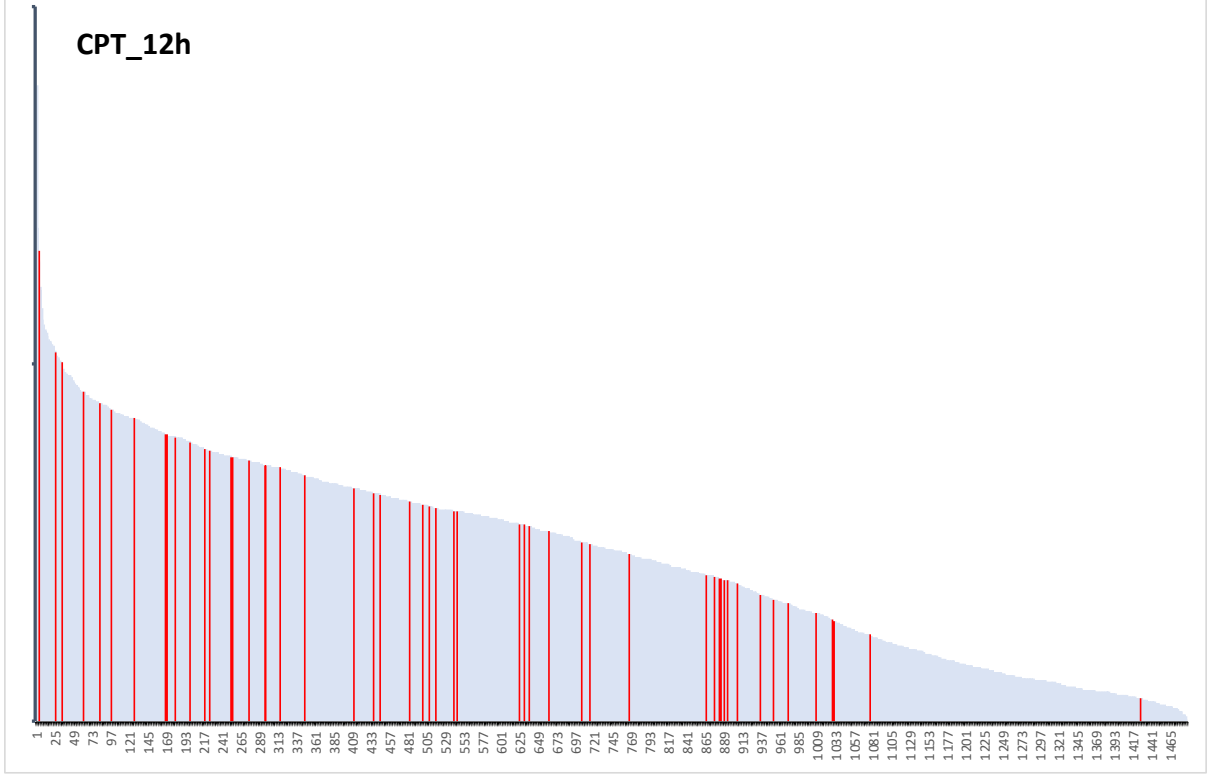
Figure S9. Proteins ranked after their abundances in data sets shown in *Figures 2* and *4*. The significantly regulated proteins are depicted in red color.




MTX_12h
1
27
53
79
105
131
157
183
209
235
261
287
313
339
365
391
417
443
469
495
521
547
573
599
625
651
677
703
729
755
781
807
833
859
885
911
937
963
989
1015
1041
1067
1093
1119
1145
1171
1197
1223
1249
1275
1301
1327
1353
1379
1405
1431
1457
1483
1509
1535
1561


MTX_24h
1
44
87
130
173
216
259
302
345
388
431
474
517
560
603
646
689
732
775
818
861
904
947
990
1033
1076
1119
1162
1205
1248
1291
1334
1377
1420
1463
1506
1549
1592


MTX_48h
1
30
59
88
117
146
175
204
233
262
291
320
349
378
407
436
465
494
523
552
581
610
639
668
697
726
755
784
813
842
871
900
929
958
987
1016
1045
1074
1103
1132
1161
1190
1219
1248
1277
1306
1335
1364
1393
1422
1451
1480
1509
1538
1567
1596
1625
1654
1683
1712
1741
1770
1799


CPT_12h
1
25
49
73
97
121
145
169
193
217
241
265
289
313
337
361
385
409
433
457
481
505
529
553
577
601
625
649
673
697
721
745
769
793
817
841
865
889
913
937
961
985
1009
1033
1057
1081
1105
1129
1153
1177
1201
1225
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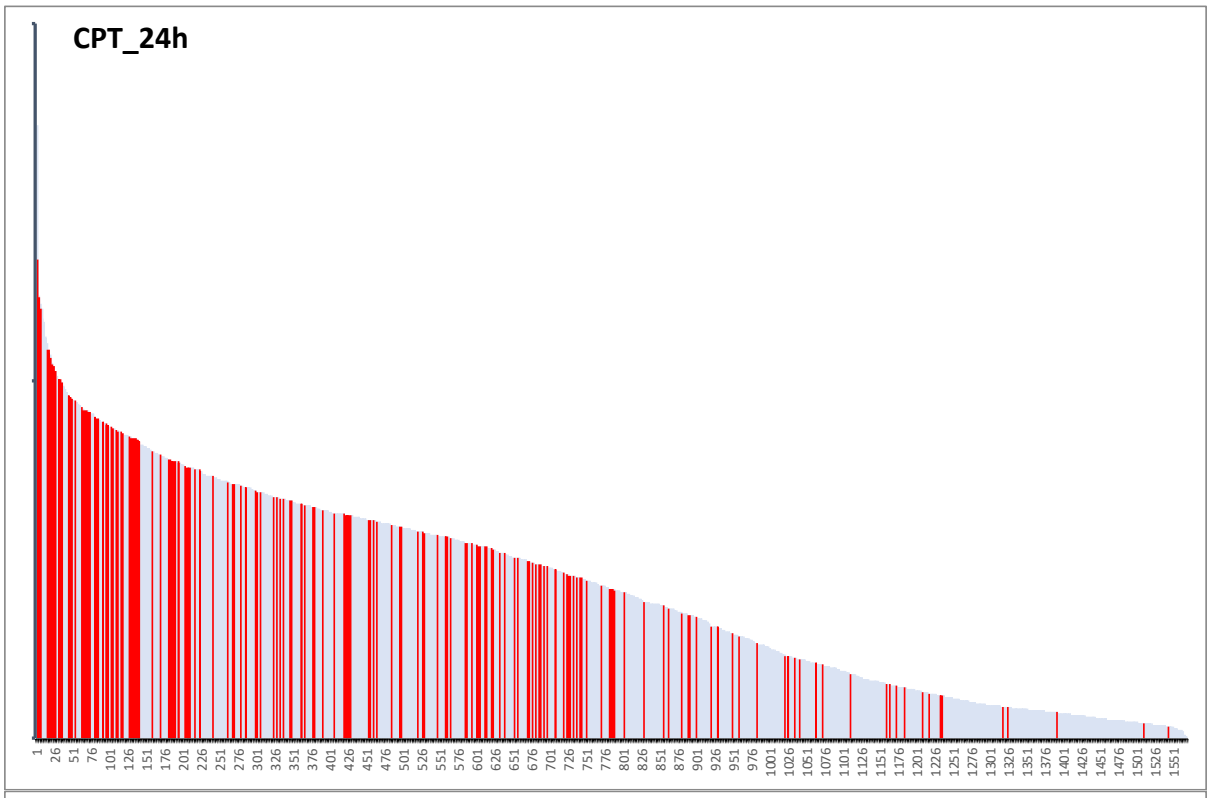
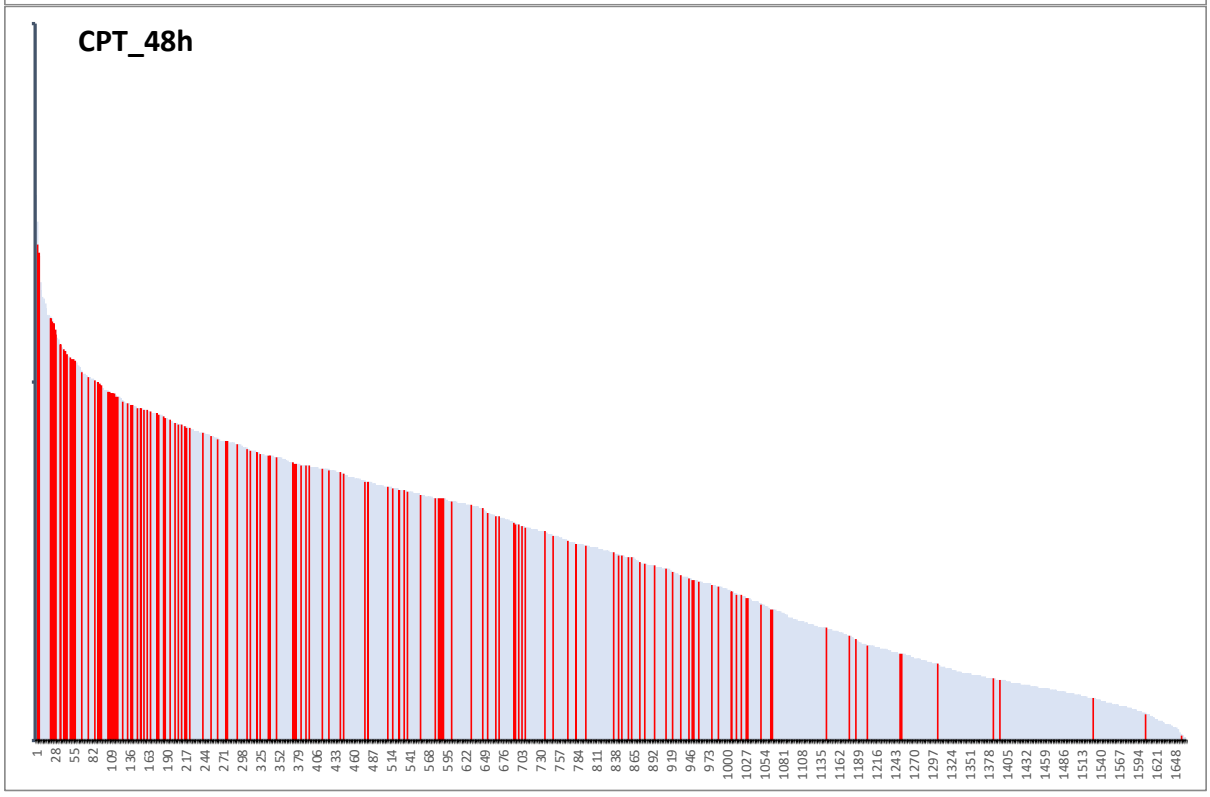
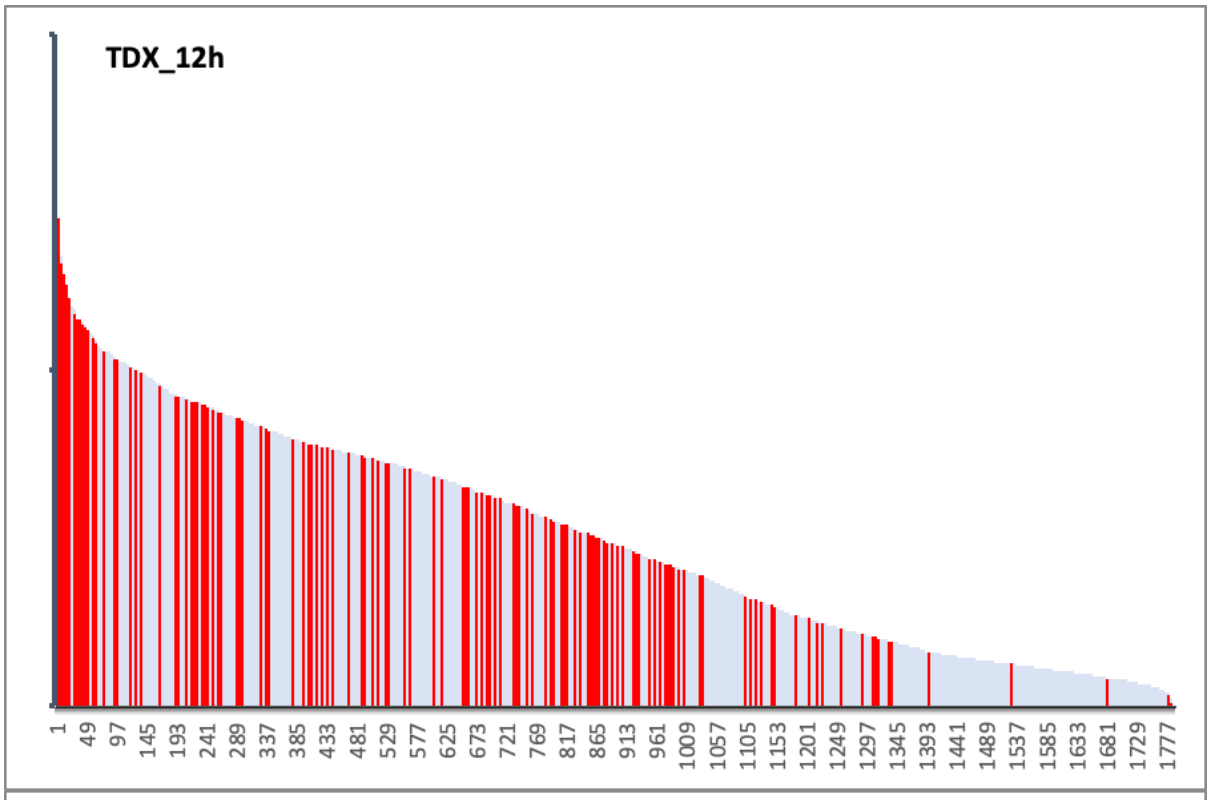
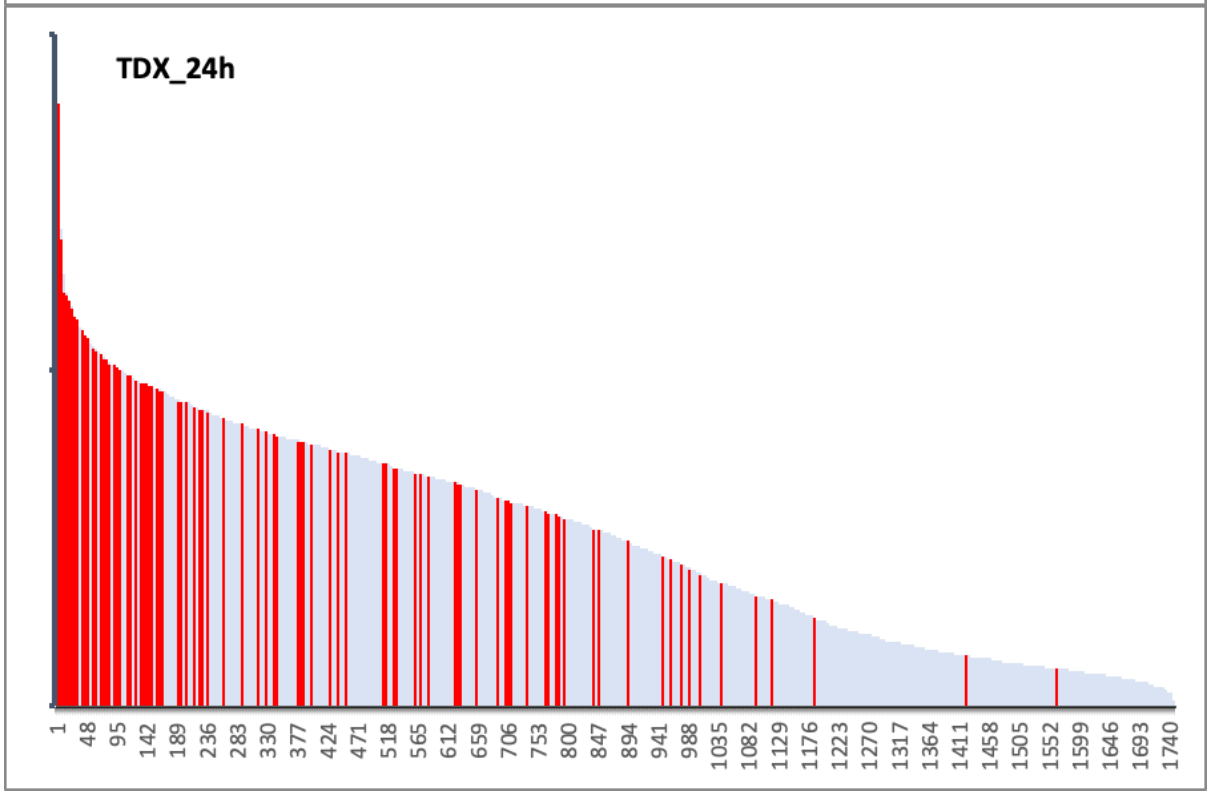
1273
1297
1321
1345
1369
1393
1417
1441
1465


CPT_24h


CPT_48h


TDX_12h
1
49
97
145
193
241
289
337
385
433
481
529
577
625
673
721
769
817
865
913
961
1009
1057
1105
1153
1201
1249
1297
1345
1393
1441
1489
1537
1585
1633
1681
1729
1777


TDX_24h
1
48
95
142
189
236
283
330
377
424
471
518
565
612
659
706
753
800
847
894
941
988
1035
1082
1129
1176
1223
1270
1317
1364
1411
1458
1505
1552
1599
1646
1693
1740

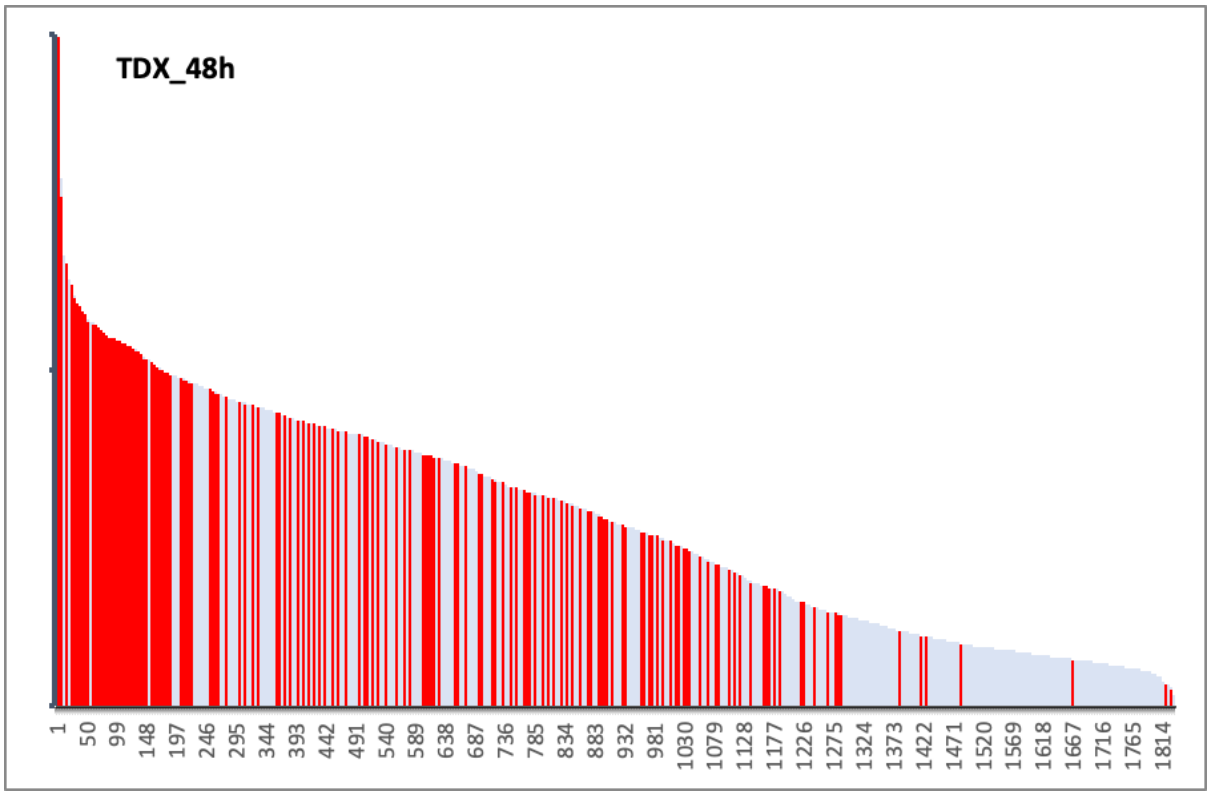

TDX_48h
1
50
99
148
197
246
295
344
393
442
491
540
589
638
687
736
785
834
883
932
981
1030
1079
1128
1177
1226
1275
1324
1373
1422
1471
1520
1569
1618
1667
1716
1765
1814

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.

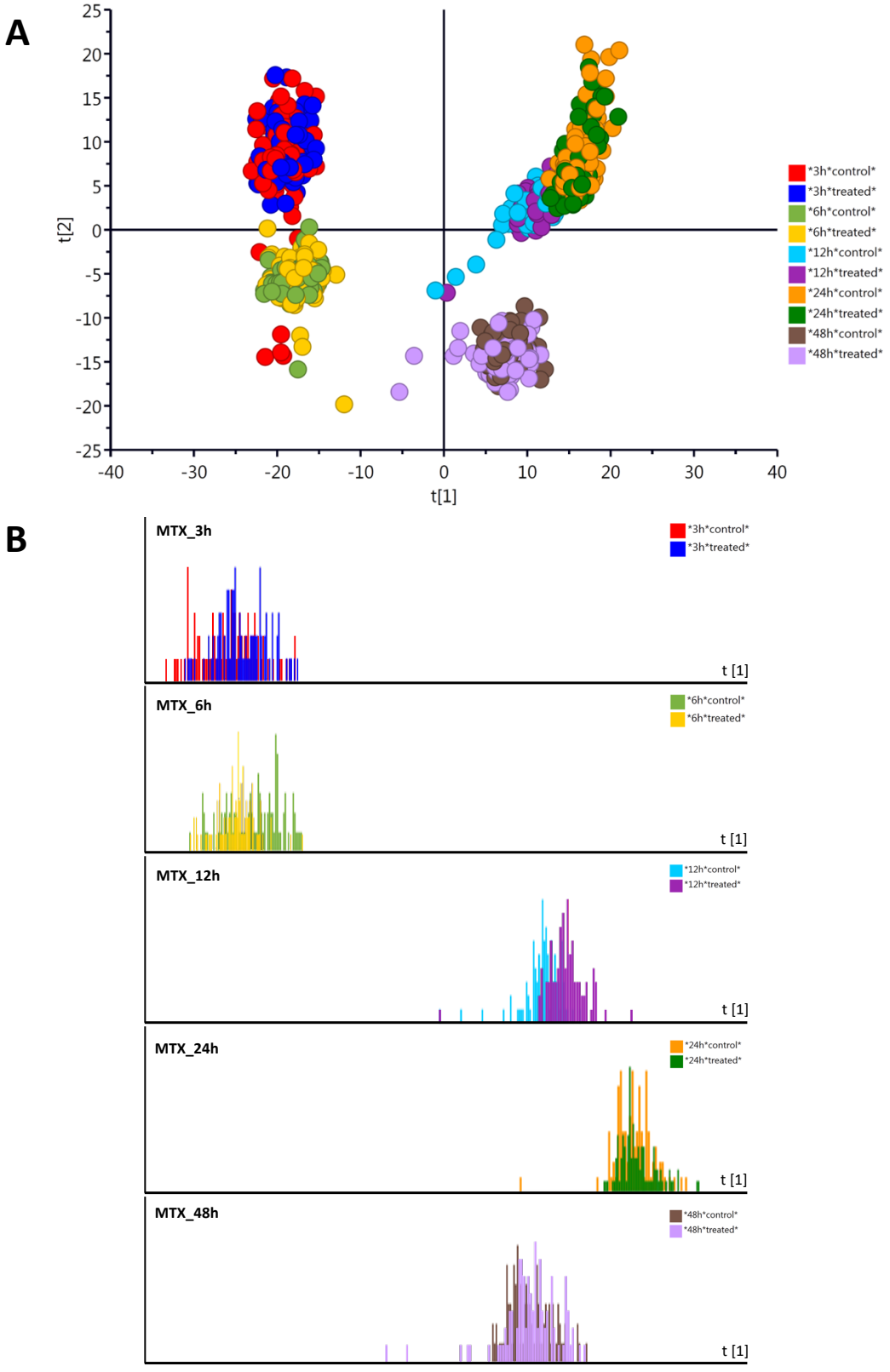
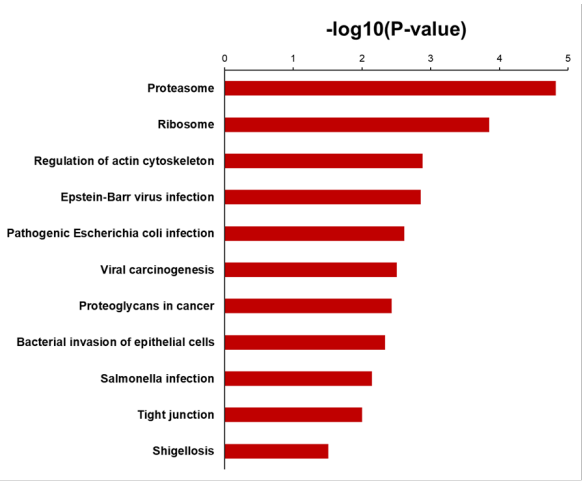
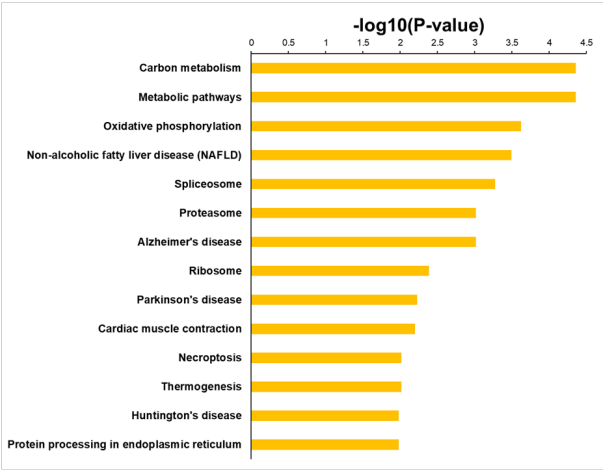


Figure S11. Pathway analysis of 179 proteins with significantly different abundances in G1 versus G2 subpopulations at 12 h past MTX treatment revealed that they preferentially belong to metabolic pathways and carbon metabolism (enriched in G2), as well as ribosome- and proteasome-related pathways (enriched in G1).



Pathways enriched in G1 (101 proteins)



Pathways enriched in G2 (78 proteins)