

Treatment of Neuroblastoma Cells with Inhibitors of Protein Disulfide Isomerase Upregulates NQO1 Activity

Dennis Özcelik

Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2,
2100 Copenhagen, Denmark

Email: dennis.ozcelik@sund.ku.dk

Keywords

Neurodegeneration, chaperone, microarray expression profile, redox regulation,
neuropharmacology, small molecules

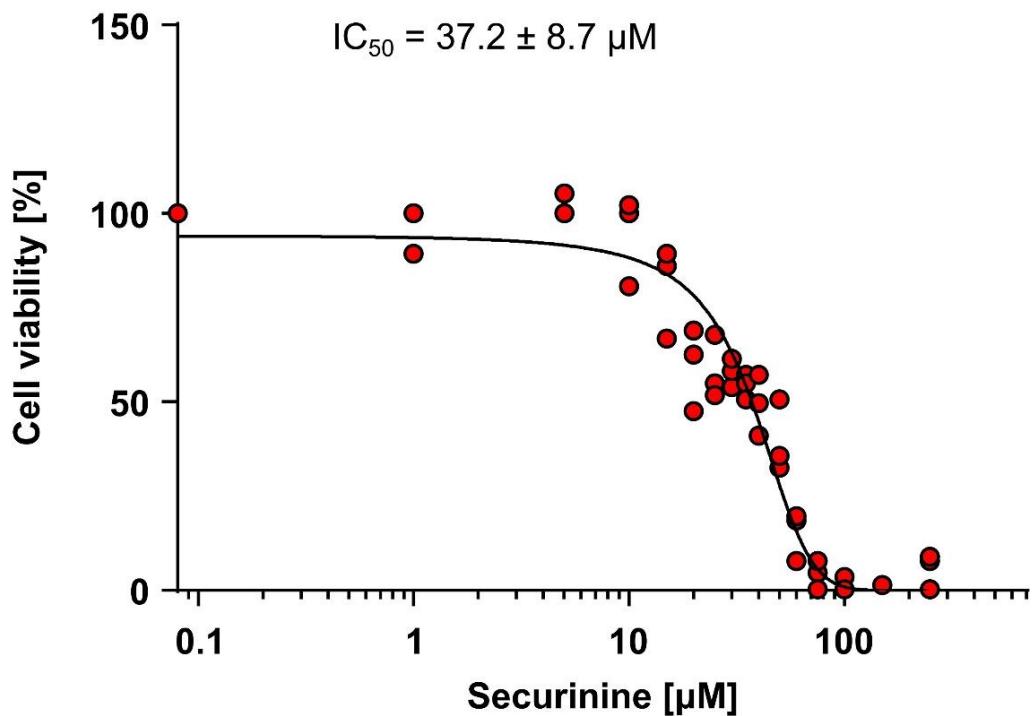
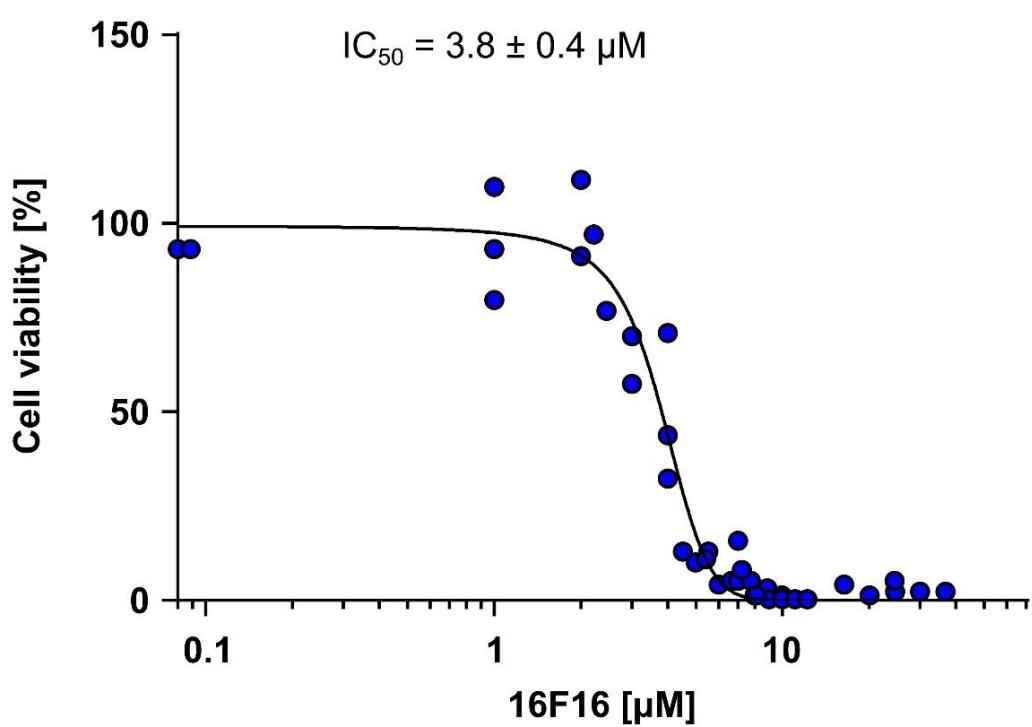
A**B**

Figure S1. SH-SY5Y neuroblastoma cells were incubated for 24 h with increasing concentrations of securinine (**A**) or 16F16 (**B**), respectively. Each solid circle represents a single, independent biological experiment.

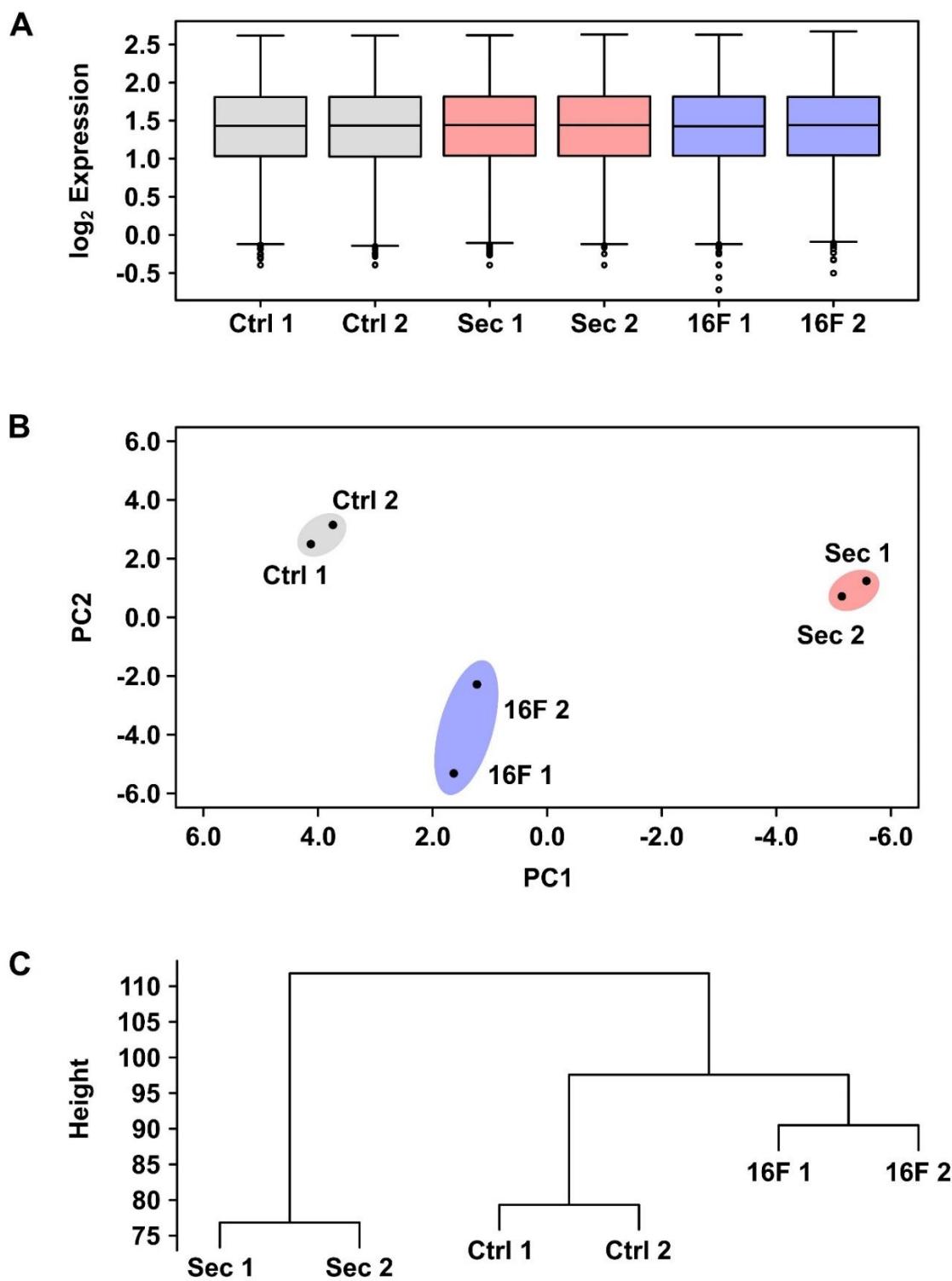


Figure S2. (A) Gene expression signals of individual microarrays after normalization. (B) Principal component analysis (PCA) shows similarities in gene expression profiles. Each black circle in the similarity matrix represents an individual microarray. (C) Dendrogram represents global hierarchical clustering of microarray data.

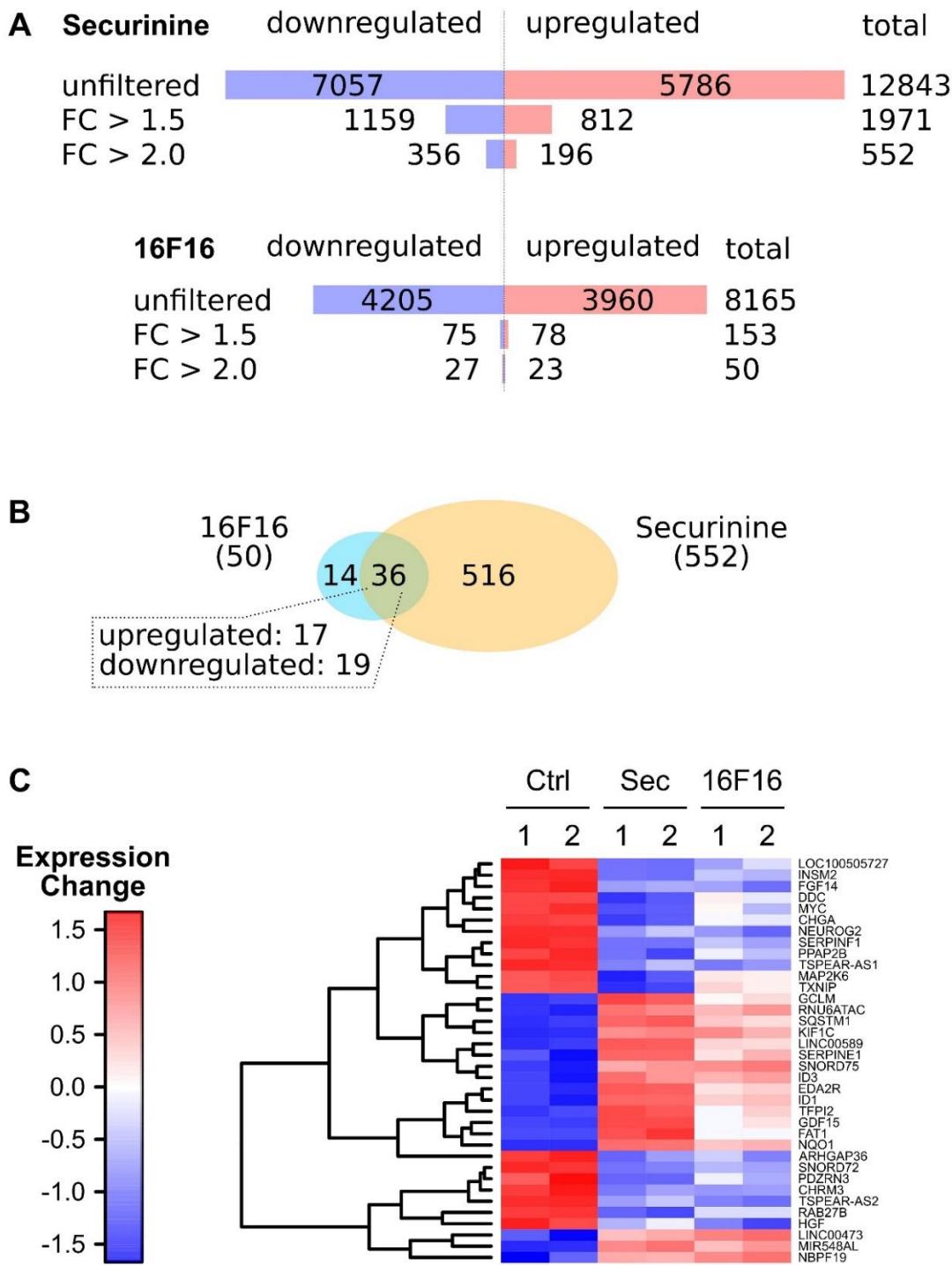


Figure S3. (A) Scheme depicts numbers of DEGs identified in the microarray data sets of samples treated with securinine (top) or 16F16 (bottom) before filtering and after selecting for 1.5-fold change or 2-fold change in gene expression levels. (B) Venn diagram depicts overlapping DEGs between treatments with securinine and 16F16. (C) Heatmap and hierarchical clustering of 36 DEGs (cut-off > 2-fold change) identified in all six microarray data sets.

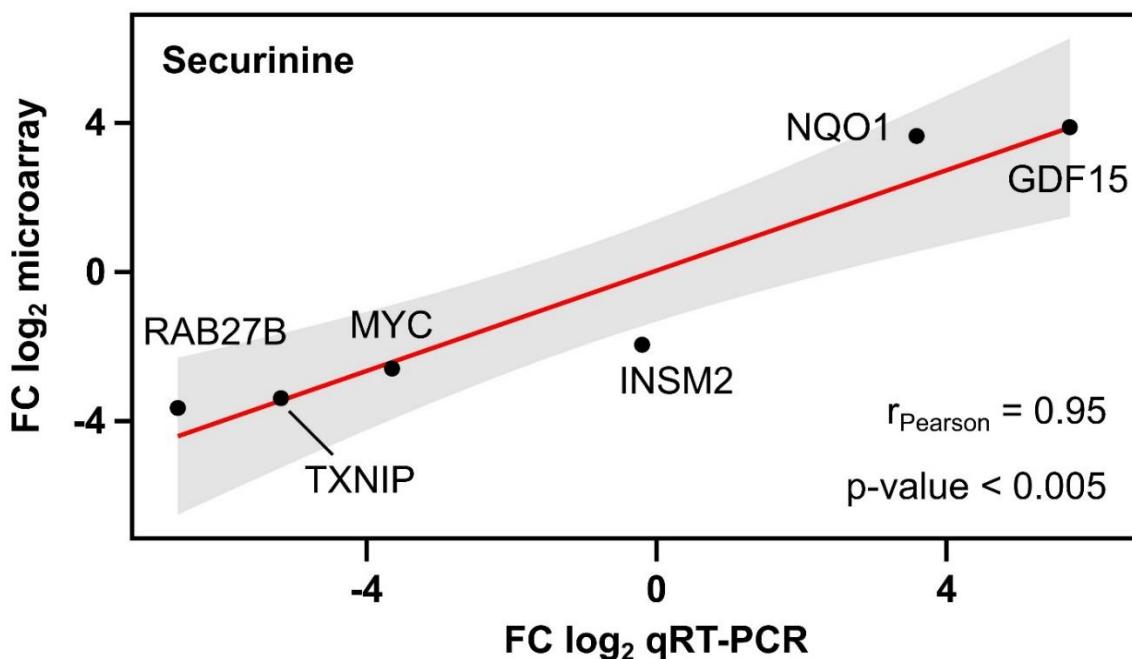
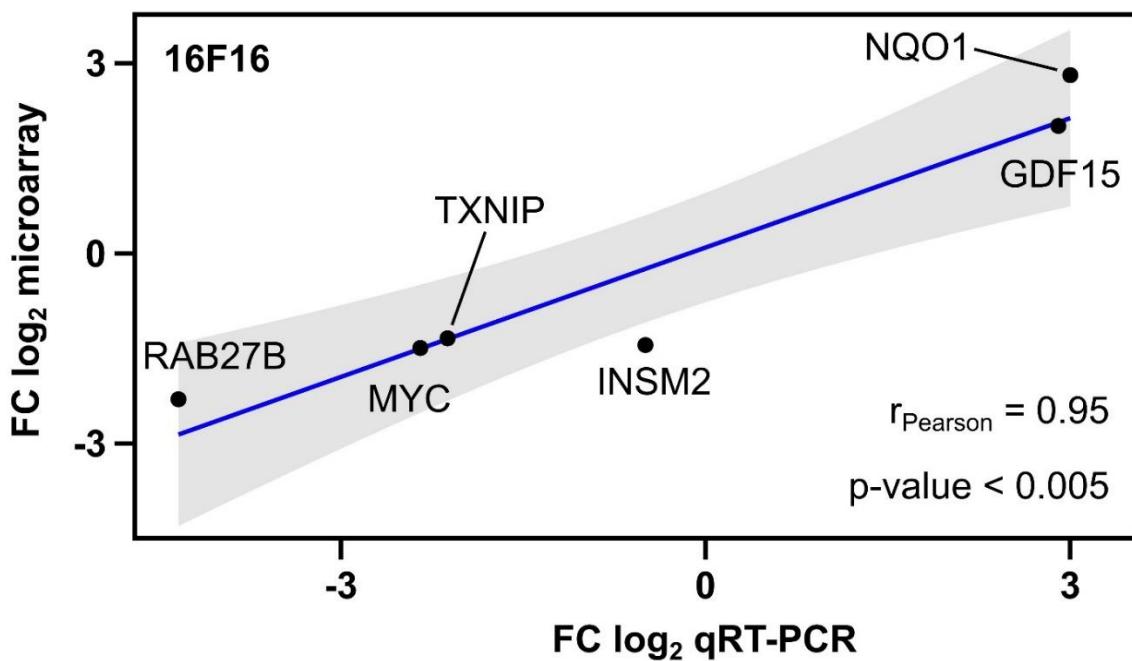
A**B**

Figure S4. Pearson's product-moment correlation coefficient was used to analyze the relationship between gene expression changes determined by microarray and qRT-PCR for either securin treatment (**A**) or 16F16 treatment (**B**). A linear regression curve is shown in red (for securin) or blue (for 16F16); the 95 % confidence interval is depicted as a grey area.

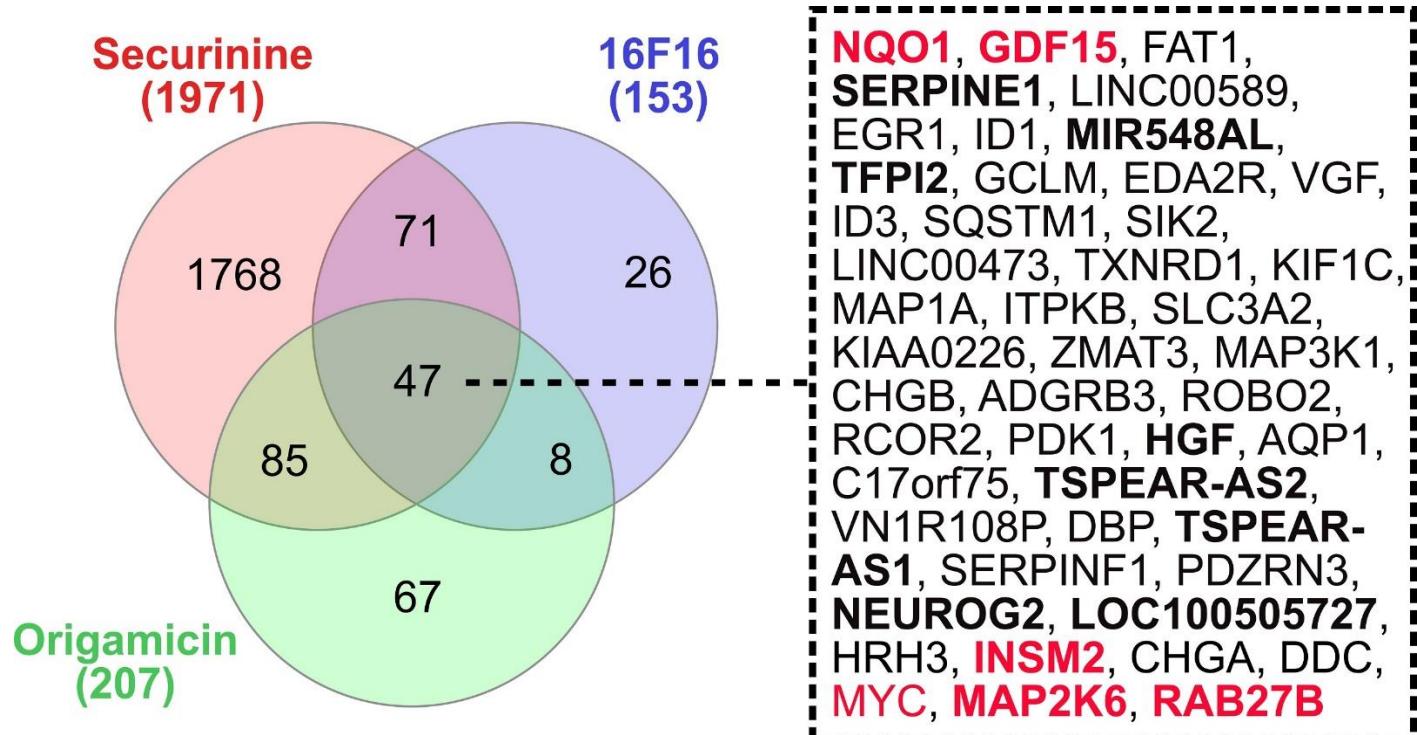


Figure S5. Differentially expressed genes (DEGs) in SH-SY5Y cells upon treatment with the PDI inhibitors of this study—securinine and 16F16—and origamicin (another study¹) The Venn diagram shows DEGs with at least 1.5-fold change. The box on the right present the DEGs commonly found in all three treatments. A fold-change of at least 2 is indicated in bold. DEGs that were validated experimentally are highlighted in red.

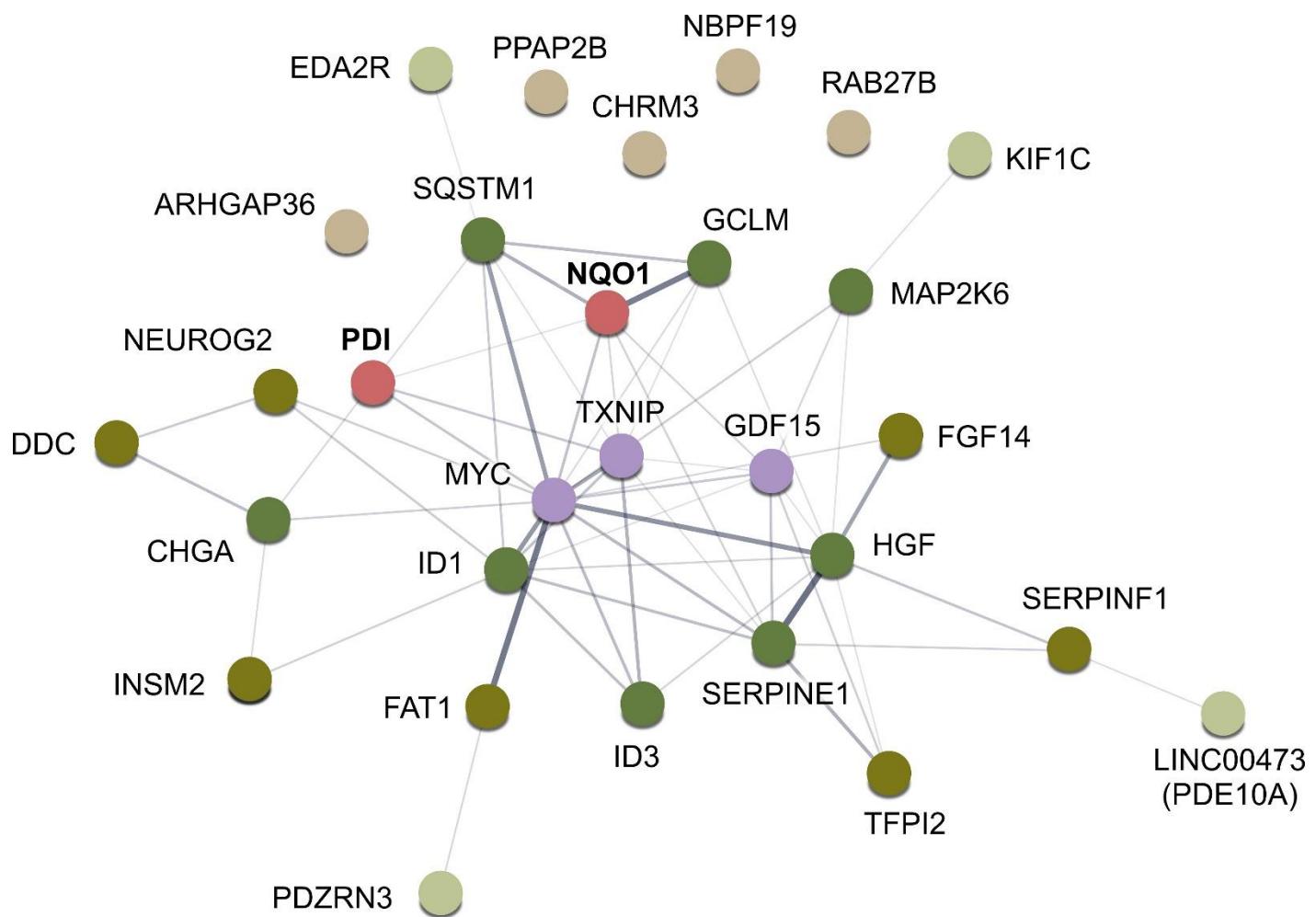


Figure S6. Prediction of protein-protein interactions of commonly identified DEGs in SH-SY5Y cells upon treatment with securinine, and 16F16 using the STRING database². The interaction map shows DEGs with at least 2-fold change. Line thickness indicates strength of data support with a minimum interaction score of 0.200.

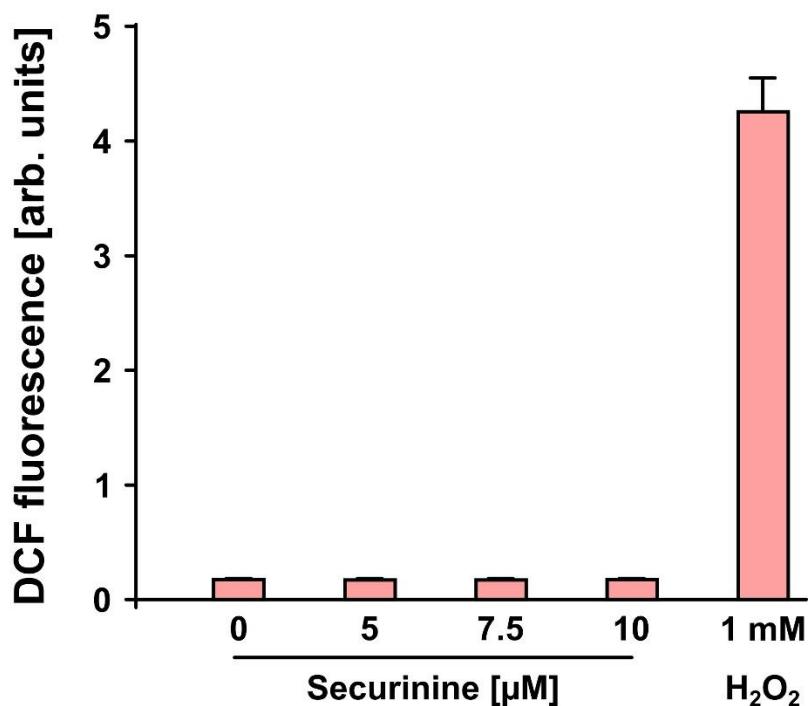
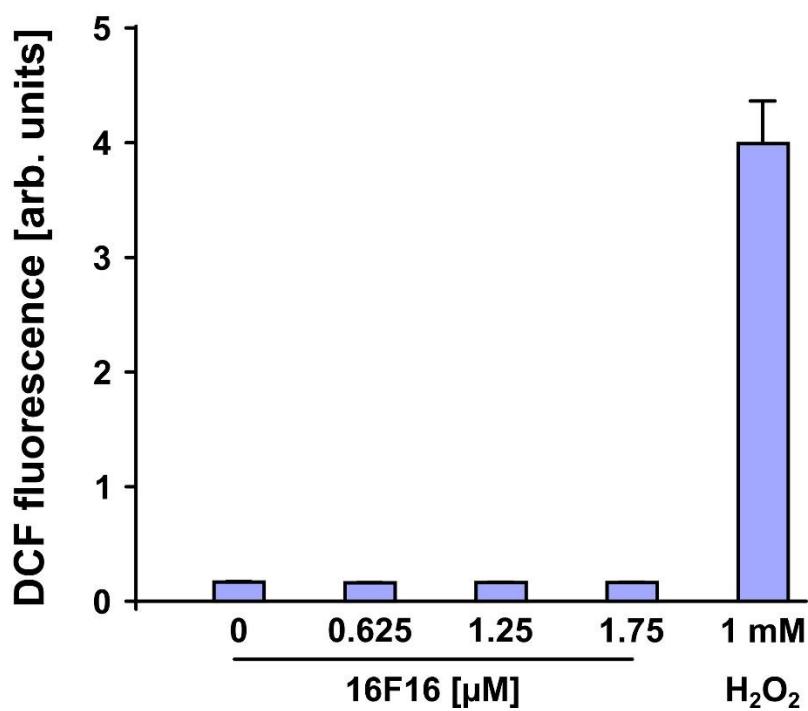
A**B**

Figure S7. The redox status of SH-SY5Y cells after treatment with either securinine (**A**) or 16F16 (**B**) was assessed via oxidation of DCFDA. H₂O₂ (1 mM) served as positive control. Error bars represent standard deviation of three independent experiments.

Supplementary Information

Table S1. Genes with at least 2-fold expression change in both PDI inhibitor treatment regiments

| Gene Symbol | HGNC Gene Title | Securinine | | | 16F16 | | |
|--------------|--|---------------------|------------------|------------------|---------------------|---------|--------------|
| | | log ₂ FC | p-value | adj. p-value | log ₂ FC | p-value | adj. p-value |
| NQO1 | NAD(P)H dehydrogenase, quinone 1 | 3.6476 | 0.0000 | 0.0001 | 2.8131 | 0.0000 | 0.0257 |
| GDF15 | growth differentiation factor 15 | 3.8847 | 0.0000 | 0.0001 | 2.0117 | 0.0000 | 0.0365 |
| FAT1 | FAT atypical cadherin 1 | 2.9316 | 0.0000 | 0.0006 | 1.3823 | 0.0000 | 0.0265 |
| SERPINE1 | serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 | 2.8454 | 0.0000 | 0.0021 | 1.9800 | 0.0001 | 0.0422 |
| LINC00589 | long intergenic non-protein coding RNA 589 | 2.4933 | 0.0000 | 0.0012 | 1.5690 | 0.0000 | 0.0365 |
| NBPF19 | neuroblastoma breakpoint family, member 19 | 2.4861 | 0.0001 | 0.0042 | 2.8665 | 0.0002 | 0.0496 |
| ID1 | inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | 2.3000 | 0.0000 | 0.0014 | 1.5191 | 0.0000 | 0.0365 |
| MIR548AL | microRNA 548al | 2.2745 | 0.0000 | 0.0031 | 1.7743 | 0.0000 | 0.0365 |
| TFPI2 | tissue factor pathway inhibitor 2 | 2.0354 | 0.0000 | 0.0012 | 1.1222 | 0.0001 | 0.0415 |
| GCLM | glutamate-cysteine ligase, modifier subunit | 1.9219 | 0.0000 | 0.0012 | 1.1194 | 0.0000 | 0.0365 |
| EDA2R | ectodysplasin A2 receptor | 1.8044 | 0.0000 | 0.0012 | 1.1751 | 0.0000 | 0.0365 |
| ID3 | inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | 1.5346 | 0.0000 | 0.0029 | 1.3682 | 0.0001 | 0.0368 |
| SQSTM1 | sequestosome 1 | 1.5045 | 0.0000 | 0.0021 | 1.0157 | 0.0000 | 0.0365 |
| RNU6ATAC | RNA, U6atac small nuclear (U12-dependent splicing) | 1.2886 1.1530 | 0.0000 0.0000 | 0.0021 0.0024 | 1.0663 | 0.0000 | 0.0363 |
| LINC00473 | long intergenic non-protein coding RNA 473 | 1.1065 | 0.0001 | 0.0053 | 1.4160 | 0.0001 | 0.0376 |
| KIF1C | kinesin family member 1C | 1.0780 | 0.0001 | 0.0042 | 1.0096 | 0.0001 | 0.0368 |
| SNORD75 | small nucleolar RNA, C/D box 75 | 1.0057 | 0.0001 | 0.0053 | 1.0175 | 0.0001 | 0.0368 |
| HGF | hepatocyte growth factor (hepapoitin A; scatter factor) | -1.0683 | 0.0012 | 0.0189 | -1.4711 | 0.0002 | 0.0471 |
| ARHGAP36 | Rho GTPase activating protein 36 | -1.1189 | 0.0000 | 0.0024 | -1.0130 | 0.0000 | 0.0368 |
| FGF14 | fibroblast growth factor 14 | -1.1516 | 0.0000 | 0.0028 | -1.2650 | 0.0000 | 0.0365 |
| SNORD72 | small nucleolar RNA, C/D box 72 | -1.1635 | 0.0001 | 0.0060 | -1.0084 | 0.0002 | 0.0422 |
| TSPEAR-AS2 | TSPEAR antisense RNA 2 | -1.2422 | 0.0001 | 0.0048 | -1.3948 | 0.0000 | 0.0363 |
| CHRM3 | cholinergic receptor, muscarinic 3 | -1.2570 | 0.0000 | 0.0033 | -1.0878 | 0.0001 | 0.0408 |
| TSPEAR-AS1 | TSPEAR antisense RNA 1 | -1.4352 | 0.0000 | 0.0031 | -1.7121 | 0.0000 | 0.0335 |
| SERPINF1 | serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 | -1.5028 | 0.0000 | 0.0021 | -1.2488 | 0.0001 | 0.0368 |
| PDZRN3 | PDZ domain containing ring finger 3 | -1.5080 | 0.0002 | 0.0072 | -1.0481 | 0.0001 | 0.0421 |
| NEUROG2 | neurogenin 2 | -1.5655 | 0.0000 | 0.0023 | -1.8318 | 0.0000 | 0.0265 |
| PPAP2B | phosphatidic acid phosphatase type 2B | -1.5693 | 0.0000 | 0.0027 | -1.0480 | 0.0000 | 0.0365 |
| LOC100505727 | uncharacterized LOC100505727 | -1.7248 | 0.0000 | 0.0018 | -1.4389 | 0.0000 | 0.0363 |
| INSM2 | insulinoma-associated 2 | -1.9511 | 0.0000 | 0.0012 | -1.4460 | 0.0000 | 0.0365 |
| CHGA | chromogranin A | -2.0832 | 0.0000 | 0.0019 | -1.3246 | 0.0000 | 0.0265 |
| DDC | dopa decarboxylase (aromatic L-amino acid decarboxylase) | -2.1011 | 0.0000 | 0.0013 | -1.0465 | 0.0002 | 0.0422 |
| MYC | v-myc avian myelocytomatosis viral oncogene homolog | -2.5890 | 0.0000 | 0.0012 | -1.4931 | 0.0002 | 0.0496 |
| MAP2K6 | mitogen-activated protein kinase kinase 6 | -2.6750 | 0.0000 | 0.0023 | -1.1492 | 0.0000 | 0.0365 |
| TXNIP | thioredoxin interacting protein | -3.3847 | 0.0000 | 0.0006 | -1.3395 | 0.0001 | 0.0396 |
| RAB27B | RAB27B, member RAS oncogene family | -3.6471 | 0.0000 | 0.0014 | -2.3015 | 0.0000 | 0.0265 |

Supplementary Information

Table S2. Selected genes for qRT-PCR validation

| Gene Symbol | HGNC Gene Title | Securinine | | 16F16 | |
|-------------|---|---------------------------|-----------------------------|---------------------------|-----------------------------|
| | | log ₂ FC array | log ₂ FC qRT-PCR | log ₂ FC array | log ₂ FC qRT-PCR |
| NQO1 | NAD(P)H dehydrogenase, quinone 1 | 3.6476 | 3.5889 | 2.8131 | 3.0018 |
| GDF15 | growth differentiation factor 15 | 3.8847 | 5.7024 | 2.0117 | 2.9036 |
| TXNIP | Thioredoxin-interacting protein | -3.3847 | -5.1826 | -1.3395 | -2.1221 |
| INSM2 | insulinoma-associated 2 | -1.9511 | -0.1988 | -1.4460 | -0.4942 |
| MYC | v-myc avian myelocytomatosis viral oncogene homolog | -2.5890 | -3.6492 | -1.4931 | -2.3464 |
| RAB27B | RAB27B, member RAS oncogene family | -3.6471 | -6.6056 | -2.3015 | -4.3365 |

Supplementary Information

Table S3. List of oligonucleotides used in this study

| Gene | Direction | Sequence |
|--------|-----------|-------------------------------|
| NQO1 | forward | 5'-CTGCCATCATGCCTGACTAA-3' |
| | reverse | 5'-TGCAGATGTACGGTGTGGAT-3' |
| GDF15 | forward | 5'-CTCCAGATTCCGAGAGTTGC-3' |
| | reverse | 5'-AGAGATAACGCAGGTGCAGGT-3' |
| TXNIP | forward | 5'-ACTCGTGTCAAAGCCGTTAGG-3' |
| | reverse | 5'-TCCCTGCATCCAAGCACTT-3' |
| INSM2 | forward | 5'-TCCCTCCTCTGACCTCCGA-3' |
| | reverse | 5'-ATCAGGAGTGGGAGCGGGTC-3' |
| MYC | forward | 5'-TGCTGCCAAGAGGGTCAAGT-3' |
| | reverse | 5'-GTGTGTTCGCCTCTTGACATT-3' |
| RAB27B | forward | 5'-TGGCAACAAGGCAGACCTACCA-3' |
| | reverse | 5'-CTCCACATTCTGTCCAGTTGCTG-3' |

Supplementary References

1. Özcelik, D.; Pezacki, J. P., Small Molecule Inhibition of Protein Disulfide Isomerase in Neuroblastoma Cells Induces an Oxidative Stress Response and Apoptosis Pathways. *ACS Chem Neurosci* 2019, 10 (9), 4068-4075.
2. Szklarczyk, D.; Gable, A. L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N. T.; Morris, J. H.; Bork, P.; Jensen, L. J.; Mering, C. V., STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019, 47 (D1), D607-D613.