# APPENDIX FIGURES AND TABLE
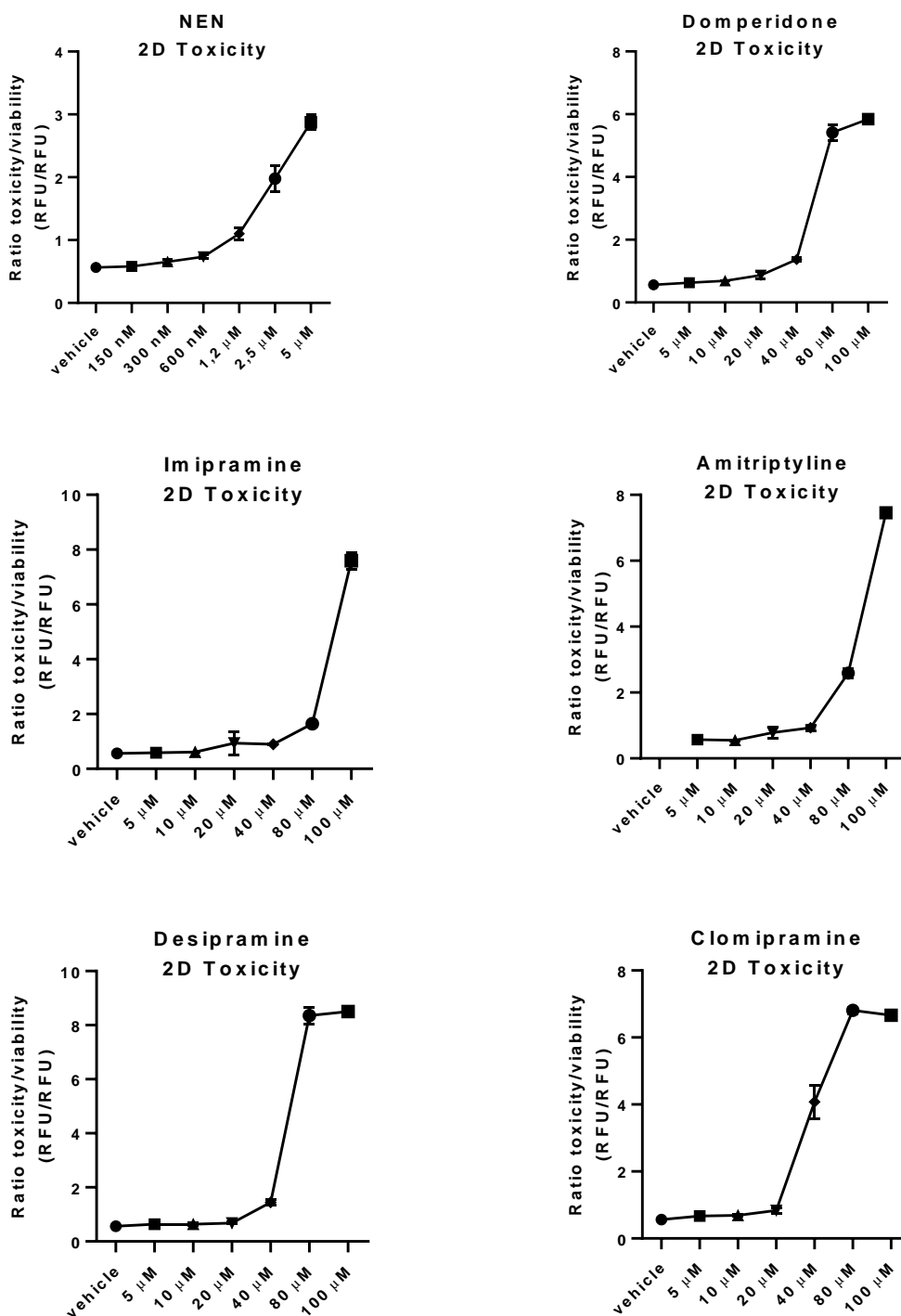
**Table of content**

# Appendix Figure S1: Dose-response analyses for the individual drugs



Legend: HCT116 cells were treated with increasing concentrations of NEN and the individual drugs as indicated. Graphs shows the ratio of toxicity and viability measured after a 24h treatment (N=3). The concentrations used for determination of cytotoxicity effects throughout the study were NEN 1.2 µM; Domperidone (Domp), Imipramine (Imi), Desipramine (Desi) and Amitriptyline (Ami), each 30 µM; Clomipramine (Clomi) 20 µM.

# Appendix Figure S2: GO gene set enrichment analysis (GSEA) of transcriptome data
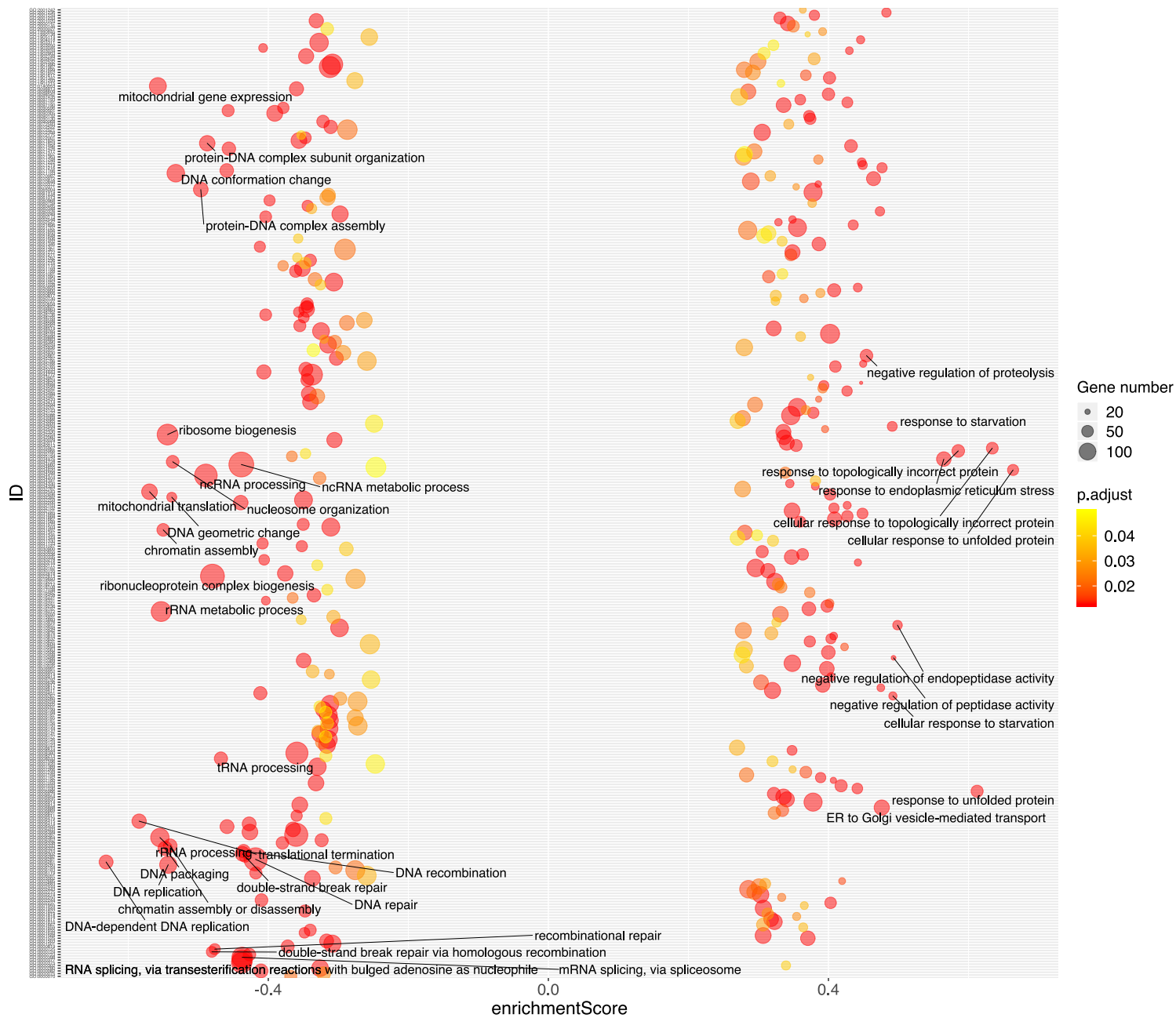


Legend: GO gene set enrichment analysis (GSEA) of microarray data from HCT116 cells upon double treatment with NEN (1.2µM) and Domperidone (30µM) for 16 hours vs. vehicle control treatment (N=3).

# Appendix Figure S3: Heat map of differentially expressed genes (DEGs) from the transcriptome analysis
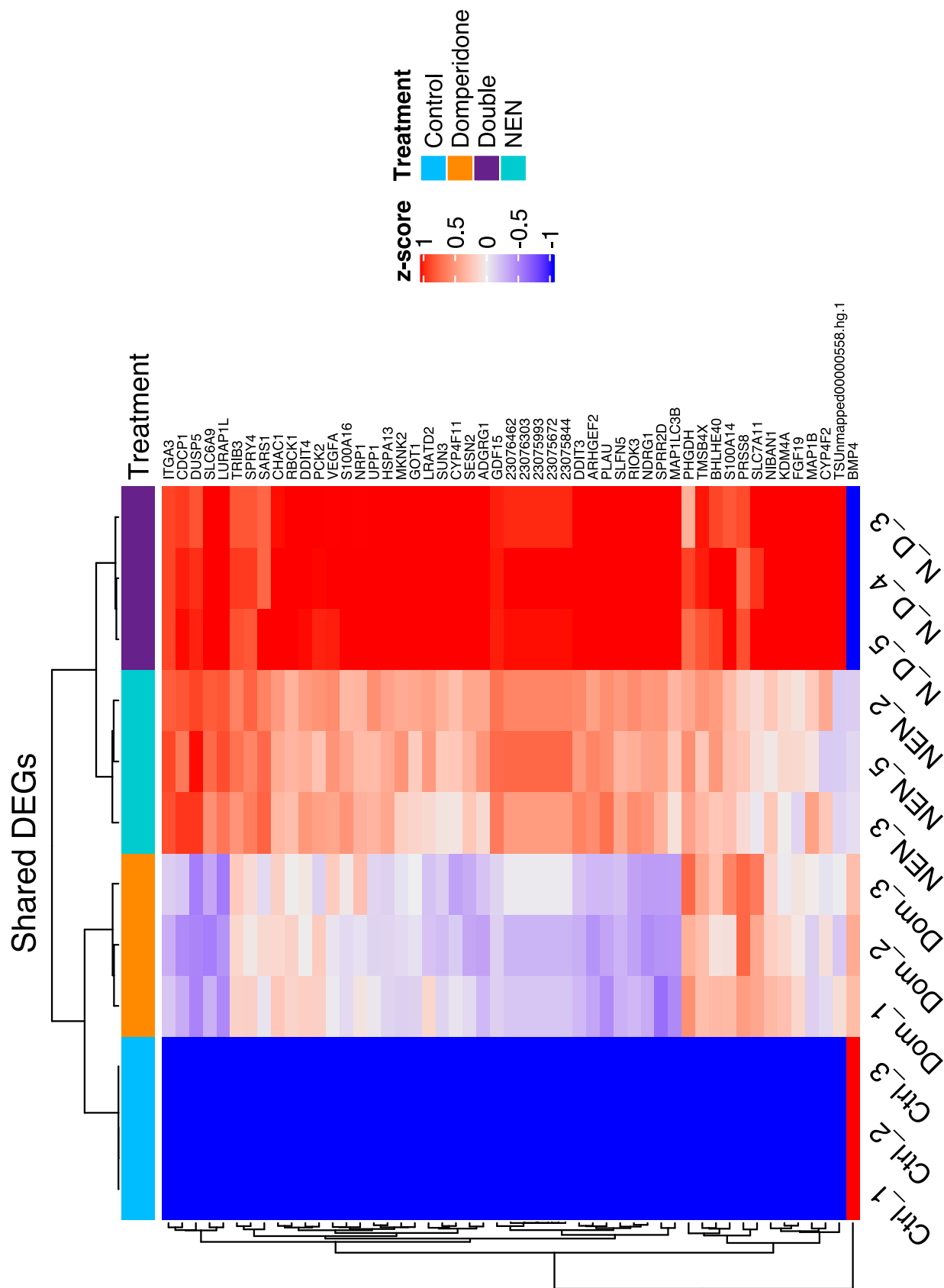


Legend: Differentially expressed genes determined from the microarray analysis of HCT116 cells upon treatment with vehicle control (Ctrl), NEN (1.2μM), Domperidone (Dom, 30μM) or double treatment with NEN and Domperidone for 16 hours. Genes showing synergistic induction upon double treatment in comparison to single on control treatment are shown.

# Appendix Figure S4A: Induction of ISR proteins upon drug treatments
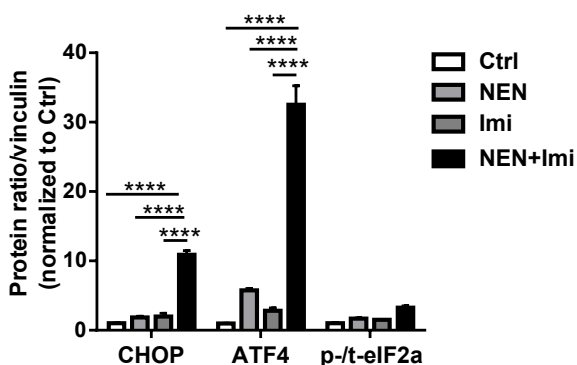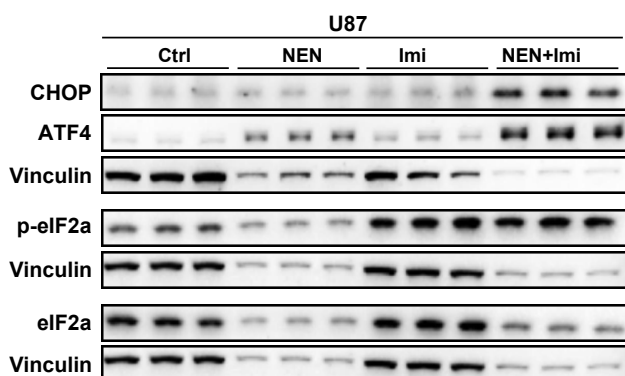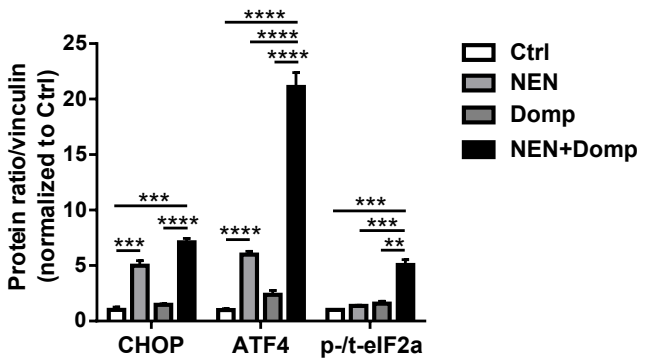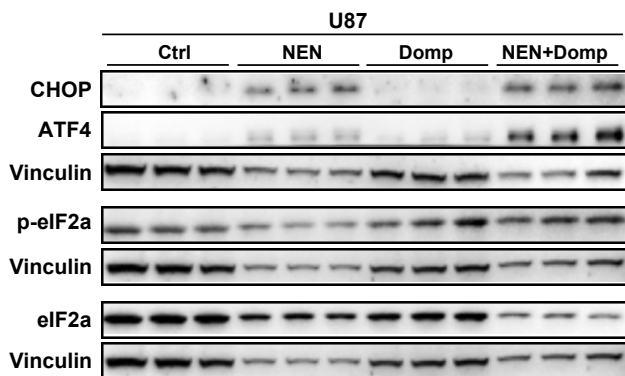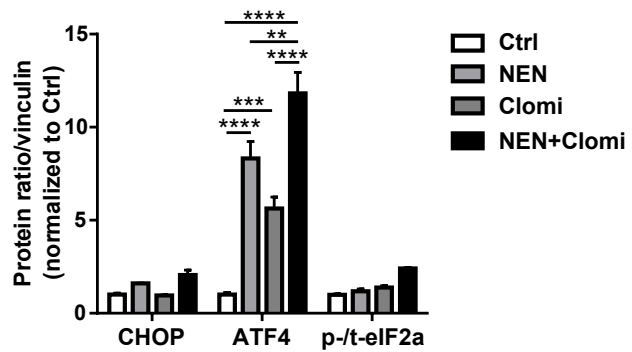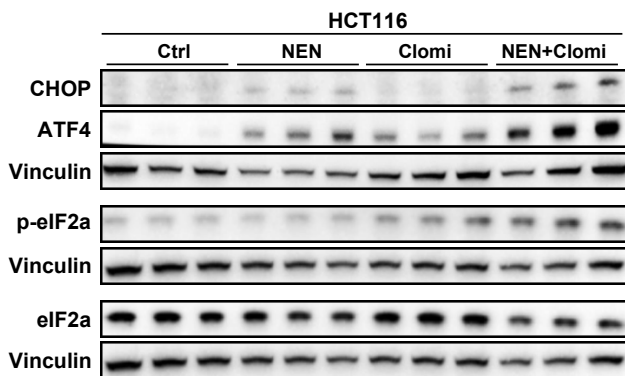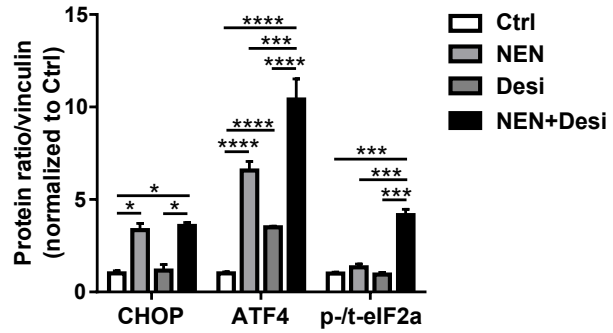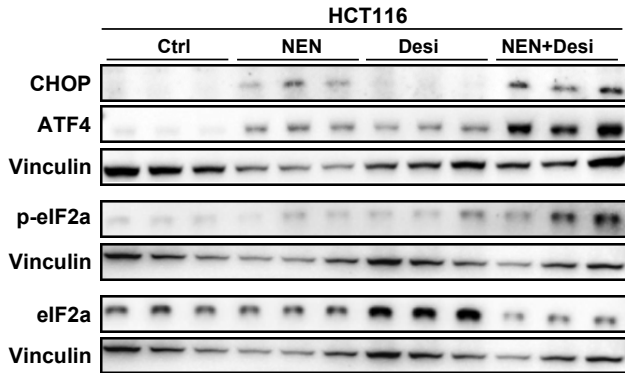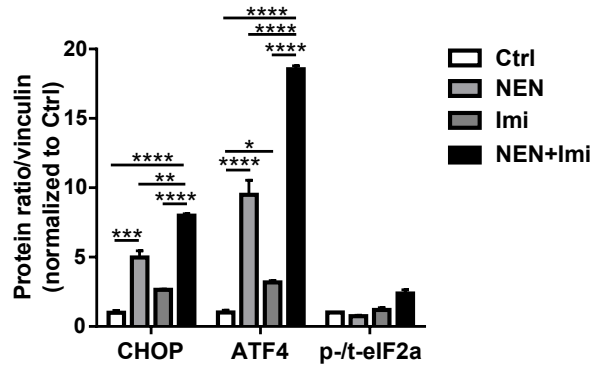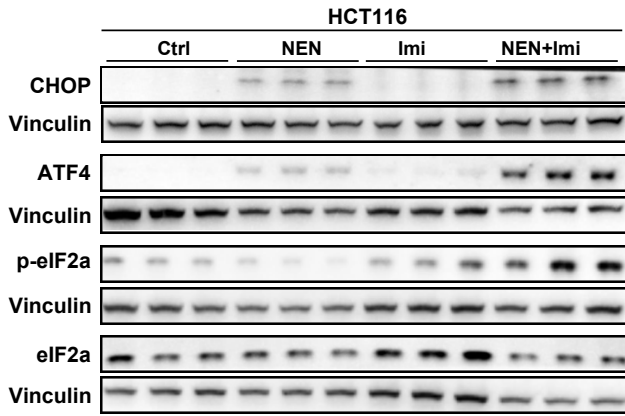
# Appendix Figure S4B: Induction of ISR proteins upon drug treatments
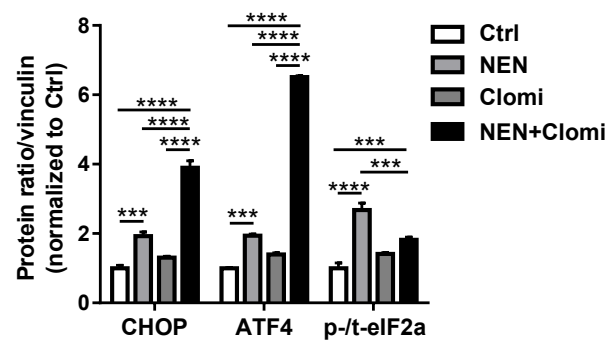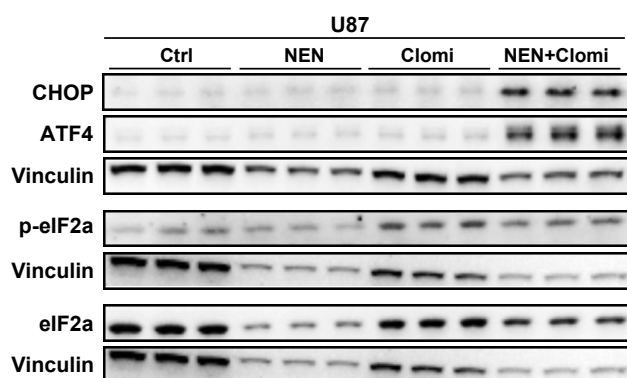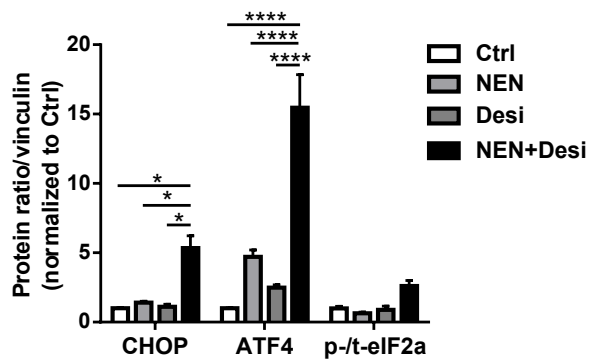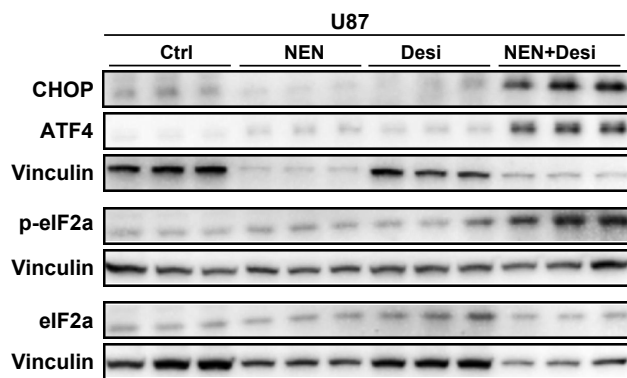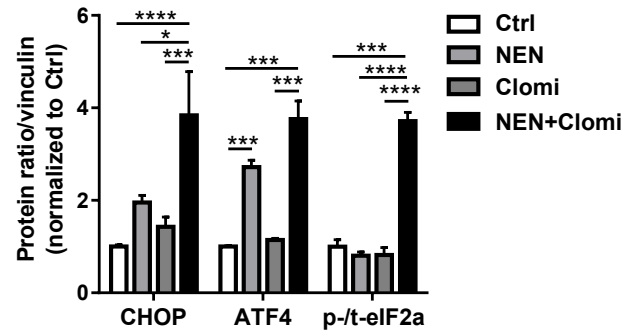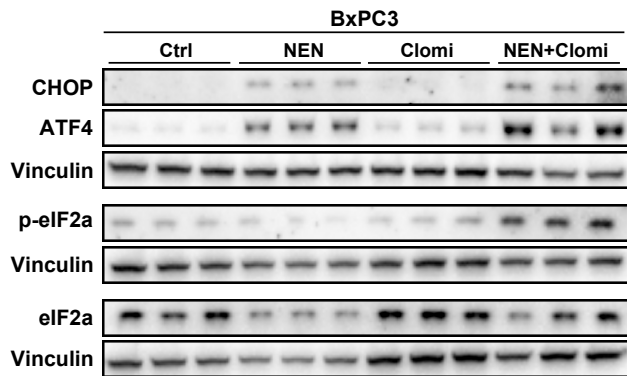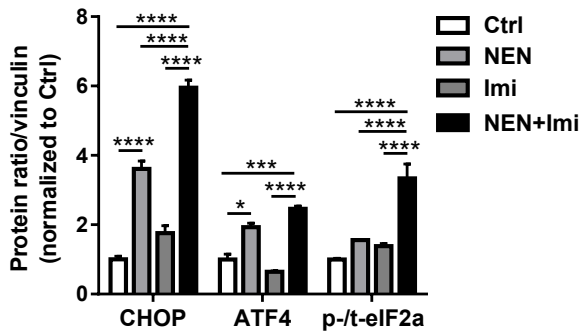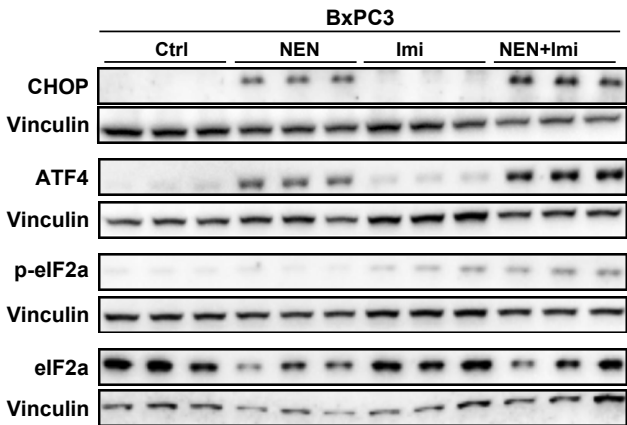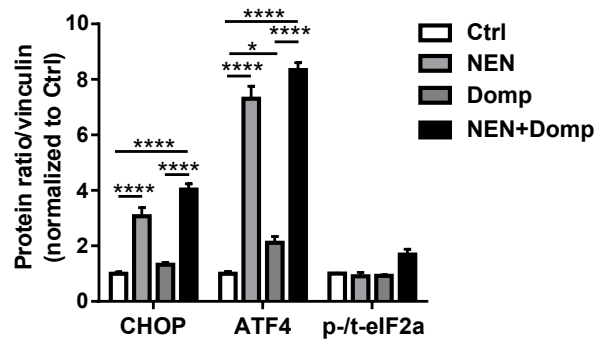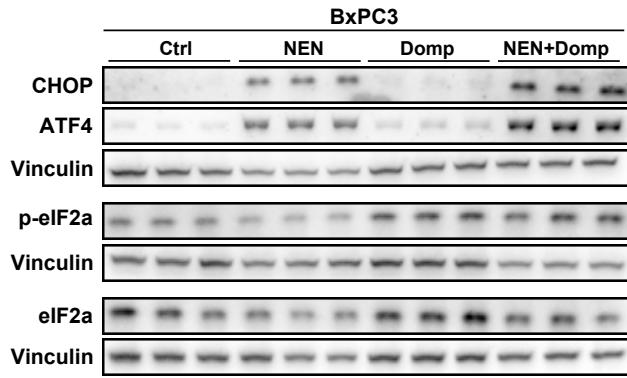
# Appendix Figure S4A and for B

Legend:

A Immunoblot analysis including corresponding densitometric quantifications of CHOP, ATF4, phosphorylated (p-) and t-) eIF2alpha (eIF2a) of HCT116 and U87 cells treated as indicated for 16h or 24h, respectively (NEN 1.2 µM; Domperidone (Domp), Imipramine (Imi), Desipramine (Desi), each 30 µM; Clomipramine (Clomi) 20 µM).

B Immunoblot analysis including corresponding densitometric quantifications of CHOP, ATF4, phosphorylated (p-) and t-) eIF2alpha (eIF2a) of BxPC3 and U87 cells treated as indicated for 24h (NEN 1.2 µM; Domperidone (Domp), Imipramine (Imi), Desipramine (Desi), each 30 µM; Clomipramine (Clomi) 20 µM).

Data information: Data are presented as mean (SD) (N=3) and were analyzed two-way ANOVA with Tukey post-hoc test. * $p < 0.05$; ** $p < 0.01$;*** $p < 0.001$; **** $p < 0.0001$.

# Appendix Figure S5: WikiPathways gene set enrichment analysis (GSEA) of transcriptome data
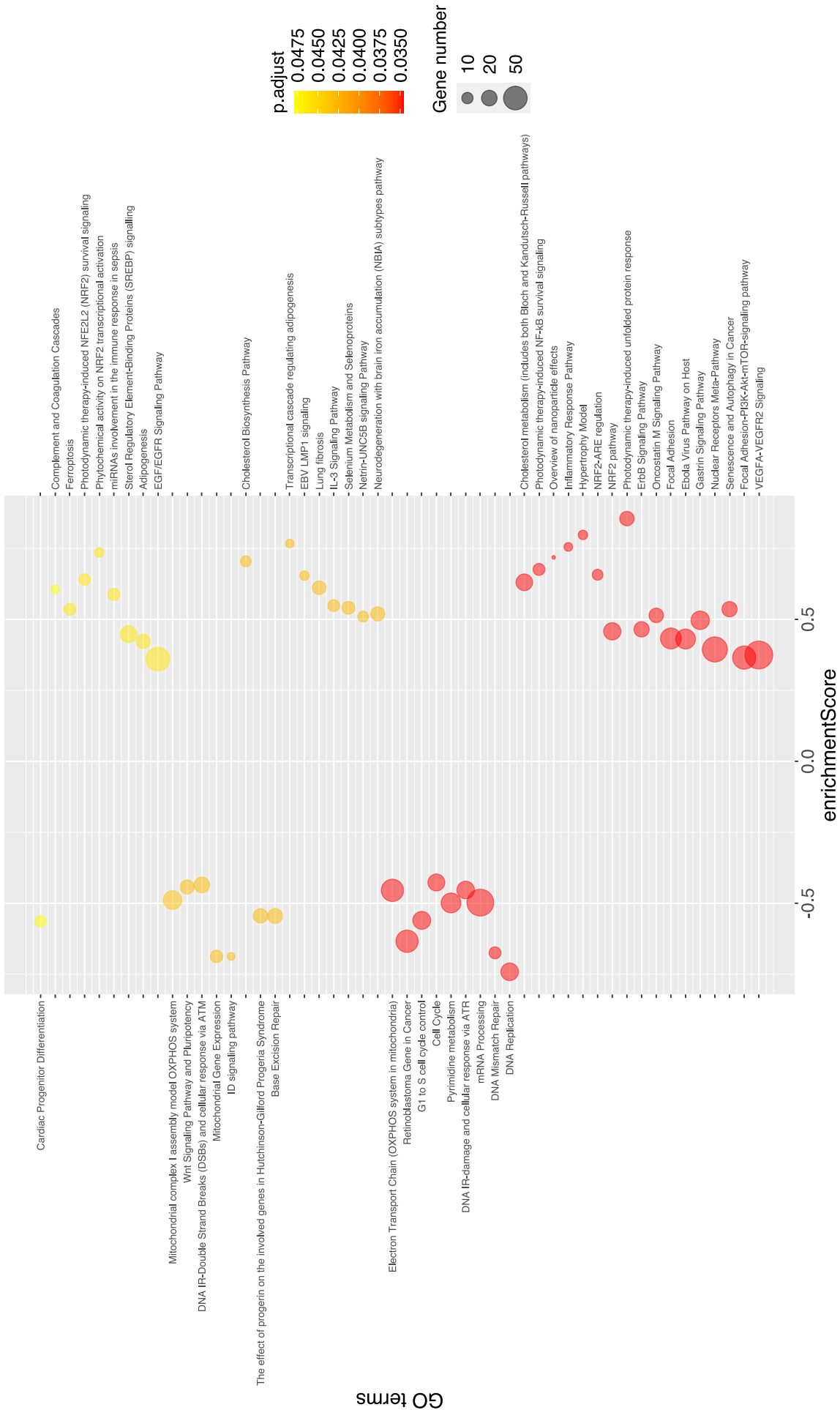


Legend: Wikipathway gene set enrichment analysis (GSEA) of microarray data from HCT116 cells upon double treatment with NEN (1.2µM) and Domperidone (30µM) for 16 hours vs. vehicle control treatment (N=3).

Appendix Figure S6: Heat map of CLEAR network gene expression from the transcriptome analysis
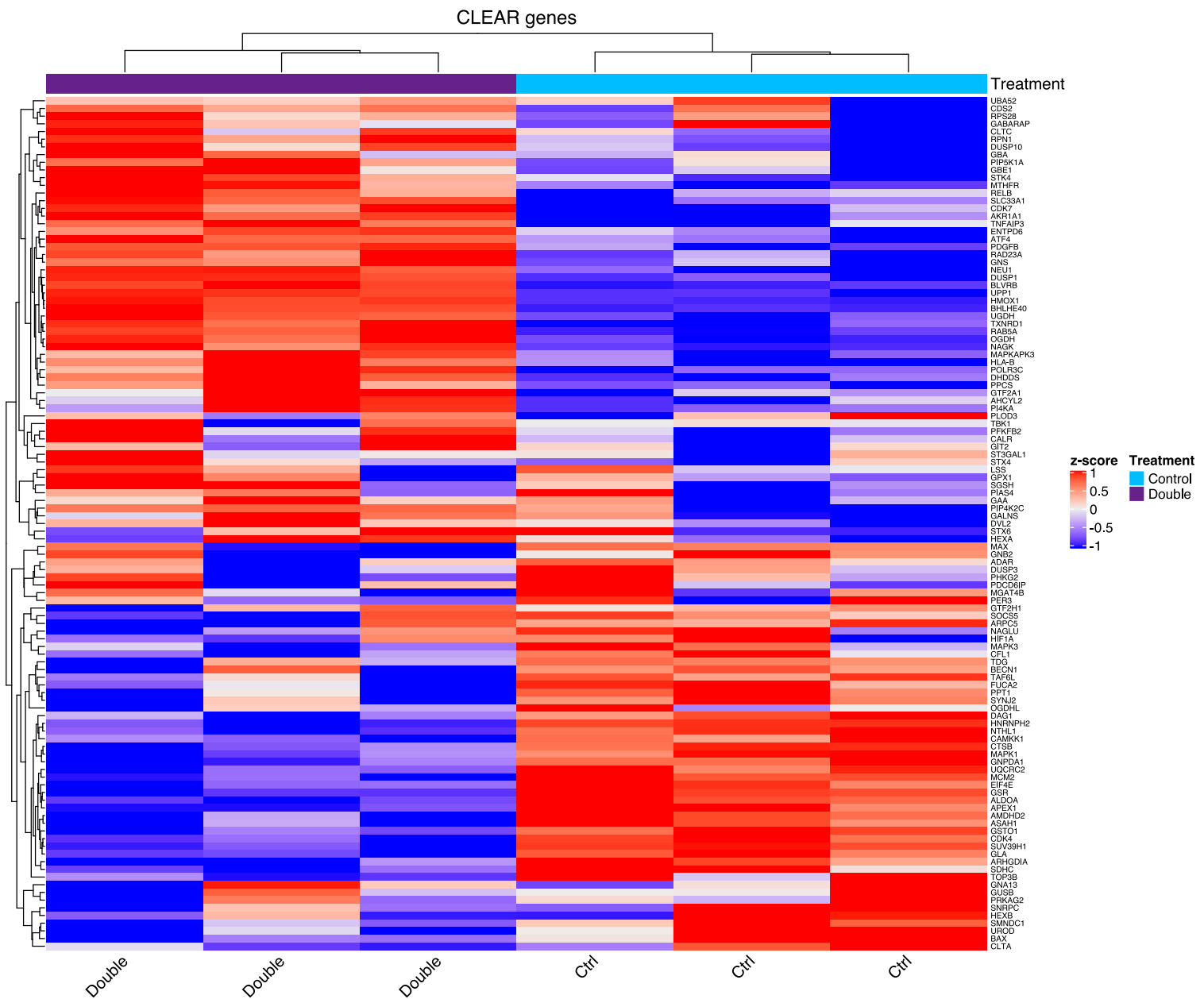
Legend: Expression of genes defining the CLEAR network as reported in Palmieri et al. (2011, Hum Mol Genet 20: 3852-3866, Table 2) from the microarray analysis of HCT116 cells upon treatment with vehicle control (Ctrl), NEN (1.2µM), Domperidone (Dom, 30µM) or double treatment with NEN and Domperidone for 16 hours. Of the 127 gene, 16 were missing from the expression matrix (probably filtered out due to low expression or they were missing from the Affymetrix chip).

Appendix Figure S7: Volcano blot of DEGs from the transcriptome analysis



**Double treatment vs Control**
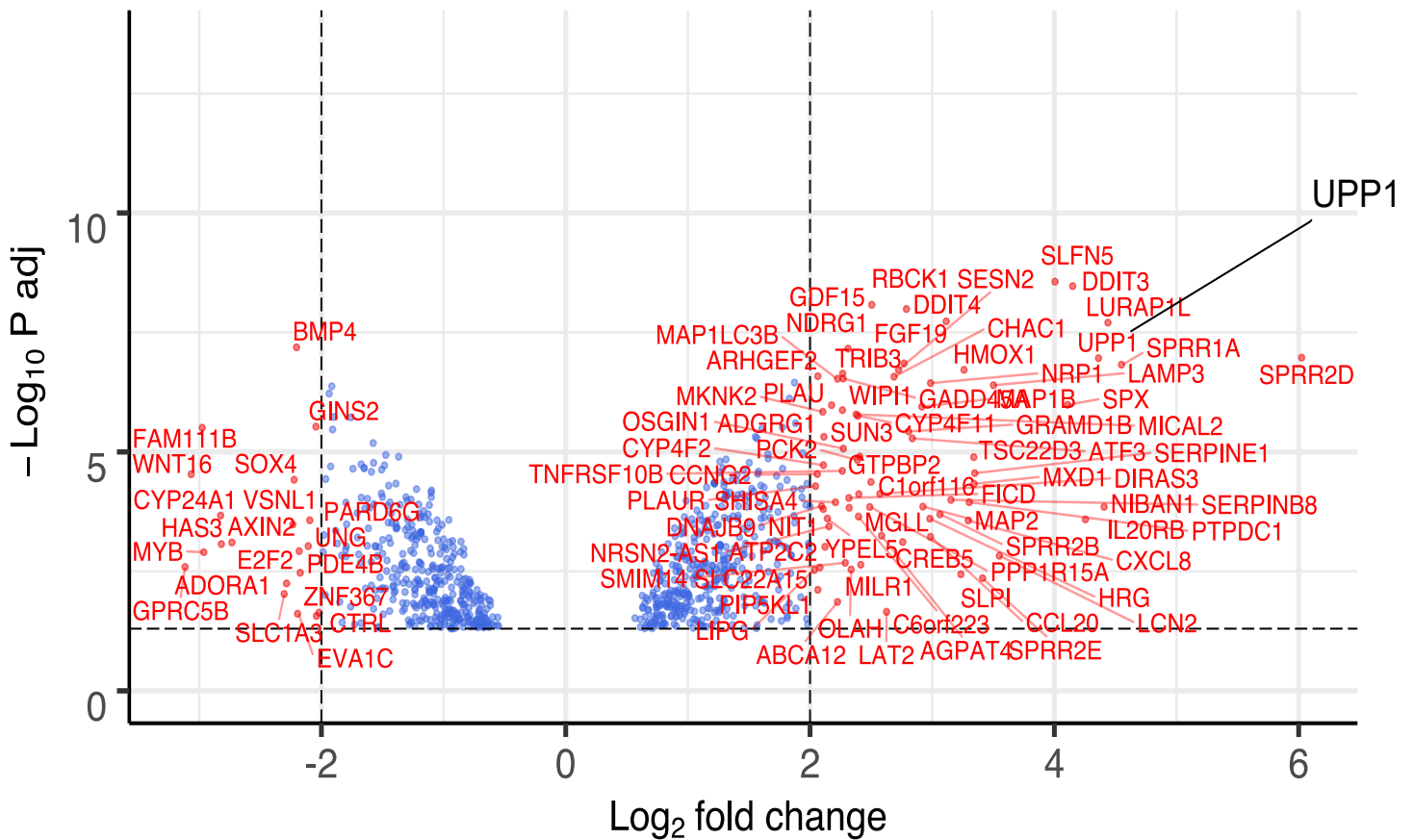
Total = 857 genes

Legend: Volcano blot of differentially expressed genes determined from the microarray analysis from HCT116 cells upon double treatment with NEN (1.2μM) and Domperidone (30μM) for 16 hours vs. vehicle control treatment (N=3). UPP1 is highlighted as one of the strongest upregulated genes upon combined drug treatment.

# Appendix Figure S8: Induction of UPP1 upon drug treatments
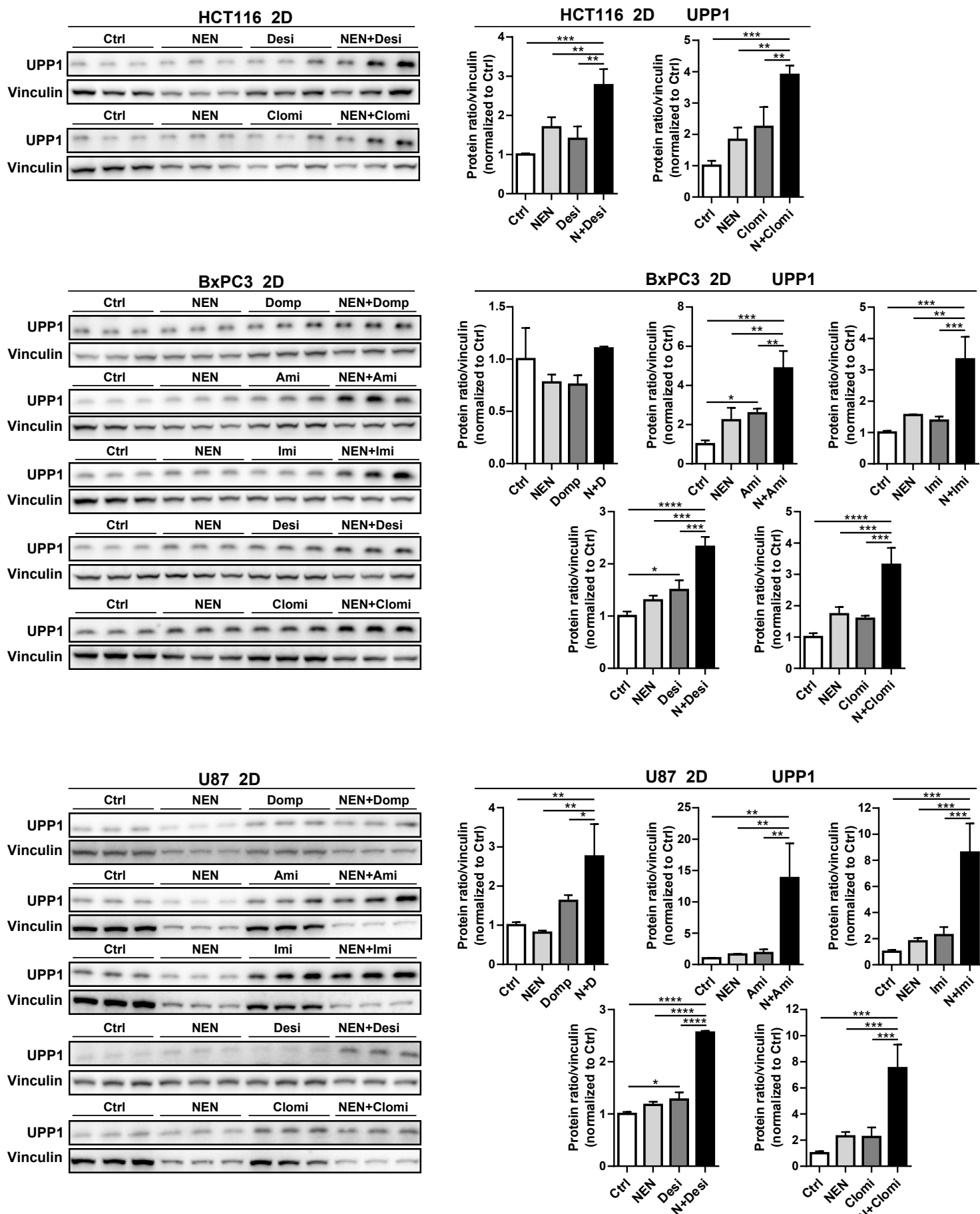


Legend:Immunoblot analysis including corresponding densitometric quantifications of UPP1 in HCT116, BxPC3 and U87 cells treated as indicated for 16h (HCT116) or 24h (BxPC3, U87) (NEN 1.2 μM Domepridone (Domp), Imipramine (Imi), Amitiptylin (Ami), 30μM each, and Clomipramine 20μM). Protein bands were normalized to vinculin (loading control) and were analyzed by one-way ANOVA and Tukey post-hoc test.
* p<0.05; **p<0.01; ***p<0.001; **** p<0.0001.

**APPENDIX TABLE S1**

**Statistical tests and exact p-values**

| Figure panel | Test and post hoc test | p-value (main test) | Groups for post hoc comparisons | Adjusted p-values for multiple comparisons |
|---|---|---|---|---|
| Figure 1A | two-way ANOVA with Tukey post-hoc test | < 0.0001 | NEN:Ctrl vs. NEN:2-DG<br>FCCP:Ctrl vs. FCCP:2-DG | 0.0001<br>0.0337 |
| Figure 1D | HCT116:<br>two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Domp vs. NEN:Domp<br>Ctrl:Imi vs. NEN:Imi<br>Ctrl:Ami vs. NEN:Ami<br>Ctrl:Desi vs. NEN:Desi<br>Ctrl:Clomi vs. NEN:Clomi | < 0.0001<br>< 0.0001<br>< 0.0001<br>< 0.0001<br>< 0.0001 |
| | U87:<br>two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Ctrl vs. NEN:Ctrl<br>Ctrl:Domp vs. NEN:Domp<br>Ctrl:Imi vs. NEN:Imi<br>Ctrl:Ami vs. NEN:Ami<br>Ctrl:Desi vs. NEN:Desi<br>Ctrl:Clomi vs. NEN:Clomi | 0.0332<br>< 0.0001<br>< 0.0001<br>0.0001<br>< 0.0001<br>< 0.0001 |
| | BxPC3:<br>two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Domp vs. NEN:Domp<br>Ctrl:Imi vs. NEN:Imi<br>Ctrl:Ami vs. NEN:Ami<br>Ctrl:Desi vs. NEN:Desi<br>Ctrl:Clomi vs. NEN:Clomi | < 0.0001<br>< 0.0001<br>< 0.0001<br>0.4315 (ns)<br>< 0.0001 |
| Figure 1E | HCT116:<br>one-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl vs. NEN<br>Ctrl vs. N + D<br>Ctrl vs. N + D + zVAD<br>Ctrl vs. Staurospor.<br>N + D vs. N + D + zVAD<br>N + D vs. Staurospor. | 0.0002<br>< 0.0001<br>< 0.0001<br>< 0.0001<br>0.0007<br>0.0052 |
| | U87:<br>Kruskal-Wallis and Dunn´s post-hoc test | 0.0075 | Ctrl vs. N + D<br>Domp vs. N + D | 0.0206<br>0.0050 |

| Figure 2A | HCT116; CHOP: (upper left graph) one-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl vs. N+D<br>NEN vs. N+D<br>Domp vs. N+D | < 0.0001<br>< 0.0001<br>< 0.0001 |
|---|---|---|---|---|
| | (upper right graph) Kruskal-Wallis and Dunn´s post-hoc test | < 0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+Imi<br>Ctrl vs. NEN+Ami<br>Ctrl vs. NEN+Desi<br>Ctrl vs. NEN+Clomi | 0.0355<br>0.0049<br>0.0003<br>0.0001<br>0.0004 |
| | HCT116; ATF4: (lower left graph) one-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl vs. N+D<br>NEN vs. N+D<br>Domp vs. N+D | < 0.0001<br>< 0.0001<br>0.0004 |
| | (lower right graph) Kruskal-Wallis and Dunn´s post-hoc test | < 0.0001 | Ctrl vs. NEN+Imi<br>Ctrl vs. NEN+Ami<br>Ctrl vs. NEN+Desi<br>Ctrl vs. NEN+Clomi | 0.0088<br>0.0017<br>0.0003<br>0.0011 |
| Figure 2C | two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Crtl-siRNA vs. N+D:Crtl-siRNA<br>N+D:Crtl-siRNA vs. N+D:siATF4<br>N+D:Crtl-siRNA vs. N+D:siCHOP | < 0.0001<br><br>0.1369 (ns)<br><br>< 0.0001 |
| Figure 2D | one-way ANOVA with Tukey post-hoc test | GADD34 <0.0001<br><br>CHOP<br><br><br>ATF4 | GADD34<br>Ctrl vs. N+D<br>N+D vs. N+D+ISRIB<br>CHOP<br>Ctrl vs. N+D<br>N+D vs. N+D+ISRIB<br>ATF4<br>Ctrl vs. N+D<br>N+D vs. N+D+ISRIB | <br><0.0001<br>0.0001<br><br>< 0.0001<br>< 0.0001<br><br>0.0002<br>0.0046 |
| Figure 2E | two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Ctrl vs. NEN:Domperidone<br>NEN:Domperidone vs. NEN:ISRIB | < 0.0001<br><br>< 0.0001 |
| Figure 2F | two-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl:NEN+Domp vs. ISRIB:NEN+Domp<br>Ctrl:NEN+Imi vs. ISRIB:NEN+Imi<br>Ctrl:NEN+Ami vs. ISRIB:NEN+Ami<br>Ctrl:NEN+Desi vs. ISRIB:NEN+Desi<br>Ctrl:NEN+Clomi vs. ISRIB:NEN+Clomi | < 0.0001<br><br>< 0.0001<br><br>< 0.0001<br><br>< 0.0001<br><br>< 0.0001 |

| Figure | Test | p-value | Comparison | p-value |
|---|---|---|---|---|
| Figure 3A | TFE3: (left graph):<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. Domp<br>Ctrl vs. N+D<br>NEN vs. N+D<br>Domp vs. N+D | < 0.0001<br>0.0118<br><0.0001<br><0.0001<br><0.0001 |
| | TFE3 (right graph):<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN+Imi<br>Ctrl vs. NEN+Ami<br>Ctrl vs. NEN+Desi<br>Ctrl vs. NEN+Clomi | 0.0052<br>0.0482<br><0.0001<br><0.0001 |
| | CD68 (left graph):<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. Domp<br>Ctrl vs. N+D | 0.0002<br><0.0001<br><0.0001 |
| | CD68 (right graph):<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN+Imi<br>Ctrl vs. NEN+Ami<br>Ctrl vs. NEN+Desi<br>Ctrl vs. NEN+Clomi | <0.0001<br><0.0001<br><0.0001<br><0.0001 |
| Figure 3B | Lamp1 (top panel):<br>Kruskal-Wallis with Dunn´s post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+Domp<br>NEN vs. NEN+Domp<br>Domp vs. NEN+Domp | 0.0008<br><0.0001<br>0.0057<br><0.0001 |
| | LC3 (middle panel):<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+Domp<br>NEN vs. Domp<br>NEN vs. NEN+Domp<br>Domp vs. NEN+Domp | <0.0001<br><0.0001<br>0.0468<br><0.0001<br><0.0001 |
| | p62 (lower panel):<br>Kruskal-Wallis with Dunn´s post-hoc test | <0.0001 | Ctrl vs. NEN<br>NEN vs. Domp<br>Domp vs. NEN+Domp | 0.0008<br><0.0001<br><0.0001 |
| Figure 3C | LC3-II/I protein ratio:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+Domp<br>NEN vs. NEN+Domp | 0.0387<br><0.0001<br><0.0001 |
| | p62 levels:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN+Domp<br>NEN vs. NEN+Domp | <0.0001<br>0.0002 |
| Figure 3D | Kruskal-Wallis with Dunn´s post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+Domp | 0.0461<br>0.0007 |
| Figure 4B | UPP1, left graph:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. Domp<br>Ctrl  vs. N+D<br>NEN vs. N+D | 0.0002<br>0.0002<br><0.0001 |

| | | | | |
|---|---|---|---|---|
| | UPP1, right graph: one-way ANOVA with Tukey post-hoc test | <0.0001 | Domp vs. N+D | <0.0001 |
| | | | Ctrl vs. NEN+Imi | <0.0001 |
| | | | Ctrl vs. NEN+Ami | <0.0001 |
| | | | Ctrl vs. NEN+Desi | <0.0001 |
| | | | Ctrl vs. NEN+Clomi | <0.0001 |
| Figure 4C | two-way ANOVA with Tukey post-hoc test | 0.0016 (treatment) 0.0137 (KD) | Ctrl:siCtrl vs. N+D:siCtrl | 0.0024 |
| | | | N+D:siCtrl vs. N+D+ISRIB:siCtrl | 0.0041 |
| | | | N+D:siCtrl vs. N+D:siTFE3/MITF | 0.0071 |
| Figure 4D | two-way ANOVA with Tukey post-hoc test | <0.0001 | siCtrl:Ctrl vs. siCtrl:N+D | <0.0001 |
| | | | siCtrl:N+D vs. siUPP1:N+D | <0.0001 |
| Figure 4E | 3D Toxicity (left graph): two-way ANOVA with Tukey post-hoc test | <0.0001 | siCtrl:Ctrl vs. siCtrl:NEN+Domp | <0.0001 |
| | | | siCtrl:NEN+Domp vs. siUPP1:NEN+Domp | 0.0001 |
| | 2D Toxicity (right graph): two-way ANOVA with Tukey post-hoc test | <0.0001 | siCtrl:Ctrl vs. siUPP1:NEN+Domp | <0.0001 |
| | | | siCtrl:NEN+Domp vs. siUPP1:NEN+Domp | <0.0001 |
| Figure 4H | two-way ANOVA with Tukey post-hoc test | | siCtrl:NEN+Imi vs. siUPP1:NEN+Imi | 0.0003 |
| | | | siCtrl:NEN+Ami vs. siUPP1:NEN+Ami | <0.0001 |
| | | | siCtrl:NEN+Desi vs. siUPP1:NEN+Desi | 0.0004 |
| Figure 5A | DHODH, Left graph: Kruskal-Wallis with Dunn´s post-hoc test | <0.0001 | Ctrl vs. NEN | 0.0067 |
| | | | Ctrl vs. N+D | 0.1246 |
| | DHODH, right graph: one-way ANOVA with Tukey post-hoc test | | Ctrl vs. NEN | <0.0001 |
| | | | Ctrl vs. Imi | <0.0001 |
| | | | Ctrl vs. NEN+Imi | <0.0001 |
| | | | Ctrl vs. Ami | <0.0001 |
| | | | Ctrl vs. NEN+Ami | <0.0001 |
| | | | Ctrl vs. NEN+Desi | <0.0001 |
| | | | Ctrl vs. Clomi | 0.0061 |
| | | | Ctrl vs. NEN+Clomi | <0.0001 |
| Figure 5B | two-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl+Jnki:Ctrl vs. NEN:Domp+A77 | <0.0001 |

4

| | | | | |
|---|---|---|---|---|
| | | | NEN:Domp vs. NEN:Domp+A77 | <0.0001 |
| | | | NEN:Domp+A77 vs. NEN+Jnki:Domp+A77 | <0.0001 |
| Figure 5D | Left graph: two-way ANOVA with Tukey post-hoc test | <0.0001 | NEN:Imipramine vs. NEN:Imi+A77 | <0.0001 |
| | | | NEN:Desipramine vs. NEN:Desi+A77 | <0.0001 |
| | | | NEN:Amitriptyline vs. NEN:Ami+A77 | <0.0001 |
| | Right graph: two-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl:Clomi+A77 vs. NEN:Clomi+A77 | <0.0001 |
| | | | NEN:Clomi vs. NEN:Clomi+A77 | <0.0001 |
| Figure 6C | two-way ANOVA with Tukey post-hoc test | 0.0002 | Ctrl:Ctrl vs. Ctrl:NEN+Domp | <0.0001 |
| | | | Ctrl:NEN+Domp vs. 0.3%CD:NEN+Domp | 0.0004 |
| | | | Ctrl:NEN+Domp vs. 1.5%CD:NEN+Domp | <0.0001 |
| | | | 0.01%CD:Ctrl vs. 0.01%CD:NEN+Domp | 0,0007 |
| | | | 0.06%CD:Ctrl vs. 0.06%CD:NEN+Domp | 0,0004 |
| Figure 6D | two-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl:NEN+Domp vs. CD:NEN+Domp | <0.0001 |
| | | | Ctrl:NEN+Imi vs. CD:NEN+Imi | 0.0009 |
| | | | Ctrl:NEN+Ami vs. CD:NEN+Ami | <0.0001 |
| | | | Ctrl:NEN+Desi vs. CD:NEN+Desi | <0.0001 |
| | | | Ctrl:NEN+Clomi vs. CD:NEN+Clomi | <0.0001 |
| Figure 6E | CHOP: one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. N+D | <0.0001 |
| | | | N+D vs. N+D+CyD | <0.0001 |
| | TFE3: one-way ANOVA with Tukey post-hoc test | 0.0030 | Ctrl vs. N+D | 0.0014 |
| | CD68: one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. N+D | <0.0001 |
| | | | N+D vs. N+D+CyD | 0.0323 |
| | NPC1: | | | |

| | | | | |
|---|---|---|---|---|
| | one-way ANOVA with Tukey post-hoc test | 0.0001 | Ctrl vs. N+D<br>N+D vs. N+D+CyD | <0.0001<br>0.0078 |
| | UPP1:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. N+D<br>N+D vs. N+D+CyD | <0.0001<br><0.0001 |
| | DHODH:<br>one-way ANOVA with Tukey post-hoc test | 0.0038 | Ctrl vs. N+D | 0.0082 |
| Figure 7A | PDO-42 (left):<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN 1,2+A<br>Imi30 vs. NEN1,2 + I<br>Desi30 vs. NEN1,2+D<br>NEN2,5 vs. NEN2,5+C20 | 0.0001<br>0.0001<br><0.0001<br>0.0041 |
| | PDO-48 (right):<br>one-way ANOVA with Tukey post-hoc test | 0.0003 | Ami vs. NEN 1,2+A<br>Desi vs. NEN1,2+D<br>NEN 1,2 vs. NEN1,2+C<br>NEN 2,5 vs. NEN 2,5+A<br>NEN 2,5 vs. NEN 2,5+I<br>NEN 2,5 vs. NEN 2,5+D<br>NEN 2,5 vs. NEN 2,5+C | 0.0331<br>0.0456<br>0.0257<br>0.0016<br>0.0003<br>0.0001<br><0.0001 |
| Figure 7B | PDO-42:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Upper left graph:<br>Ctrl  vs. N+A<br>Ctrl vs. N+A+Paclitaxel 2nM<br>Ctrl vs. N+Paclitaxel 20nM<br>Ctrl vs. N+A+Paclitaxel 20nM<br>Lower left graph:<br>Ctrl vs. N+I+Paclitaxel 2nM<br>Ctrl vs. N+Paclitaxel 20nM<br>Ctrl vs. N+I+ Paclitaxel 20nM<br>Ctrl vs. Pacli 200 | 0.0372<br><0.0001<br>0.0016<br><0.0001<br>0.0010<br><br>0.0019<br>0.0005<br><br><0.0001<br>0.0003 |
| | PDO-48:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Upper right graph<br>Ctrl vs. N+A+Paclitaxel 2nM<br>Ctrl vs. Pacli 20<br>Ctrl vs. N+Paclitaxel 20nM<br>Ctrl vs. N+A+Paclitaxel 20nM<br>Ctrl vs. Pacli 200<br>Lower left graph:<br>Ctrl vs. Pacli 20 | 0.0154<br>0.0006<br><0.0001<br><br><0.0001<br><0.0001<br><br>0.0008 |

| Figure | Test | p-value | Comparison | p-value |
|---|---|---|---|---|
| | | | Ctrl vs. N+Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. N+I+ Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. Pacli 200 | <0.0001 |
| Figure EV1A | BxPC3 (upper graph): two-way ANOVA with Tukey post-hoc test | < 0.0001 | NEN:Ctrl vs. NEN:2-DG NEN:Ctrl vs. NEN:2-DG FCCP:Ctrl vs. FCCP:2-DG | <0.0001 <0.0001 <0.0001 |
| | U87 (lower graph): two-way ANOVA with Tukey post-hoc test | < 0.0001 | NEN:Ctrl vs. NEN:2-DG NEN:Ctrl vs. NEN:2-DG FCCP:Ctrl vs. FCCP:2-DG | <0.0001 <0.0001 <0.0001 |
| Figure EV1B | one-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl vs. 2-DG Ctrl vs. NEN+2-DG NEN vs. 2-DG NEN vs. NEN+2-DG 2-DG vs. NEN+2-DG | <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 |
| Figure EV1C | one-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl 1% vs. NEN 10% NEN 10% vs. NEN 1% | <0.0001 <0.0001 |
| Figure EV1D | HCT116, upper graph: two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Domp vs. NEN:Domp Ctrl:Imi vs. NEN:Imi Ctrl:Ami vs. NEN:Ami Ctrl:Desi vs. NEN:Desi Ctrl:Clomi vs. NEN:Clomi | <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 |
| | BxPC3, lower graph: two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Imi vs. NEN:Imi Ctrl:Ami vs. NEN:Ami Ctrl:Desi vs. NEN:Desi Ctrl:Clomi vs. NEN:Clomi | <0.0001 <0.0001 <0.0001 <0.0001 |
| Figure EV1E | HCT116, upper graph: two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Ctrl vs. NEN:Ctrl Ctrl:Domp vs. NEN:Domp Ctrl:Imi vs. NEN:Imi Ctrl:Ami vs. NEN:Ami Ctrl:Desi vs. NEN:Desi Ctrl:Clomi vs. NEN:Clomi | <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001 |
| | U87, lower graph: two-way ANOVA with Tukey post-hoc test | < 0.0001 | Ctrl:Domp vs. NEN:Domp Ctrl:Imi vs. NEN:Imi Ctrl:Ami vs. NEN:Ami Ctrl:Desi vs. NEN:Desi | <0.0001 <0.0001 <0.0001 <0.0001 |

| | | | Ctrl:Clomi vs. NEN:Clomi | <0.0001 |
|---|---|---|---|---|
| Figure EV1F | one-way ANOVA+Welch´s correction with Dunnet´s post hoc test | < 0.0001 | Ctrl vs. Staurosp | <0.0001 |
| Figure EV2A | one-way ANOVA with Dunnet´s post hoc test | | Ctrl vs. NEN+Imi | 0.0096 |
| | | | Ctrl vs. NEN+Ami | 0.0002 |
| | | | Ctrl vs. NEN+Desi | <0.0001 |
| | | | Ctrl vs. NEN+Clomi | <0.0001 |
| Figure EV2C | HCT116, left graph: two-way ANOVA with Tukey post-hoc test | <0.0001 | CHOP:Ctrl vs. CHOP:NEN | 0.0003 |
| | | | CHOP:Ctrl vs. CHOP:NEN+Domp | <0.0001 |
| | | | ATF4:Ctrl vs. ATF4:NEN+Domp | 0.0007 |
| | | | p-/t-eIF2a:Ctrl vs. p-/t-eIF2a:NEN+Domp | 0.0006 |
| | | | p-/t-eIF2a:NEN vs. p-/t-eIF2a:NEN+Domp | 0.0005 |
| | HCT116, middle graph: two-way ANOVA with Tukey post-hoc test | <0.0001 | CHOP:Ctrl vs. CHOP:NEN | 0.0010 |
| | | | CHOP:Ctrl vs. CHOP:NEN+Ami | <0.0001 |
| | | | CHOP:NEN vs. CHOP:NEN+Ami | 0.0062 |
| | | | CHOP:Ami vs. CHOP:NEN+Ami | <0.0001 |
| | | | ATF4:Ctrl vs. ATF4:NEN+Ami | <0.0001 |
| | | | ATF4:NEN vs. ATF4:NEN+Ami | <0.0001 |
| | | | ATF4:Ami vs. ATF4:NEN+Ami | 0.0002 |
| | | | p-/t-eIF2a:Ctrl vs. p-/t-eIF2a:NEN+Ami | <0.0001 |
| | | | p-/t-eIF2a:NEN vs. p-/t-eIF2a:NEN+Ami | <0.0001 |
| | | | p-/t-eIF2a:Ami vs. p-/t-eIF2a:NEN+Ami | <0.0001 |
| | U87, right graph: two-way ANOVA with Tukey post-hoc test | <0.0001 | CHOP:Ctrl vs. CHOP:NEN+Ami | <0.0001 |
| | | | CHOP:NEN vs. CHOP:NEN+Ami | <0.0001 |
| | | | CHOP:Ami vs. CHOP:NEN+Ami | <0.0001 |
| | | | ATF4:Ctrl vs. ATF4:NEN | <0.0001 |
| | | | ATF4:Ctrl vs. ATF4:NEN+Ami | <0.0001 |

| | | | ATF4:NEN vs. ATF4:NEN+Ami | <0.0001 |
| | | | ATF4:Ami vs. ATF4:NEN+Ami | <0.0001 |
| | | | p-/t-eIF2a:Ctrl vs. p-/t-eIF2a:NEN+Ami | <0.0001 |
| | | | p-/t-eIF2a:NEN vs. p-/t-eIF2a:NEN+Ami | 0.0266 |
| | | | p-/t-eIF2a:Ami vs. p-/t-eIF2a:NEN+Ami | 0.0012 |
| Figure EV2D | Unpaired t test with Welch's correction | | Left graph: Ctrl vs. siATF4 | 0.0015 |
| | | | Right graph: Ctrl vs. siChop | 0.0064 |
| Figure EV2E | two-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl:NEN+Imi vs. ISRIB:NEN+Imi | <0.0001 |
| | | | Ctrl:NEN+Ami vs. ISRIB:NEN+Ami | <0.0001 |
| | | | Ctrl:NEN+Desi vs. ISRIB:NEN+Desi | <0.0001 |
| | | | Ctrl:NEN+Clomi vs. ISRIB:NEN+Clomi | 0.0020 |
| Figure EV2F | two-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl:NEN+Domp vs. ISRIB:NEN+Domp | <0.0001 |
| | | | Ctrl:NEN+Imi vs. ISRIB:NEN+Imi | <0.0001 |
| | | | Ctrl:NEN+Ami vs. ISRIB:NEN+Ami | <0.0001 |
| | | | Ctrl:NEN+Desi vs. ISRIB:NEN+Desi | <0.0001 |
| | | | Ctrl:NEN+Clomi vs. ISRIB:NEN+Clomi | 0.0017 |
| Figure EV3A | MITF, left graph: one-way ANOVA with Tukey post-hoc test | 0.0006 | Ctrl vs. NEN | 0.0009 |
| | | | Ctrl vs. Domp | 0.0024 |
| | | | Ctrl vs. N+D | 0.0012 |
| | MITF, right graph: one-way ANOVA with Tukey post-hoc test | 0.0001 | Ctrl vs. NEN+Clomi | 0.0220 |
| | SQSTM1, left graph: one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN | 0.0031 |
| | | | Ctrl vs. Domp | <0.0001 |
| | | | Ctrl vs. N+D | <0.0001 |
| | | | NEN vs. N+D | <0.0001 |
| | | | Domp vs. N+D | 0.0004 |
| | SQSTM1, right graph: one-way | <0.0001 | Ctrl vs. NEN+Imi | <0.0001 |
| | | | Ctrl vs. NEN+Ami | 0.0007 |

| | | | | |
|---|---|---|---|---|
| | ANOVA with Tukey post-hoc test | | Ctrl vs. NEN+Desi<br>Ctrl vs. NEN+Clomi | <0.0001<br><0.0001 |
| | NPC1, left graph:<br>one-way ANOVA with Tukey post-hoc test | 0.0098 | Ctrl vs. Domp<br>Ctrl vs. N+D | 0.0077<br>0.0405 |
| | NPC1, right graph:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN+Imi<br>Ctrl vs. NEN+Ami<br>Ctrl vs. NEN+Desi<br>Ctrl vs. NEN+Clomi | <0.0001<br><0.0001<br><0.0001<br><0.0001 |
| Figure EV3C | LC3-II/I protein ratio:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+IMI<br>NEN vs. NEN+IMI | 0.0007<br><0.0001<br><0.0001 |
| | p62 levels:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+IMI<br>NEN vs. NEN+IMI | 0.0402<br>0.0003<br>0.0030 |
| Figure EV3D | LC3-II/I protein ratio:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+AMI<br>NEN vs. NEN+AMI | 0.0005<br><0.0001<br><0.0001 |
| | p62 levels:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. NEN+AMI<br>NEN vs. NEN+AMI | 0.0551 (ns)<br>0.0006<br>0.0077 |
| Figure EV4A | one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. N+D<br>NEN vs. N+D<br>Domp vs. N+D | <0.0001<br><0.0001<br><0.0001 |
| Figure EV4C | UPP1, left graph:<br>one-way ANOVA with Tukey post-hoc test | <0.0001 | Ctrl vs. NEN<br>Ctrl vs. Domp<br>Ctrl vs. N+D<br>NEN vs. N+D<br>Domp vs. N+D | 0.0123<br>0.0006<br><0.0001<br>0.0003<br>0.0050 |
| | UPP1, middle graph:<br>one-way ANOVA with Tukey post-hoc test | 0.0017 | Ctrl vs. N+Ami<br>NEN vs. N+Ami<br>Ami vs. N+Ami | 0.0017<br>0.0079<br>0.0053 |
| | UPP1, right graph:<br>one-way ANOVA with Tukey post-hoc test | 0.0002 | Ctrl vs. N+Imi<br>NEN vs. N+Imi<br>Ami vs. N+Imi | 0.0003<br>0.0010<br>0.0011 |

| Figure EV4F | two-way ANOVA with Tukey post-hoc test | <0.0001 | siCtrl:NEN+Domp vs. siUPP1:NEN+Domp | 0.0034 |
| | | | siCtrl:NEN+Imi vs. siUPP1:NEN+Imi | 0.0211 |
| | | | siCtrl:NEN+Ami vs. siUPP1:NEN+Ami | 0.0135 |
| | | | siCtrl:NEN+Desi vs. siUPP1:NEN+Desi | 0.0196 |
| | | | siCtrl:NEN+Clomi vs. siUPP1:NEN+Clomi | 0.1278 (ns) |
| Figure EV5A | PDO-B42: one-way ANOVA with Tukey post-hoc test | <0.0001 | Upper left graph: Ctrl vs. N+D+Paclitaxel 2nM | 0.0053 |
| | | | Ctrl vs. N+Paclitaxel 20nM | 0.0275 |
| | | | Ctrl vs. N+D+ Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. Pacli 200 | 0.0203 |
| | | | Lower left graph: Ctrl vs. N+C+Paclitaxel 2nM | 0.0113 |
| | | | Ctrl vs. N+C+Paclitaxel 20nM | <0.0001 |
| | PDO-48: one-way ANOVA with Tukey post-hoc test | <0.0001 | Upper right graph: Ctrl vs. Pacli 20 | 0.0068 |
| | | | Ctrl vs. N+Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. N+D+Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. Pacli 200 | <0.0001 |
| | | | Lower right graph: Ctrl vs. N+C+Paclitaxel 2nM | 0.0010 |
| | | | Ctrl vs. Pacli 20 | 0.0024 |
| | | | Ctrl vs. N+Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. N+C+Paclitaxel 20nM | <0.0001 |
| | | | Ctrl vs. Pacli 200 | <0.0001 |
| Figure EV5B | Left graph, CHOP: one-way ANOVA with Dunnet´s post hoc test | 0,0148 | Ctrl vs. NEN+Ami | 0,0316 |
| | | | NEN vs. NEN+Ami | 0,0578 (ns) |
| | | | Ami vs. NEN+Ami | 0,0345 |
| | Middle graph, UPP1: one-way ANOVA with Dunnet´s post hoc test | 0.0061 | Ctrl vs. NEN+Ami | 0.0307 |
| | | | NEN vs. NEN+Ami | 0.1257 |
| | | | Ami vs. NEN+Ami | 0.0184 |
| | Right graph, PUMA: one-way ANOVA with Dunnet´s post hoc test | 0,0039 | Ctrl vs. NEN+Ami | 0,0058 |
| | | | NEN vs. NEN+Ami | 0,1306 |
| | | | Ami vs. NEN+Ami | 0,1430 |