# Supplementary Information

**Human Retinal Organoid Model of Disease-Relevant Photoreceptor Cell Death Amenable to Drug Screening**

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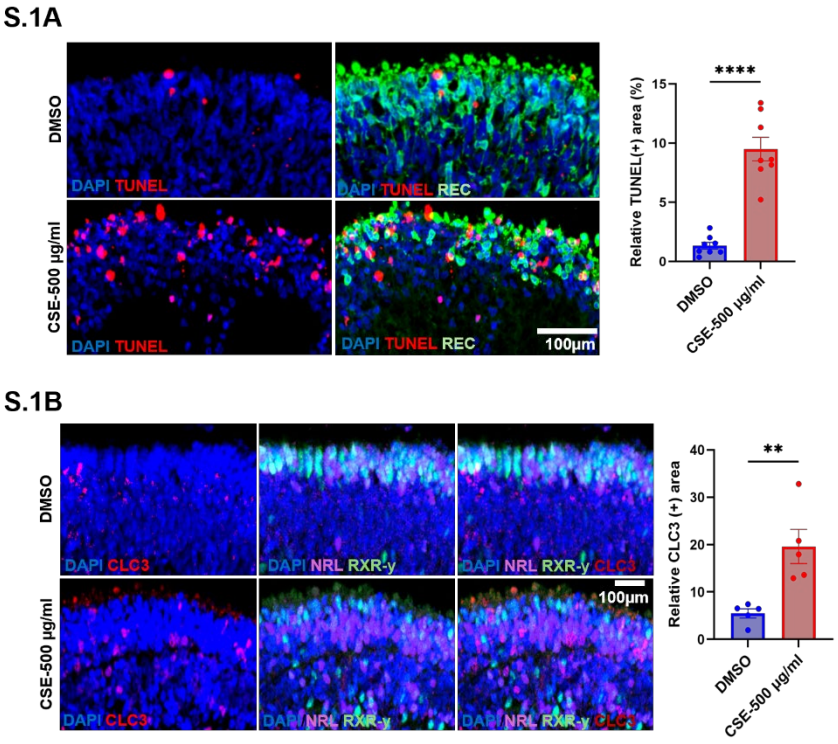
**Figure S1. Apoptosis induction in CSE-treated ROs**



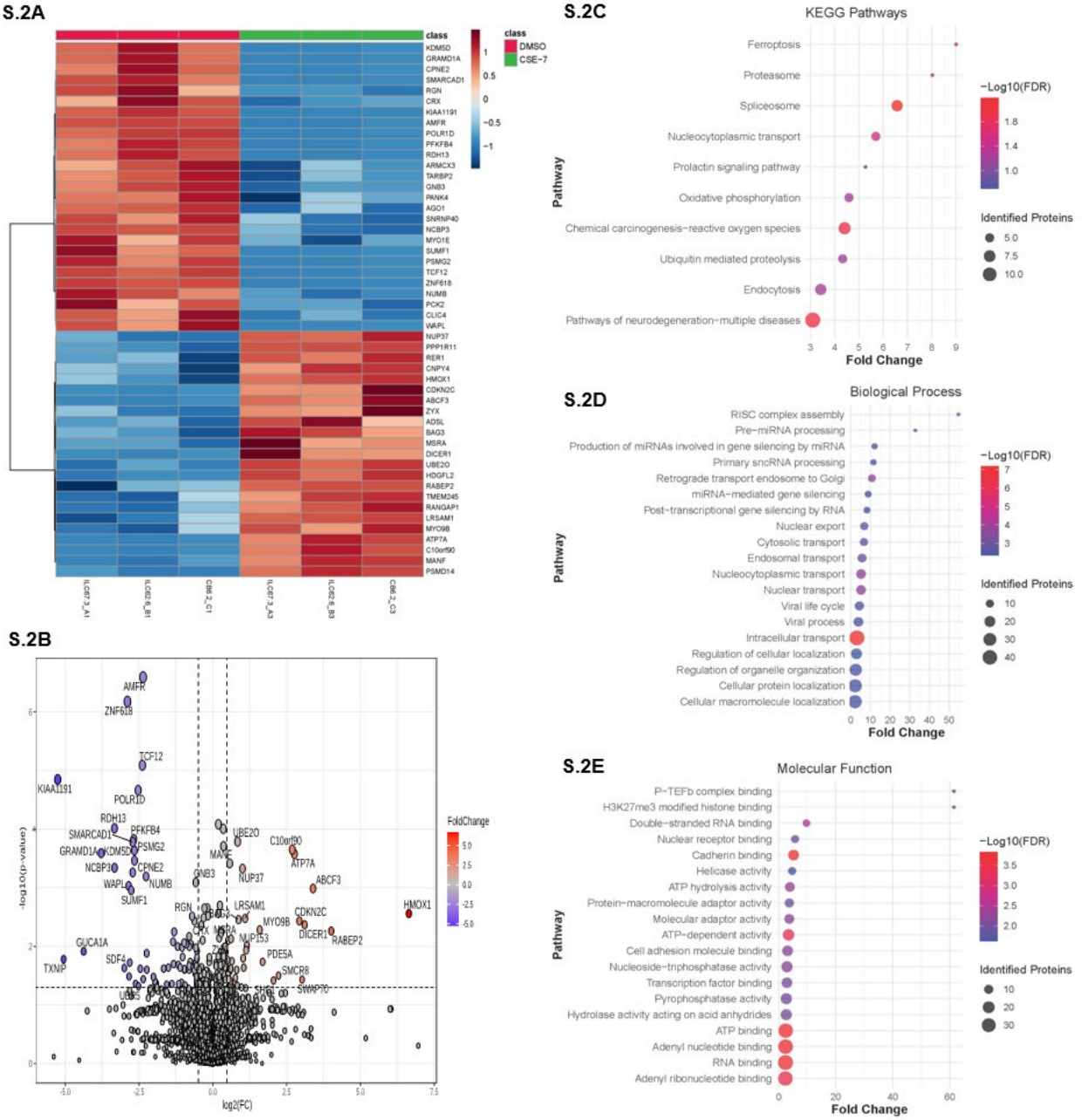

ROs were treated with vehicle control (DMSO) or CSE-500 µg/ml for 5 days to evaluate the extent of apoptotic cell death:

A. A representative confocal micrographs of D180 RO sections stained with TUNEL to examine DNA fragmentation, along with recoverin (REC) staining to identify photoreceptor cells. Scale bar: 100µm. TUNEL staining quantification shows a statistically significant increase in cell death in CSE-treated ROs compared to vehicle controls (DMSO). Relative TUNEL (+) area: DMSO: 1.342±0.28 (n=8), and CSE-500 µg/ml: 9.501±0.988 (n=8). Error bars indicate Mean±SEM; T-test; ****p<0.0001.

B. Immunofluorescence staining was performed on D180 RO sections using antibodies for CLC3 and photoreceptor markers NRL (Rod) and RXR-γ (Cone). Colocalization of CLC3 and photoreceptor markers was also analyzed. Scale bar: 100µm. Bar graph represents the quantification of relative CLC-3-labeled area/DAPI. A significant increase in CLC3-mediated

photoreceptor cell death was observed in CSE-treated ROs compared to vehicle controls (DMSO). DMSO= 5.483±0.95 (n=5), and CSE-500 µg/ml= 19.622±3.599 (n=5). Error bar shows Mean±SEM; T-test; **p<0.005.

**Figure S2. Proteomic analysis of ROs treated with CSE-750 µg/ml or DMSO for 48 h**




LC-MS proteomic analysis of ROs treated with CSE-750 µg/ml show very similar results to those observed in ROs treated with CSE-500 µg/ml, along with several additional DEPs.

A. Heatmap and hierarchical clustering representation of the top 50 DEPs across three independent hiPSC cell lines (ILC67.3, ILC62.6, and CB6.2), comparing DMSO and CSE-

750 µg/ml treatments. Each column represents an individual sample, and clustering reflects distinct proteomic signatures induced by CSE exposure.

B. Volcano plot illustrating differential protein expression between CSE (750 µg/mL) and vehicle (DMSO) control-treated ROs. The x-axis represents the log2 fold change (log2FC), and the y-axis represents the statistical significance (−log10 p-value). A total of 80 significant proteins (Fold Change ≥ 1.4, $p < 0.05$) were plotted. Black dots indicate non-significant changes; blue and red dots indicate significantly downregulated and upregulated proteins, respectively.

C. KEGG pathway enrichment analysis of DEPs revealed significant enrichment in pathways associated with ferroptosis, metabolic pathways, neurodegeneration, and others in CSE-treated ROs compared to vehicle (DMSO) control-treated ROs.

D. Gene Ontology (GO) Biological Process analysis identified key functional categories significantly associated with DEPs in CSE-treated ROs, highlighting pathways involved in autophagy, mRNA processing, and cellular processes.

E. GO Molecular Function analysis revealed enrichment in molecular activities such as antioxidant activity, receptor binding, and protein transport among the DEPs following CSE exposure.

**Table S1.** Primary Antibody Information

| **Primary antibody** | **Company/State/Country** | **Catalog Number** | **Dilution** |
|---|---|---|---|
| Recoverin | Millipore, Burlington, MA, USA | AB5585 | 1:1000 |
| Human NRL | R&D Systems; NE, Minneapolis, MN, USA. | AF2945 | 1:300 |
| RXR-γ (A-2) | Santa Cruz, CA, USA | Sc-365252 | 1:100 |
| Cleaved Caspase 3 (Asp175) | Cell Signaling Technology; Danvers, MA, USA | 9664 | 1:500 |
| Cleaved Caspase 9 (Asp330) | Cell Signaling Technology; Danvers, MA, USA | 7237 | 1:500 |

**Table S2. Secondary Antibody information**

| **Secondary antibody** | **Company** | **Catalog Number** | **Dilution** |
|---|---|---|---|
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 | Thermo Fisher Scientific; Waltham, MA, USA | A21207 | 1:2000 |
| Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 | Thermo Fisher Scientific; Waltham, MA, USA | A21203 | 1:2000 |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 | Thermo Fisher Scientific; Waltham, MA, USA | A21206 | 1:2000 |
| Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 | Thermo Fisher Scientific; Waltham, MA, USA | A32766 | 1:2000 |
| Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 | Thermo Fisher Scientific; Waltham, MA, USA | A21447 | 1:2000 |