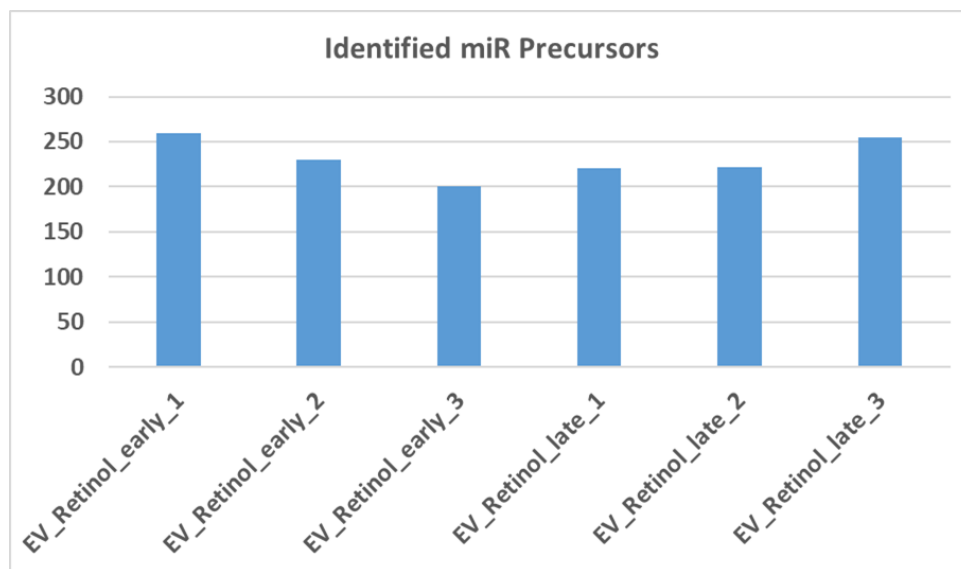


# Cannabidiol-Loaded Retinal Organoid-Derived Extracellular Vesicles Protect Oxidatively Stressed ARPE-19 Cells

## Supplementary Materials

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Supplementary Figure S1. The number of miRNAs identified using miRNA sequencing.



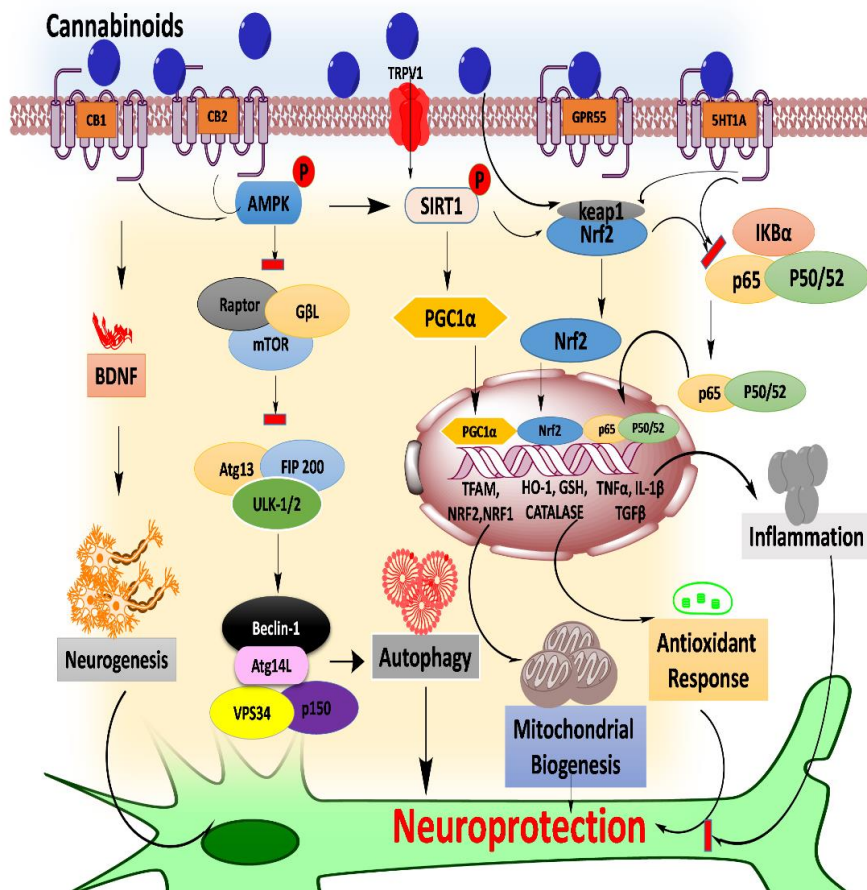
**Supplementary Table S1.** Primary and secondary antibodies.

| <b>Protein Name</b> | <b>Host</b> | <b>Source</b>                | <b>Catalog number</b> | <b>Dilution</b>                                         |
|---------------------|-------------|------------------------------|-----------------------|---------------------------------------------------------|
| AMPK                | Mouse       | Santa Cruz<br>Biotechnology  | SC-398861             | 1:1000 (Western blotting)<br>1:200 (Immunofluorescence) |
| Beta-actin          | Rabbit      | Cell Signaling<br>Technology | 4970S                 | 1:1000                                                  |
| Catalase            | Mouse       | Santa Cruz<br>Biotechnology  | 271803                | 1:200                                                   |
| Complex 1           | Mouse       | Invitrogen                   | 43-8800               | 1:200                                                   |
| HO1                 | Rabbit      | Cell Signaling<br>Technology | 43966S                | 1:1000                                                  |
| mTOR                | Rabbit      | Cell Signaling<br>Technology | 2972                  | 1:1000                                                  |
| NRF1                | Rabbit      | Cell Signaling<br>Technology | 46743S                | 1:1000                                                  |
| NQO1                | Mouse       | Santa Cruz<br>Biotechnology  | SC-32793              | 1:1000                                                  |
| Parkin              | Mouse       | Santa Cruz<br>Biotechnology  | SC-32282              | 1:1000                                                  |
| P38                 | Rabbit      | Cell Signaling<br>Technology | 9212                  | 1:200                                                   |
| Sirt1               | Rabbit      | Cell Signaling<br>Technology | 9475S                 | 1:1000                                                  |
| SOD2                | Rabbit      | Cell Signaling<br>Technology | 13141S                | 1:1000 (Western blotting)<br>1:200 (Immunofluorescence) |
| TFAM                | Mouse       | Santa Cruz<br>Biotechnology  | 166965                | 1: 200                                                  |

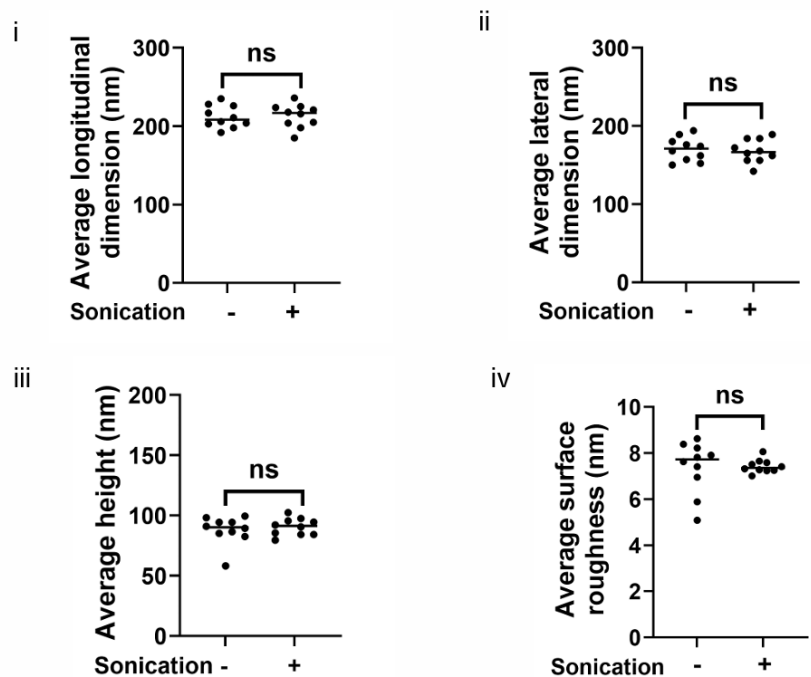
|                               |        |                          |        |                            |
|-------------------------------|--------|--------------------------|--------|----------------------------|
| Mouse anti-rabbit IgG-CFL 488 | Mouse  | Santa Cruz Biotechnology | 516248 | 1:200 (Immunofluorescence) |
| Rabbit anti-mouse IgG-PE      | Rabbit | Santa Cruz Biotechnology | 358926 | 1:200 (Immunofluorescence) |
| Rabbit anti-mouse IgG-HRP     | Rabbit | Santa Cruz Biotechnology | 358914 | 1:5000 (Western blotting)  |
| Mouse anti-rabbit IgG-HRP     | Mouse  | Santa Cruz Biotechnology | 2357   | 1:5000 (Western blotting)  |

**Supplementary Figure S2. Plausible mechanism of action of cannabinoids to protect peripheral neurons.** Cannabinoids have been found to activate TRPV1 ion channels, GPR55, and 5HT1A receptors, including CB1 and CB2 receptors. Upon activation, these receptors regulate the downstream AMPK, Nrf2, NF- $\kappa$ B, and BDNF cell signaling pathways. All these orchestrated events collectively improve the neurogenesis, increase the autophagosome formation, augment mitochondrial biogenesis and function, and enhance the antioxidant response for decreasing the inflammation in different conditions of neuropathy[1]. AMPK: adenosine monophosphate-activated protein kinase; Atg: anti-thymocyte globulin; BDNF: brain-derived neurotrophic factor; FIP200: FAK family kinase-interacting protein of 200 kDa; GSH: glutathione; HO1: heme oxygenase 1; mTOR: mechanistic target of rapamycin; SIRT1: silent mating type information regulation 2 homolog 1; NRF: nuclear respiratory factor; Nrf2: nuclear factor erythroid 2 (NFE2)-related factor 2; NF- $\kappa$ B: nuclear factor kappa-light-chain-enhancer of activated B cells; PGC-1 $\alpha$ : peroxisome proliferator-activated receptor gamma coactivator 1-alpha; TFAM: mitochondrial transcription factor; Ulk1: unc-51 like autophagy activating kinase; VPS34: vacuolar protein sorting.

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**Supplementary Figure S3. Topographical measurements by atomic force microscopy show EVs remain unchanged after sonication.** Quantification of retinal organoid EVs and sonicated EVs showing average longitudinal dimension (i), average lateral dimension (ii), average height (iii), and average surface roughness (iv).



## Reference

[1] P. Arthur, A.K. Kalvala, S.K. Surapaneni, M.S. Singh, Applications of cannabinoids in neuropathic pain: an updated review, *Critical Reviews™ in Therapeutic Drug Carrier Systems* 41(1) (2024).