| **Supplementary Table 1: MicroFlow Cytometer Settings** | | | | |
|---|---|---|---|---|
| **Platform** | Apogee A50 MP | S/N 0027 | | |
| | | | | |
| **Parameter** | **Setting** | | | |
| Sample Flow Rate | 3.01 µL/min | | | |
| Pressure | 150 units | | | |
| Acquisition time | 60 sec | | | |
| Sample Dilution | 25x with PBS | | | |
| Volume/well | 250 µL | | | |
| Sample volume | 10 µL | | | |
| Diluent volume | 250µL | | | |
| Event Trigger | LALs unless stated otherwise | | | |
| | | | | |
| **Channel** | **Laser Power (mW)** | **PMT** | **Gain** | **Threshold** |
| 405nm | 75 | | | |
| 488nm | 50 | | | |
| 561nm | N/A | | | |
| 638nm | 75 | | | |
| 405-SALS | | 342 | 1.0 | 20 |
| 405-LALS | | 350 | 1.0 | 48 |
| 405-Red | | 350 | 1.0 | |
| 405-Green | | 500 | 1.0 | |
| 488-Green | | 450 | 1.0 | |
| 488-Orange | | 550 | 1.0 | |
| 488-Red | | 580 | 1.0 | |
| 561-Orange | | NA | 1.0 | |
| 561-Red | | NA | 1.0 | |
| 638-Red | | 550 | 1.0 | |
| 638-Far Red | | NA | 1.0 | |
| | | | | |
| **Beads** | **Product number** | **Lot number** | **Expiration** | |
| Monitoring | Apogee 1493 | CAL0093<br>CAL0095 | 09/08 2022<br>13/02/2023 | |
| | | | | |

| **Supplementary Table 2: LM10 NTA Settings** | | | | |
|---|---|---|---|---|
| **Platform** | LM10 | | | |
| | | | | |
| **Parameter** | **Setting** | | | |
| Software | NTA 3.0 0064 | | | |
| ~Temp (°C) | 18-25 | **Sample Dilution** 25x | | |
| Camera settings | Type = sCMOS Level =12 | | | |
| Slider shutter = 600 | Slider gain = 350 | Shutter/ms = 26.6 | Frame rate = 18.8 | |
| Histogram Upper = 8325 | | Histogram Lower = 0 | | |
| Detection Settings | Avg completed tracks samples =3000 <based on sub sample of 40 samples> | | Avg completed tracks medias =14 <based on sub sample of 12 samples> | |
| Threshold = 7 | Blur = Auto | Vibration correction applied | Particles per frame = 10-100 | |
| Size distribution - Number weighting - With Percentiles | | | | |
| Size distribution - Surface area weighting - With Percentiles | | | | |
| Size distribution - Volume weighting - With Percentiles | | | | |
| | | | | |
| **Beads** | **Product number** | **Lot number** | **Expiration** | |
| NIST Polystyrene | Thermo Scientific 3000 Series | 3060A: Lot40223<br>3200A: Lot 39557<br>3400A: Lot 40720 | See lot specific dates;minimum 2 yrs | |
| Nanosight 100nm Polystyrene size standard | Nanosight stock solutions | N/A | N/A | |

| **Supplementary Table 3: MIFlowCyt / MISEV Compliant Items** | |
|---|---|
| **Requirement** | **Please Include Requested Information** |
| 1.1. Purpose | To assess cellular export capacity of Huntington aggregates using an expressed form of polyglutamine expanded huntingtin (GFP-tagged 72Qhuntingtinexon1) in cells. |
| 1.2. Keywords | microflow cytometry, extracellular vesicle, molecular chaperone, CSPα, DnaJC5, Huntington's disease |
| 1.3. Experiment variables | Conditioned media, expression of polyglutamine expanded huntingtin (GFP-tagged 72Qhuntingtinexon1) and other vectors in CAD neural cells. Specific details in Methods. |
| 1.4. Organization name and address | Hotchkiss Brain Institute<br>Department of Biochemistry and Molecular Biology<br>Cumming School of Medicine, University of Calgary<br>3330 Hospital Dr. N.W.<br>Calgary, Alberta, Canada T2N 4N1 |
| 1.5. Primary contact name and email address | Hotchkiss Brain Institute<br>Department of Biochemistry and Molecular Biology<br>Cumming School of Medicine, University of Calgary<br>3330 Hospital Dr. N.W.<br>Calgary, Alberta, Canada T2N 4N1 |
| 1.6. Date or time period of experiment | 2017 - 2020 |
| 1.7. Conclusions | Our data links the molecular chaperone, CSPα, and the packaging of pathogenic misfolded huntingtin into extracellular vesicles for elimination |
| 1.8. Quality control measures | 1493 Apogee Bead Mix, NIST 200nm bead standard |
| 2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description | Conditioned (serum free) DMEM media from CAD neural cells collected 48hrs post transfection. |
| 2.1.1.2. Biological sample source description | Palmitoylated GFP positive PC3 cells (PC3 ATC: CRL-1435) were generated using a palmitoylation signal genetically fused in-frame to the N terminus of EGFP (previously described: Lai et al, 2015). |
| 2.1.1.3. Biological sample source organism description | |
| 2.1.2.2. Environmental sample location | |
| 2.3. Sample treatment description | Conditioned (serum free) DMEM media from CAD neural cells collected 48hrs post transfection.<br>1. Samples were subjected to a single 300xg centrifugation for 5minutes prior freezing (-80°C) and subsequent analysis by micfroflow cytometry or NTA;<br>2. For some samples, prior to microflow cytometry analysis, EVs were treated with Cell Mask Deep Red plasma membrane stain (ThermoFisher C10046) (0.1X final concentration);<br>3. Palmitoylated GFP positive PC3 cells (PC3 ATC: CRL-1435) were generated using a palmitoylation signal genetically fused in-frame |

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|---|---|
| | to the N terminus of EGFP (previously described: Lai et al, 2015 Nature Communications). Cells were permitted to grow to confluency in serum free DMEM (T175flasks, ~20mLs per flask) and EVs collected in the supernatant following a single 300xg centrifugation for 5minutes prior freezing (-80°C).<br>4. Following 300xg centrifugation for 5 minutes, samples from CAD cells were precipitated using ExoQuick (SBI) prior to SDS-PAGE;<br>5. For imaging, EVs were labels with ExoGlow Protein Red (SBI) as per manufacturers instructions and then applied to recipient CAD cells for 24hrs. Cells were washed 3X prior to imaging. |
| 2.4. Fluorescence reagent(s) description | 1. Cell Mask Deep Red plasma membrane stain (ThermoFisher C10046), used at a final concentration of 0.1x (optimal concentration defined by titration). |
| 3.1. Instrument manufacturers, model | 1. Apogee A50 MicroPlus Microflow cytometer (S/N 0027): Apogee Flow Systems<br>2. LM10 NTA (Malvern Panalytical NanoSight LM10HSBF Nanoparticle Characterization System) |
| 3.3. Instrument configuration and settings | See Tables |
| 4.1. List-mode data files | *Data files will be submitted to http://flowrepository.org |
| 4.2. Compensation description | No compensation |
| 4.3. Data transformation details | |
| 4.4.1. Gate description | Defined by unstained controls |
| 4.4.2. Gate statistics | Data provided as concentration (events/uL) |
| 4.4.3. Gate boundaries | Defined by unstained controls |