nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: No software

Data analysis	edgeR package (version 3.30.3) limma/voom function (version 3.44.3) WGCNA (version 1.72) mixOmics (version 6.24) Seurat (version 4.06) PHATE (version 1.0.4) slingshot (version 2.8.0) Mclust (version 6.0) tradeSeq package (version 1.14.0) ANCOM-BC (version 2.02). stats (version 3.6.2) IMPUTE2 (version 2.3.2) GENESIS (version 2.30) STAR (version 2.7.1) gprofiler2 (version 0.2.3) GSVA (version 1.44) Harmony (version 1.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The human snRNAseq and bulk RNAseq dataset is available in GEO under accession GSE242198. All other datasets are provided in the supplementary material. The customized code and object used for analysis in this manuscript is provided here: https://github.com/Al-Dalahmah-lab/HD_astrocytes_paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	All data on brain donor sex was reported in the supplementary information.
Reporting on race, ethnicity, or other socially relevant groupings	Our differential gene expression analysis corrected for individual donor, batch, sex, and age. Ethnicities or gender were not available or considered. No other socially relevant groupings were relevant to this study.
Population characteristics	see above
Recruitment	This is a retrospective study - no recruitment was done
Ethics oversight	All brain donors consented to donating their brains to research. The study of human post-mortem material has been determined by our IRB as non-human subjects. No additional IRB approval was necessary.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size used in this study was based on prior studies using snRNAseq and Huntington disease. No power analysis was conducted to determine sample size. All IHC and in vitro experiments were performed according to previously published studies. We modeled all n's in this study according to previously published studies including ours, https://doi.org/10.1038/s41467-022-35388-x, where the n's were sufficient to detect biologically significant effects. we empirically found that performing the experiment at least 3 times or in three independent trials was sufficient to discover medium-size effects using paired t-test. For IHC experiments, we empirically found that a minimum of n = 5 was

	sufficient to discover medium size effects in astrocytes. Where possible, we increased the n according to the availability of brain tissue material.
Data exclusions	No data was excluded
Replication	We replicated all in vitro studies at least 3 times in independent trials. The reported data reflects biological replicates.
Randomization	No randomization was employed
Blinding	All analysis were blind to the condition during data collection. Unblinding was done when we the data was analyzed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	The following primary antibodies were used: Rabbit ALDH1L1 (1:100, EnCor, Cat#RPCA-ALDH1L1), Chicken GFAP (1:1000, Abcam, Cat#4674), Goat Clusterin (1:200, Thermo fisher, cat#PA5-46931), Mouse MT (1:100, abcam, cat#ab12228) Rabbit MT3 (1:200, Novus Biologicals, cat#NPP1-89772), Mouse CD44 (1:50, Millipore, cat#SAB1405590), Rabbit CD44 (1:100, Abcam, Cat#ab101531), Rabbit MT3 (1:100, Millipore, Cat#HPA004011), Mouse anti Metallothionein (Detects MT1 and MT2 proteins – Abcam, Ab12228, 1:100) Secondary antibodies conjugated to fluorophores: anti-mouse Alexa Fluor 488, 568, and 633, anti-rabbit Alexa Fluor 488, 594, anti-chicken Alexa Fluor 488 and 647, and anti-goat Alexa Fluor 488, 568, 633; all from goat or donkey (1:500, ThermoFisher Scientific, Eugene, OR). These antibodies can be viewed here: www.abcam.com www.sigmaaldrich.com www.thermofisher.com www.novusbio.com www.encor.com
Validation	All antibodies were commercially available and validated by the manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human astrocytes (Sciencell© cat#1800) were cultured in Astrocyte culture medium (Sciencell © cat#1801) according to vendor's protocols on poly L-Lysine coated cell culture plates. HMC3 cells were obtained from the ATCC cat# CRL-3304 and cultured according to the vendors guidelines. Neuro-2a cells were obtained (Sigma cat # 89121404) and cultured according to the vendors guidelines. This was previously deposited in the ATCC and became commercially available. HMC3 cells were obtained from the ATCC (cat#CRL-3304) and cultured according to vendors protocols. The following patient-derived primary fibroblast lines were converted to neurons: ND30013 - male, AG04194 - female, and AG04230 - male.
Authentication	The cell lines were validated by the manufacturer. We performed qPCR and RNAseq analysis to confirm expression of cell-type specific genes (astrocytes and microglia). We also performed functional on HMC3 cells to confirm their microglial identifiy. We confirmed neurons were authenticated using morphologic studies and qPCR/IHC analysis.
Mycoplasma contamination	We confirm no mycoplasma infections were detected in our cultures
Commonly misidentified lines (See ICLAC register)	None

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	We performed flow analysis to confirm viability of neurons co-cultured with astrocytes and to quantify HMC3 phagocytosis of fluorescent beads with co-cultured with astrocytes as described in our methods section
Instrument	BD Bioscience LSRII flowcytometer
Software	De Novo Software FCS express
Cell population abundance	We did not sort cells to retrieve them, we only analyzed cells populations using flow cytometry
Gating strategy	Standard gating strategy was used, by using FCS and SSC to gate out cell debris, and FSC height vs FSC to gate on singlets. Unstained cells were used to set thersholds for FITC-positve or PI-positive signal in each flow cytometry run. Cells with signal > 10^3 were considered positive.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.