**snRNAseq Methods Section**

**High-throughput generation of single-nucleus transcriptomes from frozen human brain using cellular barcodes and droplet capture.** Nuclei will be isolated from frozen postmortem brain tissue using a procedure adapted in our laboratory for frozen human brain (see **Preliminary Studies**) from Ref.1-5. Postmortem middle temporal gyrus will be mechanically dissociated, filtered, and resuspended. DAPI-positive nuclei will be sorted using Fluorescence Activated Cell Sorting. Single nuclei will be captured using the  Chromium Controller from 10x Genomics2,3,6. cDNA libraries will be generated with the Single Cell 3′ Library & Gel Bead kit version 3 and sequenced on an Illumina NextSeq500 using 150-bp paired-end sequencing. Based on our Preliminary Studies, at least 5,000 cells will be captured per sample, for a total of more than 495,000 cells over the 99 temporal cortex samples, and sequenced with at least 150 million reads per sample (see **Preliminary Studies**). To minimize bias, for all RNA processing and RNA-Seq steps, case and control samples were processed in parallel by researchers blinded to the diagnosis.

**Single-nucleus bioinformatics analysis.** Gene counts will be obtained by aligning reads to the human genome (GRCh38) using CellRanger software (version 5.0.1)4,5. Transcriptome annotation will be based on ENSEMBL v98 (reference available from the CellRanger website). Libraries will be aggregated from all samples using cellranger aggr.

**Quality control for cells and expressed genes.** For quality control and downstream analysis we will use the R package Seurat7. In order to remove low quality cells from the dataset, we will exclude from downstream analysis any cells that have **1**, a total number of unique molecular identifier (UMI) counts greater than three median average deviations above the dataset median; ***2***, greater than 5% of their reads mapping to the mitochondrial chromosome, because in a broken cell cytoplasmic RNA will be lost, while RNAs that are enclosed in the mitochondria will be retained8; ***3****,* less than 200 unique genes detected, based on previous studies4,9. Finally, genes detected in less than three cells will be excluded. After applying these filtering steps, the resulting dataset will be used for downstream analysis.

**Cell type annotation and clustering.** We will further normalize and cluster the data using the standard Seurat workflow (see: https://satijalab.org/seurat/articles/pbmc3k\_tutorial.html)7. Principal component analysis will be performed, the Harmony algorithm used to correct for batch effects (and integrate data from different scRNA-seq runs where necessary) and an Elbow plot used to identify the significant principle components and define a cut off for the ‘dimensionality’ of the dataset i.e. the number of principle components to carry forward10. Clustering (using the Seurat functions ‘FindNeighbours(), which uses the K-nearest neighbors algorithm and FindClusters(), using the Louvain algorithm11) will then be performed over the number of PCs we define to be significant and UMAP will be run over the significant principal components for cluster visualization. We will then find cell cluster markers by identifying the top genes differentially expressed in each cluster vs all other clusters. The top 500 ranking genes will be extracted for each cluster and tested for overlap with known marker genes, and we will assign each cluster a cell-type label by assessing the expression levels of sets of marker genes across the clusters. Clusters having abnormally high number of total counts and mixed expression of markers from different cell-types will be tagged as potential doublets and not considered for downstream analyses12.

1. Habib, N., Avraham-Davidi, I., Basu, A., Burks, T., Shekhar, K., Hofree, M., Choudhury, S.R., Aguet, F., Gelfand, E., Ardlie, K., Weitz, D.A., Rozenblatt-Rosen, O., Zhang, F. & Regev, A. Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat Methods* **14**, 955-958 (2017).

2. Tasic, B., Menon, V., Nguyen, T.N., Kim, T.K., Jarsky, T., Yao, Z., Levi, B., Gray, L.T., Sorensen, S.A., Dolbeare, T., Bertagnolli, D., Goldy, J., Shapovalova, N., Parry, S., Lee, C., Smith, K., Bernard, A., Madisen, L., Sunkin, S.M., Hawrylycz, M., Koch, C. & Zeng, H. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nat Neurosci* **19**, 335-46 (2016).

3. Tasic, B., Yao, Z., Graybuck, L.T., Smith, K.A., Nguyen, T.N., Bertagnolli, D., Goldy, J., Garren, E., Economo, M.N., Viswanathan, S., Penn, O., Bakken, T., Menon, V., Miller, J., Fong, O., Hirokawa, K.E., Lathia, K., Rimorin, C., Tieu, M., Larsen, R., Casper, T., Barkan, E., Kroll, M., Parry, S., Shapovalova, N.V., Hirschstein, D., Pendergraft, J., Sullivan, H.A., Kim, T.K., Szafer, A. *et al.* Shared and distinct transcriptomic cell types across neocortical areas. *Nature* **563**, 72-78 (2018).

4. Mathys, H., Davila-Velderrain, J., Peng, Z., Gao, F., Mohammadi, S., Young, J.Z., Menon, M., He, L., Abdurrob, F., Jiang, X., Martorell, A.J., Ransohoff, R.M., Hafler, B.P., Bennett, D.A., Kellis, M. & Tsai, L.H. Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* (2019).

5. Jakel, S., Agirre, E., Mendanha Falcao, A., van Bruggen, D., Lee, K.W., Knuesel, I., Malhotra, D., Ffrench-Constant, C., Williams, A. & Castelo-Branco, G. Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature* **566**, 543-547 (2019).

6. Zheng, G.X., Terry, J.M., Belgrader, P., Ryvkin, P., Bent, Z.W., Wilson, R., Ziraldo, S.B., Wheeler, T.D., McDermott, G.P., Zhu, J., Gregory, M.T., Shuga, J., Montesclaros, L., Underwood, J.G., Masquelier, D.A., Nishimura, S.Y., Schnall-Levin, M., Wyatt, P.W., Hindson, C.M., Bharadwaj, R., Wong, A., Ness, K.D., Beppu, L.W., Deeg, H.J., McFarland, C., Loeb, K.R., Valente, W.J., Ericson, N.G., Stevens, E.A., Radich, J.P. *et al.* Massively parallel digital transcriptional profiling of single cells. *Nat Commun* **8**, 14049 (2017).

7. Hao, Y., Hao, S., Andersen-Nissen, E., Mauck III, W., Zheng, S., Butler, A., Lee, M., Wilk, A., Darby, C., Zagar, M., Hoffman, P., Stoeckius, M., Papalexi, E., Mimitou, E., Jain, J., Srivastava, A., Stuart, T., Fleming, L., Yeung, B., Rogers, A., McElrath, J., Blish, C., Gottardo, R., Smibert, P., Satija, R. Integrated analysis of multimodal single-cell data. *BioRxiv* (2020).

8. Bacher, R. & Kendziorski, C. Design and computational analysis of single-cell RNA-sequencing experiments. *Genome Biol* **17**, 63 (2016).

9. Chen, G., Ning, B. & Shi, T. Single-Cell RNA-Seq Technologies and Related Computational Data Analysis. *Front Genet* **10**, 317 (2019).

10. Korsunsky, I., Fan, J., Slowikowski, K., Zhang, F., Wei, K., Baglaenko, Y., Brenner, M., Loh, P., Raychaudhuri, S. Fast, sensitive, and accurate integration of single cell data with Harmony. *BioRxiv* (2019).

11. Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W., Hao, Y., Stoeckius, M., Smibert, P., Satija, R. Comprehensive Integration of Single-Cell Data. *Cell*  **177**(7), 1888-1902 (2019).

12. Lake, B.B., Chen, S., Sos, B.C., Fan, J., Kaeser, G.E., Yung, Y.C., Duong, T.E., Gao, D., Chun, J., Kharchenko, P.V. & Zhang, K. Integrative single-cell analysis of transcriptional and epigenetic states in the human adult brain. *Nat Biotechnol* **36**, 70-80 (2018).