SUPPLEMENTAL METHODS

Systematic review

Protocol Registration

Methodology and inclusion criteria were specified in advance and documented in a protocol, which was registered in PROSPERO (ID: CRD42020188720).

Eligibility Criteria

Inclusion criteria were (1) original research journal articles; (2) English language; (3) human brain post-mortem morphological (quantitative, semi-quantitative, or qualitative) studies of astrocytes in AD versus control brains (including immunohistochemical and immuno-electron microscope studies). Exclusion criteria were (1) non-original journal articles (e.g., reviews, opinion articles, editorial comments); (2) non-English language; (3) non-human studies (e.g., studies using animal models, *in vitro* primary astrocyte cultures or astrocytoma cell lines, computational analyses and mathematical models); (4) human plasma/serum and CSF biomarker studies or PET imaging studies; (5) human brain post-mortem non-morphological studies such as biochemical (Western blot, ELISA, mass spectrometry) or molecular biology (RT-qPCR, microarray, RNA-seq) studies; (4) human brain post-mortem morphological electron microscopy studies (i.e., if no immuno-gold antibodies were used).

Information Sources

Three databases were inquired: National Center for Biotechnology Information (NCBI) PubMed, American Psychiatry Association (APA) PsycInfo, and the Web of Science – Science Citation Index Expanded (WoS-SCIE).

Search Strategies

All three databases were interrogated on the same day (March 15th, 2020) with no date filters applied. For PubMed, we used the MeSH Terms 'Alzheimer Disease' and 'Astrocytes' with the Boolean 'AND', along with filters for English language, human studies, and journal articles, as follows: ("Alzheimer Disease"[MeSH Terms] AND "Astrocytes"[MeSH Terms]) AND ((journalarticle[Filter]) AND (humans[Filter]) AND (english[Filter])). For APA PsycInfo, we used the terms 'Alzheimer's disease' and 'Astrocytes' with the Boolean 'AND' and applied filters for human studies, English language, and journal articles. For WoS-SCIE, we conducted individual searches for 'Alzheimer's disease' and 'Astrocyte' and then combined them with the Boolean 'AND', with the appropriate filters for journal articles in English. To maximize article retrieval, we refrained from adding other search terms such as 'post-mortem', 'autopsy', 'immunohistochemistry', 'immunoreactivity', or 'reactive', at the expense of applying the above eligibility criteria to a larger number of articles. In addition, we carefully examined the reference lists of eligible articles to capture additional eligible articles which may have been excluded at the search, screening, or eligibility steps.

Study Selection

We collected n = 1,234 available PubMed IDs (PMIDs) of the articles retrieved by querying the three databases, to which we manually added n = 3 articles (two from the APA PsycInfo search and one from the WoS-SCIE search) lacking PMIDs, as well as n = 54 additional records selected by scanning of reference lists, for a total of n = 1,291 records identified. We then removed duplicated or unavailable records in R to obtain n = 1,067 unique articles. The screening of these 1,067 articles based on title and abstract was conducted by two authors (LV-N and AS-P), each of whom screened approximately half of the references. A list of 100 references was generated by a

random sampling of the 1,067 references and screened by both LV-N and AS-P to investigate interrater agreement. There was a 91% agreement between both observers with a Cohen's kappa coefficient of 0.79. Articles that passed screening were downloaded and further examined by LV-N and AS-P for adherence to eligibility criteria. Eligible articles were those meeting all inclusion criteria and none of the exclusion criteria.

Data Collection Process

Data collected included: PMIDs; first author; year of publication; study marker; sample size, age, sex and post-mortem interval for AD and control groups; sample size, age, and sex for all other neuropathological diagnosis groups; type of the study (qualitative, semi-quantitative or quantitative); quantitative method in quantitative studies (e.g., area fraction, densitometry, stereology-based); main cell type expressing the marker; other cell types or features stained; brain region(s); tissue characteristics (e.g., free floating, fresh frozen, formalin-fixed paraffin-embedded); antibody characteristics (monoclonal versus polyclonal, clone if monoclonal, concentration, brand name, catalogue number, and epitope when available); immunohistochemistry visualization technique (chromogenic/peroxidase, immunofluorescence, or both); and colocalization with GFAP. To consolidate changing nomenclatures over the span of this systematic review, astrocyte markers are referred to by both UniProtKB recommended names (14) and HUGO Gene Nomenclature Committee symbols (15) (of the genes encoding each protein). In the case of proteins with more than one subunit or isoform for which the specificity of the antibody was not described by the original publication, only the symbol corresponding to the catalytic subunit or predominant isoform was selected.

Bioinformatics analyses

Unless otherwise indicated, all bioinformatics analyses were performed in the R programming language and statistical computing environment (version 4.0.3).

Pathway Enrichment Analysis (PEA)

For each database (i.e., Gene Ontology (GO): Biological Process, GO: Molecular Function, GO: Cellular Component, and Reactome), the top 100 most significant pathways by false discovery rate (FDR) *q*-value were calculated. To reduce redundancy of these pathways and aggregate terms describing the same biological phenomenon, pathways were grouped by hierarchical clustering based on the Jaccard similarity coefficient (i.e., the ratio of the intersection over the union of the constituent genes of each pair of pathways). Aggregated pathways were labelled with the common biological processes denominated by the individual pathways.

Protein-Protein Interaction (PPI) Network Analysis

In the PPI network, the nodes (representing proteins) are coloured based on a functional protein classification decided *a priori* (see below). The thickness of each edge represents the strength of the evidence supporting an interaction between the parent nodes, and the minimum required interaction score was set at a medium confidence (0.400). All active interaction sources were used (including text mining, experimental, knowledge base, co-expression, neighbourhood, gene fusion, and co-occurrence), and isolated nodes (with degree ≤ 2) were hidden from visualization for clarity. The connectivity of each protein within the network was evaluated by eigenvalue centrality to identify specific hub proteins which may play modulatory and/or regulatory roles in the network.

Transcription Factor Enrichment Analysis (TFEA)

We leveraged publicly available ChIP-seq databases to perform TFEA using TFEA.ChIP and Enrichr. Specifically, for TFEA.ChIP, we used a set of 2,759 ChIP-seq experiments from the ReMap 2018 repository (23) representing potential gene targets for 487 human transcriptional regulators, where ChIP-seq peaks were associated with potential target genes using the GeneHancer database (21,24). For Enrichr, we used consensus target genes for transcription factors present in both the ENCODE project (25) and the ChIP Enrichment Analysis (ChEA) database (26). Transcription factor enrichment was represented via volcano plots of significance versus effect size for each ChIP-seq experiment, and bar graphs of enrichment scores per transcription factor, respectively.

Comparison with Transcriptomic and Proteomic Studies

Finally, we compared the ADRA protein set with recent transcriptomic and proteomic studies on human control and AD brains, including: (1) a microarray expression profiling dataset of laser-capture microdissected GFAP-immunoreactive astrocytes from the temporal neocortex of $n = 6$ Braak I/II, $n = 6$ Braak III/IV, and $n = 6$ Braak V/VI subjects (27); (2) a single-nuclei RNA-seq study on the entorhinal cortex of $n = 6$ AD and $n = 6$ control subjects (28); (3) the Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) Consortium bulk brain proteomic study on the dorsolateral prefrontal cortex (BA9) of 419 individuals from four cohorts, encompassing $n = 91$ control (Braak 0-III and cognitively normal), $n = 98$ asymptomatic AD (Braak III-VI, but cognitively normal), and $n = 230$ AD dementia subjects (Braak IV-VI and cognitively impaired), with 3,334 proteins identified in > 50% subjects (29); and (4) Cohort 1 of the AMP-AD Consortium CSF proteomic study, which includes $n = 147$ control and $n = 150$ AD subjects (total $n = 297$ subjects) (29).

After performing enrichment analysis using Fisher's exact test, we created heatmaps to illustrate the expression levels of the transcripts and proteins overlapping between the ADRA protein set and each of the -omics datasets. Specifically, the heatmaps of the Simpson et al. microarray and Grubman et al. snRNA-seq datasets represent the z-scores of both upregulated and downregulated transcripts across all astrocytes within each subject. Meanwhile, the heatmap of the Johnson et al. bulk brain proteomic study depicts the z-score of each protein averaged across all subjects with the same Braak NFT stage within each diagnostic group (i.e., control, asymptomatic AD, and AD dementia), and the heatmap of the Johnson et al. CSF proteomic study illustrates the z-scores averaged by deciles of CSF $A\beta_{42}$/p-tau ratio as a proxy for the severity of AD neuropathological changes.

SUPPLEMENTAL FIGURES

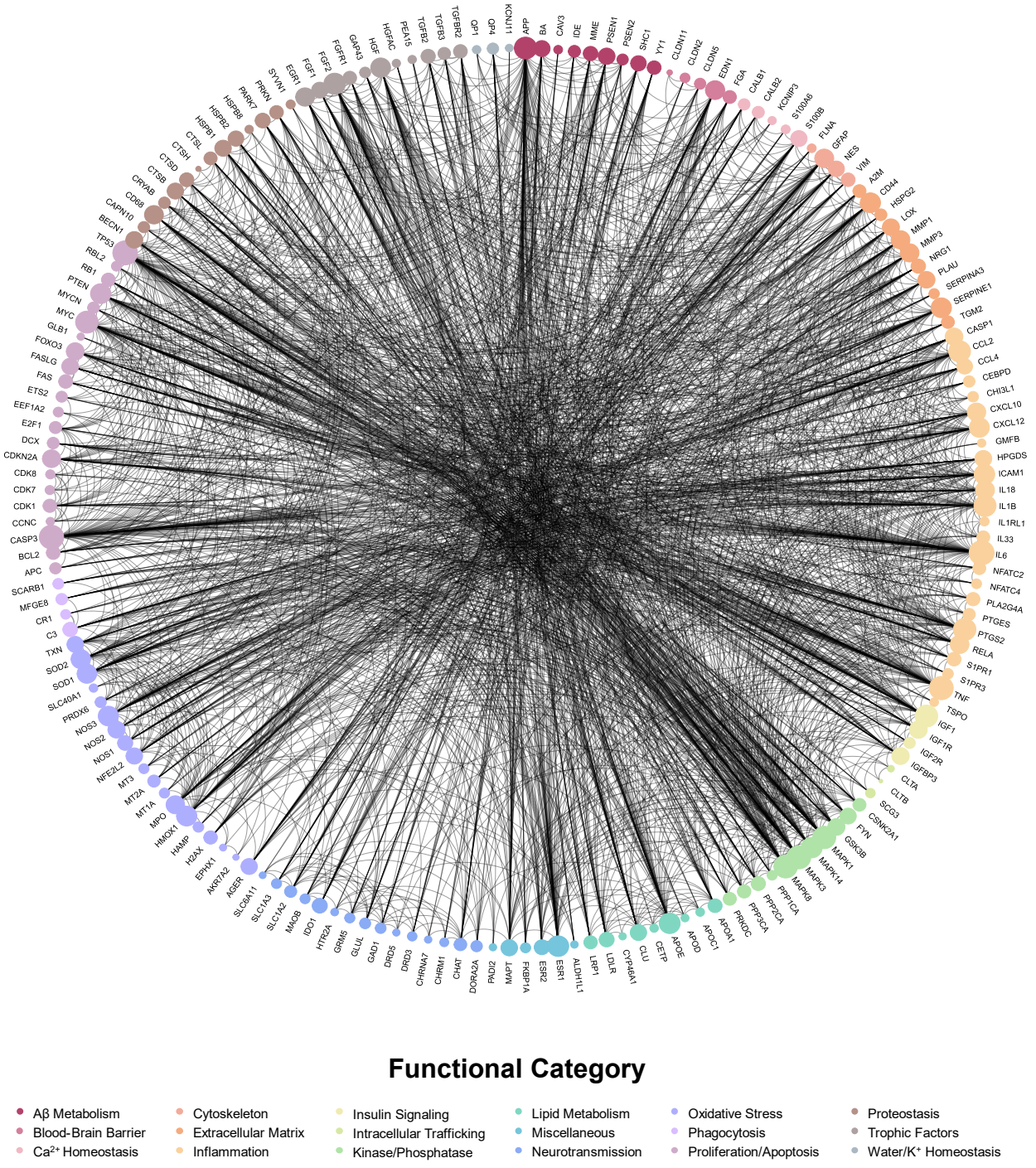

Figure S1. Circos plot depicting interconnectivity within the Alzheimer's disease reactive astrocyte (ADRA) protein set. The interconnectivity between the 196 constituents of the ADRA protein set and across functional categories is demonstrated via a Circos plot, where each node is a protein marker (magnify to read labels) and each colour represents a functional group.

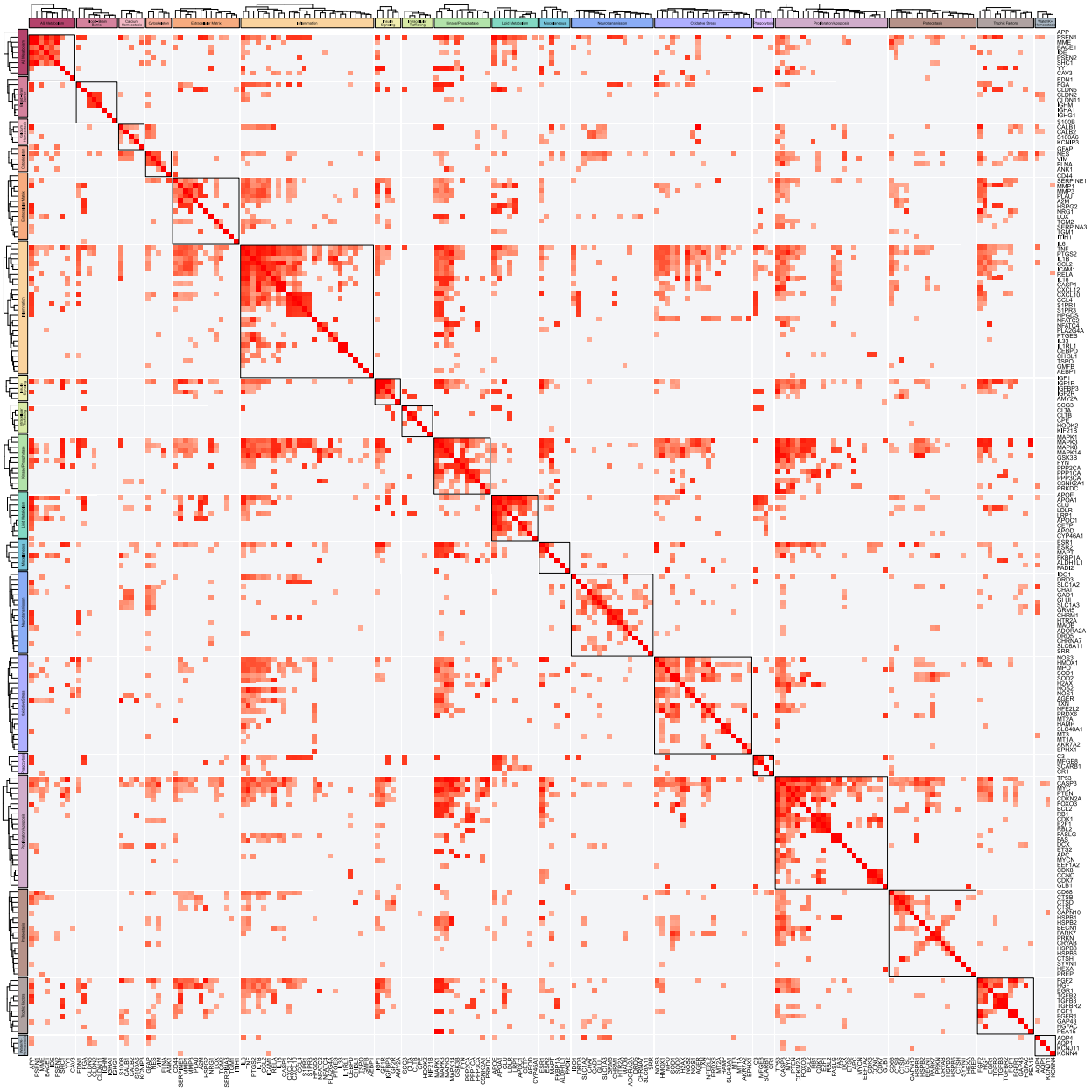

Figure S2. Heatmap of protein-protein interaction (PPI) network. Heatmap of the PPI network of the 196 ADRA proteins grouped by expert-annotated functional category. Note that markers belonging to the same functional group are highly connected, but substantial interactions are also observed between markers from different functional categories (e.g., oxidative stress and inflammation, proliferation/apoptosis and kinase/phosphatase). Please magnify to read the individual protein and functional category labels.

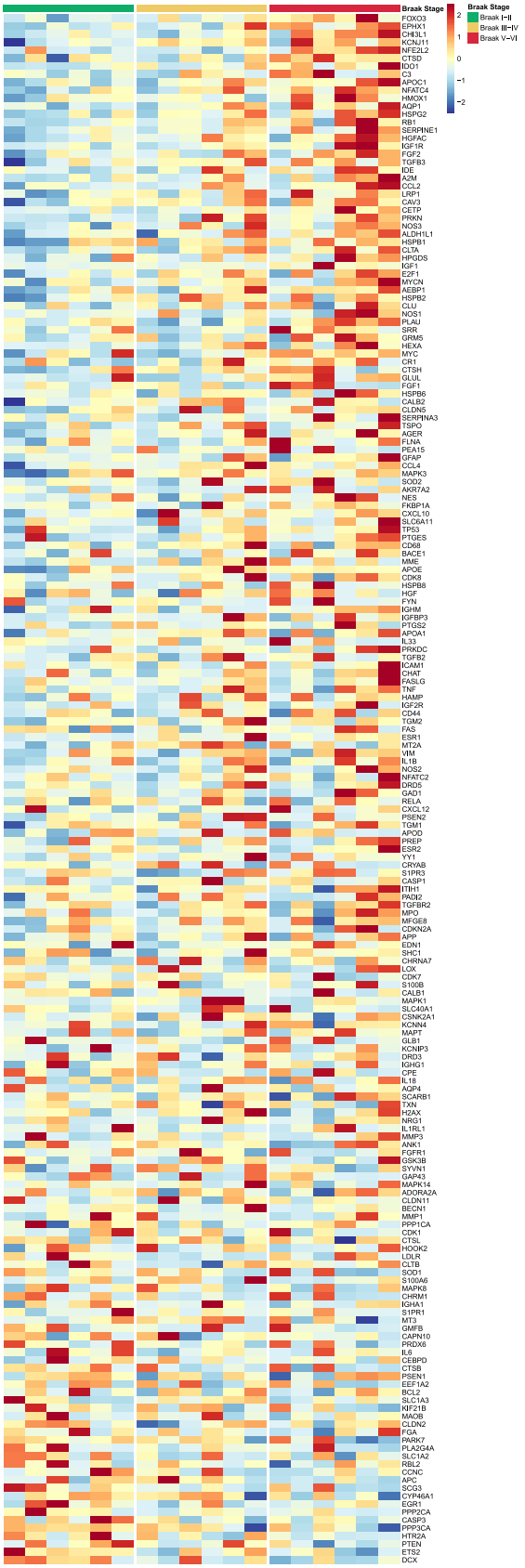

Figure S3. Full heatmap of the overlap between the ADRA protein set and a microarray dataset of laser-capture microdissected GFAP+ astrocytes. Heatmap shows the z-scores of gene expression of all available ADRA markers across the 18 subjects (n = 6 Braak I/II, n = 6 Braak III/IV, and n = 6 Braak V/VI) included in a microarray study of laser-capture microdissected GFAP+ astrocytes from the temporal neocortex (Simpson et al., 2011). Please magnify to read the individual protein labels.

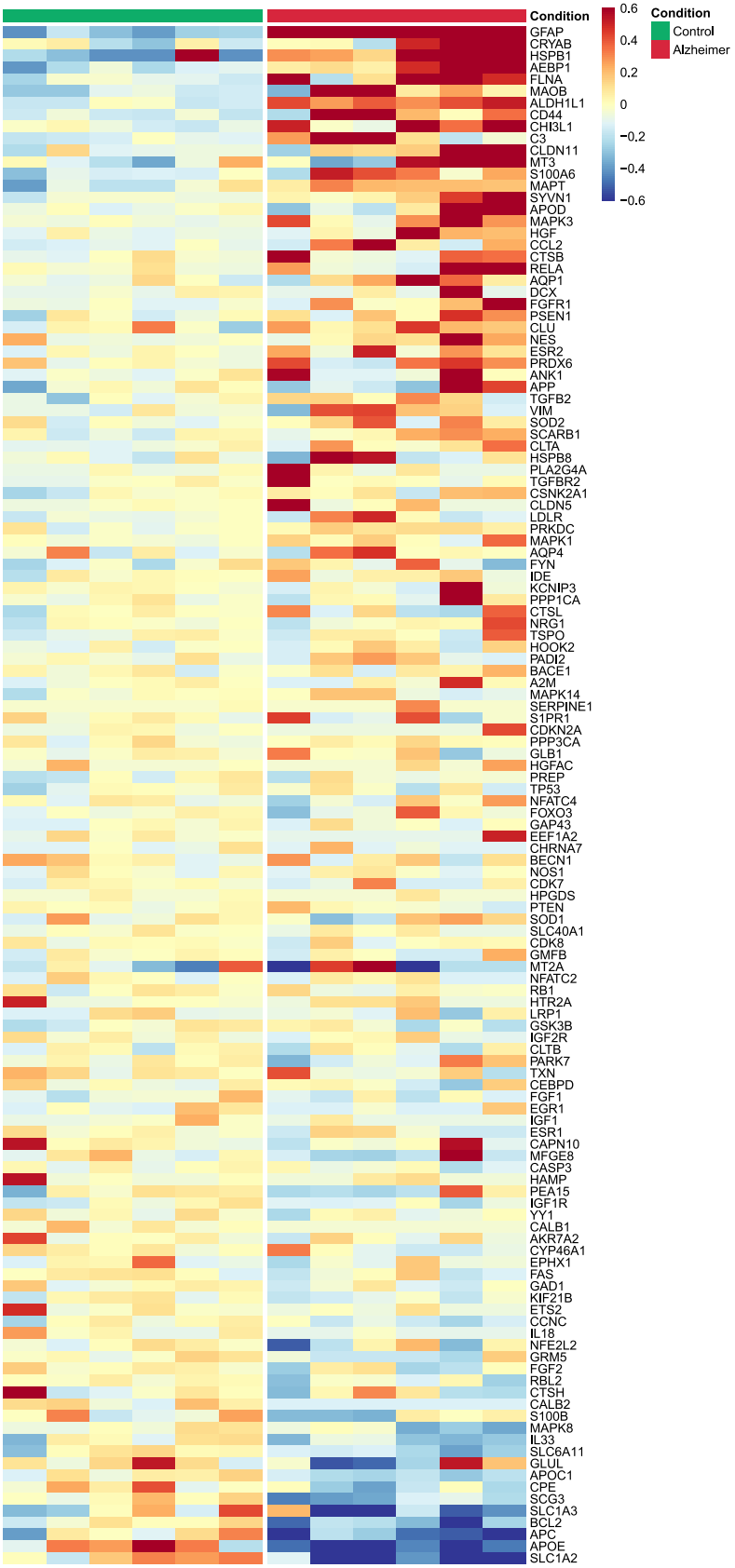

Figure S4. Full heatmap of the overlap between the ADRA protein set and a human astrocyte single-nuclei RNA-seq dataset. Heatmap shows the z-scores of gene expression of all available ADRA markers across all 12 subjects ($n = 6$ control and $n = 6$ AD) included in a single-nuclei RNA-seq study from the entorhinal cortex (Grubman et al., 2019). Please magnify to read the individual protein labels.

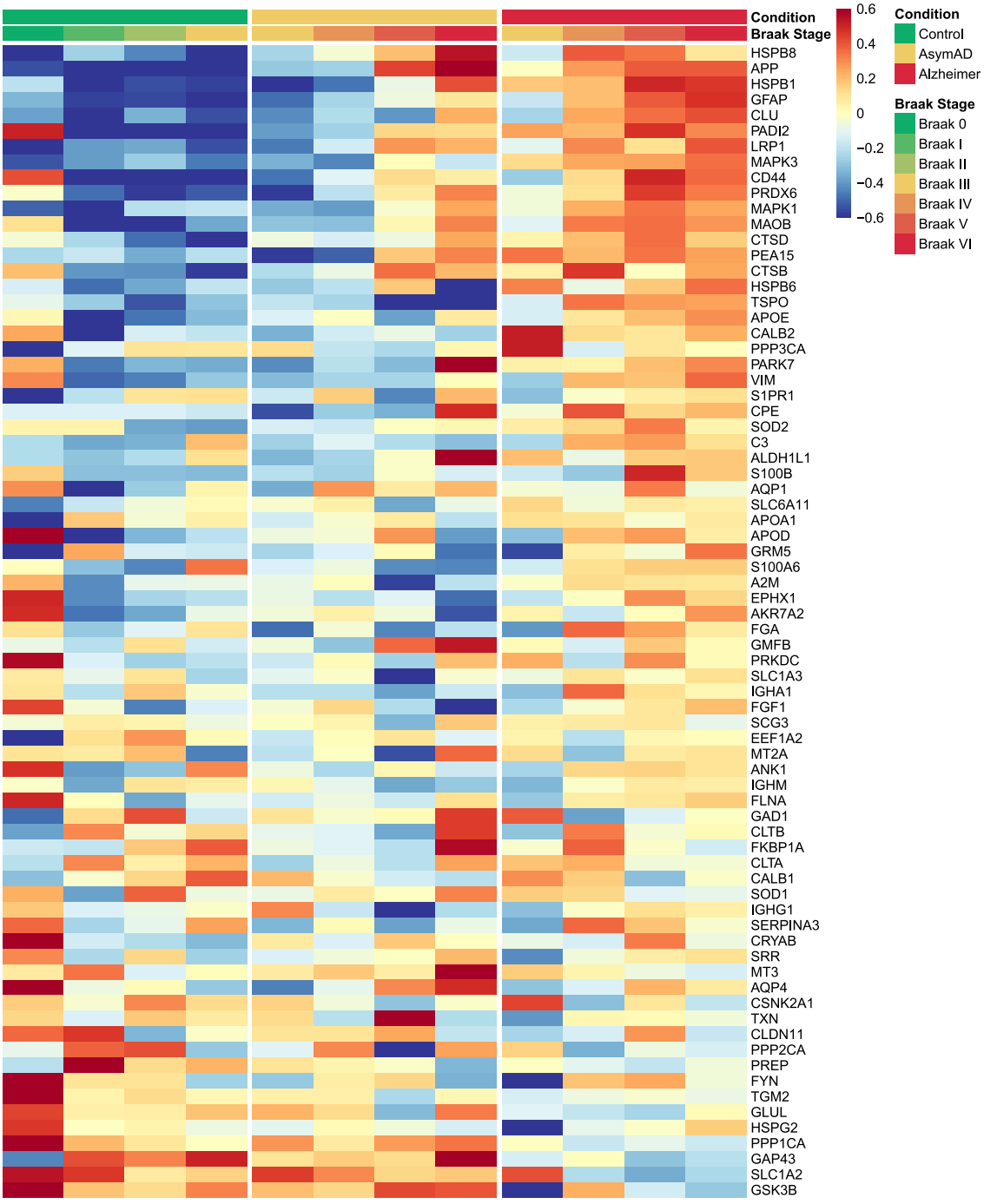

Figure S5. Full heatmap of the overlap between the ADRA protein set and a human bulk brain proteomic dataset. Heatmap illustrates the z-scores of protein expression of all available ADRA markers averaged by Braak NFT stage within each diagnostic group ($n = 91$ control, $n = 98$ asymptomatic AD, and $n = 230$ AD dementia subjects) described in the AMP-AD Consortium bulk brain proteomic dataset (Johnson et al., 2020).

SUPPLEMENTAL TABLE LEGENDS

Table S1. Studies and markers included in the systematic review. Detailed information collected from studies included in the systematic review. Studies are grouped by the functional categories of their respective markers. Colour code corresponds to Figures 3 and S1.

Table S2. Detailed pathway enrichment analysis results. Output of the pathway enrichment analyses against the curated pathway databases (i.e., Gene Ontology (GO): Biological Process (BP), GO: Cellular Component (CC), GO: Molecular Function (MF), and Reactome). Pathways are grouped by hierarchical clustering based on the Jaccard similarity coefficient and expert-annotated.

Table S3. Detailed transcription factor enrichment analysis results. Output of the TFEA.ChIP and Enrichr transcription factor enrichment analyses.