

DNA methylation patterns in the frontal lobe white matter of multiple system atrophy, Parkinson's disease, and progressive supranuclear palsy: A cross-comparative investigation

Megha Murthy^{1,2}, Katherine Fodder^{1,3}, Yasuo Miki⁴, Naiomi Rambarack^{1,2}, Eduardo De Pablo Fernandez^{1,2,5}, Lasse Pihlstrøm⁶, Jonathan Mill⁷, Thomas T Warner^{1,2,5}, Tammarn Lashley³, Conceição Bettencourt^{1,3,*}

1. Queen Square Brain Bank for Neurological Disorders, UCL Queen Square Institute of Neurology, London, UK.
2. Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK.
3. Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK.
4. Department of Neuropathology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki, Japan.
5. Reta Lila Weston Institute, UCL Queen Square Institute of Neurology, London, UK.
6. Department of Neurology, Oslo University Hospital, Oslo, Norway
7. Department of Clinical and Biomedical Sciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, UK.

***Corresponding Author:**

Conceição Bettencourt, PhD

Department of Neurodegenerative Disease

UCL Queen Square Institute of Neurology

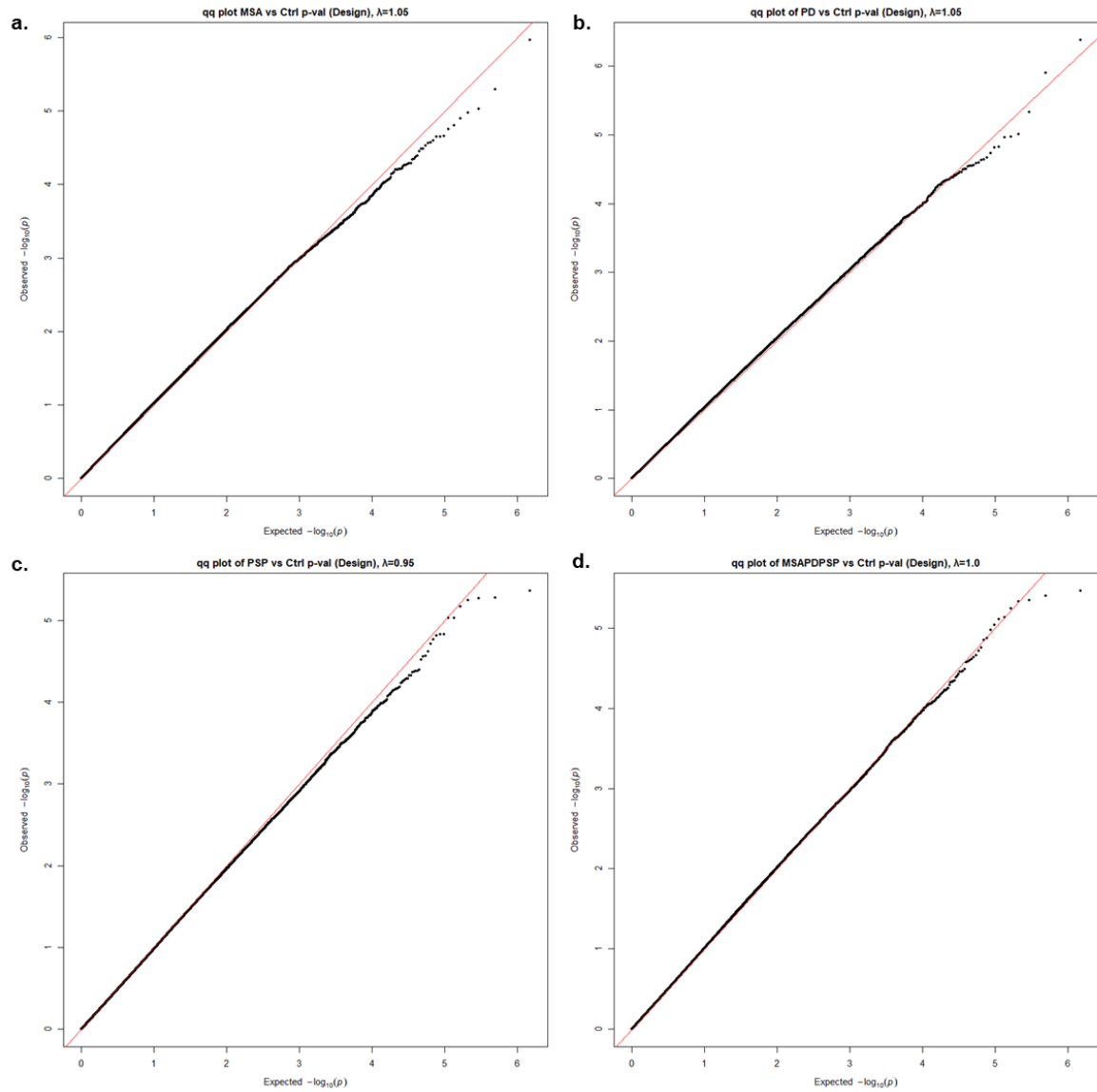
1 Wakefield Street

London WC1N 1PJ

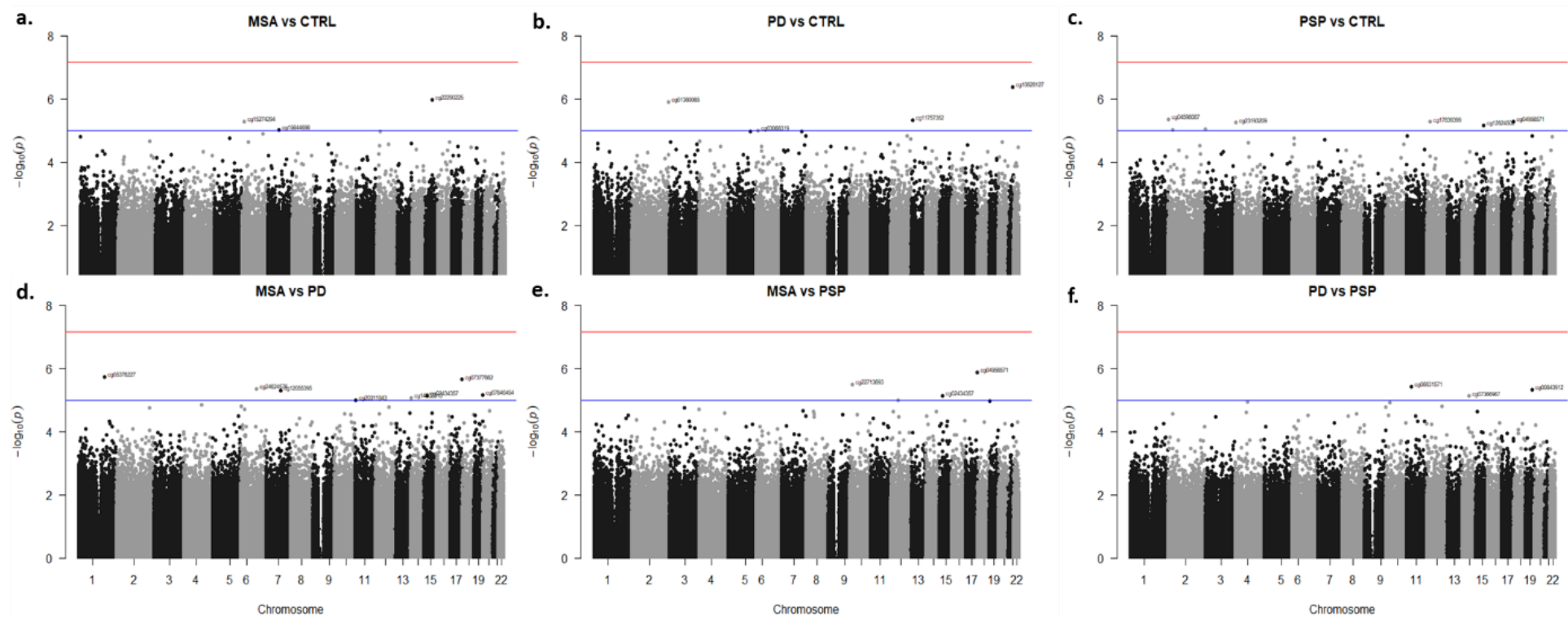
United Kingdom

Email c.bettencourt@ucl.ac.uk

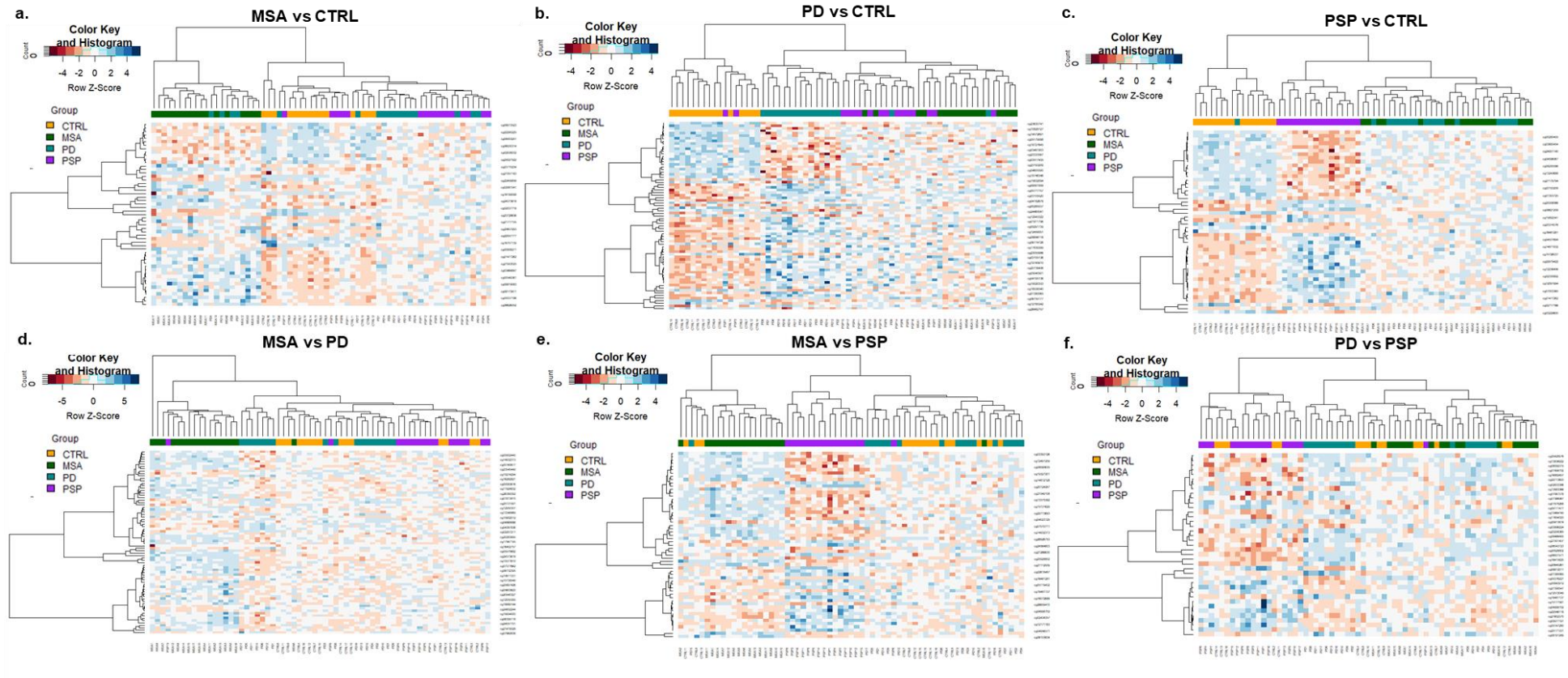
Supplementary Figures



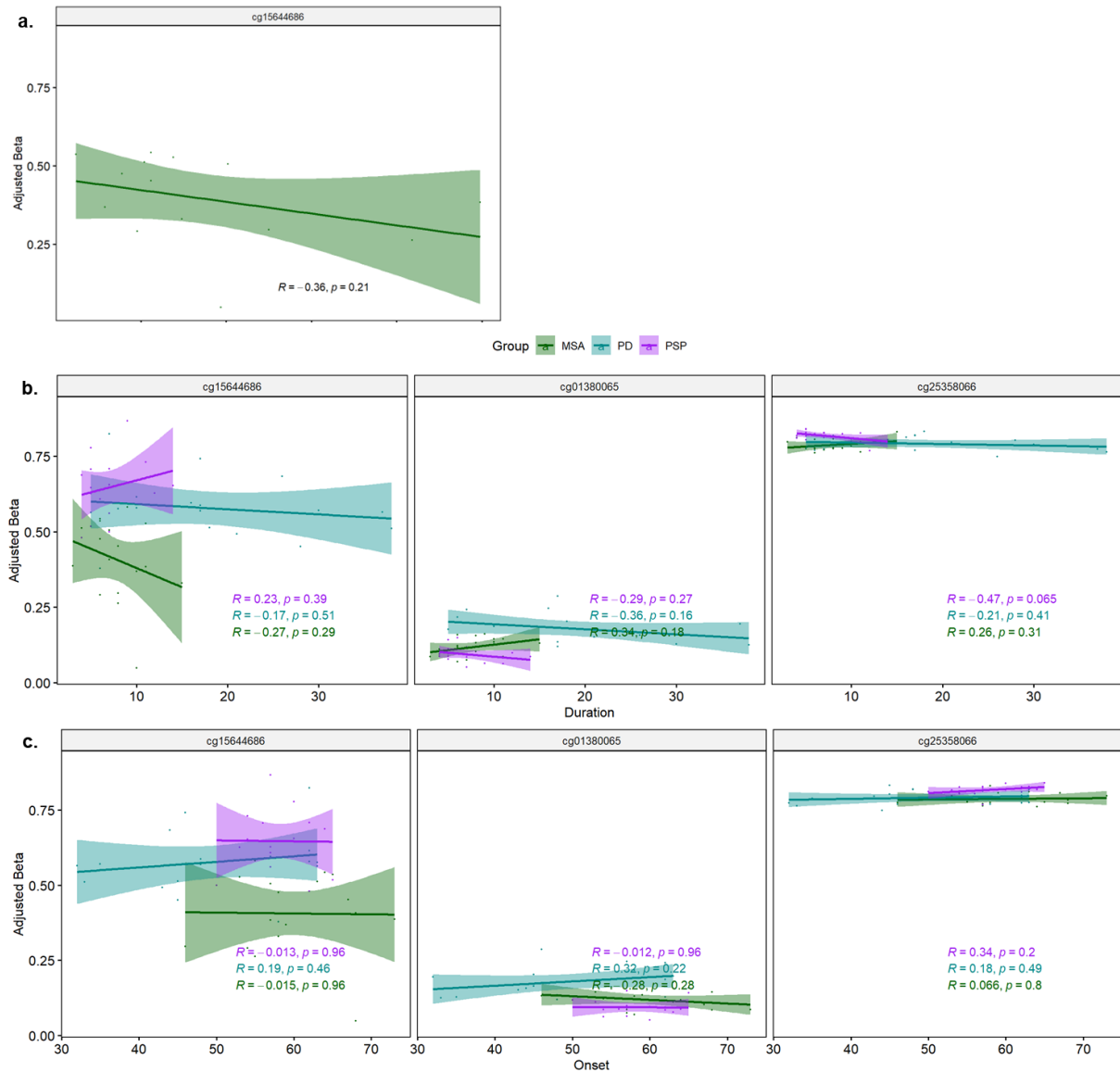
Supplementary fig. S1 Quantile-quantile (Q-Q) plots for the disease group specific case-control EWAS (a) Q-Q plot for MSA shows an estimated inflation factor (λ) of 1.05, (b) Q-Q plot for PD shows an estimated inflation factor (λ) of 1.05, (c) Q-Q plot for PSP shows an estimated inflation factor (λ) of 0.95, (d) Q-Q plot for all disease groups shows an estimated inflation factor (λ) of 1.0. CTRL – controls, MSA – multiple system atrophy, PD – Parkinson’s disease, PSP – progressive supranuclear palsy



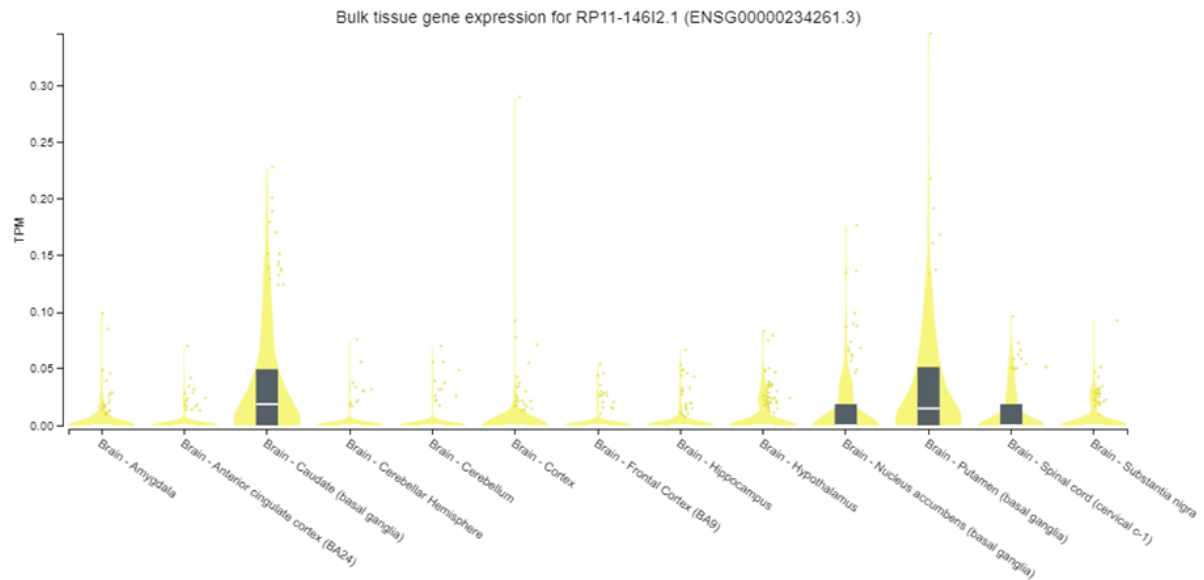
Supplementary fig. S2 Manhattan plots showing the p-value distributions of the differentially methylated CpGs for the different comparisons, (a.) MSA vs CTRL, (b.) PD vs CTRL, (c.) PSP vs CTRL, (d.) MSA vs PD, (e.) MSA vs PSP, (f.) PD vs PSP. The red line indicates genome-wide significance threshold based on Bonferroni-corrected p-values ($p = 6.8 \times 10^{-8}$), and the blue line indicates a less stringent suggestive significance threshold of $p = 1 \times 10^{-5}$. CTRL – controls, MSA – multiple system atrophy, PD – Parkinson’s disease, PSP – progressive supranuclear palsy



Supplementary fig. S3 Heatmap of the topmost significant differentially methylated loci ($p < 0.0001$) identified in (a.) MSA compared to controls (b.) PD compared to controls (c.) PSP compared to controls, (d.) MSA compared to PD, (e.) MSA compared to PSP, and (f.) PD compared to PSP. The rows represent CpGs, columns represent samples, and the colours represent the direction as well as the magnitude of effect (adjusted β values) in all the samples

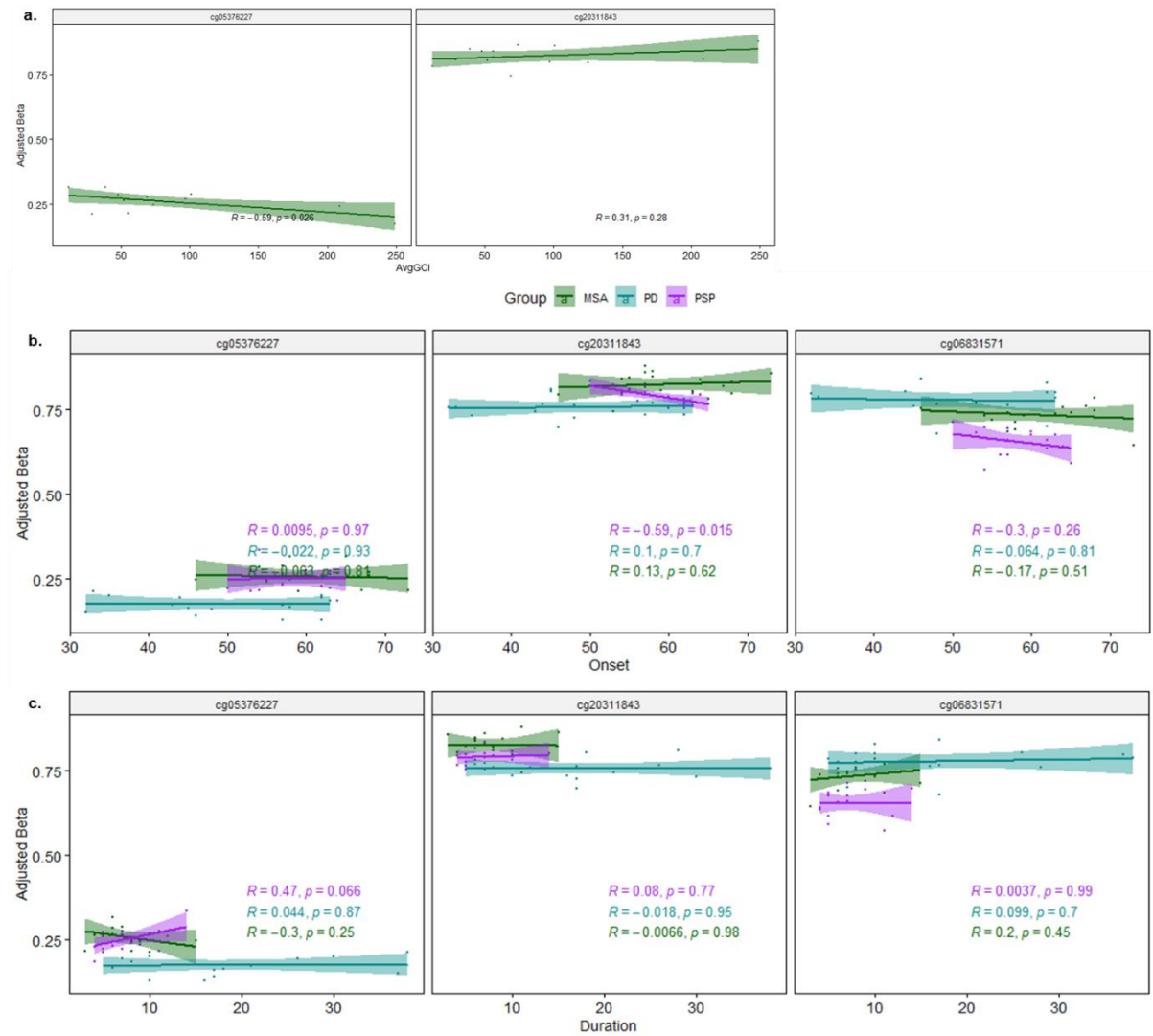


Supplementary fig. S4 Correlation between differential methylation levels and disease associated traits for the DMPs cg15644686 (*BCL7B*), cg01380065 (*UBE2F*), and cg25358066 (*D2HGDH*) in the different disease comparisons. Scatter plot and trend line (Pearson's correlation) showing correlation between methylation levels and (a) average GCI, (b) disease duration, and (c) disease onset. MSA – multiple system atrophy (mixed subtype), PD – Parkinson's disease, PSP – progressive supranuclear palsy, AvgGCI – average number of glial cytoplasmic inclusions

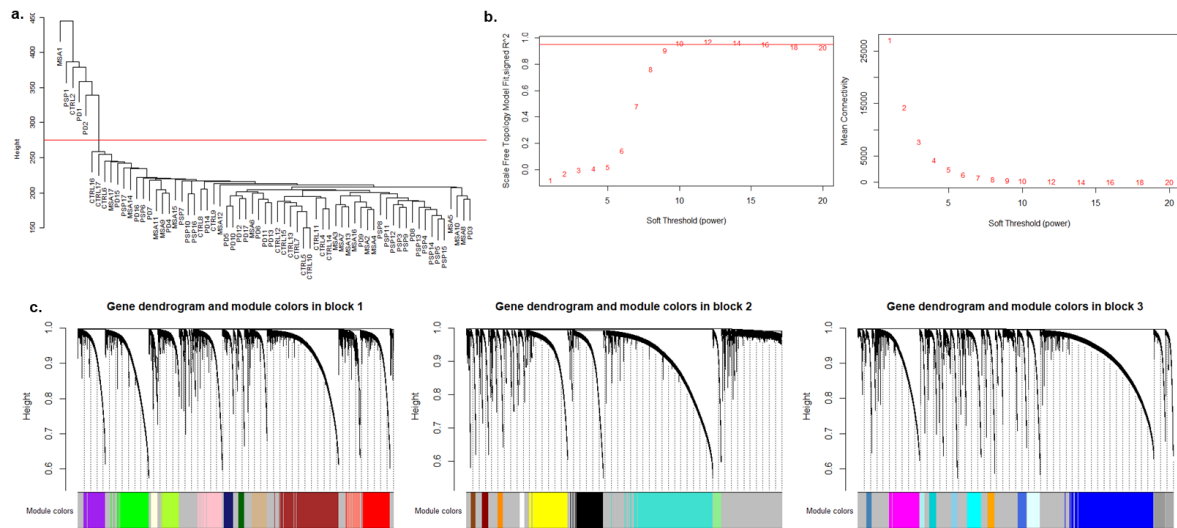


Supplementary fig. S5 Bulk tissue expression levels for the lincRNA encoded by the novel transcript ENSG00000234261 (where the CpG cg15274294 maps to) in different regions within the healthy brain

Gene expression data from GTEx analysis release V8 obtained from the GTEx Portal on 01/03/24 (<https://www.gtexportal.org/home/gene/ENSG00000234261>).



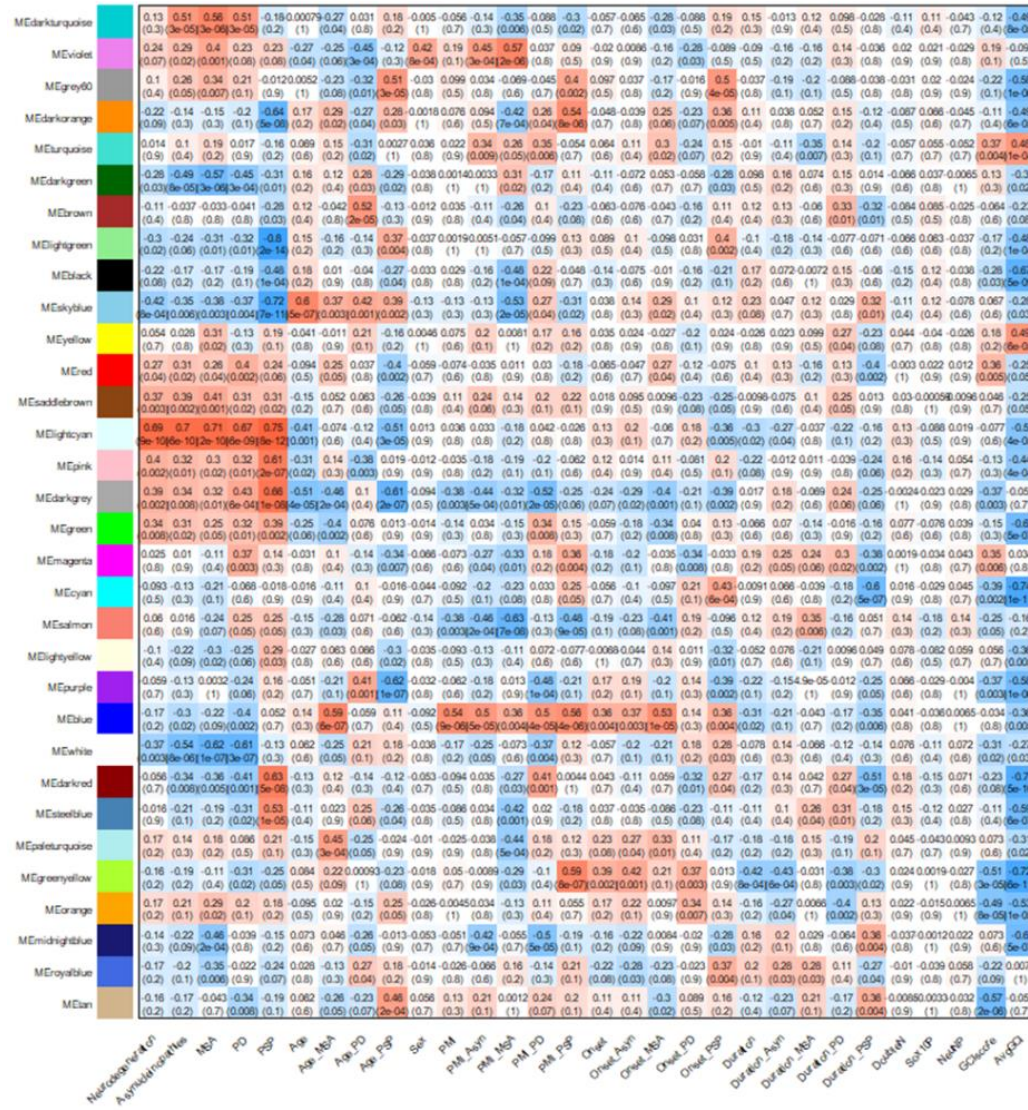
Supplementary fig. S6 Correlation between differential methylation levels and disease associated traits for the DMPs *cg05376227* (*FMO6P*), *cg20311843* (*OR51A7*) in MSA vs PD and *cg06831571* (Chr11 – IGR) in PD vs PSP; scatter plot and trend line (Pearson's correlation) showing correlation between methylation levels and (a) average GCI, (b) disease onset, and (c) disease duration. MSA– multiple system atrophy (mixed subtype), PD – Parkinson's disease, PSP – progressive supranuclear palsy, AvgGCI – average number of glial cytoplasmic inclusions



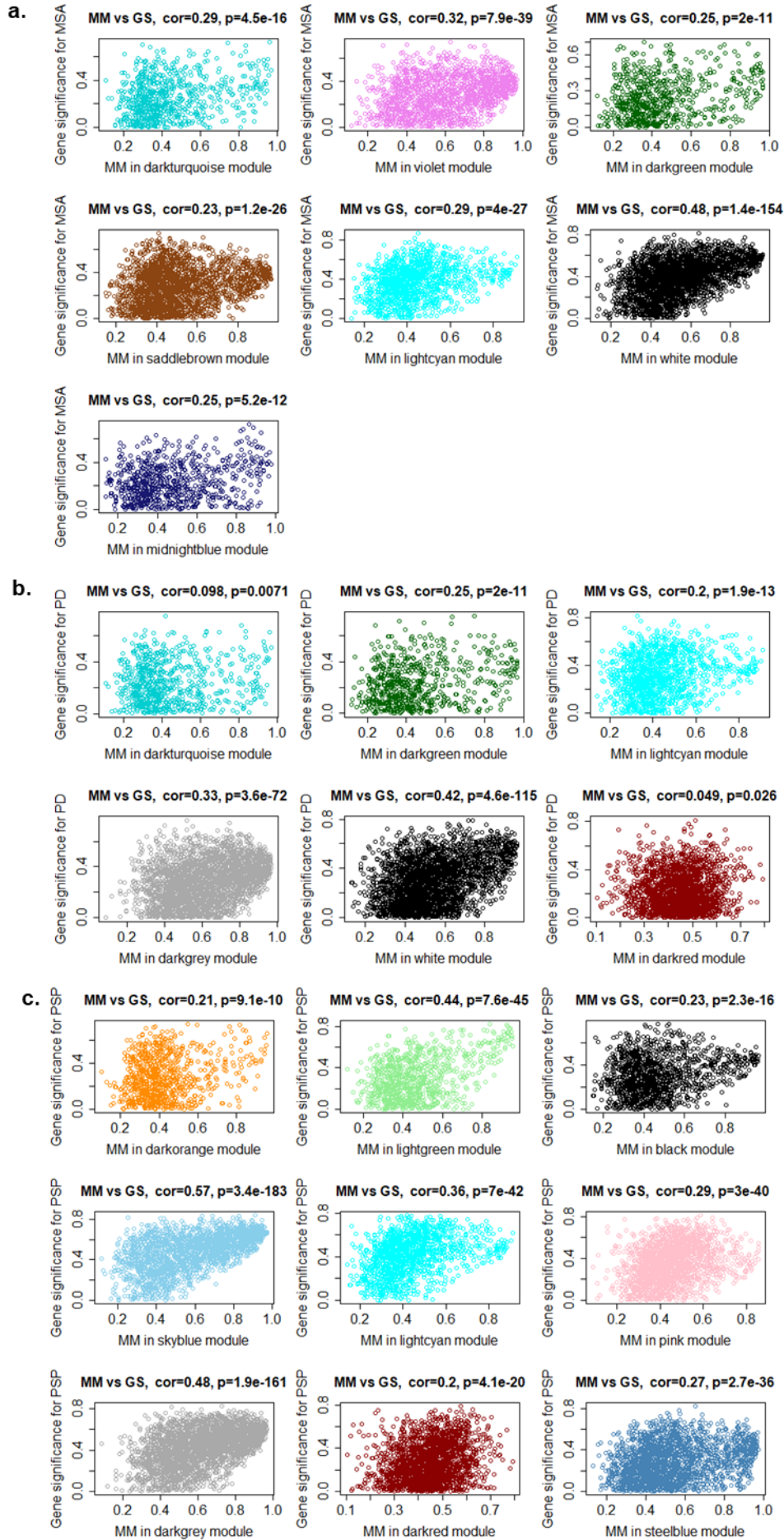
Supplementary fig. 7 Cluster trees and scale-free topology criteria for the comethylation network: (a) Sample clustering to detect outliers, (b) scale-free topology (SFT) plot for choosing the soft-thresholding power β for the signed weighted correlation network; left: SFT index R^2 (y-axis) as a function of different powers β (x-axis), right: mean connectivity (y-axis) as a function of the power β (x-axis), (c) hierarchical clustering dendrogram generated using blockwise module detection

a.

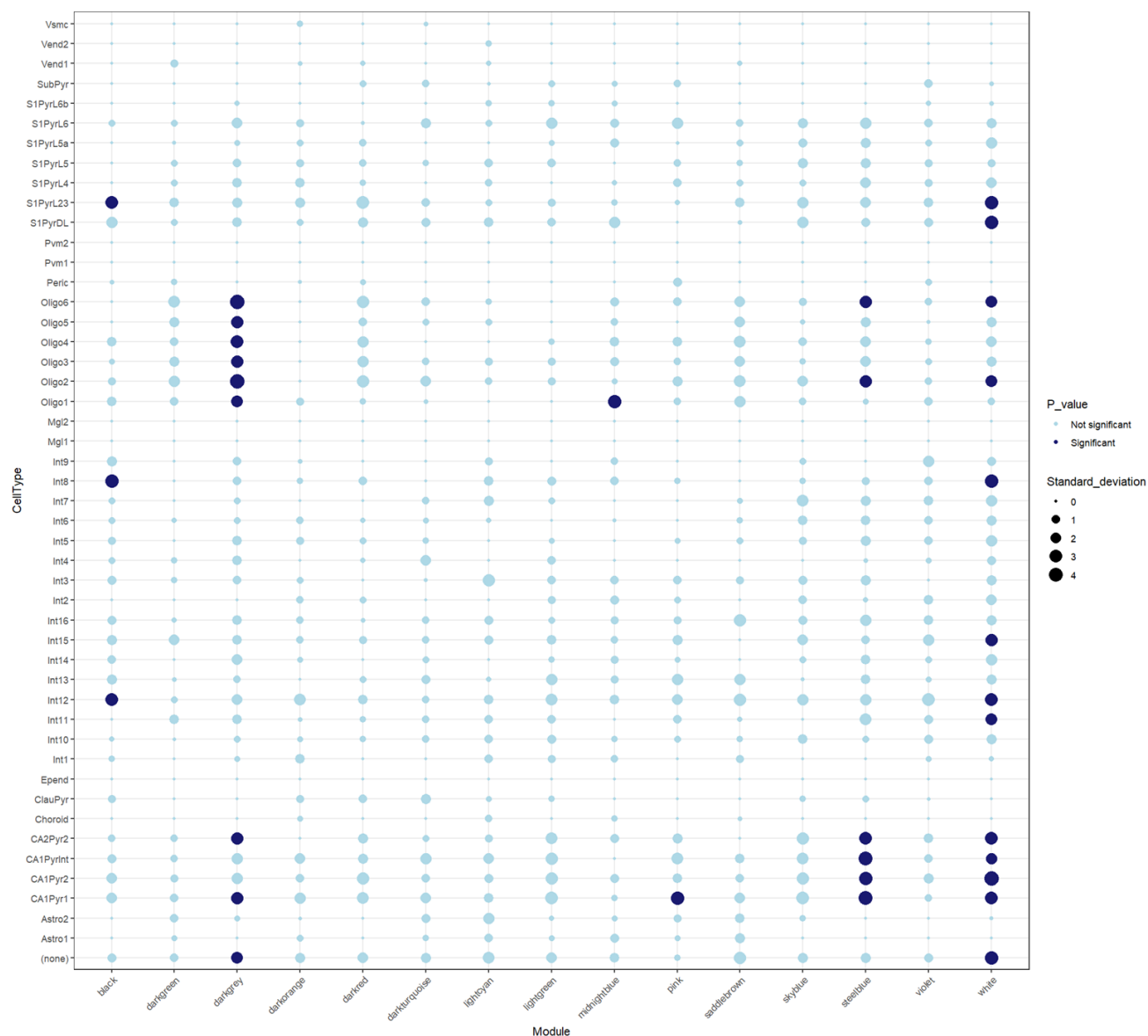
Module-trait relationships (k-means)



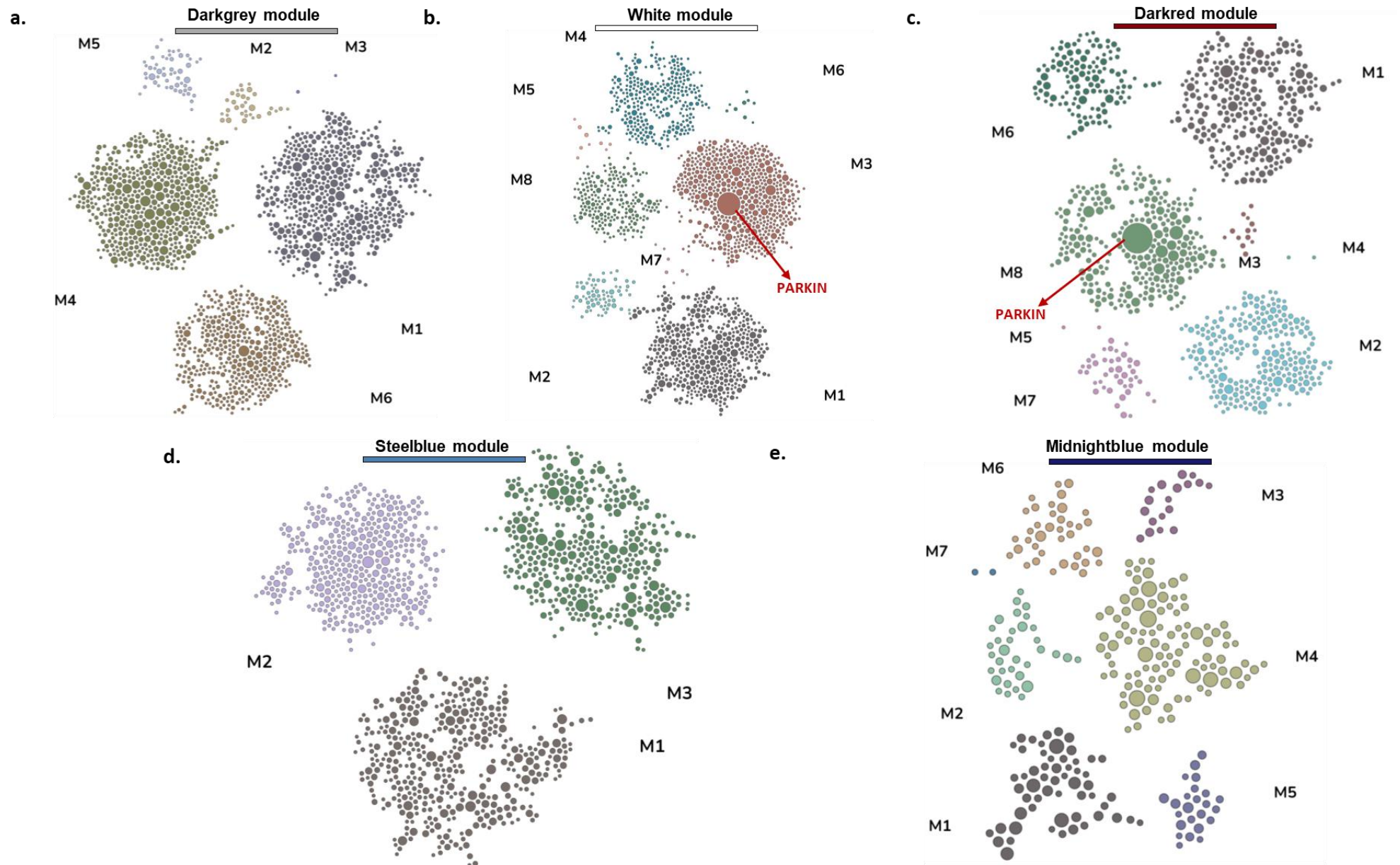
Supplementary fig. 8 Module-trait correlations and gene significance (a) heatmap showing the module-trait correlations and p values for all disease associated clinical/pathological traits, (b) gene significance of the different modules in all three disease groups (neurodegeneration), in MSA and PD (α -synucleinopathies), and in the individual disease groups – MSA, PD, and PSP. MSA– multiple system atrophy, PD – Parkinson’s disease, PSP – progressive supranuclear palsy



Supplementary fig. S9 Correlation between gene significance and module membership (kME) for modules significantly associated (adj.p $\leq$ 0.001) with (a) MSA, (b) PD, and (c) PSP. MSA– multiple system atrophy, PD – Parkinson’s disease, PSP – progressive supranuclear palsy, GS – gene significance, MM – module membership.



Supplementary fig. S10 Cell-type enrichment for the WGCNA modules associated with one or more disease groups. Enrichment for the different brain cell subtypes performed using the package EWCE and associated single-cell transcriptomic data which uses mouse to human homologs of genes associated with various cell types; dark blue circles represent significantly enriched cell types with adjusted p < 0.05 after Bonferroni corrections; the size of the circles represents the number of standard deviations (SD) from the mean



Supplementary fig. 11 Frontal lobe specific functional network analysis on the oligodendrocyte-enriched modules created using HumanBase (<https://hb.flatironinstitute.org/>) for the (a) darkgrey module associated with all three disease groups (MSA, PD, PSP), (b) white module significantly associated with α -synucleinopathies (MSA and PD), (c) darkred and (d) steelblue modules negatively associated with α -synucleinopathies (MSA and PD) but positively associated with PSP, and (e) midnightblue module exclusively associated with MSA. Both white and darkred modules showed PARKIN as the hubgene in their submodules. MSA – multiple system atrophy, PD – Parkinson’s disease, PSP – progressive supranuclear palsy