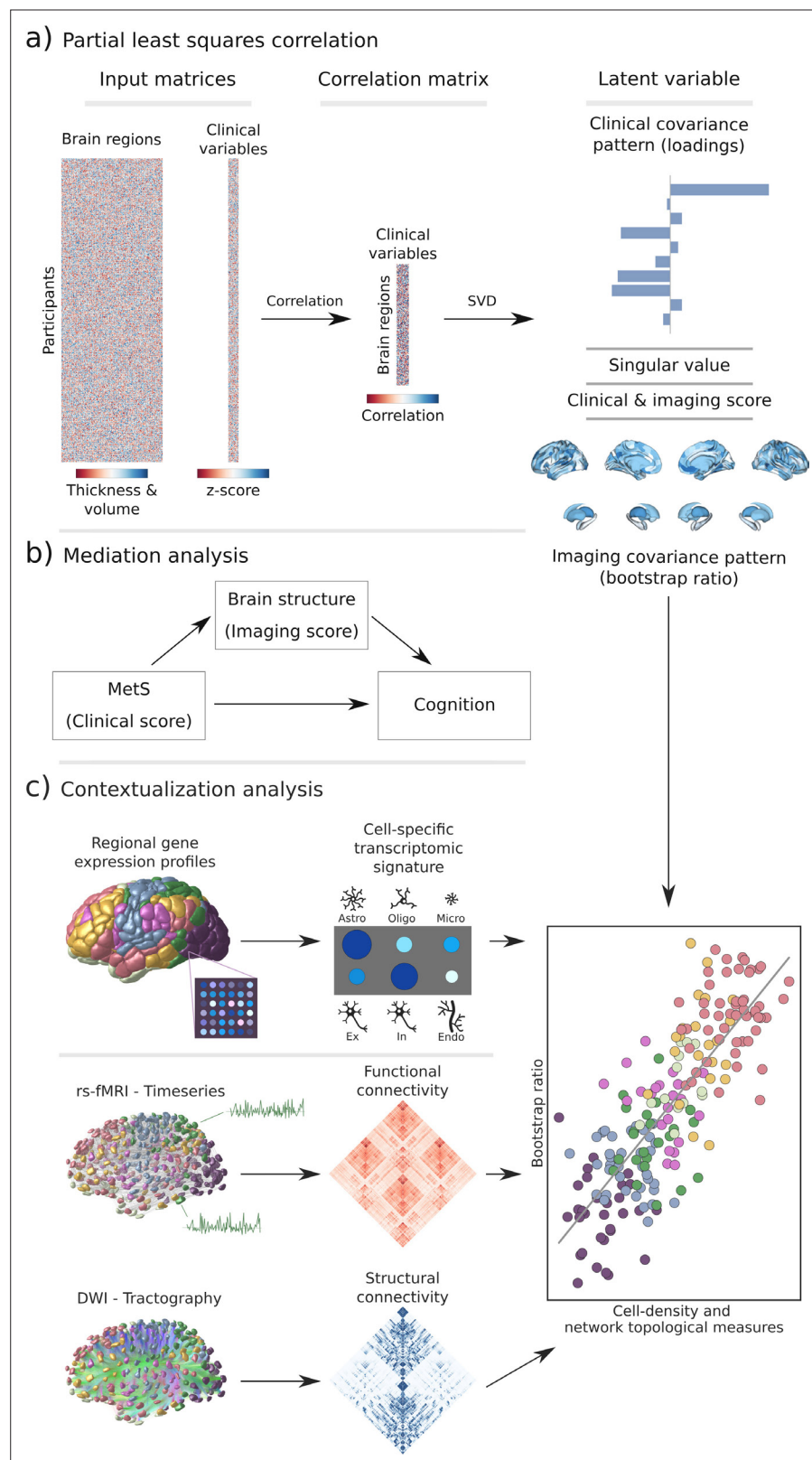# Figures and figure supplements

A latent clinical-anatomical dimension relating metabolic syndrome to brain structure and cognition

Marvin Petersen *et al.*

Figure 1. Methodology. (**a**) Illustration of the partial least squares correlation analysis. Starting from two input matrices containing per-subject information of regional morphological measures as well as clinical data (demographic and metabolic syndrome (MetS)-related risk factors) a correlation matrix is computed. This matrix is subsequently subjected to singular value decomposition resulting in a set of mutually orthogonal latent

Figure 1 continued on next page

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variables. Latent variables each consist of a left singular vector (here, clinical covariance profile), singular value, and right singular vector (here, imaging covariance profile). In addition, subject-specific clinical and imaging scores are computed. (**b**) The interplay between MetS, brain structure, and cognition was investigated in a post-hoc mediation analysis. We tested whether the relationship between the clinical score, representing MetS severity, and different cognitive test performances was statistically mediated by the imaging score. (**c**) Contextualization analysis. Upper row: based on microarray gene expression data, the densities of different cell populations across the cortex were quantified. Middle and lower row: based on functional and structural group-consensus connectomes based on data from the Human Connectome Project, metrics of functional and structural brain network topology were derived. Cell density as well as connectomic measures were related to the bootstrap ratio via spatial correlations. Modified from *Petersen et al., 2022b*; *Zeighami et al., 2019*. Abbreviations: Astro – astrocytes; DWI – diffusion-weighted magnetic resonance imaging; Endo – endothelial cells; Ex – excitatory neuron populations (Ex1-8); In – inhibitory neuron populations (In1-8); Micro – microglia; Oligo – oligodendrocytes; rs-fMRI – resting-state functional magnetic resonance imaging; SVD – singular value decomposition.
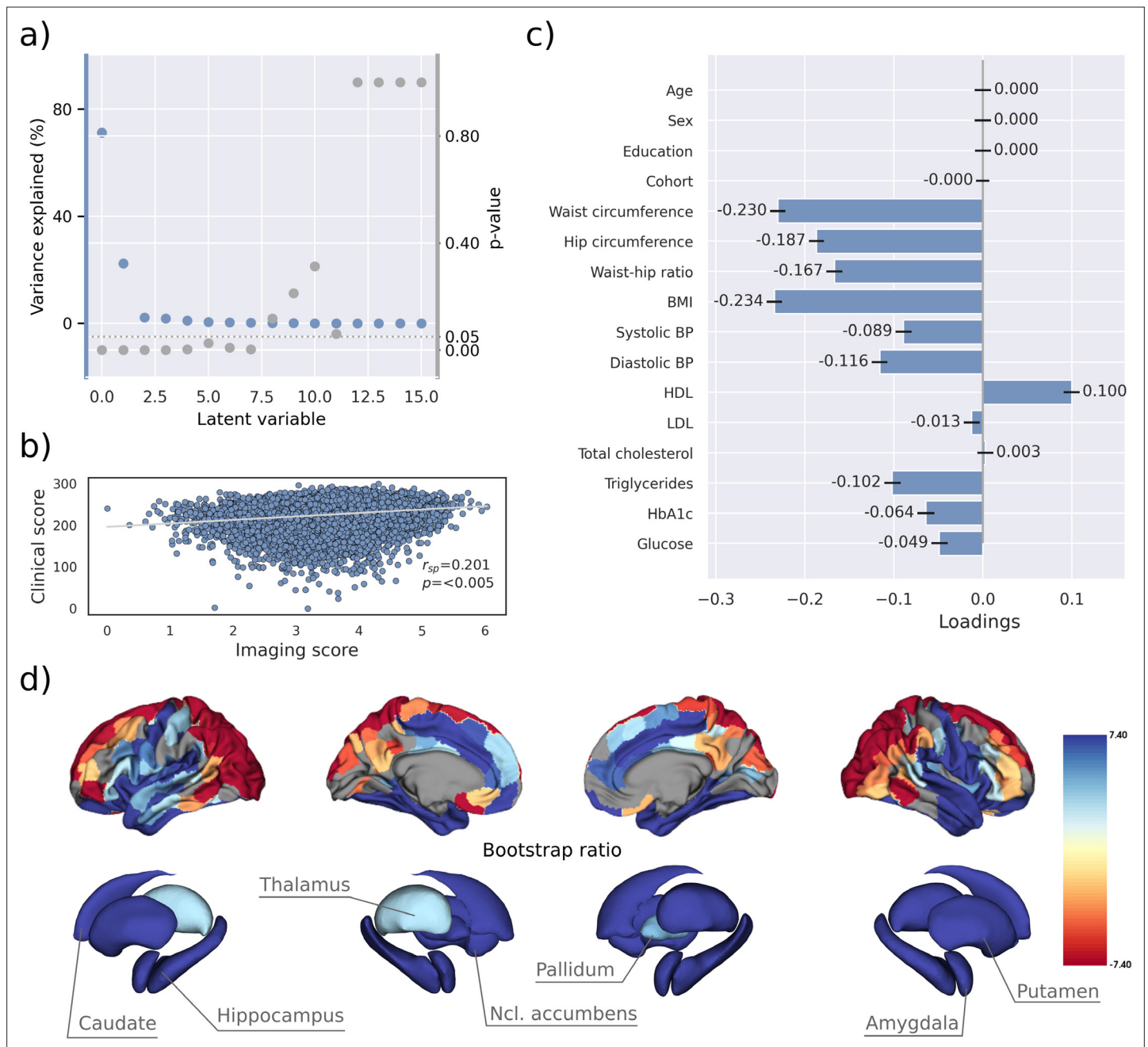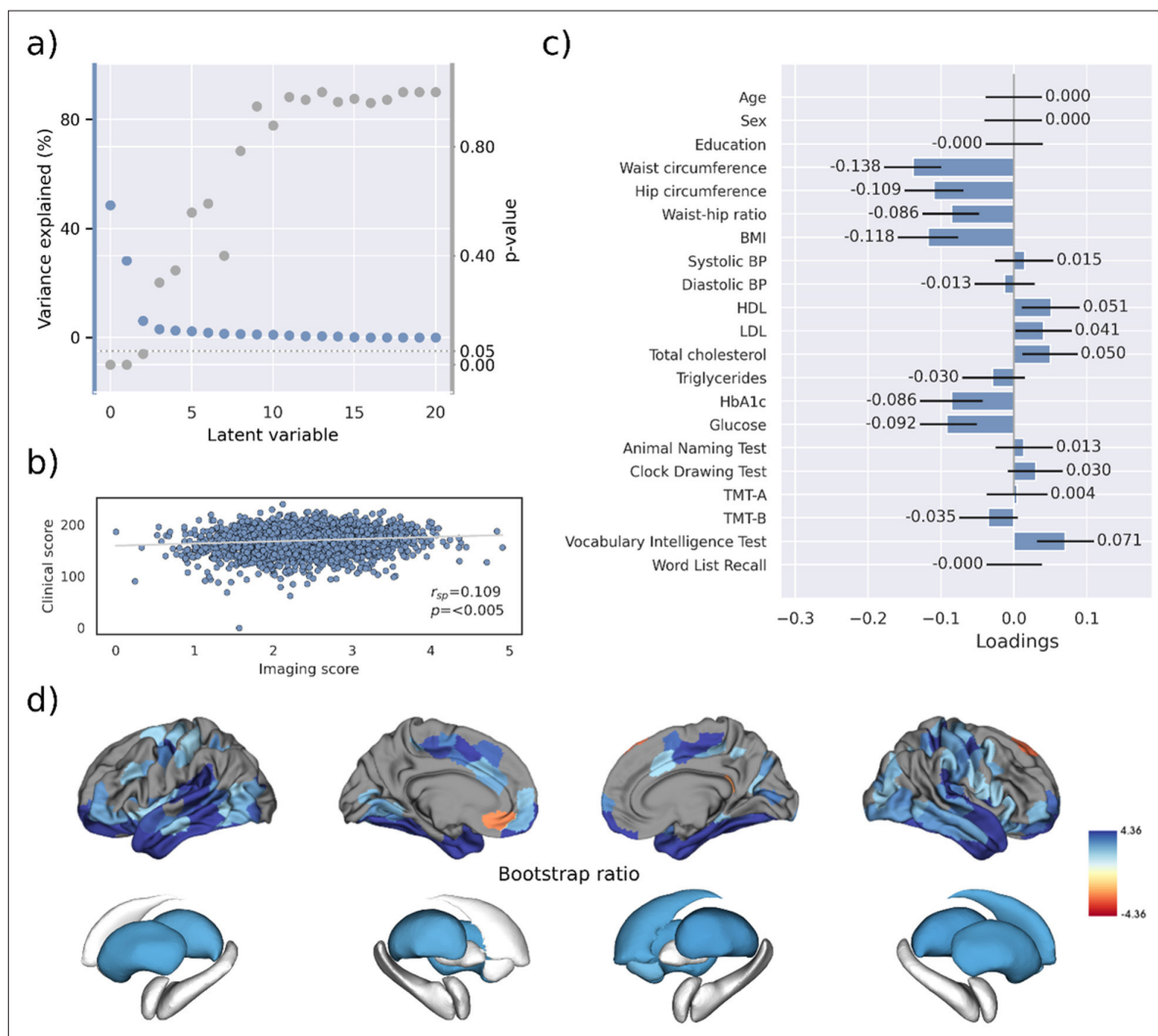
Figure 2. Partial least squares correlation analysis (PLS). (**a**) Explained variance and p-values of latent variables. (**b**) Scatter plot relating subject-specific clinical and imaging PLS scores. Higher scores indicate higher adherence to the respective covariance profile. (**c**) Clinical covariance profile. 95% confidence intervals were calculated via bootstrap resampling. Note that confound removal for age, sex, education, and cohort was performed prior to the PLS. (**d**) Imaging covariance profile represented by bootstrap ratio. A high positive or negative bootstrap ratio indicates high contribution of a brain region to the overall covariance profile. Regions with a significant bootstrap ratio (>1.96 or <−1.96) are highlighted by colors. Abbreviations: BMI – Body mass index, HDL – high-density lipoprotein, LDL – low-density lipoprotein, r_{sp} - Spearman correlation coefficient.
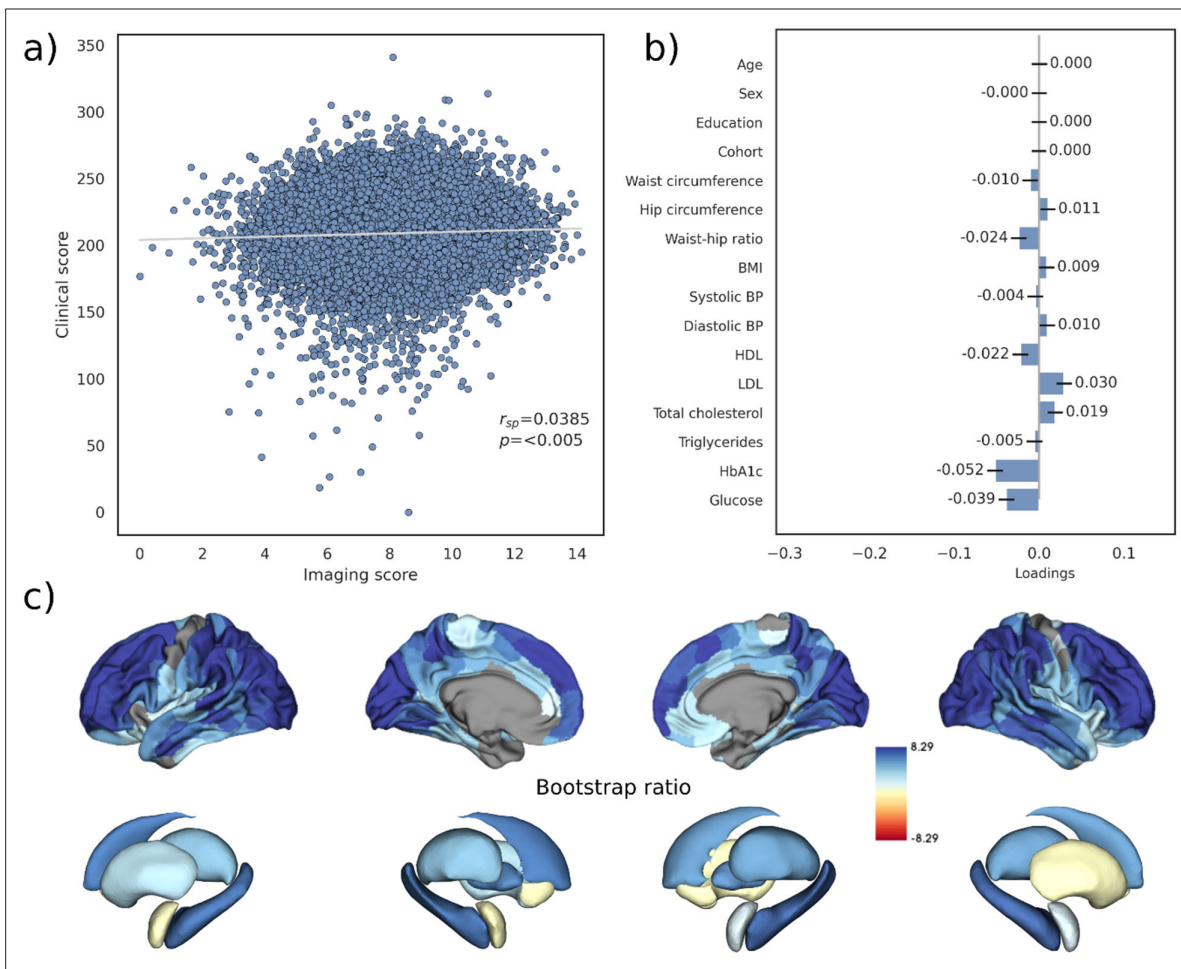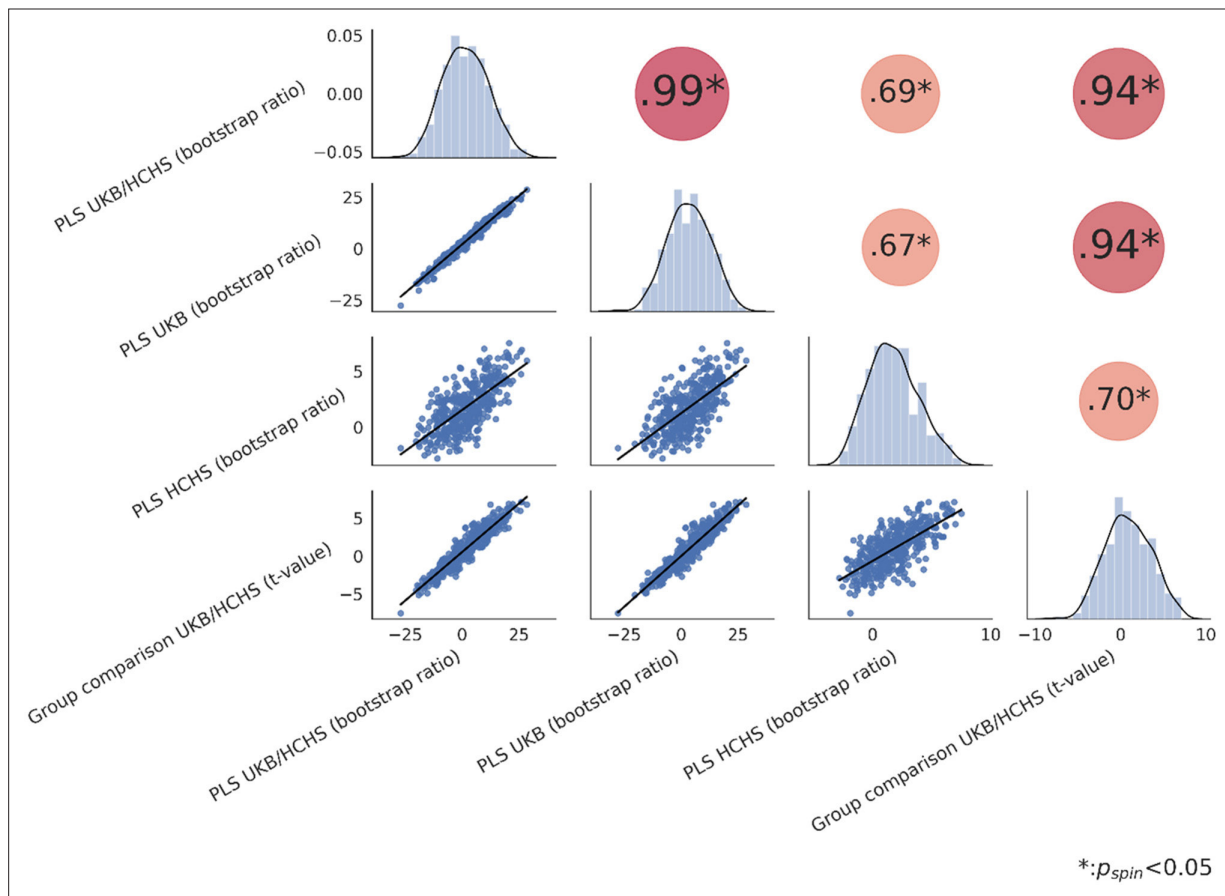
Figure 2—figure supplement 1. Partial least squares correlation analysis – Latent variable 2. The figure presents the results of latent variable 2 of the partial least squares correlation analysis. (**a**) Scatter plot relating subject-specific clinical and imaging PLS scores. (**b**) Clinical covariance profile. (**c**) Imaging covariance profile represented by bootstrap ratio. A high positive or negative bootstrap ratio indicates high contribution of a brain region to the overall covariance profile. Regions with a significant bootstrap ratio (>1.96 or <–1.96) are highlighted by colors. Abbreviations: BMI – Body mass index, HDL – high-density lipoprotein, LDL – low-density lipoprotein, r_{sp} - Spearman correlation coefficient.
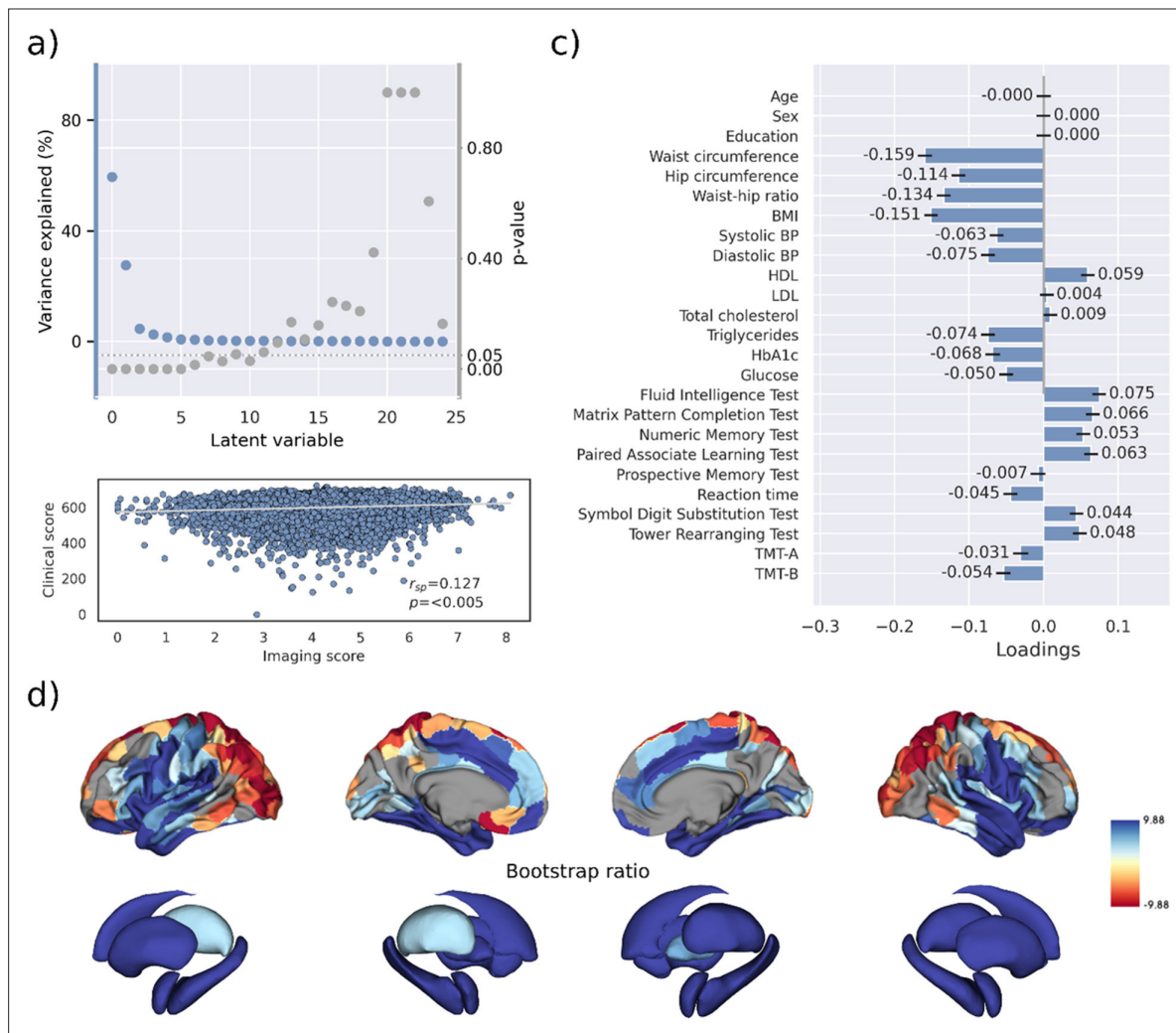
Figure 2—figure supplement 2. Partial least squares correlation analysis – UK Biobank (including cognitive test results). Partial least squares correlation analysis of the UK Biobank subsample including cognitive test results. (**a**) Explained variance and p-values of latent variables. (**b**) Scatter plot relating subject-specific clinical and imaging scores. Higher scores indicate higher adherence to the respective covariance profile. (**c**) Clinical covariance profile. 95% confidence intervals were calculated via bootstrap resampling. Note that confound removal for age, sex, and education was performed prior to the PLS. (**d**) Bootstrap ratio representing the covarying brain morphology pattern. A high positive or negative bootstrap ratio indicates high contribution of a brain region to the overall covariance profile. Regions with a significant bootstrap ratio (>1.96 or <–1.96) are highlighted by colors. Abbreviations: BMI – Body mass index, HDL – high-density lipoprotein, LDL – low-density lipoprotein, r_{sp} – Spearman correlation coefficient; p – p-value; TMT-A – Trail Making Test A; TMT-B – Trail Making Test B.

Figure 2—figure supplement 3. Partial least squares correlation analysis – Hamburg City Health Study (HCHS) (including cognitive test results). Partial least squares correlation analysis of the HCHS subsample including cognitive test results. (**a**) Explained variance and p-values of latent variables. (**b**) Scatter plot relating subject-specific clinical and imaging scores. Higher scores indicate higher adherence to the respective covariance profile. (**c**) Clinical covariance profile. 95% confidence intervals were calculated via bootstrap resampling. Note that confound removal for age, sex, and education was performed prior to the PLS. (**d**) Bootstrap ratio representing the covarying brain morphology pattern. A high positive or negative bootstrap ratio indicates high contribution of a brain region to the overall covariance profile. Regions with a significant bootstrap ratio (>1.96 or <–1.96) are highlighted by colors. Abbreviations: BMI – Body mass index, HDL – high-density lipoprotein, LDL – low-density lipoprotein, r_{sp} – Spearman correlation coefficient; p – p-value; TMT-A – Trail Making Test A; TMT-B – Trail Making Test B.

Figure 2—figure supplement 4. Spatial correlation of effect size maps. Spatial correlation matrix of all Schaefer 400-parcellated metabolic syndrome effect maps (bootstrap ratio and t-statistic). The upper triangle of the matrix displays Spearman correlations with dot size and color representing the magnitude of the coefficients. Asterisks highlight significant correlations after spin permutation testing and false discovery rate correction. The diagonal shows kernel density plots. The lower triangle illustrates the variables' linear relationships via regression plots. Abbreviations: HCHS – Hamburg City Health Study, PLS – Partial least squares correlation analysis; r_{sp} - Spearman correlation coefficient; p_{spin} – false discovery rate-corrected p-value derived from spin permutations; UKB – UK Biobank.
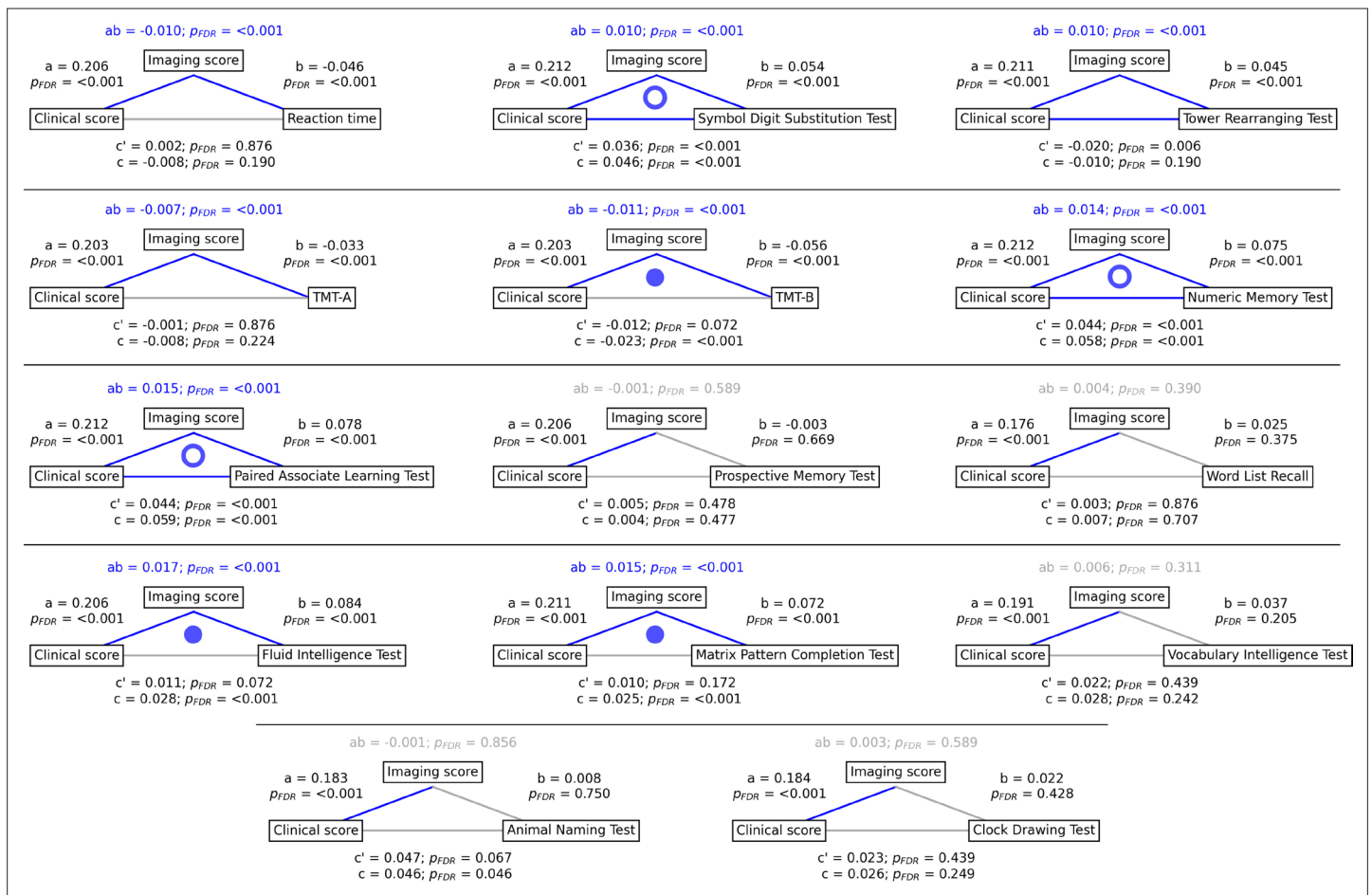
Figure 3. Mediation analysis results. Mediation effects of subject-specific imaging PLS scores on the relationship between metabolic syndrome (MetS) represented by the clinical PLS score and cognitive test performances. Path plots display standardized effects and p-values: (**a**) clinical score to imaging score, (**b**) imaging score to cognitive score, (ab) indirect effect (**c'**) direct effect, and (**c**) total effect. Significant paths are highlighted in blue; non-significant in light gray. If the indirect effect ab was significant, the text for ab is highlighted in blue. A blue dot in the path plot indicates if a relationship is significantly mediated, i.e., the indirect effect ab was significant and the direct effect c' was reduced or non-significant compared to the total effect c. An empty dot indicates a partial mediation, and a full dot indicates a full mediation. Abbreviations: p_{FDR} - false discovery rate-corrected p-values; PLS – partial least squares correlation; TMT-A – Trail Making Test A; TMT-B – Trail Making Test B.
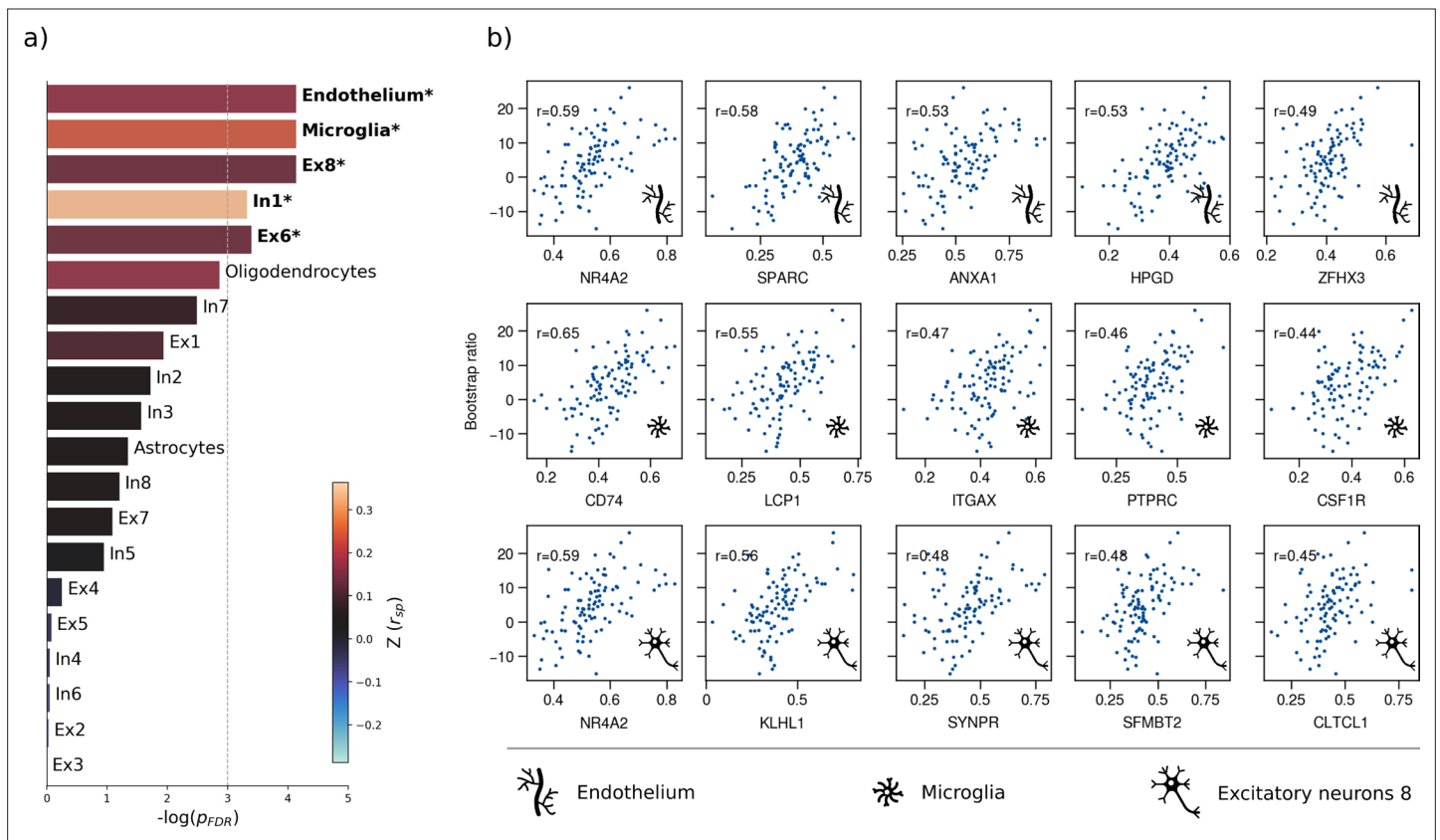
Figure 4. Virtual histology analysis. The regional correspondence between metabolic syndrome (MetS) effects (bootstrap ratio) and cell type-specific gene expression profiles was examined via an ensemble-based gene category enrichment analysis. (**a**) Barplot displaying spatial correlation results. The bar height displays the significance level. Colors encode the aggregate z-transformed Spearman correlation coefficient relating the Schaefer100-parcellated bootstrap ratio and respective cell population densities. Asterisks indicate statistical significance. The significance threshold of $p_{FDR} < 0.05$ is highlighted by a vertical dashed line. (**b**) Scatter plots illustrating spatial correlations between MetS effects and exemplary cortical gene expression profiles per cell population significantly associated across analyses – i.e., endothelium, microglia, and excitatory neurons type 8. Top 5 genes most strongly correlating with the bootstrap ratio map were visualized for each of these cell populations. Icons in the bottom right of each scatter plot indicate the corresponding cell type. A legend explaining the icons is provided at the bottom. First row: endothelium; second row: microglia; third row: excitatory neurons type 8. Virtual histology analysis results for the bootstrap ratios of latent variables 2 and 3 are shown in **Figure 4—figure supplement 1**. A corresponding plot illustrating the contextualization of the t-statistic derived from group statistics is shown in **Figure 4—figure supplement 2**. Abbreviations: $-log(p_{FDR})$ – negative logarithm of the false discovery rate-corrected p-value derived from spatial lag models (**Dukart et al., 2021**; **Burt et al., 2018**); r – Spearman correlation coeffient. $Z(r_{sp})$ – aggregate z-transformed Spearman correlation coefficient.
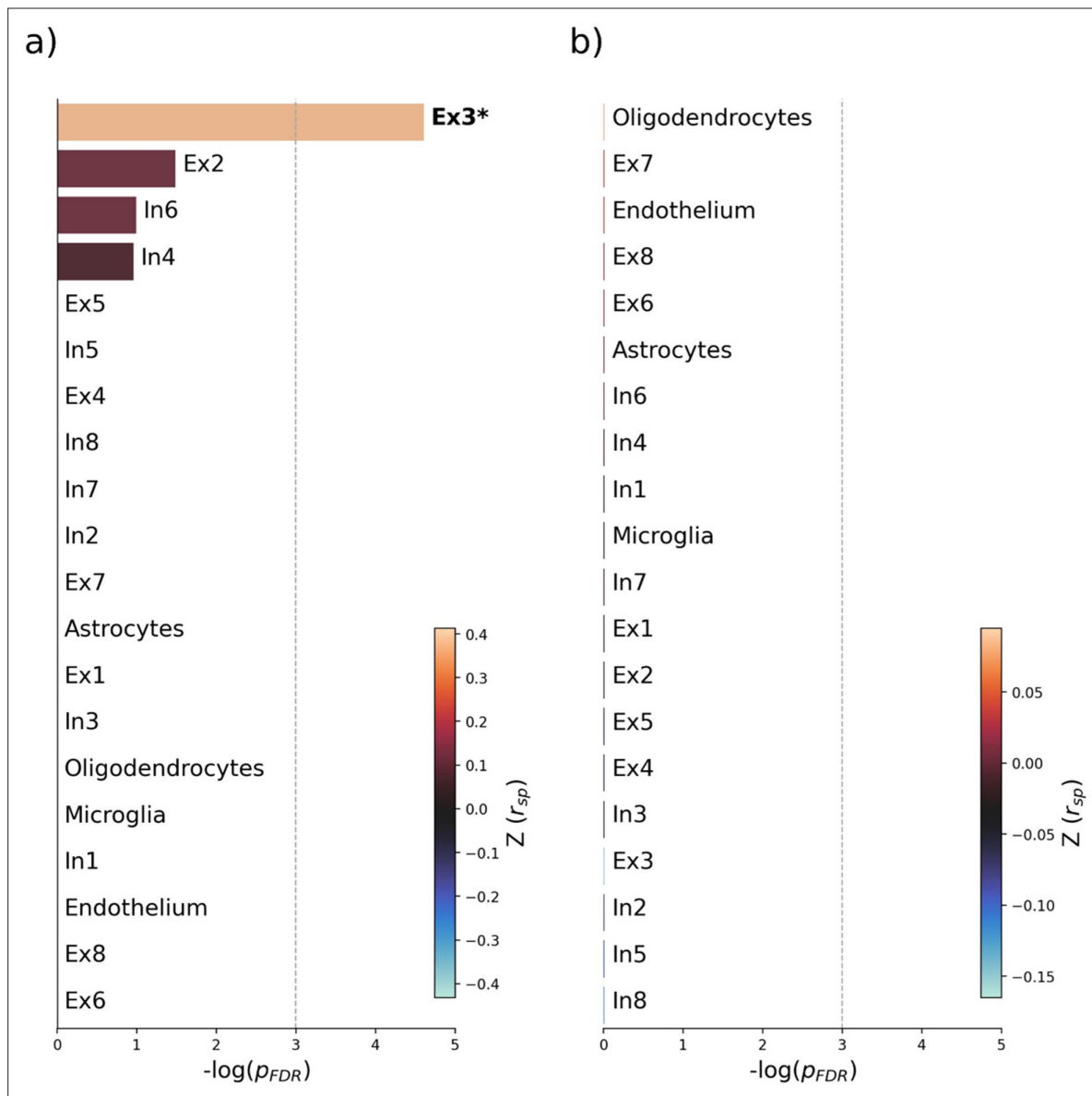
Figure 4—figure supplement 1. Virtual histology analysis of latent variables 2 and 3. Virtual histology analysis of the bootstrap ratio maps of latent variables 2 and 3 from the partial least squares (PLS) main analysis. Barplots display spatial correlation results of the bootstrap ratio of latent variables 2 and 3 and respective cell population densities computed via ensemble-based gene category enrichment analysis. (**a**) Results corresponding with the bootstrap ratio of latent variable 2. (**b**) Results corresponding with the bootstrap ratio of latent variable 3. Abbreviations: $-log\left(p_{FDR}\right)$ – negative logarithm of the false discovery rate-corrected p-value derived from spatial lag models; r – Spearman correlation coefficient. $Z\left(r_{sp}\right)$ – aggregate z-transformed Spearman correlation coefficient.
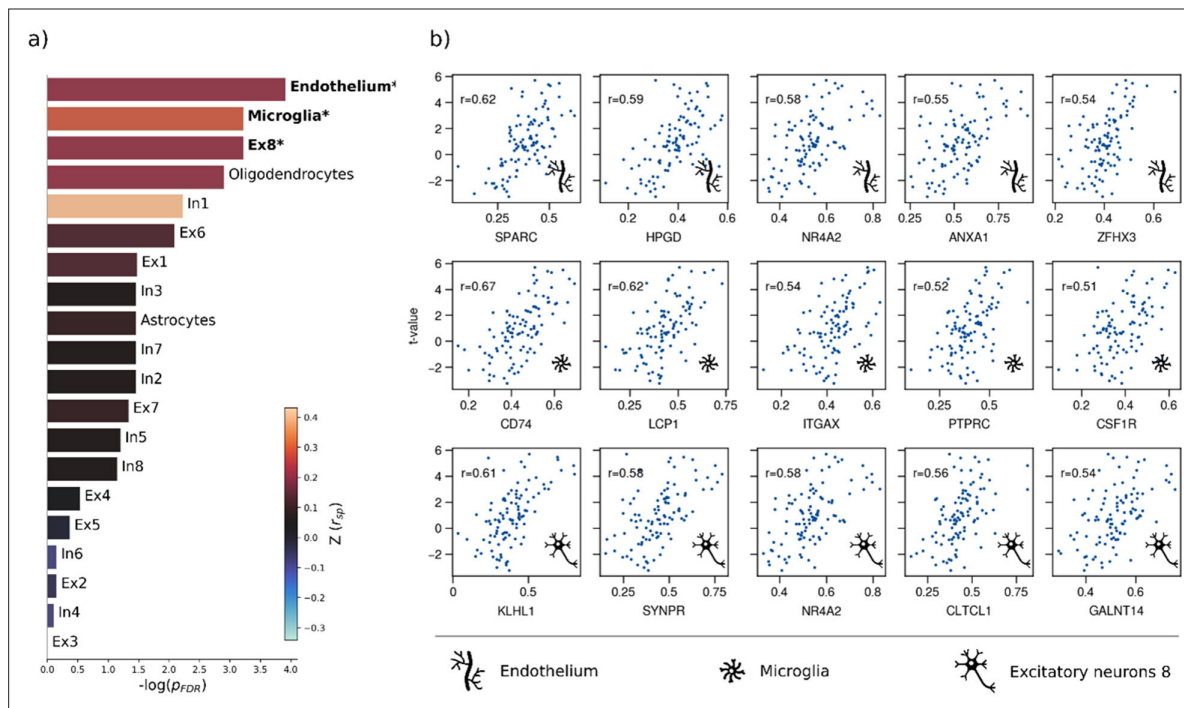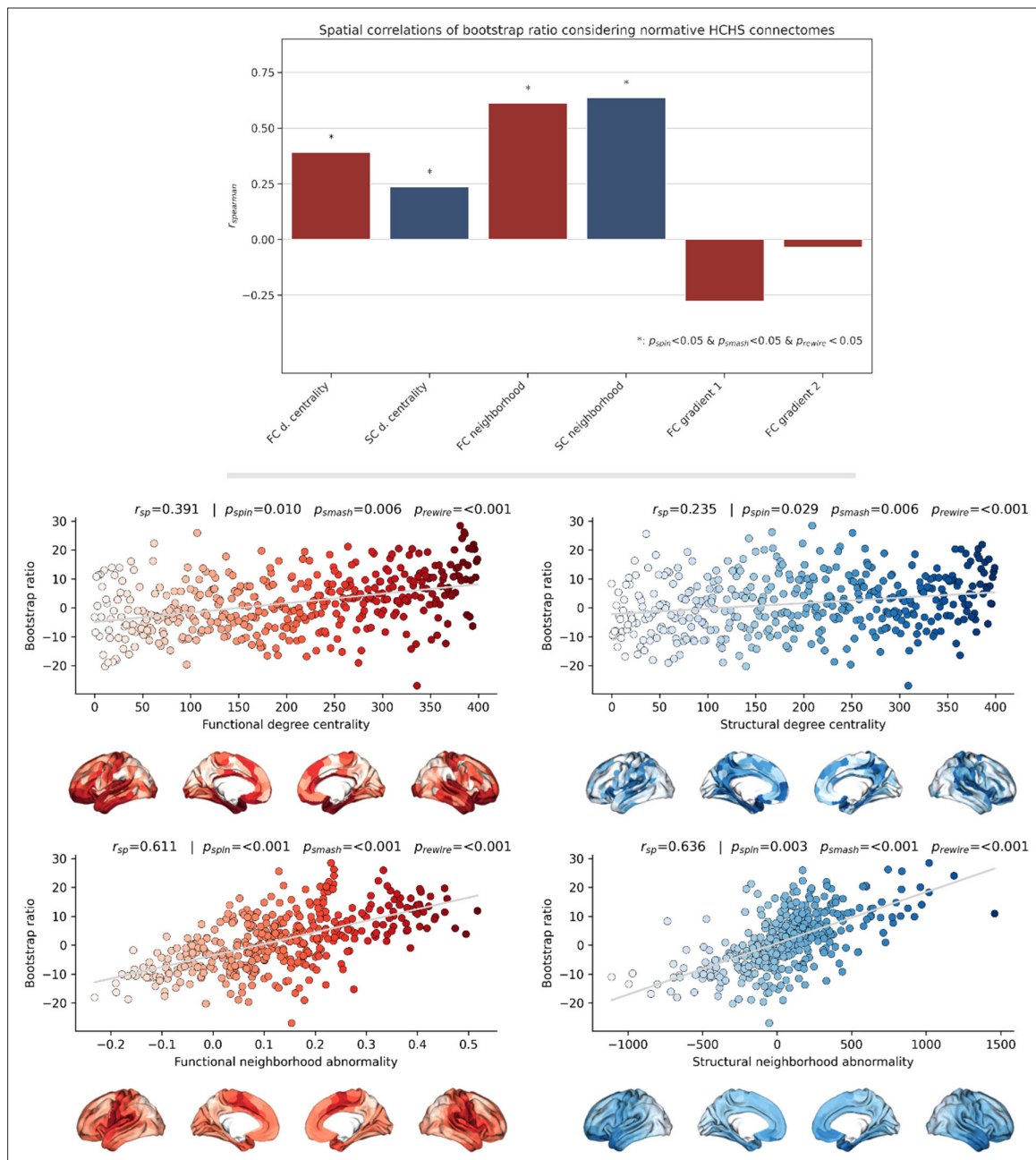
Figure 4—figure supplement 2. Sensitivity virtual histology analysis based on t-statistic map from group comparison. Virtual histology analysis of the t-statistic map derived from group comparison between individuals with metabolic syndrome and controls. (**a**) Barplot displaying spatial correlation results of the bootstrap ratio and respective cell population densities computed via ensemble-based gene category enrichment analysis. (**b**) Scatter plots illustrating per significantly associated cell population exemplary genes with top 5-highest correlation coefficients with the t-statistic map per significantly associated cell population across analyses (i.e. endothelium, microglia, excitatory neurons 8). Icons in the bottom right of each scatter plot indicate the corresponding cell type. First row: endothelium; second row: microglia; third row: excitatory neurons type 8. Abbreviations: $-log\left(p_{FDR}\right)$ – negative logarithm of the false discovery rate-corrected p-value derived from spatial lag models; r – Spearman correlation coefficient. $Z\left(r_{sp}\right)$ – aggregate z-transformed Spearman correlation coefficient.
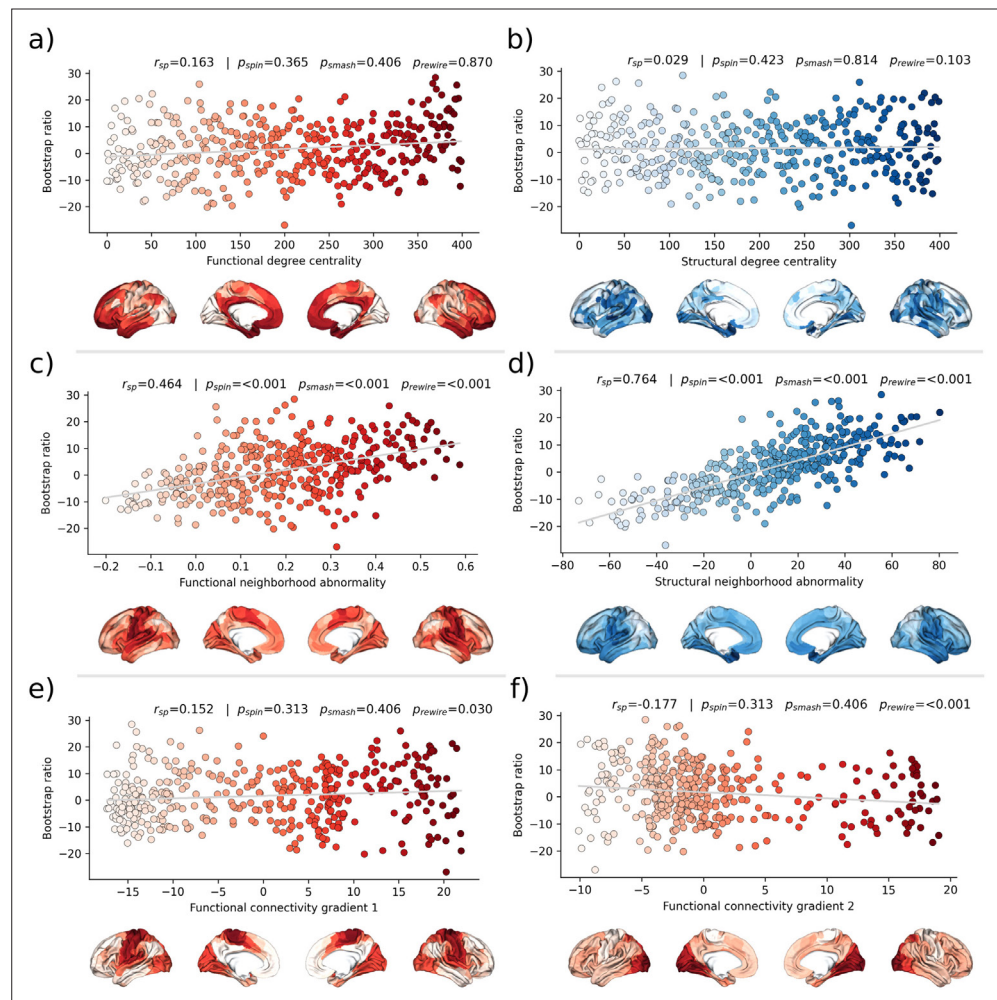
Figure 5. Brain network contextualization. Spatial correlation results derived from relating Schaefer 400×7-parcellated maps of metabolic syndrome (MetS) effects (bootstrap ratio) to network topological indices (red: functional connectivity, blue: structural connectivity). Scatter plots that illustrate the spatial relationship are supplemented by surface plots for anatomical localization. The color coding of cortical regions and associated dots corresponds. (**a and b**) Functional and structural degree centrality rank. (**c and d**) Functional and structural neighborhood abnormality. (**e and f**) Intrinsic functional network hierarchy represented by functional connectivity gradients 1 and 2. Complementary results concerning t-statistic maps derived from group comparisons between MetS subjects and controls are presented in *Figure 5—figure supplement 1*. Corresponding results after reperforming the analysis with HCHS-derived group-consensus connectomes are presented in *Figure 5—figure supplement 2*. Abbreviations: HCHS – Hamburg City Health Study; p_{rewire} - p-value derived from network rewiring (*Maslov et al., 2004*); p_{smash} - p-value derived from brainSMASH surrogates (*Burt et al., 2020*); p_{spin} - p-value derived from spin permutation results (*Alexander-Bloch et al., 2018*); r_{sp} - Spearman correlation coefficient.
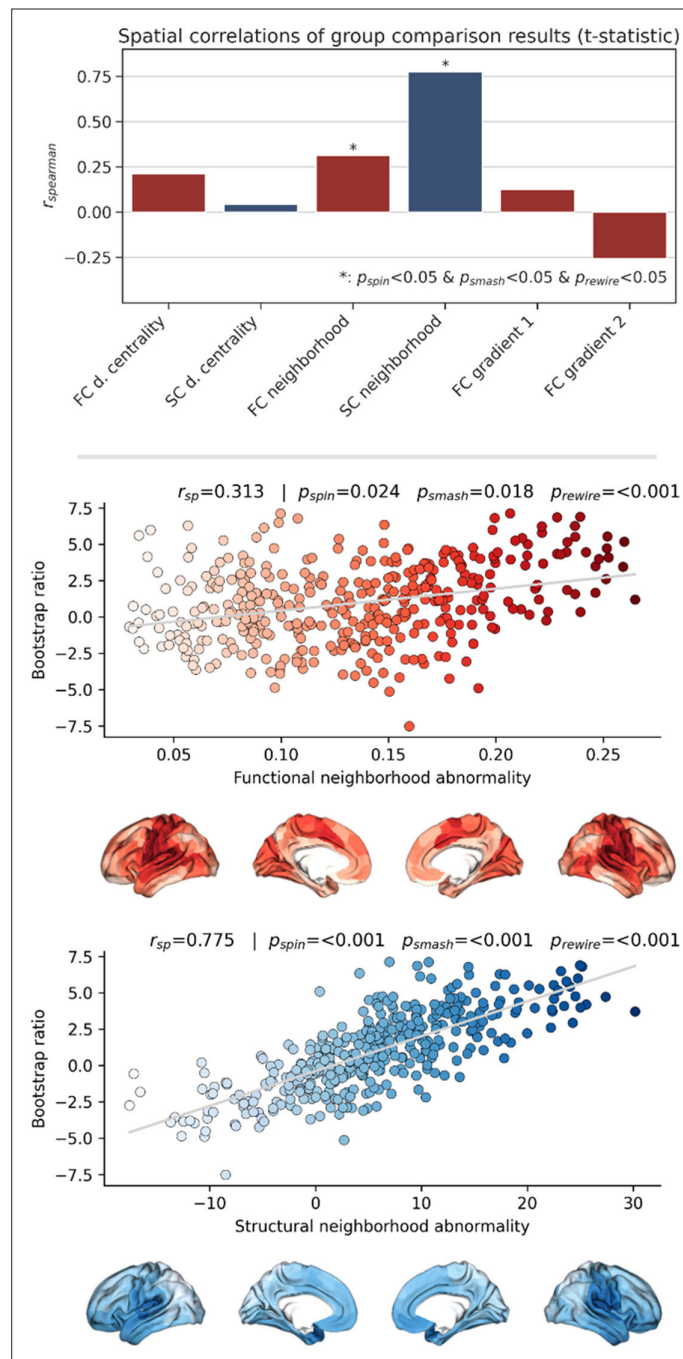
Figure 5—figure supplement 1. Sensitivity network contextualization analysis based on t-statistic map derived from group comparison. Brain network contextualization analysis of group statistics results. Results are presented for t-statistics maps derived from group statistics considering the pooled sample of UK Biobank subjects and Hamburg City Health Study (HCHS) subjects. The upper row barplot summarizes the analysis results displaying the Spearman correlation with regard to each investigated index. Asterisks indicate statistical significance with respect to spin, brainSMASH, and network rewiring null models. The middle and lower rows display scatter plots of the significant association of the t-statistics map and the functional and structural neighborhood abnormality, respectively. The scatter plots are supplemented by surface plots for anatomical localization. Abbreviations: p_{rewire} - p-value derived from network rewiring (**Maslov et al., 2004**); p_{smash} - p-value derived from brainSMASH surrogates (**Burt et al., 2020**); p_{spin} - p-value derived from spin permutation results (**Alexander-Bloch et al., 2018**); r_{sp} - Spearman correlation coefficient.

Figure 5—figure supplement 2. Sensitivity network contextualization analysis based on group-consensus connectomes from the Hamburg City Health Study. Brain network contextualization analysis of partial least squares correlation results (bootstrap ratio) based on group-consensus connectomes from the Hamburg City Health Study. Results are presented for bootstrap ratio maps derived from partial least squares correlation analysis considering the pooled sample. The upper row bar plot summarizes the analysis results displaying the Spearman correlation with regard to each investigated index. Asterisks indicate statistical significance with respect to spin, brainSMASH, and network rewiring null models. Scatter plots that illustrate the significant spatial relationships are presented below. The middle row displays the relationship of the bootstrap ratio map and the ranked functional and structural degree centrality. The lower row illustrates the association of the bootstrap ratio map and the functional and structural neighborhood abnormality. Abbreviations: p_{rewire} - p-value derived from network rewiring (**Maslov et al., 2004**); p_{smash} - p-value derived from brainSMASH surrogates (**Burt et al., 2020**); p_{spin} - p-value derived from spin permutation results (**Alexander-Bloch et al., 2018**); r_{sp} - Spearman correlation coefficient.
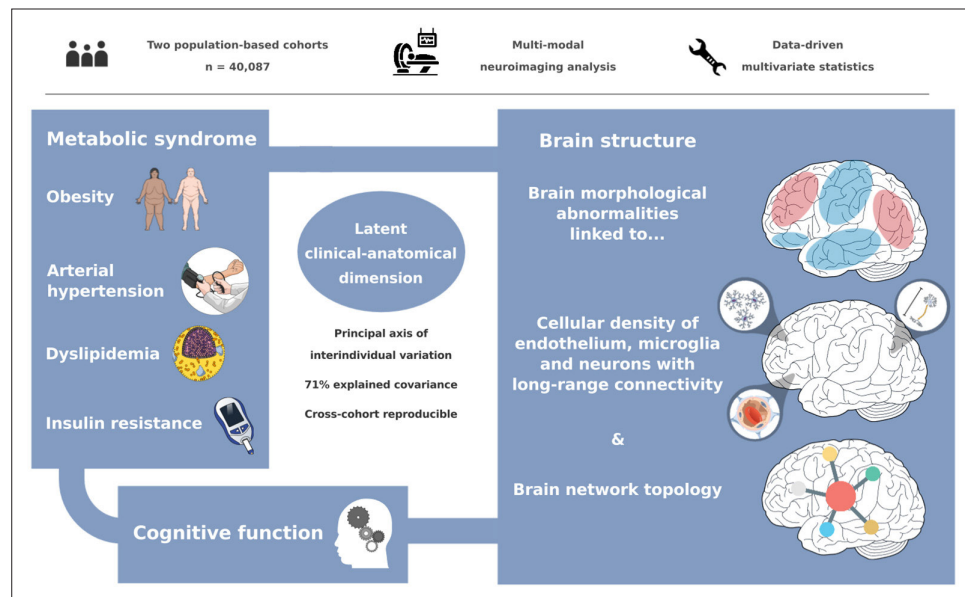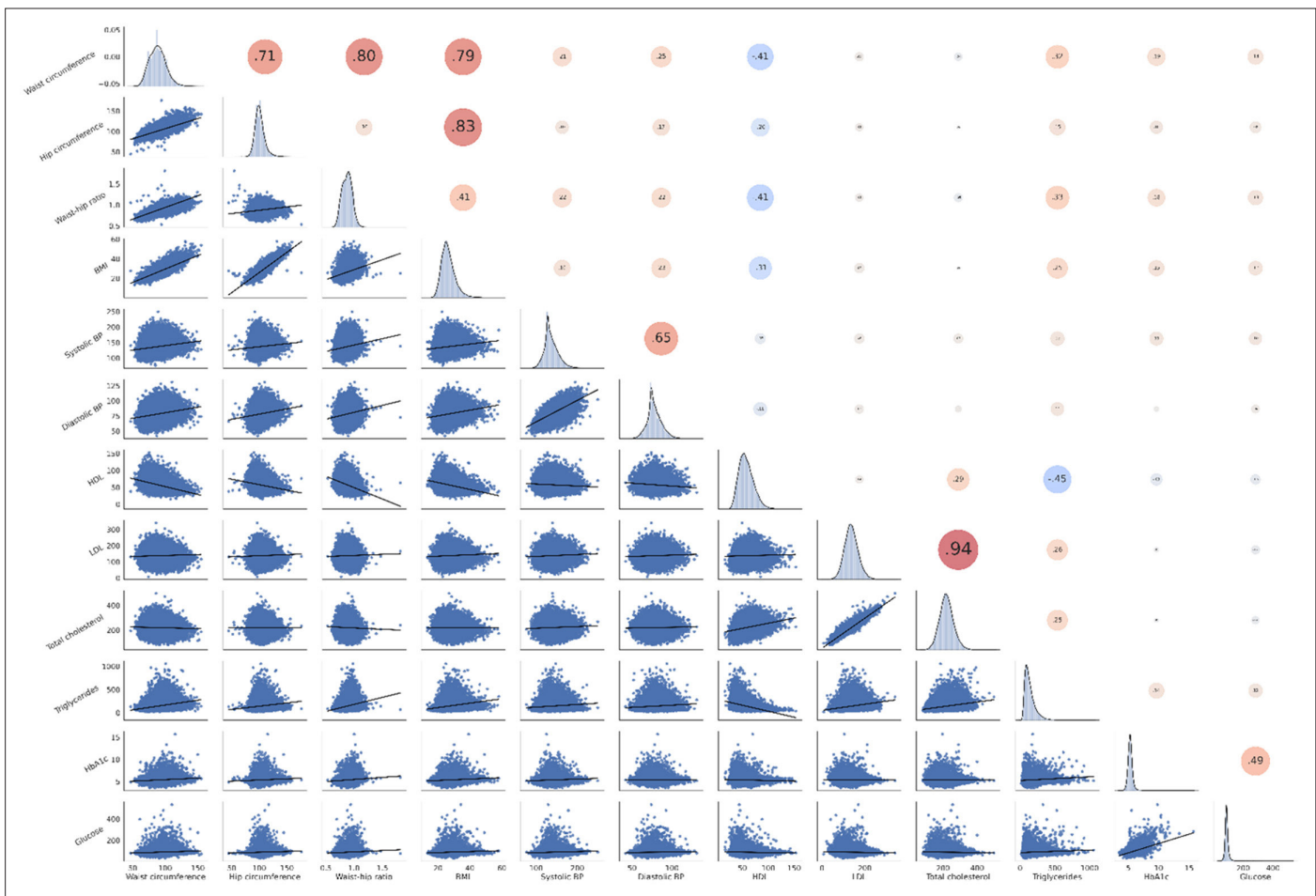
Figure 6. Graphical abstract.
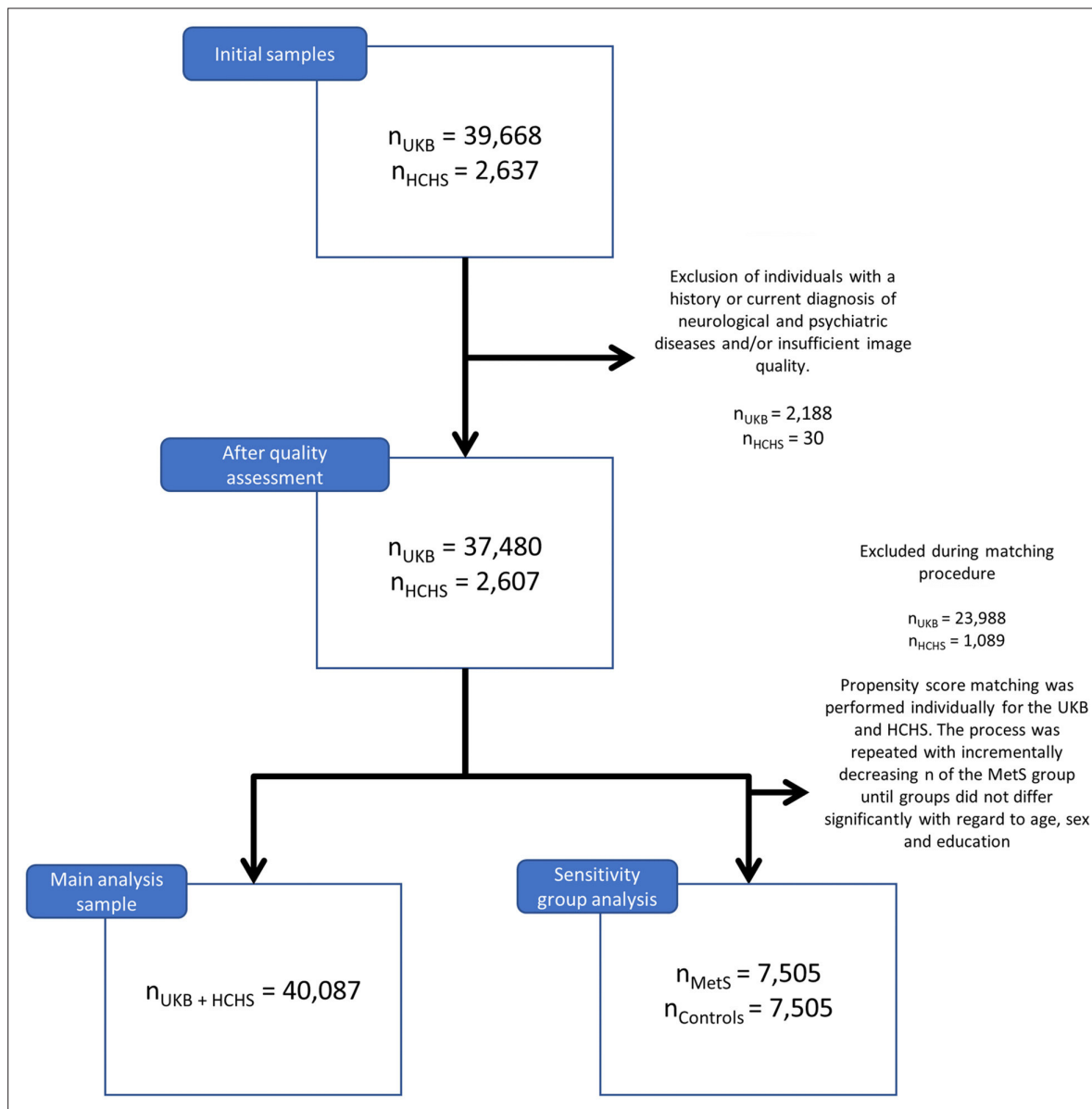
**Appendix 1—figure 1.** Flowchart sample selection procedure.

Appendix 1—figure 2. Correlation matrix of metabolic syndrome-related risk factors. The upper triangle of the matrix displays Pearson correlations with dot size and color representing the magnitude of the coefficients. The diagonal shows kernel density plots. The lower triangle illustrates the variables' linear relationships via regression plots. Of note, non fasting plasma glucose was investigated in this analysis. Abbreviations: BP – blood pressure.
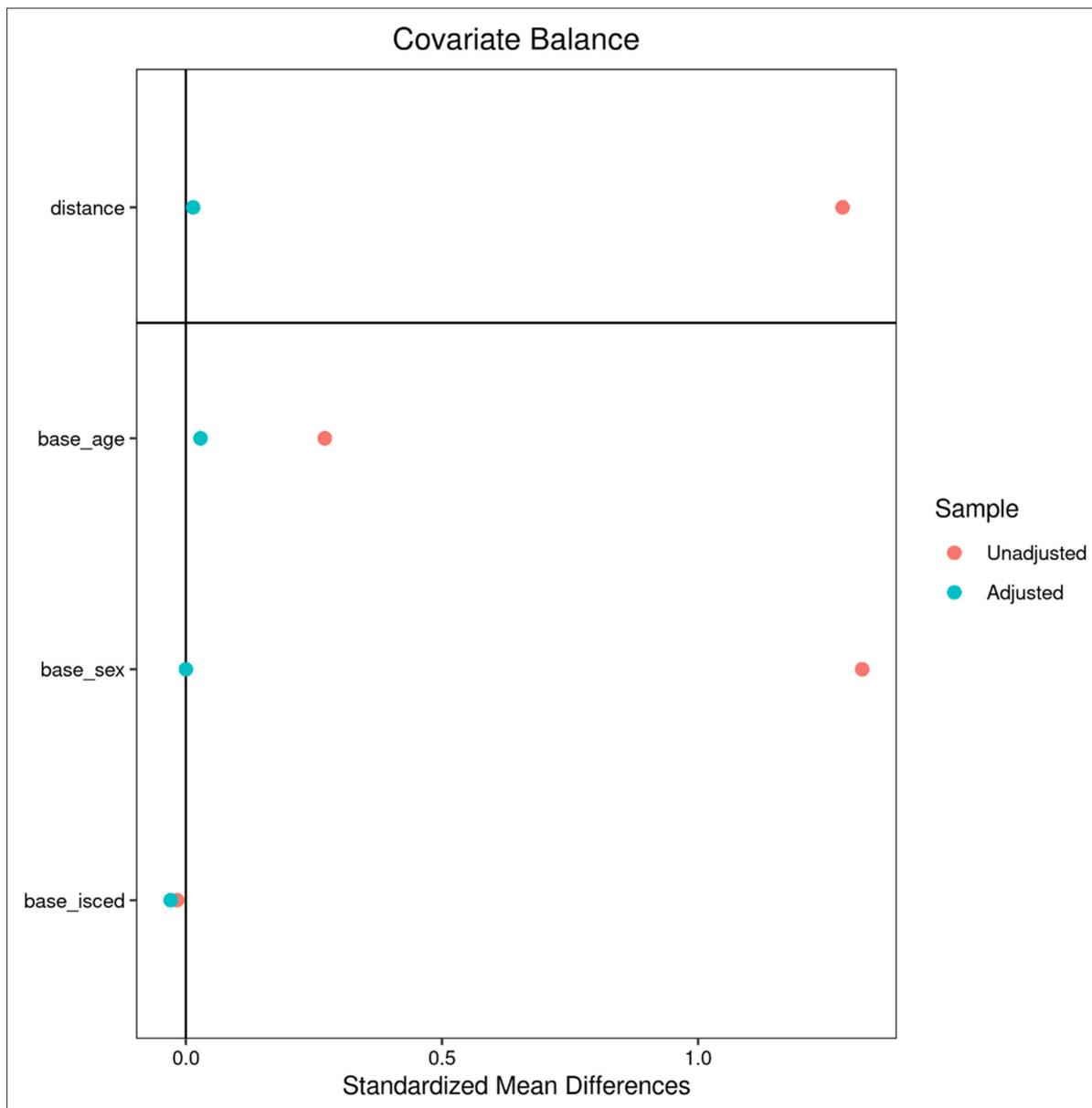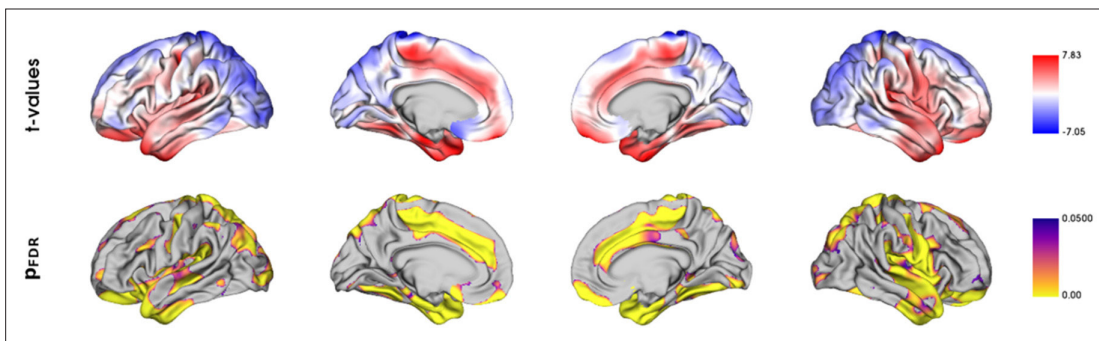
Appendix 2—figure 1. Matching - UK Biobank.
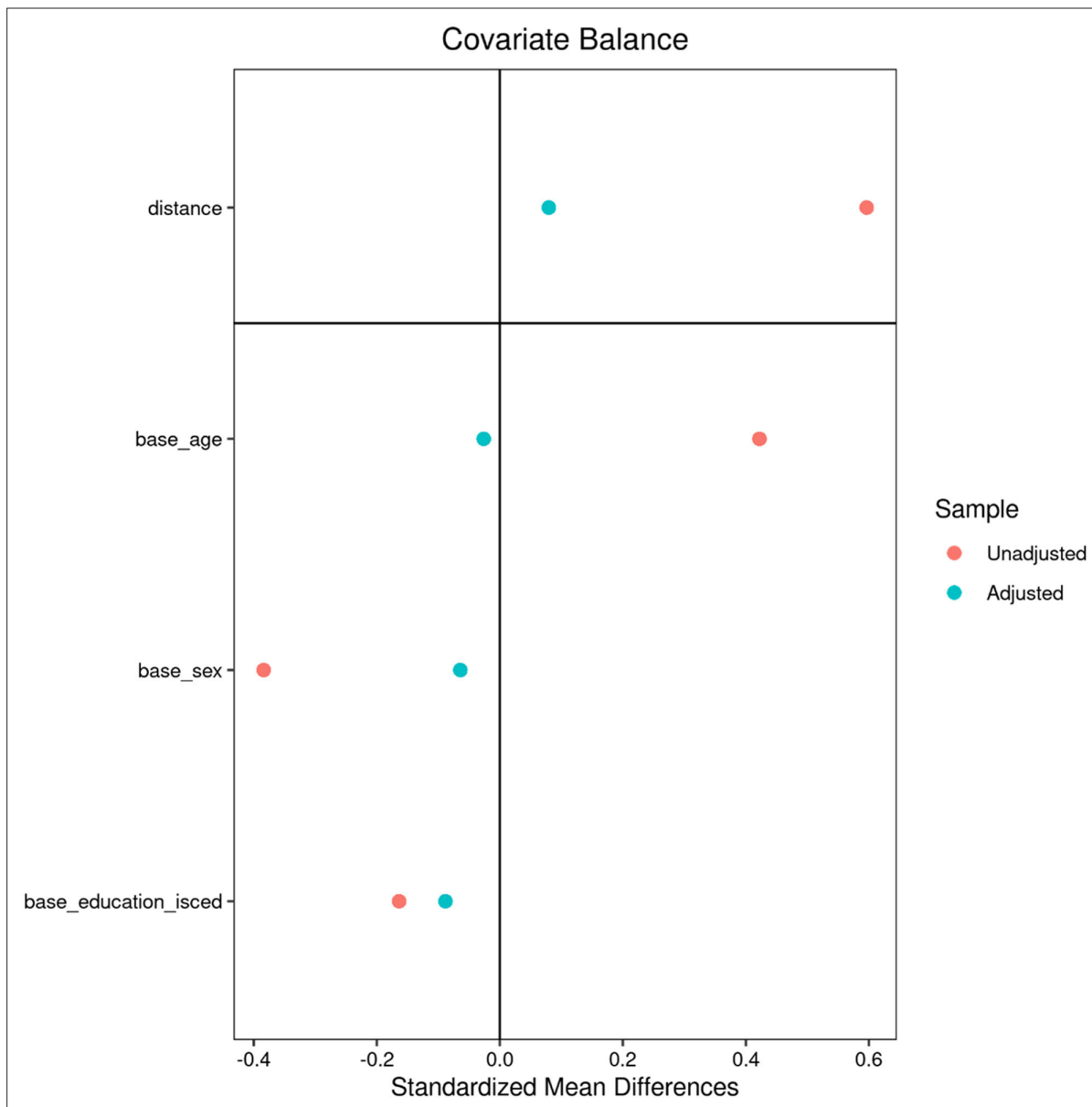
Appendix 2—figure 2. Matching – Hamburg City Health Study.
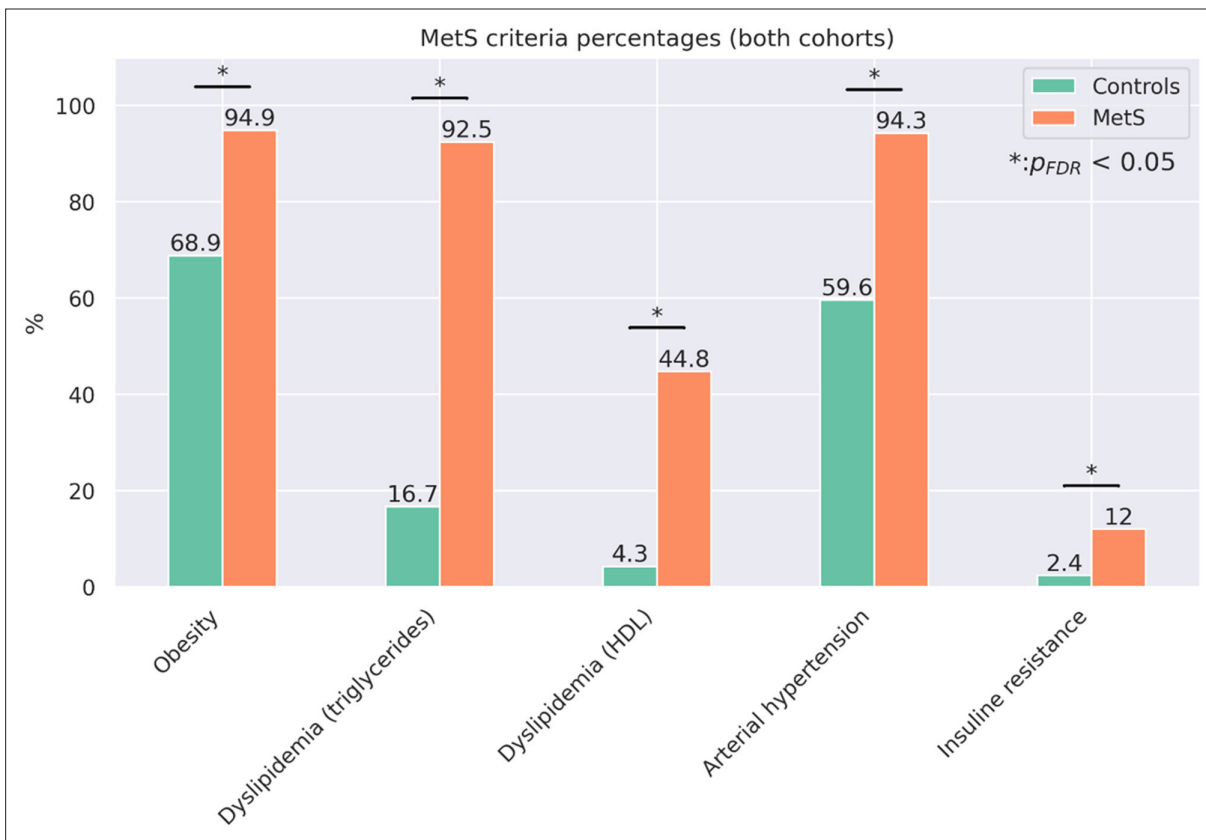
Appendix 2—figure 3. Proportion of metabolic syndrome criteria. Barplots indicate the percentage amount of metabolic syndrome (MetS) criteria that apply by group for the pooled sample. Significant group differences in χ 2-tests are highlighted by asterisks.

Appendix 2—figure 4. Vertix-wise group comparison of cortical thickness. Vertex-level group comparison between individuals with metabolic syndrome and matched controls. Resulting surface maps of standardized *t*-statistic estimates encode the group-differences between patients and controls, with lower cortical thickness in the metabolic syndrome (MetS) group being represented by a positive *t* and lower by a negative *t*. The vertex-wise *t*-statistic map was Schaefer-parcellated for the downstream analyses.