Changes in the Public IgM Repertoire and its Idiotypic Connectivity in Alzheimer's Disease and Frontotemporal Dementia

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Abstract

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are prevalent neurodegenerative disorders. Early diagnosis is challenging due to the lack of definitive biomarkers and reliance on invasive procedures. Immune biomarkers, particularly those reflecting the interaction between the central nervous system (CNS) and the peripheral immune system, have shown promise for non-invasive detection through blood samples. This study investigates the reactivity of serum IgM and IgG from AD and FTD patients against a library of mimotopes representing public IgM reactivities in healthy donors. Serum samples from AD, FTD, and other neurodegenerative dementias (ND), and controls were tested on peptide microarrays. The samples were pooled to mitigate individual variability. The reactivity data were analyzed using graphs to represent the cross-reactivity networks. The analysis revealed distinct reactivity patterns for the studied groups. Public IgM reactivities showed significant correlations with neurodegenerative conditions, with AD and FTD exhibiting loss or gain of specific IgM reactivities. Graph analysis highlighted significant differences in graph density, clustering, and assortativity parameters between disease and control groups. Idiotypic reactivities, particularly in IgM, were more connected in healthy controls compared to those with neurodegenerative diseases. Furthermore, clusters of reactivities showed significant distinctions between AD and FTD, with IgG reactivities providing additional differentiation. A number of self proteins related to neurodegeneration proved to have sequences homologous to disease associated mimotopes. Thus, the public IgM repertoire, characterized by its broad reactivity and inherent autoreactivity, offers valuable insights into the immunological alterations in neurodegenerative diseases. The study supports the potential of IgM and IgG reactivity profiles as another compartment of non-invasive biomarkers for early diagnosis and differentiation of AD and FTD.

1. Introduction

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are among the most prevalent neurodegenerative disorders affecting the elderly population (Musa et al., 2020). Even the basic pathogenic mechanisms of these diseases are still debated (Castellani et al., 2022) but it is clear that they involve complex system-level interactions including immunological mechanisms (Chen and Colonna, 2022). A better understanding of the pathogenesis of neurodegenerative disease serves the development of potential therapy approaches and better and earlier diagnosis. The diagnosis of AD and FTD remains a significant challenge due to the lack of definitive biomarkers and the need for invasive procedures like cerebrospinal fluid (CSF) analysis or positron emission tomography (PET) scans (Gifford et al., 2023; Shue et al., 2024). Recent advancements in plasma biomarkers and computational approaches have opened up new avenues for the early diagnosis of these neurodegenerative diseases. Immune biomarkers, particularly those related to the cross-talk between the central nervous system (CNS) and the immune system, have emerged as promising tools for the detection of AD and FTD (Krix et al., 2024). These biomarkers can be easily accessed through blood samples, making them more accessible and cost-effective compared to traditional methods. The multitude of hypothetical biomarkers justify the use of machine learning approaches combining them with further information (e.g., age, sex, APOE genotype) into predictive models supporting an earlier diagnosis.

Among the promising immunological biomarkers, antibodies have a unique role because of their high-resolution molecular recognition. Previous studies have demonstrated that patients with AD exhibit unique IgG antibody binding patterns compared to healthy controls and those with other types of dementia. For instance, Restrepo et al. (2011) explored the application of a peptide microarray containing 10,000 random sequence peptides to profile the antibody repertoire of AD patients and transgenic mice models. The study found that AD patients and mice with cerebral amyloidosis displayed distinct immunoprofiles, suggesting the utility of immunosignature technique in AD diagnosis (Restrepo et al., 2011). Some sets of IgG autoantibodies with better-defined autoantigen specificities have shown great promise for the development of multi-disease biomarkers including for early detection of AD (DeMarshall et al., 2017; DeMarshall et al., 2023; Nagele et al., 2011). Unlike the highly specific IgG which is usually the product of an adaptive immune response, the IgM repertoire abounds in constitutively produced natural antibody and at least 20% of them are physiologically autoreactive (Imkeller and Wardemann, 2018). Since it is also more diverse than its IgG counterpart and better overlapping with the public reactivities (Galson et al., 2015), the actual individual IgM repertoire is better poised as a sensor of the internal environment before the development of overt autoimmunity. With this working hypothesis, we started developing a diagnostic platform applicable to a very broad range of pathologies that is based on applying IgOme approaches (Ryvkin et al., 2012) for extracting patterns of diagnostic public IgM reactivities (Ferdinandov et al., 2023; Pashov et al., 2019; Pashova-Dimova et al., 2023; Pashova et al., 2022; Shivarov et al., 2020).

In this manuscript, we present our findings on the reactivity of serum IgM and IgG from AD and FTD patients to a large library of mimotopes of the public IgM reactivities found in healthy donors. In addition, the cross-reactivities to the same mimotopes in the IgG repertoire were tested hoping to estimate the degree of the adaptive immune response contribution to the observed patterns of binding. We focus on the differences in antibody reactivity graphs generated from the binding data as a formalism for cross-reactivity networks. Somewhat surprisingly, losing or gaining public IgM reactivities in AD and FTD

correlated with their cross-reactivity with human immunoglobulin CDR3 sequences seen as potential idiotopes.

2. Materials and methods

2.1. Patients and sera

Patients were recruited at Clinic of Neurology, Medical University - Sofia. The diagnosis of AD was made according to the NIA-AA criteria (McKhann et al., 2011). A diagnosis of behavioural variant of FTD and primary progressive aphasia (PPA) was considered based on current revised diagnostic guidelines (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). A group of patients with other neurodegenerative dementias was also included. Serra were collected from three groups of 8 patients diagnosed with AD, FTD, or other neurodegenerative dementias and compared to sera form 6 sex and age matched control group patients without dementia (C). All participants or their caregivers gave written informed consent to participation in the study. The informed consent forms and study protocols were approved by the local ethics committee, UH "Alexandrovska", and performed in harmony with the Helsinki Declaration.

2.2. IgM and IgG isolation

The serum samples were thawed and incubated at 37°C for 30 min to dissolve the IgM aggregates. After spinning for 10 min at 20 000 × g the IgM concentration was determined by ELISA and the sera were diluted to 10 µg/ml IgM. The samples used in the study were limited to 5 per diagnosis so some of the samples had to be pooled. Pooling creates correlation between the samples which made cross validation and machine learning impossible. Since this limitation was inevitable, it was decided that all samples will be pools of two patients' appropriately diluted sera as an intermediate averaging of reactivities in the groups and giving a slight advantage to public reactivities. The pooling schemes in terms of serum number were: AD – 1+6, 2+7, 3+8, 4+8, and 5+2, FTD - 1+6, 2+7, 3+8, 4+1, and 5+2, ND - 1+6, 2+7, 3+8, 4+1, and 5+2, and C – 1+3, 2+6, 3+4, 4+2, 5+1.

The blood group of the patients was detected by a reverse blood group typing of the sera using test erythrocytes kit Biotestcell (BioRad).

2.3. Peptide microarray binding assay

2.3.1. Sequence selection

Previously we have studied a library of 2×10^5 peptide mimotopes of the public IgM of healthy donors (Pashov et al., 2019). The filtering of public reactivities is assured by using IgM isolated from a blood plasma pool from 10 000 individuals which dilutes substantially the private IgM specificities. Using a Gibbs sampling approach (Andreatta et al., 2013) (https://services.healthtech.dtu.dk/services/GibbsCluster-2.0/), the sequences were clustered optimally in 790 clusters (Pashov et al., 2019). Here we further analyzed this grouping by constructing a graph of the clusters. For each primary cluster the neighboring clusters were determined based on sequence proximity. The latter was based on the next best cluster information for each of the sequences of a given cluster as reported by the algorithm. If a cluster had more than n sequences close to the same alternative cluster, the two clusters were connected by an edge. Most (675) of the clusters remained disconnected but some formed the graph components connecting 2 to 10 clusters. The threshold n was adjusted to produce less

than 730 components (a number defined by the peptide array format). These components were considered the secondary clusters (n=726). The singletons in this graph representing primary clusters and the Gibbs Sampling algorithm provided the most relevant representative sequence from each cluster. Each of the secondary clusters was converted to an igome graph of sequences (Pashova et al., 2022) and the vertex with the highest eigencentrality was used to define the representative sequence. In this way, a set of 726 peptides that sampled symmetrically the space of the 7-mer mimotopes of the public IgM reactivities were selected and used to synthesize the peptide arrays.

2.3.2. Peptide microarrays design

Custom peptide microarrays with 726 7 amino acid residues long linear peptides (see 2.3.1) were custom produced by PEPperPRINT™ (Heidelberg, Germany). The peptides were synthesized in situ attached to the functionalized chip surface by their C-terminus and through a GSGSG spacer. The layout of the microarrays contained the respective peptide spots duplicated in random positions.

Supplementary Table 1 shows the sequences of the peptides used. The peptide arrays were blocked for 60 min using PBS, pH 7.4, and 0.05% Tween 20 with 1% BSA with constant shaking; washed 3 × 1 min with PBS, pH 7.4, and 0.05% Tween 20 followed by incubation with sera in dilutions equivalent to 0.01 mg/mL IgM (established by determining the immunoglobulin concentrations by ELISA) on an orbital shaker overnight at 4°C. After washing 3 × 1 min, the arrays were incubated with secondary antibodies at room temperature, washed, rinsed with distilled water, and dried by spinning in a vertical position in empty 50 mL test tubes at 100×g for 2 min. Two different secondary antibodies were used to measure simultaneously the binding of IgG and IgM at wavelengths of detection respectively 544 nm and 647 nm.

2.4. Microarray data analysis

2.4.1. Acquisition, cleaning and normalization

Innoscan 1100 (Innopsys, Carbonne, France) microarray scanner was used to acquire the stained microarray fluorescence images. MAPIX software was used for the subsequent densitometry. The analysis of the densitometry data was performed using publicly available packages from the R statistical environment (*Bioconductor, Biostrings, limma, pepStat, igraph, clvalid, gplots, future.apply, FactoMIneR, factoextra, stringi, stringdist, rBLAST, rgl,* etc.) as well as in-house developed R functions. The data and the scripts of the analysis are deposited at https://github.com/ansts/ADFTDigome. The cross-reactivity graph strategy was previously described (Ferdinandov et al., 2023). Here it is used with some modifications. Briefly, the raw data was cleaned based on the flags set during densitometry. The background was defined based on the gradients of staining between the randomly positioned duplicate peptide spots and subtracted (Pashov et al., 2019).

The logarithms of the data were normalized relative to the amino acid residue composition using the pepstat Bioconductor package as described previously (Pashov et al., 2019). This compensates for binding which is dependent on the overall charge and hydrophobicity rather than on the sequence. Further, the data was normalized between arrays using the *limma*::normalizeCyclicLoess function with the "affy" method applied in 3 iterations separately for the IgM and IgG datasets. At the end of this stage the datasets were based on 722 mimotopes.

2.4.2. Graph representation

Each of the isotype sets was used to generate a separate cross-reactivity graph. The criterion for cross-reactivity between two peptides used to define the edges of the graphs was a similarity measure. It was based on the reciprocal of the Manhattan distance between the respective profiles of reactivities with the 20 samples. Each pair of profiles was first scaled and centered using the median and the interquartile range. If M is the n×k matrix of the n reactivity values with k samples and $i1$ and $i2$ are two rows corresponding to two peptides (profiles), the quartiles of each row M_i being

$$Q^i_{0.25}, Q^i_{0.5}, Q^i_{0.75} = quantile(M_i, \{0.25, 0.5, 0.75\})$$

the similarity measure is calculated as:

$$s(M) = \frac{1}{\sum_{j:Q_{0.2}<D_j<Q_{0.8}} D_j}, D_j = \left| \frac{2\left(M_{i1,j} - Q^{(1)}_{0.5}\right)}{Q^{(1)}_{0.75} - Q^{(1)}_{0.25}} - \frac{2\left(M_{i2,j} - Q^{(2)}_{0.5}\right)}{Q^{(2)}_{0.75} - Q^{(2)}_{0.25}} \right| .$$

Only the D values between the quantiles 0.2 and 0.8 were used. The filtering out of the extreme values of D is done to prevent the decision about cross-reactivity being taken on the basis of just a couple of similar values. The concept of defining a cross-reactivity by testing multiple individual repertoires depends on the multiplicity of samples with correlated reactivities. The criterion was tested using the data form (Ferdinandov et al., 2023). The ROC curve and the selected threshold for connecting vertices of the graphs were analyzed as previously described (Ferdinandov et al., 2023). The threshold selected ensured specificity of 0.9 and sensitivity of 0.6, thus emphasizing only the most probable cross-reactivities (Fig. 1).

An edge in a reactivity graph G can be interpreted as a cross-reactivity relation between two peptides. Since the cross-reactivity is inferred from similarity of the reactivity profiles across samples, the same peptide can be cross-reactive with two other peptides based on different parts of the profile. The two respective edges will represent potentially different antibody reactivities. Therefore, the topology of the reactivity graph does not represent the map of cross reactivities with the necessary detail unless the qualitative differences between the edges are figured in. To improve the clustering so that it can be used to formalize the concept of latent antibody reactivities, a second graph – GE, was constructed with vertices representing the edges in G. GE was not a line graph of G. The vertices in GE (originally edges in G) are connected not when they have a common incident vertex (like in a line graph) but when they stand for correlating profiles D_j. Given the reactivity graph G, and the raw data matrix M, from which G is constructed,

$$E_i = \{\, ends(G, e) \mid e \in E(G) \,\}$$

are the endpoints of each edge where $ends(G, e)$ represents the pair of vertices at the ends of edge e in graph G. The distance matrix

$$A = \begin{pmatrix} d_{11} & \cdots & d_{1m} \\ \vdots & \ddots & \vdots \\ d_{m1} & \cdots & d_{mn} \end{pmatrix}$$

where the distances $d_{\{jk\}} = \left|\mu(M[l_j, :]) - \mu(M[l_k, :])\right|$ and μ is the vector of column-wise means of each row pair, l_j and l_k being the two pairs of indices of the respective pairs of profiles *j* and *k*. After setting

the diagonal of A to zero, this became effectively the adjacency matrix of the GE graph. The weights of the edges in this graph (as distances) are given by the elements of A. Next, a cluster size optimization was performed by varying the threshold w_i for edge deletion. The threshold c_t was determined by maximizing the product of the cluster sizes s_k of the clusters larger than 5 vertices:

$$c_t = arg \max_{w_i} \left(log_{10} \left(\prod_k t_k \mid t_k > 5 \right) \right)$$

The edges with weights greater than the optimal threshold c_t were deleted. The ultimate form of the graph GE, thus obtained, was clustered to obtain clusters of what are respectively edges in the context of the original graph G – so they are labeled edge clusters. Every edge cluster was defined in the terms of the vertices - its end points in the original graph G. The final grouping of reactivities was obtained as intersections of the G clusters with the vertex clustering derived from the GE edge clusters. The groups of reactivity obtained are not a partition (like the clusters) but a cover (they overlap) because different edges can have overlapping vertices as endpoints.

To test the significance of various graph parameters (like size of large component, number of clusters, assortativity of vertex parameters, degree and maximal clique distributions, etc.) the baseline values for these parameters were estimated using 2 sets of 100 random graphs generated using the same algorithm but starting with the scrambled raw data matrices for IgM and IgG reactivities respectively. All graph analysis was performed using the igraph package.

Let $\mathbf{1}_{dia_l}$ be the indicator function of the diagnosis group $\mathrm{dia}_l \in \{\text{"A"},\text{"C"},\text{"F"},\text{"ND"}\}$ and let $r_i = [r_{i1}, r_{i1}, \dots, r_{ik}]$ represent the *i-th* row (*i-th* mimotope) of the reactivity data matrix M. The rows are ranked: $\mathbf{rank}(r_i) = \mathbf{r}_i^{\mathrm{rank}} = [r_{i1}^{\mathrm{rank}}, r_{i2}^{\mathrm{rank}}, \dots, r_{ik}^{\mathrm{rank}}]$, r_{ij}^{rank} denotes the *j-th* element of the *i-th* row. Then, the sum of ranks for group dia_l for the *i-th* row is:

$$S_{i,dia_l} = \sum_{j=1}^{m} r_{ij}^{rank} \cdot \mathbf{1}_{dia_l}(l),$$

Where $\mathbf{1}_{dia_l}(l)$ is 1 if $dia_j = \mathbf{dia}_k$ and 0 otherwise. The S_{i,dia_l} was used to quantitate the degree of association of each reactivity with each diagnosis.

For the visualization, the graphs were embedded using the eigenvectors of the graph Laplacian of each isotype graph (spectral embedding). The eigenvectors that corresponded to the k smallest eigenvalues were used. The cut off to determine k was based on the irregular increase in the ordered eigenvalues (Suppl. Fig. 2). The exact position of the vertices in the two-dimensional projections are derived by UMAP embedding from k dimensions to two dimensions. UMAP was considered appropriate for the final 2D embedding in this case because applying it to a relatively low dimensional data reduces the distortion of distances. The 3D visualization was done with the help of the rgl package. The graph clustering was done using the Leiden algorithm with modularity optimization and resolution parameter 1.

2.5. External data sources

The set of human immunoglobulin heavy chain junction regions that are found in the NCBI protein database (1 012 520 different entries) were used. The junction regions include the D gene product and

correspond to the most diverse portion of the HCDR3 (heavy chain complementarity determining region 3). Form them, 4 433 252 unique overlapping 7-residues sequence frames were derived to compare the mimotopes and find potential linear idiotopes among the latter. The comparison to a given mimotope sequence was done by finding the number of HCDR3 region 7-mer sequences within a longest common subsequence distance of 2, i.e. – with no more than 1 mismatch. A set of 217 442 7-mer sequences from a previously published analysis (Matochko et al., 2014; Matochko and Derda, 2013) of the same PDL (Ph.D -7, New England Biolabs) without prior selection was used as a background non-selected library to bootstrap the frequency of idiotopes among mimotopes. To perform protein BLAST (using the rBLAST package), the human RefSeq protein data base from NCBI was used (136 194 sequences). The filter was set to 1 mismatch. The criterion of editing distance of 2 (one deletion, one insertion) was previously established (Pashova et al., 2022) based on data from the Biopanning data bank (http://i.uestc.edu.cn/bdb/) (He et al., 2016) summarizing results from phage display screening with monoclonal antibodies.

2.6. Statistics

In this study, the groups were compared using non-parametric tests – the Wilcoxon rank sum test and Dunn's test for multiple comparisons with Benjamini-Hochberg (BH) adjustment of the p values where necessary. Principal component analysis (PCA) was used for data exploration and dimensionality reduction using the *FactoMineR* and *factoextra* packages. Each reactivity was correlated to the blood group antigen score. The blood group antigen score equaled the number of pooled sera coming from patients expressing that antigen. Thus, it took only 3 values: 0, 1, and 2. Correlations from non-significant regressions were considered equal to 0.

3. Results

An array of 722 representative mimotopes of public IgM reactivities in healthy donors (Pashov et al., 2019) were used to probe the repertoires of serum IgM and IgG from patients with neurodegenerative diseases. To this end, samples from AD (n=8), FTD (n=8), ND (n=8), and controls without clinical dementia (n=6) were pooled in pairs to yield respectively 4 groups of 5 serum samples. These were tested on the IgM mimotopes in oriented planar peptide microarrays (Suppl. Table 1).

3.1. The reactivity data distinguishes diagnoses and shows correlations

After cleaning and normalization, the data from serum IgM and IgG binding to the array of public IgM mimotopes showed considerable correlation between all samples (Suppl. Fig. 1). Amino acid residue composition bias is most probably not the cause because the data was normalized with that respect. Some part of this correlation may be due to testing public reactivities that reflect constant characteristics of the repertoire. Another contribution may be from the between-array normalization. In the overview of the data analyzed by PCA the latent variable underlying this correlation was isolated in the first component. Excluding this factor, the other components separated the samples by diagnosis (Fig.2).

Figure 3 shows the correlations between the mean reactivity values for the different diagnoses. There was a weak positive correlation between IgG and IgM reactivities respectively for AD, NDD, and the control group as well as for the blood group antigens but not for FTD. Among the disease groups, AD correlated with FTD in the IgM but not the IgG set. The strongest anti-correlations were between the control group reactivities and the FTD / AD reactivities.

Correlation with blood group antigens was checked because, in a previous study, a major clustering of the IgM reactivities to self-antigen linear epitopes was found to correlate with the blood group (Pashova et al., 2022). The mimotopes' blood group A antigen cross-reactivity correlated weakly with ND separately in the IgM and the IgG data sets while blood group B cross-reactivity with both the IgM and the IgG sets correlated weakly with AD in the IgG but not in the IgM set. Also, blood group B cross-reactivity correlated with FTD separately within each isotype data set.

3.2. Graph representation of mimotope reactivities for a detailed analysis

The analysis of the correlation between the reactivities in AD, FTD, NDD, and C as well as the PCA indicated that the public IgM reactivity repertoire contains information relevant to the neurodegenerative disease under study. To extract further detail, the cross-reactivity data for both isotypes was represented as graphs along the lines of the methodology published recently (Ferdinandov et al., 2023; Pashova-Dimova et al., 2023). The method for inferring cross-reactivities between peptides was improved measuring the similarities between profiles across the array of samples (see Materials and methods for a detailed description). The threshold of profile similarity for connecting two mimotope reactivities with an edge was adjusted to yield a large connected component of the graph with at least 700/722 reactivities. The connected components of the graphs were visualized in 2D using a twostep spectral embedding (Suppl. Fig. 2). A 3D final projection is also provided which is of even higher fidelity to the 4(5)D spectral embedding (Supplementary files IgM_AD_FTD_NDD_C.mp4 and IgG_AD_FTD_NDD_C.mp4). The visualizations illustrate a very clear distinction between the public reactivities lost (high in the controls) and those gained in the patients with dementia. Furthermore, there is also a clear distinction between the diagnoses, especially in the IgG graph.

3.3. Reactivity graph structure and parameters

To estimate the significance of some descriptive parameters of the graphs, the baseline values were simulated by using graphs generated by the same algorithm but based on the scrambled raw data matrices for each isotype. The graph density (the number of edges relative to the maximal number of possible edges) and the size of the large connected component were higher for both the IgM and the IgG graphs then for the random graphs (Fig. 4). Similarly, the degree distributions were shifted slightly to the right (Suppl. Fig. 3) and the maximal clique size distributions showed more cliques but with similar distribution as compared to the random graphs (Suppl. Fig. 4).

Next the information about classes of public reactivities encoded in the topology of the graphs and their relation to neurodegenerative diseases was studied. Clusters of vertices were determined in the graphs based on modularity (regions with denser edges within than to other parts of the graph) using the Leiden algorithm (Traag et al., 2019) (https://github.com/TomKellyGenetics/leiden). The IgM graph clusters were 47 with 13 consisting of single vertices and largest being of 60 vertices. For the IgG graph these values were respectively 60, 22 and 33. The IgG graph clusters appeared more and of smaller size but these differences were not significant. Compared to the random graphs both the IgM and the IgG graphs had significantly lower number of clusters (Fig. 4) which means that the repertoire of public reactivities at this coarse resolution exhibits large scale modularity with relatively few clusters. Interestingly, compared to the random graphs, the modularity of the clustering was very different between IgM (high) and IgG (low) although the values for both graphs were rather similar. This can be interpreted as the public IgM repertoire clusters in relatively small number tight cluster while the IgG reactivities to the IgM mimotopes form slightly more and significantly less well-defined clusters.

The vertices of the graphs were assigned attributes corresponding to the rank sums of the mimotopes' reactivities or each diagnosis. Thus, for instance, mimotopes with high reactivities in the control and low in the disease groups will have a high-rank sum associated with the control group. The attributes reflected also blood group antigen cross-reactivity and cross-labeling – assigning the IgM graph vertices the IgG attributes by diagnosis and vice versa. The latter approach was applied to check if the graph of each isotype reflects the diagnosis-associated relations of the other.

The degree of correlation of the graph structure with a quantitative attribute is measured by assortativity – the measure of the extent to which neighboring vertices have similar magnitude of the attribute. Figure 5 shows the comparison between the assortativities of several attributes for both graphs compared to the respective sets of random graphs. The highest assortativity was found for the control group-related reactivities (i.e. – those that are lost in disease). The assortativity of the correlation with blood group antigen was comparable but somewhat lower and the cross-labeling attribute assortativities were markedly lower reaching insignificant levels in FTD (for IgM) and NDD (for IgG). The correlation between the reactivity profiles across isotypes (GMCR) did not show significant assortativity, thus, the IgM/IgG cross-reactivity for these IgM mimotopes was not reflected directly in the graph structure.

3.4. Idiotypy and neurodegenerative disease

In our previous studies we stumbled upon a significantly high proportion of homologies to HCDR3 region sequences from other human antibodies in the public IgM reactivity mimotopes (Pashov et al., 2019;

Pashova et al., 2022). Such HCDR3 homologues of mimotopes could be considered, at least potentially, as idiotopes. Since the IgM mimotope array used in this study was shown previously to be significantly enriched in idiotipic mimotopes, we decided to check if this idiotope reactivities correlated with reactivities associated with neurodegenerative diseases. The nearest neighbors for each mimotope among a library of 4×10^6 7-residue frames from 10^6 human antibodies were determined using as a criterion at most 1 mismatch. Under this condition, 483/722 mimotopes had at least one nearest neighbor among the J region frames and some had up to 187 which are probably parts of germline genes. Next, the number of nearest neighbors among idiotopes (NNNI) that splits the data into groups maximally correlated with diagnosis was determined. To that end, the mimotope set was repeatedly split into two groups relative to each value of the sorted NNNI. At each step, the two groups of NNNI low and NNNI high mimotopes were compared using the Wilcoxon rank sum test. Figure 6 shows that for IgM 2 NNNI was the optimal criterion to detect maximal correlation. The correlation was positive for the reactivities lost in the disease groups (high in the control group) and negative for the reactivities high in AD. The group of NDD had also a positive correlation although much lower than the group C and the FTD group had a similar to AD but much lower negative correlation. For IgG these correlations were mostly insignificant. Unexpectedly and contrary to the IgM, the control group in IgG had small but significant negative correlation while the NDD group had still small positive correlation, all at a higher NNNI split point. Thus, the public IgM reactivities lost in the neurodegenerative diseases tend to be more idiotypically connected in contrast to, in particular, those gained in AD but also to some extent those found in FTD.

3.5. Public reactivity clusters associated with neurodegenerative diseases

The mean reactivities by diagnosis in each of the vertex clusters defined in 3.3. were compared using Dunn test (BH adjusted $p<0.01$). The clusters found to have significant differences between at least two diagnoses were 17/47 for the IgM graph and 17/60 for the IgG one. To summarize these results each significant cluster was represented by the 6 differences between mean reactivities for the 4 diagnoses. The data was subjected to PCA and plotted as a biplot (Fig.7) which shows simultaneously the correlations between the axes as well as the mapping of the clusters onto the same space. The IgM graph clusters show differences mostly along the axis defined by differences the controls vs AD and FTD with a weak correlation between the control reactivities and the NDD patients. The IgG clusters complemented these distinctions by differences between AD and FTD. The symmetric distribution of the clusters around the origin shows that there are approximately equal numbers of cluster representing positive or negative differences.

The repertoire probing with just 722 mimotopes provides too coarse a picture. The reactivity graphs clustering summarized the cross-reactivities based on profile similarities. The respective underlying reactivities are encoded in the pattern of similarities between the samples that generate an edge. So, although the reactivities in each cluster were generally related, augmenting the grouping by a further clustering of the edges would provide a higher resolution. Indeed, when each cluster was subdivided into (overlapping) subclusters connected by highly correlated edges, a new more detailed clustering was obtained. It consisted of 211 clusters (sizes 3-47) for the IgM graph and 236 clusters (sizes 3-26) for the IgG graph. Applying similar strategy for statistical analysis as for the vertex clusters with a significance level of $p<1e-3$ after BH adjustment, 44 highly significant secondary clusters were found for the IgM graph and 21 – for IgG (Fig. 8). At an even higher level of significance ($p<1e-5$) 18 IgM clusters and 11 IgG clusters remained. Many of these clusters had the same type of difference between the diagnoses so they could be grouped in 7 groups for the IgM and 4 groups for the IgG (Suppl. Table 2). As concluded above, IgM reactivities did not differentiate between AD and FTD but IgG clusters did. Since the final clusters of

mimotopes were overlapping, the relations between them were examined using a venn diagram (Suppl. Figure 5). The clusters with higher reactivity in the controls were only from the IgM set while those with disease associated reactivities tended to overlap between IgM and IgG. The IgG reactivities high in disease grouped in more diverse clusters. Interestingly, the few reactivities overlapping between IgG and IgM clusters belonged to clusters with opposing characteristics (eg. – FTD reactivity is higher than that in the controls in IgG but the opposite is true for the igM set). These are the few examples of a possible change due to an isotype switch in an ongoing auto-immune response.

3.6. Mapping the NNNI to clusters

Since the disease associated reactivities were clearly correlated to idiotypy at least in the IgM data set, the mean NNNI of each of the final clusters were compared (Fig.9). As expected, for IgM the clusters of reactivities higher in AD and FTD had lower NNNI then those lost in the dementia patients (C) or the overall mean for the remaining (not significant) clusters (labeled ALL). Among the reactivities lost in dementia as compared to the controls (high in C) only those that in NDD were higher than C had significantly more idiotope neighbors then the bulk (ALL). In IgG, surprisingly, only the clusters with reactivity higher in FTD then in the controls had significantly higher NNNI.

3.7. Mapping the altered reactivity mimotopes to potential self antigen linear epitopes

The reactivity of public antibodies, especially IgM, could be often identified as directed to carbohydrate epitopes on bacteria. Still, many of these reactivities probably come from natural antibodies which have no nominal antigen in the sense of adaptively evolved specificity and are inherently poly/autospecific. Also, it is almost impossible to deconvolute conformational protein epitopes corresponding to mimotopes selected by a bulk repertoire. With these considerations, the only accessible specificity interpretation of the studied mimotopes was to checked them against potential linear epitopes in the human proteome. BLAST search for sequences which had at most one mismatch with any of the studied mimotopes yielded 184/722 sequences with hits in 2182 proteins and 46/197 sequences with hits in 67 proteins for the mimotopes selected in the ultimate disease associated clusters (Suppl. Table 3). Among the 67 putative targets, 31 were involved in brain metabolism and signaling. The proteins LRPs (LRP1B, LRP4, LRP6, and proLRP), sortilin, TIA-1, and BMAL1 are directly contributing to the pathogenesis of neurodegenerative disorders. The LRPs are endocytic receptors for the internalization of ApoE-containing lipoproteins and sortilin is an endocytic receptor for the uptake of diverse proteins, including ApoE (Asaro et al., 2021; Carlo et al., 2013), amyloid beta (Saadipour et al., 2013), and α-synuclein (Ishiyama et al., 2023). These receptors had homologies with mimotopes part of a reactivity group overexpressed in IgG from FTD and NDD patients. TIA-1 - a cytoplasmic scaffold protein of stress granules, was homologous to a mimotope of reactivities overexpressed in IgM from NDD and FTD patients. BMAL1 – a transcription factor which is important regulator of circadian rhythm, has a mimotope in a group of reactivities overexpressed in IgM from AD and FTD. Some other potentially targeted proteins with involvement in neurodegeneration are SMG7 (mRNA quality control), Tenascin C (neuroinflammation and association with core amyloid-beta plaques), FGF17 (cognitive processes), IRS2 (Insulin receptor signaling), Lon protease (protein quality control), UBXN1 (protein quality control), ZFYVE26 (autophagy), PlexinD1 (neuronal guidance) (Suppl. Table 4).

4. Discussion

The presented study aimed at exploring the capacity of the public IgM antibody repertoire to detect and react to different types of dementia. To this end, the public IgM repertoire and the IgG counterparts that are cross-reactive with it, were analyzed using high throughput binding assays and cross-reactivity graphs. The immune system's role in neurodegenerative diseases has been supported by extensive evidence – both in terms of cellular immunity (Chen et al., 2018; Reagin and Funk, 2023; Yang et al., 2022), inflammatory mechanisms (Baulch et al., 2020; Foley et al., 2024; Shue et al., 2024), and antibody based biomarkers (DeMarshall et al., 2022; DeMarshall et al., 2023; Kheirkhah et al., 2020; Restrepo et al., 2013). Particularly, with respect to the antibody repertoire, several groups have demonstrated the utility of IgG adaptive immunity responses as a source of biomarkers including for early detection (DeMarshall et al., 2016; Reddy et al., 2011; Restrepo et al., 2013). The main paradigm underlying these studies was basically autoimmunity, possibly a “protective” one (Yoles et al., 2001). At the same time others considered the role of constitutively expressed natural antibodies (Rothstein, 2016) although in most cases the term naturally occurring antibodies is used for antibodies elicited by self-antigens (Britschgi et al., 2009; Rosenmann et al., 2006; Szabo et al., 2008).

Enticed by the successful mining of the antibody repertoires for AD biomarkers (DeMarshall et al., 2015; DeMarshall et al., 2016; Restrepo et al., 2013), we looked for public IgM reactivities with the same properties. A recently developed approach (Ferdinandov et al., 2023; Pashov et al., 2019; Pashova et al., 2022) was applied. It consists of combining IgOme repertoire analysis (Ryvkin et al., 2012) with the immunosignature concept (Restrepo et al., 2013). Indeed, we could demonstrate distinct patterns of public IgM and their cross-reactive IgG counterparts in AD, FTD, and NDD patients compared to controls. The loss or gain of specific IgM reactivities in AD and FTD indicates alterations in the natural and possibly the induced IgM repertoire. The loss of constitutive natural antibody reactivities has been observed before (Ferdinandov et al., 2023; Pashova et al., 2022). It probably represents a temporary exhaustion due to binding to some overexpressed self-antigens in terms of quantitative tolerance thresholds (Hennings et al., 2011). The opposite, reactivities increased in disease, is most easily explained as an adaptive immune response.

The concept of public clones has two aspects. Antibodies can be shared between individuals as clonotypes or just as a convergent specificity. In the latter, broader context, the IgM repertoire is not only more diverse but also organized in larger public specificity classes compared to IgG or IgA (Arora and Arnaout, 2022; Galson et al., 2015; Wu et al., 2022). It is also safe to assume that natural antibodies and public IgM overlap without being synonymous. Sometimes the IgM compartment is considered predominantly consisting of ill-defined polyreactivities or antibodies which are simply sticky (Ausserwöger et al., 2022; Cunningham et al., 2021)). The cross-reactivity graphs provided a framework for visualization and analysis of the IgM network of cross-reactivities inferred only from high-throughput binding assays. It proved its potential to extract meaningful information from IgM polyreactivity. Spectral embedding ensured a well-defined visualization which can provide insight to the graph topology. The study found the cross-reactivity graphs to be dense and clustered in a relatively small number of clusters. It is possible that this is the image of the classes of overlapping reactivities discussed by Arora&Arnout (Arora and Arnaout, 2022). It is seen at a relatively coarse resolution due to the low ratio of the diversity of the mimotope library used to the antibody diversity. The clusters had a higher modularity in IgM indicating that their structure is less distinct in the IgG graph probably due to the addressing IgM mimotopes with IgG. Thus, the IgG reactivities were either randomly cross-reactive, natural IgG antibodies, or isotype switched versions of the observed IgM.

There was a low positive correlation between the IgM and IgG reactivities within each group except FTD. The controls in IgM on one side and AD/FTD in IgG on the other were negatively correlated. This favored the conclusion about a detectable reflection of the IgM repertoire into the IgG. While the occurrence of particular reactivities in IgM is primary, the same reactivities in IgG should come mostly as a result of isotype switching. This conclusion is reinforced by the patterns of overlap between the ultimate clusters seen in the venn diagram (Suppl. Fig. 5). Thus, as much as some of the probes were mimotopes of self-proteins, the observed correlations might be due to an autoimmune response to neurodegeneration.

The characteristic AD and FTD cross-reactivities patterns were manifested as high assortativity – neighboring mimotopes had similar levels of reactivity in a given diagnosis. The highest assortativity was observed for the control group emphasizing the phenomenon of loss of IgM reactivities that are common for the different neurodegenerative diseases. In the IgM network, the key distinction was between the controls (sightly correlating with ND) vs AD and FTD. The latter were not well separated in the clusters. The good distinction between AD and FTD was apparent only in the IgG data set. Interestingly, FTD associated IgG not only differed from those in AD but unlike in IgM they were correlated with ND. Unlike AD, FTD IgG patterns lacked the characteristic anticorrelation with the IgM in controls and appeared of higher idiotypic connectivity out of line with the other observations. These facts point to the occurrence of new IgG reactivities in FTD which are unrelated to the repertoire of IgM.

The BLAST search of the human proteome produced hits of proteins, potential targets of the reactivities found associated with dementia. Many of those putative autoantigens are implicated in neurodegeneration. This is especially true for the endocytic receptors LRPs and sortilin which, although very different in structure, share ApoE and ApoE/amyloid beta complexes as ligands for internalization and subsequent endolysosomal degradation (Cam et al., 2004; Chow et al., 2021; Kanekiyo and Bu, 2014; Zhang et al., 2020) (Asaro et al., 2020). The clearance of amyloid beta from brain was shown to depend on the level of expression of LRPs (Castellano et al., 2012) and sortilin-deficient mice present elevated levels of APOE and increased amyloid beta accumulation (Carlo et al., 2013). The interaction of sortilin with ApoE variants determines the direction of neuronal lipid metabolism. Binding of ApoE3 is neuroprotective through sustaining normal lipid metabolism and anti-inflammatory state. Binding of ApoE4, on the other hand, leads to disruption of intracellular pathway for lipid transport, generation of neuromediators and a pro-inflammatory state in the brain (Asaro et al., 2021). In addition, sortilin is also a receptor for progranulin (PGRN), which is recognized as a cause of FTD (fronto-temporal dementia) when it is mutated or insufficient (Rhinn et al., 2022). Another way in which sortilin is involved in neurodegenerative mechanisms is its interaction with BACE1 (the enzyme that cleaves amyloid beta precursor protein, APP). Increased expression of sortilin in brains of AD patients leads to increased BACE1-mediated cleavage of APP and amyloid beta aggregation (Finan et al., 2011). Additionally, LRP4 and LRP6 are engaged in Wnt/beta-catenin signaling, an important pathway promoting cell proliferation during early development, which is tightly controlled during aging and suppressed in neurodegeneration (Inestrosa and Toledo, 2008). LRP6 is a co-receptor while LRP4 is negative modulator (competitor for the co-receptor function) of Wnt/beta-catenin activation (Ahn et al., 2017). Antibodies to these LRPs may contribute to the pathology of neurodegeneration by causing a loss of control over the Wnt/beta-catenin pathway. A tau-related neurodegenerative factor - TIA-1 was also identified as a target. It is an RNA-binding protein and a scaffold for the formation of stress granules (SG) in response to cellular stress. SG include arrested from translation mRNA, the 40S ribosome subunit and other RNA-binding proteins. TIA-1 interacts with tau, and this interaction promotes tau misfolding and accumulation into SGs (Ash et al., 2021; Vanderweyde et al.,

2016). Downregulation of TIA-1 restrains the inclusion of tau in pathological and persistent stress granules, while overexpression of TIA1 stimulates tau-related degeneration (Apicco et al., 2018; Maziuk et al., 2018). Additionally, another factor etiologically related to neurodegeneration and associated with sleep disturbances in AD patients was found among the targets. This is the circadian transcription factor BMAL1, which is the main driver of circadian rhythm and is involved in the rhythmic transcription of about 5 to 10 % of expressed genes (Hughes et al., 2009). BMAL1 has been shown to be aberrantly methylated and irregularly expressed in the brains of AD patients (Cronin et al., 2017; Hulme et al., 2020). Interestingly, amyloid-beta can induce degradation of BMAL1, thus damaging the oscillation of the expression of its target genes (Song et al., 2015). Similar effects could be induced by specific antibodies. However, it is worth noting that BMAL1 and TIA-1, like most of the other identified proteins, are intracellular antigens and are accessible either upon cellular disintegration by necrosis or to antibodies with cell-penetrating capability.

A number of previously discussed biomarkers for FTD and AD like Nfl, progranulin, TDP-43, or GFA (Duran-Aniotz et al., 2021) were not among the potential targets of the antibody reactivities observed and most probably this is due to the coarse repertoire probing. The mimotope library was not designed for a comprehensive coverage but as an optimal sparse probe of the public IgM reactivities. Yet, it did produce a number of relevant hits.

Previously we found that the public IgM mimotope library is enriched in homologues to HCDR3 sequences of human immunoglobulins. Looking into this correlation here, the public IgM reactivities lost in AD and FTD proved significantly cross-reactive with idiotopes. On the contrary, the IgM reactivities increased in AD (and partially those increased in FTD) were much less idiotypically connected. The correlation between idiotypic connectivity and disease-specific reactivities points to a potential mechanism of an AD associated immune dysregulation probably shaped by the induced autoimmunity. Interestingly, DeMarshall, et al. defined a diagnostic autoantibody reactivity panel for AD including auto-light chain kappa antibodies (DeMarshall et al., 2023). Another interesting aspect is revealed in relation to the recent finding on the pivotal role of the peripheral immune system in the brain-gut-periphery axis potentially shaping the trajectory of AD (Han et al., 2018; Iban-Arias et al., 2024). The intriguing link necessitating further studies is through the role of the microbiome in shaping the IgM repertoire (Li et al., 2020) and the possibility that the idiotypic connectivity is at least partially if not mainly determined during repertoire selection (Detours et al., 1996; Kazatchkine et al., 1994).

With respect to the identified potential self-antigen targets, only 9/65 different proteins had putative linear epitopes recognized by IgM in the controls which is in contrast to the prevalence of idiotypic connections in IgM controls as compared to IgG. YWTDSSR from LRP1B had 7 idiotope neighbors. Interestingly, it was found in the IgM cluster of reactivities increased in FTD and ND relative to the controls but is not so prominent in AD. Overall, there is certain tendency for the IgM reactivities lost in AD and FTD to be idiotypically connected while the reactivities overexpressed in the dementia patients tended to be related to self-antigens involved in neurodegenerative processes while being less idiotypically connected.

The differences in antibody public IgM reactivity profiles highlight another repertoire compartment with a considerable potential as non-invasive biomarkers for early disease diagnosis. Understanding the immunological changes in AD and FTD could also inform therapeutic strategies. Despite the promising findings, this study has several limitations. The selected clusters of mimotopes defined an antibody reactivity space in which the patient groups by diagnosis were easily separable. Still, the experimental setting with small groups and overlapping serum pools precluded model validation so no attempt was

made to train a classifier. Still, this is clearly possible with a better suited data set. Future studies with larger cohorts and individual sample analysis are needed to validate these findings. Additionally, the study primarily focused on linear mimotopes, and conformational epitopes should also be considered in future research to capture the full complexity of antibody-antigen interactions. Conformational epitope information is actually encoded in the clusters of linear mimotopes but is hard to deconvolute without knowing in advance at least the target protein if not the exact epitope.

In conclusion, this study provides a compelling case for the utility of public, idiotypically connected IgM reactivity profiles as biomarkers for AD and FTD. The application of cross-reactivity graphs to high throughput antibody binding data offers a powerful tool for uncovering complex patterns and networks associated with disease states.

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7. Figure Legends

Figure 1. Tuning the similarity measure used to construct the graph. A and B - Examples of profile pairs of cross-reactive (A) and not cross-reactive peptides (B), C - ROC curve illustrating the capacity of the similarity criterion to classify 4150 pairs of peptides overlapping in 11/15 positions as a model of cross-reactive peptide pairs compared to a set of 10,000 pairs of dissimilar peptides sharing a longest common subsequence of fewer than 3/15 position (data from (Ferdinandov et al., 2023)). The tradeoff between sensitivity and specificity chosen log10(s)=-1.1 (specificity = 0.905, sensitivity = 0.604, AUC=0.848)**.**

Figure 2. Principle component analysis (PCA) of the binding data of IgM (top) and IgG (bottom) from patients with AD (red), FTD (green), NDD (blue), and healthy controls (black) to an array of 722 7-mer mimotopes of human public IgM reactivities. The PCA analysis was used to characterize the reactivities by their patterns across patients, so the layout is transposed and "individuals" refers to individual reactivities while the variables are the respective serum samples. Left – correlation of serum reactivities mapped to the first two components – the first component correlates highly with all sera and accounts for 60% of the variation. Most probably, it is not related to composition biases because the raw data has been normalized for the physicochemical properties of the amino acid residues in the peptides. Right – individual mimotope reactivities color coded for the 4 diagnostic groups studied, mapped to factors 2 and 4 (IgM) and 2 and 3 (IgG) as an example of the capacity of the data to differentiate the patient groups.

Figure 3. Heatmap of the correlations between the mean IgM and IgG reactivities in the AD (A), FTD (F), NDD (ND), and control (C) samples, the correlation of the IgM and IgG reactivities with blood group A (BlGr

A/M or G) or B (BlGr B/M or G) antigen presence, and the number of nearest idiotope neighbors (Id NN). The latter is calculated as the number of nearest neighbors among 4.4×10^6 7 residues sequence frames derived from 10^6 human immunoglobulin J region available in the NCBI data base. A nearest neighbor sequence is defined as one with a longest common subsequence of 6/7 residues. The mimotope reactivities in the patient groups were used after controlling for the common factor (Fig.1) by taking the residuals of a linear regression on the means across all samples. The IgM reactivities for AD, NDD, and the controls were somewhat correlated to their IgG counterparts but this was not true for FTD. Blood group A (IgG and IgM) reactivities, control samples, NDD and the idiotope reactivities clustered separately from AD and FTD reactivities and blood group B (IgG and IgM) reactivities.

Figure 4. Some parameters of the IgM and IgG reactivity graphs. Each parameter has been bootstrapped by generating 100 graphs constructed using the same algorithm but using scrambled matrices of the raw data. The antibody reactivity graphs are dense and clustered into fewer clusters than the random graphs but the modularity of the clustering is very different for IgM (higher) and IgG (lower) the that of the random graphs. This indicates that the IgM clusters are better defined that the IgG ones.

Figure 5. Assortativity of the properties associated with the vertices (mimotopes) for the IgM (left) and the IgG (right) reactivity graphs. The assortativity measures the degree to which vertices with similar values are connected, respectively the property segregates the mimotopes in the space of their reactivities. The values for the studied sera are compared to the values obtained for random graphs (100 graphs constructed using the same algorithm but using scrambled matrices of the raw data) and using the same labeling of the vertices. The highest assortativity is observed for the control samples (C) but also for the AD (A) and FTD (F). Cross-labeling the vertices using the reactivity magnitude seen in IgM for the IgG graph and vice versa gives much lower assortativity indicating that the patterns of clustering of the same reactivities differ between IgM and IgG when tested with IgM mimotopes.

Figure 6. Correlations between IgM and IgG reactivity averaged by diagnosis and number of nearest neighbors among idiotopes. Red – AD (A), green – FTD (F), blue – NDD (ND), and black – controls (C). Wilcoxon rank sum test was used to compare the reactivities in the peptides dichotomously grouped by splitting the mimotope set relative to the number of neighboring idiotopes of each peptide. The test was performed at each split point indicated on the abscissa. The resulting statistic z-score is on the ordinate. The shaded area indicates significance level $p\geq0.05$ so the portions of the curves outside of the shaded area correspond to significant difference. The insets show the Spearman's correlation coefficient for the reactivity vs number of idiotope neighbors. For IgM, even 2 idiotope neighbors are sufficient to show the maximal correlation. The IgM reactivities higher in the controls (i.e. – lost in the other patients) show the highest positive correlation with idiotypy while those increased in AD – the highest negative correlation. The IgG reactivities hardly show any correlation with the controls having some negative correlation with having more than 4 idiotope neighbors.

Figure 7. Biplot from the PCA results on the differences between the mean reactivities by diagnosis in the established clusters in the IgM and the IgG graphs. Only the clusters for which the differences are significant at the level $p<0.05$ after adjusting for false discovery rate using the Benjamini-Hochberg correction are used. IgM shows a simpler separation of the clusters mostly between those with high reactivity in AD and FTD and those with low reactivity in the patients (high in the controls). The IgG clusters show higher diversity some differentiating AD and FTD or NDD but their differences were generally smaller than those in the IgM clusters.

Figure 8. Biplot from the PCA results on the differences between the mean reactivities by diagnosis in the established combined (vertex and edge) clusters in the IgM and the IgG graphs. The statistical parameters are the same as for Fig.7. Again, no IgM cluster differentiate AD and FTD while IgG clusters do. The cluster numbers are composed of the vertex cluster separated from the subdividing edge cluster by a period. Most of the subdivisions of a given vertex cluster still cluster together but some are mapped away illustrating the utility of the edge cluster-based refinement which also increases the significance of the differences in the clusters.

Figure 9. Distribution of the decimal logarithms of the number of idiotope neighbors by cluster groups. Since many of the significant clusters had the same pattern of reactivity across diagnoses, they were reunited to form cluster groups with the same difference in the reactivity between diagnoses. These cluster groups are analyzed for the distribution of the number of idiotope neighbors per peptide. For comparison the set of tested mimotopes not included in the selected clusters was added as a separate group (All). The groups are shown sorted by increasing mean number of idiotope neighbors. Dunn test showed that, for the IgM graph, the clusters to the left of the red asterisk have less idiotope neighbors that the rest. Also, the groups with higher expression in the controls as compared to AD and FTD (C>F.A) had more idiotope neighbors than the mean for those not included in the significant clusters (black asterisk). For IgG only the reactivities inxcreased in FTD had more idiotope neighbors than those not included in the significant clusters.

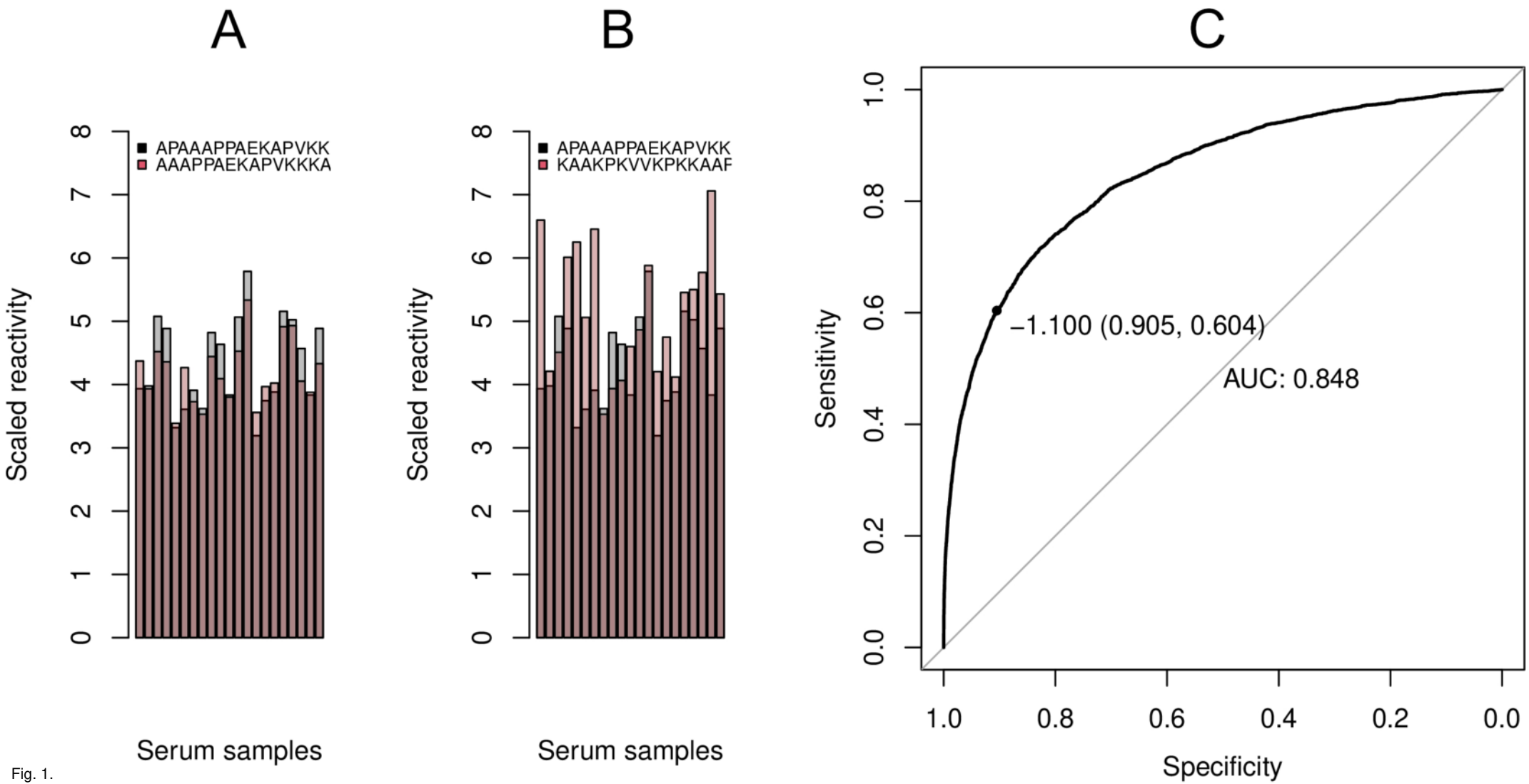
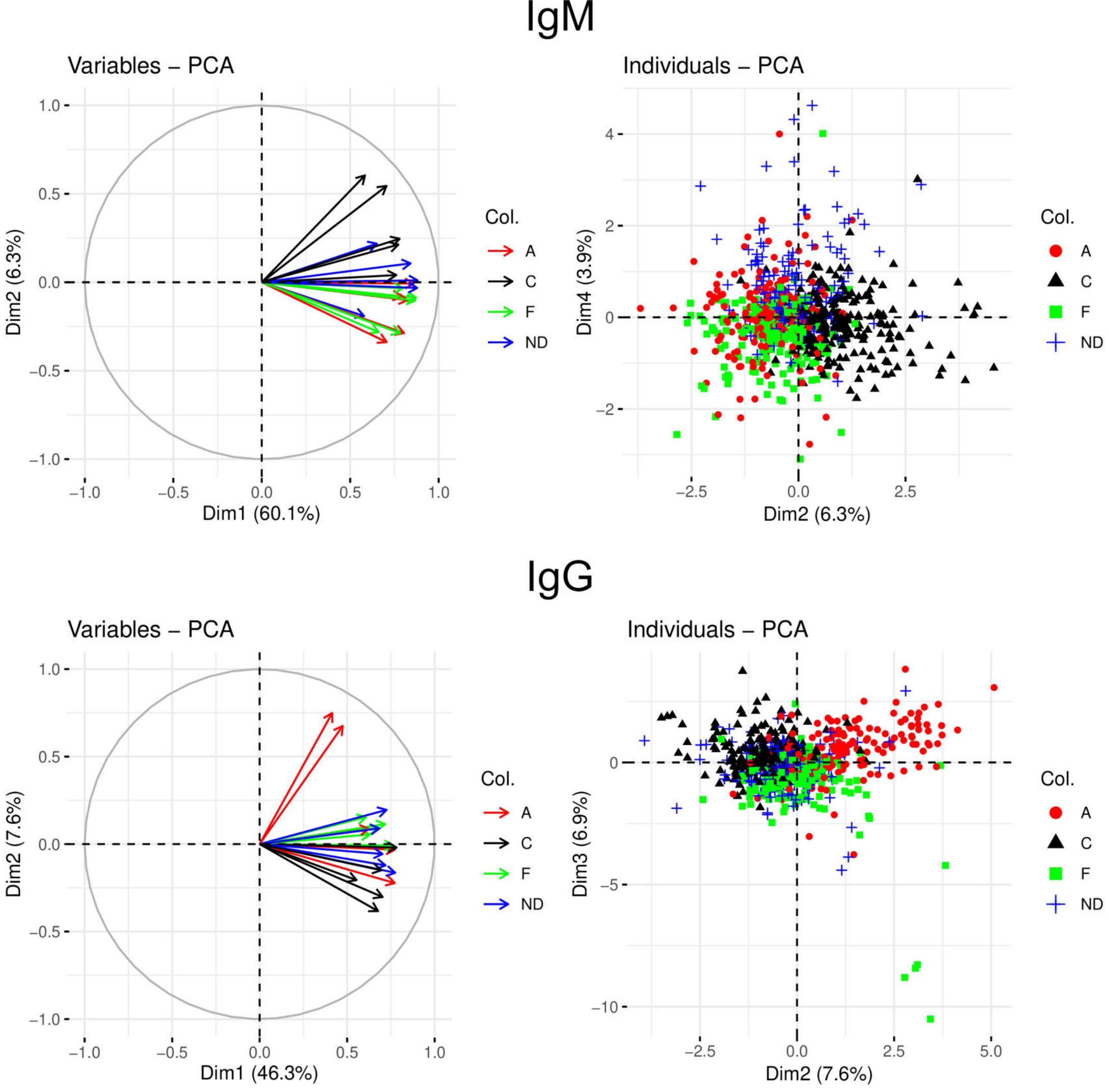

A
APAAAPPAEKAPVKK
AAAPPAEKAPVKKKA
Scaled reactivity
Serum samples
B
APAAAPPAEKAPVKK
KAAKPKVVKPKKAAF
Scaled reactivity
Serum samples
C
Sensitivity
Specificity
−1.100 (0.905, 0.604)
AUC: 0.848

Fig. 1.


IgM
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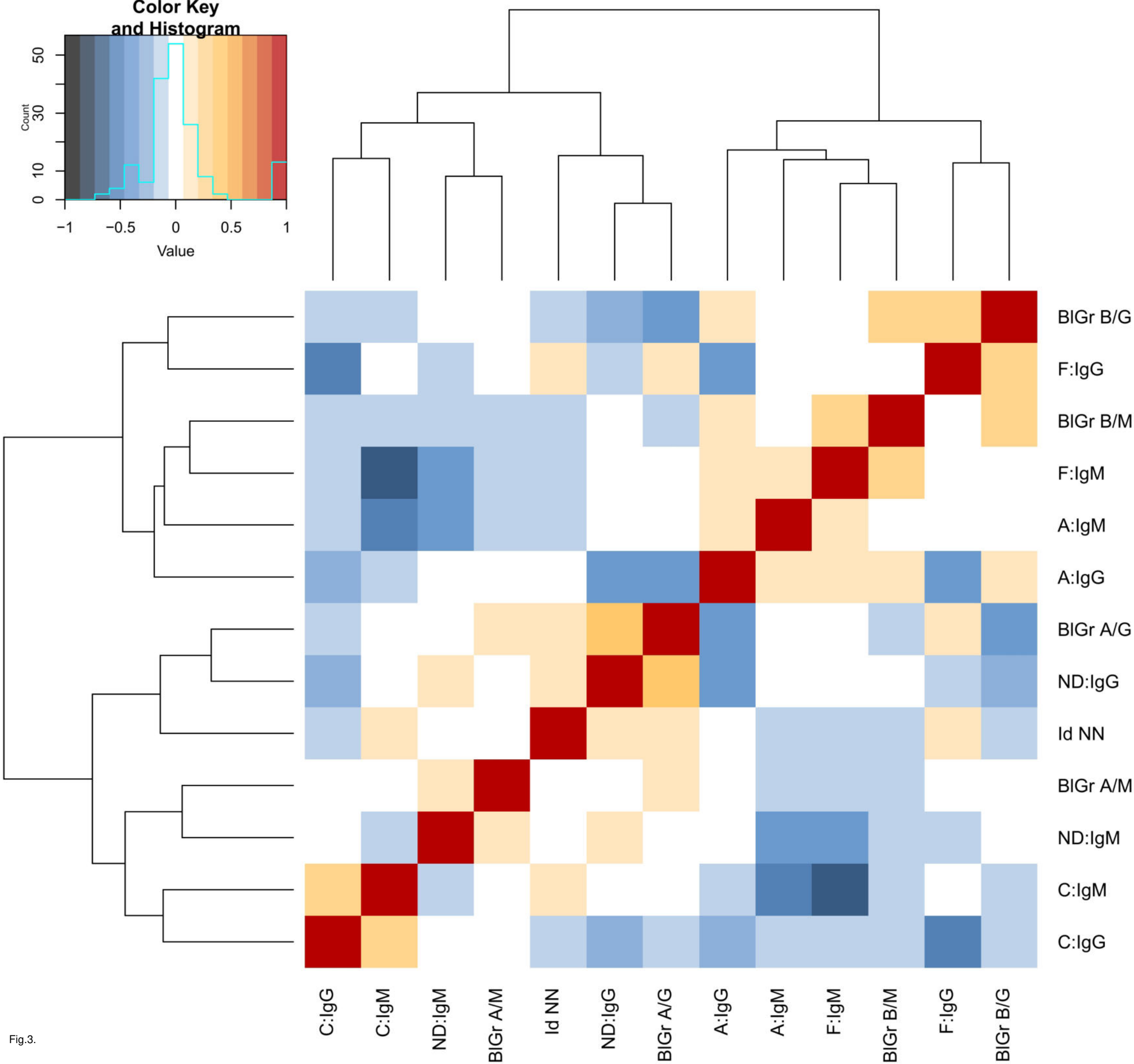
Dim1 (60.1%)
Dim2 (6.3%)
Col.
A
C
F
ND
Individuals – PCA
Dim2 (6.3%)
Dim4 (3.9%)
Col.
A
C
F
ND
IgG
Variables – PCA
Dim1 (46.3%)
Dim2 (7.6%)
Col.
A
C
F
ND
Individuals – PCA
Dim2 (7.6%)
Dim3 (6.9%)
Col.
A
C
F
ND

Fig. 2.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and Histogram
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10
30
50
−1
−0.5
0
0.5
1
Value
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F:IgG
BlGr B/M
F:IgM
A:IgM
A:IgG
BlGr A/G
ND:IgG
Id NN
BlGr A/M
ND:IgM
C:IgM
C:IgG
C:IgG
C:IgM
ND:IgM
BlGr A/M
Id NN
ND:IgG
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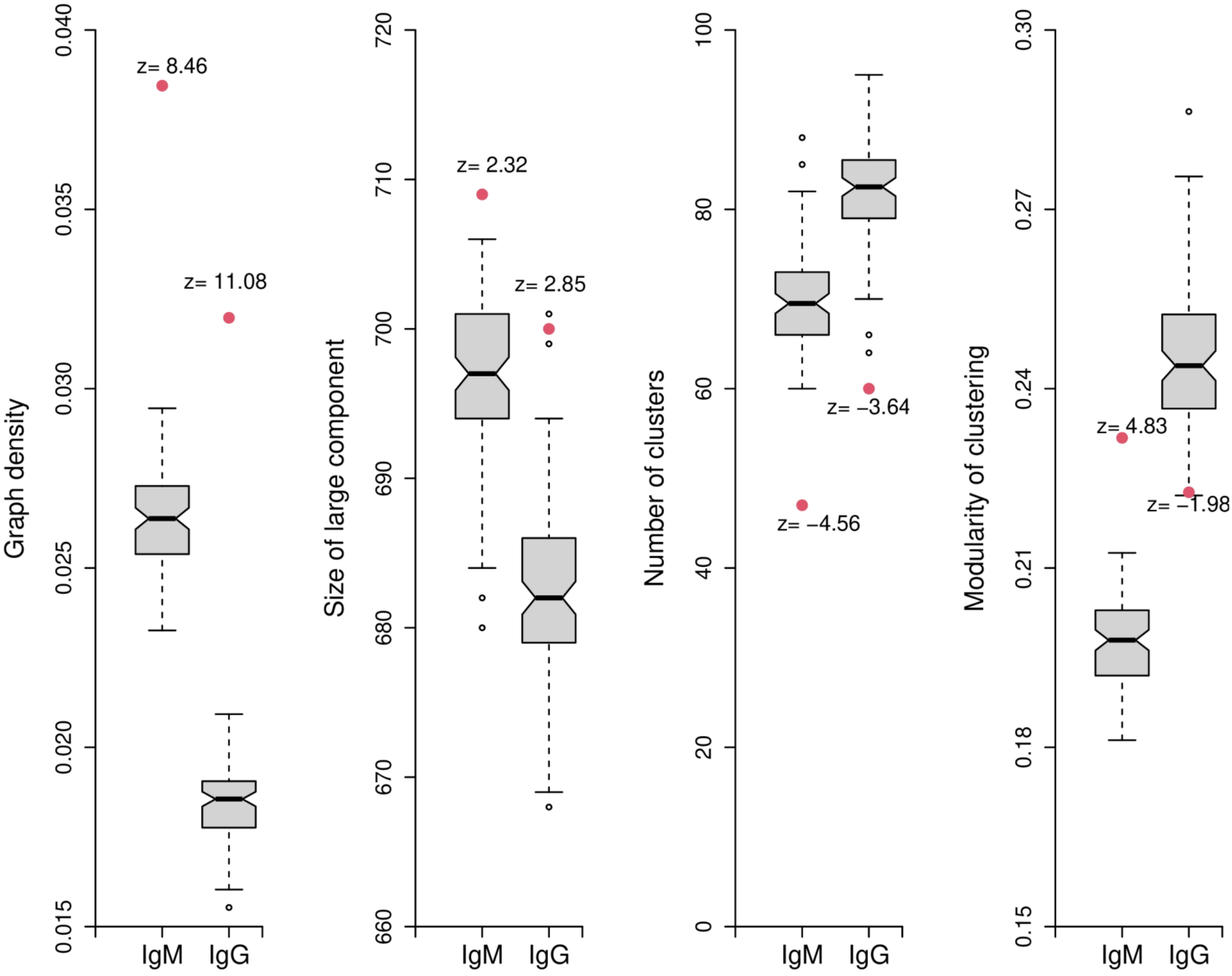
A:IgG
A:IgM
F:IgM
BlGr B/M
F:IgG
BlGr B/G

Fig.3.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0.035
0.040
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z= 11.08
IgM
IgG
Size of large component
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670
680
690
700
710
720
z= 2.32
z= 2.85
IgM
IgG
Number of clusters
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20
40
60
80
100
z= −4.56
z= −3.64
IgM
IgG
Modularity of clustering
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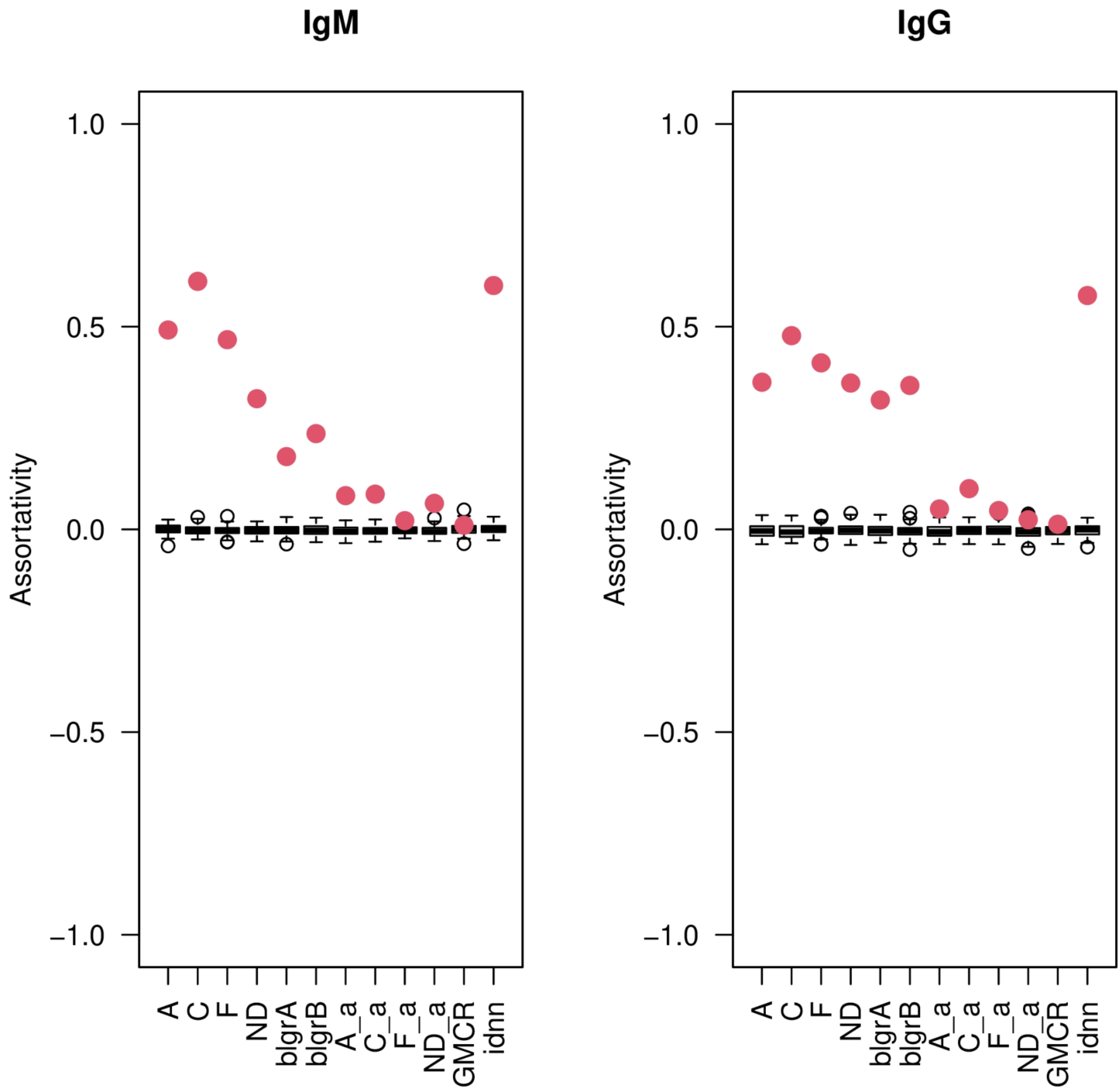
IgM
IgG

Fig. 4.


IgM
IgG
Assortativity
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0.0
−0.5
−1.0
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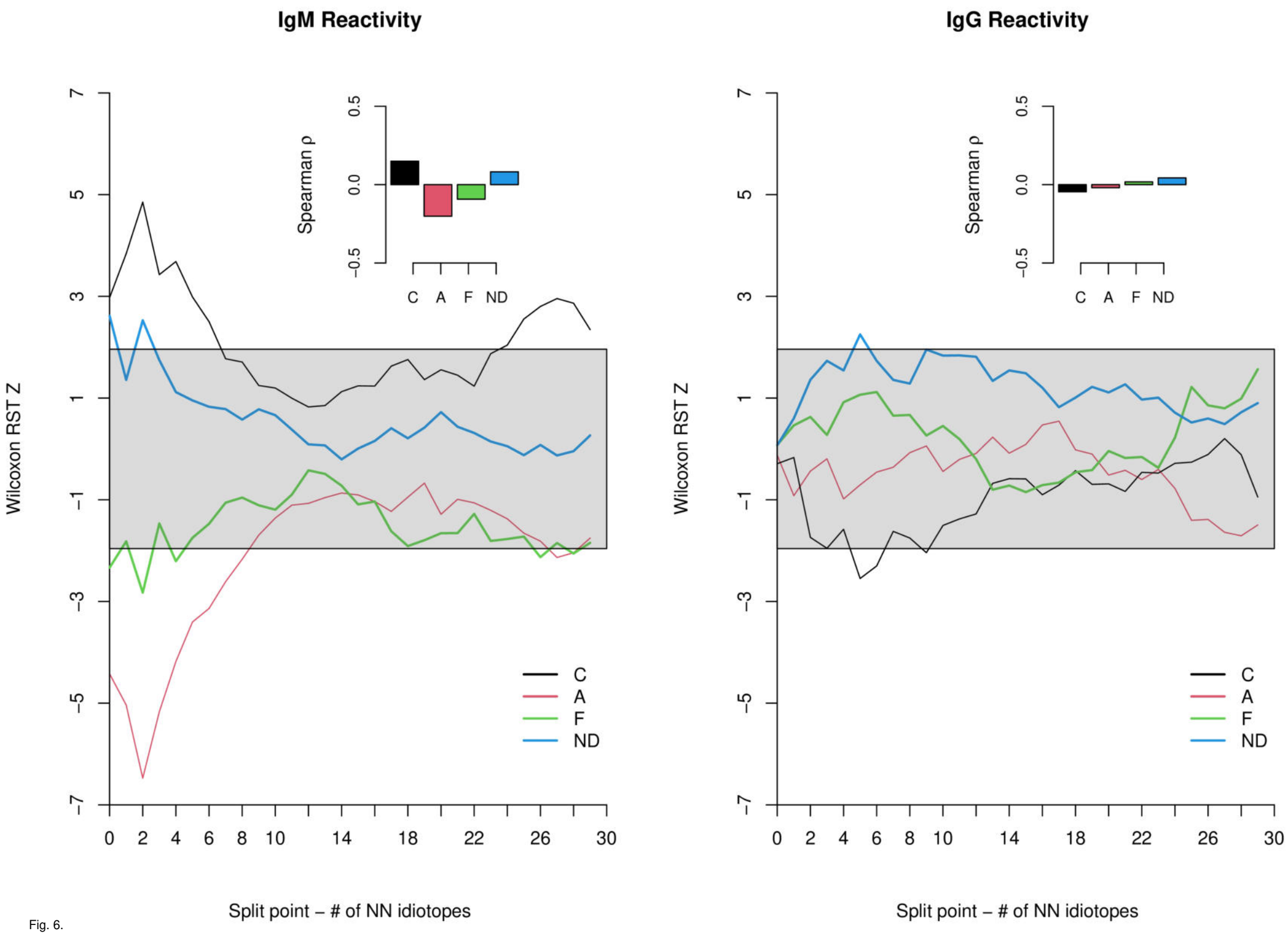
C
F
ND
blgrA
blgrB
A_a
C_a
F_a
ND_a
GMCR
idnn

Fig. 5.


IgM Reactivity
IgG Reactivity
Wilcoxon RST Z
Split point – # of NN idiotopes
Spearman ρ
C
A
F
ND
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5
3
1
−1
−3
−5
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6
8
10
14
18
22
26
30
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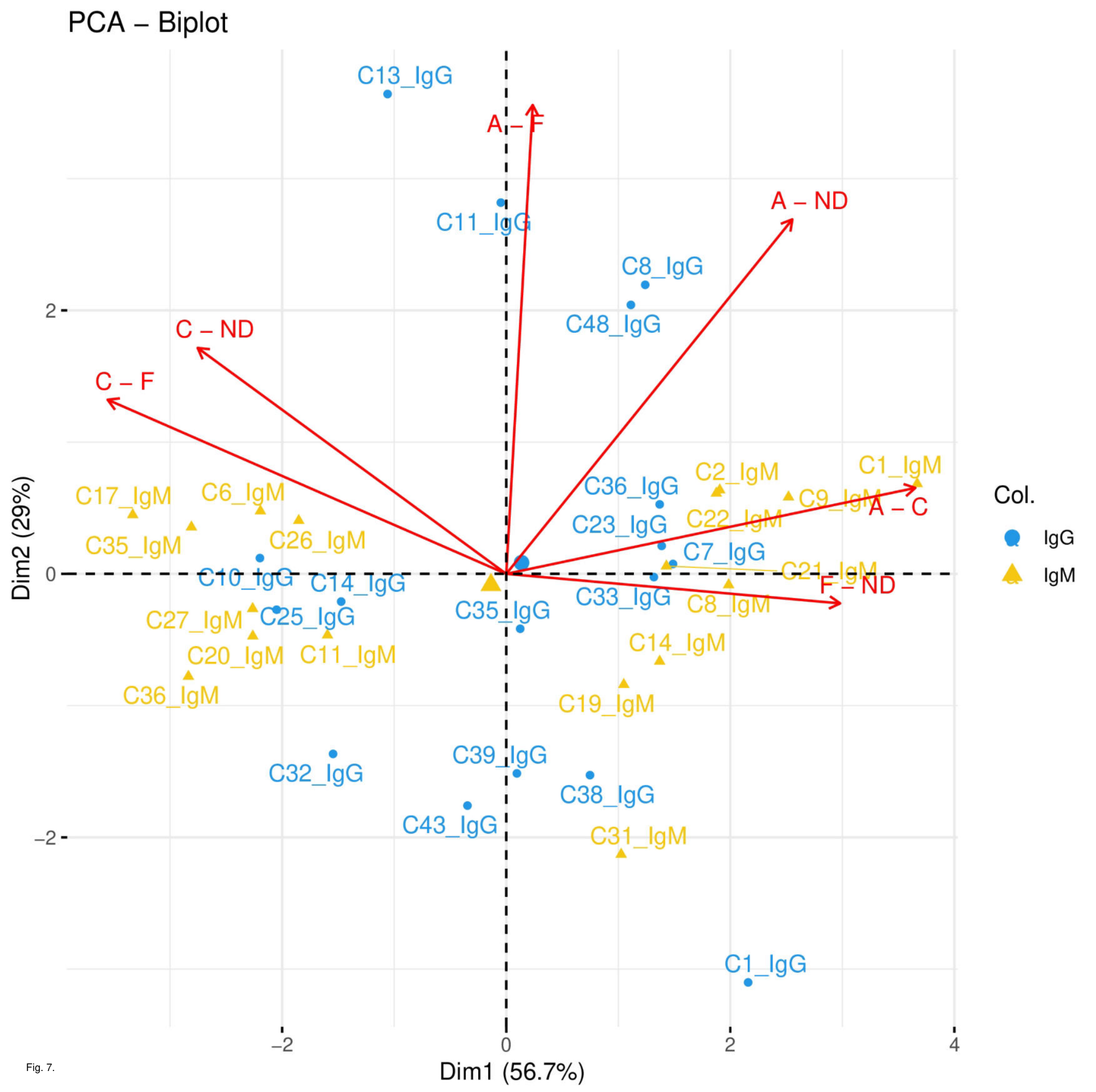
0.0
−0.5

Fig. 6.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A – F
A – ND
C11_IgG
C8_IgG
C48_IgG
C – ND
C – F
C17_IgM
C6_IgM
C35_IgM
C26_IgM
C36_IgG
C2_IgM
C1_IgM
C9_IgM
A – C
C23_IgG
C22_IgM
C7_IgG
C21_IgM
F – ND
C10_IgG
C14_IgG
C33_IgG
C8_IgM
C27_IgM
C25_IgG
C35_IgG
C14_IgM
C20_IgM
C11_IgM
C36_IgM
C19_IgM
C39_IgG
C32_IgG
C38_IgG
C43_IgG
C31_IgM
C1_IgG
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Dim1 (56.7%)
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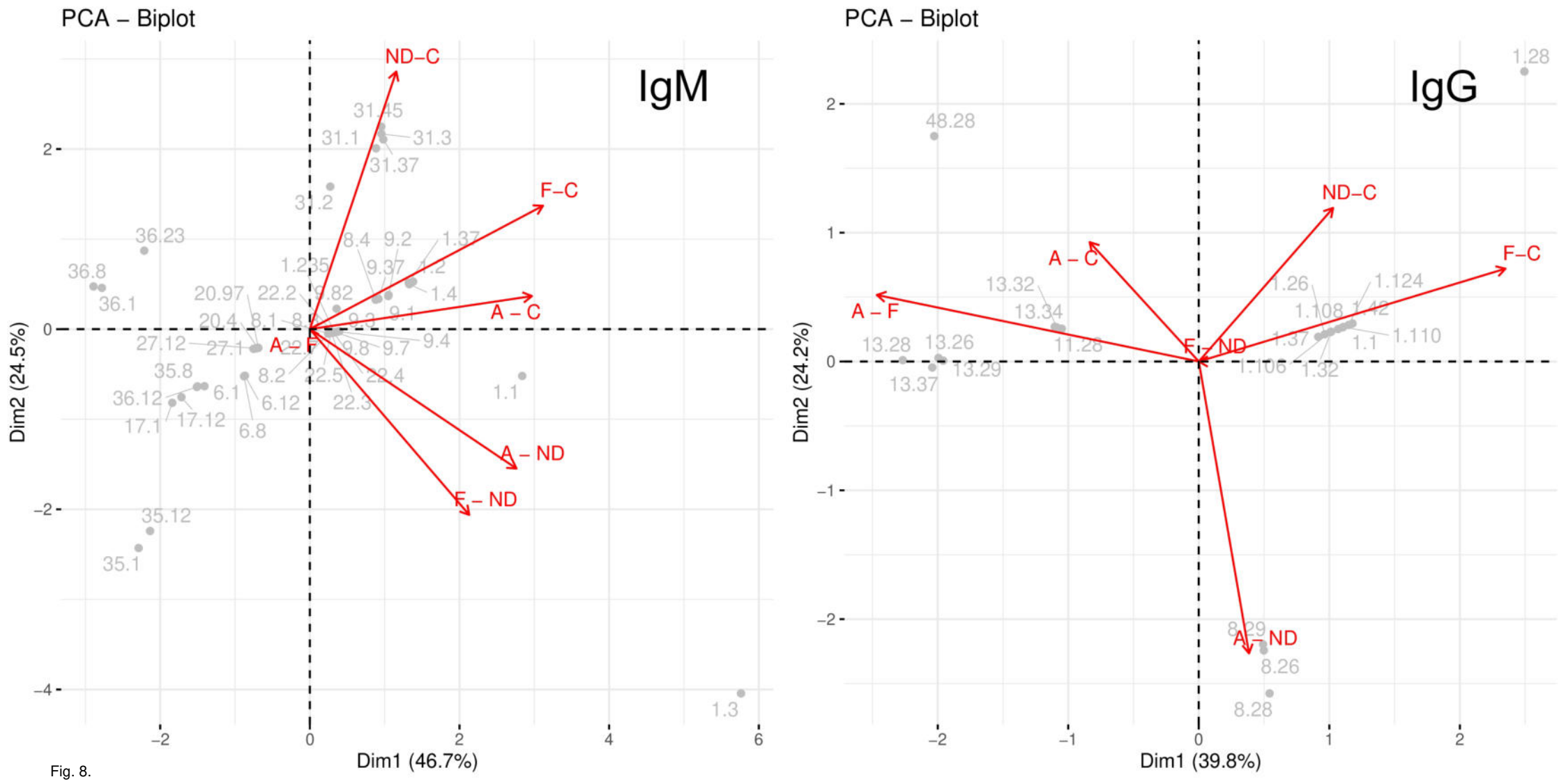
0
−2
−2
0
2
4
Col.
IgG
IgM

Fig. 7.


PCA – Biplot
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ND–C
F–C
A – C
A – ND
F – ND
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IgG
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A – C
F–C
A – F
F – ND
A – ND
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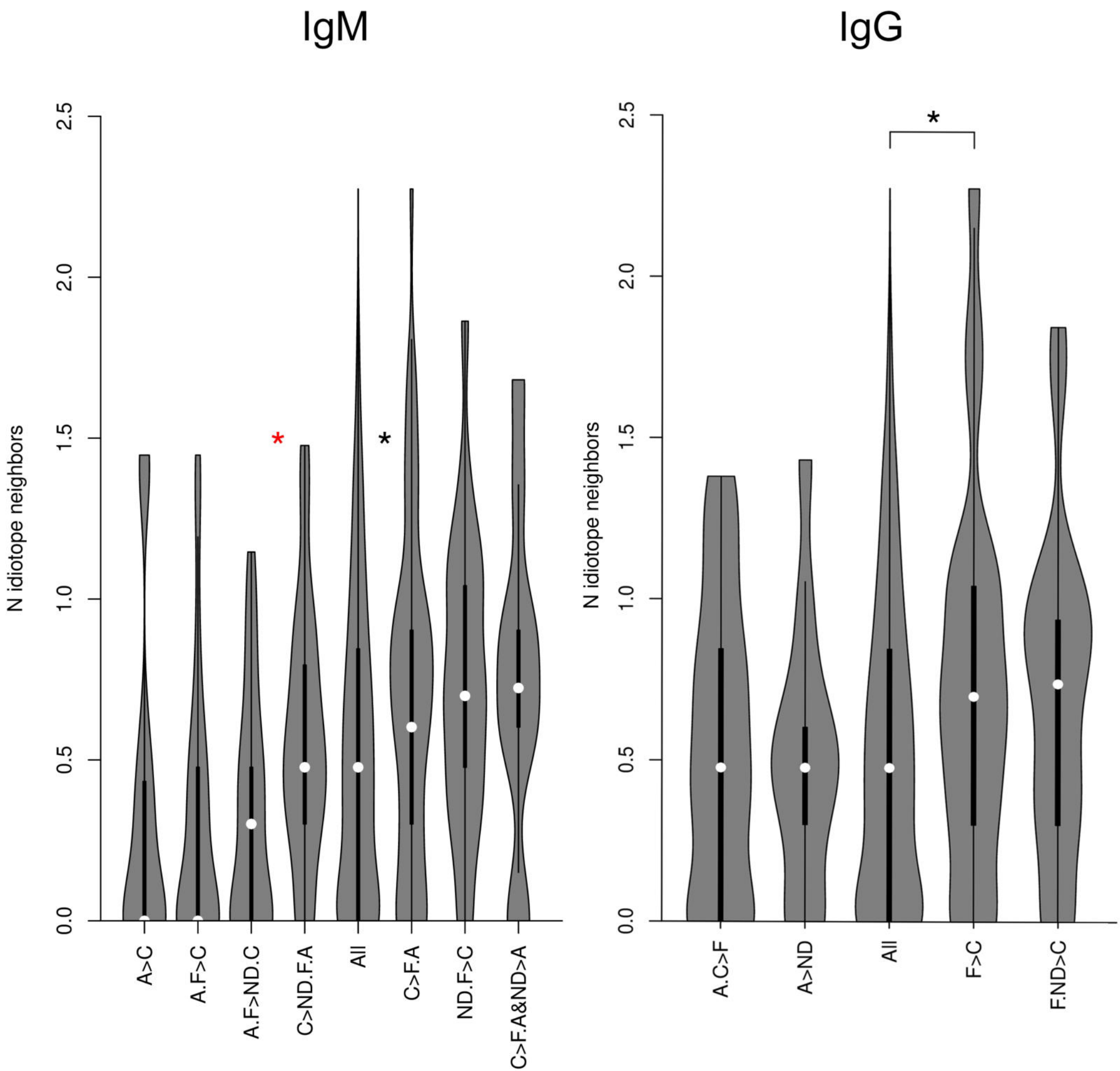
Dim2 (24.2%)

Fig. 8.


IgM
IgG
N idiotope neighbors
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0.5
1.0
1.5
2.0
2.5
*
A>C
A.F>C
A.F>ND.C
C>ND.F.A
All
C>F.A
ND.F>C
C>F.A&ND>A
A.C>F
A>ND
F>C
F.ND>C

Fig. 9.

Supplementary Materials

Supplementary Table 1. Sequences of the peptide mimotopes used in the study.

AAANSRM	ARDLGNV	EEPERWV	GKTLSDV	GYPGLWS	KPSNPEW	NGEPLIP	RTTCDHC	TGSPHTA	TTMSLVT
AAGNASL	ARPAEMS	EERHYPE	GKYFIRN	HAKSIGQ	KPTTGLY	NHWQLRW	RTTPPHY	TGSSWLV	TTPIRWP
AAGSYLP	ARSVHPI	EEVQIPV	GLCLDCR	HAMWPSR	KPVEWRV	NKQHEVP	RWDPFPA	TGTPWSP	TTPMVPW
AAIHQIR	ASVYCGR	EGLLDRN	GLCSSRL	HAQMQFV	KPYFLPH	NLAPRPH	RWIDKVP	TGVTRDS	TTPNFIL
AAISQRY	ATAFKIG	EGNKPMN	GLDIKLH	HDESFFR	KQMLPFR	NPALWCC	SAITHAL	THLAQDV	TTSSTLR
AAMDRLM	ATRADYF	EGWAREP	GLENLSH	HDGLIPS	KQPCILK	NPHHVTR	SAYWNAF	THPELAL	TTTVHLT
AANINLR	ATRGQLH	EHFRHWF	GLKSWPP	HDIQSWH	KWADLAR	NPVQAHY	SCWWCPE	THPTSSF	TTYDQSP
AASHVVT	AVAIDPR	EHIAKVF	GLLRPSP	HDLMWHR	KWPEYKY	NRYPTQI	SDAPWYN	THRWSLS	TVAHGKQ
AATQLWW	AVDGTDR	EHLDRRS	GLPITQT	HDYENRG	LDGPRPH	NTCDICW	SGALITG	THSNREV	TVCGWAC
AAYKGEE	AVGSPRS	EHSLETE	GMHLSNW	HEEQVMM	MDARPAT	NVAGLLS	SGTATLR	THVWFPS	TVLLPRD
ACCQDRM	AVGYPVR	EHVPRIL	GMPHYRE	HEIGSQL	MDCCAGG	QAFPFHP	SGWNEMV	THWENSG	TVPYRFF
ACRLQCN	AVPDLRW	EHWEAPY	GMPTRTF	HGLLERD	MDTDALT	QAMMKLR	SHELFVS	TIPRSLV	TVTRFPL
ADDACPR	AVRFSPY	ELRLIVV	GMSHLNN	HGPRCCW	MDTFTML	QANTSVR	SHQRYLS	TITSSWS	TVVAPVP
ADDRSSW	AVTDRWI	ELVRTND	GNCGQPF	HGSSAFR	MDWKVTP	QDGRDPL	SHTQKLL	TIWGADF	TVVPNGF
ADGPGMI	AVTQNYH	ELWEKTN	GNDFPPV	HGTMYPD	MEGEYWH	QDMPRLP	SITKPGN	TIWHPGL	TWPGGMR
ADMDTQA	AVVNKLI	EPLHRFI	GNRVAYV	HGTQRSH	MEMRDPY	QDQICHC	SMHLGFI	TKHIGSG	TYAEPVR
ADPGYHS	AWARHES	EPVIPRS	GPAVTTS	HGYFRFS	MEPQVII	QETPFFA	SMSFASP	TKIPLWS	TYNPRDS
ADPRNKI	AWKWDFI	EPVTSYL	GPCCRSV	HKDLWPG	MEREIPH	QEVQTVI	SPDDLRV	TKIYPQH	TYPSRPI
ADRLSFS	AWRVIPI	EQSAWRE	GPGFNKT	HKVTDVF	METFTAG	QEWHWKV	SPIDTWS	TKNSMPW	VASGTAG
ADSNLSR	AWSTGRT	ERLTCEF	GPGSQAT	HLGPYLR	MGASSFL	QFTMATF	SPIGSPR	TKTVTER	VASTSTP
ADTCWAC	AWTESLP	ETVFWRM	GPKTMLN	HLTCPKC	MGIRQND	QHDTQIM	SPNWLRL	TLARTAP	VATNPAL
ADVARTH	AWVNDLQ	ETWIGPI	GPLNLQM	HMATHPW	MGQIRYN	QIAWIRQ	SPVQSHC	TLEEFPF	VERMYTP
ADYQPPR	AYEPSLI	EVERPQI	GPLPNMD	HMKAFAR	MGTPKED	QIYAPFI	SPWSLIN	TLHMTQT	VERSRYL
AEECNIC	AYVDPRY	EVGKPHI	GPNTCHC	HMKQNSP	MGTQQDP	QLPGGSA	SQGHLKT	TLHNPRW	VERTLSY
AEHIRAF	CCLAWDP	EVPYDRR	GPPGVSR	HNAFRYD	MGVQTEV	QLWASYP	SQGYSMH	TLIPPRA	VGLTFTQ
AEKSARL	CLRYTNC	GAEWRNG	GPPLTWK	HNAIVFG	MHKNYGQ	QMQINLD	SQTHKVV	TLITSFW	VPRPMSD
AENNGSL	CNMPSRC	GAHMRWQ	GPRIPPL	HPDFWPI	MHKQINP	QPNKHFD	SRFVEPP	TLPMHHQ	VQGLTPG
AESKLAY	CPASKCW	GALQSAT	GPTNTAR	HPGCSAR	MHQEQCR	QPRVVIF	STFWKFP	TLRPWEP	VQNMWPV
AFPPWRV	CPFGCKN	GAPKHWL	GPWRSVH	HPGWAWQ	MIHDKRY	QPSPMFL	STLERLS	TMDNYFV	VWPQIIG
AGGFEWR	CPSHGCT	GARMISD	GPYTLSL	HPLLSNS	MLCSKCQ	QQLPFIW	STMQVFR	TMHEILP	VYKPKNP
AGGTPRD	CPWWCEE	GASLRPG	GQAGGLI	HPLRHSG	MLKPQVK	QSDWSSF	STYMTNF	TMMGVPR	WAERASL
AGTSRCN	CWARSVG	GATGAYN	GQHQGRI	HPNYYGL	MLRTADT	QSGHNQR	SWAMRGN	TMPDPWT	WAKGMCC
AGVAPRL	CWRSPQS	GATGSLP	GQIALSS	HPPAGIL	MMGTQRN	QSGRAPQ	SWHFQKN	TMRYPDP	WAPRIIA
AGWSPVN	CWYPSCM	GATGSYP	GQIDKIP	HPSGSSL	MMQRMSP	QSRHPEP	SWQAPFY	TNGPLSV	WCTRCFQ
AHNRTLS	DAEGFTK	GCASFAR	GQNVTAP	HQCHNCW	MMSEWQA	QSSMLER	SYPRYAW	TNGQTQS	WCVHCVQ
AHNTLRD	DAFIHTR	GCCAMHW	GQTCPCT	HQKQDLL	MPAEFLR	QTFQVLS	SYQCCAW	TNNWPFL	WDKCRDA
AHNWWFD	DAGPCRP	GCCGADP	GQVFTYP	HRLNAEW	MPCQGCS	QTGKSLH	TAAFAFT	TNPHGDT	WDMRSSF
AHPRNYS	DAHVRLA	GCSNCLY	GRWSDSY	HRWPDVM	MPDGRVY	QTGSSFP	TADGLNI	TNVHLQS	WDRNIHL
AHSIPFR	DALNAFR	GDEARDG	GSDSSDY	HTMSNPS	MPDMRVP	QTHLFSS	TAFAIKL	TNWDTTV	WERDCCT
AKGWHYP	DAPLSIK	GDLKHGN	GSGPCCS	HTQPCVD	MPFKTGH	QTMRTWN	TAGRVDS	TPGKYPA	WGGLDVT
AKVFHGR	DARADLL	GEHLLLR	GSIIFHR	HTQSHKF	MPFTDFR	QTSNRSY	TALGSRG	TPLPRAV	WGIKLRH
AKVPYTE	DARSGKW	GEHRWPW	GSYEAIG	HTRADVV	MPHKNDF	QTSSVFR	TALMILP	TPQNSLP	WGKPTLR
ALERTSK	DAVSCAR	GEPVYNP	GTATTLP	HTRTTLS	MPHLDPF	QTVEWYR	TALPVTS	TPRLVTT	WGNQYRI
ALNVATL	DCSRHPC	GEPWRYE	GTGHHVY	HTTLVYP	MPHTPRD	QTVIMRD	TANHQHL	TPSPDLW	WGPWIIP
ALTKNHT	DEGWCNC	GGAHLVT	GTGLLPT	HVNGFSK	MPIRGPM	QTYLLTP	TANNHKF	TPSRYVV	WGTTRVA
ALYALPR	DGASNLP	GGAWRES	GTHLLTR	HVNGTVQ	MPMSPPS	QTYVSRY	TANTFTI	TPTLFPQ	WHERPWY
AMFPRND	DGGLIRI	GGCQTGL	GTIPGQP	HVPLNNP	MPTYVNR	QVATYSY	TAPRDPV	TPTSSRL	WHGVQNI
AMFTPIK	DGWEAAS	GGIPCCT	GTPVLSH	HVRLPWT	MQCPNDC	QVHMHLK	TARDSAH	TPVFHGC	WHQTSRM
AMMSGFI	DHCFARR	GGKHASW	GTQNKTL	HWEPMRN	MQQTSST	QVIPFNH	TARFYGT	TPYPPFP	WMPQVSF
AMPDKLT	DHCGAPC	GGKPYLV	GTSKFDL	HWRYSPY	MQSTGHQ	QVKIPLS	TCAASLL	TQADPYR	WNGPWRI
AMPIIYG	DHQVRFT	GGRLDST	GTSMIVP	HYTCPQC	MQVSMIP	QVNPPLS	TDCTDGW	TQEYWRG	WPLMLMP
AMWDKPR	DHRMLSK	GGTLVPP	GTTAGTM	IAEMWTA	MRDPIYF	QVPSFWY	TDEIHQM	TQGFQTM	WPSQIGH
ANAPLLM	DHRNSIR	GHANPPD	GTTQVLG	IAGCHHC	MRGDLVP	QVTYMIV	TDERNFL	TQIWNSQ	WPYFSLG
ANFPSPW	DIRYHGY	GHARLSP	GTTSLAG	IANRAEQ	MRLDPDE	QYMSIPQ	TDRANWW	TQLSGVP	WQFKGLA
ANLYLPW	DKAEIWH	GHCISRP	GTTSLLS	IGLPSYS	MRPFVPC	RAADEYS	TDRVRAQ	TQMYITS	WQGRPPQ
ANQIHND	DPAGHLN	GHCRMNM	GVDRSRF	IPRIPYT	MRTDGVL	RADIYAP	TEAKLYR	TQNYAAI	WRDASMP
ANYTQRL	DPGLRYS	GHGKLSR	GVDSNWR	IPRLSPL	MSGTHQT	RAWPEAY	TEKPKNW	TQQAYLH	WRSHPQM
APGNCCR	DQPHVWN	GHLPVWS	GVLPMRF	IPTLHRP	MTDMSLL	RCCHNVF	TELKEMI	TQQTLNP	WSLAKYT
APNPYIG	DRALPSR	GHQDKSG	GVLQAAG	IRVVESG	MTPFFPV	RDMEFSN	TENWVHQ	TRCLTSC	WSPNHTS
APSNKFT	DRCCVLD	GHQSWLG	GVPQNSN	KAELIKP	MTPHNLP	RDVLDVY	TEPDYRE	TRMPATI	WTGFPLR
APTKFSL	DRHASYH	GHQVPDL	GVQMRML	KANCCRV	MTPYTQK	RFPVDQH	TEQLMRT	TSAPSYS	WTKGEHF
APWFPIG	DRLELGQ	GHSLKPR	GVRCPPQ	KENAWWR	MTVGNRL	RIAQNHP	TFATAGP	TSGLEKL	WTKVPSP
APWVTGQ	DSGCGHQ	GHWWKEG	GWCRTPM	KEVPPFR	MVKNYAD	RLDDTSY	TFQARSM	TTDARIH	WTNPFPQ
APYKSYR	DTSKAGR	GIDPGVP	GWHAIVH	KHDSISF	MVNAPWY	RLPHPLP	TGADNYR	TTDIPAR	WTPPQLR
AQALNMR	DTTLQNS	GIHTMPH	GWQHPNL	KHLLLWP	MVVDSRS	RLPMPDS	TGCFGPC	TTDRTMM	WVGQLLR

AQDCCLN	EAFQMIA	GILTPNQ	GWSYPWV	KLPARIP	MYGVDQN	RMEITNL	TGDVVLP	TTELLVA	WVKPWWP
AQGDINR	EAHYRGP	GIQGHPL	GWTESFL	KLVDLYA	MYTDPGW	RPDMLRY	TGFHGHK	TTFRLPD	WYPKHVF
AQIKHVL	EAIDPIY	GIVSYPG	GYALDPD	KMAPGPS	NALLDCT	RPFVYEY	TGHFSLR	TTHVSTG	YAGQRSW
AQLKVST	EALHWAK	GIWRDPW	GYASPPQ	KPCDCPP	NAQCCGT	RPMLQIV	TGINKTY	TTIMLPR	YPRWITE
AQMKREP	EANSIAF	GKHCWSC	GYAWITD	KPCTPCH	NAQTTVS	RPSTFHL	TGMKSPG	TTLPLPT	YQNMPWI
AQPSILR	EDCKWCR	GKHITMW	GYCQLCR	KPGSGWP	NAVPLRY	RSTDLYT	TGNWLLR	TTLSPKN	YTQCPKC
AQSMEFV	EEGLIRG	GKPPLHA	GYCRESC	KPLYVHL	NEPRDTK	RTNHMTP	TGPIAPT	TTMCCSL	YTSTLNP
ARDFTMS									YWTDSSR

Supplemental Table 2. Mimotope clusters with significantly different expression in the different diagnoses after the final clustering.

IgM							IgG			
C>ND.F.A	**C>F.A&ND>A**	**C>F.A**	**ND.F>C**	**A.F>ND.C**	**A>C**	**A.F>C**	**A.C>F**	**A>ND**	**F.ND>C**	**F>C**
DQPHVWN	GAPKHWL	AENNGSL	AWSTGRT	AAANSRM	ACRLQCN	ARDFTMS	AFPPWRV	KHLLLWP	ARDFTMS	ARDFTMS
GEHLLLR	GILTPNQ	DIRYHGY	DGASNLP	ARDFTMS	CNMPSRC	GSGPCCS	AMMSGFI	MGVQTEV	DRHASYH	DRHASYH
GHQVPDL	GIVSYPG	DPAGHLN	DPGLRYS	DTTLQNS	HAMWPSR	MDTDALT	ANFPSPW	RPSTFHL	MGASSFL	GSDSSDY
GHSLKPR	GPGFNKT	GAEWRNG	MEPQVII	GKHITMW	MPMSPPS	MDTFTML	AVGSPRS	RTNHMTP	MRDPIYF	MGASSFL
GIQGHPL	GPGSQAT	GATGAYN	MRPFVPC	GSGPCCS	MYTDPGW	MDWKVTP	CNMPSRC	SPWSLIN	MRPFVPC	MRDPIYF
GNCGQPF	GPPLTWK	GCCAMHW	RTTCDHC	HEEQVMM	NHWQLRW	MEMRDPY	EPVIPRS	TFATAGP	MTPFFPV	MRPFVPC
GTSKFDL	HPNYYGL	GCSNCLY	TGSPHTA	HMATHPW	QDMPRLP	MIHDKRY	ETVFWRM	THVWFPS	QLWASYP	MTPFFPV
GWTESFL	TSGLEKL	GEPVYNP	TGTPWSP	HTMSNPS	QTGSSFP	RDMEFSN	GMPTRTF	TTMSLVT	QSDWSSF	QLWASYP
GYAWITD	WGKPTLR	GGCQTGL	TLRPWEP	MDARPAT	SMSFASP	SRFVEPP	GPWRSVH	TVTRFPL	QTSSVFR	QSDWSSF
HDYENRG	WGIKLRH	GHLPVWS	TNWDTTV	MDTFTML	TTPIRWP	TDEIHQM	GVLPMRF	VERMYTP	QYMSIPQ	QYMSIPQ
HGPRCCW		GHQDKSG	TKIPLWS	MDWKVTP	MHKQINP	TTMCCSL	HWEPMRN	WGNQYRI	SMSFASP	SMSFASP
HQKQDLL		GLDIKLH	TGADNYR	MHQEQCR	WTNPFPQ	WDKCRDA	KQMLPFR	WRSHPQM	TALGSRG	TALGSRG
KPTTGLY		GLENLSH	TLHMTQT	MIHDKRY	RWIDKVP	MQCPNDC	NHWQLRW	WSLAKYT	TGSSWLV	TFQARSM
MRTDGVL		GMHLSNW	WRDASMP	MQCPNDC	VERMYTP	MTDMSLL	SPNWLRL		THRWSLS	THRWSLS
TQLSGVP		GQTCPCT	TEQLMRT	QVTYMIV	HYTCPQC	MVNAPWY	SQGYSMH		TQIWNSQ	TQIWNSQ
WDRNIHL		GRWSDSY	TYNPRDS	RDMEFSN	CPSHGCT	AAANSRM	TARFYGT		TFQARSM	KHDSISF
WPSQIGH		GSDSSDY	GTGLLPT	SGWNEMV	DHQVRFT	AAMDRLM	THLAQDV		YWTDSSR	GVPQNSN
WSPNHTS		GVDRSRF	QTYLLTP	SRFVEPP	GTTSLLS	ADMDTQA	YAGQRSW		TARDSAH	YQNMPWI
TGHFSLR		GVDSNWR	RSTDLYT	TDEIHQM	IANRAEQ	DTTLQNS	TPSPDLW		QTYLLTP	AAANSRM
WQFKGLA		GWSYPWV	TARFYGT	TTMCCSL	MQQTSST	GKHITMW	GWCRTPM		YQNMPWI	AWSTGRT
		HDGLIPS	TGVTRDS	ADMDTQA	SGALITG	HMATHPW	TTPIRWP			TGSSWLV
		MRGDLVP		MVNAPWY	MPCQGCS	HTMSNPS	ADDRSSW			QTSSVFR
		TNPHGDT		QEWHWKV		HWEPMRN	TEPDYRE			TPQNSLP
		GWQHPNL		VQNMWPV		QEWHWKV	QVNPPLS			
		DQPHVWN		WDKCRDA		QVTYMIV	GWSYPWV			
		GHSLKPR		WDMRSSF		QEVQTVI	TPLPRAV			
		GTSKFDL		AAMDRLM		MHQEQCR	DTSKAGR			
		GWTESFL		HRWPDVM		WDMRSSF	TGTPWSP			
		GYAWITD		MDTDALT		MDARPAT	GPRIPPL			
		HDYENRG		MTDMSLL		VQNMWPV				
		HQKQDLL		QEVQTVI		ACRLQCN				
		KPTTGLY				CNMPSRC				
		MRTDGVL				CPSHGCT				
		TQLSGVP				CWYPSCM				
		WSPNHTS				DHQVRFT				
		SHELFVS				GHCISRP				
		WQFKGLA				GTSMIVP				
		EAHYRGP				HAMWPSR				
		GAPKHWL				HYTCPQC				
		GDLKHGN				MMSEWQA				
		GILTPNQ				MPCQGCS				
		GIVSYPG				MPMSPPS				
		GMPTRTF				MQQTSST				
		GPGSQAT				MYTDPGW				
		GPNTCHC				NHWQLRW				
		GPPLTWK				QDMPRLP				
		GYPGLWS				RWIDKVP				
		HPNYYGL				VERMYTP				
		TIWHPGL				SGALITG				
		TSGLEKL				TTLPLPT				
		WGIKLRH				TTMSLVT				
		WGKPTLR				QTGSSFP				
		GPGFNKT				WTNPFPQ				
						SMSFASP				
						TTPIRWP				
						EGNKPMN				
						GLKSWPP				
						IANRAEQ				
						MHKQINP				
						HWRYSPY				

Supplementary Table 3. Proteins containing potential linear epitopes homologous to the mimotopes with differentially expressed antibody reactivities in AD and FTD.

Cluster type	Milmotope Sequence	Protein name/ protein sequence	Localization	Associated process	Function and relation to neurodegeneration	Reference
F.ND>C:IgG	YWTDSSR	Low-density lipoprotein, receptor-related protein 1B (LRP1B), YWIDSSR, YWTDHSR	Membrane	Endocytosis	Receptor for ApoE-carring lipoproteins. LRP1B interacts with APP, leading to a decrease in APP processing to Aβ.	(Real et al., 2022), (Haas et al., 2011),(Cam et al., 2004)
F.ND>C:IgG	YWTDSSR	Low-density lipoprotein, receptor-related protein 4 (LRP4), YWTDSGTSR	Membrane	Endocytosis	Promotes Aβ uptake by interacting with ApoE. Involved in maintenance of the synapse between motor neuron and skeletal muscle, as well for the synapse function in the CNS.	(Pohlkamp et al., 2015; Zhang et al., 2020), (Gomez et al., 2014), (Aoki et al., 2020), (Kim et al., 2008)
F.ND>C:IgG	YWTDSSR	Low-density lipoprotein receptor-related protein 6 (LRP6), YWTDWSR	Membrane	Endocytosis, Wnt signaling	Co-receptor of synaptic Wnt signaling. Maintain the functional integrity of the synaptic connections in the adult brain.	(Chow et al., 2021; Wood, 2023), (Buechler and Salinas, 2018)
F>C:IgG, F.ND>C:IgG	MRDPIYF	Sortilin SORT1 RDPIYF	Membrane	Endocytosis	ApoE receptor	(Saadipour et al., 2013), (Asaro et al., 2020), (Ruan et al., 2018) (Hu et al., 2017a)
ND.F>C:IgM	TEQLMRT	Cytotoxic granule associated RNA binding protein TIA-1 TEQLMR	Intracellular	Post-transcriptional regulation of Gene expression	Interaction between TIA1 and tau lead to insoluble tau aggregates in stress granules. Reduced levels of TIA1 restrain the inclusion of tau in pathological and persistent stress granules while overexpression of TIA1 stimulates tau-related degeneration. High expression in GS where binds to and controls multitude of specific transcripts during B-cell positive selection.	(Vanderweyde et al., 2016), (Osma-Garcia et al., 2023), (Maziuk et al., 2018)
A.F>C:IgM	GLKSWPP	Basic helix-loop-helix ARNT-like protein 1 BMAL1 LKSWPP	Intracellular	Regulation of transcription	Circadian transcription factor which is important regulator of circadian rhythm. DNA methylation and rhythmic expression of Bmal1 is aberrant in brains of AD.	(Cronin et al., 2017; Hulme et al., 2020)
A.C>F:IgG ND.F>C:IgM	TGTPWSP	Nonsense-mediated mRNA decay factor SMG7	Intracellular	Nonsense-mediated	SMG7 is part of the mRNA decay complex. It directs aberrant mRNA for degradation by SMG6	(Boehm et al., 2021),

		GTPWSP		mRNA decay; RNA quality control	endonuclease, also part of the complex. Inhibition of the mRNA decay complex results in massive transcriptomic alterations.	(Unterholzner and Izaurralde, 2004), (Weskamp and Barmada, 2018)
A.C>F:IgG	GPRIPPL	Fibroblast growth factor 17, FGF 17, GPRIPPL	Secreted	Signalling	Enriched in brain; Required for normal brain development; FGF17 improves memory in aging brains; blocking of FGF17 impairs cognitive processes.	(Iram et al., 2022)
A.F>C:IgM A.F>ND.C:IgM	MDTDALT	Plexin-D1 MDTDTLT	Membrane	Cell signaling, Neuronal guidance	Glycoprotein receptor for semaphorin 3E. Plexin-D1 signaling positively mediates synapse formation and have a role in ensuring the specificity of synapse formation. A missense mutation variant in PLXND1 gene is enriched among AD patients.	(Wang et al., 2015), (Balabanski et al., 2021), (Burk et al., 2017),(Pecho-Vrieseling et al., 2009),(Ding et al., 2011)
A.F>C:IgM A.F>ND.C:IgM	MQCPNDC	Tenascin X QCPNDC	Extracellular	Cell adhesion	Regulate tissue structures through anti-adhesive interaction with collagen.	(Aktar et al., 2018), (Okuda-Ashitaka and Matsumoto, 2023)
A.F>C:IgM A.F>ND.C:IgM	MQCPNDC	Tenascin C MSCPNDC	Extracellular	Cell adhesion	Anti-adhesive effects. Blood level of Tenascin C may increase in AD patients. Tenascin C can be found deposited together with amyloid beta plaques in AD patients.	(Soares et al., 2012) ,(Mi et al., 2016)
A.F>C:IgM A>C:IgM	MPMSPPS	Insulin receptor substrate 2 IRS2 MPMSPAS	Intracellular	Insulin/IGF-1 signaling pathway	Signaling through Insulin receptor/IRS2 is associated with the amount of amyloid beta accumulation in brain.	(Schubert et al., 2003), (Sano et al., 2023), (Ochiai et al., 2021)
A.F>C:IgM A>C:IgM	MPMSPPS	Zinc finger and BTB domain-containing protein 5, ZBTB5 LPMSPPS	Intracellular	Transcription factor	Participates in cell fate decision as repressor of cell cycle arrest gene p21. Proto-oncogene that stimulates cell proliferation in presence of p53 and apoptosis in absence in p53.	(Choi et al., 2020; Koh et al., 2009)
A.F>C:IgM	GLKSWPP	Catenin alpha-1 GLKGWPP	Intracellular	Cell adhesion; Wnt signaling suppression	Beyond its involvement in adherent junctions alpha-catenin is reported to repress Wnt/beta catenin target genes.	(Choi et al., 2013)
A.C>F:IgG	SPNWLRL	Brain mitochondrial carrier protein 1, UCP5 PNWLRL	Intracellular	Mitochondrial energy metabolism	Passes protons through the inner mitochondrial membrane, thus controlling ATP synthesis from ADP. Improper functioning lead to elevated reactive oxygene species and mitochondrial dysfunction.	(Ramsden et al., 2012; Tracy et al., 2022; Wang et al., 2020)
C>F.A:IgM	GRWSDSY	Actin-binding protein IPP, Profilin I GRWSDS	Intracellular	Regulation of the actin cytosceleton	Localized at synaptic sites in the brain and is involved in activity dependent mechanisms at dendritic spine synapses.	(Neuhoff et al., 2005)
C>F.A:IgM	GCCAMHW	Adhesion G protein-coupled receptor E1, ADGRE1, GCCVMHSW	Membrane	Adhesion	Plays a regulatory role in the creation of contact sites between the endoplasmic reticulum and mitochondria.	(Rasul et al., 2021)

A.C>F:IgG A.F>C:IgM	HWEPMRN	E3 ubiquitin-protein ligase TRIM4, HWEPLR	Intracellular	Innate immune signaling	Activator of innate immune response triggered by RIG-I	(Yan et al., 2014),(Fernández-de Frutos et al., 2019)
A.F>C:IgM A>C:IgM	HAMWPSR	Proteasome adapter and scaffold protein ECM29 HAMWNSR	Intracellular	Protein degradation	Involved in the regulation of the proteasomal distribution during neuronal maturation. Under oxidative stress promotes proteasome disassembly.	(Wang et al., 2017), (Lee et al., 2020),
A.F>C:IgM A.F>ND.C:IgM	MTDMSLL	Intermembrane lipid transfer protein VPS13C MTDMSIL	Intracellular	Transfer of lipids; vesicle trafficking	Mediates the transfer of lipids between membranes at organelle contact sites. Mutatins in VPS13C might relate to impaired lysosomal degradation.	(Kumar et al., 2018), (Hancock-Cerutti et al., 2022)
A.F>C:IgM A>C:IgM A>ND:IgG	VERMYTP	lon protease homolog, mitochondrial VERMYDVTP	Intracellular	Hydrolysis of mitochondrial proteins	Degradation of oxidatively damaged proteins.	(Bezawork-Geleta et al., 2015)
A.F>ND.C:IgM	SGWNEMV	TBC1 domain family TBC1D SGAWNEMV	Intracellular	Various	Some members of the family may be indirectly related to ND. For example TBC1D24 regulates autophagy; TBC1D1 regulate GLUT4 trafficing and glucose uptake via VPS13C.	(Finelli and Oliver, 2017), (Hook et al., 2020)
C>ND.F.A:IgM	GHQVPDL	RNA/RNP complex-1-interacting phosphatase, DUSP11, GHQVPD	Intracellular	RNA metabolism	Reduce the exposure of intracellular RNA to RIG-1, thus preventing activation of the innate immune response.	(Choi and Sullivan, 2021)
A.F>C:IgM A.F>ND.C:IgM	AAMDRLM	UBX domain-containing protein 1 UBXN1 AAMDWLM	Intracellular	Protein degradation	Inhibit NFκB signaling. Regulatory adaptor for proteasome degradation of ubiquitylated proteins	(Hu et al., 2017b), (Ganji et al., 2018)
A.F>C:IgM	TTLPLPT	Zinc finger FYVE domain-containing protein 26, ZFYVE26, TTLPLPT	Intracellular	Autophagy	ZFYVE26 mutations lead to defects in the fusion between autophagosomes and endosomes.	(Vantaggiato et al., 2019), (Khundadze et al., 2013)
A.F>C:IgM A.F>ND.C:IgM	QVTYMIV	Dynein axonemal intermediate chain 3 QITYMI	Intracellular	Cellular transport	Transports intracellular cargos towards the minus end of microtubules.	(Chen et al., 2014)
A.F>C:IgM	HWRYSPY	Rho GTPase- activating protein 32, ARHGAP32, HYRYSPY	Intracellular	Signaling, energy metabolism	Dysregulation of Rho GTPases, regulated by ARHGAP32, has been implicated in conditions like Alzheimer's disease.	(Aguilar et al., 2017)
F.ND>C:IgG F>C:IgG	TALGSRG	Sigma non-opioid intracellular receptor 1 TALGSRG	Intracellular	Calcium signaling	During healthy aging, the density of σ1R increases, but in diseases like Alzheimer's, there appears to be a reduction in σ1R expression.	(Prasanth et al., 2021)

A.F>C:IgM A.F>ND.C:IgM	GSGPCCS	Tumor necrosis factor receptor superfamily SGPCCS	Membrane	TNF signaling	For example, Death receptor 6 (DR6, TNFRSF21) has been implicated in Alzheimer's disease pathogenesis. It interacts with the amyloid precursor protein APP.	(Ren et al., 2022)
A.F>C:IgM A>C:IgM	HYTCPQC	Zinc finger protein 214 ZNF231 YTCPEC	Intracellular	Regulation of transcription	Brain-specific gene that is implicated in the neurodegenerative disease MSA.	(Hashida et al., 1998)
A.F>C:IgM A.F>ND.C:IgM	RDMEFSN	Leucine-rich repeat-containing protein 66 RDLEFSN	Membrane	Cell adhesion	-	-
F>C:IgG ND.F>C:IgM	AWSTGRT	S arrestin WSTGRT	intracellular	Signaling	Rho signal termination; S-arrestin is immunogenic	(Baldin et al., 2019), (Velez et al., 2016)
C>F.A&ND>A:IgM C>F.A:IgM	GAPKHWL	Signal peptide, CUB and EGF-like domain-containing protein 2, GAPKHWM	Secreted	Signaling	Enhancer of proteolytic truncation for soluble Shh	(Jakobs et al., 2014)
A.F>C:IgM A>C:IgM	MPMSPPS	Transmembrane serine protease 9, LPMSPPS	Membrane	-	TMPRSS9 may induce changes in cell adhesion and contribute to induce cancer cell invasion	(Fontanil et al., 2014)
A.F>C:IgM A.F>ND.C:IgM	HMATHPW	Trinucleotide repeat-containing gene 18 protein TNRC18, HLATHPW	Intracellular	Chromatin regulation	mediate the silencing of ERV class I (ERV1)	(Zhao et al., 2023)
A.F>C:IgM A>C:IgM	HYTCPQC	Zinc finger protein 311, ZNF311, HYGCPQC	Intracellular	-	-	-
A.F>C:IgM A.F>C:IgM A.F>ND.C:IgM A>C:IgM	WDMRSSF HYTCPQC WDMRSSF HYTCPQC	Zinc finger protein 532 DMRSSF, YTCPEC	Intracellular	DNA-binding	Transcriptional activation of the expression ZNF532 is poor biomarker for laryngeal carcinoma progression.	(Fan et al., 2022)
F.ND>C:IgG F>C:IgG	QSDWSSF SDWSSF	zinc finger protein-like 1, ZFPL1	Membrane, Intracellular (different isoforms)	-	structural component of the Golgi apparatus; Identified as a significantly different and down regulated in FTD as compared to controls	(Bronner et al., 2009)
ND.F>C:IgM	DPGLRYS	Sorting nexin-29, SNX29 DPGLRYS	Intracellular	Cell trafficking	Involved in intacellular trafficking of proteins; regulator of myocyte differentiation	(Vieira et al., 2021)
ND.F>C:IgM	GTGLLPT	Protein PRRC1 GTGLLPT	Membrane, Intracellular	Cell trafficking	The cellular function of PRRC1 is unknown; facilitates ER-to-Golgi trafficking of proteins	(Huang et al., 2021)
A>ND:IgG	WRSHPQM	Nuclear cap-binding protein subunit 1, WRNHPQM	Intracellular	Nuclear export	Component of the cap-binding complex (CBC)	(Kim and Taylor, 2017)

A.C>F:IgG	GWCRTPM	Mismatch repair endonuclease PMS2 GWCRTP	Intracellular	Mismatch repair	Part of mismatch repair complex. PMS2 deficiency leads to increased genomic mutation rates.	(van Oers et al., 2010)
A.C>F:IgG	GPRIPPL	Ly6/PLAUR domain-containing protein 3, LYPD3 PRIPPL	Membrane, No brain expression	Focal adhesion	Supports cell migration, associated with metastasis	(Ngora et al., 2012)
A.C>F:IgG	EPVIPRS	general transcription factor 3C, polypeptide 2, TFIIIC110 EPVVPRS	Intracellular	Transcription	Subunit of human TFIIIC complex which is specific for RNA pol III and essential for transcription of tRNA and other type 2 genes.	(Schaffer et al., 2019), (Crepaldi et al., 2013)
A>ND:IgG	SPWSLIN	Acid sphingomyelinase-like phosphodiesterase 3b/ SPWALIN	Membrane	Lipid metabolism	GPI-anchored glycoprotein, negative regulator of TLR and insulin receptor signaling.	(Heinz et al., 2015; Mitrofanova et al., 2019)
A.F>ND.C:IgM	HEEQVMM	Spliceosome RNA helicase DDX39B, HEKQVMM	Intracellular	Nuclear mRNA export	Controls the splicing of FOXP3	(Nagasawa et al., 2024)
A.F>C:IgM	CWYPSCM	Outer dense fiber protein 2, ODF2, CWFPSC	Intracellular	Structural protein	Cytoskeletal structure protein of the sperm tail, attaches to the microtubules	(Donkor et al., 2004)
A.F>C:IgM A.F>ND.C:IgM	SRFVEPP	Dynein axonemal heavy chain 2, SRFIEPP	Intracellular	Cellular movement	Generate force for the movement of microtubules in beating cilia, cell division and intracellular transport	(Schmidt and Carter, 2016)
A.C>F:IgG	SPNWLRL	Kidney mitochondrial carrier protein 1 UCP6/PNWLRL	Intracellular	Mitochondrial energy metabolism	Highly homologous to UCP5 (Brain mitochondrial carrier protein 1). Passes protons through the inner mitochondrial membrane, thus controlling ATP synthesis from ADP	(Gorgoglione et al., 2019)
A.C>F:IgG C>F.A:IgM	GWSYPWV	Proline-rich protein 23E WAYPWV	-	-	-	-
C>F.A:IgM	GRWSDSY	Olfactomedin-like protein 2B, GRWSNSY	Intracellular	-	-	-
A.F>C:IgM	GHCISRP	Maestro heat-like repeat-containing protein family GHCISRP	Intracellular	-	-	-
A.F>C:IgM	GHCISRP	Chymotrypsin-like elastase family preproprotein GHCISR	Secreted	Hydrolysis of proteins,	Expression restricted to pancreas; digestive function protease.	
F.ND>C:IgG F>C:IgG ND.F>C:IgM	MRPFVPC	Cerebral cavernous malformations 2 protein-like, MRPFIP	Intracellular	Signaling	Part of CCM signaling complex	(Whitehead et al., 2009)
A.F>C:IgM A.F>ND.C:IgM	GSGPCCS	Disintegrin and metalloproteinase domain-containing protein 18 GSGPCC	Membrane	Cell-matrix interaction	Possibly involved in spermatogenesis	-

A.F>C:IgM A.F>ND.C:IgM	GSGPCCS	Disintegrin and metalloproteinase domain-containing protein 18, GSGPCC	Membrane	Cell-matrix interaction	-	-
C>ND.F.A:IgM	GEHLLLR	Helicase with zinc finger domain 2, HELZ2, GDHLLLR	Intracellular	Regulation of transcription	Interferon stimulated gene, transcriptional coactivator, degrades mRNA of L1 retrotransposons.	(Luqman-Fatah et al., 2023)
C>F.A:IgM	GLDIKLH	Keratin, type I cytoskeletal 25, LDIKLH	-	-	-	
C>F.A:IgM	GPNTCHC	Multiple epidermal growth factor-like domains protein 11, MEGF11/PDTCHC	Membrane		Involved in neuronal mosaic arrangements. Upregulated in triple negative breast cancer.	(Chiu et al., 2020; Kay et al., 2012)
A.F>ND.C:IgM	HEEQVMM	Plakophilin-1 QEQVMM	Intracellular	Cytoskeleton/ cell membrane interactions	Linker molecule at adherens junctions.	(McGrath et al., 1997)
A.F>C:IgM A>C:IgM	HYTCPQC	Zinc finger protein 497 YTCPEC	-	-	Predicted to be involved in regulation of transcription by RNA polymerase II.	-
A.F>C:IgM A>C:IgM	HYTCPQC	zinc finger protein 500 YTCPEC	Intracellular	-	Elevated in breast cancer cells. Binds and activates p53, thus suppressing the proliferation of breast cancer cells.	(Ma et al., 2023)
A.F>C:IgM A>C:IgM	HYTCPQC	Zinc finger protein 668 YTCPEC	Intracellular	-	Tumor suppressor involved in regulation and stabilization of p53.	(Hu et al., 2011)
A.F>C:IgM A>C:IgM	HYTCPQC	Zinc finger protein 777 YTCPEC	Intracellular	Transcription	Overexpression inhibits cell proliferation	(Yuki et al., 2015)
A.F>C:IgM A>C:IgM	HYTCPQC	Zinc finger protein 835 YTCPEC	Intracellular	Transcription	Differentially expressed in colorectal cancer.	(Jovov et al., 2012)

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Supplementary Figure 1. Pairwise scatter plots of the raw data after between array and compositional bias (dependence on charge, hydrophobicity, etc. general physicochemical properties of the residues) normalization.

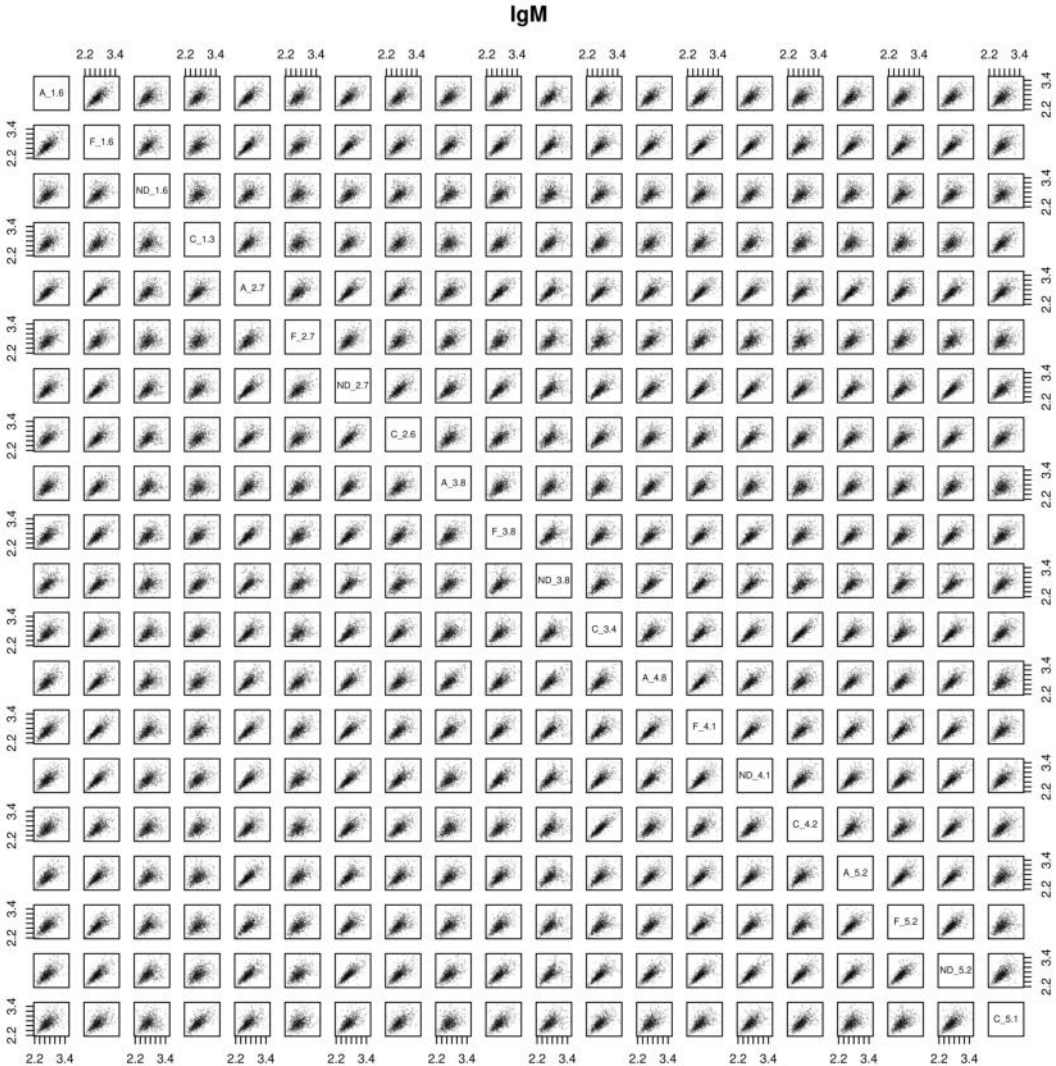
Supplementary Figure 2. Cross-reactivity graph spectral embeddings for the IgM (left) and the IgG (center) graphs. The embedding uses the eigenvectors of the graph Laplacian that correspond to the smallest eigenvalues with a cut off based on irregular increase in the ordered eigenvalues (right) – 4 dimensions for IgM and 5 dimensions for IgG. The exact position of the vertices in the two-dimensional projections are derived by UMAP transformation from 4 (5) dimensions to two dimensions. The vertices are color coded from blue (low) to red (high). The values mapped to them in this way correspond to the levels of reactivity as sums of the ranks of the indicated samples by group (AD, FTD, NDD, and control - C), or to the number of nearest neighbors among the idiotopes. The edges inherit their color from their incident vertices by averaging. A clear separation is observed between the reactivities higher in the C vs those higher in AD or FTD. NDD related reactivities differ from all. The number of idiotope neighbors are more for a cluster of vertices overlapping those with higher reactivity in the controls. IgG pathology associated reactivities seem less clustered.

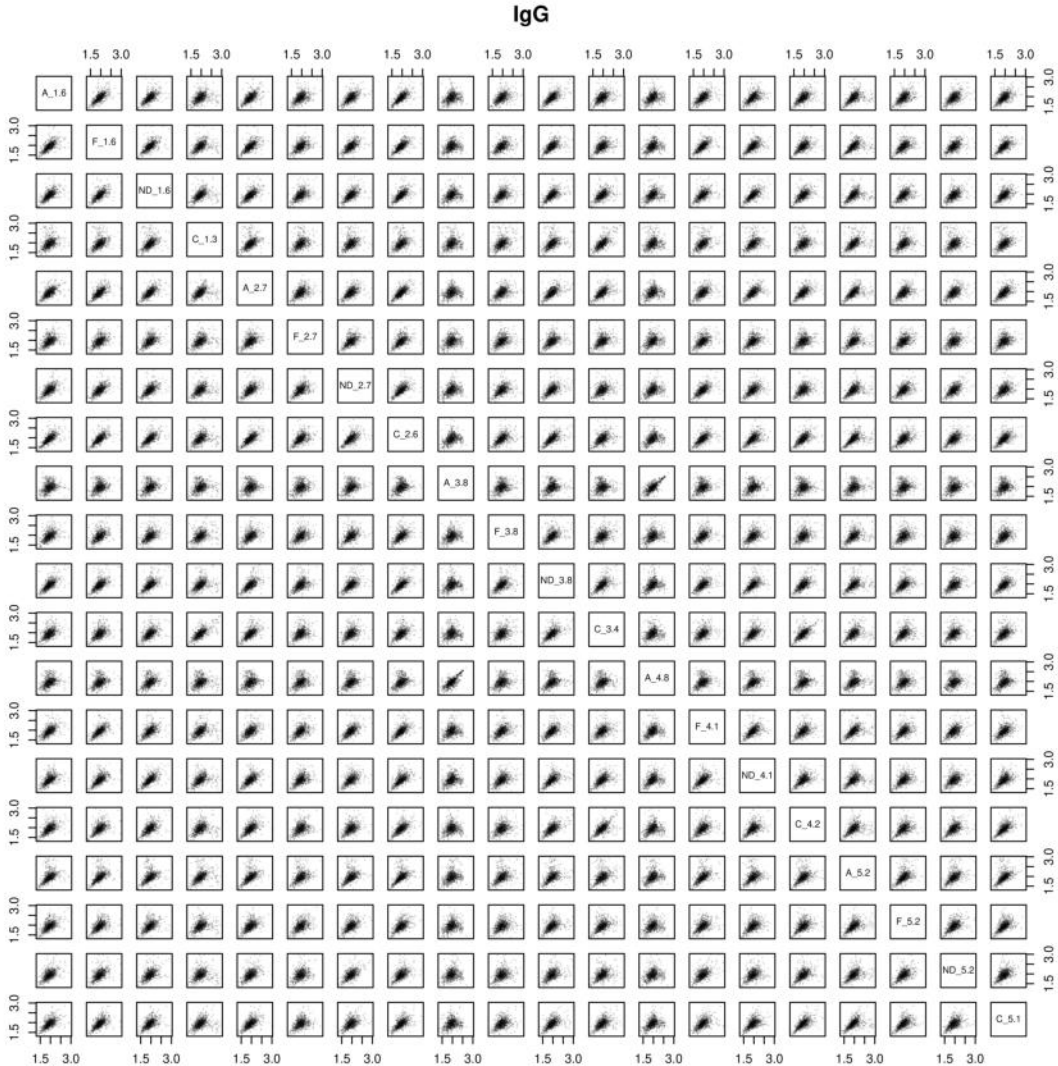
Supplementary Figure 3. Degree distribution for the IgM/IgG graphs compared to the distributions of 100 graphs generated after scrambling the respective raw data matrices. The graphs have degree distribution drawn to the right which corresponds to their higher the density as compared to randomly generated graphs.

Supplementary Figure 4. Maximal clique size distribution for the IgM and IgG graphs compared to the distributions of 100 graphs generated after scrambling the respective raw data matrices. The graphs have the same size but more cliques as compared to randomly generated graphs.

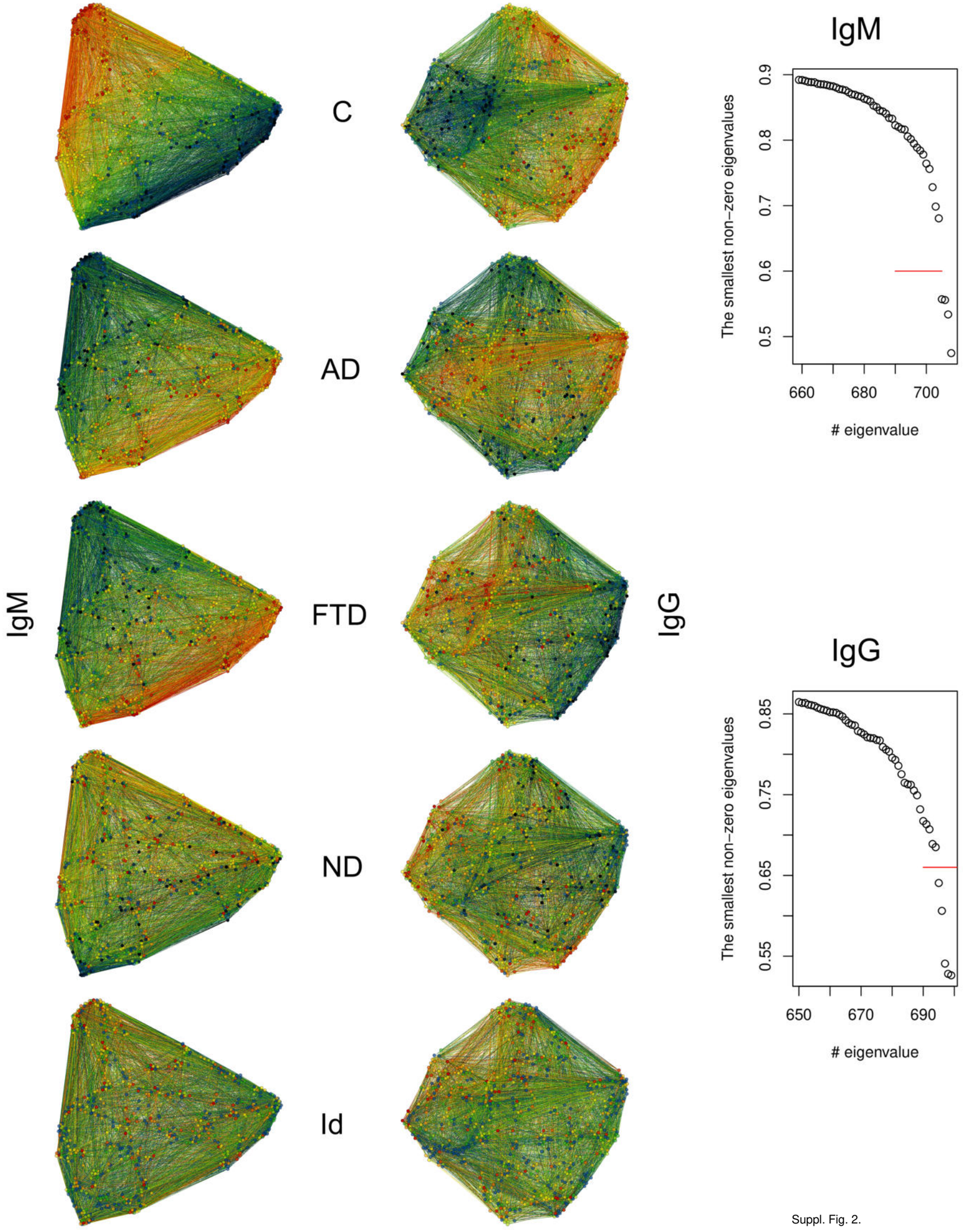
Supplementary Figure 5. Venn diagram of the overlap between the ultimate cluster groups defined by the type of significant changes in the different diagnoses (annotation example: C>F.A ND>A:IgM – the reactivity in the controls is higher than that in FTD and AD plus NDD is only higher than AD, an IgM set clusters). The numbers indicate the number of mimotopes in this section. Some overlaps are between opposing characteristics, respectively from the IgM and the IgG sets (e.g. – some reactivities higher in FTD and NDD relative to the controls in IgG are found also in the group with the opposite ratios in IgM).

The supplementary files IgM_AD_FTD_NDD_C.mp4 and IgG_AD_FTD_NDD_C.mp4 visualize 3D embedding of the files in motion. The color code is mapping the rank sums by diagnosis AD, FTD and NDD scaled respectively as the red, green and blue components of the color while the luminosity codes for the reciprocal of the rank sum of the controls. Thus, the darker or black vertices correspond to reactivities found high in the controls but lost in the neurodegenerative diseases. In the IgM graph a considerable portion of the vertices with very light colors (reactivities not found in the controls) are colored red and green yielding a spectrum of predominantly yellow hues – the overlap of AD and FTD associated reactivities. In the IgG graph AD and FTD reactivities are separated in bright red and green clusters.

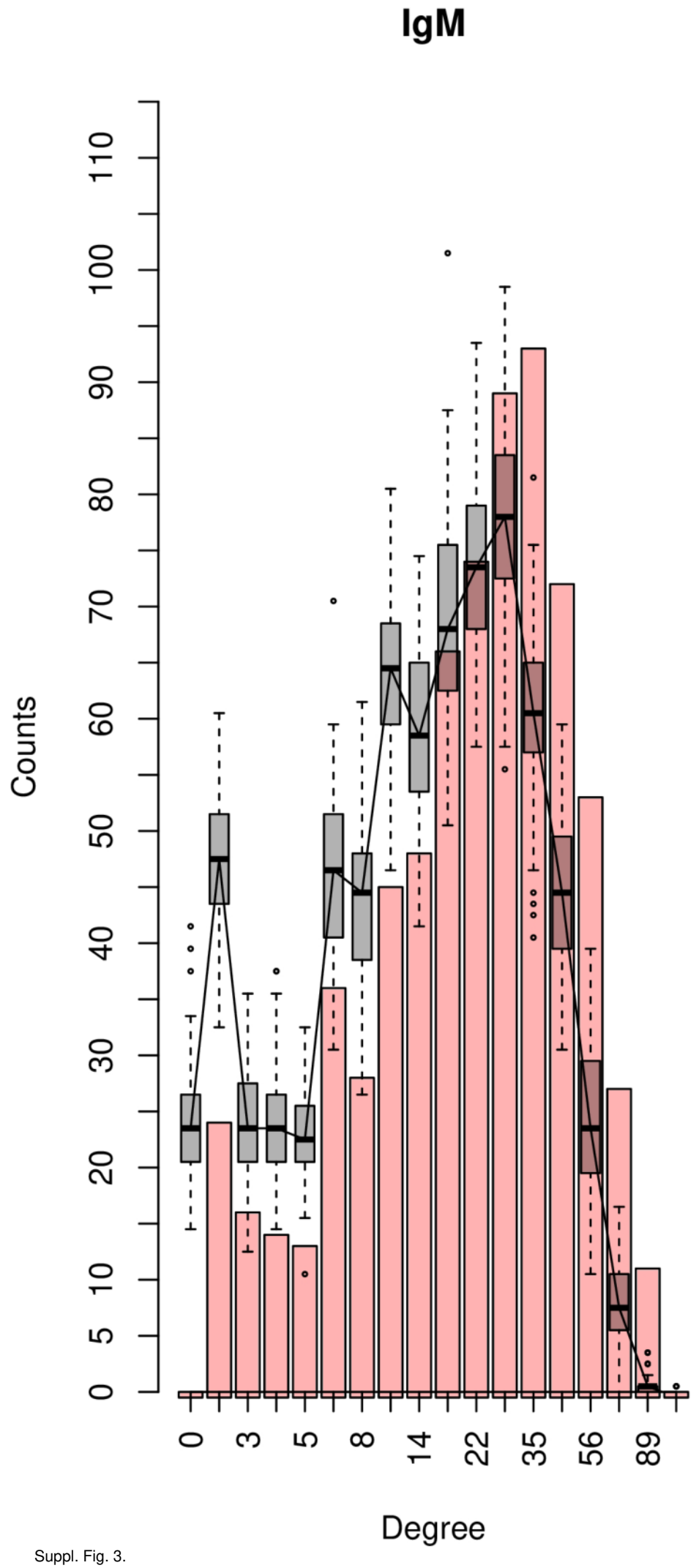

IgM
A_1.6
F_1.6
ND_1.6
C_1.3
A_2.7
F_2.7
ND_2.7
C_2.6
A_3.8
F_3.8
ND_3.8
C_3.4
A_4.8
F_4.1
ND_4.1
C_4.2
A_5.2
F_5.2
ND_5.2
C_5.1
2.2 3.4

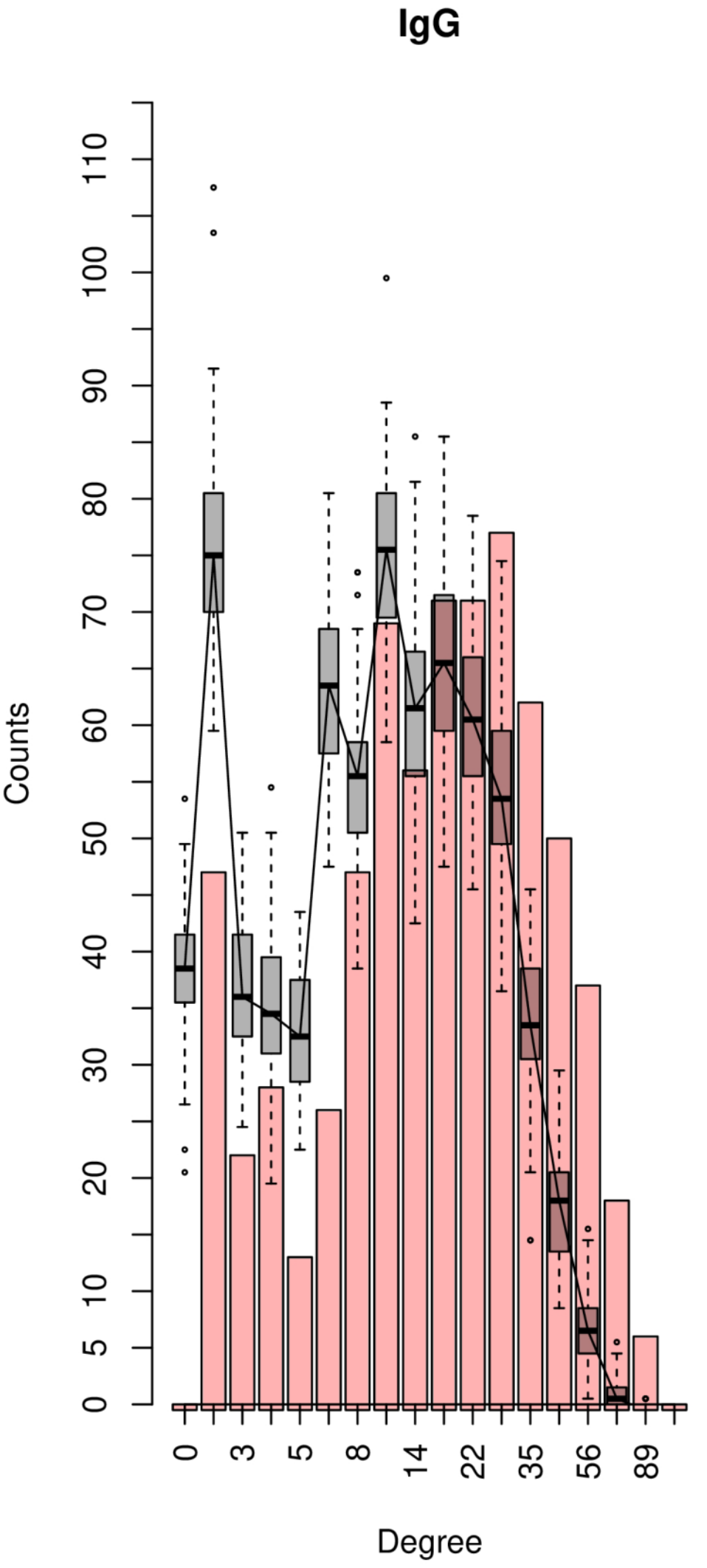

IgG
A_1.6
F_1.6
ND_1.6
C_1.3
A_2.7
F_2.7
ND_2.7
C_2.6
A_3.8
F_3.8
ND_3.8
C_3.4
A_4.8
F_4.1
ND_4.1
C_4.2
A_5.2
F_5.2
ND_5.2
C_5.1
1.5 3.0

Suppl. Fig.1.

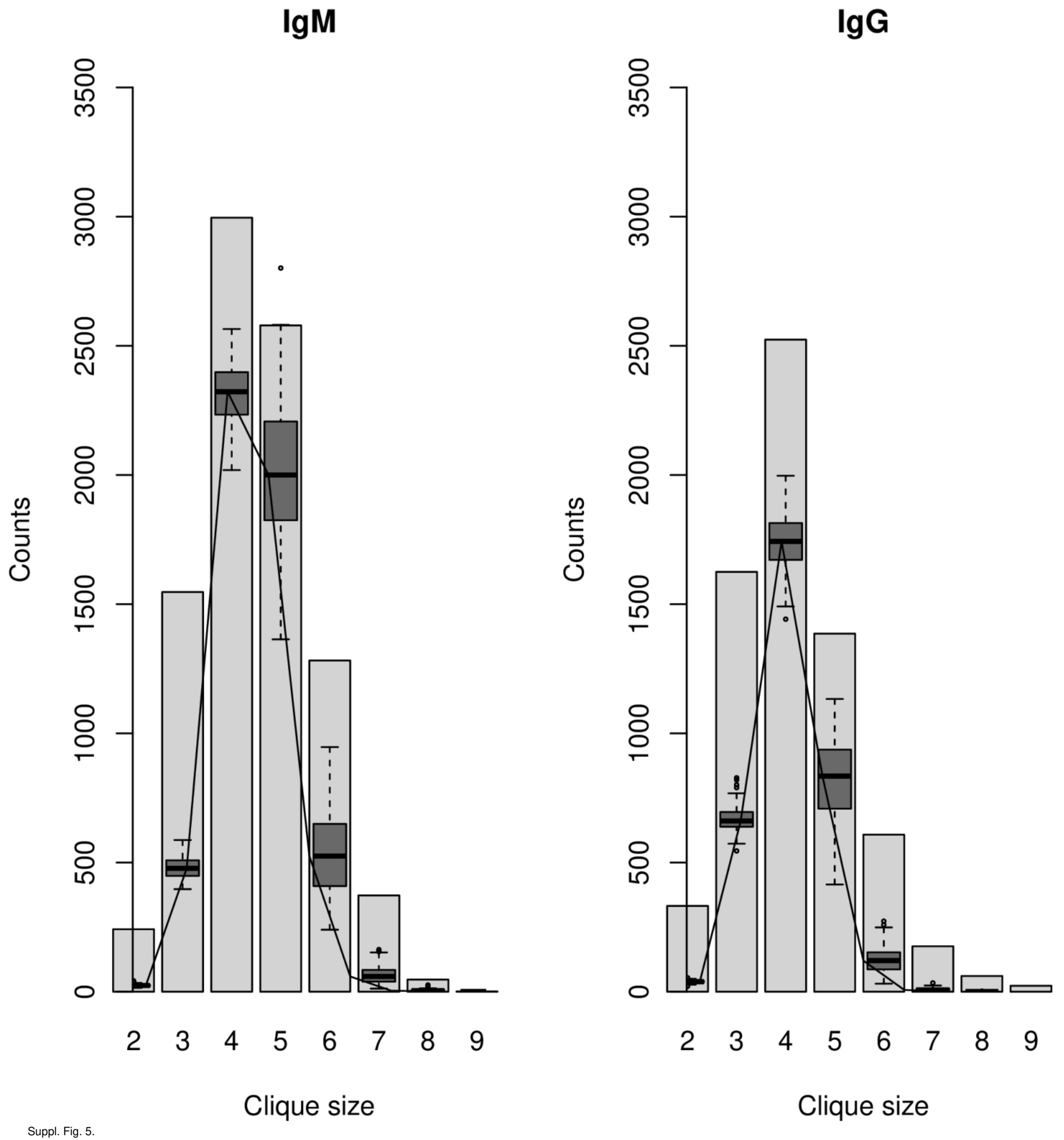

IgM
C
AD
FTD
ND
Id
IgG
IgM
The smallest non−zero eigenvalues
0.9
0.8
0.7
0.6
0.5
660
680
700
eigenvalue
IgG
The smallest non−zero eigenvalues
0.85
0.75
0.65
0.55
650
670
690
eigenvalue

Suppl. Fig. 2.

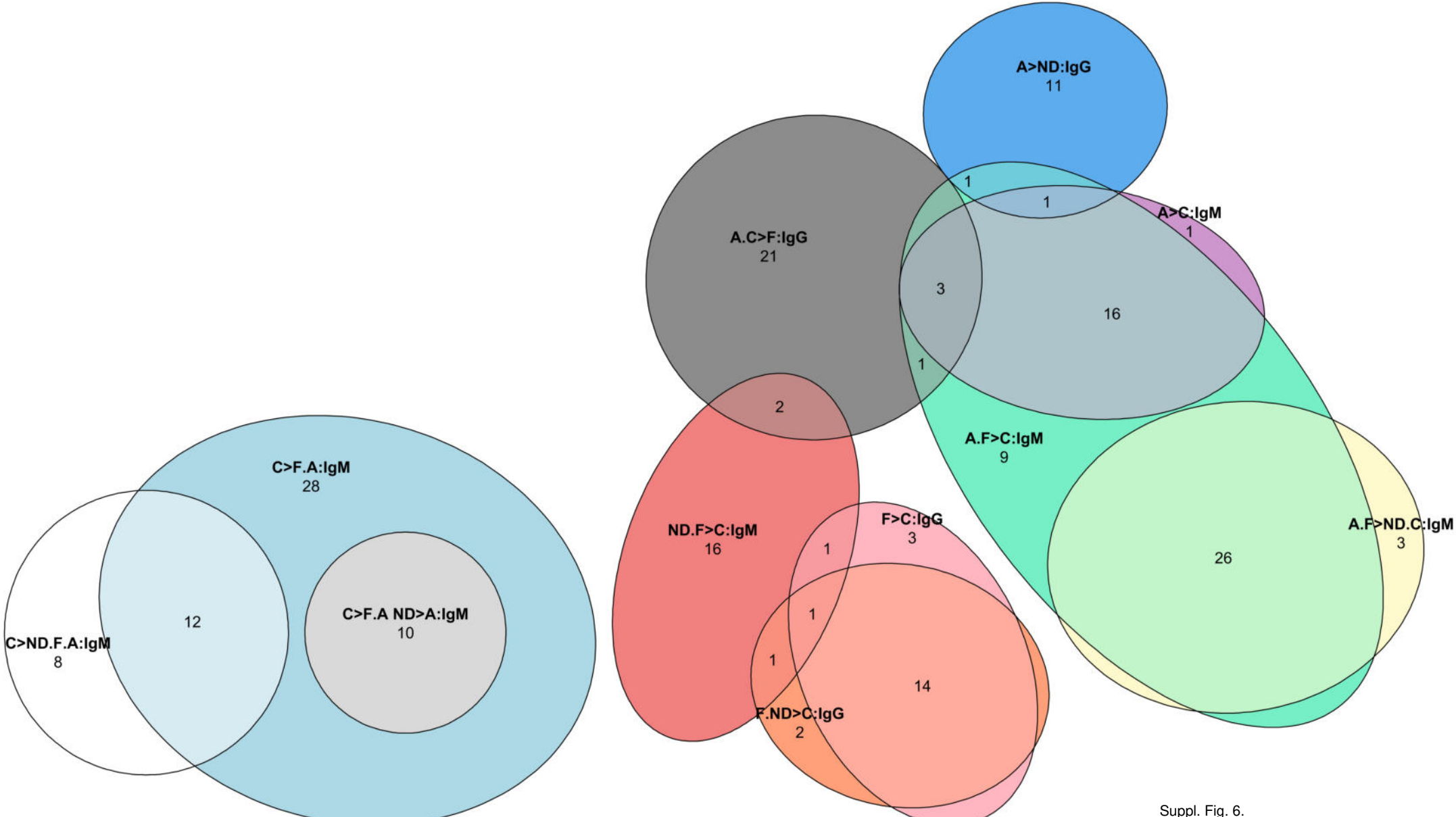

IgM
Counts
0
5
10
20
30
40
50
60
70
80
90
100
110
0
3
5
8
14
22
35
56
89
Degree


IgG
Counts
0
5
10
20
30
40
50
60
70
80
90
100
110
0
3
5
8
14
22
35
56
89
Degree

Suppl. Fig. 3.


IgM
IgG
Counts
0
500
1000
1500
2000
2500
3000
3500
2
3
4
5
6
7
8
9
Clique size

Suppl. Fig. 5.


C>F.A:IgM
28
C>ND.F.A:IgM
8
12
C>F.A ND>A:IgM
10
A>ND:IgG
11
A.C>F:IgG
21
A>C:IgM
1
1
1
3
16
1
2
A.F>C:IgM
9
ND.F>C:IgM
16
F>C:IgG
3
A.F>ND.C:IgM
3
26
1
1
1
14
F.ND>C:IgG
2

Suppl. Fig. 6.