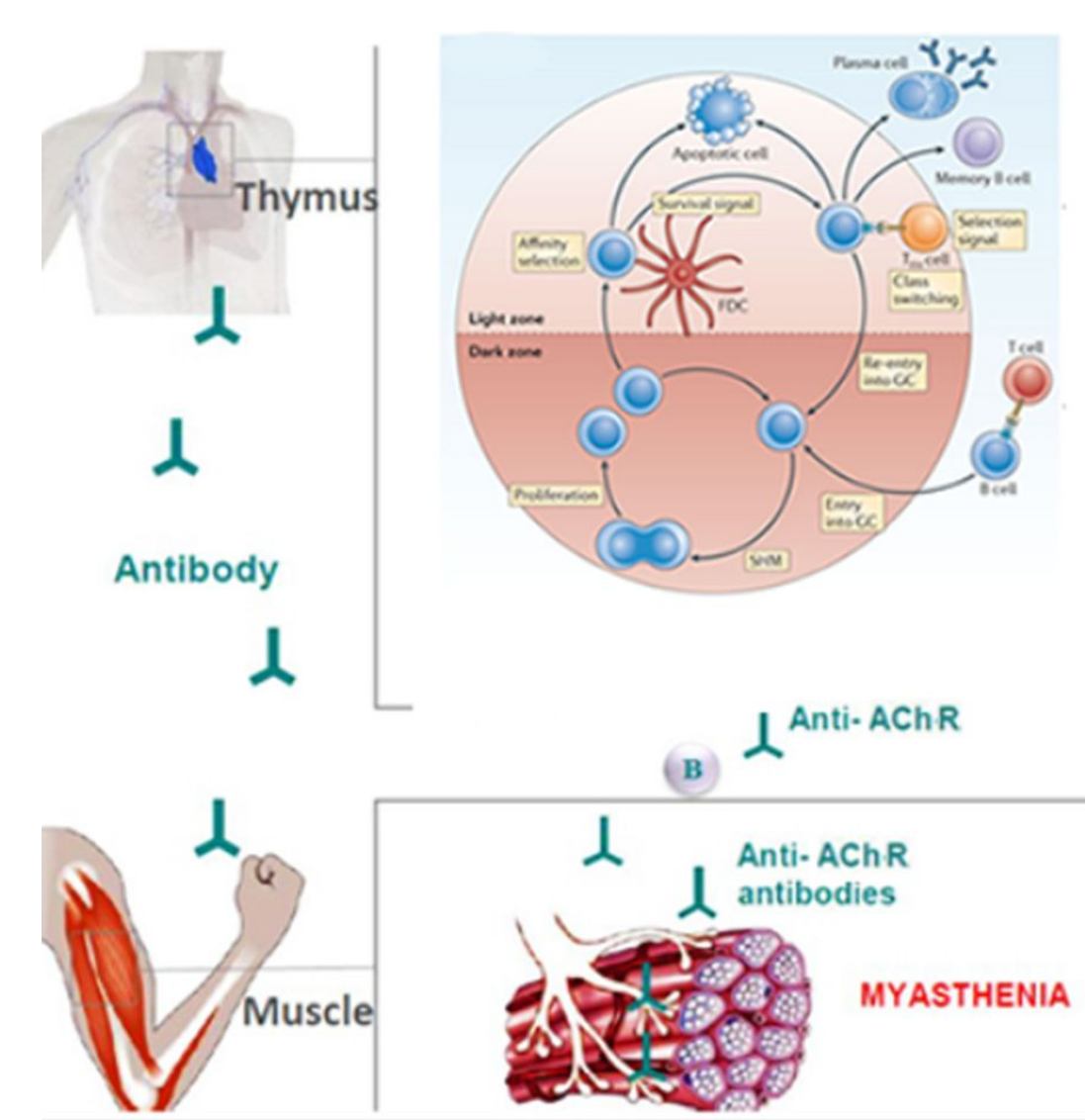


**BACKGROUND:** Myasthenia gravis (MG) is an autoimmune neuromuscular disorder caused by antibodies directed against proteins present at the post-synaptic surface of neuromuscular junction (NMJ). A characteristic pathology of patients with early onset MG is thymic hyperplasia with ectopic germinal centers (GC). However, mechanisms that trigger and maintain thymic hyperplasia are poorly characterized. Micro-RNAs (miRNA) are small, non-coding RNAs that are increasingly appreciated to be involved in the pathology of several autoimmune diseases. In order to determine the central mechanisms involved in the pathology, thymus samples from MG patients were assessed by histology and grouped based on appearance of GC compared to samples without them. MiRNA and mRNA profiles were evaluated.

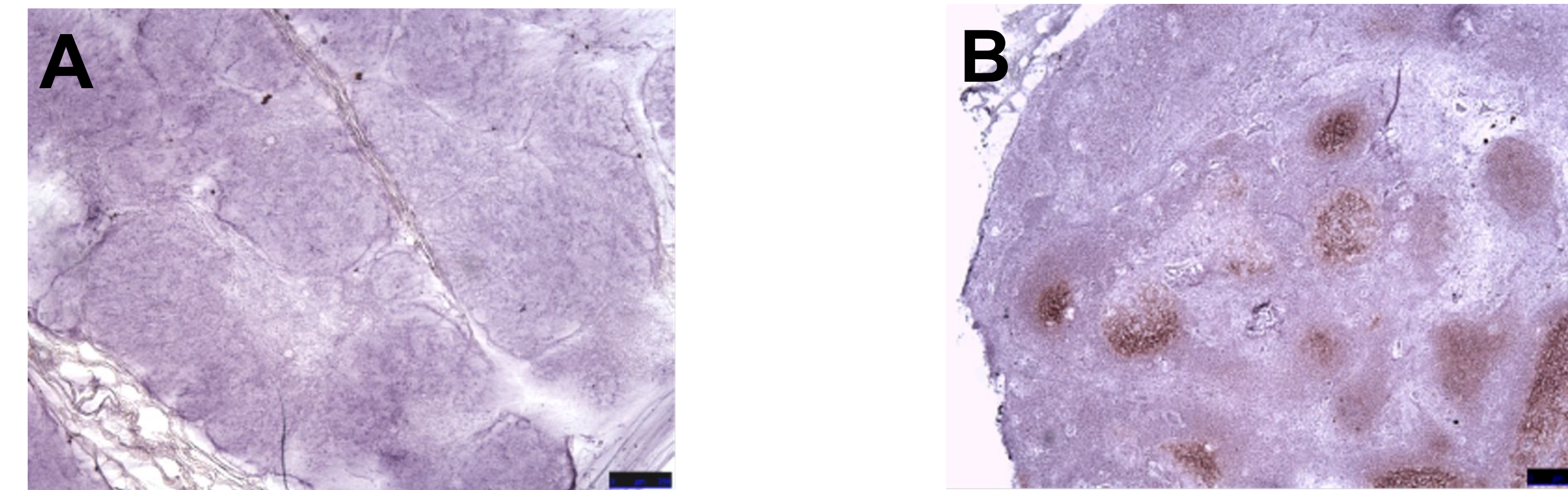
### Fig.1 Germinal center activity in MG thymus



Adapted from B. A. Heesters et. al., 2014, Nature Reviews Immunology

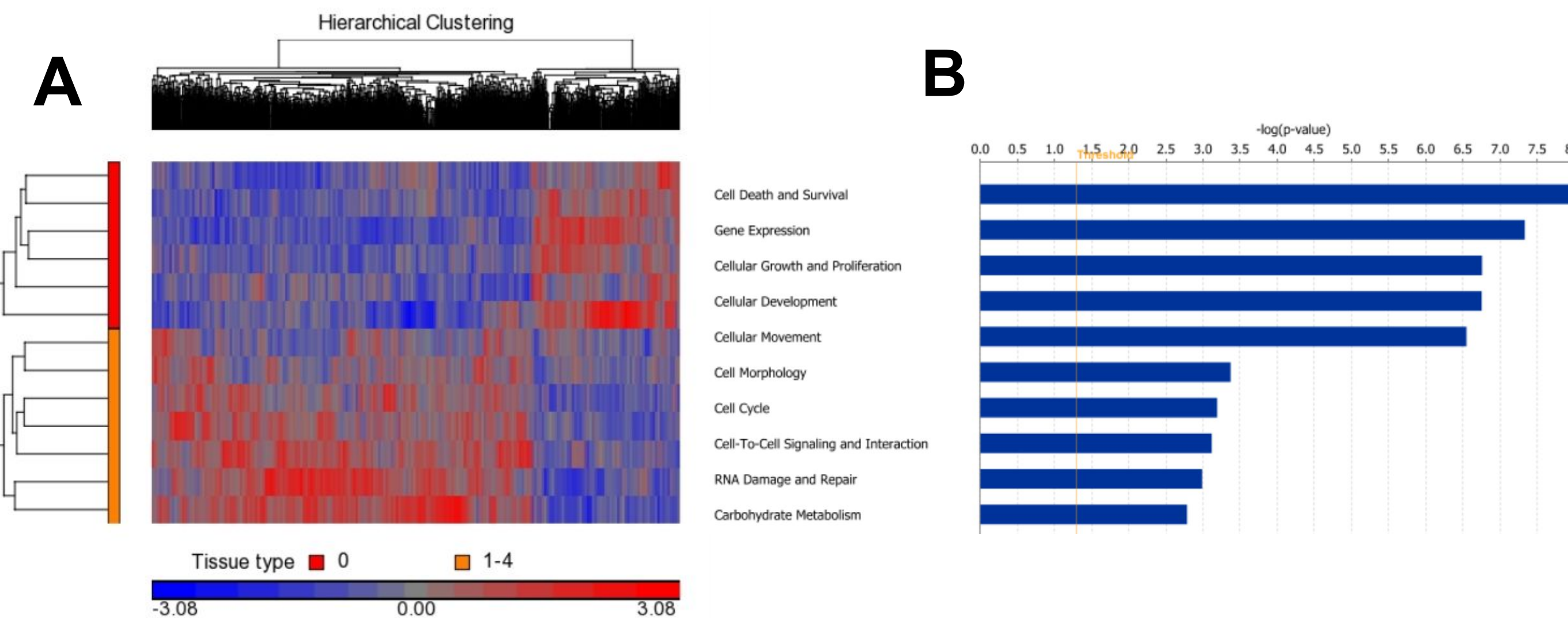
**METHODS:** Thymic specimens collected during the course of the NIH-supported study of thymectomy (MGTX, U01 NS4268) were used for histological analysis to grade the degree of thymic hyperplasia, grade 0, no observed GC and grade 1 to 4 with varying numbers of GCs. MiRNA and mRNA were evaluated using GeneChip® miRNA 4.0 Array and GeneChip® Human Transcriptome Array 2.0, respectively. Partek Genomic Suite 6.6 and Transcriptome Analysis Console 2.0 programs were used to identify candidates that were differentially expressed in grade 0 versus grades 1 to 4. ANOVA p-value <0.05 and FDR<0.05 was determined as significant. Further validation by qRT-PCR was done. Ingenuity Pathway Analysis was used to identify miRNA targets, pathways involved and reciprocal expression pairing of miRNA-mRNA. IHC was performed to check localization of proteins.

### Fig.2 Gradation of thymus samples



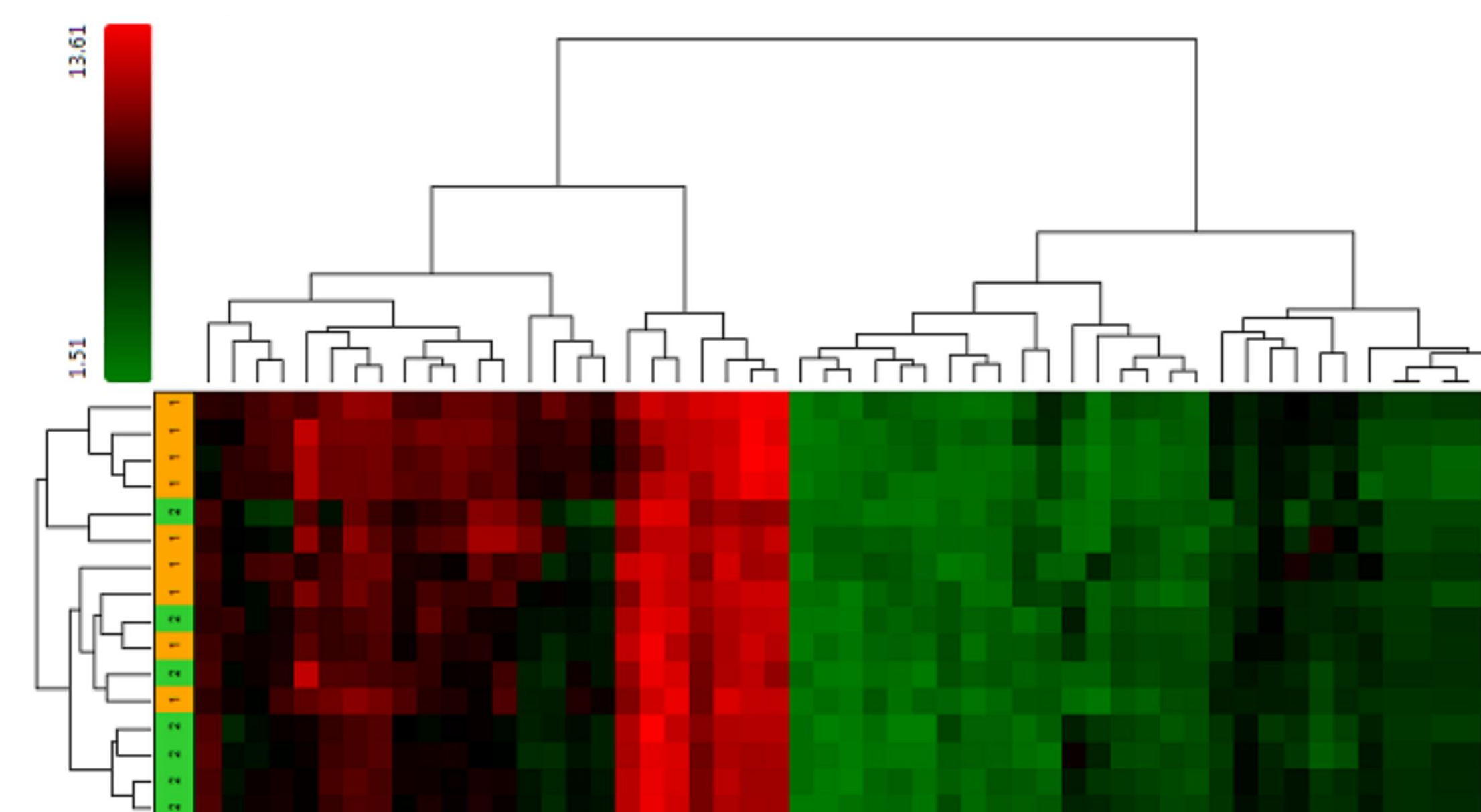
Immunohistochemistry of thymus samples using anti-human CD23 antibody and HRP conjugated secondary. A) Grade 0 sample with no GC B) Grade 4 sample with several GCs.

### Fig.3 Differential mRNA expression in MG thymus



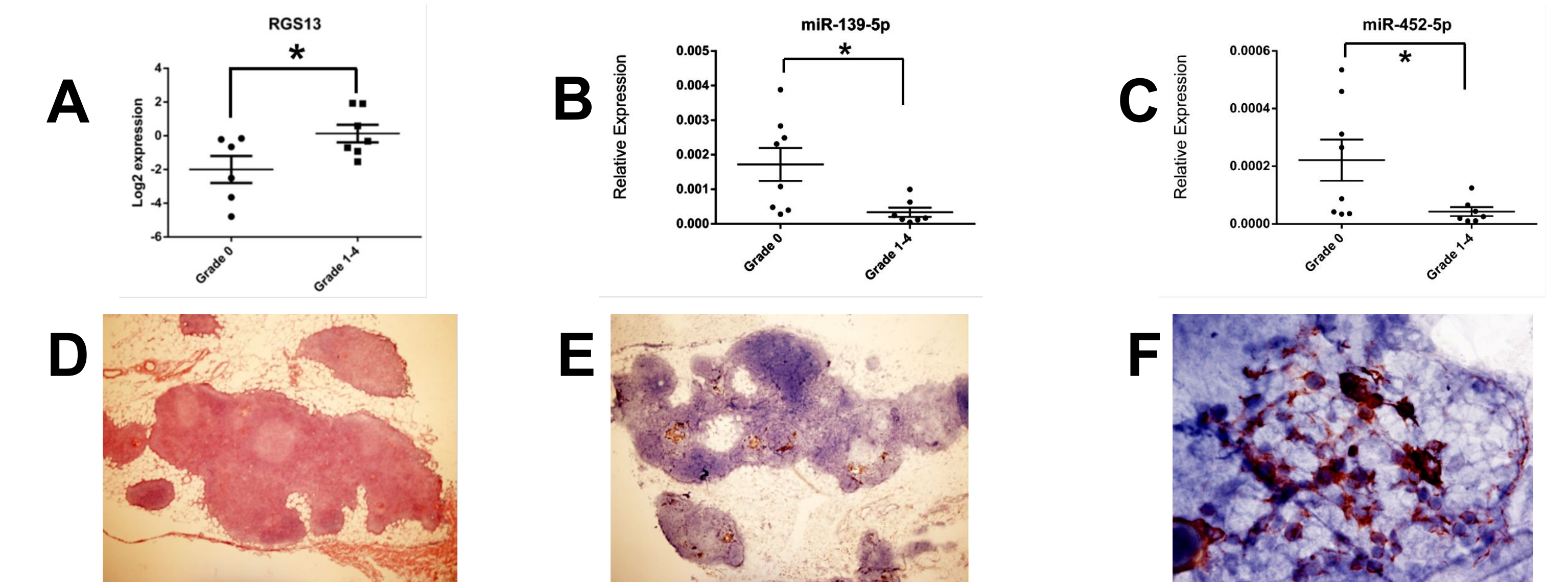
A) Hierarchical Clustering of significant genes in grade 0 vs. 1-4 (p<0.05, no fold change). B) Cellular and molecular functions associated.

### Fig.4 Cluster analysis of small RNA expression profile stratifying samples across the grades



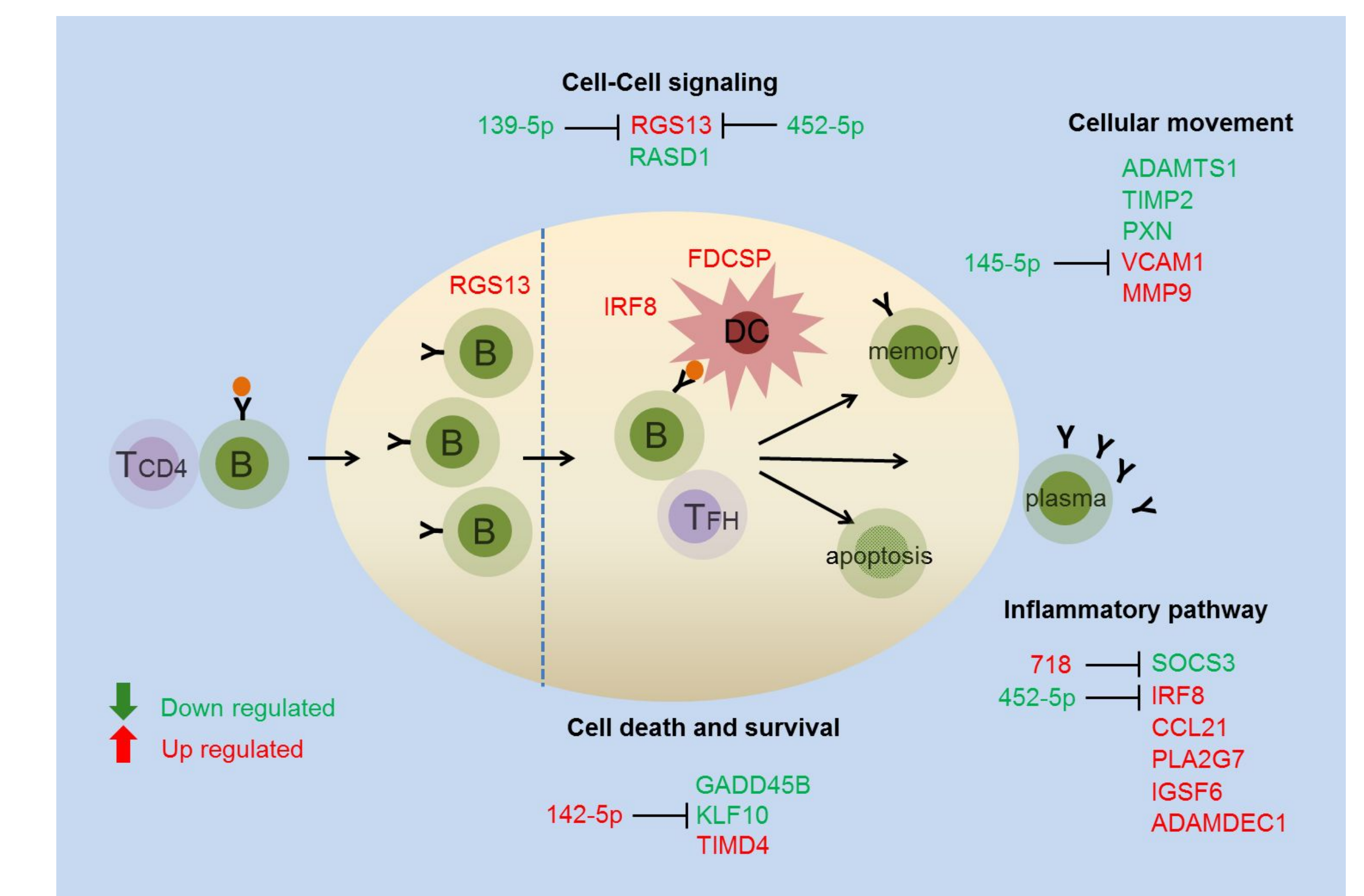
Differentially expressed small RNAs showing greater than 1.5 fold change in expression between the grades. ANOVA p<0.05

### Fig.5 Reciprocal Pairing of miRNA and mRNA



Ingenuity Pathway Analysis predicted 7 miRNA-mRNA pairs that were validated by qPCR. Increase in RGS13 (A) accompanied by decrease in miR 139-5p (B) and miR 452-5p (C) in grades 1-4. Immunohistochemistry of thymus samples using RGS13 antibody confirms its expression in GC. H and E staining 4x (D) anti RGS13-HRP 4x (E) and at 60X (F).

### Fig.6 mRNA and miRNA regulating cellular functions in germinal center formation



### CONCLUSIONS:

- ❖ Grade 0 and grade 1-4 patients have distinct mRNA and miRNA profile demonstrating separation of groups.
- ❖ Deregulation of 100 annotated mRNA and 34 mature miRNA.
- ❖ Reciprocal expression pairing of miRNA and mRNA observed.
- ❖ Maintenance of autoimmunity is supported by regulatory pathways known to be involved in neoplasia