

Figure Legends

Figure S1. Differentially expressed genes in PBMCs from controls and alpha-gal sensitized but asymptomatic participants. Volcano plots show $-\log_{10}(\text{p-value})$ and $\log_2(\text{fold change})$ in gene expression for sensitized but asymptomatic ("C.S.") participants compared to control. Highly statistically significant genes are at the top of the plot above the horizontal lines which represent p-value thresholds adjusted using the Benjamini-Hochberg method of estimating false discovery rates (FDR). Highly differentially expressed genes fall to the left and right. There are 80 genes where adjusted p-value for differential gene expression is $p < 0.05$ and 45 genes where adjusted p-value is $p < 0.02$. The 40 most statistically significant genes are labeled in the plot.

Figure S2. Differentially expressed genes common to both AGS and alpha-gal sensitized but asymptomatic subjects compared to controls. Panel (A) shows the 53 differentially expressed genes (DEG) with adjusted p-value < 0.02 in AGS vs. Control. Thirty-seven genes were upregulated (green) and 16 genes were downregulated (purple) in AGS compared to control. Panel (B) shows the 45 genes with adjusted p-value < 0.02 in sensitized but asymptomatic (C/S) compared to control. Thirty-one genes were upregulated (green) and 14 downregulated (purple). The 21 upregulated genes common to AGS and C/S vs. Controls are boxed in red; the 7 common downregulated genes are boxed in blue. (C) Venn diagram depicting differentially expressed genes common to both AGS and C/S when each group is compared to controls. Red represents upregulation and blue, downregulation.

Figure S3. Cell type profiling across all samples. Adaptive (B and T) lymphocyte populations (circled in red) are abundant in PBMC preparations from study participants. Nanostring nSolver Software uses pre-designated immune cell type transcriptional signatures within the nCounter expression panel to quantify the relative abundance of the 11 different immune cell types listed across the X-axis in the figure. In this barplot of p-values across cell types, p-values are $-\log_{10}$ transformed. Bars above the solid black line indicate statistically significant cell types at an alpha level of 0.01. Bars above the dashed line indicate statistically significant cell types at an alpha level of 0.001.

Figure S4. Circulating, activated CD1d-restricted iNKT cells are detectable in both control and alpha-gal allergic subjects. (A) Representative flow cytometry plots and (B) Summary graphs of CD3⁺hCD1dtetramer⁺iNKT cell frequency (C) CD3⁺ hCD1dtetramer negative non-iNKT T cell frequency (D) Frequency of CD69⁺ activated cells among iNKT cells and (E) Frequency of CD69⁺ activated cells among CD3⁺non-iNKT T cells detected in PBMCs from control and alpha-gal allergic subjects. Invariant natural killer T cells were identified using anti-CD3 and human CD1d tetramers loaded with the alpha-galactosylceramide analog PBS57 ("hCD1dtetramer"). p-values generated using the Mann-Whitney test with $p < 0.05$ considered significant. AGS = Alpha-gal syndrome.

Figure 1. Study Participant Characteristics for Multiplex Gene Expression Analysis Cohort. (A) Age. (B) Sex. (C) Self-reported race. (D) Symptom distribution. (E) Serum alpha-gal-specific IgE. (F) Serum total IgE. Control (n=7); Sensitized but asymptomatic ("Control/Sens," n=2); Alpha-gal syndrome ("AGS" n=15). P values were generated using an unpaired t test comparing AGS to control, with $p < 0.05$ considered significant. Due to the small sample size, the sensitized but asymptomatic group was excluded from statistical analysis.

Figure 2. Differentially expressed genes in PBMCs from alpha-gal allergic participants compared to controls. (A) Volcano plots show $-\log_{10}(\text{p-value})$ and \log_2 fold change in gene expression for AGS

participants compared to control. Highly statistically significant genes are at the top of the plot above the horizontal lines which represent p-value thresholds adjusted using the Benjamini-Hochberg method of estimating false discovery rates (FDR). Highly differentially expressed genes fall to the left and right. There are 81 genes where adjusted p-value for differential gene expression is $p < 0.05$ and 53 genes where adjusted p-value is $p < 0.02$. The 40 most statistically significant genes are labeled in the plot. (B) Graphic lists the 53 genes with adjusted p-value < 0.02 . Thirty-seven gene transcripts highlighted in green were upregulated and 16 gene transcripts highlighted in purple were downregulated in AGS compared to control.

Figure 3. Normalized expression levels of selected immune response-related genes upregulated in PBMCs from AGS subjects compared to controls. (A) Genes involved in transcriptional regulation and/or the NFkappaB pathway. (B) Genes associated with antigen presentation and MHC II surface expression. (C) Genes associated with type 2 / Th2 allergic immune responses. (D) Genes associated with itch and allergic dermatitis. P-values were generated using an unpaired t test comparing AGS to controls, with $p < 0.05$ considered significant. Due to the small sample size, the sensitized but asymptomatic ("C/S") group was excluded from statistical analysis. AGS = alpha-gal syndrome.

Figure 4. Transcriptional immune profiles for B and T cell function from control and alpha-gal sensitized subjects are largely distinct. Heatmap of the normalized gene expression data and unsupervised hierarchical clustering of genes associated with (A) T cell function and (B) B-cell function. (C) Principal component analysis of gene expression data associated with T cell function and (D) B cell function. Control (n=7); Sensitized ("CS" n=2); Alpha-gal syndrome ("AGS" n=15).

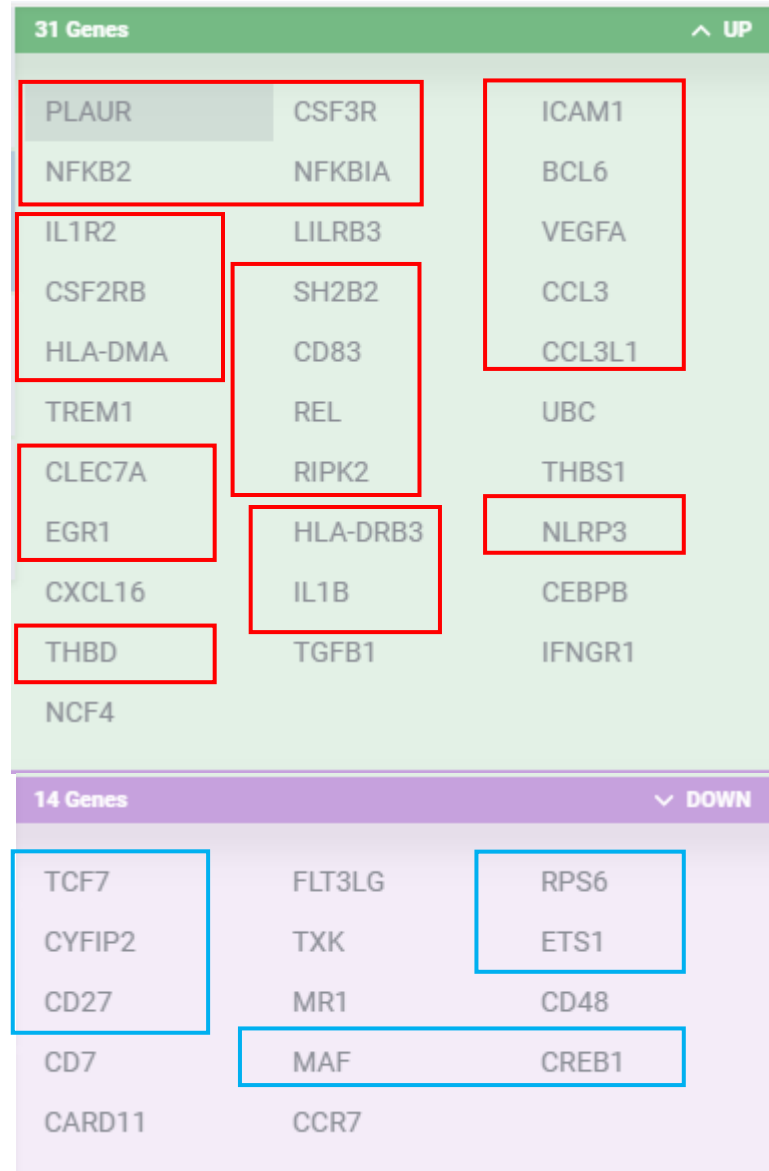
Figure 5. Normalized expression levels of select genes associated with iNKT cell development and effector function in bulk PBMCs from control, alpha-gal sensitized but asymptomatic, and AGS subjects. P-values were generated using an unpaired t test comparing AGS to controls, with $p < 0.05$ considered statistically significant. Due to the small sample size, the sensitized but asymptomatic group was excluded from statistical analysis.

Figure 6. Normalized expression levels of select genes associated with B cell function among control, alpha-gal sensitized but asymptomatic, and AGS subjects. P-values were generated using an unpaired t test comparing control to AGS, with $p < 0.05$ considered statistically significant. Due to the small sample size, the sensitized but asymptomatic group was excluded from statistical analysis.

A Alpha-gal Syndrome (AGS) vs Control



B Sensitized but asymptomatic (C/S) vs Control



C

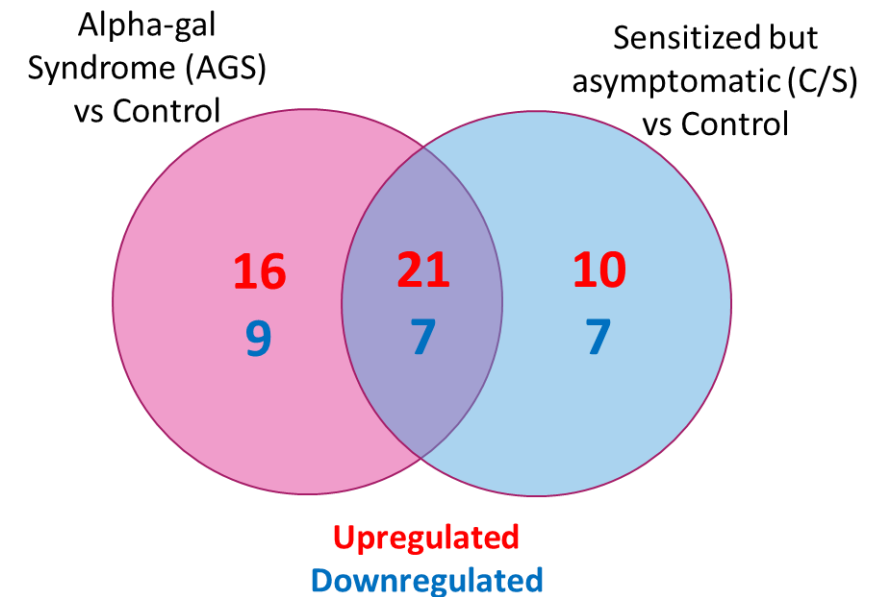


Figure S2.

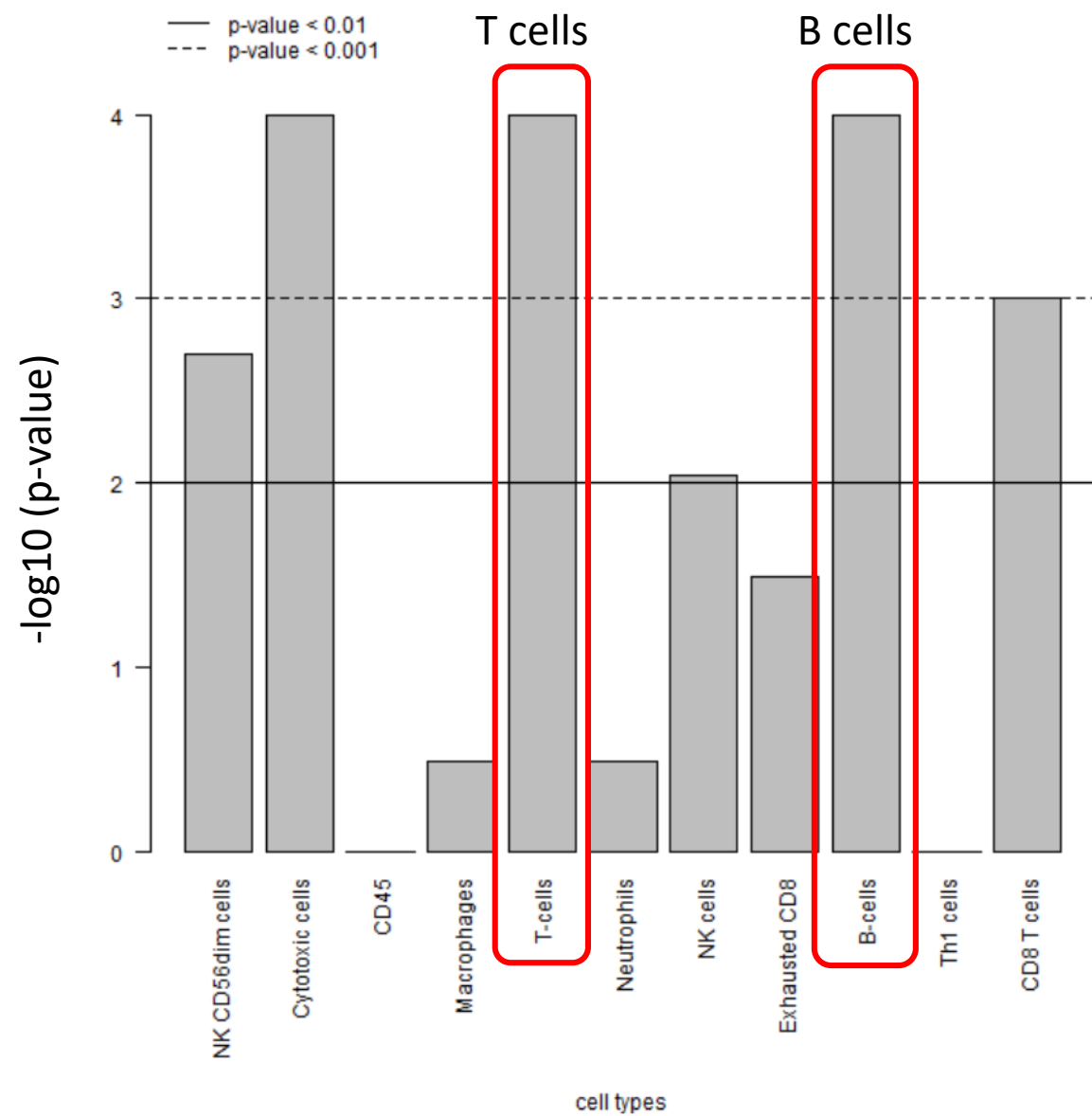
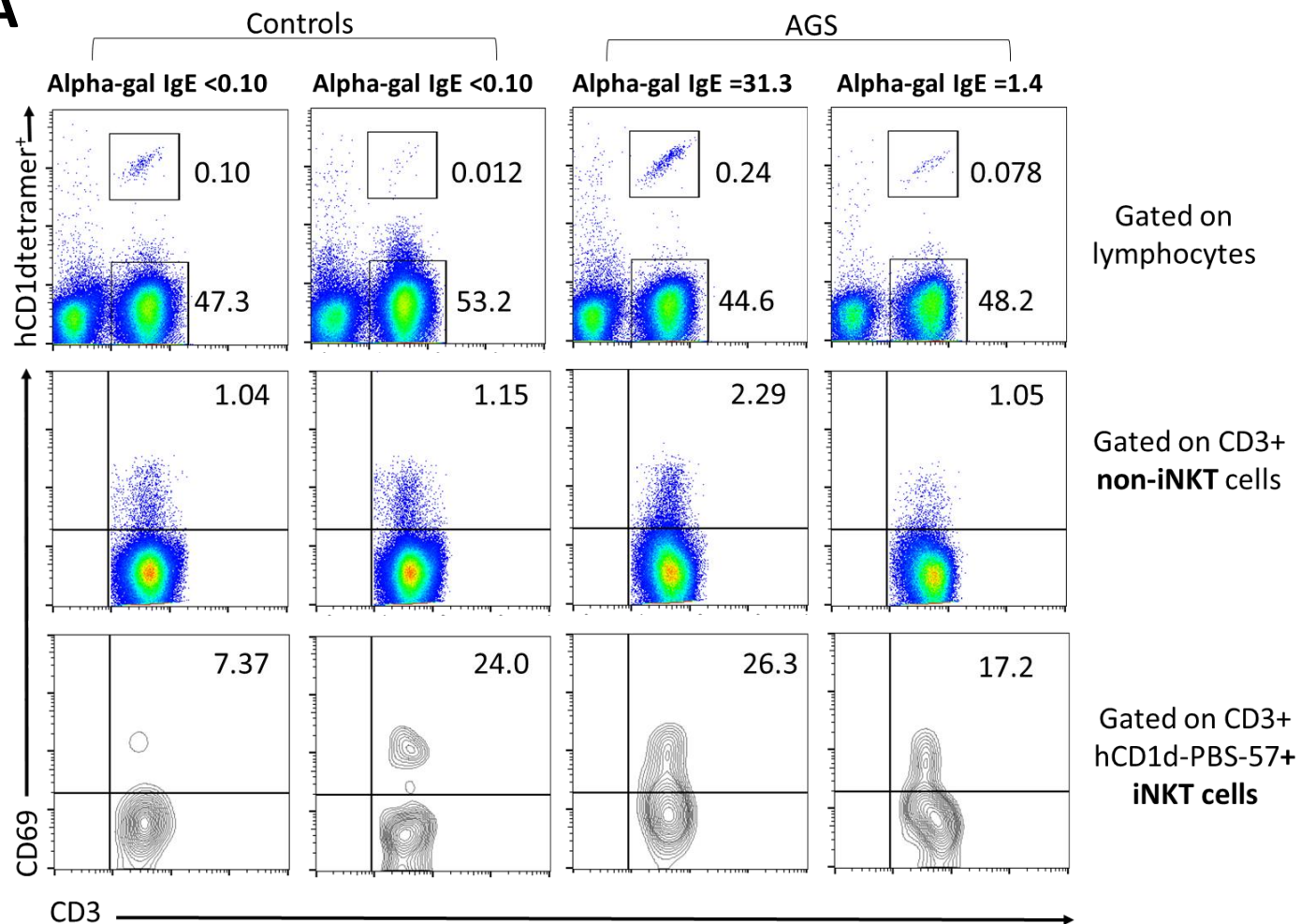
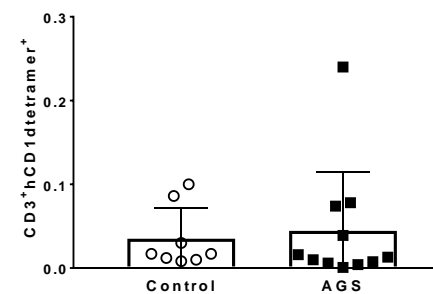
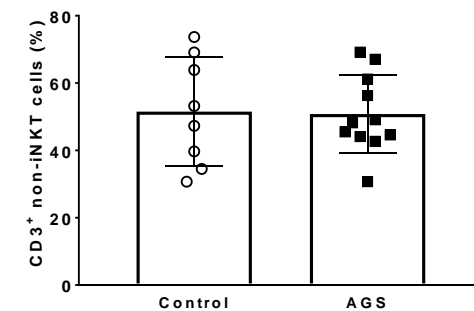
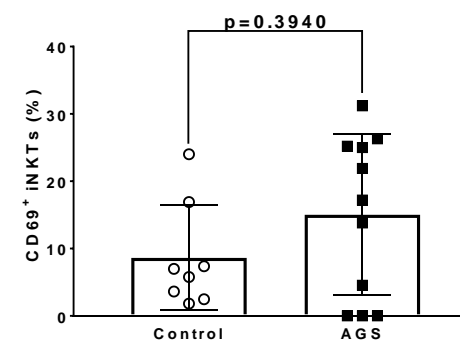
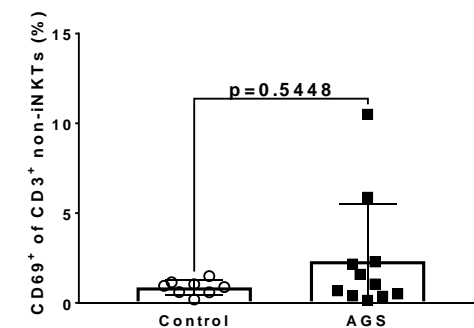
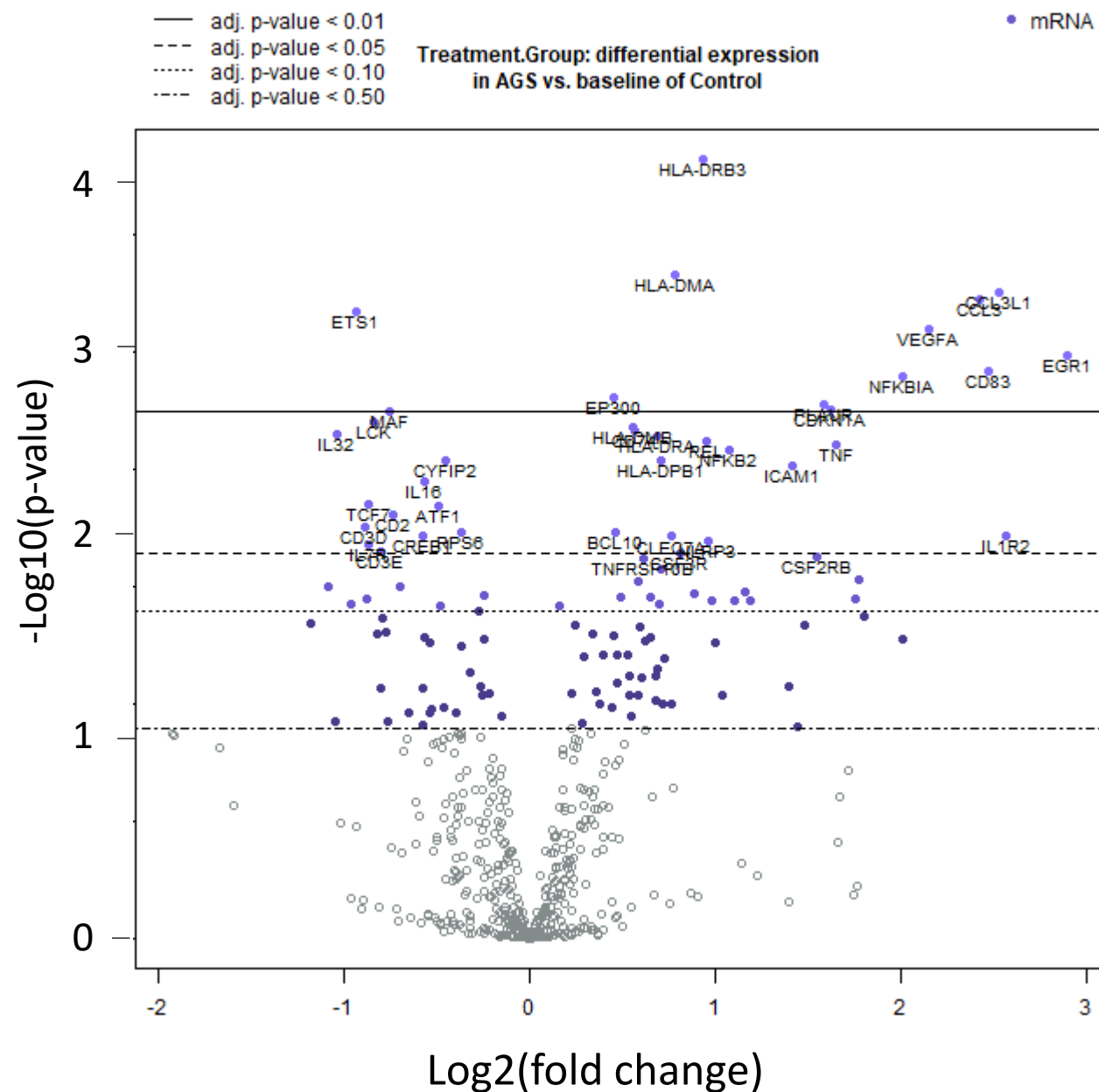


Figure S3.

A**B****C****D****E****Figure S4.**

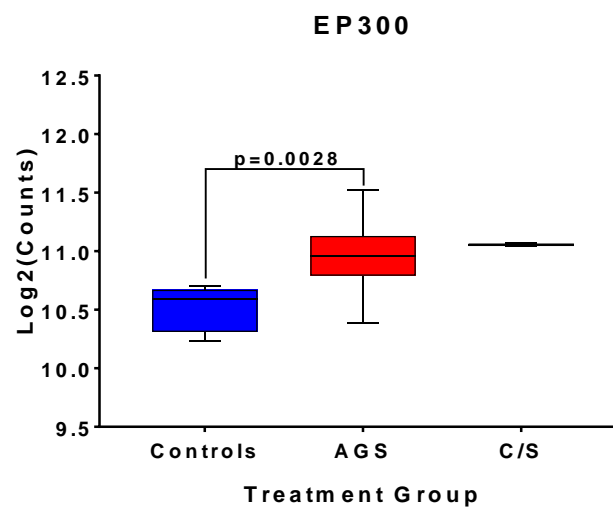
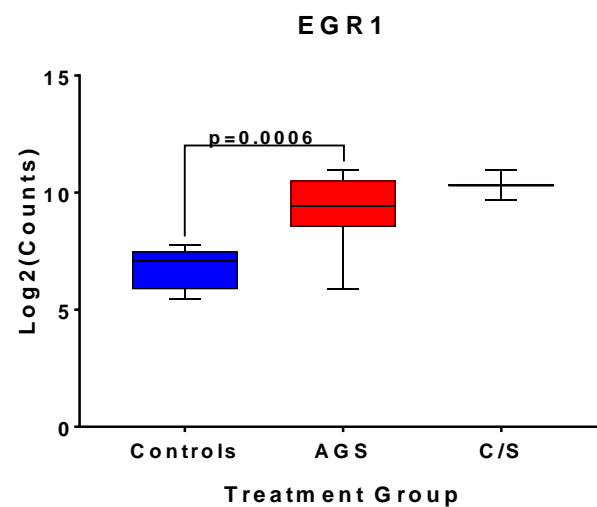
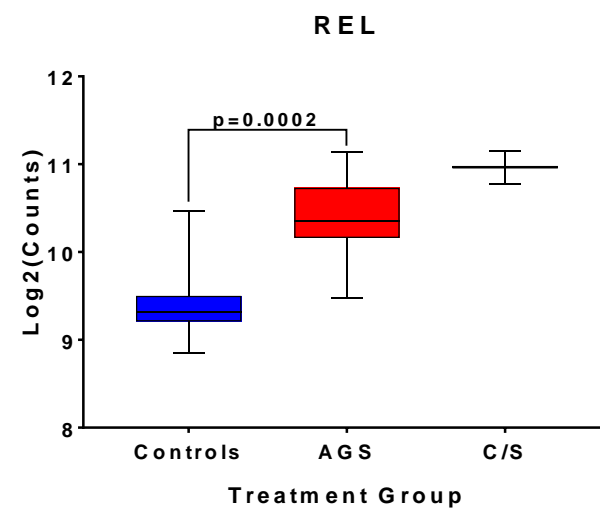
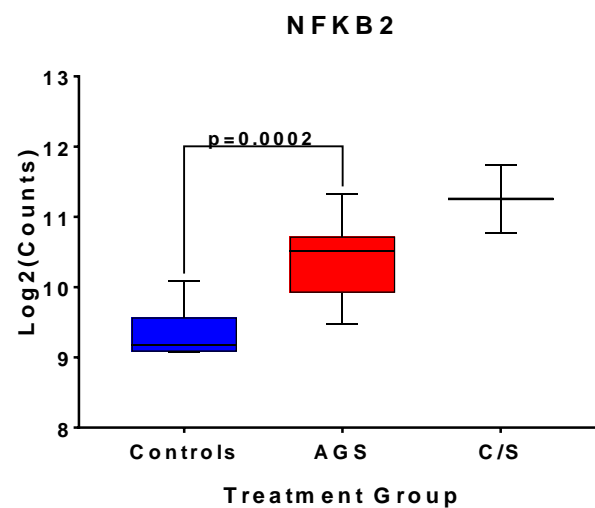
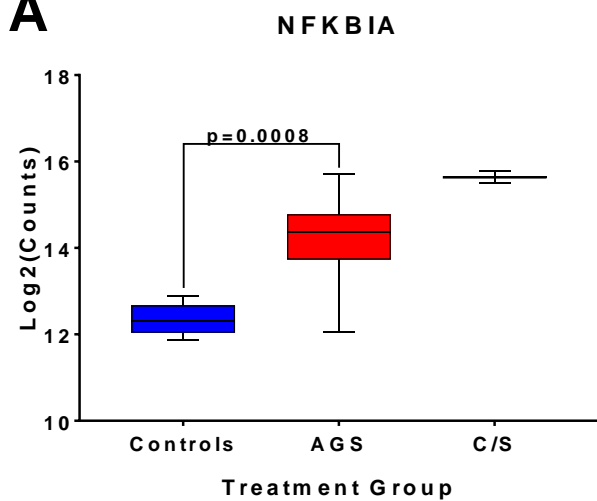
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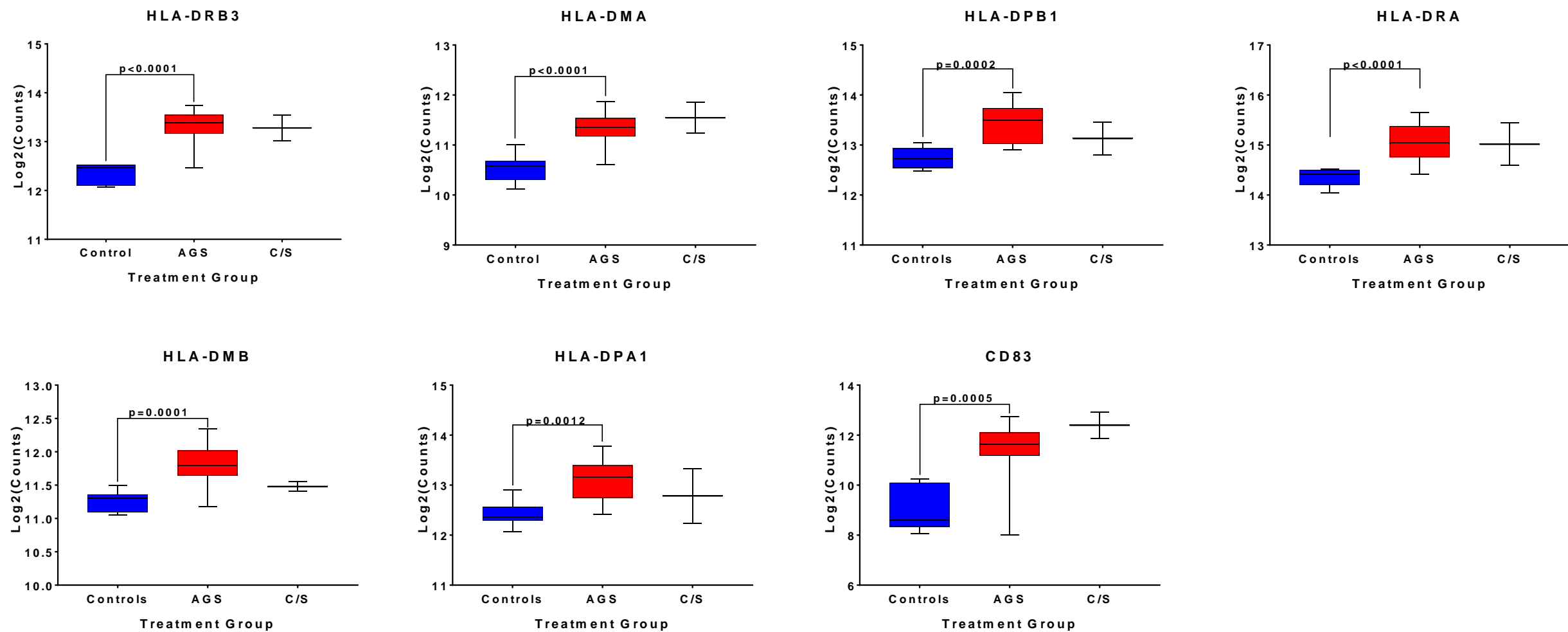


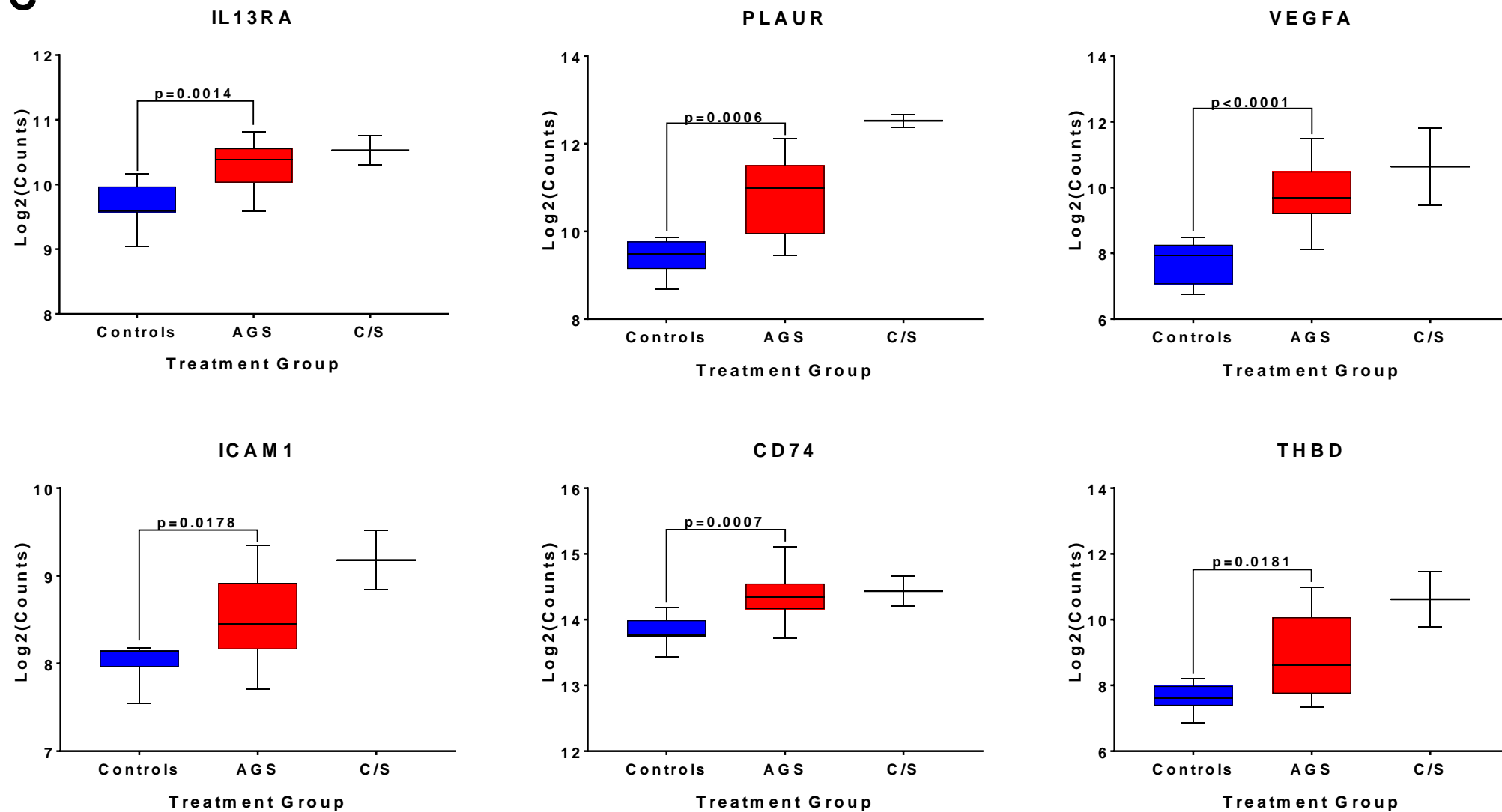
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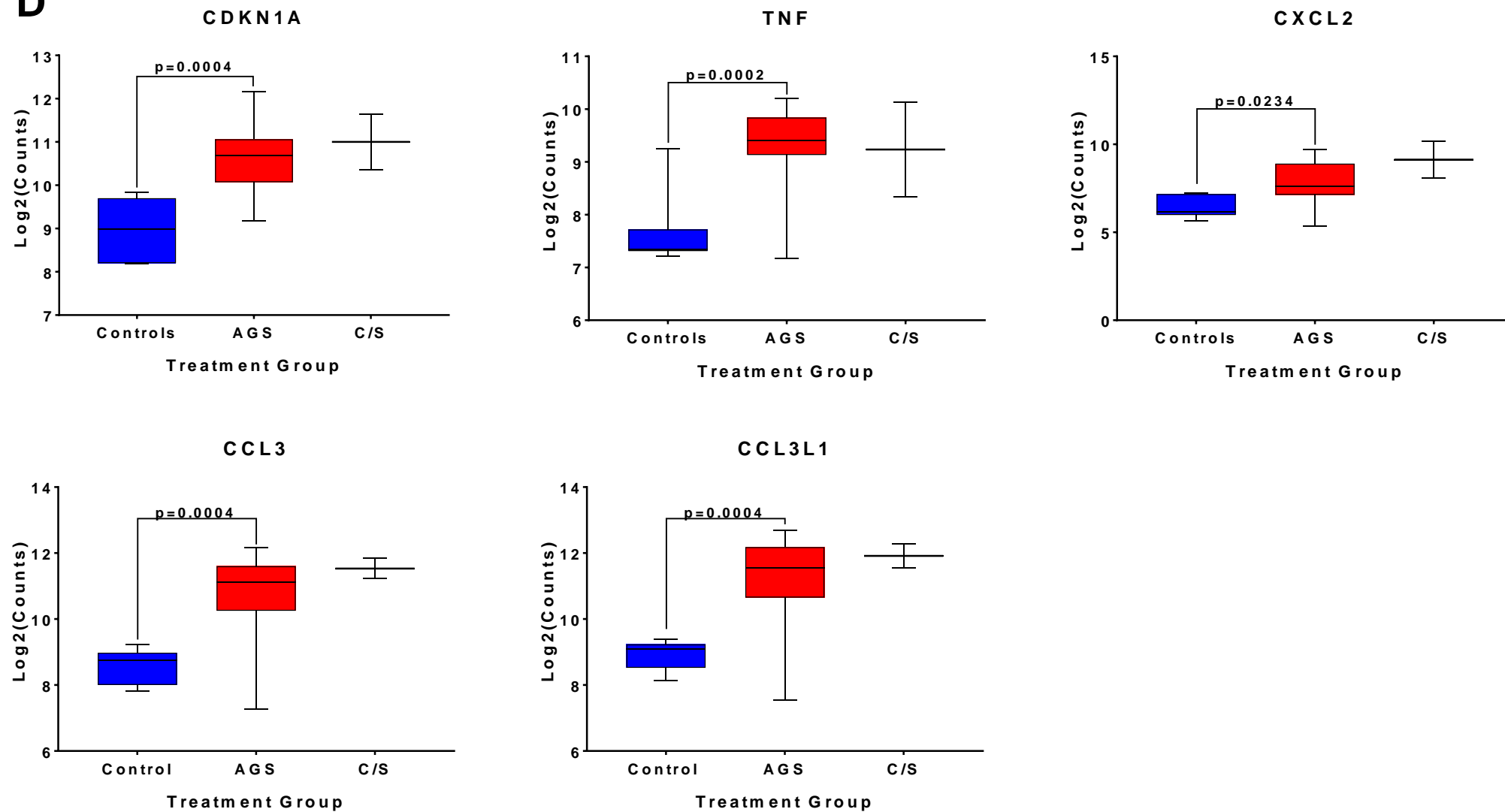
37 Genes ^ UP		
HLA-DRB3	CCL3L1	CCL3
HLA-DMA	EGR1	CD83
NFKBIA	VEGFA	PLAUR
HLA-DRA	NFKB2	HLA-DMB
ICAM1	REL	TNF
CDKN1A	HLA-DPB1	CD74
CLEC7A	CSF2RB	IL1R2
CSF3R	HLA-DPA1	NLRP3
BCL10	RIPK2	THBD
CARD9	IL13RA1	BCL6
IL1B	EP300	ICOSLG
TNFRSF10B	CXCL2	SH2B2
TNFRSF1B		
16 Genes v DOWN		
ETS1	CYFIP2	MAF
TCF7	CD3D	IL16
ATF1	RPS6	CREB1
LCK	IL32	TXK
CD27	CD2	CD8B
MAP2K4		

Figure 2.

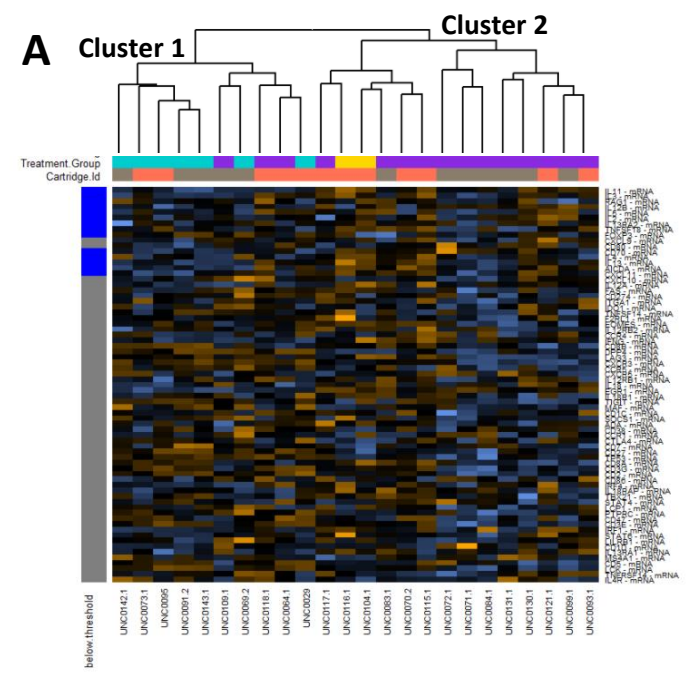
A**Figure 3A.**

B**Figure 3B.**

C**Figure 3C.**

D**Figure 3D.**

T cell function



B cell function

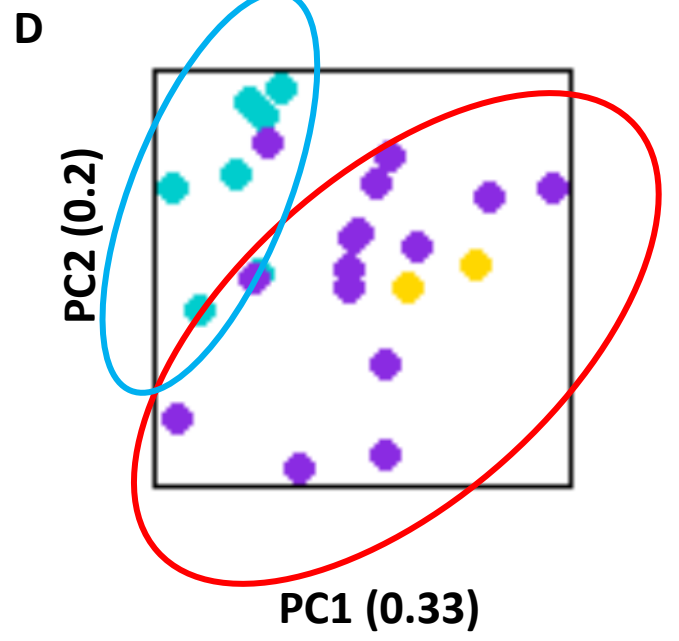
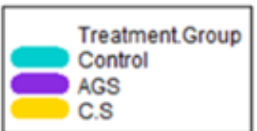
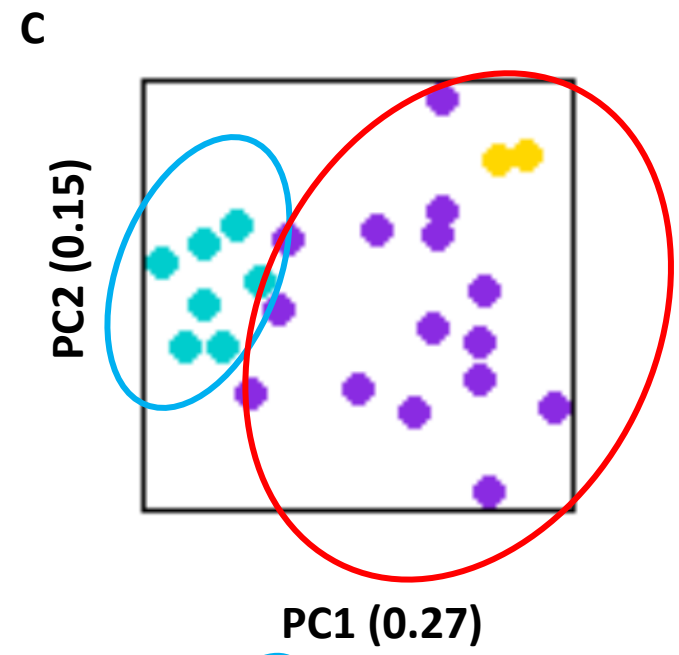
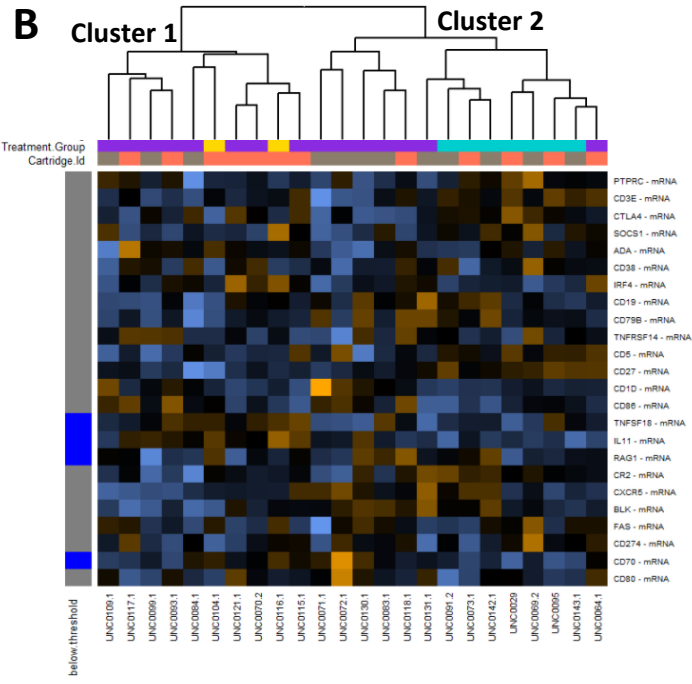


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Figure 4.

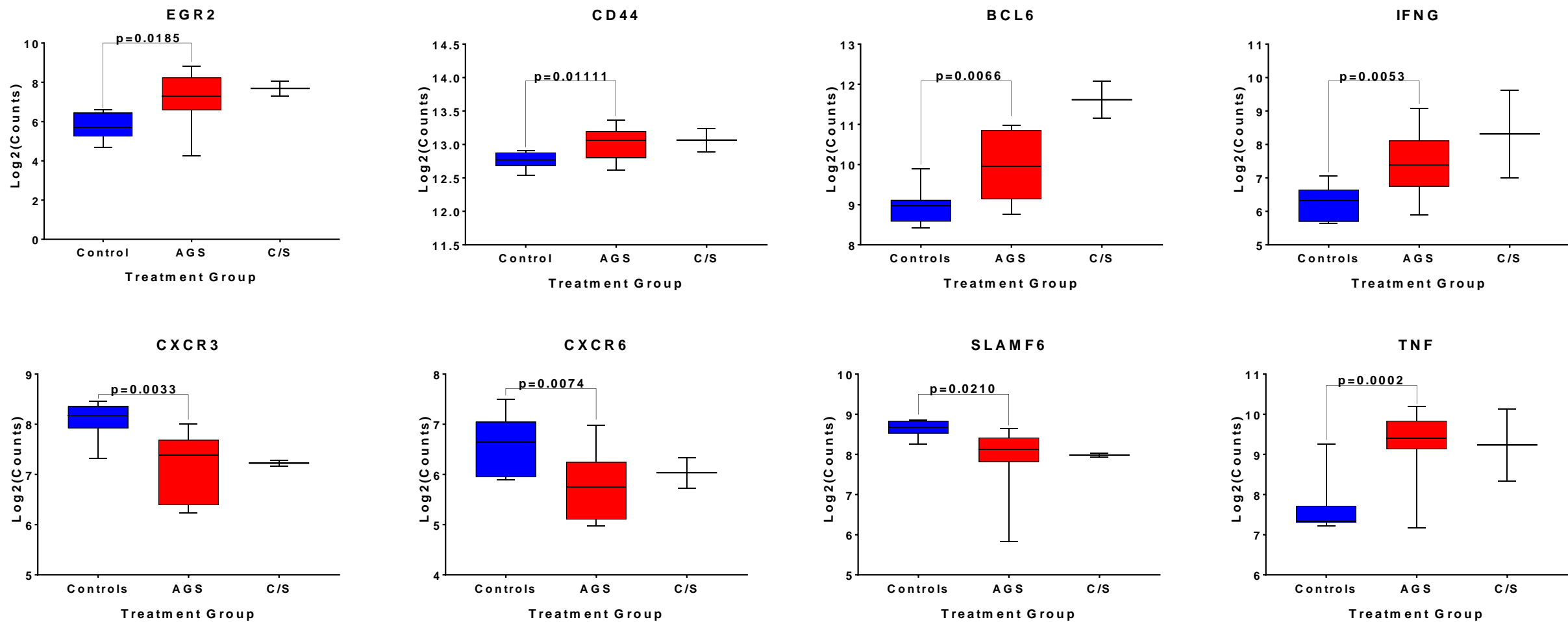


Figure 5.

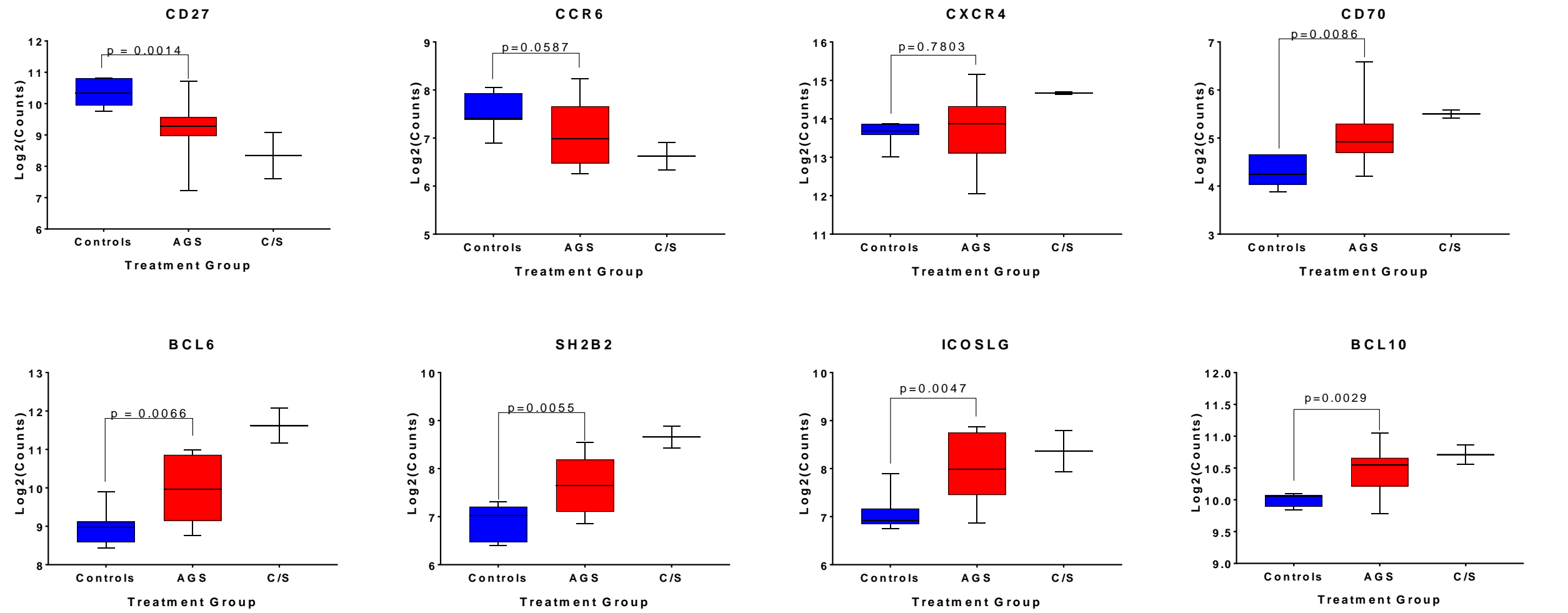


Figure 6.