Supplemental Figure Legends

Figure S1. A) CheckMate 038 clinical trial schematic. See also Figure 1 and Table 1.

Figure S2. Clinical outcomes of patients in the clinical trial included in the study. Therapy groups are nivolumab in treatment-naïve (Nivo.Naive), nivolumab in ipilimumab progression (Nivo.Prog), nivolumab plus ipilimumab combination (Ipi/Nivo). Kaplan Meier plots of overall survival and progression free survival according to therapy group. See also Figure 1 and Table 1.

Figure S3. T cell infiltration correlated and anti-correlated using Pearson correlation with 3,134 genes. Heatmaps of select genes in tumor biopsies organized by increasing T cell score and annotated by clinical features including melanoma subtype, mono versus combination therapy, ipilimumab naïve versus ipilimumab progressed, pre-treatment versus on-treatment, and response. The genes most correlated with T cell Score are shown in A) ordered bottom to top in order of increasing correlation, while B) shows the genes most anti-correlated with T cell Score ordered bottom to top in order of increasing anti-correlation. See also Figure 2.

Figure S4. A) Heatmap showing MCP-Counter immune cell deconvolution signatures for 184 samples in CheckMate 038 clinical trial sample ordered according to the T-cell score. B) Heatmap showing the Pearson correlation between the MCP-Counter immune cell types for the CheckMate 038 clinical data. C) t-SNE plot showing the clustering of the genes based on their degree of correlation or anti-correlation with an MCP-Counter immune cell types. We selected four of the most uncorrelated cell types, T cells, Myeloid Dendritic Cells, Neutrophils, and Fibroblasts, in order to try to get separate clusters. For each gene with a sufficiently strong correlation or anti-correlation with any of these four cell types ($p < 1e-4$), we plotted as distance the Pearson correlation coefficient of that gene with the closest of these four immune cell signatures. Each gene is labeled with the color of the closest cell type (T cell - Blue, Myeloid Dendritic Cell – Green, Fibroblasts – Orange, Neutrophils – Red) and the gene is labeled with a circle if the Pearson correlation is positive and an ‘x’ if it is negative. See also Figure 2.

Figure S5. Immune blockade therapy induced changes in T cell effector genes in- 184 patient samples in clinical study CheckMate 038. Box plots of T cell effector gene expression according to response to therapy using an optimal pooled t-test since data is paired and unpaired (*p value < 0.05, **p value < 0.01, ***p value < 0.001). Therapy group All Mono includes nivolumab in treatment-naïve and nivolumab in ipilimumab progression, while Ipi/Nivo is nivolumab plus ipilimumab combination. See also Figure 2.

Figure S6. Cell line identity organizes the gene expression data in the interferon-gamma induced treatment cohort. Heatmaps showing correlations for gene expression for 58 human cell lines exposed to interferon gamma for 6 hours including A) pre-treatment and post-treatment, B) pre-treatment samples, C) post-treatment samples for all genes. Each cell in the heatmap is the correlation of the fold changes for all genes for two samples, while the color of the cell reflects the Pearson correlation. Any cell darker than red is correlated with an R^2 > 0.5. For all three heatmaps, the only samples that are highly correlated with each other are samples derived from the same cell line, which are located on the diagonal. See also Figure 3.

Figure S7. Interferon-gamma-induced changes in gene expression in 58 human cell lines are highly correlated when fold changes pre- to post-treatment are compared. Heatmap showing fold change correlations for pre-treatment to 6-hour post-treatment exposure to interferon gamma for 58 human melanoma cell lines for all genes. Each cell in the heatmap is the correlation of the fold changes for all genes for two samples, while the color of the cell reflects the Pearson correlation. Any cell darker than red is correlated with an R^2 > 0.5. See also Figure 3.

Figure S8. Interferon-gamma-induced changes in gene expression in 58 human cell lines are highly conserved unless *JAK1* or *JAK2* are lost. A) Box plots showing the fold changes for 58 human cell lines pre- and post-treatment with interferon gamma for 6 hours for select interferon gamma regulated genes (Blue – unmodified cell lines, Red – *in vitro* modified cell lines). B) Heatmap of fold changes for all genes significantly up-regulated (549 genes) or down-regulated (1,176 genes) based on a paired t-test with an FDR of 0.1 after exposure to interferon gamma for 6 hours for 58 human melanoma cell lines. See also Figure 3.

Figure S9. Interferon-gamma-induced changes in gene expression in 58 human melanoma cell lines overlap previously reported and canonical interferon-gamma gene sets. Enrichment plots showing where genes from previously published interferon gamma response gene sets fall in our list of significantly up- or down-regulated genes in the 58 human melanoma cell lines after 6 hours listed with most up-regulated on the left to most down-regulated on the right and the enriched genes each showing as a black line when it occurs in the list. See also Figure 3.

Figure S10. Interferon-gamma-induced changes in gene expression in 58 human cell lines showing key up-regulated genes pre- and post-interferon-gamma treatment. Heatmaps of changes in key interferon-gamma response genes after 6 hours of treatment at 5 ng/ml from the pre-treatment (A) and the post-treatment gene expression (B). Genes are organized by class: anti-proliferative (Black), antigen presentation (White), chemoattractants (Cyan), cytotoxic effectors (Orange), feedback/signaling (Purple). See also Figure 3.

Figure S11. Immune blockade therapy induced changes in antigen presenting genes of 184 patient samples in clinical study CheckMate 038. A) Box plots of select antigen presenting gene expression according to response to therapy clinical study CheckMate 038 using an optimal pooled t-test since data is paired and unpaired (*p value < 0.05, **p value < 0.01, ***p value < 0.001). B) Box plots of Leukocyte Common Antigen expression (i.e. *PTPRC*/CD45 expression, HLA Class I genes adjusted for LCA gene expression, including HLA-A, HLA-B, and HLA-C. See also Figure 4.

Figure S12. Immune blockade therapy induced changes in antigen presenting genes in 184 patient samples in clinical study CheckMate 038. Box plots of antigen presenting gene expression according to response to therapy in clinical study CheckMate 038 using an optimal pooled t-test since data is paired and unpaired (*p value < 0.05, **p value < 0.01, ***p value < 0.001). A) MHC class I, and B) MHC class II. See also Figure 4.

Figure S13. Changes pre- to on-therapy biopsies with CRPR. A) Venn Diagram showing the number of genes significantly (p value 1.0e-4) up (or down) post-treatment CRPR relative to post-treatment PD (based on an optimal pooled t-test since data is paired and unpaired and an FDR cut-off of 0.1) and the number of genes in post-treatment CRPR relative to pre-treatment CRPR (based on an optimal pooled t-test since data is paired and unpaired and an FDR cut-off of 0.1), including the number of overlapping genes. B) shows that the number of overlapping genes between these two sets are enriched so that the up-regulated genes in one set is upregulated in the other and vice versa, while C) shows that the number of genes down-regulated genes in one set is down-regulated in the other and vice versa. See also Figure 5.

Figure S14. Systematic fold changes in the HALLMARK_GENE_SET, for each biopsy sample in CheckMate 038. The blue bar indicates the total number of genes at least 2-fold down-regulated in the sample after therapy, while the red bar indicates the total number of genes at least 2-fold up-regulated in the sample after therapy. A) Each black dot annotation on the bar is a gene from the HALLMARK GENE SET: HALLMARK_INTERFERON_GAMMA_RESPONSE where its location is the fold change for that gene. B) The black dot annotations are the HALLMARK GENE SET: HALLMARK_G2M_CHECKPOINT. Each sample is labeled with clinical data including, response, monotherapy versus combination therapy, ipilimumab naïve positive, and melanoma subtype. See also Figure 5.

Figure S15. Global changes in gene expression in 58 human melanoma cell lines in interferon-gamma response genes and cell cycle genes. For each of the 58 human melanoma cell lines in the interferon gamma 6 hour treated cohort, the blue bar indicates the total number of genes at least 2-fold down-regulated in the sample, while the red bar indicates the total number of genes at least 2-fold up-regulated in the sample. Each black dot is a gene from the gene from the set the figure is labeled with C) HALLMARK_INTERFERON_GAMMA_RESPONSE and D) HALLMARK_G2M_CHECKPOINT. See also Figure 5.

Figure S16. Global changes in gene expression in interferon-alpha and inflammatory response in CheckMate 038 patient data and interferon-gamma treated melanoma cell lines. For each sample, the blue bar indicates the total number of genes at least 2-fold down-regulated in the sample, while the red bar indicates the total number of genes at least 2-fold up-regulated in the sample. Each black dot shows the fold change for a gene from C) the HALLMARK_INTERFERON_ALPHA_RESPONSE and D) the HALLMARK_INFLAMMATORY_RESPONSE. Each patient sample is labeled with clinical data including, response, monotherapy versus combination therapy, ipilimumab naïve positive, and melanoma subtype. Human melanoma cell lines treated with interferon-gamma for 6 hours are shown similarly for B) the HALLMARK_INTERFERON_ALPHA_RESPONSE and D) HALLMARK_INFLAMMATORY_RESPONSE. v See also Figure 5.

Figure S17. Global changes in gene expression in allograft rejection and tnf-alpha signaling and inflammatory response in CheckMate 038 patient data and interferon-gamma treated melanoma cell lines. For each sample, the blue bar indicates the total number of genes at least 2-fold down-regulated in the sample, while the red bar indicates the total number of genes at least 2-fold up-regulated in the sample. Each black dot shows the fold change for a gene from C) the HALLMARK_ALLOGRAFT_REJECTION and the D) HALLMARK_TNFA_SIGNALING_VIA_NFKB. Each patient sample is labeled with clinical data including, response, monotherapy versus combination therapy, ipilimumab naïve positive, and melanoma subtype. Human melanoma cell lines treated with interferon-gamma for 6 hours are shown similarly for B) the HALLMARK_ALLOGRAFT_REJECTION and D) HALLMARK_TNFA_SIGNALING_VIA_NFKB. See also Figure 5.

Figure S18. Global changes in gene expression in cell cycle associated genes, E2F targets and mitotic spindle genes, in CheckMate 038 patient data and interferon-gamma treated melanoma cell lines. For each sample, the blue bar indicates the total number of genes at least 2-fold down-regulated in the sample, while the red bar indicates the total number of genes at least 2-fold up-regulated in the sample. Each black dot shows the fold change for a gene from C) the HALLMARK_E2F_TARGETS and D) the HALLMARK_MITOTIC_SPINDLE. Each patient sample is labeled with clinical data including, response, monotherapy versus combination therapy, ipilimumab naïve positive, and melanoma subtype. Human melanoma cell lines treated with interferon-gamma for 6 hours are shown similarly for B) the HALLMARK_E2F_TARGETS and D) HALLMARK_MITOTIC_SPINDLE. See also Figure 5.

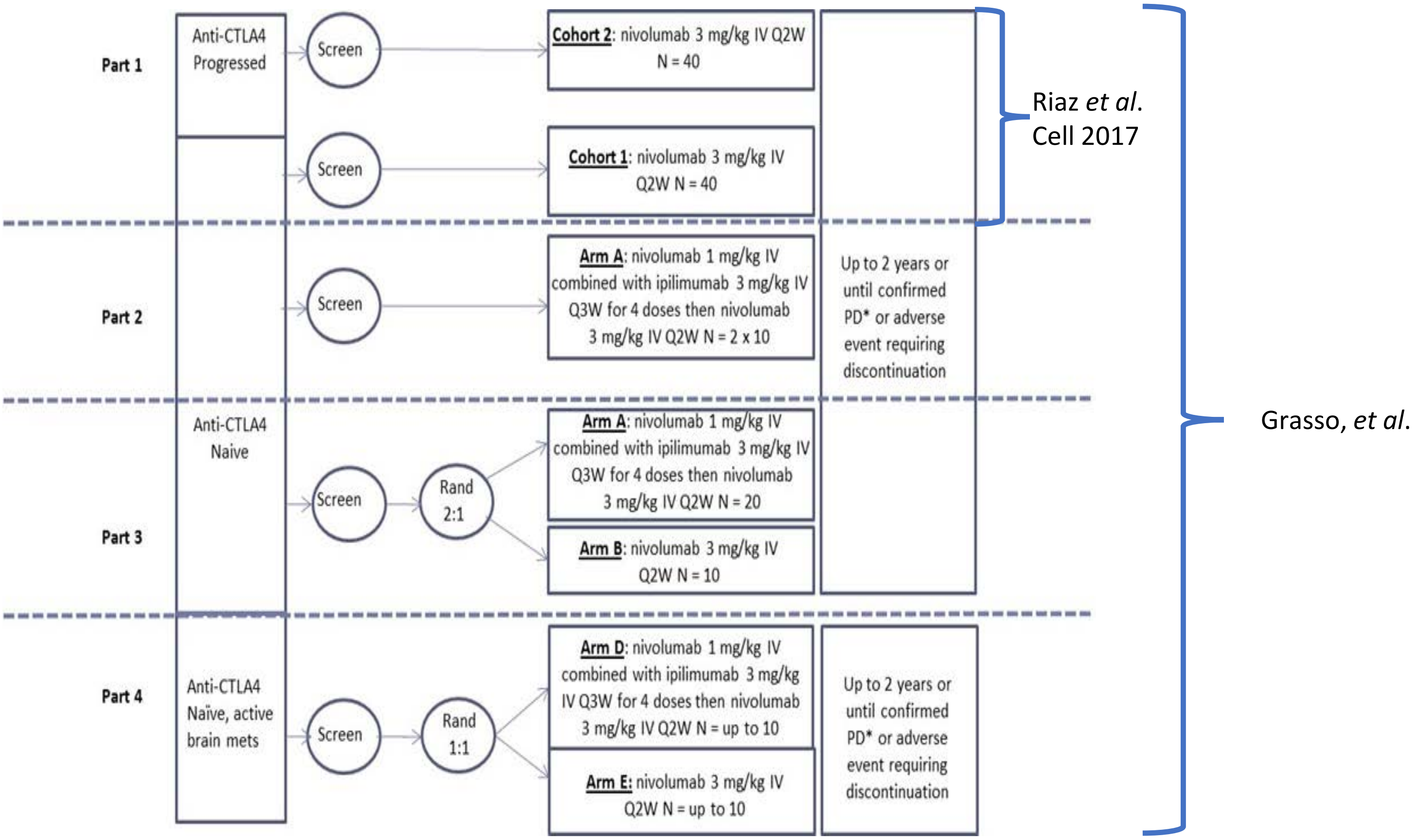
Figure S19. Global changes in gene expression of MYC targets in patient biopsies and interferon-gamma treated cell lines. For each sample, the blue bar indicates the total number of genes at least 2-fold down-regulated in the sample, while the red bar indicates the total number of genes at least 2-fold up-regulated in the sample. Each black dot shows the fold change for a gene from C) the HALLMARK_MYC_TARGETS_V1 and D) the HALLMARK_MYC_TARGETS_V2. Each patient sample is labeled with clinical data including, response, monotherapy versus combination therapy, ipilimumab naïve positive, and melanoma subtype. Human melanoma cell lines treated with interferon-gamma for 6 hours are shown similarly in B) the HALLMARK_MYC_TARGETS_V1 and D) HALLMARK_MYC_TARGETS_V2. See also Figure 6.

Figure S20. Interferon-gamma-induced changes in gene expression in 58 human melanoma cell lines include down-regulation of WNT signaling genes. A) Box plots showing the fold changes for 58 human melanoma cell lines pre- and post-treatment with interferon-gamma for 6 hours for select Wnt pathway genes (Blue - unmodified cell lines, Red – modified cell lines). B) The pre- and post-treatment gene expression levels of *FZD5*, the cell surface receptor for the WNT5A ligand initiating the down-stream Wnt signaling programming (Red = up, Blue = down). See also Figure 6.

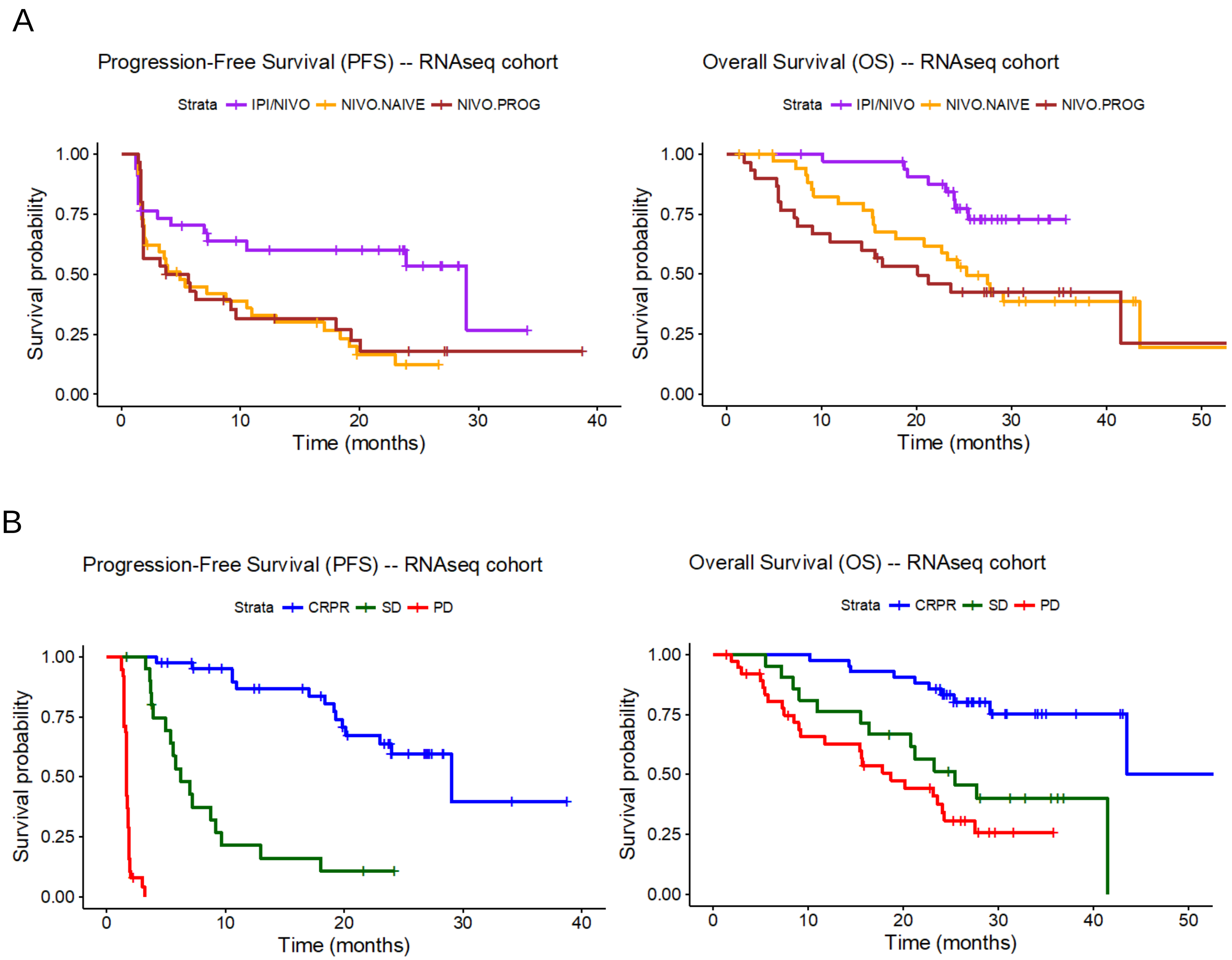
Figure S21. Interferon-gamma-induced changes in gene expression in specimens in clinical study CheckMate 038 include systematic changes in genes regulating immune exclusion. Heatmap of changes in immune exclusion up and immune exclusion down gene sets from Jerby-Arnon, et al in pre- and on-treatment biopsies. The samples are separated by whether they are pre- or on-treatment and by response. Within these figures the samples are ordered by T cell score and annotated by ipilimumab naïve status, monotherapy versus combination therapy, and melanoma subtype. Genes are organized by genes that are up-regulated in immune excluded samples (White) and genes that are down-regulated in immune excluded samples (Black). See also Figure 6.

Supplemental Figure 1

CheckMate 038 Study Design



Supplemental Figure 2



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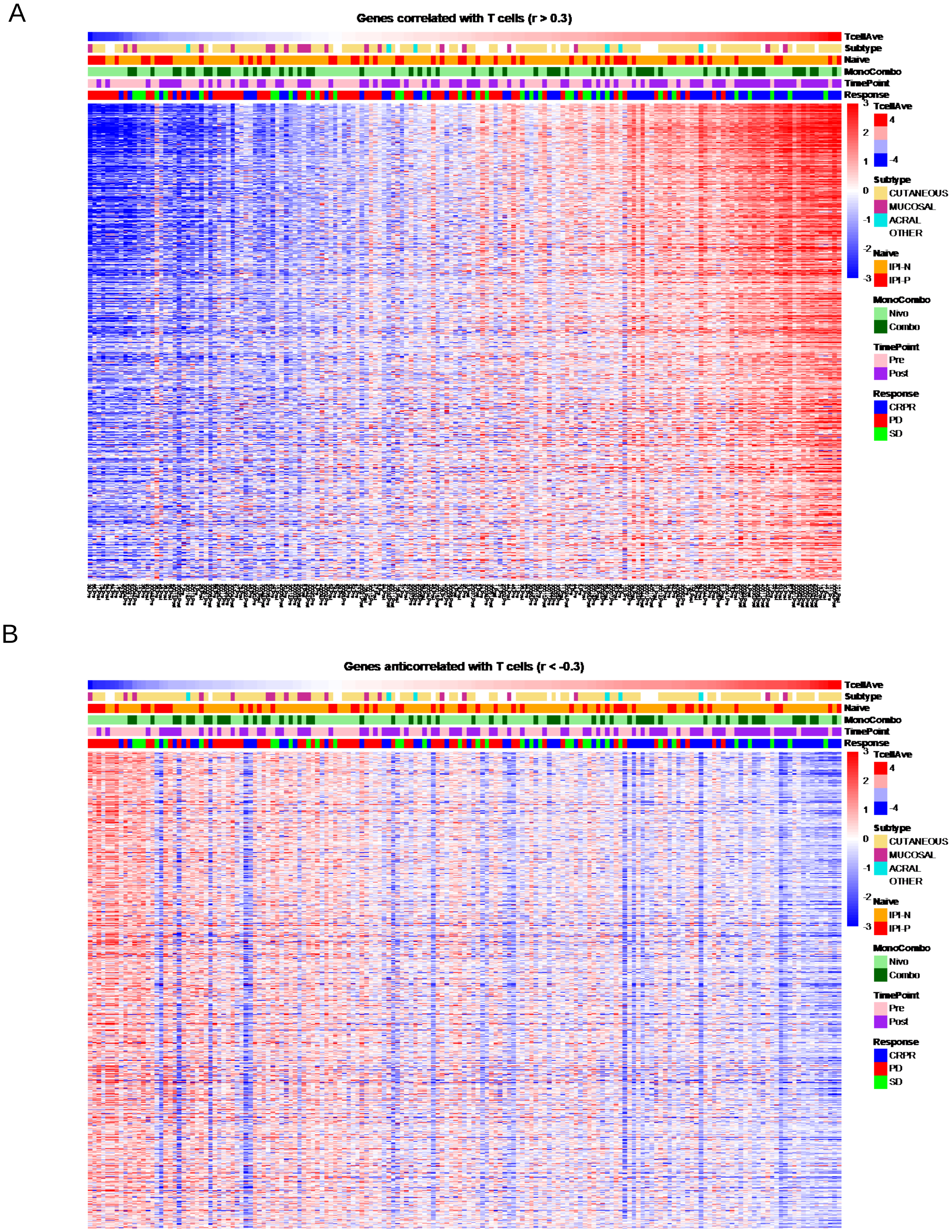

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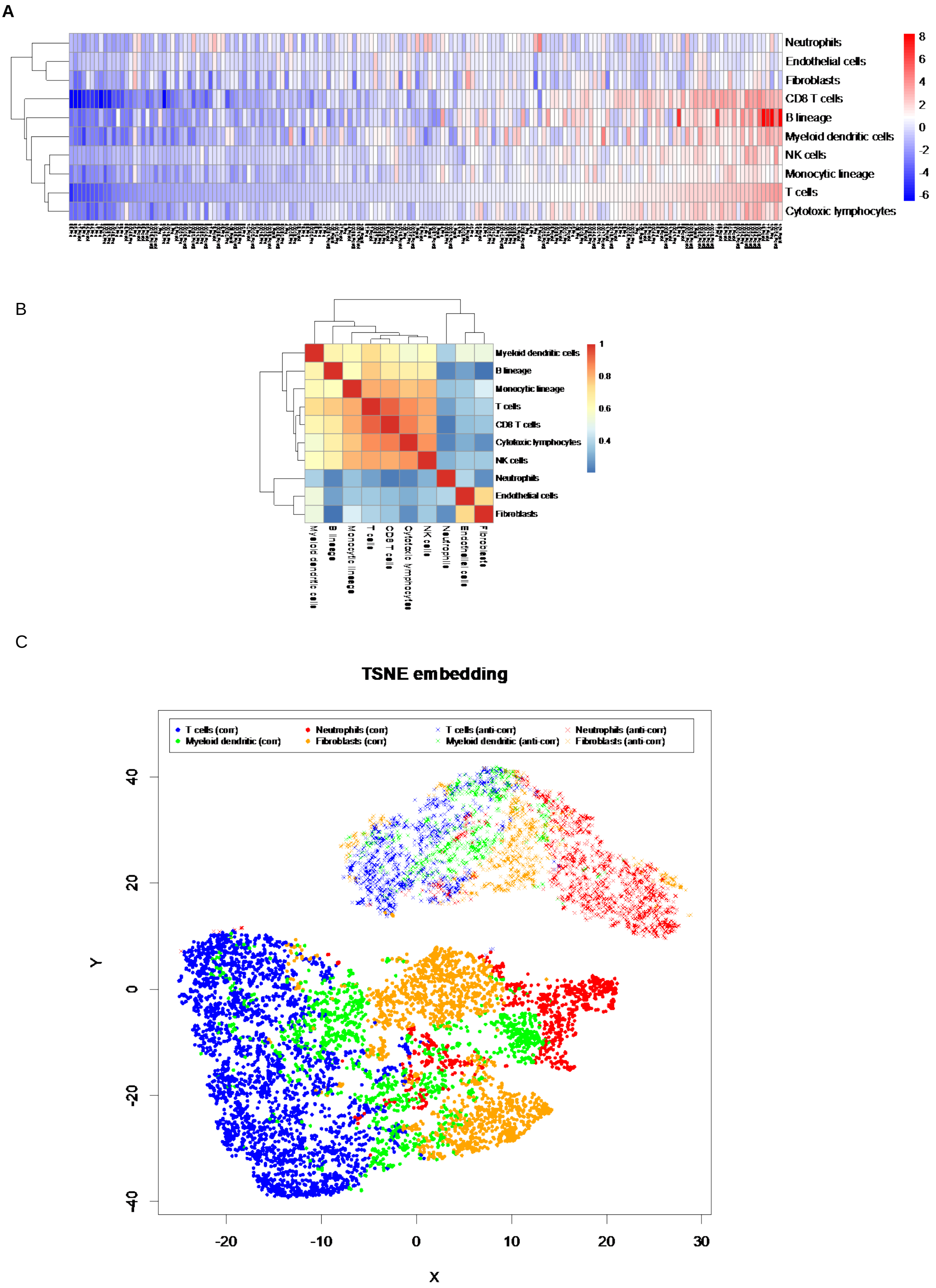

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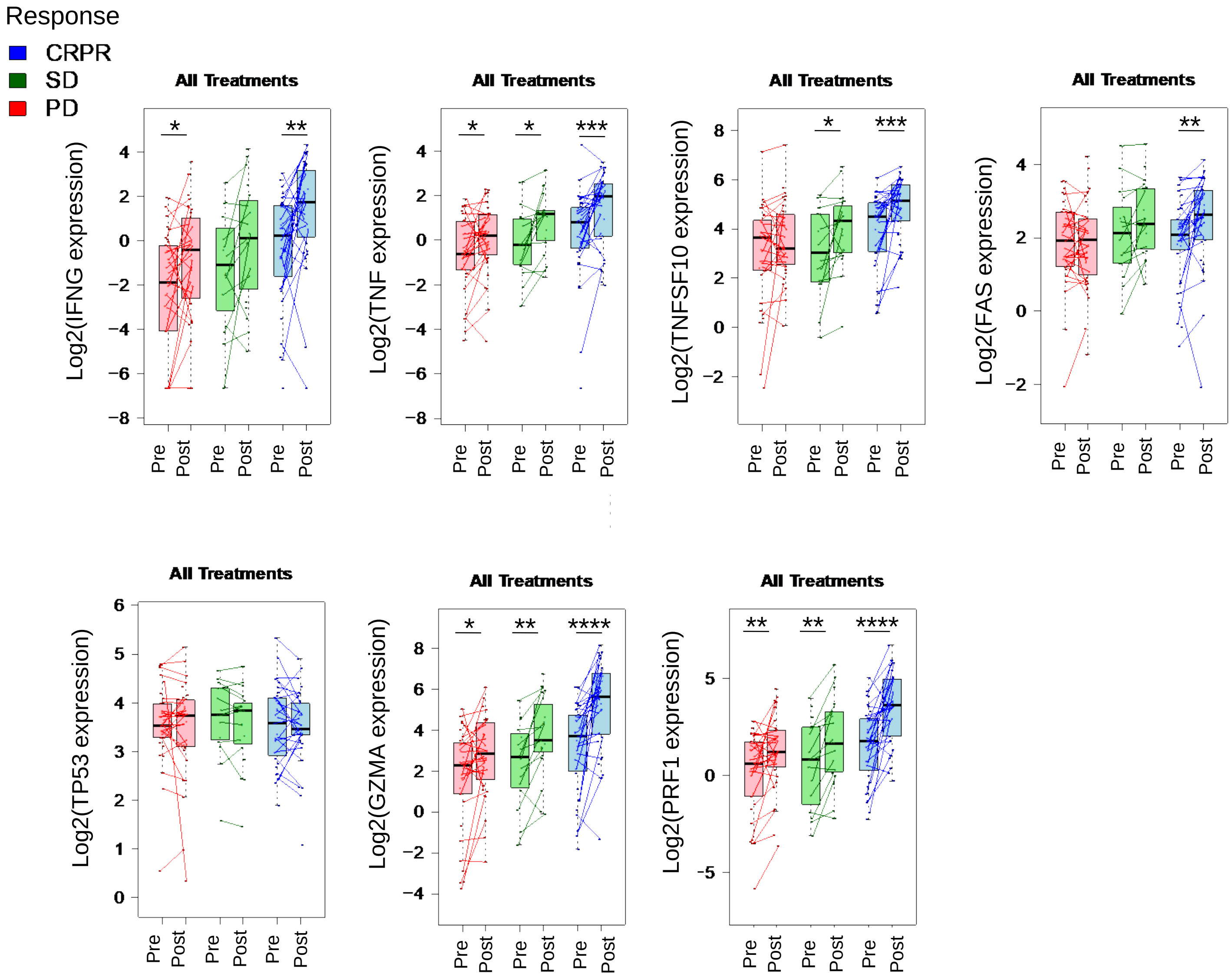

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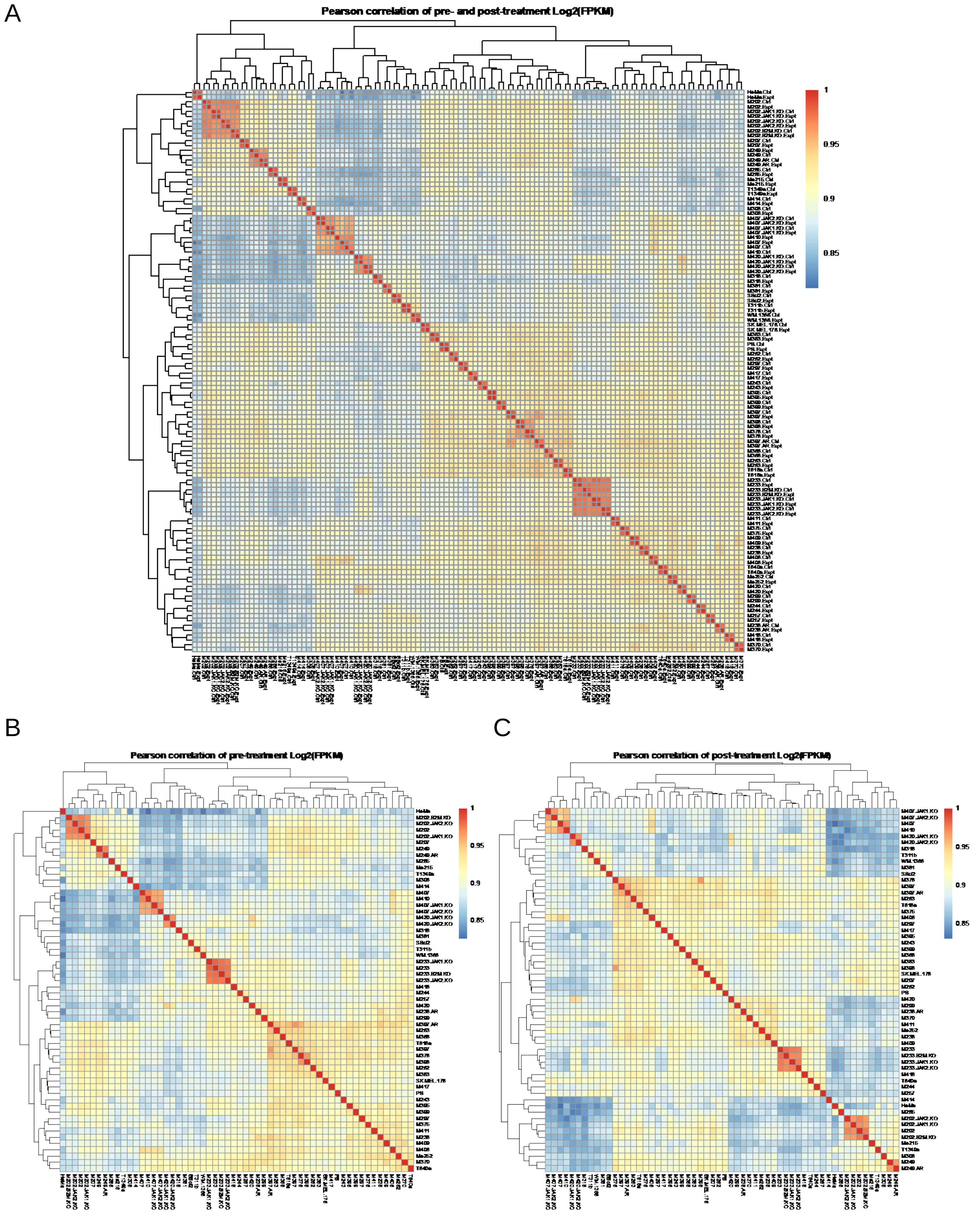

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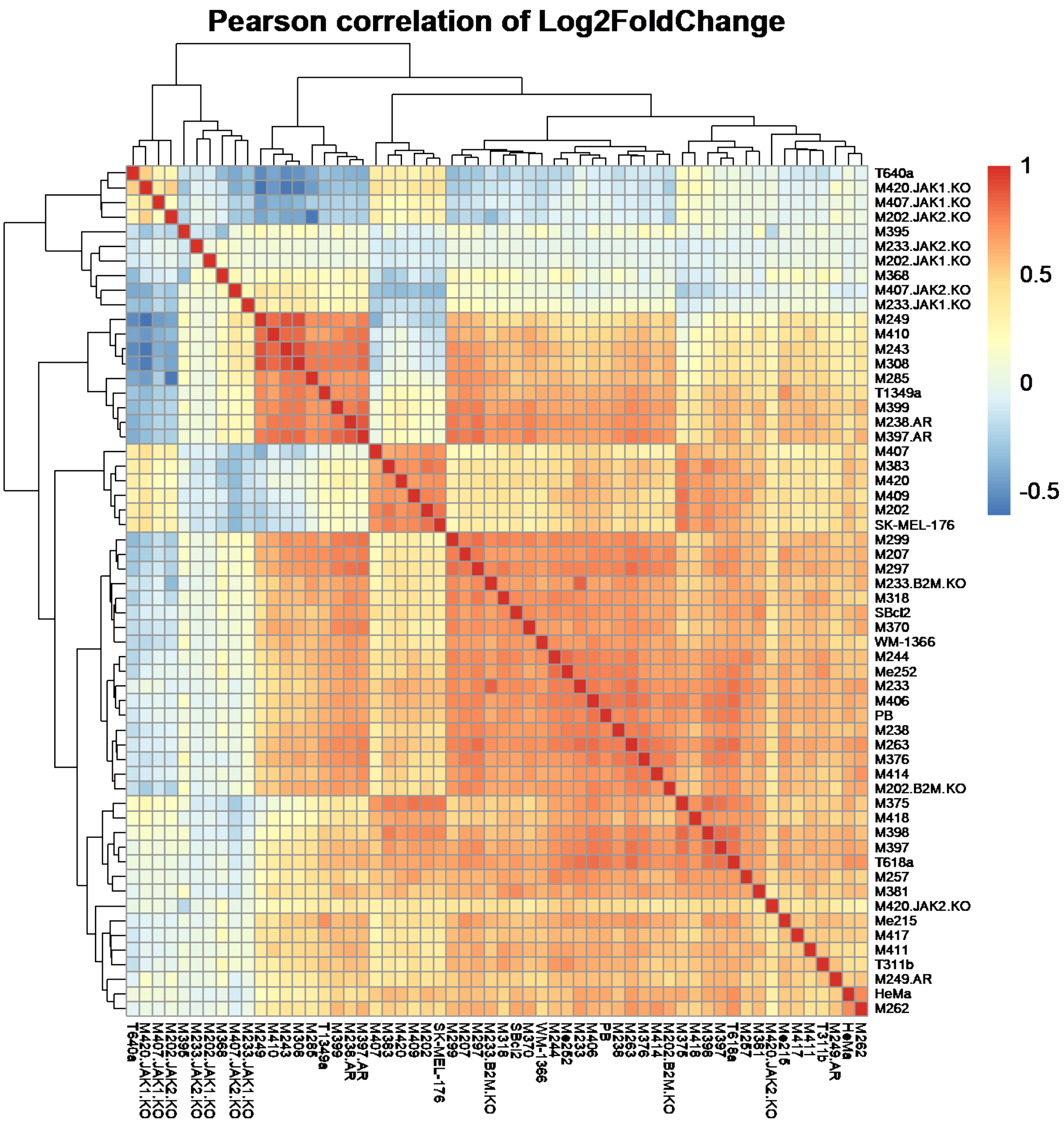
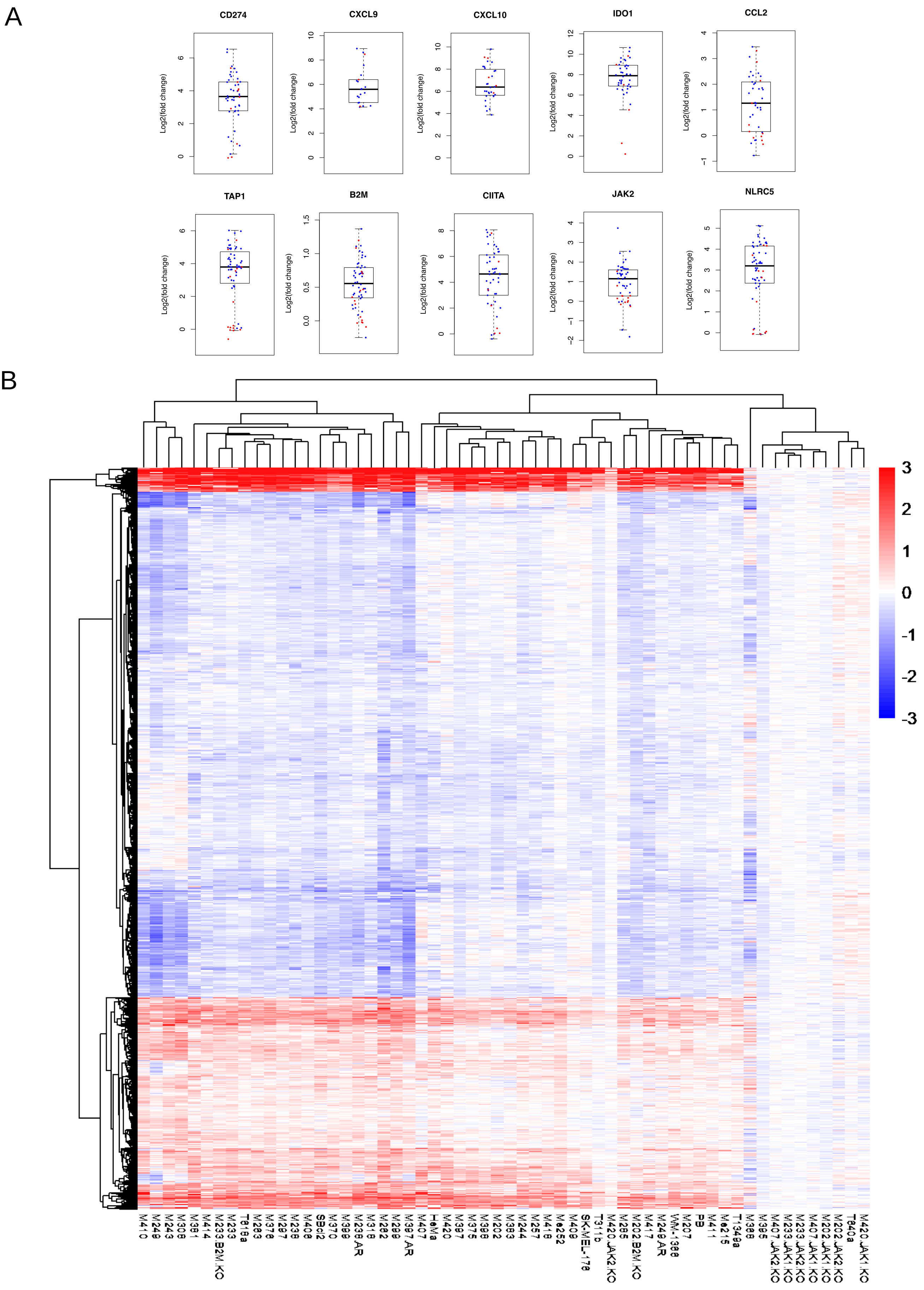


Pearson correlation of Log2FoldChange

Supplemental Figure 8



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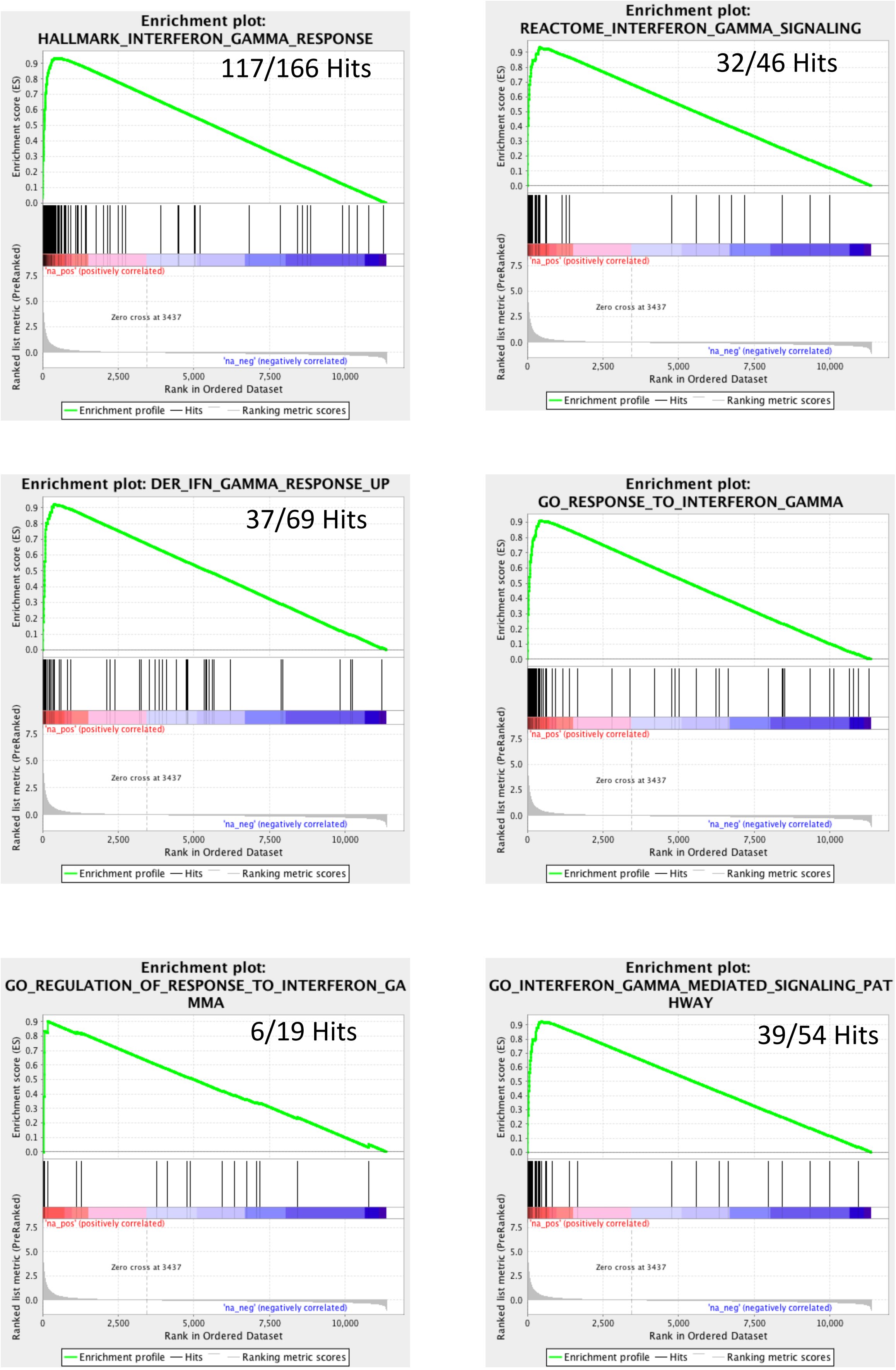
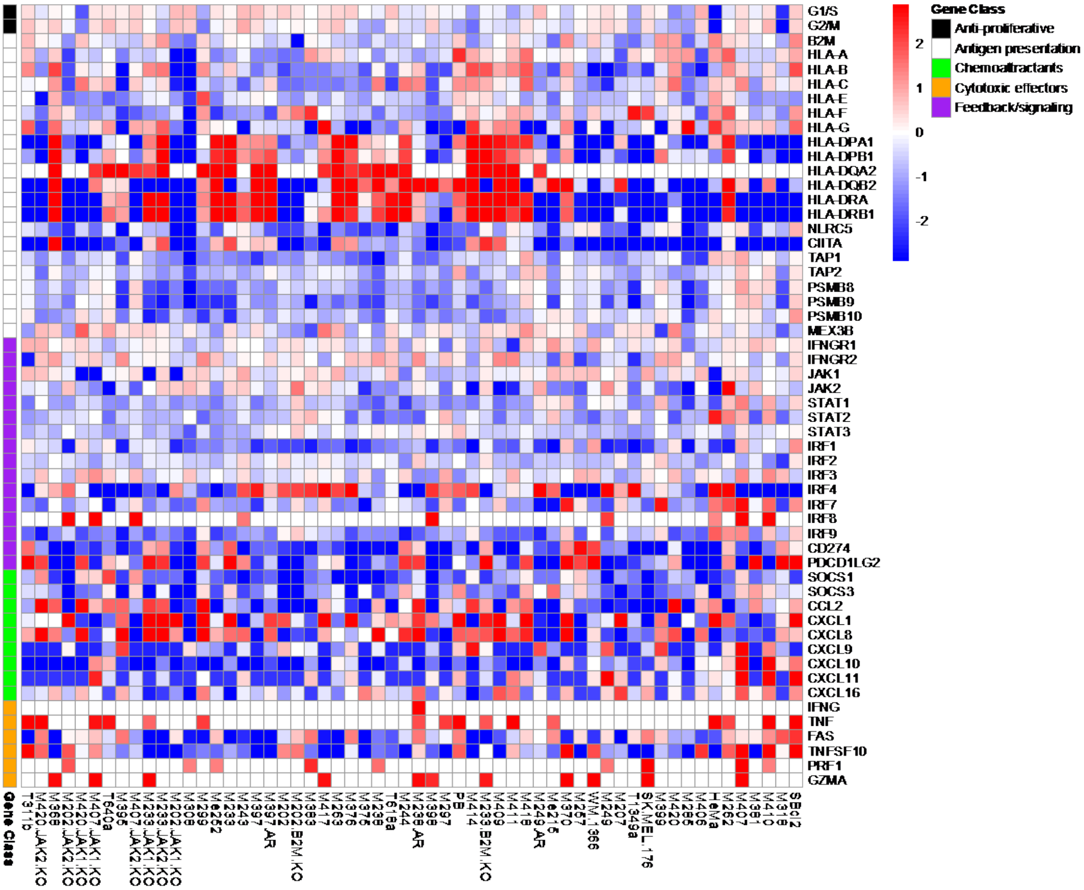


Supplemental Figure 10

A



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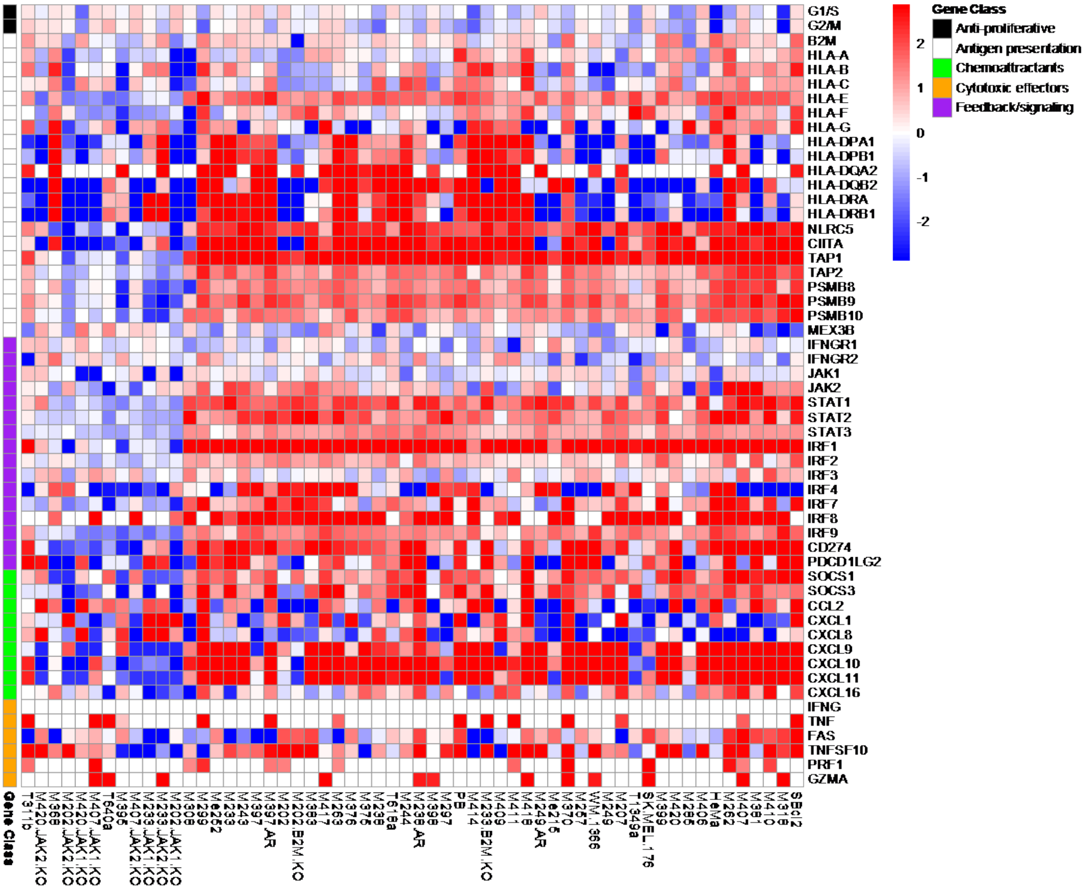
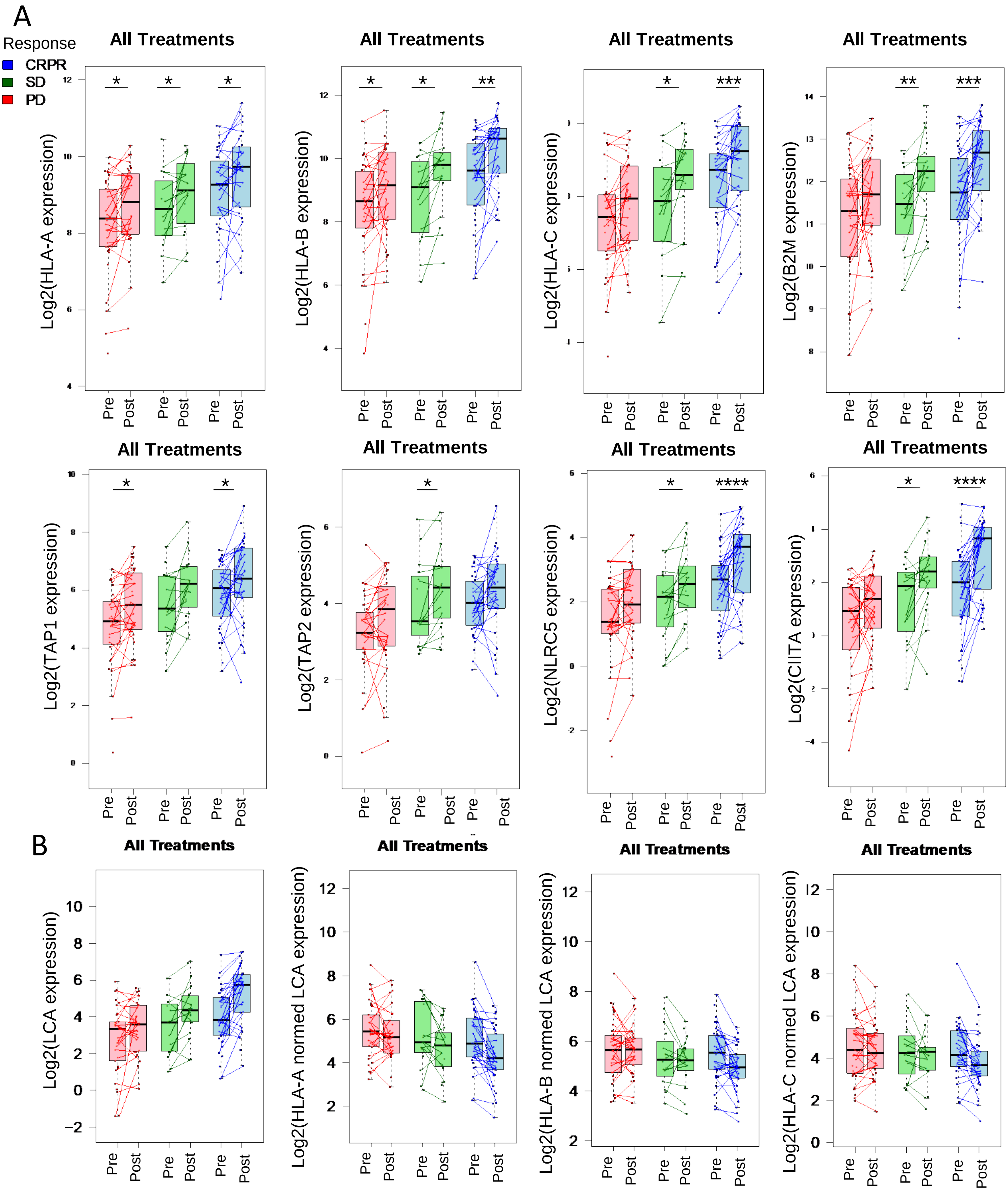
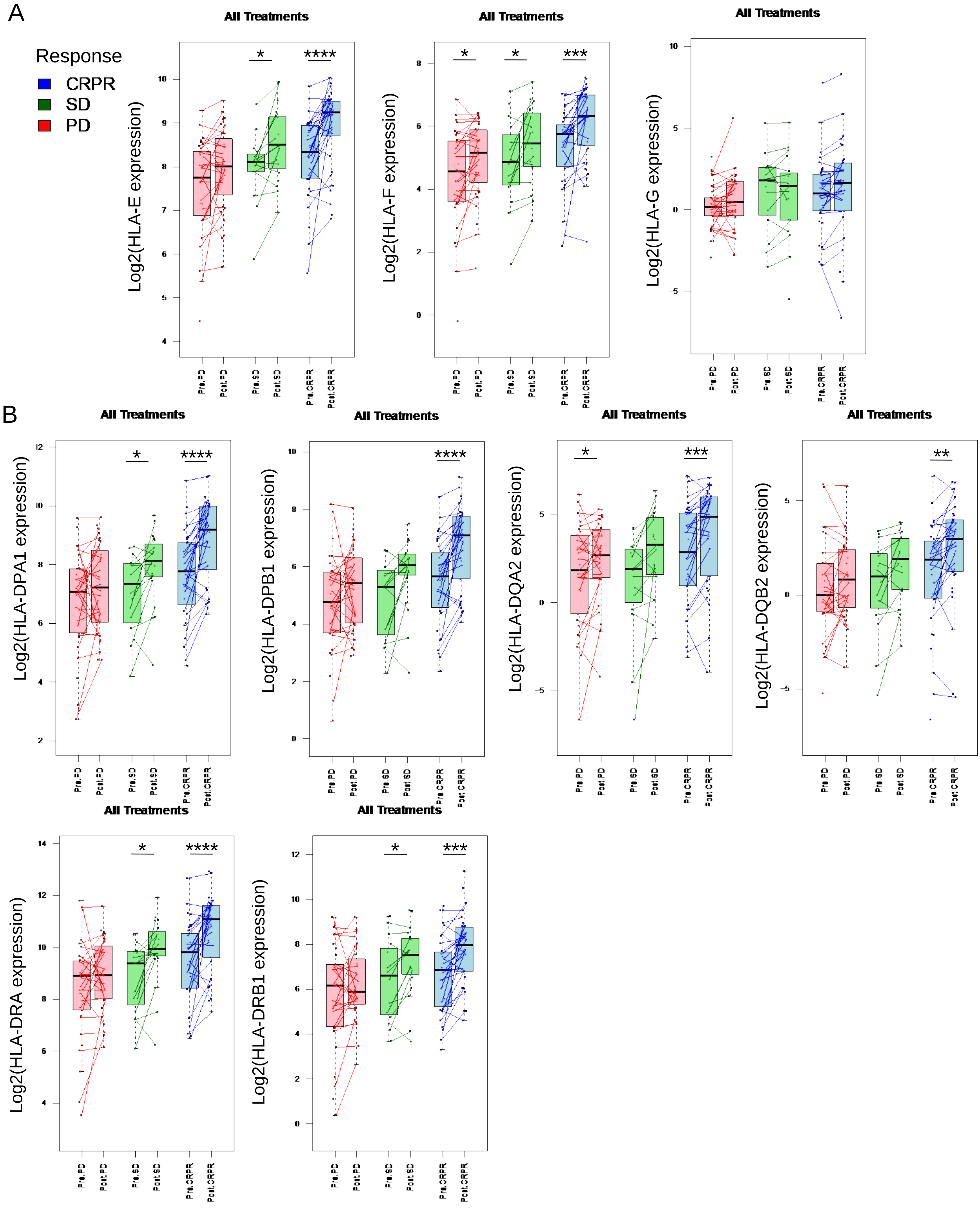


Supplemental Figure 11



Supplemental Figure 12



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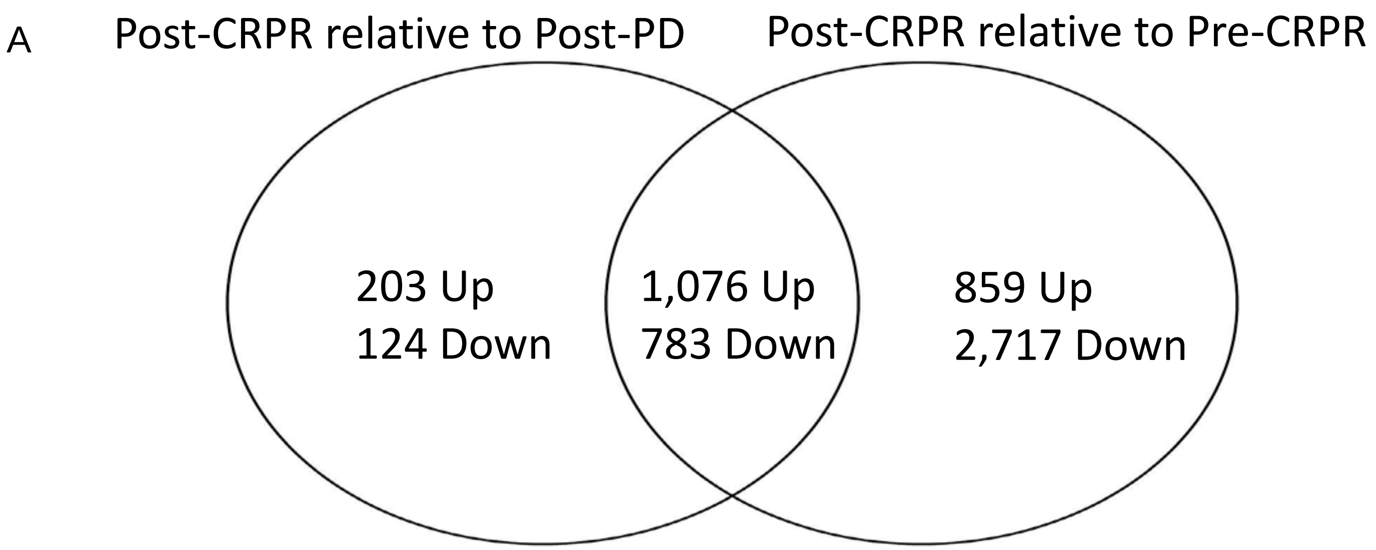

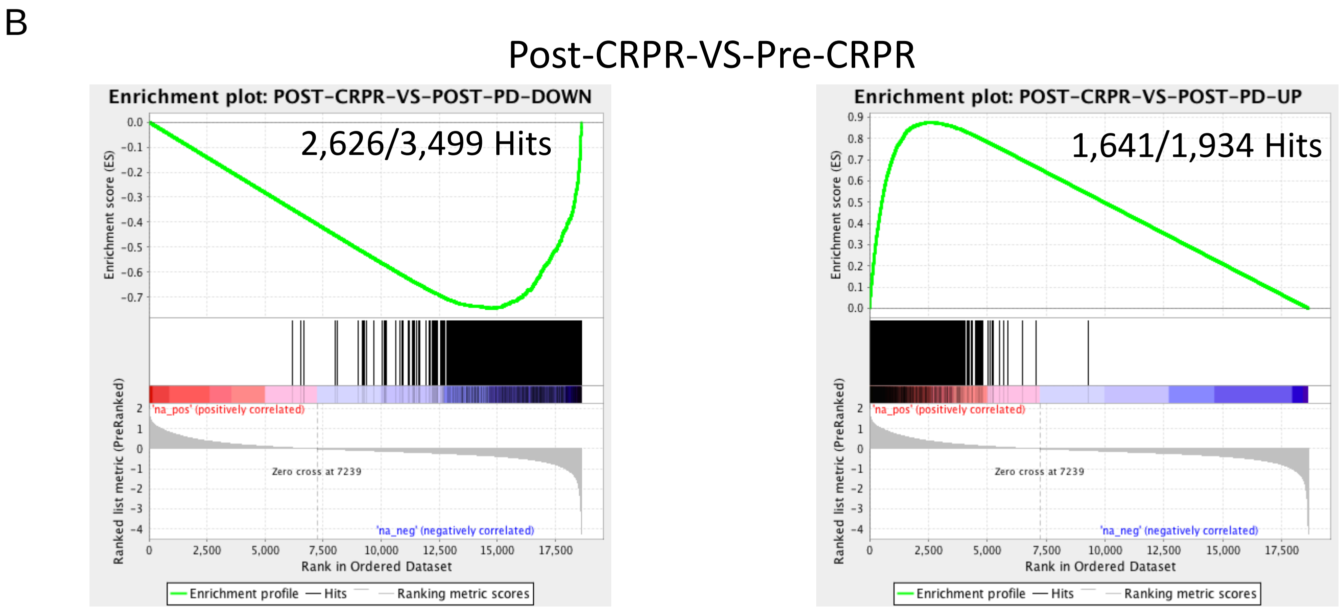

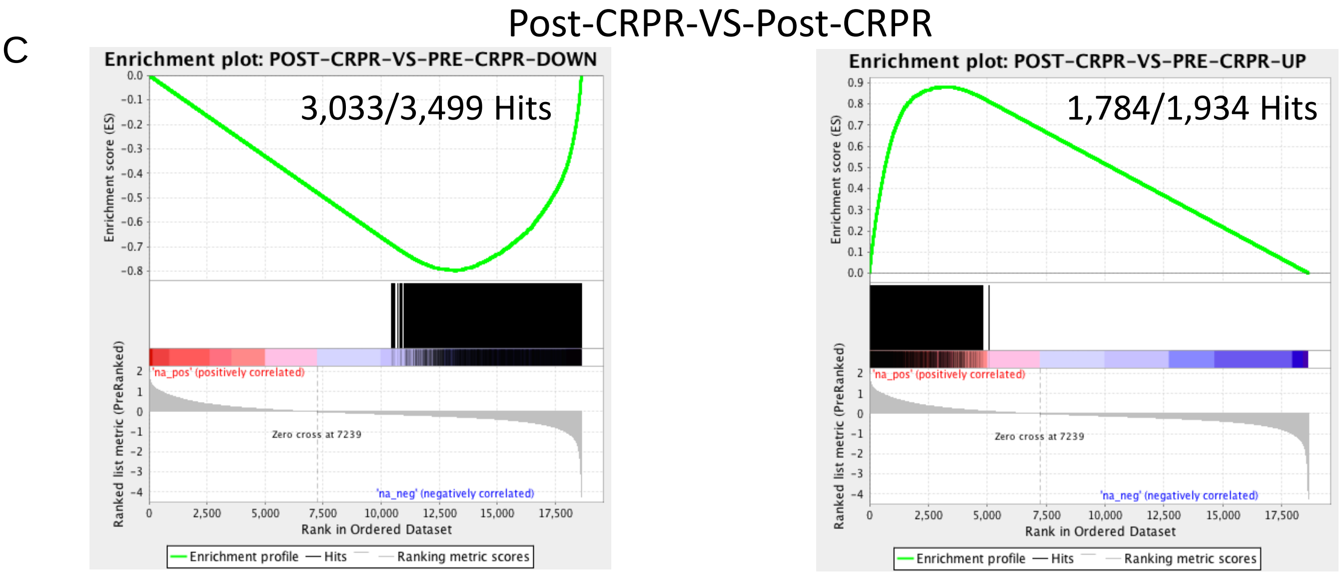

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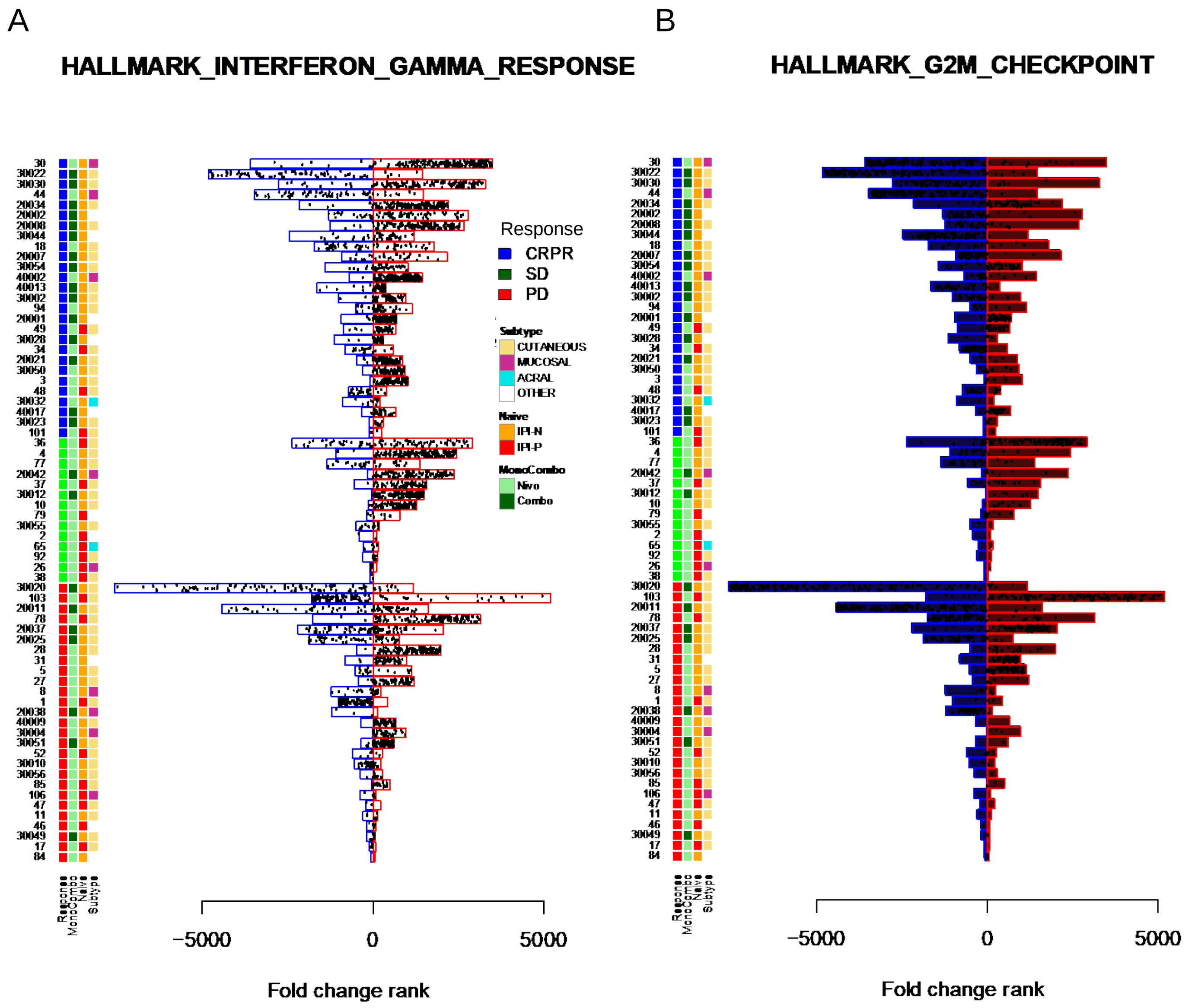

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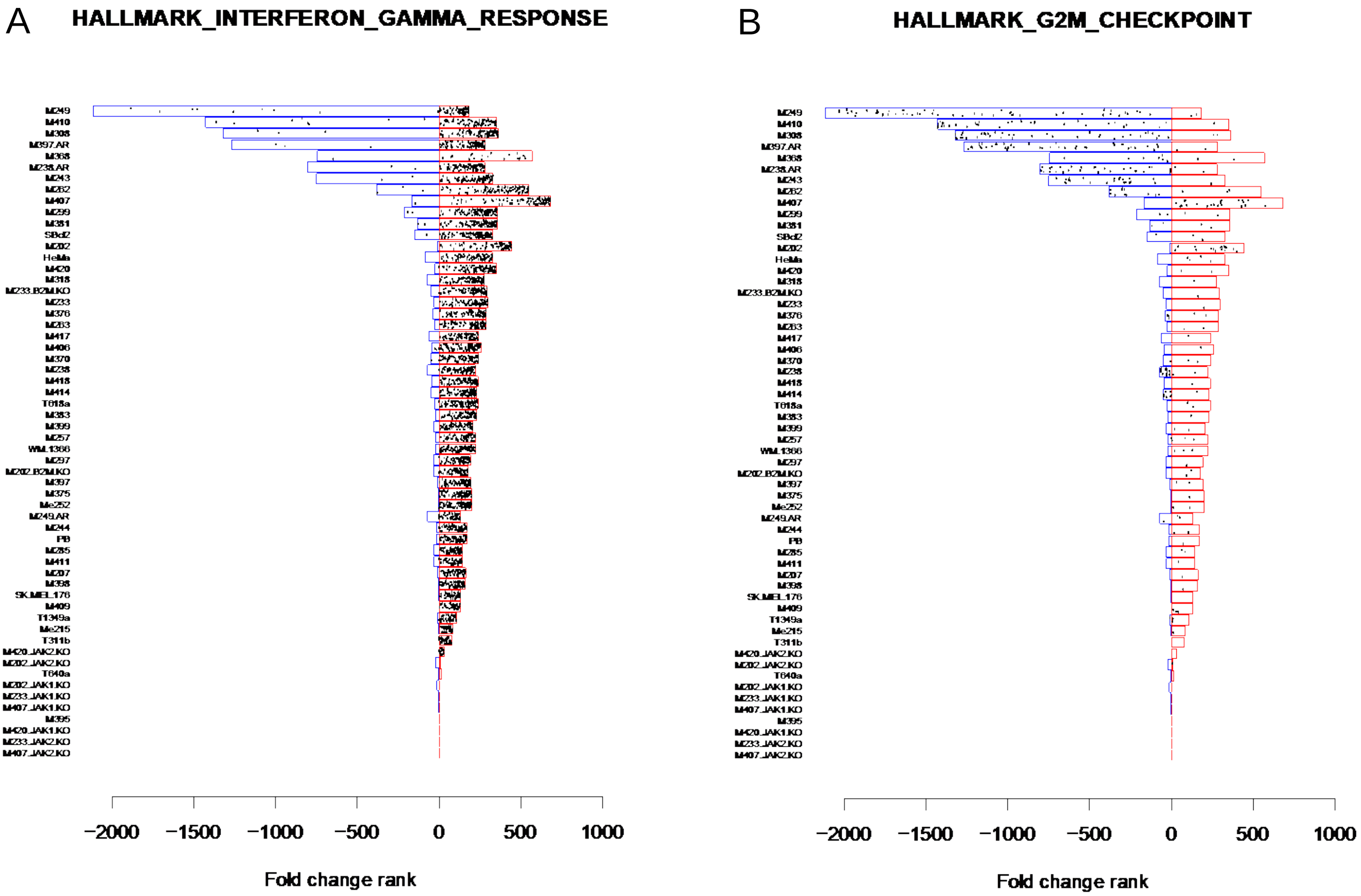

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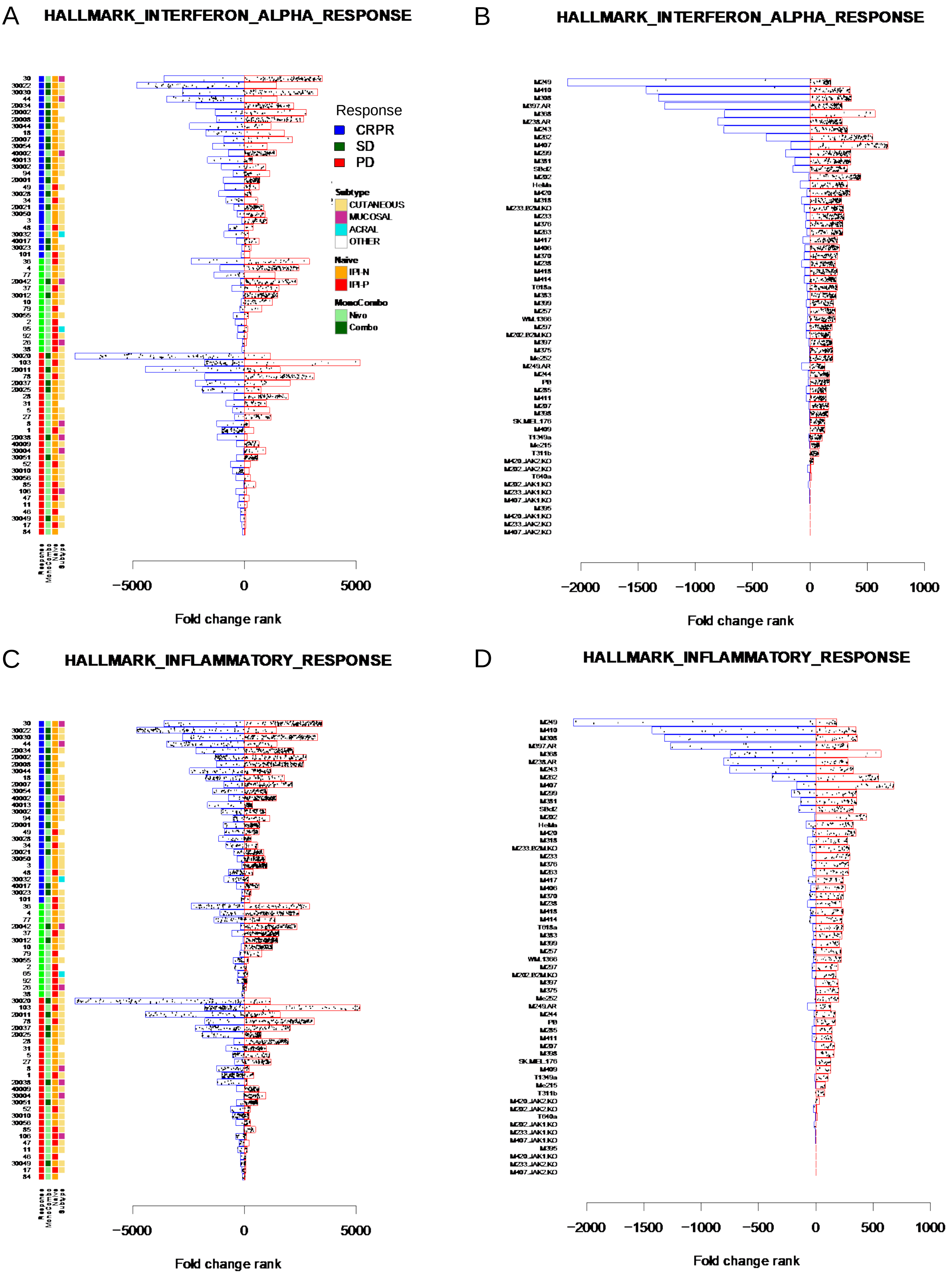

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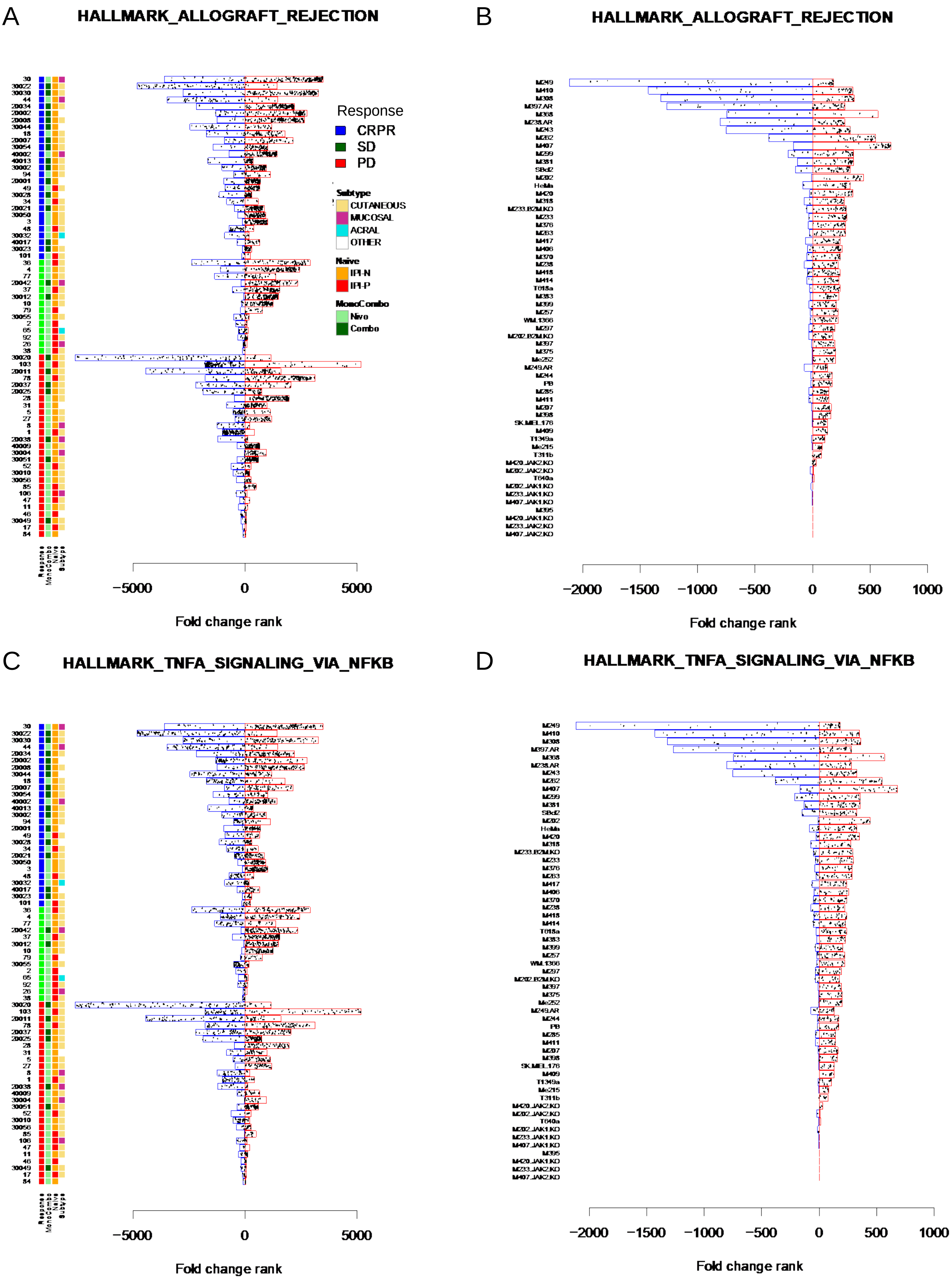

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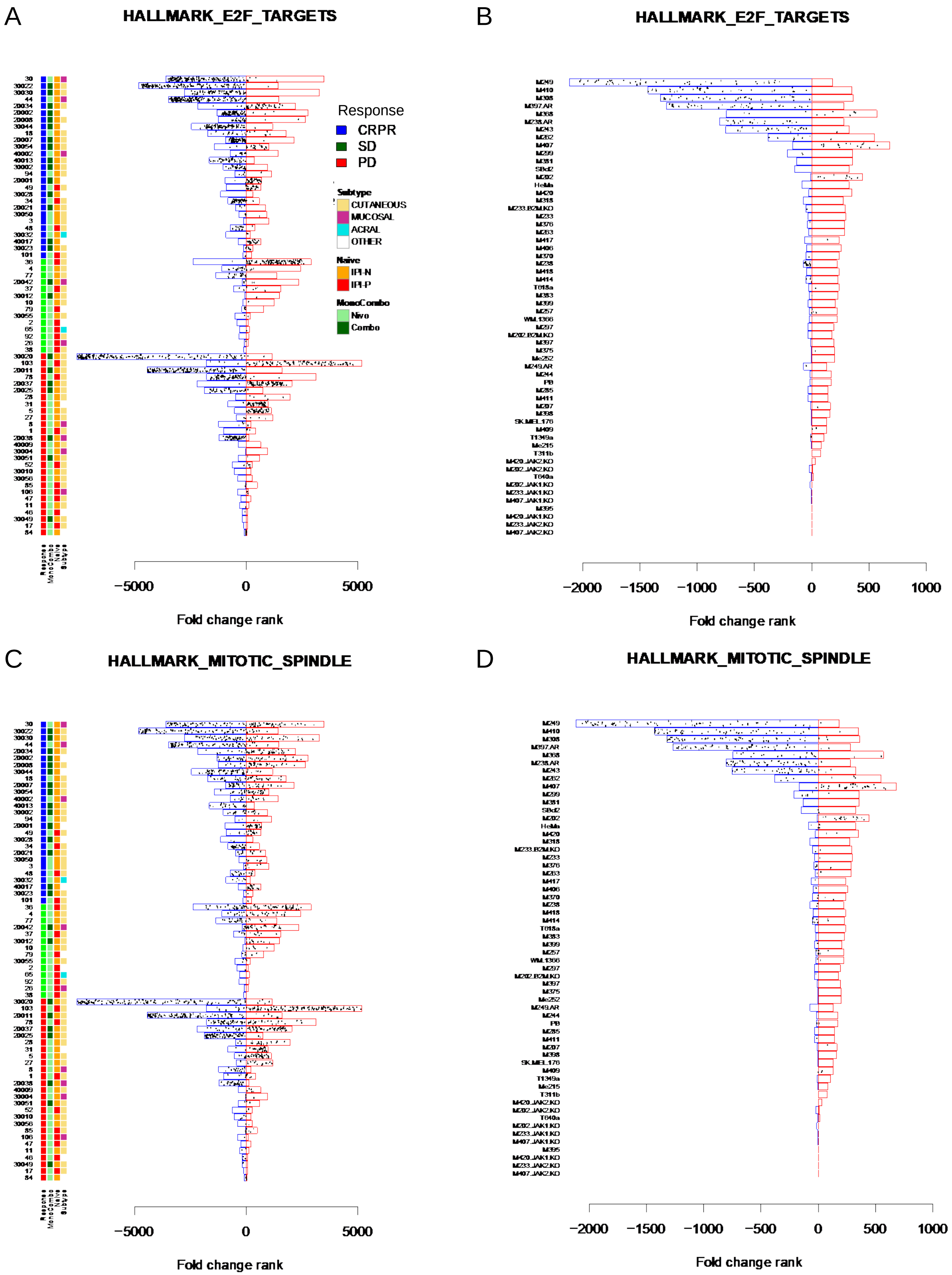

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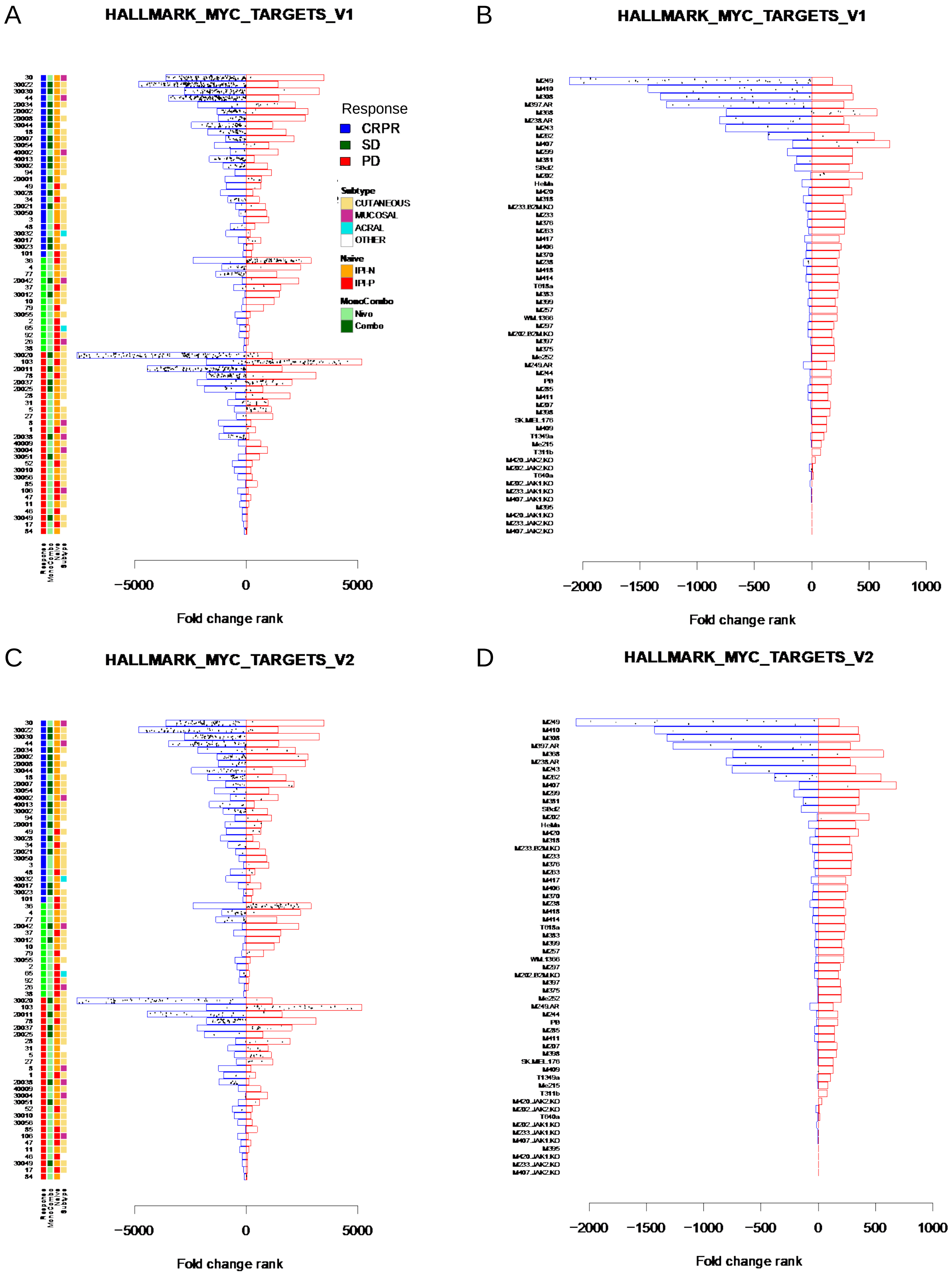

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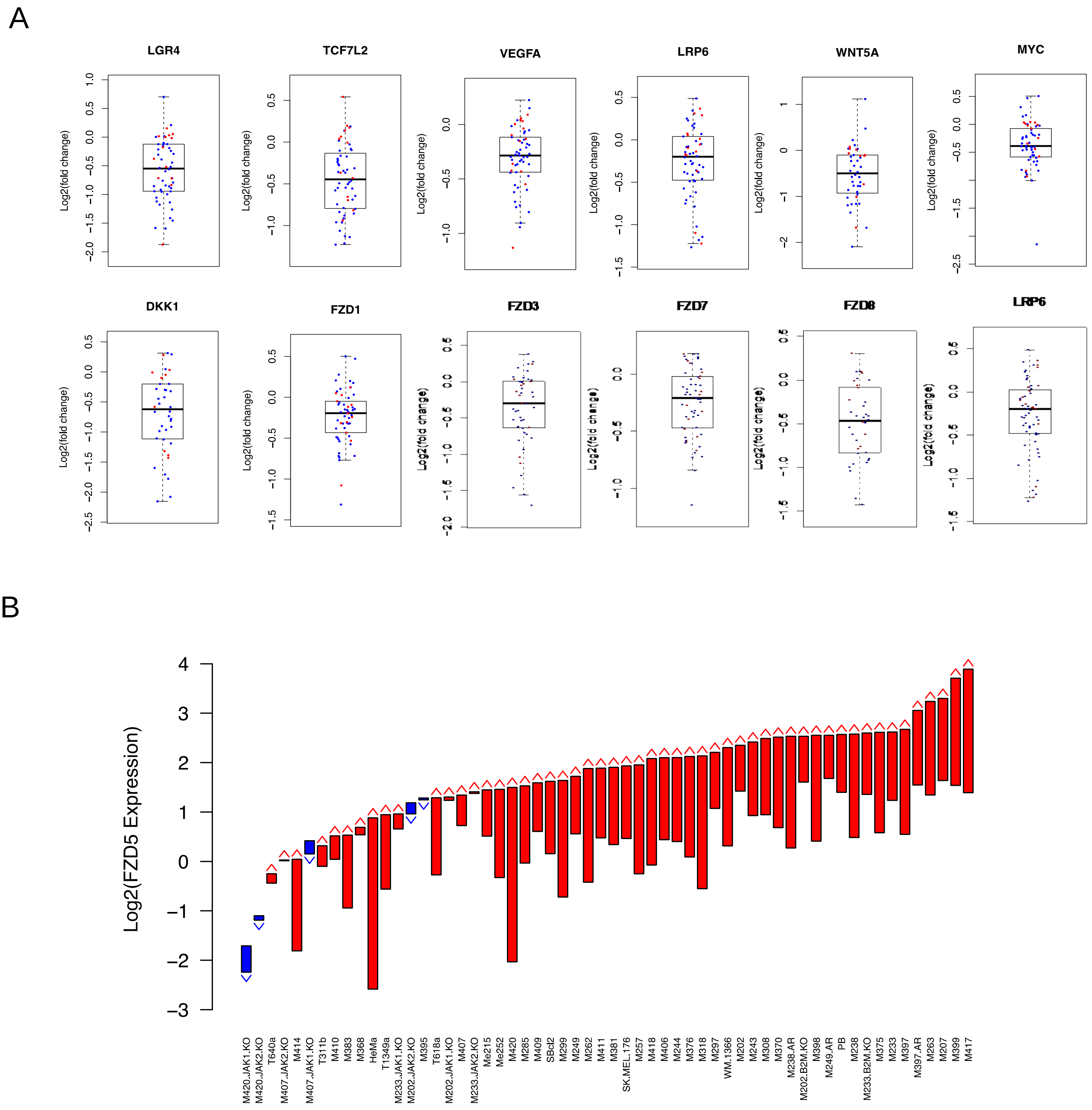

Supplemental Figure 21