Science Advances
AAAS

Supplementary Materials for

Integrated analysis of immunometabolic interactions in Down syndrome

Lucas A. Gillenwater *et al.*

Corresponding author: Joaquin M. Espinosa, joaquin.espinosa@cuanschutz.edu;
James C. Costello, james.costello@cuanschutz.edu

Sci. Adv. **10**, eadq3073 (2024)
DOI: 10.1126/sciadv.adq3073

This PDF file includes:

Figs. S1 to S7
Legends for tables S1 to S10

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S10

Fig. S1

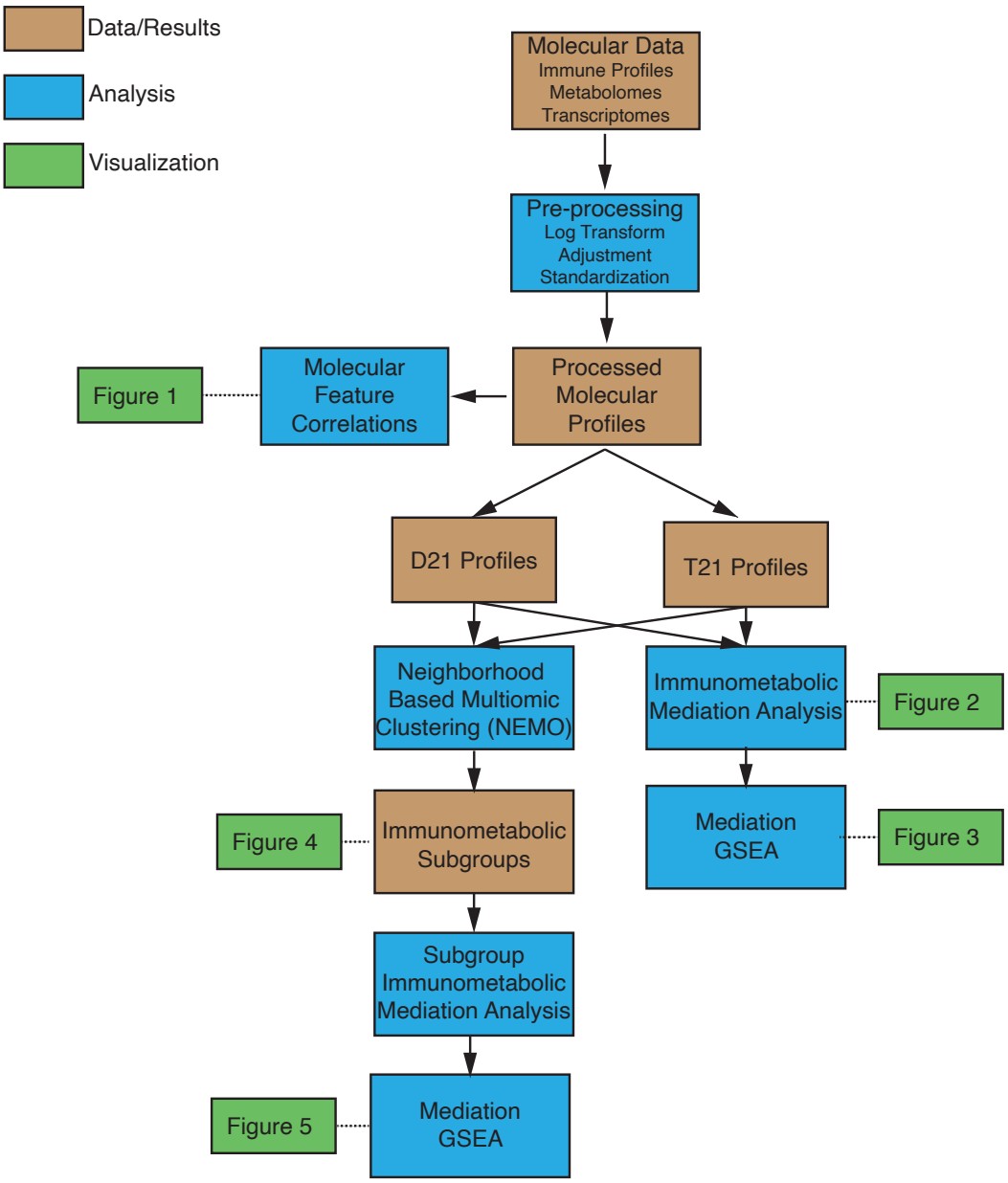


Fig. S1. Overview of immunometabolic analysis pipeline. Quantified molecular profiles were pre-processed prior to molecular feature correlation. Data was then stratified by karyotype and analyzed separately in downstream processes. Immunometabolic mediation analyses were performed on the karyotype stratified data, followed by gene set enrichment with gene set enrichment analysis (GSEA) (*44-45,70)*. Immunometabolic subgroups were identified using neighborhood based multiomic clustering (NEMO) (*71)*. Immunometabolic mediation analyses were performed on subgroups, followed by gene set enrichment with GSEA.

Fig. S2

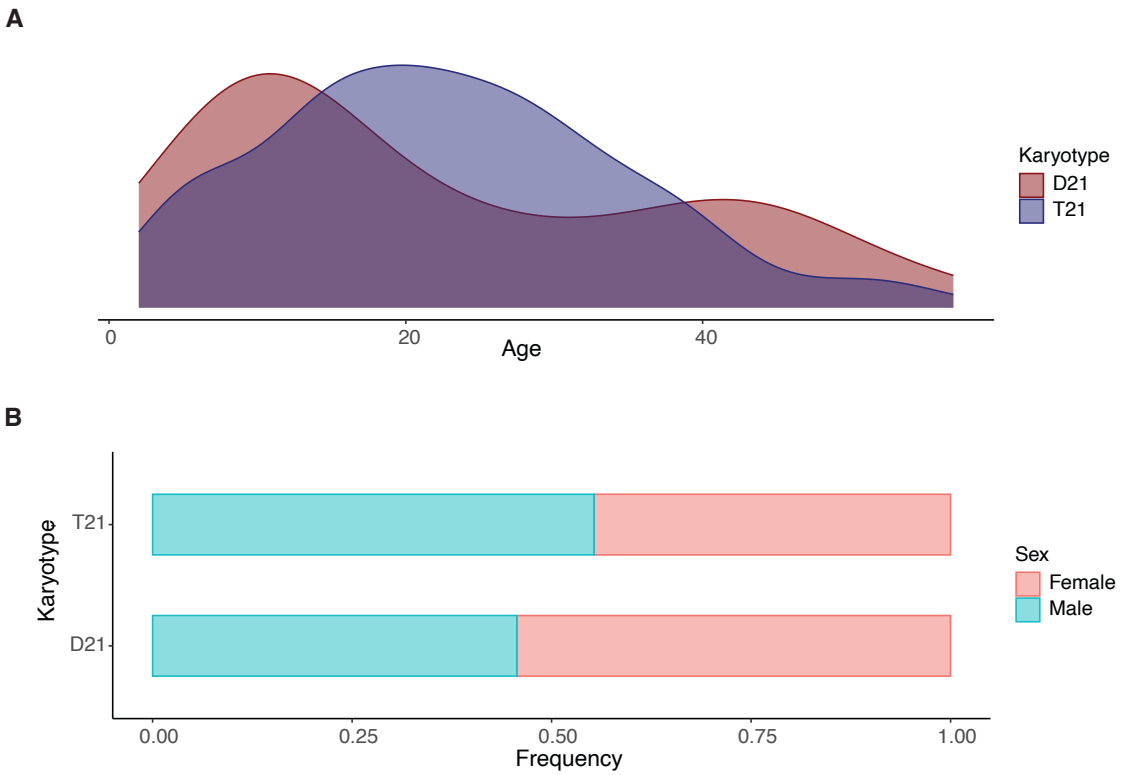


Fig. S2. Age and sex in the Human Trisome Project. (A) Density plot of the age at visit distribution for the subset of the HTP cohort with matched cytokine, metabolite, and transcriptomic data. **(B)** Bar plot of the frequency of sex by cohort.

Fig. S3

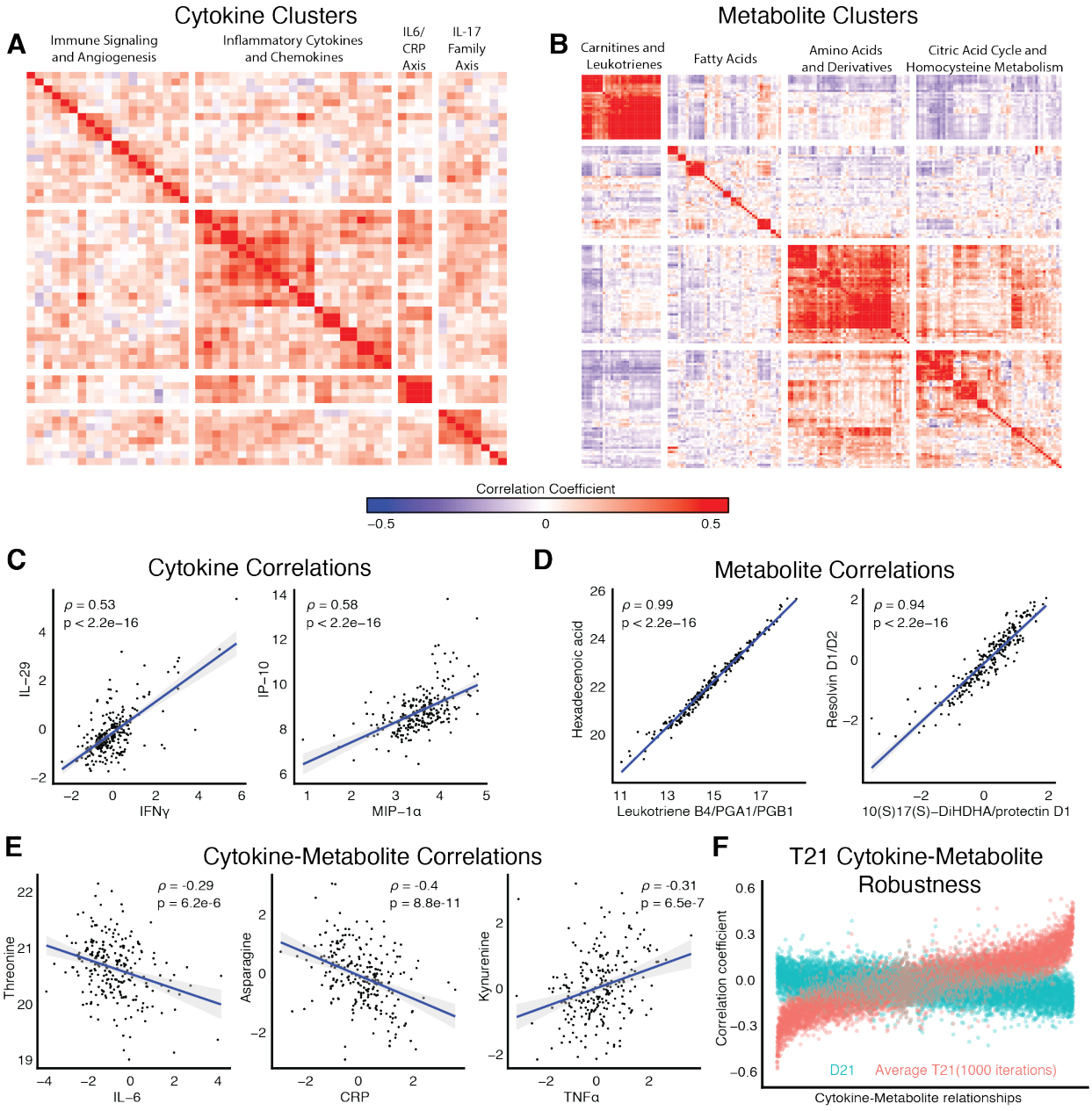


Fig. S3. Cytokine and Metabolite correlations. (A-B) Heatmaps of the Spearman correlation coefficients between standardized cytokines **(A)** and standardized metabolites **(B)**. Both axes contain identical features. **(C-E)** Scatterplots of standardized values between two cytokines (**C**), two metabolites **(D)**, and a cytokine with a metabolite **(E)**. The blue line represents the line of best fit between the features. The gray area around the lines represents the 90% confidence interval. The Spearman correlation statistics are reported. **(F)** Scatterplot of correlation coefficients between cytokines and metabolites for individuals with D21 karyotypes (blue) and the average over size matched subsets of people with T21 (red).

Fig. S4

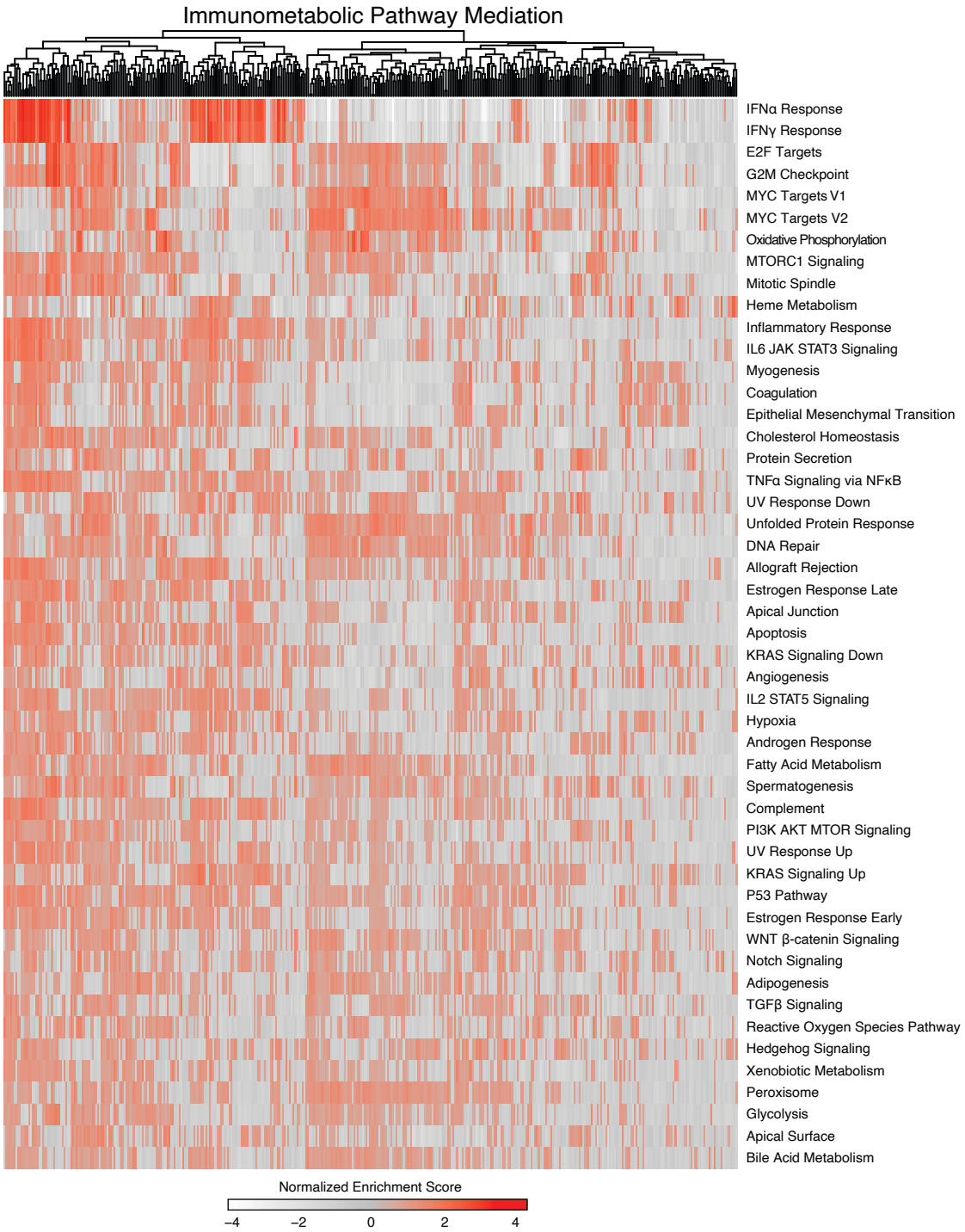


Fig. S4. Immunometabolic pathway mediation enrichment. (A) Heatmap of gene set normalized enrichment scores for significantly correlated cytokine-metabolite relationships. The gene sets are ordered from top to bottom by decreasing variance of normalized enrichment scores across cytokine metabolite relationships.

Fig. S5

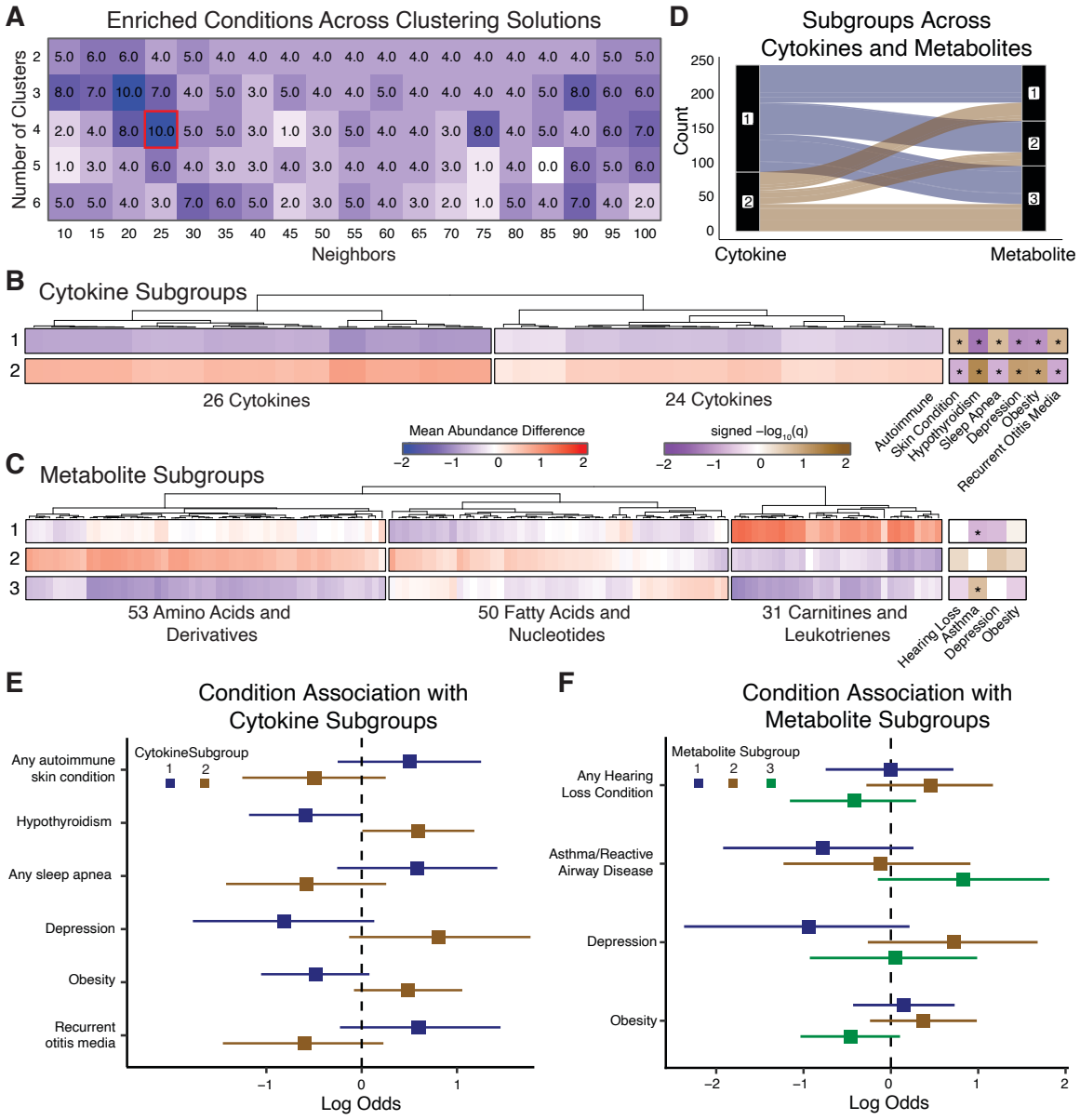
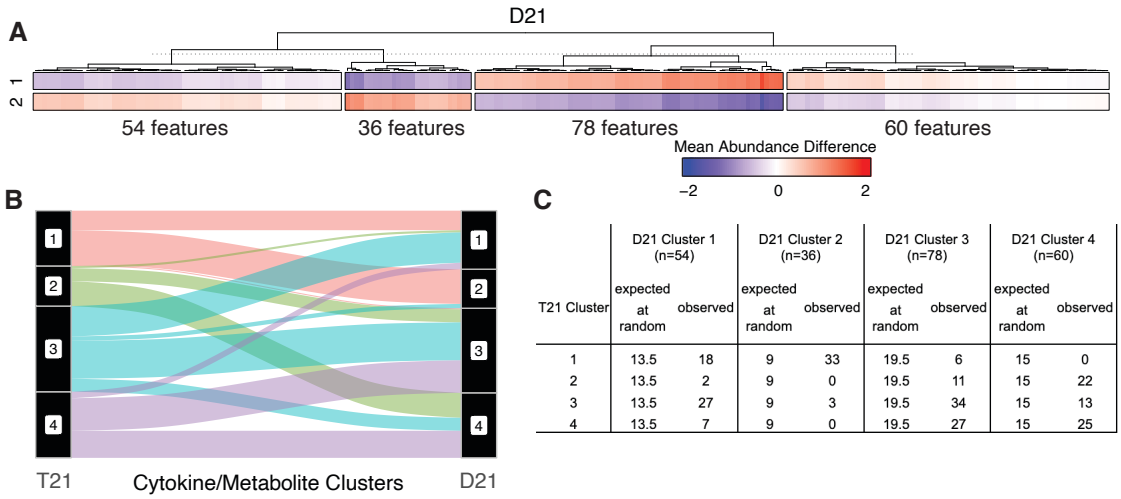


Fig. S5. Patterns across cytokine and metabolite profiles alone. (A) Heatmap of the number of enriched co-occurring conditions (q < 0.2) over the number of clusters for different nearest neighbor assignments in the NEMO algorithm. **(B-C)** Heatmaps representing the difference in mean feature abundances (IMS vs. all others) and co-occurring condition enrichment across clusters of people with DS based on cytokine profiling **(B)** and metabolite profiling **(C)**. The features along the x-axis are hierarchically clustered by the difference in mean feature abundance and grouped according to k-means clustering with a k=4. The stars indicate an FDR < 0.2. **(D)** Alluvial plot demonstrating the distribution of individuals across subgroups based on cytokine profiles or metabolite profiles. **(E-F)** Forest plots of the log odds for a reported co-occurring condition for people in each cluster based on cytokine profiling **(E)** or metabolite profiling **(F).**

Fig. S6



T21 Cluster	D21 Cluster 1 (n=54) expected at random	D21 Cluster 1 (n=54) observed	D21 Cluster 2 (n=36) expected at random	D21 Cluster 2 (n=36) observed	D21 Cluster 3 (n=78) expected at random	D21 Cluster 3 (n=78) observed	D21 Cluster 4 (n=60) expected at random	D21 Cluster 4 (n=60) observed
1	13.5	18	9	33	19.5	6	15	0
2	13.5	2	9	0	19.5	11	15	22
3	13.5	27	9	3	19.5	34	15	13
4	13.5	7	9	0	19.5	27	15	25

Fig. S6. Immunometabolic clustering in disomic individuals (D21). (**A**) Heatmap representing the difference in mean feature abundances across clusters of euploid controls based on integrated immunometabolic profiles. (**B**) Alluvial plot showing the membership between molecular clusters of cytokines and metabolites from T21 and D21 subgrouping. (**C**) Table of the expected and observed distributions of T21 cytokines-metabolites across D21 clusters.

Fig. S7

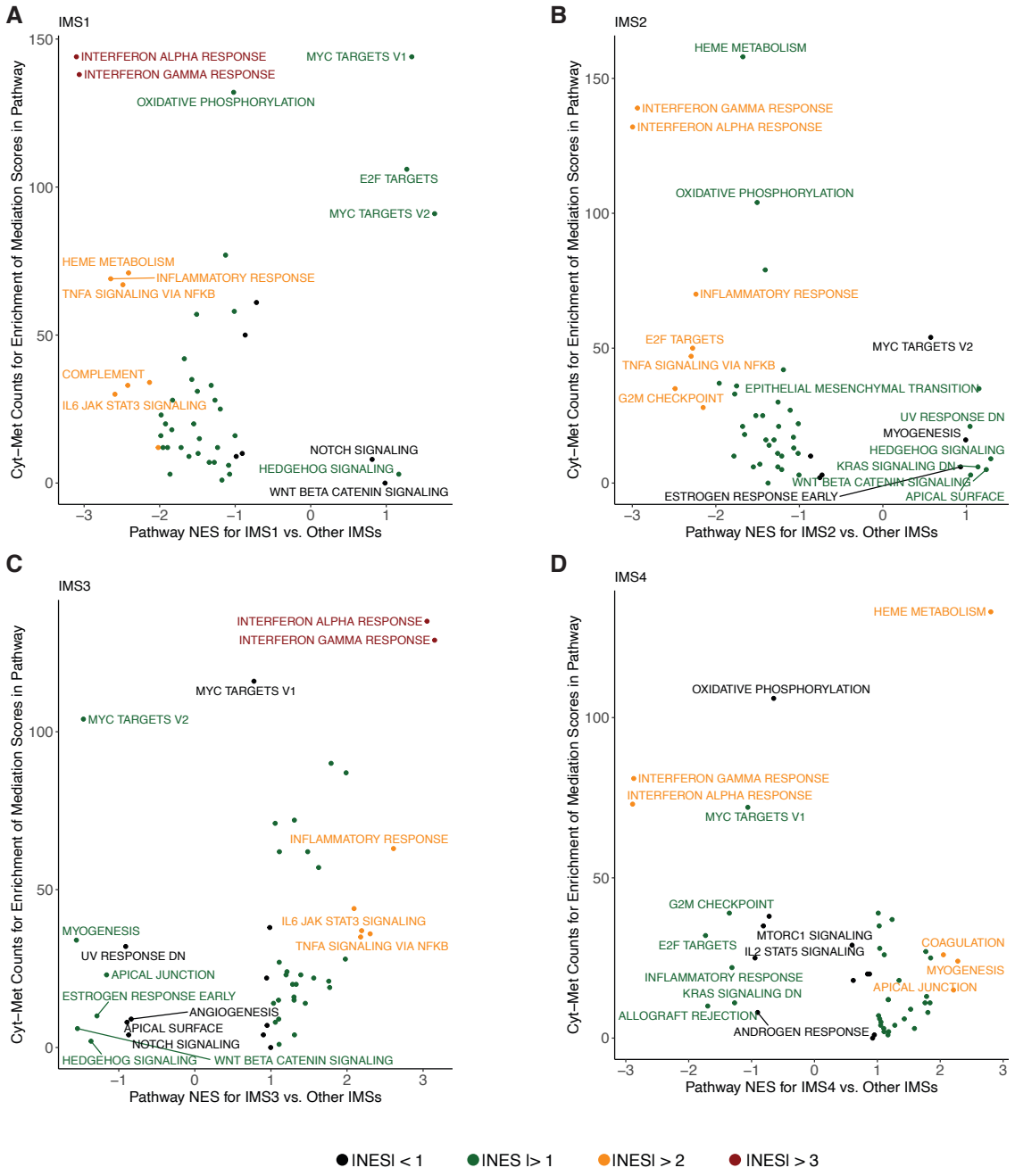


Fig. S7. Comparison of pathway enrichment for differential gene expression and mediation analysis. (**A-D**) Scatterplots of pathway normalized enrichment scores (NESs) based on differential gene expression between individuals in IMS1 and all others (**A**), IMS2 and all others (**B**), IMS3 and all others (**C**), and IMS4 and all others (**D**) compared with corresponding counts of cytokine-metabolite pairs with enrichment of mediation scores in that pathway.

Supplementary Table Legends

Table S1. Demographic Statistics. Continuous variables are summarized with mean and standard deviation and statistically compared with t-tests. Binary variables are summarized by counts and percentages and statistically compared with the chi-squared test for independence.

Tables S2-3. Differential Abundance of Cytokines/Metabolites by Karyotype. Differences in standardized abundances of cytokines (**S2**) or metabolites (**S3**) by karyotype are summarized as the mean difference and statistically compared using a Wilcoxon rank sum test. P-values were adjusted for multiple comparisons by false discovery rate (*65*).

Tables S4-5. Cytokines/Metabolites Correlations in People with T21. Spearman correlation statistics between standardized cytokine (**S4**) or metabolite (**S5**) abundances are reported.

Table S6. Cytokine Metabolite Correlations by Karyotype. Spearman correlation statistics between standardized cytokine abundances and metabolite abundances are reported. P-values were adjusted for multiple comparisons by false discovery rate (*65)*. The average correlation coefficient for iterative sub-samplings of people with T21 is reported.

Tables S7-9. NEMO Clustering Solution Evaluations. The number of enriched co-occurring conditions, differentially abundant features, minimum cluster size, and average adjusted rand index over 1000 bootstrapped iterations are reported based on immunometabolic profiles (**S7**), cytokine profiles (**S8**), or metabolite profiles (**S9**).

Table S10. Differential Gene Expression for IMSs
The log2-fold-change corresponds to the estimate of the effect, with CI.L and CI.R representing the lower and upper confidence limit, respectively. ‘t’ corresponds with the moderated t-statistic. P-values were adjusted for multiple comparisons by false discovery rate (*65*). ‘B’ represents the log-odds that the gene is differentially expressed.