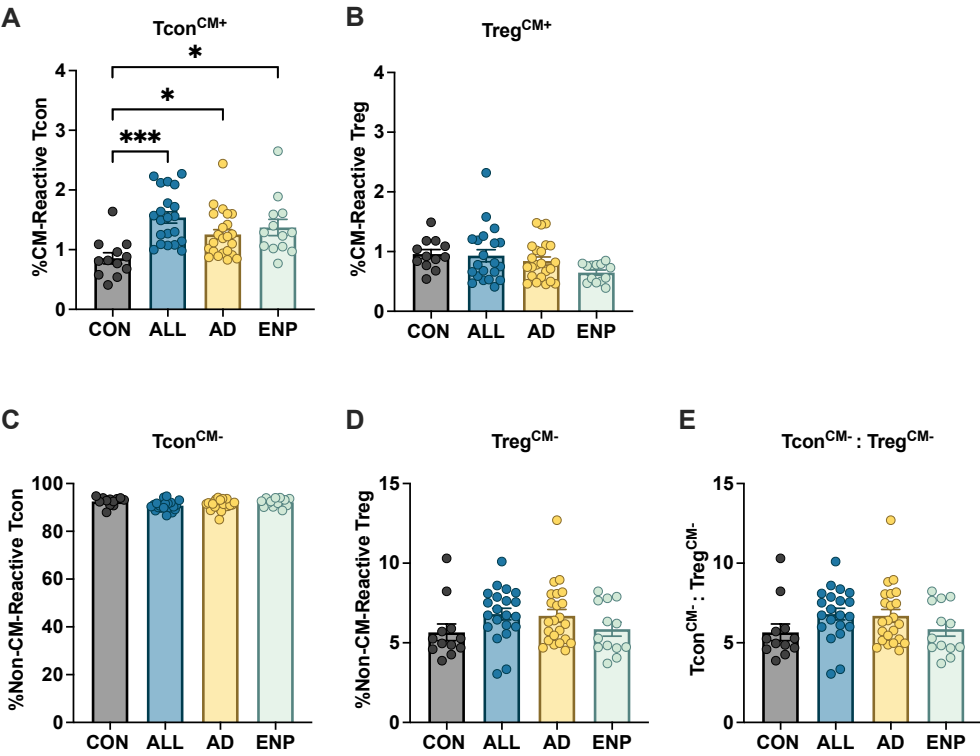


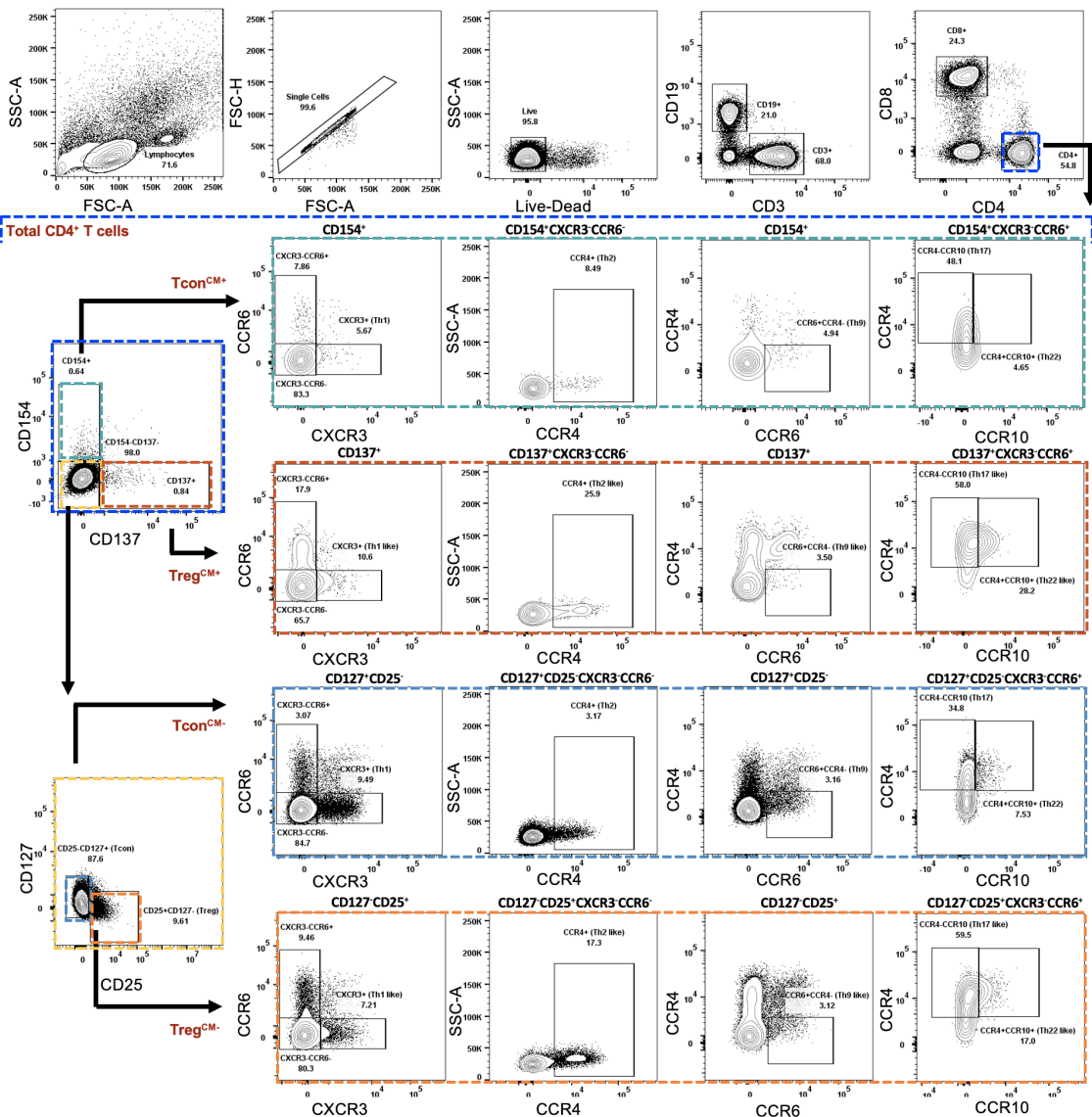
Supplementary Figure S1



Supplementary Figure S1: Characterization of conventional and regulatory CD4⁺ T cells in CMA patients. Comparison of the percentages of CM-Reactive (A) Tcon^{CM+} and (B) Treg^{CM+} cells in CMA subjects in comparison to controls. Comparative analysis of the percentages of non-CM-reactive Tcon^{CM-} (C) and Treg^{CM-} (D) in allergic subjects. (E) Non-CM-specific Tcon^{CM-}:Treg^{CM-} ratios in CMA patients. Data are represented as mean ± SEM. Quantitative variables were compared using One-way ANOVA with Tukey's test for multiple comparison. Statistical analysis is defined by * p < 0.05, ** p < 0.01 and *** p < 0.001.

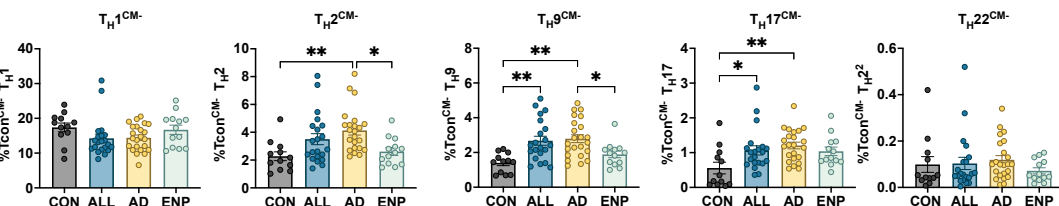
Supplementary Figure S2

PBMCs

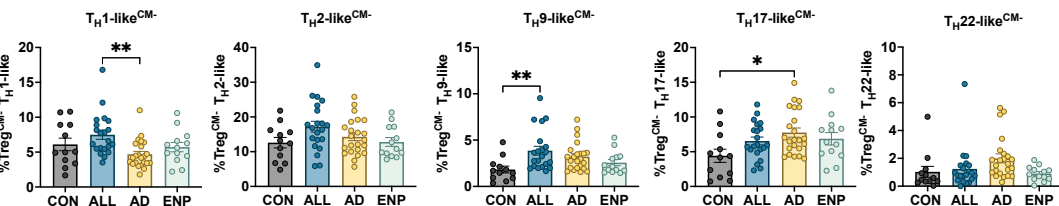


Supplementary Figure S2: Gating strategy for T_H and T_H-like sub-populations: Characteristic gating strategy for identification of cow milk Ag-reactive and Non-CM reactive T cell subsets in CMA patients analyzed by multiparametric flow cytometry analysis using a combination of surface and chemokine markers specific for different T cell subsets. CM-reactive (upper panel) and Non-CM-reactive (lower panel) - T_H1, T_H2, T_H9, T_H17 and T_H22 subsets and T_H1-like, T_H2-like, T_H9-like, T_H17-like and T_H22-like subsets were gated from total CD4⁺ T cells as demonstrated in Mahnke et al.

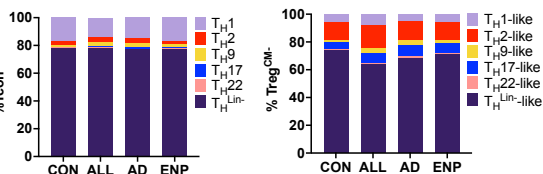
A



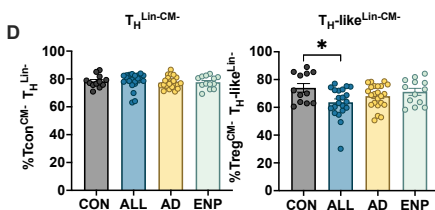
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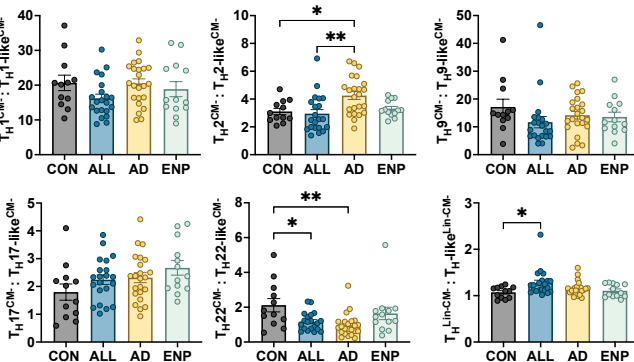
C



D



E

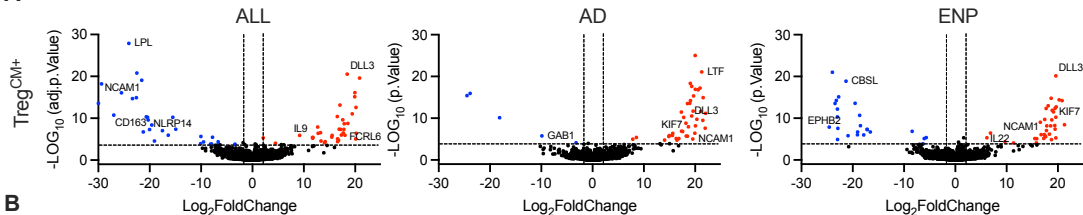


Supplementary Figure S3: Proportions of non-CM reactive T_H and T_H-like cells present among CD4⁺ T cells from CMA subjects.

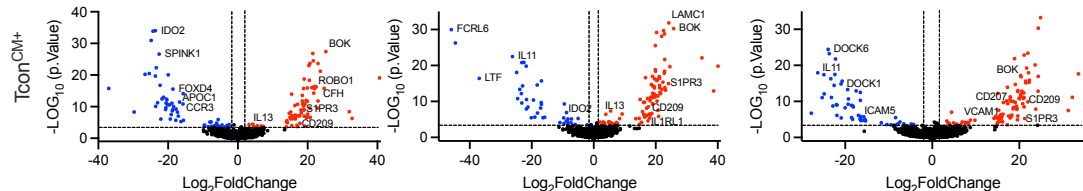
(A) Non-CM-reactive Tcon cells (CD4+CD127+CD25-CD154-) T_H cell subsets in CMA subjects: T_H1, T_H2, T_H9, T_H17 and T_H22 subsets. (B) Non-CM-reactive Treg cells (CD4+CD127-CD25+CD137-) T_H-like subsets in CMA patients: T_H1-like, T_H2-like, T_H9-like, T_H17-like and T_H22-like subsets. (C) Proportions of non-CM-reactive T_H and T_H-like cells present within Tcon and Treg subsets. (D) Non-CM-reactive T_H-Lin- subsets in CMA patients, present in Tcon and Treg subsets. (E) Ratiometric analysis of T_H/T_H-like non-CM-reactive subsets: T_H1/T_H1-like, T_H2/T_H2-like, T_H9/T_H9-like, T_H17/T_H17-like, T_H22/T_H22-like and T_H-Lin^{CM-}/T_H-Lin-like^{CM-}. Quantitative variables were compared using one-way ANOVA with Tukey's test for multiple comparison. Statistical significance is defined as *p < 0.05, **p < 0.01 and ***p < 0.001. Data are represented as mean ± SEM.

Supplementary Figure S4

A

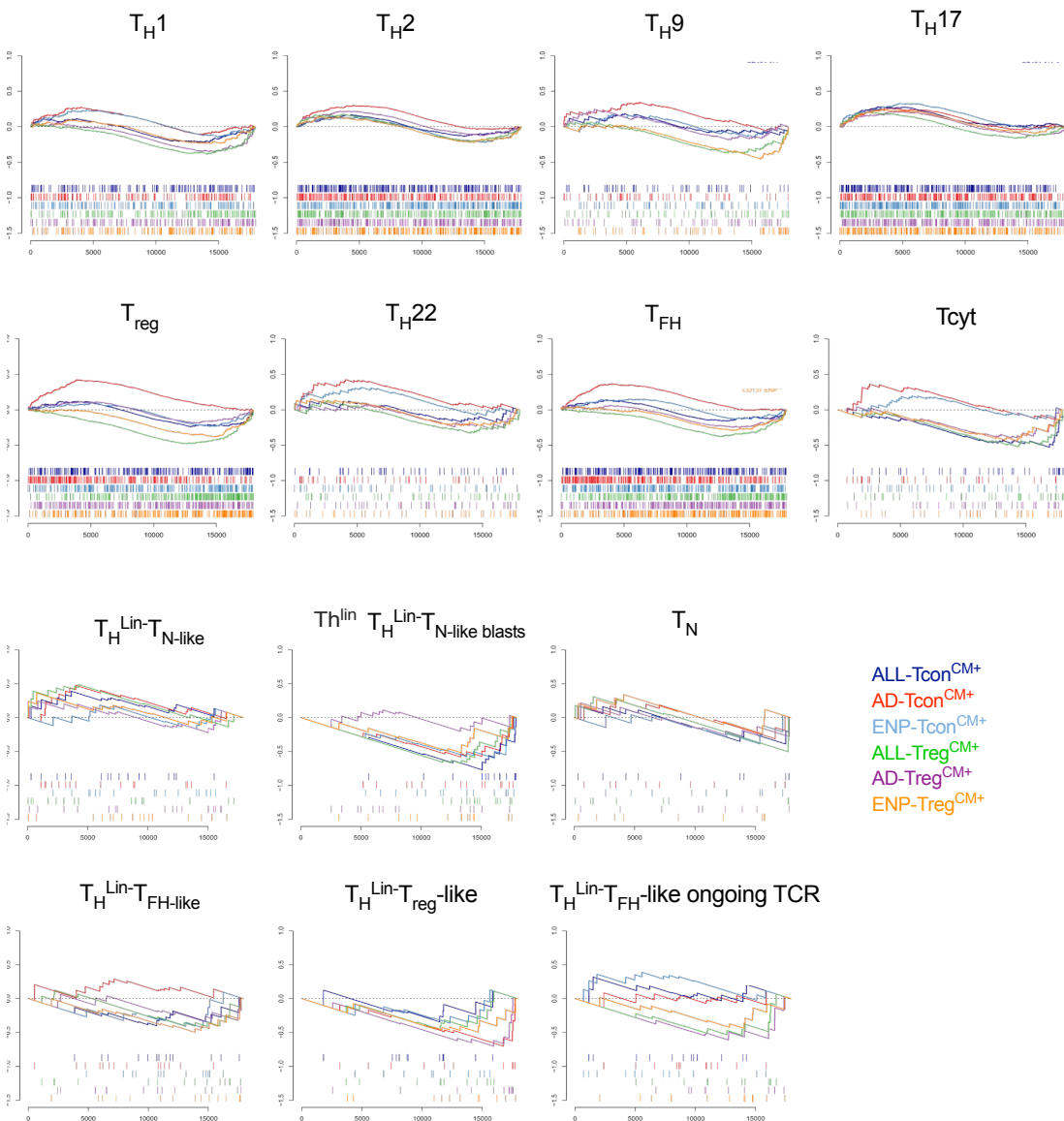


B



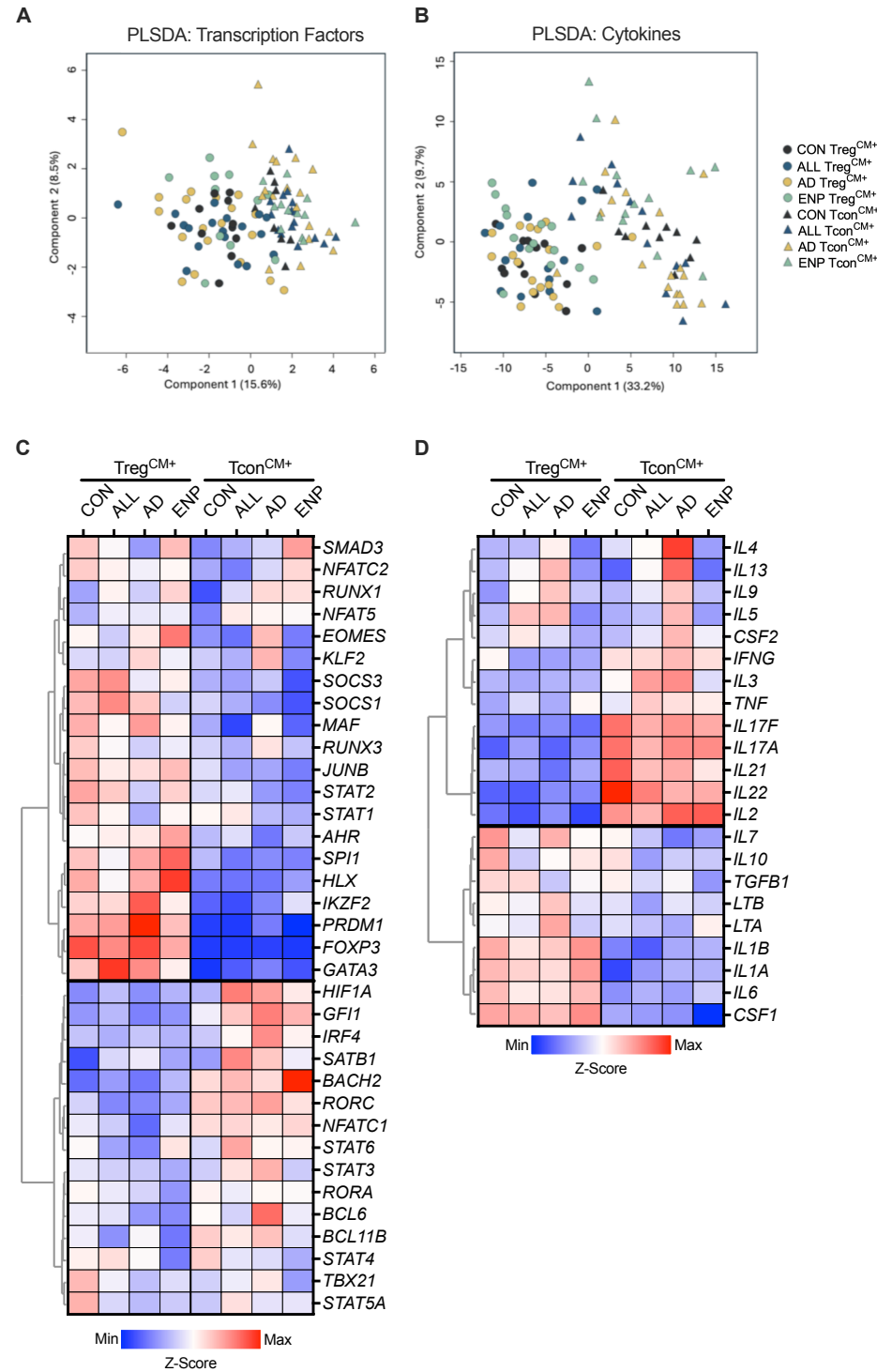
Supplementary Figure S4: Differential gene expression in CMA phenotypes vs. controls. Volcano plots showing RNA-seq data obtained from sorted Treg^{CM+} (**A**) and Tcon^{CM+} (**B**) subsets. Differential gene expression is shown comparing CMA subject group (ALL, AD or ENP) with control subjects (CON). Genes colored red indicate upregulation in CMA subject group, genes colored blue indicate downregulation. Genes with a p-value higher than an FDR threshold of 0.05 and a fold-change lower than 2 are colored black.

Supplementary Figure S5



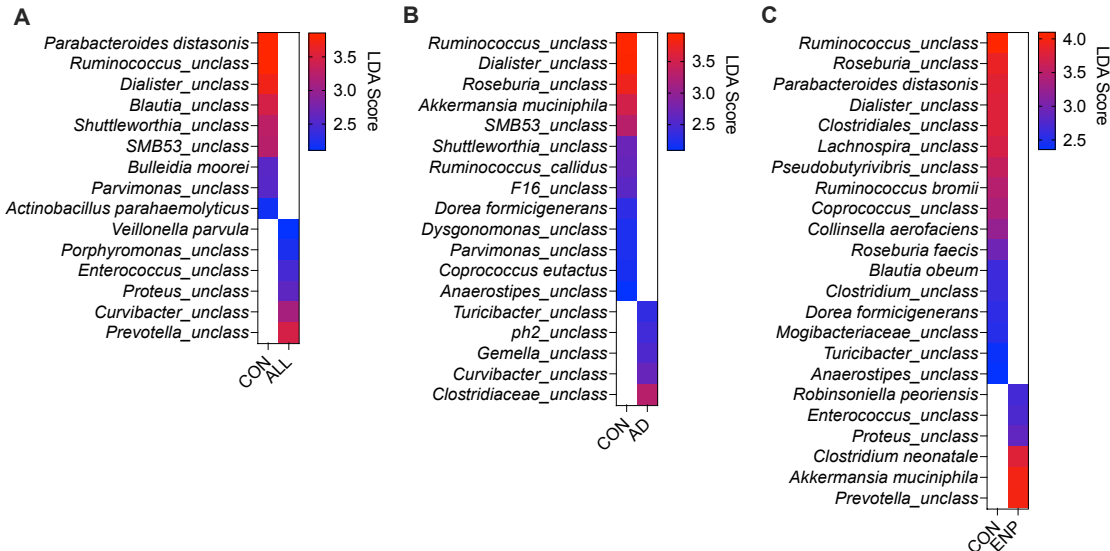
Supplementary Figure S5. Gene set enrichment analysis of T_H subsets. Gene set enrichment analysis (GSEA) of **(A)** T-helper subsets and **(B)** non-canonical hyporesponsive T-helper subsets. GSEA was conducted using RNA-seq data from sorted Treg^{CM+} and CD154⁺ Tcon^{CM+} subsets and comparisons between CON and ALL, AD or ENP groups are shown as indicated in each figure. q , false discovery rate (FDR) for NES, * <0.25 , ** <0.05 , *** <0.05 .

Supplementary Figure S6



Supplementary Figure S6. Comparison of transcription factor and cytokine expression and clustering between CD137⁺ Treg and CD154⁺ Tcon subsets. Partial least squares discriminant analysis (PLS-DA) of Treg^{CM+} and Tcon^{CM+} subsets from CON, ALL, AD and ENP subject groups using RNA-seq expression data from CD4⁺ T cell **(A)** transcription factors or **(B)** cytokines. Heat map of CD4⁺ T cell **(C)** TFs or **(D)** cytokines organized by hierarchical clustering of z-score-normalized RNAseq expression data.

Supplementary Figure S7



Supplementary Figure S7. LEfSe analysis of microbiome at species level. Identification of dominant bacteria prevalent in the different CMA phenotypes. **(A-C)** LEfSe analysis showing uniquely abundant bacteria with LDA score above 2 between **(A)** CON and ALL, **(B)** CON and AD and **(C)** CON and ENP.