

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. *FOXP3* locus activity in IPEX Treg-like cells

Read mapping to the *FOXP3* locus from sorted CD4⁺ CD25⁺ CD127^{low} cells in HD and IPEX samples from both cohorts (population RNAseq). Arrow indicates mutation. Mutated bases are colored. All samples from both cohorts are shown as well as control traces from representative Tconv samples (sorted CD4⁺ CD25⁻ CD127⁺ cells)

Fig. S2. Flow cytometric analysis of Treg-like cells in IPEX

a, b. Flow cytometric analysis of HD and IPEX CD4⁺ cells: CD25 and CD127 (a), *FOXP3* and HELIOS (b). Gated cells are HD Tregs and IPEX Treg-like cells. All samples from both cohorts are shown. See also Fig. 1.

Fig. S3. Identification of Treg-like cells in IPEX by flow-tSNE

Flow-tSNE plots of CD3⁺ CD4⁺ cells using flow cytometric expression of CD3, CD4, CD25, CD127, HELIOS, CD45RA and *FOXP3*. Color represents scaled expression of CD25, *FOXP3*, HELIOS and CD127. All samples from both cohorts are shown.

Fig. S4. IPEX Treg-like cells maintain expression of the Treg signature, but with increased noise

a. Same ranked FC plots as 2b. showing the distribution of each Treg signature gene expression in IPEX and HD donors. The y-axis displays the gene expression foldchange in each donor over the average expression in HD Tconv. Genes are ranked by the average Treg over Tconv

foldchange in HD. Regression lines are shown in blue. Age (in years) and summary statistics (intensity (index) and variability (coefficient of variation, CV) of expression the Treg signature), are shown for each sample.

b. Clinical correlations with the Treg UP index (computed in a). RAPA, rapamycin, CS, corticosteroids, CNI, calcineurin inhibitor, IV Ig, intravenous immunoglobulins. * Mann Whitney test $p < 0.05$.

c. Ranked FC plot showing the distribution of expression of the Treg signature gene in IPEX (orange) and HD (green) Treg samples. The y-axis corresponds to the expression ratio in IPEX Treg-like cells relative to the mean in HD Tregs. Genes are ranked by their average shift in IPEX samples.

d. Average expression of cytokine-encoding transcripts across Treg and Tconvs in IPEX (mean and SEM, normalized to mean of HD Tregs or Tconvs). * two-sided t-test $p < 0.05$.

Fig. S5. IPEX signature: reproducibility, independence from the Treg Up index, and clinical correlates.

a. IPEX/HD expression ratio in cohort 1 vs cohort 2 showing the reproducibility of the IPEX effect between the two cohorts.

b. Absence of correlation between the IPEX index (x-axis) and Treg UP index (y-axis)

c. Clinical correlations with the IPEX index. RAPA, rapamycin, CS, corticosteroids, CNI, calcineurin inhibitor, IV Ig, intravenous immunoglobulins. All comparison showed no significant differences (two-sided t.test).

d. Proportion of human endogenous retroviruses (HERV) mapped reads in IPEX and HD Treg and Tconv samples.

Fig. S6. scRNAseq analysis of CD4+ cells in IPEX and HD reveals a stable IPEX signature that affect all CD4+ cells (resting, activated Tconvs and Tregs)

a. Same UMAP plots as 3a., showing the reproducible segregation of HD and IPEX samples: plots are split by experiment and cohort. Individual HD and IPEX donors are highlighted in different colors.

b. Same UMAP as 3a. showing the stability of the IPEX transcriptomic signature: before and after treatment initiation (P7), over several days (B4a and B4b, four days apart) and or years (> 2 years apart, P4), and across two different experiments (B5, technical replicates)

c. UMAP1 correlates with the IPEX signature defined by population RNAseq (Pearson correlation $r = 0.75$). y-axis shows the IPEX/HD expression ratio of the IPEX signature genes (population RNAseq). The x axis shows their correlation with UMAP1 in scRNAseq.

d. Same UMAP as 3a. showing each HD and IPEX donor individually. Blue, green, and red cells represent resting Tconvs, activated Tconvs, and Tregs, respectively.

Fig. S7. Identification of resting Tconvs, activated Tconvs and Tregs in IPEX by scRNAseq

a. Single-cell biclustering heatmap of canonical resting Tconv, activated Tconv and Treg genes. Top ribbons indicate donor origin and annotations for every single cell.

b. Similar proportions of Tregs, resting and activated Tconvs in total CD4+ cells in HD and IPEX (scRNAseq).

Fig. S8. Heterogeneous Treg-like cells in IPEX (A and B types) identified by scRNAseq

a. Same UMAP as 3a. showing each HD and IPEX donor individually. Blue, green, and red cells represent resting Tconvs, activated Tconvs, and Tregs, respectively. *FOXP3*-expressing cells (RNA) are in yellow. An arrow indicates type-A IPEX Tregs, overlapping with HD Tregs.

b. Down-tuning of the IPEX signature in type A IPEX Tregs vs. type B IPEX Tregs. Volcano plot comparing the gene expression profiles of type A versus type B IPEX Tregs (p values from two-sided t.test). Up- and downregulated signature genes are highlighted (red and blue, respectively). χ^2 -test p values.

Fig. S9. Identification of resting Tconvs, activated Tconvs and Tregs in $\Delta Foxp3$ mice by scRNAseq

a. Same UMAP as 5a showing the individual samples in both experiments and the absence of batch effect.

b. Shared $\Delta Foxp3$ signature in spleen and lung Tregs. Top: $\Delta Foxp3$ vs WT expression ratio in spleen vs lung Tregs. Bottom: Volcano plot comparing the expression profile of $\Delta Foxp3$ vs WT lungs Tregs. Up- and downregulated $\Delta Foxp3$ signature genes are highlighted (red and blue, respectively). χ^2 -test p values, n =3 mice per group, population RNAseq.

c. Single-cell biclustering heatmap of the expression of canonical genes in WT and $\Delta Foxp3$ Tregs, resting Tconvs, and activated Tconvs. Top ribbons indicate mouse origin and cluster annotations for each single cell.

d. Proportion of Tregs, resting and activated Tconvs in total CD4+ cells in WT and $\Delta Foxp3$ mice (scRNAseq). * two-sided t.test p < 0.05 (n = 4 WT, 4 $\Delta Foxp3$ mice).

Fig. S10. Lower proportion of $\Delta Foxp3$ Tregs and absence of the $\Delta Foxp3$ signature in $\Delta Foxp3$ Tconvs and Tregs in mixed bone marrow chimera with WT cells.

a. $\Delta Foxp3$ signature expression in whole mice and mixed bone marrow chimera (50/50 WT and $\Delta Foxp3$) showing the downregulation of the $\Delta Foxp3$ signature expression in resting, activated $\Delta Foxp3$ Tconv and $\Delta Foxp3$ Tregs in 50/50 BMC. **** two-sided t.test $p < 10^{-4}$ (n = 4 WT mice, 4 $\Delta Foxp3$ mice, 3 BMC mice).

b. WT Tregs outcompete $\Delta Foxp3$ Tregs in in 50/50 BMC. Proportion of WT and $\Delta Foxp3$ Tregs, resting and activated Tconvs in total CD4+ cells. ** two-sided t.test $p < 10^{-2}$ (n = 3 mice).

Figure S1

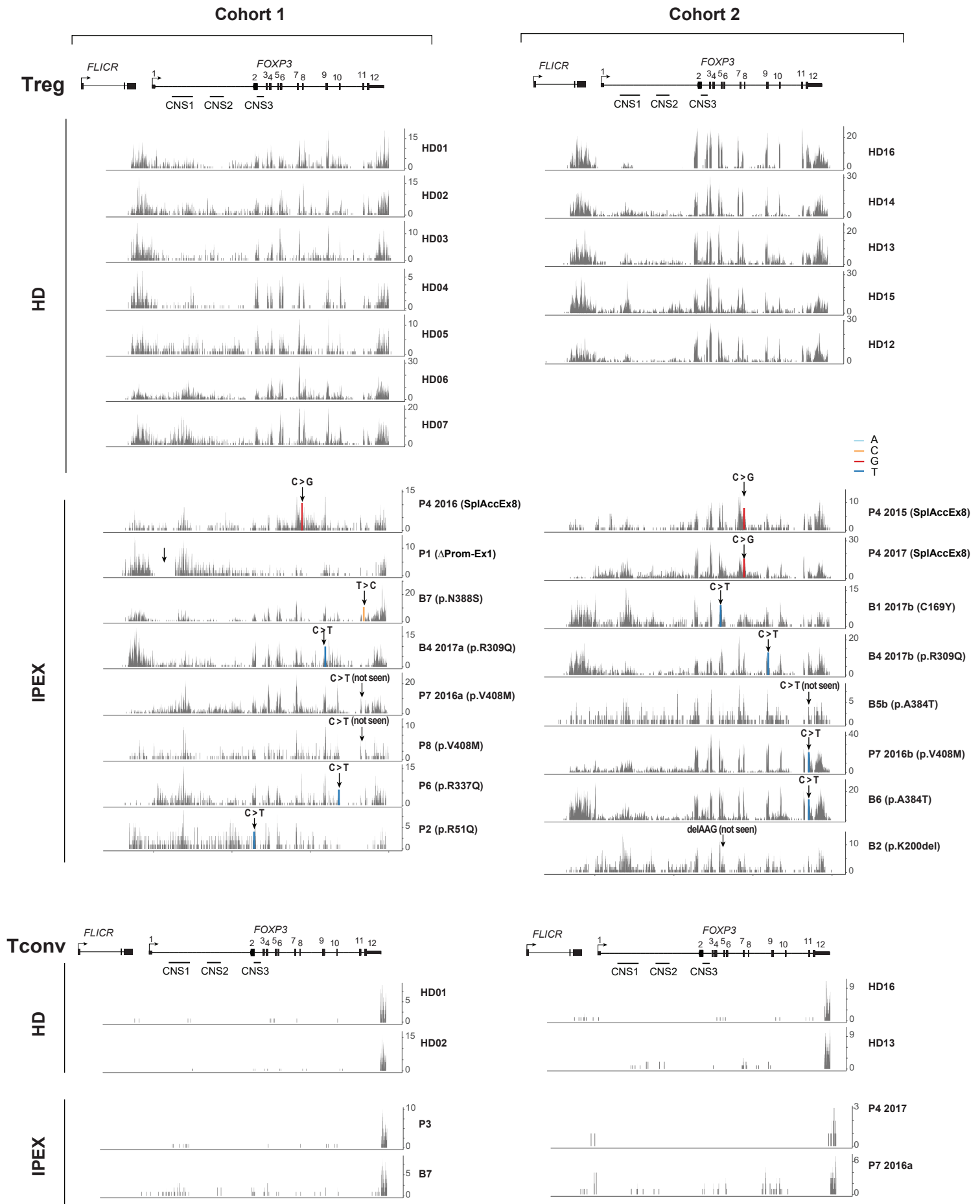


Figure S2

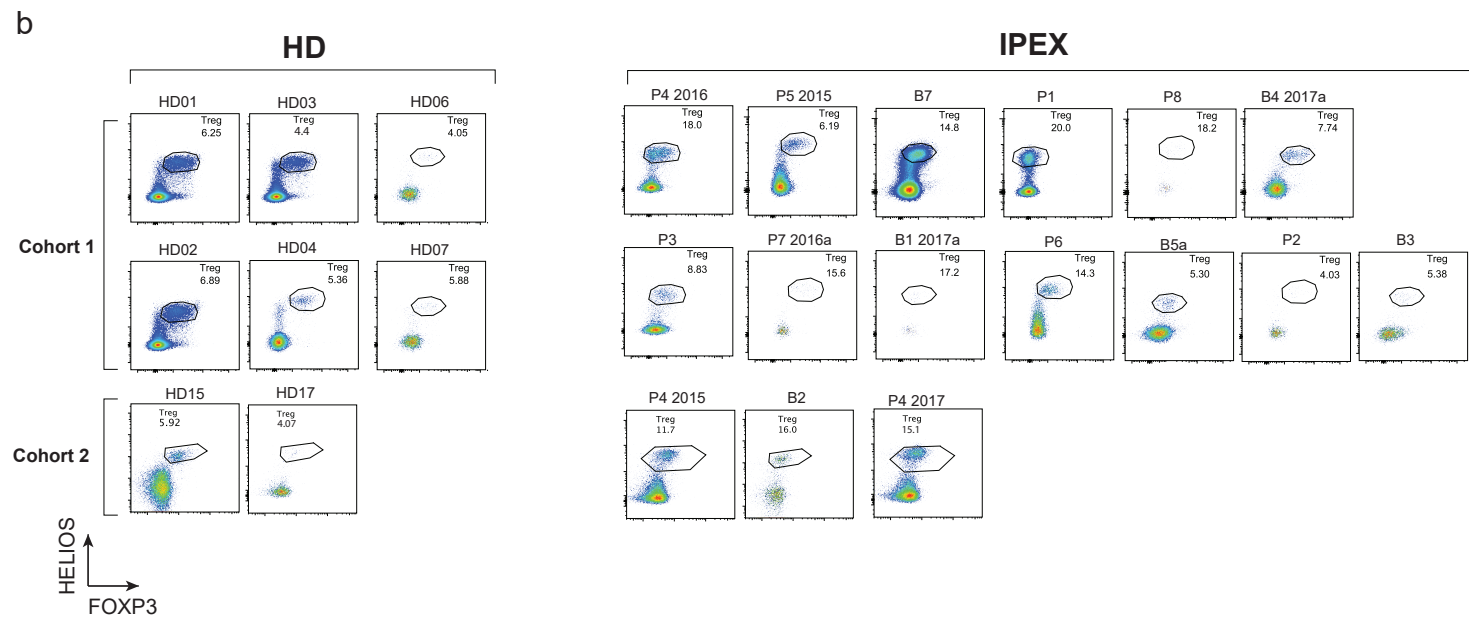
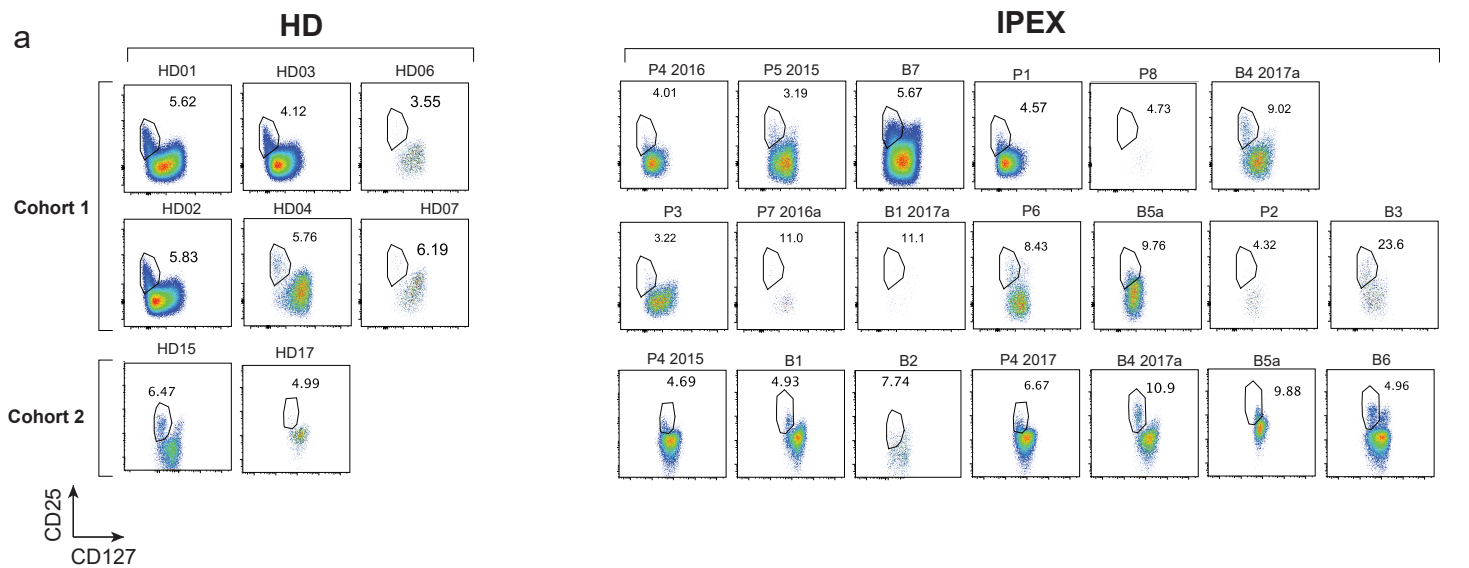


Figure S3

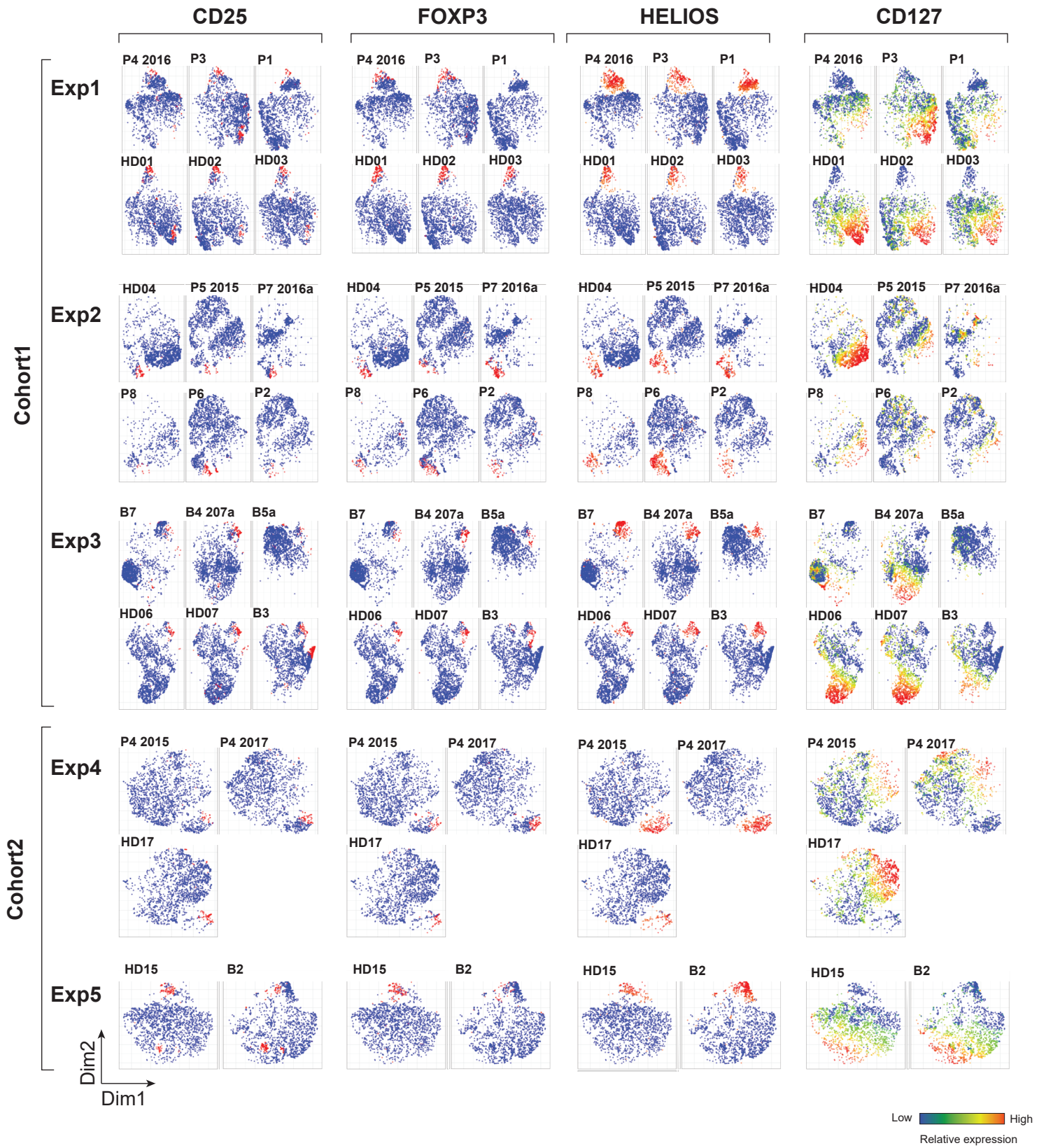
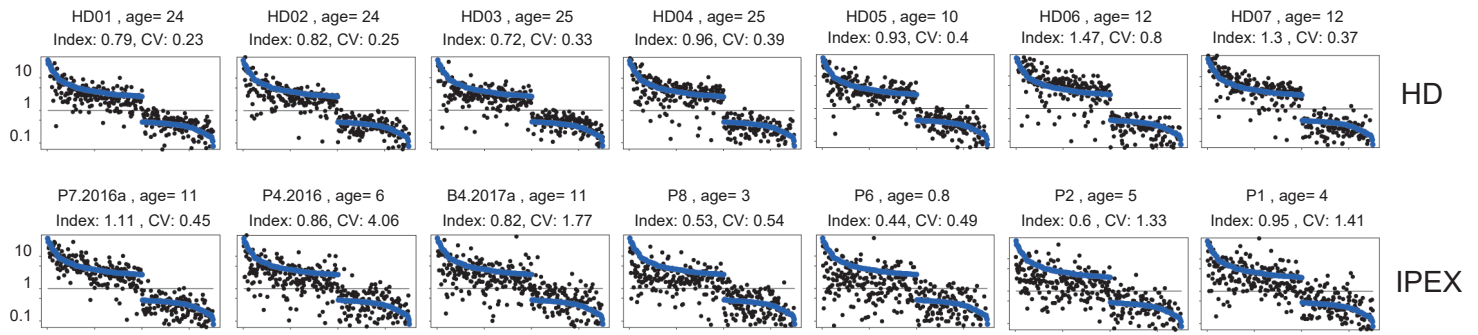
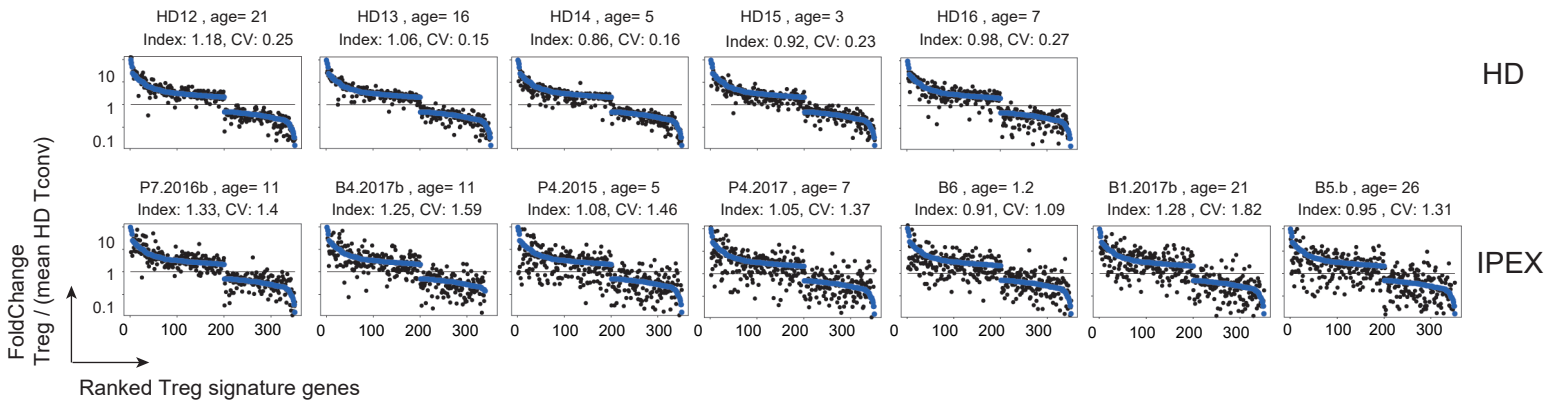


Figure S4

a Cohort 1

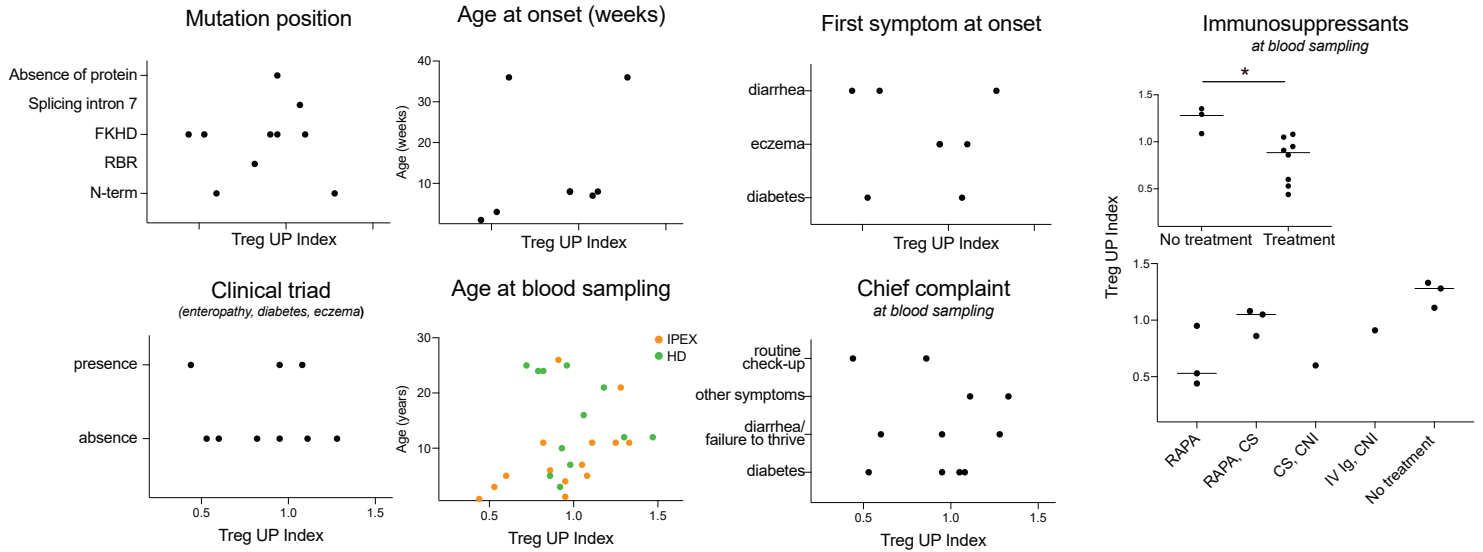


Cohort 2

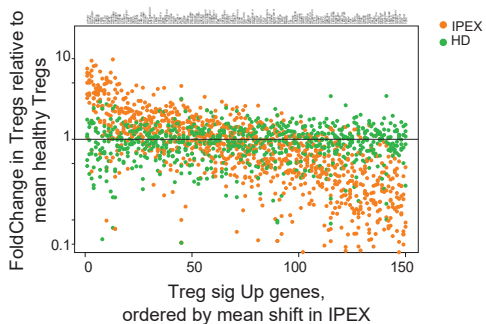


b

TREG UP INDEX



c



d

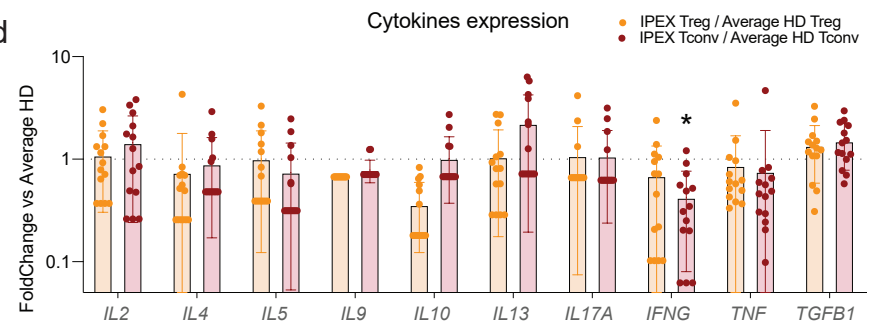
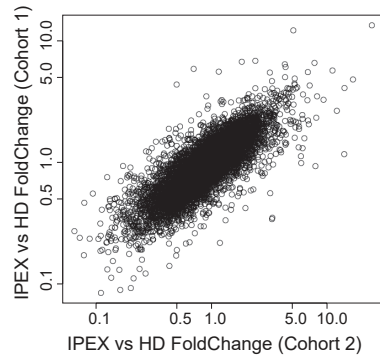
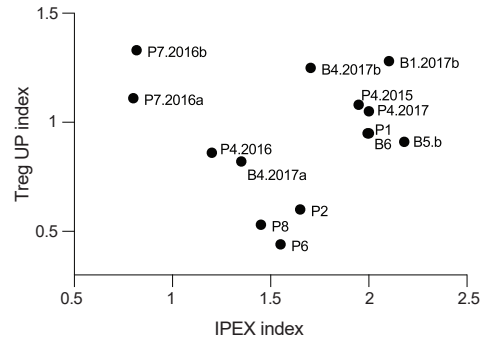


Figure S5

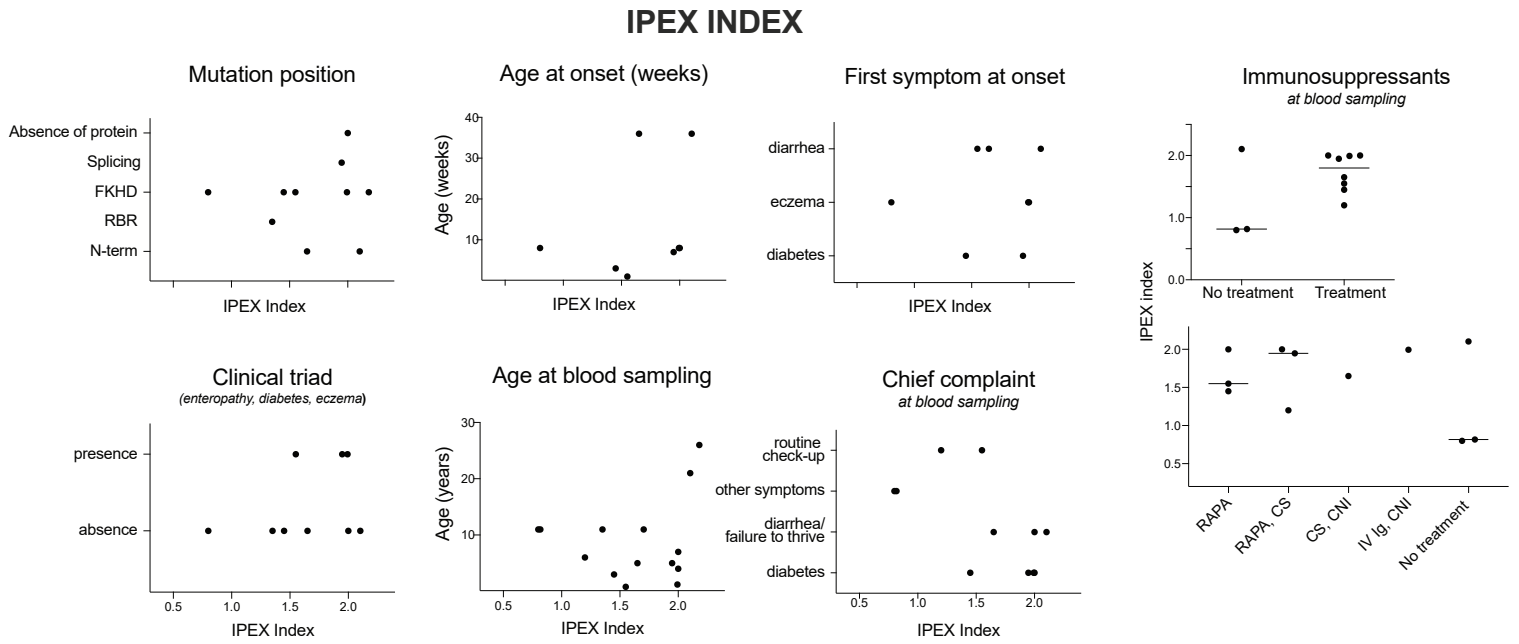
a



b



c



d

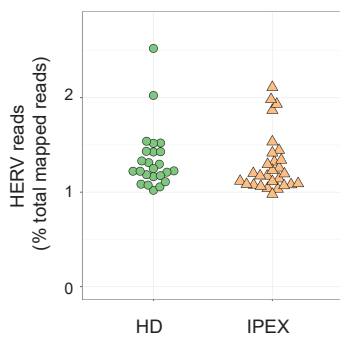


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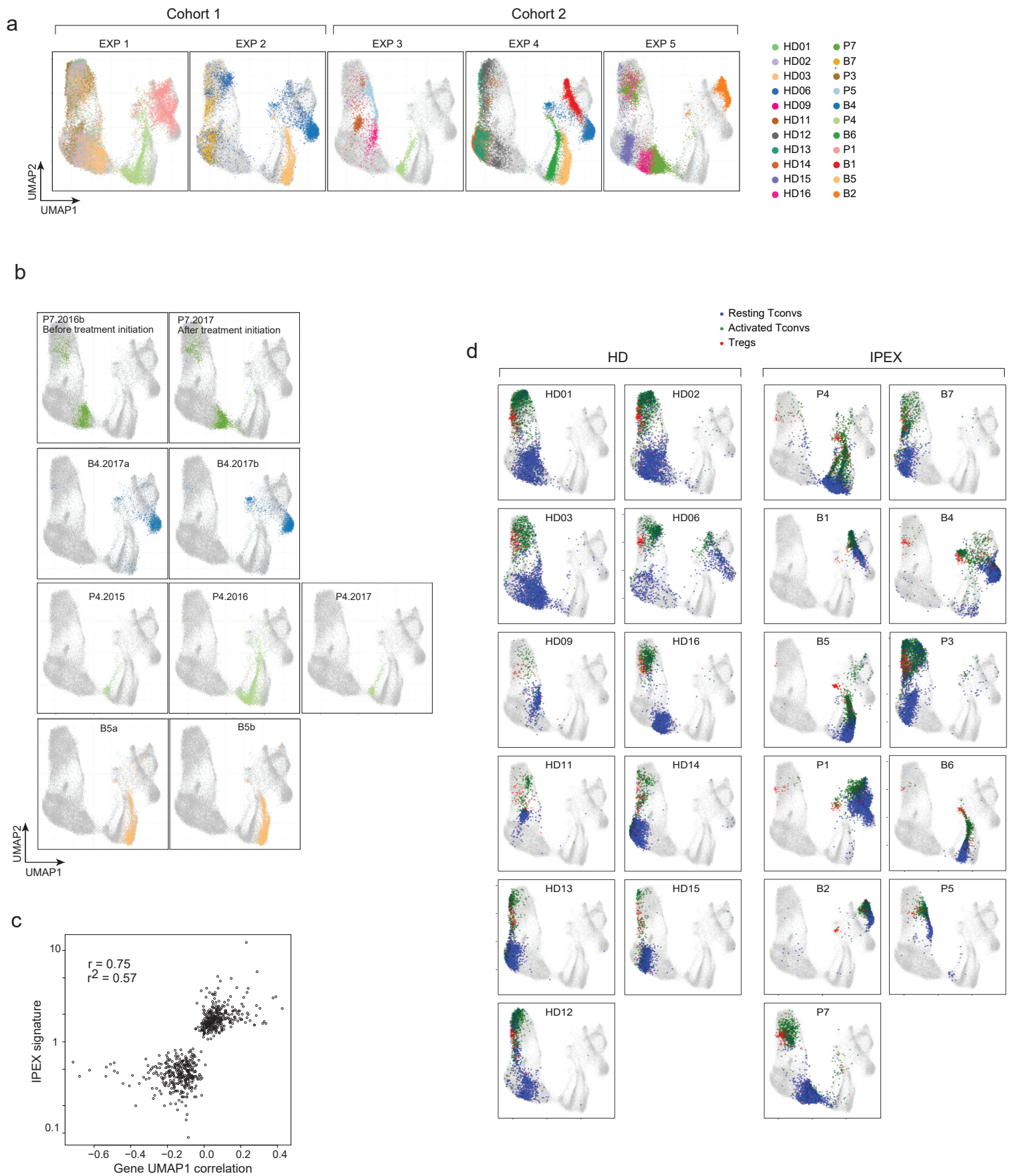


Figure S7

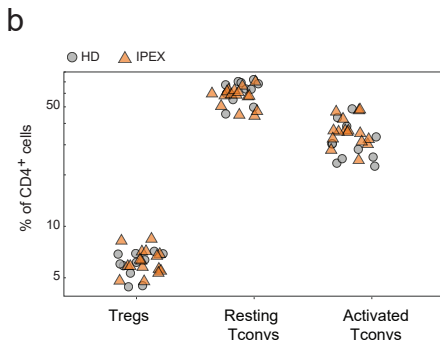
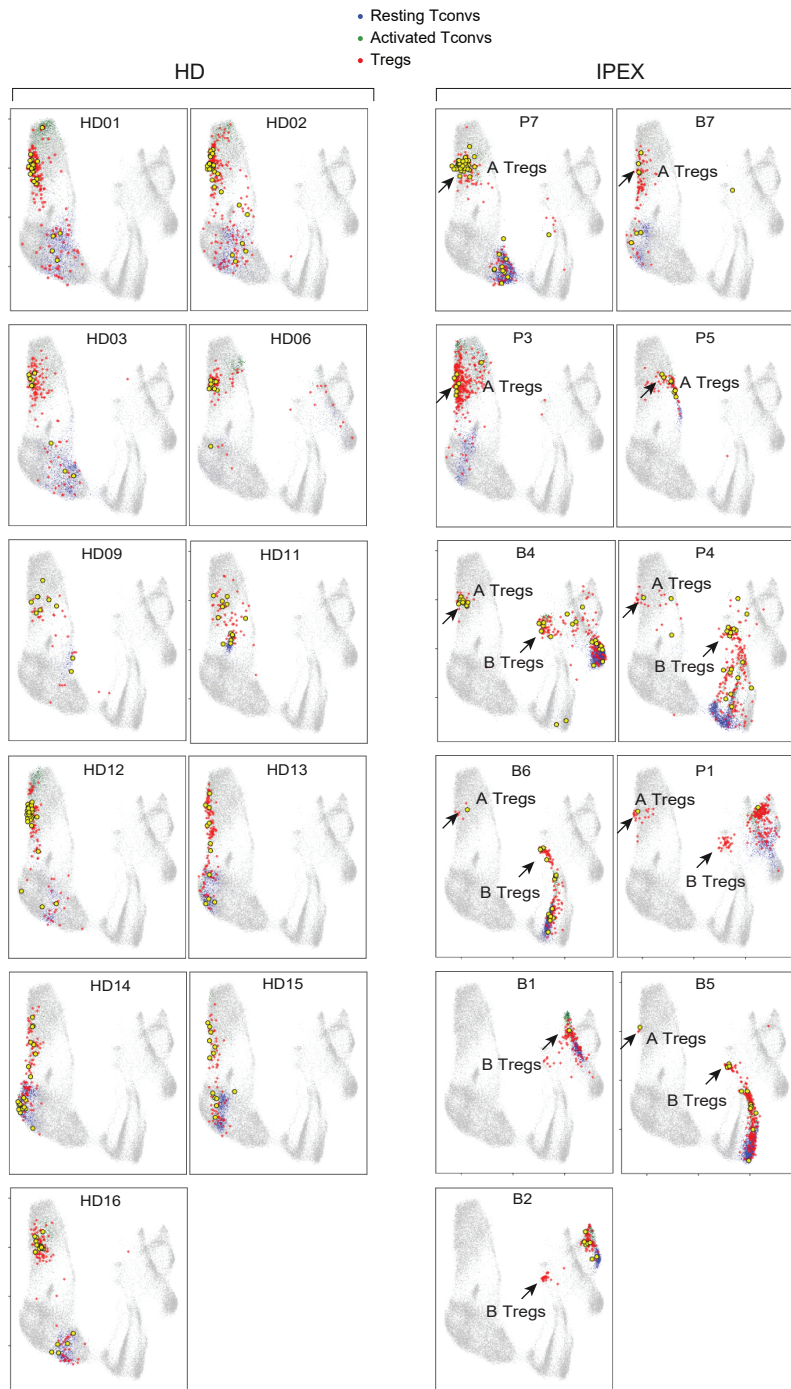


Figure S8

a



b

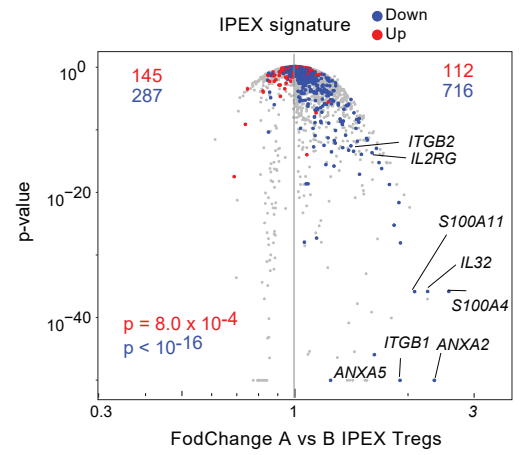


Figure S9

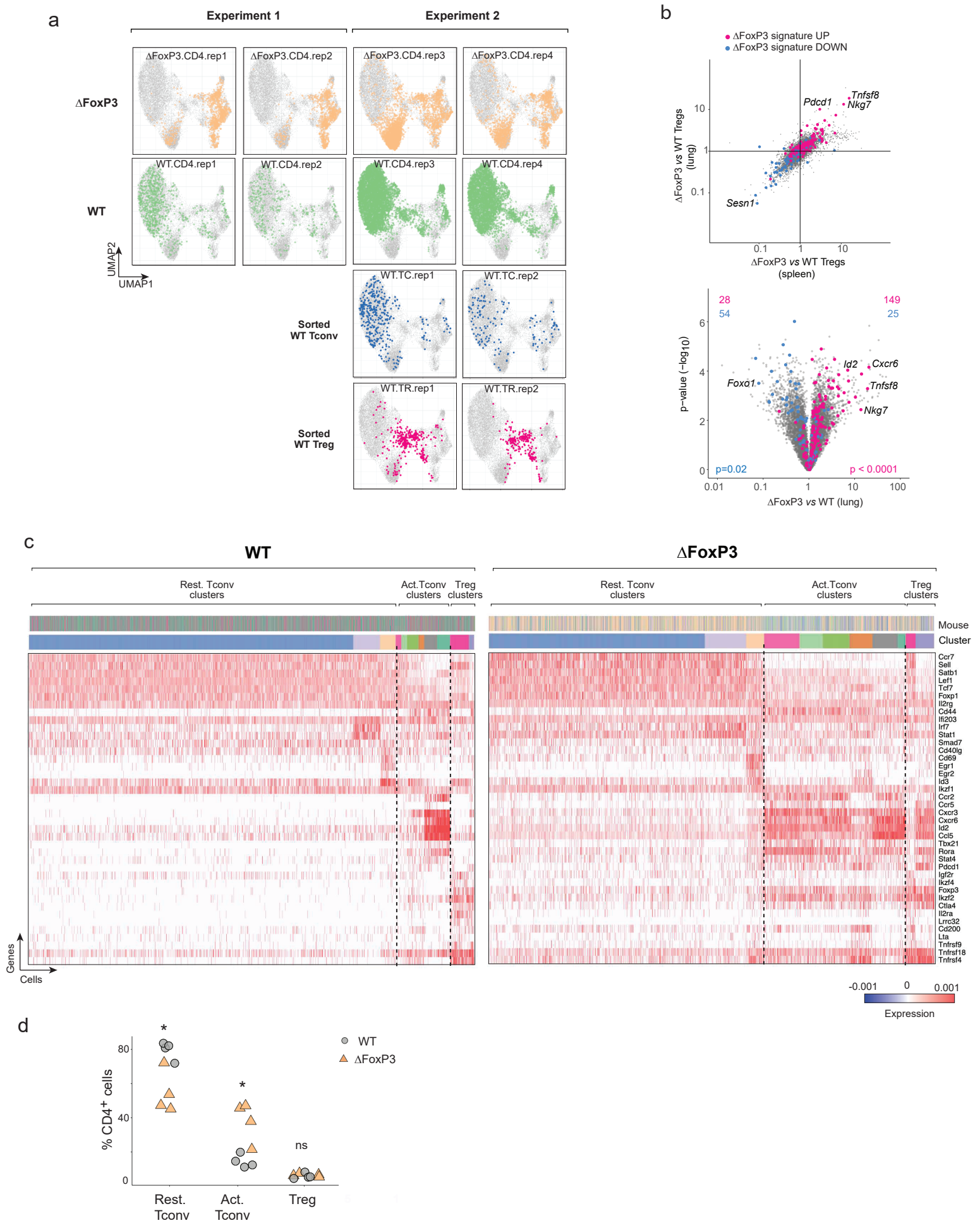


Figure S10

