Myeloid-Driven Inflammation in Prodromal Parkinson's Disease

Yoshiaki Yasumizu[1,2*,†], M. Elizabeth Deerhake[1,2*], Jeonghyeon Moon[1,], Nicholas Buitrago-Pocasangre[1], Anthony Russo[1], Haowei Wang[1], Biqing Zhu[3,4], John P. Seibyl[1], Vijaya Reddy[1], Qianchang Wang[5], Karen J Neish[5], Maria Grazia Spillantini[2,6], David A. Posner[5], Menna Clatworthy[5], Tomokazu S. Sumida[1,7], Erin E. Longbrake[1], Jesse M. Cedarbaum[1,2], Le Zhang[1,2,8,*,†], David A. Hafler*[1,2,7,9,†]

1. Department of Neurology, Yale School of Medicine, New Haven, CT, 06510, USA.
2. Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA.
3. Department of Biostatistics, Yale School of Public Health, New Haven, CT, 06510, USA.
4. Program of Computational Biology and Bioinformatics, Yale University, New Haven, CT 06510, USA.
5. Molecular Immunity Unit, University of Cambridge Department of Medicine, Cambridge, UK
6. Department of Clinical Neurosciences, University of Cambridge Department of Medicine, Cambridge, UK
7. Department of Immunobiology, Yale School of Medicine, New Haven, CT, 06510, USA.
8. Department of Neuroscience, Yale School of Medicine, New Haven, CT, 06510, USA.
9. Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA.

* Equally contributed

† Correspondence

Abstract

We hypothesized that prodromal Parkinson's Disease (PD), characterized by REM Sleep Behavior Disorder and hyposmia, is an inflammatory disorder initiated in the gut that evolves into a neurodegenerative process manifest as classical PD. To test this hypothesis, we performed single-cell RNAseq analysis of cerebrospinal fluid (CSF) and blood from 118 individuals, comparing healthy subjects with prodromal PD and manifest PD to patients with the central nervous system (CNS) autoimmune disease, multiple sclerosis (MS). Surprisingly, we identified increased numbers of immune cells in the CSF of patients with prodromal PD. Single-cell RNA sequencing revealed increases in CSF-specific microglia-like macrophages with enhanced JAK-STAT and TNFα signaling signatures in prodromal PD. CSF macrophages exhibited similar transcriptional profiles to dural macrophages and gut muscularis macrophages from α-synuclein-expressing PD model mice. Finally, shared memory T cell clones were found in humans between gut and CSF T cells. These findings uncover a myeloid-mediated TNFα inflammatory process in the CNS of patients with prodromal PD, with an immunological linkage between the gut and the CNS.

Introduction

Inflammation involving both innate and adaptive immune responses is a common feature across a wide range of central nervous system (CNS) diseases. This most often arises as a secondary response to neurodegeneration or tissue damage, although in certain adaptive T cell -mediated conditions, such as multiple sclerosis (MS), immune activation itself can play a more primary pathogenic role. Parkinson's Disease (PD) is a neurodegenerative disorder, predominantly in older populations, characterized by the progressive loss of dopaminergic and other neurons and the accumulation of α-synuclein aggregates in the brain.[1] Both genetic and pathologic evidence support the notion that CNS inflammation contributes to PD pathogenesis.[2-6] Specifically, the presence of TNFα in PD microglia[7] and the recent discovery of autoreactive α-synuclein T cells in patients with early PD[8] raises the hypothesis that autoreactive T cells drive early CNS inflammation in prodromal PD, as is seen in autoimmune, relapsing-remitting MS, with later, secondary neurodegeneration manifesting as classic PD.[9-14] However, pathological studies reveal only a minimal presence of $CD8^+$ T cells in the substantia nigra of PD patients early in disease pathogenesis

We previously identified myeloid cells in the cerebrospinal fluid (CSF) that express microglia-associated cell surface proteins, including APOE, C1Q, and TREM2, that are presumably derived from a monocyte lineage. While first observed in higher numbers in the CSF of patients with HIV,[15] they are also observed in the CSF of healthy subjects. These CSF microglia-like macrophages share gene expression patterns with border-associated macrophages located along the brain vasculature at the interface of the brain parenchyma.[16,17] Surprisingly, the frequency of this population of microglia-like macrophages was markedly decreased in the CSF of patients with autoimmune, relapsing-remitting MS as compared to age-matched healthy controls.[18] With highly effective B cell depletion therapy, this population re-emerged in the CSF of MS patients, with associated increases in myeloid TNFα expression in blood, suggesting an important role in autoimmune disease pathogenesis.[18]

One early clinical manifestation of α-synucleinopathy is Rapid Eye Movement (REM) Sleep Behavior Disorder (RBD), a parasomnia that can appear years or even decades before the onset of typical symptoms of PD or Dementia with Lewy Bodies (DLB).[19] Thus, RBD serves as an early indicator of α-synuclein-related neurodegeneration. There is strong epidemiologic data indicating a link between autoimmune disorders, such as inflammatory bowel disease and PD, adding further support for a prominent role for the immune system in mediating the inception and progression PD pathology.[20,21] In addition, mounting evidence implicates the gut as an early site of PD-related pathology, including α-synuclein accumulation and intestinal inflammation,

raising the possibility of a gut–brain axis in prodromal synucleinopathy.[22] Thus, examining whether there is CNS inflammation in RBD as a manifestation of prodromal synucleinopathy and determining the pathways mediating inflammation can provide a rationale for clinical experiments to prevent the disease and to provide more convincing evidence that an early autoimmune-like process precedes a secondary neurodegenerative form of disease.

Here, we performed single-cell RNAseq analysis of immune cells from CSF and blood from 118 individuals, including 36 with RBD, 15 with PD without RBD, 18 with PD and RBD, 15 age-matched healthy controls for PD, and 27 patients with recent onset MS that had been compared to 7 healthy individuals. We unexpectedly identified an increased number of immune cells in the CSF of patients with RBD, which was not present in patients with longer-standing PD, similar to what is observed in patients with early, autoimmune MS. However, in marked contrast to MS, single-cell RNAseq in prodromal PD revealed an increase in the frequency and cell numbers of CSF microglia-like macrophages expressing IL6-JAK-STAT3 and TNFα signaling pathways. Aspects of this inflammatory signature were shared with those of brain microglia of PD patients as well as CNS-dural sinus myeloid cells, border-associated macrophages, and muscularis macrophages from α-synuclein-expressing PD model mice.[23] These combined findings in humans and PD animal models reveal a myeloid-mediated TNFα inflammatory process in the CNS, perhaps arising from the gut during the prodromal stage of PD, suggesting a newly described pathologic process in disease etiology.

Results

Single-cell immune atlas of PBMCs and CSF in prodromal and early stage of PD

To profile the immunological changes in prodromal and across PD stages, we generated a single-cell RNAseq immune cell atlas from paired peripheral blood mononuclear cells (PBMCs) and CSF cells. Participants were categorized into four groups: RBD (n=36), PD without RBD (described as PD; n=15), PD with RBD (described as PD-RBD; n=18), 27 patients with relapsing remitting MS, age-matched healthy controls (HC; n=15 for PD, n=7 for MS) (Supplementary Tables 1-3) who were separately compared to a previously published cohort of age matched healthy controls. Clinical and demographic information was collected, and PBMCs and CSF samples were obtained from all participants. The demographic and clinical characteristics of our study population were similar to other cohorts (Supplementary Table 2). The mean ages of the subjects in the four groups ranged from 64 to 89 years; 77% were males. Of the RBD group, 19 (53%) were hyposmic, as defined by an UPSIT score below the 15th percentile for age and gender,[24] 7 (19%) evidenced reduced dopamine transporter (DaT) binding in the striatum, and 25 (69%) showed Synuclein Aggregating Activity (SAA) in their CSF.

In all, 26 (72%) of the RBD subjects met MDS Prodromal PD Likelihood Ratio criteria for prodromal PD,[25] and were thus defined as a “high-risk” population. Of the subjects who entered with a diagnosis of RBD, six were diagnosed with either PD or Lewy Body Dementia (LBD) during the course of the study.

We first examined the CSF for evidence of inflammation by quantitative analysis of approximately ~30 mL of CSF from each subject. Unexpectedly, subjects with RBD showed increased CSF cellularity compared to age-matched HC subjects (3.3 vs 1.4 cells/µL; p=0.029, ANOVA followed by Tukey-Kramer post-hoc test; Fig. 1a), indicative of inflammation in the CNS of prodromal PD patients. We then performed droplet-based single-cell RNAseq with VDJ sequencing using the 10x Genomics Chromium platform. After stringent quality control, 523,521 cells from PBMCs (73 donors) and 259,560 cells from CSF (72 donors) were used for the analysis (Figs. 1b-f, Supplementary Fig. 1a). The average number of genes and percentage of mitochondrial genes detected per cell across all samples were 1,833 genes and 2.67% in PBMCs and 1,929 genes and 1.83% in CSF, respectively, reflecting high-quality single-cell transcriptomic data across both sample types. Based on the clusters defined by unsupervised clustering, we identified major immune cell types: B cells, myeloid cells, T cells, and NK cells in both PBMCs and CSF. Subclusters within these groups were further annotated based on known marker genes, resulting in 24 clusters in PBMCs and 20 clusters in CSF (Figs. 1c-f, Supplementary Figs. 1b, c). The cell cluster annotations were validated using CellTypist [26] (Supplementary Figs. 1e,f).

In PBMCs, the proportions of immune cells were as follows: B cells 8.09%, myeloid cells 20.8%, T cells 59.6%, and NK cells 11.0% (Fig. 1d). In contrast, CSF showed distinctly different proportions consistent with our previous findings.[27] B cells 0.53%, myeloid cells 23.8%, T cells 73.0%, and NK cells 2.59% (Fig. 1f). Compared to PBMCs, CSF had a higher proportion of T cells and myeloid cells, and a lower proportion of B cells and NK cells. At the subcluster level, specific differences were observed. For example, populations such as $CD4^+$ Tnaive cells (15.9% in PBMC, 5.62% in CSF), $CD16^+$ monocytes (3.56% in PBMC, 0.09% in CSF), and $CD16^+$ NK cells (10.0% in PBMC, 0.34% in CSF) were less abundant in CSF, while clusters such as cDC2 (1.06% in PBMC, 3.98% in CSF) and $CD4^+$ T central memory (Tcm) cells (12.3% in PBMC, 41.6% in CSF) were enriched. As we previously reported, in patients with CNS HIV[15], microglia-like macrophages in the CSF (termed CSF Mac), which expressed *C1QC* and *TREM2* specifically, were the most abundant myeloid cell population in CSF. These findings suggest that, while the CSF immune environment largely consists of circulating immune cells similar to those in PBMCs, the CSF exhibits significant differences in cell composition that likely

represents a unique immune microenvironment linked to the brain and CNS borders. Thus, we successfully constructed a single-cell immune cell atlas of PBMCs and CSF in RBD and PD.

Pronounced immunological changes in prodromal PD

To identify PD-specific immune alterations, we analyzed our single-cell immune atlas to assess both shifts in cell frequencies and absolute cell numbers and alterations in gene expression. To evaluate changes in cell frequencies, we used a Bayesian model[28] to compare RBD, PD, and PD-RBD groups against age-matched healthy controls (Figs. 2a-c). RBD exhibits heterogeneity in features such as hyposmia and CSF SAA status, both of which are known to be associated with PD onset.[29] Using available demographic, clinical and imaging data, we further stratified RBD individuals based on prodromal PD likelihood using the Movement Disorders Society (MDS) online Prodromal PD Calculator.[25] This analysis revealed a significant increase in the CSF Mac population in the RBD high-probability group (Figs. 2b,c). Additionally, the absolute numbers of CSF Mac, $CD4^+$ Tnaive, Tcm, and Tem cells in CSF were increased as compared to healthy controls (Fig. 2d) while there was a decrease in the relative proportion of $CD4^+$ Tcm cells and $CD8^+$ T effector memory/terminally differentiated effector memory (em/emra) cells in the RBD group (FDR<0.05 and <0.1 respectively). As previously described,[30] we observed an increase in $CD4^+$ T naive cells in the peripheral blood of prodromal PD (Supplementary Fig. 6, *FDR*<0.05).

We next examined gene expression changes within each cluster (Supplementary Fig. 2a). Compared to PBMCs, we observed more pronounced gene expression changes in CSF, especially in $CD4^+$ T cells, $CD14^+$ monocytes, and the CSF Mac population. Notably, when stratifying RBD patients by MDS Prodromal PD probability score, the RBD high-probability group exhibited the highest number of differentially expressed genes (DEGs). These findings suggest that both cell frequency and gene expression changes are most pronounced in the RBD high-probability group, with relatively smaller differences observed in PD and PD-RBD. Furthermore, in CSF, $CD4^+$ T cells and CSF Mac demonstrated coordinated changes in both cell numbers and gene expression.

To interpret these gene expression changes, we calculated scores for each cell population across conditions using Hallmark gene sets from the Molecular Signatures Database (MSigDB) (Fig. 2e, Supplementary Figs. 2b-f, Supplementary Tables 4,5). In PBMCs, oxidative phosphorylation was broadly upregulated in T cells from RBD and PD-RBD patients, suggesting that peripheral circulating T cells maintain a homeostatic state, consistent with the observed increase in naive $CD4^+$ T cells. In CSF, the most pronounced gene program changes were detected in myeloid populations, particularly in CSF Mac. In the RBD group, significant

enrichment of pathways such as mitotic spindle (*Mean change*=0.640, p_{adj}=4.33x10^{-302}), inflammatory response (*Mean change*=0.689, p_{adj}=2.45x10^{-189}), IL6-JAK-STAT3 signaling (*Mean change*=0.447, p_{adj}=3.97x10^{-166}), and TNFα signaling via NF-κB (*Mean change*=0.807, p_{adj}=2.84x10^{-96}), were observed. These findings suggest heightened cellular proliferation with TNFα/IL6-JAK-STAT3 immune pathways in the CSF Mac population in prodromal PD.

Inflammatory signature in MS versus RBD CSF

A major paradox in the field of autoimmunity revolves around the TNFα pathway, presumably relating to the different genetic architectures of human autoimmune diseases.[31,32] Specifically, while anti-TNFα therapy is an effective treatment for Inflammatory Bowel Disease (IBD) and rheumatoid arthritis (RA), it paradoxically induces exacerbations in patients with MS.[33,34] Moreover, studies have shown an increased incidence of PD among persons with IBD but a highly significant decrease in the incidence of PD in IBD patients on anti-TNFα therapy.[20,21] Based on these observations, we hypothesized that the CSF Mac population in prodromal PD would be characterized by elevated TNFα signaling and exhibit a distinct gene expression profile compared to CSF from patients with MS. We analyzed a new single-cell dataset of CSF cells from 29 MS patients and 7 previously published age-matched healthy individuals. As recently reported in a different MS dataset,[18] we observed a marked reduction in the CSF Mac population that was confirmed with a recent meta-analysis of other MS datasets (Fig. 2c).[17,18,35] Gene set enrichment analysis revealed a pattern opposite to that observed in RBD, with strong enrichment of gene sets in T cells, B cells, and dendritic cells, while enrichment in CSF macrophages was minimal (Fig. 2e, Supplementary Table 6). Notably, TNFα signaling via NF-κB showed a negative change in MS CSF Mac (*Mean change*=-0.716, p_{adj}=4.75x10^{-5}). Furthermore, we applied (1) a detailed analysis pipeline specialized for circulating CD4^{+} T cells[36,37] and (2) TCR-based estimation of T cell activity.[38] Both approaches consistently suggested that T cells did not show evidence of global T cell activation in PD or RBD (Supplementary Note and Supplementary Fig. 6-12).

Inflammatory signature in CSF Macrophages

We conducted a detailed analysis of CSF Mac and found notable quantitative and qualitative changes. As previously described, the CSF Mac population shares transcriptomic similarity with microglia and expresses macrophage markers, such as *C1QC*, *APOE*, and *TREM2,* at higher levels compared to other myeloid populations (Figs. 3a-c).[15,17,18] Previous studies also suggested this population resembles border-associated macrophages.[16,17] To further analyze the tissue specificity of this CSF Mac population and its similarity with microglia, we integrated myeloid populations from both PBMCs and CSF with publicly available datasets

of microglia[39] and cross-tissue myeloid cells,[26] comparing their expression profiles (Supplementary Fig. 3). While the analysis as expected revealed that CSF Mac population was transcriptionally close to microglia and tissue-resident macrophages, distinct differences were also observed. For instance, *SPP1*, *P2RY12*, and *TMEM119* were more highly expressed in microglia than in CSF Mac (Supplementary Fig. 3). These findings highlight both the similarities and the divergence in expression patterns between CSF Mac and microglia. We next compared the gene expression of CSF Mac between controls and RBD. The analysis revealed upregulation of HLA class II molecules and their regulator *CIITA*, as well as increased expression of *HIF1A*, mitochondrial genes, AP1 family genes, *JAK2*, *TLR2*, and *TNFRSF1A* and *TNFRSF1B* in RBD (Figs. 3d, Supplementary Table 7). These changes were consistent with hallmark gene set analyses, which showed elevated scores for pathways such as inflammatory response and IL6-JAK-STAT3 signaling in RBD patients (Fig. 3e). Both RNA seq and flow cytometry analysis revealed an increase in integrin alpha 4 subunit (ITGA4), a key molecule for CNS homing in $CD16^+$ monocytes from the blood of RBD low-risk individuals, suggesting that monocyte migration to the CNS is more active during the prodromal phase of PD (Fig. 3f). The pattern of ITGA4 upregulation suggests that monocyte activation may occur relatively early in disease.

We further hypothesized that CSF Mac might be functionally linked to microglial changes occurring in the brains of PD patients. To test this hypothesis, we utilized publicly available single-nucleus RNAseq data profiling multiple brain regions from 100 donors, including 75 PD cases.[40] The analysis revealed that genes upregulated in CSF Mac in RBD were also significantly upregulated in the primary motor cortex and prefrontal cortex of PD brains (Supplementary Figs. 4a, b). These results suggest that CSF Mac are not only activated during the prodromal phase of PD but also reflect microglial changes occurring within the PD brain. In addition, we attempted to examine myeloid populations in the CNS border region. As dural tissue was not available, we examined dural myeloid cells from the MI2BAC knock-in PD model mice, which express both human 1–120 truncated and full-length α-synuclein in catecholaminergic neurons under the control of the tyrosine hydroxylase promoter on a mouse α-synuclein–null background, thereby lacking endogenous murine α-synuclein expression[41] (Methods). The dural macrophages expressed CSF Mac marker genes such as *Trem2*, *Apoe*, and *C1qc* (Fig. 3g,h and Supplementary Fig. 4c). We found that TNFα-related genes and MHC class II genes were upregulated in PD model mice, consistent with the changes observed in CSF Mac in prodromal PD. These data suggest that synchronized gene expression changes occur in brain parenchymal microglia, CNS border-associated macrophages, and CSF Mac in prodromal and clinical stages of PD.

Genetic prioritization of cell populations highlighted myeloid population in CSF

To determine whether integrating known genetic information with our immune cell atlas could aid in prioritizing relevant cell populations in PD, we analyzed both polygenic and rare variant components. First, we used scDRS[42] to integrate GWAS data associated with PD,[4] RBD,[43] and LBD[44] traits, evaluating the enrichment of polygenic signals in individual cell types (Supplementary Figs. 5a,b). The analysis revealed the strongest polygenic signal in CSF Mac for LBD. Note that LBD[44] is a mixed proteinopathy that demonstrates genetic, clinical, and pathological features in common with both PD and Alzheimer's disease, and approximately half of patients with RBD will go on to develop dementia with Lewy Bodies rather than motor Parkinson's disease,[45,46] consistent with it not being a pure synucleinopathy. For PD, significant associations were observed in cDC2 and pDC in blood and pDC in CSF. No significant associations were detected for RBD, although polygenic signals appeared to accumulate in myeloid populations within the CSF.

Enhanced cell-cell communication in the immune landscape of RBD

Next, we examined cell-cell interactions in the CSF using CellPhoneDB.[47] The ligand—receptor interaction prediction suggested that myeloid cells, including CSF Mac and cDC2, would exhibit robust ligand—receptor signals and that there would be more interactions between lymphocytes and myeloid cells than among lymphocytes alone (Fig. 4a). Focusing on the RBD group, where we observed the most pronounced immunological changes, we investigated cell-cell interaction pairs with substantial shifts in signaling dynamics. We found that ligands associated with signaling were moderately elevated (*FDR*<0.2) in lymphocyte subsets such as CD4 Tcm, NK CD16, and memory B cells, whereas receptor signals were enhanced (*FDR*<0.2) in myeloid subsets including cDC2 and CSF Mac (Fig. 4b). Notably, the strongest elevation in receptor mediated signals occurred among myeloid cells themselves, suggesting the presence of autonomous feedback activation. To explore these observations further, we examined autonomous ligand—receptor pairs in CSF Mac and identified several upregulated pairs in RBD, including *C3–C3AR1*, *CD55–ADGRE5*, and *LGALS9–HAVCR2* (Tim3), which are associated with immunoregulatory functions (Fig. 4c). In addition, we detected neurodegeneration-related signals mediated by amyloid precursor protein (*APP–CD74*, *APP–SORL1*) and prion protein (*PRNP–ADGRG6*).

It is also known that aggregated α-synuclein can induce the activation of myeloid cells.[48] These ligand—receptor pairs may modulate the activation state of CSF macrophages and influence the CNS microenvironment. In particular, increases in TIM-3 expression in models of Alzheimer's Disease models leads to decreased clearance of amyloid plaques[49] indicating that

TIM-3 expression may lead to decreased clearance of α-synuclein, leading to increases in myeloid cells activation (Fig. 4d). Collectively, these dynamic and comprehensive changes in cell-cell interactions appear to play a critical role in shaping the immune landscape during the prodromal stage of PD.

An Immunological Gut–Brain Axis in Prodromal PD

Building on our previous work demonstrating a gut–brain axis mediated by gut-derived T cells,[50] we analyzed paired PBMC, CSF, and gut samples from individuals with RBD, PD-RBD, MS, and HC using single-cell RNA and TCR-seq (Fig. 5a, Supplementary Figs. 4d-e). Across tissues, shared TCR clones were predominantly observed among CD8 effector memory T cells, with clonal overlap detected between PBMC and gut, as well as between PBMC and CSF. Although less frequent, shared clones spanning the gut and CSF were also identified, suggesting a continuous T cell circulation axis linking the gut, peripheral blood, and central nervous system.

Finally, a recent report suggested that intestinal macrophages modulate synucleinopathy in a murine α-synucleinopathy model.[23] Re-analysis of muscularis macrophages from these mice showed that intestinal macrophages display increased TNFα signaling pathway activity similar to CSF Mac in human RBD individuals (Fig. 5b). Taken together, these findings support a model in which gut α-synuclein aggregation and intestinal macrophage activation are immunologically linked to CSF and border-associated macrophages through shared TNFα-driven inflammatory programs, forming a coordinated gut–brain immune axis in prodromal PD (Fig. 5c).

Discussion

While emerging evidence suggests an underlying inflammatory component in PD, as demonstrated by the presence of α-synuclein autoreactive T cells in the circulation of patients with manifest and prodromal PD, pathological studies reveal only a minimal presence of $CD8^+$ T cells in the substantia nigra of PD patients early in disease pathogenesis.[51] To elucidate early mechanisms of disease pathogenesis in PD, we performed single-cell RNAseq analysis of paired CSF and blood from subjects with different stages of PD and compared them to age-matched healthy subjects and patients with MS. We found a previously unrecognized increase in CSF immune cells that is most pronounced in patients with prodromal PD (RBD). Single-cell RNAseq analysis revealed increased frequencies of a population of microglia-like macrophages, termed CSF Mac, exhibiting inflammatory responses characterized by increased expression of IL6-JAK-STAT3 and *TNFRSF1A* and *TNFRSF1B* signaling pathways, with a relative lack of T

cell activation in CSF or blood. This is in marked contrast to what is observed in MS CSF, where there is a loss of CSF microglia-like macrophages and an increase in activated T cells.

Specifically, we compared immune populations with single-cell resolution in the CSF of subjects with new-onset MS and RBD. In contrast to MS CSF where we observed a decreased frequency of CSF Mac population, this population was increased in the CSF of patients with prodromal PD, with a pronounced TNF/IL-6/JAK-STAT signature. Of note, inhibition of the JAK/STAT pathway has been implicated in exerting protective effects in PD.[52-54] We recently observed that treatment of patients with MS with B cell depletion leads to an exponential increase of this CSF Mac population with increased secretion of TNFα by circulating monocytes.[18] Taken together, these data suggest fundamental differences in the activity of TNFα pathways between prodromal PD and MS. Moreover, these experimental results are consistent with large-scale epidemiologic investigations of patients with IBD, demonstrating an increased risk of PD in patients with IBD, and that anti-TNFα agents substantially decrease the incidence of new-onset PD in this population.[20] Our observations comparing PD and MS are in line with a fundamental observation of human inflammatory diseases. Specifically, the therapeutic efficacy found with blocking an inflammatory pathway in one autoimmune disease can lead to flare-ups in other autoimmune diseases. Two prominent examples include anti-IL-17 blockade, which has strong efficacy in treating psoriasis but has the risk of exacerbating IBD[55] and, as noted anti-TNFα blockade, an effective treatment in IBD and rheumatoid arthritis, can cause exacerbations in patients with MS.[34] These dichotomous responses are likely related to the different genetic architectures of autoimmune diseases, where a risk haplotype in one disease can be protective in another.[31]

Compared to the significant changes we observed in CSF myeloid populations, relatively minor changes in T cell populations were noted in prodromal PD. Specifically, our analysis replicated previous studies[30] showing increases in circulating and CSF naïve $CD4^+$ T cells in prodromal PD and PD with reductions in the proportion of $CD4^+$ Tcm cells and $CD8^+$ Temra cells in CSF. These results are in contrast to T and B cell populations in patients with early relapsing/remitting MS, which display markedly inflammatory signals in CSF T cells and in parenchymal lesions which are relatively absent in prodromal PD. While prior studies have identified α-synuclein–reactive T cells in patients with PD [8,10,12], we did not observe evidence of global T cell expansion or activation in the CSF of prodromal PD. We hypothesize, based on PD α-synuclein mouse models, that these autoreactive T cells exhibit their pathogenic function in the gut and dural associated lymph node tissue. Notably, we identified shared T cell clones between the gut and CSF, suggesting that T cells may represent an immunologic link between

these compartments in PD. This finding aligns with α-synuclein mouse models in which aggregated α-synuclein in the gut leads to activation of both myeloid cells and reactive T cells[56]. Furthermore, the accompanying paper demonstrates the presence of activated T cells and myeloid cells with a TNFα signature in dura, suggesting that the meningeal compartment is a key anatomical immune niche in PD.[48] In this framework based on observations in both animal models and in humans with prodromal PD, we hypothesize the disease originates from α-synuclein aggregation in the gut perhaps triggered by the microbiome inducing TNFα secreting macrophages in the muscularis layer of the gut leading to: 1) propagation of α-synuclein aggregates via the vagus nerve to the brain[57]; and/or 2) trafficking of α-synuclein reactive T cells between gut and dural sinuses that then; 3) mediate a myeloid-driven inflammatory response characterized by TNF signaling across intestinal and then meningeal compartments into the CNS (figure S13).

In summary, our integrated analysis of human CSF and blood, together with observations in mouse models, supports a role for myeloid-driven inflammation in early PD pathogenesis. We identified an increase in CSF immune cells in prodromal PD, marked by expansion of CSF macrophages with TNFα and JAK/STAT pathway inflammatory signatures, in contrast to the T-cell driven inflammation observed in MS. Although α-synuclein-reactive T cells have been previously identified, we did not find evidence of global T cell activation in CSF. However, we did detect shared T cell clones between gut and CSF, alongside shared macrophage inflammatory responses in intestinal and meningeal compartments. Within this framework, TNFα pathway-associated myeloid populations emerge as a central effector in PD pathogenesis, providing a mechanistic rationale for therapeutic targeting of this pathway in prodromal PD. Based on these data, we have initiated a clinical trial of an anti-TNFα monoclonal antibody in patients with prodromal PD (ClinicalTrials.gov: NCT06996652).

Figure Legends

Figure 1: Single-cell immune atlas of PBMCs and CSF in prodromal and early stage of PD. (a) CSF cell counts. (HC: 1.4±0.7; RBD: 3.3±2.3, PD: 2.1±1.8 cells/µL; *p*=0.029, ANOVA followed by Tukey-Kramer post-hoc test) (b) Schematic overview of the project workflow. (c-f) Uniform Manifold Approximation and Projection (UMAP) plots of single-cell data (c,e) and corresponding cell frequencies (d,f) for blood (c,d) and CSF (e,f). Error bars indicate 95% confidence intervals (e,f).

Figure 2: Global characterization highlighted activation of CSF Macrophages in prodromal PD.
(a,b) Relative cell frequency differences were analyzed in Blood (a) and CSF (b) for patients with RBD, PD, and PD-RBD using scCODA[28] (a Bayesian statistical tool) relative to age matched healthy controls (HC), with RBD stratified by PD risk score (right heatmaps). See materials and methods for the details. FDR thresholds: 0.05, '**'; 0.1, '*'. Color indicates fold change in relative cell frequency. The RBD high-risk group is outlined with dotted lines. (c) Relative CSF Mac cell frequency was significantly increased in both RBD low and high patients as compared to PD and PD RBD and age-matched controls. Shown in comparison are new onset, previously reported untreated[18] MS patients, where the relative CSF Mac cell frequency was lower compared to age-matched healthy controls. For MS, Statistical testing was performed using the Mann–Whitney U test for each cell population, followed by multiple testing corrections. The adjusted p-value was 1.12×10^{-4}. (d) The absolute cell number of CSF Mac and T cell populations calculated by the proportion of the cell from scRNAseq and the cell count data. (e) Hallmark gene sets enriched in MS (upper) and RBD (lower) across cell types in comparison to HC (materials and methods). Only positively associated gene sets were visualized. See Supplementary Fig. 2 for other comparisons. Panels include only clusters containing >1000 cells for blood and >500 cells for CSF. (lower) The same cell types were retained for MS.

Figure 3: CSF macrophages activation and linked gene patterns with PD brain microglia.
(a) Myeloid cell populations in CSF on UMAP embeddings. (b) Dot plot depicting signature genes' mean expression levels and percentage of cells expressing them across clusters. Marker genes for the plot were manually selected. (c) Heatmap showing the transcriptome correlation between myeloid cell populations. (d) Volcano plot showing the differentially expressed genes in CSF Macrophage (CSF Mac) between RBD vs HC. For the visualization, genes with *mean expression*>0.5 were selected. Genes with *FDR*<0.1, *Log2 fold change*>0.2 or <-0.2 were highlighted in red. (e) Sample-wise HALLMARK gene set activity in CSF Mac. *: *FDR*<0.05. (f) Mean Fluorescence Intensity (MFI) distribution of ITGA4 in peripheral blood $CD16^{+}$ Monocytes. (*p*=0.0147). (g and h) Heatmap showing scaled mean expression of representative genes for

CSF Mac population (g) and from the Mac population isolated from the dura from human truncated αSYN expressing PD model mice (MI2BAC, labeled as truncated αSYN) and control mice (BAC, labeled as Ctrl). (h). The value was scaled for each gene.

Figure 4: Cell-cell interaction analysis showed enhanced receiver activity in CSF myeloid cell populations

(a) Heatmap showing the number of interactions inferred by CellPhoneDB.[47] The x-axis shows the sender, and the y-axis shows the receiver. (b) The activated ligand-receptor pairs in RBD compared to HC (materials and methods). The upregulated sender signals (upper) and receiver signals (lower) are shown. Interactions with *FDR*<0.2 are shown in the black lines. Potentially upregulated interactions (p<0.05) are shown in the dashed grey lines. (c) Heatmaps show fold change in disease conditions compared to healthy controls for representative CSF Mac ligand-receptor pairs. (d) Dot plot showing the expression of *HAVCR2* across CSF myeloid cell populations (upper) and in CSF Mac population across conditions (lower).

Figure 5: Immunological gut-brain axis in prodromal PD.

(a) Heatmap showing pairwise dissimilarity of T cell clonal repertoires across human tissues quantified using *1−Jaccard index*. (b) Hallmark gene sets show higher activity in Macrophages in the muscularis externa (ME) in 3KL αS transgenic mice compared to WT (Methods). Only positively associated gene sets were visualized. The dashed line at the bottom indicates *Padj* = 0.05. The data was downloaded from GSE307000 [23]. (c) Working model linking gut and CNS-border myeloid inflammation.

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Conflict of interests

D.A.H. has received research funding from Bristol-Myers Squibb, Sanofi, Genentech, and Hoffman LaRoche. He has been a consultant for Repertoire Inc, Bristol Myers Squibb, Genentech, Novartis Pharmaceuticals, and Sanofi Genzyme. J.M.C has received consulting fees from VanquaBio, Inc.

Author Contributions

L.Z., Y.Y., M.E.D., J.M.C., and D.A.H. conceptualized the study. L.Z., J.M., A.R., and H.W. performed the experiments. Y.Y., M.E.D., B.Z., L.Z., J.P.S, and V.R. analyzed the data. Q.W., M.G.S., K.J.N., D.A.P., and M.C. generated mouse scRNAseq data. Y.Y., L.Z., J.M.C., and D.A.H. wrote the manuscript. A.R., H.W., V.R., N.P., E.E.L., J.M.C., and D.A.H. collected clinical samples. Y.Y., L.Z., J.M.C., T.S.S., and D.A.H. supervised the project.

Data and Materials Availability

Codes will be available on Github (https://github.com/yyoshiaki/2025_PD_PBMCCSF_scRNAseq) upon acceptance. Raw fastq files will be available in the ASAP CRN cloud for the PD and RBD cohort, and dbGAP and GEO for the MS cohort upon acceptance. The processed scRNA-seq data will be available in CZ CELLxGENE upon acceptance.

Reference

1 Kalia, L. V. & Lang, A. E. Parkinson's disease. *Lancet* **386**, 896-912 (2015). https://doi.org/10.1016/S0140-6736(14)61393-3

2 Jo, M. G., Kim, S. H. & Yun, S. P. Hidden face of Parkinson's disease: is it a new autoimmune disease? *Neural Regen Res* (2024). https://doi.org/10.4103/NRR.NRR-D-24-01063

3 Kim, J. J. *et al.* Multi-ancestry genome-wide association meta-analysis of Parkinson's disease. *Nat Genet* **56**, 27-36 (2024). https://doi.org/10.1038/s41588-023-01584-8

4 Nalls, M. A. *et al.* Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol* **18**, 1091-1102 (2019). https://doi.org/10.1016/S1474-4422(19)30320-5

5 Qu, Y. *et al.* A systematic review and meta-analysis of inflammatory biomarkers in Parkinson's disease. *NPJ Parkinsons Dis* **9**, 18 (2023). https://doi.org/10.1038/s41531-023-00449-5

6 Yu, E. *et al.* Fine mapping of the HLA locus in Parkinson's disease in Europeans. *NPJ Parkinsons Dis* **7**, 84 (2021). https://doi.org/10.1038/s41531-021-00231-5

7 Boka, G. *et al.* Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci Lett* **172**, 151-154 (1994). https://doi.org/10.1016/0304-3940(94)90684-x

8 Lindestam Arlehamn, C. S. *et al.* alpha-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease. *Nat Commun* **11**, 1875 (2020). https://doi.org/10.1038/s41467-020-15626-w

9 Sommer, A. *et al.* Th17 Lymphocytes Induce Neuronal Cell Death in a Human iPSC-Based Model of Parkinson's Disease. *Cell Stem Cell* **24**, 1006 (2019). https://doi.org/10.1016/j.stem.2019.04.019

10 Sulzer, D. *et al.* T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature* **546**, 656-661 (2017). https://doi.org/10.1038/nature22815

11 de Fabregues, O. *et al.* Relevance of tissue-resident memory CD8 T cells in the onset of Parkinson's disease and examination of its possible etiologies: infectious or autoimmune? *Neurobiol Dis* **187**, 106308 (2023). https://doi.org/10.1016/j.nbd.2023.106308

12 Furusawa-Nishii, E. *et al.* alpha-Synuclein orchestrates Th17 responses as antigen and adjuvant in Parkinson's disease. *J Neuroinflammation* **22**, 38 (2025). https://doi.org/10.1186/s12974-025-03359-w

13 Jiang, Z. *et al.* The role of the immune system in Parkinson's disease pathogenesis: A focus on Th17 cells - A systematic review and meta-analysis. *J Neuroimmunol* **398**, 578484 (2025). https://doi.org/10.1016/j.jneuroim.2024.578484

14 Bolte, A. C. & Lukens, J. R. Th17 Cells in Parkinson's Disease: The Bane of the Midbrain. *Cell Stem Cell* **23**, 5-6 (2018). https://doi.org/10.1016/j.stem.2018.06.016

15 Farhadian, S. F. *et al.* Single-cell RNA sequencing reveals microglia-like cells in cerebrospinal fluid during virologically suppressed HIV. *JCI Insight* **3** (2018). https://doi.org/10.1172/jci.insight.121718

16 Ostkamp, P. *et al.* A single-cell analysis framework allows for characterization of CSF leukocytes and their tissue of origin in multiple sclerosis. *Sci Transl Med* **14**, eadc9778 (2022). https://doi.org/10.1126/scitranslmed.adc9778

17 Cantoni, C. *et al.* A single-cell compendium of human cerebrospinal fluid identifies disease-associated immune cell populations. *J Clin Invest* **135** (2025). https://doi.org/10.1172/JCI177793

18 Wei, J. *et al.* Transcriptomic profiling after B-cell depletion reveals central and peripheral immune cell changes in multiple sclerosis. *J Clin Invest* (2025). https://doi.org/10.1172/JCI182790

19 Postuma, R. B., Gagnon, J. F., Bertrand, J. A., Genier Marchand, D. & Montplaisir, J. Y. Parkinson risk in idiopathic REM sleep behavior disorder: preparing for neuroprotective trials. *Neurology* **84**, 1104-1113 (2015). https://doi.org/10.1212/WNL.0000000000001364

20 Peter, I. *et al.* Anti-Tumor Necrosis Factor Therapy and Incidence of Parkinson Disease Among Patients With Inflammatory Bowel Disease. *JAMA Neurol* **75**, 939-946 (2018). https://doi.org/10.1001/jamaneurol.2018.0605

21 Potashman, M. *et al.* The impact of anti-inflammatory therapy on Parkinson's disease incidence: A retrospective cohort study. *Parkinsonism Relat Disord* **130**, 107194 (2025). https://doi.org/10.1016/j.parkreldis.2024.107194

22 Berg, D. *et al.* Prodromal Parkinson disease subtypes - key to understanding heterogeneity. *Nat Rev Neurol* **17**, 349-361 (2021). https://doi.org/10.1038/s41582-021-00486-9

23 De Schepper, S. *et al.* Intestinal macrophages modulate synucleinopathy along the gut-brain axis. *Nature* (2026). https://doi.org/10.1038/s41586-025-09984-y

24 Brumm, M. C. *et al.* Updated Percentiles for the University of Pennsylvania Smell Identification Test in Adults 50 Years of Age and Older. *Neurology* **100**, e1691-e1701 (2023). https://doi.org/10.1212/WNL.0000000000207077

25 Berg, D. *et al.* MDS research criteria for prodromal Parkinson's disease. *Mov Disord* **30**, 1600-1611 (2015). https://doi.org/10.1002/mds.26431

26 Dominguez Conde, C. *et al.* Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science* **376**, eabl5197 (2022). https://doi.org/10.1126/science.abl5197

27 Hafler, D. A. *et al.* In vivo activated T lymphocytes in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *N Engl J Med* **312**, 1405-1411 (1985). https://doi.org/10.1056/NEJM198505303122201

28 Buttner, M., Ostner, J., Muller, C. L., Theis, F. J. & Schubert, B. scCODA is a Bayesian model for compositional single-cell data analysis. *Nat Commun* **12**, 6876 (2021). https://doi.org/10.1038/s41467-021-27150-6

29 Siderowf, A. *et al.* Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using alpha-synuclein seed amplification: a cross-sectional study. *Lancet Neurol* **22**, 407-417 (2023). https://doi.org/10.1016/S1474-4422(23)00109-6

30 Kouli, A. *et al.* T lymphocyte senescence is attenuated in Parkinson's disease. *J Neuroinflammation* **18**, 228 (2021). https://doi.org/10.1186/s12974-021-02287-9

31 Harroud, A. & Hafler, D. A. Common genetic factors among autoimmune diseases. *Science* **380**, 485-490 (2023). https://doi.org/10.1126/science.adg2992

32 Lincoln, M. R. *et al.* Genetic mapping across autoimmune diseases reveals shared associations and mechanisms. *Nat Genet* **56**, 838-845 (2024). https://doi.org/10.1038/s41588-024-01732-8

33 The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology* **53**, 457-465 (1999). https://doi.org/https://doi.org/10.1212/WNL.53.3.457

34 Sicotte, N. L. & Voskuhl, R. R. Onset of multiple sclerosis associated with anti-TNF therapy. *Neurology* **57**, 1885-1888 (2001). https://doi.org/10.1212/wnl.57.10.1885

35 Wei, J. *et al.* Systems Analysis of Immune Changes after B-cell Depletion in Autoimmune Multiple Sclerosis. *bioRxiv* (2024). https://doi.org/10.1101/2024.02.07.576204

36 Yasumizu, Y. *et al.* Single-cell transcriptome landscape of circulating CD4(+) T cell populations in autoimmune diseases. *Cell Genom* **4**, 100473 (2024). https://doi.org/10.1016/j.xgen.2023.100473

37 Yasumizu, Y. PBMC/CSF single-cell RNAseq CD4+ T cell reference mapping. *protocol.io* (2025). https://doi.org/dx.doi.org/10.17504/protocols.io.q26g7mqj1gwz/v1

38 Zhang, L. *et al.* Lineage tracking reveals dynamic relationships of T cells in colorectal cancer. *Nature* **564**, 268-272 (2018). https://doi.org/10.1038/s41586-018-0694-x

39 Olah, M. *et al.* Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Commun* **11**, 6129 (2020). https://doi.org/10.1038/s41467-020-19737-2

40 N, M. P. *et al.* A multi-region single nucleus transcriptomic atlas of Parkinson's disease. *Sci Data* **11**, 1274 (2024). https://doi.org/10.1038/s41597-024-04117-y

41 Longhena, F. *et al.* Alpha-synuclein aggregation and dopaminergic neuron death in a new mouse model of Parkinson’s disease expressing human full-length and C-terminally truncated 1- 120 alpha-synuclein. *bioRxiv*, 2025.2004.2013.647581 (2025). https://doi.org/10.1101/2025.04.13.647581

42 Zhang, M. J. *et al.* Polygenic enrichment distinguishes disease associations of individual cells in single-cell RNA-seq data. *Nat Genet* **54**, 1572-1580 (2022). https://doi.org/10.1038/s41588-022-01167-z

43 Krohn, L. *et al.* Genome-wide association study of REM sleep behavior disorder identifies polygenic risk and brain expression effects. *Nat Commun* **13**, 7496 (2022). https://doi.org/10.1038/s41467-022-34732-5

44 Chia, R. *et al.* Genome sequencing analysis identifies new loci associated with Lewy body dementia and provides insights into its genetic architecture. *Nat Genet* **53**, 294-303 (2021). https://doi.org/10.1038/s41588-021-00785-3

45 Postuma, R. B. *et al.* Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. *Brain* **142**, 744-759 (2019). https://doi.org/10.1093/brain/awz030

46 Joza, S. *et al.* Prodromal dementia with Lewy bodies in REM sleep behavior disorder: A multicenter study. *Alzheimers Dement* **20**, 91-102 (2024). https://doi.org/10.1002/alz.13386

47 Efremova, M., Vento-Tormo, M., Teichmann, S. A. & Vento-Tormo, R. CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes. *Nat Protoc* **15**, 1484-1506 (2020). https://doi.org/10.1038/s41596-020-0292-x

48 Schonhoff, A. M. *et al.* Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. *Nat Commun* **14**, 3754 (2023). https://doi.org/10.1038/s41467-023-39060-w

49 Kimura, K. *et al.* Immune checkpoint TIM-3 regulates microglia and Alzheimer's disease. *Nature* **641**, 718-731 (2025). https://doi.org/10.1038/s41586-025-08852-z

50 Yoshida, T. M. *et al.* The subfornical organ is a nucleus for gut-derived T cells that regulate behaviour. *Nature* **643**, 499-508 (2025). https://doi.org/10.1038/s41586-025-09050-7

51 Galiano-Landeira, J., Torra, A., Vila, M. & Bove, J. CD8 T cell nigral infiltration precedes synucleinopathy in early stages of Parkinson's disease. *Brain* **143**, 3717-3733 (2020). https://doi.org/10.1093/brain/awaa269

52 Qin, H. *et al.* Inhibition of the JAK/STAT Pathway Protects Against alpha-Synuclein-Induced Neuroinflammation and Dopaminergic Neurodegeneration. *J Neurosci* **36**, 5144-5159 (2016). https://doi.org/10.1523/JNEUROSCI.4658-15.2016

53 Lashgari, N. A. *et al.* The involvement of JAK/STAT signaling pathway in the treatment of Parkinson's disease. *J Neuroimmunol* **361**, 577758 (2021). https://doi.org/10.1016/j.jneuroim.2021.577758

54 Yan, Z., Gibson, S. A., Buckley, J. A., Qin, H. & Benveniste, E. N. Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases. *Clin Immunol* **189**, 4-13 (2018). https://doi.org/10.1016/j.clim.2016.09.014

55 Fauny, M. *et al.* Paradoxical gastrointestinal effects of interleukin-17 blockers. *Ann Rheum Dis* **79**, 1132-1138 (2020). https://doi.org/10.1136/annrheumdis-2020-217927

56 Garretti, F. *et al.* Interaction of an alpha-synuclein epitope with HLA-DRB1(*)15:01 triggers enteric features in mice reminiscent of prodromal Parkinson's disease. *Neuron* **111**, 3397-3413 e3395 (2023). https://doi.org/10.1016/j.neuron.2023.07.015

57 Kim, S. *et al.* Transneuronal Propagation of Pathologic alpha-Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron* **103**, 627-641 e627 (2019). https://doi.org/10.1016/j.neuron.2019.05.035

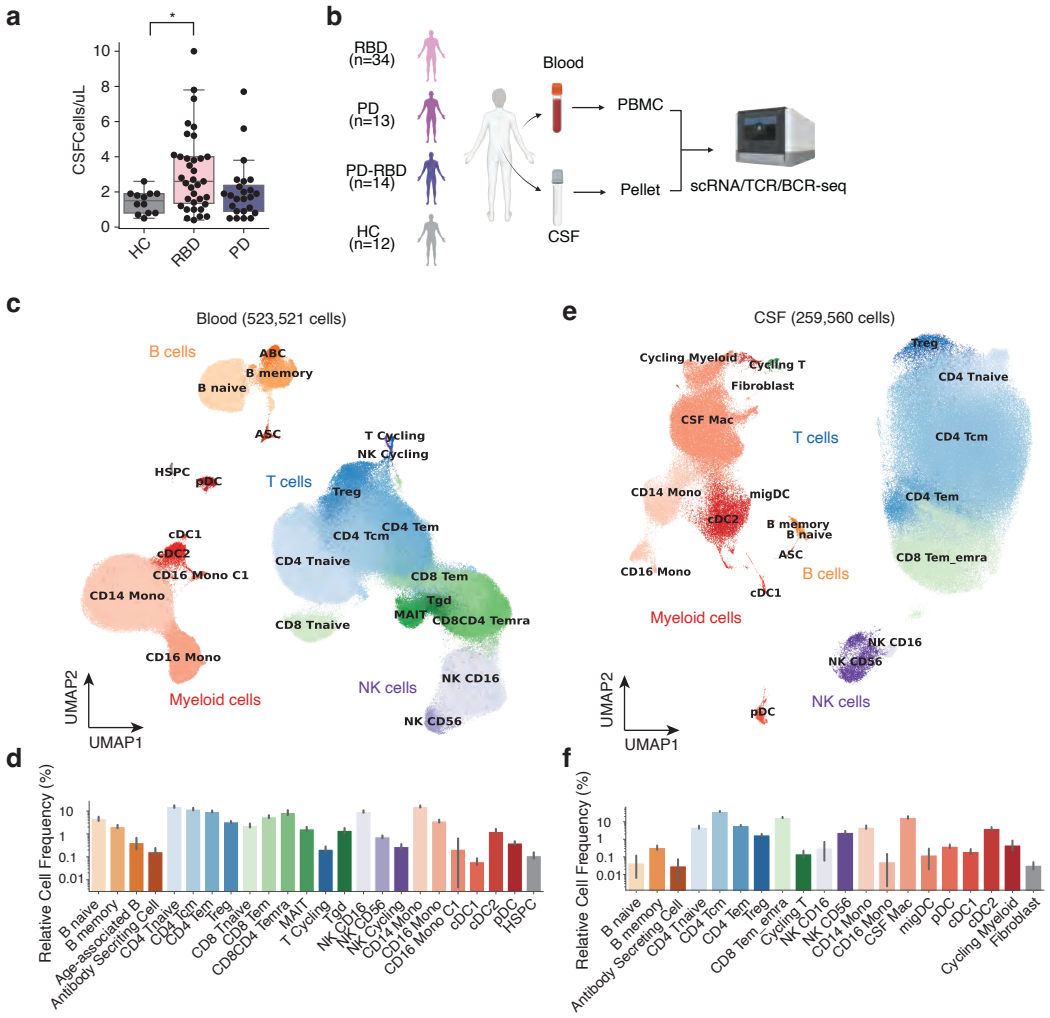

a
CSFCells/uL
HC
RBD
PD
b
RBD (n=34)
PD (n=13)
PD-RBD (n=14)
HC (n=12)
Blood
PBMC
Pellet
CSF
scRNA/TCR/BCR-seq
c
Blood (523,521 cells)
B cells
T cells
Myeloid cells
NK cells
UMAP1
UMAP2
e
CSF (259,560 cells)
T cells
B cells
Myeloid cells
NK cells
d
Relative Cell Frequency (%)
f
Relative Cell Frequency (%)

Figure 1

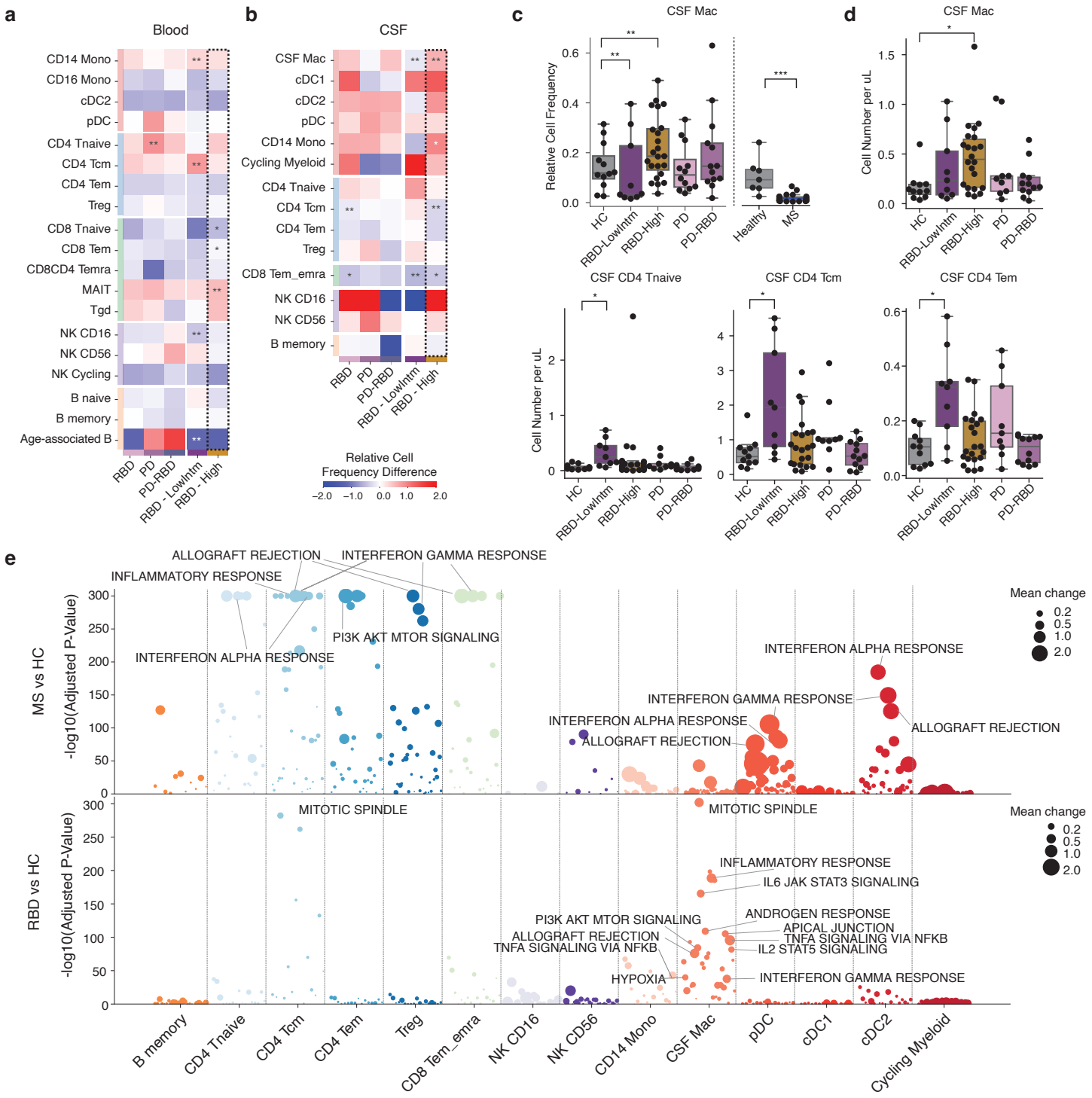

a
Blood
CD14 Mono
CD16 Mono
cDC2
pDC
CD4 Tnaive
CD4 Tcm
CD4 Tem
Treg
CD8 Tnaive
CD8 Tem
CD8CD4 Temra
MAIT
Tgd
NK CD16
NK CD56
NK Cycling
B naive
B memory
Age-associated B
RBD
PD
PD-RBD
RBD - LowIntm
RBD - High
b
CSF
CSF Mac
cDC1
cDC2
pDC
CD14 Mono
Cycling Myeloid
CD4 Tnaive
CD4 Tcm
CD4 Tem
Treg
CD8 Tem_emra
NK CD16
NK CD56
B memory
RBD
PD
PD-RBD
RBD - LowIntm
RBD - High
Relative Cell Frequency Difference
−2.0 −1.0 0.0 1.0 2.0
c
CSF Mac
Relative Cell Frequency
HC
RBD-LowIntm
RBD-High
PD
PD-RBD
Healthy
MS
CSF CD4 Tnaive
CSF CD4 Tcm
CSF CD4 Tem
Cell Number per uL
d
CSF Mac
Cell Number per uL
e
ALLOGRAFT REJECTION
INTERFERON GAMMA RESPONSE
INFLAMMATORY RESPONSE
PI3K AKT MTOR SIGNALING
INTERFERON ALPHA RESPONSE
MS vs HC
-log10(Adjusted P-Value)
Mean change
0.2
0.5
1.0
2.0
MITOTIC SPINDLE
RBD vs HC
IL6 JAK STAT3 SIGNALING
ANDROGEN RESPONSE
APICAL JUNCTION
TNFA SIGNALING VIA NFKB
IL2 STAT5 SIGNALING
HYPOXIA
B memory
CD4 Tnaive
CD4 Tcm
CD4 Tem
Treg
CD8 Tem_emra
NK CD16
NK CD56
CD14 Mono
CSF Mac
pDC
cDC1
cDC2
Cycling Myeloid

Figure 2

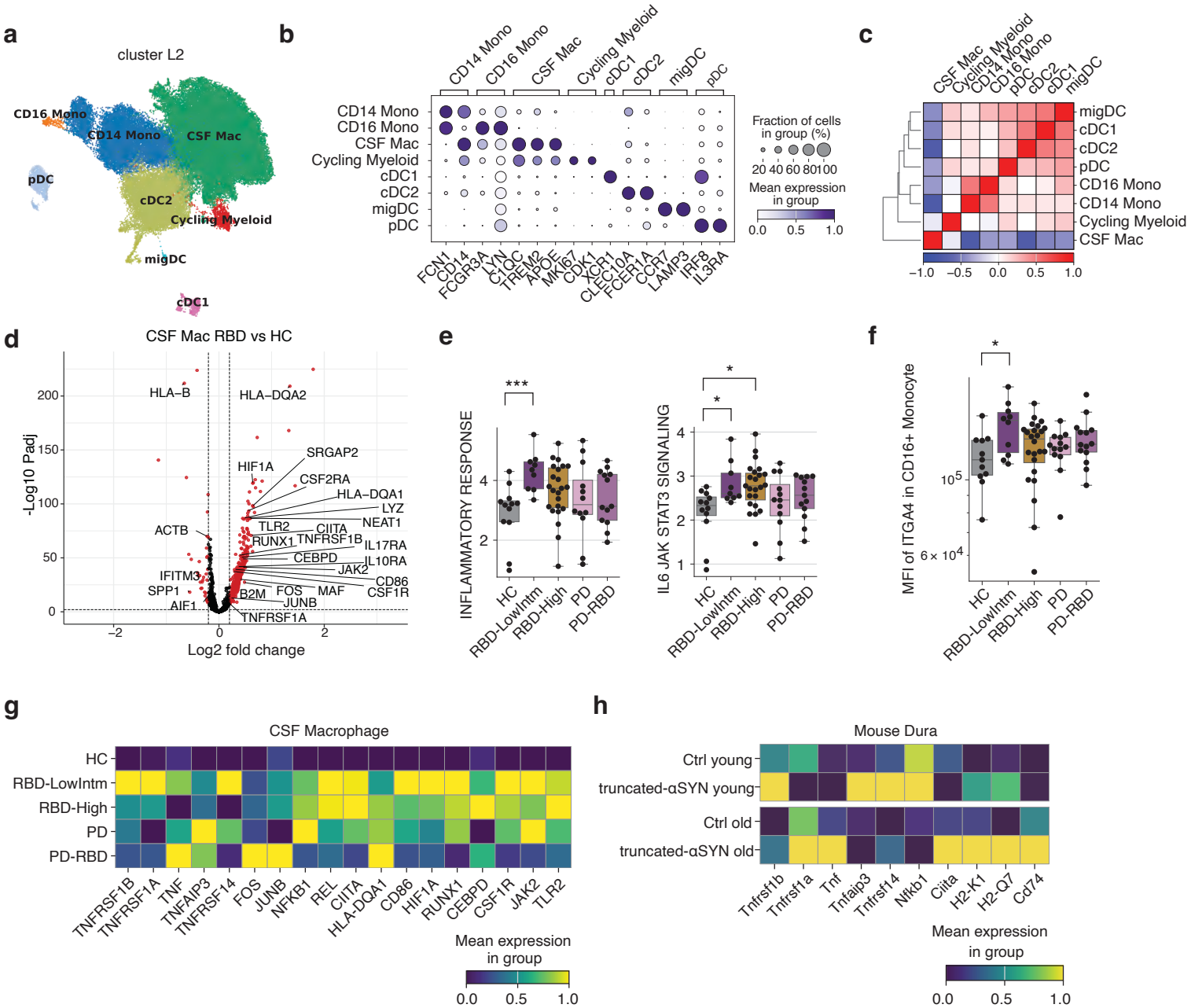

a
cluster L2
CD16 Mono
CD14 Mono
CSF Mac
pDC
cDC2
Cycling Myeloid
migDC
cDC1
b
CD14 Mono
CD16 Mono
CSF Mac
Cycling Myeloid
cDC1
cDC2
migDC
pDC
FCN1
CD14
FCGR3A
LYN
C1QC
TREM2
APOE
MKI67
CDK1
XCR1
CLEC10A
FCER1A
CCR7
LAMP3
IRF8
IL3RA
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
0.0 0.5 1.0
c
CSF Mac
Cycling Myeloid
CD14 Mono
CD16 Mono
pDC
cDC2
cDC1
migDC
−1.0 −0.5 0.0 0.5 1.0
d
CSF Mac RBD vs HC
-Log10 Padj
Log2 fold change
HLA–B
HLA–DQA2
SRGAP2
HIF1A
CSF2RA
HLA–DQA1
LYZ
NEAT1
ACTB
TLR2
CIITA
RUNX1
TNFRSF1B
IL17RA
CEBPD
IL10RA
JAK2
IFITM3
CD86
SPP1
B2M
FOS
MAF
CSF1R
AIF1
JUNB
TNFRSF1A
e
INFLAMMATORY RESPONSE
IL6 JAK STAT3 SIGNALING
HC
RBD-LowIntm
RBD-High
PD
PD-RBD
f
MFI of ITGA4 in CD16+ Monocyte
HC
RBD-LowIntm
RBD-High
PD
PD-RBD
g
CSF Macrophage
HC
RBD-LowIntm
RBD-High
PD
PD-RBD
TNFRSF1B
TNFRSF1A
TNF
TNFAIP3
TNFRSF14
FOS
JUNB
NFKB1
REL
CIITA
HLA-DQA1
CD86
HIF1A
RUNX1
CEBPD
CSF1R
JAK2
TLR2
Mean expression in group
0.0 0.5 1.0
h
Mouse Dura
Ctrl young
truncated-αSYN young
Ctrl old
truncated-αSYN old
Tnfrsf1b
Tnfrsf1a
Tnf
Tnfaip3
Tnfrsf14
Nfkb1
Ciita
H2-K1
H2-Q7
Cd74
Mean expression in group
0.0 0.5 1.0

Figure 3

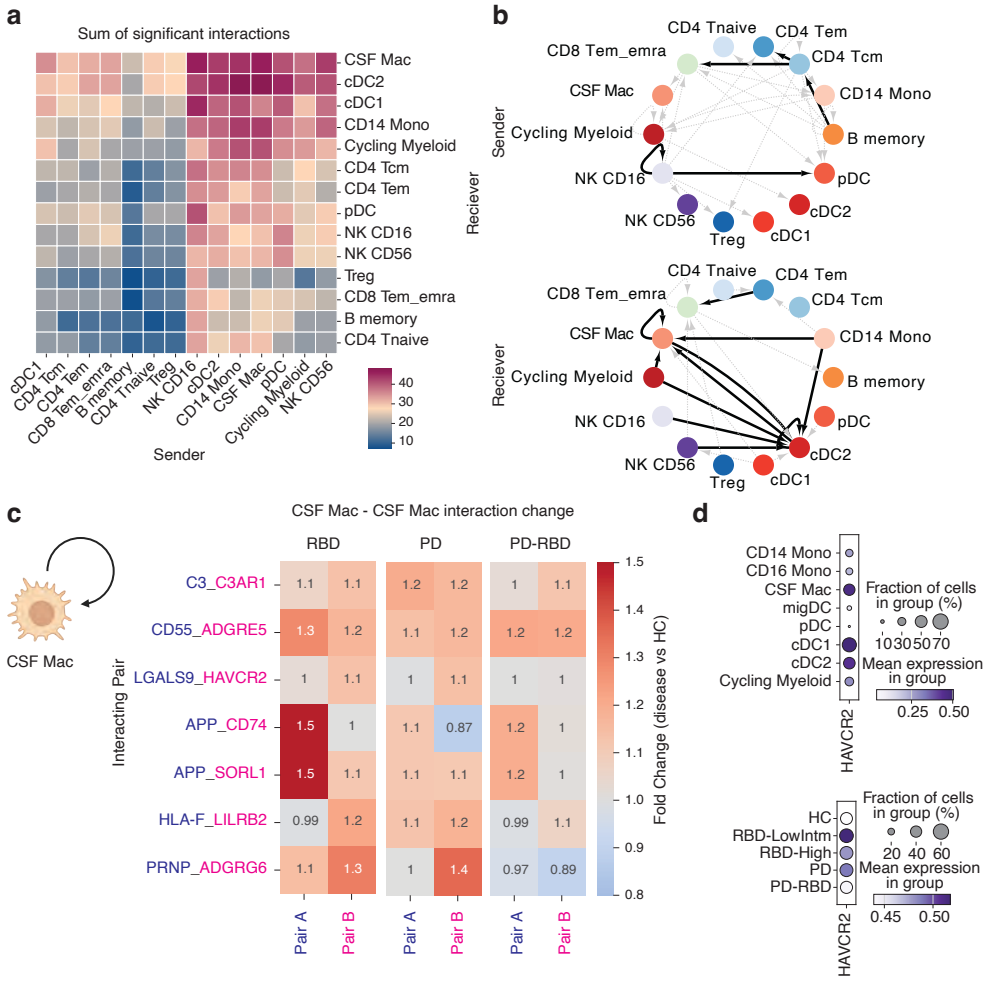

a
Sum of significant interactions
CSF Mac
cDC2
cDC1
CD14 Mono
Cycling Myeloid
CD4 Tcm
CD4 Tem
pDC
NK CD16
NK CD56
Treg
CD8 Tem_emra
B memory
CD4 Tnaive
Sender
40
30
20
10
b
Sender
Reciever
CD4 Tnaive
CD4 Tem
CD8 Tem_emra
CD4 Tcm
CSF Mac
CD14 Mono
Cycling Myeloid
B memory
NK CD16
pDC
NK CD56
cDC2
Treg
cDC1
c
CSF Mac - CSF Mac interaction change
CSF Mac
RBD
PD
PD-RBD
Interacting Pair
C3_C3AR1
CD55_ADGRE5
LGALS9_HAVCR2
APP_CD74
APP_SORL1
HLA-F_LILRB2
PRNP_ADGRG6
Pair A
Pair B
Fold Change (disease vs HC)
d
CD14 Mono
CD16 Mono
CSF Mac
migDC
pDC
cDC1
cDC2
Cycling Myeloid
HAVCR2
Fraction of cells in group (%)
10 30 50 70
Mean expression in group
0.25 0.50
HC
RBD-LowIntm
RBD-High
PD
PD-RBD
20 40 60
0.45 0.50

Figure 4

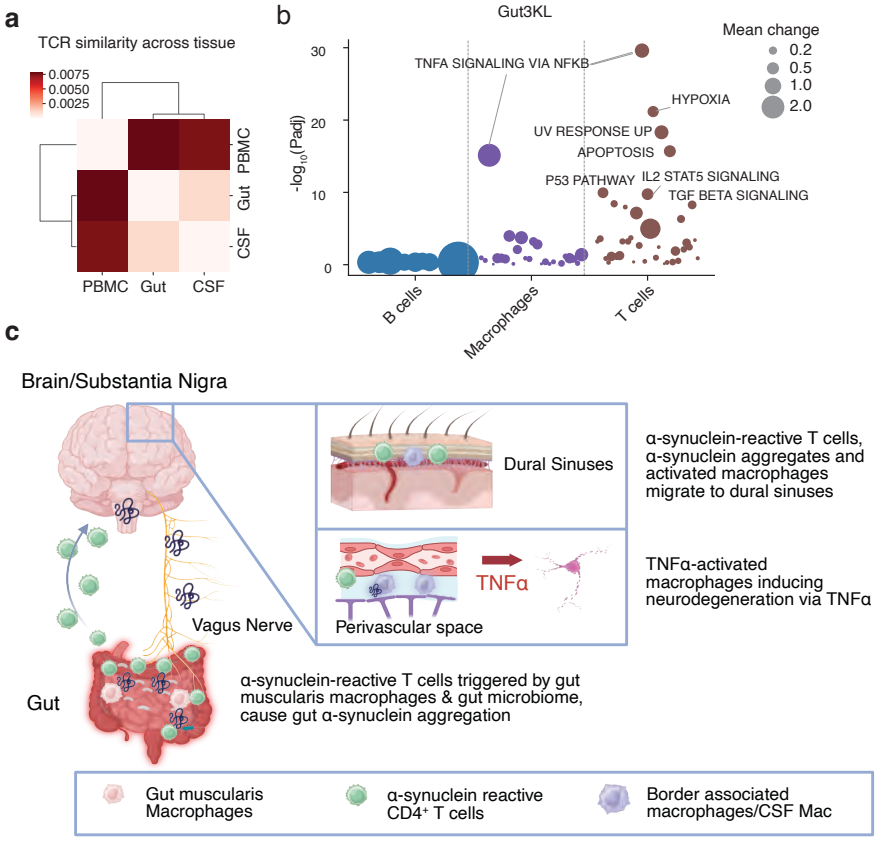
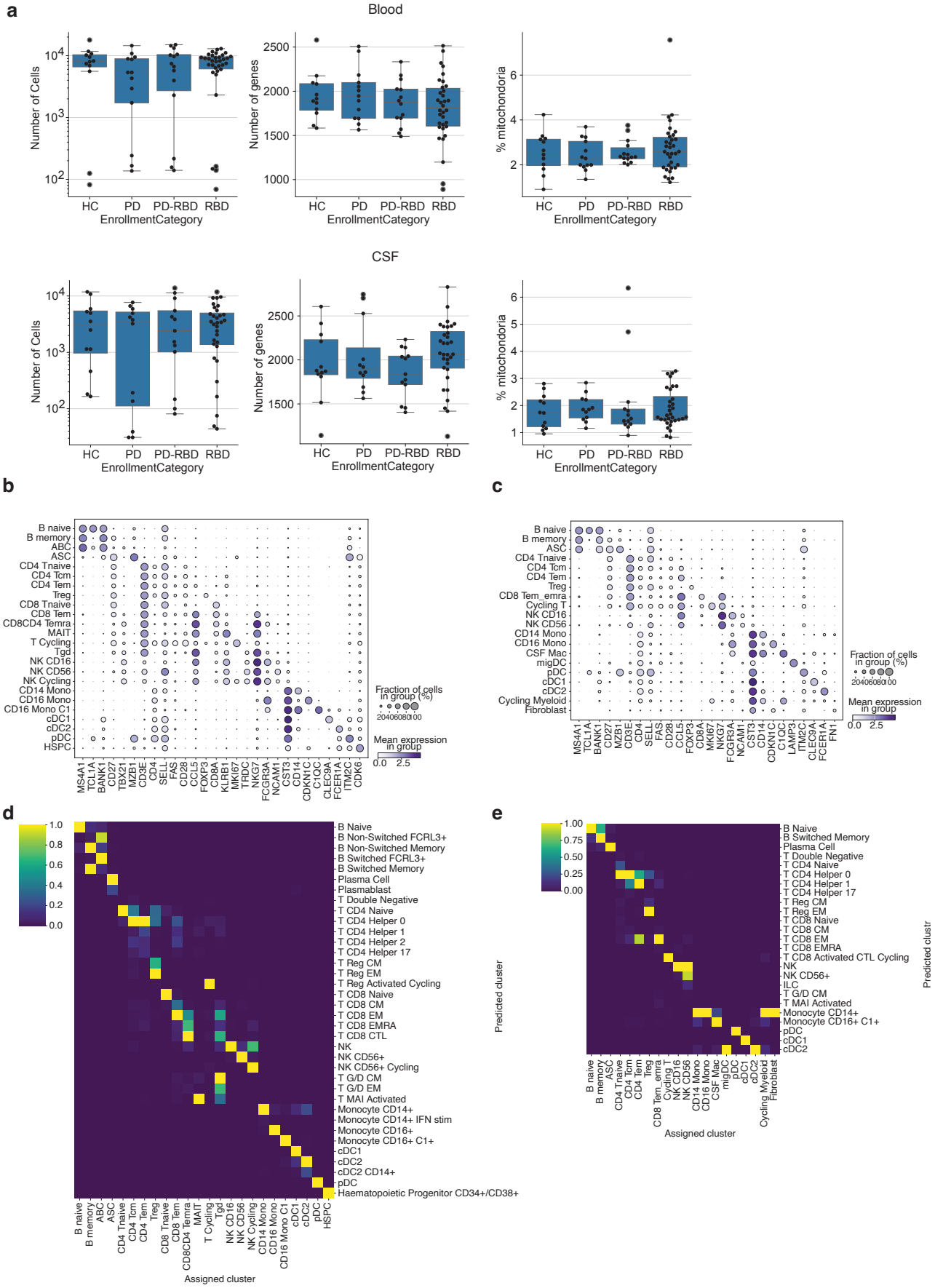
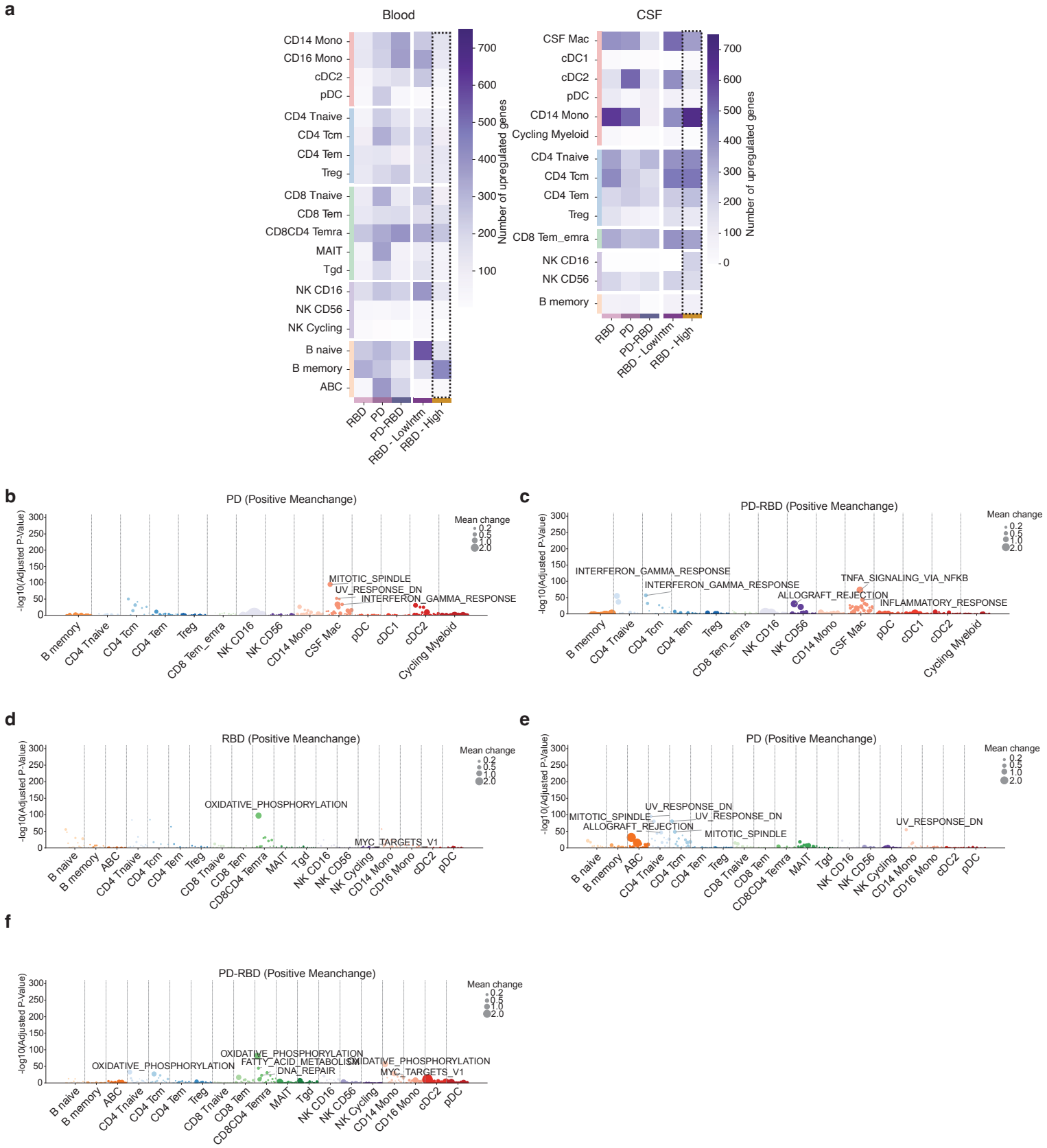

a
TCR similarity across tissue
0.0075
0.0050
0.0025
PBMC
Gut
CSF
b
Gut3KL
Mean change
0.2
0.5
1.0
2.0
TNFA SIGNALING VIA NFKB
HYPOXIA
UV RESPONSE UP
APOPTOSIS
P53 PATHWAY
IL2 STAT5 SIGNALING
TGF BETA SIGNALING
-log10(Padj)
B cells
Macrophages
T cells
c
Brain/Substantia Nigra
Dural Sinuses
α-synuclein-reactive T cells, α-synuclein aggregates and activated macrophages migrate to dural sinuses
TNFα
Perivascular space
TNFα-activated macrophages inducing neurodegeneration via TNFα
Vagus Nerve
Gut
α-synuclein-reactive T cells triggered by gut muscularis macrophages & gut microbiome, cause gut α-synuclein aggregation
Gut muscularis Macrophages
α-synuclein reactive CD4+ T cells
Border associated macrophages/CSF Mac

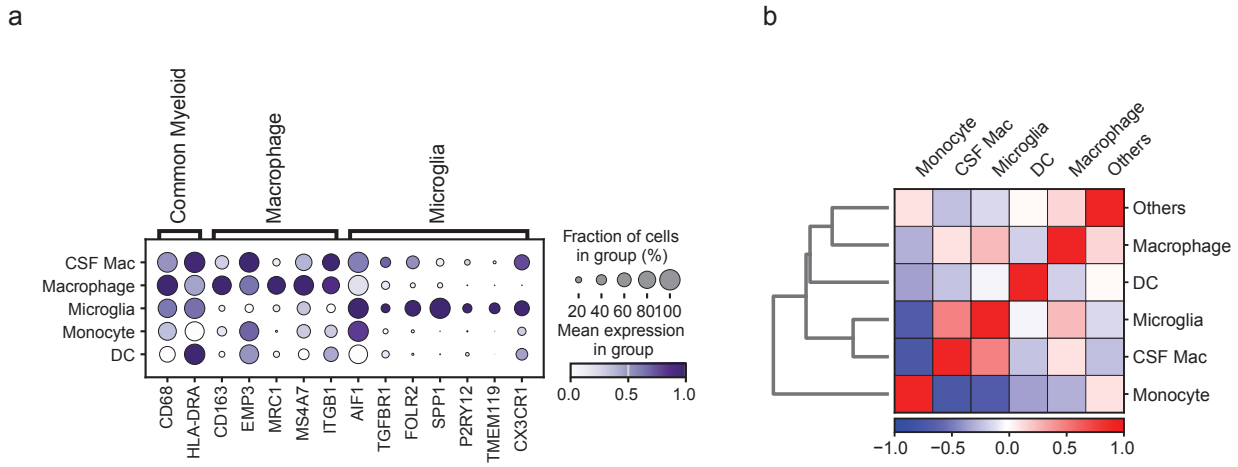
Figure 5



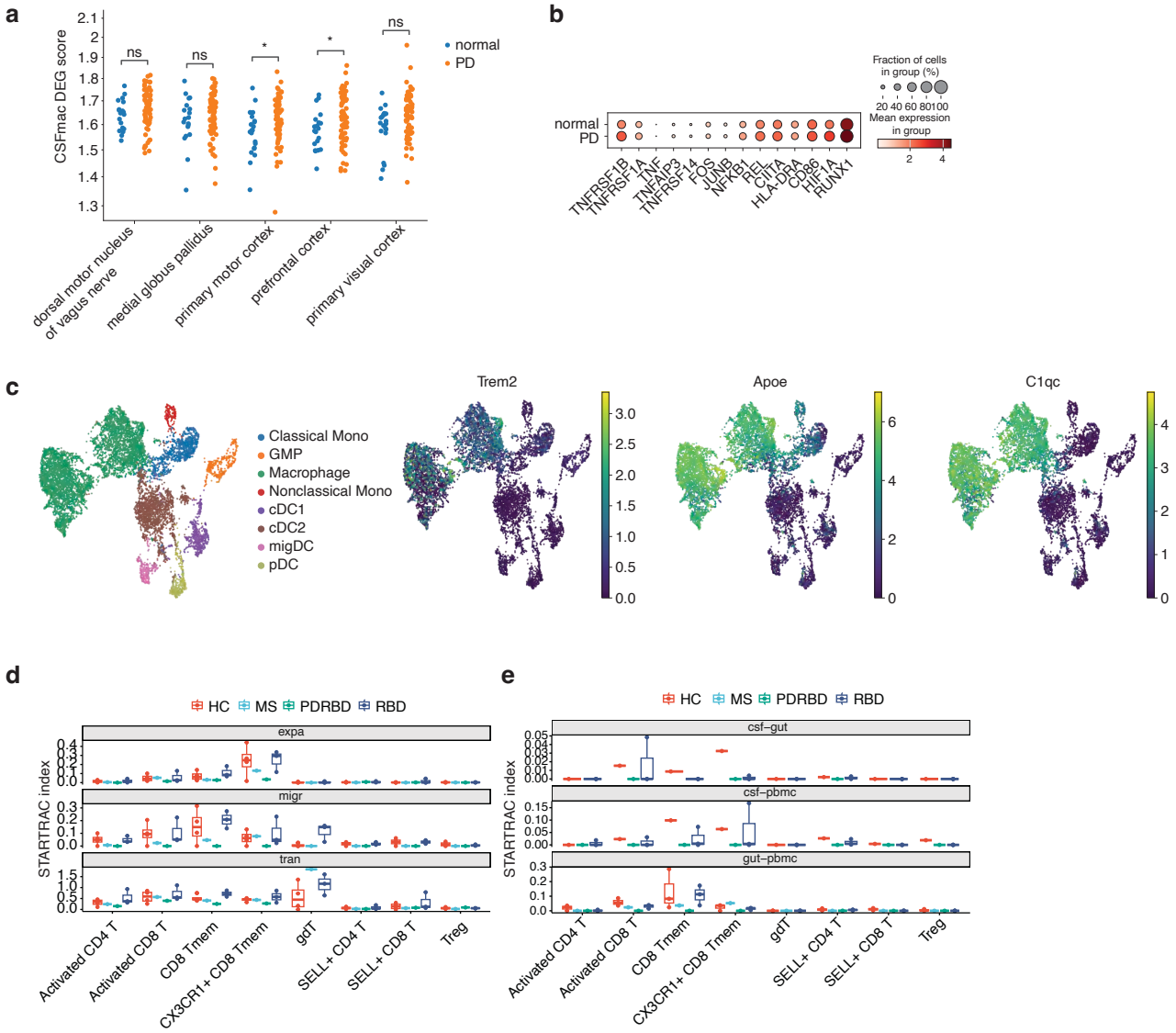
Supplementary Figure 1



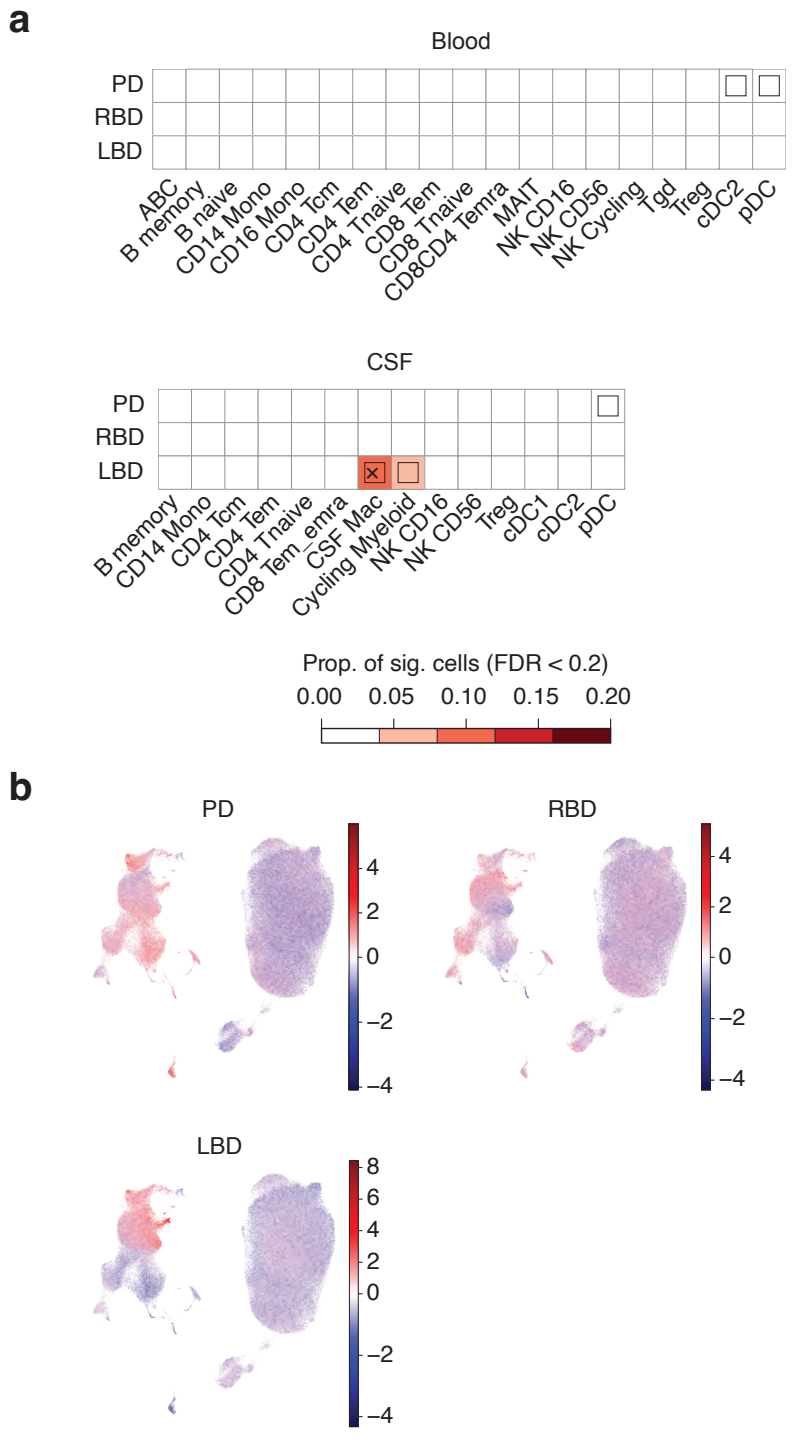
Supplementary Figure 2



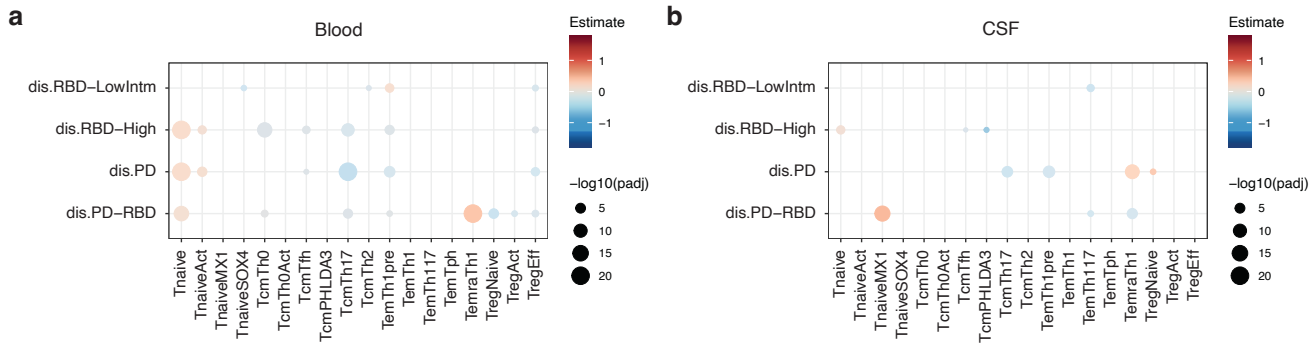
Supplementary Figure 3



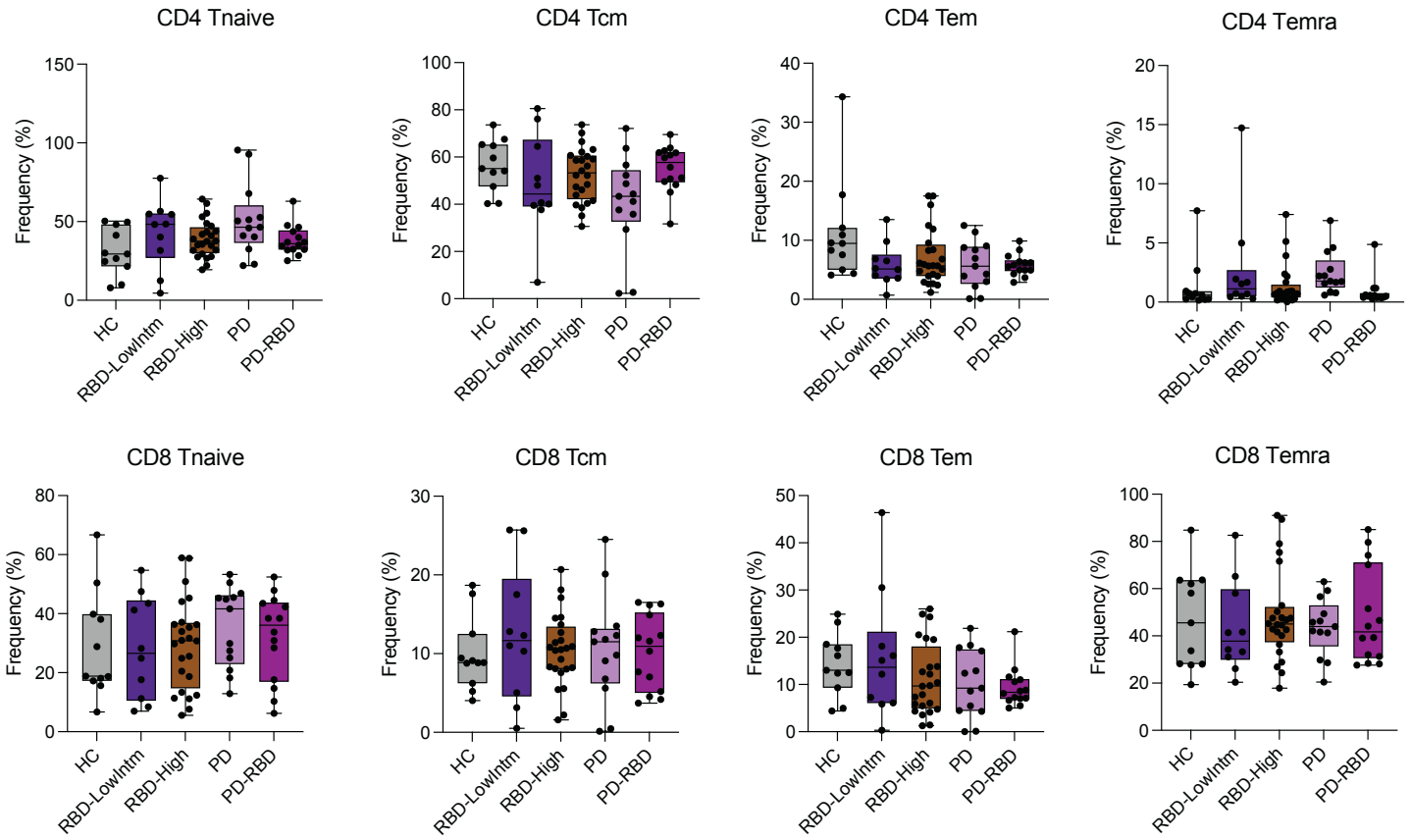
Supplementary Figure 4



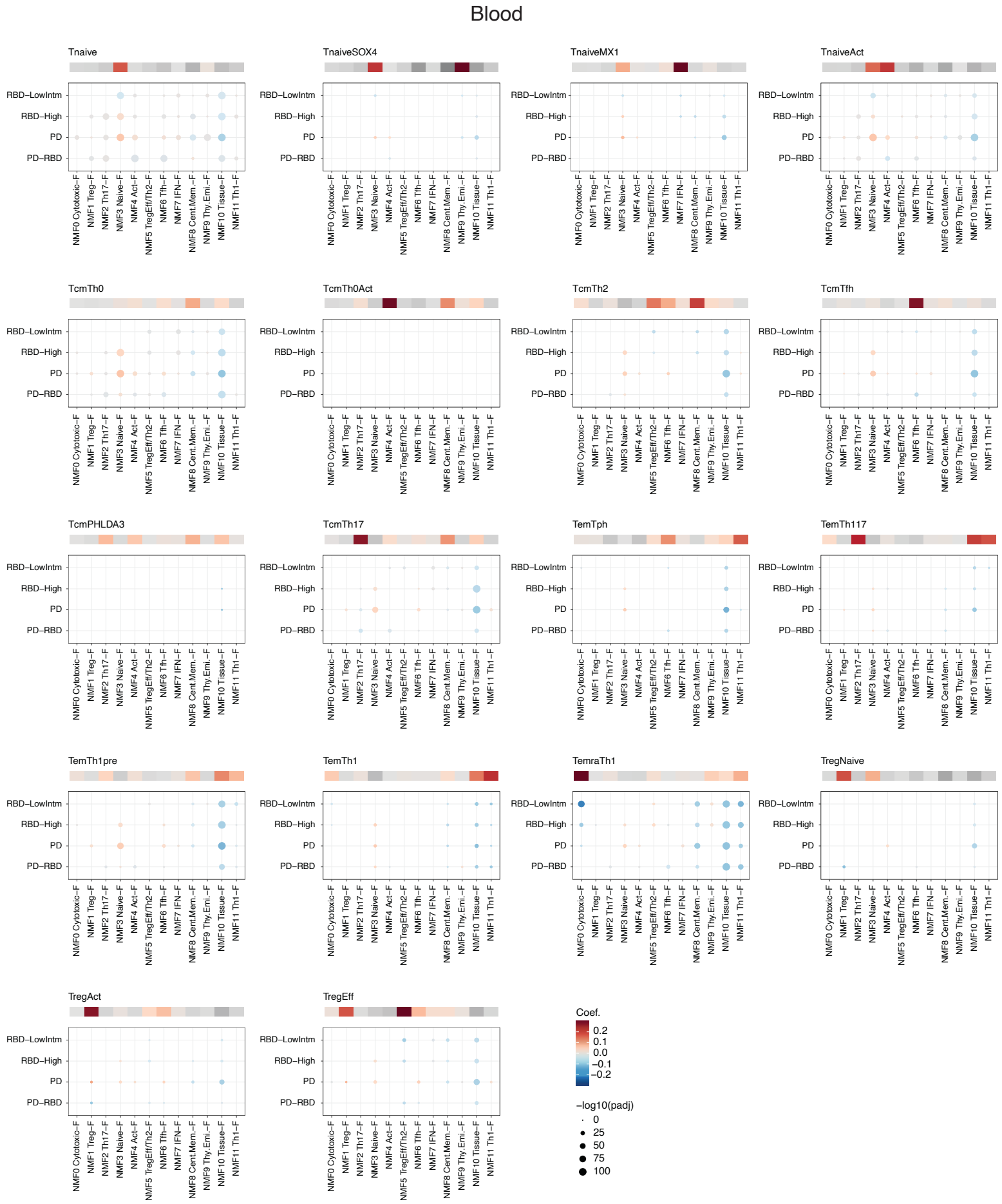
Supplementary Figure 5



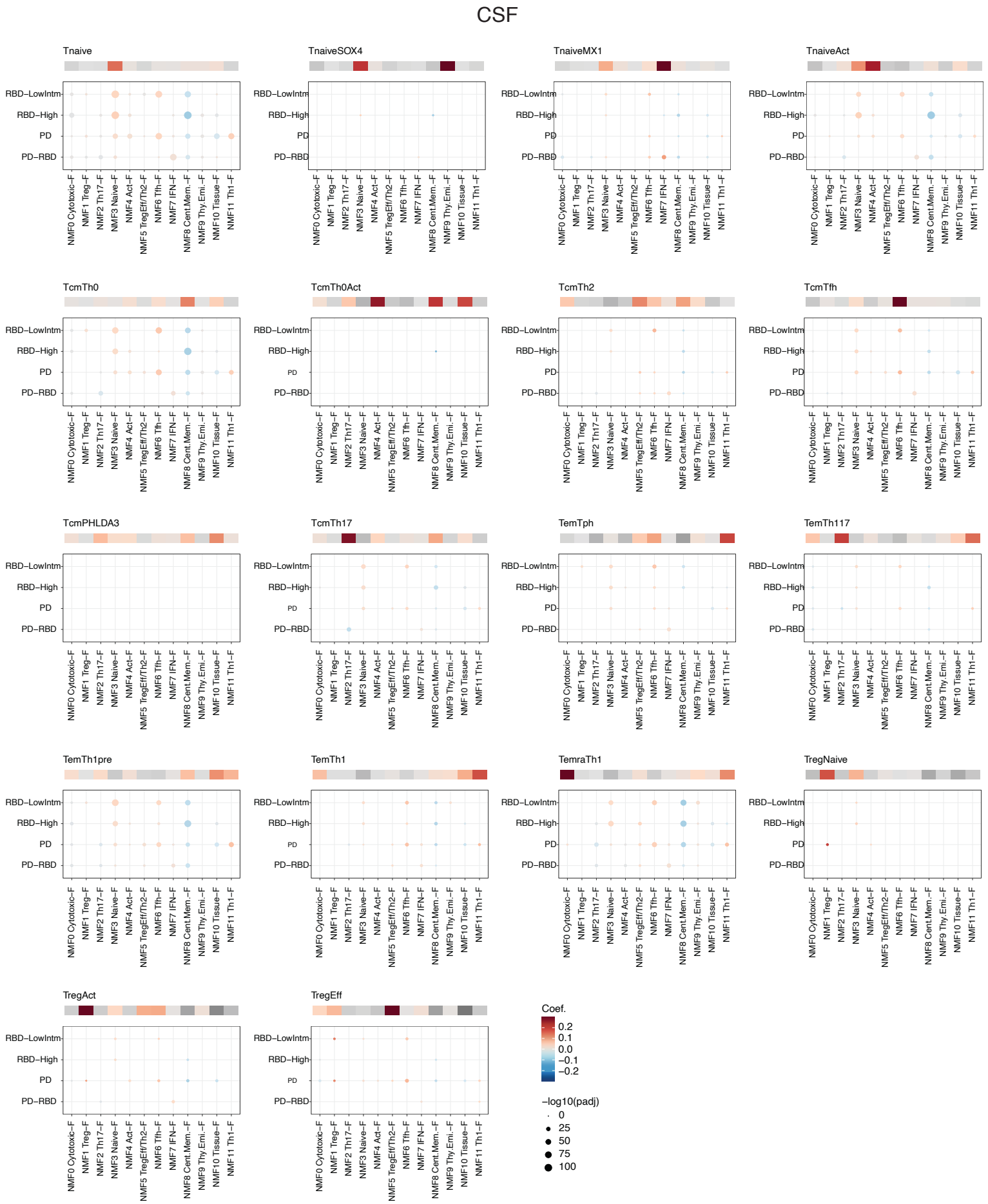
Supplementary Figure 6



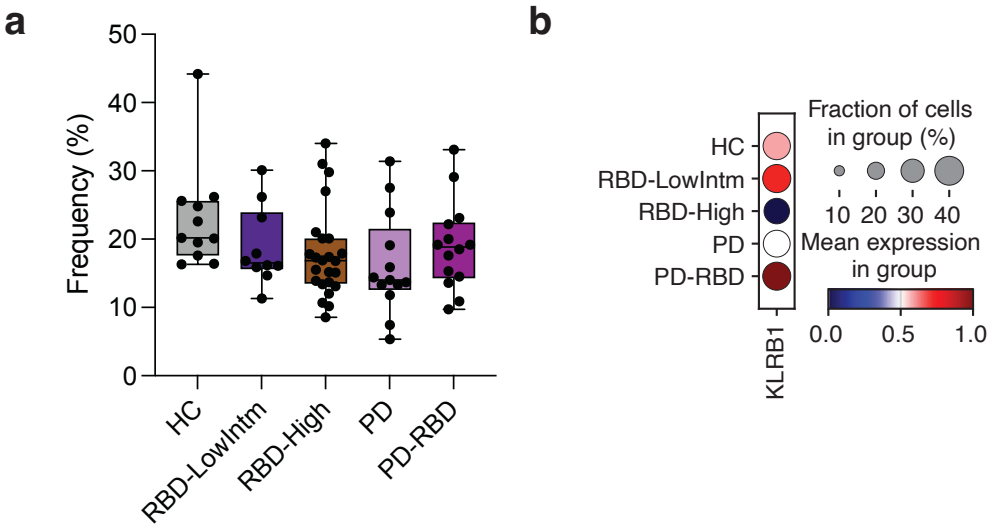
Supplementary Figure 7



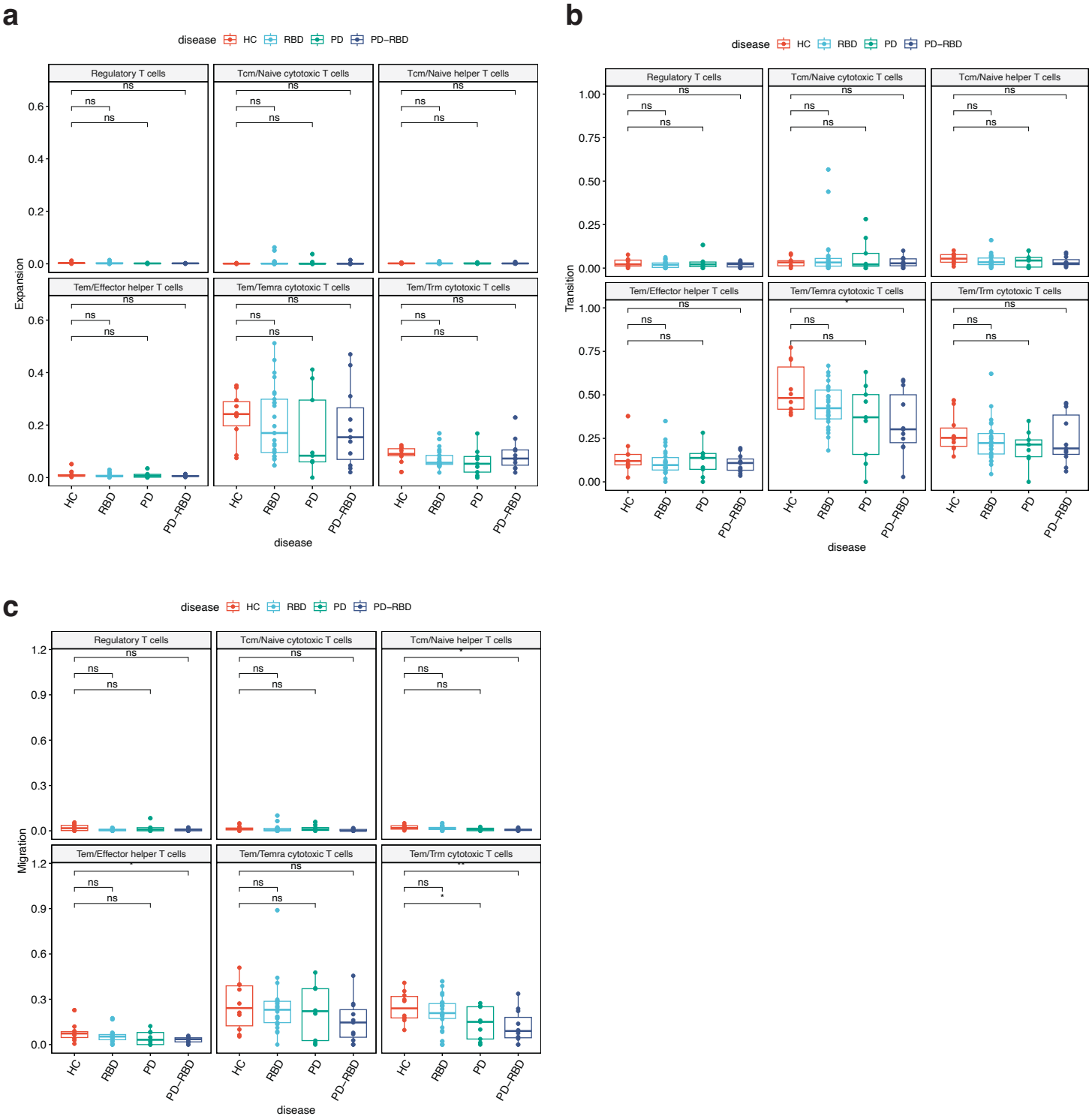
Supplementary Figure 8



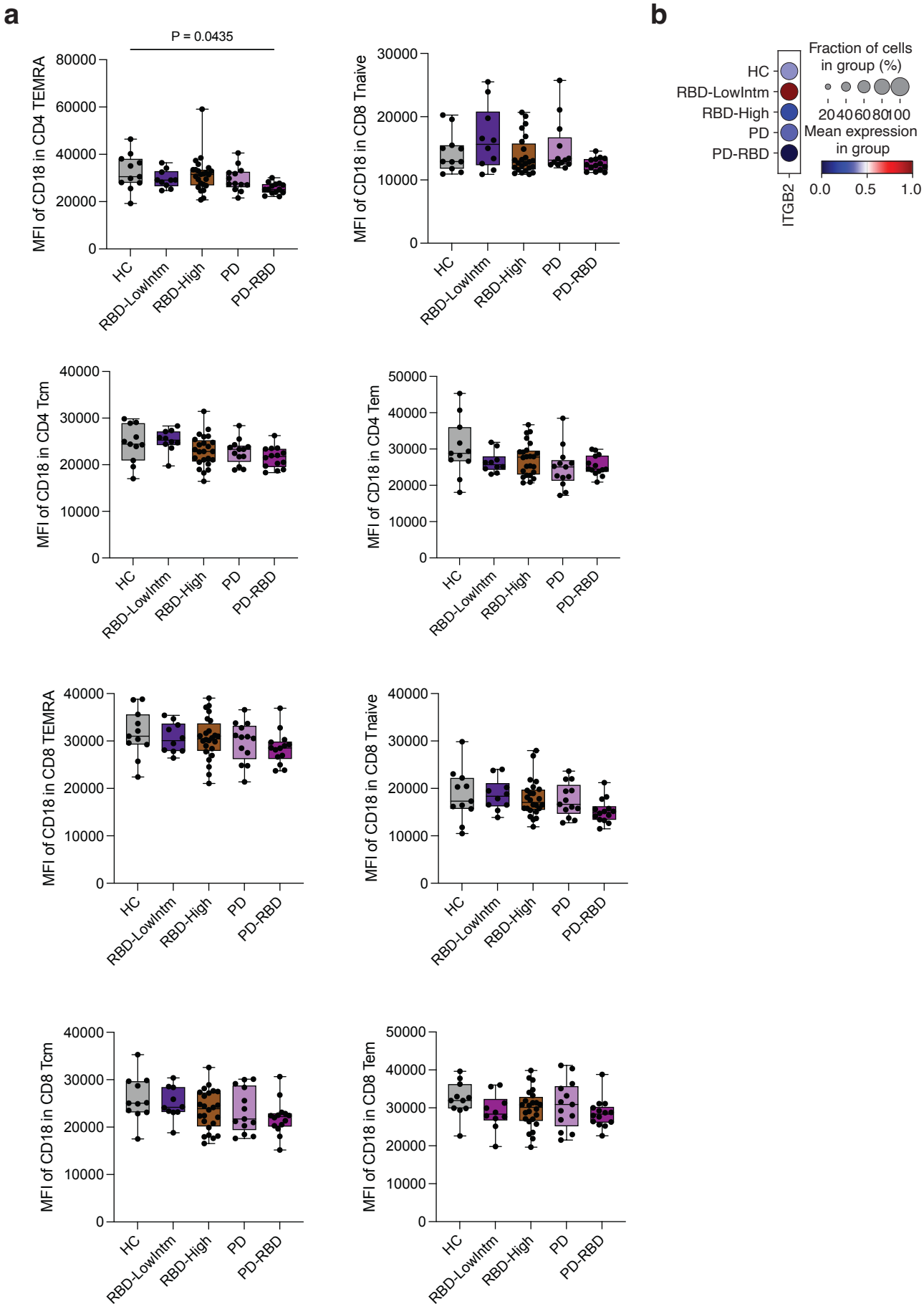
Supplementary Figure 9



Supplementary Figure 10



Supplementary Figure 11



Supplementary Figure 12