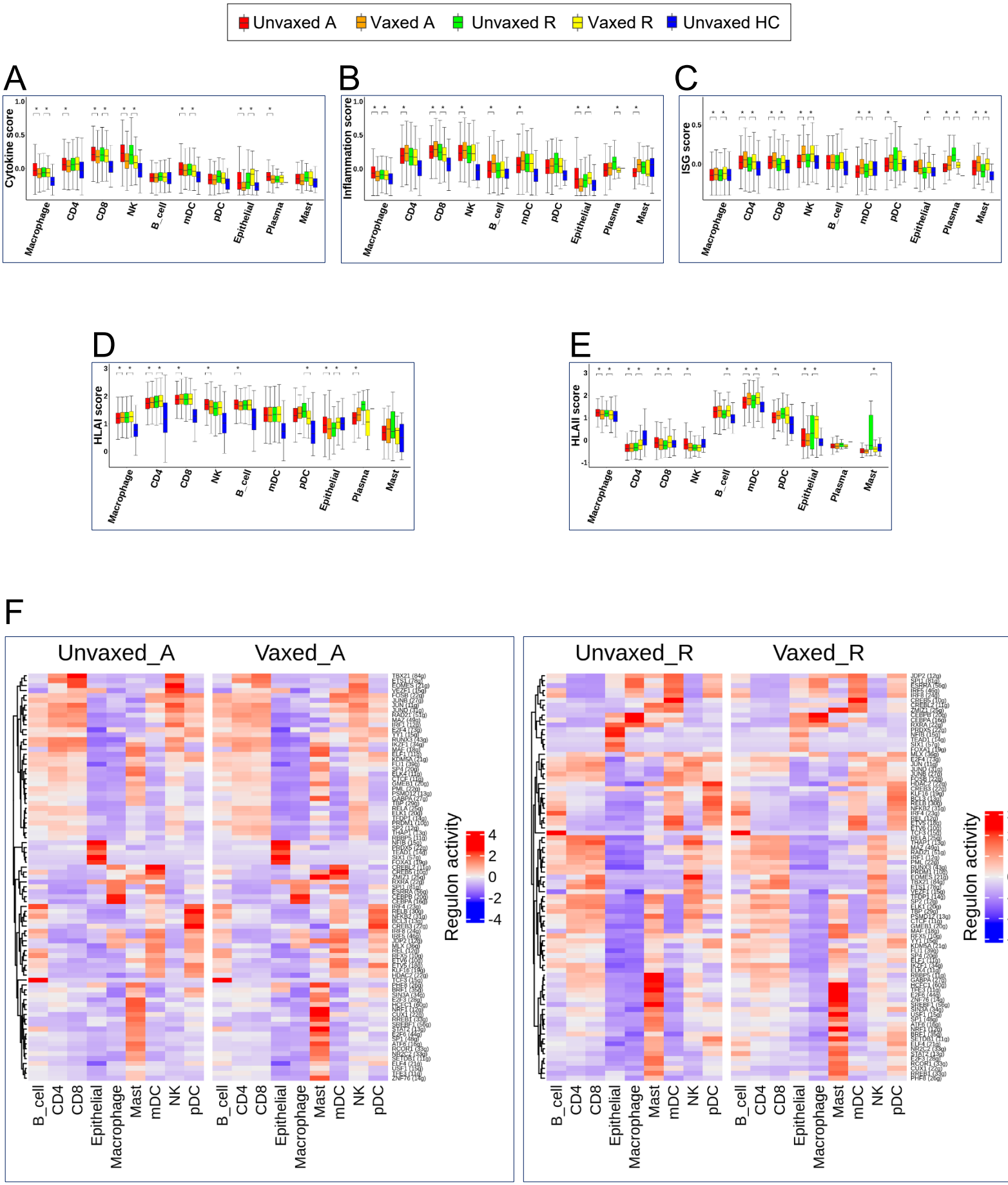
Supplementary Figure 1



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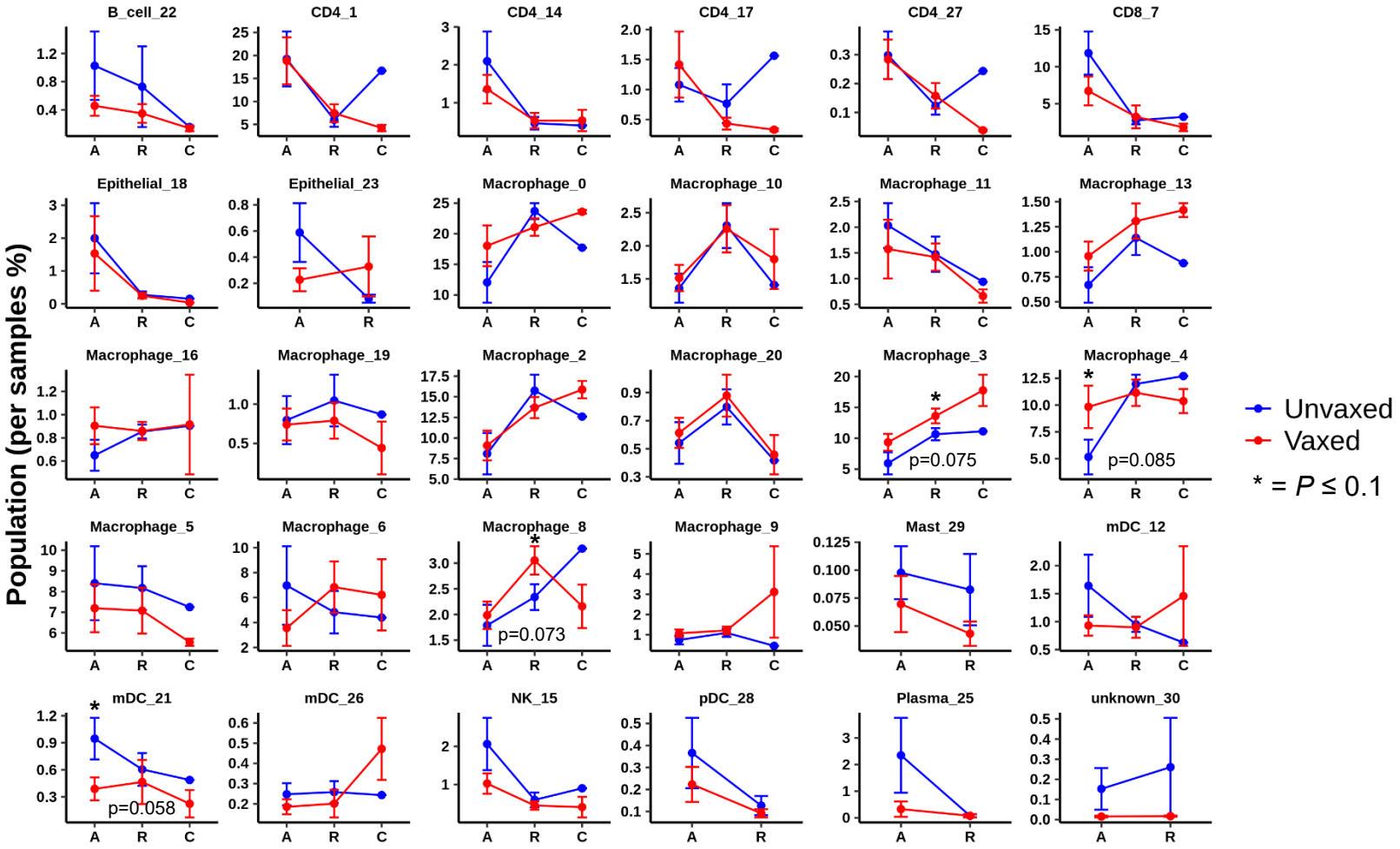
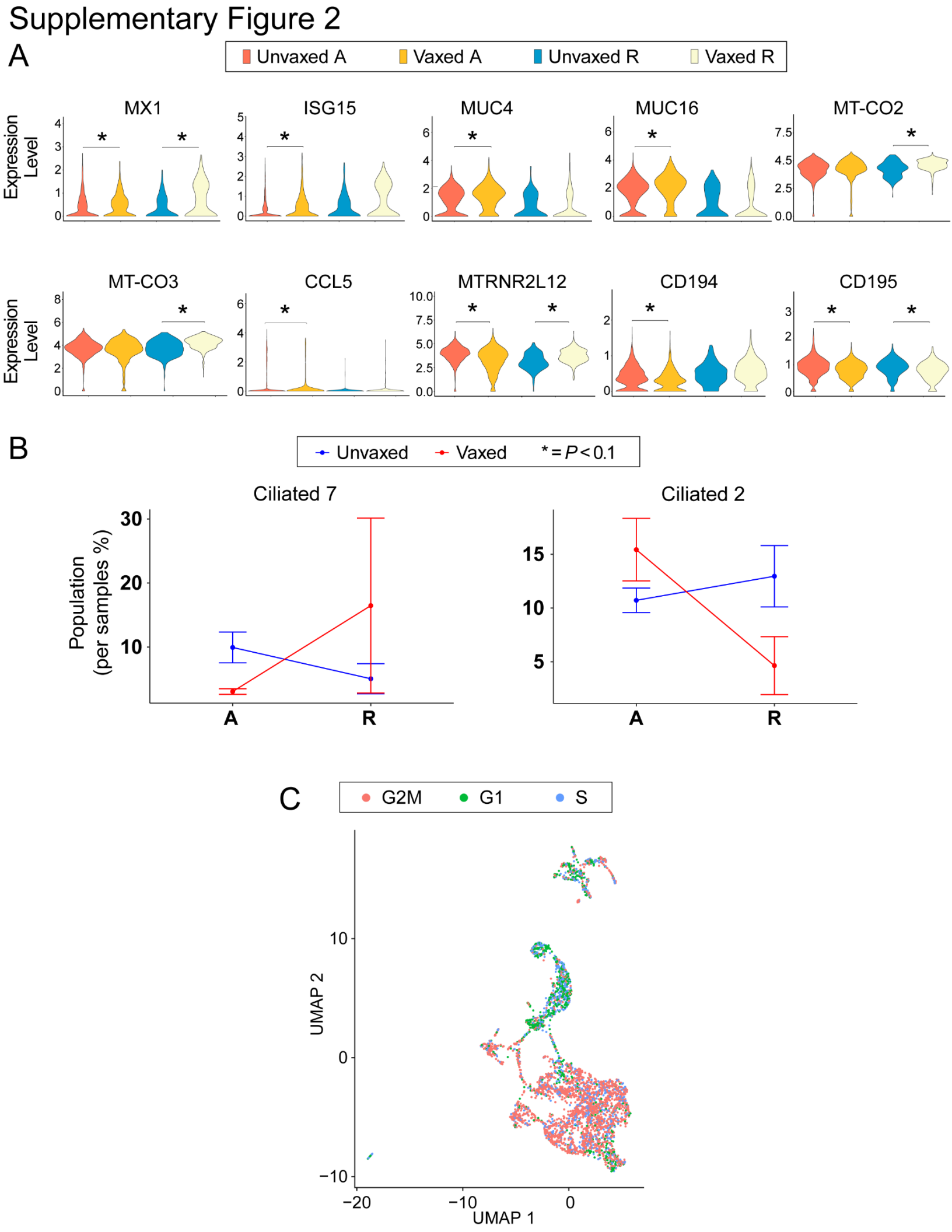


Supplementary Figure 1: Natural and breakthrough SARS-CoV-2 infection differentially shape lung microenvironment in acute and recovery phases. **a-e)** Box plots displaying gene set scores for (a) cytokine and chemokine signaling, (b) inflammatory processes, (c) interferon stimulated genes (ISG), human leukocyte antigens (HLA): major histocompatibility complex (MHC) class I, and MHC class II genes in lung cells from natural (unvaccinated/unvaxed) and breakthrough (vaccinated/vaxed) infections at acute (A) and recovery (R) phases. Comparisons are made between unvaxed A and vaxed A and unvaxed R and vaxed R. * represents $P \leq 0.05$. Unvaxed healthy control (HC) is provided as reference only. (**f**) Heatmap displaying regulon activity in all lung cell types from unvaxed and vaxed during A and R phases. (**g**) Proportion of all lung cells types (per sample, %) in unvaxed (blue) and vaxed (red) at A, R and convalescence (C) phases. The numbers following "cells" indicate the respective clusters of the lung cells. Comparisons are made between unvaxed A and vaxed A, unvaxed R and vaxed R, and unvaxed C and vaxed C. * represents $P \leq 0.1$. Unvaccinated (n = 8; A = 8, R = 8, C = 1), vaccinated (n = 11; A = 11, R = 11, C = 2) and healthy control (HC, n = 4).

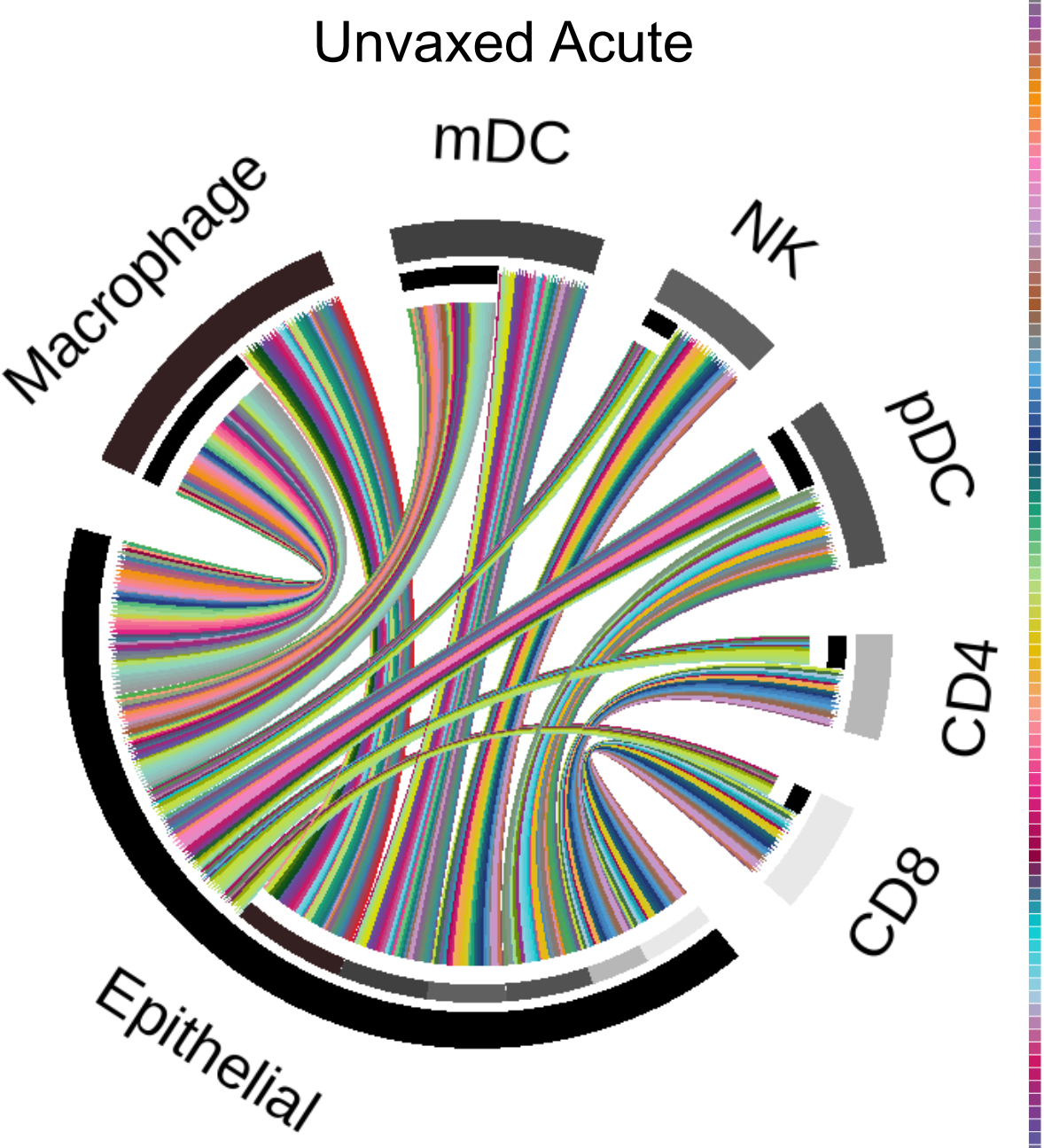
Supplementary Figure 2



Supplementary Figure 2: Breakthrough SARS-CoV-2 infection promotes enhanced lung epithelial repair and antiviral response compared to persistent cell activation in natural infection.

Violin plots showing expression of genes (*MX1, ISG15, MUC4, MUC16, MT-CO2, MT-CO3, CCL5, MTRNR2L12*) and proteins (CD194, CD195) in total epithelial cells from natural (unvaccinated /unvaxed) and breakthrough (vaccinated/vaxed) infection at Acute (A) and Recovery (R) phases. Comparisons are made between unvaxed A and vaxed A and unvaxed R and vaxed R. * represents $P \leq 0.05$. (**b**) Proportion of ciliated epithelial cells (per sample, %) in unvaxed (blue) and vaxed (red) at A and R phases. The numbers following "ciliated cells" indicate the respective clusters of the ciliated cells. Comparisons are made between unvaxed A vs vaxed A and unvaxed R vs vaxed R. * represents $P \leq 0.1$ (**c**) Uniform Manifold Approximation and Projection (UMAP) embedding of lung epithelial cell subtypes in different cell cycle states. Unvaccinated ($n = 8$; A = 8, R = 8) and vaccinated ($n = 11$; A = 11, R = 11).

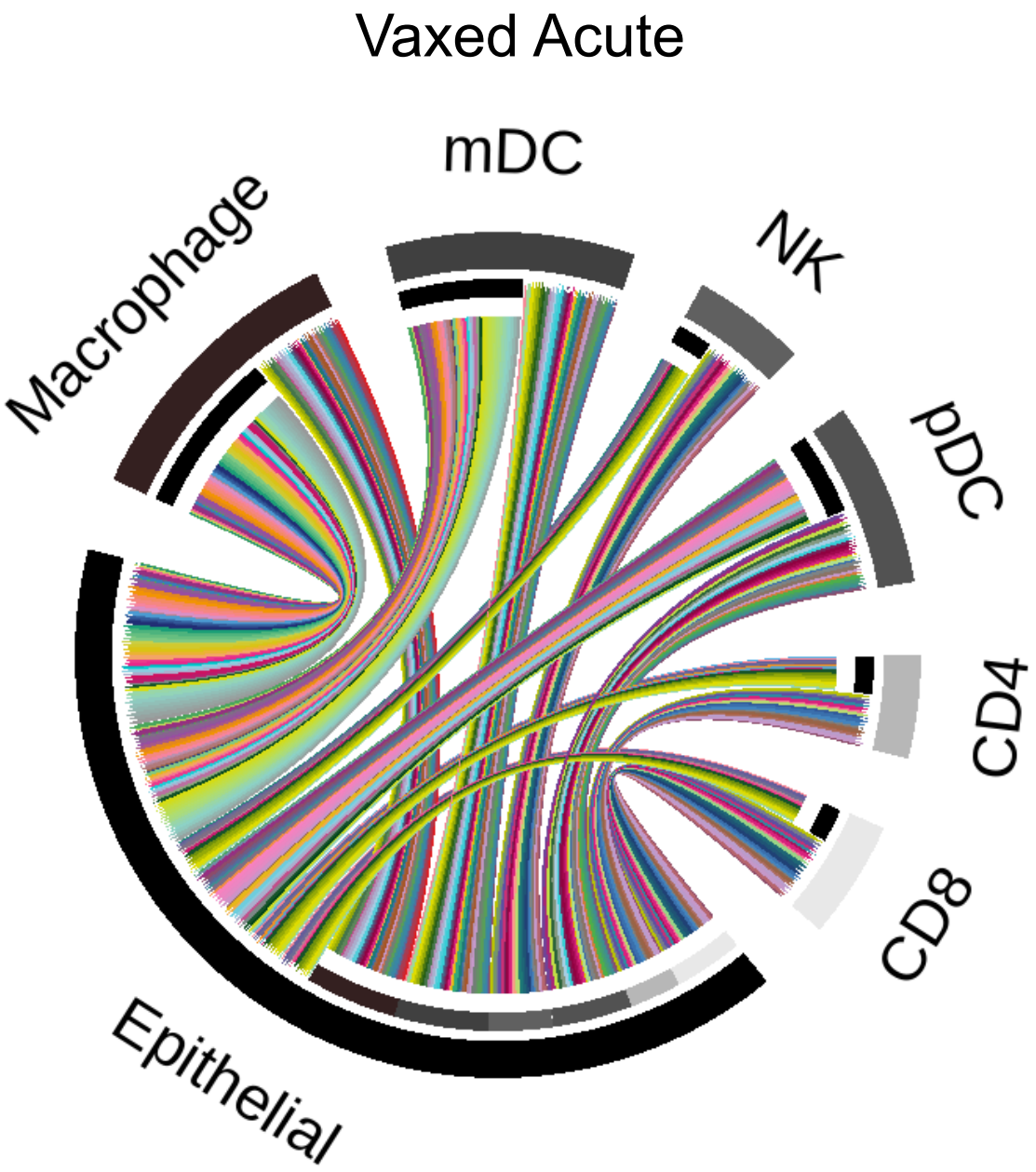
Supplementary Figure 3A



interactions

- APP - FPR2
- APP - TREM2 - receptor
- SAA1 - FPR2
- APP - SORL1
- APP - TNFRSF21
- APOE - TREM2 - receptor
- CLU - TREM2 - receptor
- CDH1 - integrin - a2b1 - complex
- APP - CD74
- APP - PLXNA4
- APLP2 - PLXNA4
- ENTPD1 - ADORA2B
- AGRN - PTPRS
- ANXA1 - FPR3
- HEBP1 - FPR3
- ANXA1 - FPR1
- AREG - EGFR
- EREG - EGFR
- HBEGF - EGFR
- BAG6 - NCR3
- COL8A2 - integrin - a2b1 - complex
- BST2 - LILRA4
- EREG - ERBB4
- HBEGF - ERBB4
- COL9A2 - integrin - a2b1 - complex
- COL9A3 - integrin - a2b1 - complex
- COL24A1 - integrin - a2b1 - complex
- COL26A1 - integrin - a2b1 - complex
- FN1 - integrin - a2b1 - complex
- CDH1 - integrin - aEb7 - complex
- COL4A5 - integrin - a1b1 - complex
- COL21A1 - integrin - a1b1 - complex
- CDH1 - KLRG1
- CADM1 - CADM1
- CRTAM - CADM1
- CADM1 - NECTIN3
- CXCL2 - DPP4
- CCL17 - CCR4
- CCL19 - ACKR4
- ALCAM - CD6
- PODXL2 - SELL
- PODXL - SELL
- SELL - SELPLG
- CD40LG - CD40
- COL28A1 - integrin - a1b1 - complex
- C3 - integrin - aMb2 - complex
- ICAM1 - integrin - aMb2 - complex
- FN1 - integrin - a3b1 - complex
- THBS1 - integrin - a3b1 - complex
- ICAM1 - integrin - aXb2 - complex
- FN1 - integrin - a4b1 - complex
- PLAUR - integrin - a4b1 - complex
- C3 - C3AR1
- C3 - C5AR2
- FN1 - integrin - aVb1 - complex
- integrin - aVb5 - complex - FN1
- TGM2 - ADGRG1
- COL4A5 - ADGRG6
- ICAM1 - integrin - aLb2 - complex
- IFNG - Type - II - IFNR
- CXCL1 - CXCR2
- IL1B - IL1 - receptor
- DSC2 - DSG2
- EFNA1 - EPHA1
- EFNA5 - EPHA1
- EFNA1 - EPHA2
- EFNA5 - EPHA2
- EFNA1 - EPHA4
- EFNA5 - EPHA4
- IL1 - receptor - IL1RN
- IL7 - IL7 - receptor
- CD8 - receptor - LCK
- SEMA3A - PlexinA1 - complex3
- SEMA3A - PlexinA2 - complex1
- FLT3LG - FLT3
- GAS6 - AXL
- HBEGF - ERBB2
- HLA-E - CD94:NKG2A
- HLA-E - KLRC1
- PlexinA3 - complex1 - SEMA3A
- ICAM1 - ITGAL
- SPN - ICAM1
- SEMA3A - PlexinA4 - complex1
- WNT5B - FZD3 - LRP5
- LipoxinA4 - byALOX5 - FPR2
- BMPR1B - BMPR2 - BMPR1A
- KLRB1 - CLEC2D
- LAIR1 - LILRB4
- Cholesterol - byCEL - RORA
- Cholesterol - byDHCR24 - RORA
- Cholesterol - byLIPA - RORA
- Cholesterol - byDHCR24 - RORC
- CD58 - CD2
- MDK - ALK
- MDK - PTPRZ1
- NELL2 - ROBO3
- NTN1 - UNC5B
- NTN4 - UNC5B
- SCT - VIPR1
- PECAM1 - CD38
- SEMA3A - NRP1
- SEMA3C - NRP2
- SEMA3F - NRP2
- VEGFA - NRP2
- Cholesterol - byLIPA - RORC
- Desmosterol - byDHCR7 - NR1H3
- LeukotrieneC4 - byLTC4S - CYSLTR1
- LRFN4 - PTPRS
- LRFN4 - PTPRF
- SLITRK6 - PTPRS
- TIGIT - NECTIN2
- NECTIN3 - TIGIT
- NECTIN1 - NECTIN4
- LipoxinA4 - byALOX5 - CYSLTR1
- LTC4 - byLTC4S - CYSLTR1
- ProstaglandinD2 - byAKR1C3 - PTGDR
- ProstaglandinD2 - byAKR1C3 - PTGFR
- SEMA4D - PLXNB1
- SEMA7A - PLXNC1
- SLIT1 - ROBO1
- SLIT2 - ROBO1
- TNFSF10 - TNFRSF10D
- TNFSF10 - TNFRSF10A
- TNFSF10 - TNFRSF10B
- TNFSF12 - TNFRSF12A
- LTB - LTBR
- ProstaglandinF2a - byAKR1B1 - PTGFR
- CX3CL1 - CX3CR1
- IL1RAP - PTPRF
- APLP2 - PIGR
- CD1B - complex - IL6ST
- TENM4 - ADGRL1
- Glutamate - byGLS - and - SLC1A3 - GRIK2
- Glutamate - byGLS - and - SLC1A7 - GRIK2
- Glutamate - byGLS - and - SLC1A3 - GRM7
- Glutamate - byGLS - and - SLC1A7 - GRM7

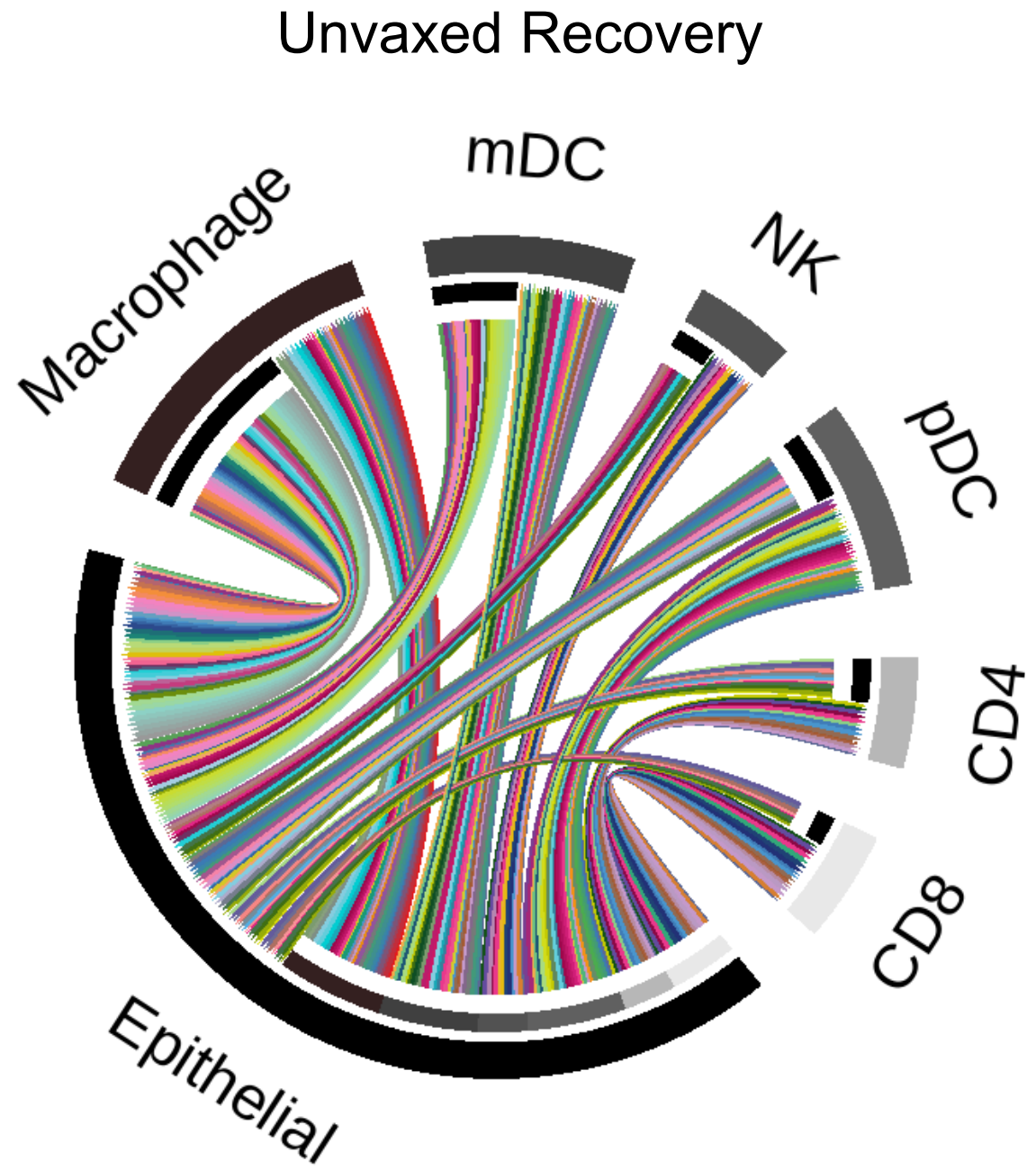
Supplementary Figure 3B



interactions

- APP - FPR2
- APP - TREM2 - receptor
- SAA1 - FPR2
- APP - SORL1
- APP - TNFRSF21
- APOE - TREM2 - receptor
- CLU - TREM2 - receptor
- CDH1 - integrin - a2b1 - complex
- APP - CD74
- APP - PLXNA4
- APLP2 - PLXNA4
- ENTPD1 - ADORA2B
- AGRN - PTPRS
- ANXA1 - FPR3
- HEBP1 - FPR3
- ANXA1 - FPR1
- AREG - EGFR
- EREG - EGFR
- HBEGF - EGFR
- TGFA - EGFR
- BAG6 - NCR3
- COL1A1 - integrin - a2b1 - complex
- BST2 - LILRA4
- EREG - ERBB4
- HBEGF - ERBB4
- LTA - TNFRSF14
- COL8A2 - integrin - a2b1 - complex
- COL9A2 - integrin - a2b1 - complex
- COL9A3 - integrin - a2b1 - complex
- COL24A1 - integrin - a2b1 - complex
- COL26A1 - integrin - a2b1 - complex
- FN1 - integrin - a2b1 - complex
- LAMC1 - integrin - a2b1 - complex
- CDH1 - integrin - aEb7 - complex
- COL4A5 - integrin - a1b1 - complex
- COL21A1 - integrin - a1b1 - complex
- CDH1 - KLRG1
- CADM1 - CADM1
- CRTAM - CADM1
- CADM1 - NECTIN3
- CXCL2 - DPP4
- CCL17 - CCR4
- RARRES2 - CCRL2
- ALCAM - CD6
- PODXL2 - SELL
- PODXL - SELL
- SELL - SELPLG
- CD40LG - CD40
- CTLA4 - CD86
- CEACAM8 - CEACAM6
- COL28A1 - integrin - a1b1 - complex
- C3 - integrin - aMb2 - complex
- ICAM1 - integrin - aMb2 - complex
- FN1 - integrin - a3b1 - complex
- THBS1 - integrin - a3b1 - complex
- ICAM1 - integrin - aXb2 - complex
- FN1 - integrin - a4b1 - complex
- FN1 - integrin - aVb1 - complex
- FN1 - integrin - aVb5 - complex
- integrin - aLb2 - complex - ICAM1
- TGM2 - ADGRG1
- COL4A5 - ADGRG6
- IFNG - Type - II - IFNR
- IL1B - IL1 - receptor
- DSC2 - DSG2
- EFNA5 - EPHA1
- EFNA5 - EPHA2
- EFNA5 - EPHA4
- IL1 - receptor - IL1RN
- IL7 - IL7 - receptor
- LCK - CD8 - receptor
- WNT5B - FZD4 - LRP5
- FLT3LG - FLT3
- GAS6 - MERTK
- LGALS3 - MERTK
- GAS6 - AXL
- HBEGF - ERBB2
- HLA-E - CD94:NKG2A
- HLA-E - KLRC1
- FZD8 - LRP6 - WNT5B
- ICAM1 - ITGAL
- SPN - ICAM1
- SEMA3A - PlexinA2 - complex1
- SEMA3A - PlexinA3 - complex1
- SEMA3A - PlexinA4 - complex1
- WNT5B - FZD8 - LRP5
- KLRB1 - CLEC2D
- LAIR1 - LILRB4
- WNT5B - FZD3 - LRP5
- WNT5B - FZD3 - LRP6
- WNT5B - FZD4 - LRP6
- WNT5B - FZD6 - LRP5
- WNT5B - FZD6 - LRP6
- LipoxinA4 - byALOX5 - FPR2
- CD58 - CD2
- MDK - ALK
- MDK - PTPRZ1
- BMPR1B - BMPR2 - BMPR1A
- NELL2 - ROBO3
- NTN1 - UNC5B
- SCT - VIPR1
- PECAM1 - CD38
- SEMA3A - NRP1
- SEMA3C - NRP2
- SEMA3F - NRP2
- VEGFA - NRP2
- PROC - PROCR
- Cholesterol - byCEL - RORA
- Cholesterol - byDHCR24 - RORA
- Cholesterol - byLIPA - RORA
- LRFN4 - PTPRS
- LRFN4 - PTPRF
- SLITRK4 - PTPRS
- SLITRK6 - PTPRS
- PVR - CD96
- TIGIT - PVR
- TIGIT - NECTIN2
- NECTIN3 - TIGIT
- NECTIN1 - NECTIN4
- RARRES2 - CMKLR1
- Cholesterol - byDHCR24 - RORC
- KITLG - KIT
- Cholesterol - byLIPA - RORC
- LeukotrieneC4 - byLTC4S - CYSLTR1
- LipoxinA4 - byALOX5 - CYSLTR1
- SEMA4D - PLXNB1
- SEMA7A - PLXNC1
- SLIT1 - ROBO1
- SLIT2 - ROBO1
- CD96 - NECTIN1
- TNFSF10 - TNFRSF10D
- TNFSF10 - TNFRSF10A
- TNFSF10 - TNFRSF10B
- TNFSF12 - TNFRSF12A
- LTB - LTBR
- LTC4 - byLTC4S - CYSLTR1
- LeukotrieneC4 - byLTC4S - CYSLTR2
- LTC4 - byLTC4S - CYSLTR2
- ProstaglandinD2 - byAKR1C3 - PTGDR
- ProstaglandinD2 - byAKR1C3 - PTGFR
- ProstaglandinF2a - byAKR1B1 - PTGFR
- CX3CL1 - CX3CR1
- IL1RAP - PTPRF
- APLP2 - PIGR
- CD1B - complex - IL6ST
- Glutamate - byGLS - and - SLC1A3 - GRIK2
- Glutamate - byGLS - and - SLC1A7 - GRIK2
- Glutamate - byGLS - and - SLC1A3 - GRM5
- Glutamate - byGLS - and - SLC1A7 - GRM5
- Glutamate - byGLS - and - SLC1A3 - GRM7
- Glutamate - byGLS - and - SLC1A7 - GRM7

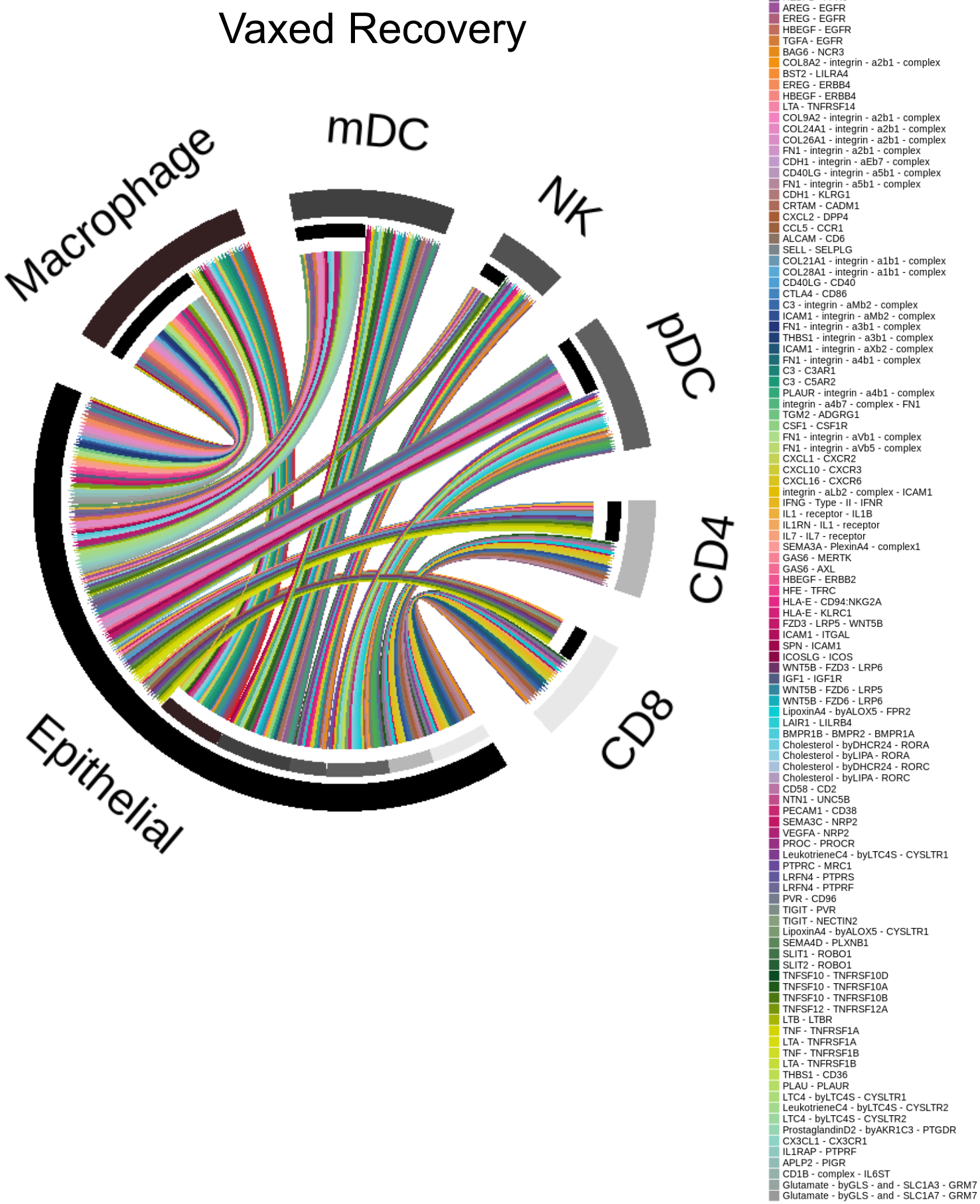
Supplementary Figure 3C



interactions

- APP - FPR2
- ANXA1 - FPR2
- APP - TREM2 - receptor
- SAA1 - FPR2
- APP - SORL1
- APP - TNFRSF21
- CLU - TREM2 - receptor
- CDH1 - integrin - a2b1 - complex
- APP - CD74
- APP - PLXNA4
- APLP2 - PLXNA4
- ENTPD1 - ADORA2B
- AGRN - PTPRS
- ANXA1 - FPR3
- HEBP1 - FPR3
- ANXA1 - FPR1
- AREG - EGFR
- EREG - EGFR
- HBEGF - EGFR
- BAG6 - NCR3
- COL8A2 - integrin - a2b1 - complex
- BST2 - LILRA4
- EREG - ERBB4
- HBEGF - ERBB4
- LTA - TNFRSF14
- COL9A2 - integrin - a2b1 - complex
- COL24A1 - integrin - a2b1 - complex
- FN1 - integrin - a2b1 - complex
- CDH1 - integrin - aEb7 - complex
- CD40LG - integrin - a5b1 - complex
- FN1 - integrin - a5b1 - complex
- CDH1 - KLRG1
- CXCL2 - DPP4
- CCL3 - CCR1
- CCL5 - CCR1
- CCL3 - CCR5
- CCL4 - CCR5
- ALCAM - CD6
- SELL - SELPLG
- COL21A1 - integrin - a1b1 - complex
- COL28A1 - integrin - a1b1 - complex
- CD40LG - CD40
- ICAM1 - integrin - aMb2 - complex
- FN1 - integrin - a3b1 - complex
- ICAM1 - integrin - aXb2 - complex
- FN1 - integrin - a4b1 - complex
- PLAUR - integrin - a4b1 - complex
- FN1 - integrin - aVb1 - complex
- integrin - aVb5 - complex - FN1
- TGM2 - ADGRG1
- ICAM1 - integrin - aLb2 - complex
- CXCL5 - CXCR2
- EFNA5 - EPHA1
- EFNA5 - EPHA2
- Type - II - IFNR - IFNG
- IL1B - IL1 - receptor
- IL1 - receptor - IL1RN
- IL1A - IL1 - receptor - inhibitor
- IL7 - IL7 - receptor
- FLT3LG - FLT3
- GAS6 - AXL
- HBEGF - ERBB2
- HLA-E - CD94:NKG2A
- HLA-E - KLRC1
- PlexinA1 - complex3 - SEMA3A
- ICAM1 - ITGAL
- SPN - ICAM1
- SEMA3A - PlexinA2 - complex1
- SEMA3A - PlexinA3 - complex1
- SEMA3A - PlexinA4 - complex1
- WNT5B - FZD3 - LRP5
- WNT5B - FZD3 - LRP6
- KLRB1 - CLEC2D
- LAIR1 - LILRB4
- WNT5B - FZD6 - LRP5
- WNT5B - FZD6 - LRP6
- LipoxinA4 - byALOX5 - FPR2
- CD58 - CD2
- OSM - LIFR
- MDK - PTPRZ1
- NELL2 - ROBO3
- NTN1 - UNC5B
- NTN4 - UNC5B
- OSM - OSMR
- PECAM1 - CD38
- SEMA3A - NRP1
- SEMA3C - NRP2
- VEGFA - NRP2
- PROC - PROCR
- BMPR1B - BMPR2 - BMPR1A
- Cholesterol - byDHCR24 - RORA
- Cholesterol - byLIPA - RORA
- Cholesterol - byDHCR24 - RORC
- Cholesterol - byLIPA - RORC
- LRFN4 - PTPRS
- LRFN4 - PTPRF
- SLITRK6 - PTPRS
- PVR - CD96
- TIGIT - PVR
- TIGIT - NECTIN2
- NECTIN1 - NECTIN4
- LeukotrieneC4 - byLTC4S - CYSLTR1
- LipoxinA4 - byALOX5 - CYSLTR1
- LTC4 - byLTC4S - CYSLTR1
- ProstaglandinD2 - byAKR1C3 - PTGDR
- SEMA4D - PLXNB1
- SLIT1 - ROBO1
- SLIT2 - ROBO1
- TNFSF10 - TNFRSF10A
- TNFSF10 - TNFRSF10B
- TNFSF12 - TNFRSF12A
- LTB - LTBR
- TNF - TNFRSF1B
- LTA - TNFRSF1B
- THBS1 - CD36
- ProstaglandinD2 - byAKR1C3 - PTGFR
- ProstaglandinF2a - byAKR1B1 - PTGFR
- ProstaglandinE2 - byPTGES2 - PTGER3
- ProstaglandinE2 - byPTGES3 - PTGER3
- CX3CL1 - CX3CR1
- IL1RAP - PTPRF
- APLP2 - PIGR
- CD1B - complex - IL6ST
- Glutamate - byGLS - and - SLC1A3 - GRIK2
- Glutamate - byGLS - and - SLC1A7 - GRIK2
- Glutamate - byGLS - and - SLC1A3 - GRM5
- Glutamate - byGLS - and - SLC1A7 - GRM5
- Glutamate - byGLS - and - SLC1A3 - GRM7
- Glutamate - byGLS - and - SLC1A7 - GRM7

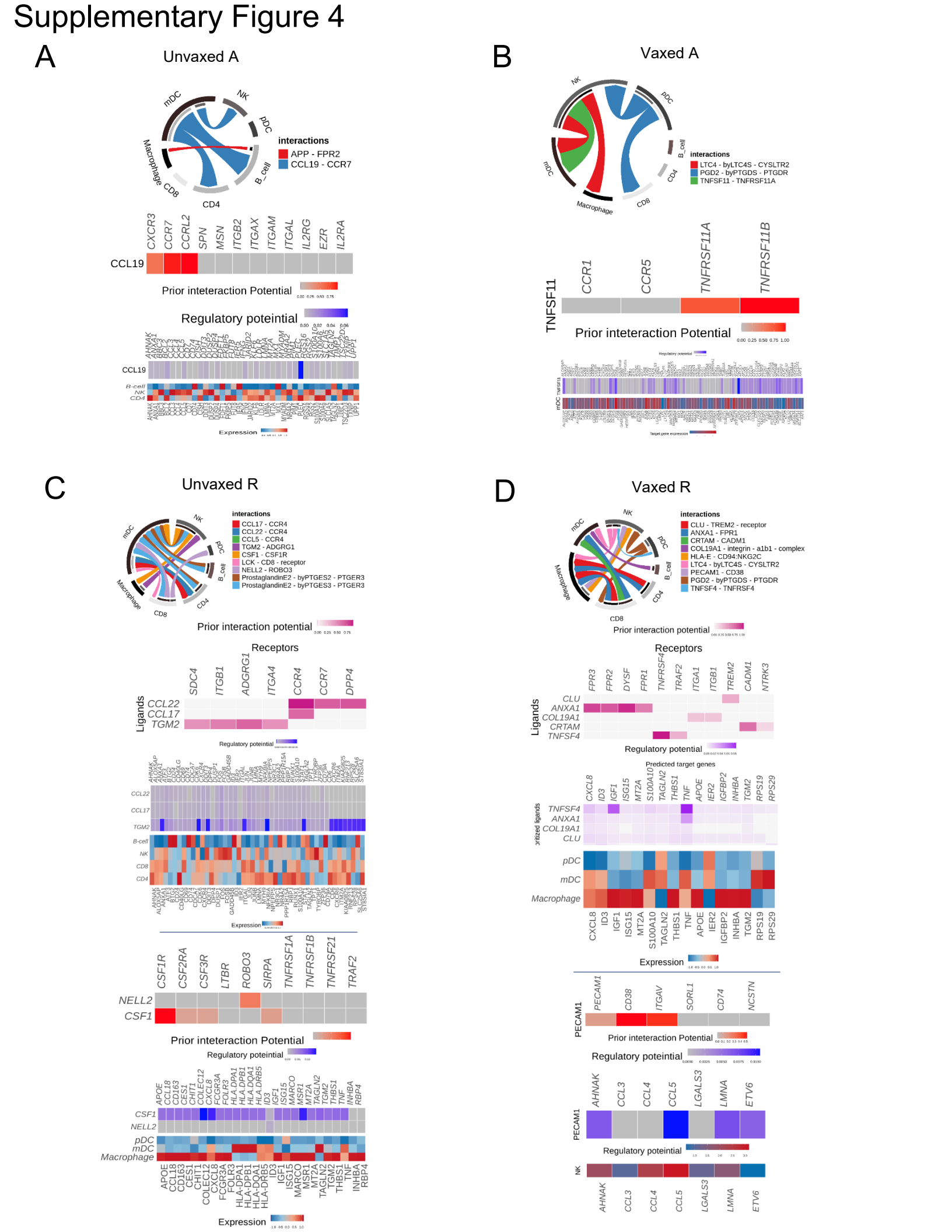
Supplementary Figure 3D



Supplementary Figure 3: Breakthrough SARS-CoV-2 infection exhibits greater lung epithelial and immune cell crosstalk.

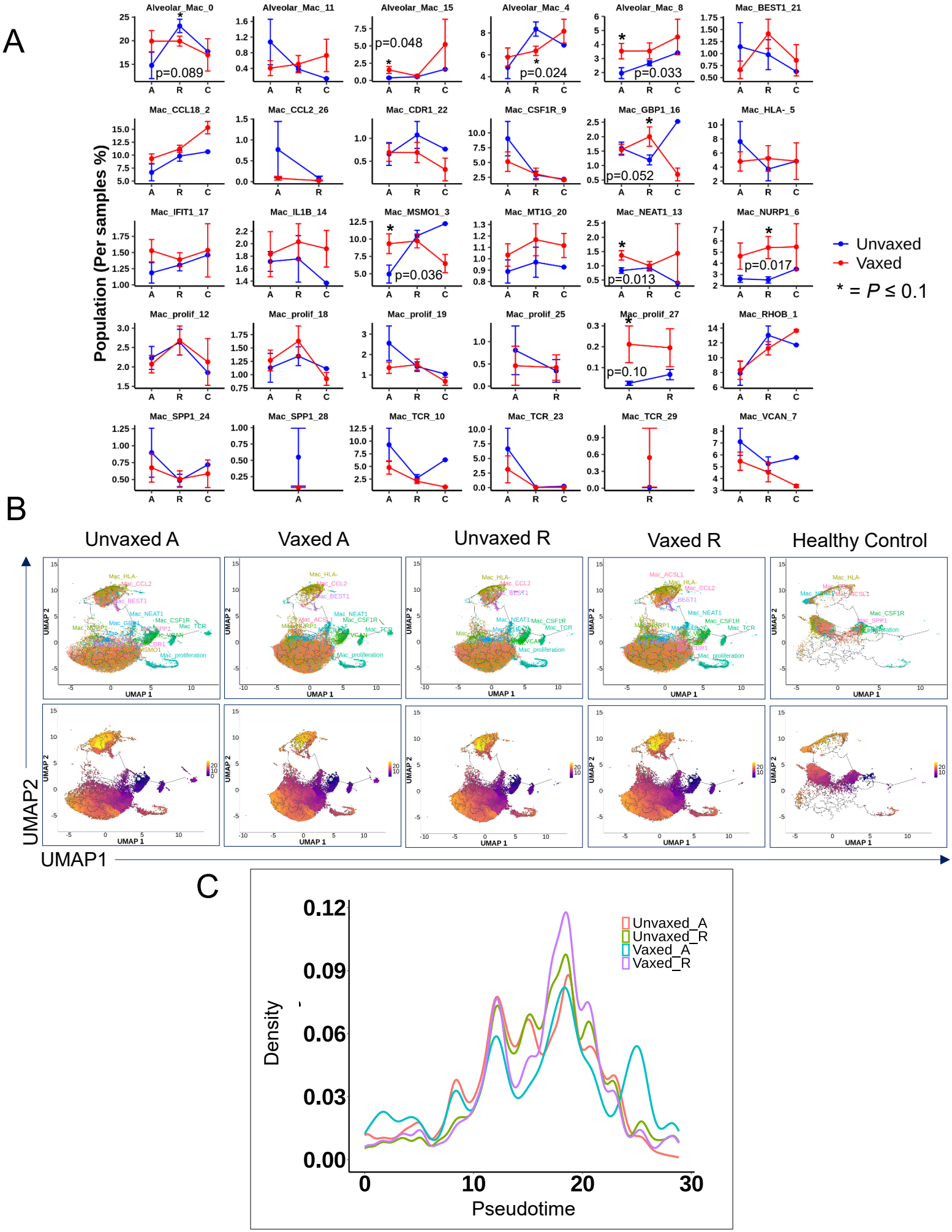
Circos plots depicting the ligand-receptor pairs between lung epithelial cells and immune cell as senders and receivers in natural (unvaccinated) and breakthrough (vaccinated) infection at Acute (A) and Recovery (R) phases. All significant interaction are shown. Ligands occupy the lower semicircle, and corresponding receptors are on the upper semicircle. Ligands and receptors are colored by the cell type. Ribbons connect edge of circle with edge thickness proportional to edge weight. Cross talk between lung epithelial cells and immune cells in (**a**) unvaccinated A, (**b**) vaccinated A, (**c**) unvaccinated R, and (**d**) vaccinated R phases.

Supplementary Figure 4



Supplementary Figure 4: Breakthrough SARS-CoV-2 infection exhibits greater lung innate and adaptive immune cell crosstalk. Circos plots of top ligand-receptor pairs with heatmap showing cell-cell interaction between APC and lymphocytes. (**a**) Natural infection (Unvaccinated Acute/Unvaxed A): two unique interactions were identified. *CCL19* from myeloid dendritic cells (mDCs) binding to *CCR7* on B cells, CD4 T cells, and NK cells. Differential gene expression analysis revealed high expression of plectin (*PLEC*) in CD4+ T cells, indicating the interaction (*CCL19: CCR7*) may be crucial for cytoskeleton organization and cell motility. (**b**) Breakthrough infection (Vaccinated A/Vaxed-A): key interactions included receptor activator of nuclear factor-κB ligand (*RANKL/TNFSF11*) from NK cells binding RANK (*TNFRSF11A*) on mDCs enhancing immune response modulation and leukotriene C4 (*LTC4*) from NK cells interacting with *CYSLTR2* on macrophages and mDCs, boosting their phagocytic capacity. Further, plasmacytoid dendritic cells (pDC) derived prostaglandin D2 (*PGD2*) signaling initiated an anti-inflammatory response through the prostaglandin D2 receptor (*PTGDR*) on NK and CD8 T cells, demonstrating the role of vaccination in training immune cells for effective viral clearance and minimizing inflammation. (**c**) Unvaccinated Recovery (Unvaxed R): interactions involving NK derived colony stimulating factor 1 (*CSF1*) signaling to mDCs and alveolar macrophage predicted *CXCL8* gene induction, supporting cytokine production and migration of mDCs. Release of *PGE2* from NK, T and B cells acts through PGE2-prostaglandin E receptor 3 (*PTGER3*) to enhance the maturation, cytokine production, and migration of mDCs. RANTES (*CCL5*) released by mDCs interacts with *CCR4* on CD4 T cells. This interaction might facilitate the recruitment of CD4+ T cells, leading to adaptive immune responses. (**d**) Vaccinated R (vaxed R): multiple interactions occur among antigen presenting cells (APCs) and lymphocytes. An example includes annexin 1 (*ANXA1*) signaling between T cells and formyl peptide receptor 1 (*FPR1*) on mDCs or macrophages modulates TNF and platelet endothelial cell adhesion molecule -1 (*PECAM1*) gene expression with *CD38* regulating NK cell functions. The coordinated immune interactions, including CD8+ cytotoxic and regulatory T cell molecule (*CRTAM*) and mDC cell adhesion molecule 1 (*CADM1*) and NK cell-derived leukotriene C4 (*LTC4*) interaction with cysteinyl leukotriene receptor 2 (*CYSLTR2*) signaling, enhanced APCs migration to the lung and supports an anti-inflammatory response through PGD2 signaling. These interactions illustrate the enhanced immune coordination and regulation induced by vaccination. Unvaccinated (n = 8; A = 8, R = 8) and vaccinated (n = 11; A = 11, R = 11). P <0.05

Supplementary Figure 5

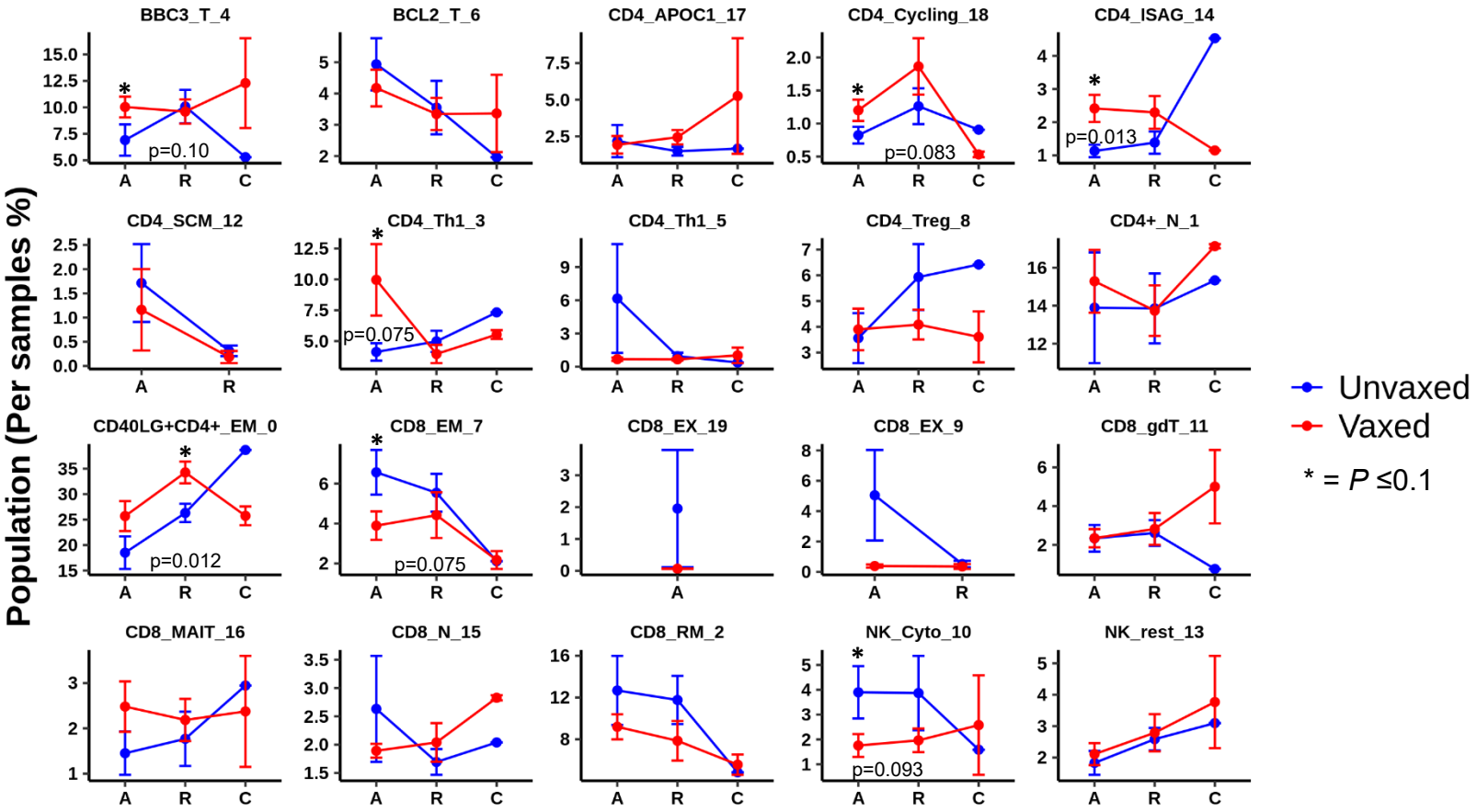


Supplementary Figure 5: Breakthrough SARS-CoV-2 infection drives a pro-inflammatory to reparative shift in lung macrophage phenotype from acute to recovery phase.

(a) Proportion of macrophage sub cell type (per sample, %) in unvaccinated (unvaxed, blue) and vaccinated (vaxed, red) at acute (A) recovery (R) and convalescence (C) phases. The numbers following different macrophages (Mac) indicate the respective clusters of the macrophage. Comparisons are made between unvaxed A and vaxed A, unvaxed R and vaxed R and unvaxed C and vaxed C. * represents $P \leq 0.1$. Unvaccinated (n = 8; A = 8, R = 8, C = 1), vaccinated (n = 11; A = 11, R = 11, C = 2) and healthy control (HC, n = 4). (**b**) Uniform Manifold Approximation and Projection (UMAP) embedding of macrophage colored by identities of 20 sub-cell subtypes (top panel) and their pseudotime trajectory (bottom panel) derived from unvaxed, vaxed persons at A, R and C phases and healthy controls (HC). (**c**) Cell density across pseudotime for macrophages from unvaxed and vaxed during the A and R phases. Unvaccinated (n = 8; A = 8, R = 8, C = 1), vaccinated (n = 11; A = 11, R = 11, C = 2) and healthy control (HC, n = 4).

Supplementary Figure 6

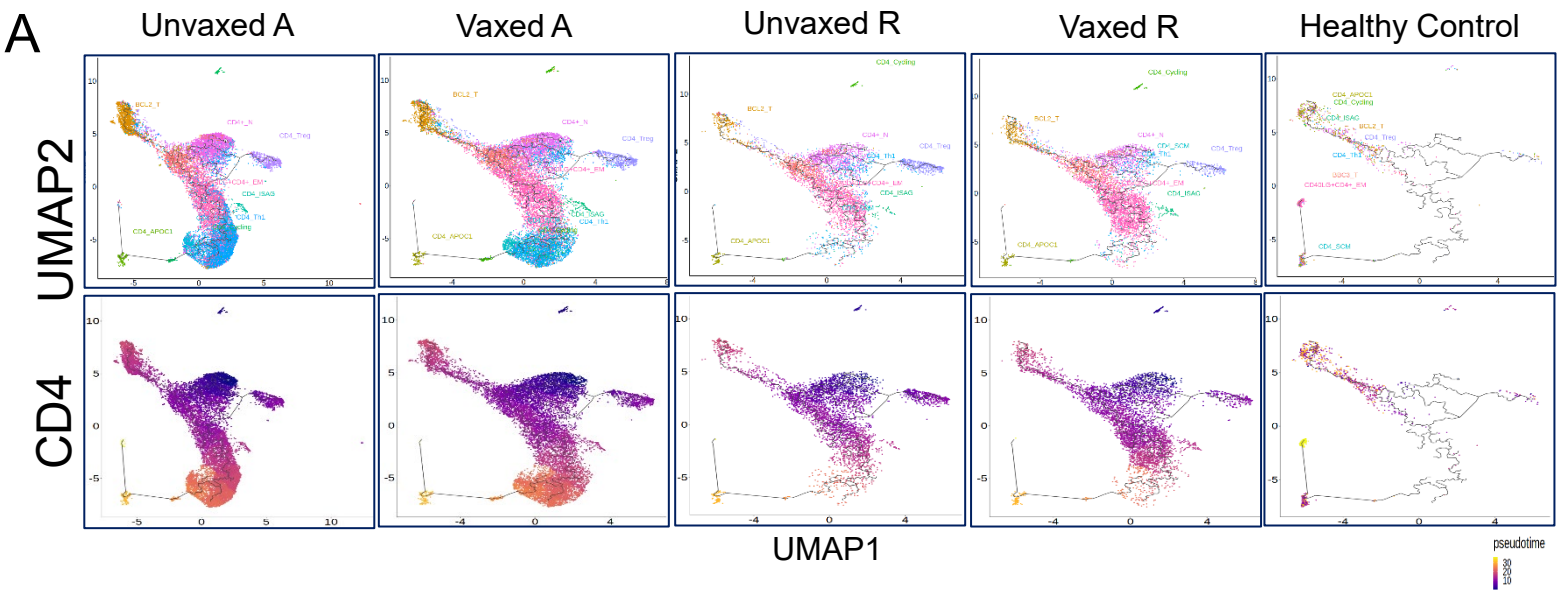
A

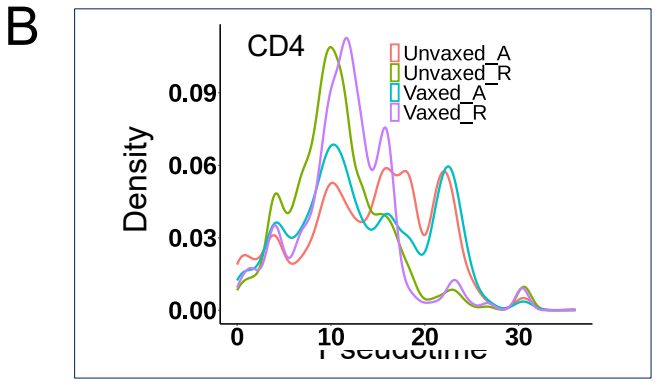


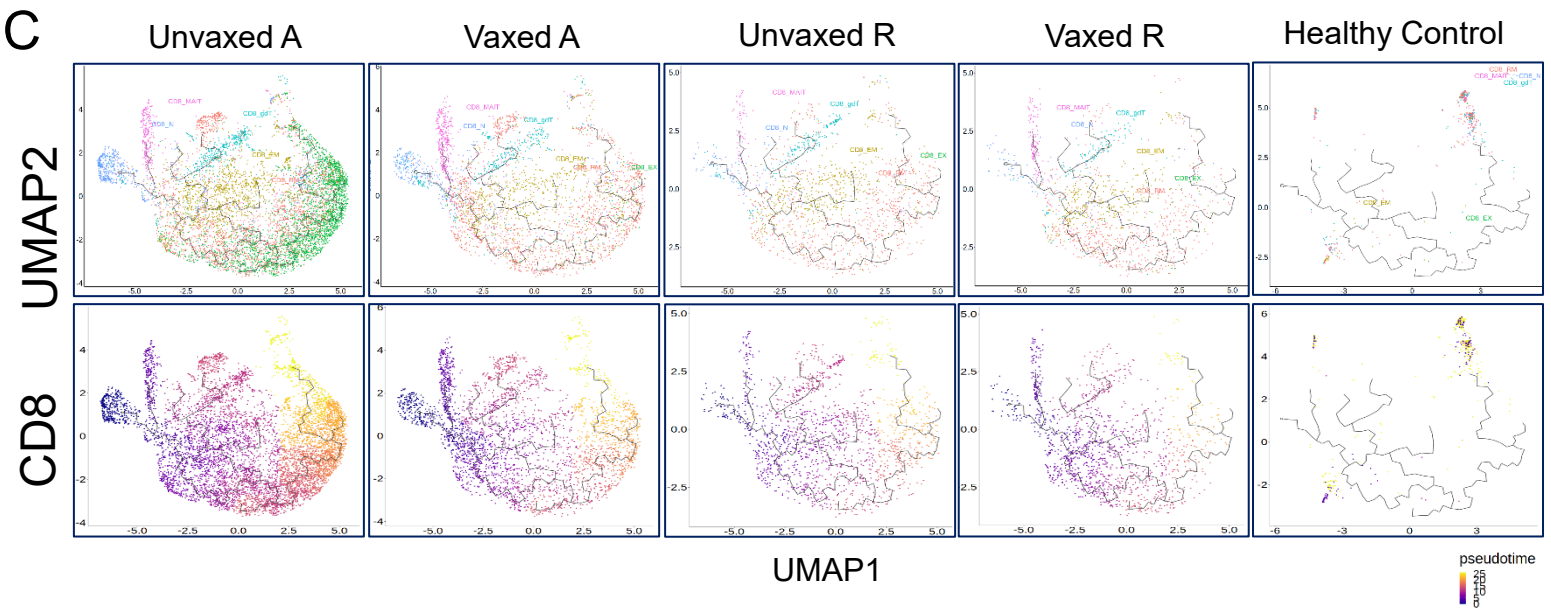
Supplementary Figure 6: Breakthrough SARS-CoV-2 infection induce proliferative and antiviral CD4+ T cell phenotypes acutely and immune regulatory phenotypes in recovery whereas natural infection elicits cytotoxic CD8+ T cell and NK cell phenotypes.

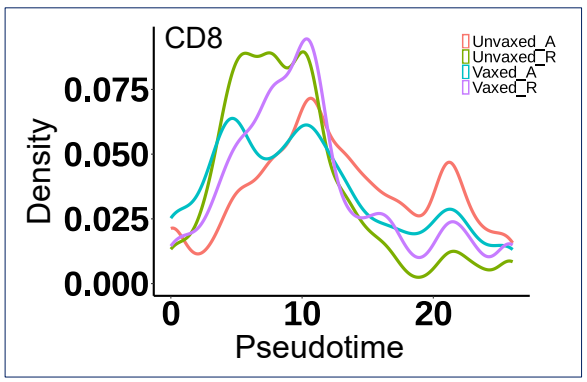
(**a**) Proportion of T and N K sub cell type (per sample, %) in unvaccinated (unvaxed, blue) and vaccinated (vaxed, red) at acute (A) recovery (R) and convalescence (C) phases. The numbers following different T and NK cells indicate the respective clusters of the T and NK cells. Comparisons are made between unvaxed A and vaxed A, unvaxed R and vaxed R, and unvaxed C and vaxed-C (* = $P \leq 0.1$). Unvaccinated (n = 8; A = 8, R = 8, C = 1), vaccinated (n = 11; A = 11, R = 11, C = 2).

Supplementary Figure 7







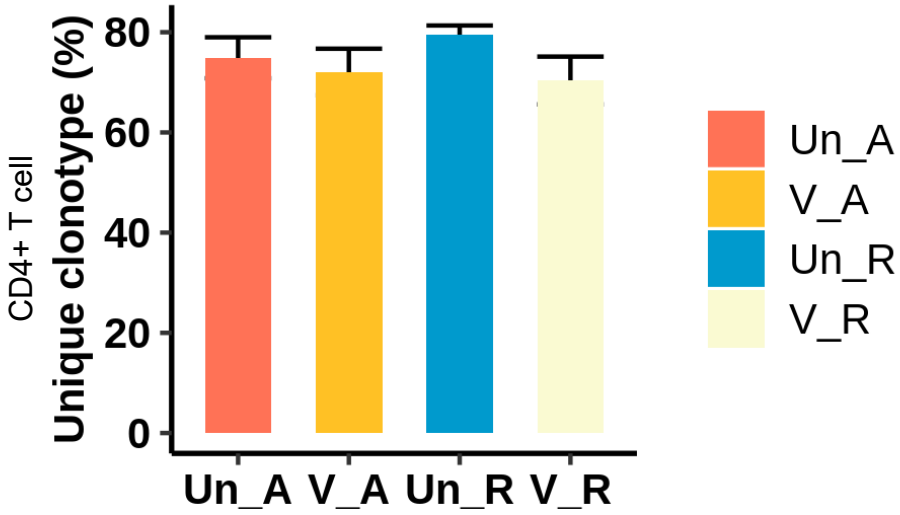


Supplementary Figure 7: CD4+ and CD8+ T cell differentiation paths distinguish breakthrough from natural SARS-CoV-2 infection.

a) Uniform Manifold Approximation and Projection (UMAP) embedding of CD4+ T cells colored by identities of 11 sub cell types (top panel) and their pseudotime trajectory (bottom panel) derived from natural (unvaccinated/unvaxed), breakthrough (vaccinated/vaxed) infection at acute (A), recovery (R) and convalescence (C) phases and healthy control (HC). (**b**) Cell density across pseudotime for CD4+ T cells from unvaxed and vaxed persons during the A and R phases. **(c)** UMAP embedding of CD8+ T cells colored by identities of 6 sub cell subtypes (top panel) and their pseudotime trajectory (bottom panel) derived from unvaxed, vaxed persons at A, R and C phases and HC. **(d)** Cell density across pseudotime for CD8+ T cells from unvaxed and vaxed persons during the A and R phases. Unvaccinated (n = 8; A = 8, R = 8) and vaccinated (n = 11; A = 11, R = 11) and HC, n = 4.

Supplementary Figure 8

A



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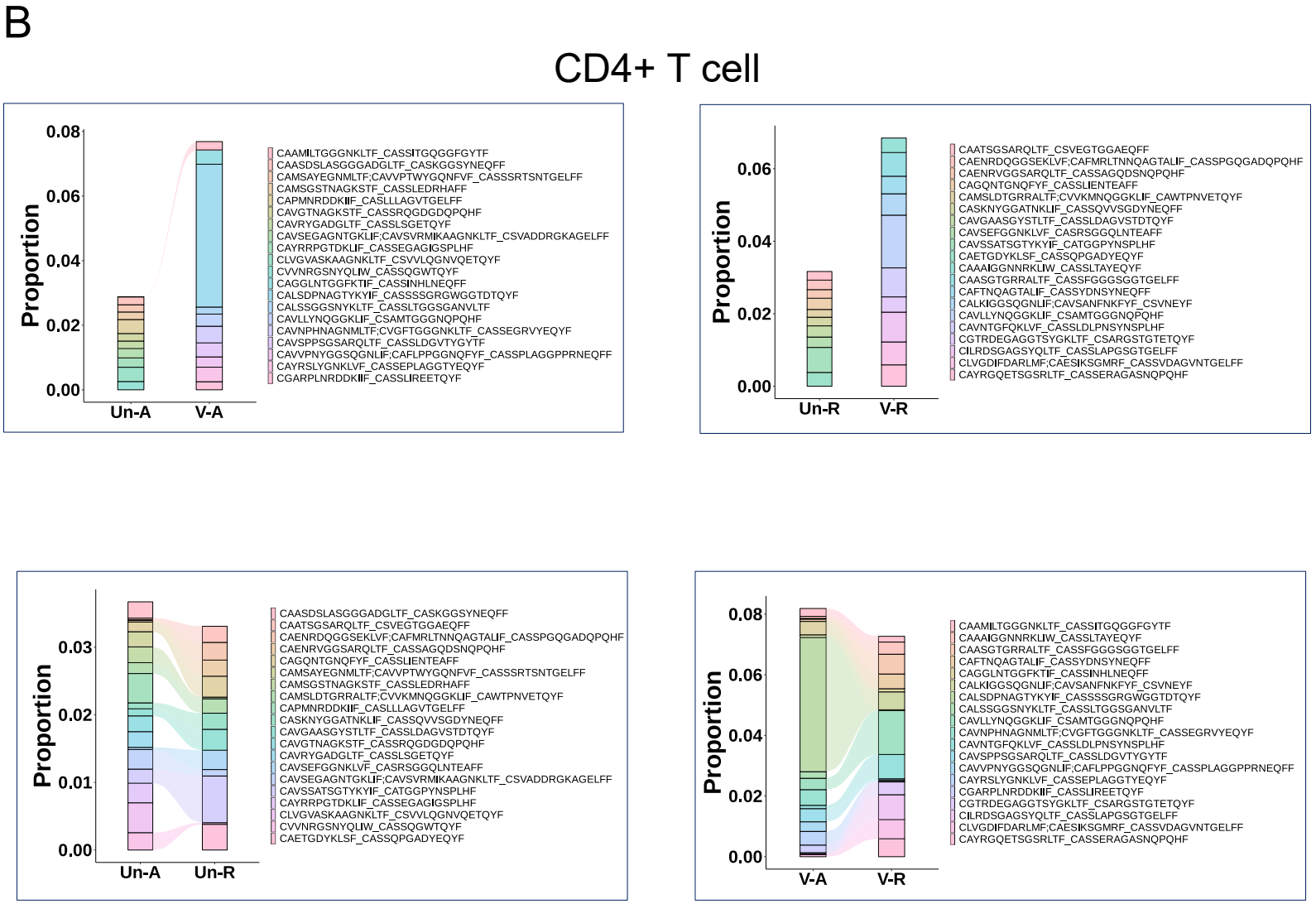
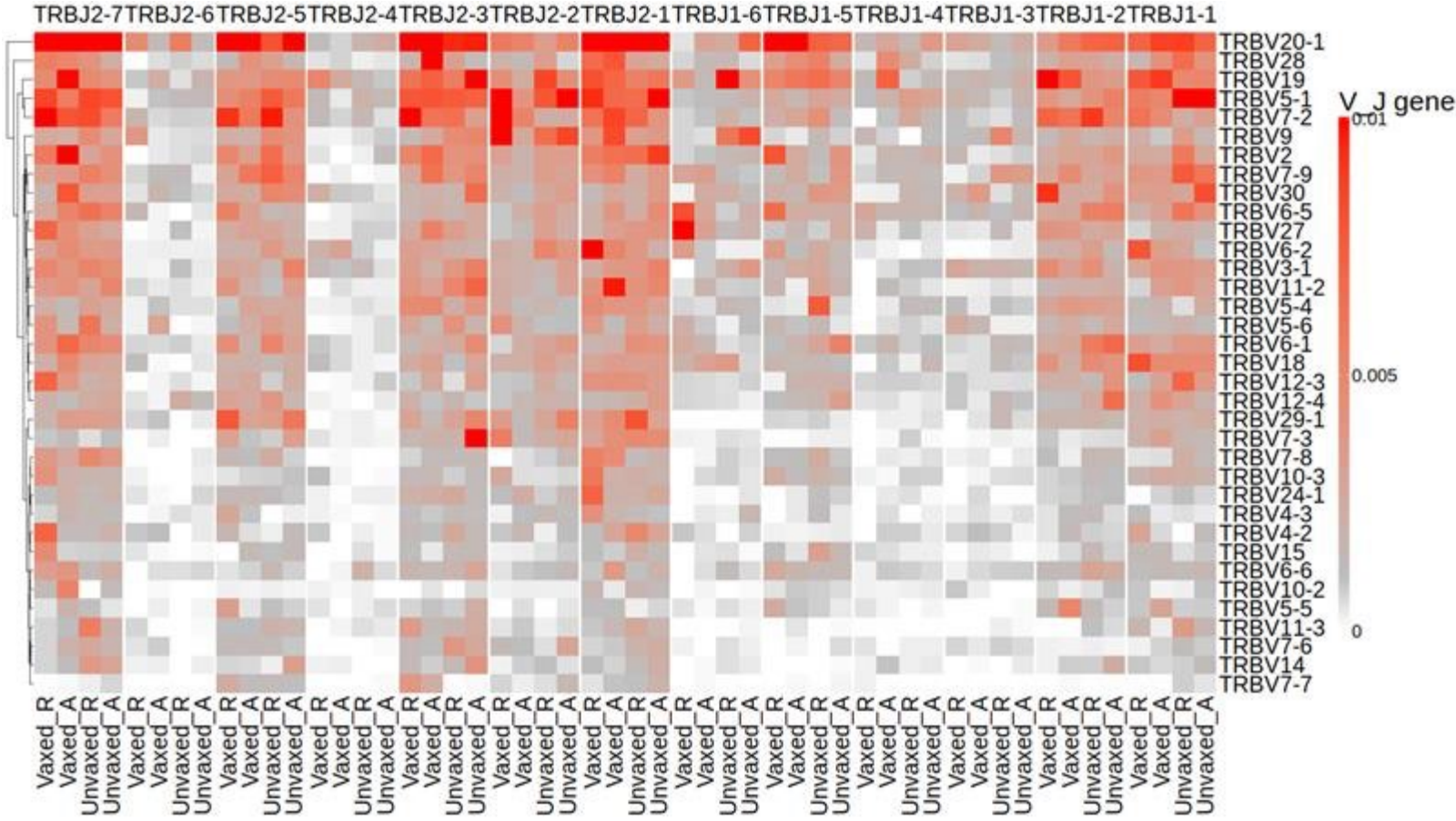


Supplementary Figure 8

C

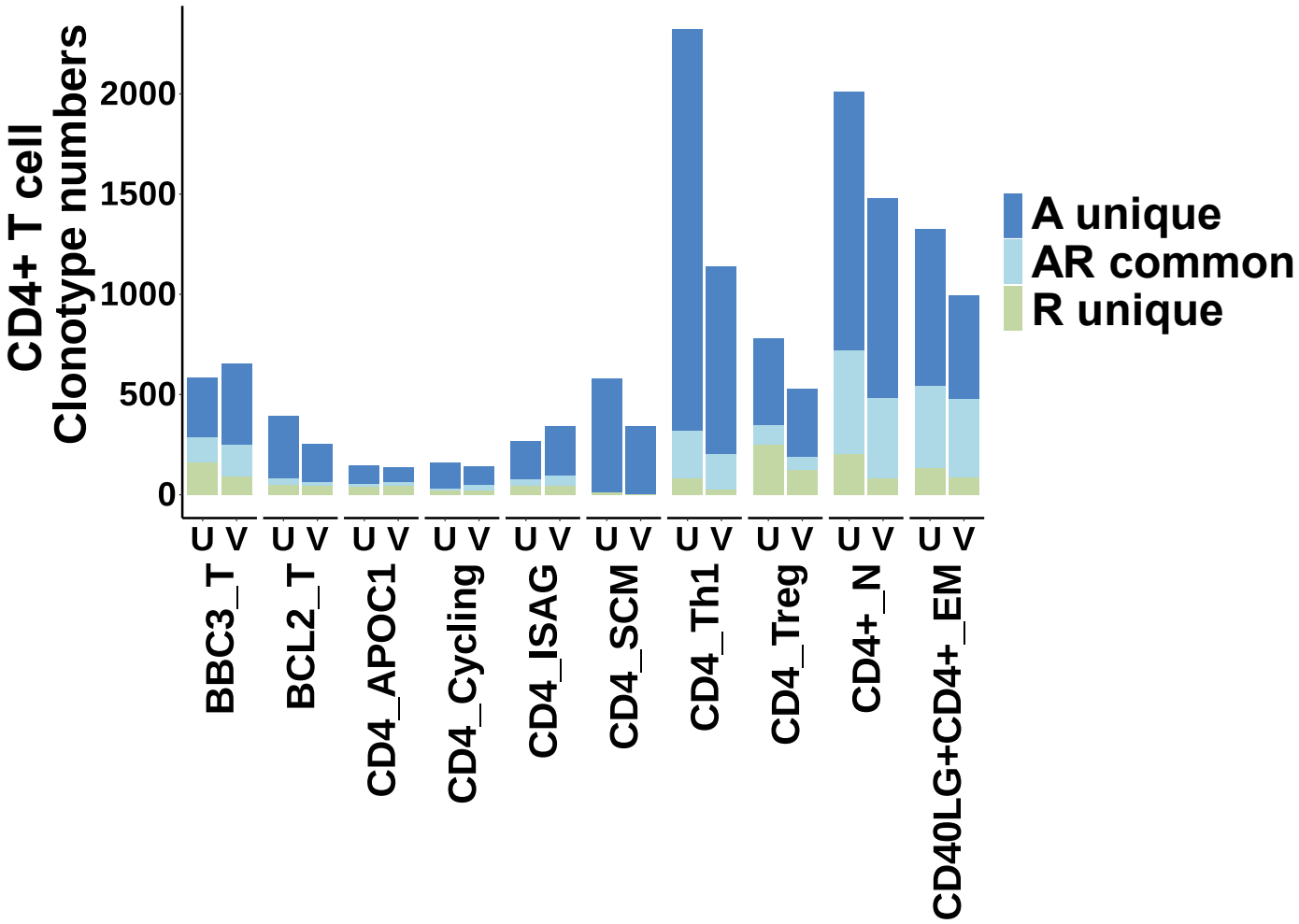


D


Unvaxed CD4+ T Cell
Vaxed CD4+ T Cell
Unvaxed Th1 CD4+ T Cell
Vaxed Th1 CD4+ T Cell
Unvaxed CD40LG+CD4+ T Cell
Vaxed CD40LG+ CD4+ T Cell
Acute
Recovery
Convalescence

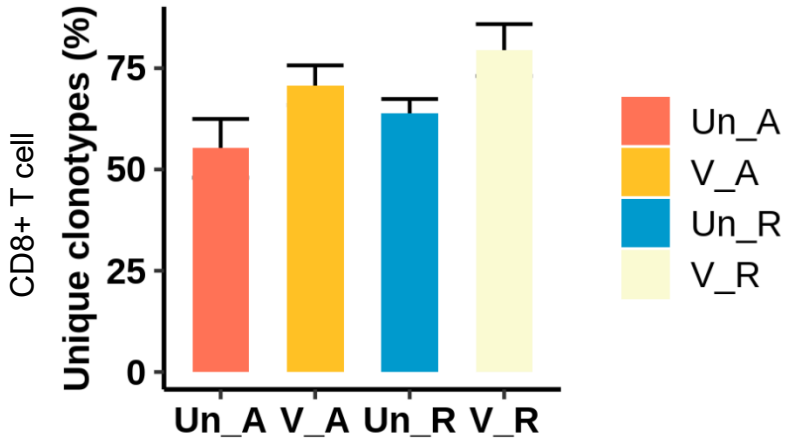
Supplementary Figure 8

E

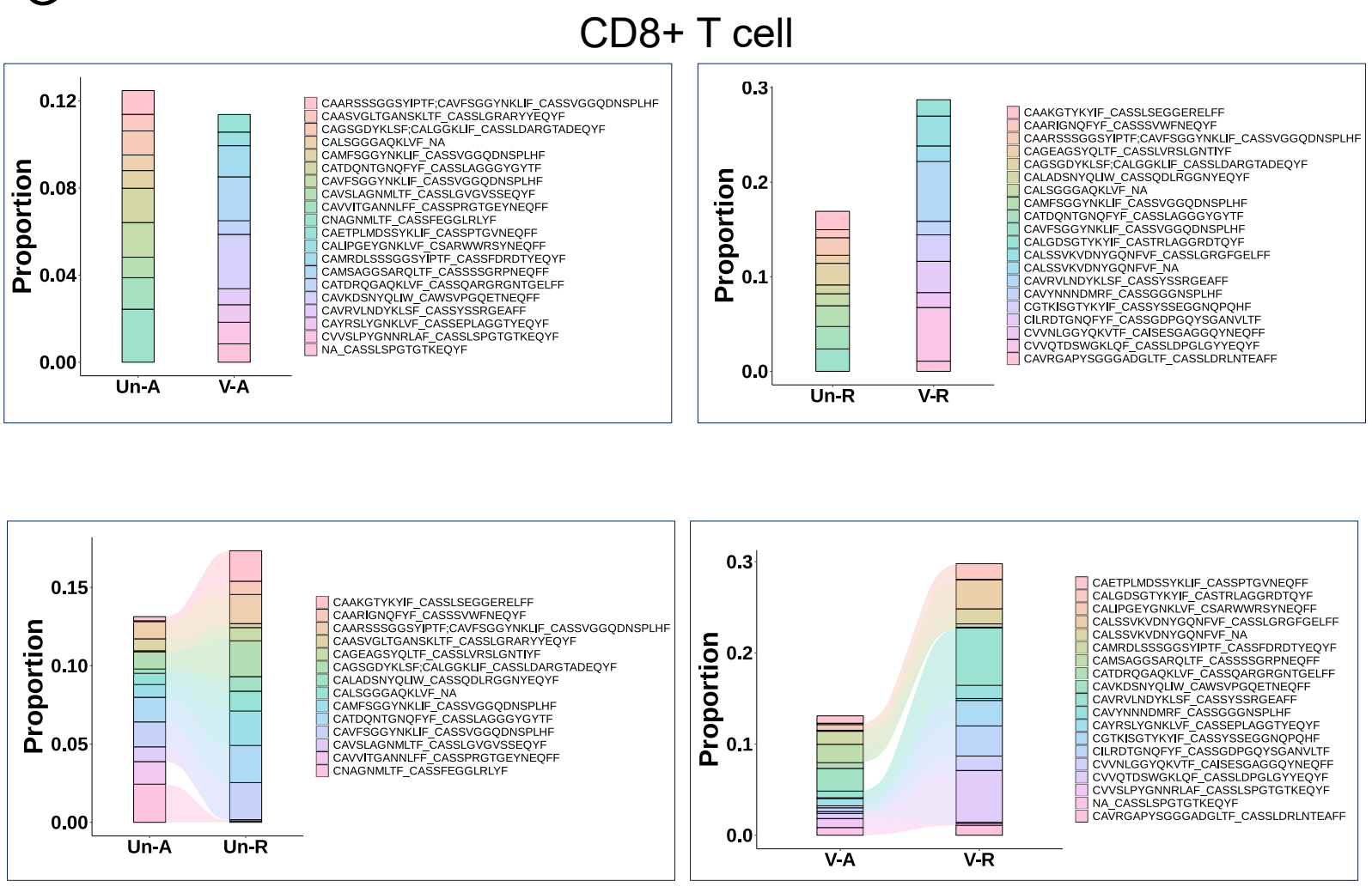


Supplementary Figure 8

F

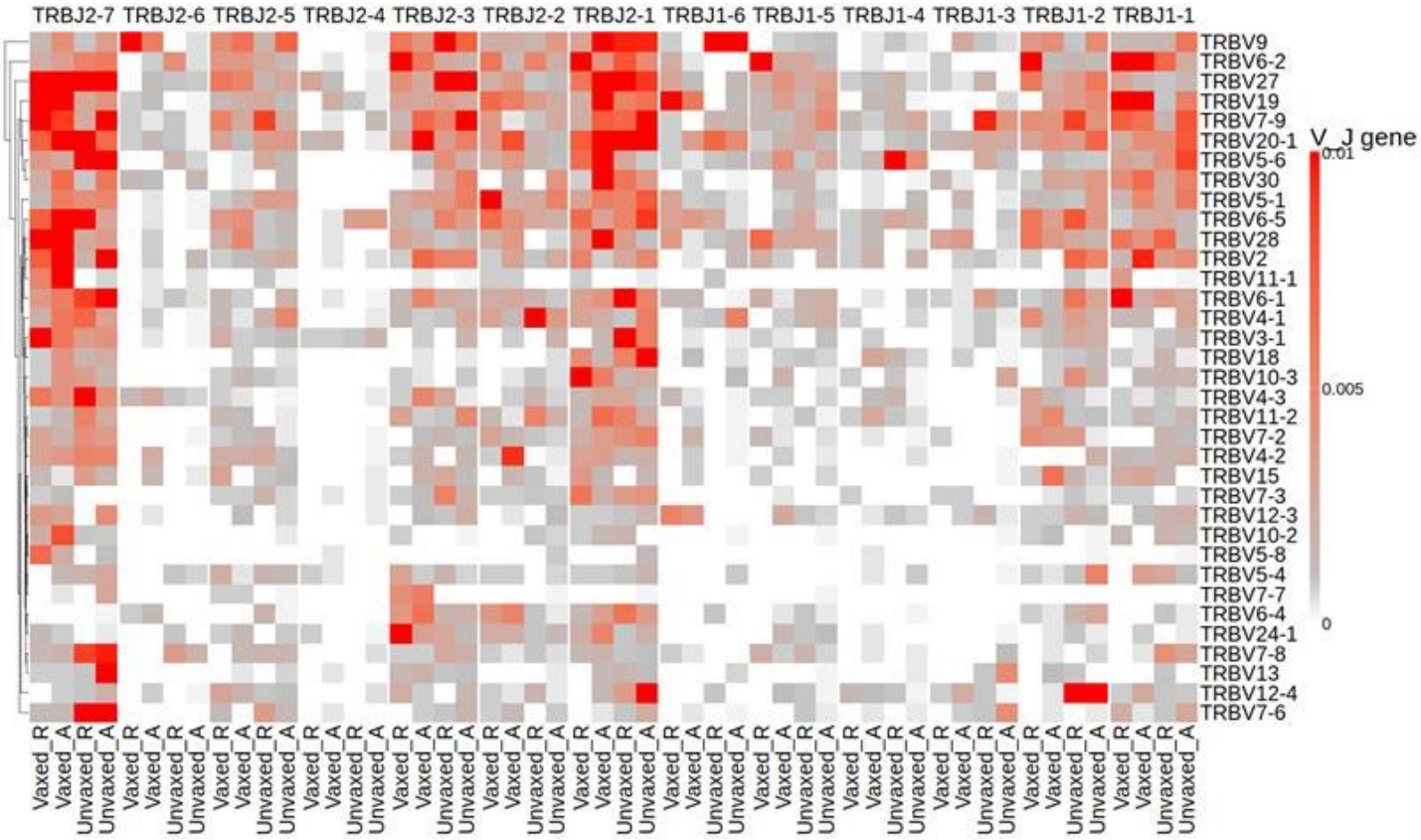


G

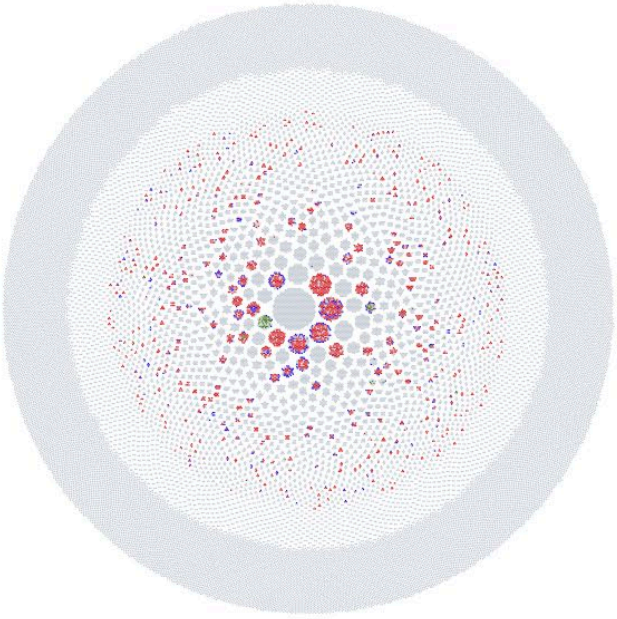


H

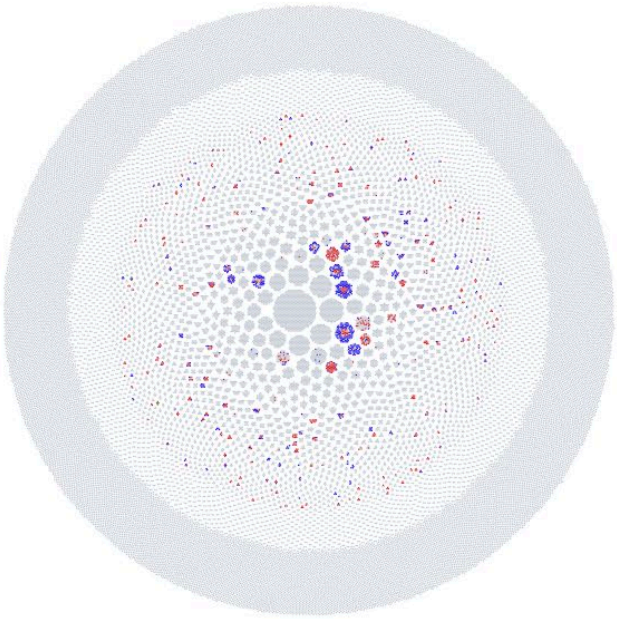
CD8+ T Cell: TCR β



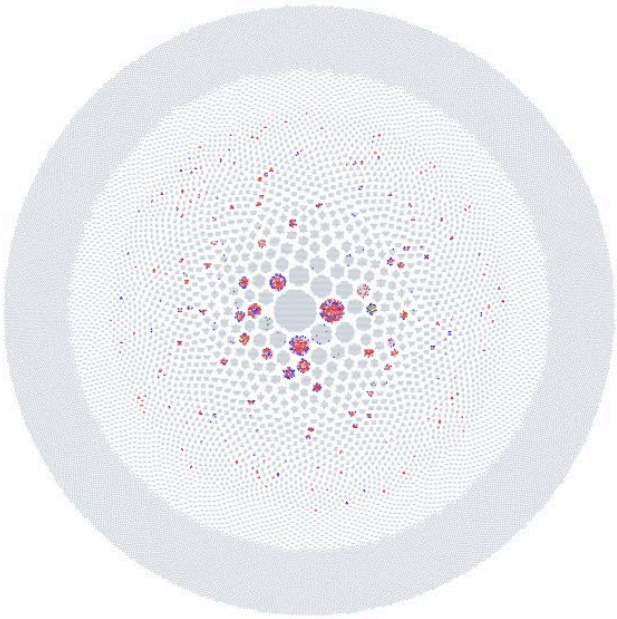
Unvaxed CD8+ T Cell



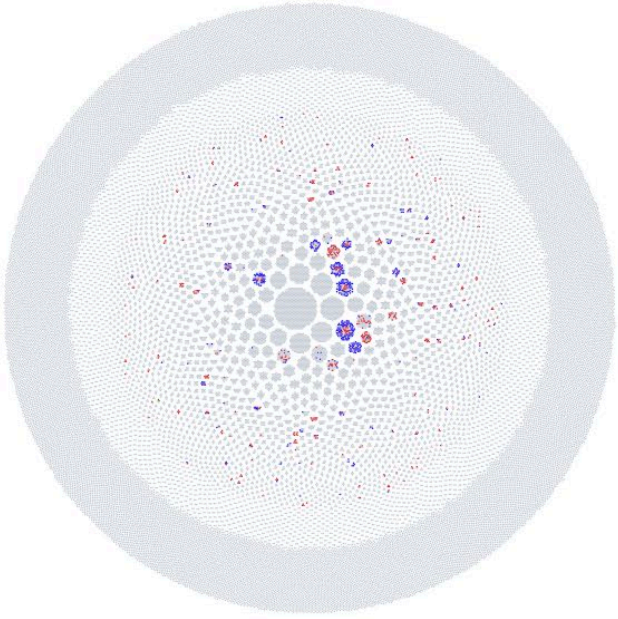
Vaxed CD8+ T Cell



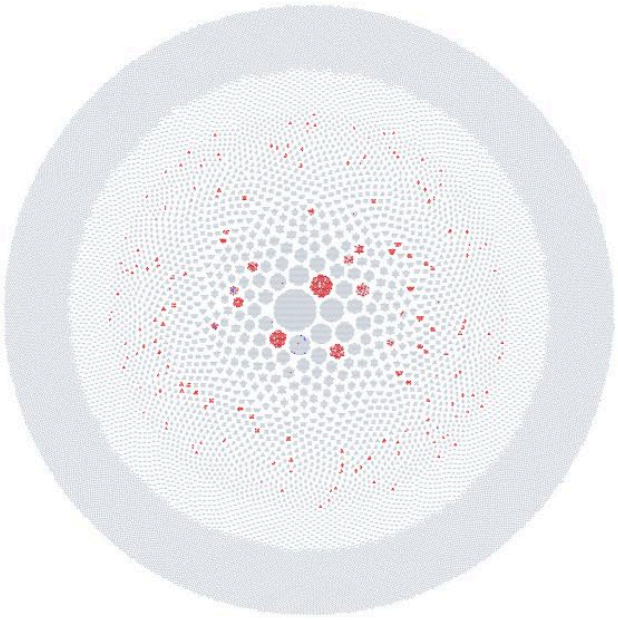
Unvaxed Resident Memory CD8+ T Cell



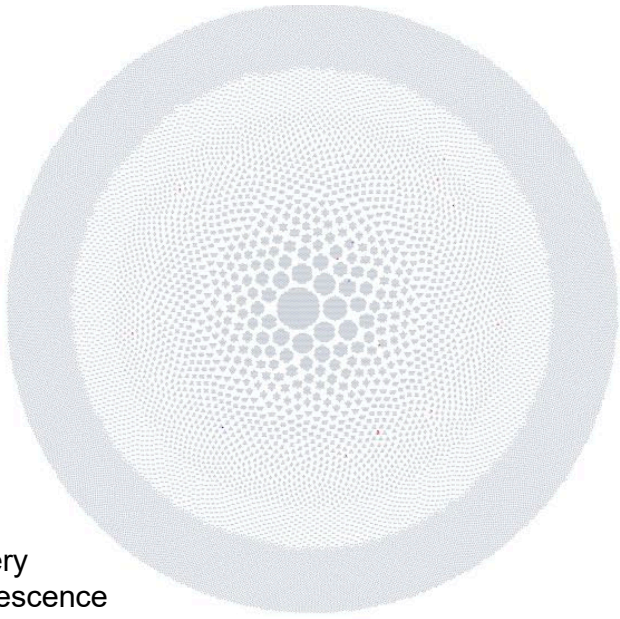
Vaxed Resident Memory CD8+ T Cell



Unvaxed Exhausted CD8+ T Cell



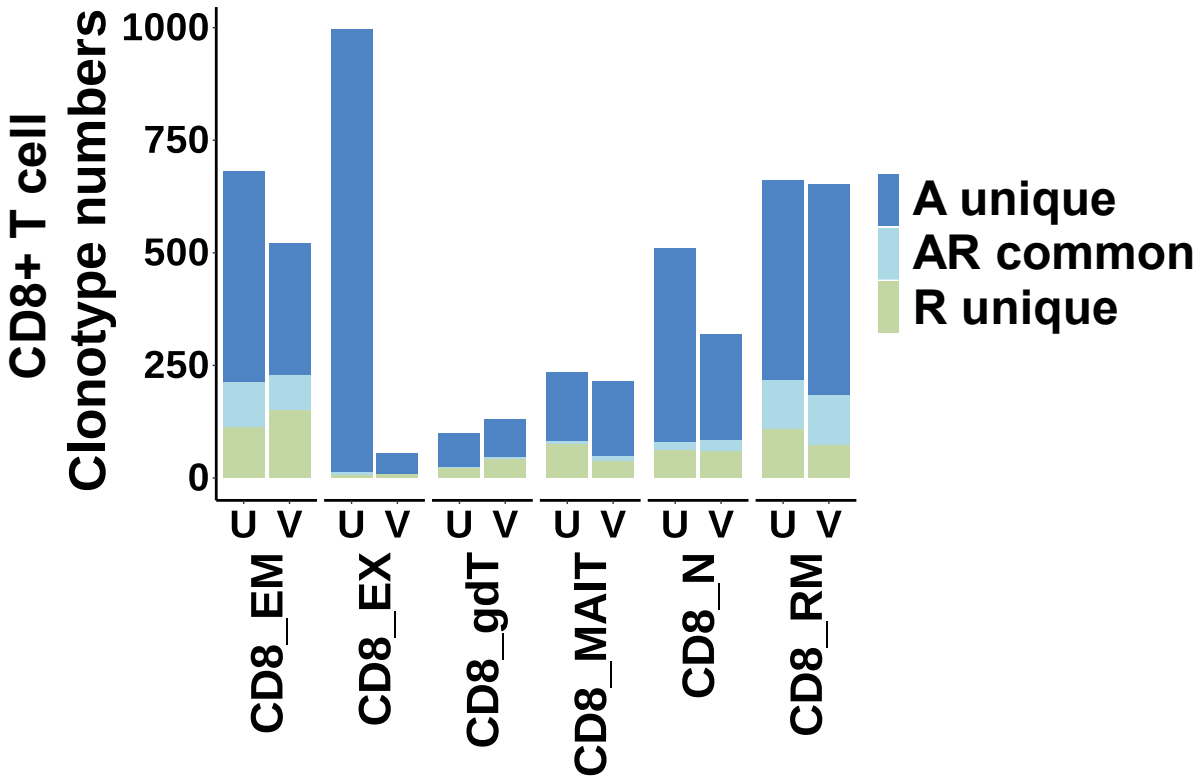
Vaxed Exhausted CD8+ T Cell



Acute
Recovery
Convalescence

Supplementary Figure 8

J



Supplementary Figure 8: Divergent CD4+ and CD8+ T cell clonotypes in breakthrough and natural SARS-CoV-2 infection.

a) Percentage of unique CD4+ T cell receptor (TCR) clones. (**b**) Relative abundance of the top 10 clonotype profiles of CD4+ T cell receptors. Stack bars showing TCR Complementarity-Determining Region 3 (CDR3) αβ sequences in natural (unvaccinated/Un/unvaxed) and breakthrough (vaccinated/V/vaxed) SARS-CoV-2 infection at acute (A) and recovery (R) phases. Only one TCR sequence CAAMILTGGGNKLTF(CDR3α):CASSITGQGGFGYTF (CDR3β) was shared between unvaxed and vaxed at A phase. The CDR3β sequence CASSITGQGGFGYTF was predicted to be 92% specific to SARS-CoV-2 according to the True Match in Immune Epitope Database. **(c)** Heatmap depicting frequency of CD4+ T cell TRβV and TRβJ usages in the unvaxed A, vaxed A, unvaxed R and vaxed R person. **(d)** Clonotype distribution plot depicting immune repertoire diversity in CD4+ T cell between unvaxed and vaxed person at A, R convalescence (C) phases. Clonotype distribution plot showing total CD4+ T cells (top panel), Th1 CD4+ T Cell (middle panel) and CD40LG+CD4+ T Cell (lower panel). Each node/ circle represents a cell with a unique clonotype. The size of circle reflects the number of cells within that clonotype. Larger circle shows most abundant clonotype. Unexpanded clonotype (gray dots), 2-time expanded clonotypes (white dots) and 3 or more expanded clonotypes are shown in red (unvaxed) and blue (vaxed). **(e)** Stacked bar showing CD4 T+ cell sub cell type clonotypes persistence from A to R phase in unvaxed (U) and vaxed (V). **(f)** Percentage of unique CD8+ TCR clones. **(g)** Relative abundance of top 10 CD8+ TCR clonotype profiles. **(h)** Heatmap depicting frequency of CD8+ T cell TRβV and TRβJ usages in the unvaxed A, vaxed A, unvaxed R and vaxed R persons. **(i)** Clonotype distribution plot depicting immune repertoire diversity in CD8+ T cell between unvaxed and vaxed patients at A, R convalescence (C) phases. Clonotype distribution plot showing total CD8+ T cells (top panel), resident memory CD8+ T Cell (middle panel) and exhausted CD8+ T Cell (lower panel). Each node / circle represents a cell with a unique clonotype. The size of circle reflects the number of cells within that clonotype. Larger circle shows most abundant clonotype. Unexpanded clonotype (gray dots), 2-time expanded clonotypes (white dots) and 3 or more expanded clonotypes are shown in red (unvaxed) and blue (vaxed). (j) Stacked bar showing CD8 T+ cell sub cell type clonotypes persistence from A to R phases in unvaxed (U) and vaxed (V). Comparisons are made between unvaxed A and vaxed A, unvaxed-R vs vaxed-R. Unvaccinated (*n* = 8; A = 8, R = 8, C = 1) and vaccinated (*n* = 11; A = 11, R = 11, C = 2).