A

UMAP 2
UMAP 1

Cell assignment
[% of B cells]
- activated [6.77%]
- germinal centre [3.38%]
- mem resting [54%]
- naive [34.6%]
- plasma cell [1.32%]

B

% of B cells sharing BCRs with d12 GC B

FNA | PBMC | IgD−enriched PBMC

day replicate

d0 A, d0 B, d5 A, d5 B, d12 A, d12 B, d12 C, d28 A, d28 B, d60 A, d60 B, d12, d28, d60, d12, d28, d60

C

UMAP 2
UMAP 1

Cell assignment
- resting mem
- *FCRL5*+ B mem
- naive
- plasma cell

D

Circulating IgD− B cell clusters

plasma cell
naive
FCRL5+ B mem
resting mem

Scaled expression
-1.0 -0.5 0.0 0.5 1.0

% of cells expressing
- 0
- 25
- 50
- 75

Gene: MARCKS, CPNE5, COCH, PLPP5, YBX3, TCL1A, ENC1, ZBTB32, ITGAX, FCRL5, CD86, TBX21, BCL6, PRDM1, PAX5, XRCC6, AICDA, CCR7, SELL, CXCR4, CXCR3, TNFRSF13B, FCER2, CD27, CD24

E

UMAP 2
UMAP 1

Cell assignment
- resting mem
- *FCRL5*+ B mem
- naive
- plasma cell

F

% GC derived B cells

FCRL5+ B mem, naive, plasma cell, resting mem

Cellular phenotype in the circulation @ d28

Figure 5 - Germinal center emigrant memory B cells are FCRL5^{+}

(A) UMAP of B cells (n=27265 cells) from fine needle aspirates (FNAs) of draining axillary lymph nodes from a single healthy volunteer on days 0, 5, 12, 28 and 60 after quadrivalent influenza vaccine (QIV), as reported by Turner *et al.*.

(B) The B cell receptors detected in germinal center (GC) B cells on day 12 after QIV immunization are shared with earlier LN B cells, and are detectable in peripheral blood mononuclear cells (PBMC) that have been enriched for B cell memory (IgD-) at days 28 and 60 post-vaccine. Sharing of a B cell receptor (BCR) requires: i. identical IGHV and IGHJ usage ii. identical heavy chain CDR3 length iii. identical IGLV and IGLJ usage and iv. identical light chain CDR3 length.

(C) UMAP of circulating B cells (n=21568 cells) from IgD- enriched PBMCs at days 0, 5, 12, 28 and 60 after QIV. Clusters identified by Louvain clustering, and annotated based on (D).

(D) Dotplot showing the expression of key genes, defined from the HA-specific B cell sequencing (Figure 2B), used to annotate the clusters identified in (C).

(E) UMAP of circulating QIV-specific B cells from IgD- enriched PBMCs at day 28 which share a germinal center BCR, n=38 cells.

(F) The percentage of QIV-specific B cells, present in the circulation at day 28, as in (E) is shown for each B cell cluster.

SessionInfo

```
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: CentOS Linux 7 (Core)
##
## Matrix products: default
## BLAS:   /bi/apps/R/3.6.1/lib64/R/lib/libRblas.so
## LAPACK: /bi/apps/R/3.6.1/lib64/R/lib/libRlapack.so
##
## locale:
##  [1] LC_CTYPE=en_GB.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_GB.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_GB.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
##  [1] grid       parallel  stats4    stats     graphics  grDevices utils
##  [8] datasets   methods   base
##
## other attached packages:
##  [1] pheatmap_1.0.12           ggmsa_0.0.4
##  [3] plyr_1.8.5                destiny_3.0.1
##  [5] Seurat_3.2.2              edgeR_3.28.0
##  [7] limma_3.42.0              gtable_0.3.0
##  [9] forcats_0.4.0             stringr_1.4.0
## [11] purrr_0.3.3               readr_1.3.1
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## [15] tidyverse_1.3.0           org.Hs.eg.db_3.10.0
## [17] AnnotationDbi_1.48.0      SingleR_1.0.6
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## [23] BH_1.72.0-2               RcppArmadillo_0.10.1.2.0
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## [27] scater_1.14.5             SingleCellExperiment_1.8.0
## [29] cowplot_1.0.0             pals_1.6
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## [33] magrittr_1.5              ggplot2_3.3.2
## [35] MultiAssayExperiment_1.12.1 SummarizedExperiment_1.16.0
## [37] DelayedArray_0.12.0       BiocParallel_1.20.0
## [39] matrixStats_0.55.0        Biobase_2.46.0
## [41] GenomicRanges_1.38.0      GenomeInfoDb_1.22.0
## [43] IRanges_2.20.1            S4Vectors_0.24.1
## [45] BiocGenerics_0.32.0
##
## loaded via a namespace (and not attached):
##    [1] rsvd_1.0.2              vcd_1.4-4
##    [3] ica_1.0-2               class_7.3-15
##    [5] flowViz_1.48.0          lmtest_0.9-37
##    [7] crayon_1.3.4            laeken_0.5.0
##    [9] MASS_7.3-51.4           nlme_3.1-141
##   [11] backports_1.1.5         reprex_0.3.0
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