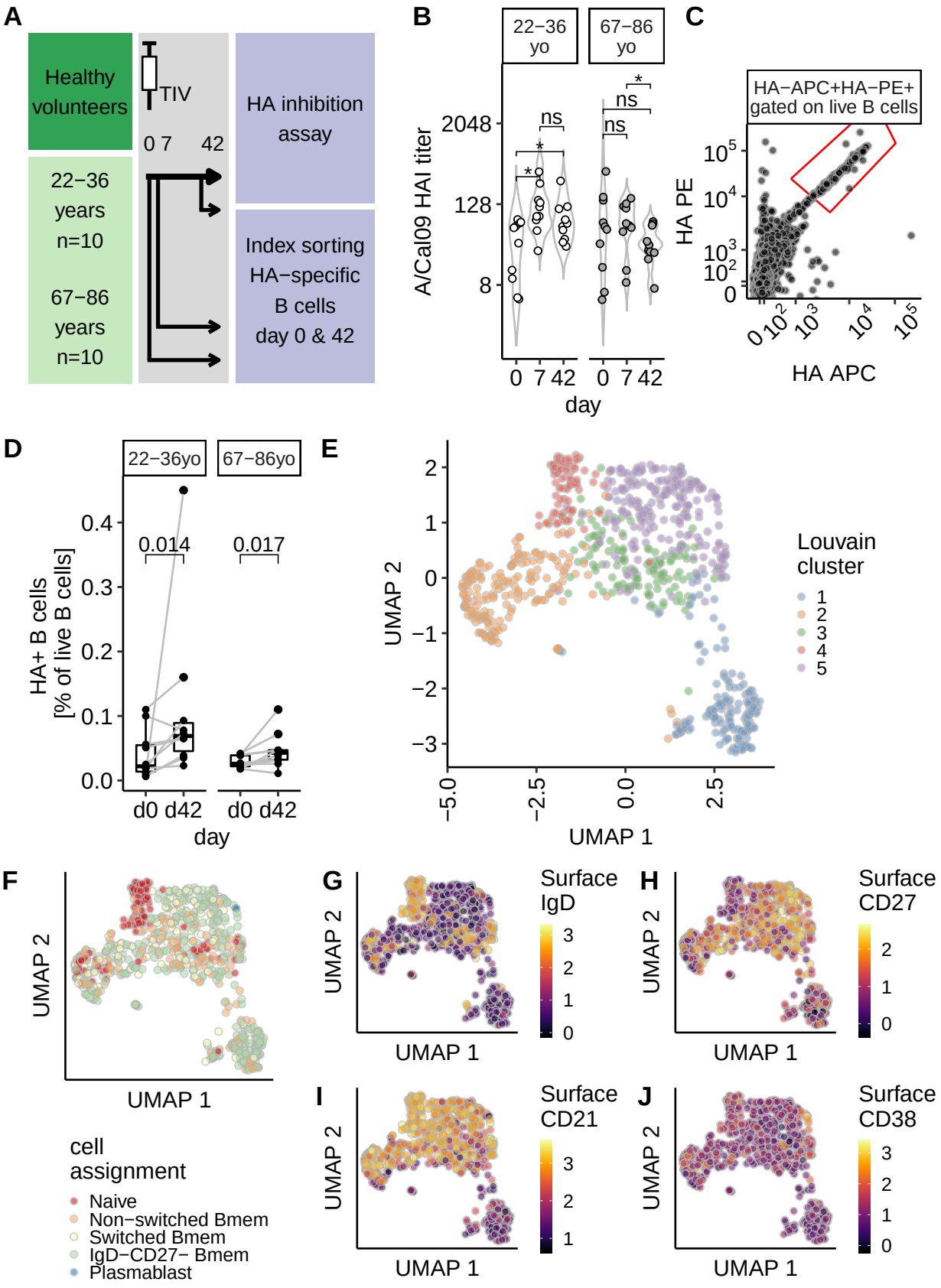
A
Healthy volunteers
22−36 years n=10
67−86 years n=10
TIV
0 7 42
HA inhibition assay
Index sorting HA−specific B cells day 0 & 42
B
22−36 yo
67−86 yo
A/Cal09 HAI titer
2048
128
8
ns
*
day
C
HA−APC+HA−PE+ gated on live B cells
HA PE
HA APC
D
22−36yo
67−86yo
HA+ B cells [% of live B cells]
0.014
0.017
day
E
UMAP 2
UMAP 1
Louvain cluster
F
cell assignment
Naive
Non−switched Bmem
Switched Bmem
IgD−CD27− Bmem
Plasmablast
G
Surface IgD
H
Surface CD27
I
Surface CD21
J
Surface CD38

Figure 1 – Single cell sequencing of haemagluttinin specific B cells to study the aged vaccine response

(A) Study design. Venepuncture performed on days 0 (just prior to immunization), 7 and 42. Peripheral blood mononuclear cells were isolated on the day of venepuncture and cryopreserved for later index sorting experiments.

(B) Haemagglutinin inhibition (HAI) assay titers from days 0, 7 and 42 are shown for 22-36 year old or 67-86 year old volunteers as open or greyed circles respectively (n=10 in both groups).

(C) Recombinant biotinylated haemagglutinin conjugated with streptavidin-PE or -APC allows the identification of haemagglutinin specific B cells. Gated on live, singlet CD19+ lymphocytes. Full gating strategy is shown in Supplementary Figure 1A.

(D) The proportion of haemagglutinin binding B cells increases after vaccination, for both 22-36 year olds and 67-86 year olds. Proportion expressed as percentage of live B cells, that do not bind free streptavidin.

(E) UMAP embedding of single cell RNA sequencing from A/Cal09-specific B cells, n=771 cells. UMAP projection based on the first 40 principal components using the features with the top 10% variance, after removal of low quality cells (Supplementary Figure 1) and size normalization by deconvolution. Louvain clustering reveals 5 clusters.

(F) Cell identity assignment based on published transcriptional profiles of 29 human immune subsets including the following B cell subsets: IgD+CD27- 'naive', IgD+CD27+ 'non-switched Bmem', IgD-CD27+ 'switched Bmem', IgD-CD27- 'IgD-CD27-', IgD-CD27+CD38+ plasmablasts 'PB' and plasmacytoid DCs. The following numbers of cells were identified: naive 120 cells; non-switched Bmem 165 cells; Switched Bmem 144 cells; IgD-CD27- Bmem 337 cells; Plasmablast 3 cells and plasmacytoid DC 2 cell. No cells were defined as T cells or members of other lymphoid or myeloid lineages. (G-J) UMAP embedding as in (E), showing the logicle transformed index sort surface expression of IgD (G), CD27 (H), CD21 (I) and CD38 (J) proteins. In (B), P values from paired Mann-Whitney tests (on log2 transformed data) are summarised: $P>0.05$ ns; $0.05>P>0.01$ *; $0.01>P<0.001$ **. In (D), samples from the same individual are indicated with a grey line and P values shown are from a paired two-tailed Mann-Whitney test. In (G)-(J), the scales reflect the decimal log of the logicle transformed fluorescence intensity value.

SessionInfo

```
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## 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
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##  [4] LC_COLLATE=C         LC_MONETARY=C        LC_MESSAGES=C
##  [7] LC_PAPER=C           LC_NAME=C            LC_ADDRESS=C
## [10] LC_TELEPHONE=C       LC_MEASUREMENT=C     LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
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##
## other attached packages:
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##  [3] purrr_0.3.3                 readr_1.3.1
##  [5] tidyr_1.0.0                 tibble_3.0.4
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##  [9] AnnotationDbi_1.48.0        SingleR_1.0.6
## [11] rstatix_0.6.0               ggcyto_1.18.0
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## [31] matrixStats_0.55.0          Biobase_2.46.0
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## [35] IRanges_2.20.1              S4Vectors_0.24.1
## [37] BiocGenerics_0.32.0
##
## loaded via a namespace (and not attached):
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##   [3] AnnotationHub_2.18.0         BiocFileCache_1.10.2
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##  [15] modelr_0.1.5                 RcppParallel_4.4.4
##  [17] colorspace_1.4-1             rvest_0.3.5
##  [19] rappdirs_0.3.1               blob_1.2.0
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