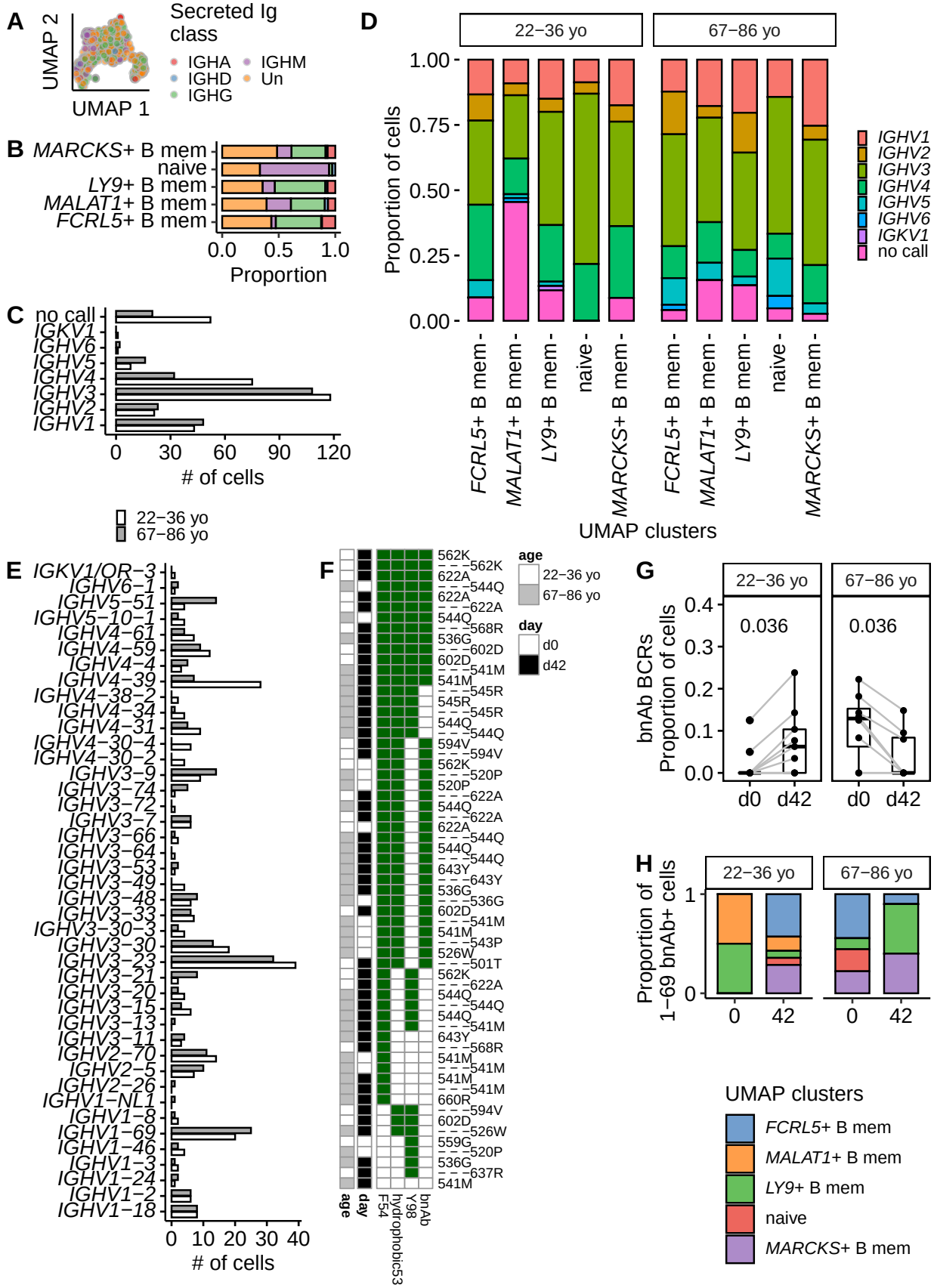
A
UMAP 2
UMAP 1
Secreted Ig class
IGHA
IGHD
IGHG
IGHM
Un
B
MARCKS+ B mem
naive
LY9+ B mem
MALAT1+ B mem
FCRL5+ B mem
0.0 0.5 1.0
Proportion
C
no call
IGKV1
IGHV6
IGHV5
IGHV4
IGHV3
IGHV2
IGHV1
0 30 60 90 120
# of cells
22−36 yo
67−86 yo
D
22−36 yo
67−86 yo
1.00
0.75
0.50
0.25
0.00
Proportion of cells
FCRL5+ B mem
MALAT1+ B mem
LY9+ B mem
naive
MARCKS+ B mem
IGHV1
IGHV2
IGHV3
IGHV4
IGHV5
IGHV6
IGKV1
no call
UMAP clusters
E
IGKV1/OR−3
IGHV6−1
IGHV5−51
IGHV5−10−1
IGHV4−61
IGHV4−59
IGHV4−4
IGHV4−39
IGHV4−38−2
IGHV4−34
IGHV4−31
IGHV4−30−4
IGHV4−30−2
IGHV3−9
IGHV3−74
IGHV3−72
IGHV3−7
IGHV3−66
IGHV3−64
IGHV3−53
IGHV3−49
IGHV3−48
IGHV3−33
IGHV3−30−3
IGHV3−30
IGHV3−23
IGHV3−21
IGHV3−20
IGHV3−15
IGHV3−13
IGHV3−11
IGHV2−70
IGHV2−5
IGHV2−26
IGHV1−NL1
IGHV1−8
IGHV1−69
IGHV1−46
IGHV1−3
IGHV1−24
IGHV1−2
IGHV1−18
0 10 20 30 40
# of cells
F
562K
622A
622A
544Q
544Q
622A
568R
536G
602D
541M
541M
545R
545R
544Q
544Q
594V
562K
594V
520P
622A
544Q
622A
622A
544Q
544Q
544Q
643Y
643Y
536G
536G
602D
541M
541M
543P
526W
501T
562K
622A
544Q
544Q
544Q
541M
643Y
568R
541M
541M
541M
541M
660R
594V
602D
526W
559G
520P
536G
637R
541M
age
22−36 yo
67−86 yo
day
d0
d42
age
day
F54
hydrophobic53
Y98
bnAb
G
22−36 yo
67−86 yo
0.036
0.036
bnAb BCRs
Proportion of cells
0.4
0.3
0.2
0.1
0.0
d0
d42
d0
d42
H
22−36 yo
67−86 yo
Proportion of
1−69 bnAb+ cells
1
0
0
42
0
42
UMAP clusters
FCRL5+ B mem
MALAT1+ B mem
LY9+ B mem
naive
MARCKS+ B mem

Figure 3 – Differences in heavy chain V segment usage, including broadly neutralizing *IGHV1-69*, in aged individuals after TIV immunization

(A) UMAP embedding as previously, showing secreted immunoglobulin classes. Isotypes are combined to immunoglobulin class (eg. IgG1, IgG2, IgG3, IgG4 grouped as IGHG): IGHA n=48 cells; IGHD n=12 cells; IGHG n=217 cells; IGHM n=112 cells; Un, unassigned n=272 cells.

(B) For each UMAP cluster, the proportion of secreted immunoglobulin classes are plotted. Each immunoglobulin class is indicated with the same shading as in (A).

(C) V segment family usage in the immunoglobulin heavy chain at day 42 for younger and older individuals. P=0.002, by Fisher's test.

(D) V segment family usage by HA-specific immunoglobulin heavy chains at day 42 for younger and older individuals expressed as the proportion of the cells separated by each UMAP cluster.

(E) V allele usage in the immunoglobulin heavy chain at day 42 for younger and older individuals. P=0.016, by Fisher's test.

(F) Heatmap summarising the presence of biophysical attributes characteristic of broadly neutralizing antibody for *IGHV1-69* B cells. The presence of F54, a hydrophobic residue at 53 and a tyrosine at 97, 98 or 99 are shown. An antibody is expected to be a bnAb if F54, there is a hydrophobic residue at position 53 and a Y within the CDR3. Presence of a characteristic is shown by a green box and empty boxes reflect its absence. On the left hand side of the panel, the age group of the cell is shown (cells from 18-36 year olds and 65-98 year olds in white and grey respectively) and the day of the sample is shown (day 0 and day 42 in white and black respectively). The individual identifiers are listed next to each row and some individuals.

(G) The proportion of B cells from each study day that encode *IGHV1-69* bnAbs. Individual B cells were filtered based on productive heavy chains, and only those individuals with paired data from day 0 and day 42 are shown (requires > 1 succesfully filtered B cell at both days). BnAbs were defined as shown in (F). Grey lines link the same individual. Paired 2 tailed Mann-Whitney P values are shown.

(H) The proportion of *IGHV1-69* B cells within each UMAP cluster for both age groups and on days 0 and 42 after TIV.

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
## [13] tidyr_1.0.0                 tibble_3.0.4
## [15] tidyverse_1.3.0             org.Hs.eg.db_3.10.0
## [17] AnnotationDbi_1.48.0        SingleR_1.0.6
## [19] rstatix_0.6.0               ggcyto_1.18.0
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## [23] BH_1.72.0-2                 RcppArmadillo_0.10.1.2.0
## [25] flowCore_1.52.1             scran_1.14.5
## [27] scater_1.14.5               SingleCellExperiment_1.8.0
## [29] cowplot_1.0.0               pals_1.6
## [31] ggpubr_0.2.4                dplyr_1.0.2
## [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] rappdirs_0.3.1               ggthemes_4.2.0
##   [3] bit64_0.9-7                  knitr_1.26
##   [5] irlba_2.3.3                  rpart_4.1-15
##   [7] data.table_1.12.8            RCurl_1.95-4.12
##   [9] generics_0.0.2               RSQLite_2.1.4
##  [11] RANN_2.6.1                   proxy_0.4-23
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