



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 successfully 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_US.UTF-8 LC_NUMERIC=C LC_TIME=C
##  [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
## [8] methods base
##
## other attached packages:
##  [1] pheatmap_1.0.12 readr_1.3.1
##  [3] MultiAssayExperiment_1.12.1 stringr_1.4.0
##  [5] ggmsa_0.0.4 ggpubr_0.2.4
##  [7] magrittr_1.5 scater_1.14.5
##  [9] ggplot2_3.3.2 dplyr_1.0.2
## [11] plyr_1.8.5 SingleCellExperiment_1.8.0
## [13] SummarizedExperiment_1.16.0 DelayedArray_0.12.0
## [15] BiocParallel_1.20.0 matrixStats_0.55.0
## [17] Biobase_2.46.0 GenomicRanges_1.38.0
## [19] GenomeInfoDb_1.22.0 IRanges_2.20.1
## [21] S4Vectors_0.24.1 BiocGenerics_0.32.0
##
## loaded via a namespace (and not attached):
##  [1] viridis_0.5.1 BiocSingular_1.2.0 tidyr_1.0.0
##  [4] viridisLite_0.3.0 DelayedMatrixStats_1.8.0 GenomeInfoDbData_1.2.2
##  [7] vipor_0.4.5 yaml_2.2.0 pillar_1.4.7
## [10] lattice_0.20-38 glue_1.4.2 digest_0.6.23
## [13] RColorBrewer_1.1-2 ggsignif_0.6.0 XVector_0.26.0
## [16] gridtext_0.1.4 colorspace_1.4-1 cowplot_1.0.0
## [19] htmltools_0.4.0 Matrix_1.2-17 pkgconfig_2.0.3
## [22] zlibbioc_1.32.0 purrr_0.3.3 scales_1.1.0
## [25] tibble_3.0.4 generics_0.0.2 farver_2.0.1
## [28] ellipsis_0.3.0 withr_2.1.2 crayon_1.3.4
## [31] ggtext_0.1.1 evaluate_0.14 xml2_1.2.2
## [34] beeswarm_0.2.3 tools_3.6.1 hms_0.5.2
## [37] lifecycle_0.2.0 munsell_0.5.0 irlba_2.3.3
## [40] Biostrings_2.54.0 compiler_3.6.1 rsvd_1.0.2
## [43] rlang_0.4.10 grid_3.6.1 RCurl_1.95-4.12
## [46] BiocNeighbors_1.4.1 bitops_1.0-6 labeling_0.3
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## [52] reshape2_1.4.3 R6_2.4.1 gridExtra_2.3
## [55] knitr_1.26 stringi_1.4.3 ggbeeswarm_0.6.0
## [58] Rcpp_1.0.3 vctrs_0.3.6 tidyselect_1.1.0
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## [61] xfun_0.11
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