



**Figure 4 – Somatic hypermutation is reduced in haemagglutinin specific memory B cells from aged individuals after TIV immunisation**

- (A) The number of nucleotide mutations within the antibody heavy chain are shown for each cell for day 0 and 42 and for older and younger individuals.
- (B) and (C) The number of nucleotide mutations within the antibody heavy chain are shown for each region at day 0 and for older and younger individuals for FR (framework regions, B) or CDR (complementarity determining regions, C).
- (C) The ratio of replacement:silent mutations within the antibody heavy chain are shown for each cell for day 0 and 42 and for older and younger individuals. The replacement:silent ratio was calculated:  $\frac{\# \text{ replacement mutations}}{(\# \text{ silent mutations} + 0.01)}$ , as many cells had zero silent mutations and  $\geq 1$  replacement mutations.
- (D) and (F) The ratio of replacement:silent mutations within the heavy chain is shown for each cell from day 0 from both age groups.
- (E) and (H) The number of mutations in the antibody heavy chain at day 0 plotted by age group for each UMAP cluster, for FR (G) or CDR (H).
- (F) and (J) The ratio of replacement:silent mutations the antibody heavy chain at day 0 plotted by age group for each UMAP cluster, for FR (I) or CDR (J). In (G)-(J), each UMAP cluster is labelled and the colors correspond to its appearance in Figures 1-3. For (E, F, I & J), the ratio of replacement:silent mutations is calculated as in (D), and plotted as a pseudocolor. In (A)-(J) *P* values from two tailed unpaired Mann-Whitney tests are shown. Where data are transformed for plotting (E, F, I & J), the test was performed on the untransformed data. The boxplots show the median, and inter-quartile range (IQR), with whiskers extending to the furthest data point, up to a maximum of 1.5x IQR. In (E, F, I & J), the boxplots correspond to the median and IQR of the transformed data.

The MW  $P$  value between CDR R:S and FR R:S (for day 42 cells):  $1.3 \times 10^{-35}$ .

## 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] parallel stats4      stats      graphics  grDevices  utils      datasets
## [8] methods    base
##
## other attached packages:
##  [1] ggpubr_0.2.4           magrittr_1.5
##  [3] scatter_1.14.5         ggplot2_3.3.2
##  [5] SingleCellExperiment_1.8.0 stringr_1.4.0
##  [7] readr_1.3.1            MultiAssayExperiment_1.12.1
##  [9] SummarizedExperiment_1.16.0 DelayedArray_0.12.0
## [11] BiocParallel_1.20.0    matrixStats_0.55.0
## [13] Biobase_2.46.0         GenomicRanges_1.38.0
## [15] GenomeInfoDb_1.22.0    IRanges_2.20.1
## [17] S4Vectors_0.24.1      BiocGenerics_0.32.0
## [19] dplyr_1.0.2
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.3             rsvd_1.0.2             lattice_0.20-38
##  [4] digest_0.6.29          R6_2.4.1               plyr_1.8.5
##  [7] evaluate_0.14          pillar_1.4.7           zlibbioc_1.32.0
## [10] rlang_0.4.10           irlba_2.3.3            Matrix_1.2-17
## [13] rmarkdown_2.14         labeling_0.3            BiocNeighbors_1.4.1
## [16] gridtext_0.1.4         RCurl_1.95-4.12        munsell_0.5.0
## [19] compiler_3.6.1         vipor_0.4.5            BiocSingular_1.2.0
## [22] xfun_0.31              pkgconfig_2.0.3        ggbeeswarm_0.6.0
## [25] htmltools_0.5.2        ggtext_0.1.1           tidyselect_1.1.0
## [28] tibble_3.0.4           gridExtra_2.3          GenomeInfoDbData_1.2.2
## [31] viridisLite_0.3.0     crayon_1.3.4           withr_2.1.2
## [34] bitops_1.0-6           grid_3.6.1             gtable_0.3.0
## [37] lifecycle_0.2.0       scales_1.1.0           stringi_1.4.3
## [40] farver_2.0.1           ggsignif_0.6.0         XVector_0.26.0
## [43] reshape2_1.4.3        viridis_0.5.1          xml2_1.2.2
## [46] DelayedMatrixStats_1.8.0 ellipsis_0.3.0         generics_0.0.2
```

## [49]	vctrs_0.3.6	cowplot_1.0.0	tools_3.6.1
## [52]	glue_1.4.2	beeswarm_0.2.3	markdown_1.1
## [55]	purrr_0.3.3	hms_0.5.2	fastmap_1.1.0
## [58]	yaml_2.2.0	colorspace_1.4-1	knitr_1.26