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 42 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: # replacement mutations / (# silent mutations + 0.01), as many cells had zero silent mutations and >=1 replacement mutations.

(D) and (F) The ratio of replacement:silent mutations within the heavy chain is shown for each cell from day 42 from both age groups.

(E) and (H) The number of mutations in the antibody heavy chain at day 42 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 42 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 pseudolog. 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): 1.3×10^{-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
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## locale:
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##  [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
## [21] flowWorkspace_3.34.1       ncdfFlow_2.32.0
## [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
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## [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
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