



Figure 2 – Transcriptional landscape is altered between young and old HA-specific memory B cells

- (A) Heatmap showing expression of the top 15 features from t-tests distinguishing a UMAP cluster from *any* other cluster ($\text{FDR} < 0.01$, \log_2 fold change > 2). Each row is a feature ($n=76$) and its gene symbol is shown. Each column is a single cell ($n=771$). Cells are ordered by UMAP cluster, as shown in the color bar above the heatmap. The 15 features with the largest fold-changes were selected with tied positions allowed, and a feature could appear in more than one comparison. Features that did not map to a gene symbol and duplicated features were removed prior to plotting. Log2 expression values are row-normalized and centered.
- (B) Dotplot showing the expression of selected genes in each UMAP cluster. The size of the dot reflects the proportion of cells within that cluster which express the gene of interest. The color of each dot is scaled according to the scaled normalised expression of the given gene in that cluster. Genes were selected as follows: the top 2 genes from a t-test comparison between each cluster from all other clusters ($\text{L2FC} > 0.5$, $\text{FDR} < 0.25$); biologically relevant B cell genes - selected genes from (A), B cell transcription factors, DNA repair proteins, B cell chemokine receptors and other B cell surface receptors.
- (C) Diffusion co-efficient (DC) based pseudotime analysis from A/Cal09-specific B cells from day 42. Cells are shaded based on their position in pseudotime. Nodes are plotted in red and paths are shown by straight lines.
- (D) Pseudotime analysis as in (C), with colors determined by the UMAP clusters in (A) and defined in Figure 1.
- (E) Boxplots of the numbers of cells within each UMAP cluster comparing day 0 and day 42 cell numbers sorted for 22-36 year old and 67-86 year old individuals. UMAP clusters are named by putative surface marker genes shown in (B). Clusters are labelled with putative surface marker genes shown in (B). P values from 2 tailed paired Mann-Whitney tests are shown, after Benjamini-Hochberg correction for 5 tests.
- (F) Volcano plots from differential abundance analysis for the whole study ('all'), or the two age groups individually. Shown are $-\log_{10}$ (Benjamini-Hochberg) FDR and \log_2 fold change (L2FC). Grey dashed lines are plotted at $-\log_{10}(0.05)$ and at $\text{L2FC} \pm 0.5$.

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] grid parallel stats4 stats graphics grDevices utils
## [8] datasets methods base
##
## other attached packages:
## [1] forcats_0.4.0 stringr_1.4.0
## [3] dplyr_1.0.2 purrr_0.3.3
## [5] readr_1.3.1 tidyr_1.0.0
## [7] tibble_3.0.4 tidyverse_1.3.0
## [9] rstatix_0.6.0 SingleR_1.0.6
## [11] destiny_3.0.1 Seurat_3.2.2
## [13] edgeR_3.28.0 limma_3.42.0
## [15] gtable_0.3.0 org.Hs.eg.db_3.10.0
## [17] AnnotationDbi_1.48.0 ggpubr_0.2.4
## [19] magrittr_1.5 MultiAssayExperiment_1.12.1
## [21] scanr_1.14.5 scater_1.14.5
## [23] ggplot2_3.3.2 SingleCellExperiment_1.8.0
## [25] SummarizedExperiment_1.16.0 DelayedArray_0.12.0
## [27] BiocParallel_1.20.0 matrixStats_0.55.0
## [29] Biobase_2.46.0 GenomicRanges_1.38.0
## [31] GenomeInfoDb_1.22.0 IRanges_2.20.1
## [33] S4Vectors_0.24.1 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 data.table_1.12.8
## [7] rpart_4.1-15 RCurl_1.95-4.12
## [9] generics_0.0.2 cowplot_1.0.0
## [11] RSQLite_2.1.4 RANN_2.6.1
## [13] proxy_0.4-23 future_1.20.1
## [15] bit_1.1-14 xml2_1.2.2
## [17] spatstat.data_1.4-3 lubridate_1.7.4
## [19] httpuv_1.5.2 assertthat_0.2.1
## [21] viridis_0.5.1 xfun_0.11
## [23] hms_0.5.2 evaluate_0.14
## [25] promises_1.1.0 fansi_0.4.0
## [27] DEoptimR_1.0-8 caTools_1.17.1.3
```

## [29] dbplyr_1.4.2	readxl_1.3.1
## [31] igraph_1.2.4.2	DBI_1.1.0
## [33] htmlwidgets_1.5.1	ellipsis_0.3.0
## [35] RSpectra_0.16-0	backports_1.1.5
## [37] markdown_1.1	RcppParallel_4.4.4
## [39] deldir_0.1-23	vctr_0.3.6
## [41] TTR_0.23-6	ROCR_1.0-7
## [43] abind_1.4-5	RcppEigen_0.3.3.7.0
## [45] withr_2.1.2	robustbase_0.93-5
## [47] vcd_1.4-4	sctransform_0.3.2.9000
## [49] xts_0.12-0	goftest_1.2-2
## [51] cluster_2.1.0	ExperimentHub_1.12.0
## [53] lazyeval_0.2.2	laeken_0.5.0
## [55] crayon_1.3.4	labeling_0.3
## [57] pkgconfig_2.0.3	nlme_3.1-141
## [59] vipor_0.4.5	nnet_7.3-12
## [61] rlang_0.4.10	globals_0.13.1
## [63] lifecycle_0.2.0	miniUI_0.1.1.1
## [65] BiocFileCache_1.10.2	modelr_0.1.5
## [67] rsvd_1.0.2	AnnotationHub_2.18.0
## [69] cellranger_1.1.0	polyclip_1.10-0
## [71] RcppHNSW_0.2.0	lmtest_0.9-37
## [73] Matrix_1.2-17	carData_3.0-3
## [75] boot_1.3-23	zoo_1.8-8
## [77] reprex_0.3.0	beeswarm_0.2.3
## [79] ggribes_0.5.2	pheatmap_1.0.12
## [81] png_0.1-7	viridisLite_0.3.0
## [83] bitops_1.0-6	KernSmooth_2.23-15
## [85] blob_1.2.0	DelayedMatrixStats_1.8.0
## [87] parallelly_1.21.0	ggsignif_0.6.0
## [89] scales_1.1.0	memoise_1.1.0
## [91] plyr_1.8.5	hexbin_1.28.0
## [93] ica_1.0-2	gplots_3.0.1.1
## [95] gdata_2.18.0	zlibbioc_1.32.0
## [97] compiler_3.6.1	dqrng_0.2.1
## [99] RColorBrewer_1.1-2	pcaMethods_1.78.0
## [101] fitdistrplus_1.1-1	cli_2.0.0
## [103] XVector_0.26.0	listenv_0.8.0
## [105] patchwork_1.0.0	pbapply_1.4-2
## [107] ggplot.multistats_1.0.0	MASS_7.3-51.4
## [109] mgcv_1.8-28	tidyselect_1.1.0
## [111] stringi_1.4.3	yaml_2.2.0
## [113] BiocSingular_1.2.0	locfit_1.5-9.1
## [115] ggrepel_0.8.1	tools_3.6.1
## [117] future.apply_1.6.0	rio_0.5.16
## [119] rstudioapi_0.10	foreign_0.8-72
## [121] gridExtra_2.3	smoother_1.1
## [123] scatterplot3d_0.3-41	farver_2.0.1
## [125] Rtsne_0.15	digest_0.6.23
## [127] BiocManager_1.30.10	ggtext_0.1.1
## [129] shiny_1.4.0	gridtext_0.1.4
## [131] Rcpp_1.0.3	car_3.0-5
## [133] broom_0.7.3	BiocVersion_3.10.1
## [135] later_1.0.0	RcppAnnoy_0.0.14

## [137] httr_1.4.1	colorspace_1.4-1
## [139] rvest_0.3.5	fs_1.3.1
## [141] tensor_1.5	ranger_0.11.2
## [143] reticulate_1.14	splines_3.6.1
## [145] uwot_0.1.5	statmod_1.4.32
## [147] spatstat.utils_1.17-0	sp_1.3-2
## [149] plotly_4.9.1	xtable_1.8-4
## [151] jsonlite_1.6	spatstat_1.64-1
## [153] R6_2.4.1	pillar_1.4.7
## [155] htmltools_0.4.0	mime_0.7
## [157] glue_1.4.2	fastmap_1.0.1
## [159] VIM_4.8.0	BiocNeighbors_1.4.1
## [161] class_7.3-15	interactiveDisplayBase_1.24.0
## [163] codetools_0.2-16	lattice_0.20-38
## [165] curl_4.3	ggbeeswarm_0.6.0
## [167] leiden_0.3.4	gtools_3.8.1
## [169] zip_2.0.4	openxlsx_4.1.4
## [171] survival_2.44-1.1	rmarkdown_2.0
## [173] munsell_0.5.0	e1071_1.7-3
## [175] GenomeInfoDbData_1.2.2	haven_2.2.0
## [177] reshape2_1.4.3	