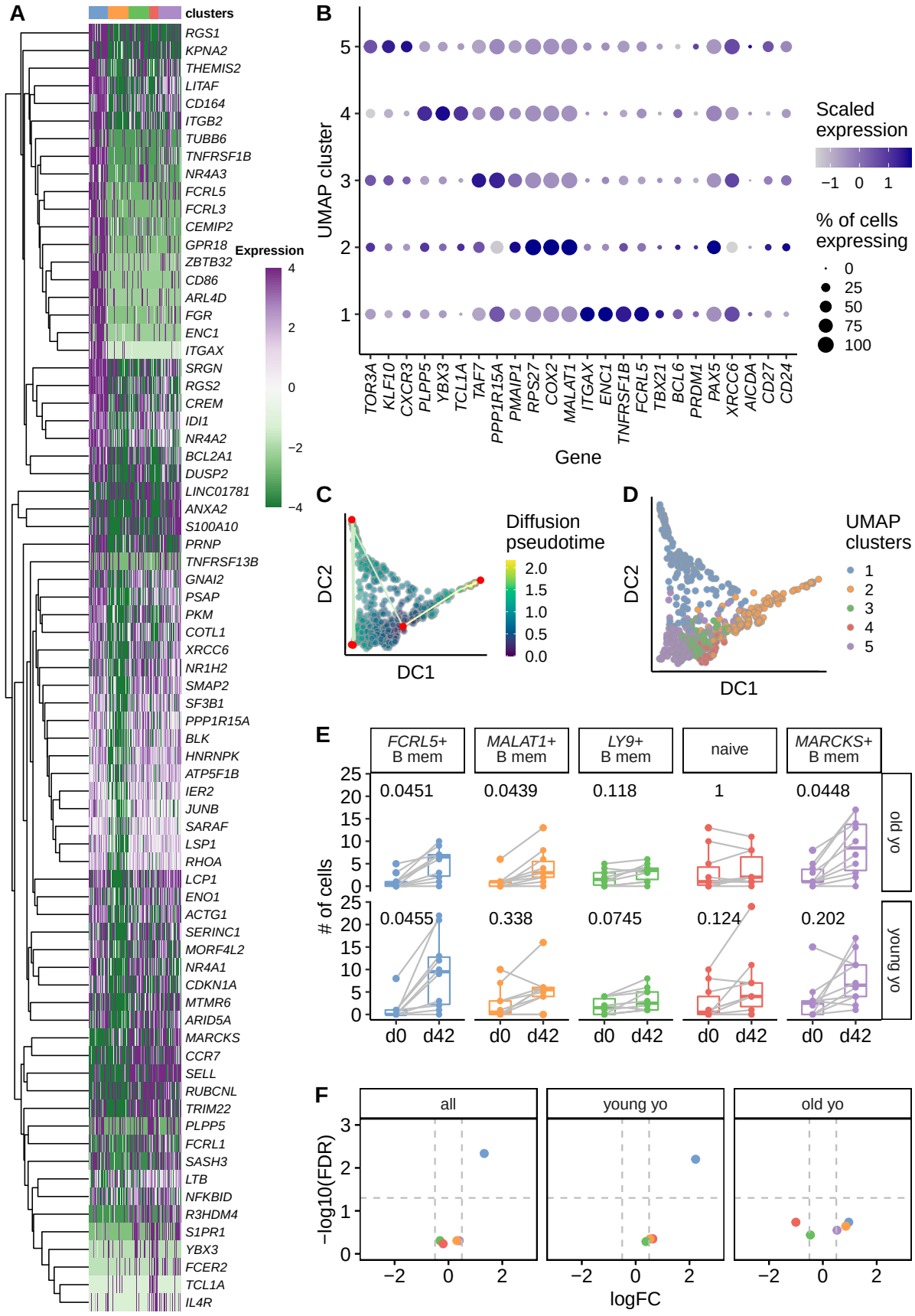
A
clusters
RGS1
KPNA2
THEMIS2
LITAF
CD164
ITGB2
TUBB6
TNFRSF1B
NR4A3
FCRL5
FCRL3
CEMIP2
GPR18
ZBTB32
CD86
ARL4D
FGR
ENC1
ITGAX
SRGN
RGS2
CREM
IDI1
NR4A2
BCL2A1
DUSP2
LINC01781
ANXA2
S100A10
PRNP
TNFRSF13B
GNAI2
PSAP
PKM
COTL1
XRCC6
NR1H2
SMAP2
SF3B1
PPP1R15A
BLK
HNRNPK
ATP5F1B
IER2
JUNB
SARAF
LSP1
RHOA
LCP1
ENO1
ACTG1
SERINC1
MORF4L2
NR4A1
CDKN1A
MTMR6
ARID5A
MARCKS
CCR7
SELL
RUBCNL
TRIM22
PLPP5
FCRL1
SASH3
LTB
NFKBID
R3HDM4
S1PR1
YBX3
FCER2
TCL1A
IL4R
Expression
4
2
0
−2
−4
B
UMAP cluster
5
4
3
2
1
TOR3A
KLF10
CXCR3
PLPP5
YBX3
TCL1A
TAF7
PPP1R15A
PMAIP1
RPS27
COX2
MALAT1
ITGAX
ENC1
TNFRSF1B
FCRL5
TBX21
BCL6
PRDM1
PAX5
XRCC6
AICDA
CD27
CD24
Gene
Scaled expression
−1 0 1
% of cells expressing
0
25
50
75
100
C
DC2
DC1
Diffusion pseudotime
2.0
1.5
1.0
0.5
0.0
D
DC2
DC1
UMAP clusters
1
2
3
4
5
E
FCRL5+ B mem
MALAT1+ B mem
LY9+ B mem
naive
MARCKS+ B mem
old yo
young yo
# of cells
0.0451
0.0439
0.118
1
0.0448
0.0455
0.338
0.0745
0.124
0.202
d0
d42
F
all
young yo
old yo
−log10(FDR)
logFC

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 (FDR<0.01, log2 fold change>2). Each row is a feature (n=73) and its gene symbol is shown. Each column is a single cell (n=774). Cells are ordered by UMAP cluster, as shown in the color bar above the heatmap. The 15 features with the larged 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 (L2FC>0.5, 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 young year old and old 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 -log10 (Benjamini-Hochberg) FDR and log2 fold change (L2FC). Grey dashed lines are plotted at -log10(0.05) and at 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_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] 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               png_0.1-7
## [11] SingleR_1.0.6               destiny_3.0.1
## [13] Seurat_3.2.2                edgeR_3.28.0
## [15] limma_3.42.0                gtable_0.3.0
## [17] org.Hs.eg.db_3.10.0         AnnotationDbi_1.48.0
## [19] ggpubr_0.2.4                magrittr_1.5
## [21] MultiAssayExperiment_1.12.1 scran_1.14.5
## [23] scater_1.14.5               ggplot2_3.3.2
## [25] SingleCellExperiment_1.8.0  SummarizedExperiment_1.16.0
## [27] DelayedArray_0.12.0         BiocParallel_1.20.0
## [29] matrixStats_0.55.0          Biobase_2.46.0
## [31] GenomicRanges_1.38.0        GenomeInfoDb_1.22.0
## [33] IRanges_2.20.1              S4Vectors_0.24.1
## [35] 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_0.31
```

```
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##  [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                vctrs_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] ggridges_0.5.2               pheatmap_1.0.12
##  [81] viridisLite_0.3.0            bitops_1.0-6
##  [83] KernSmooth_2.23-15           blob_1.2.0
##  [85] DelayedMatrixStats_1.8.0     parallelly_1.21.0
##  [87] ggsignif_0.6.0               scales_1.1.0
##  [89] memoise_1.1.0                plyr_1.8.5
##  [91] hexbin_1.28.0                ica_1.0-2
##  [93] gplots_3.0.1.1               gdata_2.18.0
##  [95] zlibbioc_1.32.0              compiler_3.6.1
##  [97] dqrng_0.2.1                  RColorBrewer_1.1-2
##  [99] pcaMethods_1.78.0            fitdistrplus_1.1-1
## [101] cli_2.0.0                    XVector_0.26.0
## [103] listenv_0.8.0                patchwork_1.0.0
## [105] pbapply_1.4-2                ggplot.multistats_1.0.0
## [107] MASS_7.3-51.4                mgcv_1.8-28
## [109] tidyselect_1.1.0             stringi_1.4.3
## [111] yaml_2.2.0                   BiocSingular_1.2.0
## [113] locfit_1.5-9.1               ggrepel_0.8.1
## [115] tools_3.6.1                  future.apply_1.6.0
## [117] rio_0.5.16                   rstudioapi_0.10
## [119] foreign_0.8-72               gridExtra_2.3
## [121] smoother_1.1                 scatterplot3d_0.3-41
## [123] farver_2.0.1                 Rtsne_0.15
## [125] digest_0.6.29                BiocManager_1.30.10
## [127] ggtext_0.1.1                 shiny_1.4.0
## [129] gridtext_0.1.4               Rcpp_1.0.3
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```
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## [143] splines_3.6.1                 uwot_0.1.5
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## [149] xtable_1.8-4                  jsonlite_1.6
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## [173] e1071_1.7-3                   GenomeInfoDbData_1.2.2
## [175] haven_2.2.0                   reshape2_1.4.3
```