ACTA (Anybody Can Titrate Antibody) Report v3.6.11

2026-11-12

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A. MiFlowCyt metadata

1 Experiment Overview

MiFlowCyt 1.0. Structured to MiFlowCyt but not self-contained: purpose, experiment variables, organization, conclusions, specimen source and collection, and instrument QC are recorded in the ELN and referenced here. Not a compliant submission document on its own.

Category	Value
Purpose	See ELN EXP1402 (ELN)
Keywords	(not supplied)
Experiment variables	Stain volume (uL) per antibody; see Reagents. See ELN EXP1402 (ELN)
Organization	See ELN EXP1402 (ELN)
Primary contact	JD
Acquisition date	2026-09-17
Conclusions	See ELN EXP1402 (ELN)
Quality control	6 automated check(s) per antibody group; see the QC section.

2 Flow Sample/Specimen Details

Category	Value
Test material	HD Human PBMC
Cells per well (e6)	1
Well volume (uL)	100
Source	See ELN EXP1402 (ELN)
Collection	See ELN EXP1402 (ELN)
SOP	(not supplied)
Fixed	TRUE
Permeabilised	TRUE
Protocol	See ELN EXP1402 (ELN)

2.4 Fluorescence Reagent Description

CD4

Channel	Markername
SSC-H	—
SSC-A	—
FSC-H	—
FSC-A	—
SSC-B-H	—
SSC-B-A	—
BUV496-A	CD4
BUV805-A	CD3
Alexa Fluor 532-A	CD8
FVD eFluor 780-A	Viability

CD8

Channel	Markername
SSC-H	—
SSC-A	—
FSC-H	—
FSC-A	—
SSC-B-H	—
SSC-B-A	—
BUV496-A	CD4
BUV805-A	CD3
Alexa Fluor 532-A	CD8
FVD eFluor 780-A	Viability

Table 1: Costain panel 'Iteration1' from 'Master_Panel_Inventory.xlsx': 2 of 2 reagents matched.

Antigen	Fluorochrome	Matched to	Verdict	Catalogue	Vendor	Clone	Type
CD3	BUV805	CD3	matched	612893	BD	SK7	Base
Live Dead eFluor 780 (LD) (Viability)	efluor780	Viability	matched	65-0865-14	TS	NA	Base

3 Instrument Details

Category	Value
Manufacturer and model	Aurora
Detectors with a recorded gain	6
Instrument QC	See ELN EXP1402 (ELN)

Detector voltages ($PnV)

detector	marker	voltage
SSC-H	NA	127
SSC-A	NA	127
FSC-H	NA	125
FSC-A	NA	125
SSC-B-H	NA	126
SSC-B-A	NA	126

4 Data Analysis Details

Everything below is MiFlowCyt part 4 – what was done to the events between acquisition and the numbers in this report, in the order it happened: events read, compensation, transformation, gating, and the software that did it.

4.1 List-mode data files: downsampling

Info!Downsample_to: *(blank)*

No downsampling performed.

Events read: 1,165,915 across 20 file(s) (smallest 57,334, largest 59,542).

4.2 Compensation Description

Info!compensation: *(blank)*

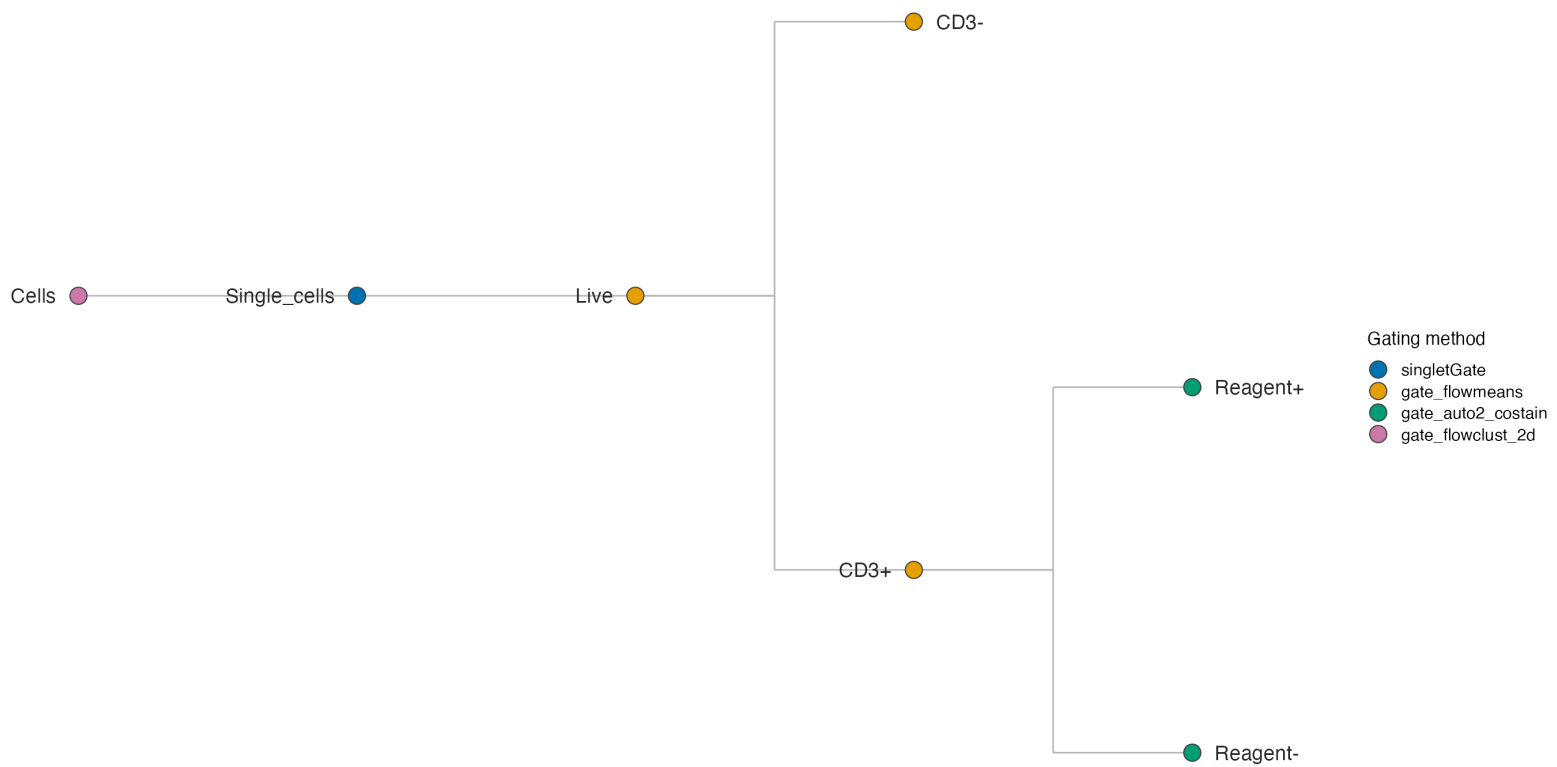
Identity matrix was applied – the data were not compensated.

4.3 Data Transformation Details

Argument	CD4	CD8
maxValue (from $PnR)	4194304	4194304
channelRange	9000	9000
pos	4.5	4.5
neg	0	0
widthBasis	-10	-10

4.4 Gating Details

Gating scheme



4.4.2 Gate Statistics

Count, percent of parent, median fluorescence intensity and robust SD were collected for 36 population/well combinations and written to the **Pop_stats** sheet of the titration export beside this report.

In `gating_template`, mark `stats_export` as TRUE to make sure that population's stats are exported.

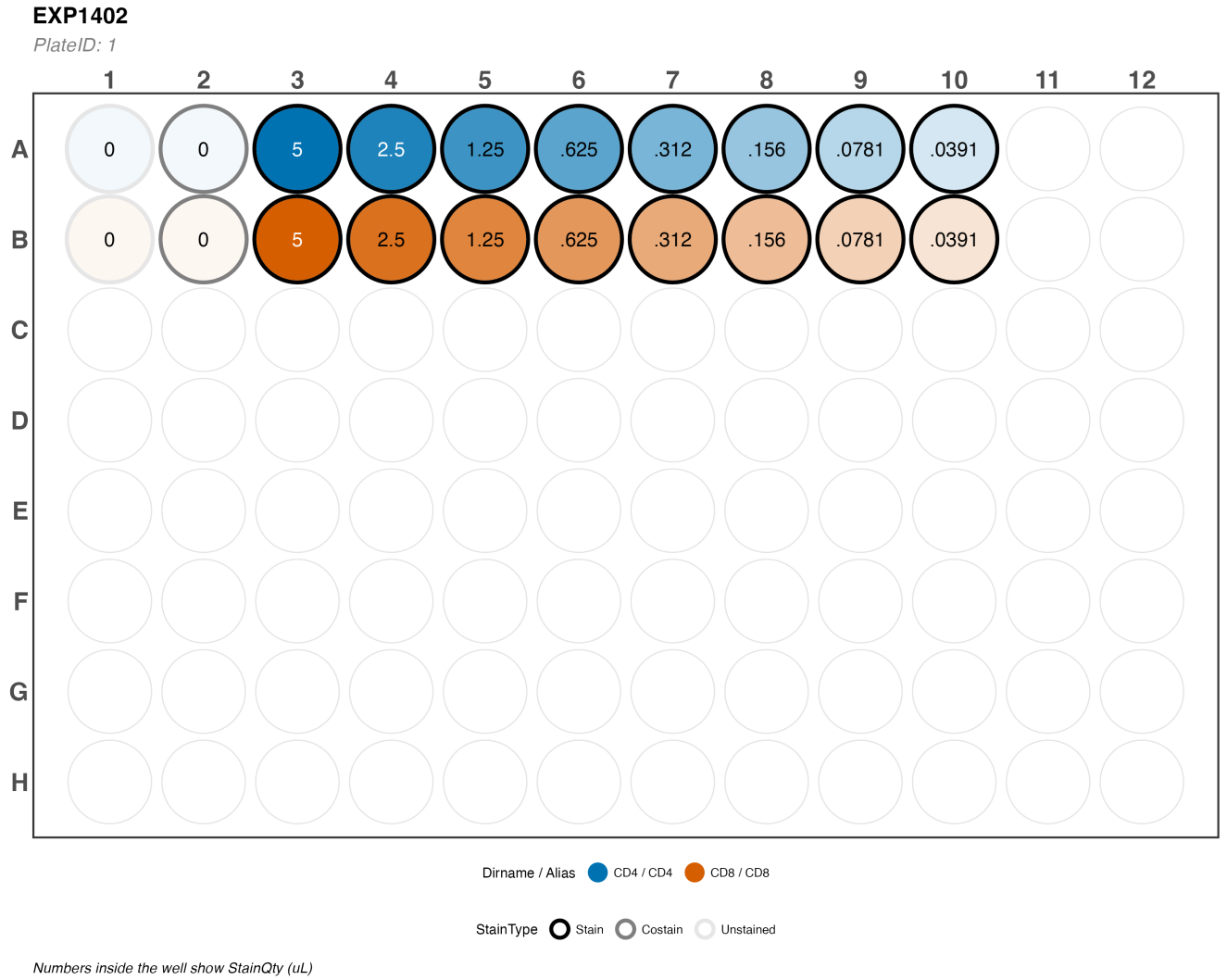
4.5 Analysis software

Category	Value
ACTA version	3.6.11
R version	R version 4.5.1 (2025-06-13)

This pipeline can be driven from R without the app: see **Automating ACTA** in `README.md` in the script folder.

B. Titration results

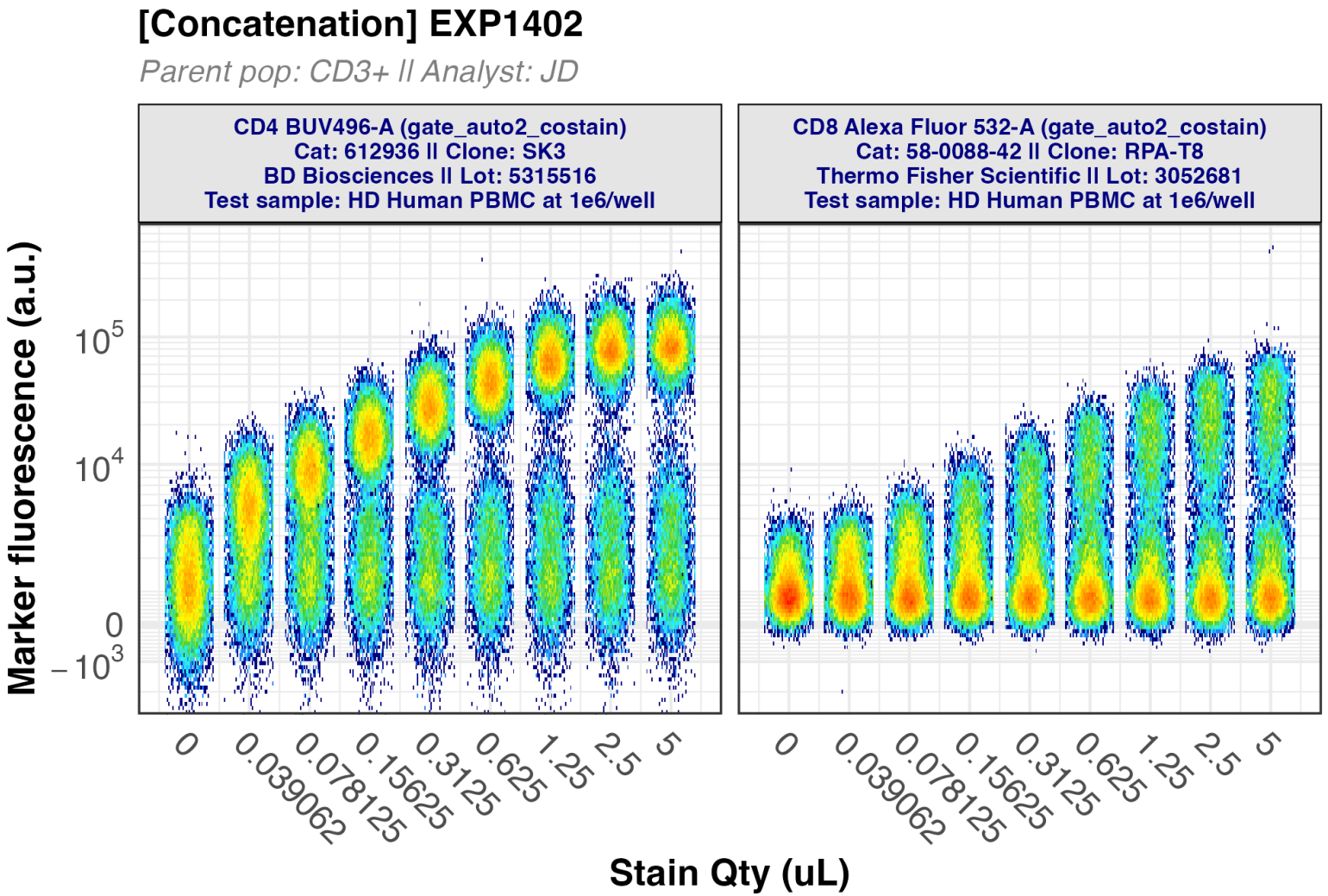
1 Plate map



2 QC

Check	CD4	CD8
Median FSC-A of Cells is at least 50% of the median of all events	**PASS**	**PASS**
More than 50% Live cells	**PASS**	**PASS**
More than 80% single cells	**PASS**	**PASS**
Costain SI is the lowest in the group	**FAIL**	**FAIL**
Co-stain is less than 5% positive	**PASS**	**PASS**
Every population has at least 100 events in every well (except costain)	**PASS**	**PASS**
Overall	**FAIL**	**FAIL**

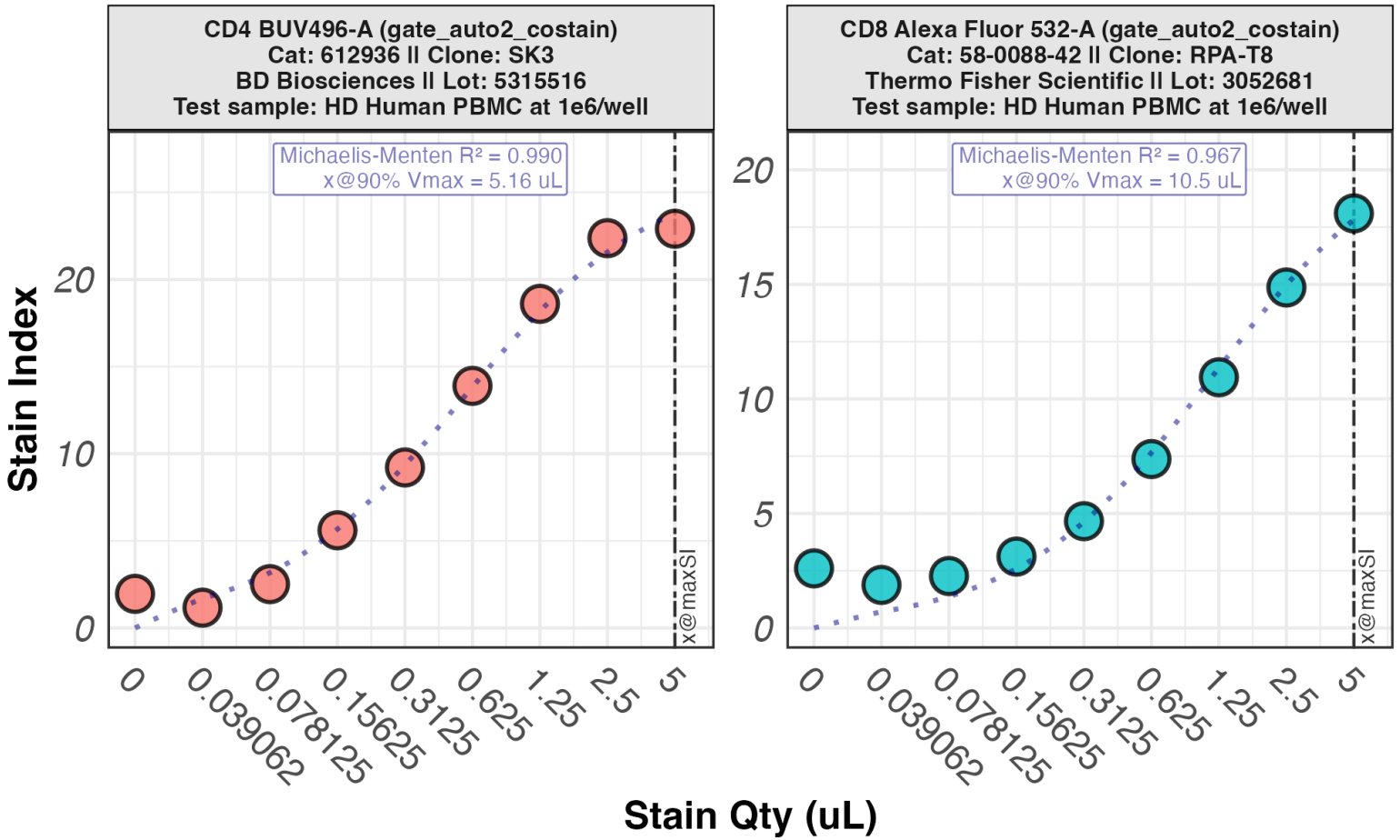
3 Resolution of populations



Concatenated events, up to 20,000 per FCS.

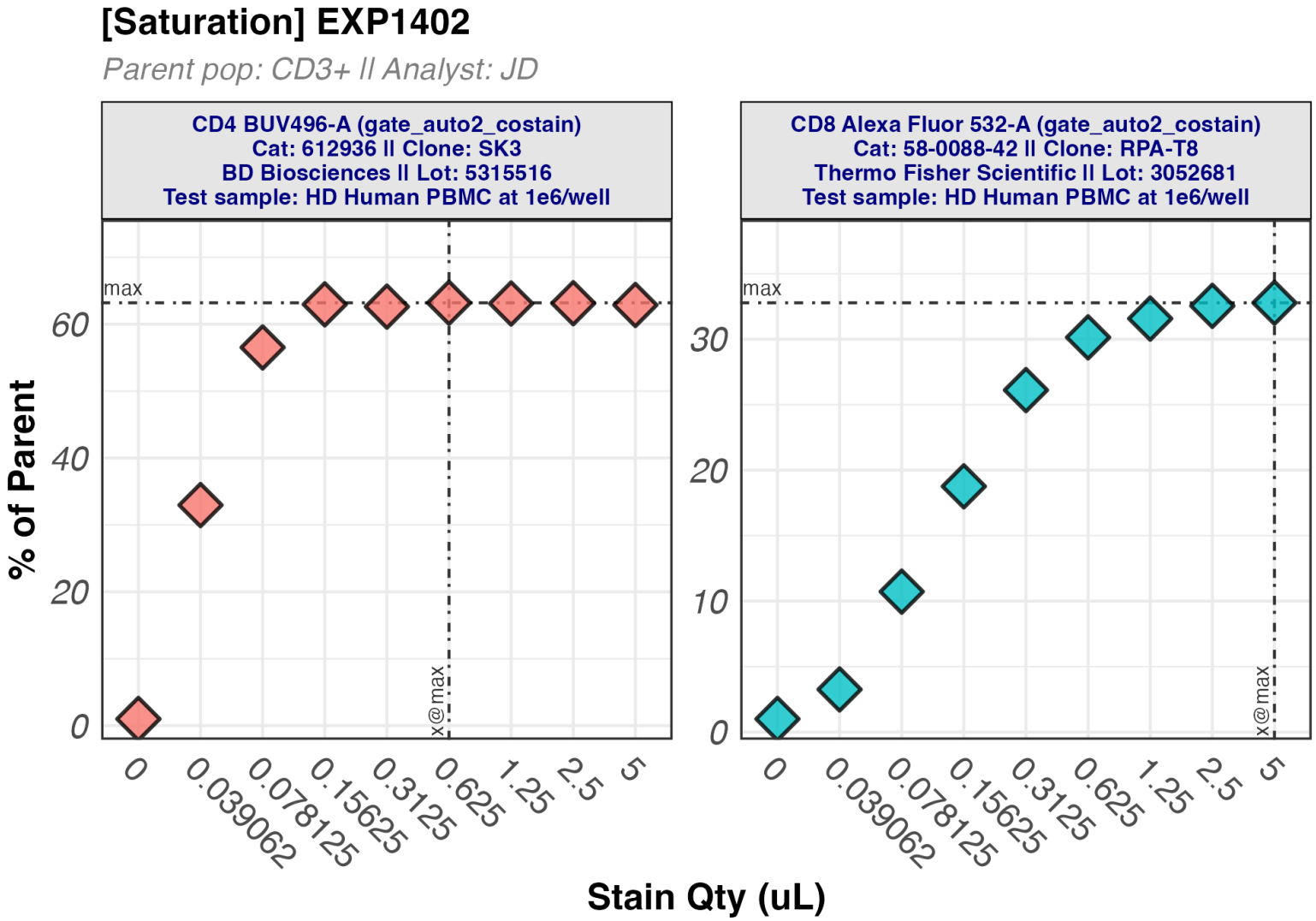
[Stain Index] EXP1402

Parent pop: CD3+ ll Analyst: JD



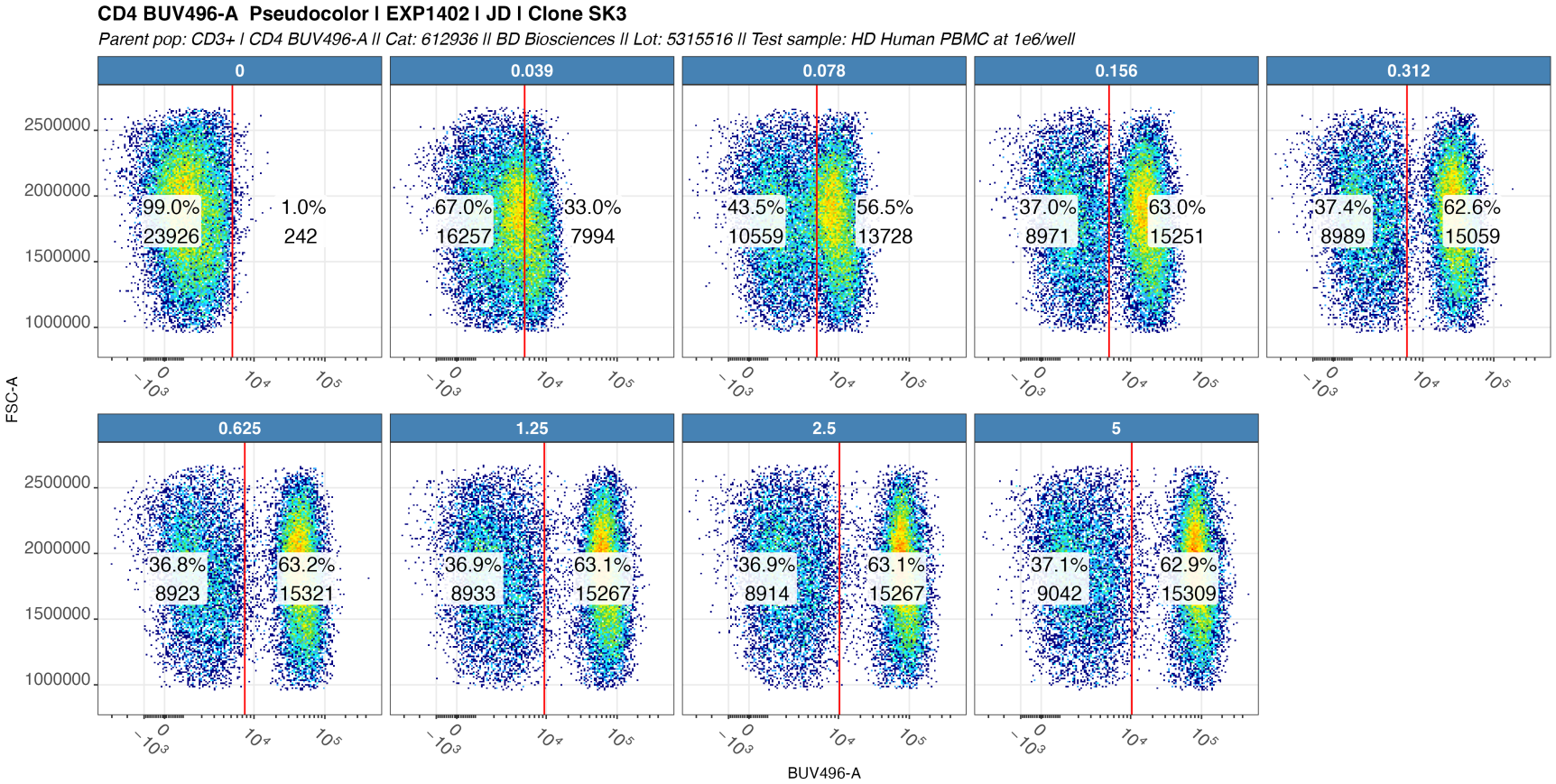
MFI calculated on untransformed (linear) values. ll Compensation: —

4 Saturation of positive population proportion



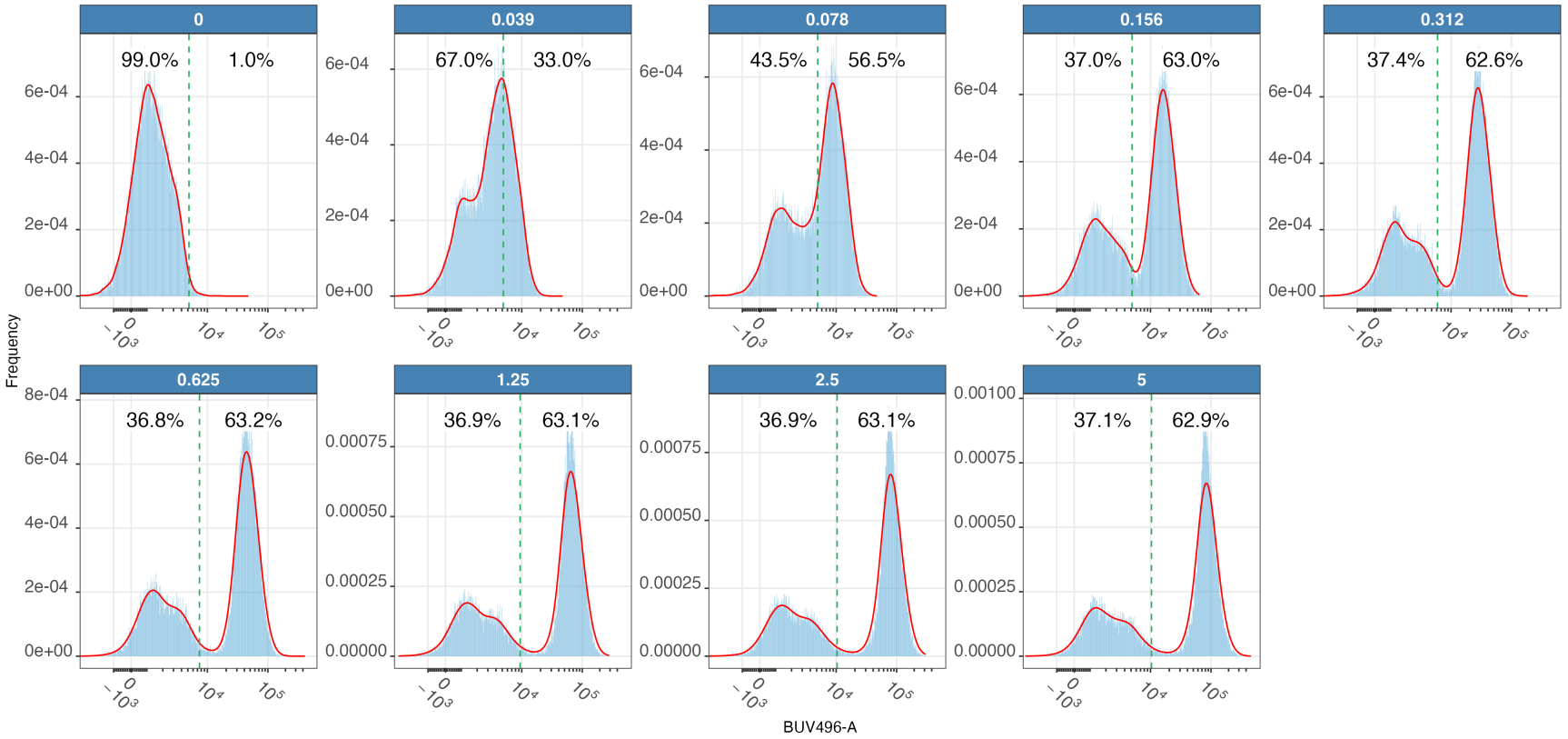
5 Layouts for each antibody

5.1 CD4


CD4 BUV496-A Pseudocolor | EXP1402 | JD | Clone SK3
Parent pop: CD3+ | CD4 BUV496-A || Cat: 612936 || BD Biosciences || Lot: 5315516 || Test sample: HD Human PBMC at 1e6/well
0
99.0%
23926
1.0%
242
0.039
67.0%
16257
33.0%
7994
0.078
43.5%
10559
56.5%
13728
0.156
37.0%
8971
63.0%
15251
0.312
37.4%
8989
62.6%
15059
0.625
36.8%
8923
63.2%
15321
1.25
36.9%
8933
63.1%
15267
2.5
36.9%
8914
63.1%
15267
5
37.1%
9042
62.9%
15309
FSC-A
BUV496-A
Gating method: gate_auto2_costain || args: cluster='neg', quantile=0.99, K=2 || collapse: FALSE || groupBy: Dirname || pp: pp_auto2_costain || pp args: costain_quantile=0.99, sat_threshold=0.90, dip_pval=0.05
Compensation: —

CD4 BUV496-A Histogram | EXP1402 | JD | Clone SK3

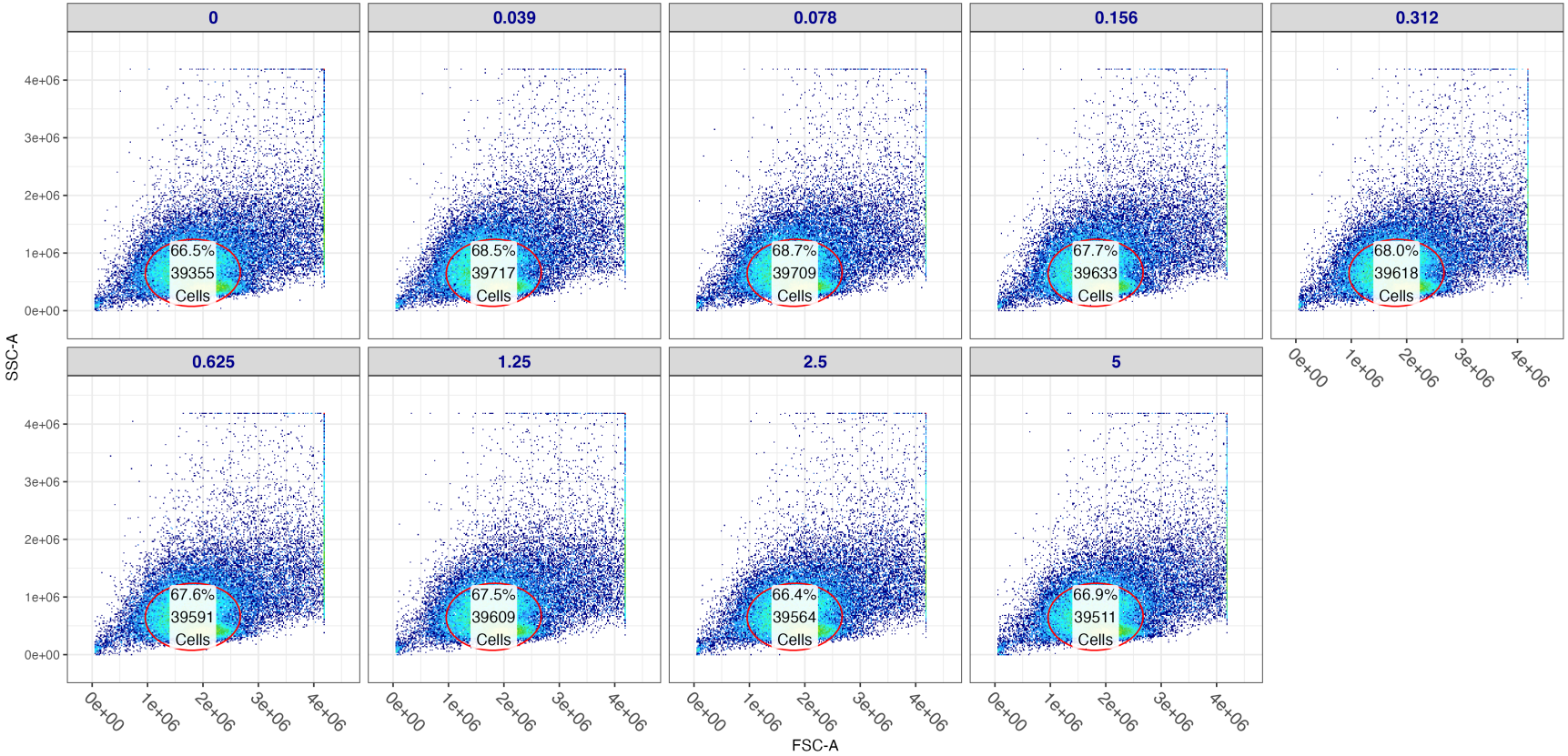
Parent pop: CD3+ || CD4 BUV496-A || Cat: 612936 || BD Biosciences || Lot: 5315516 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_auto2_costain || args: cluster='neg', quantile=0.99, K=2 || collapse: FALSE || groupBy: Dirname || pp: pp_auto2_costain || pp args: costain_quantile=0.99, sat_threshold=0.90, dip_pval=0.05
Compensation: —

CD4 BUV496-A : Cells | EXP1402 | JD | Clone SK3

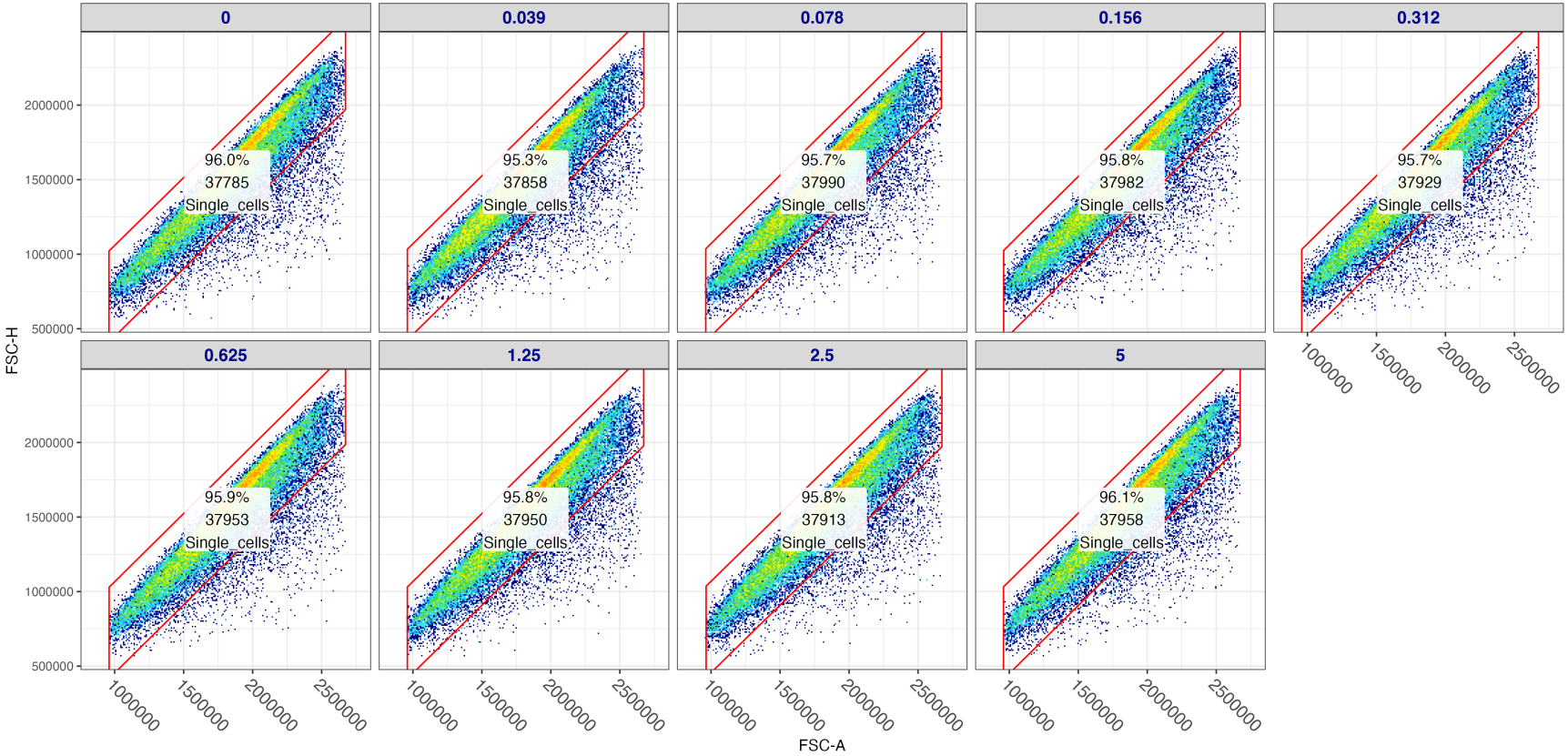
Parent pop: root | CD4 BUV496-A || Cat: 612936 || BD Biosciences || Lot: 5315516 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_flowclust_2d || args: K=2, quantile=0.75, min=NULL, max=NULL || collapse: TRUE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

CD4 BUV496-A : Single_cells | EXP1402 | JD | Clone SK3

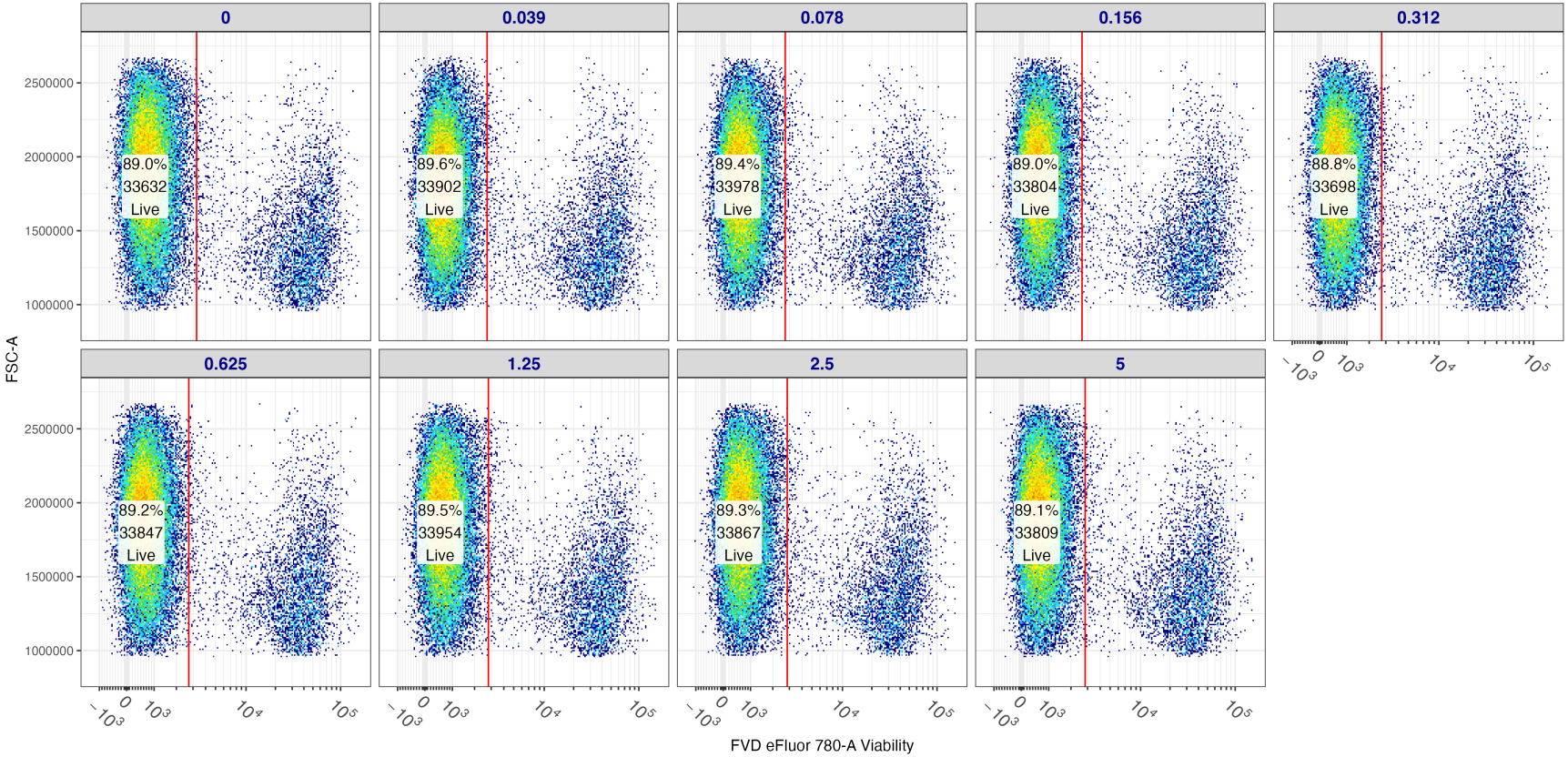
Parent pop: Cells | CD4 BUV496-A || Cat: 612936 || BD Biosciences || Lot: 5315516 || Test sample: HD Human PBMC at 1e6/well



Gating method: singletGate || args: prediction_level=0.99, wider_gate=FALSE || collapse: FALSE || groupBy: Dirname || pp: — || pp args: — Compensation: —

CD4 BUV496-A : Live | EXP1402 | JD | Clone SK3

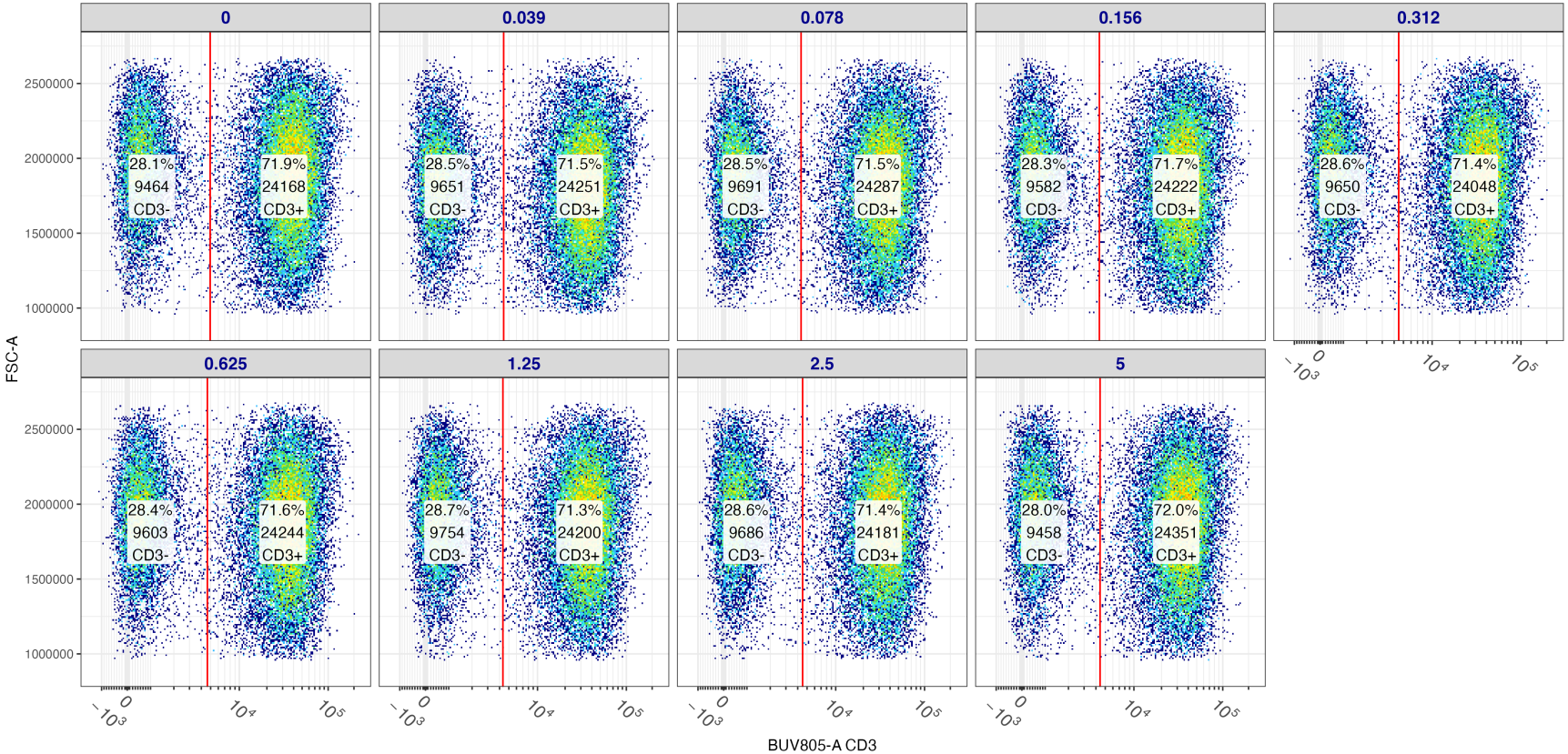
Parent pop: Single_cells | CD4 BUV496-A || Cat: 612936 || BD Biosciences || Lot: 5315516 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_flowmeans || args: K=2, cluster='neg', quantile=0.99 || collapse: FALSE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

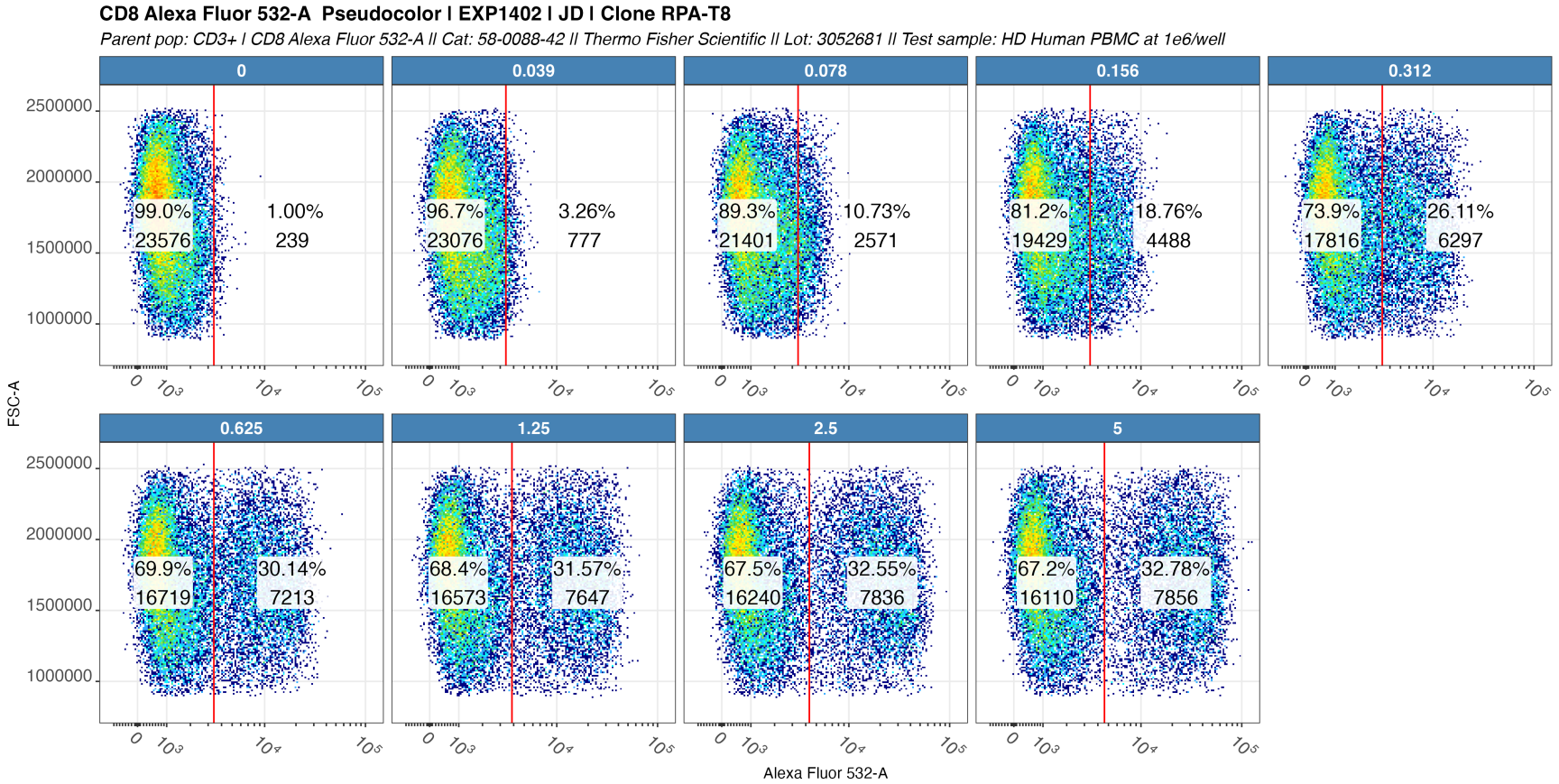
CD4 BUV496-A : CD3 | EXP1402 | JD | Clone SK3

Parent pop: Live | CD4 BUV496-A || Cat: 612936 || BD Biosciences || Lot: 5315516 || Test sample: HD Human PBMC at 1e6/well



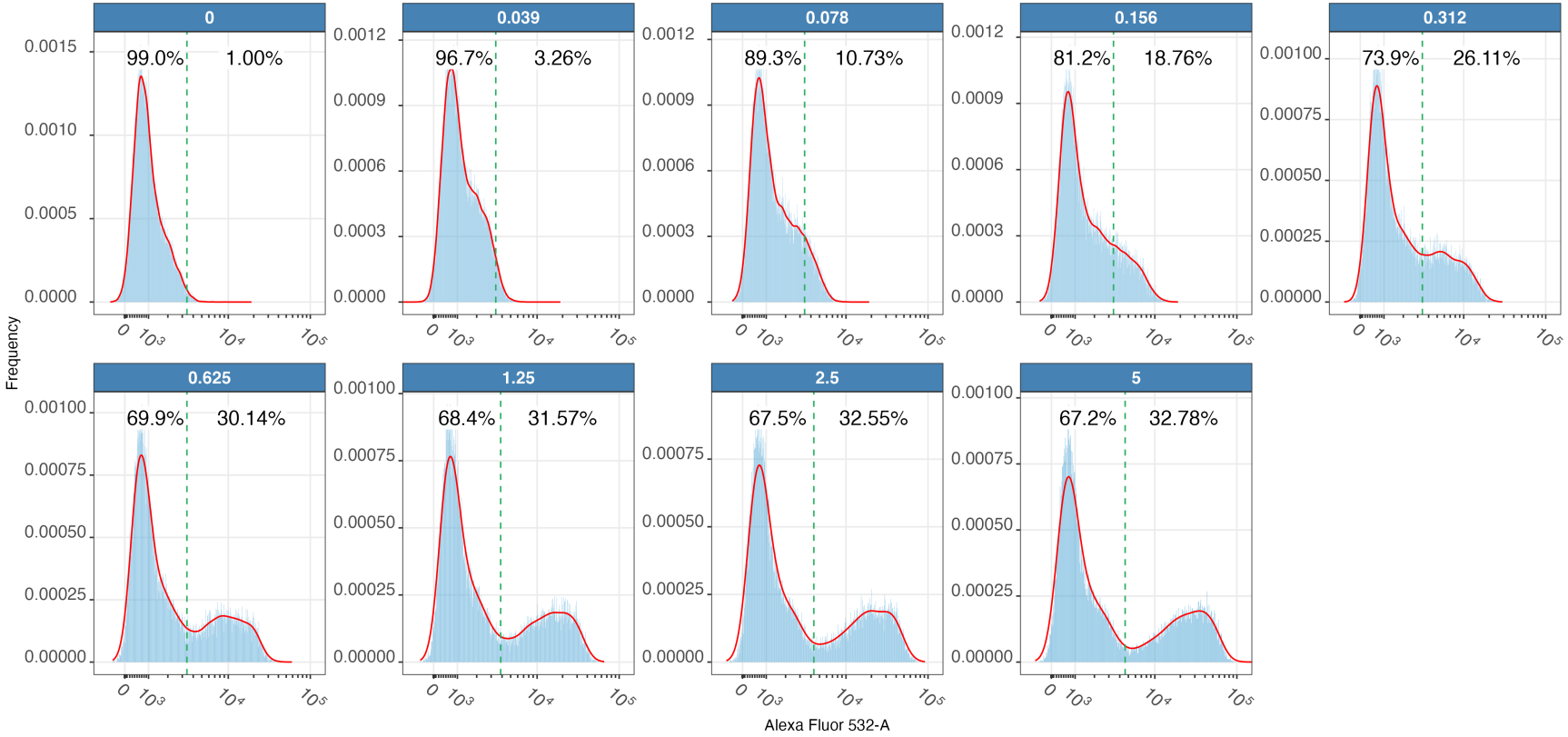
Gating method: gate_flowmeans || args: K=2, cluster='neg', quantile=0.99 || collapse: FALSE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

5.2 CD8


CD8 Alexa Fluor 532-A Pseudocolor | EXP1402 | JD | Clone RPA-T8
Parent pop: CD3+ | CD8 Alexa Fluor 532-A || Cat: 58-0088-42 || Thermo Fisher Scientific || Lot: 3052681 || Test sample: HD Human PBMC at 1e6/well
0
99.0%
23576
1.00%
239
0.039
96.7%
23076
3.26%
777
0.078
89.3%
21401
10.73%
2571
0.156
81.2%
19429
18.76%
4488
0.312
73.9%
17816
26.11%
6297
0.625
69.9%
16719
30.14%
7213
1.25
68.4%
16573
31.57%
7647
2.5
67.5%
16240
32.55%
7836
5
67.2%
16110
32.78%
7856
FSC-A
Alexa Fluor 532-A
Gating method: gate_auto2_costain || args: cluster='neg', quantile=0.99, K=2 || collapse: FALSE || groupBy: Dirname || pp: pp_auto2_costain || pp args: costain_quantile=0.99, sat_threshold=0.90, dip_pval=0.05
Compensation: —

CD8 Alexa Fluor 532-A Histogram | EXP1402 | JD | Clone RPA-T8

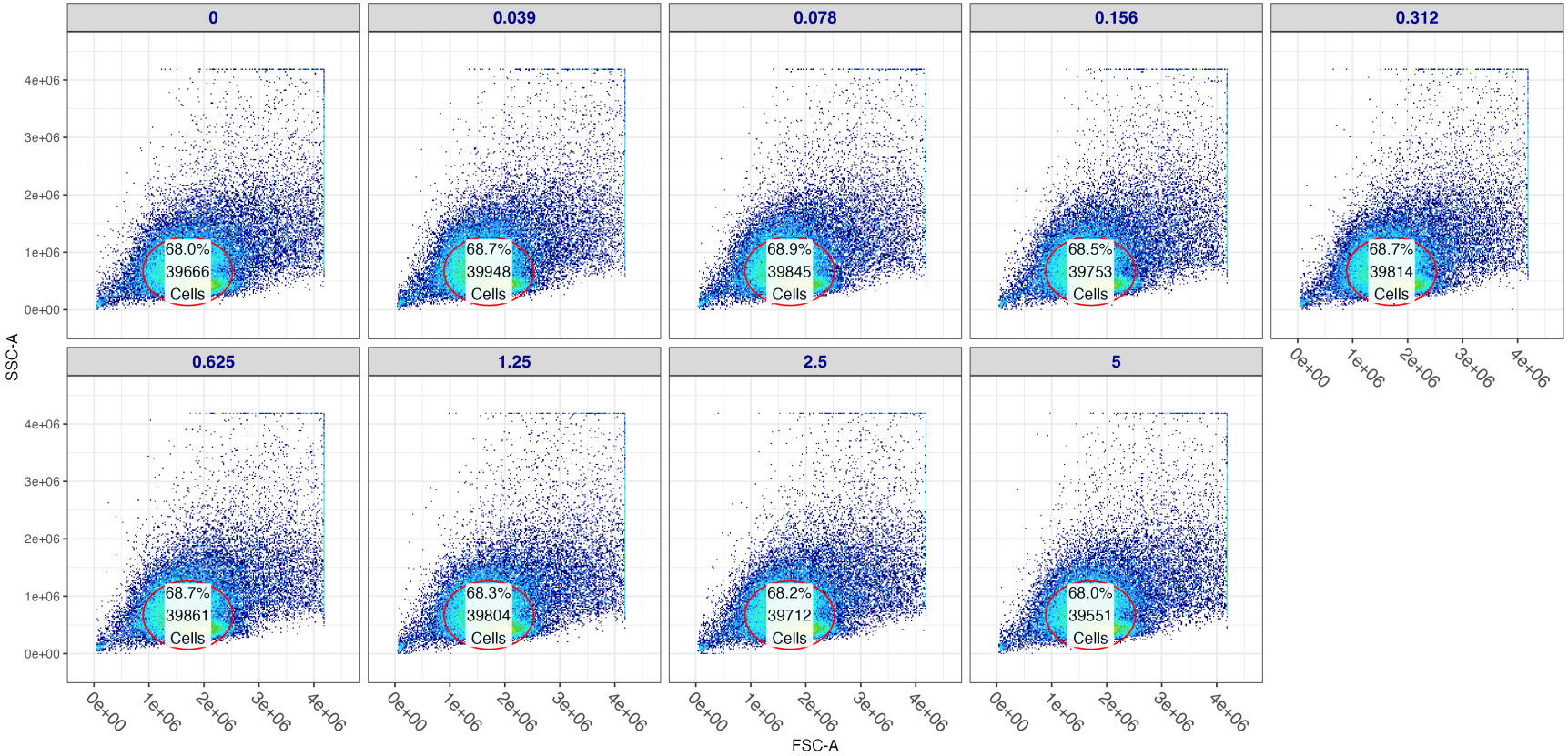
Parent pop: CD3+ || CD8 Alexa Fluor 532-A || Cat: 58-0088-42 || Thermo Fisher Scientific || Lot: 3052681 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_auto2_costain || args: cluster='neg', quantile=0.99, K=2 || collapse: FALSE || groupBy: Dirname || pp: pp_auto2_costain || pp args: costain_quantile=0.99, sat_threshold=0.90, dip_pval=0.05
Compensation: —

CD8 Alexa Fluor 532-A : Cells | EXP1402 | JD | Clone RPA-T8

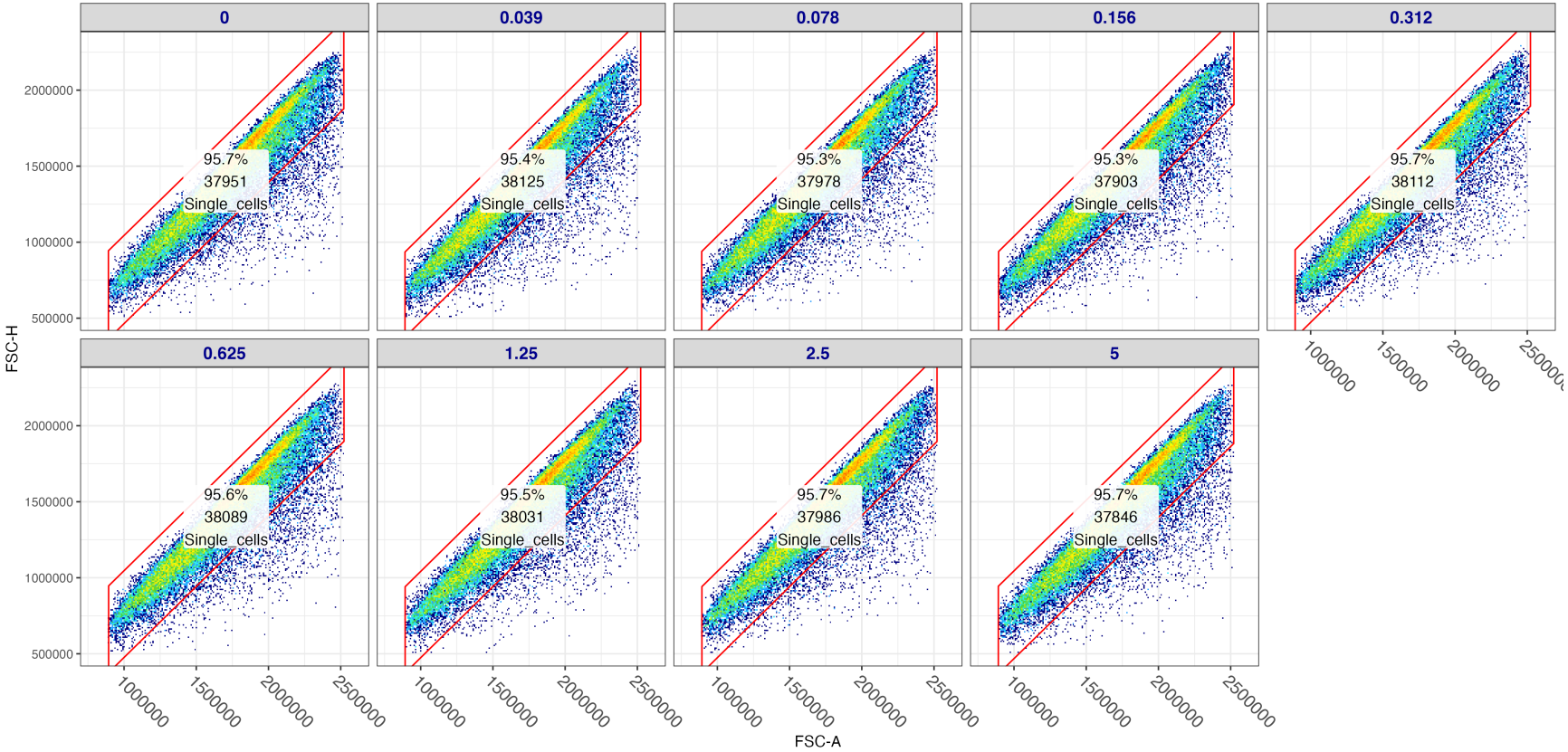
Parent pop: root | CD8 Alexa Fluor 532-A || Cat: 58-0088-42 || Thermo Fisher Scientific || Lot: 3052681 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_flowclust_2d || args: K=2, quantile=0.75, min=NULL, max=NULL || collapse: TRUE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

CD8 Alexa Fluor 532-A : Single_cells | EXP1402 | JD | Clone RPA-T8

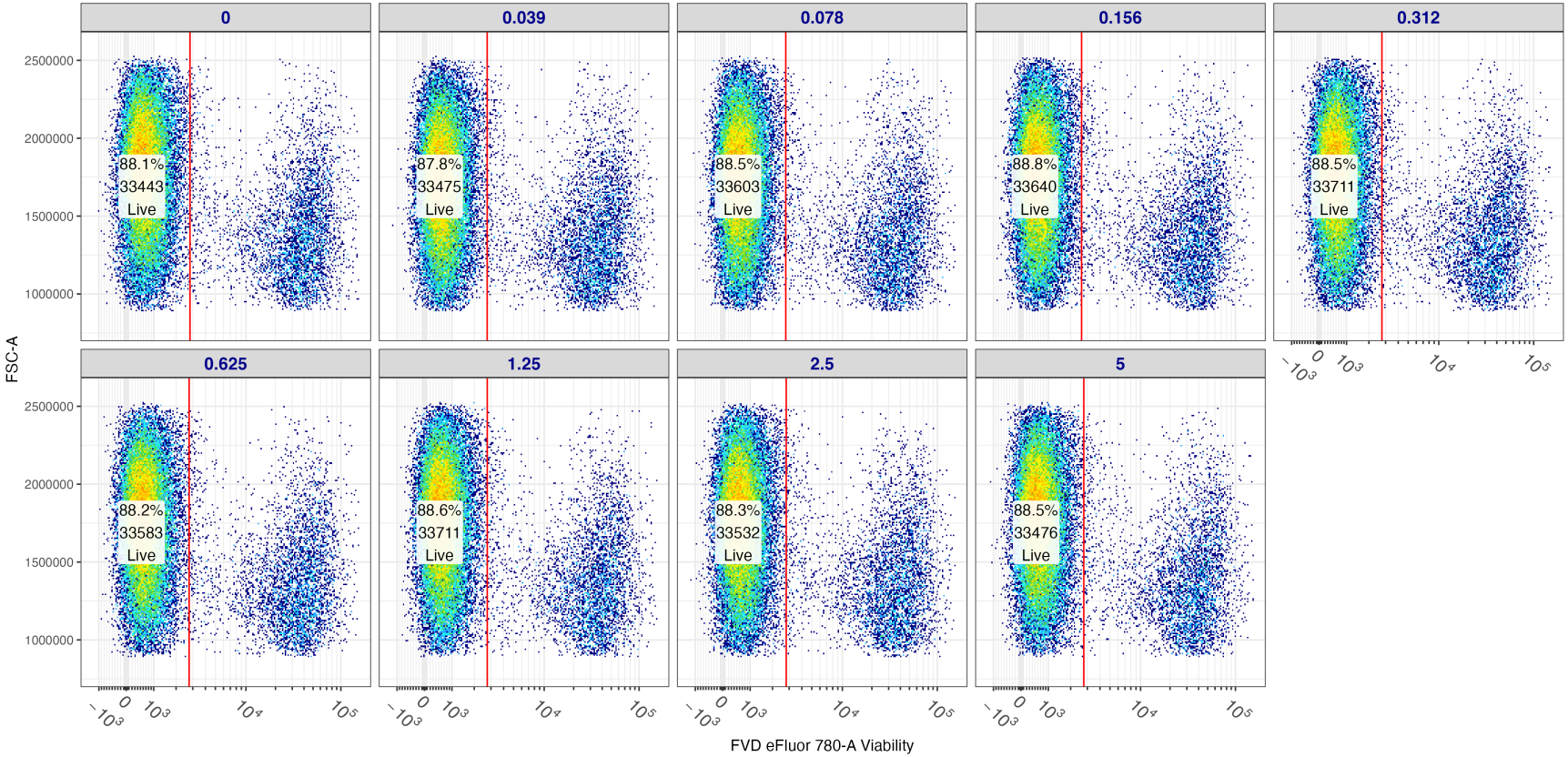
Parent pop: Cells | CD8 Alexa Fluor 532-A || Cat: 58-0088-42 || Thermo Fisher Scientific || Lot: 3052681 || Test sample: HD Human PBMC at 1e6/well



Gating method: singletGate || args: prediction_level=0.99, wider_gate=FALSE || collapse: FALSE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

CD8 Alexa Fluor 532-A : Live | EXP1402 | JD | Clone RPA-T8

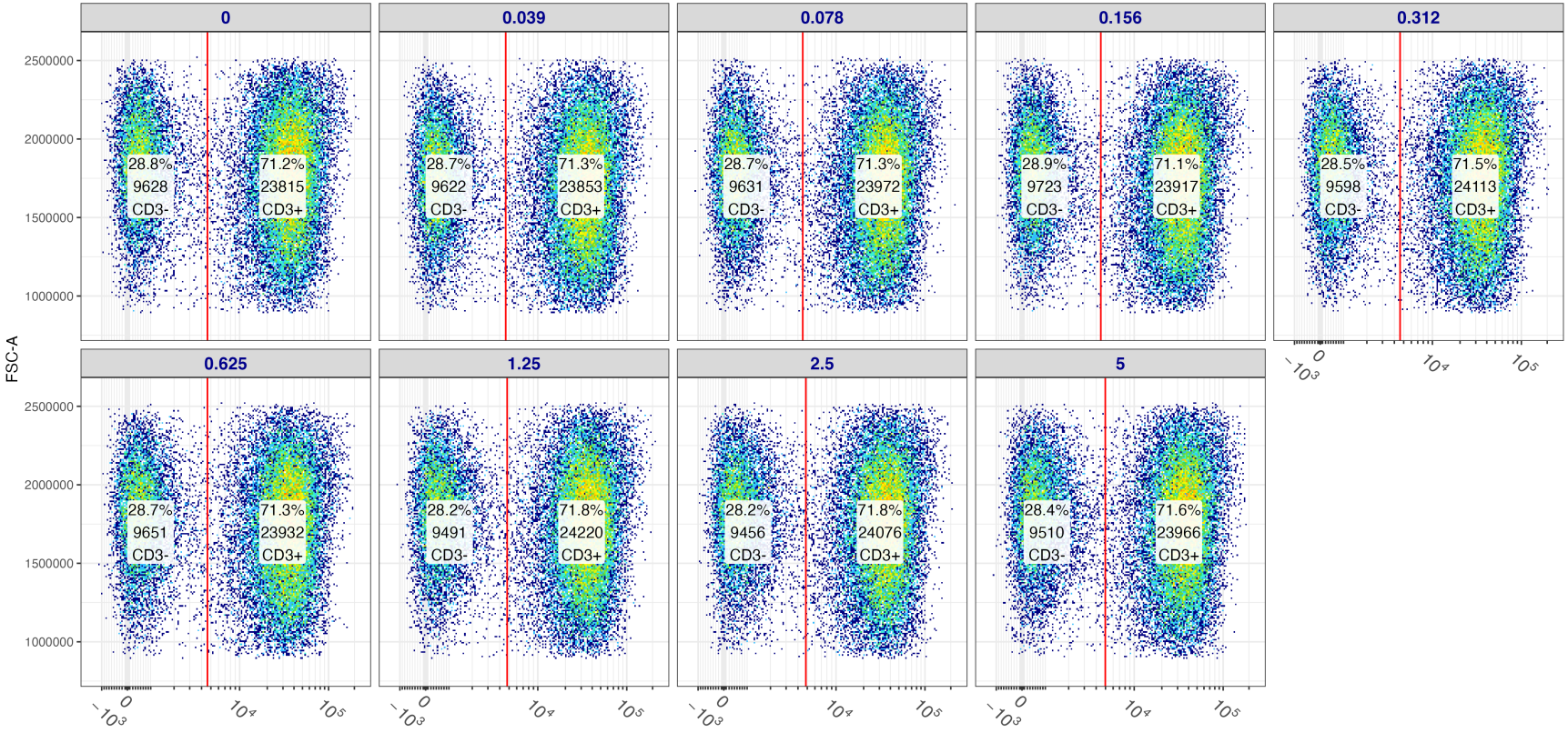
Parent pop: Single_cells | CD8 Alexa Fluor 532-A || Cat: 58-0088-42 || Thermo Fisher Scientific || Lot: 3052681 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_flowmeans || args: K=2, cluster='neg', quantile=0.99 || collapse: FALSE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

CD8 Alexa Fluor 532-A : CD3 | EXP1402 | JD | Clone RPA-T8

Parent pop: Live | CD8 Alexa Fluor 532-A || Cat: 58-0088-42 || Thermo Fisher Scientific || Lot: 3052681 || Test sample: HD Human PBMC at 1e6/well



Gating method: gate_flowmeans || args: K=2, cluster='neg', quantile=0.99 || collapse: FALSE || groupBy: Dirname || pp: — || pp args: —
Compensation: —

6 Info

Table 3: Info sheet: script-level settings of this run

Variable	Value	Source
General		
Template_Version	3.6.8	Info sheet
working_directory	(not set)	not set
compensation	(not set)	not set
miflowcyt_export (L)	FALSE	Info sheet
dashboard_dir	(not set)	not set
titration_export_dir	(not set)	not set
dashboard_tabbed_by	(not set)	not set
MM_R2_threshold	0.9	Info sheet
Downsample_to (N)	(not set)	not set
flowjo_transformation_arg	channelRange=9000, pos=4.5, neg=0, widthBasis=-10	Info sheet
keywords	(not set)	not set
TiterPubs		
titerpubs_export	TRUE	Info sheet
scrub_confidential	TRUE	Info sheet
Organization/Affiliation	(not set)	not set
Submitter_Name	(not set)	not set
Submitter_ORCID	(not set)	not set
Submitter_Email	<redacted-email>	Info sheet
Quality_Control_Measures	(not set)	not set
Collection_Software	(not set)	not set
Target_Species	Human	Info sheet
Cell_Type_Fallback	(not set)	not set
Sample_Details	(not set)	not set
Tissue_Fallback	(not set)	not set
References	https://doi.org/10.5281/zenodo.24181307	Info sheet
Protocol		
Incubation_Time	30	Info sheet
Incubation_Temperature_C	4	Info sheet
Equipment		
Equipment_Make	(not set)	not set
Equipment_Model	(not set)	not set
Provenance		
master_panel_inventory_path	Master_Panel_Inventory.xlsx	run folder
Protocol		
Preparation_Protocol	(not set)	not set
Protocol_reference	https://www.biolegend.com/en-us/protocols/intracellular-flow-cytometry-staining-protocol	Info sheet

Table 3: Info sheet: script-level settings of this run *(continued)*

Variable	Value	Source
TiterPubs		
scrub_eln_placeholder	EXP1402	Info sheet

7 Session Information

Script version: ACTA v3.6.11

Render timestamp: 2026-11-12 20:26:16 PST

Operator: JD

Layout workbook: 1402_Ab_Titration_Instructions.xlsx

```
- Session info -------------------------------------------------------------
 setting  value
 version  R version 4.5.1 (2025-06-13)
 os       macOS Tahoe 26.7.2
 system   aarch64, darwin20
 ui       X11
 language (EN)
 collate  C.UTF-8
 ctype    C.UTF-8
 tz       America/Los_Angeles
 date     2026-11-12
 pandoc   3.9.0.2 @ /opt/homebrew/bin/ (via rmarkdown)
 quarto   NA

- Packages -----------------------------------------------------------------
 package       * version   date (UTC) lib source
 ACTA          * 3.6.11    2026-11-13 [1] Bioconductor
 askpass         1.2.1     2024-10-04 [2] CRAN (R 4.5.0)
 BH            * 1.90.0-1  2025-12-14 [2] CRAN (R 4.5.2)
 Biobase         2.70.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
 BiocGenerics    0.56.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
 BiocManager   * 1.30.27   2025-11-14 [2] CRAN (R 4.5.2)
 bslib           0.11.0    2026-05-16 [2] CRAN (R 4.5.2)
 cachem          1.1.0     2024-05-16 [2] CRAN (R 4.5.0)
 cellranger      1.1.0     2016-07-27 [2] CRAN (R 4.5.0)
 cli             3.6.6     2026-04-09 [2] CRAN (R 4.5.2)
 cytolib         2.22.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
 data.table      1.18.4    2026-05-06 [2] CRAN (R 4.5.2)
 DEoptimR        1.2-0     2026-06-07 [2] CRAN (R 4.5.2)
 digest          0.6.39    2025-11-19 [2] CRAN (R 4.5.2)
```

```
diptest       * 0.77-2    2025-08-20 [2] CRAN (R 4.5.0)
dplyr         * 1.2.1     2026-04-03 [2] CRAN (R 4.5.2)
evaluate        1.0.5     2025-08-27 [2] CRAN (R 4.5.0)
farver          2.1.2     2024-05-13 [2] CRAN (R 4.5.0)
fastmap         1.2.0     2024-05-15 [2] CRAN (R 4.5.0)
feature         1.2.16    2026-05-11 [2] CRAN (R 4.5.2)
flowClust       3.48.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
flowCore      * 2.22.1    2026-01-01 [2] https://bioc-release.r-universe.dev (R 4.5.2)
flowMeans     * 1.70.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
flowWorkspace * 4.22.1    2026-01-01 [2] https://bioc-release.r-universe.dev (R 4.5.2)
generics        0.1.4     2025-05-09 [2] CRAN (R 4.5.0)
ggcyto        * 1.39.1    2026-02-25 [2] Github (RGLab/ggcyto@e5ed286)
ggplate       * 0.4.0     2026-09-01 [2] CRAN (R 4.5.2)
ggplot2       * 4.0.3     2026-04-22 [2] CRAN (R 4.5.2)
glue            1.8.1     2026-04-17 [2] CRAN (R 4.5.2)
graph           1.88.1    2025-12-08 [2] Bioconductor 3.22 (R 4.5.2)
gridExtra       2.3       2017-09-09 [2] CRAN (R 4.5.0)
gtable          0.3.6     2024-10-25 [2] CRAN (R 4.5.0)
hexbin          1.28.5    2024-11-13 [2] CRAN (R 4.5.0)
htmltools       0.5.9     2025-12-04 [2] CRAN (R 4.5.2)
jquerylib       0.1.4     2021-04-26 [2] CRAN (R 4.5.0)
jsonlite        2.0.0     2025-03-27 [2] CRAN (R 4.5.0)
kableExtra    * 1.4.0     2024-01-24 [2] CRAN (R 4.5.0)
KernSmooth      2.23-26   2025-01-01 [2] CRAN (R 4.5.1)
knitr         * 1.51      2025-12-20 [2] CRAN (R 4.5.2)
ks              1.15.2    2026-05-09 [2] CRAN (R 4.5.2)
labeling        0.4.3     2023-08-29 [2] CRAN (R 4.5.0)
lattice         0.22-9    2026-02-09 [2] CRAN (R 4.5.2)
lifecycle       1.0.5     2026-01-08 [2] CRAN (R 4.5.2)
lubridate     * 1.9.5     2026-02-04 [2] CRAN (R 4.5.2)
magrittr        2.0.5     2026-04-04 [2] CRAN (R 4.5.2)
Matrix          1.7-5     2026-03-21 [2] CRAN (R 4.5.2)
matrixStats     1.5.0     2025-01-07 [2] CRAN (R 4.5.0)
mclust        * 6.1.2     2025-10-31 [2] CRAN (R 4.5.0)
mvtnorm         1.4-1     2026-06-06 [2] CRAN (R 4.5.2)
ncdfFlow      * 2.56.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
openCyto      * 2.22.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
openxlsx      * 4.2.8.1   2025-10-31 [2] CRAN (R 4.5.0)
otel            0.2.0     2025-08-29 [2] CRAN (R 4.5.0)
```

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pcaPP           2.0-5     2024-08-19 [2] CRAN (R 4.5.0)
pdftools        3.9.0     2026-05-14 [2] CRAN (R 4.5.2)
pillar          1.11.1    2025-09-17 [2] CRAN (R 4.5.0)
pkgconfig       2.0.3     2019-09-22 [2] CRAN (R 4.5.0)
plyr            1.8.9     2023-10-02 [2] CRAN (R 4.5.0)
pracma          2.4.6     2025-10-22 [2] CRAN (R 4.5.0)
purrr           1.2.2     2026-04-10 [2] CRAN (R 4.5.2)
qpdf            1.4.1     2025-07-02 [2] CRAN (R 4.5.0)
R6              2.6.1     2025-02-15 [2] CRAN (R 4.5.0)
ragg          * 1.5.2     2026-03-23 [2] CRAN (R 4.5.2)
RBGL            1.86.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
RColorBrewer    1.1-3     2022-04-03 [2] CRAN (R 4.5.0)
Rcpp            1.1.1-1.1 2026-04-24 [2] CRAN (R 4.5.2)
readxl        * 1.5.0     2026-05-16 [2] CRAN (R 4.5.2)
Rgraphviz       2.54.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
rlang           1.2.0     2026-04-06 [2] CRAN (R 4.5.2)
rmarkdown       2.31      2026-03-26 [2] CRAN (R 4.5.2)
robustbase      0.99-7    2026-02-05 [2] CRAN (R 4.5.2)
RProtoBufLib    2.22.0    2025-10-29 [2] Bioconductor 3.22 (R 4.5.1)
rrcov           1.7-7     2025-04-21 [2] CRAN (R 4.5.0)
rstudioapi    * 0.19.0    2026-06-11 [2] CRAN (R 4.5.2)
S4Vectors       0.48.1    2026-04-04 [2] https://bioc-release.r-universe.dev (R 4.5.3)
S7              0.2.2     2026-04-22 [2] CRAN (R 4.5.2)
sass            0.4.10    2025-04-11 [2] CRAN (R 4.5.0)
scales          1.4.0     2025-04-24 [2] CRAN (R 4.5.0)
sessioninfo   * 1.2.4     2026-06-04 [2] CRAN (R 4.5.2)
stringi         1.8.7     2025-03-27 [2] CRAN (R 4.5.0)
stringr       * 1.6.0     2025-11-04 [2] CRAN (R 4.5.0)
svglite         2.2.2     2025-10-21 [2] CRAN (R 4.5.0)
systemfonts     1.3.2     2026-03-05 [2] CRAN (R 4.5.2)
textshaping     1.0.5     2026-03-06 [2] CRAN (R 4.5.2)
this.path     * 2.8.0     2026-04-10 [2] CRAN (R 4.5.2)
tibble          3.3.1     2026-01-11 [2] CRAN (R 4.5.2)
tidyr         * 1.3.2     2025-12-19 [2] CRAN (R 4.5.2)
tidyselect      1.2.1     2024-03-11 [2] CRAN (R 4.5.0)
timechange      0.4.0     2026-01-29 [2] CRAN (R 4.5.2)
vctrs           0.7.3     2026-04-11 [2] CRAN (R 4.5.2)
viridisLite     0.4.3     2026-02-04 [2] CRAN (R 4.5.2)
withr           3.0.2     2024-10-28 [2] CRAN (R 4.5.0)
```

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 xfun            0.58      2026-06-01 [2] CRAN (R 4.5.2)
 XML             3.99-0.23 2026-03-20 [2] CRAN (R 4.5.2)
 xml2            1.5.2     2026-01-17 [2] CRAN (R 4.5.2)
 yaml            2.3.12    2025-12-10 [2] CRAN (R 4.5.2)
 zip             3.0.0     2026-06-10 [2] CRAN (R 4.5.2)

 [1] ~/ACTA_release/lib3611
 [2] /Library/Frameworks/R.framework/Versions/4.5-arm64/Resources/library
 * -- Packages attached to the search path.

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```