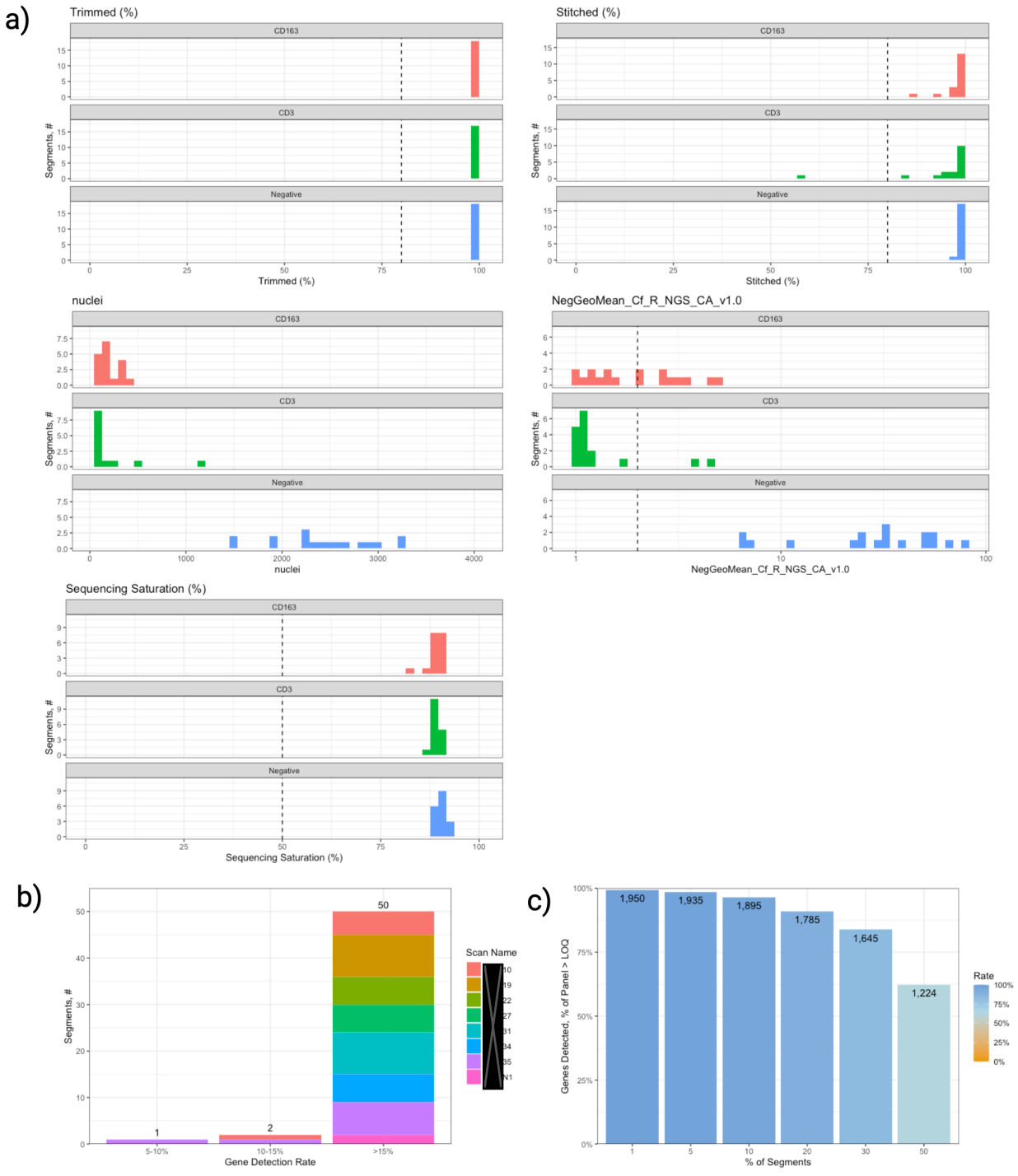
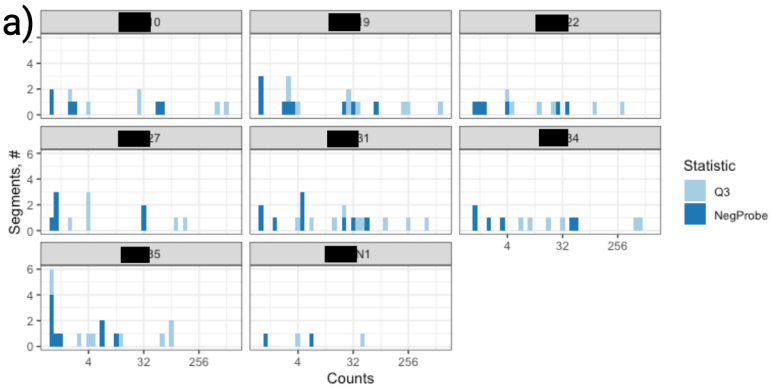
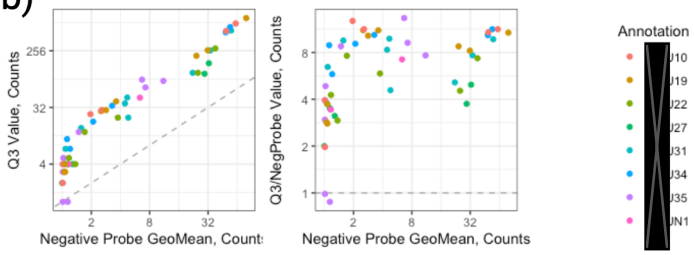

a)
Trimmed (%)
Stitched (%)
nuclei
NegGeoMean_Cf_R_NGS_CA_v1.0
Sequencing Saturation (%)
CD163
CD3
Negative
Segments, #
Trimmed (%)
Stitched (%)
nuclei
NegGeoMean_Cf_R_NGS_CA_v1.0
Sequencing Saturation (%)
b)
Segments, #
Gene Detection Rate
5-10%
10-15%
>15%
Scan Name
c)
Genes Detected, % of Panel > LOQ
% of Segments
1,950
1,935
1,895
1,785
1,645
1,224
Rate

Supplemental Figure 1: Canine GeoMx Quality Control Parameters
a) Distributions of various quality control measures including percentages of trimmed, stitched, nuclei, sequencing saturation, and negative counts. b) Distribution of segments with respect to gene detected rate c) Total number of genes detected in different percentages of segments

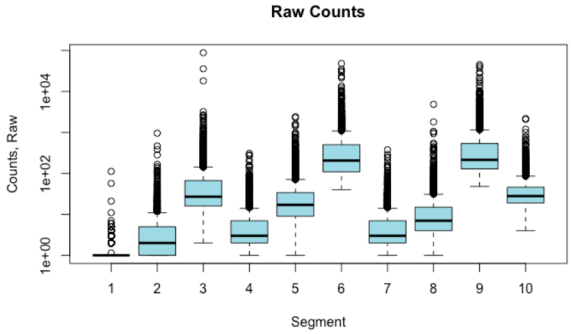
a)


Segments, #
Counts
Statistic
Q3
NegProbe

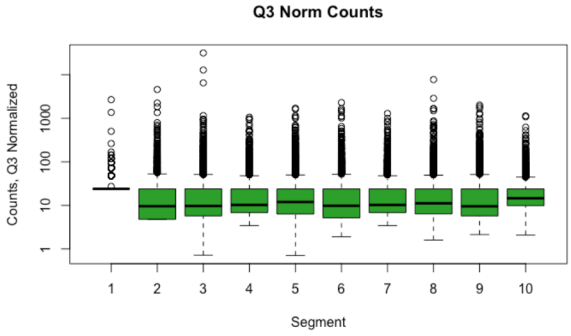
b)


Q3 Value, Counts
Negative Probe GeoMean, Count
Q3/NegProbe Value, Counts
Negative Probe GeoMean, Counts
Annotation

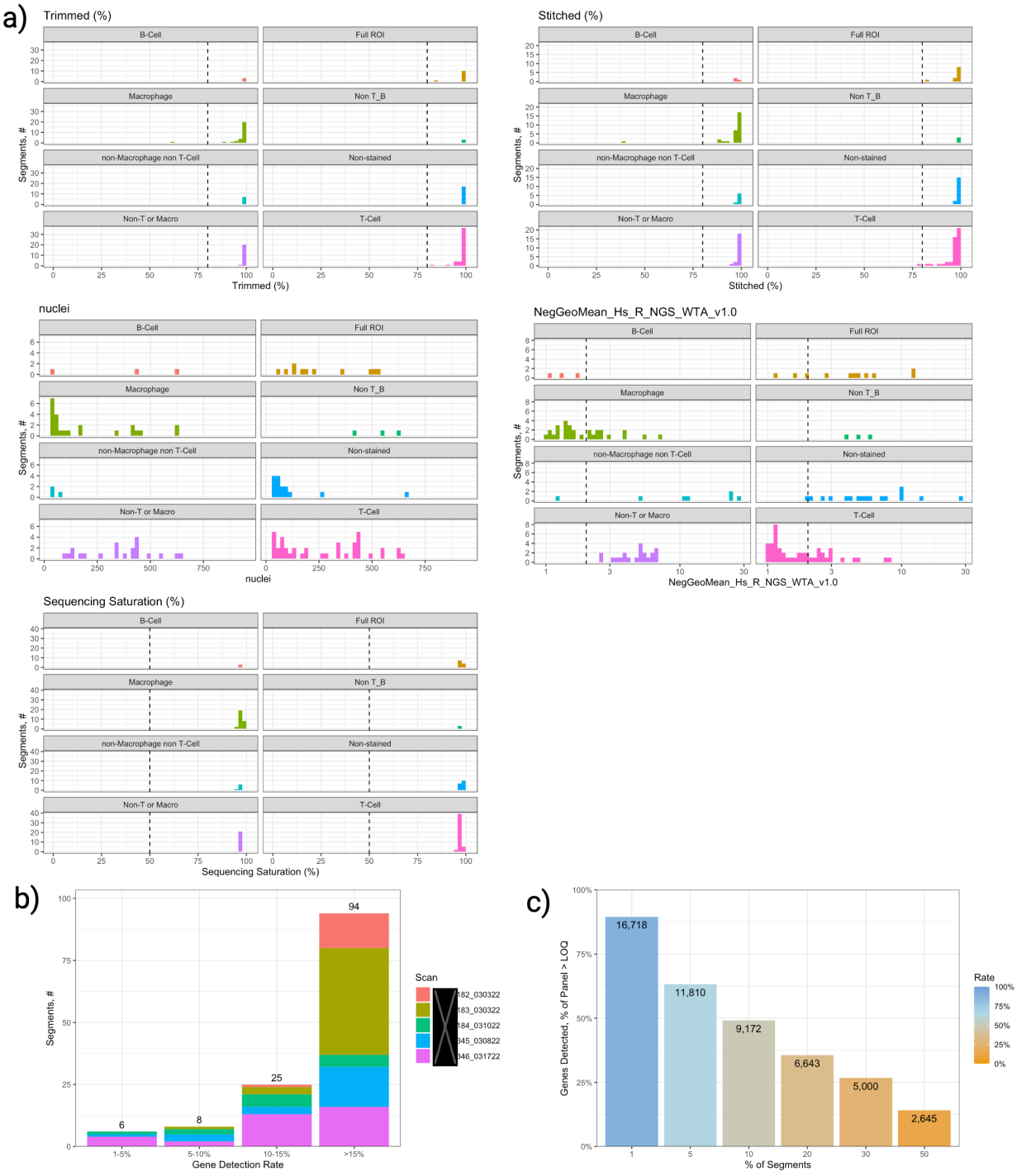
c)


Raw Counts
Counts, Raw
Segment

d)

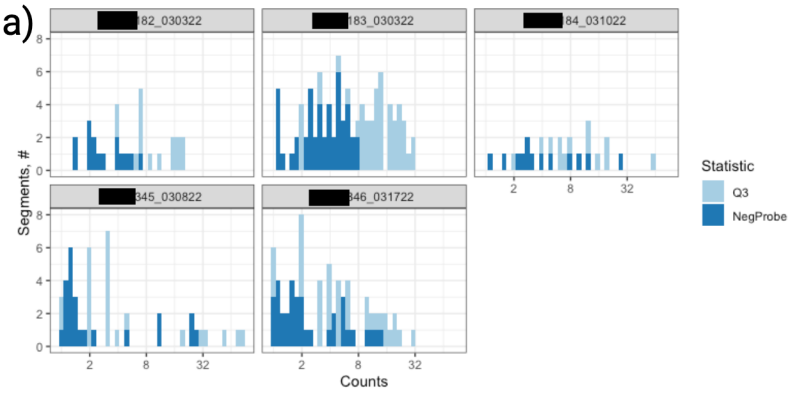

Q3 Norm Counts
Counts, Q3 Normalized
Segment

Supplemental Figure 2: Canine GeoMx Q3 Normalization
a) Relationship between upper quartile (Q3) of the counts in each segment with the geometric mean of the negative control probes at the distribution level b) Relationship between Q3 of the counts in each segment with the geometric mean of the negative control probes at the per segment level c) Representative individual segment count data before normalization d) Representative individual segment count data after Q3 normalization

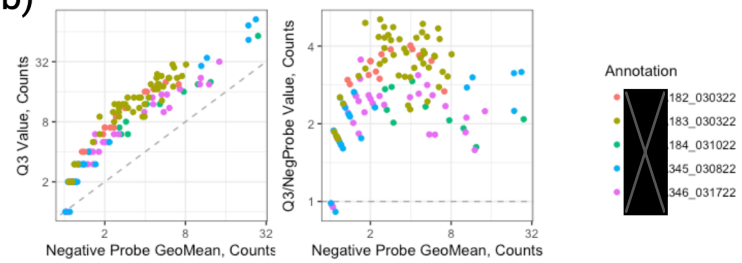

a)
Trimmed (%)
Stitched (%)
nuclei
NegGeoMean_Hs_R_NGS_WTA_v1.0
Sequencing Saturation (%)
B-Cell
Full ROI
Macrophage
Non T_B
non-Macrophage non T-Cell
Non-stained
Non-T or Macro
T-Cell
Segments, #
Trimmed (%)
Stitched (%)
nuclei
NegGeoMean_Hs_R_NGS_WTA_v1.0
Sequencing Saturation (%)
b)
Segments, #
Gene Detection Rate
1-5%
5-10%
10-15%
>15%
6
8
25
94
Scan
182_030322
183_030322
184_031022
345_030822
346_031722
c)
Genes Detected, % of Panel > LOQ
% of Segments
16,718
11,810
9,172
6,643
5,000
2,645
Rate
100%
75%
50%
25%
0%

Supplemental Figure 3: Human GeoMx Quality Control Parameters
a) Distributions of various quality control measures including percentages of trimmed, stitched, nuclei, sequencing saturation, and negative counts. b) Distribution of segments with respect to gene detected rate c) Total number of genes detected in different percentages of segments

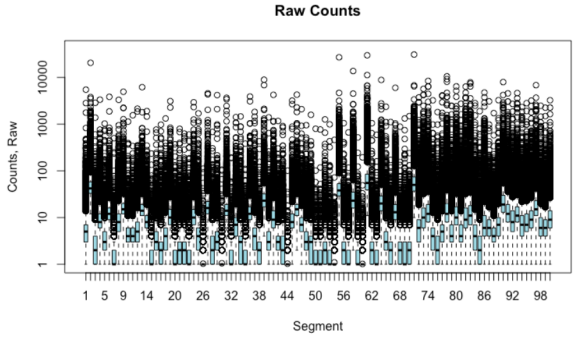
a)


[redacted]82_030322
[redacted]83_030322
[redacted]84_031022
[redacted]345_030822
[redacted]346_031722
Segments, #
Counts
Statistic
Q3
NegProbe

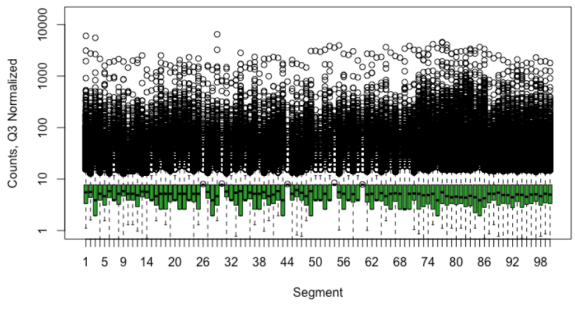
b)


Q3 Value, Counts
Negative Probe GeoMean, Counts
Q3/NegProbe Value, Counts
Negative Probe GeoMean, Counts
Annotation
182_030322
183_030322
184_031022
345_030822
346_031722

c)

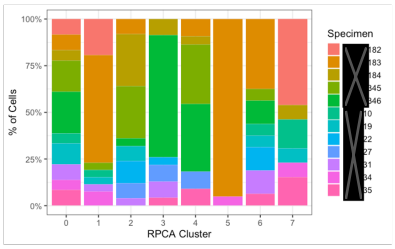

Raw Counts
Counts, Raw
Segment

d)

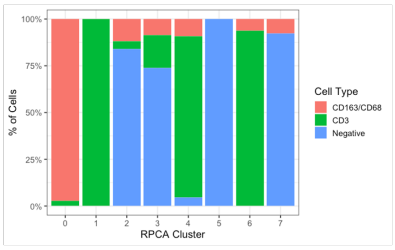

Q3 Norm Counts
Counts, Q3 Normalized
Segment

Supplemental Figure 4: Human GeoMx Q3 Normalization
a) Relationship between upper quartile (Q3) of the counts in each segment with the geometric mean of the negative control probes at the distribution level b) Relationship between Q3 of the counts in each segment with the geometric mean of the negative control probes at the per segment level c) Representative individual segment count data before normalization d) Representative individual segment count data after Q3 normalization

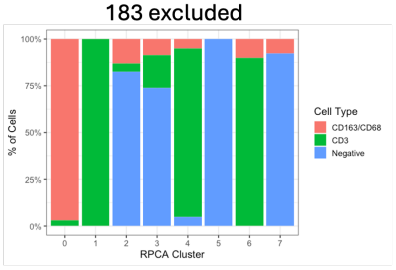
A



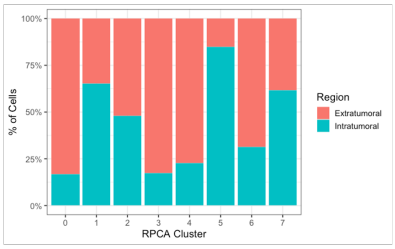
B



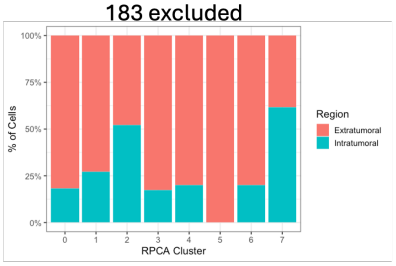
183 excluded



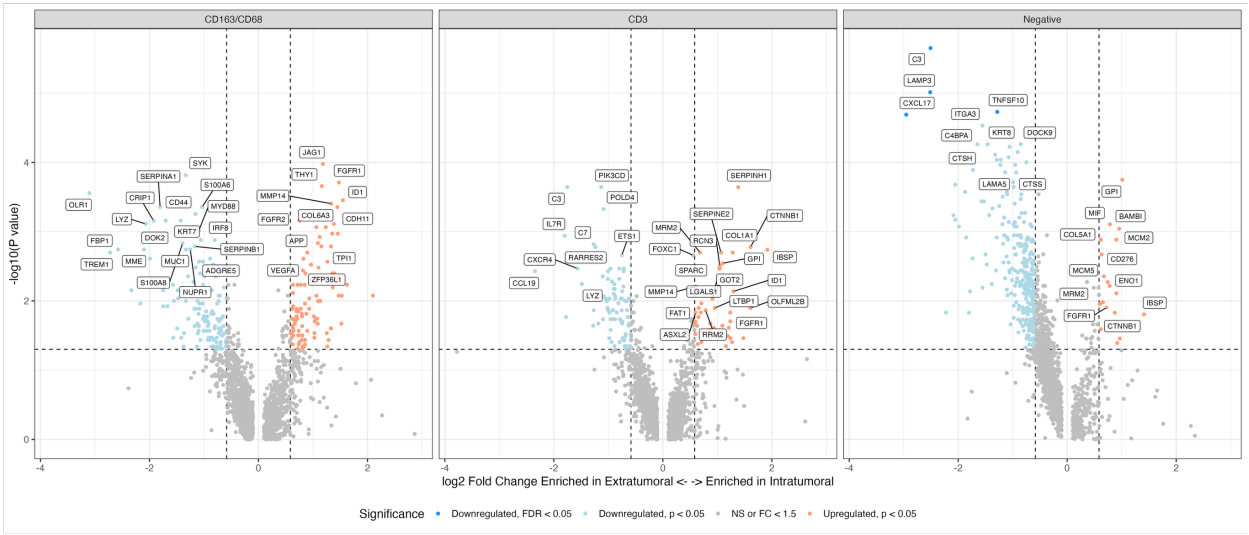
C



183 excluded



D



Supplemental Figure 5: Exclusion of Fibroblastic OS Subtype (183)
a) RPCA cluster distribution by specimen contribution demonstrates majority of cells in cluster 1 are derived from specimen 183 b) RPCA cluster distribution by cell type with all specimens (left) vs. without 183 (right) maintains predominant CD3 T cell identity c) RPCA cluster distribution by region with all specimens (left) vs. without 183 (right) demonstrates a drop in intratumoral T cells in cluster 1 which is comparable to other T cell clusters (4 and 6) d) Volcano plots of significant DEGs without 183 maintain majority of the same DEGs in CD3 T cells