Flow Cytometry Comparison

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Flow Cytometry Comparison with Nanostring Results

Goal: Are Nanostring and Flow results comparable?

Problem Setup:

- Nanostring profiles all RNA from all cells
- Flow Cytometry data profiles protein at single cell level based on percent compositions at different cell type proportions

Sample Characteristics: - Select for samples that have same phenotype characteristics between both flow and nanostring (As it is not the exact same samples) - Flow Cytometry sample organizes by Sample and fraction

Analysis setup: - compare based on protein:rna comparison (pearson correlation between nanostring and flow) - compare based on cell type calling (pearson correlation between the cell type nanostring mean values and flow calls) - focus on cd45 cells (pearson correlation between nanostring mean values and flow calls)

Nanostring Sample Setup

Table 1: Nanostring Fractions

Fraction	n
IFNy	1
IgG	1
S1	4
S2	3
S3	2
NA	1

Table 2: Flow Fractions

fraction	n
S1	25
S2	4
S3	26

Cell Types Present for Flow Cytometry and Nanostring

Table 3: Nanostring Cell Types

x
monocytes
T.cells
CD4..T.cells
CD8..T.cells
B.cells
CD56.CD16. cytotoxic.NK
DCs

Table 4: Flow Cell Types

Cell.Type	n
	712
B-cells	4
CD45	1
CD8 T cells	2
Cytotoxic cells	8
DC	4
Exhausted CD8	4
Macrophages	2
Mast cells	2
Neutrophils	3
NK CD56dim cells	3
NK cells	2
T-cells	5
Th1 cells	1
Treg	1

What markers are shared between Flow and Nanostring

- Flow Marker Labels do not all match Nanostring labels
- Double Checked each marker for surrogates
- not present: EPCAM, CD16, CD3, CD16
- substitutes: PTPRC = CD45 ITGAX = CD11c CD56 = NCAM1 CD8 = CD8A and CD8B PDCD1 = PD1 CD274 = PD-L1

Markers Found in Both Flow and Nanostring
CD14
CD4
CD19
PTPRC
CD8A
CD8B
NCAM1

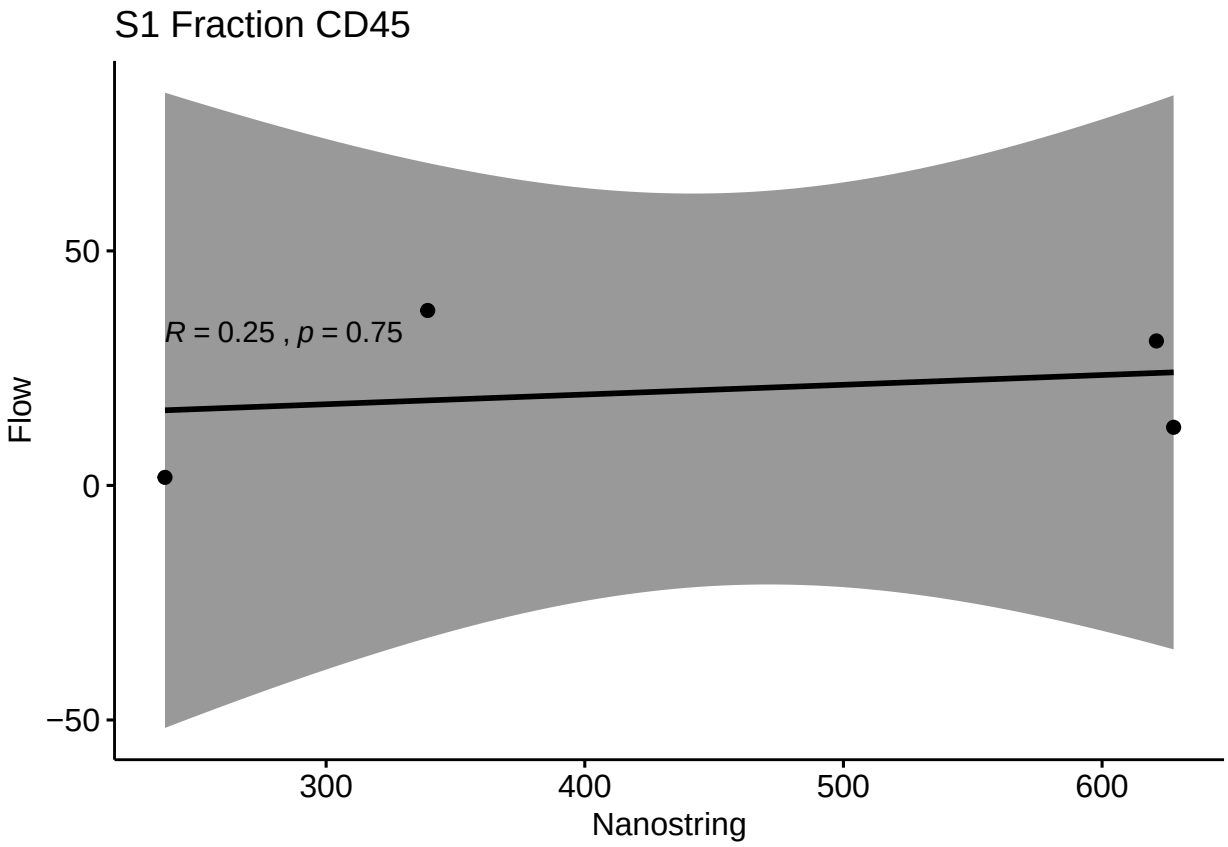
Markers Found in Both Flow and Nanostring
ITGAX
PDCD1
CD274

Correlation Between Nanostring & Flow single marker

- S1 and S3 with all or alive only cells
- Difficulty rests in how flow quantifies cells
- % of CD45+ is not comparable to CD45 RNA expression
  - Need the raw Flow values for the total protein fluorescent could be a better comparison
  - Alternate ways to normalize both Flow and Nanostring data
- Nanostring is unable to filter out dead cells
- Flow results though will give indication if there is a correlation and possibly where a threshold could be
- Current Results: multiple targets = average Nanostring values
  - if negative for flow then did not include nanostring values
  - If targets not available, skipped

S1 All Cells

```
## `geom_smooth()` using formula 'y ~ x'
```

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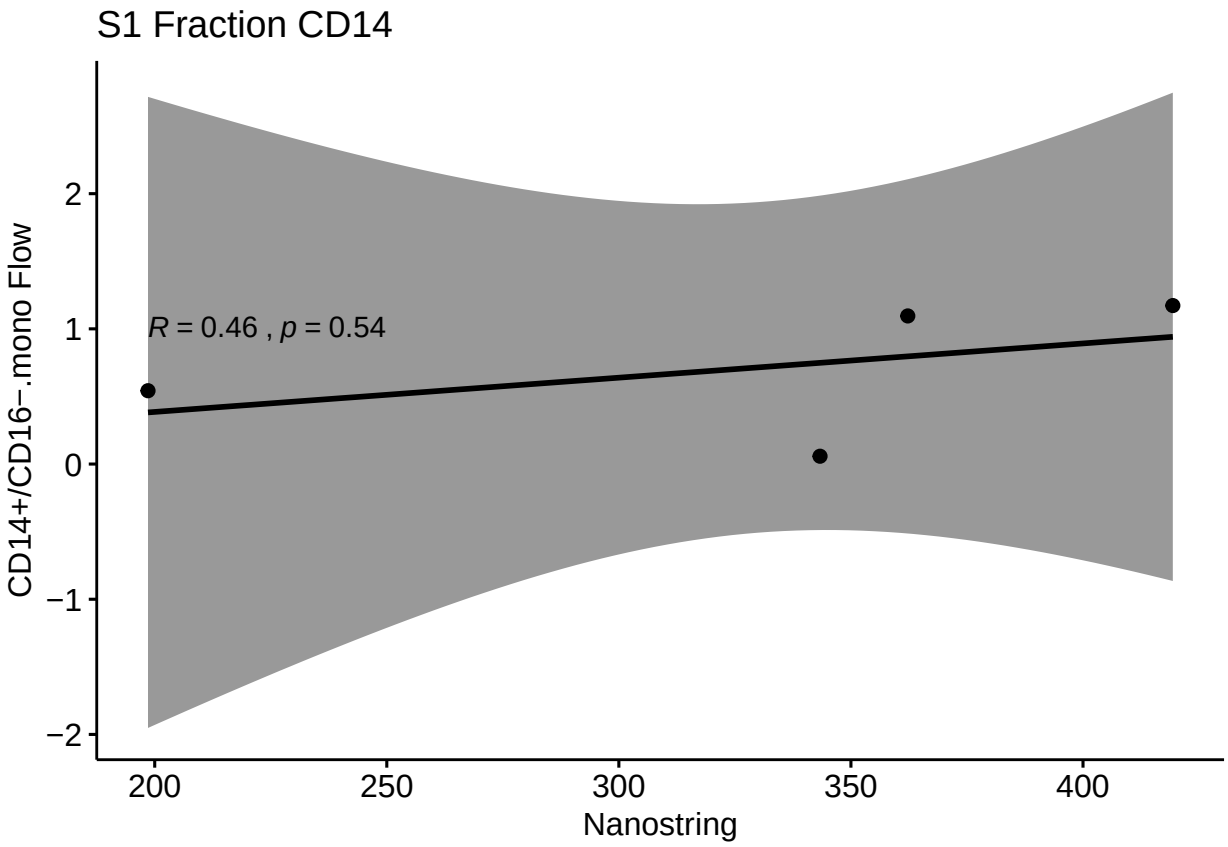


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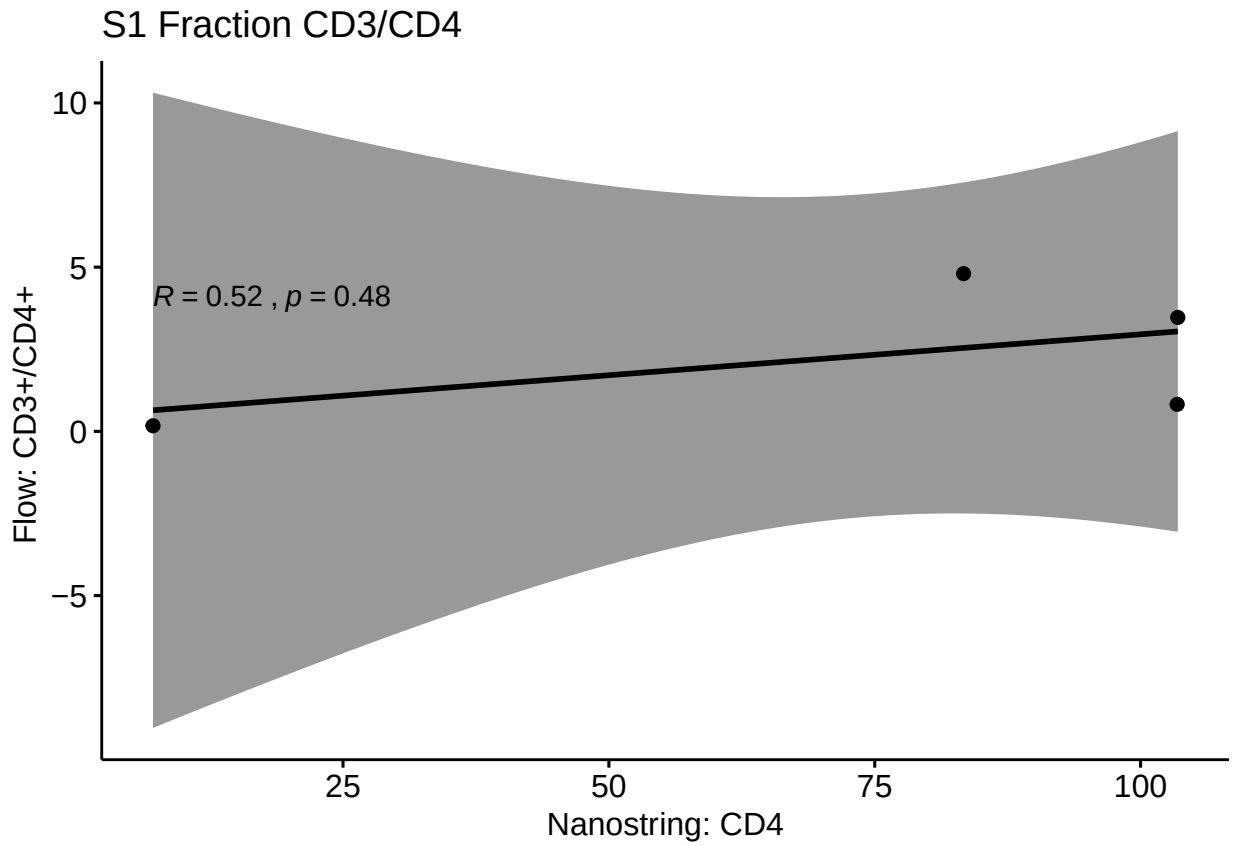


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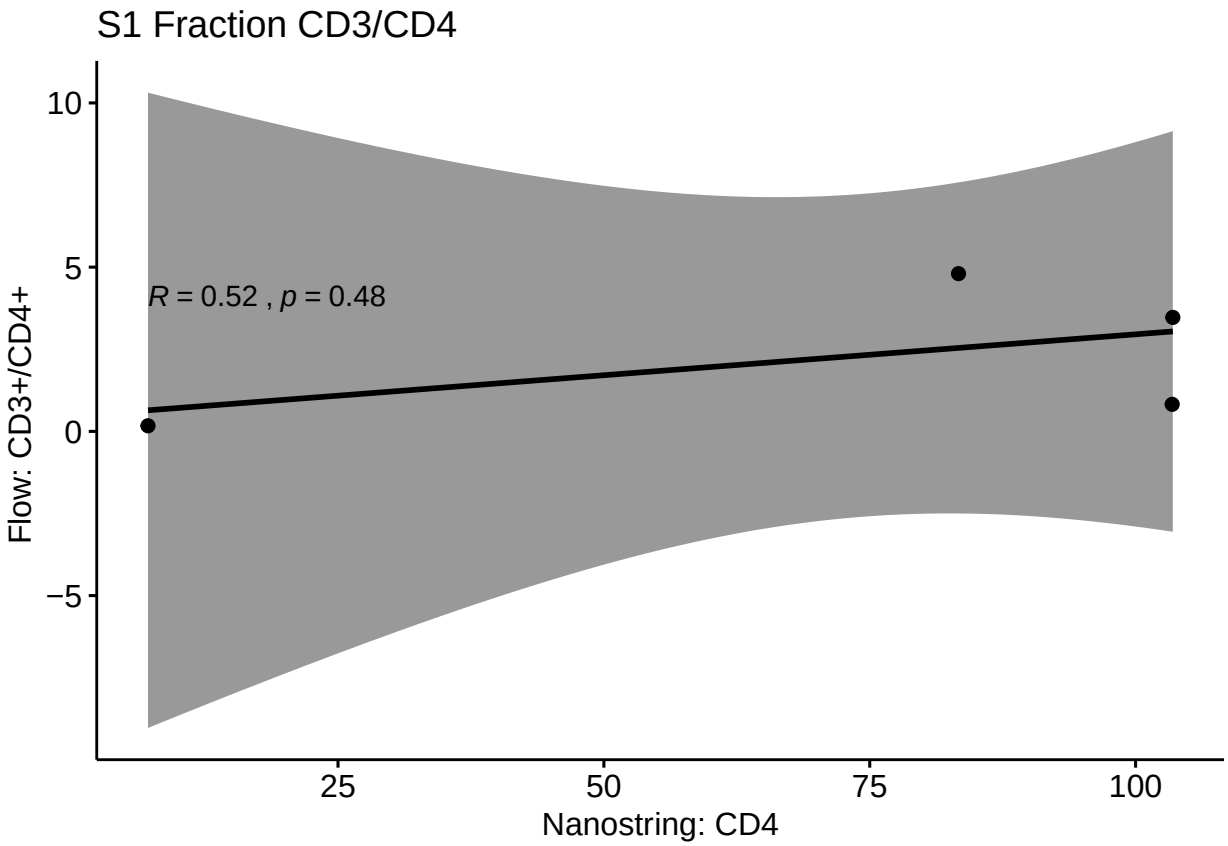

S1 Excluding Dead Cells

```
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```

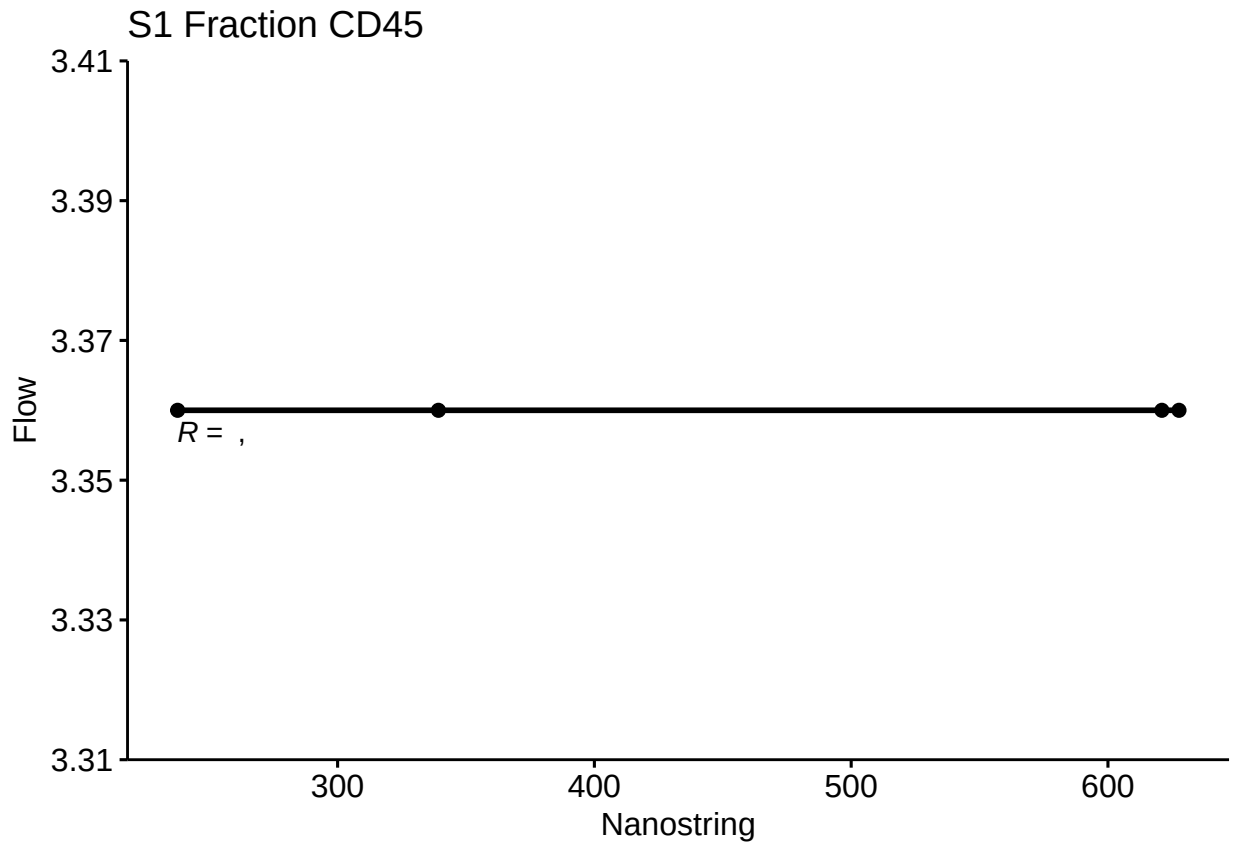


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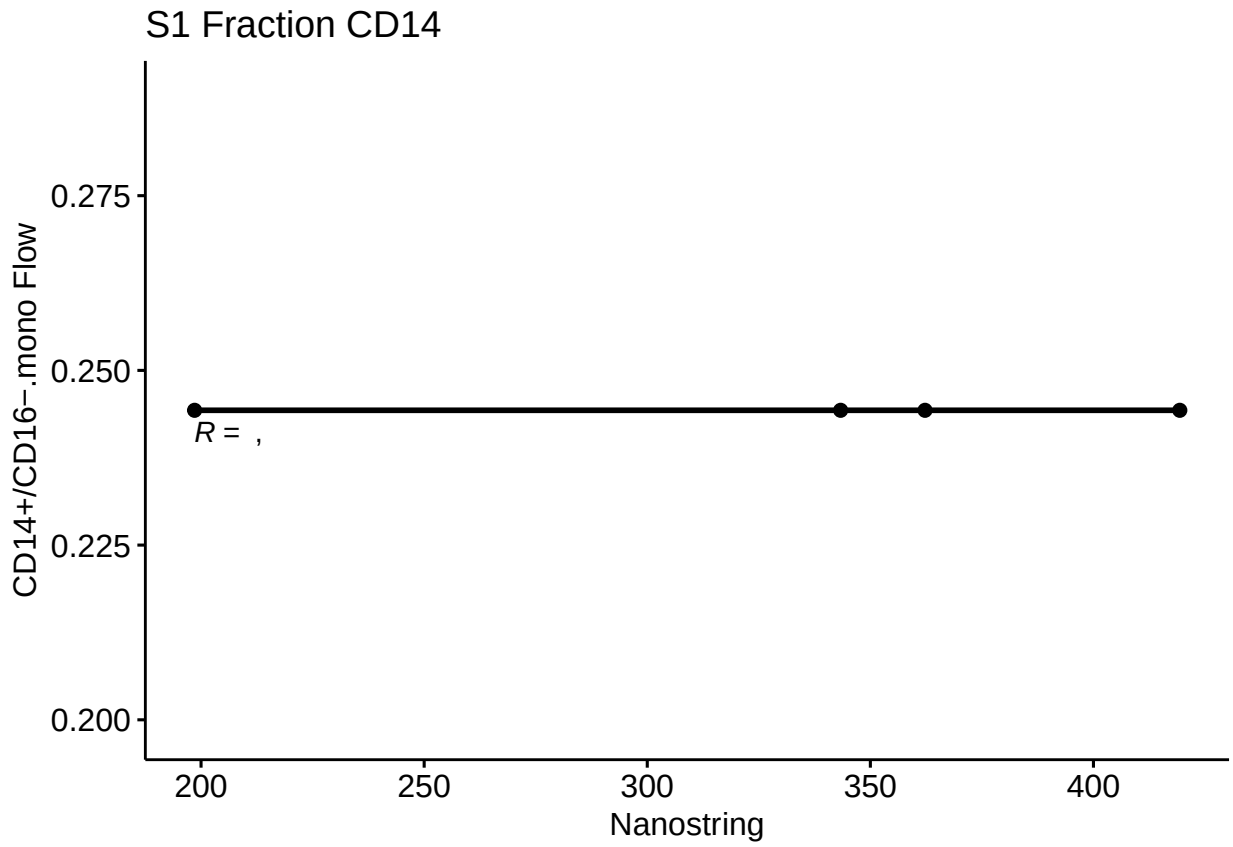


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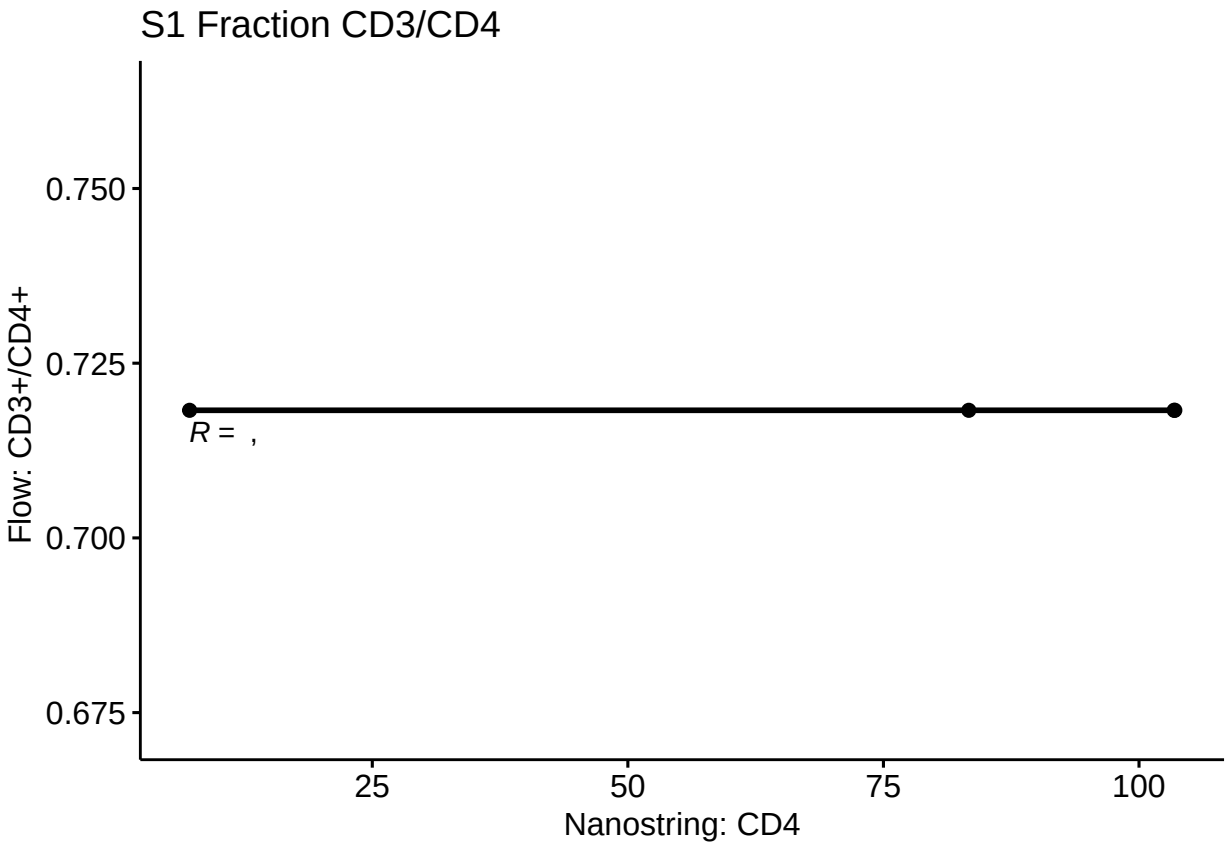


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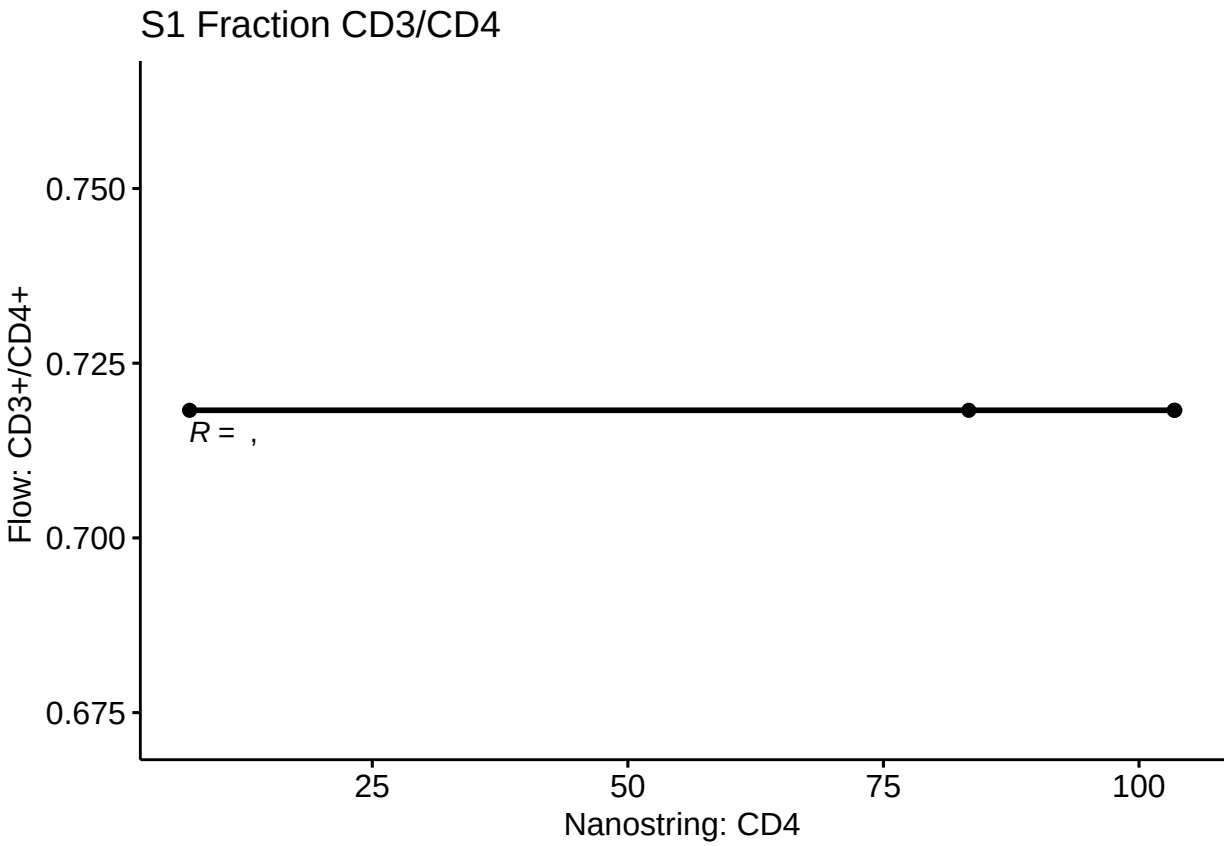

S3 all cells

```
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```

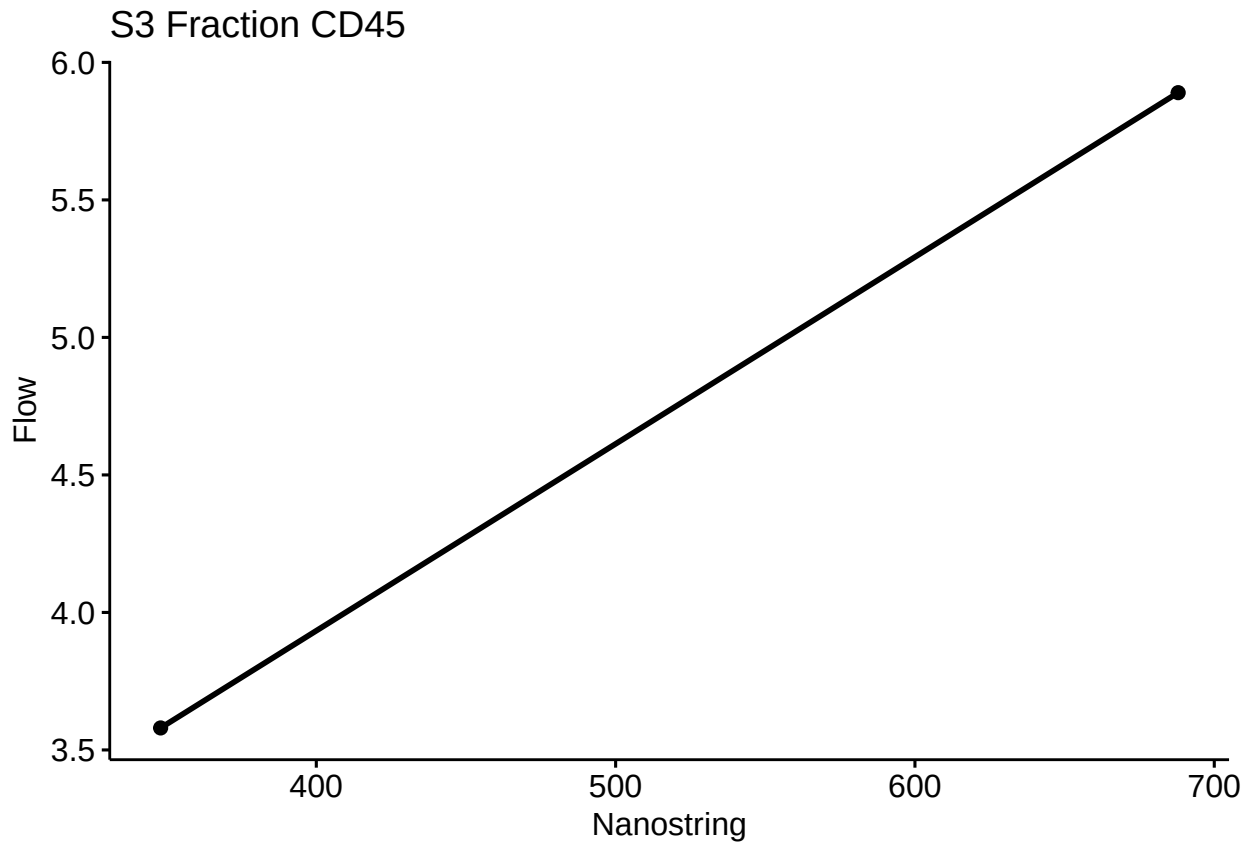


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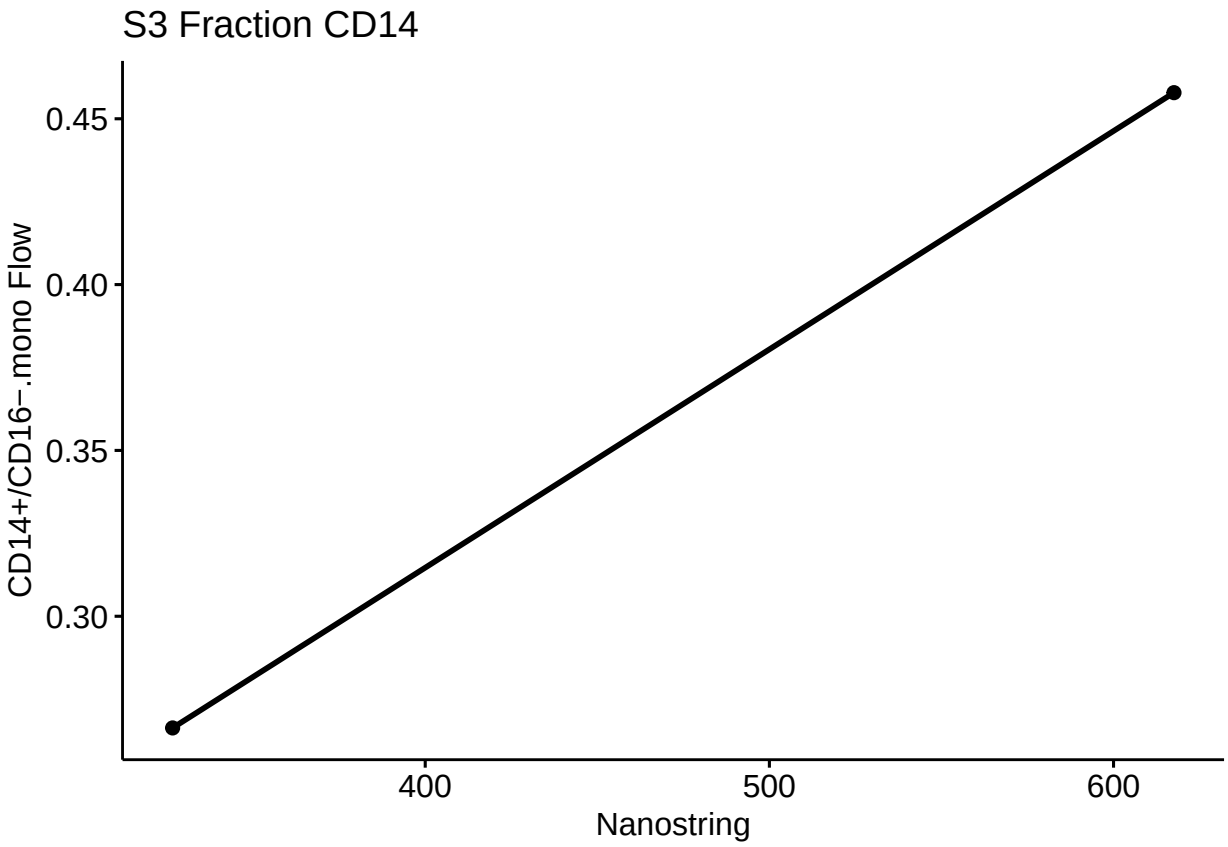


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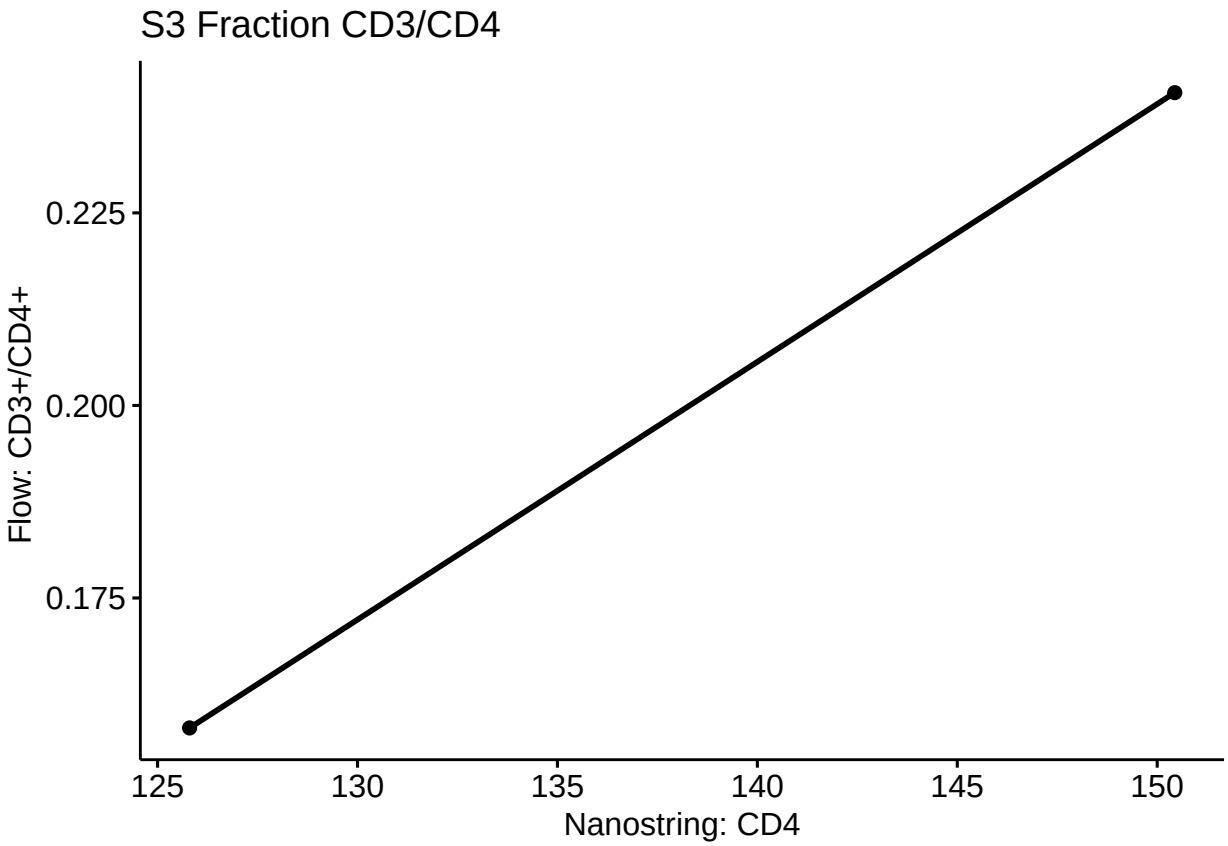


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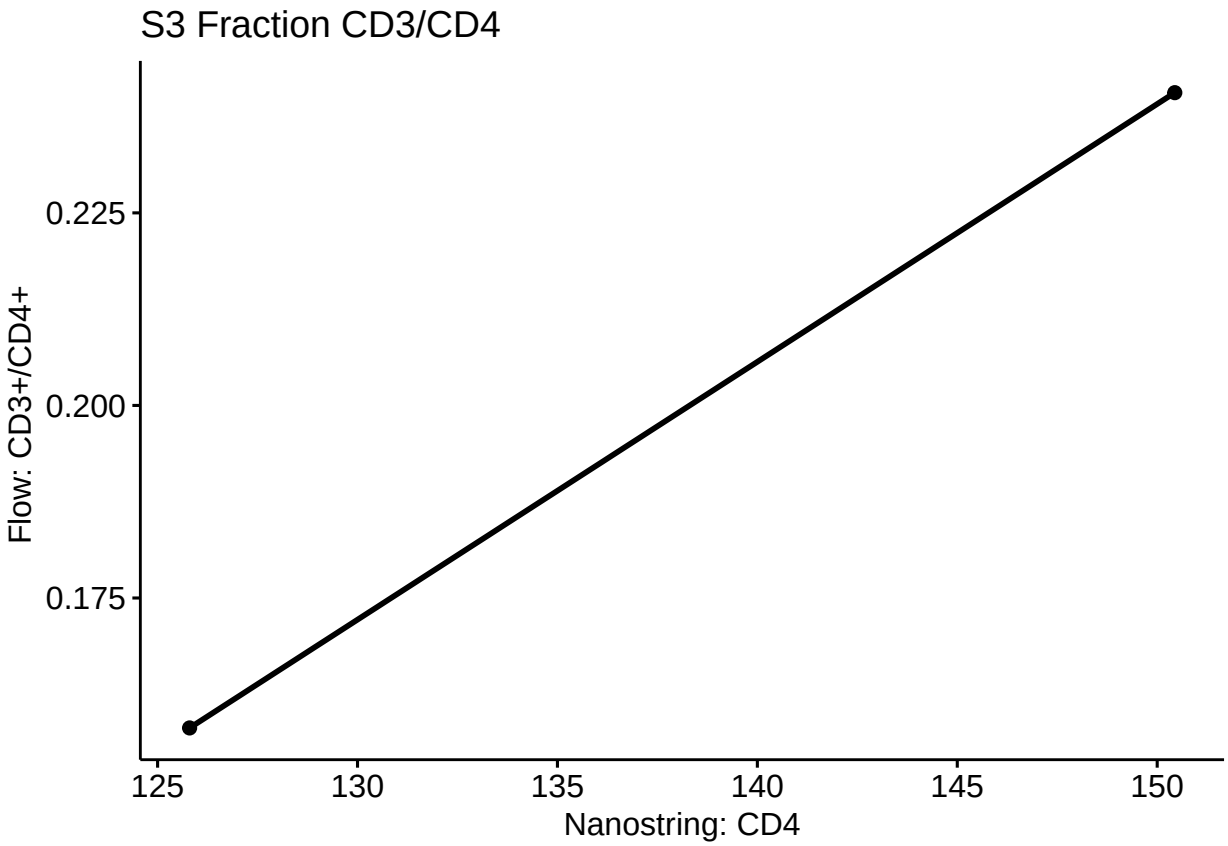

S3 Excluding Dead cells

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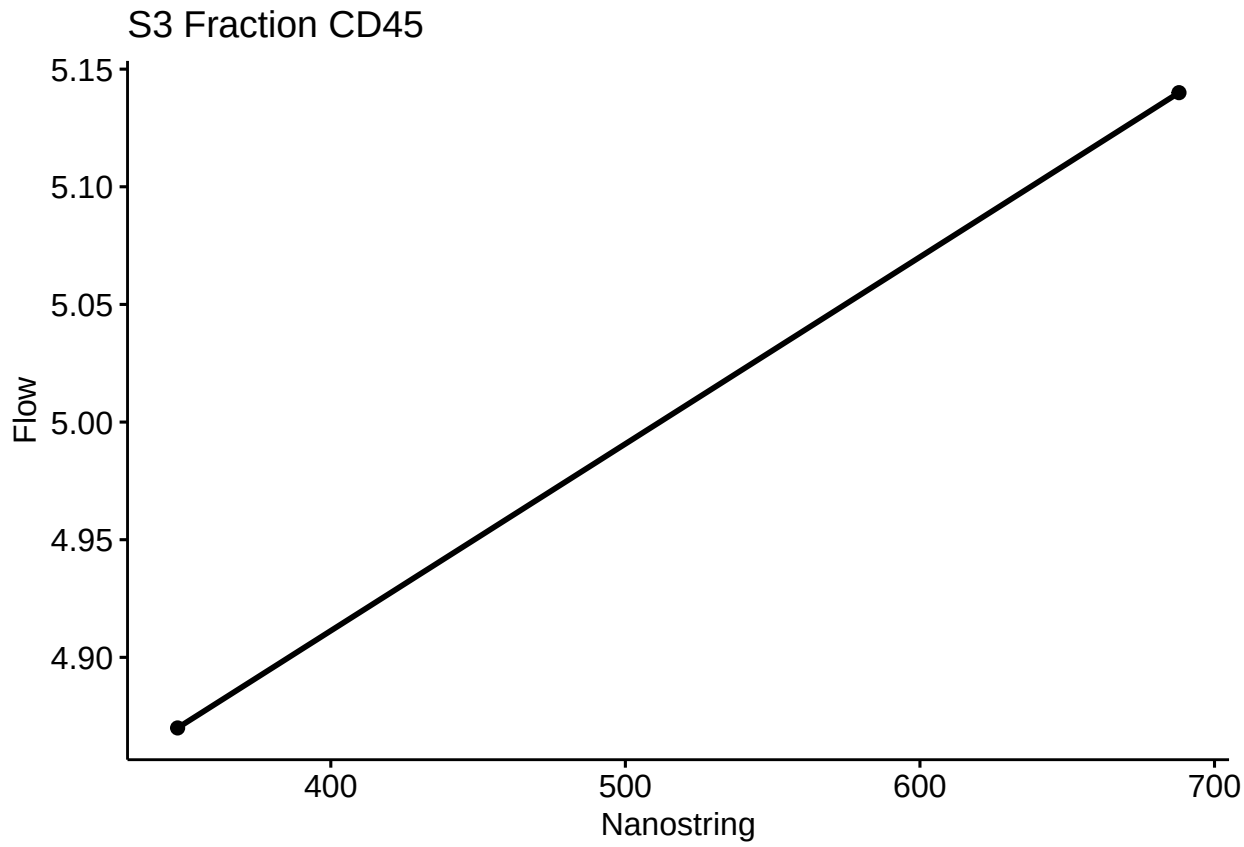


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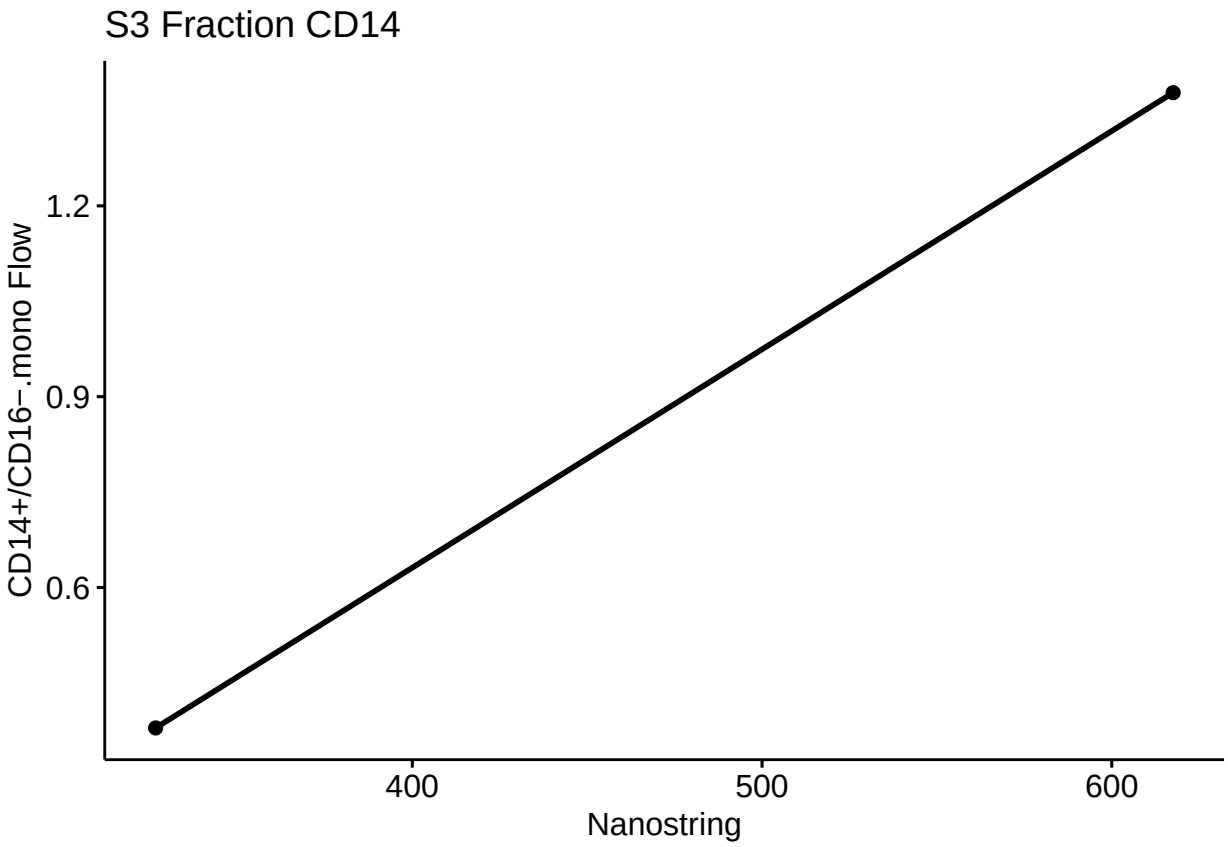


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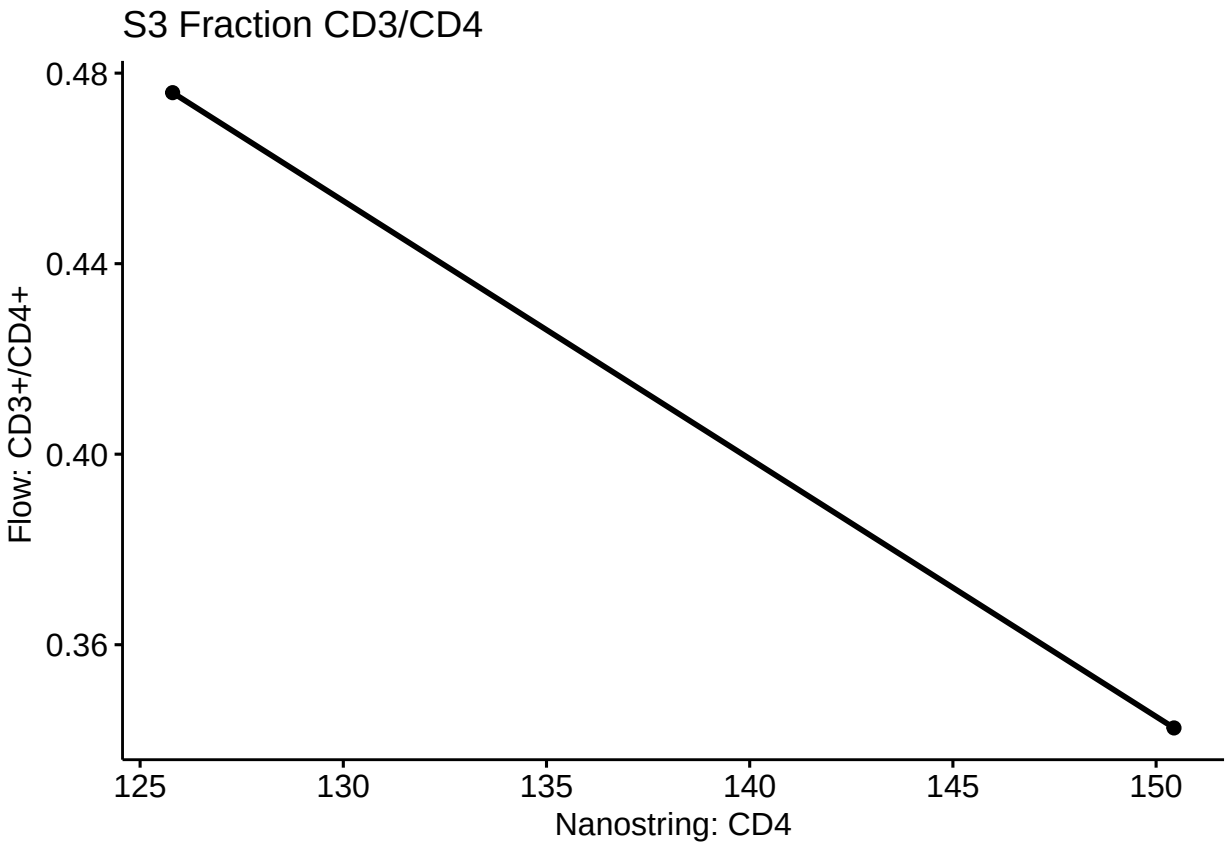


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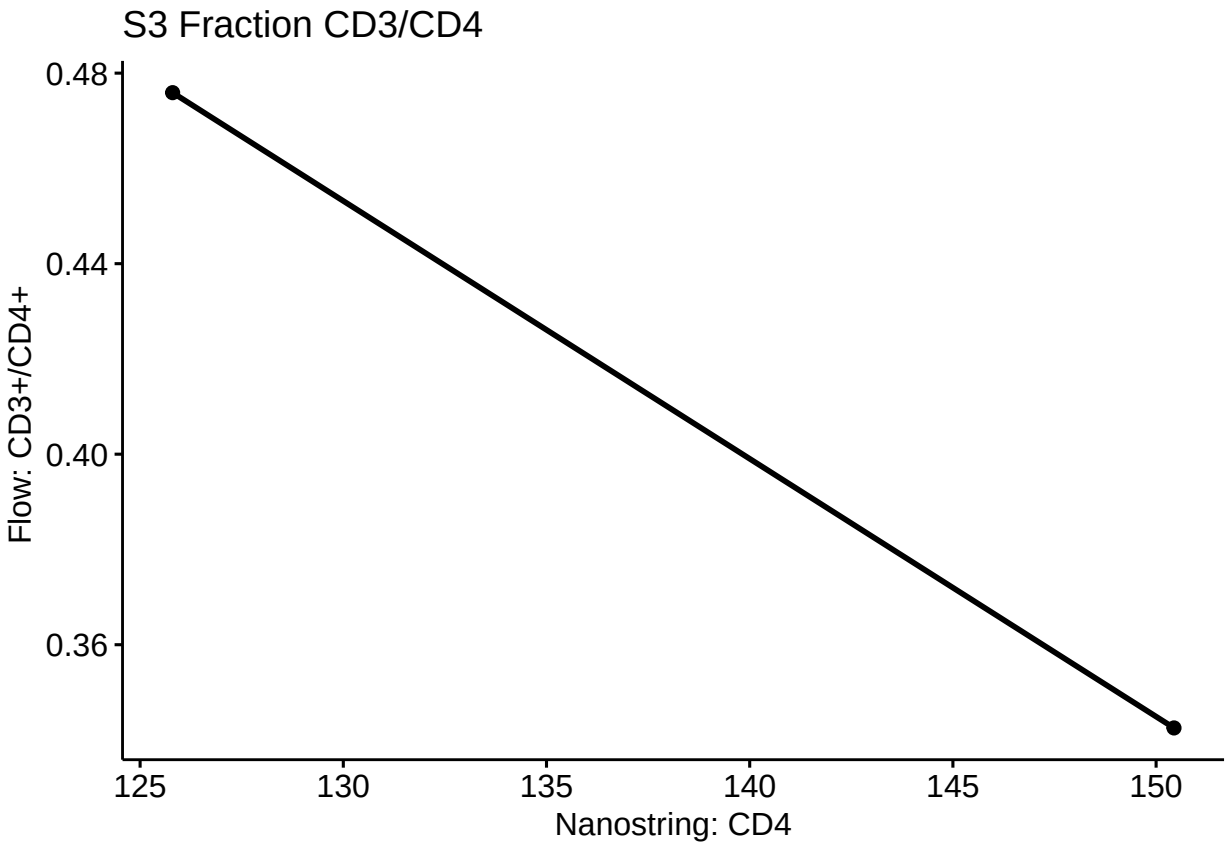
S3 Fraction CD3/CD4
0.48
0.44
0.40
0.36
Flow: CD3+/CD4+
125
130
135
140
145
150
Nanostring: CD4