TRIAMF: A New Method for Delivery of Cas9 Ribonucleoprotein Complex to Human Hematopoietic Stem Cells

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Figure S1. Representative distribution of diameters of untreated and 2-day post TRIAMF treated cells measured by Vi-CELL XR cell counter. Representative distribution of the forward scatter of the CD34+ and CD34+/CD90+ cells that were untreated, treated with TRIAMF in the absence of RNP (TRIAMF Mock) or presence of RNP (TRIMAF+RNP).

Figure S2. B2M knockout efficiency in total, $CD34^+$ or $CD34^+/CD90^+$ populations of untreated, mock treated (TRIAMF Mock), and TRIAMF+RNP treated HSPCs.

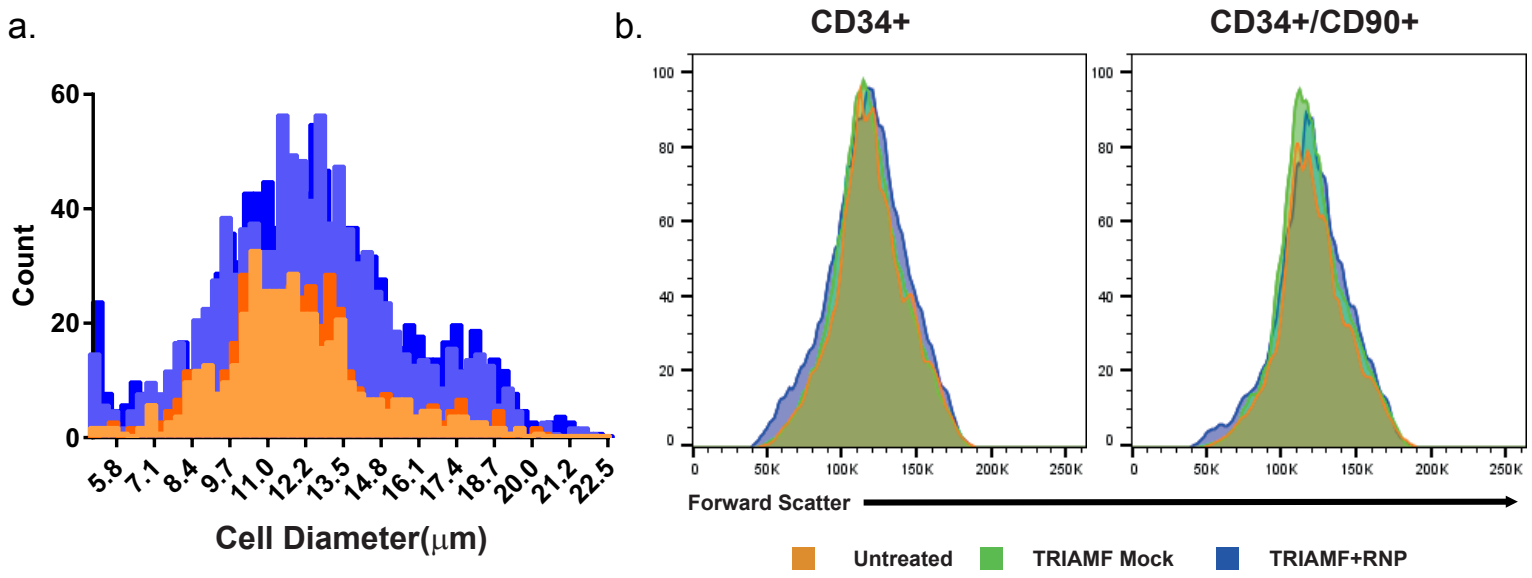
Figure S3. B2M knockout efficiency determined by NGS as a function of (a) membrane pore diameter; (b) applied pressure; (c) RNP concentration and (d) cell density (n=6, 3 donors with duplicate of each donor). (e) B2M knockout efficiency remained the same with further increased cell density from 10^8 to $2x10^8$ cells/ml in 50 μl volume processed by TRIAMF using 25 μM of RNP, 7 μm thick membrane with 8 μm pore diameter under 5 PSI nitrogen pressure (n=2). The samples are identical to the ones in Fig.1. *** $p < 0.001$, ** $p < 0.01$, one-way analysis of variance (ANOVA) and Tukey's multiple comparison test.

Figure S4. HSPC recovery rates after TRIAMF or electroporation in the absence of RNP. $5x10^4$ viable cells seeded 48 hours post treatment and expanded for 7 days. Percentage of $CD34^+$ and $CD34^+/CD90^+$ are shown in (a) and the total cell number and the number of either $CD34^+$ or $CD34^+/CD90^+$ cells are shown in (b). n=4 single donor, biological duplicate with technical duplicate each, ** $p < 0.01$, one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. Bars represent standard deviation.

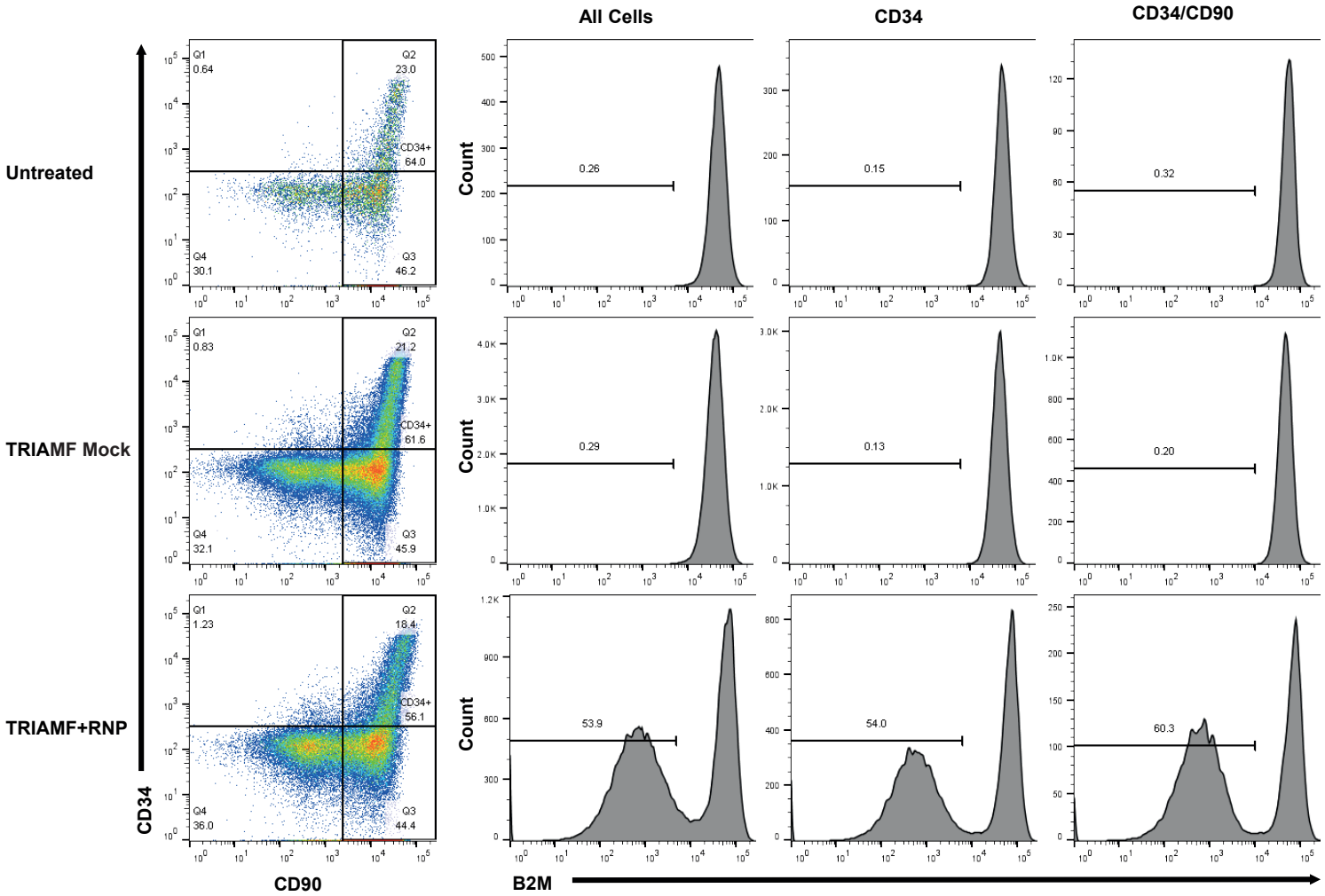
Figure S5. Representative gating scheme of $CD235a^+/CD71^{-/low}$ cells after 3 weeks erythroid differentiation/maturation in experimental groups used in Fig. 2 and 3.

Figure S6. B2M knockout efficiency as determined by NGS (a) and cell recovery (b) of untreated, mock electroporated (Neon Mock) and RNP electroporated (Neon+RNP) HSPCs. 0.14 million of ex vivo expanded HSPCs were mixed with 1 μM RNP, brought to 10 μl final volume with Buffer T and electroporated with the Neon electroporator using the same conditions as in Fig. S4.

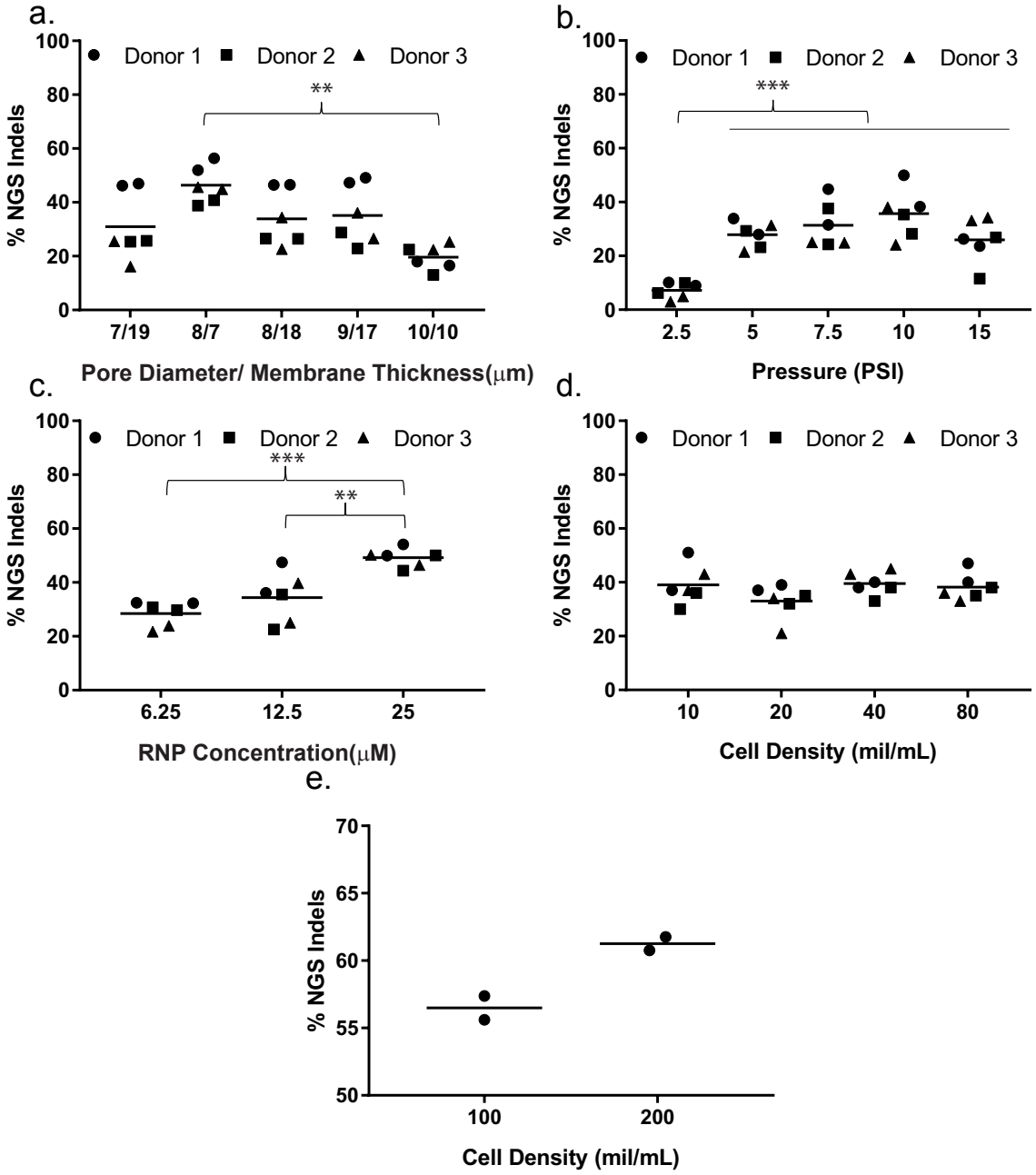
S1


a.
Count
Cell Diameter(μm)
5.8
7.1
8.4
9.7
11.0
12.2
13.5
14.8
16.1
17.4
18.7
20.0
21.2
22.5
b.
CD34+
CD34+/CD90+
Forward Scatter
Untreated
TRIAMF Mock
TRIAMF+RNP

S2

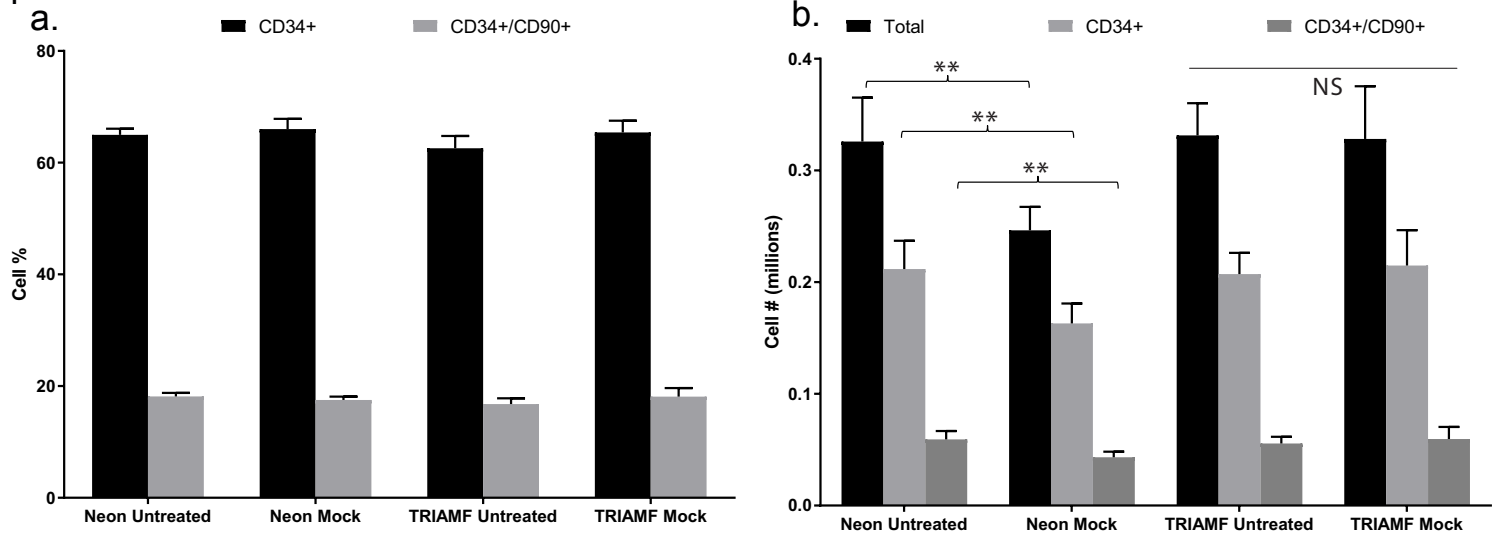

All Cells
CD34
CD34/CD90
Untreated
TRIAMF Mock
TRIAMF+RNP
CD34
CD90
B2M
Count
0.26
0.15
0.32
0.29
0.13
0.20
53.9
54.0
60.3

S3

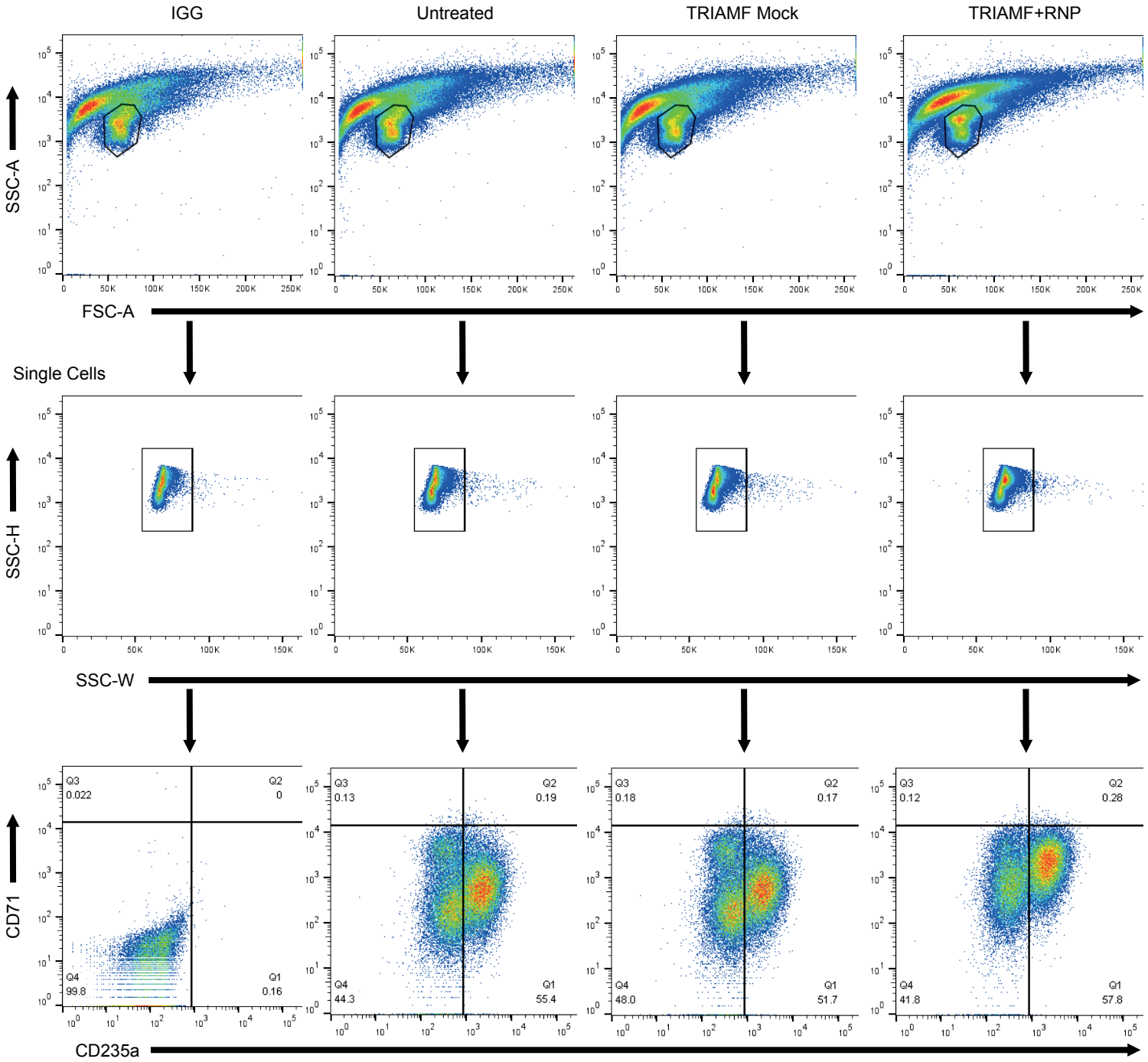

a.
Donor 1
Donor 2
Donor 3
% NGS Indels
**
7/19
8/7
8/18
9/17
10/10
Pore Diameter/ Membrane Thickness(μm)
b.
***
2.5
5
7.5
10
15
Pressure (PSI)
c.
***
**
6.25
12.5
25
RNP Concentration(μM)
d.
10
20
40
80
Cell Density (mil/mL)

e.
% NGS Indels
70
65
60
55
50
100
200
Cell Density (mil/mL)

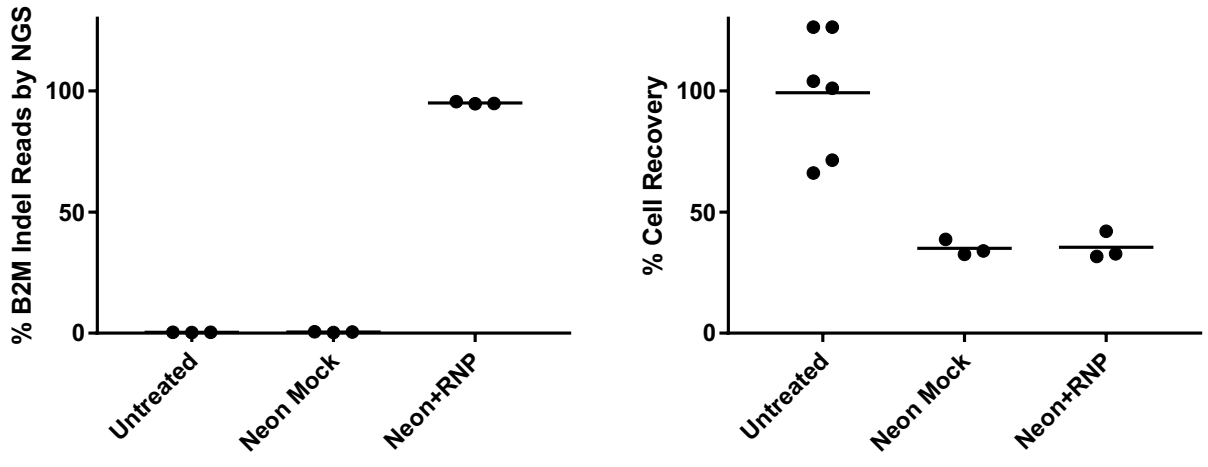
S4


a.
CD34+
CD34+/CD90+
Cell %
Neon Untreated
Neon Mock
TRIAMF Untreated
TRIAMF Mock
b.
Total
CD34+
CD34+/CD90+
**
**
**
NS
Cell # (millions)
Neon Untreated
Neon Mock
TRIAMF Untreated
TRIAMF Mock

S5


IGG
Untreated
TRIAMF Mock
TRIAMF+RNP
SSC-A
FSC-A
Single Cells
SSC-H
SSC-W
CD71
CD235a
Q3 0.022
Q2 0
Q4 99.8
Q1 0.16
Q3 0.13
Q2 0.19
Q4 44.3
Q1 55.4
Q3 0.18
Q2 0.17
Q4 48.0
Q1 51.7
Q3 0.12
Q2 0.28
Q4 41.8
Q1 57.8

S6


% B2M Indel Reads by NGS
100
50
0
Untreated
Neon Mock
Neon+RNP
% Cell Recovery
100
50
0
Untreated
Neon Mock
Neon+RNP