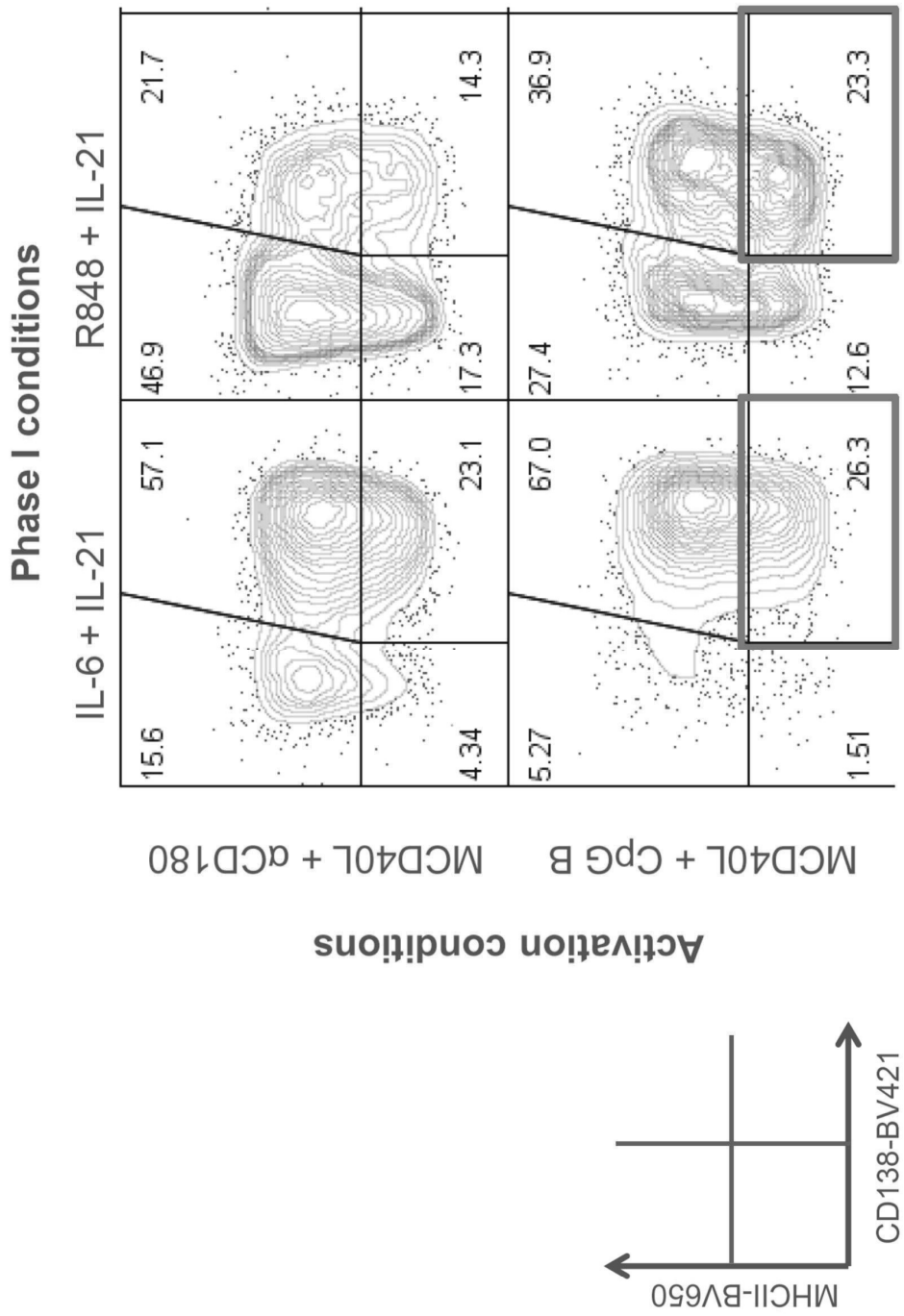
Phase I conditions
IL-6 + IL-21
R848 + IL-21
15.6
57.1
46.9
21.7
4.34
23.1
17.3
14.3
5.27
67.0
27.4
36.9
1.51
26.3
12.6
23.3
Activation conditions
MCD40L + CpG B
MCD40L + αCD180
MHCII-BV650
CD138-BV421

FIG. 1

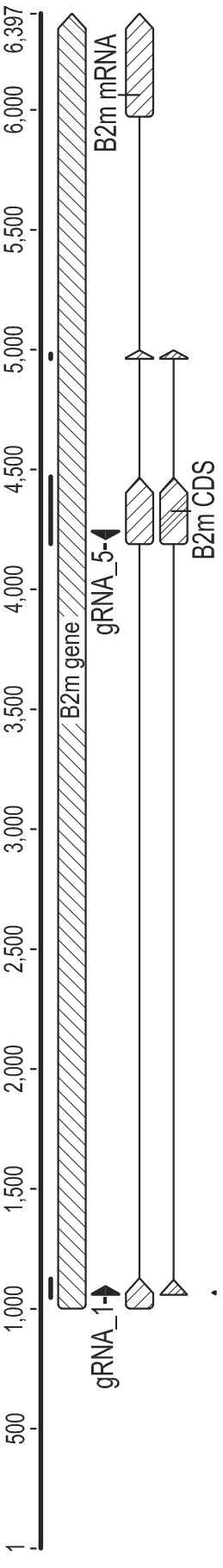
1
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,000
6,397
B2m gene
B2m mRNA
B2m CDS
gRNA_5-
gRNA_1-

FIG. 2

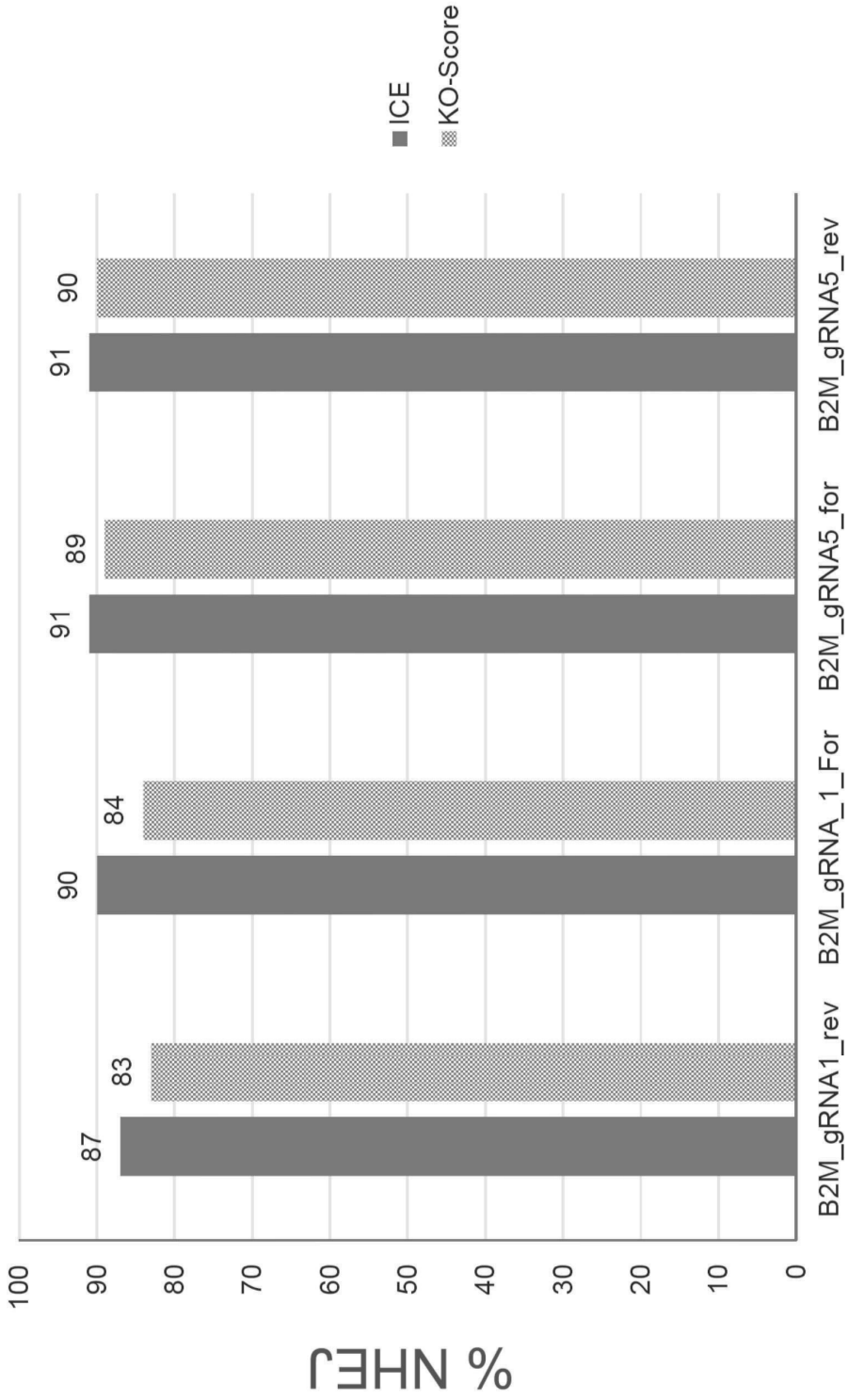
ICE
KO-Score
% NHEJ
100
90
80
70
60
50
40
30
20
10
0
87
83
90
84
91
89
91
90
B2M_gRNA1_rev
B2M_gRNA_1_For
B2M_gRNA5_for
B2M_gRNA5_rev

FIG. 3

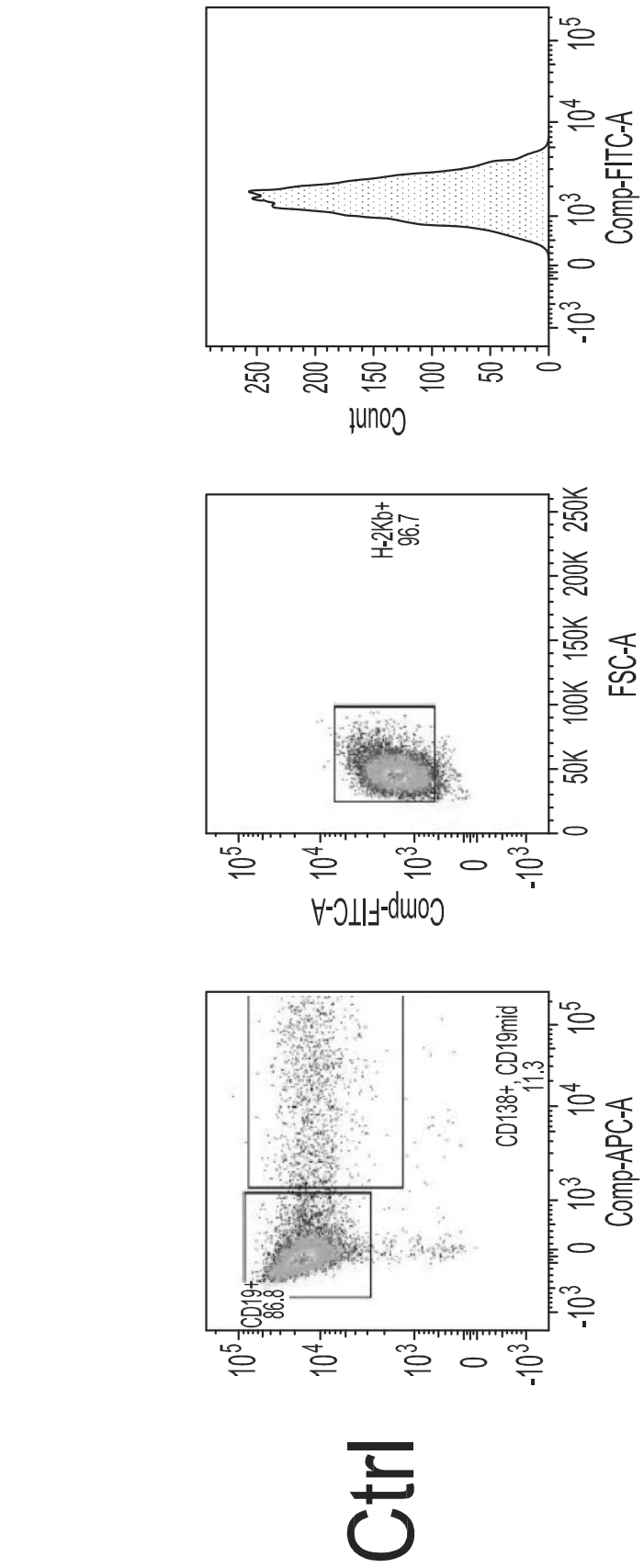
Ctrl
CD19+
86.8
CD138+, CD19mid
11.3
Comp-APC-A
Comp-FITC-A
H-2Kb+
96.7
FSC-A
Count
Comp-FITC-A

FIG. 4A

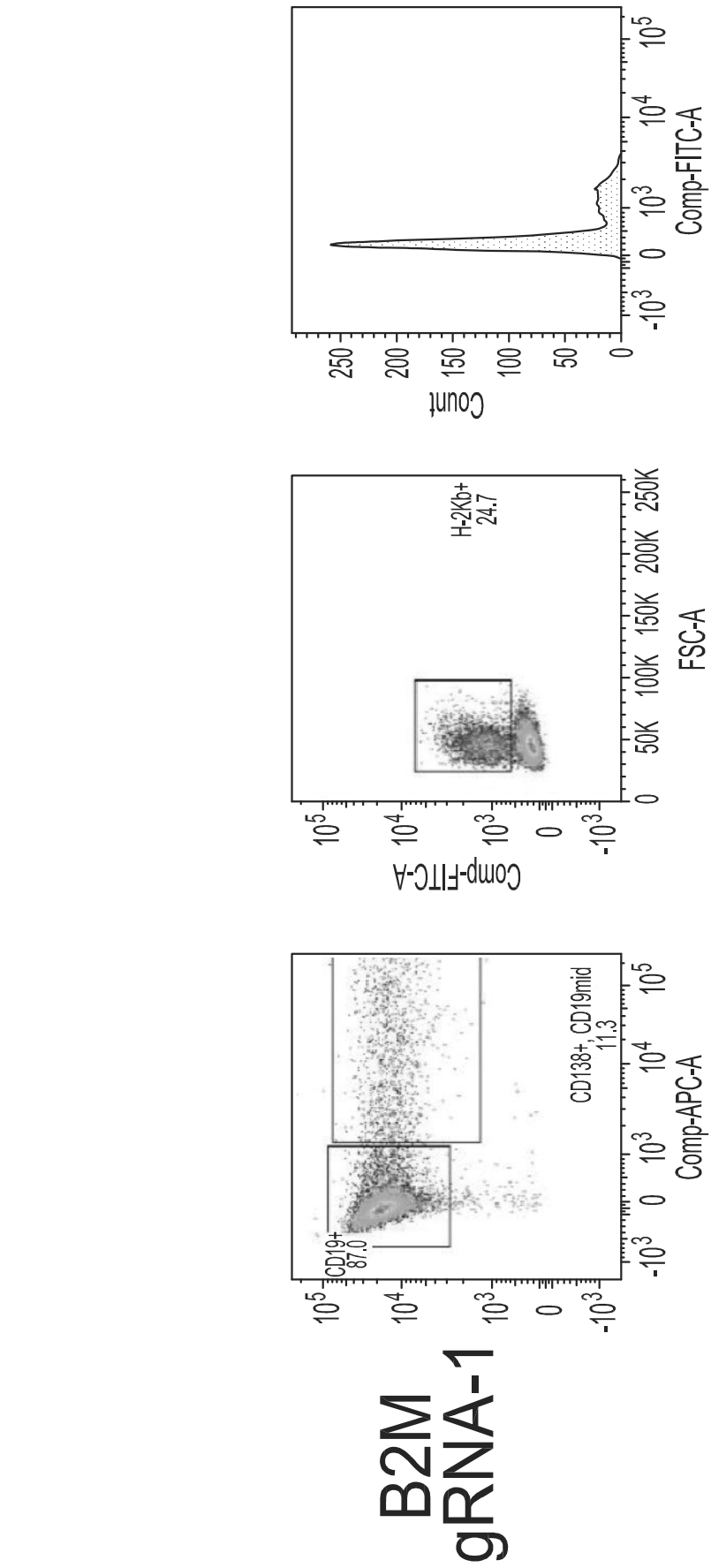

FIG. 4A
(Continued)

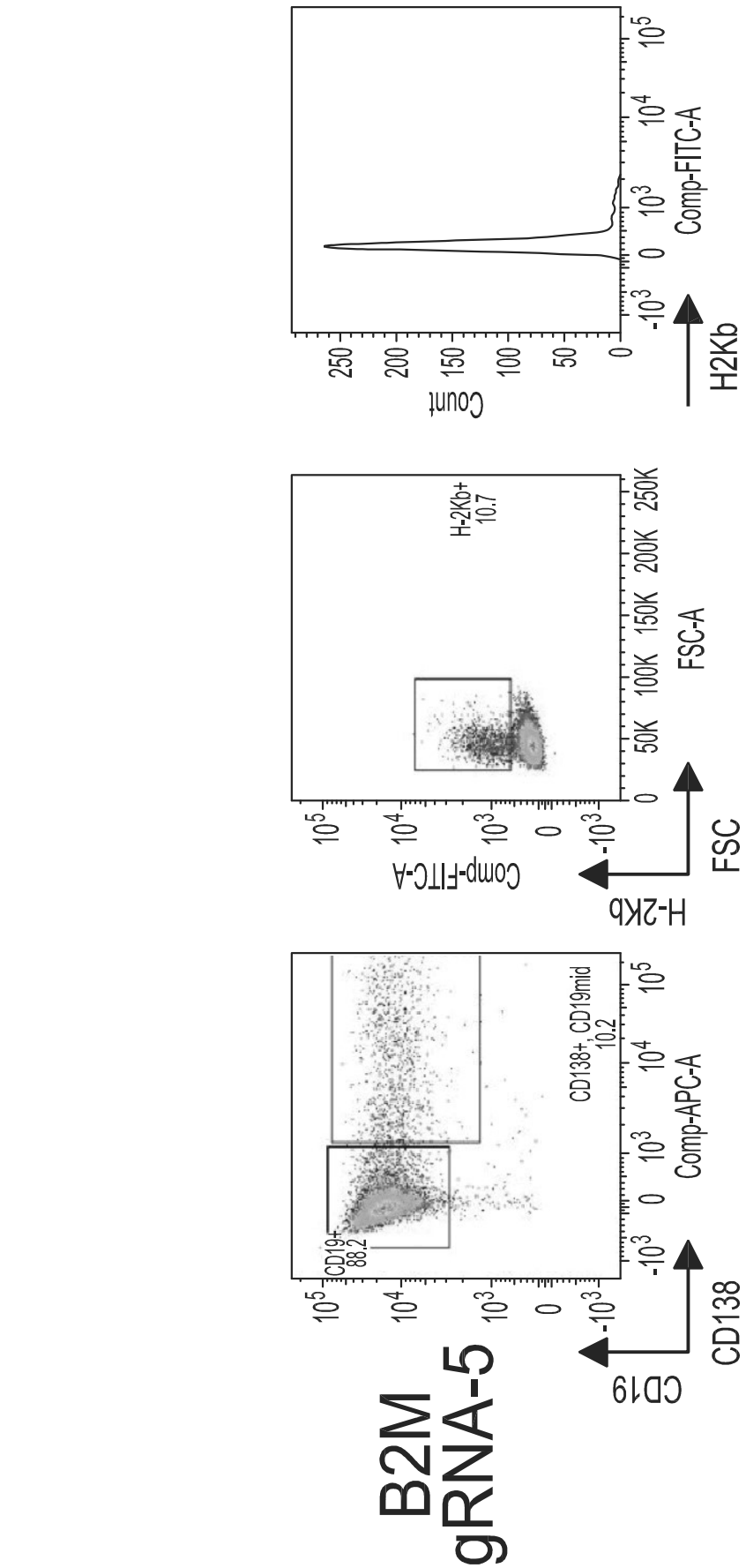

FIG. 4A
(Continued)

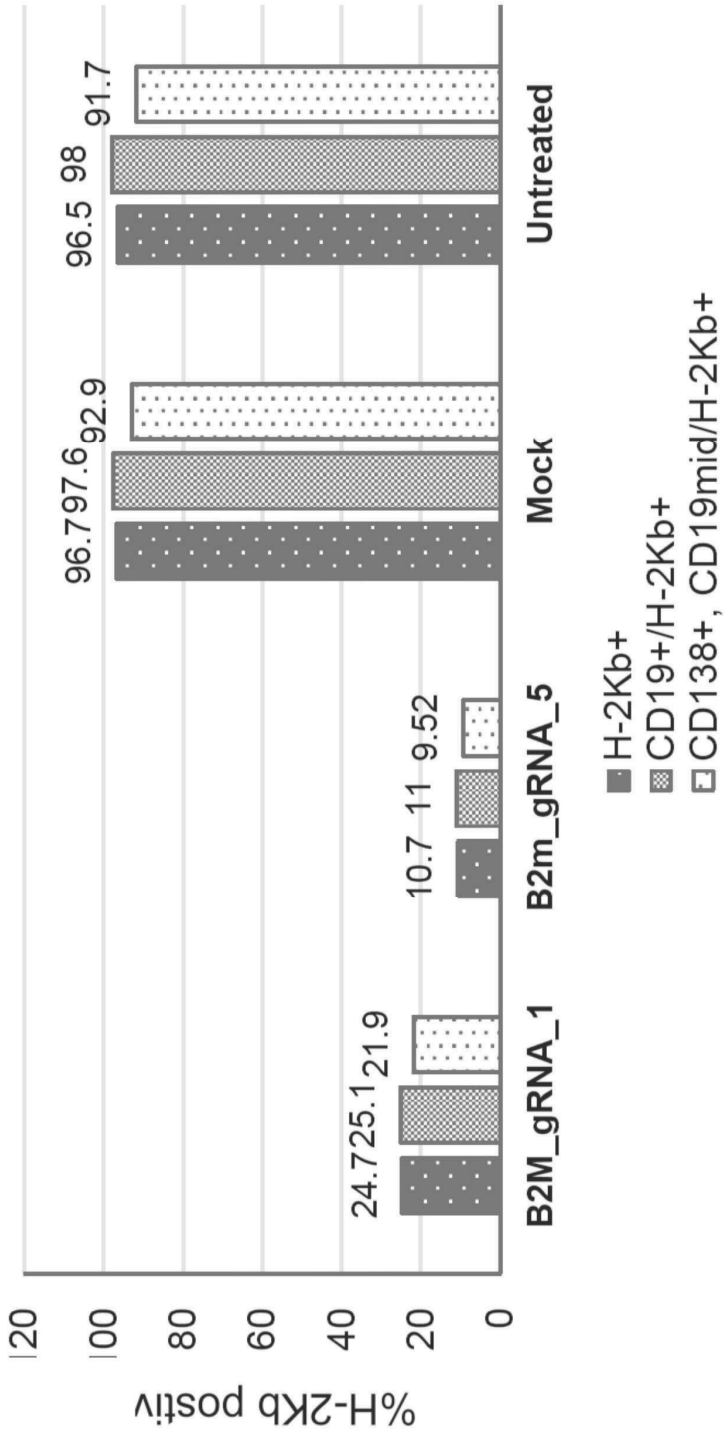
%H-2Kb positiv
120
100
80
60
40
20
0
24.7
25.1
21.9
10.7
11
9.52
96.7
97.6
92.9
96.5
98
91.7
B2M_gRNA_1
B2m_gRNA_5
Mock
Untreated
H-2Kb+
CD19+/H-2Kb+
CD138+, CD19mid/H-2Kb+

FIG. 4B

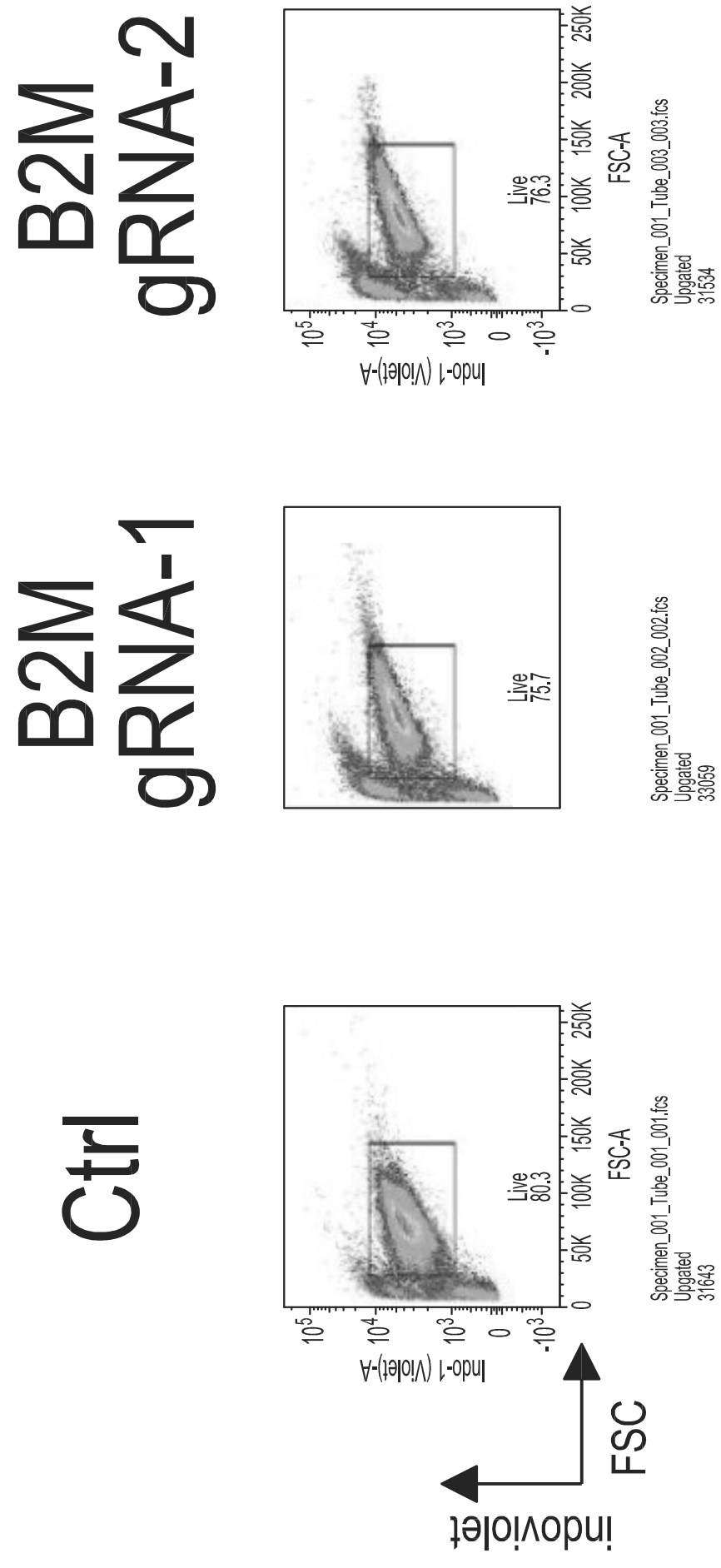
Ctrl
B2M gRNA-1
B2M gRNA-2
indoviolet
FSC
Indo-1 (Violet)-A
FSC-A
Live 80.3
Live 75.7
Live 76.3
Specimen_001_Tube_001_001.fcs
Upgated
31643
Specimen_001_Tube_002_002.fcs
Upgated
33059
Specimen_001_Tube_003_003.fcs
Upgated
31534

FIG. 4C

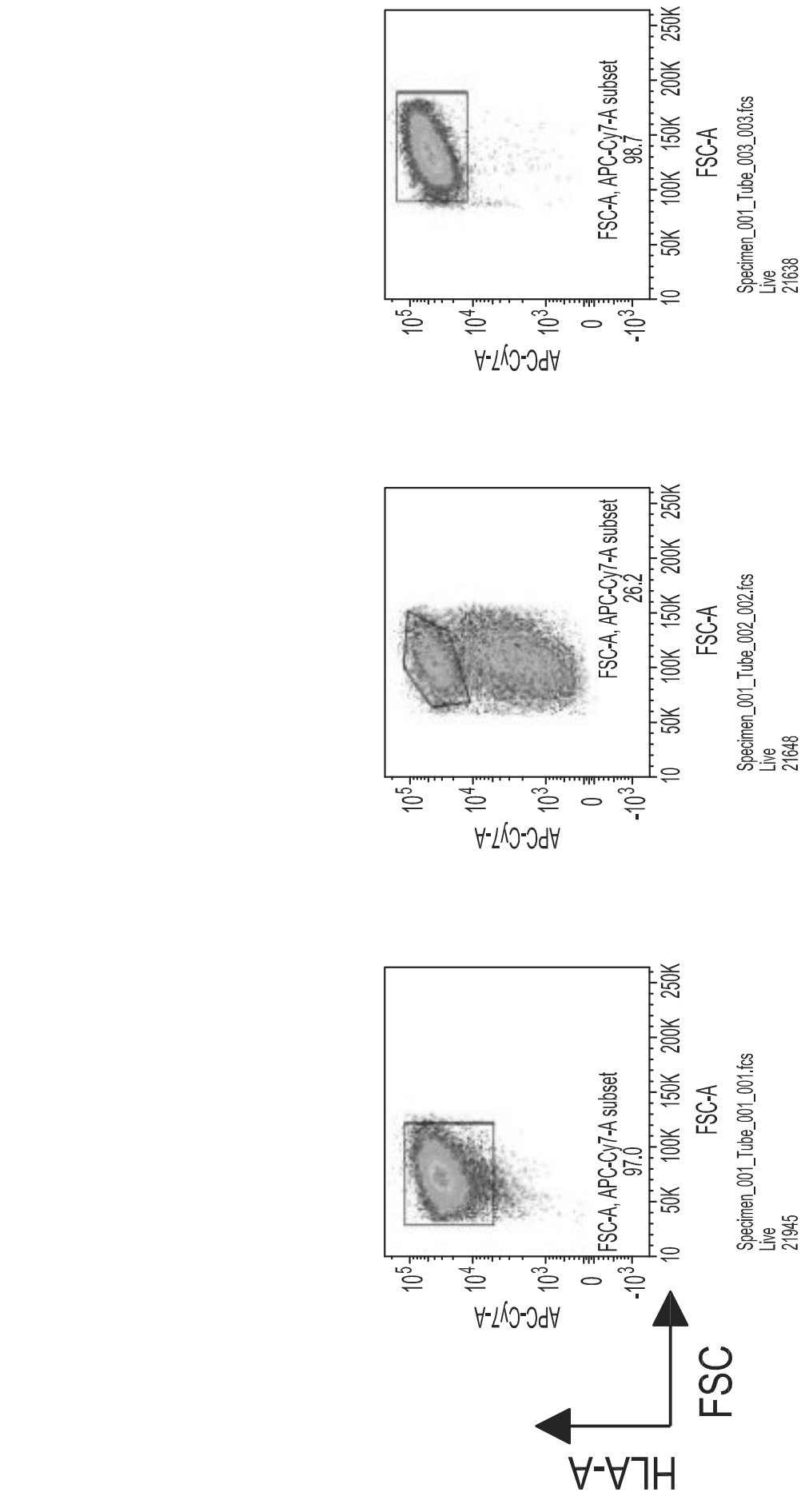

FIG. 4C
(Continued)

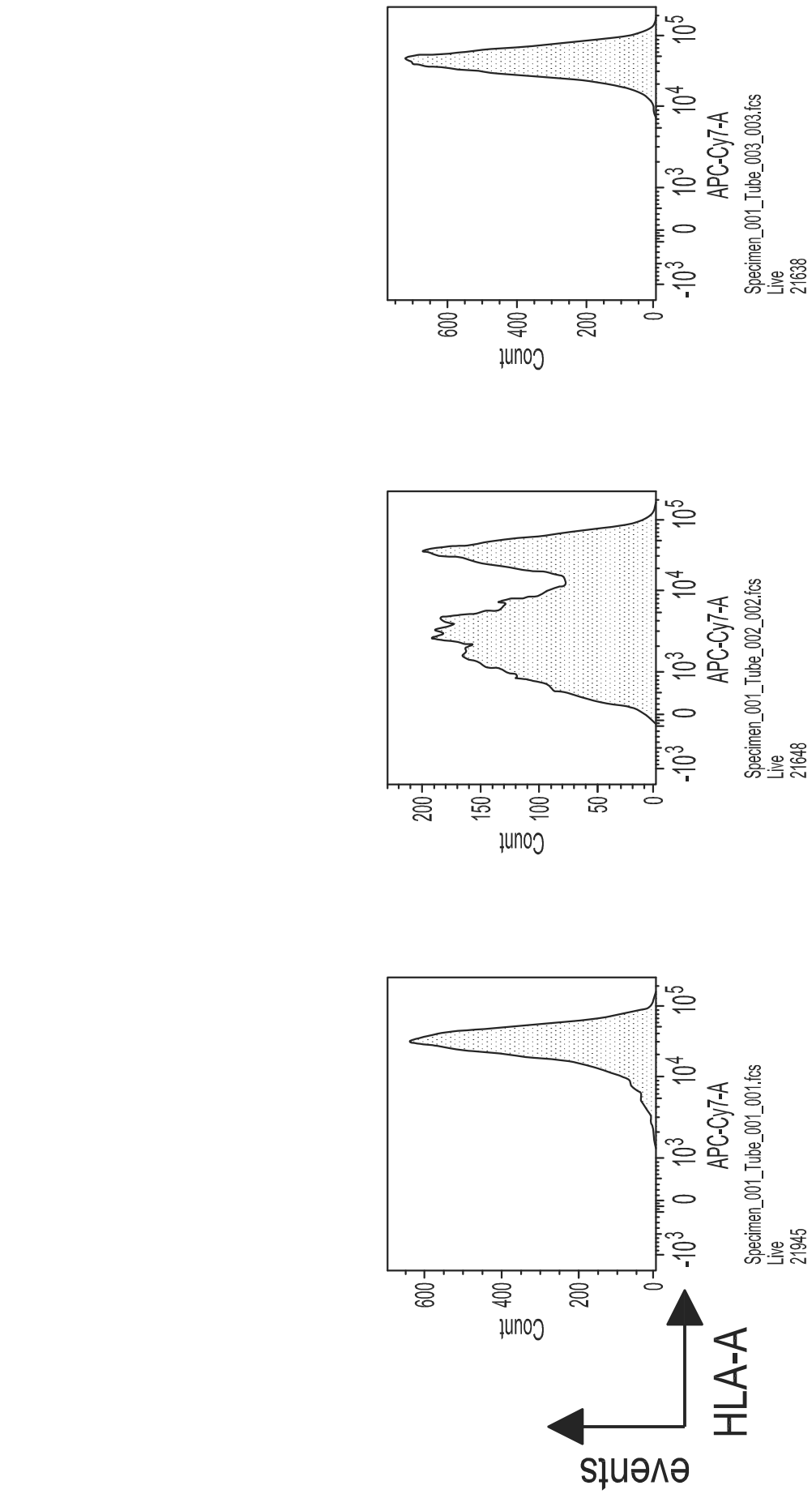

FIG. 4C
(Continued)

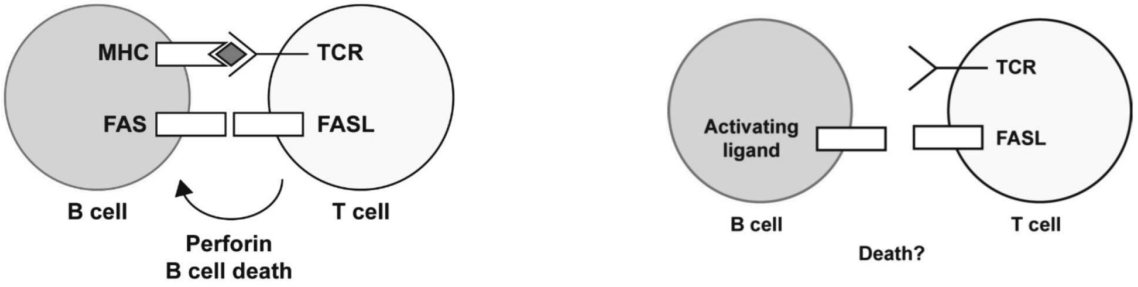
MHC
TCR
FAS
FASL
B cell
T cell
Perforin
B cell death
TCR
Activating ligand
FASL
B cell
T cell
Death?

FIG. 5A

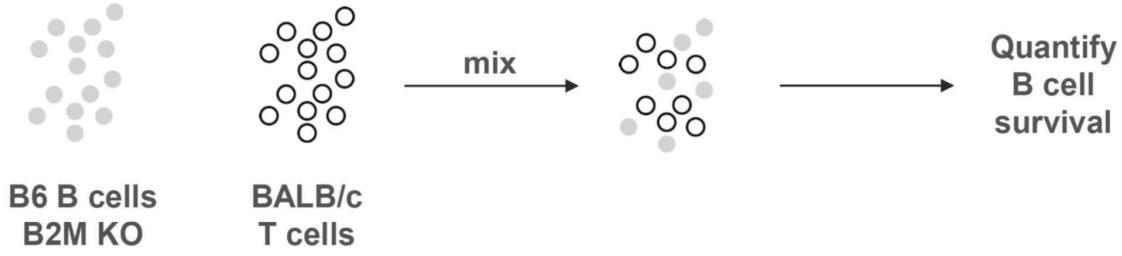
mix
Quantify B cell survival
B6 B cells B2M KO
BALB/c T cells

FIG. 5B

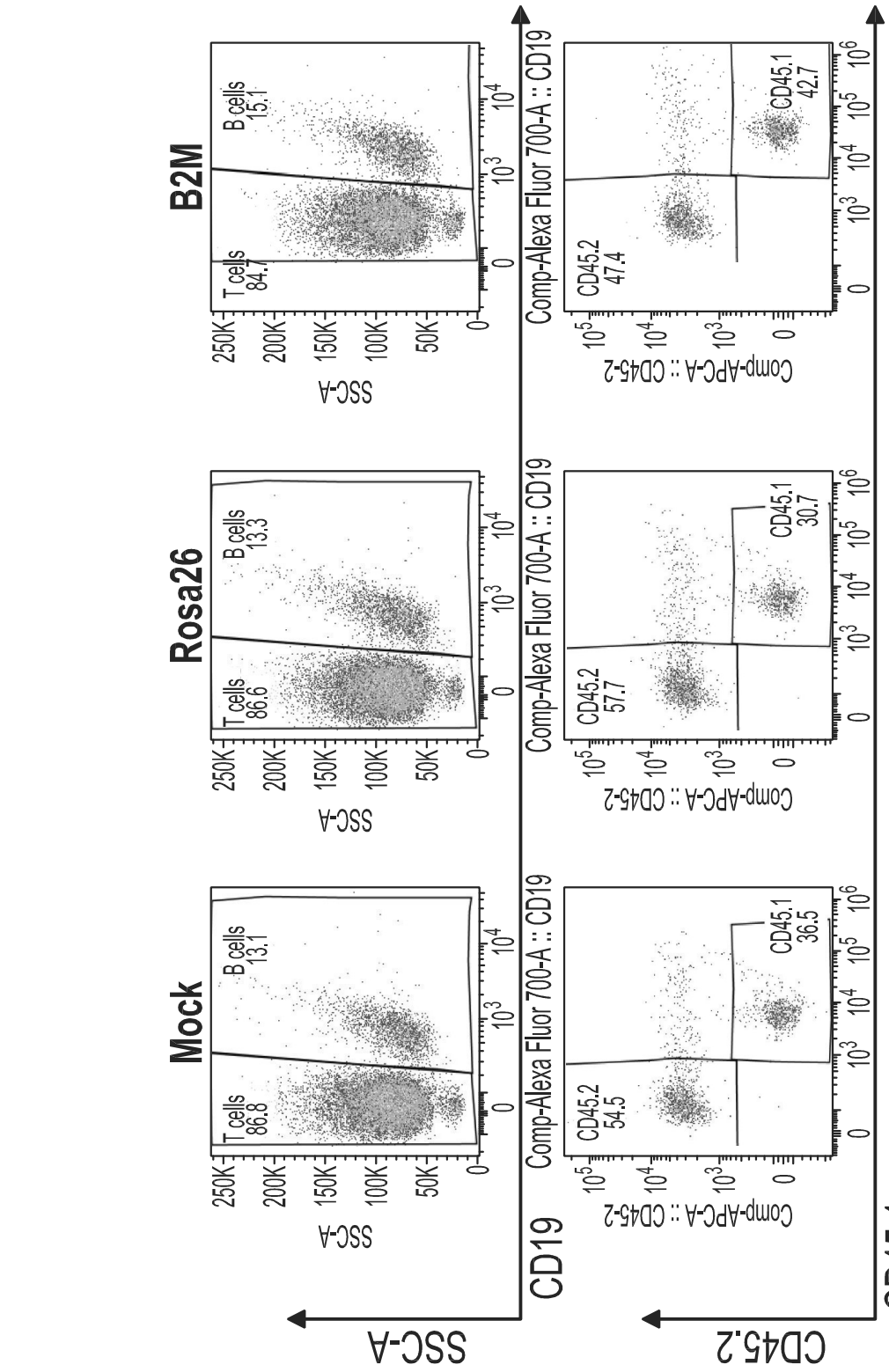
Gating strategy:
lymphocytes ->
singlets ->
CD19+ ->
CD45.1 vs CD45.2
Mock
Rosa26
B2M
SSC-A
CD19
Comp-Alexa Fluor 700-A :: CD19
CD45.2
Comp-APC-A :: CD45-2
CD45.1
T cells
86.8
B cells
13.1
CD45.2
54.5
CD45.1
36.5
T cells
86.6
B cells
13.3
CD45.2
57.7
CD45.1
30.7
T cells
84.7
B cells
15.1
CD45.2
47.4
CD45.1
42.7

FIG. 6A

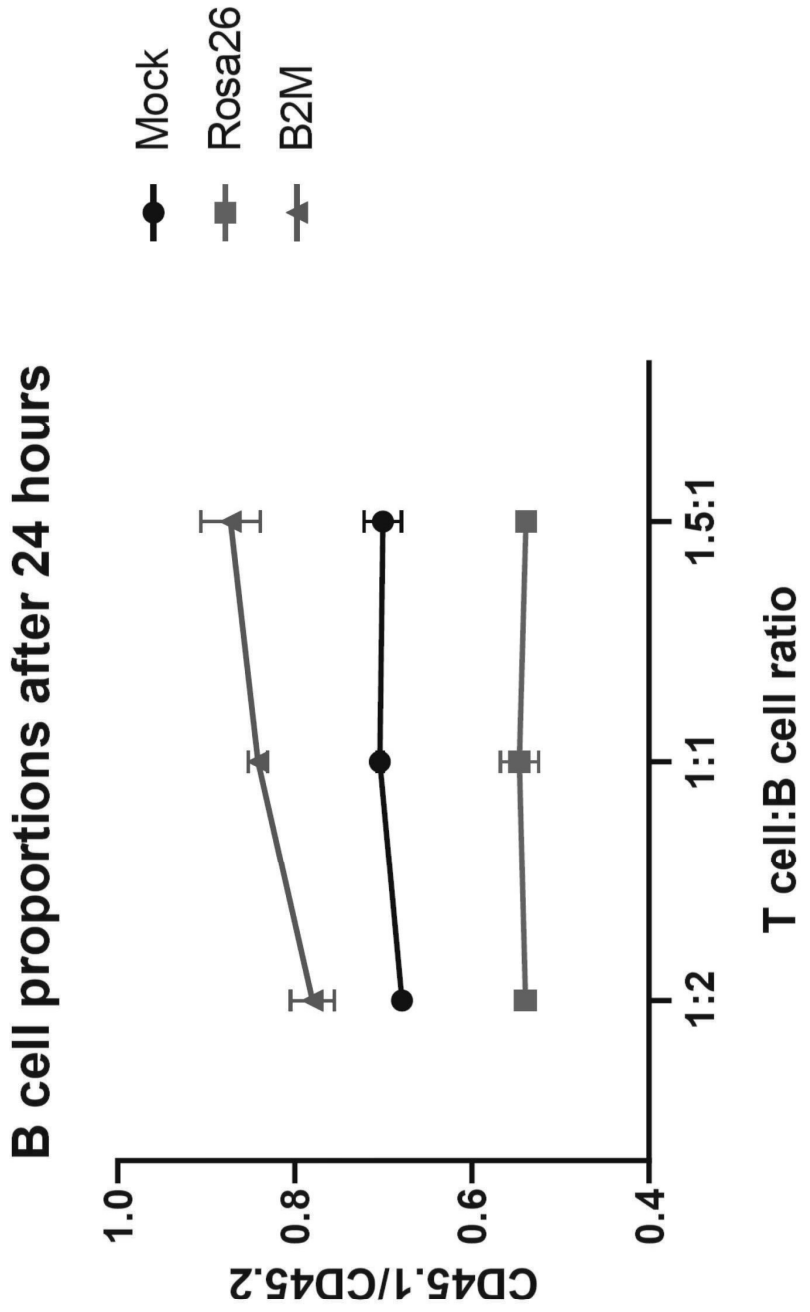
B cell proportions after 24 hours
Mock
Rosa26
B2M
CD45.1/CD45.2
1.0
0.8
0.6
0.4
1:2
1:1
1.5:1
T cell:B cell ratio

FIG. 6B

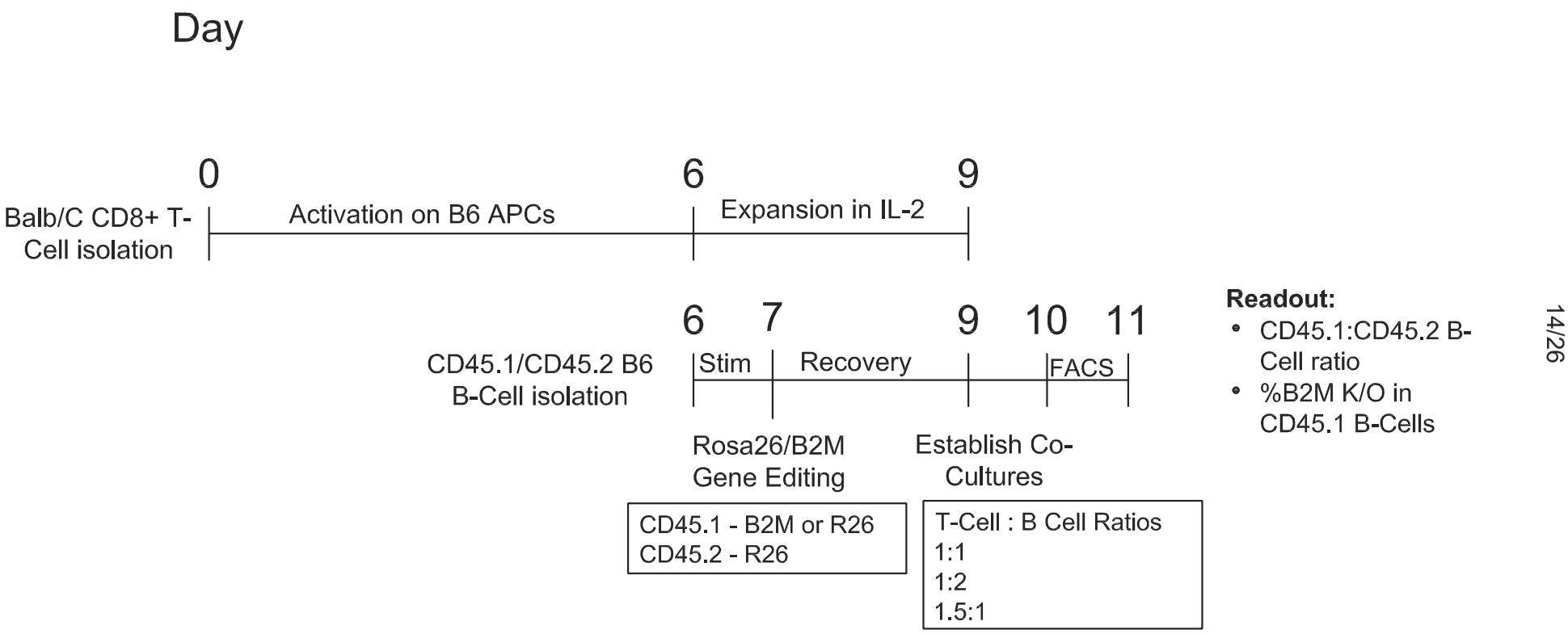
Day
0
6
9
Balb/C CD8+ T-Cell isolation
Activation on B6 APCs
Expansion in IL-2
6
7
9
10
11
CD45.1/CD45.2 B6 B-Cell isolation
Stim
Recovery
FACS
Rosa26/B2M Gene Editing
Establish Co-Cultures
CD45.1 - B2M or R26
CD45.2 - R26
T-Cell : B Cell Ratios
1:1
1:2
1.5:1
Readout:
• CD45.1:CD45.2 B-Cell ratio
• %B2M K/O in CD45.1 B-Cells

FIG. 7

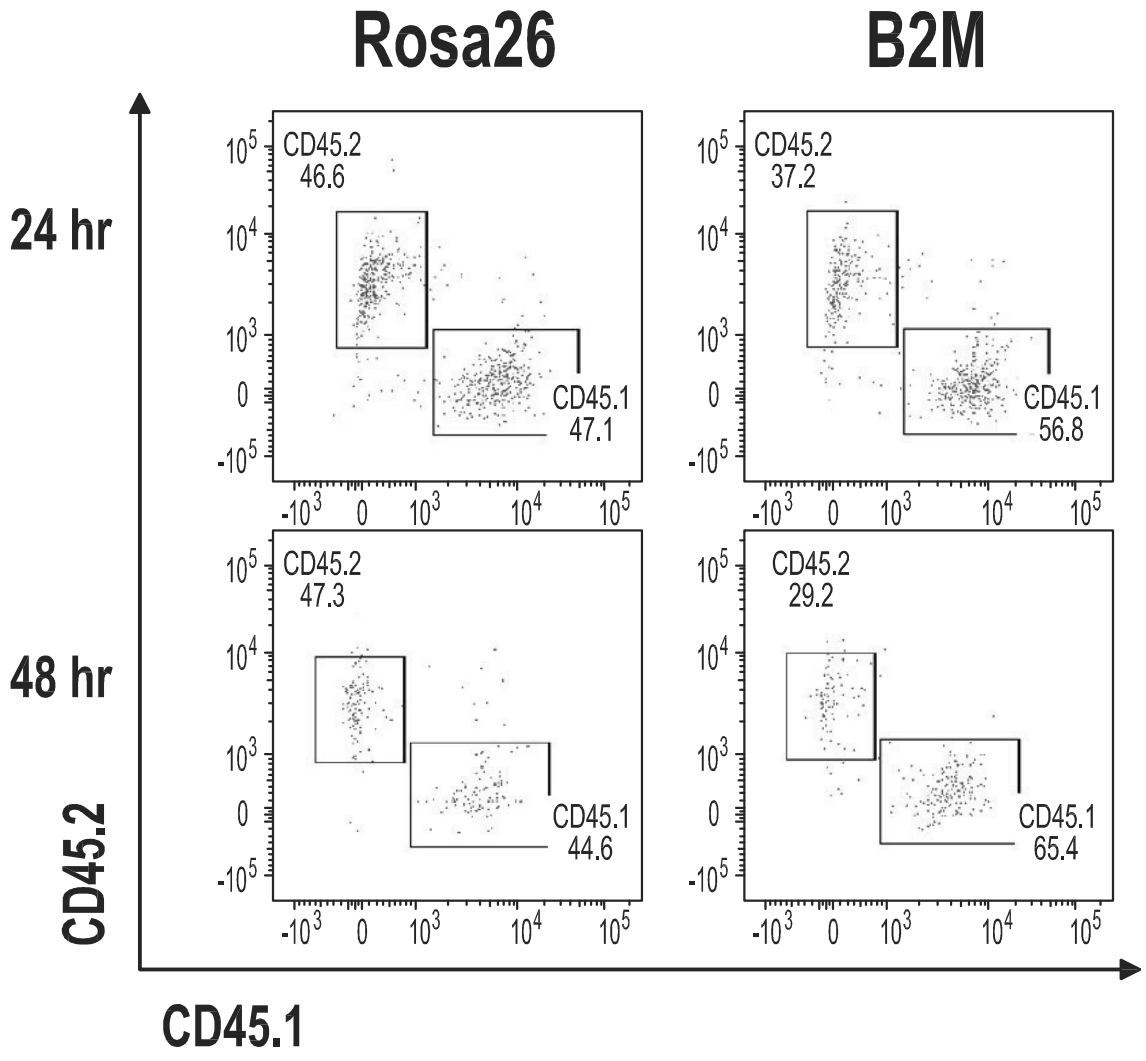
Rosa26
B2M
24 hr
48 hr
CD45.2
CD45.1
CD45.2
46.6
CD45.1
47.1
CD45.2
37.2
CD45.1
56.8
CD45.2
47.3
CD45.1
44.6
CD45.2
29.2
CD45.1
65.4

FIG. 8A

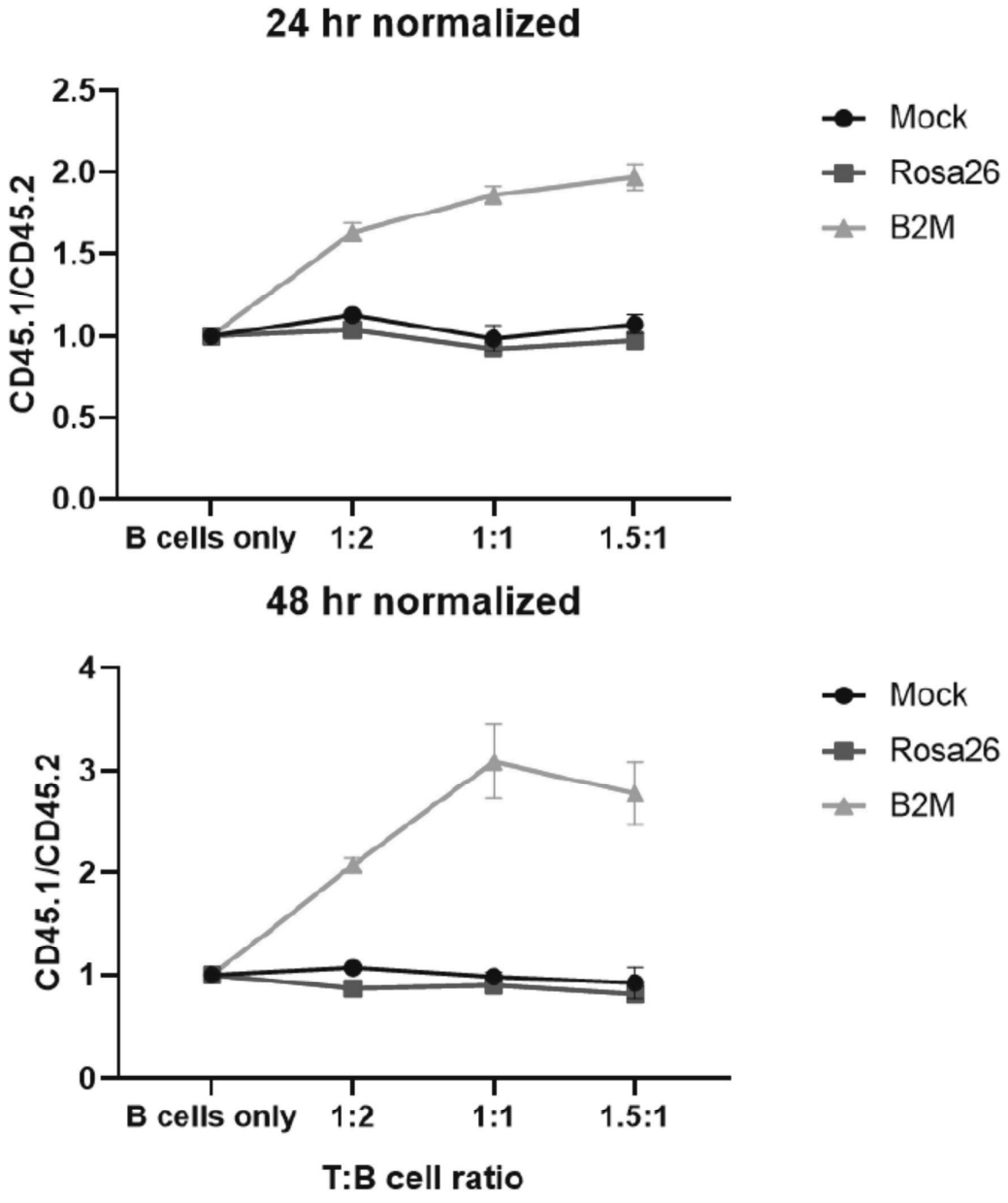
24 hr normalized
CD45.1/CD45.2
2.5
2.0
1.5
1.0
0.5
0.0
B cells only
1:2
1:1
1.5:1
Mock
Rosa26
B2M
48 hr normalized
CD45.1/CD45.2
4
3
2
1
0
B cells only
1:2
1:1
1.5:1
T:B cell ratio
Mock
Rosa26
B2M

FIG. 8B

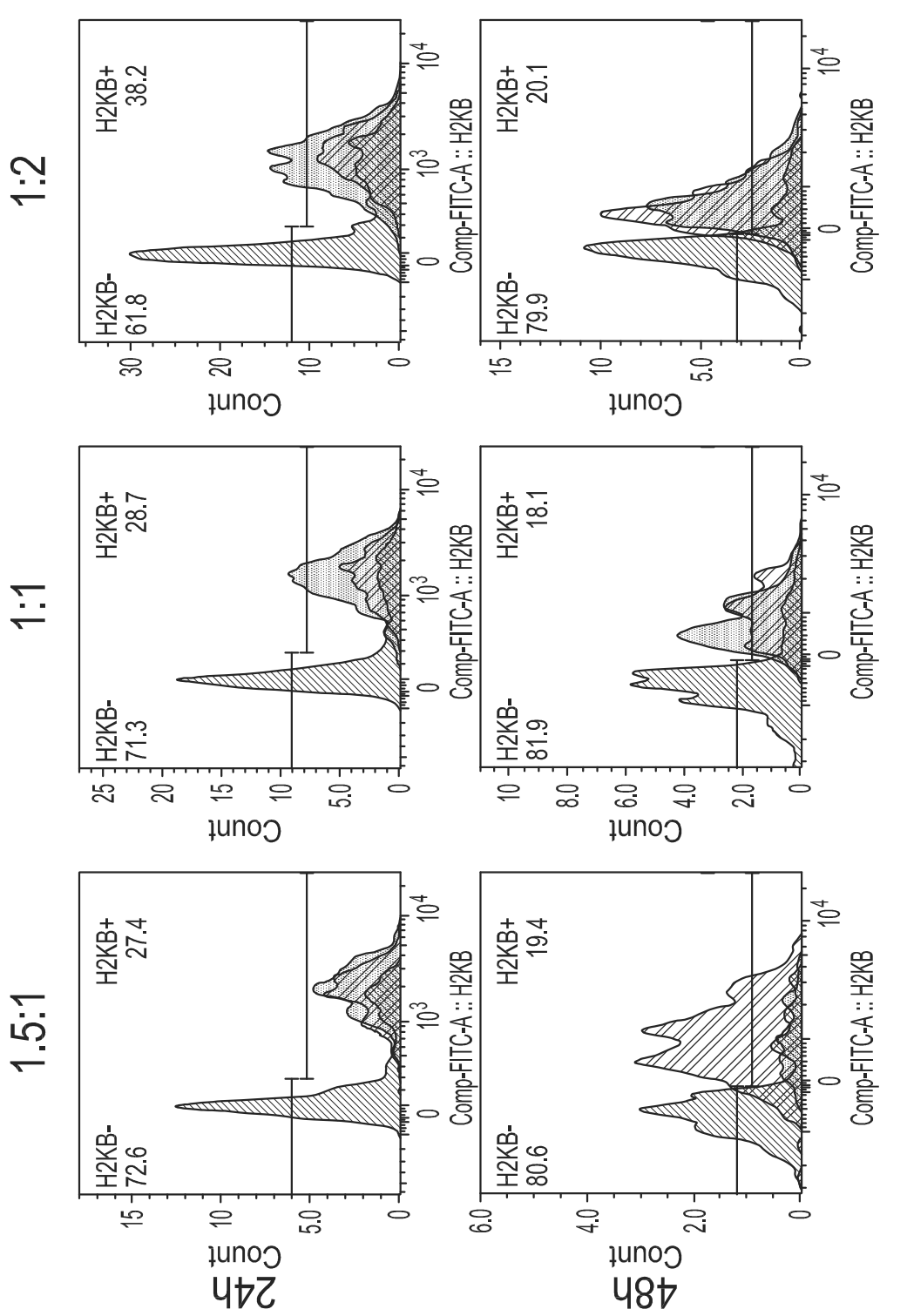
1.5:1
1:1
1:2
24h
48h
Count
Comp-FITC-A :: H2KB
H2KB-
72.6
H2KB+
27.4
H2KB-
71.3
H2KB+
28.7
H2KB-
61.8
H2KB+
38.2
H2KB-
80.6
H2KB+
19.4
H2KB-
81.9
H2KB+
18.1
H2KB-
79.9
H2KB+
20.1
B2M
Mock
Rosa26

FIG. 9A

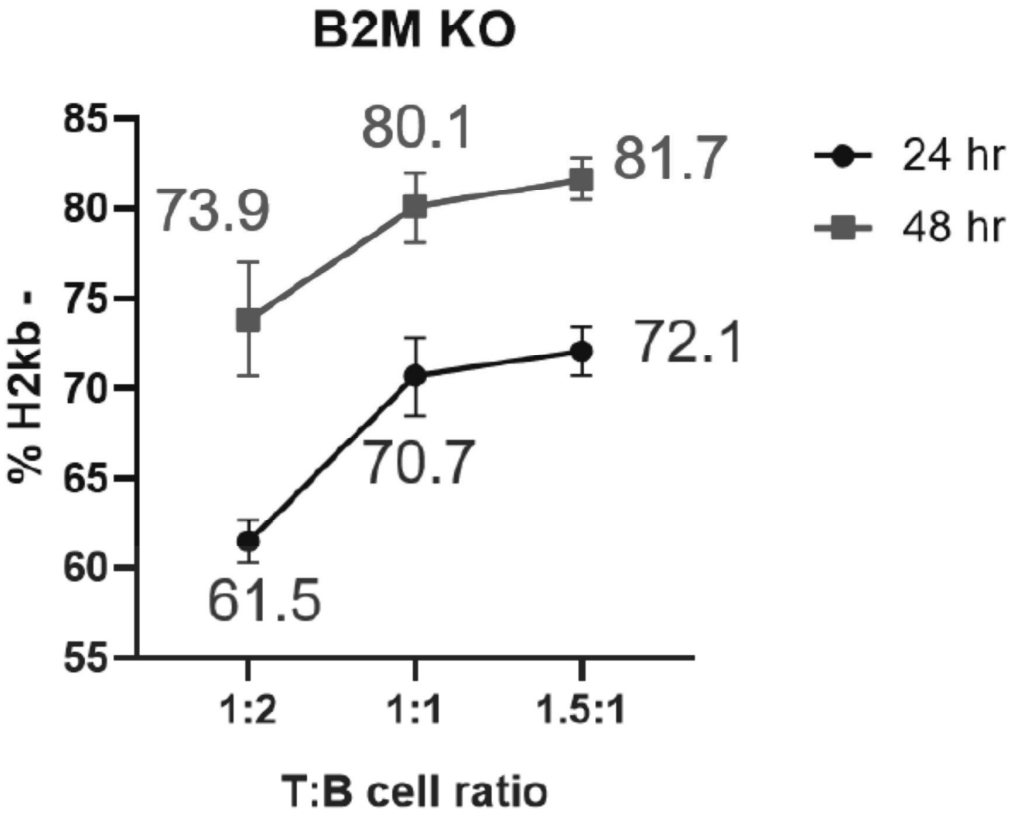
B2M KO
% H2kb -
85
80
75
70
65
60
55
80.1
81.7
73.9
72.1
70.7
61.5
1:2
1:1
1.5:1
T:B cell ratio
24 hr
48 hr

FIG. 9B

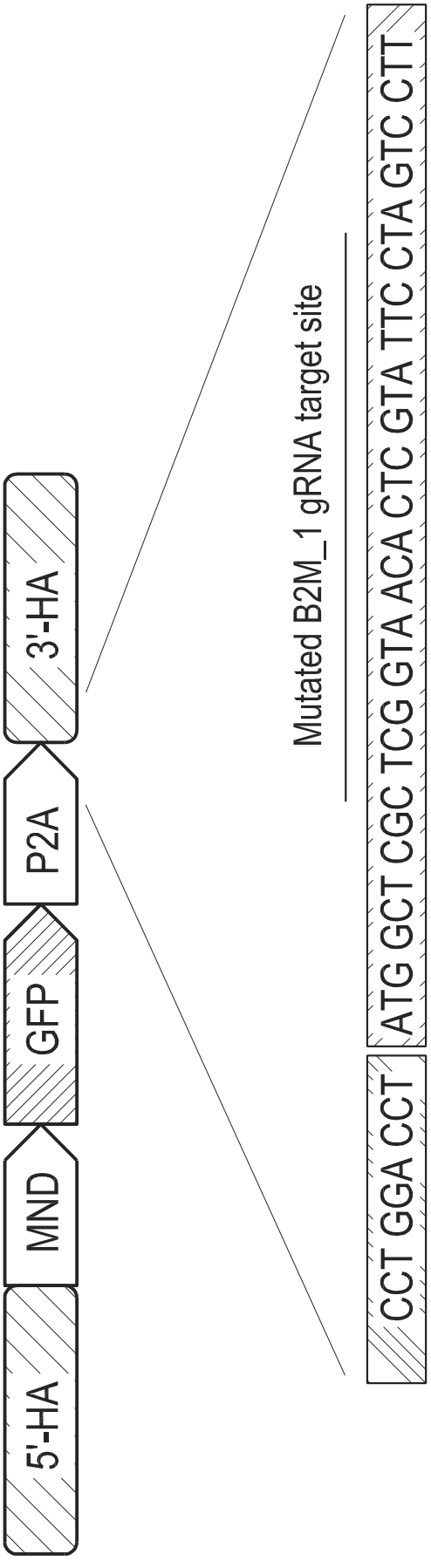
5'-HA
MND
GFP
P2A
3'-HA
Mutated B2M_1 gRNA target site
CCT GGA CCT
ATG GCT CGC TCG GTA ACA CTC GTA TTC CTA GTC CTT
P2A
B2M CDS

FIG. 10

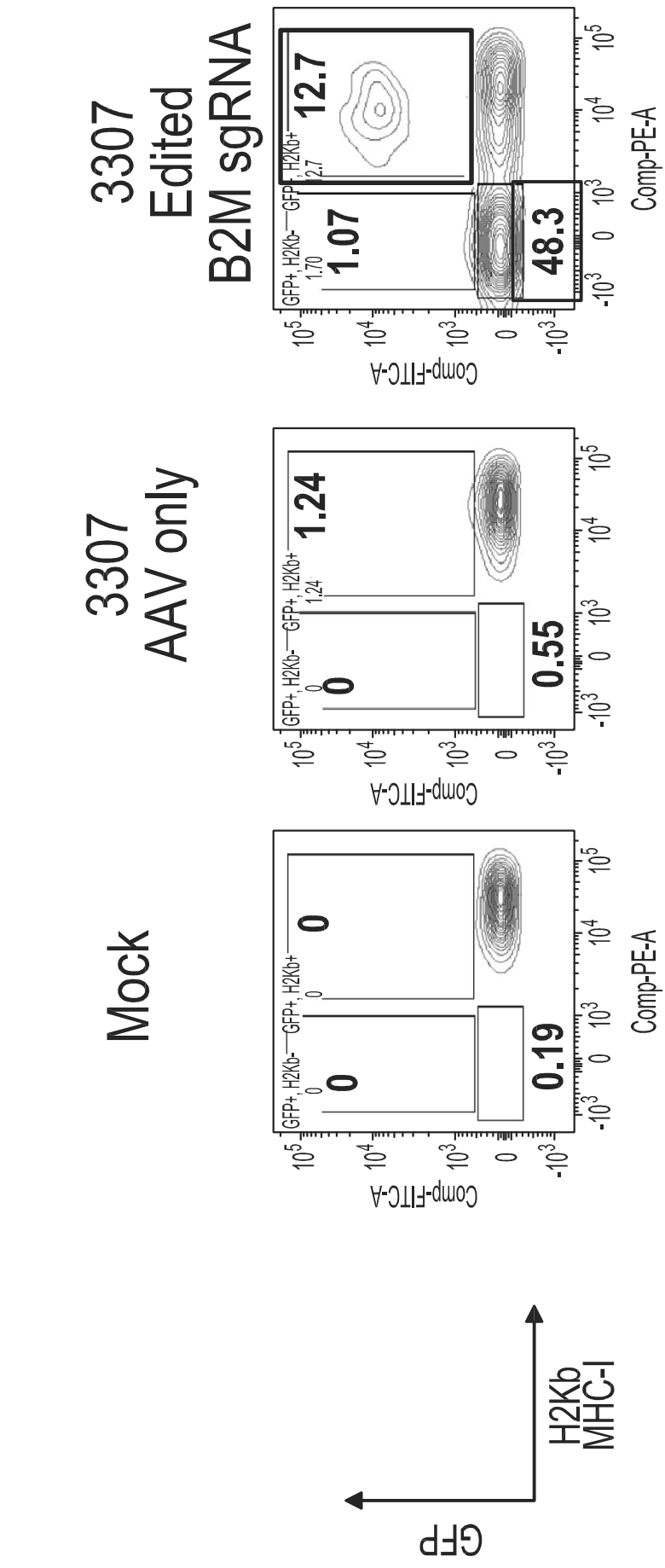
3307
Edited
B2M sgRNA
3307
AAV only
Mock
GFP
H2Kb
MHC-I
Comp-FITC-A
Comp-PE-A
GFP+, H2Kb-
GFP+, H2Kb+
0
0.19
0.55
1.24
48.3
1.07
12.7

FIG. 11

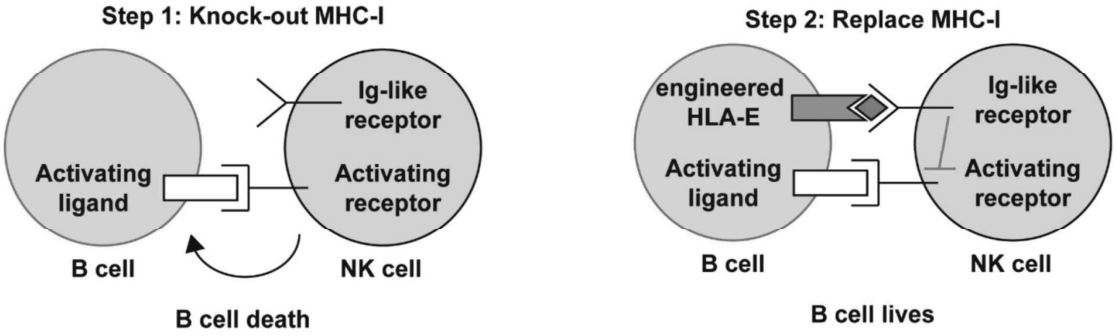

FIG. 12A

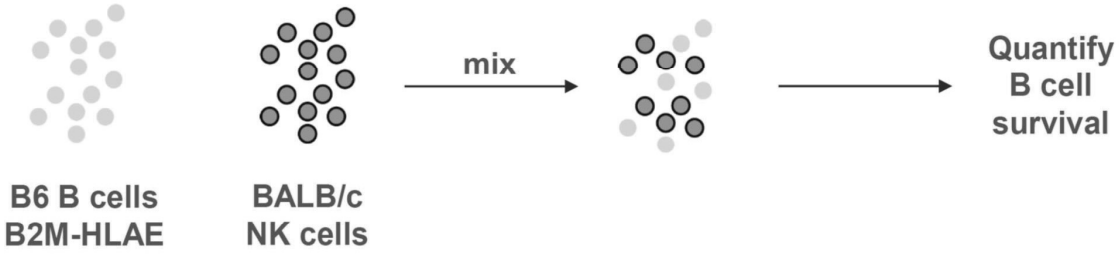

FIG. 12B

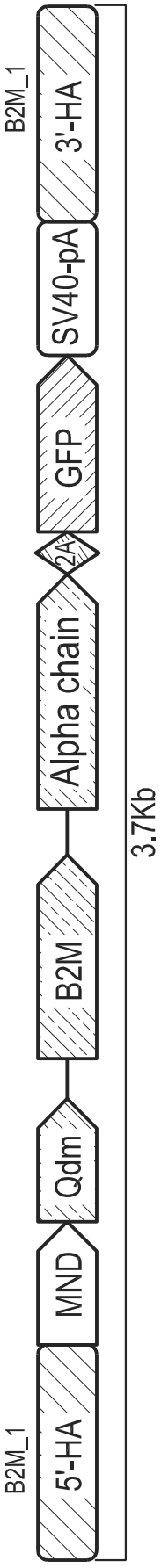

FIG. 13

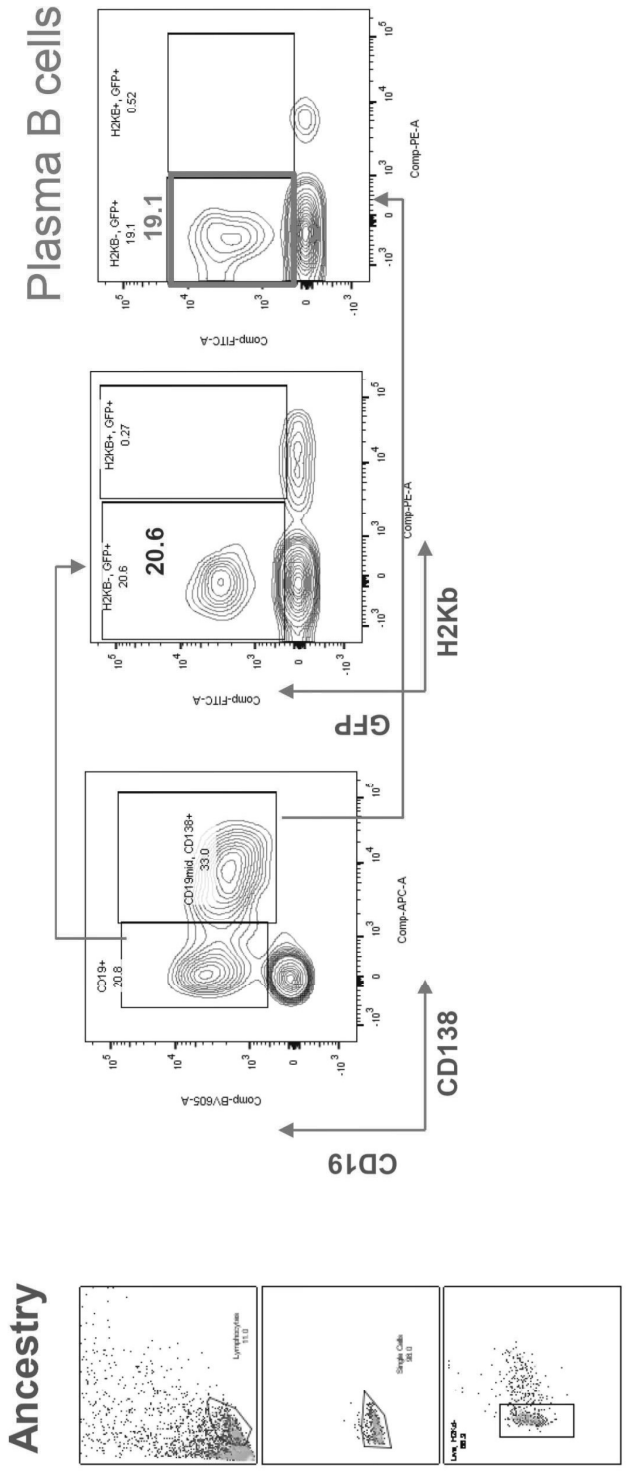
Ancestry
Plasma B cells
CD19
CD138
GFP
H2Kb
CD19+
20.8
CD19mid, CD138+
33.0
Comp-BV605-A
Comp-APC-A
H2KB-, GFP+
20.6
20.6
H2KB+, GFP+
0.27
H2KB-, GFP+
19.1
19.1
H2KB+, GFP+
0.52
Comp-FITC-A
Comp-PE-A

FIG. 14

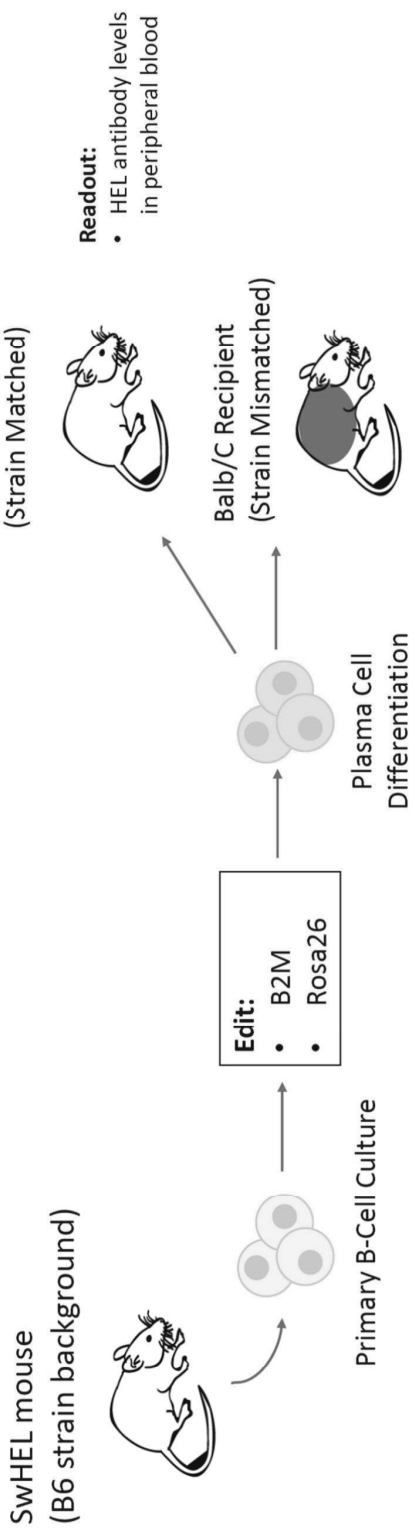
SwHEL mouse
(B6 strain background)
Primary B-Cell Culture
Edit:
• B2M
• Rosa26
Plasma Cell
Differentiation
B6 Recipient
(Strain Matched)
Balb/C Recipient
(Strain Mismatched)
Readout:
• HEL antibody levels
in peripheral blood

FIG. 15A


Primary Murine SW/HEL
C57/B6 Plasma Cells
Transplant Recipients
Balb/C Mice
Engineering Steps:
• MHC-I and/or MHC-II Knockout (CRISPR)
• HLA-E Trimer expression (LV)
• CD24 or CD47
Outcome measures:
• Allogeneic cell survival
• HEL mAb production

FIG. 15B

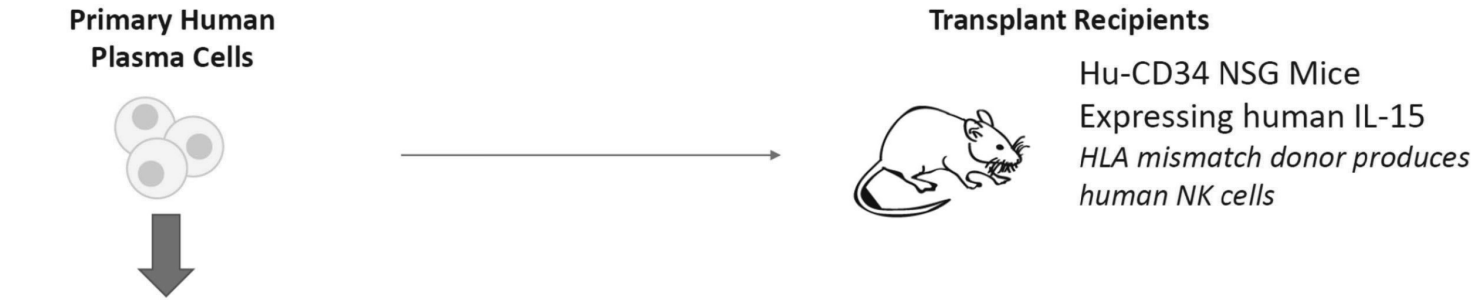
Primary Human
Plasma Cells
Transplant Recipients
Hu-CD34 NSG Mice
Expressing human IL-15
HLA mismatch donor produces
human NK cells
Engineering Steps:
• MHC-I and/or MHC-II Knockout (CRISPR)
• HLA-E Trimer expression (LV)
• CD24 or CD47
• Luciferase reporter
Outcome measures:
• Allogeneic cell survival
• Endogenous mAb production
• Luciferase tracking

FIG. 16