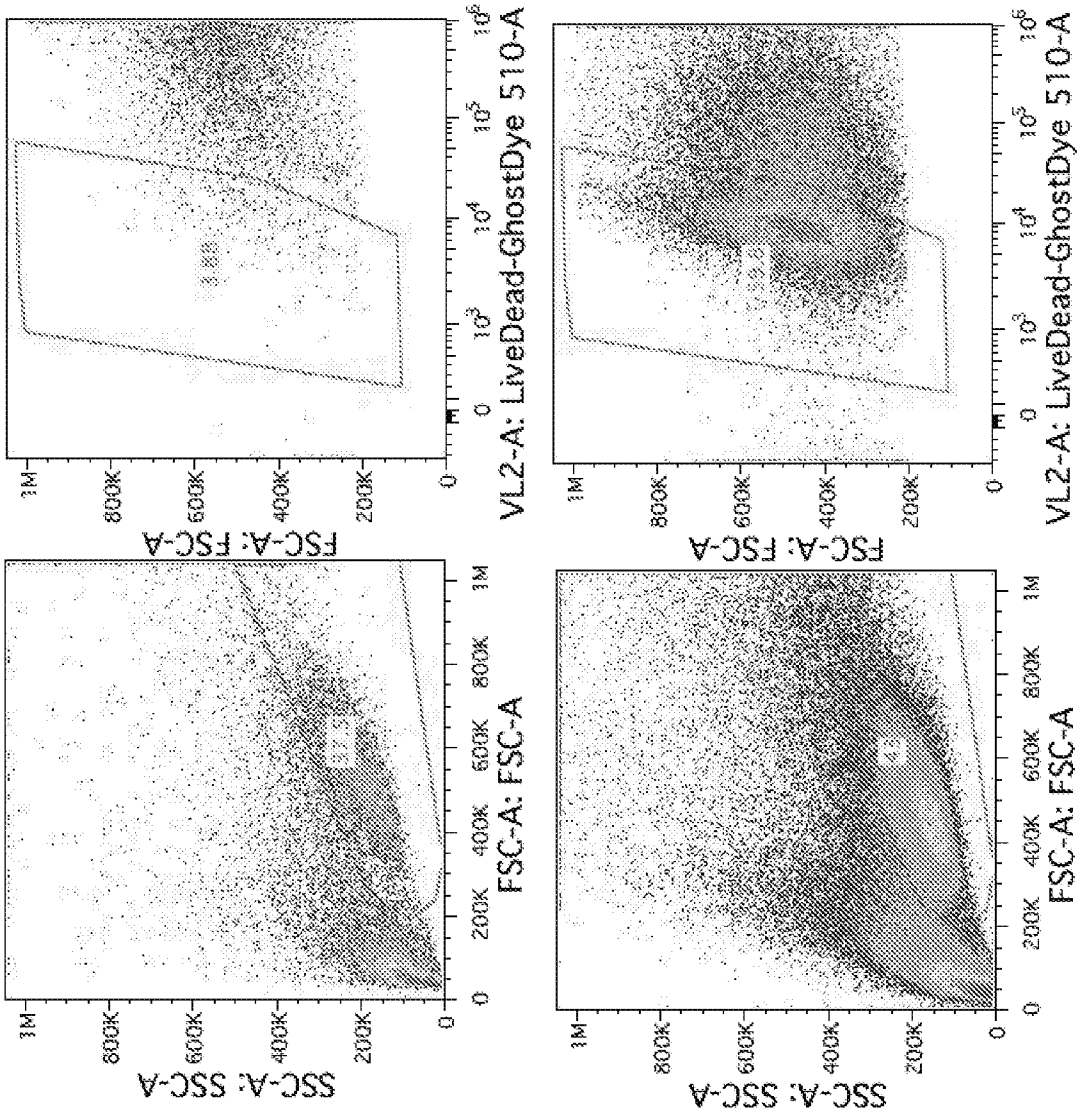

Plasmid Only
Plasmid + RNP
SSC-A: SSC-A
FSC-A: FSC-A
FSC-A: FSC-A
VL2-A: LiveDead-GhostDye 510-A
R1
R2

Figure 1

RNP + Plasmid Rescues Cell Viability

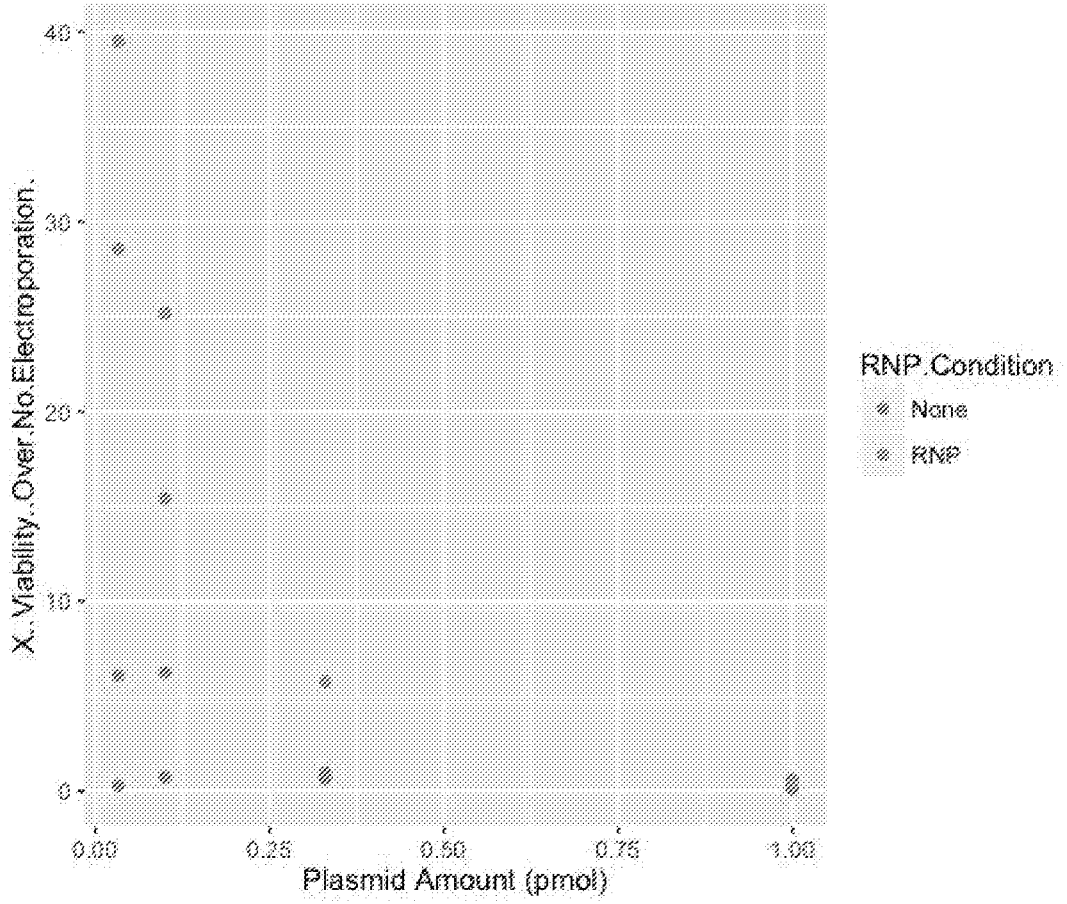


Figure 2

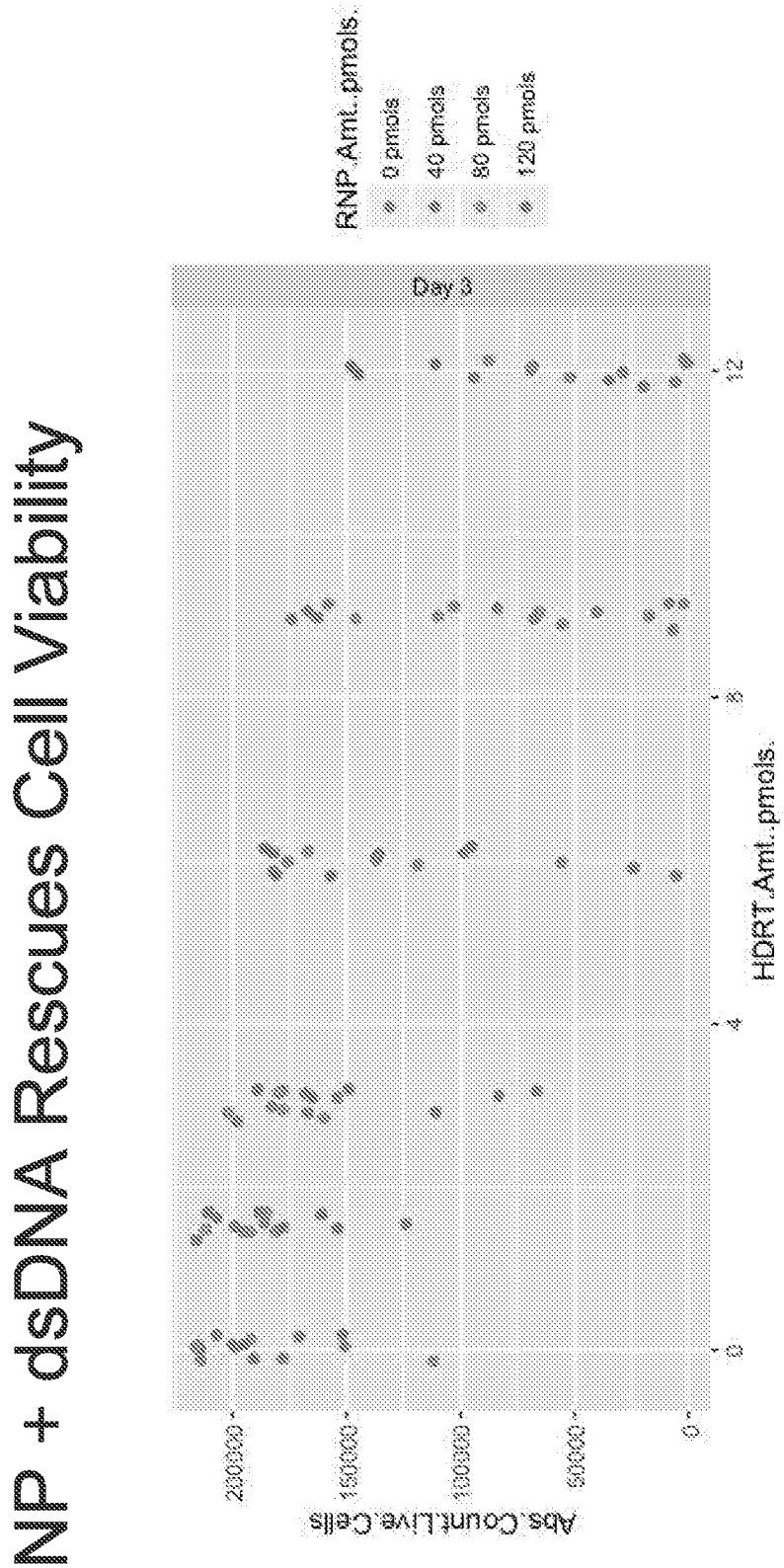

RNP + dsDNA Rescues Cell Viability
Abs.Count.Live.Cells
HDRT.Amt..pmols.
Day 3
RNP.Amt..pmols.
0 pmols
40 pmols
80 pmols
120 pmols

Figure 3

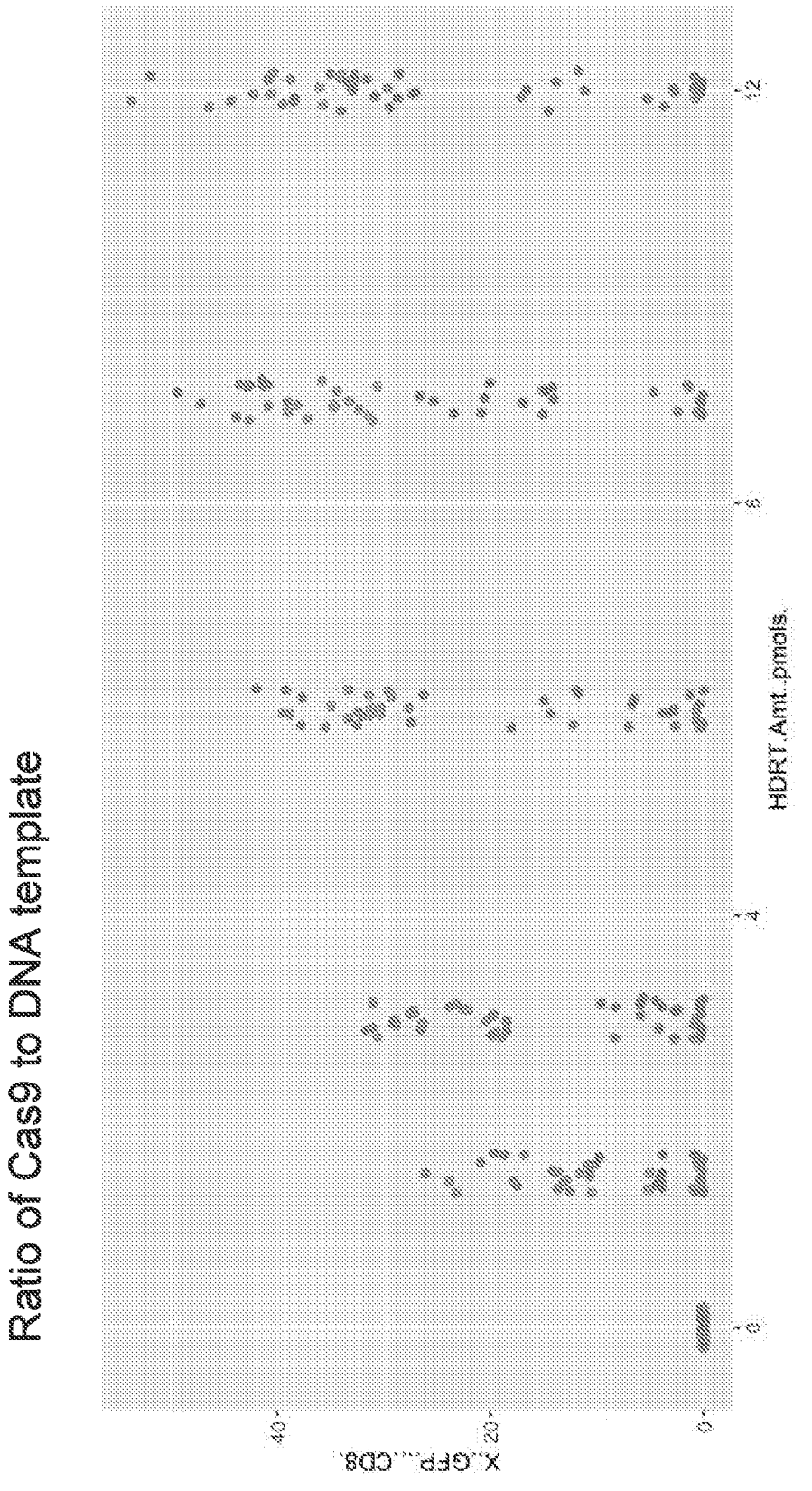

Ratio of Cas9 to DNA template
X..GFP...CDS.
40
20
0
0
4
8
12
HDRT.Amt..pmols.
RNP.Amt..pmols.
0 pmols
40 pmols
80 pmols
120 pmols

Figure 4

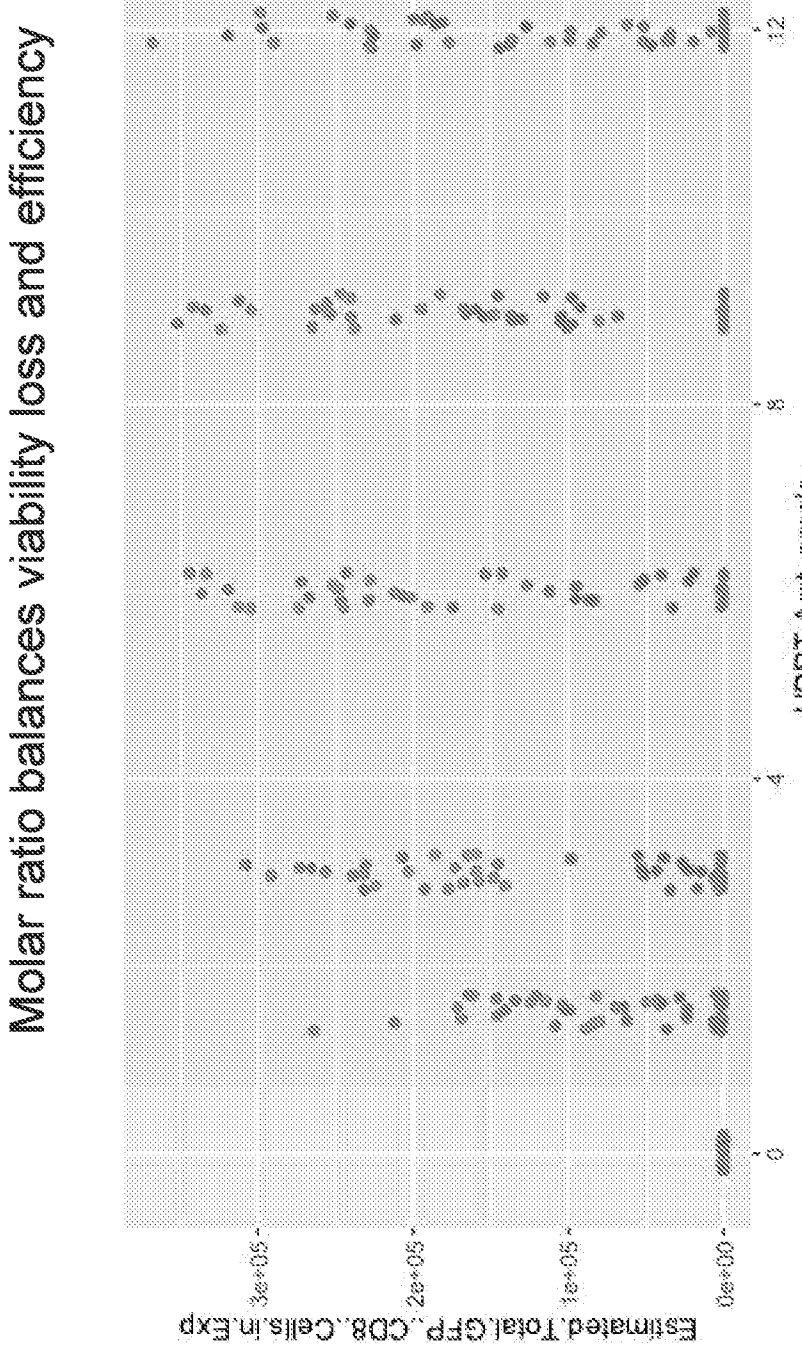

Molar ratio balances viability loss and efficiency
Estimated.Total.GFP..CD8..Cells.in.Exp
0e+00
1e+05
2e+05
3e+05
0
4
8
12
HDRT.Amt..pmols.
RNP.Amt..pmols.
0 pmols
40 pmols
80 pmols
120 pmols

Figure 5

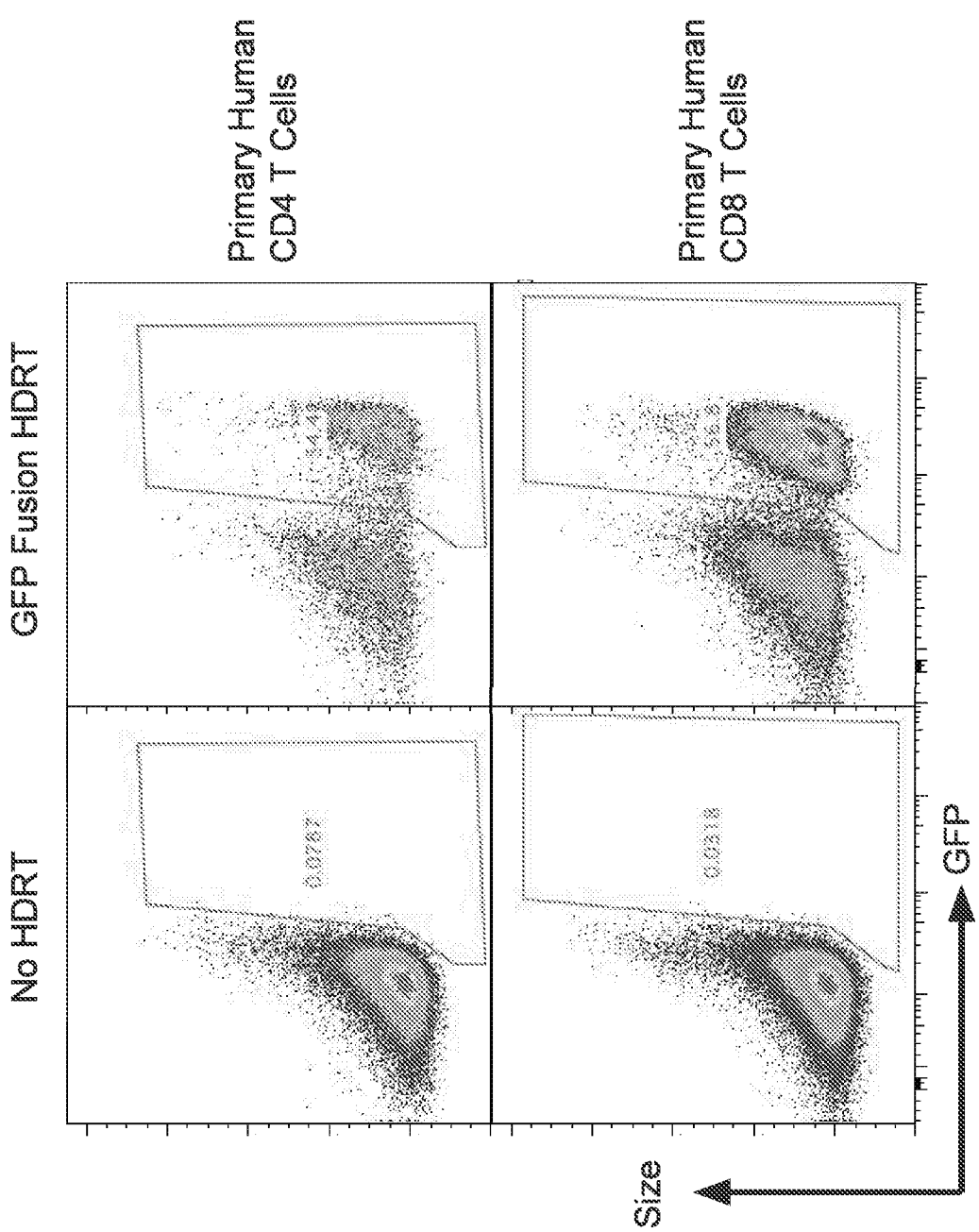

No HDRT
GFP Fusion HDRT
Primary Human CD4 T Cells
Primary Human CD8 T Cells
Size
GFP

Figure 6

But- dsDNA templates cause small amounts of off target integrations

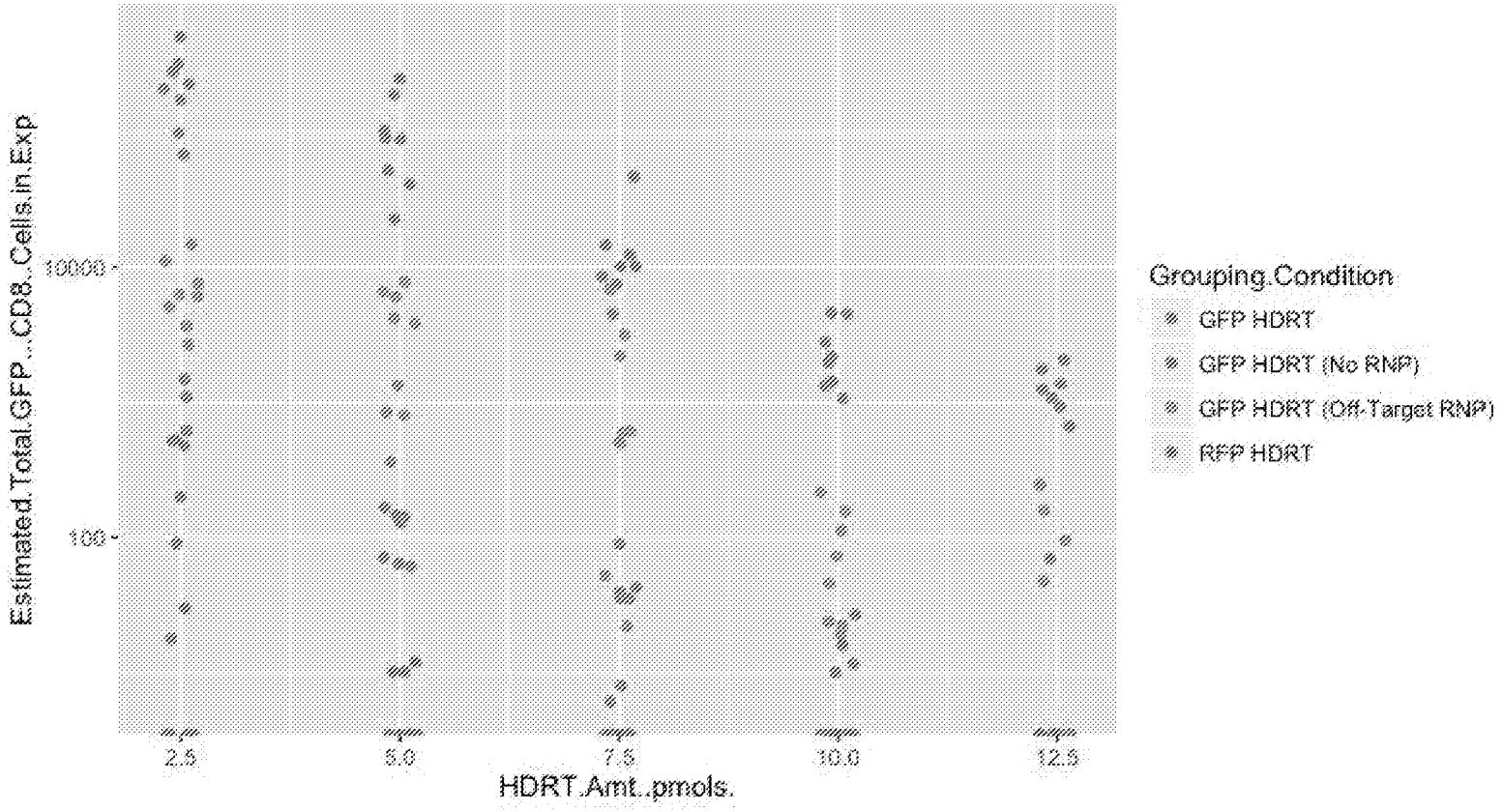


Figure 7

Reduced off-target integrations using long ssDNA

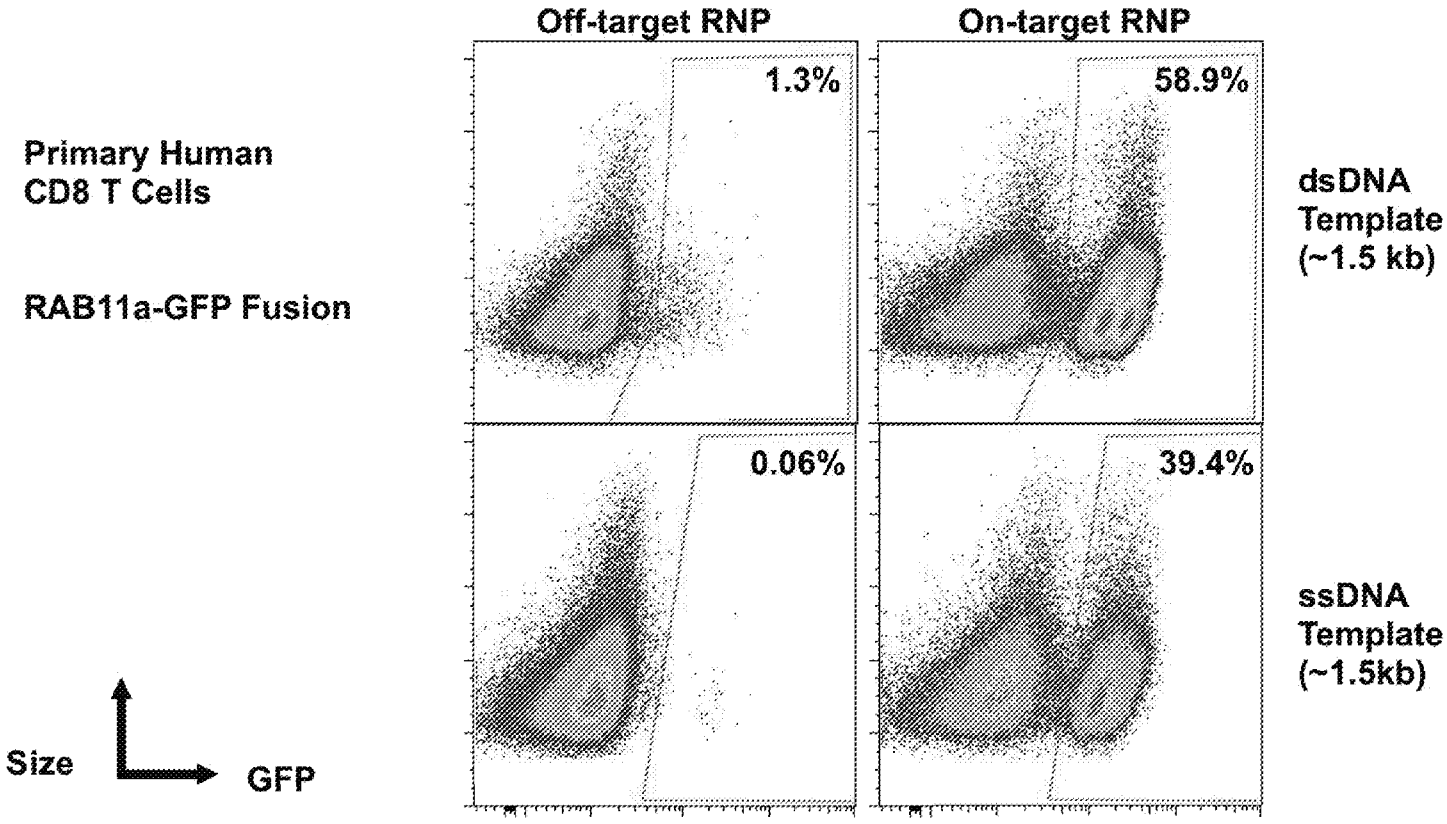


Figure 8

UCSF | Marson Lab

Cas9 nickases prevent off-target ds breaks, still allow high efficiency insertions

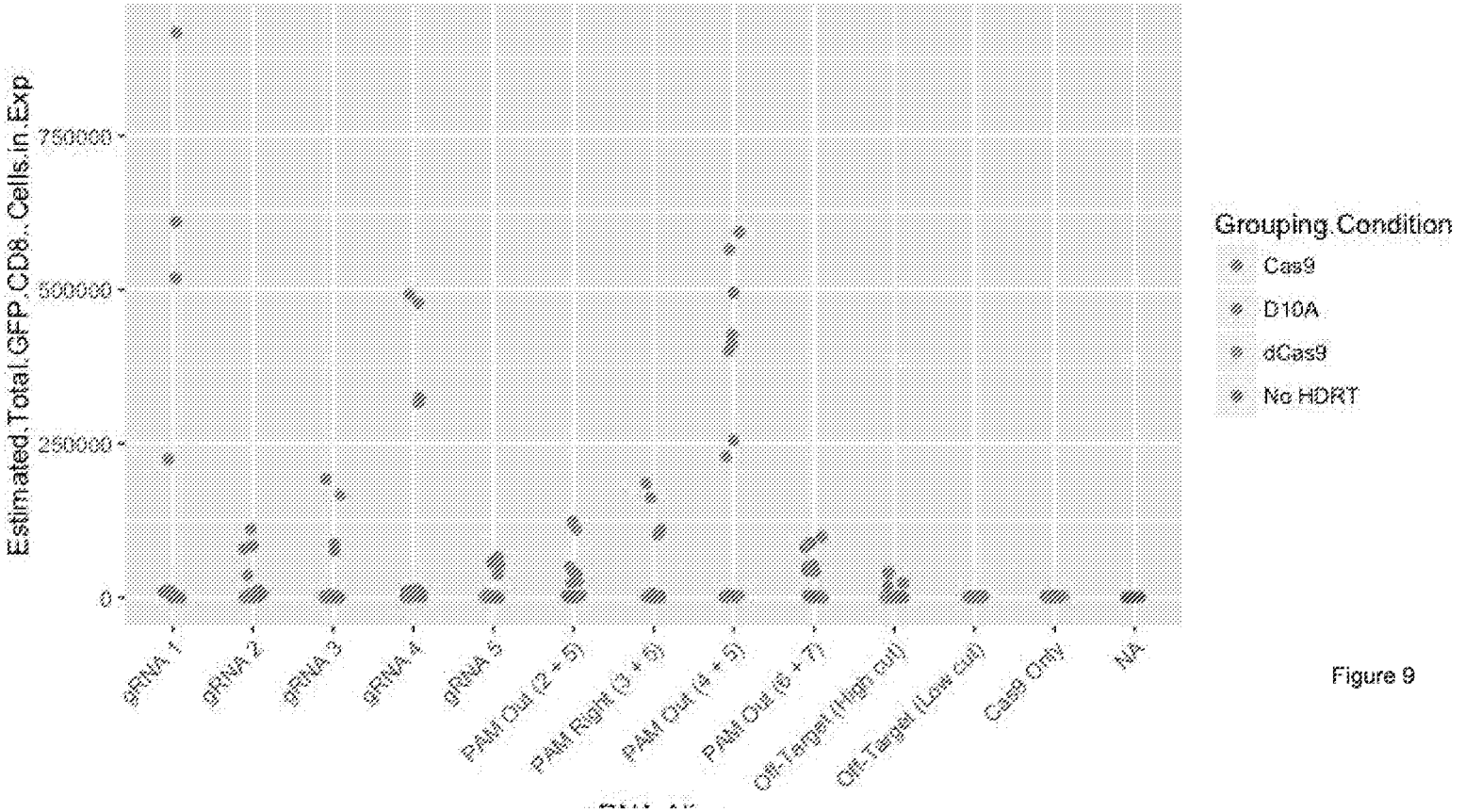


Figure 9

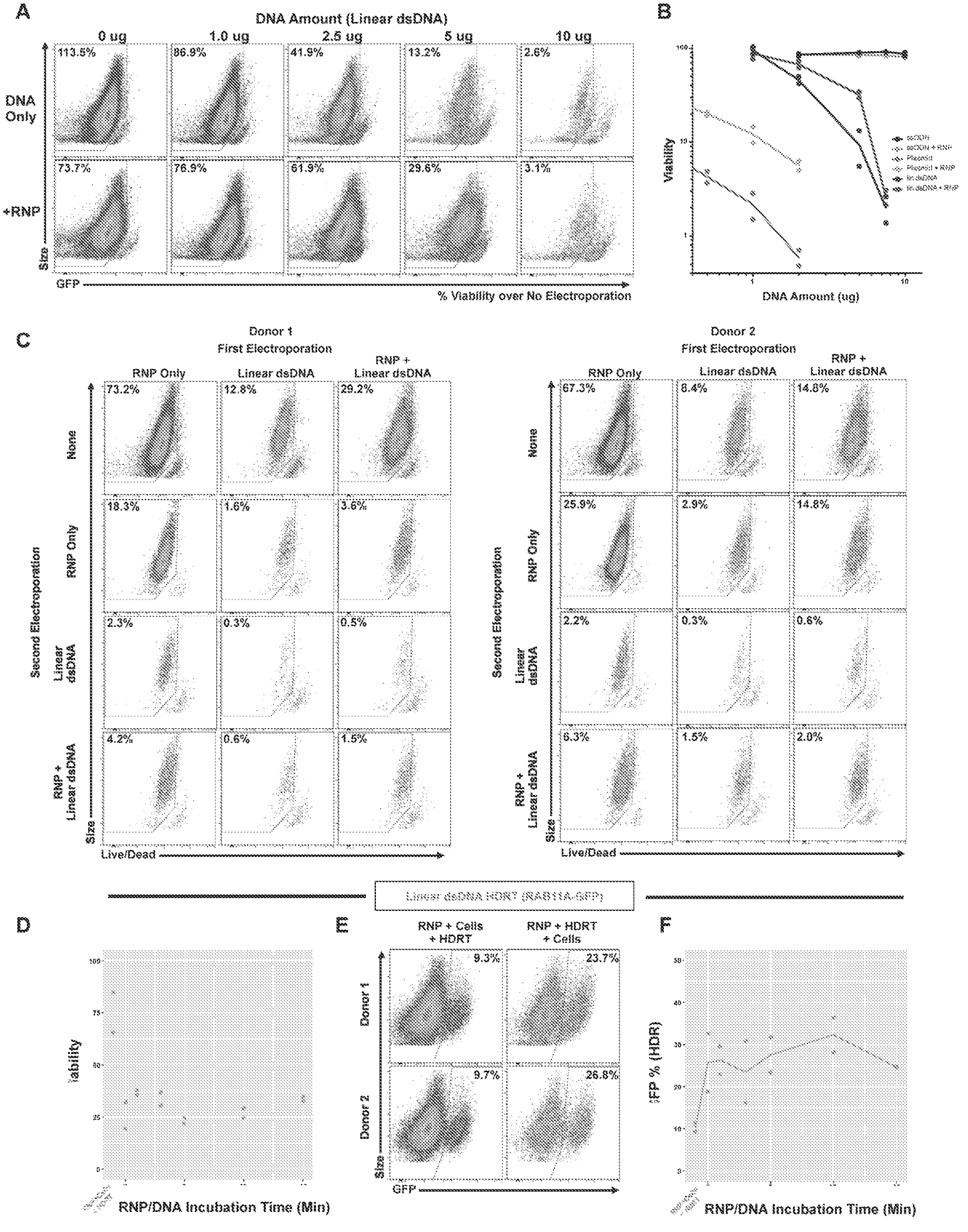

A
DNA Amount (Linear dsDNA)
0 ug
1.0 ug
2.5 ug
5 ug
10 ug
DNA Only
+RNP
113.5%
86.9%
41.9%
13.2%
2.6%
73.7%
76.9%
61.9%
29.6%
3.1%
Size
GFP
% Viability over No Electroporation
B
Viability
DNA Amount (ug)
ssODN
ssODN + RNP
Plasmid
Plasmid + RNP
lin dsDNA
lin dsDNA + RNP
C
Donor 1
First Electroporation
RNP Only
Linear dsDNA
RNP + Linear dsDNA
Second Electroporation
None
RNP Only
Linear dsDNA
RNP + Linear dsDNA
73.2%
12.8%
29.2%
18.3%
1.6%
3.6%
2.3%
0.3%
0.5%
4.2%
0.6%
1.5%
Size
Live/Dead
Donor 2
First Electroporation
RNP Only
Linear dsDNA
RNP + Linear dsDNA
Second Electroporation
None
RNP Only
Linear dsDNA
RNP + Linear dsDNA
67.3%
8.4%
14.8%
25.9%
2.9%
14.8%
2.2%
0.3%
0.6%
6.3%
1.5%
2.0%
Size
Live/Dead
Linear dsDNA HDRT (RAB11A-GFP)
D
Viability
RNP/DNA Incubation Time (Min)
E
RNP + Cells + HDRT
RNP + HDRT + Cells
Donor 1
Donor 2
9.3%
23.7%
9.7%
26.8%
Size
GFP
F
GFP % (HDR)
RNP/DNA Incubation Time (Min)

Figure 10

Figure 11

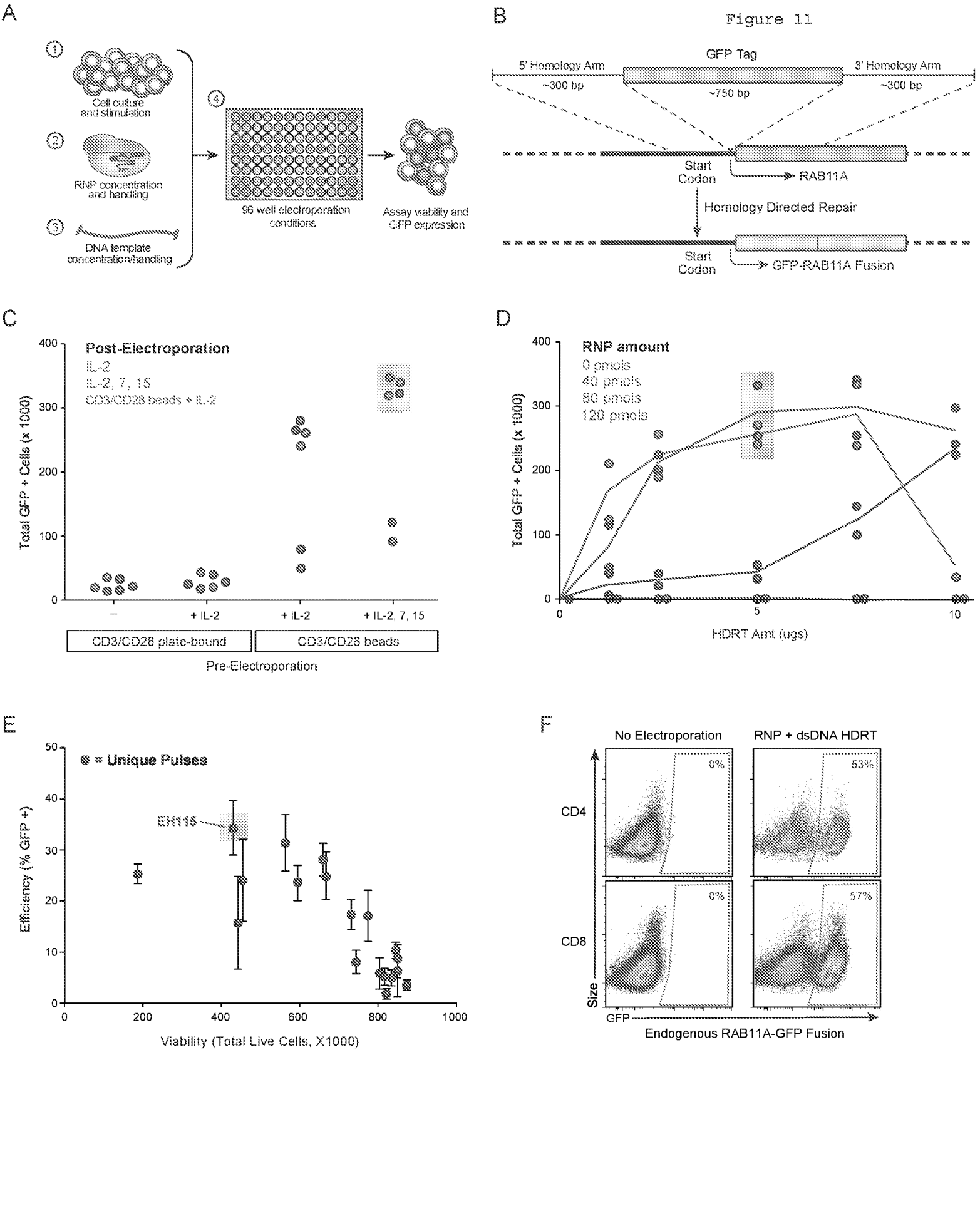
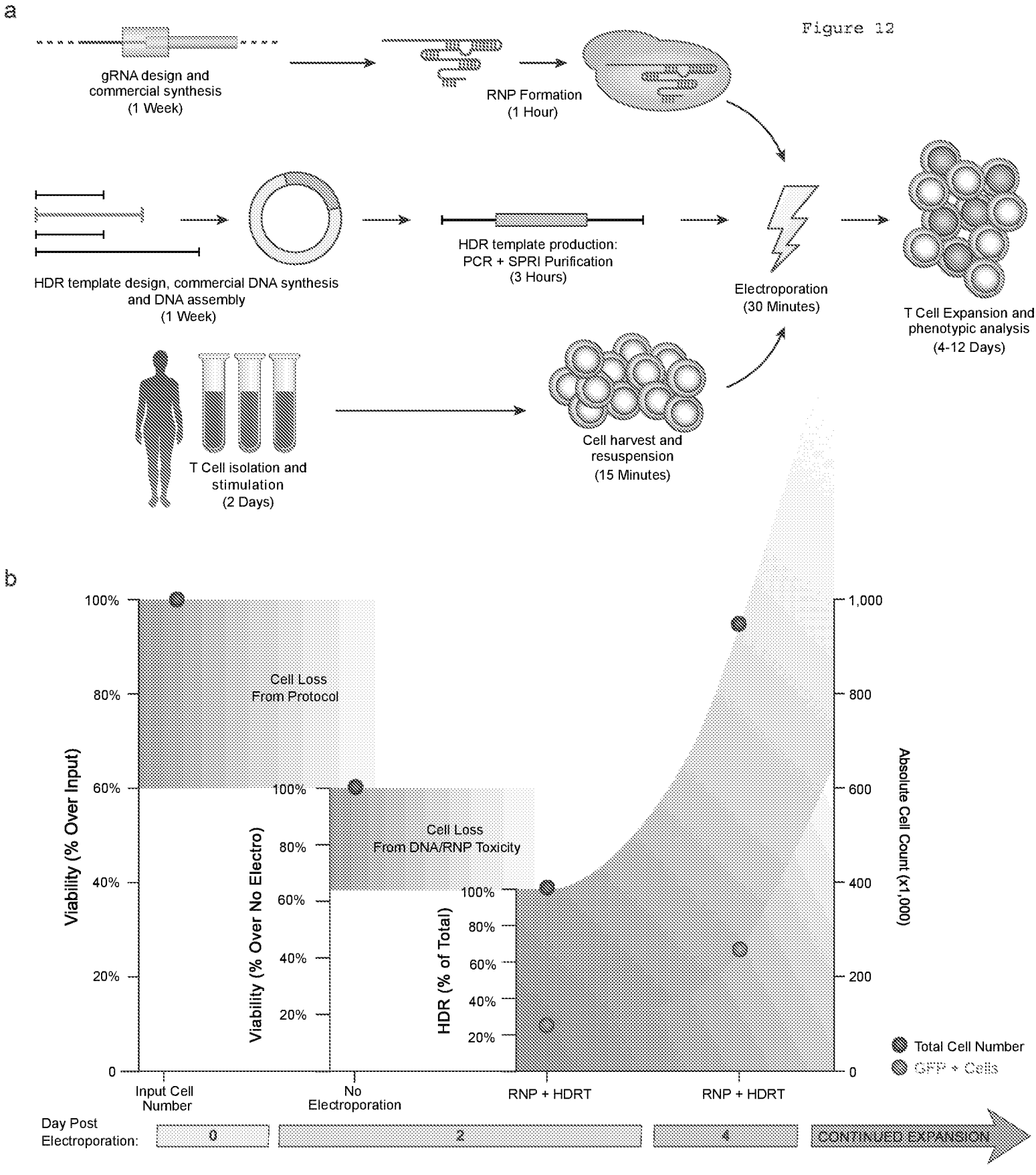

A
Cell culture and stimulation
RNP concentration and handling
DNA template concentration/handling
96 well electroporation conditions
Assay viability and GFP expression
B
5' Homology Arm
~300 bp
GFP Tag
~750 bp
3' Homology Arm
~300 bp
Start Codon
RAB11A
Homology Directed Repair
Start Codon
GFP-RAB11A Fusion
C
Post-Electroporation
IL-2
IL-2, 7, 15
CD3/CD28 beads + IL-2
Total GFP + Cells (x 1000)
– + IL-2 + IL-2 + IL-2, 7, 15
CD3/CD28 plate-bound
CD3/CD28 beads
Pre-Electroporation
D
RNP amount
0 pmols
40 pmols
80 pmols
120 pmols
Total GFP + Cells (x 1000)
HDRT Amt (ugs)
E
= Unique Pulses
EH115
Efficiency (% GFP +)
Viability (Total Live Cells, X1000)
F
No Electroporation
RNP + dsDNA HDRT
0%
53%
0%
57%
CD4
CD8
Size
GFP
Endogenous RAB11A-GFP Fusion

Figure 12


a
gRNA design and commercial synthesis (1 Week)
RNP Formation (1 Hour)
HDR template design, commercial DNA synthesis and DNA assembly (1 Week)
HDR template production: PCR + SPRI Purification (3 Hours)
Electroporation (30 Minutes)
T Cell Expansion and phenotypic analysis (4-12 Days)
T Cell isolation and stimulation (2 Days)
Cell harvest and resuspension (15 Minutes)
b
Viability (% Over Input)
Cell Loss From Protocol
Viability (% Over No Electro)
Cell Loss From DNA/RNP Toxicity
HDR (% of Total)
Absolute Cell Count (x1,000)
Total Cell Number
GFP + Cells
Input Cell Number
No Electroporation
RNP + HDRT
RNP + HDRT
Day Post Electroporation:
0
2
4
CONTINUED EXPANSION

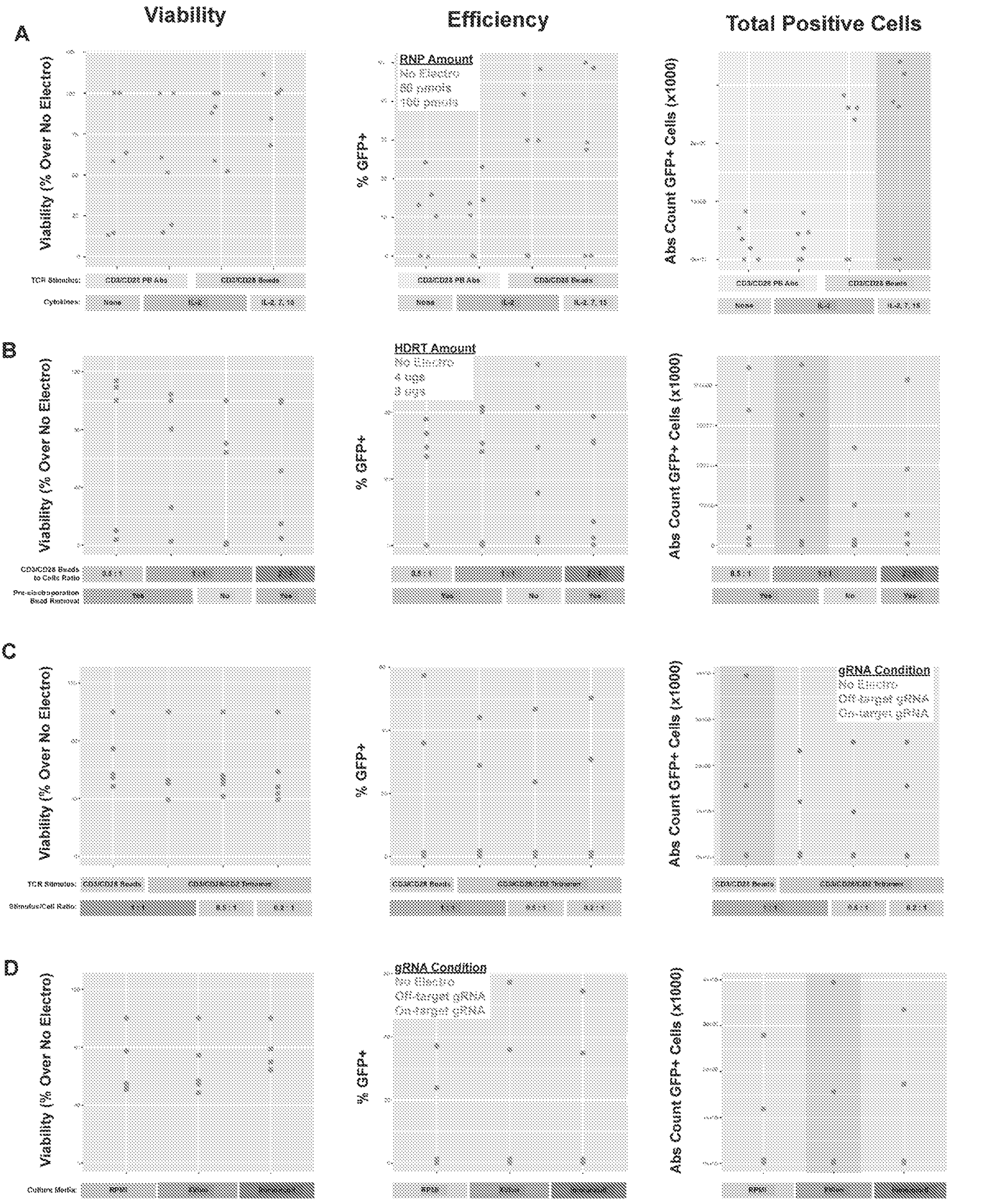

Viability
Efficiency
Total Positive Cells
A
B
C
D
Viability (% Over No Electro)
% GFP+
Abs Count GFP+ Cells (x1000)
RNP Amount
No Electro
80 pmols
160 pmols
HDRT Amount
No Electro
4 ugs
8 ugs
gRNA Condition
No Electro
Off-target gRNA
On-target gRNA
TCR Stimulus:
Cytokines:
CD3/CD28 Beads to Cells Ratio
Pre-electroporation Bead Removal
Stimulus/Cell Ratio:
Culture Media:

Figure 13

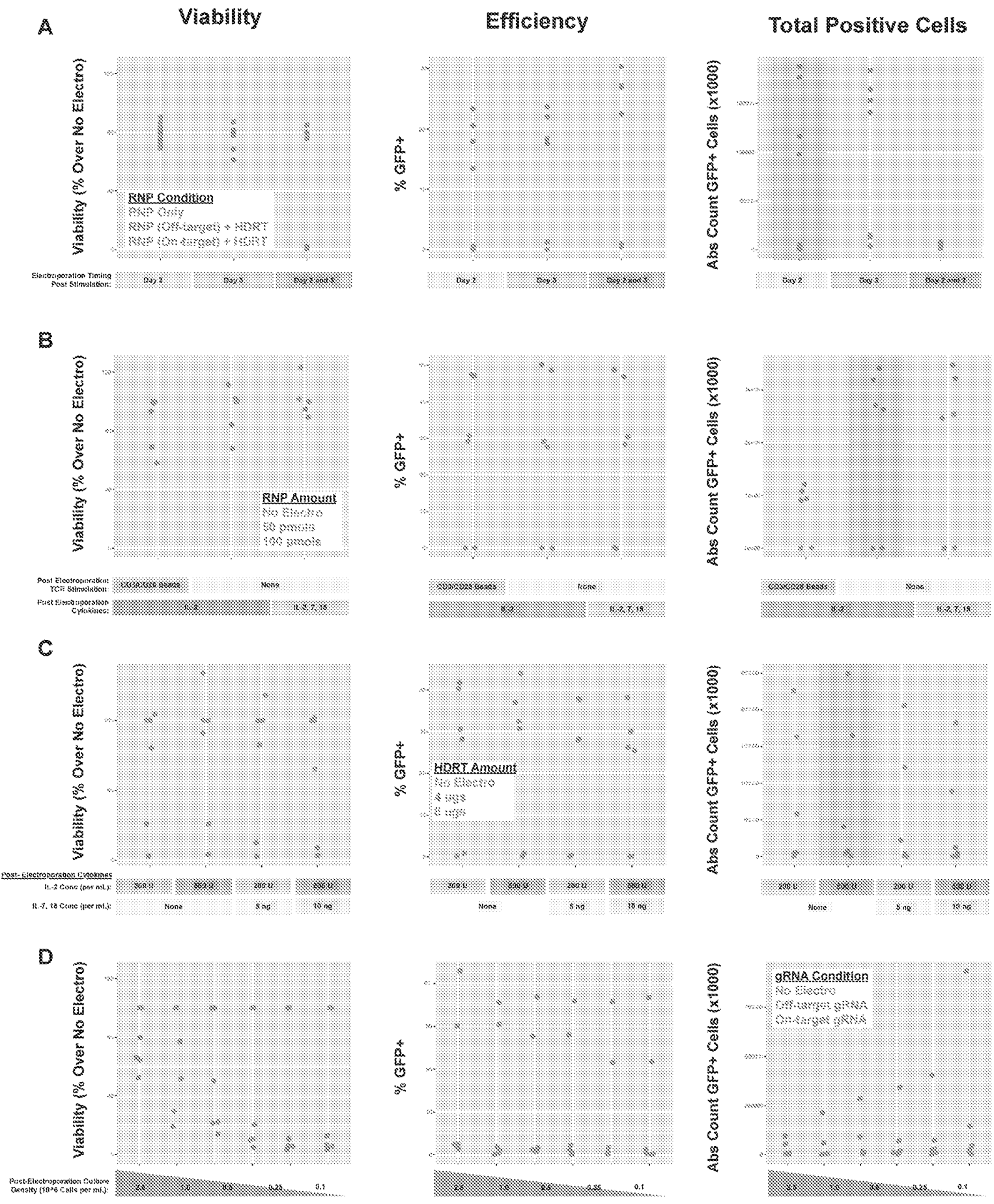
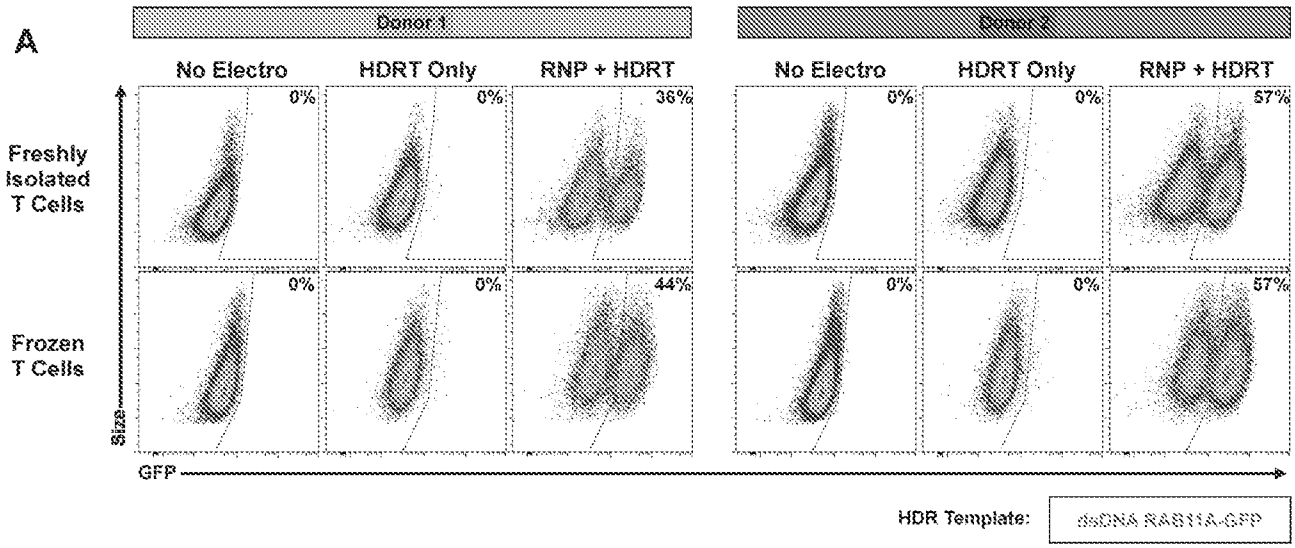

A
Viability
Efficiency
Total Positive Cells
Viability (% Over No Electro)
% GFP+
Abs Count GFP+ Cells (x1000)
RNP Condition
RNP Only
RNP (Off-target) + HDRT
RNP (On-target) + HDRT
Electroporation Timing Post Stimulation:
Day 2
Day 3
Day 2 and 3
B
RNP Amount
No Electro
50 pmols
100 pmols
Post Electroporation TCR Stimulation:
CD3/CD28 Beads
None
Post Electroporation Cytokines:
IL-2
IL-2, 7, 15
C
HDRT Amount
No Electro
4 ugs
8 ugs
Post- Electroporation Cytokines
IL-2 Conc (per mL):
IL-7, 15 Conc (per mL):
None
5 ng
10 ng
D
gRNA Condition
No Electro
Off-target gRNA
On-target gRNA
Post-Electroporation Culture Density (10^6 Cells per mL):
0.1

Figure 14


A
Donor 1
Donor 2
No Electro
HDRT Only
RNP + HDRT
No Electro
HDRT Only
RNP + HDRT
Freshly Isolated T Cells
Frozen T Cells
0%
0%
36%
0%
0%
57%
0%
0%
44%
0%
0%
57%
Size
GFP
HDR Template:
dsDNA RAB11A-GFP

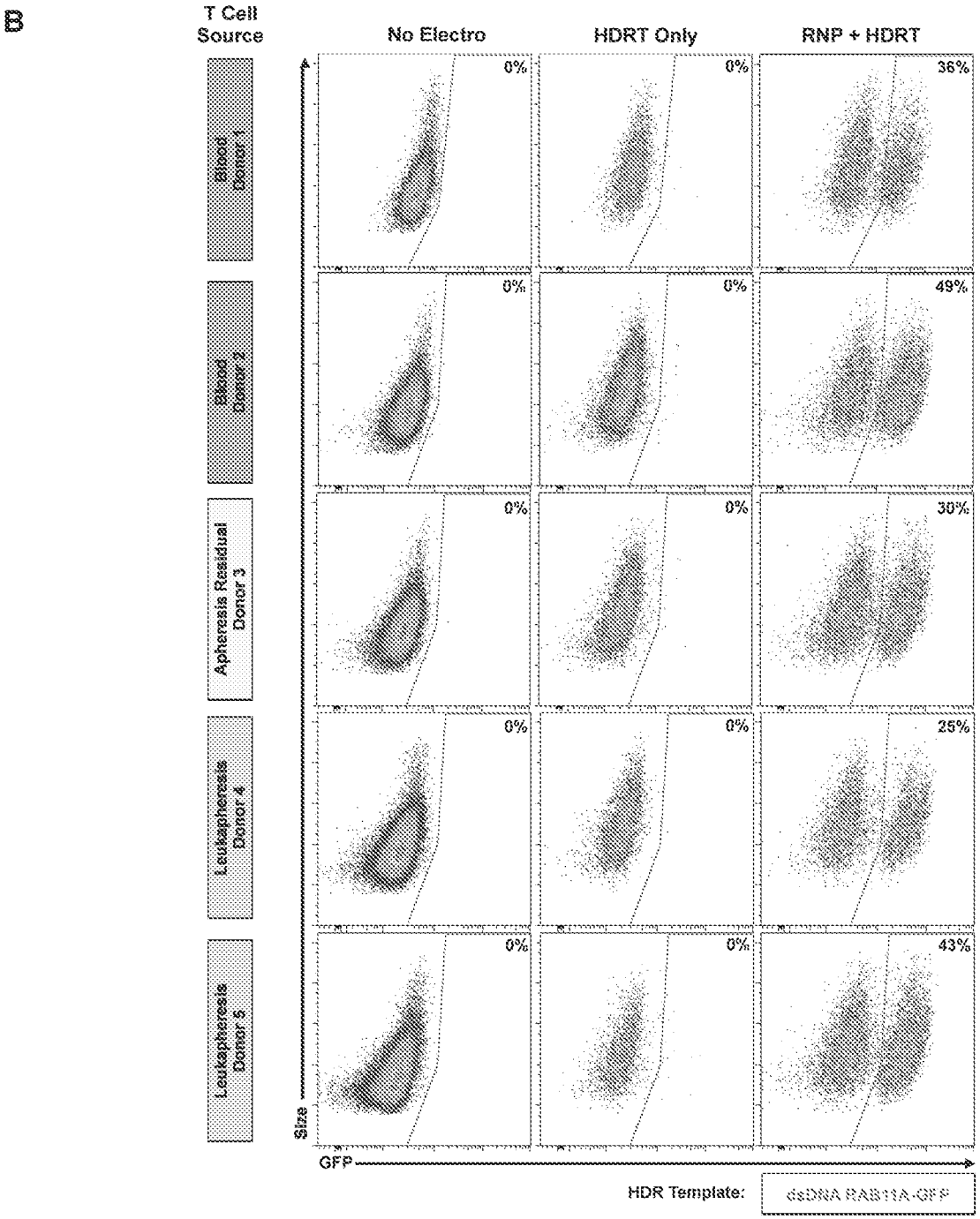
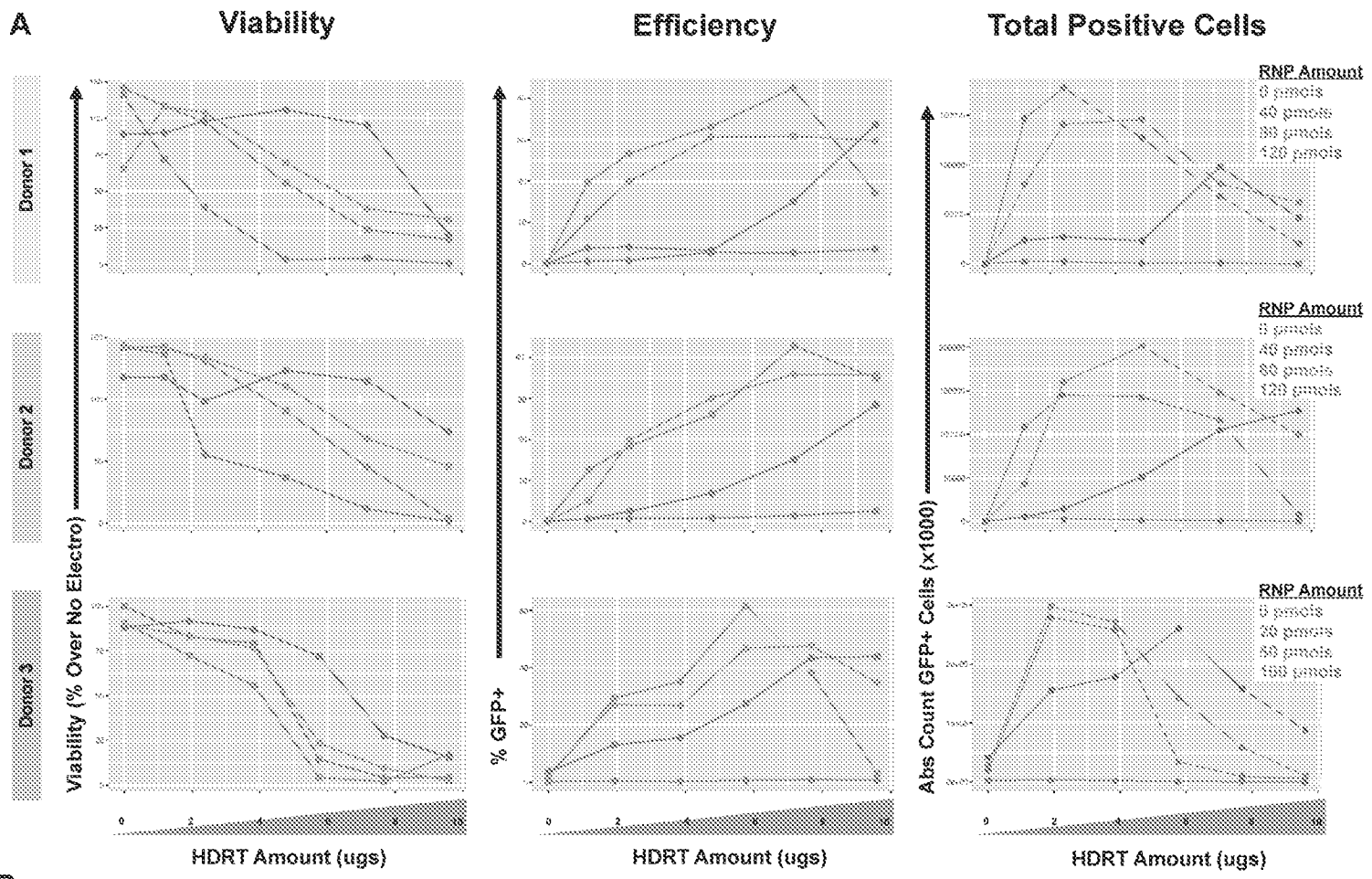

B
T Cell Source
No Electro
HDRT Only
RNP + HDRT
Blood Donor 1
Blood Donor 2
Apheresis Residual Donor 3
Leukapheresis Donor 4
Leukapheresis Donor 5
0%
0%
36%
0%
0%
49%
0%
0%
38%
0%
0%
25%
0%
0%
43%
Size
GFP
HDR Template:
dsDNA RAB11A-GFP

Figure 15


A
Viability
Efficiency
Total Positive Cells
Donor 1
Donor 2
Donor 3
Viability (% Over No Electro)
% GFP+
Abs Count GFP+ Cells (x1000)
RNP Amount
0 pmols
40 pmols
80 pmols
120 pmols
RNP Amount
0 pmols
40 pmols
80 pmols
120 pmols
RNP Amount
0 pmols
20 pmols
50 pmols
100 pmols
HDRT Amount (ugs)
HDRT Amount (ugs)
HDRT Amount (ugs)

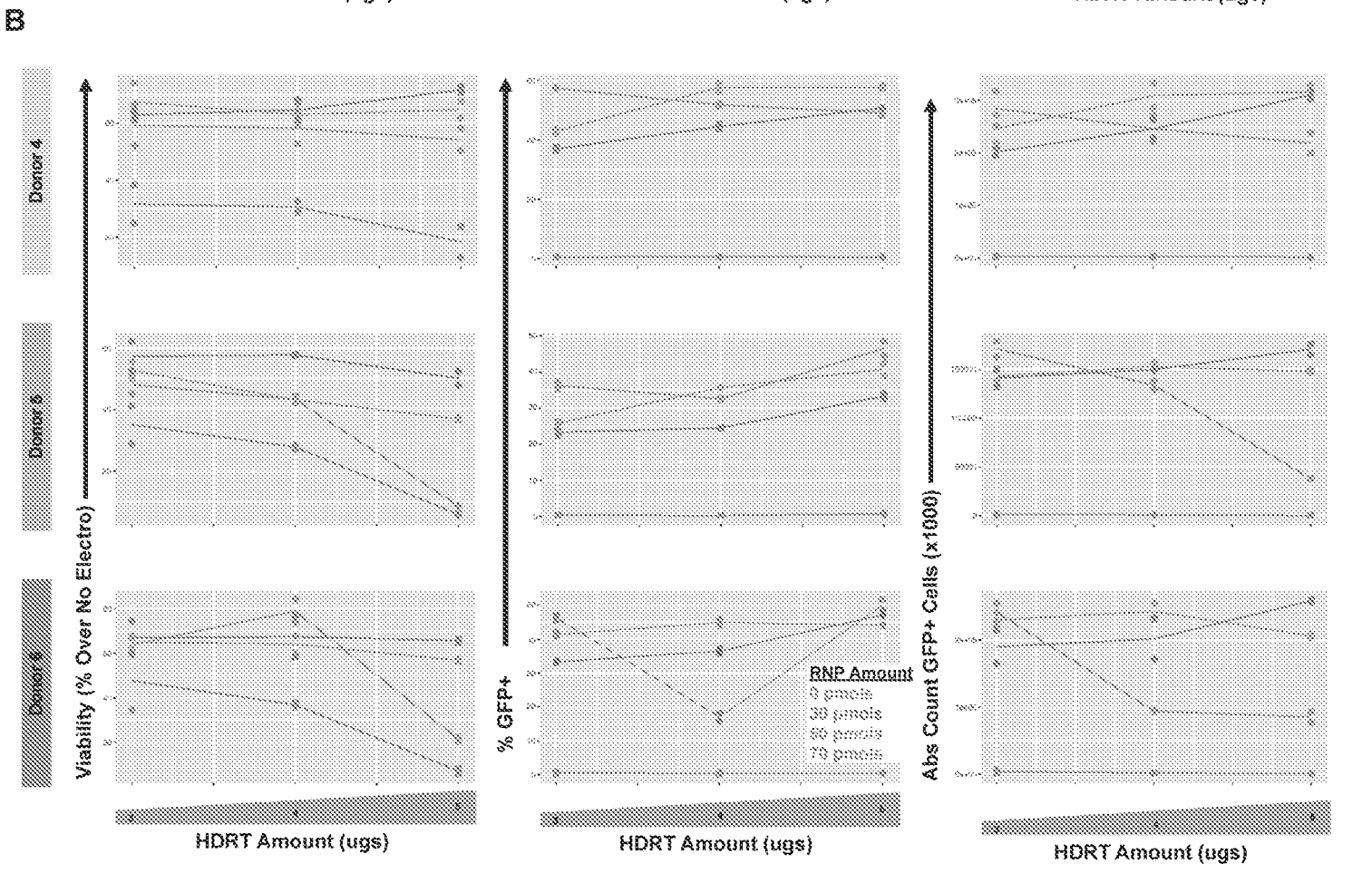
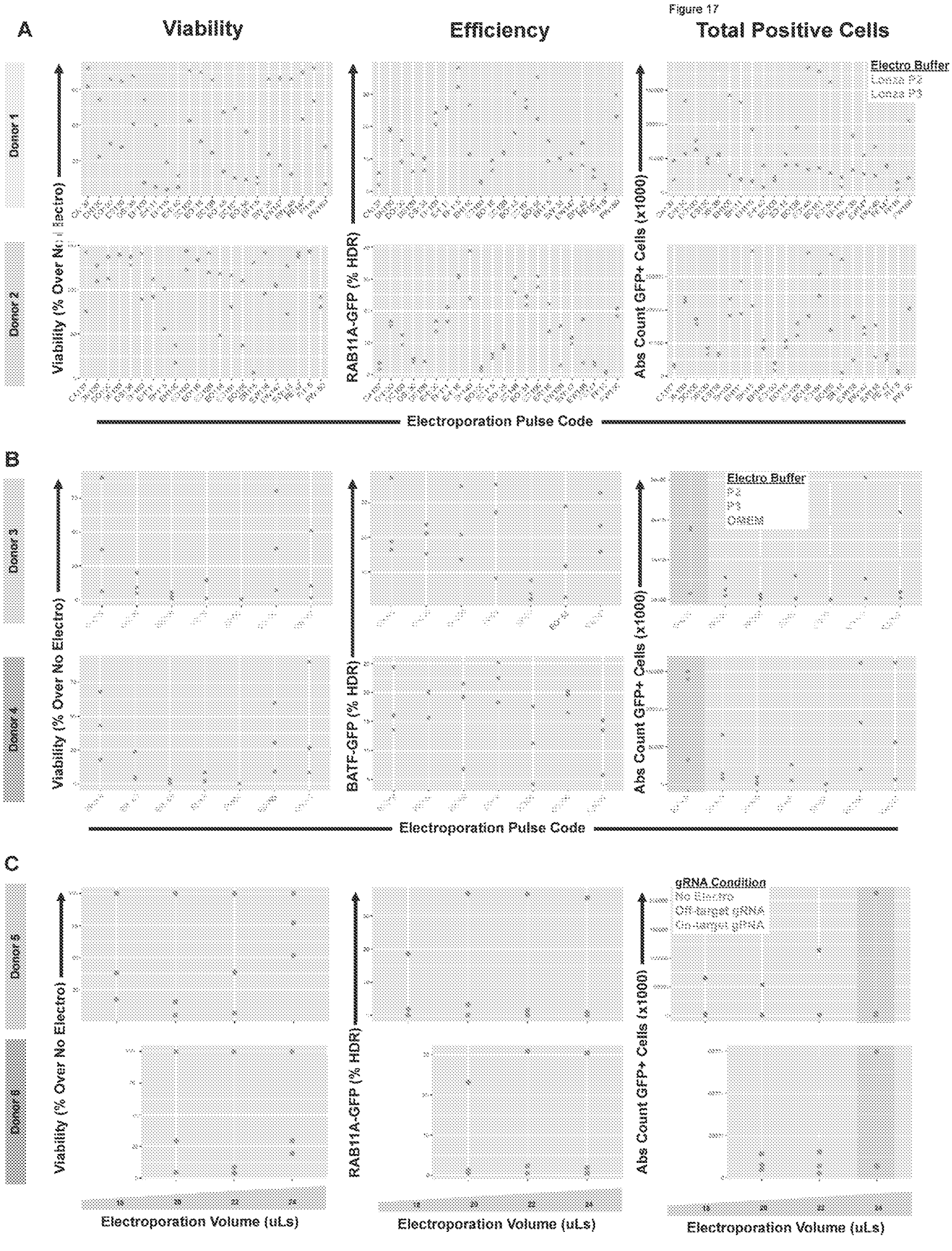

B
Donor 4
Donor 5
Donor 6
Viability (% Over No Electro)
% GFP+
Abs Count GFP+ Cells (x1000)
RNP Amount
0 pmols
30 pmols
50 pmols
70 pmols
HDRT Amount (ugs)
HDRT Amount (ugs)
HDRT Amount (ugs)

Figure 16

Figure 17


A
Viability
Efficiency
Total Positive Cells
Donor 1
Donor 2
Viability (% Over No Electro)
RAB11A-GFP (% HDR)
Abs Count GFP+ Cells (x1000)
Electro Buffer
Lonza P2
Lonza P3
Electroporation Pulse Code
B
Donor 3
Donor 4
Viability (% Over No Electro)
BATF-GFP (% HDR)
Abs Count GFP+ Cells (x1000)
Electro Buffer
P2
P3
OMEM
Electroporation Pulse Code
C
Donor 5
Donor 6
Viability (% Over No Electro)
RAB11A-GFP (% HDR)
Abs Count GFP+ Cells (x1000)
gRNA Condition
No Electro
Off-target gRNA
On-target gRNA
Electroporation Volume (uLs)
Electroporation Volume (uLs)
Electroporation Volume (uLs)

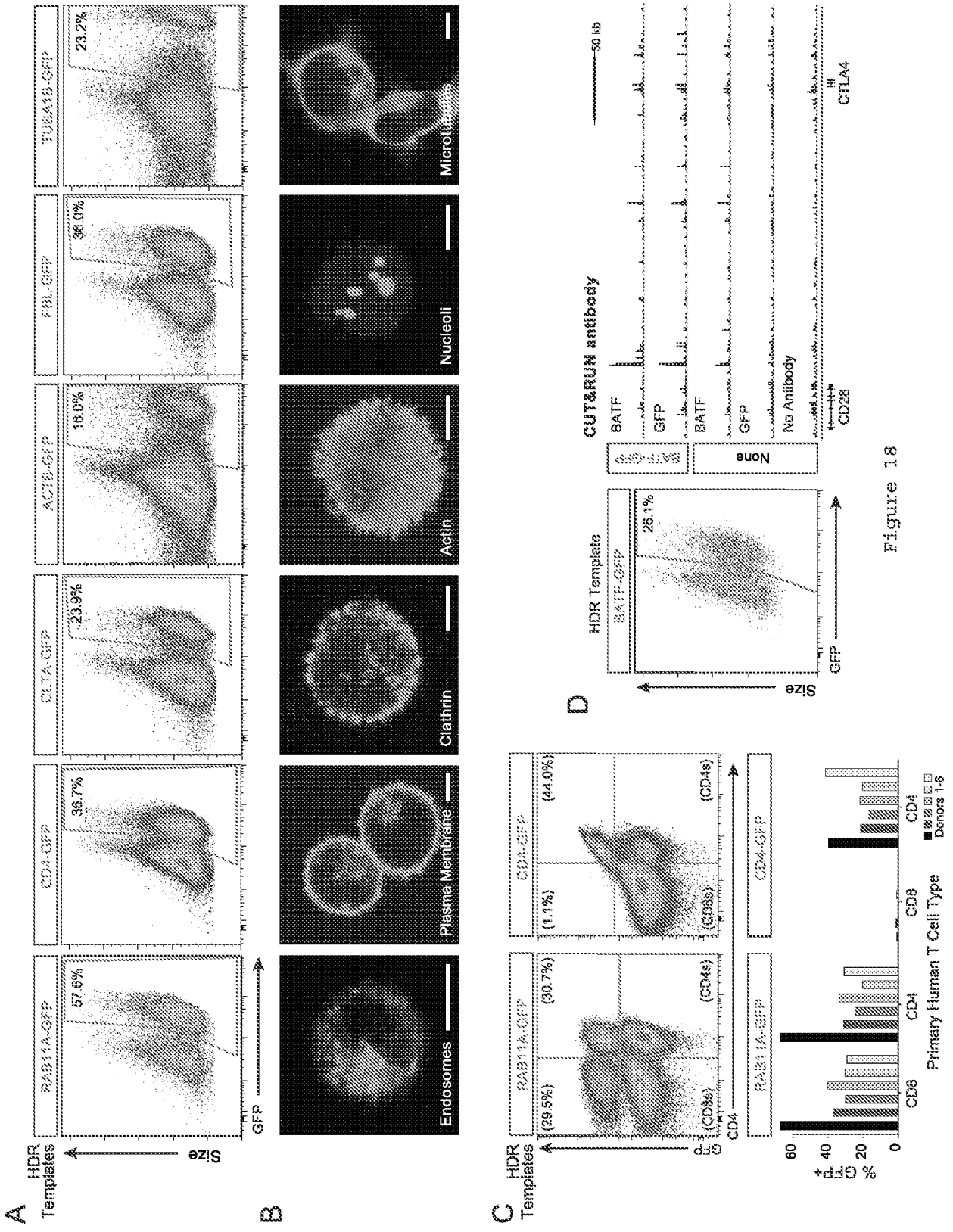

A
HDR Templates
RAB11A-GFP
CDA-GFP
CLTA-GFP
ACTB-GFP
FBL-GFP
TUBA1B-GFP
57.6%
36.7%
23.9%
16.0%
36.0%
23.2%
Size
GFP
B
Endosomes
Plasma Membrane
Clathrin
Actin
Nucleoli
Microtubules
C
HDR Templates
RAB11A-GFP
CDA-GFP
(29.5%)
(30.7%)
(1.1%)
(44.0%)
CD45
CD4
(CD8s)
(CD4s)
RAB11A-GFP
CD4-GFP
% GFP+
60
40
20
0
CD8
CD4
CD8
CD4
Primary Human T Cell Type
Donors 1-6
D
HDR Template
BATF-GFP
26.1%
Size
GFP
CUT&RUN antibody
BATF
GFP
No Antibody
None
50 kb
CD28
CTLA4

Figure 18

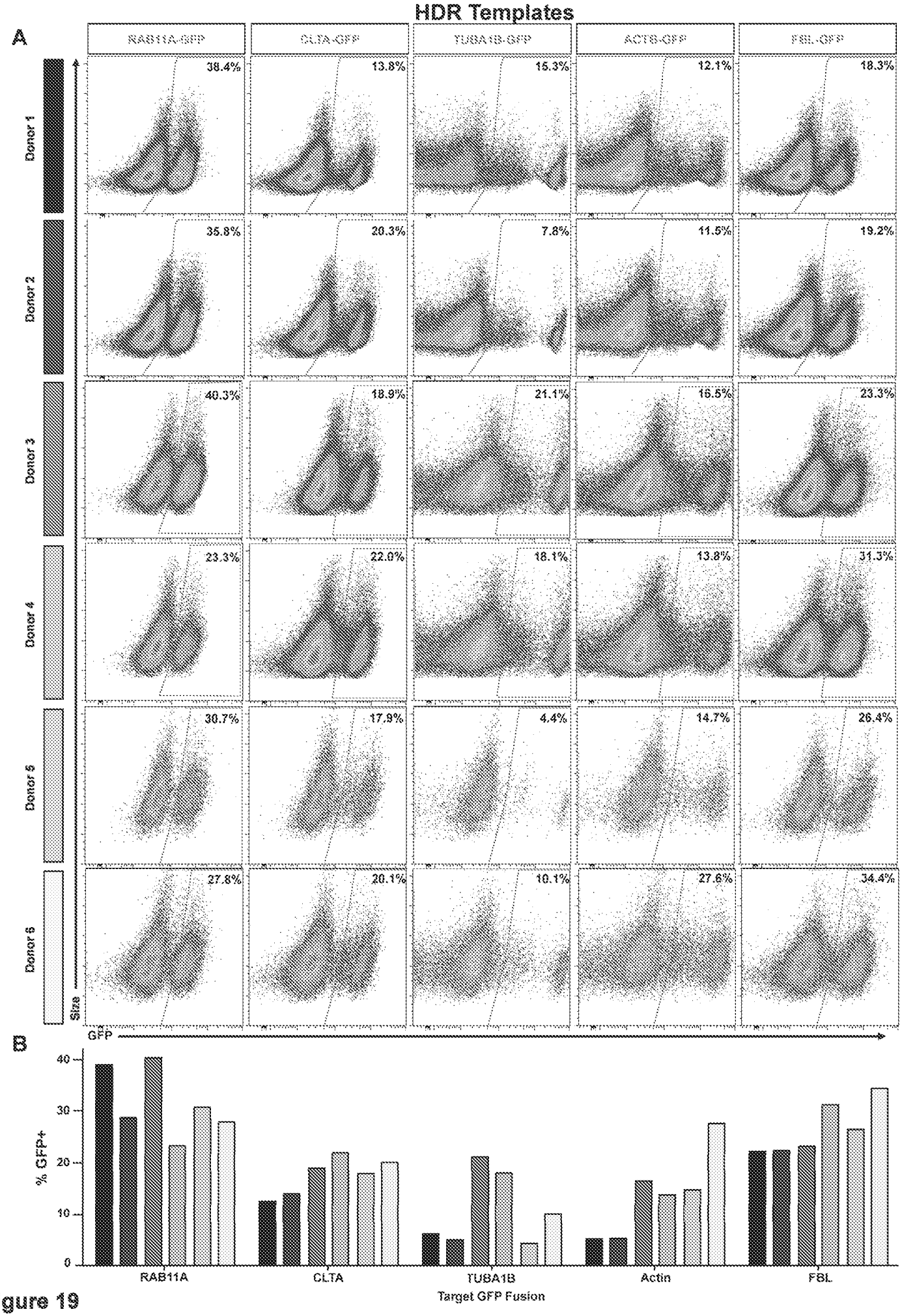

HDR Templates
A
RAB11A-GFP
CLTA-GFP
TUBA1B-GFP
ACTB-GFP
FBL-GFP
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
38.4%
13.8%
15.3%
12.1%
18.3%
35.8%
20.3%
7.8%
11.5%
19.2%
40.3%
18.9%
21.1%
16.5%
23.3%
23.3%
22.0%
18.1%
13.8%
31.3%
30.7%
17.9%
4.4%
14.7%
26.4%
27.8%
20.1%
10.1%
27.6%
34.4%
Size
GFP
B
% GFP+
0
10
20
30
40
RAB11A
CLTA
TUBA1B
Actin
FBL
Target GFP Fusion

Figure 19

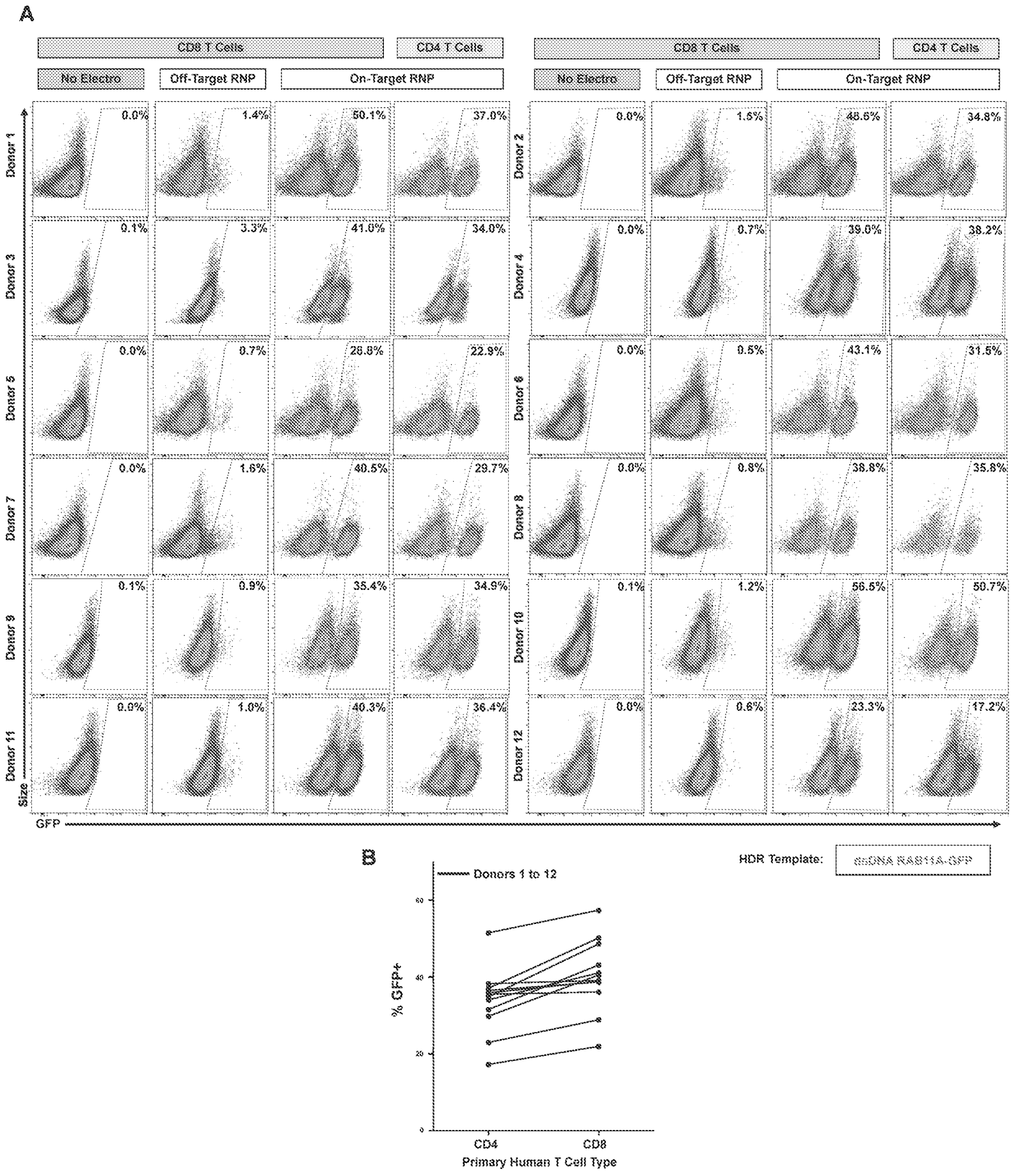

A
CD8 T Cells
CD4 T Cells
No Electro
Off-Target RNP
On-Target RNP
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
Donor 7
Donor 8
Donor 9
Donor 10
Donor 11
Donor 12
0.0%
1.4%
50.1%
37.0%
0.0%
1.5%
48.6%
34.8%
0.1%
3.3%
41.0%
34.0%
0.0%
0.7%
39.0%
38.2%
0.0%
0.7%
26.8%
22.9%
0.0%
0.5%
43.1%
31.5%
0.0%
1.6%
40.5%
29.7%
0.0%
0.8%
38.8%
35.8%
0.1%
0.9%
35.4%
34.9%
0.1%
1.2%
56.5%
50.7%
0.0%
1.0%
40.3%
36.4%
0.0%
0.6%
23.3%
17.2%
Size
GFP
B
HDR Template:
ssDNA RAB11A-GFP
Donors 1 to 12
% GFP+
60
40
20
0
CD4
CD8
Primary Human T Cell Type

Figure 20

A

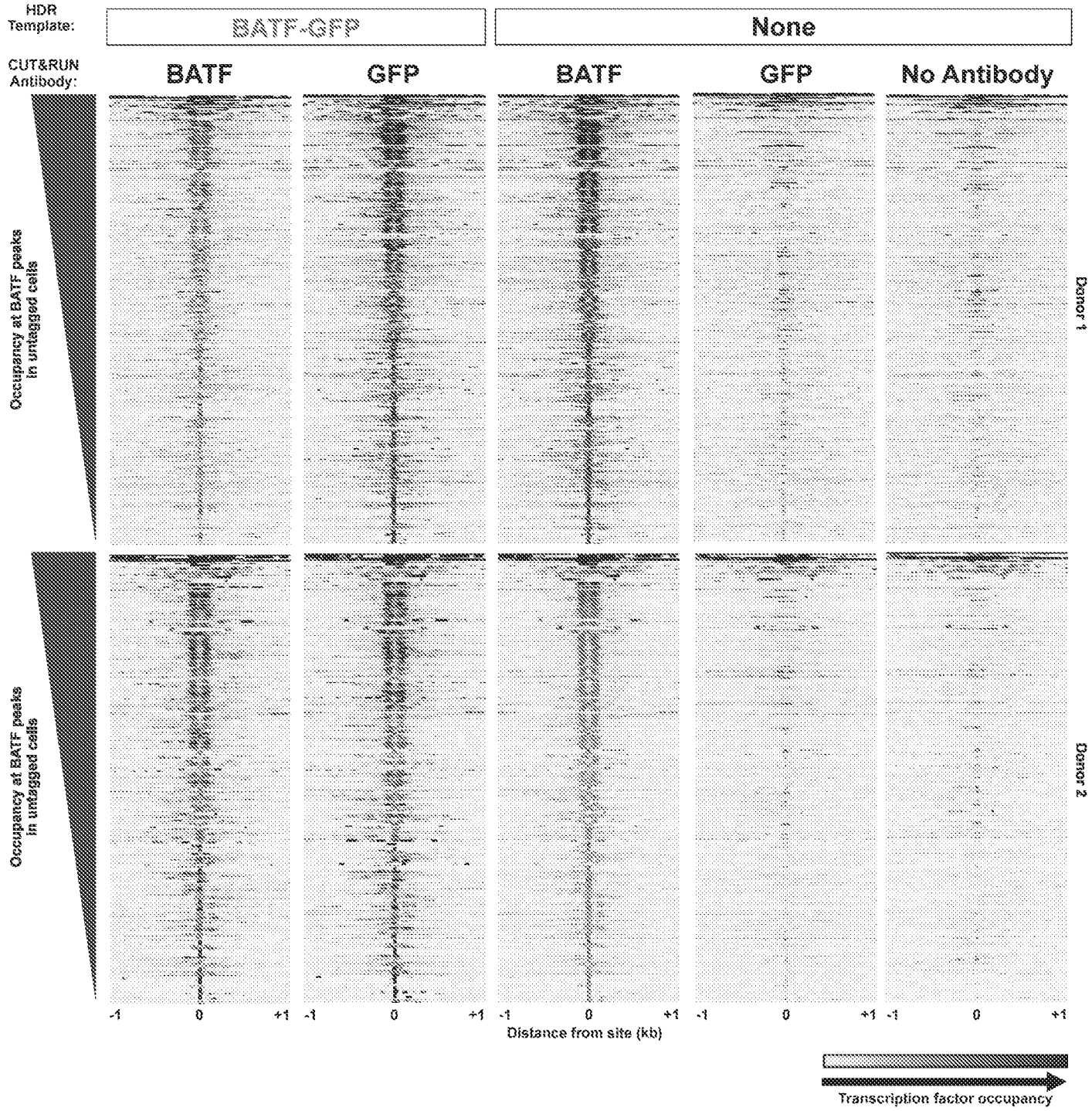

HDR Template:
BATF-GFP
None
CUT&RUN Antibody:
BATF
GFP
BATF
GFP
No Antibody
Occupancy at BATF peaks in untagged cells
Donor 1
Occupancy at BATF peaks in untagged cells
Donor 2
-1
0
+1
Distance from site (kb)
Transcription factor occupancy

FIGURE 21

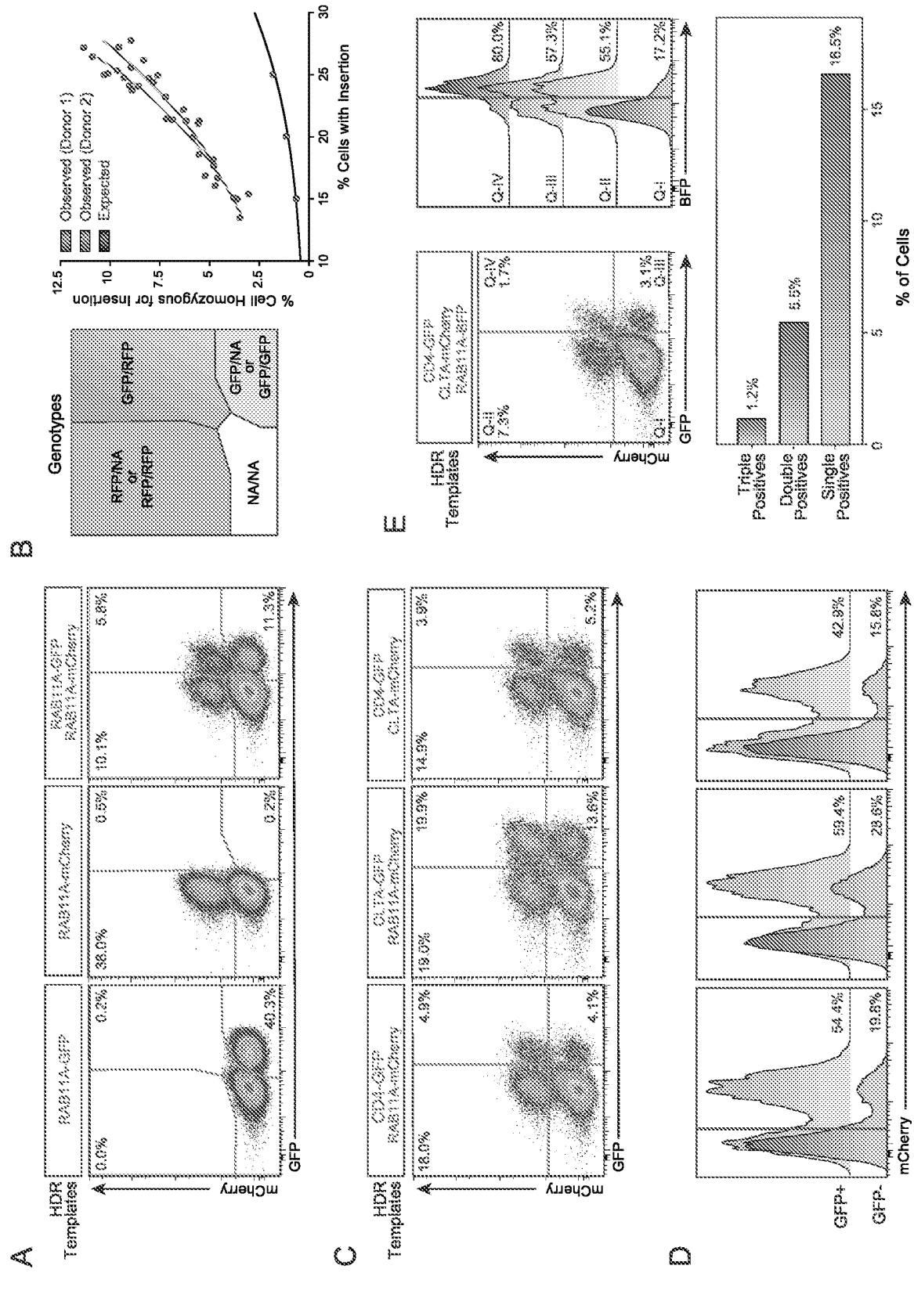
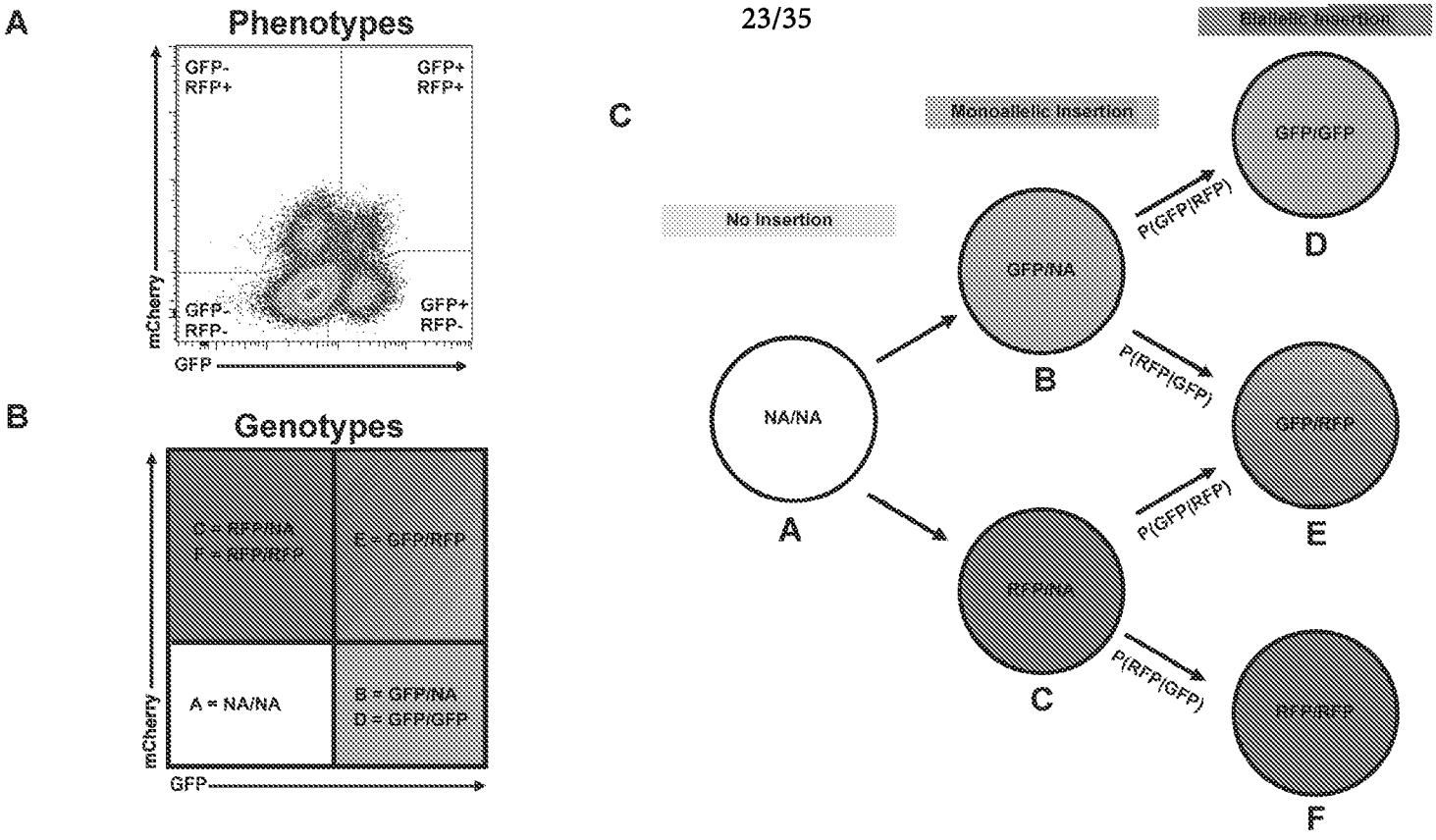
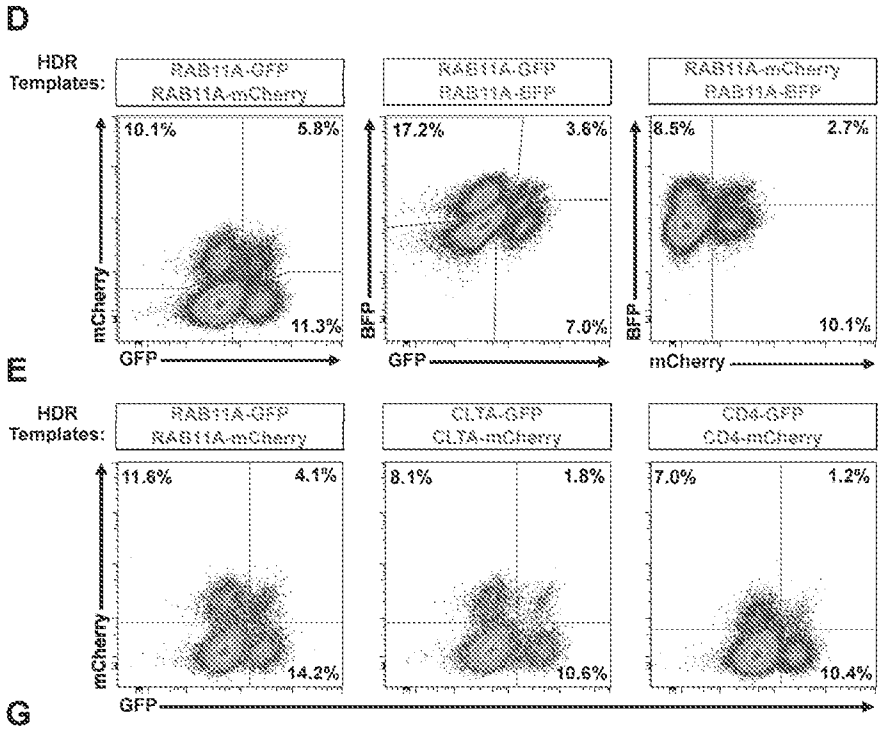
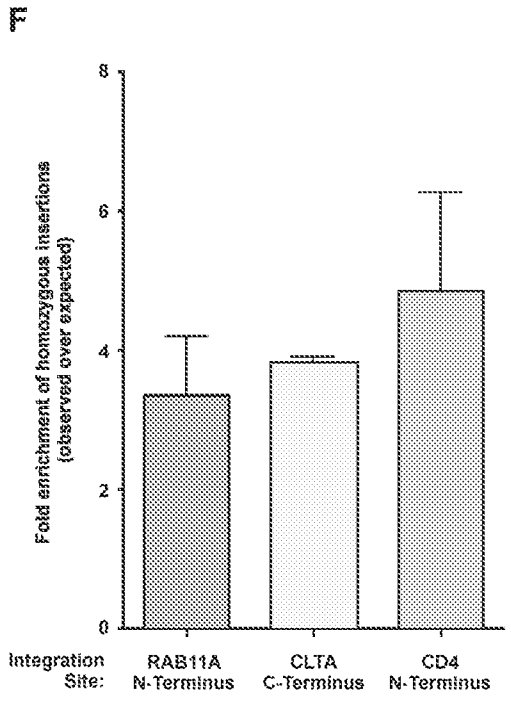

A
HDR Templates
RAB11A-GFP
RAB11A-mCherry
mCherry
GFP
10.0%
0.2%
40.3%
38.0%
0.5%
0.2%
10.1%
5.8%
11.3%
C
HDR Templates
CD4-GFP
RAB11A-mCherry
CLTA-GFP
RAB11A-mCherry
CD4-GFP
CLTA-mCherry
18.0%
4.9%
4.1%
18.0%
19.9%
13.6%
14.9%
3.9%
5.2%
B
Genotypes
RFP/NA or RFP/RFP
GFP/RFP
NA/NA
GFP/NA or GFP/GFP
% Cell Homozygous for Insertion
% Cells with Insertion
Observed (Donor 1)
Observed (Donor 2)
Expected
D
GFP+
GFP-
mCherry
54.4%
19.8%
59.4%
28.6%
42.9%
15.6%
E
HDR Templates
CD4-GFP
CLTA-mCherry
RAB11A-BFP
Q-II
7.3%
Q-IV
1.7%
Q-I
3.1%
Q-III
88.0%
Q-III
57.3%
Q-II
55.1%
Q-I
17.2%
mCherry
GFP
BFP
Triple Positives
Double Positives
Single Positives
1.2%
6.5%
16.5%
% of Cells

Figure 22


A
Phenotypes
GFP-
RFP+
GFP+
RFP+
GFP-
RFP-
GFP+
RFP-
mCherry
GFP
B
Genotypes
C = RFP/NA
F = RFP/RFP
E = GFP/RFP
A = NA/NA
B = GFP/NA
D = GFP/GFP
mCherry
GFP
C
Monoallelic Insertion
Biallelic Insertion
No Insertion
NA/NA
A
GFP/NA
B
RFP/NA
C
GFP/GFP
D
GFP/RFP
E
RFP/RFP
F
P(GFP|RFP)
P(RFP|GFP)
P(GFP|RFP)
P(RFP|GFP)


D
HDR Templates:
RAB11A-GFP
RAB11A-mCherry
RAB11A-GFP
RAB11A-BFP
RAB11A-mCherry
RAB11A-BFP
10.1%
5.8%
11.3%
17.2%
3.6%
7.0%
8.5%
2.7%
10.1%
mCherry
GFP
BFP
GFP
BFP
mCherry
E
HDR Templates:
RAB11A-GFP
RAB11A-mCherry
CLTA-GFP
CLTA-mCherry
CD4-GFP
CD4-mCherry
11.6%
4.1%
14.2%
8.1%
1.8%
10.6%
7.0%
1.2%
10.4%
mCherry
GFP


F
Fold enrichment of homozygous insertions (observed over expected)
0
2
4
6
8
Integration Site:
RAB11A N-Terminus
CLTA C-Terminus
CD4 N-Terminus

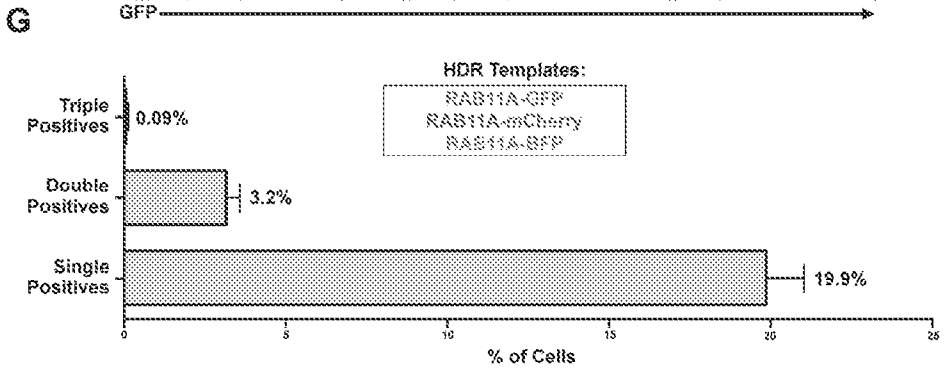
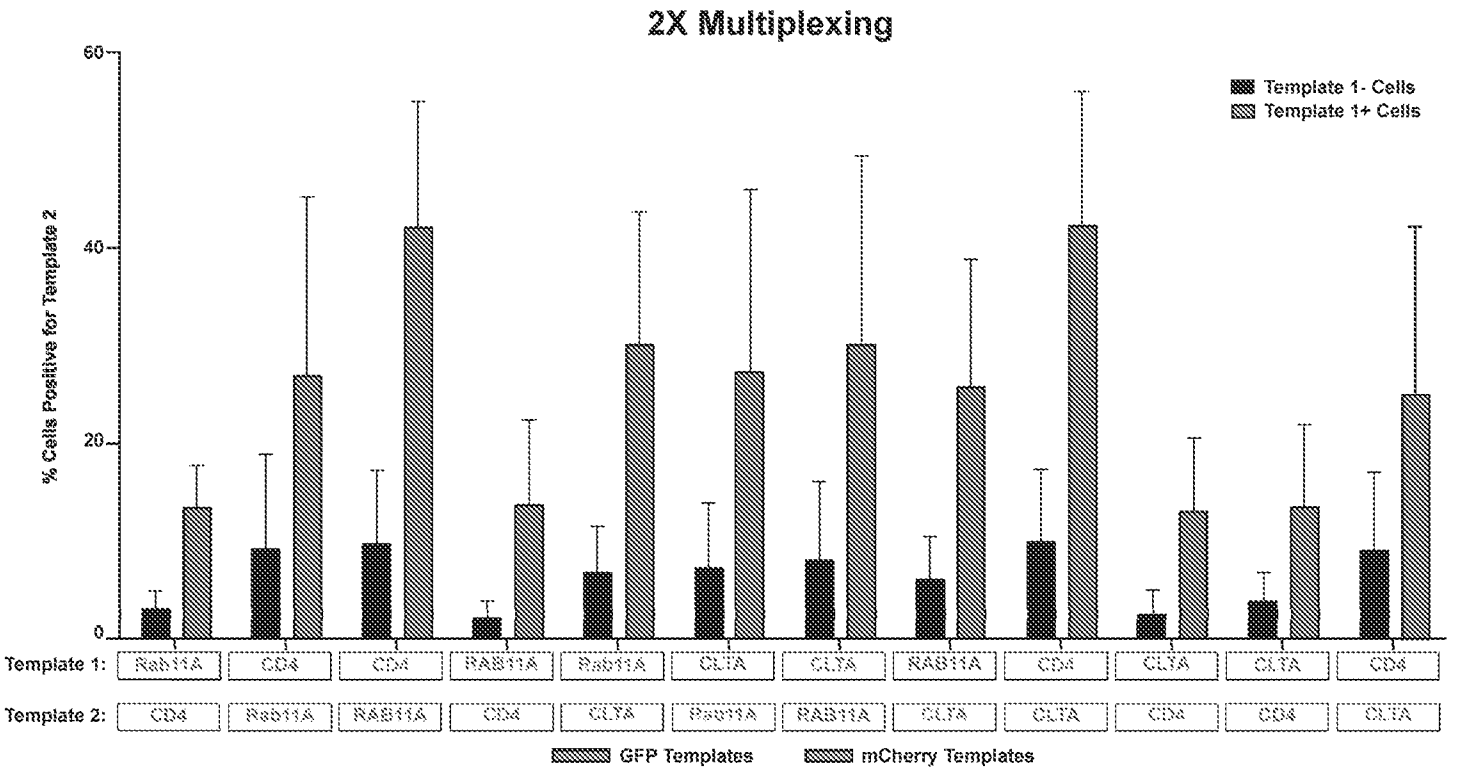

G
HDR Templates:
RAB11A-GFP
RAB11A-mCherry
RAB11A-BFP
Triple Positives
0.09%
Double Positives
3.2%
Single Positives
19.9%
0
5
10
15
20
25
% of Cells

Figure 23

A


2X Multiplexing
% Cells Positive for Template 2
Template 1- Cells
Template 1+ Cells
Template 1:
Template 2:
GFP Templates
mCherry Templates

B

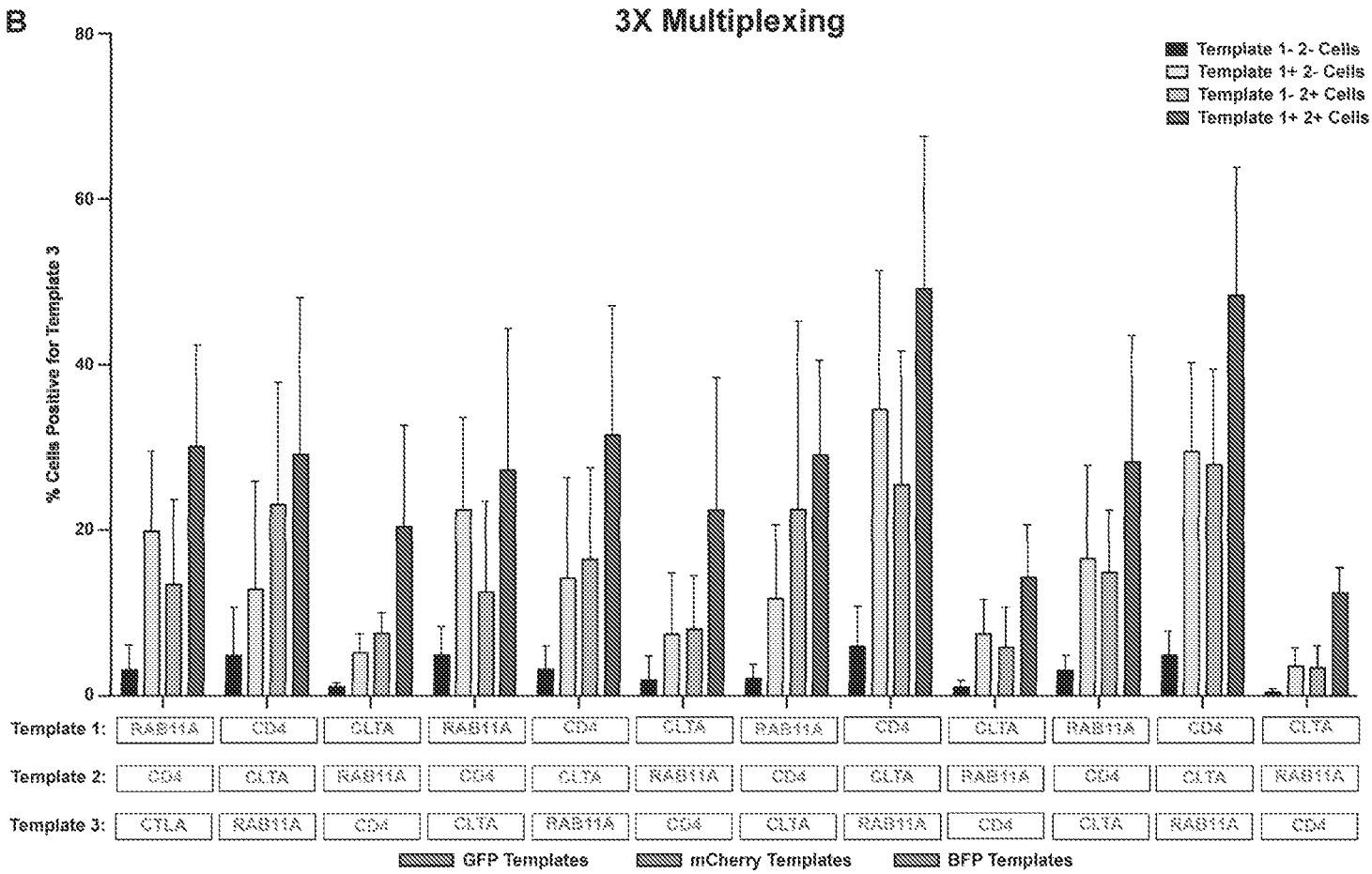

3X Multiplexing
% Cells Positive for Template 3
Template 1- 2- Cells
Template 1+ 2- Cells
Template 1- 2+ Cells
Template 1+ 2+ Cells
Template 1:
Template 2:
Template 3:
GFP Templates
mCherry Templates
BFP Templates

Figure 24

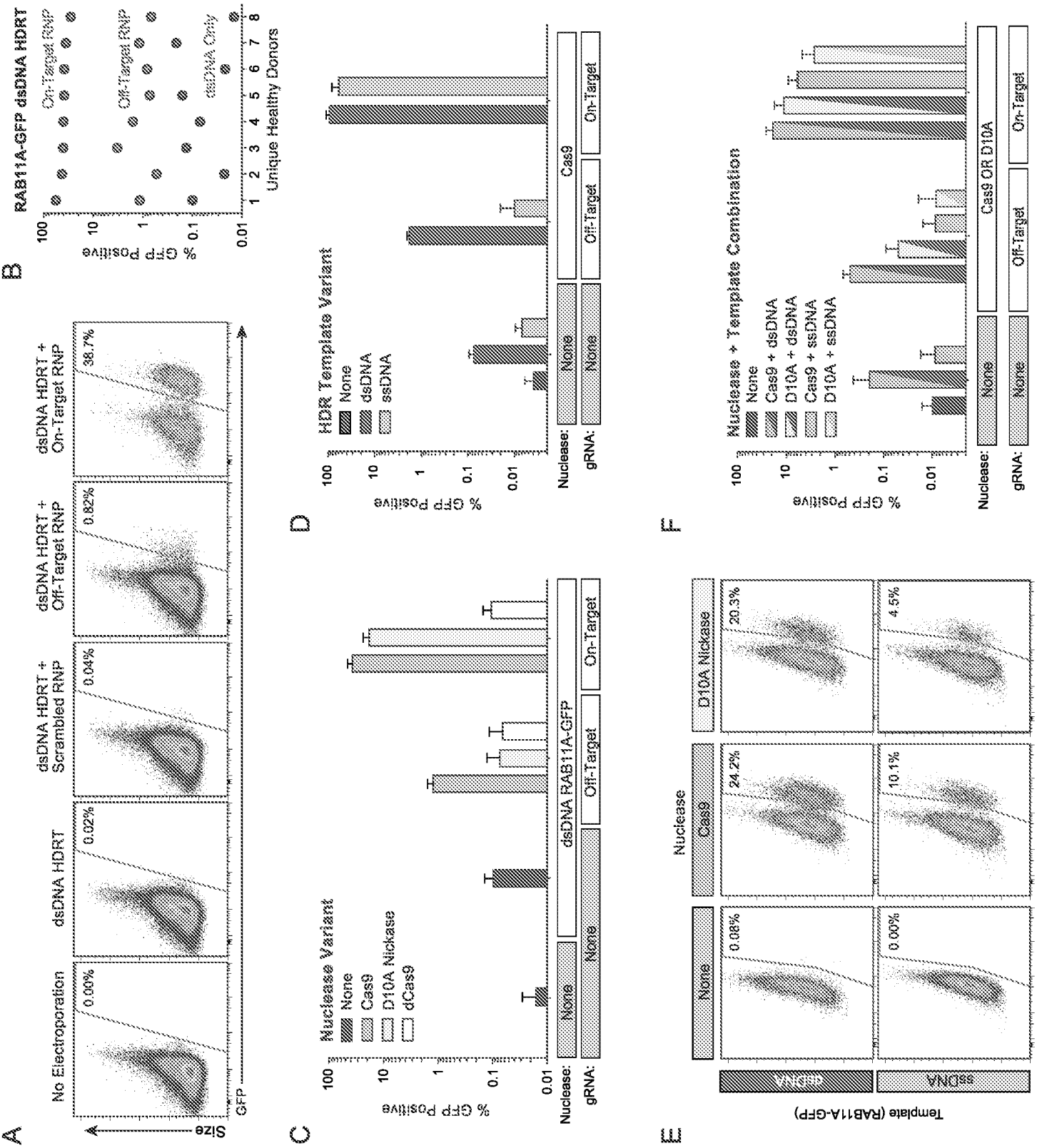

A
No Electroporation
dsDNA HDRT
dsDNA HDRT + Scrambled RNP
dsDNA HDRT + Off-Target RNP
dsDNA HDRT + On-Target RNP
0.00%
0.02%
0.04%
0.82%
38.7%
Size
GFP
B
RAB11A-GFP dsDNA HDRT
On-Target RNP
Off-Target RNP
dsDNA Only
% GFP Positive
Unique Healthy Donors
C
Nuclease Variant
None
Cas9
D10A Nickase
dCas9
% GFP Positive
dsDNA RAB11A-GFP
Nuclease:
gRNA:
Off-Target
On-Target
D
HDR Template Variant
None
dsDNA
ssDNA
% GFP Positive
Cas9
E
Nuclease
D10A Nickase
0.08%
24.2%
20.3%
0.00%
10.1%
4.5%
dsDNA
ssDNA
Template (RAB11A-GFP)
GFP
F
Nuclease + Template Combination
None
Cas9 + dsDNA
D10A + dsDNA
Cas9 + ssDNA
D10A + ssDNA
% GFP Positive
Cas9 OR D10A

Figure 25

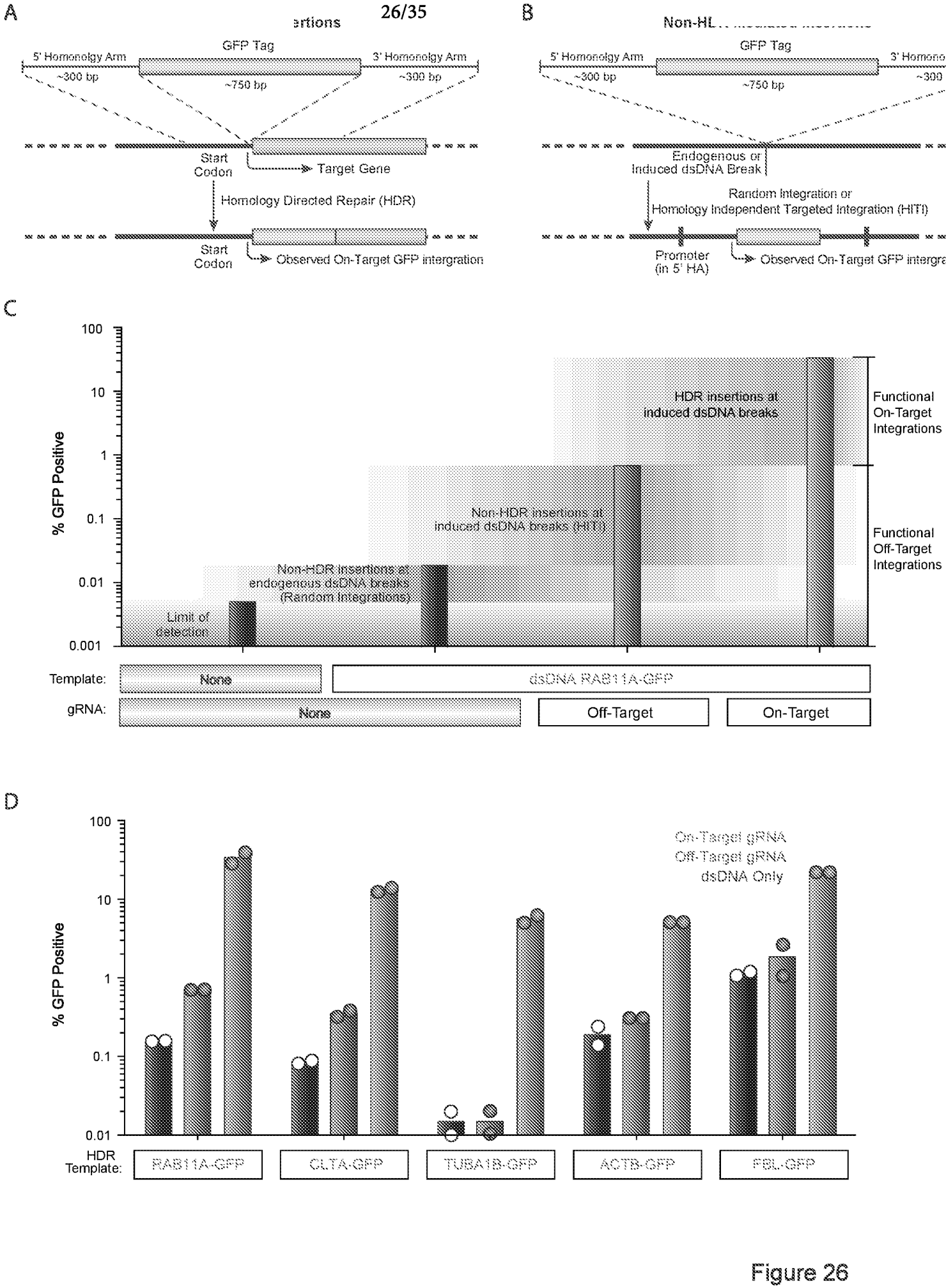

A
ertions
5' Homology Arm
~300 bp
GFP Tag
~750 bp
3' Homology Arm
~300 bp
Start Codon
Target Gene
Homology Directed Repair (HDR)
Start Codon
Observed On-Target GFP intergration
B
Non-HL
5' Homology Arm
~300 bp
GFP Tag
~750 bp
3' Homology Arm
~300 bp
Endogenous or Induced dsDNA Break
Random Integration or Homology Independent Targeted Integration (HITI)
Promoter (in 5' HA)
Observed On-Target GFP intergration
C
% GFP Positive
100
10
1
0.1
0.01
0.001
HDR insertions at induced dsDNA breaks
Non-HDR insertions at induced dsDNA breaks (HITI)
Non-HDR insertions at endogenous dsDNA breaks (Random Integrations)
Limit of detection
Functional On-Target Integrations
Functional Off-Target Integrations
Template:
None
dsDNA RAB11A-GFP
gRNA:
None
Off-Target
On-Target
D
% GFP Positive
100
10
1
0.1
0.01
On-Target gRNA
Off-Target gRNA
dsDNA Only
HDR Template:
RAB11A-GFP
CLTA-GFP
TUBA1B-GFP
ACTB-GFP
FBL-GFP

Figure 26

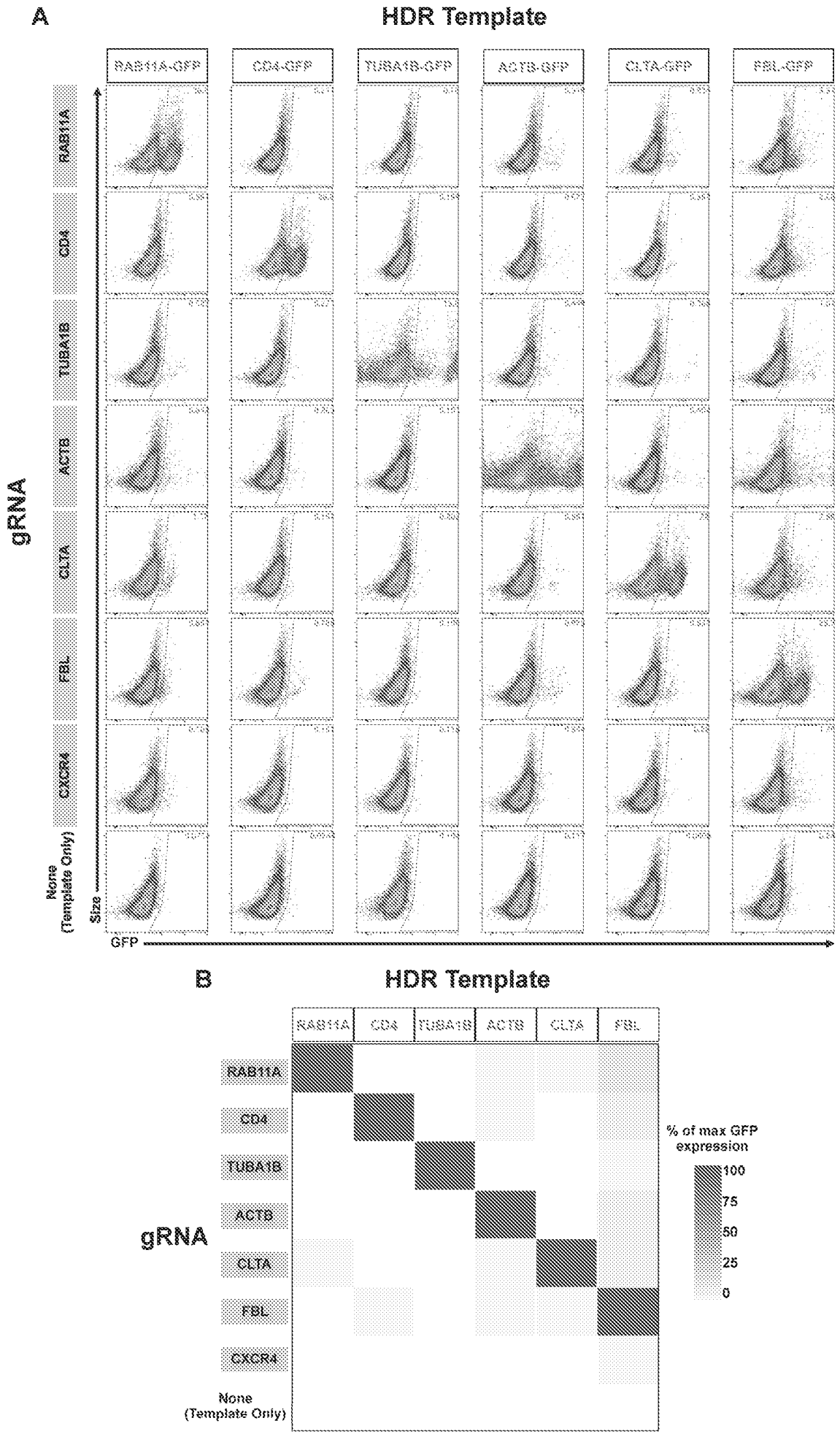

A
HDR Template
RAB11A-GFP
CD4-GFP
TUBA1B-GFP
ACTB-GFP
CLTA-GFP
FBL-GFP
gRNA
RAB11A
CD4
TUBA1B
ACTB
CLTA
FBL
CXCR4
None (Template Only)
Size
GFP
B
HDR Template
RAB11A
CD4
TUBA1B
ACTB
CLTA
FBL
gRNA
CXCR4
None (Template Only)
% of max GFP expression
100
75
50
25
0

Figure 27

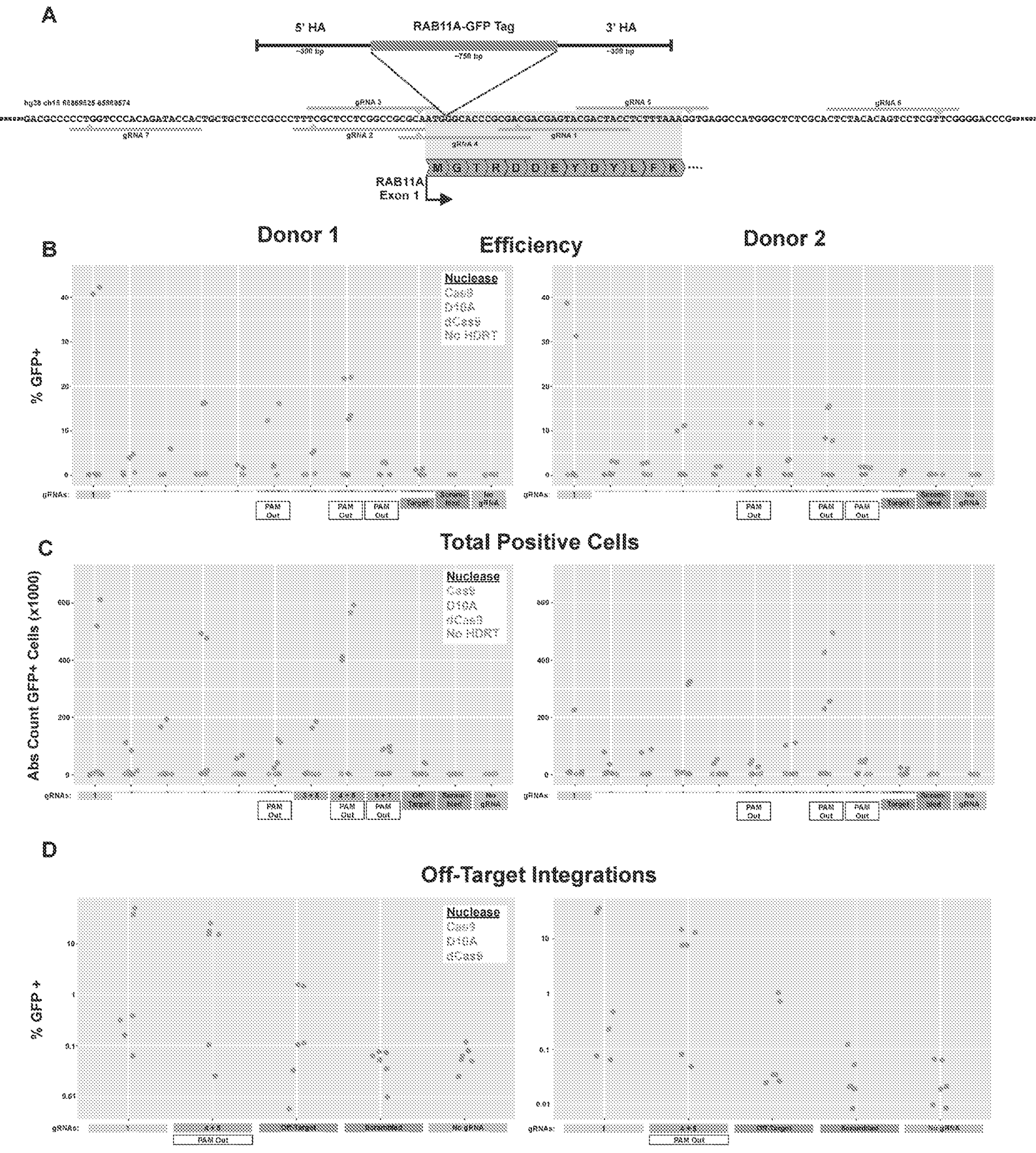

A
5' HA
RAB11A-GFP Tag
3' HA
~300 bp
~750 bp
~300 bp
gRNA 3
gRNA 5
gRNA 6
gRNA 7
gRNA 2
gRNA 4
gRNA 1
RAB11A
Exon 1
B
Donor 1
Efficiency
Donor 2
% GFP+
Nuclease
Cas9
D10A
dCas9
No HDRT
gRNAs:
PAM Out
C
Total Positive Cells
Abs Count GFP+ Cells (x1000)
D
Off-Target Integrations
% GFP +

Figure 28

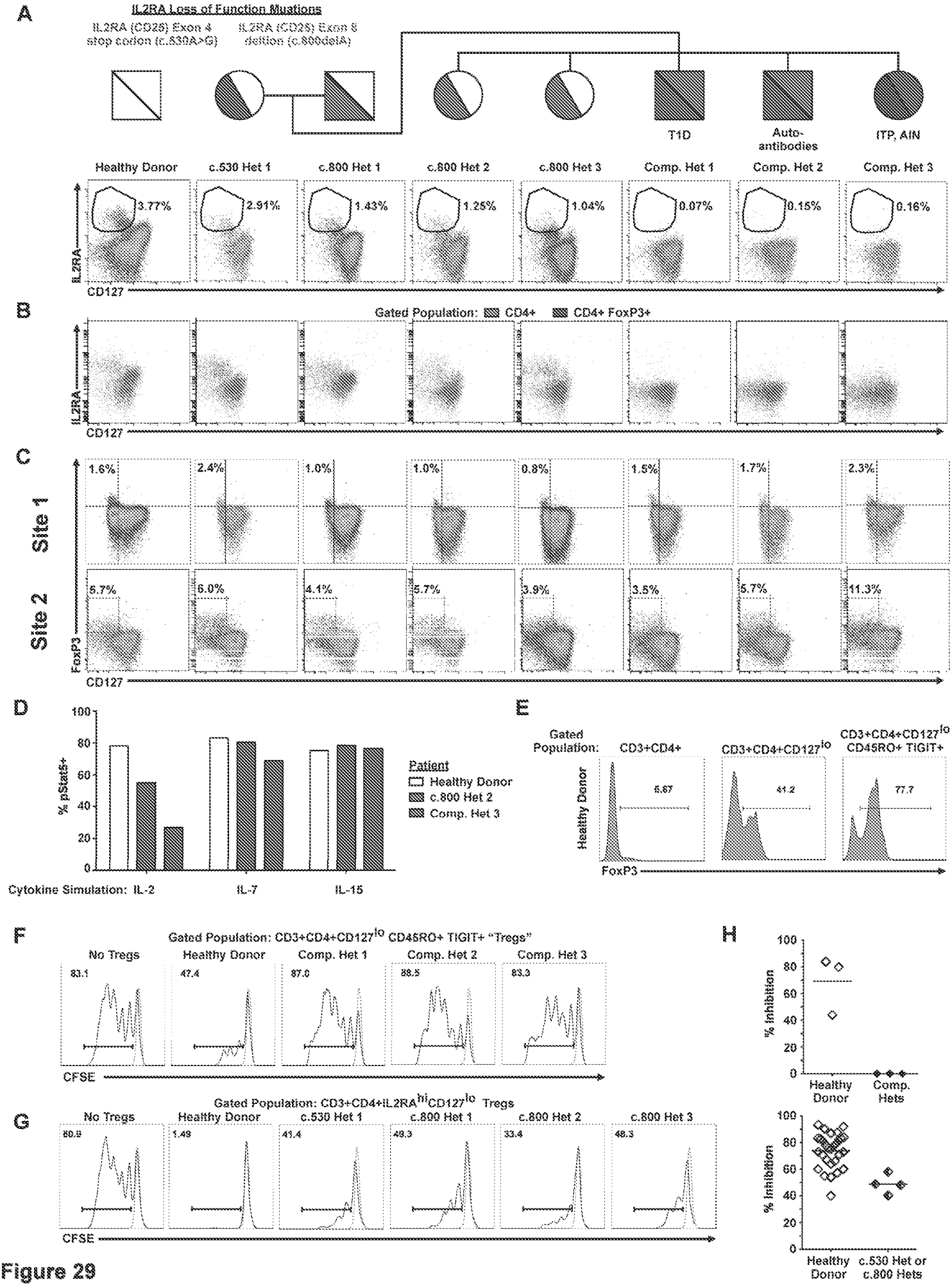
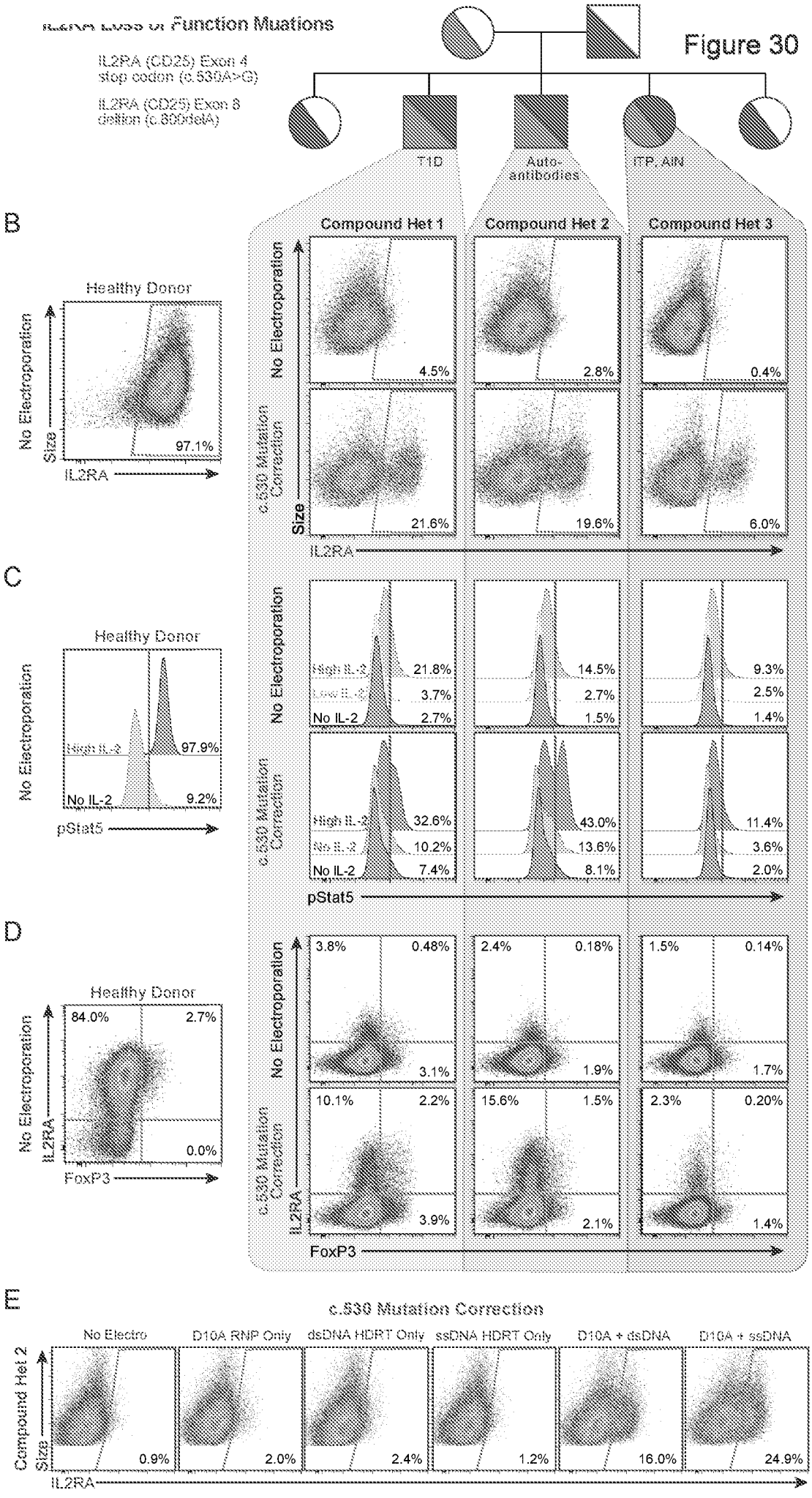

A
IL2RA Loss of Function Mutations
IL2RA (CD25) Exon 4 stop codon (c.530A>G)
IL2RA (CD25) Exon 8 deltion (c.800delA)
T1D
Auto-antibodies
ITP, AIN
Healthy Donor
c.530 Het 1
c.800 Het 1
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
3.77%
2.91%
1.43%
1.25%
1.04%
0.07%
0.15%
0.16%
IL2RA
CD127
B
Gated Population: CD4+ CD4+ FoxP3+
C
Site 1
Site 2
1.6%
2.4%
1.0%
1.0%
0.8%
1.5%
1.7%
2.3%
5.7%
6.0%
4.1%
5.7%
3.9%
3.5%
5.7%
11.3%
FoxP3
CD127
D
% pStat5+
Patient
Healthy Donor
c.800 Het 2
Comp. Het 3
Cytokine Simulation: IL-2
IL-7
IL-15
E
Gated Population:
CD3+CD4+
CD3+CD4+CD127lo
CD3+CD4+CD127lo CD45RO+ TIGIT+
Healthy Donor
6.67
41.2
77.7
FoxP3
F
Gated Population: CD3+CD4+CD127lo CD45RO+ TIGIT+ "Tregs"
No Tregs
Healthy Donor
Comp. Het 1
Comp. Het 2
Comp. Het 3
83.1
47.4
87.0
88.5
83.3
CFSE
G
Gated Population: CD3+CD4+IL2RAhiCD127lo Tregs
No Tregs
Healthy Donor
c.530 Het 1
c.800 Het 1
c.800 Het 2
c.800 Het 3
80.9
1.49
41.4
48.3
33.4
48.3
CFSE
H
% Inhibition
Healthy Donor
Comp. Hets
% Inhibition
Healthy Donor
c.530 Het or c.800 Hets

Figure 29


IL2RA Loss of Function Mutations
IL2RA (CD25) Exon 4 stop codon (c.530A>G)
IL2RA (CD25) Exon 8 deltion (c.800delA)
T1D
Auto-antibodies
ITP, AIN
Figure 30
B
Healthy Donor
No Electroporation
Size
IL2RA
97.1%
Compound Het 1
Compound Het 2
Compound Het 3
No Electroporation
c.530 Mutation Correction
4.5%
2.8%
0.4%
21.6%
19.6%
6.0%
C
Healthy Donor
High IL-2
No IL-2
97.9%
9.2%
pStat5
21.8%
14.5%
9.3%
3.7%
2.7%
2.5%
2.7%
1.5%
1.4%
32.6%
43.0%
11.4%
10.2%
13.6%
3.6%
7.4%
8.1%
2.0%
D
Healthy Donor
84.0%
2.7%
0.0%
FoxP3
3.8%
0.48%
3.1%
2.4%
0.18%
1.9%
1.5%
0.14%
1.7%
10.1%
2.2%
3.9%
15.6%
1.5%
2.1%
2.3%
0.20%
1.4%
E
c.530 Mutation Correction
No Electro
D10A RNP Only
dsDNA HDRT Only
ssDNA HDRT Only
D10A + dsDNA
D10A + ssDNA
Compound Het 2
0.9%
2.0%
2.4%
1.2%
16.0%
24.9%

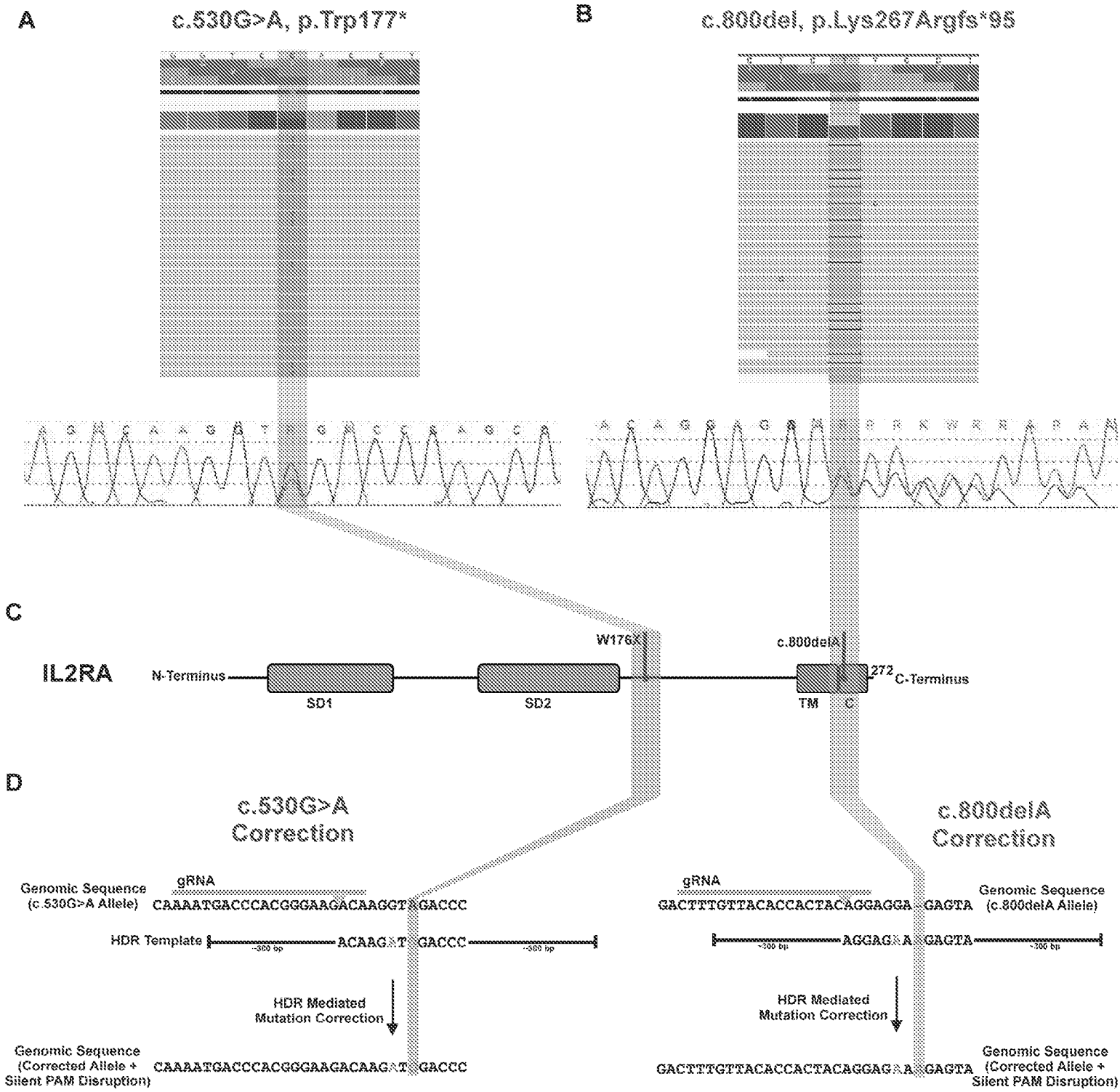

A
c.530G>A, p.Trp177*
B
c.800del, p.Lys267Argfs*95
C
IL2RA
N-Terminus
SD1
SD2
W176X
c.800delA
272
C-Terminus
TM
C
D
c.530G>A
Correction
c.800delA
Correction
Genomic Sequence
(c.530G>A Allele)
gRNA
CAAAATGACCCACGGGAAGACAAGGTGACCC
HDR Template
~300 bp
ACAAGAT
GACCC
~300 bp
HDR Mediated
Mutation Correction
Genomic Sequence
(Corrected Allele +
Silent PAM Disruption)
CAAAATGACCCACGGGAAGACAAGATGACCC
gRNA
GACTTTGTTACACCACTACAGGAGGAGAGTA
Genomic Sequence
(c.800delA Allele)
~300 bp
AGGAGAAGAGTA
~300 bp
HDR Mediated
Mutation Correction
GACTTTGTTACACCACTACAGGAGAAGAGTA
Genomic Sequence
(Corrected Allele +
Silent PAM Disruption)

Figure 31

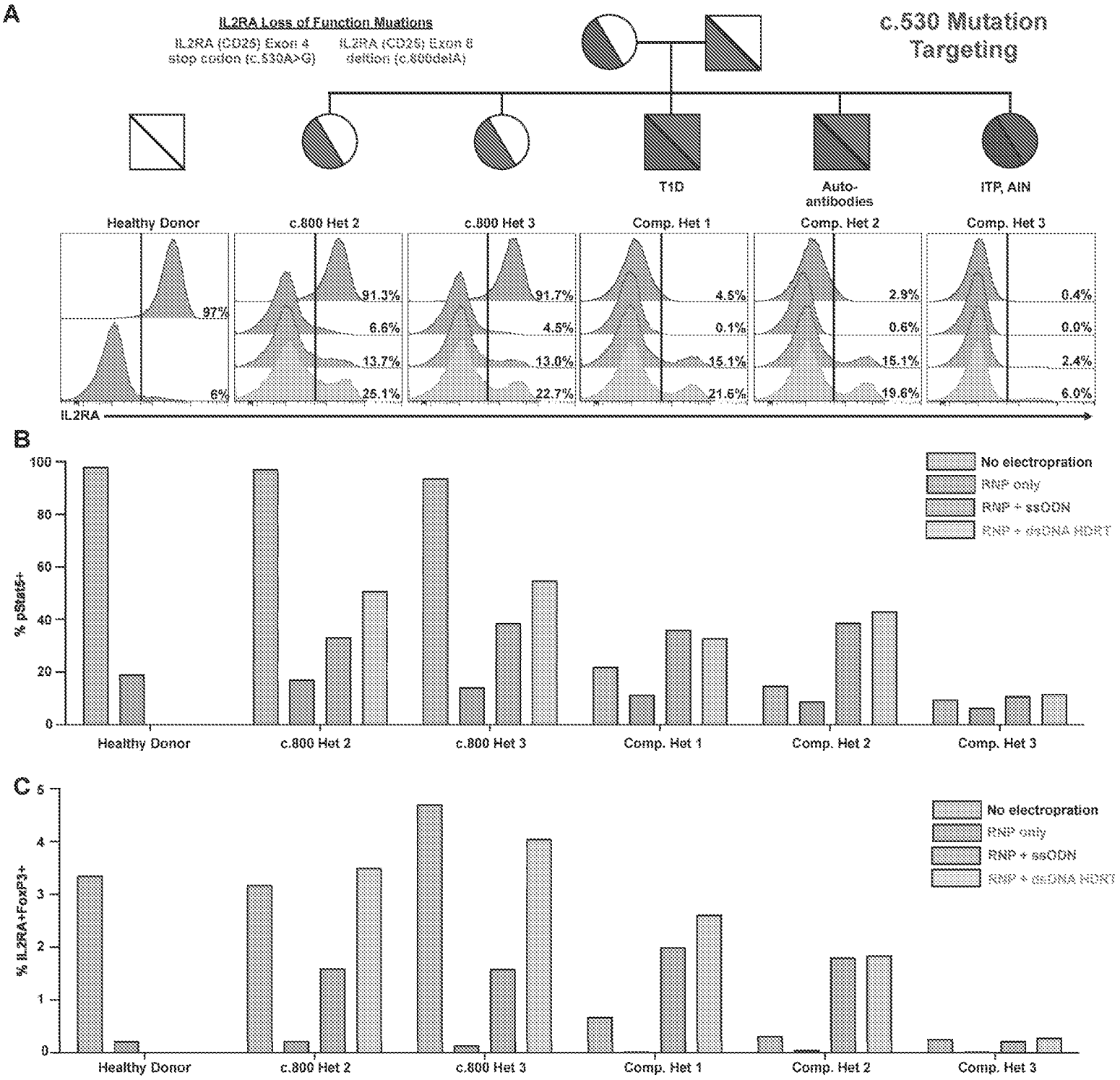

A
IL2RA Loss of Function Mutations
IL2RA (CD25) Exon 4 stop codon (c.530A>G)
IL2RA (CD25) Exon 8 deltion (c.800delA)
c.530 Mutation Targeting
T1D
Auto-antibodies
ITP, AIN
Healthy Donor
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
97%
6%
91.3%
6.6%
13.7%
25.1%
91.7%
4.5%
13.0%
22.7%
4.5%
0.1%
15.1%
21.6%
2.9%
0.6%
15.1%
19.6%
0.4%
0.0%
2.4%
6.0%
IL2RA
B
% pStat5+
No electropration
RNP only
RNP + ssODN
RNP + dsDNA HDRT
Healthy Donor
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
C
% IL2RA+FoxP3+
No electropration
RNP only
RNP + ssODN
RNP + dsDNA HDRT
Healthy Donor
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3

Figure 32

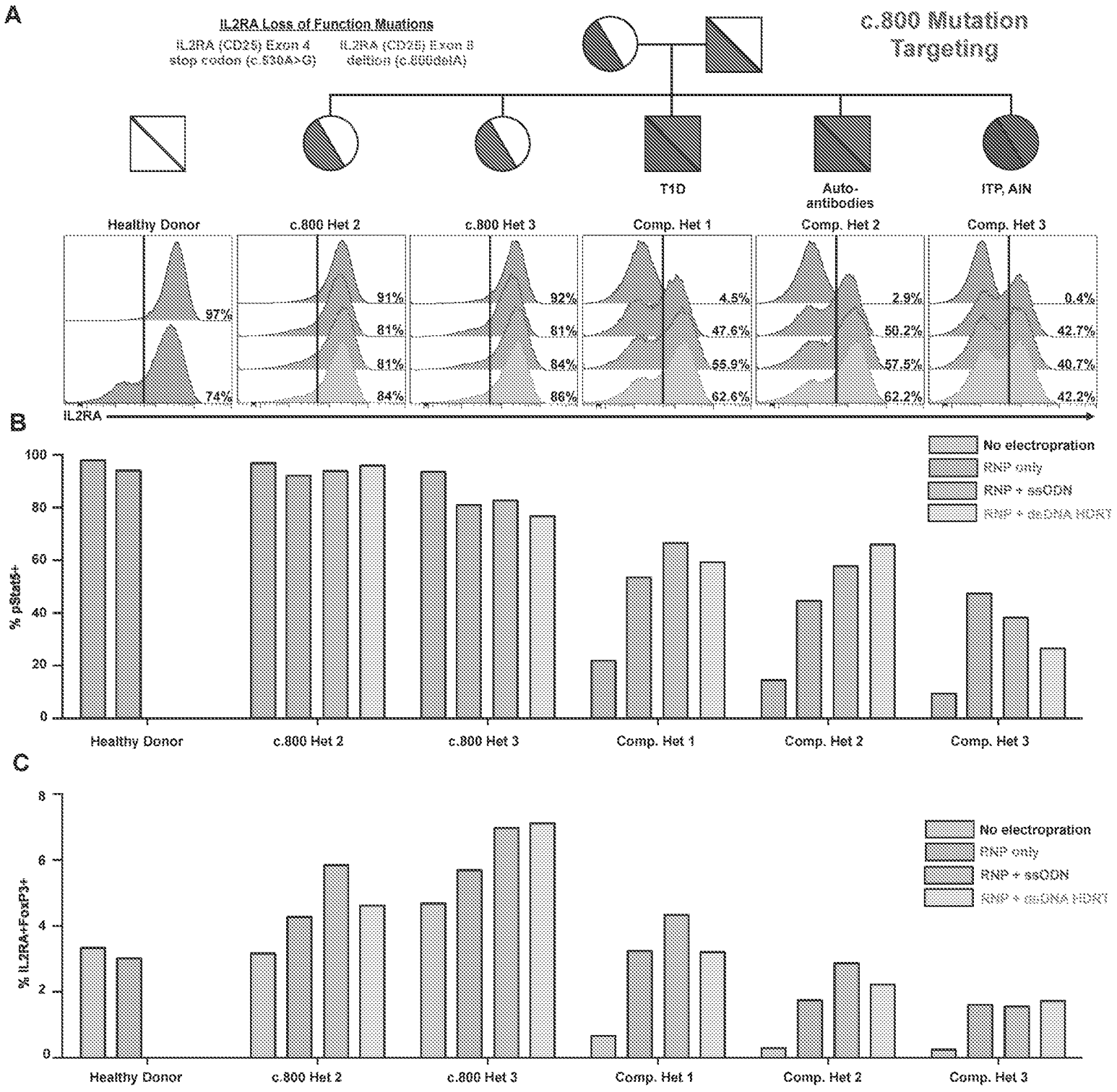
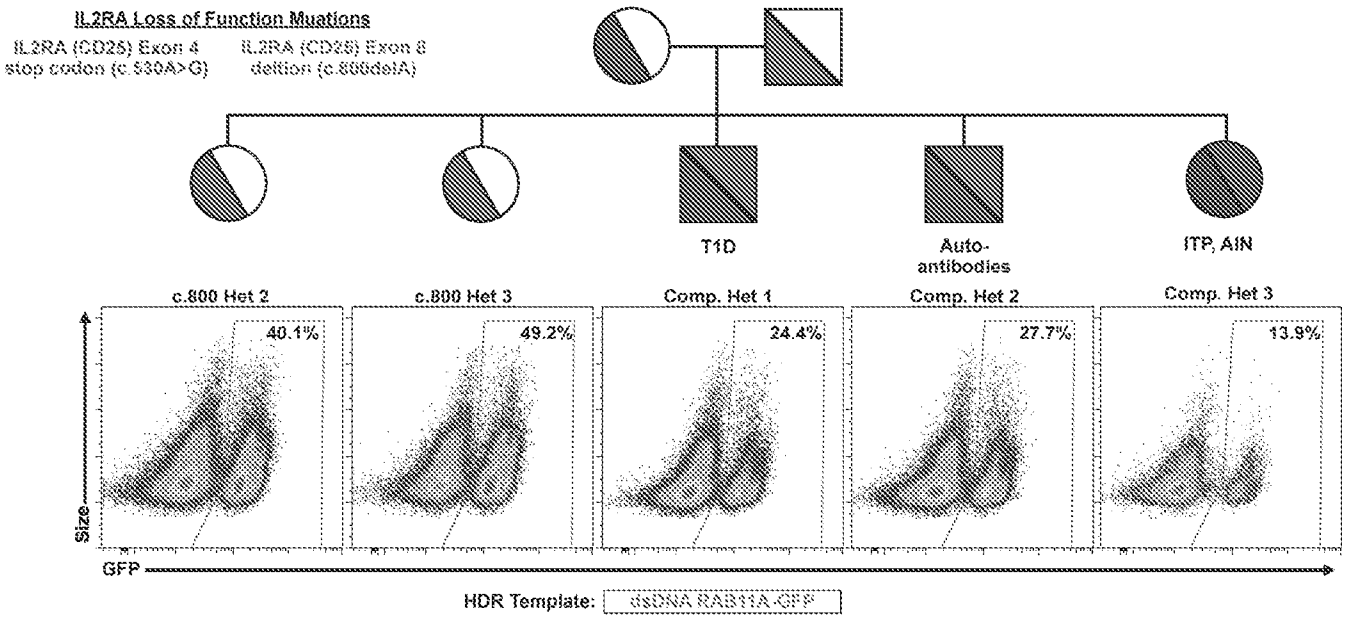

A
IL2RA Loss of Function Mutations
IL2RA (CD25) Exon 4 stop codon (c.530A>G)
IL2RA (CD25) Exon 8 deltion (c.666delA)
c.800 Mutation Targeting
T1D
Auto-antibodies
ITP, AIN
Healthy Donor
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
97%
74%
91%
81%
81%
84%
92%
81%
84%
86%
4.5%
47.6%
55.9%
62.6%
2.9%
50.2%
57.5%
62.2%
0.4%
42.7%
40.7%
42.2%
IL2RA
B
% pStat5+
No electropration
RNP only
RNP + ssODN
RNP + dsDNA HDRT
Healthy Donor
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
C
% IL2RA+FoxP3+
No electropration
RNP only
RNP + ssODN
RNP + dsDNA HDRT
Healthy Donor
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3

Figure 33

A


IL2RA Loss of Function Muations
IL2RA (CD25) Exon 4 stop codon (c.530A>G)
IL2RA (CD25) Exon 8 deltion (c.800delA)
T1D
Auto-antibodies
ITP, AIN
c.800 Het 2
c.800 Het 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
40.1%
49.2%
24.4%
27.7%
13.9%
Size
GFP
HDR Template: dsDNA RAB11A-GFP

B

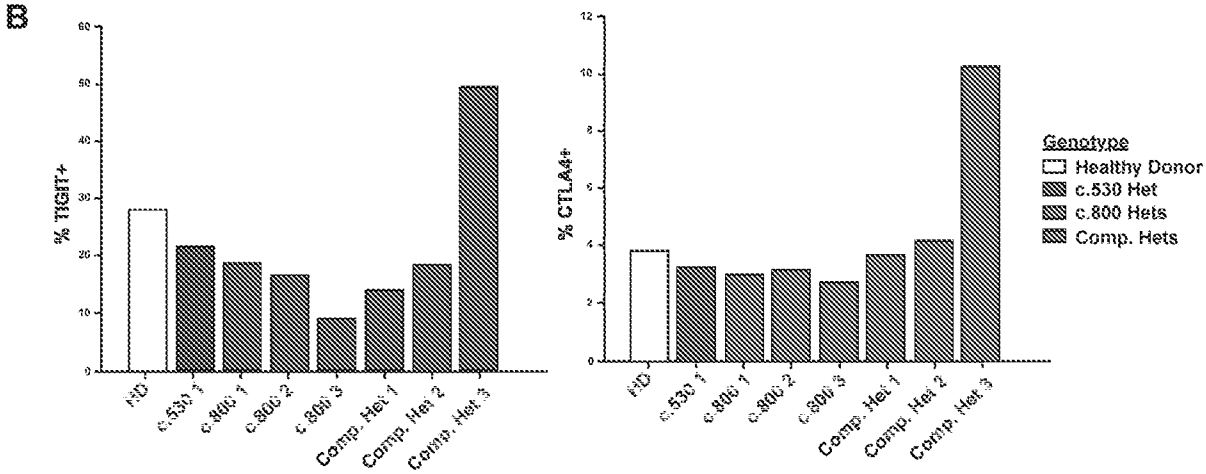
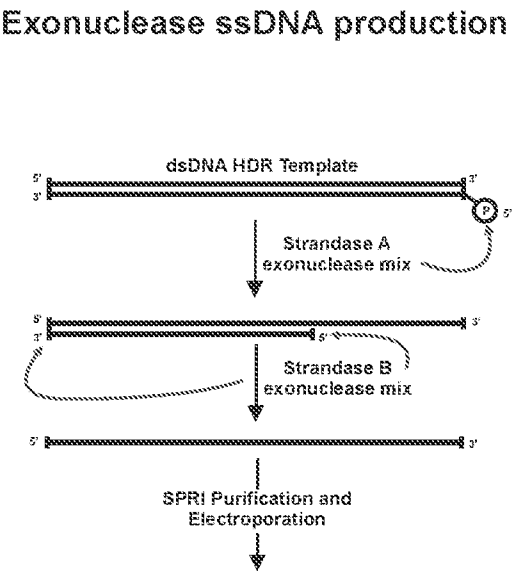
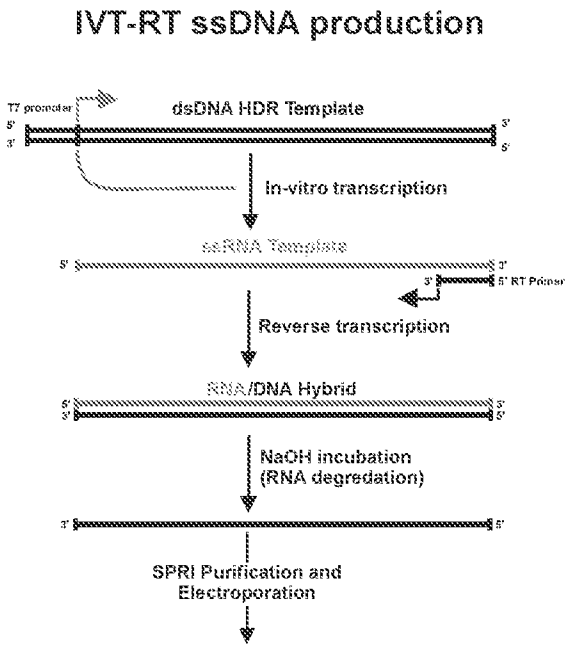

% TIGIT+
% CTLA4+
HD
c.530 1
c.800 1
c.800 2
c.800 3
Comp. Het 1
Comp. Het 2
Comp. Het 3
Genotype
Healthy Donor
c.530 Het
c.800 Hets
Comp. Hets

Figure 34

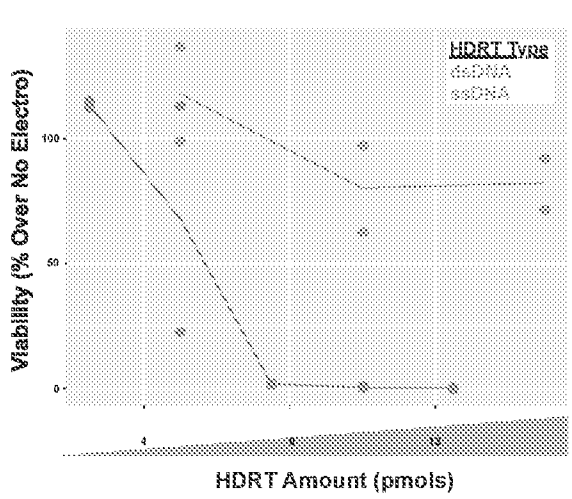
A



B



C



D

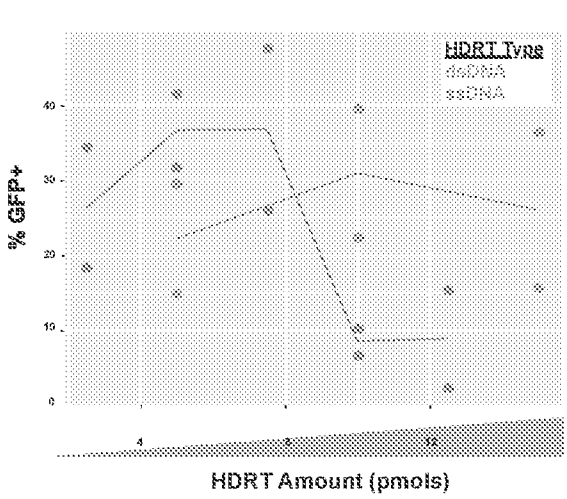


Figure 35