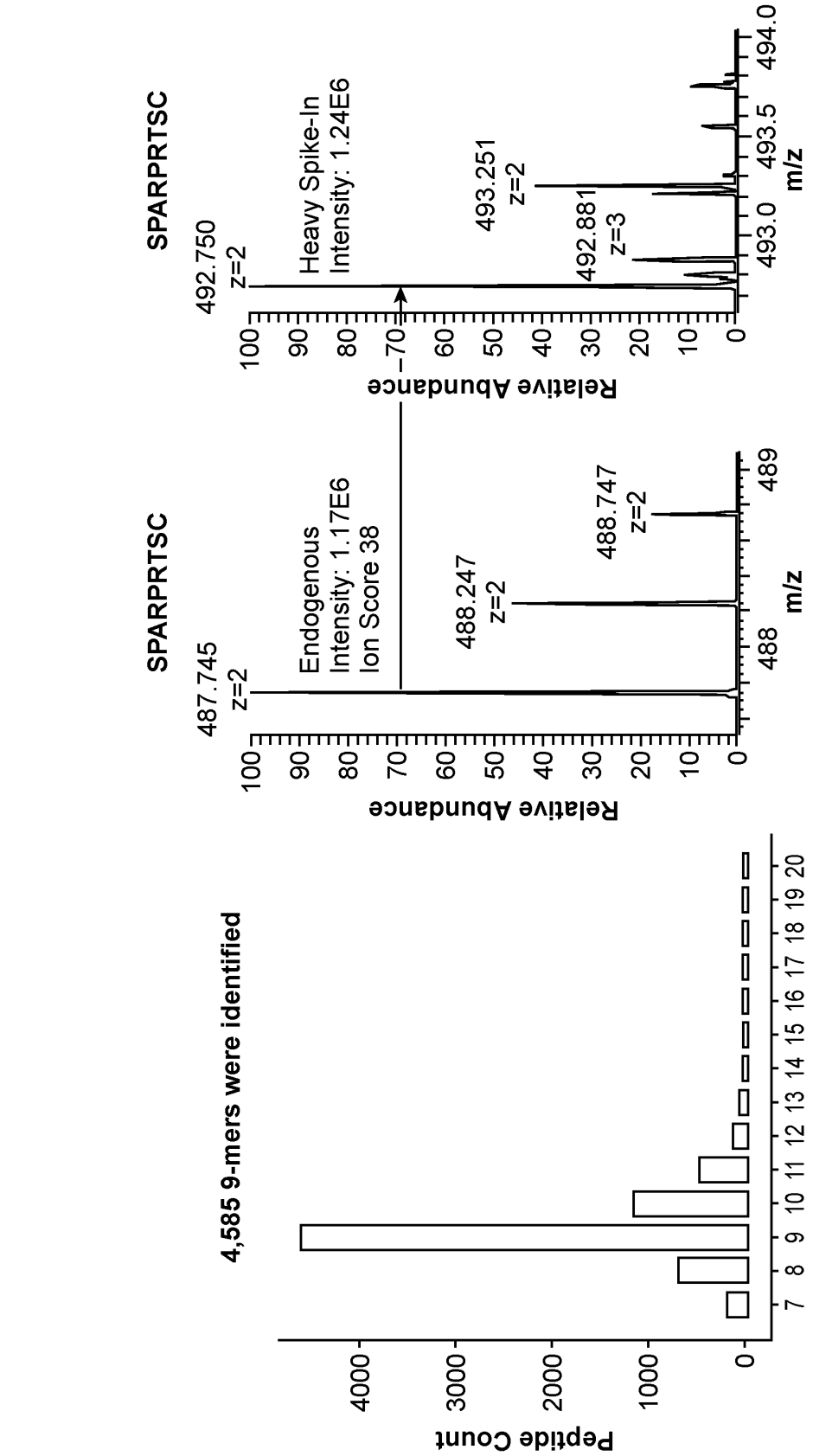

4,585 9-mers were identified
Peptide Count
Peptide Length
SPARPRTSC
487.745
z=2
Endogenous
Intensity: 1.17E6
Ion Score 38
488.247
z=2
488.747
z=2
Relative Abundance
m/z
SPARPRTSC
492.750
z=2
Heavy Spike-In
Intensity: 1.24E6
493.251
z=2
492.881
z=3
Relative Abundance
m/z
Estimated Copy number ~ 550

FIG. 1A

FIG. 1B

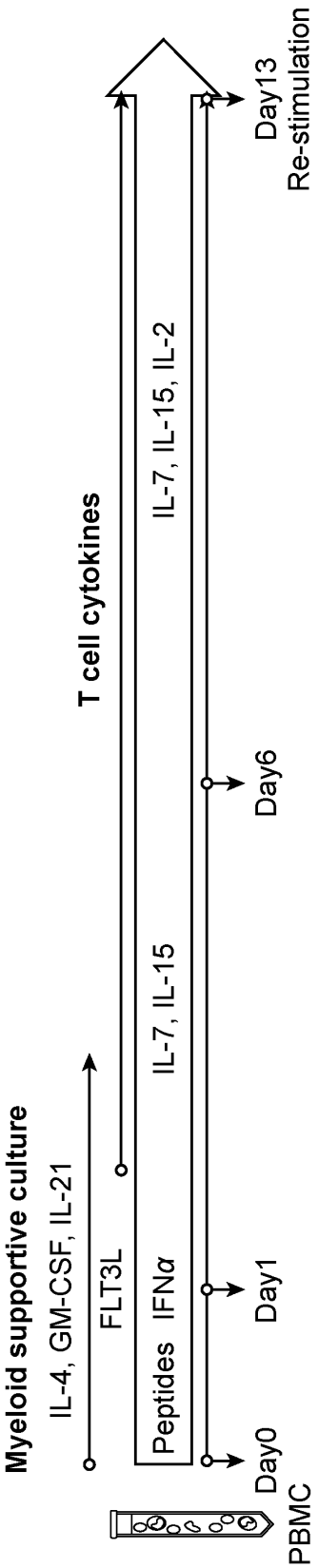

Myeloid supportive culture
IL-4, GM-CSF, IL-21
FLT3L
Peptides IFNα
IL-7, IL-15
T cell cytokines
IL-7, IL-15, IL-2
Day0
Day1
Day6
Day13
Re-stimulation
PBMC

FIG. 2A

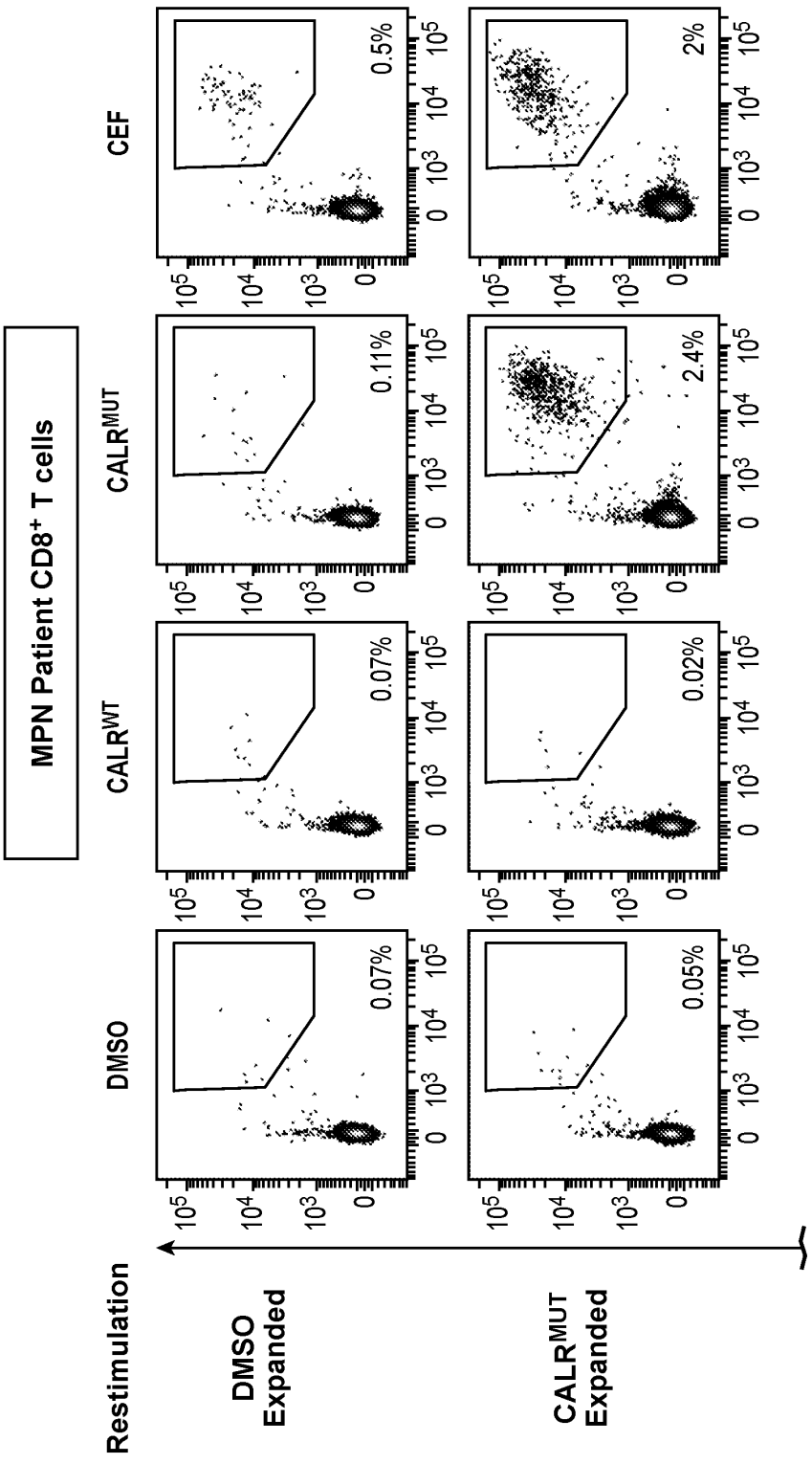

MPN Patient CD8+ T cells
DMSO
CALRWT
CALRMUT
CEF
Restimulation
DMSO Expanded
CALRMUT Expanded
0.07%
0.07%
0.11%
0.5%
0.05%
0.02%
2.4%
2%

FIG. 2B

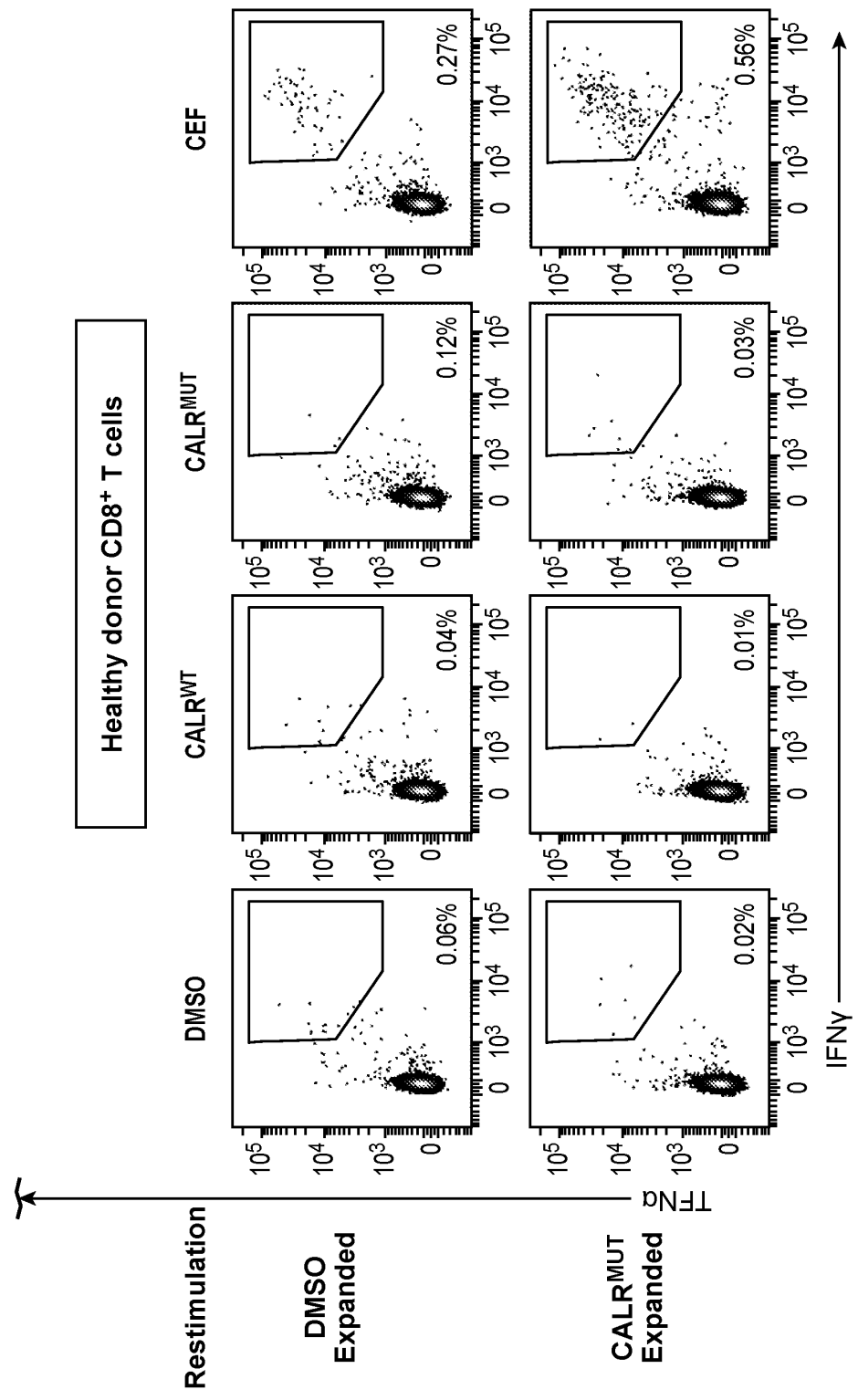

Healthy donor CD8+ T cells
DMSO
CALRWT
CALRMUT
CEF
Restimulation
DMSO Expanded
CALRMUT Expanded
TFNα
IFNγ
0.06%
0.04%
0.12%
0.27%
0.02%
0.01%
0.03%
0.56%

FIG. 2B (Cont.)

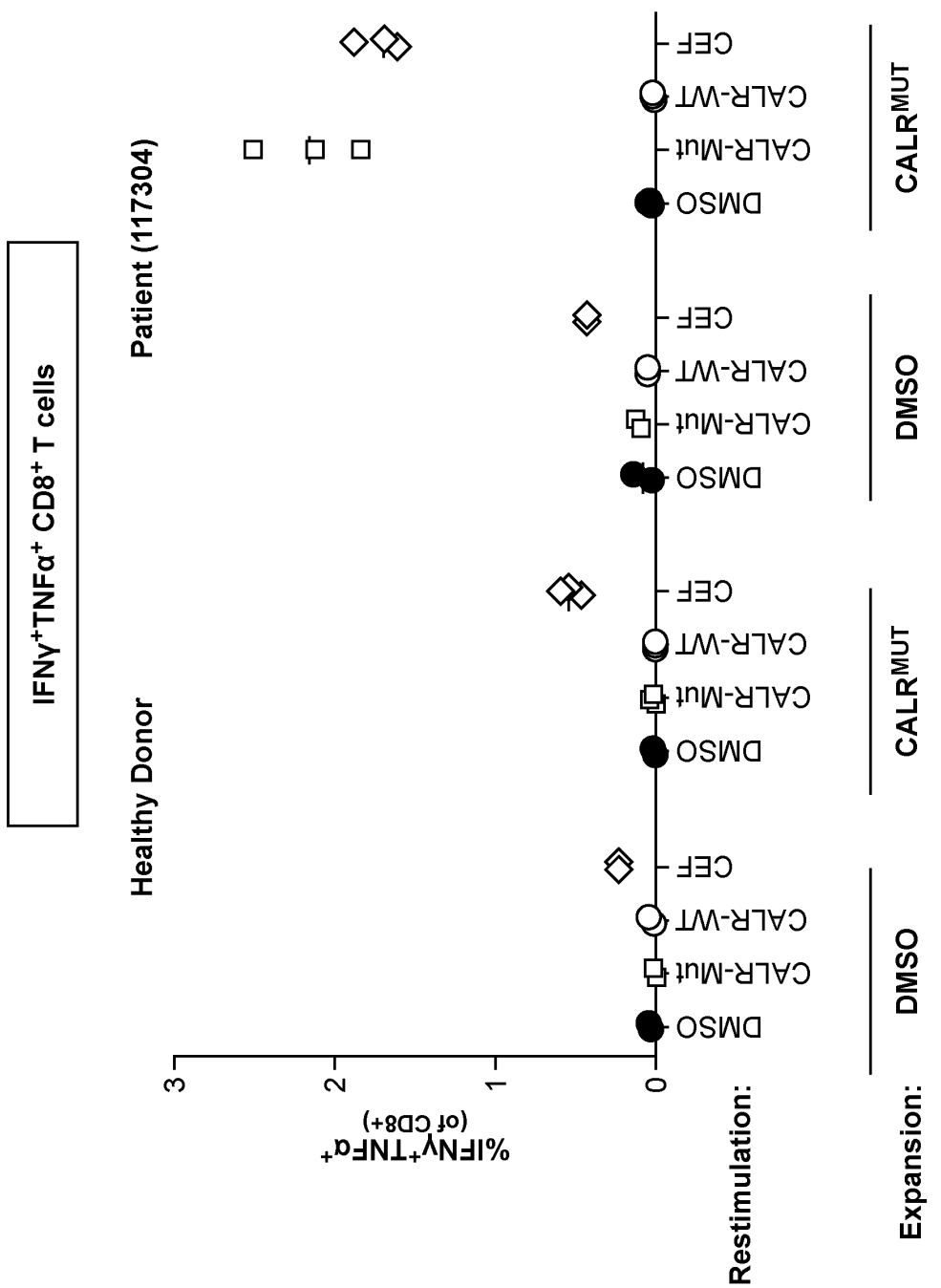

IFNγ+TNFα+ CD8+ T cells
Healthy Donor
Patient (117304)
%IFNγ+TNFα+ (of CD8+)
0
1
2
3
Restimulation:
DMSO
CALR-Mut
CALR-WT
CEF
Expansion:
DMSO
CALRMUT
DMSO
CALRMUT

FIG. 2C

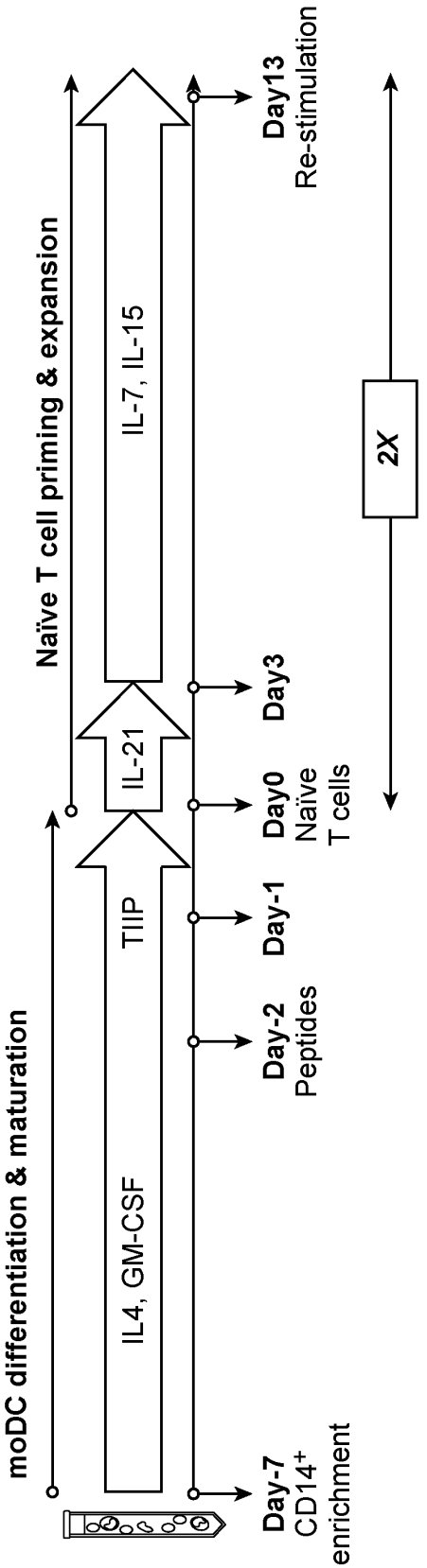

moDC differentiation & maturation
Naïve T cell priming & expansion
IL4, GM-CSF
TIIP
IL-21
IL-7, IL-15
2X
Day-7
CD14+
enrichment
Day-2
Peptides
Day-1
Day0
Naïve
T cells
Day3
Day13
Re-stimulation

FIG. 3A

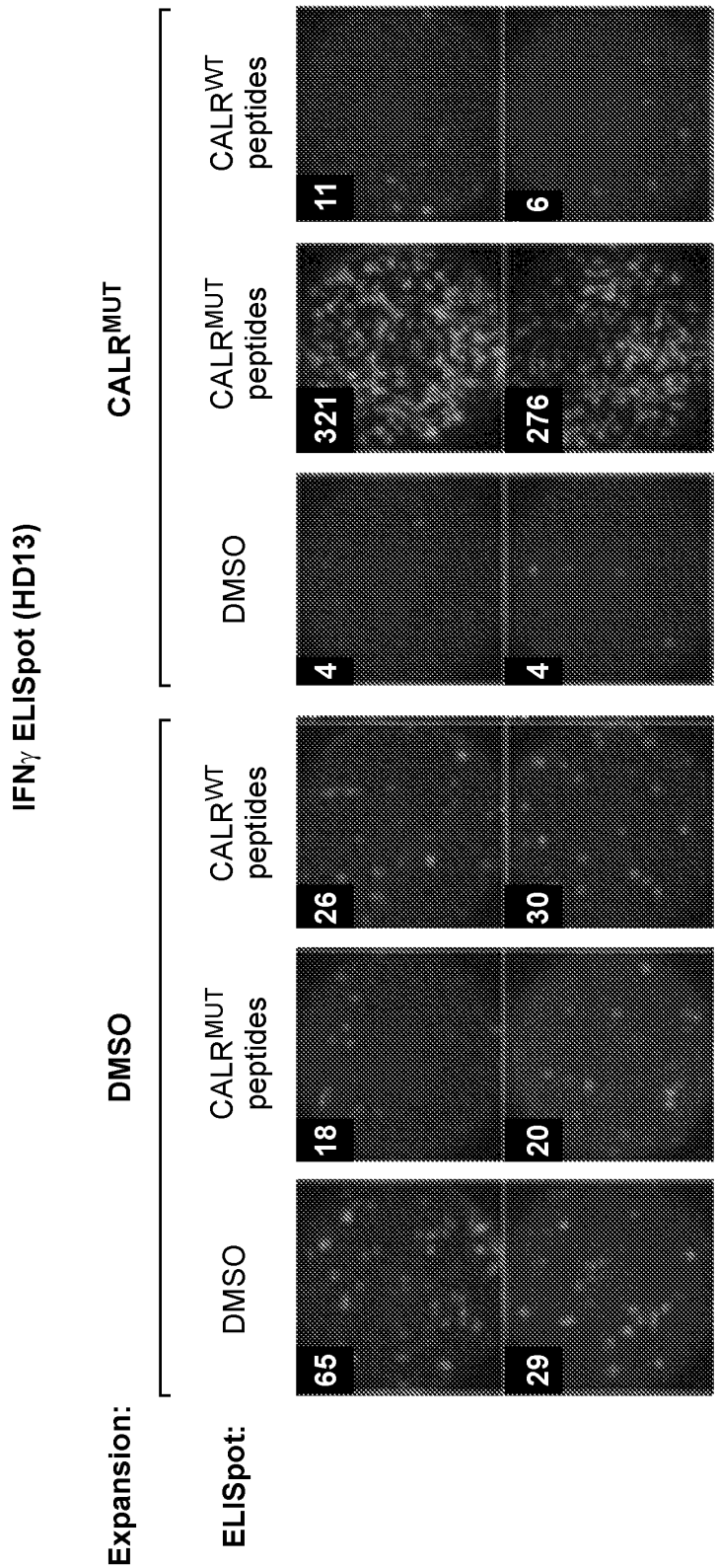

IFNγ ELISpot (HD13)
Expansion:
DMSO
CALR^MUT
ELISpot:
DMSO
CALR^MUT peptides
CALR^WT peptides
DMSO
CALR^MUT peptides
CALR^WT peptides
65
18
26
4
321
11
29
20
30
4
276
6

FIG. 3B

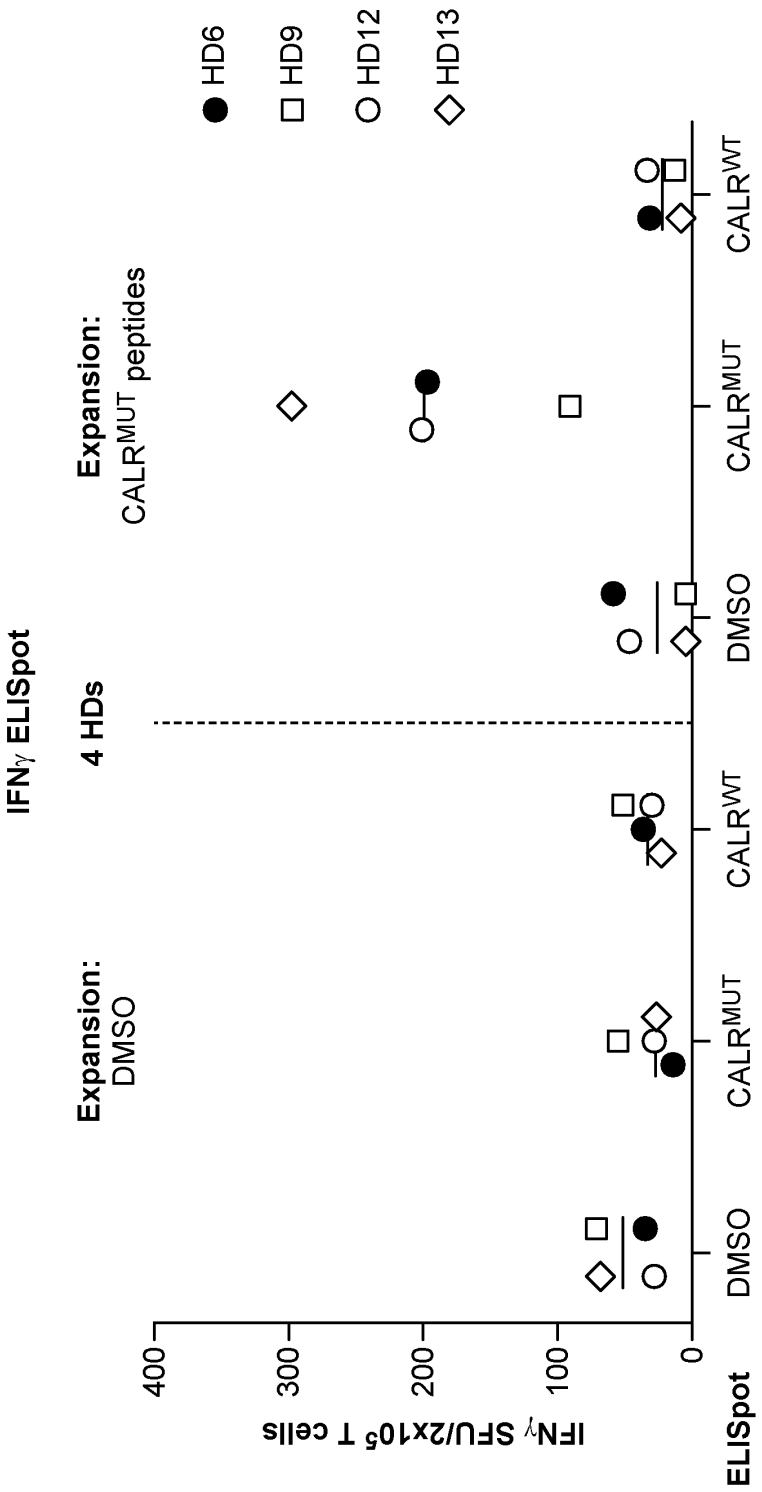

IFNγ ELISpot
4 HDs
Expansion:
DMSO
Expansion:
CALR^MUT peptides
HD6
HD9
HD12
HD13
IFNγ SFU/2x10^5 T cells
400
300
200
100
0
DMSO
CALR^MUT
CALR^WT
DMSO
CALR^MUT
CALR^WT
ELISpot

FIG. 3C

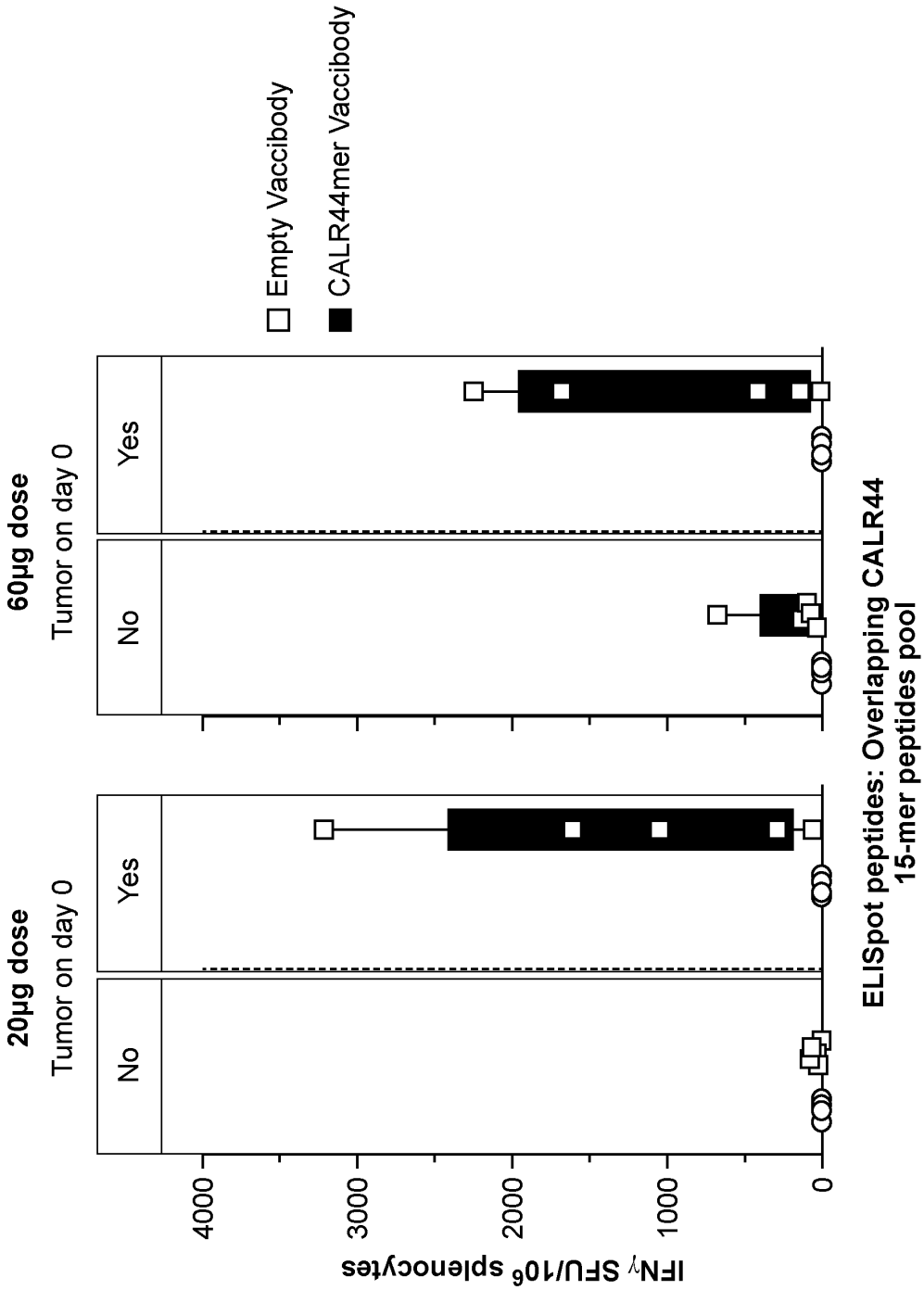

Empty Vaccibody
CALR44mer Vaccibody
60µg dose
Tumor on day 0
No
Yes
20µg dose
Tumor on day 0
No
Yes
IFNγ SFU/10^6 splenocytes
0
1000
2000
3000
4000
ELISpot peptides: Overlapping CALR44
15-mer peptides pool

FIG. 4

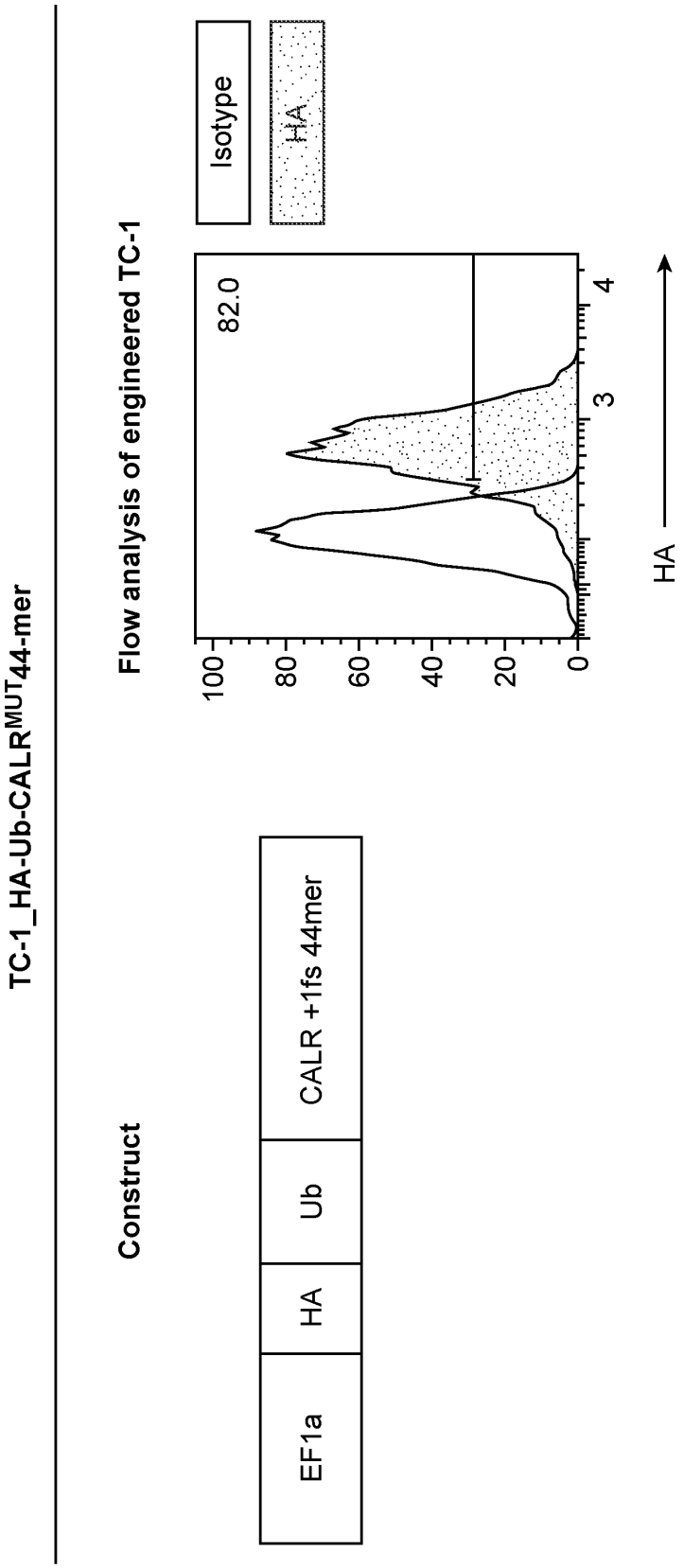

TC-1_HA-Ub-CALR^MUT44-mer
Flow analysis of engineered TC-1
Isotype
HA
82.0
100
80
60
40
20
0
3
4
HA
Construct
EF1a
HA
Ub
CALR +1fs 44mer

FIG. 5A

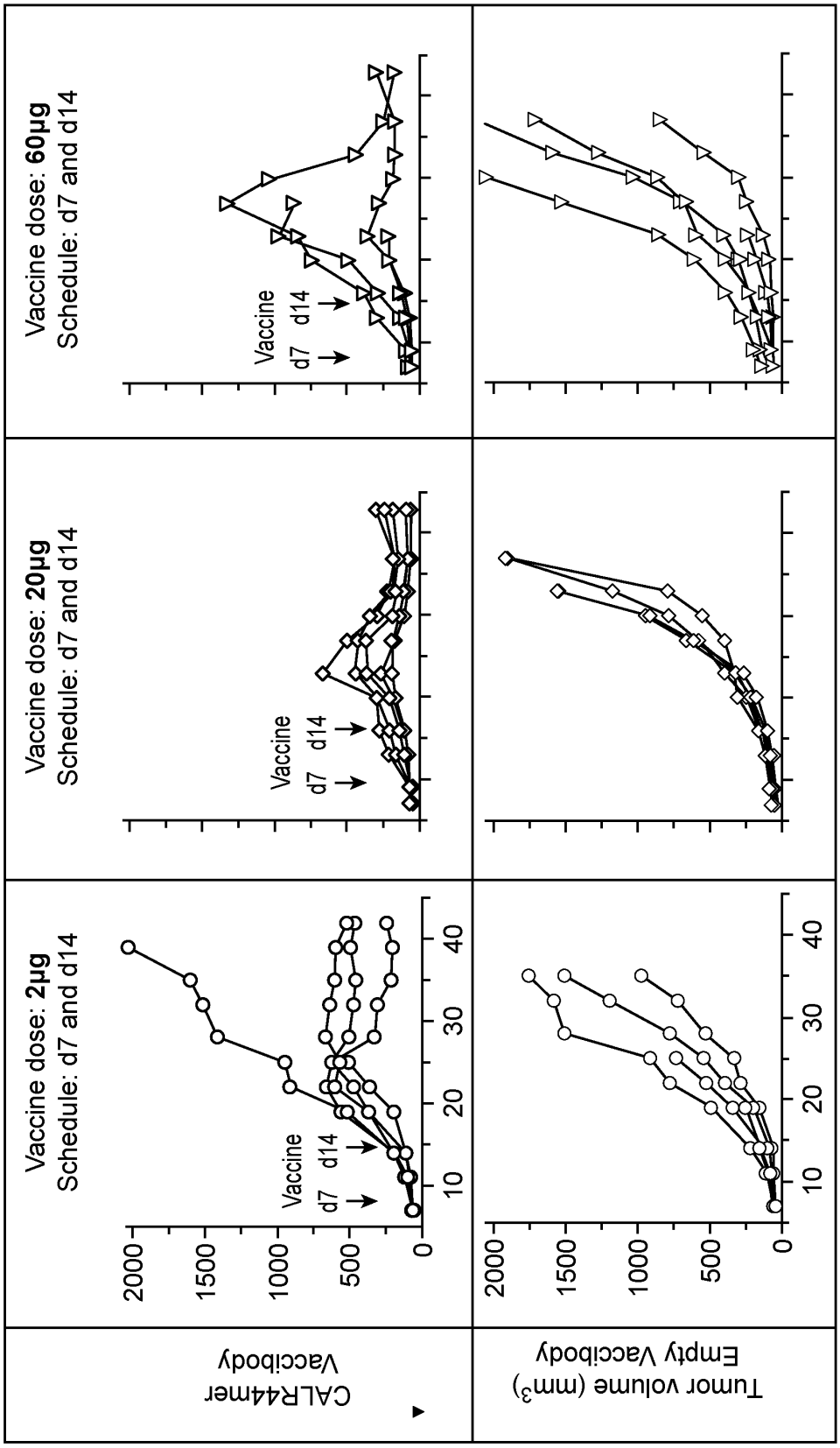

Vaccine dose: 2µg
Schedule: d7 and d14
Vaccine dose: 20µg
Schedule: d7 and d14
Vaccine dose: 60µg
Schedule: d7 and d14
Vaccine
d7 d14
CALR44mer Vaccibody
Empty Vaccibody
Tumor volume (mm3)
0
500
1000
1500
2000
10
20
30
40
Days post-tumor implantation

FIG. 5B