

Figure 1

Process 2A: about 22 days from initiation of First Expansion – Step E

1. STEP A

Obtain Patient Tumor Sample

2. STEP B

Fragmentation
Cryopreservation of Tumor Tissue Fragments
First Expansion
3 days to 14 days

3. STEP C

First Expansion to Second Expansion Transition
No Storage and Closed System

4. STEP D

Second Expansion
IL-2, OKT-3, and antigen-presenting feeder cells
Closed System

5. STEP E

Harvest TILS from Step D
Closed System

6. STEP F

Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 2A

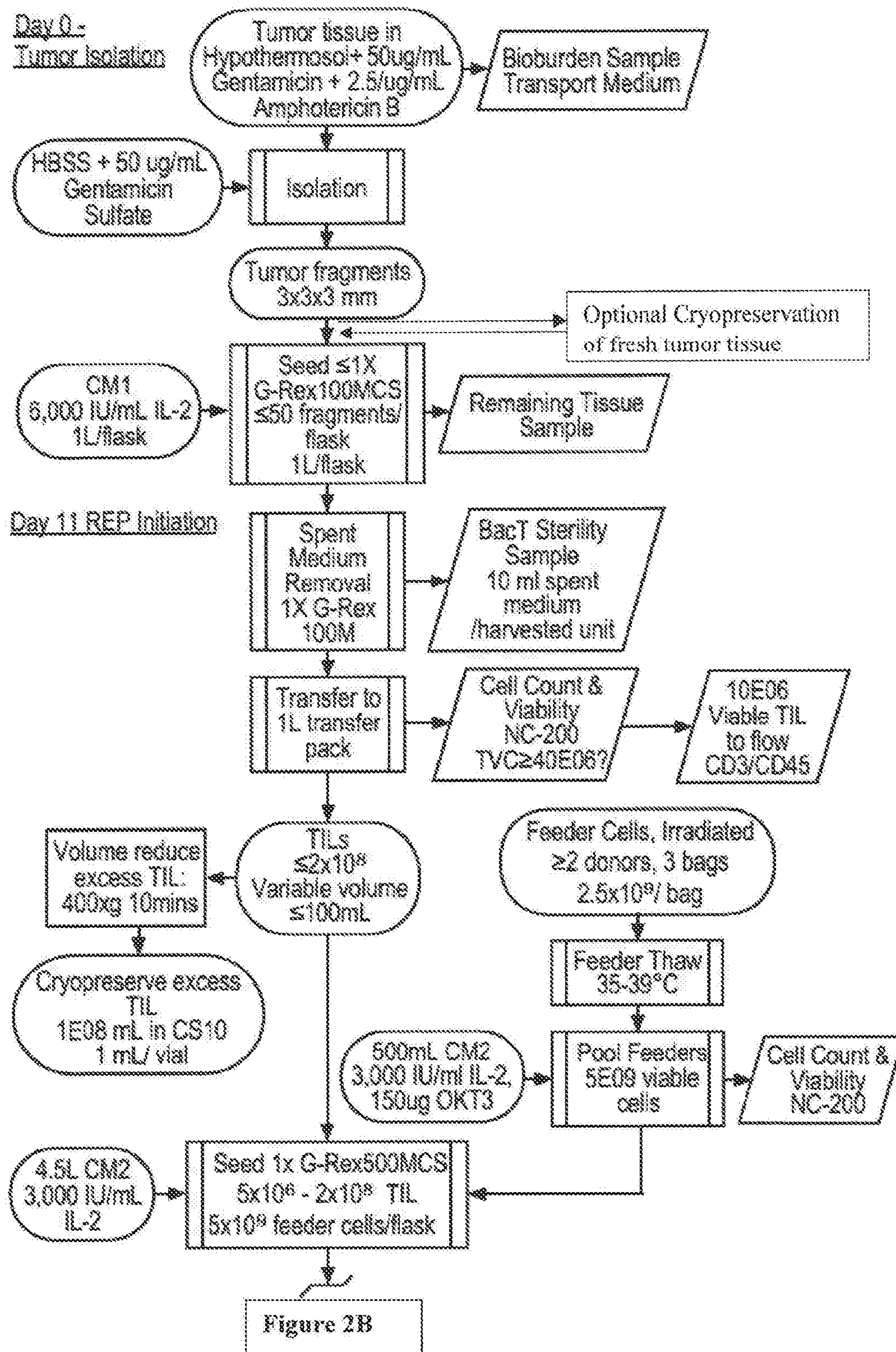


Figure 2B

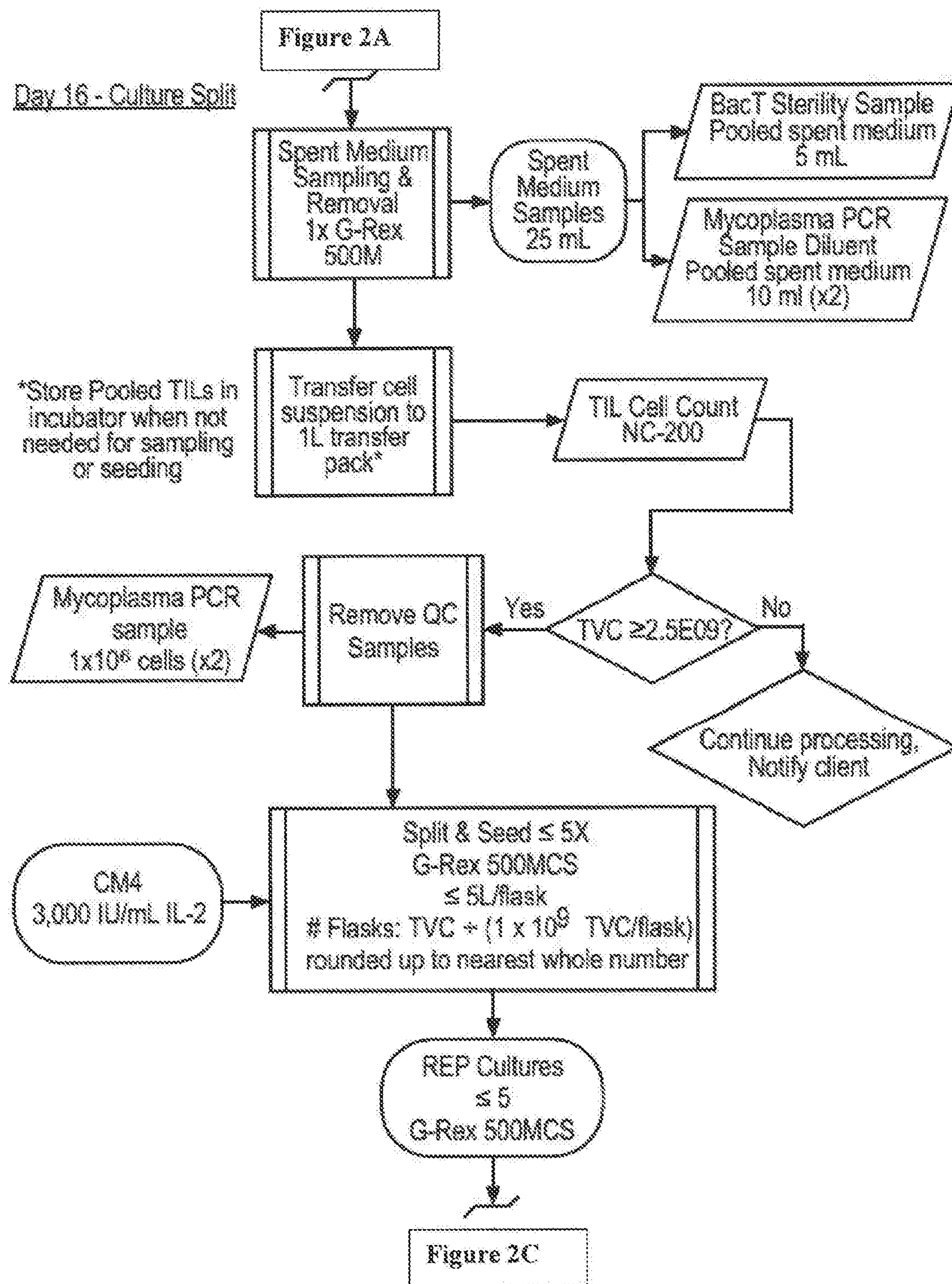
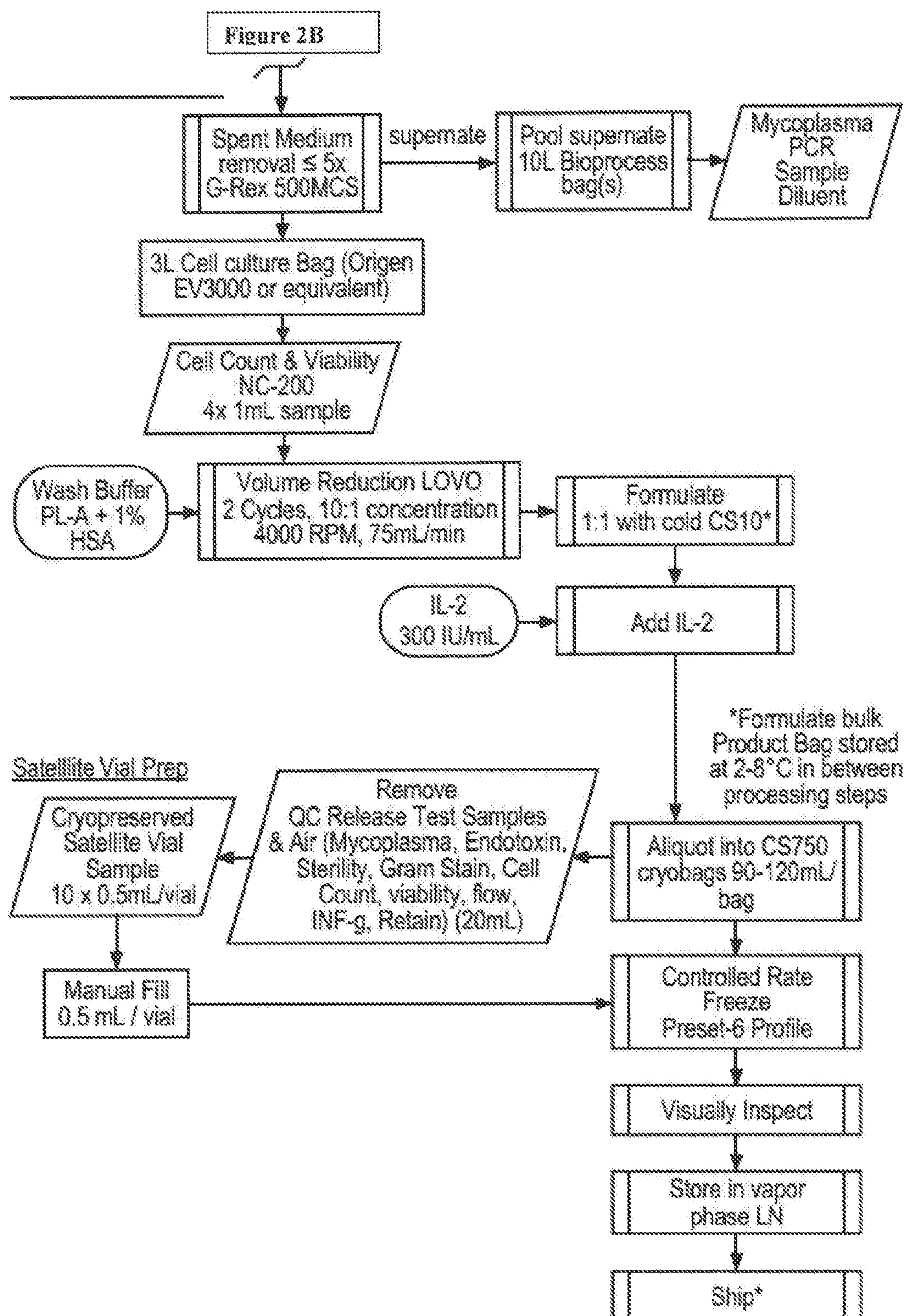


Figure 2C



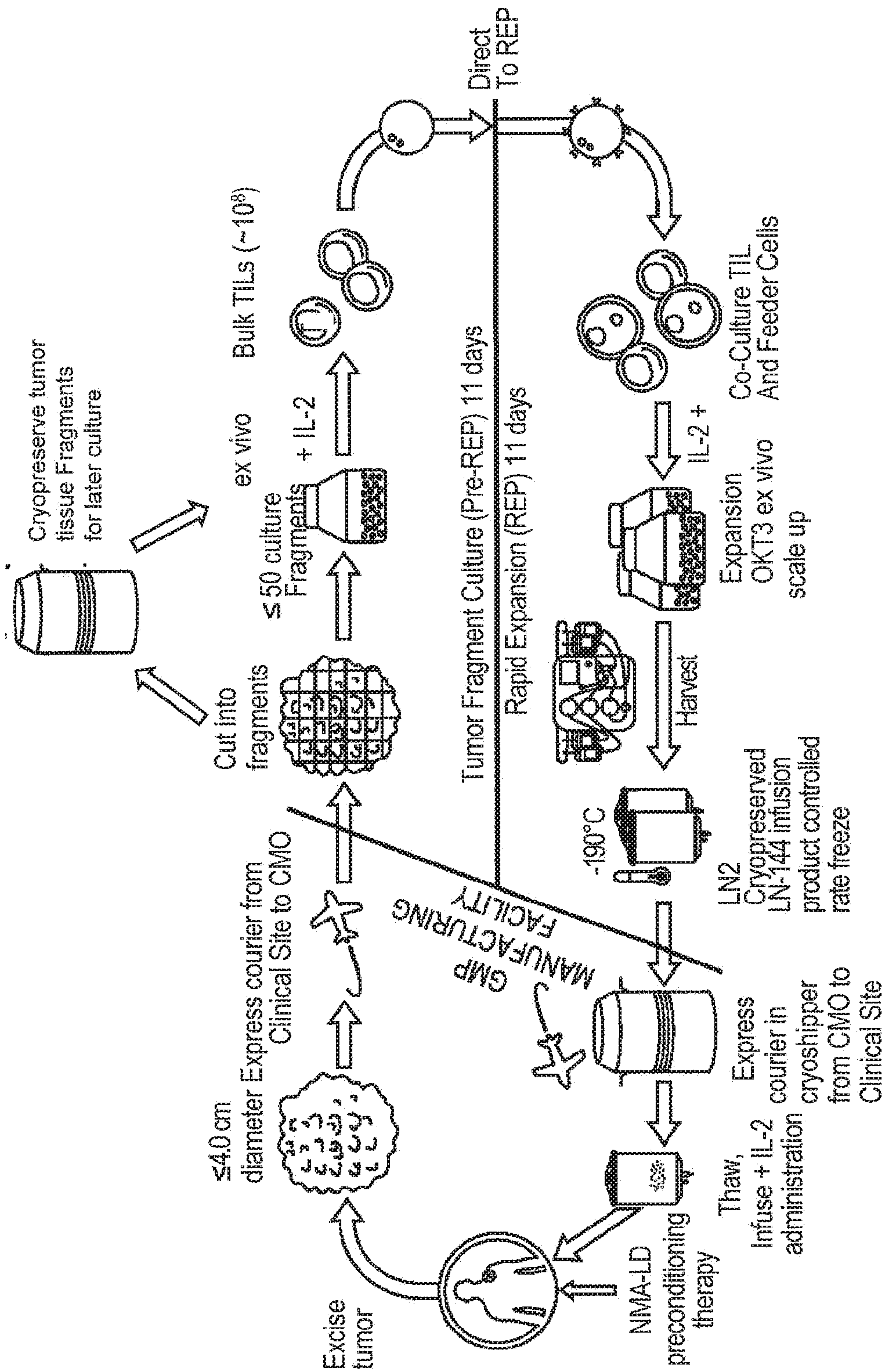


Figure 3

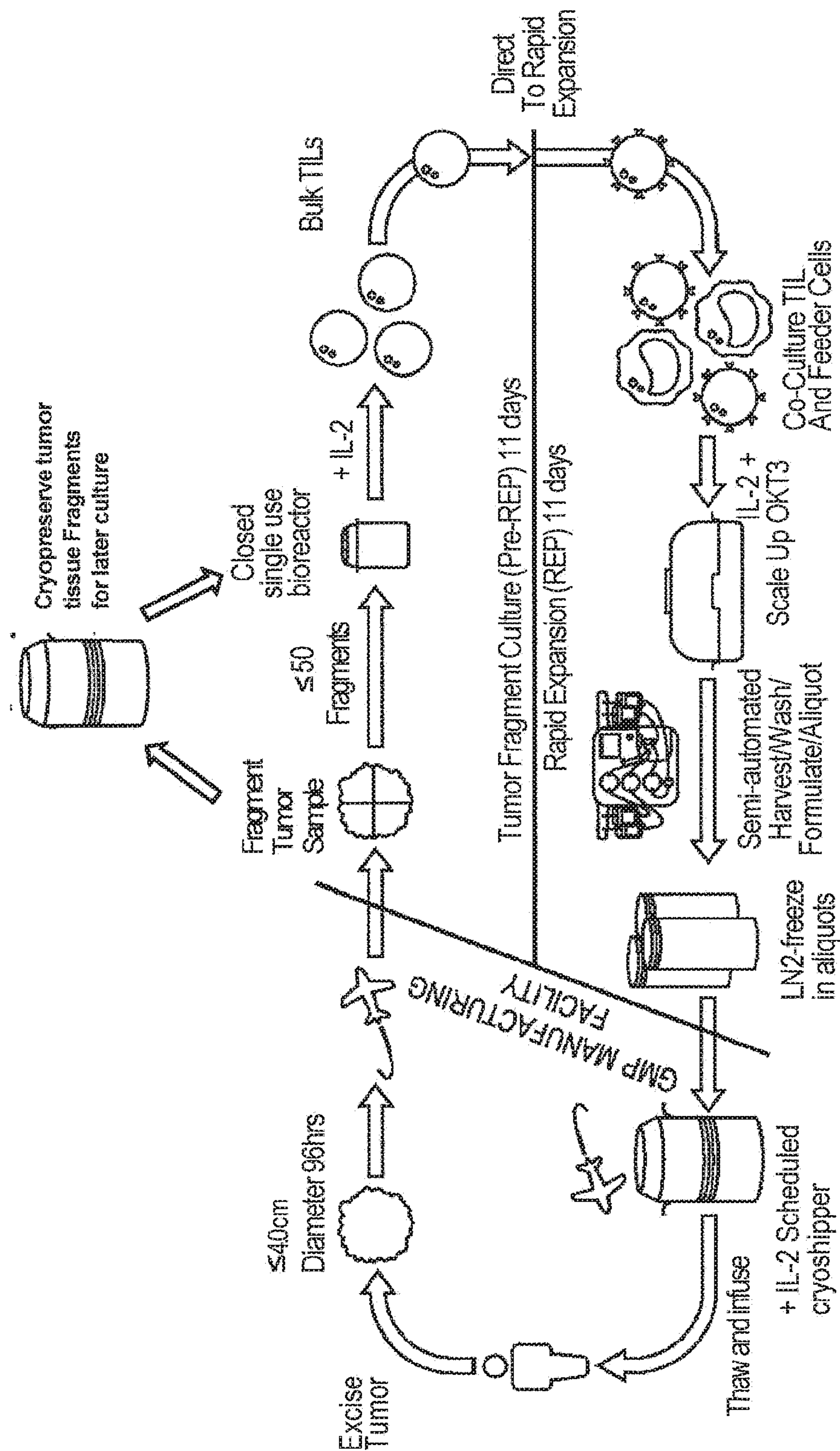


Figure 4

Figure 5

Process 1C 43 – 55 Days for Steps A - E	Process 2A About 22 Days from initiation of preREP to Step E
1. Step A Obtain Patient Tumor Sample	1. Step A Obtain Patient Tumor Sample
2. Step B Fragmentation and First Expansion 11 days to 21 days	2. Step B Fragmentation, optional cryopreservation of tumor fragments, and then First Expansion 11 days to 21 days (beginning from start of first expansion culture)
3. Step C First Expansion to Second Expansion Transition Optional Storage until Selection	3. Step C First Expansion to Second Expansion Transition No Storage and Closed System
4. Step D Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Optionally repeat one or more times	4. Step D Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Closed System
5. Step E Harvest TILs from Step D	5. Step E Harvest TILs from Step D Closed System
6. Step F Final Formulation and/or Transfer to Infusion Bag	6. Step F Final Formulation and/or Transfer to Infusion Bag Optional cryopreservation

Figure 6

Process Step	Process 1C Embodiment	Process 2A Embodiment	Advantages
Pre-REP	4 fragments per 10 GREX-10 flasks 11-21 day duration	40 fragments per 1 GREX-100M flask 11 day duration	- Increased tumor fragments per flask - Shortened culture time - Reduced number of steps - Amenable to closed system
Pre-REP to REP Transition	- Pre-REP TIL are frozen until phenotyped for selection then thawed to proceed to the REP (~day 30) - REP requires $>40 \times 10^6$ TIL	- Pre-REP TIL directly move to REP on day 11 - REP requires 25-200 $\times 10^6$ TIL	- Shortened pre-REP-to-REP process - Reduced number of steps - Eliminated phenotyping selection - Amenable to closed system
REP	6 GREX-100M flasks on REP day 0 5×10^5 TIL and 5×10^8 PBMC feeders per flask on REP day 0 - Split to 18-36 flasks on REP day 7 14 day duration	1 GREX-500M flask on day 11 25-200 $\times 10^8$ TIL and 5×10^9 PBMC feeders on day 11 - Split to ≤ 6 GREX-500M flasks on day 16 11 day duration	- Reduced number of steps - Shorter REP duration - Closed system transfer of TIL between flasks - Closed system media exchanges
Harvest	TIL harvested via centrifugation	TIL harvested via LOVO automated cell washing system	- Reduced number of steps - Automated cell washing - Closed system - Reduced loss of product during wash
Final Formulation	- Fresh product in Hypothermosol - Single infusion bag - Limited shipping stability	- Cyropreserved product in Plasmalyte-A + 1% HSA and CS10 stored in LN₂ - Multiple aliquots - Longer shipping stability	- Shipping flexibility - Flexible patient scheduling - More timely release testing
Overall Estimated Process Time	43-55 days	22 days	Faster turnaround to patient

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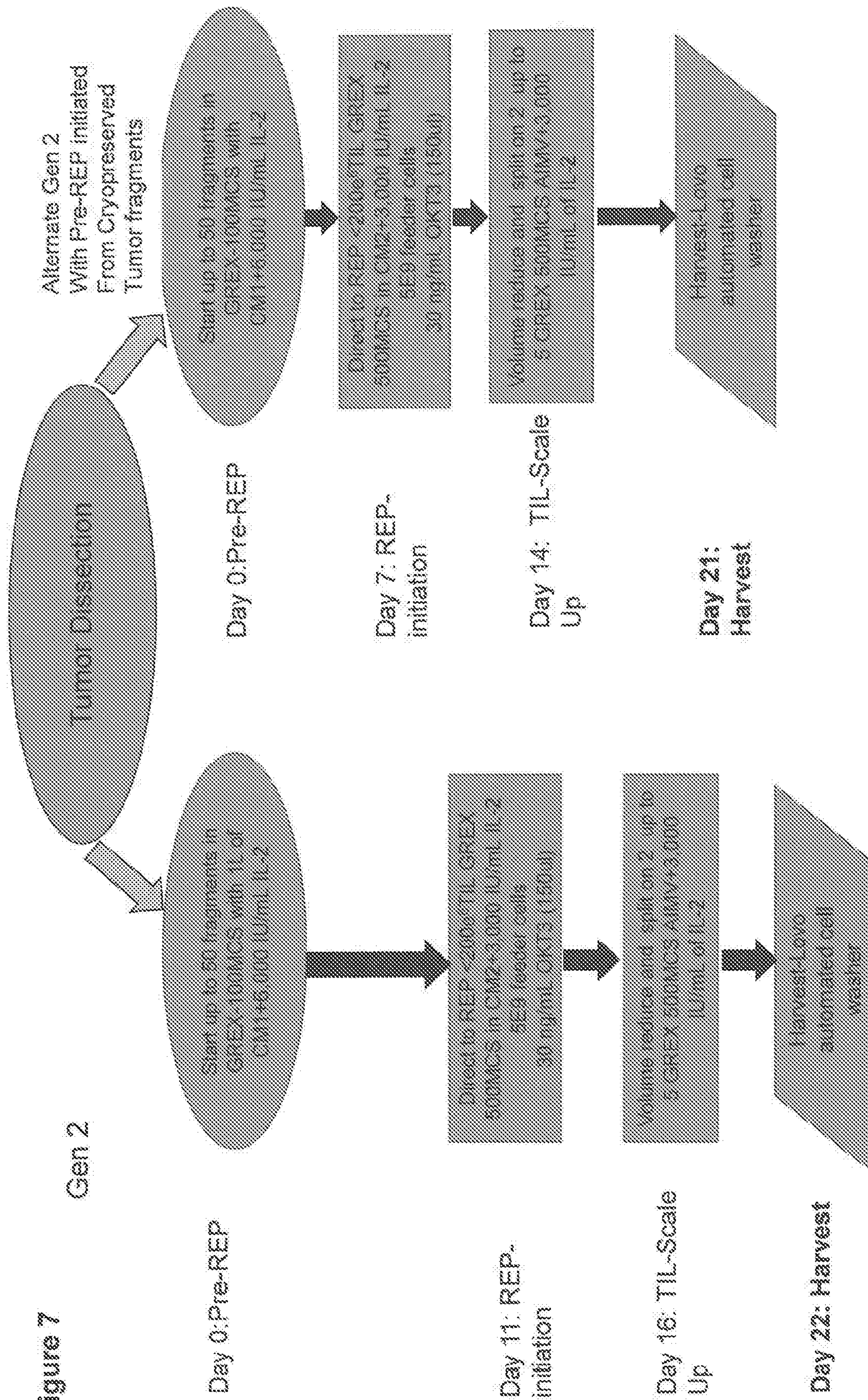


Figure 8

- Pre-REP TVC from Gen2 were 100e6 on Day 11
- Pre-REP TVC from Gen 2.2 were 0.8e6 on Day 7
- Pre-REP yield from Gen 2.2 were ~ 1% of Gen 2 control

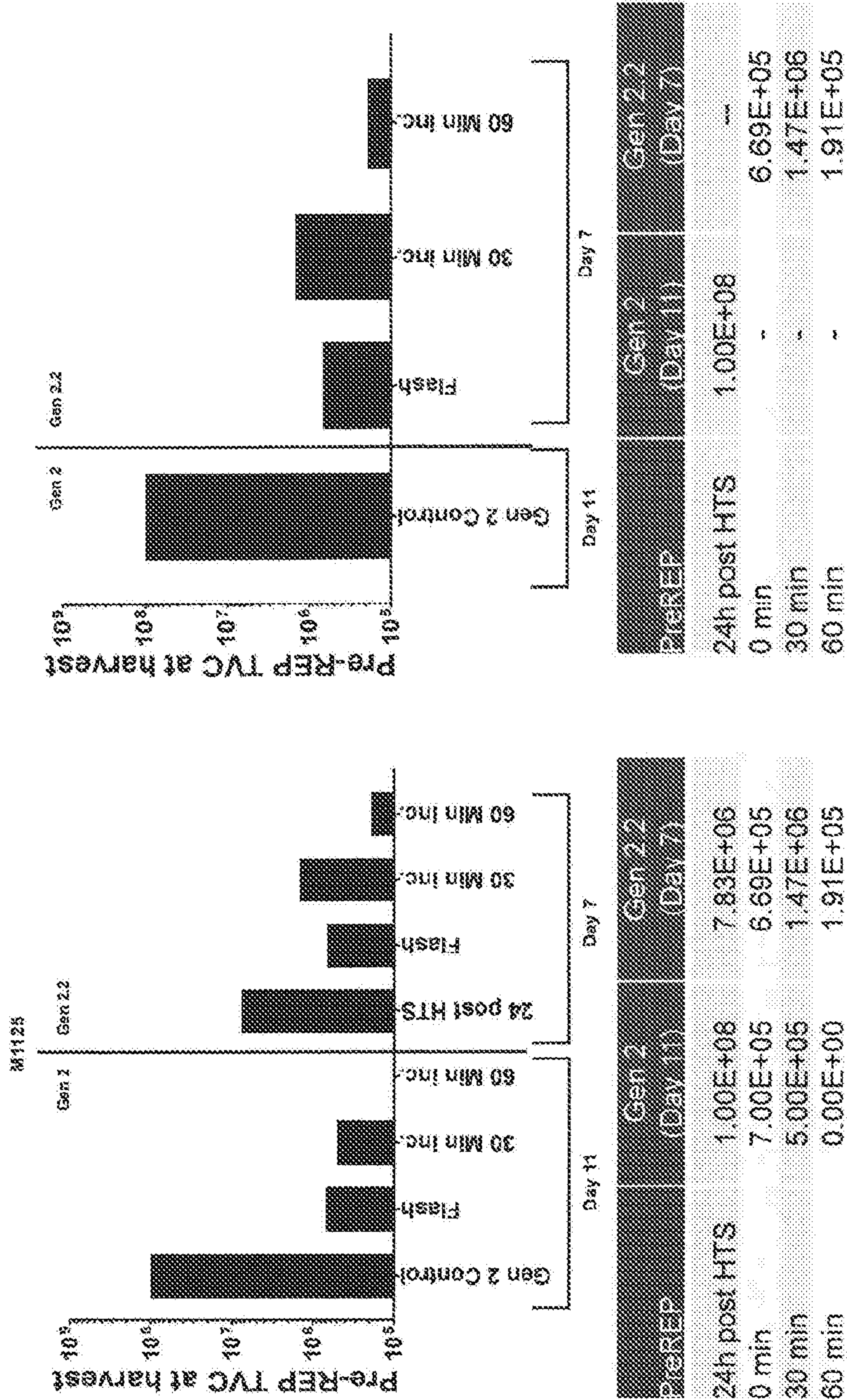


Figure 9

- Total REP TVC from Gen 2 were 2.5e9 on Day 22
- Total REP TVC from Gen 2.2 were 2.2, 2, 2, e9 on Day 21
- Total REP yield from Gen 2.2 were ~ 86% of Gen 2

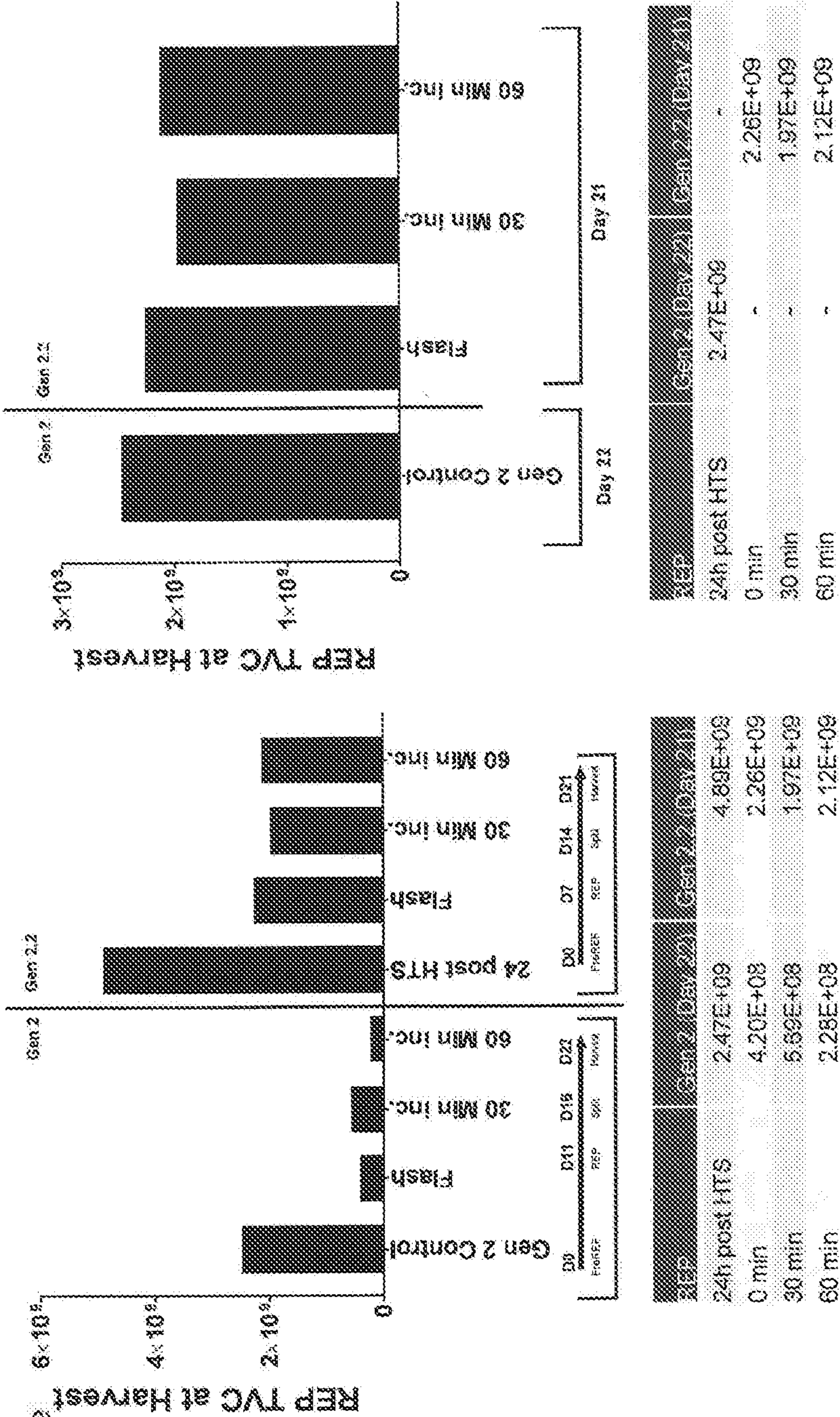


Figure 10

- REP TIL from Gen2 produced 6180 pg/mL IFN γ on Day 22
- REP TIL from Gen2.2 produced 10520, 12612, 11618 pg/mL IFN γ on Day 22

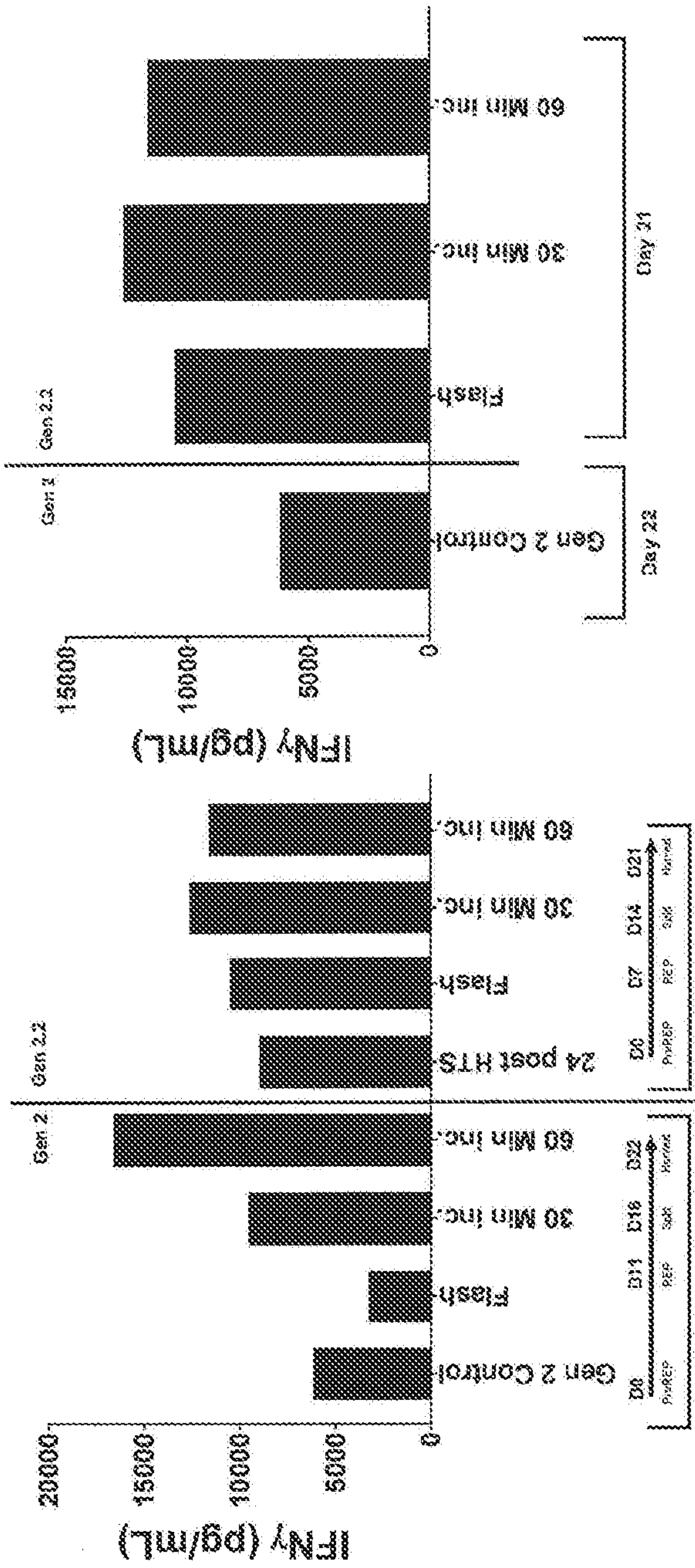


Figure 11
Tumor Processing
Example 3

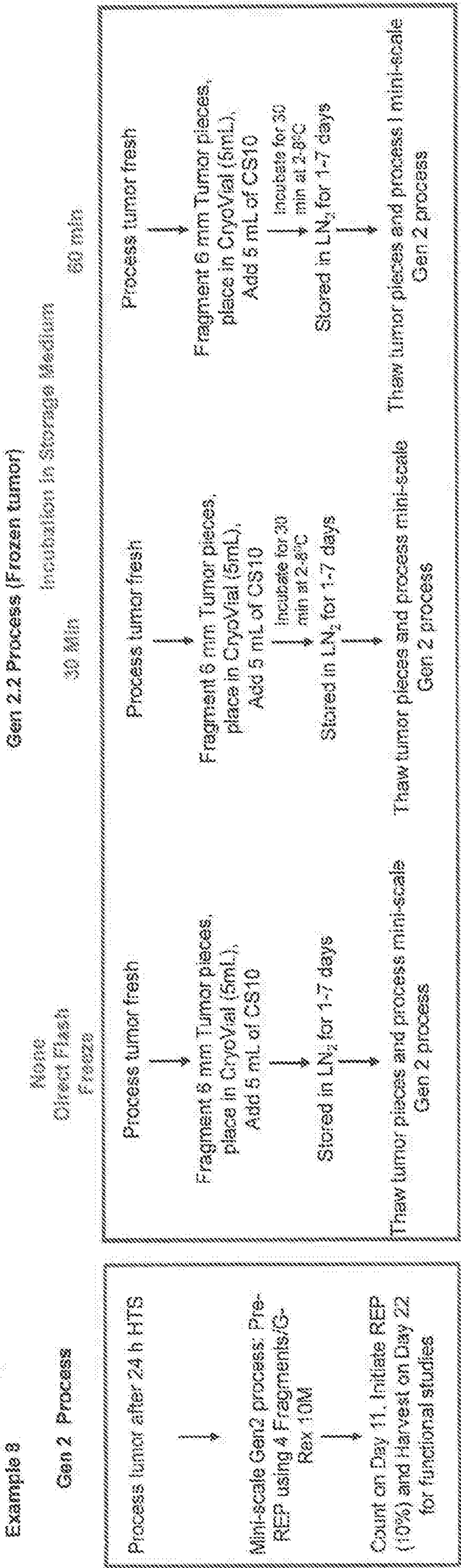


Figure 12
1/100 Scale TIL Production

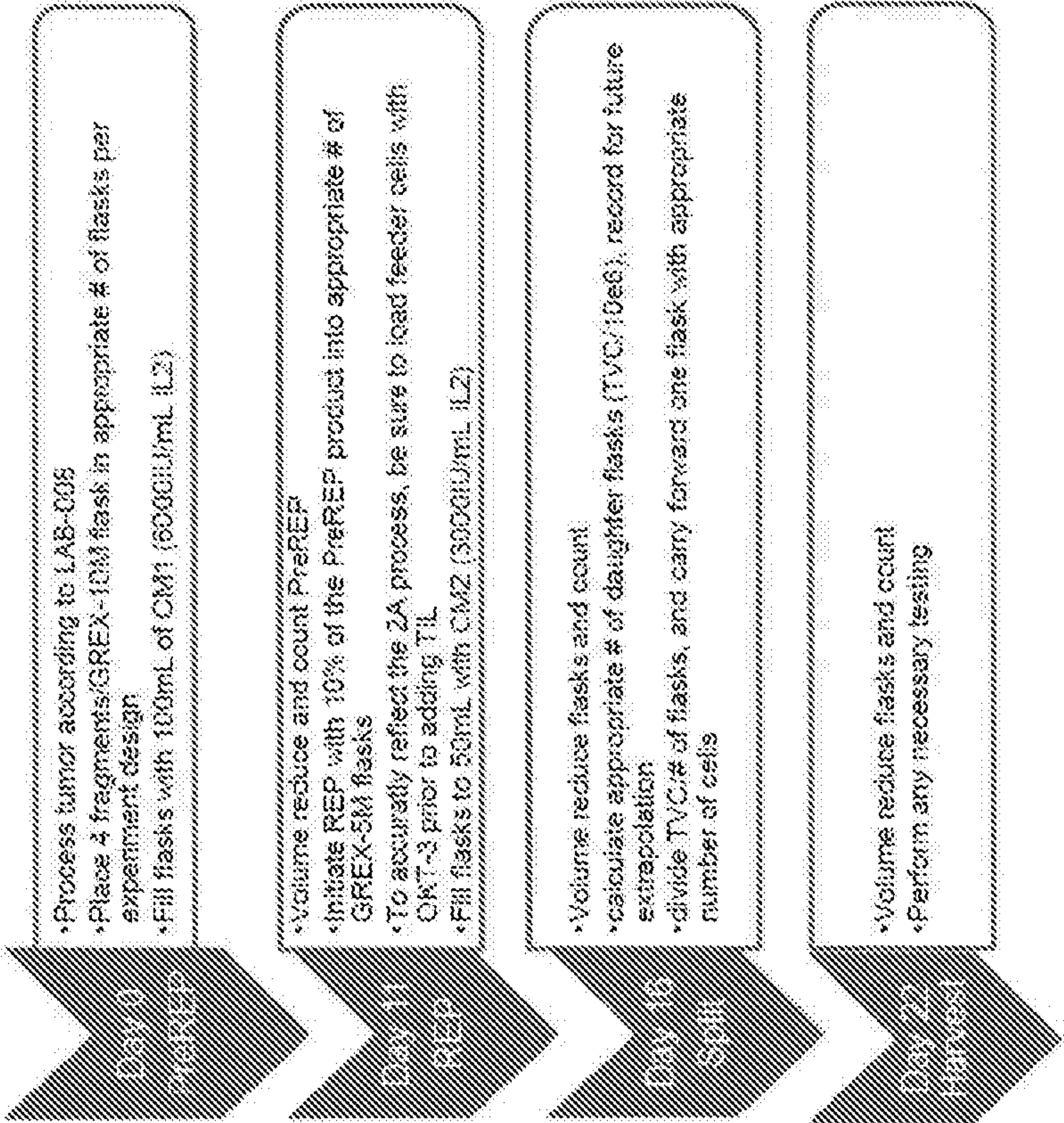


FIG. 13A

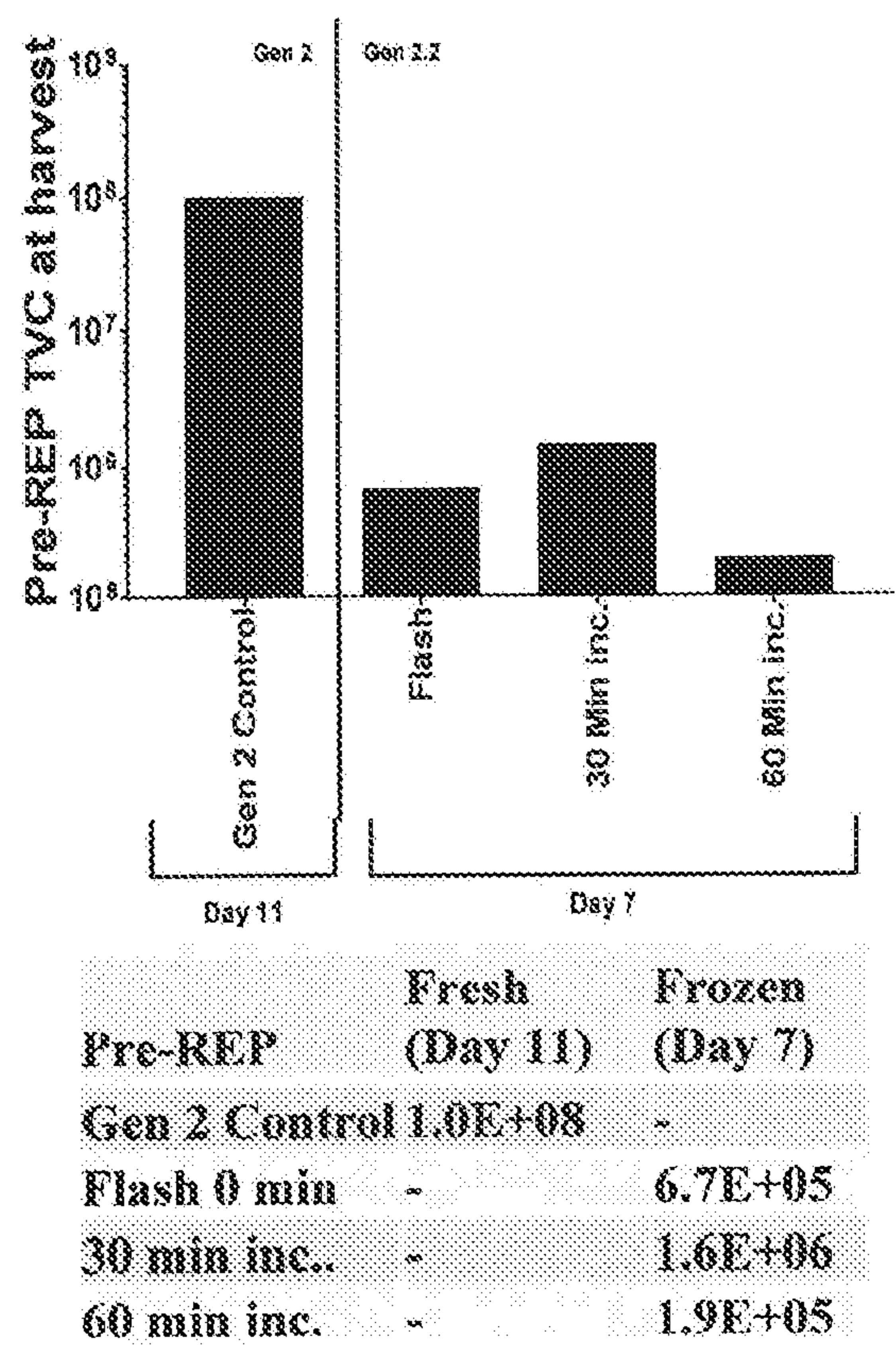


FIG. 13B

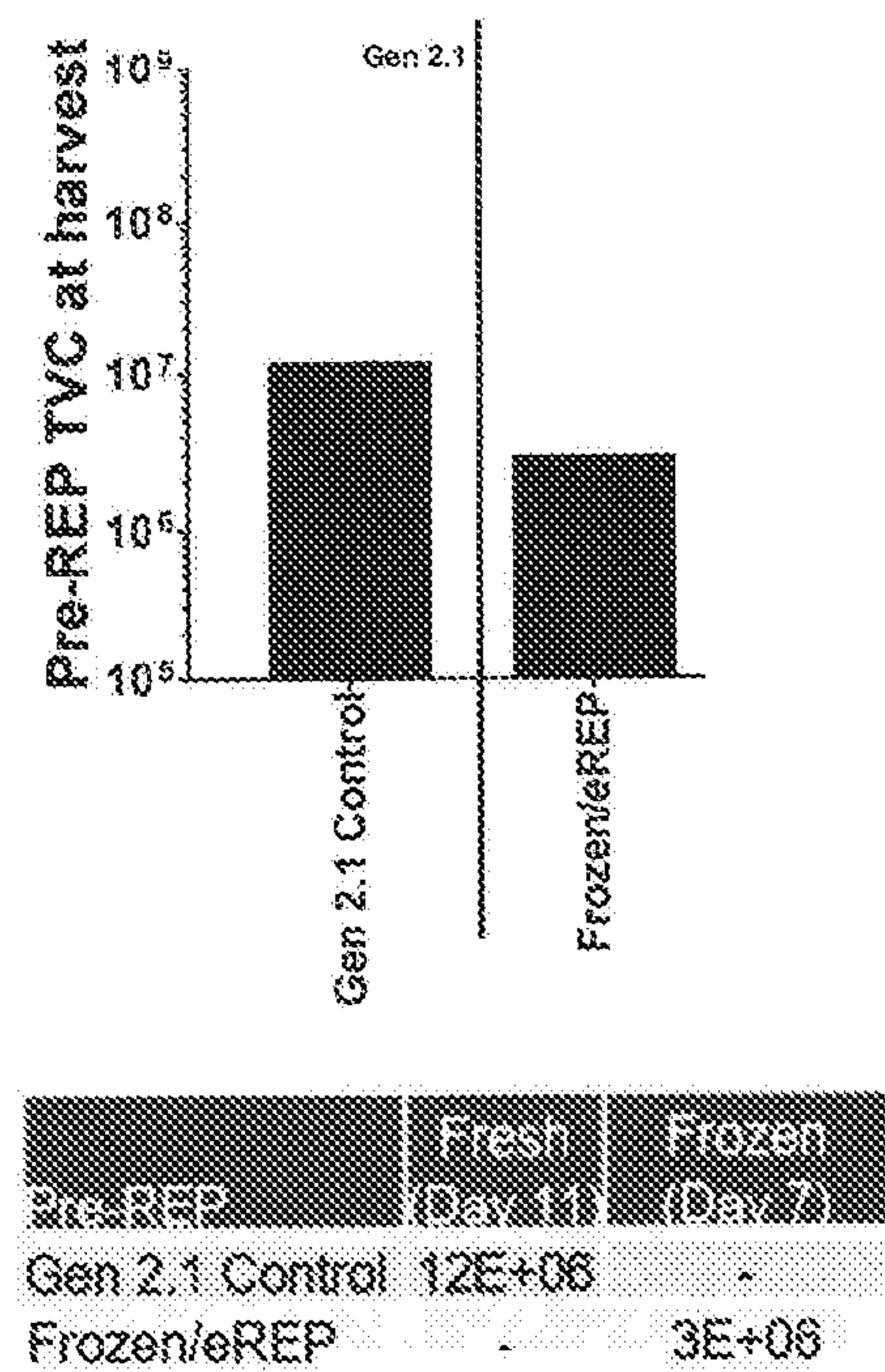


FIG. 13C

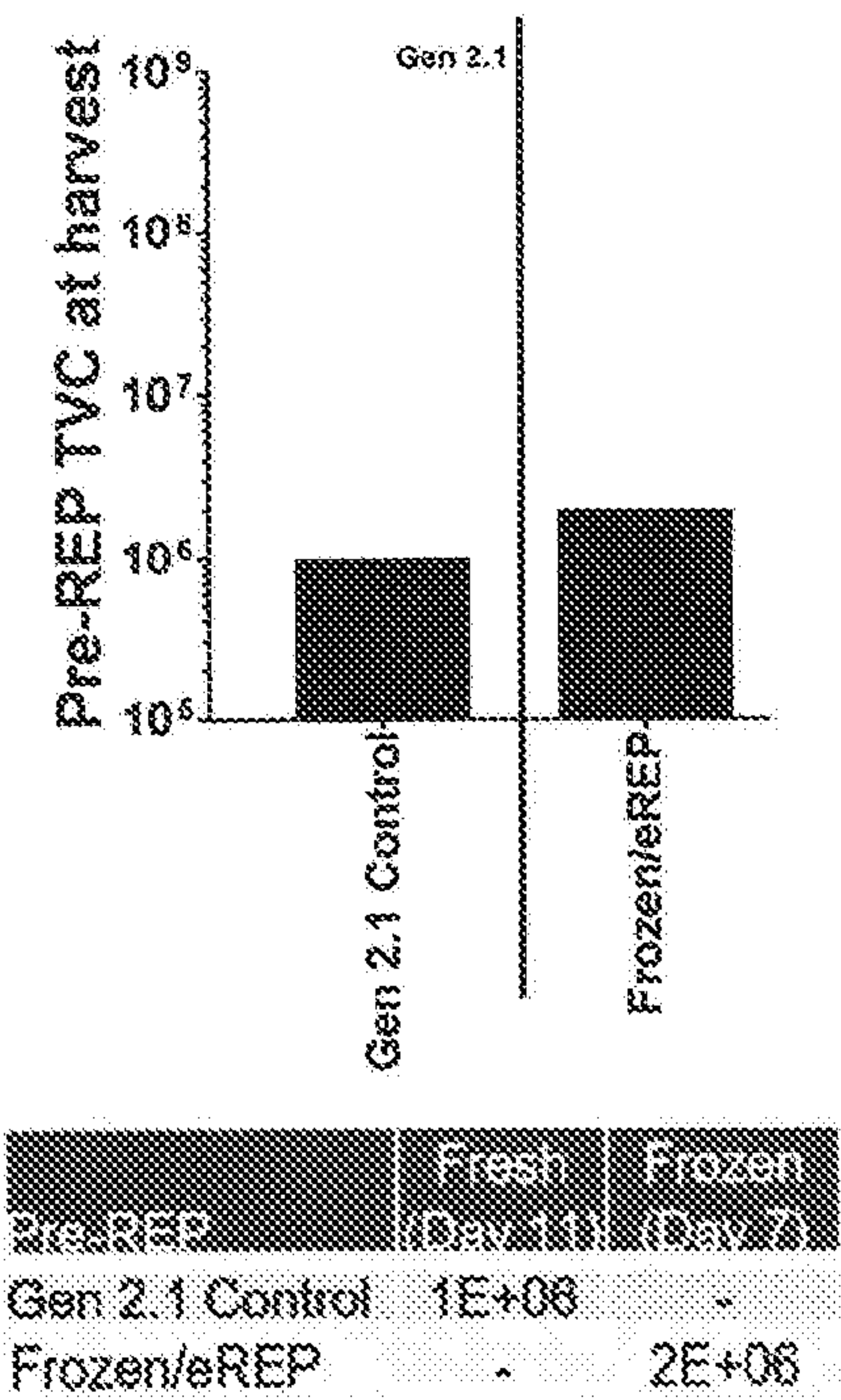


FIG. 13D

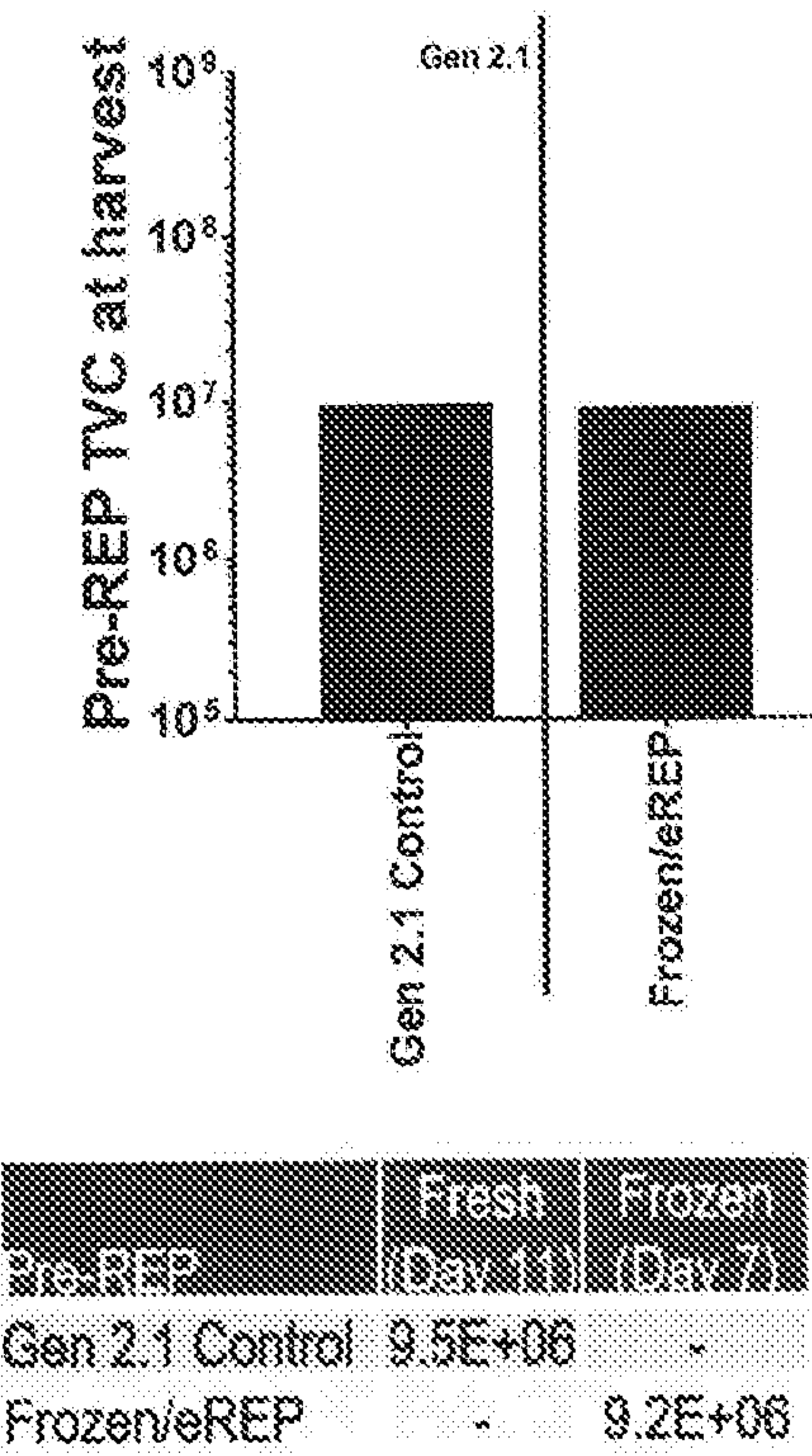


FIG. 13E

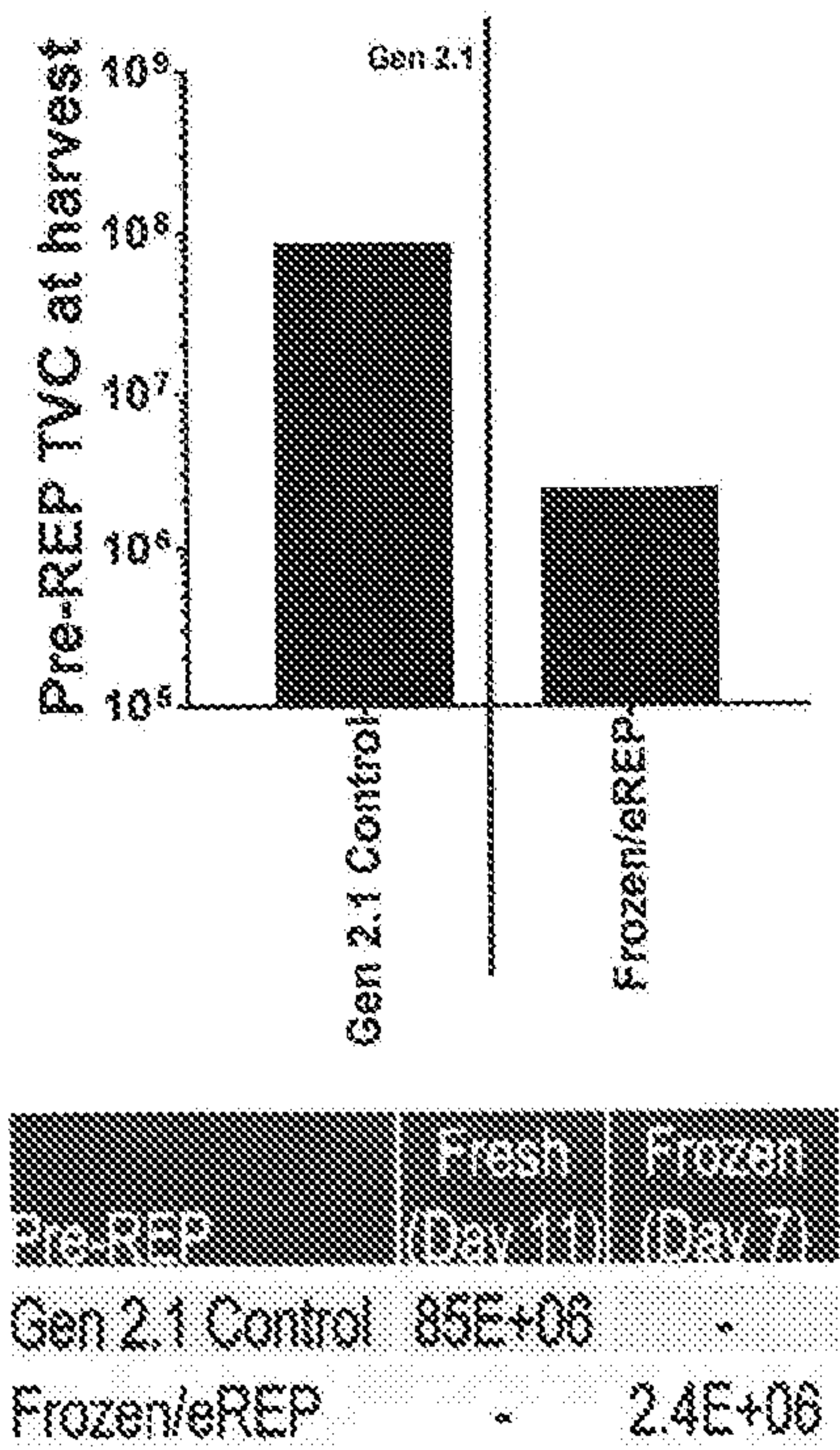
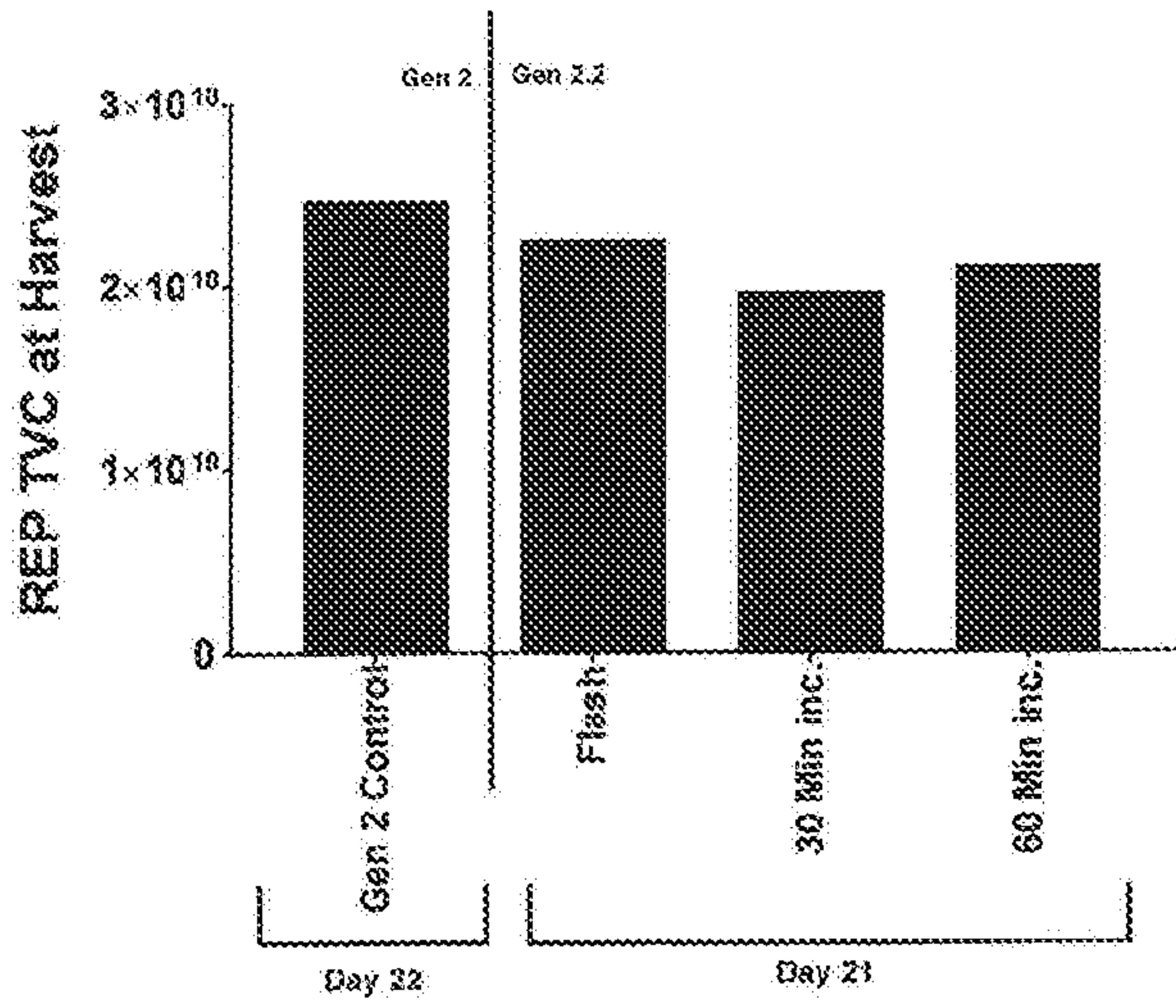
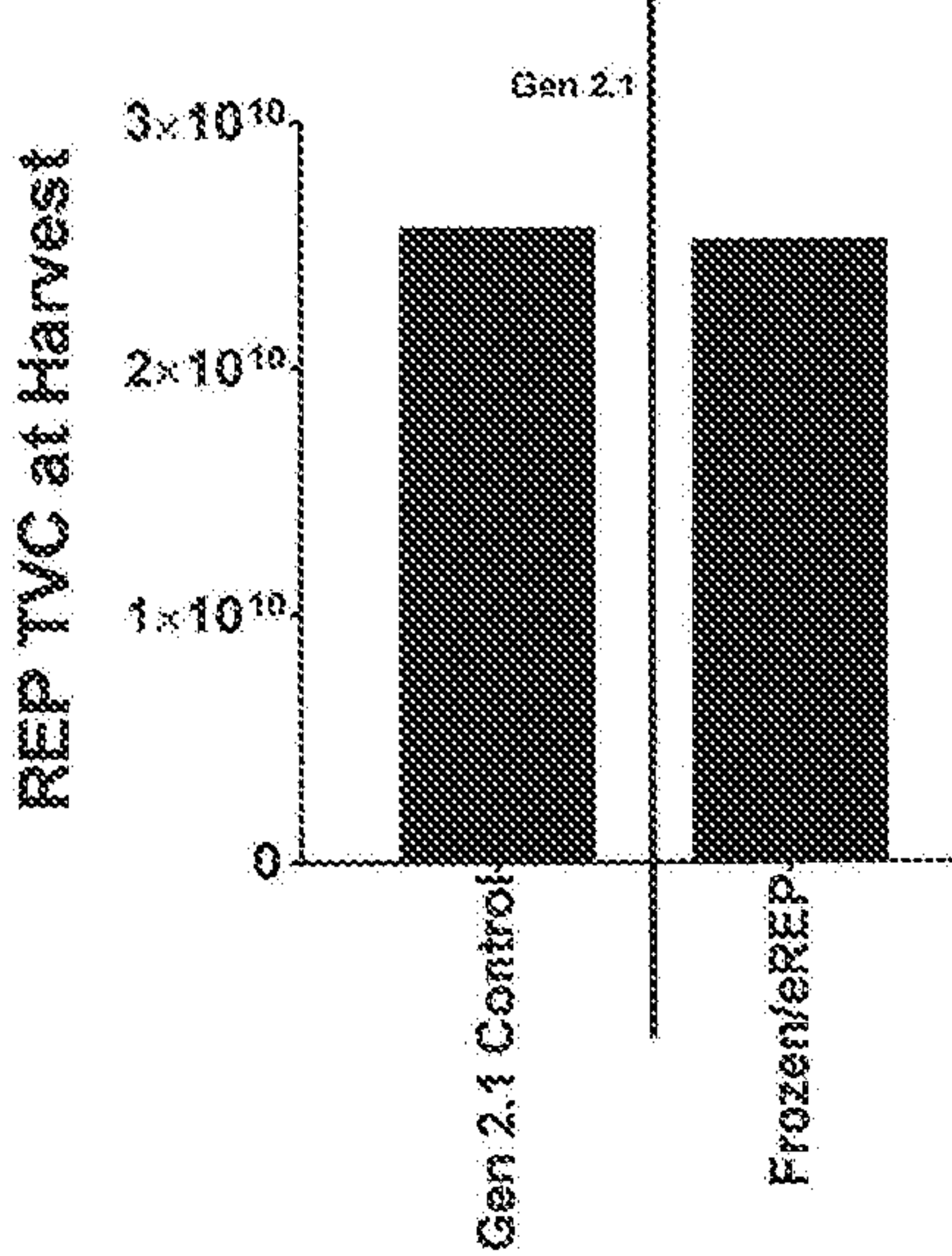


FIG. 14A



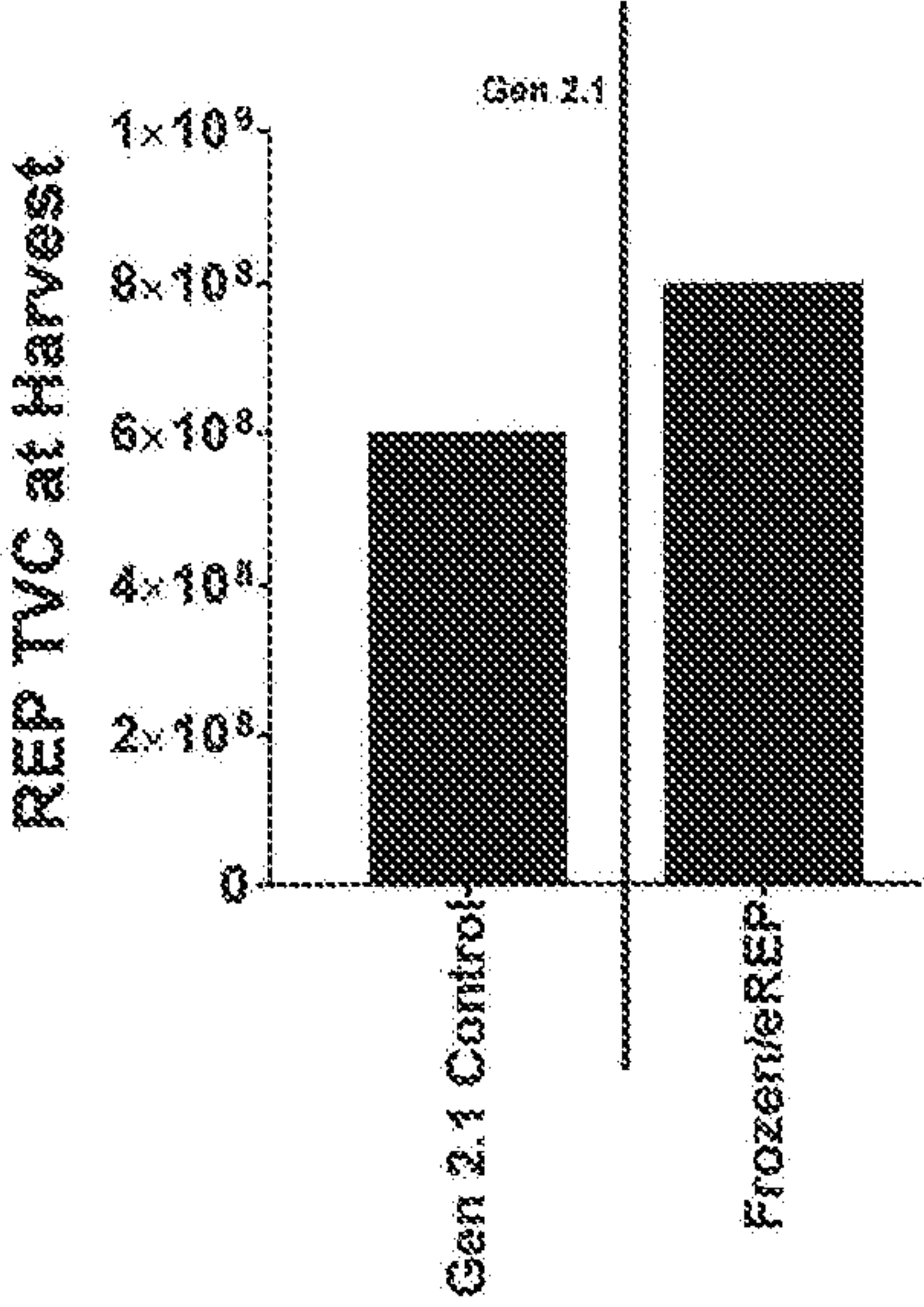
Total REP Yield	Gen 2 (Day 22)	Frozen/Early REP (Day 21)
Gen 2 Control	24.7E+09	-
Flash 0 min	-	22.6E+09
30 min inc.	-	19.7E+09
60 min inc.	-	21.2E+09

FIG. 14B



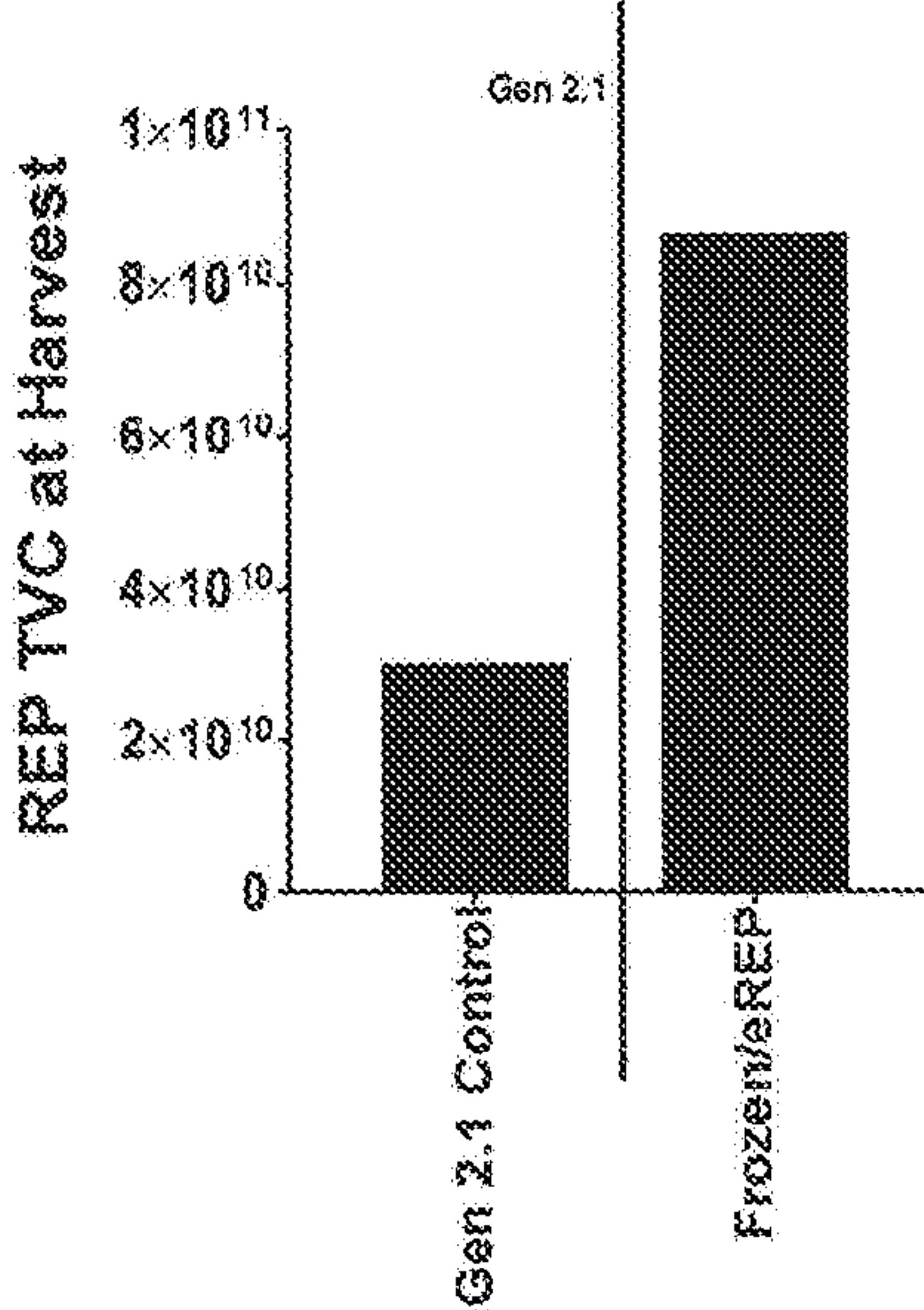
Total REP Yield	Gen 2 (Day 22)	Frozen/Early REP (Day 21)
Gen 2.1 Control	25.8E+09	-
Frozen/eREP	-	25.3E+09

FIG. 14C



Total REP Yield	Gen 2 (Day 22)	Frozen/Early REP (Day 21)
Gen 2.1 Control	0.6E+09	-
Frozen/eREP	-	0.8E+09

FIG. 14D



Total REP Yield	Gen 2 (Day 22)	Frozen/Early REP (Day 21)
Gen 2.1 Control	30E+09	-
Frozen/eREP	-	87E+09

FIG. 14E

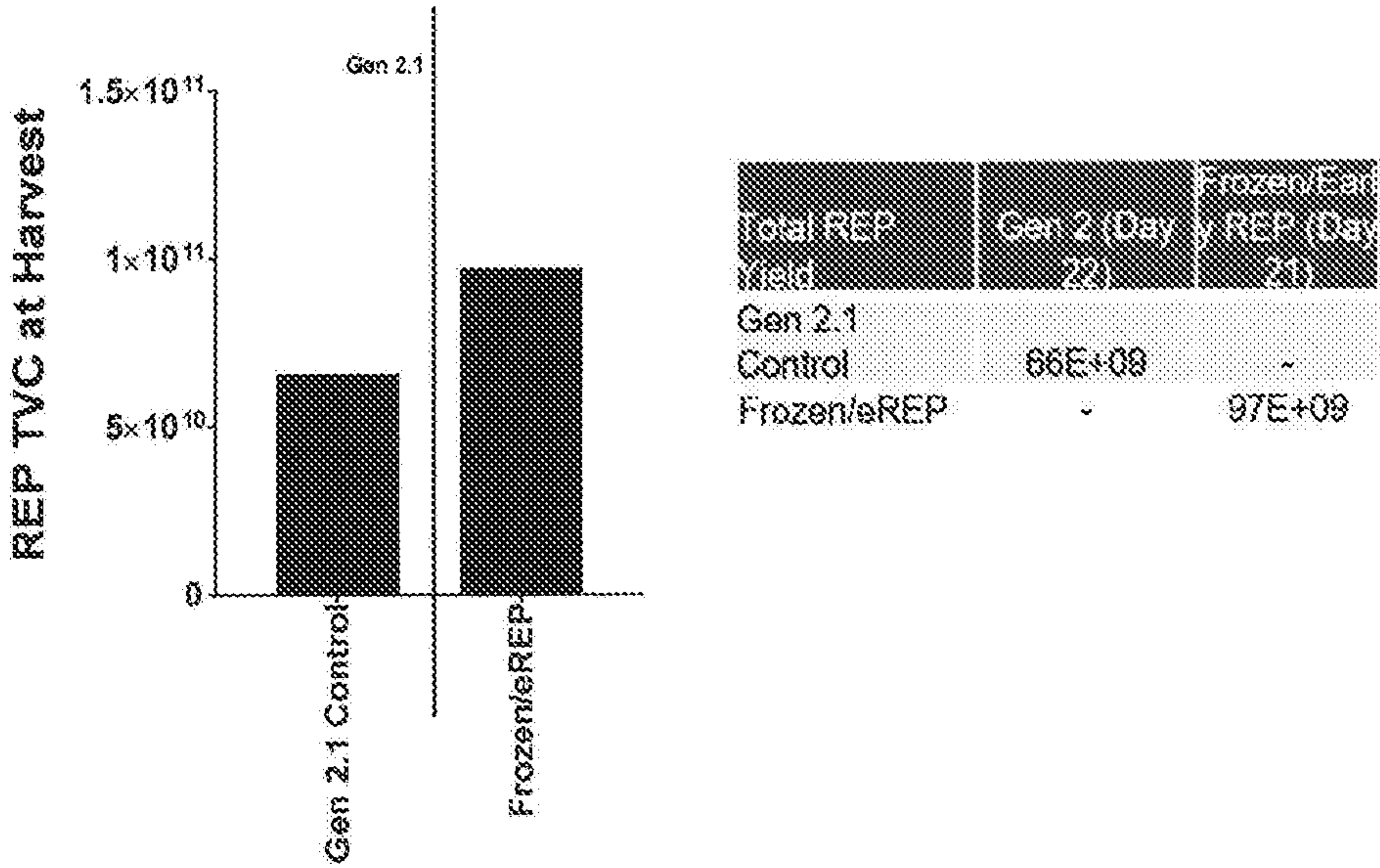


FIG. 15

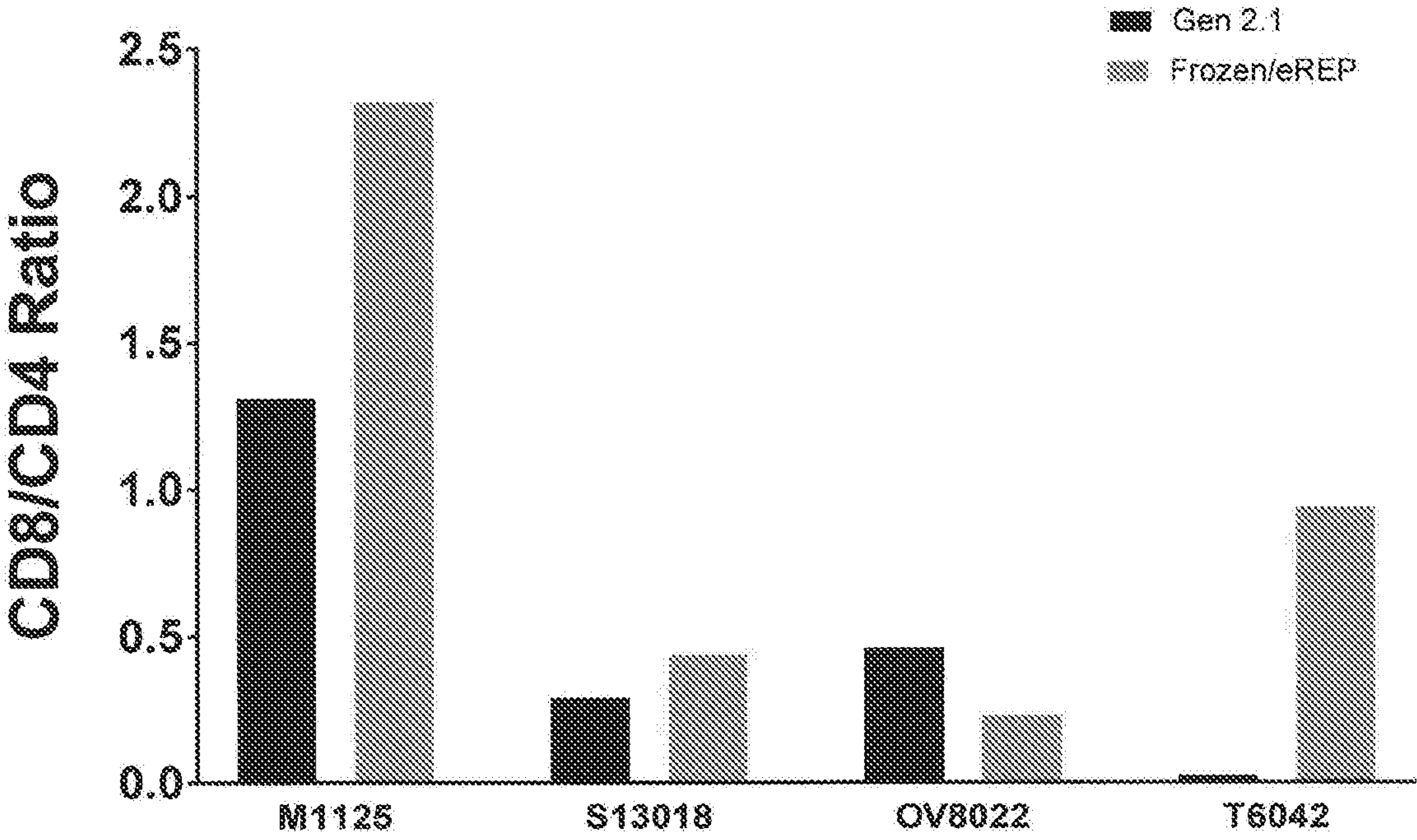


FIG. 16A

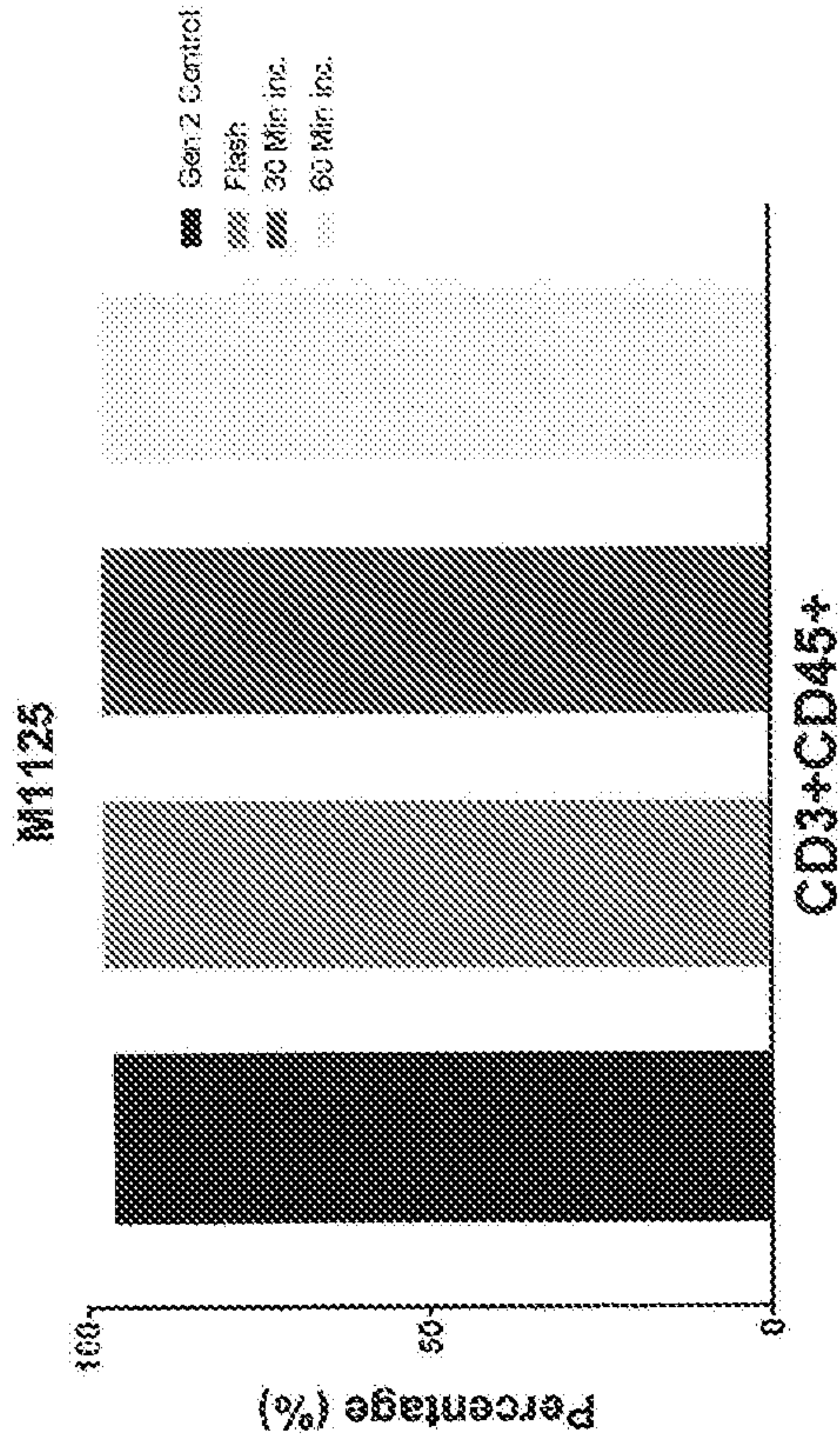


FIG. 16C

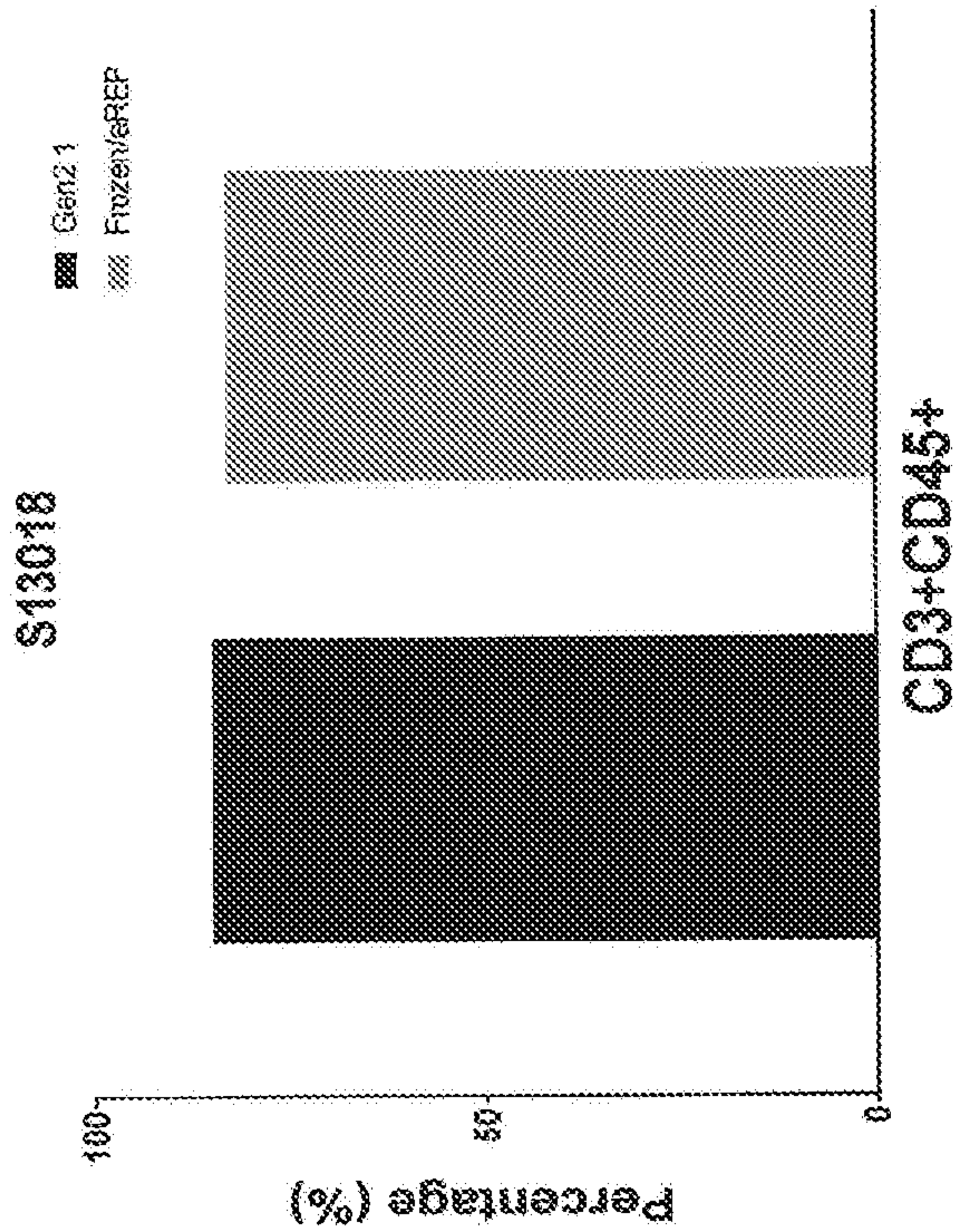


FIG. 16B

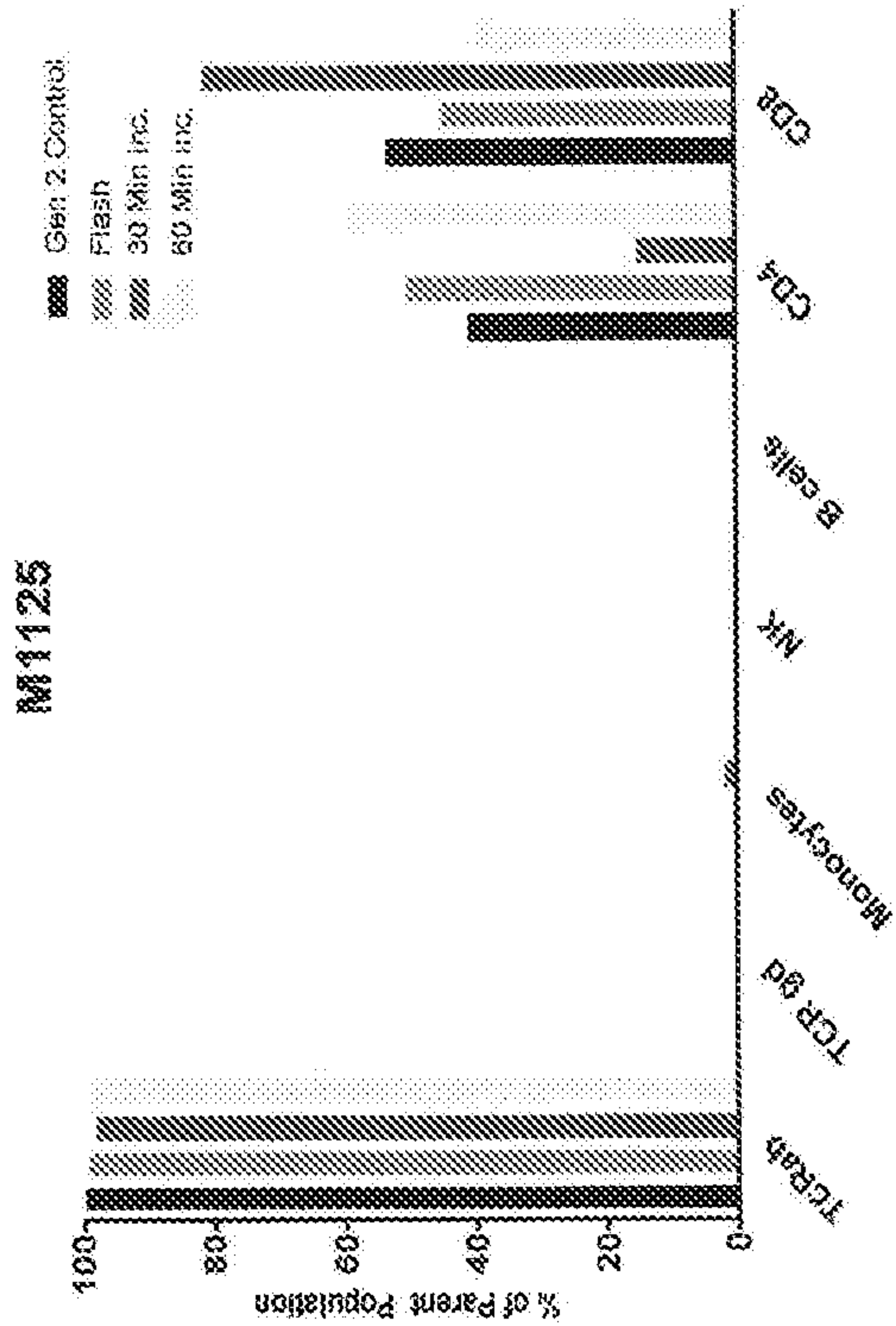


FIG. 16D

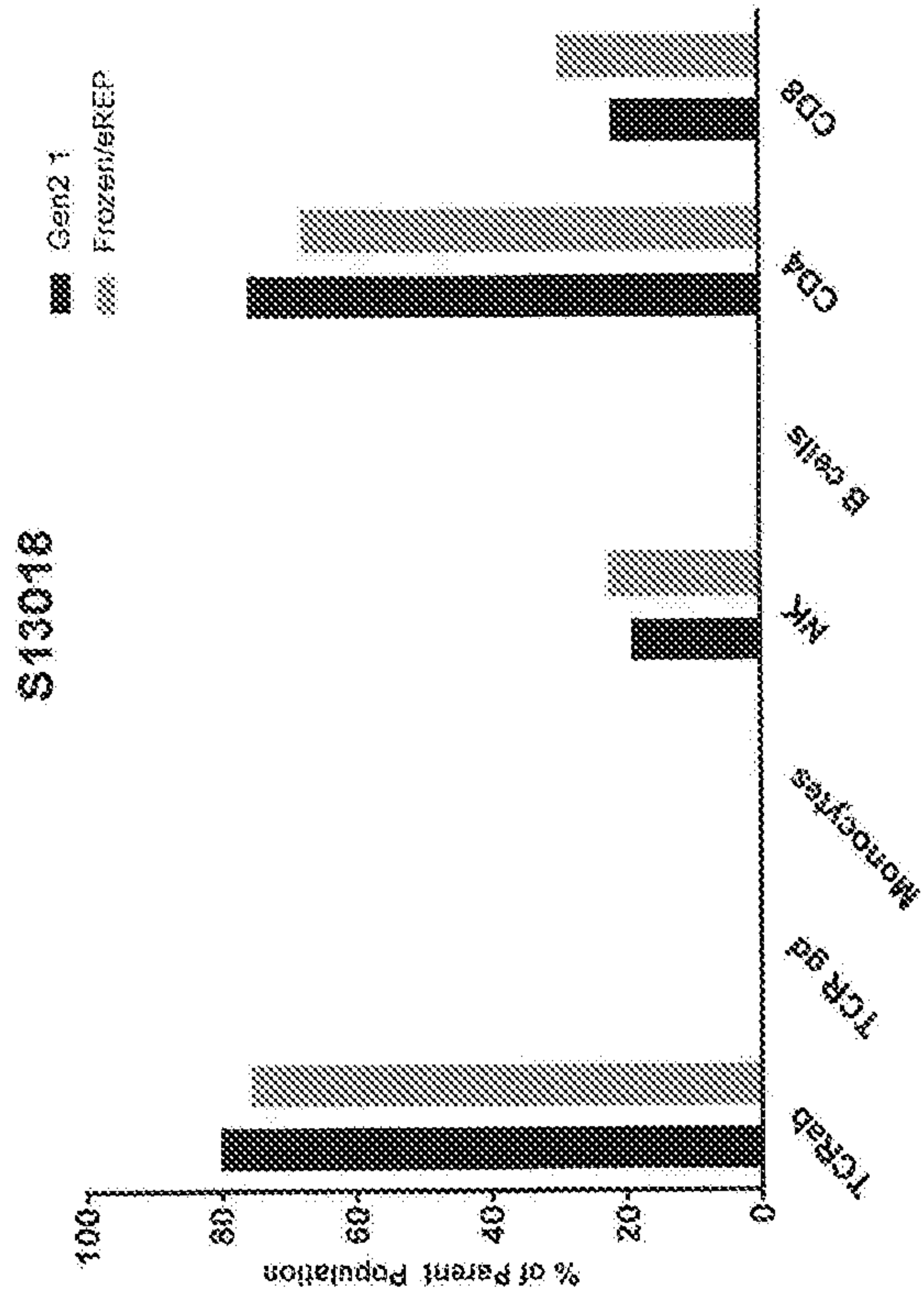


FIG. 16G

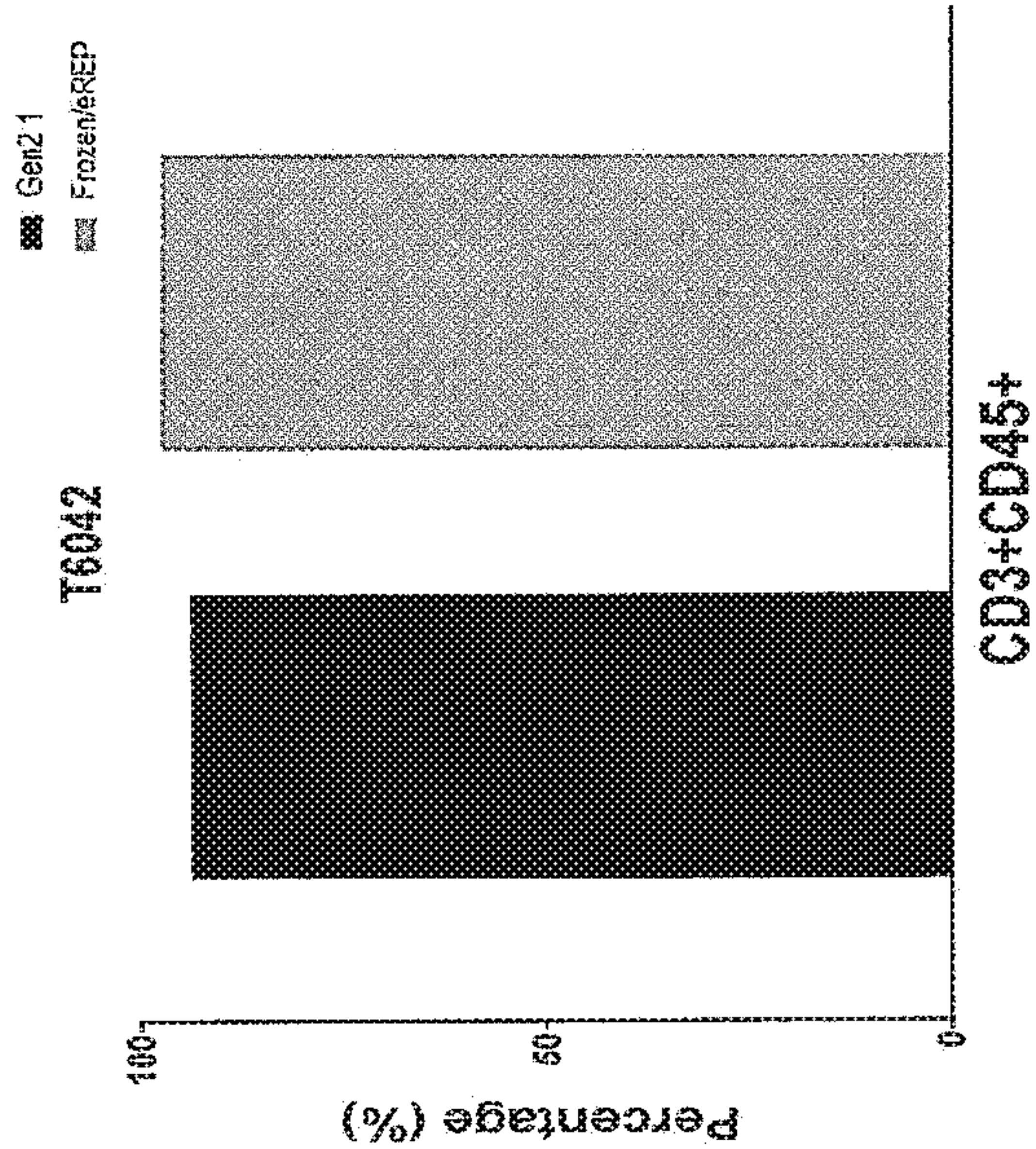


FIG. 16E

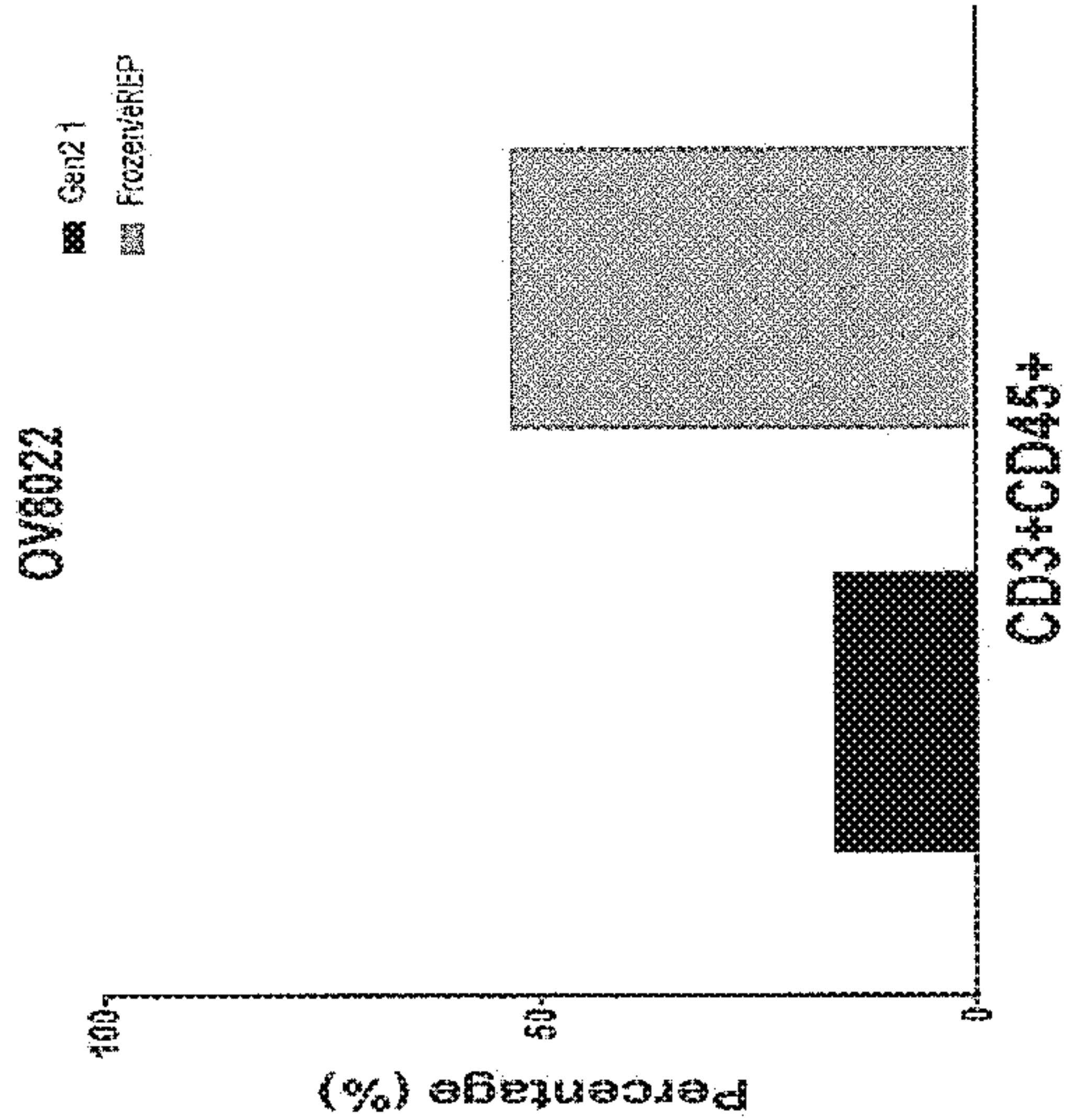


FIG. 16H

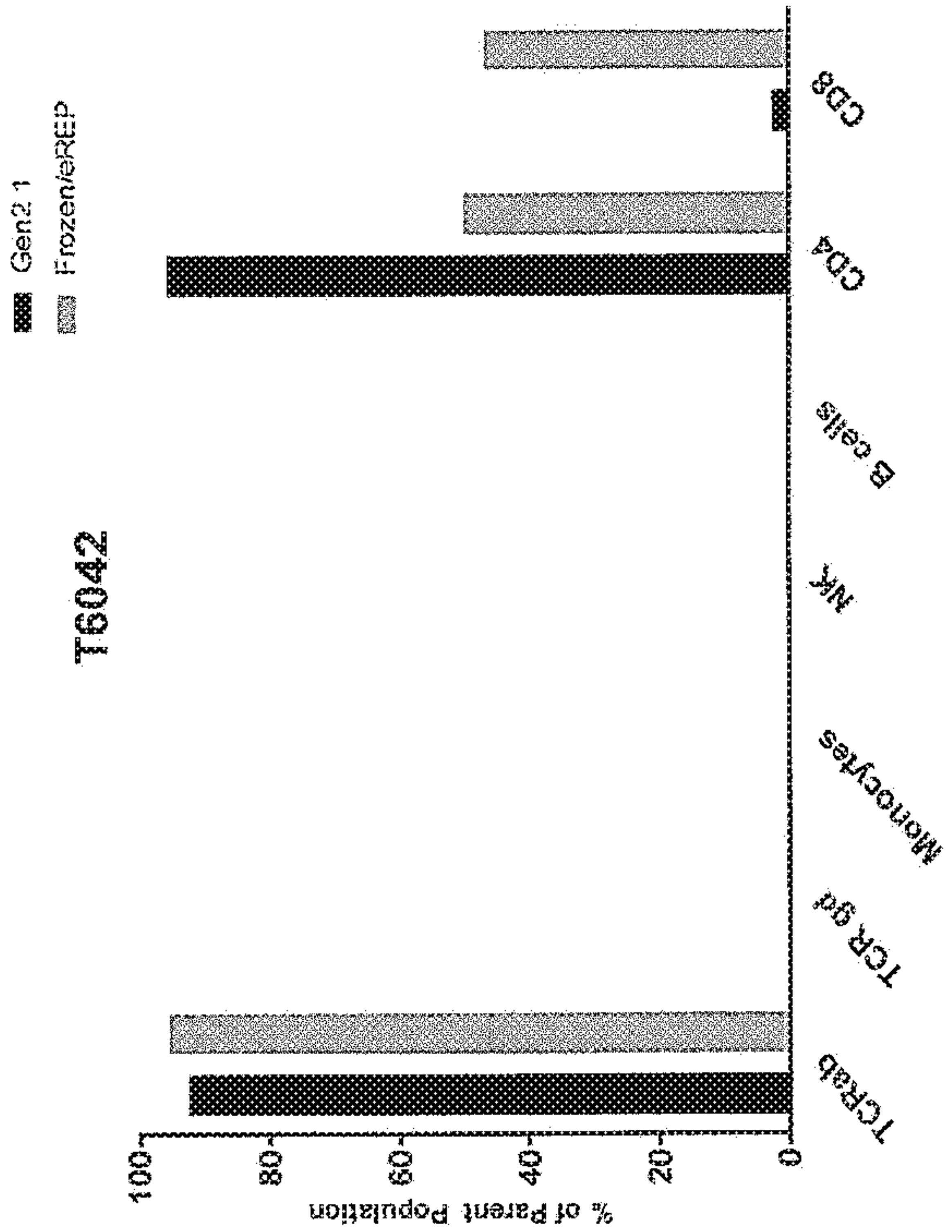


FIG. 16F

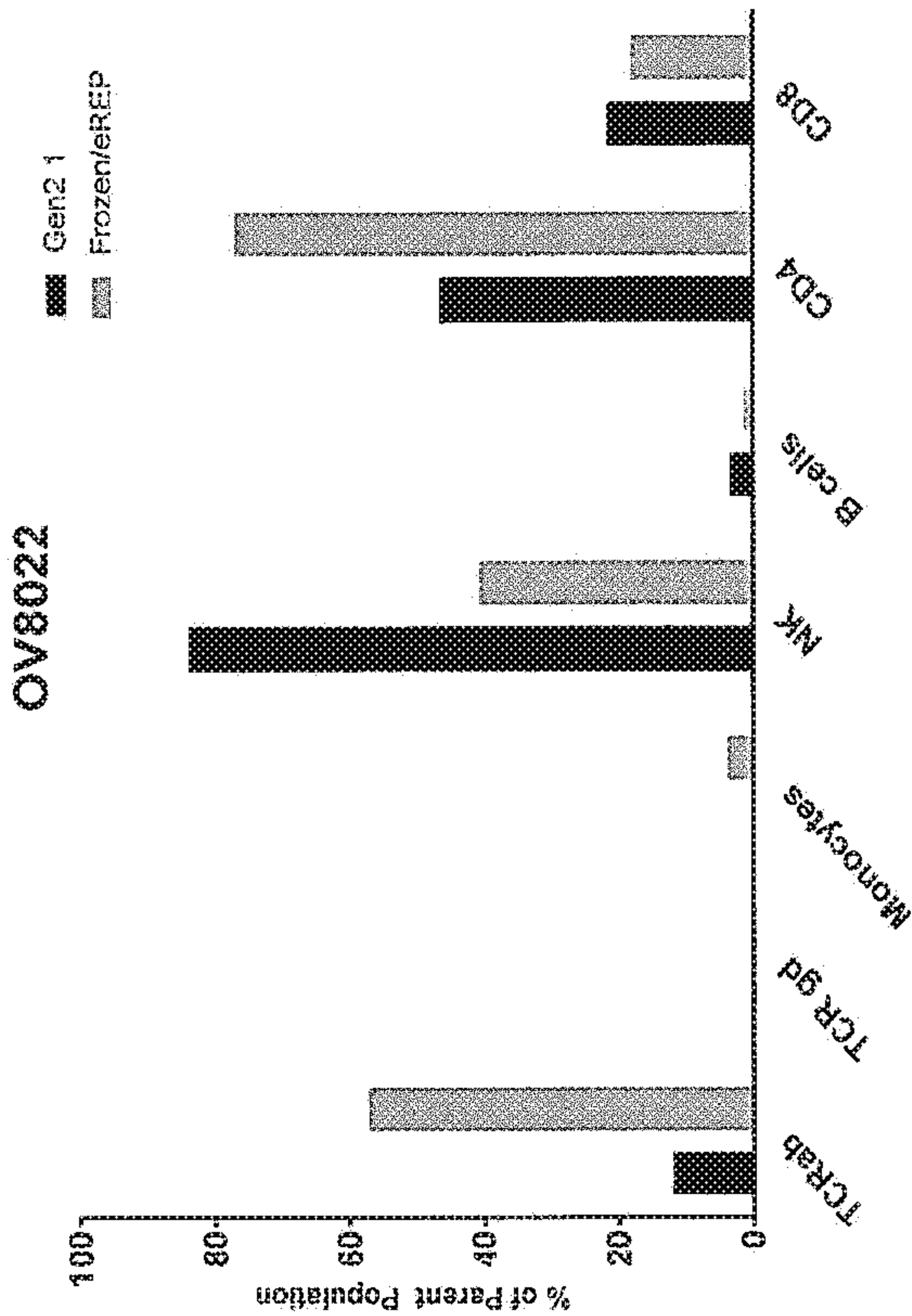


FIG. 17A

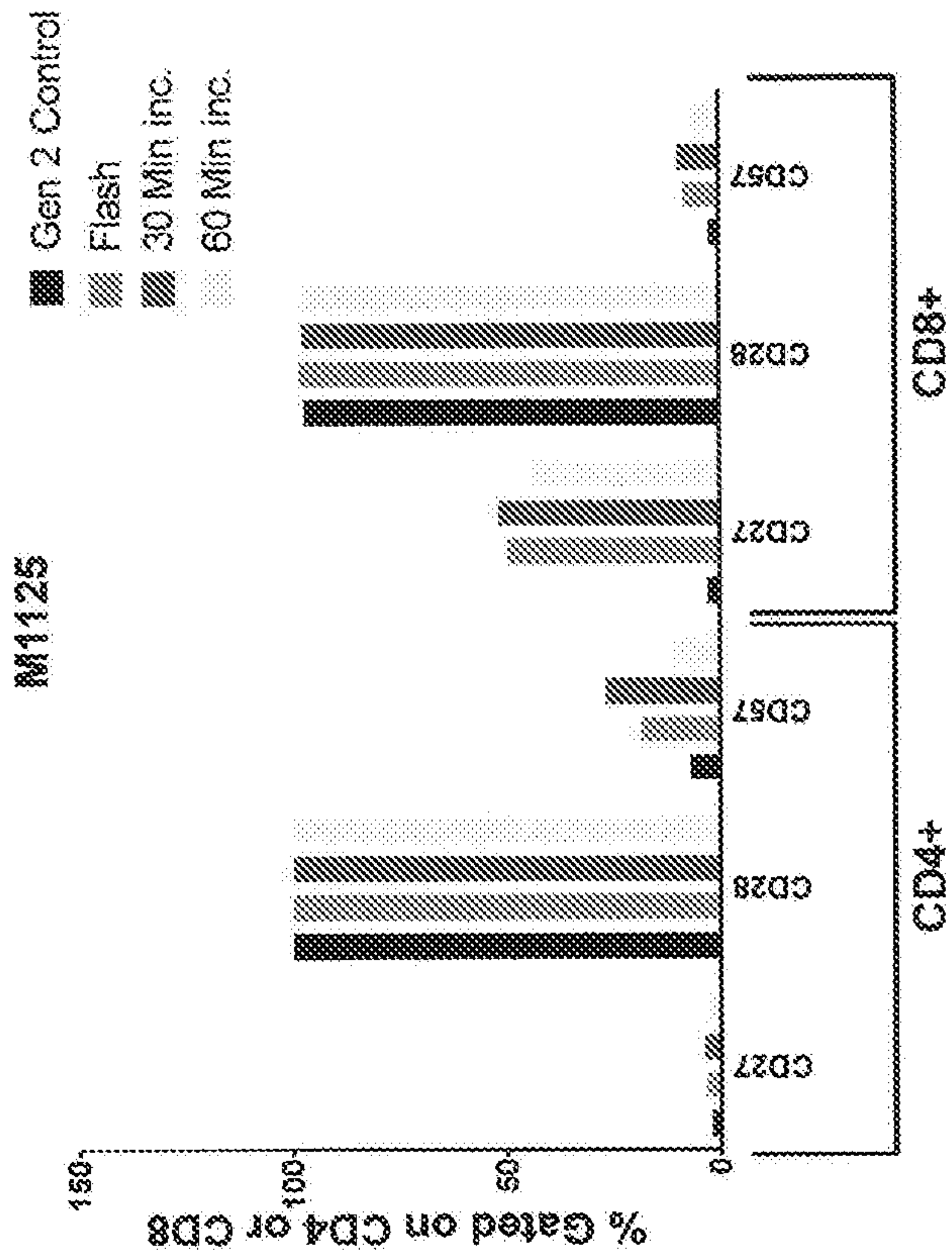
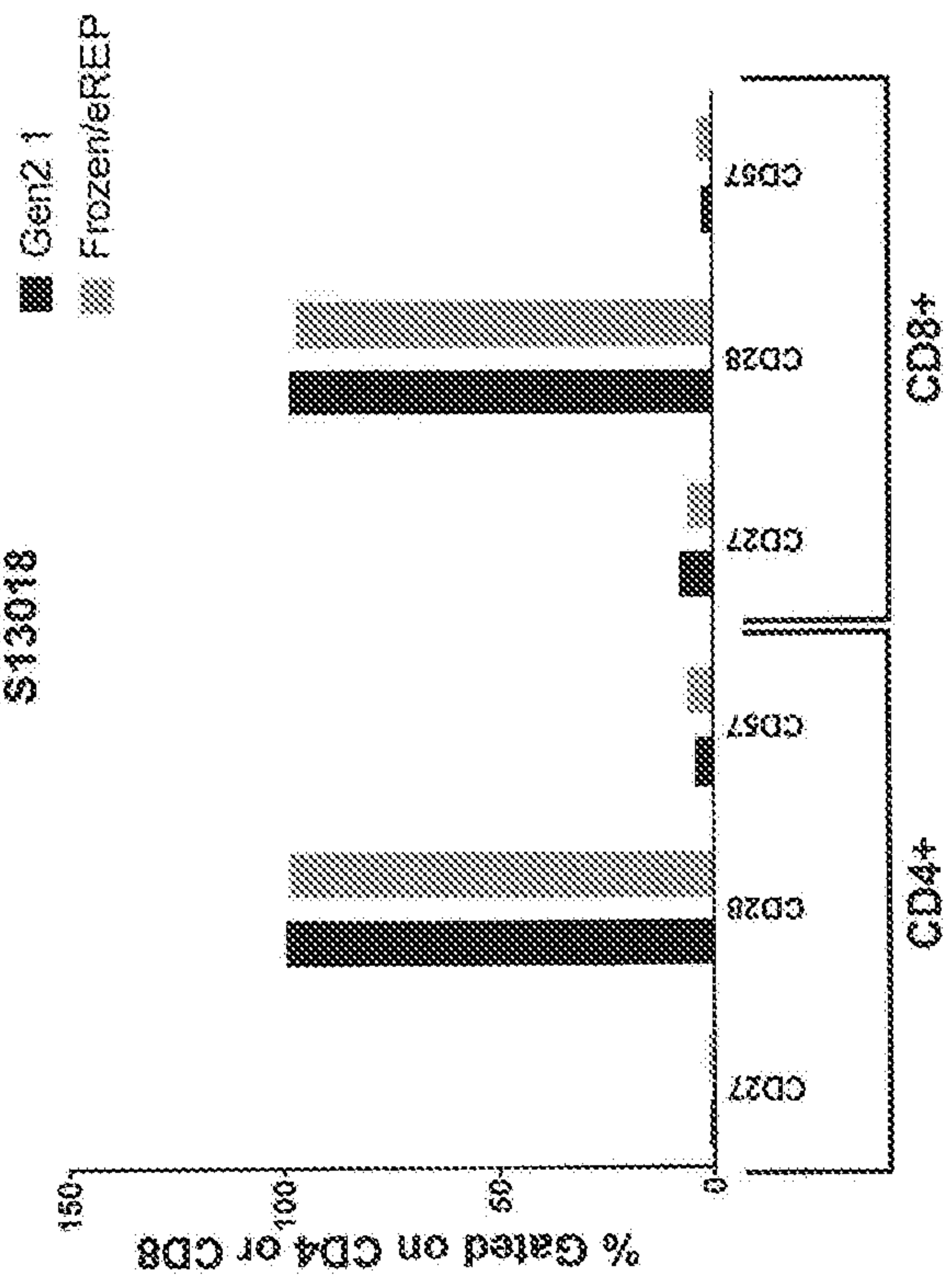


FIG. 17B



T6042

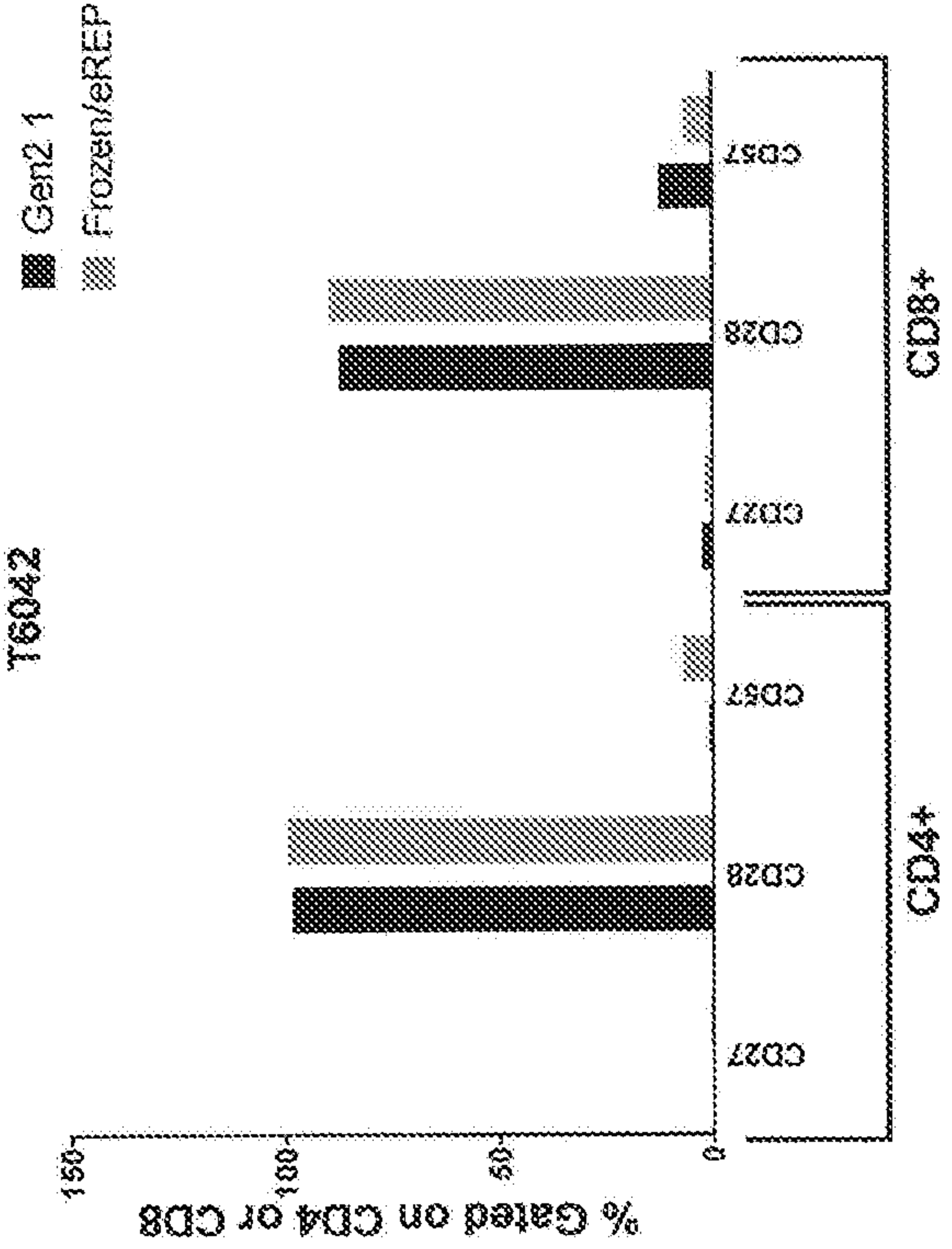


FIG. 17C

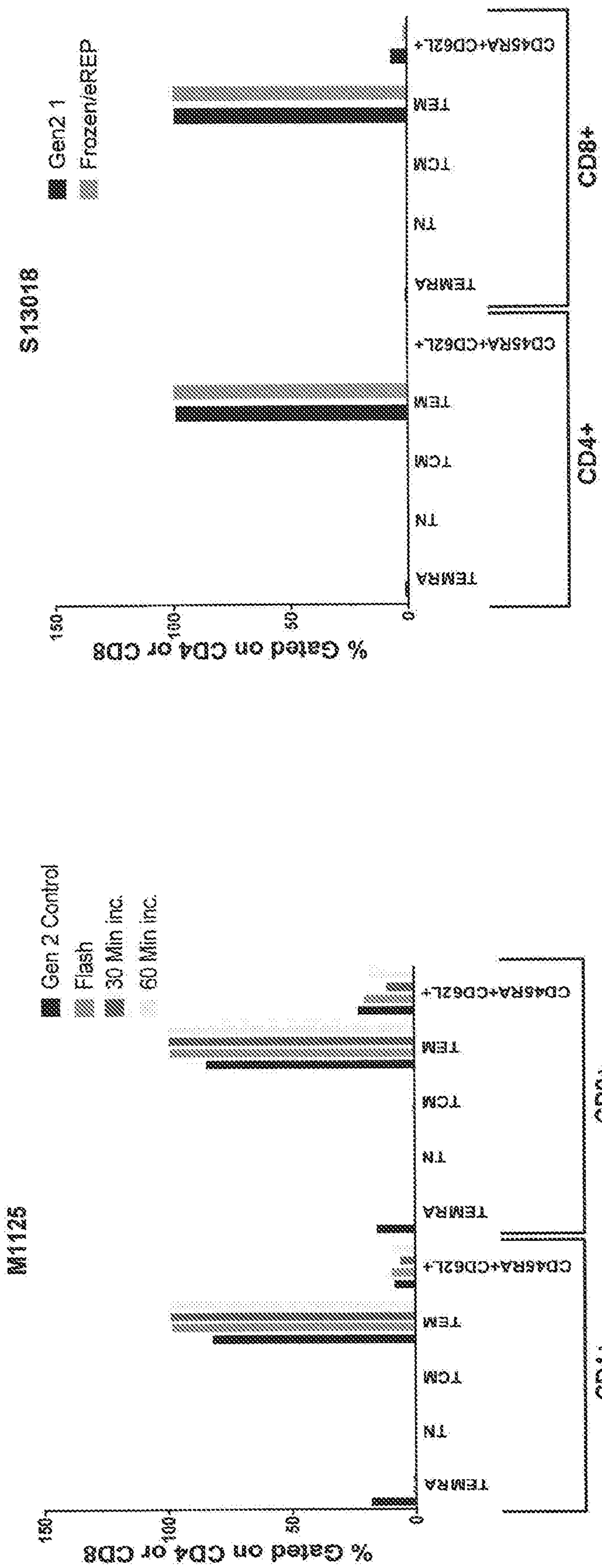


FIG. 18A

T6042

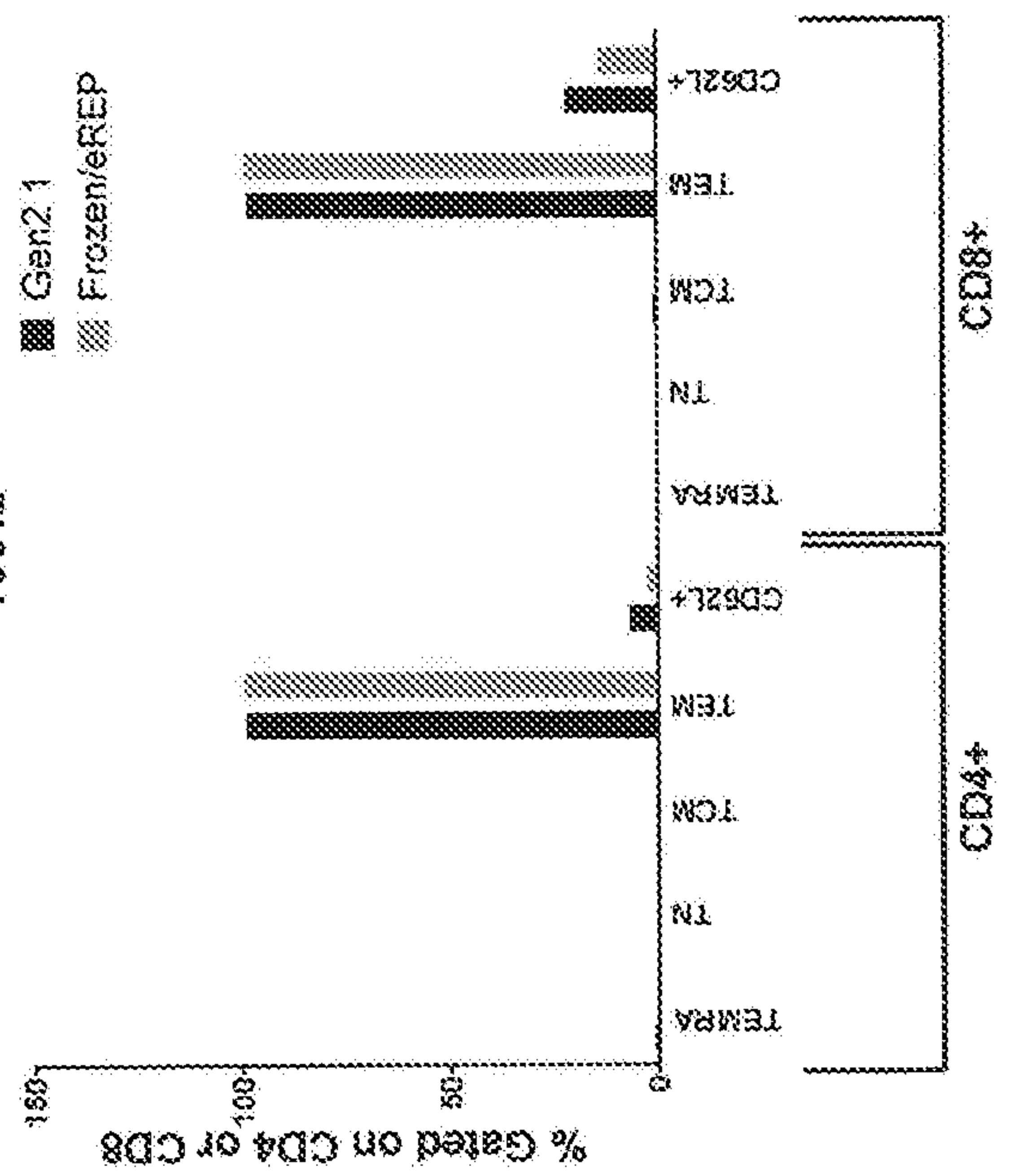


FIG. 18B

FIG. 18C

S13018

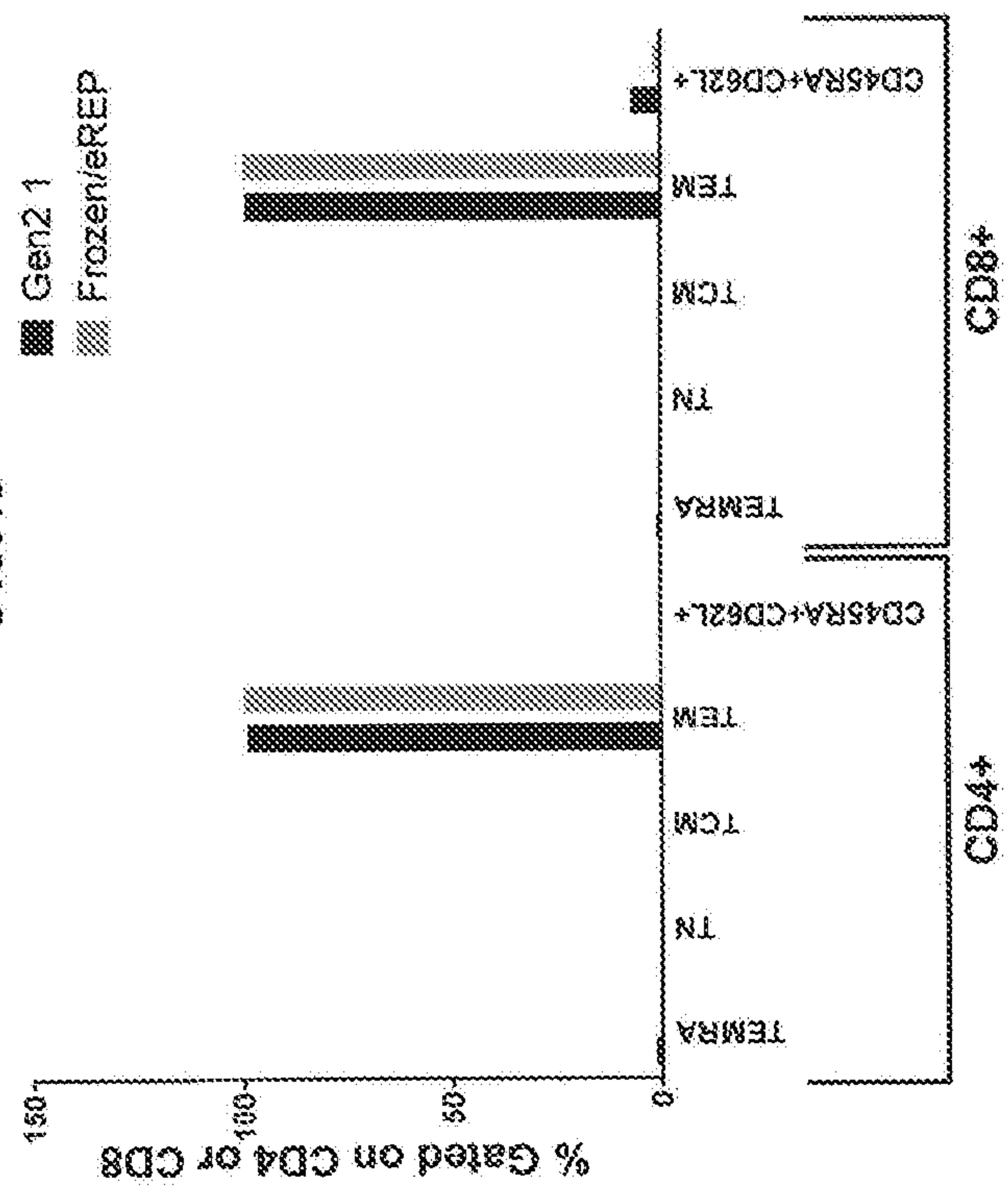


FIG. 19B

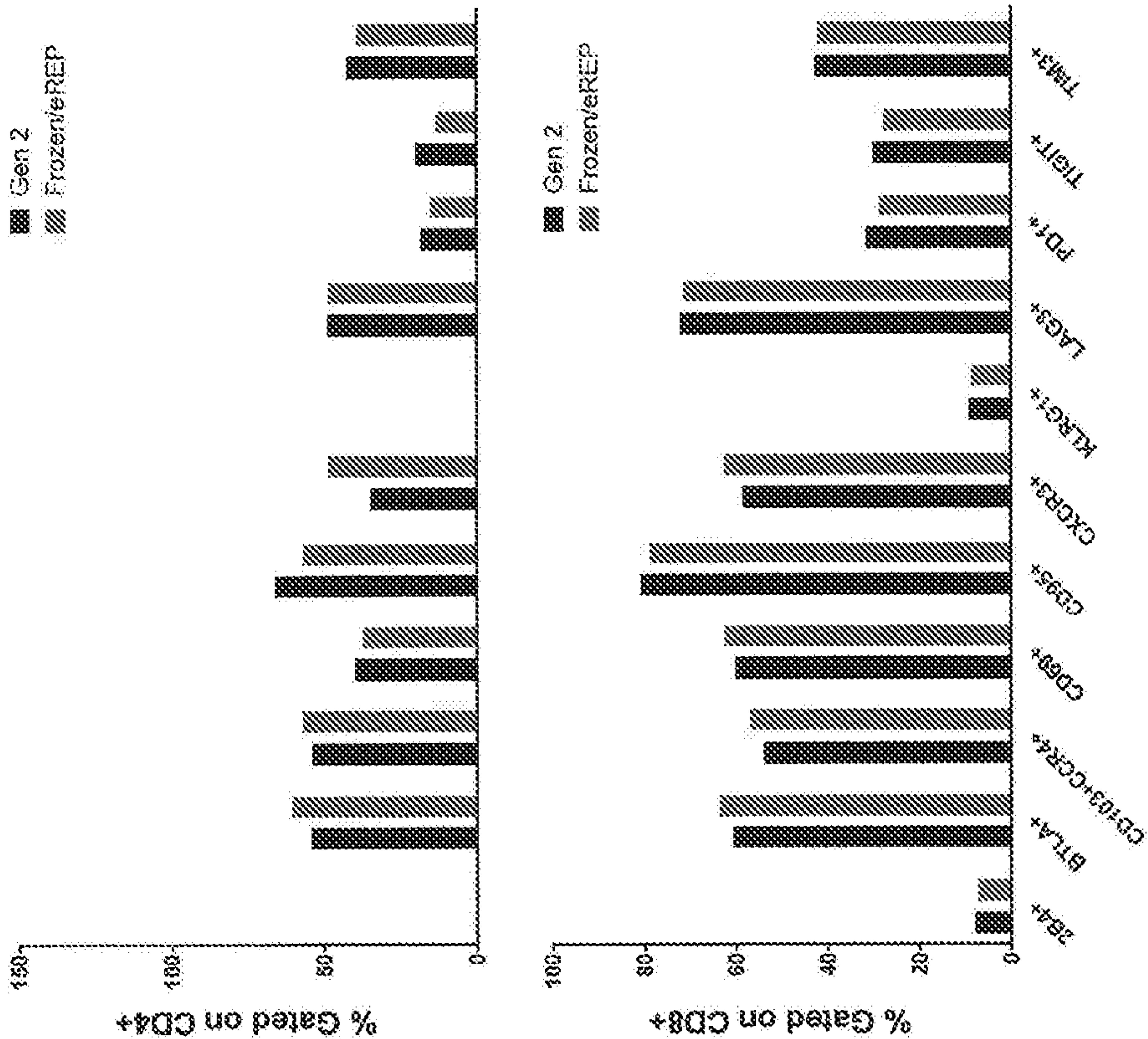


FIG. 19A

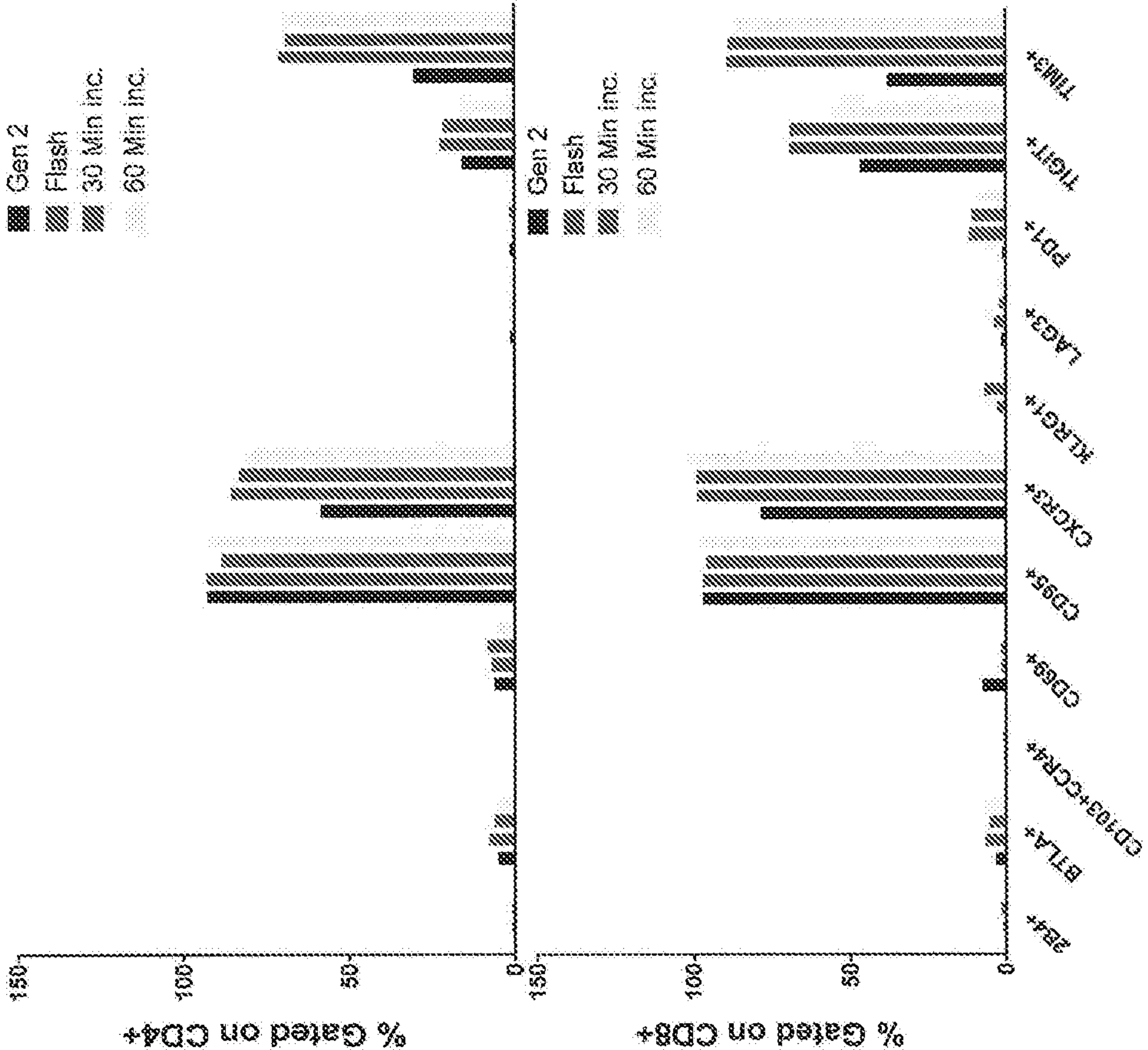


FIG. 19C

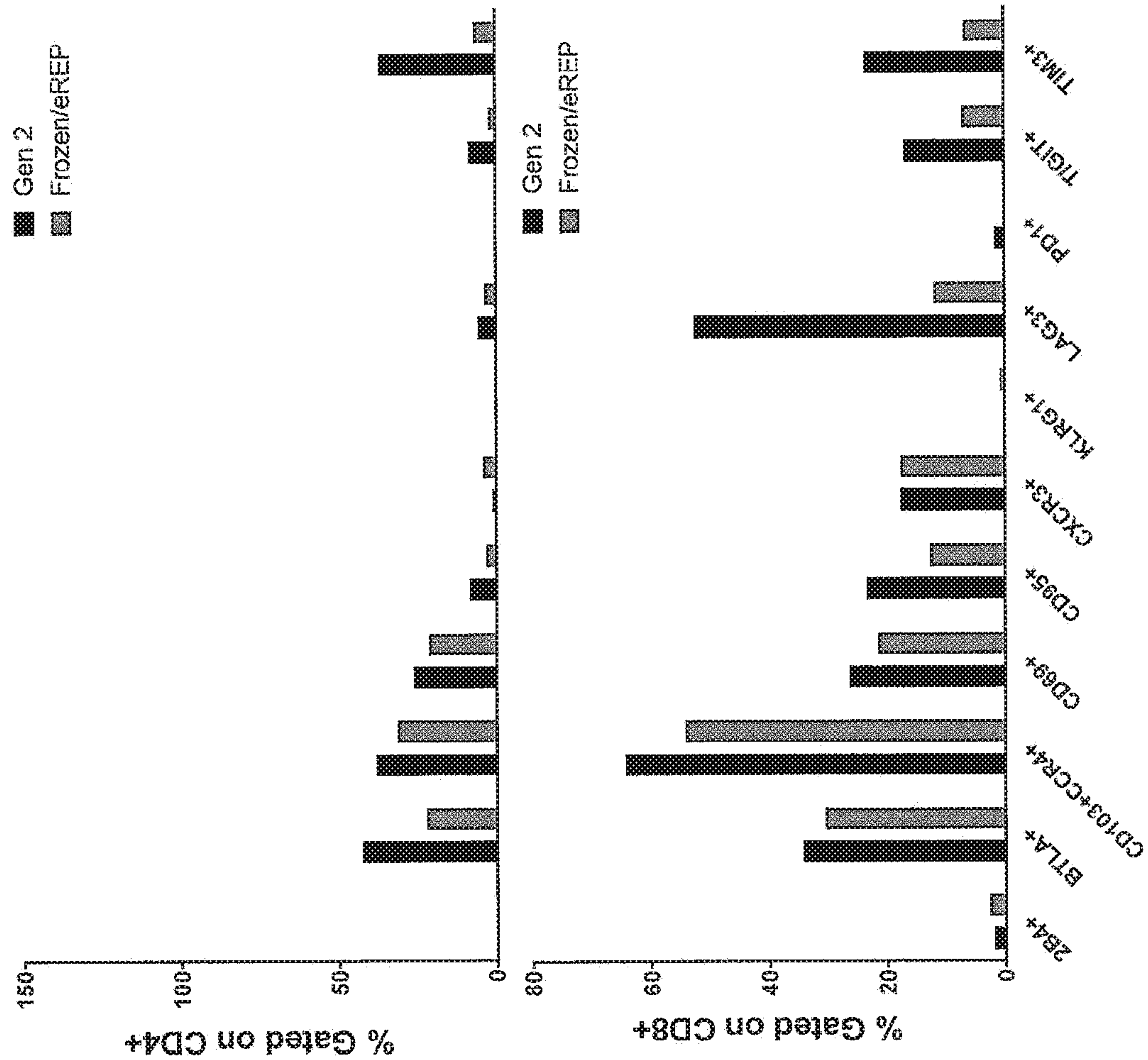


FIG. 20B

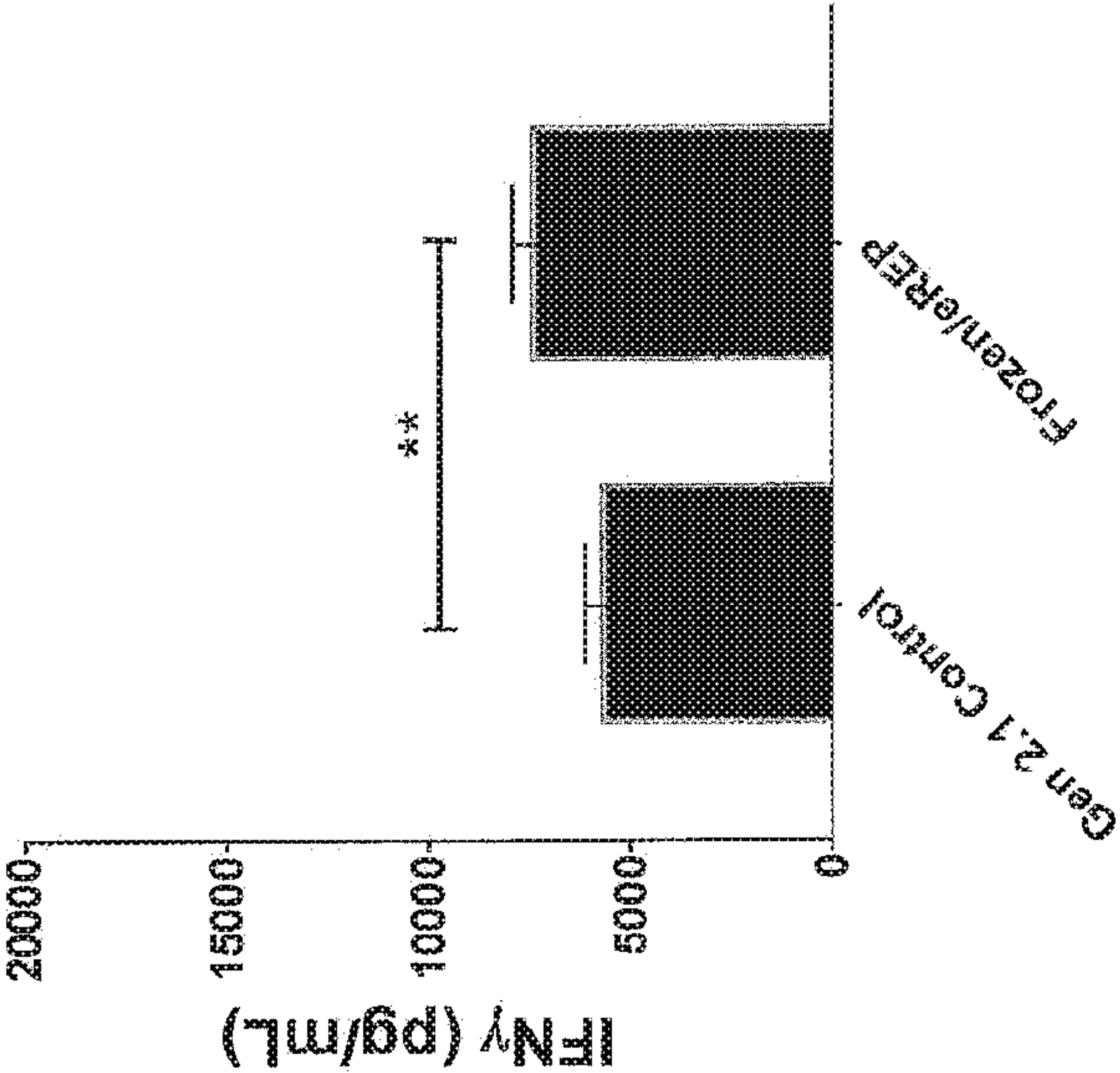


FIG. 20A

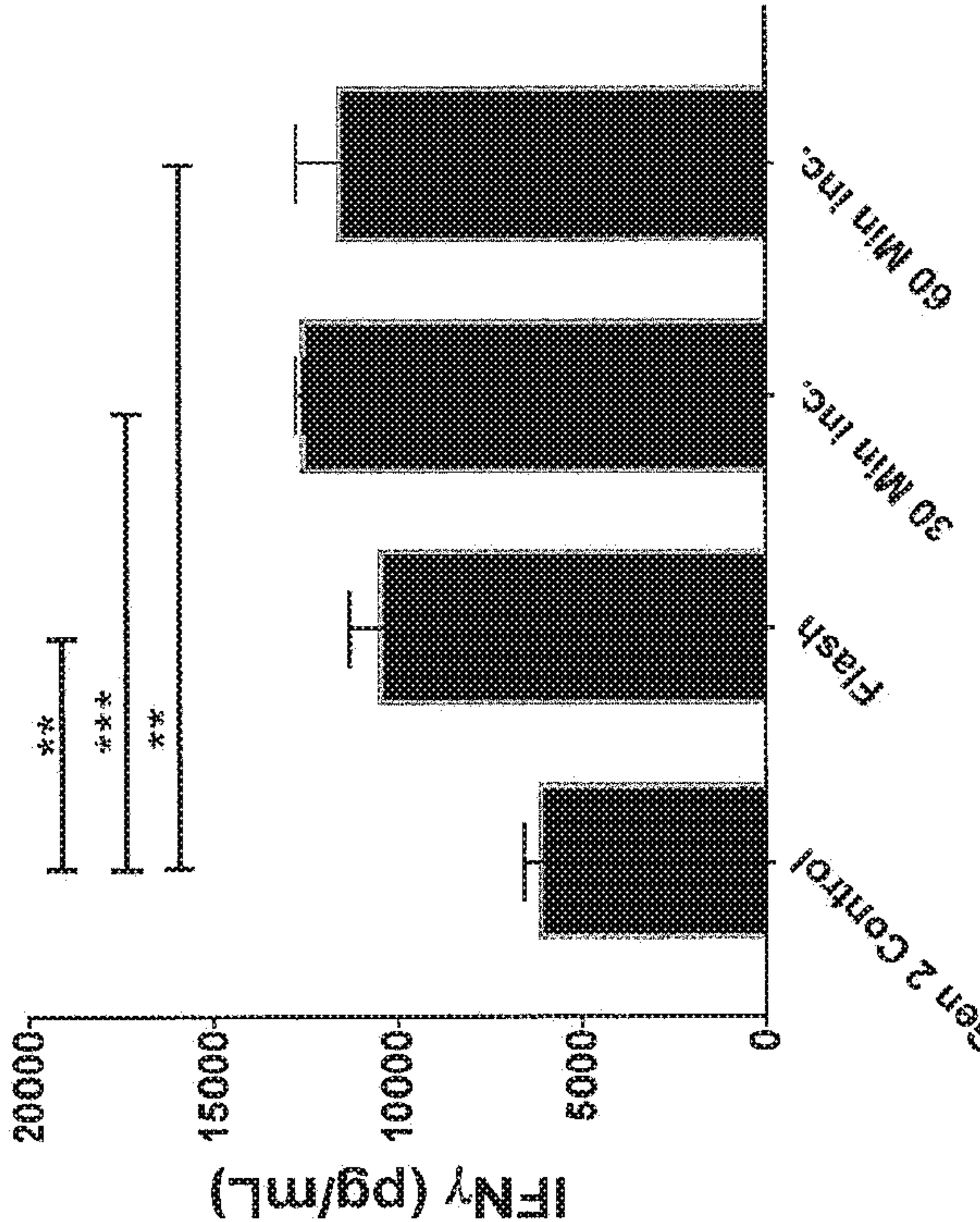


FIG. 20C

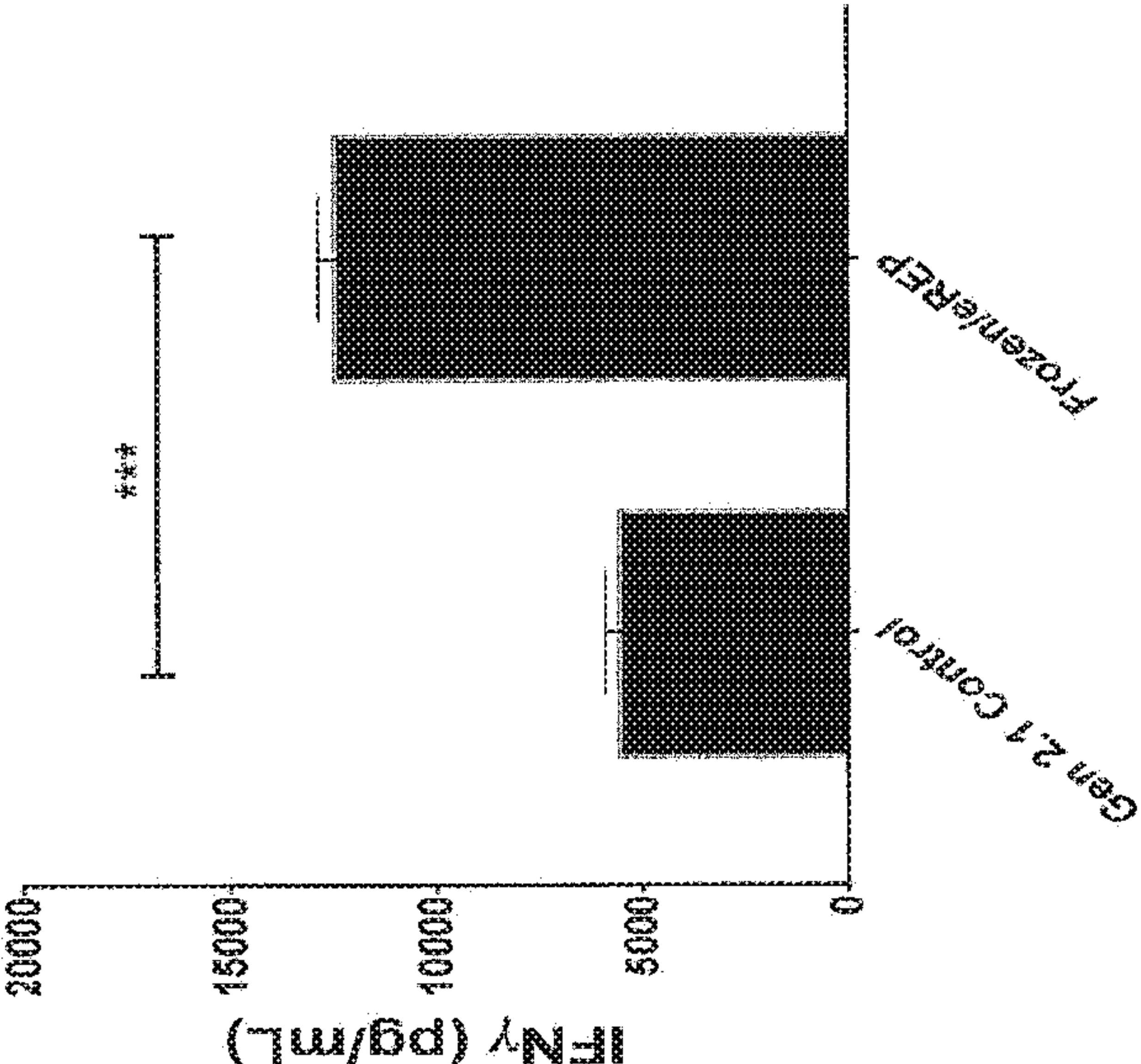


FIG. 21A

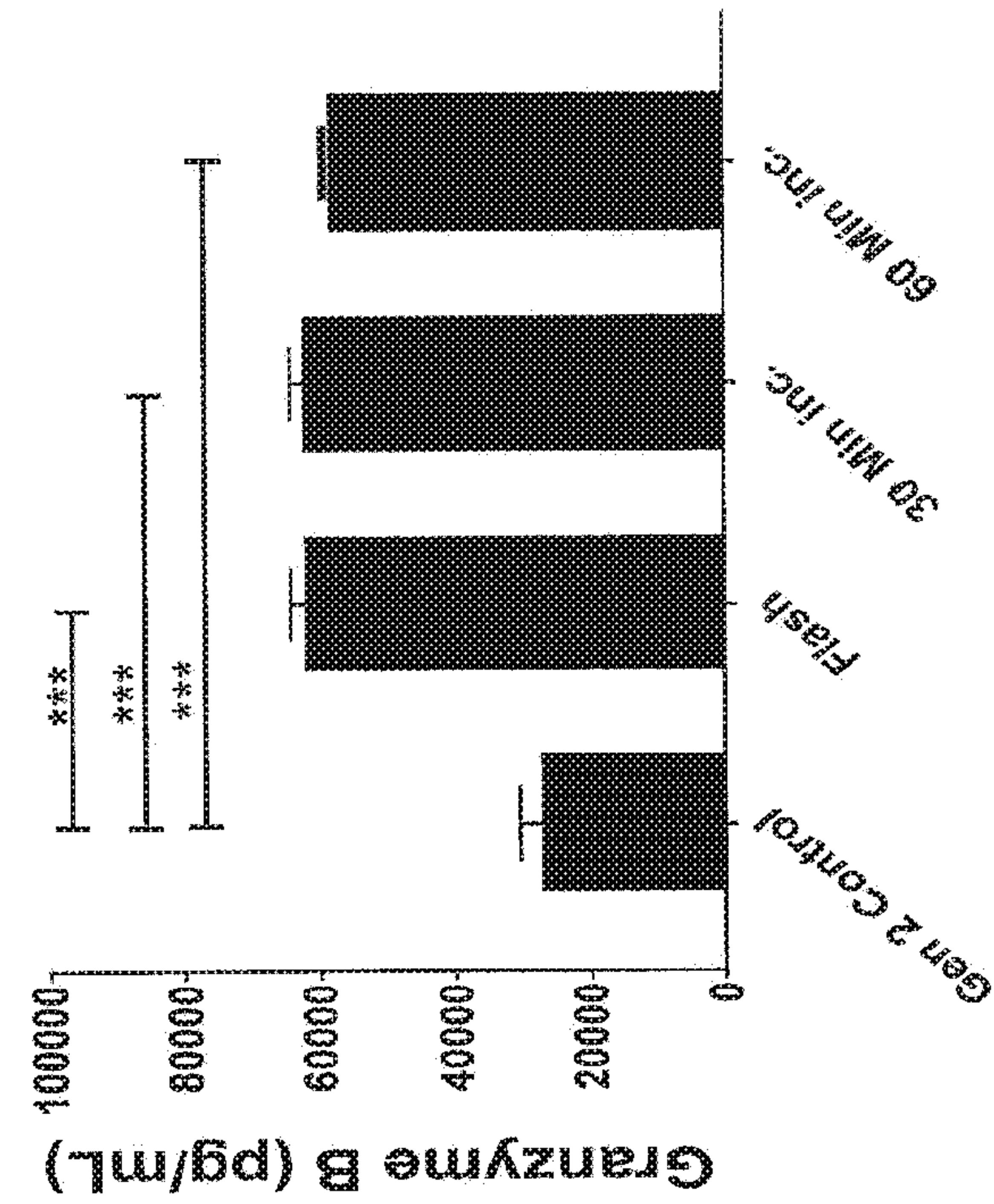


FIG. 21B

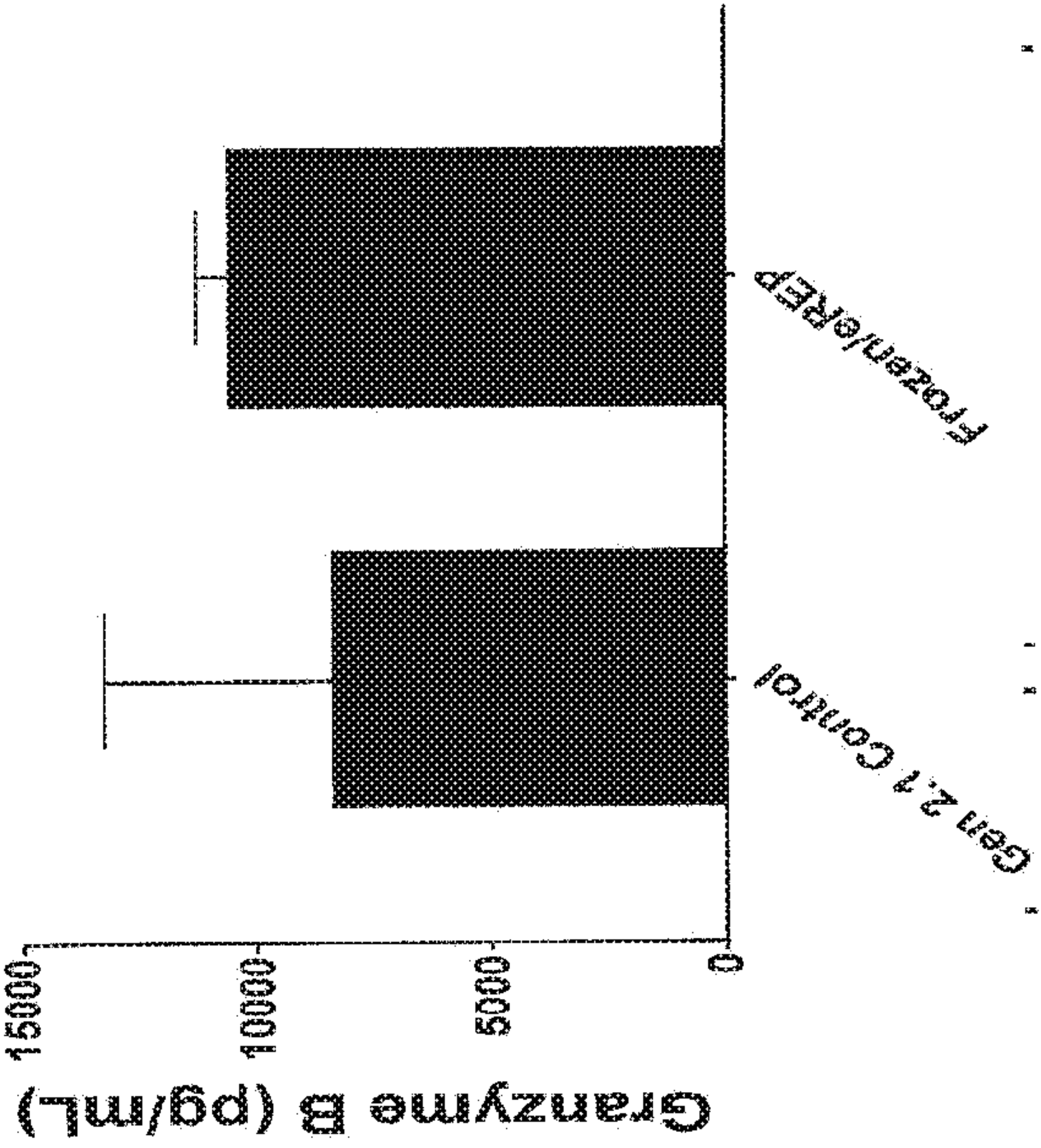


FIG. 21C

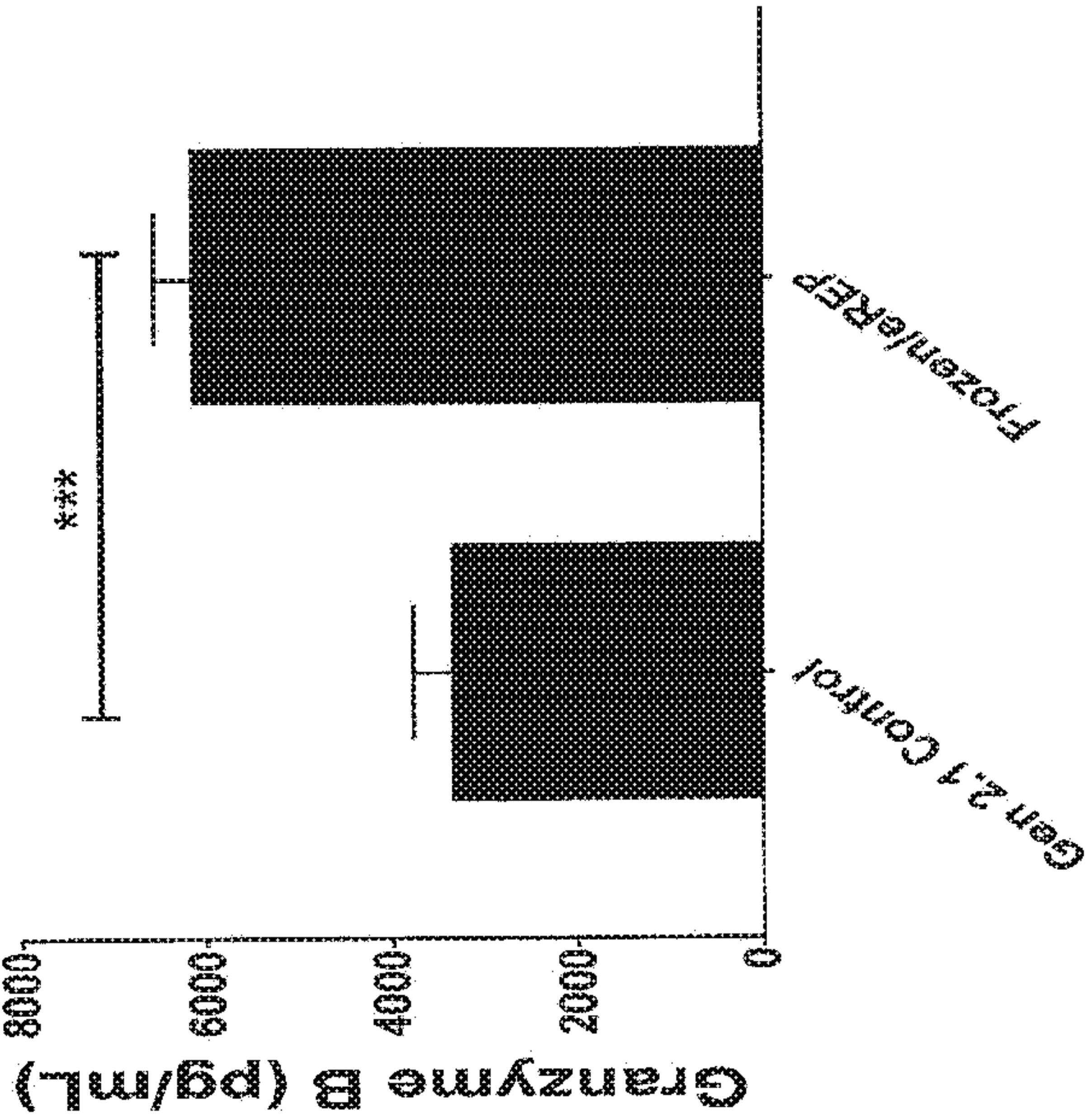


FIG. 22B

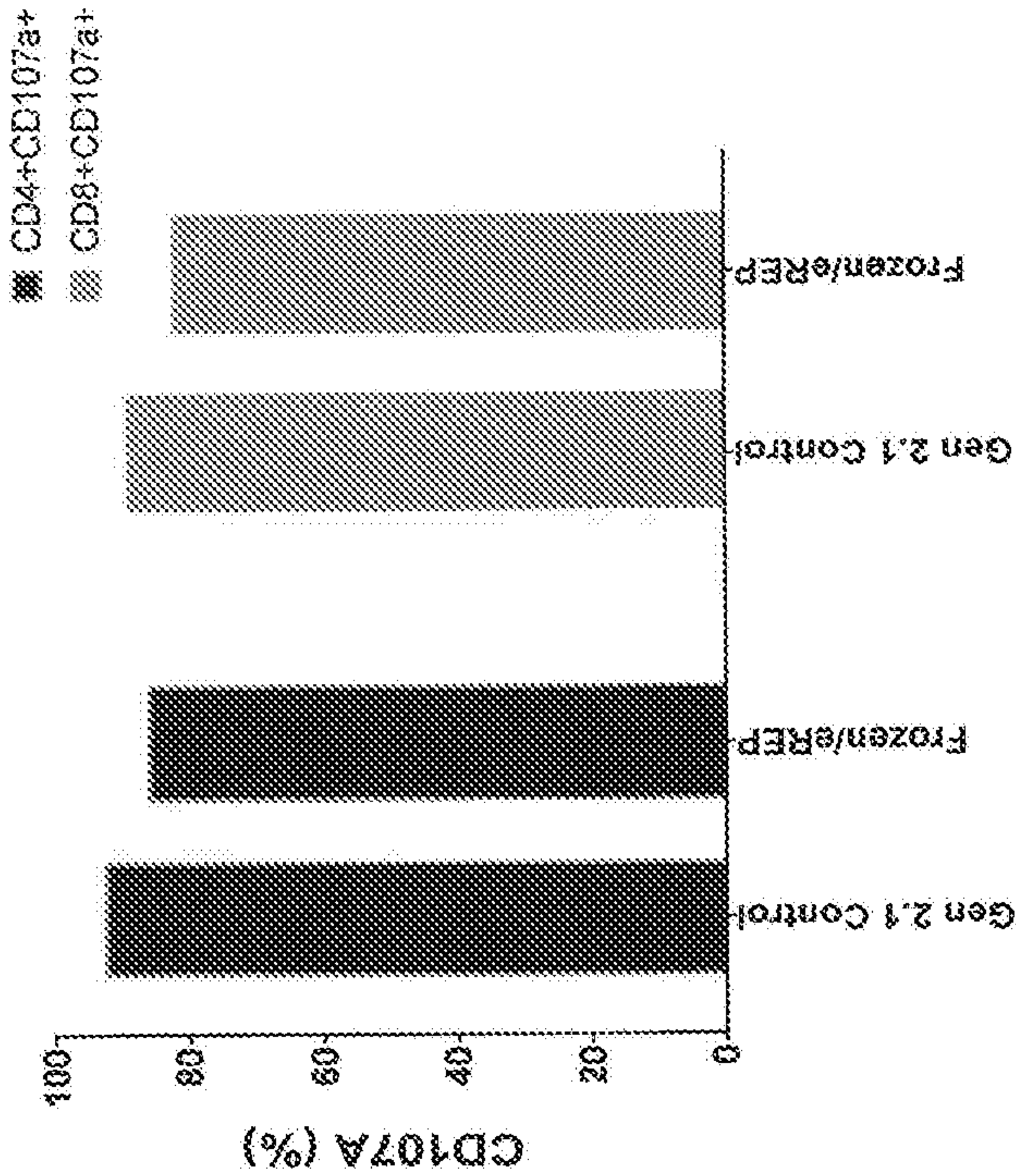
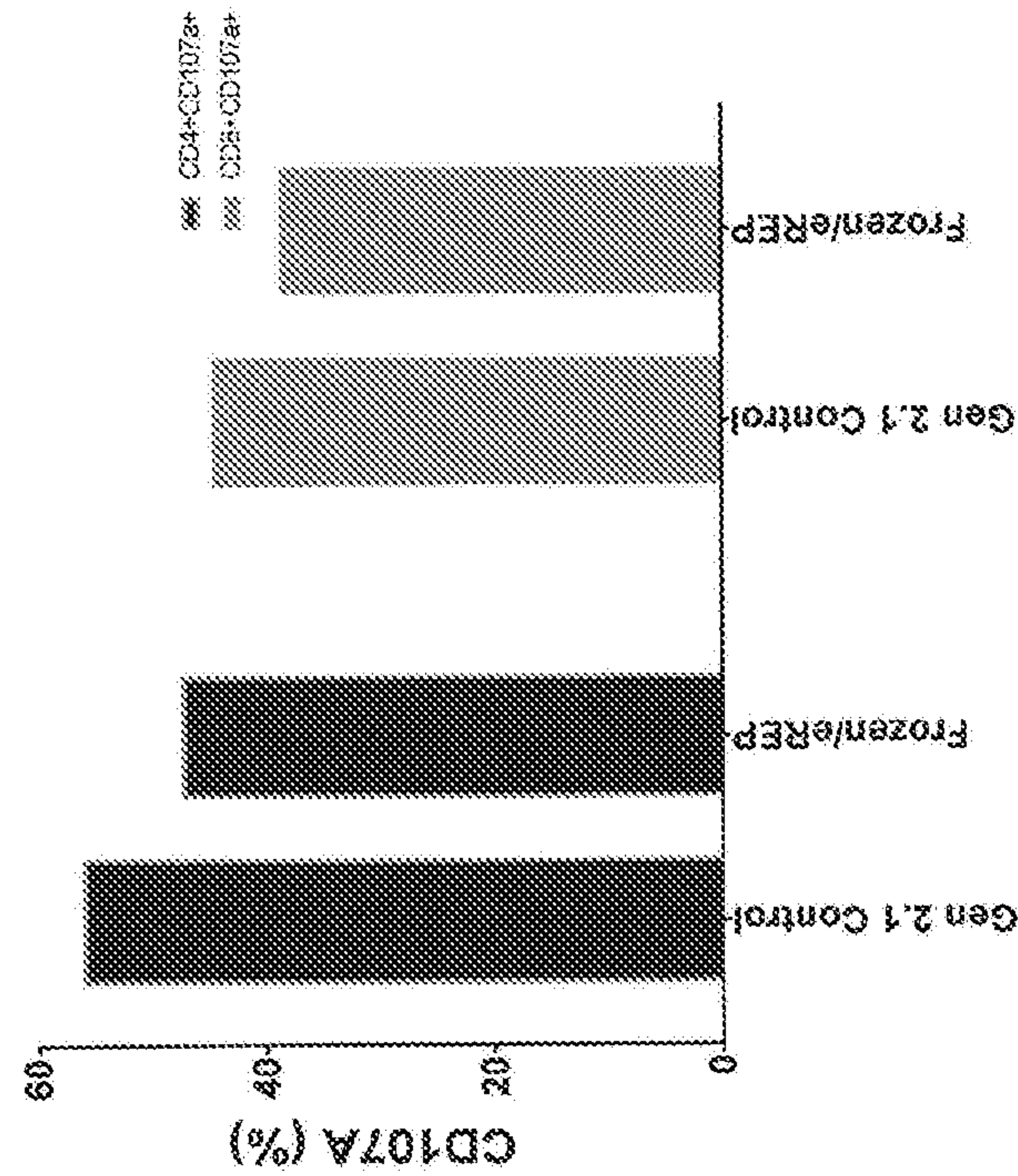


FIG. 22C

FIG. 22A

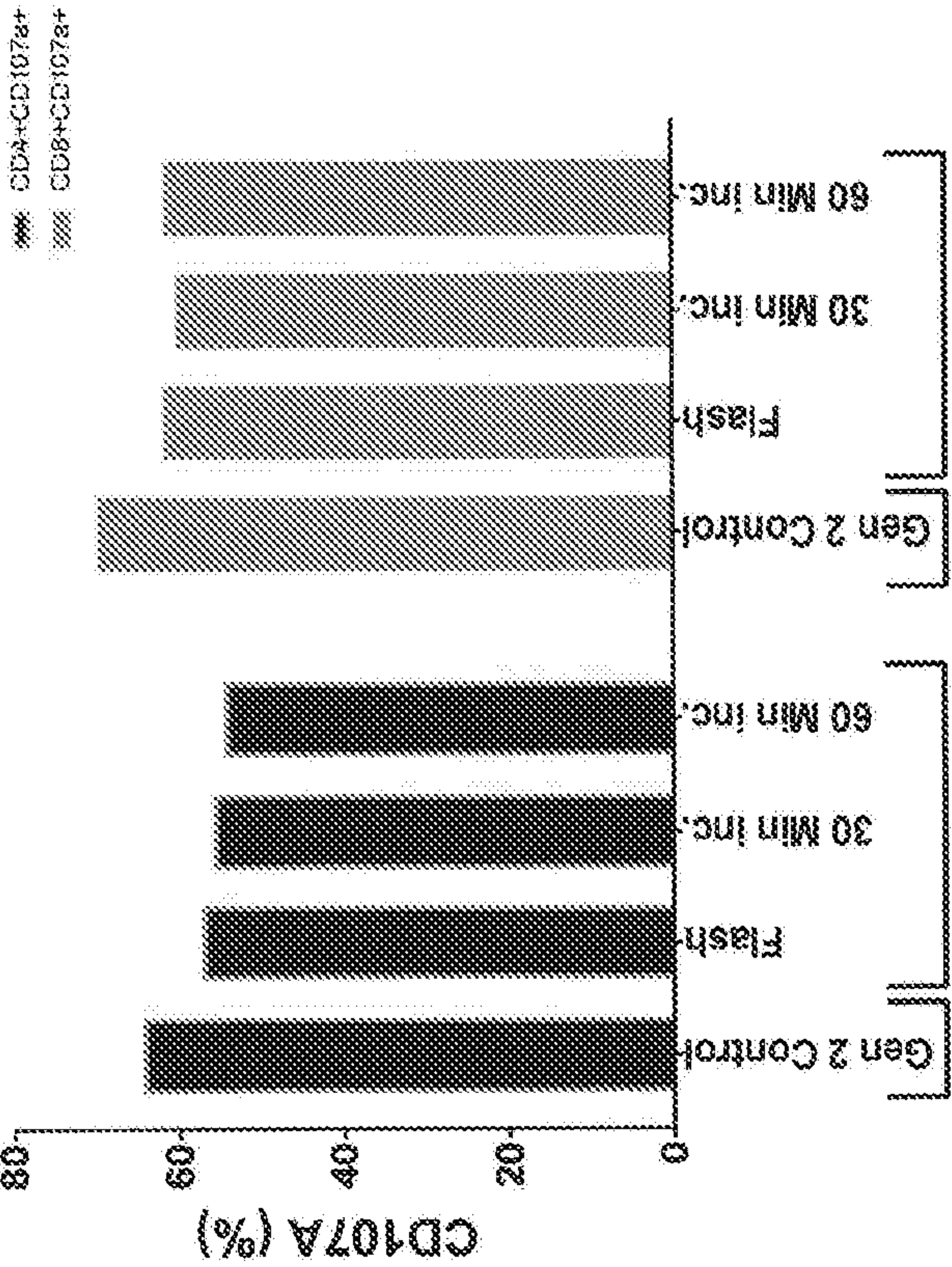


FIG. 23A

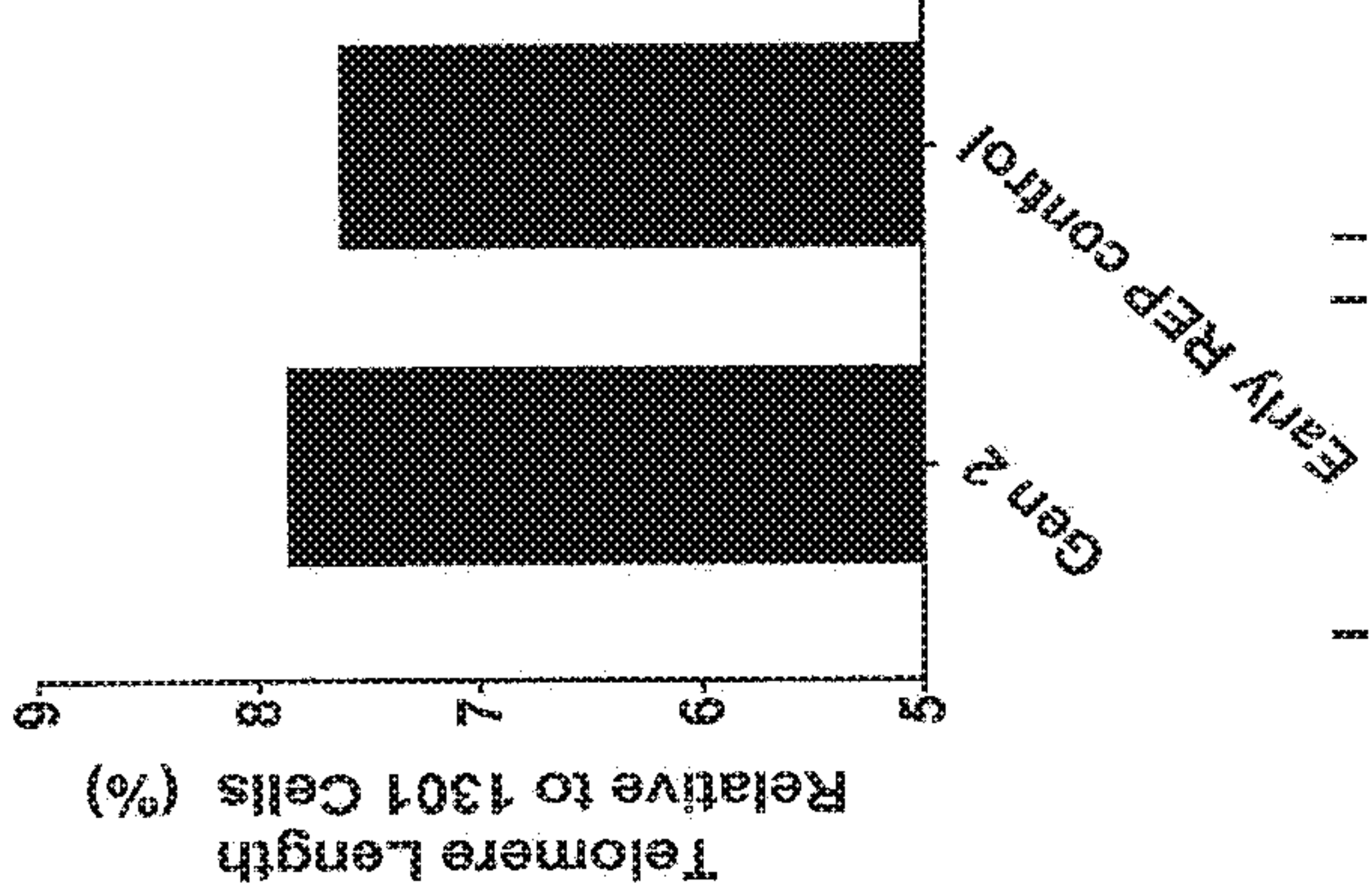
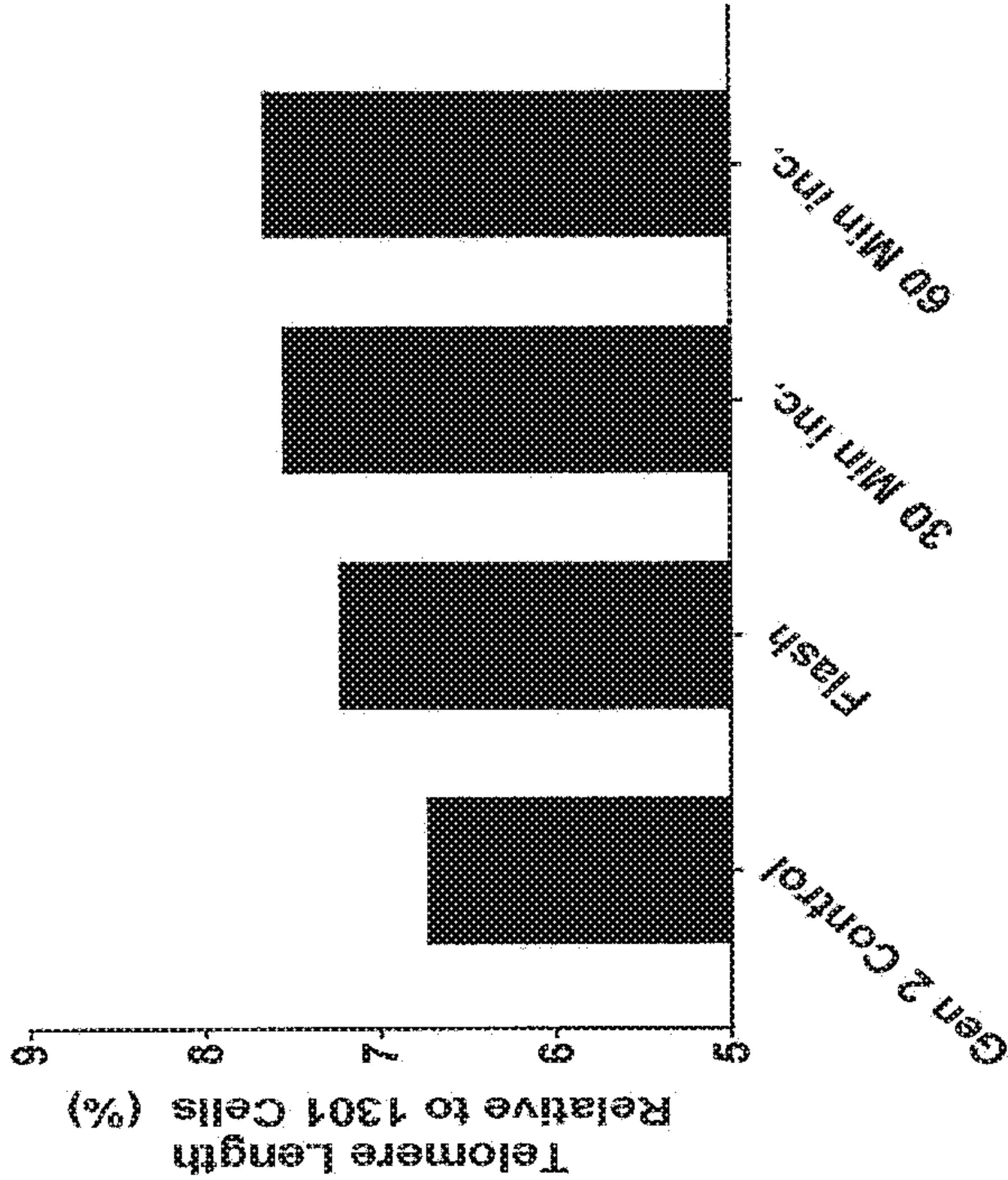
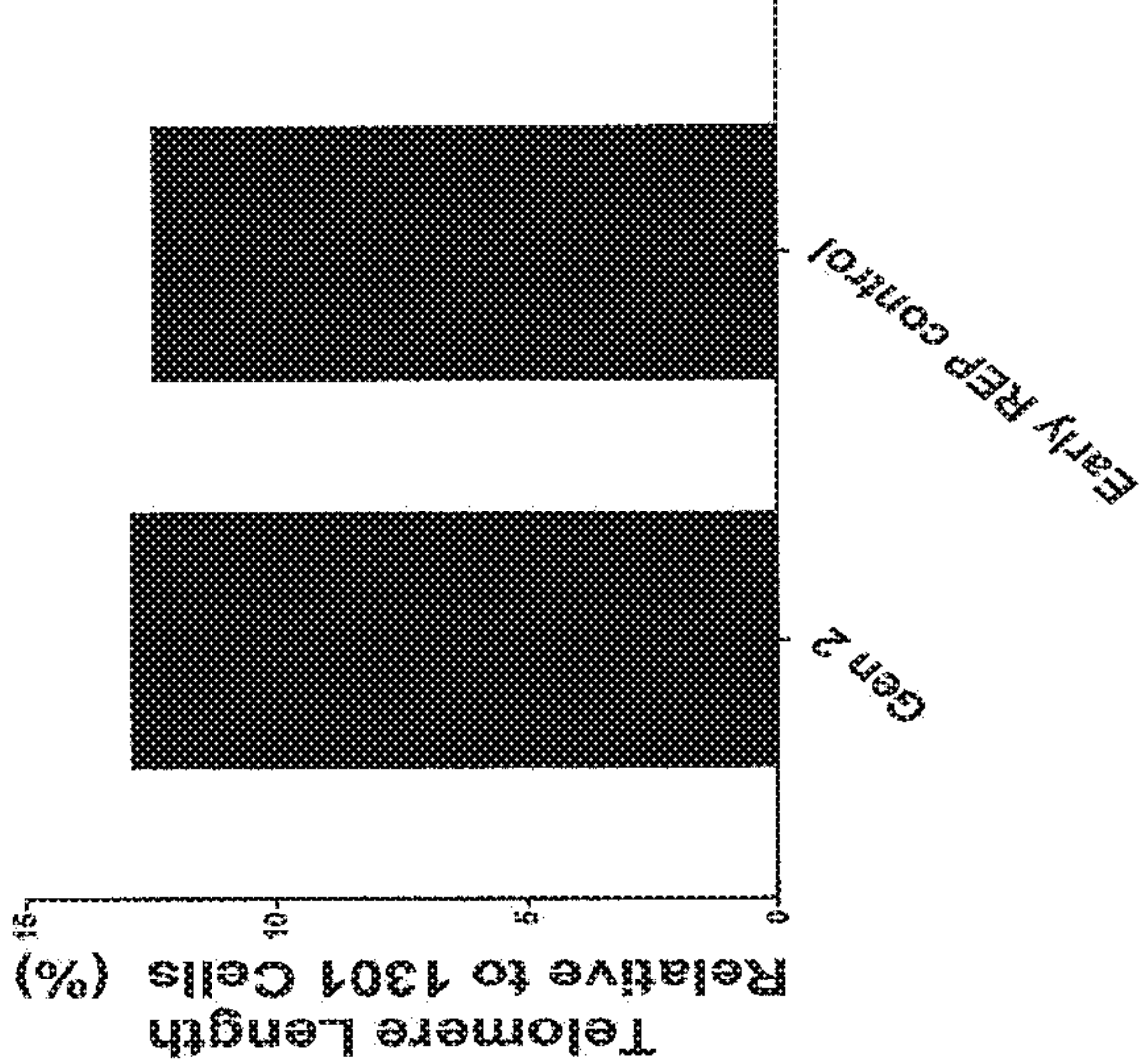
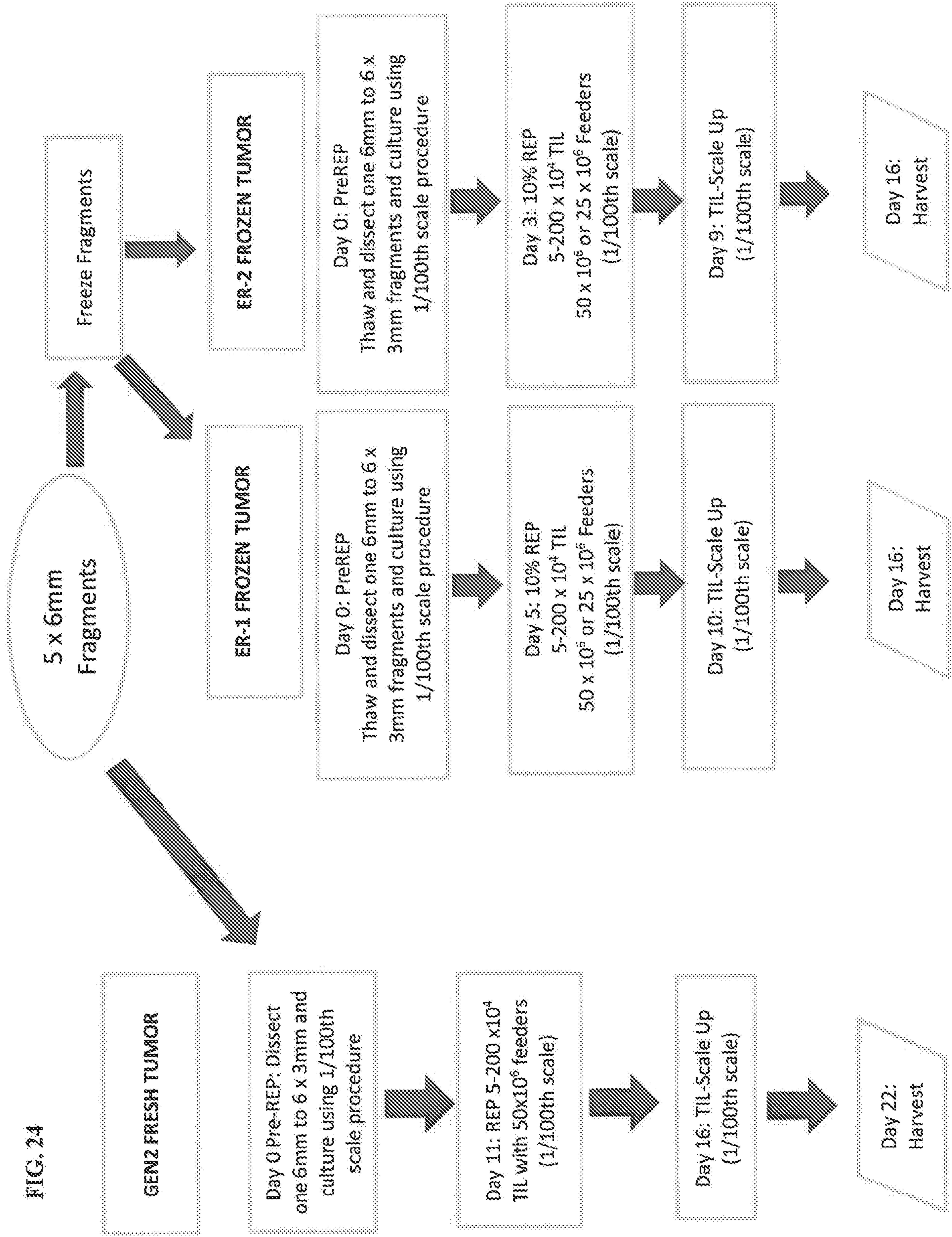


FIG. 23C





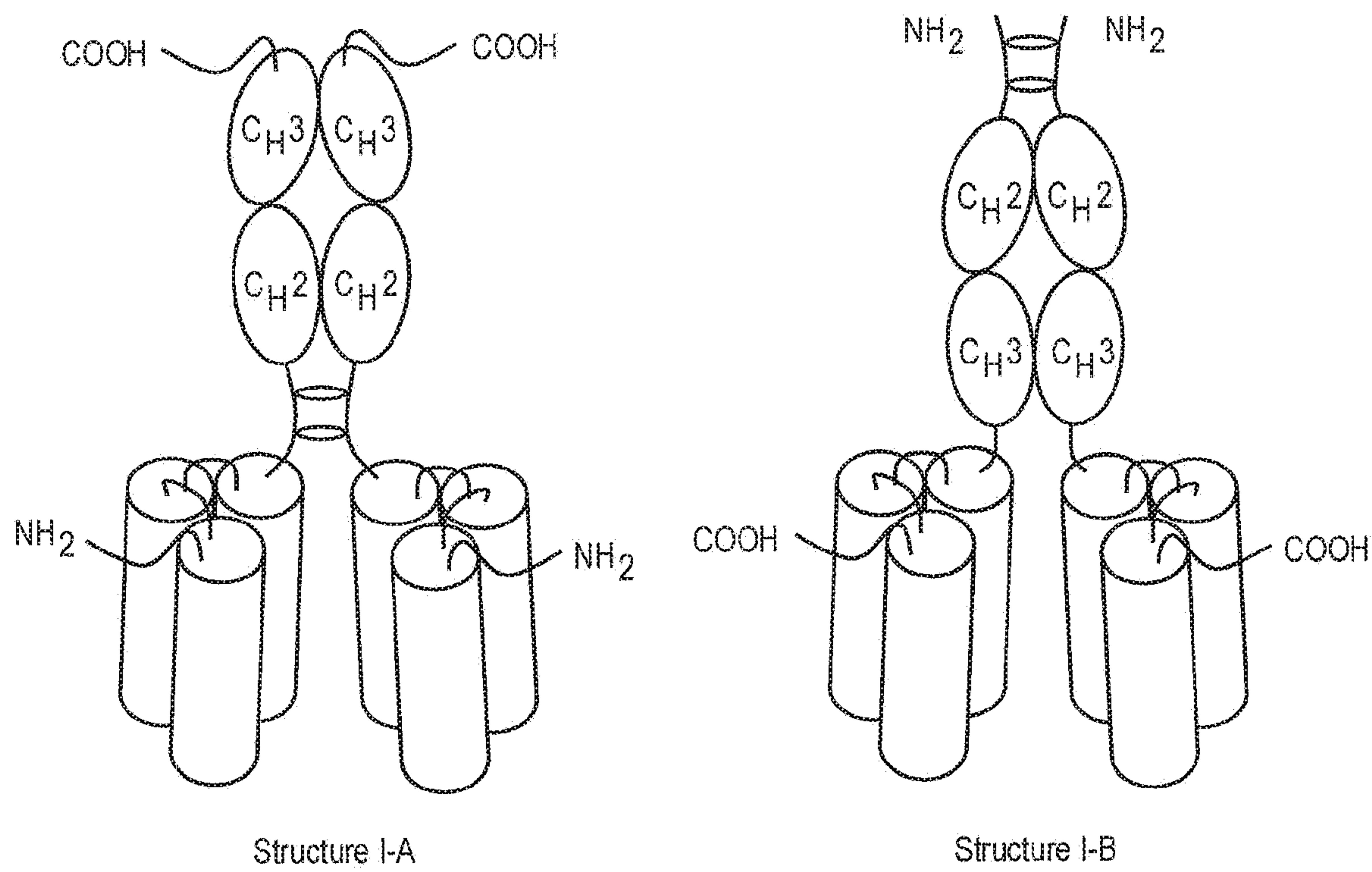


FIG. 25