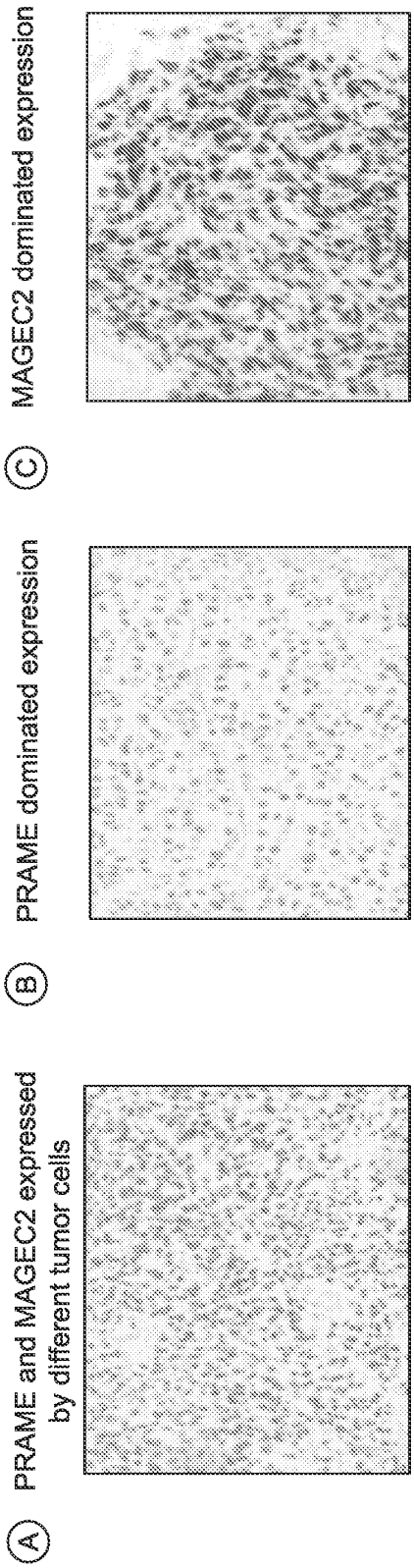

A
PRAME and MAGEC2 expressed by different tumor cells
B
PRAME dominated expression
C
MAGEC2 dominated expression

Figure 1

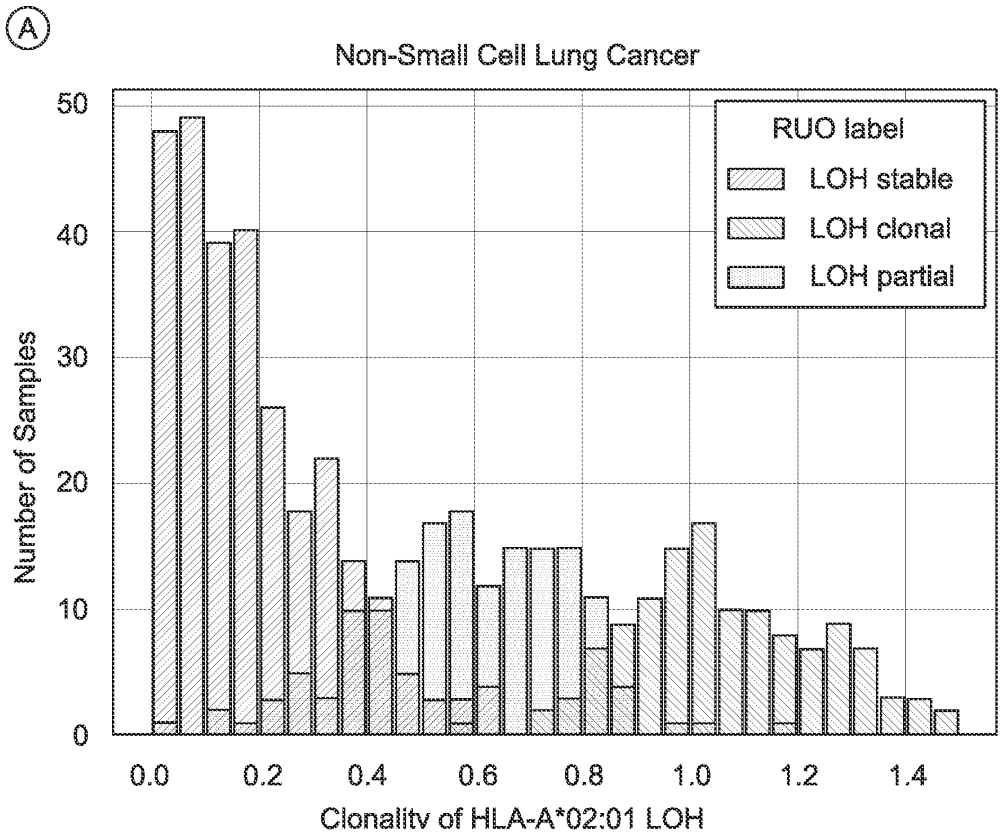

A
Non-Small Cell Lung Cancer
RUO label
LOH stable
LOH clonal
LOH partial
Number of Samples
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Clonality of HLA-A*02:01 LOH

Figure 2

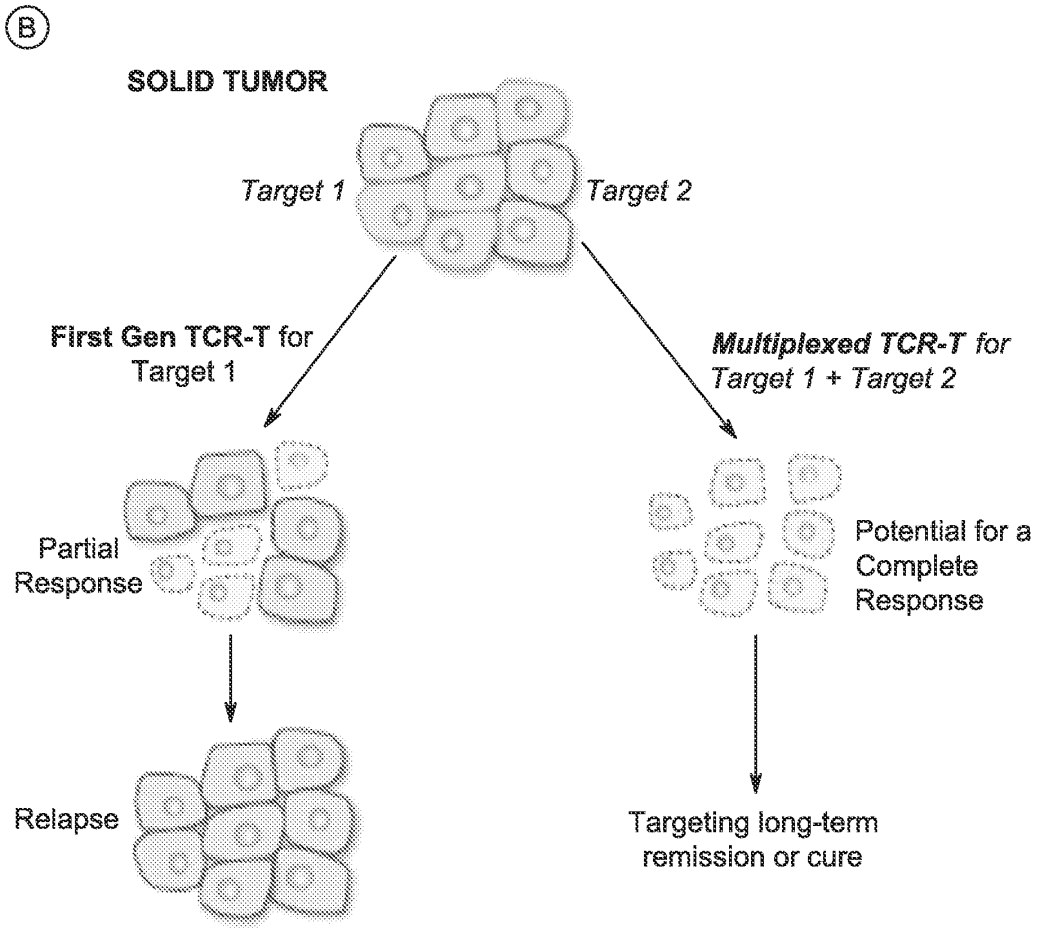


Figure 2 (Cont.)

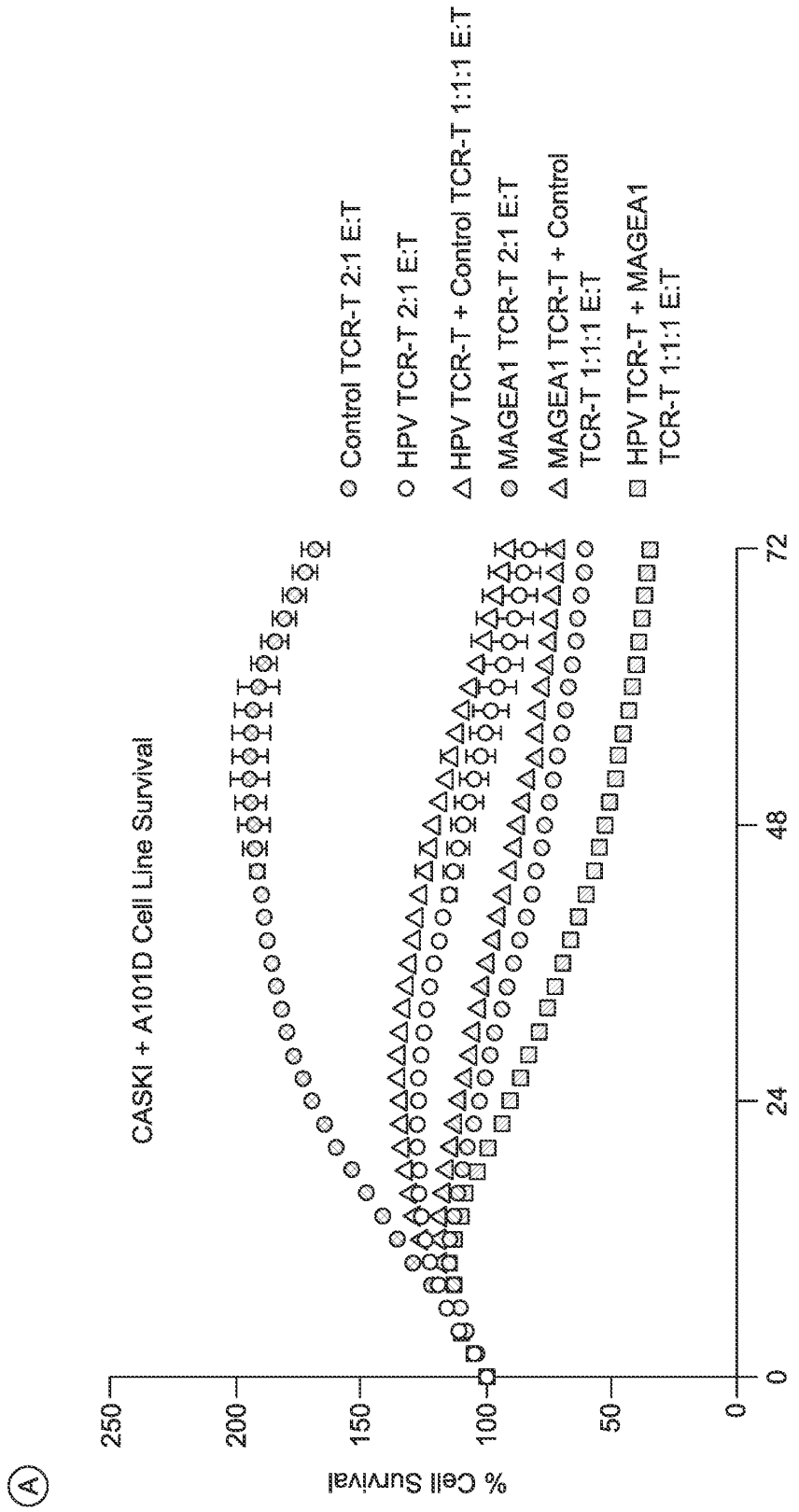

Control TCR-T 2:1 E:T
HPV TCR-T 2:1 E:T
HPV TCR-T + Control TCR-T 1:1:1 E:T
MAGEA1 TCR-T 2:1 E:T
MAGEA1 TCR-T + Control TCR-T 1:1:1 E:T
HPV TCR-T + MAGEA1 TCR-T 1:1:1 E:T
CASKI + A101D Cell Line Survival
% Cell Survival
250
200
150
100
50
0
0
24
48
72
A

Figure 3

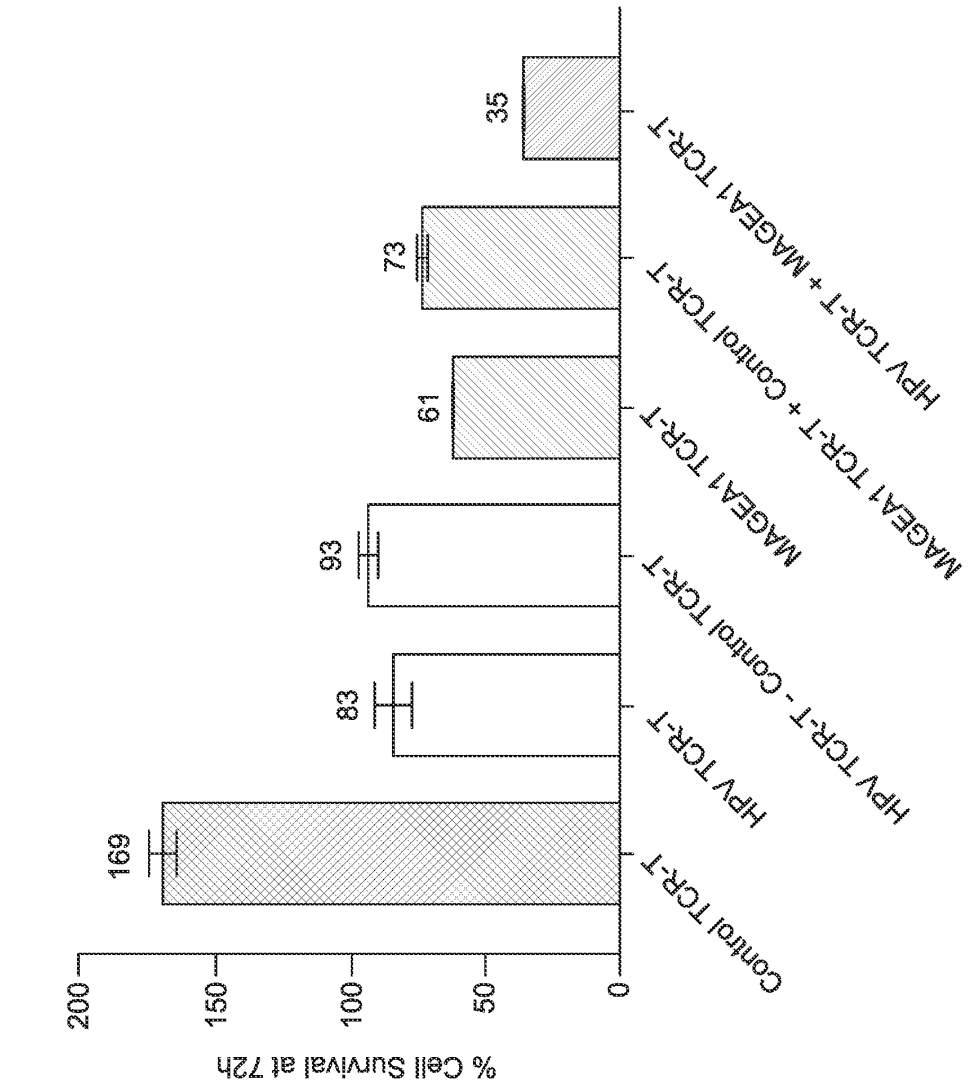
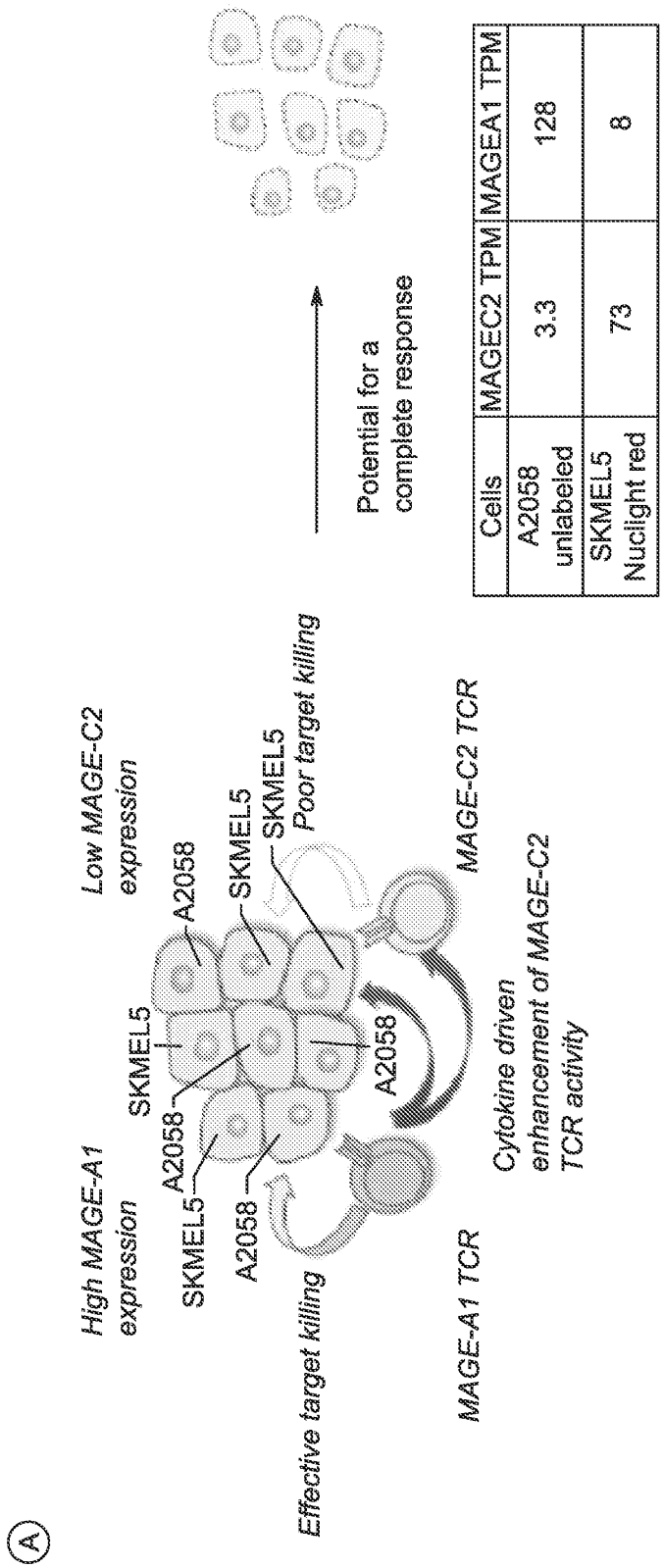


Figure 3 (Cont.)



Cells	MAGEC2 TPM	MAGEA1 TPM
A2058 unlabeled	3.3	128
SKMEL5 Nuclight red	73	8

Figure 4

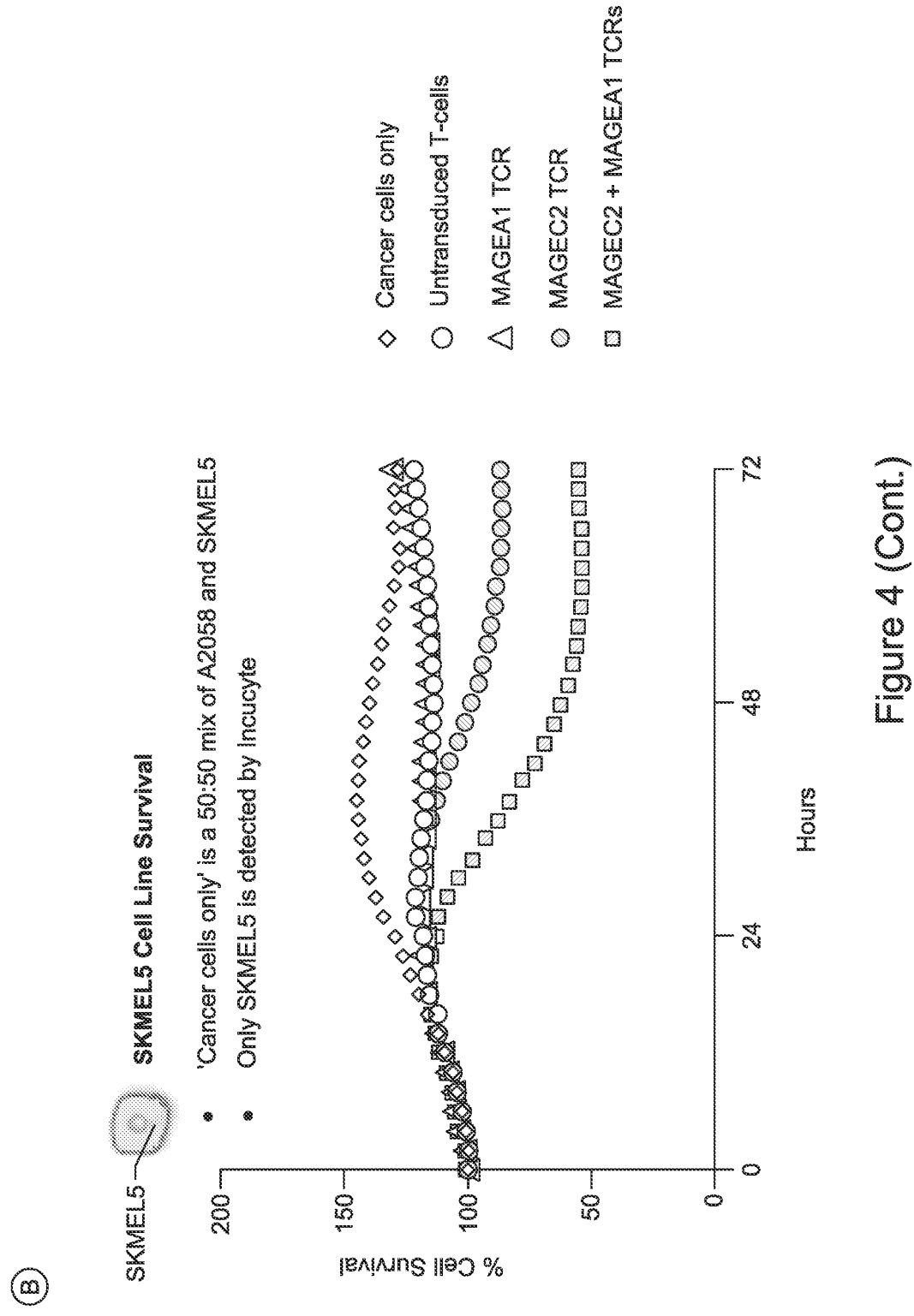


Figure 4 (Cont.)

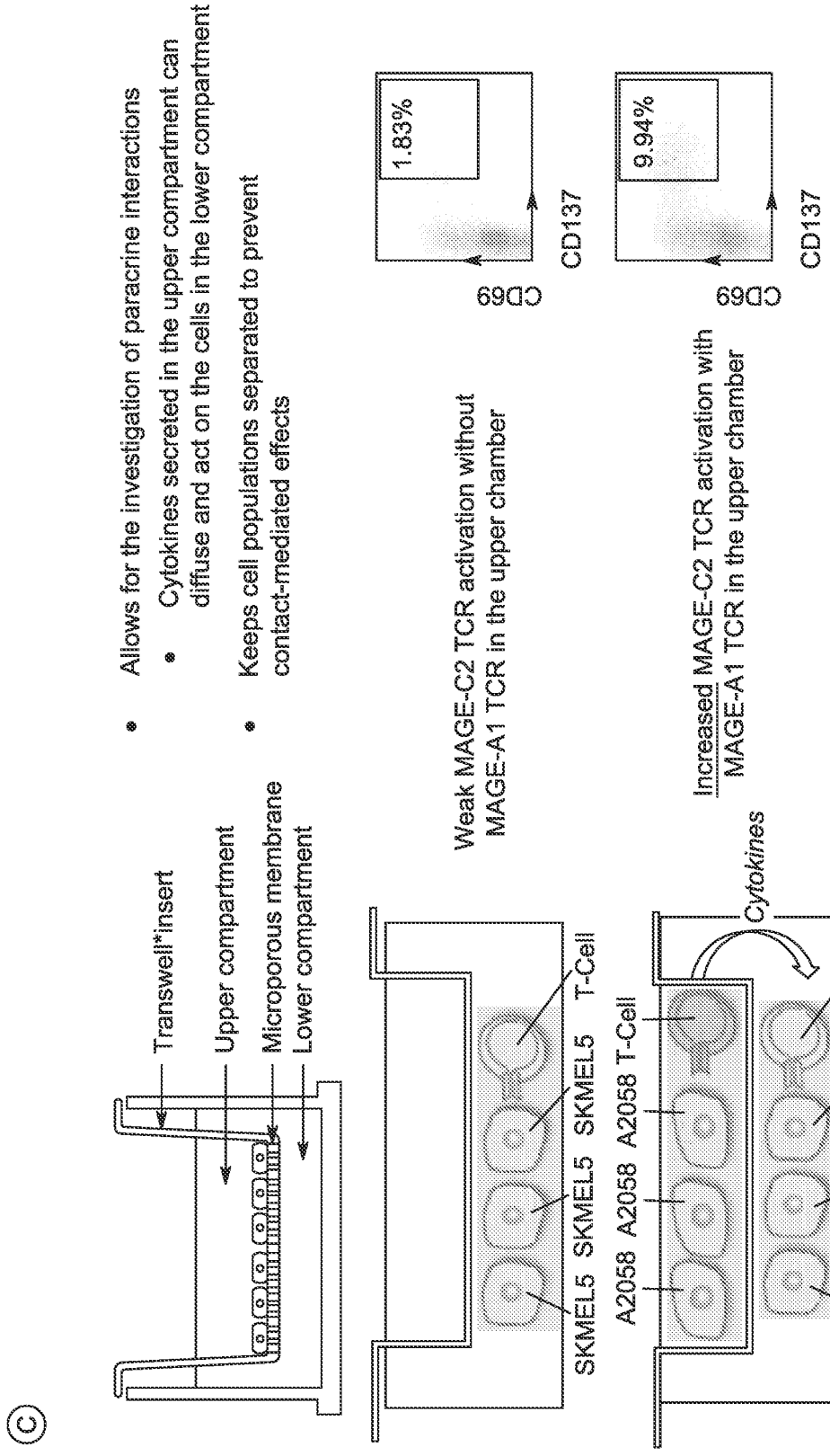


Figure 4 (Cont.)

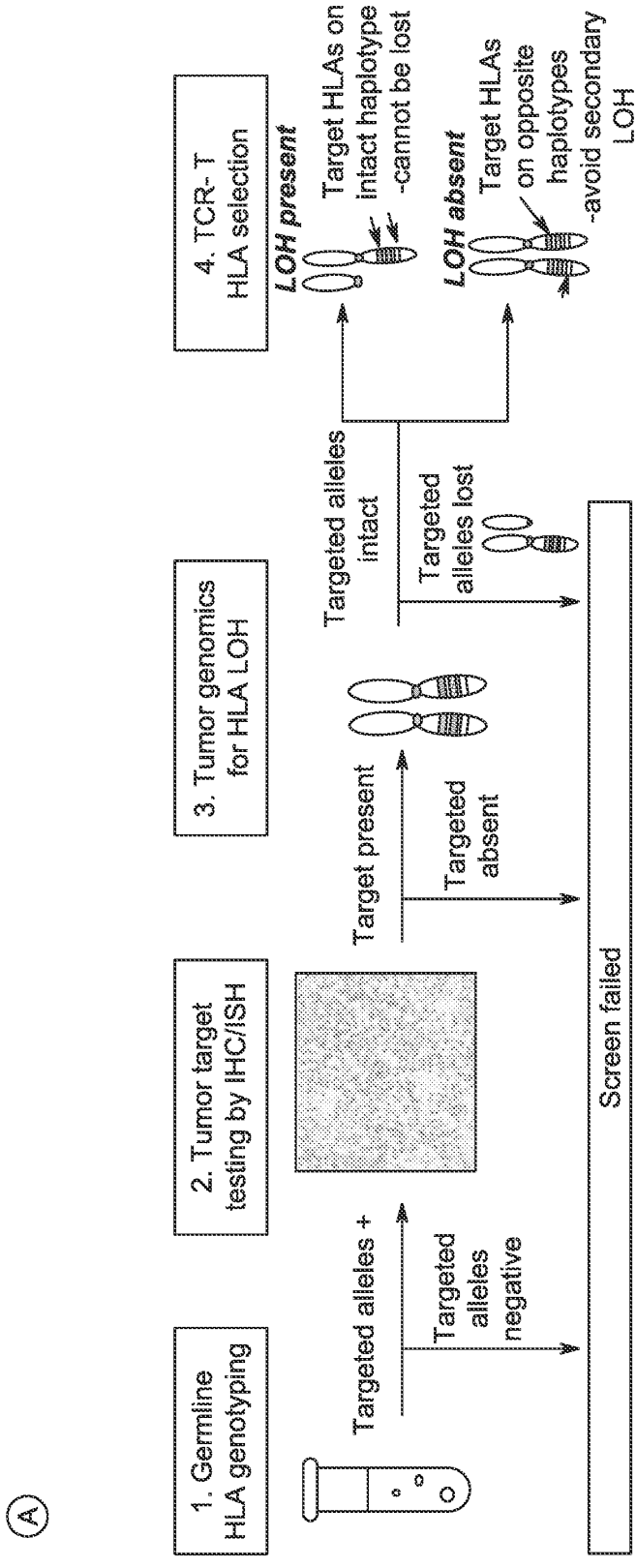

A
1. Germline HLA genotyping
Targeted alleles +
Targeted alleles negative
2. Tumor target testing by IHC/ISH
Target present
Targeted absent
3. Tumor genomics for HLA LOH
Targeted alleles intact
Targeted alleles lost
4. TCR- T HLA selection
LOH present
Target HLAs on intact haplotype -cannot be lost
LOH absent
Target HLAs on opposite haplotypes -avoid secondary LOH
Screen failed

Figure 5

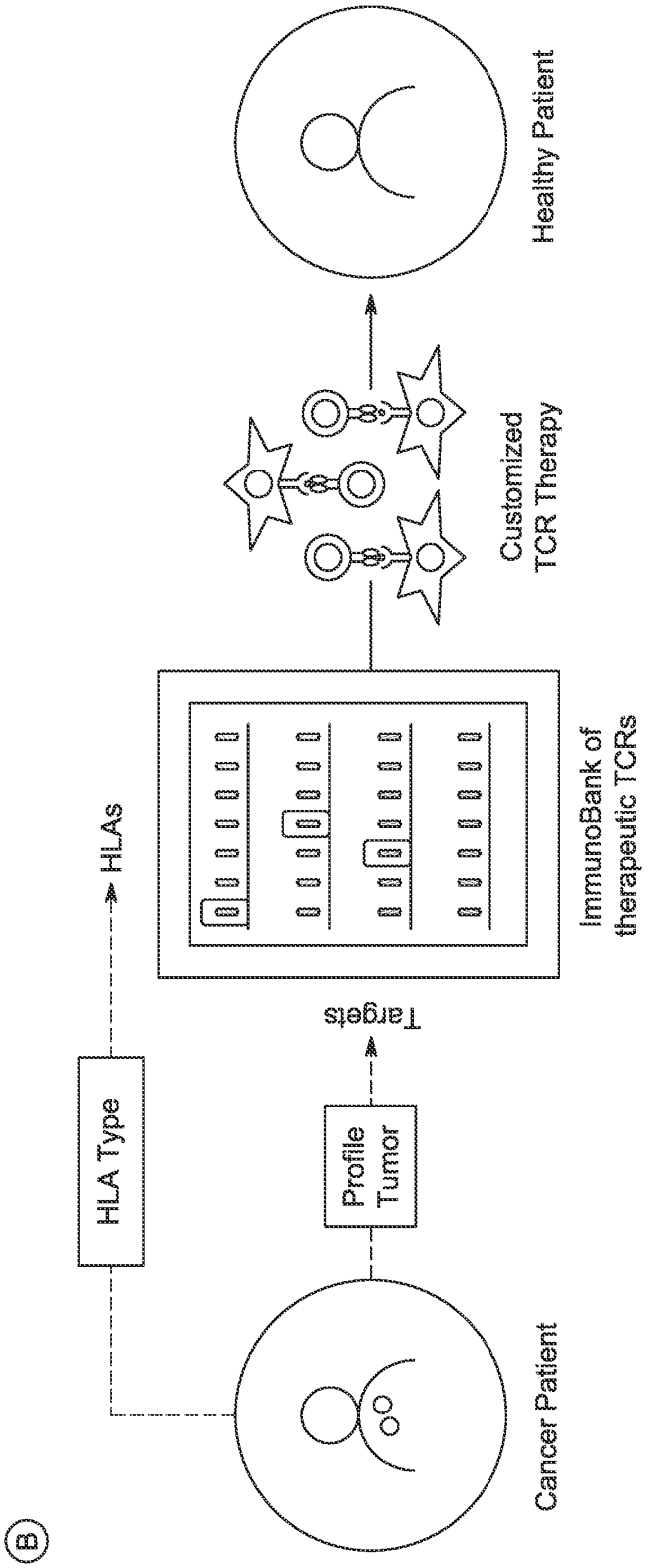


Figure 5 (Cont.)

Background Adoptive Cell Transfer with genetically engineered T cells holds great promise for treating solid tumors. To date, clinical investigations of TCR-T cell therapies have targeted one antigen at a time and have produced encouraging response rates ranging from 30- 50%. Unfortunately, complete responses have been rare, and responses are often short-lived. One possible reason why patients rapidly relapse is that their tumors exhibit substantial heterogeneity of antigen expression: not every cancer cell within a tumor expresses the target of a mono TCR therapy and, even when they do, the target is expressed at variable levels among the individual tumor cells. This suggests that TCR-T targeting one antigen could allow the cells lacking the treated antigen to escape and drive relapse.

TScan Approach To address antigen heterogeneity, we are developing multiplexed TCR-T cell therapy in which a patient is treated with multiple TCR-T cell products, chosen from a collection of highly active TCRs and matched to the patient's tumor antigens and HLA type. One of these antigens, MAGE-A1, was identified as the target of expanded tumor infiltrating T-cells from a head & neck cancer patient using TScan's screening technology. The other one, PRAME, is highly expressed in a variety of cancers. Using our ReceptorScan platform, we developed two high affinity TCRs that recognize HLA-A*02:01-restricted epitopes from MAGE-A1 and PRAME and assessed the benefits of combining these two TCR-T cell products using a variety of pre-clinical models.

Results Multiplexed TCR-T mimics the natural oligoclonal T cell response to cancer and provides a way to address solid tumor heterogeneity. Individually, both TCRs show strong cytotoxic activity in vitro when co-cultured with HLA-matched cancer cell lines expressing endogenous MAGE-A1 and PRAME. Additionally, in xenograft mouse models, each TCR was able to control the growth of tumors expressing their cognate antigens. To test whether the two TCRs exhibit additive or synergistic activity, a mixture of two different cell lines expressing either MAGE-A1 or PRAME were grown as xenograft tumors in mice, mimicking the observed heterogeneity of these targets in human tumors. Notably, when treated with multiplexed MAGE-A1/PRAME TCR-T, the mice achieved longer lasting tumor control compared to either singleplexed treatment alone. These findings support the hypothesis that multiplexed TCR-T has the potential to overcome antigen heterogeneity, which may contribute to the observed lack of durability in clinical trials of singleplexed TCR-T therapy.

Clinical application To address solid tumor heterogeneity in the clinic, we are building an ImmunoBank of therapeutic TCRs that recognize different targets presented on different HLA alleles. We submit that selecting multiplexed TCR-Ts that target intact antigens and HLA alleles in patient tumors should synergistically overcome solid tumor heterogeneity, and we are designing trials to test this hypothesis clinically.

Figure 6

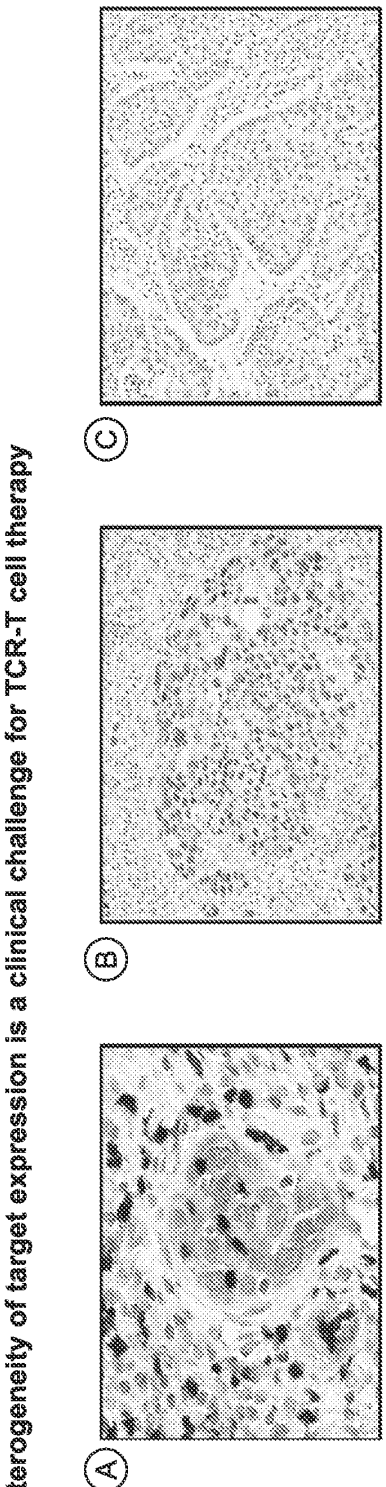


Figure 6 (Cont.)

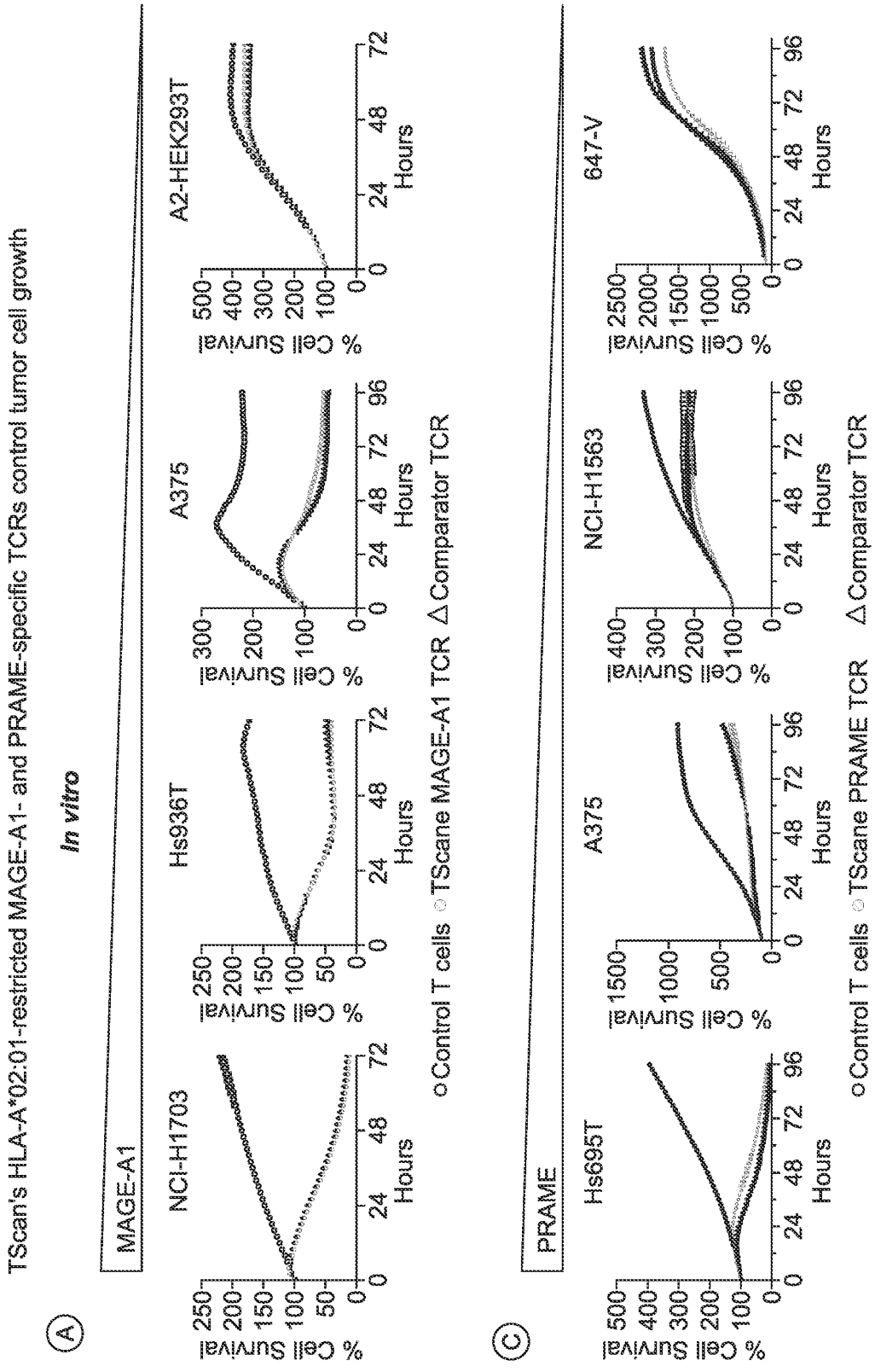


Figure 6 (Cont.)

Nuclight Red-labeled HLA-A*02:01$^+$ cancer cell lines with varying levels of endogenous MAGE-A1 (**A**) and PRAME (**C**) were grown in the presence of TScane's MAGE-A1- and PRAME-specific TCR-T cells, respectively. TCR-T-mediated cytotoxicity was assessed using Incucyte® over a period of three days. NCG immunodeficient mice (Charles River) were implanted subcutaneously with human HLA-A*02:01$^+$ tumor cell lines with endogenous expression of MAGE-A1 (U266B1) (**B**) or PRAME (Hs695T) (**D**). Once the tumors reached 100 mm^3, 20 million cognate TCR-T cells were administered twice for MAGE-A1 and once for PRAME on the indicated days.

Figure 6 (Cont.)

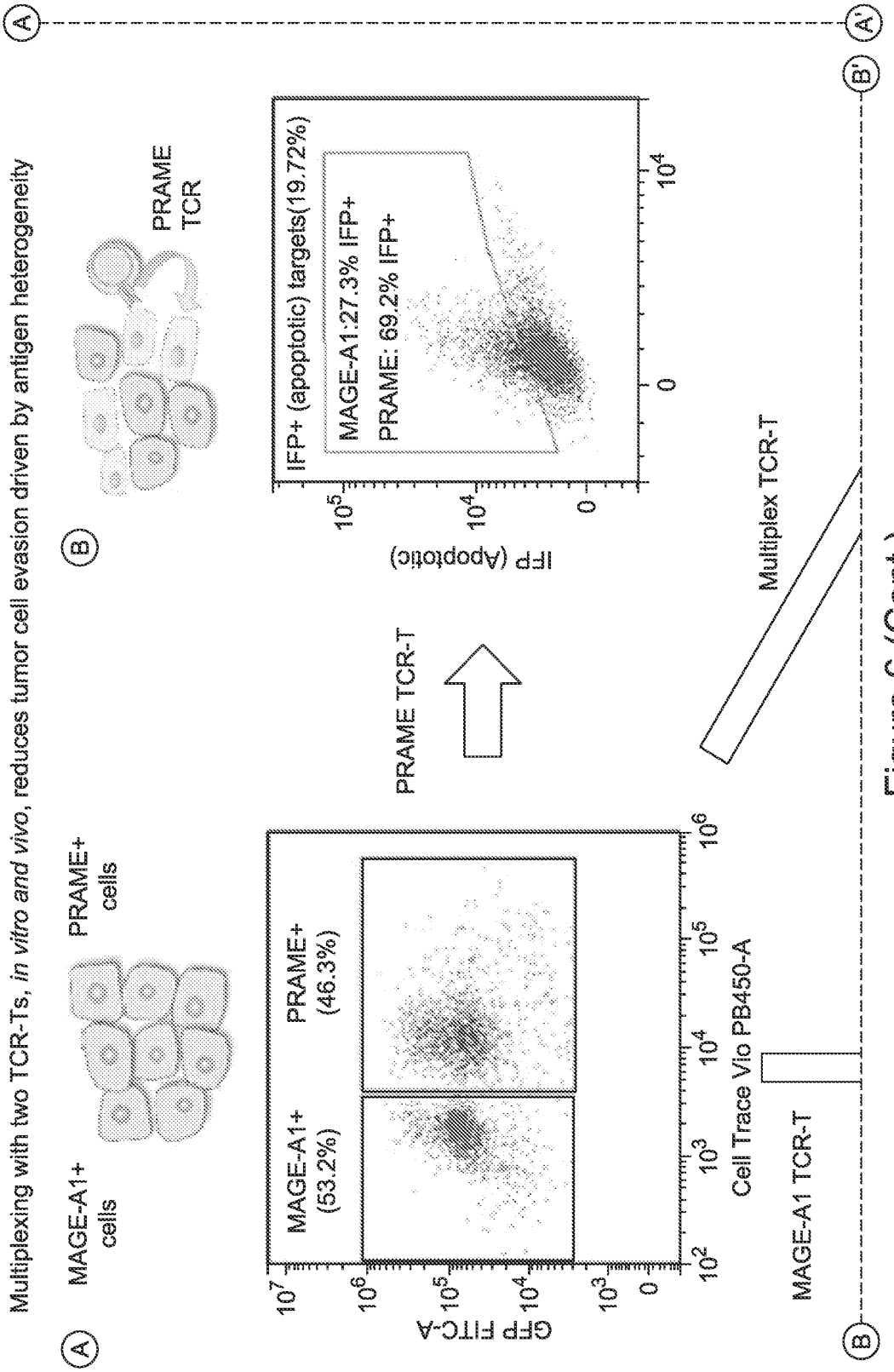
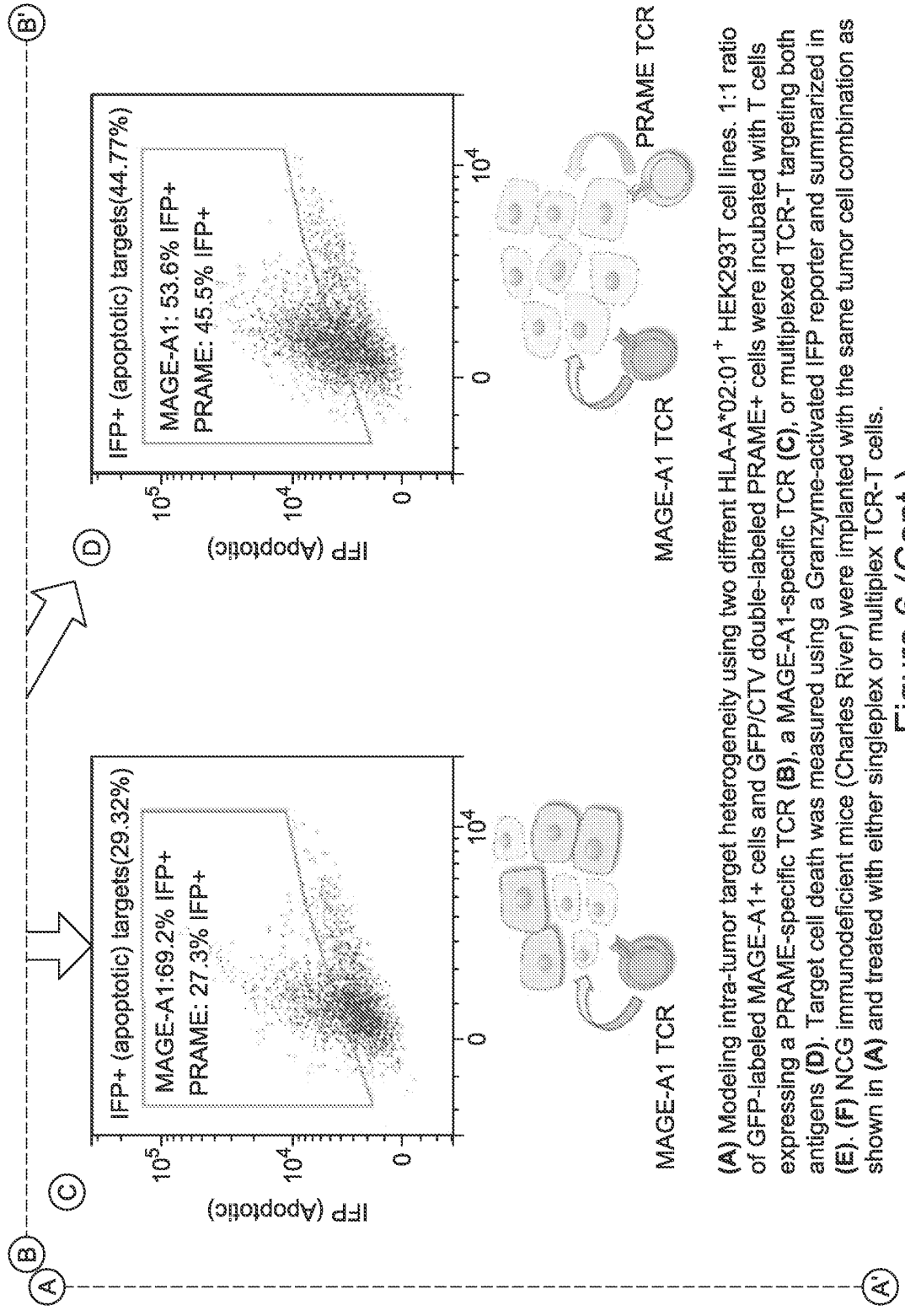


Figure 6 (Cont.)



(A) Modeling intra-tumor target heterogeneity using two diffrent HLA-A*02:01$^+$ HEK293T cell lines. 1:1 ratio of GFP-labeled MAGE-A1+ cells and GFP/CTV double-labeled PRAME+ cells were incubated with T cells expressing a PRAME-specific TCR **(B)**, a MAGE-A1-specific TCR **(C)**, or multiplexed TCR-T targeting both antigens **(D)**. Target cell death was measured using a Granzyme-activated IFP reporter and summarized in **(E)**. **(F)** NCG immunodeficient mice (Charles River) were implanted with the same tumor cell combination as shown in **(A)** and treated with either singleplex or multiplex TCR-T cells.

Figure 6 (Cont.)

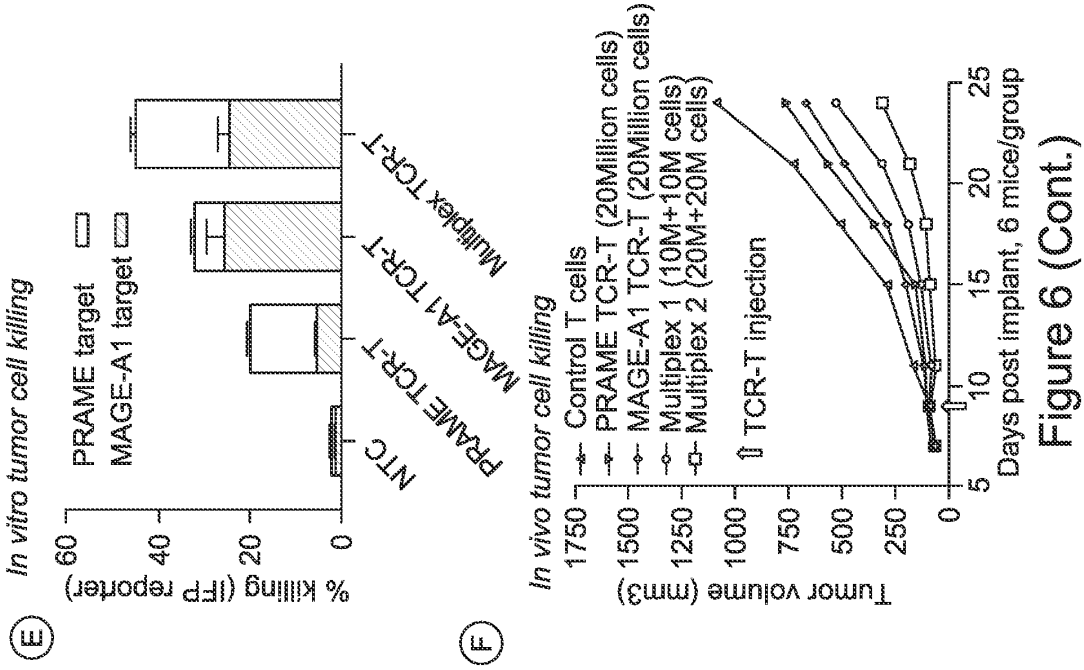
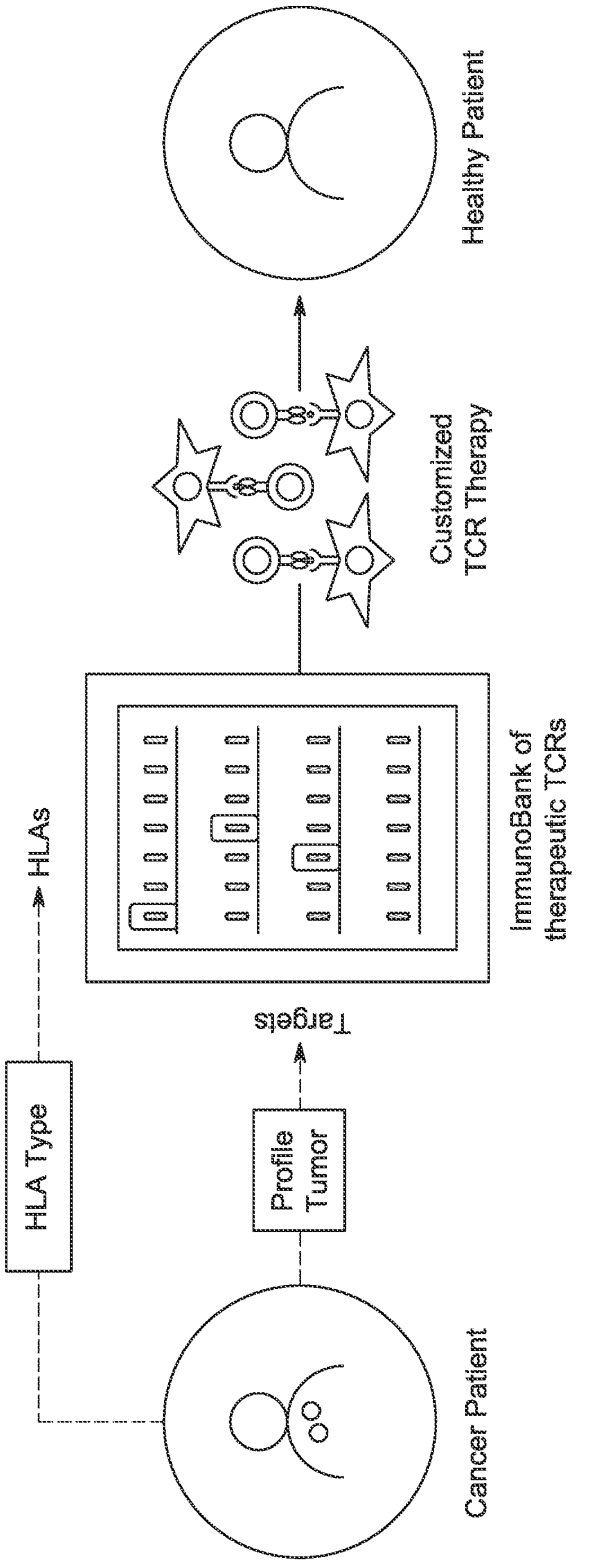


Figure 6 (Cont.)



TScan is building **customized, multiplexed TCR-T cell therapies** for solid tumors. For each patient, two or three therapeutic TCRs will be selected from the ImmunoBank that best match the targets and HLAs expressed in their tumors. By **multiplexing** across both targets and HLAs, this strategy is designed to **prevent resistance** arising from either target loss or HLA loss.

Figure 6 (Cont.)

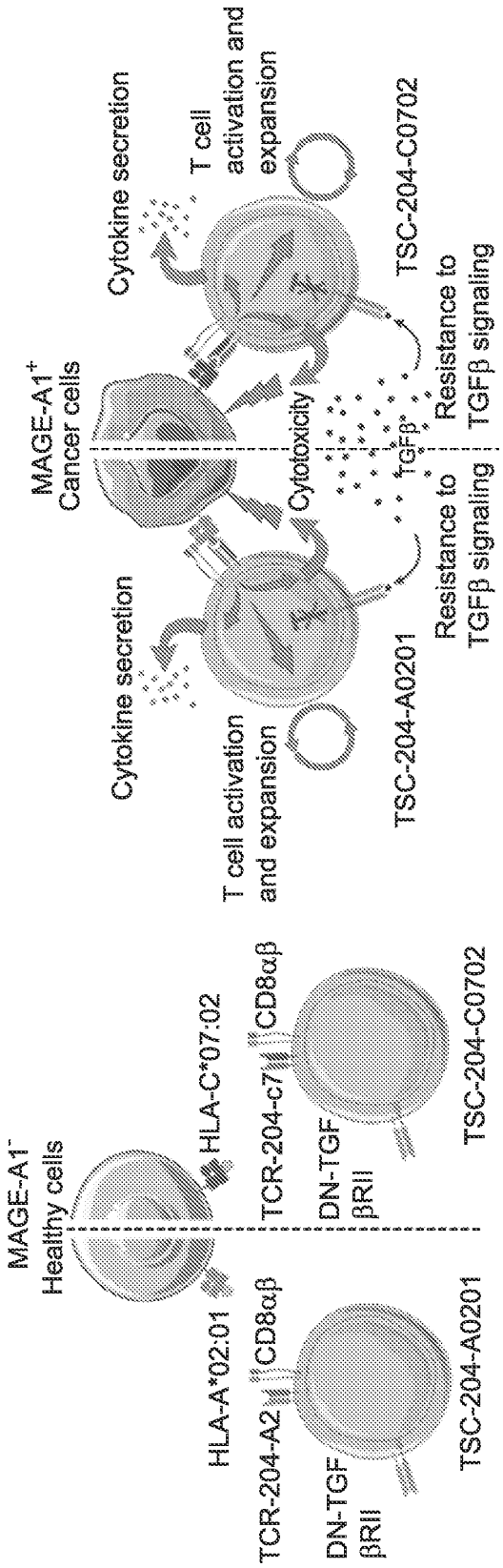

MAGE-A1⁻
Healthy cells
HLA-A*02:01
HLA-C*07:02
TCR-204-A2
CD8αβ
TCR-204-c7
CD8αβ
DN-TGF βRII
DN-TGF βRII
TSC-204-A0201
TSC-204-C0702
MAGE-A1⁺
Cancer cells
Cytokine secretion
T cell activation and expansion
Cytotoxicity
TGFβ
Resistance to TGFβ signaling
TSC-204-A0201
TSC-204-C0702
Cytokine secretion
T cell activation and expansion
Resistance to TGFβ signaling

Figure 7

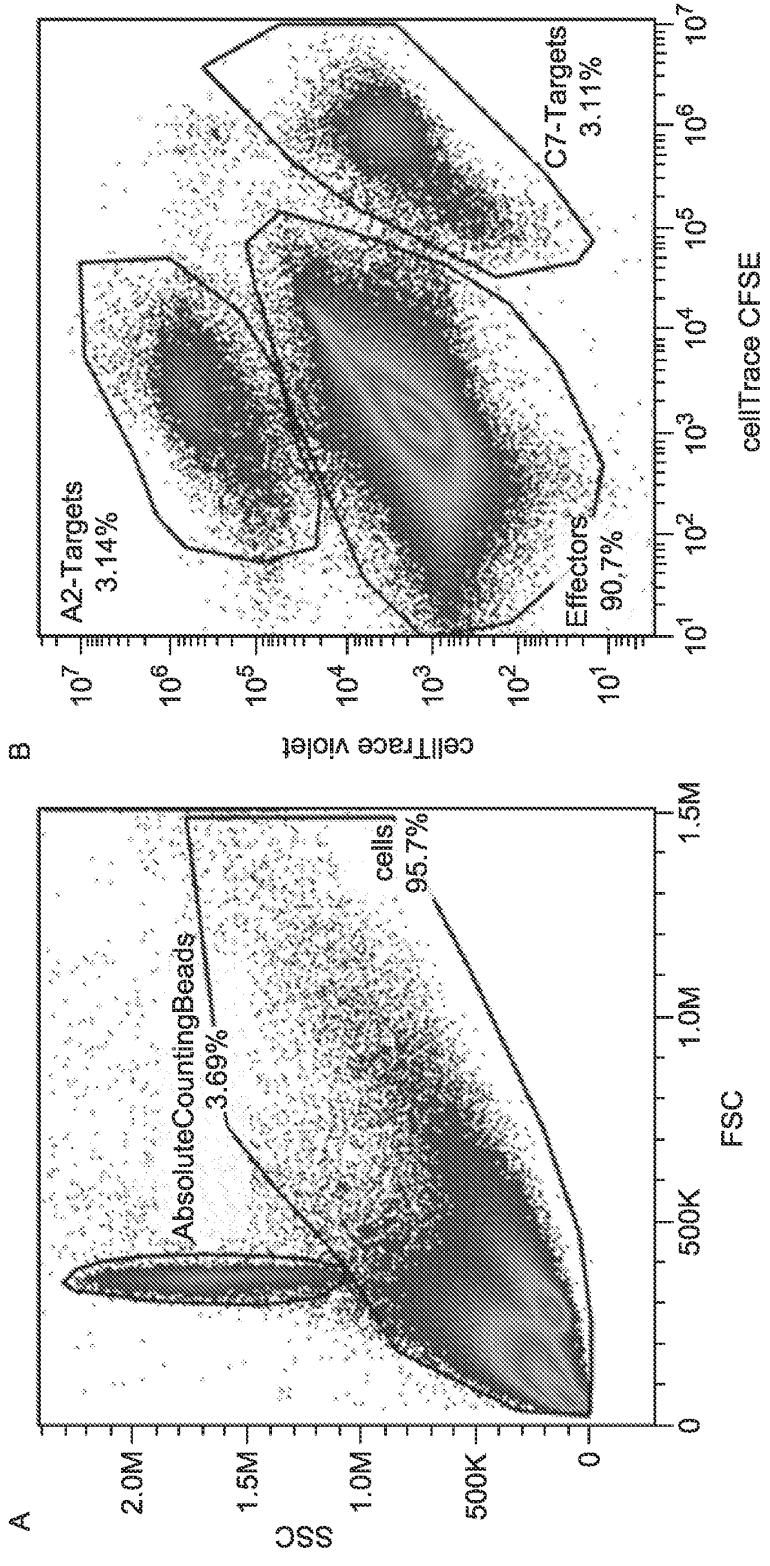

A
SSC
2.0M
1.5M
1.0M
500K
0
FSC
0
500K
1.0M
1.5M
AbsoluteCountingBeads
3.69%
cells
95.7%
B
cellTrace violet
cellTrace CFSE
A2-Targets
3.14%
C7-Targets
3.11%
Effectors
90,7%

Figure 8

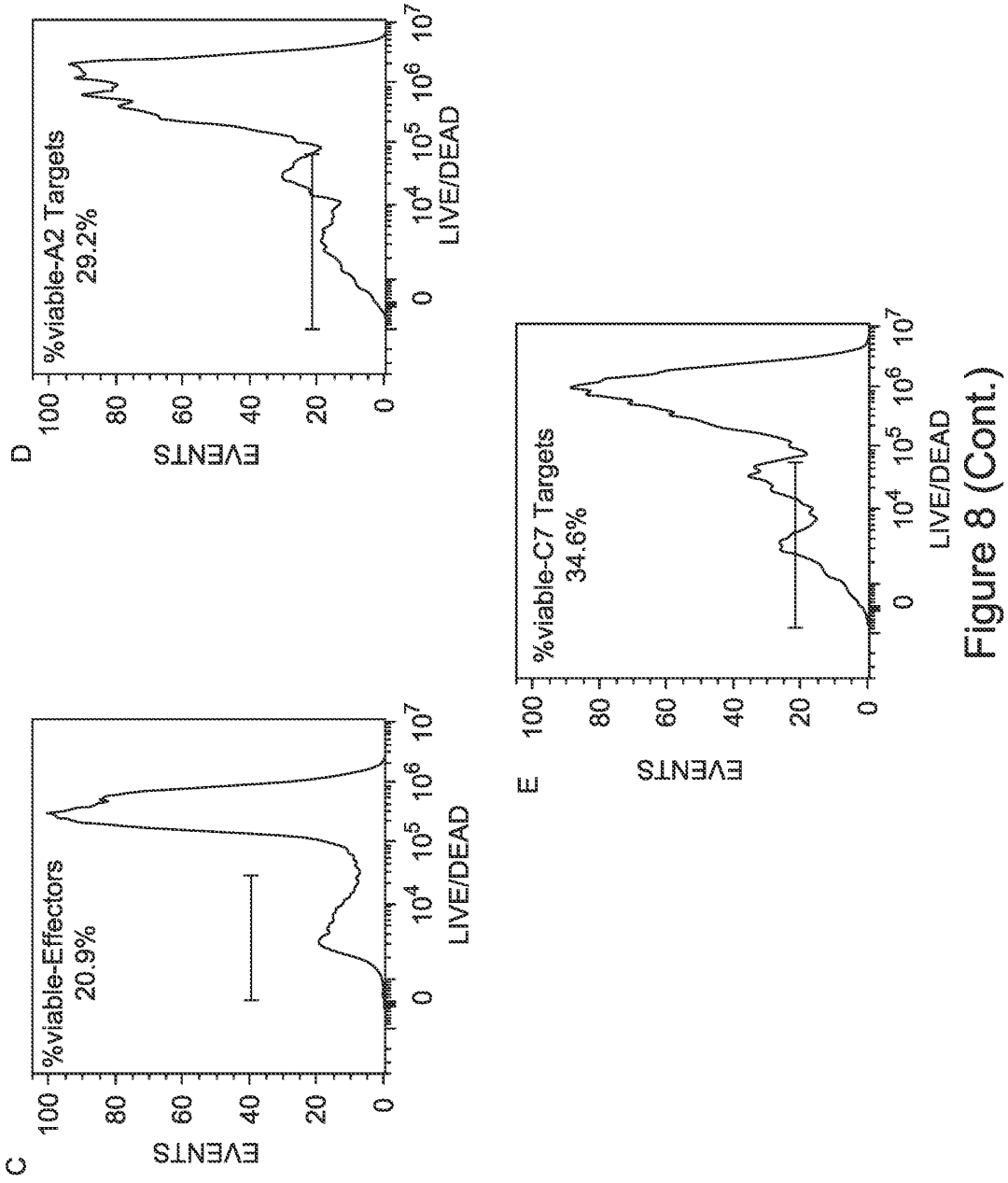


Figure 8 (Cont.)

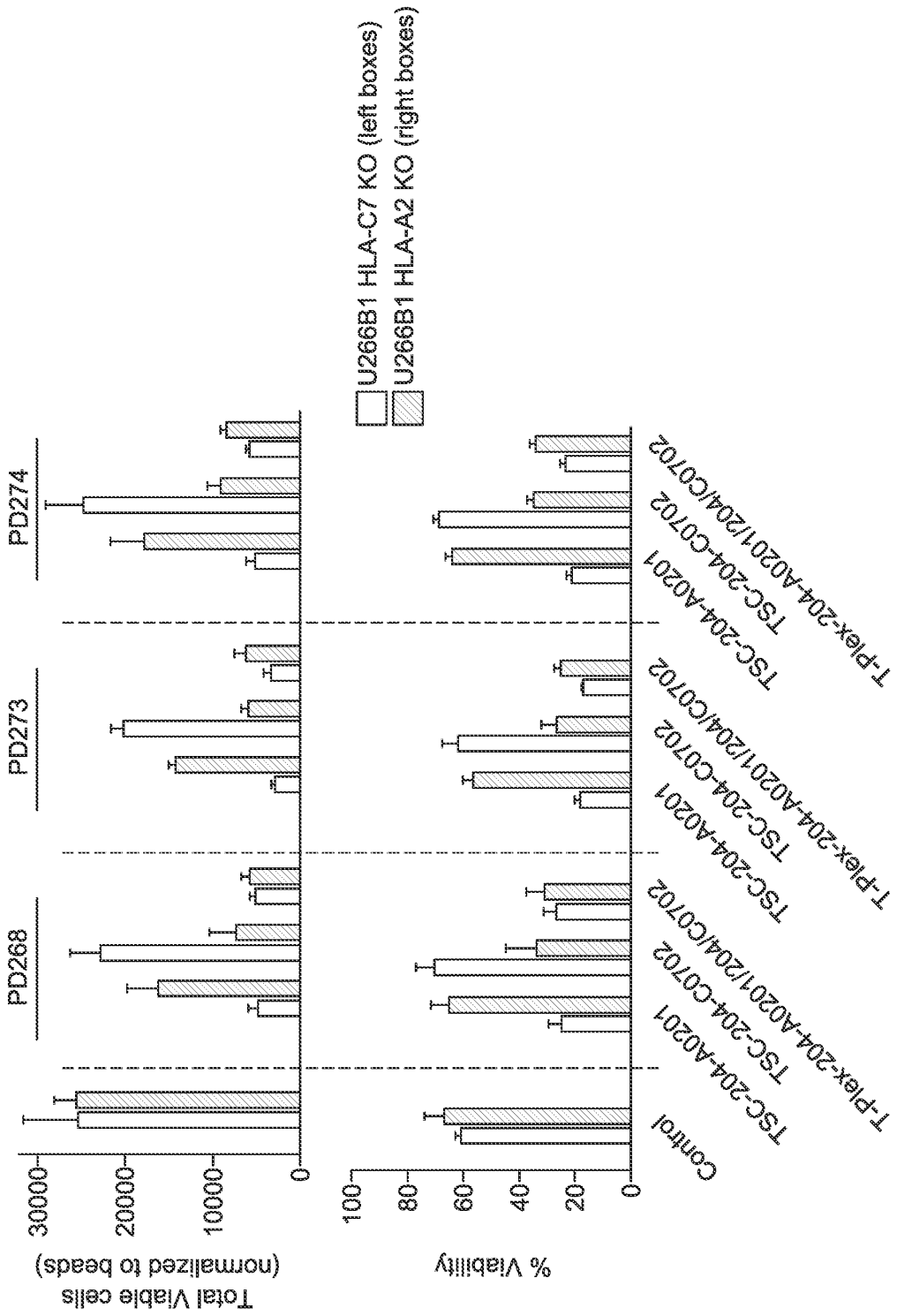

U266B1 HLA-C7 KO (left boxes)
U266B1 HLA-A2 KO (right boxes)
PD268
PD273
PD274
Total Viable cells
(normalized to beads)
30000
20000
10000
0
% Viability
100
80
60
40
20
0
Control
TSC-204-A020I
TSC-204-C070Z
T-Plex-204-A020I/204/C070Z

Figure 9