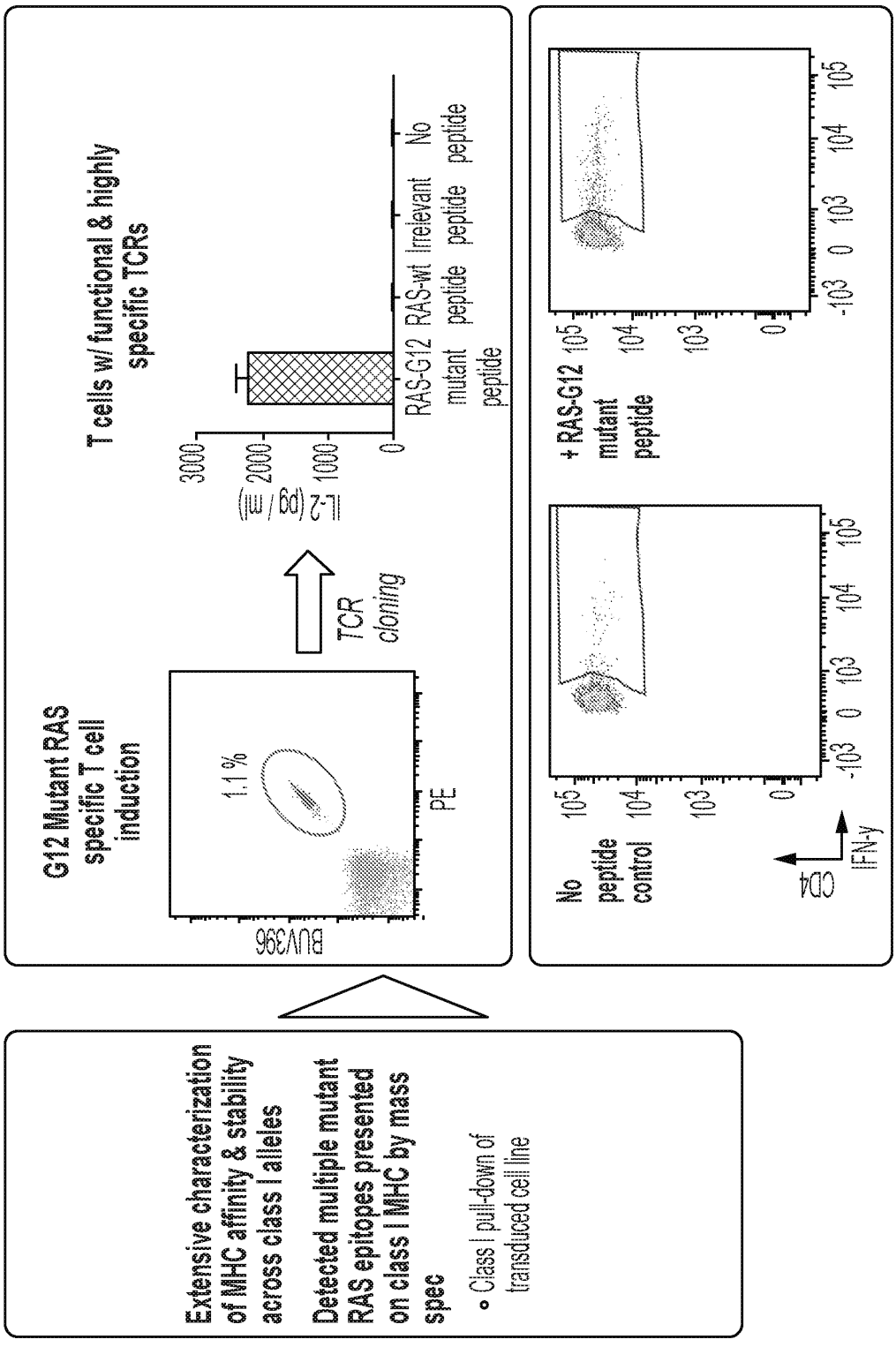
Extensive characterization of MHC affinity & stability across class I alleles
Detected multiple mutant RAS epitopes presented on class I MHC by mass spec
• Class I pull-down of transduced cell line
G12 Mutant RAS specific T cell induction
BUV396
1.1 %
PE
TCR cloning
T cells w/ functional & highly specific TCRs
IL-2 (pg / ml)
3000
2000
1000
0
RAS-G12 mutant peptide
RAS-wt peptide
Irrelevant peptide
No peptide
No peptide control
+ RAS-G12 mutant peptide
CD4
IFN-γ
105
104
103
0
-103
0
103
104
105

FIG. 1

KRAS G12C (HLA-A*11)		
Approach	Cell Input	Peptides Detected in Construct
Lentiviral transduced 293T cells	43 Million	VVVGACGVGK

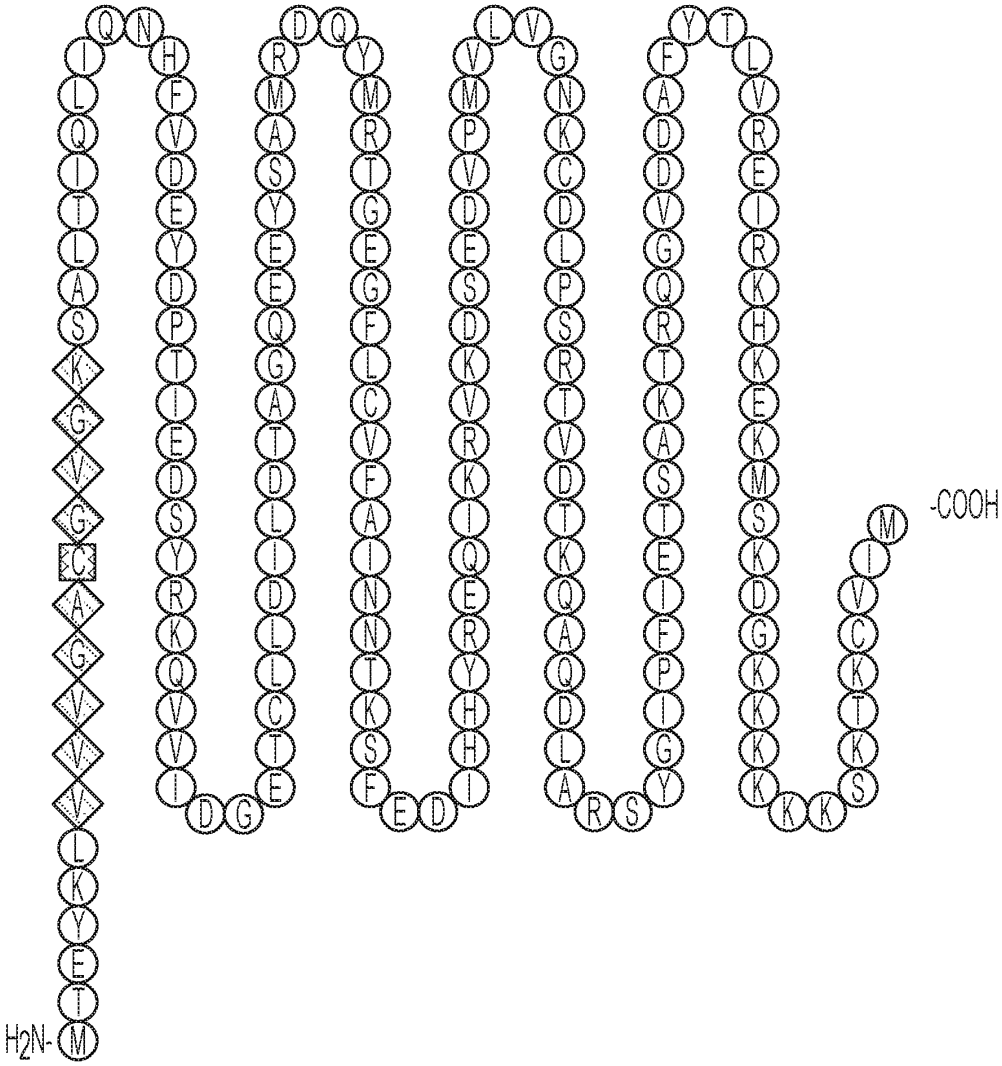

FIG. 2A

KRAS G12V (HLA-A*11)

Approach	Cell Input	Peptides Detected in Construct
Lentiviral transduced 293T cells	39-200 million	VVVGAVGVGK VVGAVGVGK

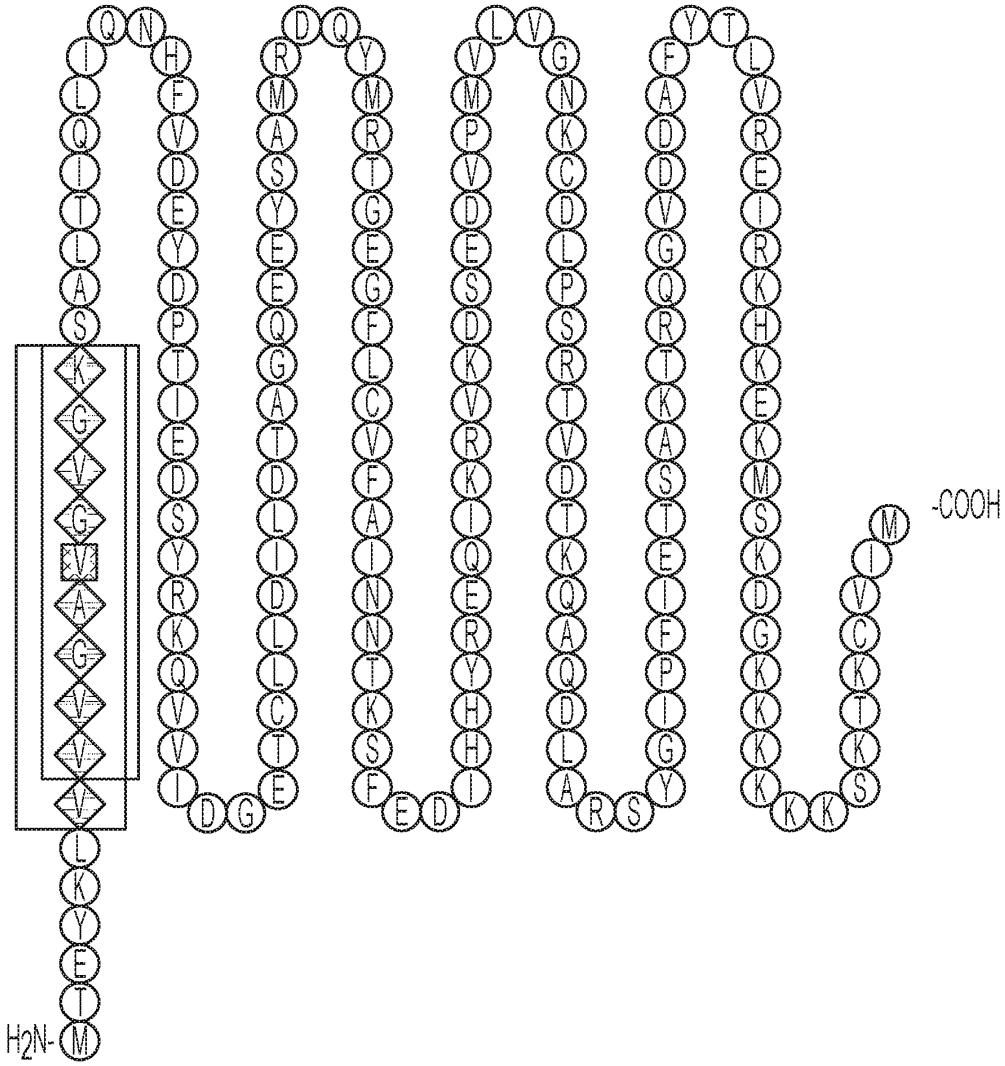

FIG. 2A
CONTINUED

Allele	Mutation	Epitope	Detected by MS?
A*02:01	G12C	LVVVGACGV	-
	G12C	KLVVVGACGV	-
	G12D	LVVVGADGV	-
	G12D	KLVVVGADGV	-
	G12V	LVVVGAVGV	-
	G12V	KLVVVGAVGV	-
A*03:01	G12C	VVGACGVGK	-
	G12C	VVVGACGVGK	✓
	G12D	VVGADGVGK	-
	G12D	VVVGADGVGK	-
	G12V	VVGAVGVGK	✓
	G12V	VVVGAVGVGK	✓
A*11:01	G12C	VVGACGVGK	-
	G12C	VVVGACGVGK	✓
	G12D	VVGADGVGK	-
	G12D	VVVGADGVGK	✓
	G12V	VVGAVGVGK	✓
	G12V	VVVGAVGVGK	✓
B*08:01	G12D	DGVGKSAL	-
	G12D	DGVGKSALT	-
	G12D	DGVGKSALTI	-

✓ Detected
- Assayed for, but not detected

FIG. 2B

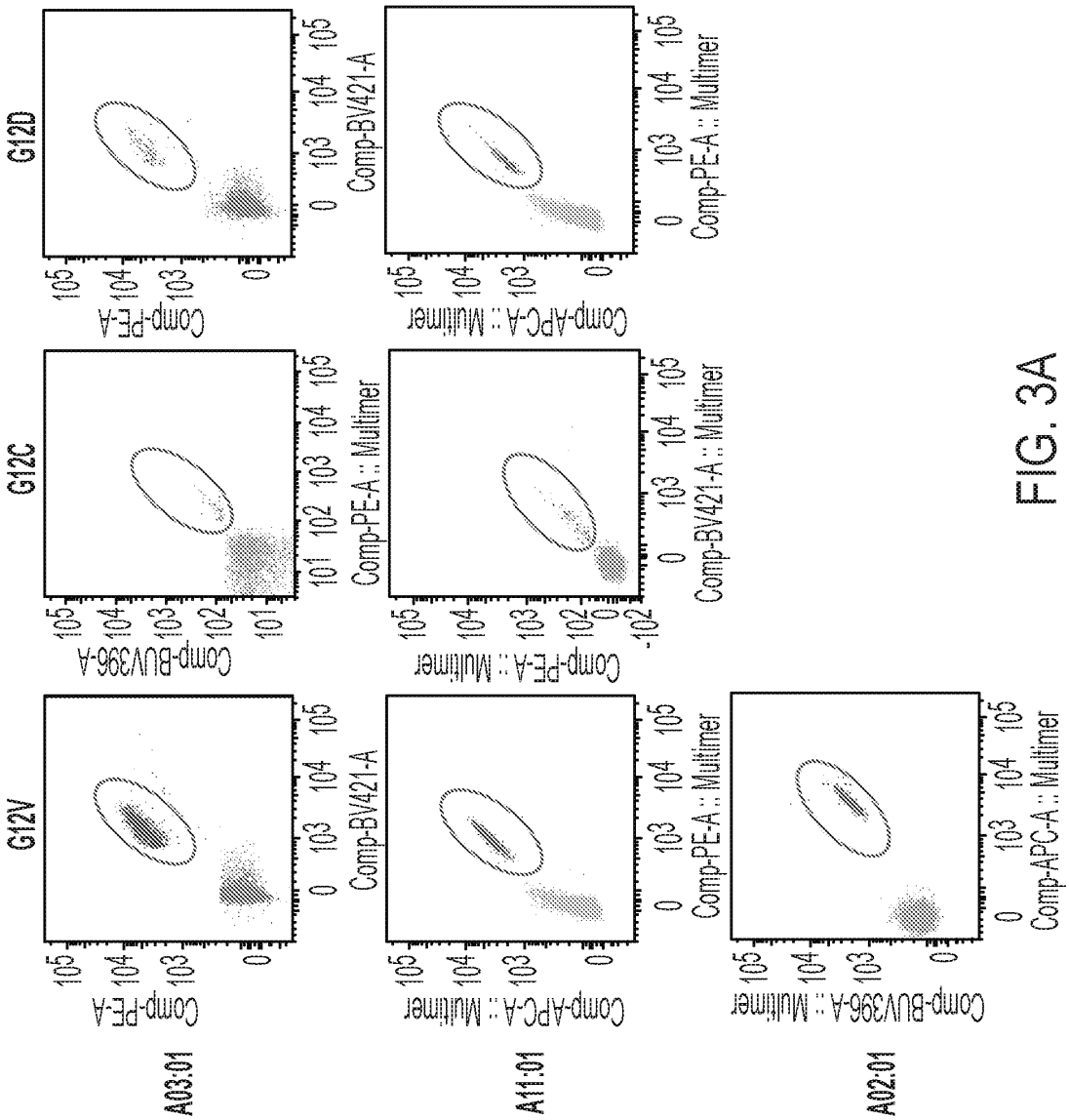
G12D
G12C
G12V
A03:01
A11:01
A02:01
Comp-PE-A
Comp-BV421-A
Comp-APC-A :: Multimer
Comp-BUV396-A
Comp-PE-A :: Multimer
Comp-BV421-A :: Multimer
Comp-APC-A :: Multimer

FIG. 3A

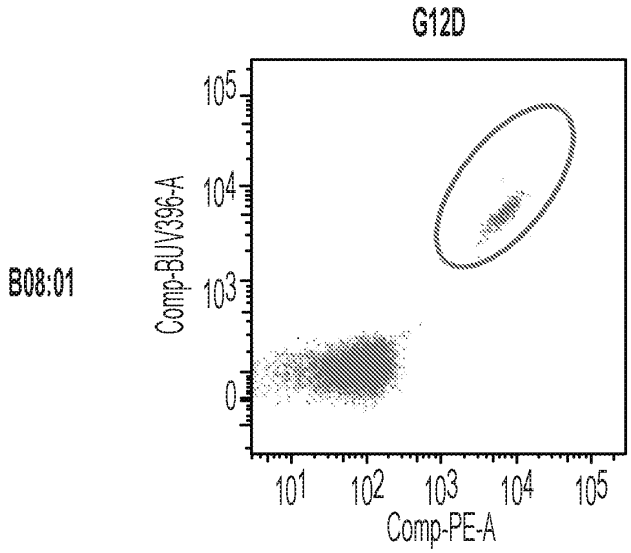

FIG. 3B

Stimulating peptide: TEYKLVVVGAVGVGKSALTI
Minimal epitope used for multimer detection: (V)VVGAVGVGK

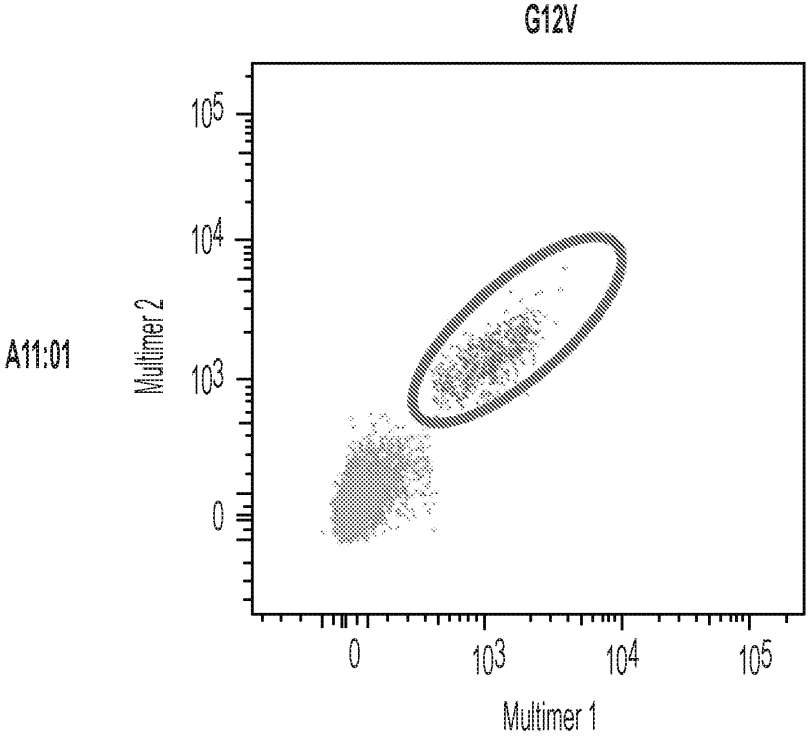

FIG. 3C

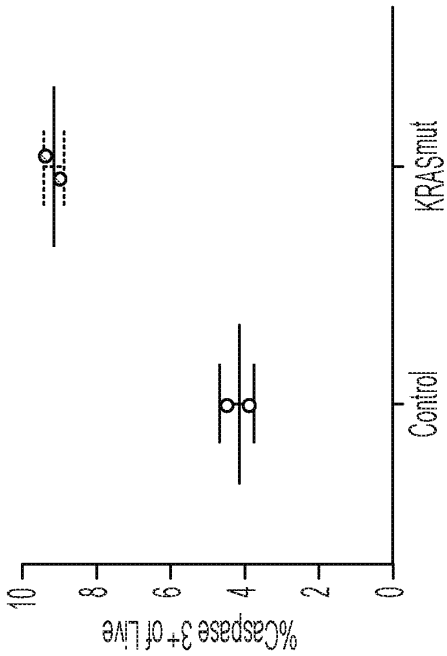
%Caspase 3+ of Live
0
2
4
6
8
10
Control
KRASmut
Target Cells

FIG. 4B

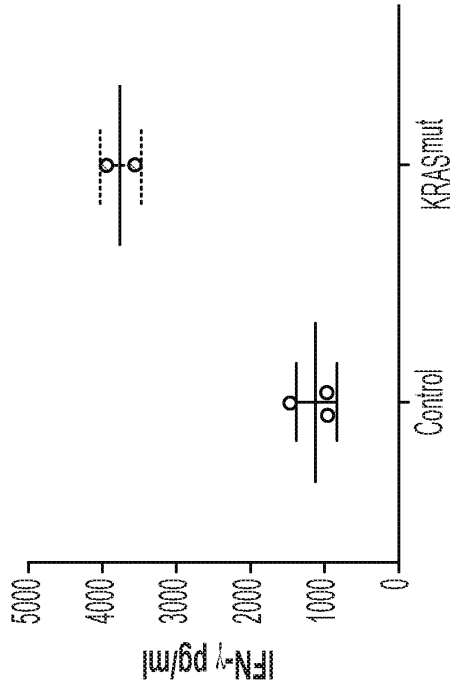
IFN-γ pg/ml
0
1000
2000
3000
4000
5000
Control
KRASmut
Target Cells

FIG. 4A

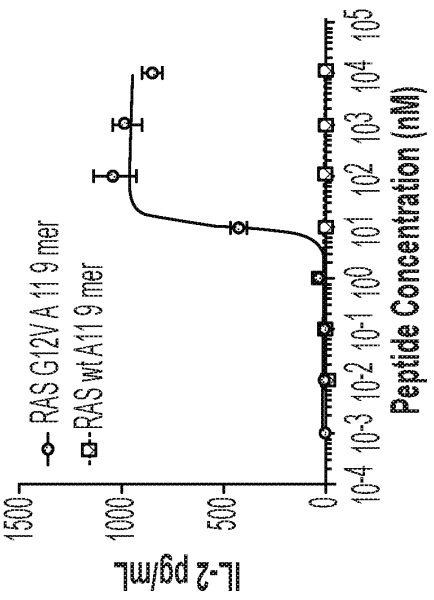

FIG. 5B

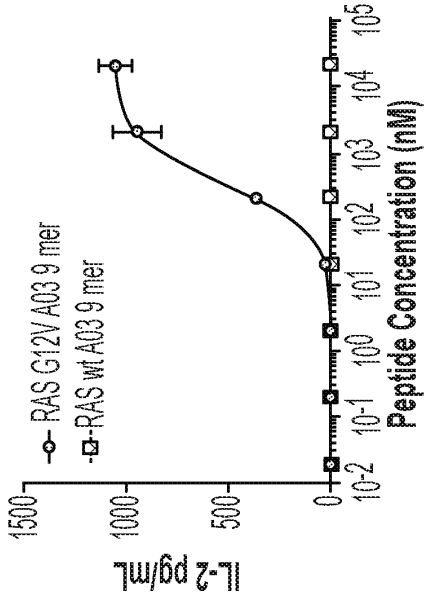

FIG. 5D

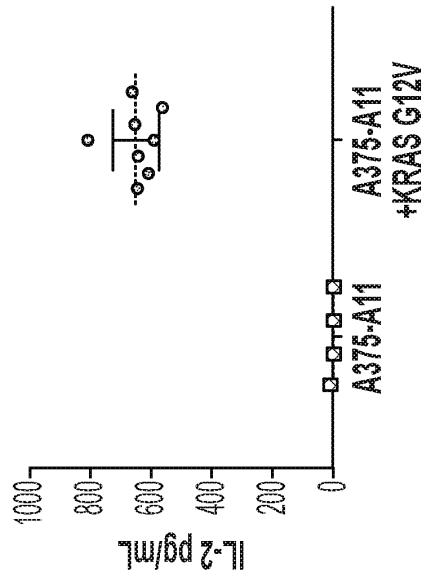

FIG. 5A

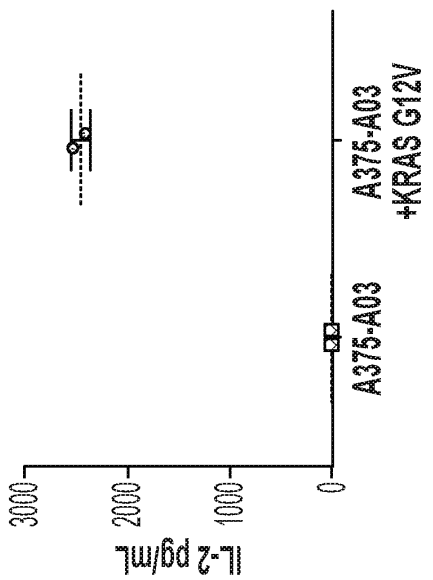

FIG. 5C

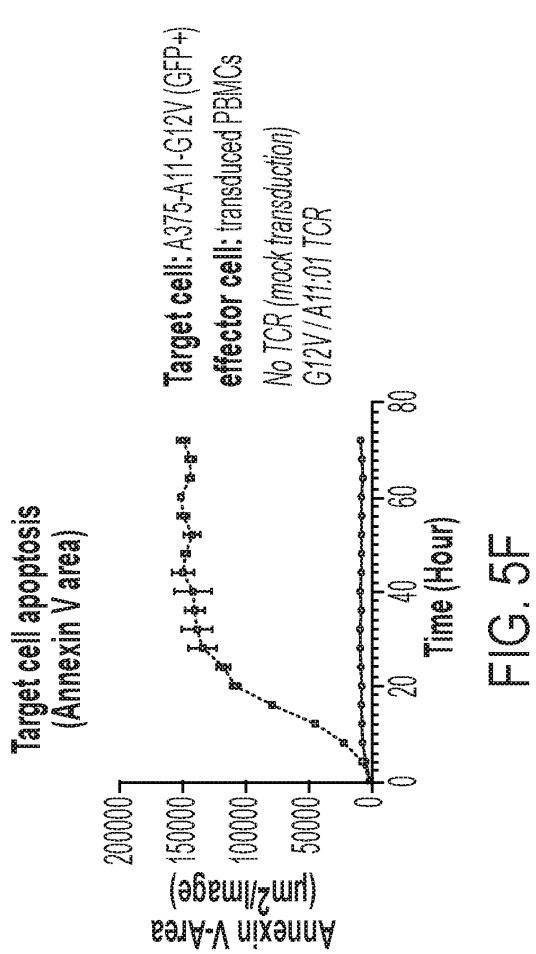

FIG. 5F

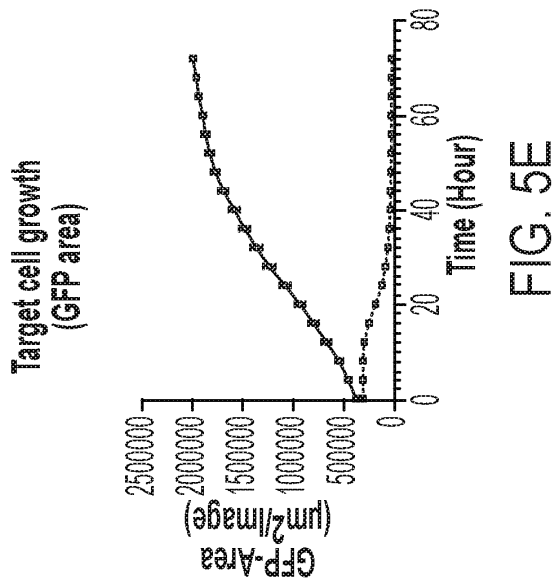

FIG. 5E

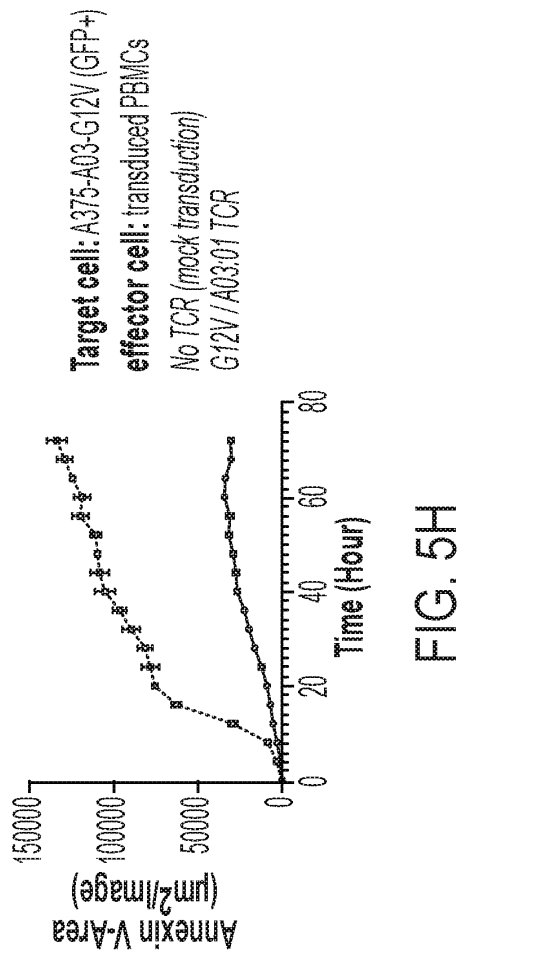

FIG. 5H

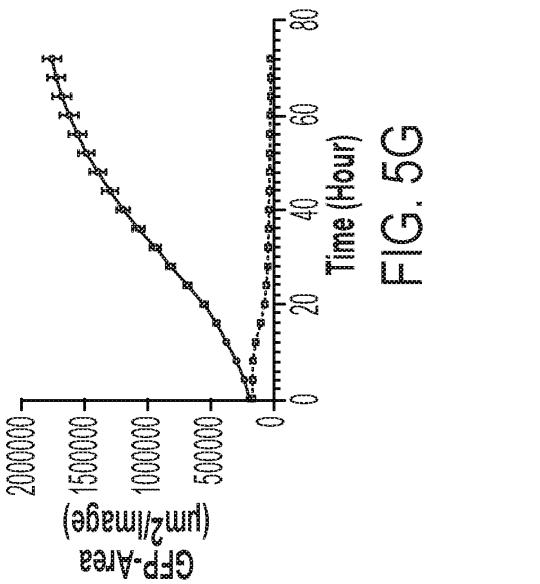

FIG. 5G

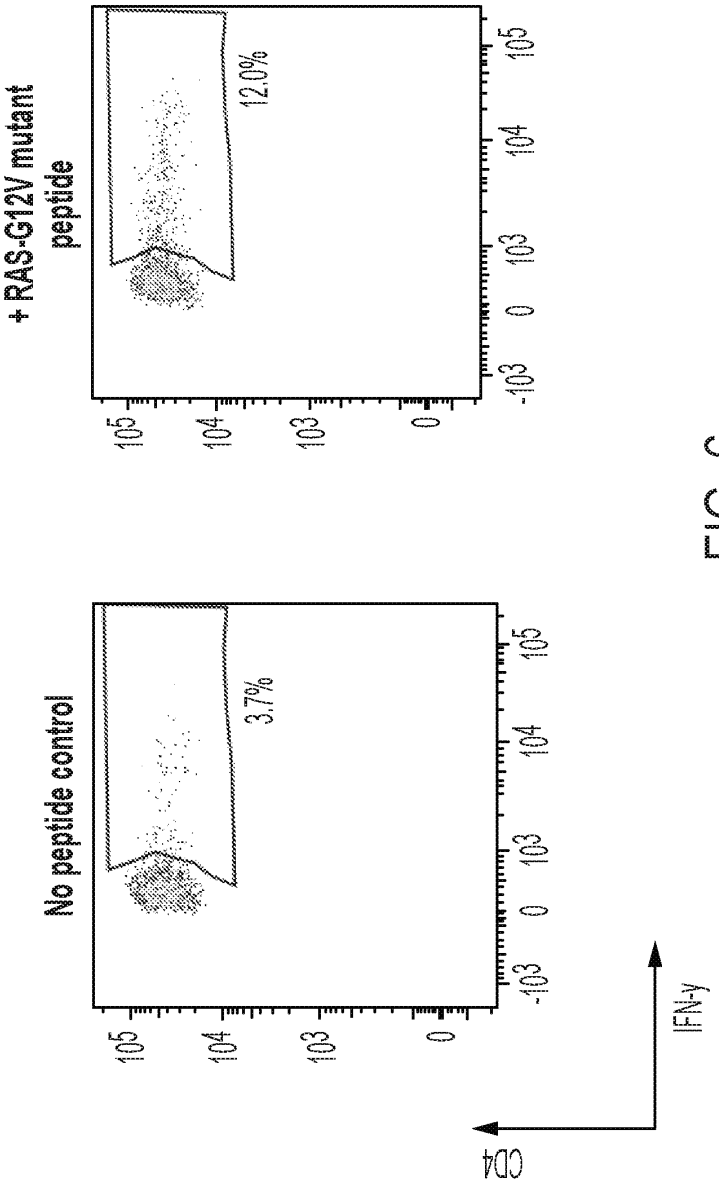
+ RAS-G12V mutant peptide
12.0%
No peptide control
3.7%
CD4
IFN-γ
105
104
103
0
-103

FIG. 6