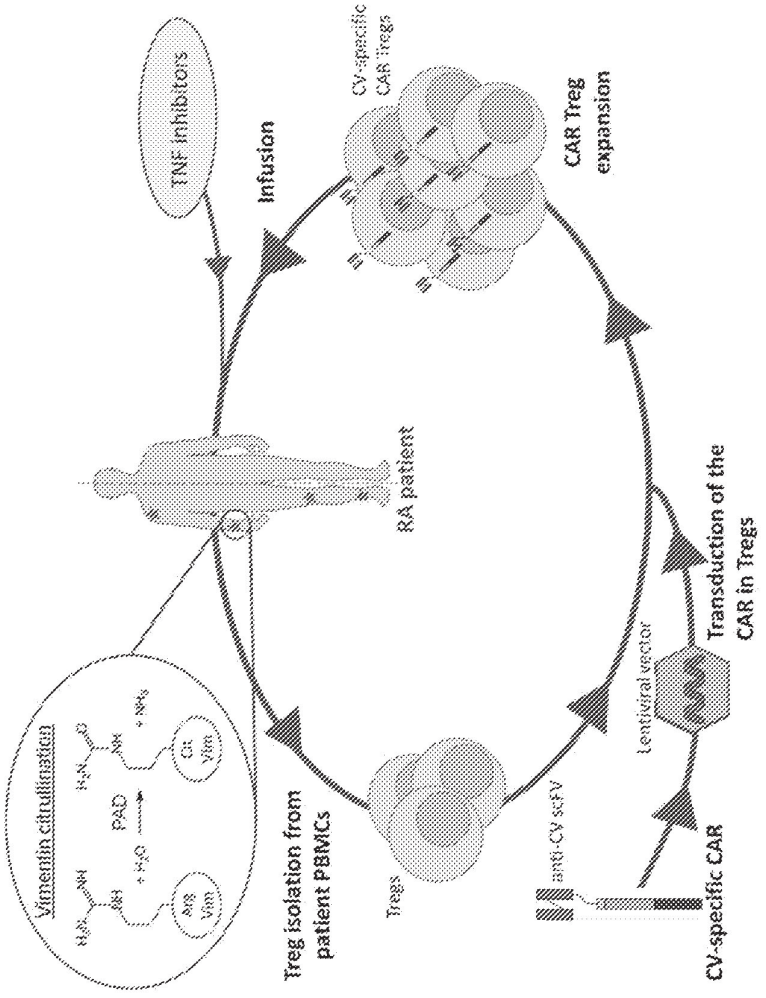

Vimentin citrullination
PAD
Arg Vim
Cit Vim
Treg isolation from patient PBMCs
Tregs
anti-CV scFV
Lentiviral vector
CV-specific CAR
Transduction of the CAR in Tregs
RA patient
CAR Treg expansion
CV-specific CAR Tregs
Infusion
TNF inhibitors

FIG. 1

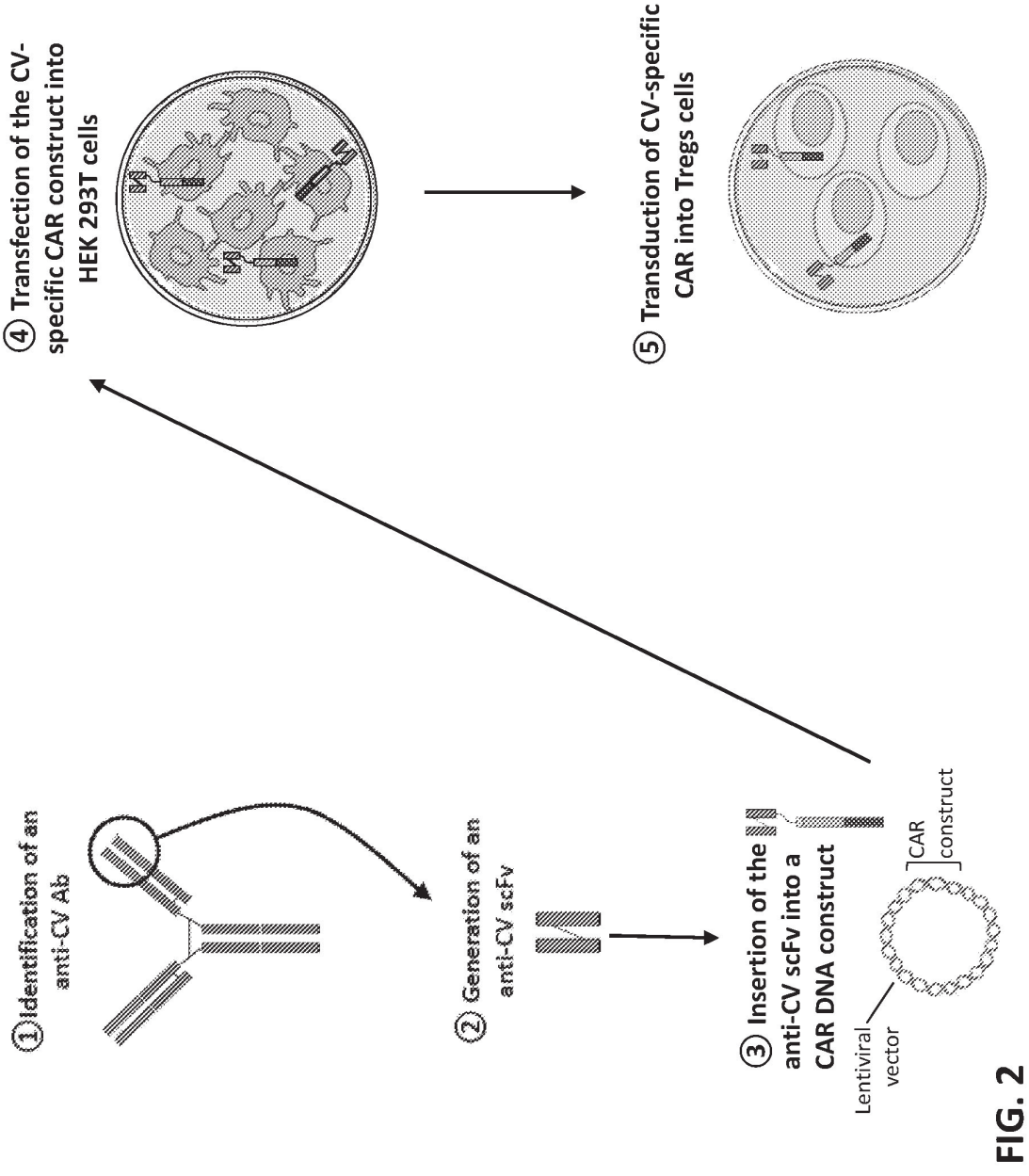

① Identification of an anti-CV Ab
② Generation of an anti-CV scFv
③ Insertion of the anti-CV scFv into a CAR DNA construct
Lentiviral vector
CAR construct
④ Transfection of the CV-specific CAR construct into HEK 293T cells
⑤ Transduction of CV-specific CAR into Tregs cells

FIG. 2

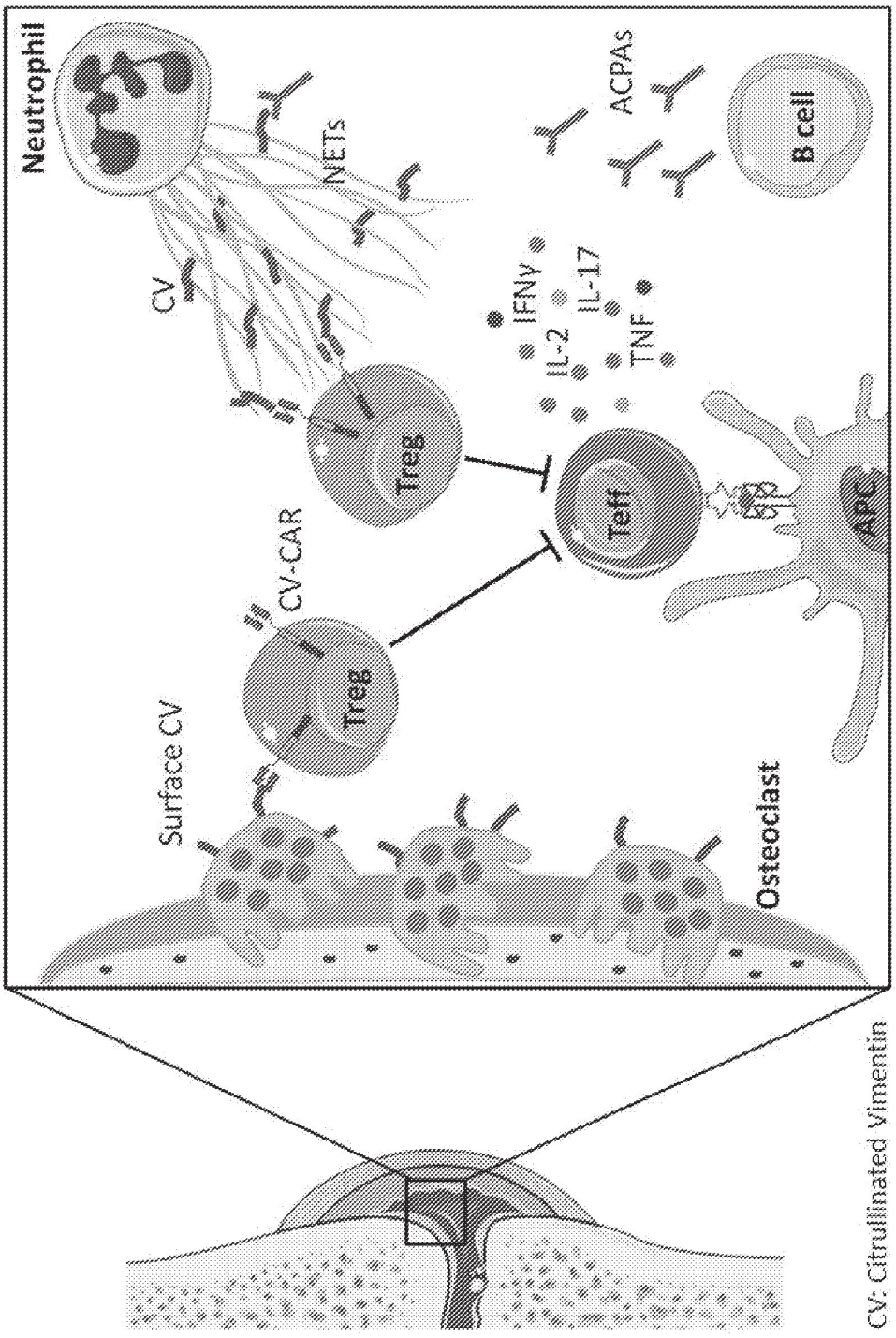
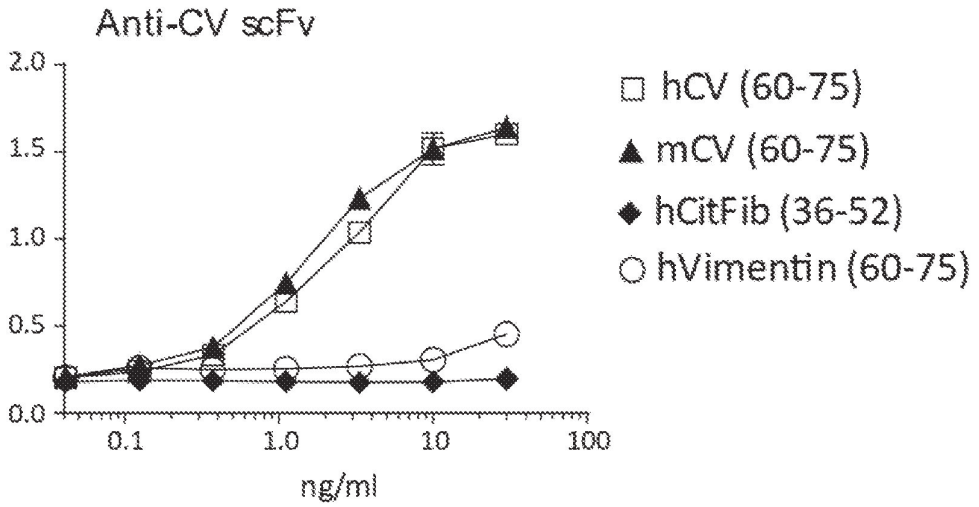
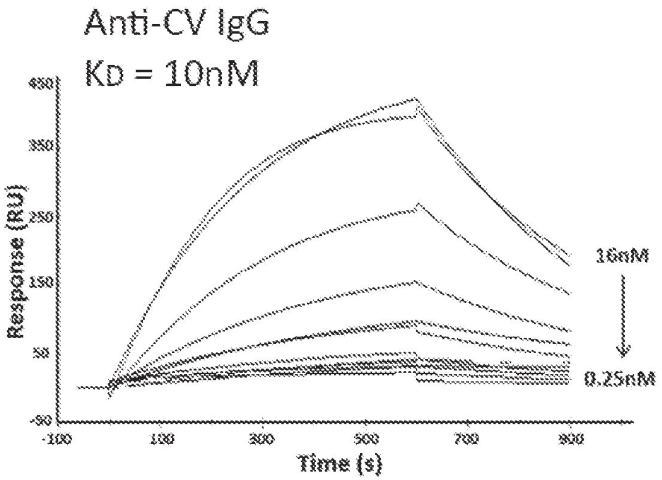

Neutrophil
CV
NETs
IFNγ
IL-2
IL-17
TNF
ACPAs
B cell
Treg
Teff
APC
CV-CAR
Treg
Surface CV
Osteoclast
CV: Citrullinated Vimentin

FIG. 3

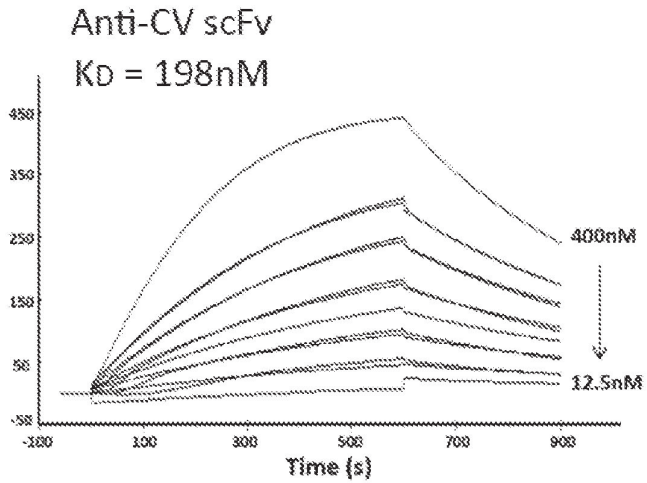
FIG. 4





ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	tc	Chi² (RU²)	U-value
5.145E+5	0.005603	1.089E-8	708.4	5.722E+7	105	1

FIG. 5A.



ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	tc	Chi² (RU²)	U-value
1.900E+4	0.003768	1.983E-7	671.9	2.340E+6	47.3	1

FIG. 5B

Human vimentin peptide 60-75	VYATRSSAVRLRSSVP
Murine vimentin peptide 60-75	AYVTRSSAVRLRSSVP

FIG. 6

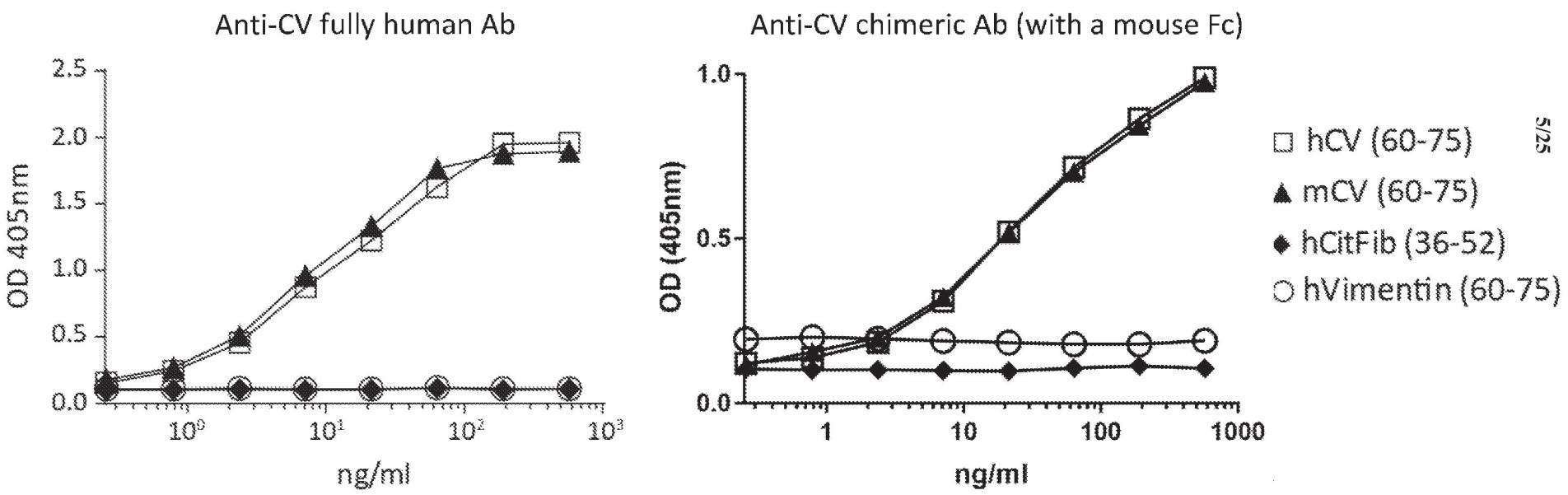


FIG. 7

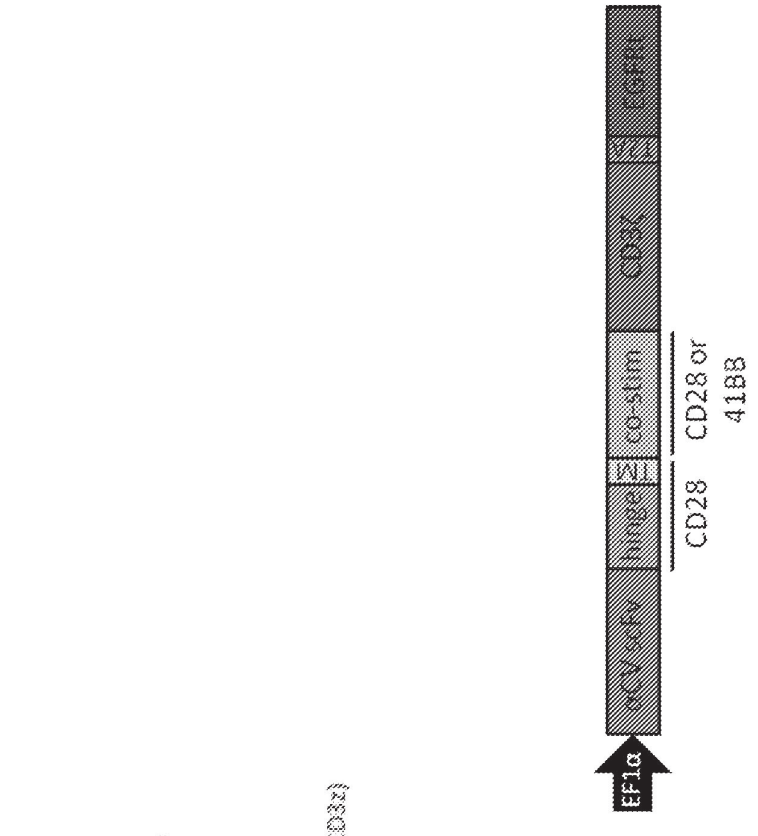

Anti-CV scFv
Hinge
Transmembrane domain (human CD28)
Costimulatory domain
Intracellular signaling domain (human CD3z)

FIG. 8A

EF1α
anti-CV scFv
hinge
co-stim
CD3z
EGFRt
CD28
CD28 or 4188

FIG. 8B

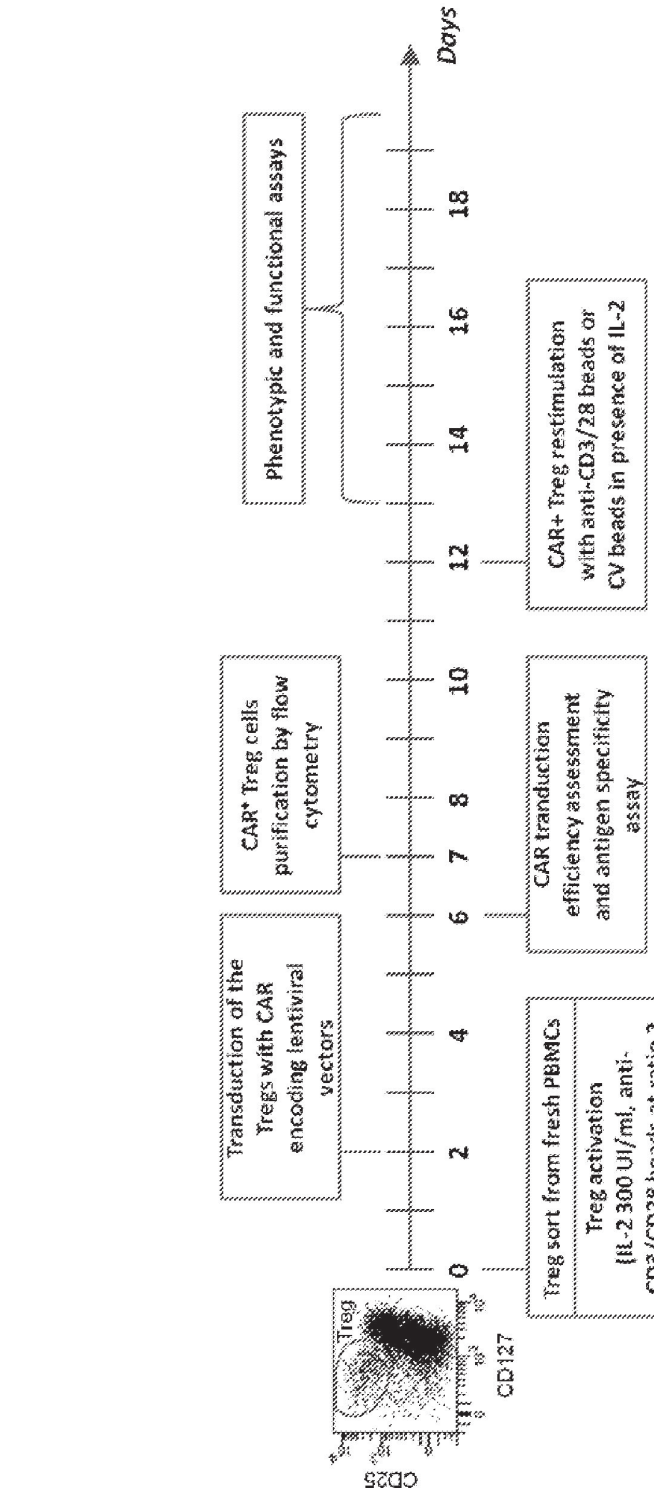
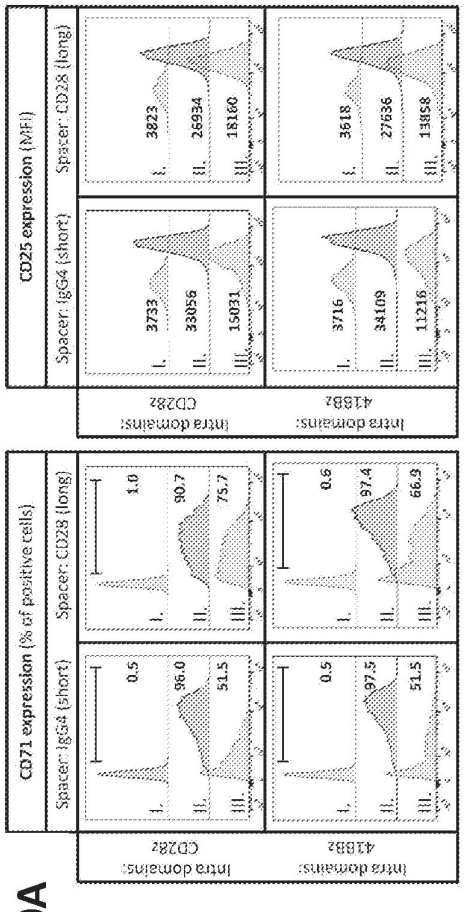

Days
0
2
4
6
7
8
10
12
14
16
18
Treg sort from fresh PBMCs
Treg activation
(IL-2 300 UI/ml, anti-CD3/CD28 beads at ratio 2 beads per cell)
Transduction of the Tregs with CAR encoding lentiviral vectors
CAR+ Treg cells purification by flow cytometry
CAR tranduction efficiency assessment and antigen specificity assay
CAR+ Treg restimulation with anti-CD3/28 beads or CV beads in presence of IL-2
Phenotypic and functional assays
Treg
CD25
CD127

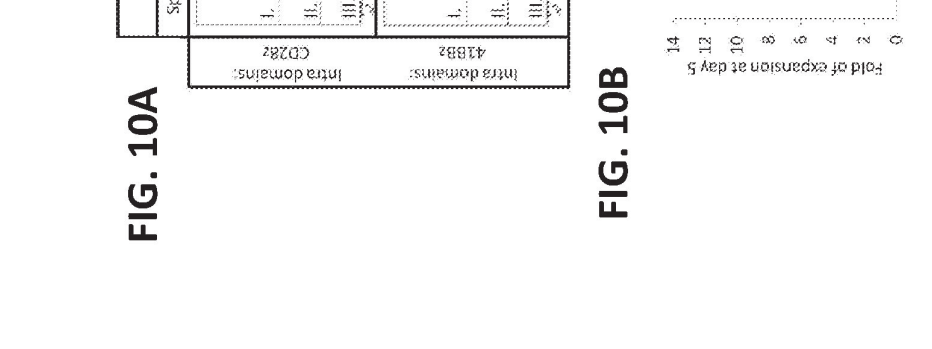
FIG. 9

FIG. 10A



I. IL-2 only II. anti-CD3/CD28 beads III. CV-SA beads

FIG. 10B



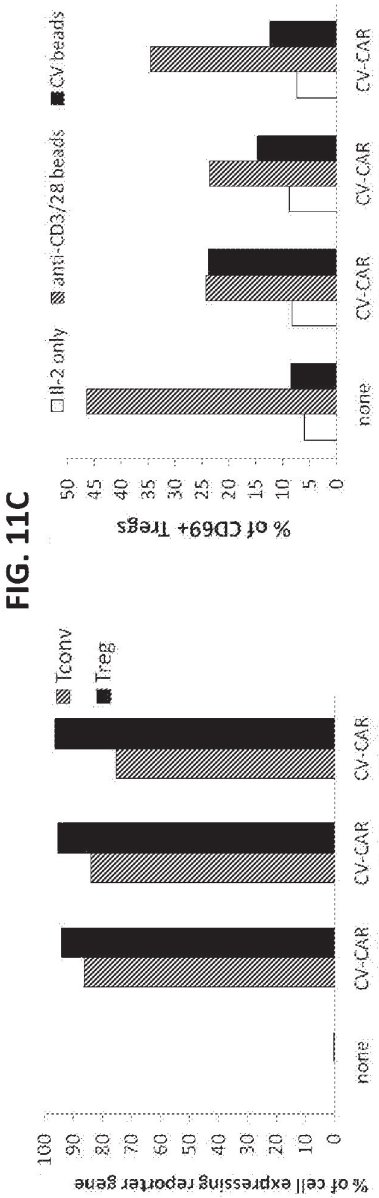


FIG. 11A

FIG. 11B

FIG. 11C

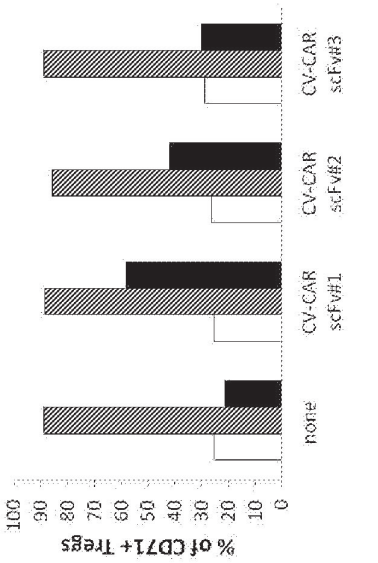


FIG. 11D

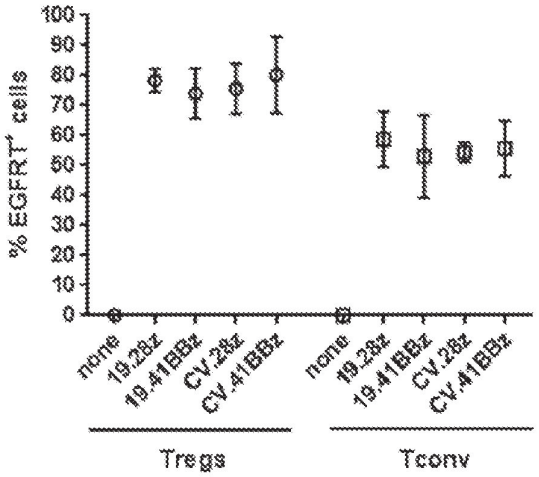


FIG. 12A

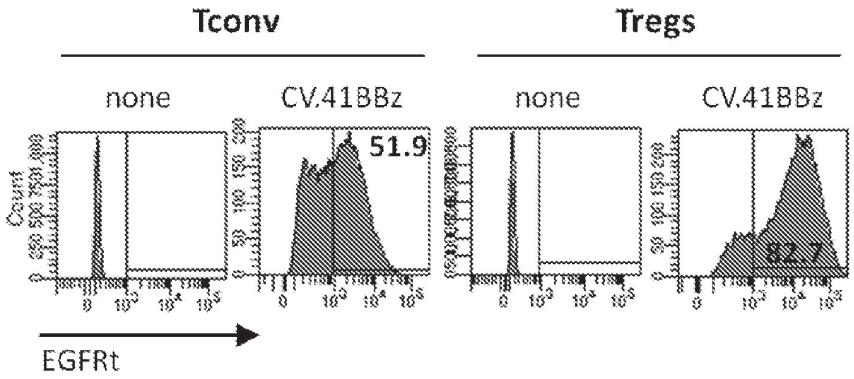


FIG. 12B

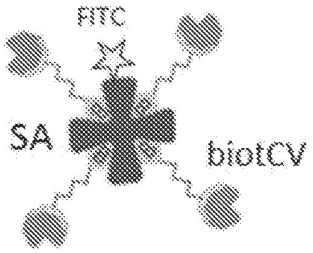


FIG. 12C

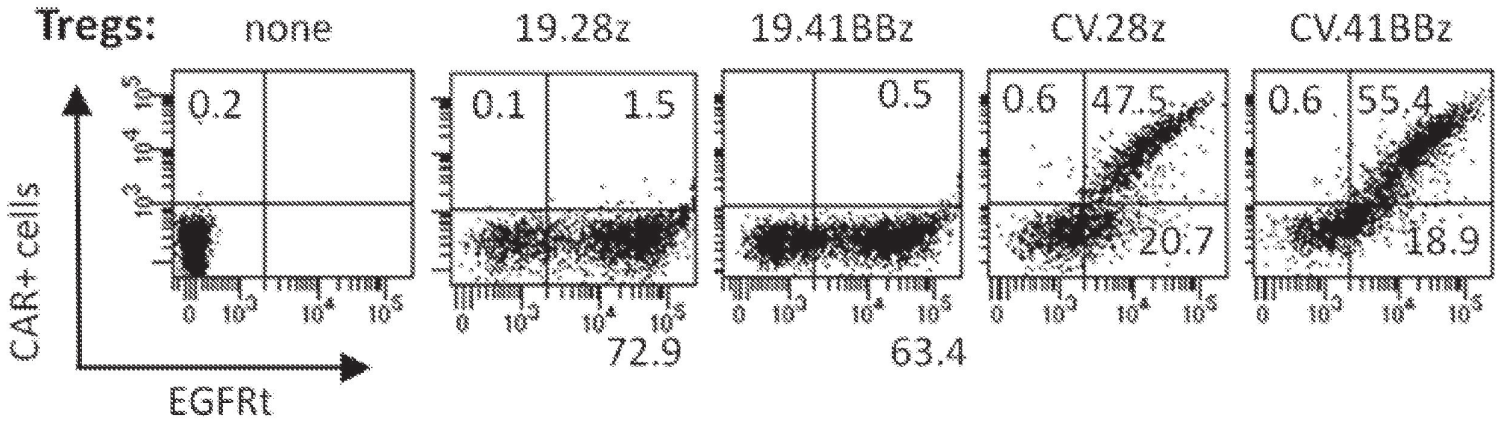


FIG. 12D

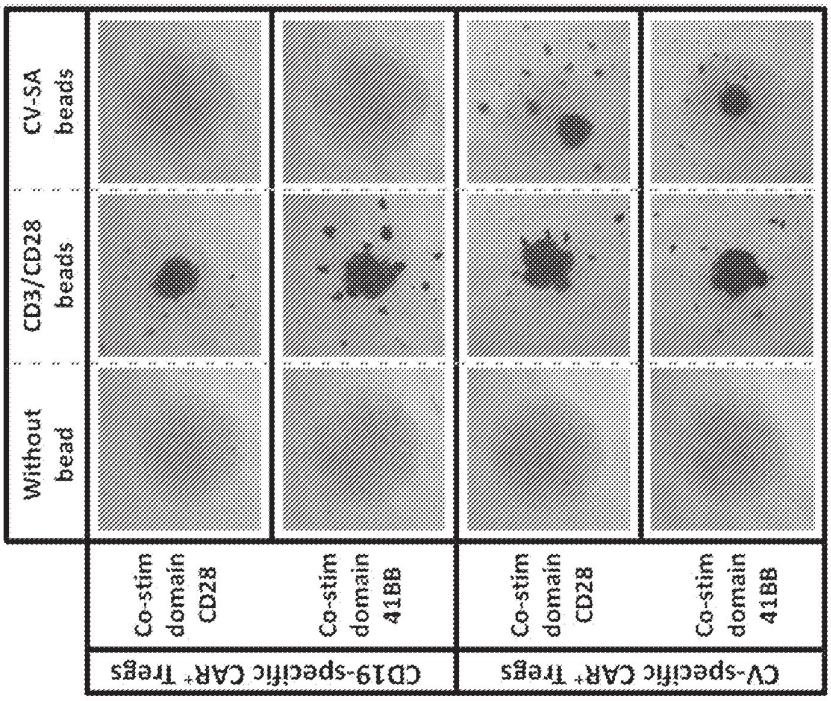


FIG. 14A

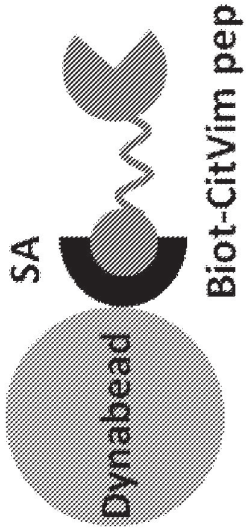


FIG. 13

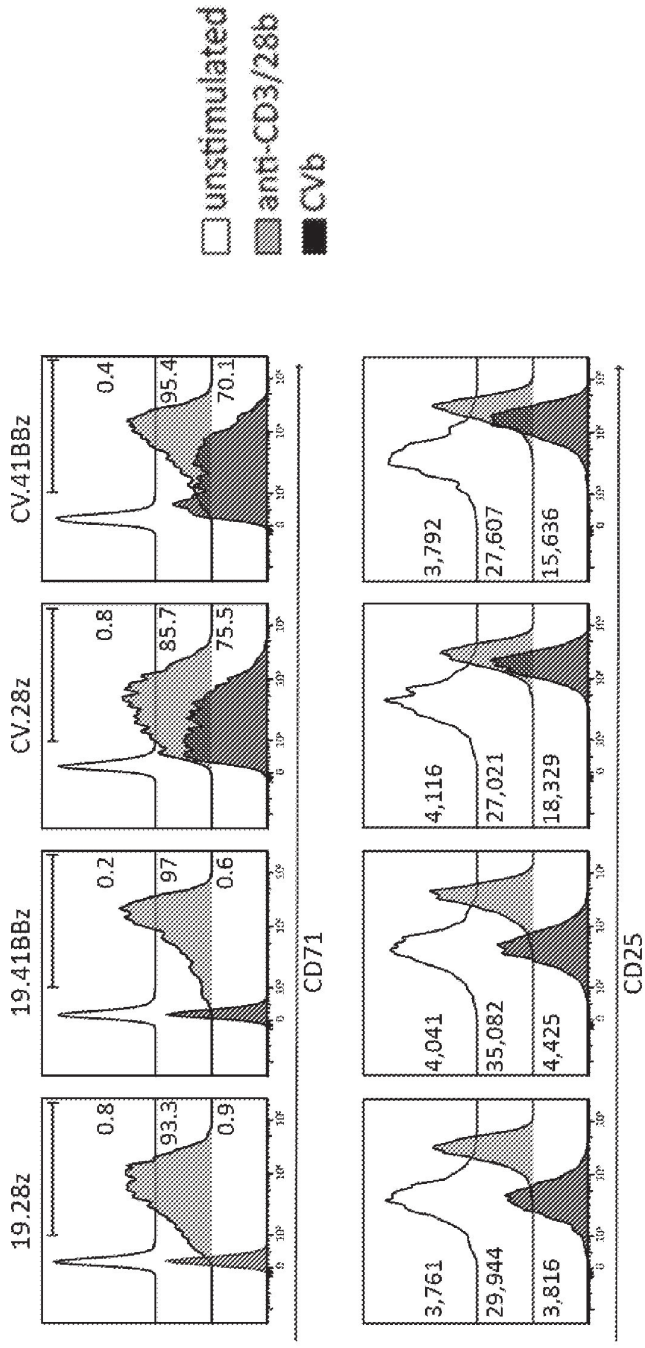


FIG. 14B

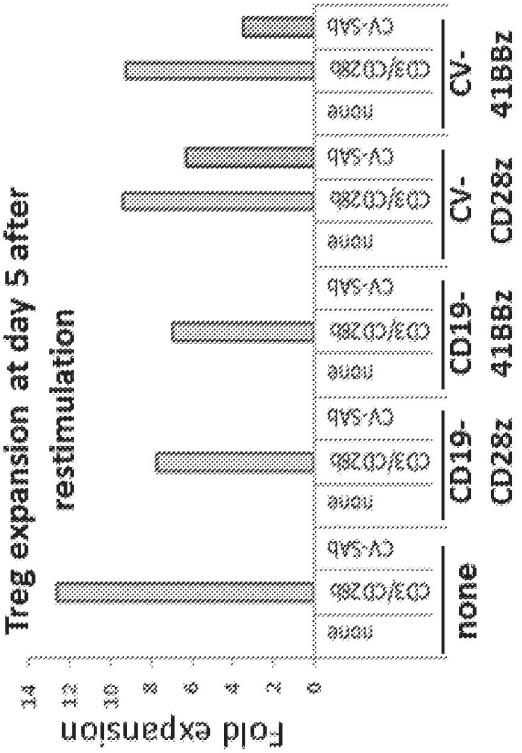


FIG. 14C

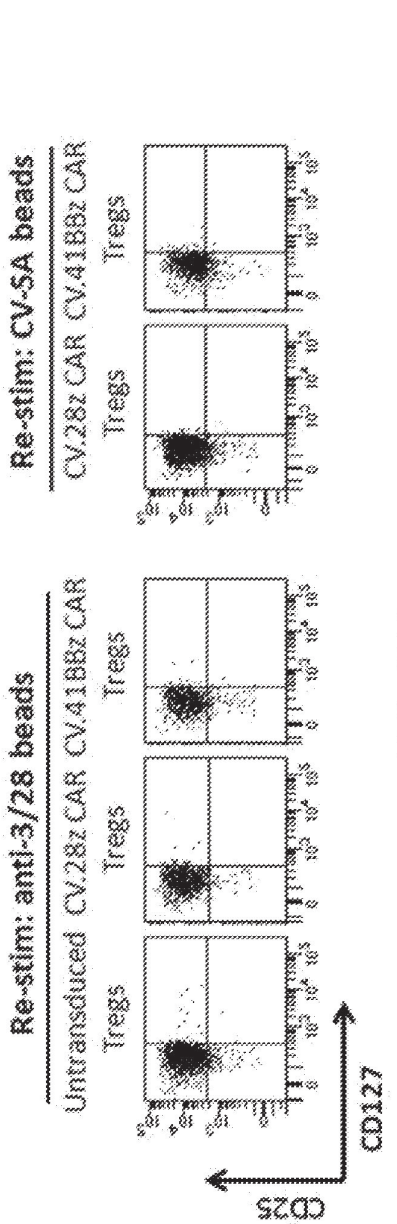


FIG. 15A

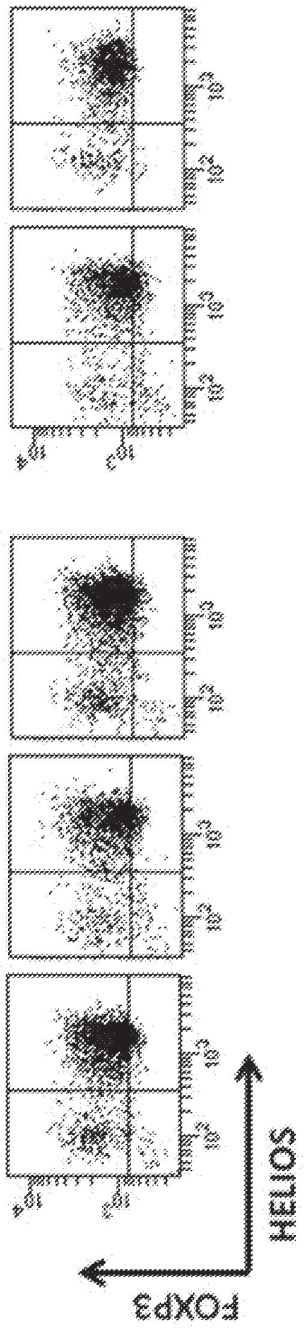


FIG. 15B

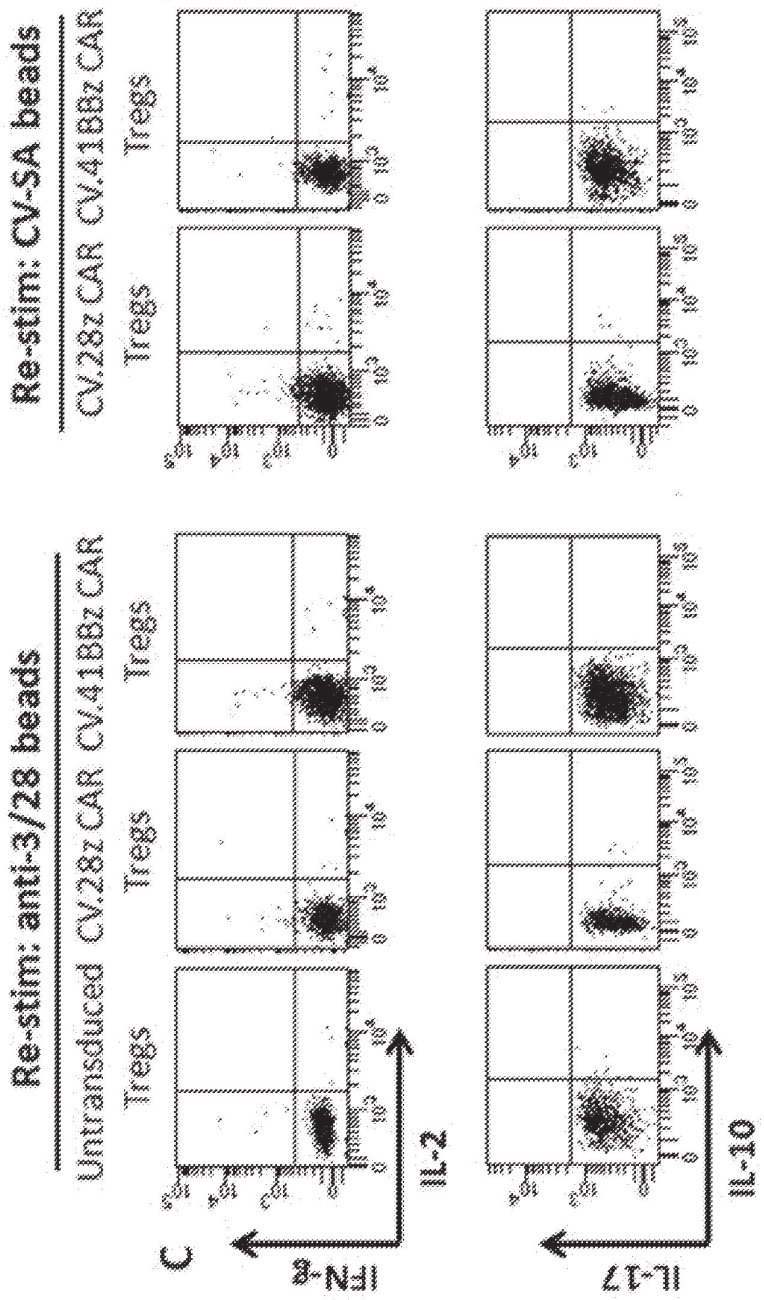

Re-stim: anti-3/28 beads
Re-stim: CV-SA beads
Untransduced
CV.28z CAR
CV.41BBz CAR
Tregs
Tregs
Tregs
Tregs
Tregs
C
IFN-ε
IL-2
IL-17
IL-10

FIG. 15C

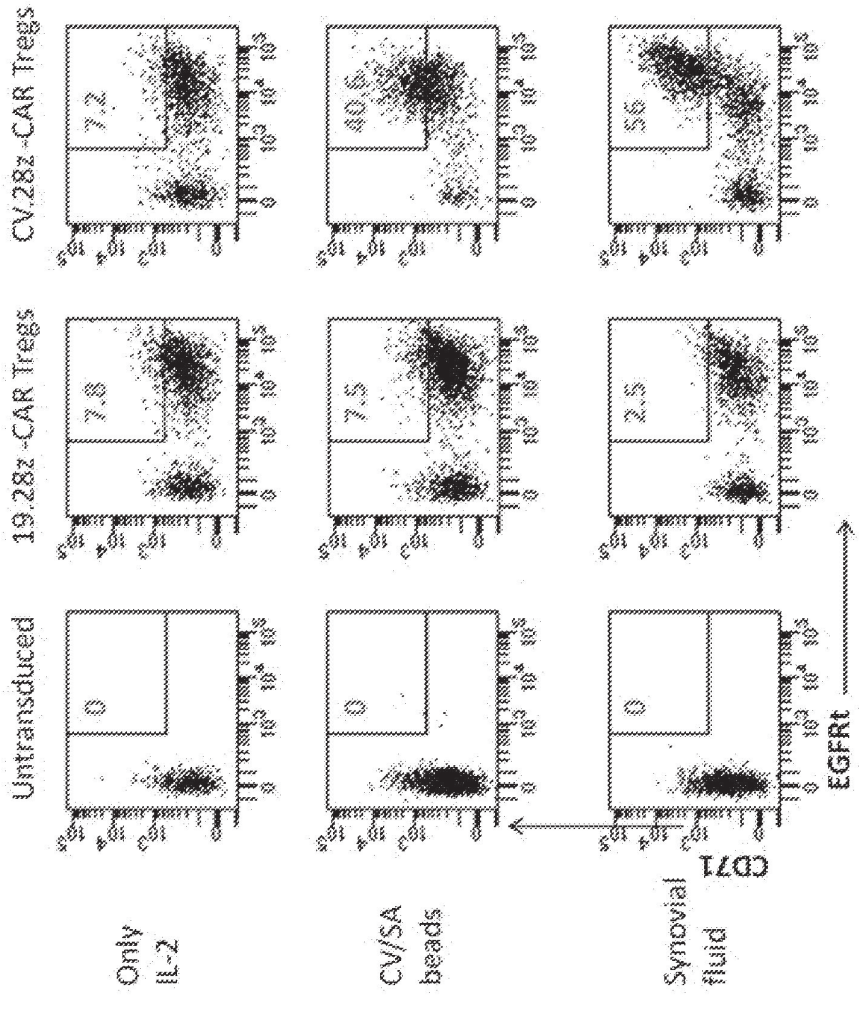

Untransduced
19.28z -CAR Tregs
CV.28z -CAR Tregs
Only IL-2
CV/SA beads
Synovial fluid
CD71
EGFRt
0
7.8
7.2
7.5
40.6
2.5
5.6

FIG. 16A

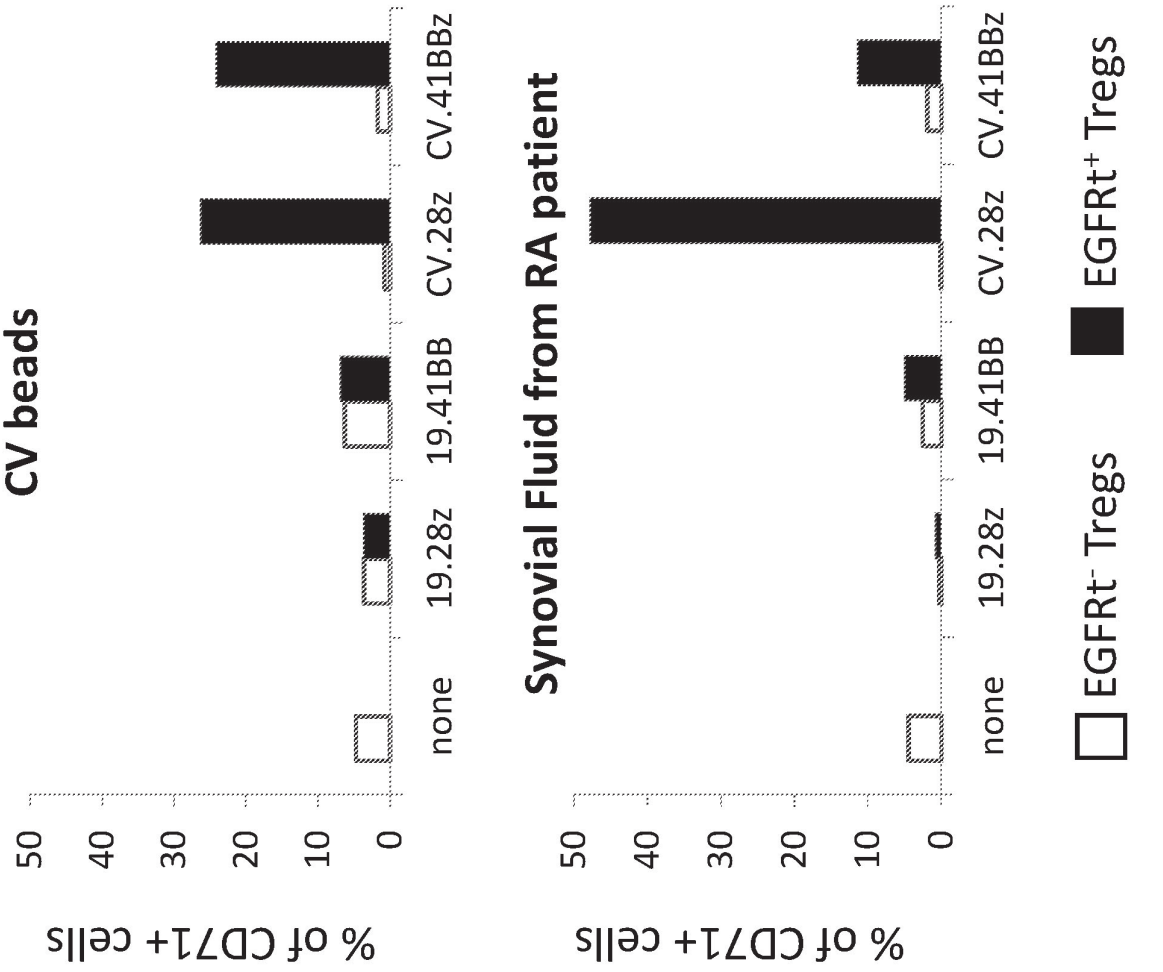

CV beads
% of CD71+ cells
50
40
30
20
10
0
none
19.28z
19.41BB
CV.28z
CV.41BBz
Synovial Fluid from RA patient
% of CD71+ cells
50
40
30
20
10
0
none
19.28z
19.41BB
CV.28z
CV.41BBz
EGFRt- Tregs
EGFRt+ Tregs

FIG. 16B

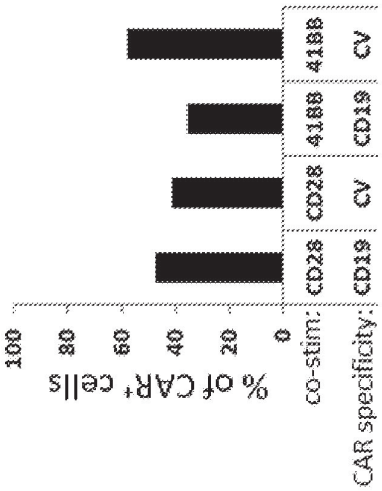


FIG. 17B

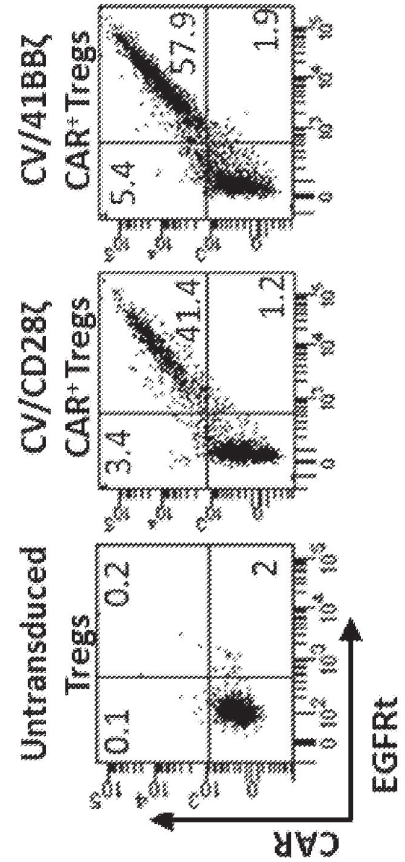


FIG. 17A

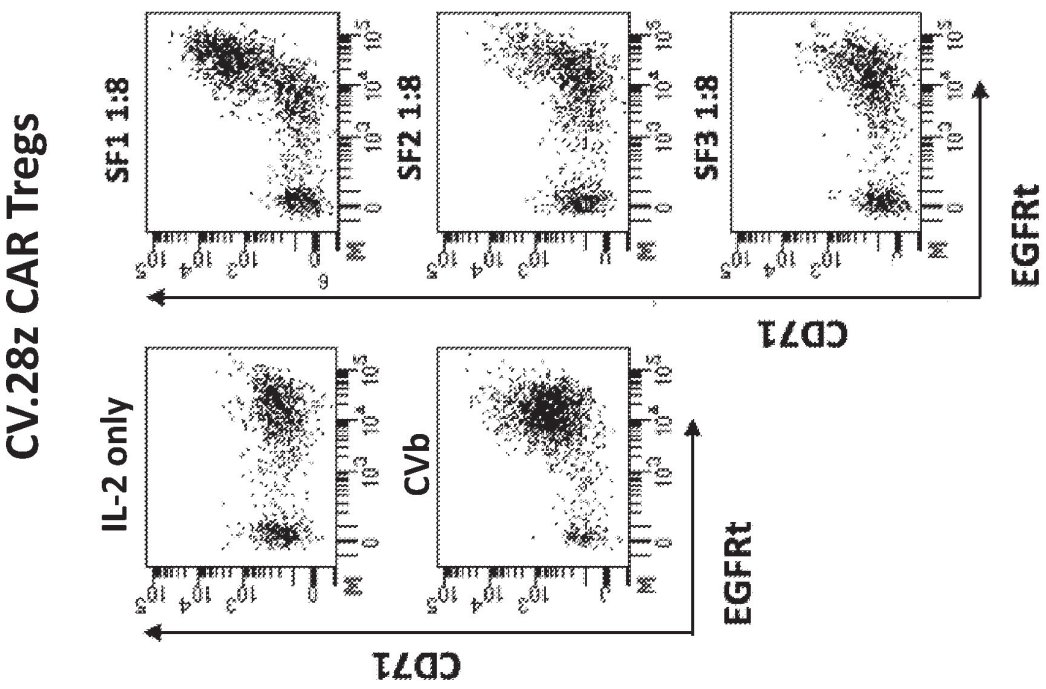


FIG. 18A

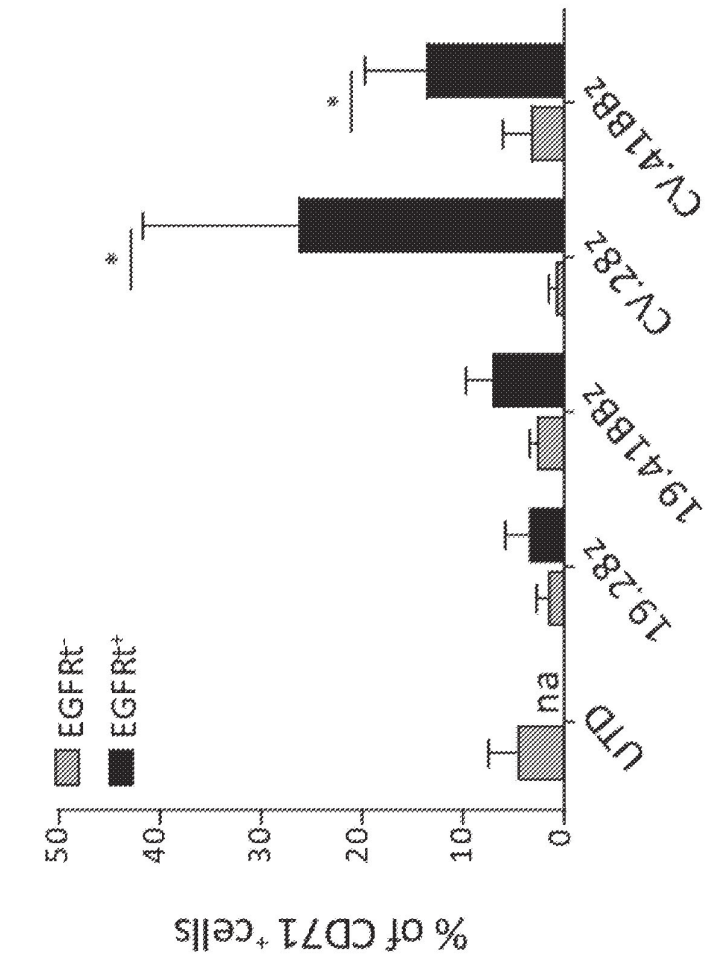


FIG. 18D

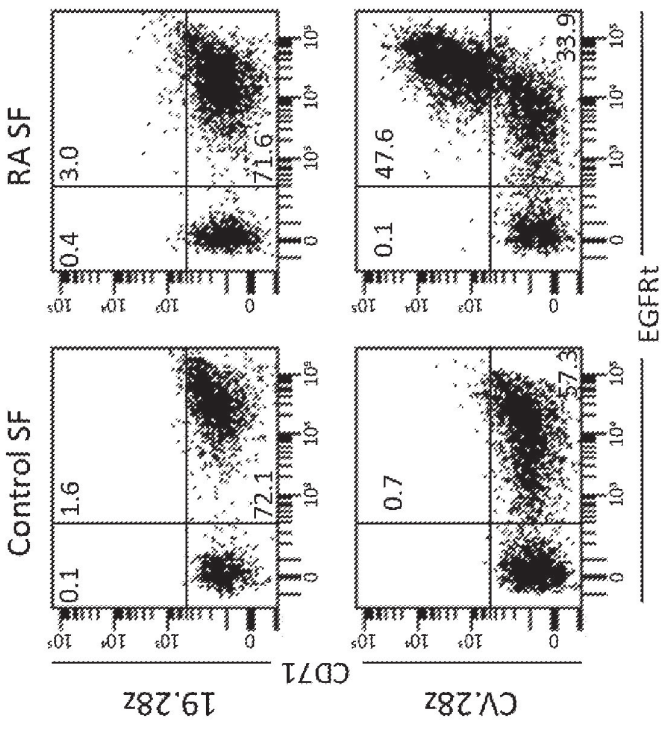


FIG. 18C

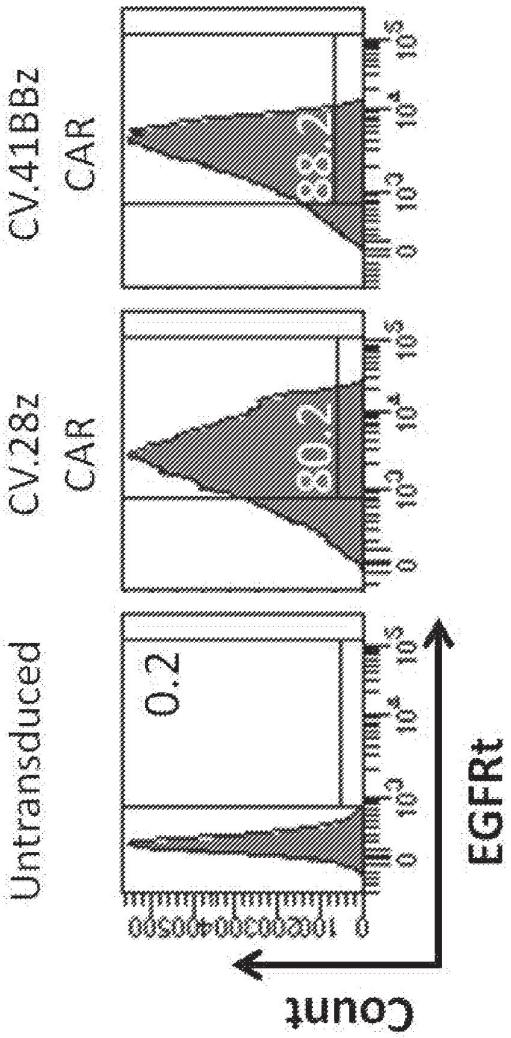


FIG. 19

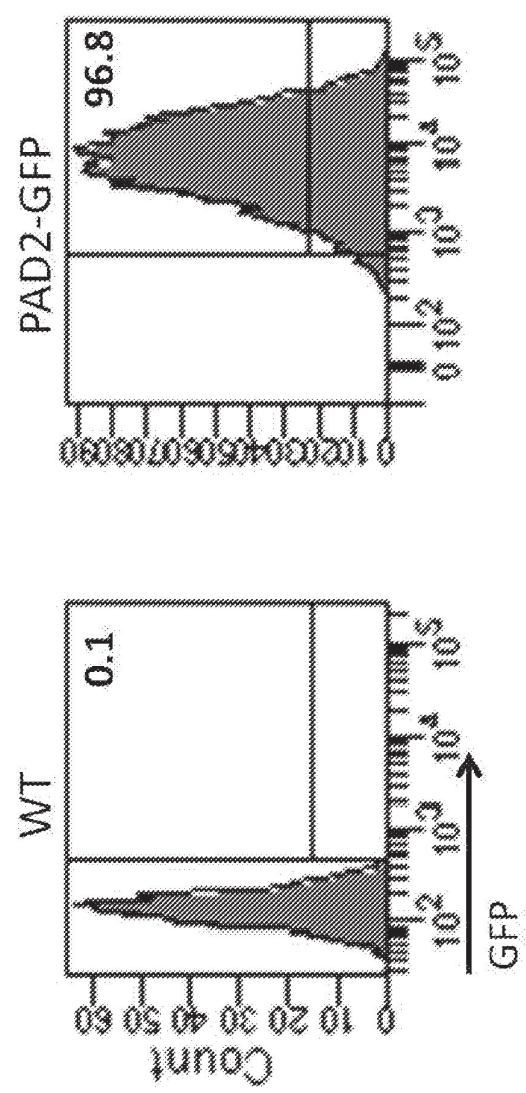


FIG. 20A

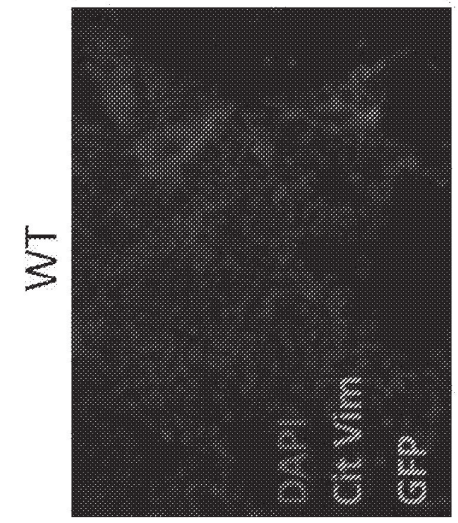


FIG. 20B

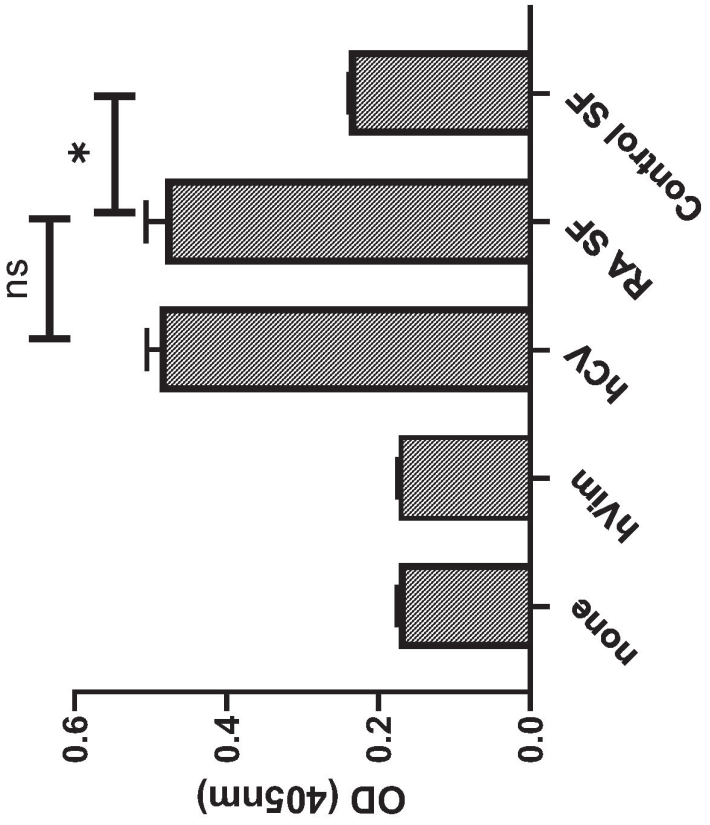


FIG. 21

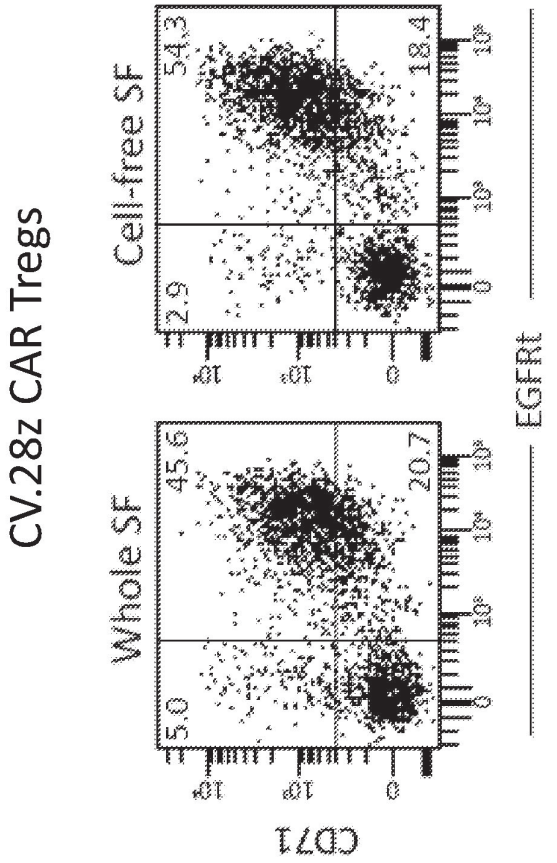


FIG. 22A

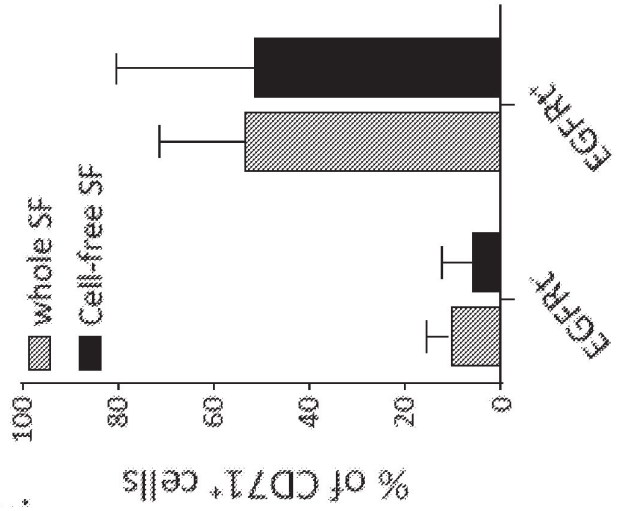


FIG. 22B

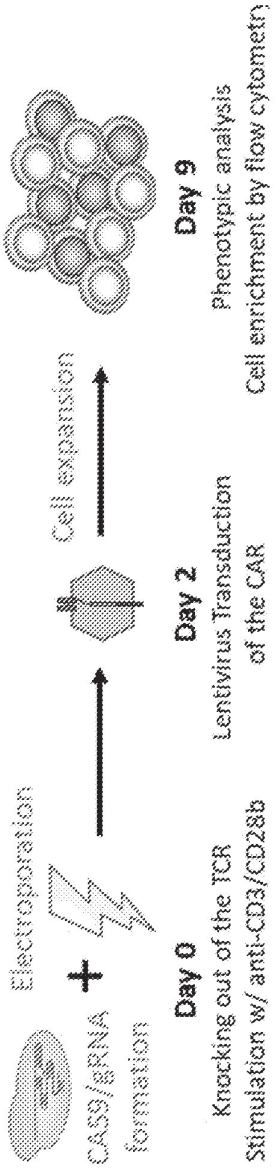


FIG. 23A

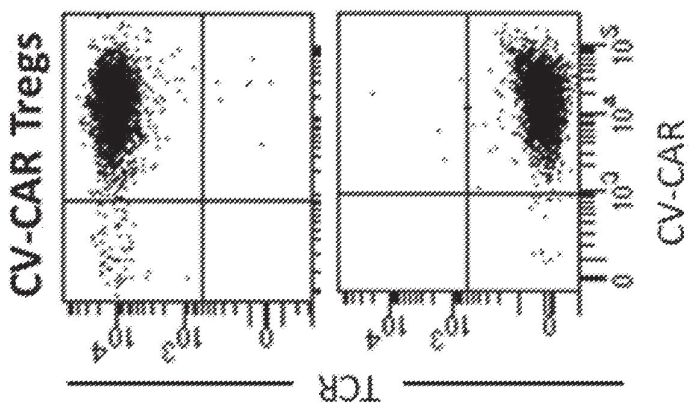


FIG. 23B

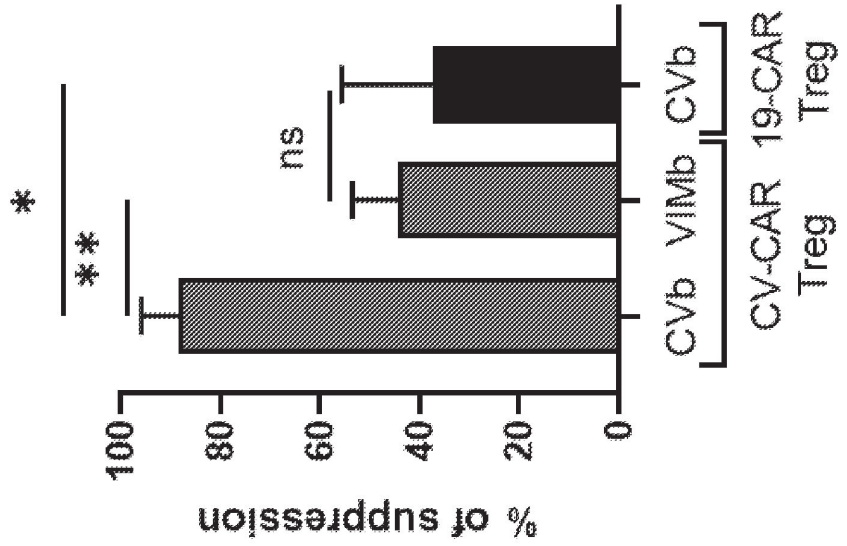


FIG. 24B

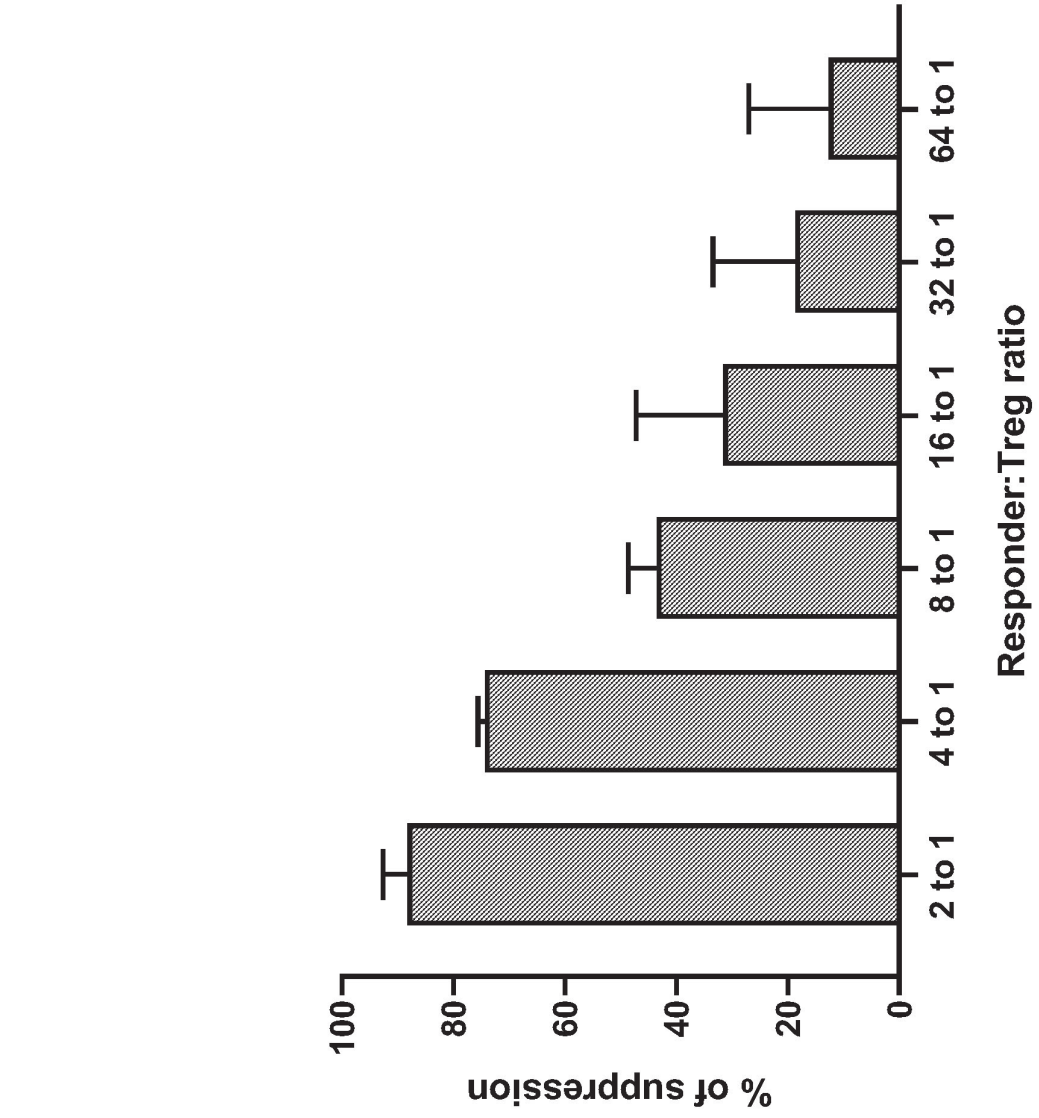


FIG. 24A