FIG. 1A

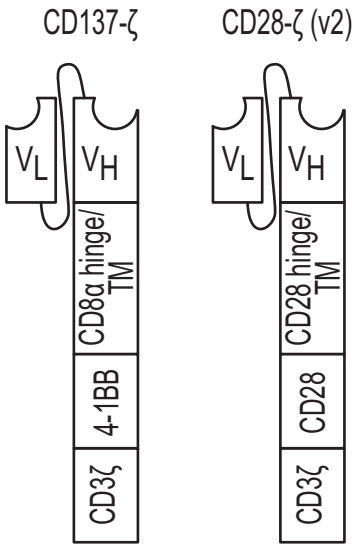
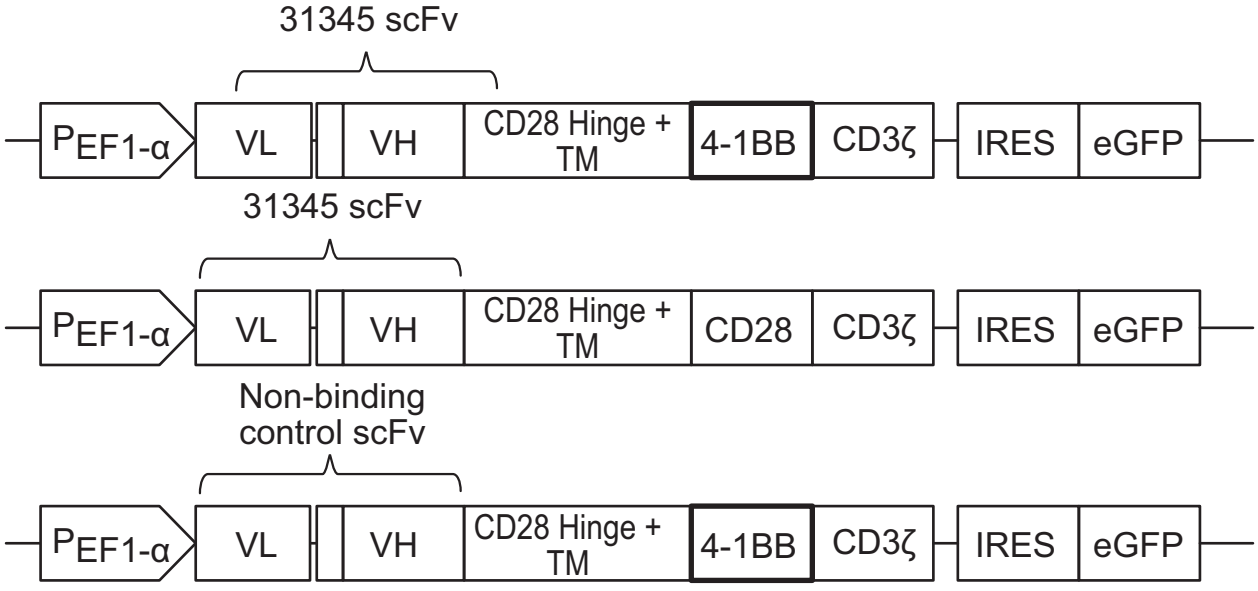


FIG. 1B



CAR construct designs

FIG. 2A

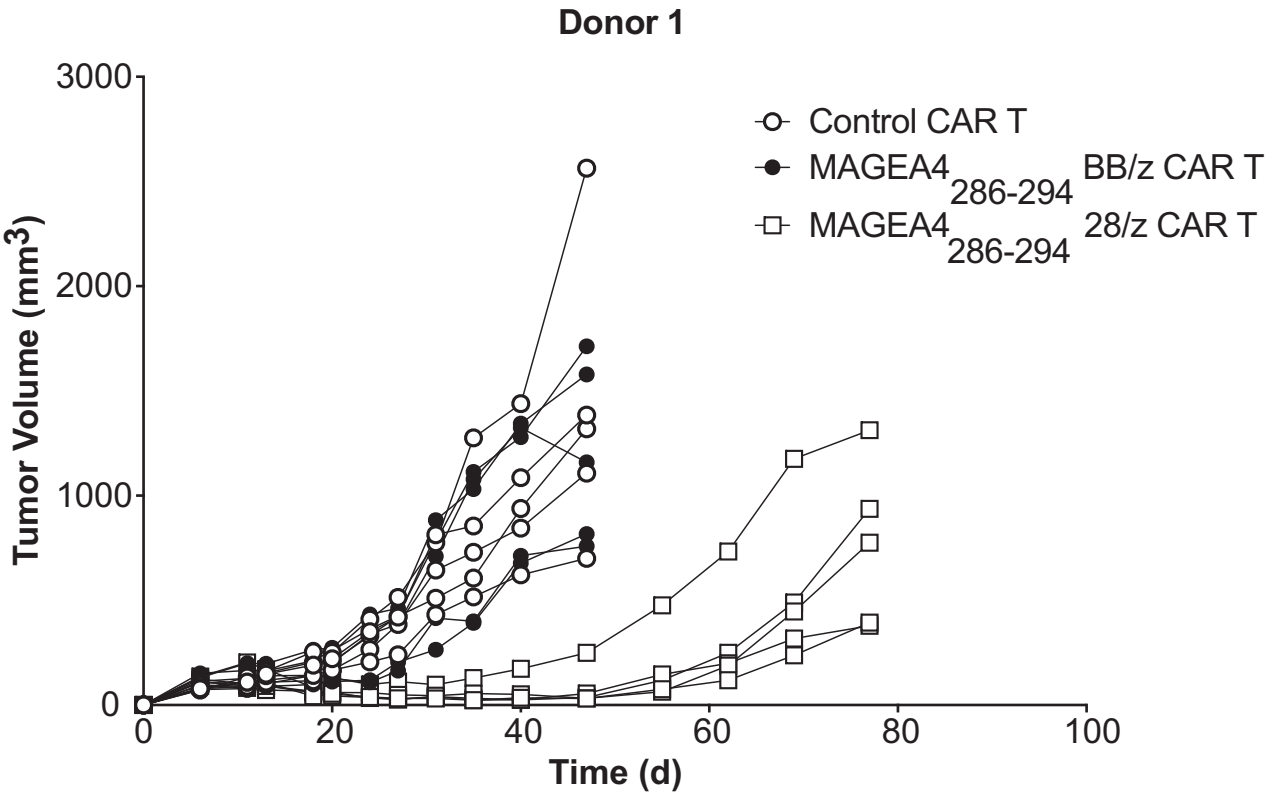


FIG. 2A Cont.

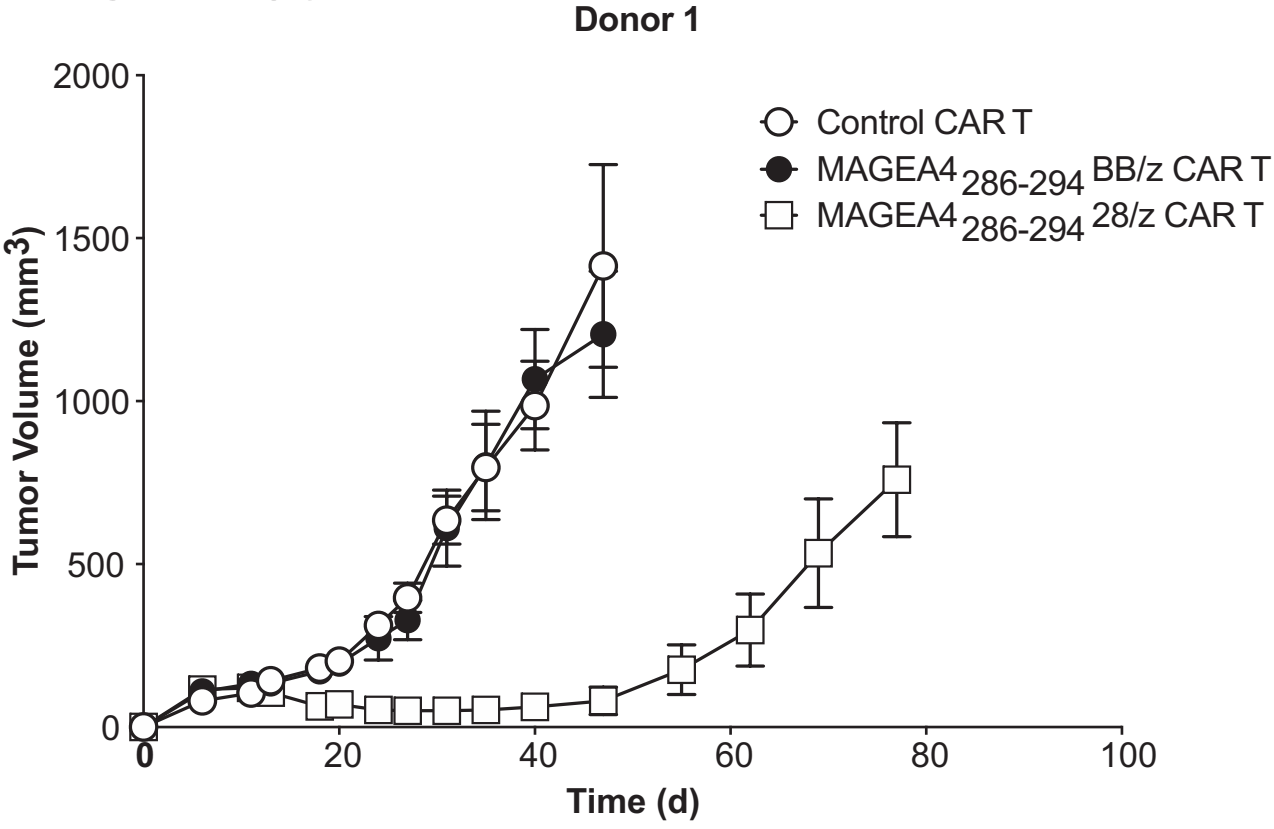


FIG. 2B

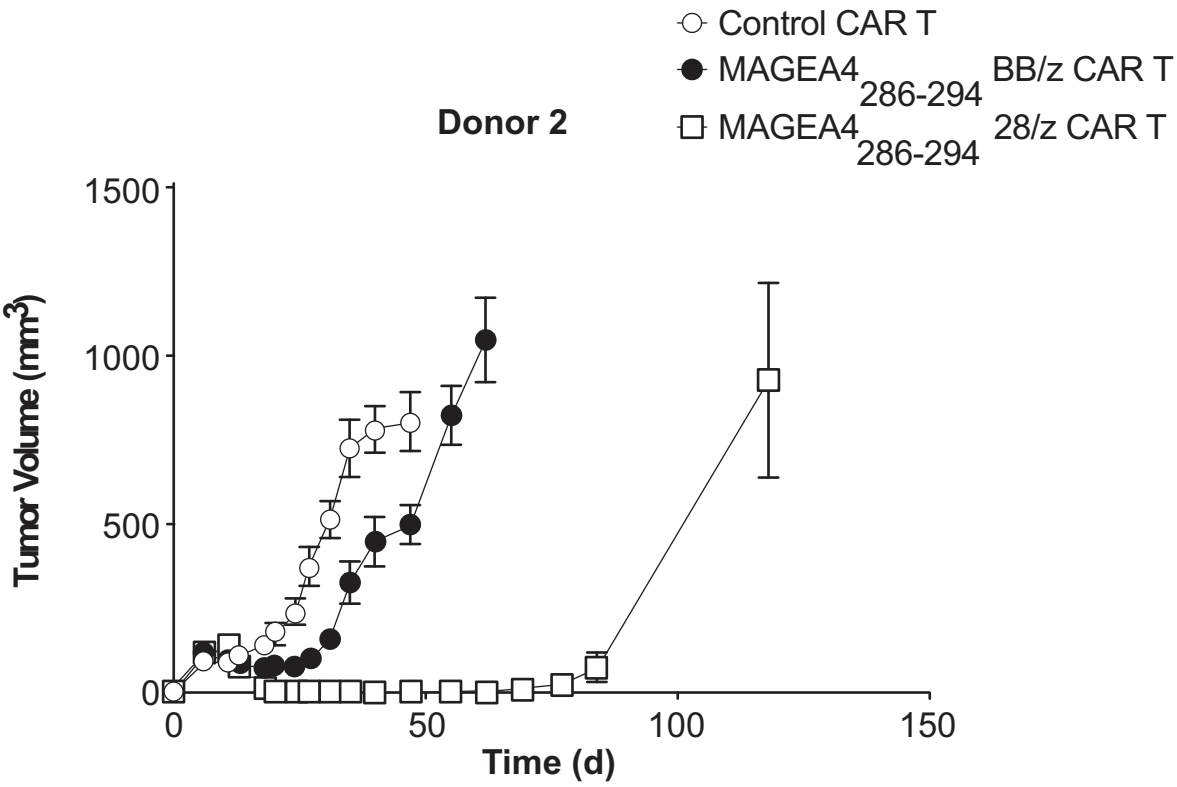


FIG.2B Cont.

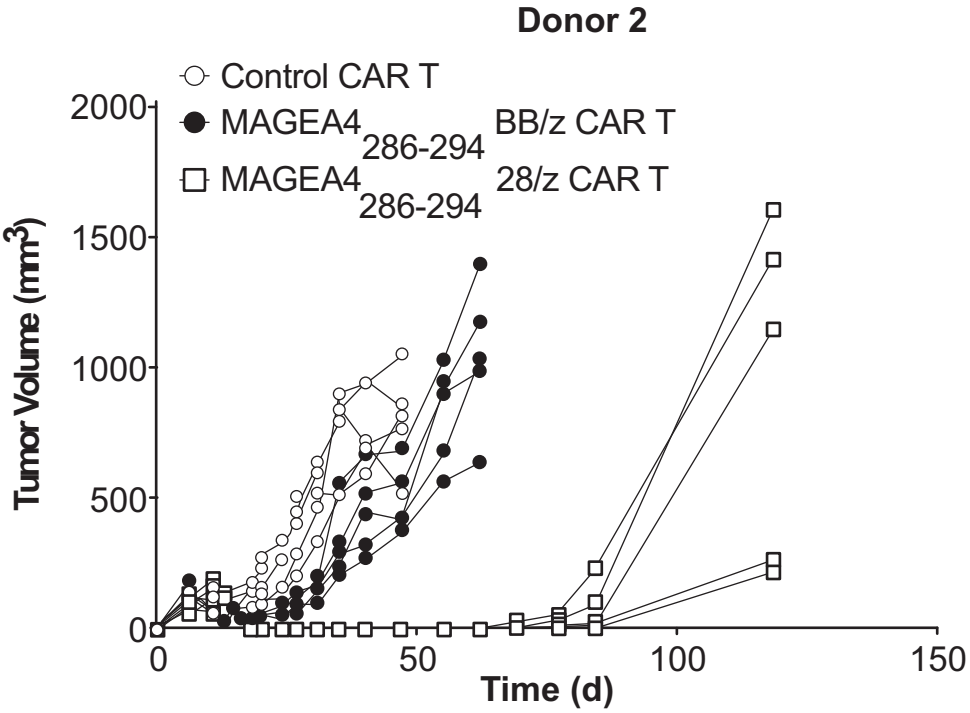


FIG. 3A

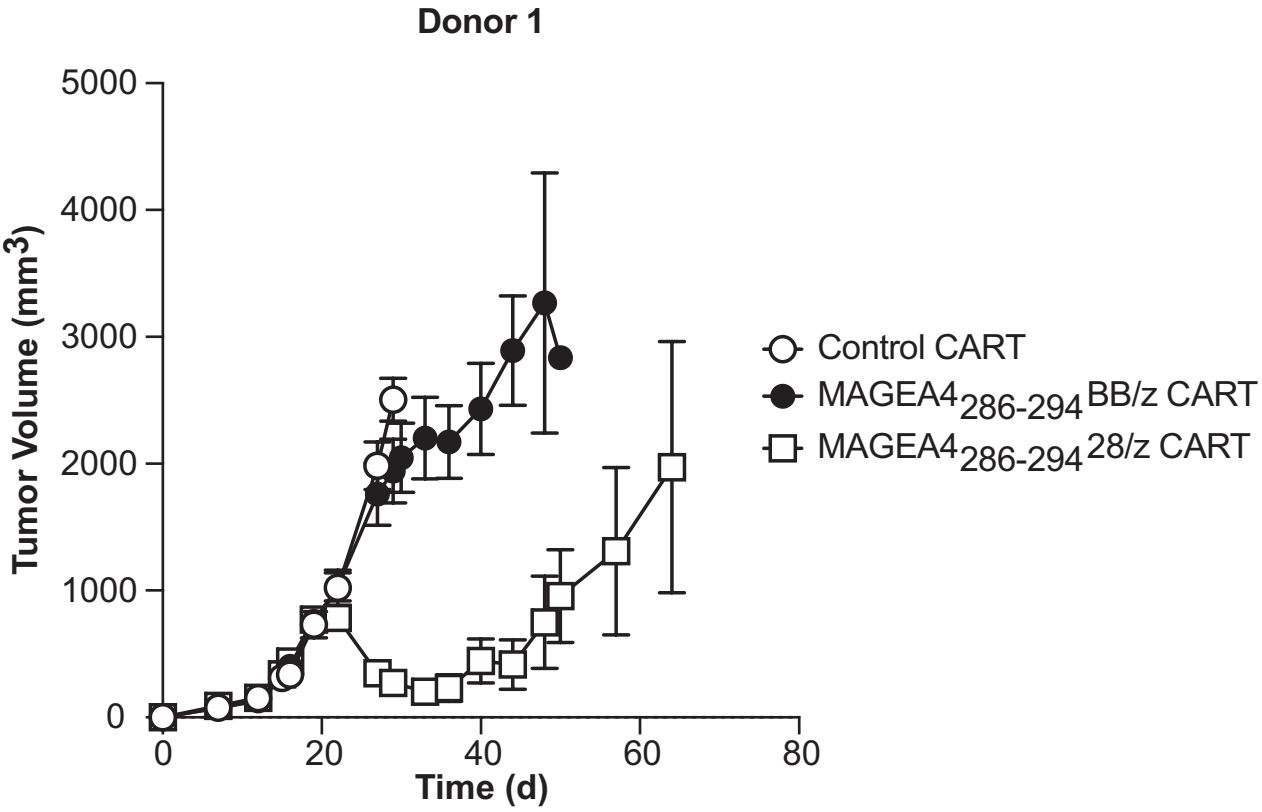


FIG. 3A Cont.

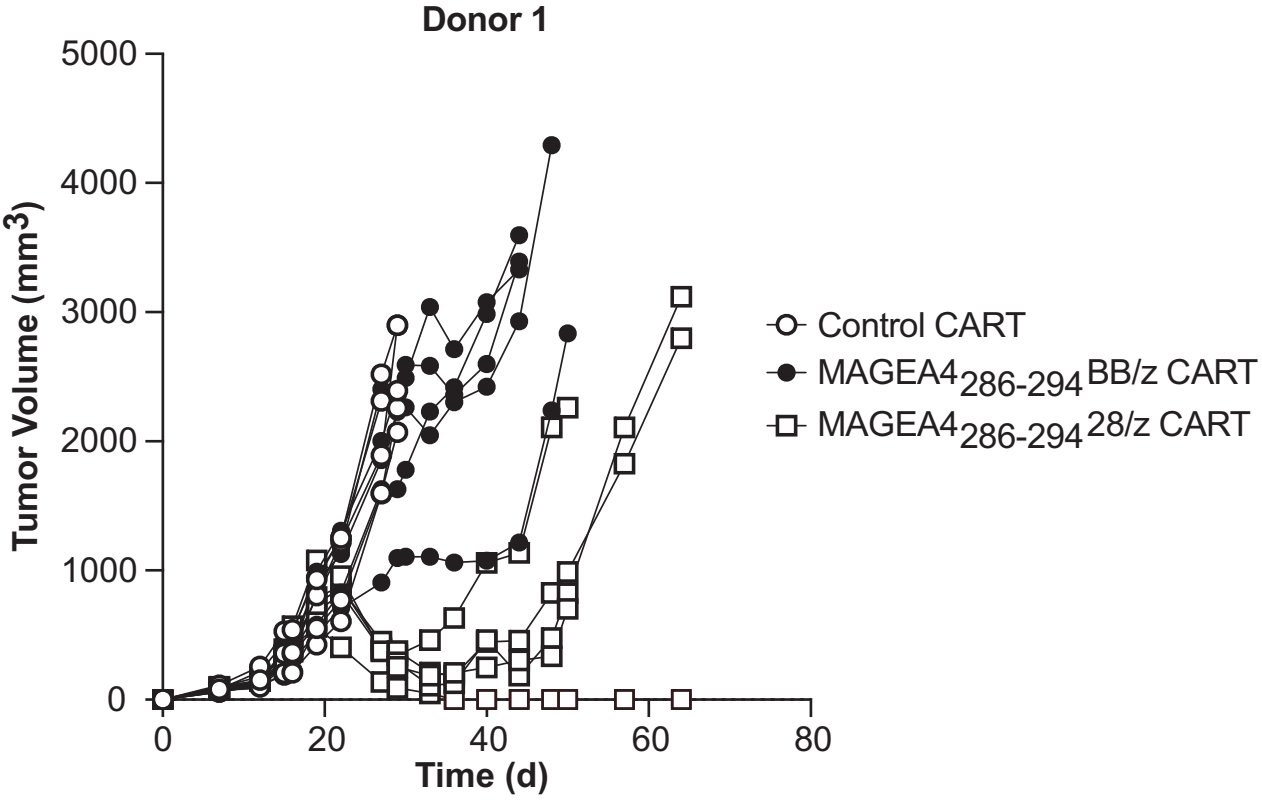


FIG. 3B

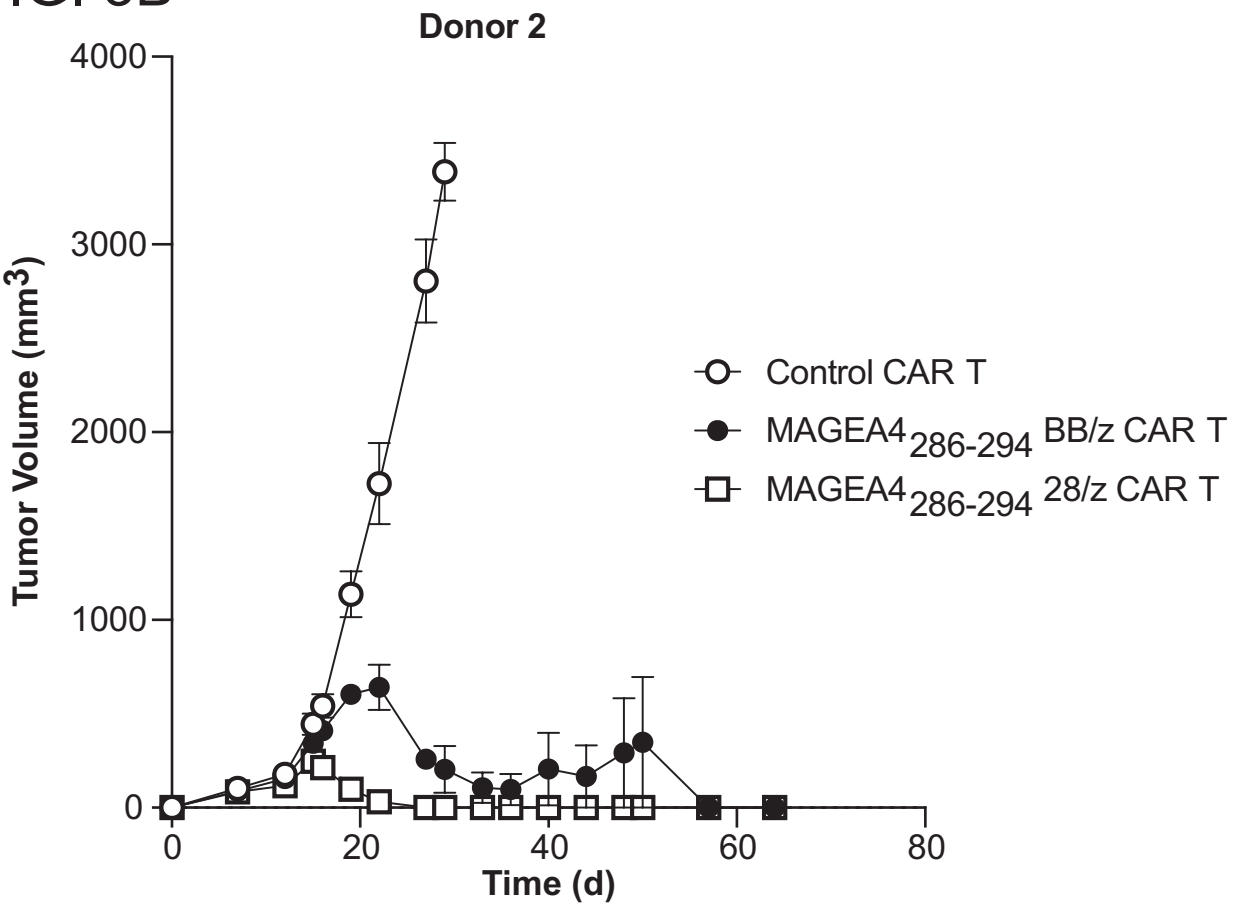


FIG. 3B Cont.

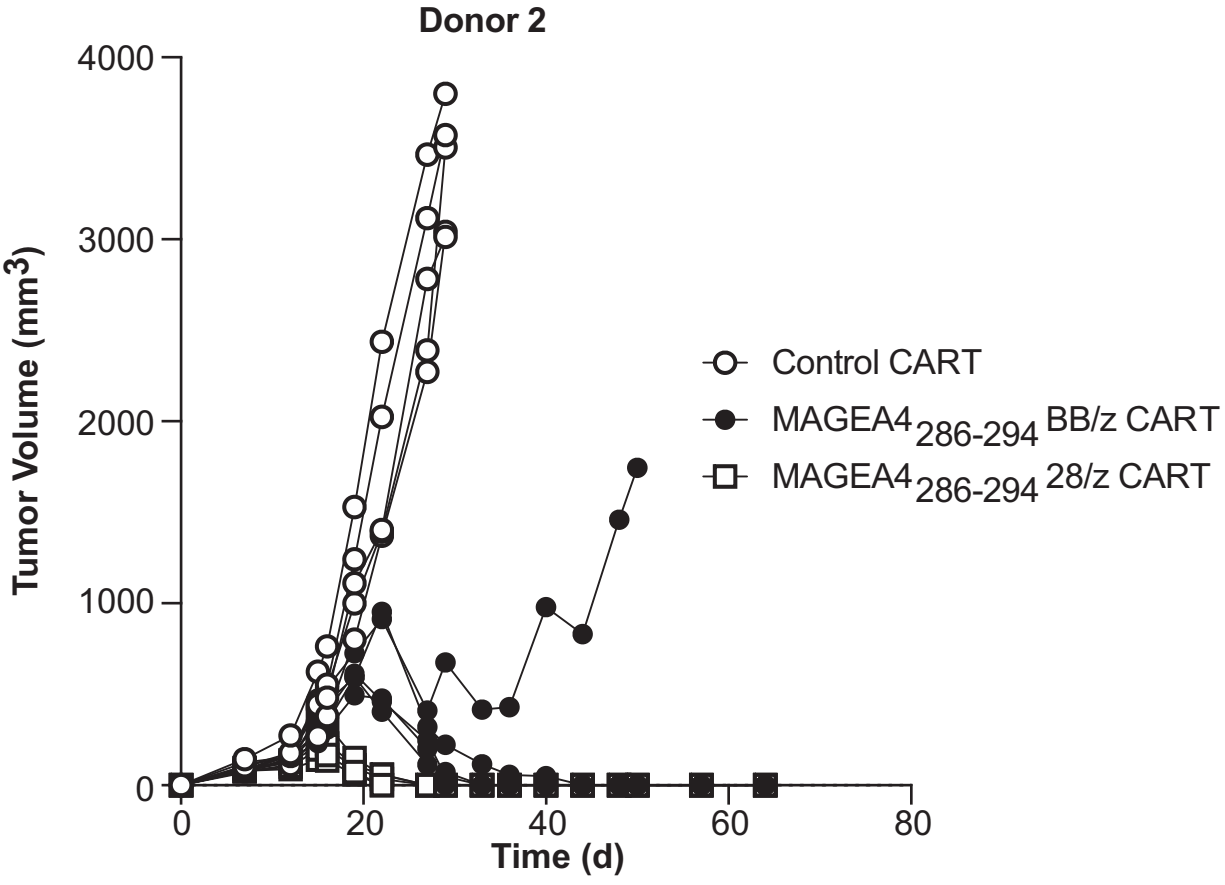


FIG. 4

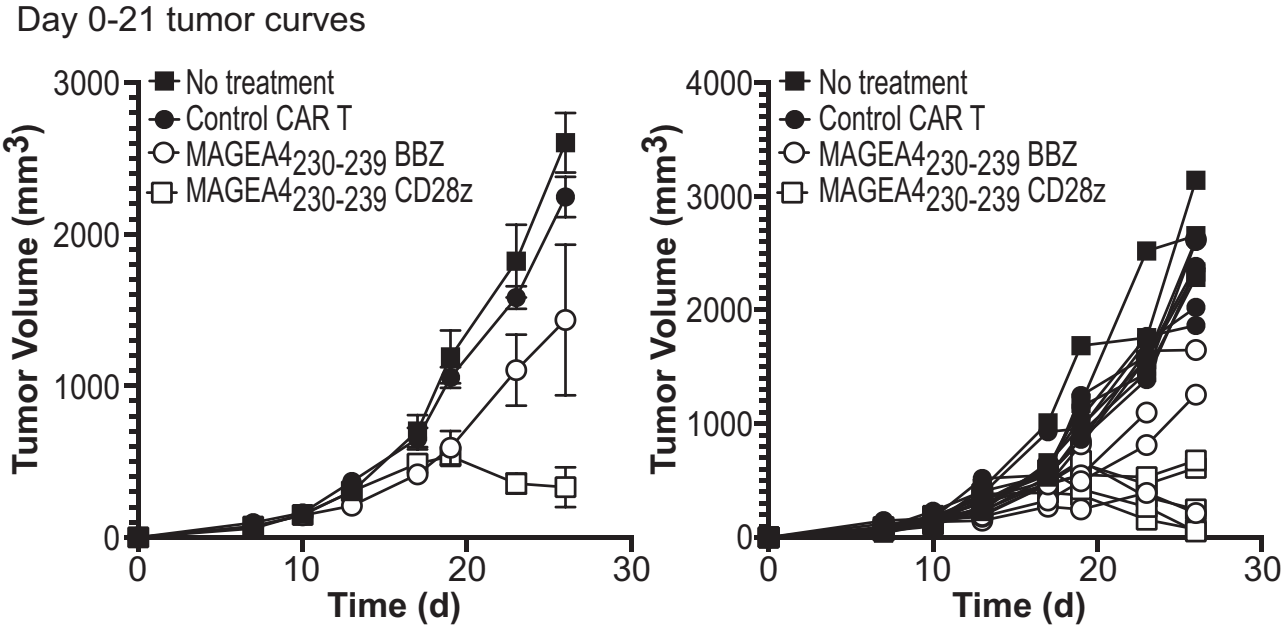


FIG. 4 Cont.

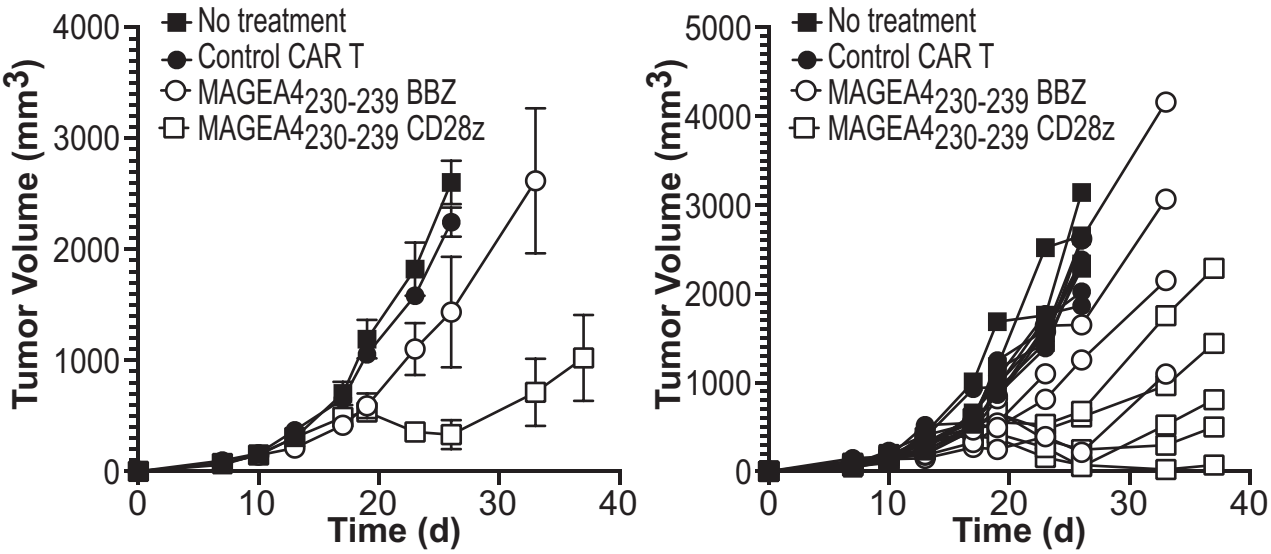


FIG. 4B

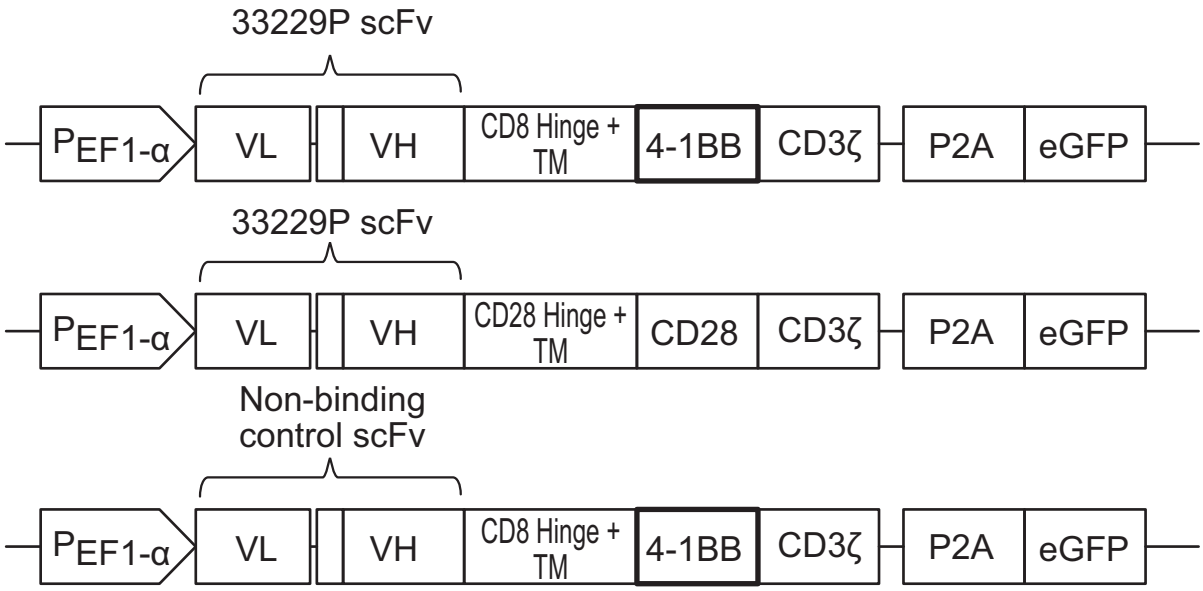

33229P scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
33229P scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP

FIG. 5A

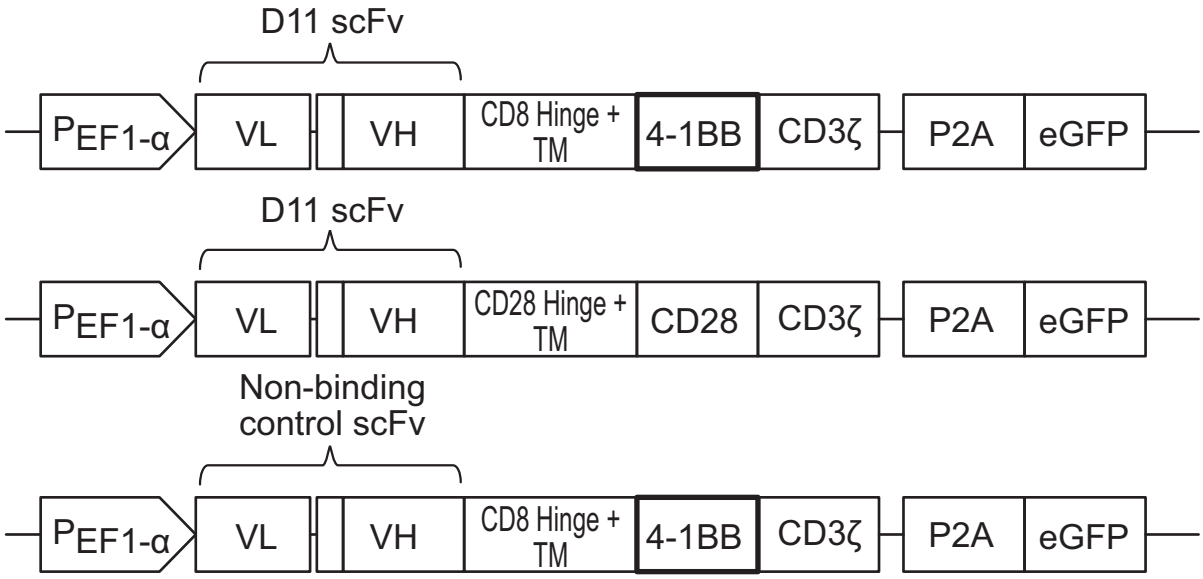
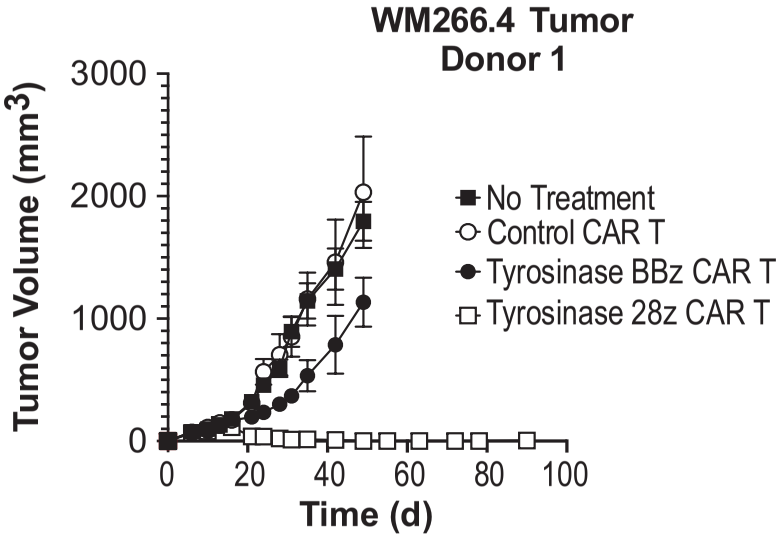
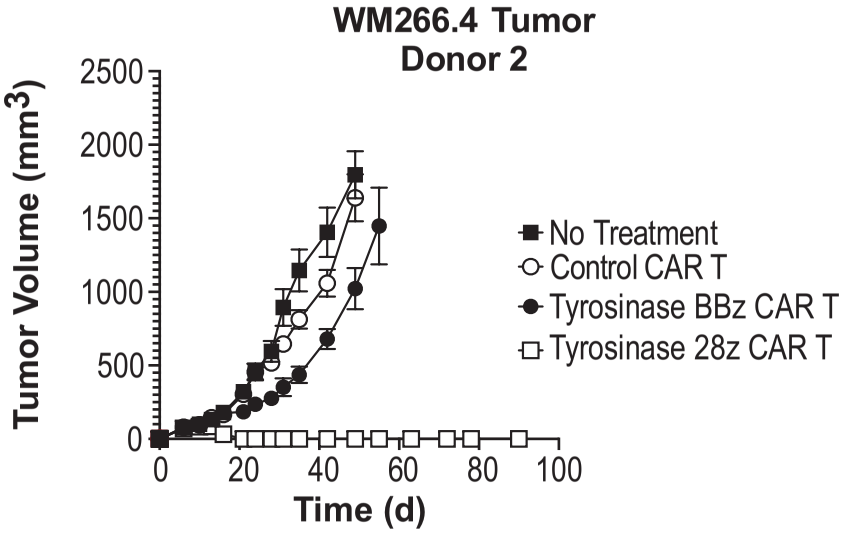
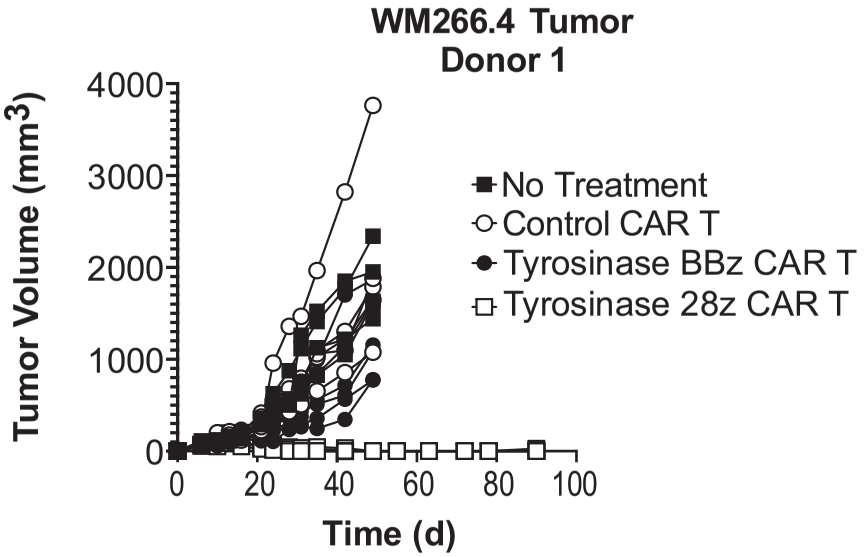

D11 scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
D11 scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP

FIG. 5B


WM266.4 Tumor
Donor 1
Tumor Volume (mm3)
3000
2000
1000
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T


WM266.4 Tumor
Donor 2
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T


WM266.4 Tumor
Donor 1
Tumor Volume (mm3)
4000
3000
2000
1000
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T

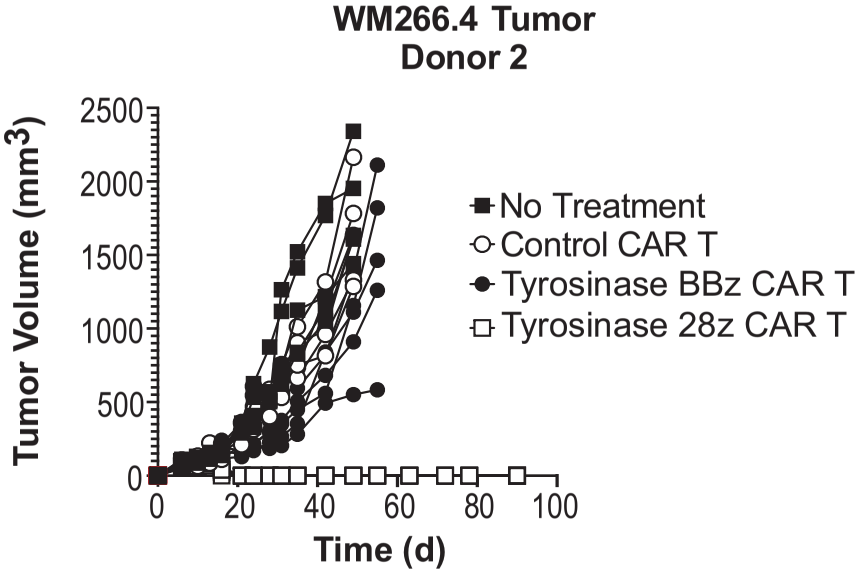
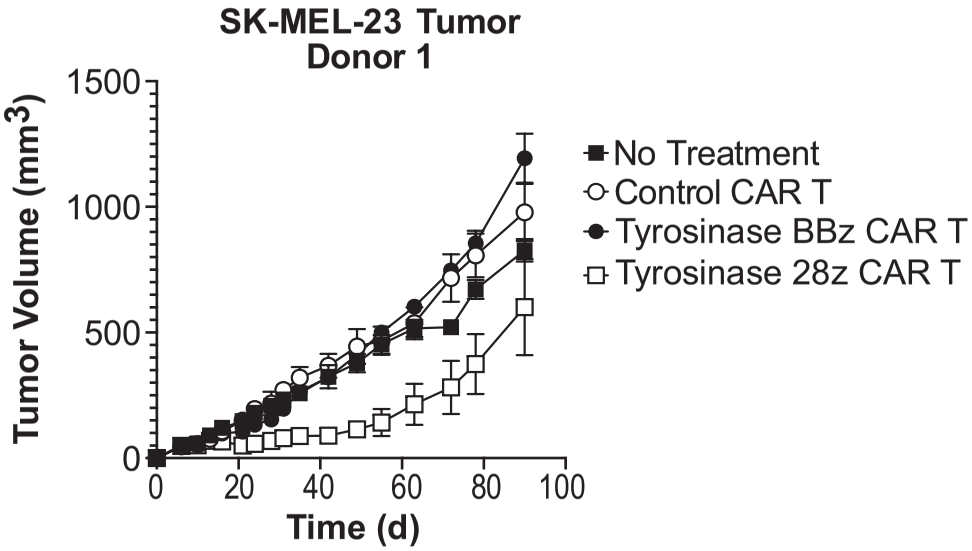
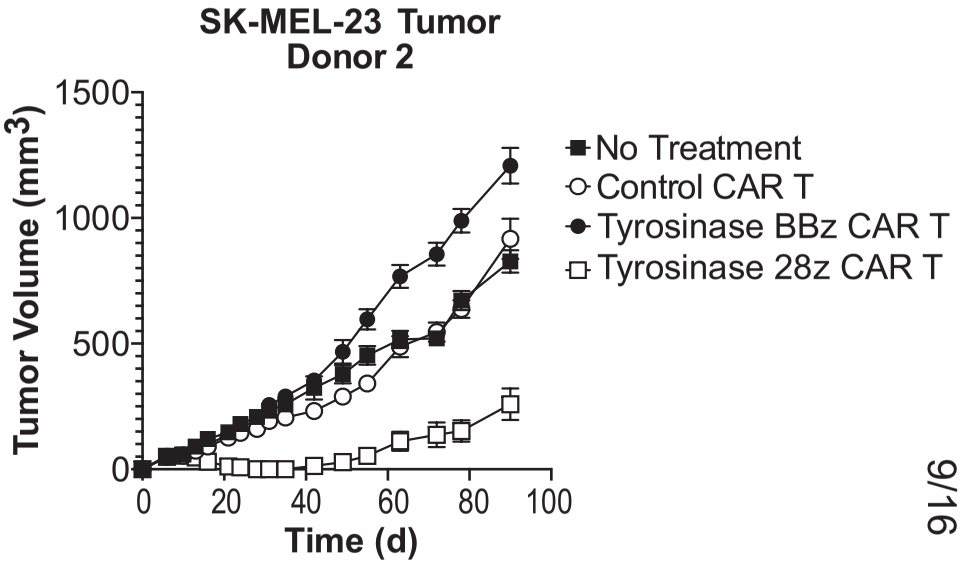
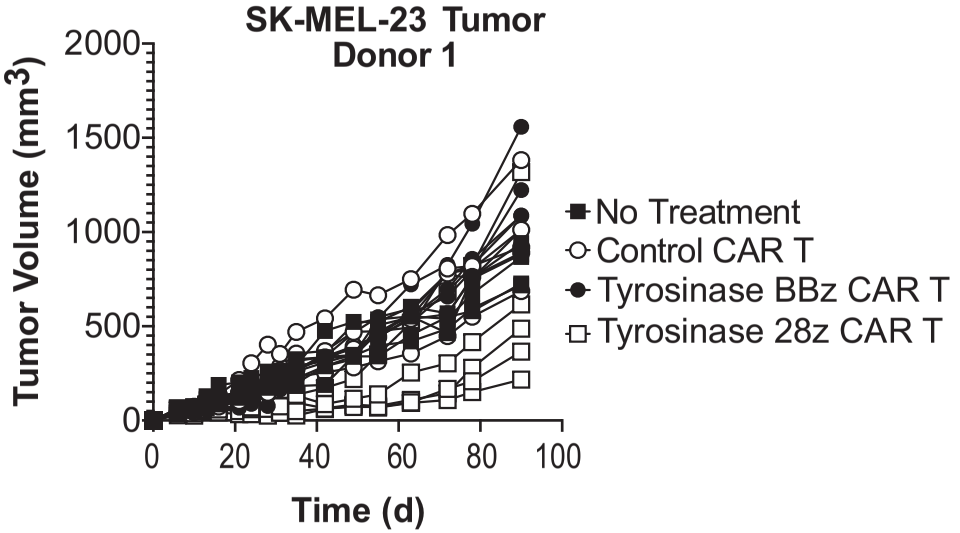

WM266.4 Tumor
Donor 2
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T

FIG. 5C


SK-MEL-23 Tumor
Donor 1
Tumor Volume (mm3)
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T


SK-MEL-23 Tumor
Donor 2
Tumor Volume (mm3)
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T


SK-MEL-23 Tumor
Donor 1
Tumor Volume (mm3)
2000
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T

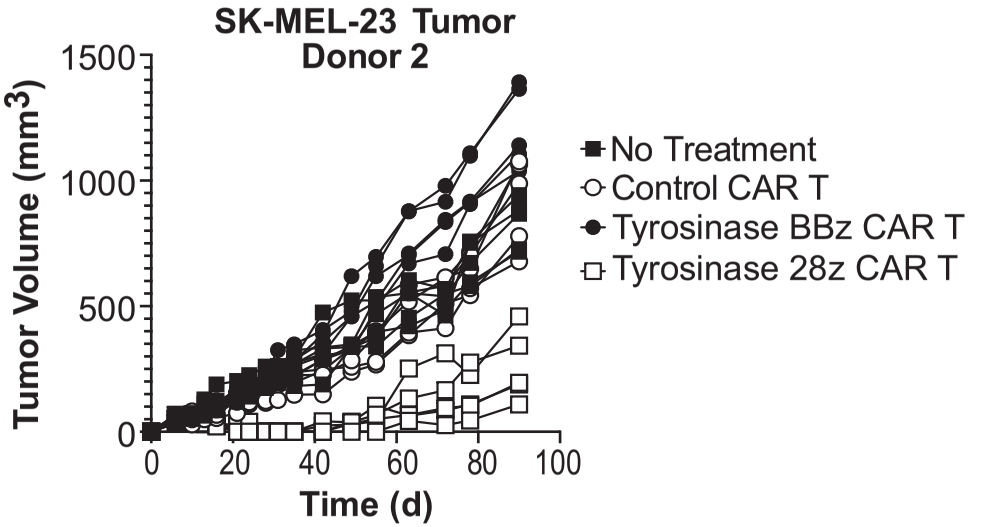

SK-MEL-23 Tumor
Donor 2
Tumor Volume (mm3)
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T

FIG. 6A

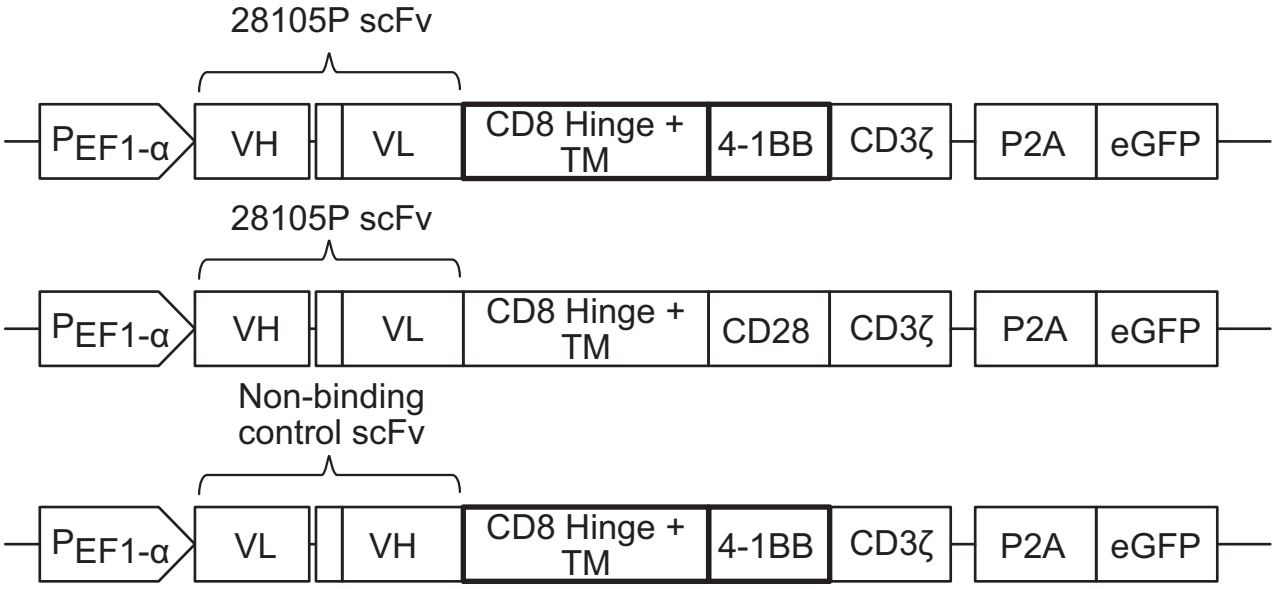

28105P scFv
PEF1-α
VH
VL
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
28105P scFv
PEF1-α
VH
VL
CD8 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP

FIG. 6B

Day 0-21 tumor curves

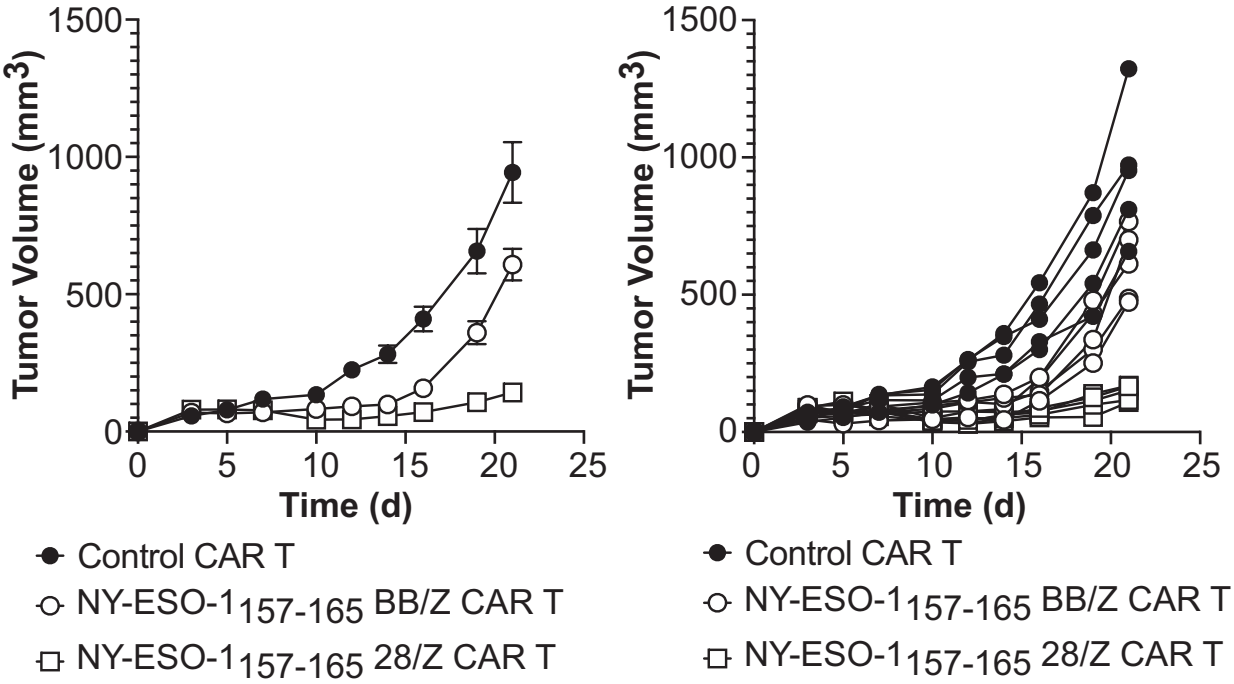


FIG. 6B Cont.

Day 0-38 tumor curves

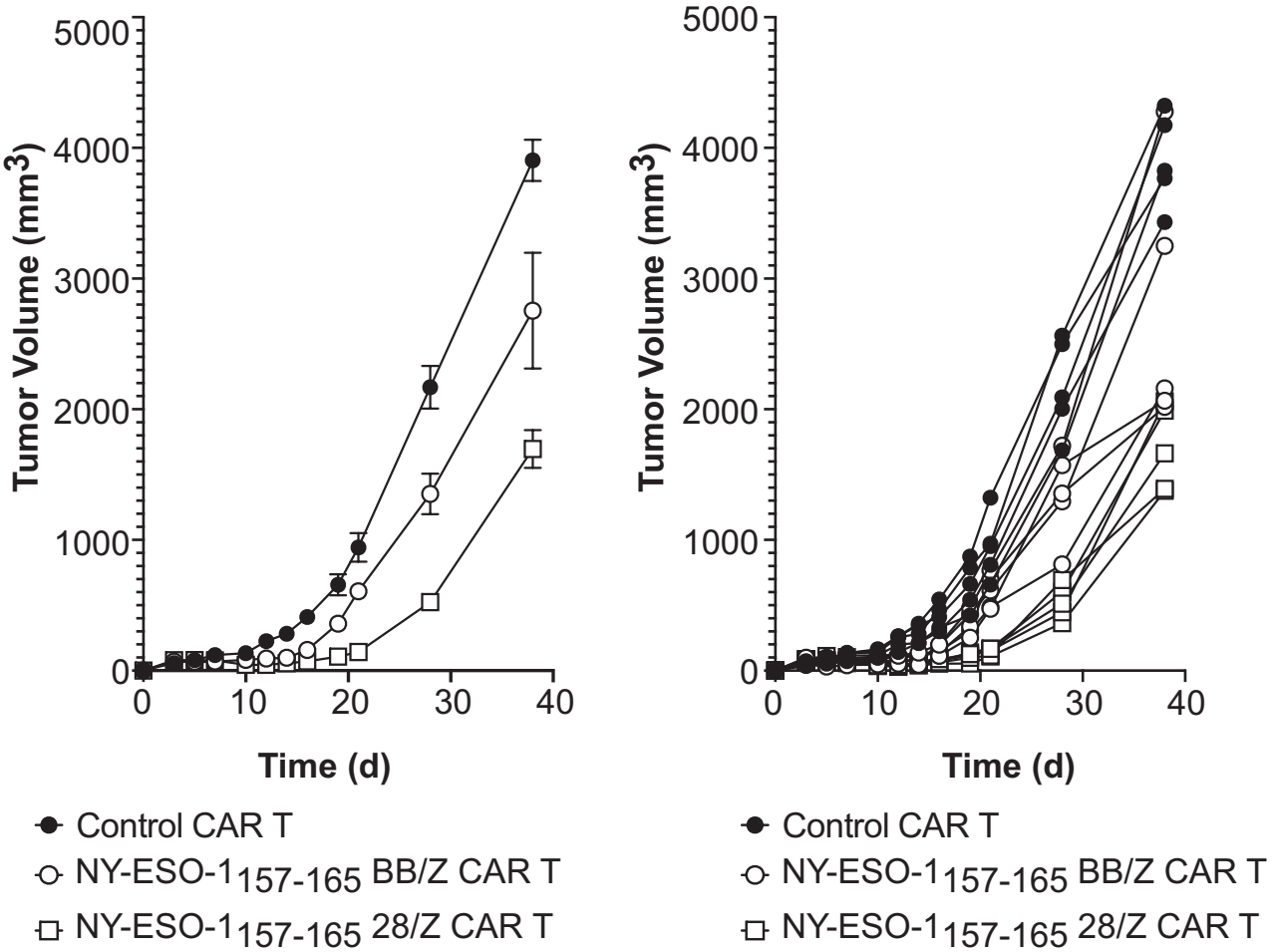


FIG. 7

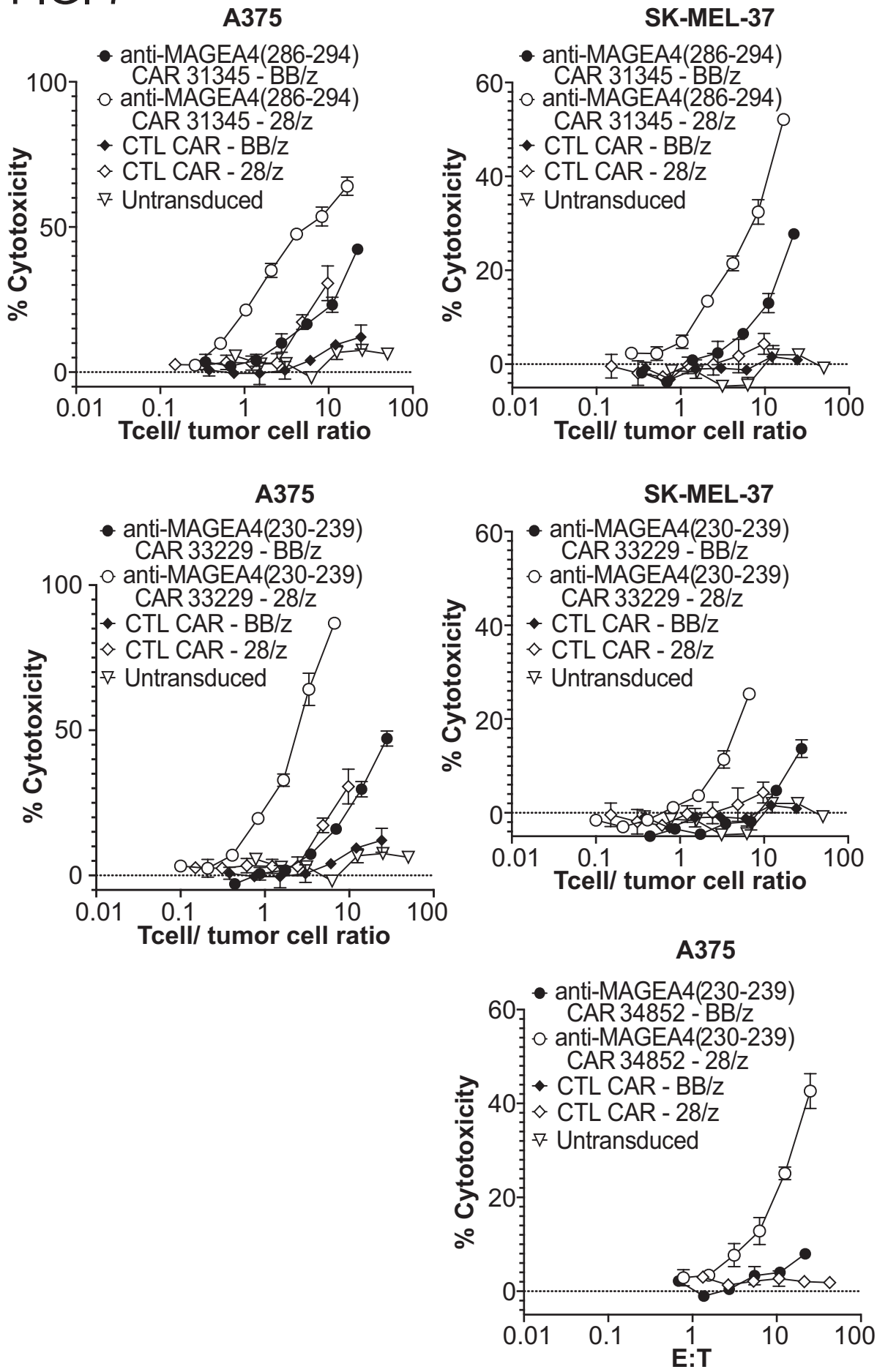

A375
anti-MAGEA4(286-294) CAR 31345 - BB/z
anti-MAGEA4(286-294) CAR 31345 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
Tcell/ tumor cell ratio
SK-MEL-37
anti-MAGEA4(286-294) CAR 31345 - BB/z
anti-MAGEA4(286-294) CAR 31345 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
Tcell/ tumor cell ratio
A375
anti-MAGEA4(230-239) CAR 33229 - BB/z
anti-MAGEA4(230-239) CAR 33229 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
Tcell/ tumor cell ratio
SK-MEL-37
anti-MAGEA4(230-239) CAR 33229 - BB/z
anti-MAGEA4(230-239) CAR 33229 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
Tcell/ tumor cell ratio
A375
anti-MAGEA4(230-239) CAR 34852 - BB/z
anti-MAGEA4(230-239) CAR 34852 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
E:T

FIG. 8

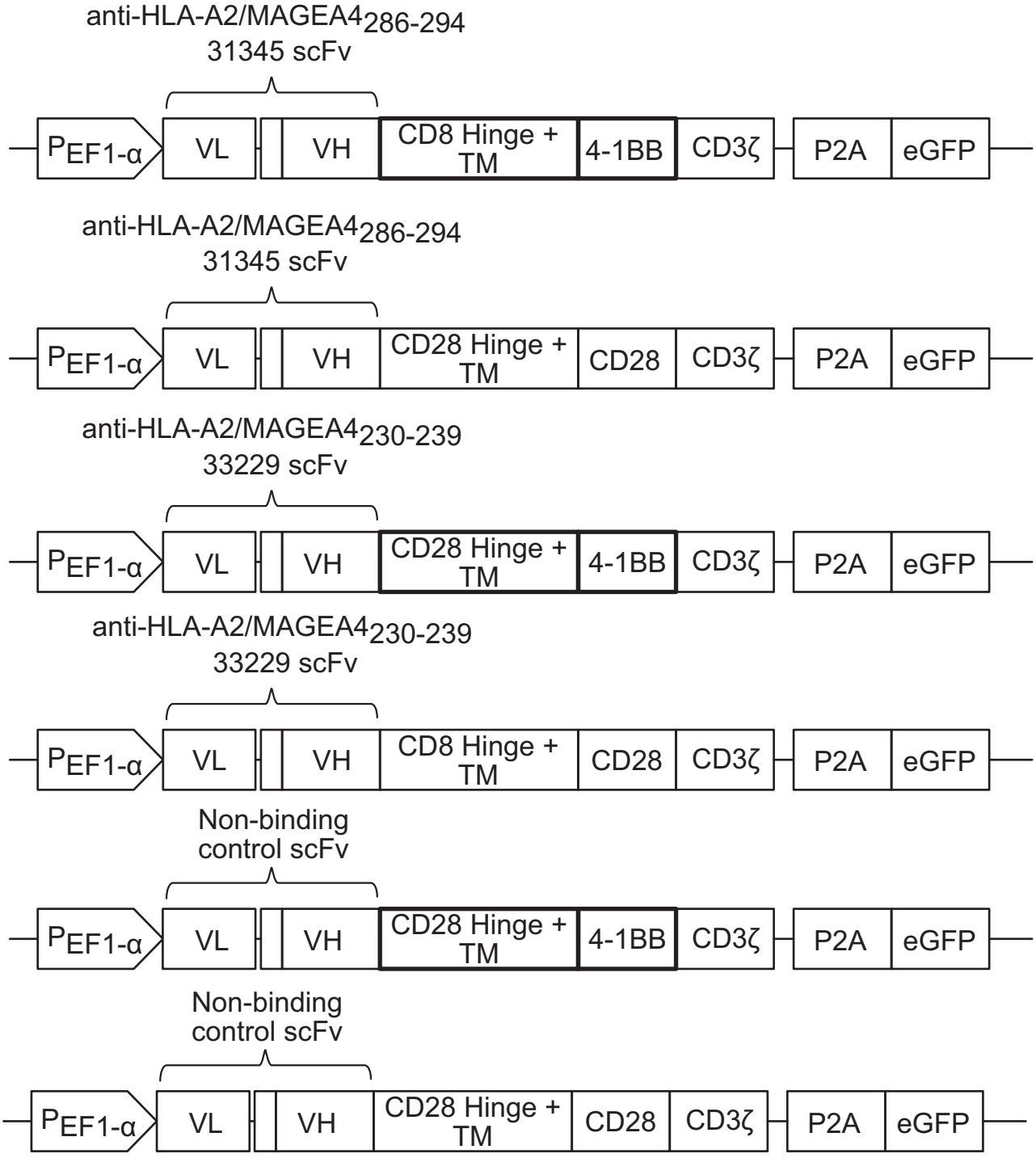

anti-HLA-A2/MAGEA4286-294
31345 scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4286-294
31345 scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4230-239
33229 scFv
PEF1-α
VL
VH
CD28 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4230-239
33229 scFv
PEF1-α
VL
VH
CD8 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD28 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP

FIG. 9

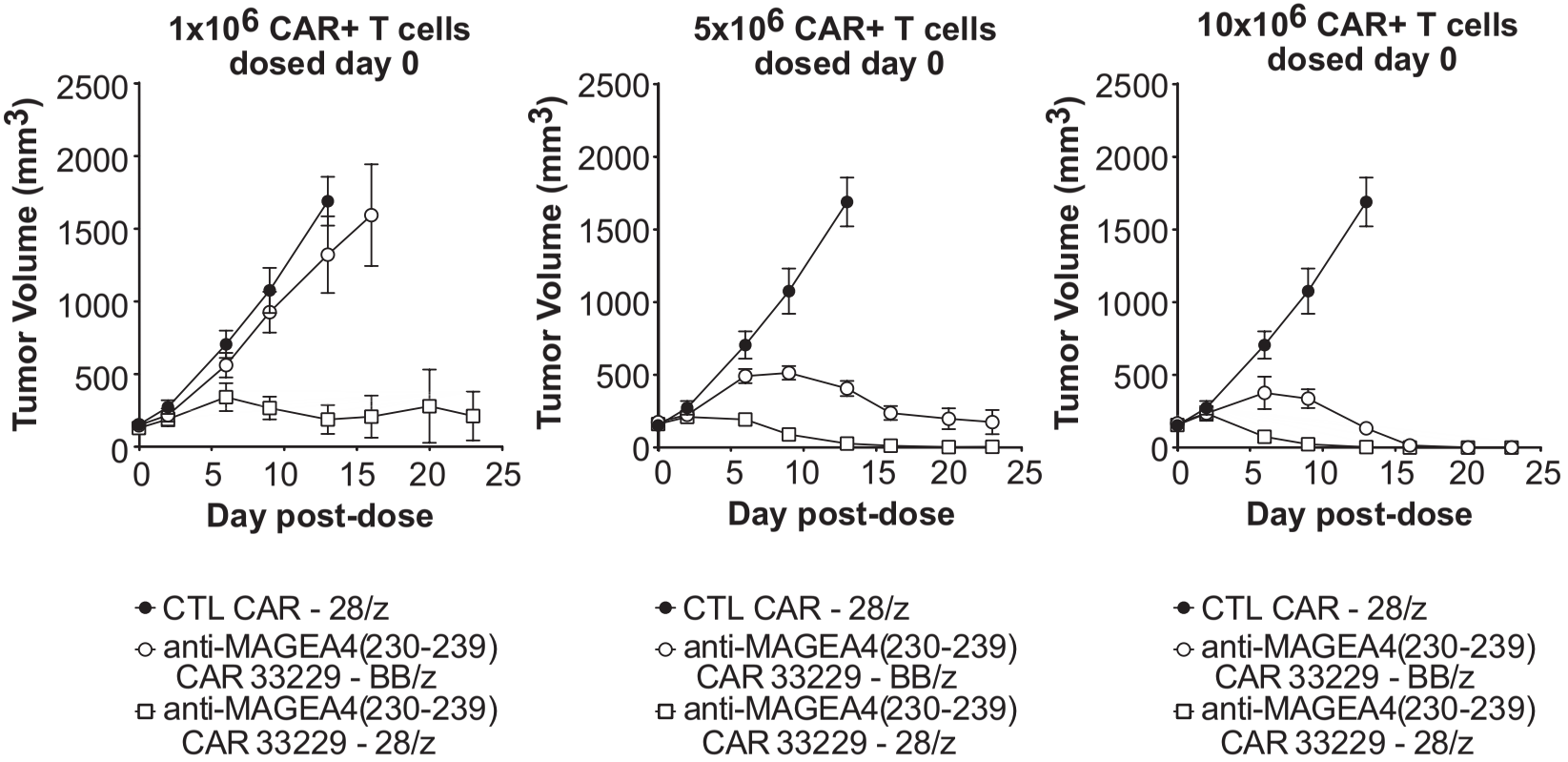

1x10^6 CAR+ T cells dosed day 0
5x10^6 CAR+ T cells dosed day 0
10x10^6 CAR+ T cells dosed day 0
Tumor Volume (mm^3)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Day post-dose
CTL CAR - 28/z
anti-MAGEA4(230-239) CAR 33229 - BB/z
anti-MAGEA4(230-239) CAR 33229 - 28/z

FIG. 10

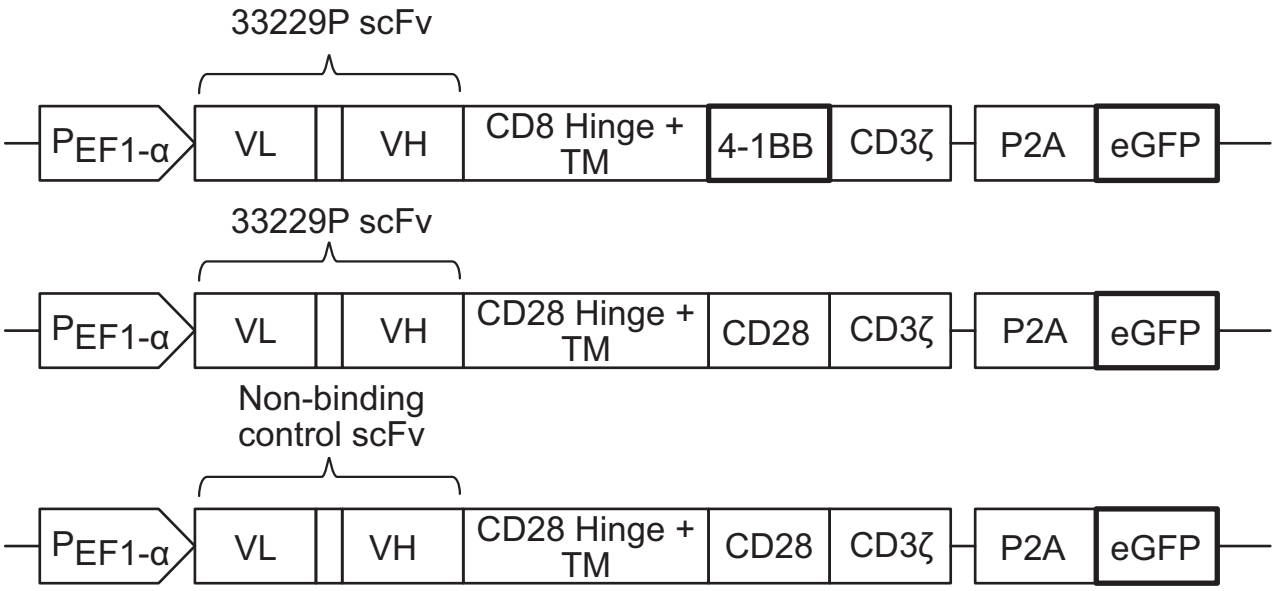

33229P scFv
P EF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
33229P scFv
P EF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
P EF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP

FIG. 11

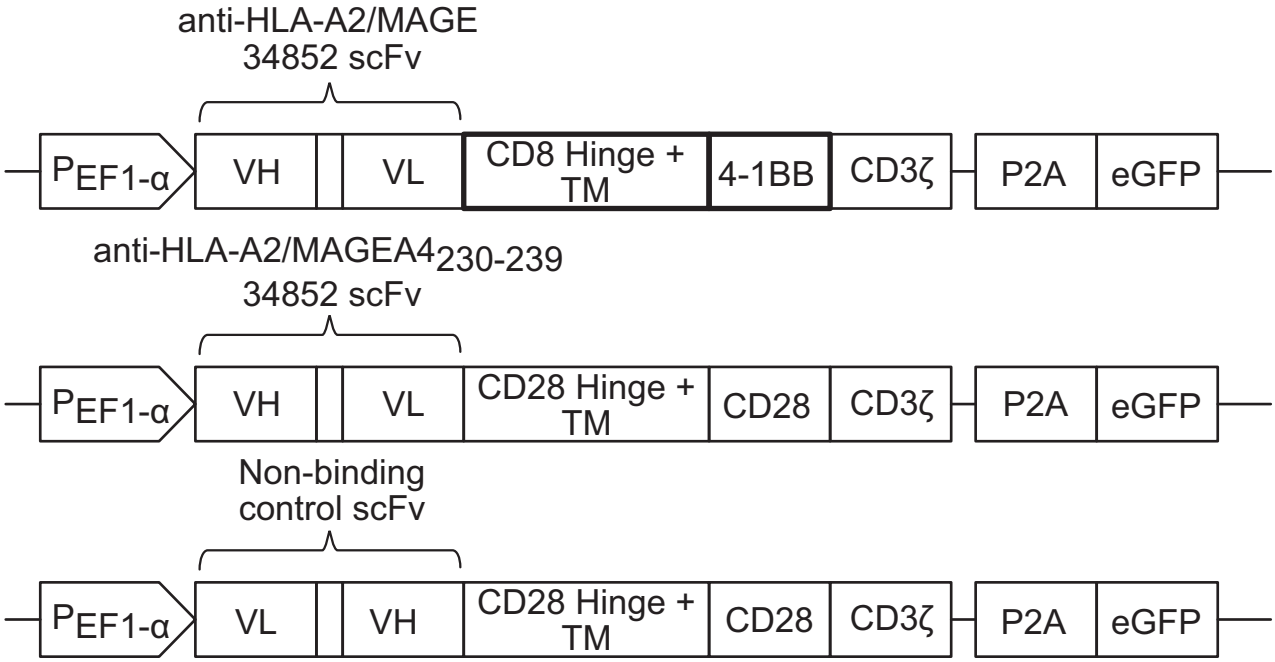
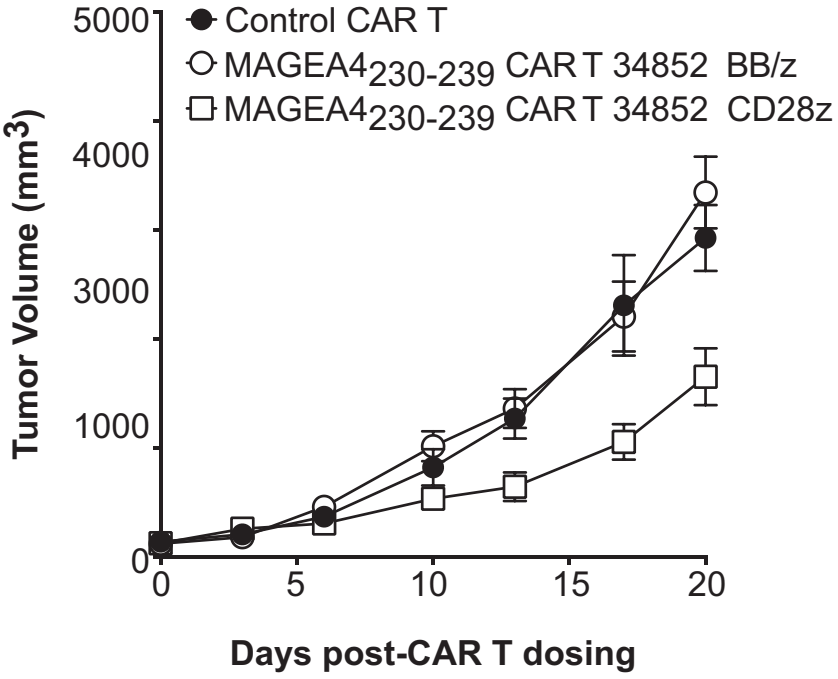

anti-HLA-A2/MAGE 34852 scFv
P EF1-α
VH
VL
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4 230-239 34852 scFv
P EF1-α
VH
VL
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
P EF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP

FIG. 12


Control CAR T
MAGEA4230-239 CAR T 34852 BB/z
MAGEA4230-239 CAR T 34852 CD28z
Tumor Volume (mm3)
5000
4000
3000
1000
0
0
5
10
15
20
Days post-CAR T dosing