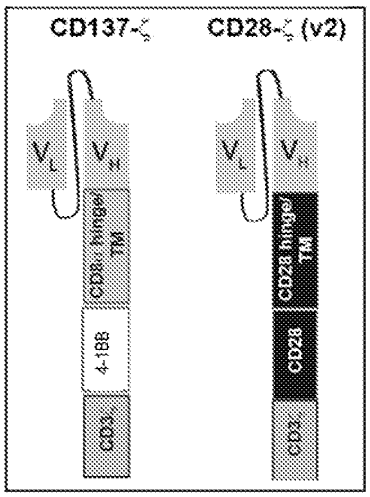
Figure 1

A


CD137-ζ
CD28-ζ (v2)
VL
VH
CD8α hinge+ TM
4-1BB
CD3ζ
CD28 hinge+ TM
CD28
CD3ζ

B

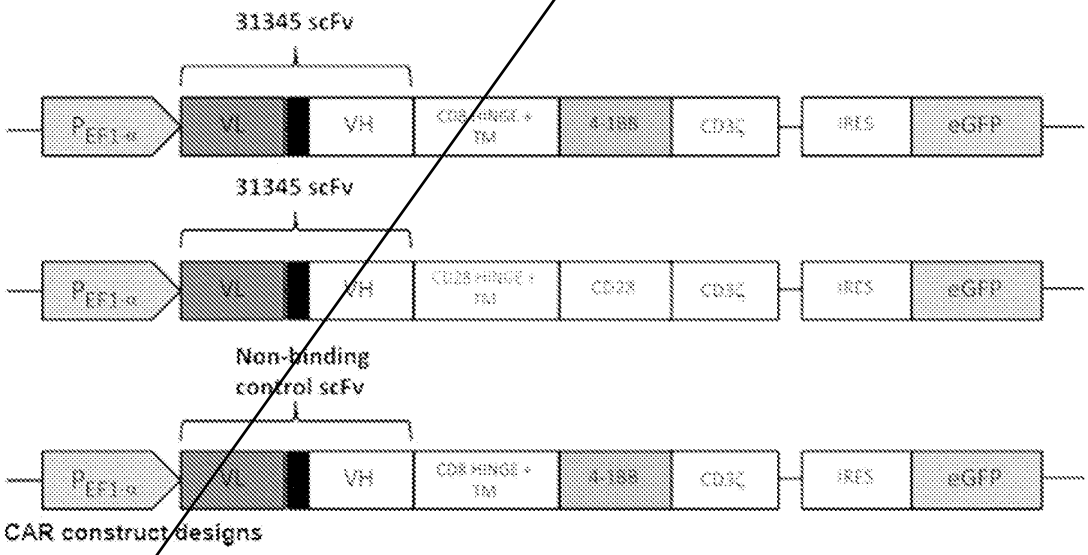

31345 scFv
PEF1α
VL
VH
CD8 HINGE + TM
4-1BB
CD3ζ
IRES
eGFP
31345 scFv
PEF1α
VH
CD28 HINGE + TM
CD28
CD3ζ
IRES
eGFP
Non-binding control scFv
PEF1α
VL
VH
CD8 HINGE + TM
4-1BB
CD3ζ
IRES
eGFP
CAR construct designs

Figure 2
A.

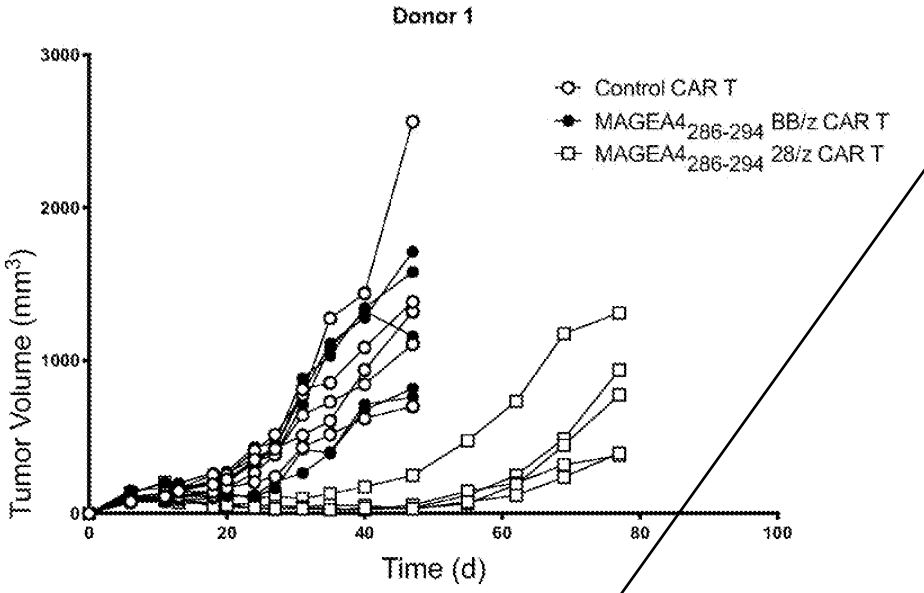


Figure 2 (cont.)
A.

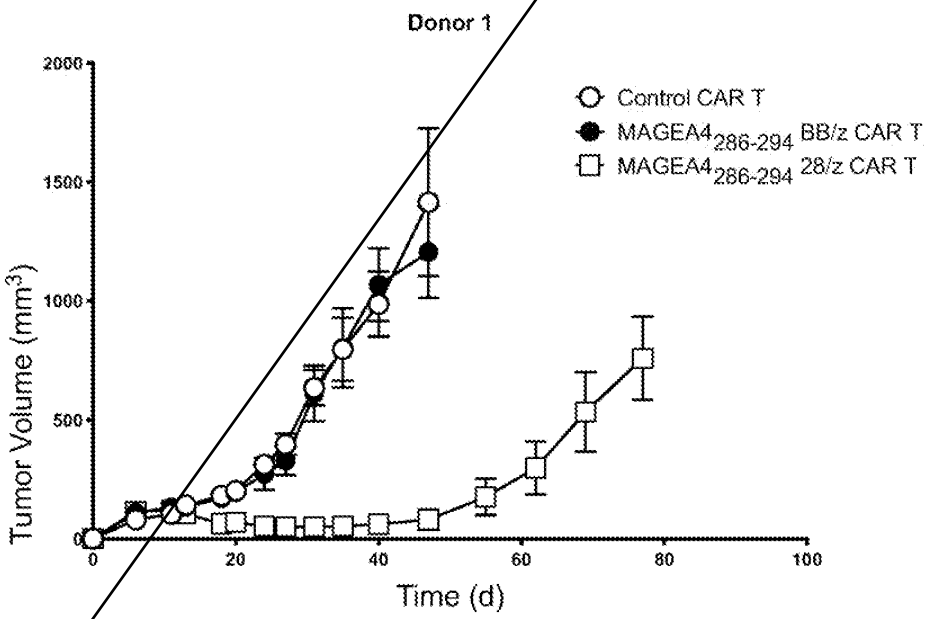
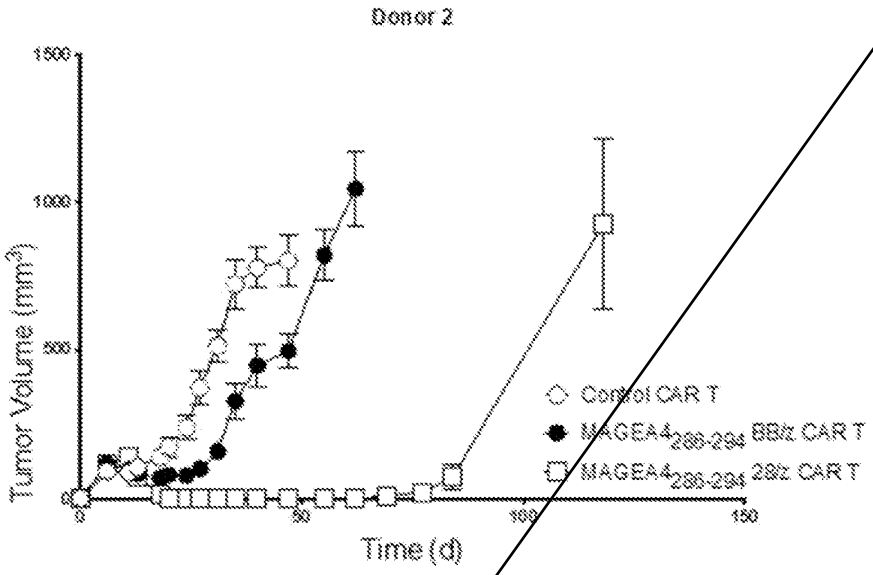


Figure 2 (cont.)
B



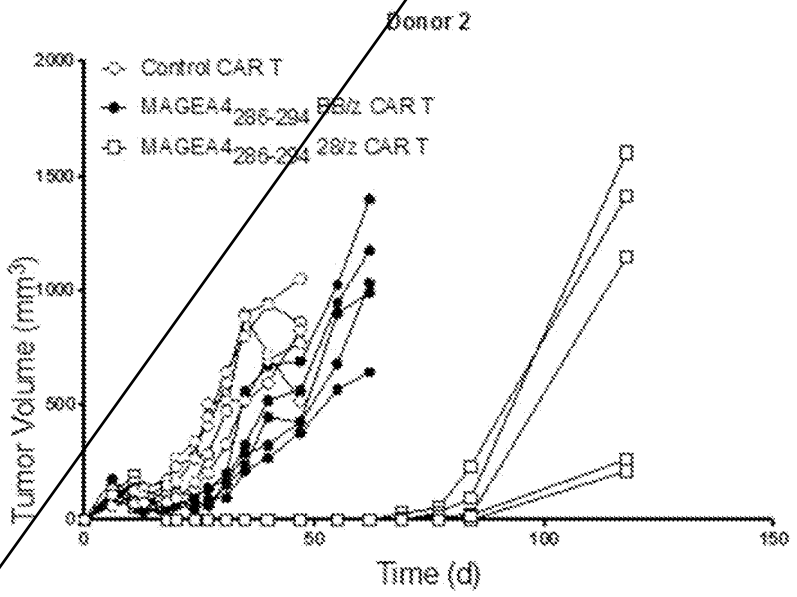


Figure 3

A

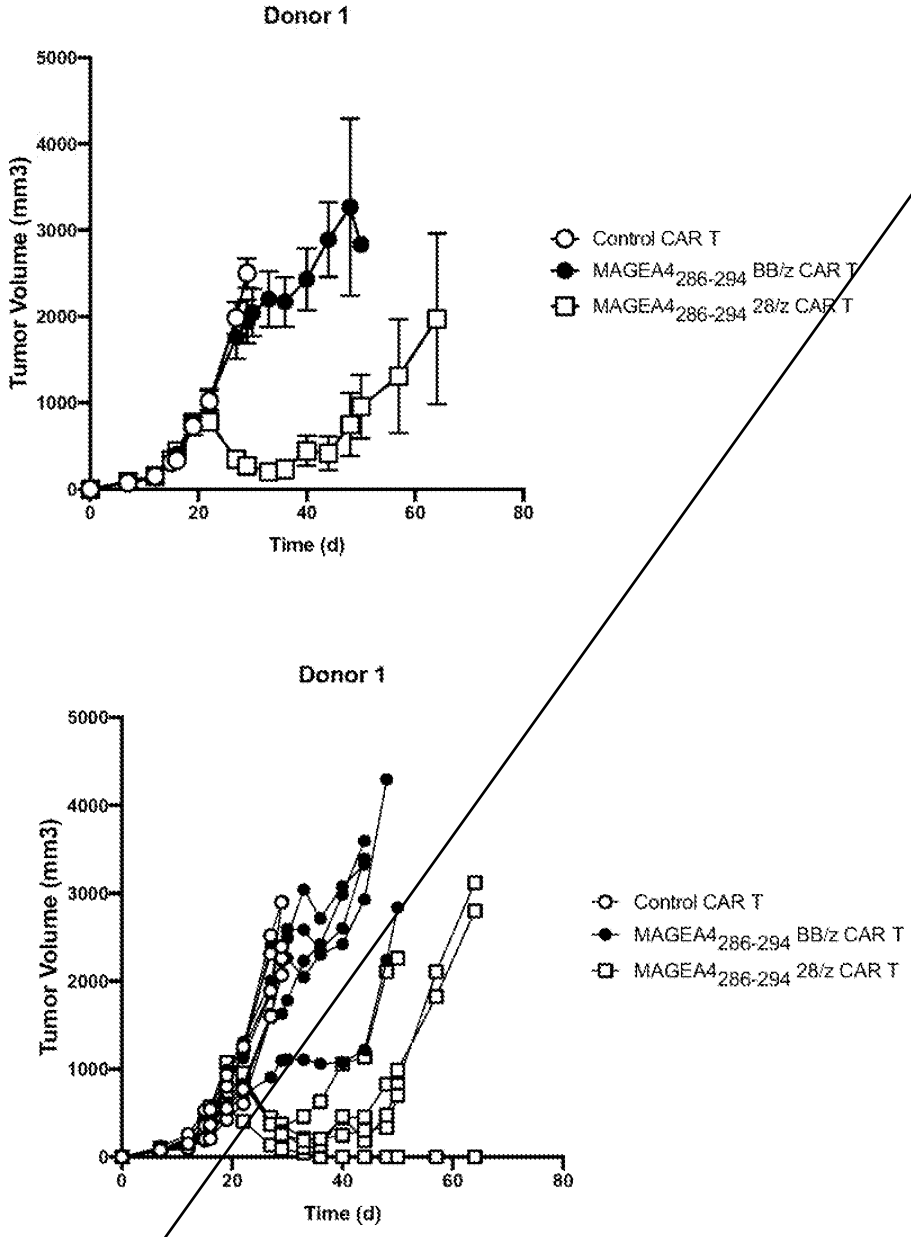

Donor 1
Tumor Volume (mm3)
5000
4000
3000
2000
1000
0
0
20
40
60
80
Time (d)
Control CAR T
MAGEA4286-294 BB/z CAR T
MAGEA4286-294 28/z CAR T
Donor 1
Tumor Volume (mm3)
5000
4000
3000
2000
1000
0
0
20
40
60
80
Time (d)
Control CAR T
MAGEA4286-294 BB/z CAR T
MAGEA4286-294 28/z CAR T

Figure 3 (cont.)
B

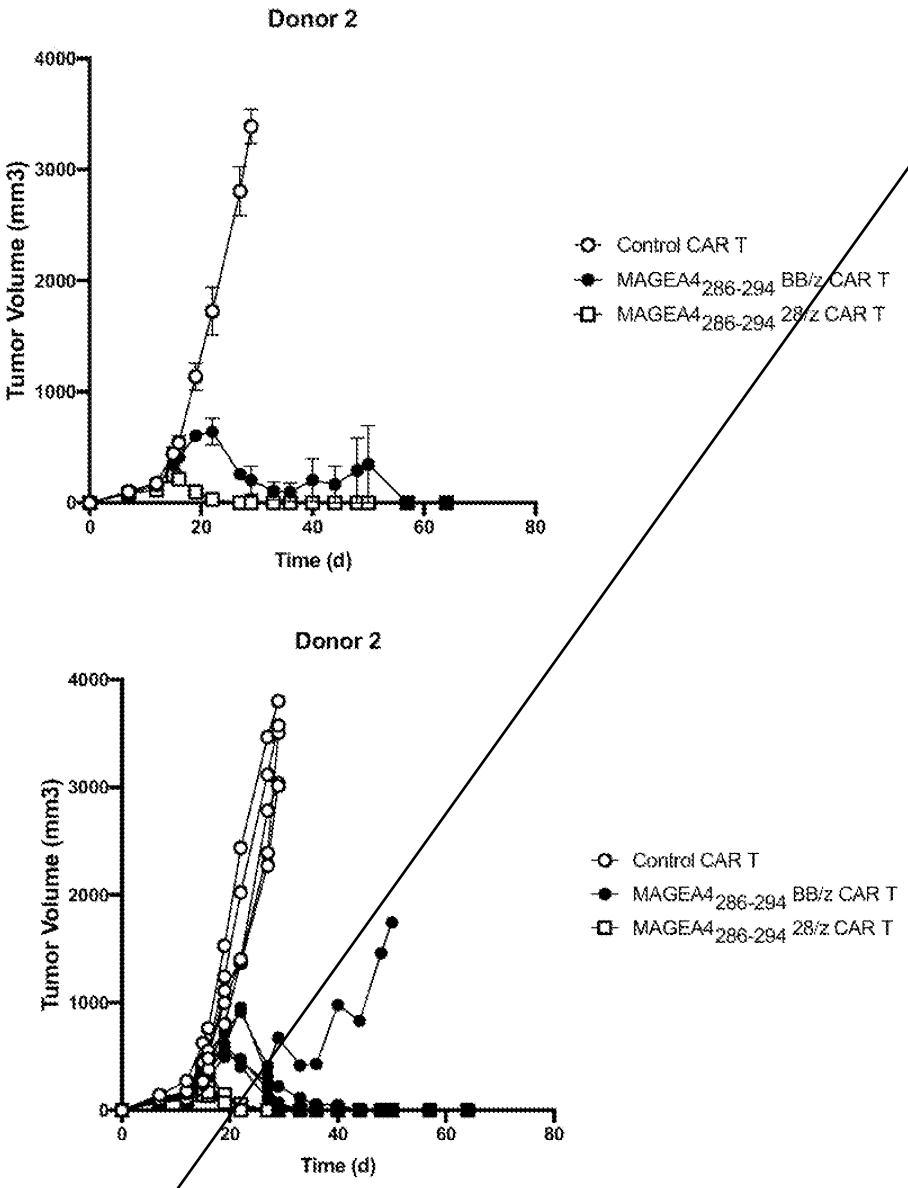
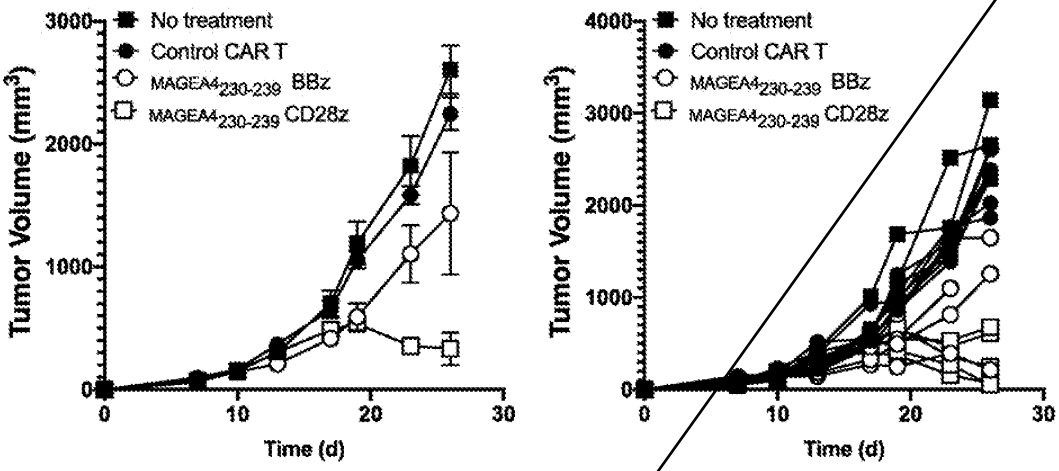


Figure 4

A. Day 0-21 tumor curves



Day 0-37 tumor curves

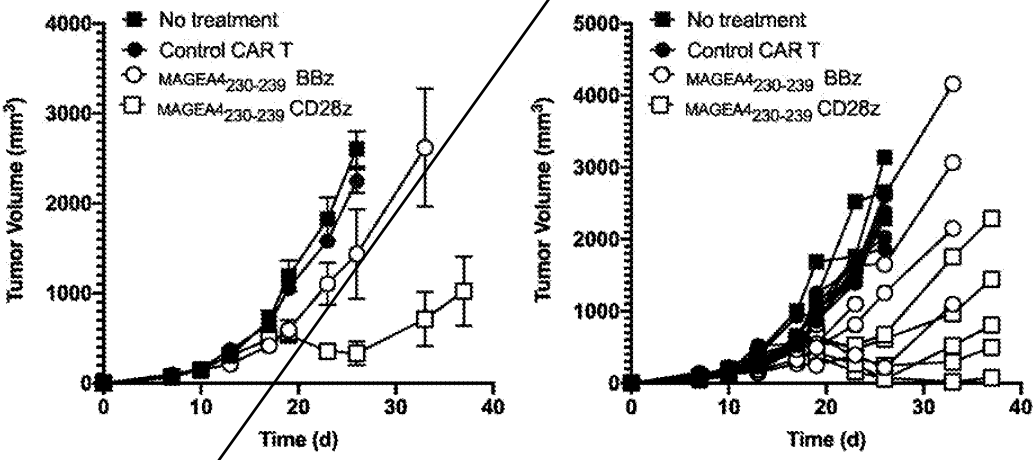


Figure 4 (cont.)

B.

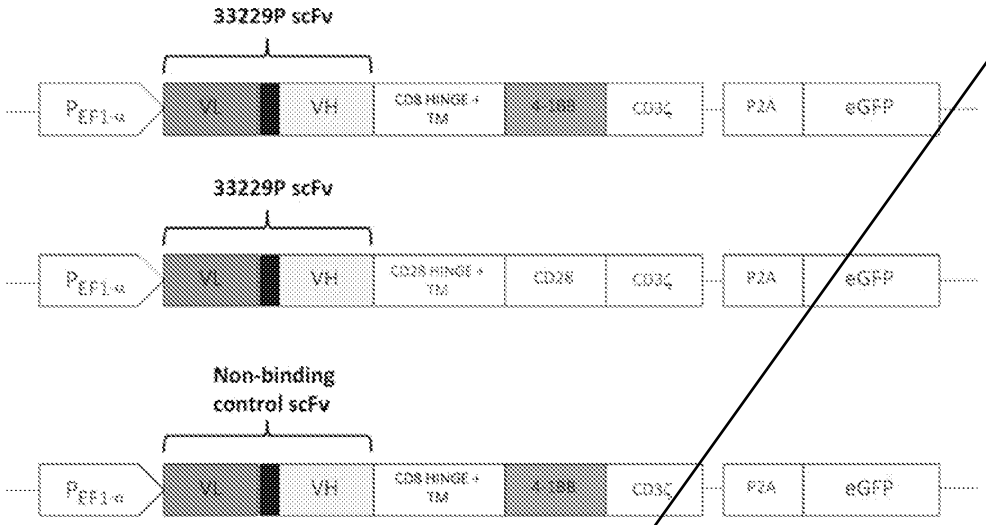


Figure 5

A

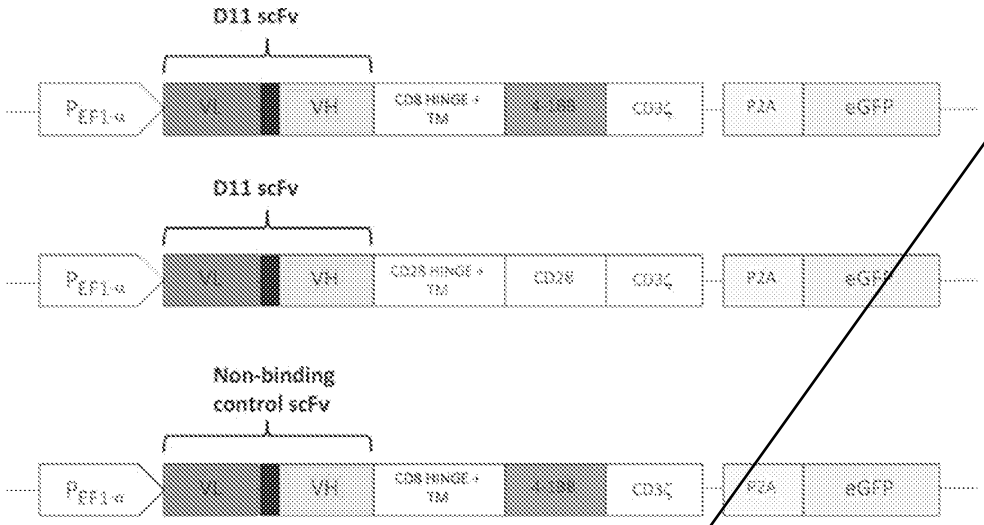

D11 scFv
$P_{EF1-\alpha}$
VL
VH
CD8 HINGE + TM
4-1BB
CD3ζ
P2A
eGFP
D11 scFv
$P_{EF1-\alpha}$
VL
VH
CD28 HINGE + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
$P_{EF1-\alpha}$
VL
VH
CD8 HINGE + TM
4-1BB
CD3ζ
P2A
eGFP

Figure 5 (cont.)

B

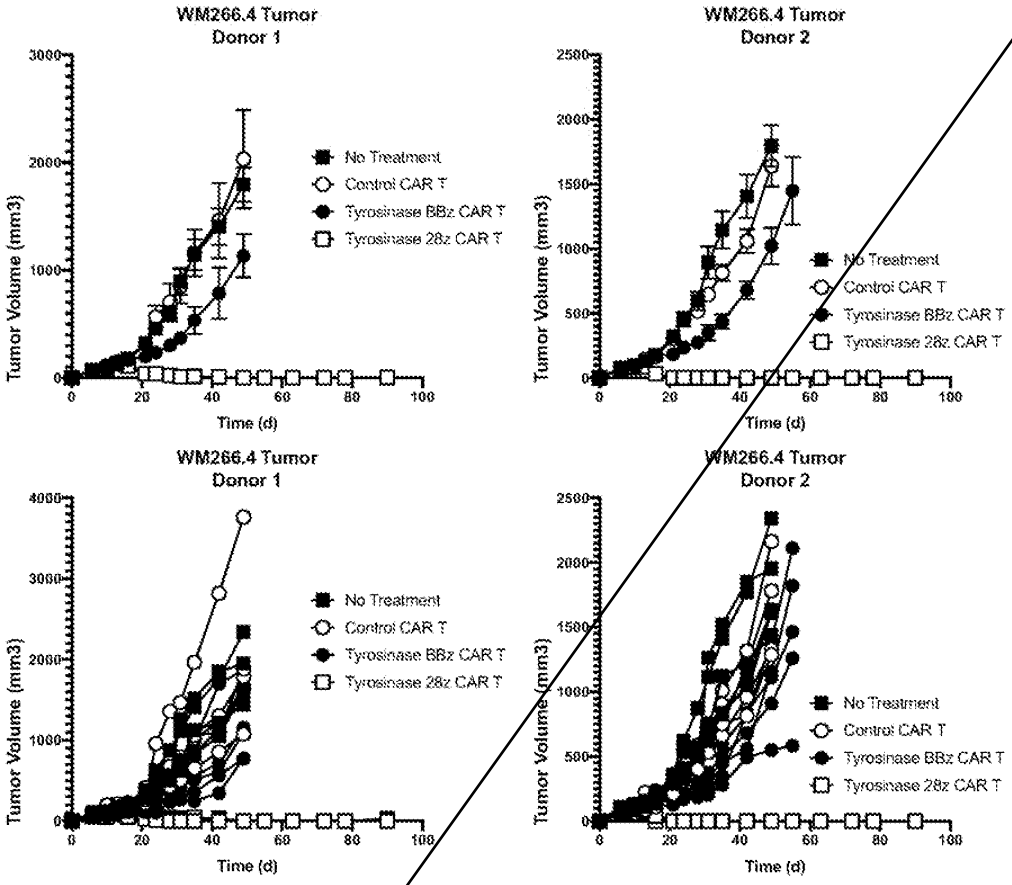


Figure 5 (cont.)

C

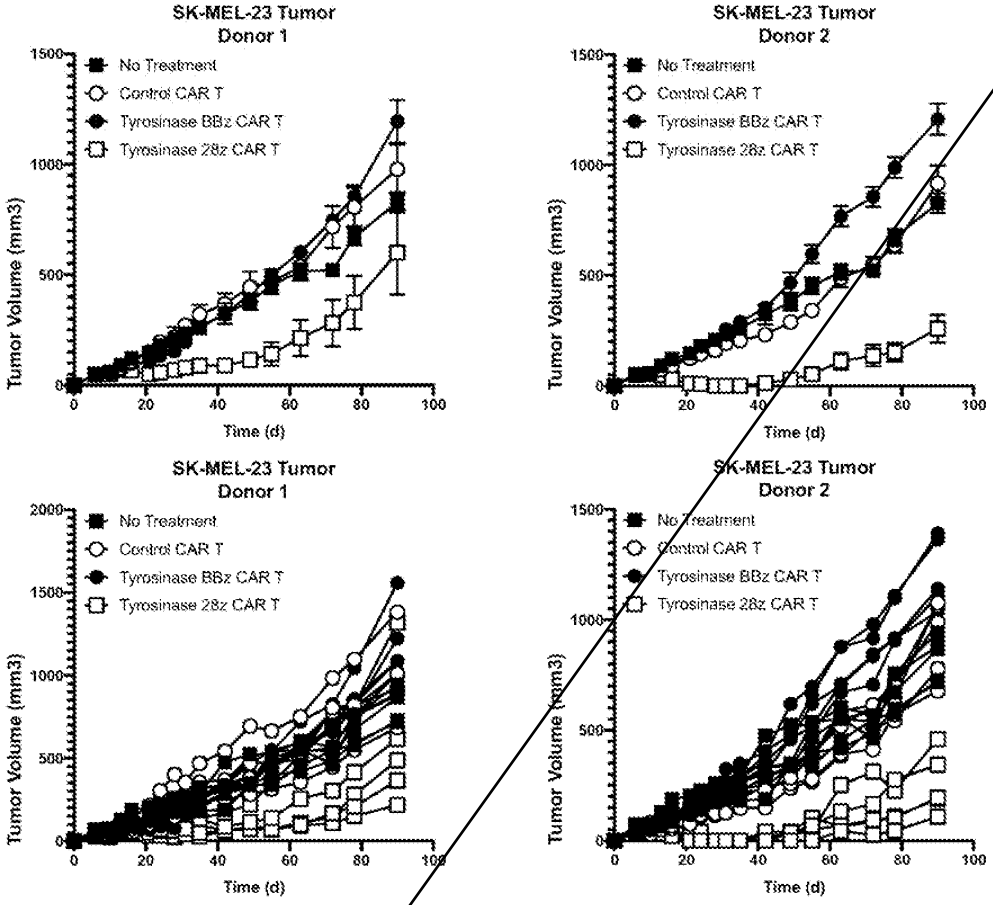


Figure 6

A.

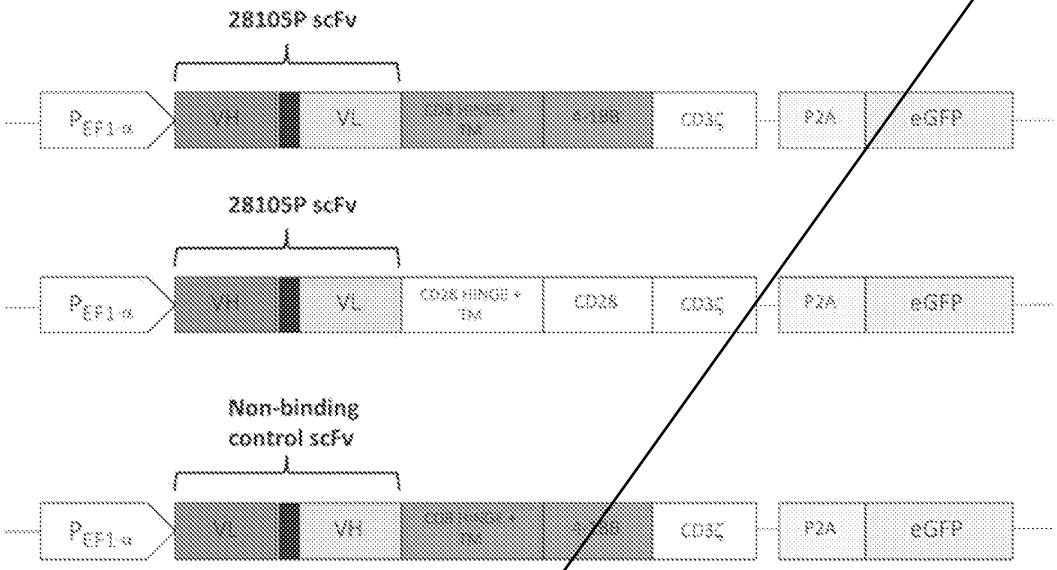
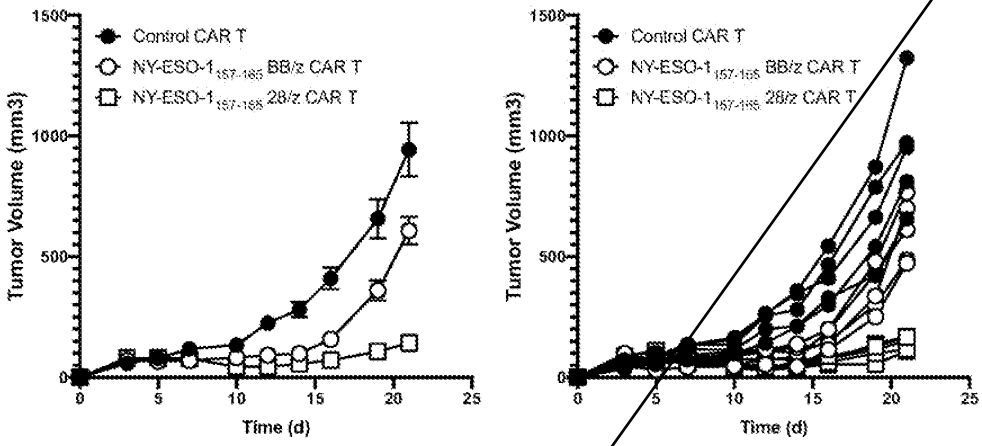

28105P scFv
$P_{EF1\alpha}$
VH
VL
CD28 HINGE + TM
CD28
CD3ζ
P2A
eGFP
28105P scFv
$P_{EF1\alpha}$
VH
VL
CD28 HINGE + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
$P_{EF1\alpha}$
VL
VH
CD28 HINGE + TM
CD28
CD3ζ
P2A
eGFP

Figure 6 (cont.)

B.

Day 0-21 tumor curves



Day 0-38 tumor curves

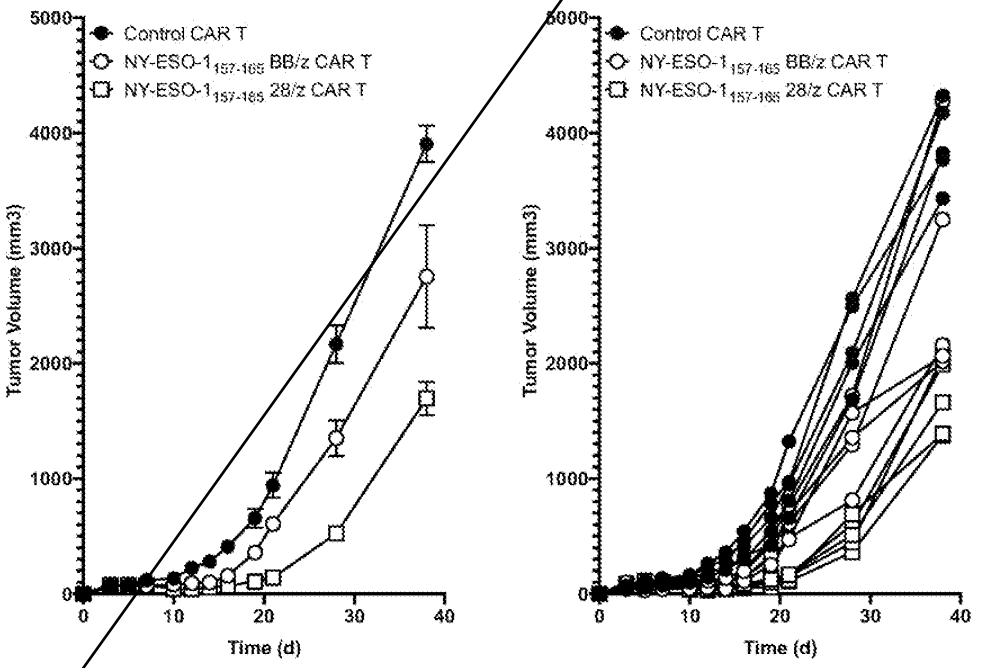


Figure 7

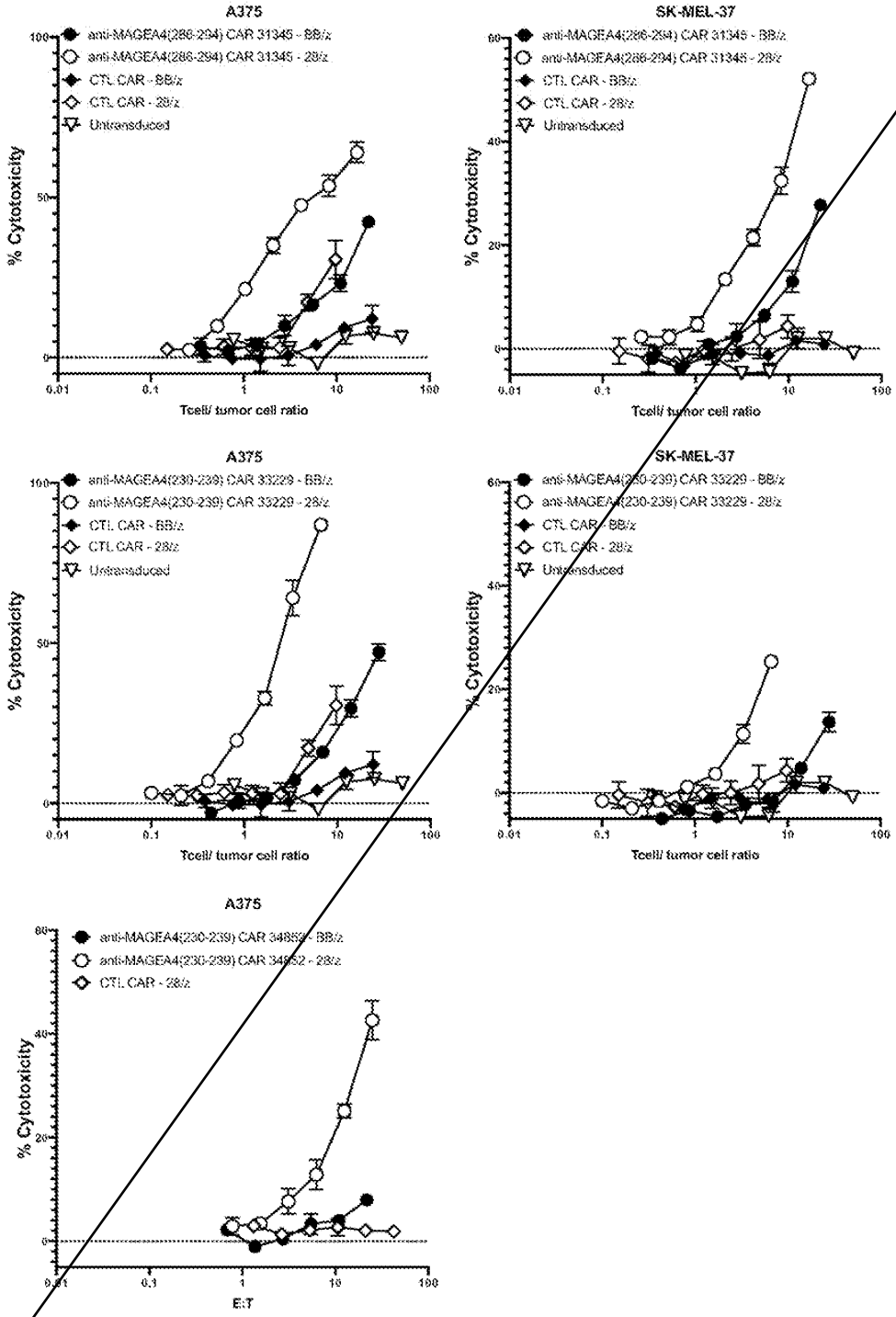

A375
anti-MAGEA4(286-294) CAR 31345 - BB/z
anti-MAGEA4(286-294) CAR 31345 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
Tcell/ tumor cell ratio
SK-MEL-37
anti-MAGEA4(230-239) CAR 33229 - BB/z
anti-MAGEA4(230-239) CAR 33229 - 28/z
A375
anti-MAGEA4(230-239) CAR 34852 - BB/z
anti-MAGEA4(230-239)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

Figure 8

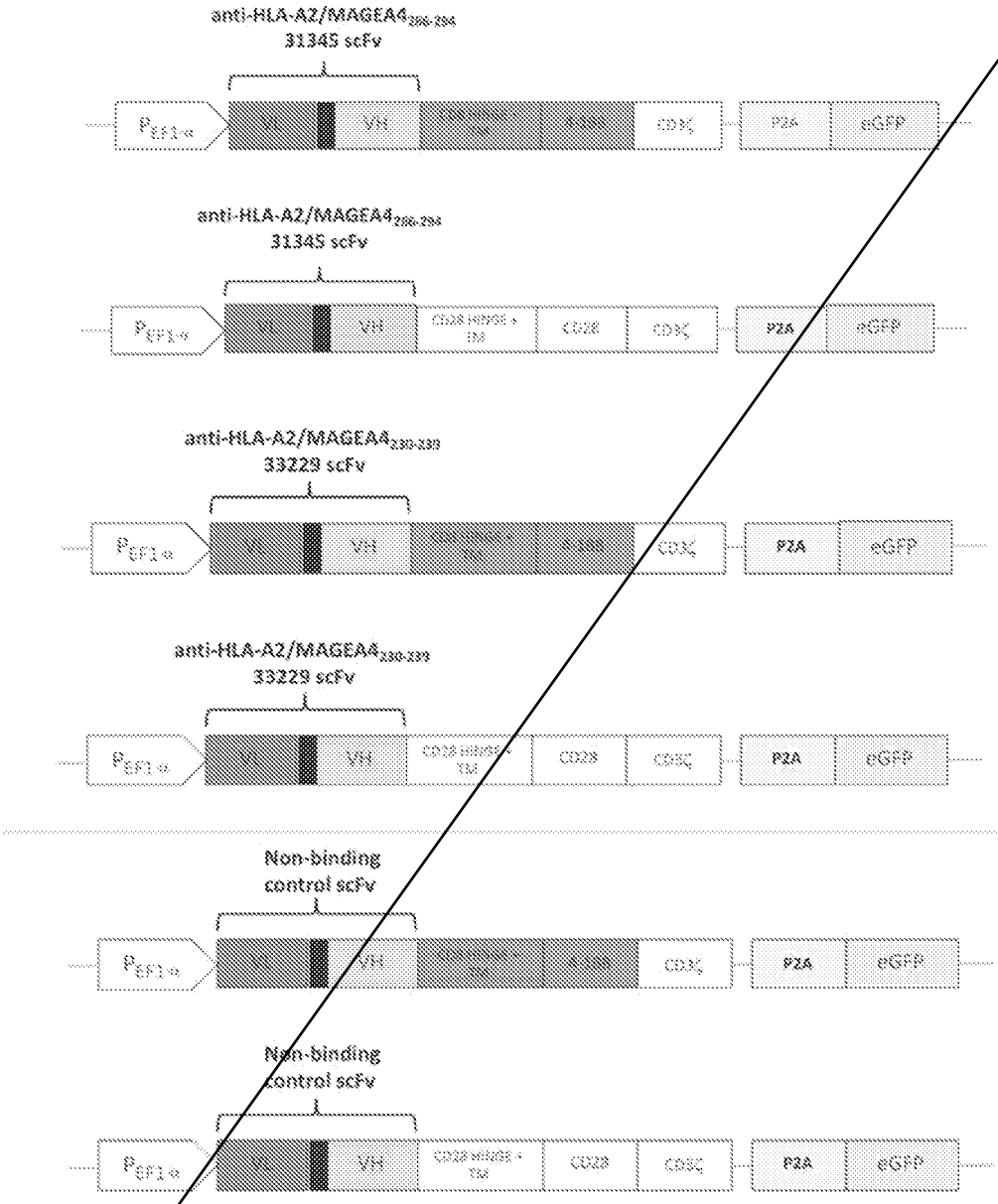

anti-HLA-A2/MAGEA4286-294
31345 scFv
VL
VH
CD28 HINGE + TM
CD28
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4230-239
33229 scFv
Non-binding control scFv

Figure 9

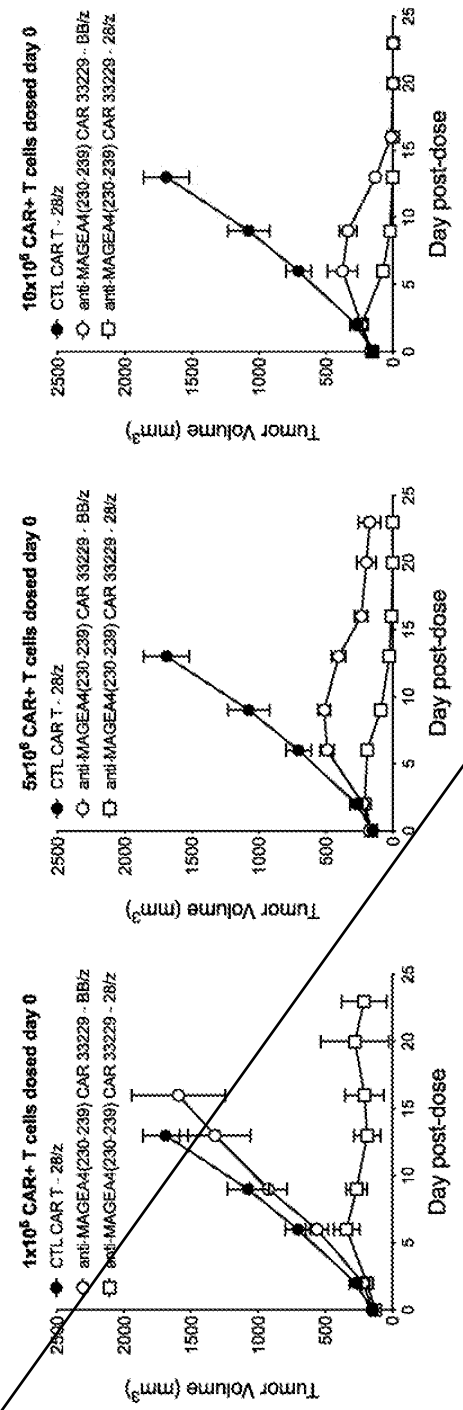

1x10^6 CAR+ T cells dosed day 0
5x10^6 CAR+ T cells dosed day 0
10x10^6 CAR+ T cells dosed day 0
CTL CAR T - 28/z
anti-MAGEA4(230-239) CAR 33229 - BB/z
anti-MAGEA4(230-239) CAR 33229 - 28/z
Tumor Volume (mm^3)
Day post-dose
0
500
1000
1500
2000
2500
0
5
10
15
20
25

Figure 10

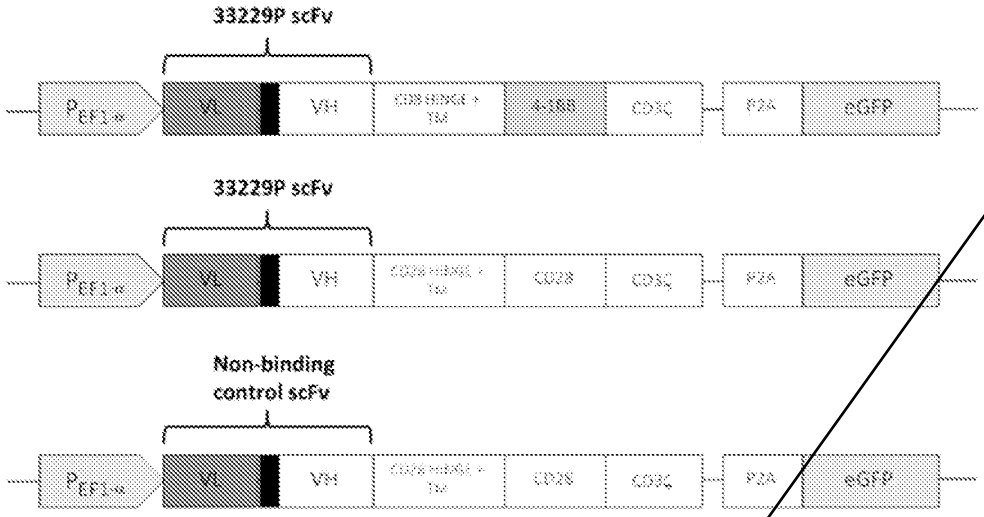

33229P scFv
VL
VH
4-1BB
CD3ζ
P2A
eGFP
33229P scFv
VL
VH
CD28
CD3ζ
P2A
eGFP
Non-binding
control scFv
VL
VH
CD28
CD3ζ
P2A
eGFP

Figure 11

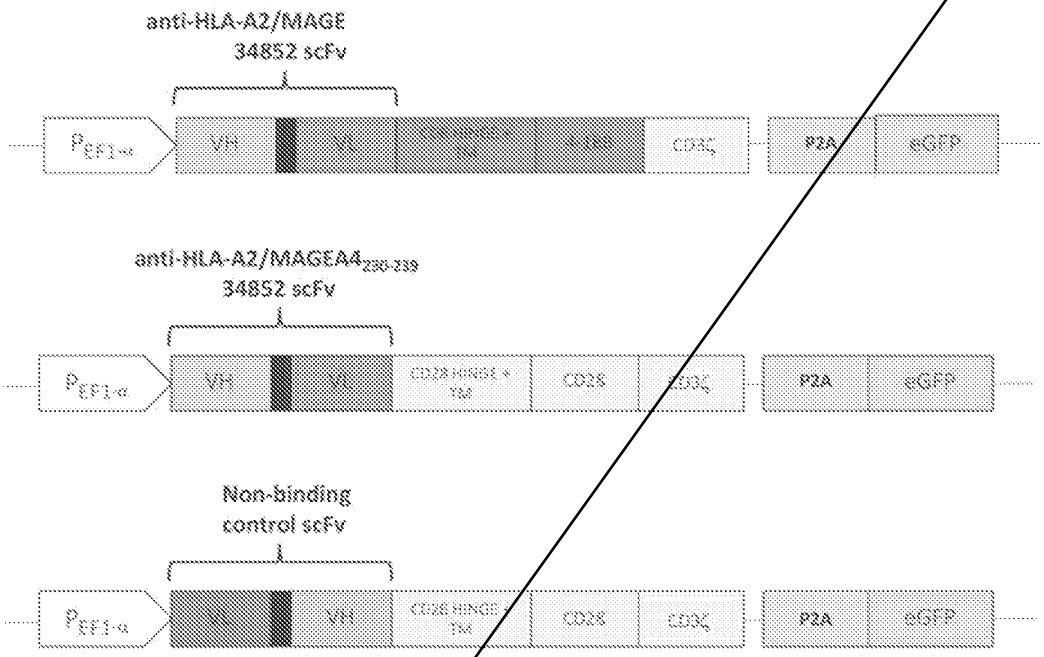


Figure 12

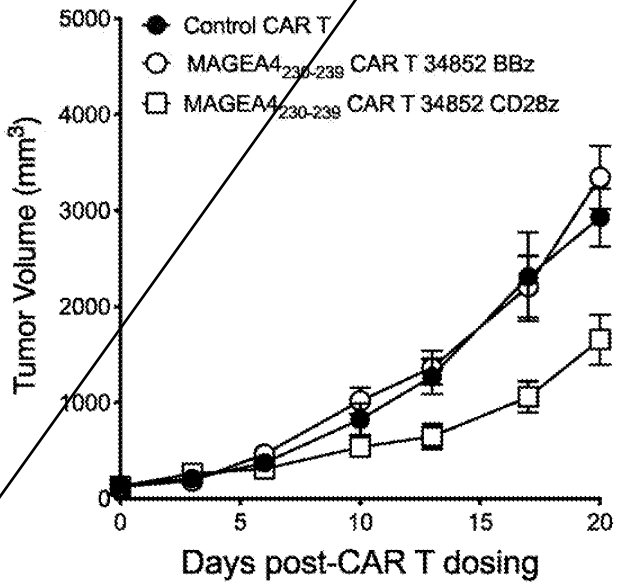


FIG. 1A

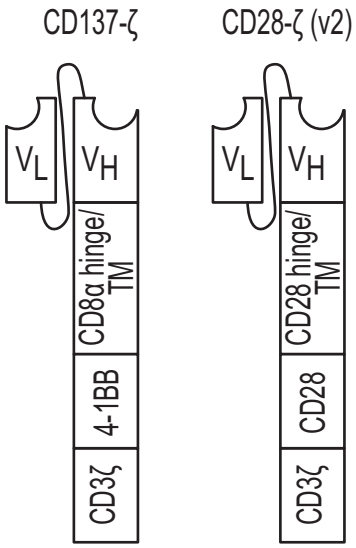
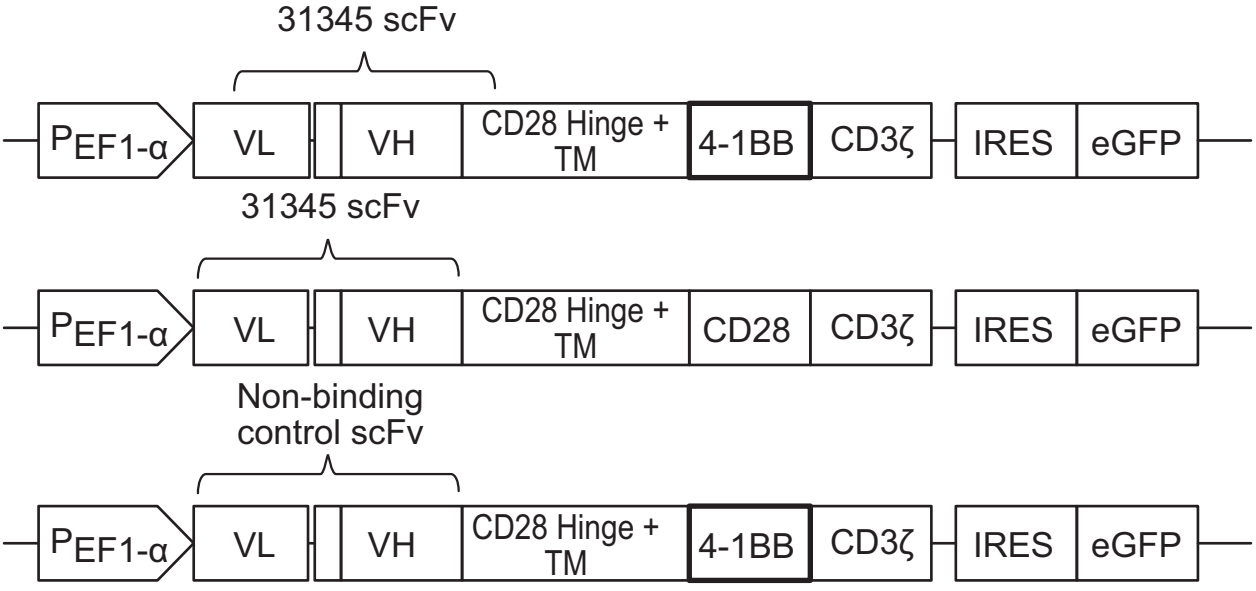


FIG. 1B



CAR construct designs

FIG. 2A

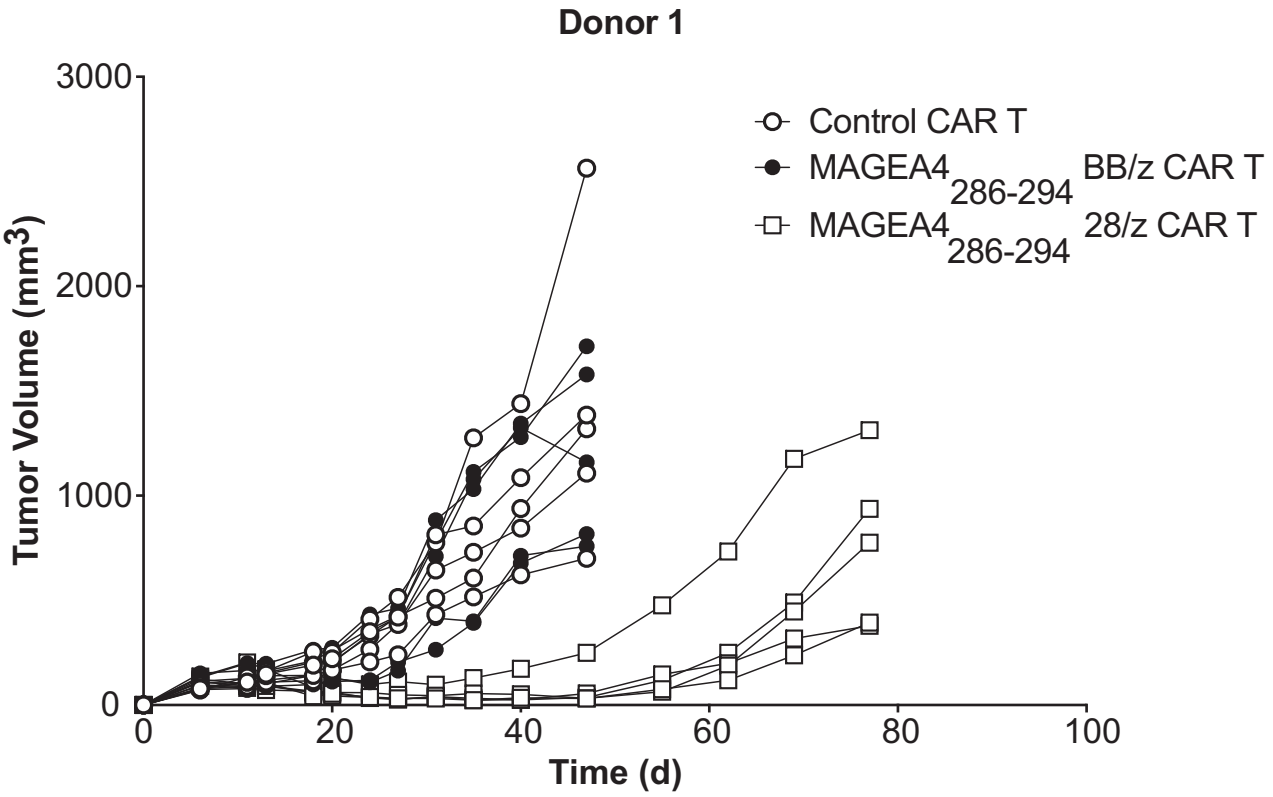


FIG. 2A Cont.

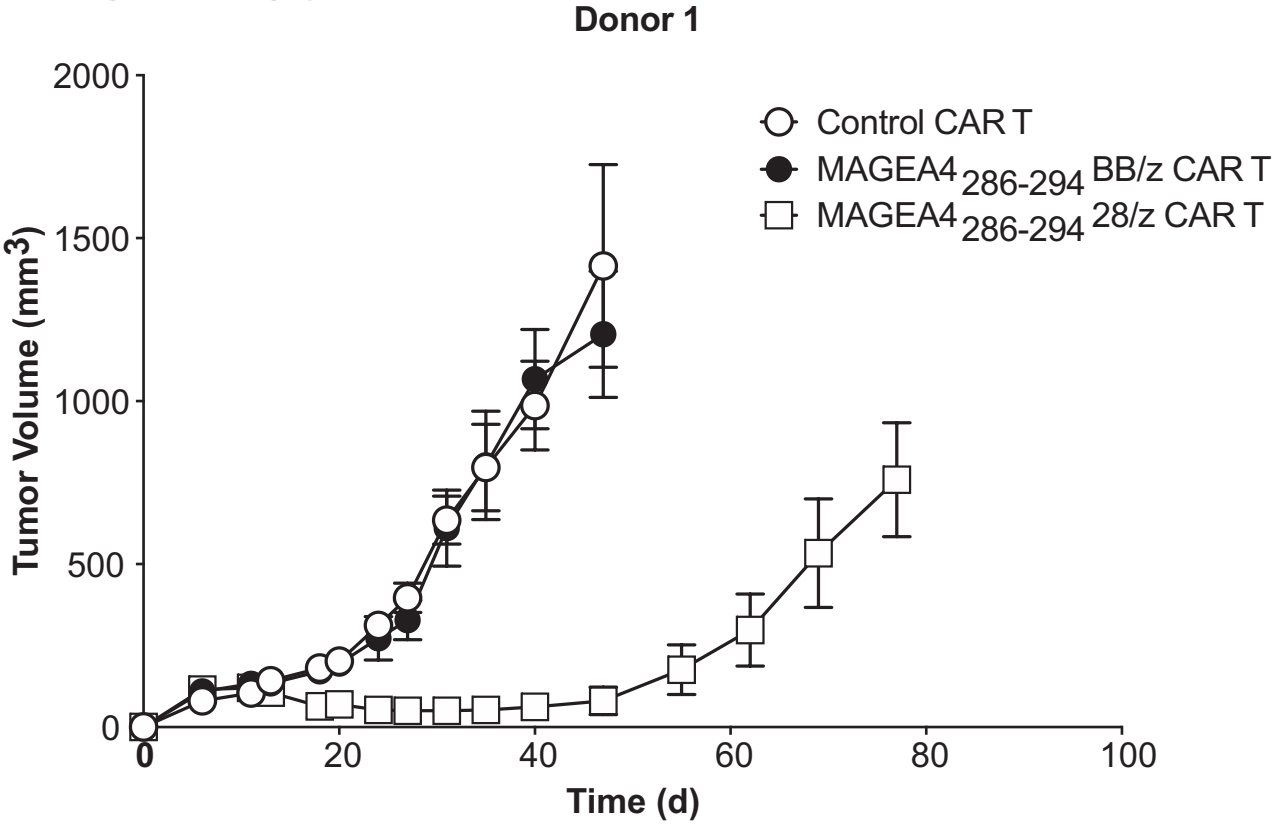


FIG. 2B

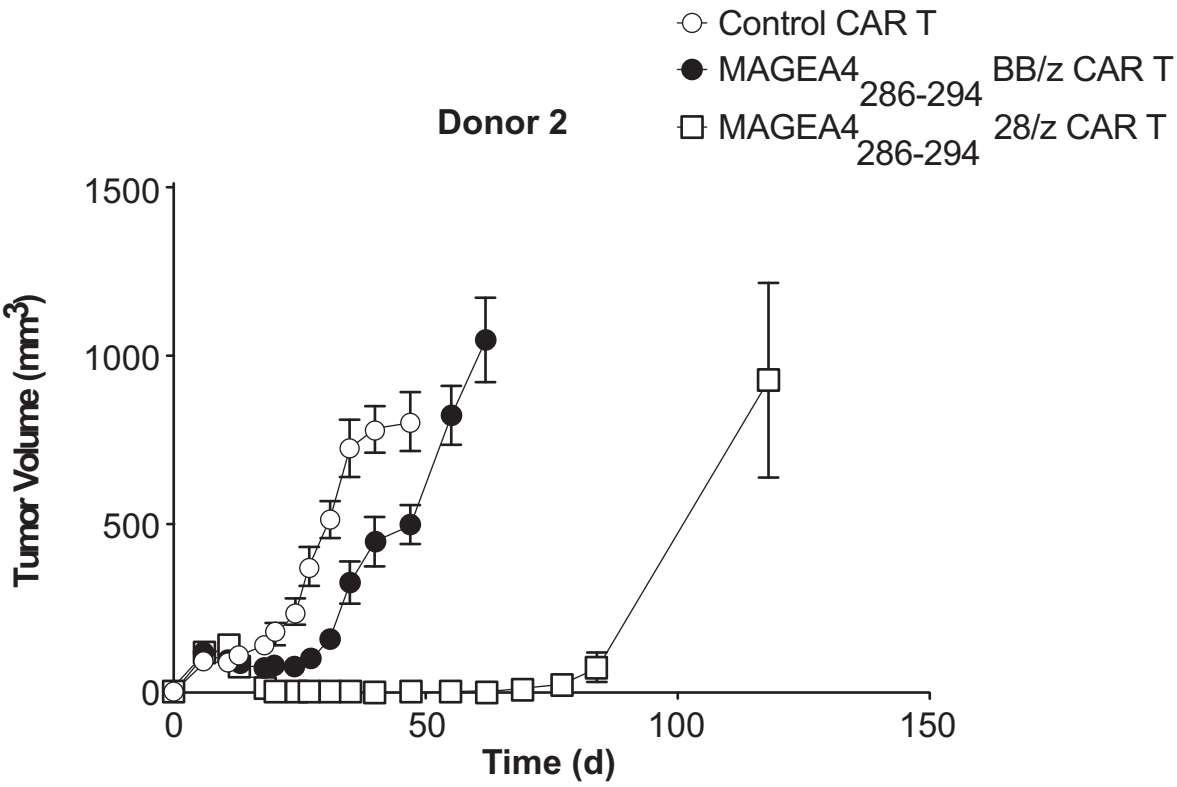


FIG.2B Cont.

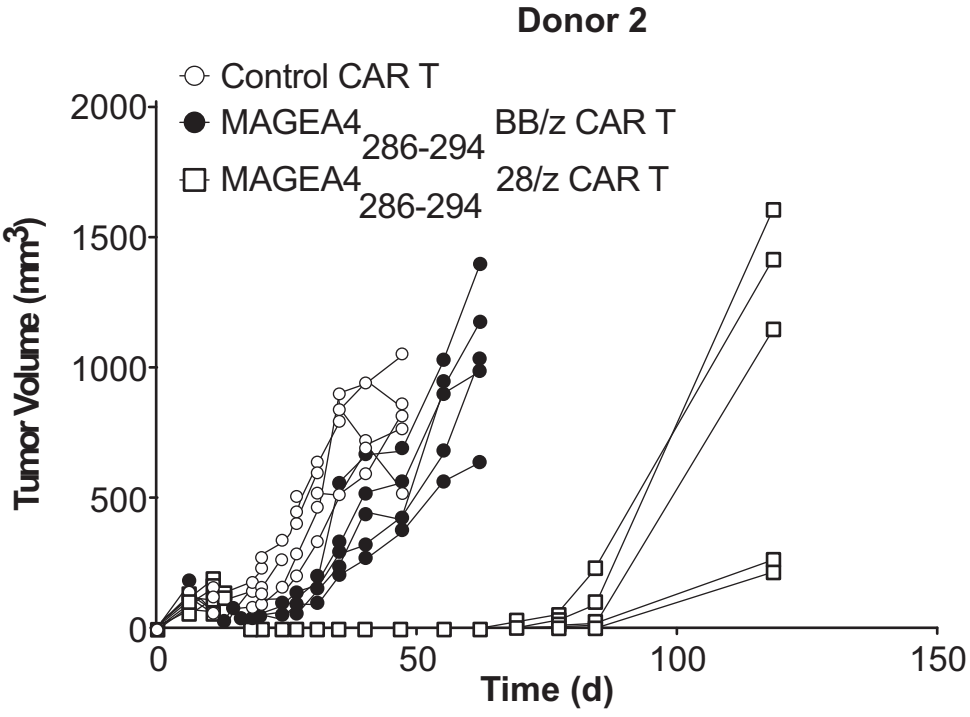


FIG. 3A

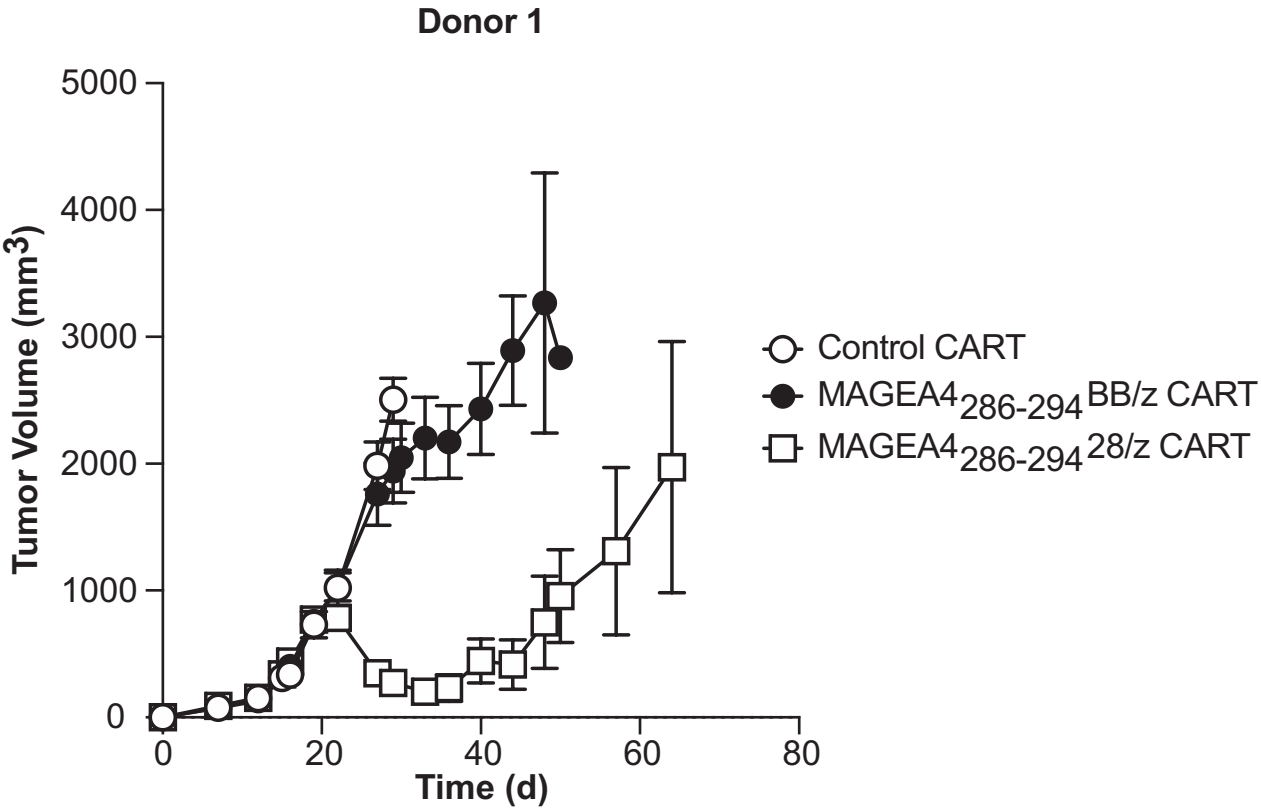


FIG. 3A Cont.

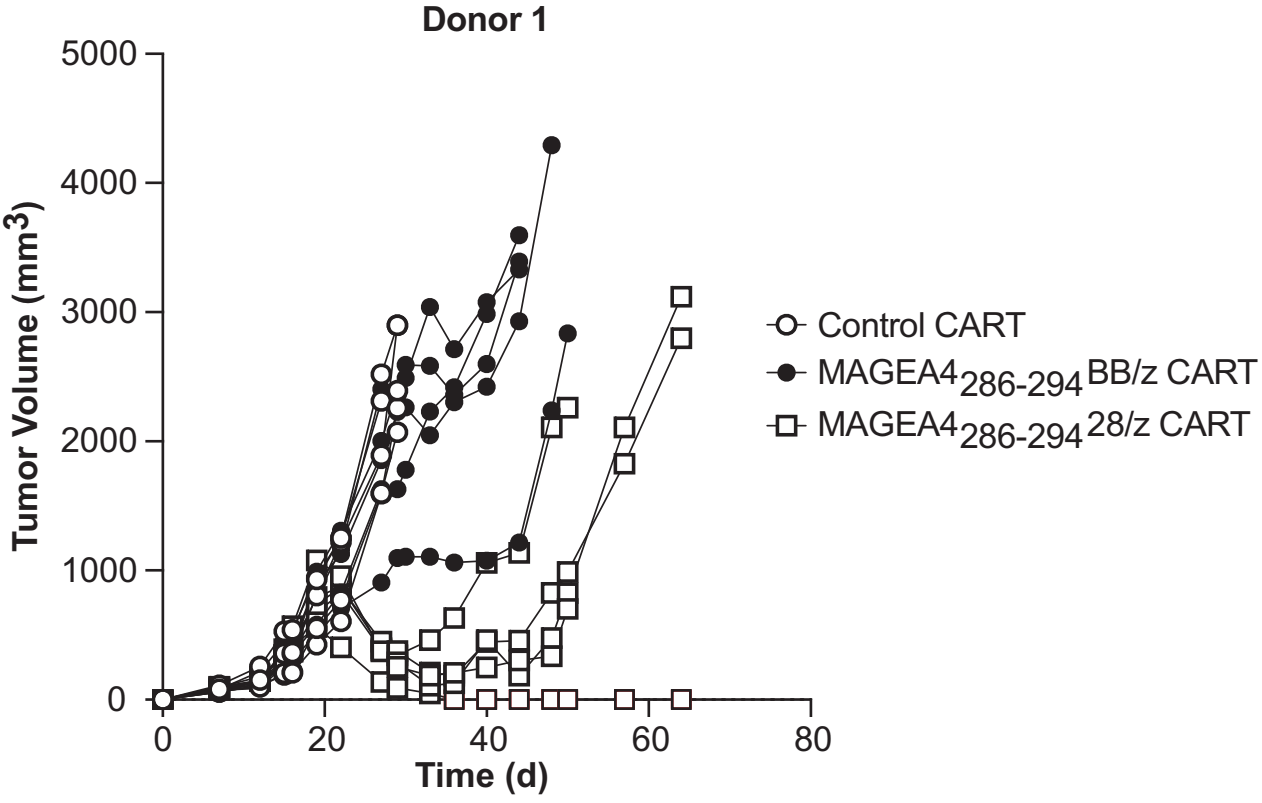


FIG. 3B

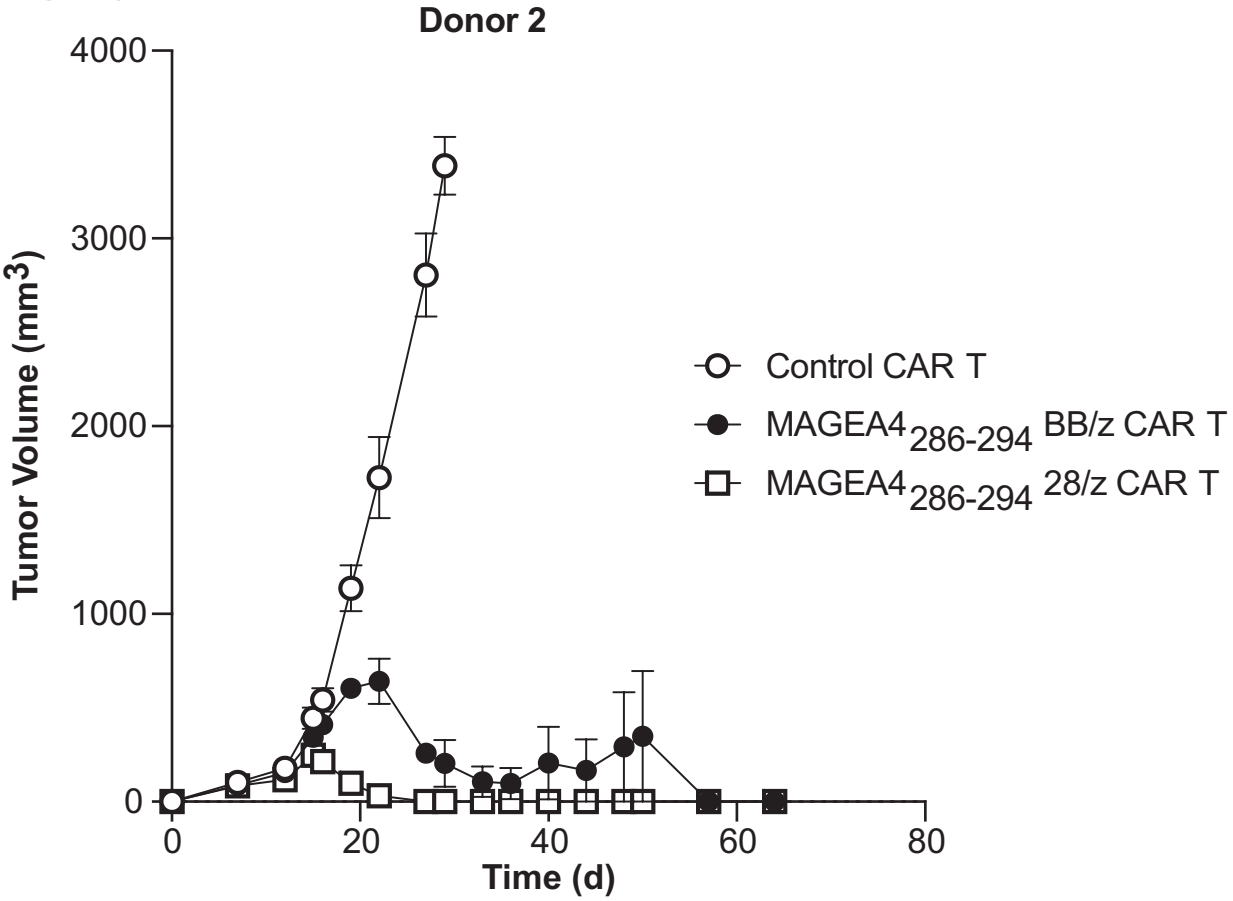


FIG. 3B Cont.

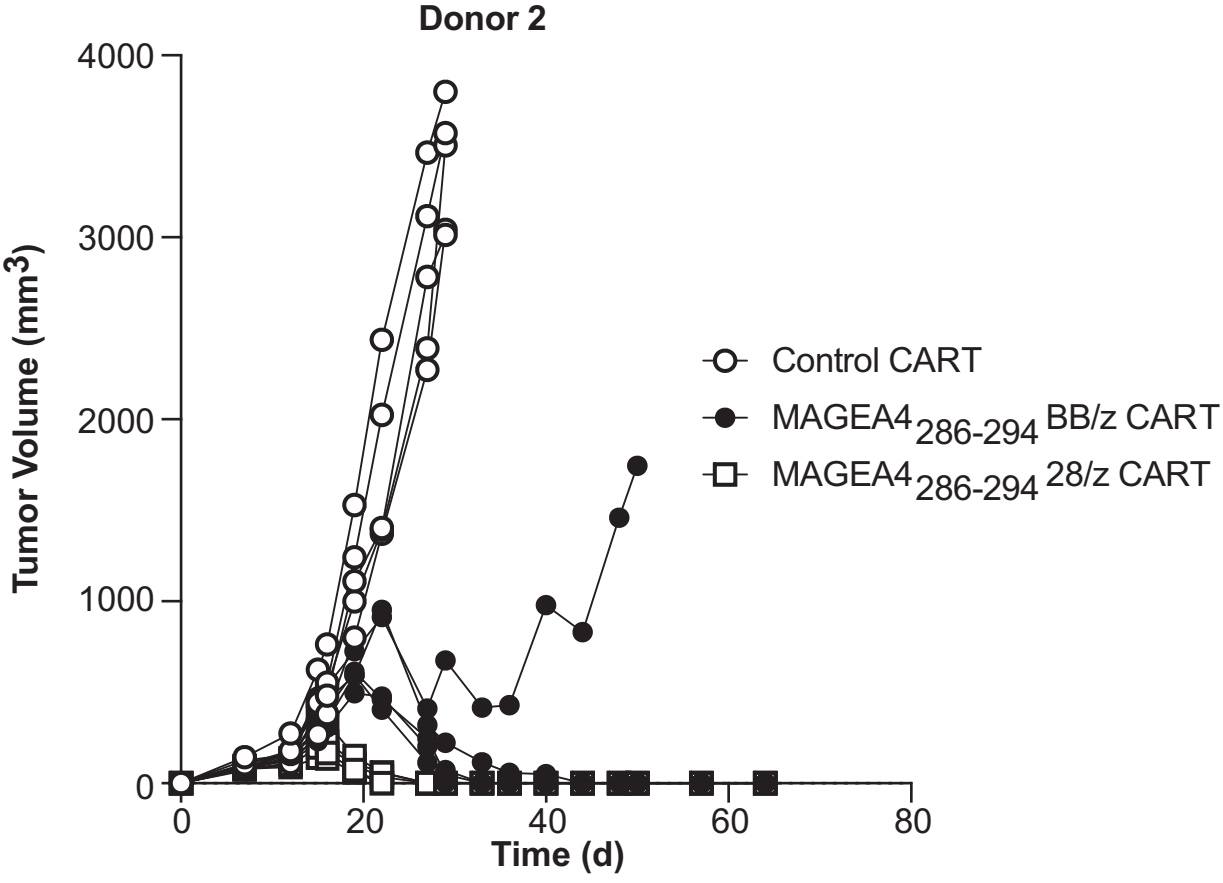


FIG. 4

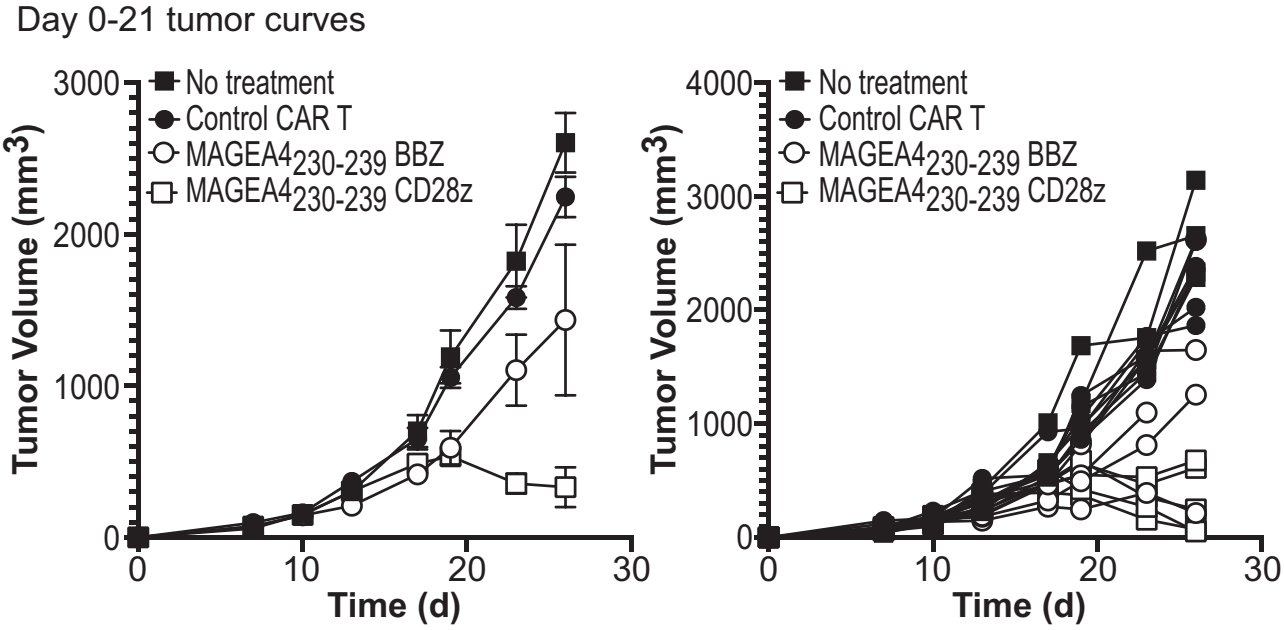


FIG. 4 Cont.

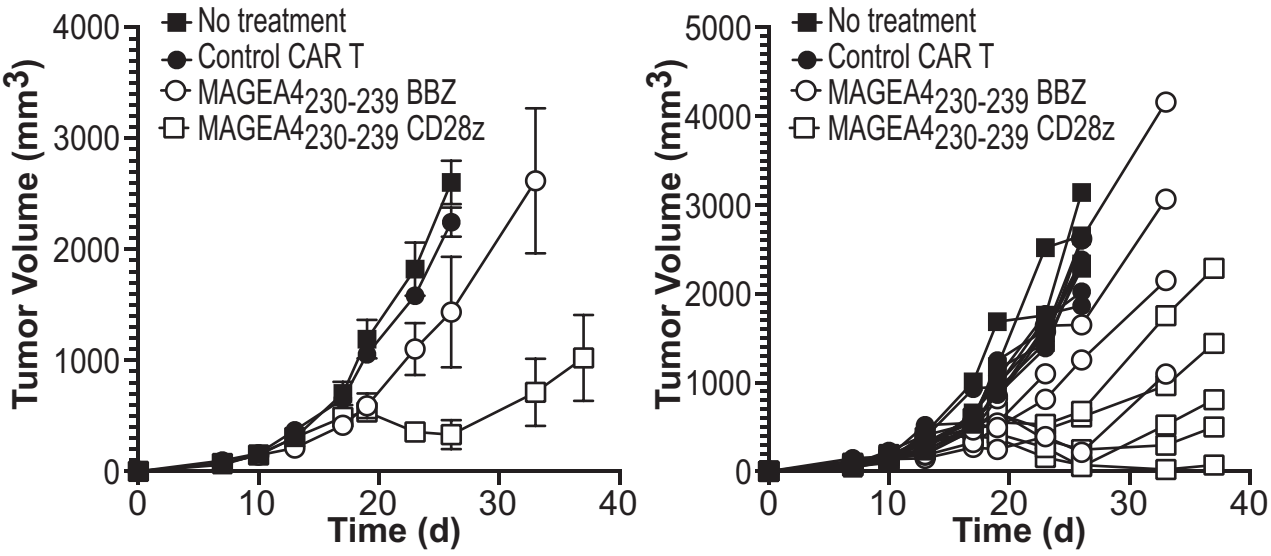


FIG. 4B

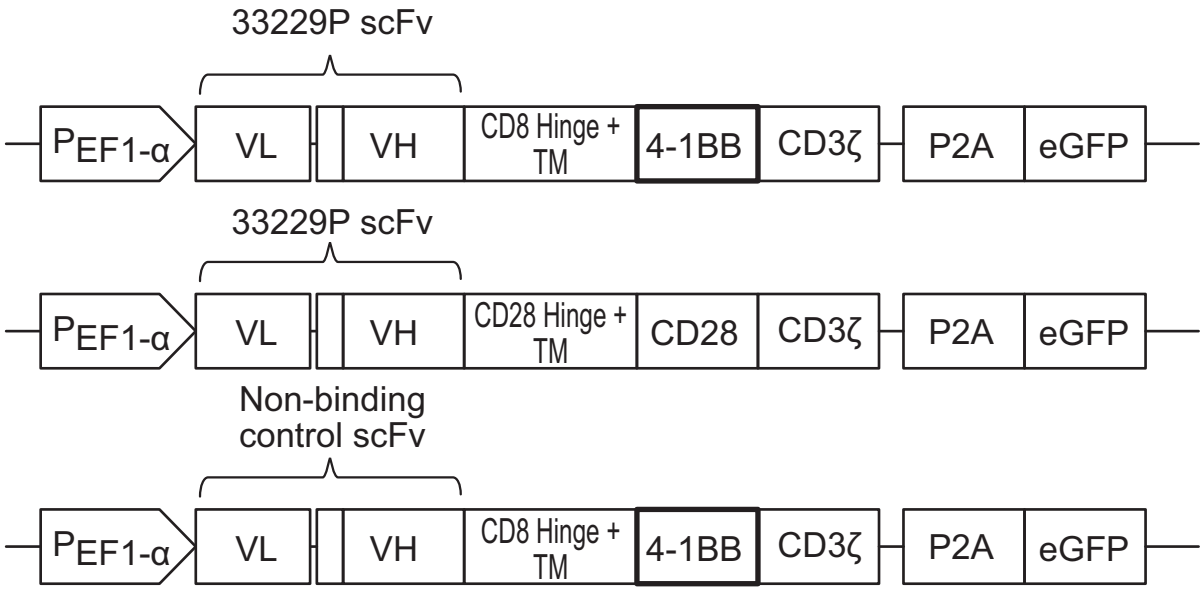

33229P scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
33229P scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP

FIG. 5A

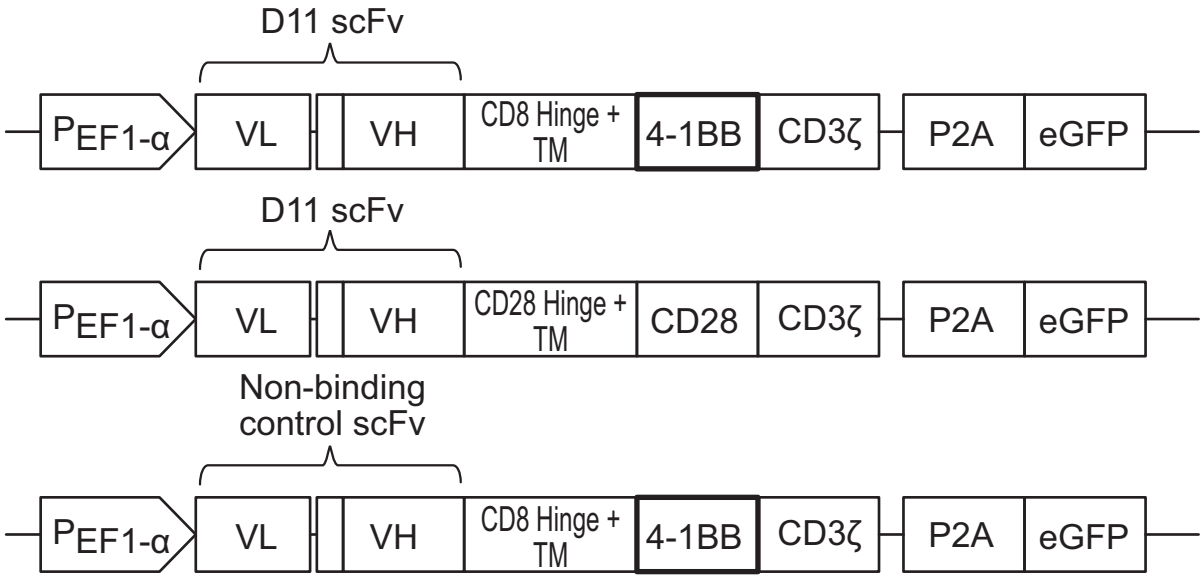
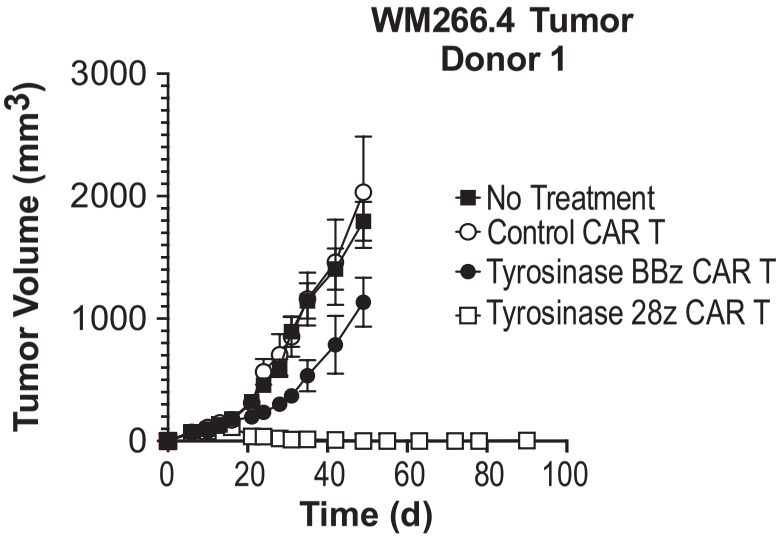
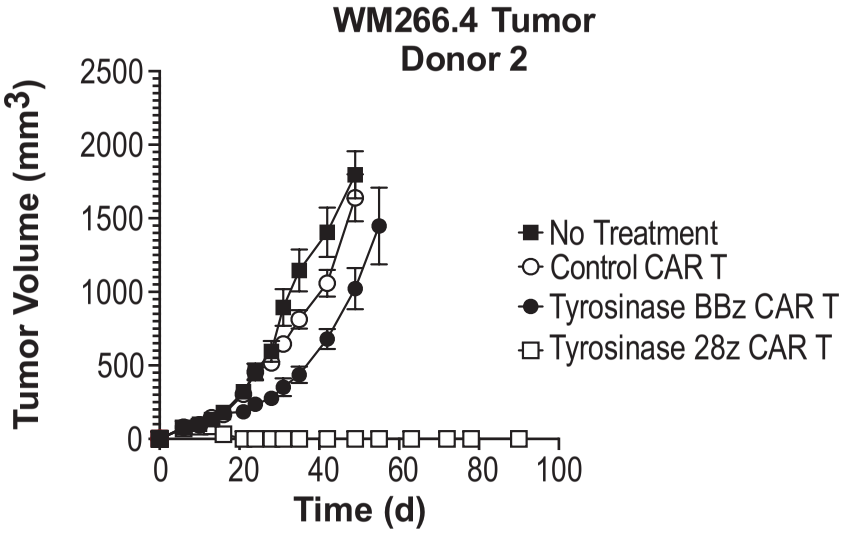
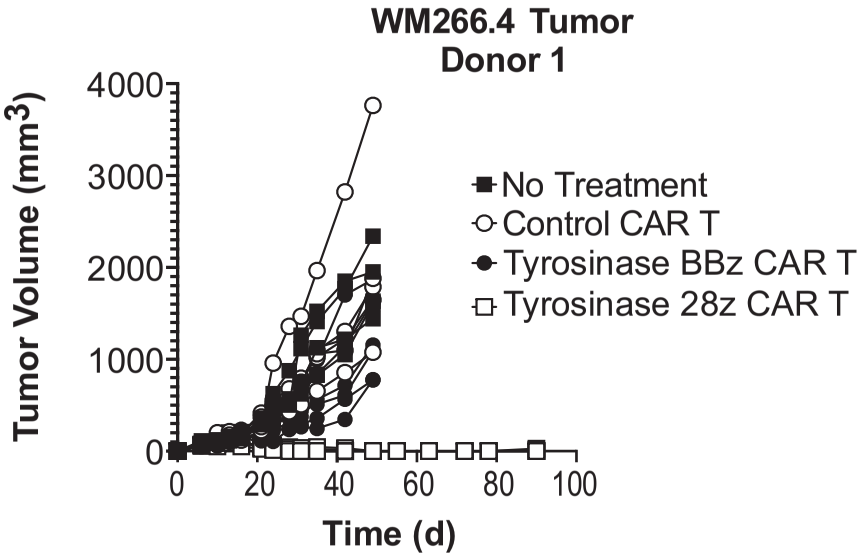

D11 scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
D11 scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP

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

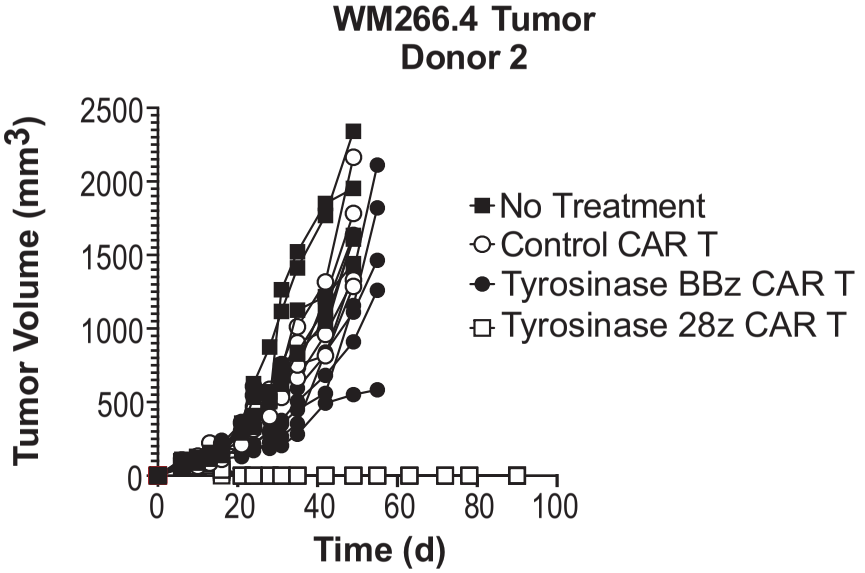
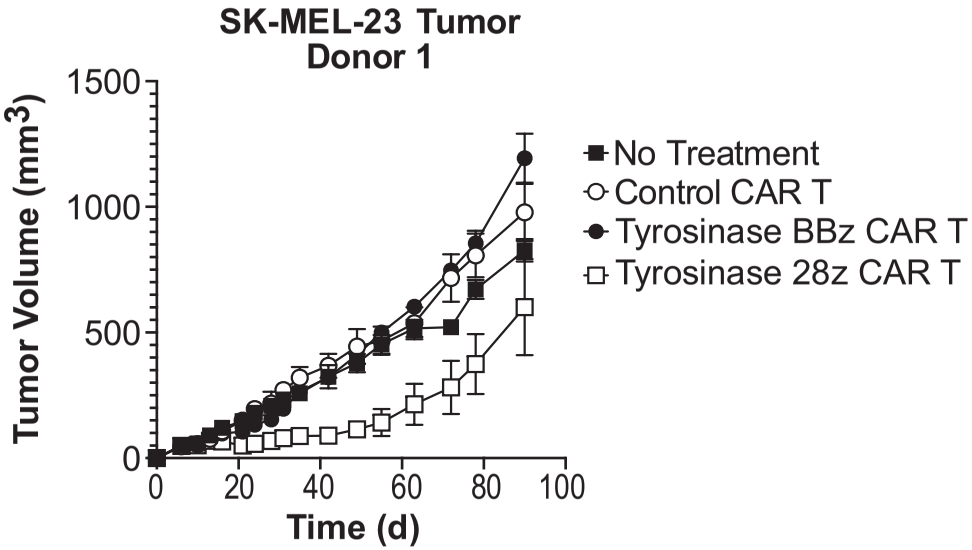
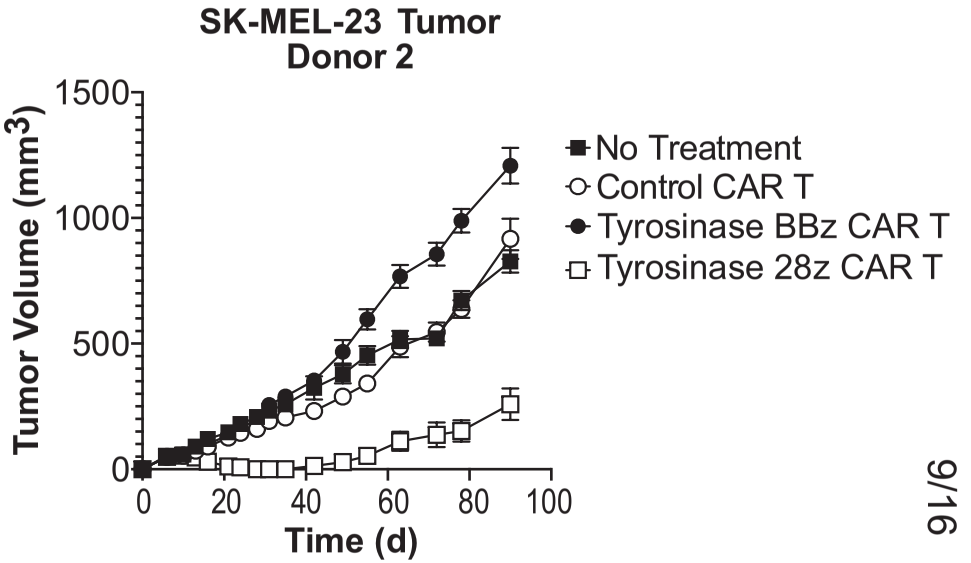
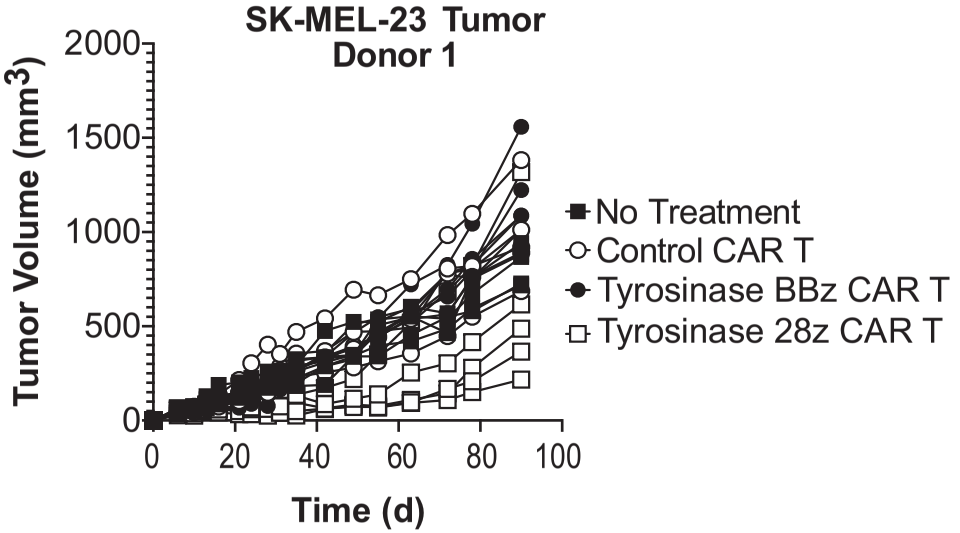

WM266.4 Tumor
Donor 2
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T

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

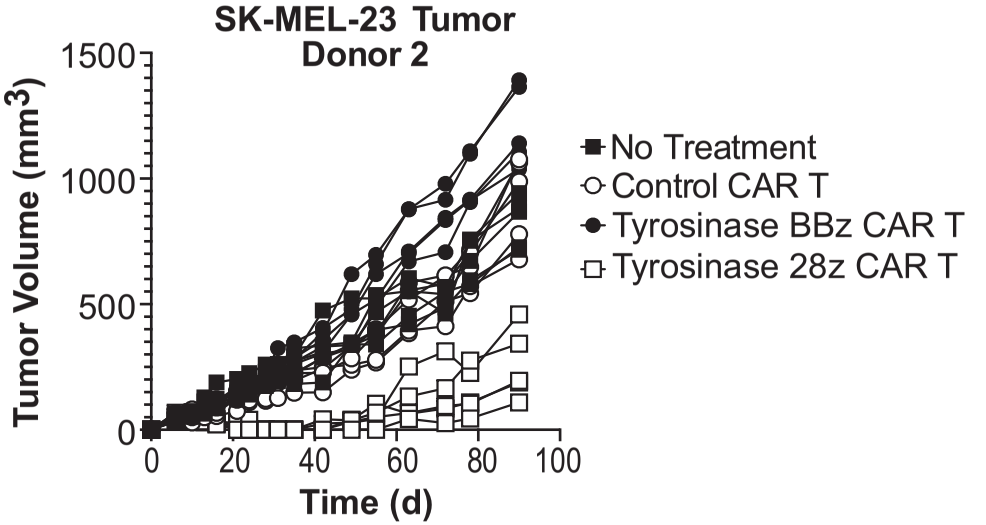

SK-MEL-23 Tumor
Donor 2
Tumor Volume (mm3)
1500
1000
500
0
0
20
40
60
80
100
Time (d)
No Treatment
Control CAR T
Tyrosinase BBz CAR T
Tyrosinase 28z CAR T

FIG. 6A

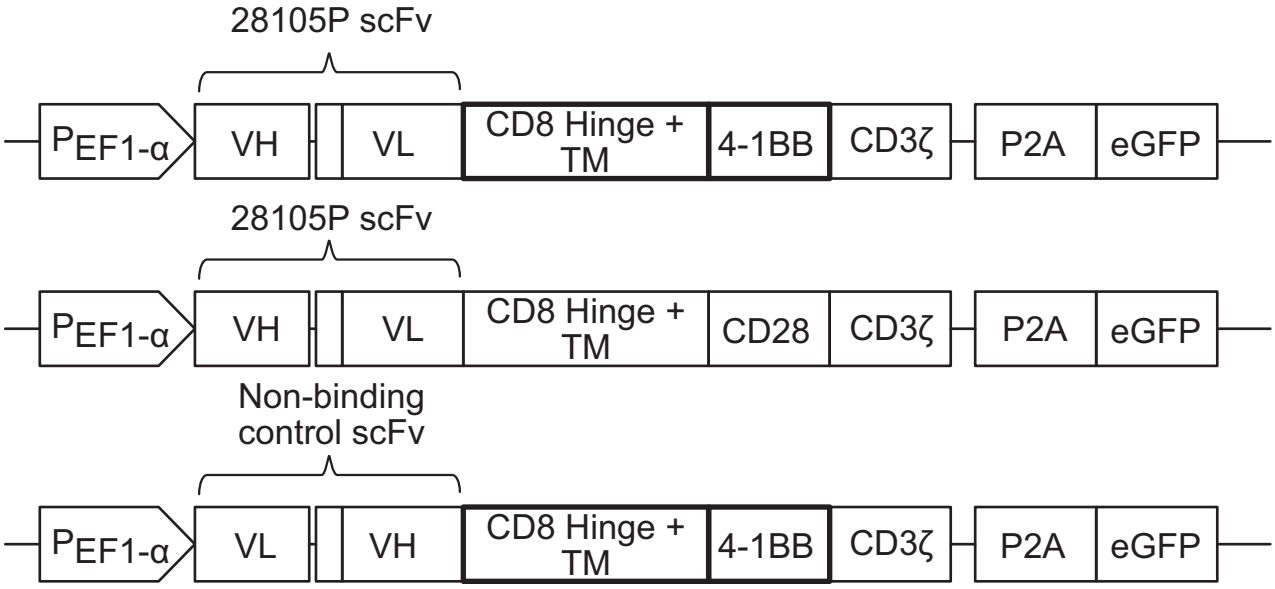

28105P scFv
PEF1-α
VH
VL
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
28105P scFv
PEF1-α
VH
VL
CD8 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP

FIG. 6B

Day 0-21 tumor curves

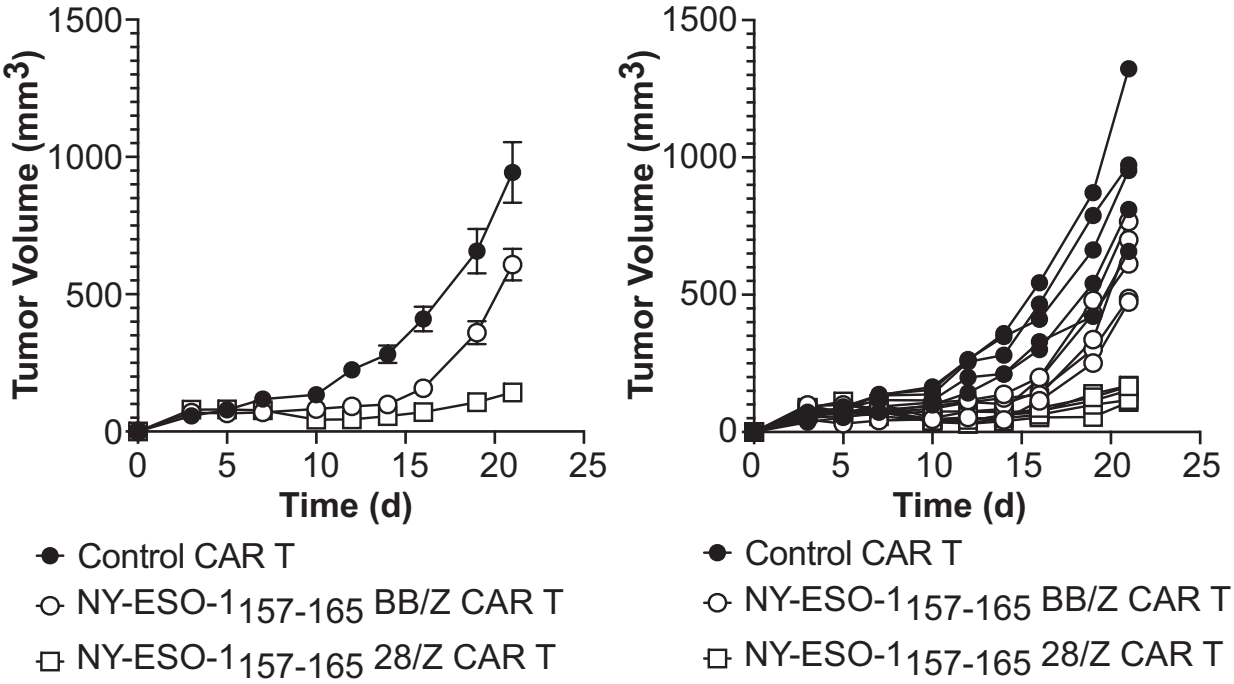


FIG. 6B Cont.

Day 0-38 tumor curves

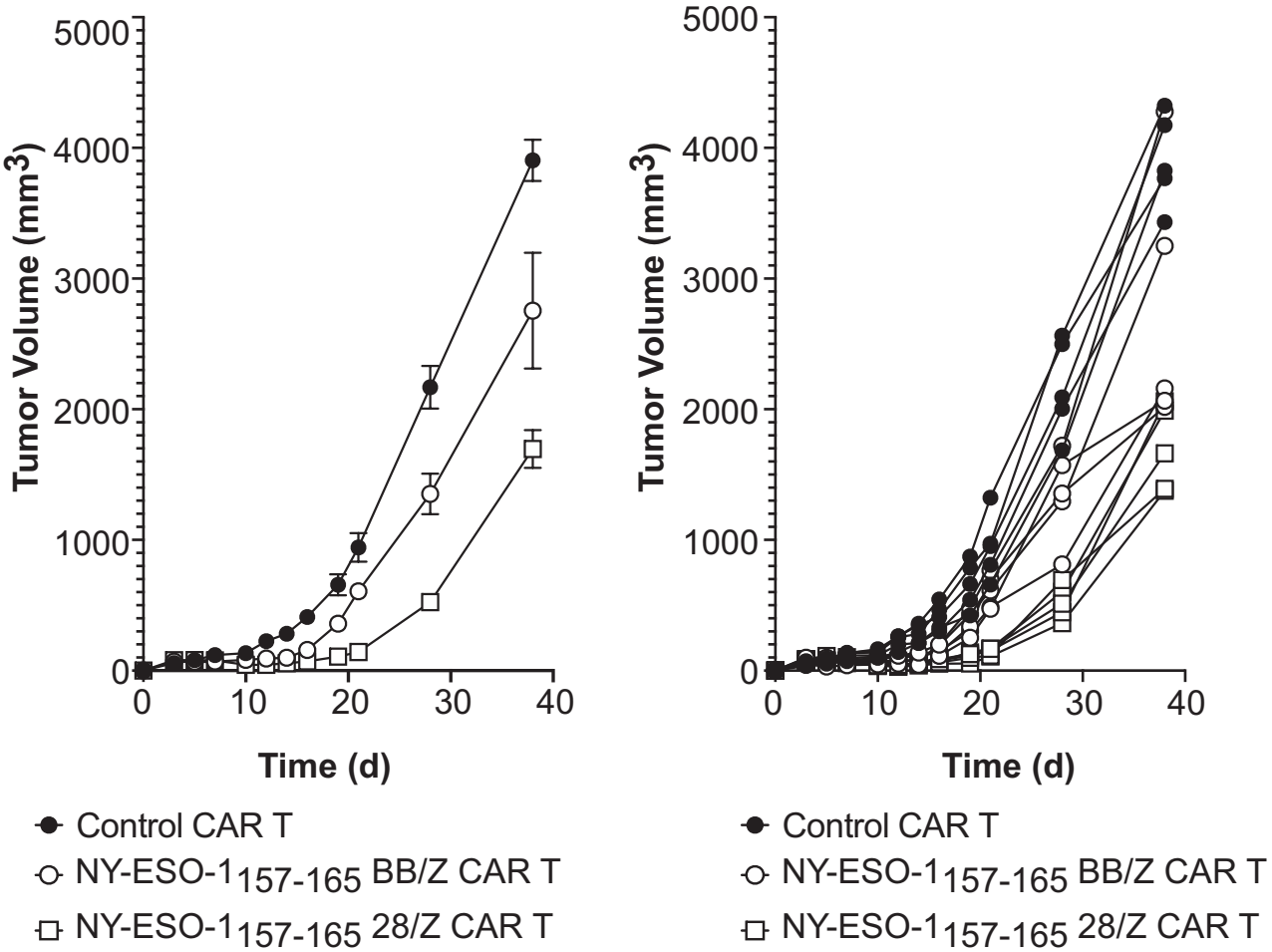


FIG. 7

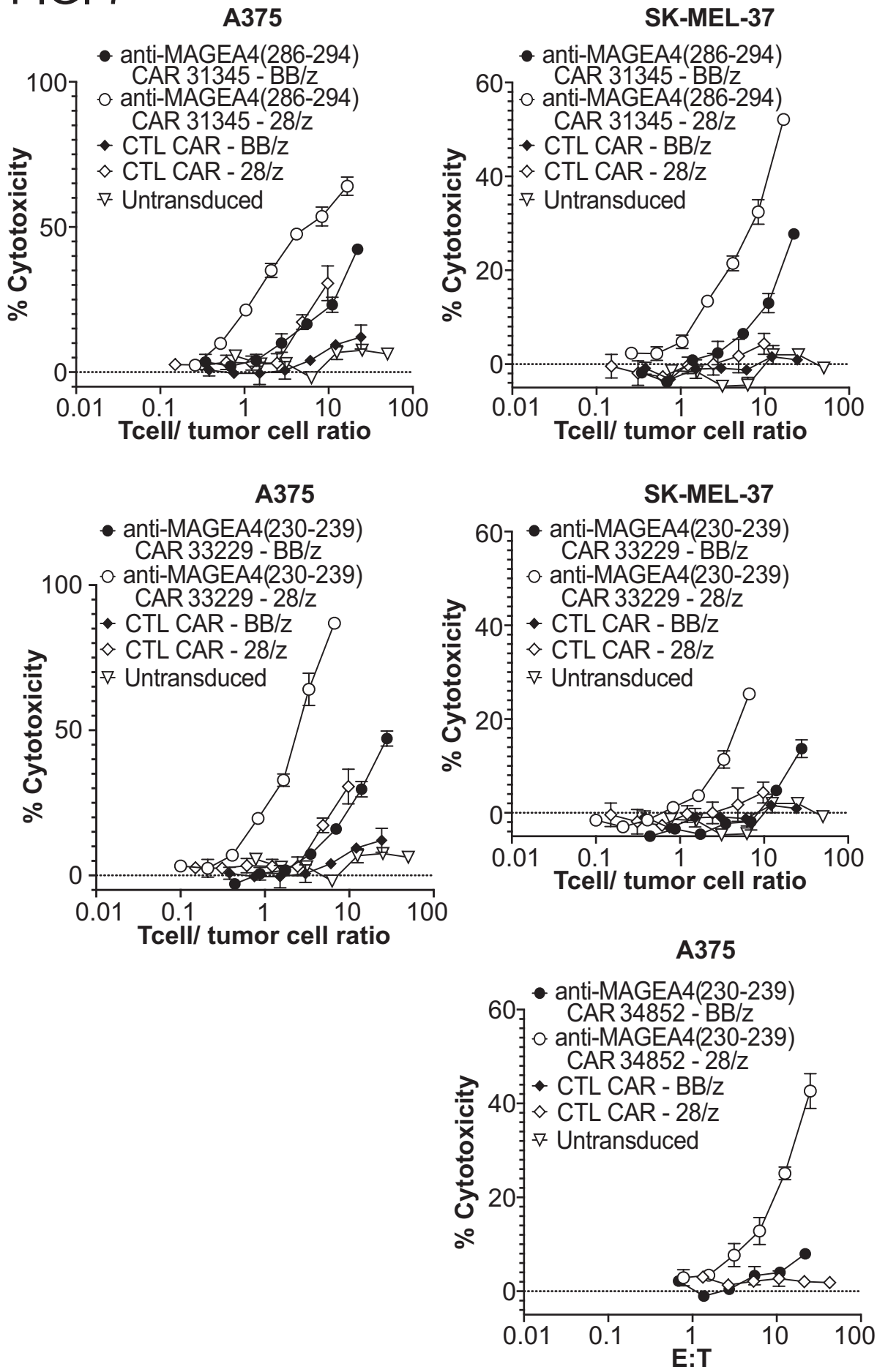

A375
anti-MAGEA4(286-294) CAR 31345 - BB/z
anti-MAGEA4(286-294) CAR 31345 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
Tcell/ tumor cell ratio
SK-MEL-37
anti-MAGEA4(286-294) CAR 31345 - BB/z
anti-MAGEA4(286-294) CAR 31345 - 28/z
CTL CAR - BB/z
CTL CAR - 28/z
Untransduced
% Cytotoxicity
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

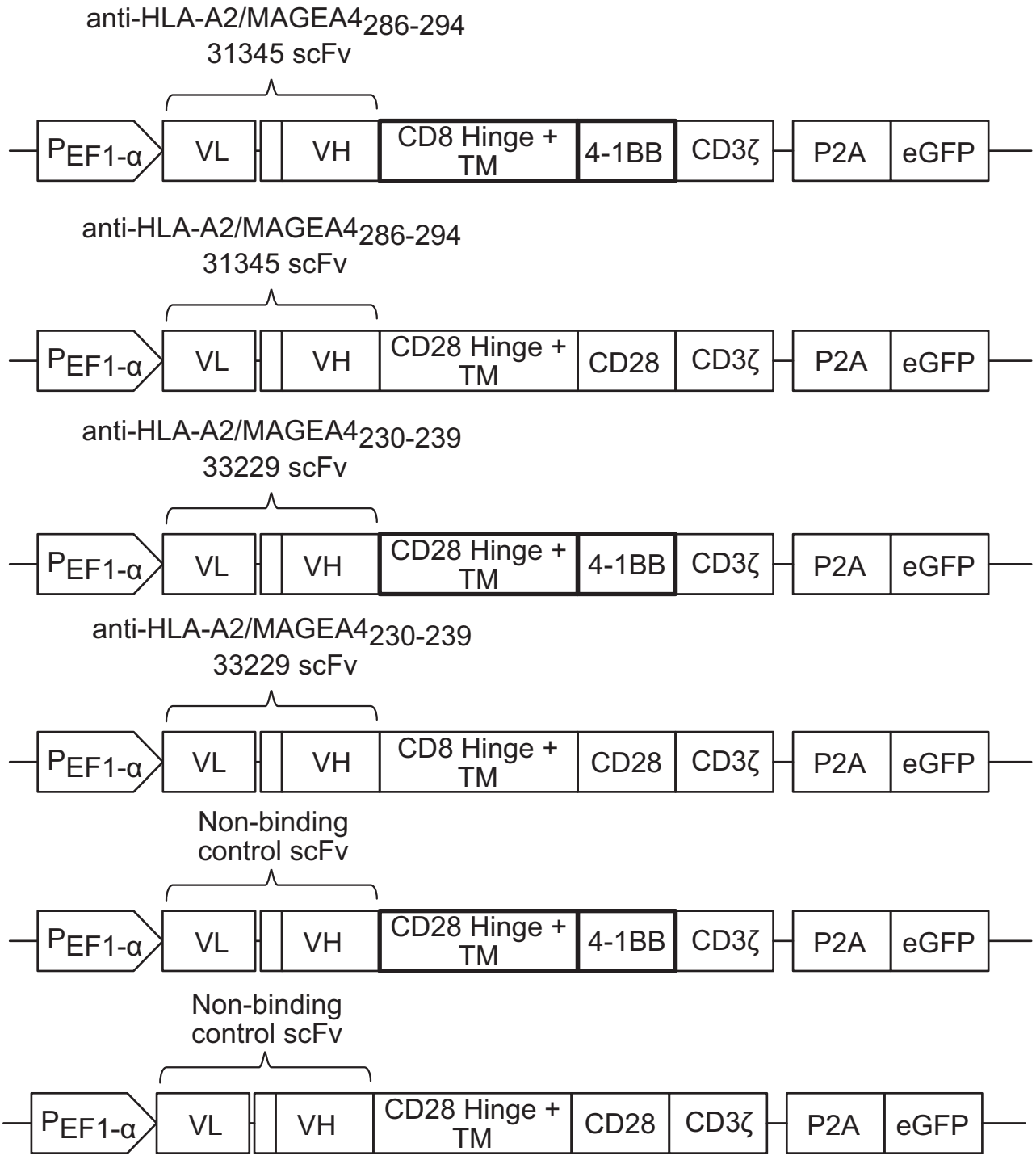

anti-HLA-A2/MAGEA4286-294
31345 scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4286-294
31345 scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4230-239
33229 scFv
PEF1-α
VL
VH
CD28 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4230-239
33229 scFv
PEF1-α
VL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

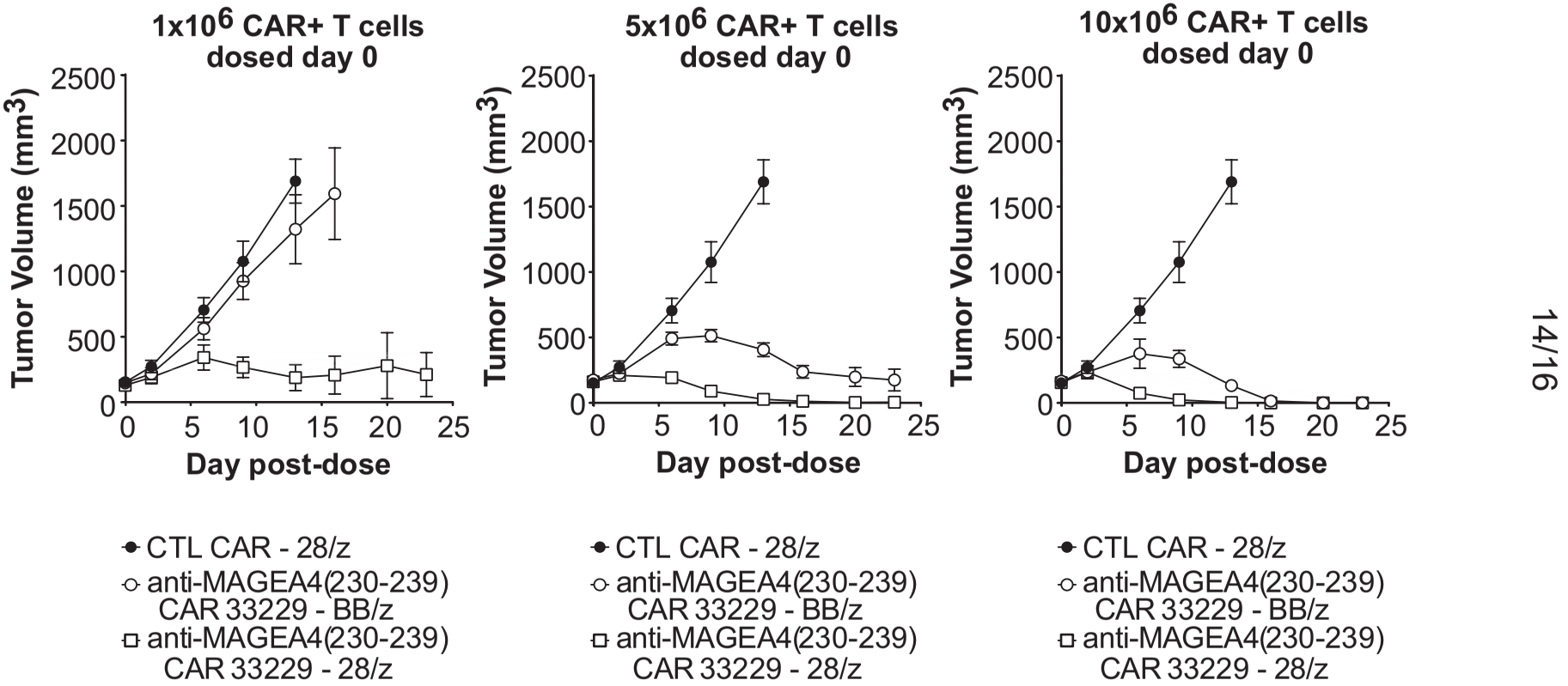

1x10^6 CAR+ T cells dosed day 0
5x10^6 CAR+ T cells dosed day 0
10x10^6 CAR+ T cells dosed day 0
Tumor Volume (mm^3)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Day post-dose
CTL CAR - 28/z
anti-MAGEA4(230-239) CAR 33229 - BB/z
anti-MAGEA4(230-239) CAR 33229 - 28/z

FIG. 10

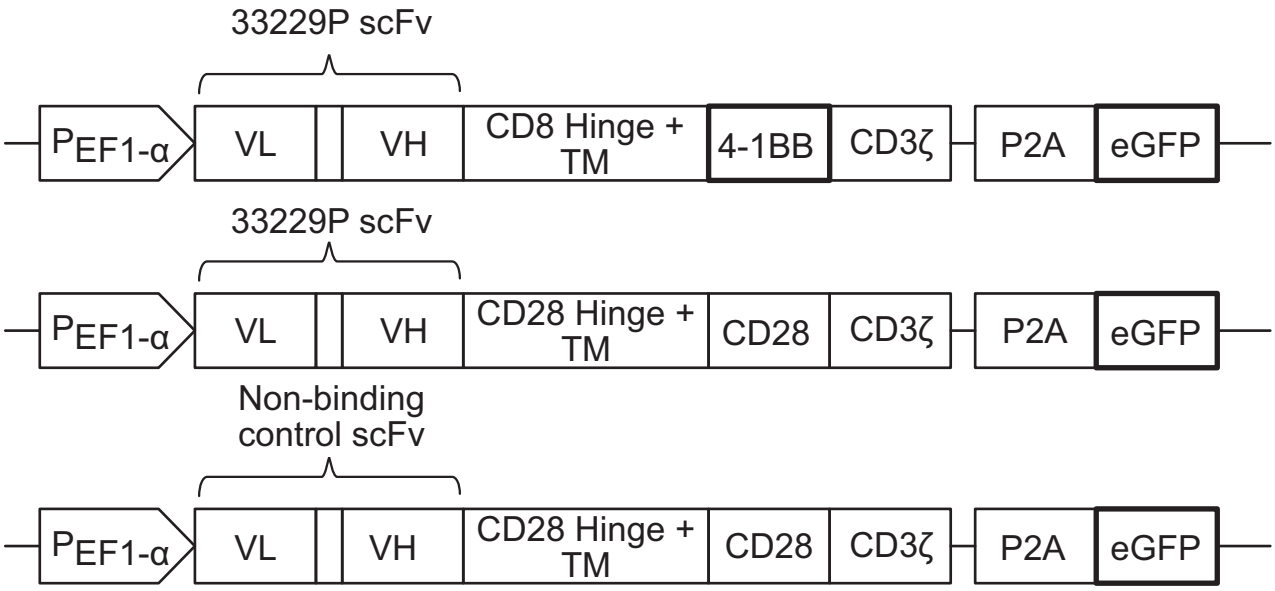

33229P scFv
PEF1-α
VL
VH
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
33229P scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP

FIG. 11

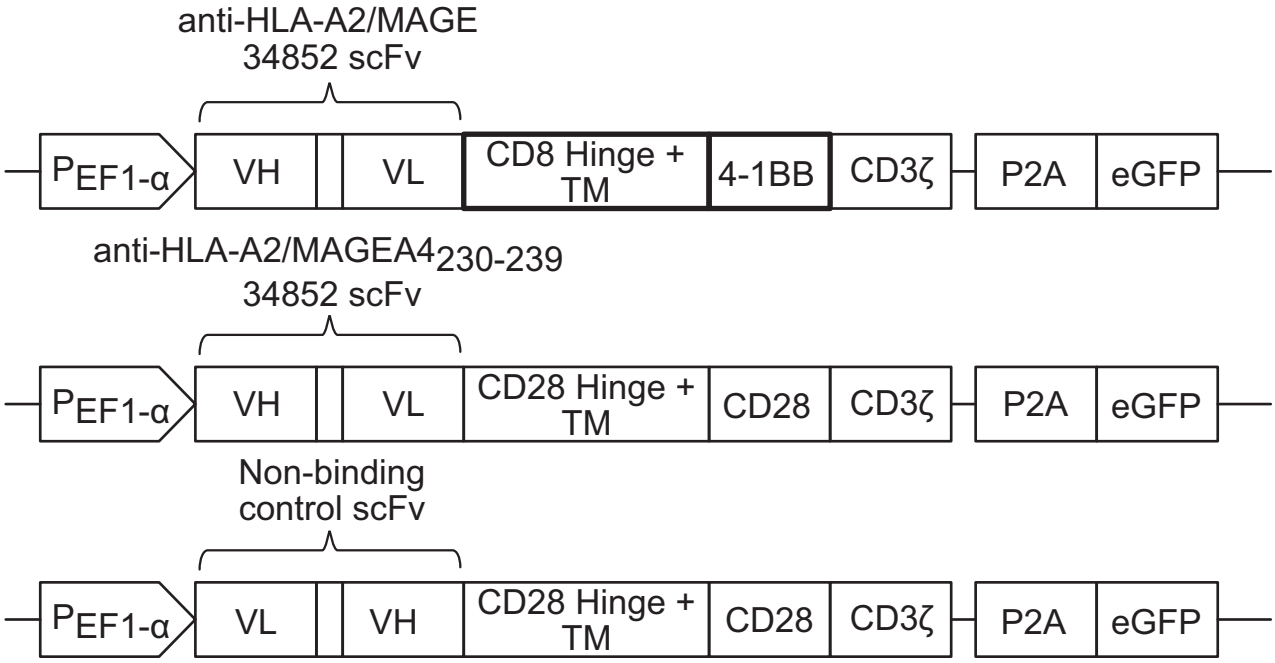
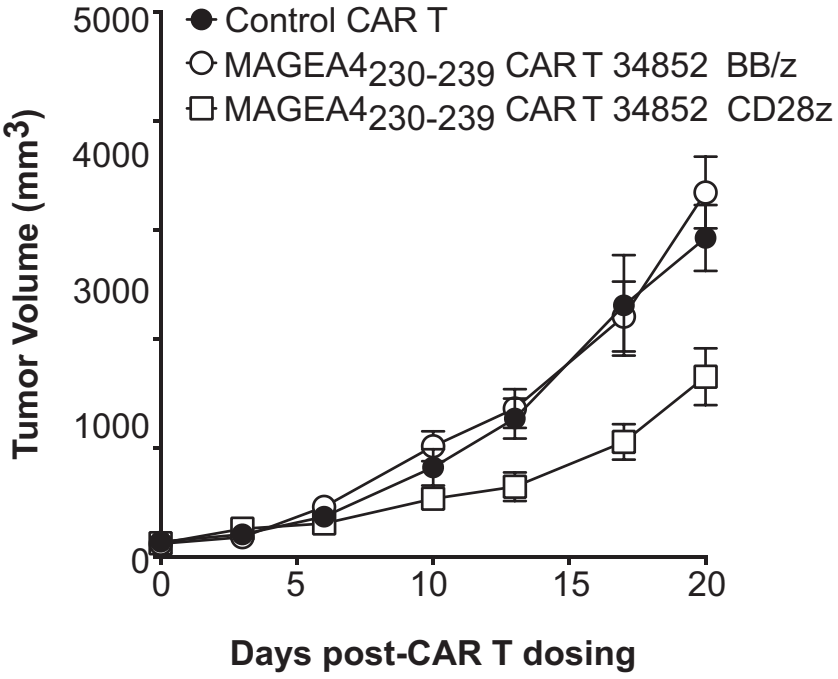

anti-HLA-A2/MAGE 34852 scFv
PEF1-α
VH
VL
CD8 Hinge + TM
4-1BB
CD3ζ
P2A
eGFP
anti-HLA-A2/MAGEA4230-239 34852 scFv
PEF1-α
VH
VL
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP
Non-binding control scFv
PEF1-α
VL
VH
CD28 Hinge + TM
CD28
CD3ζ
P2A
eGFP

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