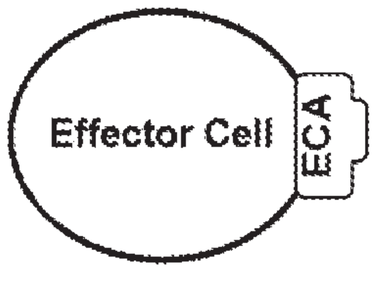
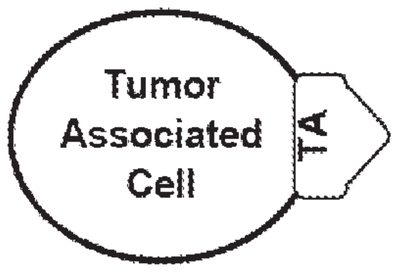
	Effector Cell Antigen	An antigen (receptor) on the surface of an Effector Cell
	Effector Cell Binding Moiety	Binding Moiety (protein domain, peptide, synthetic ligand) that binds specifically to the Effector Cell Antigen
	Effector Cell	A cell that is capable of killing or inhibiting at tumor cell or a cell that is part of tumor tissue. Effector Cells can be T-cells, NK cells.
	Tumor Antigen	An antigen (receptor) that is overexpressed on cells that from a tumor.
	Tumor Antigen Binding Moiety	A binding moiety (protein domain, peptide, synthetic ligand) that binds specifically to a tumor antigen.
	Tumor Associated Cell	A cell that is part of a tumor. This can be a tumor cell or other cell types such as stroma that form a tumor mass.
	Bulking Moiety	Bulking Moiety is a protein or polymer that has a larger size than the ECBM and the TABM. The BD can be albumin, an albumin binding domain, Fc, PEG, XTEN
		
	Release Site	Release Site is an amino acid sequence that can be cleaved by a tumor associated protease.
	Tumor Associated Protease	Tumor Associated Protease is an proteolytic enzyme that occurs in the extracellular space of tumor tissue.
	ProTIA	Protease Triggered Immune Activator
	ProTIA in pro-form	ProTIA molecule prior to protease-catalyzed trigger event
	ProTIA in apo-form	ProTIA molecule that has lost its bulking domain due to a protease-catalyzed trigger event

FIG. 1

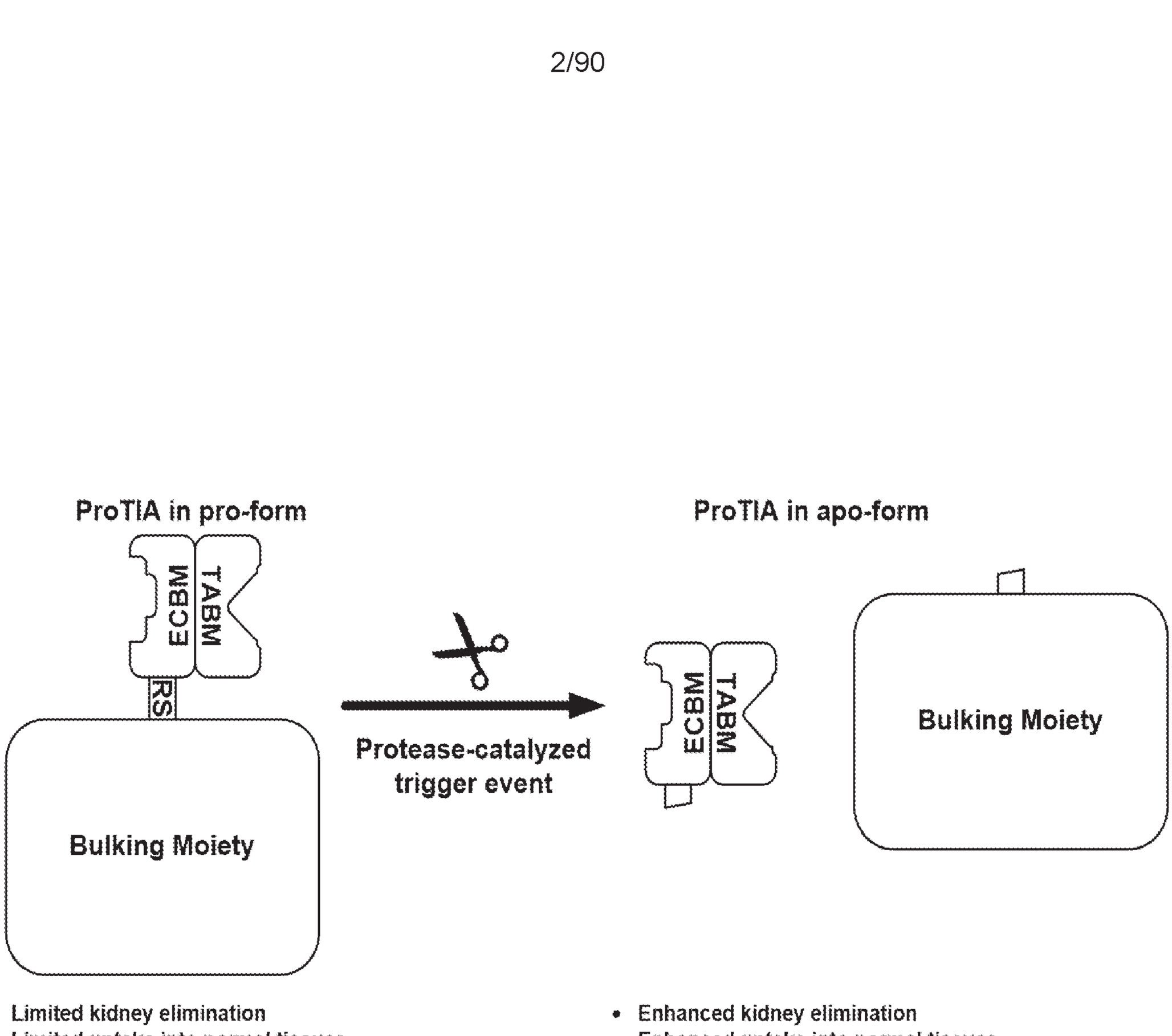

ProTIA in pro-form
ECBM
TABM
RS
Bulking Moiety
Protease-catalyzed
trigger event
ProTIA in apo-form
ECBM
TABM
Bulking Moiety
• Limited kidney elimination
• Limited uptake into normal tissues
• Limited diffusion within tissues
• Limed interaction with effector cells
• Limited interaction with tumor associated cells
• Enhanced kidney elimination
• Enhanced uptake into normal tissues
• Enhanced diffusion within tissues
• Enhanced interaction with effector cells
• Enhanced interaction with tumor associated cells

FIG. 2

FIG. 3A

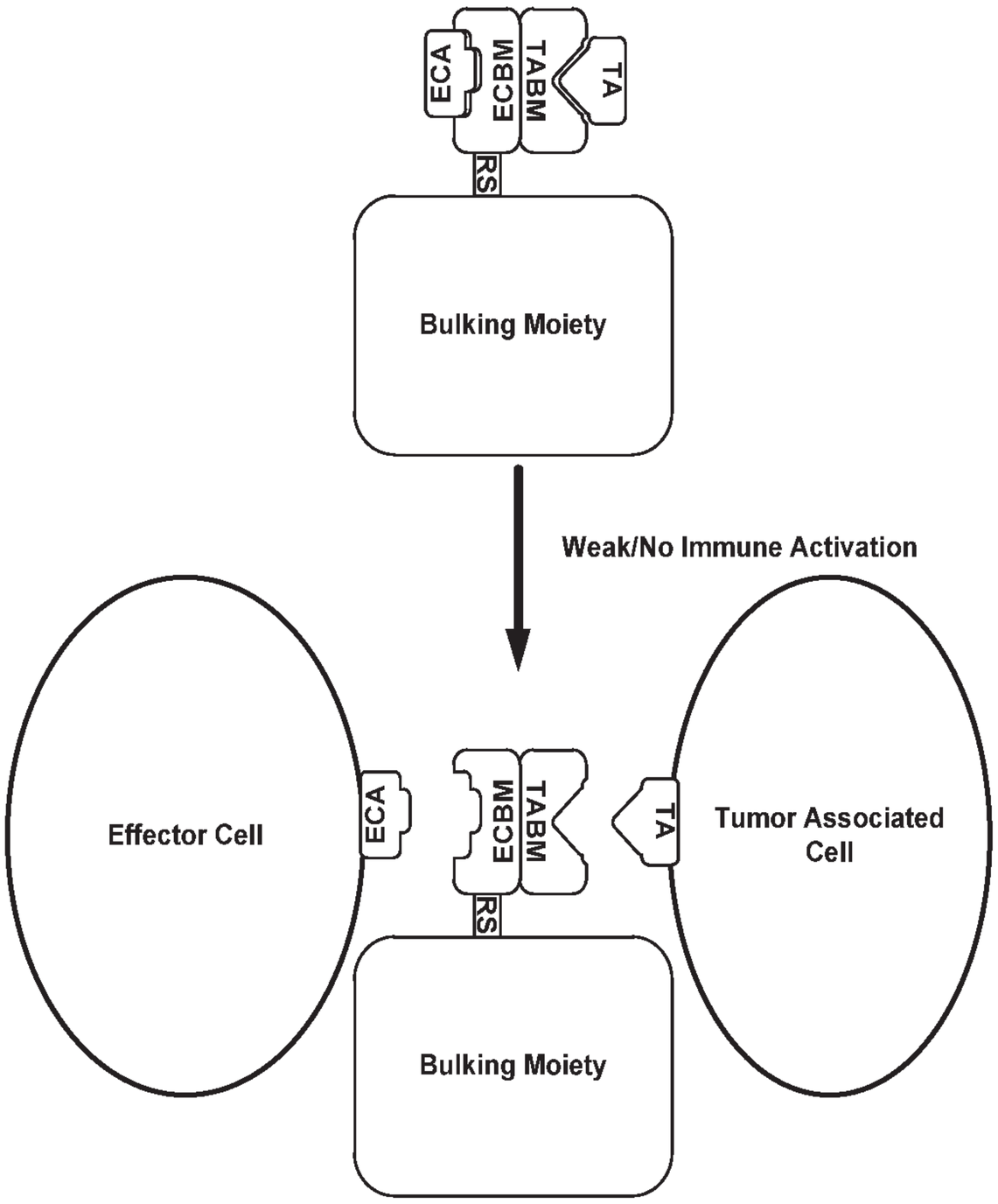


FIG. 3B

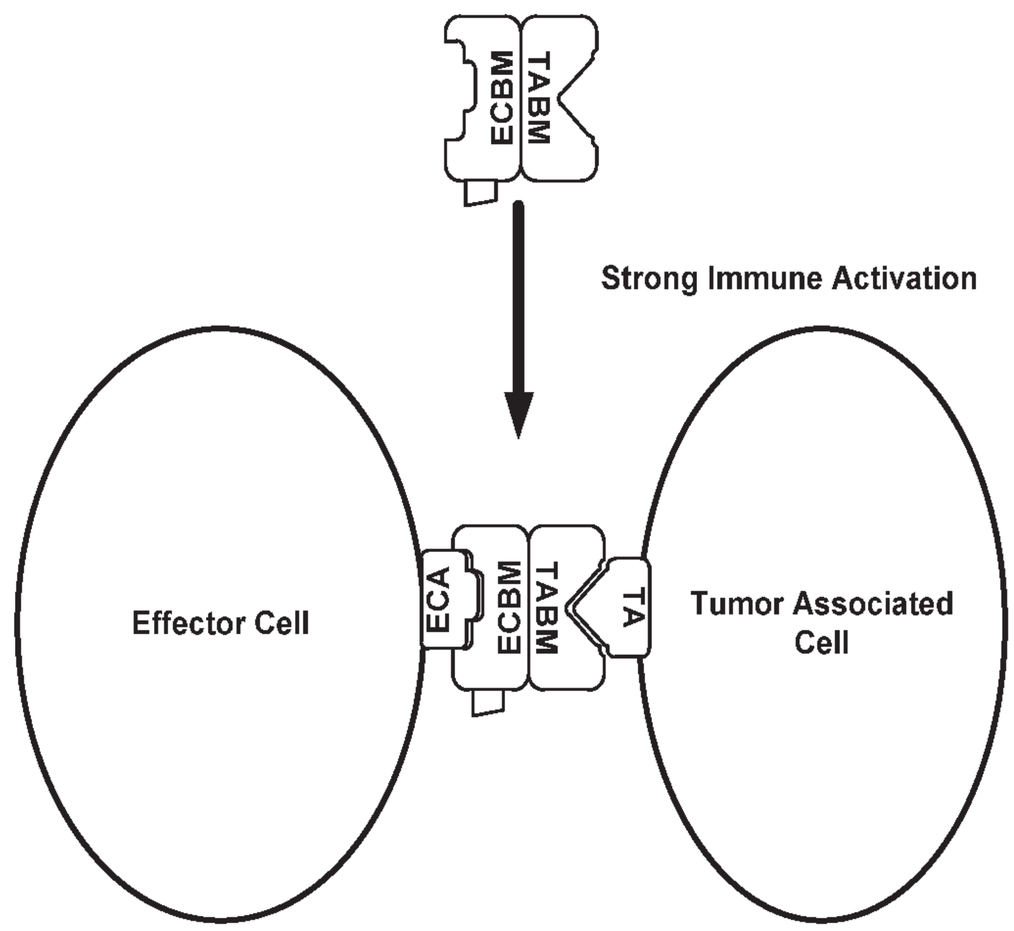


FIG. 3

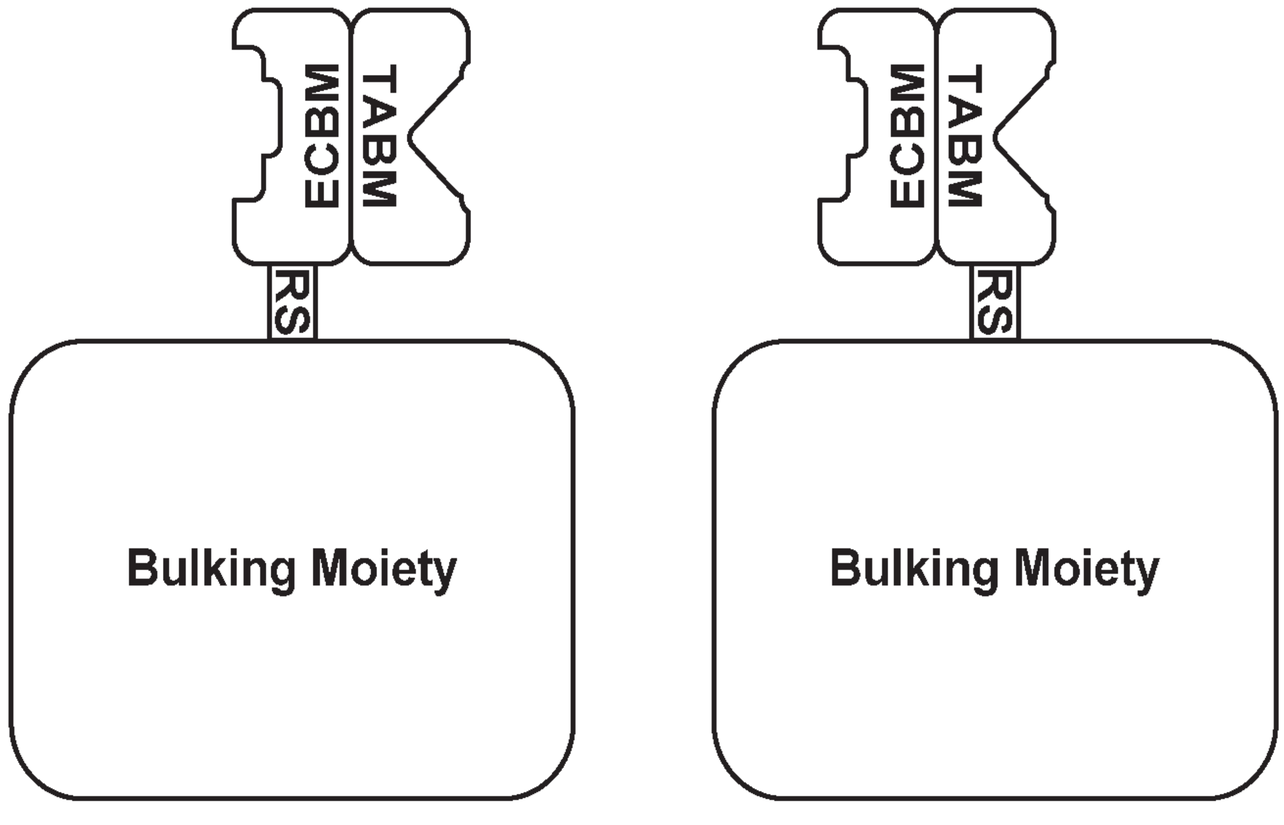


FIG. 4

FIG. 5A

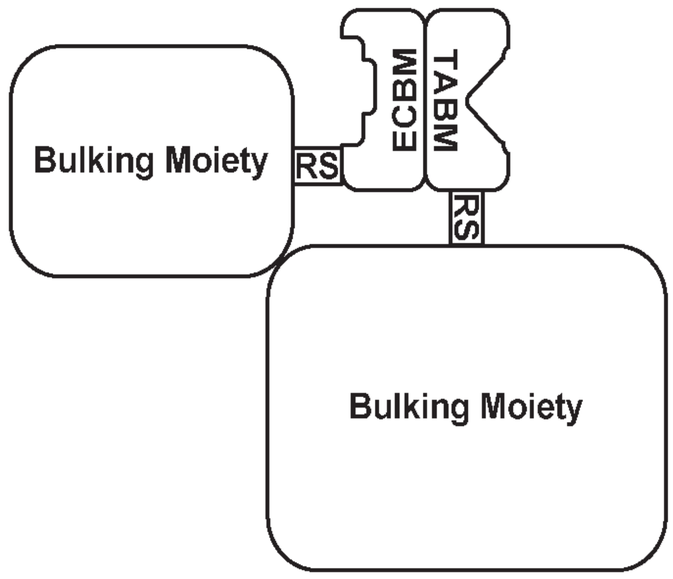


FIG. 5B

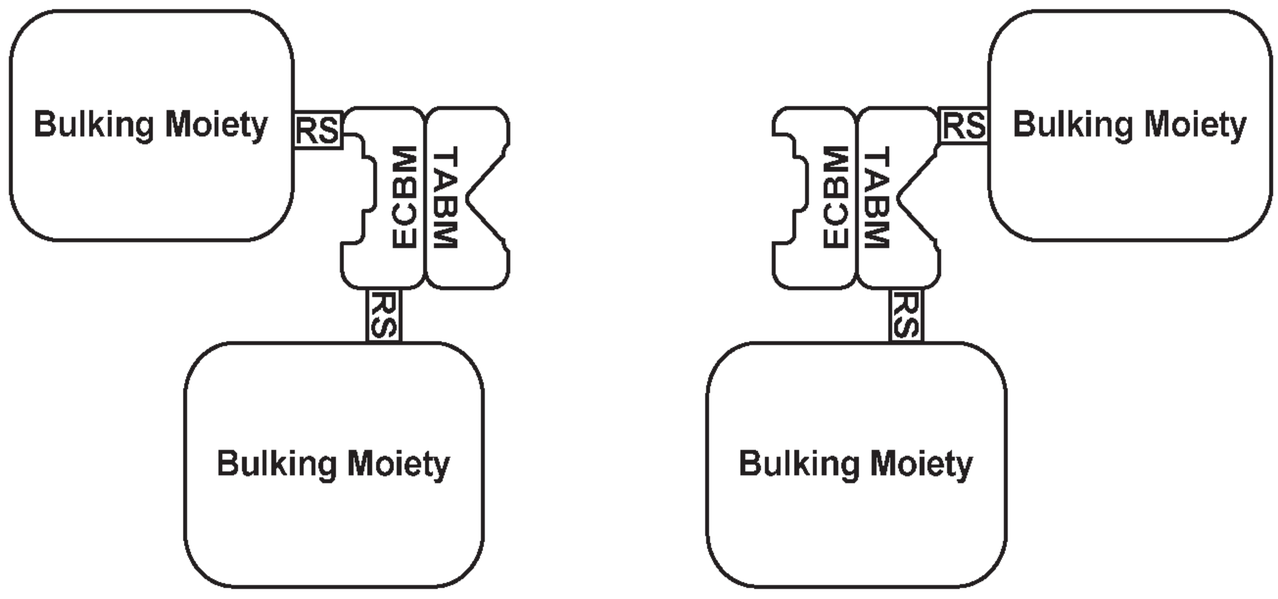


FIG. 5

FIG. 6A

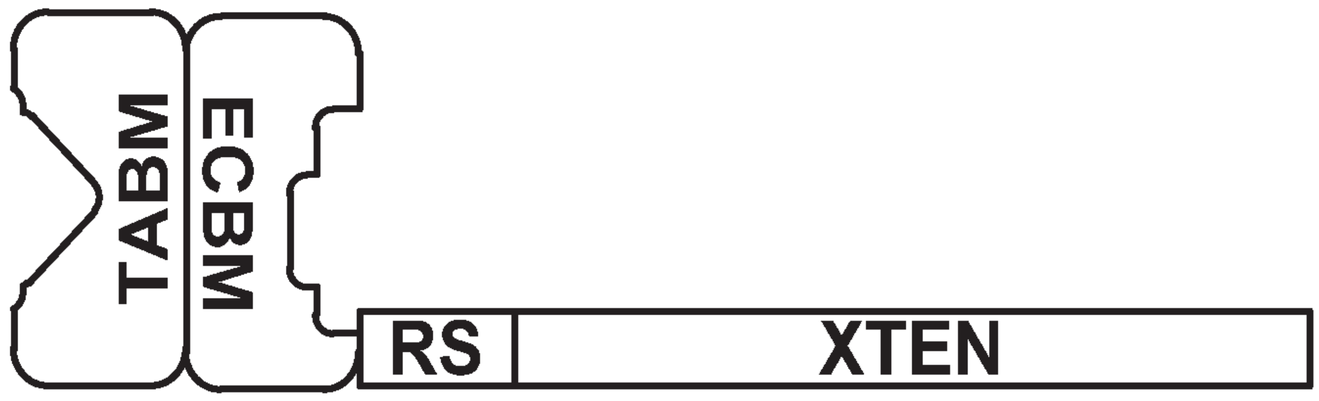


FIG. 6B

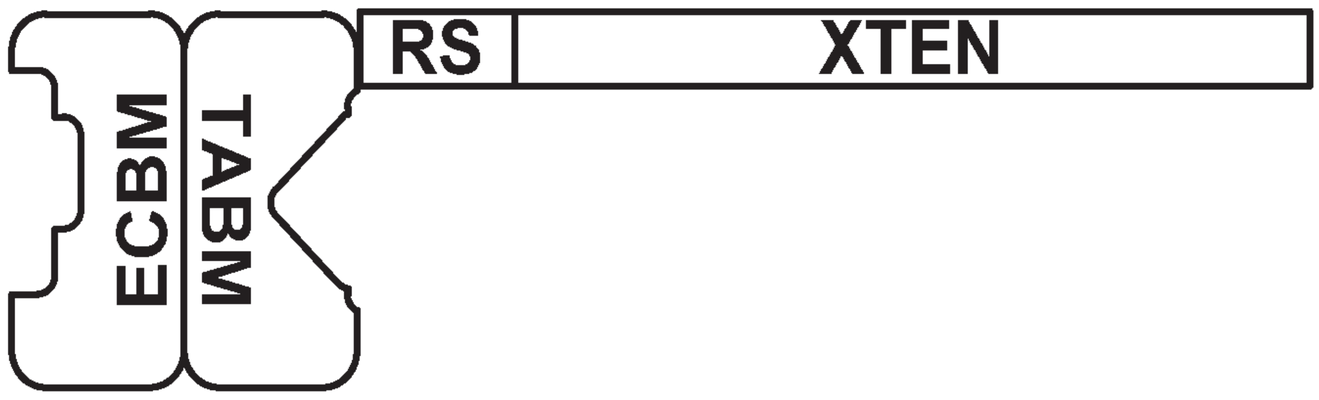


FIG. 6C



FIG. 6D



FIG. 6

FIG. 7A

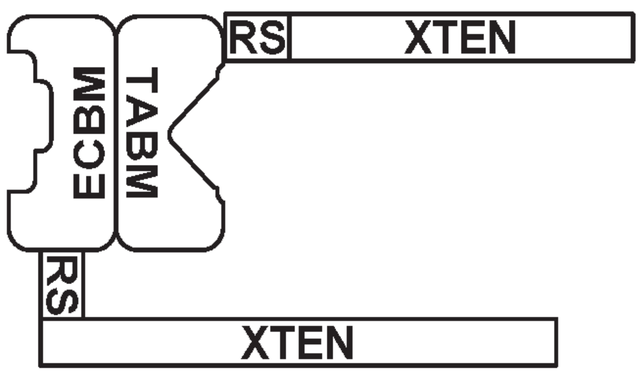


FIG. 7B

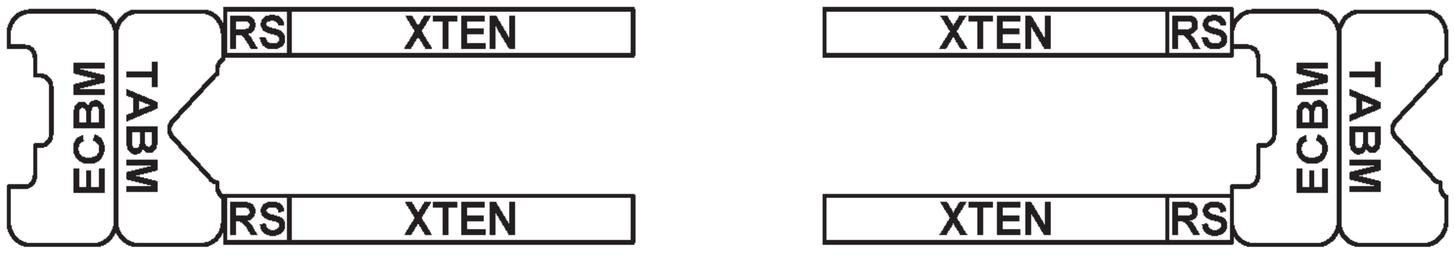
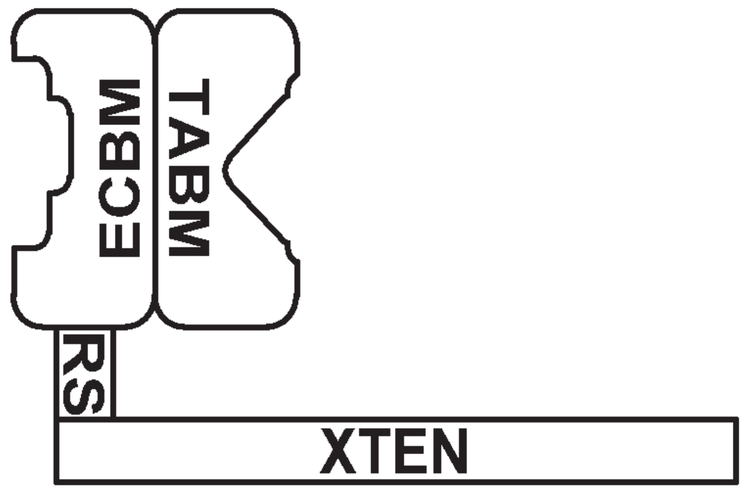


FIG. 7

FIG. 8A



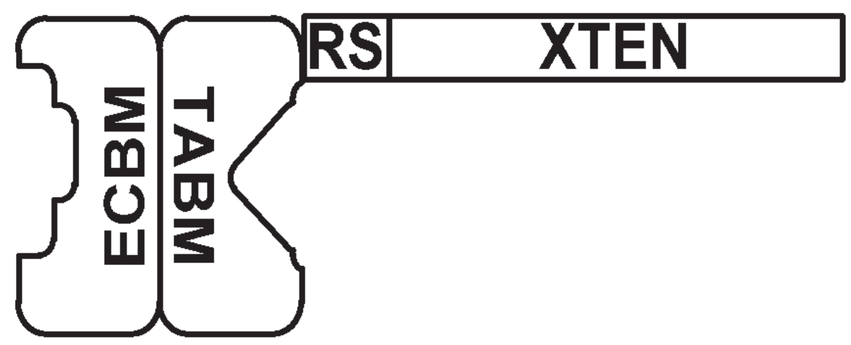
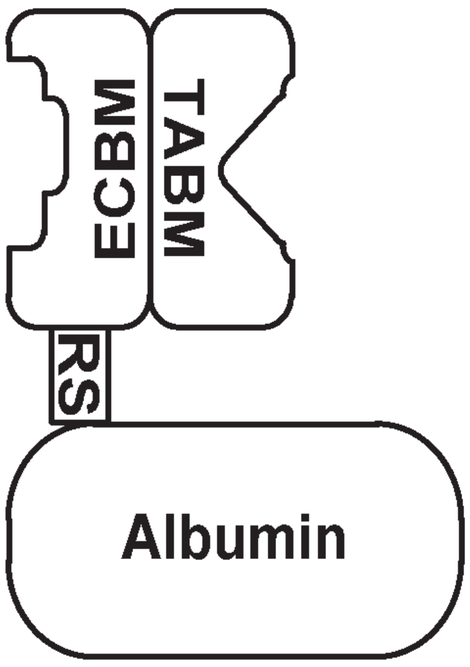


FIG. 8B



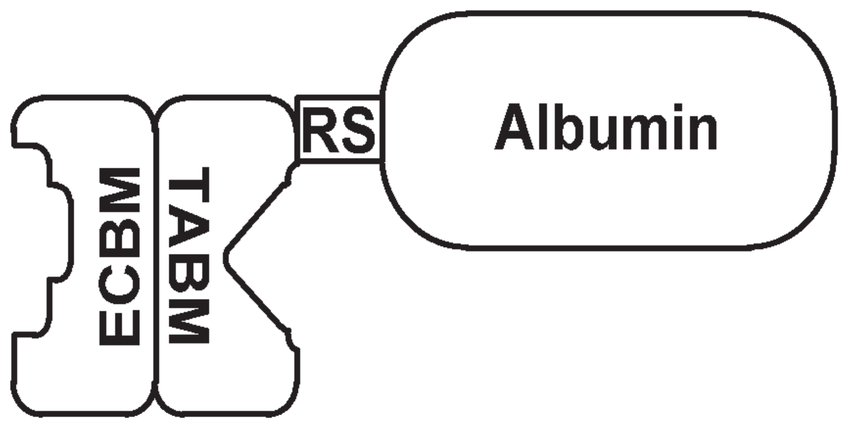
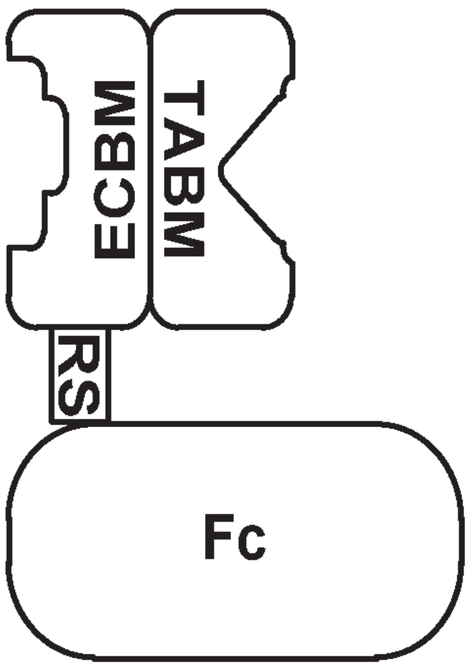


FIG. 8C



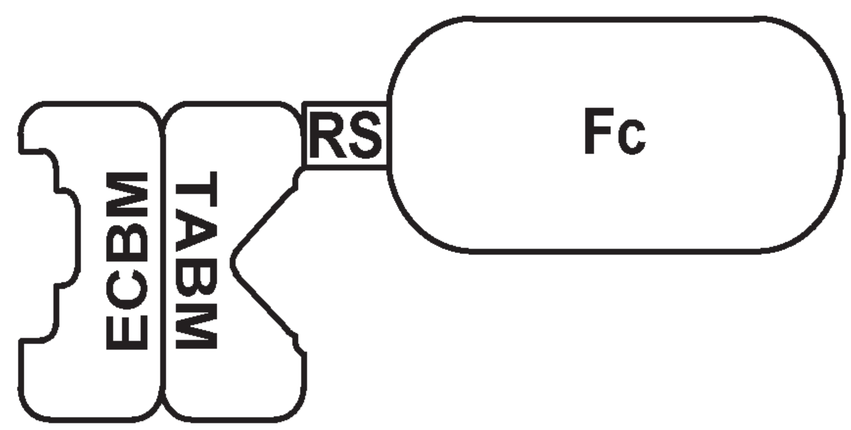


FIG. 8

FIG. 9A

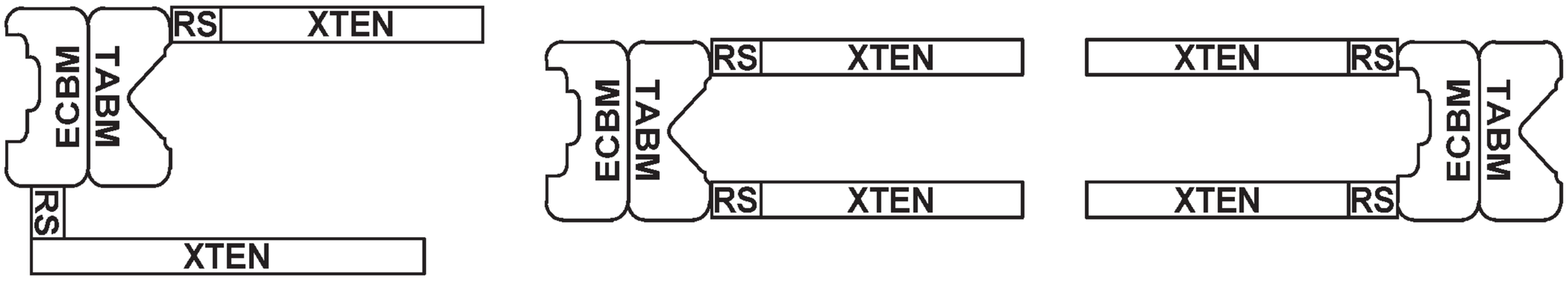


FIG. 9B

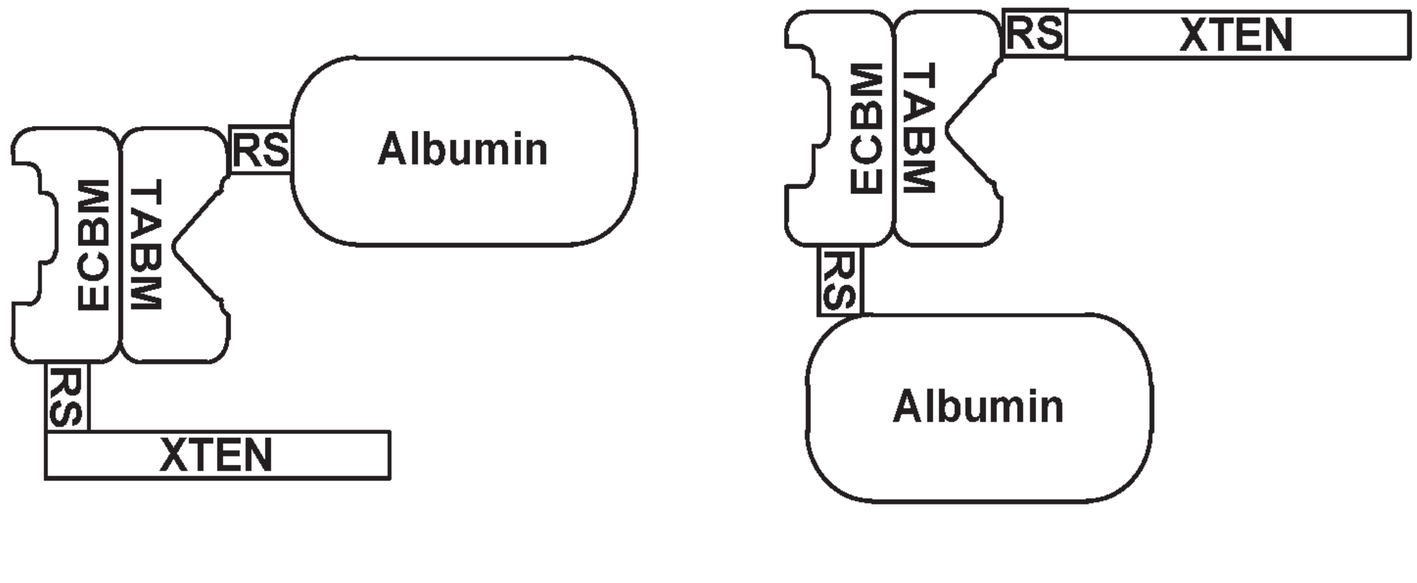


FIG. 9C

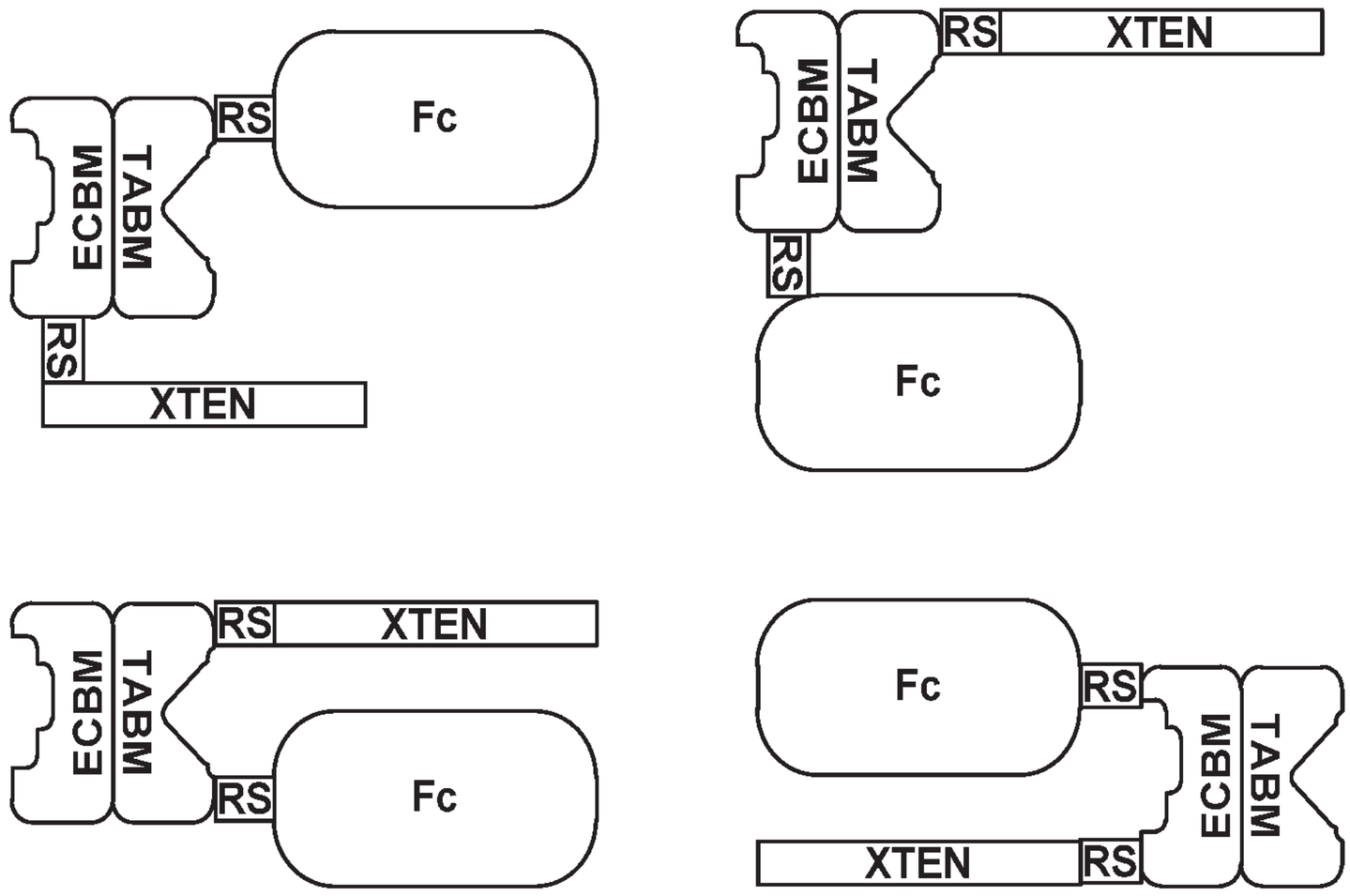


FIG. 9

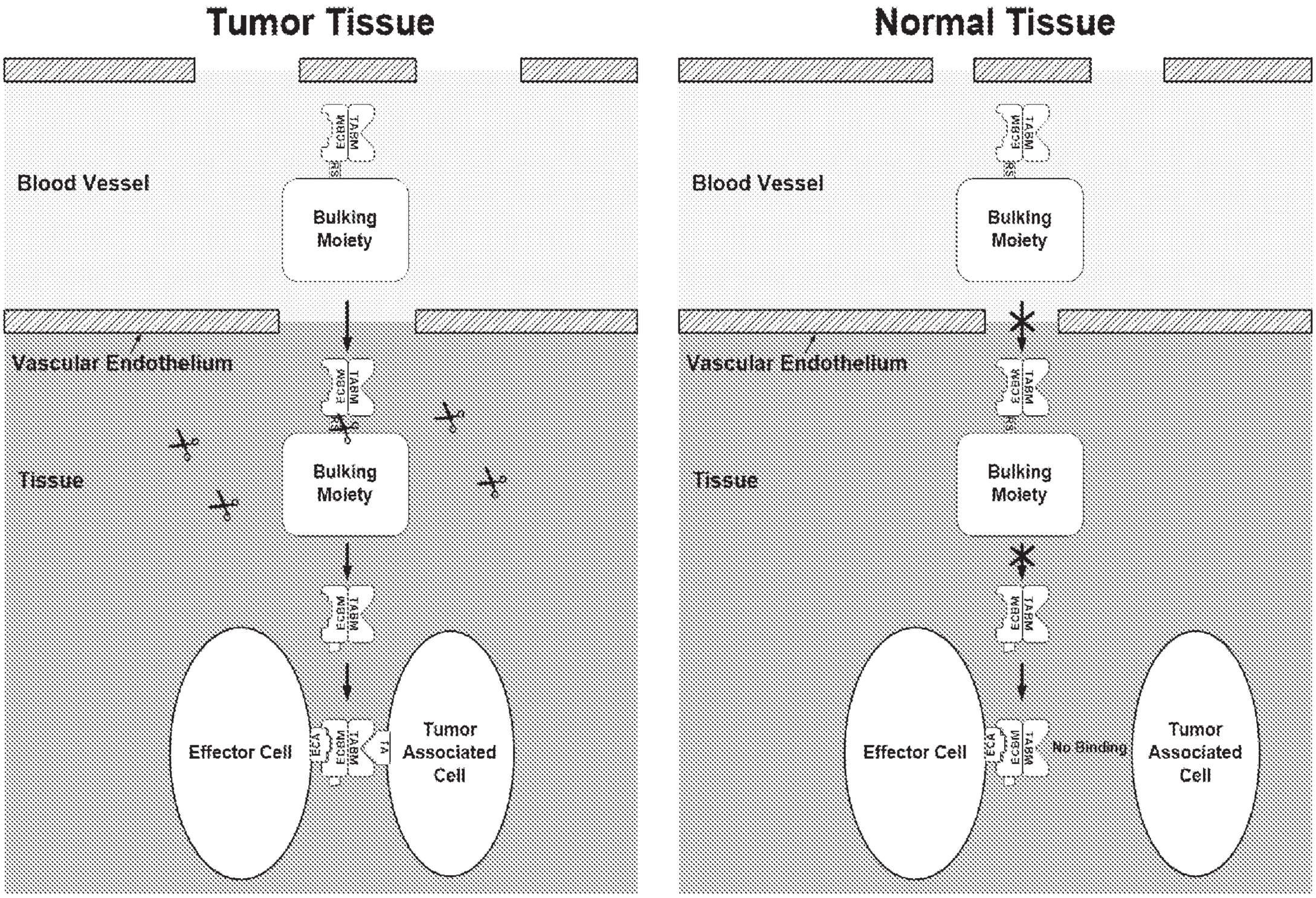

Tumor Tissue
Normal Tissue
Blood Vessel
Bulking Moiety
Vascular Endothelium
Tissue
TABM
ECBM
RS
RS3
ECA
TA
Effector Cell
Tumor Associated Cell
No Binding

FIG. 10

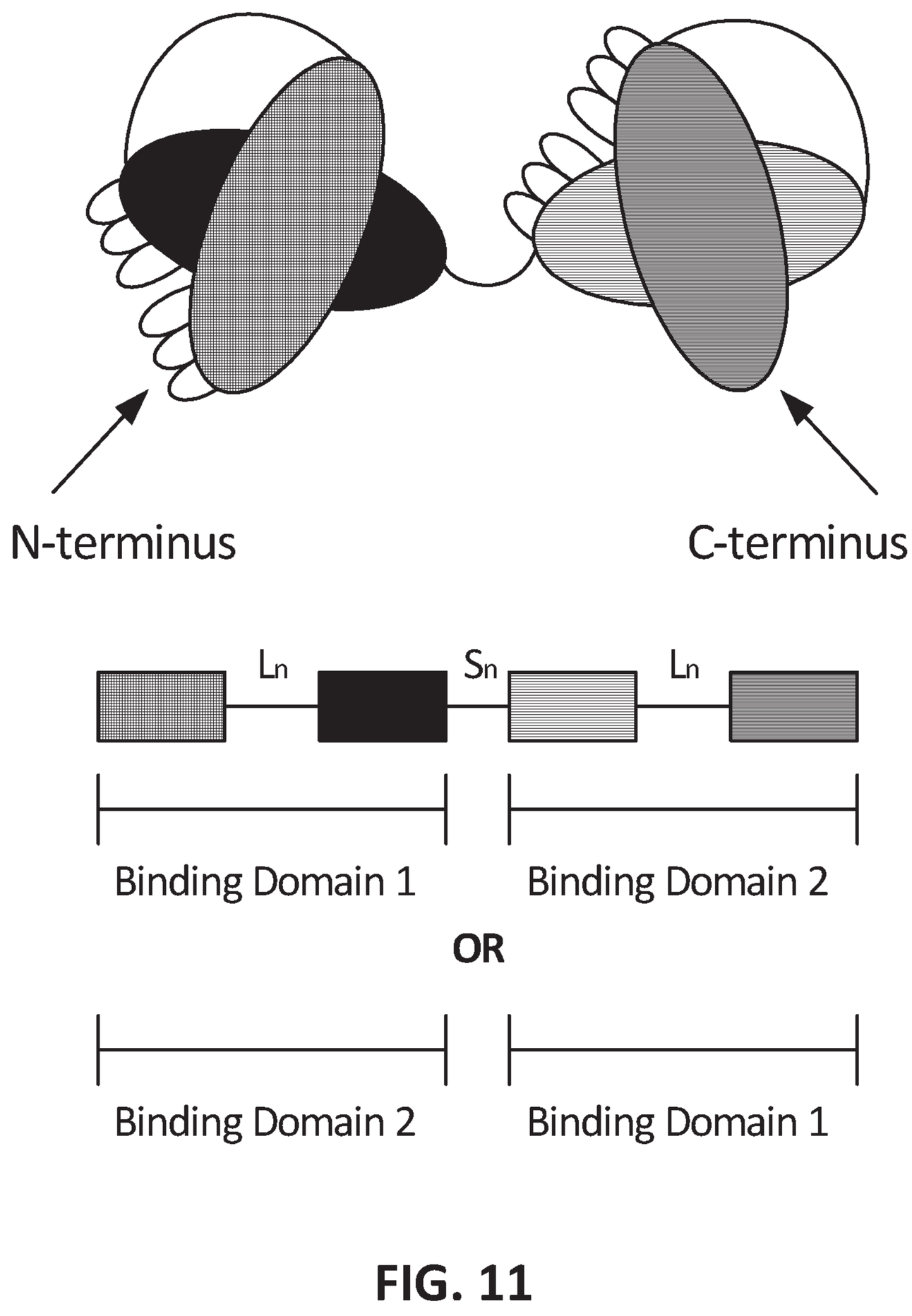
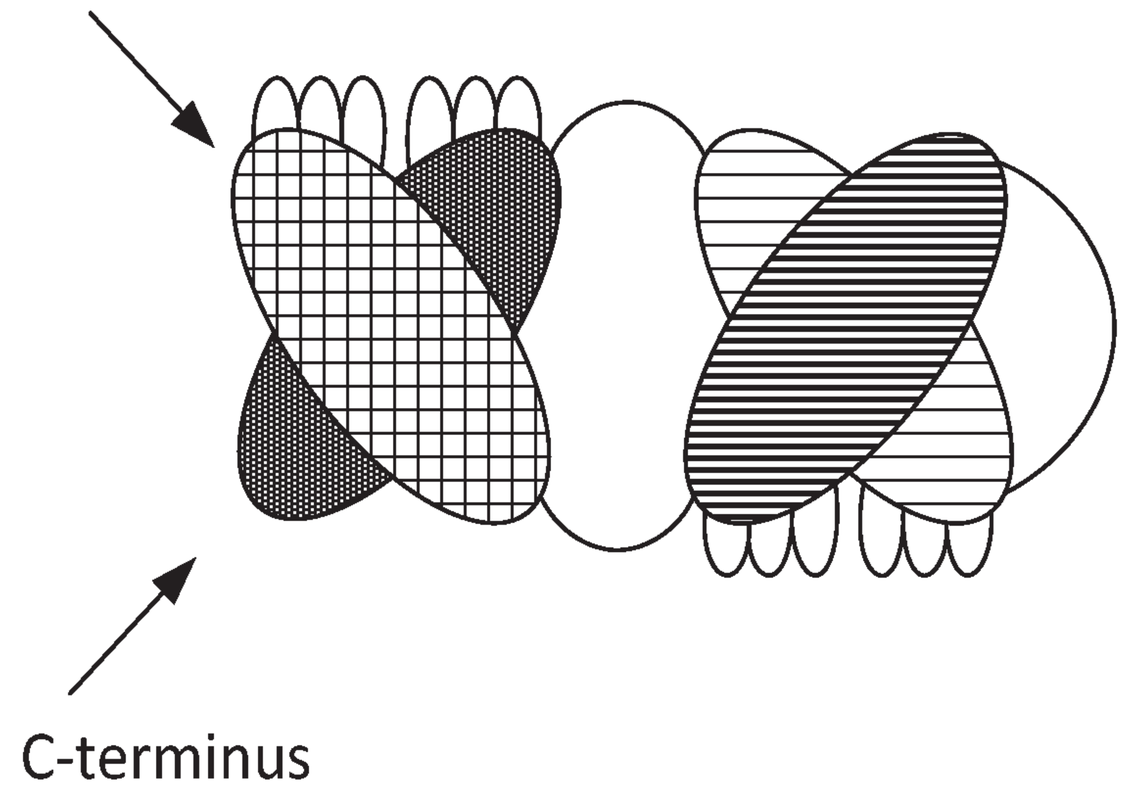

Tandem scFv Configuration
N-terminus
C-terminus
Ln
Sn
Ln
Binding Domain 1
Binding Domain 2
OR
Binding Domain 2
Binding Domain 1

FIG. 11

Diabody Configuration



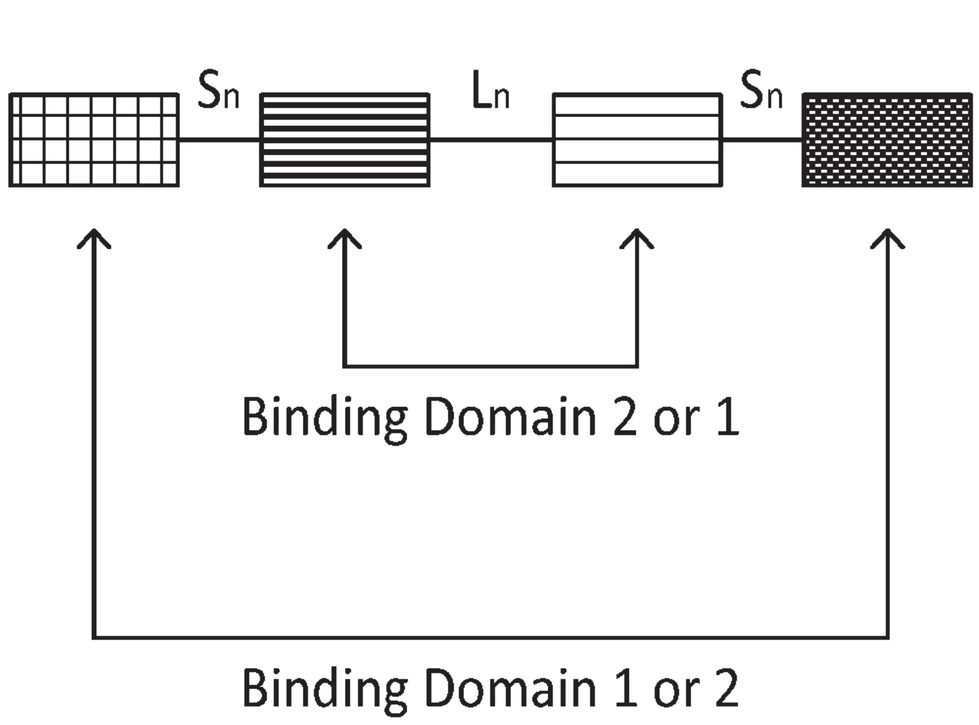


FIG. 12

FIG. 13A

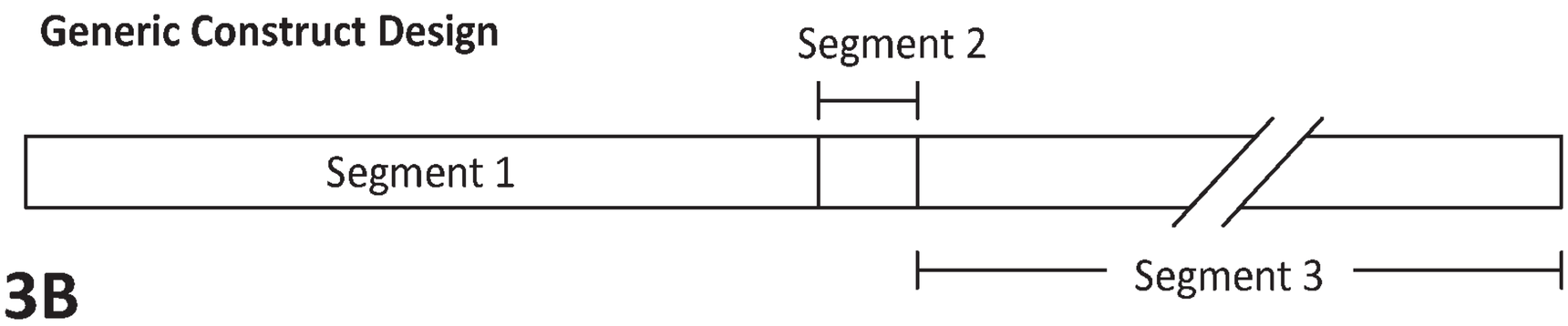


FIG. 13B

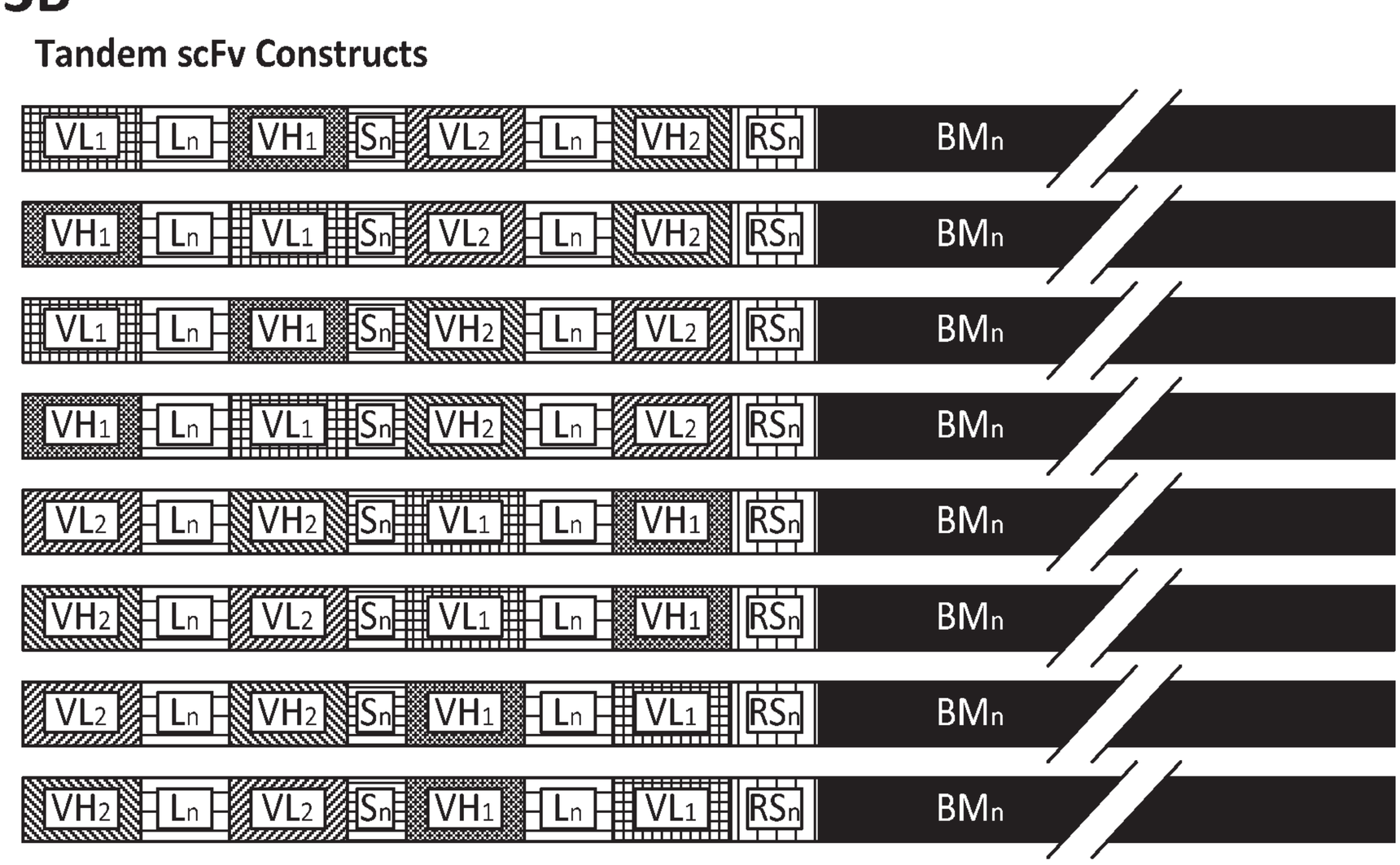


FIG. 13C

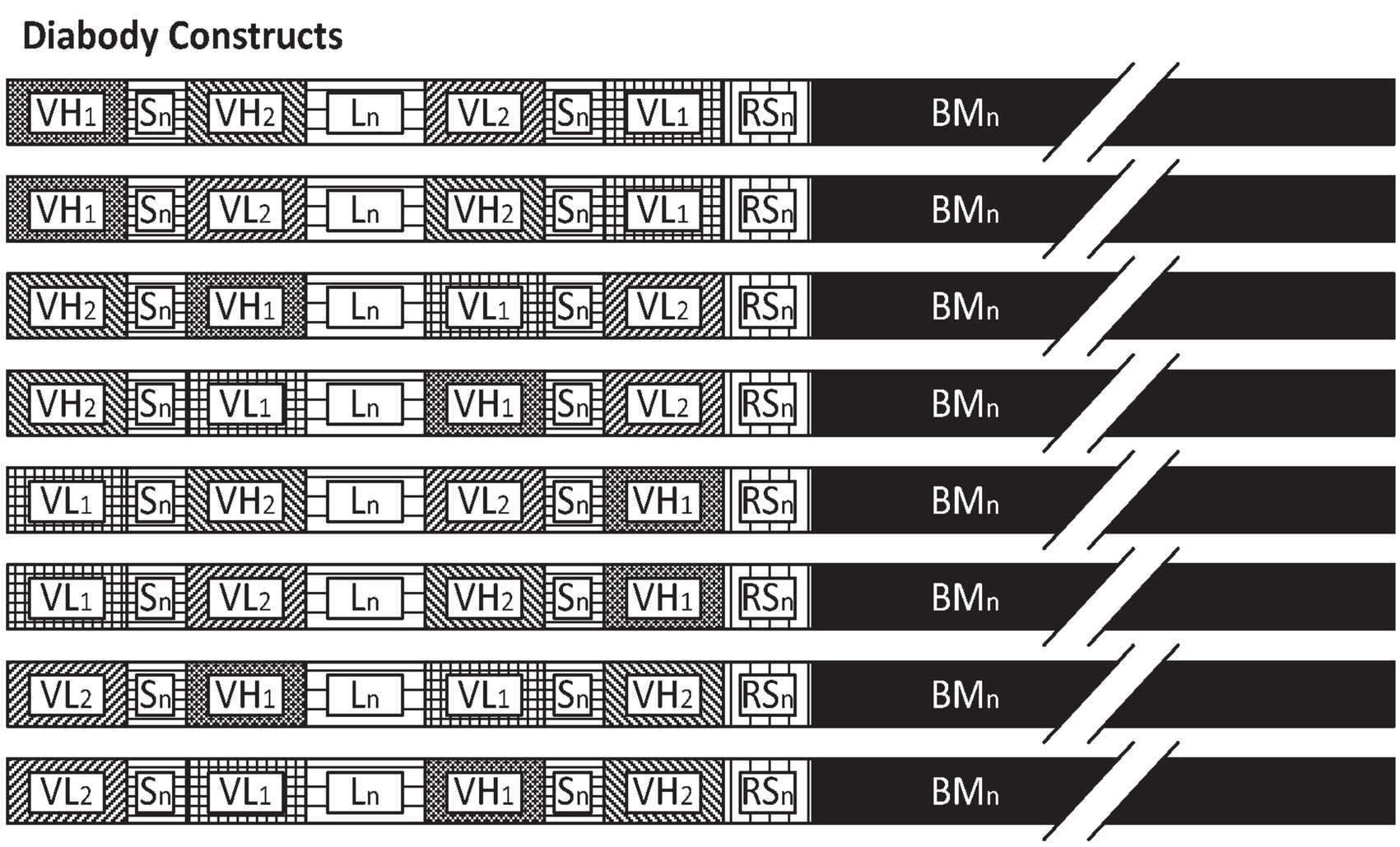


FIG. 13

FIG. 14A

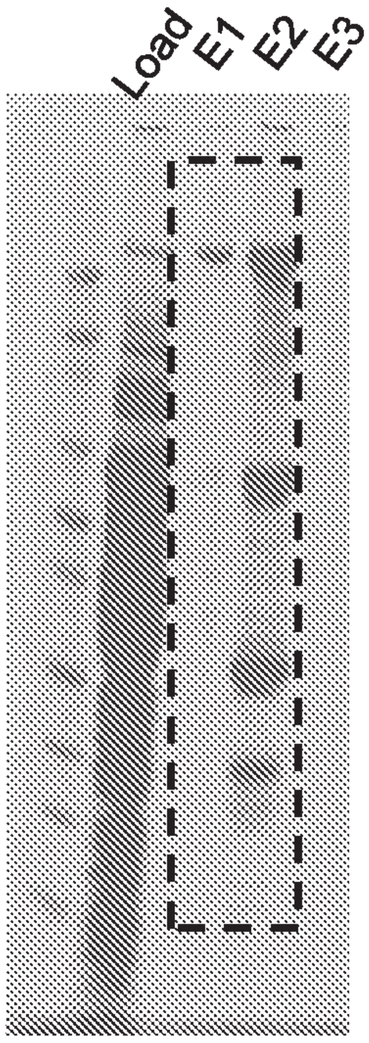


FIG. 14B

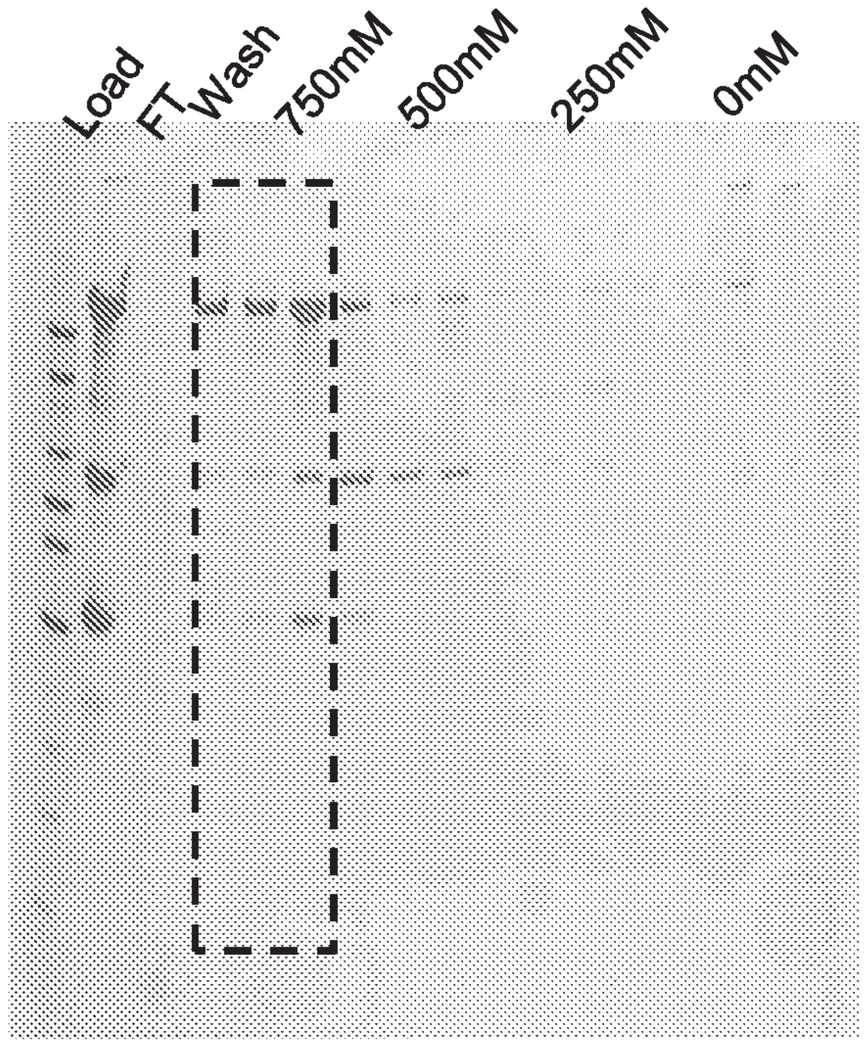


FIG. 14C

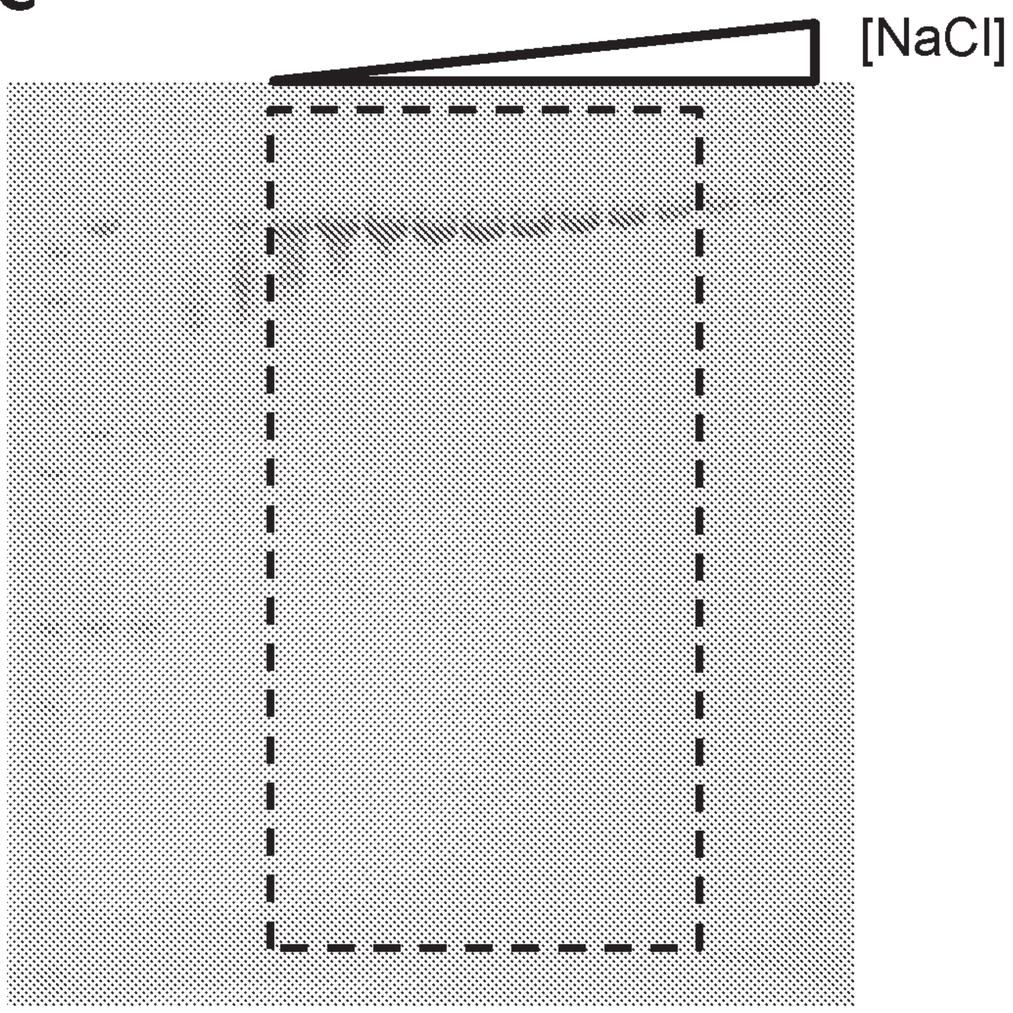


FIG. 14

FIG. 15A

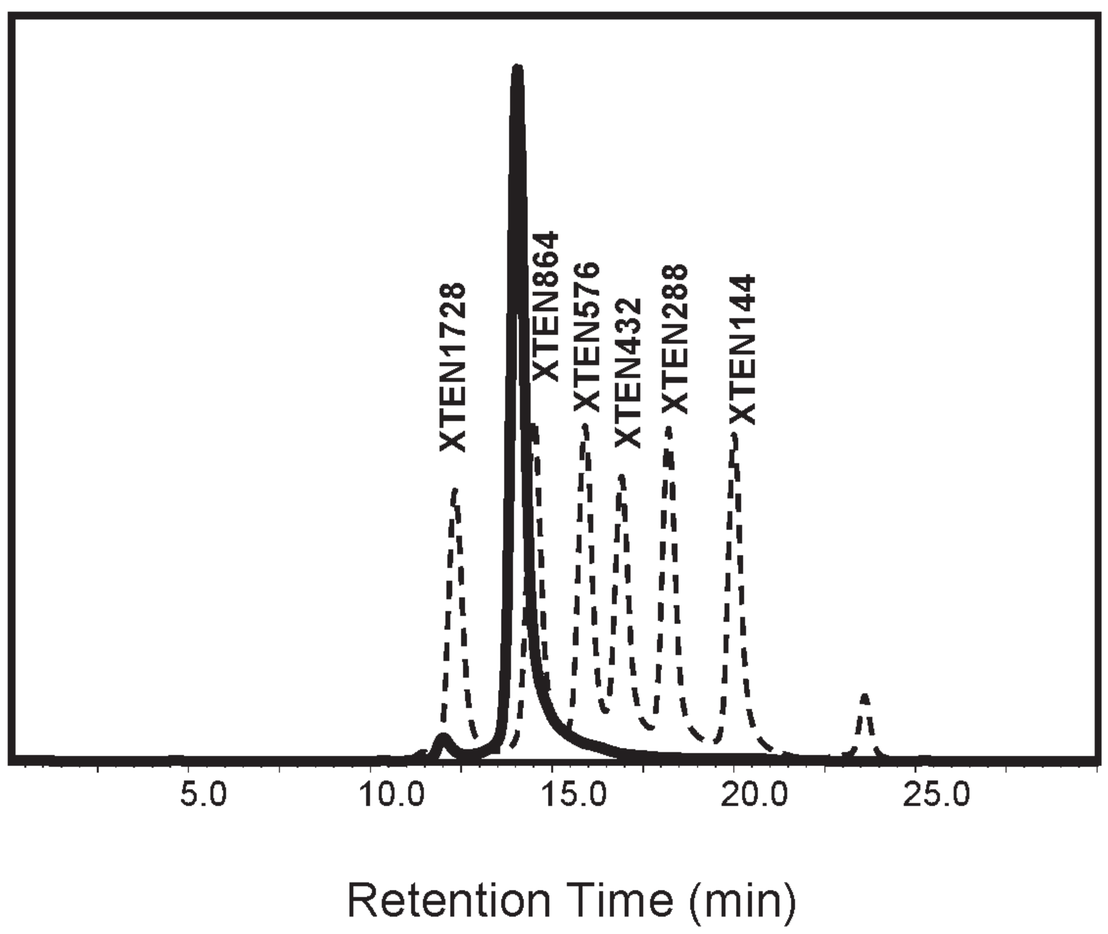


FIG. 15B

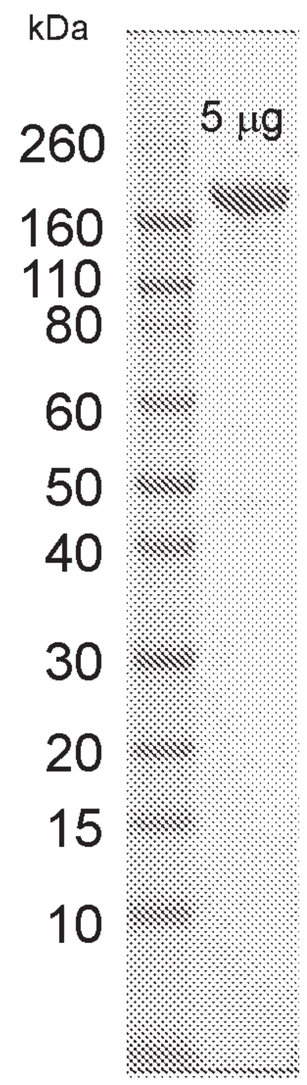


FIG. 15

FIG. 16A

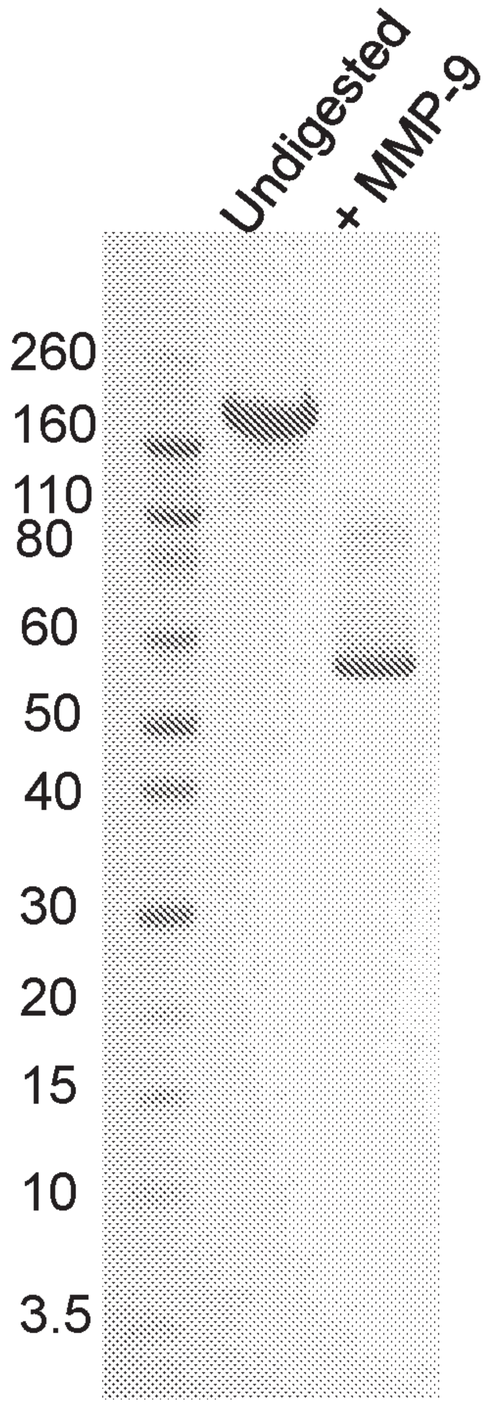


FIG. 16B

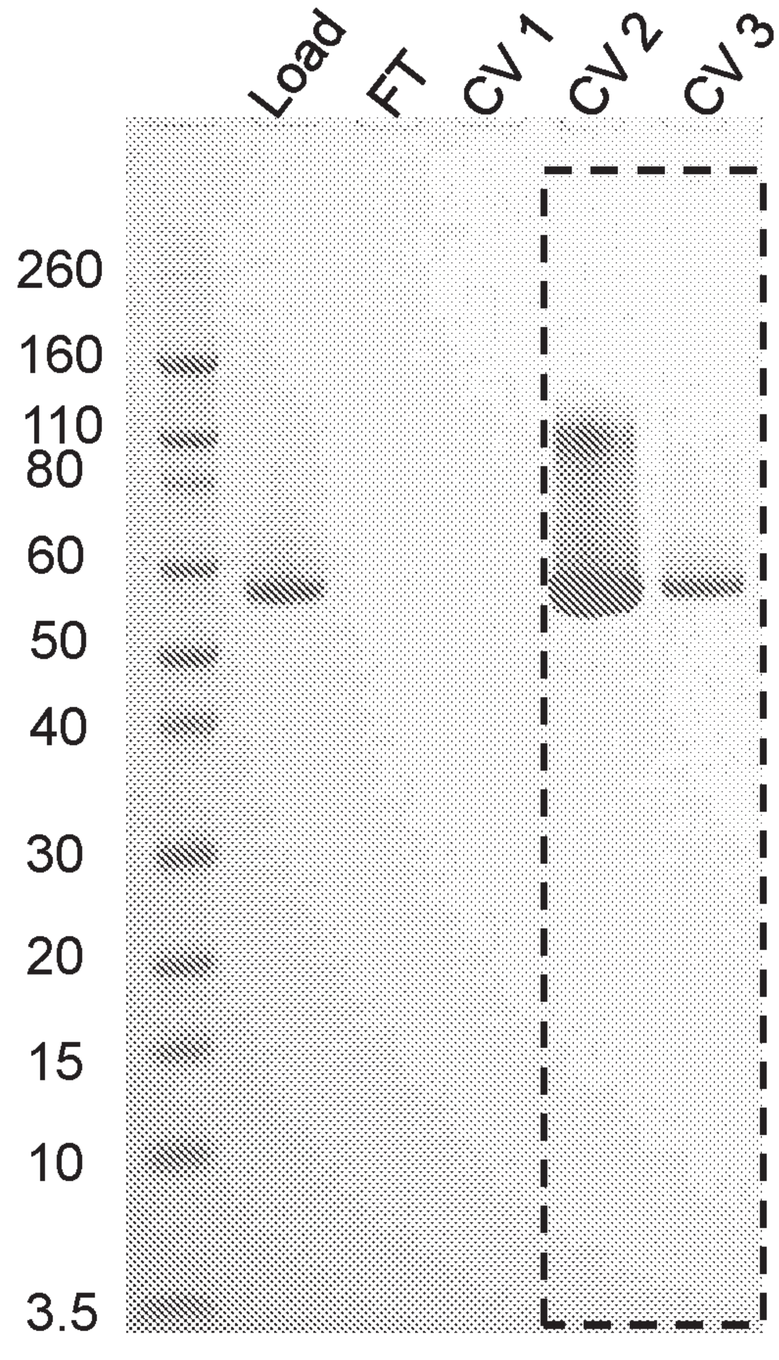


FIG. 16

FIG. 17A

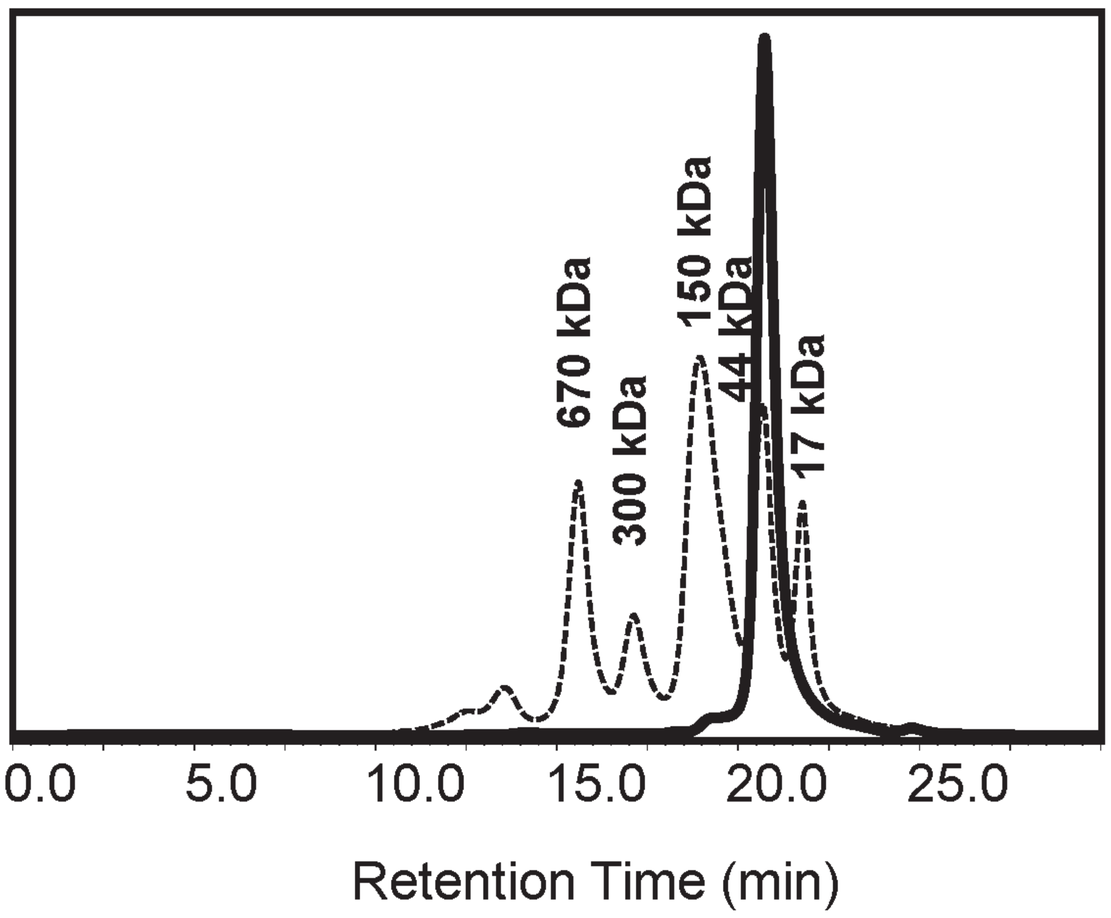


FIG. 17B

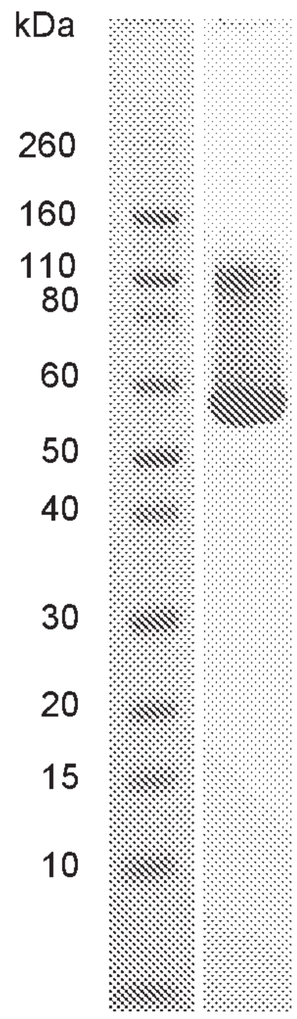


FIG. 17

FIG. 18A

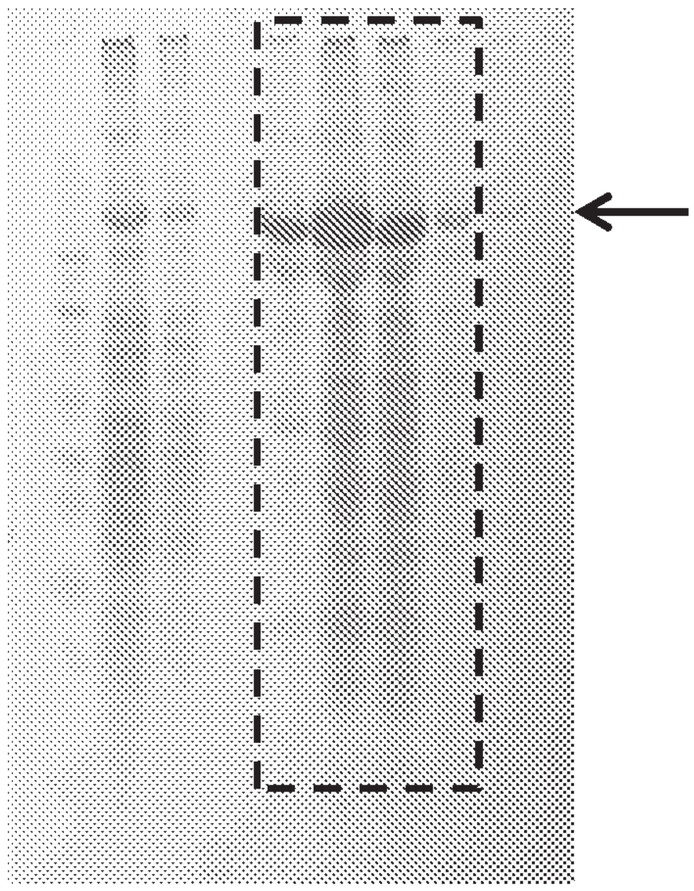


FIG. 18B

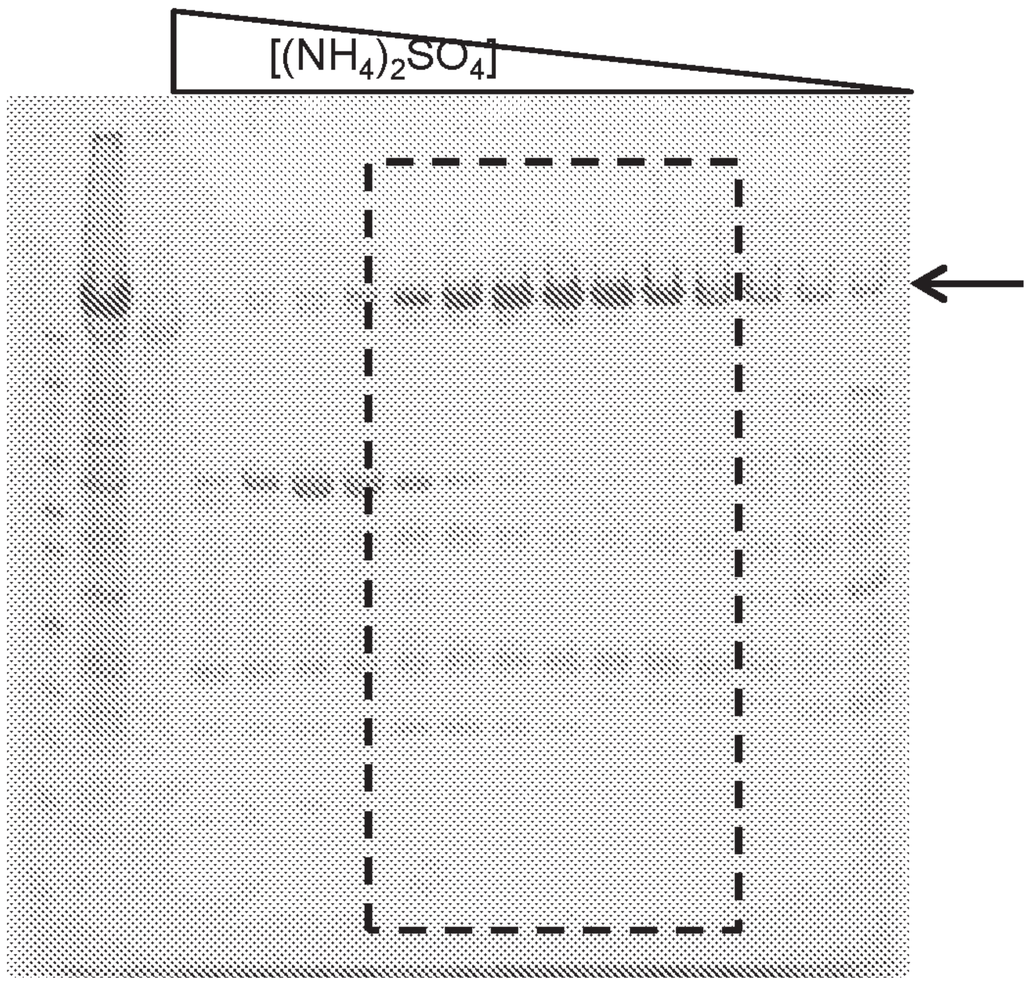


FIG. 18C

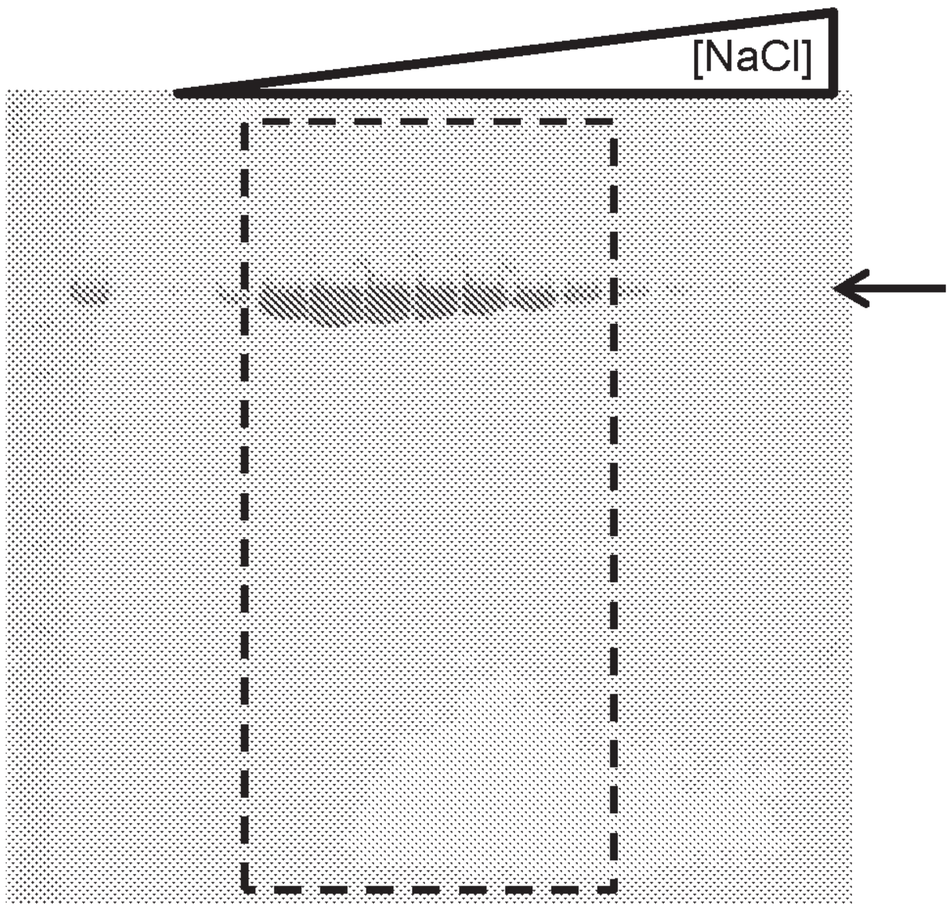


FIG. 18

FIG. 19A

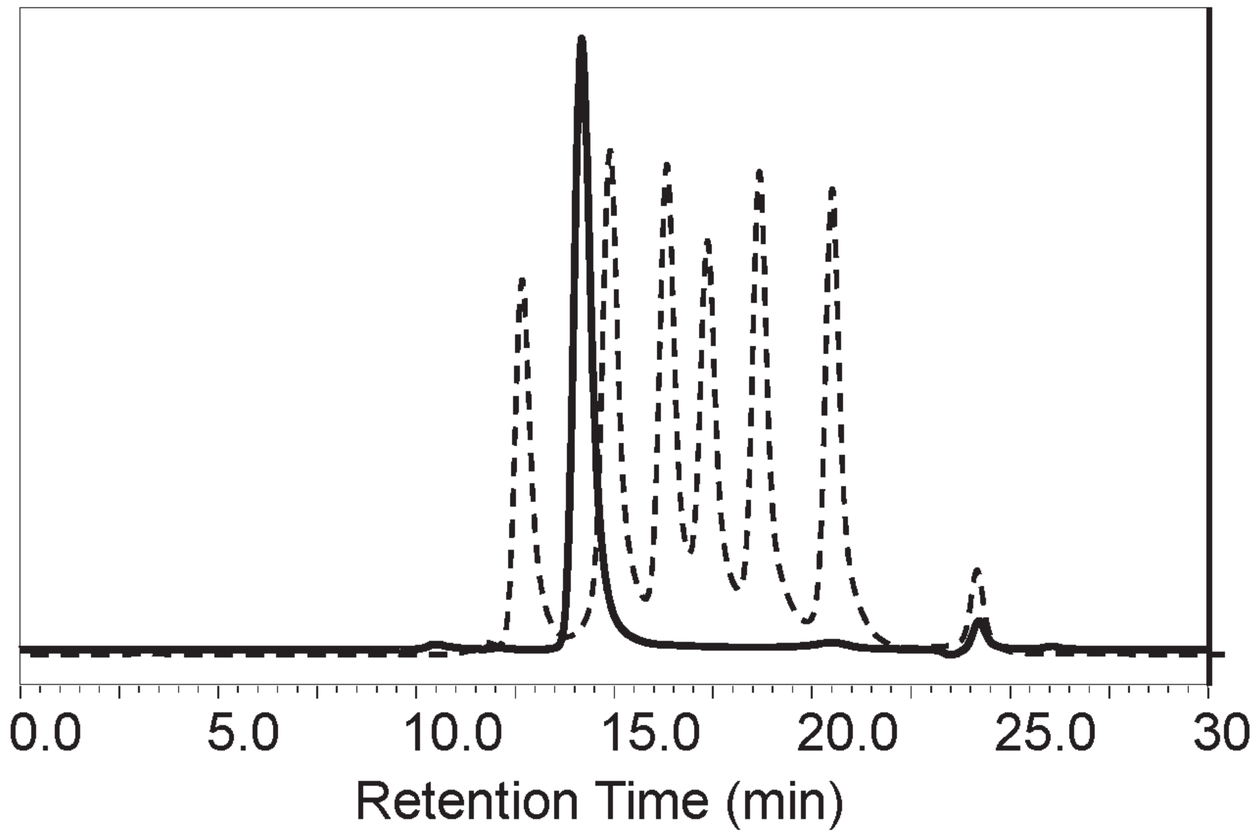


FIG. 19B

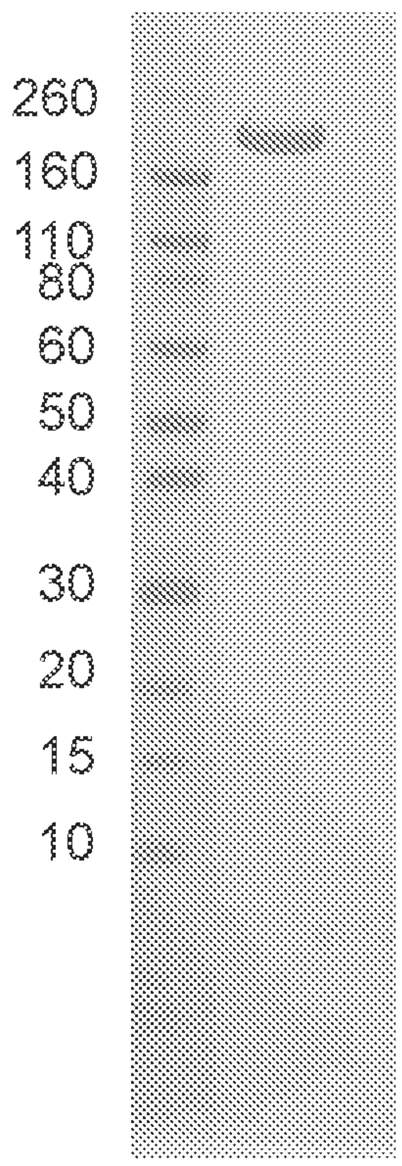


FIG. 19C

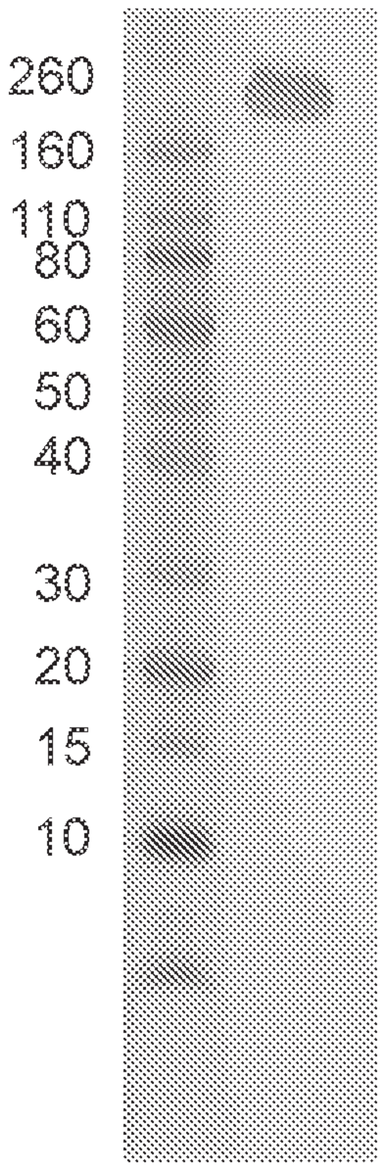


FIG. 19

FIG. 20A

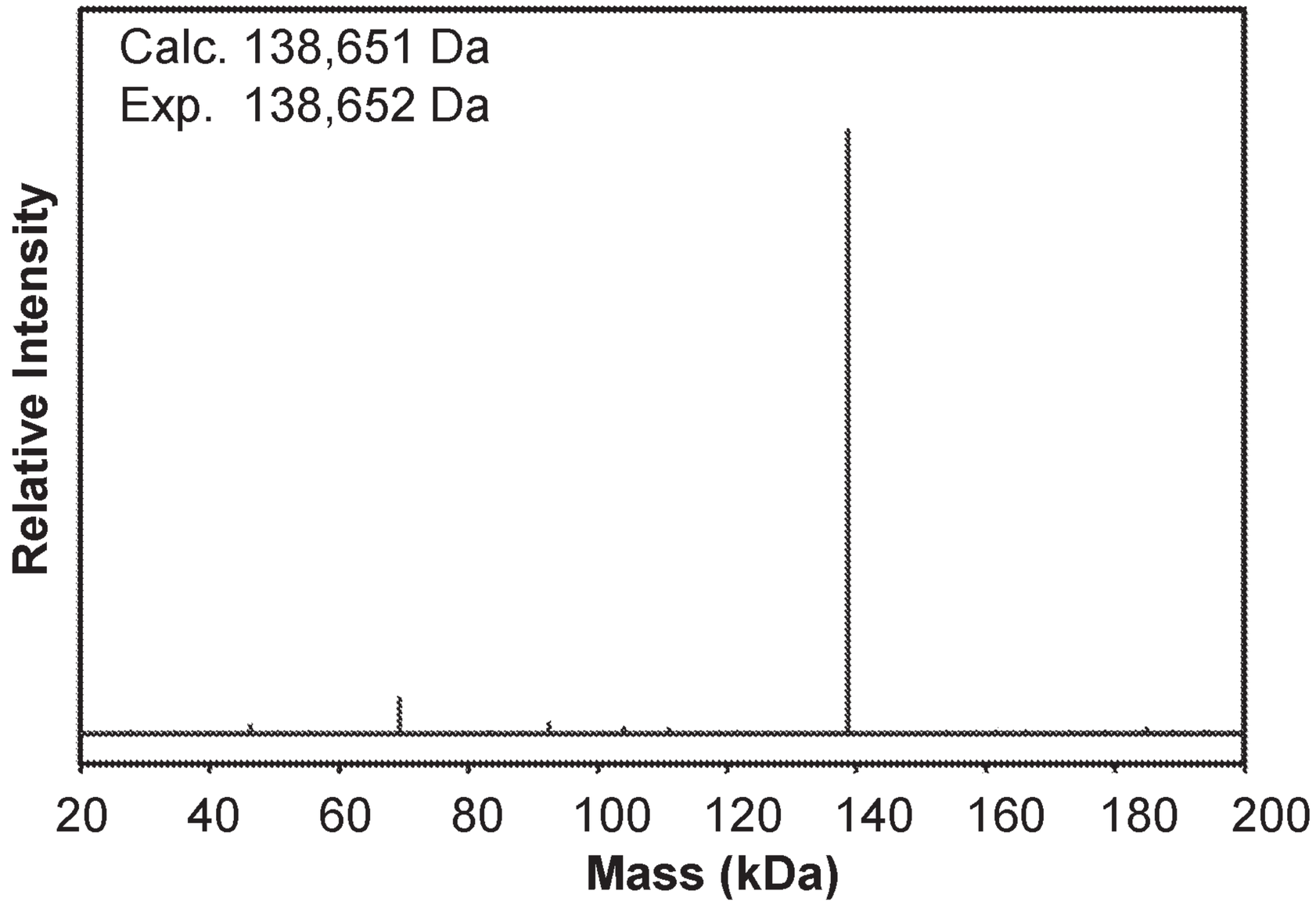


FIG. 20B

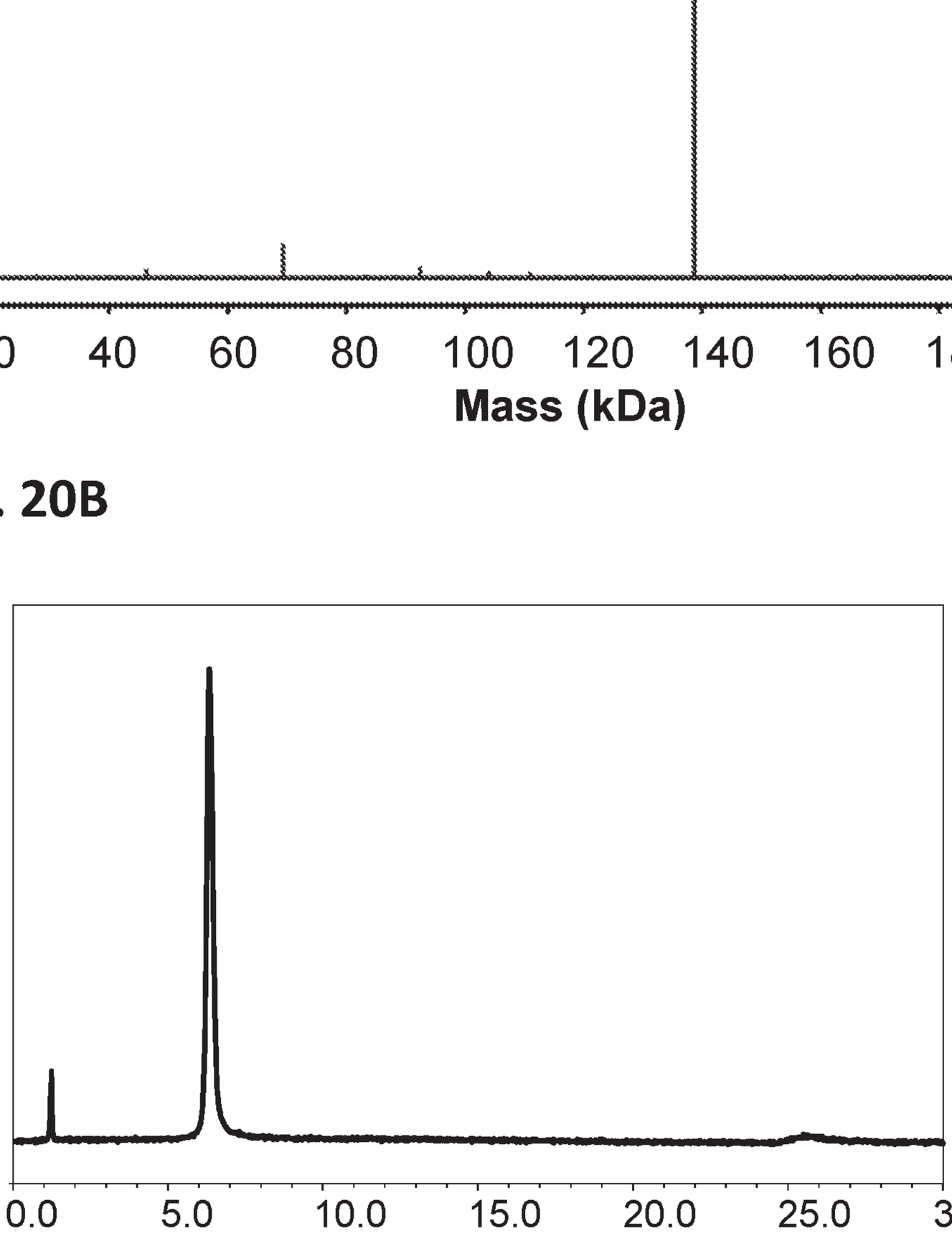


FIG. 20

FIG. 21A

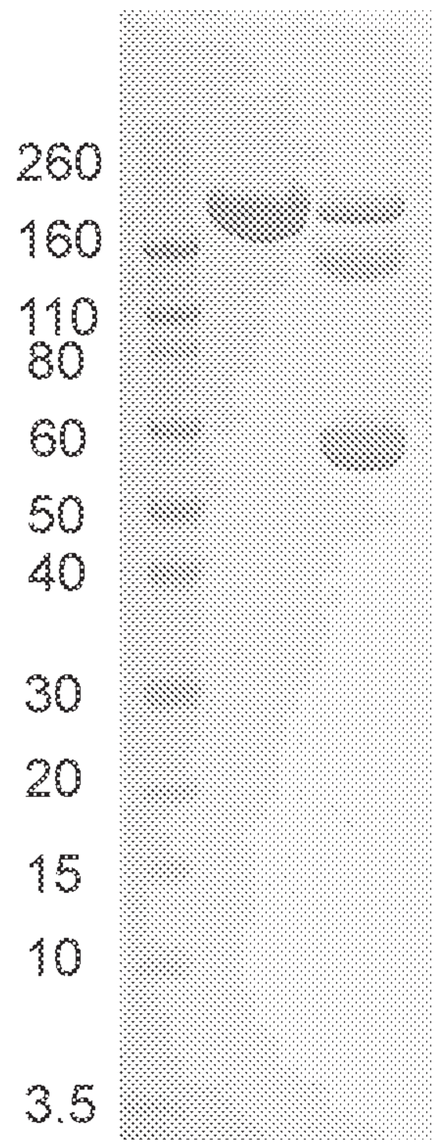


FIG. 21B

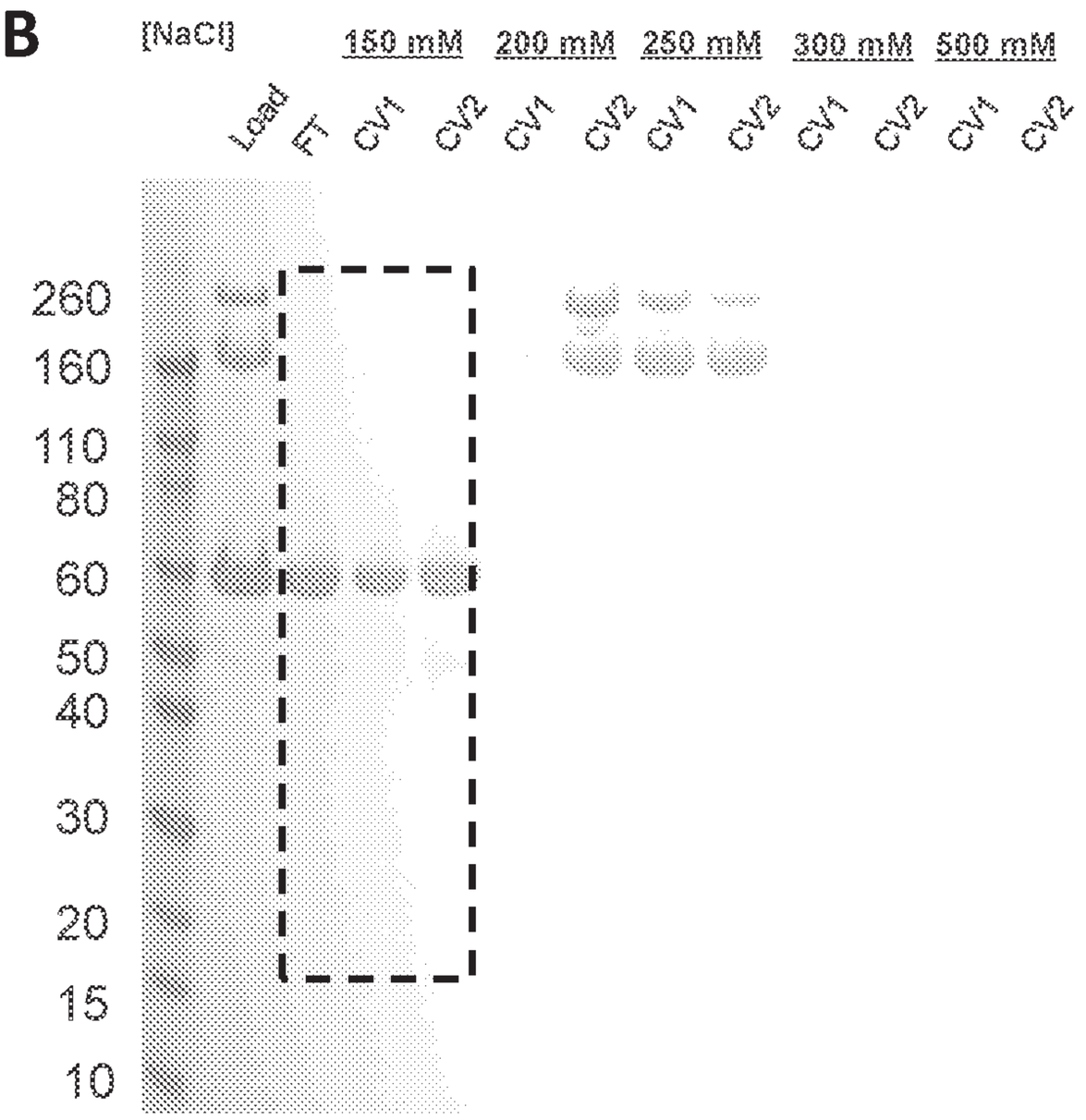


FIG. 21

FIG. 22A

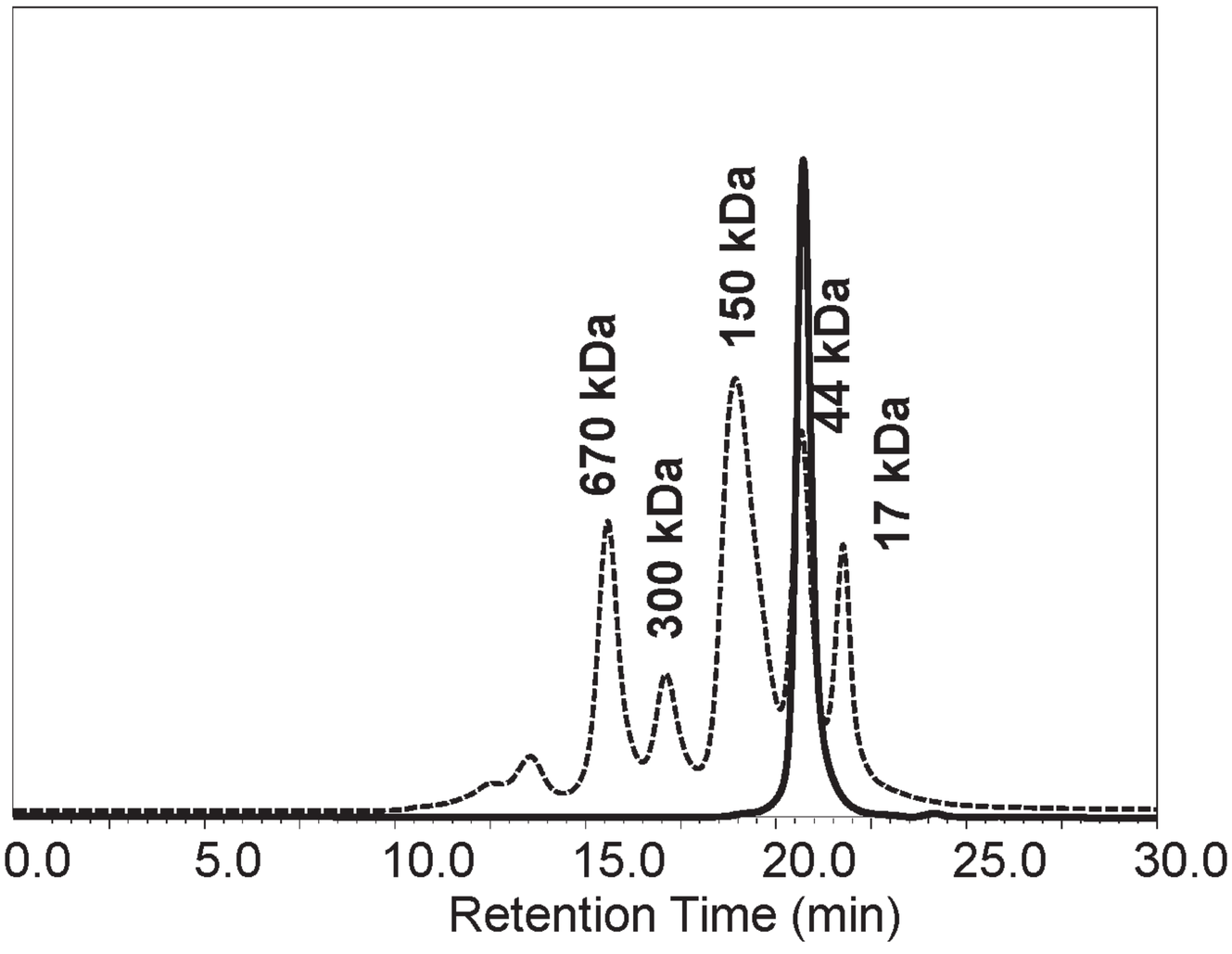


FIG. 22B

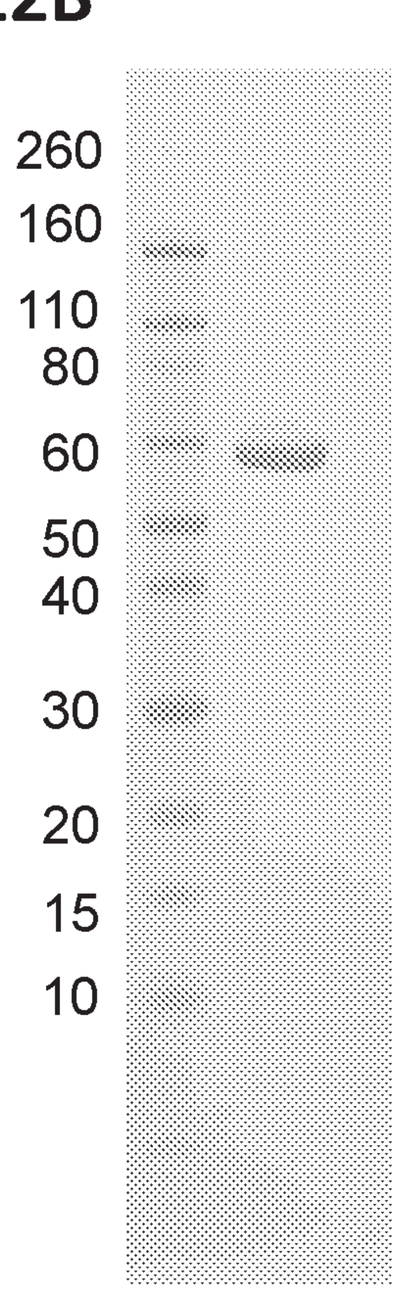


FIG. 22C

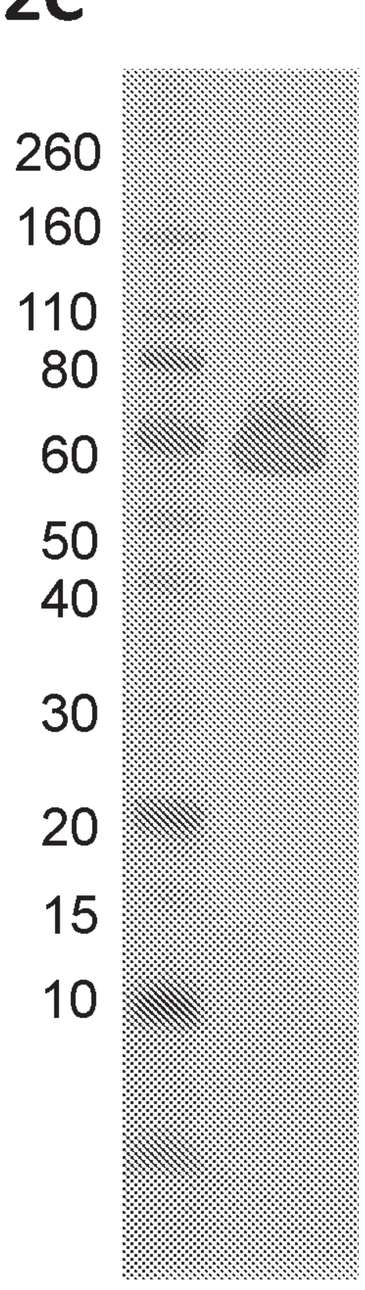


FIG. 22

FIG. 23A

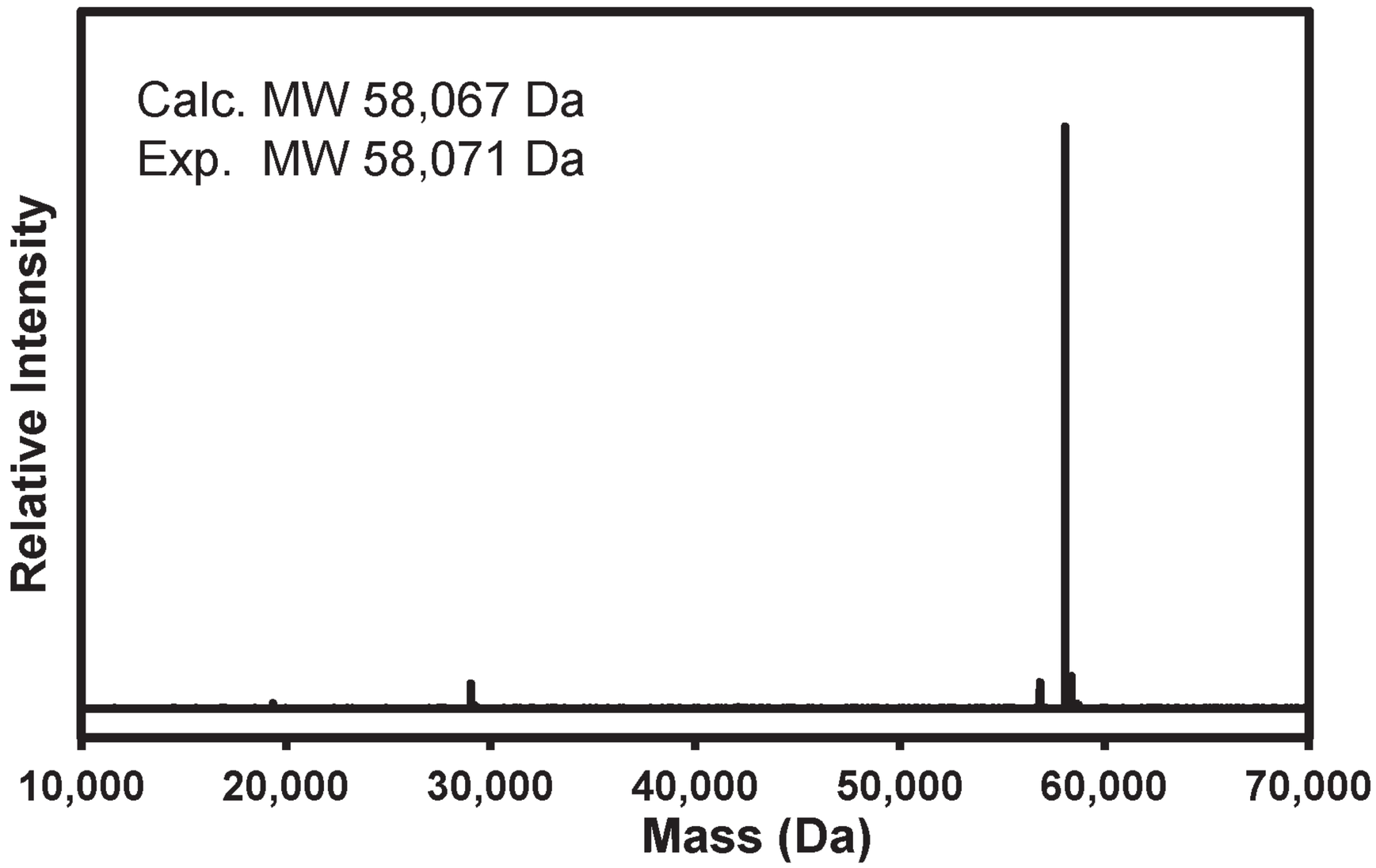


FIG. 23B

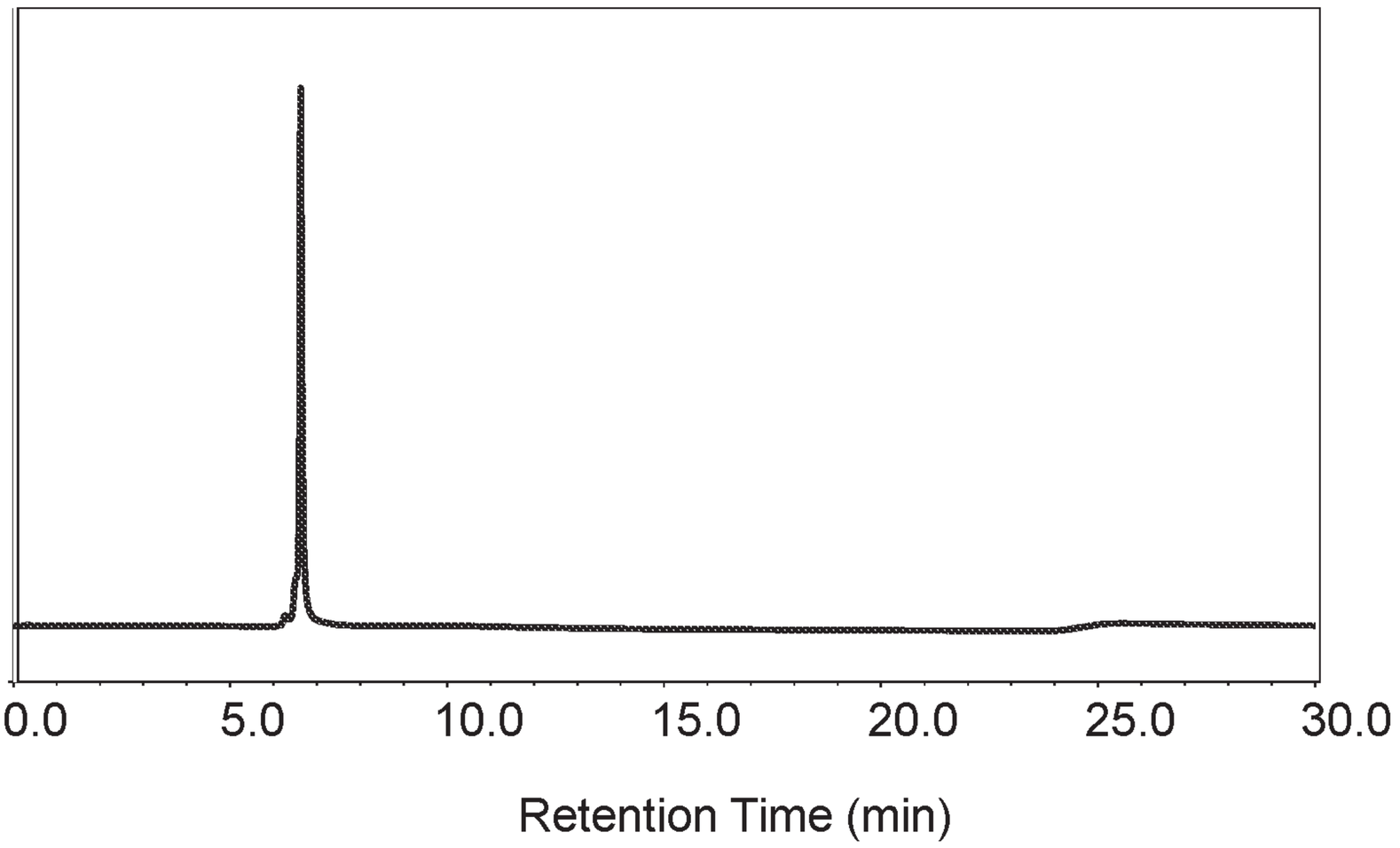


FIG. 23

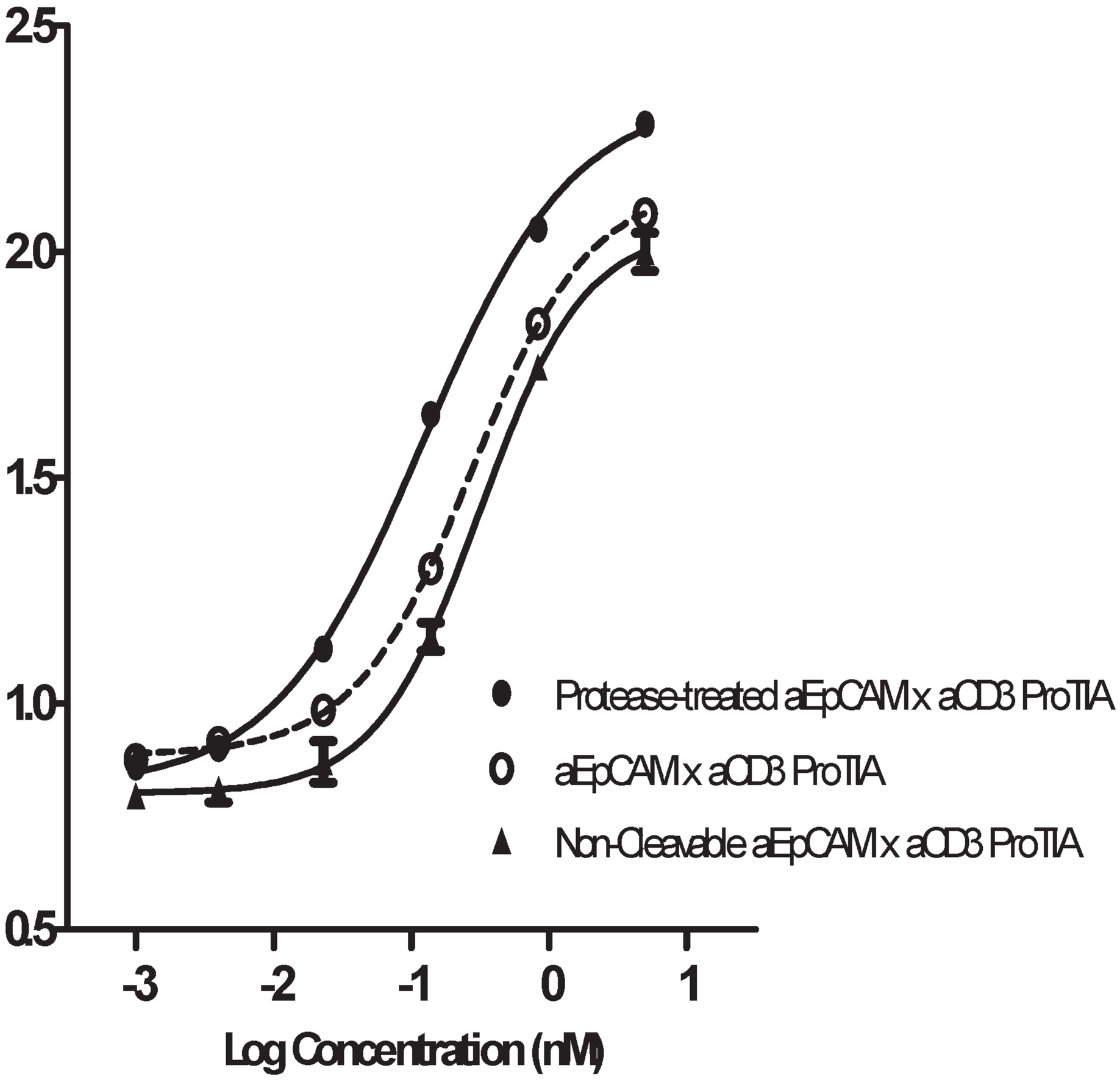

25
20
1.5
1.0
0.5
OD450nM
-3
-2
-1
0
1
Log Concentration (nM)
Protease-treated aEpCAMx aCD3 ProTIA
aEpCAMx aCD3 ProTIA
Non-Cleavable aEpCAMx aCD3 ProTIA

FIG. 24

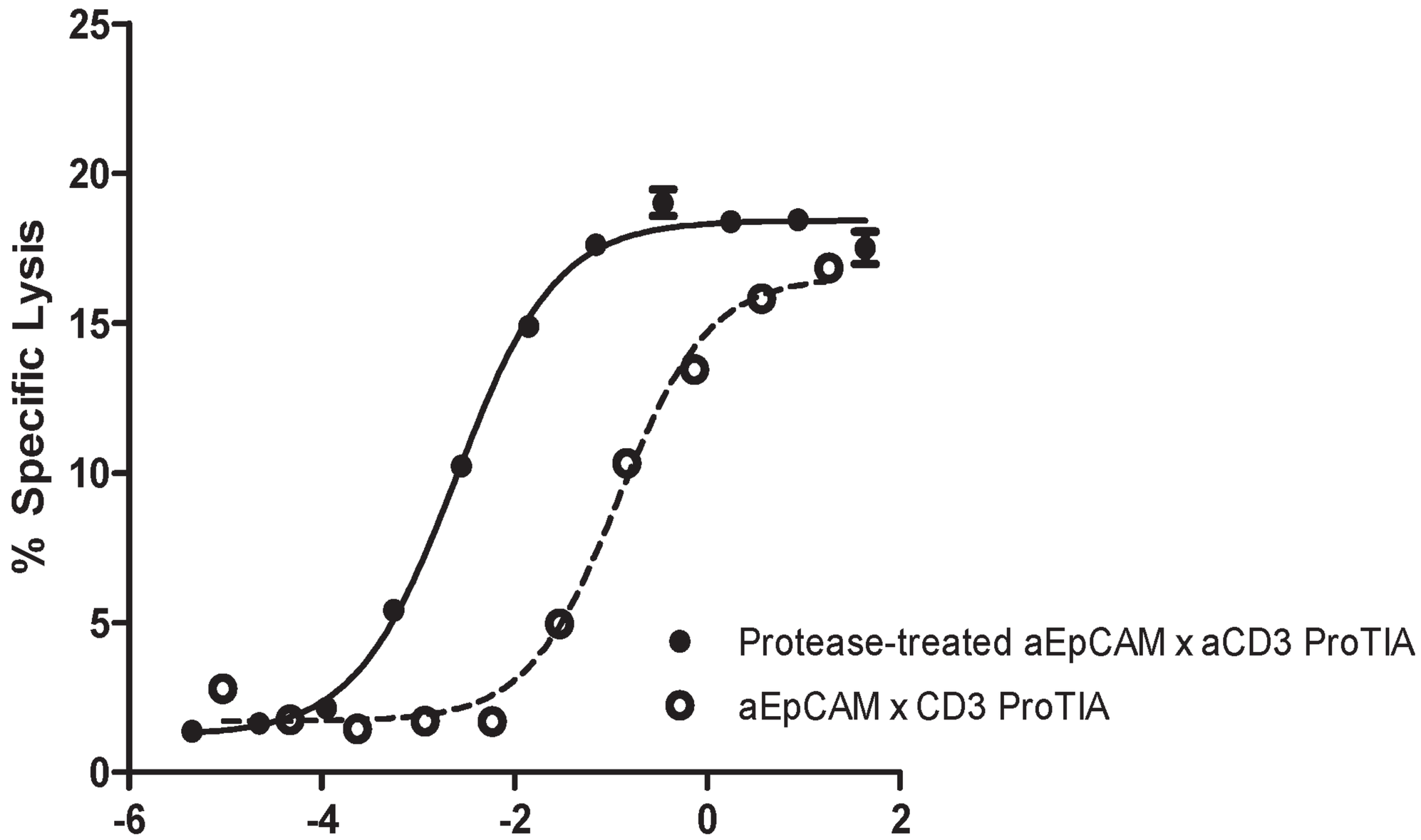

25
20
15
10
5
0
% Specific Lysis
-6
-4
-2
0
2
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x CD3 ProTIA

FIG. 25

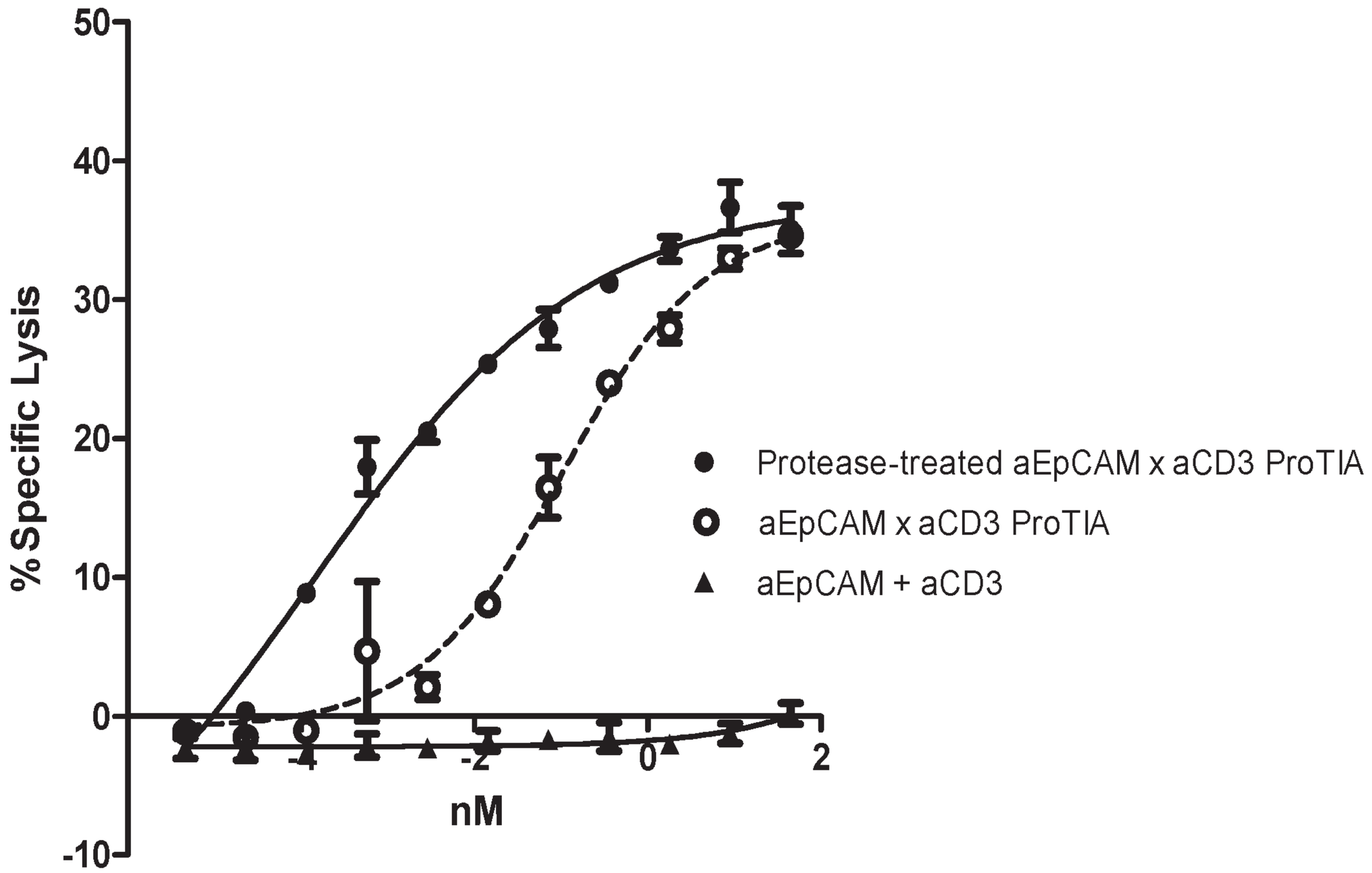

50
40
30
20
10
0
-10
% Specific Lysis
-4
-2
0
2
nM
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
aEpCAM + aCD3

FIG. 26

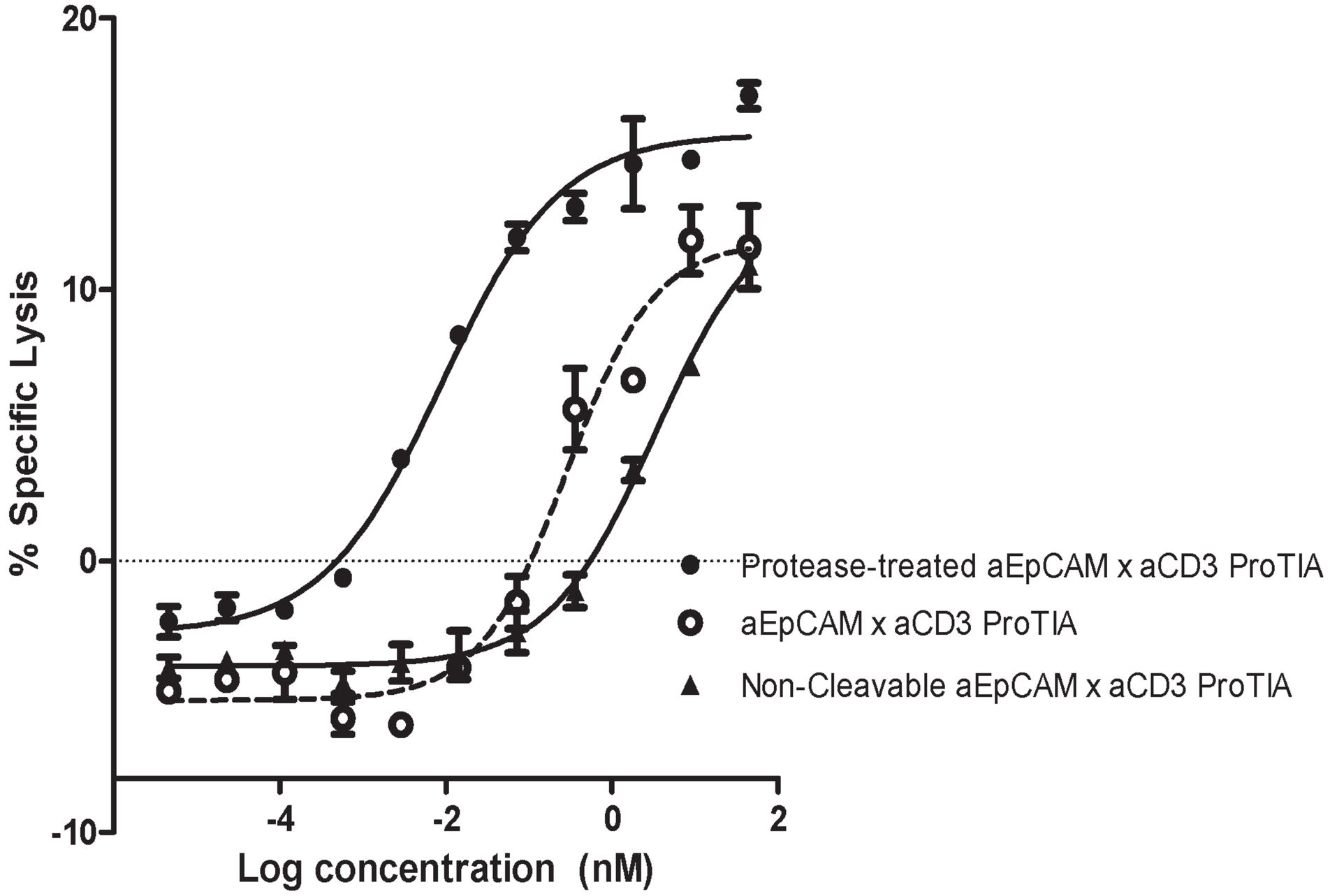

20
10
0
-10
% Specific Lysis
-4
-2
0
2
Log concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 27

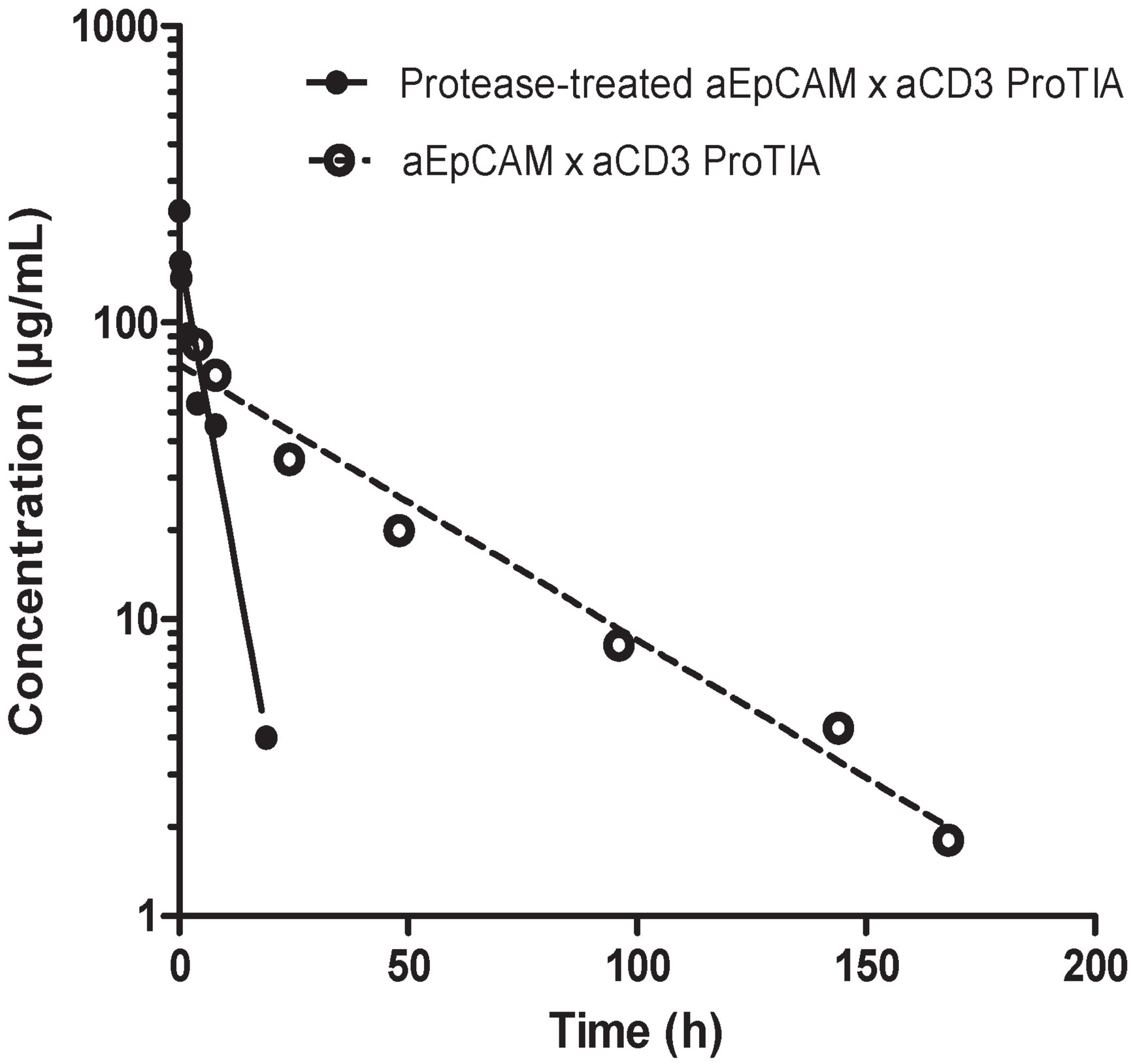

Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Concentration (µg/mL)
1000
100
10
1
0
50
100
150
200
Time (h)

FIG. 28

FIG. 29A



FIG. 29B

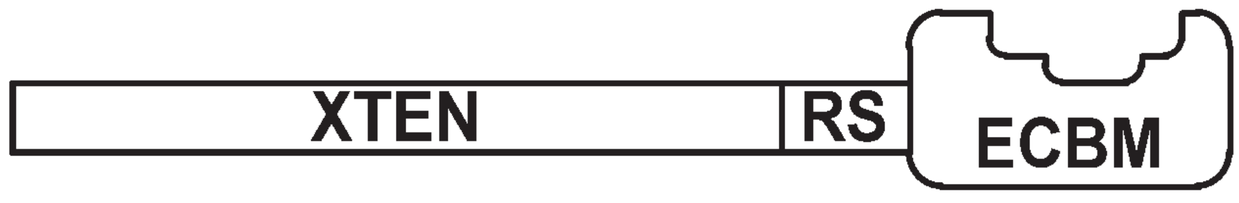


FIG. 29

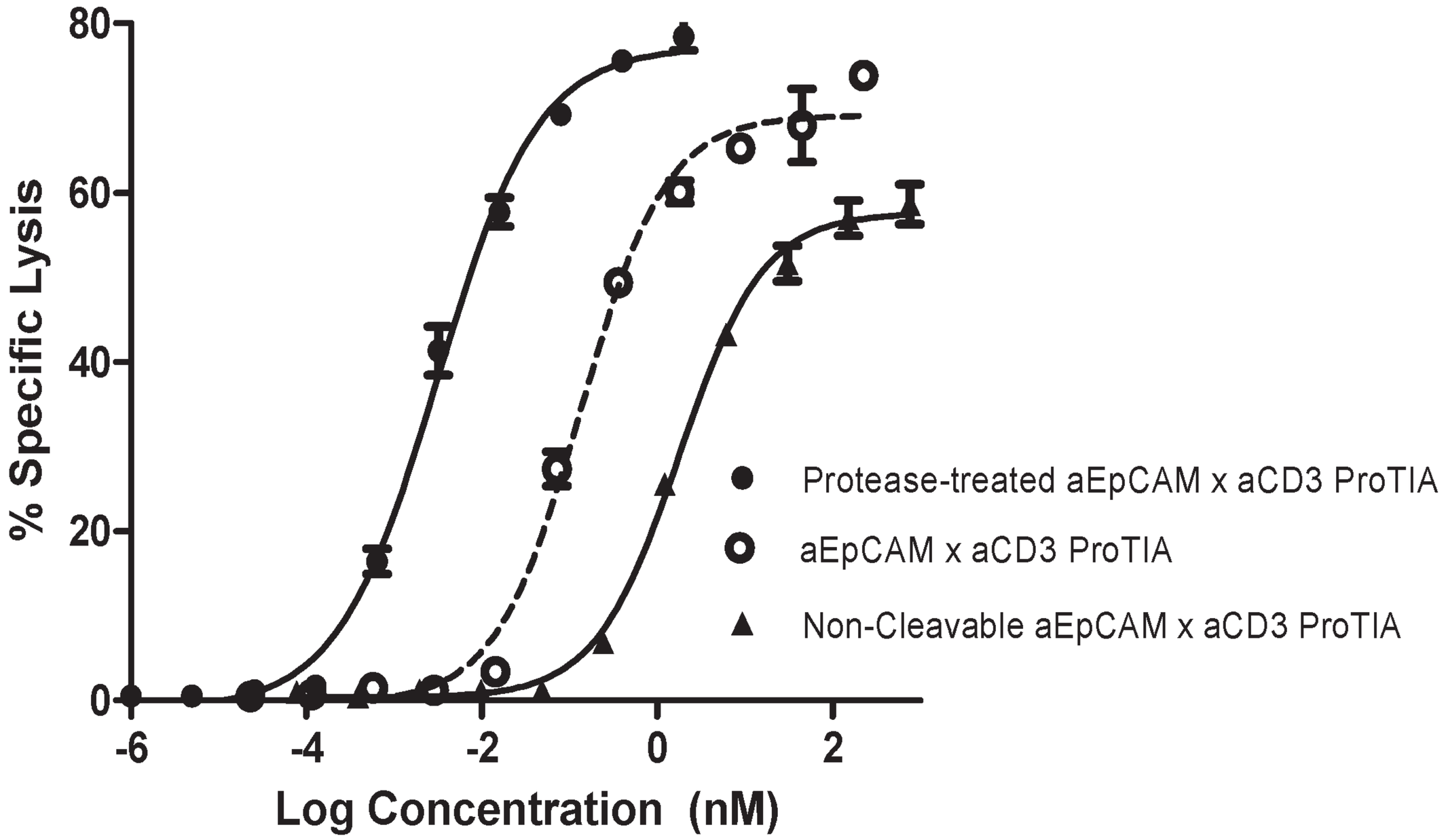

80
60
40
20
0
% Specific Lysis
-6
-4
-2
0
2
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 30

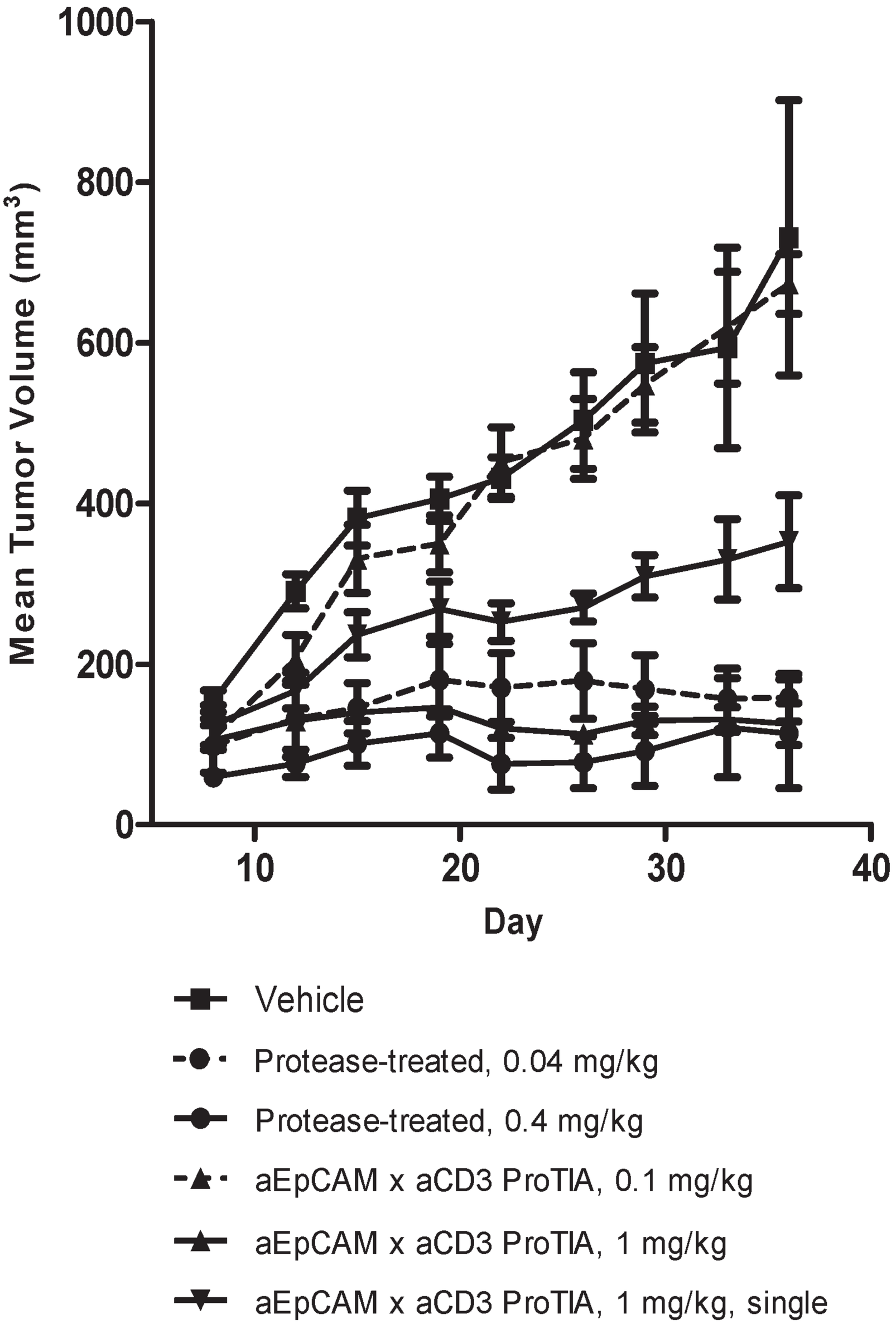

1000
800
600
400
200
0
Mean Tumor Volume (mm3)
10
20
30
40
Day
Vehicle
Protease-treated, 0.04 mg/kg
Protease-treated, 0.4 mg/kg
aEpCAM x aCD3 ProTIA, 0.1 mg/kg
aEpCAM x aCD3 ProTIA, 1 mg/kg
aEpCAM x aCD3 ProTIA, 1 mg/kg, single

FIG. 31

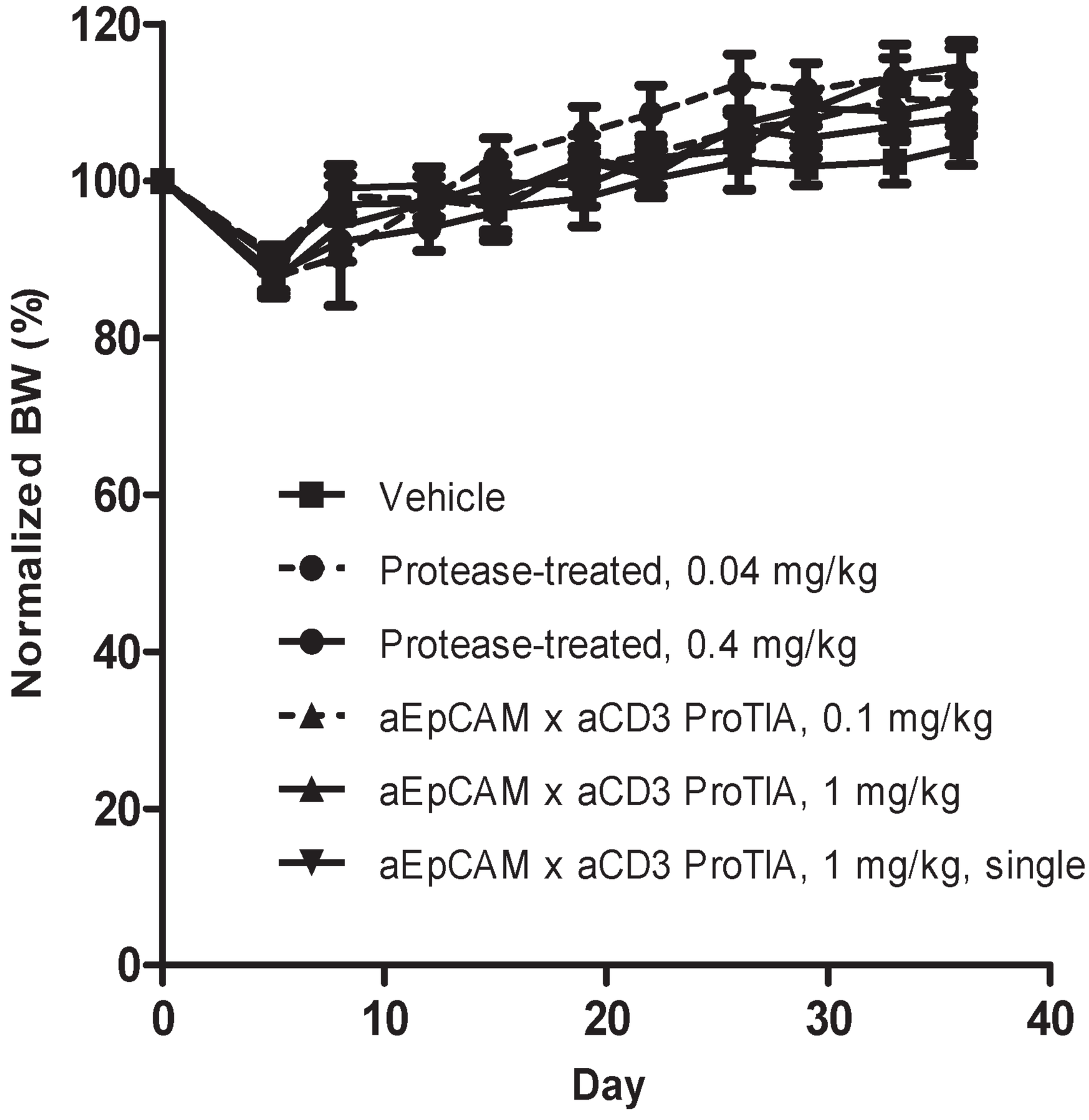

120
100
80
60
40
20
0
Normalized BW (%)
0
10
20
30
40
Day
Vehicle
Protease-treated, 0.04 mg/kg
Protease-treated, 0.4 mg/kg
aEpCAM x aCD3 ProTIA, 0.1 mg/kg
aEpCAM x aCD3 ProTIA, 1 mg/kg
aEpCAM x aCD3 ProTIA, 1 mg/kg, single

FIG. 32

FIG. 33A

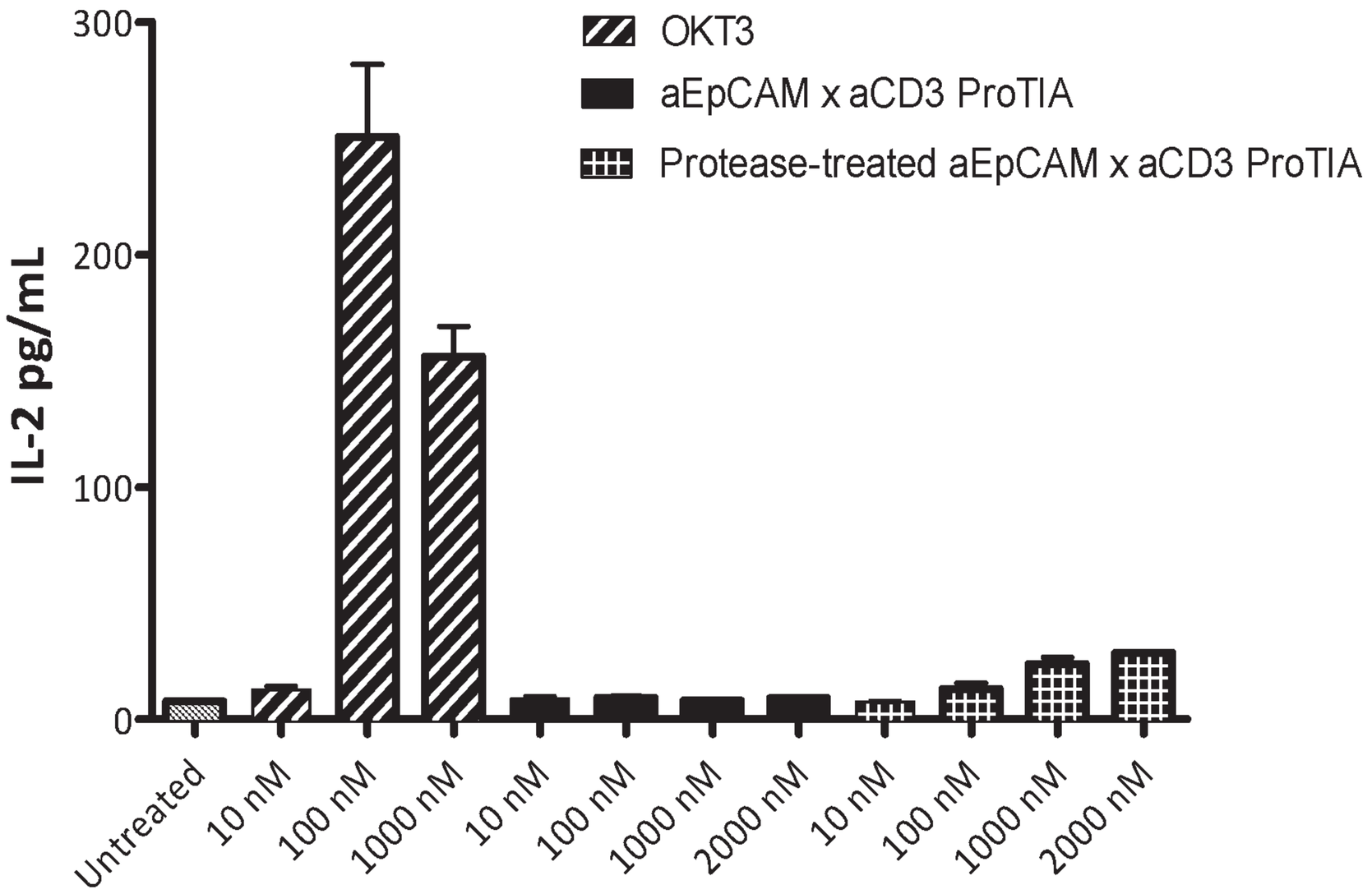


FIG. 33B

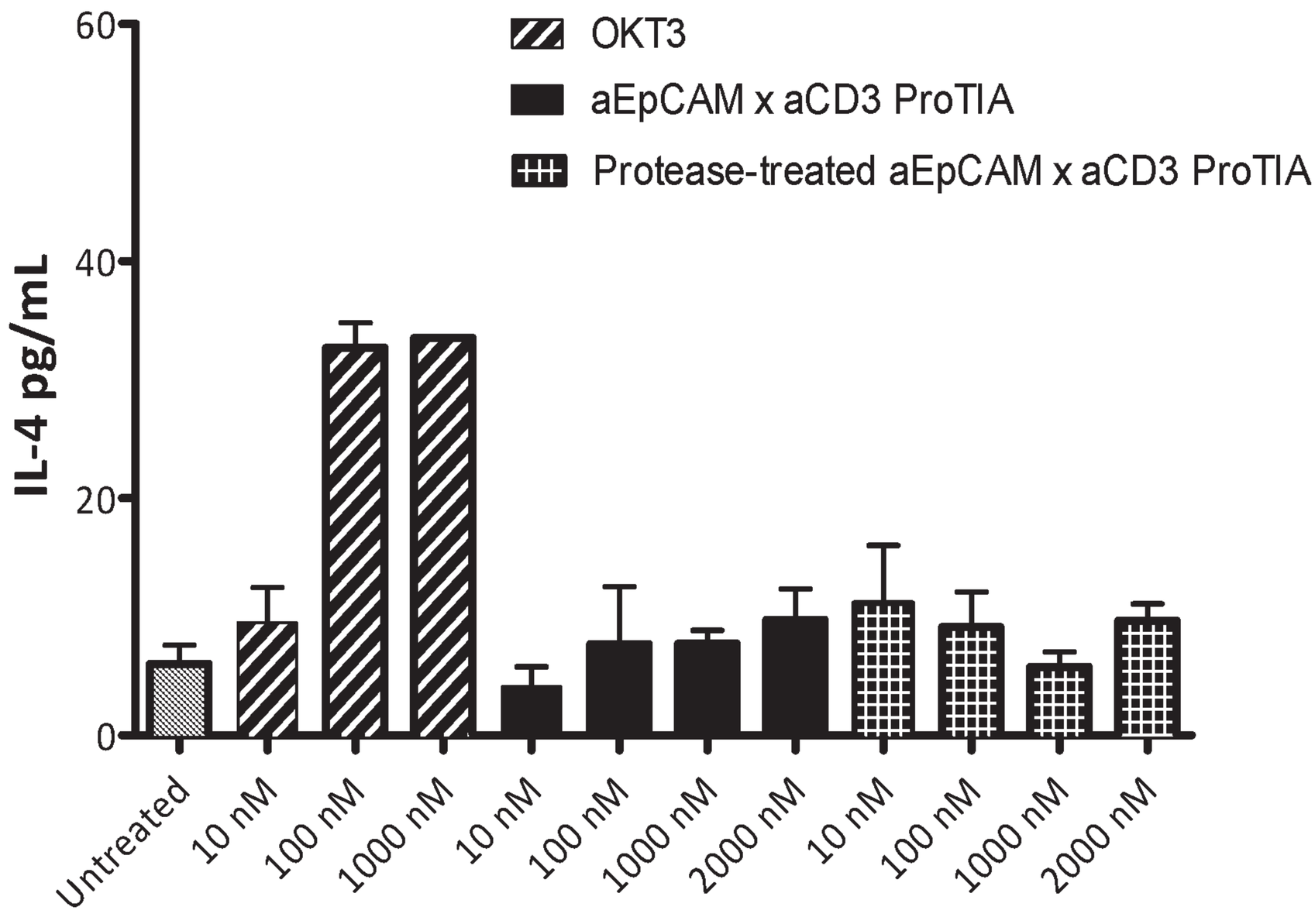


FIG. 33

FIG. 34A

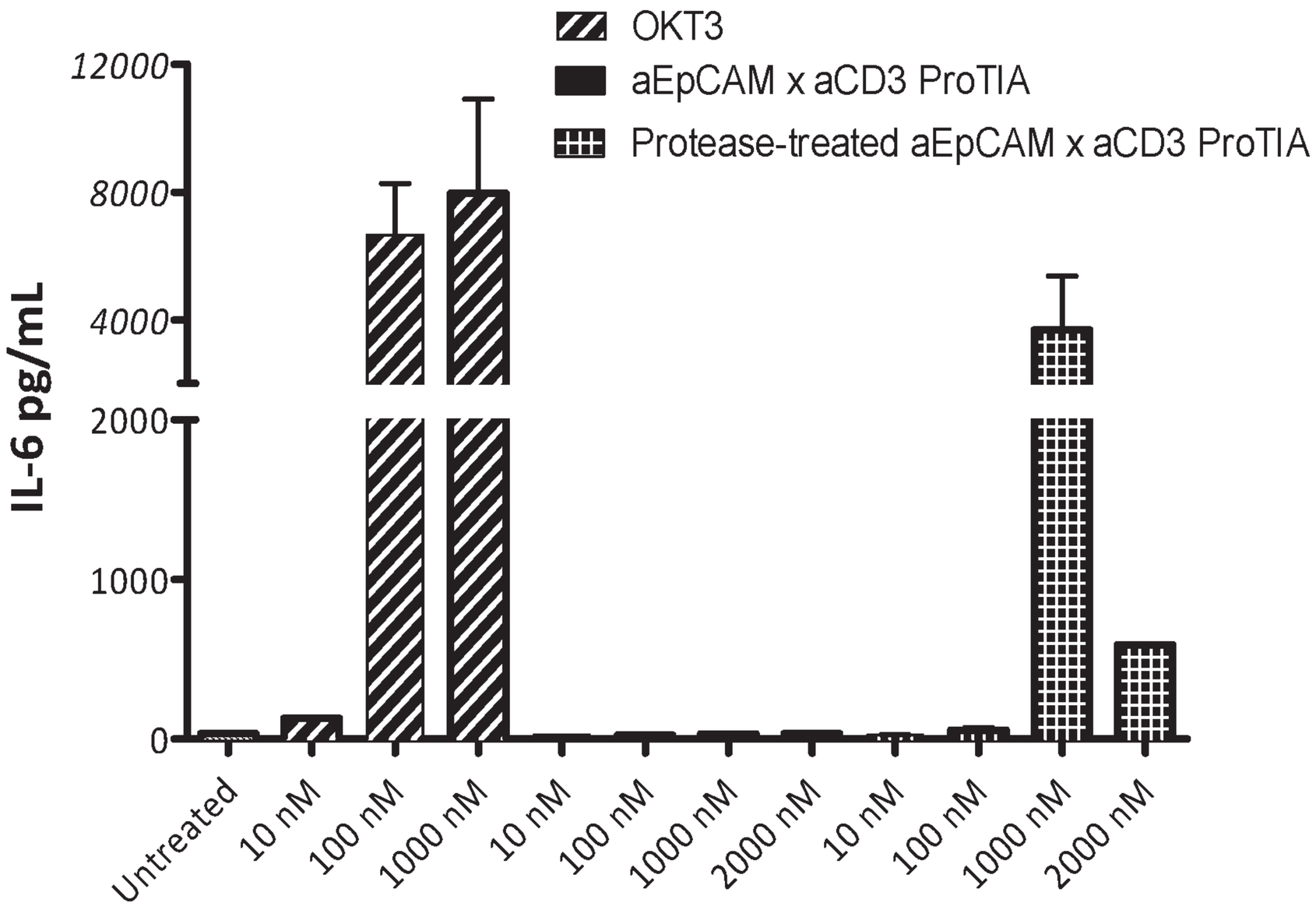


FIG. 34B

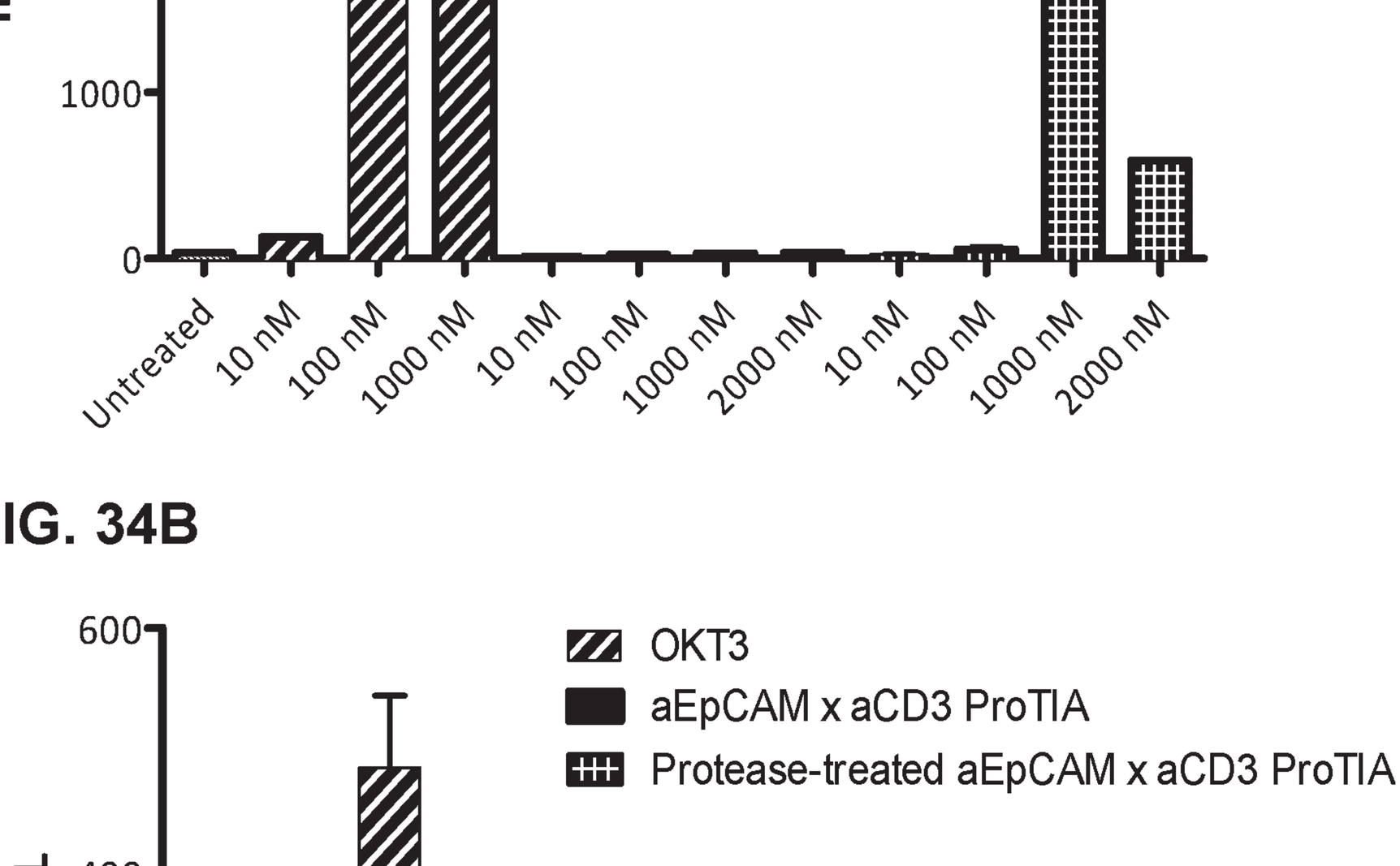


FIG. 34

FIG. 35A

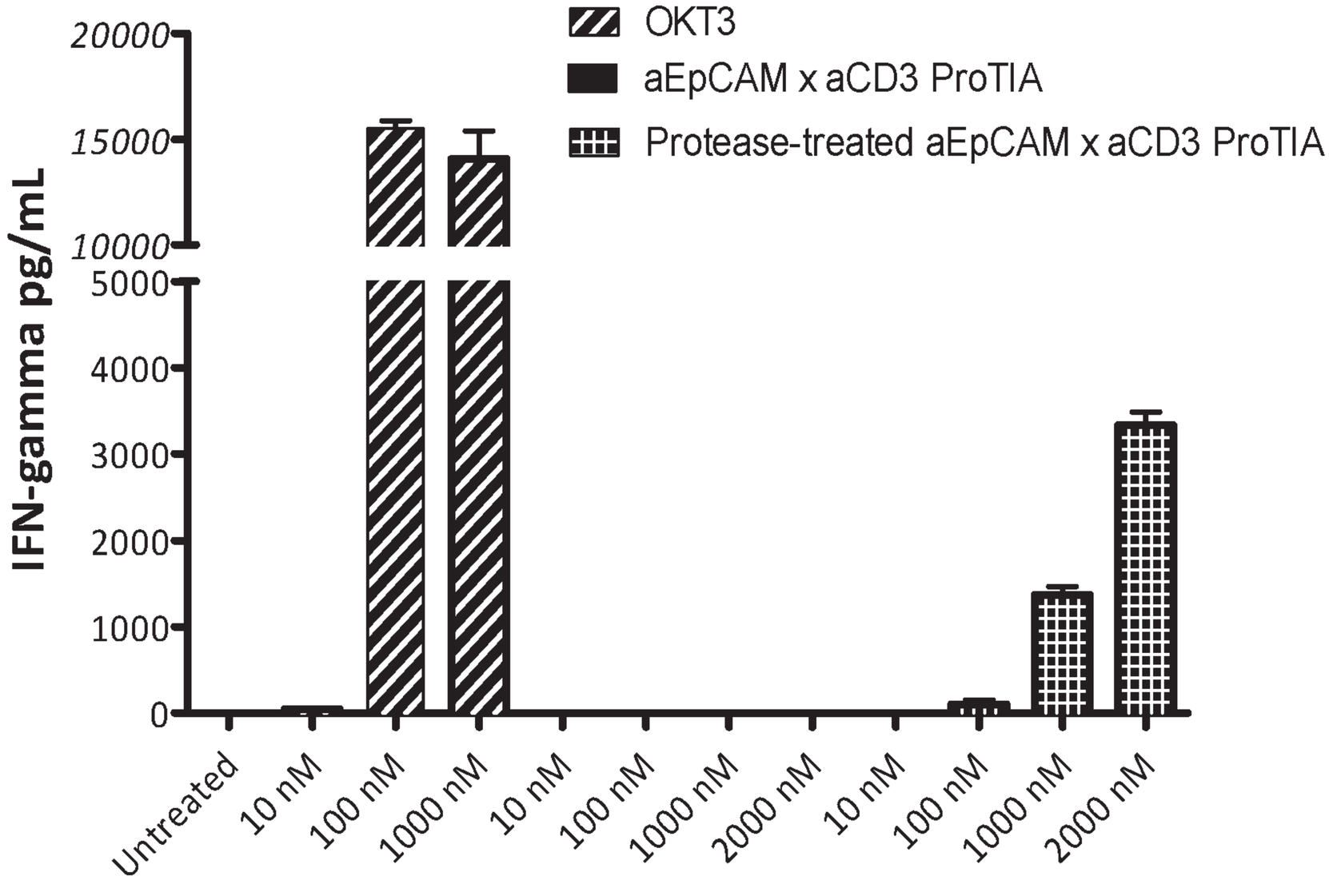


FIG. 35B

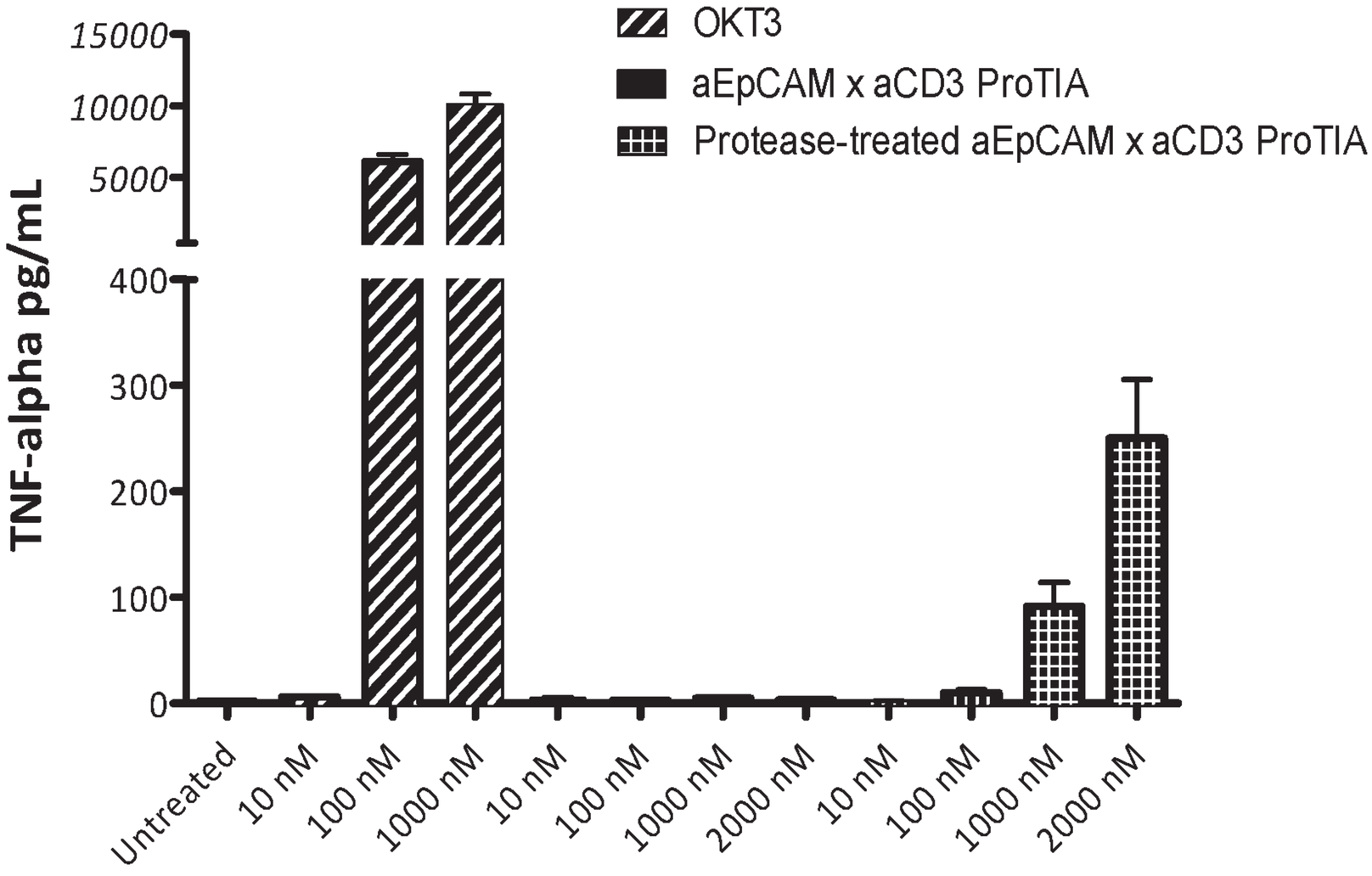


FIG. 35

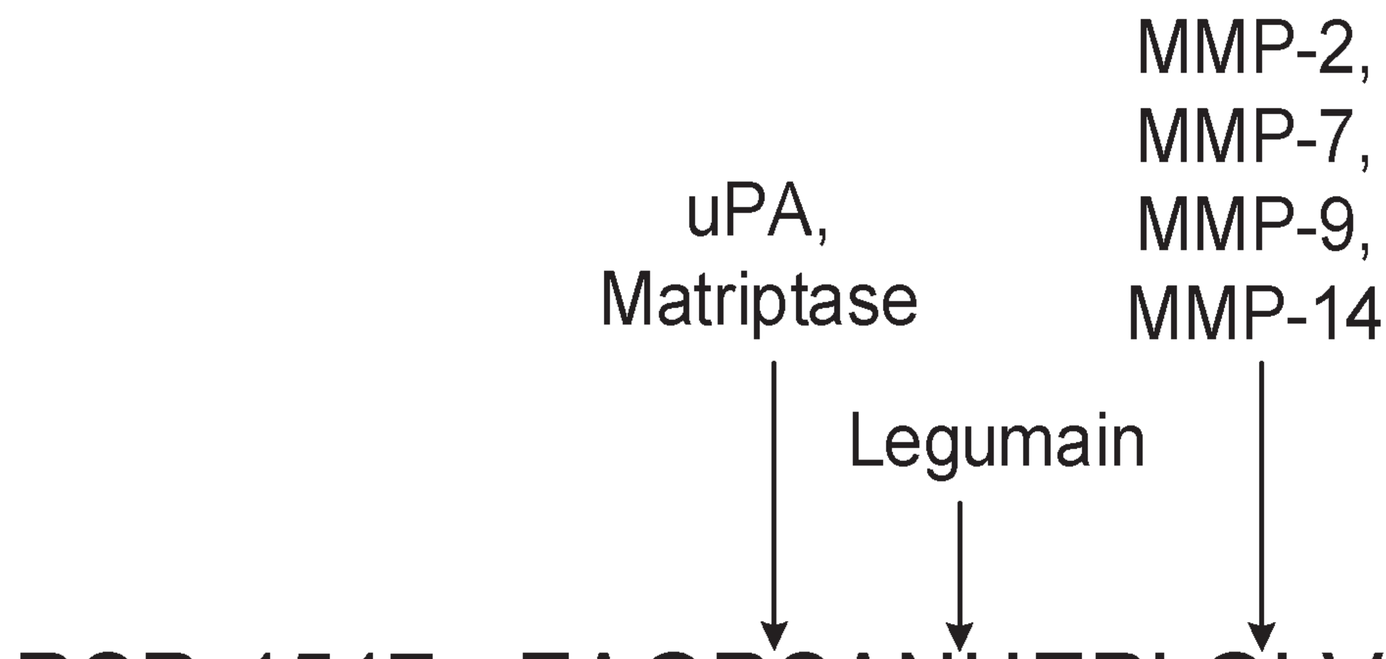

MMP-2,
MMP-7,
MMP-9,
MMP-14
uPA,
Matriptase
Legumain
RSR-1517: EAGRSANHEPLGLVAT

FIG. 36

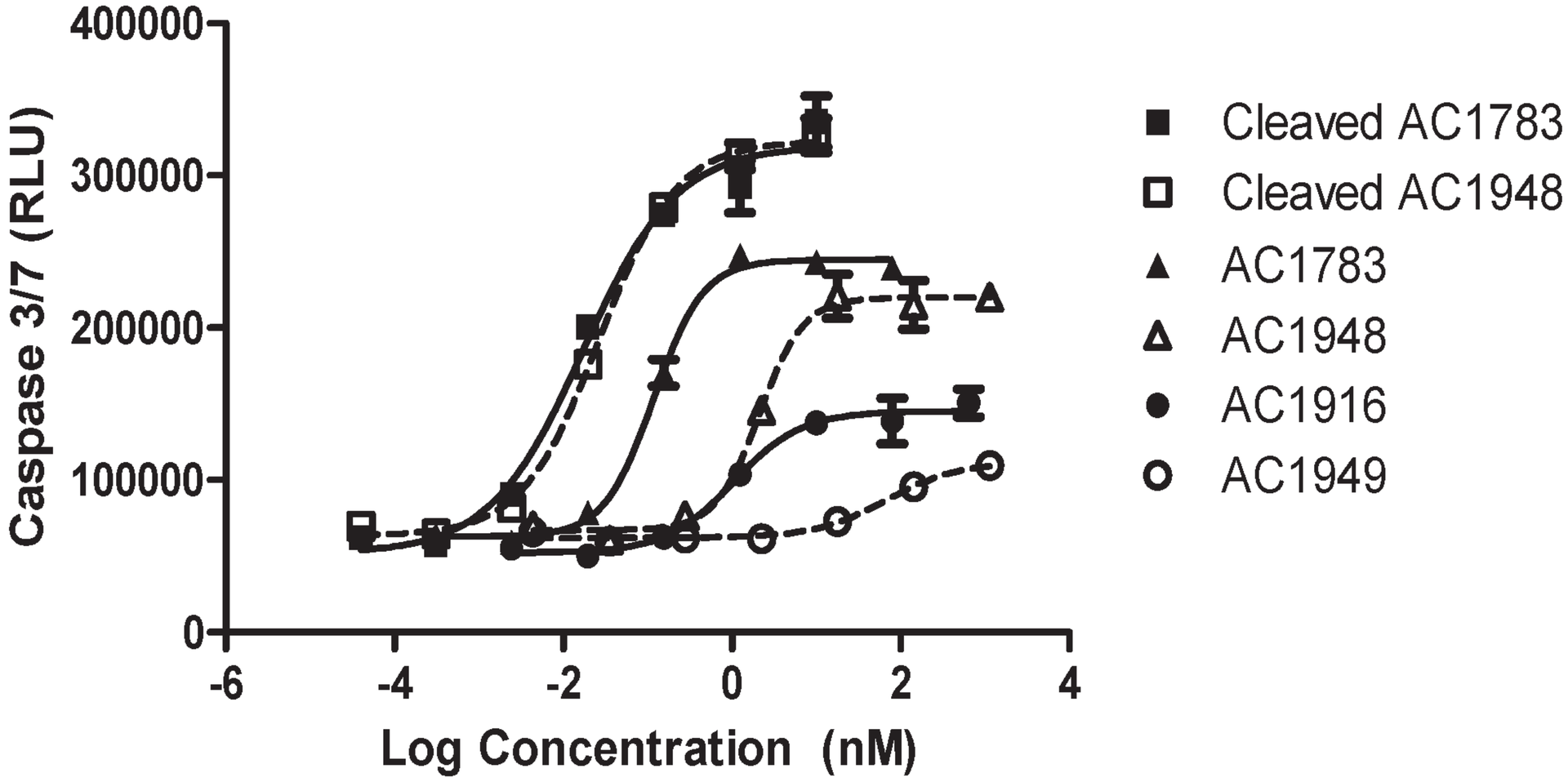

400000
300000
200000
100000
0
Caspase 3/7 (RLU)
-6
-4
-2
0
2
4
Log Concentration (nM)
Cleaved AC1783
Cleaved AC1948
AC1783
AC1948
AC1916
AC1949

FIG. 37

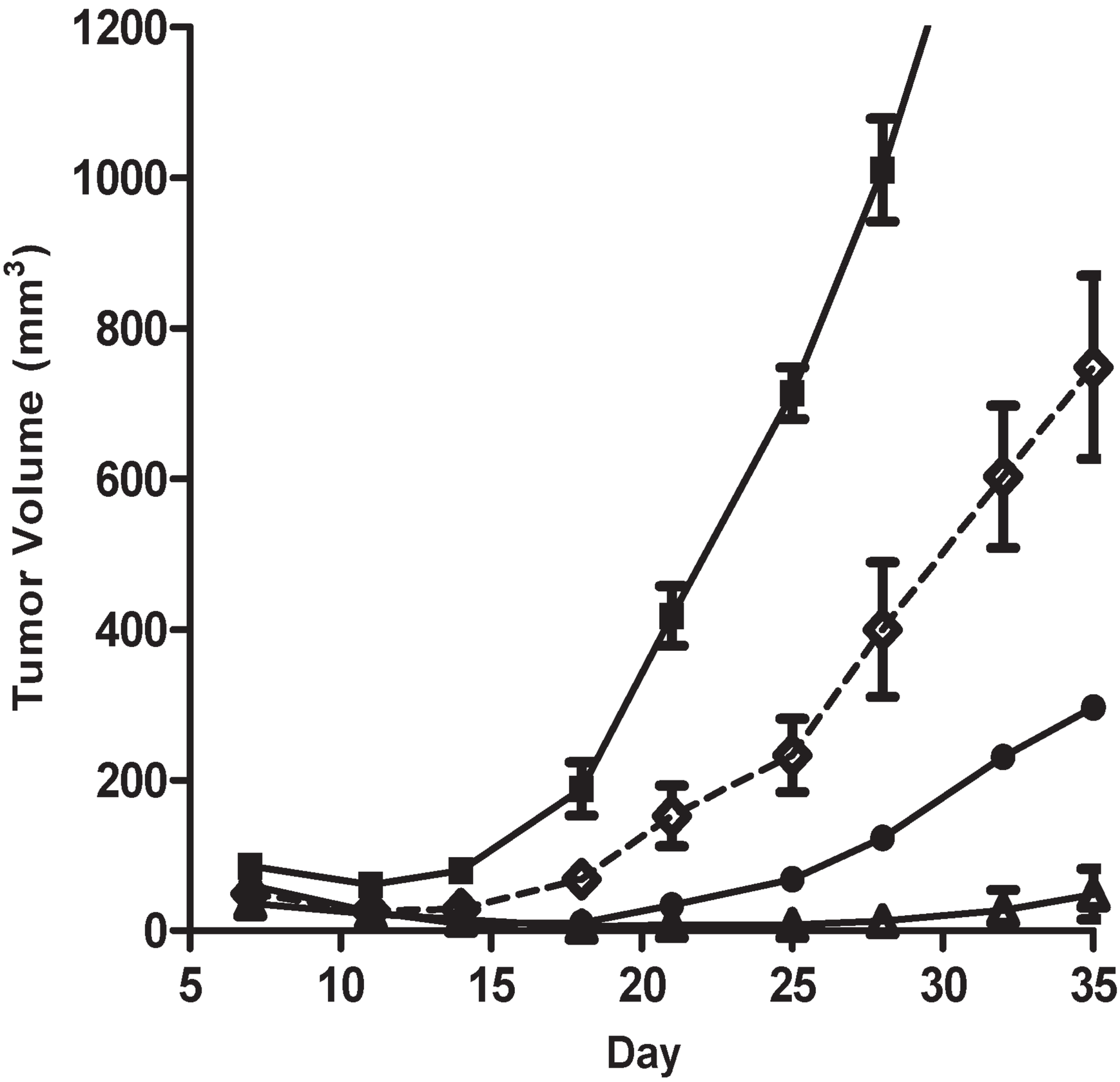

1200
1000
800
600
400
200
0
Tumor Volume (mm3)
5
10
15
20
25
30
35
Day
Vehicle
Protease treated, 0.21 mg/kg
aEpCAM x aCD3 ProTIA, 0.5 mg/kg
Non-cleavabale, 0.49 mg/kg

FIG. 38

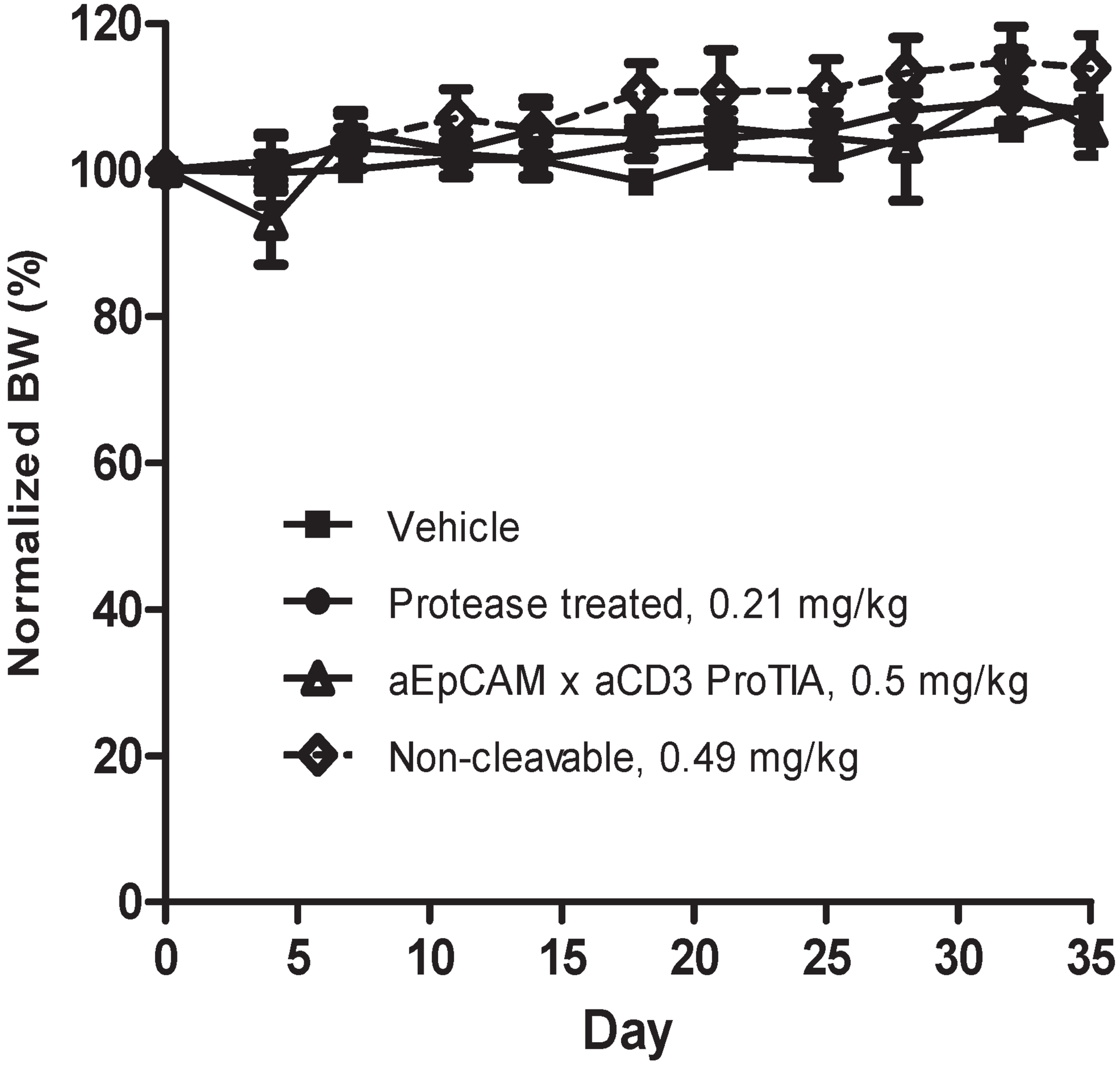

120
100
80
60
40
20
0
Normalized BW (%)
0
5
10
15
20
25
30
35
Day
Vehicle
Protease treated, 0.21 mg/kg
aEpCAM x aCD3 ProTIA, 0.5 mg/kg
Non-cleavable, 0.49 mg/kg

FIG. 39

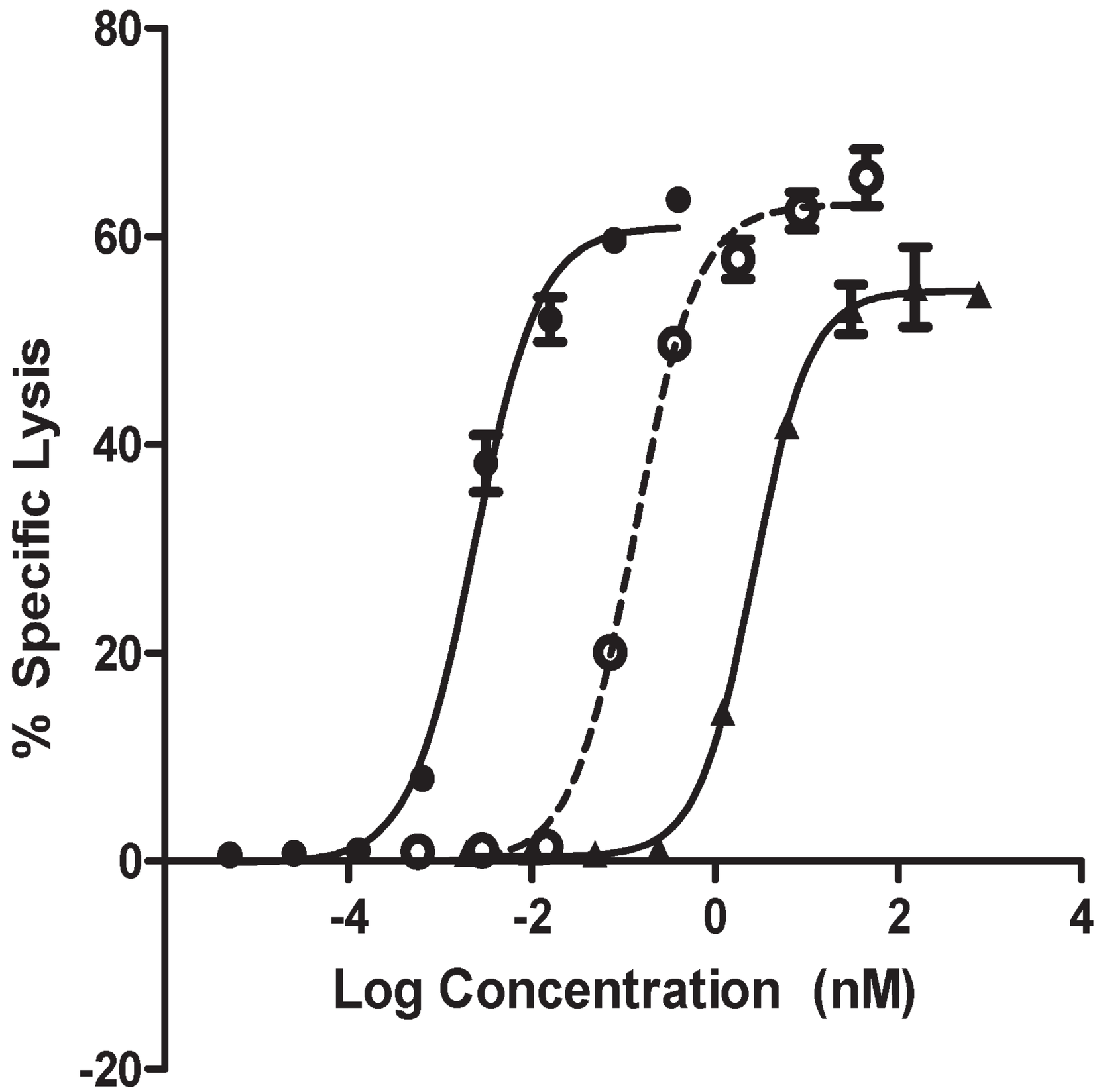

80
60
40
20
0
-20
% Specific Lysis
-4
-2
0
2
4
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 40

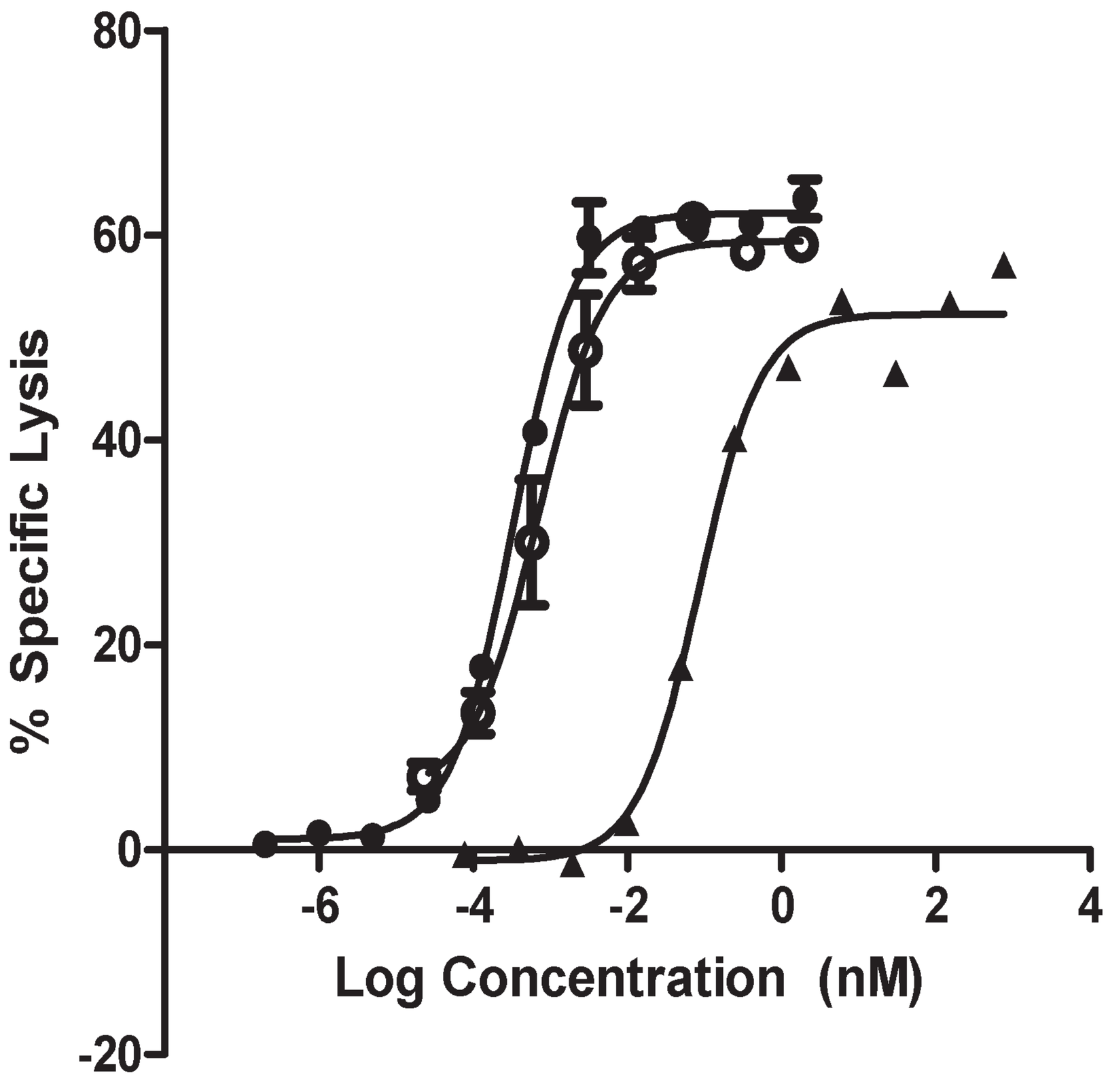

80
60
40
20
0
-20
% Specific Lysis
-6
-4
-2
0
2
4
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 41

FIG. 42A

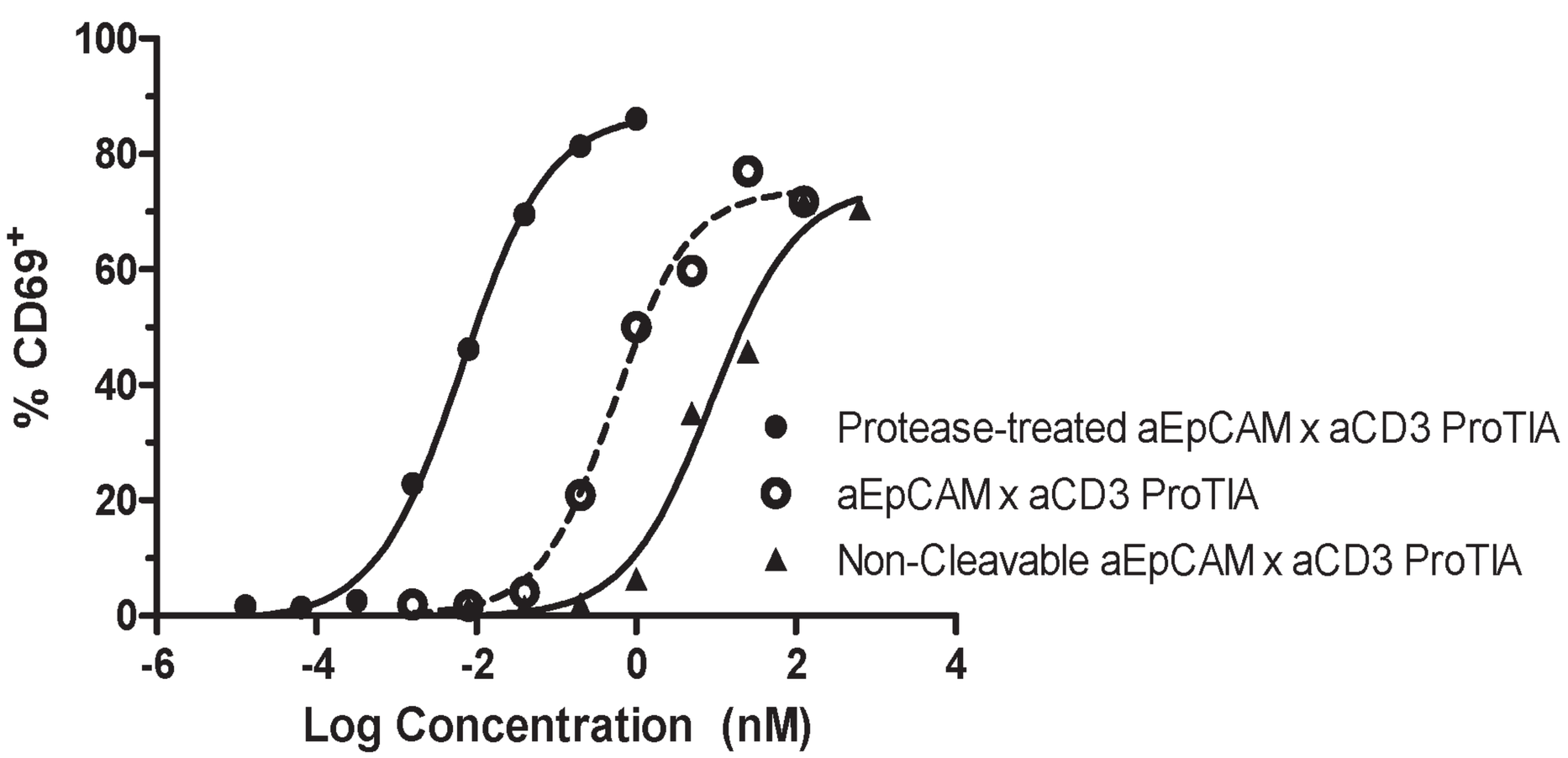


FIG. 42B

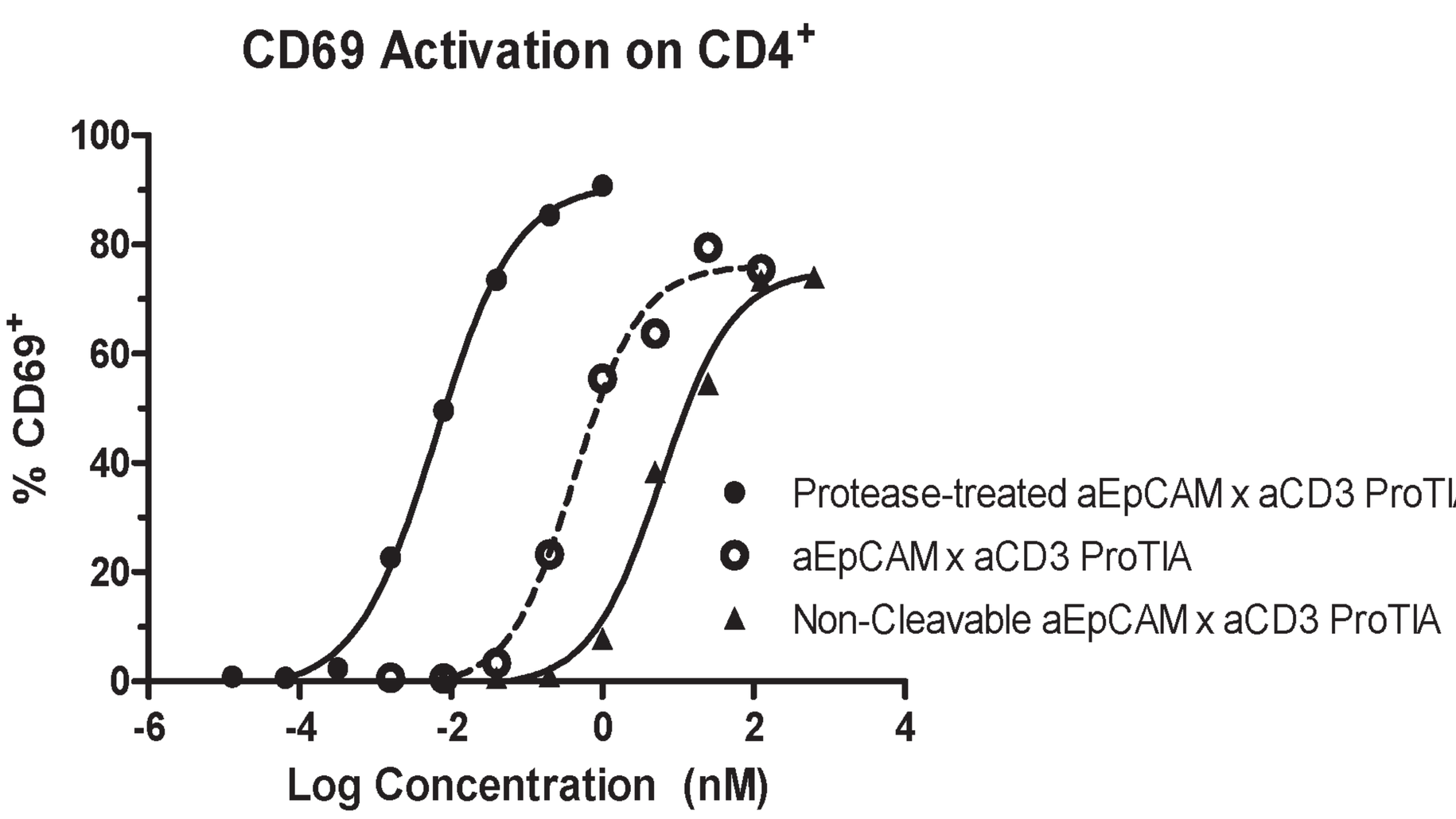


FIG. 42

FIG. 43A

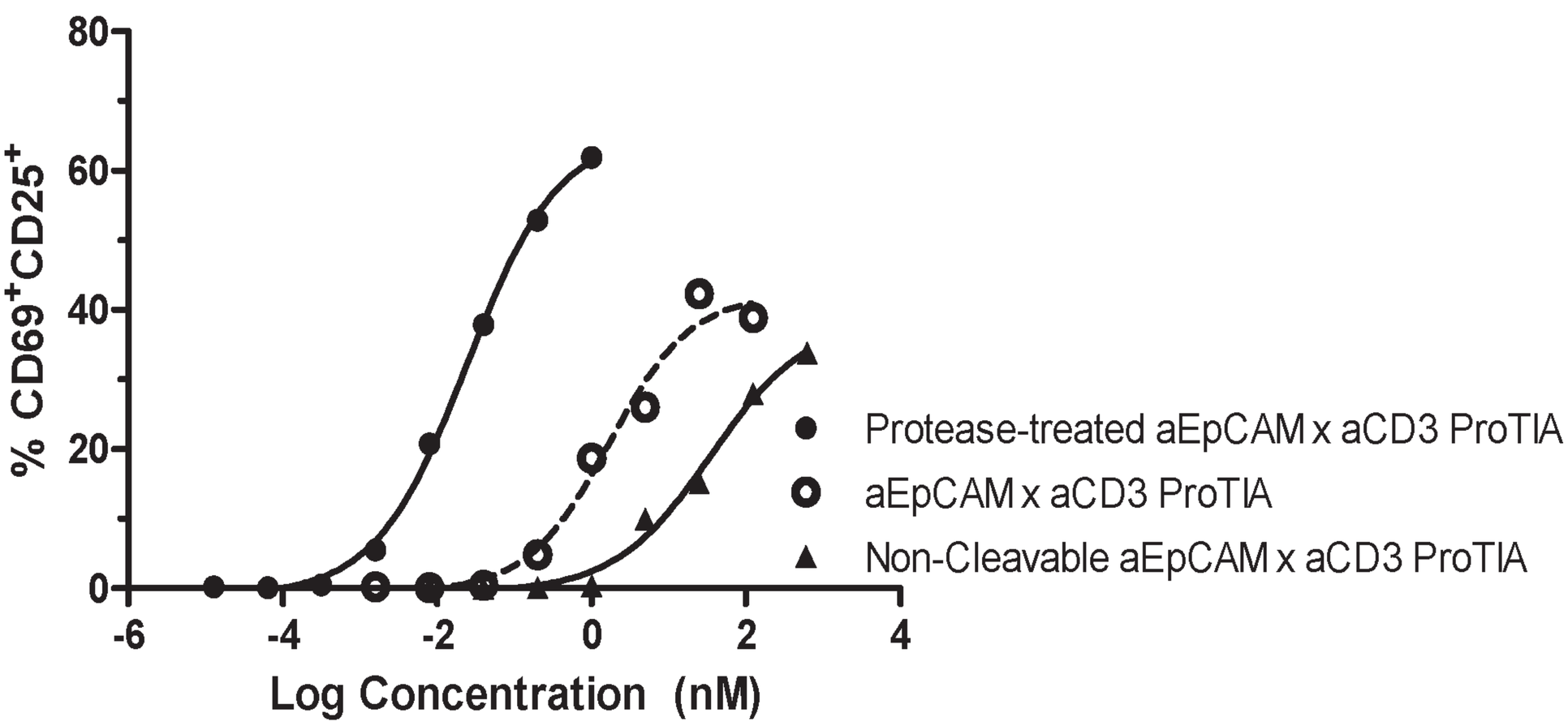


FIG. 43B

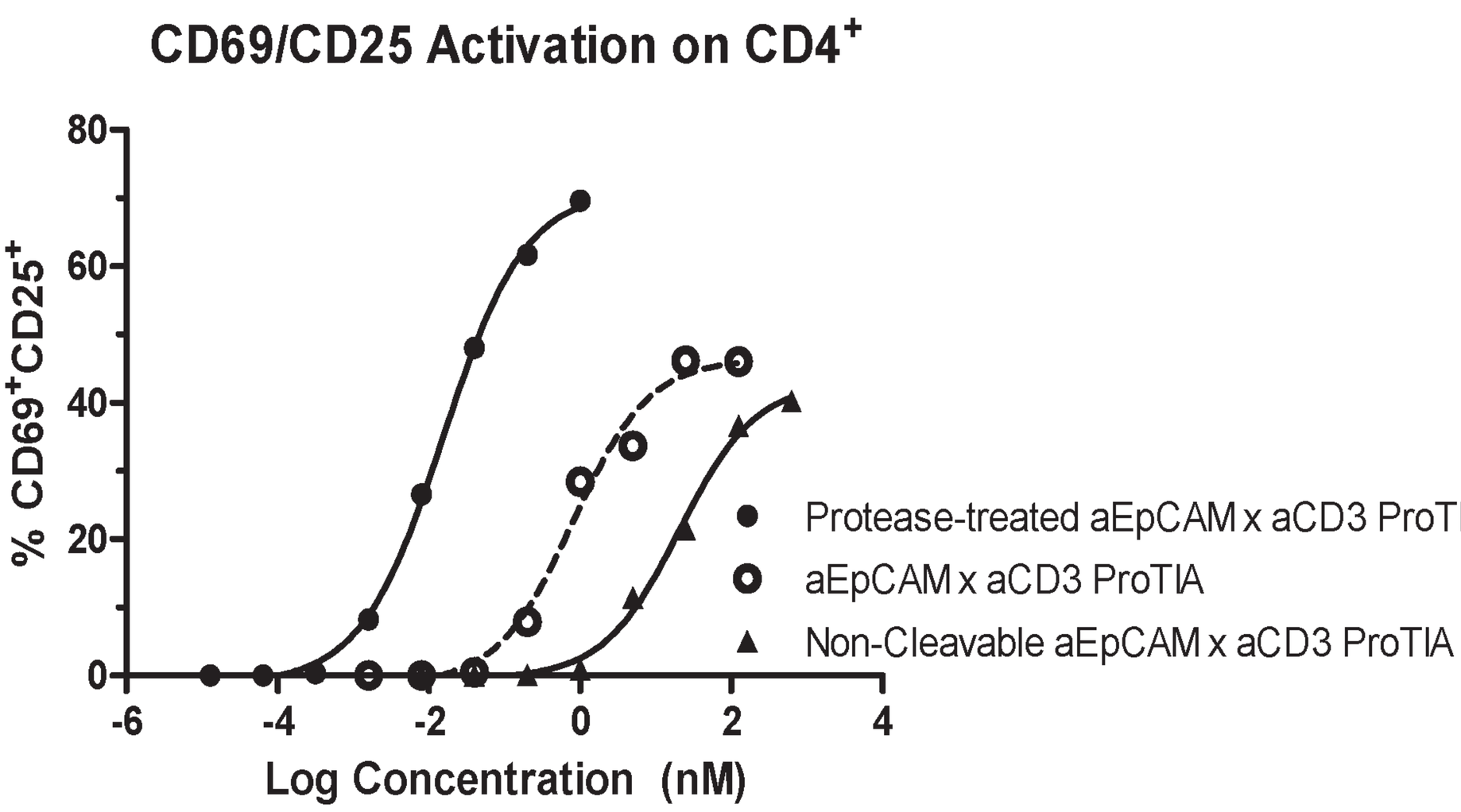


FIG. 43

FIG. 44A

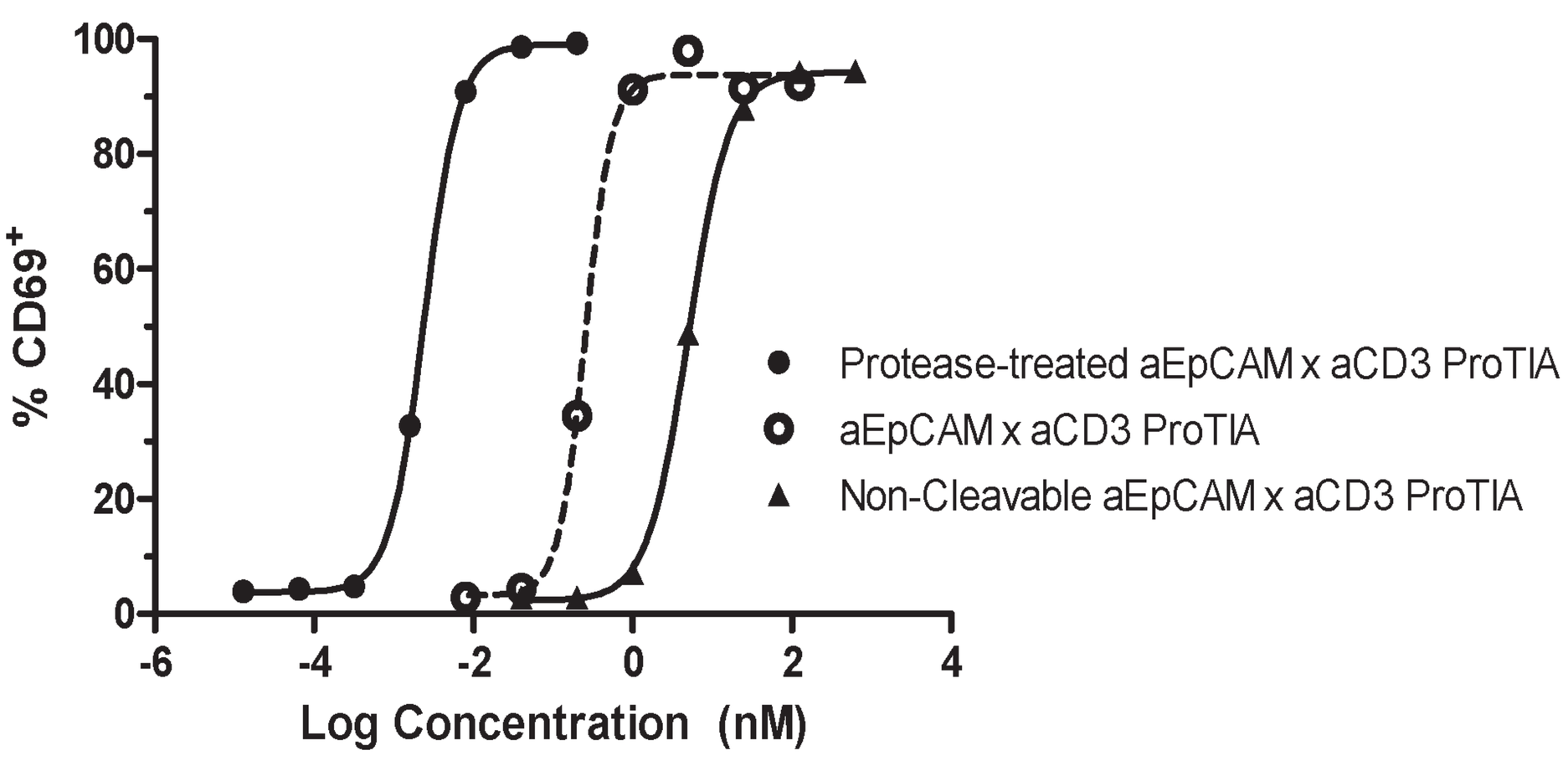


FIG. 44B

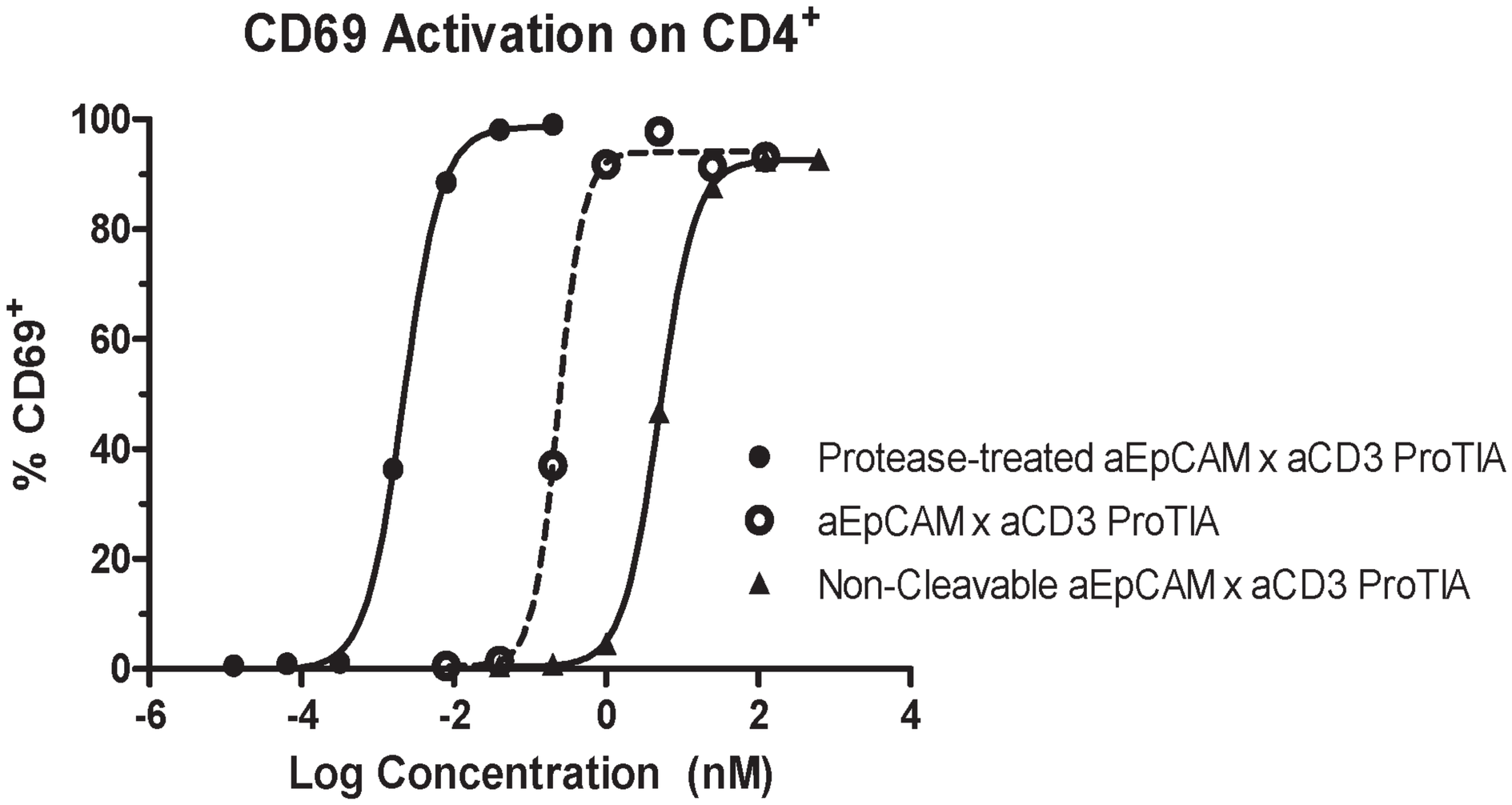


FIG. 44

FIG. 45A

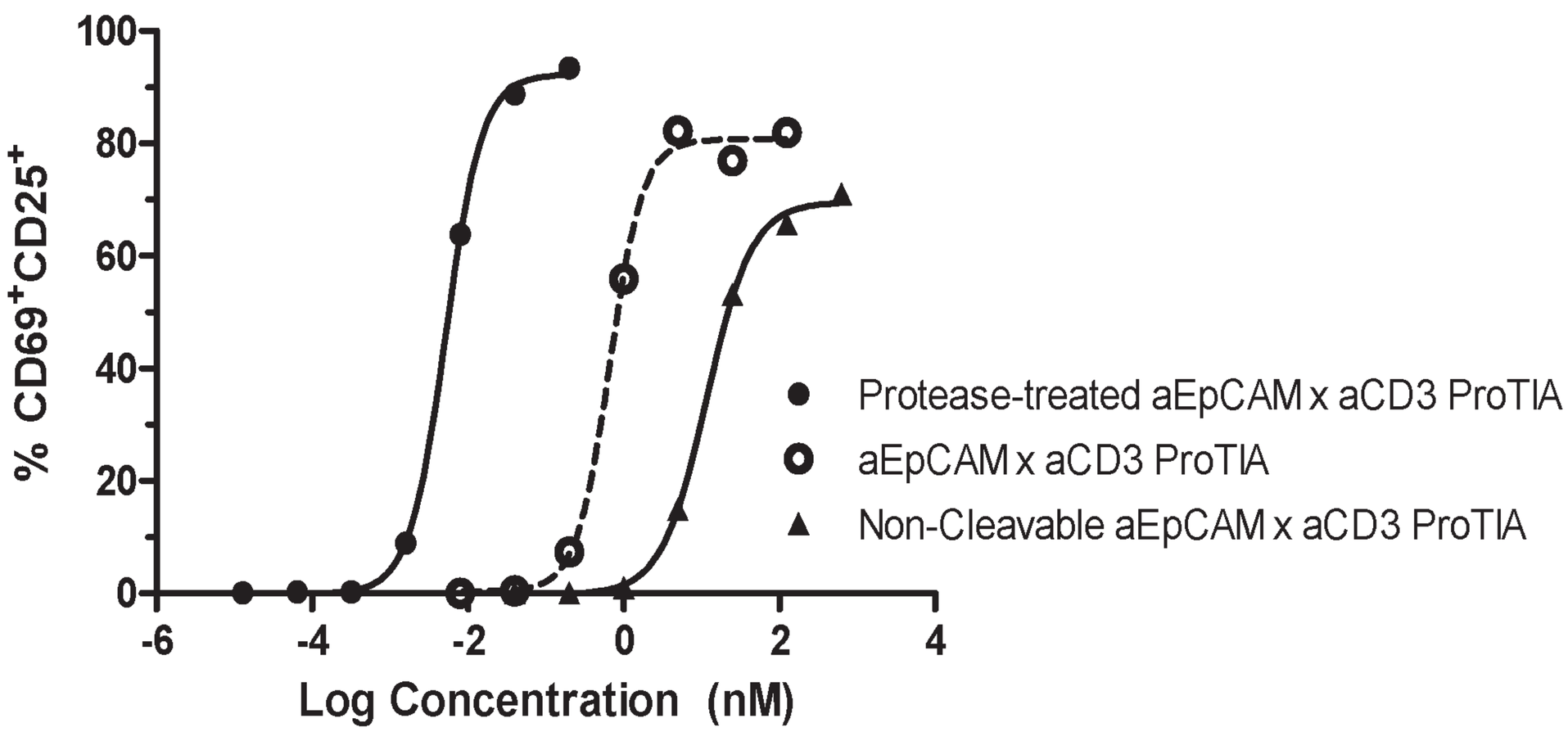


FIG. 45B

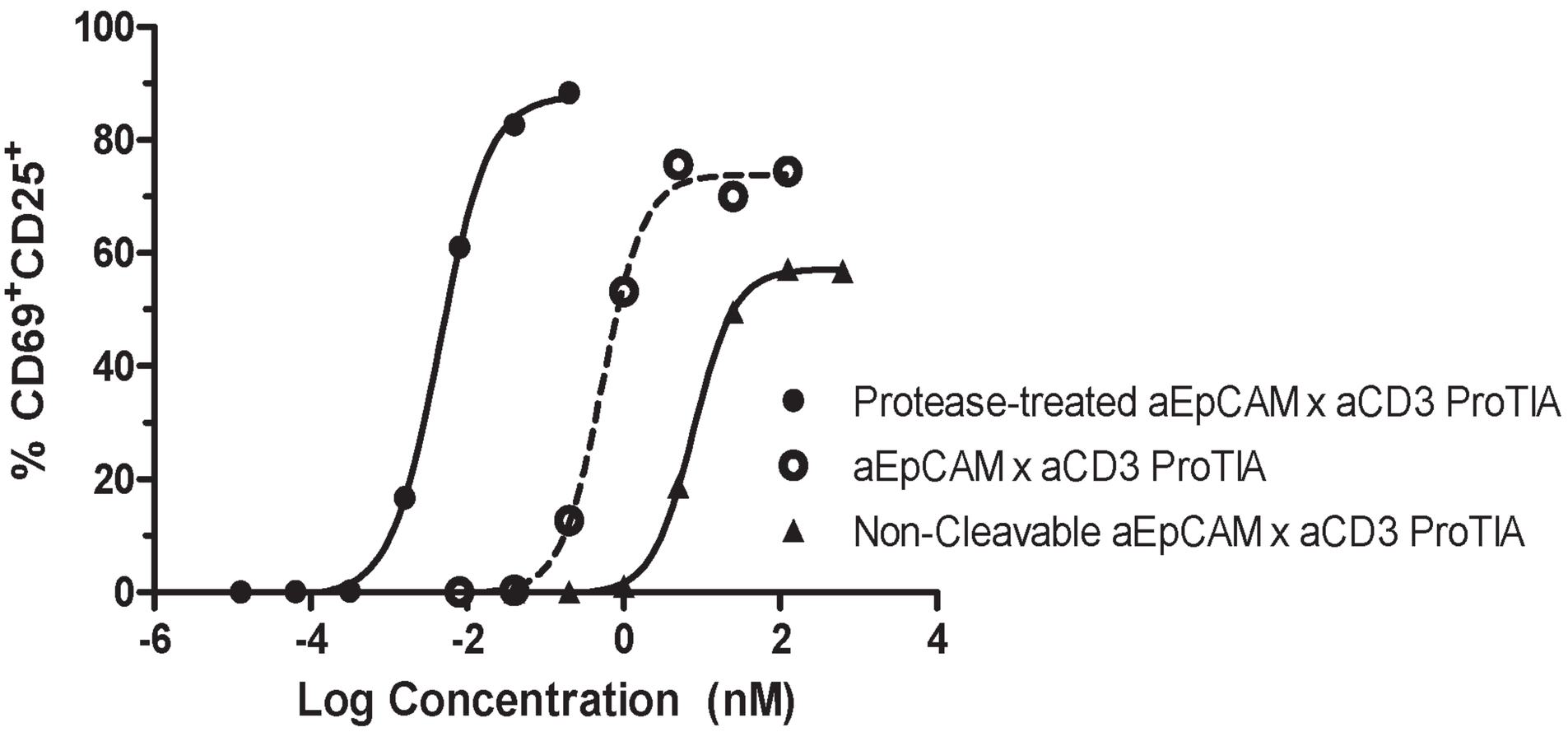


FIG. 45

FIG. 46A

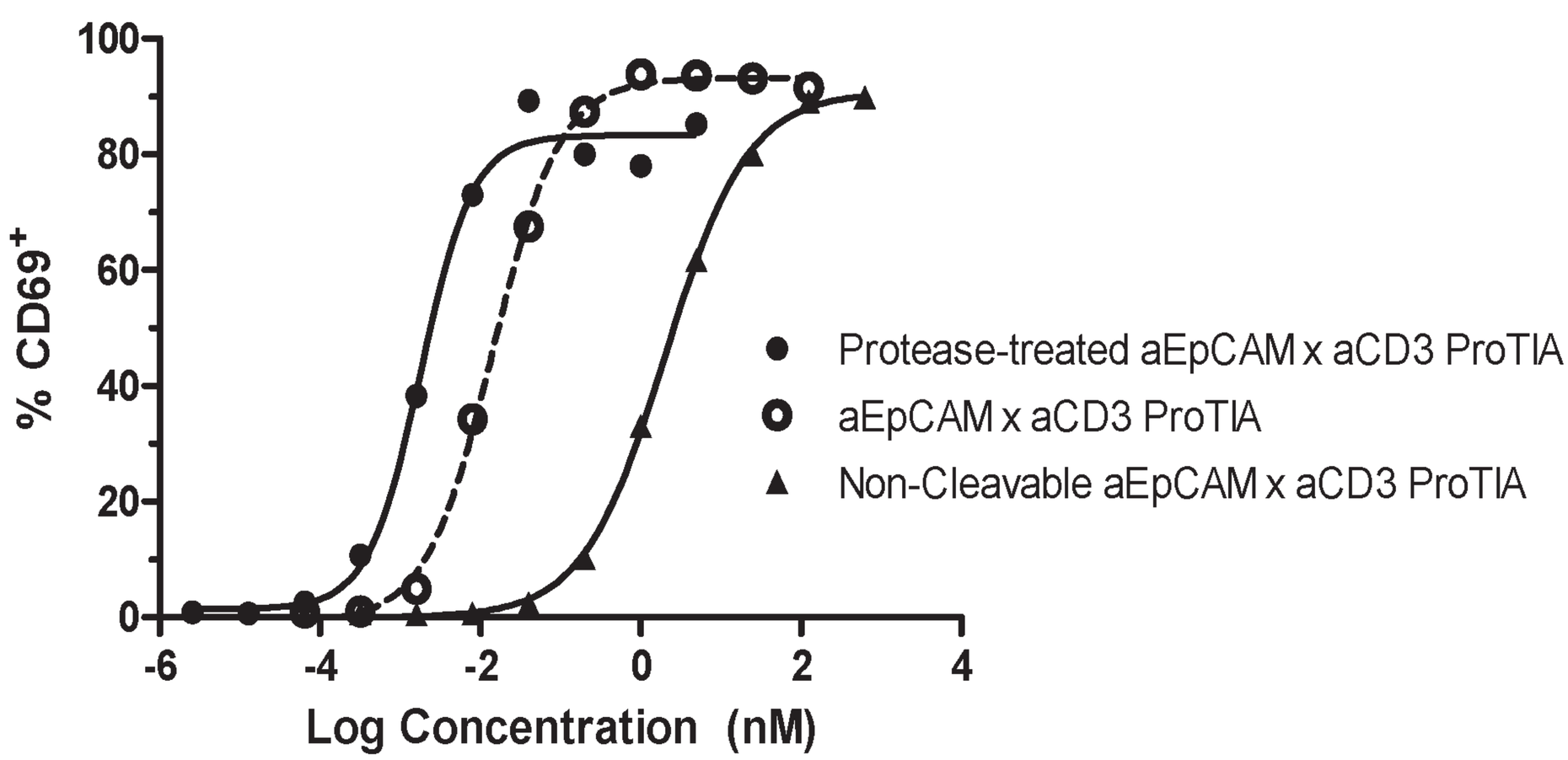


FIG. 46B

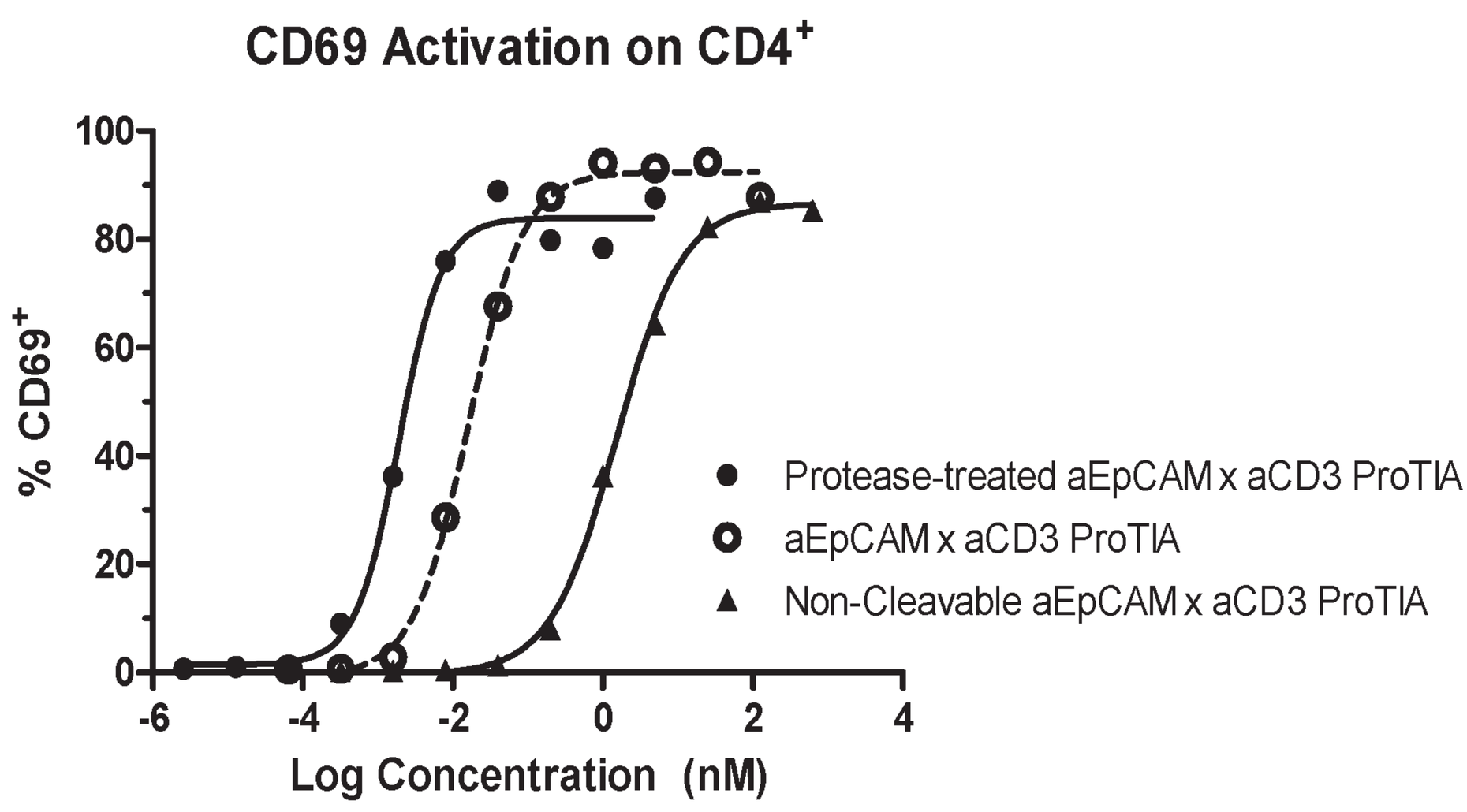


FIG. 46

FIG. 47A

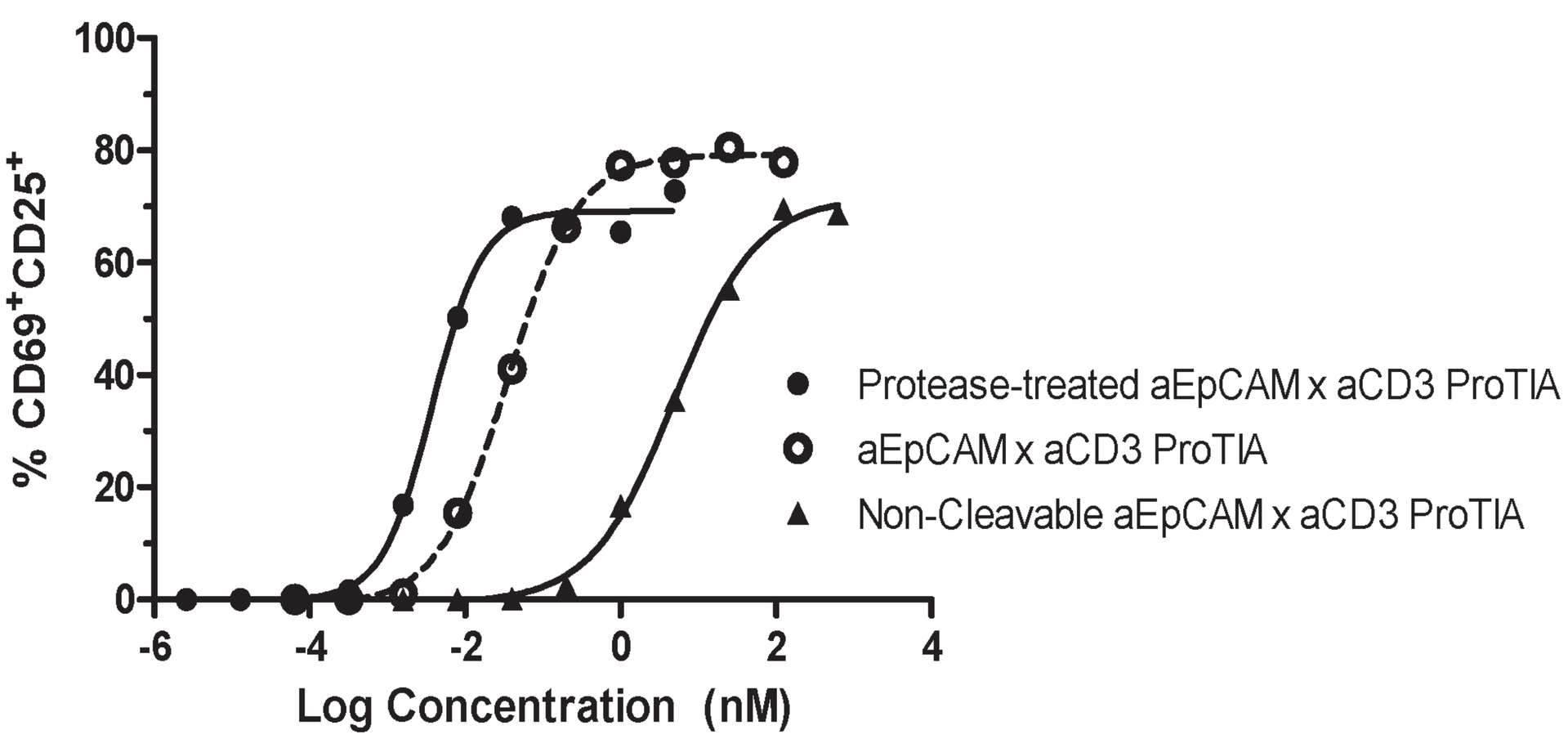


FIG. 47B

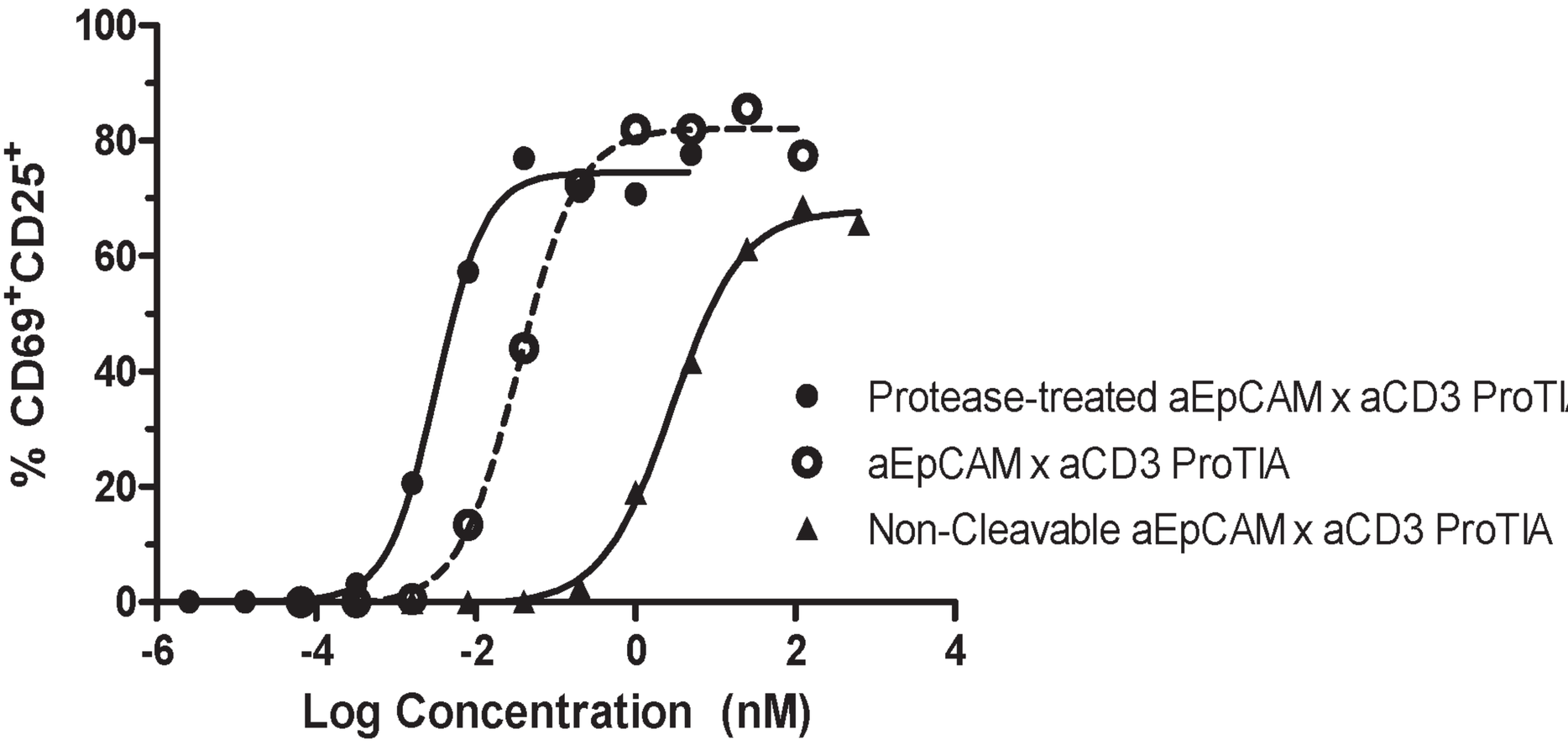


FIG. 47

FIG. 48A

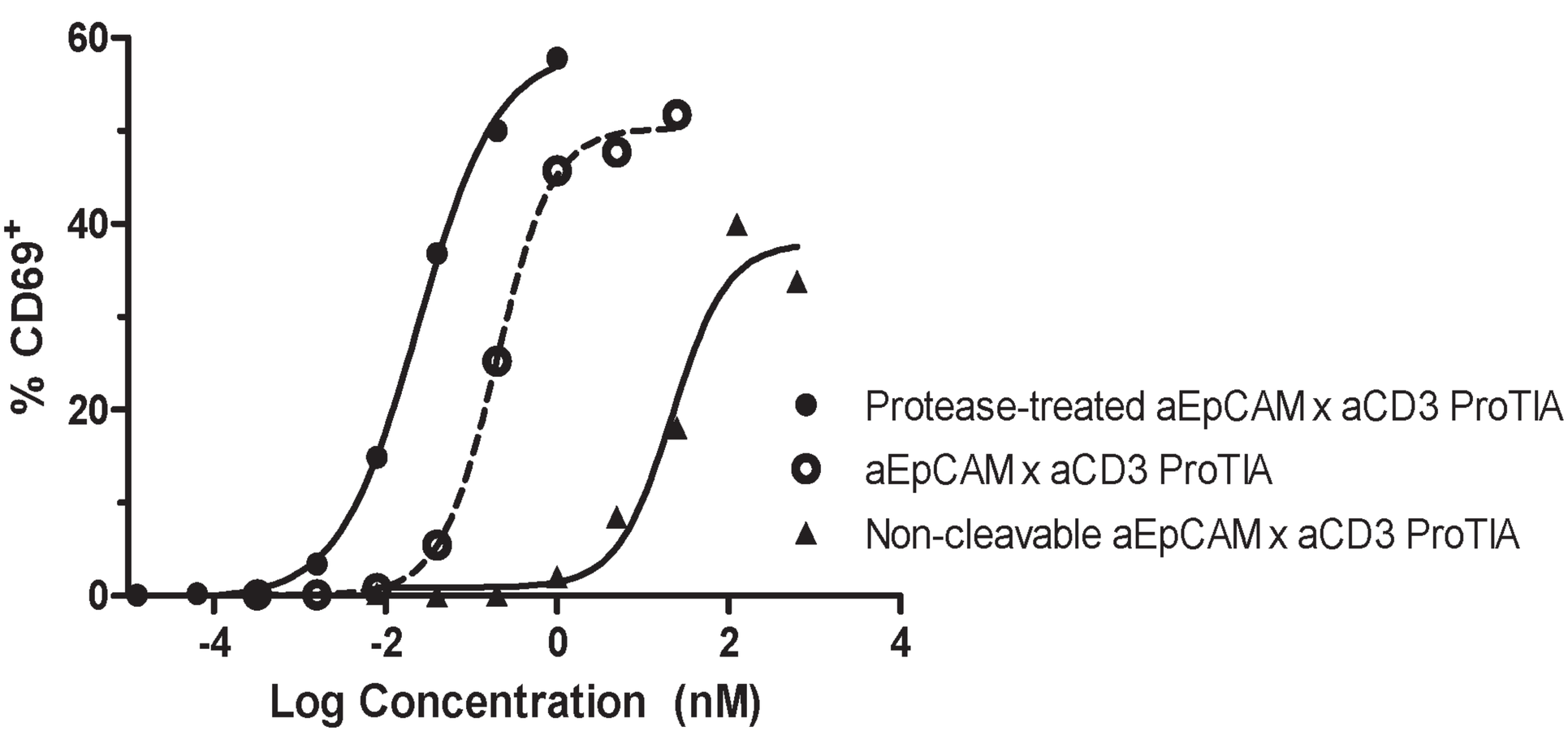


FIG. 48B

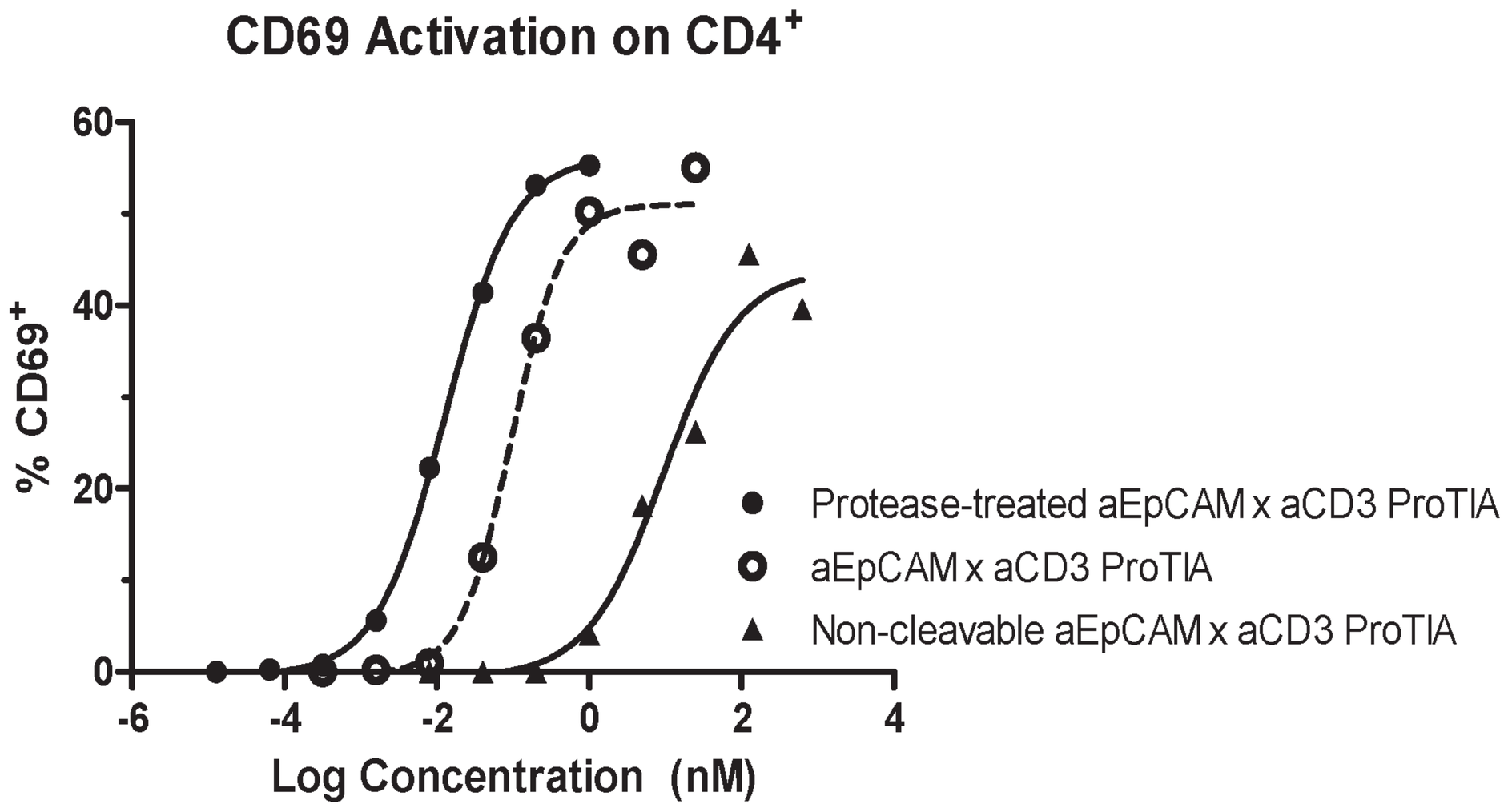


FIG. 48

FIG. 49A

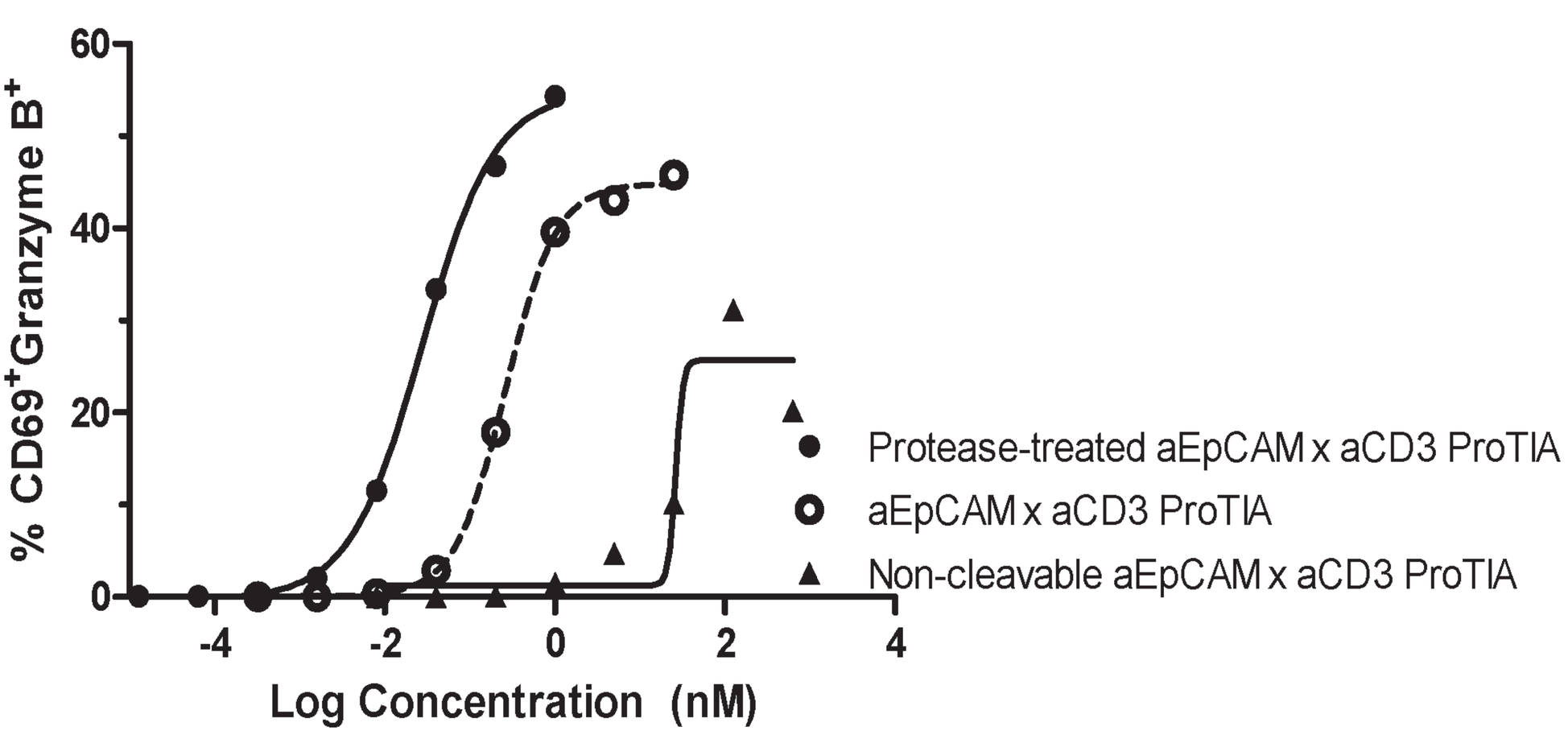


FIG. 49B

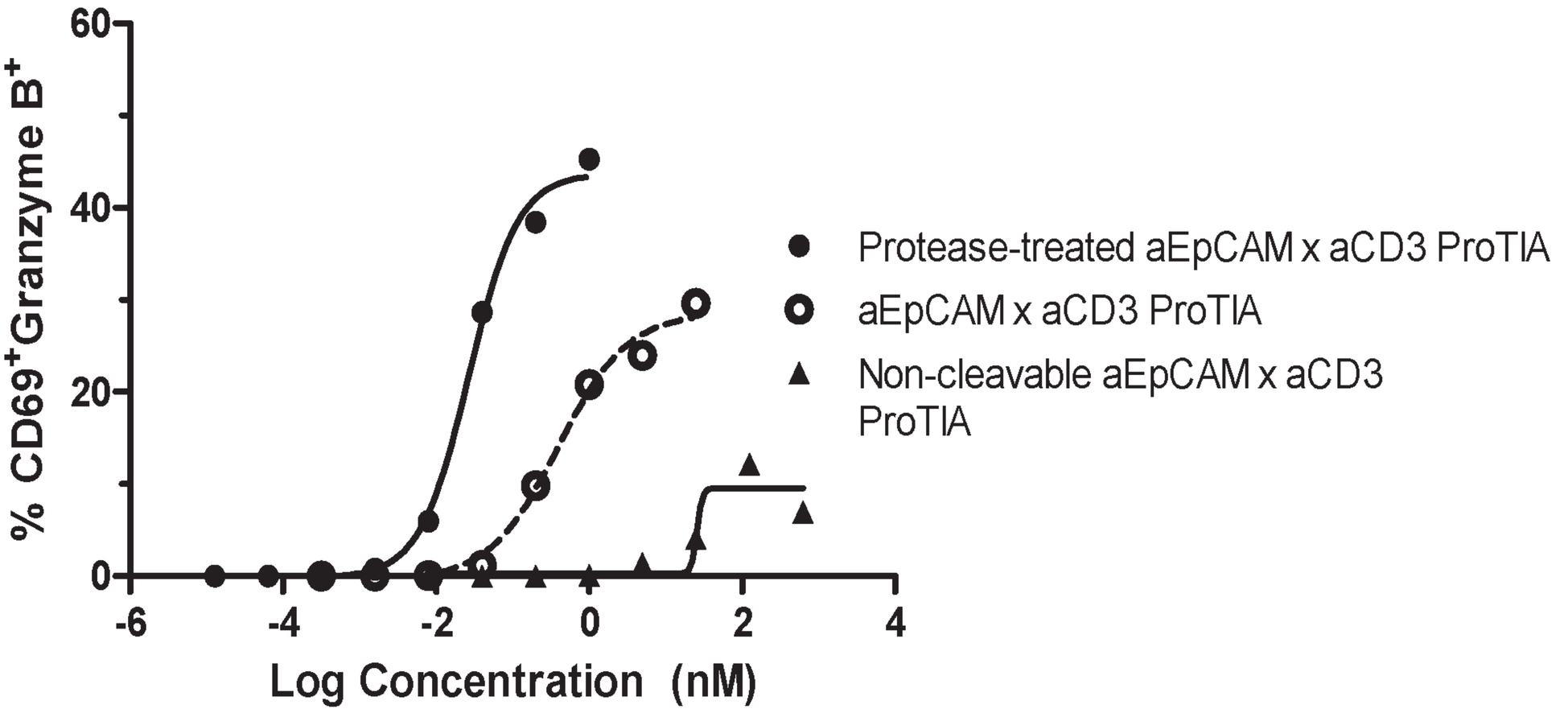


FIG. 49

FIG. 50A

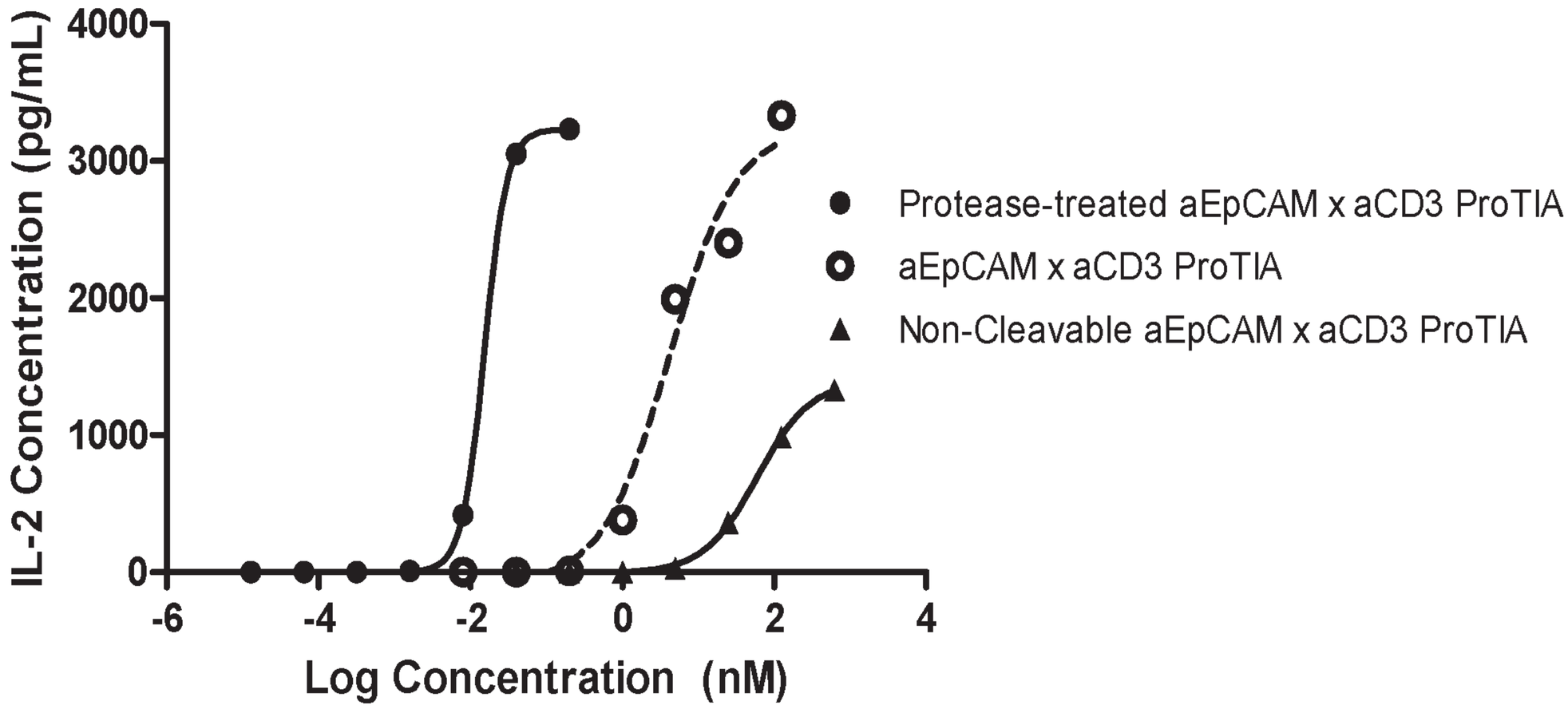


FIG. 50B

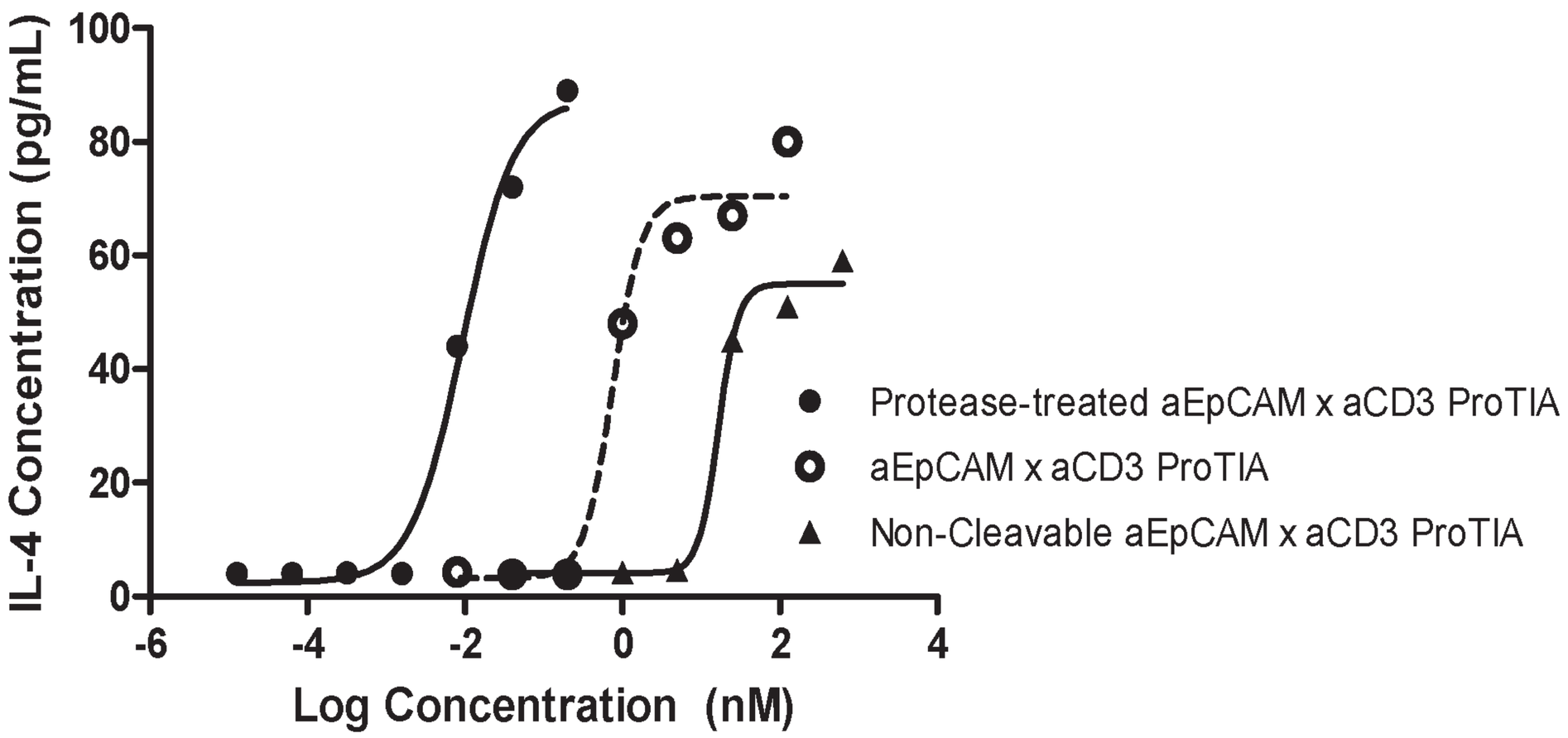


FIG. 50

FIG. 51A

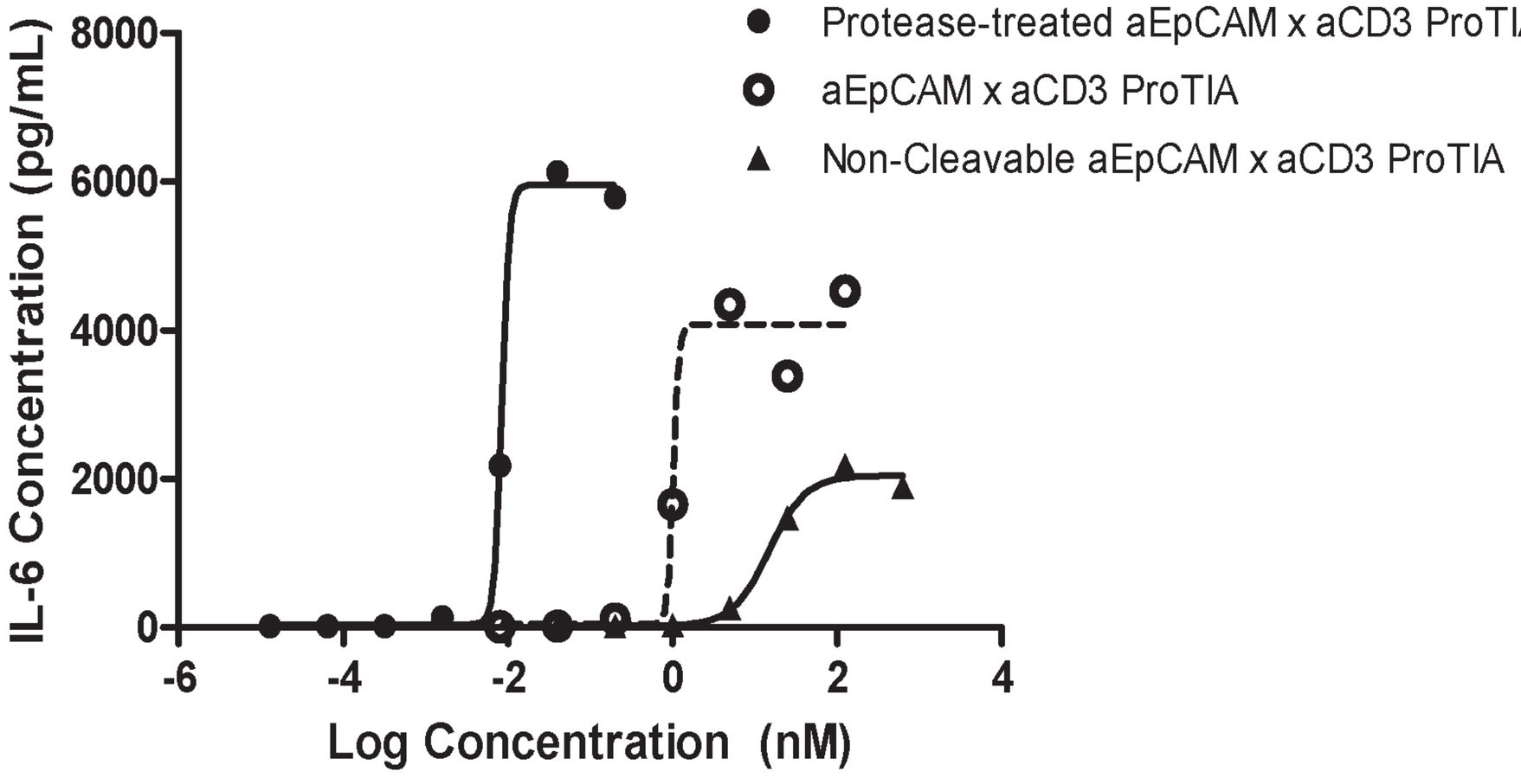


FIG. 51B

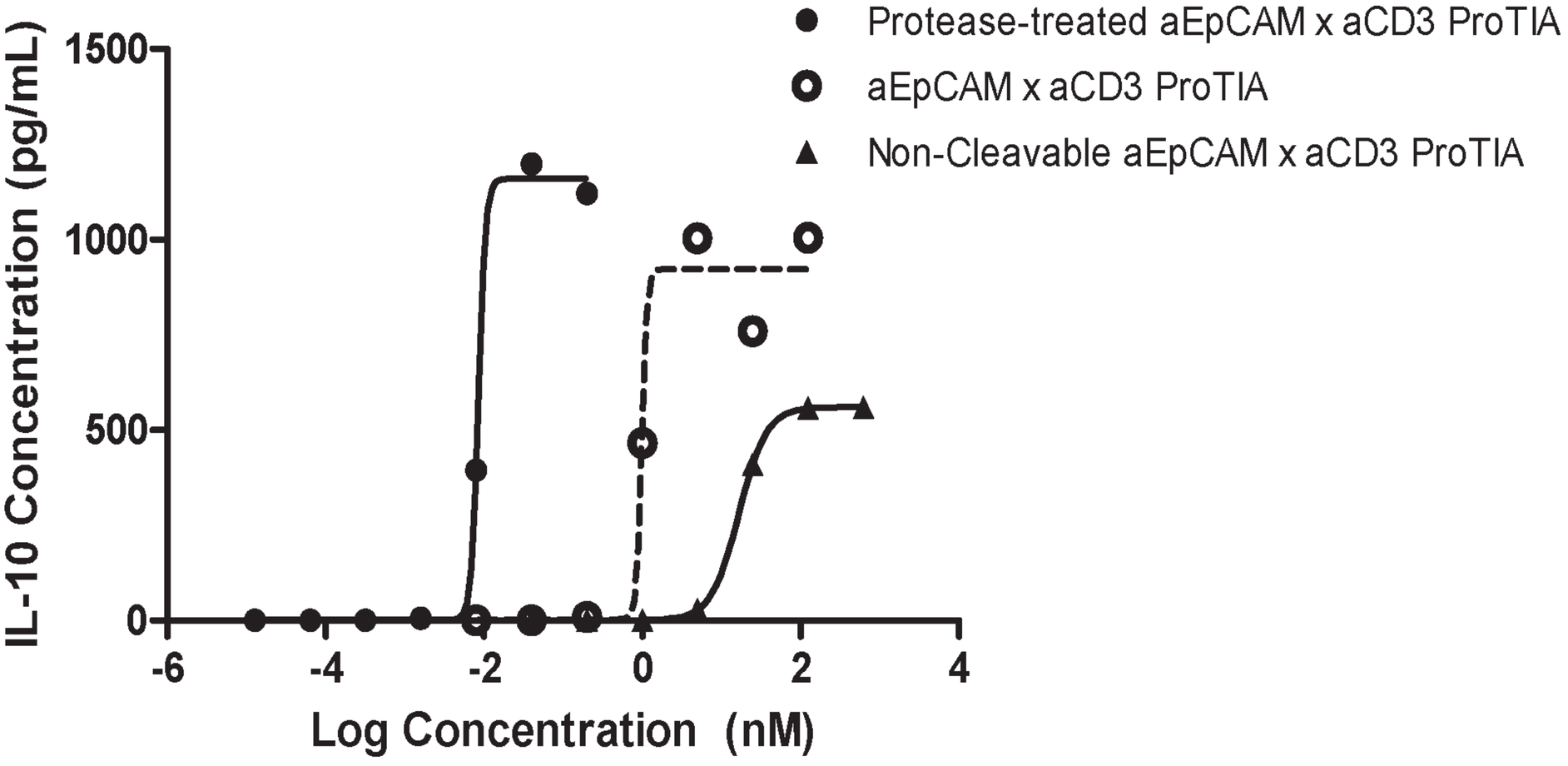


FIG. 51

FIG. 52A

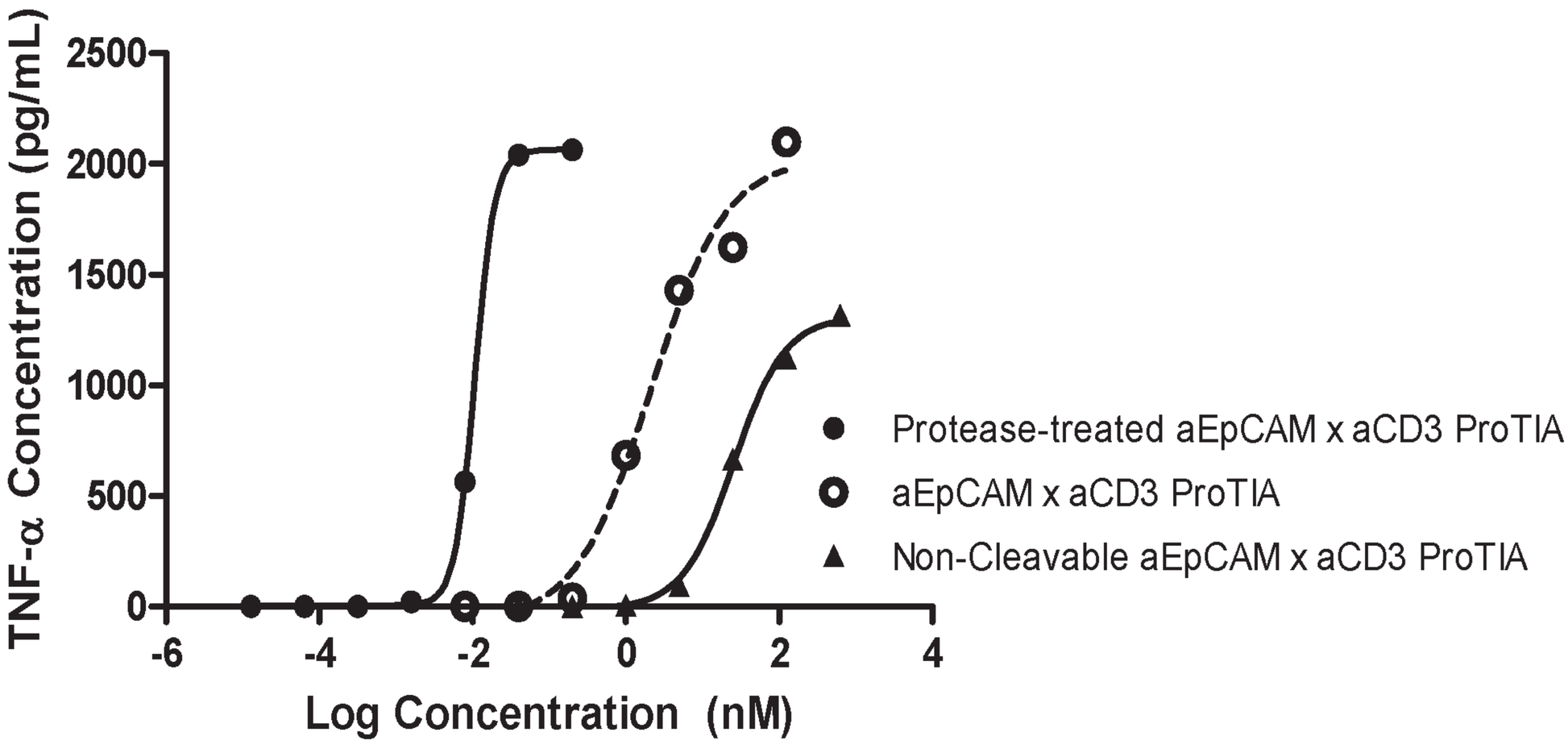


FIG. 52B

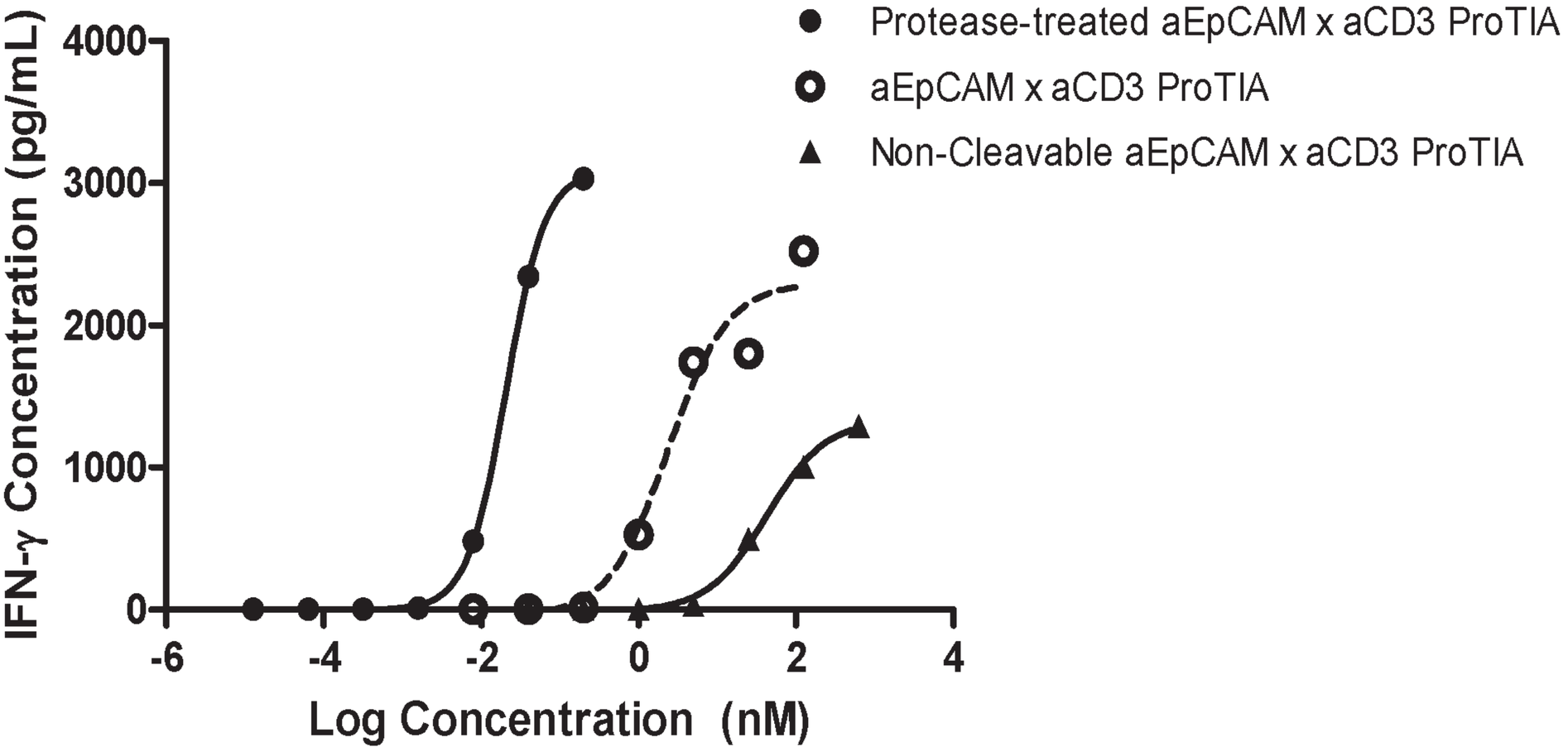


FIG. 52

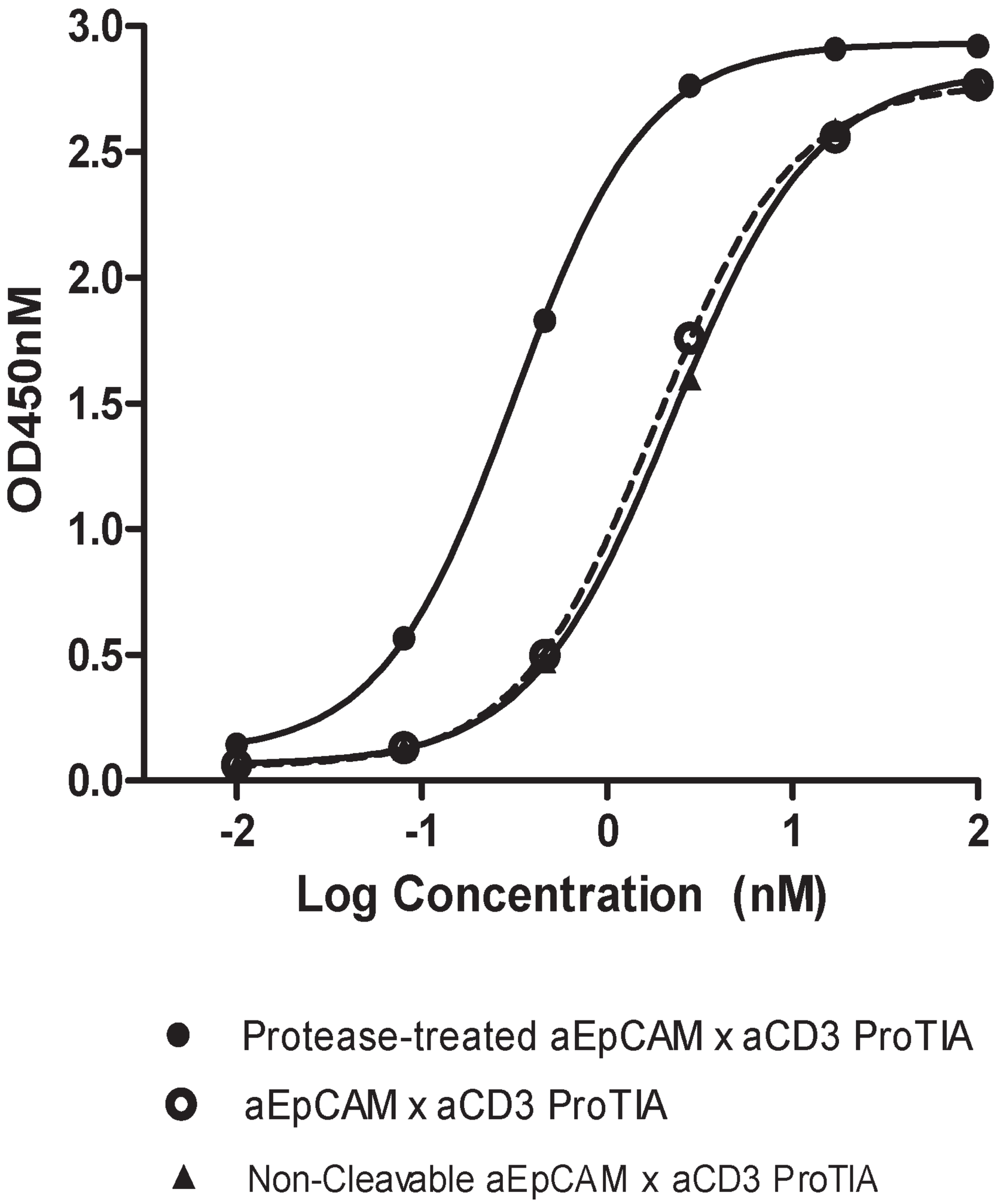
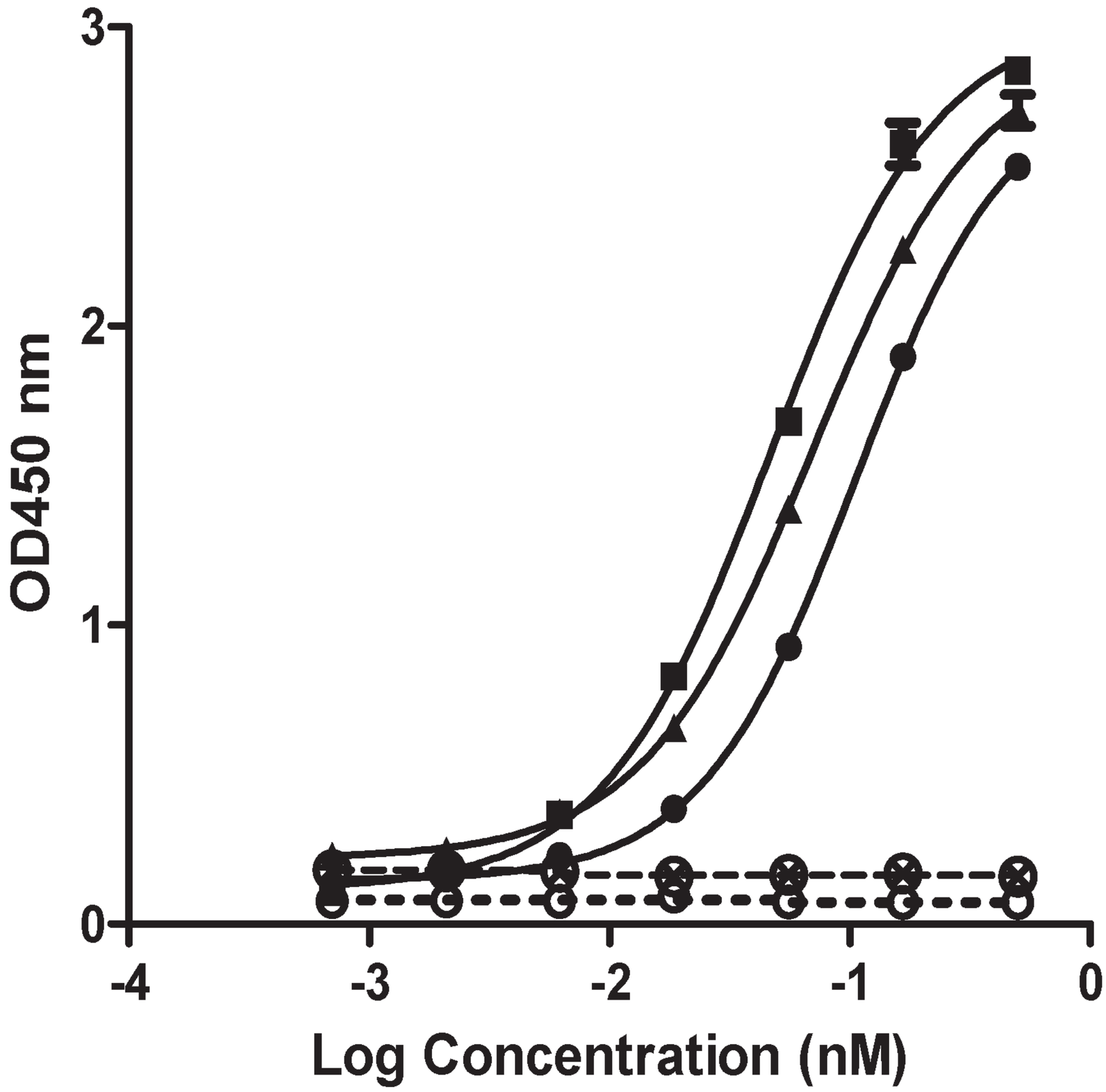

3.0
2.5
2.0
1.5
1.0
0.5
0.0
OD450nM
-2
-1
0
1
2
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 53



- ■ Protease-treated aHER2 x aCD3 ProTIA on HER2 coated on plate
- ▲ Protease-treated aCEA x aCD3 ProTIA on CEA coated on plate
- ● Protease-treated aEpCAM x aCD3 ProTIA on EpCAM coated on plate
- ○ Protease-treated aEpCAM x aCD3 ProTIA on HER2 coated on plate
- ⊗ Protease-treated aEpCAM x aCD3 ProTIA on CEA coated on plate

FIG. 54

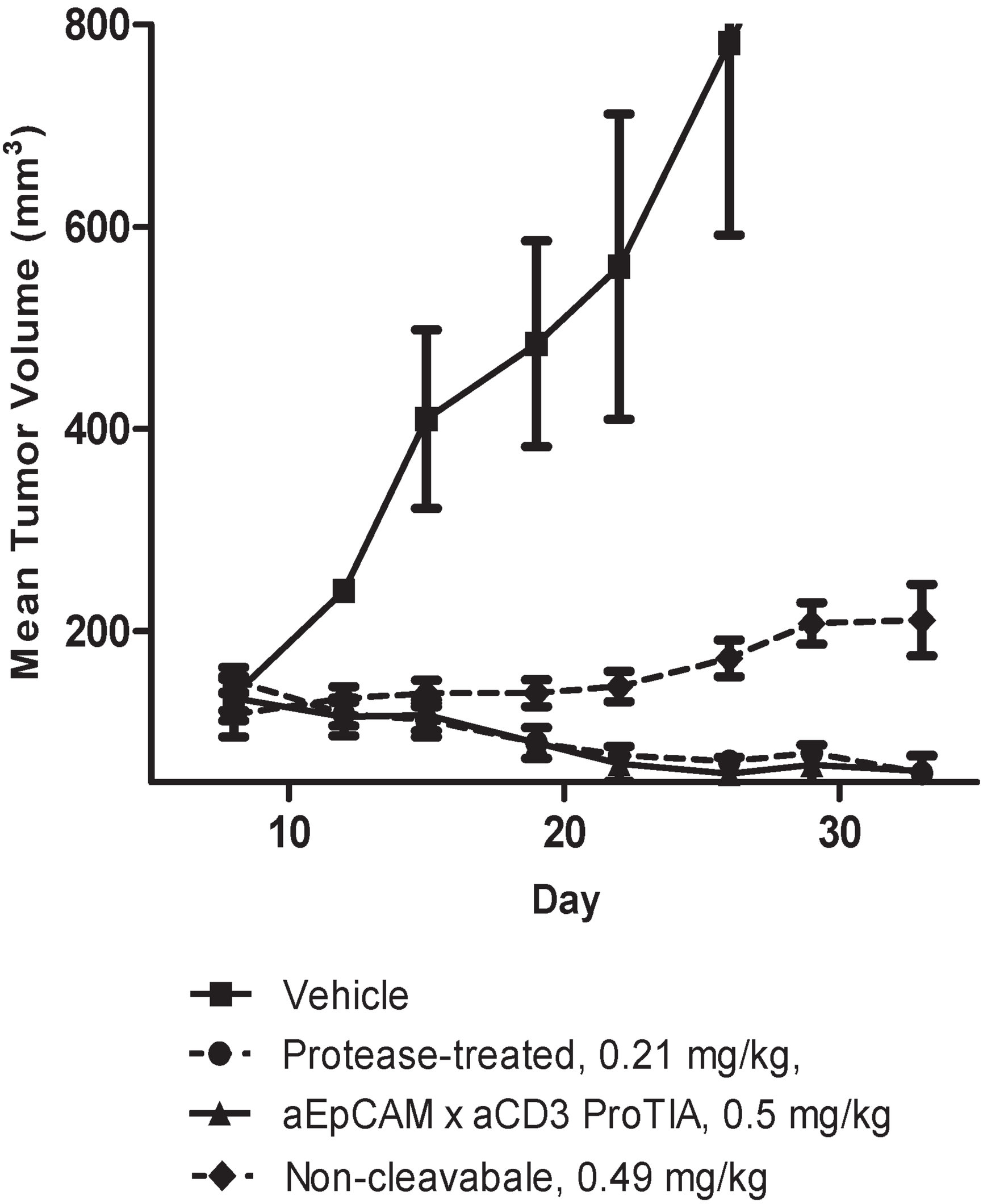

800
600
400
200
Mean Tumor Volume (mm3)
10
20
30
Day
Vehicle
Protease-treated, 0.21 mg/kg,
aEpCAM x aCD3 ProTIA, 0.5 mg/kg
Non-cleavabale, 0.49 mg/kg

FIG. 55

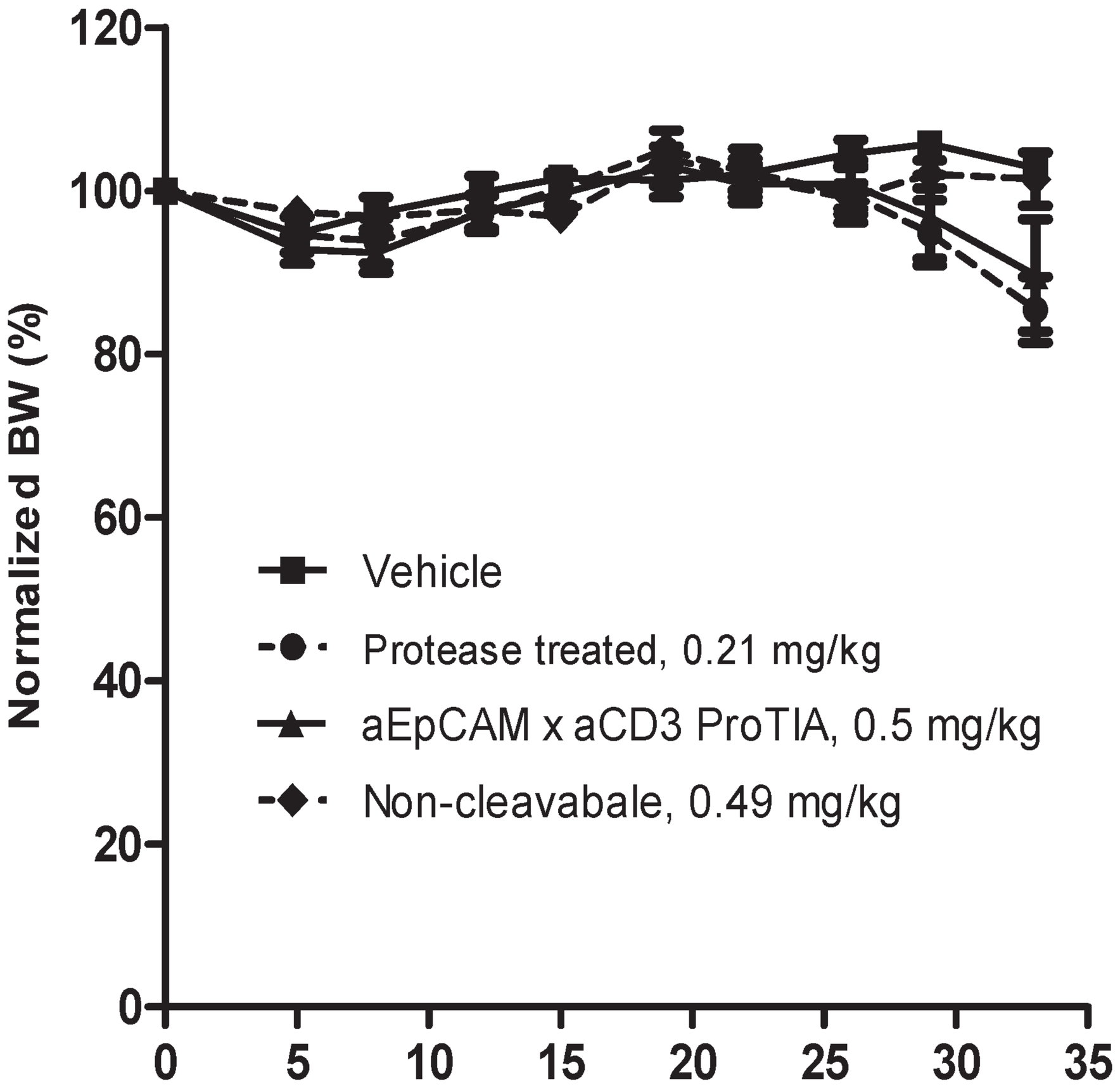

120
100
80
60
40
20
0
Normalized BW (%)
0
5
10
15
20
25
30
35
Day
Vehicle
Protease treated, 0.21 mg/kg
aEpCAM x aCD3 ProTIA, 0.5 mg/kg
Non-cleavabale, 0.49 mg/kg

FIG. 56

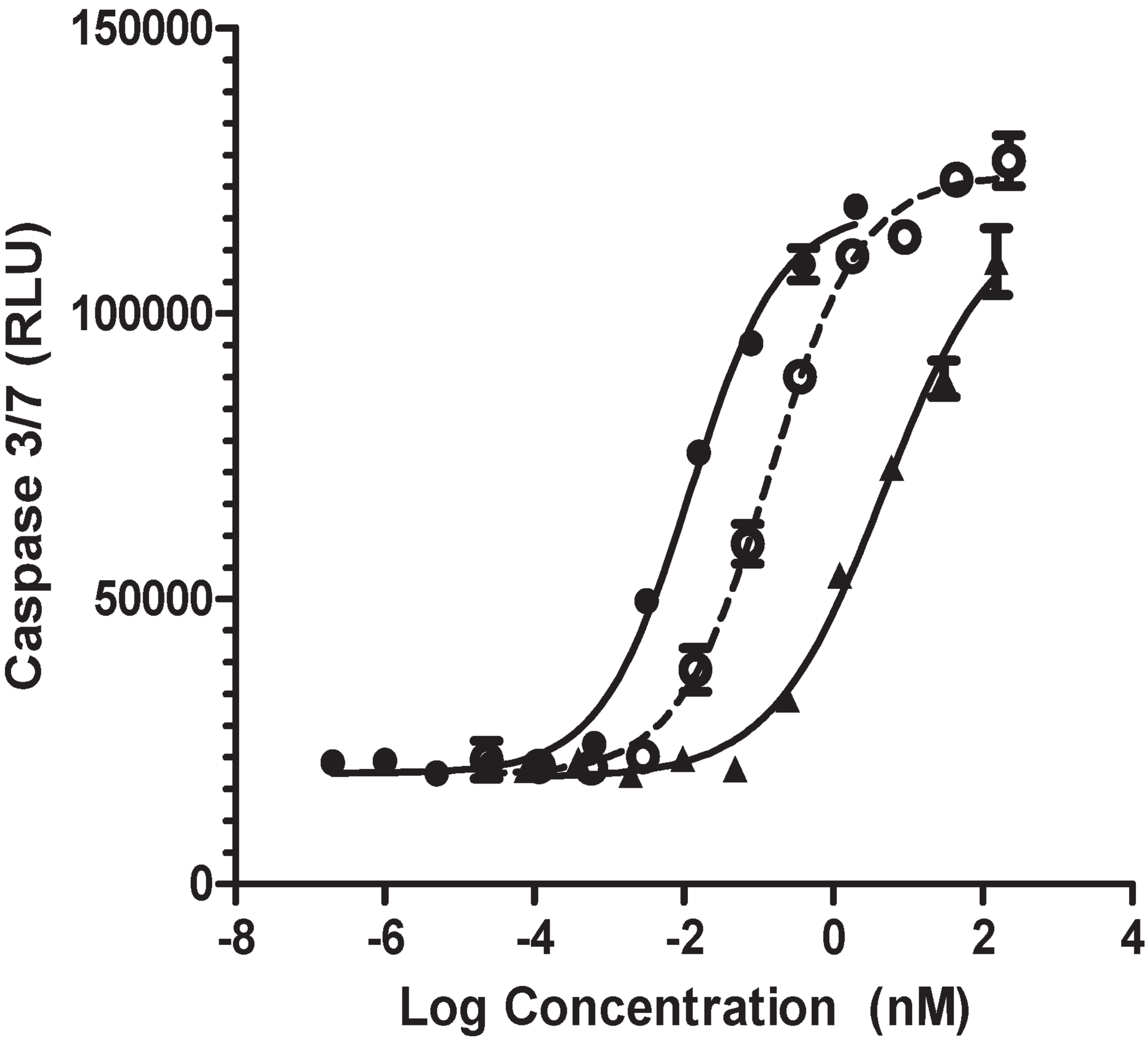

150000
100000
50000
0
Caspase 3/7 (RLU)
-8
-6
-4
-2
0
2
4
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 57

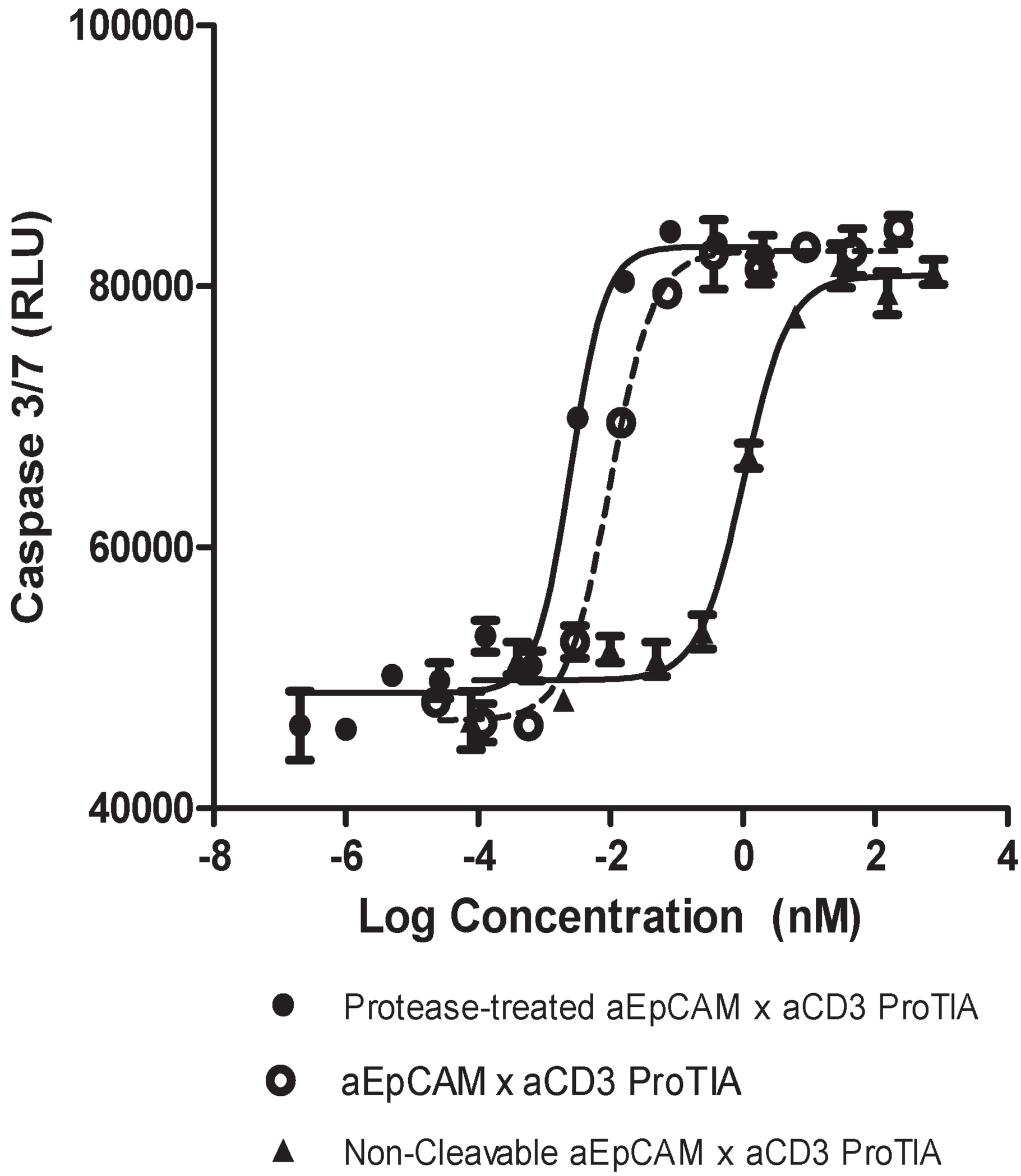

100000
80000
60000
40000
Caspase 3/7 (RLU)
-8
-6
-4
-2
0
2
4
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 58

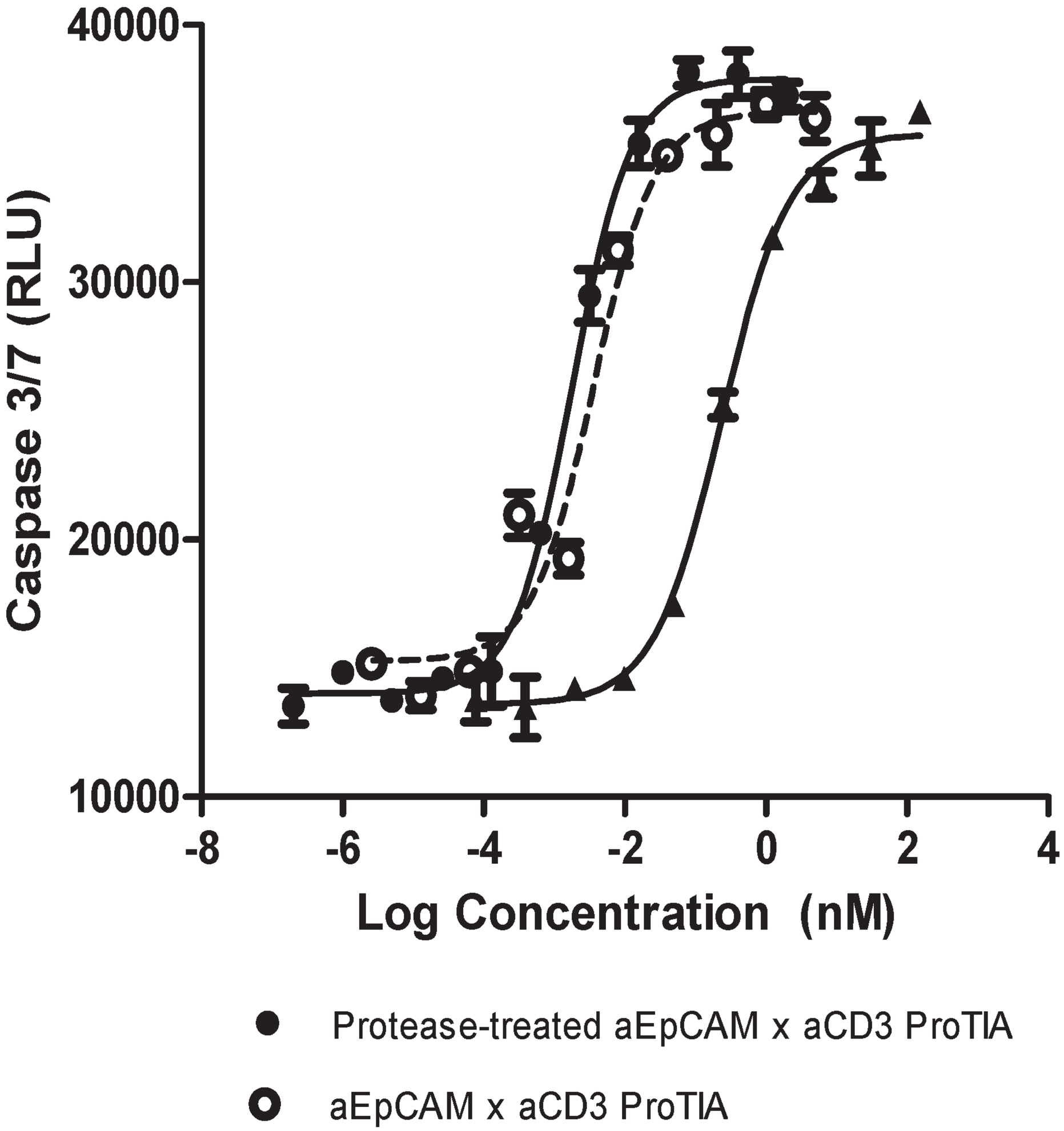

40000
30000
20000
10000
Caspase 3/7 (RLU)
-8
-6
-4
-2
0
2
4
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 59

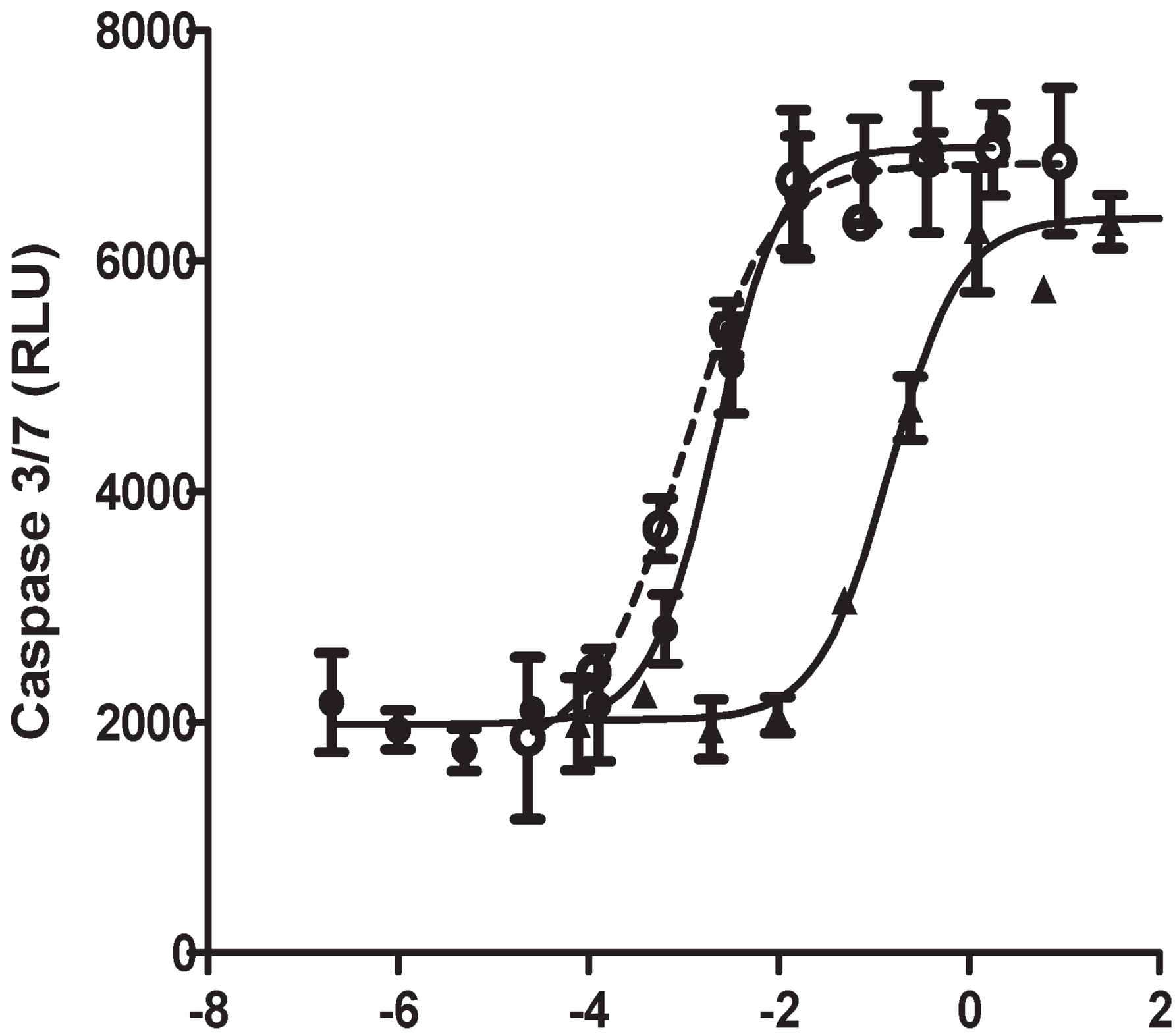

8000
6000
4000
2000
0
Caspase 3/7 (RLU)
-8
-6
-4
-2
0
2
Log Concentration (nM)
Protease-treated aEpCAM x aCD3 ProTIA
aEpCAM x aCD3 ProTIA
Non-Cleavable aEpCAM x aCD3 ProTIA

FIG. 60

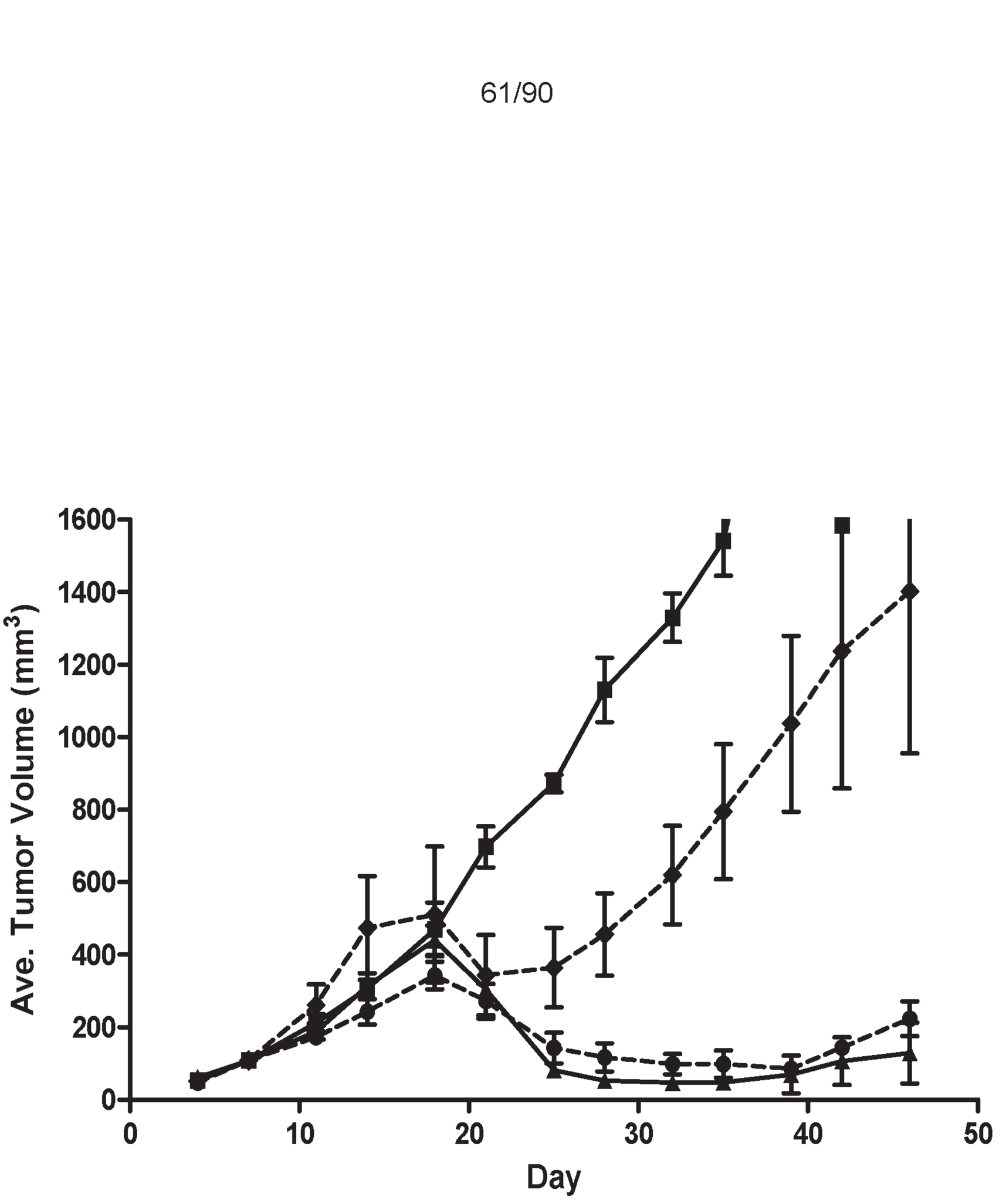

Ave. Tumor Volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Day
Vehicle
Protease-treated, 1.26 mg/kg, TIWx4
aEpCAM x aCD3 ProTIA, 3.0 mg/Kg TIWX4
Non-cleavable, 3.0 mg/Kg TIWX4

FIG. 61

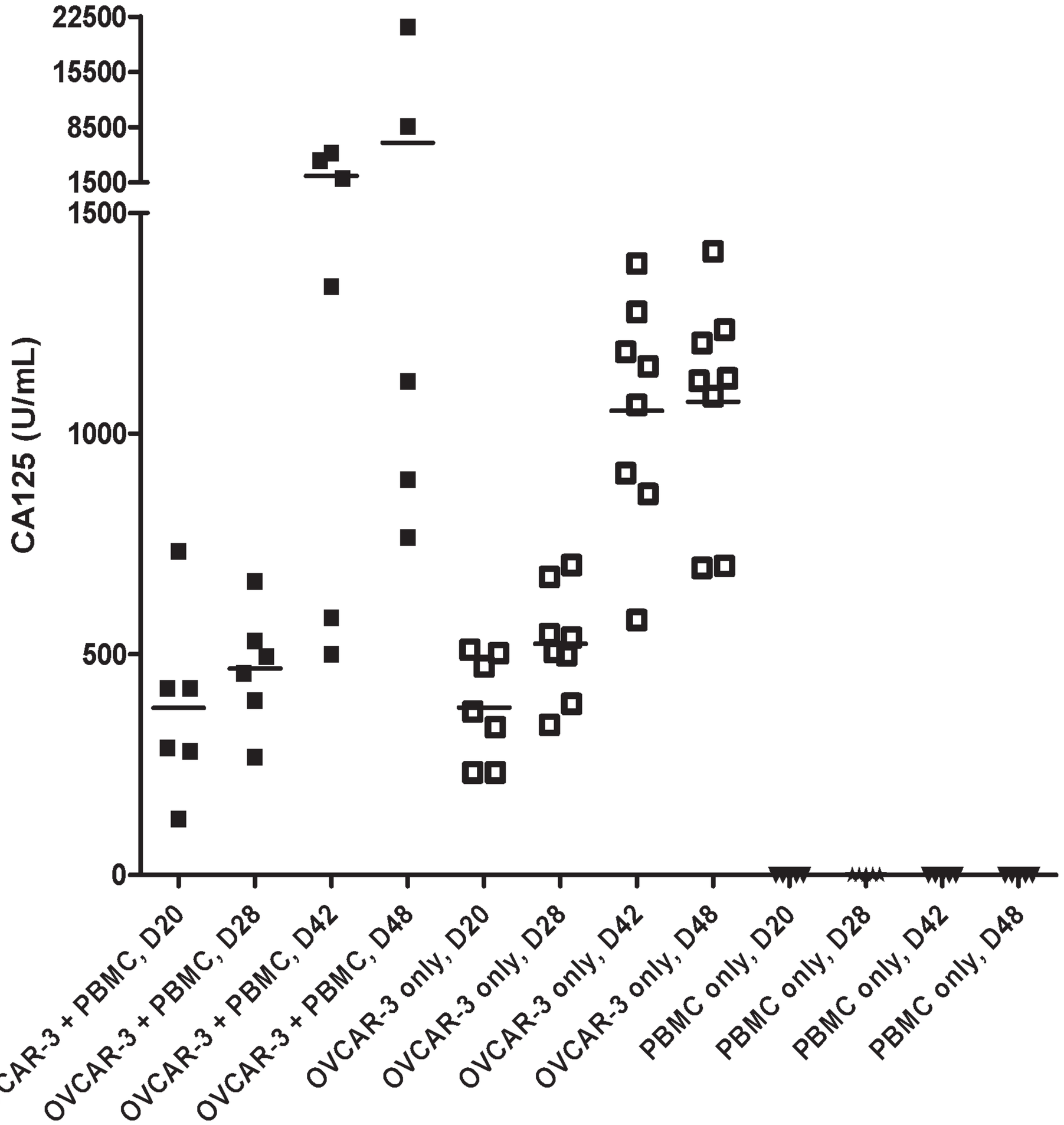

22500
15500
8500
1500
1500
1000
500
0
CA125 (U/mL)
OVCAR-3 + PBMC, D20
OVCAR-3 + PBMC, D28
OVCAR-3 + PBMC, D42
OVCAR-3 + PBMC, D48
OVCAR-3 only, D20
OVCAR-3 only, D28
OVCAR-3 only, D42
OVCAR-3 only, D48
PBMC only, D20
PBMC only, D28
PBMC only, D42
PBMC only, D48

FIG. 62

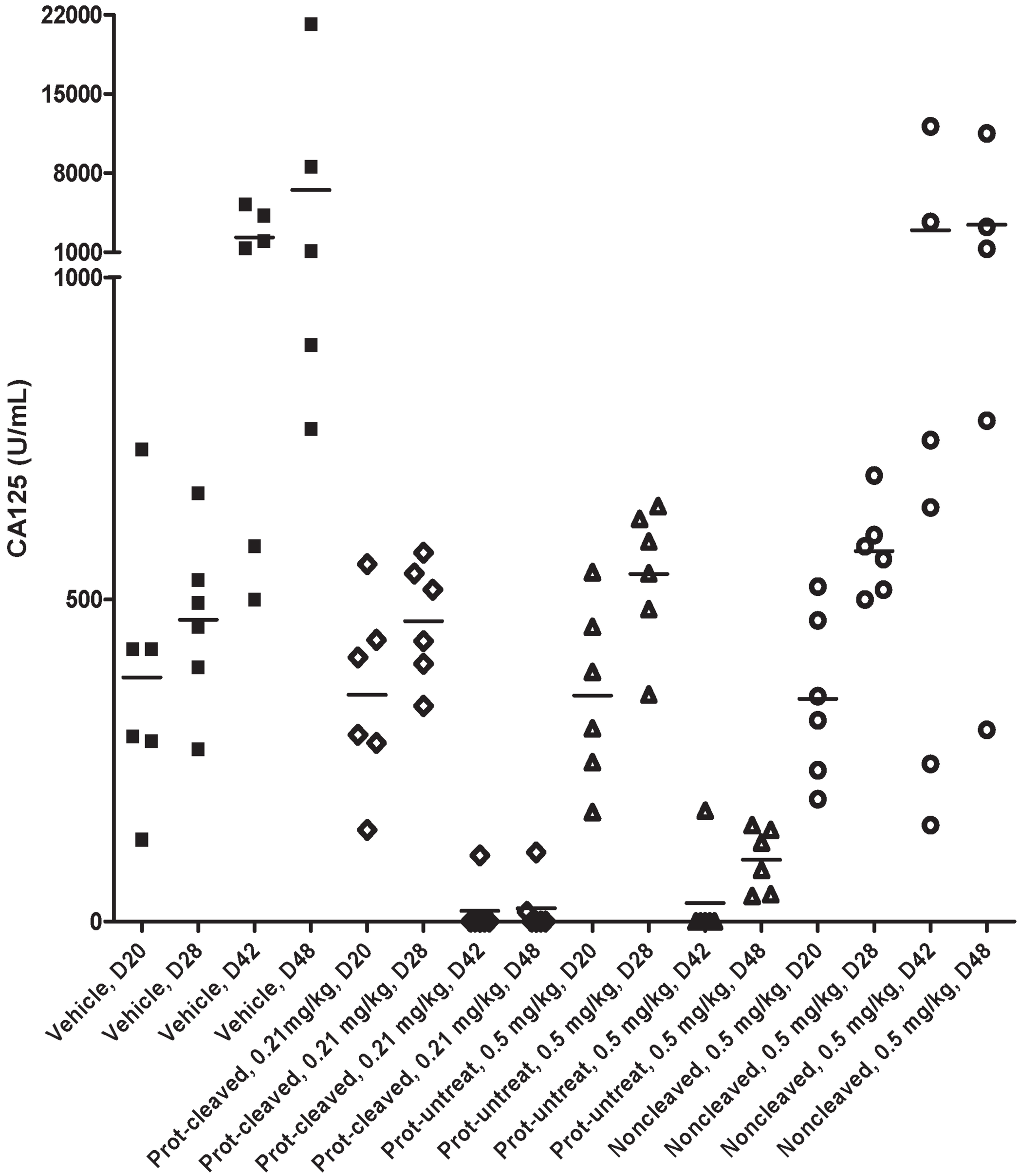

22000
15000
8000
1000
1000
500
0
CA125 (U/mL)
D20
D28
D42
D48
D20
D28
D42
D48
D20
D28
D42
D48
D20
D28
D42
D48
Vehicle,
Vehicle,
Vehicle,
Vehicle,
Prot-cleaved, 0.21mg/kg,
Prot-cleaved, 0.21 mg/kg,
Prot-cleaved, 0.21 mg/kg,
Prot-cleaved, 0.21 mg/kg,
Prot-untreat, 0.5 mg/kg,
Prot-untreat, 0.5 mg/kg,
Prot-untreat, 0.5 mg/kg,
Prot-untreat, 0.5 mg/kg,
Noncleaved, 0.5 mg/kg,
Noncleaved, 0.5 mg/kg,
Noncleaved, 0.5 mg/kg,
Noncleaved, 0.5 mg/kg,

FIG. 63

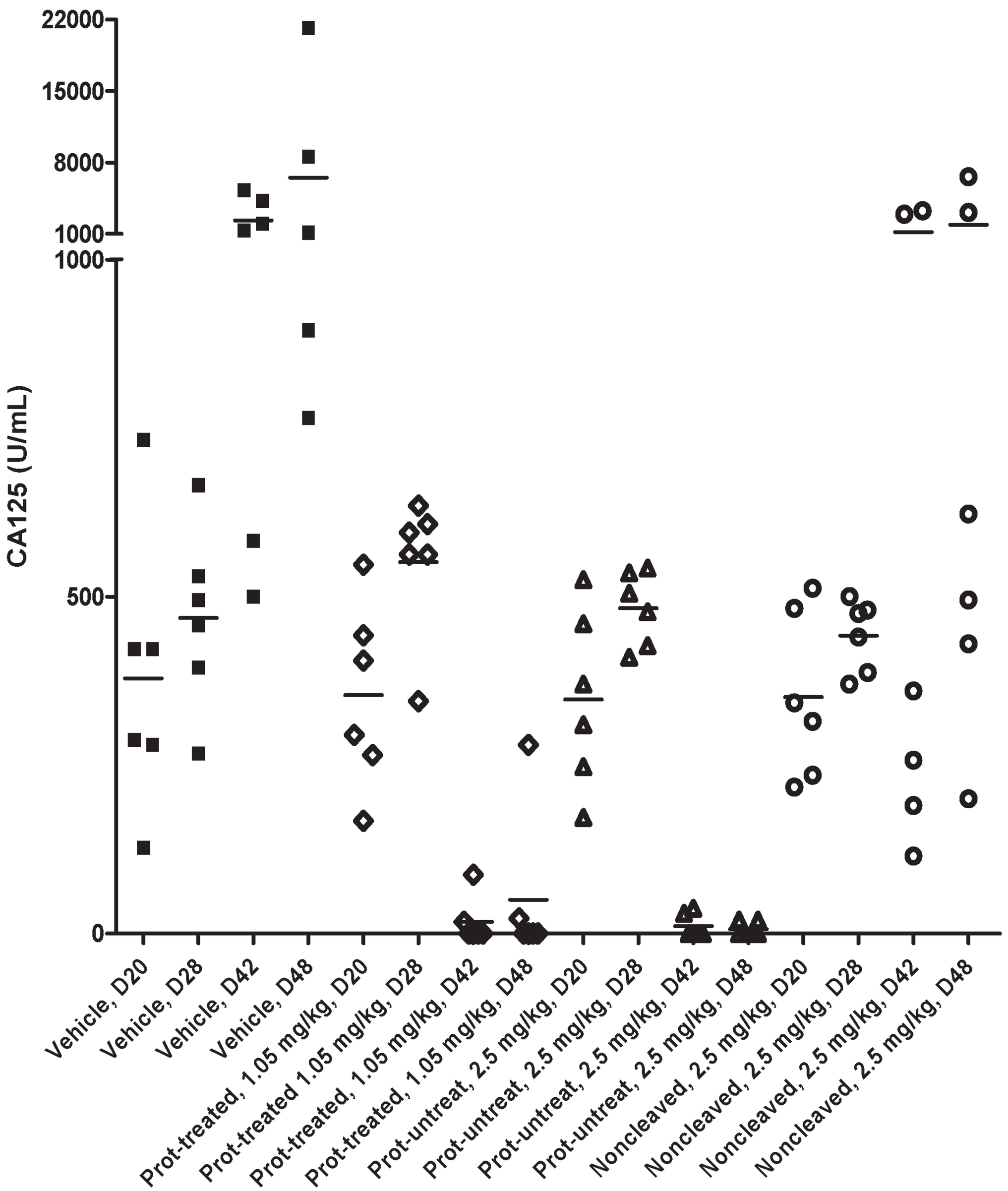

22000
15000
8000
1000
500
0
CA125 (U/mL)
D20
D28
D42
D48
Vehicle,
Prot-treated, 1.05 mg/kg,
Prot-untreat, 2.5 mg/kg,
Noncleaved, 2.5 mg/kg,

FIG. 64

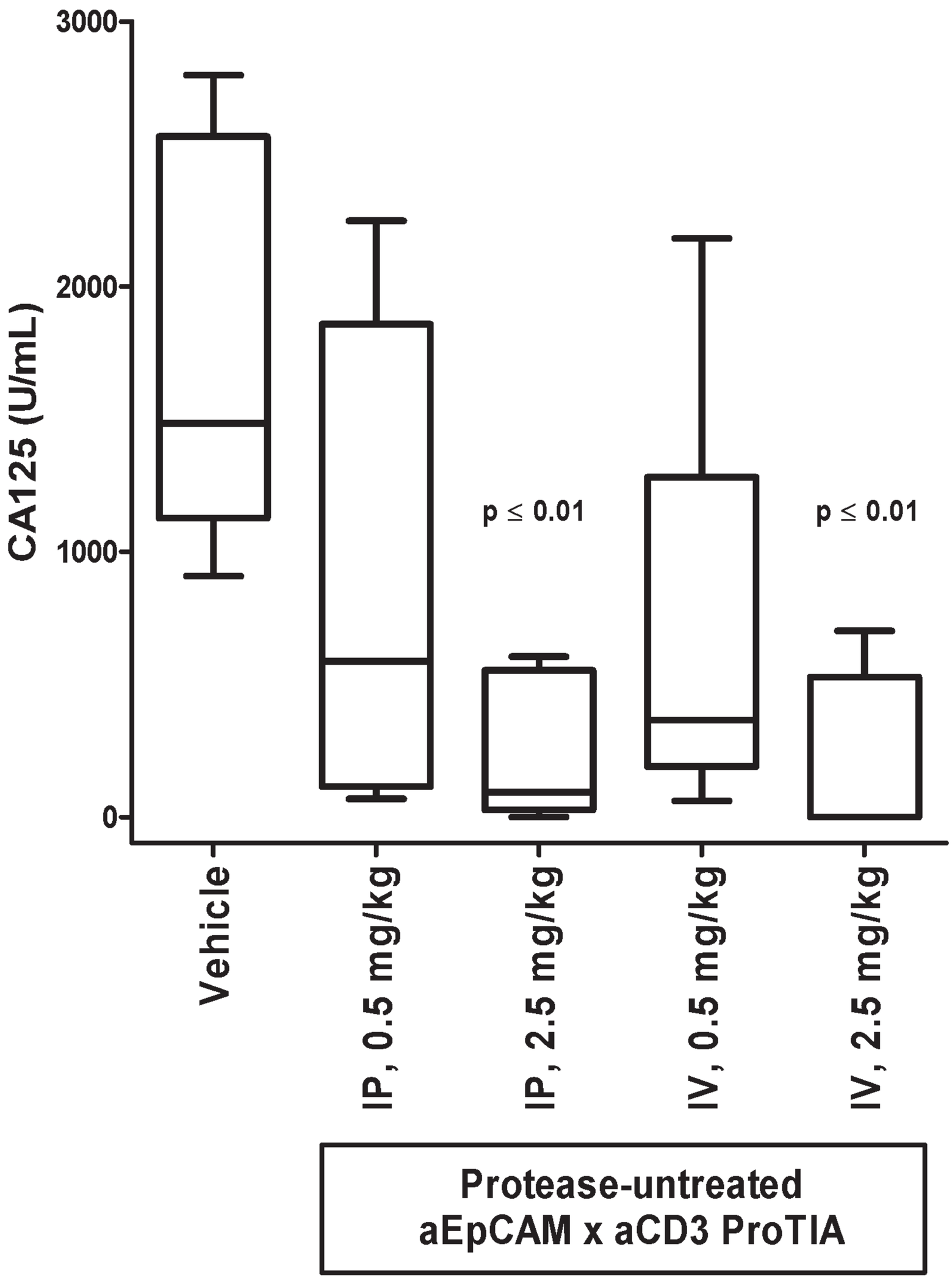

3000
2000
1000
0
CA125 (U/mL)
p ≤ 0.01
p ≤ 0.01
Vehicle
IP, 0.5 mg/kg
IP, 2.5 mg/kg
IV, 0.5 mg/kg
IV, 2.5 mg/kg
Protease-untreated
aEpCAM x aCD3 ProTIA

FIG. 65

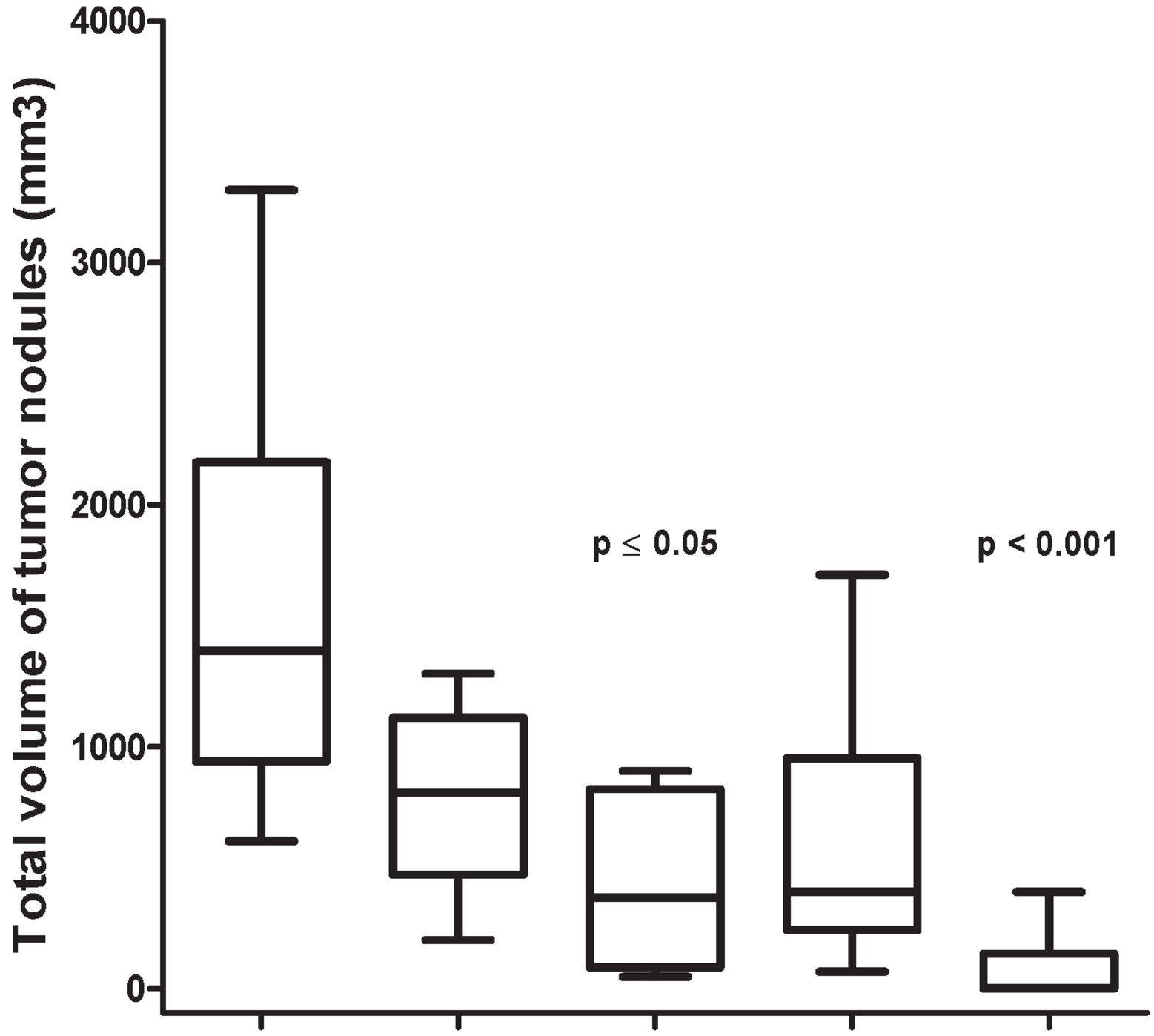

Total volume of tumor nodules (mm3)
4000
3000
2000
1000
0
p ≤ 0.05
p < 0.001
Vehicle
IP, 0.5 mg/kg
IP, 2.5 mg/kg
IV, 0.5 mg/kg
IV, 2.5 mg/kg
Protease-untreated
aEpCAM x aCD3 ProTIA

FIG. 66

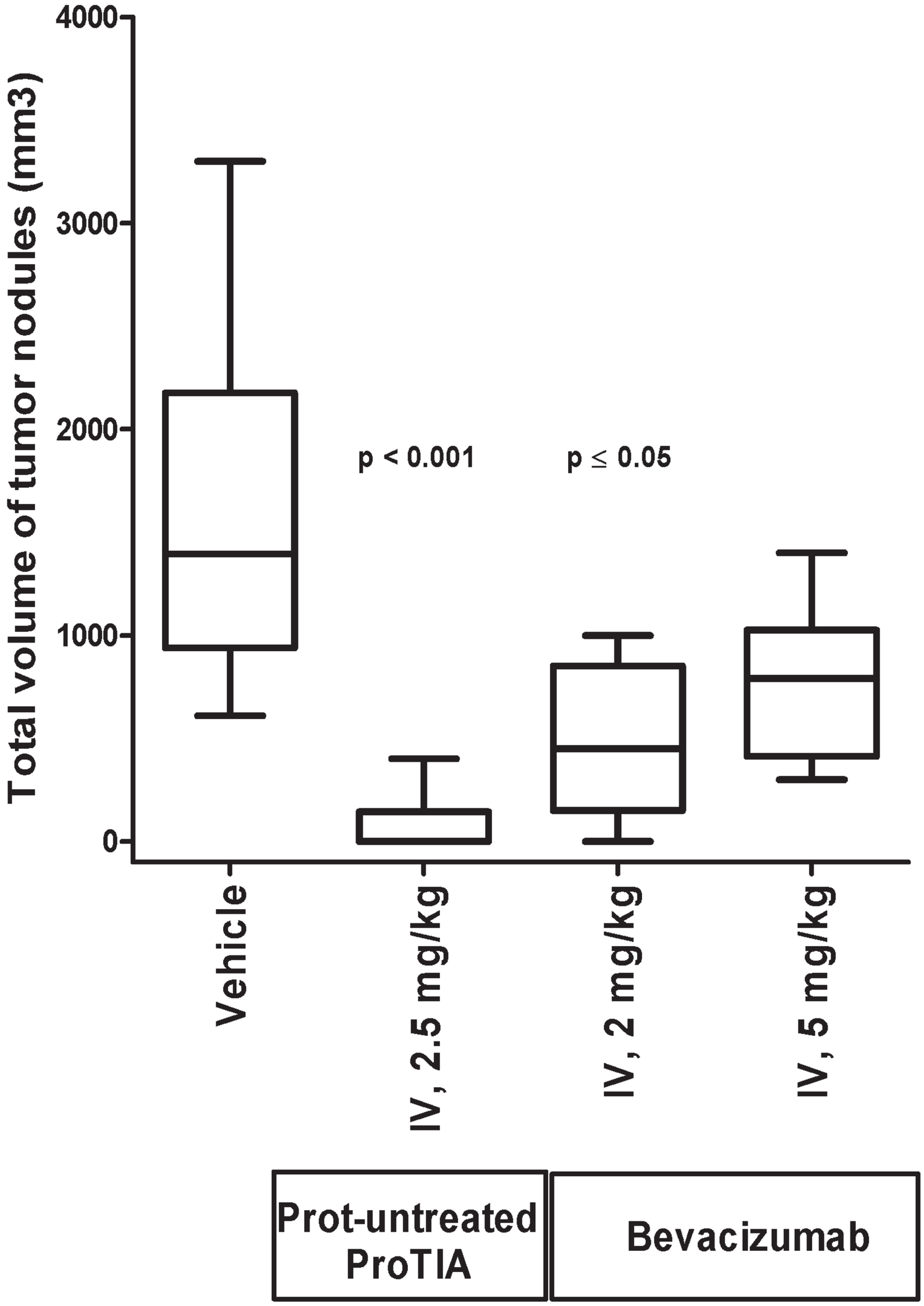

Total volume of tumor nodules (mm3)
4000
3000
2000
1000
0
p < 0.001
p ≤ 0.05
Vehicle
IV, 2.5 mg/kg
IV, 2 mg/kg
IV, 5 mg/kg
Prot-untreated ProTIA
Bevacizumab

FIG. 67

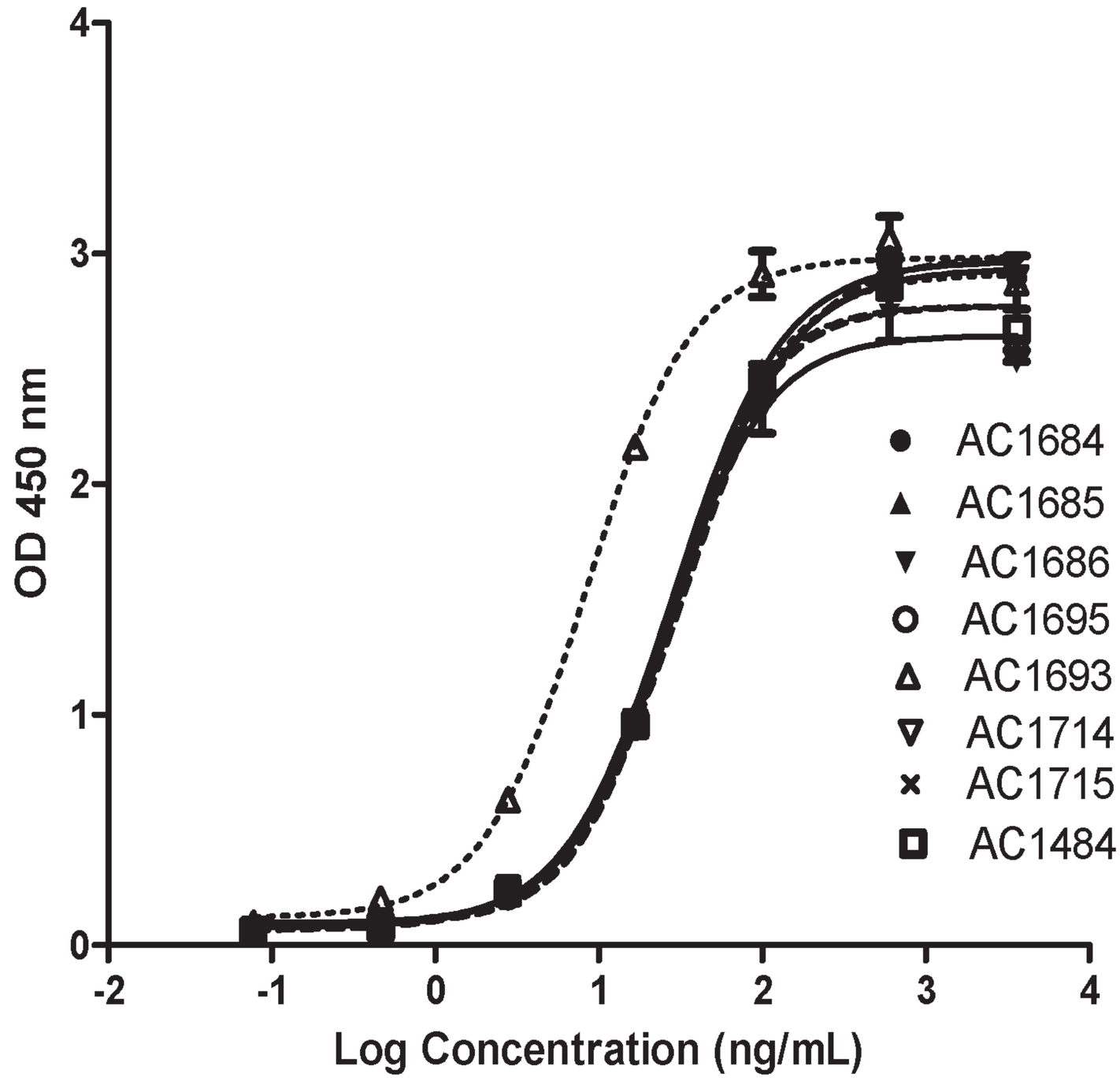

OD 450 nm
4
3
2
1
0
-2
-1
0
1
2
3
4
Log Concentration (ng/mL)
AC1684
AC1685
AC1686
AC1695
AC1693
AC1714
AC1715
AC1484

FIG. 68

FIG. 69A

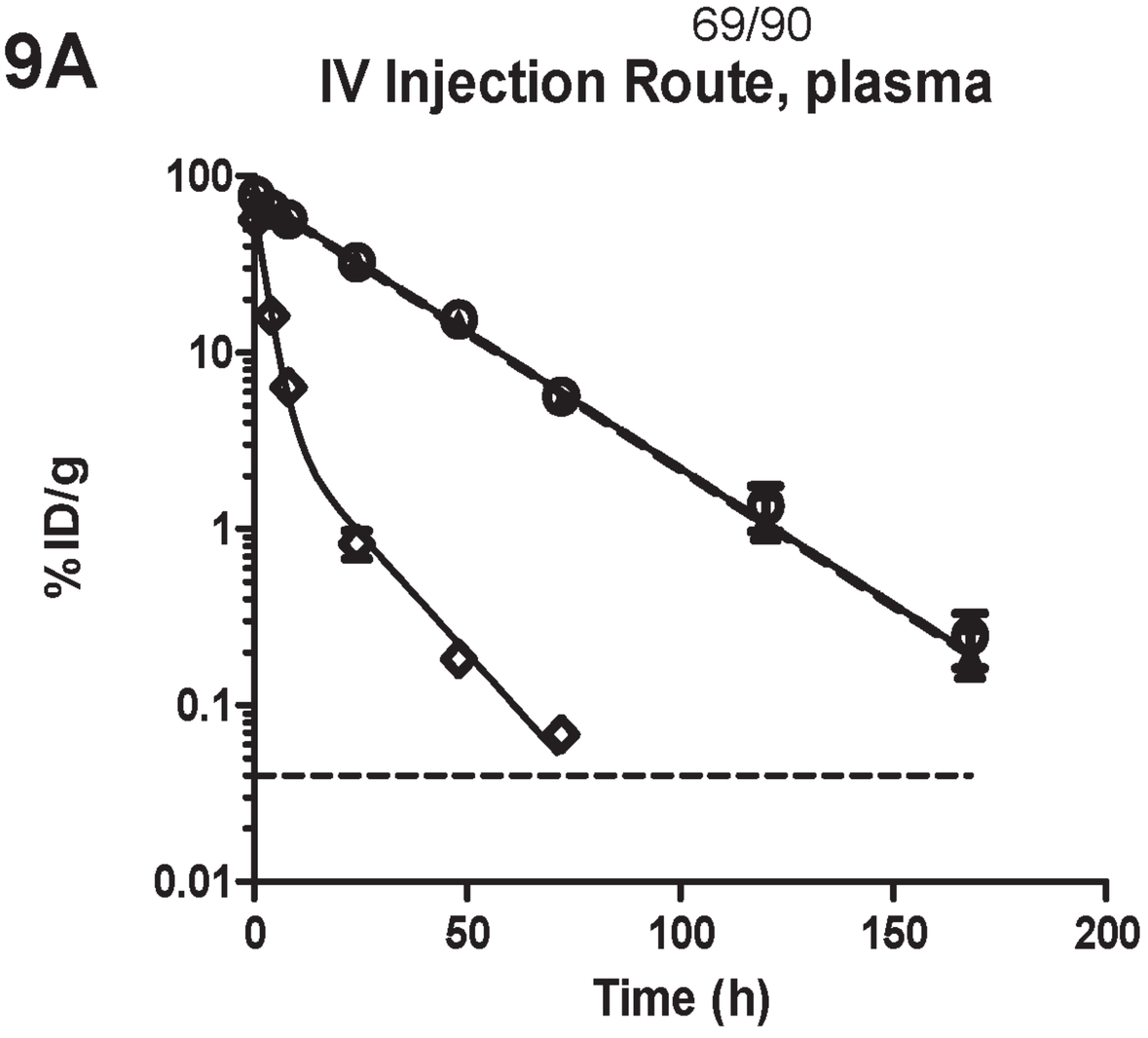


FIG. 69B

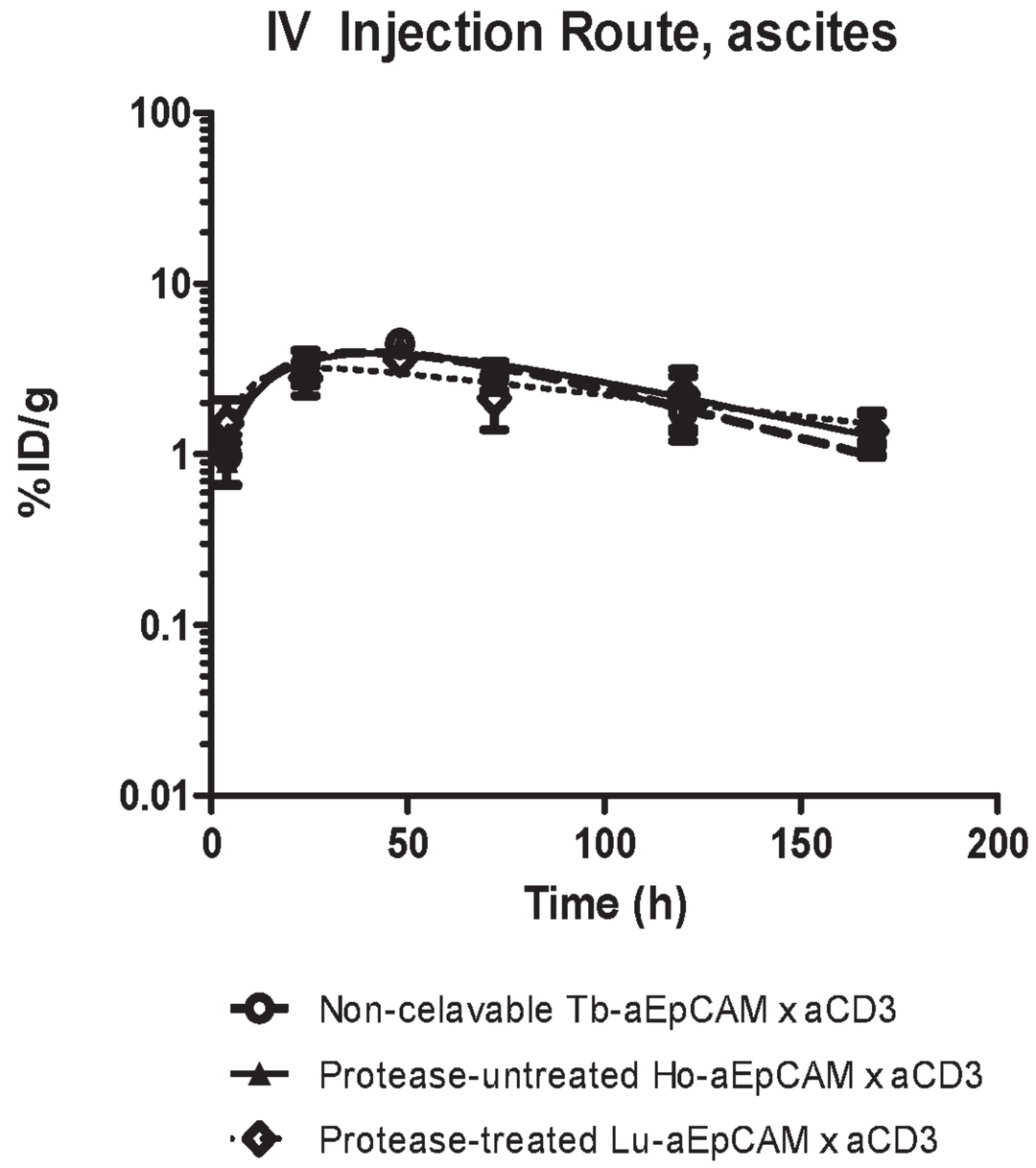


FIG. 69

FIG. 70A

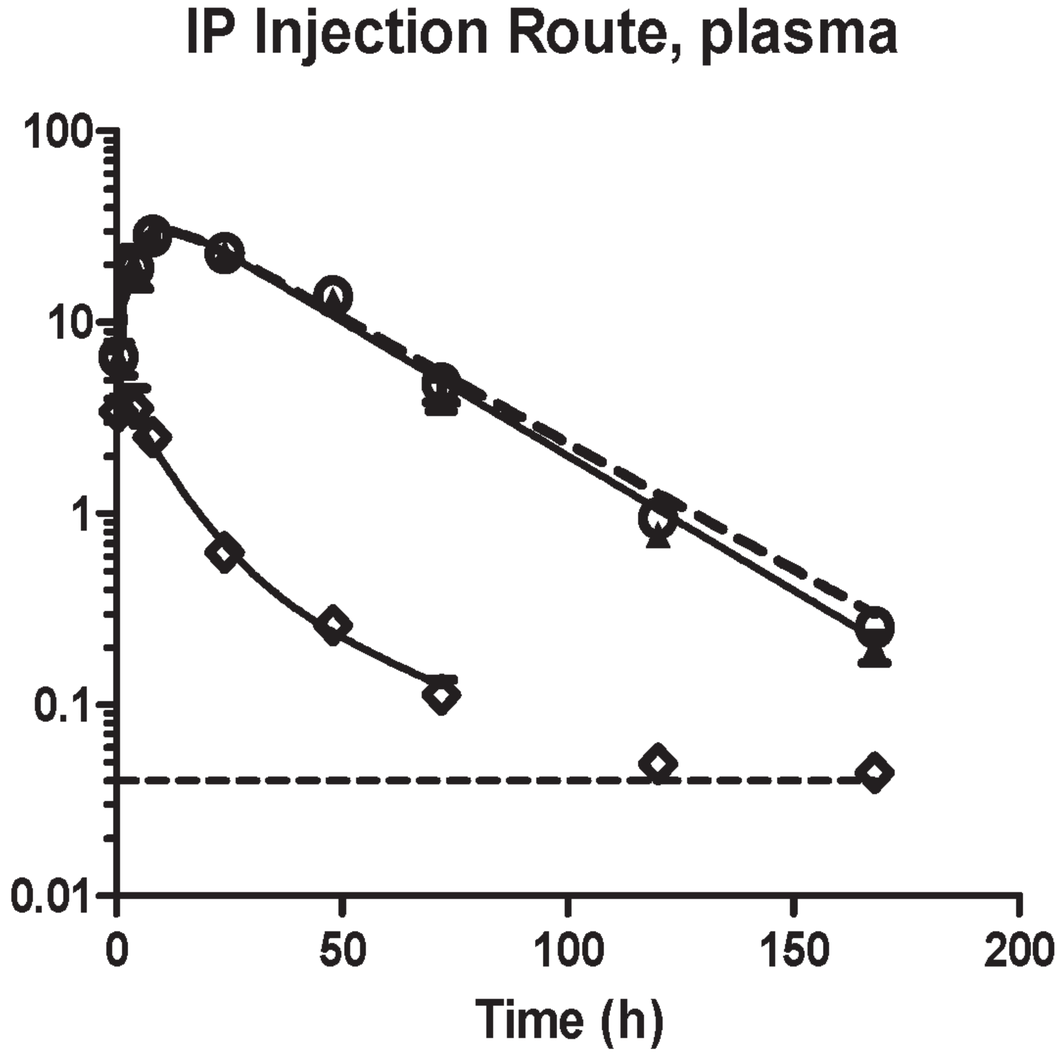


FIG. 70B

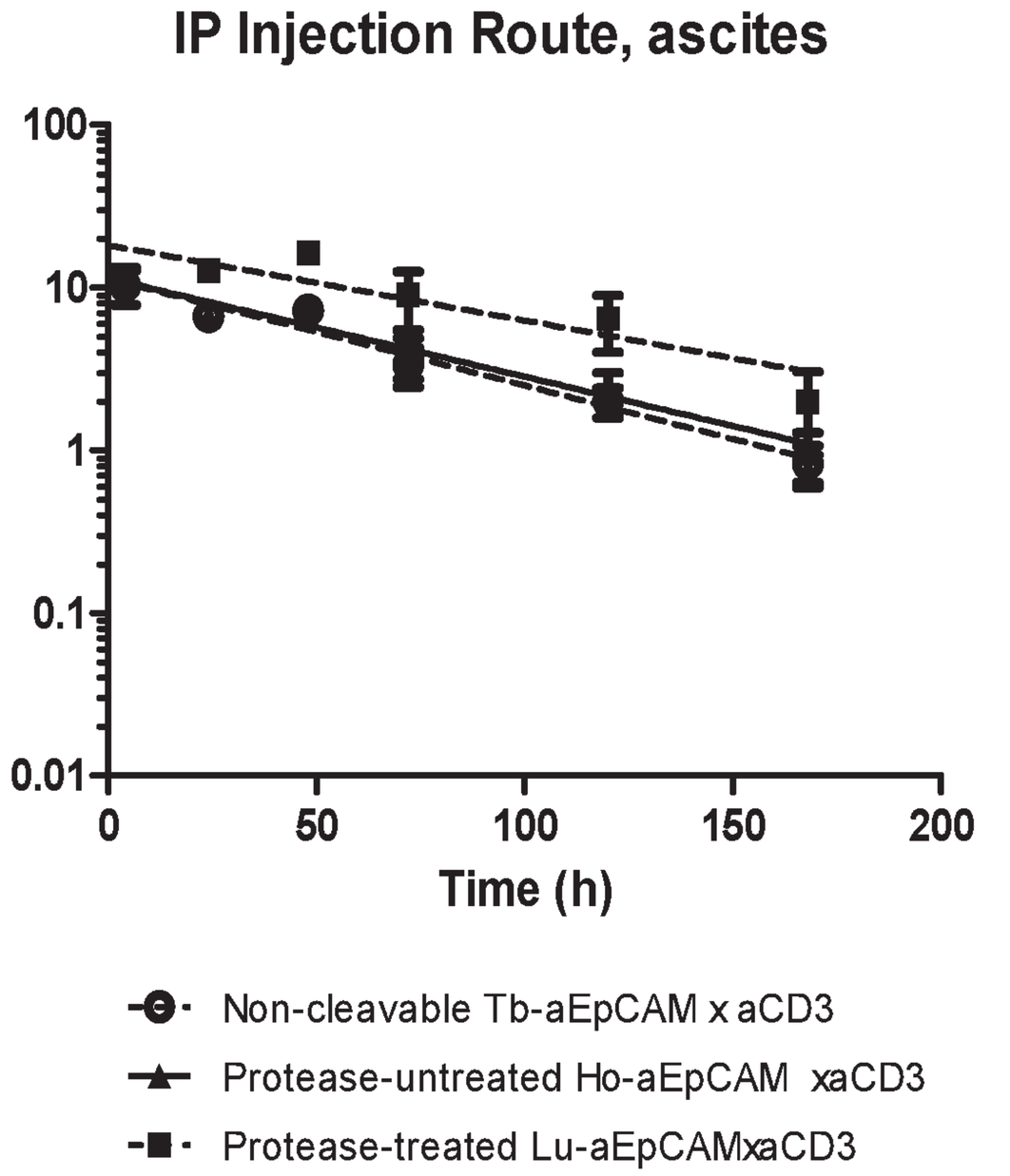


FIG. 70

FIG. 71A

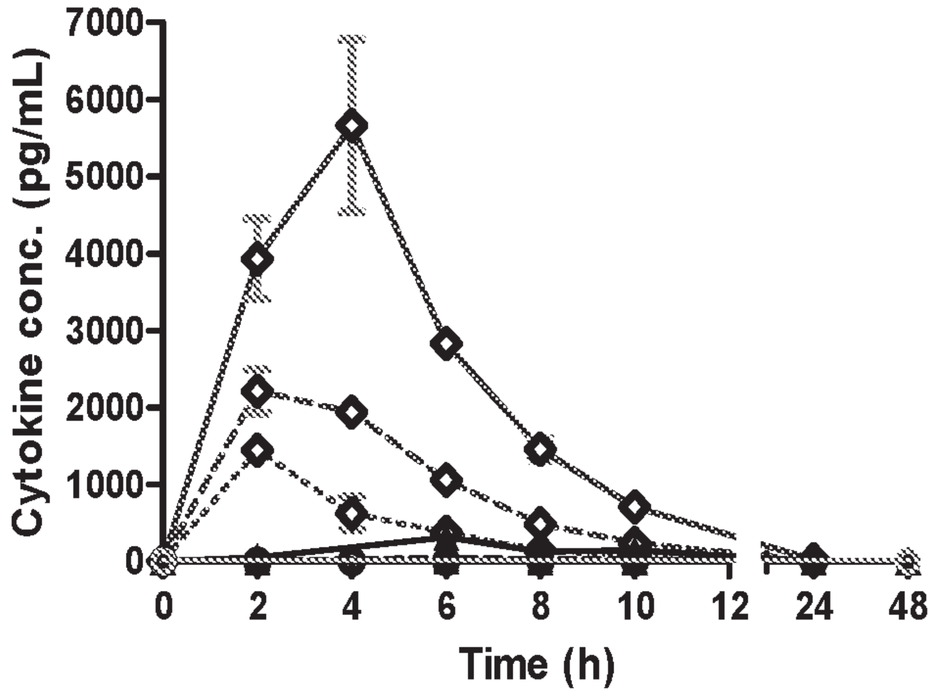


FIG. 71B

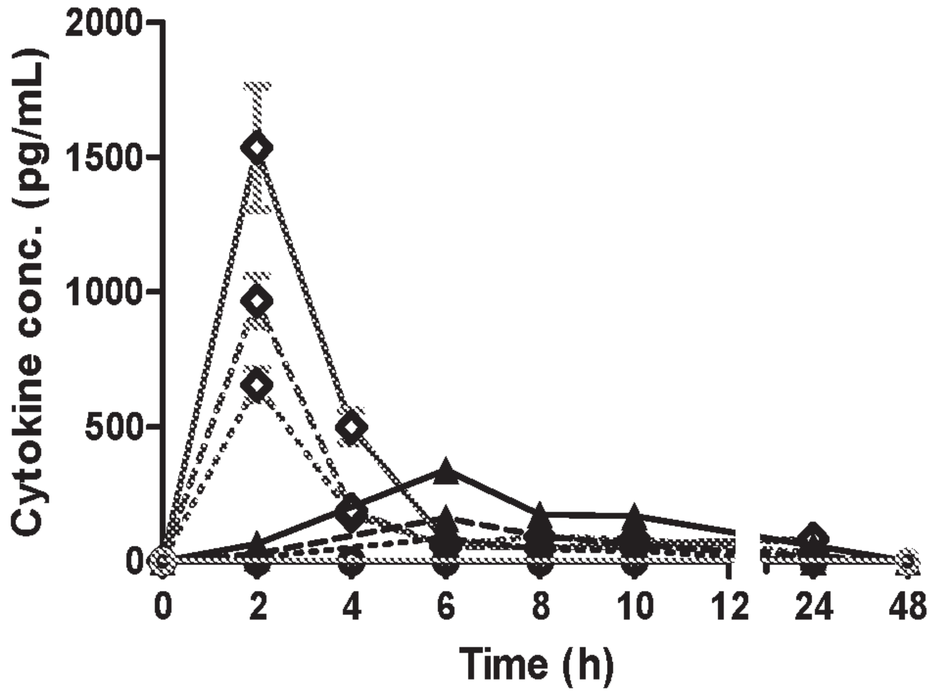


FIG. 71C

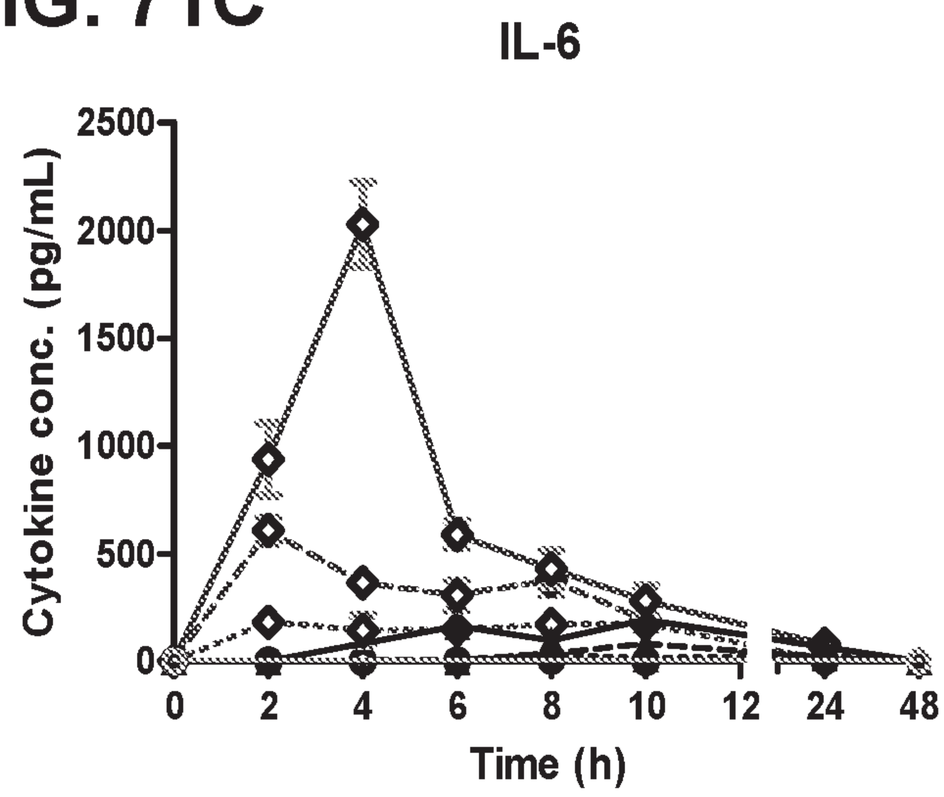


FIG. 71D

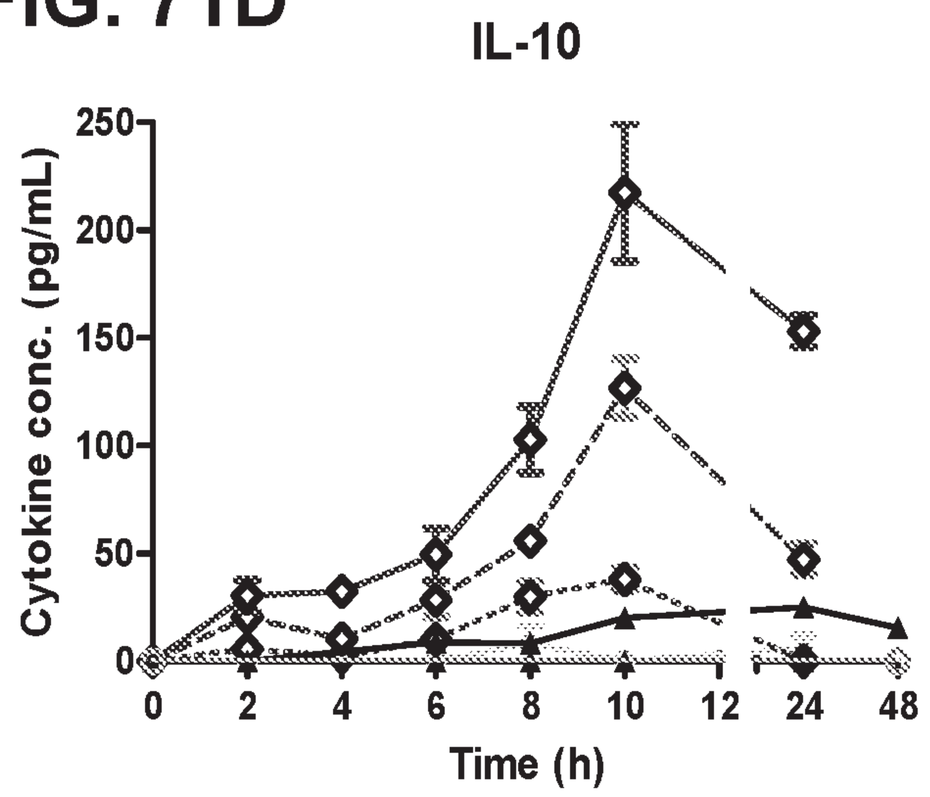


FIG. 71E

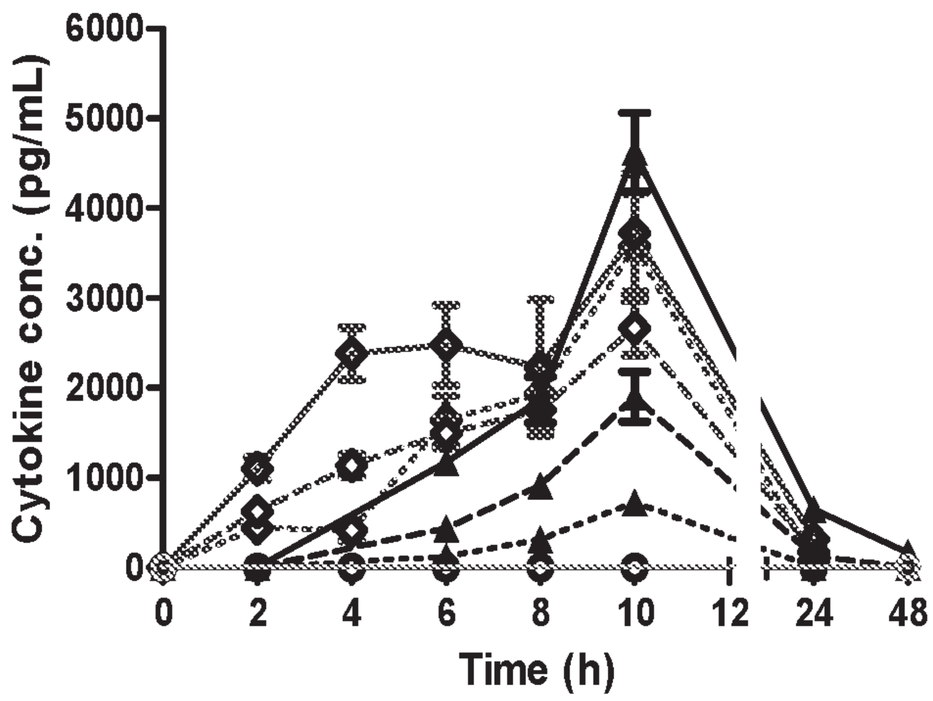
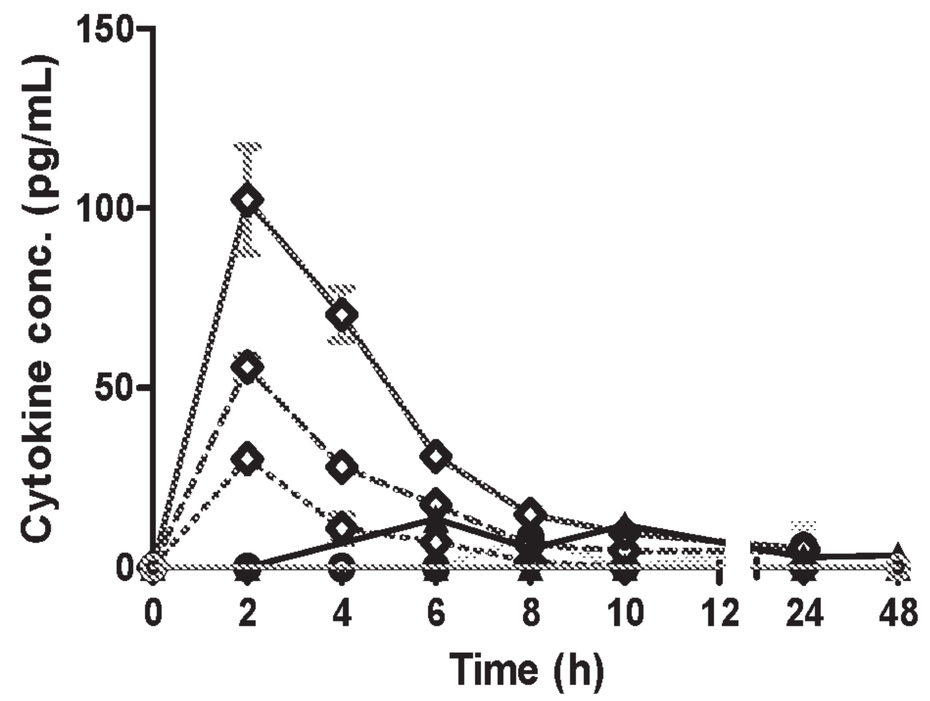


FIG. 71F



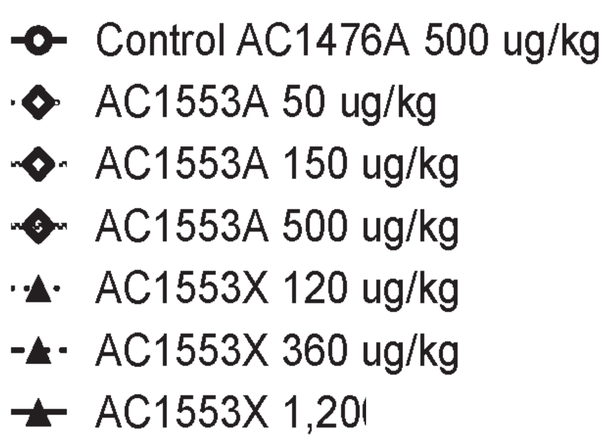


FIG. 71

AC1553A and AC1553X Treatment

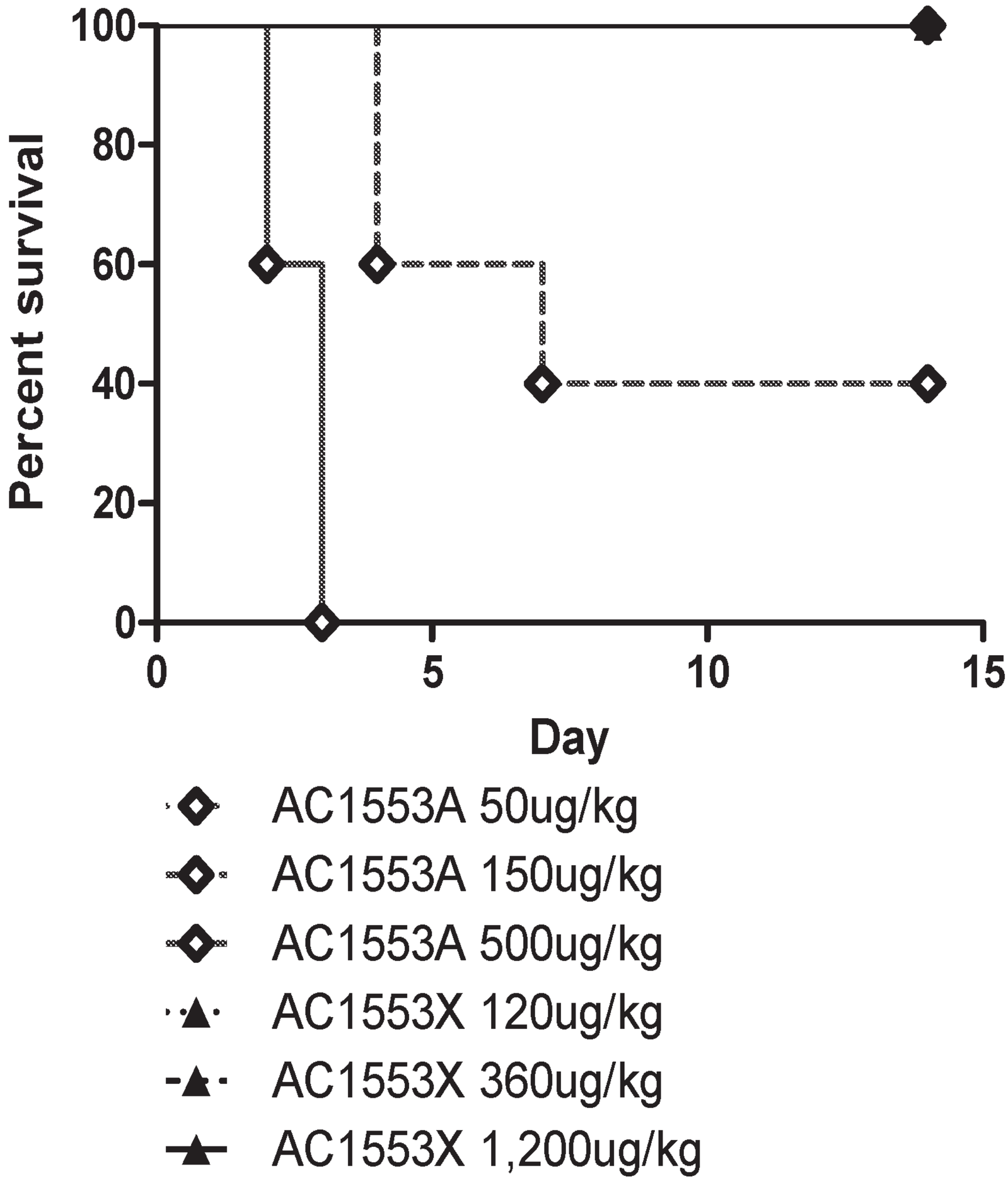


FIG. 72

FIG. 73A

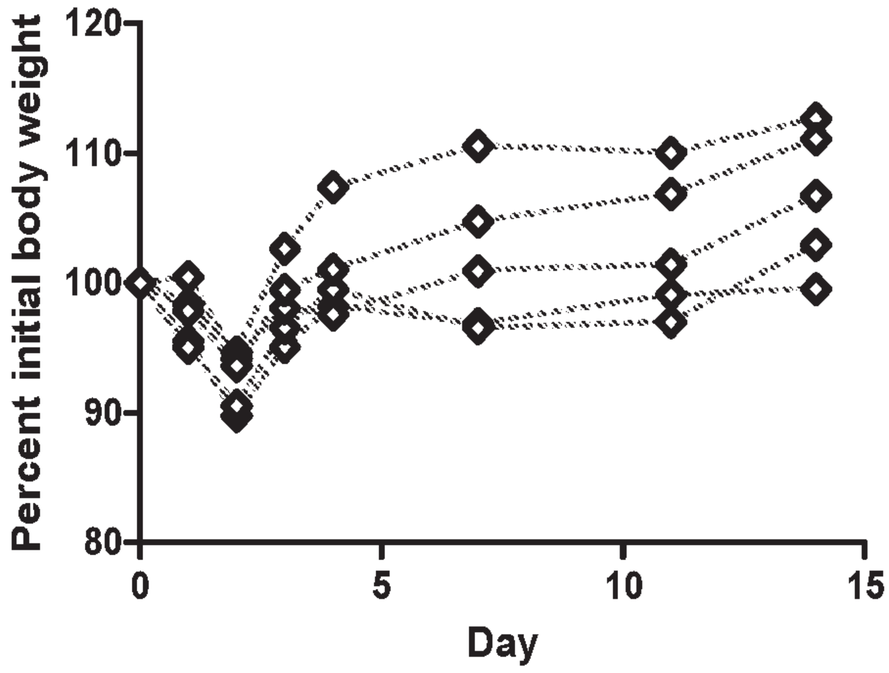


FIG. 73B

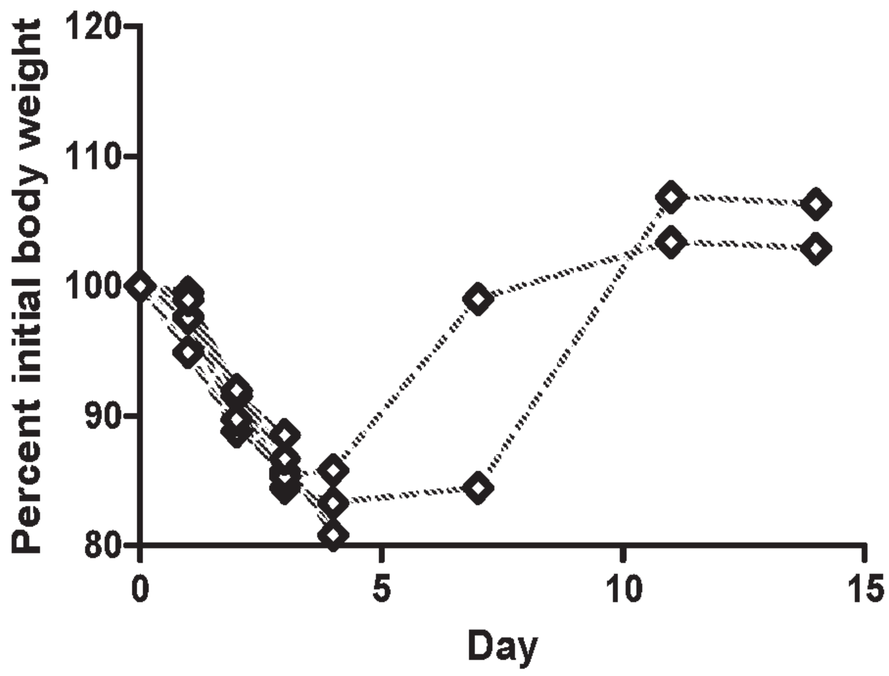


FIG. 73C

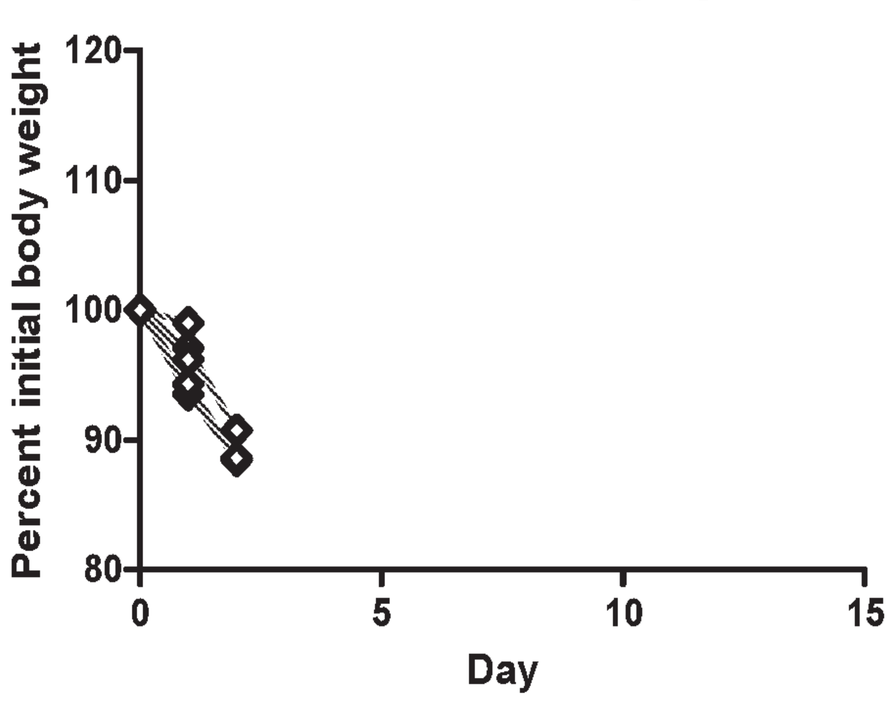


FIG. 73D

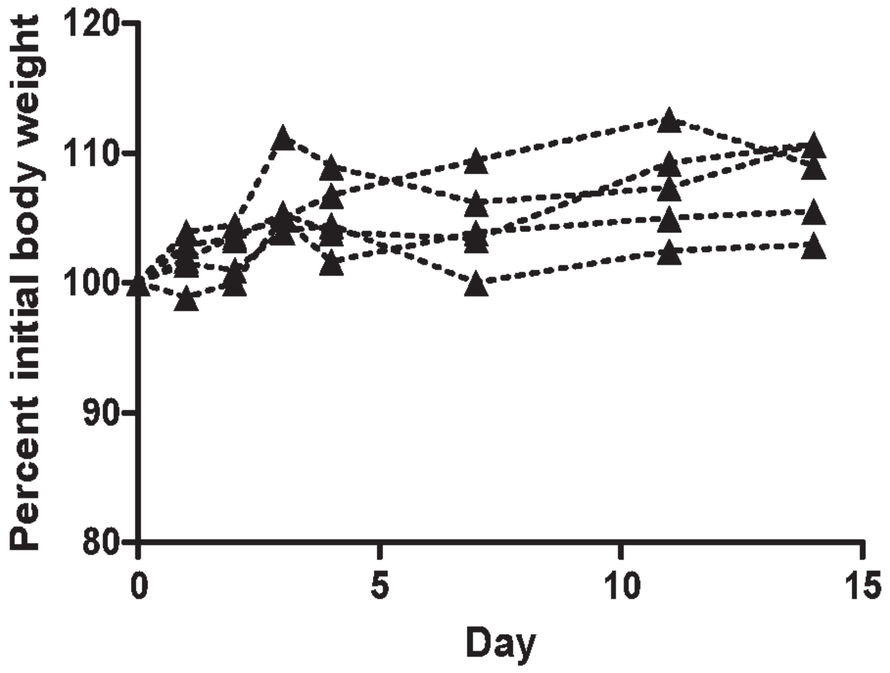


FIG. 73E

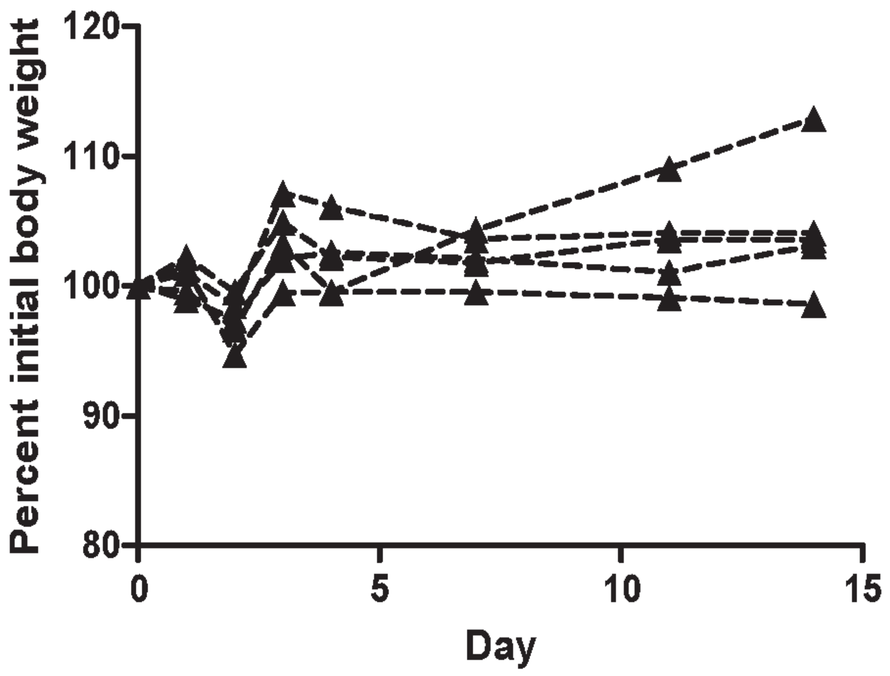


FIG. 73F

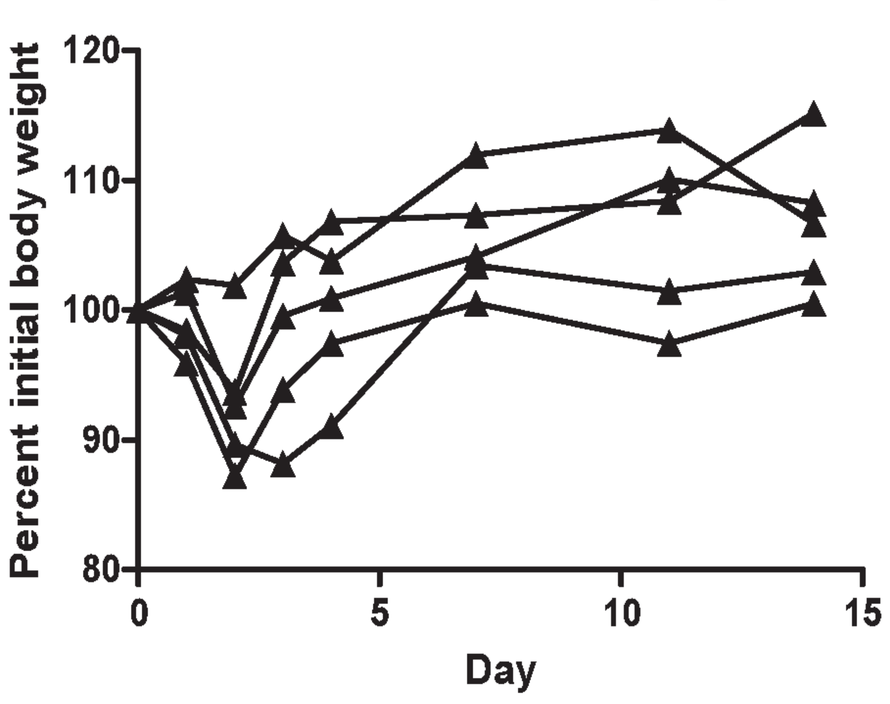


FIG. 73

FIG. 74A

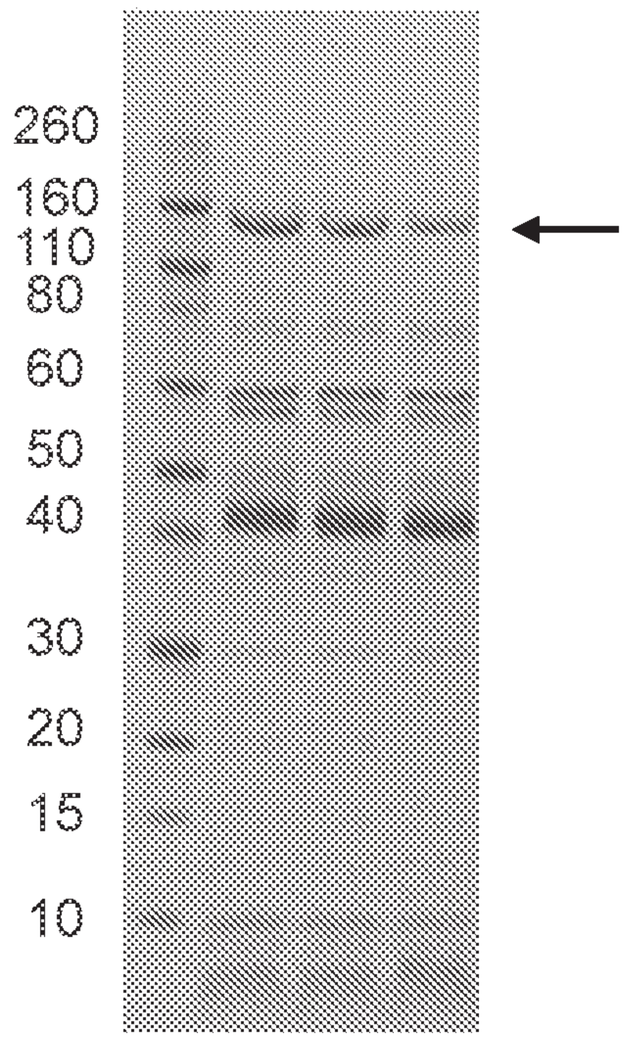


FIG. 74B

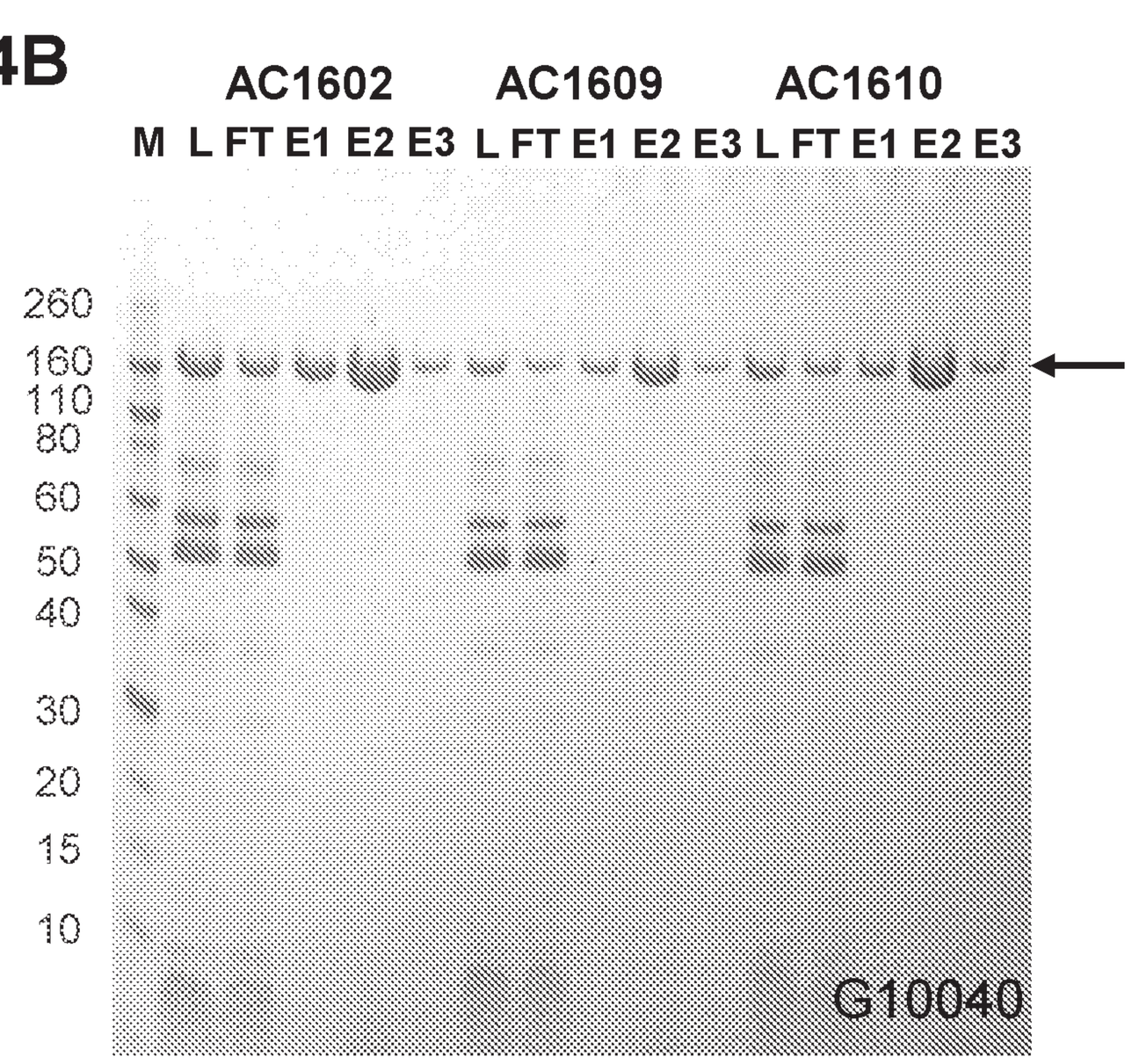


FIG. 74

FIG. 74C

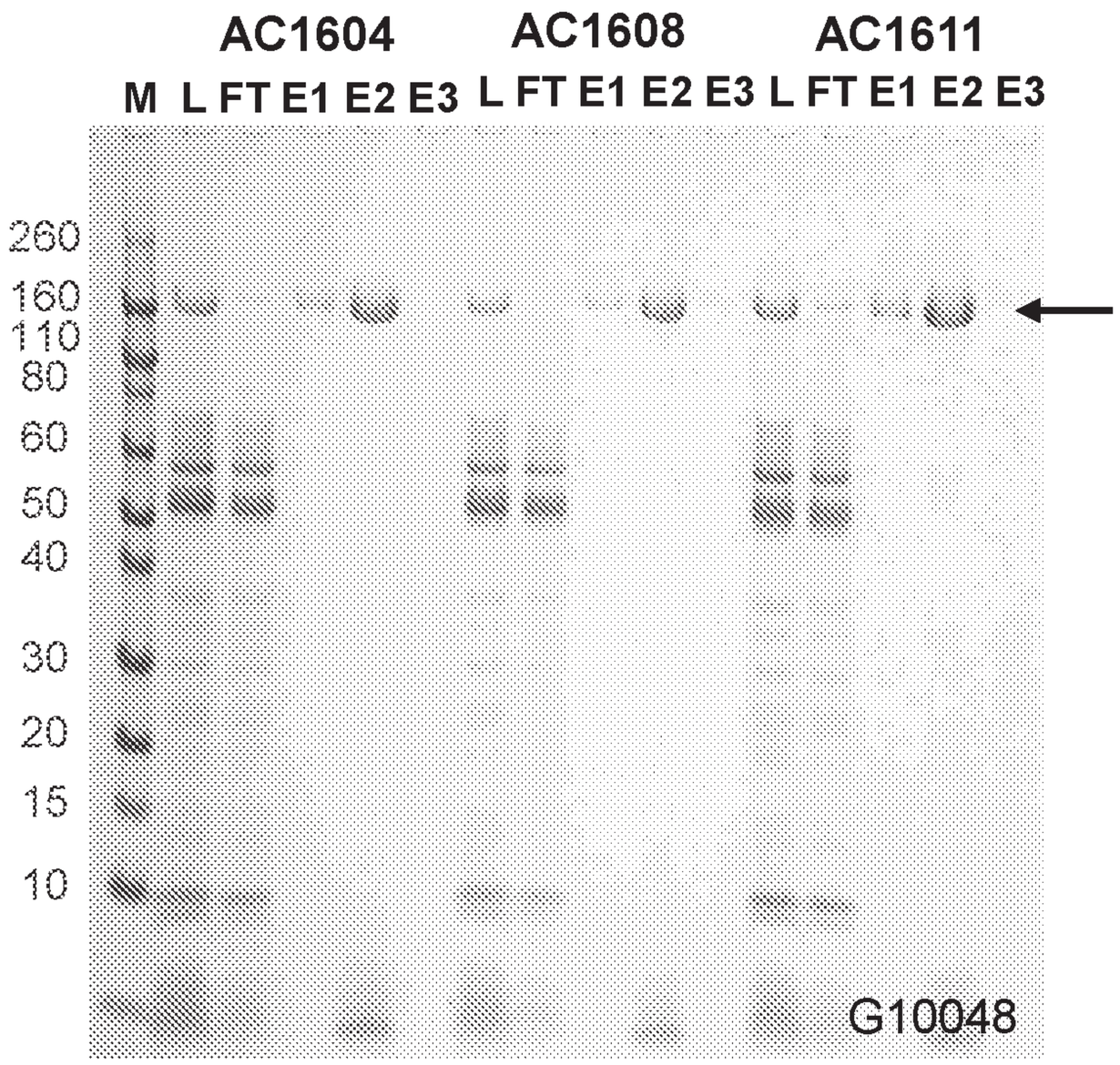


FIG. 74D

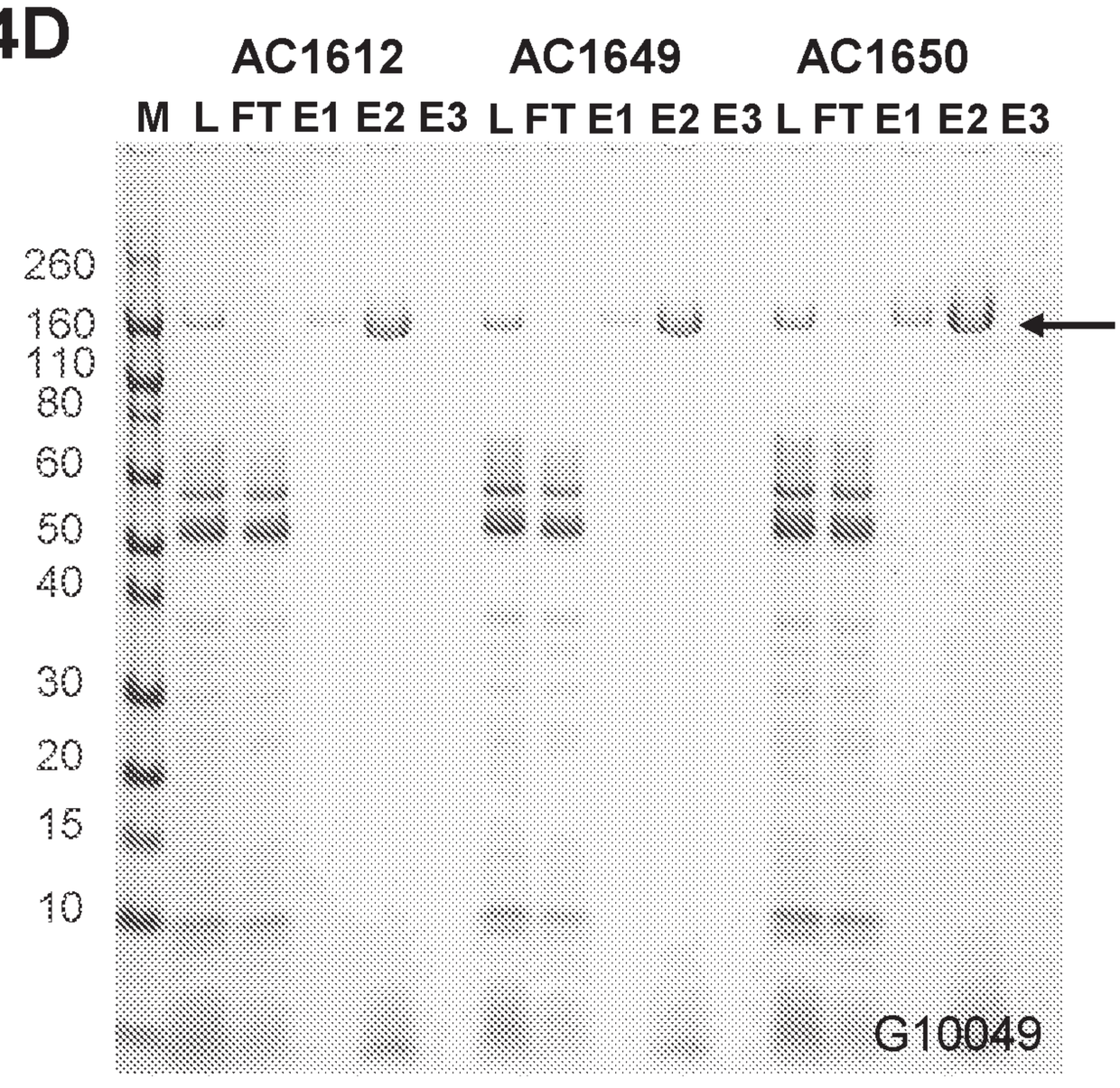


FIG. 74

FIG. 74E

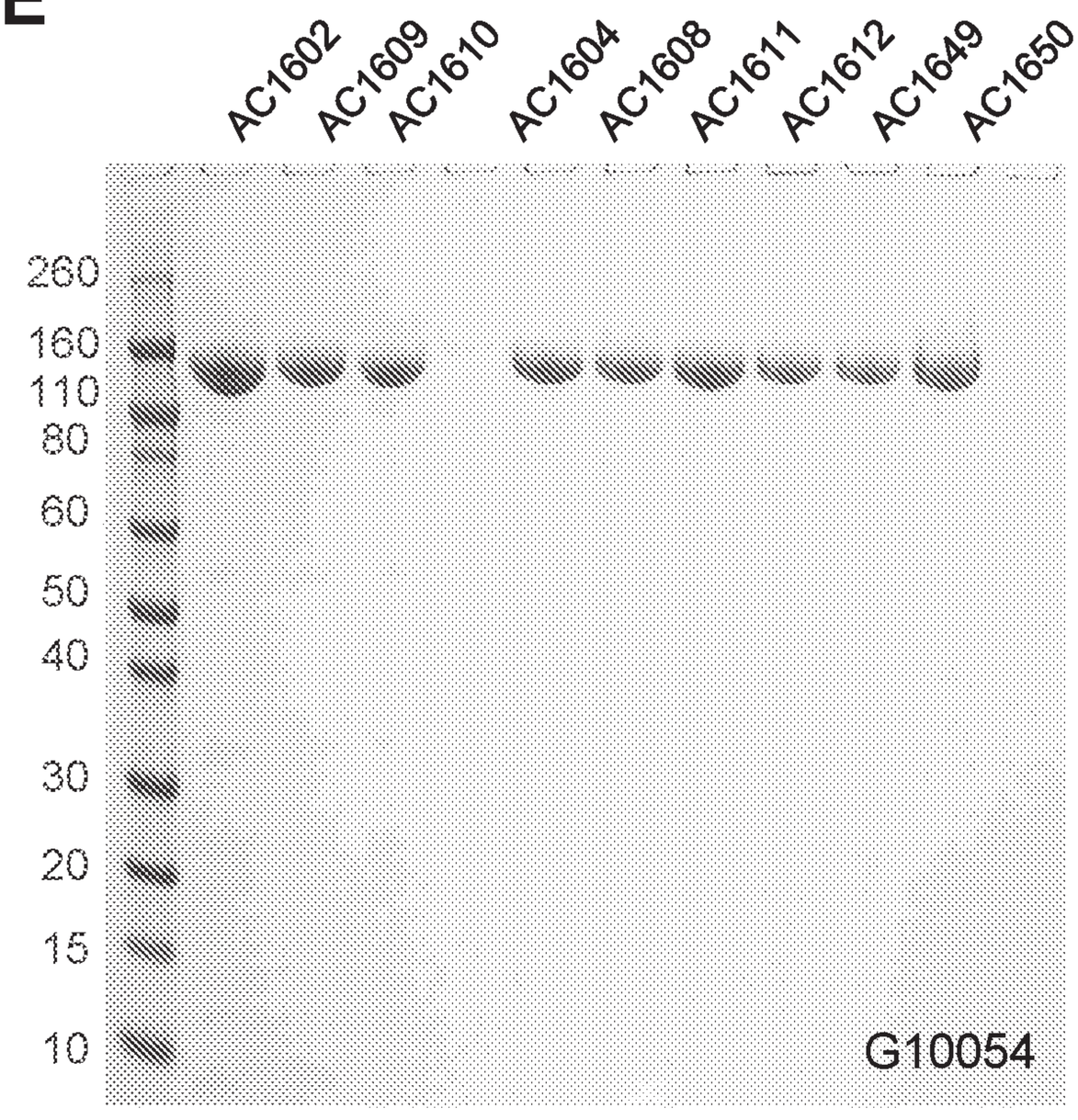


FIG. 74

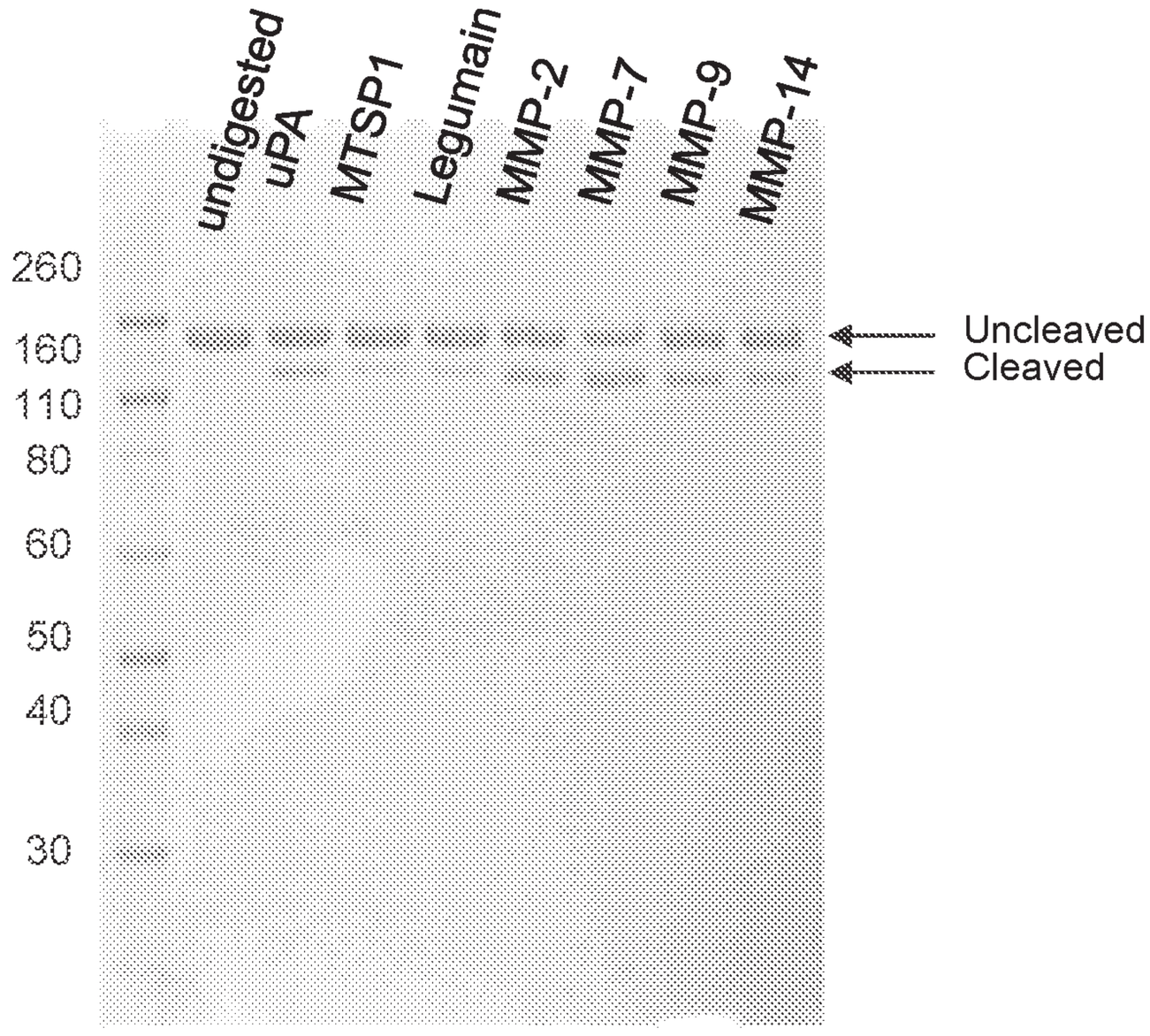

undigested
uPA
MTSP1
Legumain
MMP-2
MMP-7
MMP-9
MMP-14
260
160
110
80
60
50
40
30
Uncleaved
Cleaved

FIG. 75

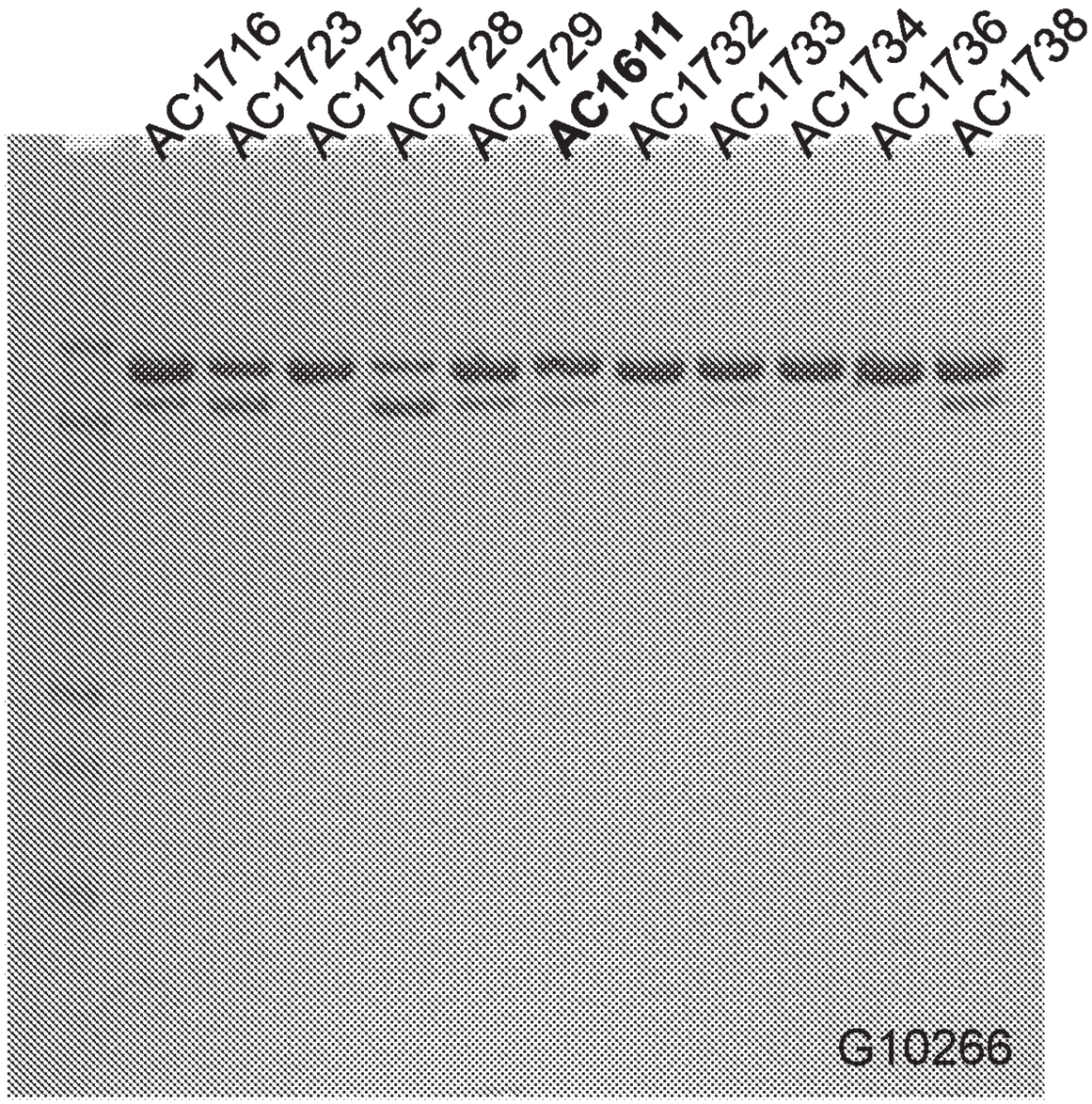

AC1716
AC1723
AC1725
AC1728
AC1729
AC1611
AC1732
AC1733
AC1734
AC1736
AC1738
G10266

FIG. 76

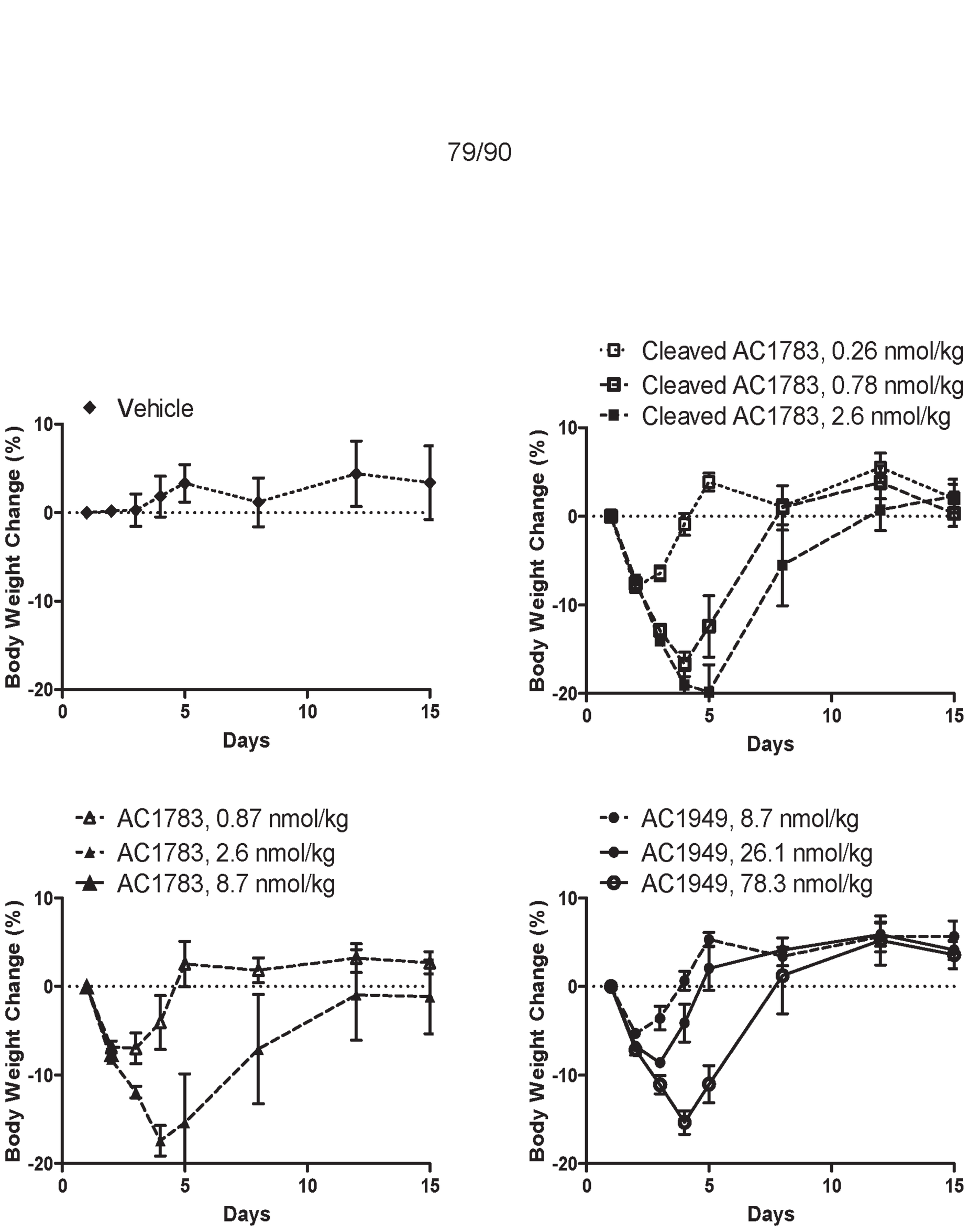

Vehicle
Cleaved AC1783, 0.26 nmol/kg
Cleaved AC1783, 0.78 nmol/kg
Cleaved AC1783, 2.6 nmol/kg
AC1783, 0.87 nmol/kg
AC1783, 2.6 nmol/kg
AC1783, 8.7 nmol/kg
AC1949, 8.7 nmol/kg
AC1949, 26.1 nmol/kg
AC1949, 78.3 nmol/kg
Body Weight Change (%)
Days
10
0
-10
-20
0
5
10
15

FIG. 77

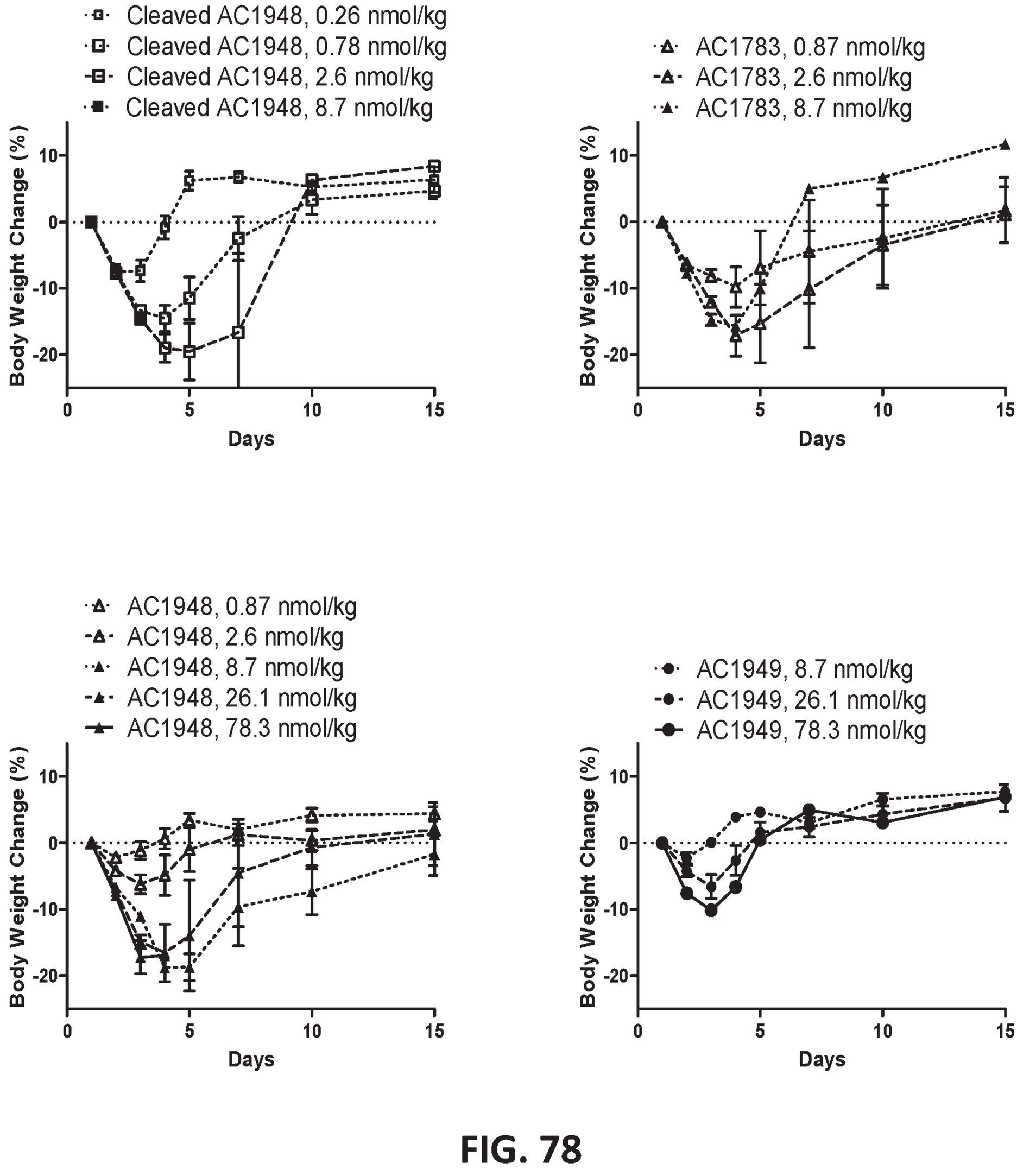

Cleaved AC1948, 0.26 nmol/kg
Cleaved AC1948, 0.78 nmol/kg
Cleaved AC1948, 2.6 nmol/kg
Cleaved AC1948, 8.7 nmol/kg
AC1783, 0.87 nmol/kg
AC1783, 2.6 nmol/kg
AC1783, 8.7 nmol/kg
AC1948, 0.87 nmol/kg
AC1948, 2.6 nmol/kg
AC1948, 8.7 nmol/kg
AC1948, 26.1 nmol/kg
AC1948, 78.3 nmol/kg
AC1949, 8.7 nmol/kg
AC1949, 26.1 nmol/kg
AC1949, 78.3 nmol/kg
Body Weight Change (%)
Days
10
0
-10
-20
0
5
10
15

FIG. 78

FIG. 79A

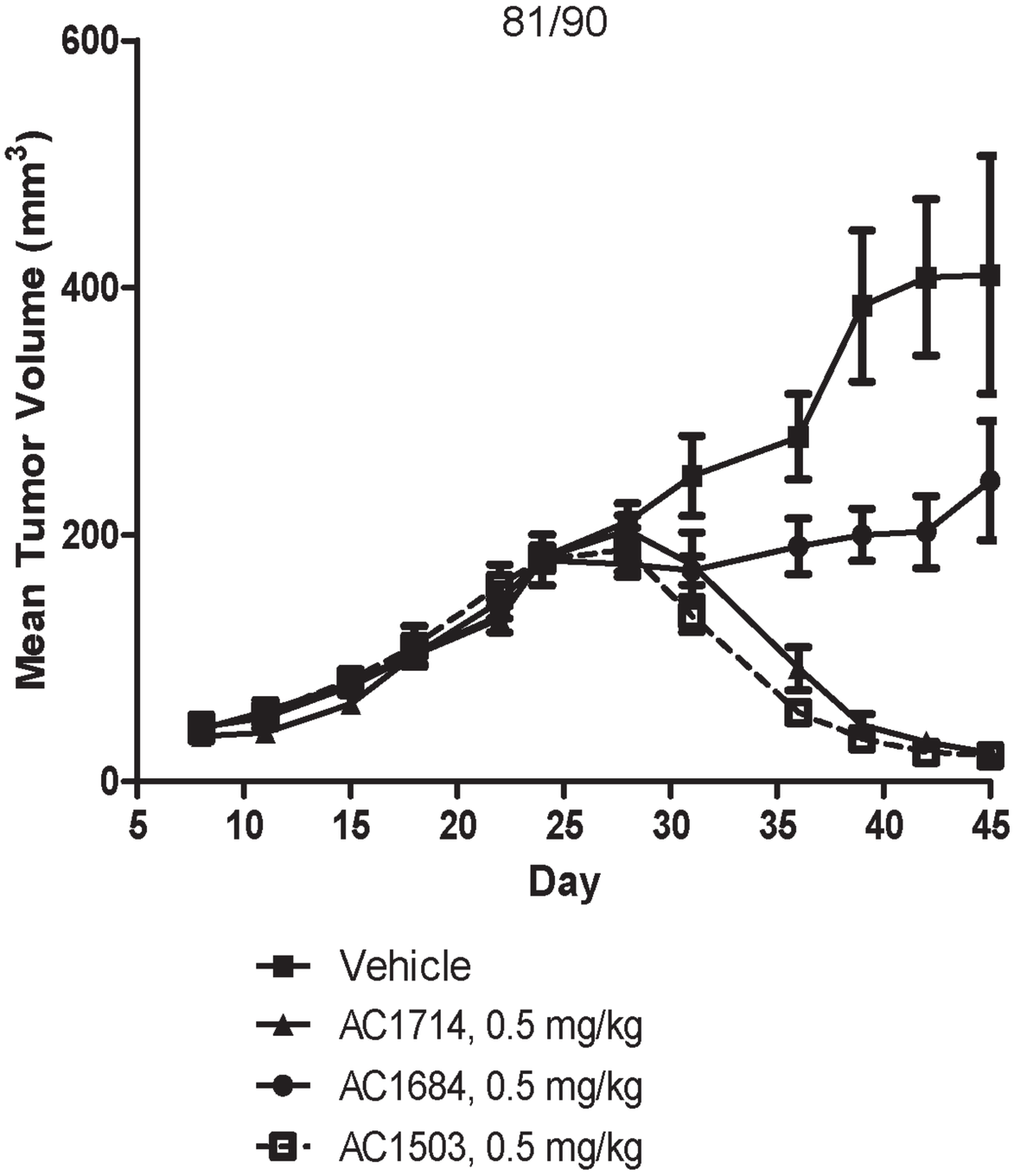


FIG. 79B

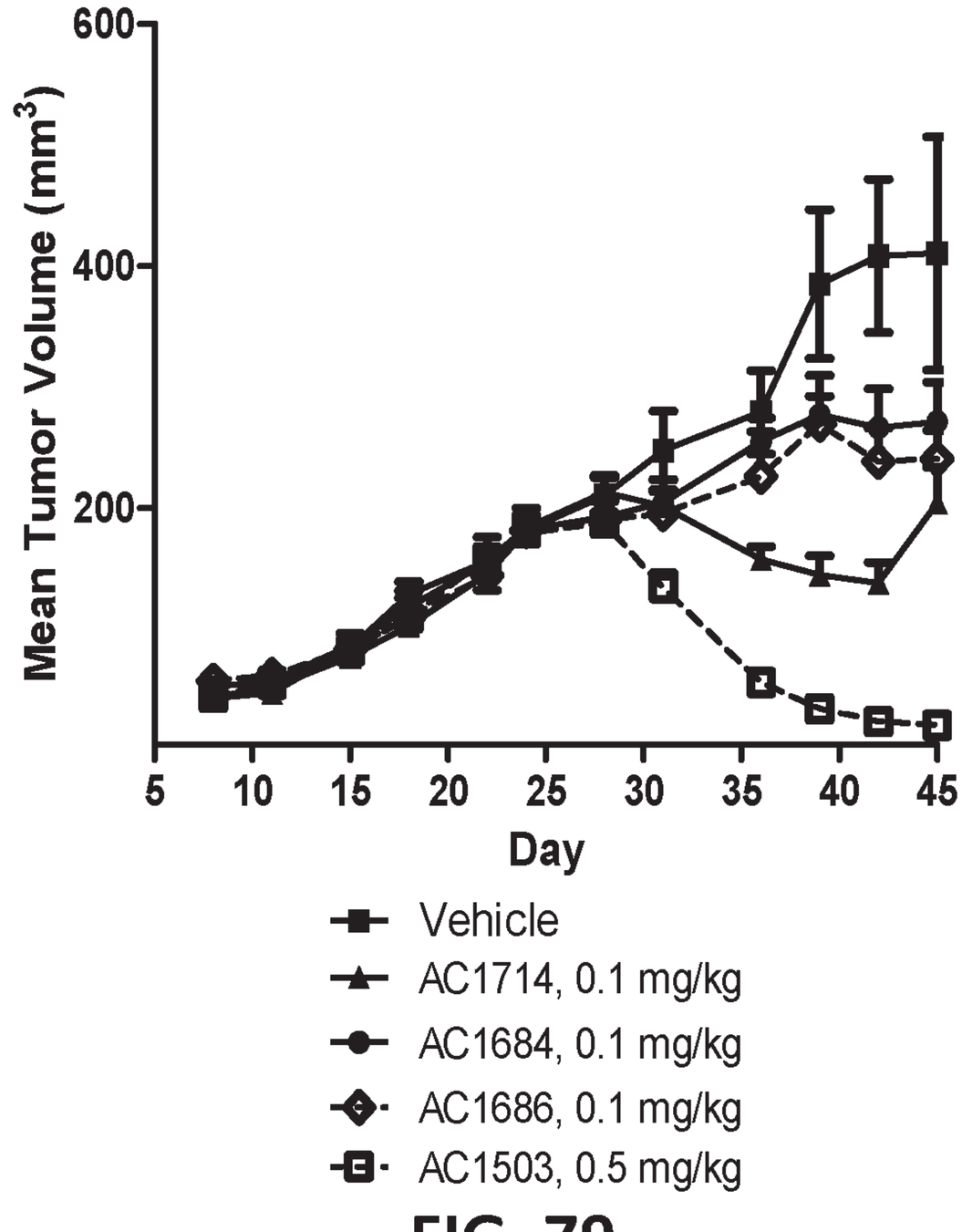


FIG. 79

FIG. 80A

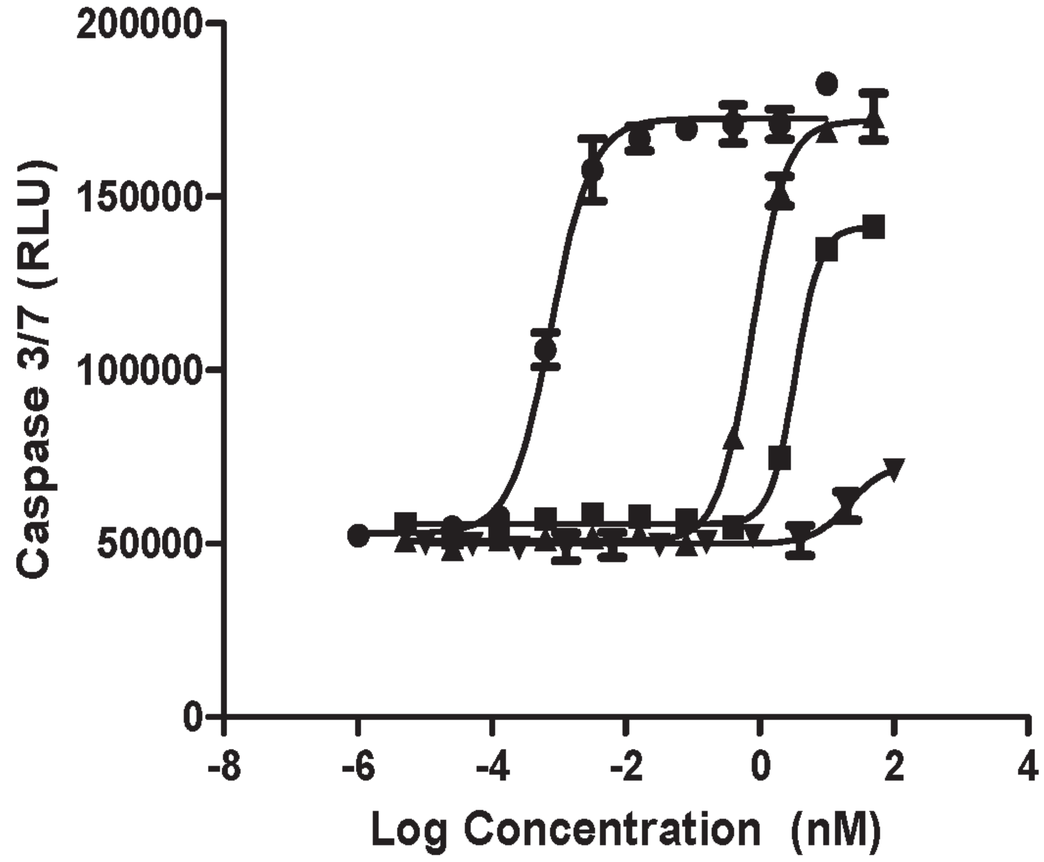


FIG. 80B

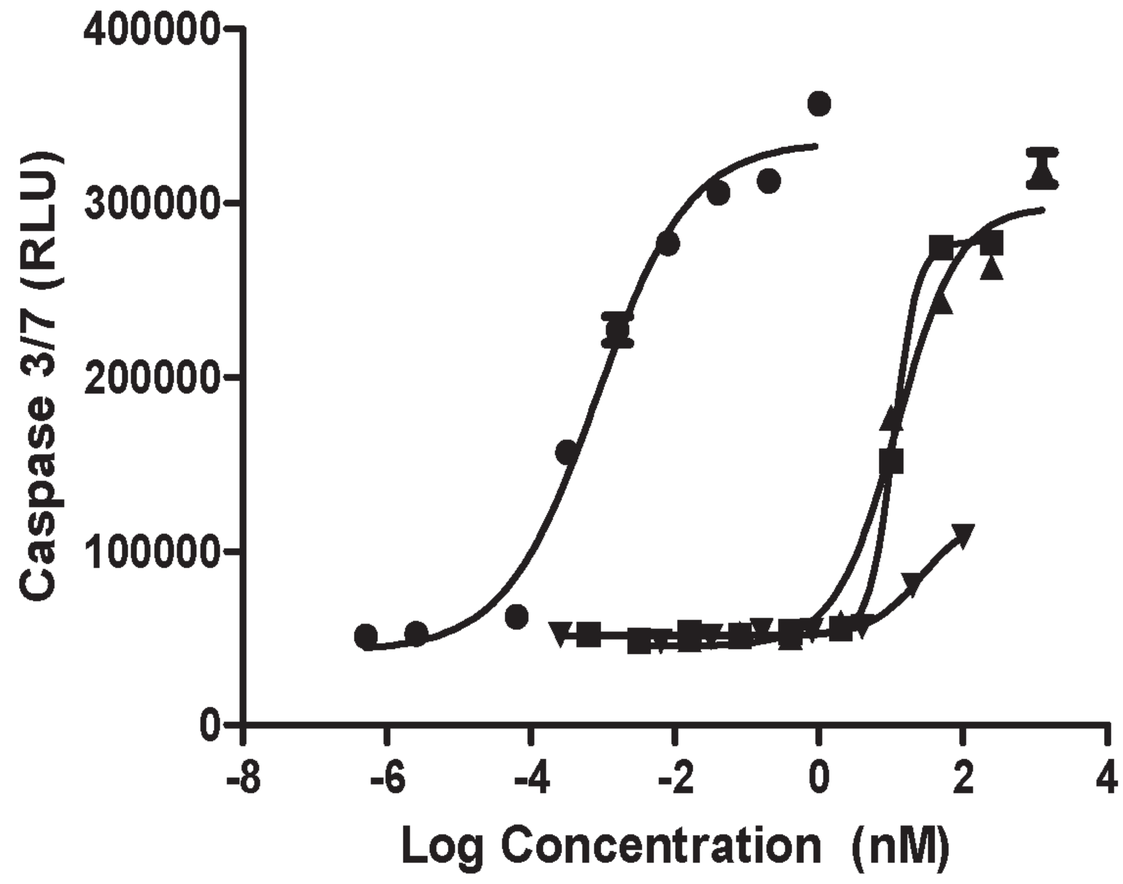


FIG. 80

FIG. 81A

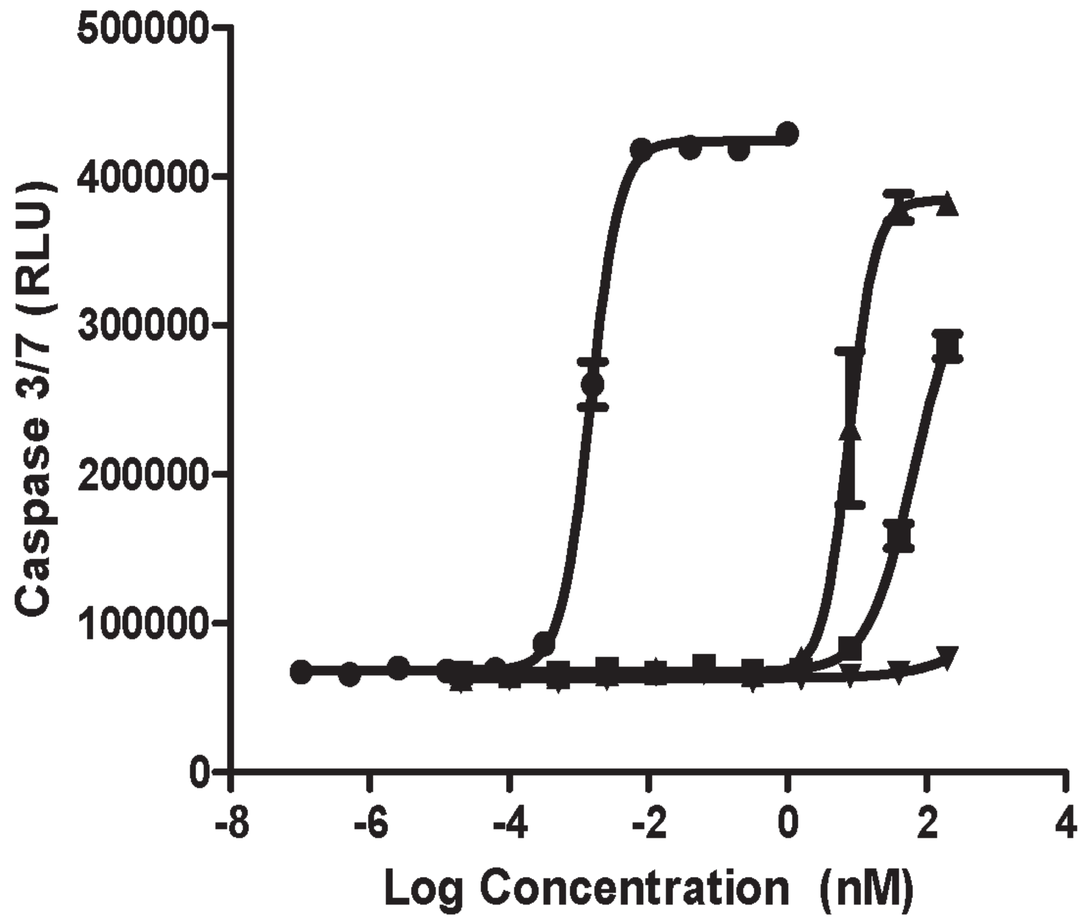


FIG. 81B

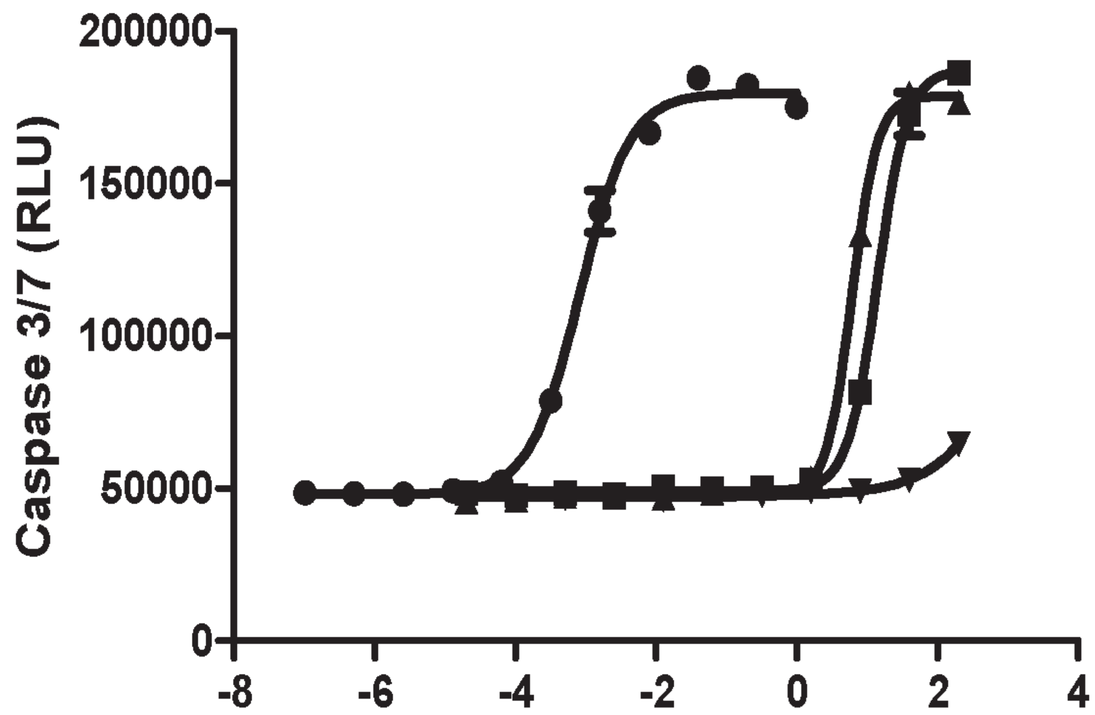


FIG. 81

FIG. 81C

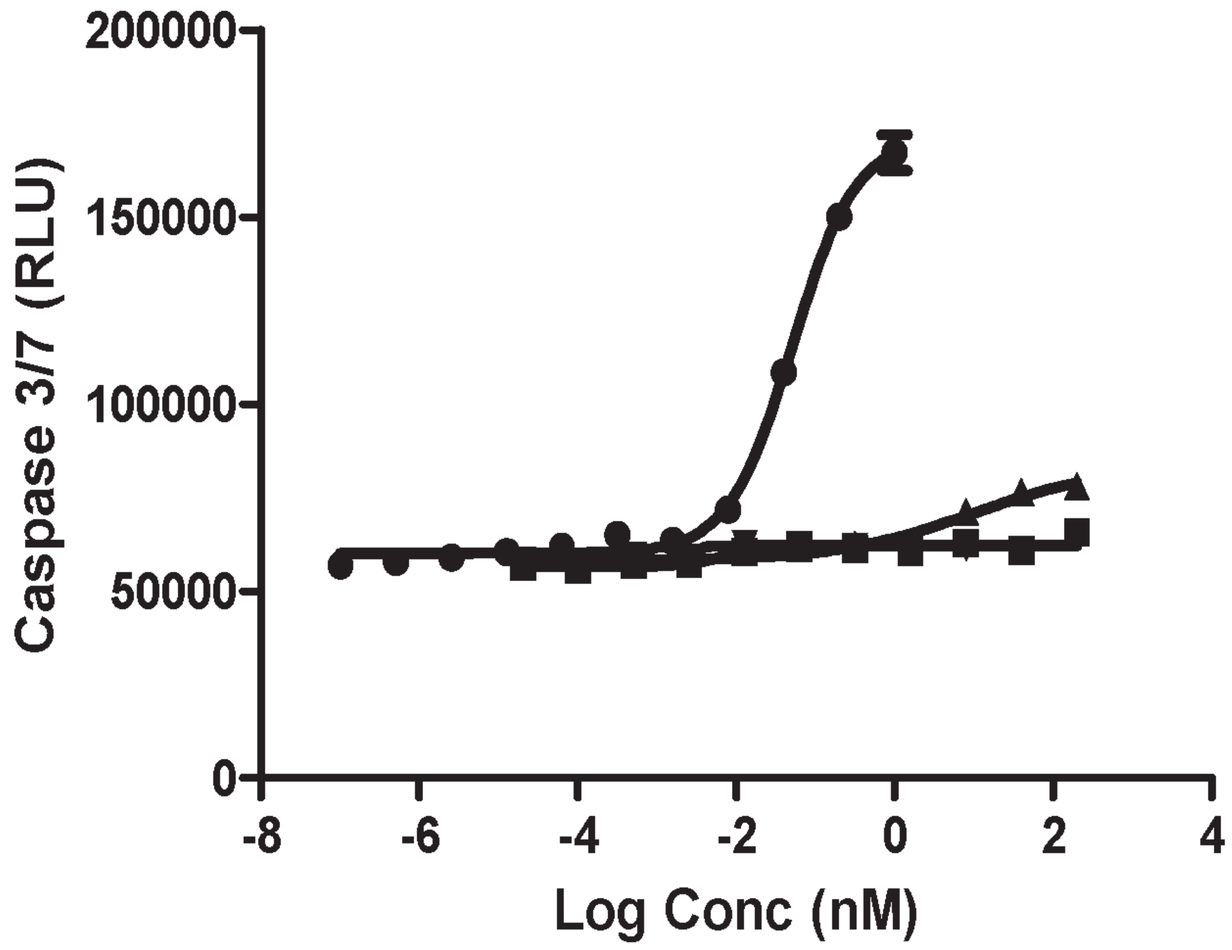


FIG. 81D

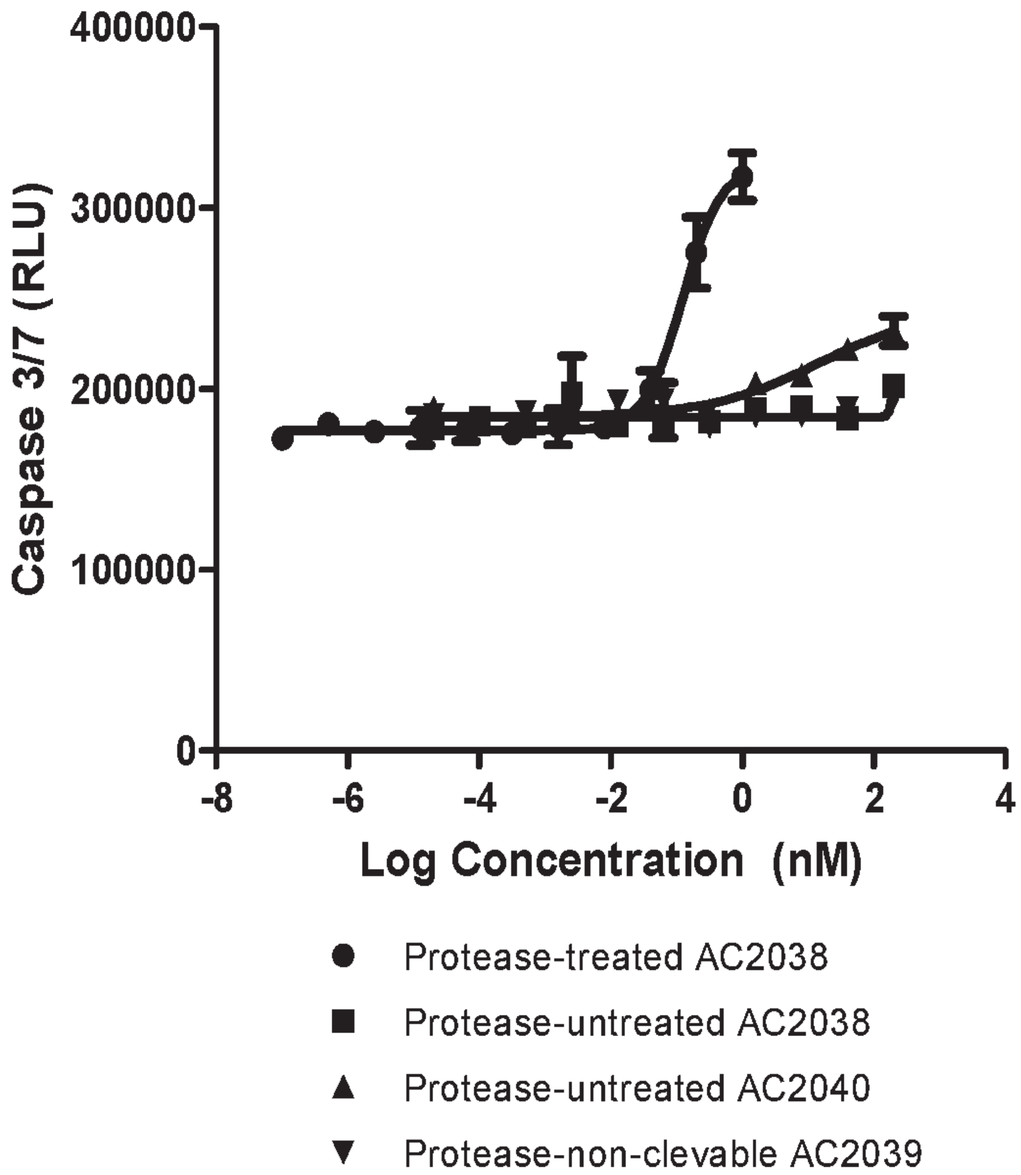


FIG. 81

FIG. 82A

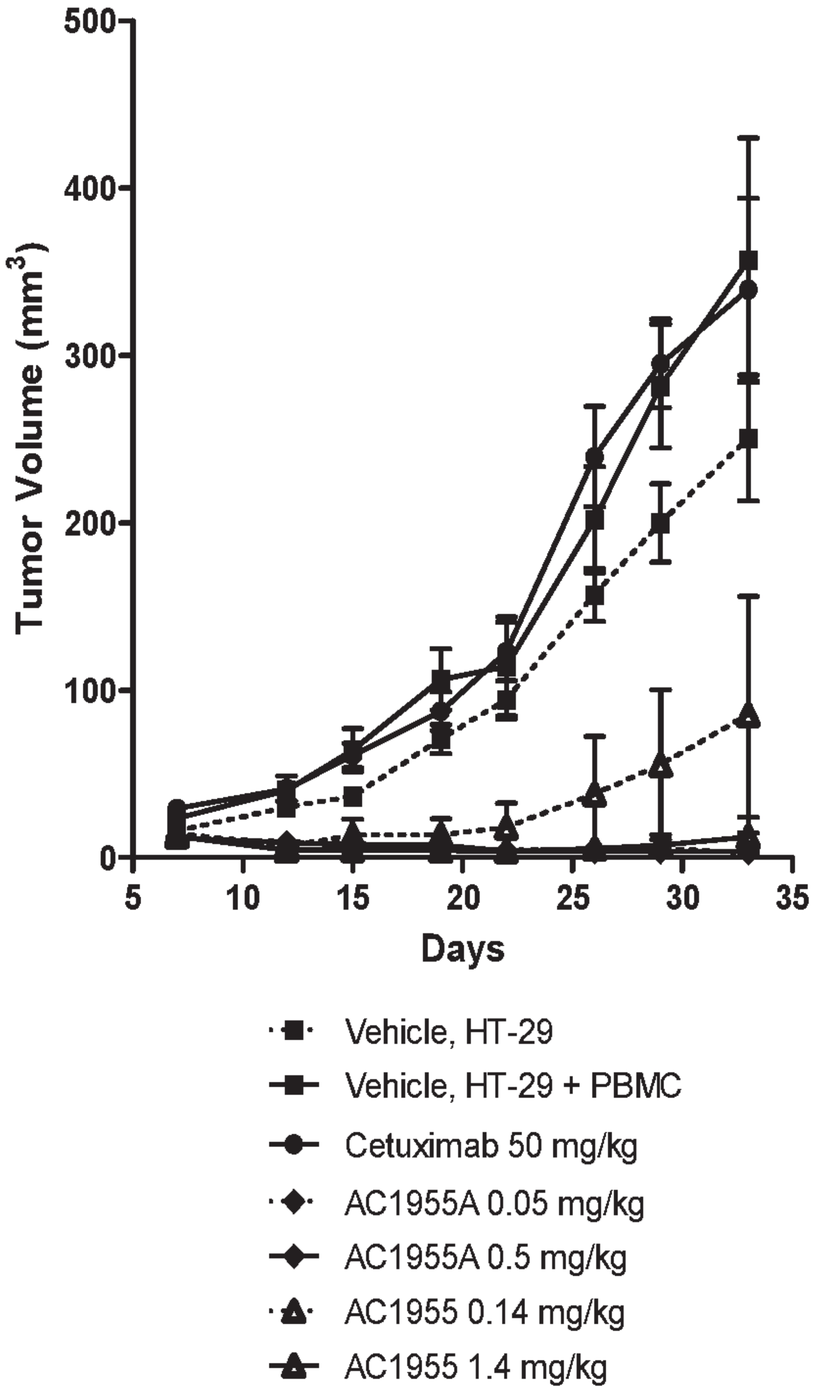


FIG. 82B

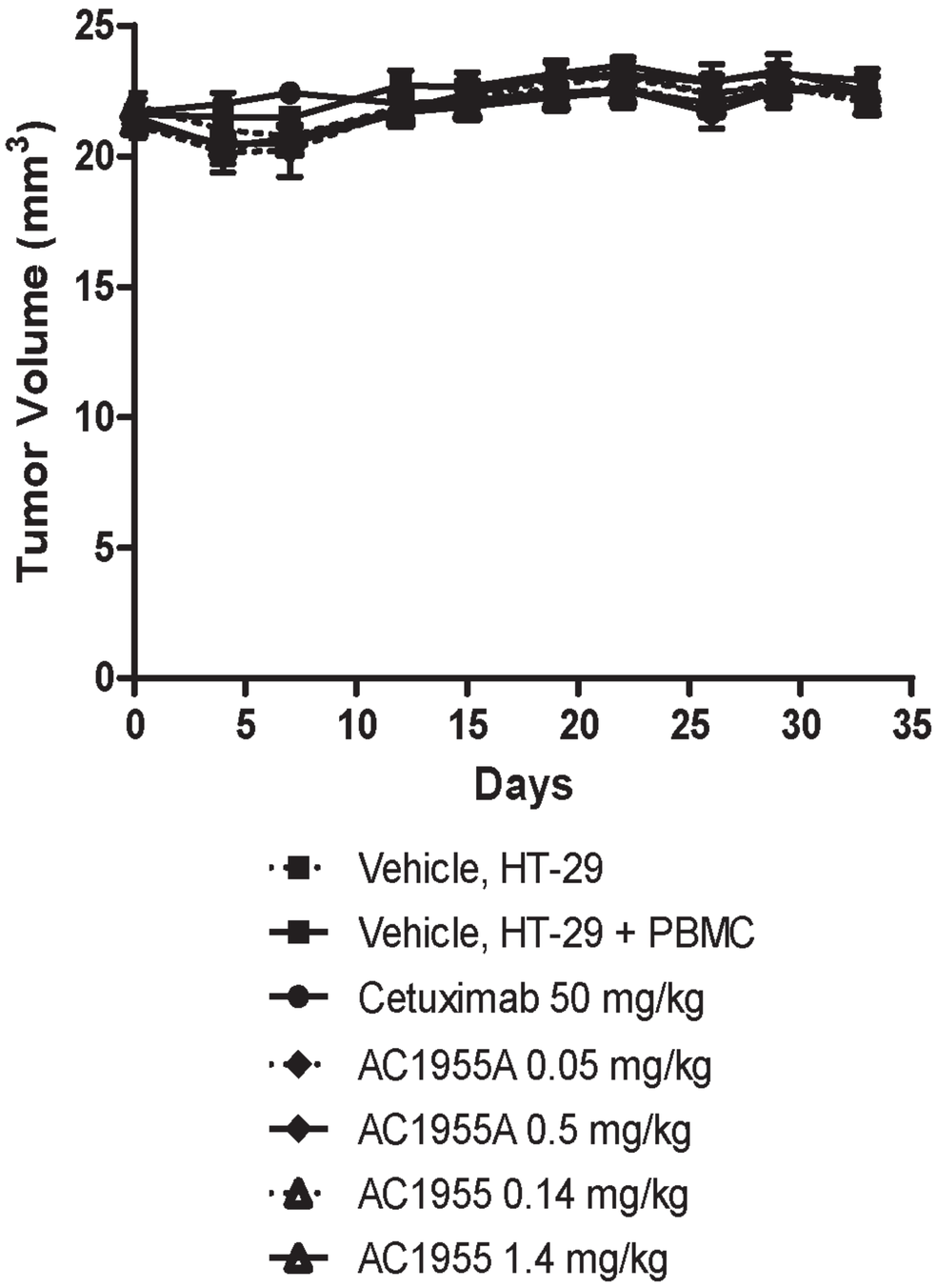


FIG. 82

FIG. 83A

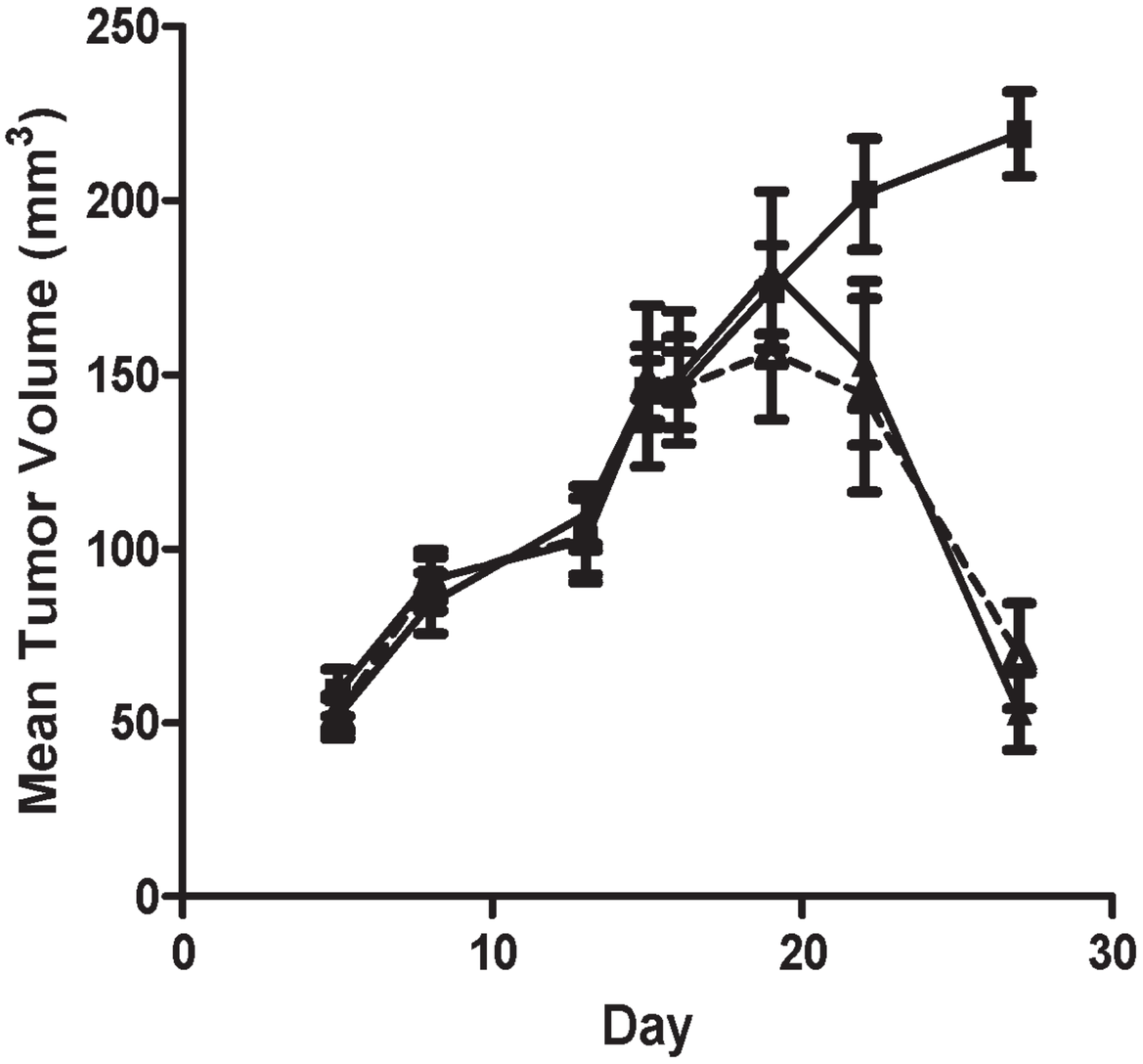


FIG. 83B

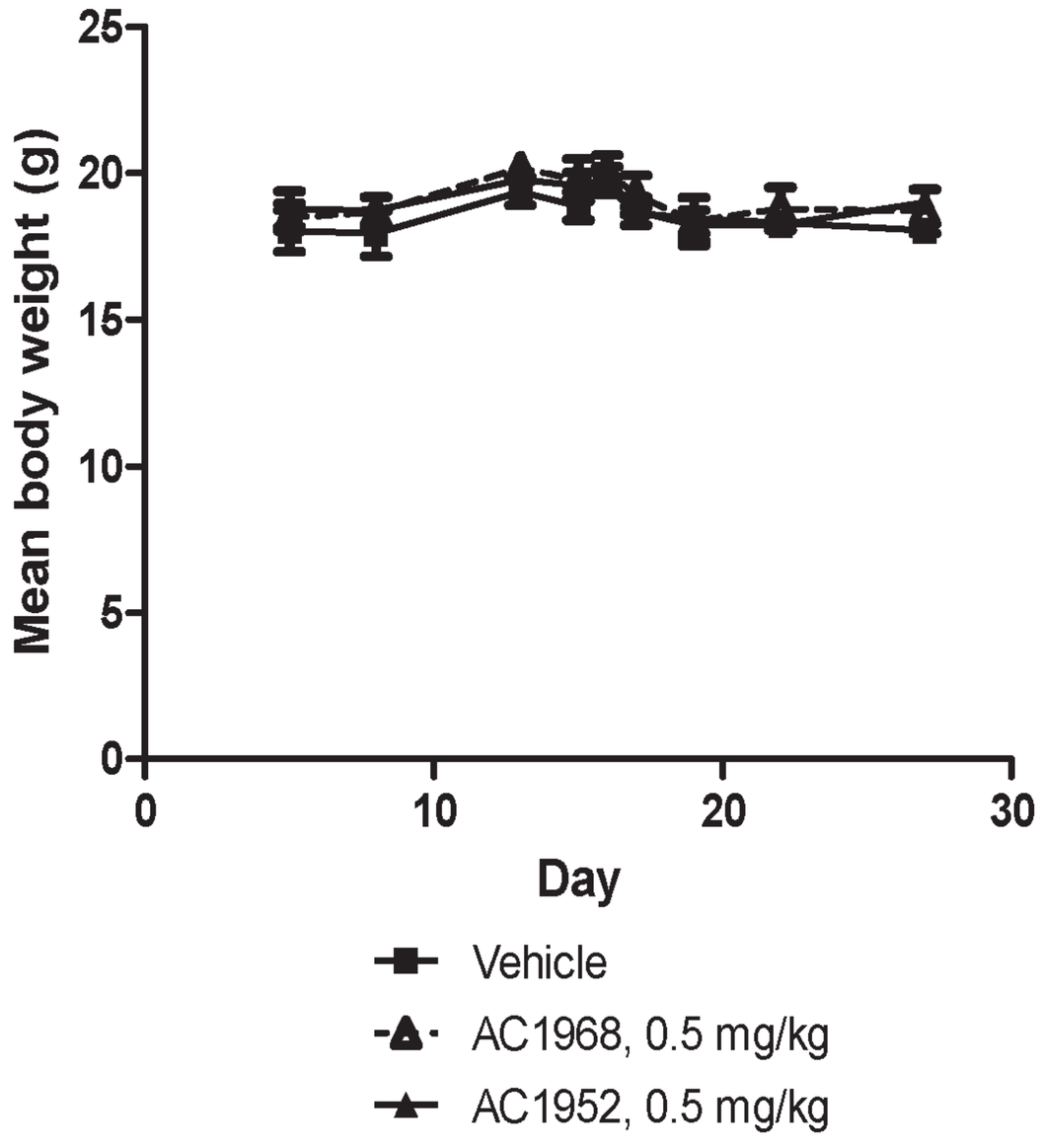


FIG. 83

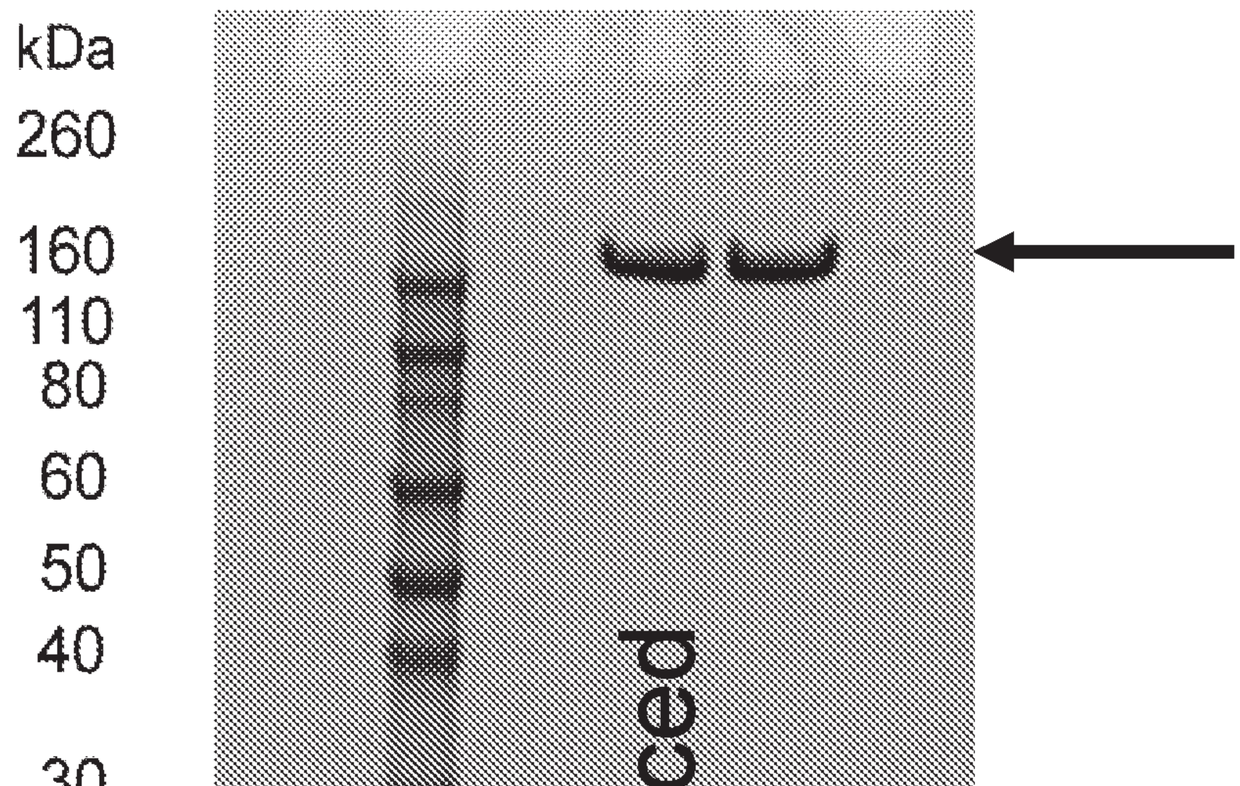

kDa
260
160
110
80
60
50
40
30
20
15
10
3.5
Non-Reduced
Reduced
Lane 1 2 3 4 5 6

FIG. 84

FIG. 85A

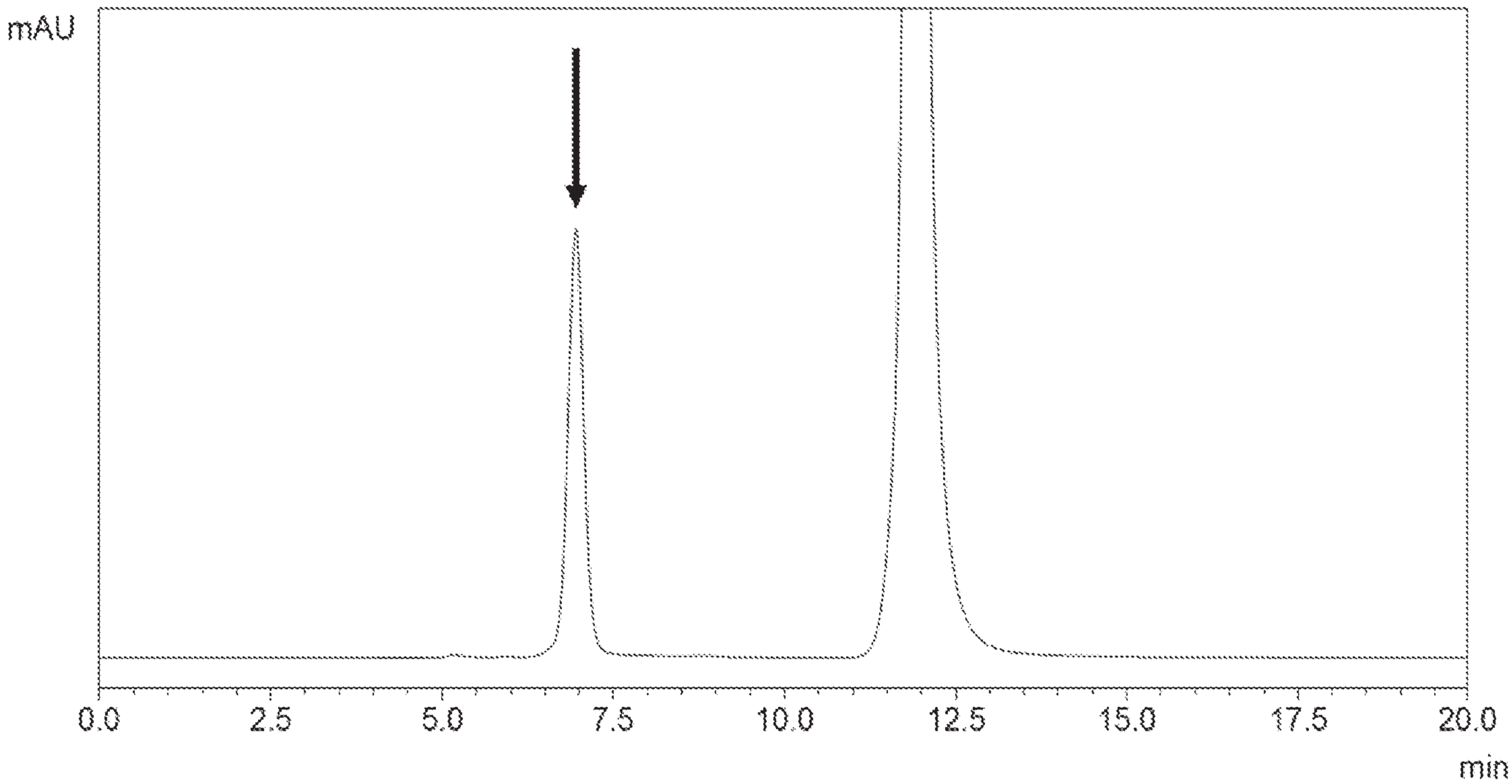


FIG. 85B

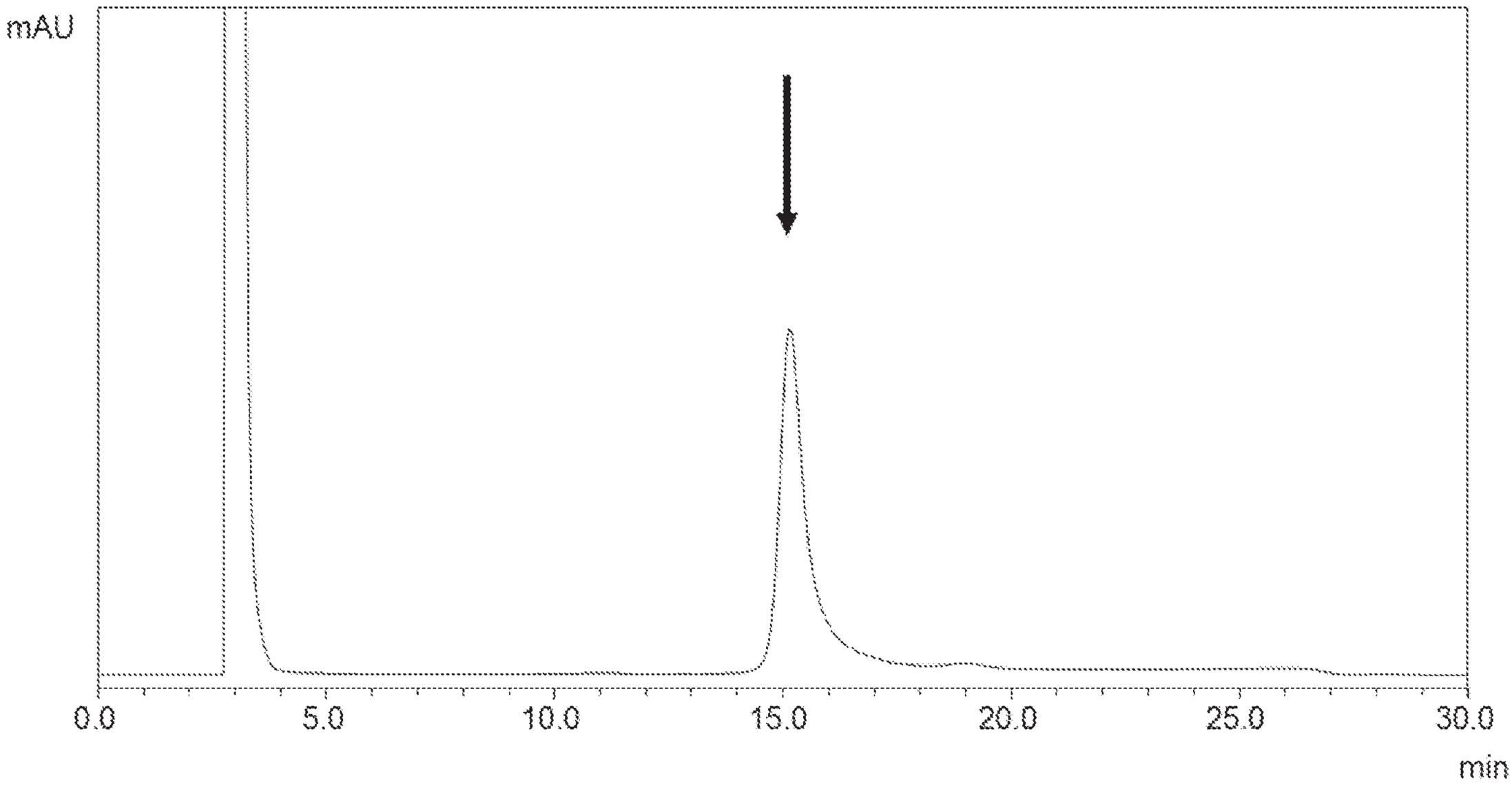


FIG. 85

FIG. 86A

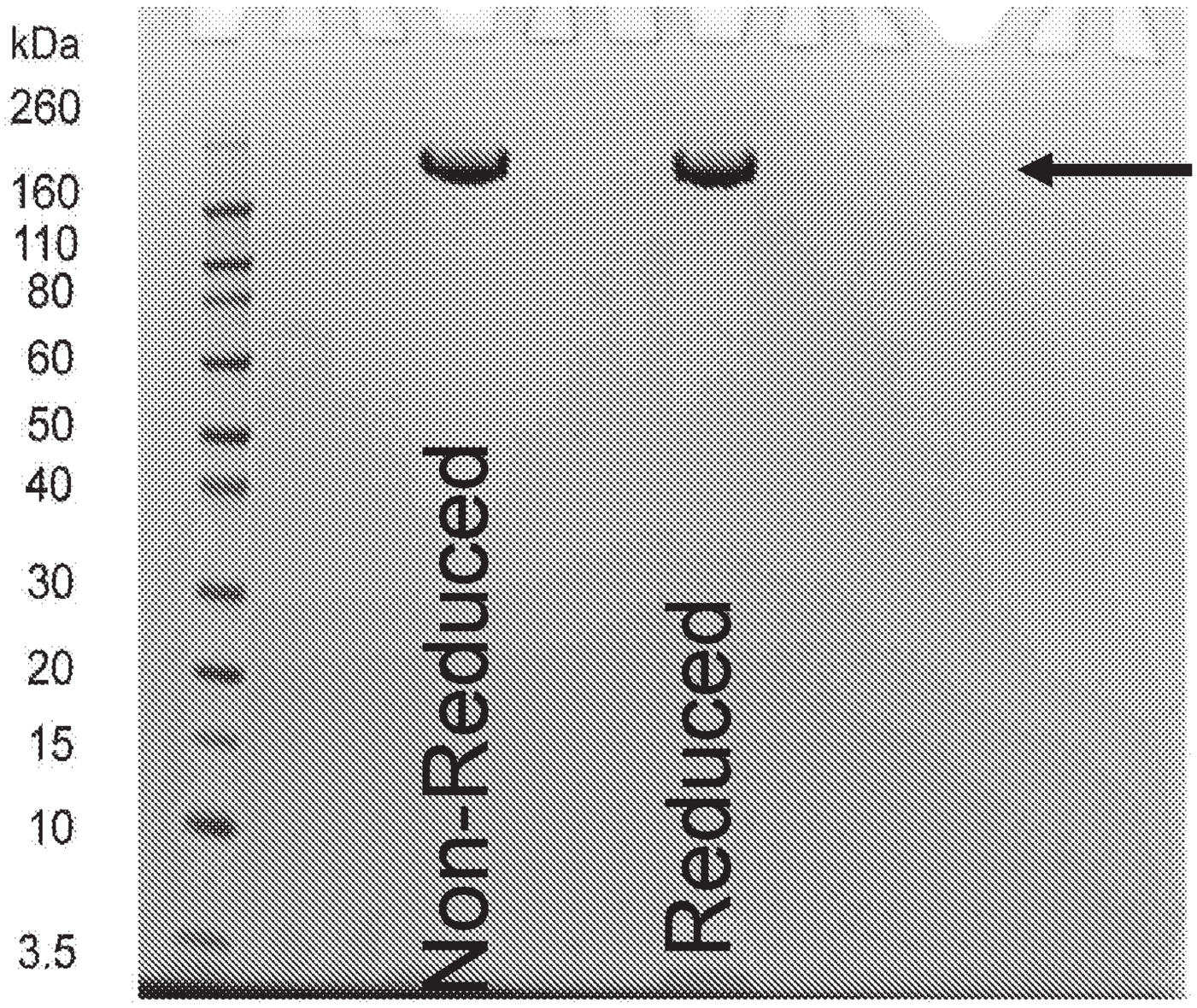


FIG. 86B

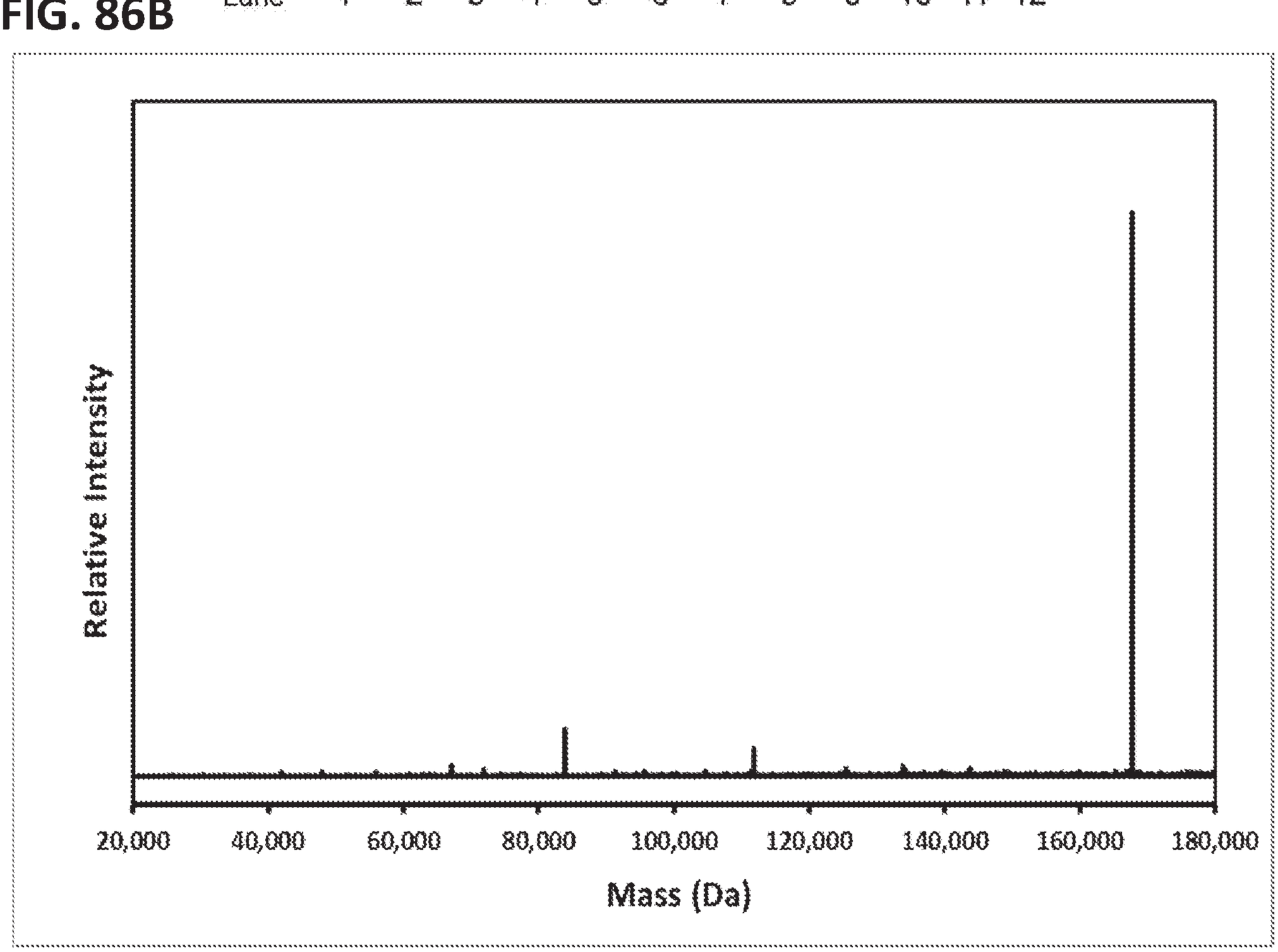


FIG. 86

FIG. 87A

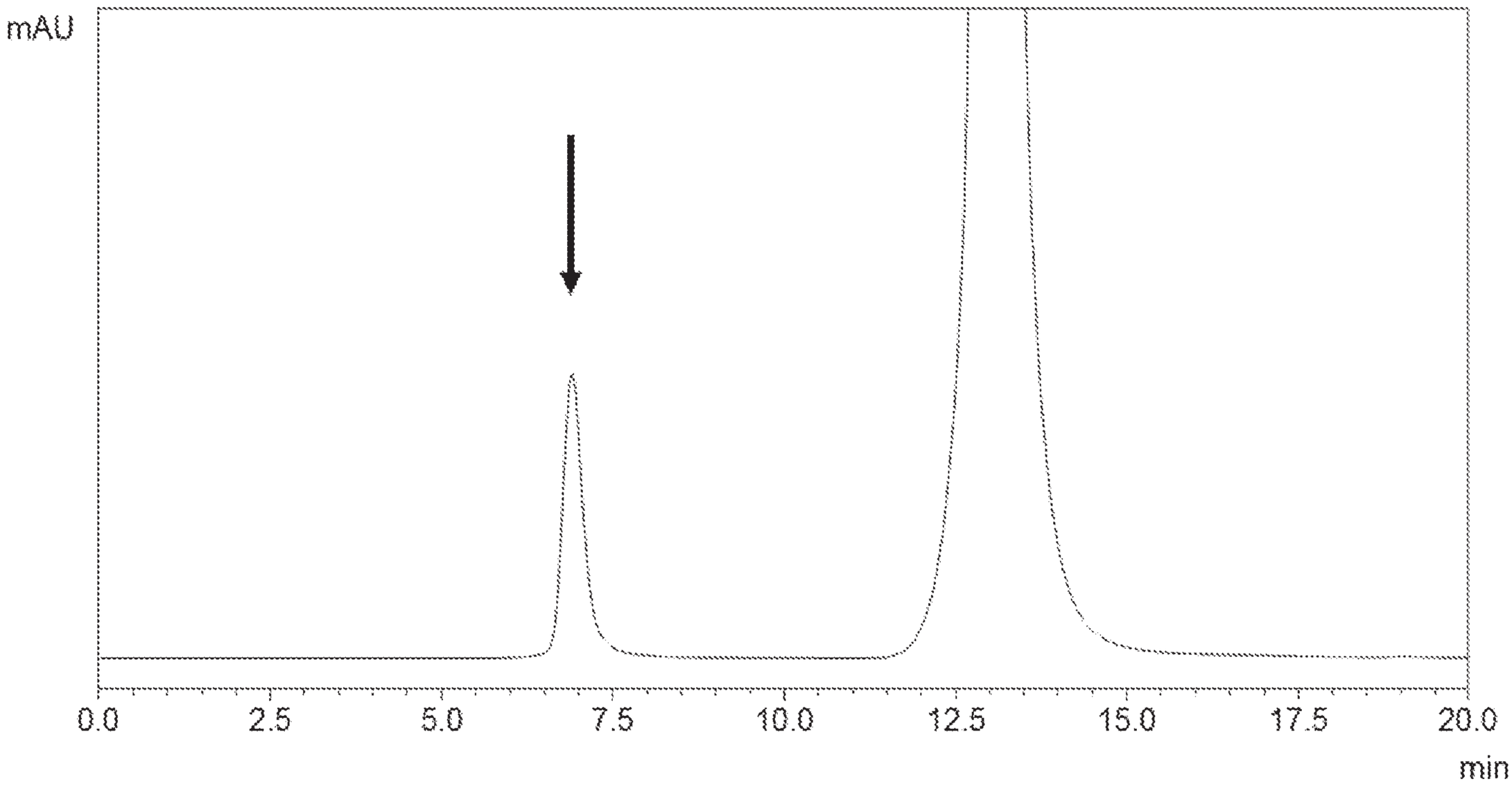


FIG. 87B

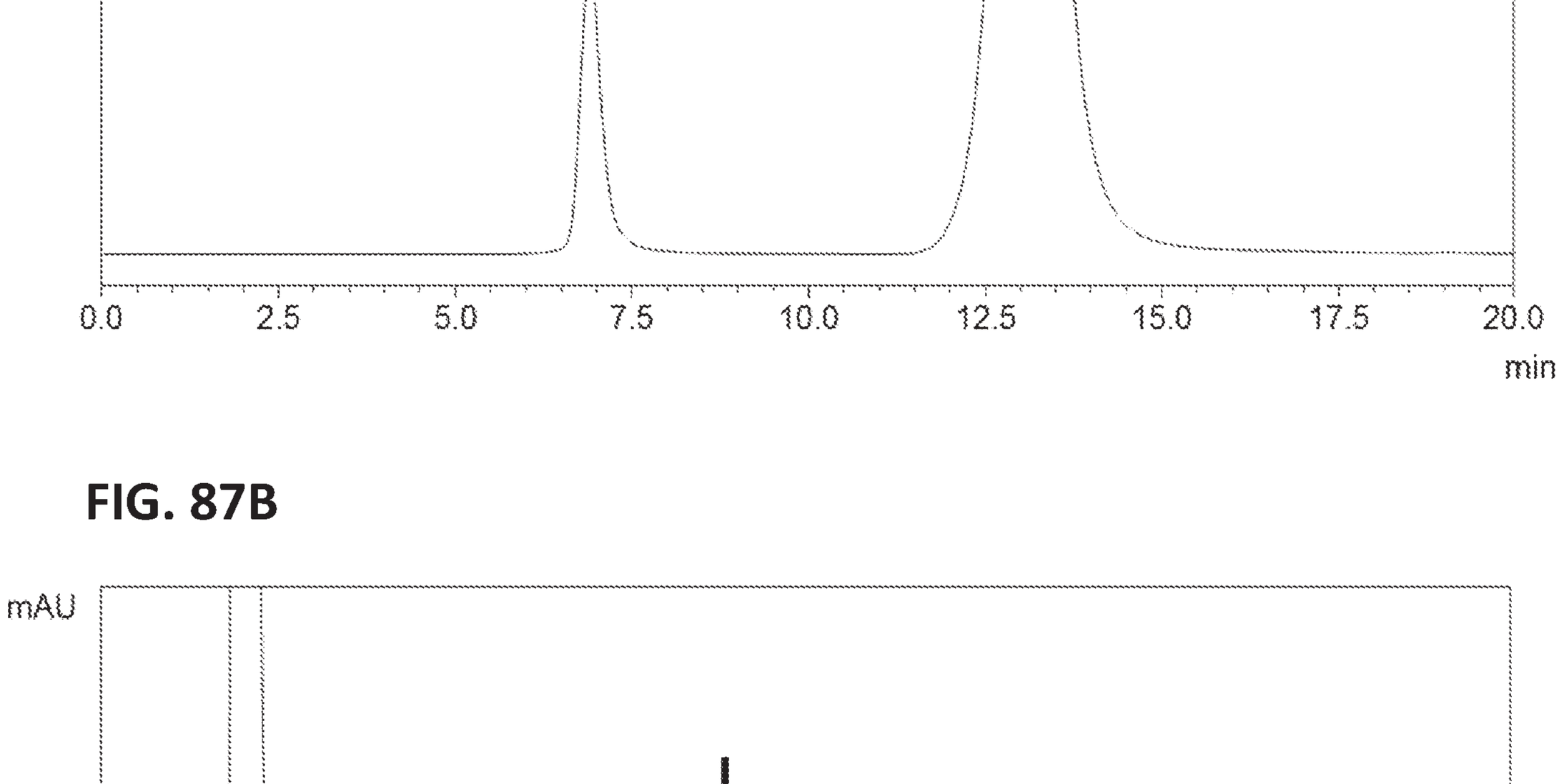


FIG. 87