Figure 1

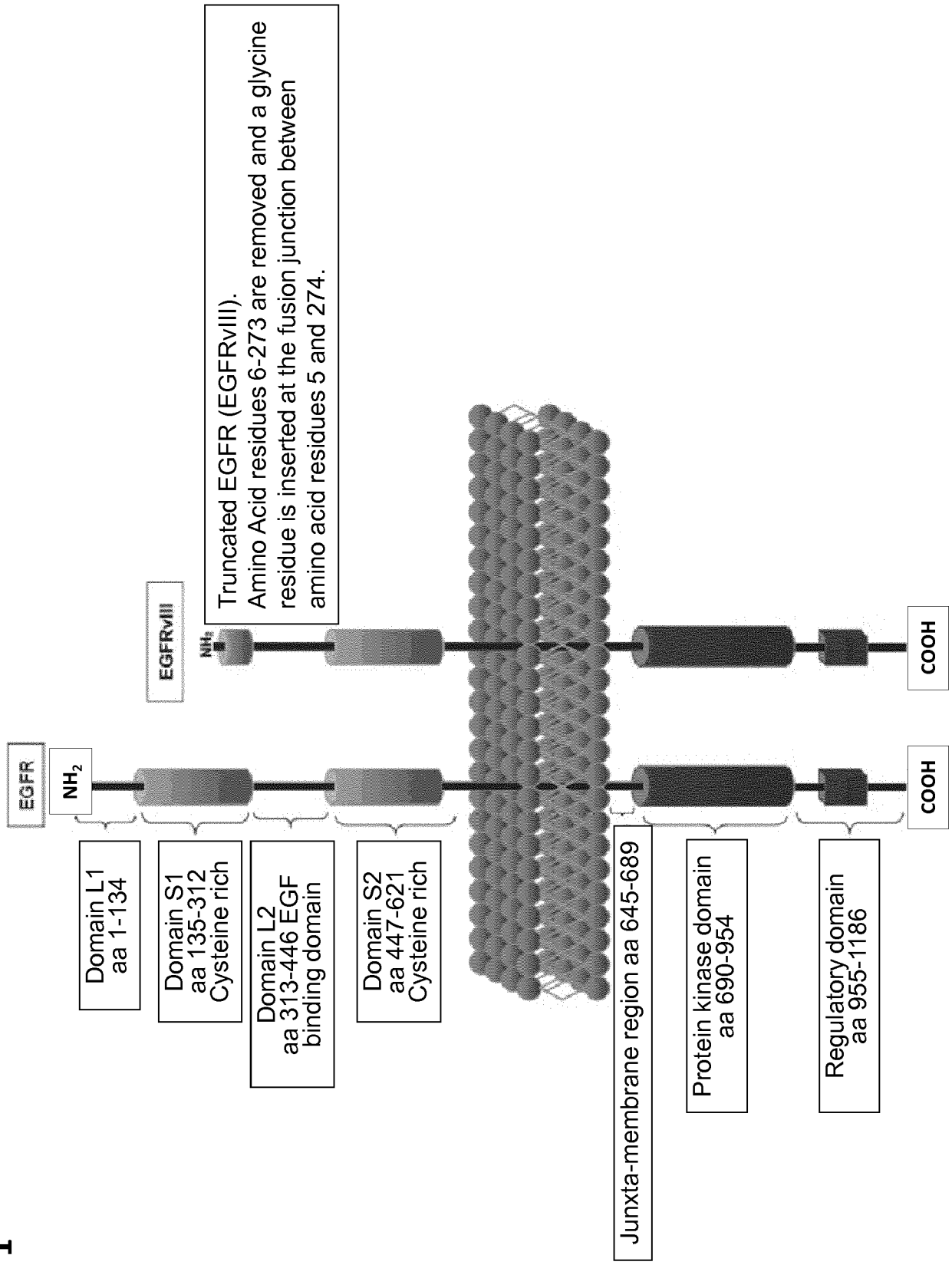

EGFR
NH2
EGFRvIII
NH2
Truncated EGFR (EGFRvIII).
Amino Acid residues 6-273 are removed and a glycine residue is inserted at the fusion junction between amino acid residues 5 and 274.
COOH
COOH
Domain L1 aa 1-134
Domain S1 aa 135-312 Cysteine rich
Domain L2 aa 313-446 EGF binding domain
Domain S2 aa 447-621 Cysteine rich
Junxta-membrane region aa 645-689
Protein kinase domain aa 690-954
Regulatory domain aa 955-1186

Figure 2

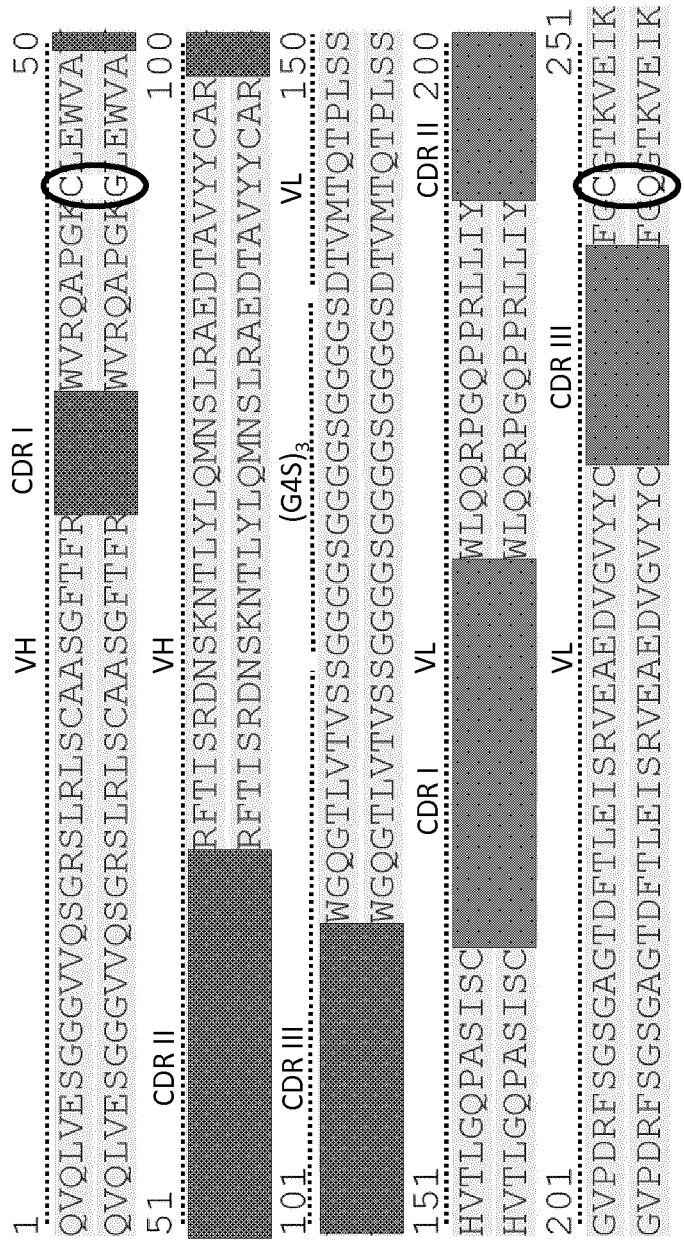
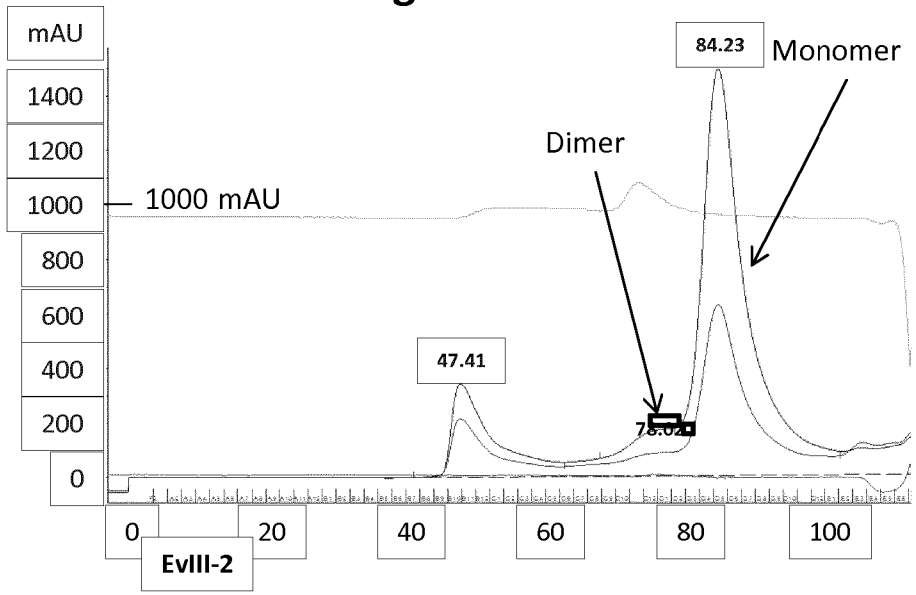
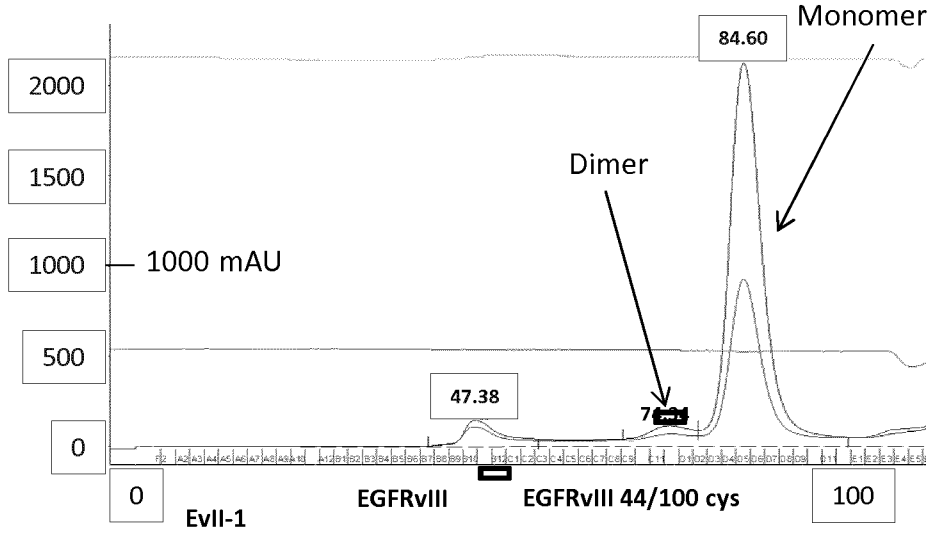

EvIII-1
EvIII-2
VH
CDR I
CDR II
CDR III
(G4S)3
VL
CDR I
CDR II
CDR III

Figure 3

SEC Chromatograms





Blue Line = OD280 nm
Red Line = OD254 nm

EGFRvIII BiTE	Monomer Yield [µg/l]	Monomer Percentage
EvIII-2	2503	90
EvIII-1	3671	93

Figure 4

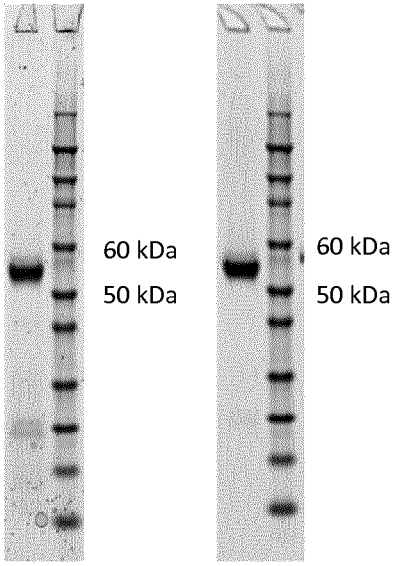


Figure 5

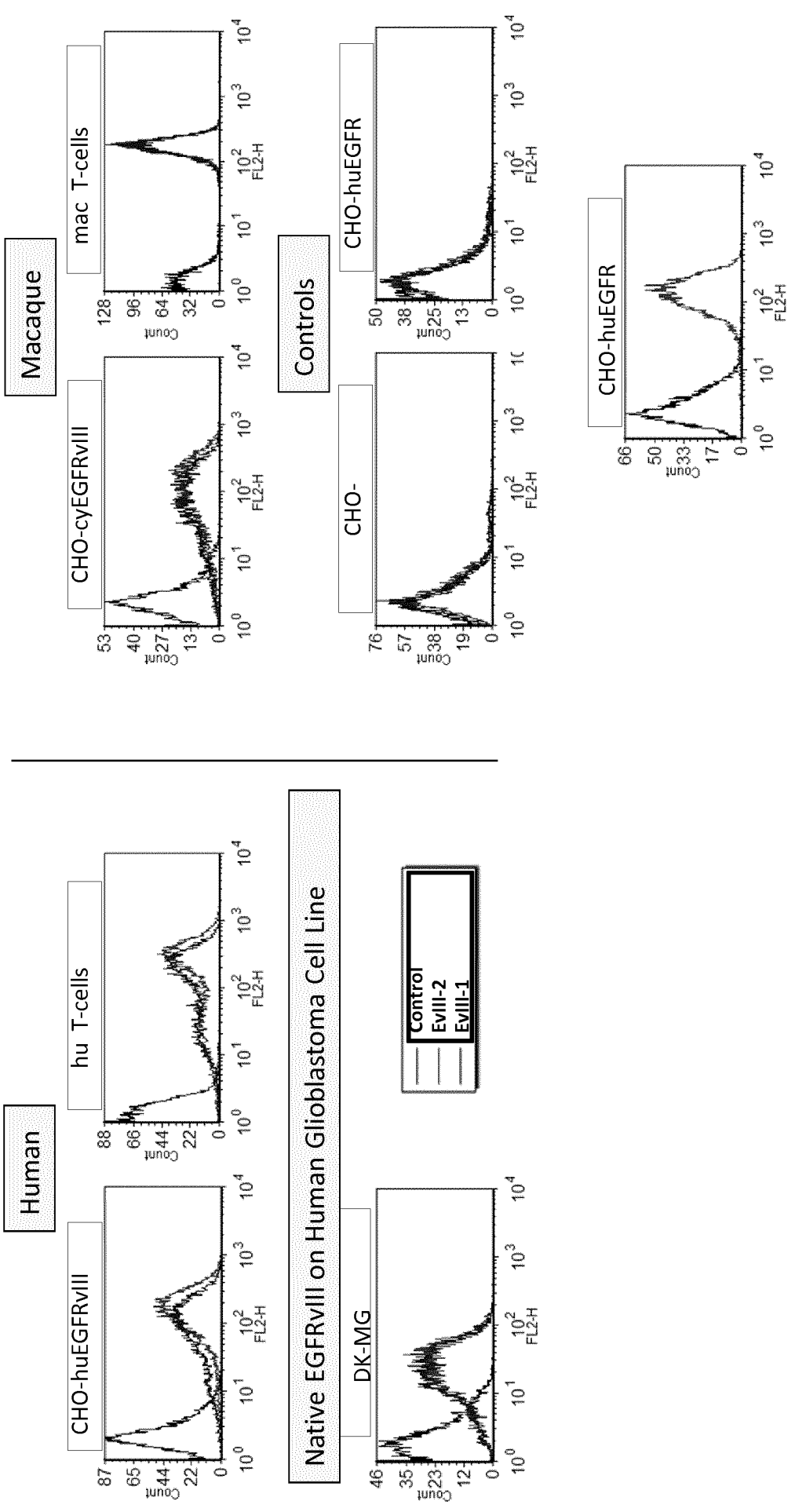

Human
Macaque
Controls
CHO-huEGFRvIII
hu T-cells
CHO-cyEGFRvIII
mac T-cells
CHO-
CHO-huEGFR
CHO-huEGFR
Native EGFRvIII on Human Glioblastoma Cell Line
DK-MG
Control
EvIII-2
EvIII-1
Count
FL2-H

Figure 6

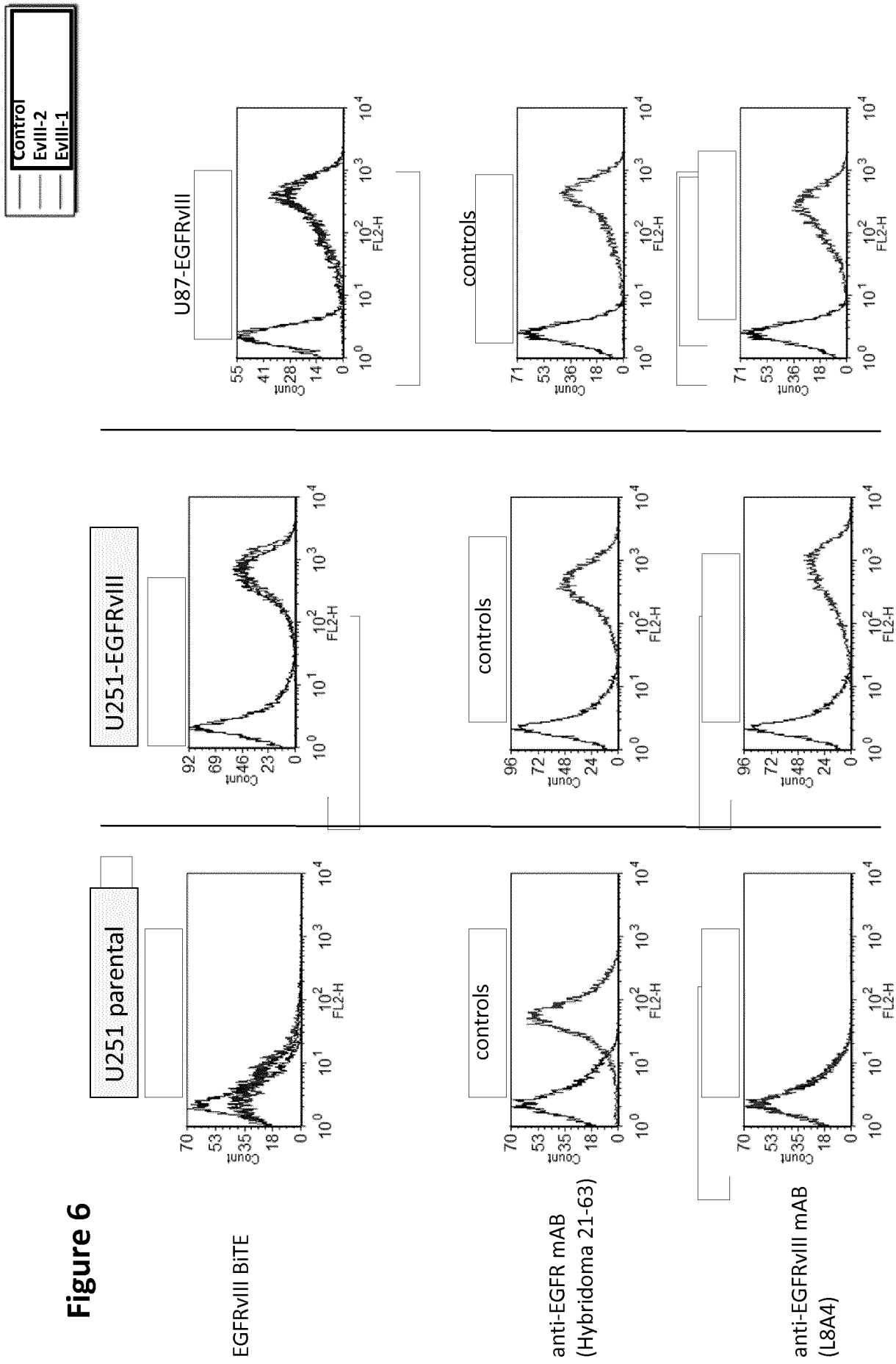
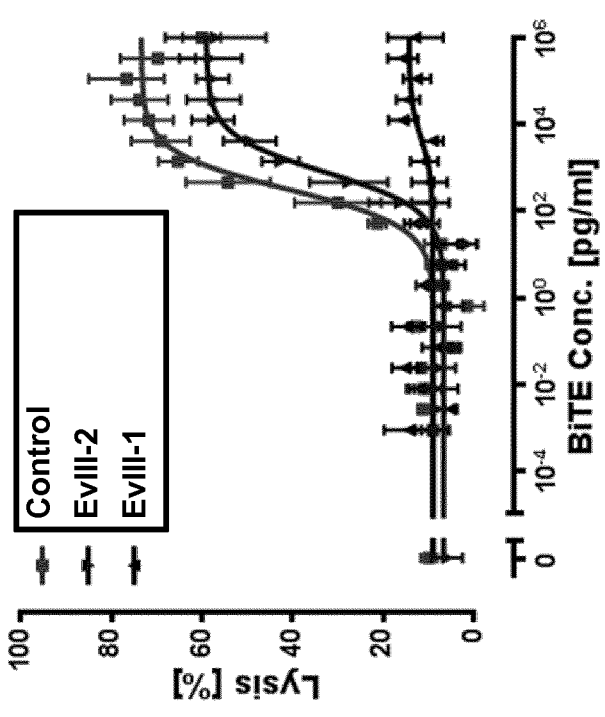

Control
EvIII-2
EvIII-1
U251 parental
U251-EGFRvIII
U87-EGFRvIII
controls
controls
controls
EGFRvIII BiTE
anti-EGFR mAB
(Hybridoma 21-63)
anti-EGFRvIII mAB
(L8A4)
Count
FL2-H

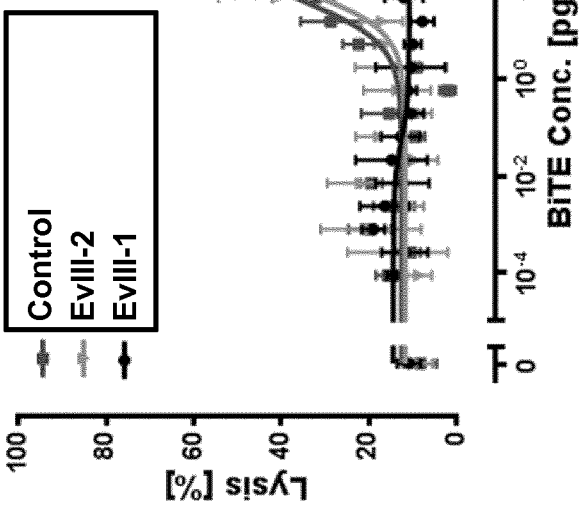
Figure 7



BiTE	EvIII-2	EvIII-1
EC50 [pg/ml]	317	783

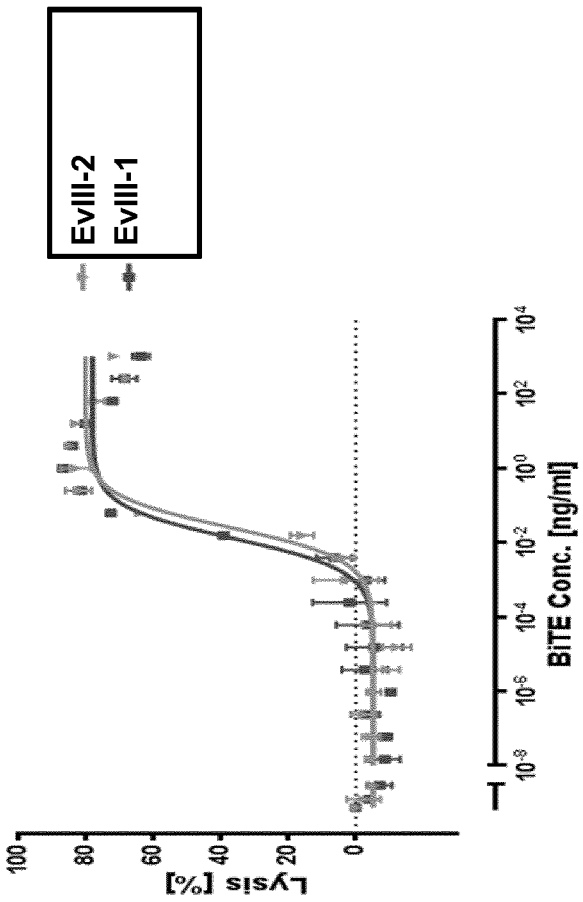
Figure 8

a)



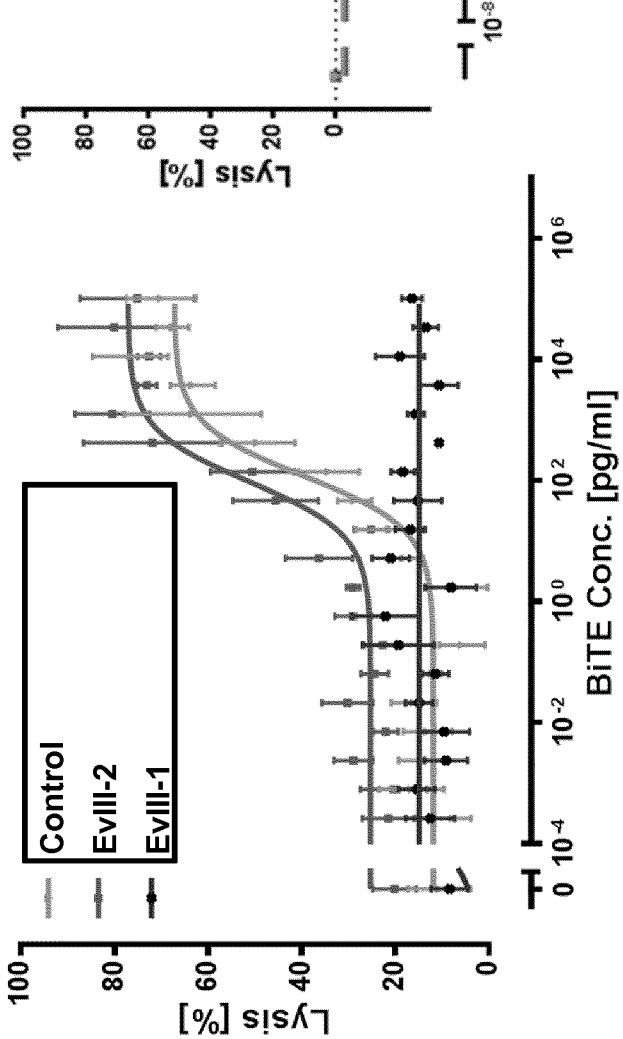
BiTE	EvIII-2	EvIII-1
EC50 [pg/ml]	39	86

b)



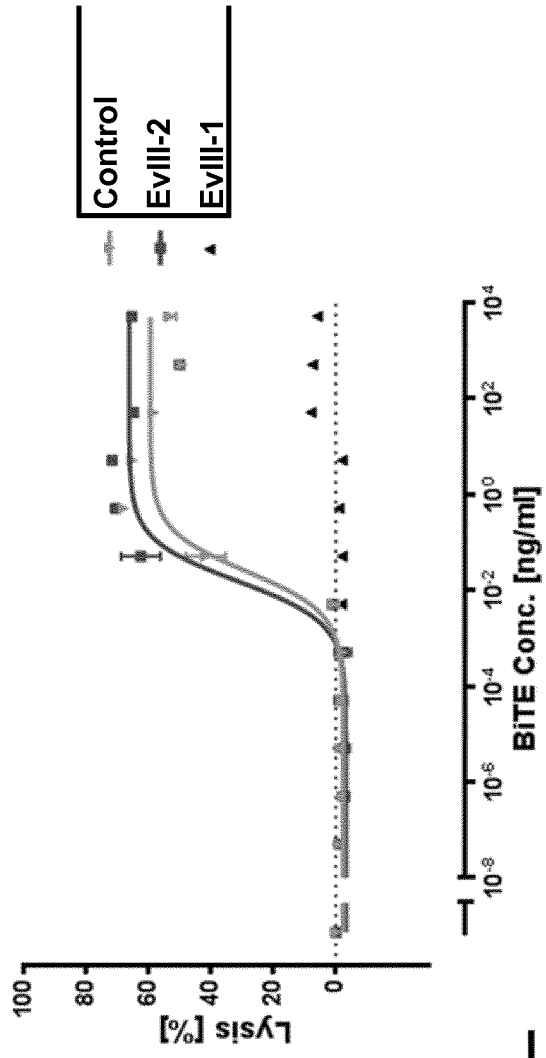
BiTE	EvIII-2	EvIII-1
EC50 [pg/ml]	13	25

Figure 9



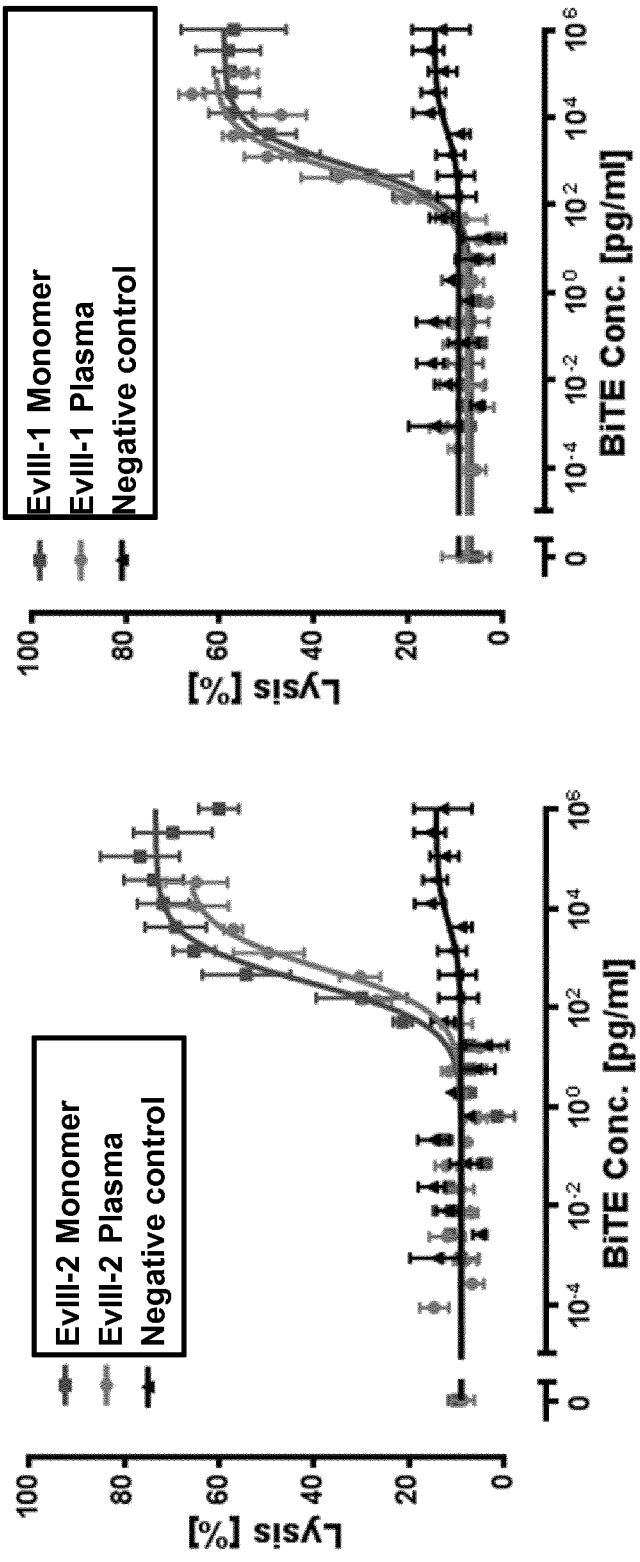
BiTE	EvIII-2	EvIII-1
EC50 [pg/ml]	94	117

Figure 10



BiTE	EvIII-2	EvIII-1
EC50 [pg/ml]	15	24

Figure 11



	EC_{50} [pg/mL]		Plasma to Control ratio (EC_{50} Plasma/EC_{50} Control)
	w/ Plasma	w/o Plasma	
EvIII-2	594	315	**1.89**
EvIII-1	586	783	**0.75**

Figure 12

EGFRvIII BiTE	AUC of Main Peak [%]
EvIII-2	100
EvIII-1	96,4

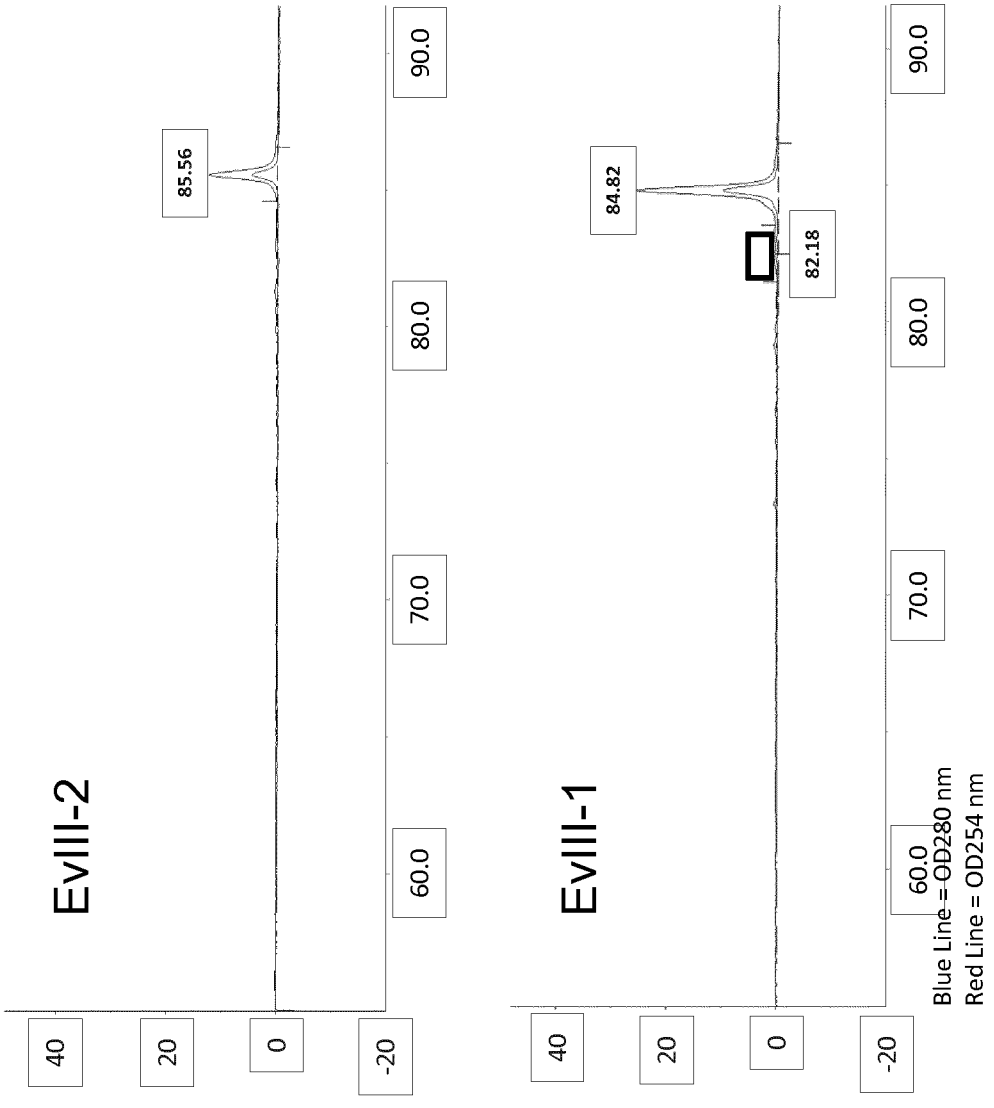


Figure 13

EGFRvIII BiTE	HIC Octyl Elution behavior
EvIII-2	3
EvIII-1	3

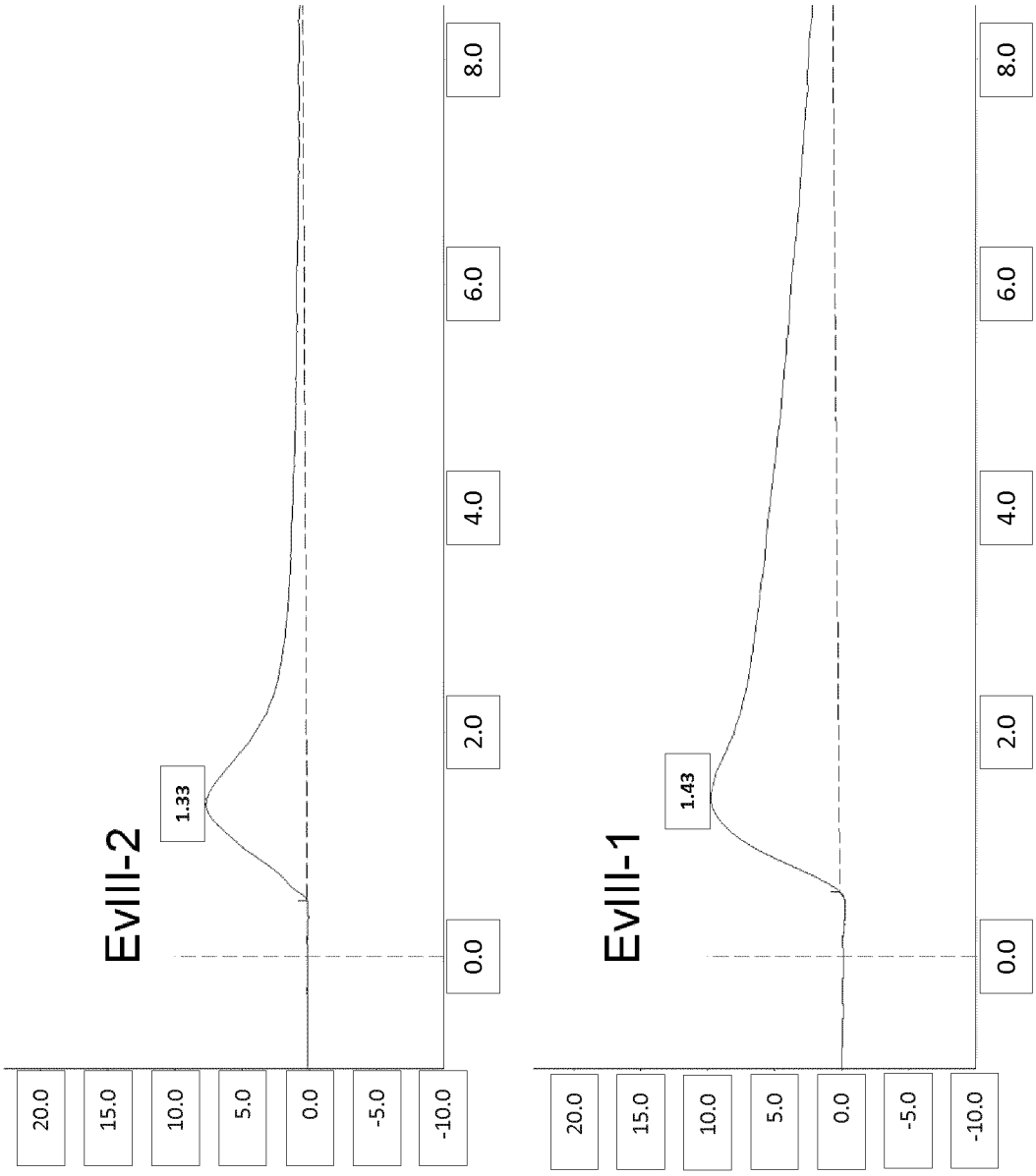


Figure 14

EGFRvIII BiTE	BiTE dimer [%]
EvIII-2	1.56
EvIII-1	0.26

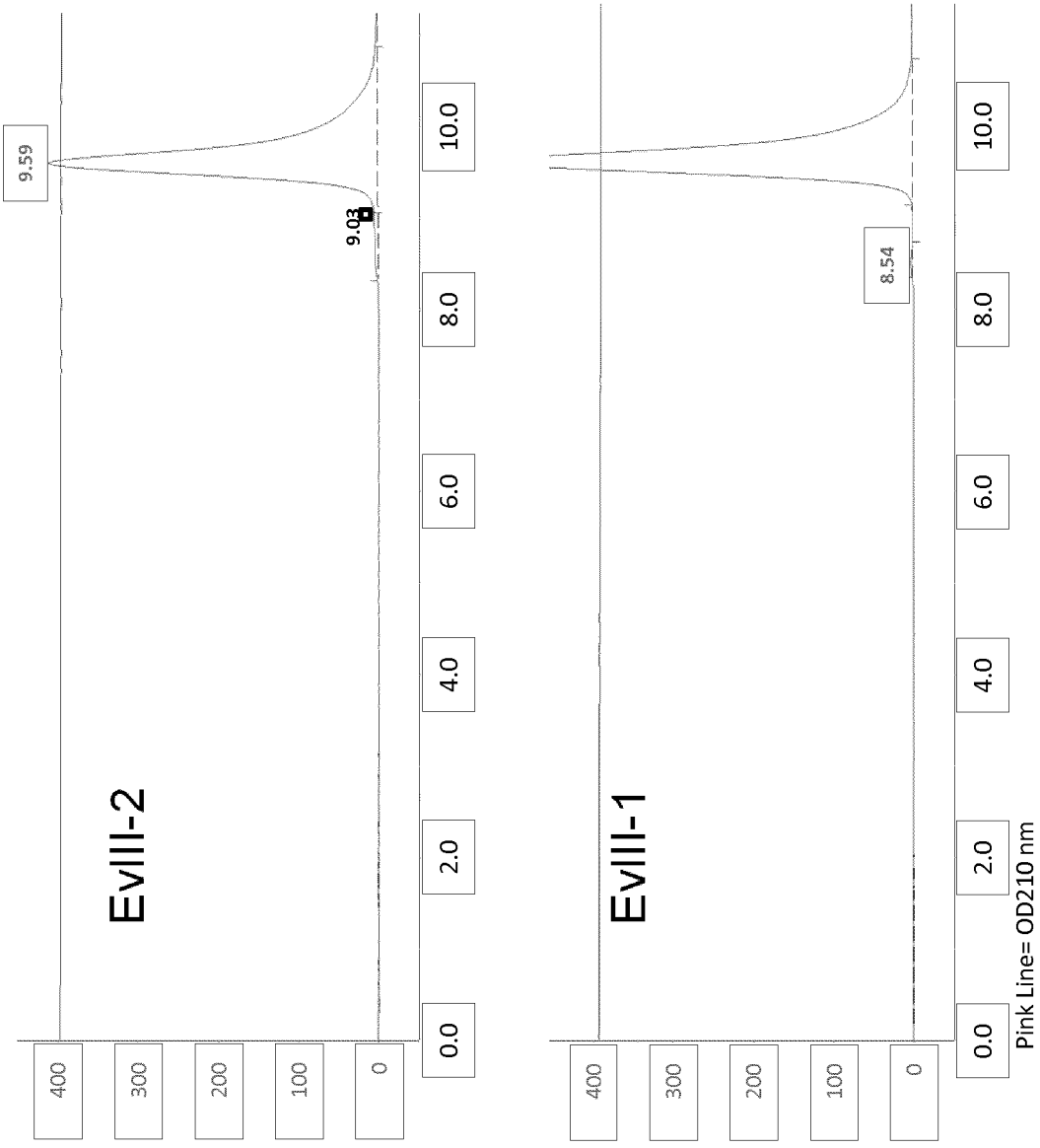
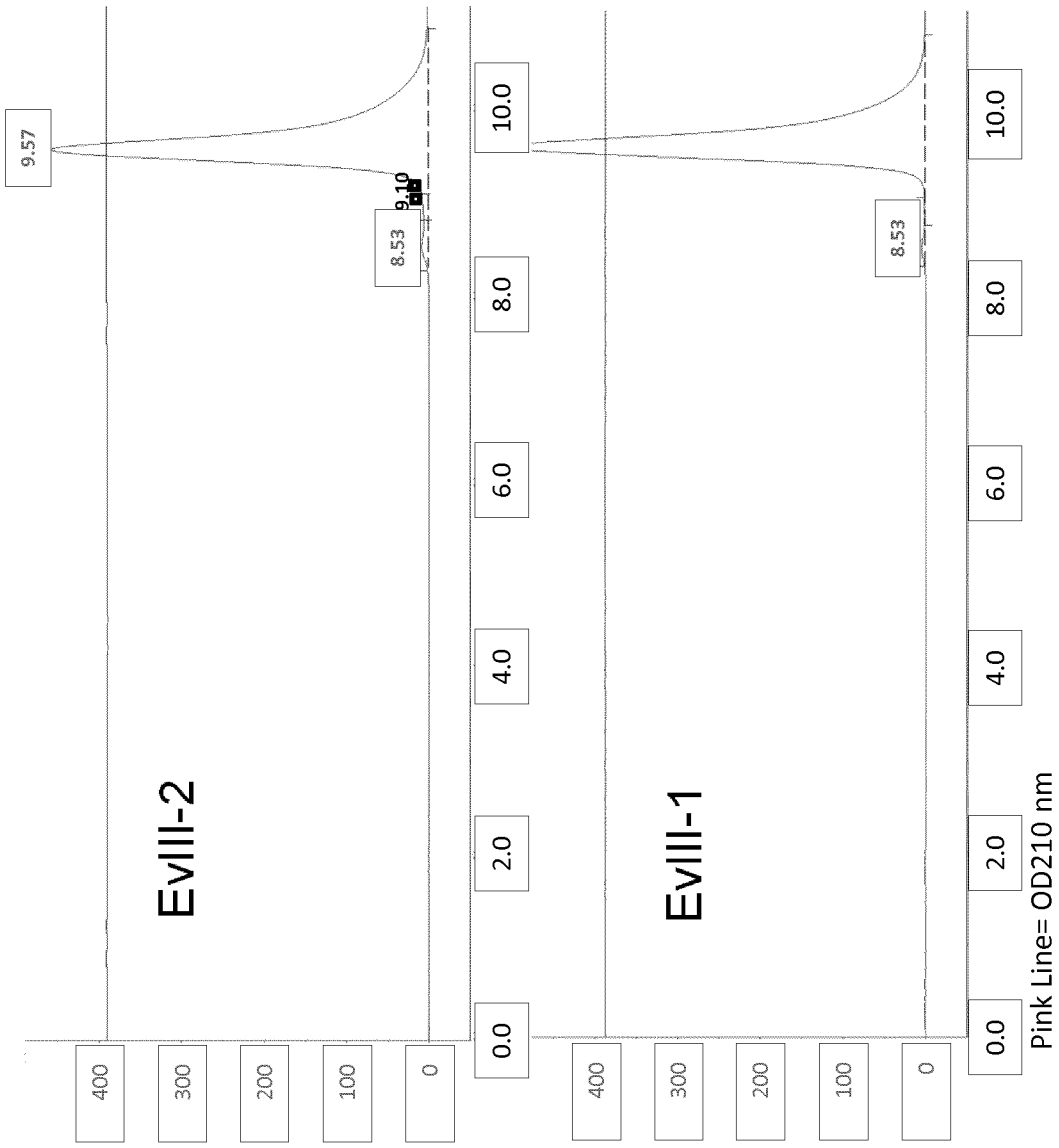


Figure 15

EGFRvIII BiTE	BiTE dimer [%]
EvIII-2	2.53
EvIII-1	0.59



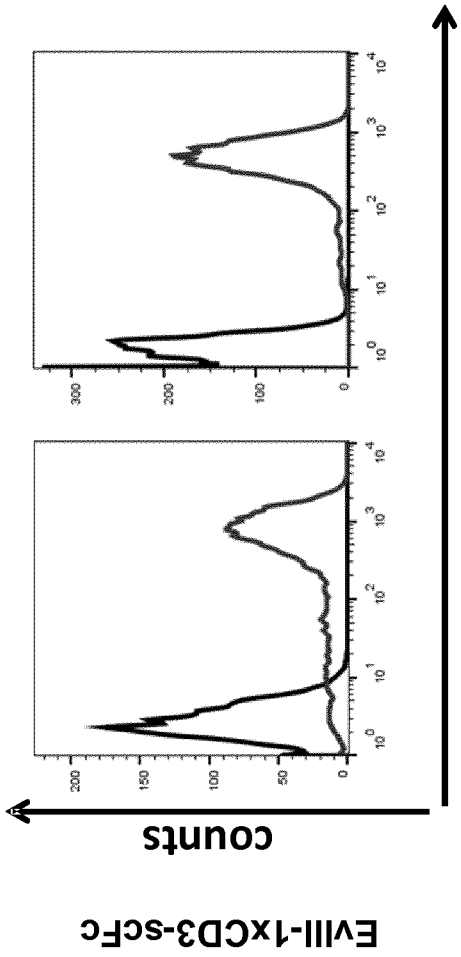


Figure 16

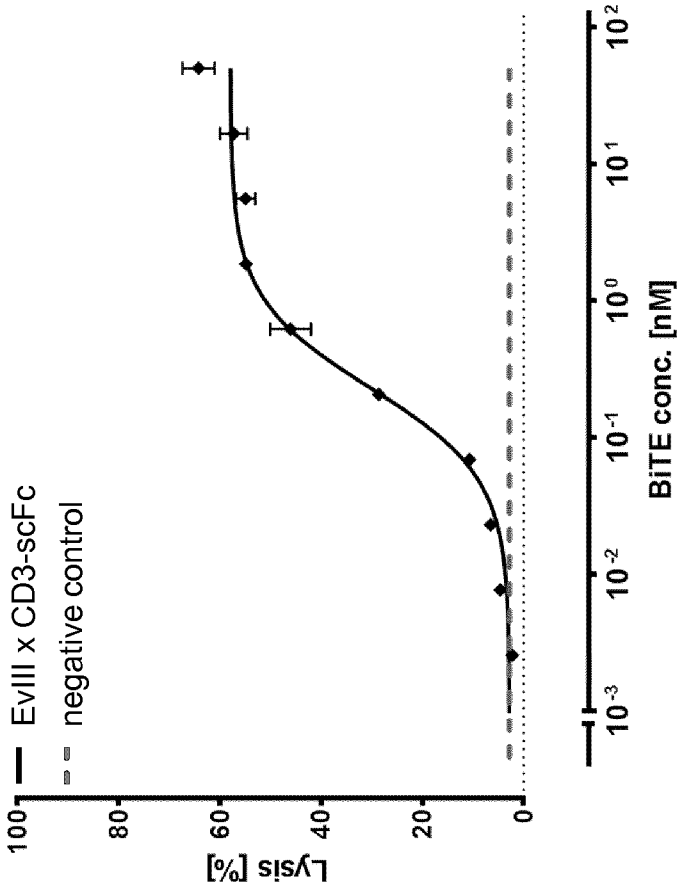


Figure 17