Figure 1
scFv C-9P21 Nucleotide sequence

CCATGGCC**CAGGTGCAGCTGCAGGAGTCCGGGGGAGGCTTAGTTCACCCTGGGGGGTCC**
NcoI |---------V_H Start (SEQ ID No.34 Start)

CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTACTGGATGCACTGGGT

CCGCCAAGCTCCAGGGAAGGGGCTGGTGTGGGTCTCACGTATTAATAGTGATGGGAGTA

GCACAAGCTACGCGGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAG

AACACGCTGTATCTGCAAATGAACAGTCTGAGAGCCGAGGACACGGCTGTGTATTACTG

TGCGAGAAAAATCTTGGGGGTGGGAGCTAGGTCTCGTCGTTACTTTGACTACTGGGGCC

AGGGAACAATGGTCACCGTCTCTTCA*AAGCTTTCAGGGAGTGCATCCGCCCCAAAACTT*
V_H End-----|HindIII--Linker Start
*GAAGAAGGTGAATTTTCAGAAGCACGCGTA***GAAACGACACTCACGCAGTCTCCAGGCAC**
Linker End ------ MluI--|------V_L Start
CCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTG

TCAGCAACTATTTAGCCTGGTACCAGCAGAAGCCTGGCCAGGCTCCCAGGCTCCTCATC

TCTGGTGCATCCAACAGGGCCACTGGCATCTCAGACAGGTTCAGTGGCAGTGGGTCTGG

GGCAGACTTCACTCTCACCATCAGCAGAGTCGAGCCTGAAGACTCAGCAGTGTATTACT

GTCAACAGTTTGATAAGTCCACGTGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA
(SEQ ID No. 34 End)V_LEnd-----|
GCGGCCGCTGGATCCGAACAAAAGCTGATCTCAGAAGAAGACCTAAACTCACATCACCA
NotI cMyc-tag
TCACCATCAC
His_6-tag

scFv C-9P21 Amino acid sequence

QVQLQESGGGLVHPGGSLRLSCAASGFTFSSYWMHWVRQAPGKGLVWVSRINSD
|---------V_H Start (SEQ ID No.35 Start)

GSSTSYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCARKILGVGARSR

RYFDYWGQGTMVTVSS*KLSGSASAPKLEEGEFSEARV***ETTLTQSPGTLSLSPGE**
V_H End-|----------Linker-------|------V_L Start
RATLSCRASQSVVSNYLAWYQQKPGQAPRLLISGASNRATGISDRFSGSGSGAD

FTLTISRVEPEDSAVYYCQQFDKSTWTFGQGTKVEIKAAAGSEQKLISEEDLNS
(SEQ ID No. 35 End)V_LEnd------|
HHHHHH

Figure 2
scFv B-1M22 Nucleotide sequence

CCATGGCC**CAGGTCCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCG**
NcoI |---------V_H Start (SEQ ID No.45 Start)

GTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGT

GCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTA

CAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACG

AGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTG

TGCGAGAGATCGGGAGAGATGGCTACAATCCGCGGGCGACTACTGGGGCCAGGGAACCC

TGGTCACTGTCTCCTCA*AAGCTTTCAGGGAGTGCATCCGCCCCAAAACTTGAAGAAGGT*
V_H End----|HindIII--Linker Start
*GAATTTTCAGAAGCACGCGTA***CAGCCTGTGCTGACTCAGTCACCCTCGGTGTCAGTGGC**
Linker End --- MluI---|------V_L Start
CCCAGGACAGACGGCCAGGATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGC

ACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCGTCTATGATGATAGCGAC

CGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCT

GACCATCAGCAGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATA

GTAGTAGTGATCATTGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGCGGCCGCT
(SEQ ID No. 45 End)V_LEnd ----| NotI
GGATCCGAACAAAAGCTGATCTCAGAAGAAGACCTAAACTCACATCACCATCACCATCAC
cMyc-tag His_6-tag

scFv B-1M22 Amino acid sequence

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPI
|---------V_H Start (SEQ ID No.46 Start)
FGTANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDRERWLQSAG

DYWGQGTLVTVSS*KLSGSASAPKLEEGEFSEARV***QPVLTQSPSVSVAPGQTARI**
V_H End- |----------Linker--- ---|------V_L Start
TCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTI

SRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVLAAAGSEQKLISEEDLNSHHH
(SEQ ID No. 46 End)V_LEnd------|
HHH

Figure 3
scFv C-1I24 Nucleotide sequence

CCATGGCC**CAGGTCCAGCTGGTACAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC**
NcoI |---------V_H Start (SEQ ID No.56 Start)

CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGT

CCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTA

ATAAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAG

AACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTG

TGCGAAAGATCTTCCGATTACCCGCGGGACAGGGGCTGACTACTGGGGCCAGGGAACCC

TGGTCACTGTCTCCTCA*AAGCTTTCAGGGAGTGCATCCGCCCCAAAACTTGAAGAAGGT*
V_H End----|HindIII--Linker Start
*GAATTTTCAGAAGCACGCGTA***CAGTCTGTCCTGATTCAGCCTGCCTCCGTGTCTGGGTC**
Linker End ---- MluI---|------V_L Start
TCCTGGACAGTCGATCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATA

ACTATGTCTCCTGGTATCAACAACACCCAGGCAAAGCCCCCAGACTCATGATTTACGAT

GTCACTAGTCGGCCCTCAGGGGTTTCGAATCGCTTCTCTGGCTCCAAGTCTGGCAACAC

GGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGTT

CATATGCAGGCAGCTACAGCGTGGTATTCGGCGGAGGGACCAAGGTCACCGTCCTAGCG
(SEQ ID No. 56 End)V_LEnd----|NotI
GCCGCTGGATCCGAACAAAAGCTGATCTCAGAAGAAGACCTAAACTCACATCACCATCA
NotI cMyc-tag His_6-tag
CCATCAC

scFv C-1I24 Amino acid sequence

QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYD
|---------V_H Start (SEQ ID No.57 Start)
GSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDLPITRGTGA

DYWGQGTLVTVSS*KLSGSASAPKLEEGEFSEARV***QSVLIQPASVSGSPGQSITI**
V_H End|----------Linker--------|------V_L Start
SCTGTSSDVGGYNYVSWYQQHPGKAPRLMIYDVTSRPSGVSNRFSGSKSGNTAS

LTISGLQAEDEADYYCSSYAGSYSVVFGGGTKVTVLAAAGSEQKLISEEDLNSH
(SEQ ID No. 57 End)V_LEnd------|
HHHHH

Figure 4
scFv D-1K21 Nucleotide sequence

CCATGGCC**CAGGTCCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCA**
NcoI |---------V_H Start (SEQ ID No.67 Start)

GTGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACTGGGT

GCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGACGGATCAACCCTAACAGTGGTG

GCACAAACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACACGTCCATC

AGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACGACACGGCCGTGTATTACTG

CGCGAGGCGTAACCTGATAGCAGCTCGTCCCCGGAATCGGGGCAGGGATGCTTTTGATA

TCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCA*AAGCTTTCAGGGAGTGCATCCGCC*
V_H End-----|HindIII--Linker Start
*CCAAAACTTGAAGAAGGTGAATTTTCAGAAGCACGCGTA***GACATCCAGATGACCCAGTC**
Linker End ------- MluI--|------V_L Start
TCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTC

AGAGTATTGGTGGCTCGTTGGCCTGGTATCAGCAGAAACCAGGGAAAGGCCCTAACCTC

CTGATCTATGCTGCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGG

ATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTCTGCAACTT

ACTACTGCCAACACTATGAAAGTTATCCCCTCTCTTTCGGCGGCGGGACCAAGCTGGAG
(SEQ ID No. 67 End)V_LEnd-----
ATCAAAGCGGCCGCTGGATCCGAACAAAAGCTGATCTCAGAAGAAGACCTAAACTCACA
------| NotI cMyc-tag
TCACCATCACCATCAC
His_6-tag

scFv D-1K21 Amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGRINPN
|---------V_H Start (SEQ ID No.68 Start)

SGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARRNLIAARPRN

RGRDAFDIWGQGTMVTVSS*KLSGSASAPKLEEGEFSEARV***DIQMTQSPSTLSAS**
V_H End- |----------Linker-------|------V_L Start
VGDRVTITCRASQSIGGSLAWYQQKPGKGPNLLIYAASTLQSGVPSRFSGSGSG

TEFTLTISSLQPEDSATYYCQHYESYPLSFGGGTKLEIKAAAGSEQKLISEEDL
(SEQ ID No. 68 End)V_LEnd------|
NSHHHHHH

Figure 5
scFv 9N10 Nucleotide sequence

CCATGGCC**CAGGTGCAGCTGCAGGAGTCCGGGGGAGGCTTAGTTCACCCTGGGGGGTCC**
NcoI |---------V_H Start (SEQ ID No.100 Start)

CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTACTGGATGCACTGGGT

CCGCCAAGCTCCAGGGAAGGGGCTGGTGTGGGTCTCACGTATTAATAGTGATGGGAGTA

GCACAAGCTACGCGGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAG

AACACGCTGTATCTGCAAATGAACAGTCTGAGAGCCGAGGACACGGCTGTGTATTACTG

TGCGAGAAAAATCTTGGGGGTGGGAGCTAGGTCTCGTCGTTACTTTGACTACTGGGGCC

AGGGAACAATGGTCACCGTCTCTTCA*AAGCTTTCAGGGAGTGCATCCGCCCCAAAACTT*
V_H End----|HindIII--Linker Start
*GAAGAAGGTGAATTTTCAGAAGCACGCGTA***GATATTGTGCTGACCCAGACTCCAGACTC**
Linker End ----- MluI--|------V_L Start
CCTGGCTGTGTCTCTGGGCGAGACGACCACCATCAACTGCAAGTCCAGCCAGAGTGTTT

TACACAGCTCCAACAATAAGAACTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCT

CCTAAGCTGCTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAG

TGGCAGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAACCTGCAGCCTGAAGATG

TGGCTTTTTACTACTGTCTGCAATATTCTACTTTTCCTCGGACGTTCGGCCAAGGGACC
(SEQ ID No. 100----
AAGGTGGAGATCAAAGCGGCCGCTGGATCCGAACAAAAGCTGATCTCAGAAGAAGACCT
-----End)V_LEnd----| NotI cMyc-tag
AAACTCACATCACCATCACCATCAC
His_6-tag

scFv 9N10 Amino acid sequence

QVQLQESGGGLVHPGGSLRLSCAASGFTFSSYWMHWVRQAPGKGLVWVSRINSD
|---------V_H Start (SEQ ID No.101 Start)
GSSTSYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCARKILGVGARSR

RYFDYWGQGTMVTVSS*KLSGSASAPKLEEGEFSEARV***DIVLTQTPDSLAVSLGE**
V_H End-|-----------Linker-------|------V_L Start
TTTINCKSSQSVLHSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGS

GSGTDFTLTISNLQPEDVAFYYCLQYSTFPRTFGQGTKVEIKAAAGSEQKLISE
(SEQ ID No. 101 End)V_LEnd-----|
EDLNSHHHHHH

Figure 6A

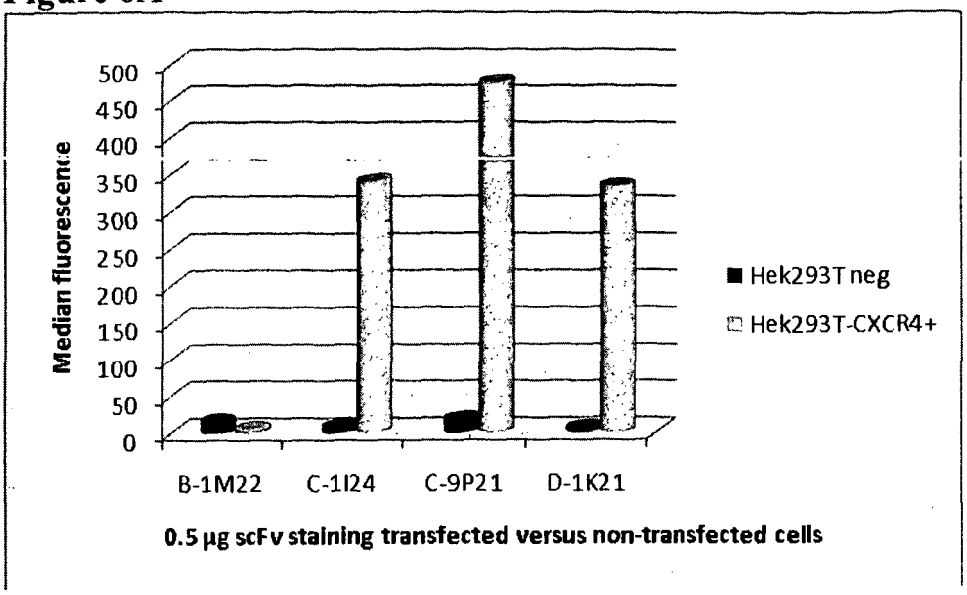


Figure 6B

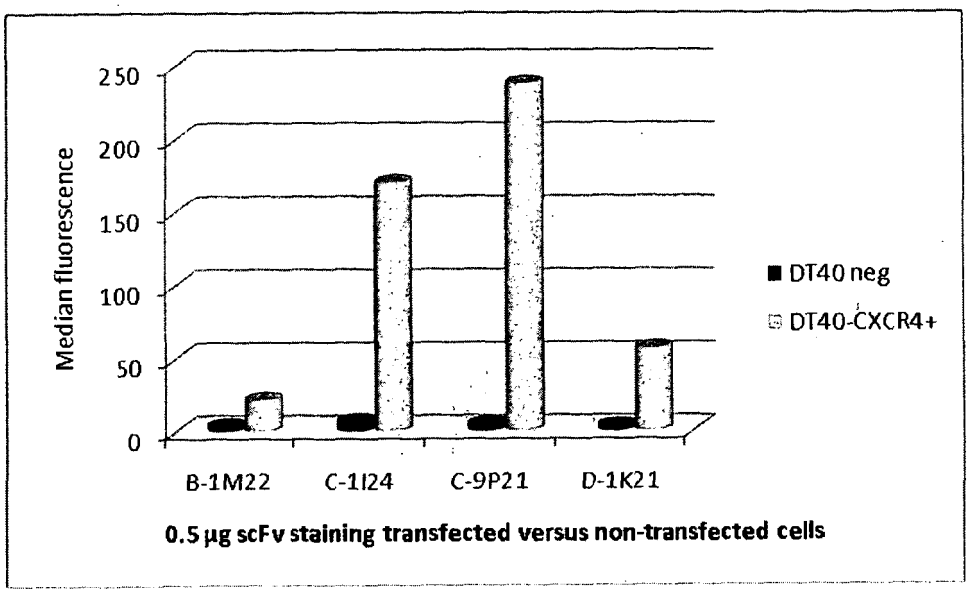


Figure 6C

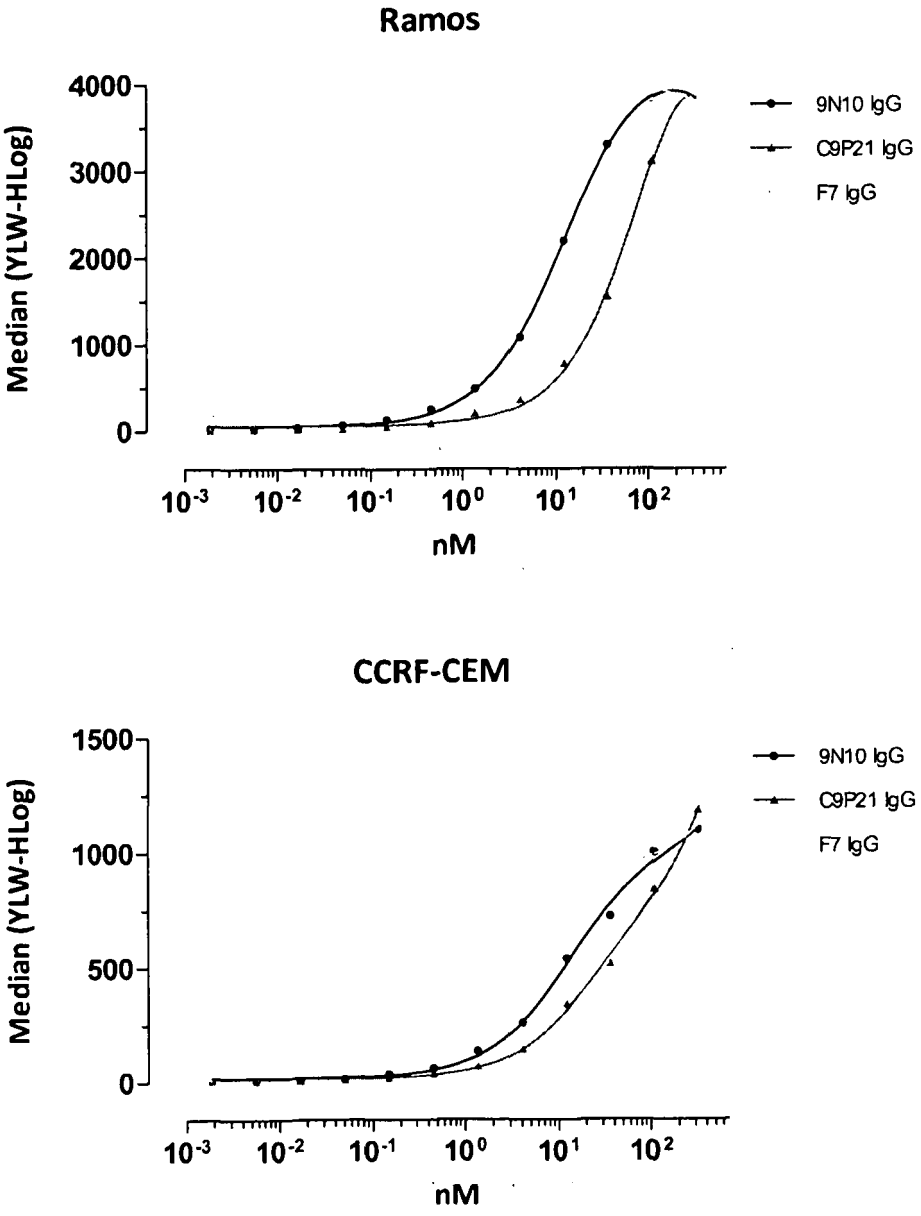

Ramos
Median (YLW-HLog)
4000
3000
2000
1000
0
10-3
10-2
10-1
100
101
102
nM
9N10 IgG
C9P21 IgG
F7 IgG
CCRF-CEM
Median (YLW-HLog)
1500
1000
500
0
10-3
10-2
10-1
100
101
102
nM
9N10 IgG
C9P21 IgG
F7 IgG

Figure 7A

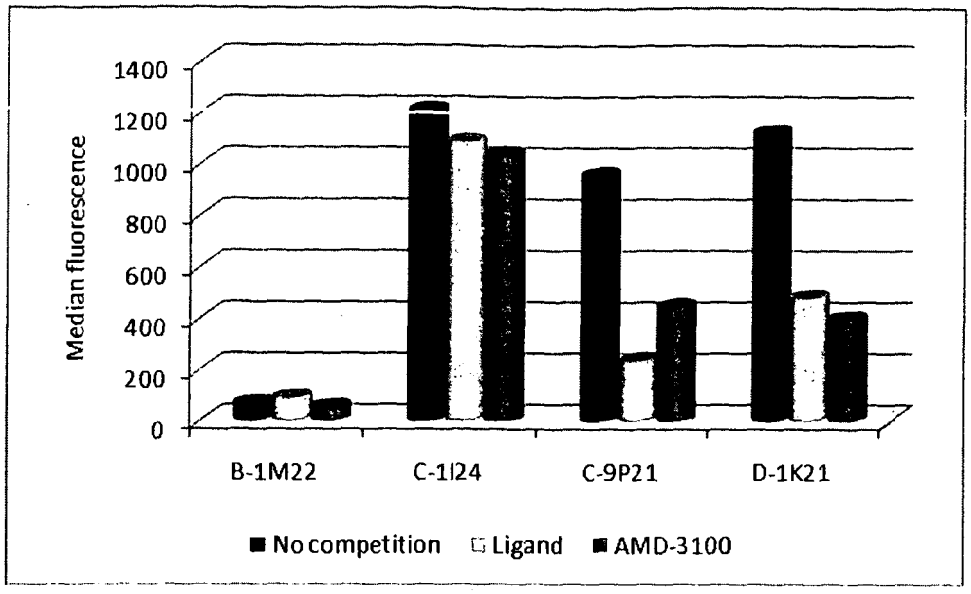


Figure 7B

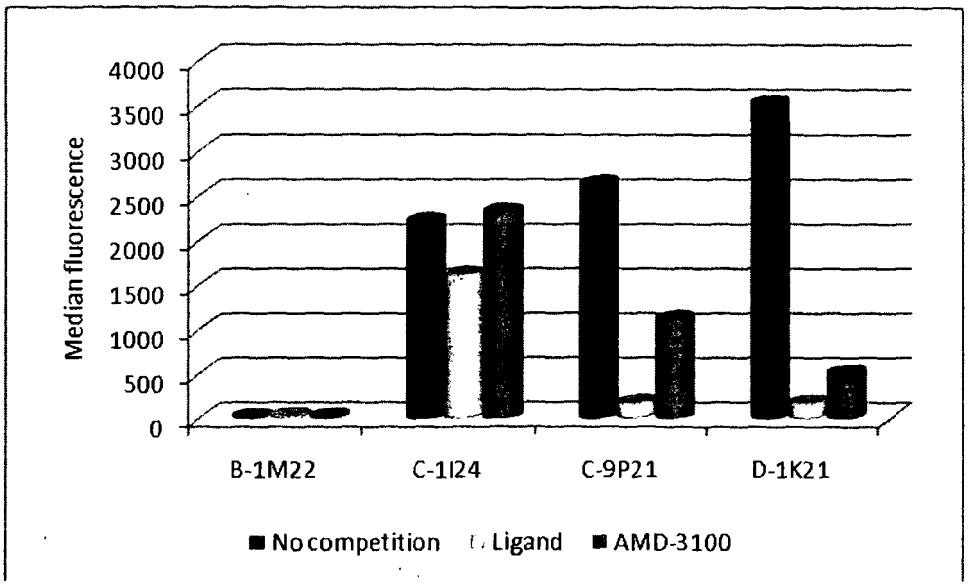
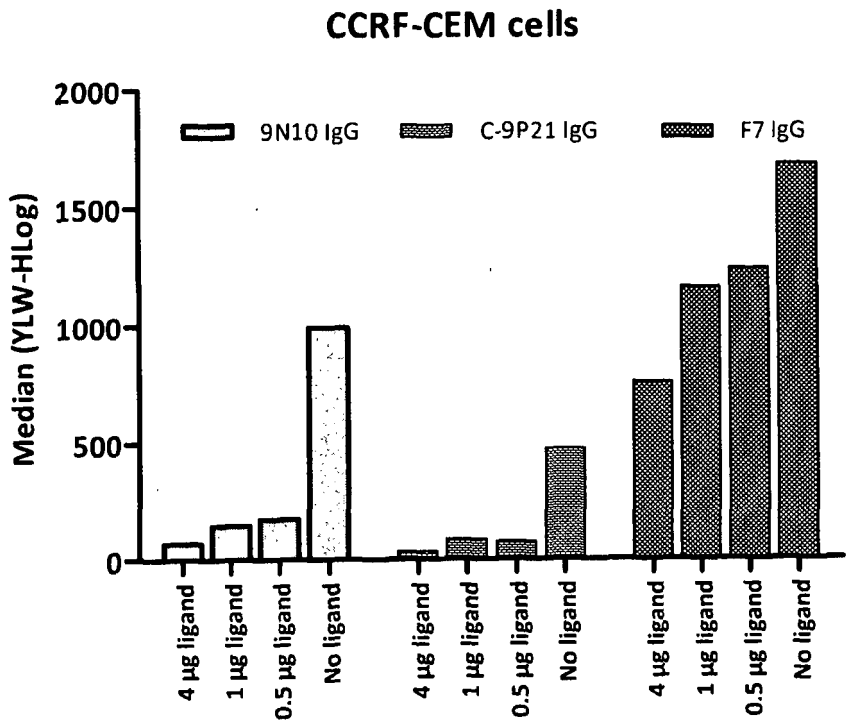


Figure 7C


CCRF-CEM cells
9N10 IgG
C-9P21 IgG
F7 IgG
Median (YLW-HLog)
2000
1500
1000
500
0
4 µg ligand
1 µg ligand
0.5 µg ligand
No ligand
4 µg ligand
1 µg ligand
0.5 µg ligand
No ligand
4 µg ligand
1 µg ligand
0.5 µg ligand
No ligand

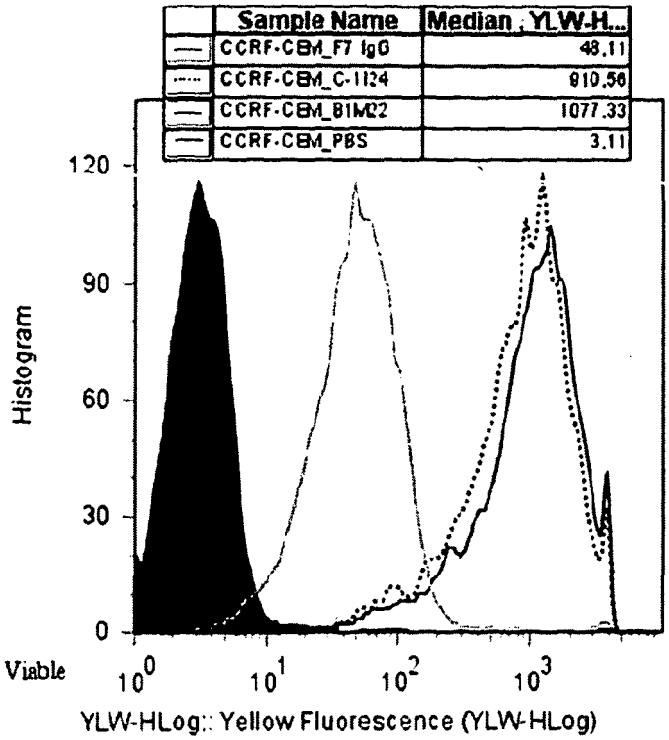


Figure 8A

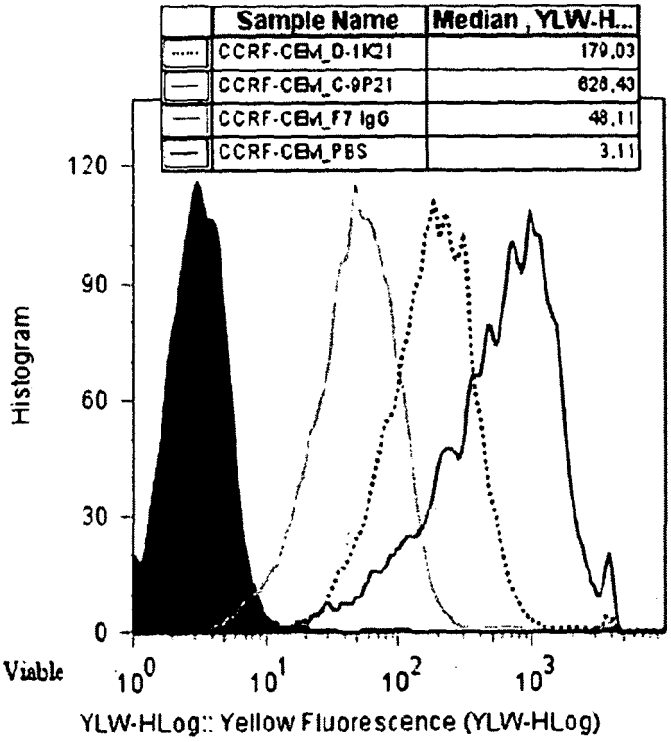


Figure 8B

Figure 9

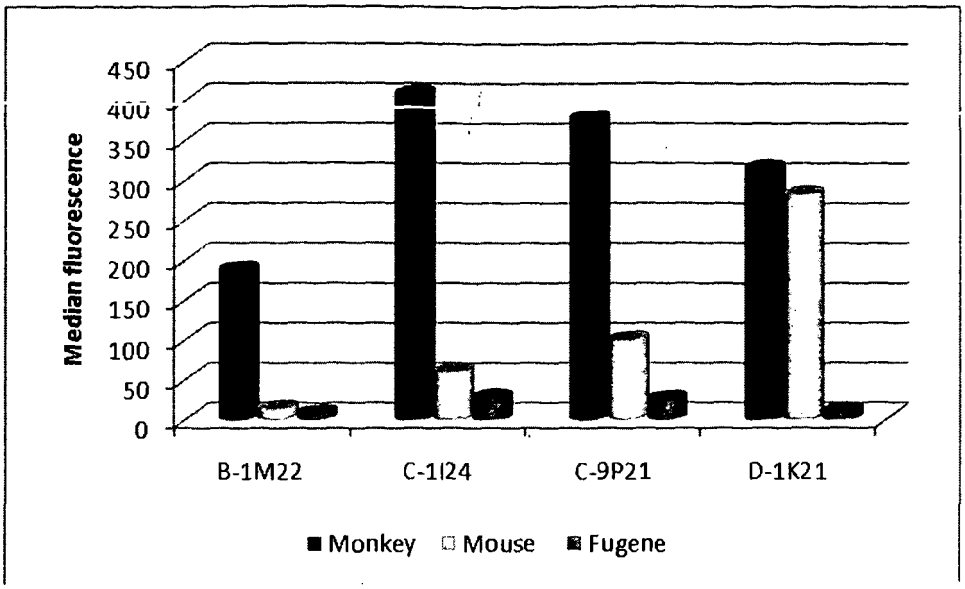

Median fluorescence
450
400
350
300
250
200
150
100
50
0
B-1M22
C-1I24
C-9P21
D-1K21
Monkey
Mouse
Fugene

Figure 10

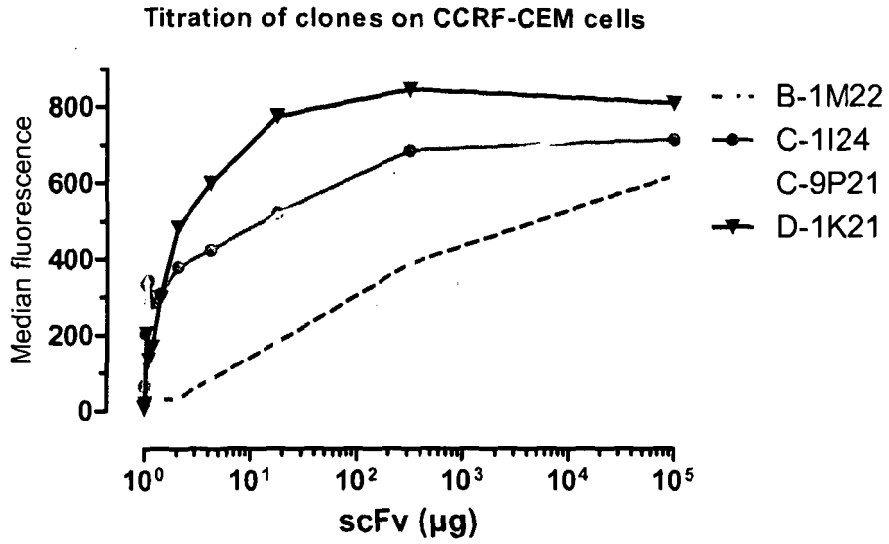

Titration of clones on CCRF-CEM cells
Median fluorescence
800
600
400
200
0
10^0
10^1
10^2
10^3
10^4
10^5
scFv (µg)
B-1M22
C-1I24
C-9P21
D-1K21

Figure 11A

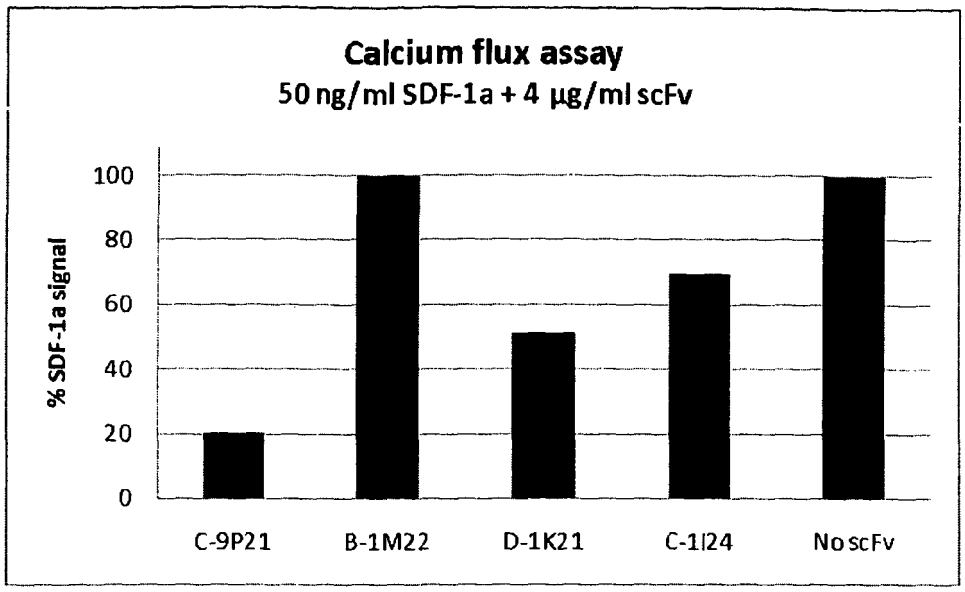


Figure 11B

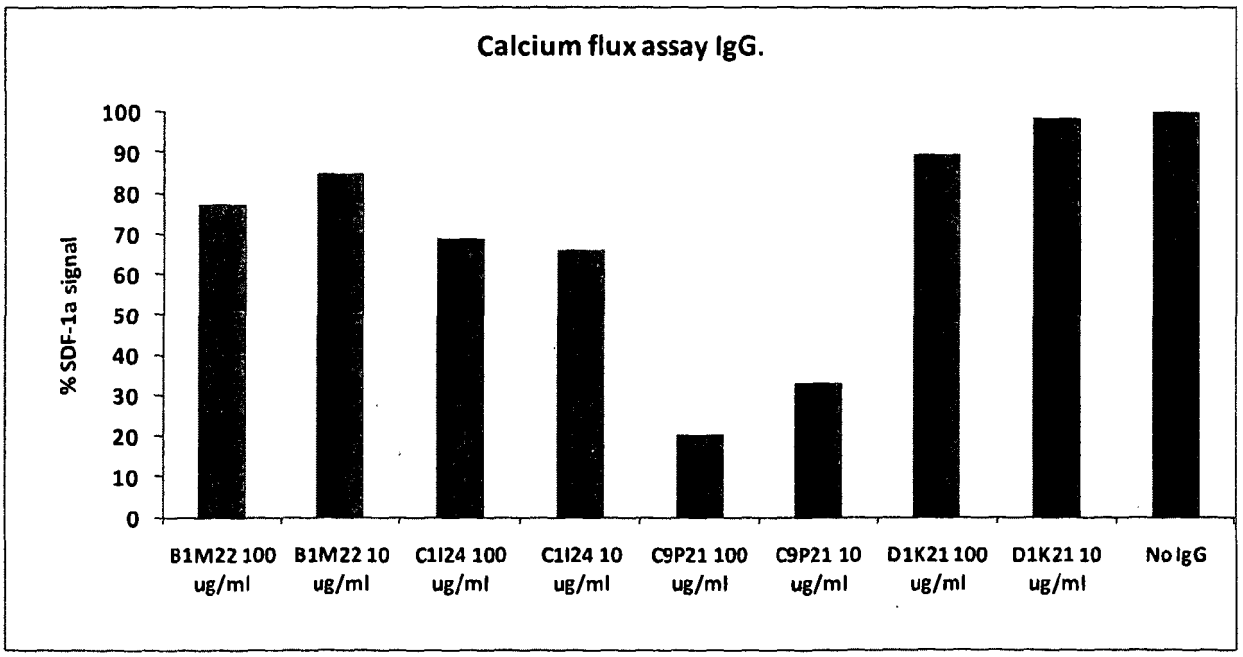


Figure 11C

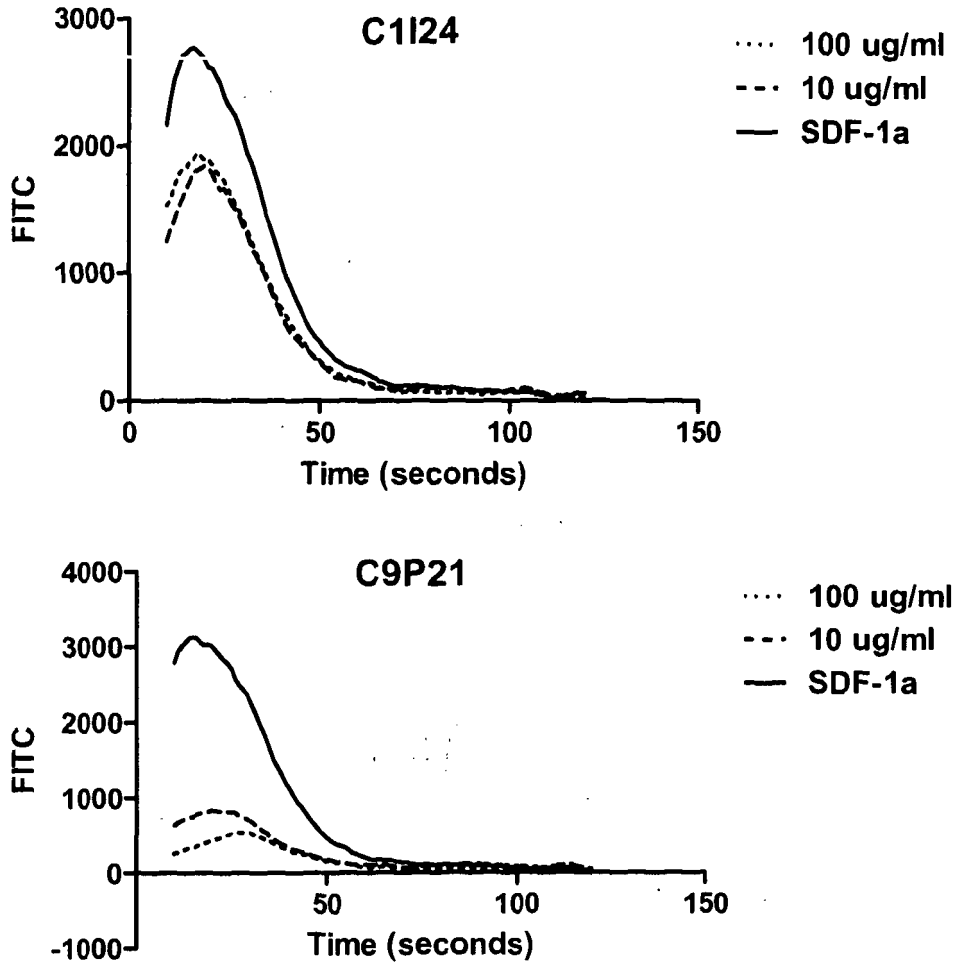

C1I24
3000
2000
1000
0
FITC
0
50
100
150
Time (seconds)
100 ug/ml
10 ug/ml
SDF-1a
C9P21
4000
3000
2000
1000
0
-1000
FITC
50
100
150
Time (seconds)
100 ug/ml
10 ug/ml
SDF-1a

Figure 11D

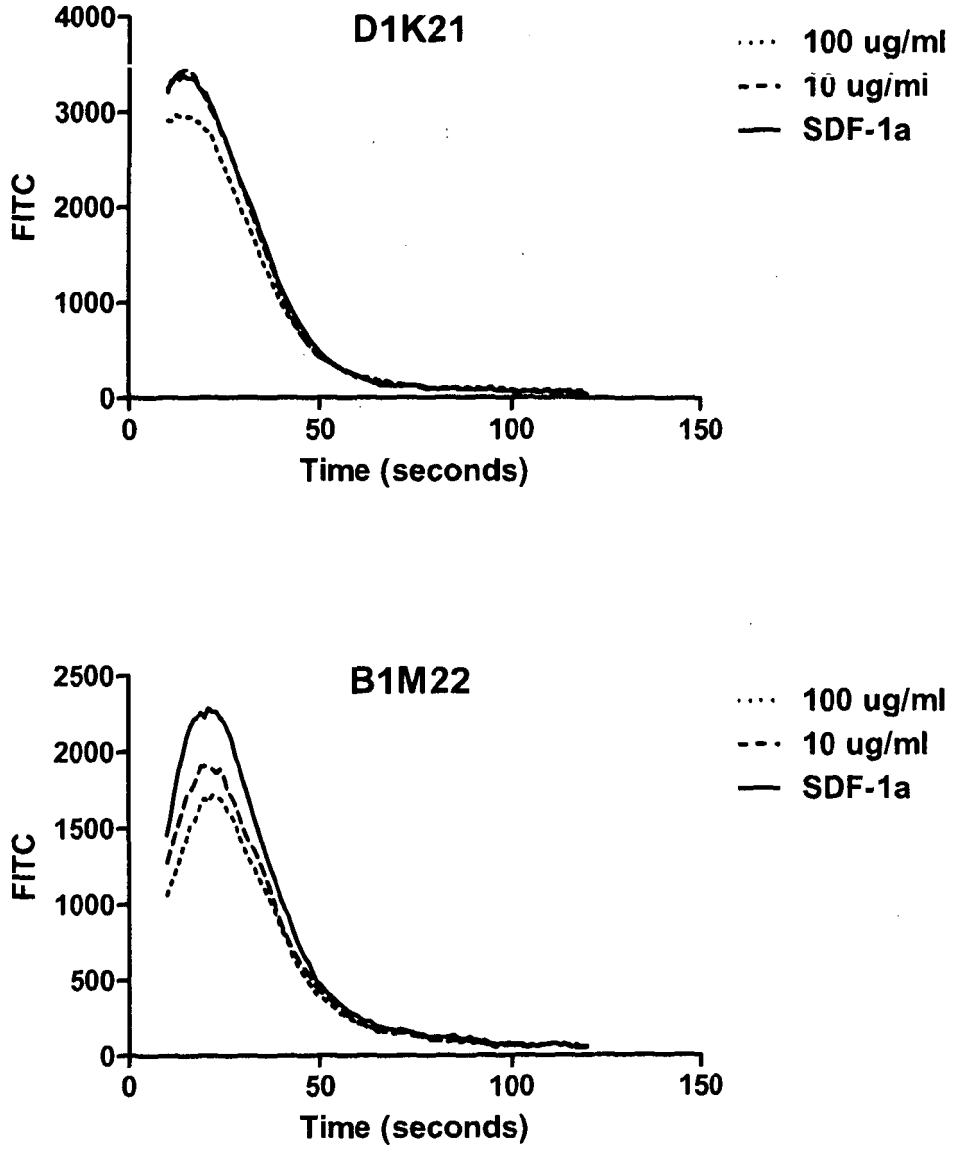

D1K21
FITC
4000
3000
2000
1000
0
0
50
100
150
Time (seconds)
100 ug/ml
10 ug/ml
SDF-1a
B1M22
FITC
2500
2000
1500
1000
500
0
0
50
100
150
Time (seconds)
100 ug/ml
10 ug/ml
SDF-1a

Figure 11E

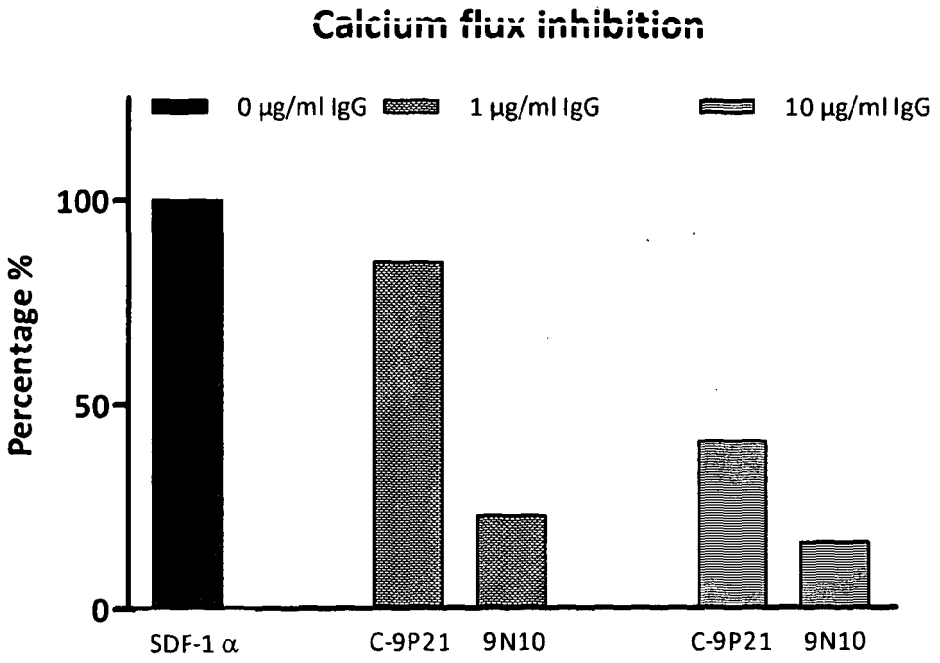

Calcium flux inhibition
0 µg/ml IgG
1 µg/ml IgG
10 µg/ml IgG
Percentage %
100
50
0
SDF-1 α
C-9P21
9N10
C-9P21
9N10

Figure 12A

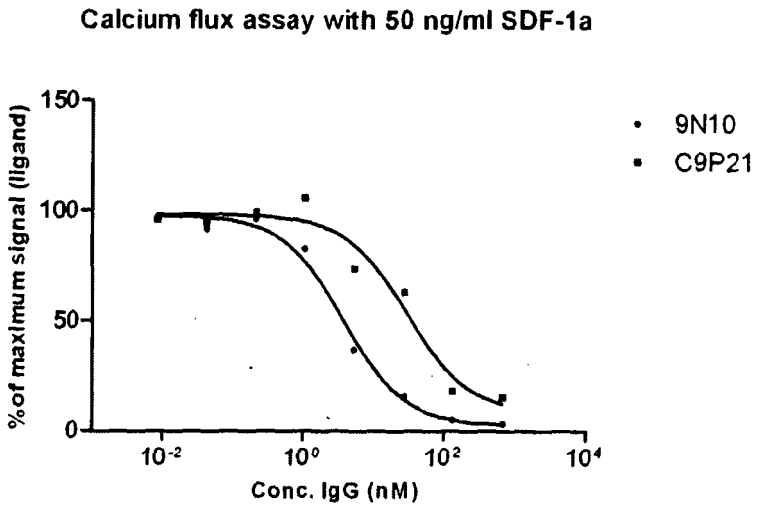

Calcium flux assay with 50 ng/ml SDF-1a
% of maximum signal (ligand)
150
100
50
0
10-2
100
102
104
Conc. IgG (nM)
9N10
C9P21

Figure 12 B

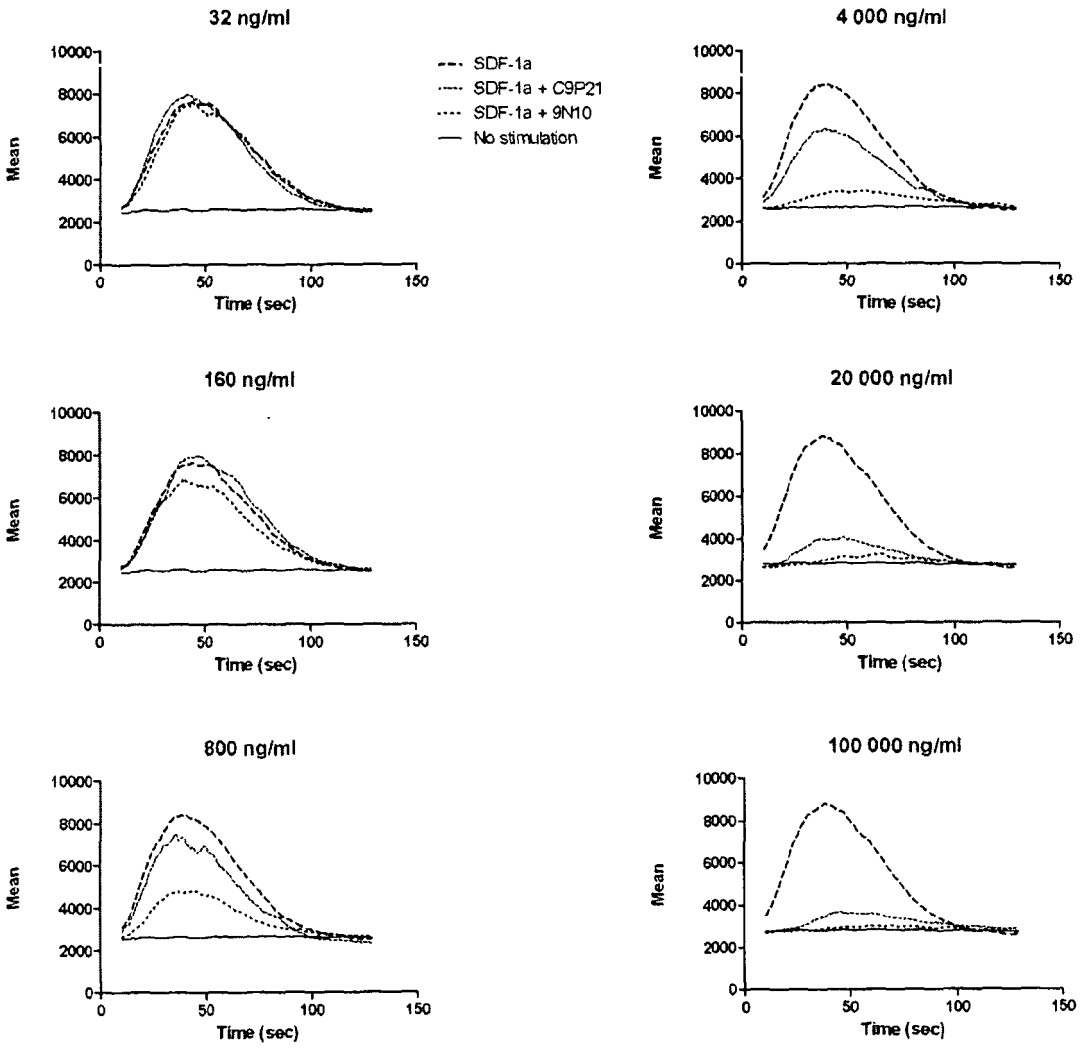

32 ng/ml
4 000 ng/ml
160 ng/ml
20 000 ng/ml
800 ng/ml
100 000 ng/ml
SDF-1a
SDF-1a + C9P21
SDF-1a + 9N10
No stimulation
Mean
Time (sec)
0
2000
4000
6000
8000
10000
50
100
150

Figure 13

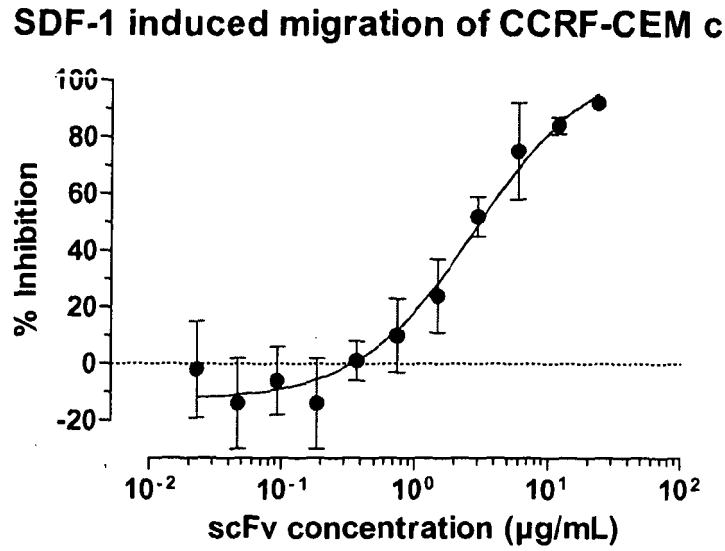

SDF-1 induced migration of CCRF-CEM cells
% Inhibition
100
80
60
40
20
0
-20
10-2
10-1
100
101
102
scFv concentration (µg/mL)

Figure 14

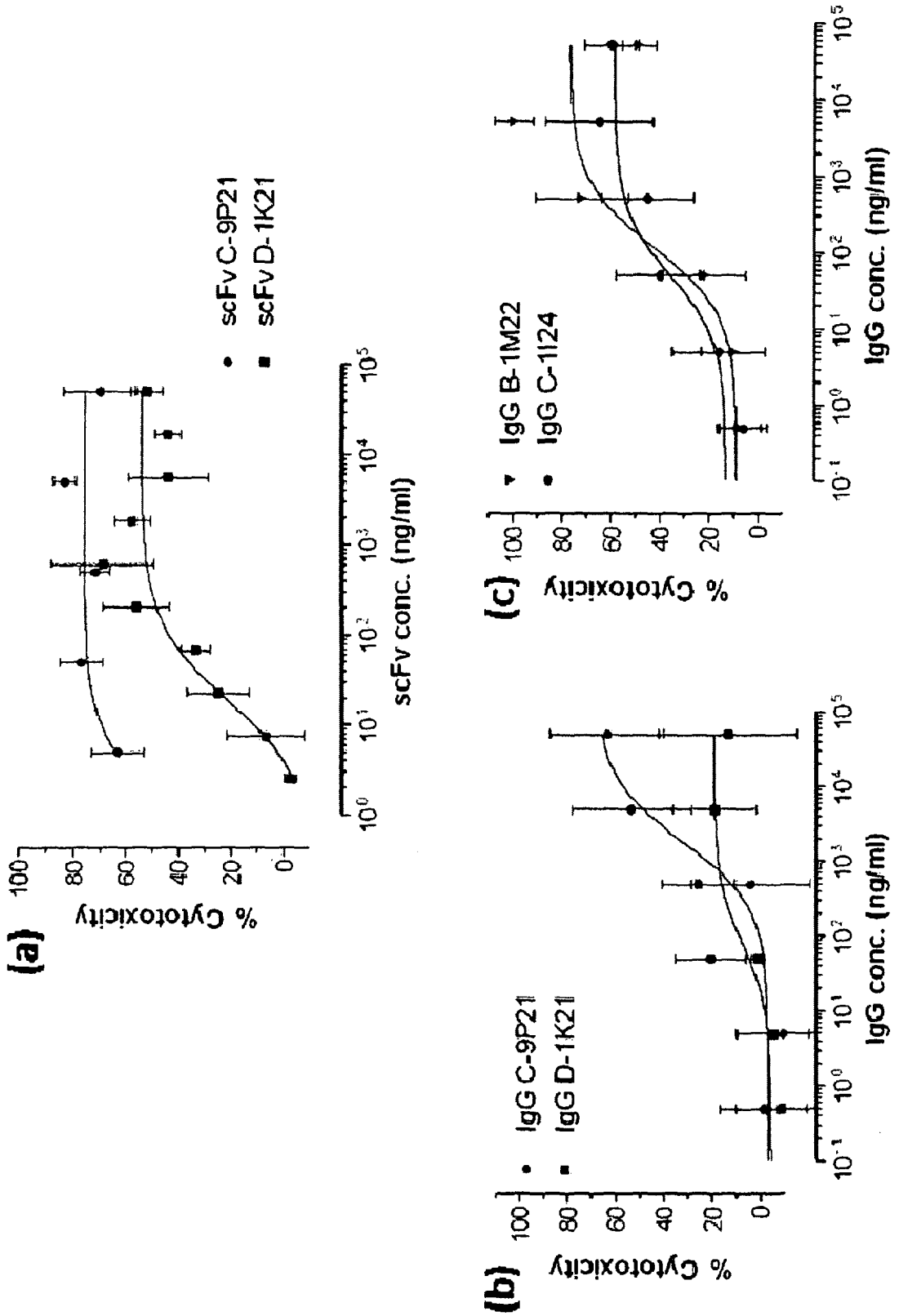

(a)
% Cytotoxicity
scFv C-9P21
scFv D-1K21
scFv conc. (ng/ml)
(b)
% Cytotoxicity
IgG C-9P21
IgG D-1K21
IgG conc. (ng/ml)
(c)
% Cytotoxicity
IgG B-1M22
IgG C-1124
IgG conc. (ng/ml)

Figure 15A

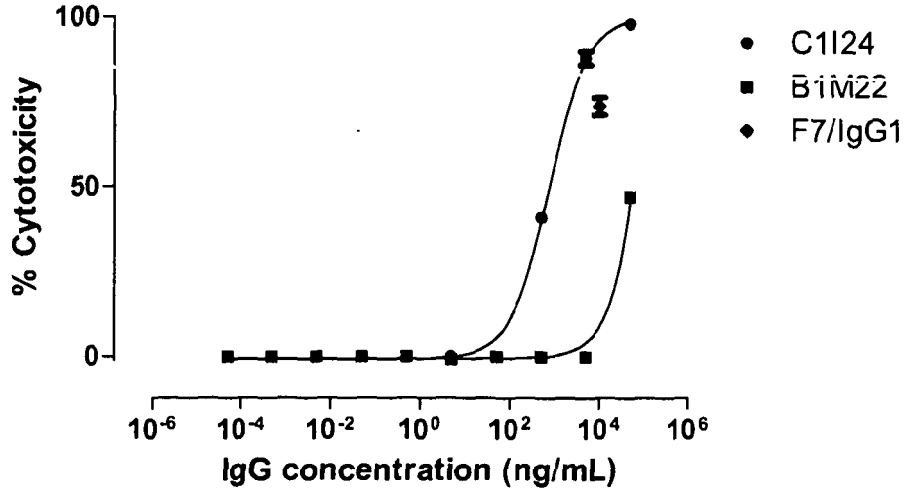


Figure 15B

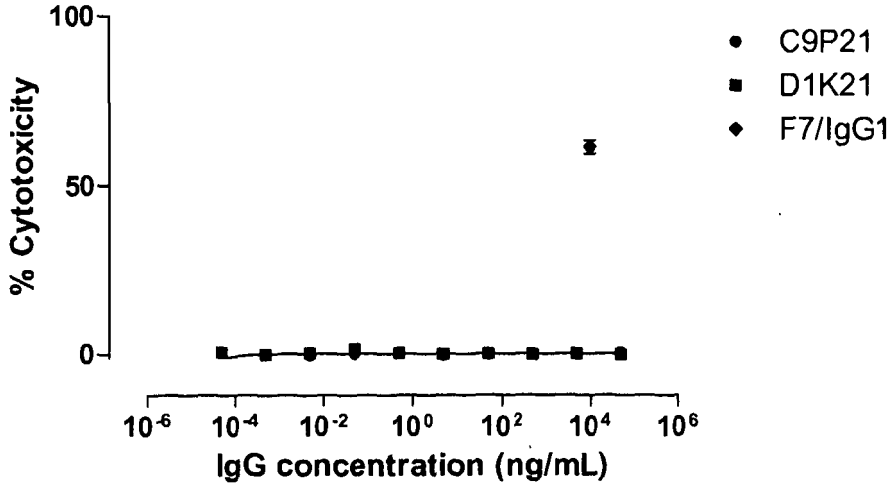


Figure 16A

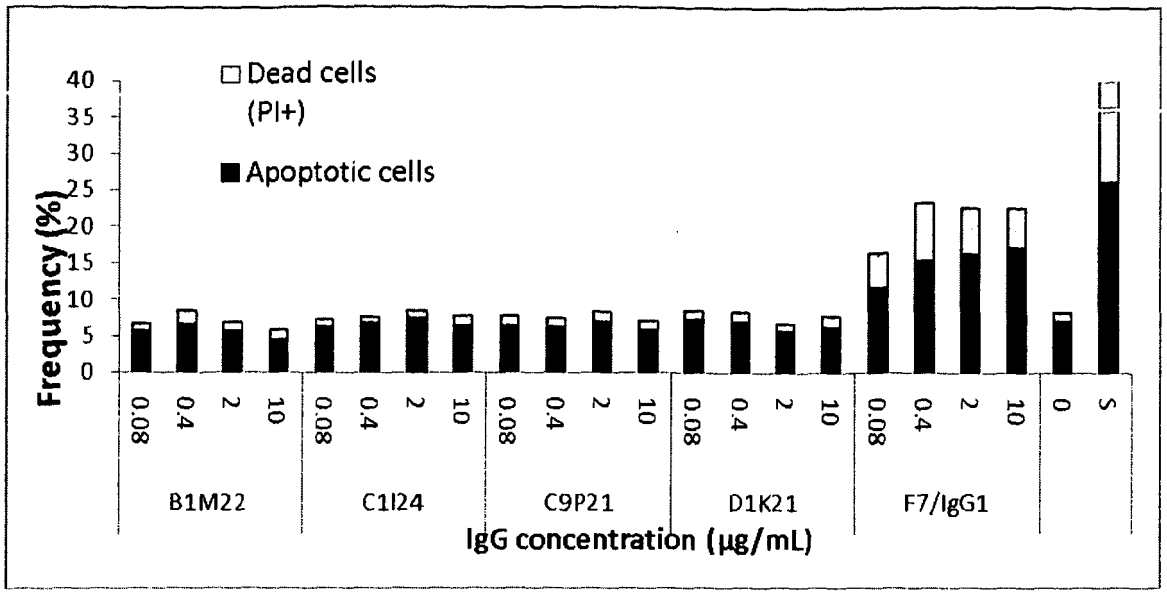


Figure 16B

