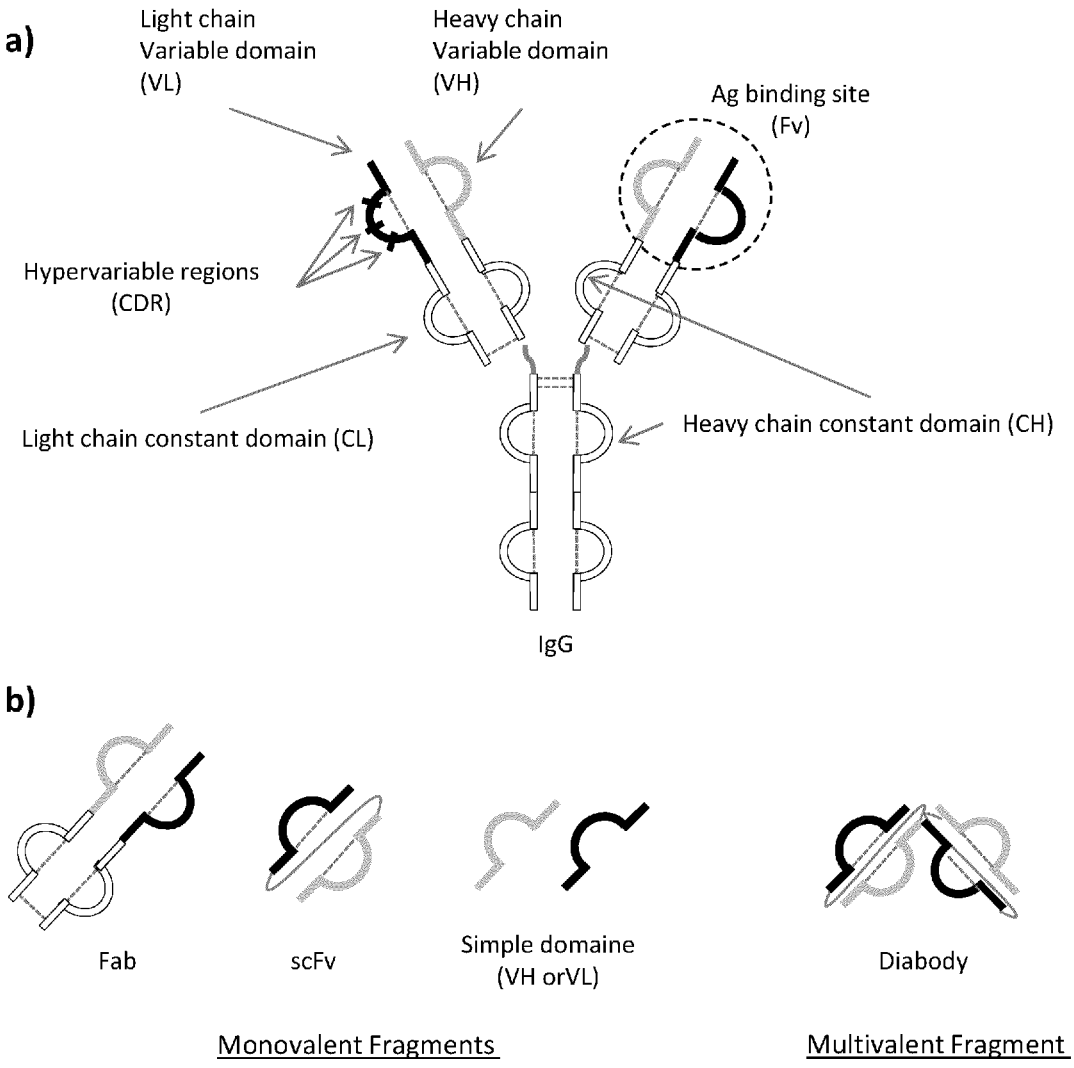

a)
Light chain
Variable domain
(VL)
Heavy chain
Variable domain
(VH)
Ag binding site
(Fv)
Hypervariable regions
(CDR)
Light chain constant domain (CL)
Heavy chain constant domain (CH)
IgG
b)
Fab
scFv
Simple domaine
(VH orVL)
Diabody
Monovalent Fragments
Multivalent Fragment

Fig. 1

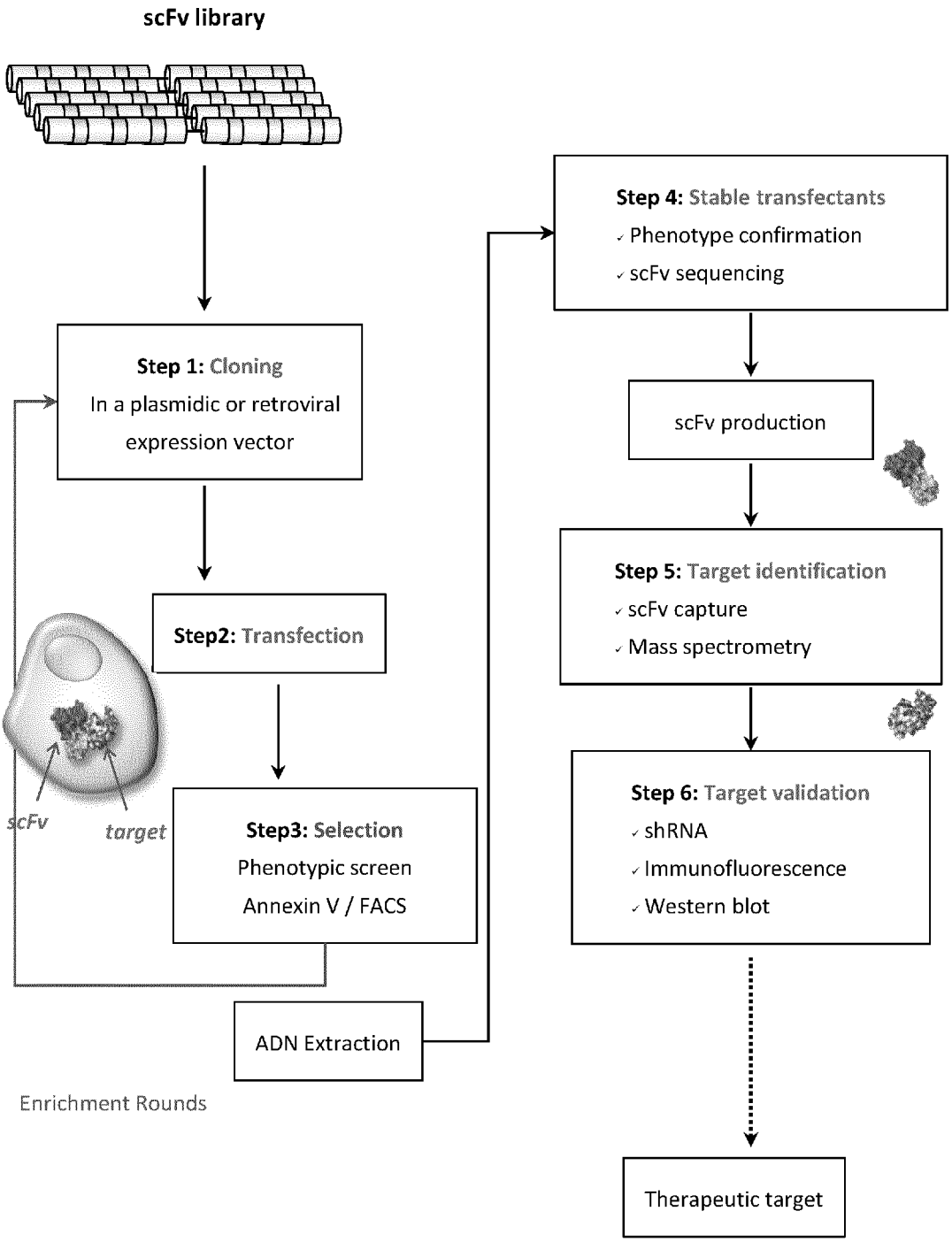
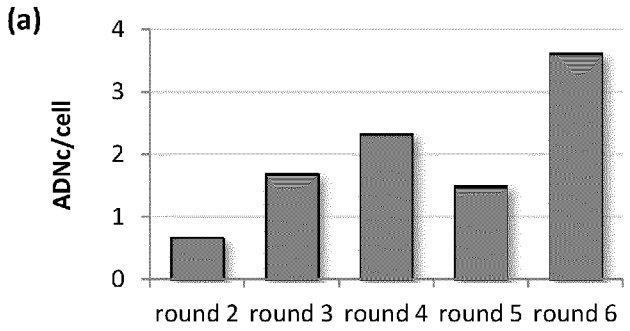

scFv library
Step 1: Cloning
In a plasmidic or retroviral
expression vector
Step2: Transfection
scFv
target
Step3: Selection
Phenotypic screen
Annexin V / FACS
ADN Extraction
Enrichment Rounds
Step 4: Stable transfectants
✓ Phenotype confirmation
✓ scFv sequencing
scFv production
Step 5: Target identification
✓ scFv capture
✓ Mass spectrometry
Step 6: Target validation
✓ shRNA
✓ Immunofluorescence
✓ Western blot
Therapeutic target

Fig. 2


(a)
ADNc/cell
4
3
2
1
0
round 2
round 3
round 4
round 5
round 6

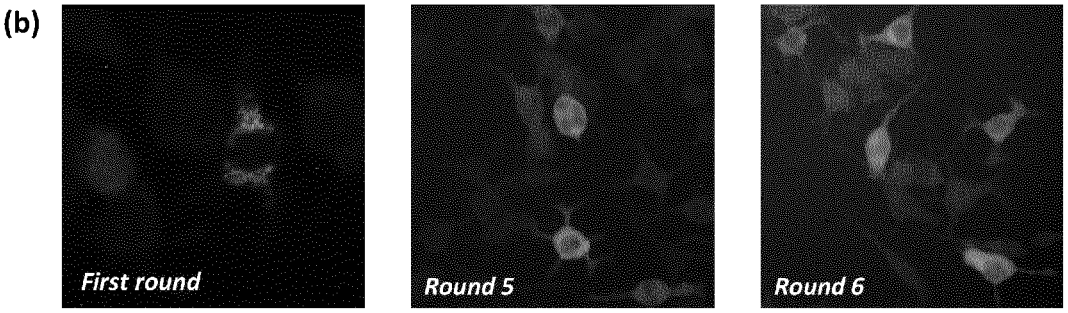
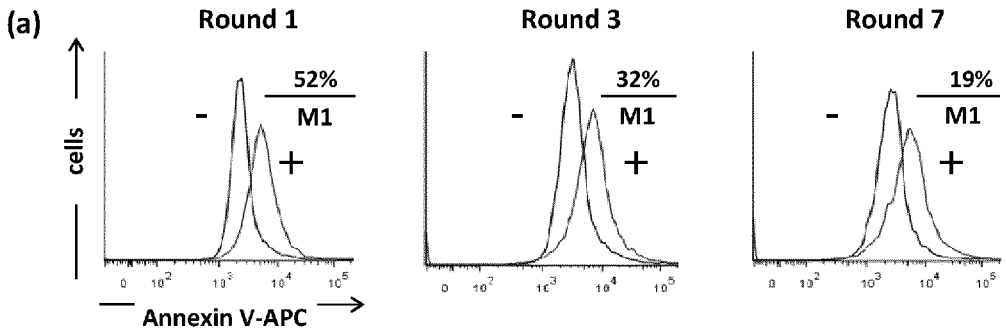

(b)
First round
Round 5
Round 6

Fig. 3


(a)
Round 1
Round 3
Round 7
cells
52%
M1
32%
M1
19%
M1
-
+
Annexin V-APC

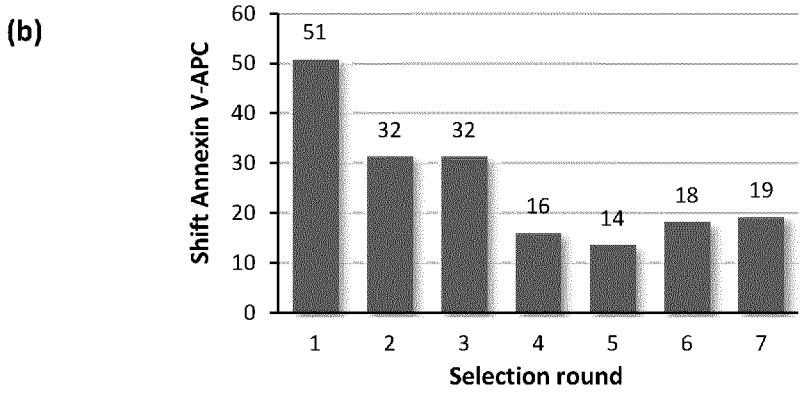

(b)
Shift Annexin V-APC
60
50
40
30
20
10
0
51
32
32
16
14
18
19
1
2
3
4
5
6
7
Selection round

Fig. 4

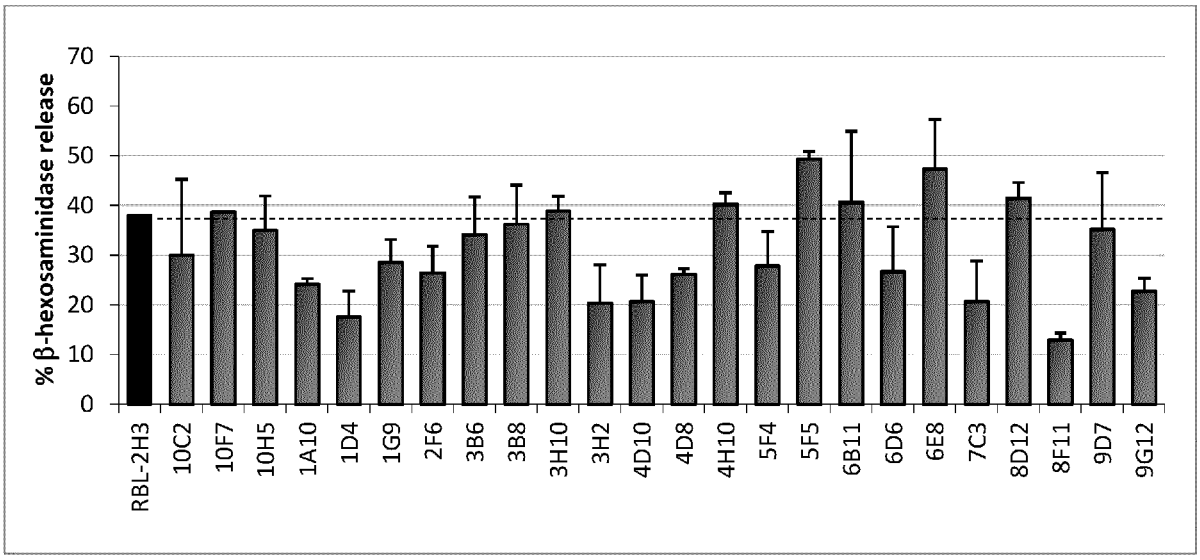

% β-hexosaminidase release
70
60
50
40
30
20
10
0
RBL-2H3
10C2
10F7
10H5
1A10
1D4
1G9
2F6
3B6
3B8
3H10
3H2
4D10
4D8
4H10
5F4
5F5
6B11
6D6
6E8
7C3
8D12
8F11
9D7
9G12

Fig. 5

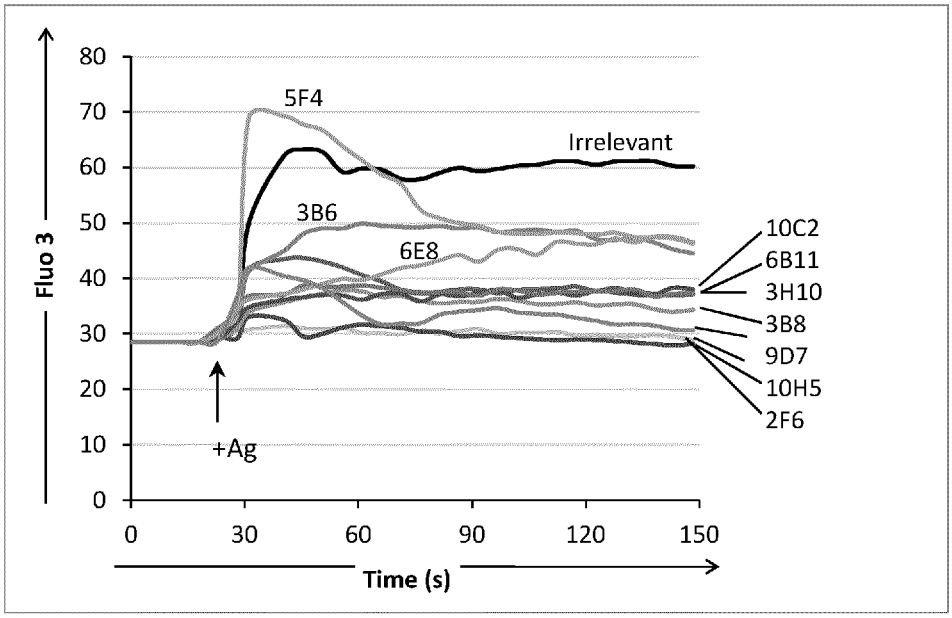
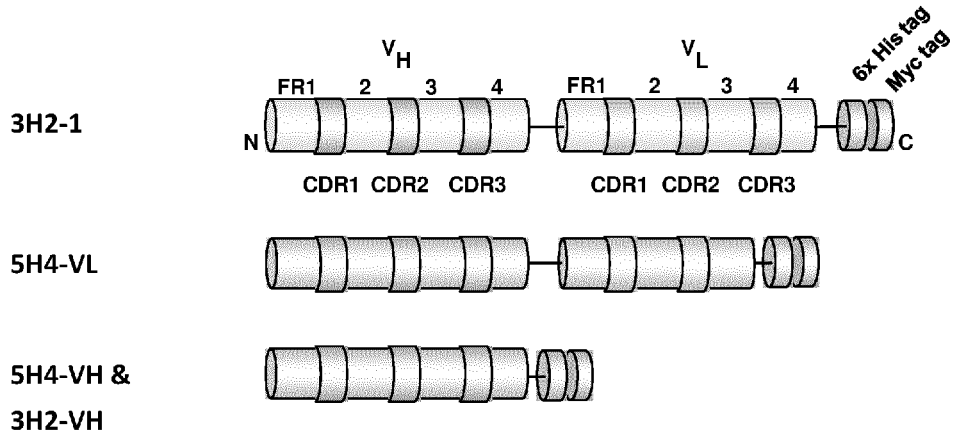

Fluo 3
80
70
60
50
40
30
20
10
0
0
30
60
90
120
150
Time (s)
5F4
Irrelevant
3B6
6E8
10C2
6B11
3H10
3B8
9D7
10H5
2F6
+Ag

Fig. 6

a)



b)

scFv clones	VH-CDR3	VL-CDR3
13R4 (original backbone)	*SSITIFGGGMDV*	*SSYTTRSTRV*
3H2-1	PIAVSDY	QTYDGSRAV
3H2-VH	GVRGGYGLDF	-
5H4	DGGLREGFDC	stop codon before CDR3

Fig. 7

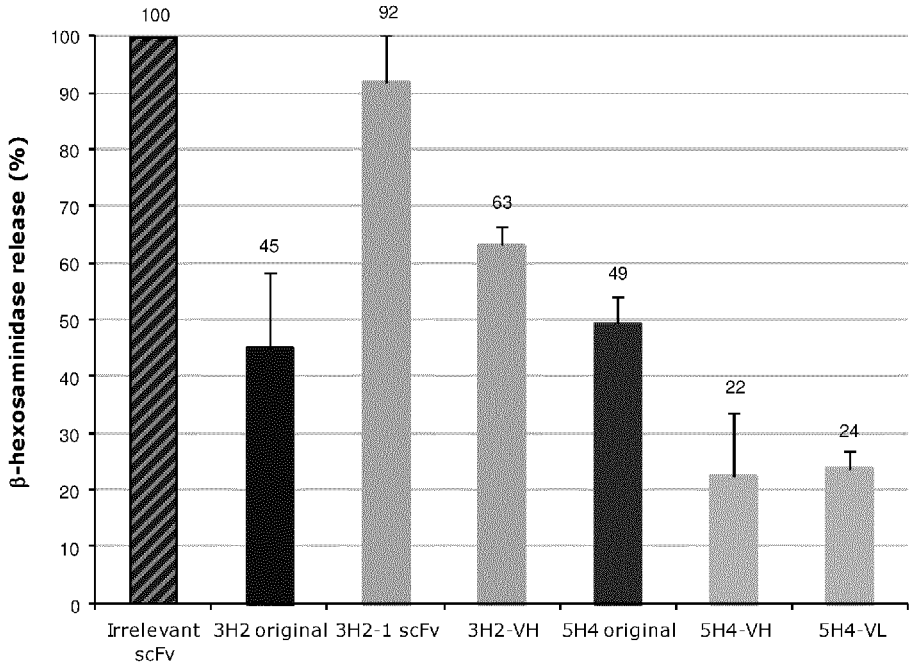

β-hexosaminidase release (%)
100
90
80
70
60
50
40
30
20
10
0
100
45
92
63
49
22
24
Irrelevant scFv
3H2 original
3H2-1 scFv
3H2-VH
5H4 original
5H4-VH
5H4-VL

Fig. 8

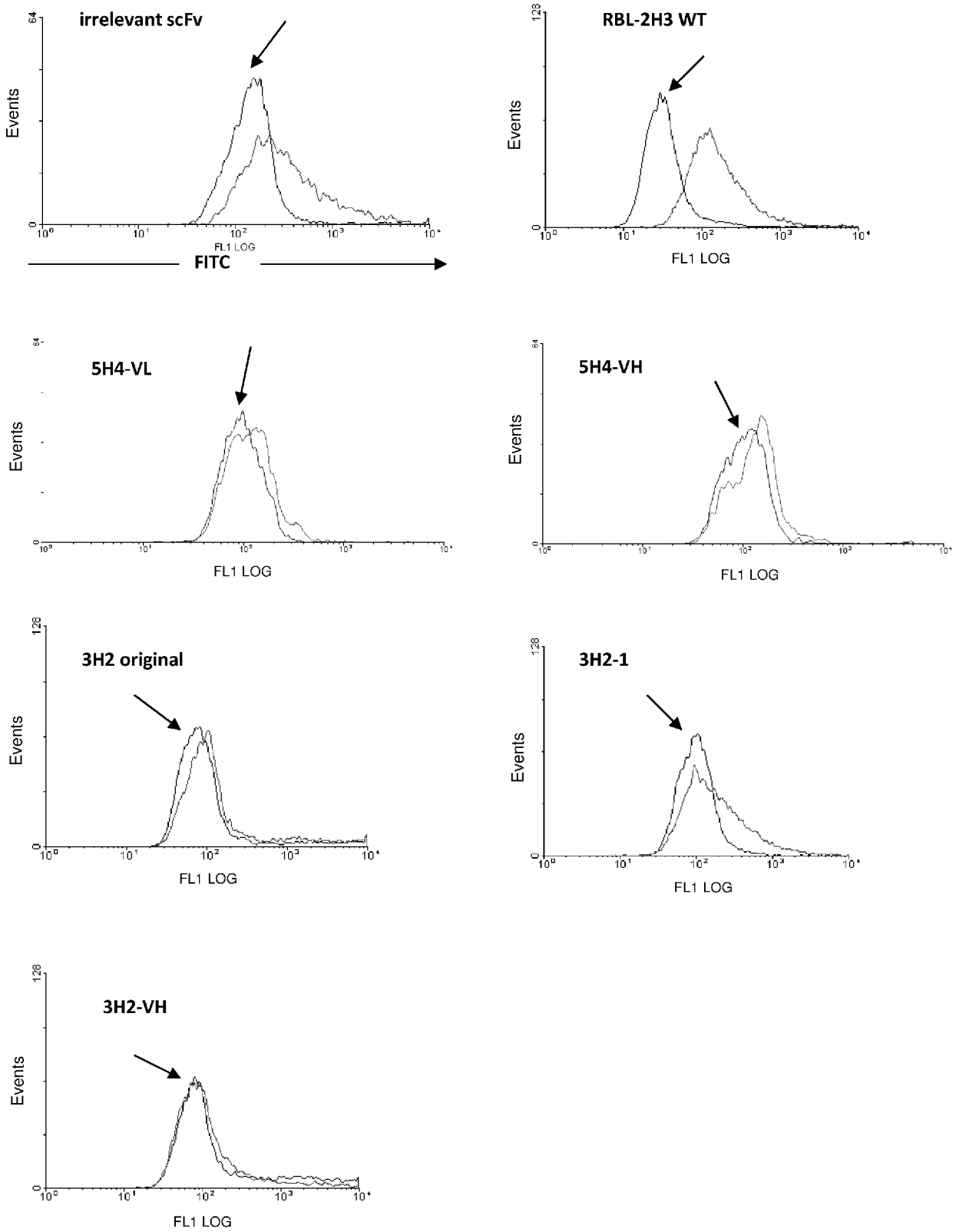

irrelevant scFv
Events
FL1 LOG
FITC
RBL-2H3 WT
Events
FL1 LOG
5H4-VL
Events
FL1 LOG
5H4-VH
Events
FL1 LOG
3H2 original
Events
FL1 LOG
3H2-1
Events
FL1 LOG
3H2-VH
Events
FL1 LOG

Fig. 9

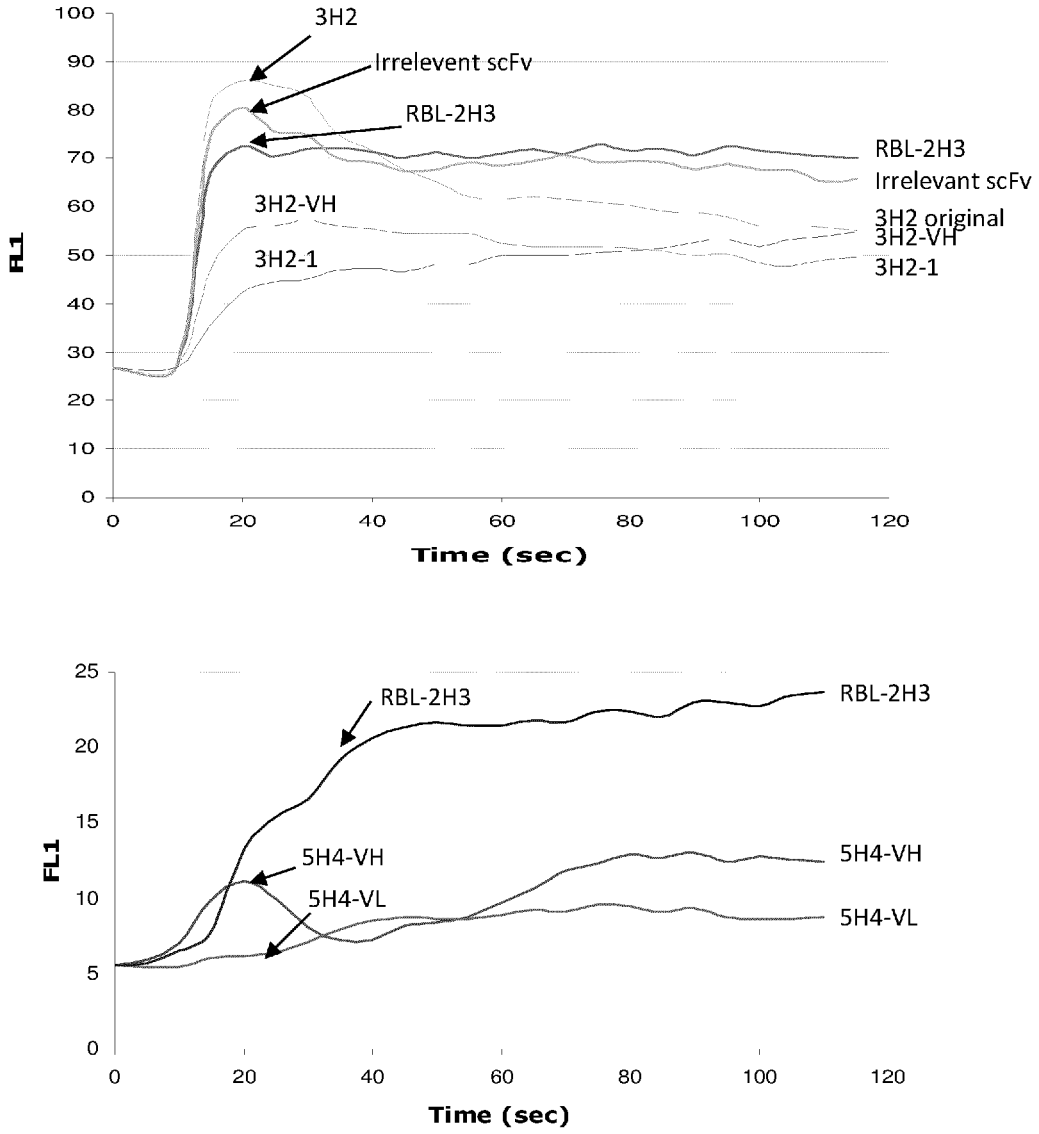

3H2
Irrelevent scFv
RBL-2H3
3H2-VH
3H2-1
RBL-2H3
Irrelevant scFv
3H2 original
3H2-VH
3H2-1
FL1
Time (sec)
RBL-2H3
5H4-VH
5H4-VL
RBL-2H3
5H4-VH
5H4-VL
FL1
Time (sec)

Fig. 10

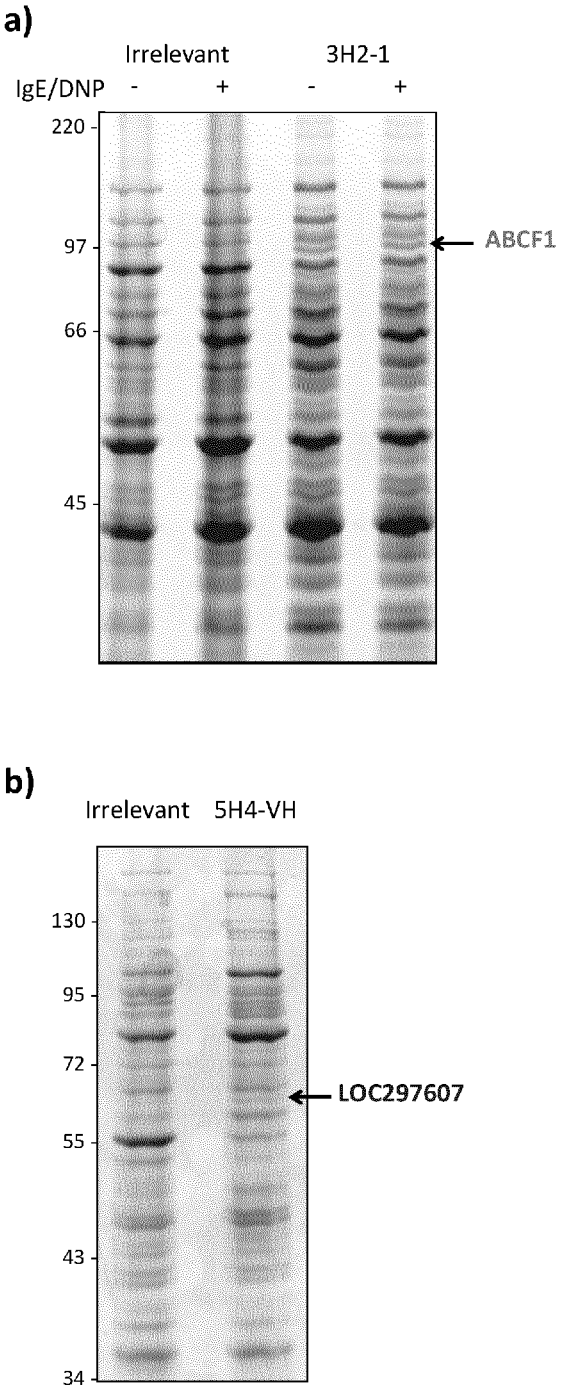

a)
Irrelevant
3H2-1
IgE/DNP - + - +
220
97
66
45
ABCF1
b)
Irrelevant 5H4-VH
130
95
72
55
43
34
LOC297607

Fig. 11

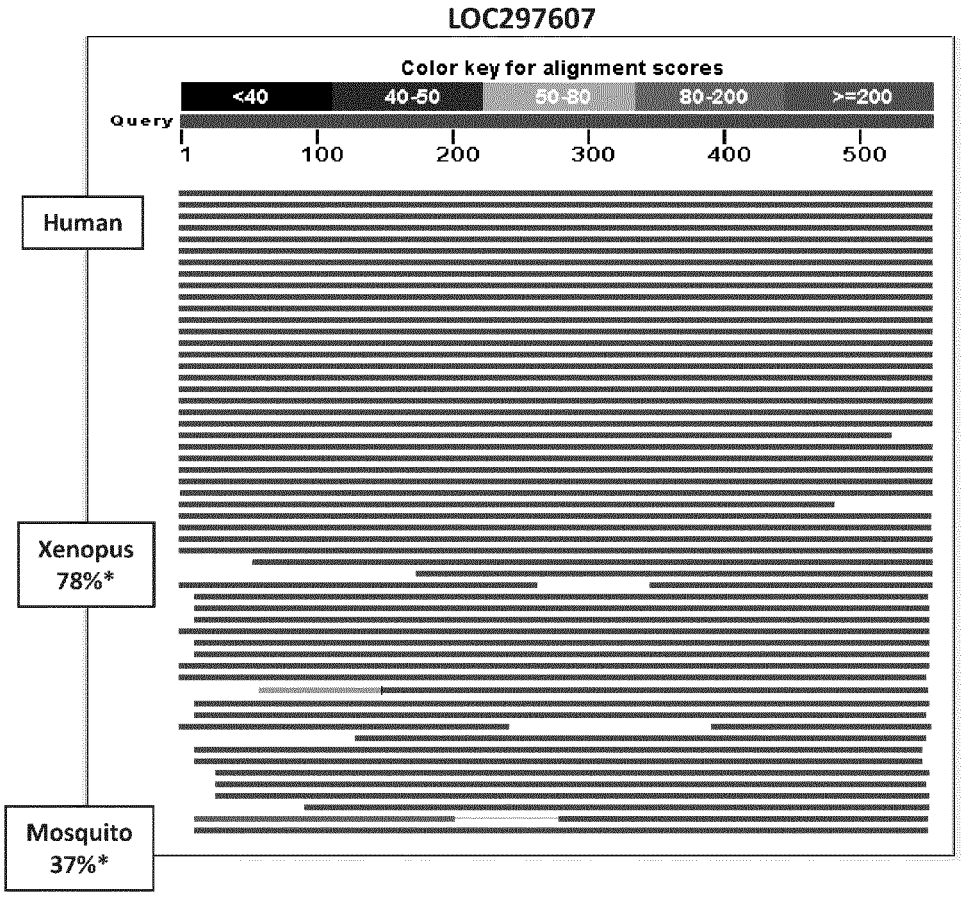
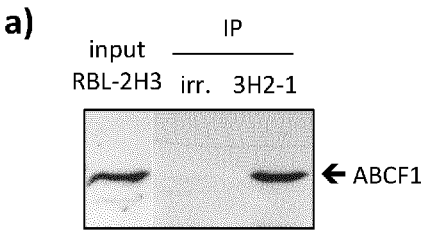

LOC297607
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
100
200
300
400
500
Human
Xenopus
78%*
Mosquito
37%*

Fig. 12


a)
input
IP
RBL-2H3
irr.
3H2-1
ABCF1

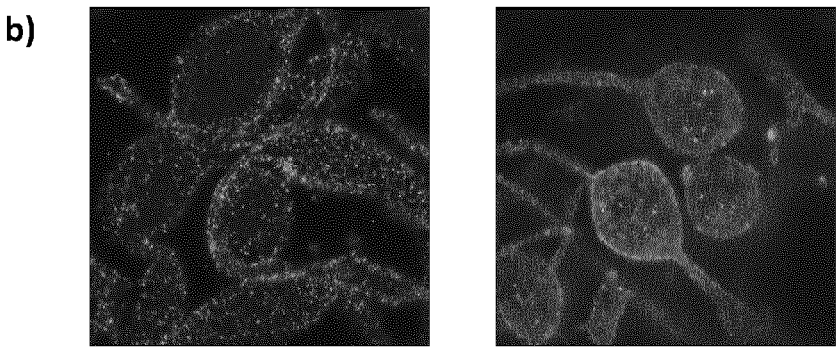

b)
3H2-Fc
Commercial anti-ABCF1

Fig. 13

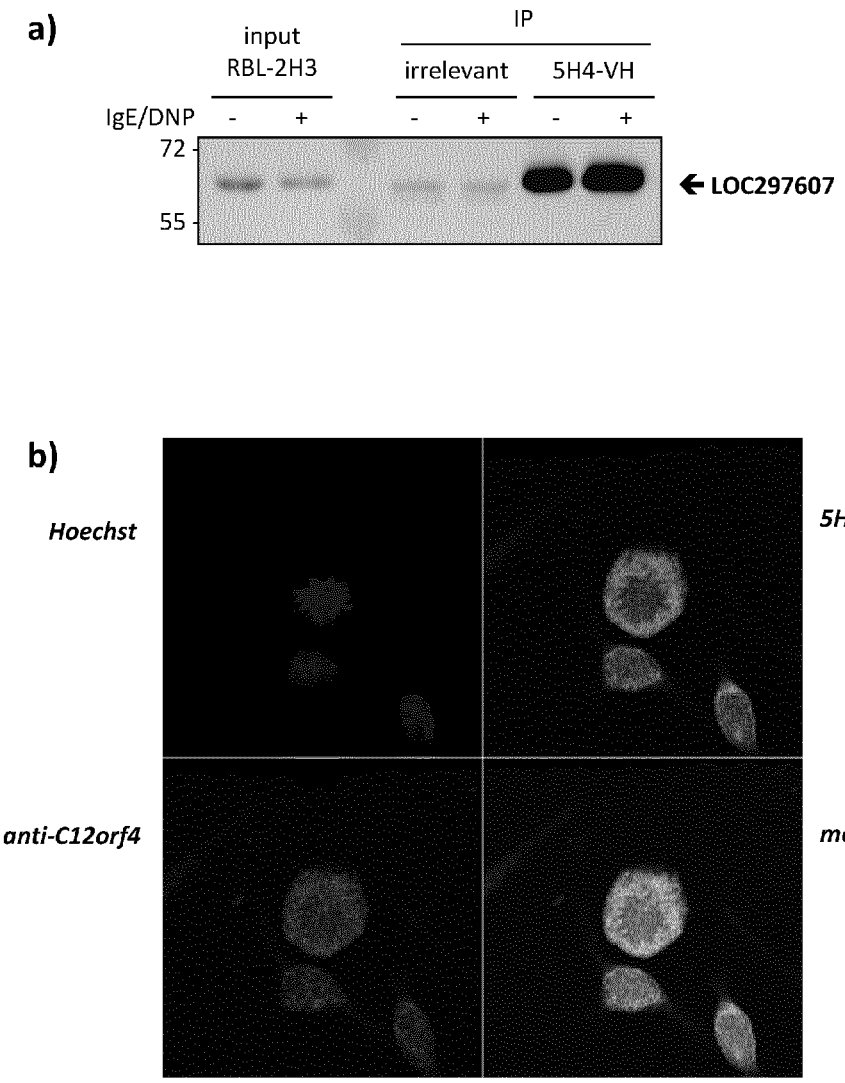

a)
input
RBL-2H3
IP
irrelevant
5H4-VH
IgE/DNP
-
+
-
+
-
+
72
55
LOC297607
b)
Hoechst
5H4-V_H
anti-C12orf4
merge

Fig. 14

```
   1 ctgacttatt gggcagttct gcgttgacag ctctaagtgt gccttggtgt ttgagtgaag
  61 agaaatcctg ctgaacaaca tgaagaaaaa ccgagaaagg ttctccagca aggaaagaga
 121 attcgtgtac aaattccaga taggaagtga gcgcttagaa ctgagagtgc ccctcaggtt
 181 tcccgtggac gagaatgcca gtcatctgca tggacgtctg atgctgctgc acagtttgcc
 241 ctgcttcata gagaatgact taaaagaagc tctggcccgg tttatagaag aagagtccct
 301 cagagaccat gacagcgatg cagaagcatg cttggaggcg gttaagtctg gtgaggtaga
 361 tctacaccag ctggcgagta catgggccaa agcgtatgct gagacaaccc tagagcatgc
 421 gagacctgag gagcctaact gggatgaaga ctttgcagat gtgtaccatg acctaatcca
 481 ttctccggcc tctgagactc tcttaaatct ggaacacaat tactttgtta gcatctcaga
 541 attgattggc gaaagagatg tggagctgaa aaagttacga gagagacaag gcattgaaat
 601 ggaaaaggtg atgcaggaac tggggaagtc gctcacagat caagatgtga actcactggc
 661 tgcccagcac tttgaatccc agcaggactt agagaataaa tggtcaaacg aactgaaaca
 721 gtctacagct atccagaagc aagaatatca ggaatgggtg ataaagcttc atcaggacct
 781 aaaaaacccc aacaacagct ccctcagtga agaaattaaa gtgcagccga gtcagttcag
 841 ggaatctgcg gacgccgctg gaaggatcta tgaggagcag aggaagctgg aagaaagctt
 901 caccattcac ctgggagccc agttgaagac catgcacaat ctgcggttgc tgagggcaga
 961 catgctggac ttctgtaagc acaagagaac gcaggggagc ggcgtgaagc tccaccggct
1021 ccagacggca ctgtcccttt actccacgtc cctctgtggc ctggttttac tagtagacaa
1081 tcggattaat tcatatagcg gcatcaaaag agattttgcc acagtttgcc aagaatgcac
1141 ggacttccac ttcccgcgga ttgaggagca actggaggtt gtccagcagg tggcgctgta
1201 cgcacgaacc cagcgcagga gcaagtgcaa ggaggcacgc gattctggaa accaaaatgg
1261 aggaagtgat gagaaatcta agaatgctga gagaaactat ttaaatattt taccaggaga
1321 attttatatt acacgtcatt ctaatctctc ggaaatccac gttgctttcc atctctgtgt
1381 ggatgacaac gtgaagtcag gaaacatcac tgctcgggat cccgctatta tgggactccg
1441 aaatatactc aaggtttgct gtacccacga tatcacaacg ataagcatcc ctctcttgct
1501 ggtgcatgat atgtcagagg aaatgactat accttggtgc ttaaggagag ctgaactggt
1561 gtttaagtgt gtcaaaggtt tcatgatgga gatggcttca tgggatggag gaatttctcg
1621 gacagtgcaa tttctggtac cacagagcat ttctgaagaa atgttttatc agctgagtaa
1681 catgctccca cagatcttcc gggtctcctc aacactcact ctgacatcca agcactaaac
1741 catacactga cacactgact gacagaagag gatttctgaa ctcctggaat cctgttgagc
1801 ggaaggtgcc tggtgggccc tgagcccccc gttaatgatg atgtacgtat ctaacattcc
1861 aaaatgtgtc tgcttcacag accagaagca gctcccagtg agctcttact cagtcctttg
1921 tggaataata gctcttcttt ctacctatat gtcctatcag gggatgatga tgctaacact
1981 taaagtgttt ctcaaacacg gacacttgta tgccattgcg tttccatgga gacttctggt
2041 ttttccaagg catgagttct gtattaggaa caggcataat cgggcatacg agtacaatct
2101 tccttctact ttatctgtca aatcaaaatt taaatataca tttcagtgta agtttcttat
2161 gaaaatgttt ttgtagttgg taaatatacc agacaaatgt gcatgttgtt ttaaattctc
2221 ctgtttcaaa gcct
```

sh1

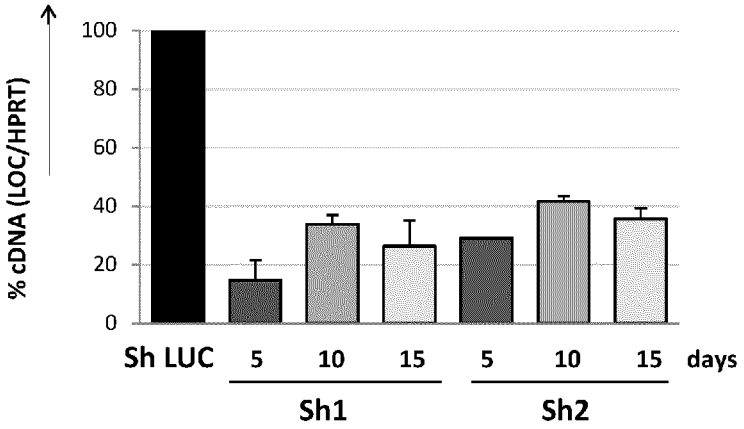
GATCCG**CATTCTAATCTCTCGGAAA**TTCAAGAGA**TTTCCGAGAGATTAGAATG**TTTTTTT**TCTAGA**G
AATT**CTCTAGA**AAAAAA**CATTCTAATCTCTCGGAAA**TCTCTTGAA**TTTCCGAGAGATTAGAATG**CG

sh2

GATCCG**AGAATTGATTGGCGAAAGA**TTCAAGAGA**TCTTTCGCCAATCAATTCT**TTTTTTT**TCTAGA**G
AATT**CTCTAGA**AAAAAA**AGAATTGATTGGCGAAAGA**TCTCTTGAA**TCTTTCGCCAATCAATTCT**CG

Fig. 15

a)


% cDNA (LOC/HPRT)
100
80
60
40
20
0
Sh LUC
5
10
15
5
10
15
days
Sh1
Sh2

b)

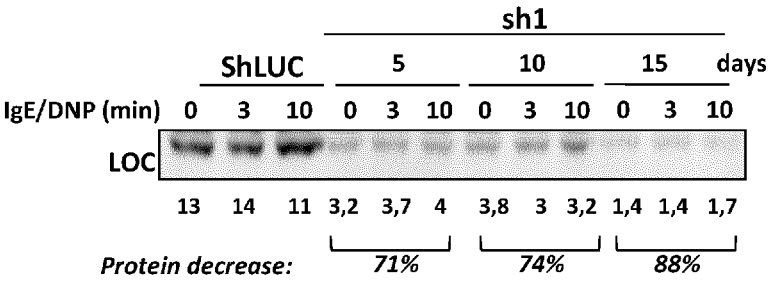
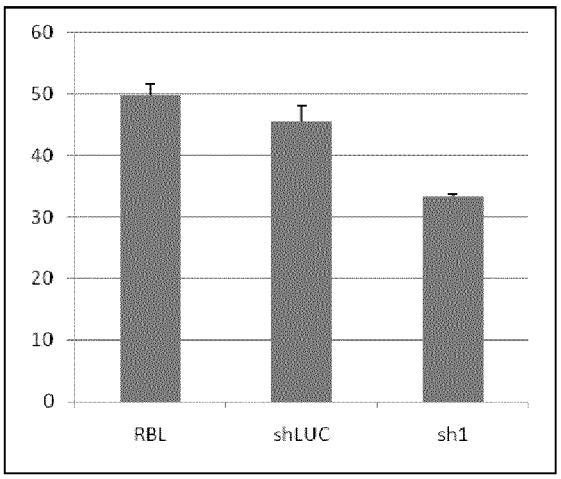

sh1
ShLUC
5
10
15
days
IgE/DNP (min) 0 3 10 0 3 10 0 3 10 0 3 10
LOC
13 14 11 3,2 3,7 4 3,8 3 3,2 1,4 1,4 1,7
Protein decrease:
71%
74%
88%

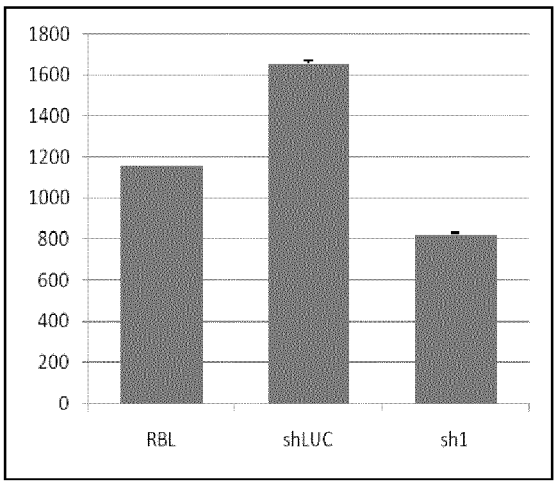
Fig. 16

a)



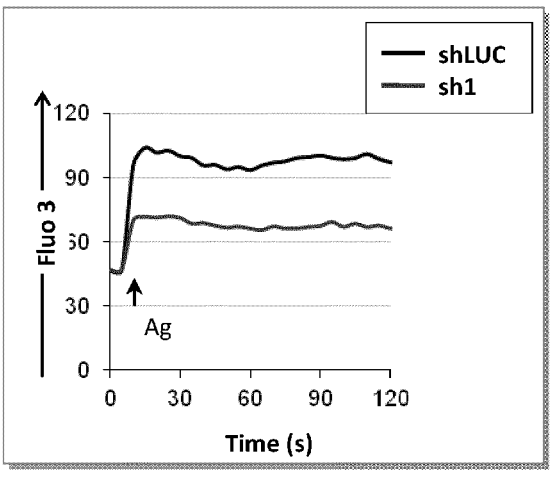
β-hexosaminidase release

b)



TNF-α secretion

c)



Calcium flux

Fig. 17

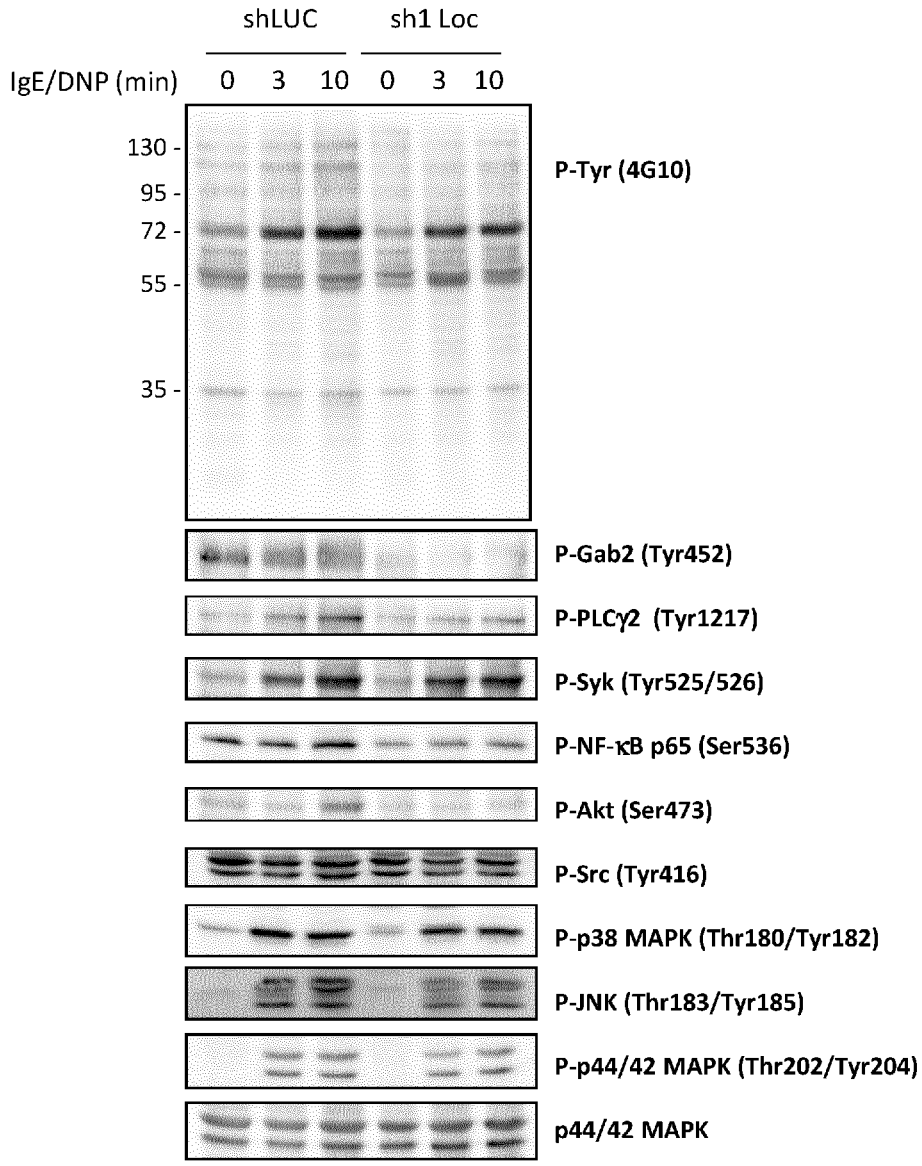

shLUC
sh1 Loc
IgE/DNP (min)
0 3 10 0 3 10
130 -
95 -
72 -
55 -
35 -
P-Tyr (4G10)
P-Gab2 (Tyr452)
P-PLCγ2 (Tyr1217)
P-Syk (Tyr525/526)
P-NF-κB p65 (Ser536)
P-Akt (Ser473)
P-Src (Tyr416)
P-p38 MAPK (Thr180/Tyr182)
P-JNK (Thr183/Tyr185)
P-p44/42 MAPK (Thr202/Tyr204)
p44/42 MAPK

Fig. 18

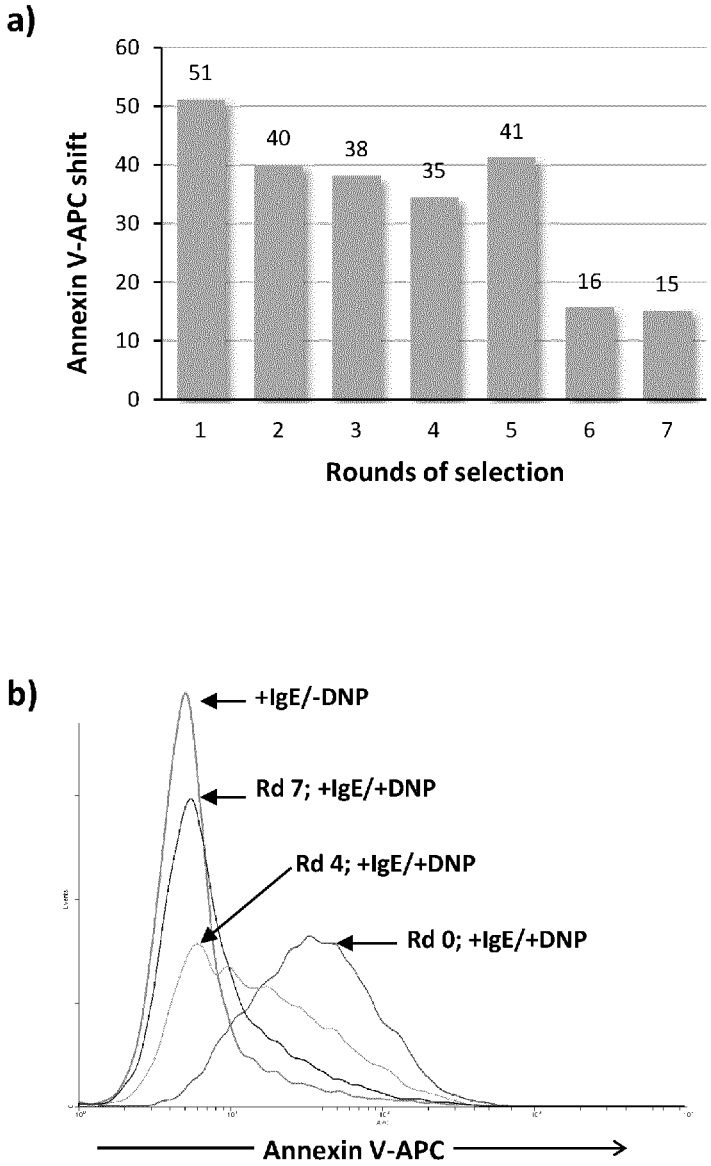

a)
Annexin V-APC shift
60
50
40
30
20
10
0
51
40
38
35
41
16
15
1
2
3
4
5
6
7
Rounds of selection
b)
+IgE/-DNP
Rd 7; +IgE/+DNP
Rd 4; +IgE/+DNP
Rd 0; +IgE/+DNP
Annexin V-APC

Fig. 19

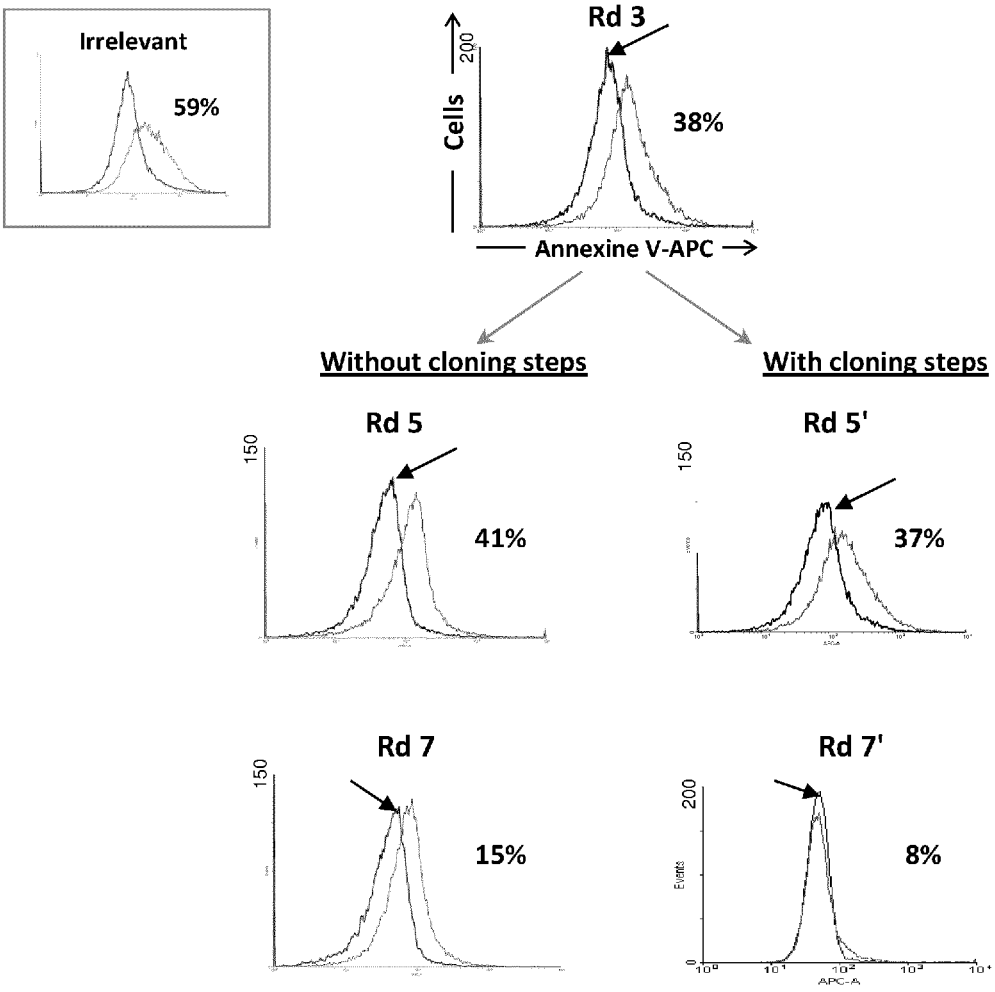
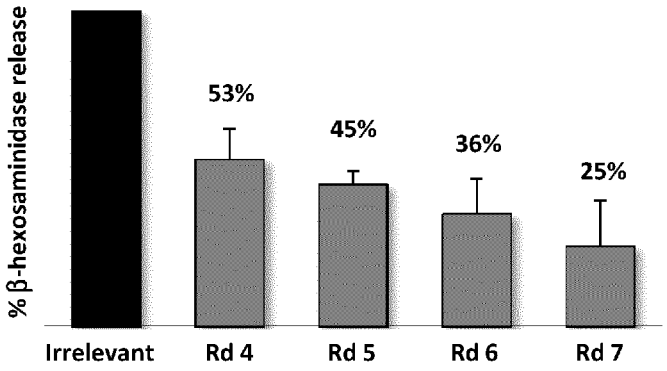

Irrelevant
59%
Rd 3
38%
Cells
200
Annexine V-APC
Without cloning steps
With cloning steps
Rd 5
41%
150
Rd 5'
37%
150
Rd 7
15%
150
Rd 7'
8%
200
Events
0
APC-A

Fig. 20

a)


% β-hexosaminidase release
53%
45%
36%
25%
Irrelevant
Rd 4
Rd 5
Rd 6
Rd 7

b)

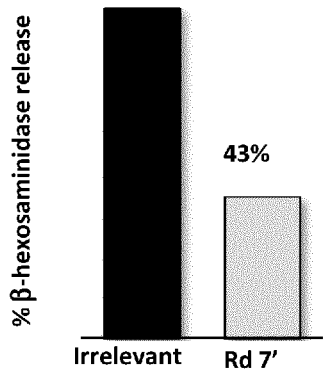

% β-hexosaminidase release
43%
Irrelevant
Rd 7'

Fig. 21

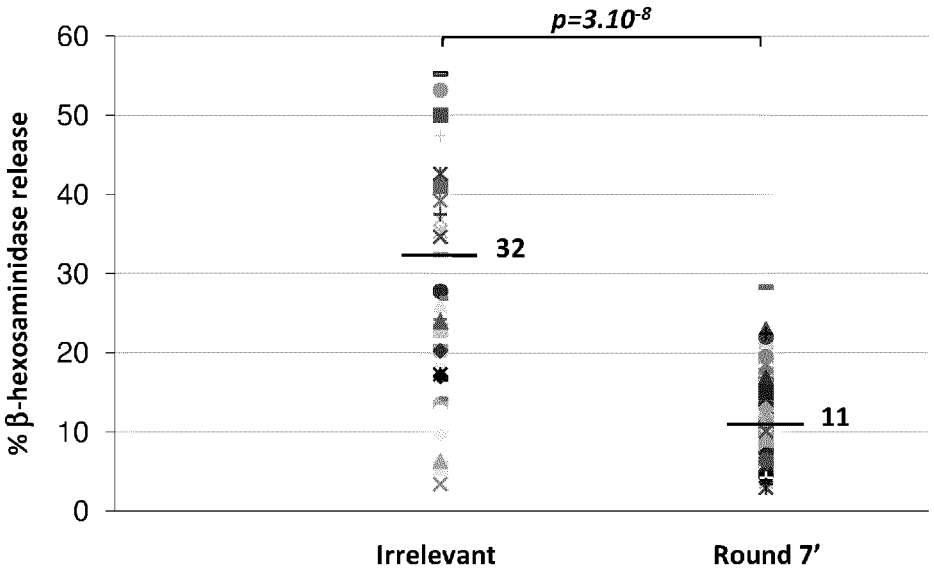

p=3.10-8
60
50
40
30
20
10
0
% β-hexosaminidase release
32
11
Irrelevant
Round 7'

Fig. 22

Cluster	VH CDR3	VL CDR3
R_1	MDCVIGSYGYGIFDT	QSFVRNSTS
R_2	GKVLKKAEYSDWLDN	QQCSKFPLT
R_3	RSASCEH	EQYDTAPPYT
R_4	GEVGFDY	QQYFSQPFT
R_5	TLECSRCGDYGFDL	HQSNTYPFT
R_6	DGLYARMYYNGSYY	QQYFSQPFT
R_7	ERRDDDGMYAYSYQFDV	Q*
R_8	DGGLREGFDC	*
R_9	NPASKCVYLEHDFEK	QTCNCLTLV
R_10	PERSAYDY	QQYSSHPLT
D_1	GDSHIIDC	QQSNILSVT
D_2	GSTAGFDY	QQDDSTPYT
D_3	EEGVDIEY	PSLNNSLTYIV

Fig. 23

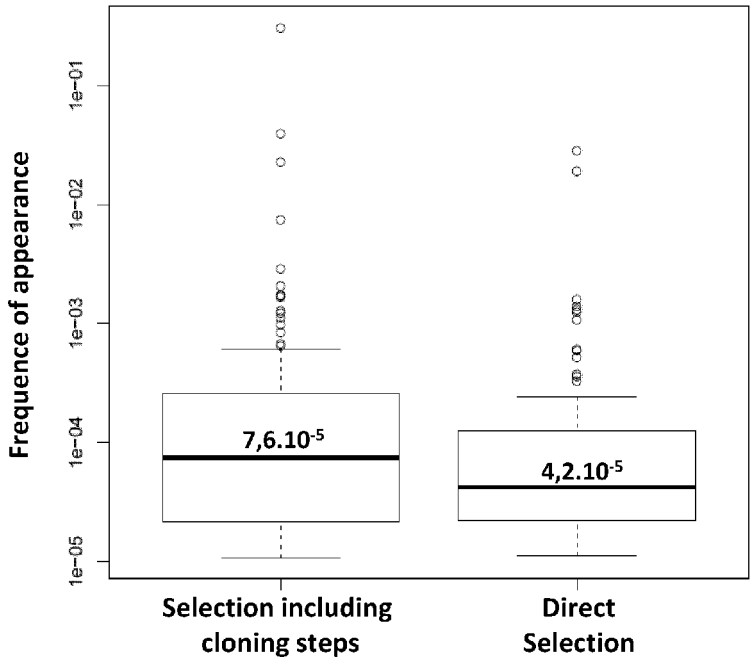

Frequence of appearance
1e-01
1e-02
1e-03
1e-04
1e-05
7,6.10-5
4,2.10-5
Selection including cloning steps
Direct Selection

Fig. 24

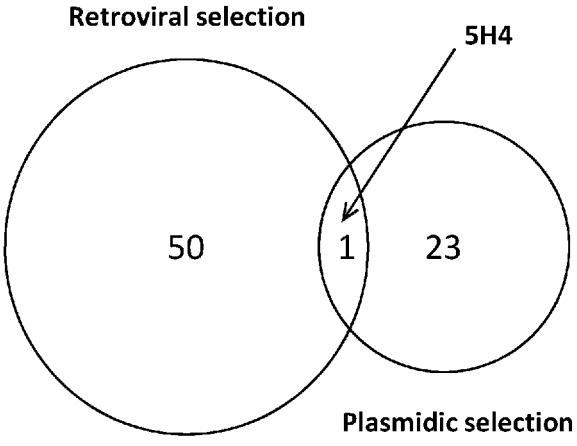

Retroviral selection
5H4
50
1
23
Plasmidic selection

Fig. 25

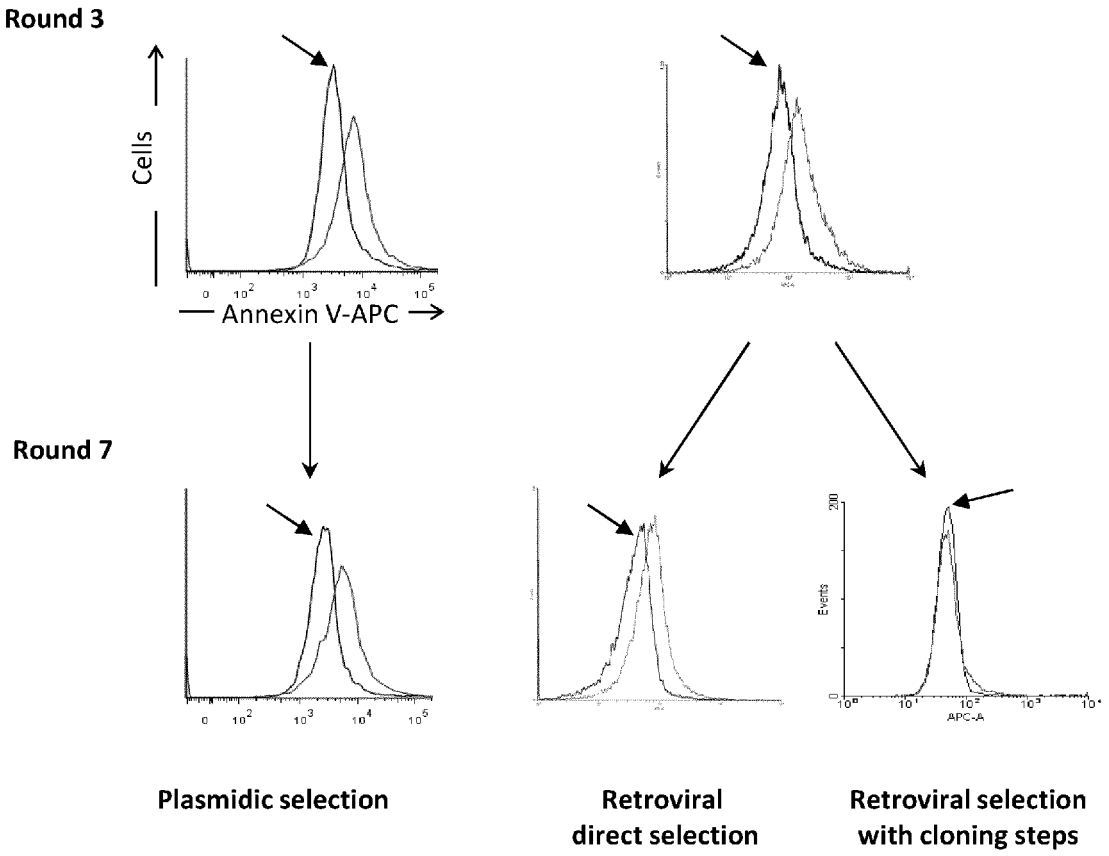

Round 3
Cells
Annexin V-APC
Round 7
Events
APC-A
Plasmidic selection
Retroviral direct selection
Retroviral selection with cloning steps

Fig. 26

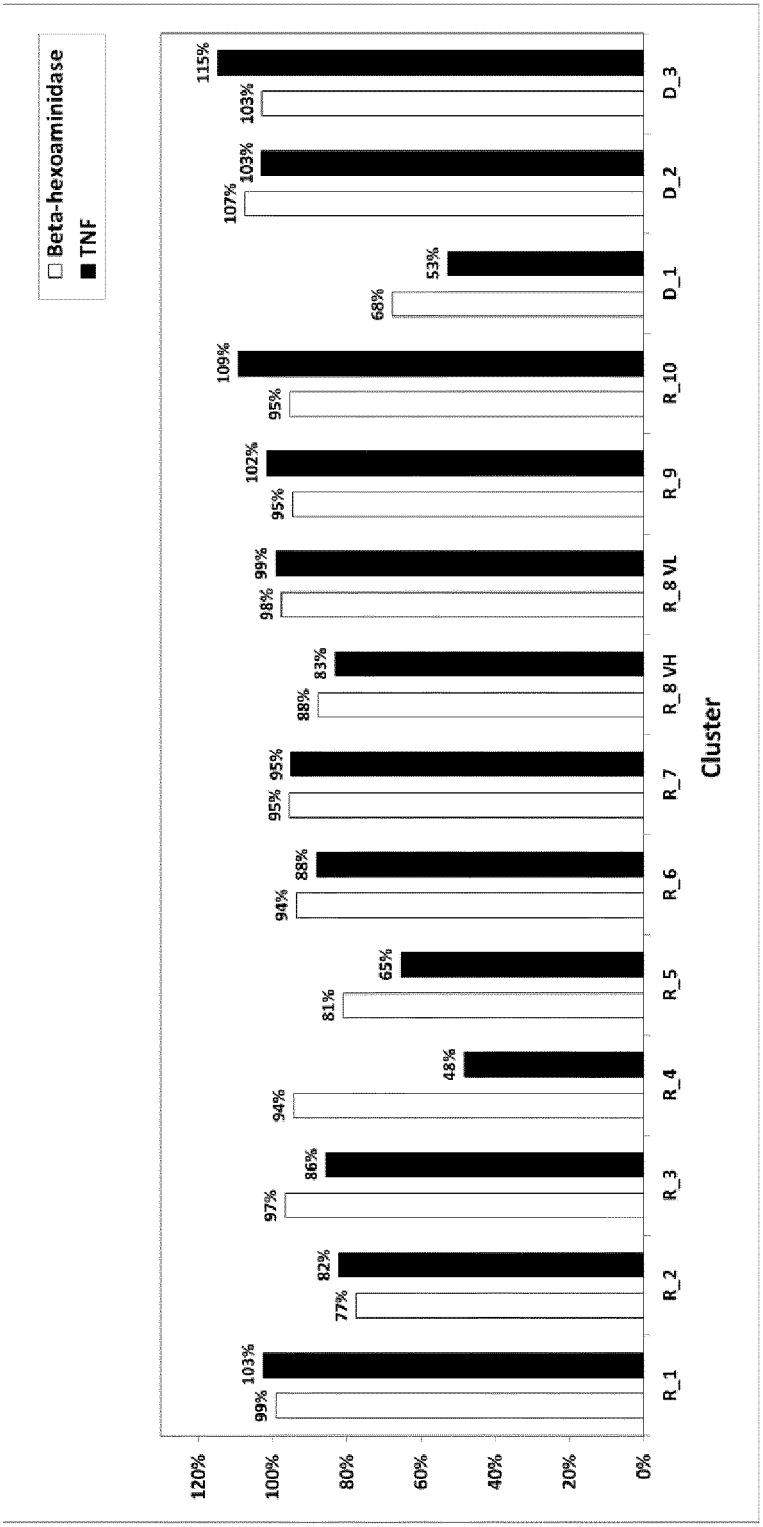

Beta-hexoaminidase
TNF
120%
100%
80%
60%
40%
20%
0%
Cluster
R_1
R_2
R_3
R_4
R_5
R_6
R_7
R_8 VH
R_8 VL
R_9
R_10
D_1
D_2
D_3
99%
103%
77%
82%
97%
86%
94%
48%
81%
65%
94%
88%
95%
95%
88%
83%
98%
99%
95%
102%
95%
109%
68%
53%
107%
103%
103%
115%

Fig. 27

```
13R4     MAEVQLVESGGSLVKPGGSLRLSCAASGFTFSNYSMNWVRQAPGKGLEWISSISGSSRYI
3H2-VH   MAEVQLVESGGSLVKPGGSLRLSCAASGFTFSNYSMNWVRQAPGKGLEWISSISGSSRYI
3H2-1    MAEVQLVESGGSLVKPGGSLRLSCAASGFTFSNYSMNWVRQAPGKGLEWISSISGSSRYI
5H4      MAEVQLVESGGSLVKPGGSLRLSCAASGFTFSNYSMNWVRQAPGKGLEWISSISGSSRYI
5H4-VH   MAEVQLVESGGSLVKPGGSLRLSCAASGFTFSNYSMNWVRQAPGKGLEWISSISGSSRYI
5H4-VL   MAEVQLVESGGSLVKPGGSLRLSCAASGFTFSNYSMNWVRQAPGKGLEWISSISGSSRYI
           <------------FR1-------------><-H1><-----FR2----><------H2
           ^VH start

13R4     YYADFVKGRFTISRDNATNSLYLQMNSLRAEDTAVYYCVRSSITIFGGGMDVWGRGTLVT
3H2-VH   YYADFVKGRFTISRDNATNSLYLQMNSLRAEDTAVYYCVRGVRGGY--GLDFWGRGTLVT
3H2-1    YYADFVKGRFTISRDNATNSLYLQMNSLRAEDTAVYYCVRPIA-----VSDYWGRGTLVT
5H4      YYADFVKGRFTISRDNATNSLYLQMNSLRAEDTAVYYCVRDGGLRE--GFDCWGRGTLVT
5H4-VH   YYADFVKGRFTISRDNATNSLYLQMNSLRAEDTAVYYCVRDGGLRE--GFDCWGRGTLVT
5H4-VL   YYADFVKGRFTISRDNATNSLYLQMNSLRAEDTAVYYCVRDGGLRE--GFDCWGRGTLVT
         -------><------------FR3---------------><----H3----><----FR4

13R4     VSSGGGGSGGGGSGGGGSQSVLTQPASVSGSPGQSITISCAGTSSDVGGYNYVSWYQQHP
3H2-VH   VSSGGGGSGGG----------------------------------------------------
3H2-1    VSSGGGGSGGGGSGGGGSQSVLTQPASVSGSPGQSITISCAGTSSDVGGYNYVSWYQQHP
5H4      VSSGGGGSGGGGSGGGGSQSVLTQPASVSGSPGQSITISCAGTSSDVGGYNYVSWYQQHP
5H4-VH   VSS-----------------------------------------------------------
5H4 VL   VSSGGGGSGGGGSGGGGSQSVLTQPASVSGSPGQSITISCAGTSSDVGGYNYVSWYQQHP
         -->               <--------FR1---------><-----L1-----><--FR2
           ^VH end         ^VL start

13R4     GKAPKLMIYEDSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTTRSTRVFG
3H2 VH
3H2-1    GKAPKLMIYEDSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCQTYDG-SRAVFG
5H4      GKAPKLMIYEDSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCPS*---------
5H4 VH
5H4-VL   GKAPKLMIYEDSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYC-------------
         --------><-L2--><---------FR3------------------><----L3--->< 

13R4     GGTKLAVLGAAAEQKLISEEDLNGAA
3H2-VH   --TKLAVLGAAAEQKLISEEDLNGAA
3H2-1    GGTKLAVLGAAAEQKLISEEDLNGAA
5H4
5H4-VH   ----------AAAEQKLISEEDLNGAA
5H4-VL   ----------AAAEQKLISEEDLNGAA
         --FR4--->    <---myc_tag-->

                ^VL end
```

Fig. 28