Fig. 1

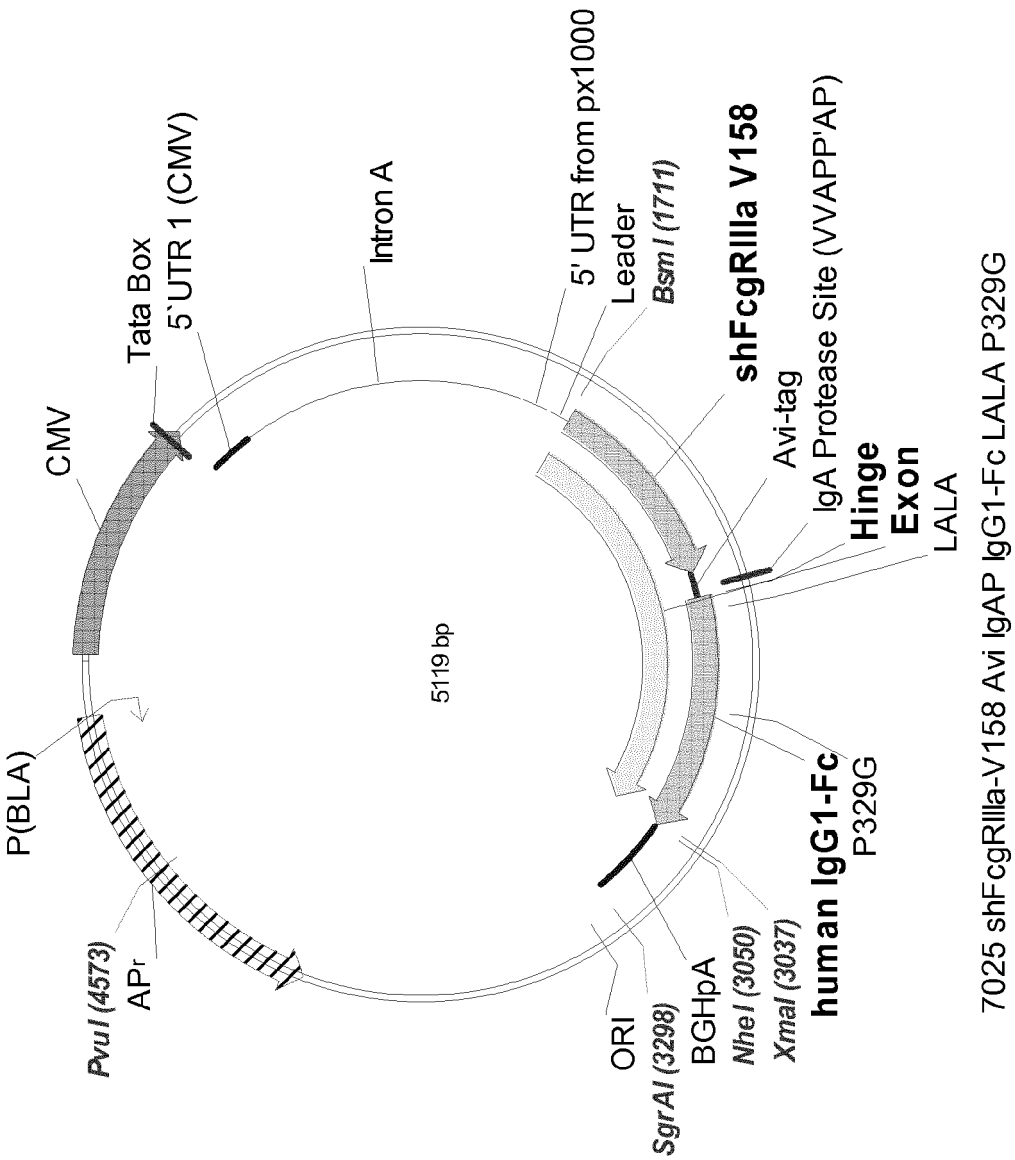
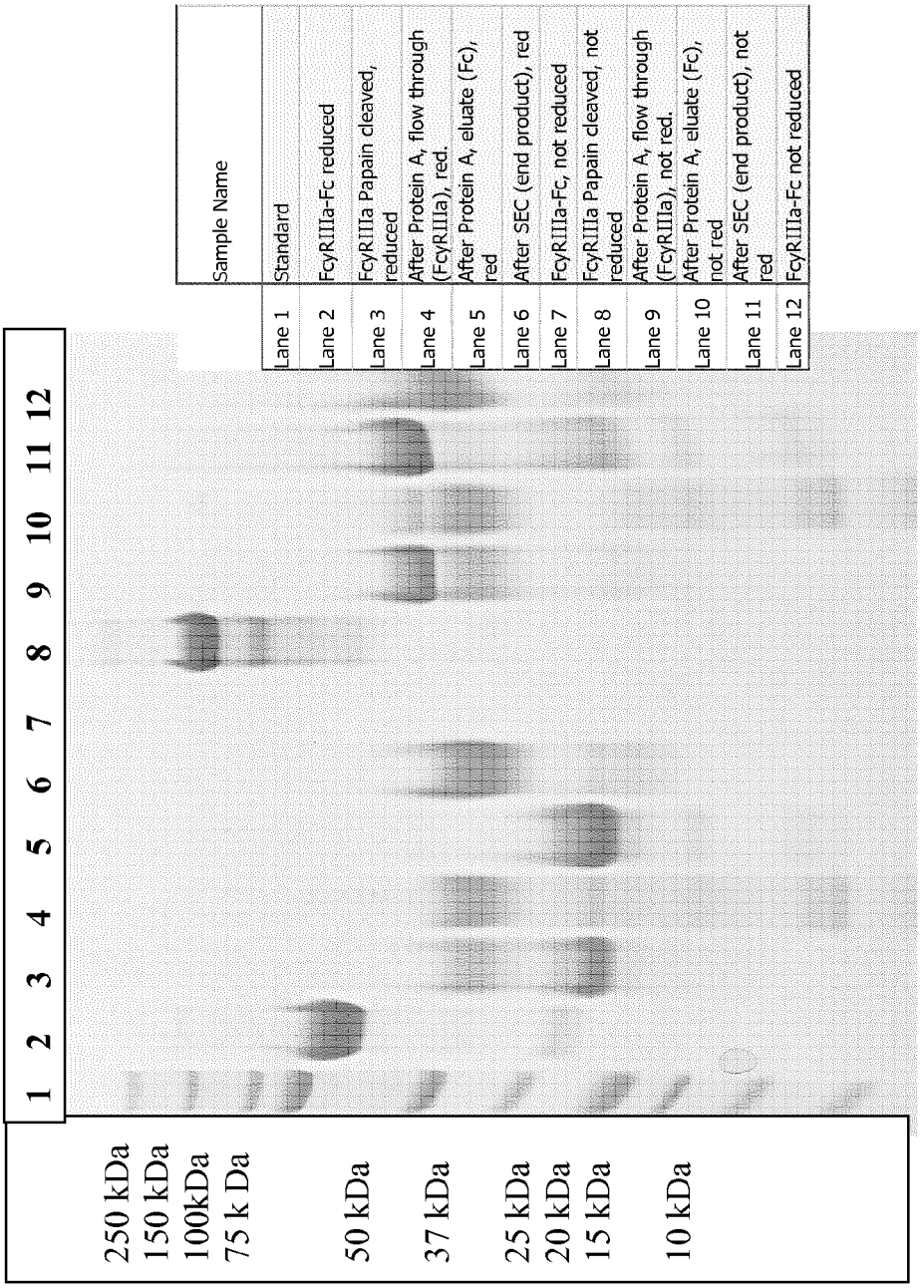

CMV
Tata Box
5`UTR 1 (CMV)
Intron A
5' UTR from px1000
Leader
BsmI (1711)
shFcgRIIIa V158
Avi-tag
IgA Protease Site (VVAPP'AP)
Hinge
Exon
LALA
P329G
human IgG1-Fc
XmaI (3037)
NheI (3050)
BGHpA
SgrAI (3298)
ORI
APr
PvuI (4573)
P(BLA)
5119 bp
7025 shFcgRIIIa-V158 Avi IgAP IgG1-Fc LALA P329G

Fig. 2



Sample Name
Lane 1 Standard
Lane 2 FcγRIIIa-Fc reduced
Lane 3 FcγRIIIa Papain cleaved, reduced
Lane 4 After Protein A, flow through (FcγRIIIa), red.
Lane 5 After Protein A, eluate (Fc), red
Lane 6 After SEC (end product), red
Lane 7 FcγRIIIa-Fc, not reduced
Lane 8 FcγRIIIa Papain cleaved, not reduced
Lane 9 After Protein A, flow through (FcγRIIIa), not red.
Lane 10 After Protein A, eluate (Fc), not red
Lane 11 After SEC (end product), not red
Lane 12 FcγRIIIa-Fc not reduced

Fig. 3

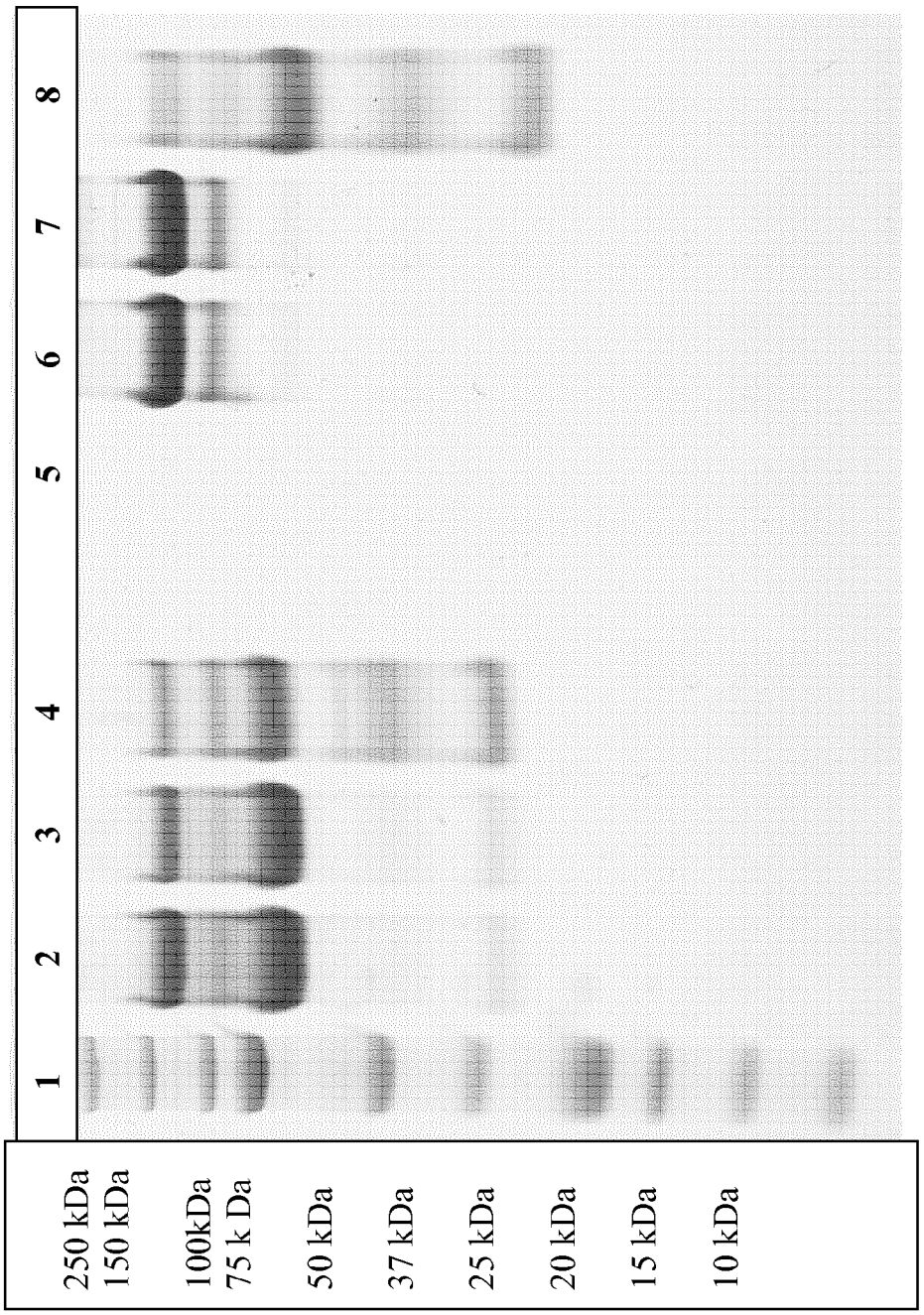

1
2
3
4
5
6
7
8
250 kDa
150 kDa
100kDa
75 k Da
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa

Fig. 4

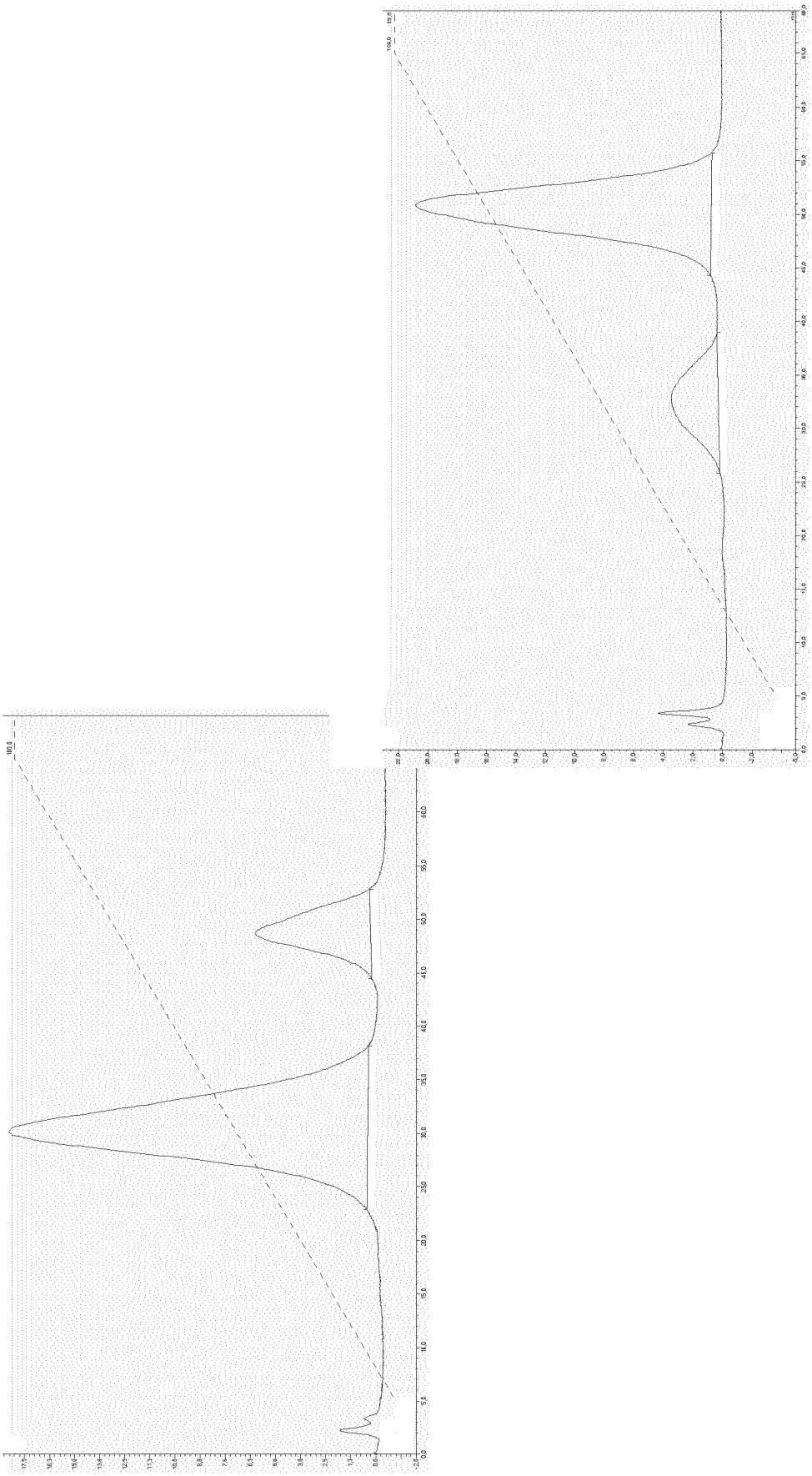
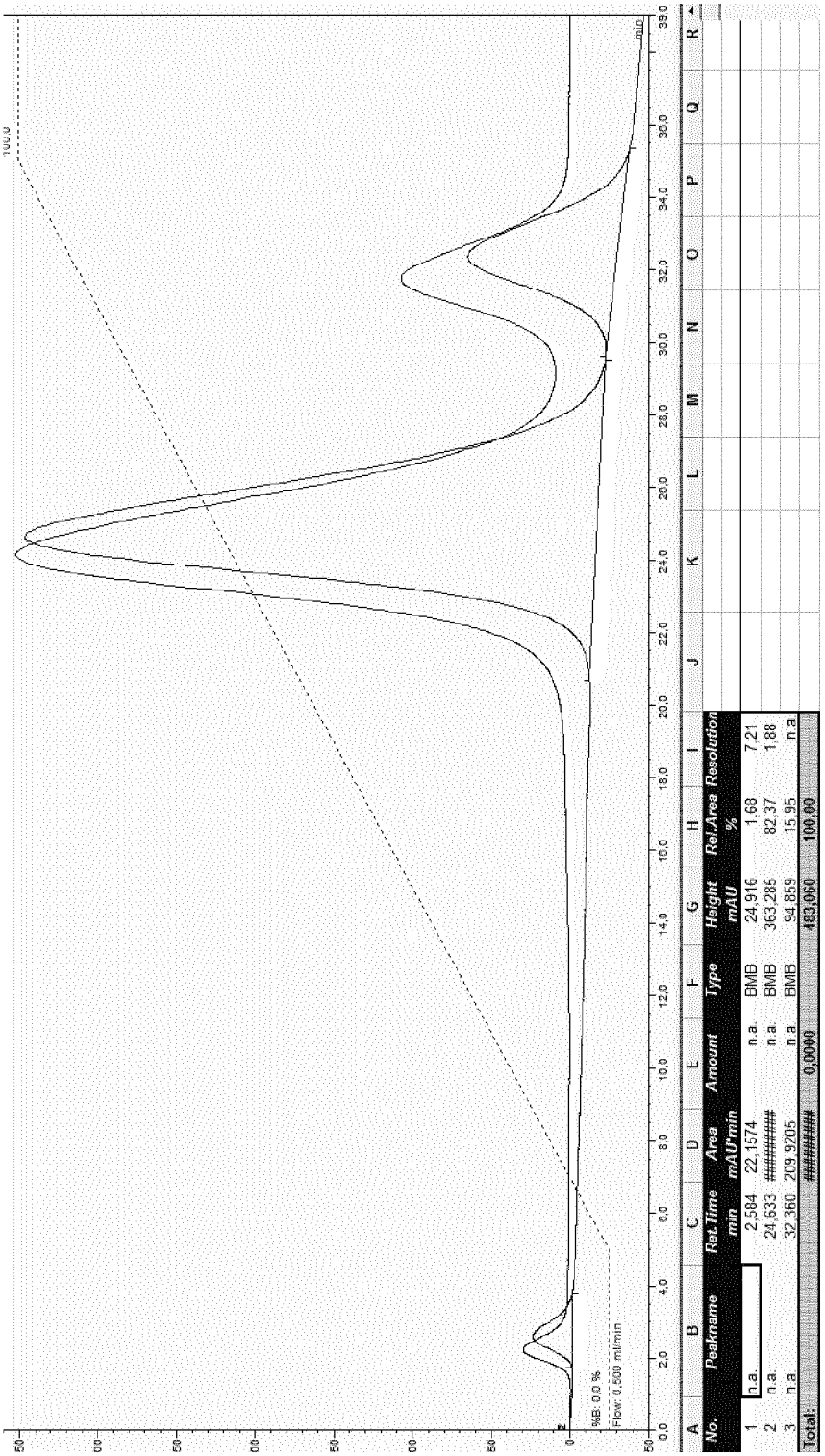


Fig. 5



No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	2.584	22.1574	n.a.	BMB	24.916	1.68	7.21
2	n.a.	24.633	########	n.a.	BMB	363.285	82.37	1.88
3	n.a	32.360	209.9205	n.a	BMB	94.859	15.95	n.a
Total:			########	0.0000		483.060	100.00	

Fig. 6

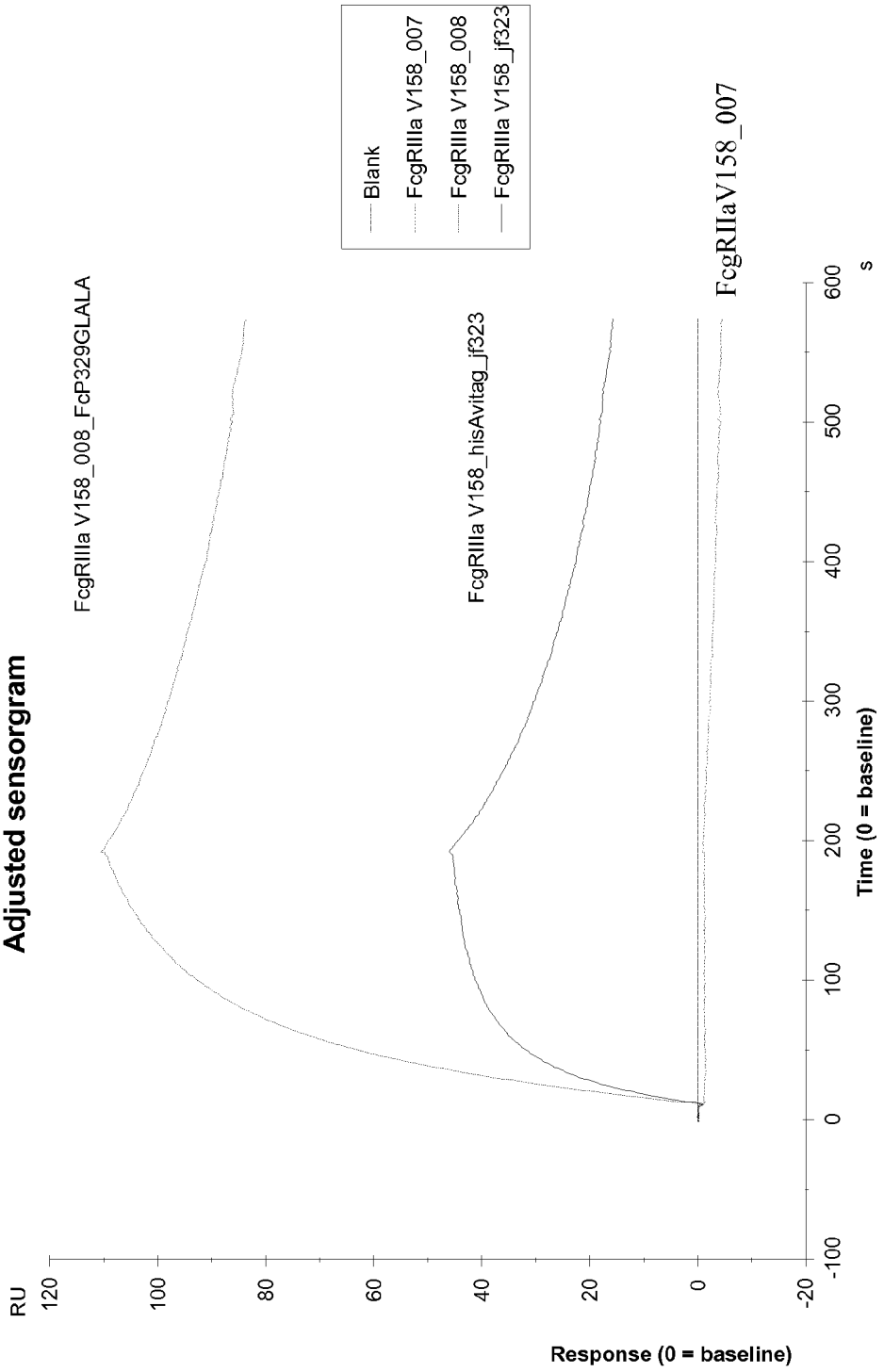

Adjusted sensorgram
Blank
FcgRIIIa V158_007
FcgRIIIa V158_008
FcgRIIIa V158_jf323
FcgRIIIa V158_008_FcP329GLALA
FcgRIIIa V158_hisAvitag_jf323
FcgRIIaV158_007
RU
120
100
80
60
40
20
0
-20
-100
0
100
200
300
400
500
600
s
Time (0 = baseline)
Response (0 = baseline)

Fig. 7a

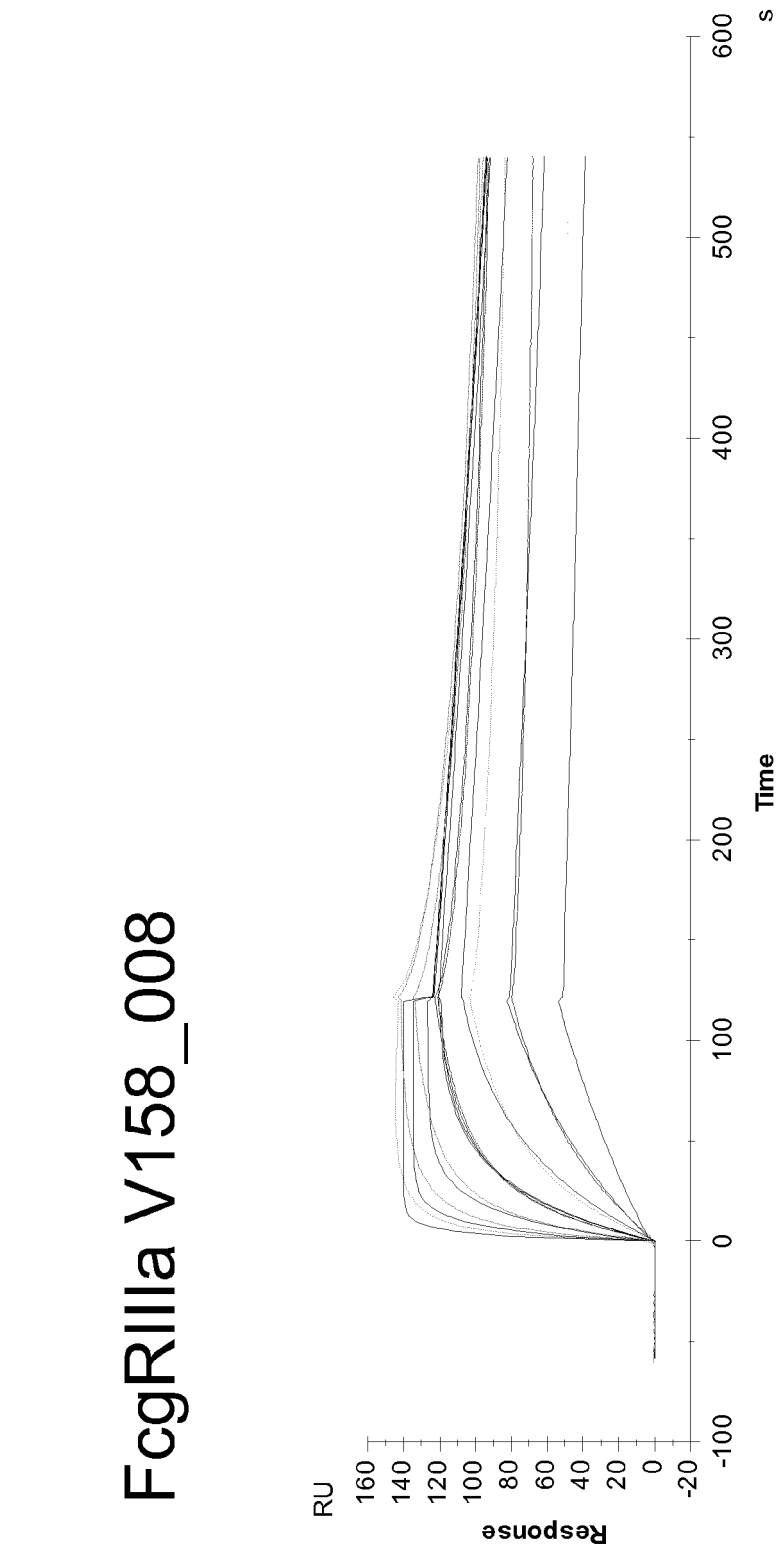

FcgRIIIa V158_008
RU
Response
160
140
120
100
80
60
40
20
0
-20
-100
0
100
200
300
400
500
600
Time
s
ka (1/Ms)
5.961E+5
kd (1/s)
6.535E-4
KD (M)
1.096E-9

Fig. 7b

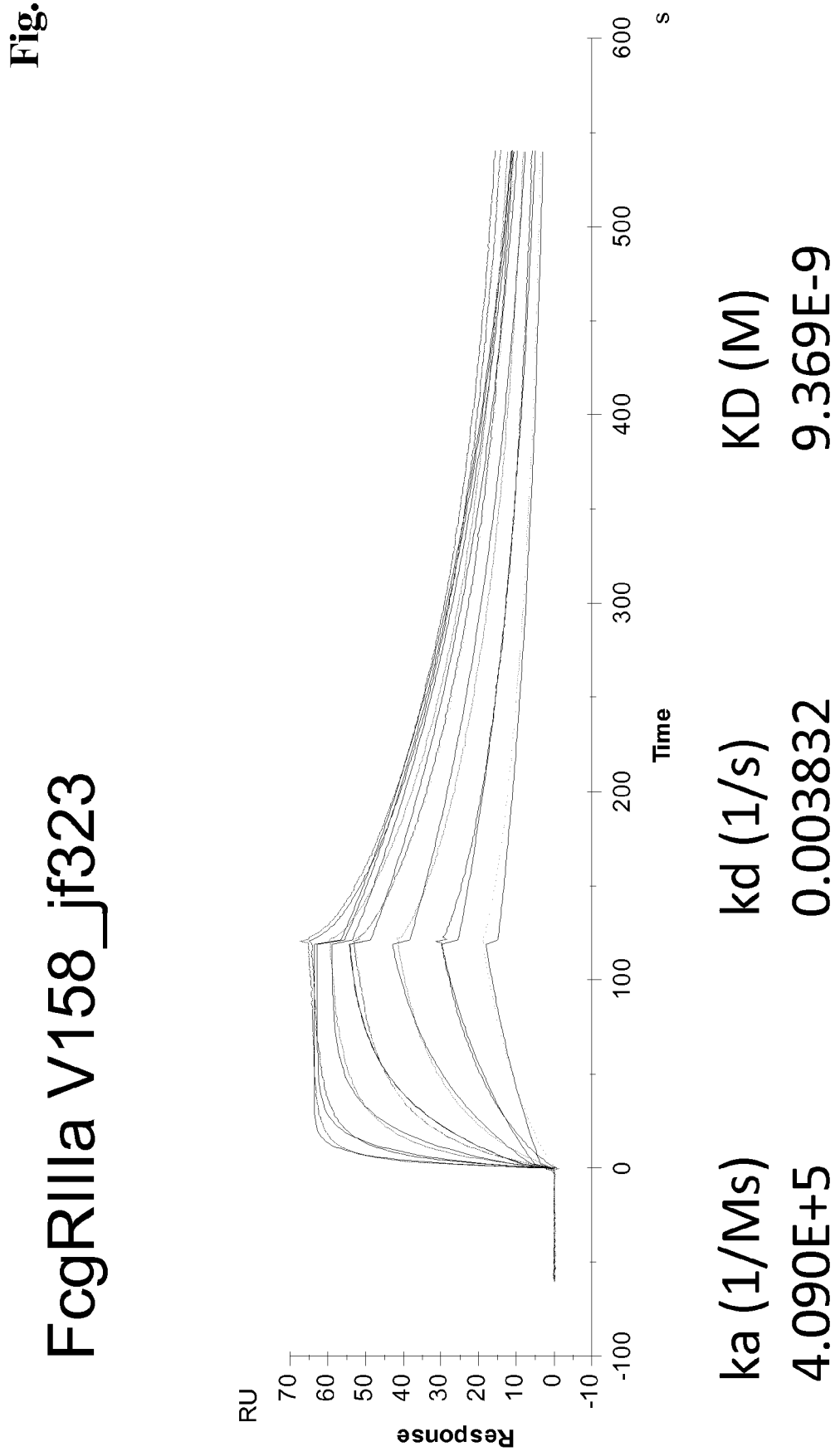

FcgRIIIa V158_jf323
Response
RU
70
60
50
40
30
20
10
0
-10
-100
0
100
200
300
400
500
600
s
Time
ka (1/Ms)
4.090E+5
kd (1/s)
0.003832
KD (M)
9.369E-9

Fig. 7c

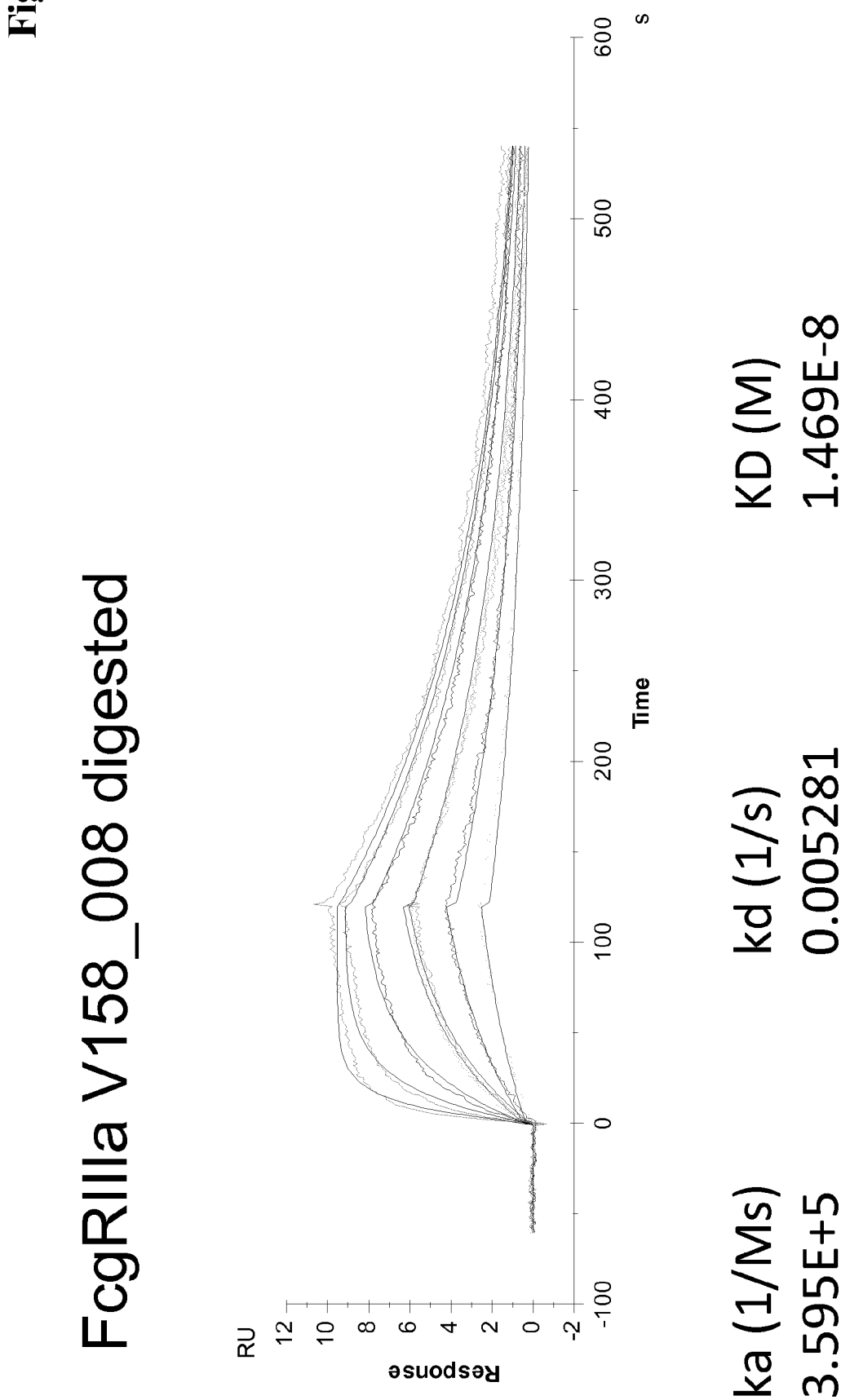
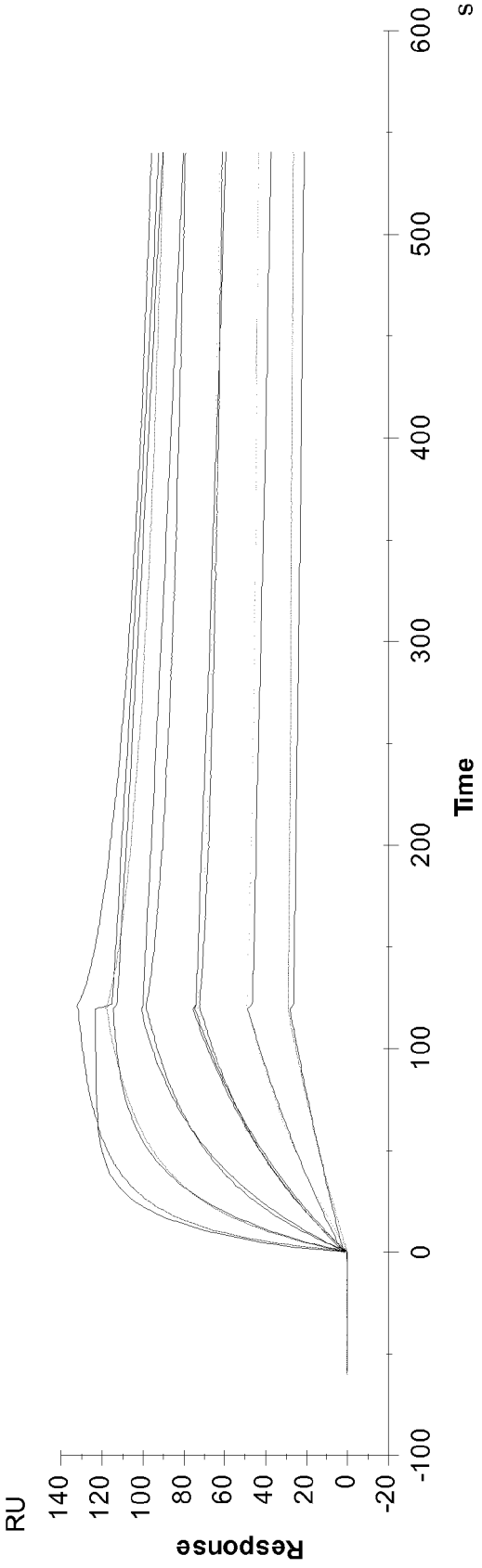

FcgRIIIa V158_008 digested
RU
Response
12
10
8
6
4
2
0
-2
-100
0
100
200
300
400
500
600
Time
s
ka (1/Ms)
3.595E+5
kd (1/s)
0.005281
KD (M)
1.469E-8

Fig. 7d

FcgRIIIa V158_008 un-digested



ka (1/Ms)	kd (1/s)	KD (M)
2.846E+5	5.276E-4	1.854E-9

Fig. 8

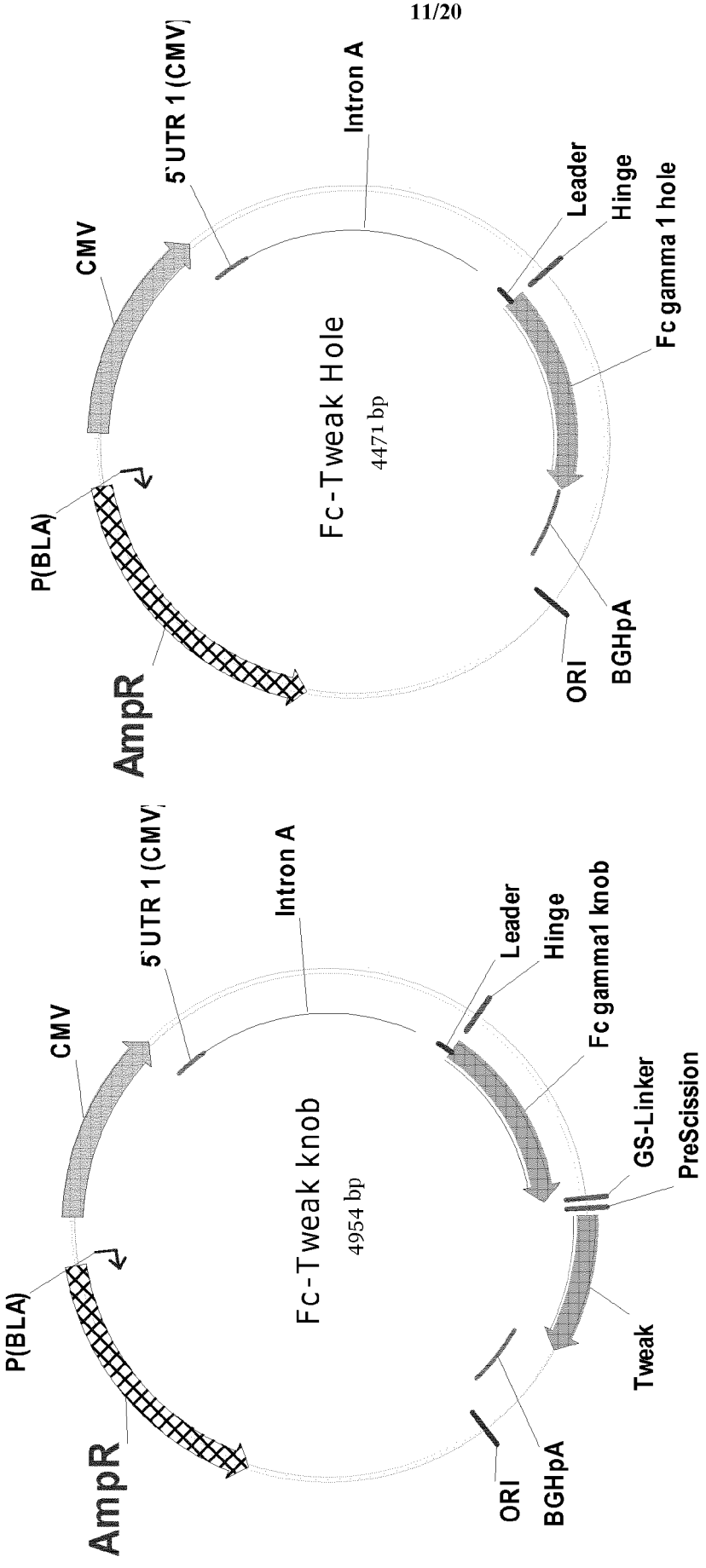

Fc-Tweak Hole
4471 bp
CMV
5'UTR 1 (CMV)
Intron A
Leader
Hinge
Fc gamma 1 hole
P(BLA)
AmpR
ORI
BGHpA
Fc-Tweak knob
4954 bp
CMV
5'UTR 1 (CMV)
Intron A
Leader
Hinge
Fc gamma1 knob
GS-Linker
PreScission
Tweak
P(BLA)
AmpR
ORI
BGHpA

Fig. 9

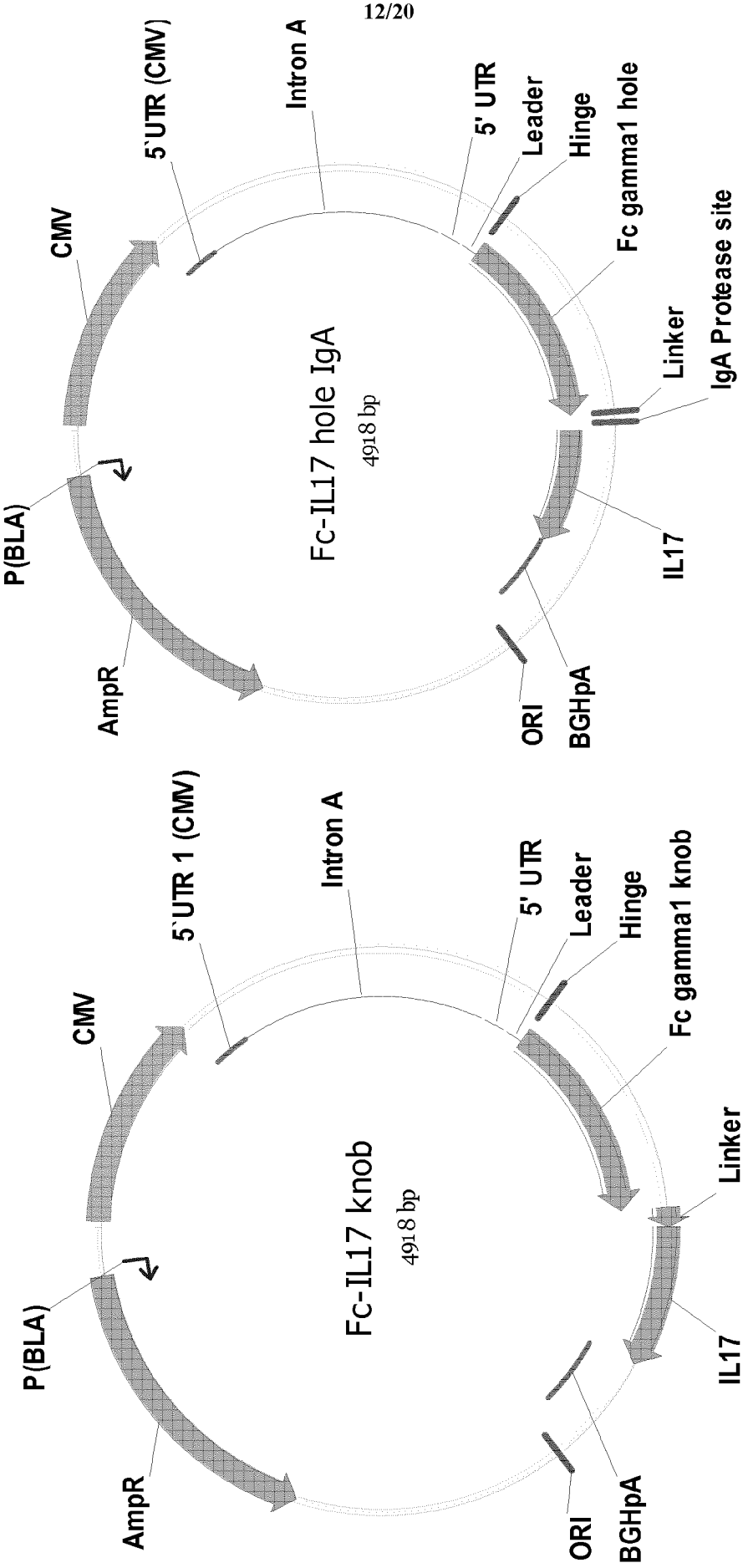

Fc-IL17 hole IgA
4918 bp
CMV
5'UTR (CMV)
Intron A
5' UTR
Leader
Hinge
Fc gamma1 hole
Linker
IgA Protease site
IL17
BGHpA
ORI
AmpR
P(BLA)
Fc-IL17 knob
4918 bp
CMV
5'UTR 1 (CMV)
Intron A
5' UTR
Leader
Hinge
Fc gamma1 knob
Linker
IL17
BGHpA
ORI
AmpR
P(BLA)

Fig. 10

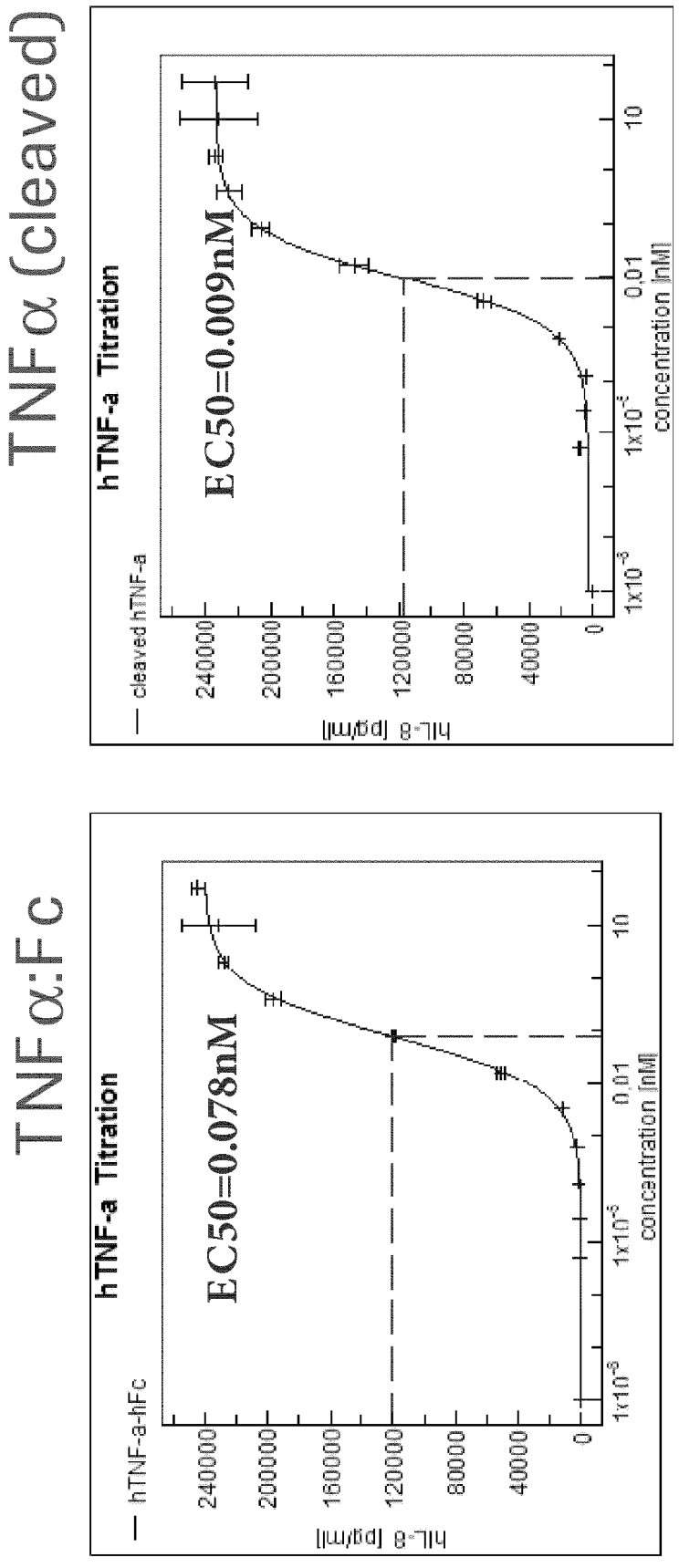

TNFα (cleaved)
hTNF-a Titration
cleaved hTNF-a
EC50=0.009nM
hIL-8 [pg/ml]
concentration [nM]
TNFα:Fc
hTNF-a Titration
hTNF-a-hFc
EC50=0.078nM
hIL-8 [pg/ml]
concentration [nM]

Fig. 11

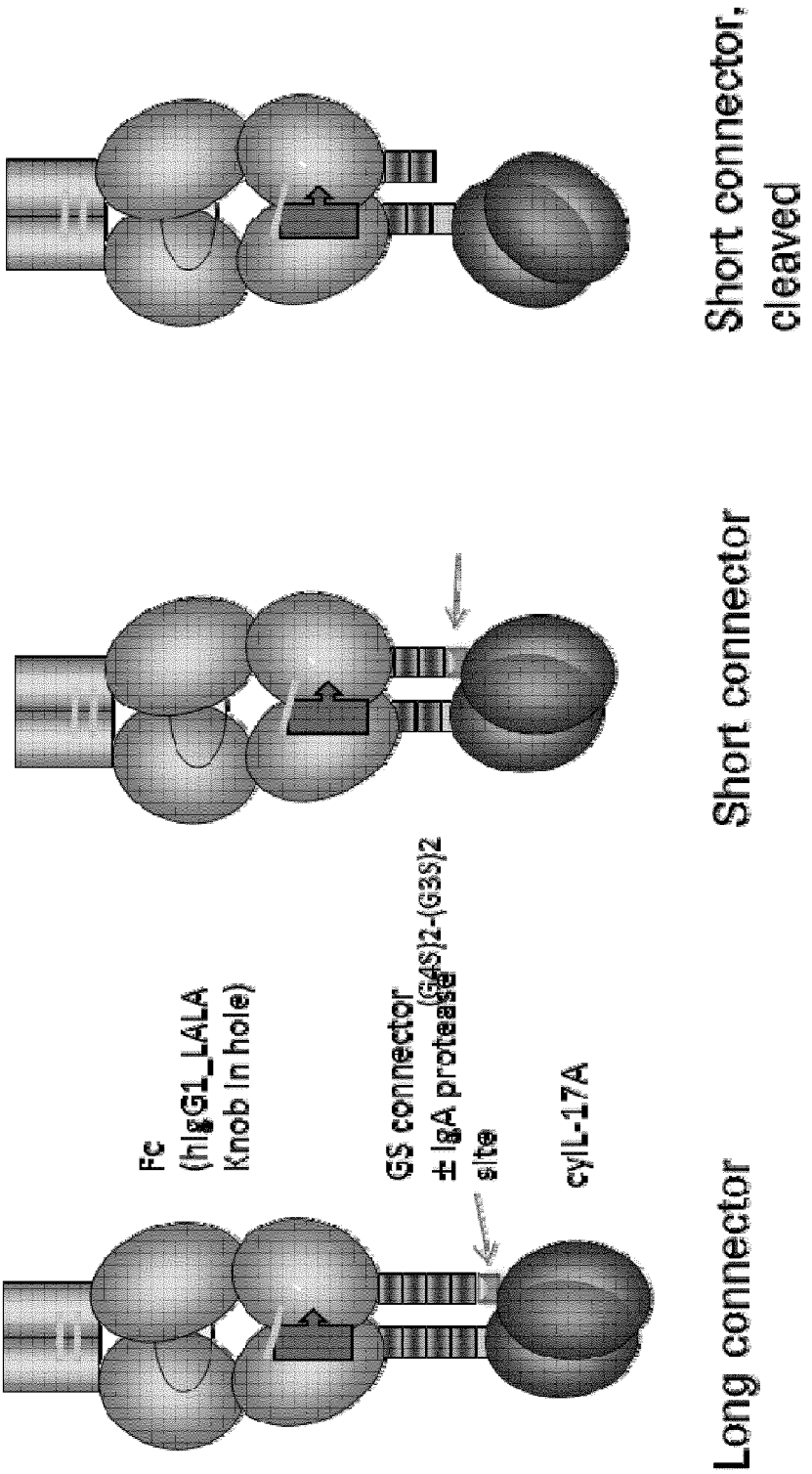
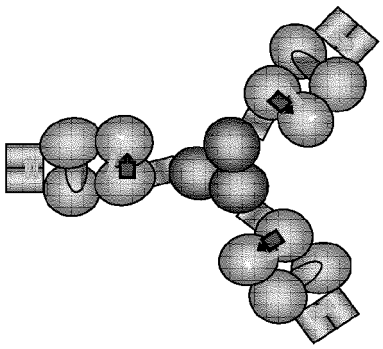

Fc
(hIgG1_LALA
Knob In hole)
GS connector
± IgA protease
site
(G4S)2-(G3S)2
cyIL-17A
Long connector
Short connector
Short connector,
cleaved

Fig. 12



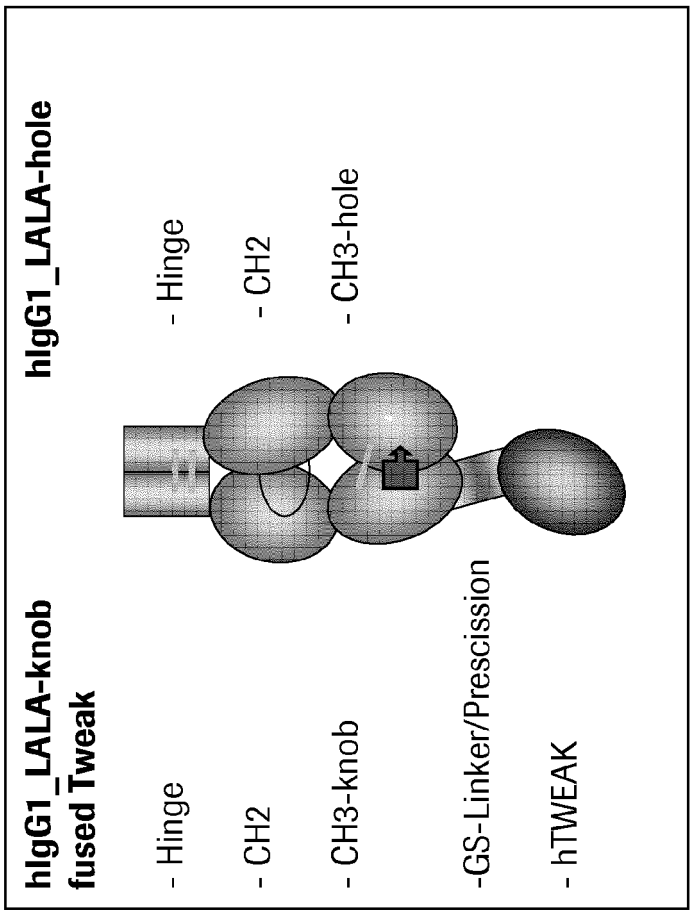

hIgG1_LALA-knob
fused Tweak
- Hinge
- CH2
- CH3-knob
-GS-Linker/Prescission
- hTWEAK
hIgG1_LALA-hole
- Hinge
- CH2
- CH3-hole

Fig. 13

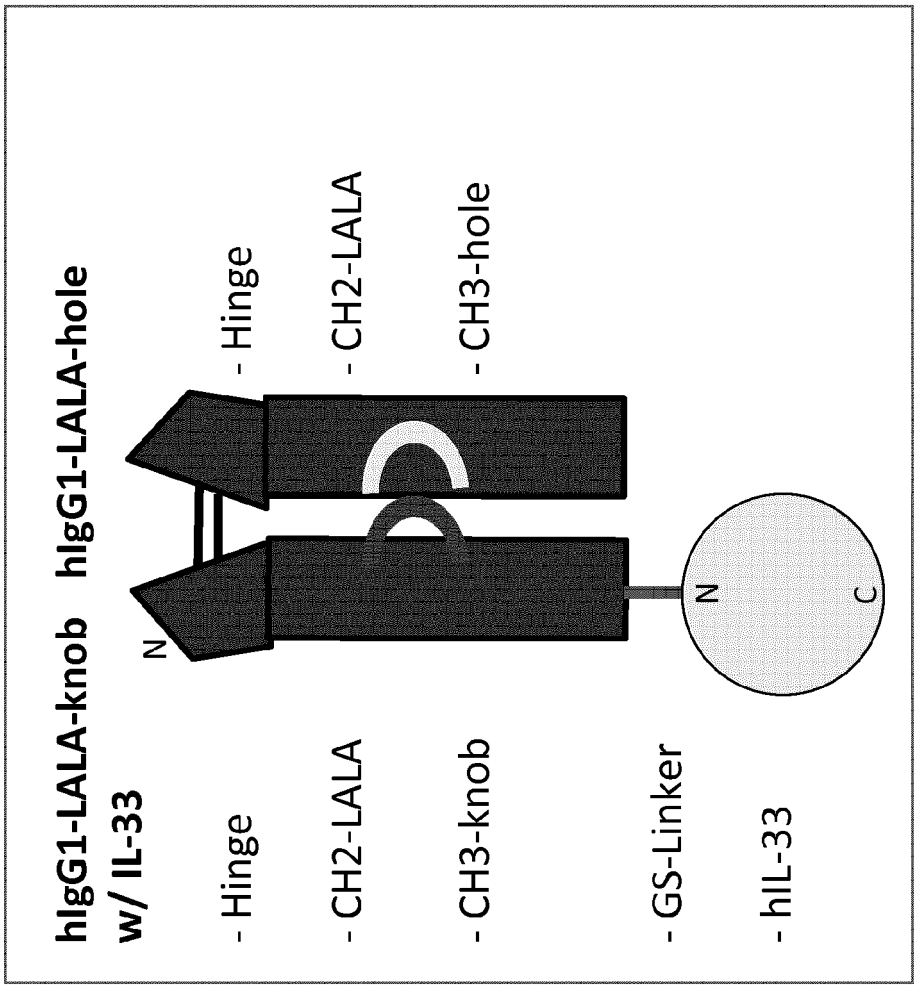

hIgG1-LALA-knob hIgG1-LALA-hole w/ IL-33
- Hinge
- CH2-LALA
- CH3-knob
- GS-Linker
- hIL-33
- Hinge
- CH2-LALA
- CH3-hole
N
N
C

Fig. 14

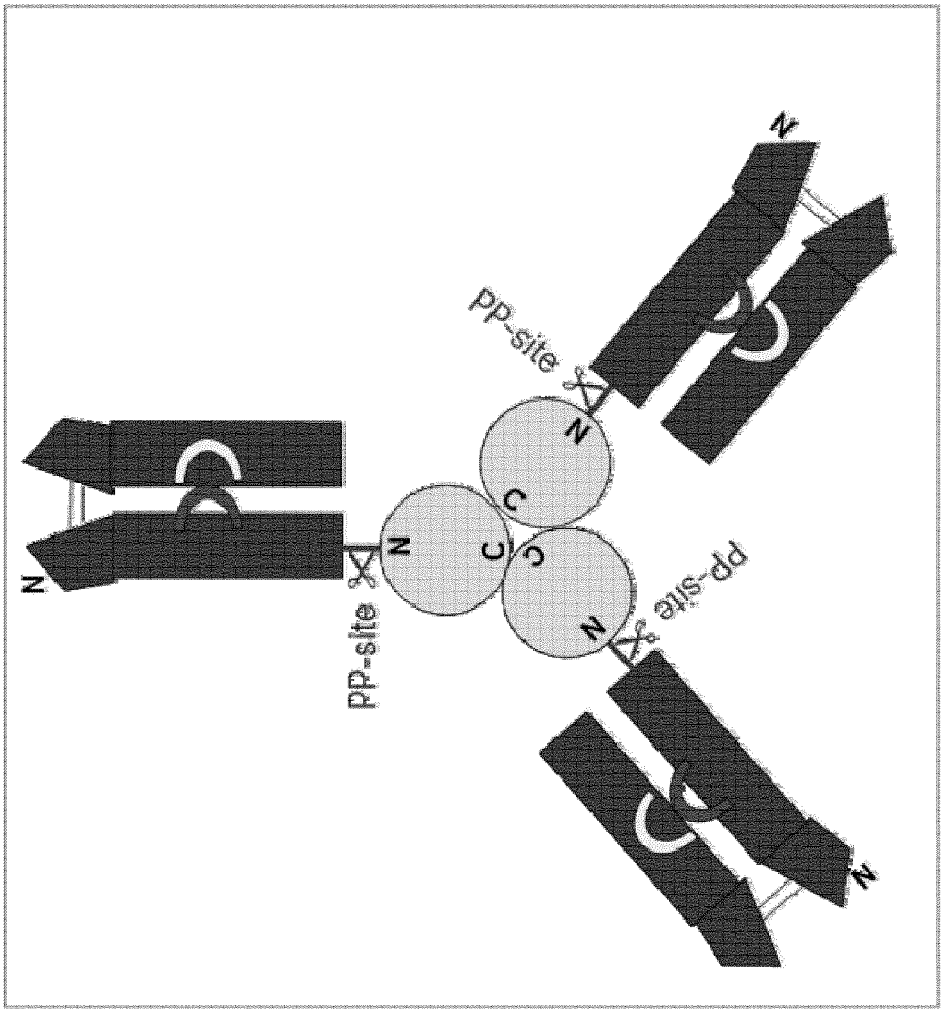


Fig. 15

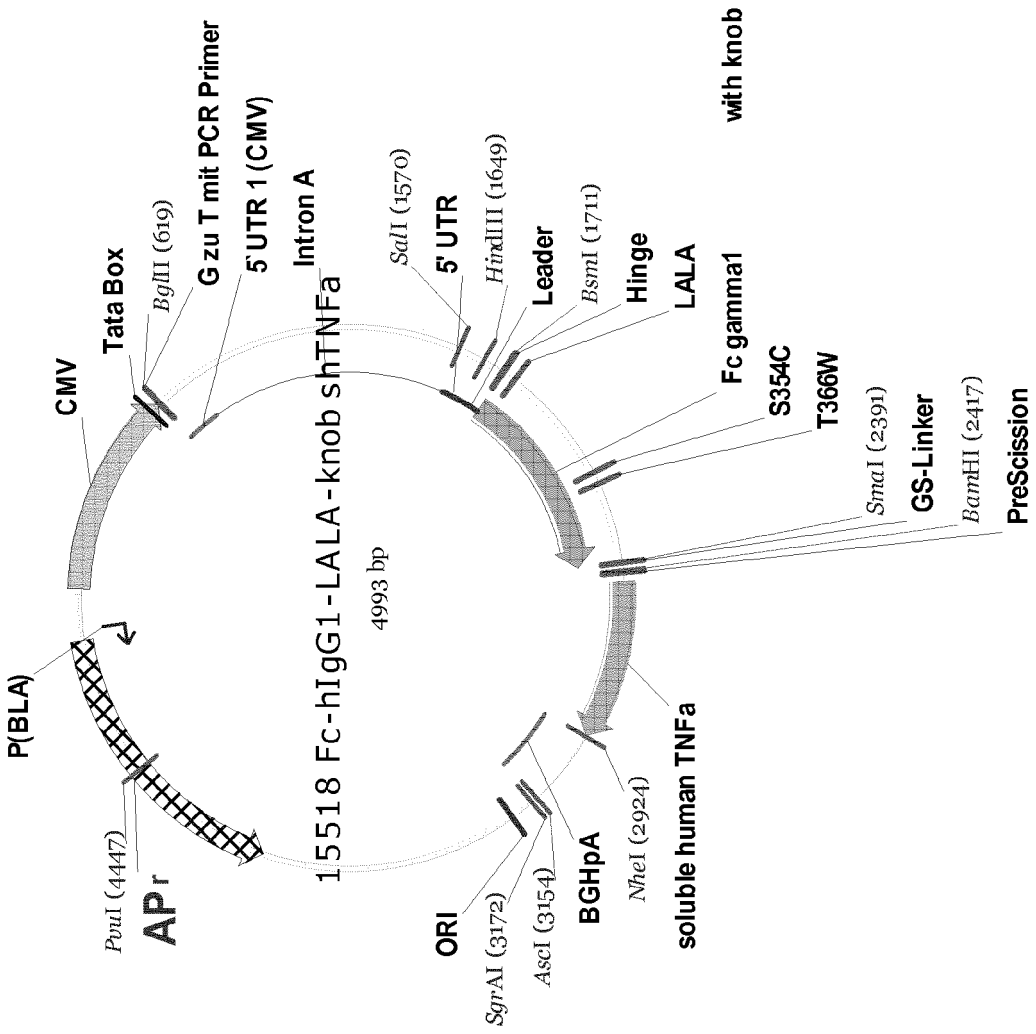

15518 Fc-hIgG1-LALA-knob shTNFa
4993 bp
CMV
Tata Box
BglII (619)
G zu T mit PCR Primer
5'UTR 1 (CMV)
Intron A
SalI (1570)
5' UTR
HindIII (1649)
Leader
BsmI (1711)
Hinge
LALA
Fc gamma1
with knob
S354C
T366W
SmaI (2391)
GS-Linker
BamHI (2417)
PreScission
soluble human TNFa
NheI (2924)
BGHpA
AscI (3154)
SgrAI (3172)
ORI
PvuI (4447)
APr
P(BLA)

Fig. 16

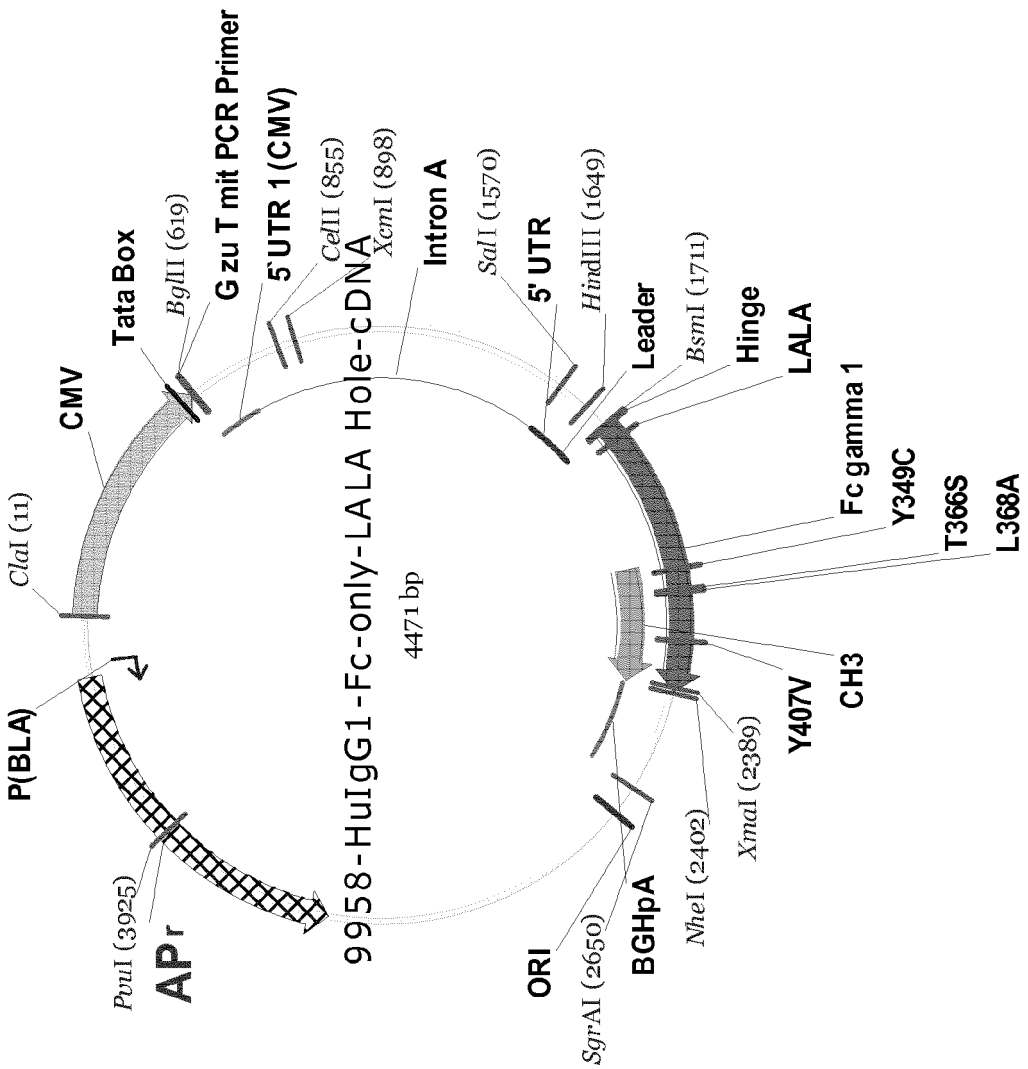

9958-HuIgG1-Fc-only-LALA Hole-cDNA
4471 bp
ClaI (11)
CMV
Tata Box
BglII (619)
G zu T mit PCR Primer
5'UTR 1 (CMV)
CelII (855)
XcmI (898)
Intron A
SalI (1570)
5' UTR
HindIII (1649)
Leader
BsmI (1711)
Hinge
LALA
Fc gamma 1
Y349C
T366S
L368A
CH3
Y407V
XmaI (2389)
NheI (2402)
BGHpA
ORI
SgrAI (2650)
APr
PvuI (3925)
P(BLA)

Fig. 17

