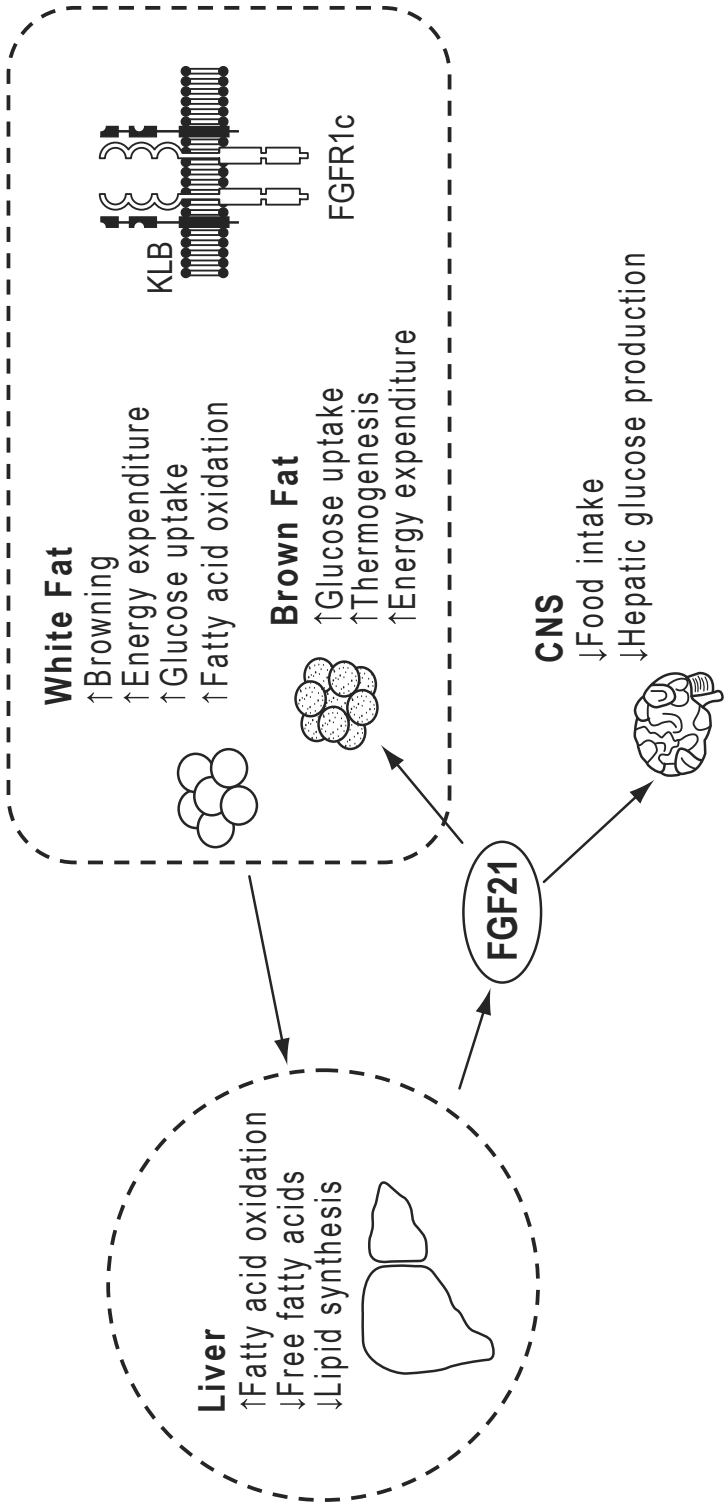

White Fat
↑Browning
↑Energy expenditure
↑Glucose uptake
↑Fatty acid oxidation
KLB
FGFR1c
Brown Fat
↑Glucose uptake
↑Thermogenesis
↑Energy expenditure
CNS
↓Food intake
↓Hepatic glucose production
FGF21
Liver
↑Fatty acid oxidation
↓Free fatty acids
↓Lipid synthesis

FIG. 1

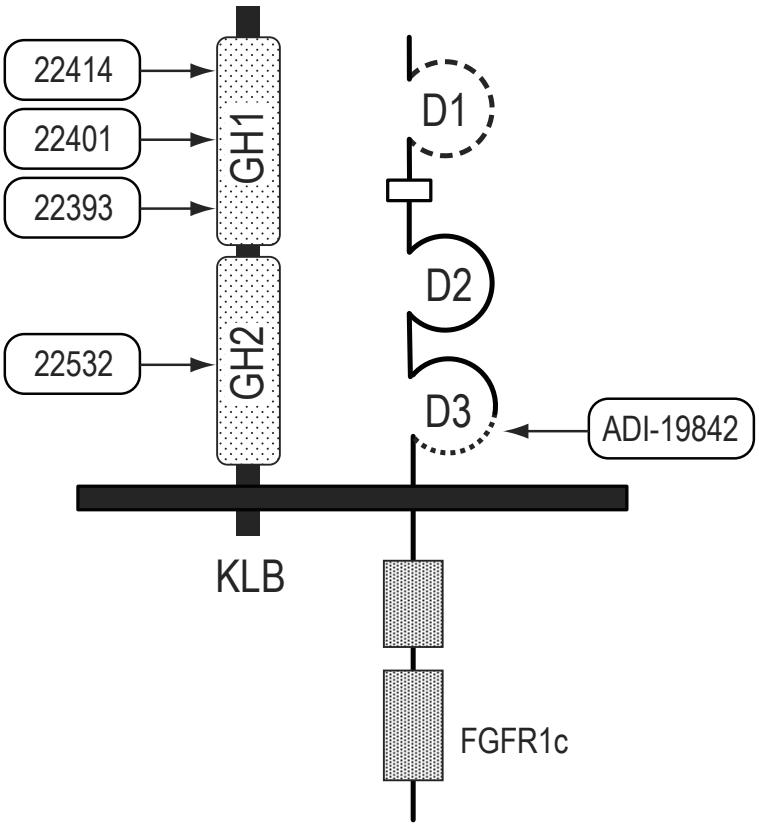


FIG. 2

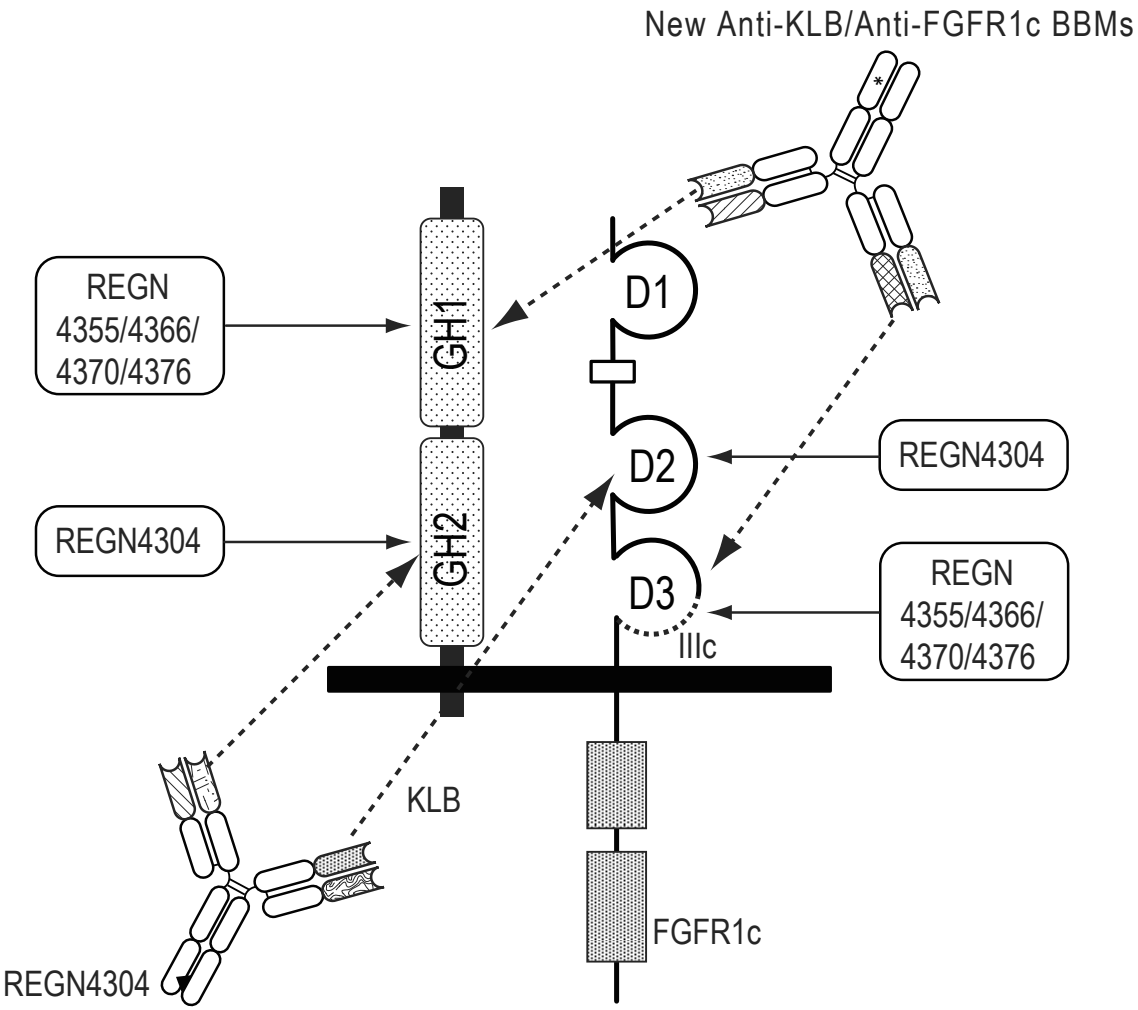

New Anti-KLB/Anti-FGFR1c BBMs
REGN 4355/4366/ 4370/4376
REGN4304
GH1
GH2
D1
D2
D3
IIIc
REGN4304
REGN 4355/4366/ 4370/4376
KLB
FGFR1c
REGN4304

FIG. 3

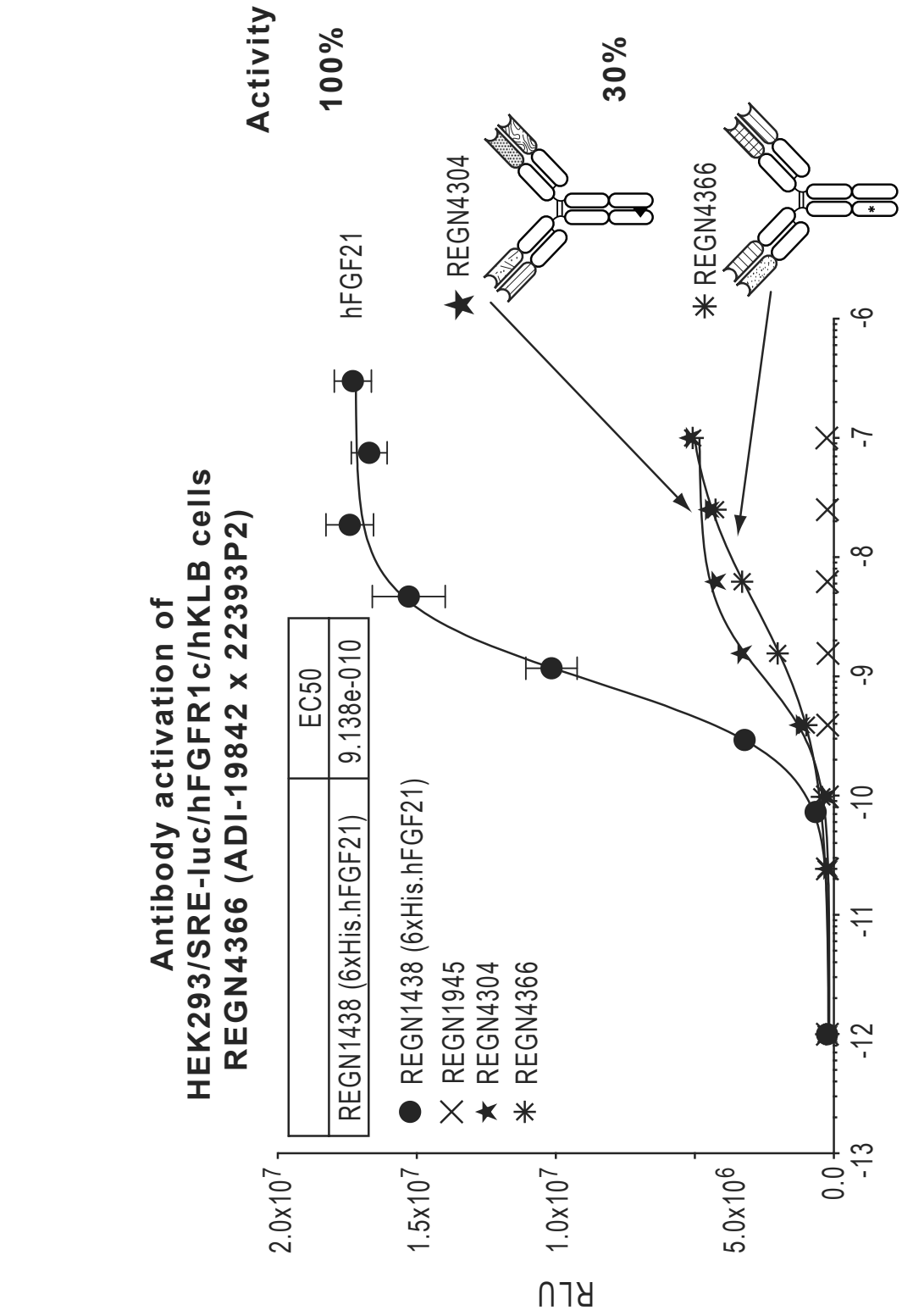

Antibody activation of
HEK293/SRE-luc/hFGFR1c/hKLB cells
REGN4366 (ADI-19842 x 22393P2)
Activity
100%
30%
hFGF21
REGN4304
REGN4366
EC50
REGN1438 (6xHis.hFGF21)
9.138e-010
REGN1438 (6xHis.hFGF21)
REGN1945
REGN4304
REGN4366
RLU
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0.0
-13
-12
-11
-10
-9
-8
-7
-6
Log 10 [M]

FIG. 4

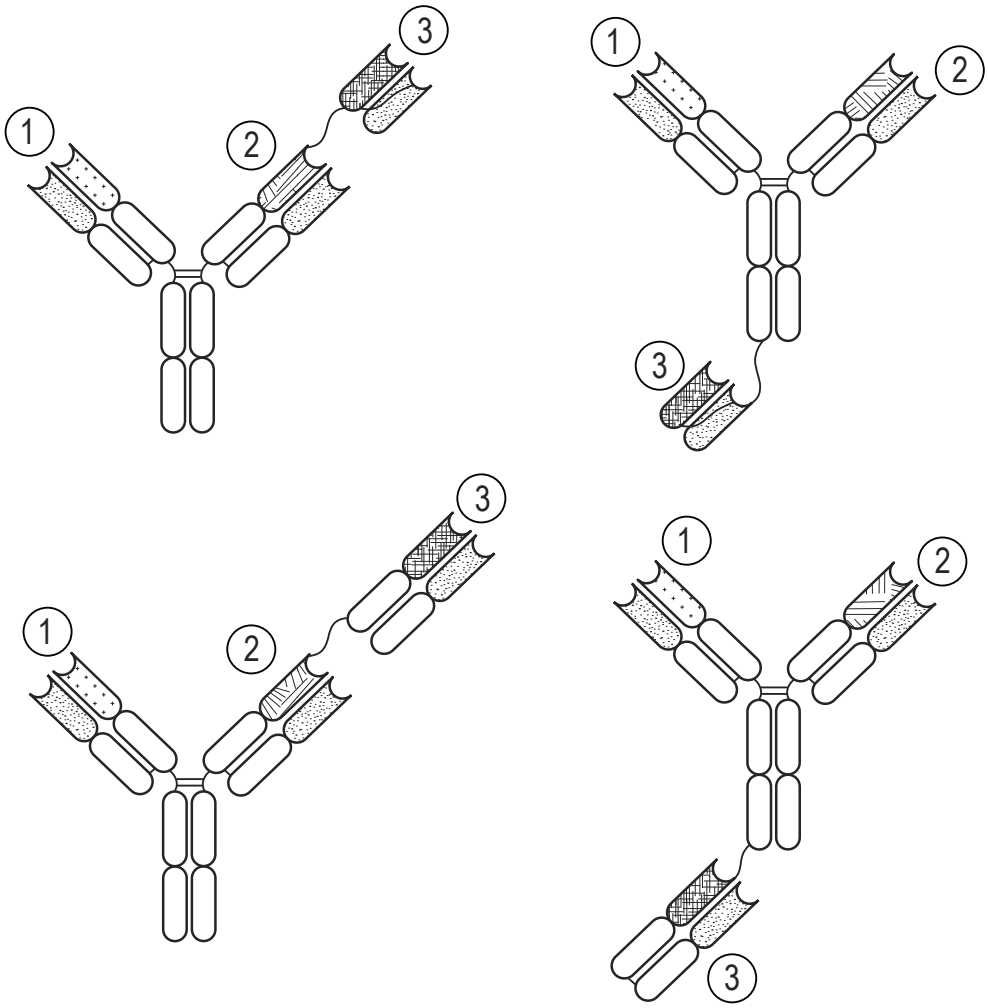


FIG. 5

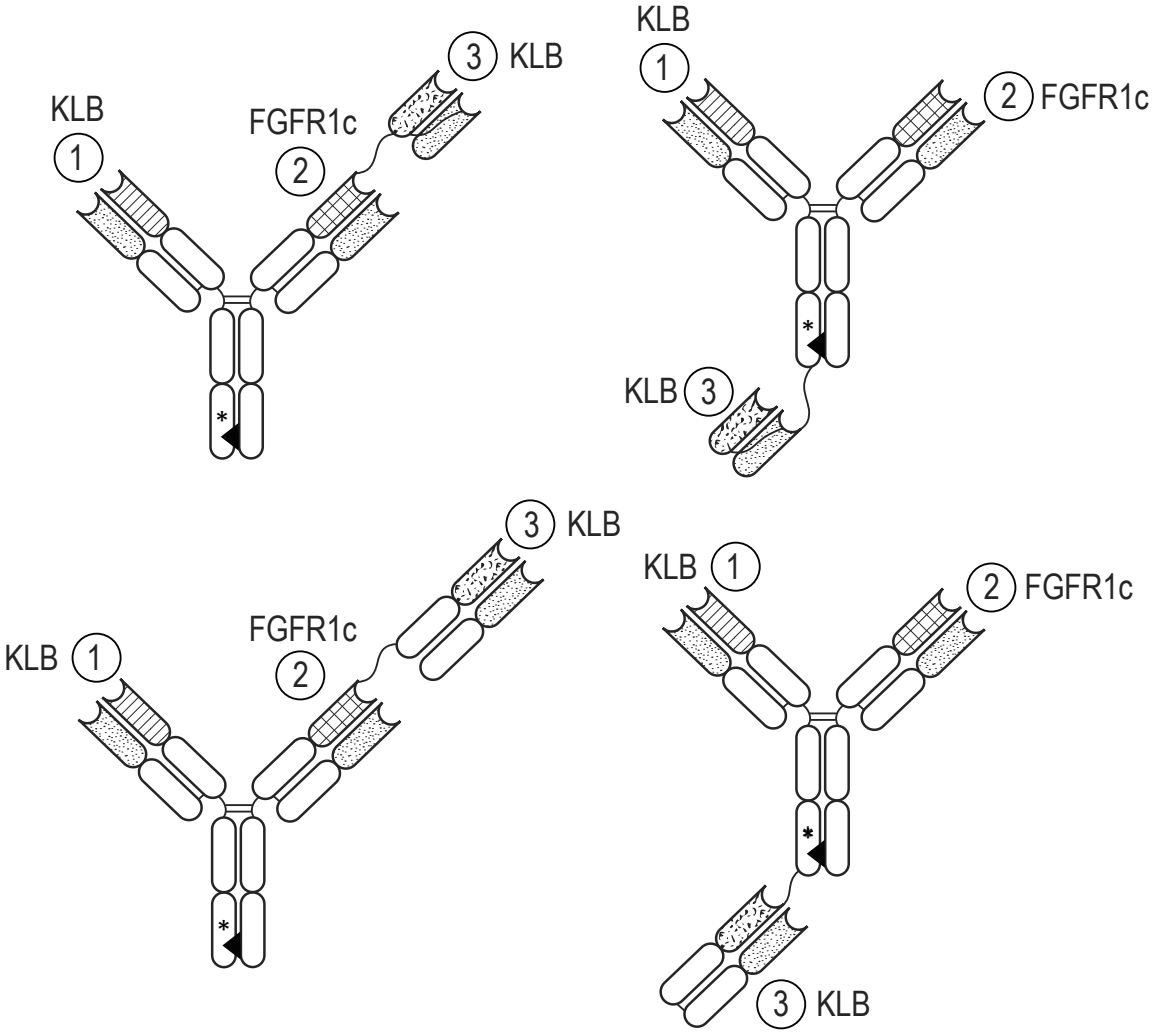

KLB
1
FGFR1c
2
3
KLB
*
KLB
1
2
FGFR1c
*
KLB
3
KLB
1
FGFR1c
2
3
KLB
*
KLB
1
2
FGFR1c
*
3
KLB

FIG. 6A

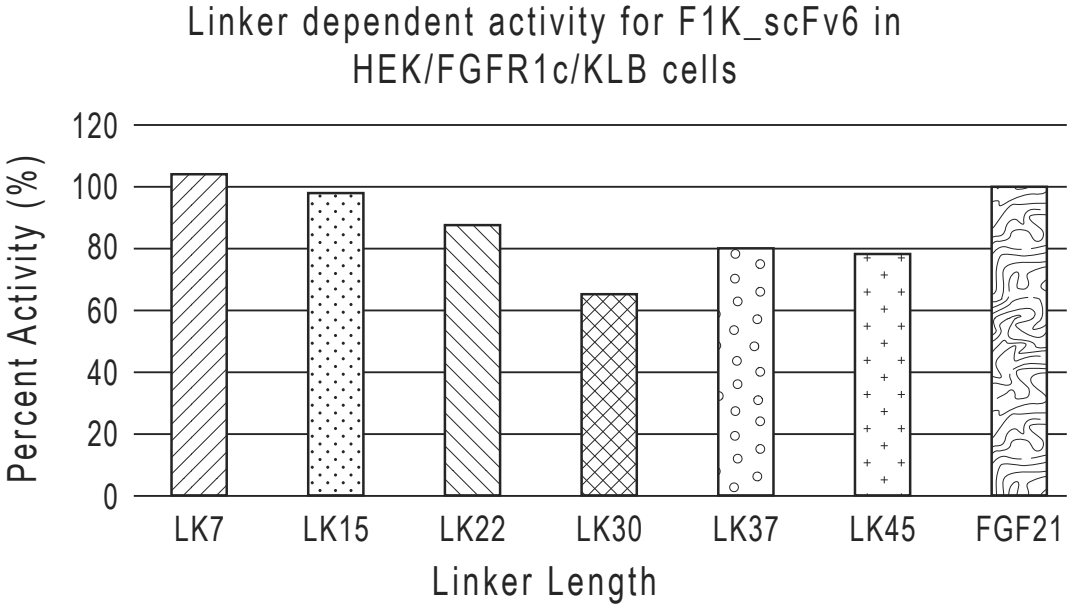

Linker dependent activity for F1K_scFv6 in HEK/FGFR1c/KLB cells
Percent Activity (%)
120
100
80
60
40
20
0
LK7
LK15
LK22
LK30
LK37
LK45
FGF21
Linker Length

FIG. 6B

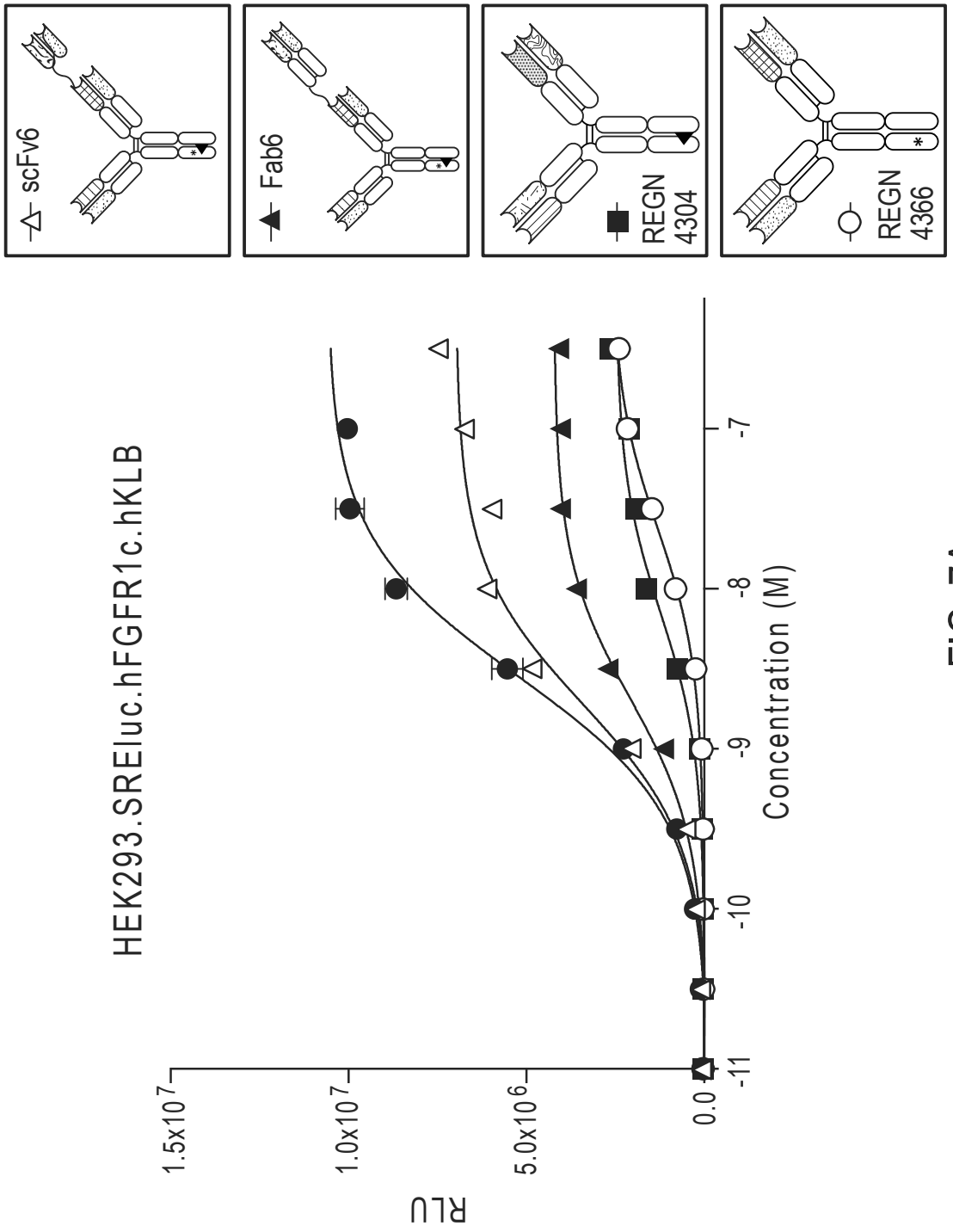
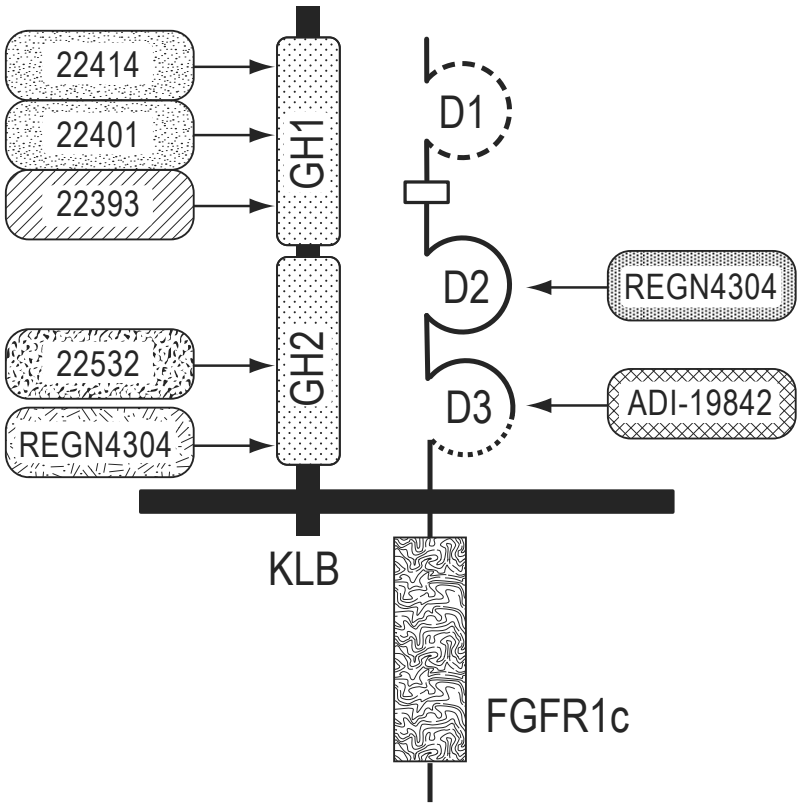

HEK293.SREluc.hFGFR1c.hKLB
RLU
1.5x10^7
1.0x10^7
5.0x10^6
0.0
-11
-10
-9
-8
-7
Concentration (M)
scFv6
Fab6
REGN 4304
REGN 4366

FIG. 7A



	KLB			*FGFR1c*	
	GH2	GH1	D3	D2	*Activity*
F1K_scFv4		22414	22393 ADI-19842		*
F1K_scFv5		22401	22393 ADI-19842		*
F1K_scFv6	22532		22393 ADI-19842		****
REGN4366			22393 ADI-19842		**
REGN4304	REGN4304			REGN4304	***

FIG. 7B

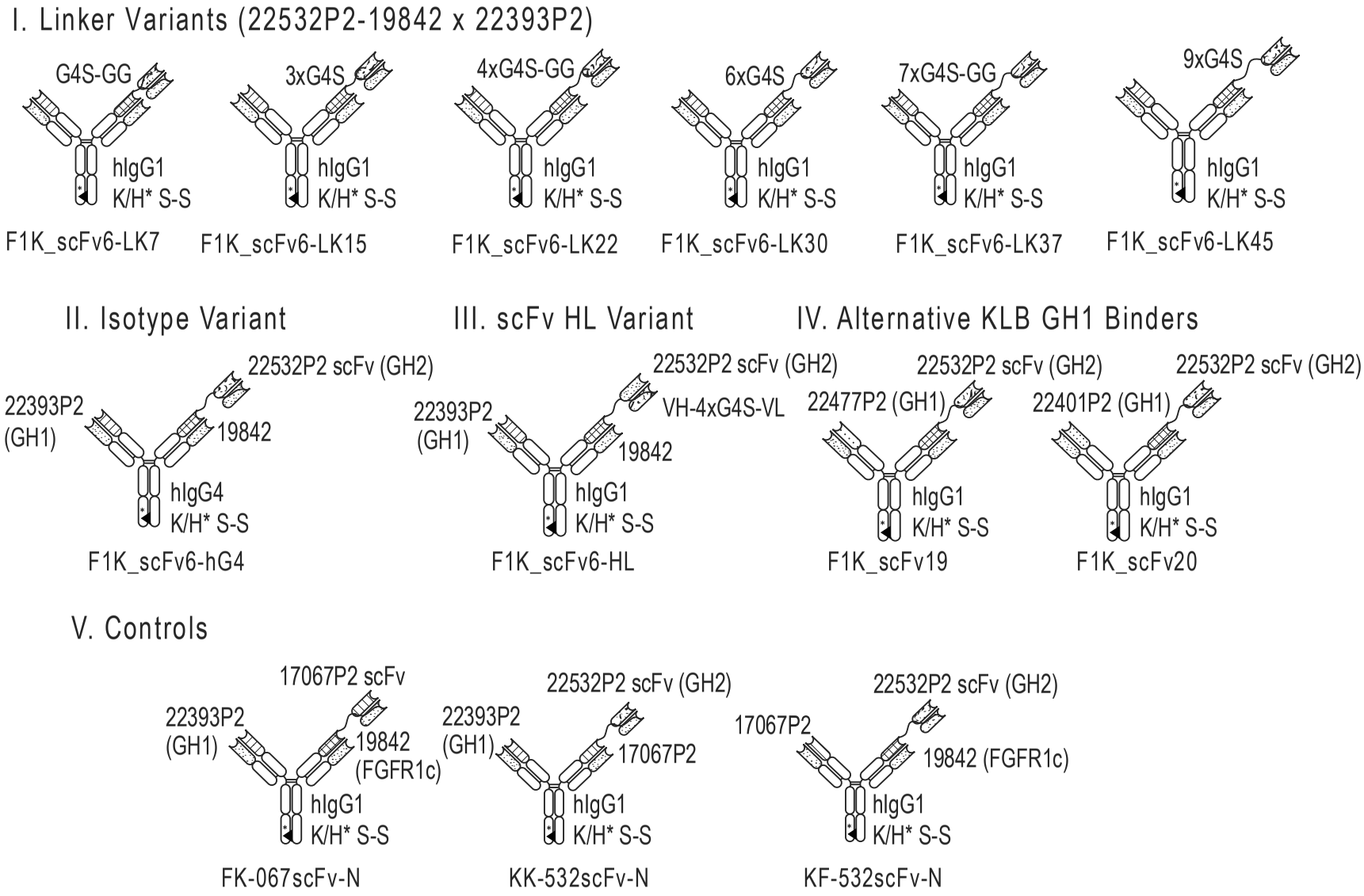

I. Linker Variants (22532P2-19842 x 22393P2)
G4S-GG
hIgG1
K/H* S-S
F1K_scFv6-LK7
3xG4S
hIgG1
K/H* S-S
F1K_scFv6-LK15
4xG4S-GG
hIgG1
K/H* S-S
F1K_scFv6-LK22
6xG4S
hIgG1
K/H* S-S
F1K_scFv6-LK30
7xG4S-GG
hIgG1
K/H* S-S
F1K_scFv6-LK37
9xG4S
hIgG1
K/H* S-S
F1K_scFv6-LK45
II. Isotype Variant
22532P2 scFv (GH2)
22393P2
(GH1)
19842
hIgG4
K/H* S-S
F1K_scFv6-hG4
III. scFv HL Variant
22532P2 scFv (GH2)
VH-4xG4S-VL
22393P2
(GH1)
19842
hIgG1
K/H* S-S
F1K_scFv6-HL
IV. Alternative KLB GH1 Binders
22532P2 scFv (GH2)
22477P2 (GH1)
hIgG1
K/H* S-S
F1K_scFv19
22532P2 scFv (GH2)
22401P2 (GH1)
hIgG1
K/H* S-S
F1K_scFv20
V. Controls
17067P2 scFv
22393P2
(GH1)
19842
(FGFR1c)
hIgG1
K/H* S-S
FK-067scFv-N
22532P2 scFv (GH2)
22393P2
(GH1)
17067P2
hIgG1
K/H* S-S
KK-532scFv-N
22532P2 scFv (GH2)
17067P2
19842 (FGFR1c)
hIgG1
K/H* S-S
KF-532scFv-N

FIG. 8A

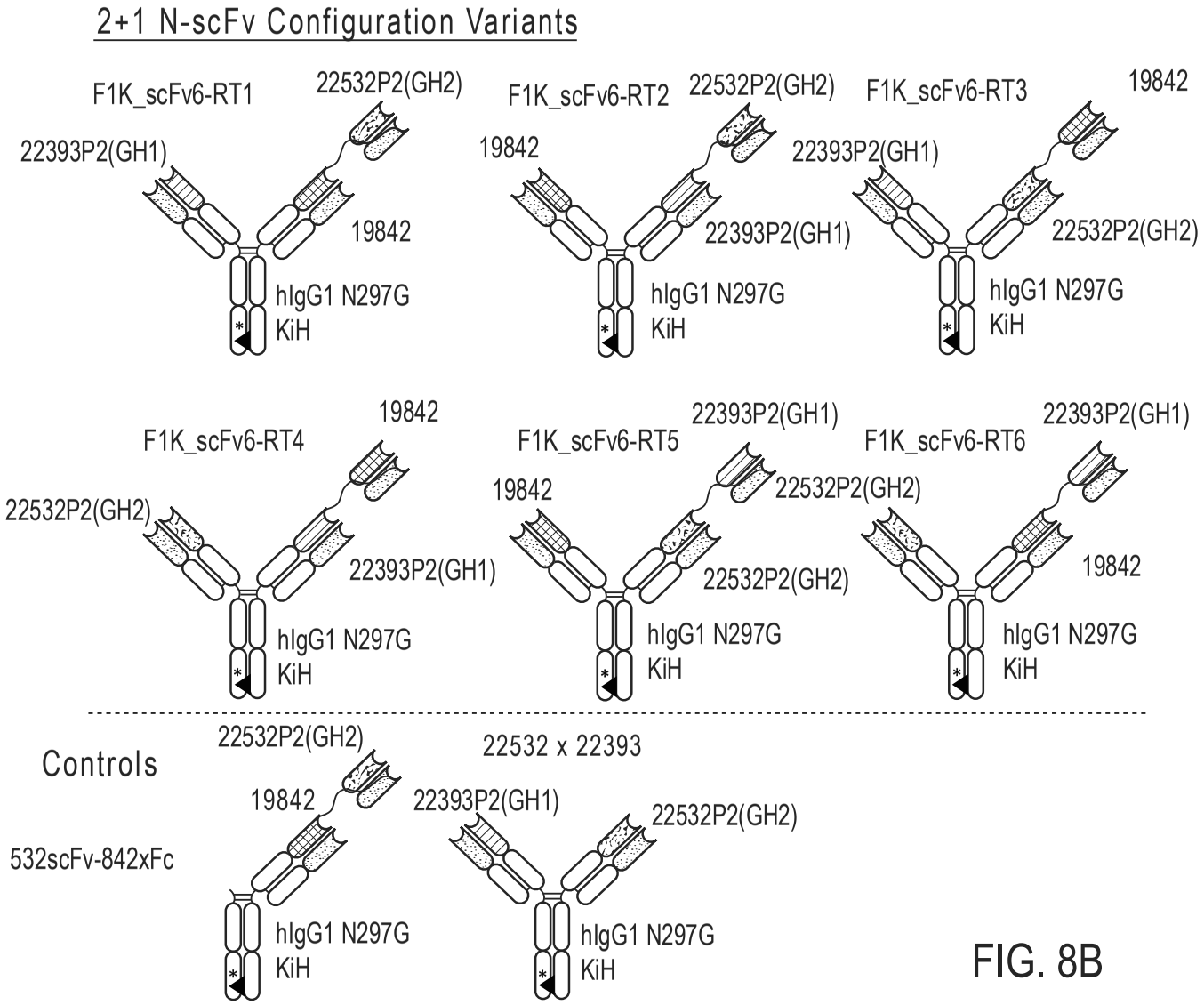
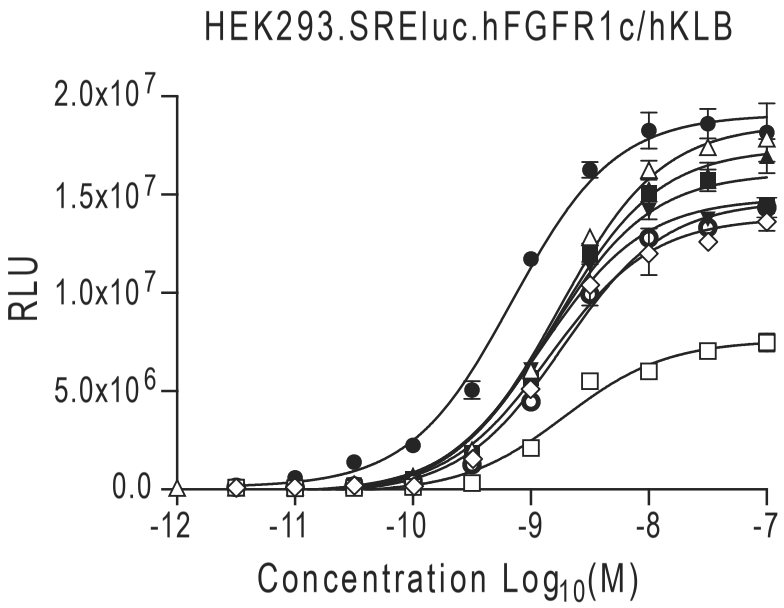
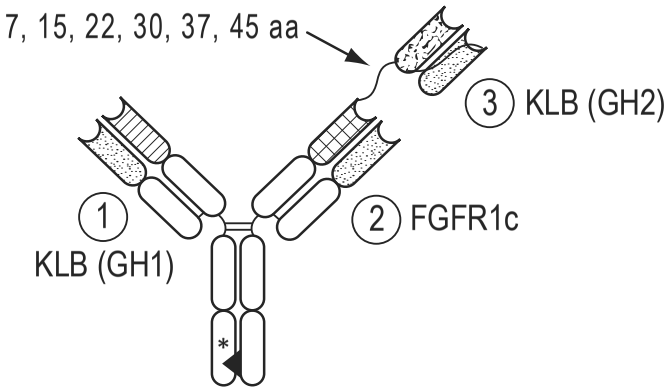

2+1 N-scFv Configuration Variants
F1K_scFv6-RT1
22532P2(GH2)
22393P2(GH1)
19842
hIgG1 N297G
KiH
F1K_scFv6-RT2
22532P2(GH2)
19842
22393P2(GH1)
hIgG1 N297G
KiH
F1K_scFv6-RT3
19842
22393P2(GH1)
22532P2(GH2)
hIgG1 N297G
KiH
F1K_scFv6-RT4
19842
22532P2(GH2)
22393P2(GH1)
hIgG1 N297G
KiH
F1K_scFv6-RT5
22393P2(GH1)
19842
22532P2(GH2)
hIgG1 N297G
KiH
F1K_scFv6-RT6
22393P2(GH1)
22532P2(GH2)
19842
hIgG1 N297G
KiH
Controls
532scFv-842xFc
22532P2(GH2)
19842
hIgG1 N297G
KiH
22532 x 22393
22393P2(GH1)
22532P2(GH2)
hIgG1 N297G
KiH

FIG. 8B





		1	2	3	Linker
●—	REGN1438	6His-FGF21			
△—	F1K_scFv6-LK7	22393	ADI-19842	22532	L20H7
▲—	F1K_scFv6-LK15	22393	ADI-19842	22532	L20H15
■—	F1K_scFv6-LK22	22393	ADI-19842	22532	L20H22
⊖—	F1K_scFv6-LK30	22393	ADI-19842	22532	L20H30
▼—	F1K_scFv6-LK37	22393	ADI-19842	22532	L20H37
◇—	F1K_scFv6-LK45	22393	ADI-19842	22532	L20H45
□—	REGN4304				

FIG. 9

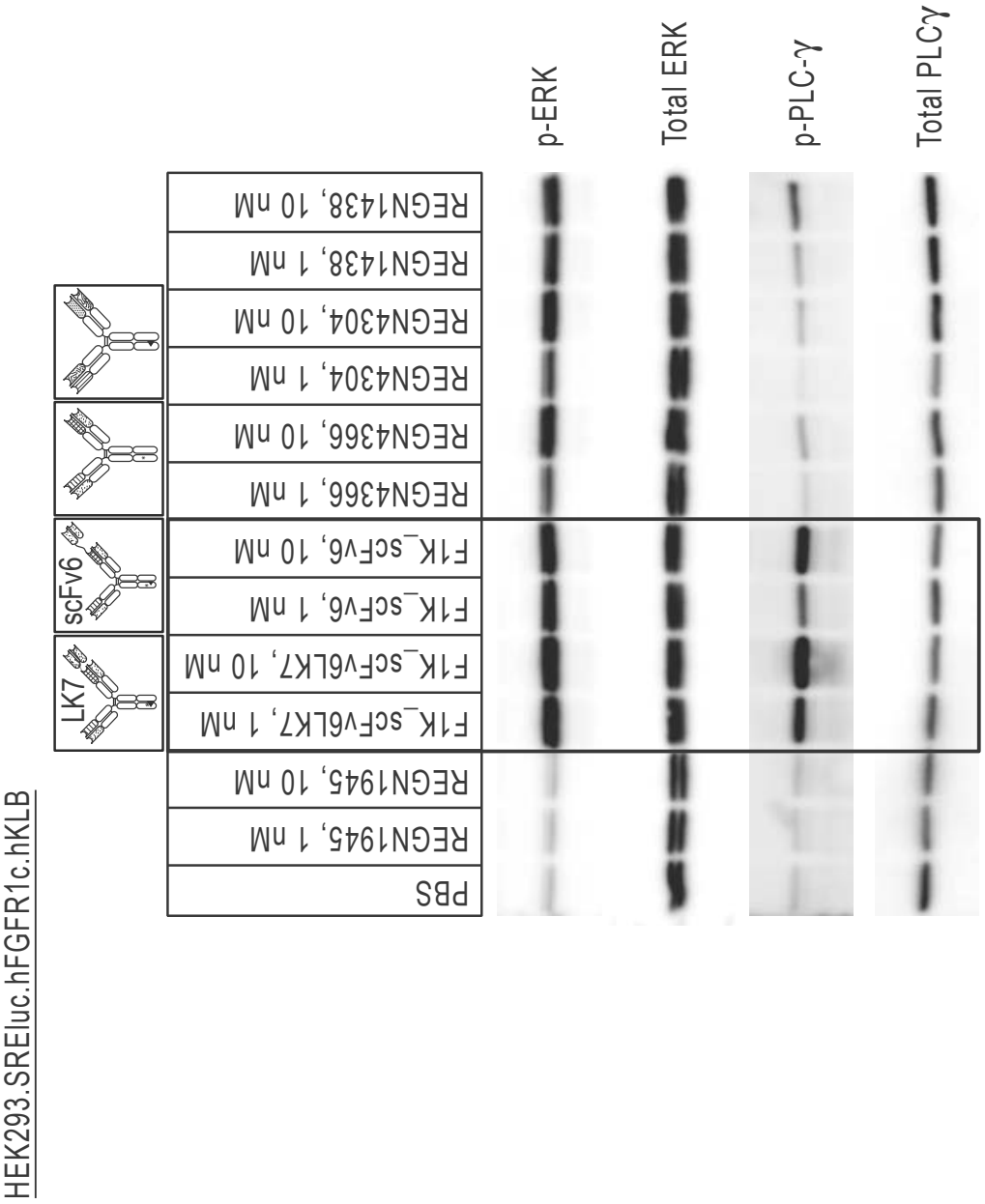

HEK293.SREluc.hFGFR1c.hKLB
LK7
scFv6
PBS
REGN1945, 1 nM
REGN1945, 10 nM
F1K_scFv6LK7, 1 nM
F1K_scFv6LK7, 10 nM
F1K_scFv6, 1 nM
F1K_scFv6, 10 nM
REGN4366, 1 nM
REGN4366, 10 nM
REGN4304, 1 nM
REGN4304, 10 nM
REGN1438, 1 nM
REGN1438, 10 nM
p-ERK
Total ERK
p-PLC-γ
Total PLCγ

FIG. 10A

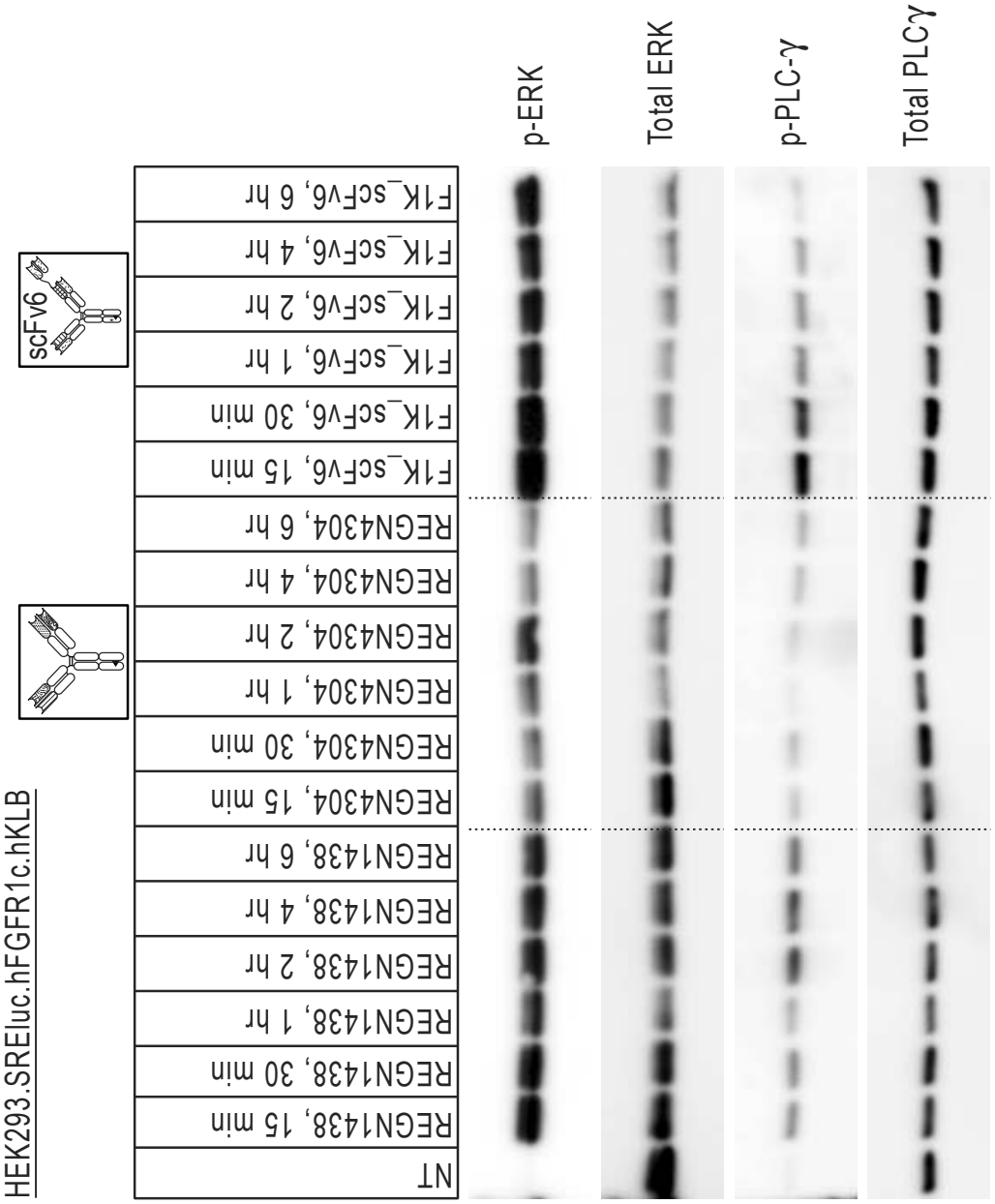

HEK293.SREluc.hFGFR1c.hKLB
NT
REGN1438, 15 min
REGN1438, 30 min
REGN1438, 1 hr
REGN1438, 2 hr
REGN1438, 4 hr
REGN1438, 6 hr
REGN4304, 15 min
REGN4304, 30 min
REGN4304, 1 hr
REGN4304, 2 hr
REGN4304, 4 hr
REGN4304, 6 hr
F1K_scFv6, 15 min
F1K_scFv6, 30 min
F1K_scFv6, 1 hr
F1K_scFv6, 2 hr
F1K_scFv6, 4 hr
F1K_scFv6, 6 hr
scFv6
p-ERK
Total ERK
p-PLC-γ
Total PLCγ

FIG. 10B

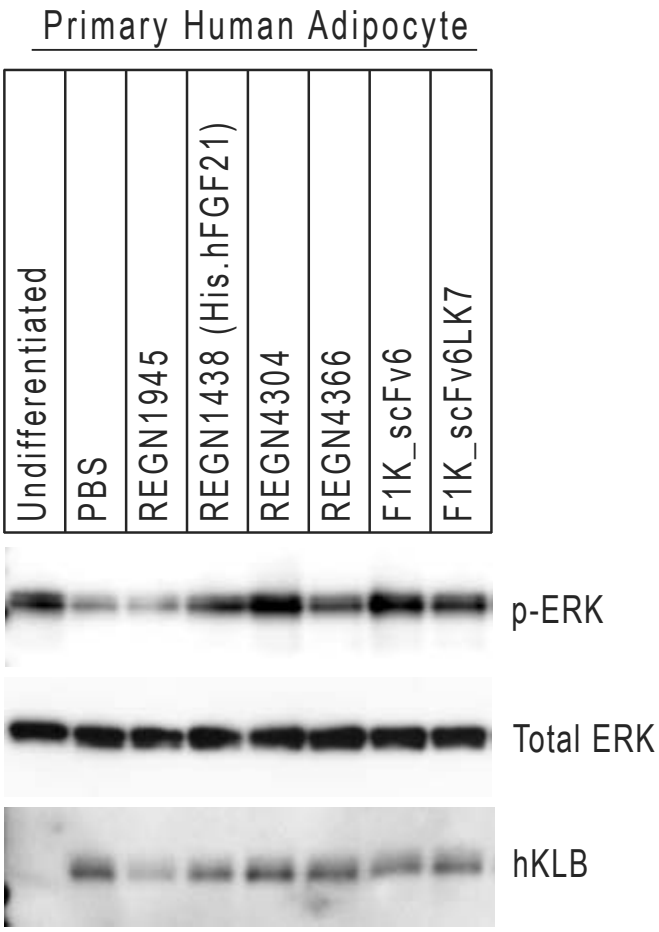

Primary Human Adipocyte
Undifferentiated
PBS
REGN1945
REGN1438 (His.hFGF21)
REGN4304
REGN4366
F1K_scFv6
F1K_scFv6LK7
p-ERK
Total ERK
hKLB

FIG. 11A

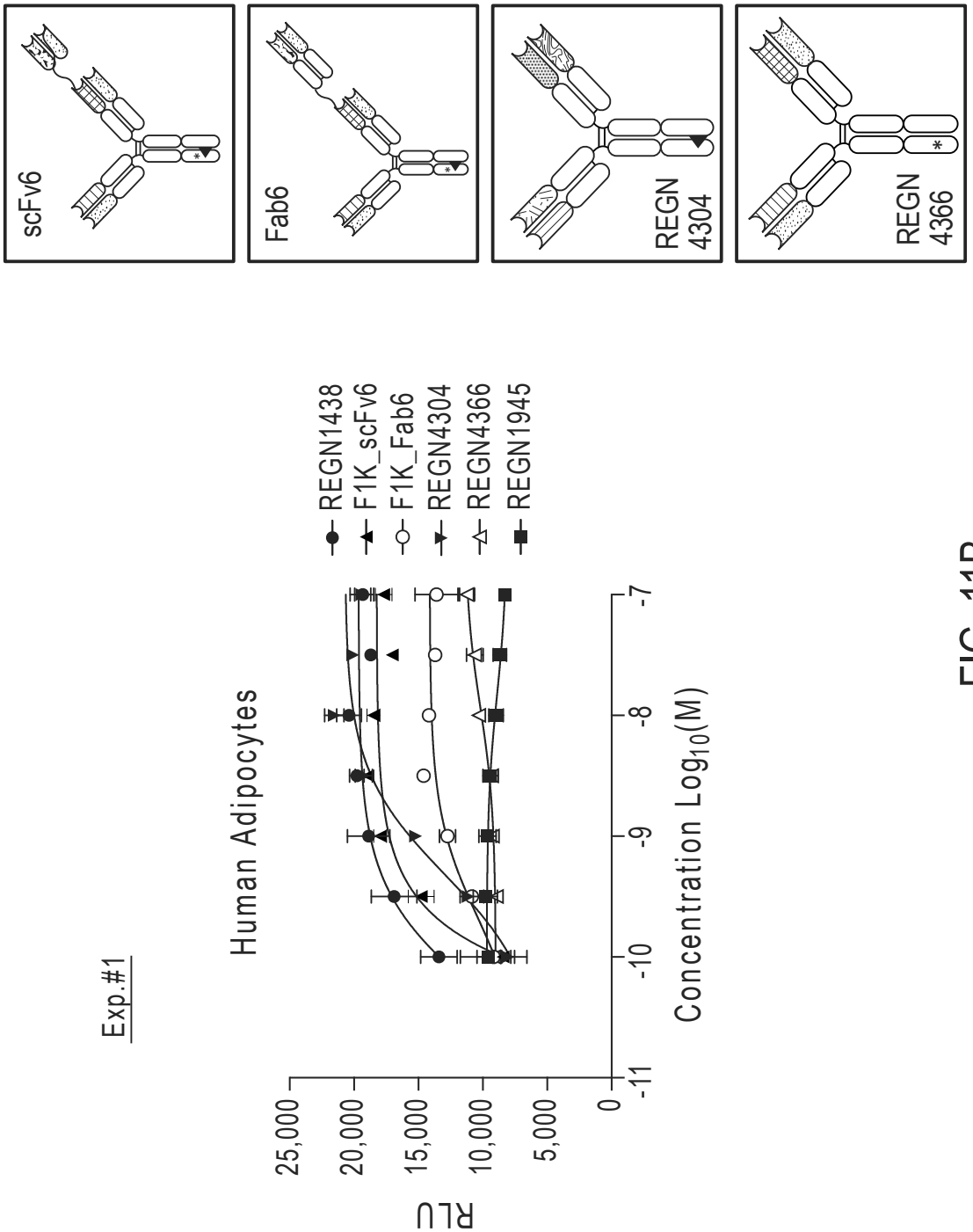

scFv6
Fab6
REGN 4304
REGN 4366
Exp.#1
Human Adipocytes
REGN1438
F1K_scFv6
F1K_Fab6
REGN4304
REGN4366
REGN1945
RLU
25,000
20,000
15,000
10,000
5,000
0
-11
-10
-9
-8
-7
Concentration Log10(M)

FIG. 11B

Exp.#2

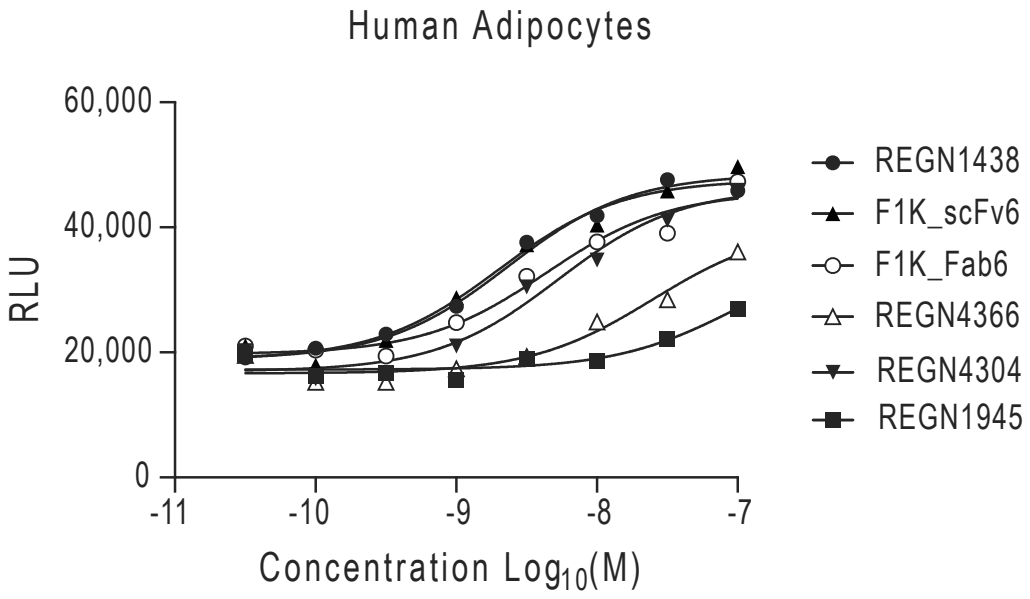

Human Adipocytes
60,000
40,000
20,000
0
RLU
-11
-10
-9
-8
-7
Concentration Log10(M)
REGN1438
F1K_scFv6
F1K_Fab6
REGN4366
REGN4304
REGN1945

FIG. 11C



FIG. 12A

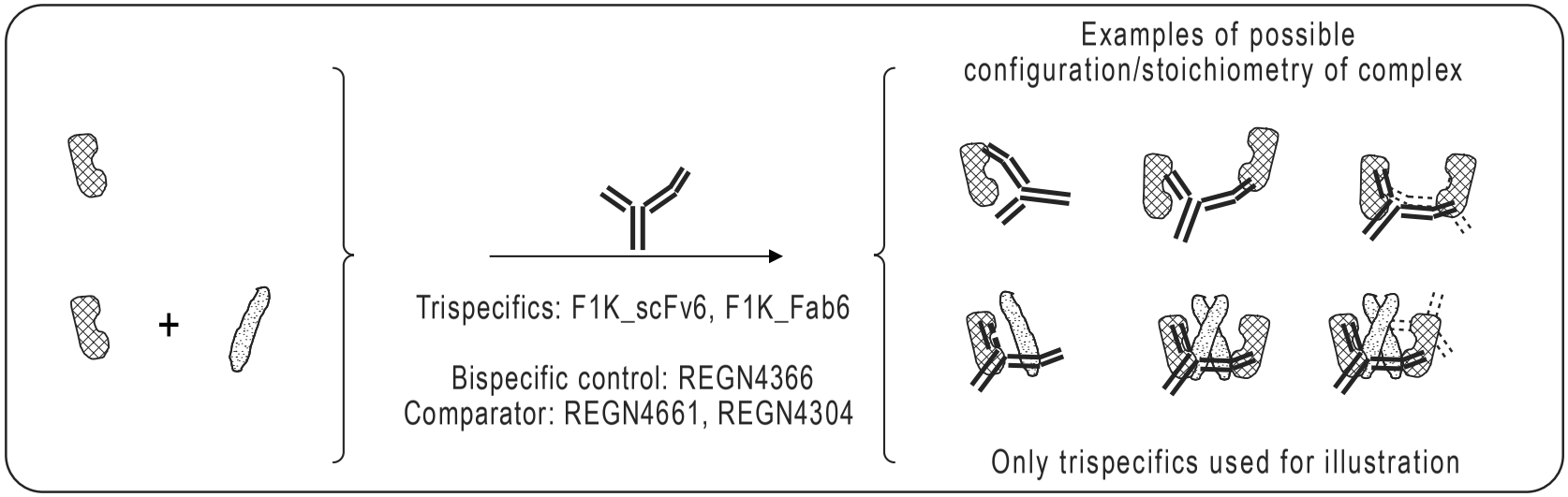
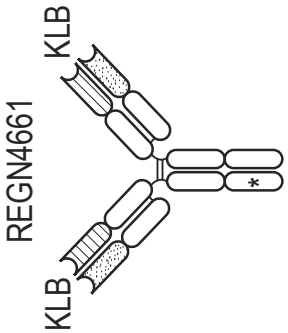


FIG. 12B

REGN4661:KLB Complex	Theoretical Molar Mass (kDa)
1:1	280
1:2	410



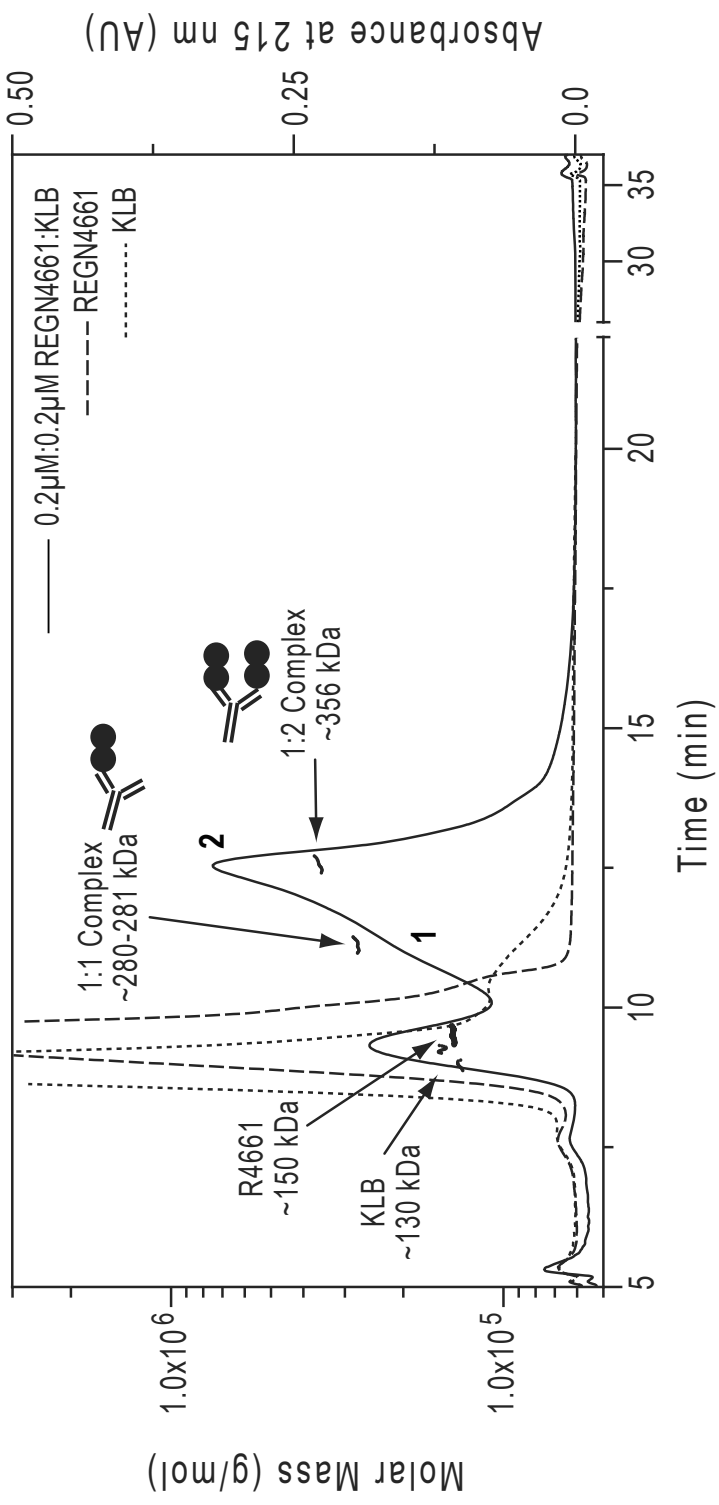
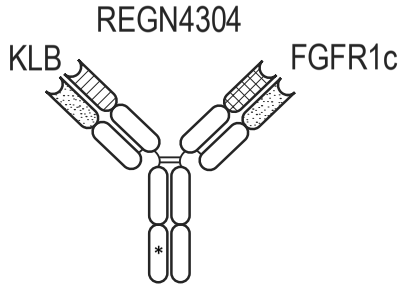


FIG. 13A



REGN4304:KLB:FGFR1c Complex	Theoretical Molar Mass (kDa)
1:1	280
1:1:1	322

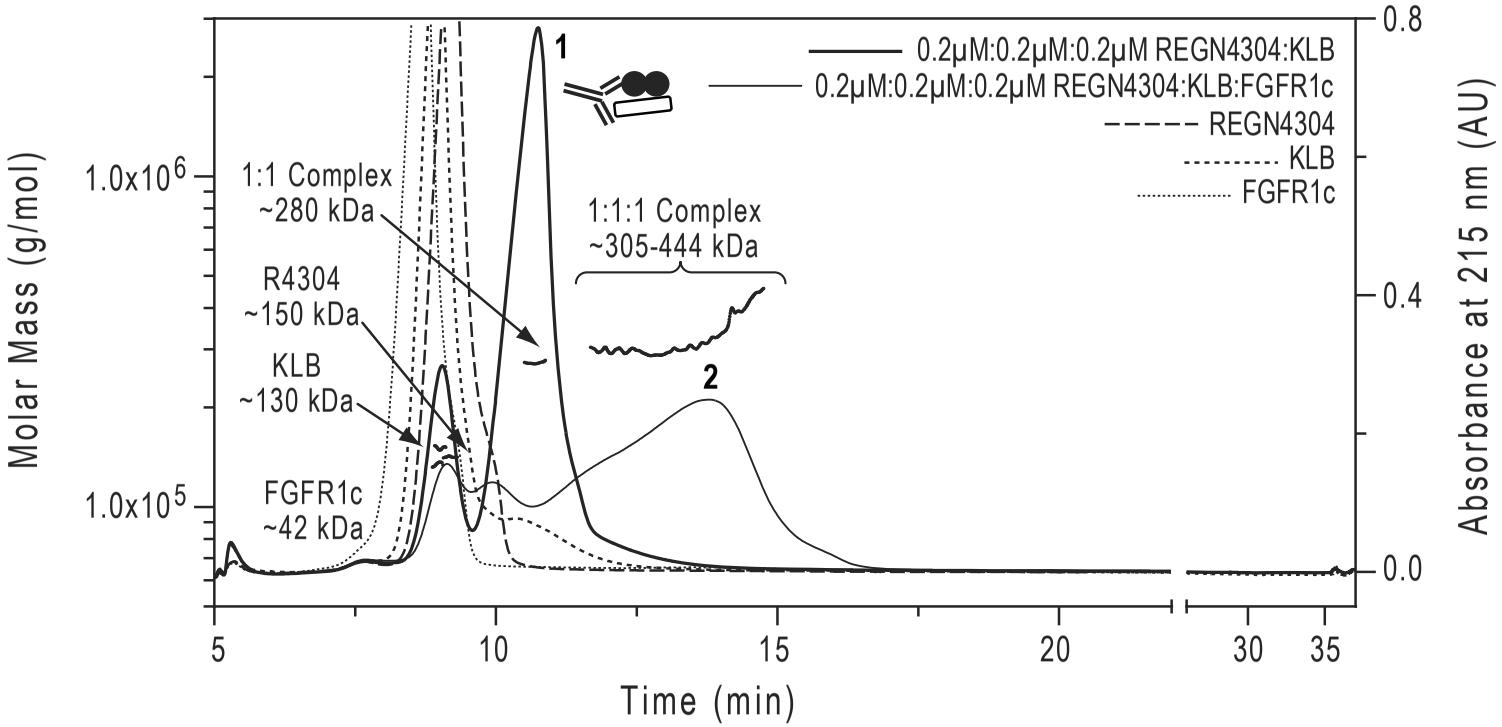


FIG. 13B

F1K_scFv6:KLB:FGFR1c Complex	Theoretical Molar Mass (kDa)
2:2	579
2:2:1	621
2:2:2	663

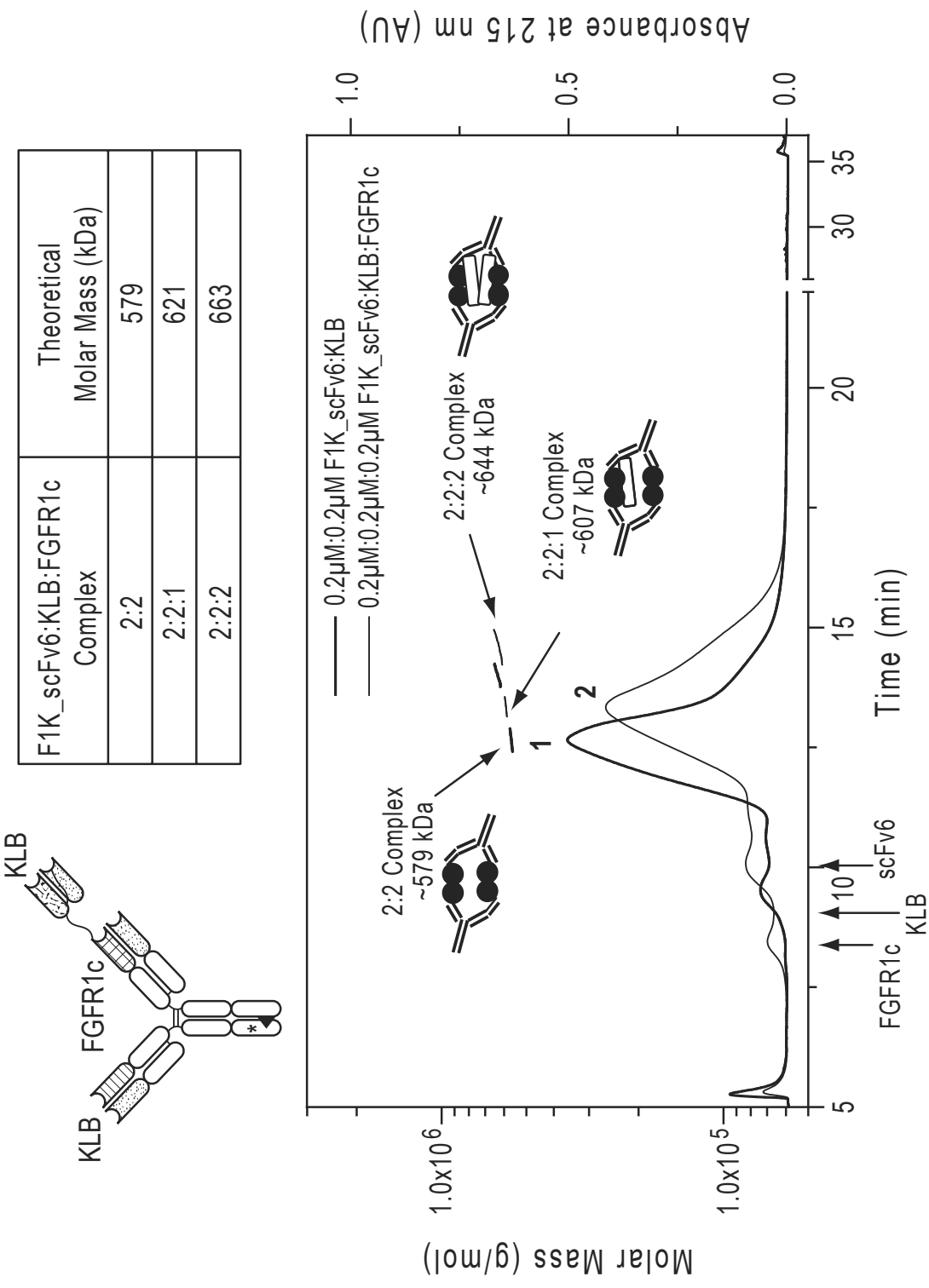
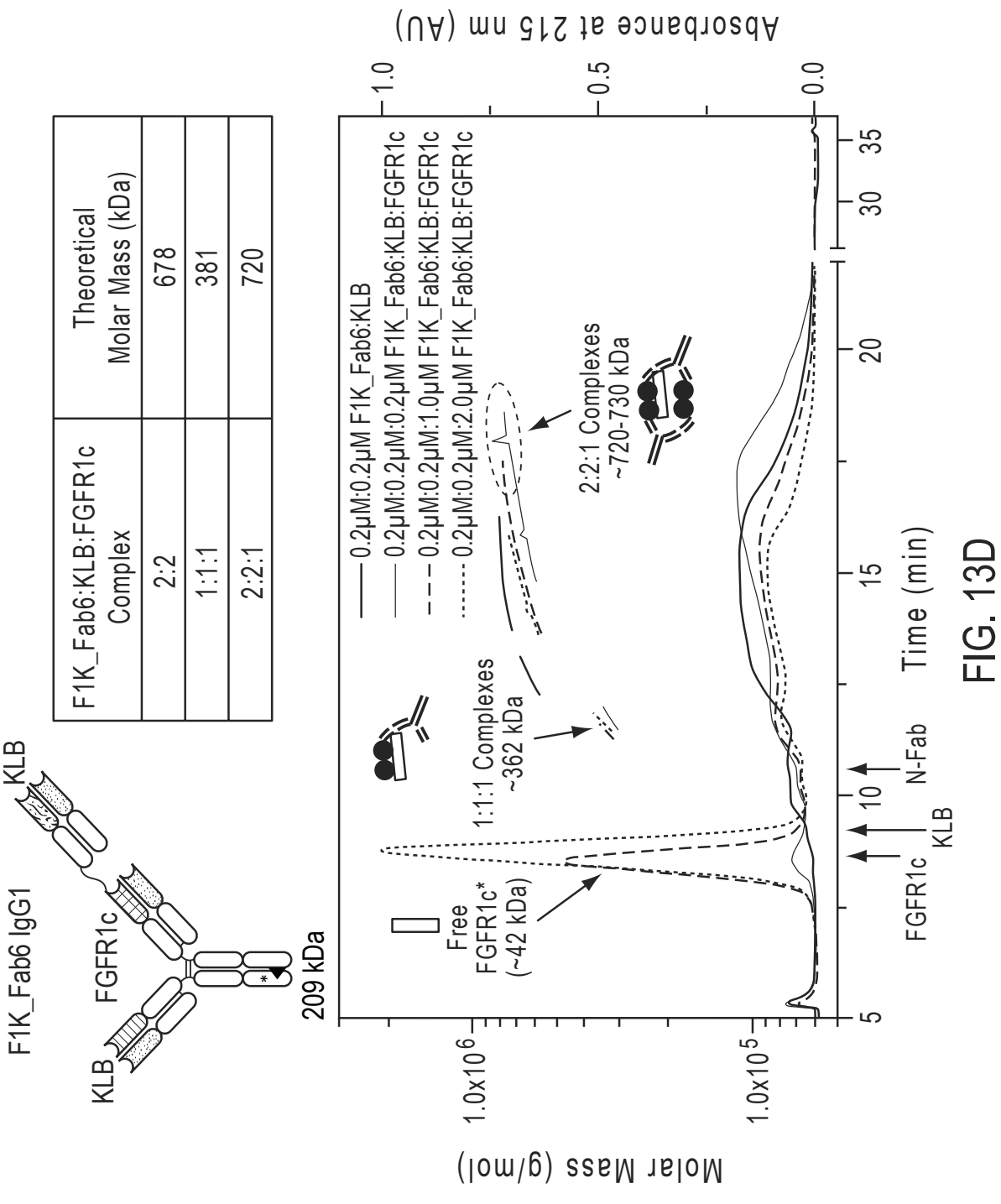


FIG. 13C



F1K_Fab6:KLB:FGFR1c Complex	Theoretical Molar Mass (kDa)
2:2	678
1:1:1	381
2:2:1	720

FIG. 13D

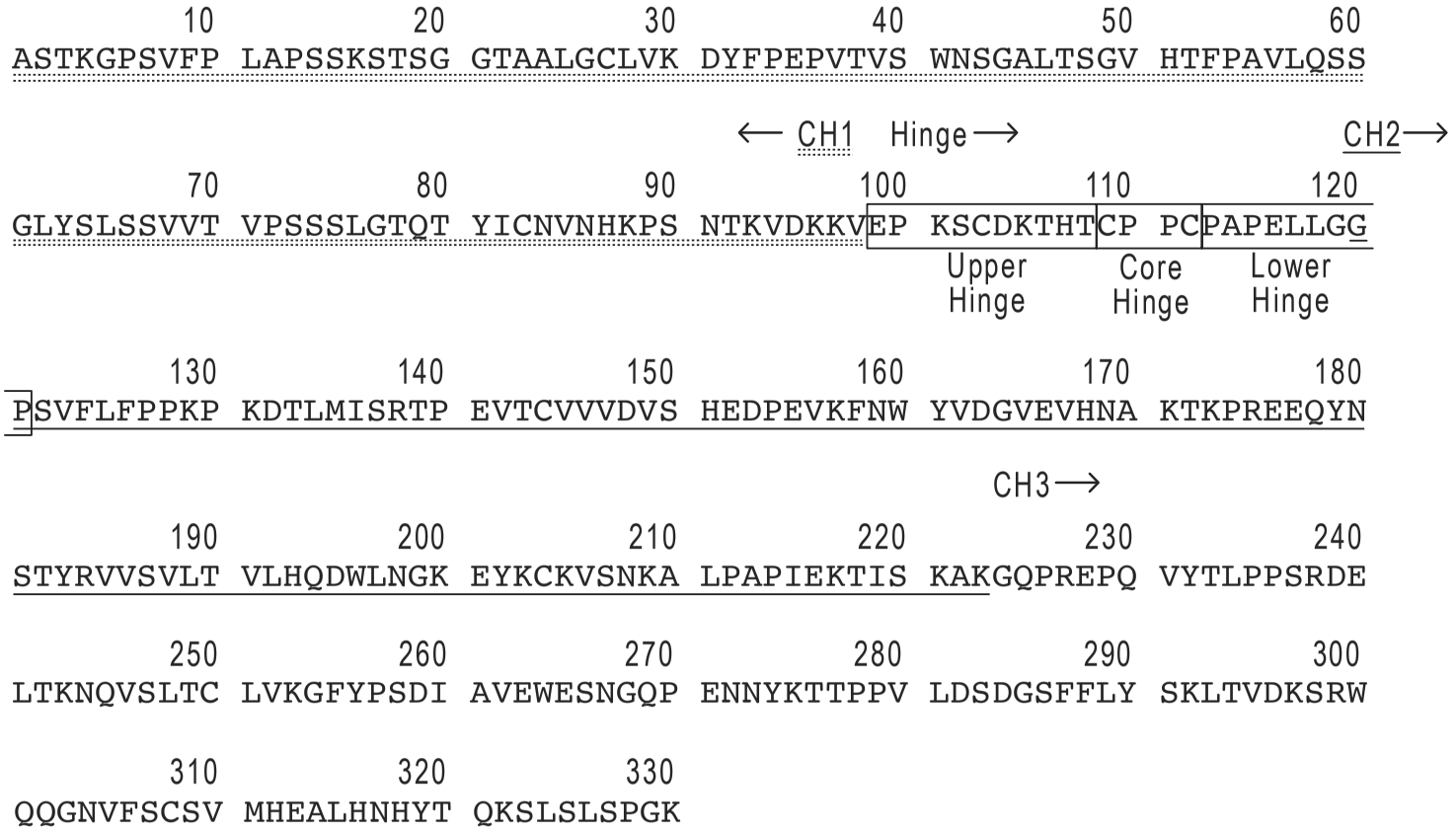

10 20 30 40 50 60
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
← CH1 Hinge → CH2 →
70 80 90 100 110 120
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG
Upper Hinge
Core Hinge
Lower Hinge
130 140 150 160 170 180
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
CH3 →
190 200 210 220 230 240
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE
250 260 270 280 290 300
LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
310 320 330
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

FIG. 14

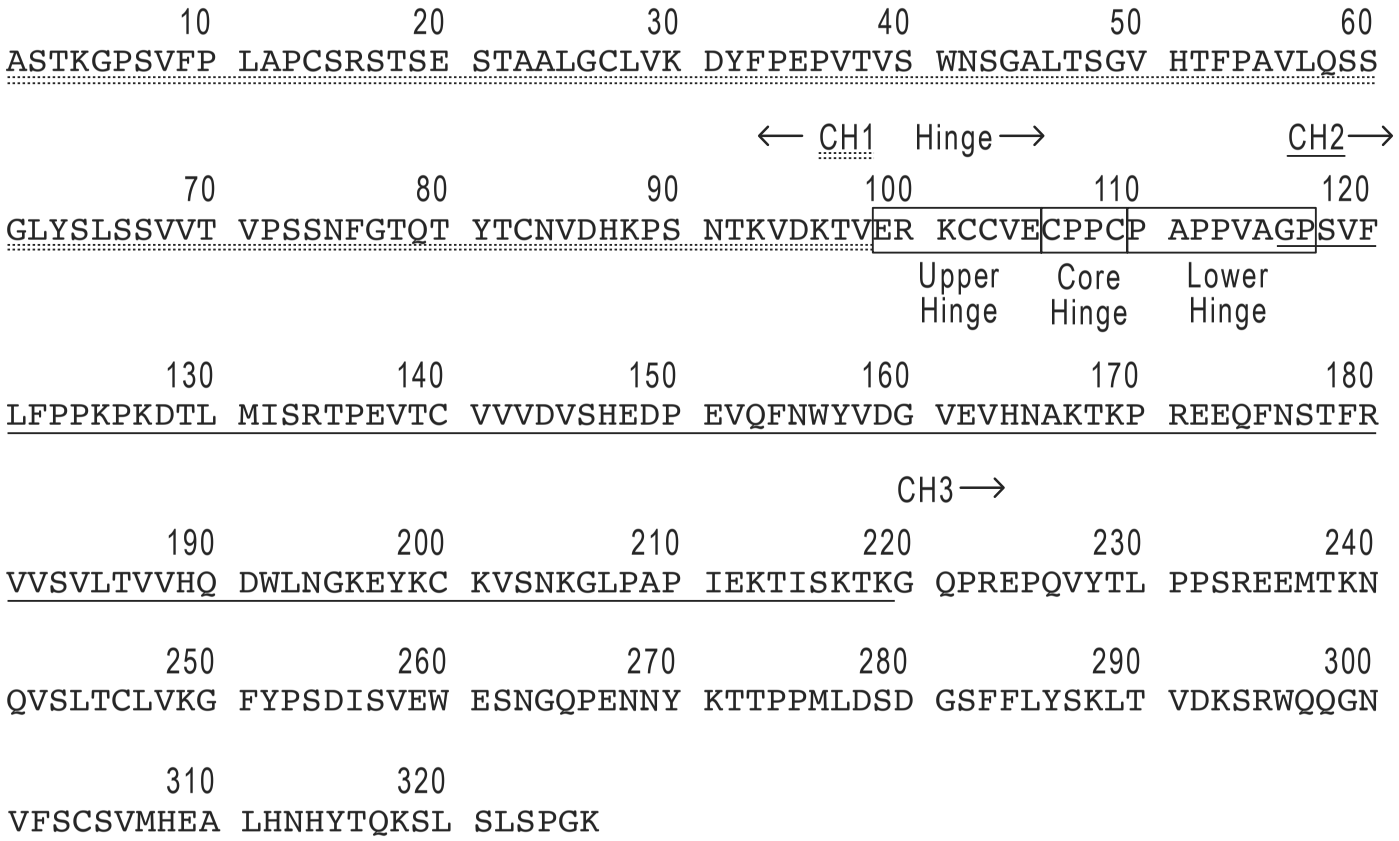

10 20 30 40 50 60
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
← CH1 Hinge → CH2 →
70 80 90 100 110 120
GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVER KCCVECPPCP APPVAGPSVF
Upper Hinge
Core Hinge
Lower Hinge
130 140 150 160 170 180
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR
CH3 →
190 200 210 220 230 240
VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN
250 260 270 280 290 300
QVSLTCLVKG FYPSDISVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN
310 320
VFSCSVMHEA LHNHYTQKSL SLSPGK

FIG. 15

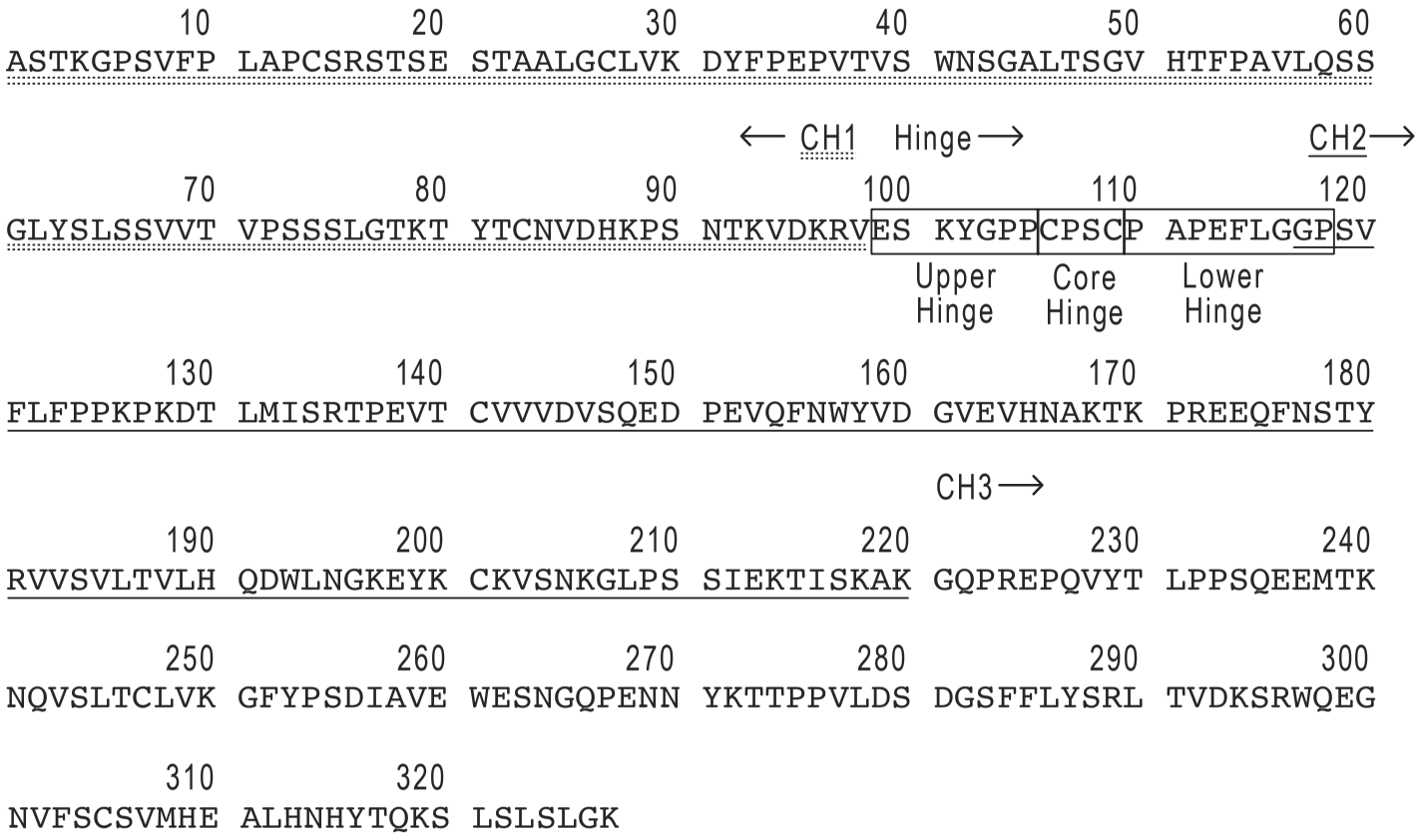

10 20 30 40 50 60
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
← CH1 Hinge →
CH2 →
70 80 90 100 110 120
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV
Upper Hinge
Core Hinge
Lower Hinge
130 140 150 160 170 180
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY
CH3 →
190 200 210 220 230 240
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK
250 260 270 280 290 300
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG
310 320
NVFSCSVMHE ALHNHYTQKS LSLSLGK

FIG. 16

	Upper Hinge	Core Hinge				Lower Hinge									CH2	CH3	Hinge SEQ ID NO:
Construct	216-225 (IgG1)	226	227	228	229	230	231	232	233	234	235	236	237	238	CH2	CH3	
hIgG1 PVA	EPKSCDKTHT	C	P	P	C	P	A	P	P	V	A	-	G	P	IgG1	IgG1	66
hIgG1s	EPKSCDKTHT	C	P	P	C	P	A	P	P	V	A	-	G	P	IgG4	IgG1	67
hIgG1	EPKSCDKTHT	C	P	P	C	P	A	P	E	L	L	G	G	P	IgG1	IgG1	68
hIgG2	ERK CCVE	C	P	P	C	P	A	P	P	V	A	-	G	P	IgG2	IgG2	69
hIgG4 S108P	ESKYGPP	C	P	P	C	P	A	P	E	F	L	G	G	P	IgG4	IgG4	70
hIgG4s	ESKYGPP	C	P	P	C	P	A	P	P	V	A	-	G	P	IgG4	IgG4	71
hIgG4	ESKYGPP	C	P	S	C	P	A	P	E	F	L	G	G	P	IgG4	IgG4	72

FIG. 17

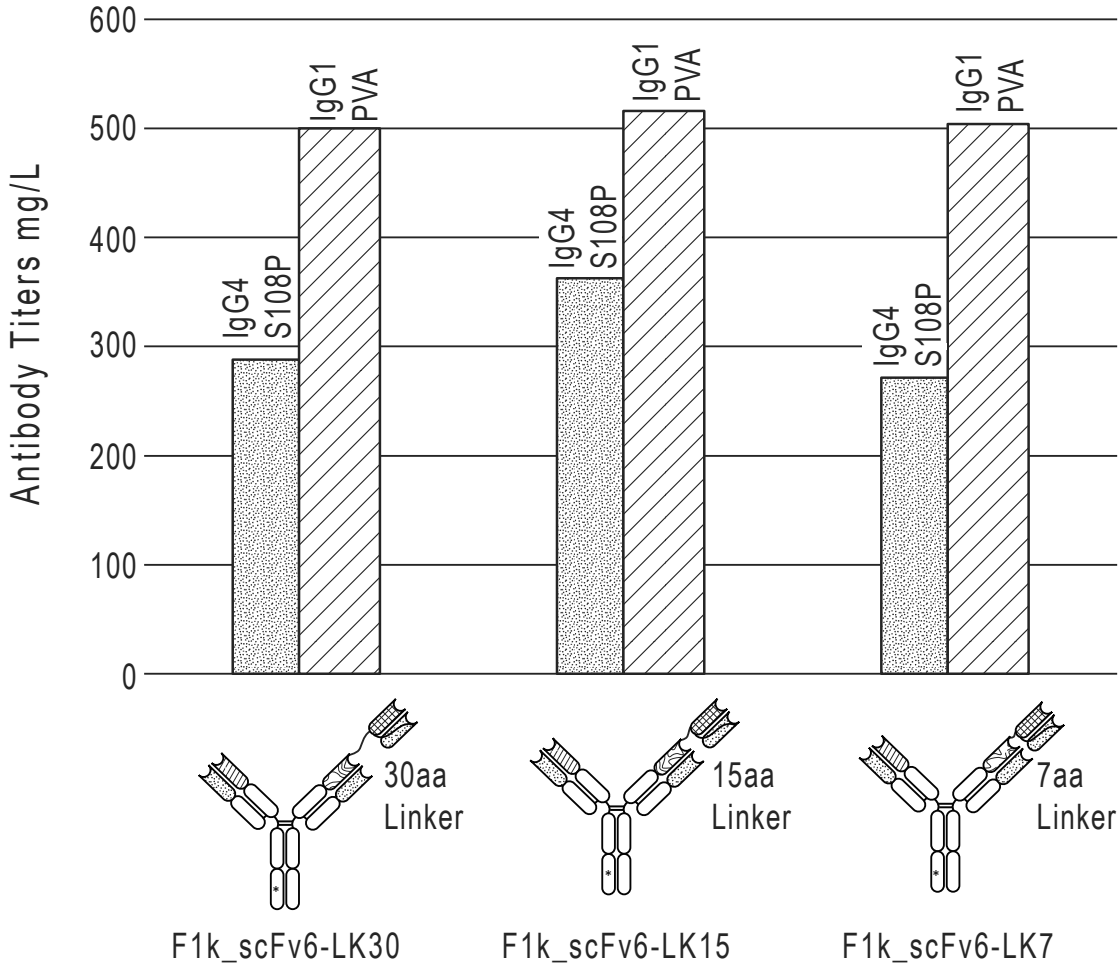

Antibody Titers mg/L
600
500
400
300
200
100
0
IgG4 S108P
IgG1 PVA
IgG4 S108P
IgG1 PVA
IgG4 S108P
IgG1 PVA
30aa Linker
15aa Linker
7aa Linker
F1k_scFv6-LK30
F1k_scFv6-LK15
F1k_scFv6-LK7

FIG. 18

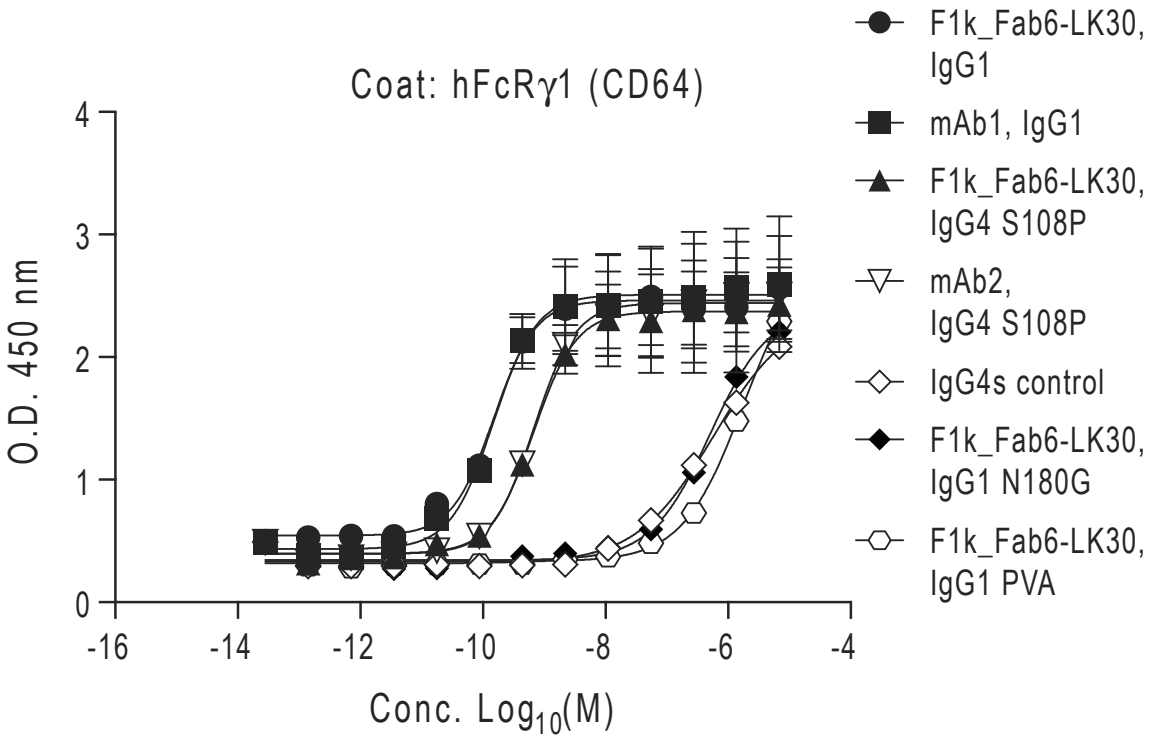


FIG. 19A

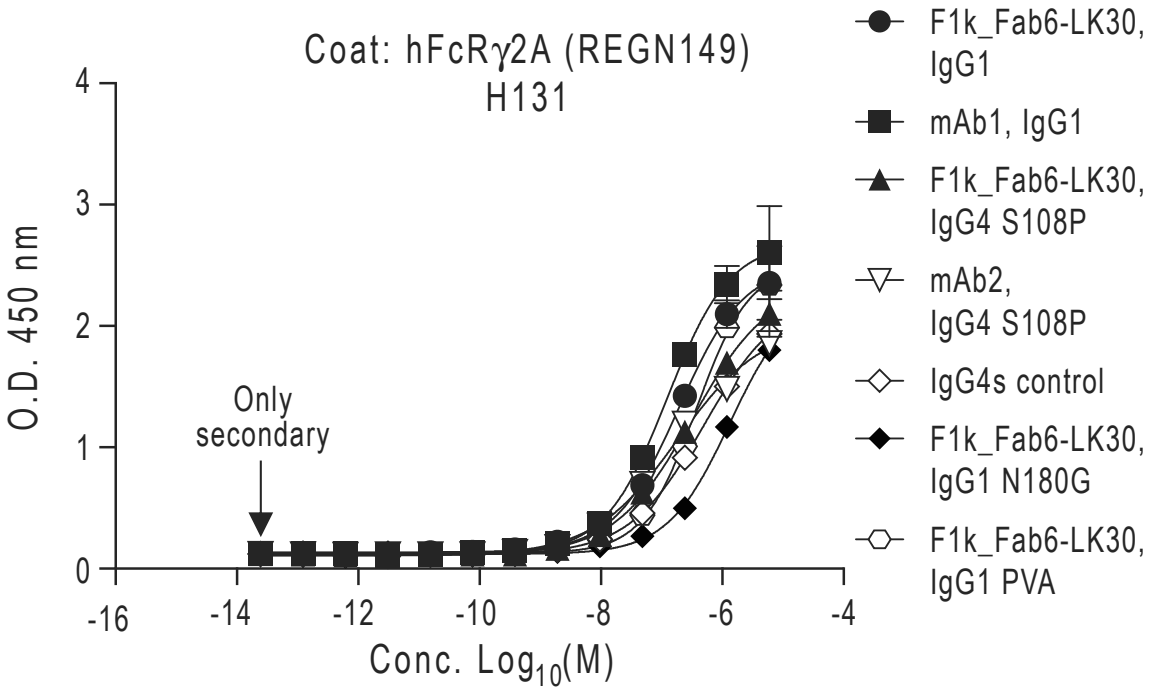


FIG. 19B

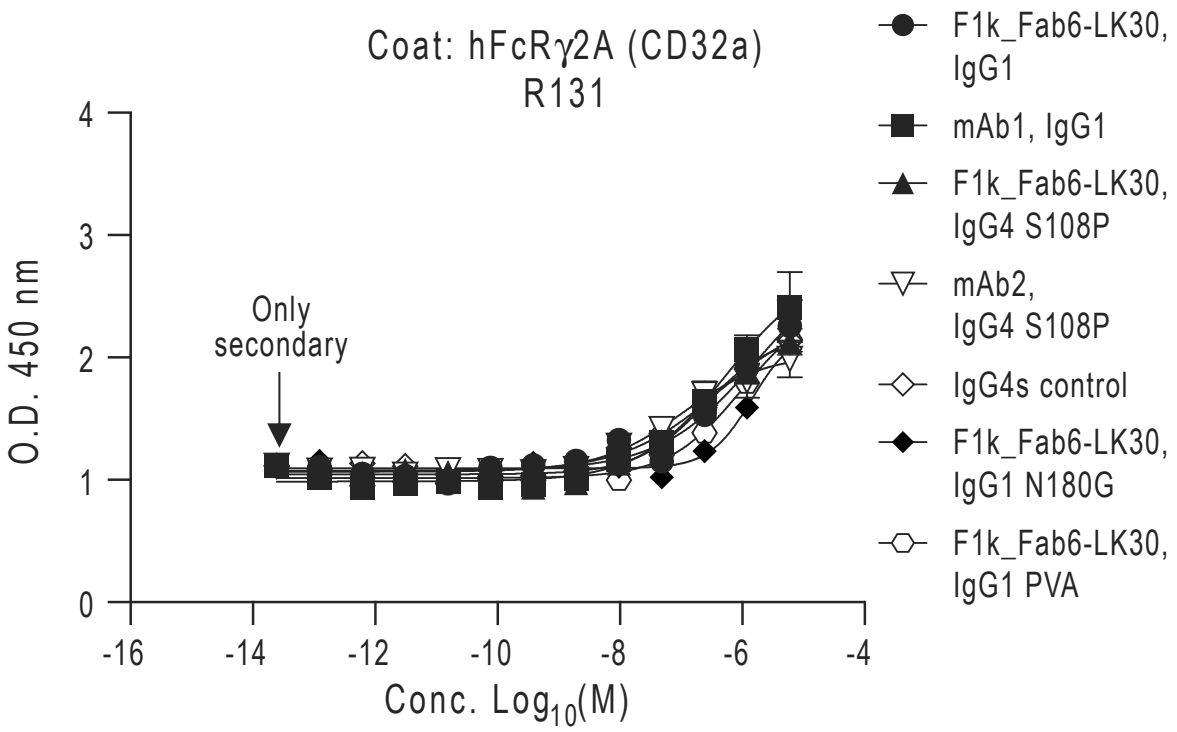


FIG. 19C

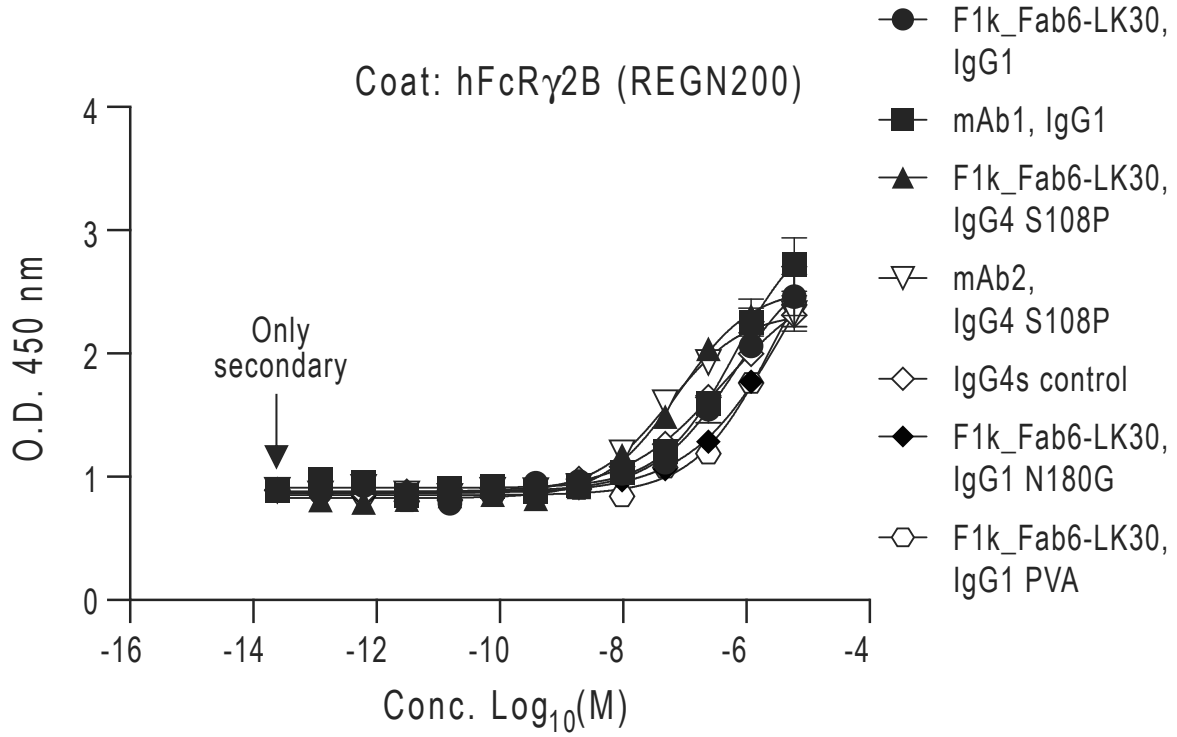


FIG. 19D

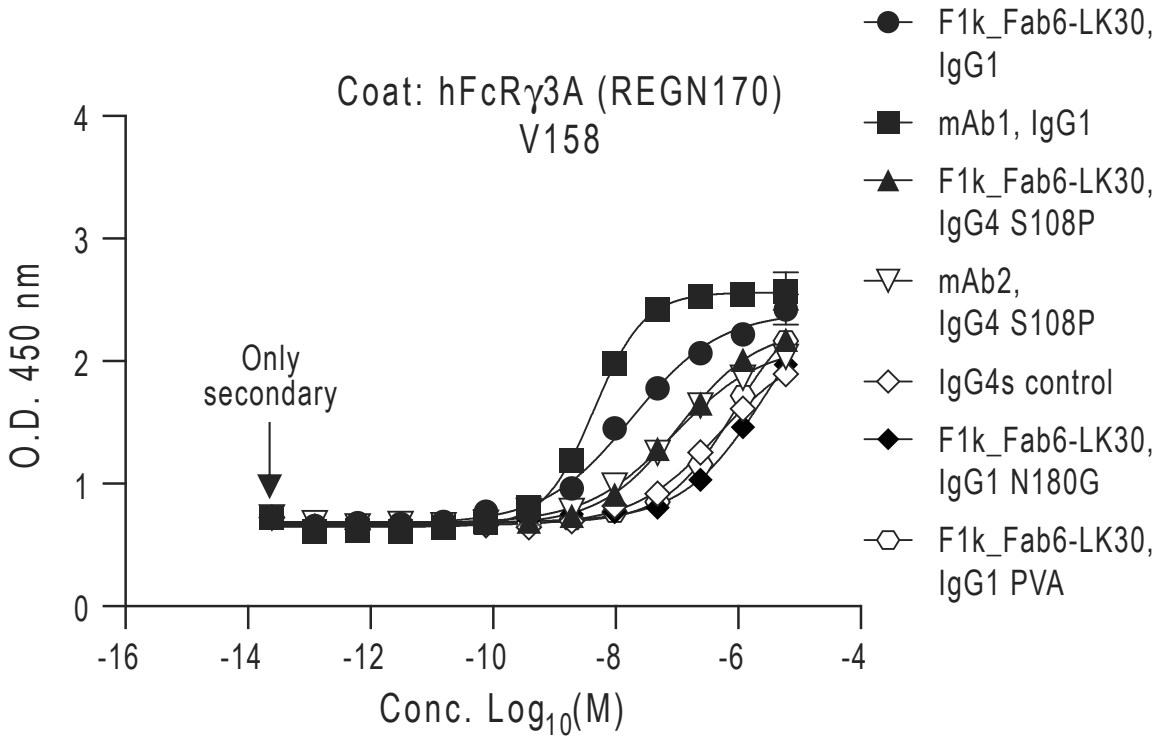


FIG. 19E

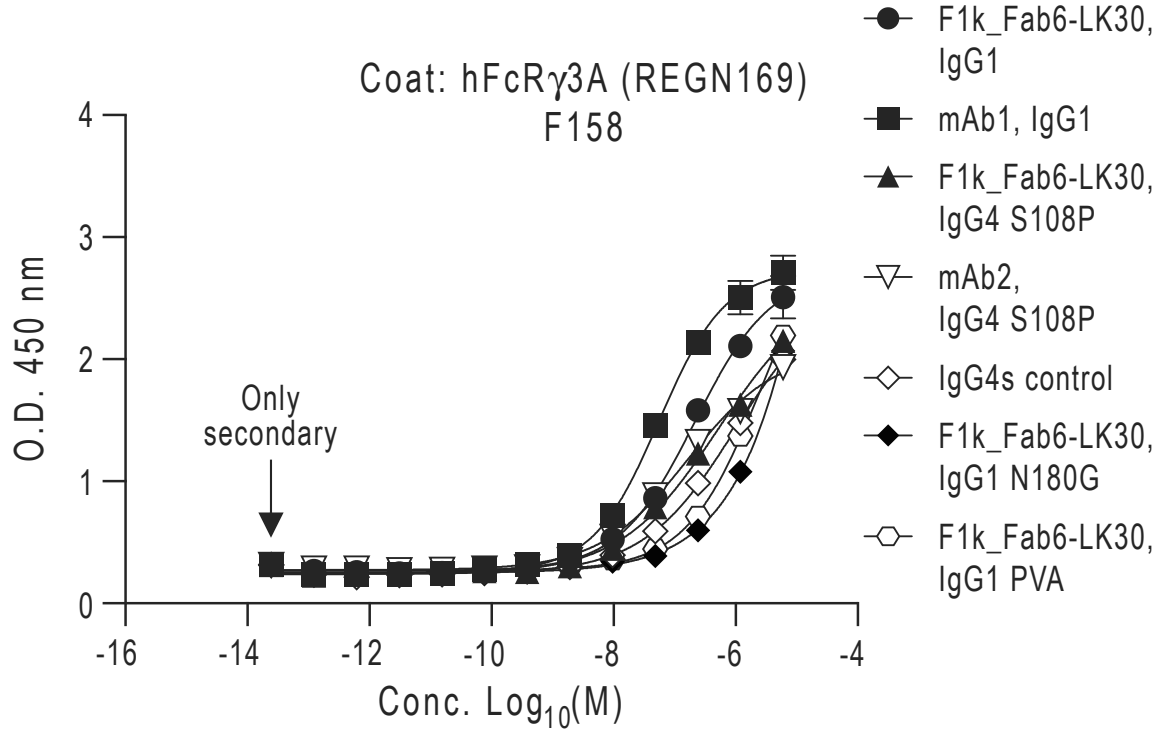


FIG. 19F

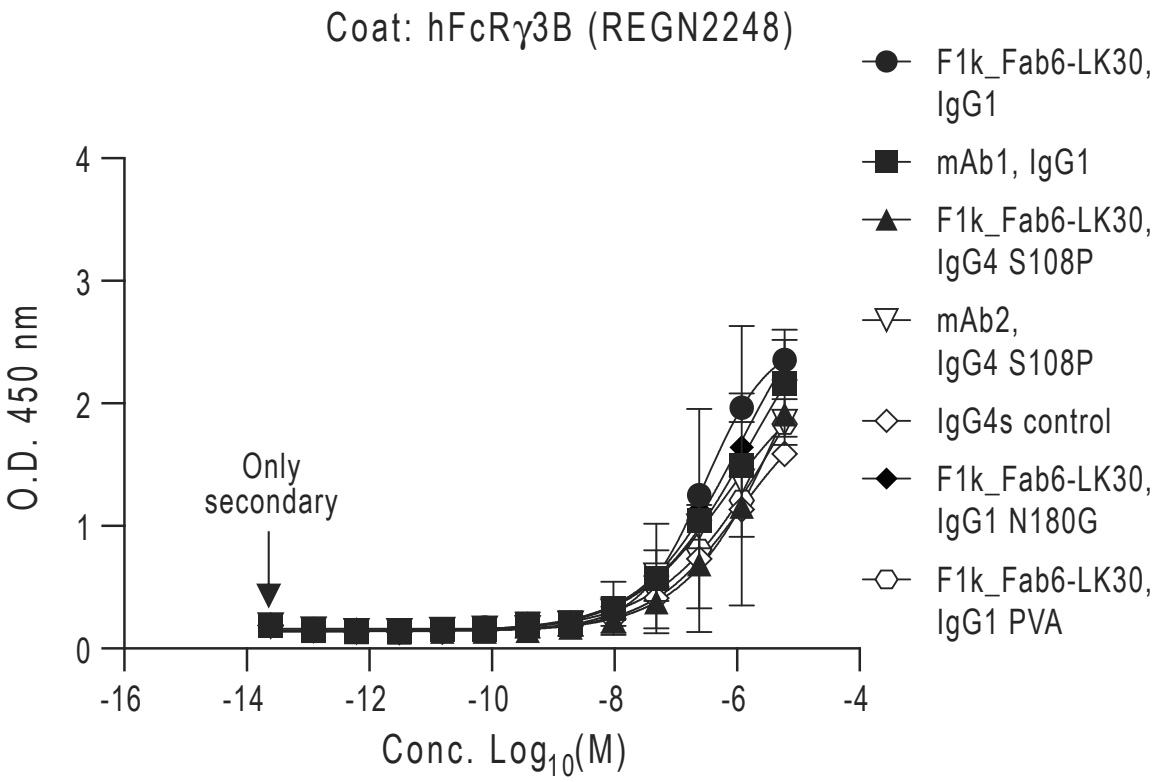

Coat: hFcRγ3B (REGN2248)
O.D. 450 nm
4
3
2
1
0
Only secondary
-16
-14
-12
-10
-8
-6
-4
Conc. Log10(M)
F1k_Fab6-LK30, IgG1
mAb1, IgG1
F1k_Fab6-LK30, IgG4 S108P
mAb2, IgG4 S108P
IgG4s control
F1k_Fab6-LK30, IgG1 N180G
F1k_Fab6-LK30, IgG1 PVA

FIG. 19G

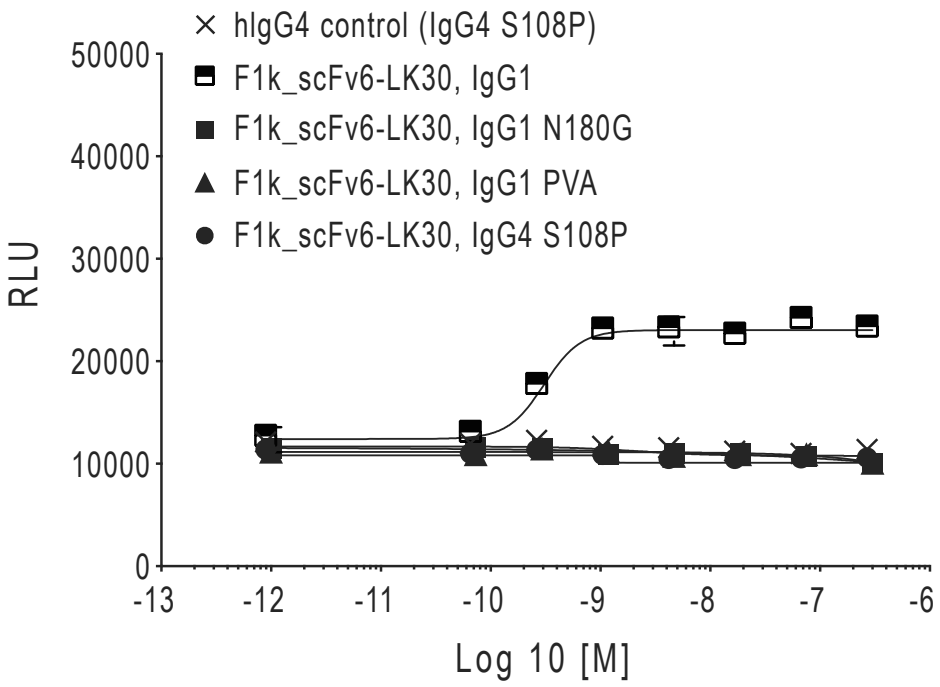

Surrogate ADCC Assay of scFv based Alternative Format Antibodies
× hIgG4 control (IgG4 S108P)
F1k_scFv6-LK30, IgG1
F1k_scFv6-LK30, IgG1 N180G
F1k_scFv6-LK30, IgG1 PVA
F1k_scFv6-LK30, IgG4 S108P
RLU
50000
40000
30000
20000
10000
0
-13
-12
-11
-10
-9
-8
-7
-6
Log 10 [M]

FIG. 20

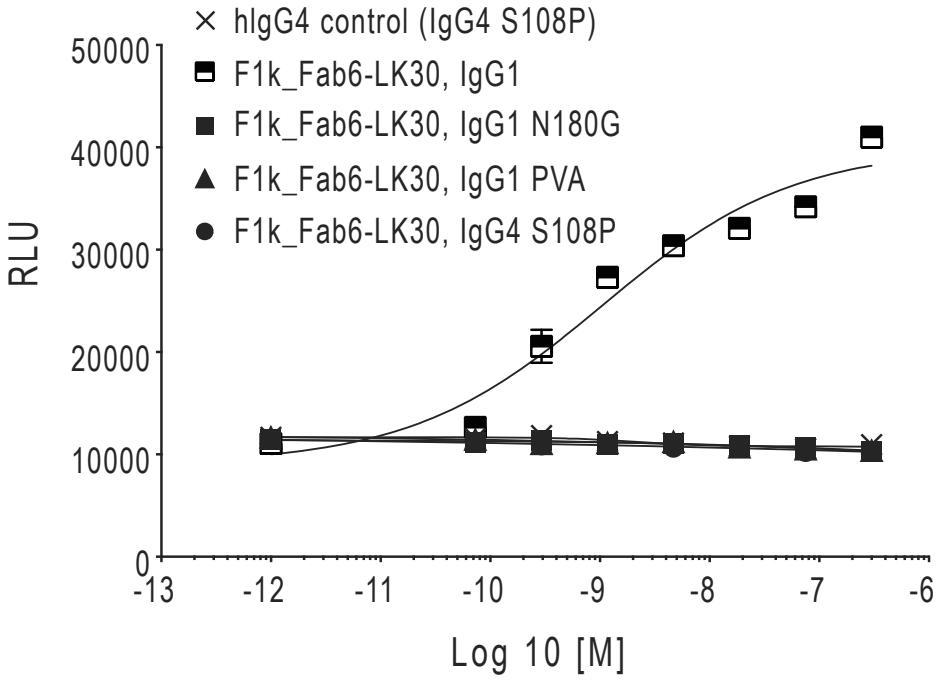

Surrogate ADCC Assay of Fab based Alternative Format Antibodies
× hIgG4 control (IgG4 S108P)
F1k_Fab6-LK30, IgG1
F1k_Fab6-LK30, IgG1 N180G
F1k_Fab6-LK30, IgG1 PVA
F1k_Fab6-LK30, IgG4 S108P
RLU
50000
40000
30000
20000
10000
0
-13
-12
-11
-10
-9
-8
-7
-6
Log 10 [M]

FIG. 21

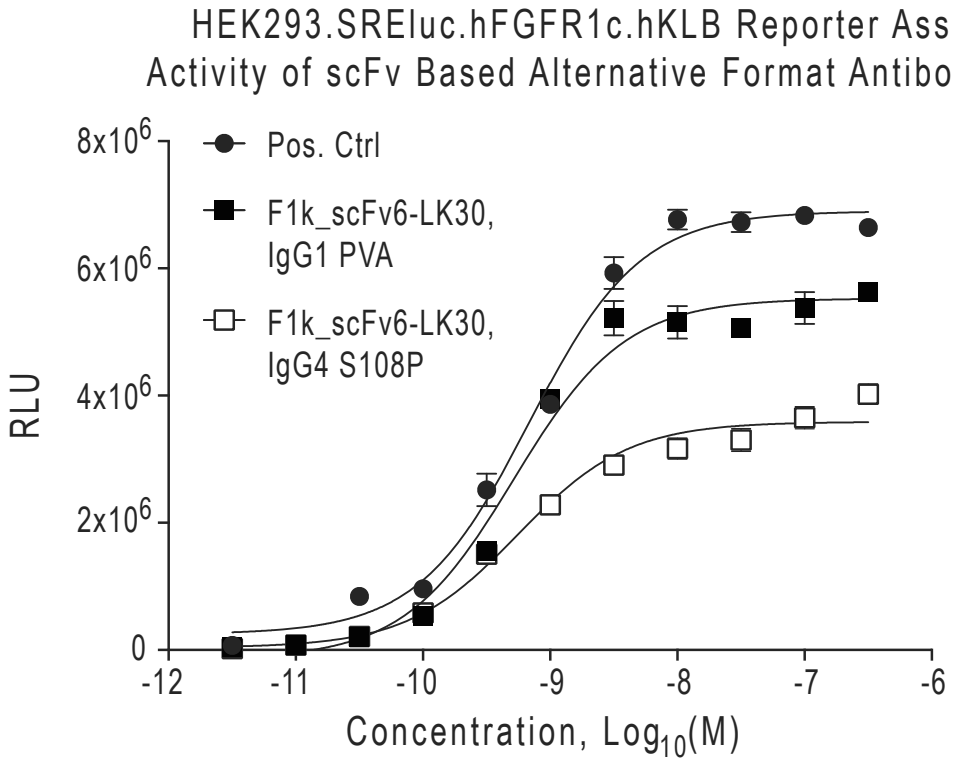

HEK293.SREluc.hFGFR1c.hKLB Reporter Assay
Activity of scFv Based Alternative Format Antibodies
Pos. Ctrl
F1k_scFv6-LK30, IgG1 PVA
F1k_scFv6-LK30, IgG4 S108P
RLU
8x10^6
6x10^6
4x10^6
2x10^6
0
-12
-11
-10
-9
-8
-7
-6
Concentration, Log10(M)

FIG. 22

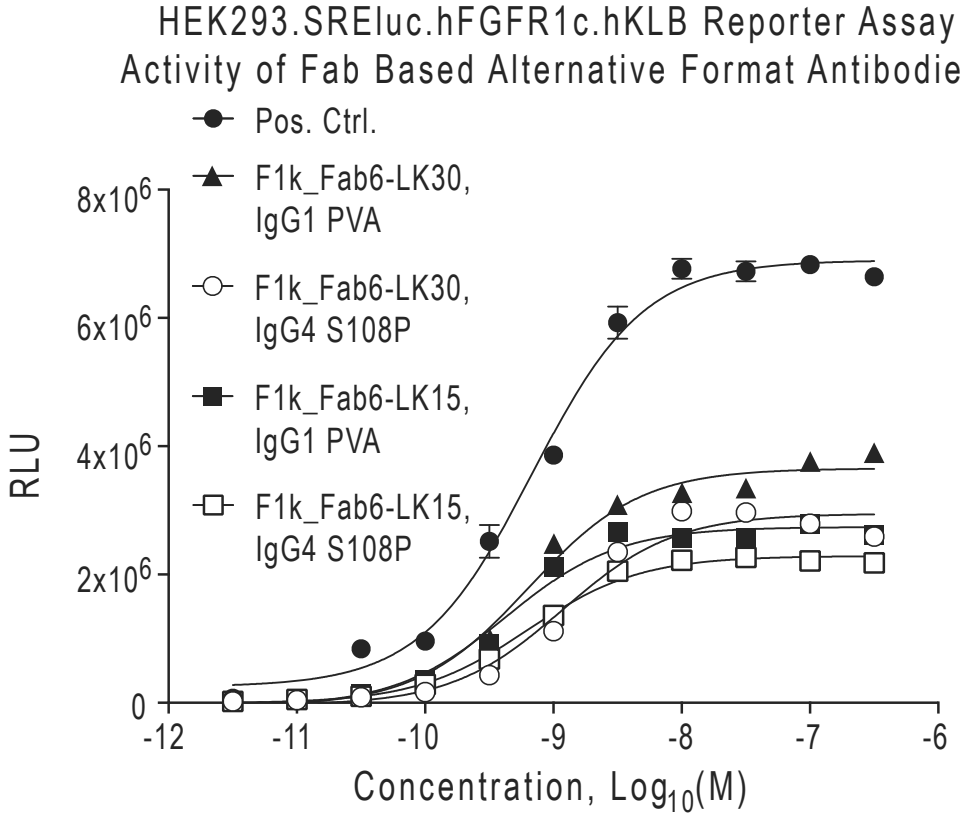
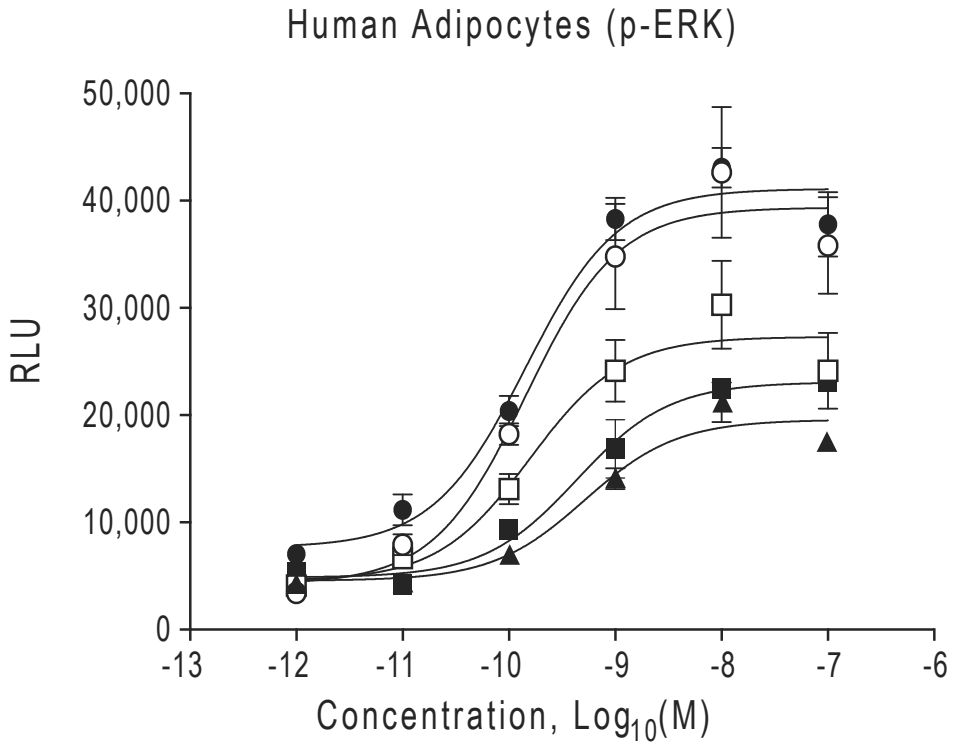

HEK293.SREluc.hFGFR1c.hKLB Reporter Assay
Activity of Fab Based Alternative Format Antibodies
Pos. Ctrl.
F1k_Fab6-LK30, IgG1 PVA
F1k_Fab6-LK30, IgG4 S108P
F1k_Fab6-LK15, IgG1 PVA
F1k_Fab6-LK15, IgG4 S108P
RLU
8x10^6
6x10^6
4x10^6
2x10^6
0
-12
-11
-10
-9
-8
-7
-6
Concentration, Log10(M)

FIG. 23



	EC50 (M)	%Activity of FGF21
His.hFGF21	1.4E-10	100.0
F1k_scFv6-LK30, IgG1 PVA	1.4E-10	95.2
F1k_Fab6-LK15, IgG1 PVA	4.3E-10	50.7
F1k_scFv6-LK30, IgG4 S108P	1.6E-10	62.2
F1k_Fab6-LK15, IgG4 S108P	5.1E-10	41.1

FIG. 24