

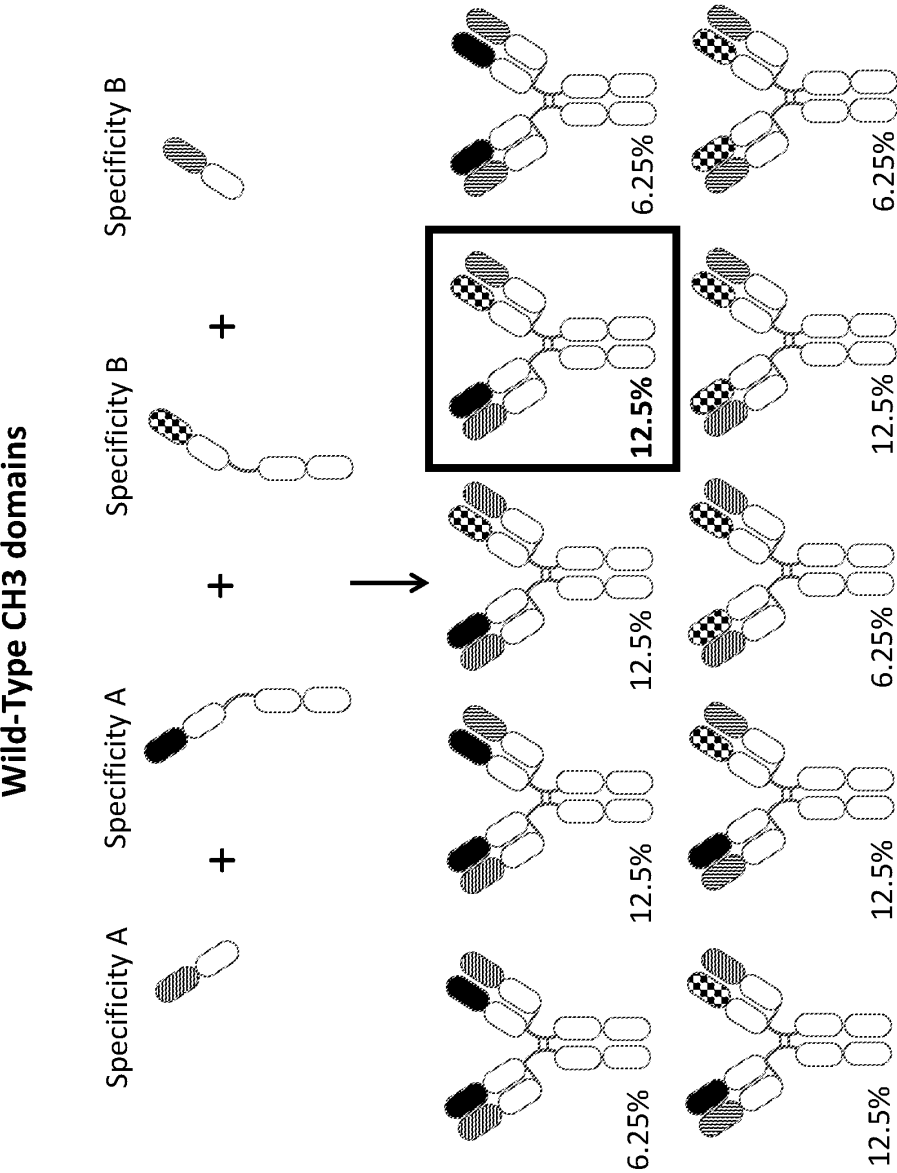


FIG. 1A

Heterodimerizing, variant CH3 domains

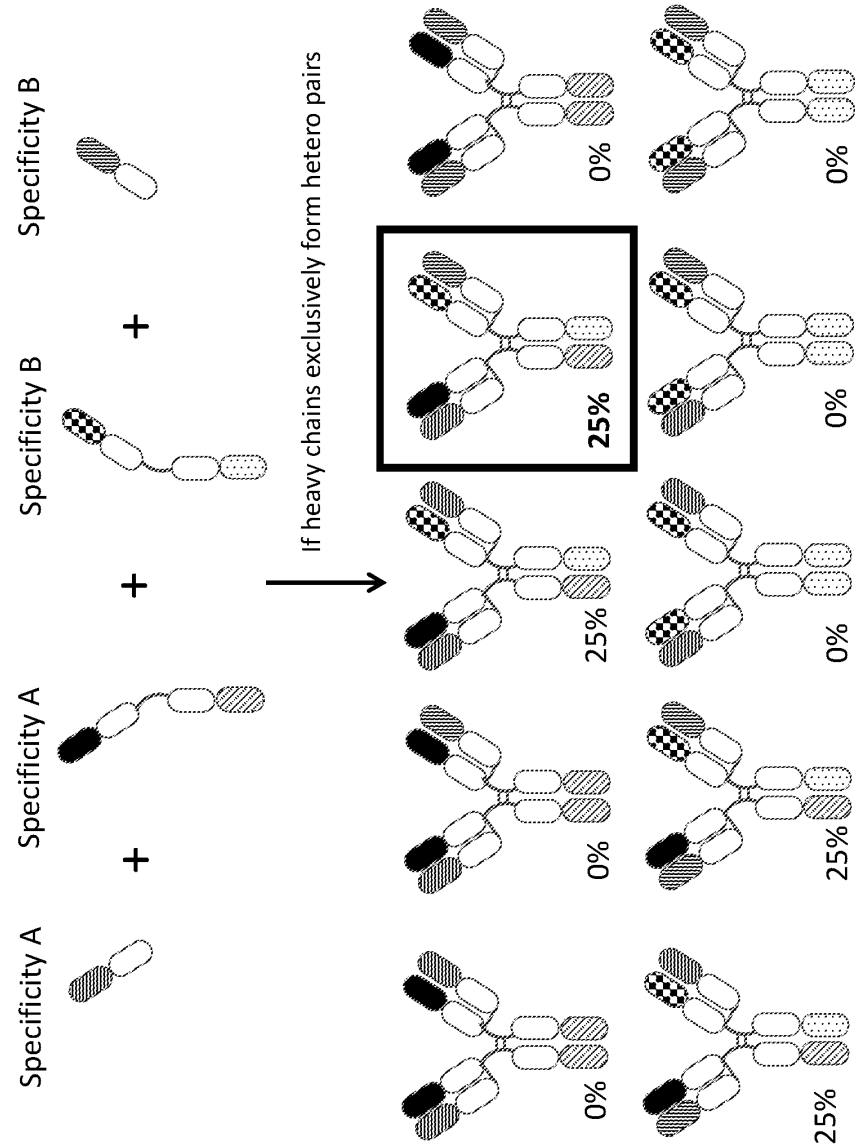


FIG. 1B

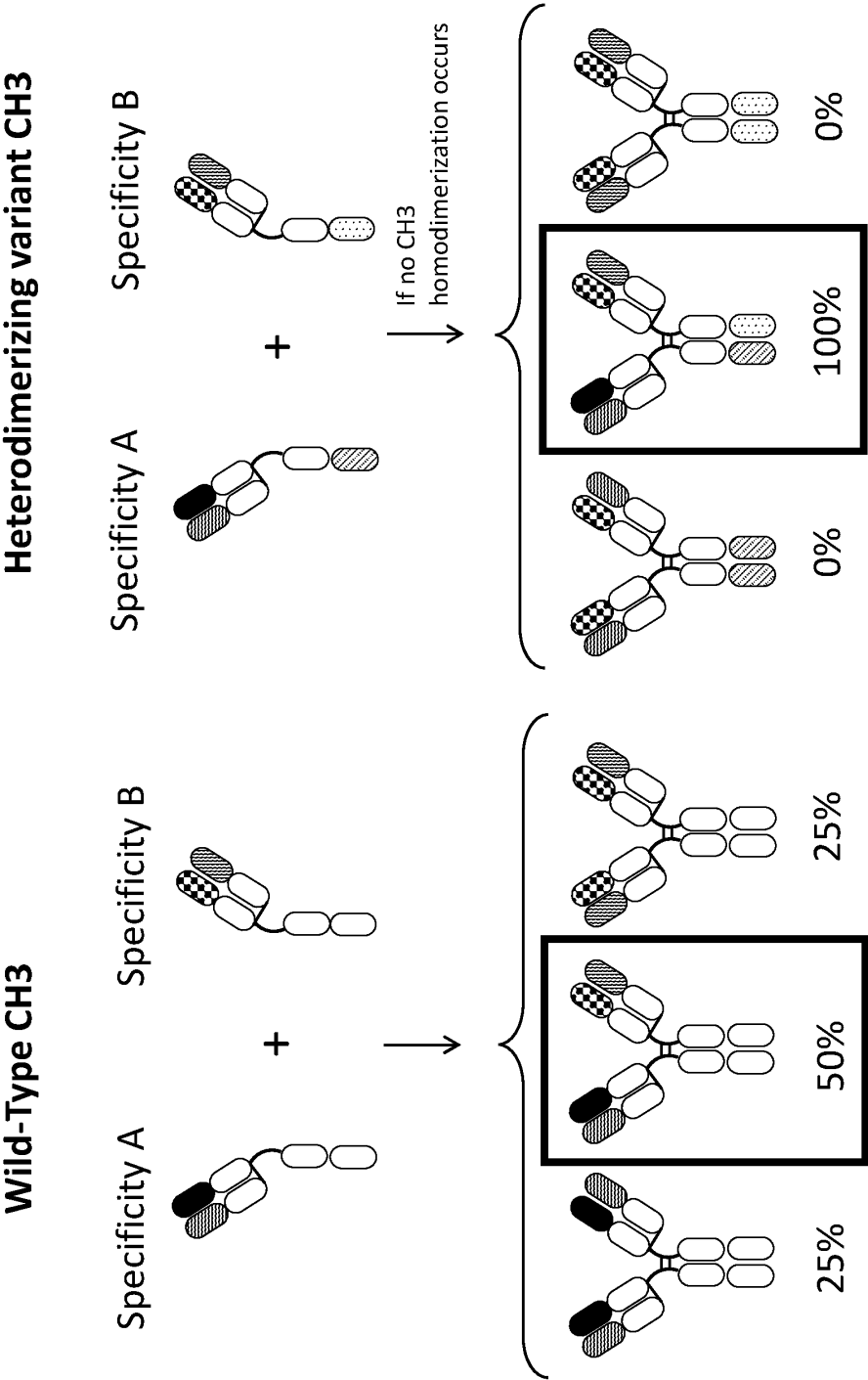


FIG. 1C

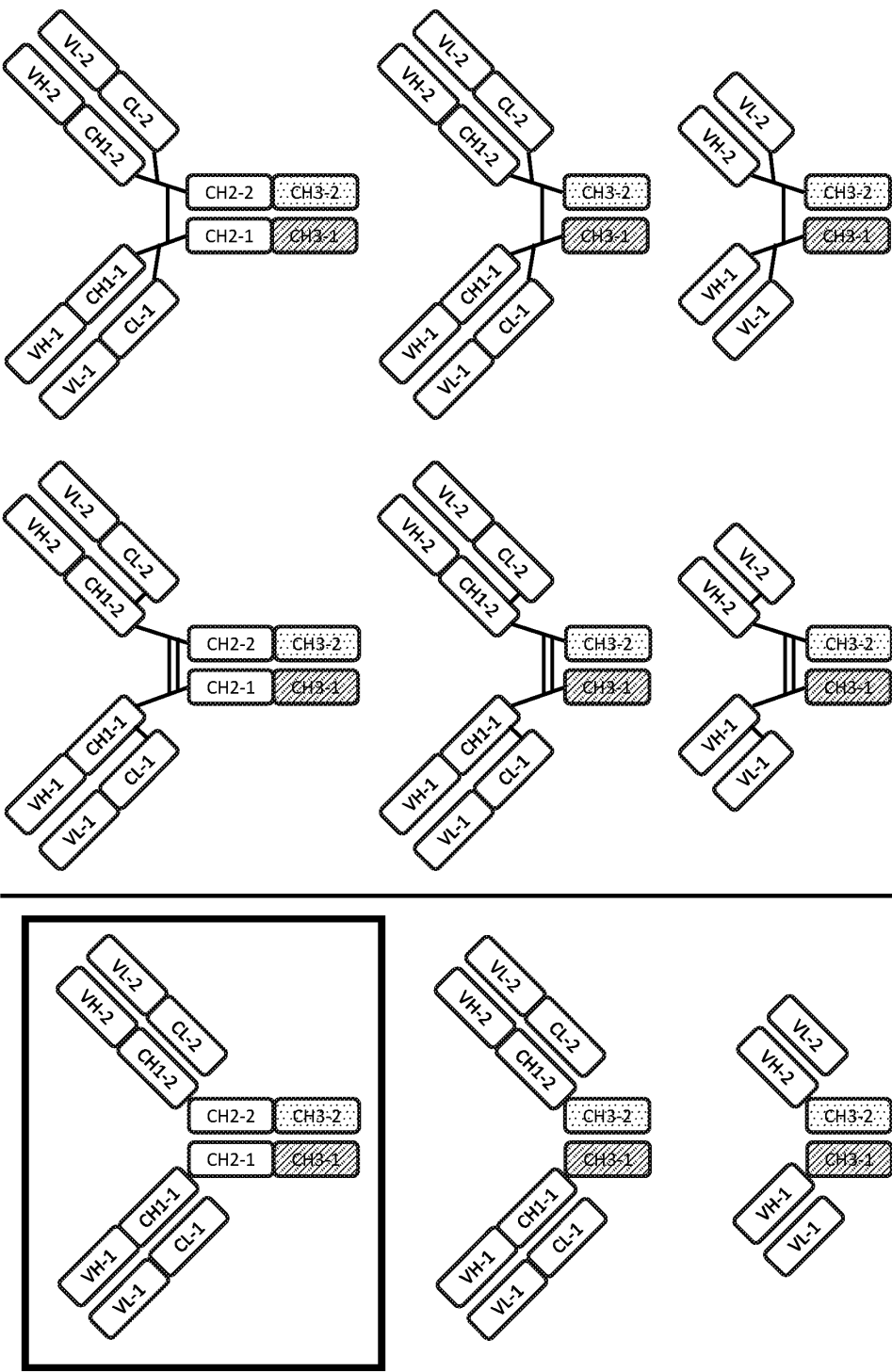


FIG. 2

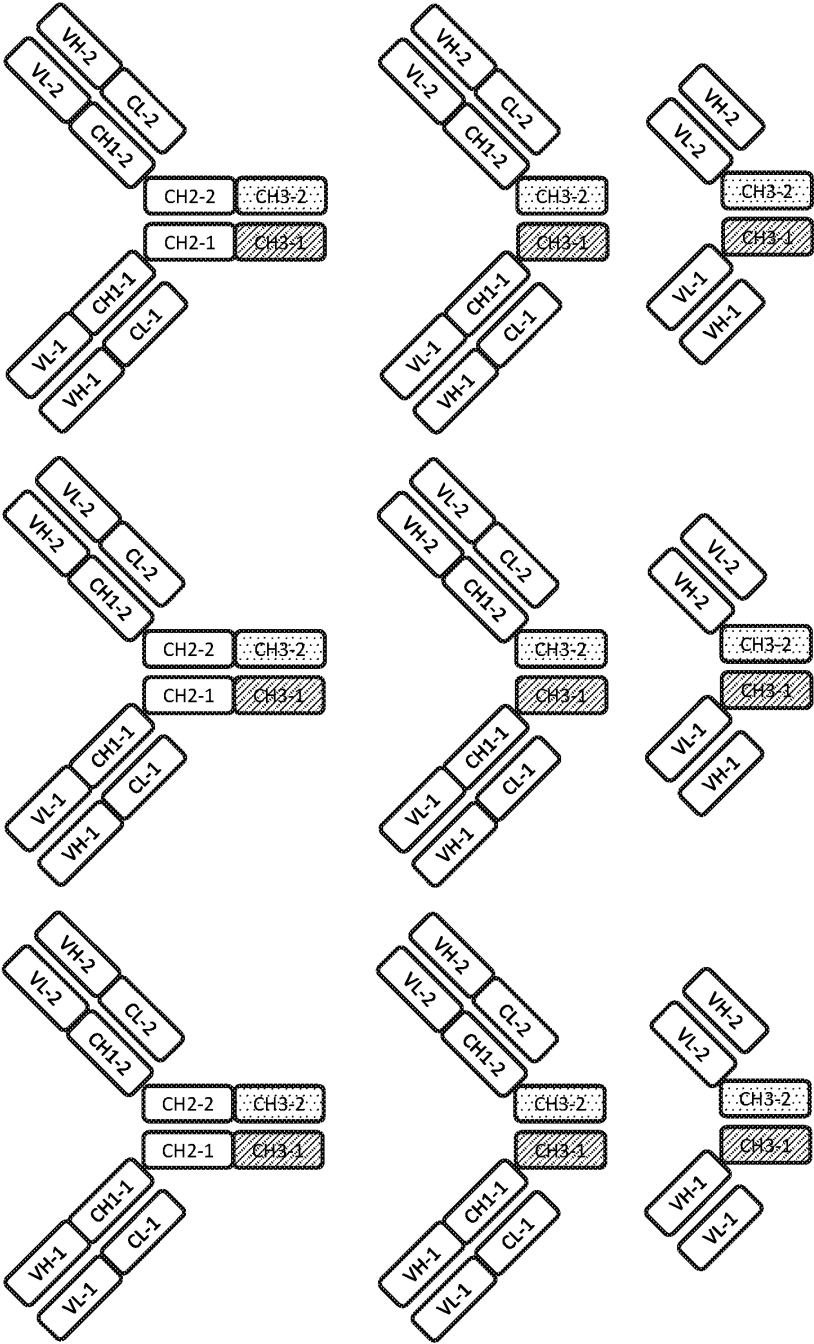


FIG. 3A

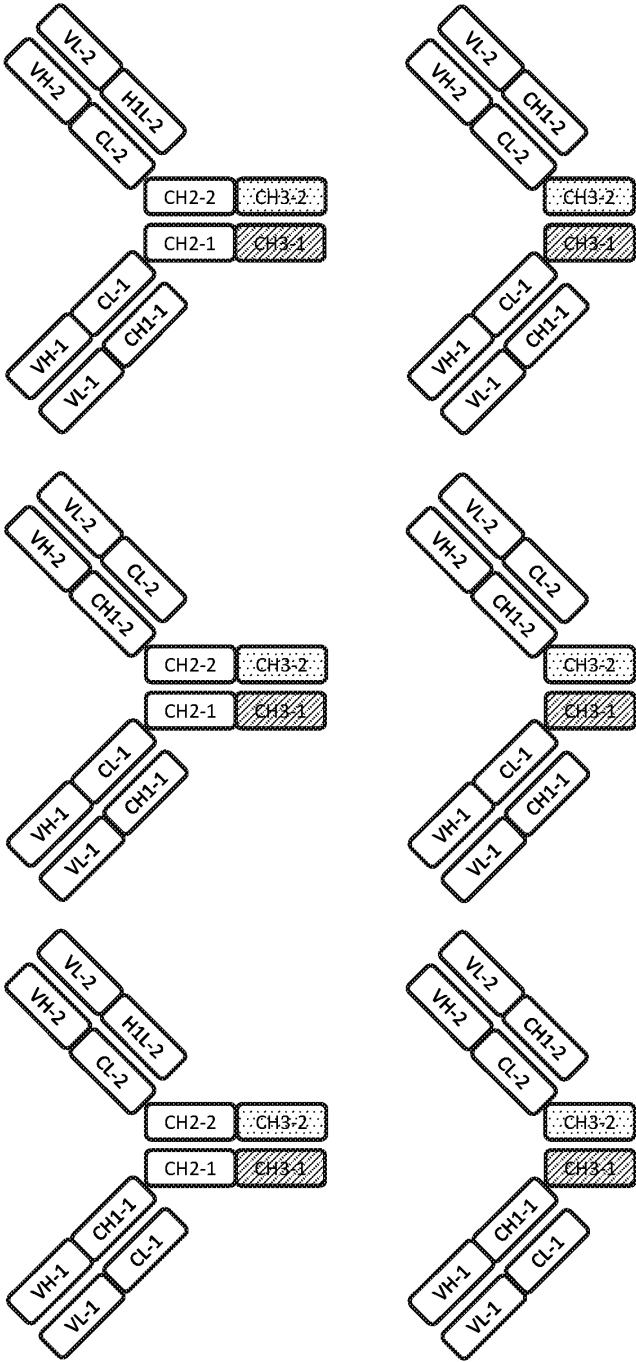


FIG. 3B

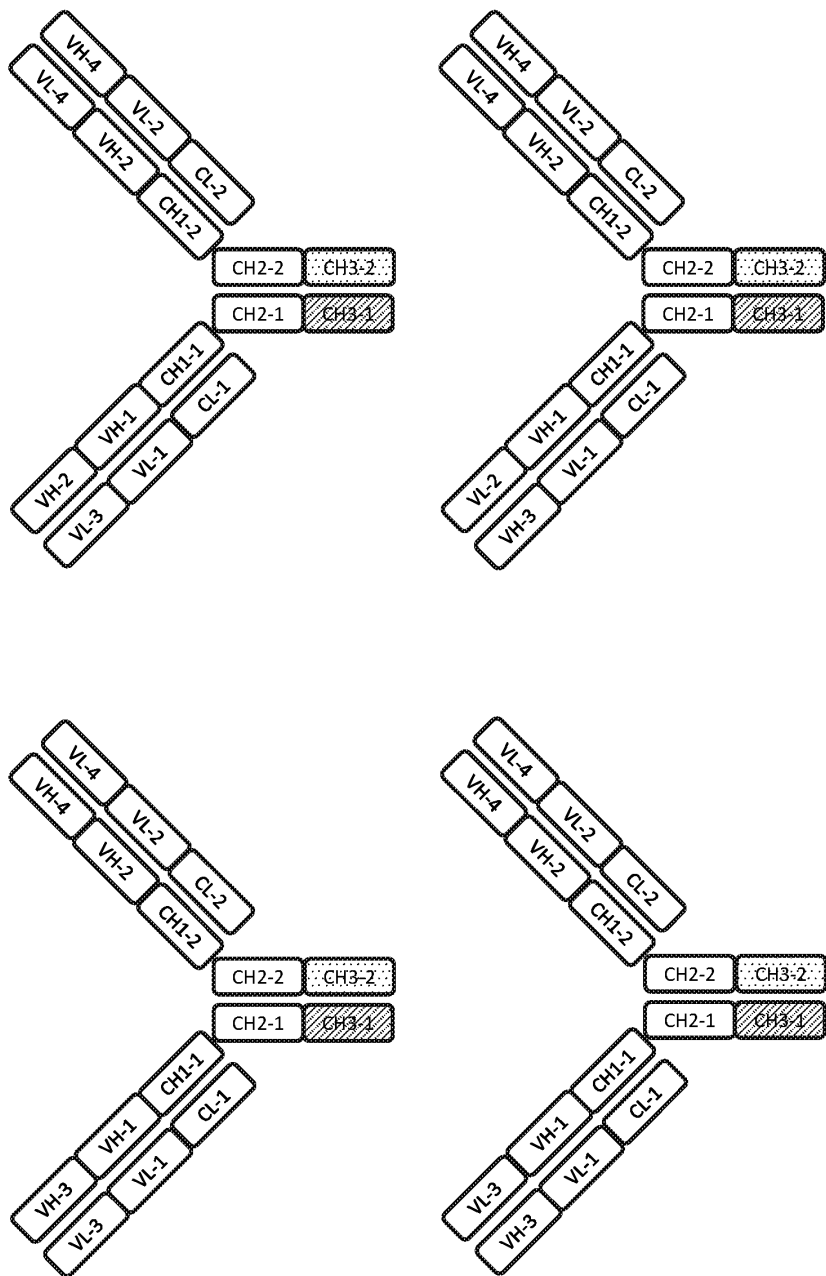


FIG. 4

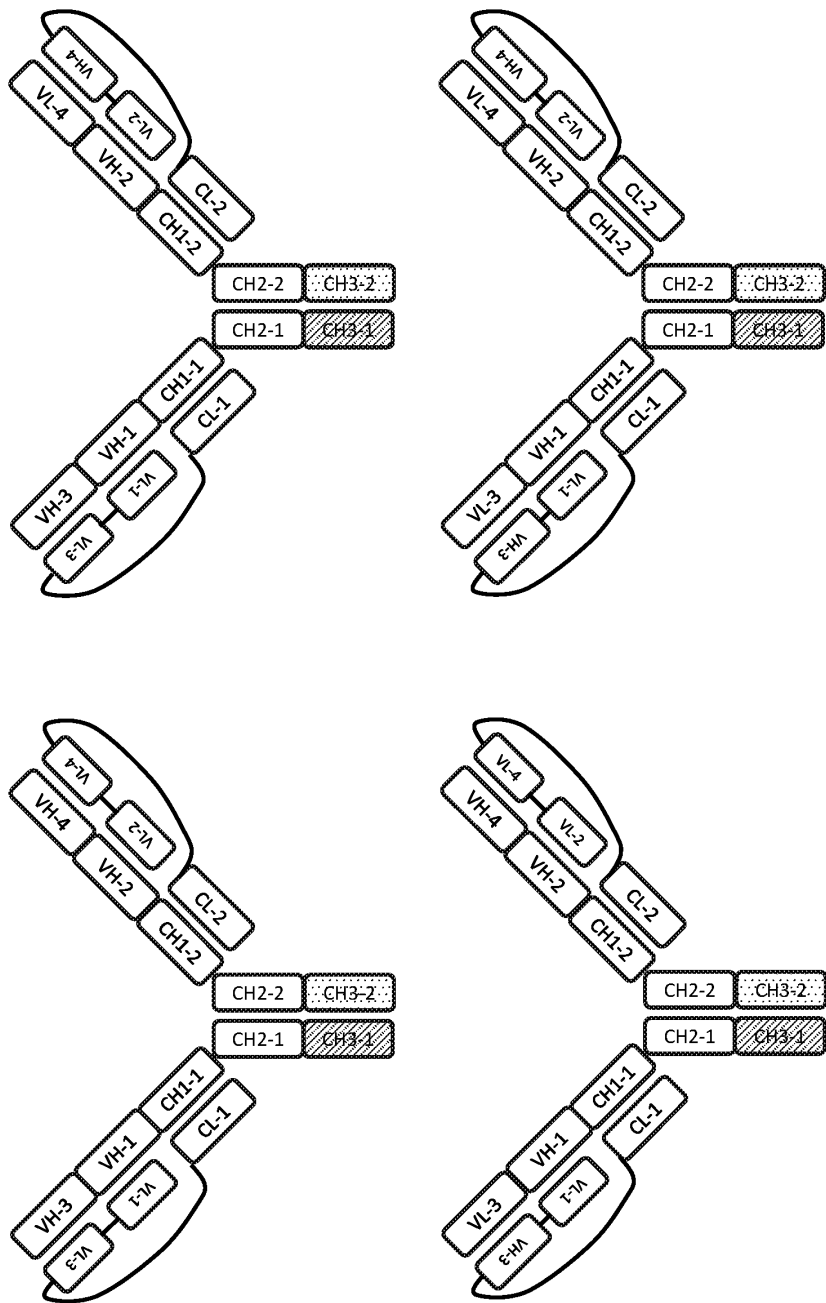


FIG. 5

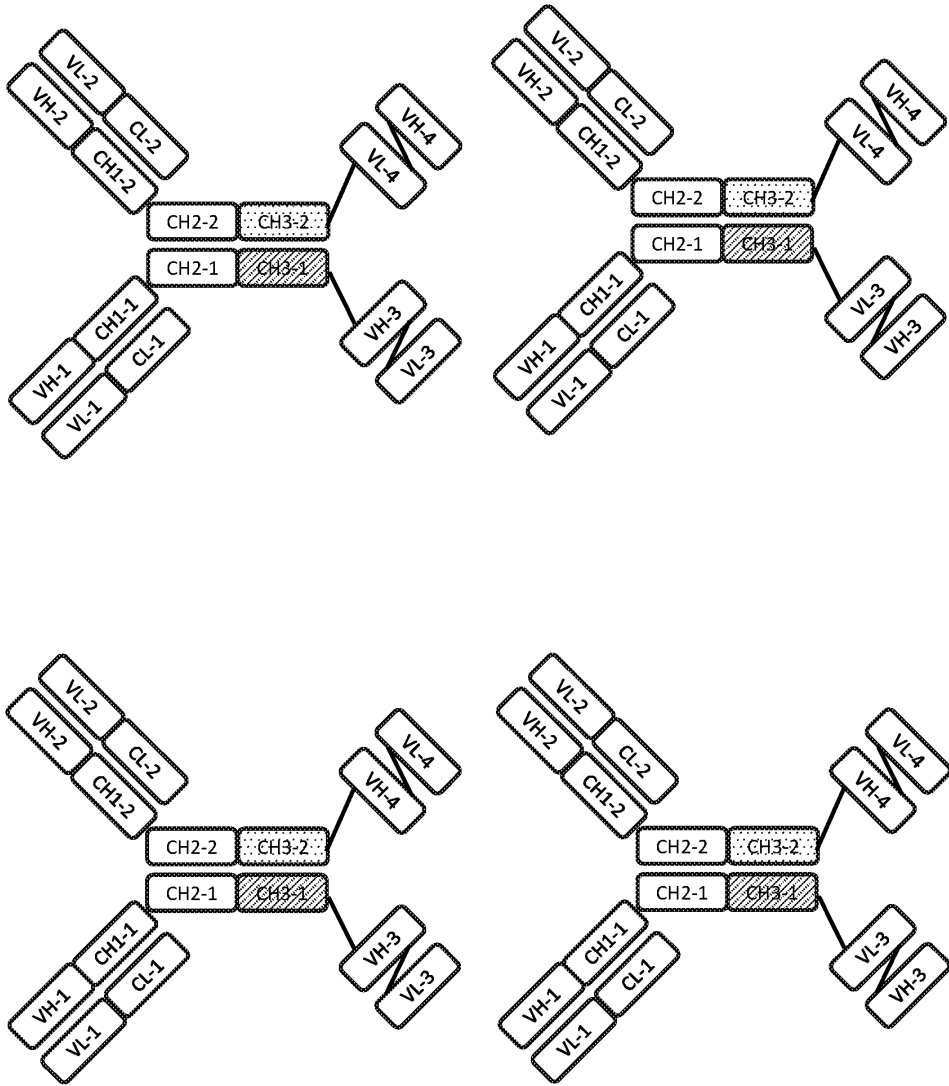


FIG. 6A

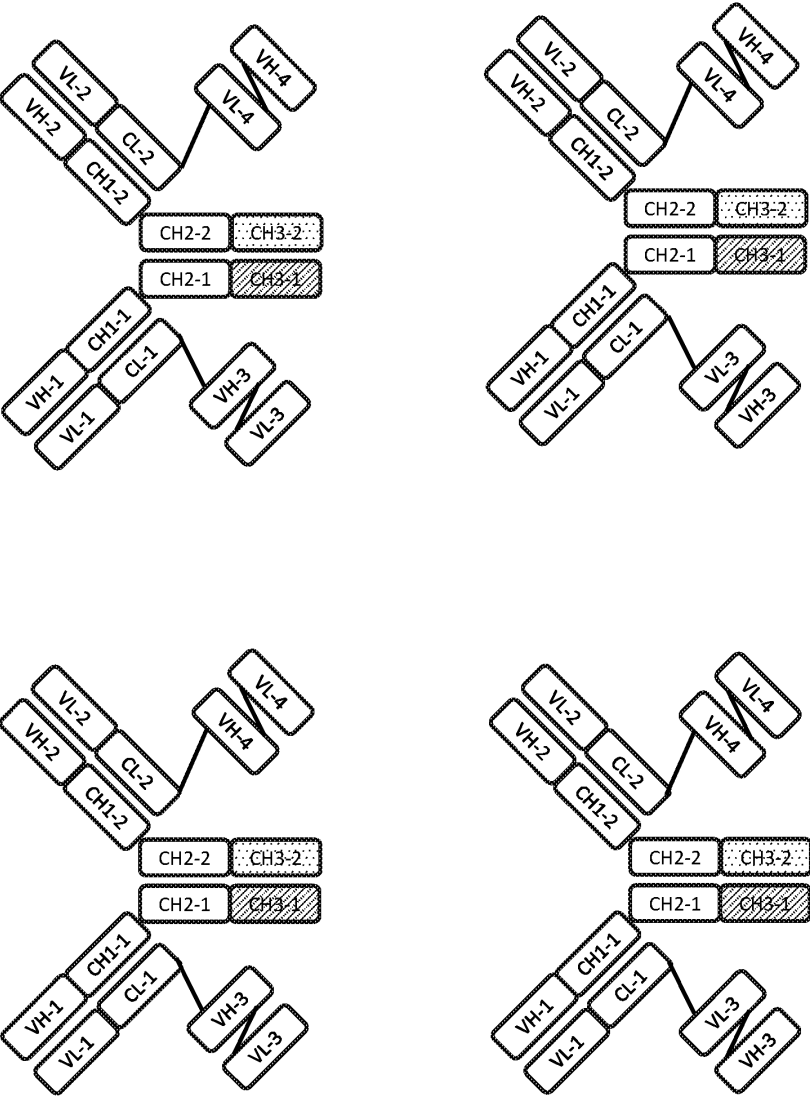


FIG. 6B

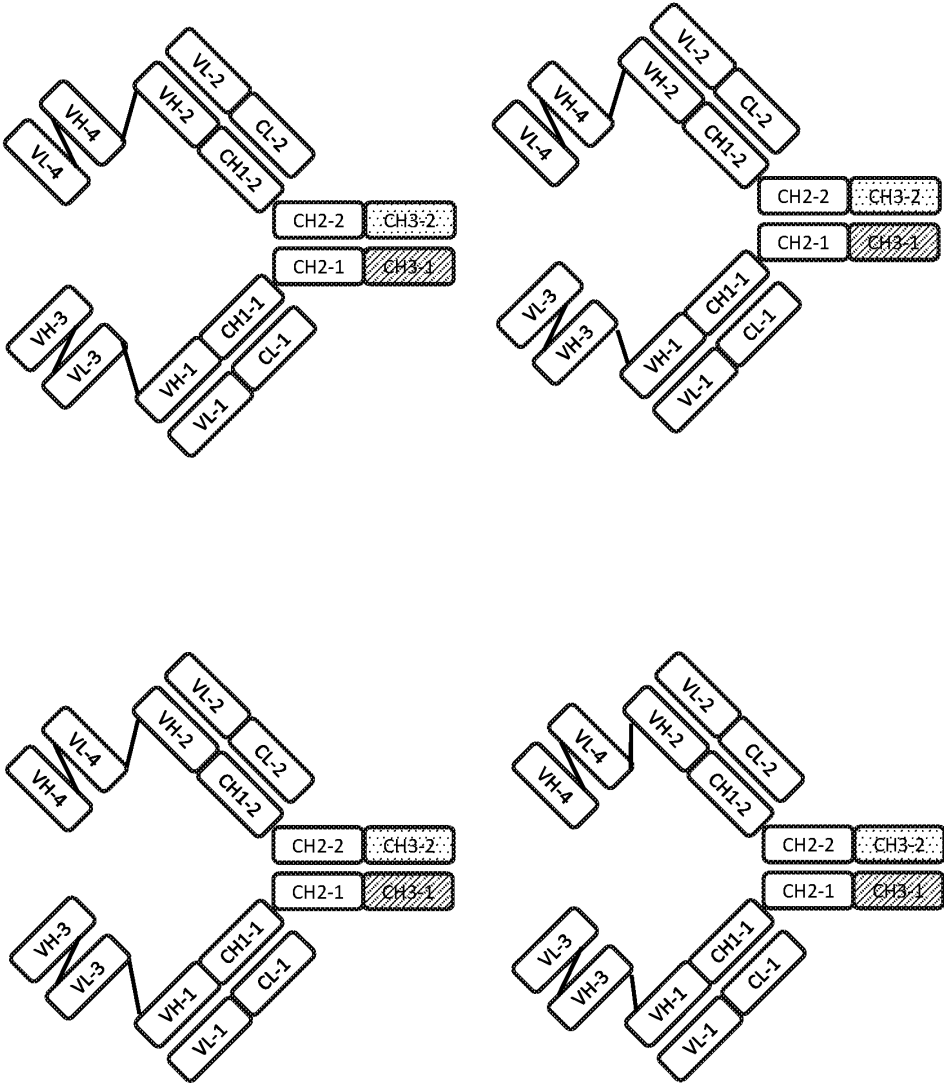


FIG. 6C

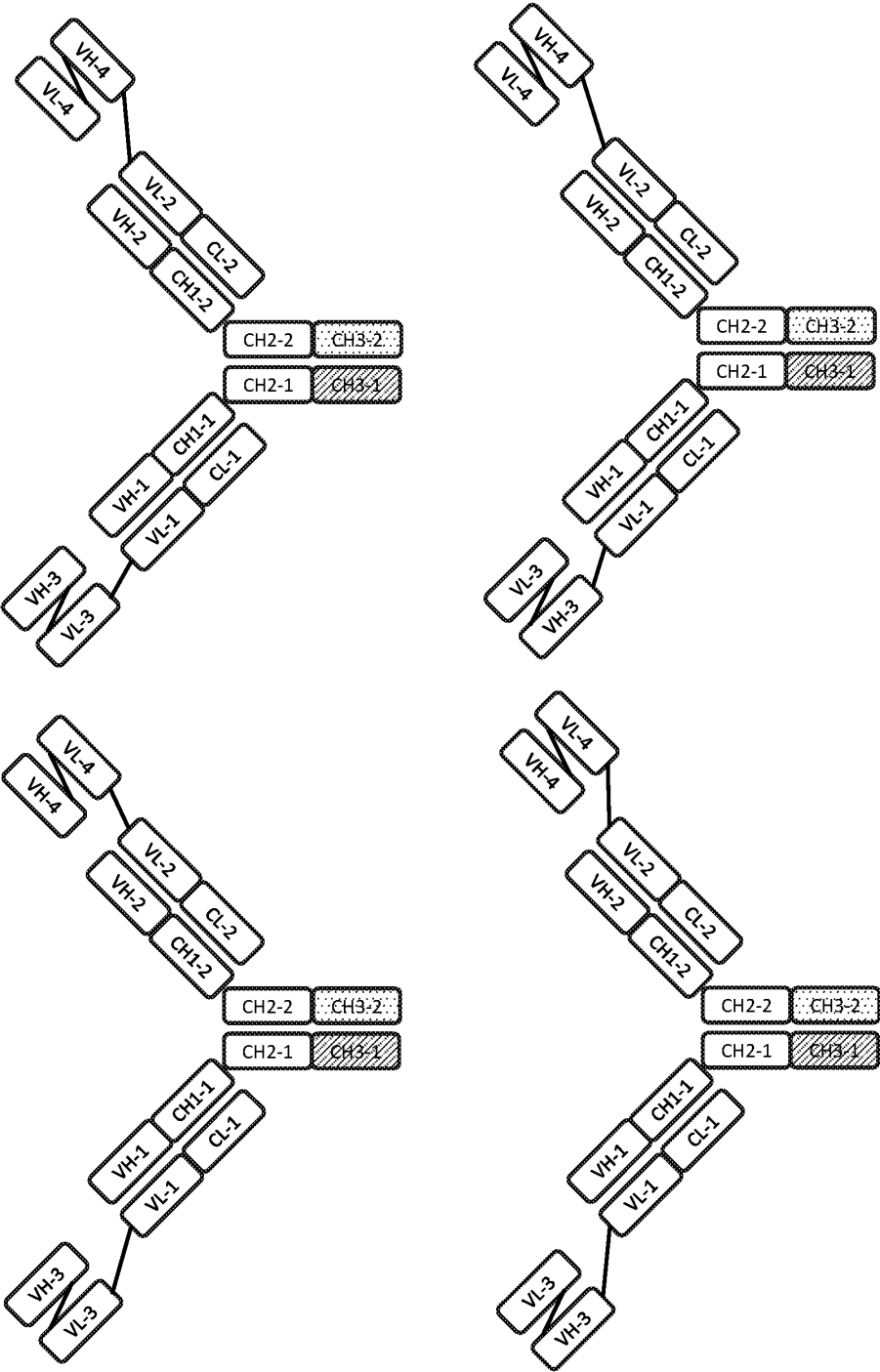


FIG. 6D

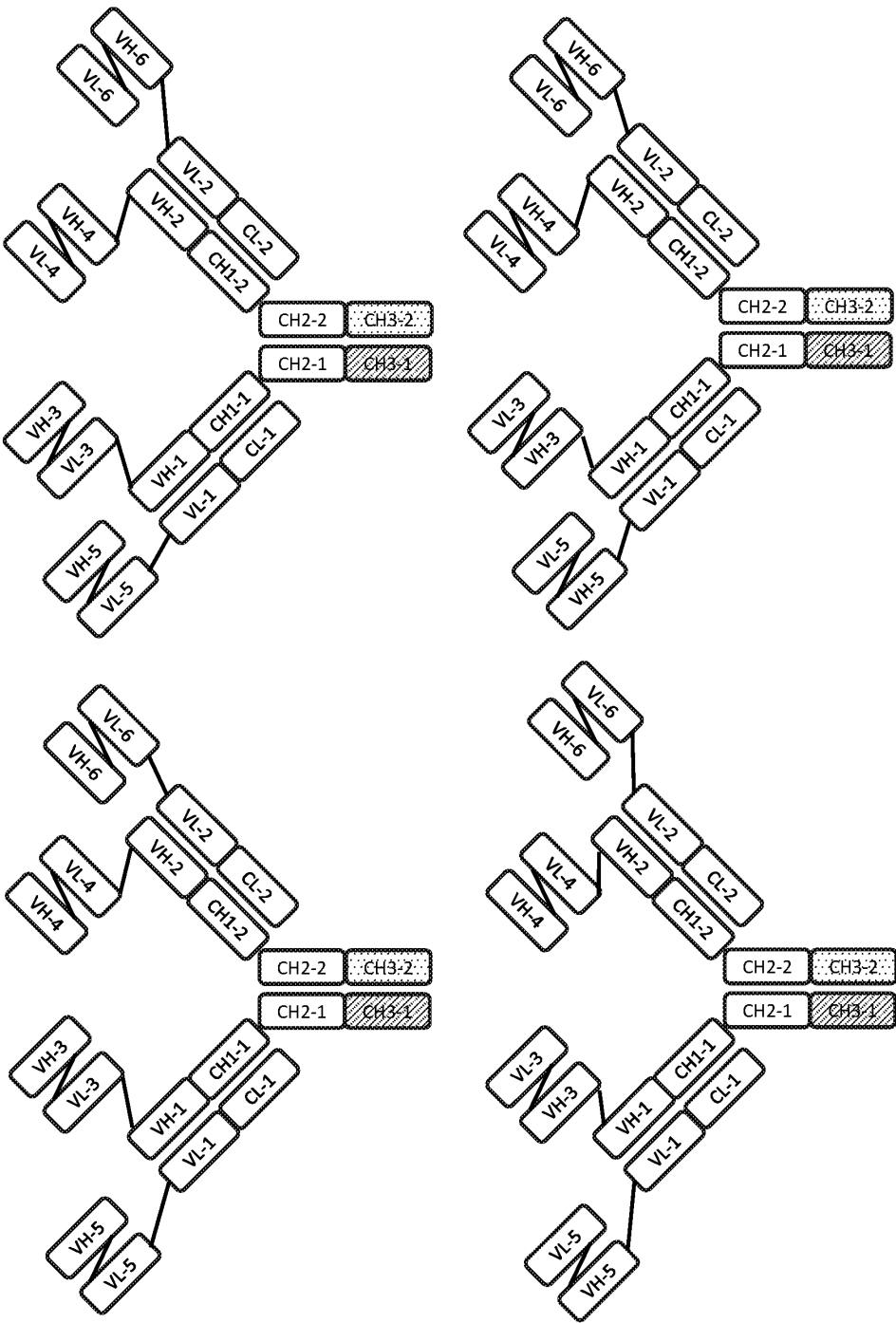


FIG. 6E

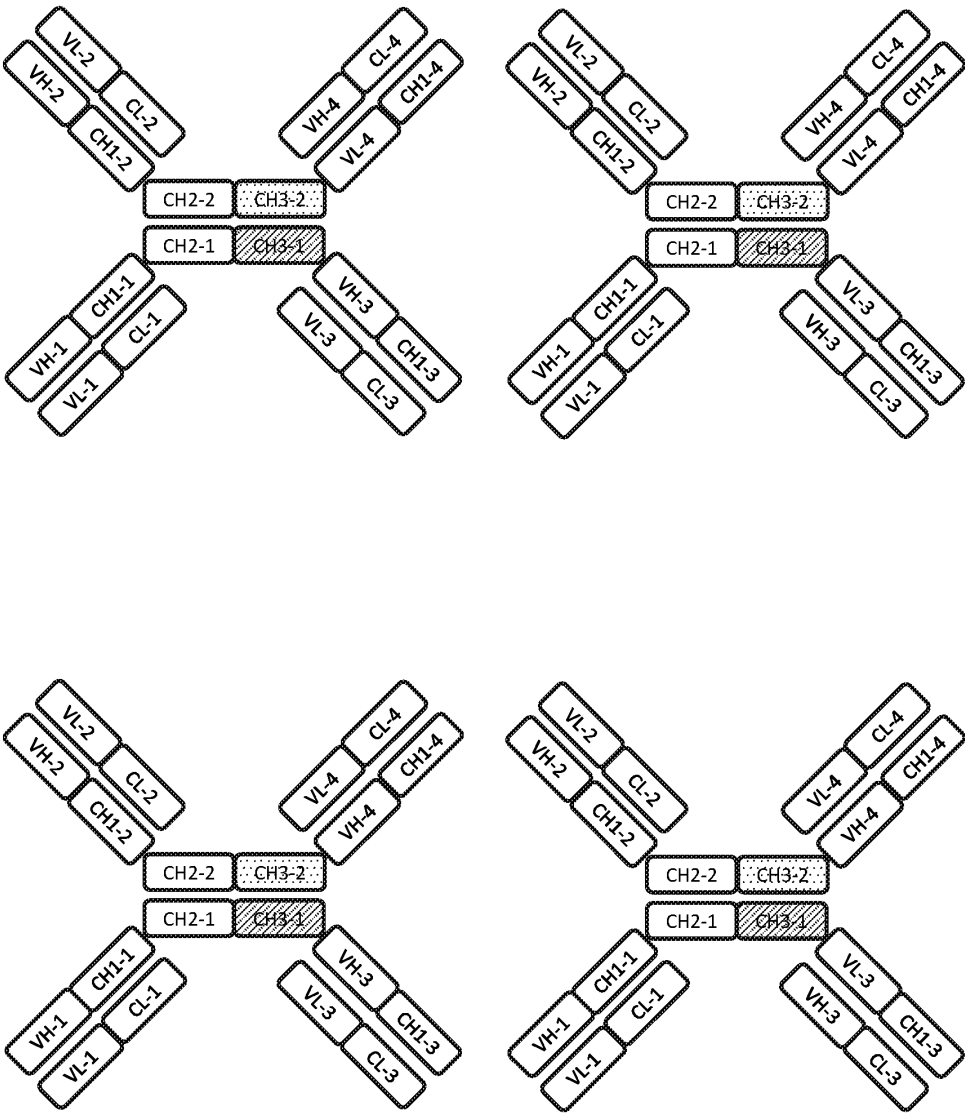


FIG. 7A

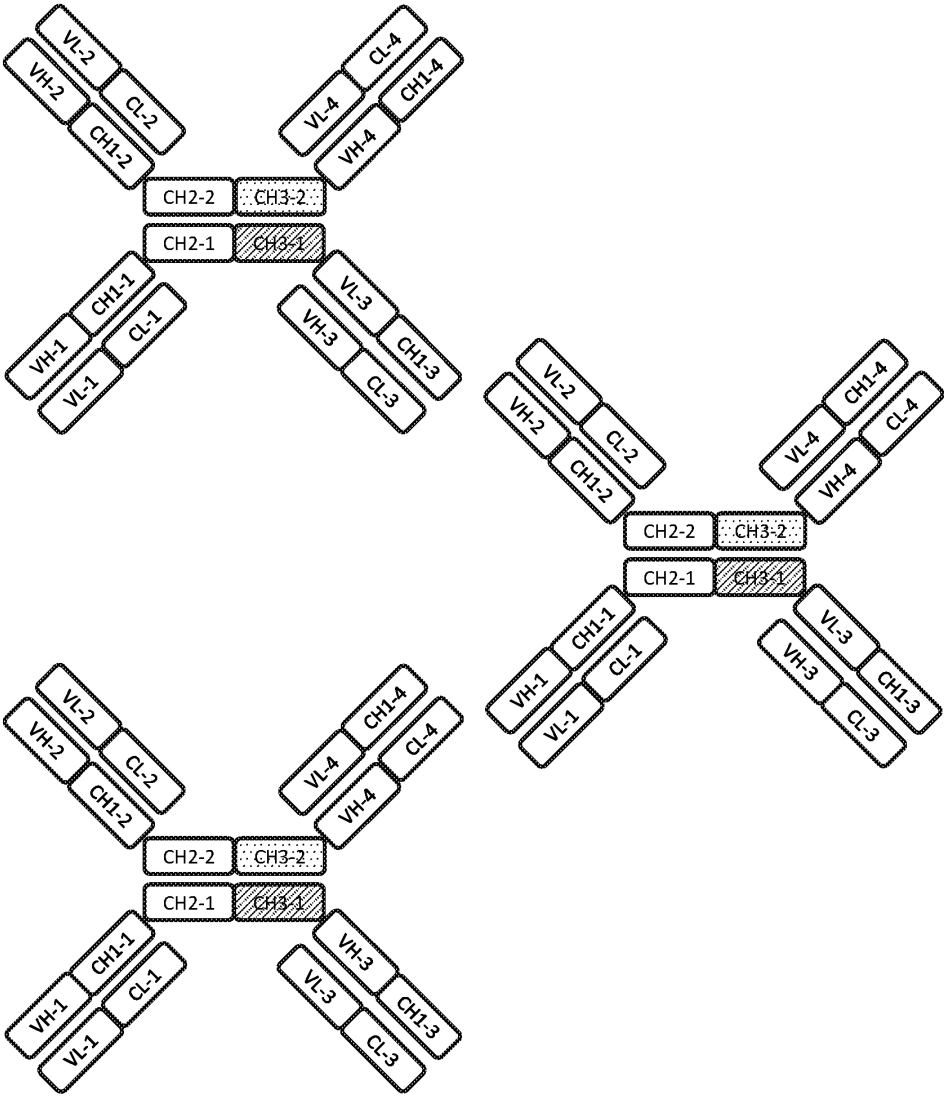


FIG. 7B

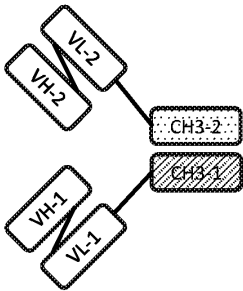
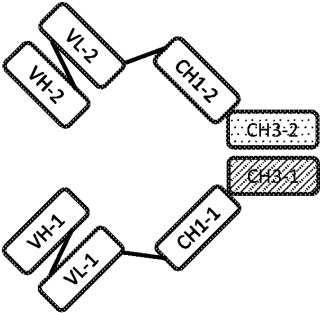
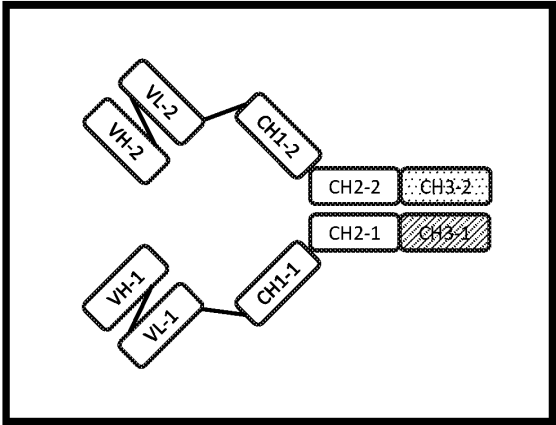


FIG. 8A

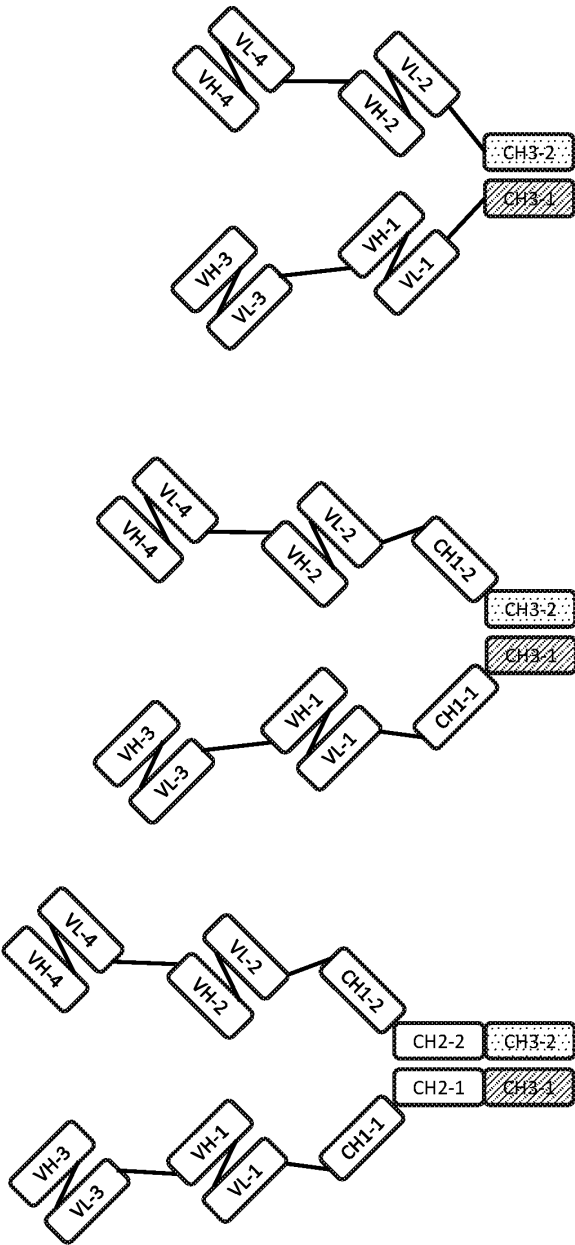


FIG. 8B

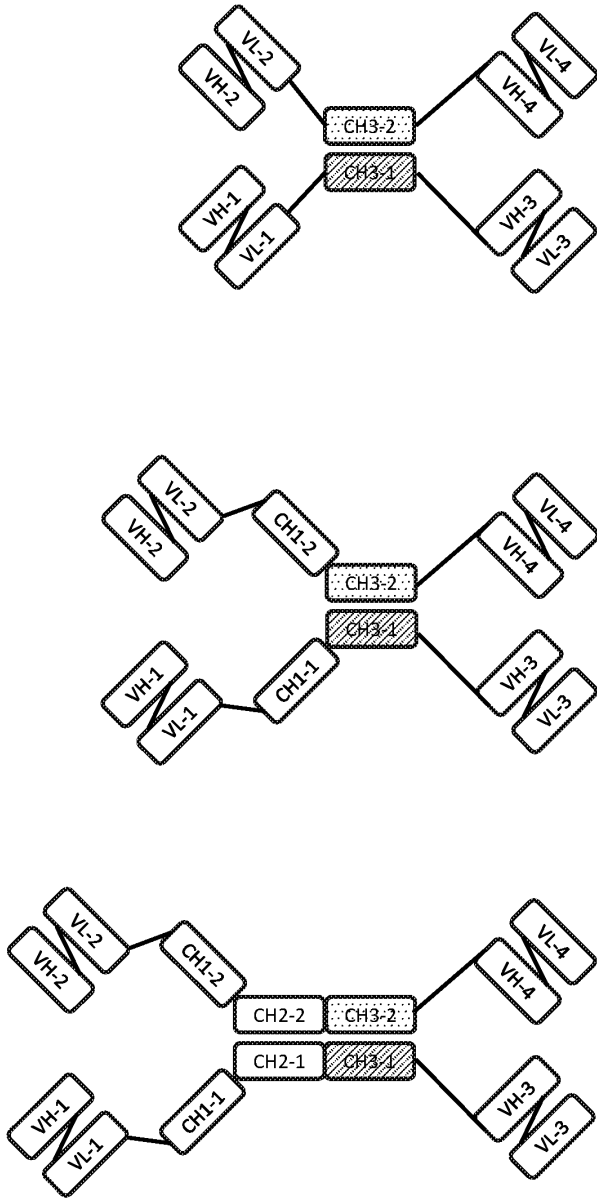


FIG. 8C

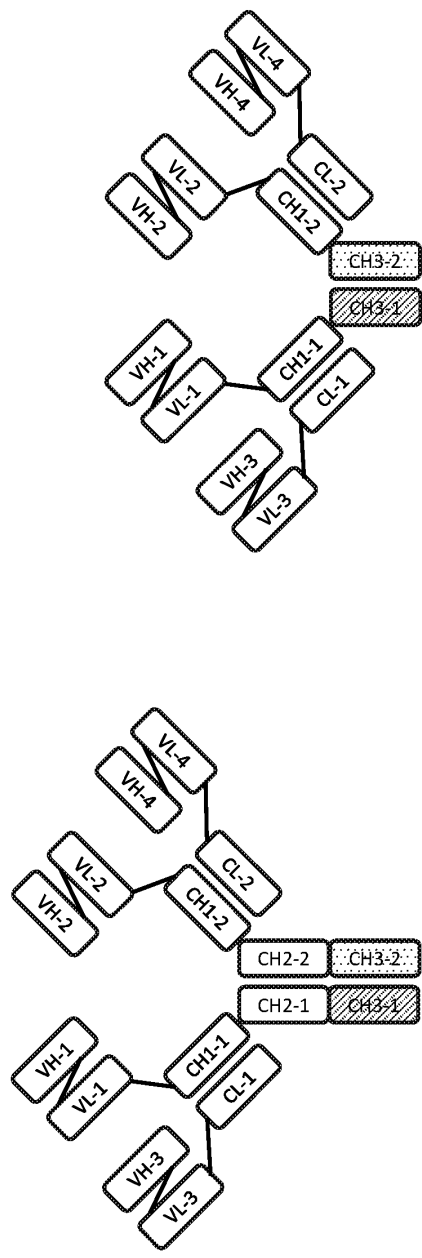


FIG. 8D

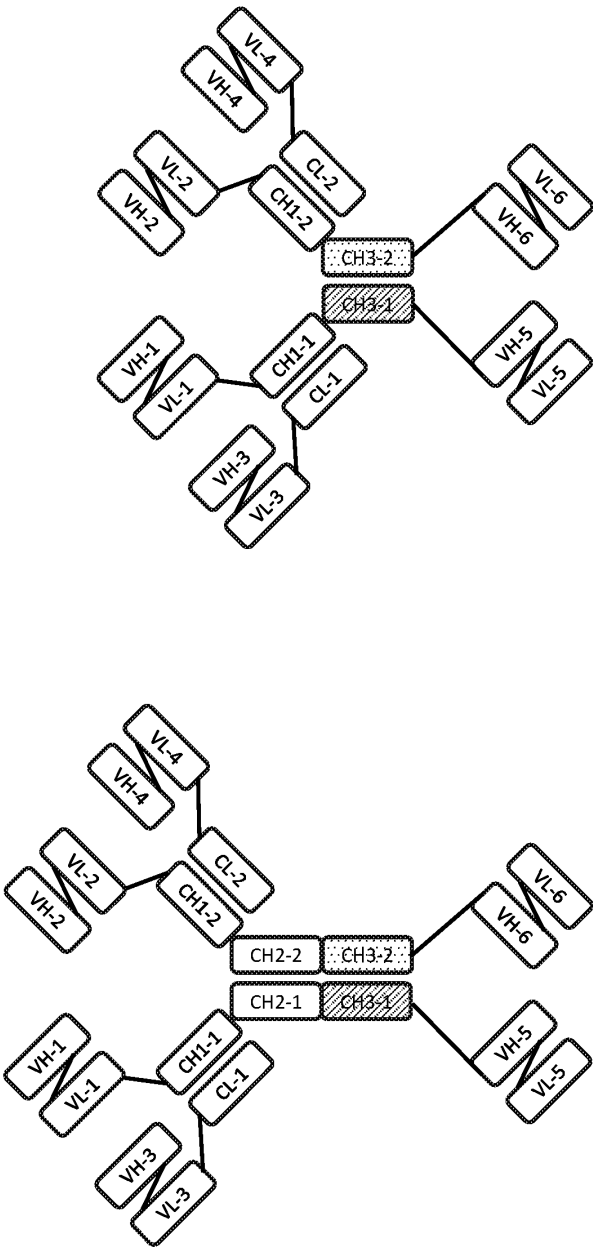


FIG. 8E

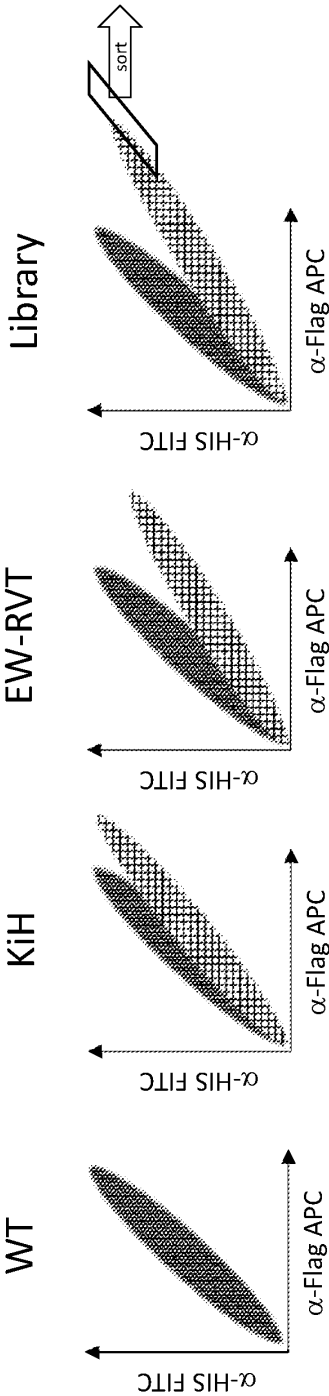


FIG. 9A

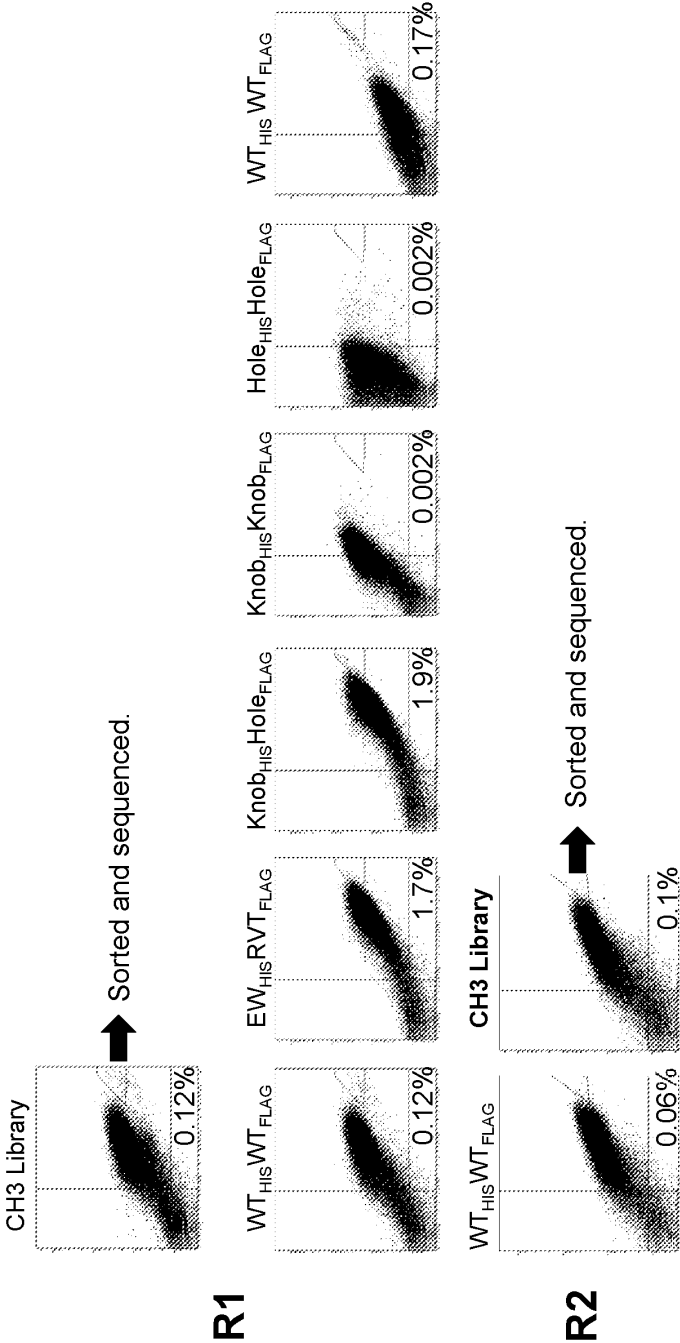


FIG. 9B

Library	Description	Theoretical Protein (DNA) Size	Transformation Efficiency
1	Knob ^{SSM} -HIS Hole ^{SSM} -FLAG	1.6x10 ⁵ (1.05x10 ⁶)	6.4x10 ⁶
2	Hole ^{SSM} -HIS Knob ^{SSM} -FLAG	1.6x10 ⁵ (1.05x10 ⁶)	9.8x10 ⁶
3	Knob-HIS Hole ^{SSM} -FLAG	8x10 ³ (3.2x10 ⁴)	4.5x10 ⁶

Construct	AA Change	AA Sequence
WT		GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
Knob	T366W	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
KnobSSM	T366X	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
Hole	T366S, L368A, Y407V	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
HoleSSM	T366X, L368X, Y407X	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK

FIG. 10A

Construct	AA Change	AA Sequence
WT		GQPREQVYTLPPSRDELTKNQVSLTCLVKGYFSPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
KnobSSM	T366X	GQPREQVYTLPPSRDELTKNQVSLTCLVKGYFSPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
HoleSSM	T366X, L368X, Y407X	GQPREQVYTLPPSRDELTKNQVSLTCLVKGYFSPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

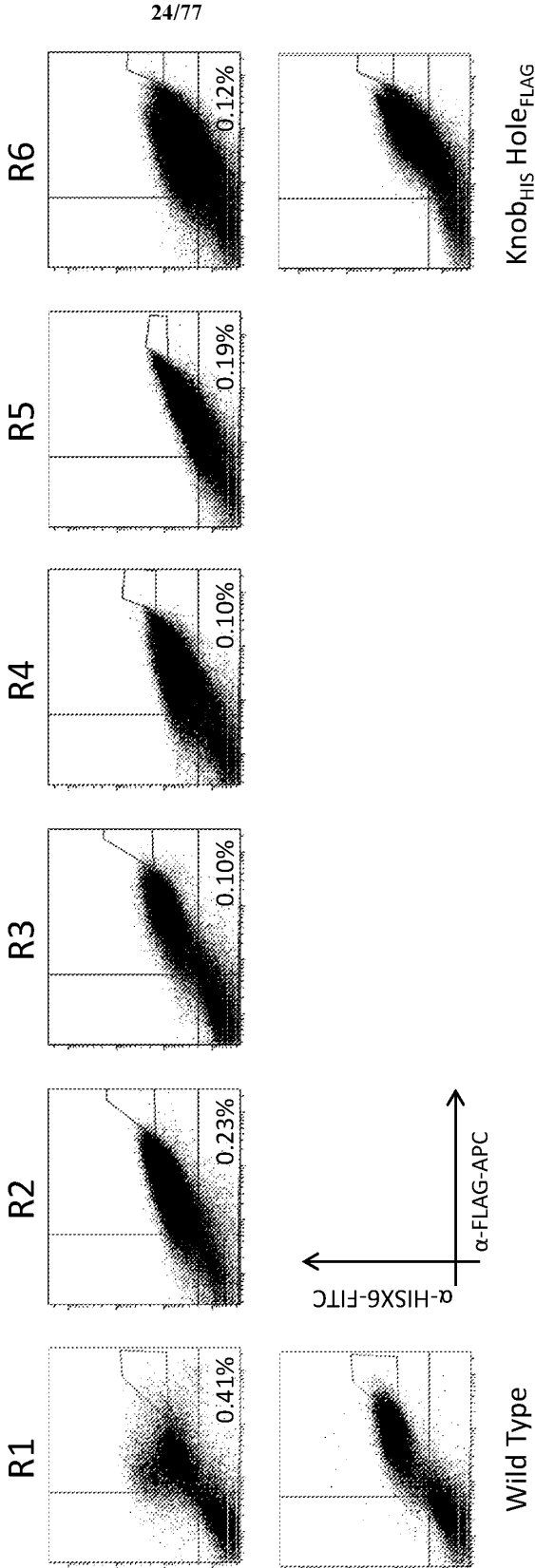


FIG. 10B

Construct	AA Change	AA Sequence
WT		GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCVMHEALHNNHYTEQKSLSPGK
Knob	T366W	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCVMHEALHNNHYTEQKSLSPGK
HoleSSM	T366X, L368X, Y407X	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCVMHEALHNNHYTEQKSLSPGK

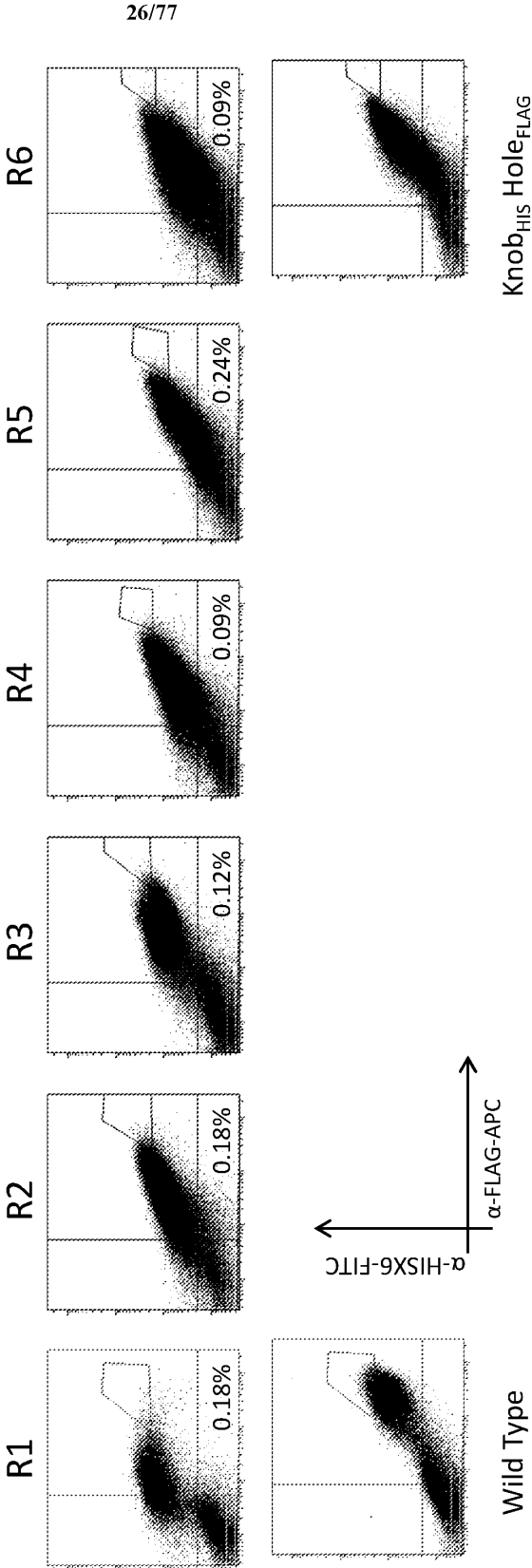


FIG. 10D

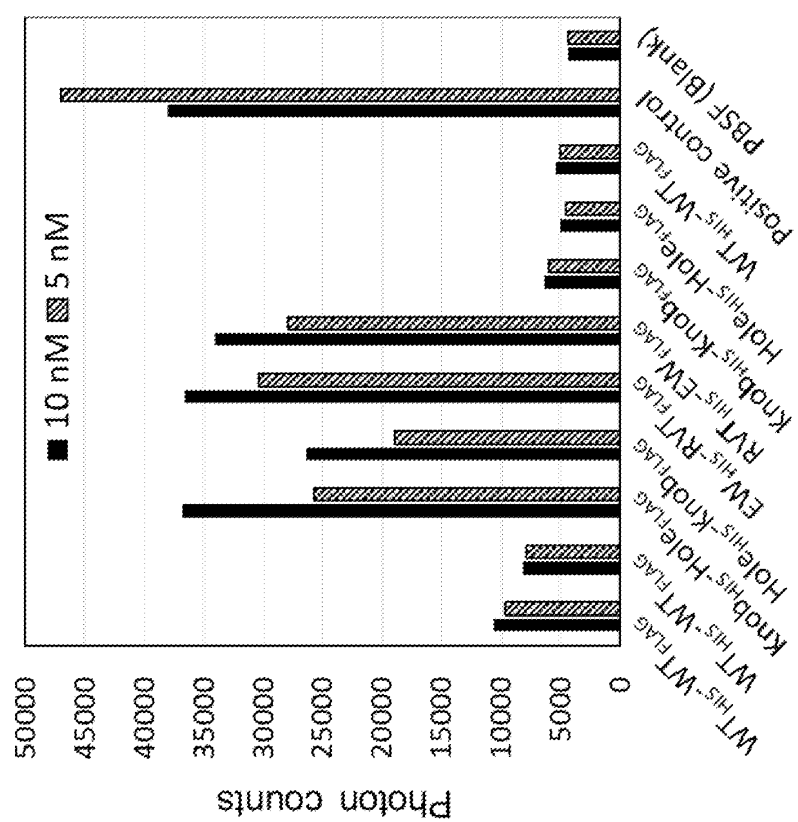
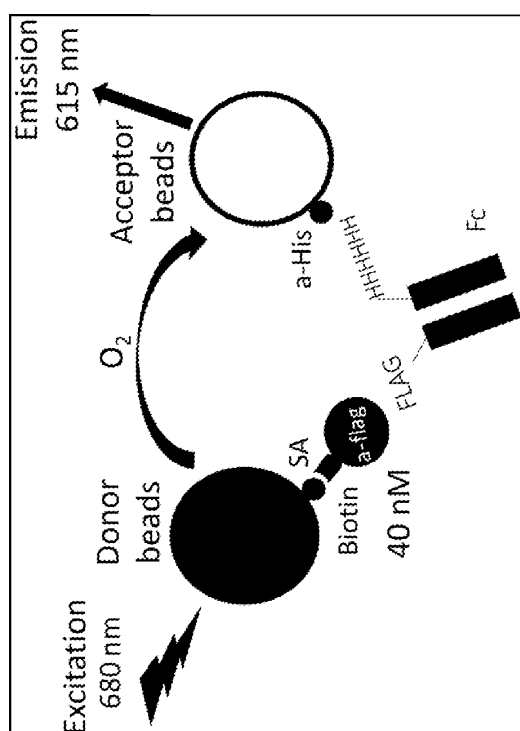


FIG. 11A

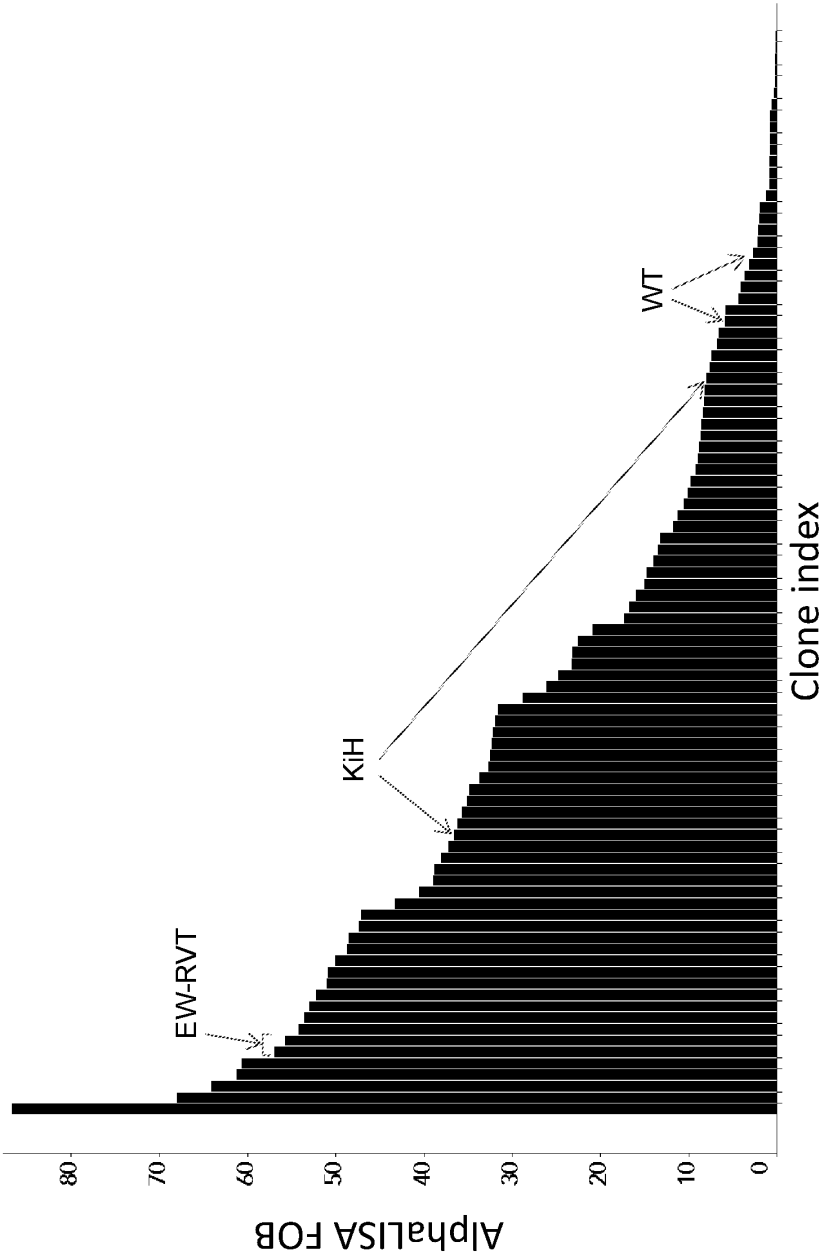


FIG. 11B

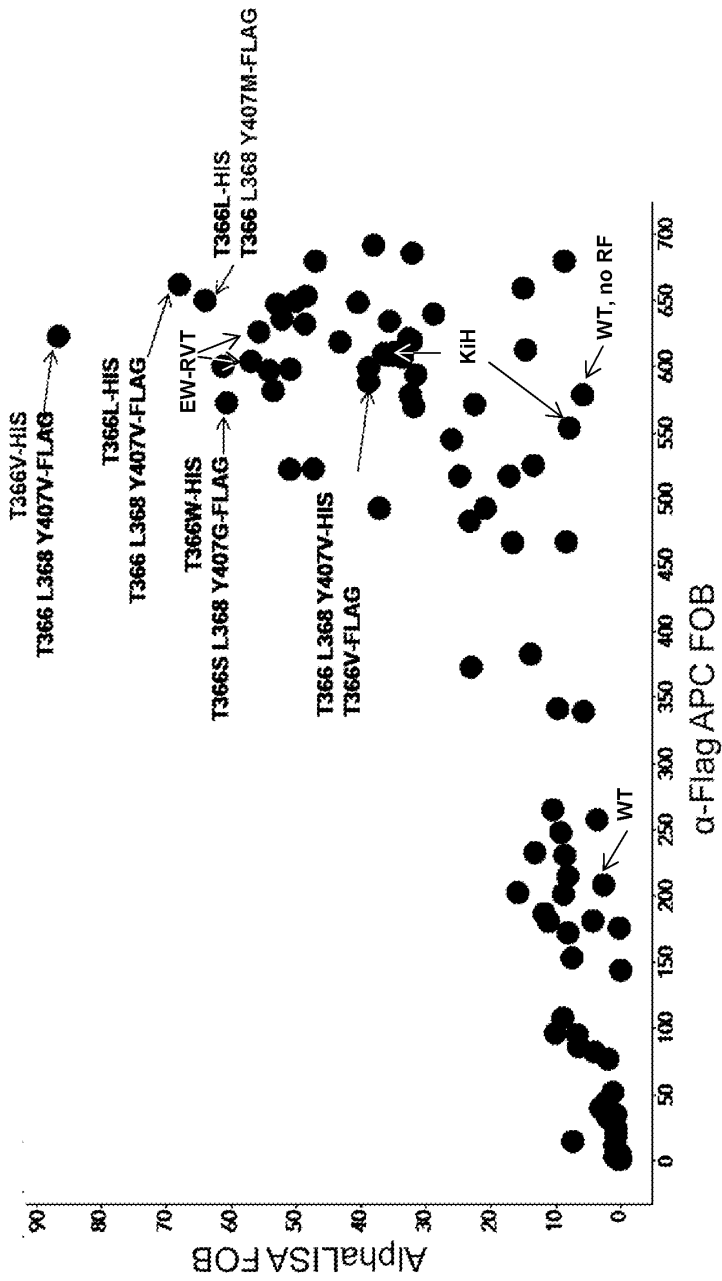


FIG. 11C

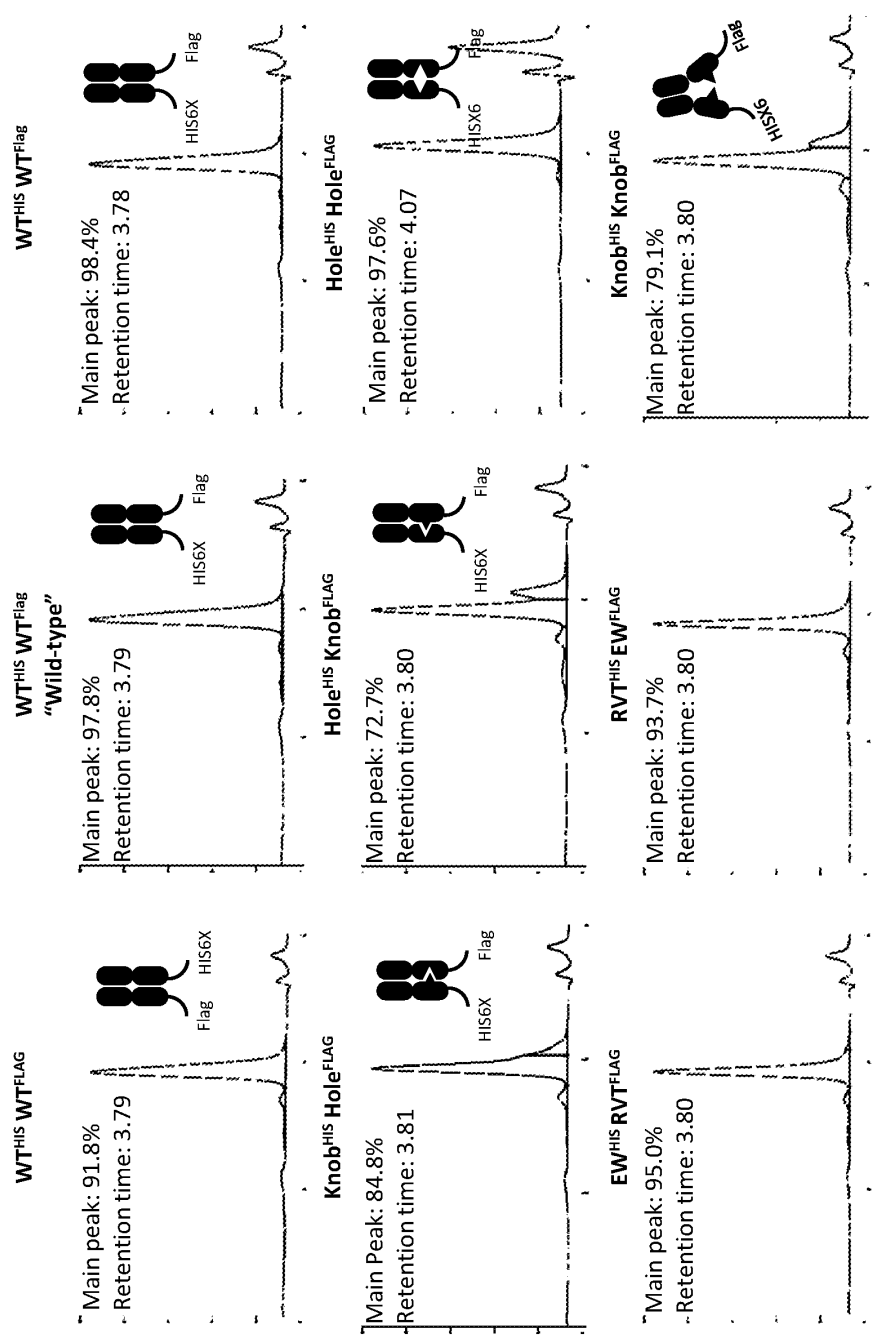


FIG. 12A

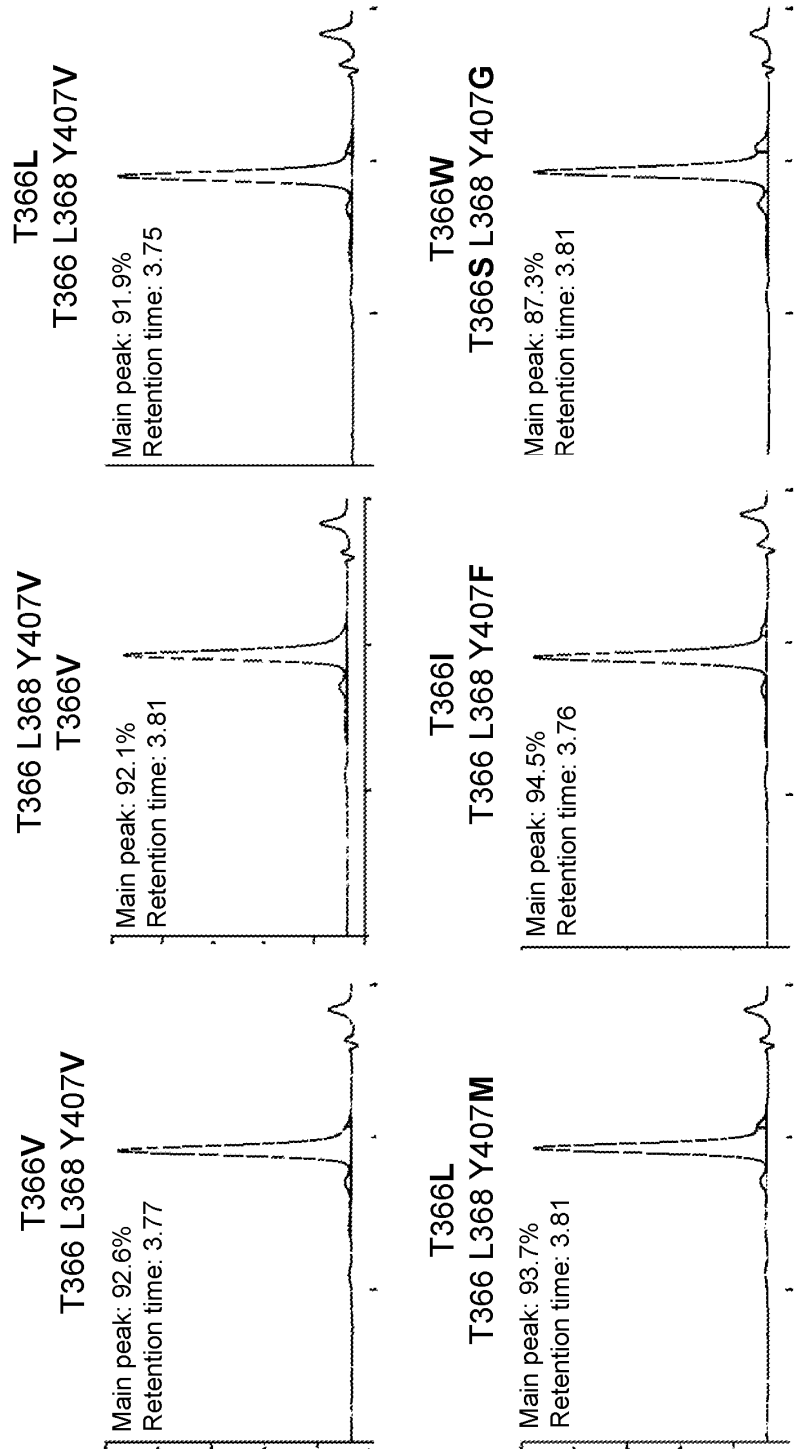


FIG. 12B

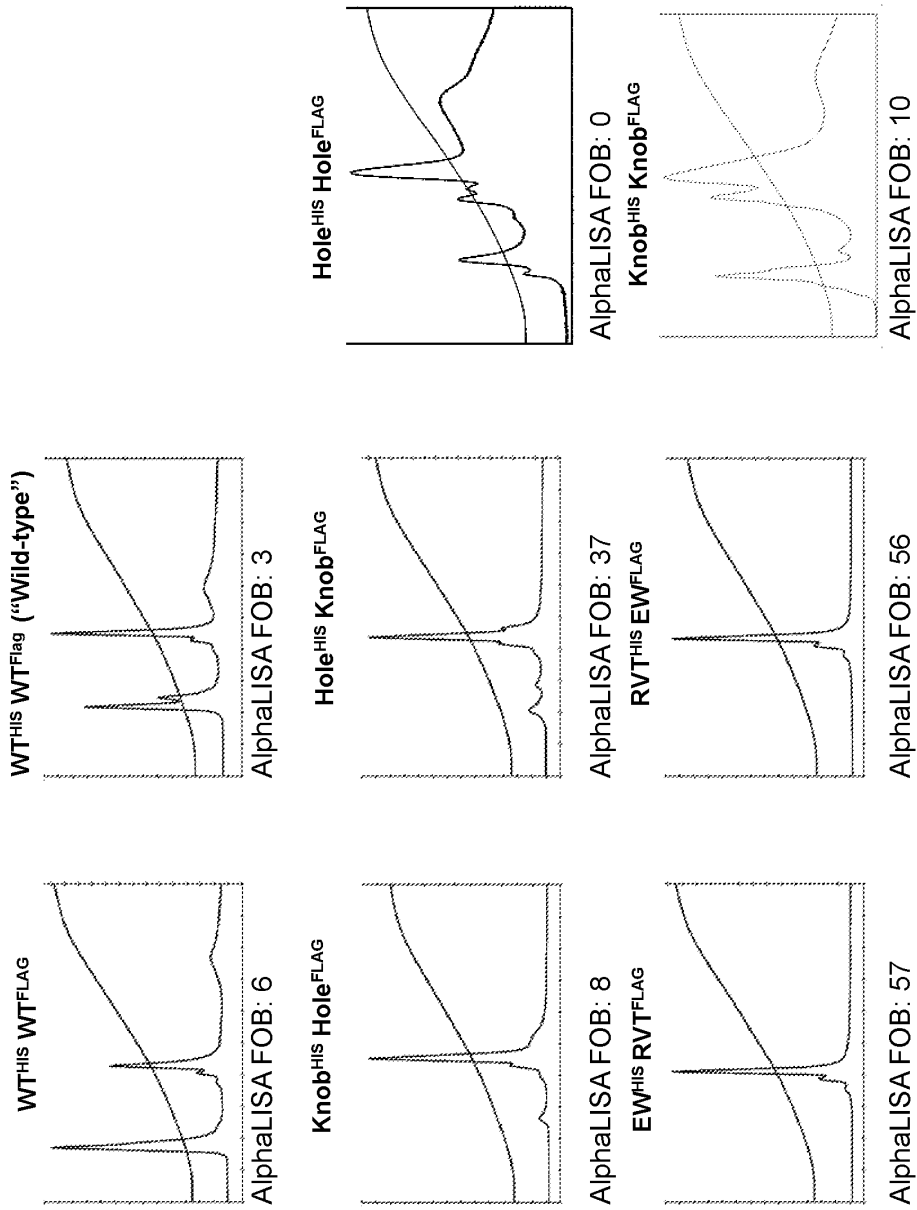


FIG. 13A

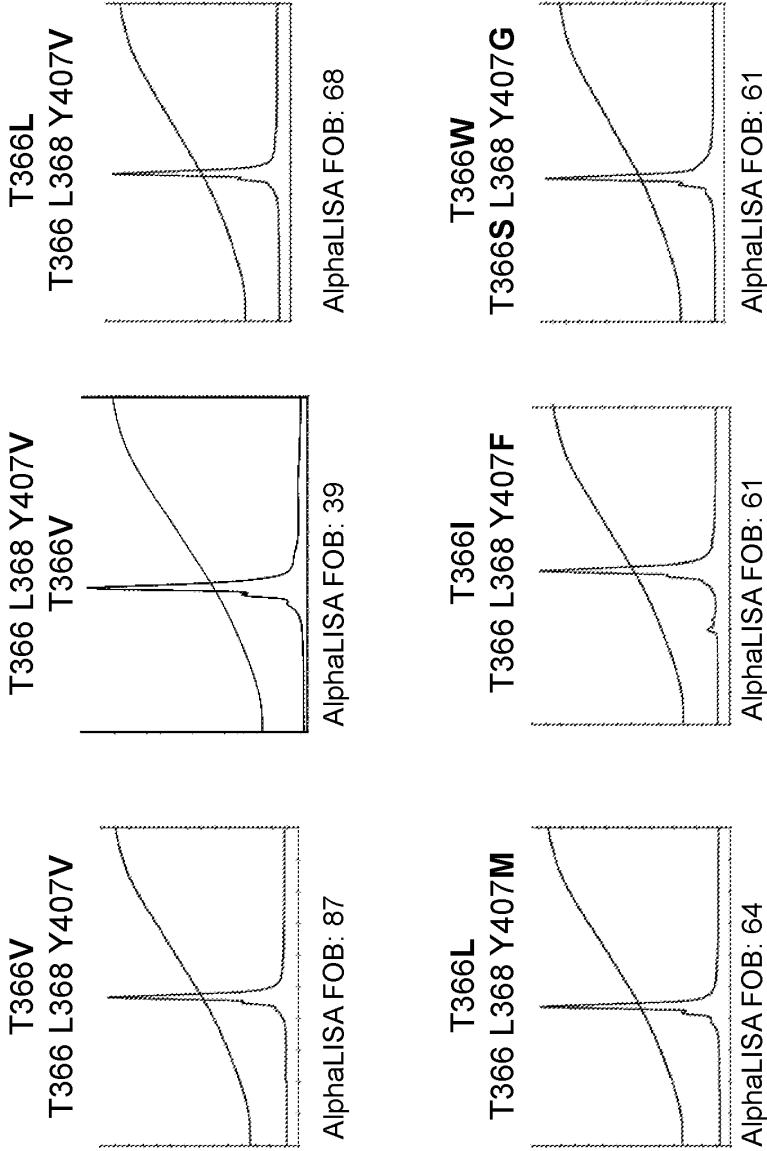


FIG. 13B

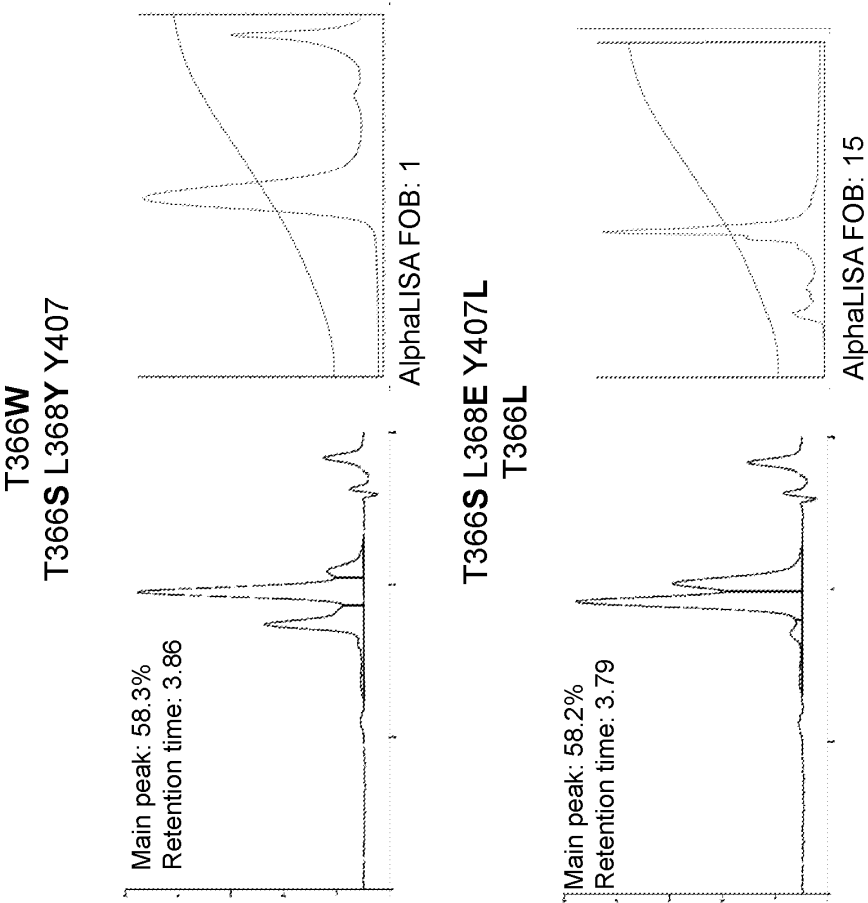


FIG. 13C

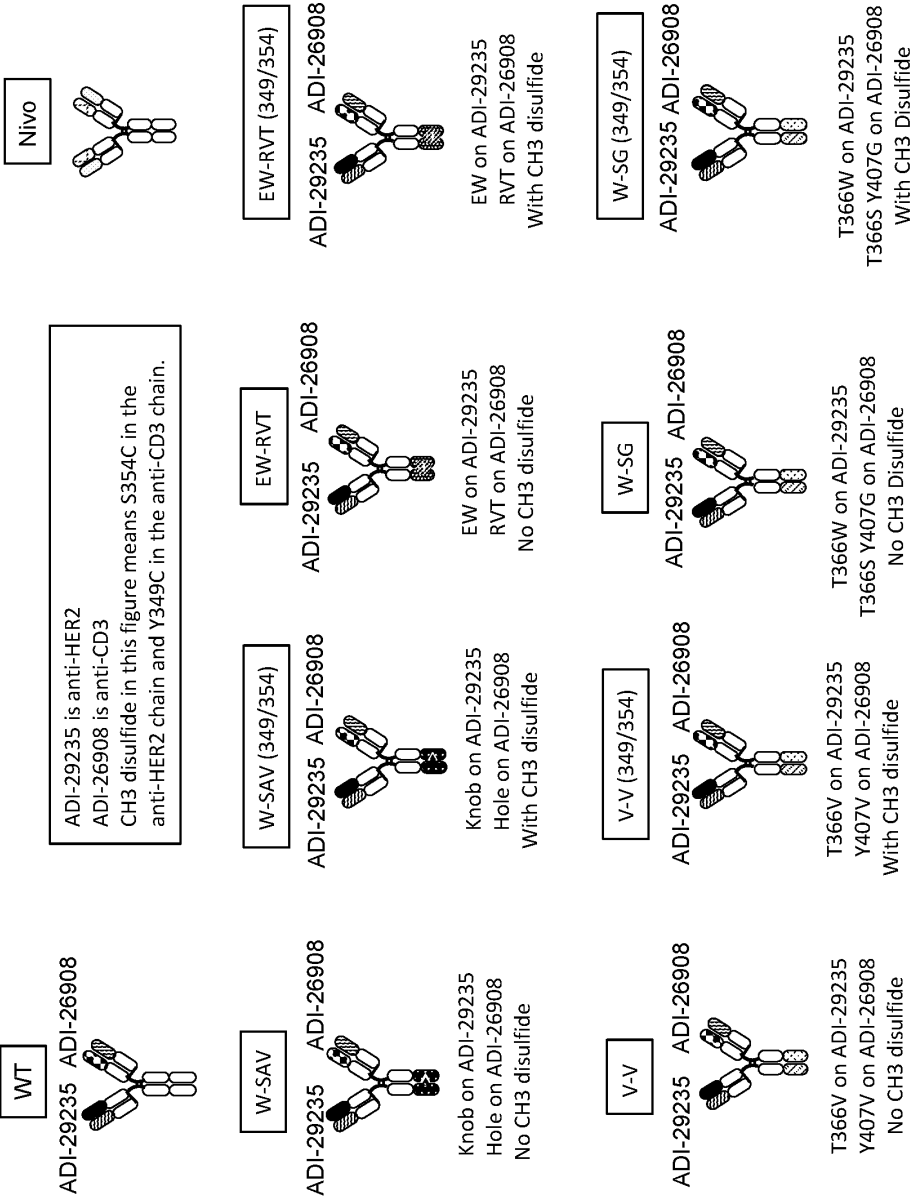


FIG. 14A

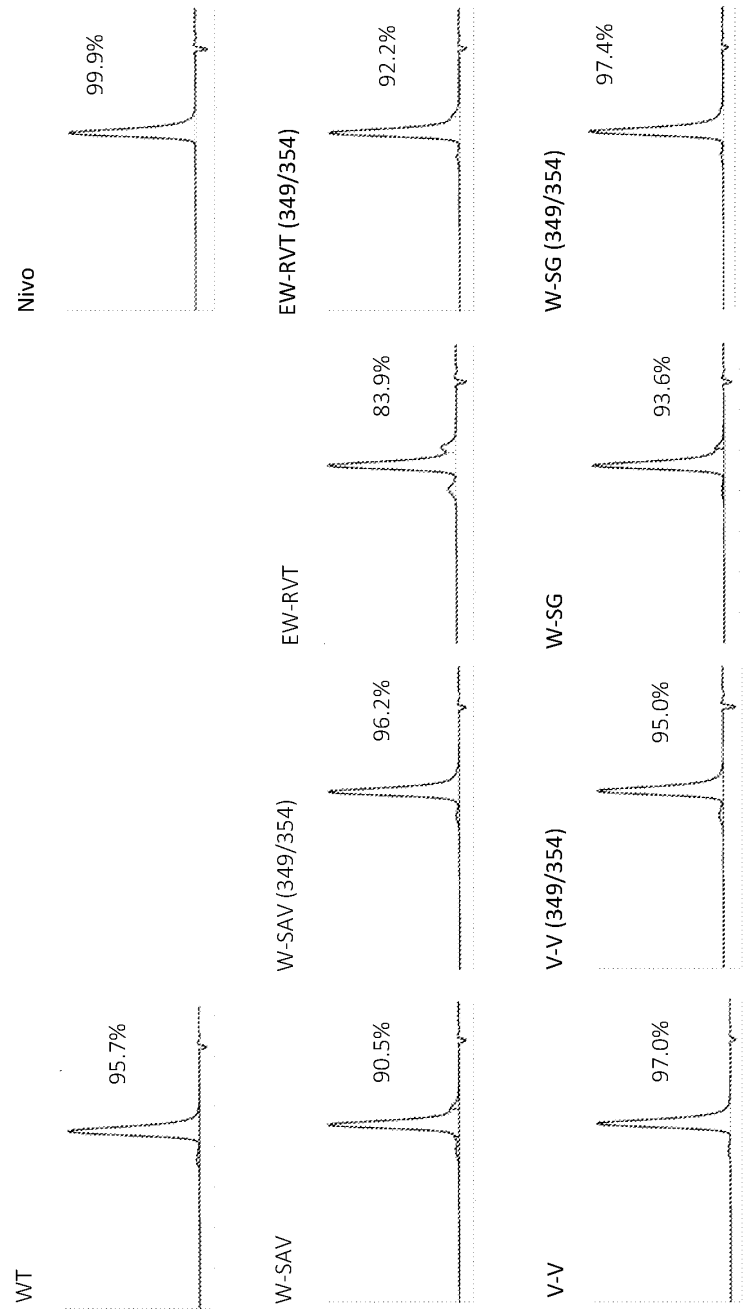


FIG. 14B

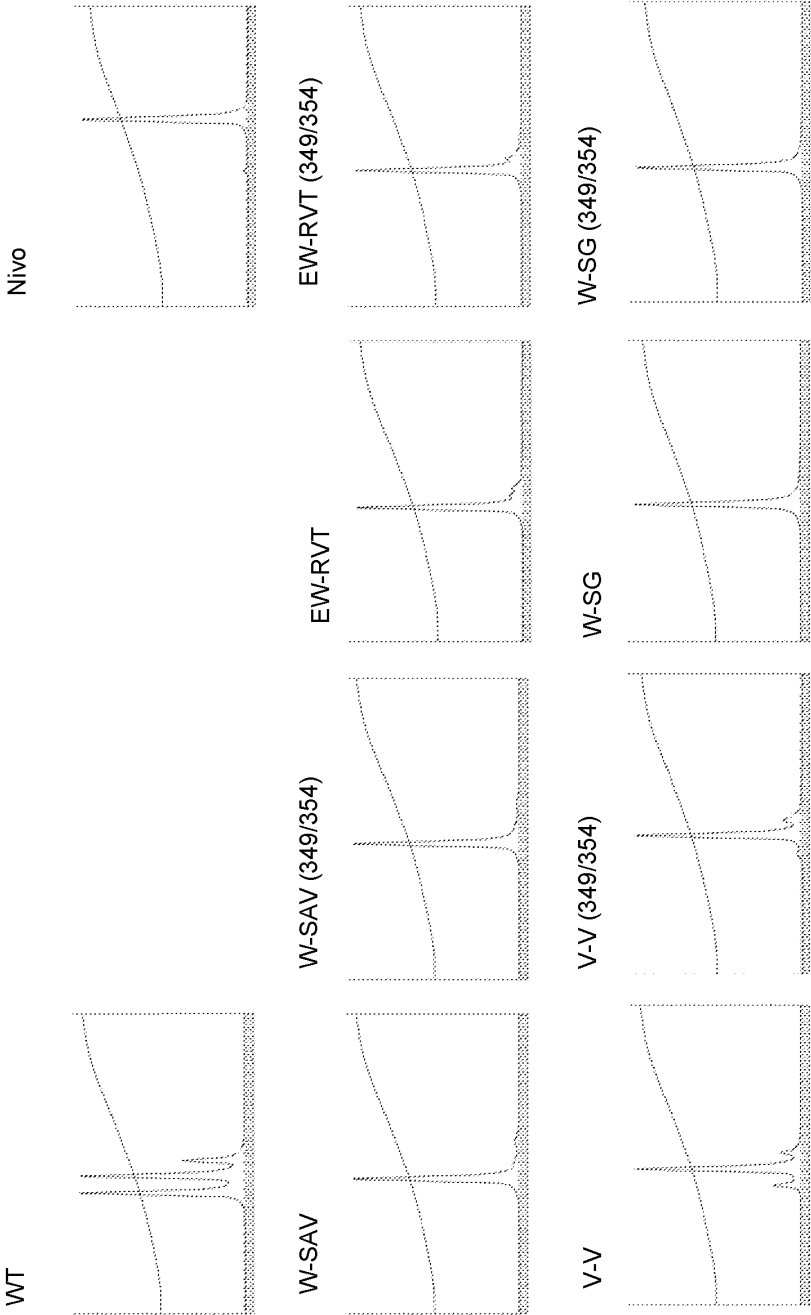


FIG. 14C

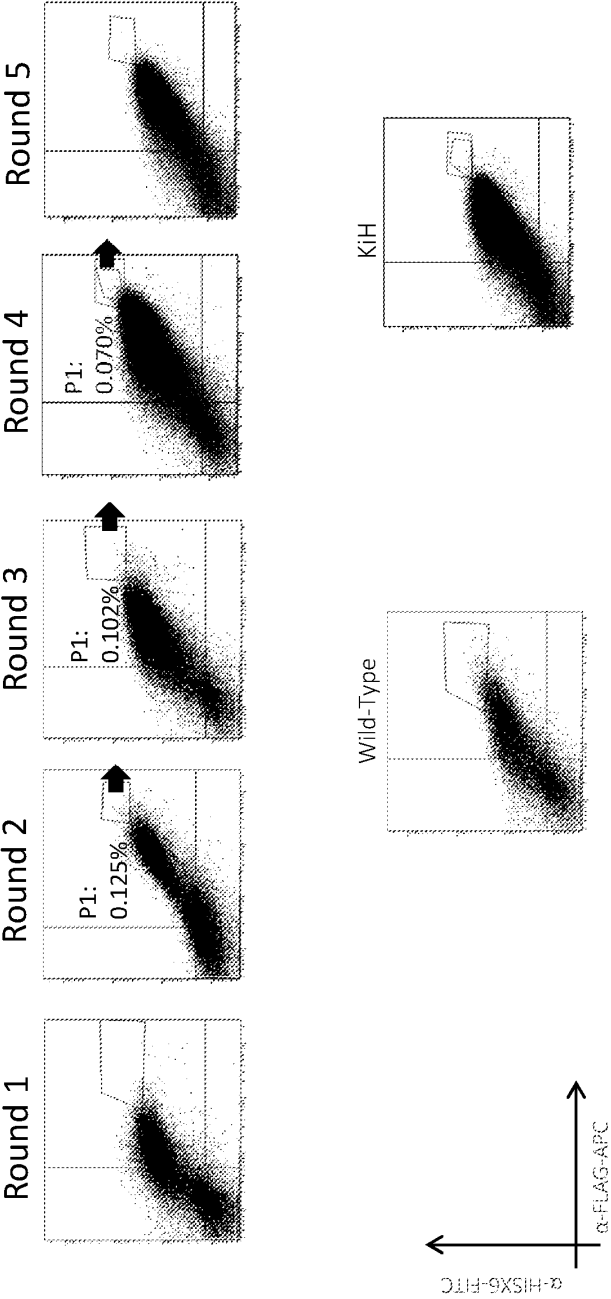


FIG. 15A

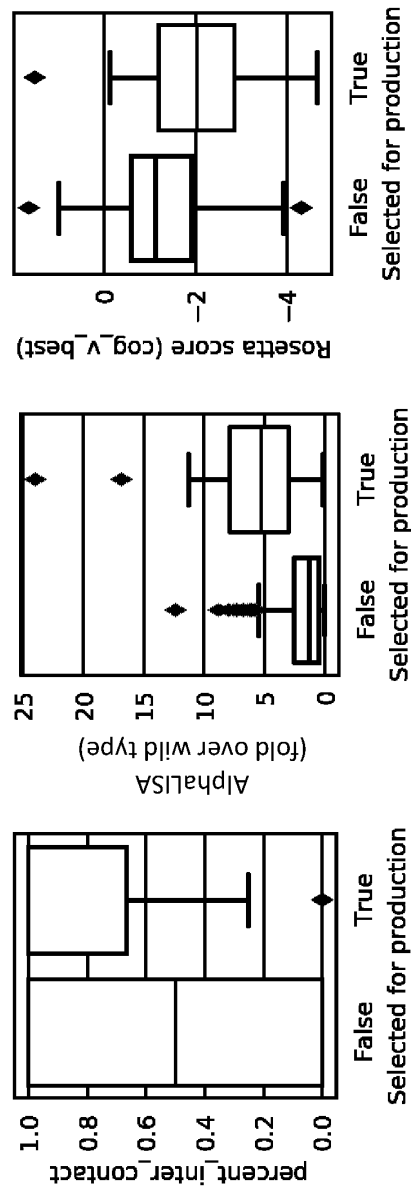


FIG. 15B

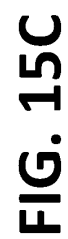


FIG. 15C

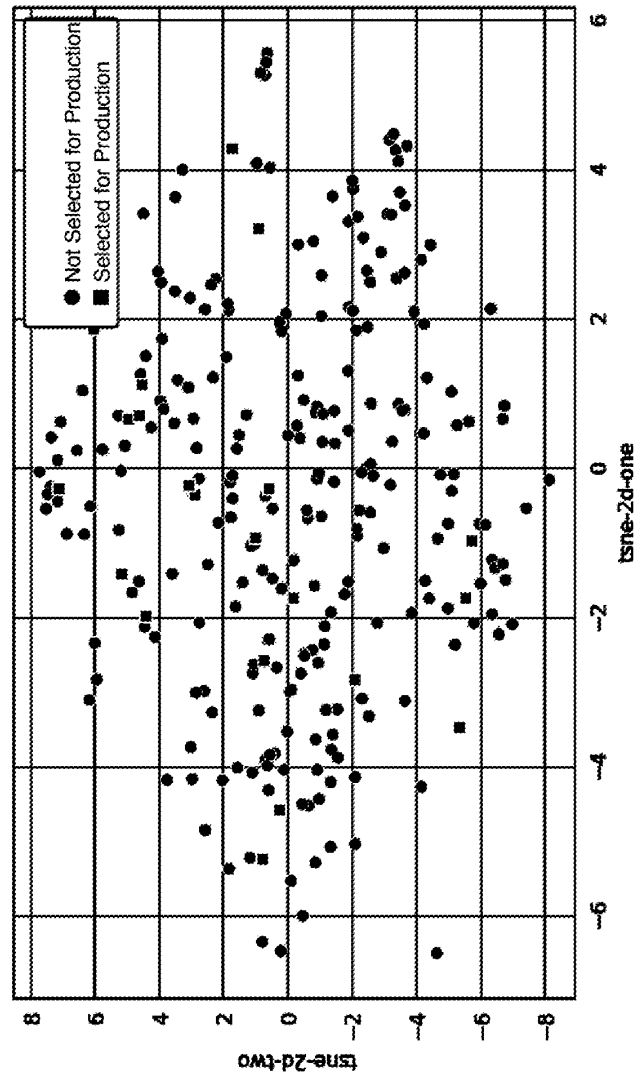
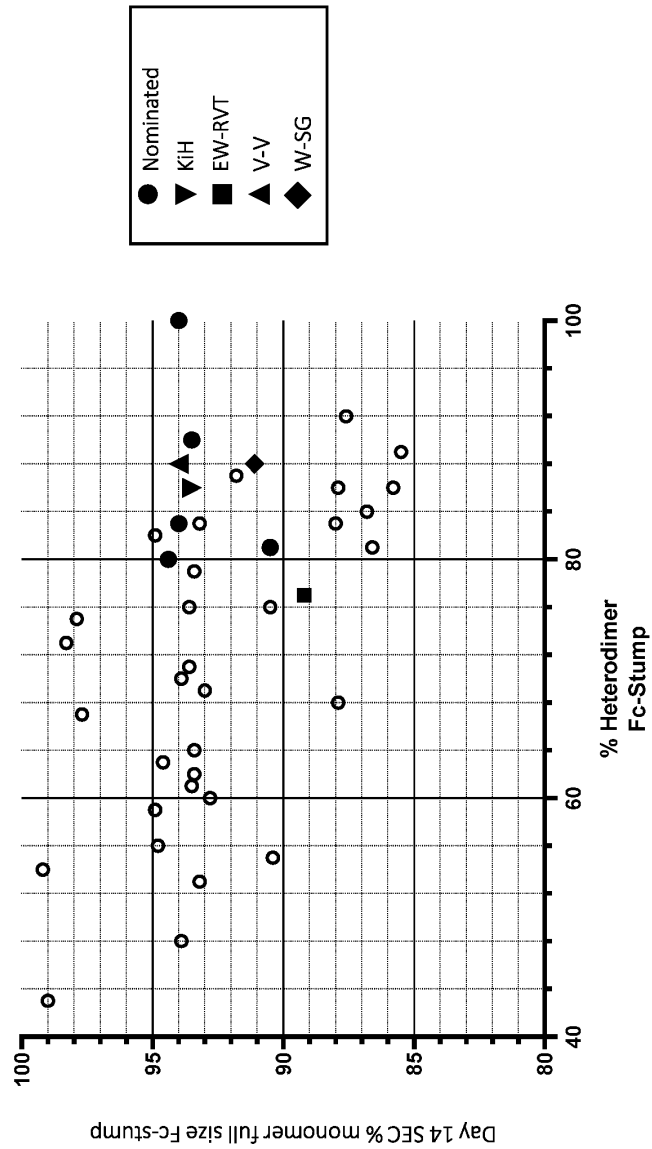


FIG. 15D



Nominated clones showed heterodimerization and stability similar to controls

FIG. 16

WT		WT		Variant CH3 set with CH3 S=S in Orientation 1		Variant CH3 set with CH3 S=S in Orientation 2		Variant CH3 set with CH3 S=S in Orientation 1	
anti-CD3	anti-HER2	anti-CD3	anti-HER2	anti-CD3	anti-HER2	anti-CD3	anti-HER2	anti-CD3	anti-HER2
anti-CD20	anti-CD3	anti-CD3	anti-CD20	anti-CD3	anti-CD20	anti-CD3	anti-CD20	anti-CD3	anti-CD20
anti-HEL	anti-BCMA (VHH)	anti-HEL	anti-BCMA (VHH)	anti-HEL	anti-BCMA (VHH)	anti-HEL	anti-BCMA (VHH)	anti-HEL	anti-BCMA (VHH)

FIG. 17A

LCMS average % heterodimer
values shown above each group

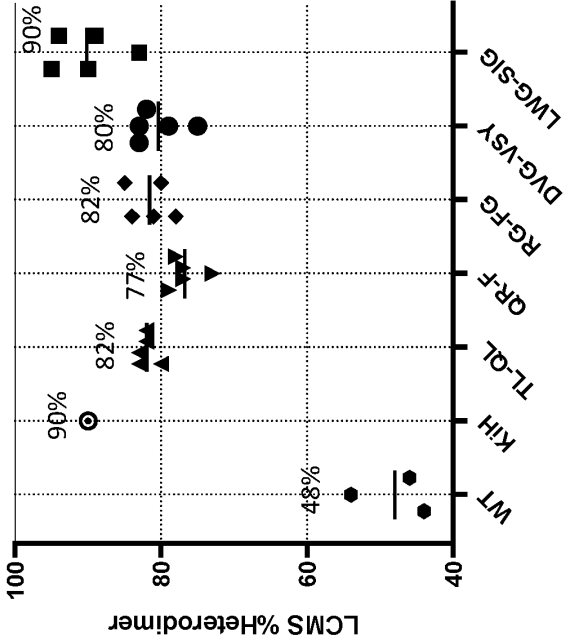


FIG. 17B

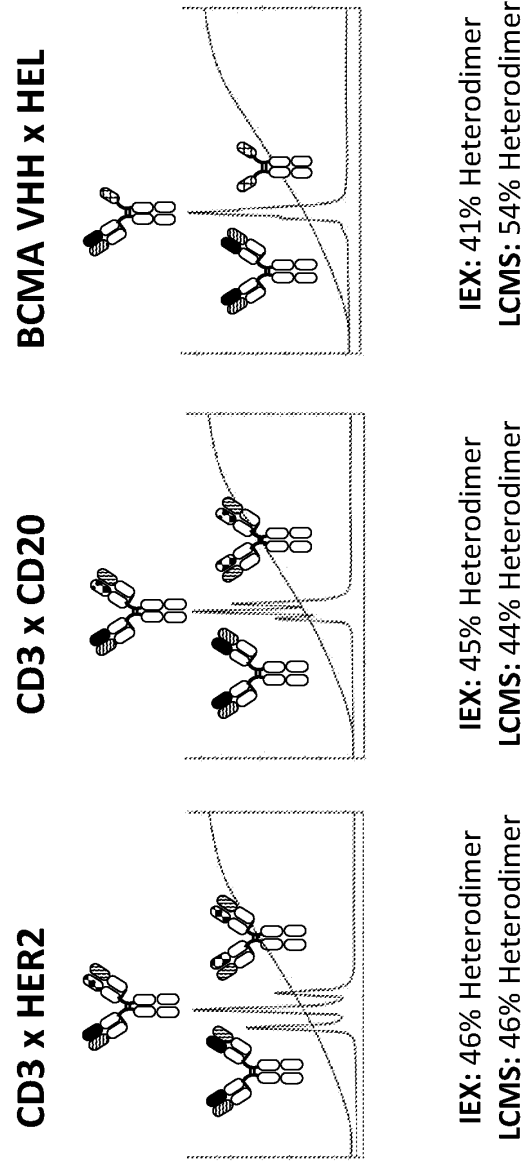


FIG. 17C

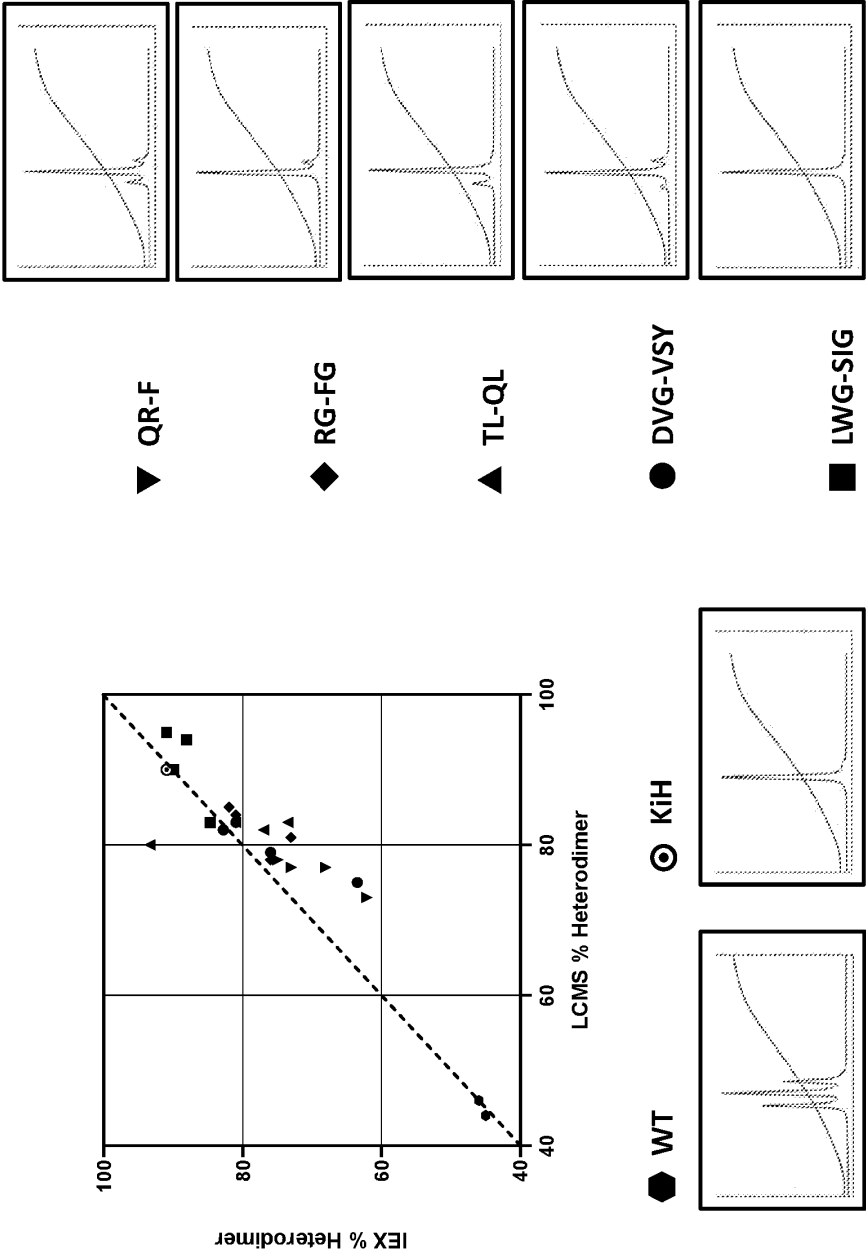


FIG. 17D

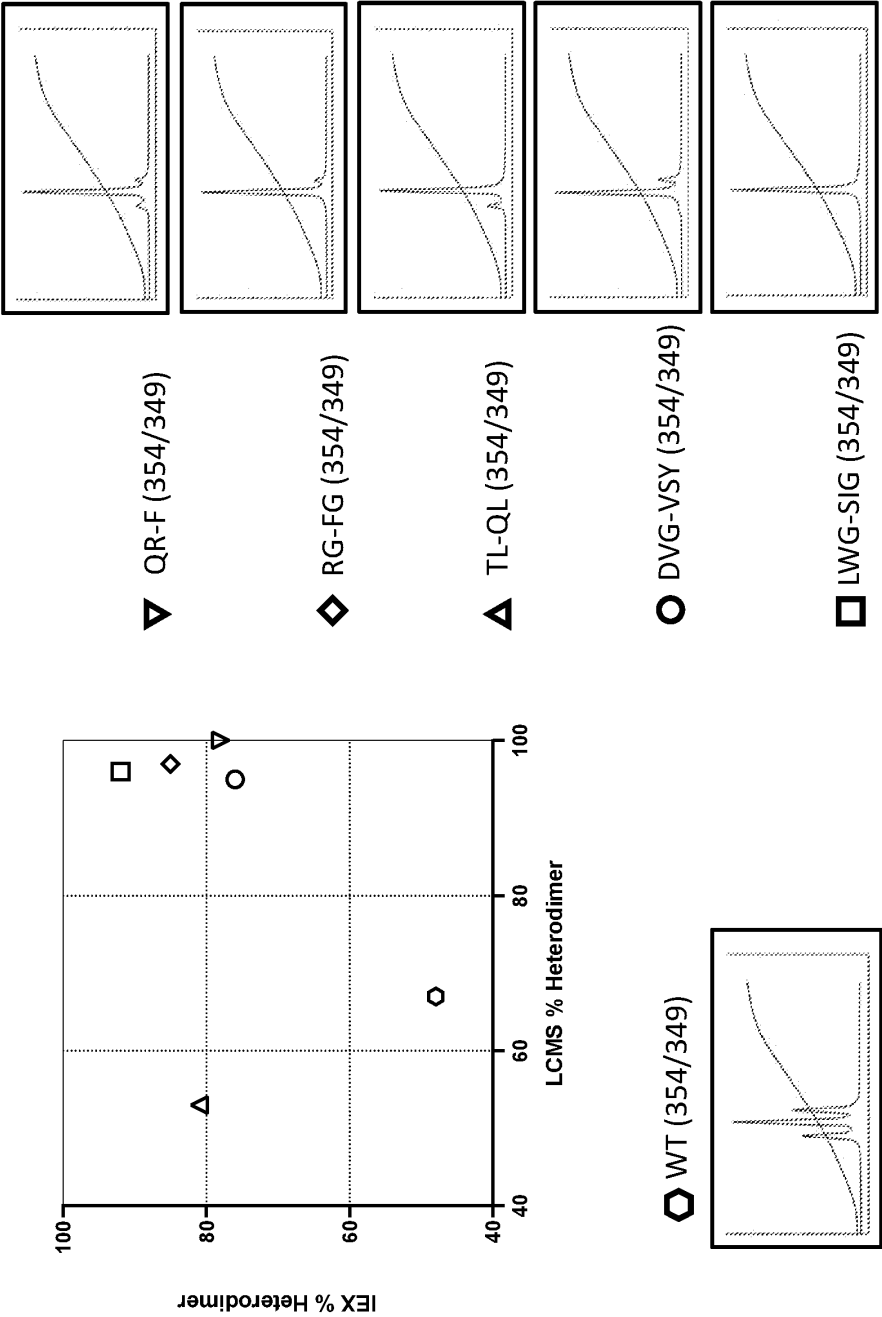


FIG. 17E

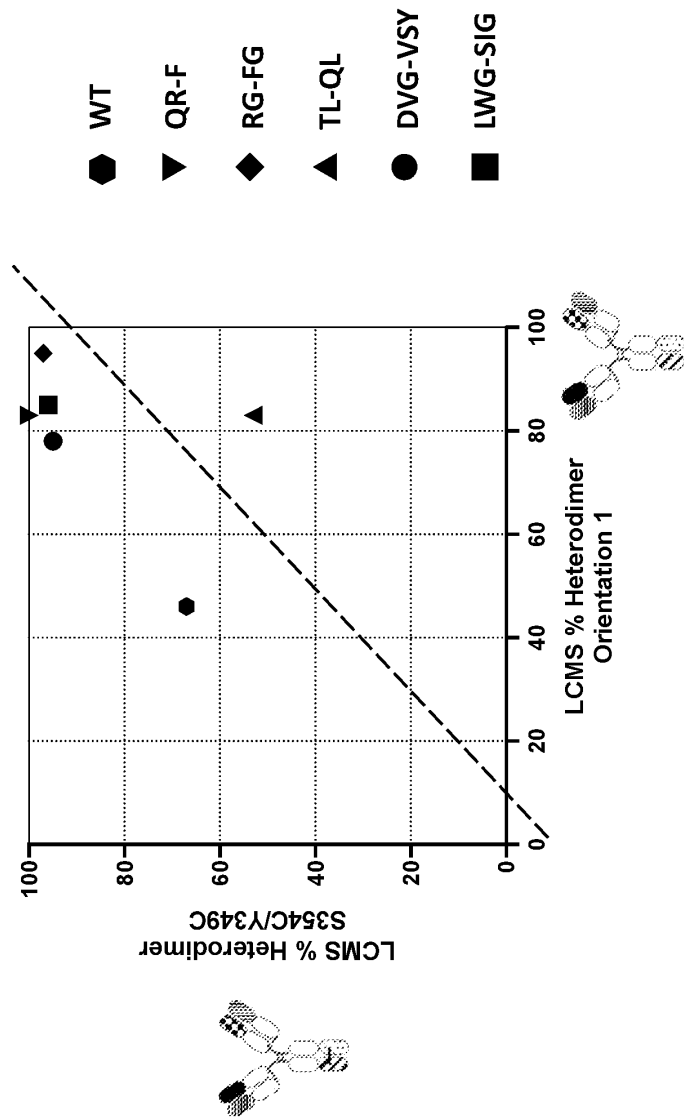


FIG. 17F

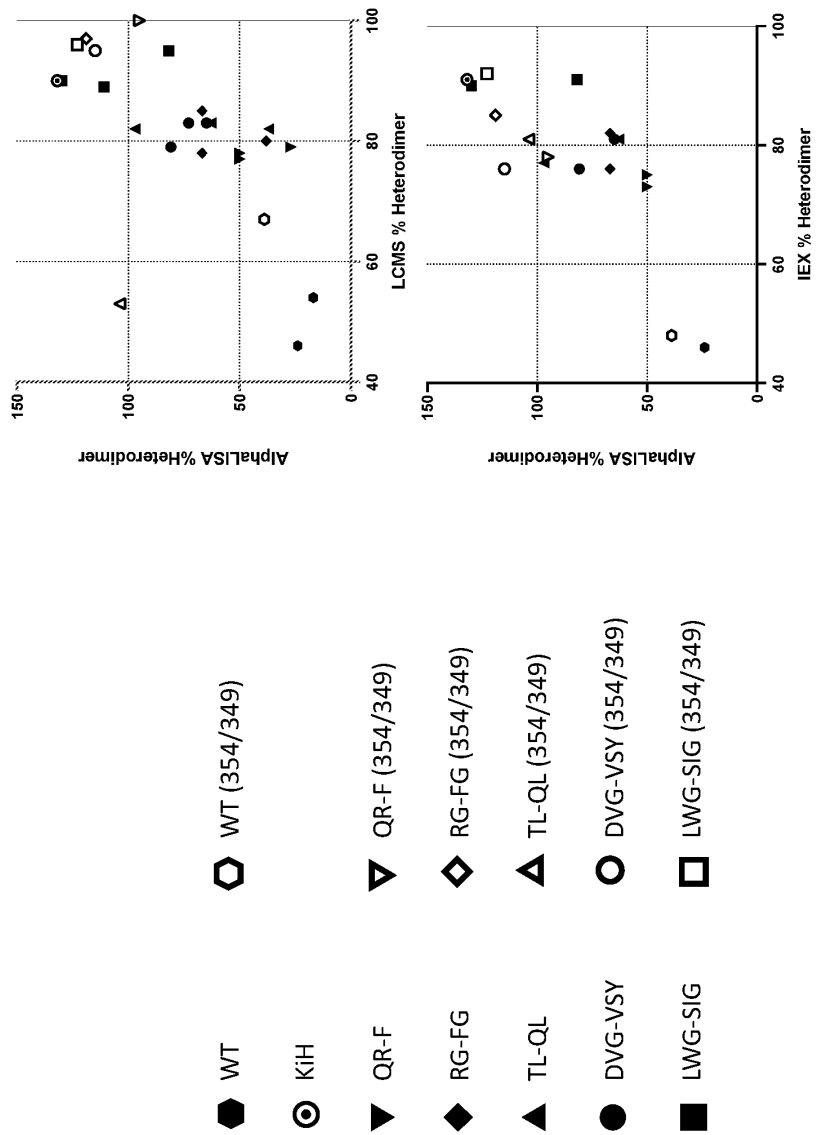


FIG. 17G

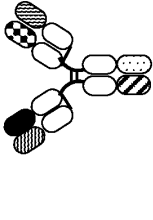
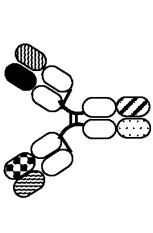
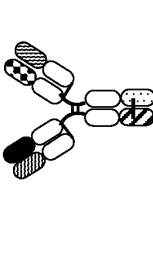
	Orientation 1	Orientation 2	Orientation 1 With CH3 S=S
			
IEX % Heterodimer	91%	90%	92%
% LCMS	LWG-SIG	90%	96%
	LWG-LWG	2%	4%
	SIG-SIG	-	-
	SIG 1/2 Ab	8%	-
AlphaLISA % Heterodimer	82%	130%	123%

FIG. 17H

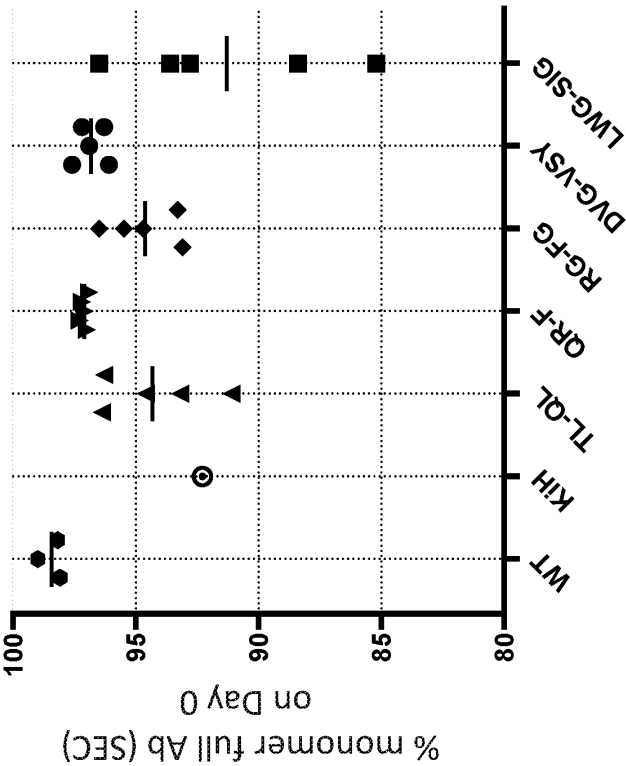
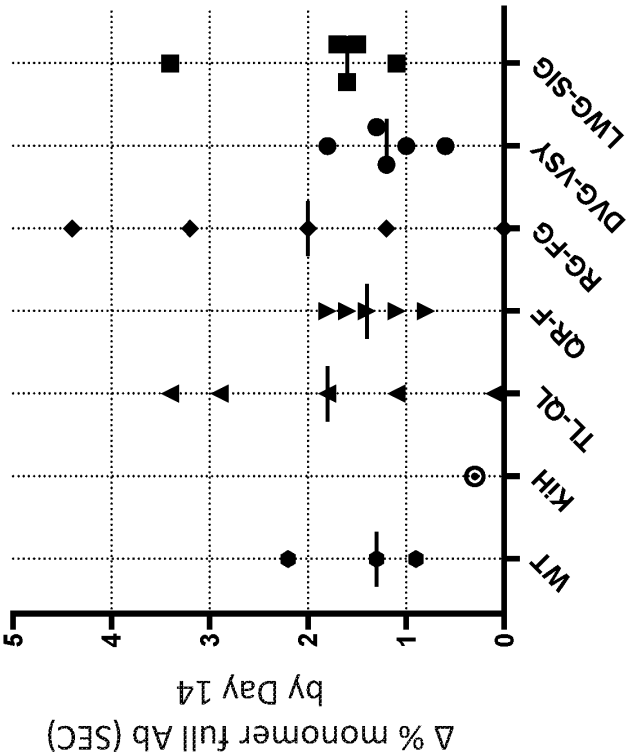


FIG. 17I

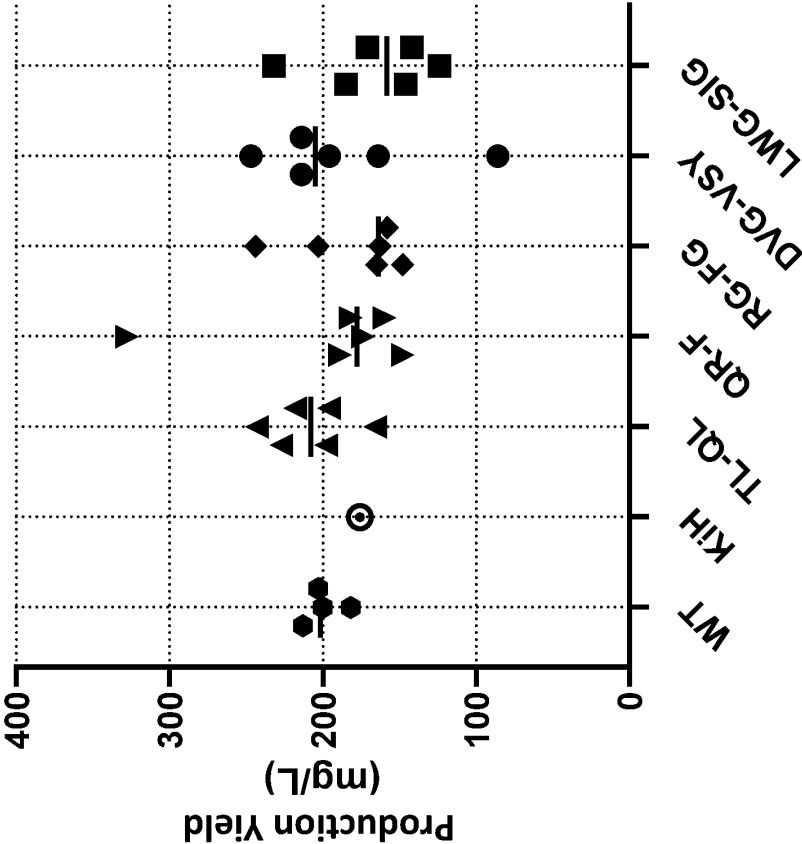


FIG. 17J

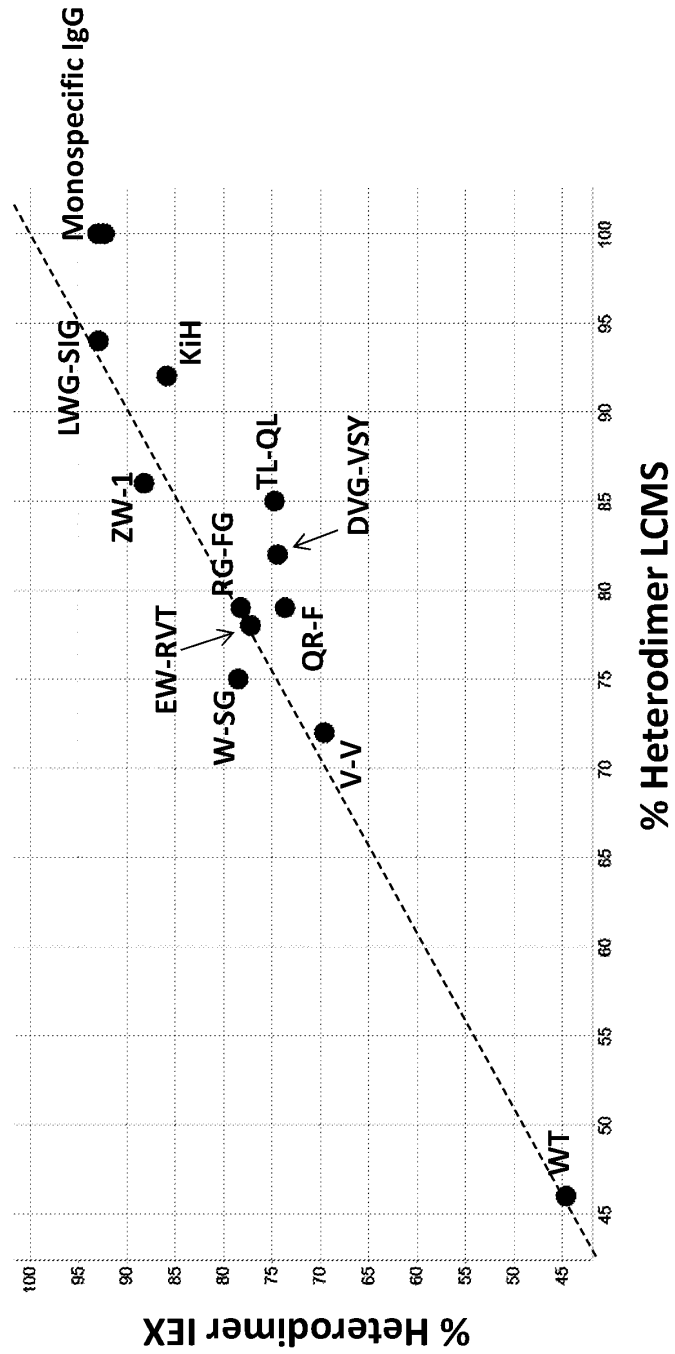


FIG. 18A

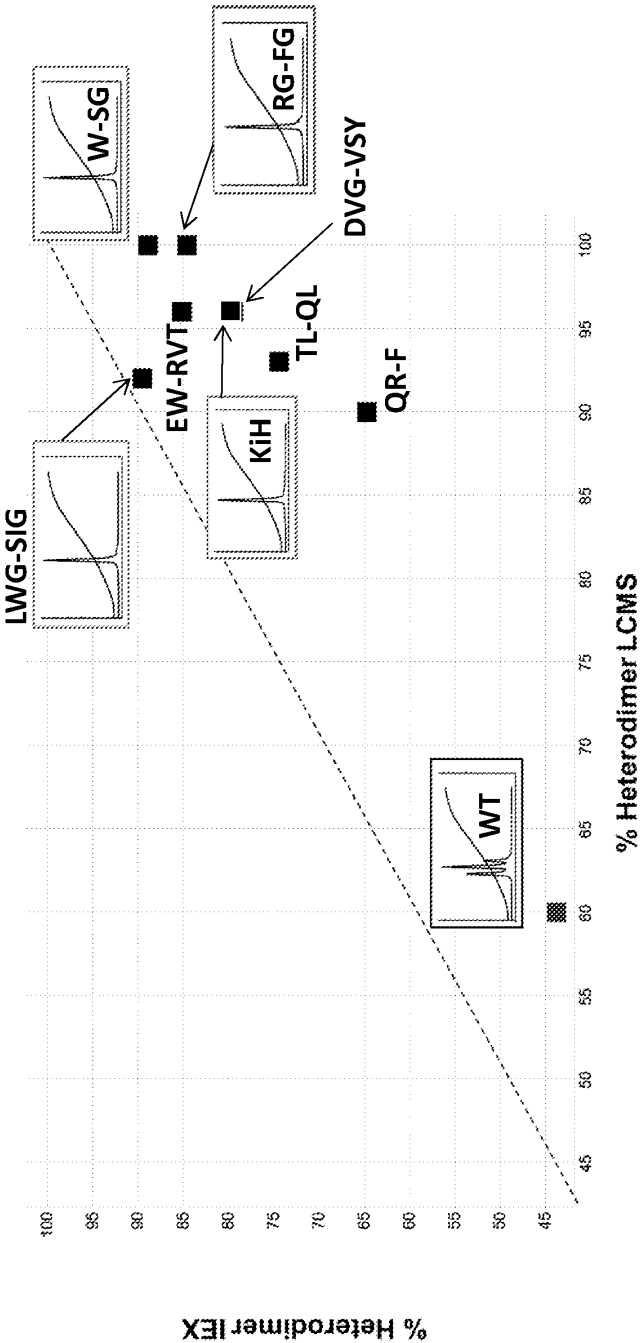


FIG. 18B

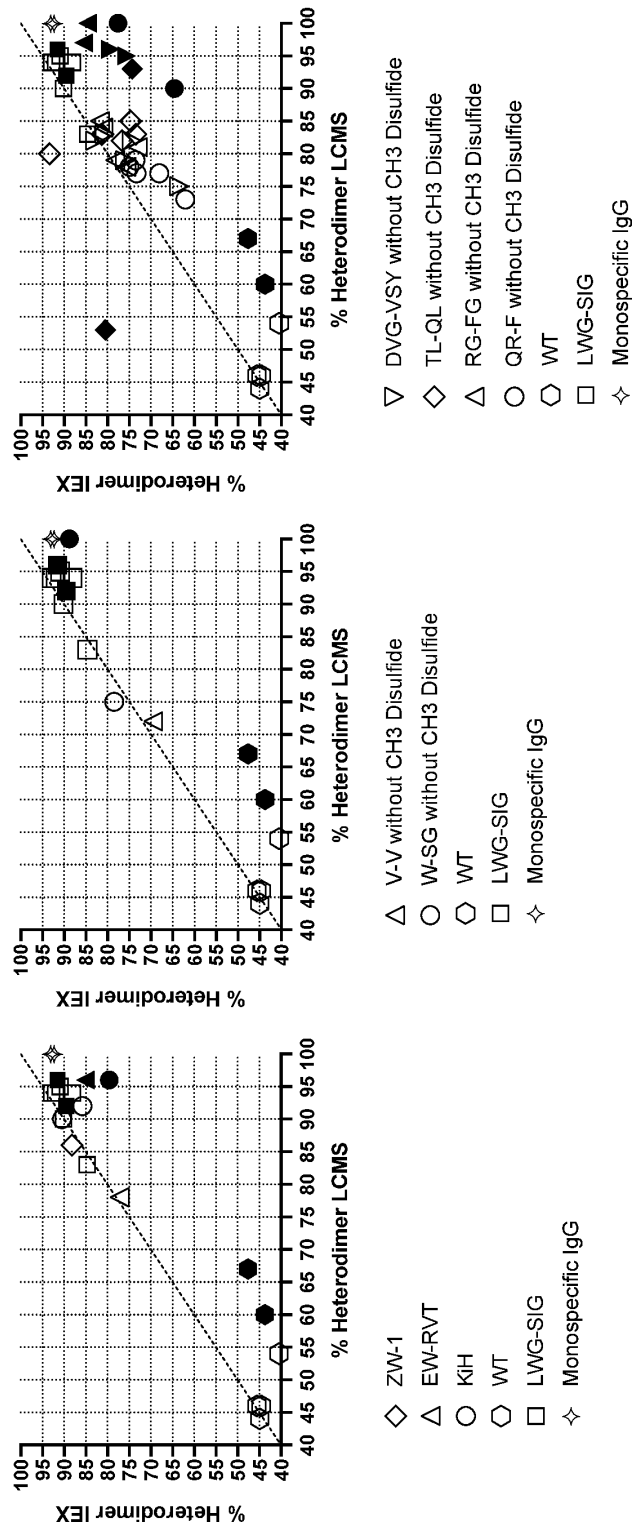


FIG. 18C

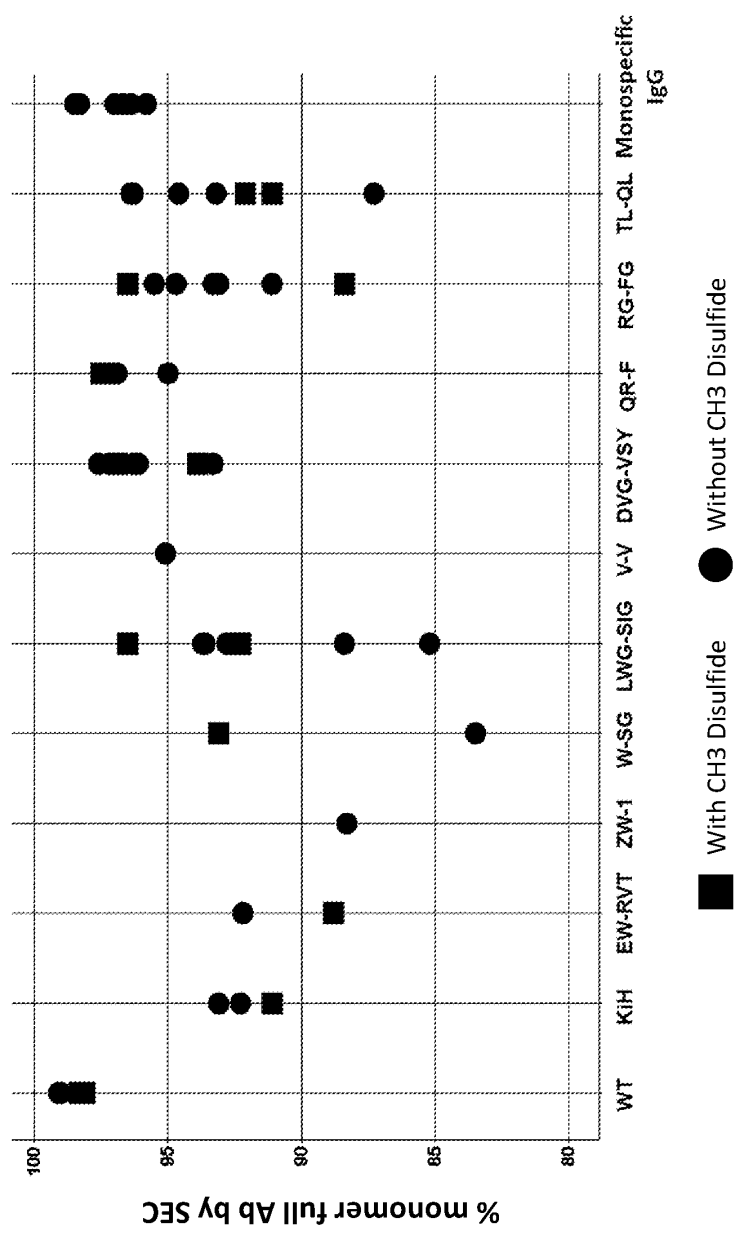
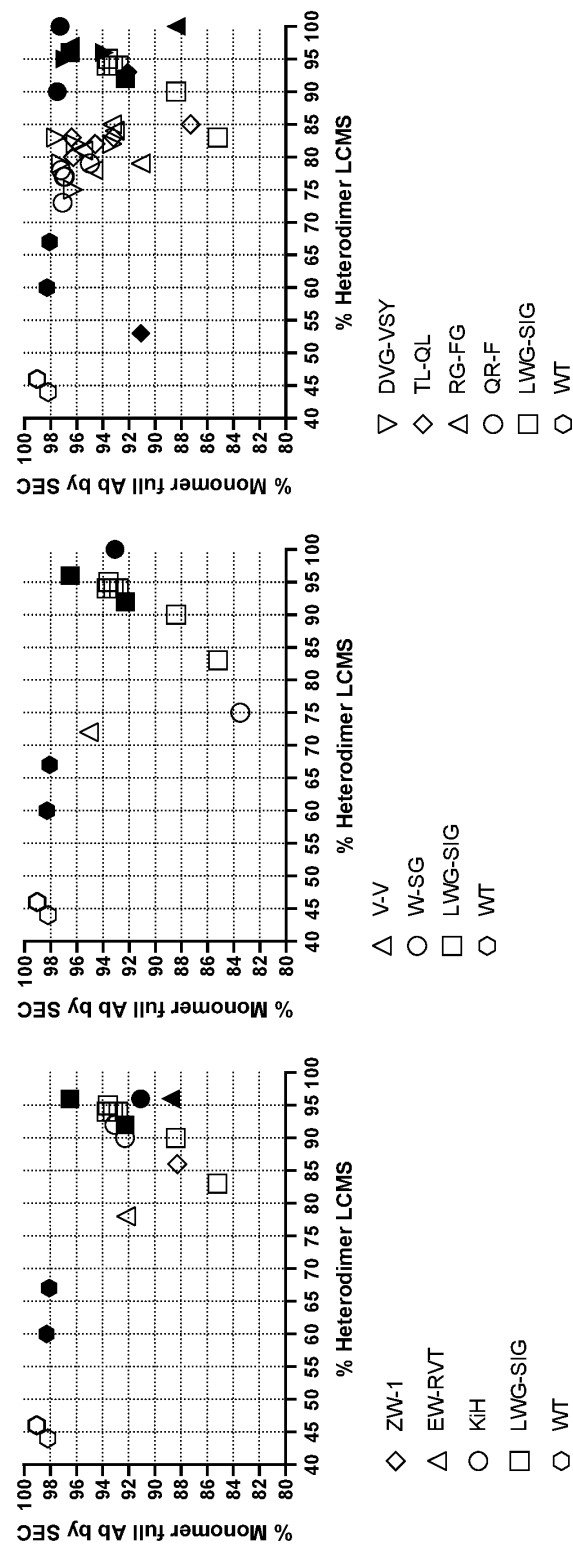


FIG. 18D



* Filled shapes indicate heterodimers with CH3 disulfide bonds

FIG. 18E

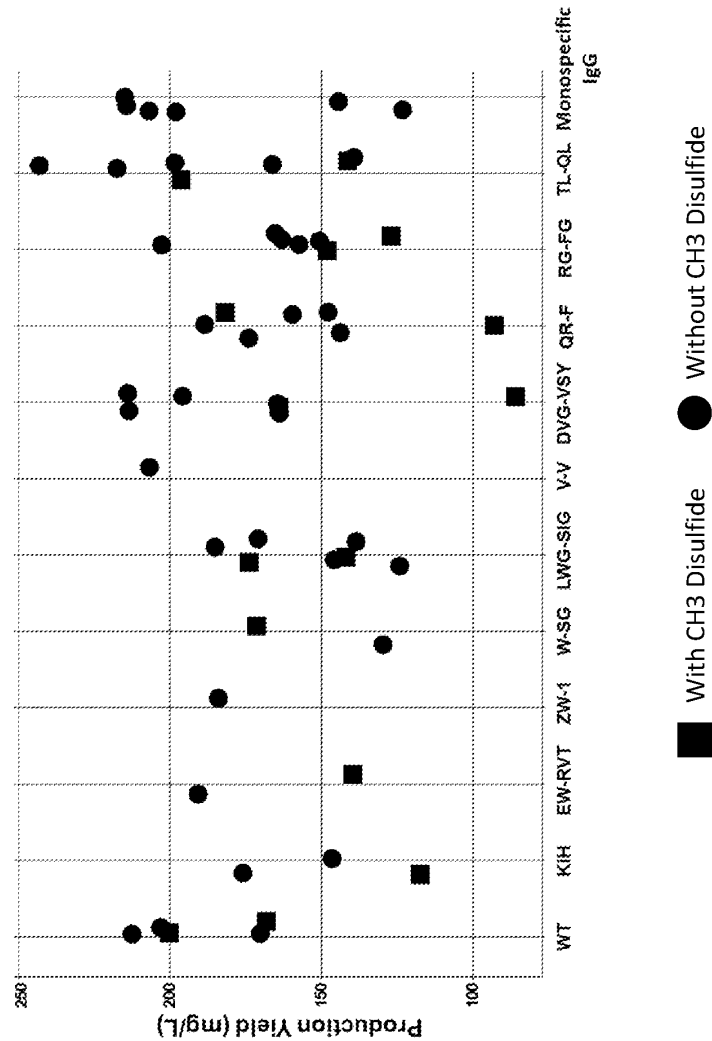


FIG. 18F

	LWG-SIG	LWG-IG
Rosetta Heterodimer Score	-5.9	-7.2
IEX % Heterodimer	92.9%	92.0%
LCMS % Heterodimer	94.0%	94.0%
SEC % Monomer	93.7%	93.3%
Production Yield (mg/L)	139	217

FIG. 18G

Cycle 1 outputs				Cycle 2 outputs				Combinations				Variation			
CH3 Set Name	Chain	SEQ ID NO:		CH3 Set Name	Chain	SEQ ID NO:		CH3 Set Name	Chain	SEQ ID NO:		CH3 Set Name	Chain	SEQ ID NO:	
W-SG	A	11		W-SG (349/354)	A	13		W-SG (354/349)	A	15		W-SG (354/349)	A	15	
	B	12			B	14			B	16			B	16	
V-V	A	21		V-V (349/354)	A	23		V-V (354/349)	A	25		V-V (354/349)	A	25	
	B	22			B	24			B	26			B	26	
QR-F	A	31		QR-F (349/354)	A	33		QR-F (354/349)	A	35		QR-F (354/349)	A	35	
	B	32			B	34			B	36			B	36	
RG-FG	A	41		RG-FG (349/354)	A	43		RG-FG (354/349)	A	45		RG-FG (354/349)	A	45	
	B	42			B	44			B	46			B	46	
TL-QL	A	51		TL-QL (349/354)	A	53		TL-QL (354/349)	A	55		TL-QL (354/349)	A	55	
	B	52			B	54			B	56			B	56	
DVG-VSY	A	61		DVG-VSY (349/354)	A	63		DVG-VSY (354/349)	A	65		DVG-VSY (354/349)	A	65	
	B	62			B	64			B	66			B	66	
LWG-SIG	A	71		LWG-SIG (349/354)	A	73		LWG-SIG (354/349)	A	75		LWG-SIG (354/349)	A	75	
	B	72			B	74			B	76			B	76	
WTL-SAVQL	A	81		WTL-SAVQL (349/354)	A	83		WTL-SAVQL (354/349)	A	85		WTL-SAVQL (354/349)	A	85	
	B	82			B	84			B	86			B	86	
WTL-SGQL	A	91		WTL-SGQL (349/354)	A	93		WTL-SGQL (354/349)	A	95		WTL-SGQL (354/349)	A	95	
	B	92			B	94			B	96			B	96	
WQL-SAVTL	A	101		WQL-SAVTL (349/354)	A	103		WQL-SAVTL (354/349)	A	105		WQL-SAVTL (354/349)	A	105	
	B	102			B	104			B	106			B	106	
WQL-SGTL	A	111		WQL-SGTL (349/354)	A	113		WQL-SGTL (354/349)	A	115		WQL-SGTL (354/349)	A	115	
	B	112			B	114			B	116			B	116	
VTL-VQL	A	121		VTL-VQL (349/354)	A	123		VTL-VQL (354/349)	A	125		VTL-VQL (354/349)	A	125	
	B	122			B	124			B	126			B	126	
VQL-VTL	A	131		VQL-VTL (349/354)	A	133		VQL-VTL (354/349)	A	135		VQL-VTL (354/349)	A	135	
	B	132			B	134			B	136			B	136	
QRQL-FTL	A	141		QRQL-FTL (349/354)	A	143		QRQL-FTL (354/349)	A	145		QRQL-FTL (354/349)	A	145	
	B	142			B	144			B	146			B	146	
VQR-VF	A	151		VQR-VF (349/354)	A	153		VQR-VF (354/349)	A	155		VQR-VF (354/349)	A	155	
	B	152			B	154			B	156			B	156	
LWG-IG	A	161		LWG-IG (349/354)	A	163		LWG-IG (354/349)	A	165		LWG-IG (354/349)	A	165	
	B	162			B	164			B	166			B	166	

FIG. 19

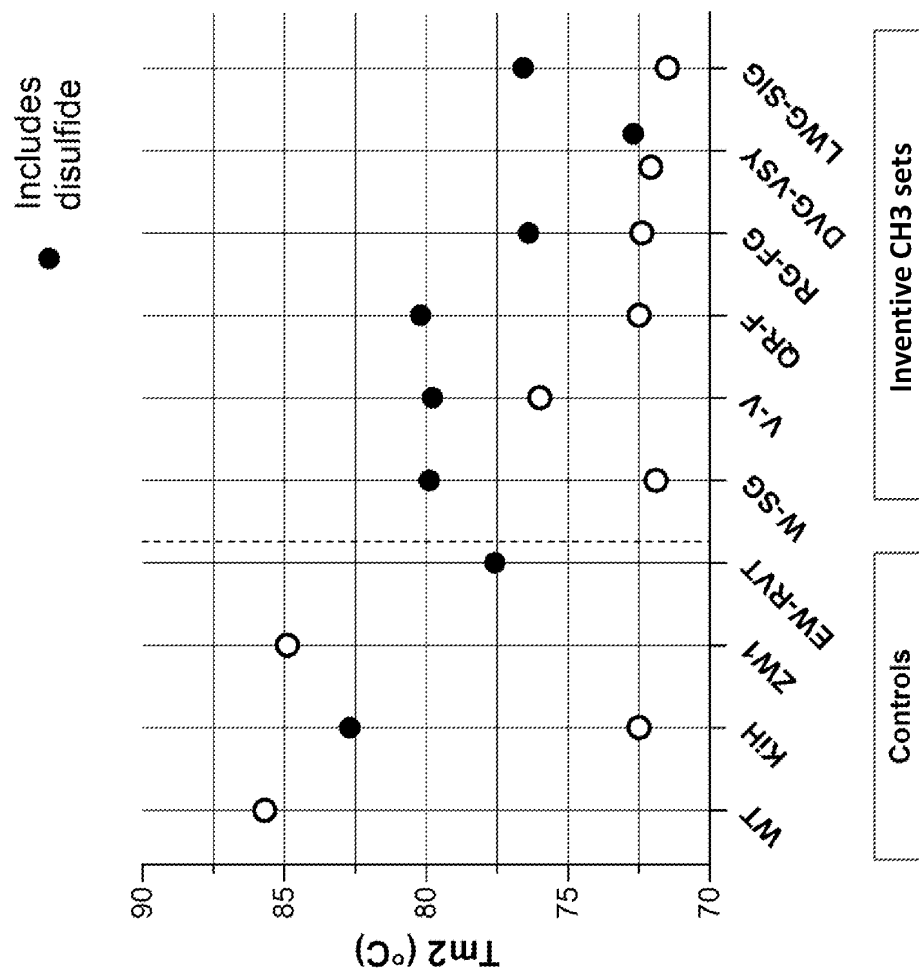


FIG. 20

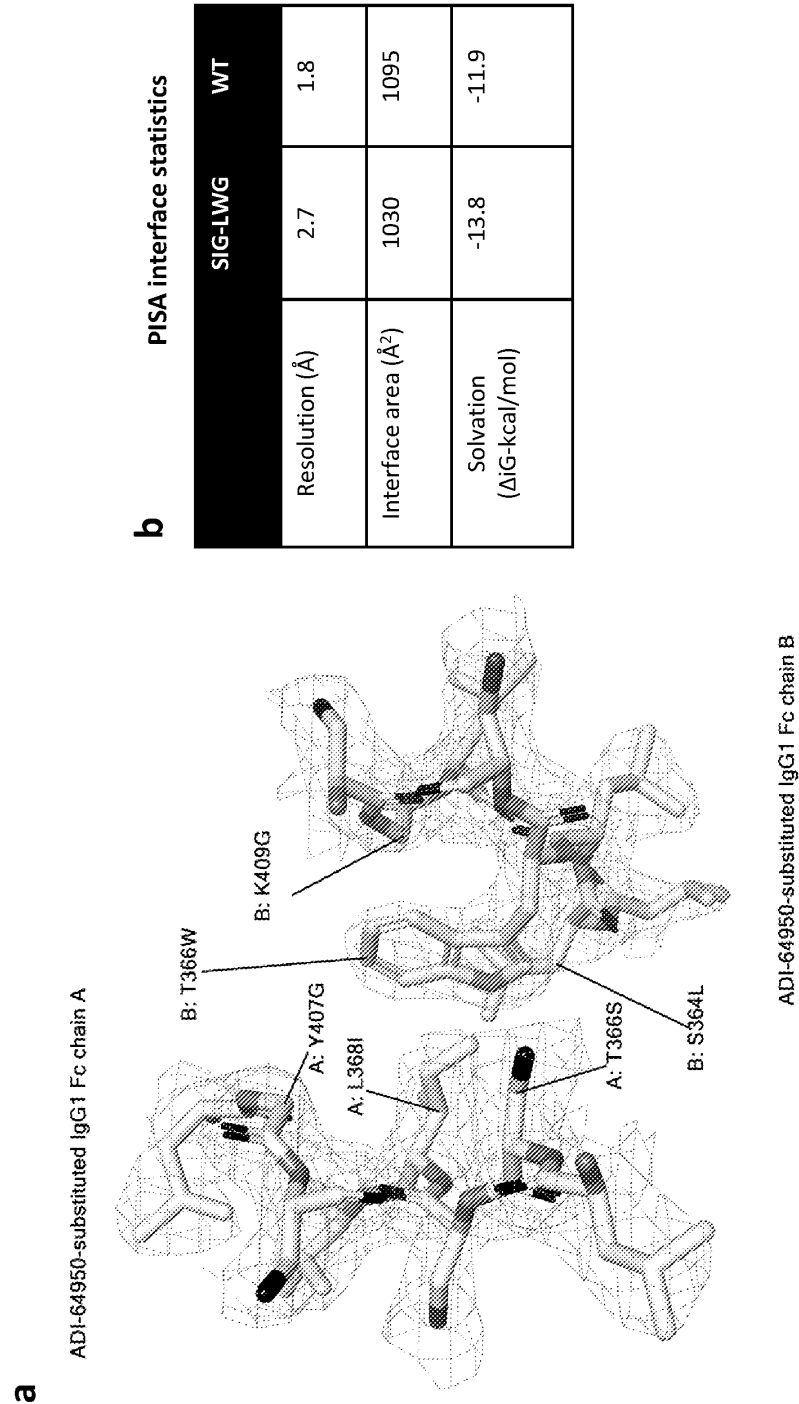
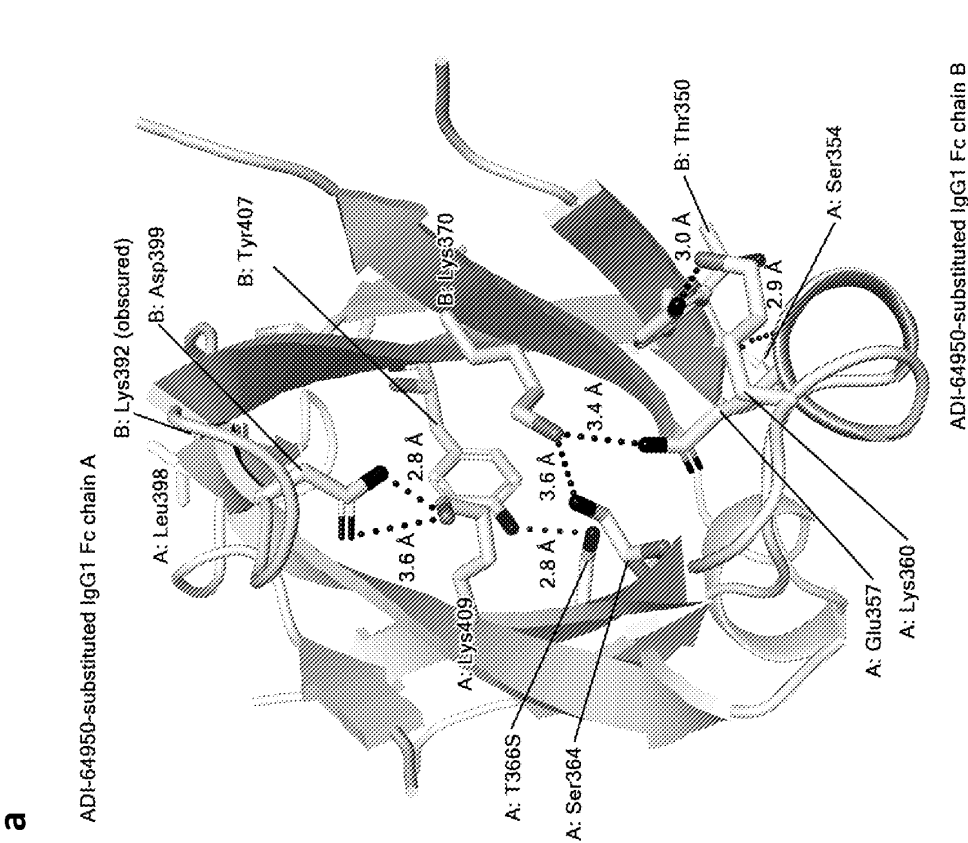


FIG. 21



b

Polar interactions

	SIG-LWG	WT
# Hydrogen bonds	7	4
# Salt bridges	3	6
Sum polar interactions	10	10

FIG. 22

ADI-64950 modeled chain A-A and B-B homodimers

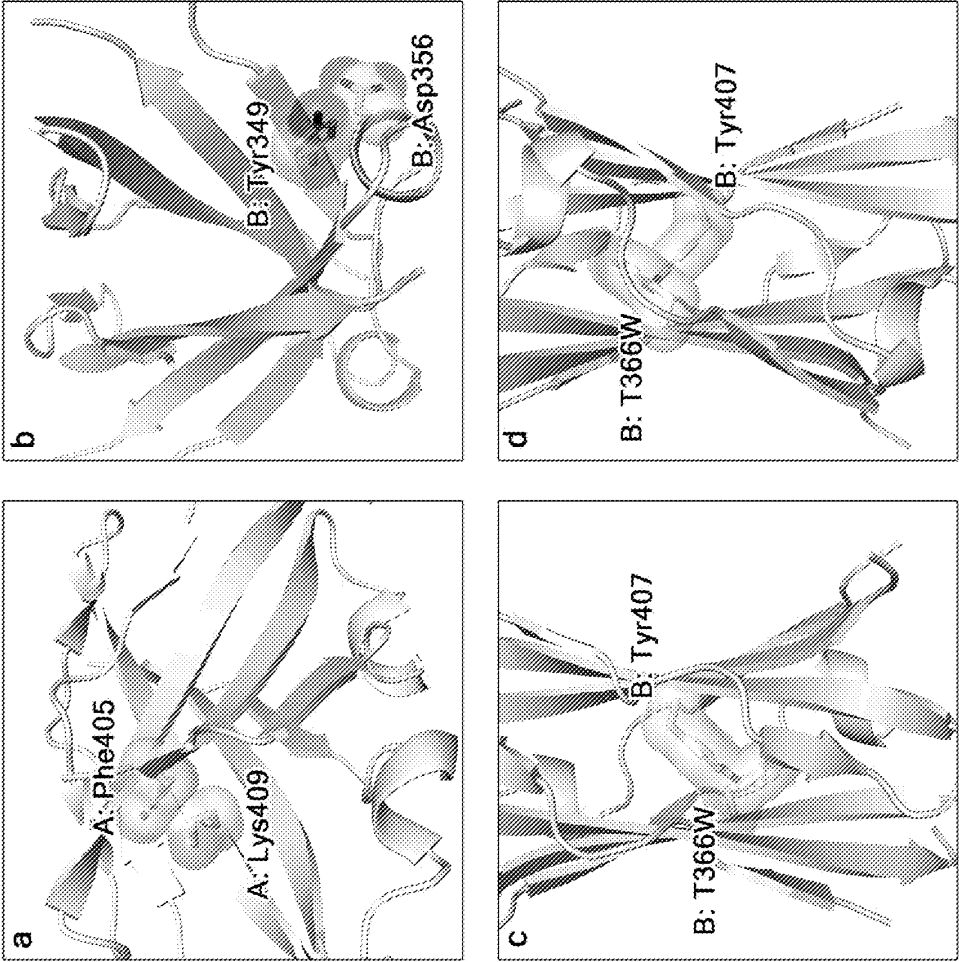


FIG. 23

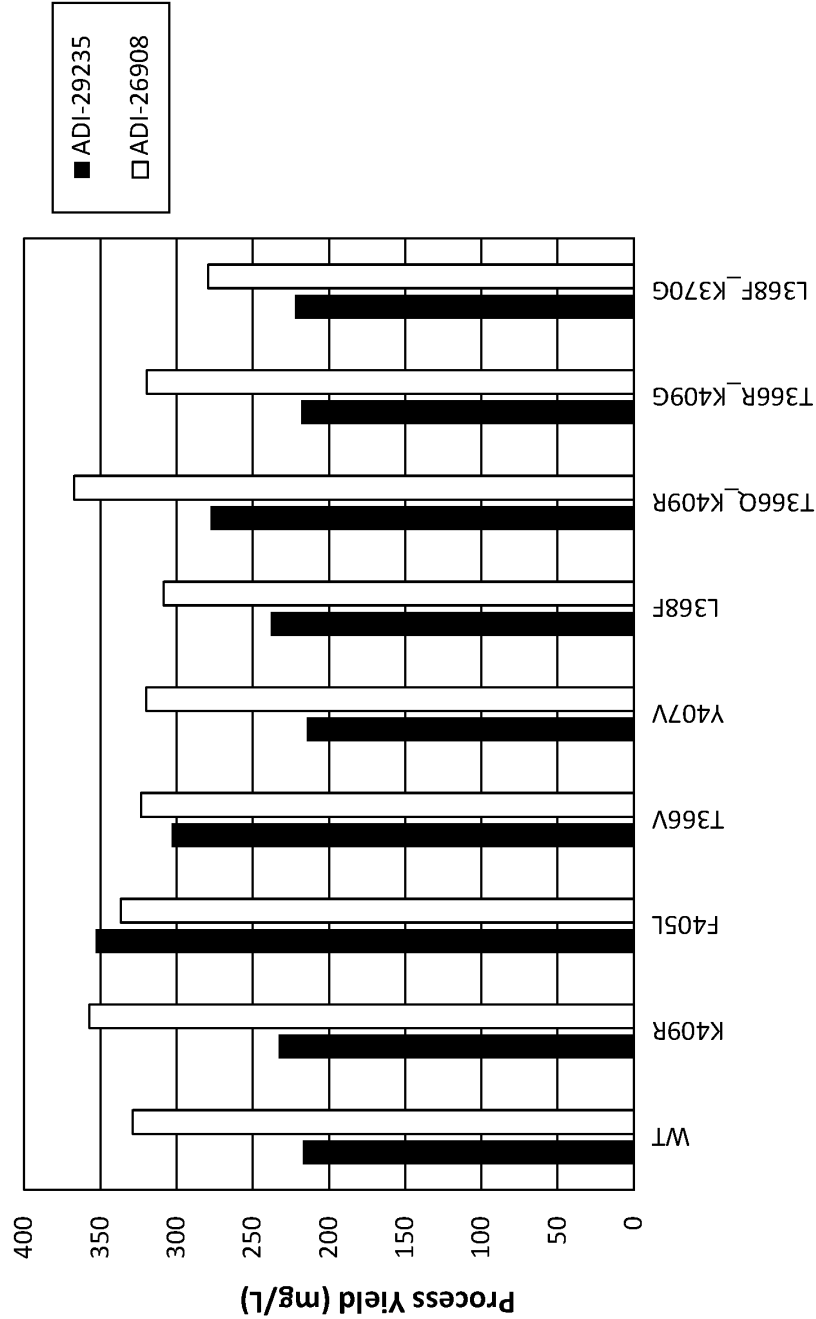


FIG. 24A

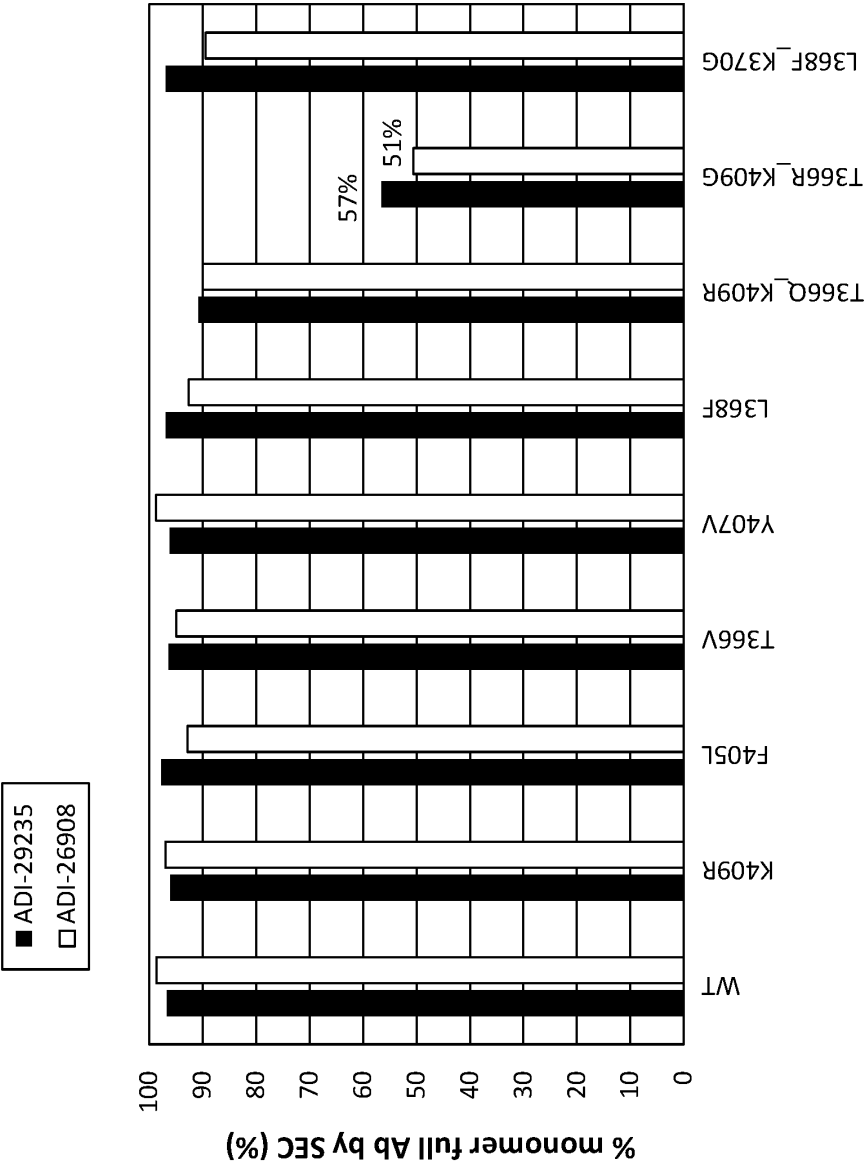


FIG. 24B

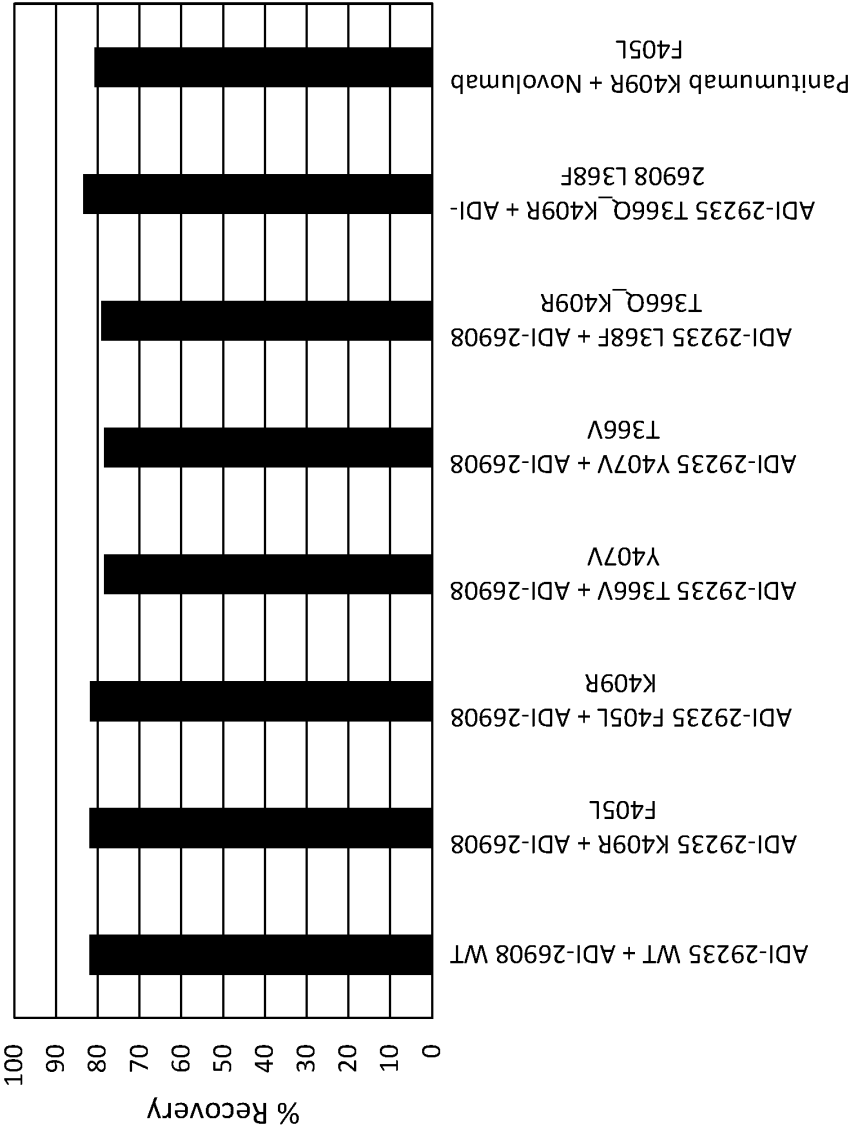


FIG. 25A

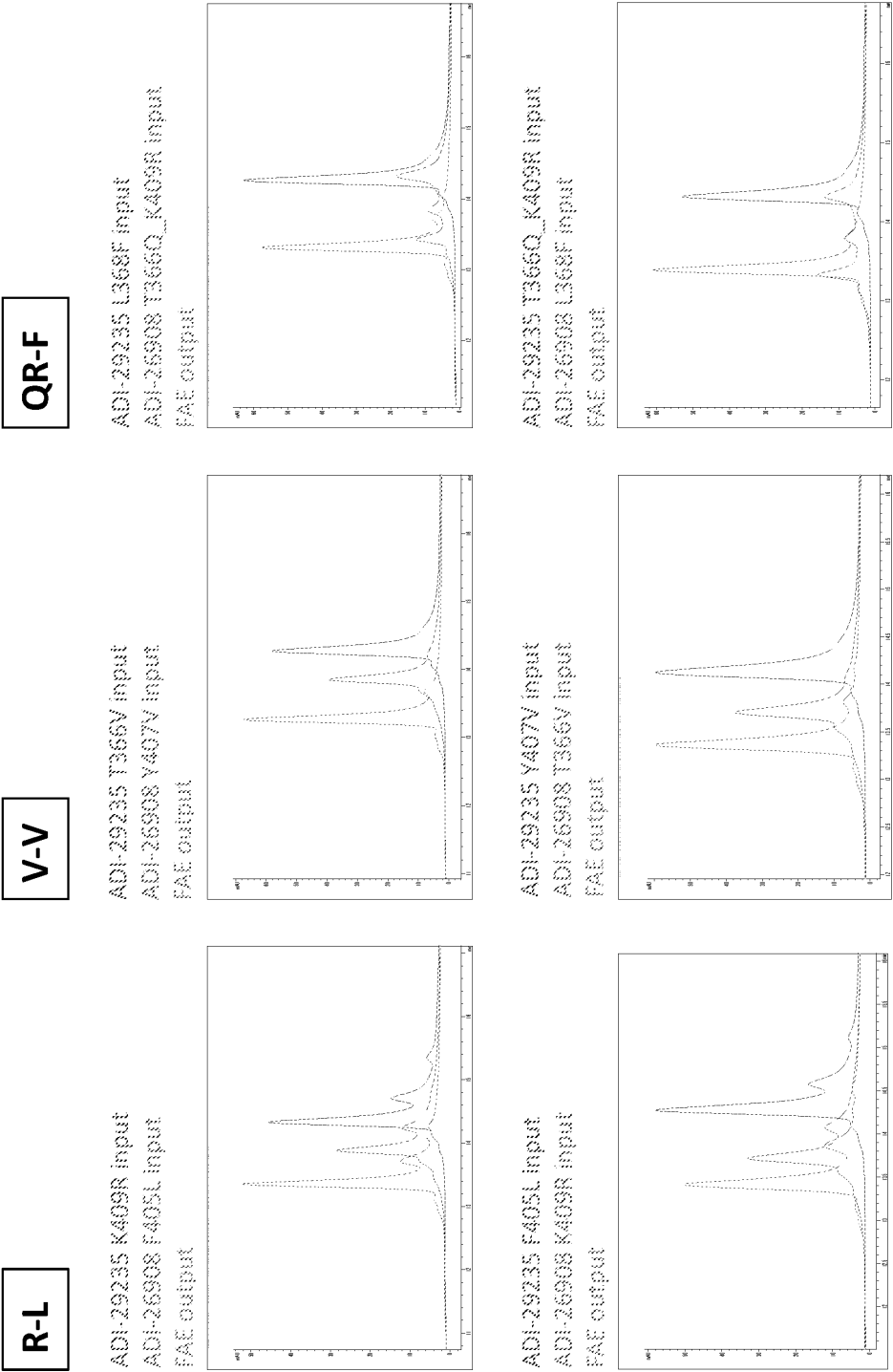


FIG. 25B

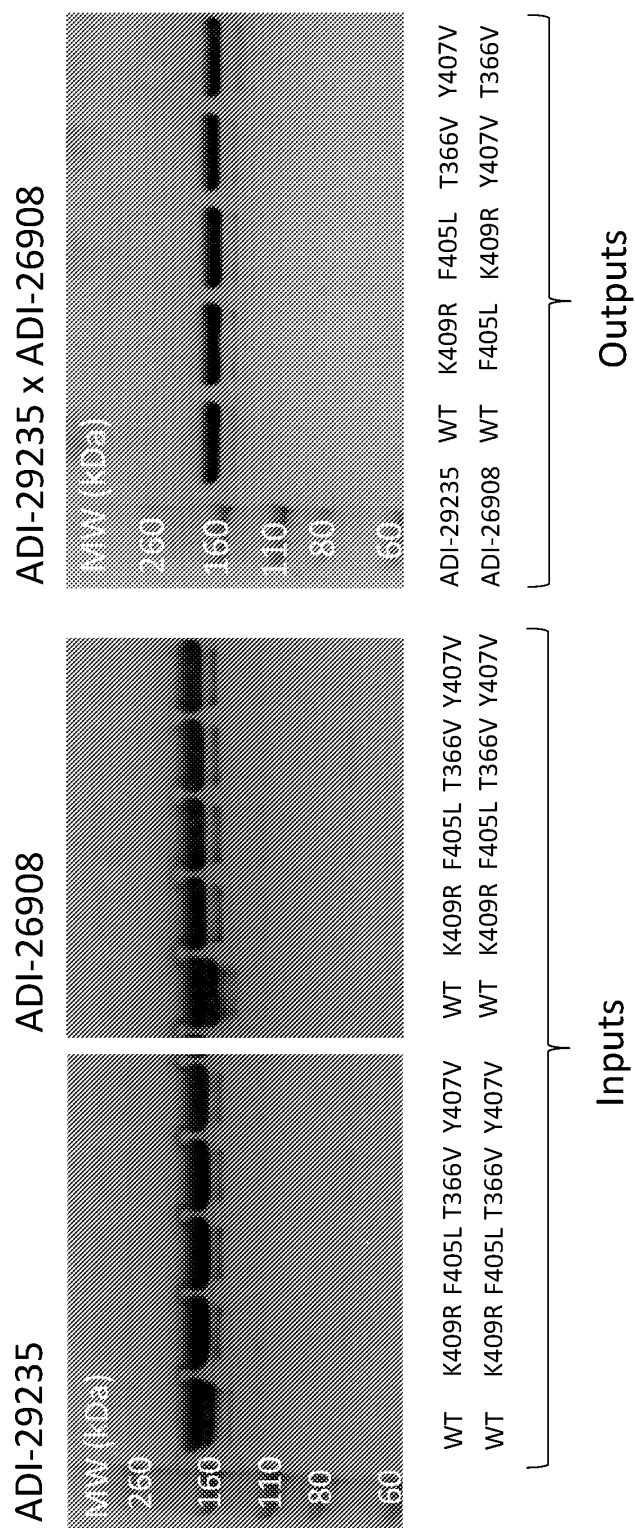


FIG. 26A

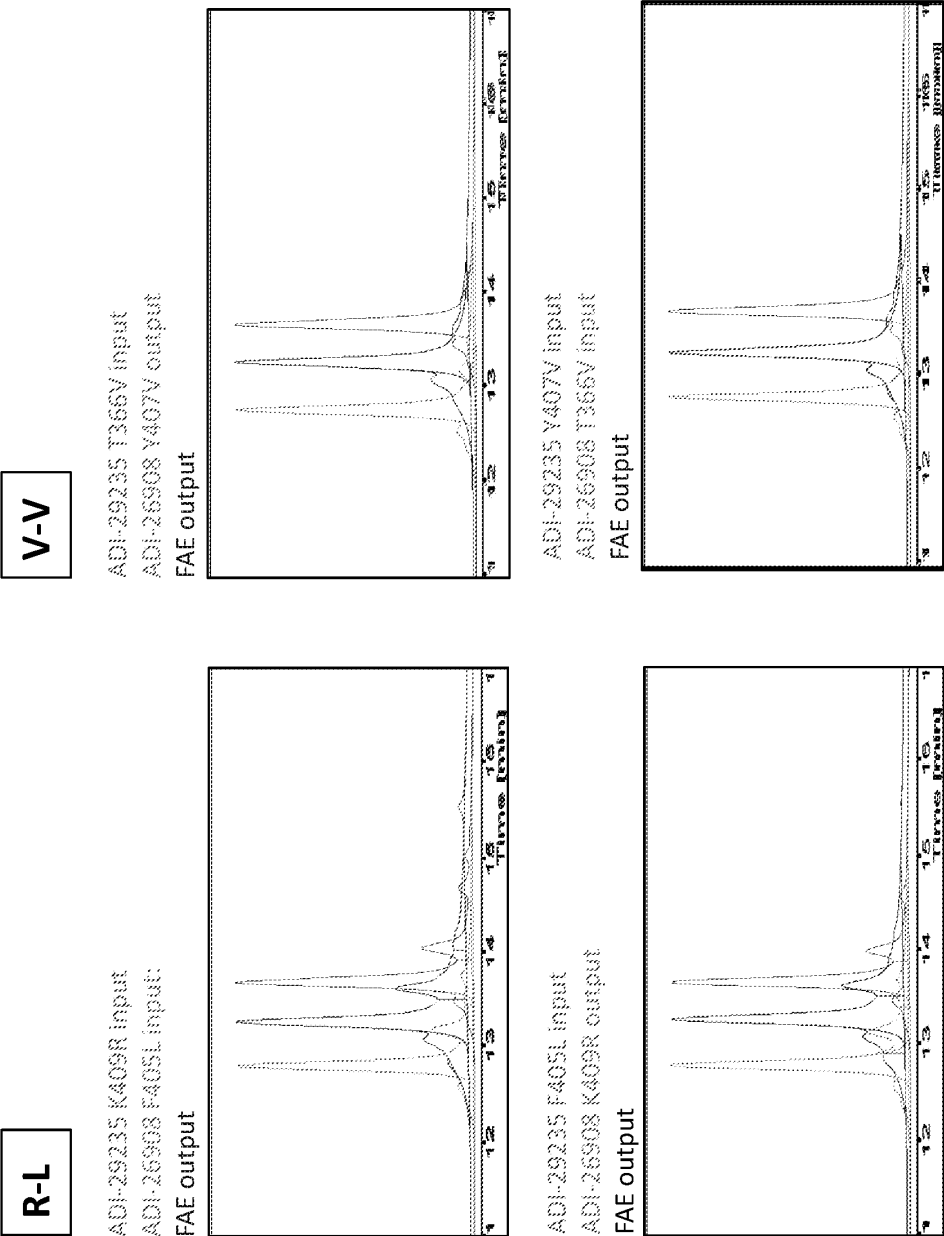


FIG. 26B

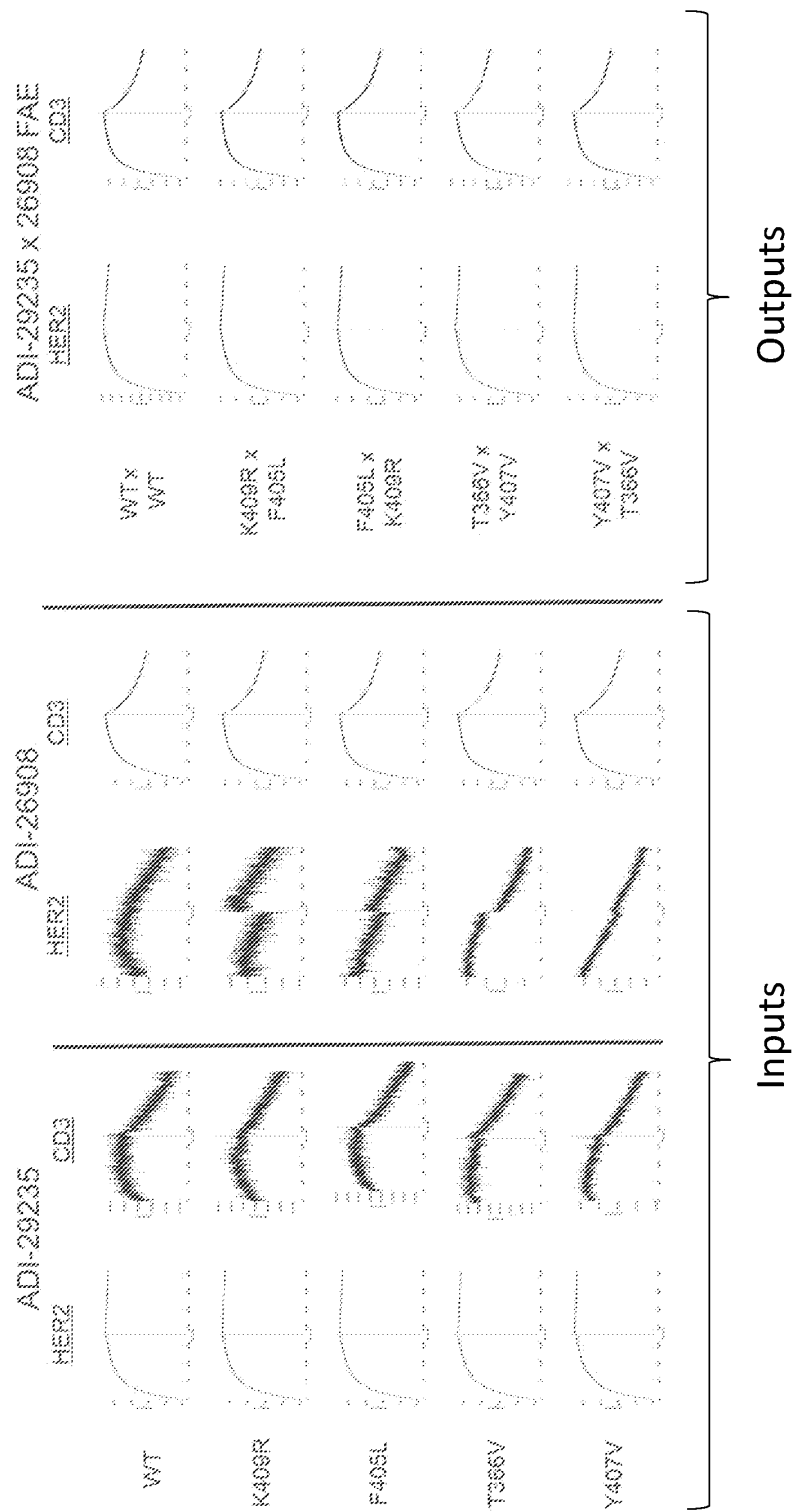


FIG. 26C

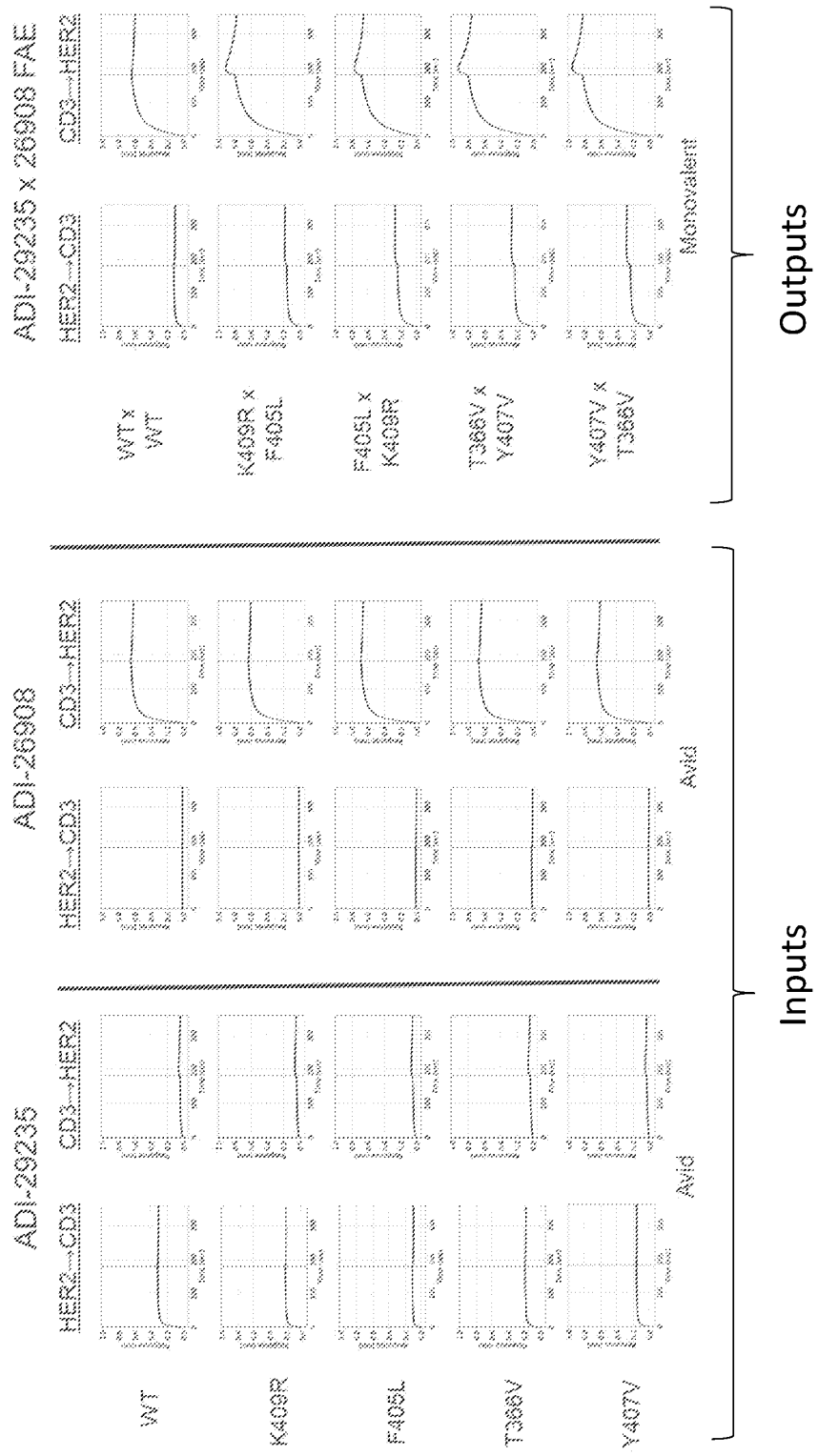


FIG. 26D

Glutathione Challenge

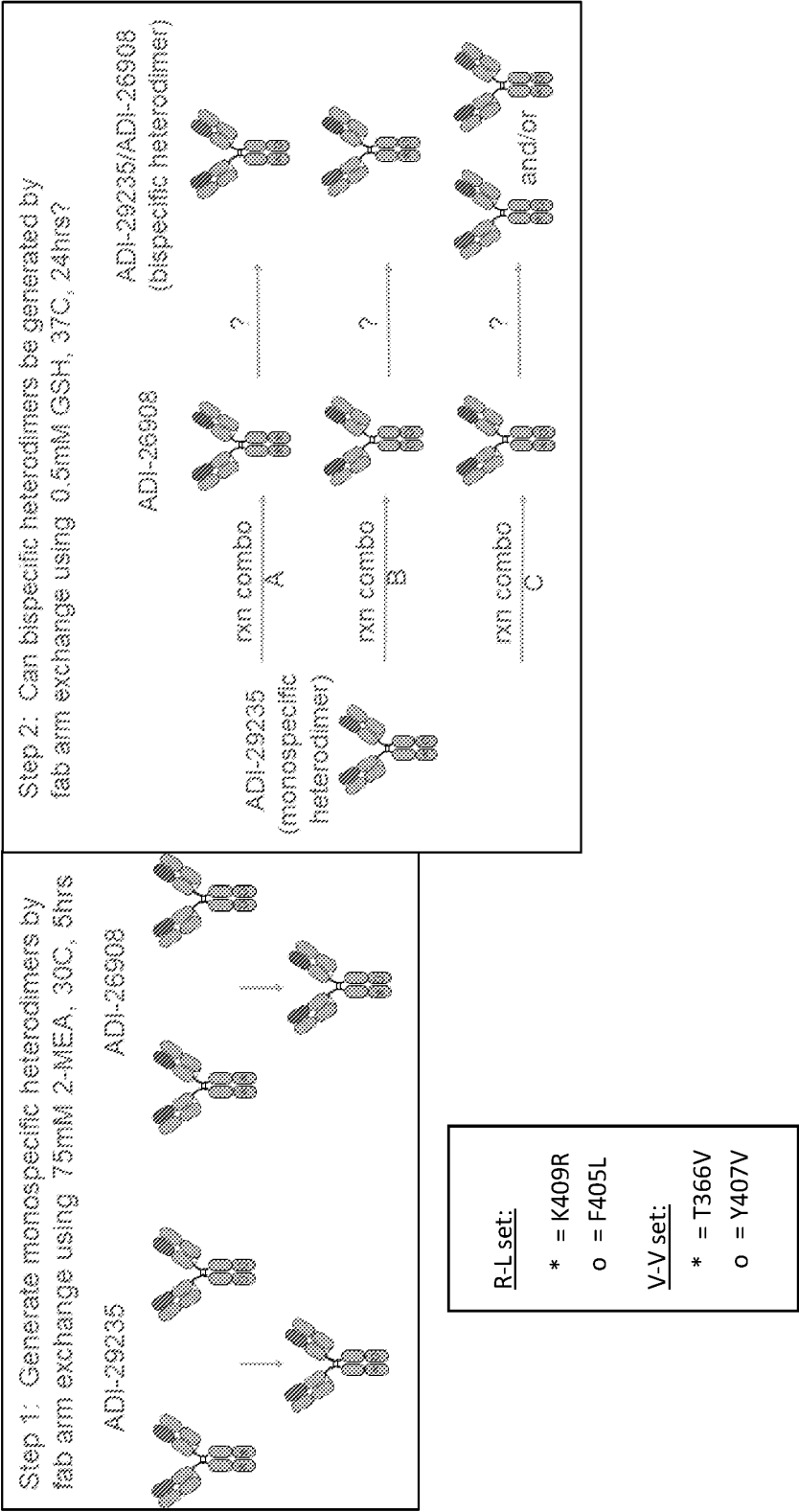


FIG. 27A

Glutathione Testing Step 1: IEX Results
Generate monospecific heterodimers by fab arm exchange using 75mM 2-MEA, 30C, 5hrs

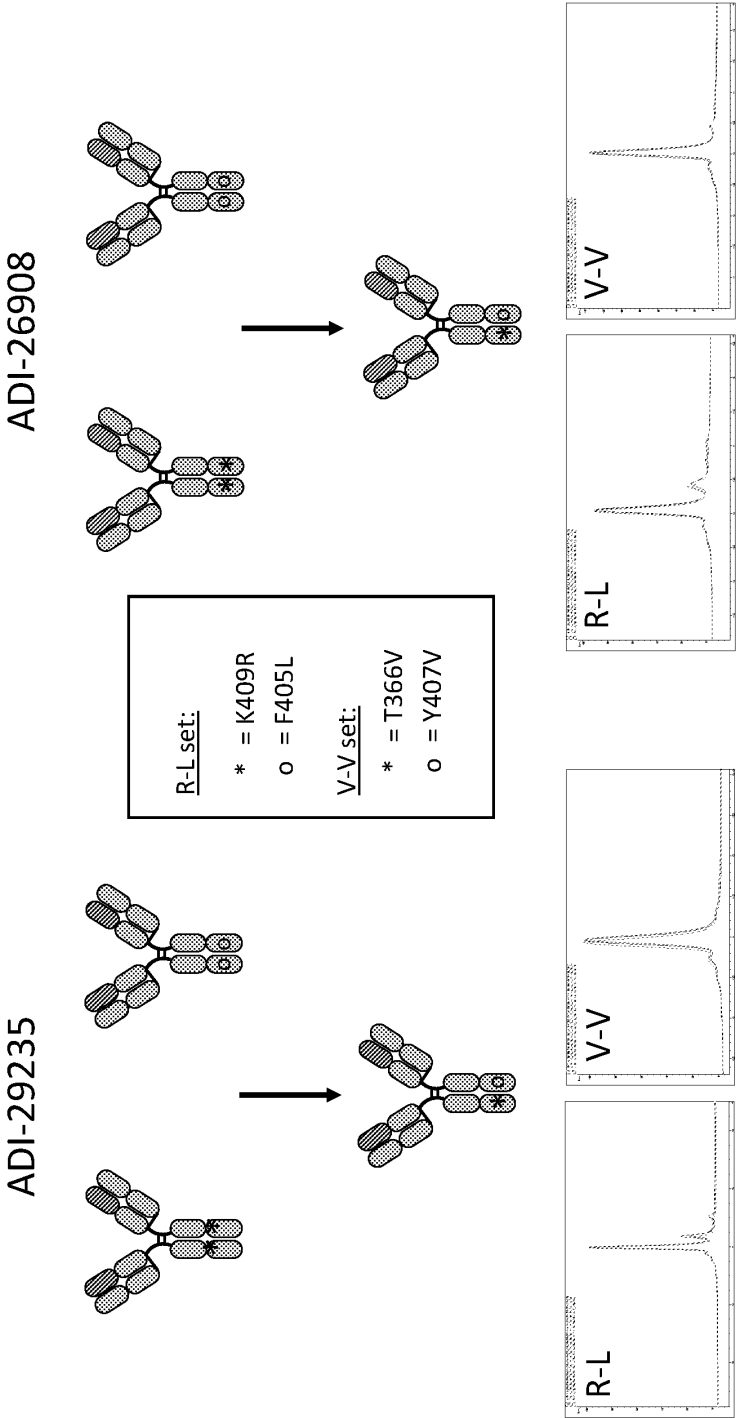


FIG. 27B

Glutathione Testing Step 2: IEX Results

Can bispecific heterodimers be generated by fab arm exchange using 0.5mM GSH, 37C, 24hrs?

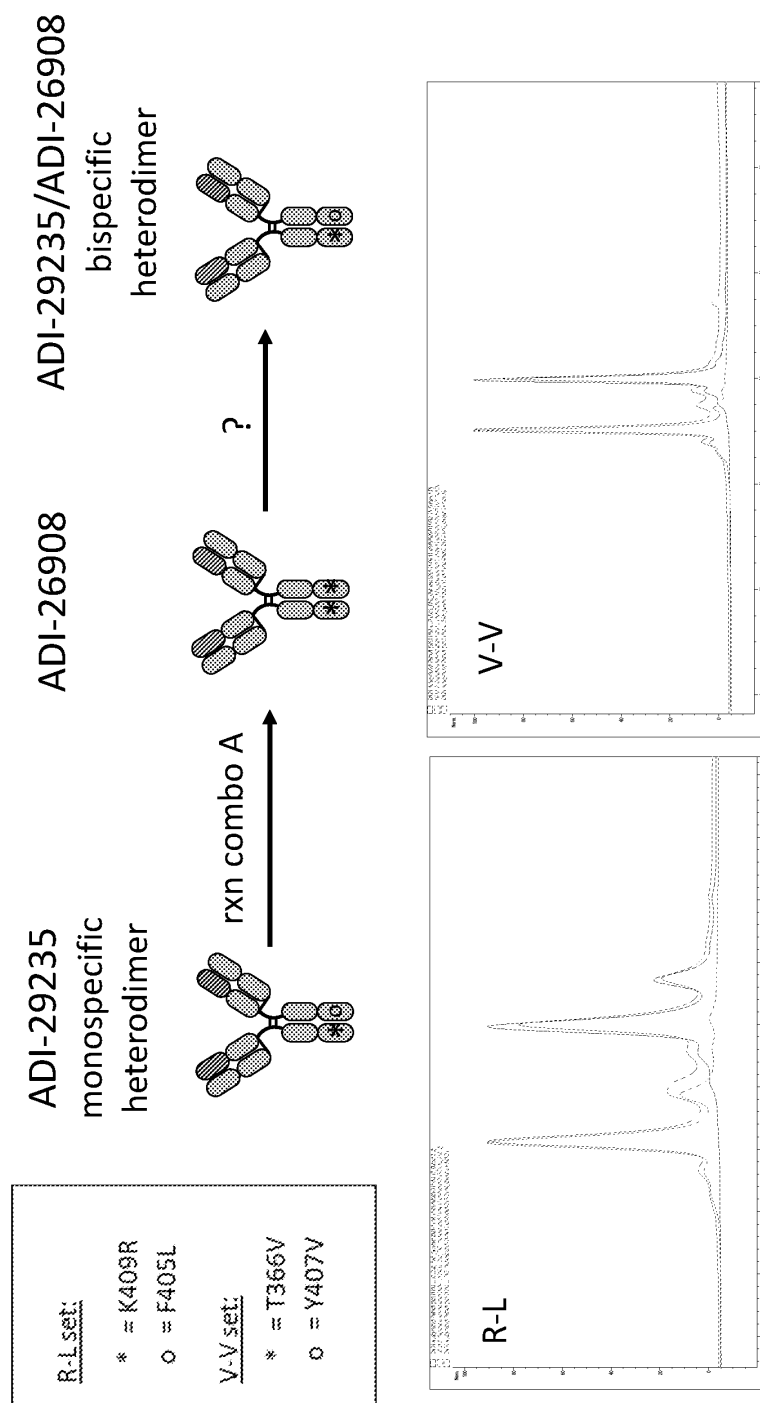


FIG. 27C

Glutathione Testing Step 2: IEX Results

Can bispecific heterodimers be generated by fab arm exchange using 0.5mM GSH, 37C, 24hrs?

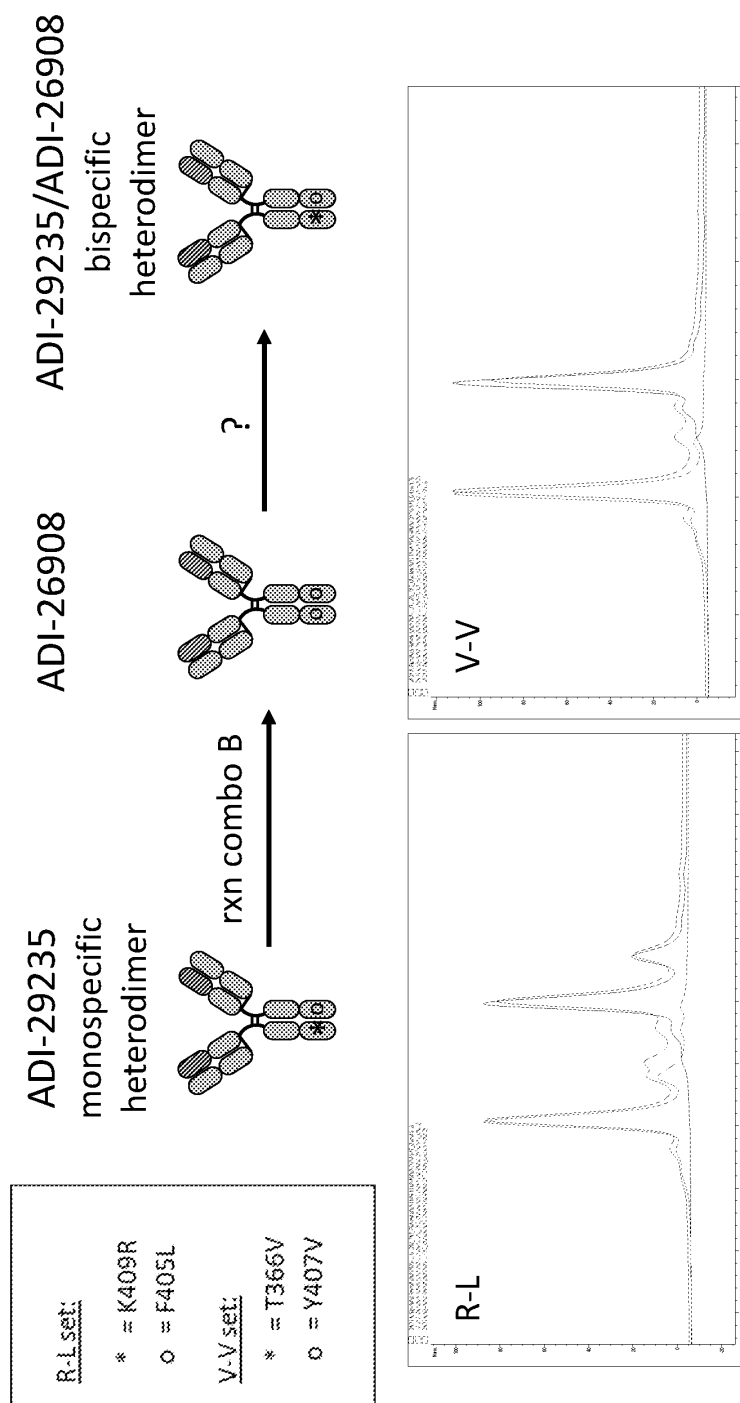


FIG. 27D

Glutathione Testing Step 2: IEX Results

Can bispecific heterodimers be generated by fab arm exchange using 0.5mM GSH, 37C, 24hrs?

