

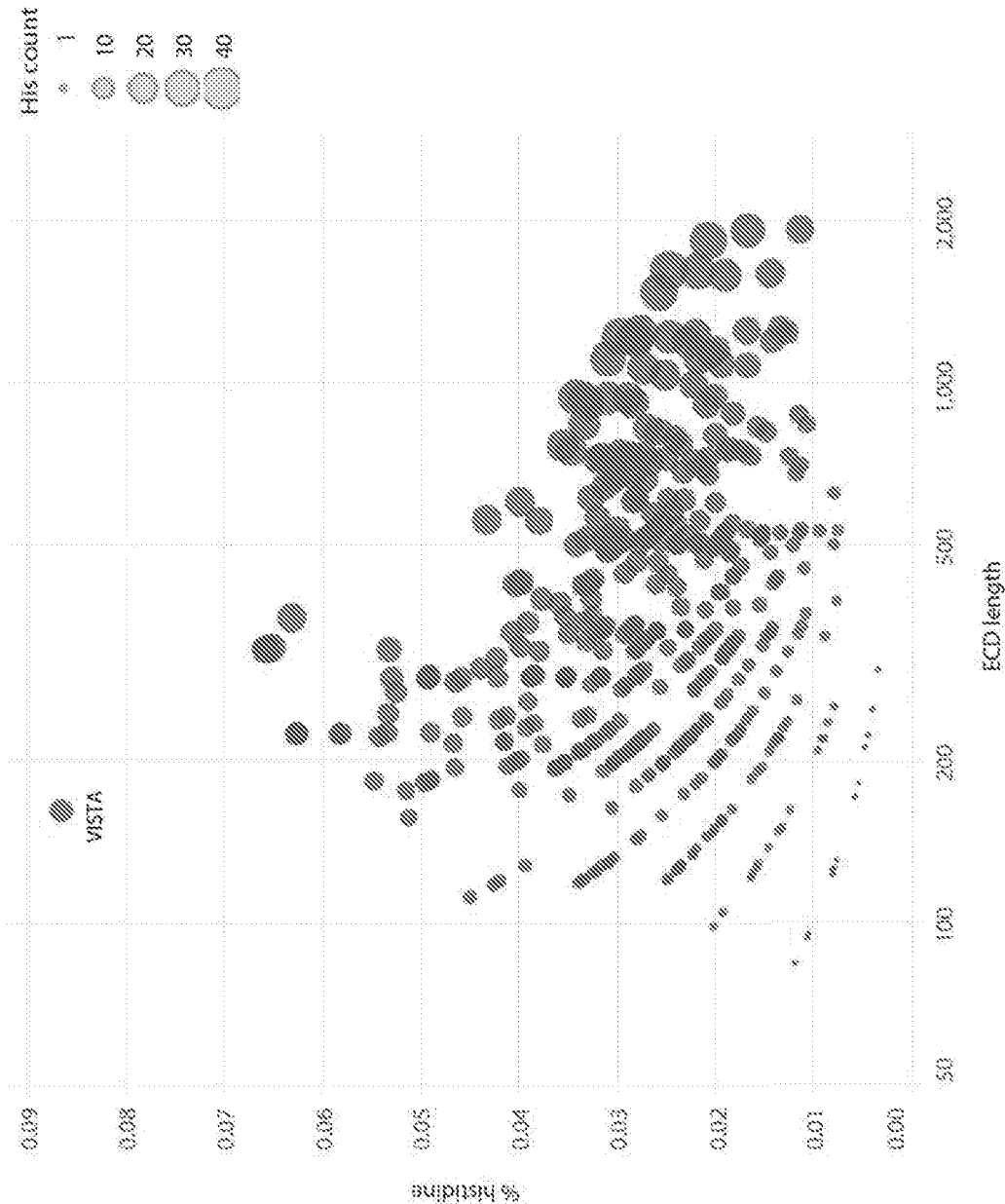


FIG. 1A

Hu/Cy/Ms conserved histidines (bold, underlined)
Hu/Cy-conserved histidines (bold)

Human NP_071436.1	Sig-	FKVATPYSLYVCPEGQNVTLTCRLLGPVDK <u>GH</u> DVTFYK <u>TW</u> YR
Cyno XP_005565644.1	Sig-	FKVATLYSLYVCPEGQNVTLTCRVFGPVDK <u>GH</u> DVTFYK <u>TW</u> YR
Mouse NP_083008.1	Sig-	FKVTTTPYSLYVCPEGQNA <u>T</u> LT <u>CR</u> ILGPVSK <u>GH</u> DV <u>TI</u> YK <u>TW</u> YL
Human NP_071436.1		SSRGEVQTCSERRPIRNLTFQD <u>LH</u> <u>HH</u> <u>GG</u> <u>H</u> QAANT <u>SH</u> <u>DL</u> AQ <u>RH</u> G <u>L</u> ES
Cyno XP_005565644.1		SSRGEVQTCSERRPIRNLTFQD <u>LH</u> <u>HH</u> <u>GG</u> <u>H</u> QAANT <u>SH</u> <u>DL</u> AQ <u>RH</u> G <u>L</u> ES
Mouse NP_083008.1		SSRGEVQ <u>MCK</u> EH <u>RPI</u> RN <u>FT</u> LQ <u>HL</u> Q- <u>HH</u> <u>GS</u> <u>H</u> LKANAS <u>SH</u> DQ <u>PQ</u> <u>KH</u> G <u>L</u> EL
Human NP_071436.1		ASD <u>HH</u> GNFSITMRNLTL <u>LD</u> SGLYCCLV <u>VEIR</u> <u>HHH</u> SE <u>HRV</u> H <u>G</u> AME <u>LQ</u> V
Cyno XP_005565644.1		ASD <u>HH</u> GNFSITMRNLTL <u>LD</u> SGLYCCLV <u>VEIR</u> <u>HHH</u> SE <u>HRV</u> H <u>G</u> AME <u>LQ</u> V
Mouse NP_083008.1		ASD <u>HH</u> GNFSITLRNVTP <u>RD</u> SGLYCCLV <u>IEL</u> KN <u>HH</u> PEQ <u>RF</u> Y <u>G</u> SM <u>ELQ</u> V
Human NP_071436.1		QTGKDAPSN <u>CV</u> YPSSSQDS <u>ENITAA</u> -TMD
Cyno XP_005565644.1		QTGKDAPSS <u>CV</u> AYPSSSQ <u>SE</u> ENITAA -TMD
Mouse NP_083008.1		QAGKGS <u>G</u> STC <u>MA</u> --S <u>NEQ</u> SD <u>S</u> ITAA -TMD

FIG. 1B

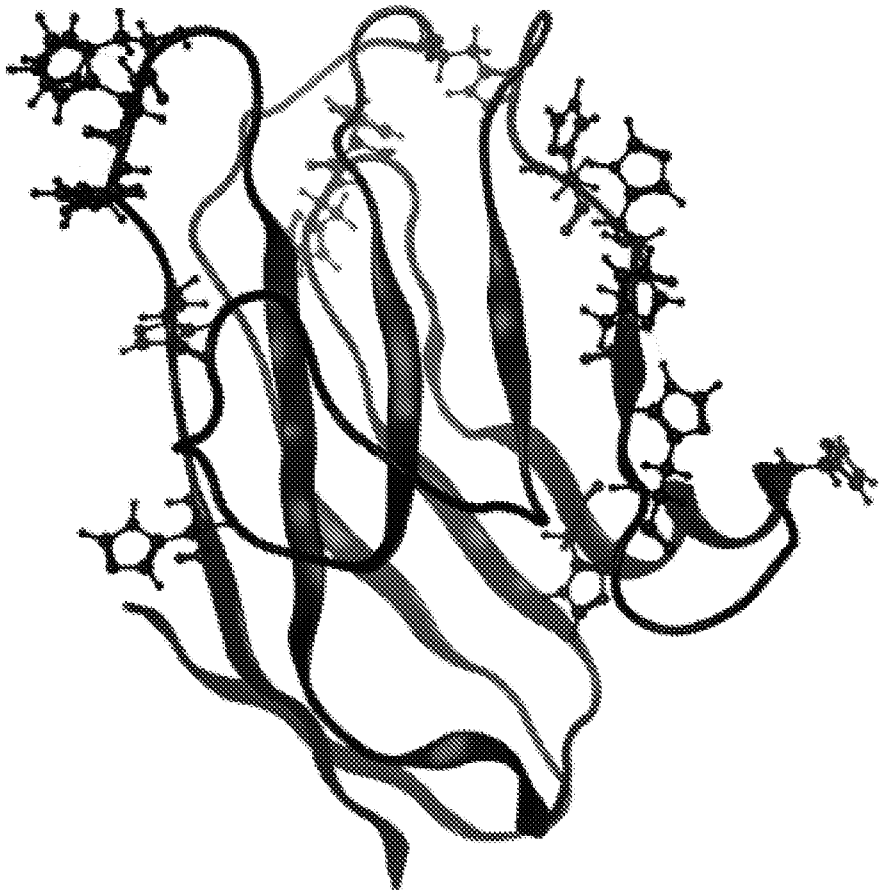


FIG. 1C

4/40

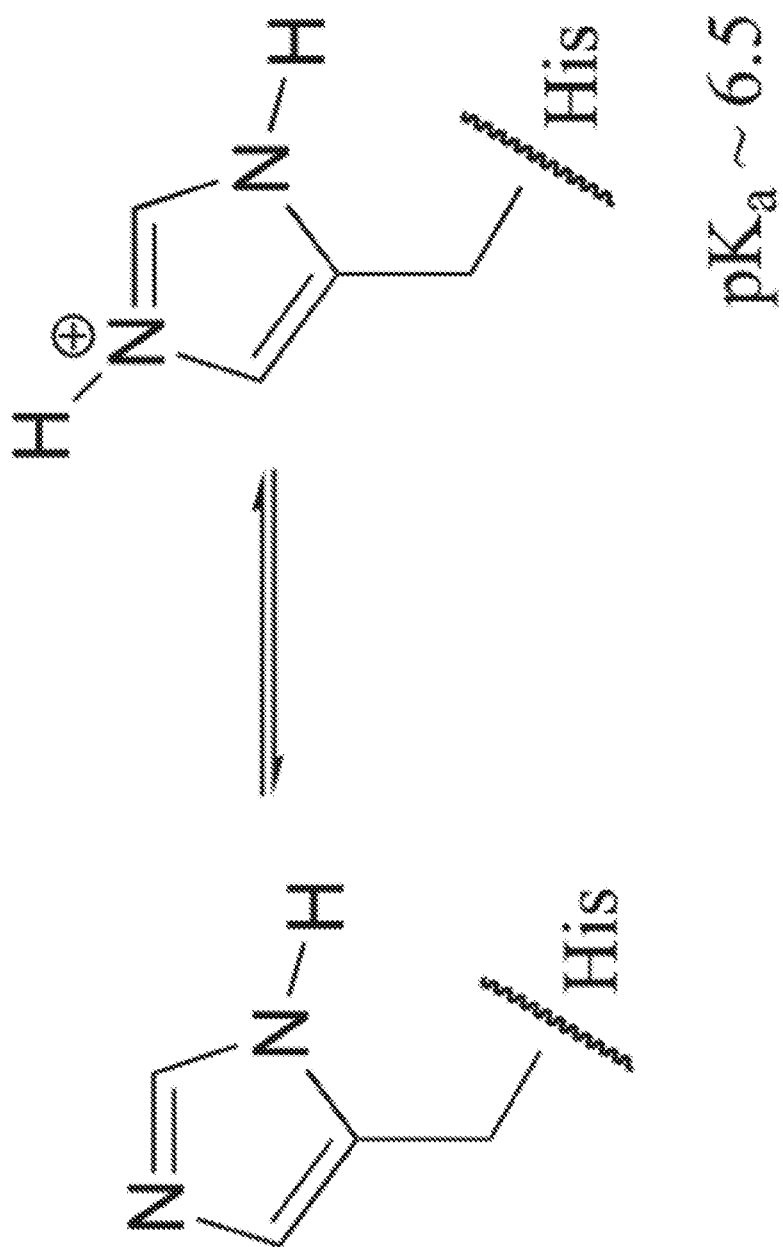


FIG. 2A

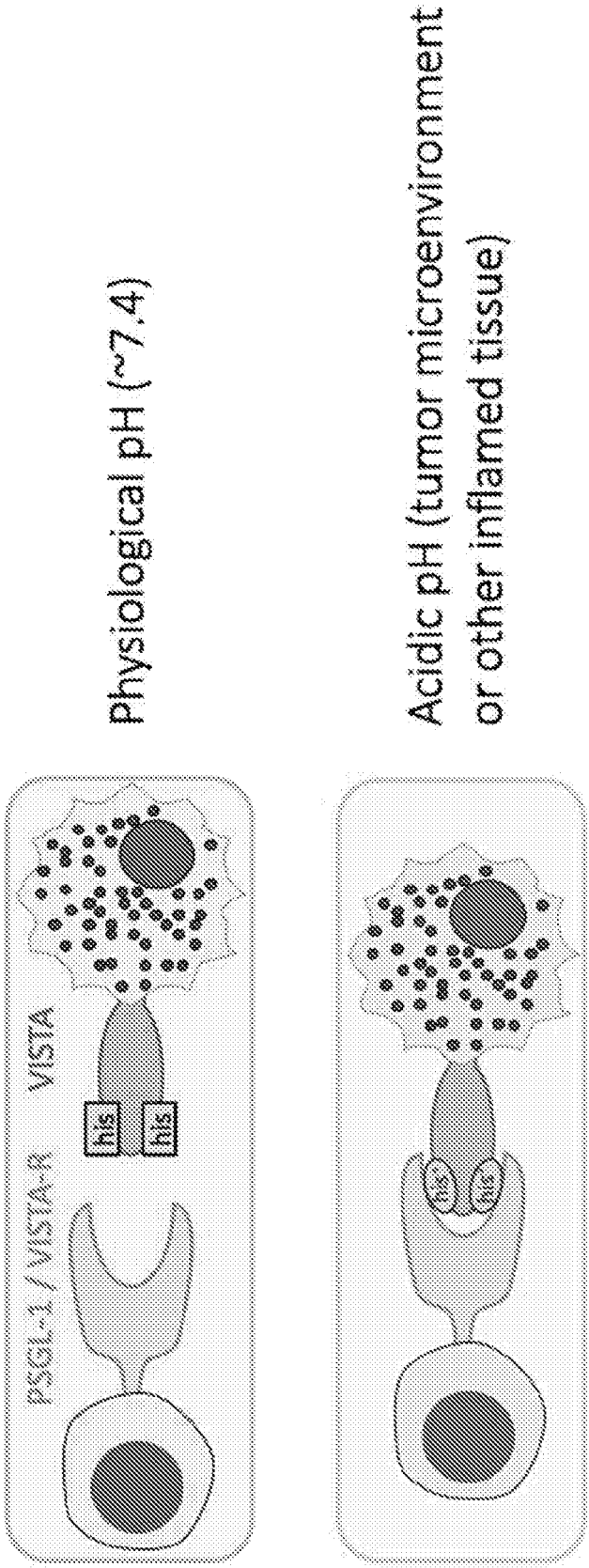


FIG. 2B

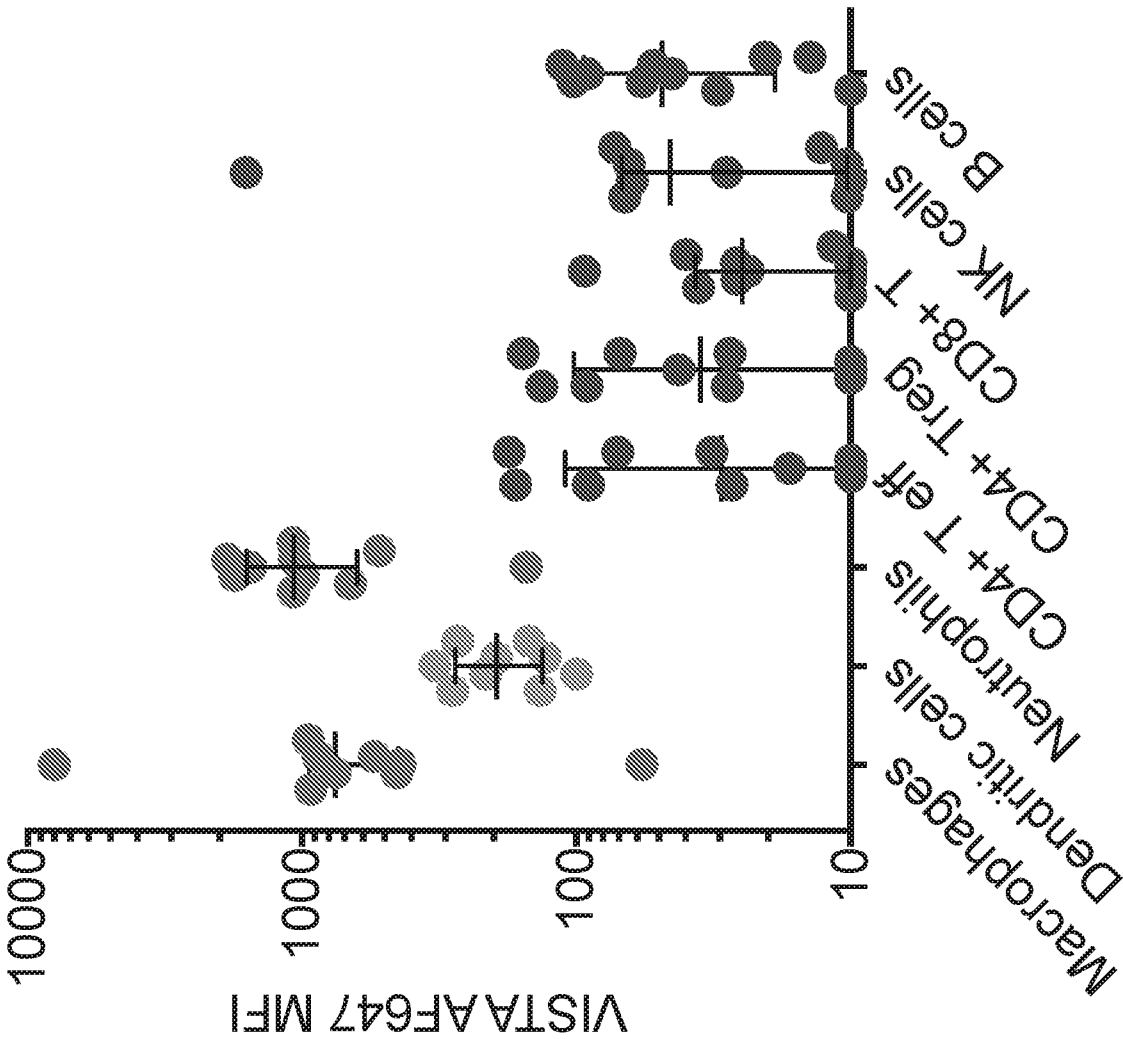


FIG. 3

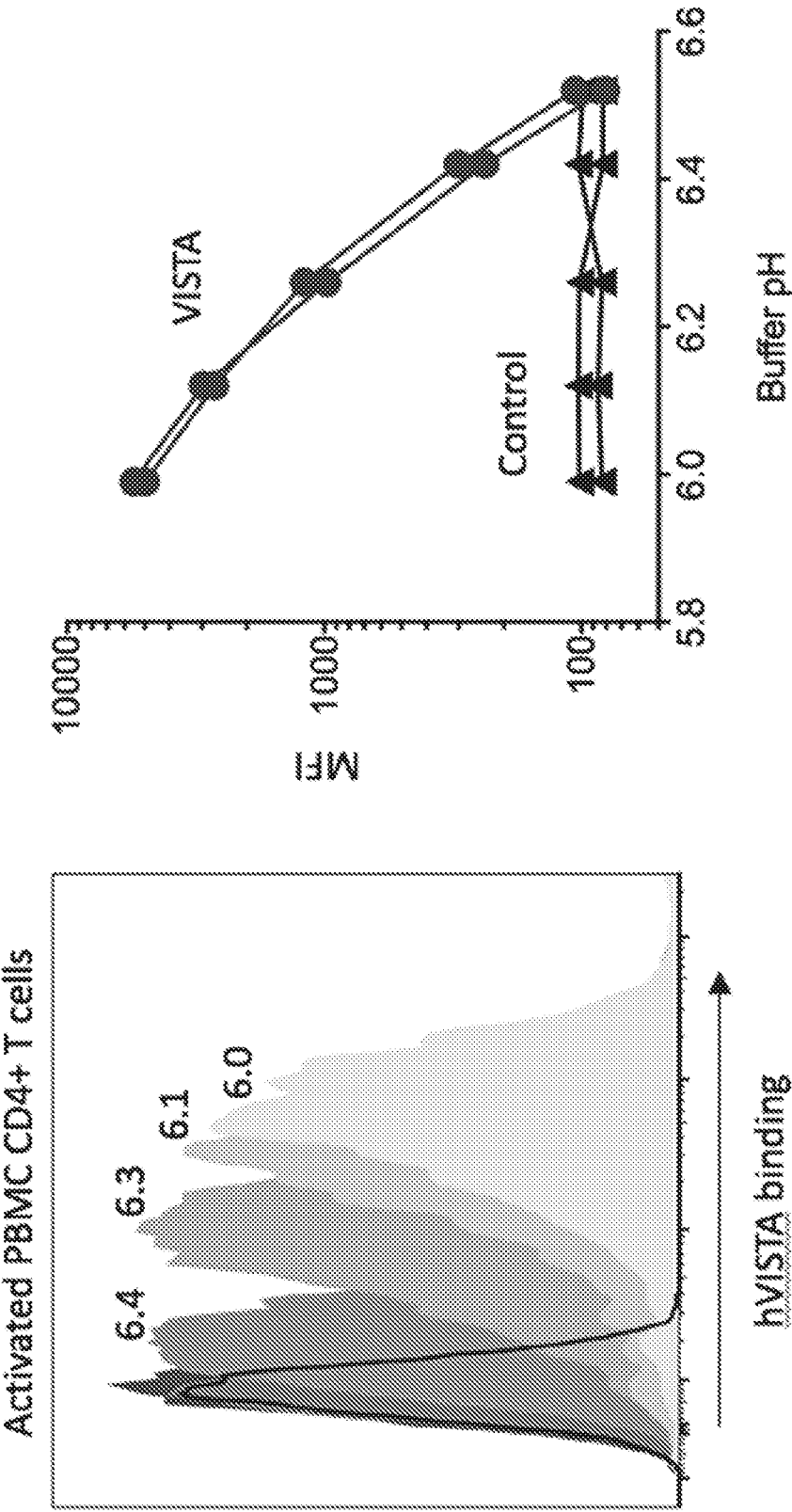


FIG. 4A

8/40

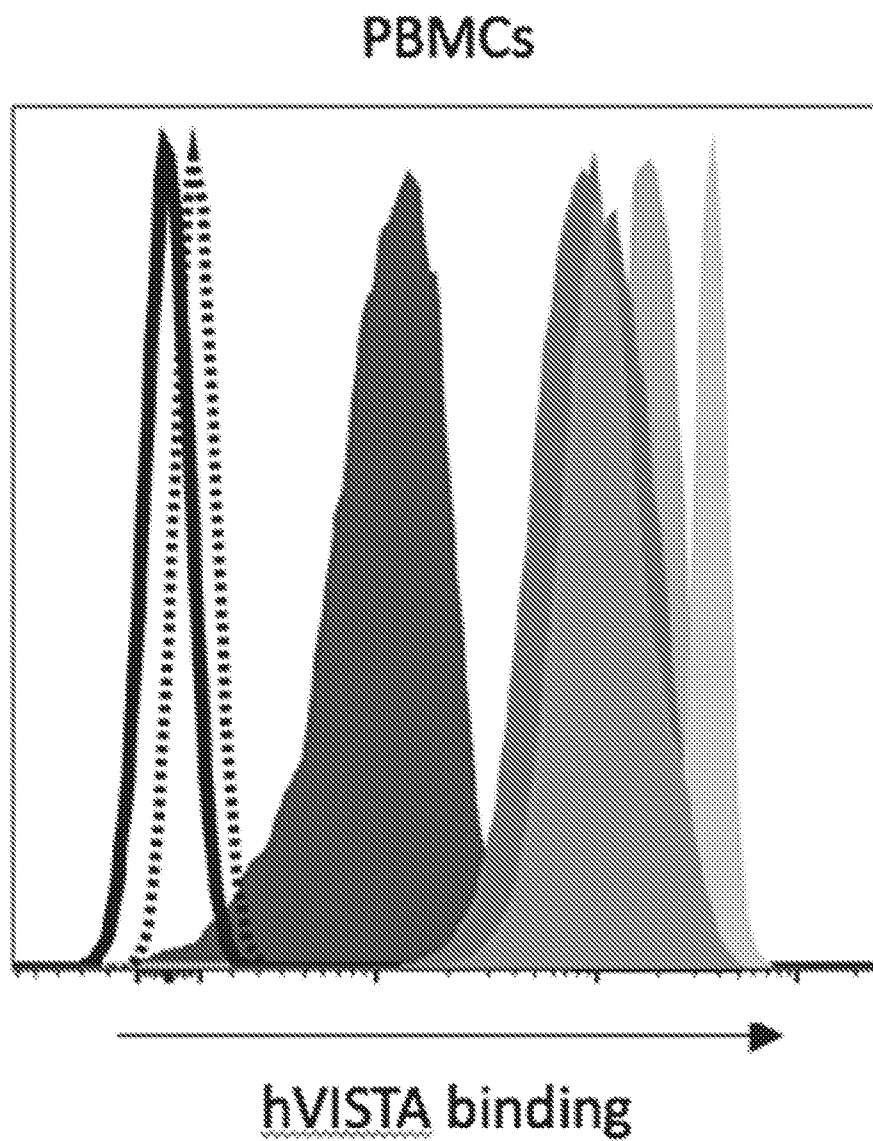


FIG. 4B

9/40

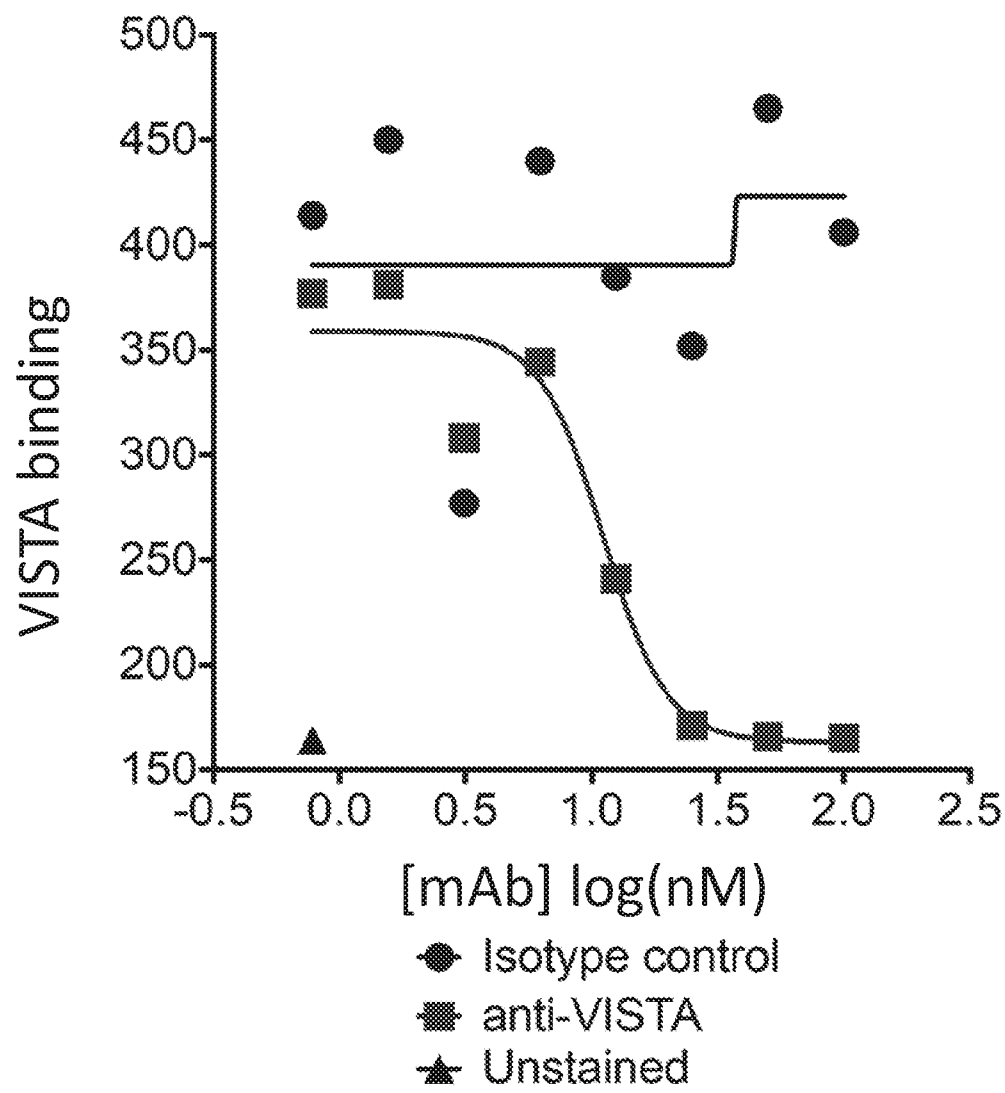


FIG. 4C

10/40

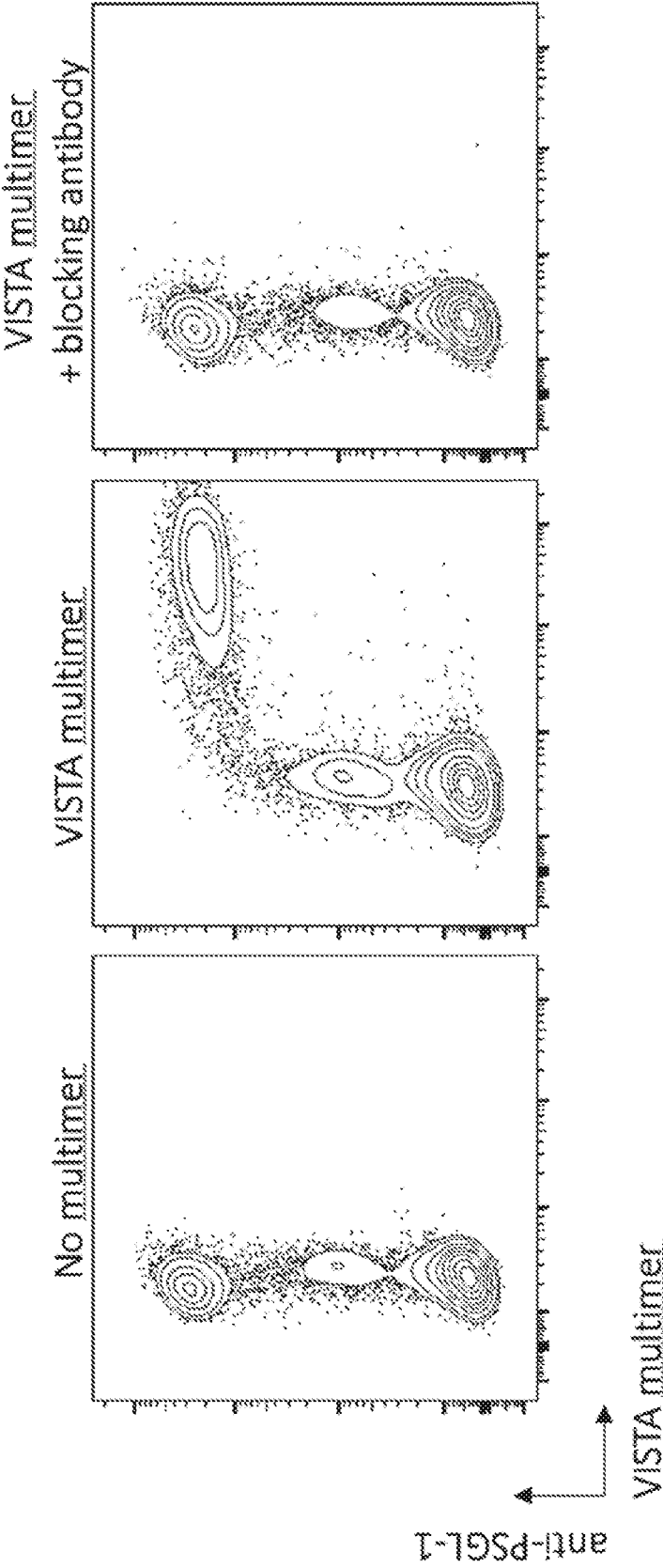


FIG. 4D

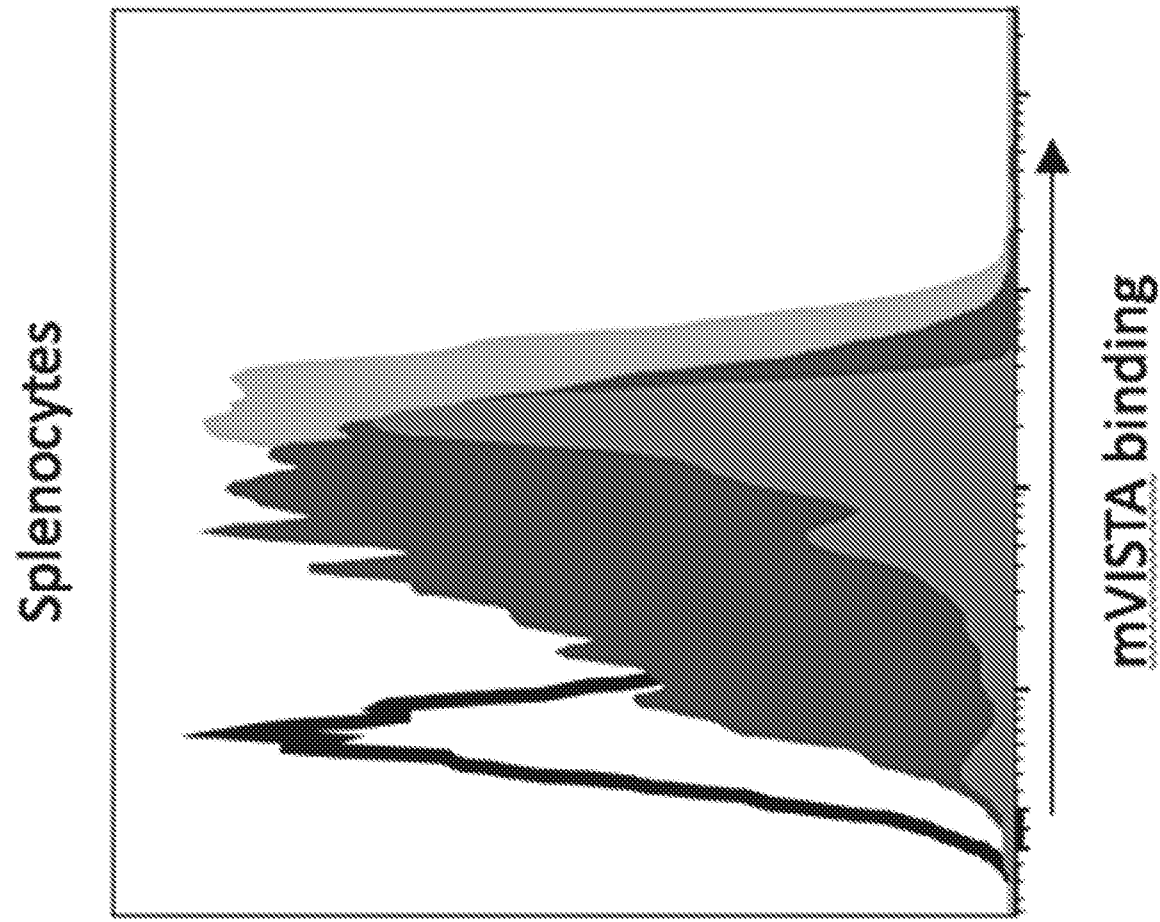


FIG. 4E

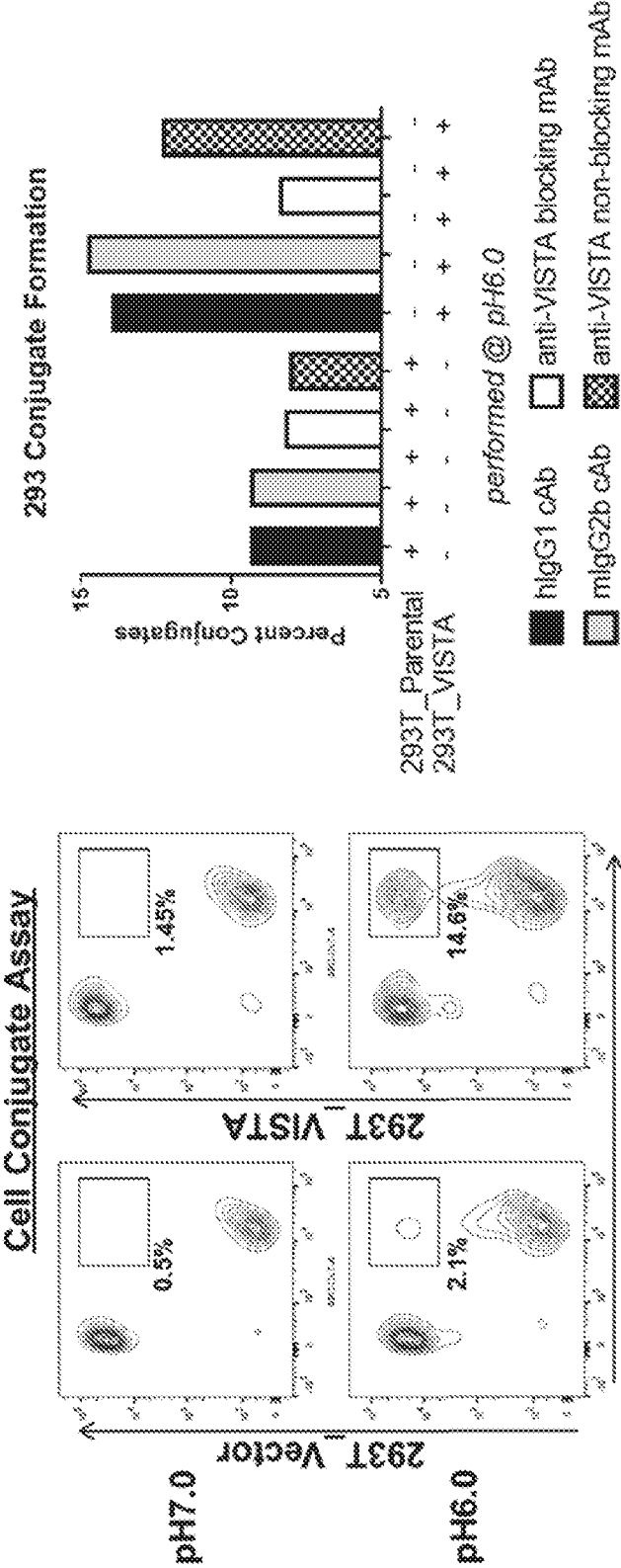


FIG. 5B

FIG. 5A

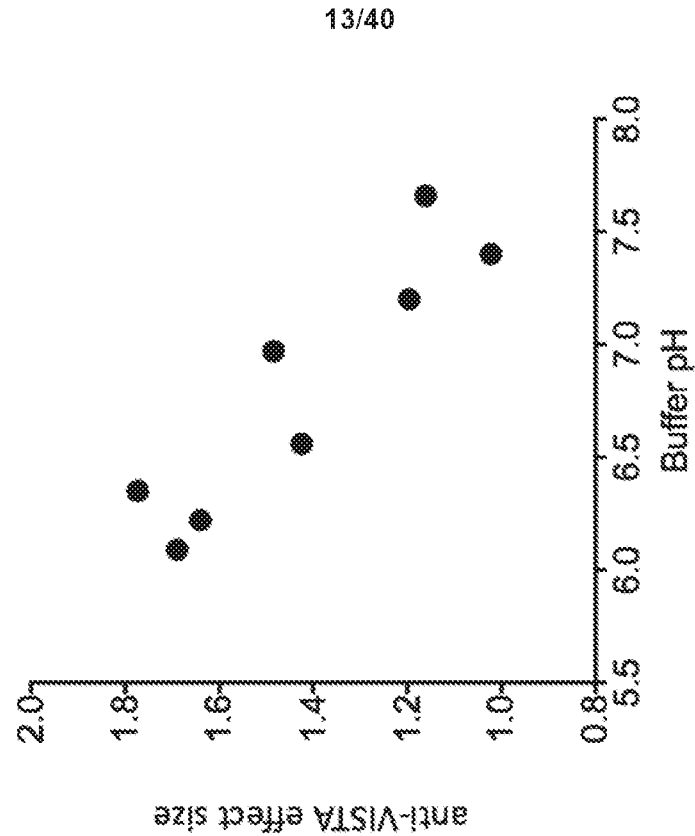


FIG. 5D

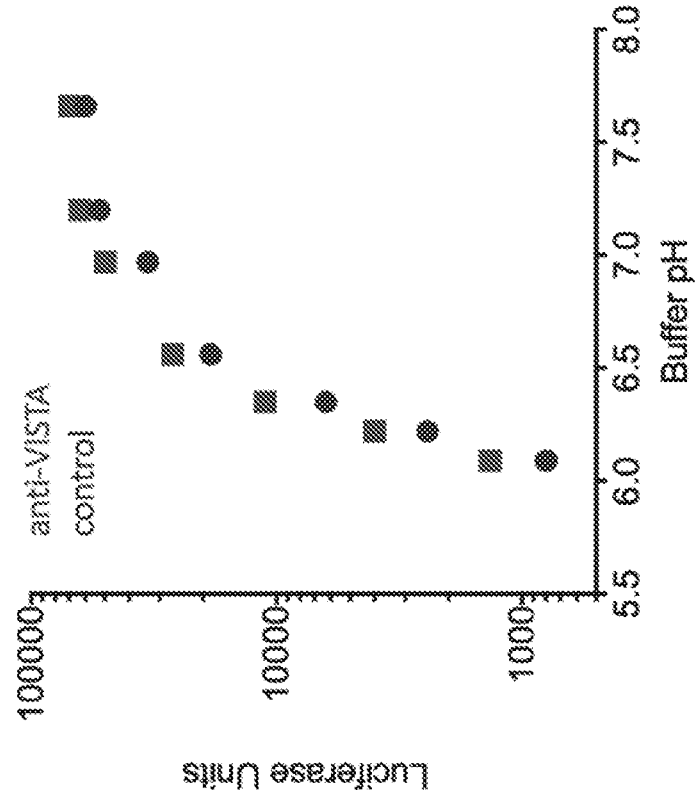


FIG. 5C

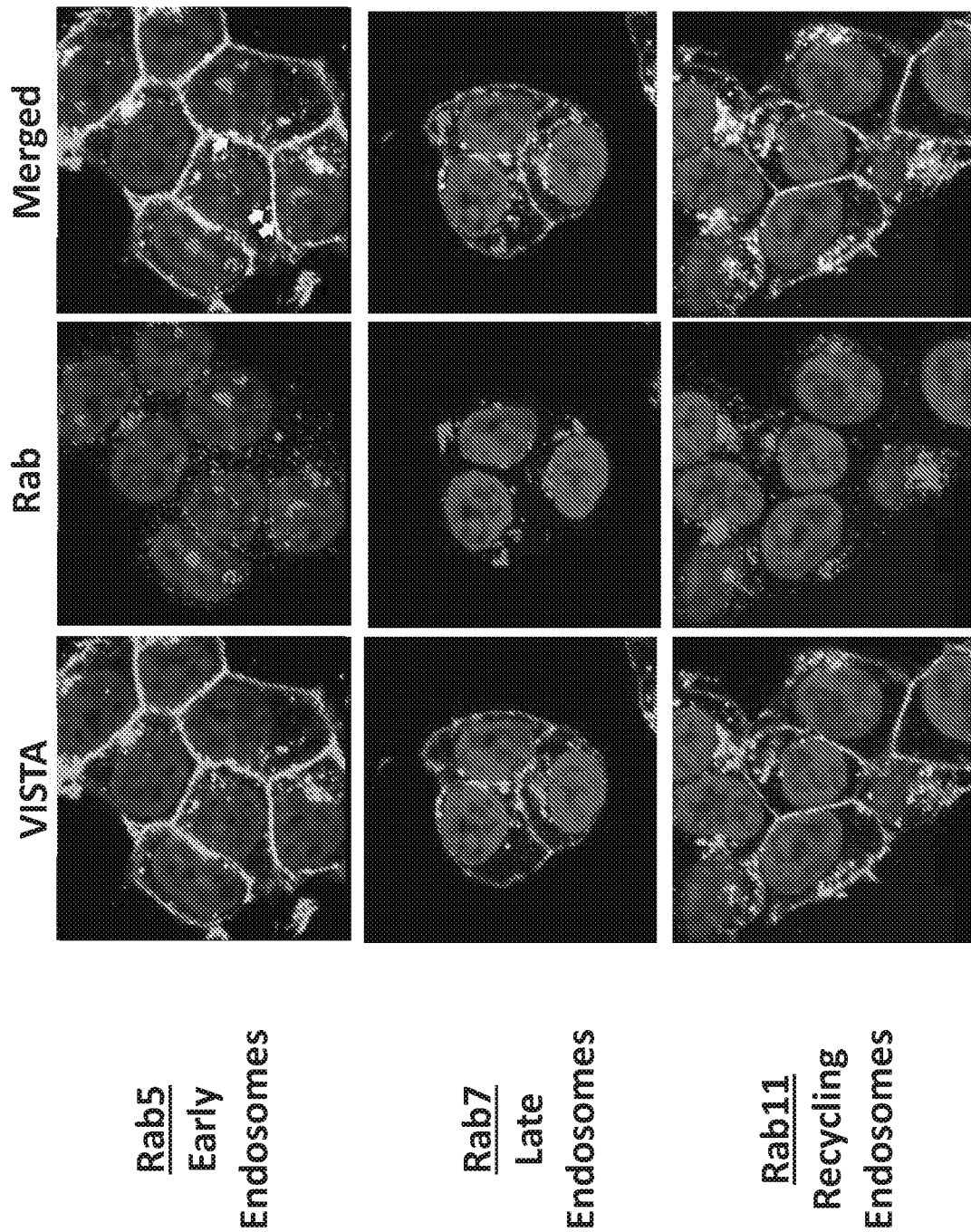


FIG. 6A

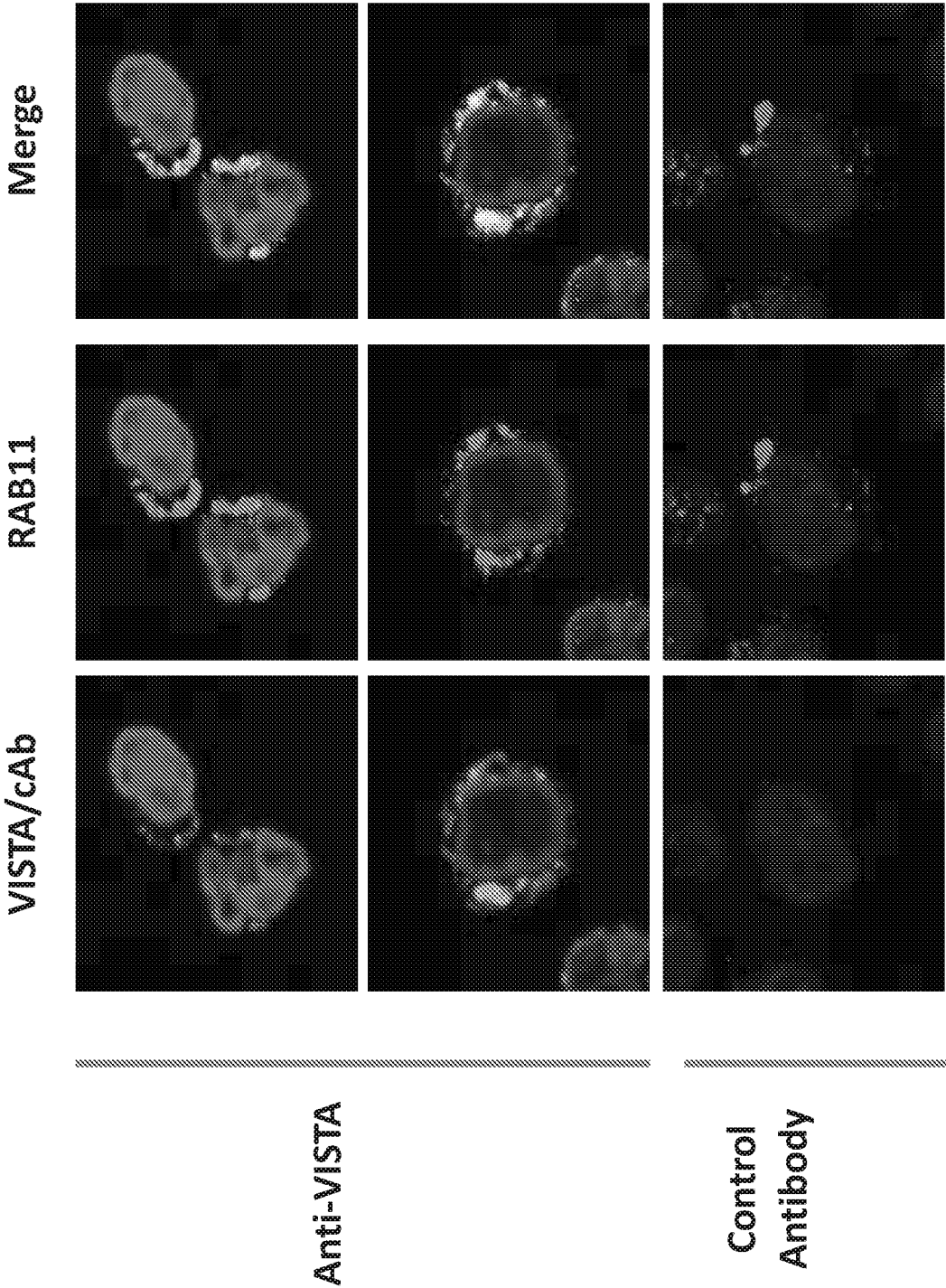


FIG. 6B

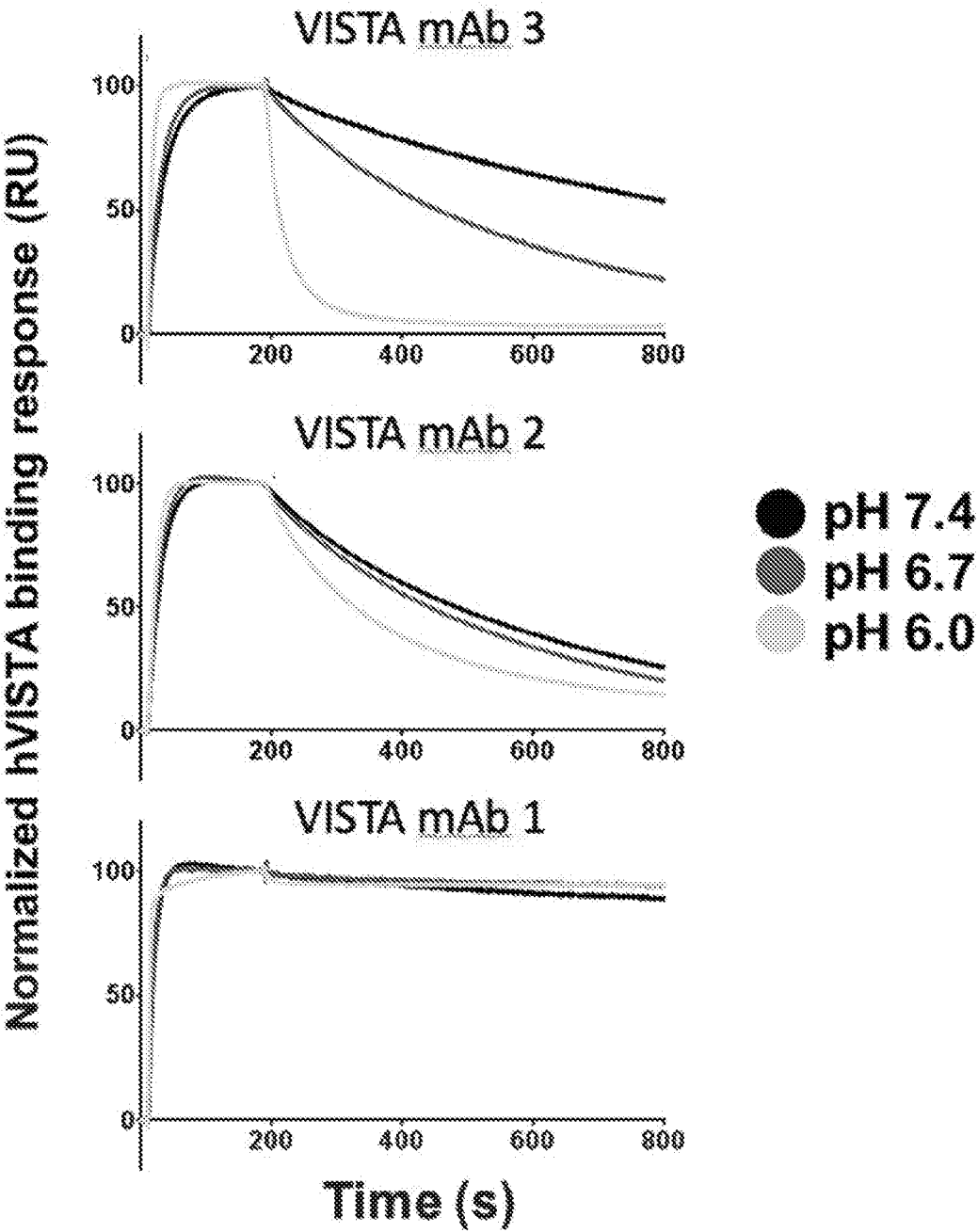


FIG. 6C

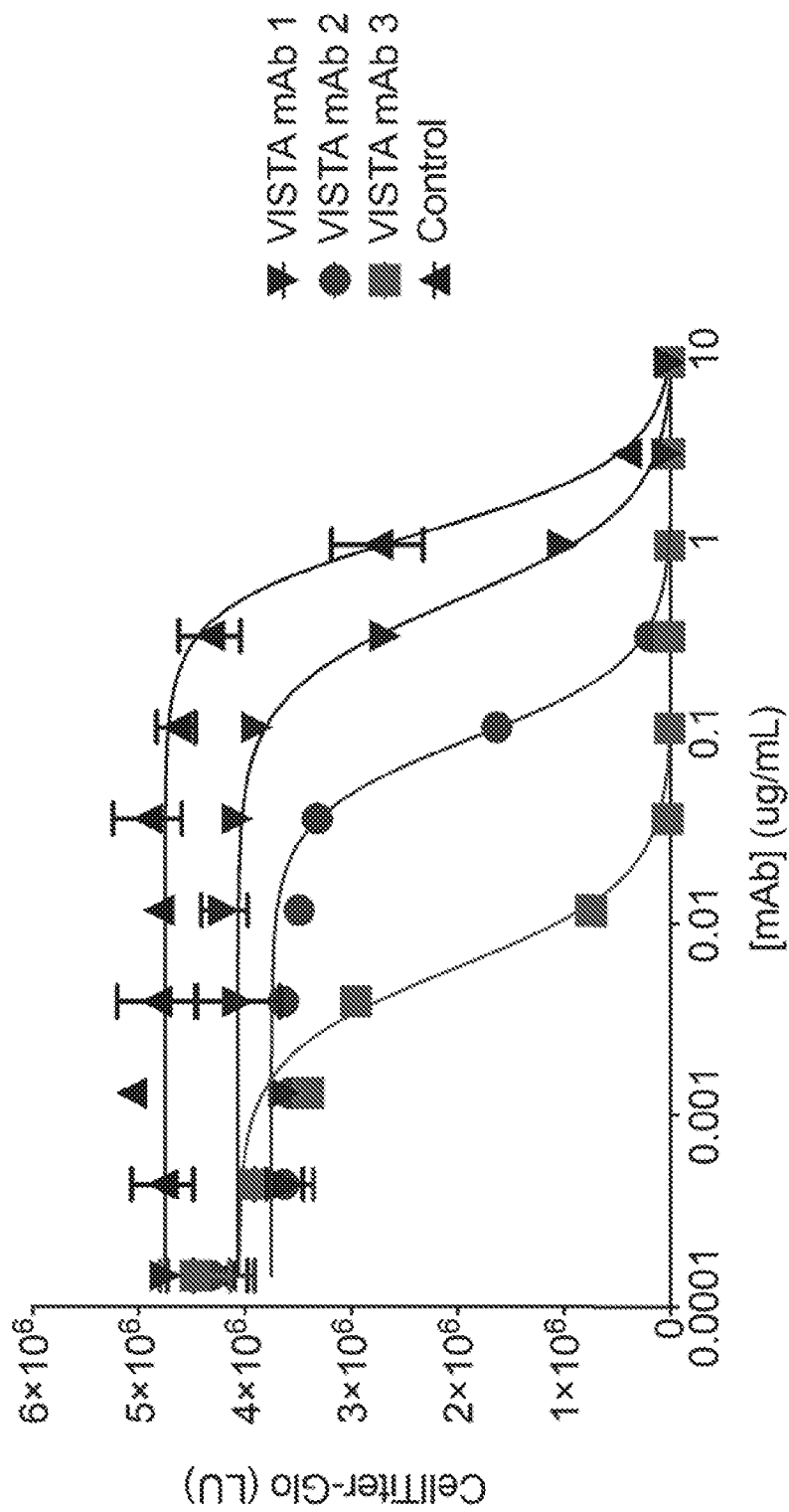


FIG. 6D

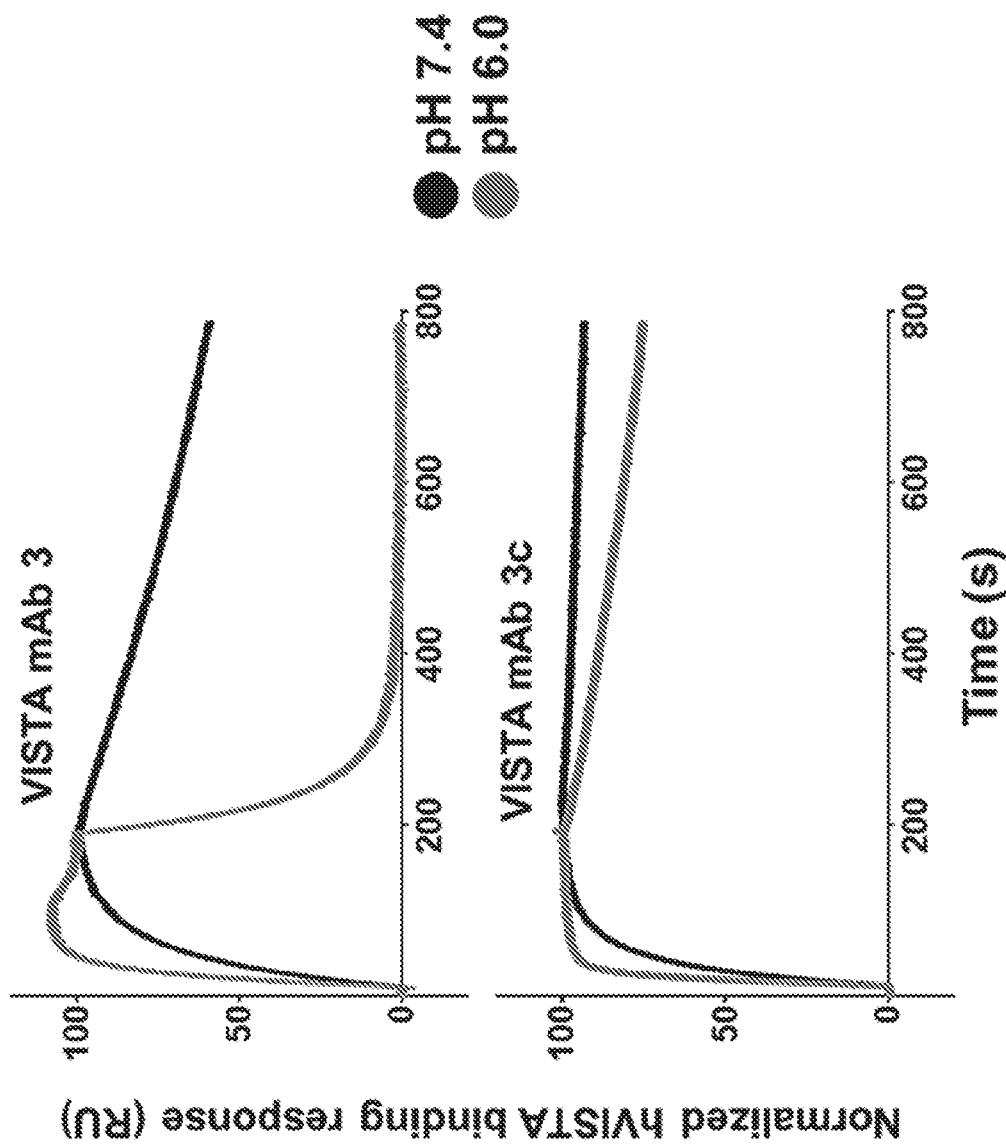


FIG. 6E

19/40

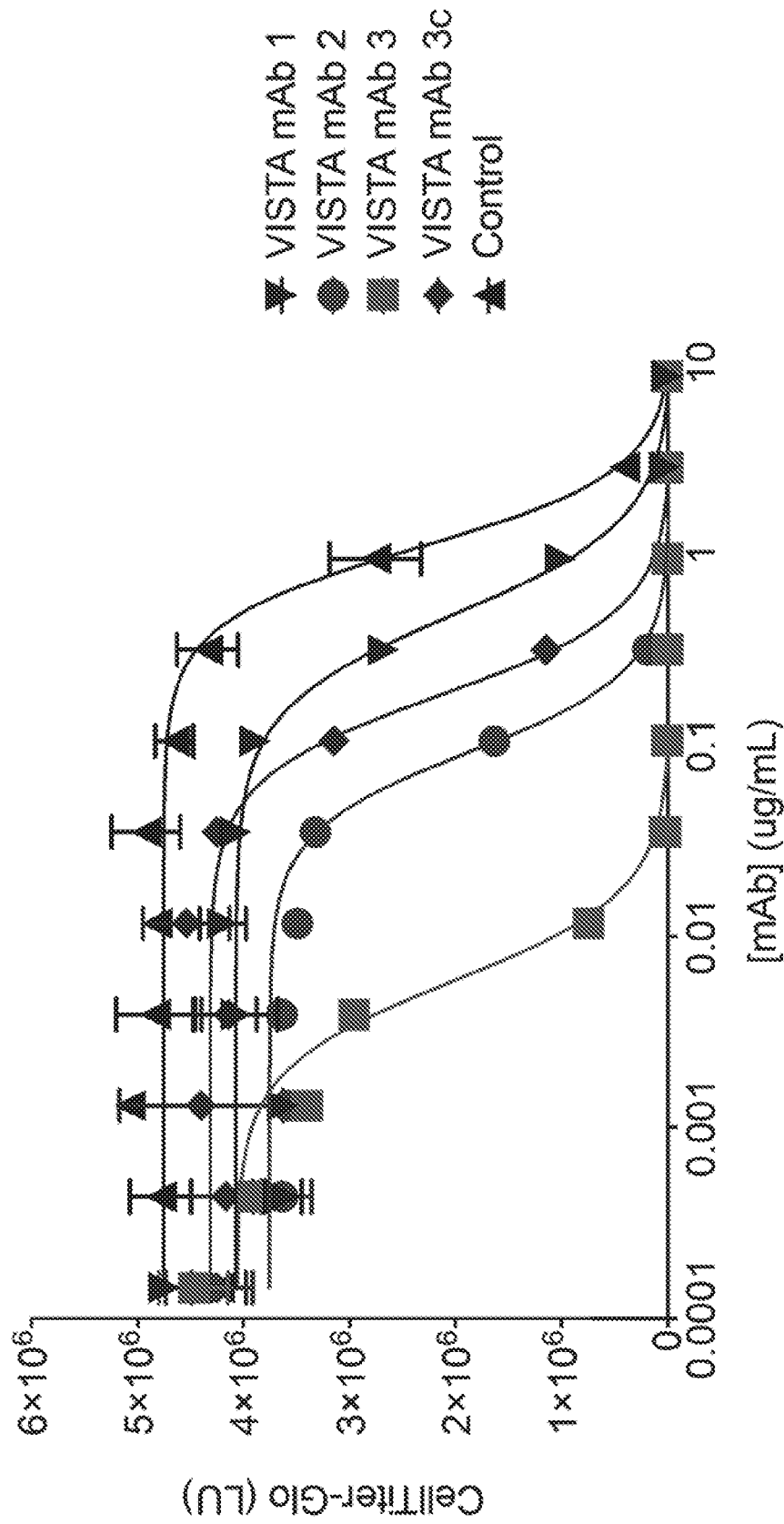


FIG. 6F

20/40

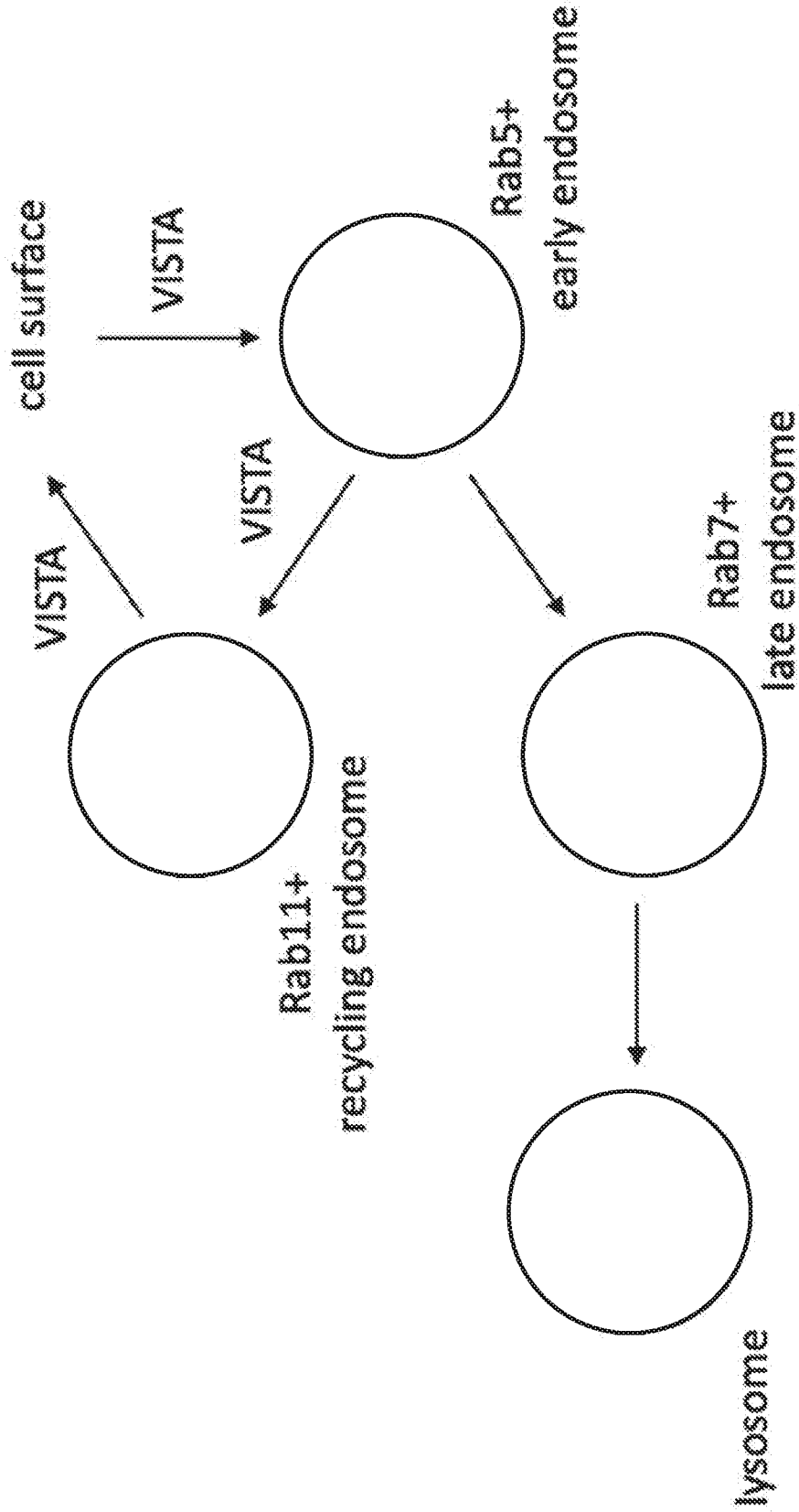


FIG. 6G

P1-061029 HCDR3 chip oligos	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV
	XPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV	EPQYSGGWIDAEIV

- 1. X = H, D, or E
- 2. Bracketed sequences were removed from synthesis to avoid introducing liabilities
- 3. A total of 647 unique sequences of P1-061029 HCDR3 with 1-2 mutations were synthesized

FIG. 7A

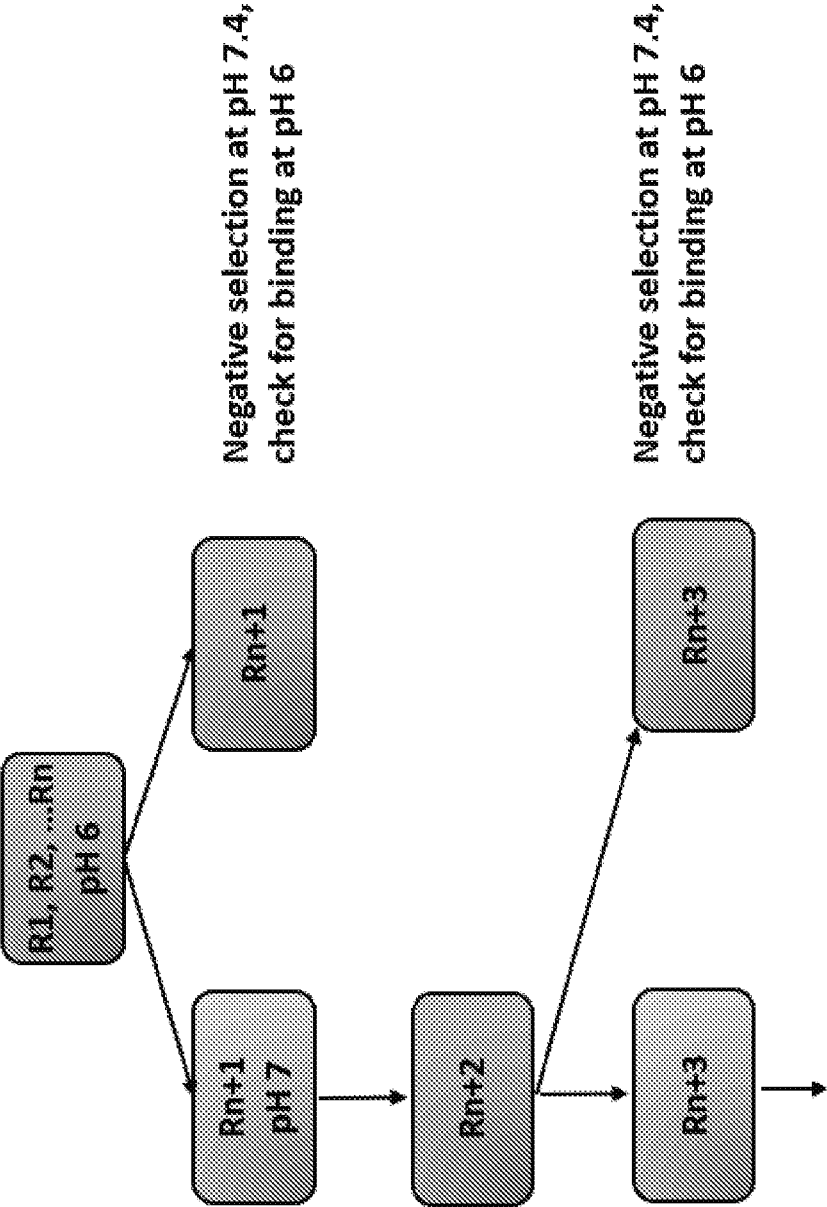


FIG. 7B

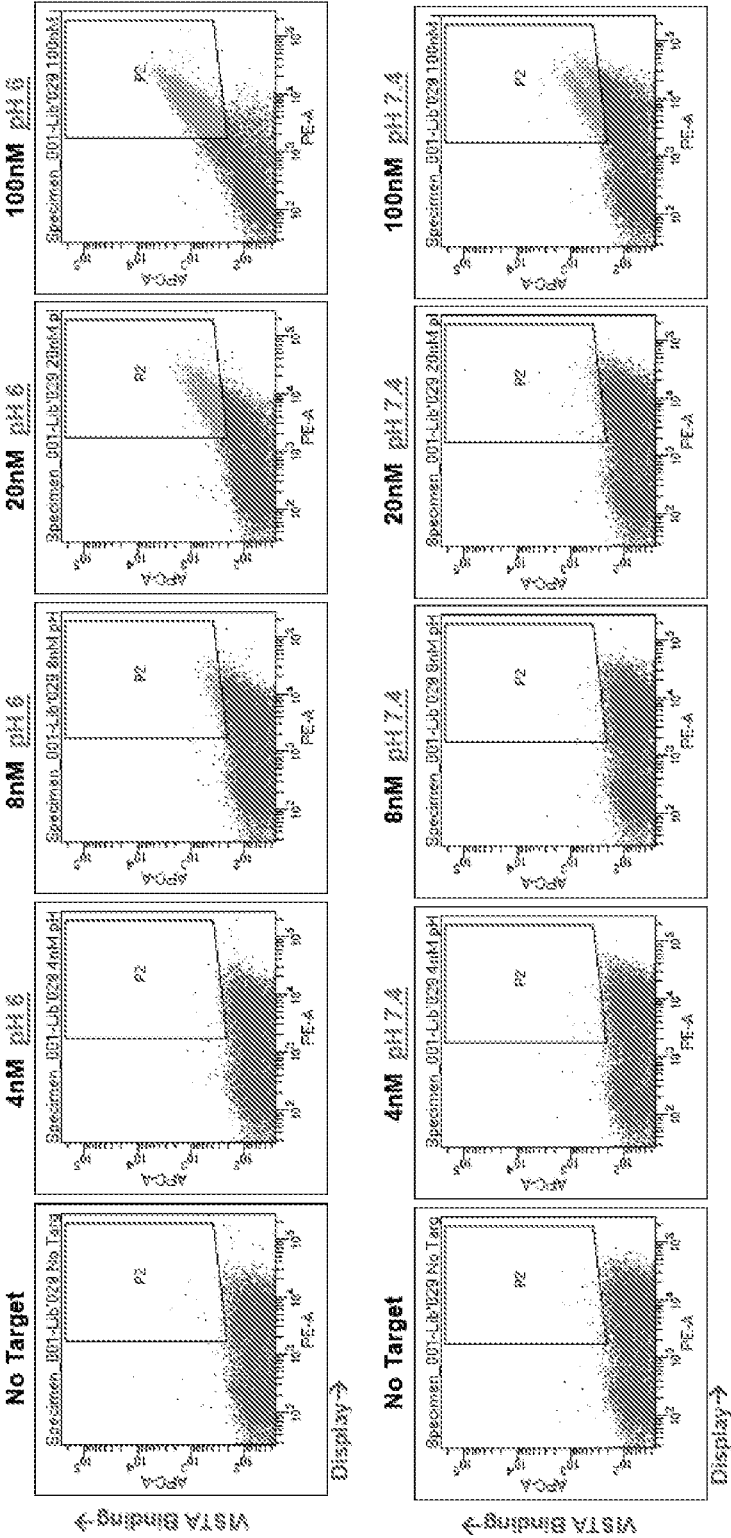


FIG. 7C

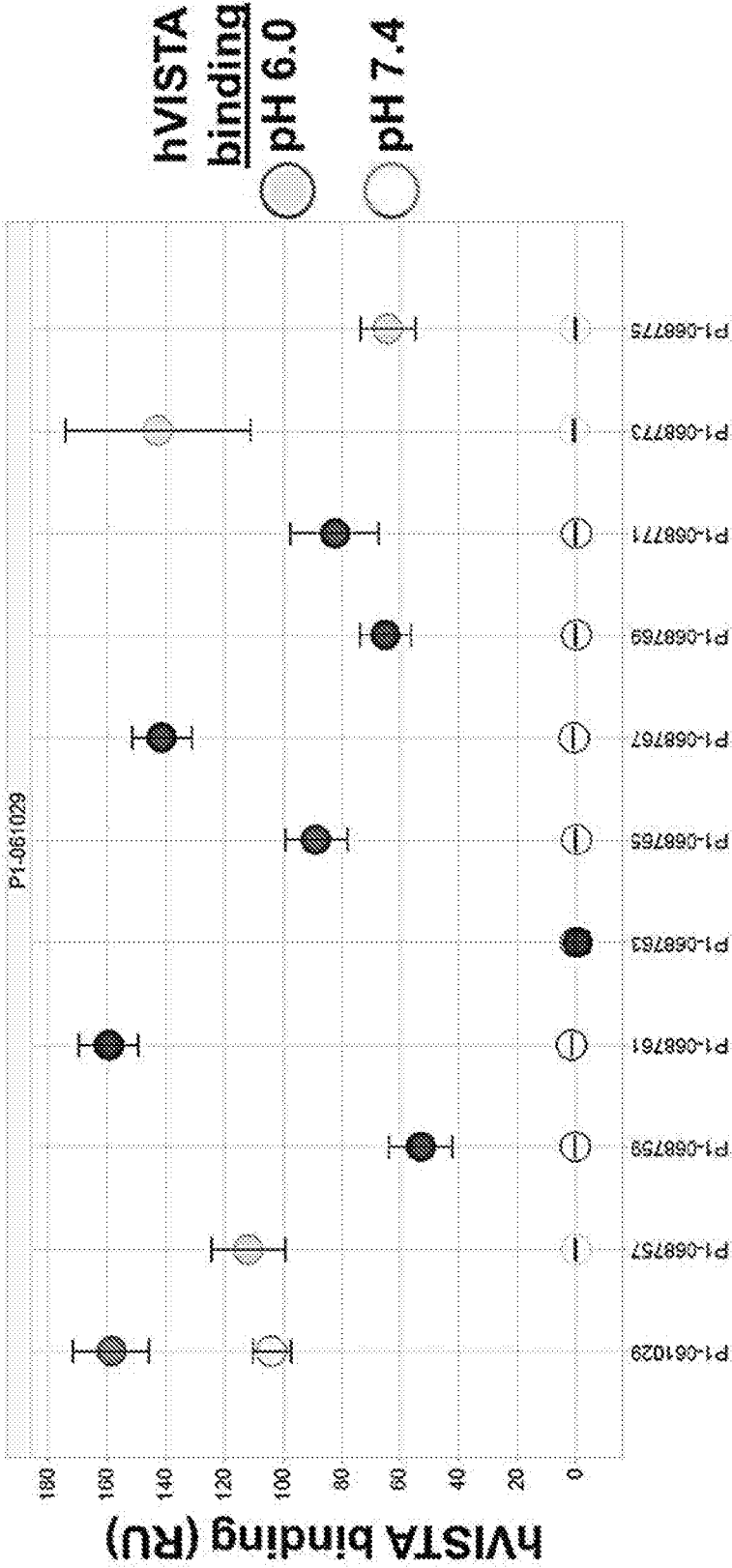


FIG. 7D

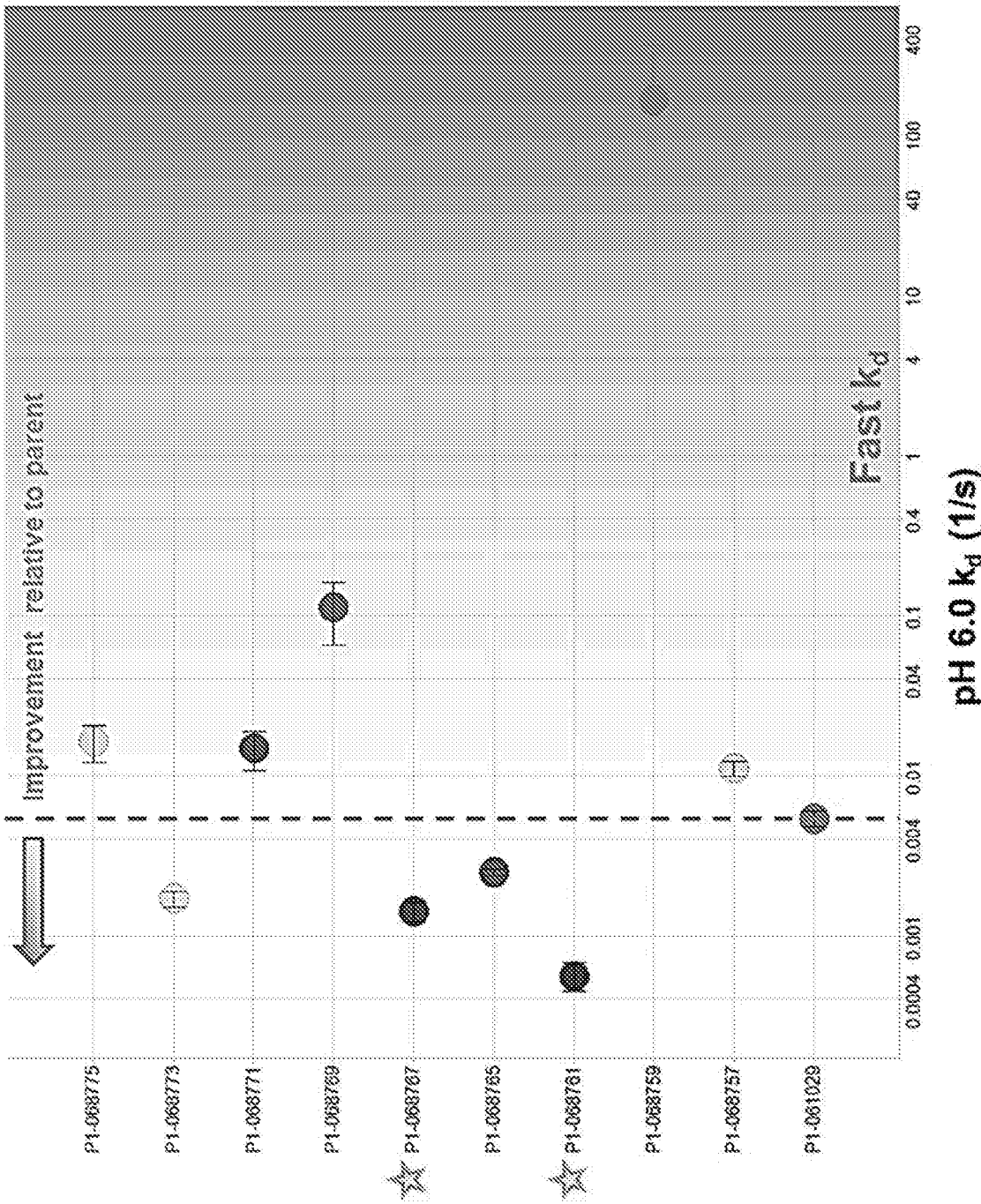


FIG. 7E

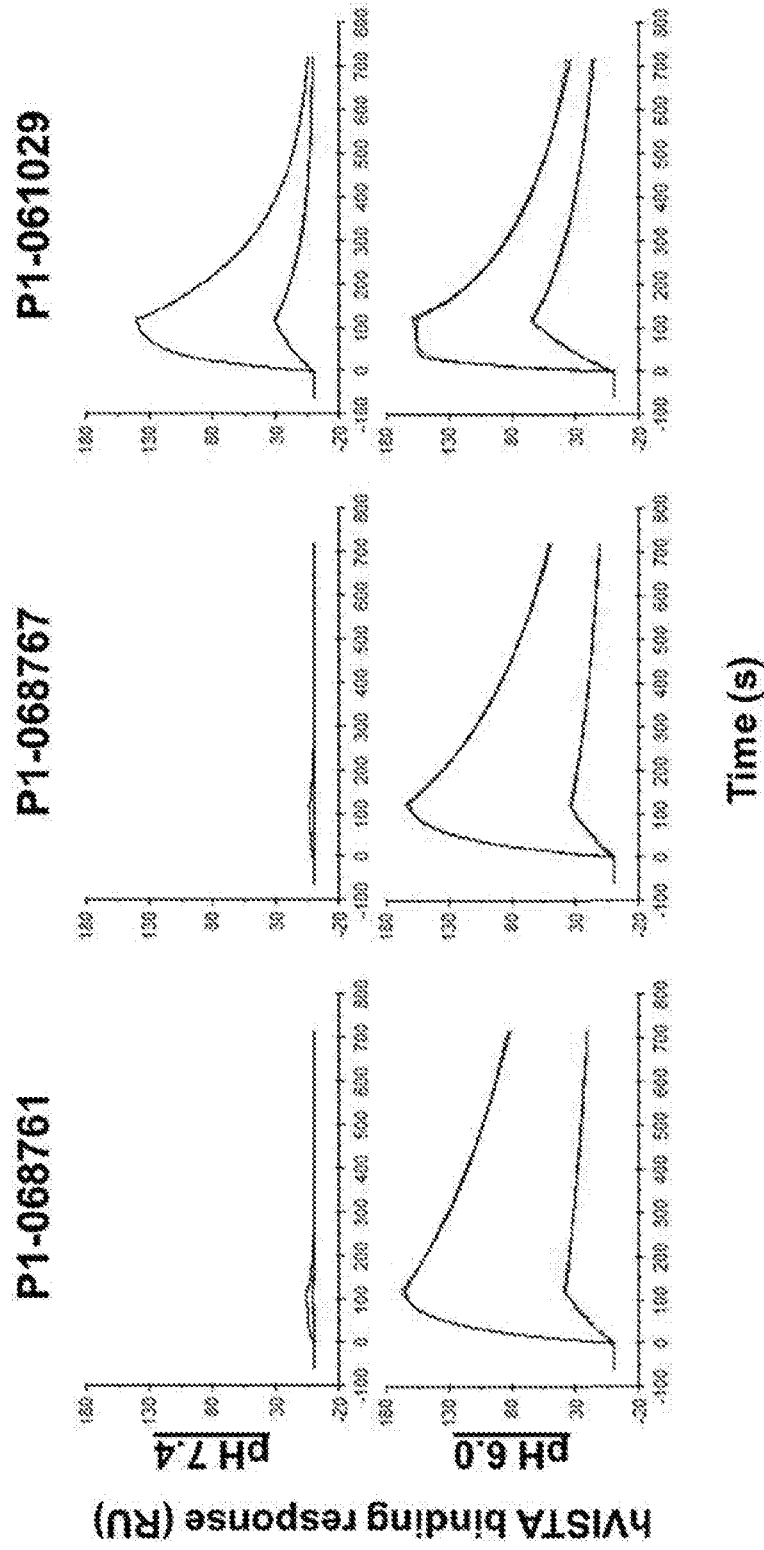


FIG. 7F

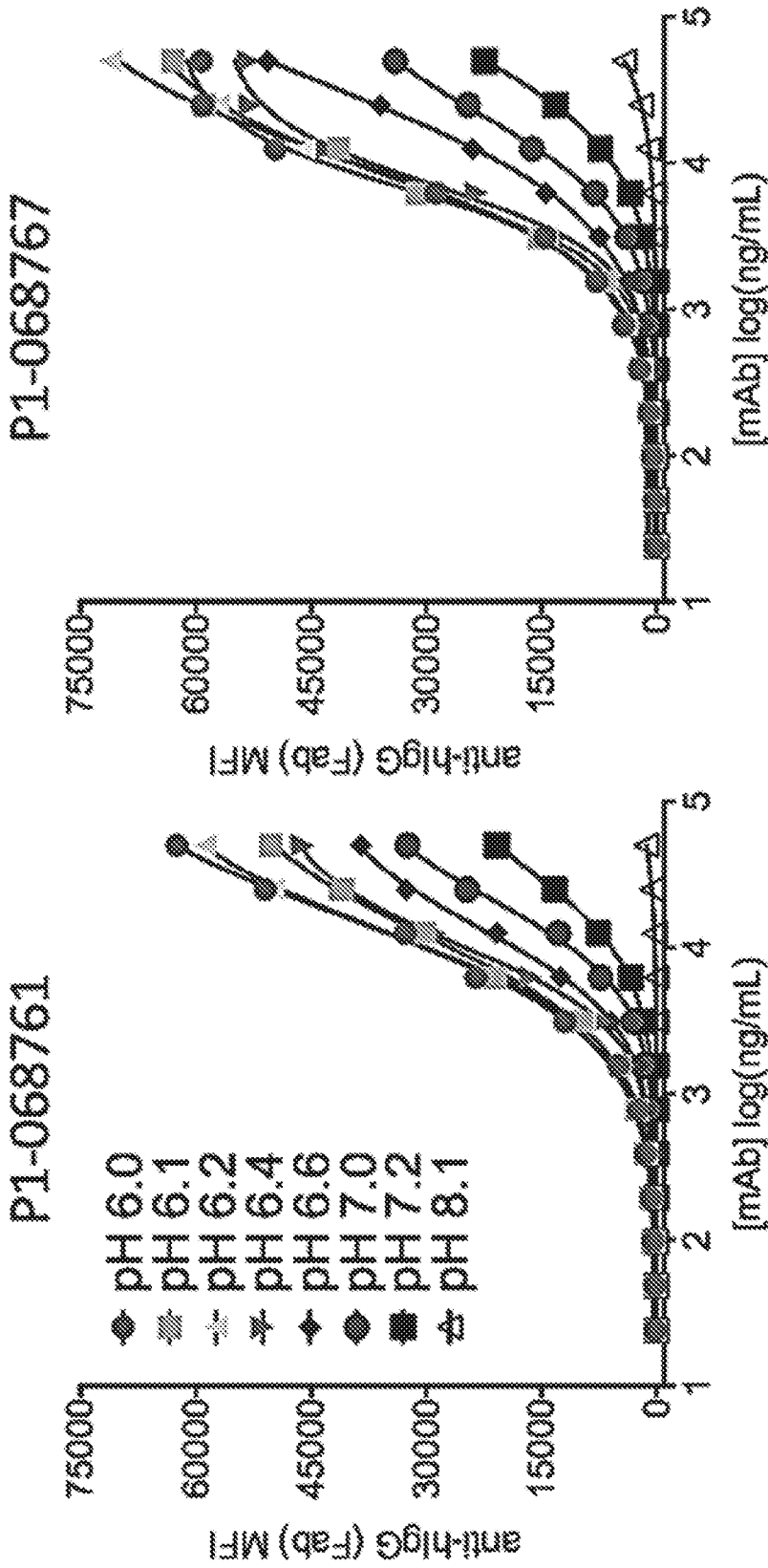


FIG. 8B

FIG. 8A

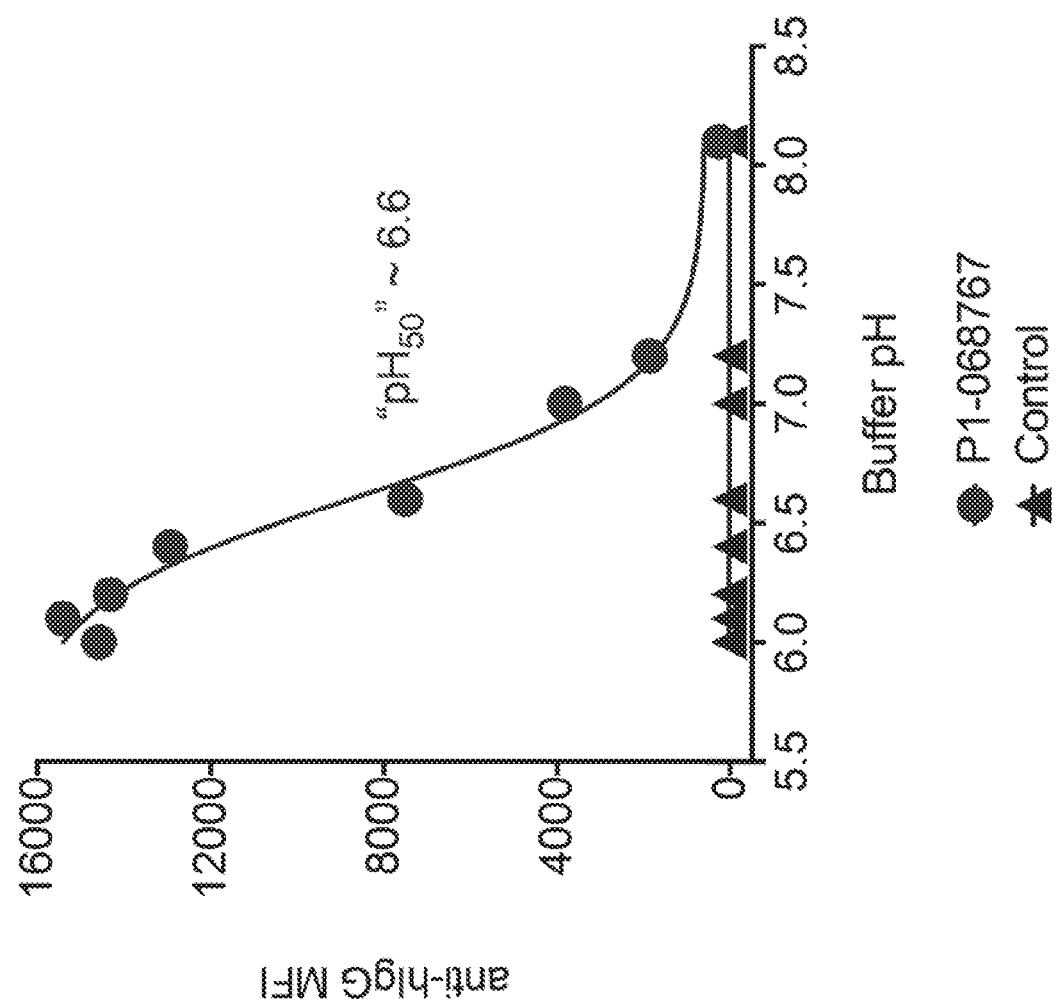


FIG. 8C

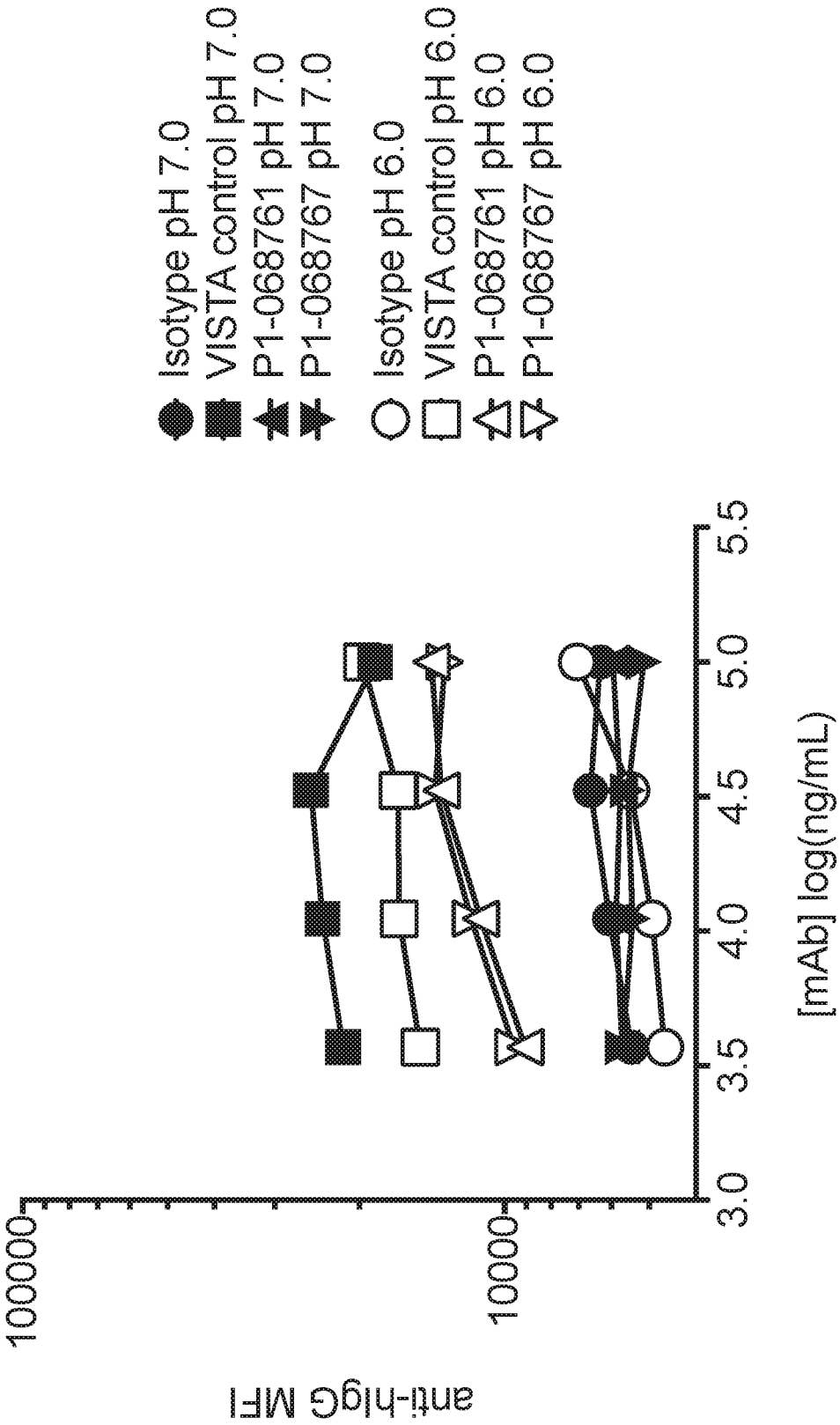


FIG. 8D

30/40

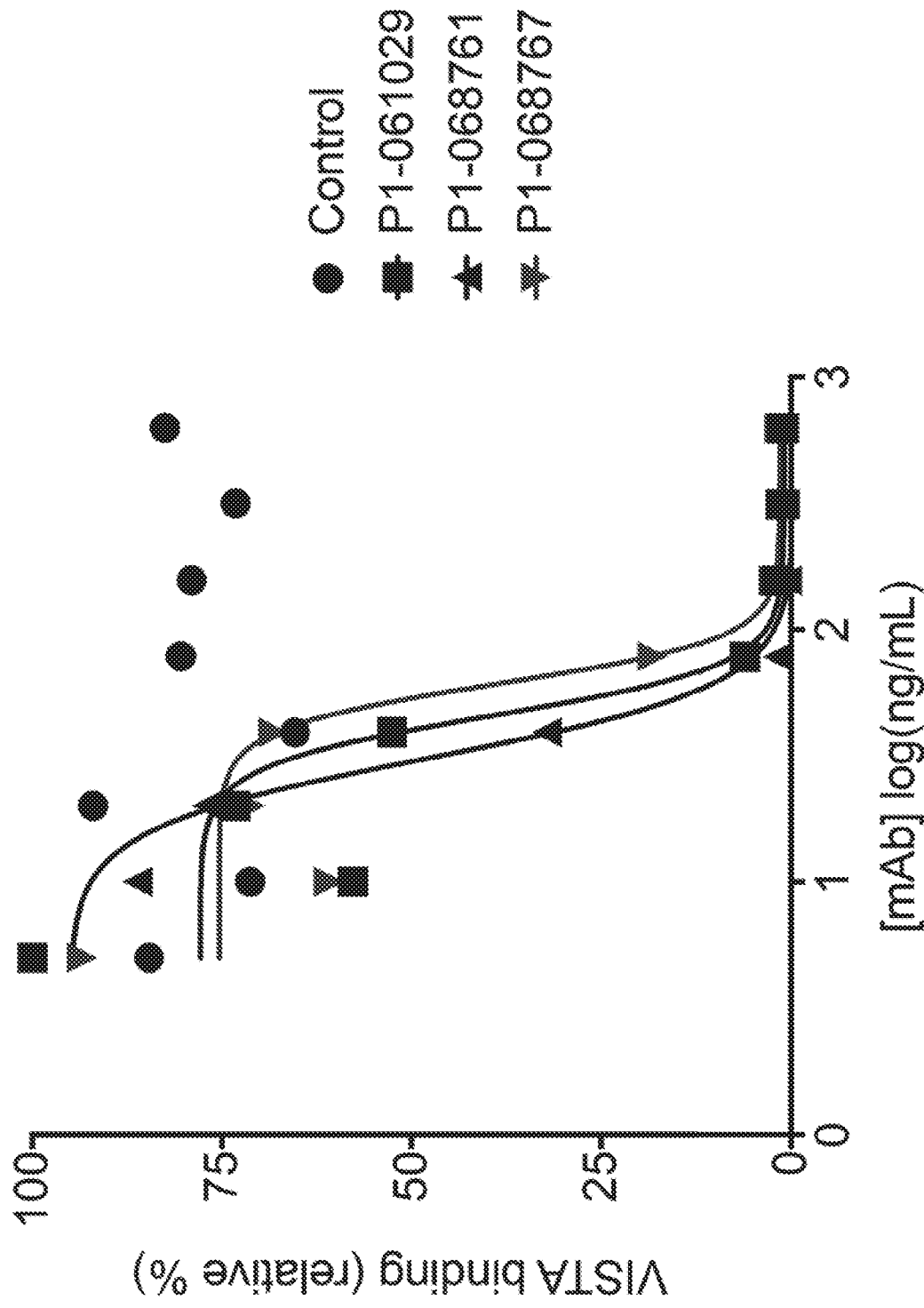


FIG. 8E

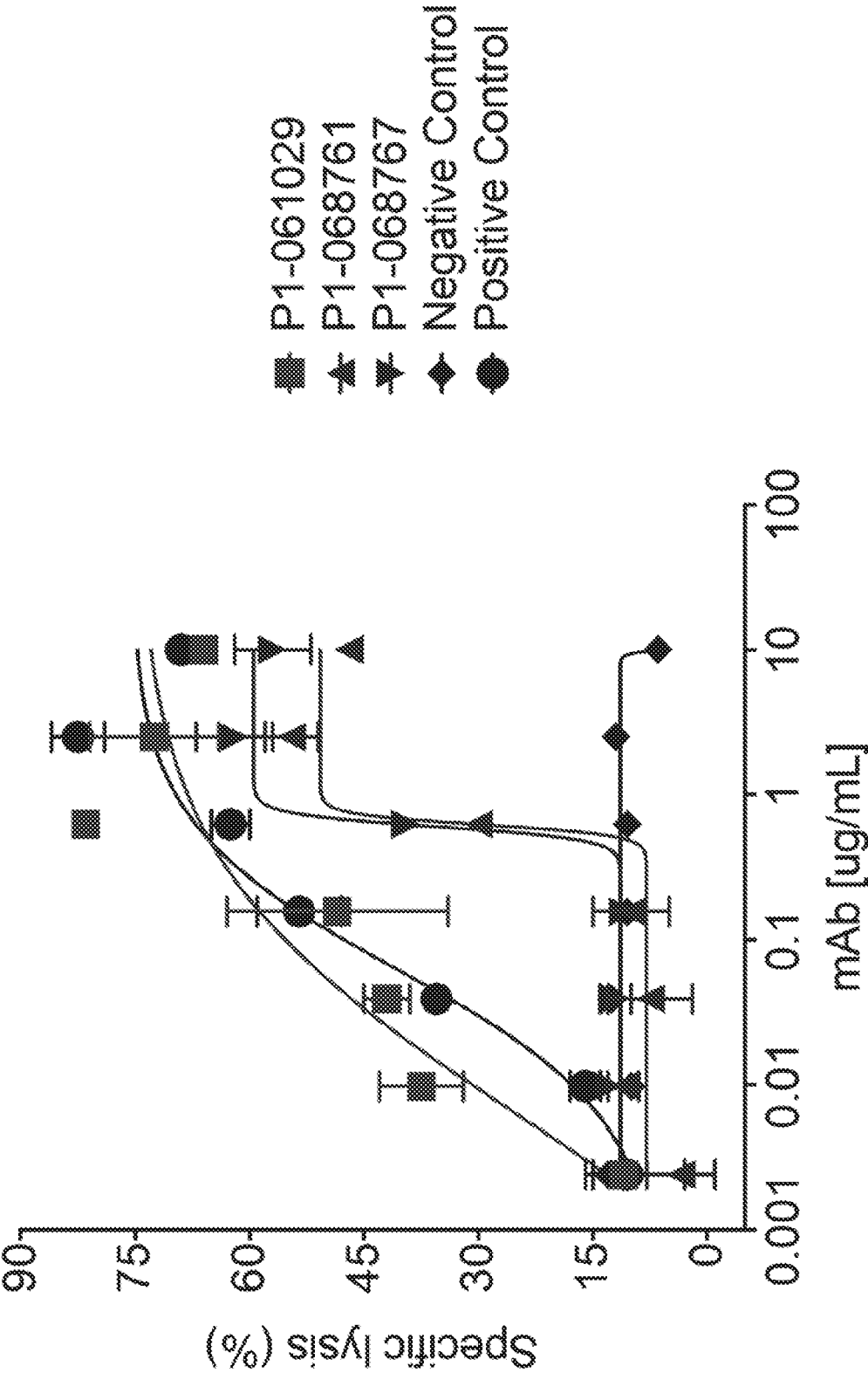


FIG. 8F

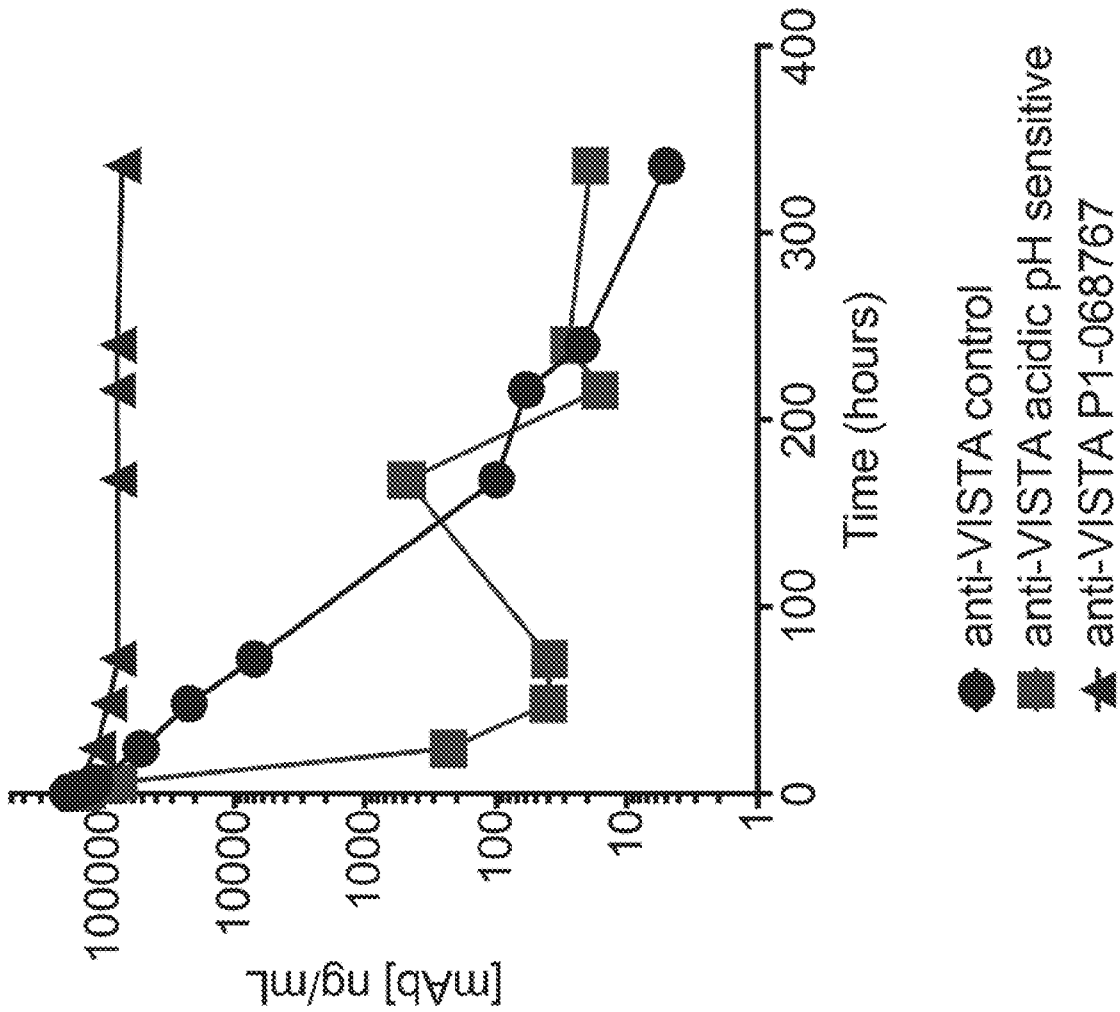


FIG. 9

Antibody	P1 ID	pH 7.4			pH 6.7			pH 6.0			HCDR1	HCDR2	HCDR3
		kd (1/s)	KD (M)	hVISTA %Rmax	kd (1/s)	KD (M)	hVISTA %Rmax	kd (1/s)	KD (M)	hVISTA %Rmax			
P1-068761_E100F	P1-070874	1.3E-03	2.0E-08	73.3	2.2E-04	1.3E-09	117.0	1.3E-04	1.9E-10	130.1	...E.E...	...E.E...	...H...
P1-068761_E55A_E100F	P1-070898	1.7E-03	1.4E-08	82.3	2.8E-04	1.5E-09	119.8	1.8E-04	2.6E-10	133.7	...E.E...	...E.E...	...H...
P1-068761_H100G_E100F	P1-070880	2.3E-03	4.1E-08	61.8	4.0E-04	2.3E-09	115.7	2.1E-04	2.3E-10	126.7	...E.E...	...E.E...	...H...
P1-068761_E30D_E100F	P1-070896	3.5E-03	4.8E-08	65.2	5.7E-04	3.7E-09	112.6	3.3E-04	4.3E-10	130.4	...E.E...	...E.E...	...H...
P1-068761_E56N_E100F	P1-070902	3.0E-03	6.5E-08	47.4	6.1E-04	6.5E-09	85.9	3.8E-04	1.4E-09	116.5	...E.E...	...E.E...	...H...
P1-068761_E32Y	P1-070866	2.5E-03	1.8E-08	102.5	6.4E-04	1.6E-09	126.9	4.1E-04	2.1E-10	123.1	...E.E...	...E.E...	...H...
P1-068761_E32Y_E55A	P1-070882	2.7E-03	2.3E-08	89.3	6.0E-04	2.0E-09	116.3	5.7E-04	3.1E-10	117.6	...E.E...	...E.E...	...H...
P1-068761_E32Y_E56N	P1-070884	3.2E-03	4.2E-08	73.7	8.3E-04	5.1E-09	104.4	7.0E-04	1.2E-09	115.8	...E.E...	...E.E...	...H...
P1-068761_E30D_E32Y	P1-070876	3.5E-03	2.1E-08	110.8	9.9E-04	2.0E-09	124.0	7.1E-04	2.4E-10	124.9	...E.E...	...E.E...	...H...
P1-068761_E32Y_H100G	P1-070886	5.5E-03	7.2E-08	68.0	1.3E-03	5.0E-09	113.4	9.2E-04	4.8E-10	120.4	...E.E...	...E.E...	...H...
P1-068761_E32Y_E100F	P1-070888	1.9E-03	3.7E-09	132.1	9.3E-04	1.1E-09	125.0	1.1E-03	2.5E-10	120.8	...E.E...	...E.E...	...H...
P1-068761	P1-068761	ND	ND	0.7	6.6E-03	7.1E-08	49.7	1.4E-03	2.6E-09	122.5	...E.E...	...E.E...	...H...
P1-068761_E55A	P1-070868	ND	ND	2.8	6.4E-03	1.2E-07	42.2	1.7E-03	3.4E-09	113.1	...E.E...	...E.E...	...H...
P1-068761_H100G	P1-070872	ND	ND	2.9	1.4E-02	2.3E-07	36.1	5.2E-03	4.4E-09	121.0	...E.E...	...E.E...	...H...
P1-068761_E56N	P1-070870	ND	ND	1.7	>1E-02	~8.2E-06	17.6	5.5E-03	1.9E-08	86.3	...E.E...	...E.E...	...H...
P1-068761_E55A_E56N	P1-070878	ND	ND	0.9	2.9E-02	2.1E-07	16.4	5.6E-03	2.1E-08	79.3	...E.E...	...E.E...	...H...
P1-068761_E30D	P1-070864	ND	ND	0.4	1.8E-02	3.7E-07	25.6	6.2E-03	8.3E-09	109.9	...E.E...	...E.E...	...H...
P1-068761_E30D_E55A	P1-070890	ND	ND	1.8	>1E-02	~1.1E-07	22.1	7.4E-03	1.2E-08	99.1	...E.E...	...E.E...	...H...
P1-061029	P1-061029	7.5E-03	3.2E-08	93.4	4.4E-03	1.1E-08	96.9	8.2E-03	4.5E-09	98.5	GFTIDDYAMR	GINWNSANIYADSVKG	VPGYSGGWIDAFDY
P1-068761_E56N_H100G	P1-070900	ND	ND	4.4	ND	ND	19.4	8.1E-03	1.0E-08	110.9	...E.E...	...E.E...	...H...
P1-068761_E30D_H100G	P1-070894	ND	ND	3.1	>1E-02	~8.1E-08	14.6	2.1E-02	1.5E-08	91.9	...E.E...	...E.E...	...H...
P1-068761_E30D_E56N	P1-070892	ND	ND	0.4	>1E-02	~3.2E-08	8.3	3.4E-02	5.0E-08	56.3	...E.E...	...E.E...	...H...

FIG. 10A

Antibody	P1 ID	pH 7.4			pH 6.7			pH 6.0			HCDR1	HCDR2	HCDR3
		kd (1/s)	KD (M)	hVISTA %Rmax	kd (1/s)	KD (M)	hVISTA %Rmax	kd (1/s)	KD (M)	hVISTA %Rmax			
P1-068767_D52N_D102V	P1-070930	6.7E-03	8.7E-08	60.0	1.8E-03	7.5E-09	101.0	1.2E-03	8.8E-10	114.6	...E...	...E...	...E...
P1-068767_D52N	P1-070906	%%	%%	3.9	6.7E-03	2.1E-07	38.9	1.5E-03	3.2E-09	116.6	...E...	...E...	...E...
P1-068767_D52N_E55A	P1-070916	%%	%%	2.3	5.5E-03	1.2E-07	33.7	1.5E-03	4.0E-09	112.0	...E...	...E...	...E...
P1-068767_E55A_D102V	P1-070932	1.0E-02	2.8E-07	35.4	3.1E-03	1.5E-08	85.7	2.0E-03	1.9E-09	111.3	...E...	...E...	...E...
P1-068767_D102V	P1-070912	9.2E-03	1.7E-07	44.7	2.8E-03	1.4E-08	98.5	2.0E-03	1.3E-09	111.9	...E...	...E...	...E...
P1-068767_E55A	P1-070908	%%	%%	2.8	8.5E-03	3.8E-07	26.8	2.3E-03	5.7E-09	108.0	...E...	...E...	...E...
P1-068767_E30D_D52N	P1-070914	%%	%%	4.5	1.1E-02	1.2E-07	35.8	2.3E-03	5.8E-09	105.7	...E...	...E...	...E...
P1-068767	P1-068767	%%	%%	1.9	7.2E-03	1.8E-07	31.1	2.4E-03	5.2E-09	111.7	...E...	...E...	...E...
P1-068767_E30D_D102V	P1-070926	1.1E-02	2.2E-07	40.8	3.5E-03	1.7E-08	95.7	3.0E-03	2.1E-09	114.8	...E...	...E...	...E...
P1-068767_E30D	P1-070904	%%	%%	5.8	%%	%%	21.0	3.2E-03	9.6E-09	99.8	...E...	...E...	...E...
P1-068767_E30D_E55A	P1-070922	%%	%%	3.5	%%	%%	22.6	3.5E-03	8.3E-09	108.8	...E...	...E...	...E...
P1-061029	P1-061029	7.5E-03	3.2E-08	93.4	4.4E-03	1.1E-08	96.9	8.2E-03	4.5E-09	98.5	GFTLDYAMH	GLMNSANI GYADSVKG	VPGYSGWIDAFDV
P1-068767_E100F_D102V	P1-070920	8.3E-03	4.1E-08	86.8	4.7E-03	1.1E-08	103.4	8.2E-03	2.7E-09	103.4	...E...	...E...	...E...
P1-068767_E55A_E100F	P1-070918	%%	%%	1.8	%%	%%	8.6	1.3E-02	6.2E-08	42.5	...E...	...E...	...E...
P1-068767_D52N_E100F	P1-070928	%%	%%	9.4	>1E-02	~3.9E-08	22.7	6.6E-02	2.0E-08	64.0	...E...	...E...	...E...
P1-068767_E100F	P1-070910	%%	%%	1.8	2.6E-02	5.2E-06	9.6	>1E-02	~4.2E-08	49.4	...E...	...E...	...E...
P1-068767_E30D_E100F	P1-070924	%%	%%	1.0	2.7E-02	1.0E-05	7.0	>1E-02	~8.2E-08	32.9	...E...	...E...	...E...

FIG. 10B

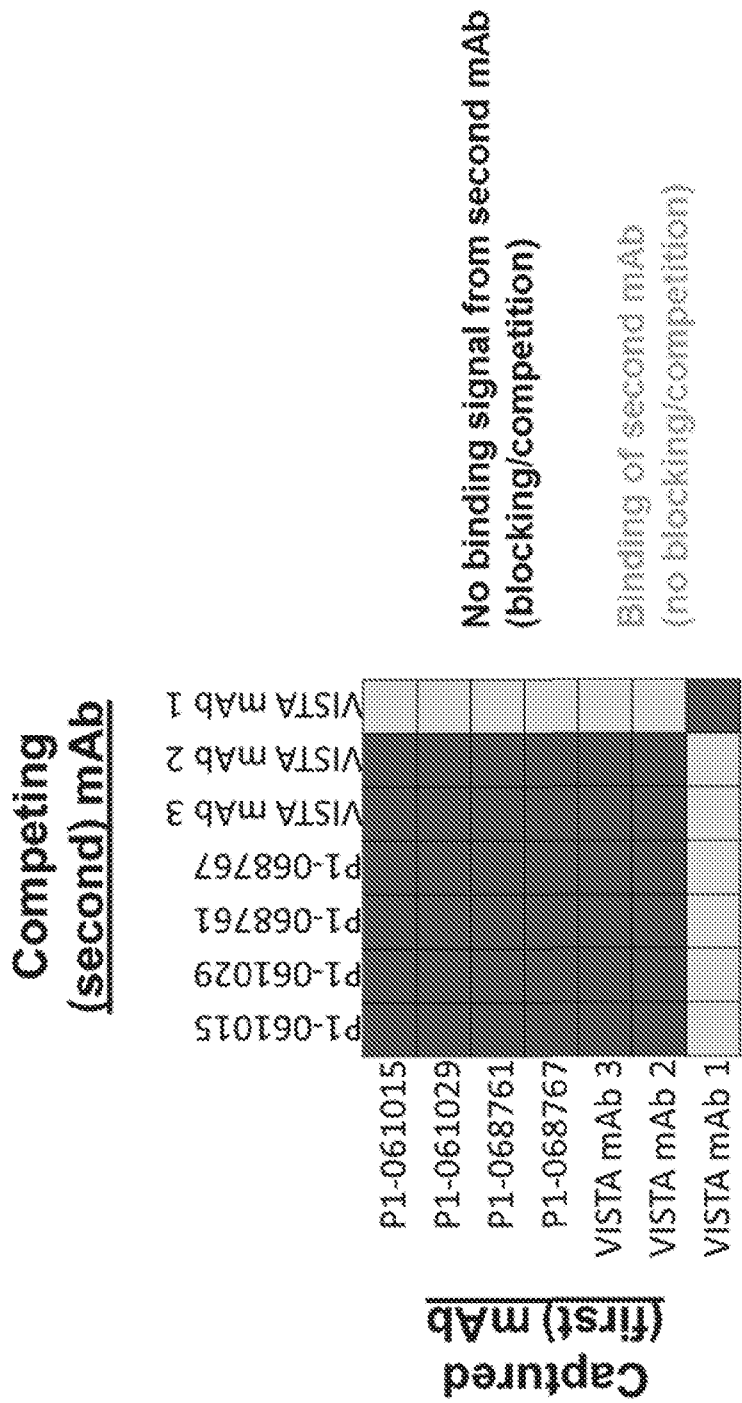


FIG. 11A

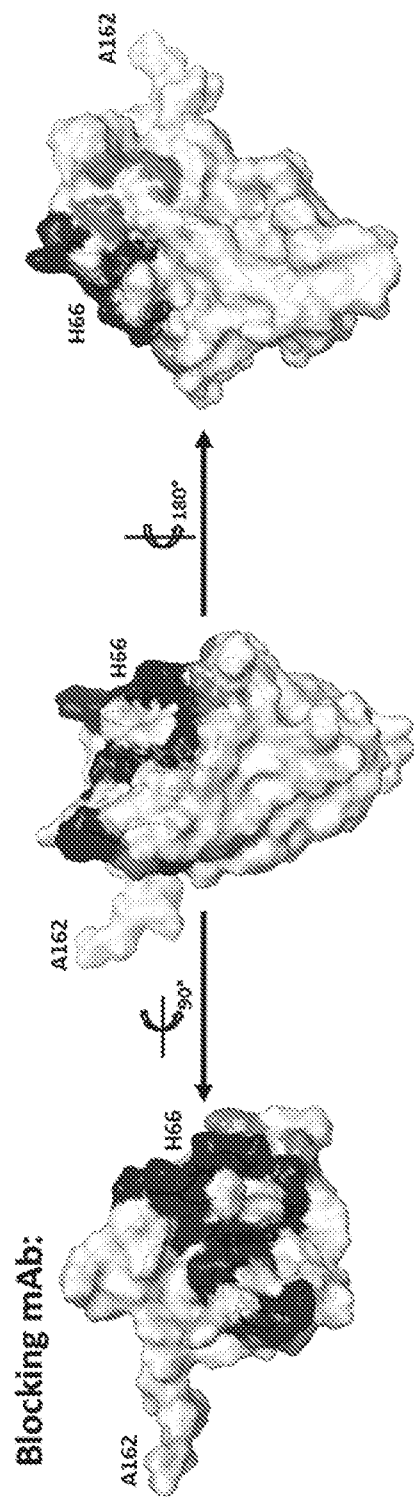
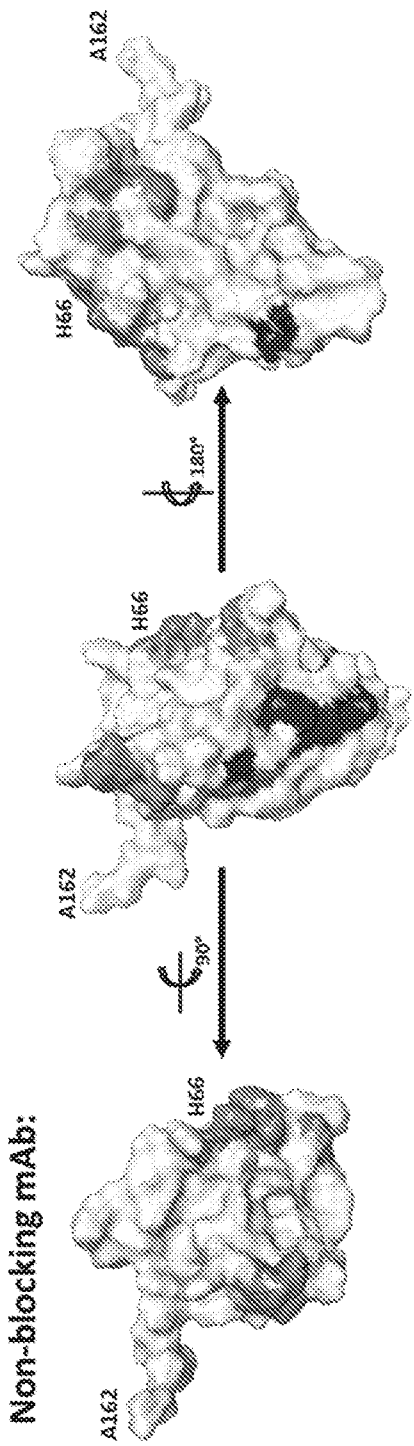


FIG. 12A

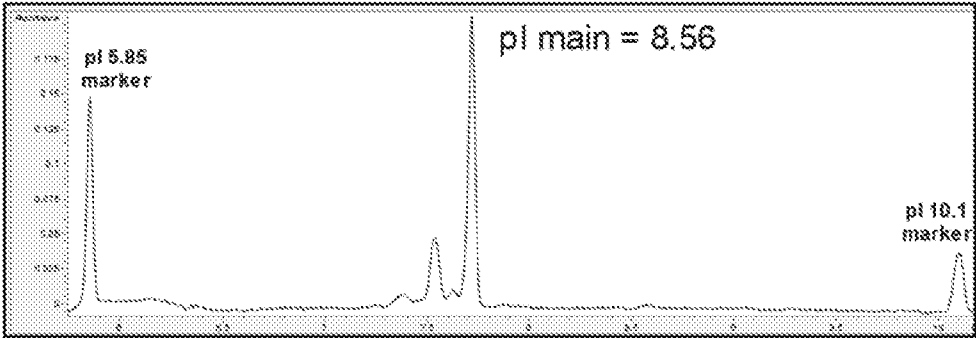


FIG. 12B

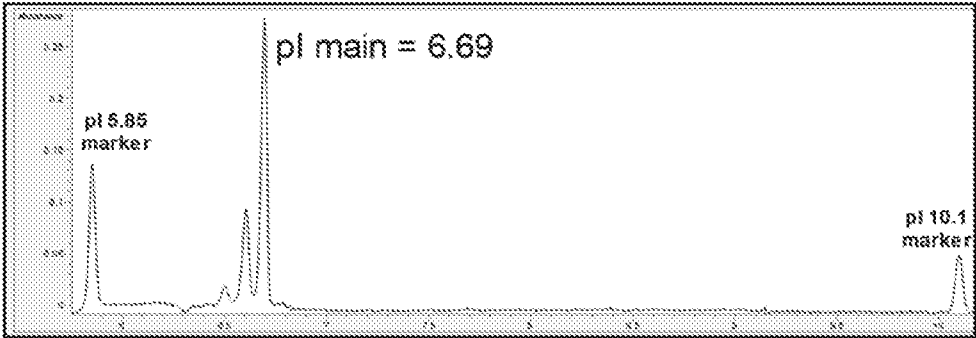
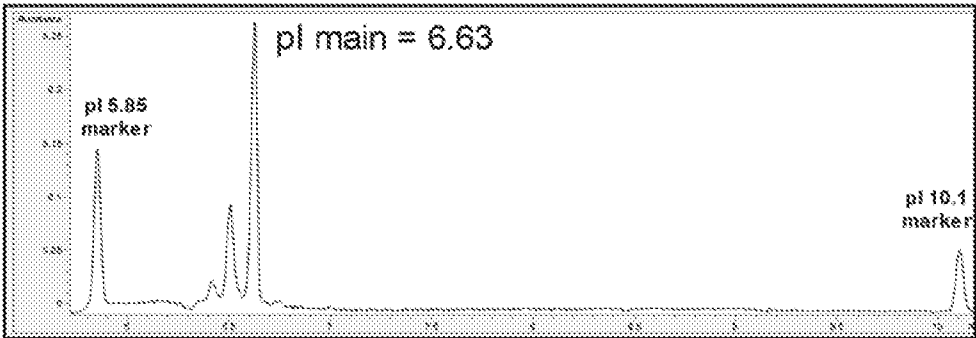


FIG. 12C



P1-061029	EVQLVESGGGLVQPEKSLRLSIAASGFTIDDDYAMHWVRQAPGKGLVWVSGINWNSANTIGYASVKGERFTISPDNAKNSILQMNLSIRTEETALYCAKVPGSYGGWIDAFDVWGQGTMTVYSS		10	20	30	40	50	60	70	80	90	100	110	120
P1-068757		E.E.....												
P1-068759		E.E.....												
P1-068761		E.E.....												
P1-068763		E.....												
P1-068765		DE.....												
P1-068767		E.....												
P1-068769		E.E.....												
P1-068771		E.E.....												
P1-068773		E.....												
P1-068775		E.E.....												
P1-069059		E.....												
P1-069061		E.....												
P1-069063		E.....												
P1-069065		E.E.....												
P1-069067														
P1-069069														
P1-069071		E.E.....												
P1-069073		E.E.....												
P1-069075		E.....												
P1-069077		E.E.....												
P1-069293		E.E.....												
P1-069298		E.....												
P1-069302														
P1-069312		E.E.....												
P1-069309		E.....												
P1-069307														
P1-070864		E.....												
P1-070866		E.....												
P1-070868		E.E.....												
P1-070870		E.E.....												
P1-070872		E.E.....												
P1-070874		E.E.....												
P1-070876														
P1-070878		E.E.....												
P1-070880		E.E.....												
P1-070882		E.....												
P1-070884		E.....												
P1-070886		E.....												
P1-070888		E.....												
P1-070890														

FIG. 13A

P1-070892	E.....	E.....	E.....	E.....	E.....
P1-070894	E.....	E.....	E.....	E.....	E.....
P1-070896	E.....	E.....	E.....	E.....	E.....
P1-070898	E.....	E.....	E.....	E.....	E.....
P1-070900	E.....	E.....	E.....	E.....	E.....
P1-070902	E.....	E.....	E.....	E.....	E.....
P1-070904	E.....	E.....	E.....	E.....	E.....
P1-070906	E.....	E.....	E.....	E.....	E.....
P1-070908	E.....	E.....	E.....	E.....	E.....
P1-070910	E.....	E.....	E.....	E.....	E.....
P1-070912	E.....	E.....	E.....	E.....	E.....
P1-070914	E.....	E.....	E.....	E.....	E.....
P1-070916	E.....	E.....	E.....	E.....	E.....
P1-070918	E.....	E.....	E.....	E.....	E.....
P1-070920	E.....	E.....	E.....	E.....	E.....
P1-070922	E.....	E.....	E.....	E.....	E.....
P1-070924	E.....	E.....	E.....	E.....	E.....
P1-070926	E.....	E.....	E.....	E.....	E.....
P1-070928	E.....	E.....	E.....	E.....	E.....
P1-070930	E.....	E.....	E.....	E.....	E.....
P1-070932	E.....	E.....	E.....	E.....	E.....

FIG. 13A (cont.)

