

Figure 1A

Immunogen	Mouse (strain, #)	EC50 hZIP12-ECD-GST	EC50 MV(-)-hZIP12
hZIP12-ECD-Fc	BALB/c 98	203446	9296
	BALB/c 99	76807	2159
	C3H 100	42903	1846
	C3H 101	46062	1516
	AIP 102	106425	3140
	AIP 103	137720	3109
	AIP 104	95828	3989
hZIP12 DNA	BALB/c 105	577.5	na
	BALB/c 106	2347	217
	C57BL/6 107	107.3	na
	C57BL/6 108	84.91	na
	AIP 109	21.06	na
	AIP 110	645.4	na
	AIP 111	50.93	na
hZIP12-VLPs	BALB/c 112	5214	97.08
	BALB/c 113	9903	136.7
	C3H 114	35759	407.2
	C3H 115	12304	245.7
MV(+)-hZIP12 1E8	C57BL/6 116	793.3	na
	C57BL/6 117	472.8	na
	C57BL/6 118	420.3	na
MV(+)-hZIP12 2G3	C57BL/6 119	806.6	na
	C57BL/6 120	198.2	na
	C57BL/6 121	122.1	na
MV(+)-hZIP12 4D6	C57BL/6 122	0.05397	na
	C57BL/6 123	18.06	na
	C57BL/6 124	666.9	na

Figure 1B

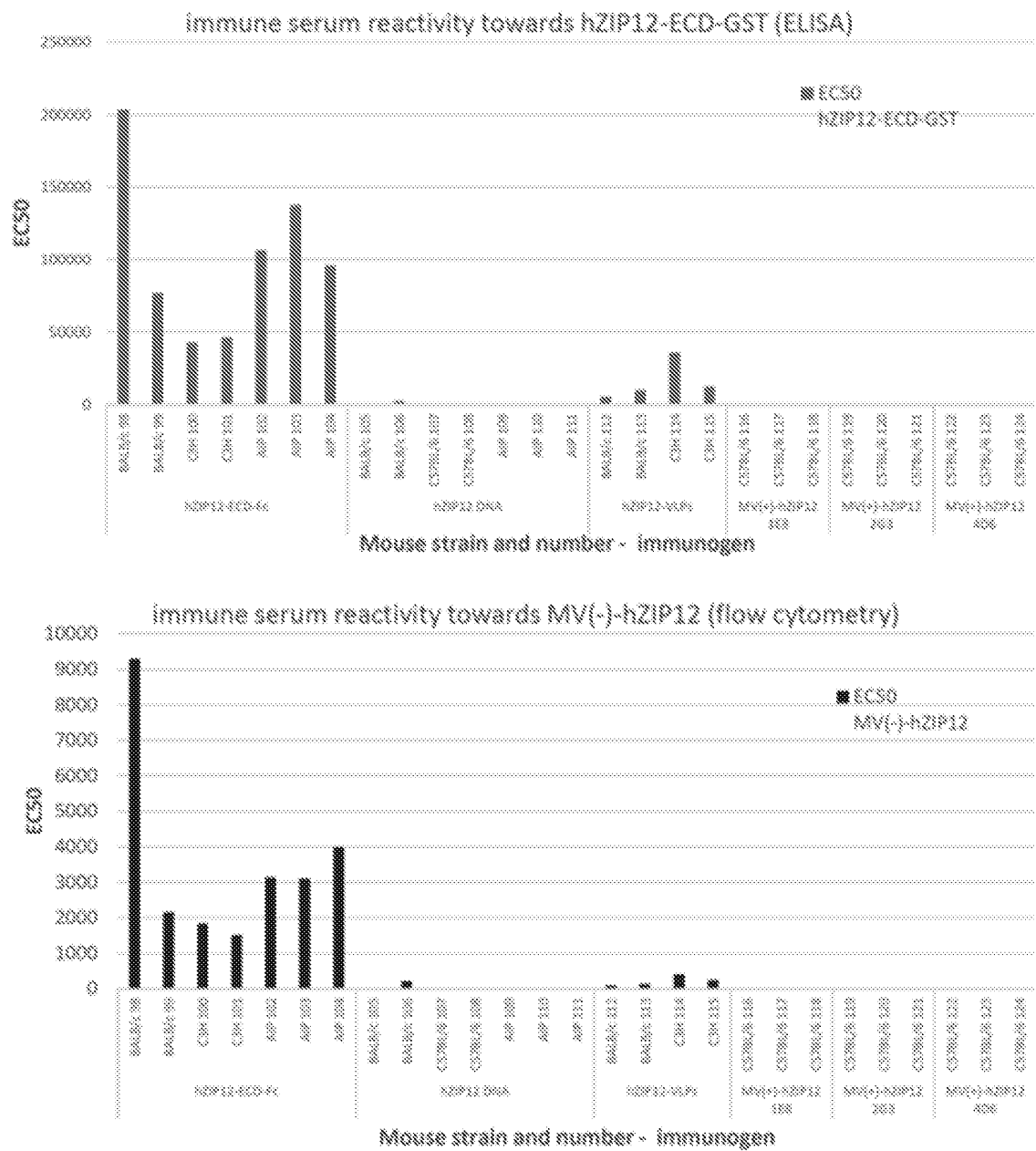


Figure 2

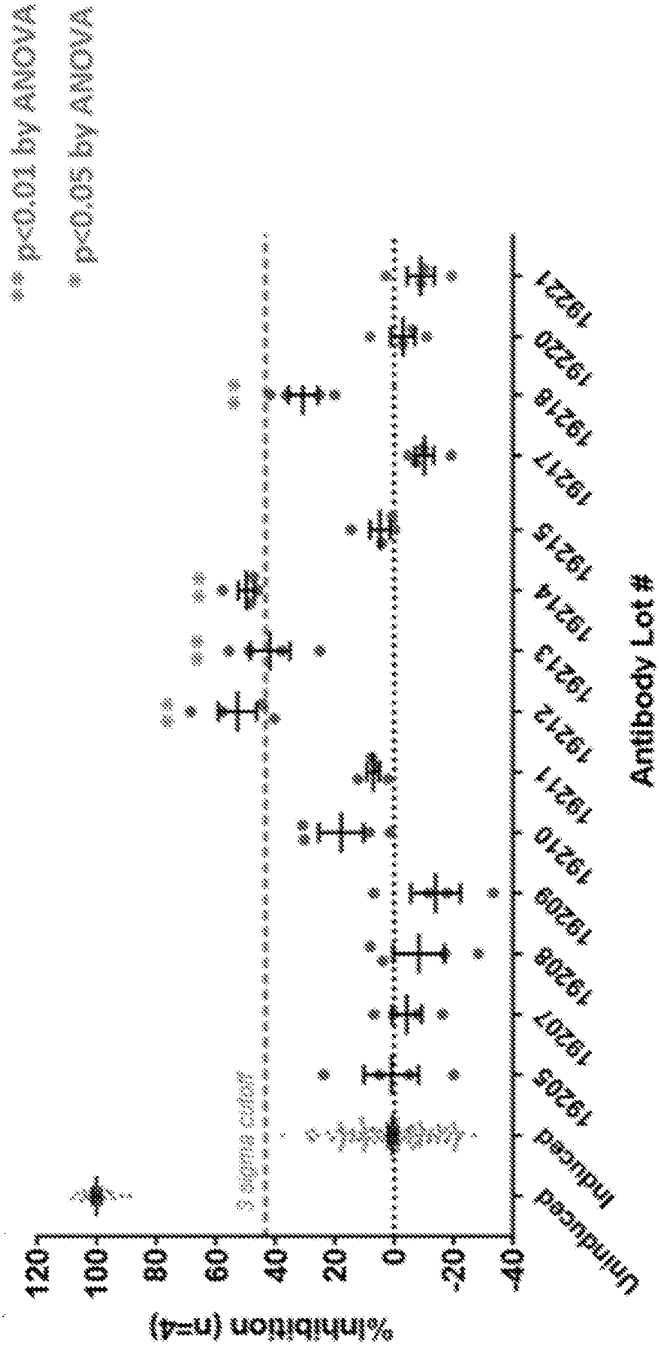


Figure 3

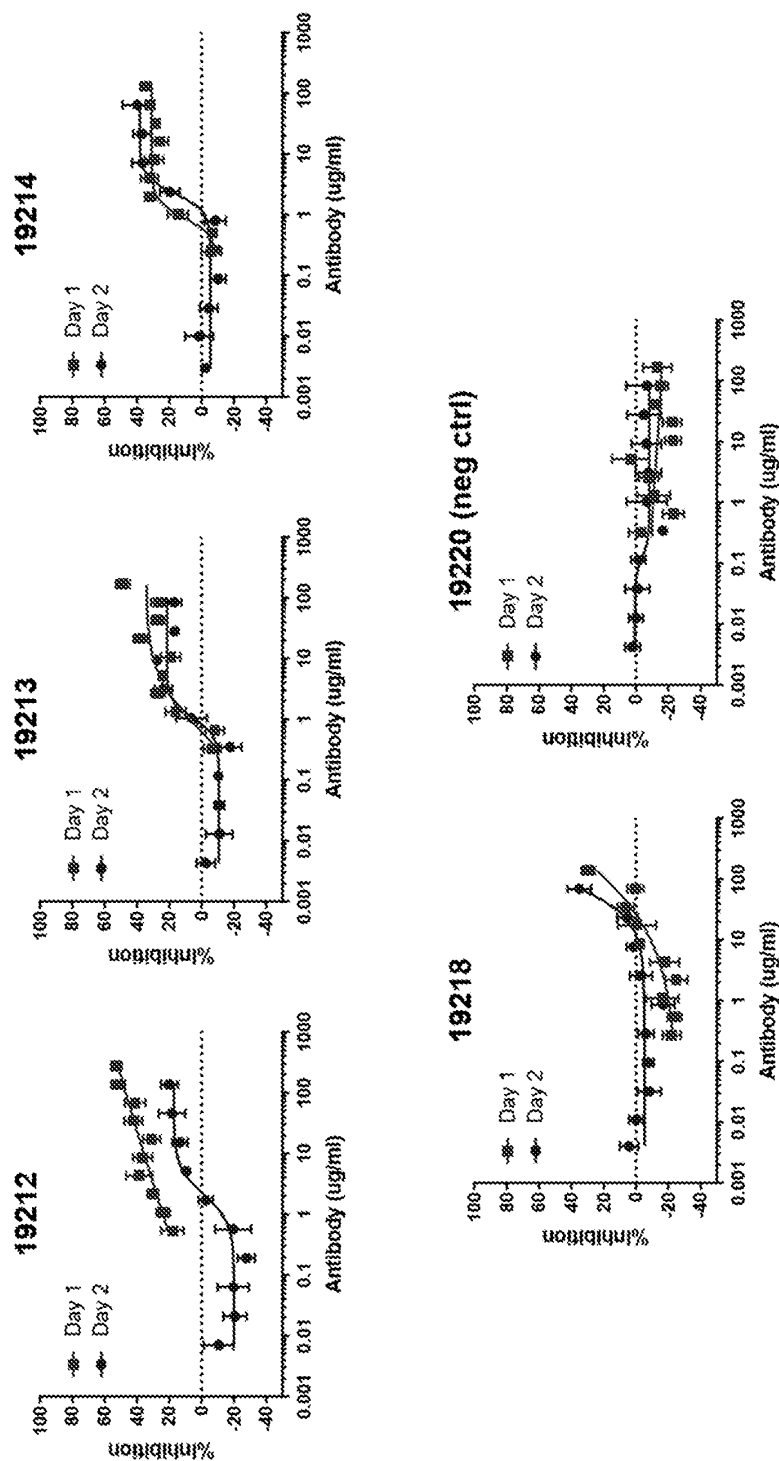


Figure 4

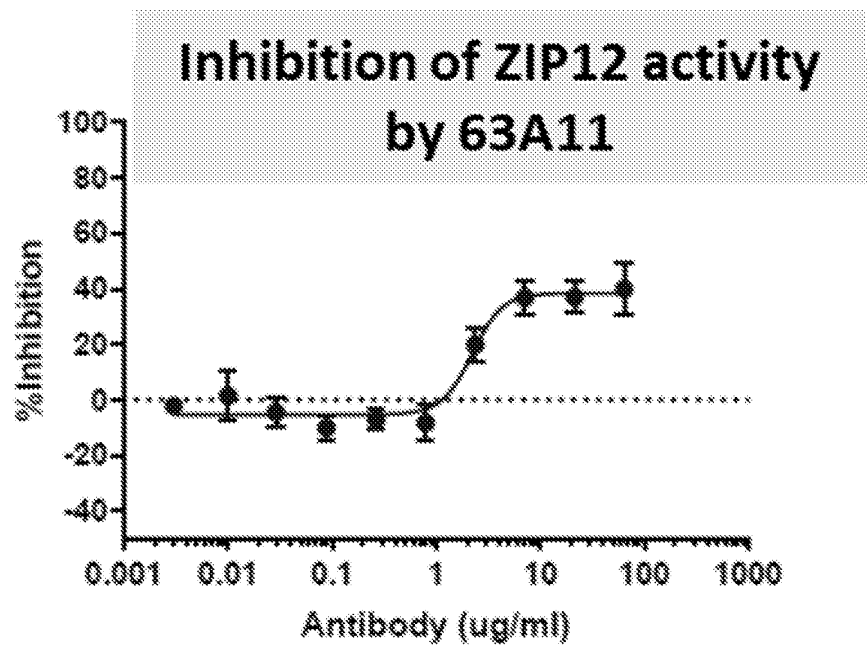


Figure 5

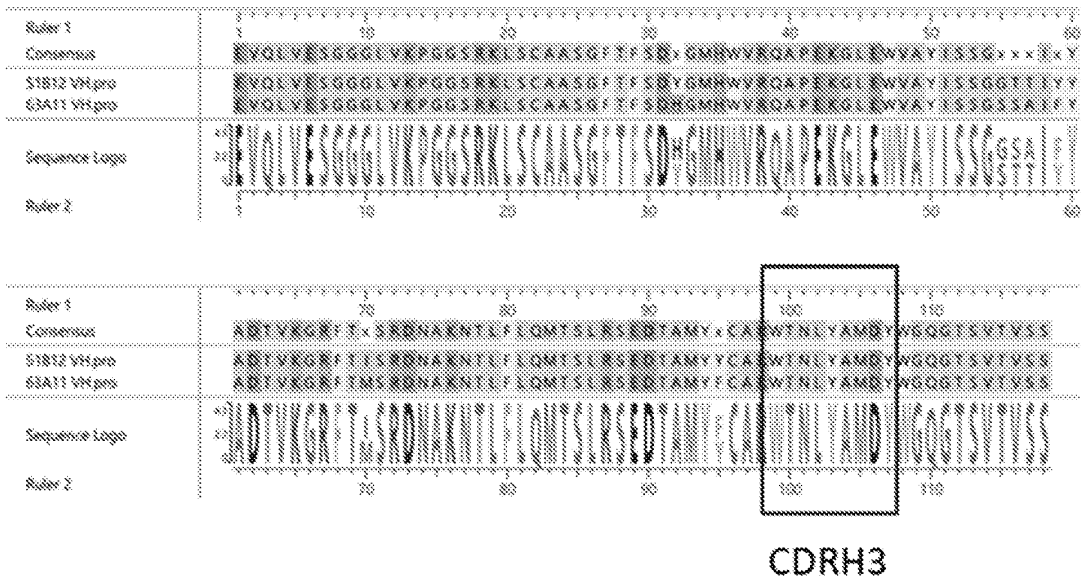


Figure 6

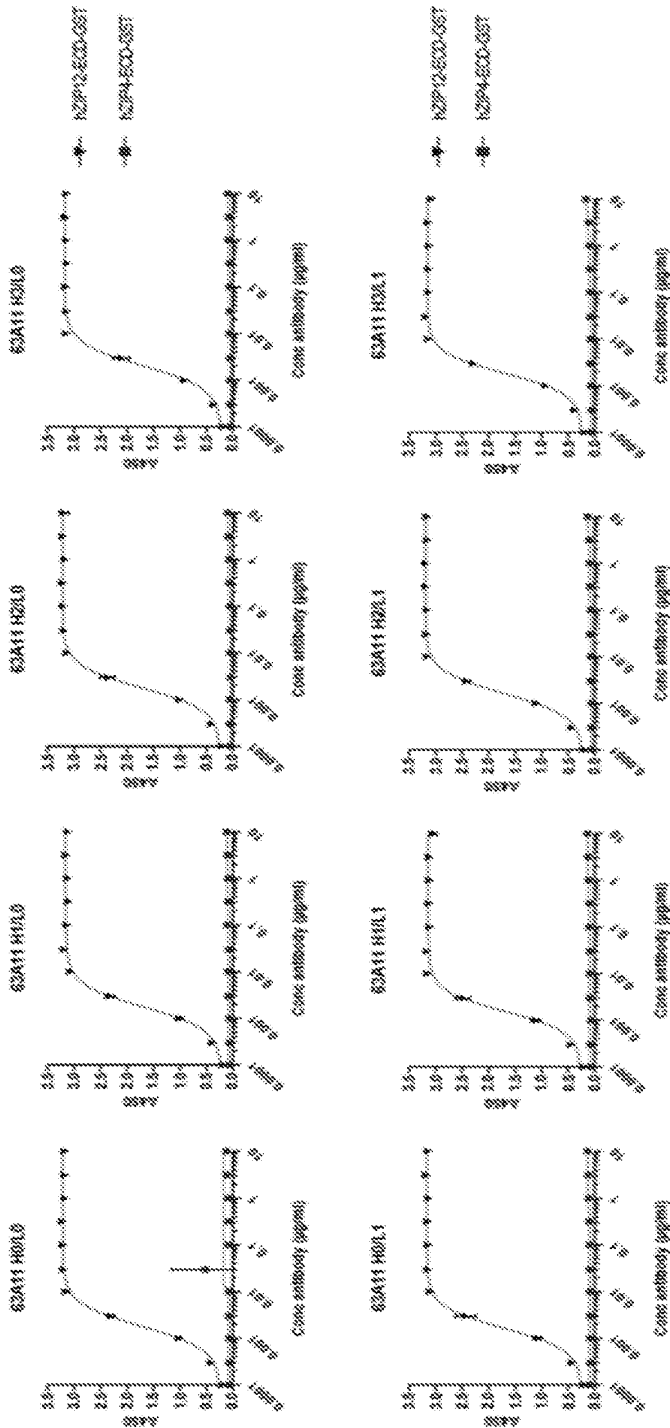


Figure 7

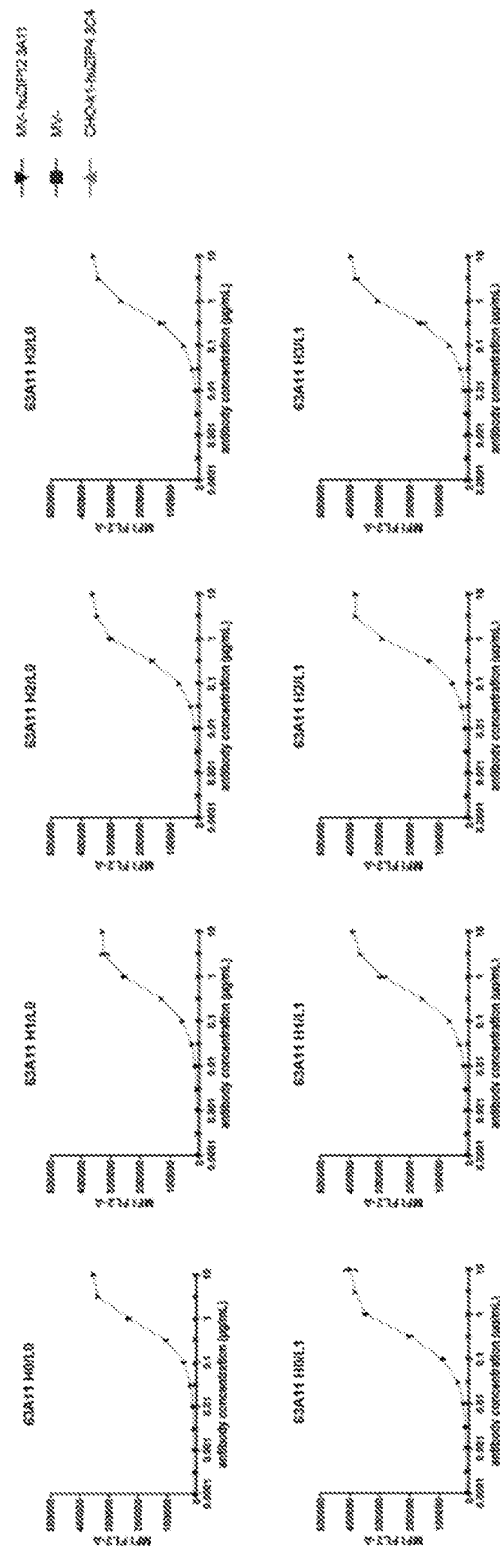


Figure 8

Sample	Concentration (mg/ml)	Volume (ml)	Yield (mg)	EC50 value (ELISA) ng/ml*	EC50 value (Flow cytometry) ng/ml#
63A11 parental	2.16	5.8	12.42	1.7	233
63A11 H0/L0	0.08	5.3	0.42	1.8	778
63A11 H0/L1	1.01	6	6.06	1.6	338
63A11 H1/L0	1.08	4.8	5.13	1.8	508
63A11 H1/L1	1.03	5.8	5.92	1.5	515
63A11 H2/L0	0.71	5	3.55	1.8	411
63A11 H2/L1	0.66	6	3.96	1.6	586
63A11 H3/L0	0.13	5.5	0.72	2.0	583
63A11 H3/L1	0.89	4.5	4.01	1.9	500

Overview of parental and humanized antibodies expressed in crude supernatant.

* ELISA EC50 refers to the antibody concentration required to obtain the half maximal signal towards hZIP12-ECD-GST

Flow cytometry EC50 refers to the antibody concentration required to obtain the half maximal signal towards MV(-)-hZIP12

Figure 9

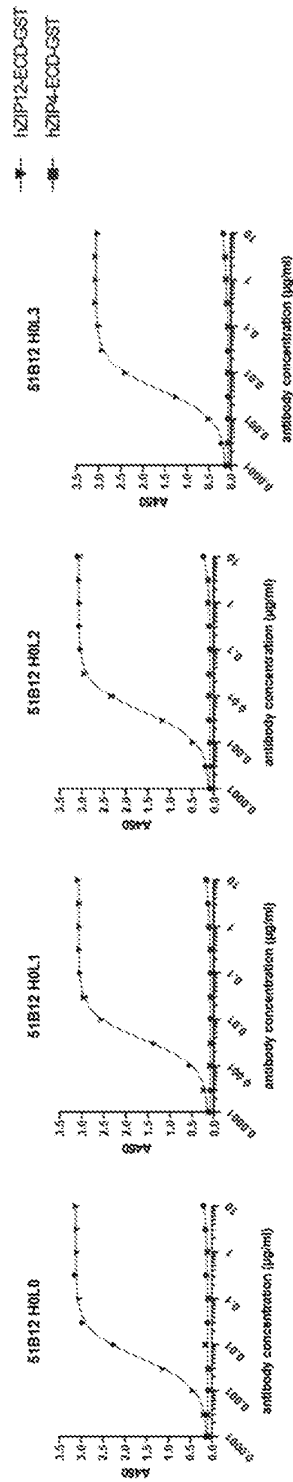


Figure 10

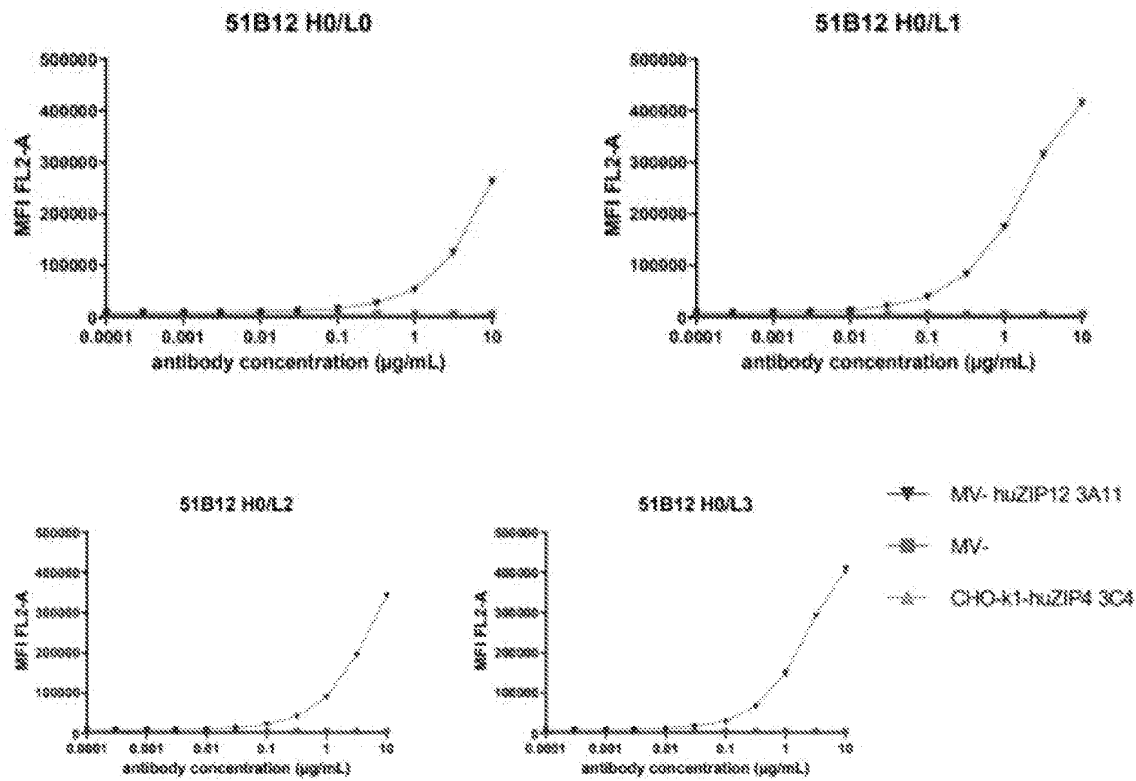


Figure 11**Table 4 Data for duplicate single cycle kinetics for clone 63A11**

Sample	Replicate	Kinetic chi ² (RU ²)	ka (1/Ms)	kd(s)	KD (M)	Rmax (RU)	Experiment I.d.
63A11_H3/_L1	1	0.24	3.83E+04	4.92E-05	1.28E-09	26.8	20GOOD025
	2	0.19	3.84E+04	5.40E-05	1.41E-09	25.3	
63A11_H2/_L1	1	0.20	3.29E+04	3.19E-05	9.70E-10	34.3	20GOOD033
	2	0.16	3.13E+04	3.57E-05	1.14E-09	29.8	
63A11_H1/_H1	1	0.17	3.14E+04	5.73E-05	1.82E-09	33.0	20GOOD025
	2	0.13	3.10E+04	6.05E-05	1.96E-09	31.7	
63A11_H0/_L1	1	0.21	3.05E+04	2.71E-05	8.88E-10	32.7	20GOOD025
	2	0.24	3.11E+04	2.76E-05	8.89E-10	30.9	
63A11_H3/_L0	1	0.14	3.12E+04	3.83E-05	1.23E-09	32.4	20GOOD033
	2	0.08	2.93E+04	4.74E-05	1.62E-09	27.3	
63A11_H2/_L0	1	0.10	3.15E+04	6.61E-05	2.10E-09	26.0	20GOOD025
	2	0.10	3.11E+04	6.36E-05	2.04E-09	24.9	
63A11_H1/_L0	1	0.12	2.96E+04	3.90E-05	1.32E-09	33.9	20GOOD033
	2	0.10	2.86E+04	4.64E-05	1.62E-09	30.2	
63A11_H0/_L0	1	0.07	2.84E+04	6.76E-05	2.38E-09	24.1	20GOOD025
	2	0.16	3.14E+04	6.14E-05	1.95E-09	23.9	
63A11 parental	1	0.24	4.64E+04	3.51E-05	7.57E-10	23.3	20GOOD025
	2	0.24	4.64E+04	3.51E-05	7.57E-10	23.3	

Figure 12**Table 7. Duplicate single cycle kinetic results for clone 51B12**

Sample	Replicate	Kinetic χ^2 (RU ²)	k_a (1/Ms)	k_{dis}	KD (M)	Rmax (RU)	Experiment i.d.
51B12 H0L0	1	0.03	7.45E+03	1.04E-05	1.39E-09	28.8	20GOOD034
	2	0.03	7.21E+03	1.00E-05	1.39E-09	27.8	
51B12 H0L1	1	0.02	7.08E+03	2.95E-05	4.17E-09	35.8	20GOOD034
	2	0.07	8.49E+03	1.55E-05	1.83E-09	35.1	
*51B12 H0L2	1	0.09	1.68E+04	1.18E-05	7.06E-10	56.6	*20GOOD033
	2	0.04	1.19E+04	2.57E-05	2.16E-09	50.6	
51B12 H0L3	1	0.25	1.67E+04	1.01E-05	6.05E-10	34.1	20GOOD034
	2	0.21	1.67E+04	1.31E-05	7.85E-10	31.9	

*20GOOD033 data for 51B12 H0L2 was used as the data obtained in 20GOOD34 was rejected.

63A11 HoL1 = 900pM

51B12 HoL3 = 700pM

No cross-reactivity with human ZIP4

Figure 13

Antibody	CDR-H1	CDR-H2	CDR-H3	CDR-L1	CDR-L2	CDR-L3	VH	VL	HC	LC
63A11 (19214)	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 7	SEQ ID NO: 8	SEQ ID NO: 9	SEQ ID NO: 41	SEQ ID NO: 42	SEQ ID NO: 6, and 49 to 52,	SEQ ID NO: 10, 53 and 54
51B12 (19213)	SEQ ID NO: 11	SEQ ID NO: 12	SEQ ID NO: 13	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 43	SEQ ID NO: 44	SEQ ID NO: 14 and 55	SEQ ID NO: 18 and 56 to 59
38F02 (19212)	SEQ ID NO: 20	SEQ ID NO: 21	SEQ ID NO: 22	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 45	SEQ ID NO: 46	SEQ ID NO: 23	SEQ ID NO: 27
89G11 (19218)	SEQ ID NO: 28	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 32	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 47	SEQ ID NO: 48	SEQ ID NO: 31	SEQ ID NO: 35

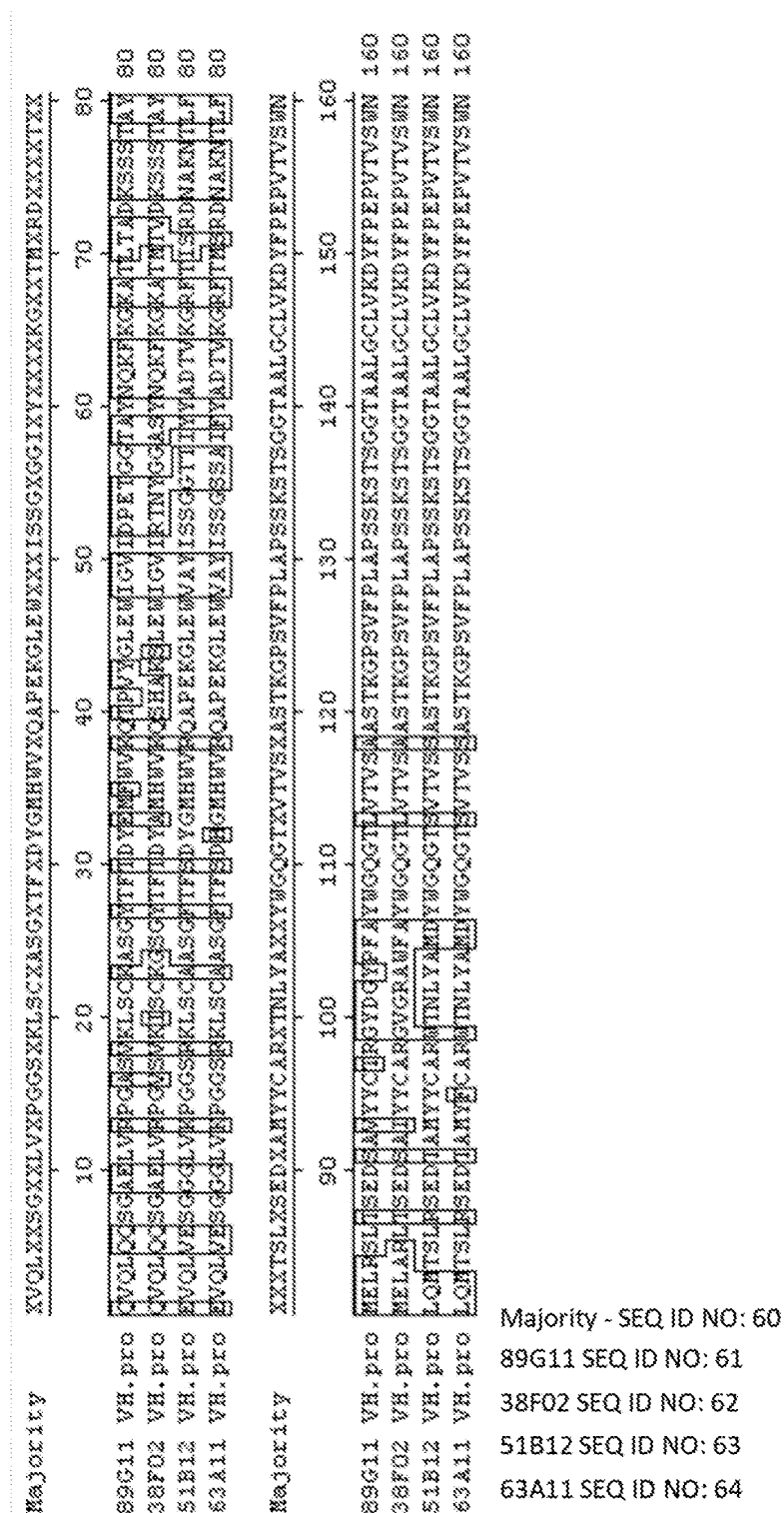


Figure 14B

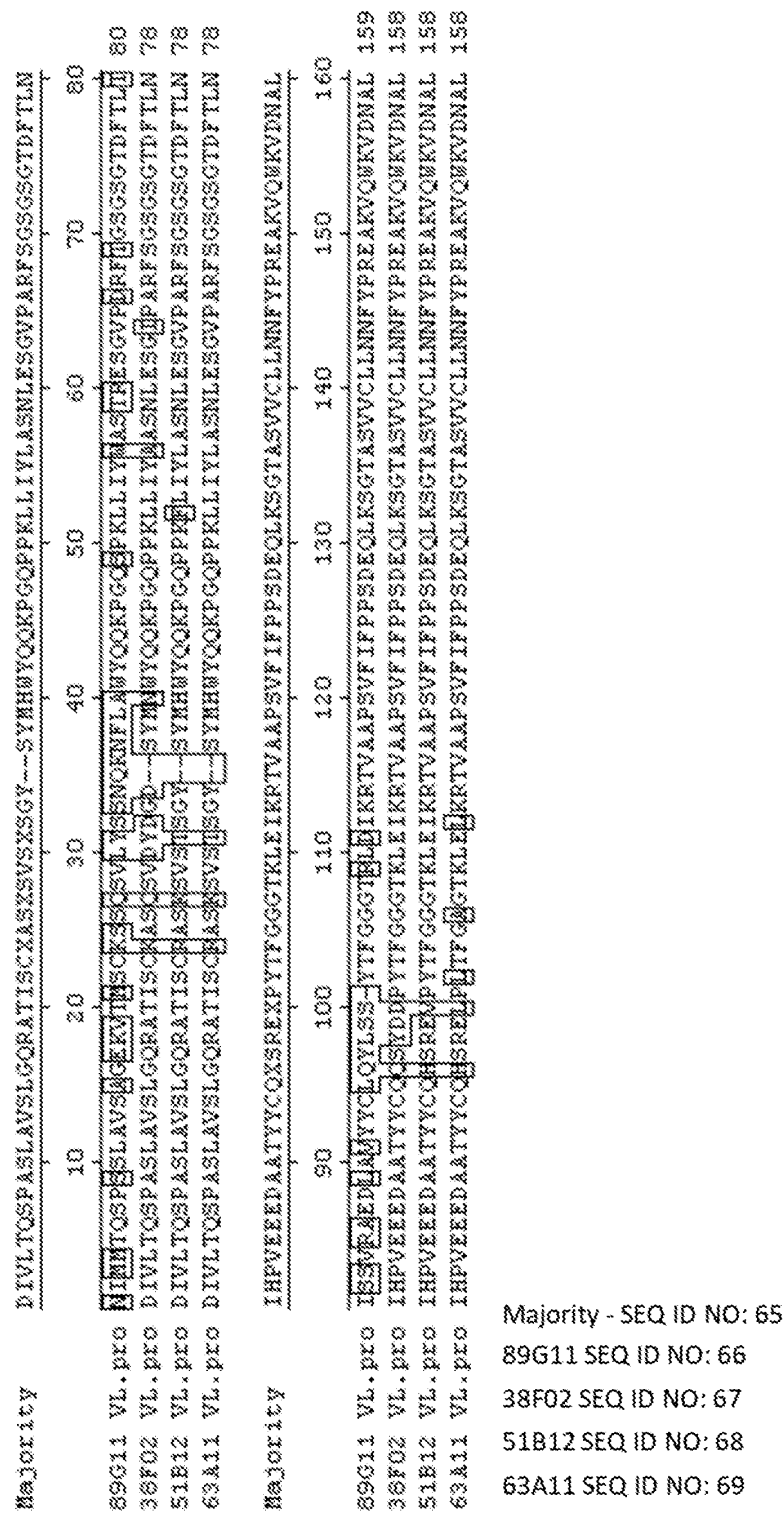
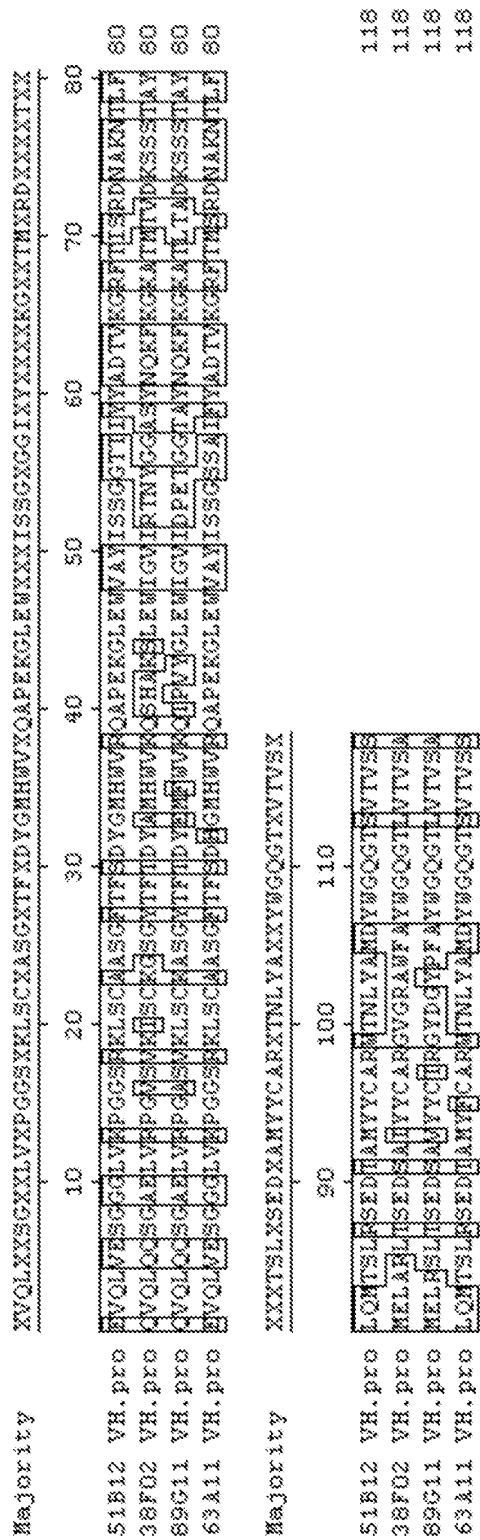
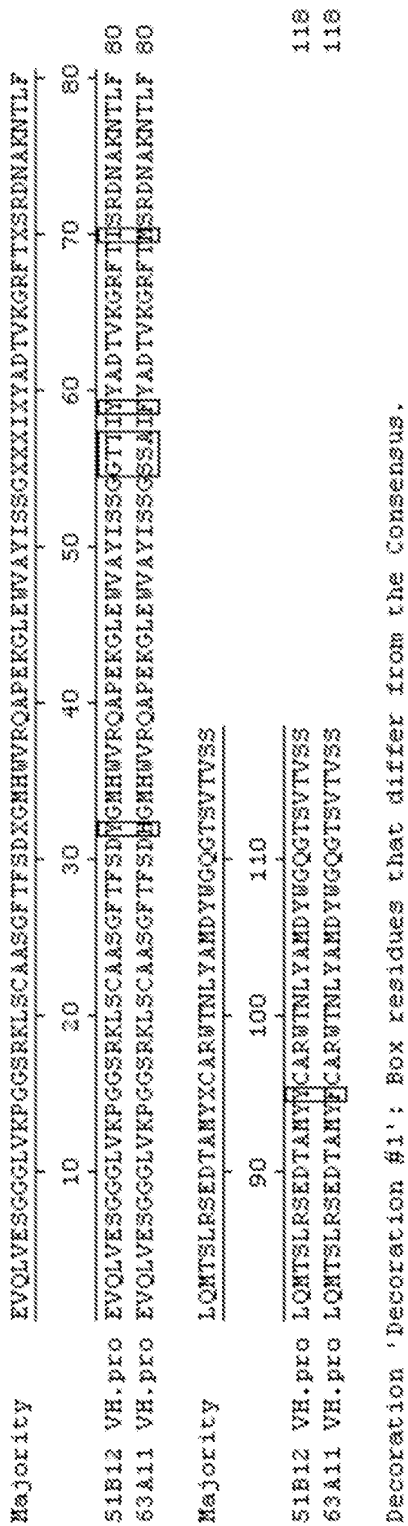


Figure 15



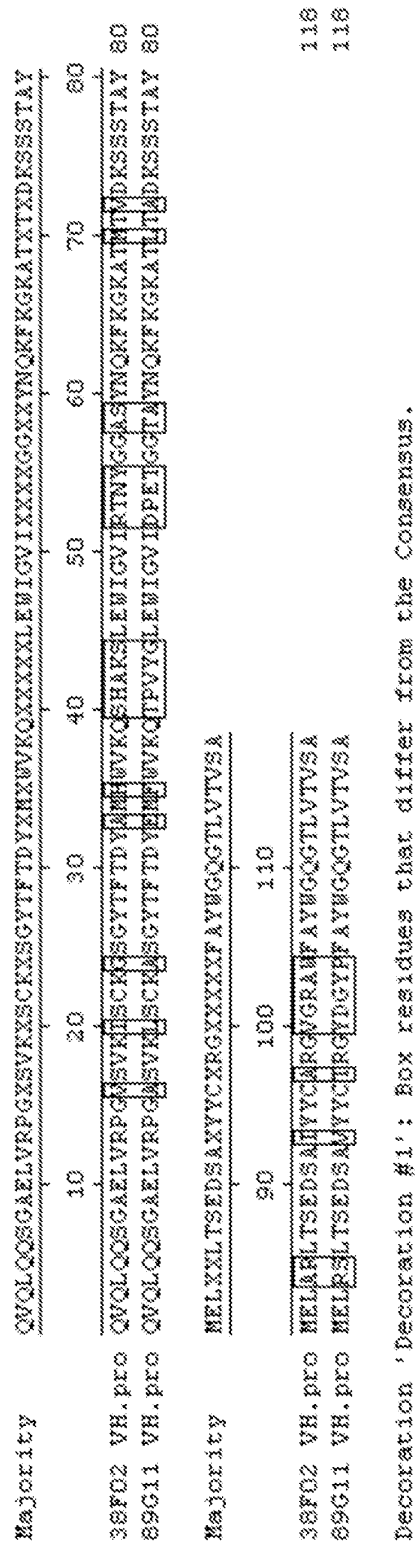
- Majority – SEQ ID NO: 70
- 51B12 – SEQ ID NO: 43
- 38F02 – SEQ ID NO: 45
- 89G11 – SEQ ID NO: 47
- 63A11 – SEQ ID NO: 41

Figure 16



Majority – SEQ ID NO: 71
51B12 – SEQ ID NO: 43
63A11 – SEQ ID NO: 41

Figure 17

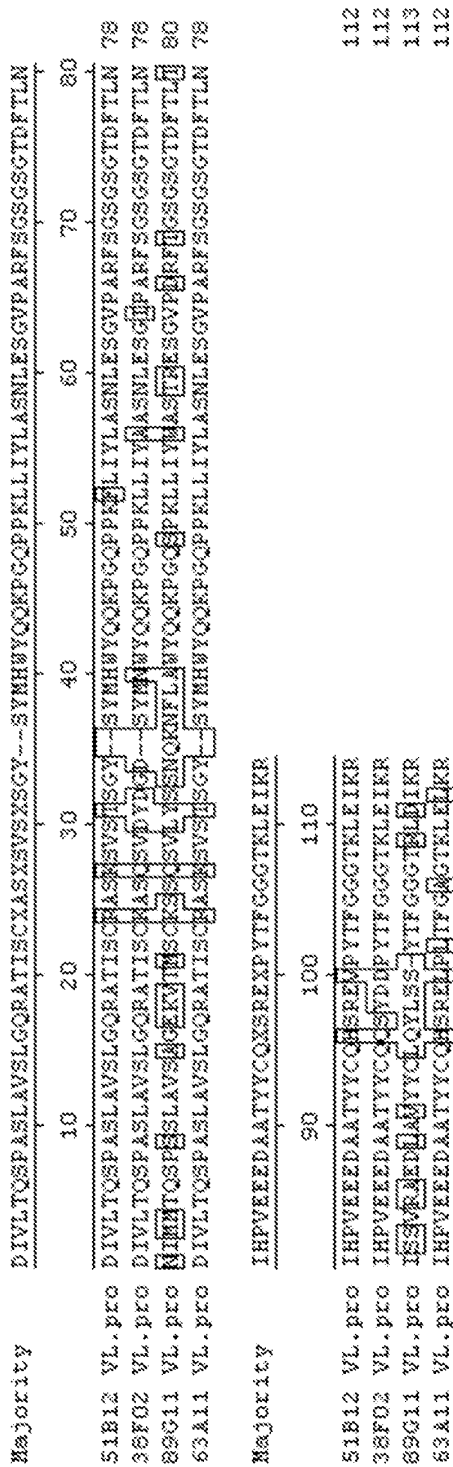


Majority – SEQ ID NO: 72

38F02 – SEQ ID NO: 45

89G11 – SEQ ID NO: 47

Figure 18



Decoration 'Decoration #1': Box residues that differ from the Consensus.

- Majority – SEQ ID NO: 73
- 51B12 – SEQ ID NO: 44
- 38F02 – SEQ ID NO: 46
- 89G11 – SEQ ID NO: 47
- 63A11 – SEQ ID NO: 48

Majority	10	20	30	40	50	60	70	80
DIVLTQSPASLAVSLGQ	RATIS	CRASKSV	STSCY	SYNHHWY	QOKPGQ	PKLLIYL	ASNLESGV	PARFSCSGTDFTLNIH
51B12 VL.pro	DIVLTQSPASLAVSLGQ	RATIS	CRASKSV	STSCY	SYNHHWY	QOKPGQ	PKLLIYL	ASNLESGV
38F02 VL.pro	DIVLTQSPASLAVSLGQ	RATIS	CRASKSV	STSCY	SYNHHWY	QOKPGQ	PKLLIYL	ASNLESGV
63A11 VL.pro	DIVLTQSPASLAVSLGQ	RATIS	CRASKSV	STSCY	SYNHHWY	QOKPGQ	PKLLIYL	ASNLESGV
Majority	PVEEEDAATYYCCHSREXPYTFGGCTLEIKR							
	90	100	110					
51B12 VL.pro	PVEEEDAATYYCCHSREXPYTFGGCTLEIKR							112
38F02 VL.pro	PVEEEDAATYYCCHSREXPYTFGGCTLEIKR							112
63A11 VL.pro	PVEEEDAATYYCCHSREXPYTFGGCTLEIKR							112

Decoration 'Decoration #1': Box residues that differ from the Consensus.

Majority – SEQ ID NO: 74
51B12 – SEQ ID NO: 44
38F02 – SEQ ID NO: 46
63A11 – SEQ ID NO: 48

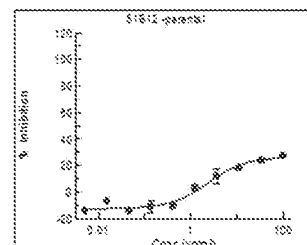
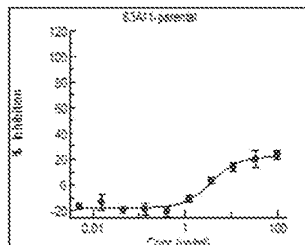
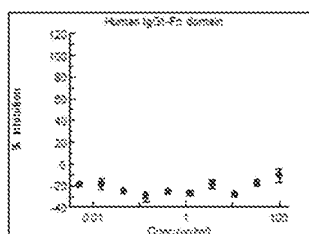
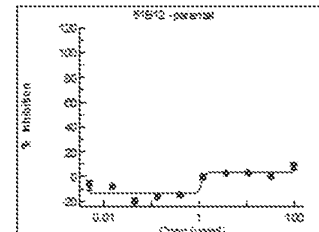
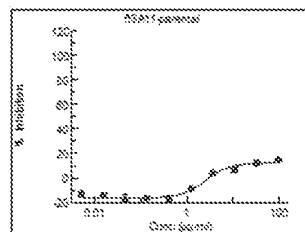
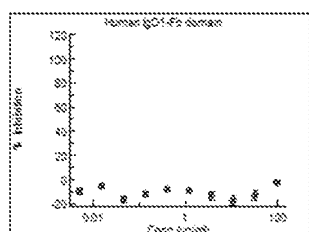
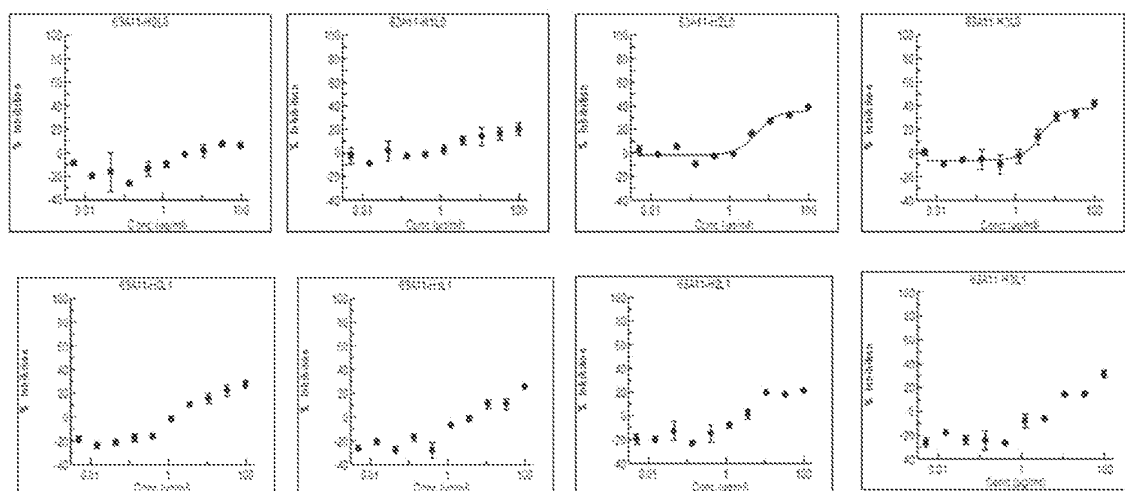
Figure 201.25uM Zn²⁺500 uM Zn²⁺

Figure 21A1.25 μM Zn^{2+} 63A11 humanised antibodies**Figure 21B**1.25 μM Zn^{2+} 51B12 humanised antibodies