

FIG. 1

% Specific Binding												
	1	2	3	4	5	6	7	8	9	10	11	12
A	108	108	98	98	102	100	101	104	101	107	96	98
B	109	102	96	81	91	100	97	98	104	101	102	102
C	100	105	96	99	106	106	97	97	107	107	102	100
D	105	107	109	107	104	106	100	105	102	98	100	0
E	107	101	108	91	110	108	112	105	104	107	99	-0
F	108	105	104	96	100	106	101	107	98	105	99	0
G	98	106	105	102	105	105	100	104	96	97	105	132
H	102	104	114	108	104	110	102	100	107	100	107	132

Total binding

Non-specific binding

Well B04 = IL330004

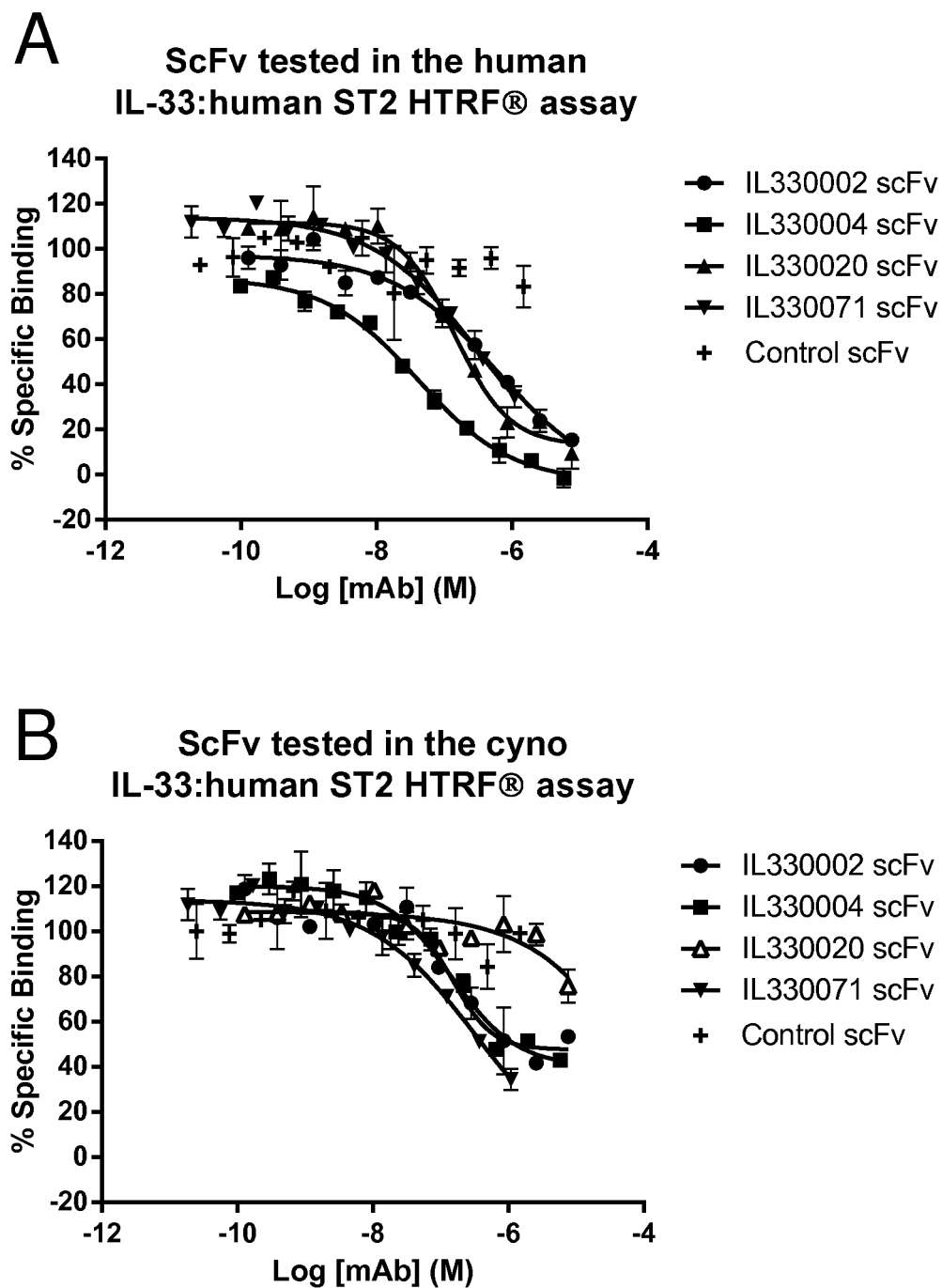


FIGURE 2

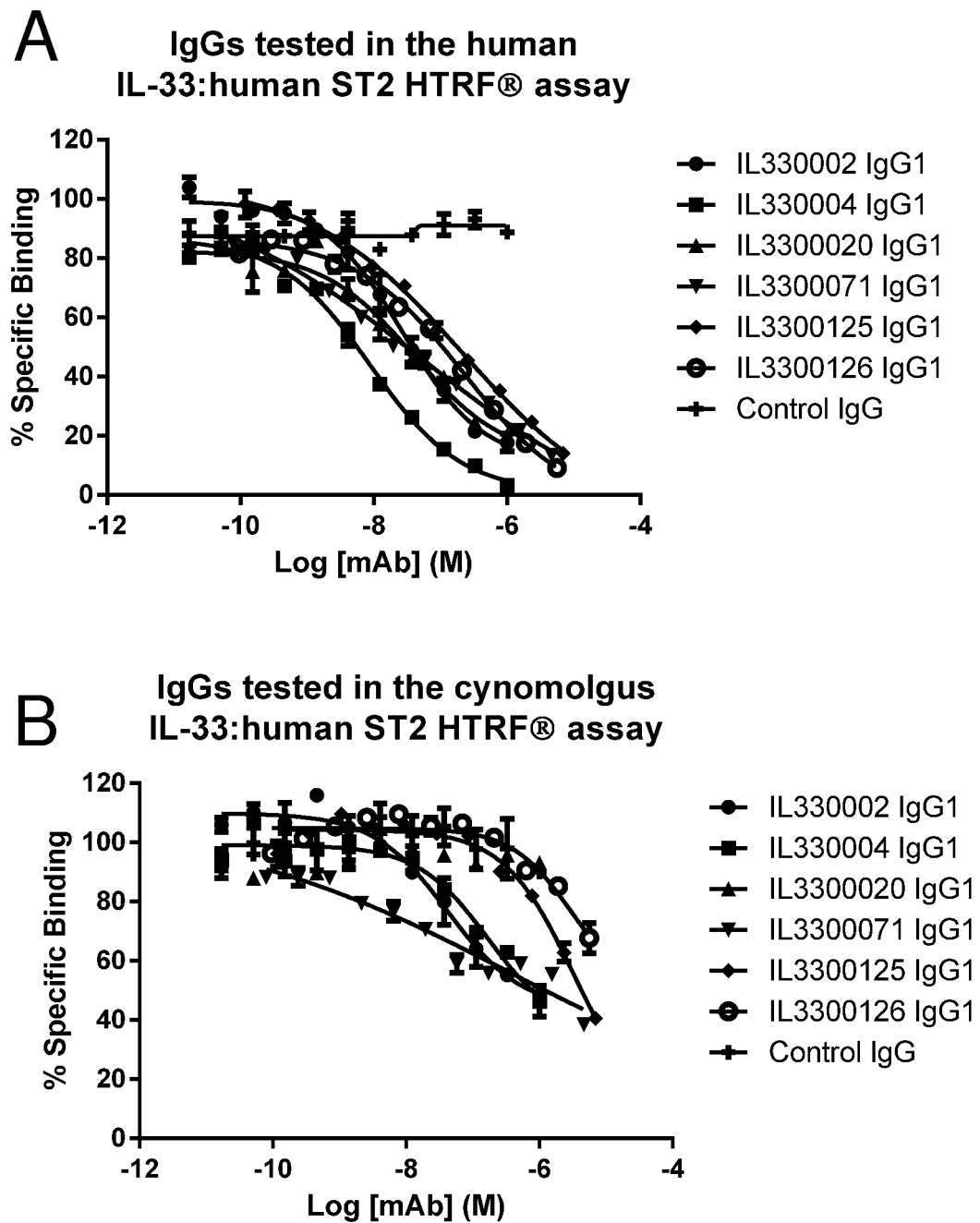


FIGURE 3

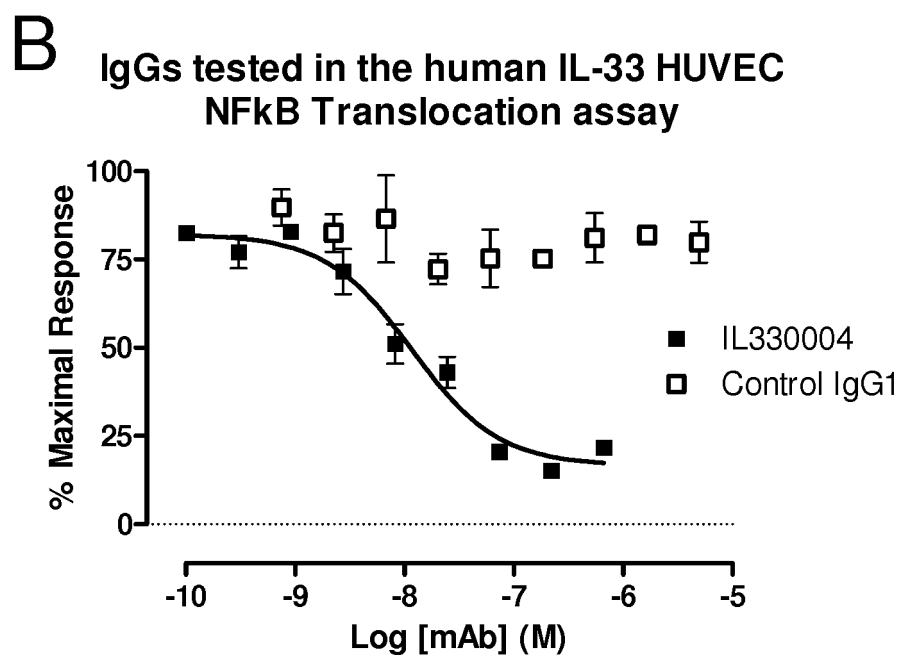
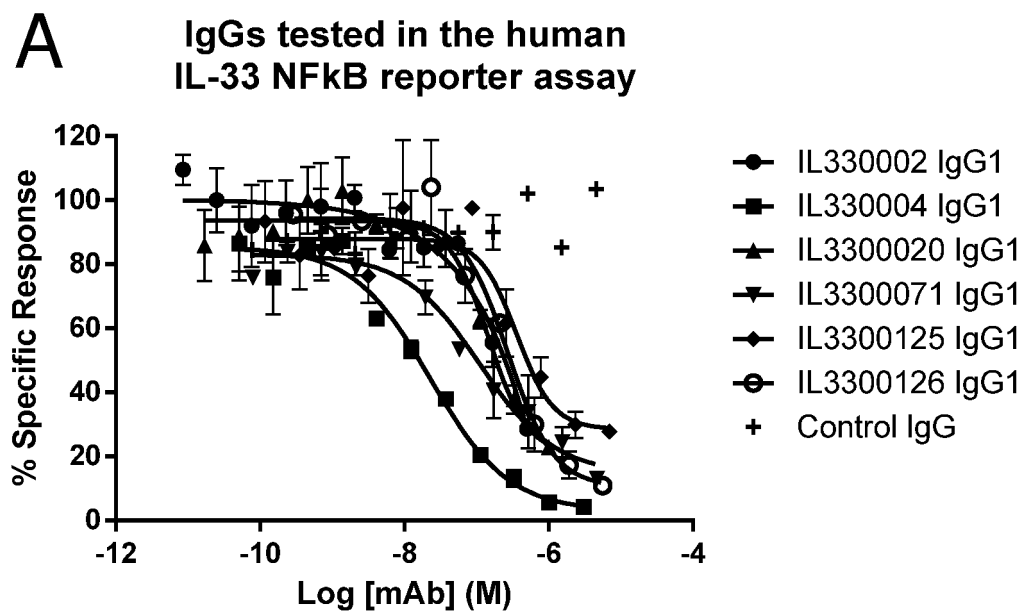


FIGURE 4

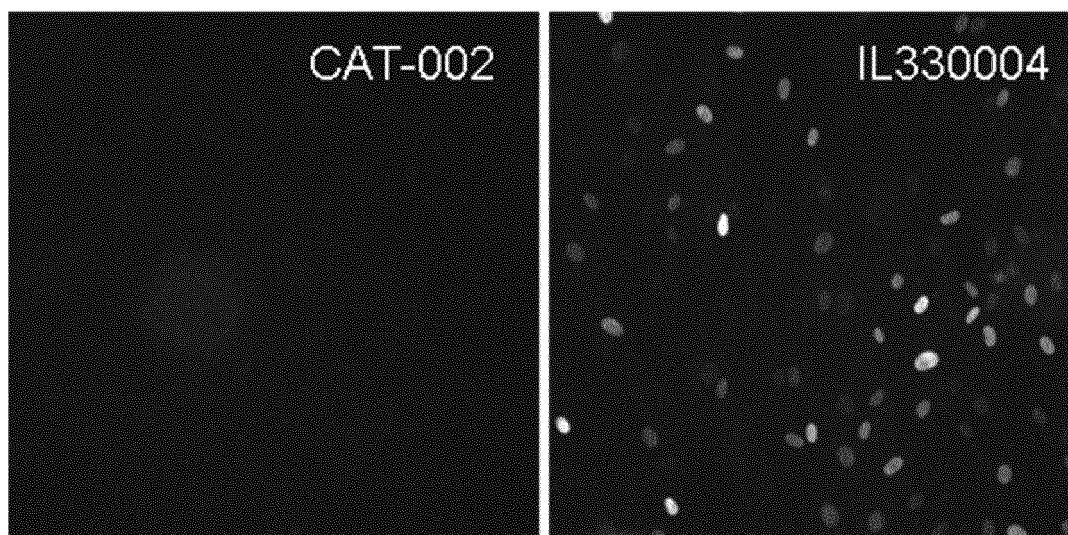


FIGURE 5

Human IL-33

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.059	0.046	0.041	0.042	0.046	0.043	0.045	0.046	0.047	0.056	0.054	1.246
B	0.056	0.042	0.039	0.045	0.046	0.049	0.045	0.047	0.048	0.048	0.043	0.921
C	0.045	0.041	0.041	1.27	0.044	0.044	0.043	0.069	0.044	0.041	0.042	0.044
D	0.051	0.039	0.038	0.039	0.039	0.041	0.04	0.04	0.041	0.04	0.043	0.033
E	0.042	0.039	0.038	0.039	0.039	0.038	0.038	0.039	0.038	0.038	0.042	0.038
F	0.05	0.039	0.038	0.045	0.039	0.039	0.04	0.041	0.039	0.039	0.263	0.035
G	0.037	0.04	0.04	0.04	0.04	0.039	0.04	0.043	0.04	0.042	0.052	0.037
H	0.051	0.052	0.046	0.053	0.049	0.048	0.048	0.051	0.041	0.05	0.07	0.04

Cyno IL-33 FH

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.072	0.059	0.061	0.081	0.048	0.041	0.038	0.041	0.044	0.041	0.044	0.148
B	0.072	0.094	0.071	0.055	0.053	0.046	0.057	0.042	0.045	0.041	0.041	0.115
C	0.074	0.098	0.076	1.193	0.08	0.042	0.041	0.066	0.1	0.062	0.045	0.064
D	0.054	0.06	0.061	0.051	0.048	0.043	0.05	0.056	0.06	0.052	0.043	0.067
E	0.087	0.067	0.059	0.065	0.045	0.063	0.095	0.039	0.073	0.043	0.061	0.072
F	0.072	0.065	0.085	0.064	0.065	0.058	0.058	0.075	0.05	0.057	0.276	0.058
G	0.068	0.054	0.074	0.065	0.049	0.045	0.052	0.042	0.059	0.04	0.073	0.092
H	0.117	0.065	0.064	0.057	0.058	0.069	0.063	0.059	0.06	0.061	0.058	0.056

Insulin

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.069	0.043	0.056	0.046	0.057	0.046	0.045	0.055	0.084	0.093	0.073	0.087
B	0.076	0.044	0.046	0.06	0.07	0.05	0.064	0.053	0.07	0.062	0.07	0.055
C	0.071	0.079	0.074	0.052	0.058	0.059	0.066	0.059	0.079	0.062	0.065	0.056
D	0.069	0.075	0.074	0.072	0.067	0.061	0.058	0.07	0.048	0.064	0.04	0.049
E	0.049	0.072	0.058	0.065	0.065	0.064	0.054	0.061	0.046	0.089	0.039	0.051
F	0.1	0.058	0.06	0.052	0.065	0.061	0.076	0.069	0.057	0.043	0.375	0.061
G	0.069	0.076	0.06	0.076	0.048	0.048	0.058	0.059	0.053	0.064	0.086	0.058
H	0.099	0.089	0.105	0.099	0.056	0.081	0.093	0.087	0.089	0.071	0.074	0.08

FIGURE 6

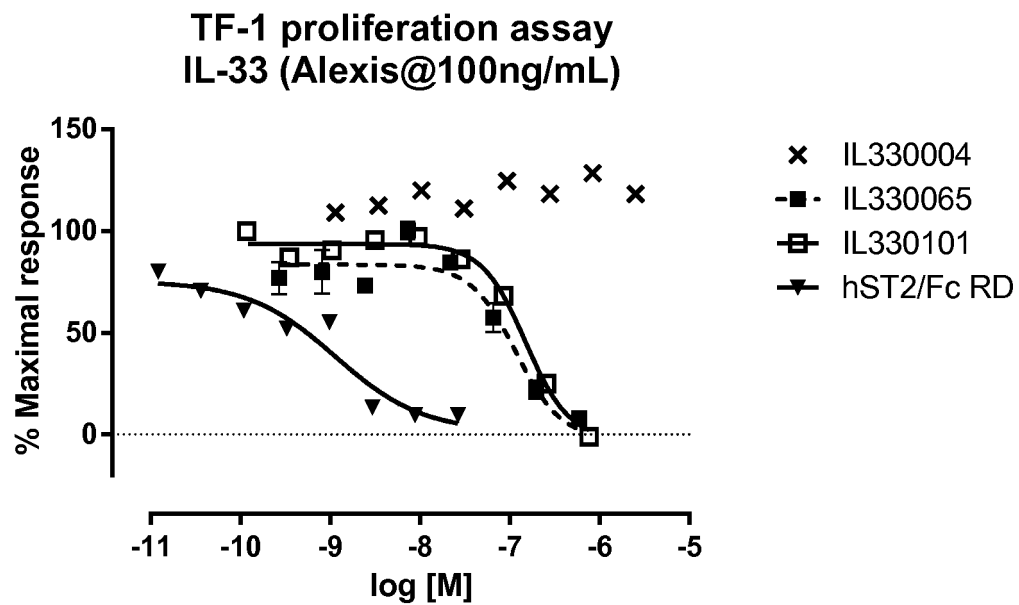
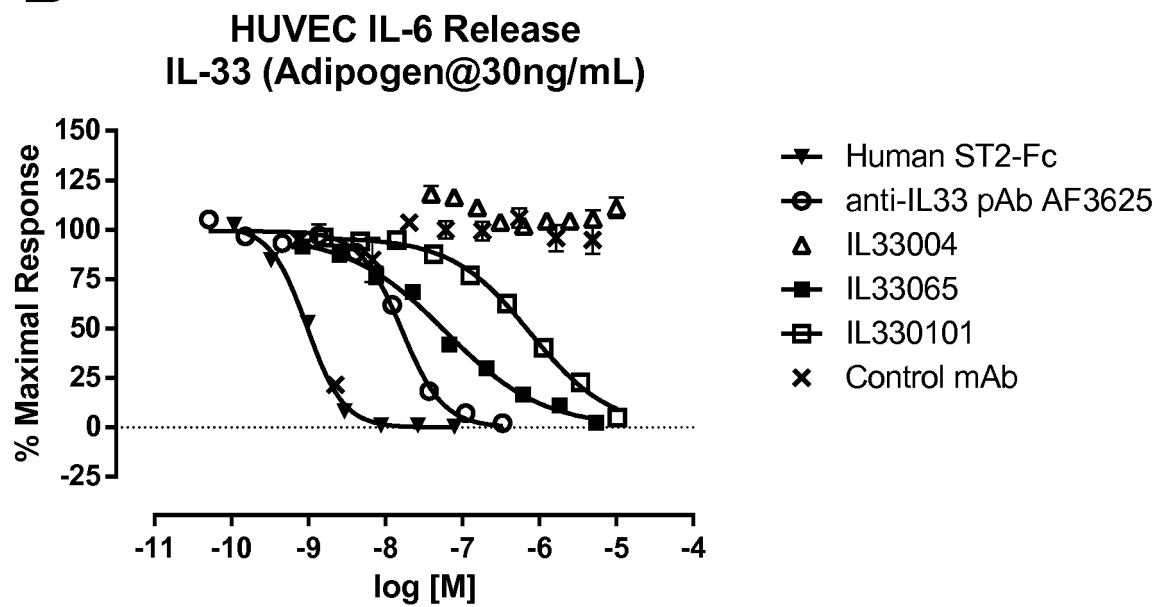
A**B**

FIGURE 7

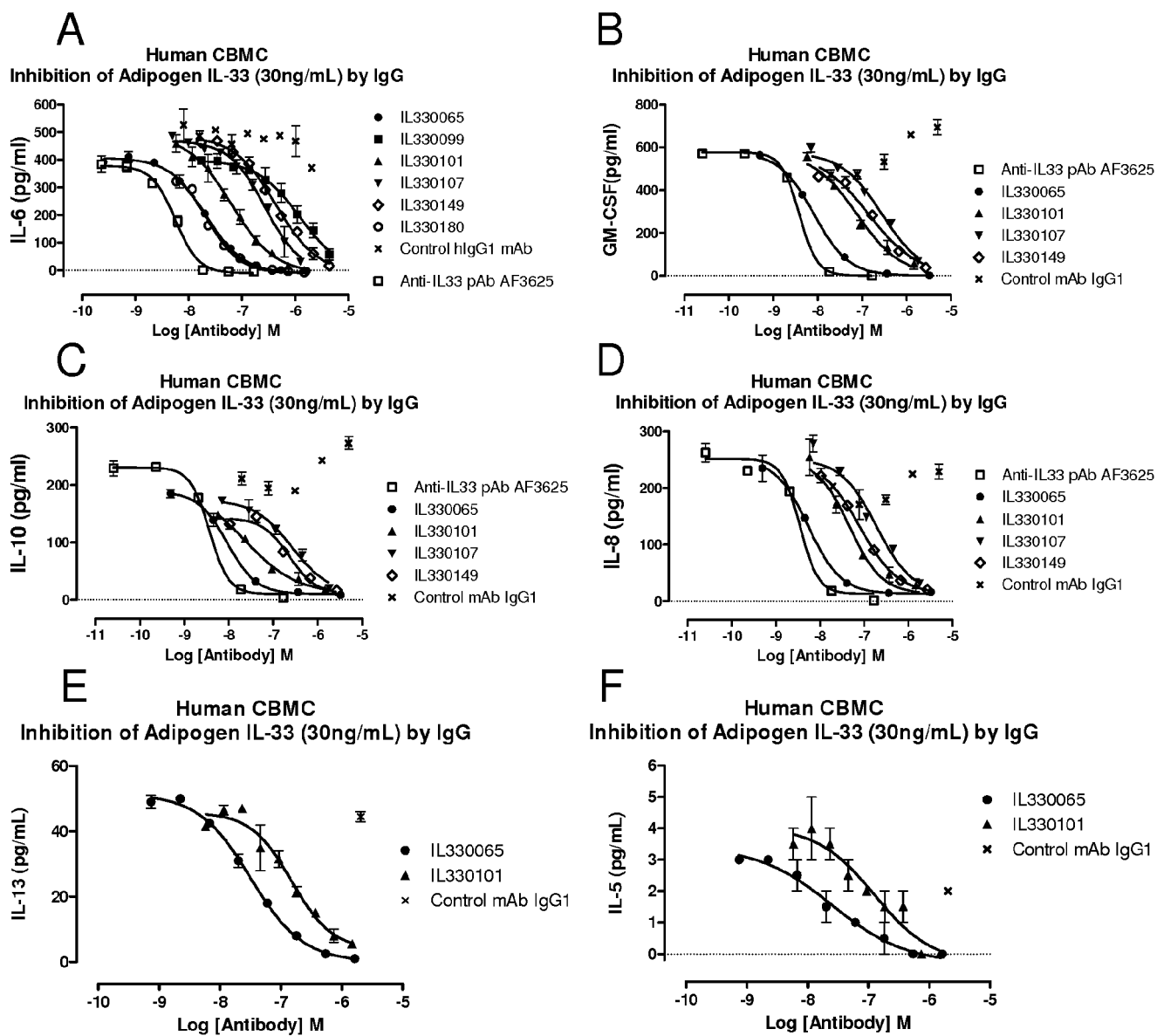
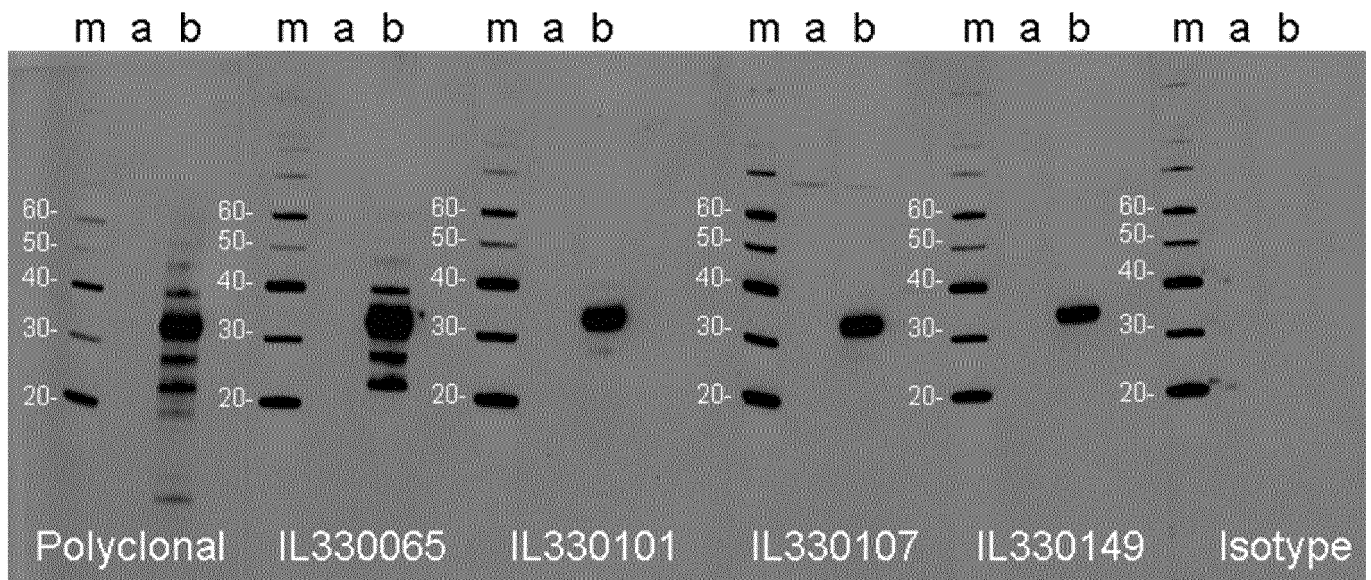


FIGURE 8



m = magic mark XP

a = mock transfected HEK-EBNA

b = FL HuIL33 transfected HEK-EBNA

FL HuIL33 predicted MW = 30kDa

Caspase3 cleaved IL33 predicted 10kDa and 20kDa fragments

FIGURE 9

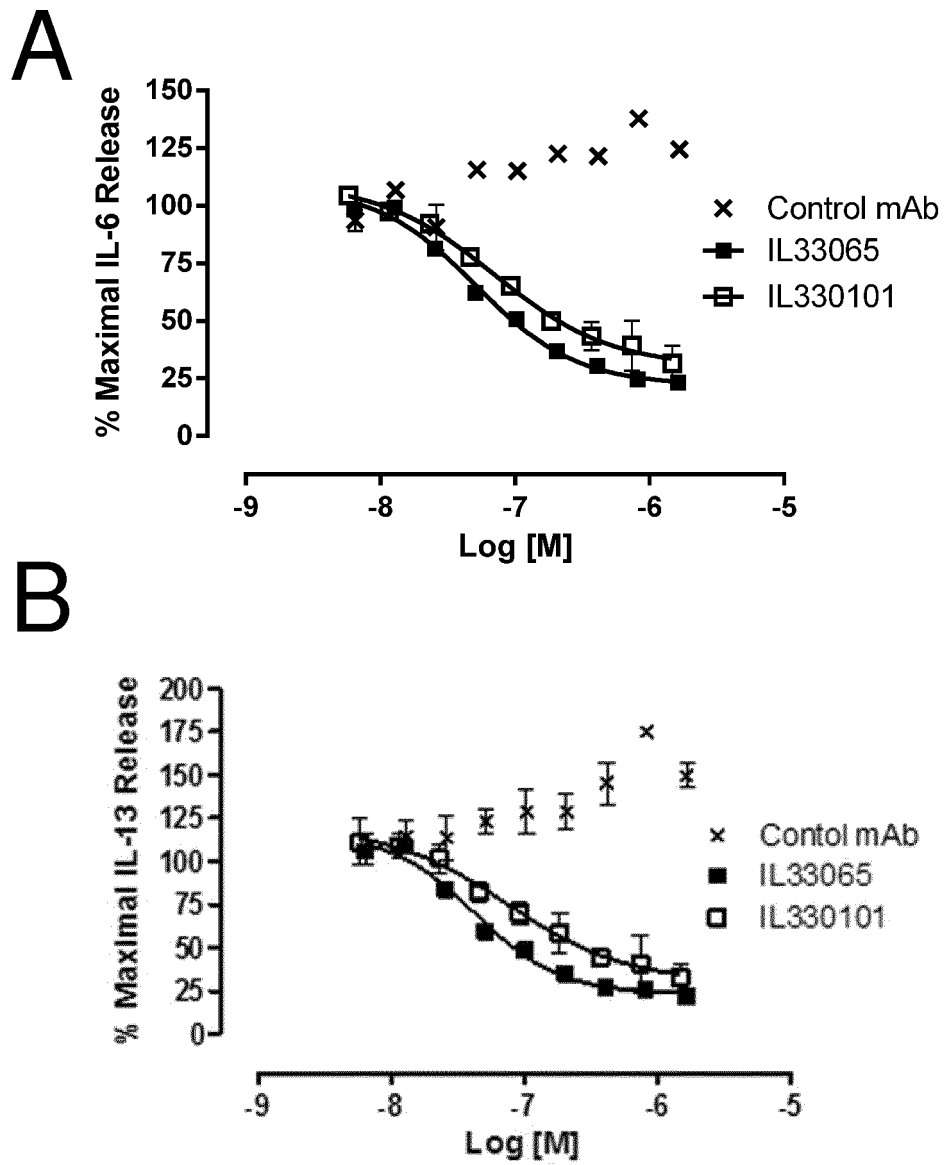


FIGURE 10

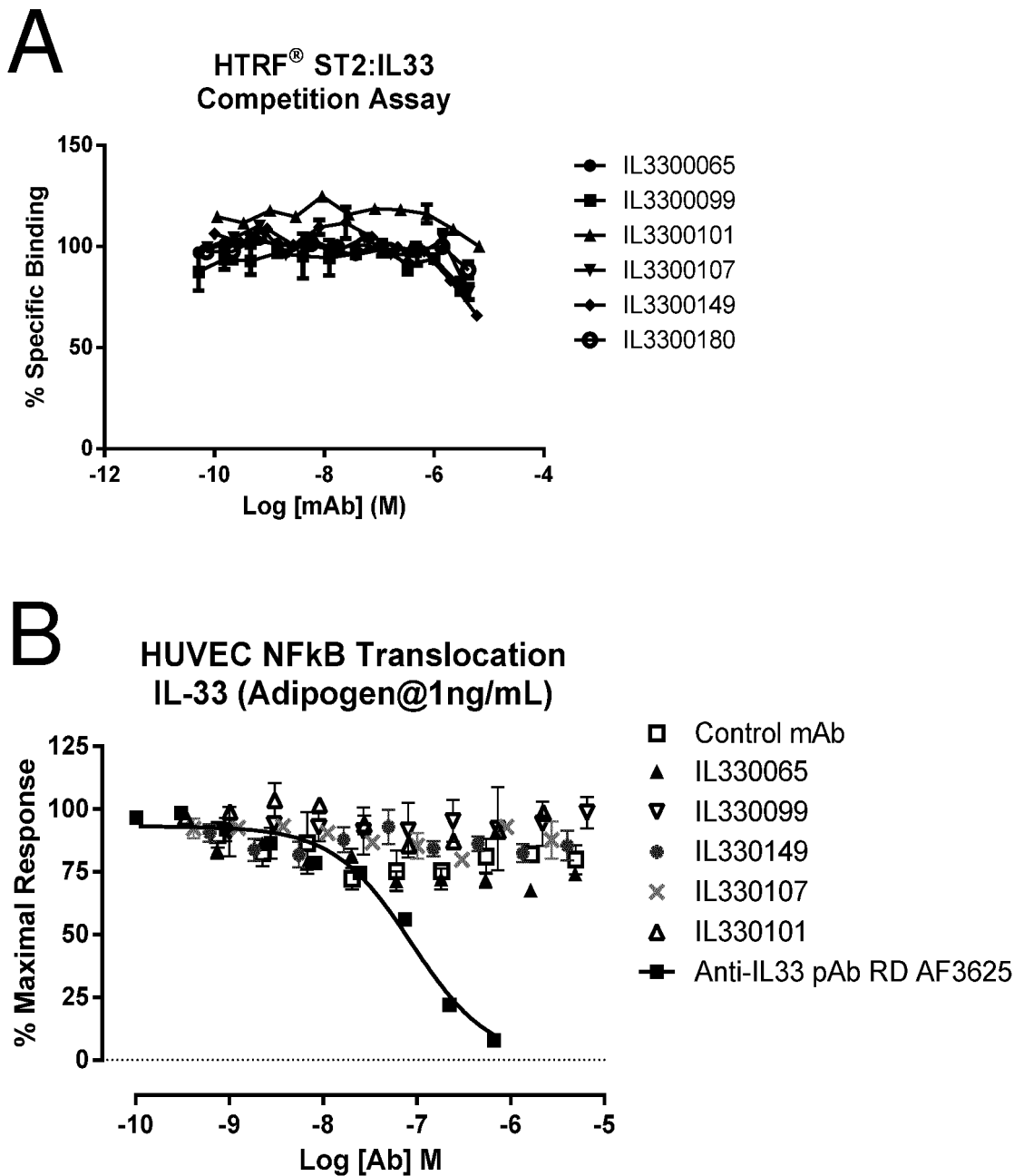


FIGURE 11

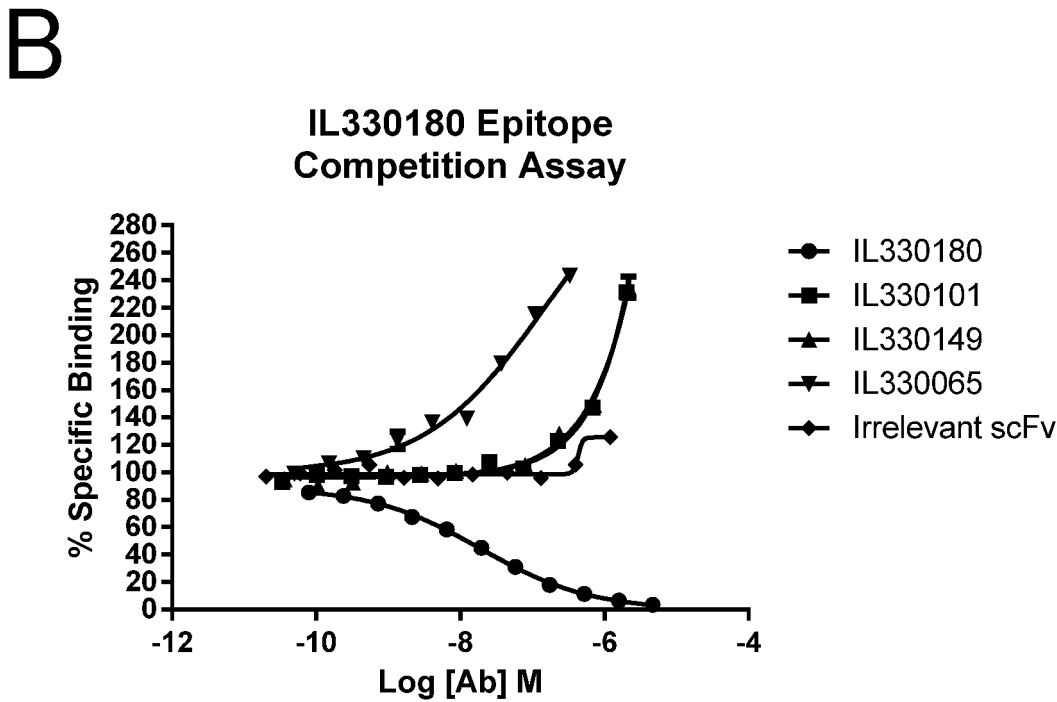
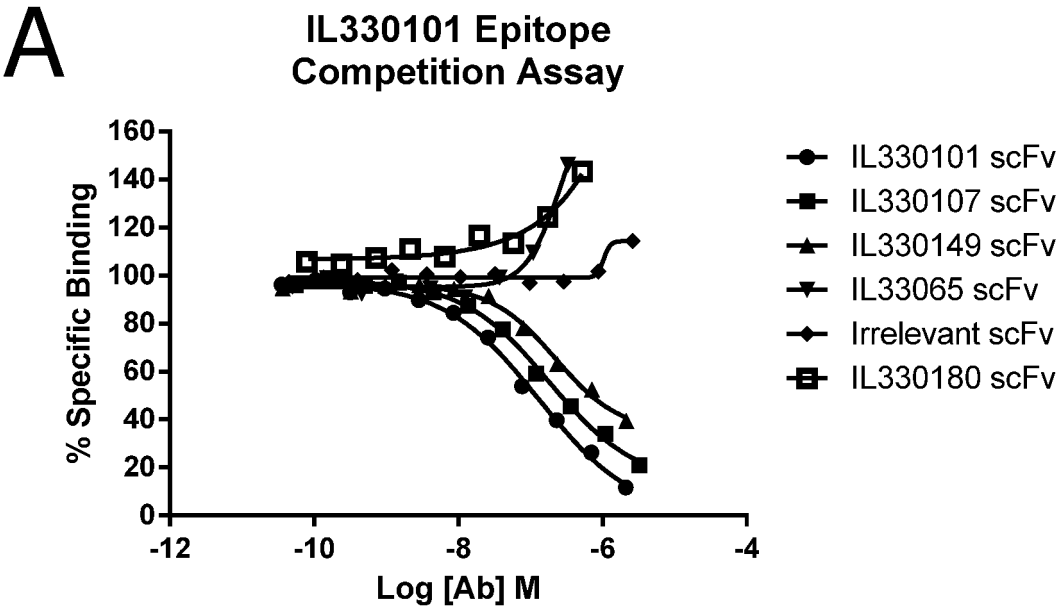


FIGURE 12

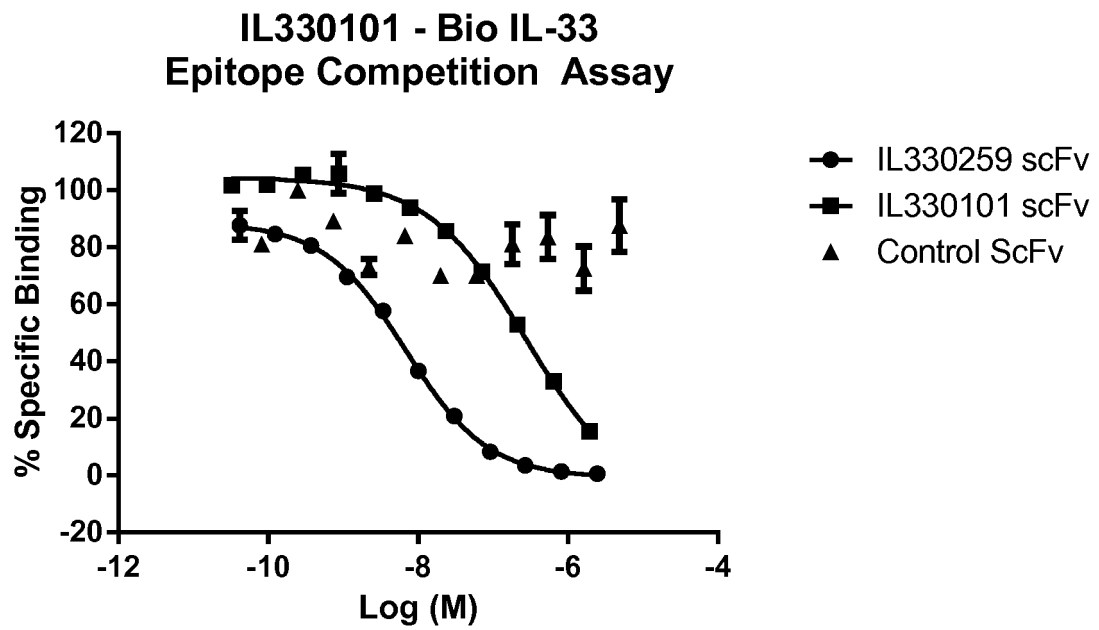


FIGURE 13

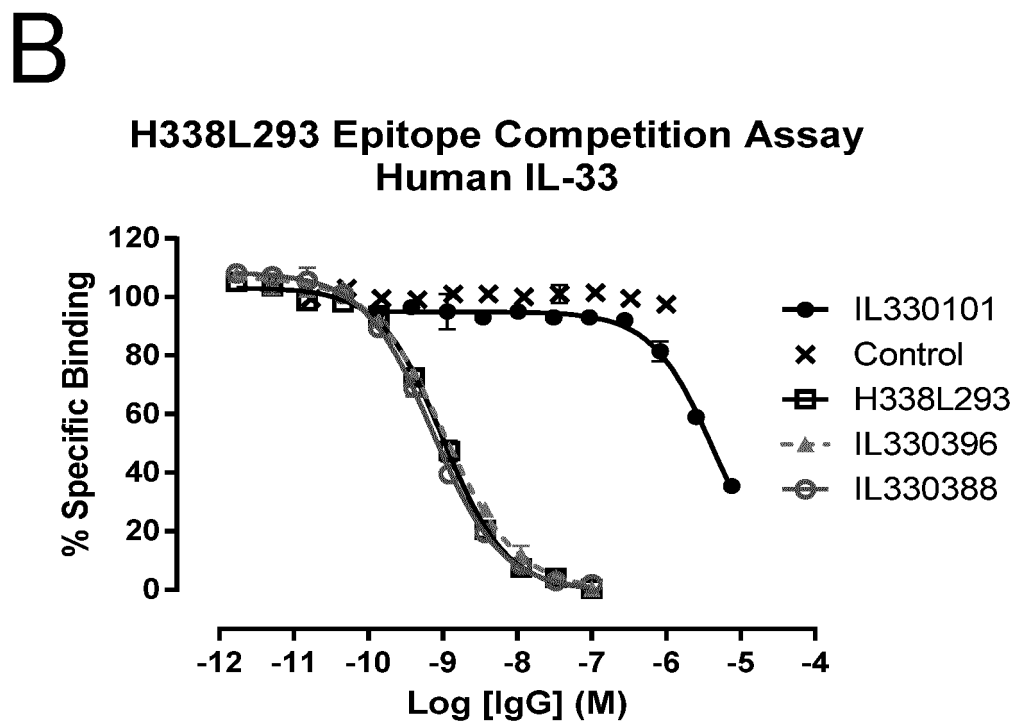
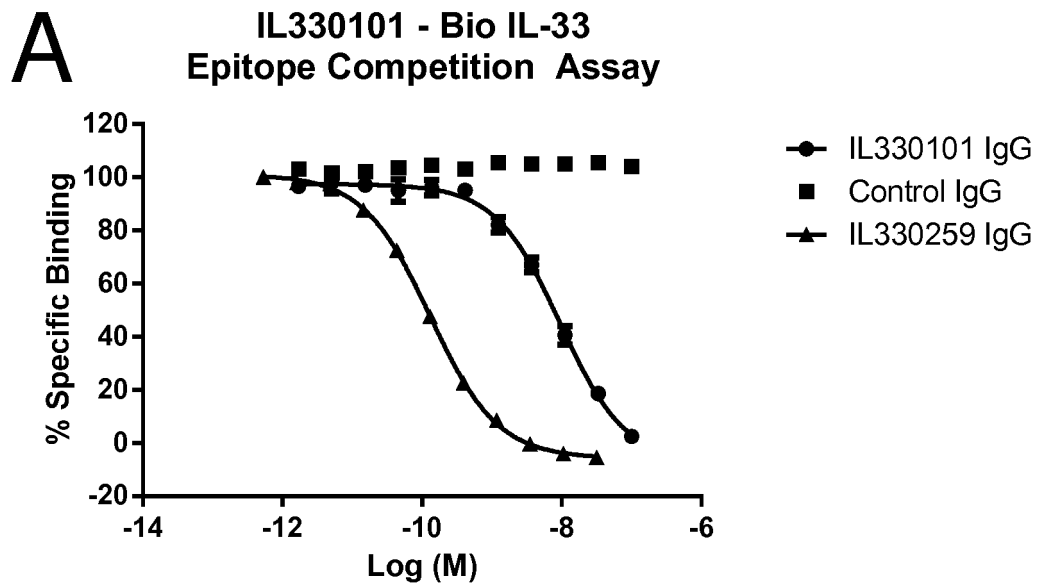


FIGURE 14

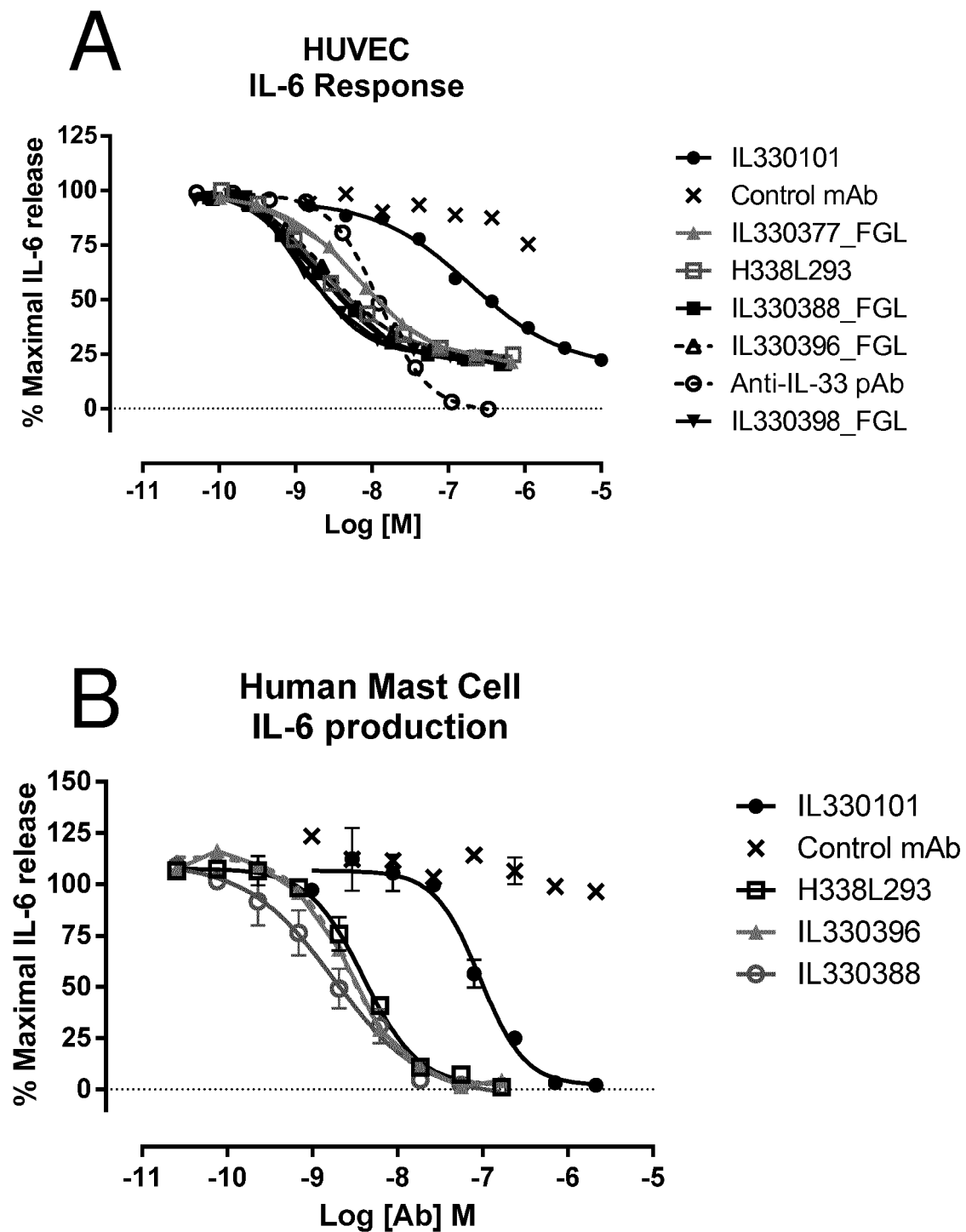


FIGURE 15

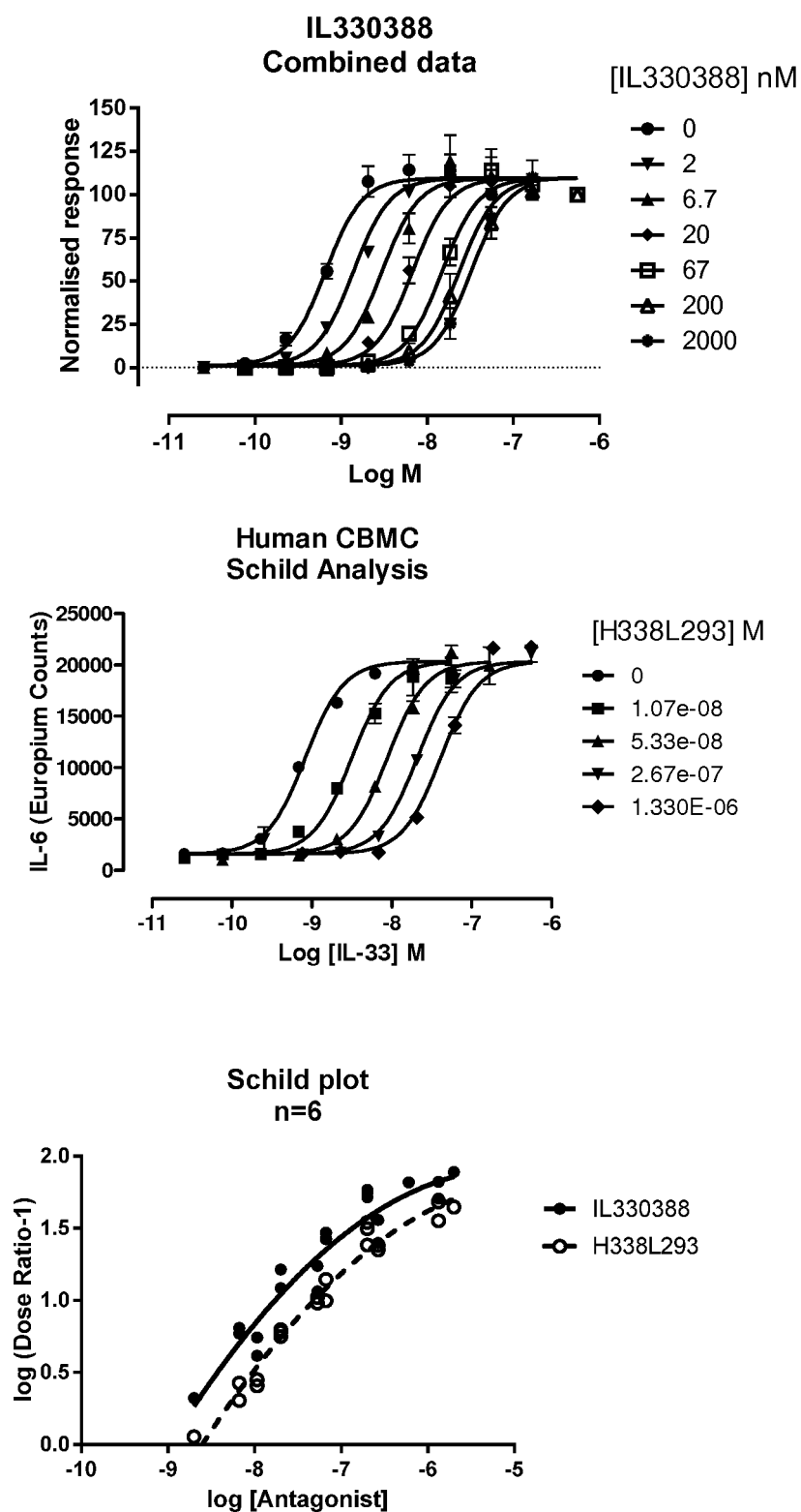


FIGURE 16

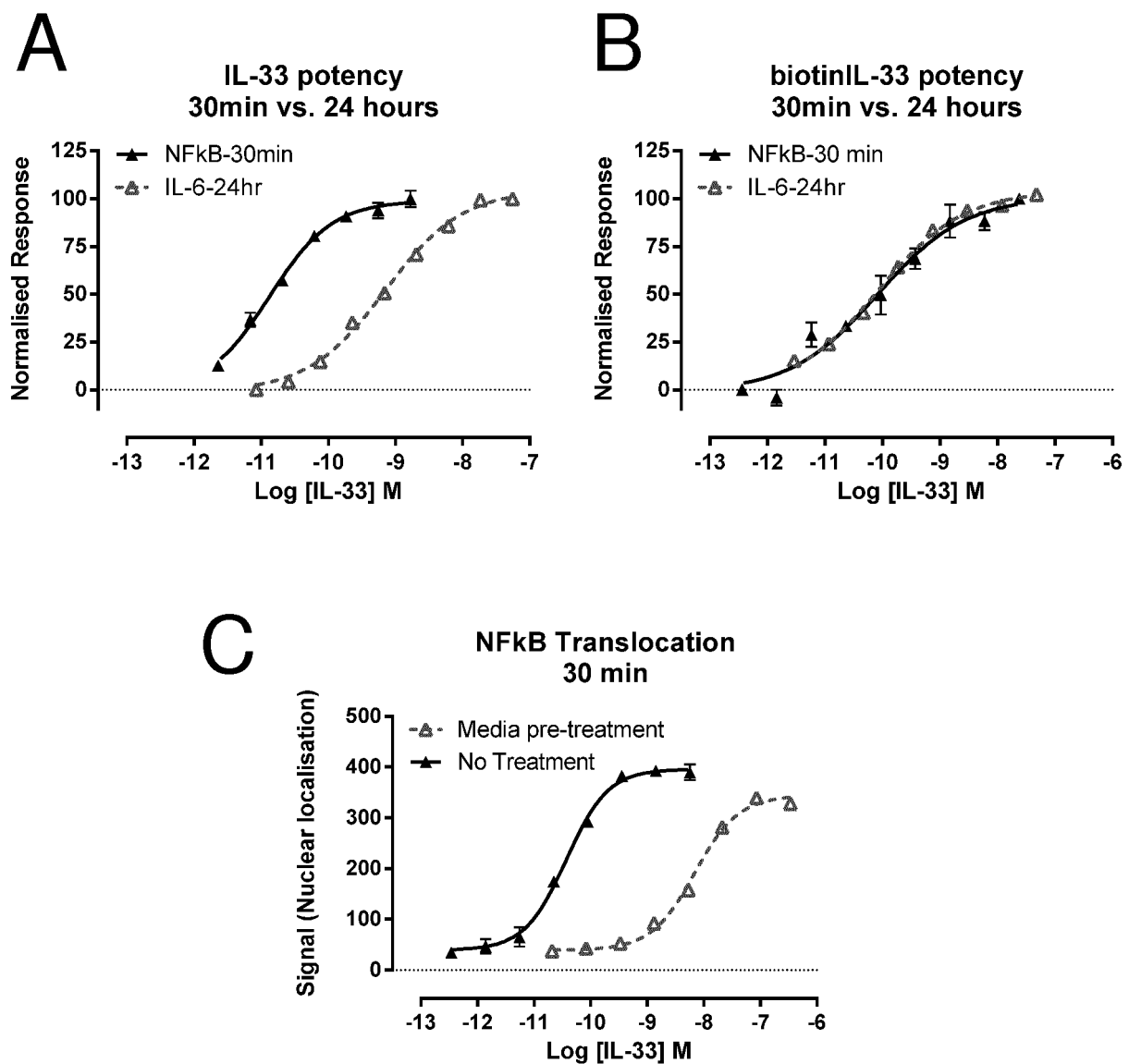


FIGURE 17

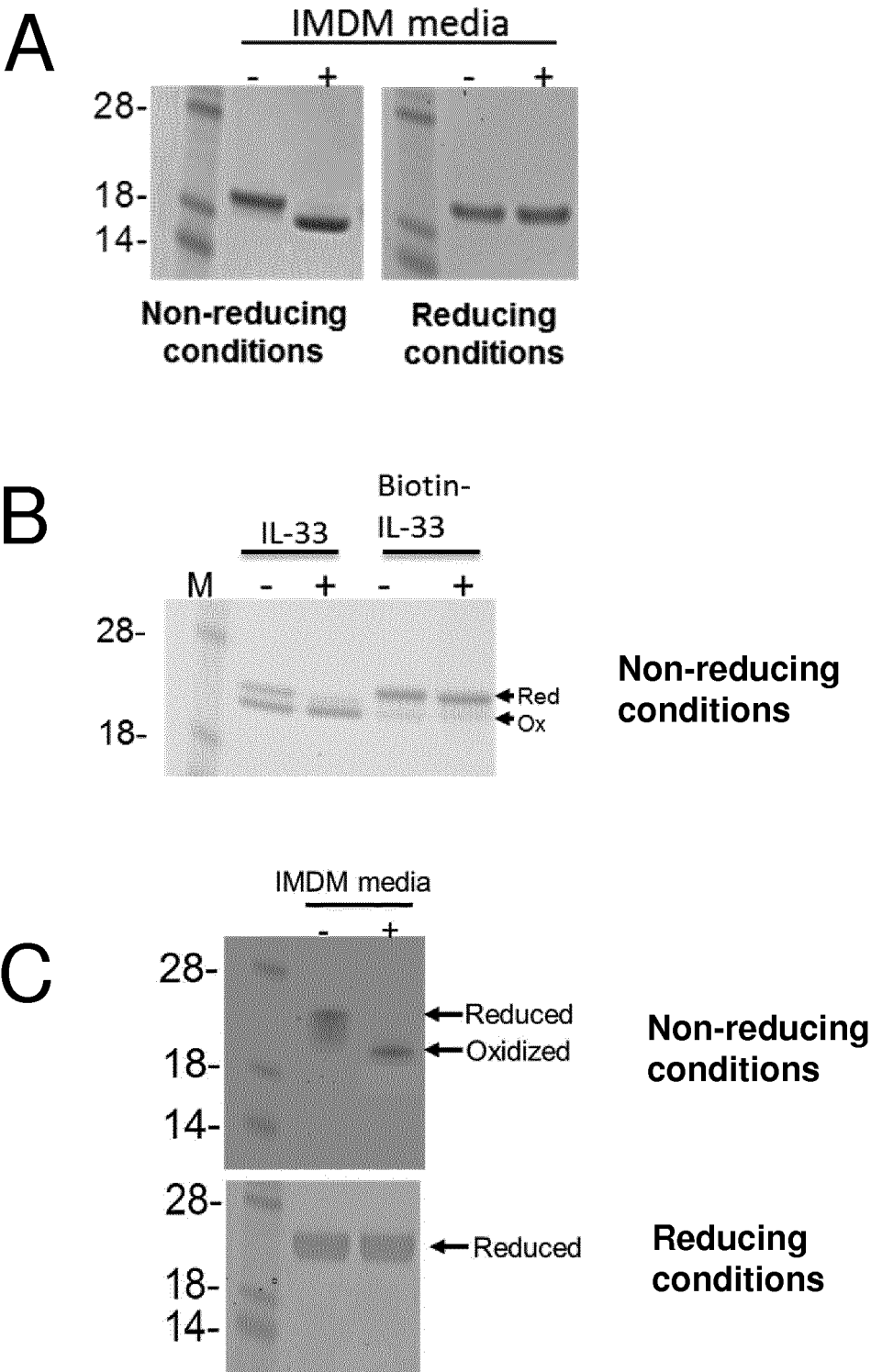


FIGURE 18

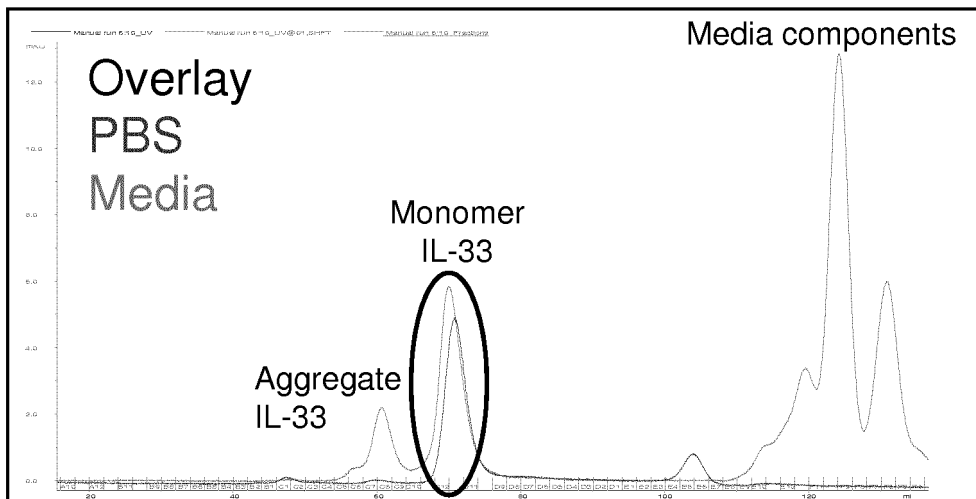
Preparation of oxidised IL-33 for mass-spec analysis

Untagged IL-33 (BK349)

18 hour incubation of IL-33 (300ug/ml) in 60% IMDM or PBS
(37°C, 5% CO₂)



SEC of alternative IL-33 preps



Mass spectrometry analysis

FIGURE 19

Mass Spec analysis – intact mass

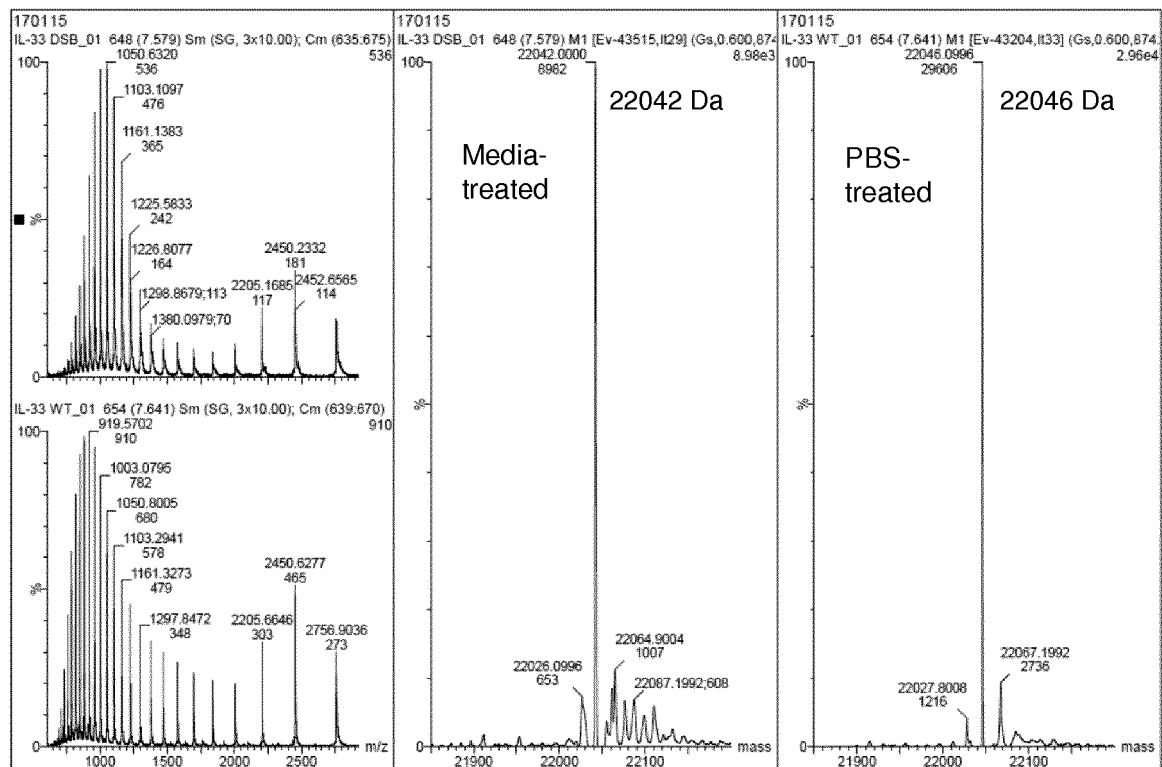


FIGURE 20

FIG. 21A

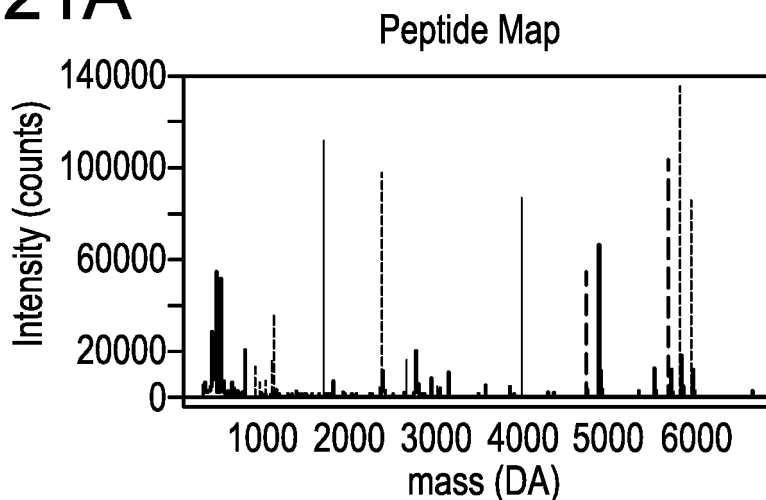


FIG. 21B

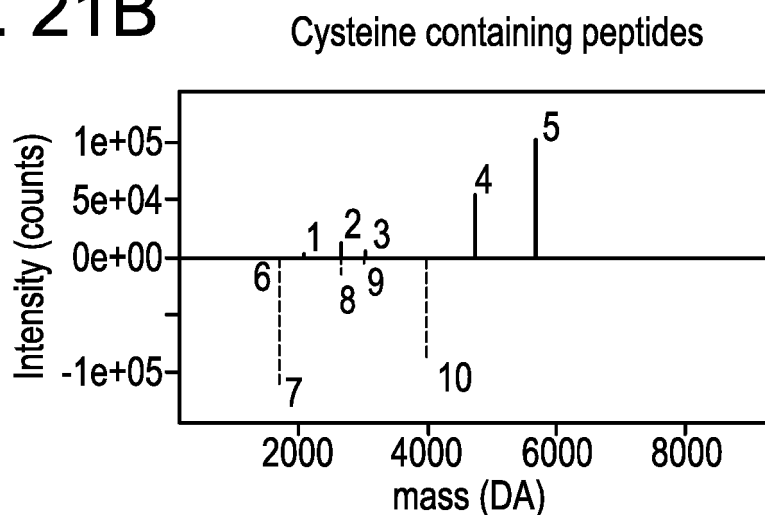


FIG. 21C

Peptides	Sequence Non-reduced Lys-C peptide map	Cys oxidation status
1	C ²⁰⁸ EK--VDSSENI C ²⁵⁹ TENILFK	SS
2	PLPDQAFFVLHNMHSN C ²²⁷ VSFE C ²³² K	SS
3	[EHSVELHK][C ²⁰⁸ EK]--VDSSENI C ²⁵⁹ TENILFK	SS
4	[C ²⁰⁸ EK][PLPDQAFFVLHNMHSN C ²²⁷ VSFE C ²³² K]--VDSSENI C ²⁵⁹ TENILFK	SS
5	[EHSVELHK][C ²⁰⁸ EK][PLPDQAFFVLHNMHSN C ²²⁷ VSFE C ²³² K]--VDSSENI C ²⁵⁹ TENILFK	SS
Reduced Lys-C peptide map		
6	[EHSVELHK][C ²⁰⁸ EK]	SH
7	VDSSENI C ²⁵⁹ TENILFK	SH
8	PLPDQAFFVLHNMHSN C ²²⁷ VSFE C ²³² K	SH
9	[C ²⁰⁸ EK][PLPDQAFFVLHNMHSN C ²²⁷ VSFE C ²³² K]	SH
10	[EHSVELHK][C ²⁰⁸ EK][PLPDQAFFVLHNMHSN C ²²⁷ VSFE C ²³² K]	SH

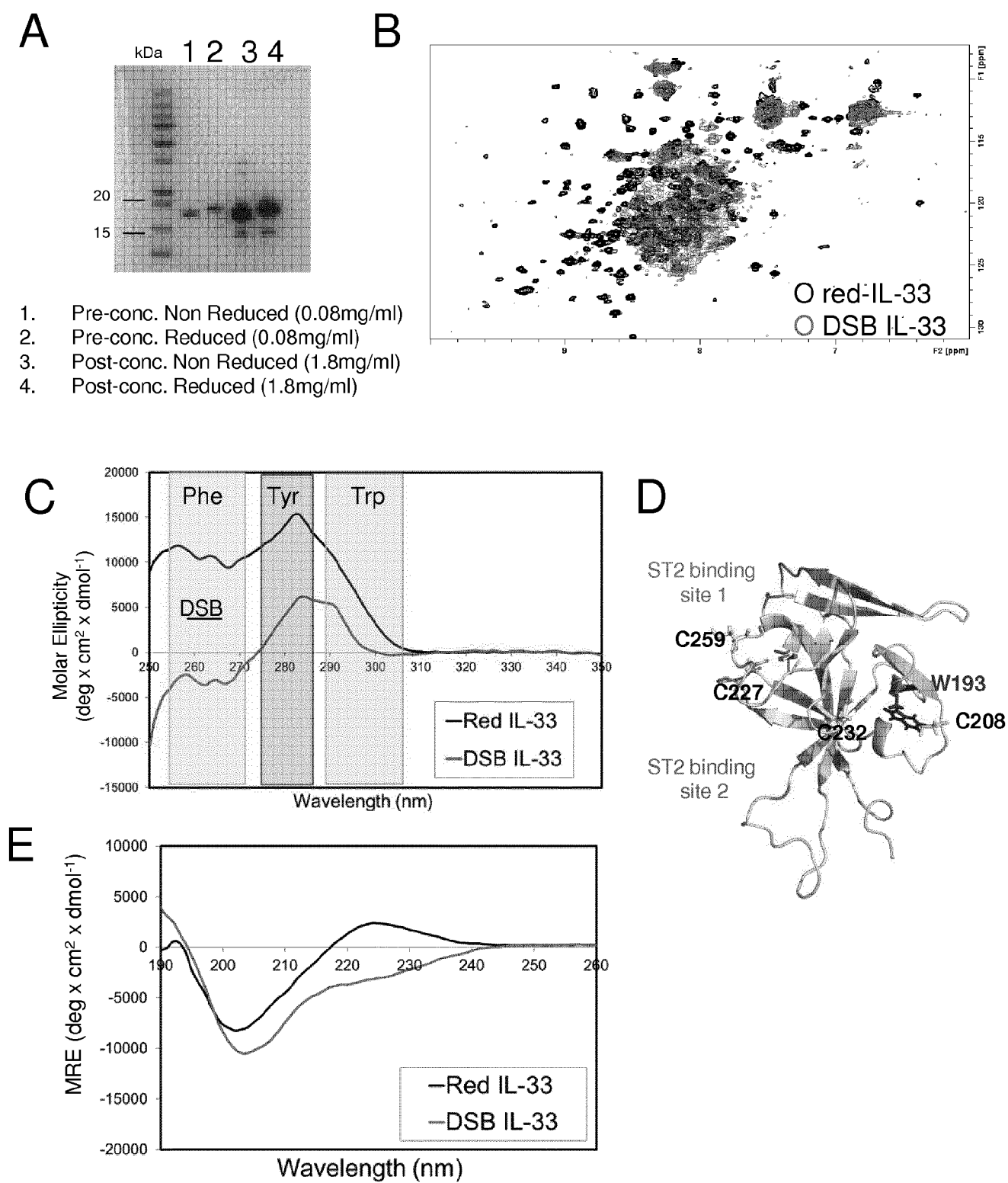


FIGURE 22

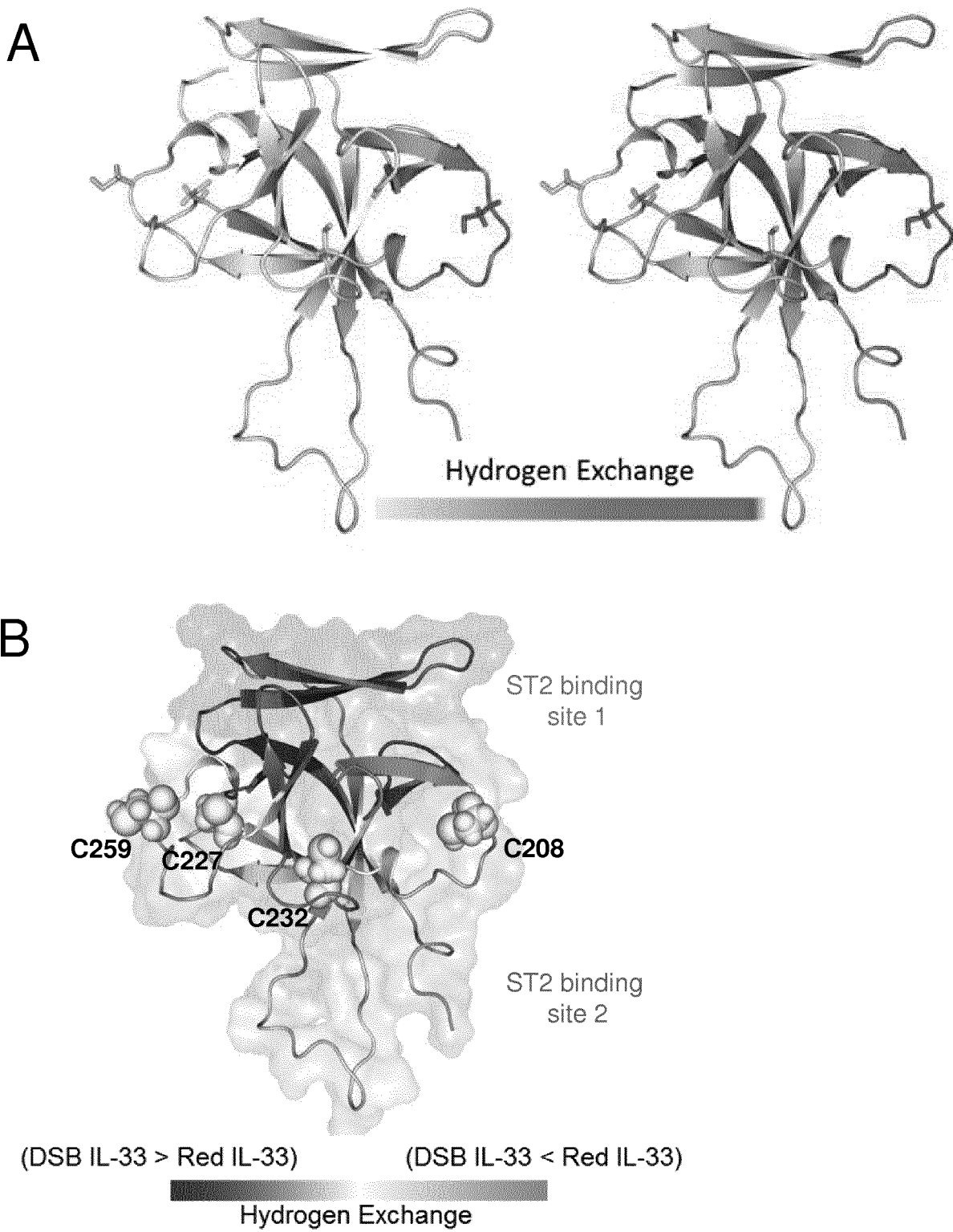
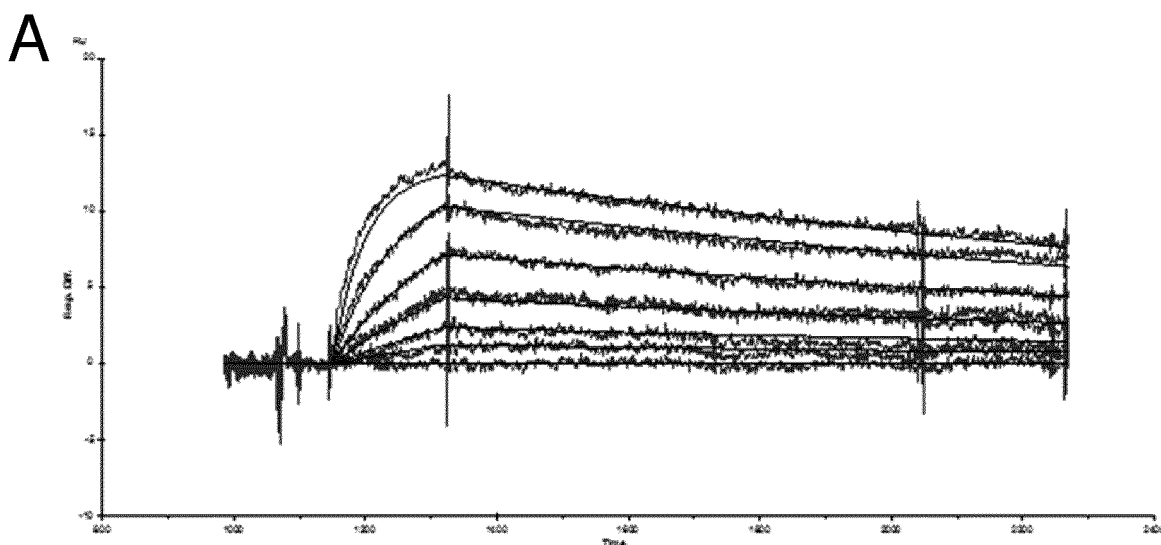


FIGURE 23

Reduced IL33 binding to ST2

Sensorgrams from 7.8nM to 0.24nM are shown giving a K_D of 0.2nM,



B

DSB IL33 binding to ST2

Sensorgrams from 500nM to 0.24nM with no obvious binding

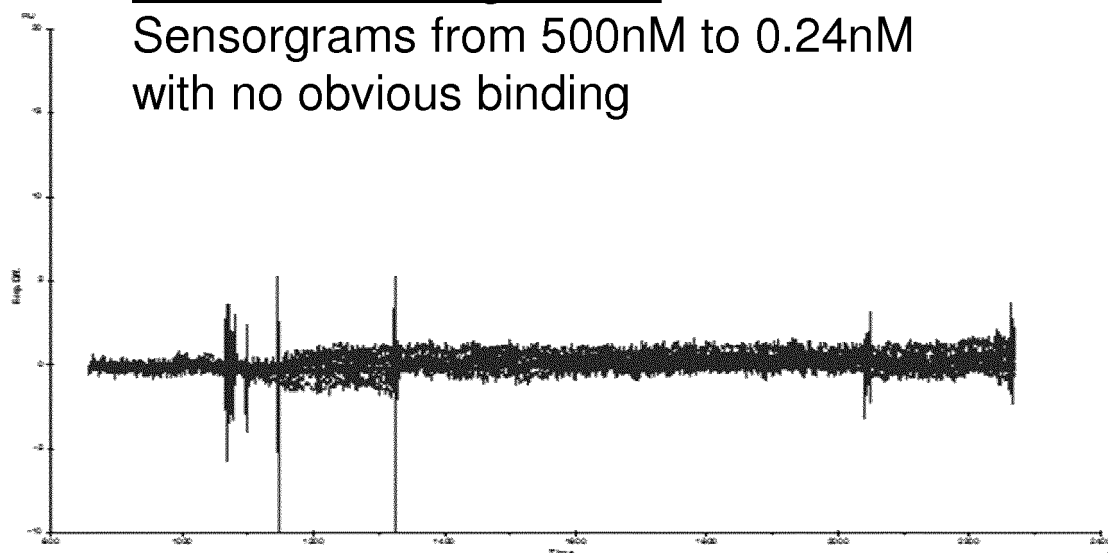
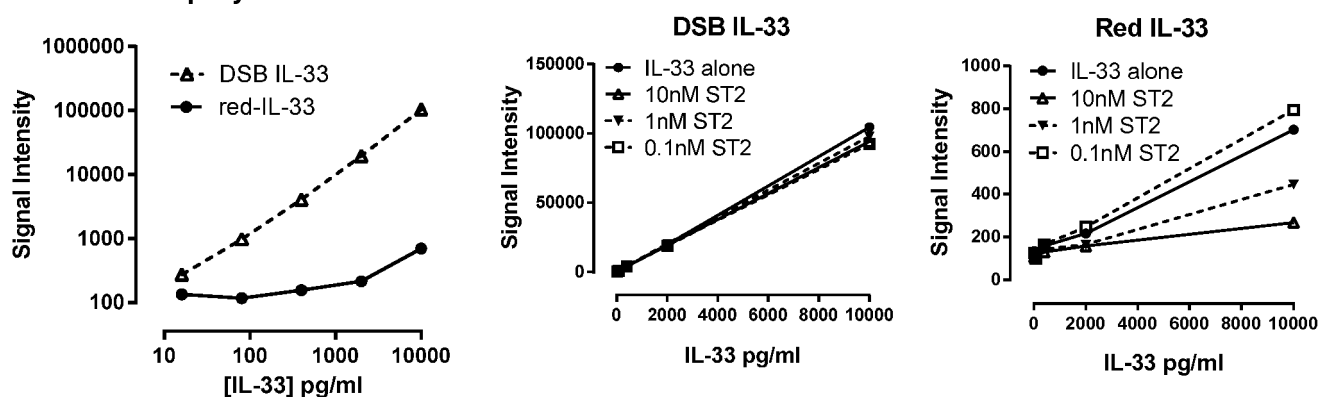
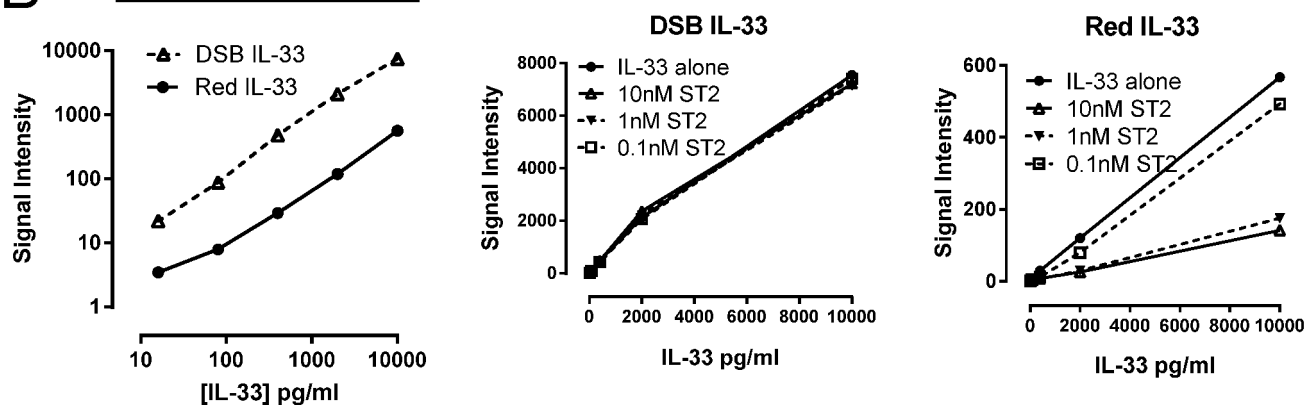


FIGURE 24

A RnD Systems – human IL-33 Goat polyclonal capture Goat polyclonal detection



B Luminex-human IL-33



C RnD Systems – mouse IL-33 Goat polyclonal capture Goat polyclonal detection

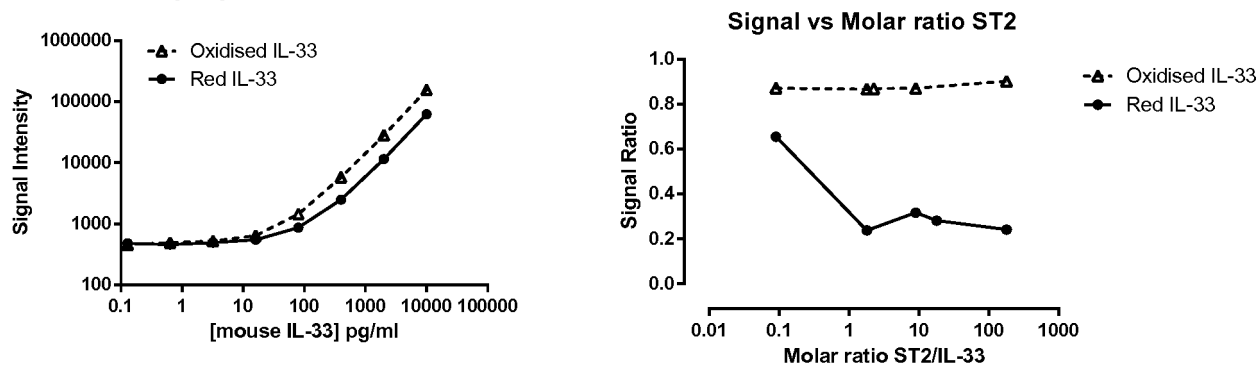


FIGURE 25

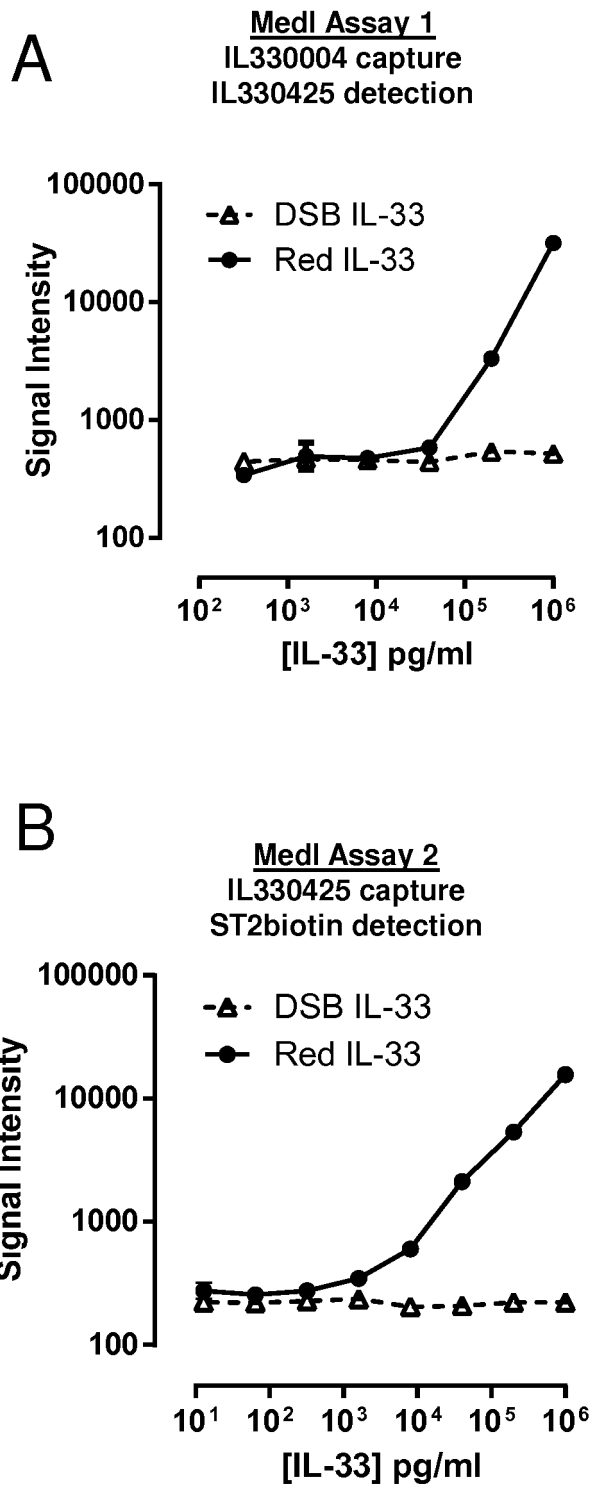


FIGURE 26

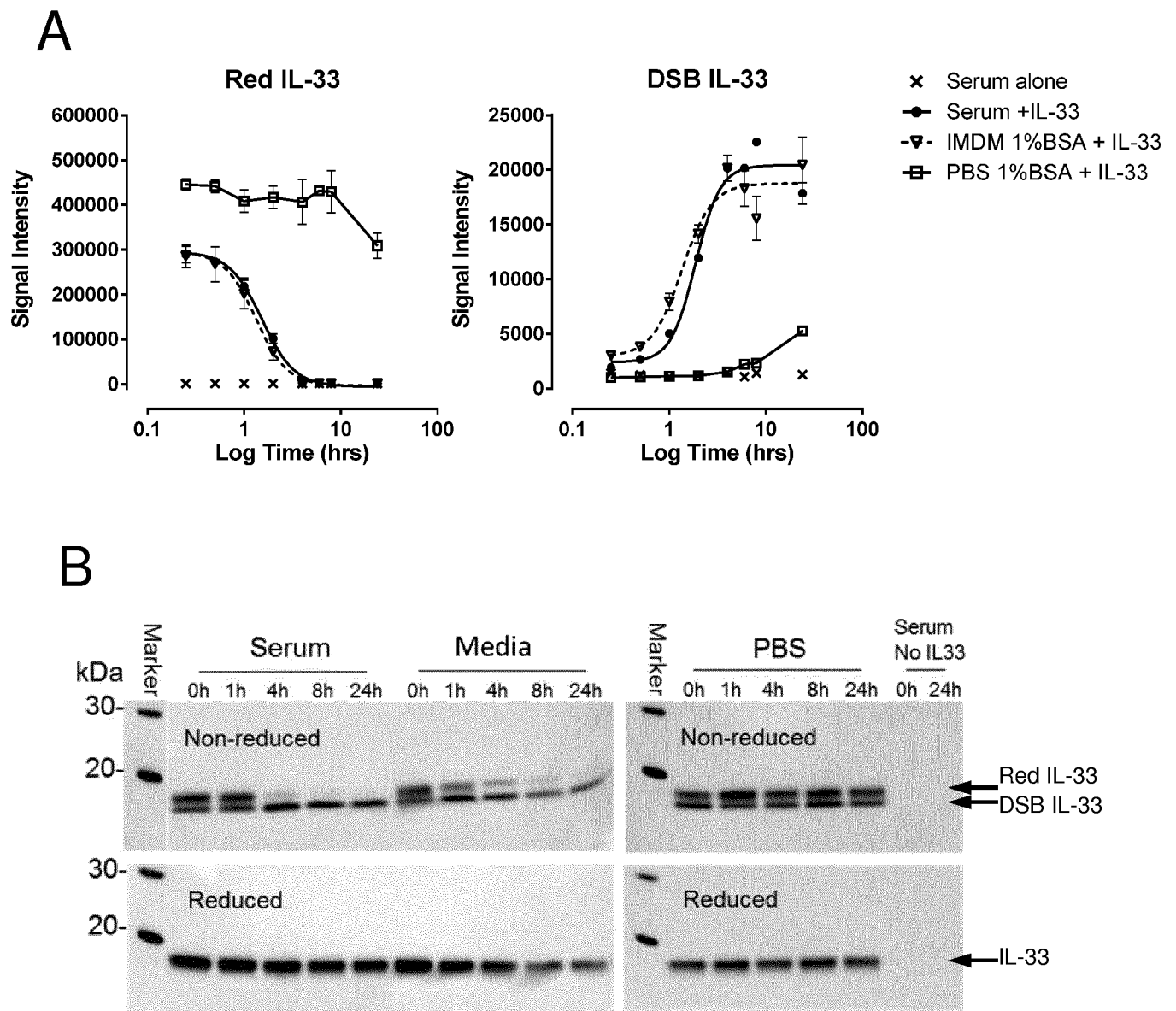
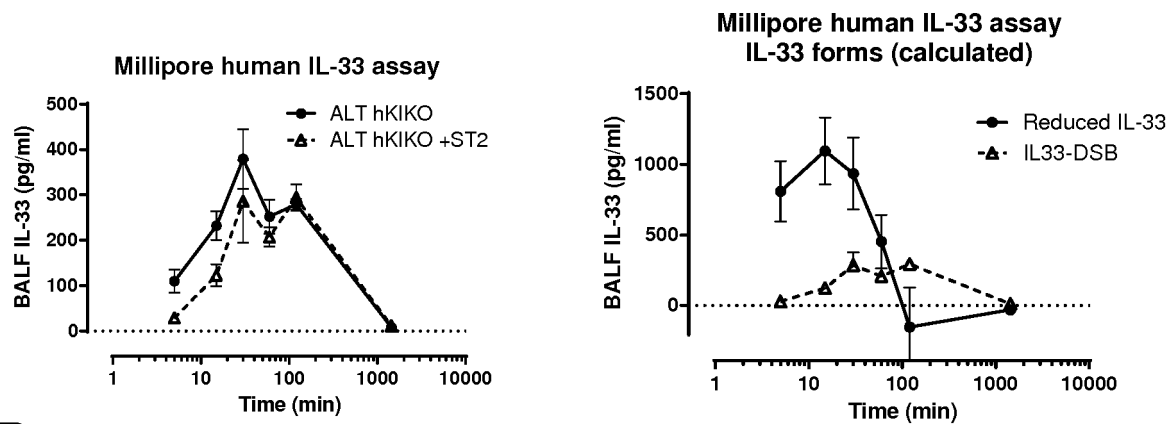
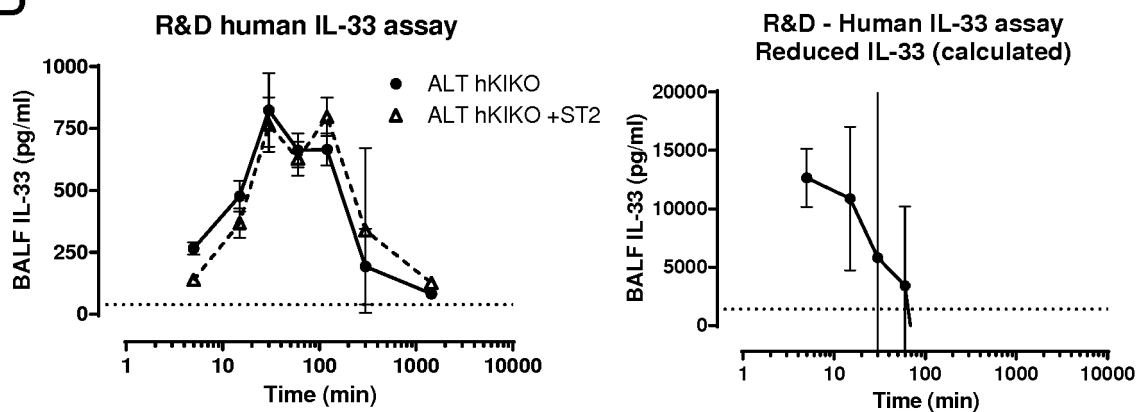


FIGURE 27

A



B



C

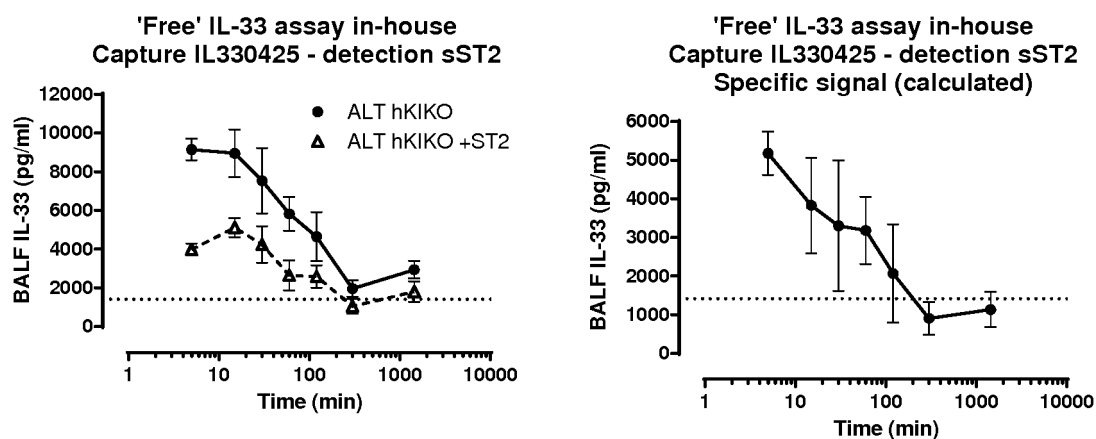


FIGURE 28

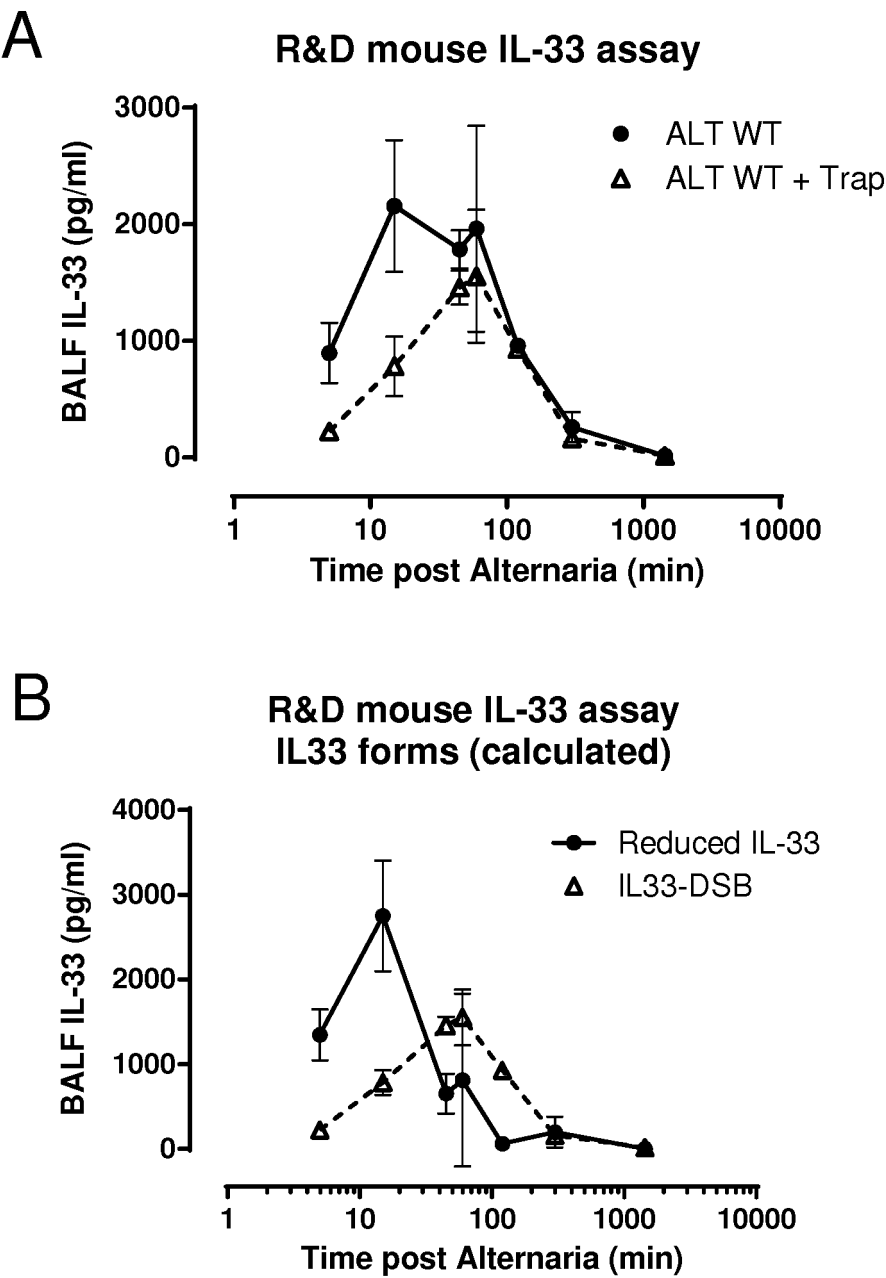


FIGURE 29

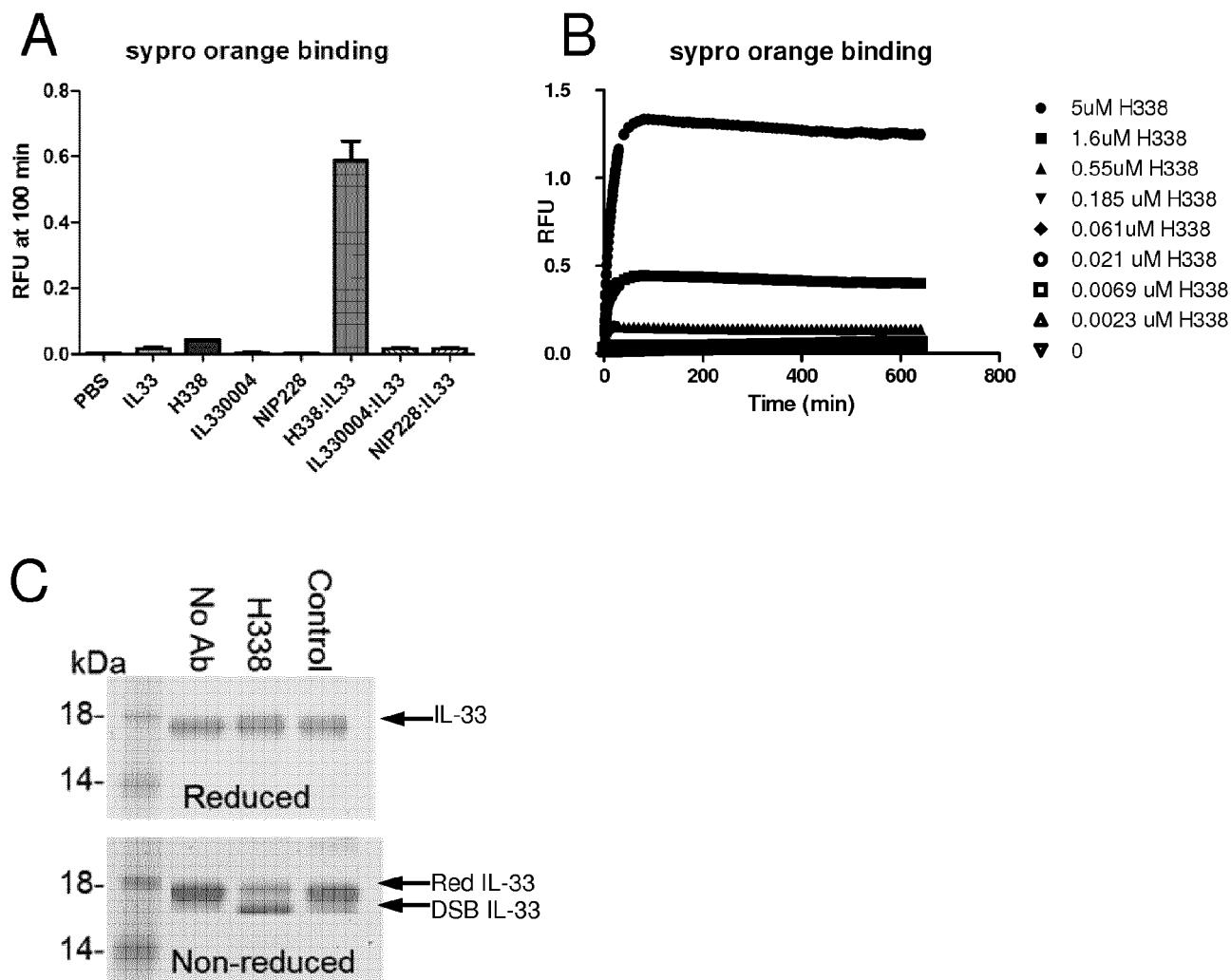


FIGURE 30

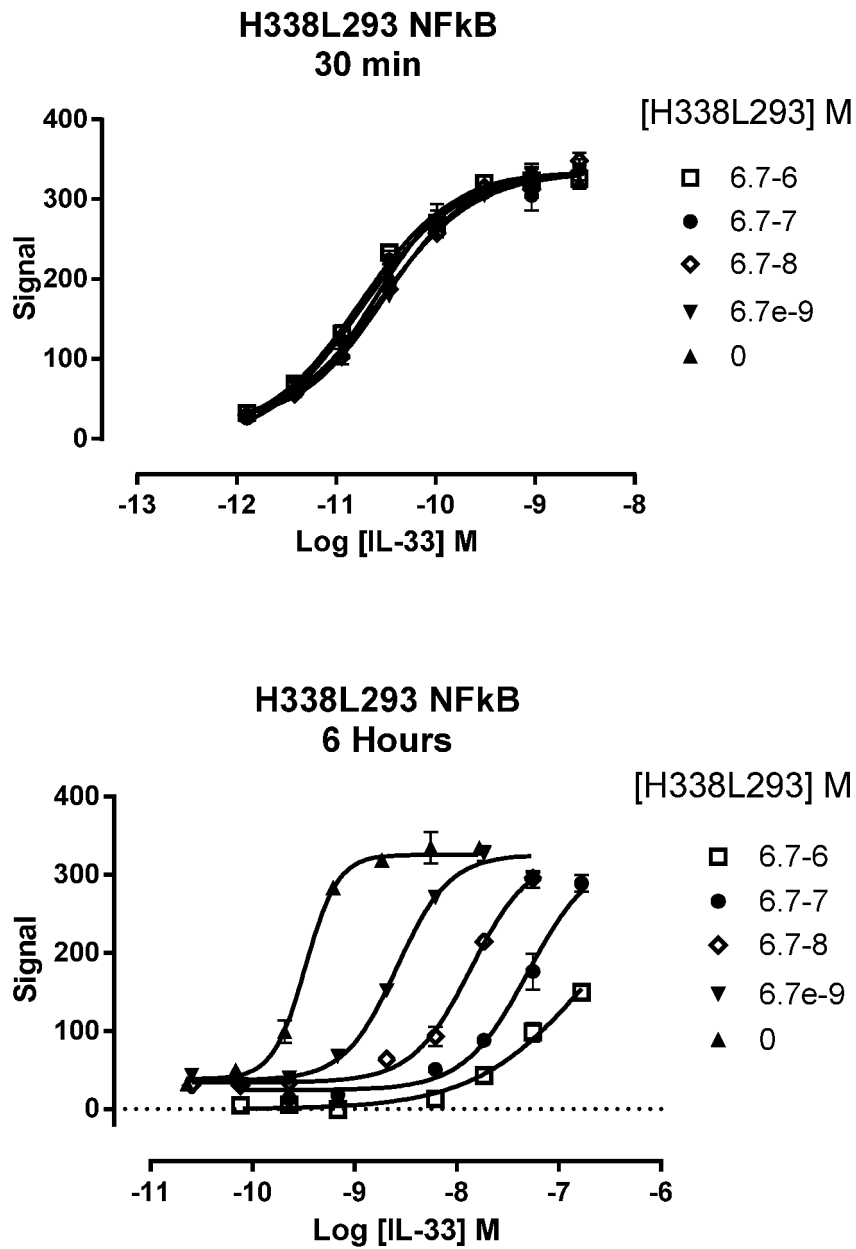
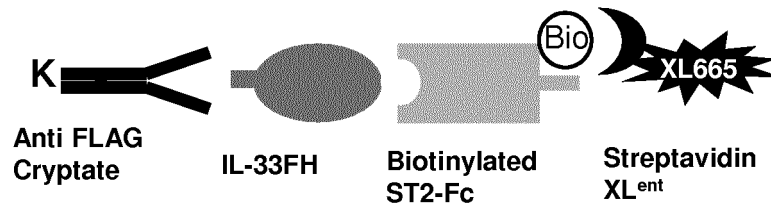
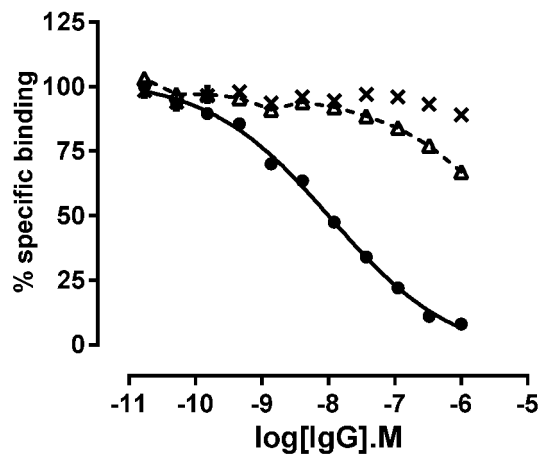


FIGURE 31

IL33-ST2 Binding Assay



Competition



IL-33:mAb pre-incubation

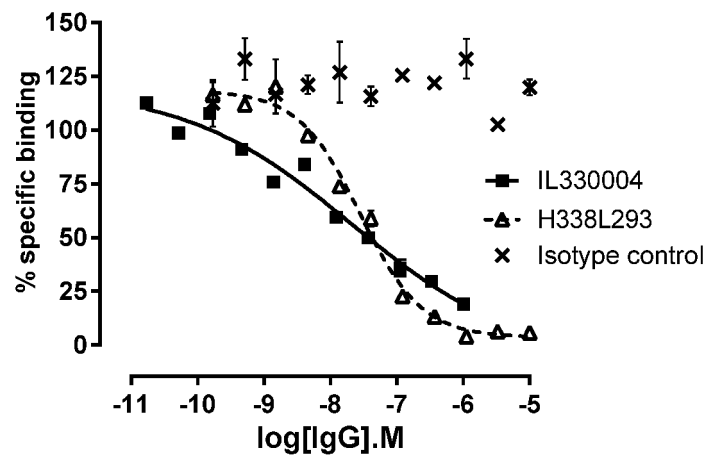
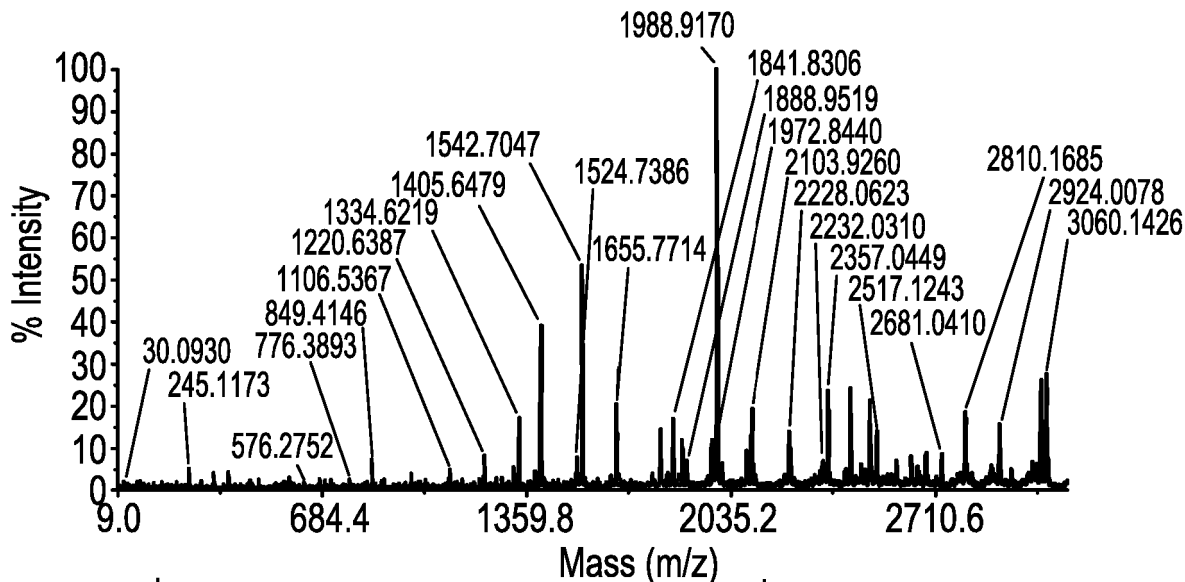


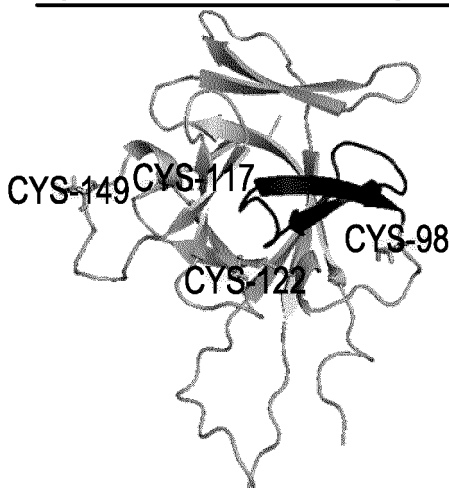
FIGURE 32

FIG. 33A

MALDI-TOF MS-MS fragmentation analysis of 3,206 Da precursor ion

MLMVTLSPTKDFWLHANNKEHSVELHK

b				v				b				v			
1	M	27													
245.1318	2	L	26	3074.5880				1800.9175	15	H	13	1542.7772			
376.1723	3	M	25	2961.5040				1871.9547	16	A	12	1405.7183			
475.2407	4	V	24	2830.4635				1985.9976	17	N	11	1334.6811			
576.2884	5	T	23	2731.3951				2100.0405	18	N	10	1220.6382			
689.3725	6	L	22	2630.3474				2228.1355	19	K	9	1106.5953			
776.4045	7	S	21	2517.2633				2357.1781	20	E	8	978.5003			
873.4573	8	P	20	2430.2313				2494.2370	21	H	7	849.4577			
974.5049	9	T	19	2333.1785				2581.2690	22	S	6	712.3988			
1102.5999	10	K	18	2232.1309				2680.3374	23	V	5	625.3668			
1217.6268	11	D	17	2104.0359				2809.3800	24	E	4	526.2984			
1364.6953	12	F	16	1989.0090				2922.4641	25	L	3	397.2558			
1550.7746	13	W	15	1841.9405				3059.5230	26	H	2	284.1717			
1663.8586	14	L	14	1655.8612				---	27	K	1	147.1128			

FIG. 33BLSPTKDFWLHANNKEHSVELHK

SUBSTITUTE SHEET (RULE 26)

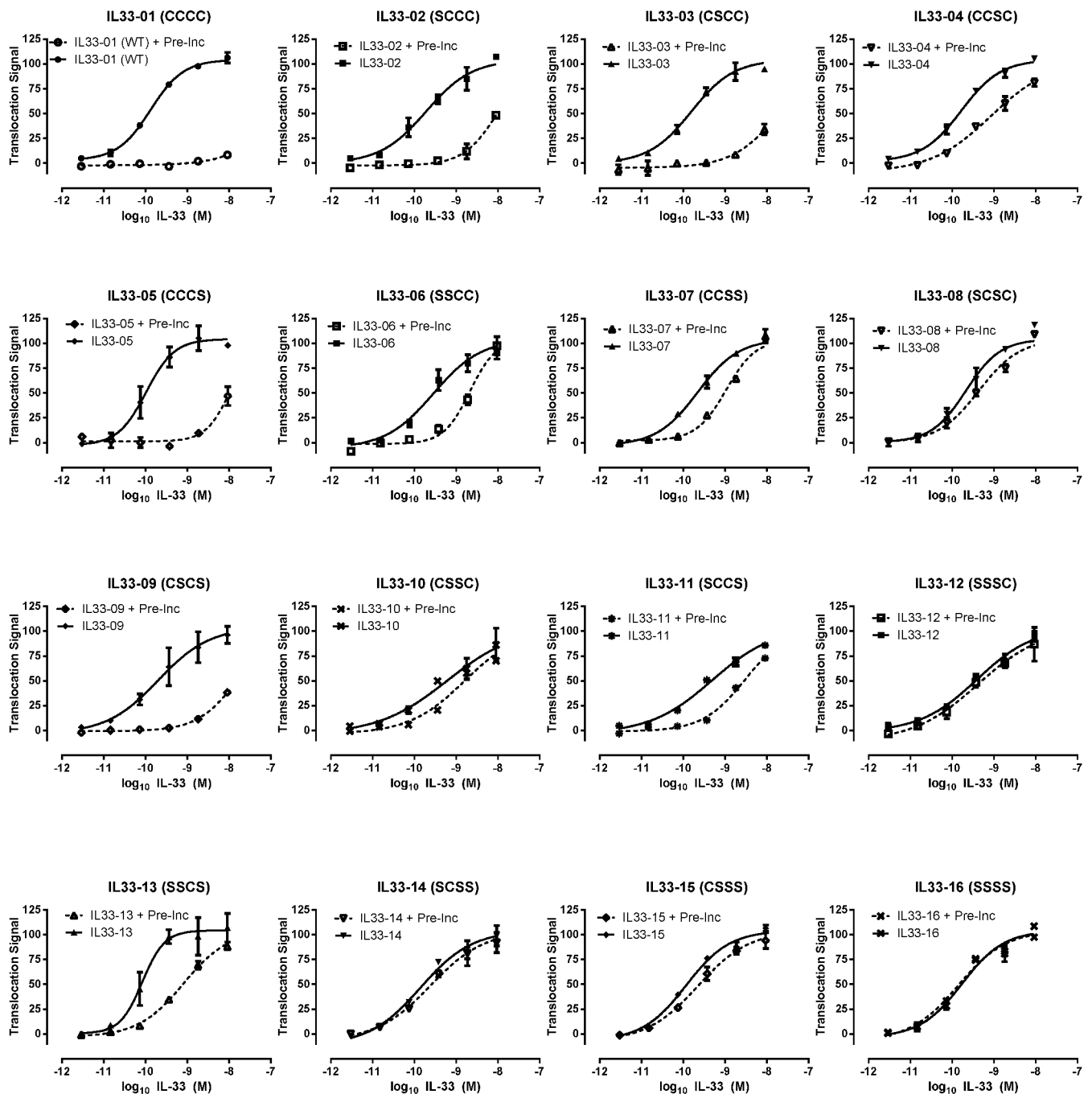


FIGURE 34

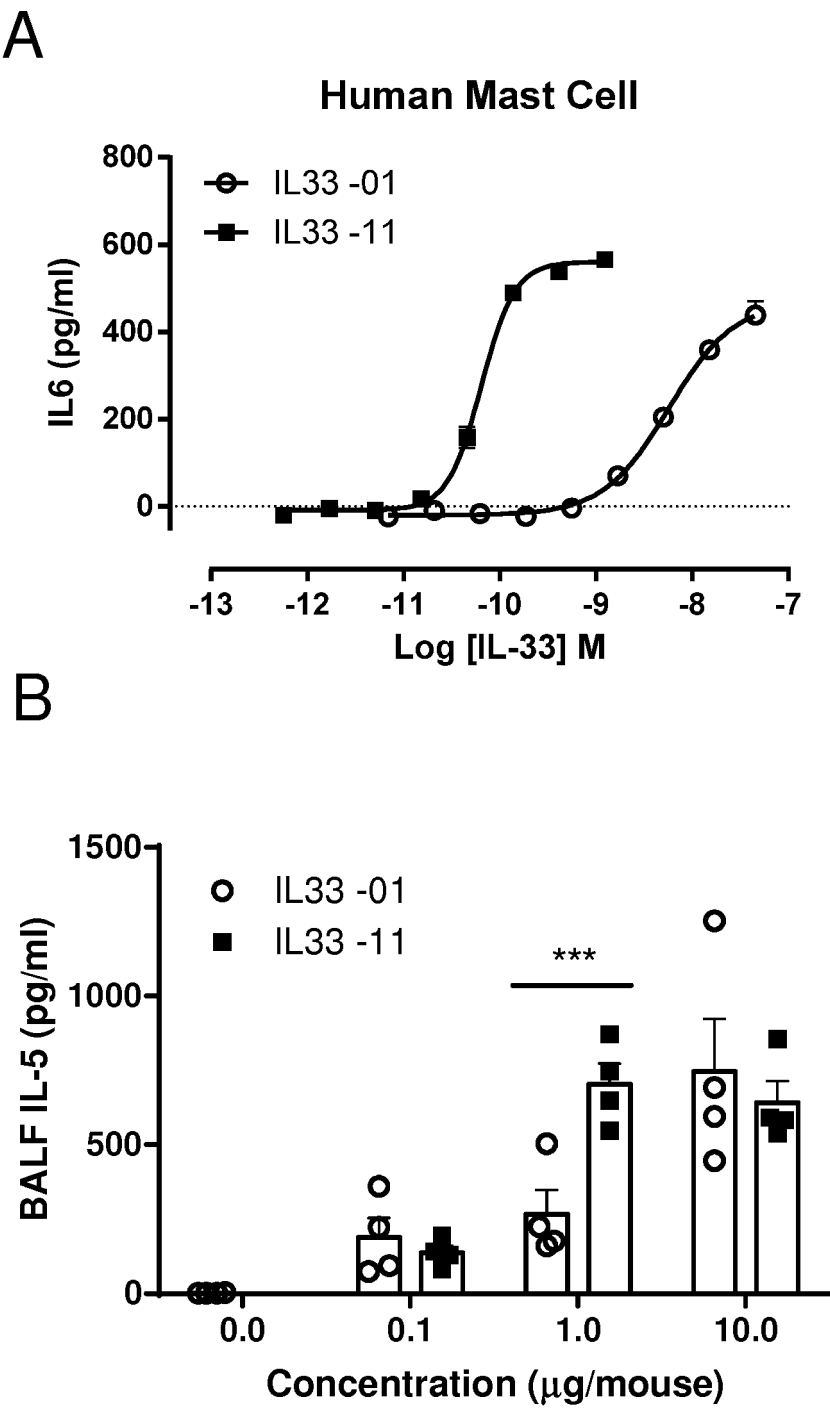


FIGURE 35

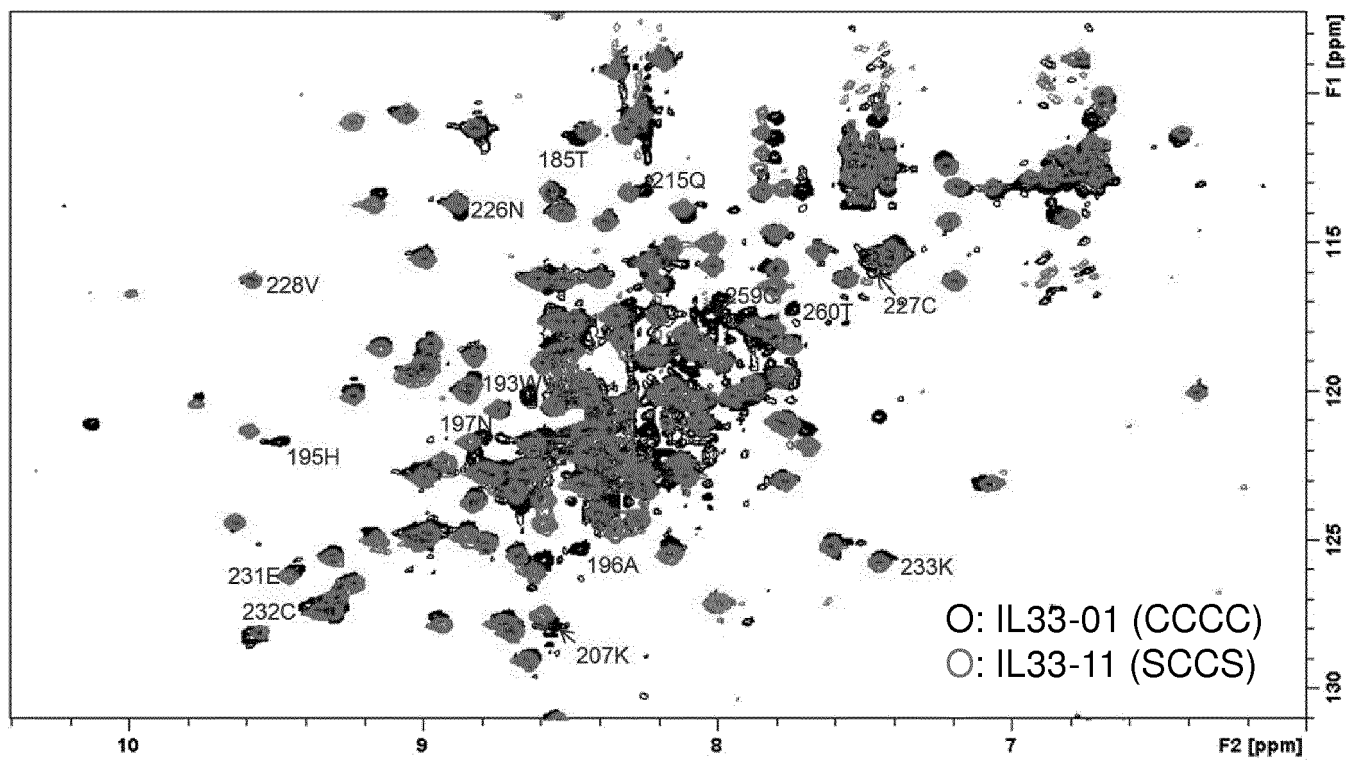


FIGURE 36

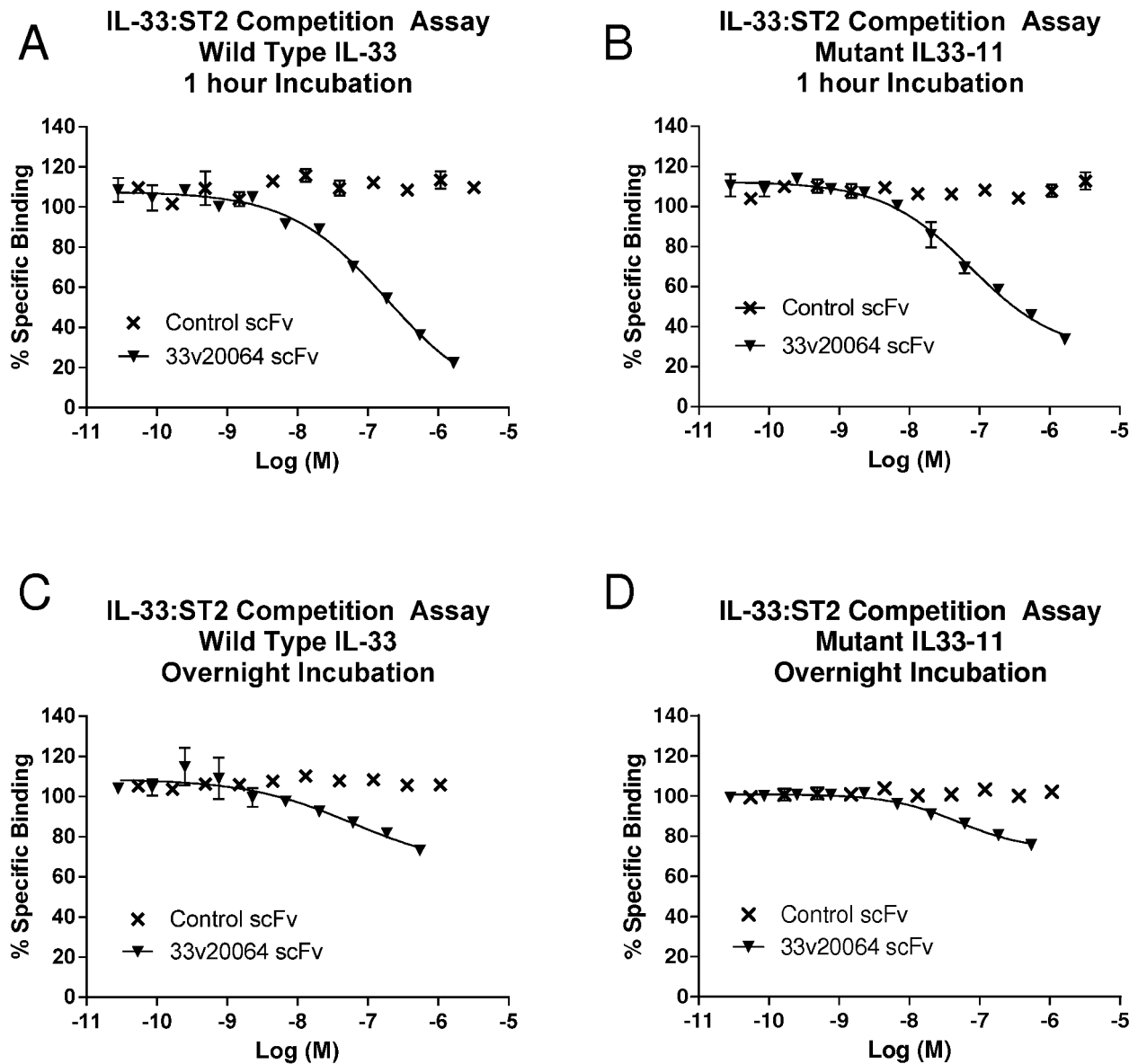


FIGURE 37

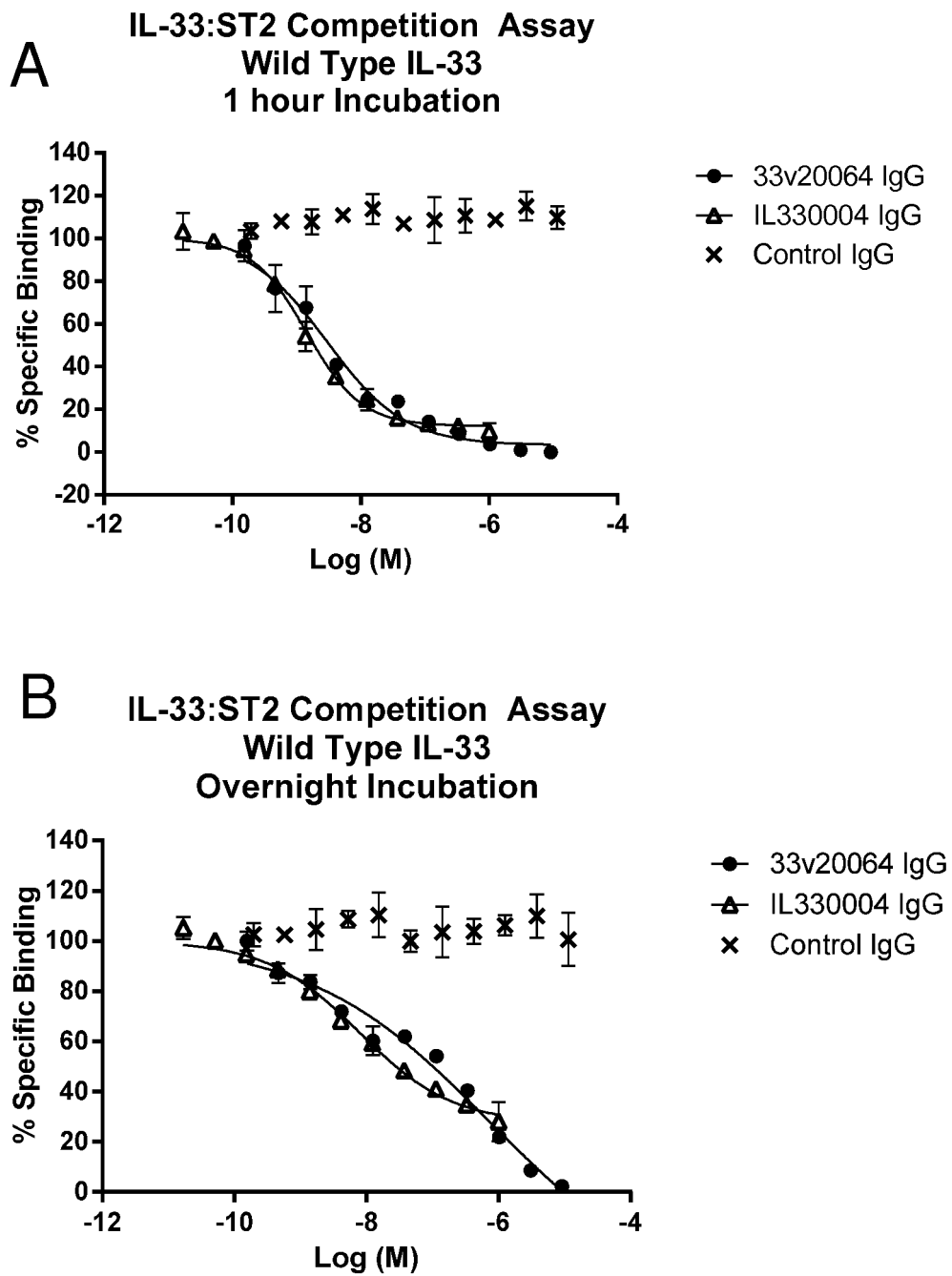


FIGURE 38

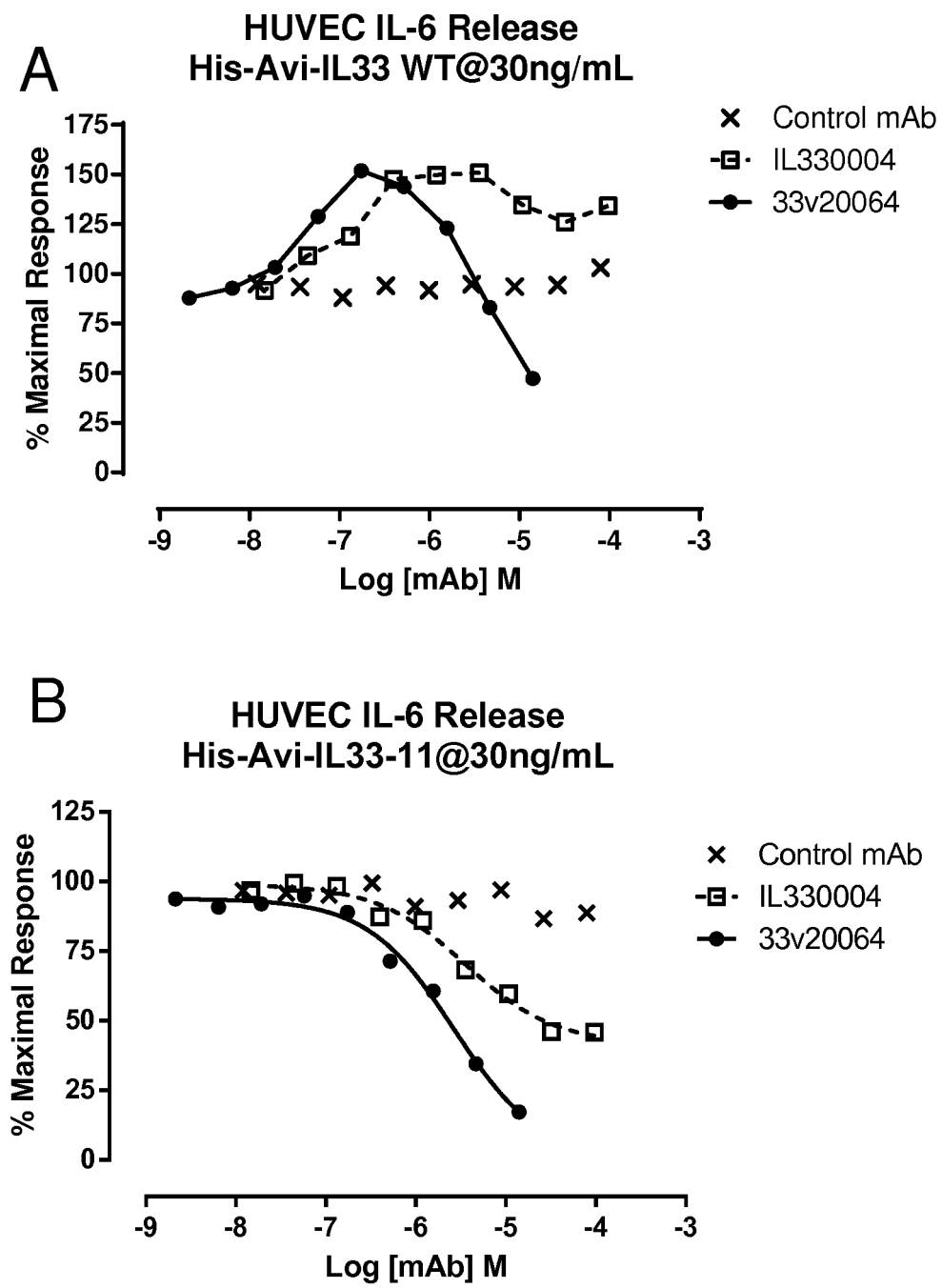


FIGURE 39

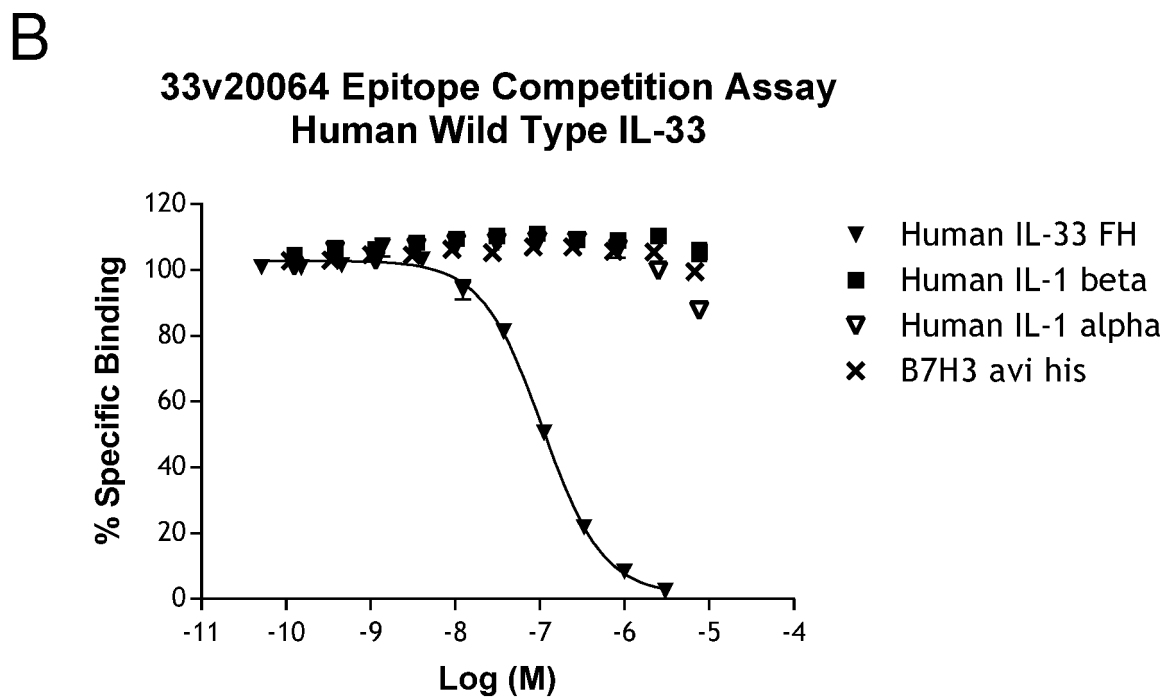
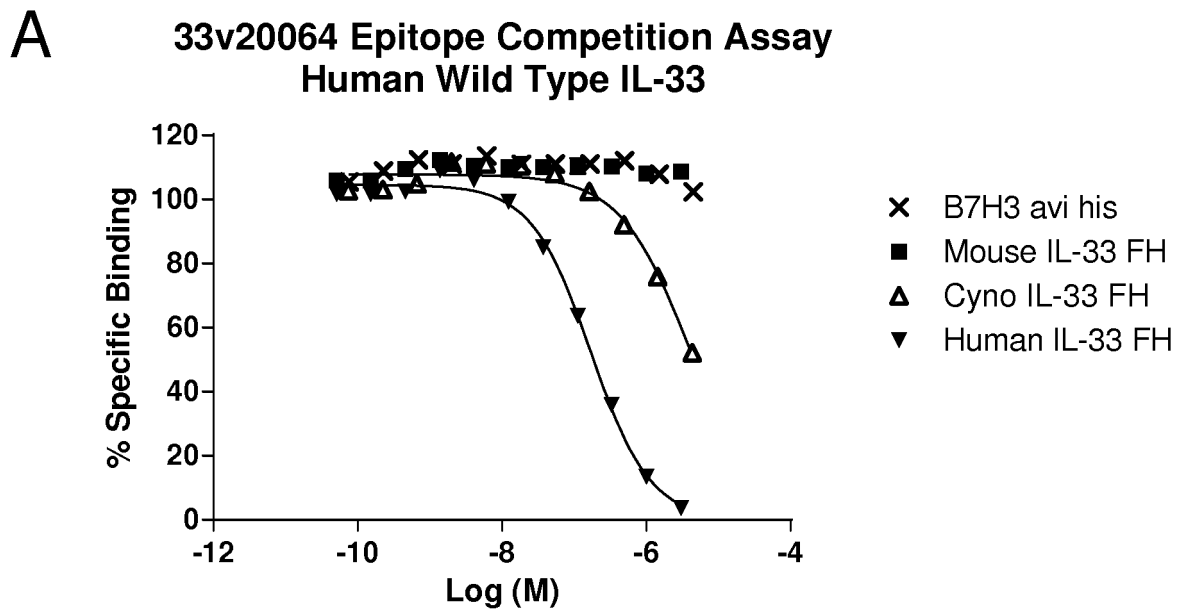


FIGURE 40

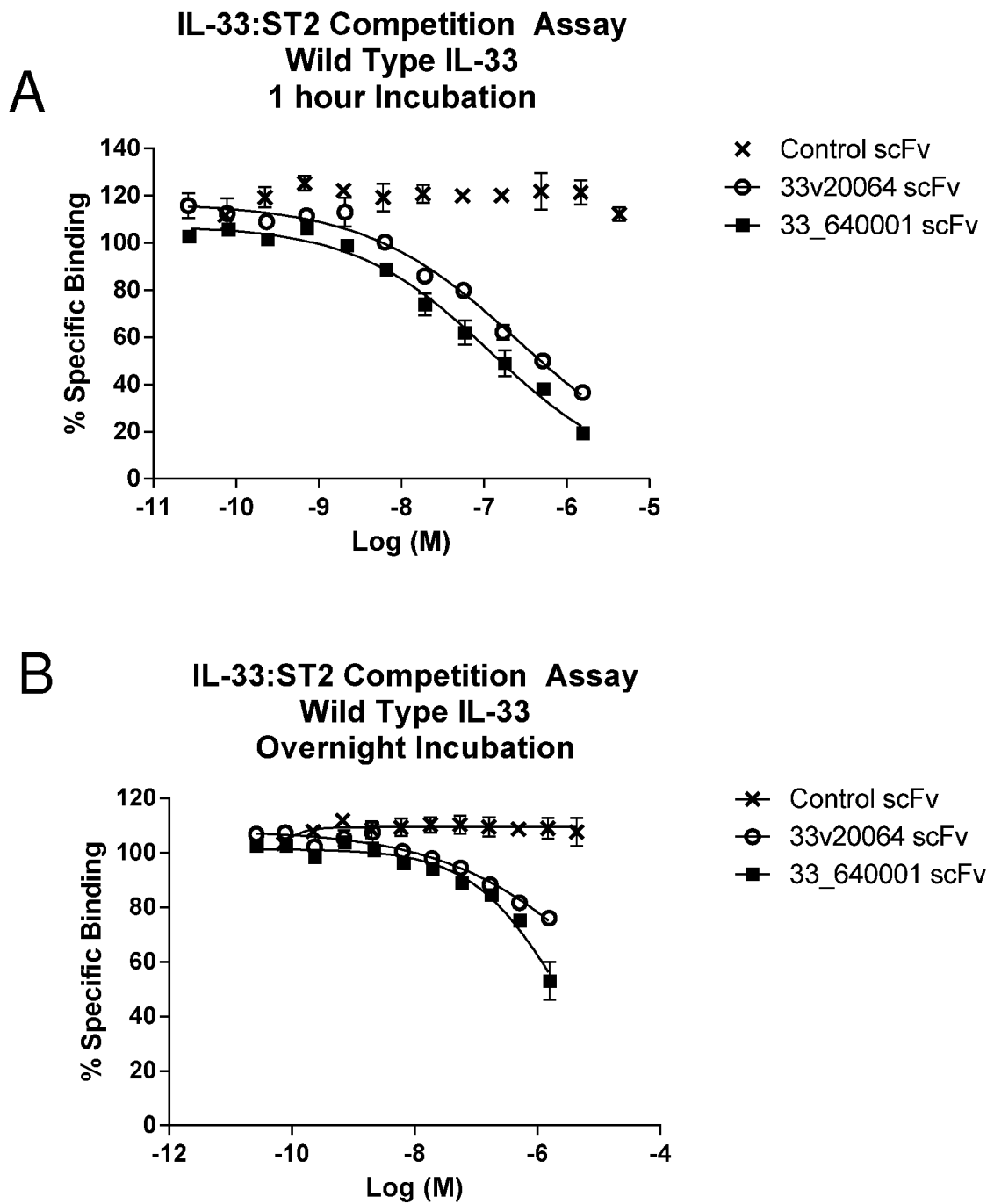


FIGURE 41

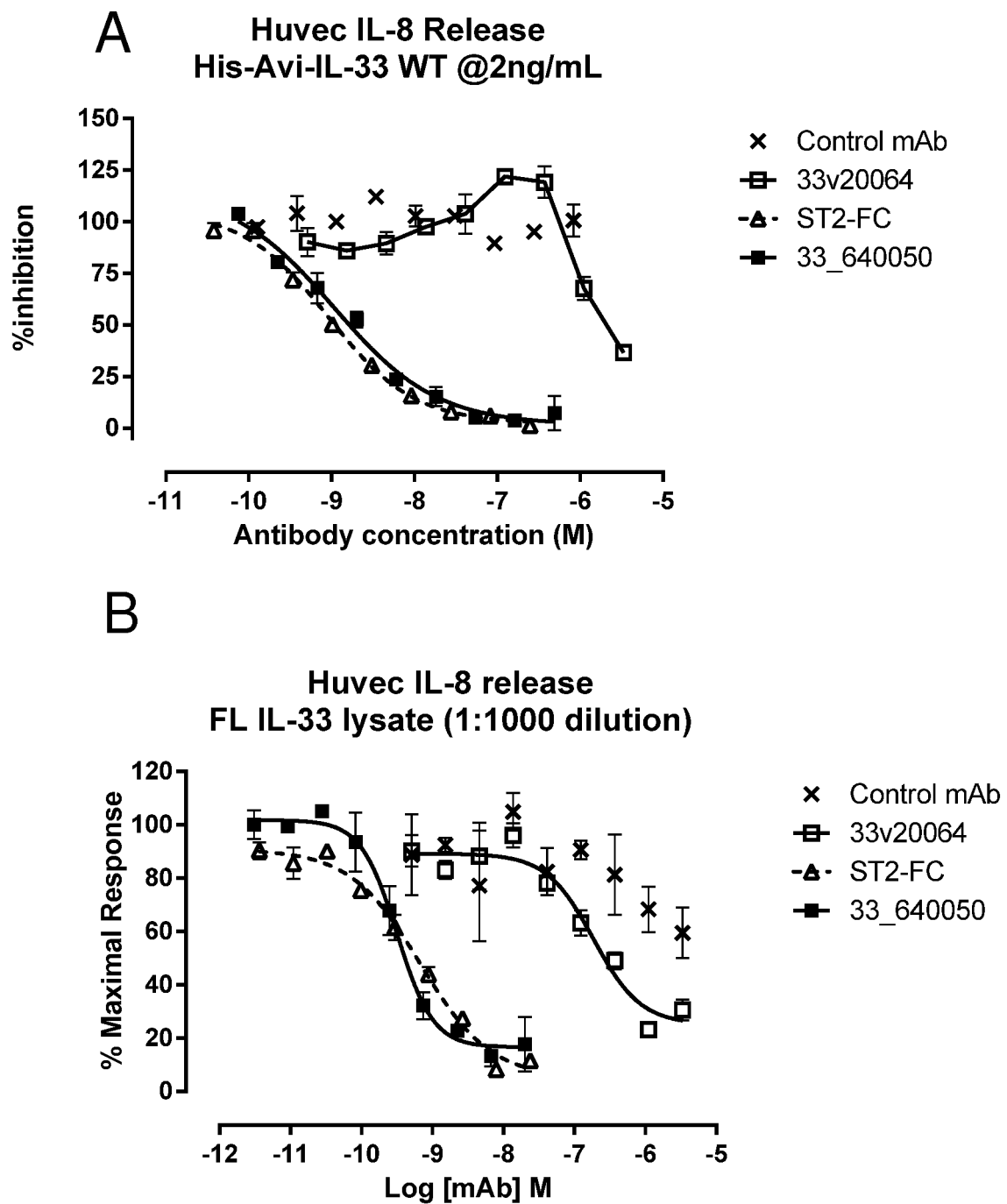
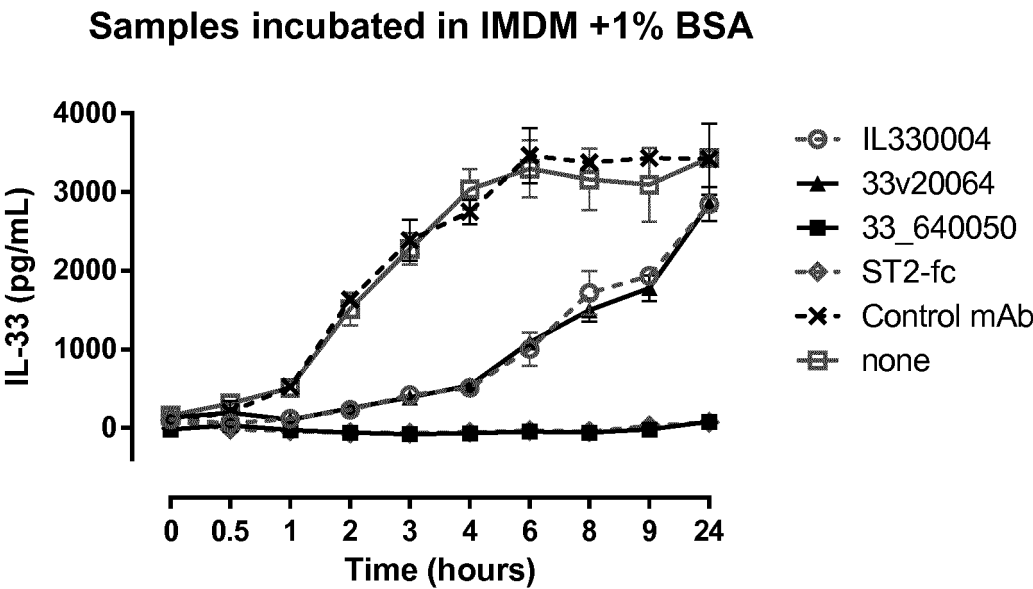


FIGURE 42

A



B

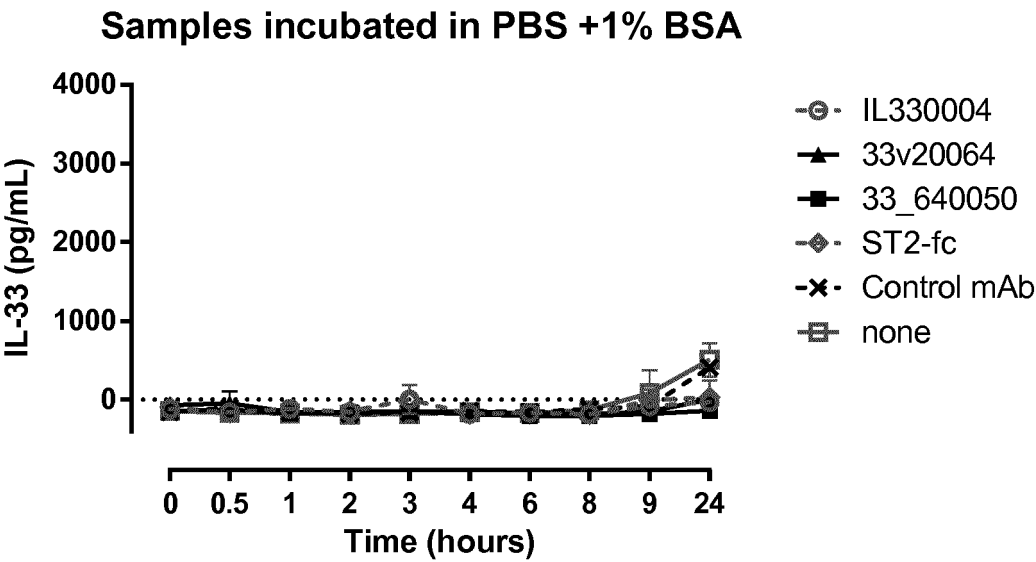


FIGURE 43

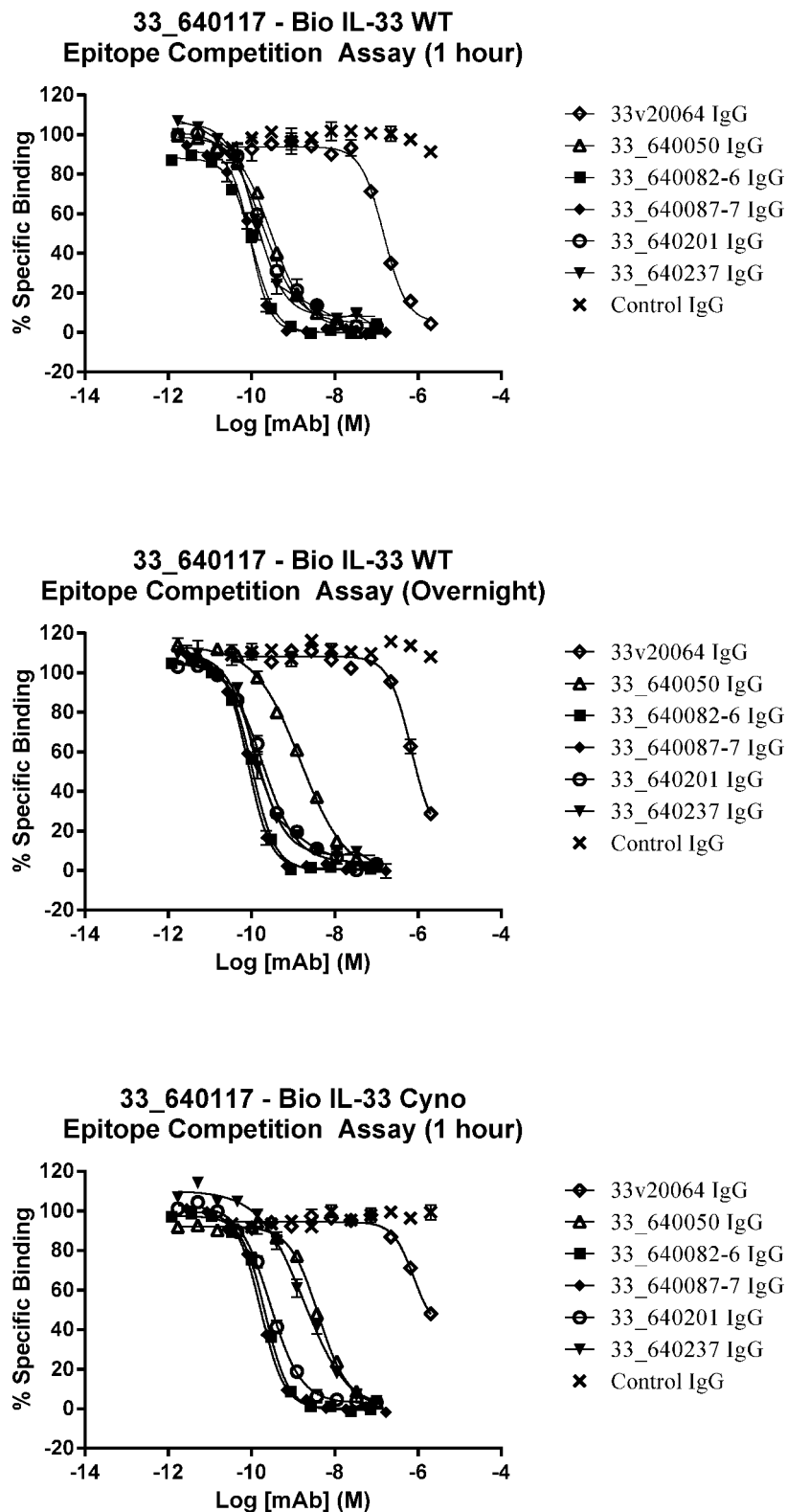


FIGURE 44

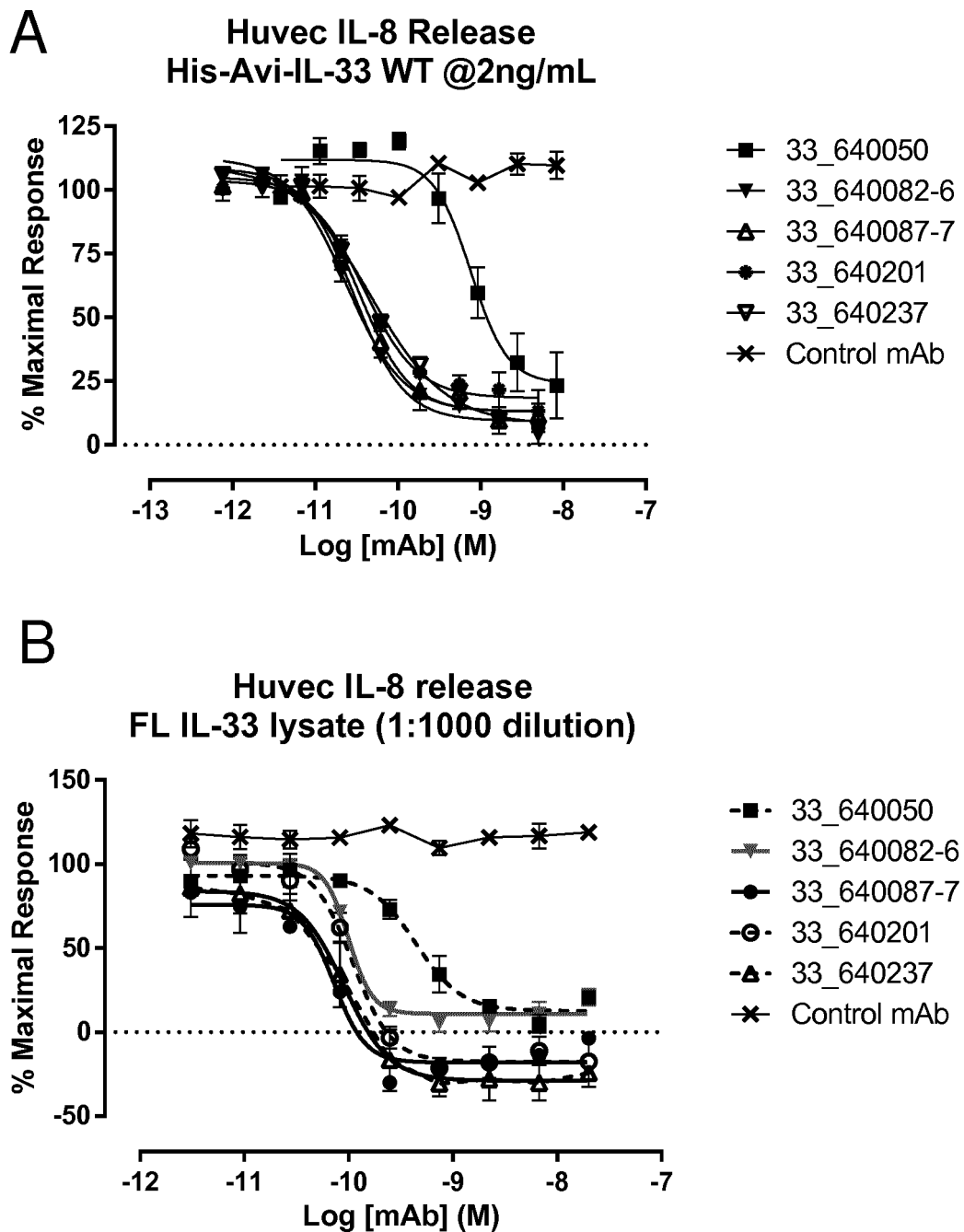


FIGURE 45

WT mouse

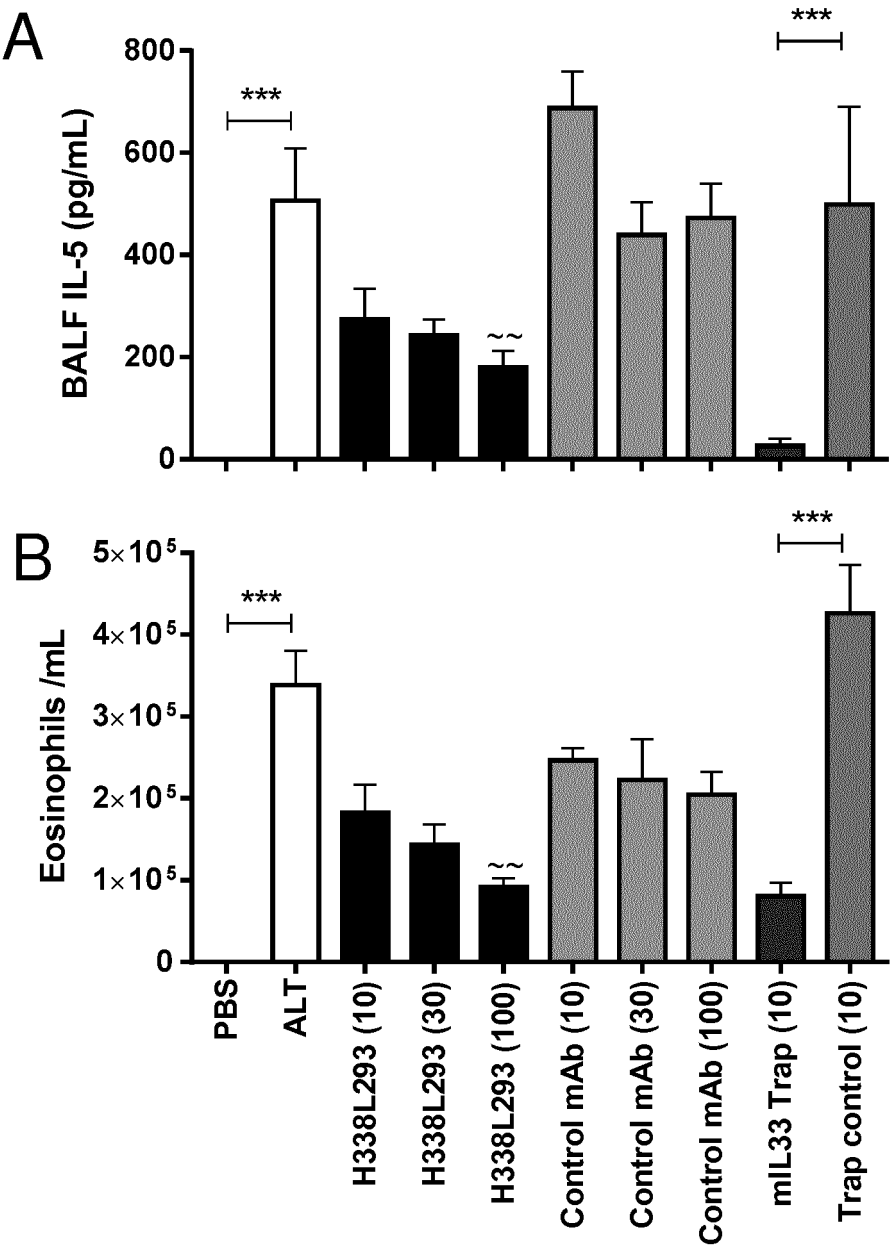


FIGURE 46

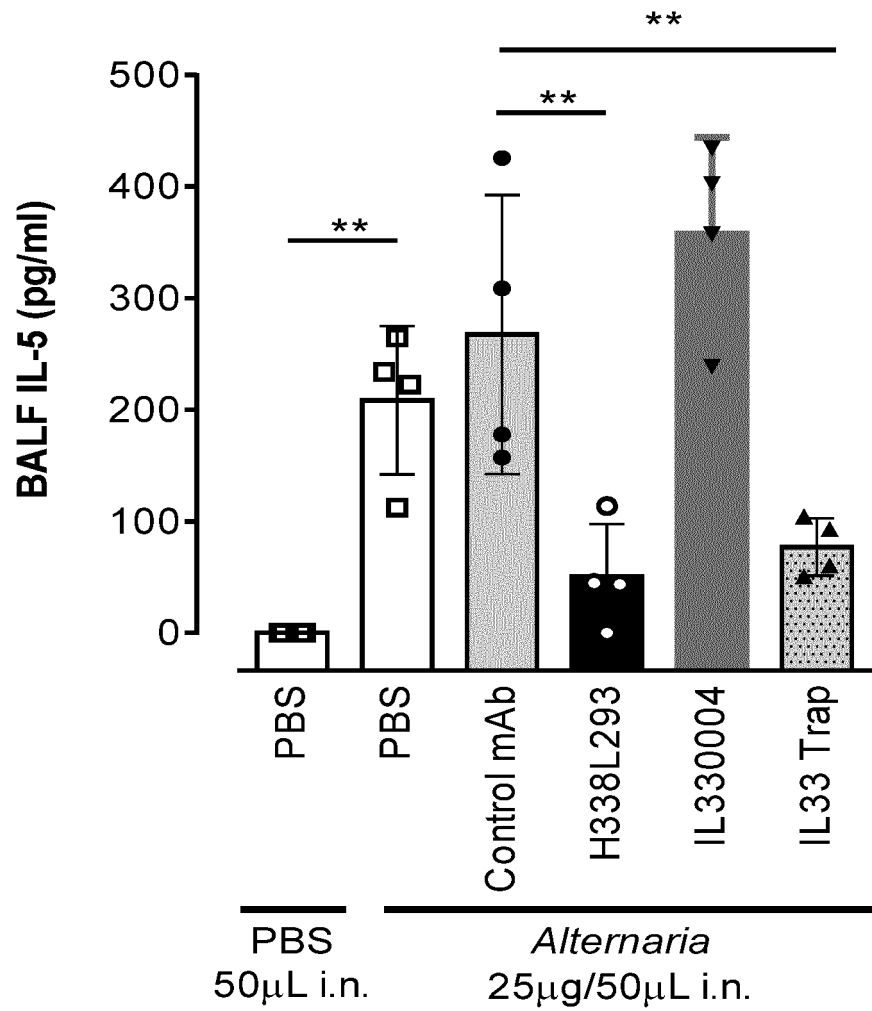
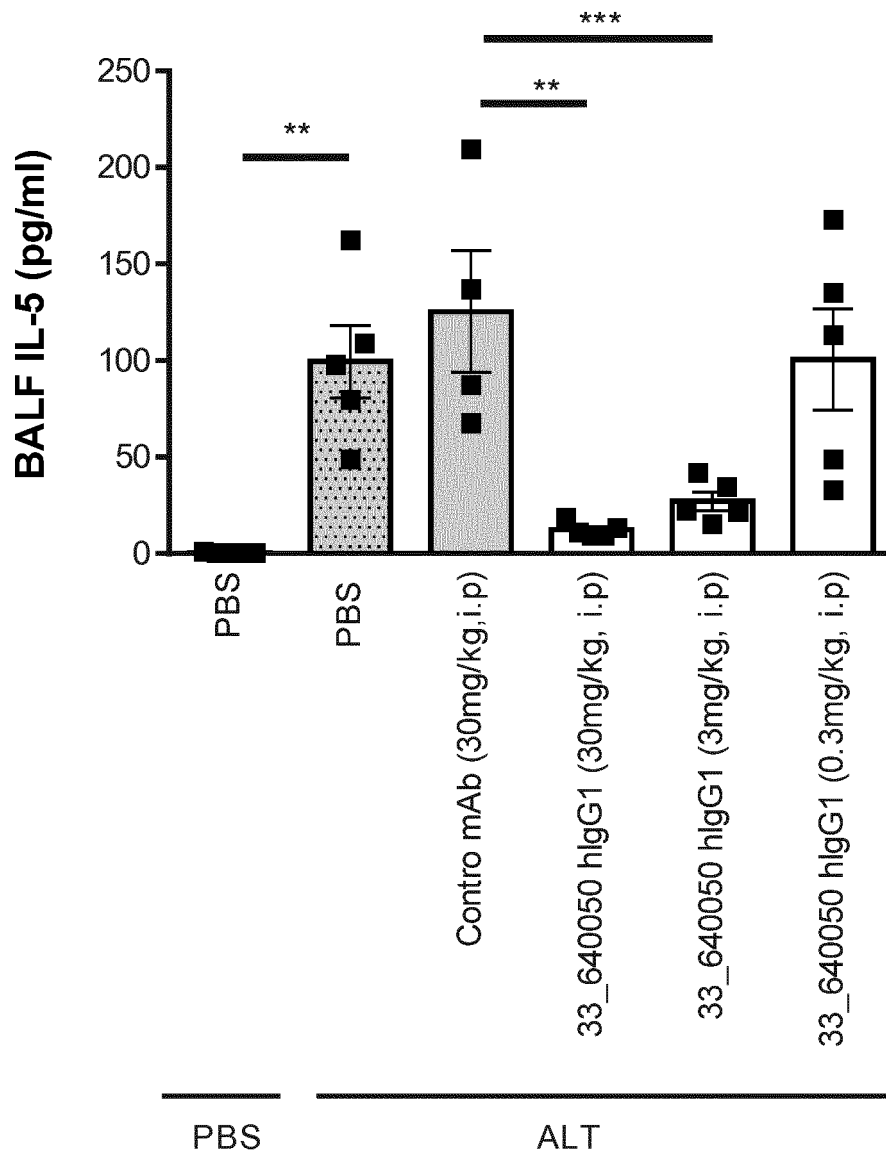
Humanized IL-33 mouse

FIGURE 47

Humanized IL-33 mouse



One-way ANOVA with Bonferroni's multiple comparisons test.
*** $p < 0.001$, ** $p < 0.01$

FIGURE 48

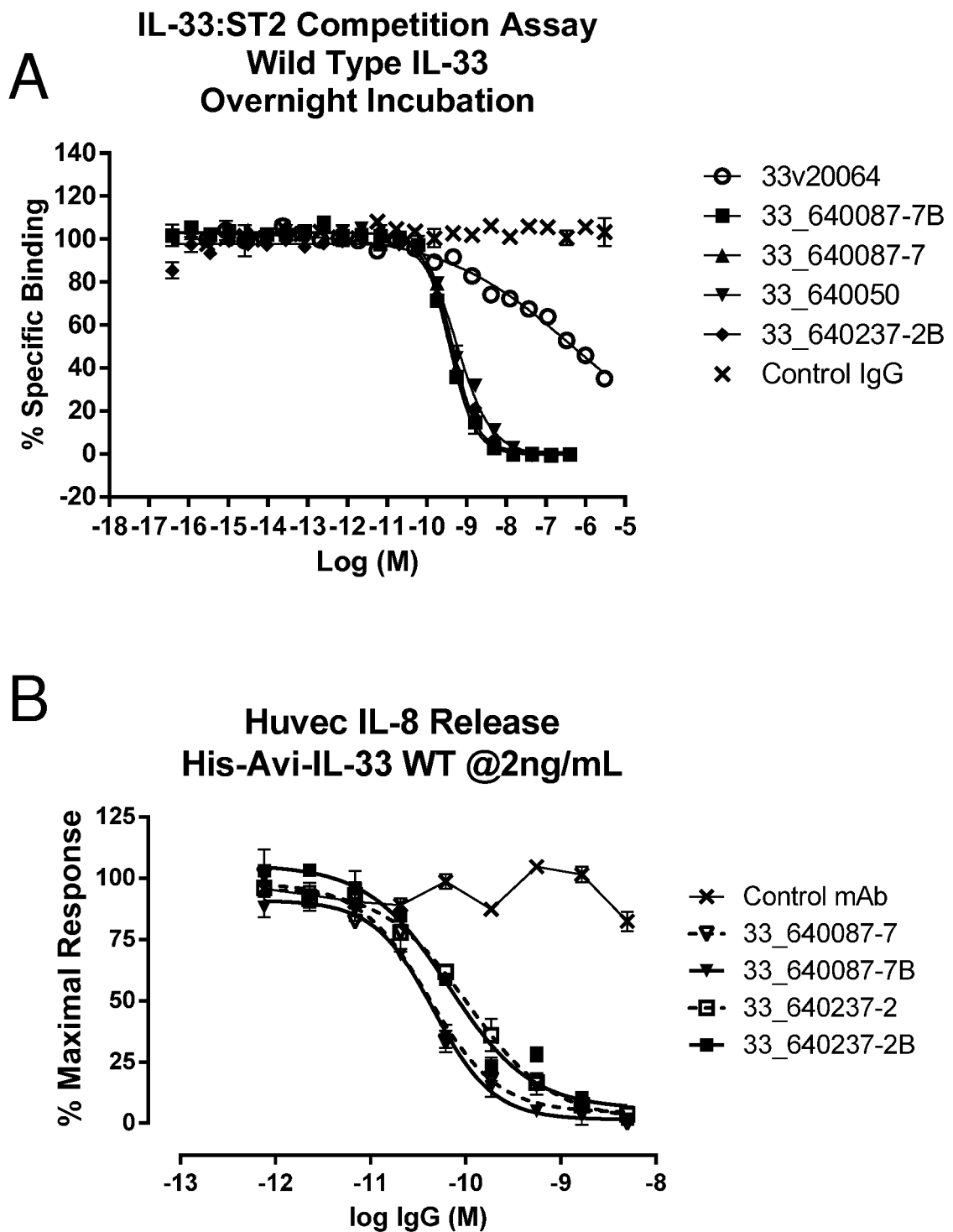


FIGURE 49

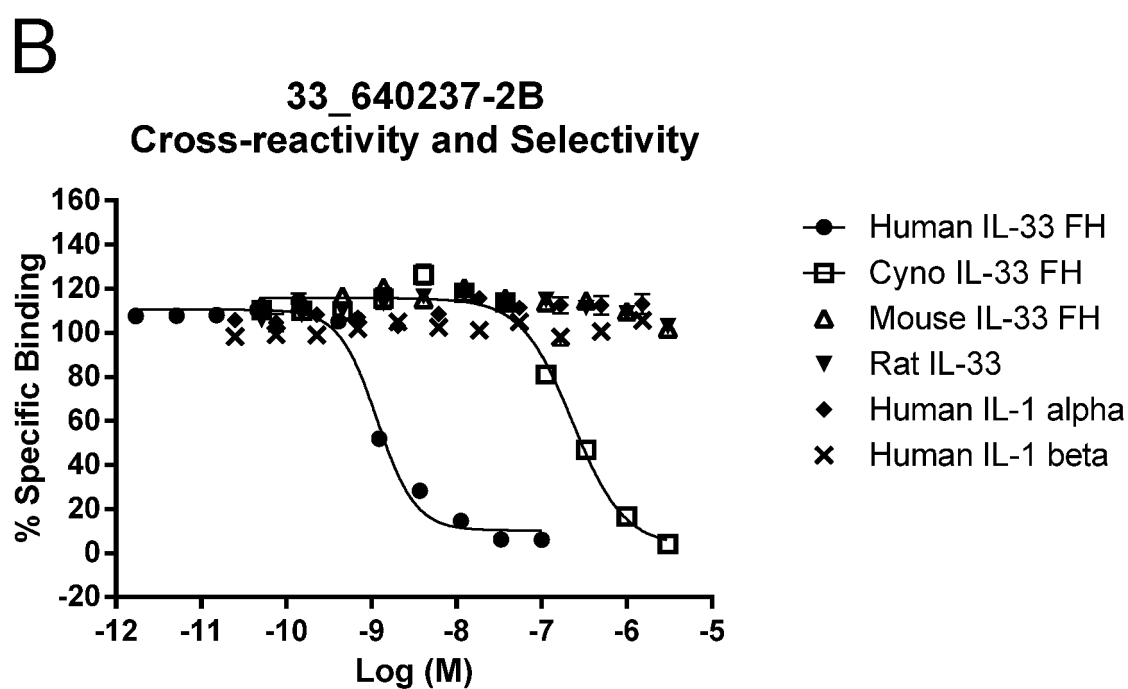
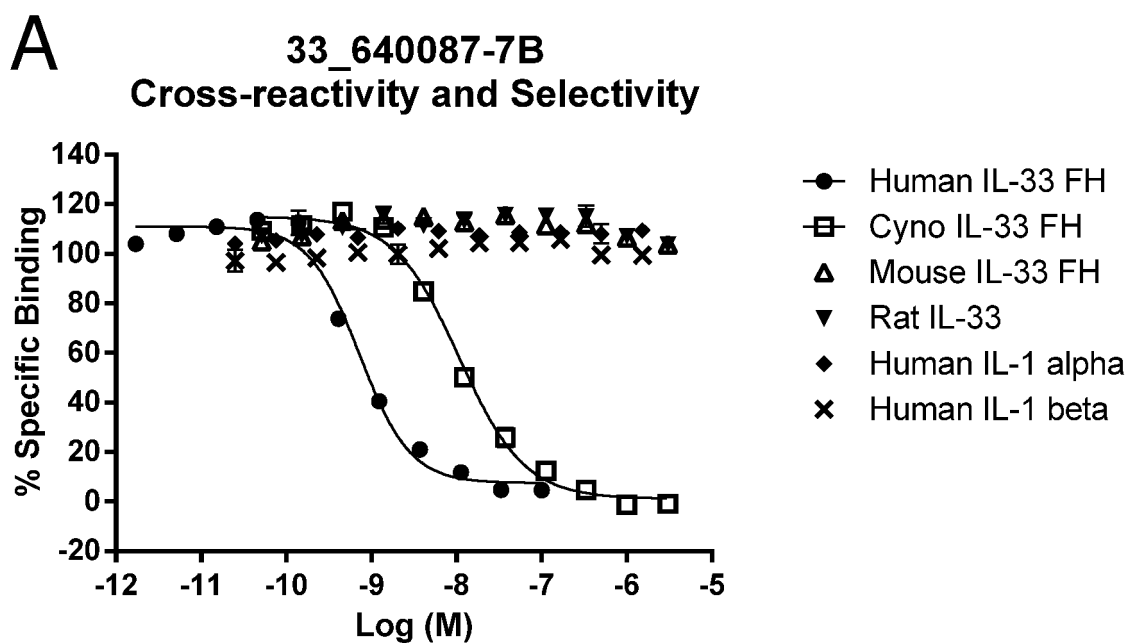


FIGURE 50

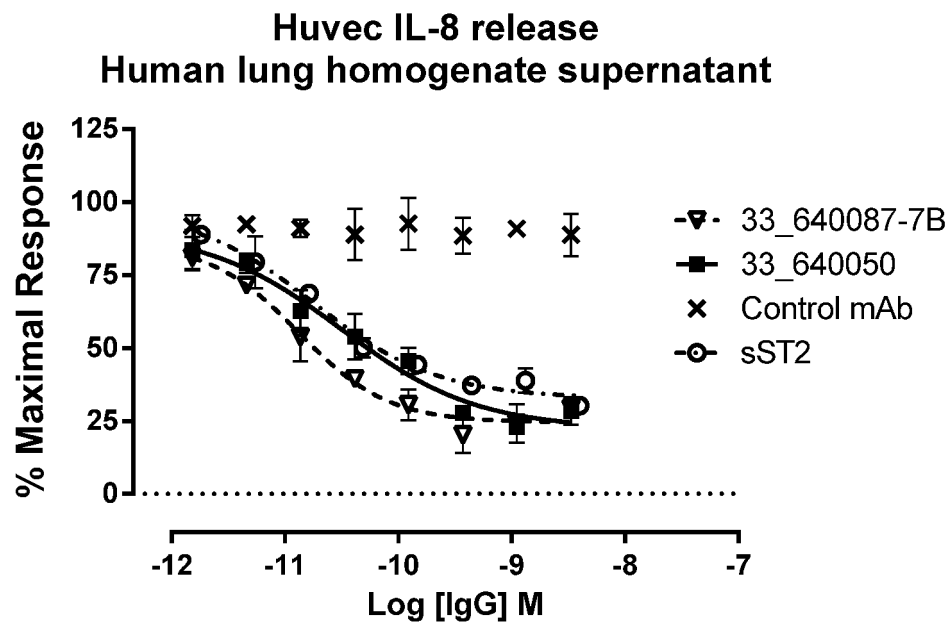
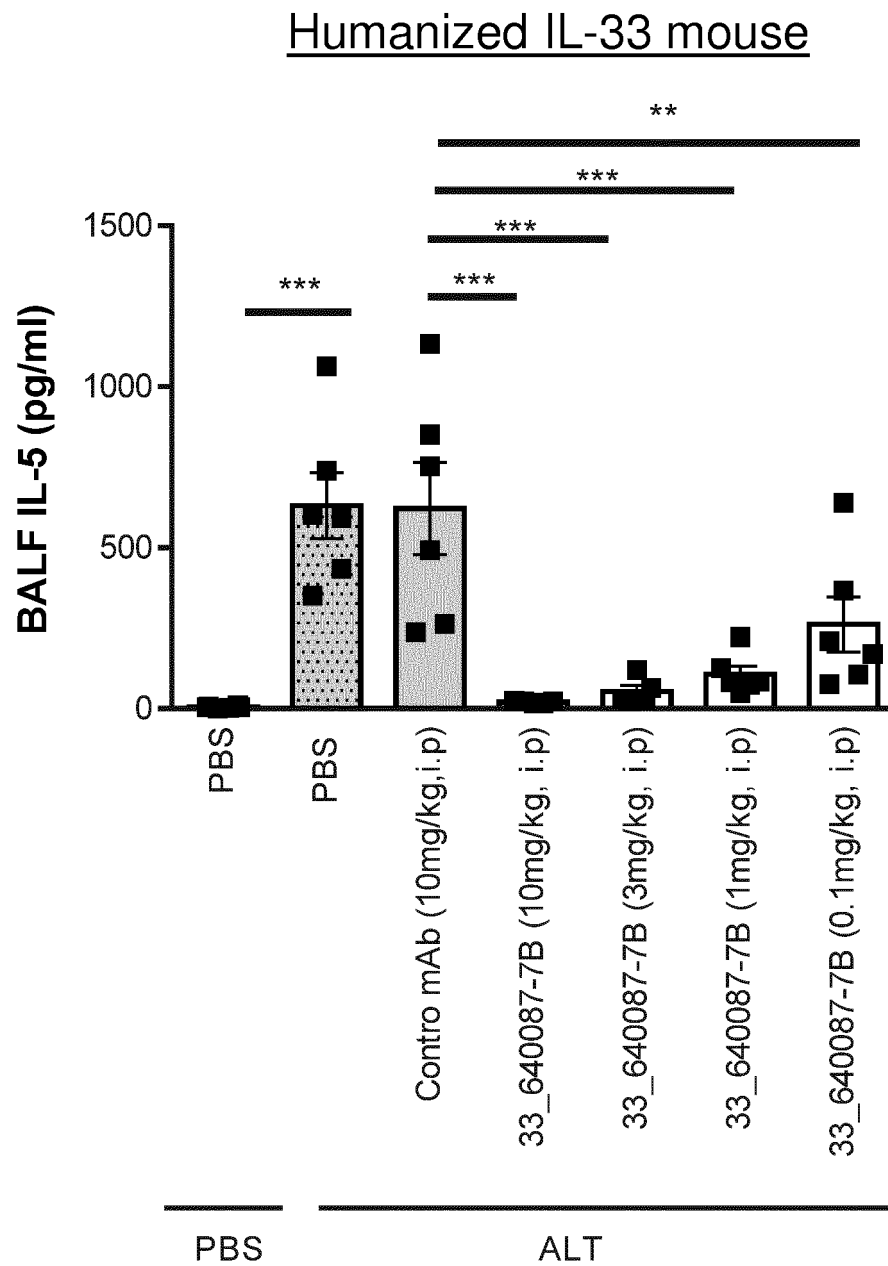


FIGURE 51



One-way ANOVA with Bonferroni's multiple comparisons test.
*** $p < 0.001$, ** $p < 0.01$

FIGURE 52

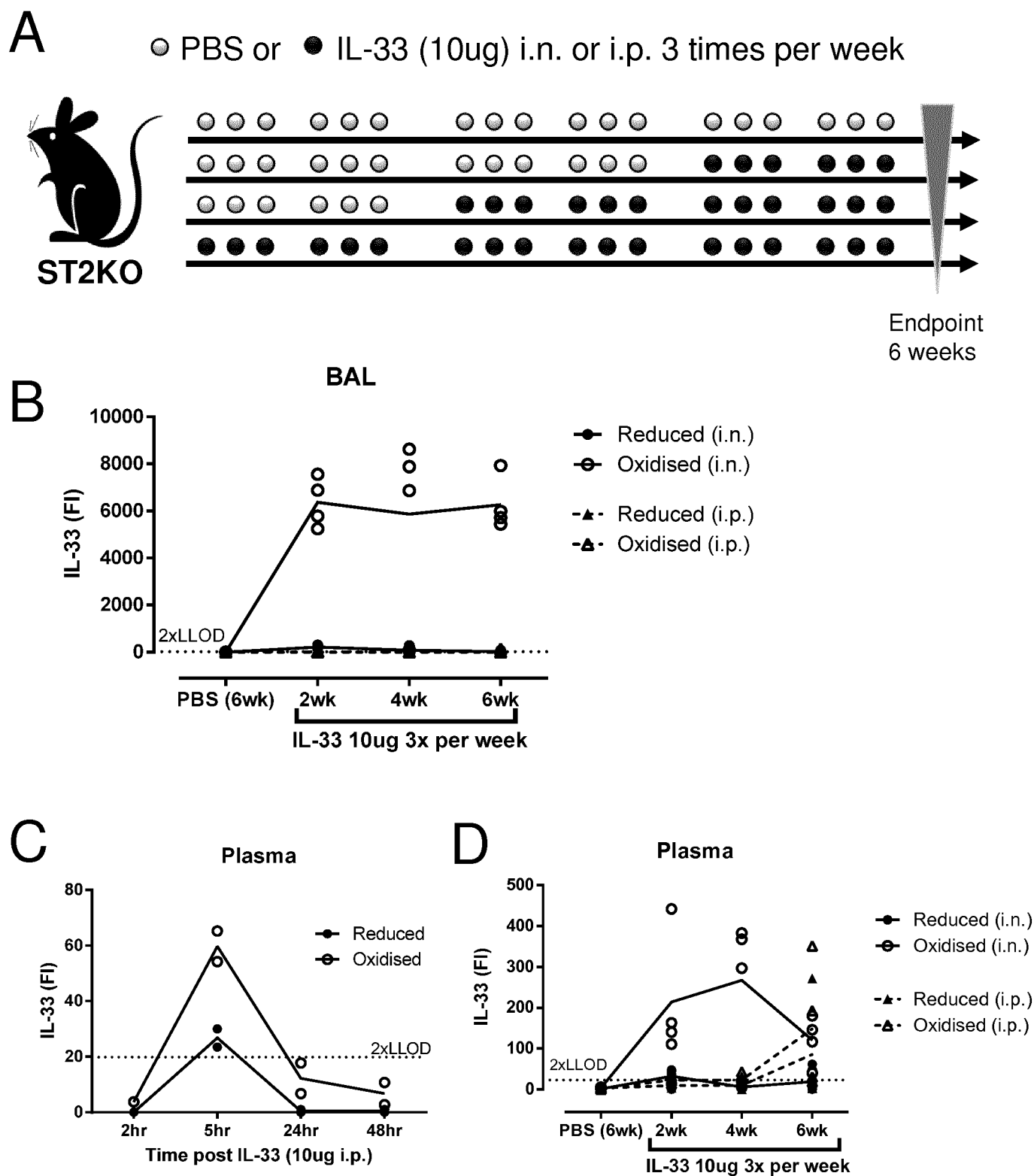
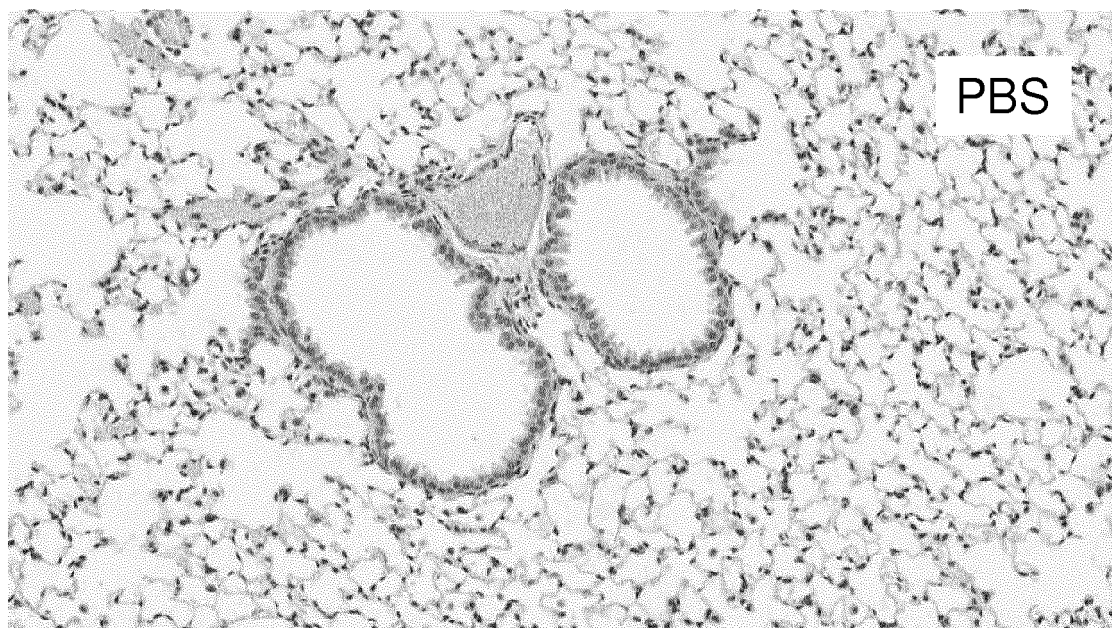
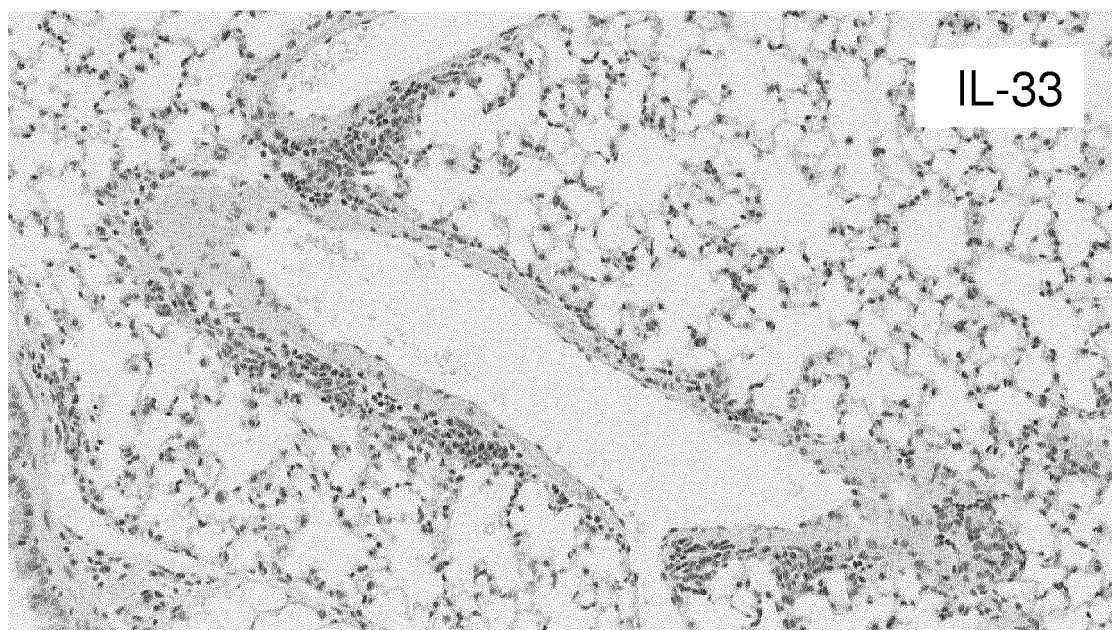


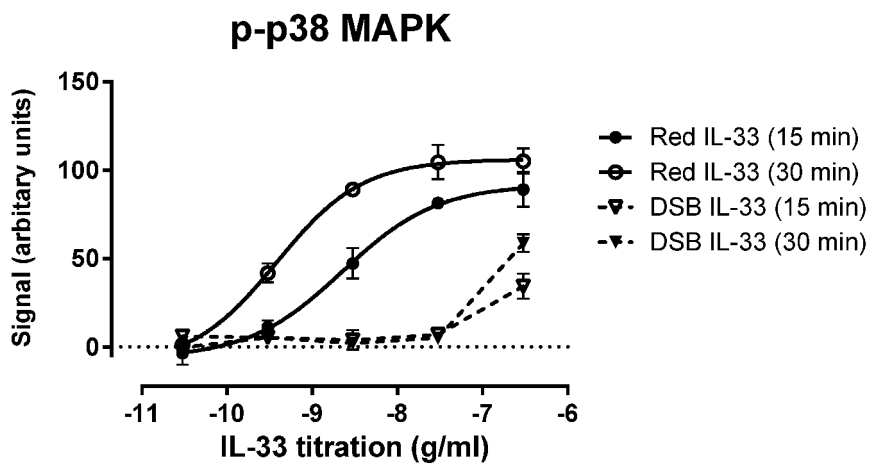
FIGURE 53

A**B**

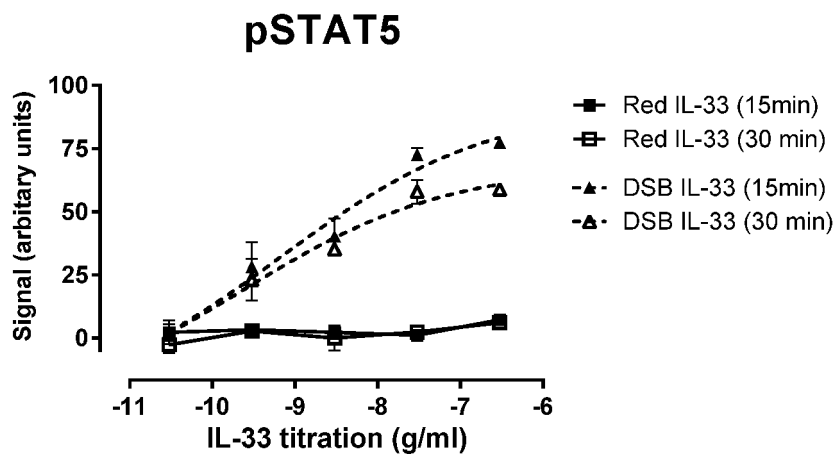
6 week intranasal dosing of IL-33 to ST2-deficient mice leads to perivascular inflammation

FIGURE 54

A



B



C

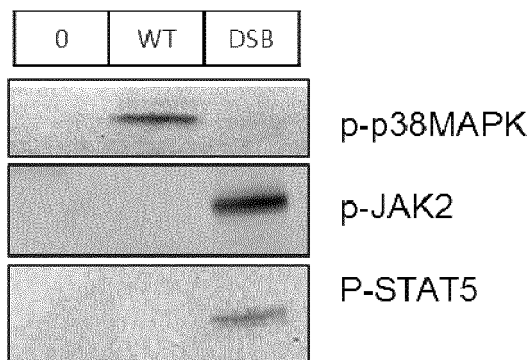
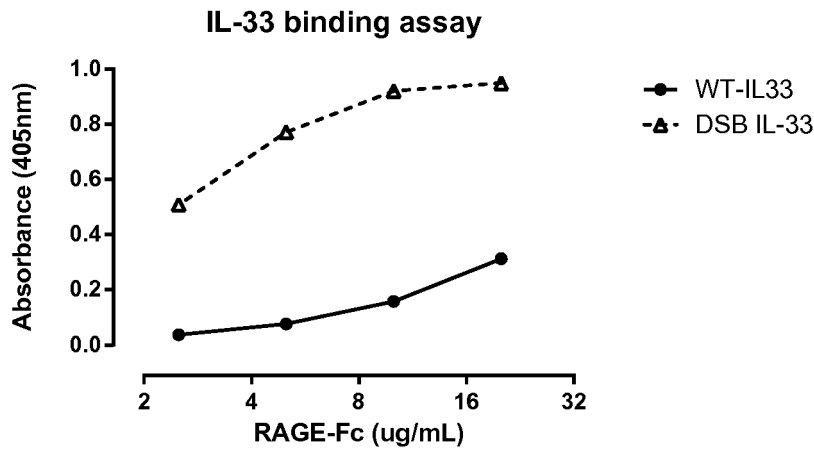
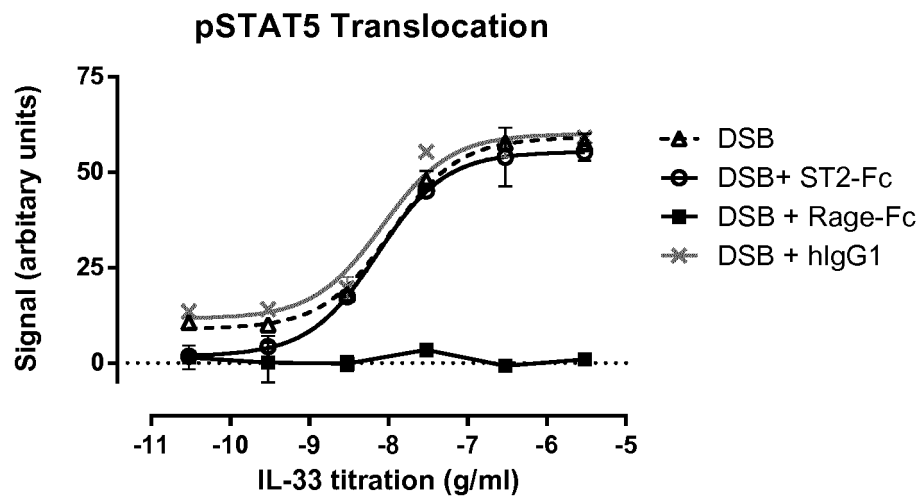


FIGURE 55

A



B



C

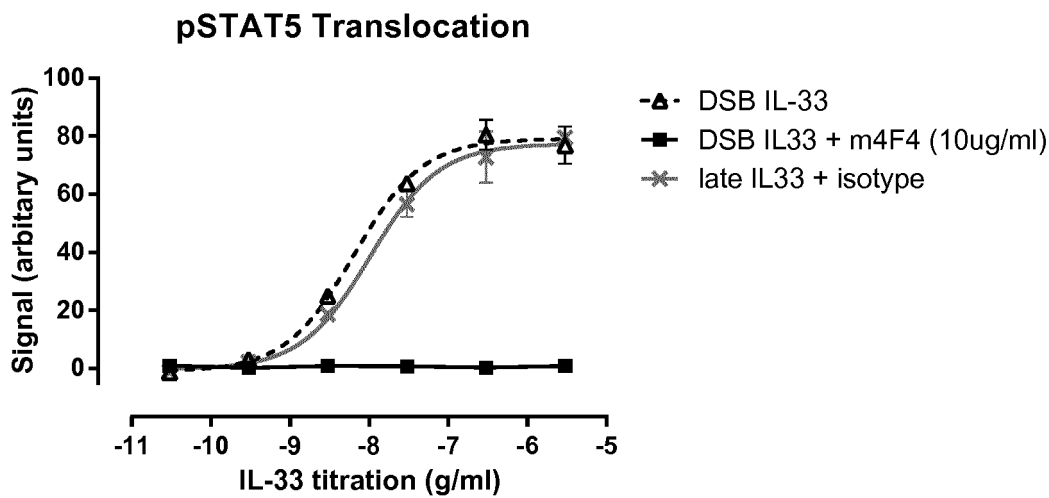


FIGURE 56

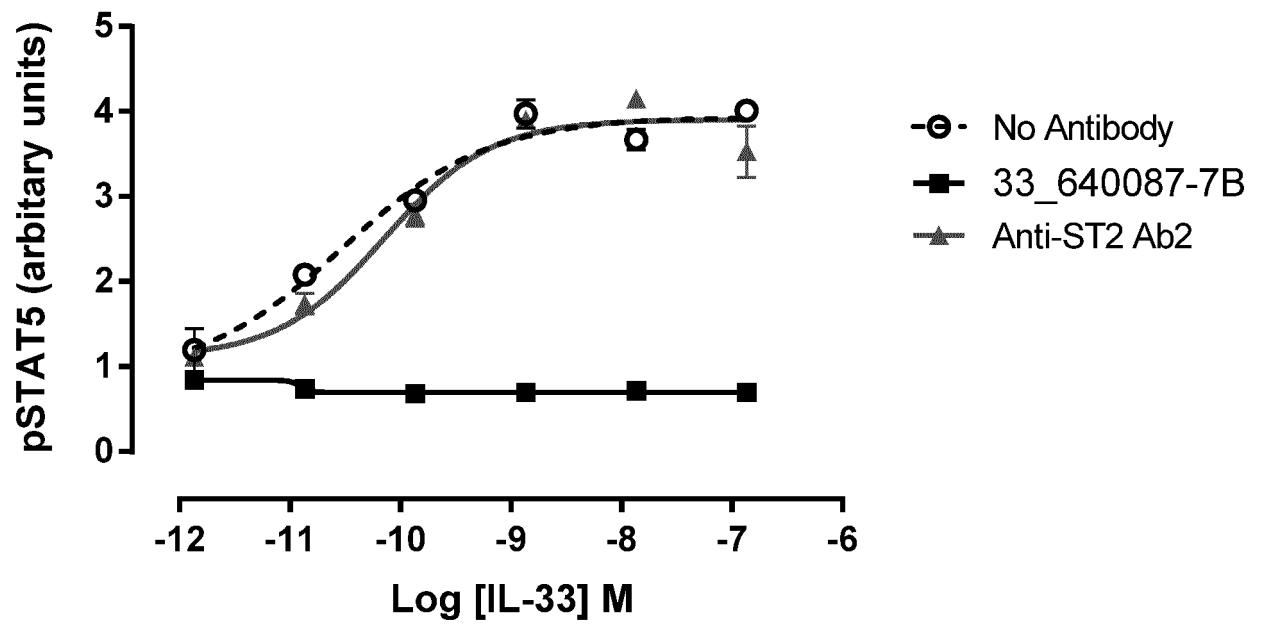
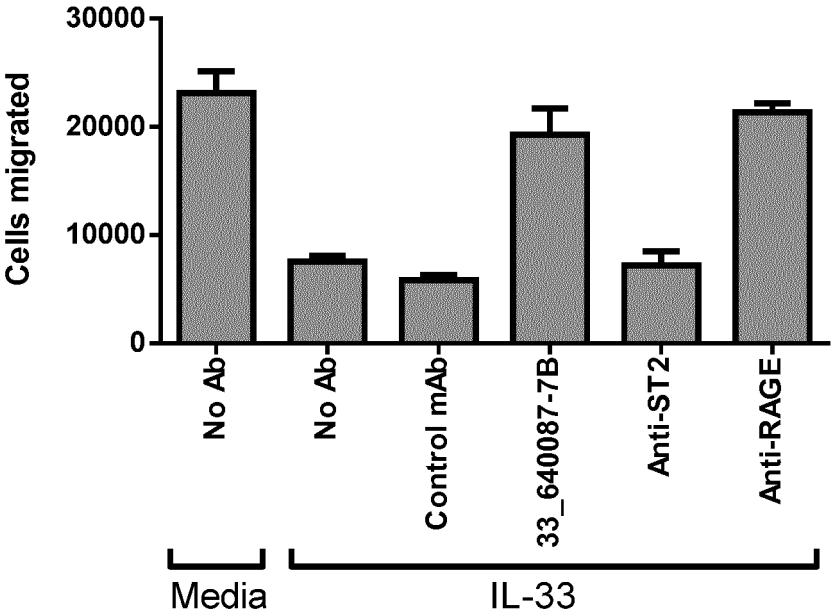


FIGURE 57

A



B

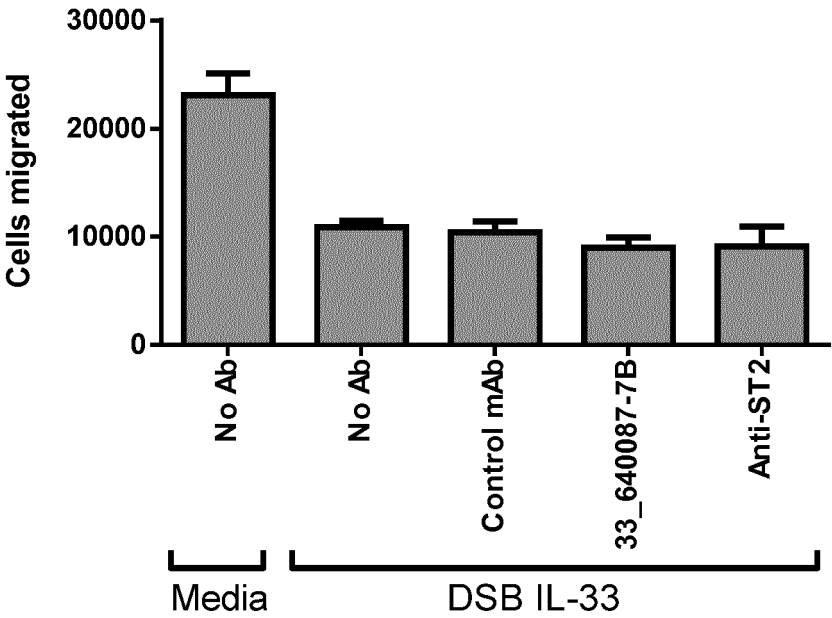


FIGURE 58