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Figure 1A

NKG2D .wt	FLNSLFNQEVQIPLTESYCGPCPNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKV
NKG2D .YA	FLNSLFNQEVQIPLTESYCGPCPNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKV
NKG2D .AF	FLNSLFNQEVQIPLTESYCGPCPNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKV

Y152

NKG2D .wt	YSKEDQDLLKLKVS ¹ YHWMGLVHIPTNGSWQWEDGSILSPNLLTIEMQKGDICALYASSFK
NKG2D .YA	YSKEDQDLLKLKVS ² YHWMGLVHIPTNGSWQWEDGSILSPNLLTIEMQKGDICALYASSFK
NKG2D .AF	YSKEDQDLLKLKVS ³ YHWMGLVHIPTNGSWQWEDGSILSPNLLTIEMQKGDICALYASSFK

Y199

NKG2D .wt	GYIENCSTPNTYICMQRTV
NKG2D .YA	GYIENCSTPNTYICMQRTV
NKG2D .AF	GYIENCSTPNTYICMQRTV

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Figure 1B

ULBP2 α 1 α 2	DPHSLCYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK
ULBP2.R80W	EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK
ULBP2.S3	EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK
ULBP2.C	EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK
ULBP2.R	EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK
ULBP2.AA	EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK
ULBP2.AB	EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLLHYDCGNKTVTPVSPGLGKKLNVTAWK

R80

ULBP2 α 1 α 2	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF
ULBP2.R80W	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF
ULBP2.S3	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF
ULBP2.C	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF
ULBP2.R	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF
ULBP2.AA	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF
ULBP2.AB	AQNPVLREVVDILTEQLMDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIF

M154

ULBP2 α 1 α 2	LLFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTLEPS
ULBP2.R80W	LLFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTLEPS
ULBP2.S3	LLFDSEKRMWTTVHPGARKMKEKWENDKVVATKLYLWSMGDCIGWLEDFLMGMDSTLEPS
ULBP2.C	LLFDSEKRMWTTVHPGARKMKEKWENDKVVATILWQTSMGDCIGWLEDFLMGMDSTLEPS
ULBP2.R	LLFDSEKRMWTTVHPGARKMKEKWENDKVVATLLWGWSMGDCIGWLEDFLMGMDSTLEPS
ULBP2.AA	LLFDSEKRMWTTVHPGARKMKEKWENDKVVATMFWWSMGDCIGWLEDFLMGMDSTLEPS
ULBP2.AB	LLFDSEKRMWTTVHPGARKMKEKWENDKVVATLMWQWSMGDCIGWLEDFLMGMDSTLEPS

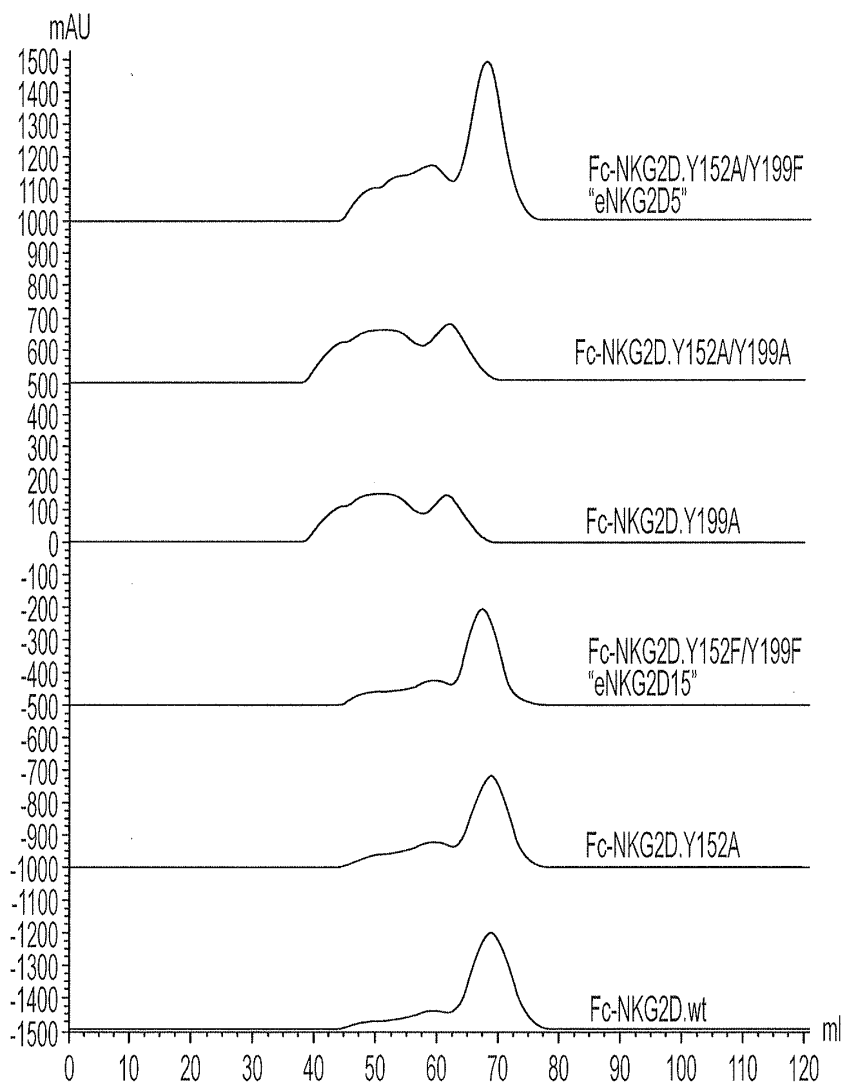
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Figure 2

eNKG2D variant	SEQ ID NO (ectodomain)	SEQ ID NO (Fc-fusion)	Y152 substitution	Y199 substitution	Protein aggregation (Akta SEC)
wild-type	17	40			Low
Y152A	18	41	A		Low
Y199A	19	42		A	High
Y152A/Y199A	20	43	A	A	High
1	21	44		F	Low
2	22	45	S		Low
3	23	46	T		Low
4	24	47	V		Low
5	25	48	A	F	Low
6	26	49	L	F	Intermediate
7	27	50	S	F	Low
8	28	51	T	F	Low
9	29	52	V	F	Intermediate
10	30	53		D	High
11	31	54		E	High
12	32	55	D	D	High
13	33	56	E	E	High
14	34	57	L		High
15	35	58	F	F	Low

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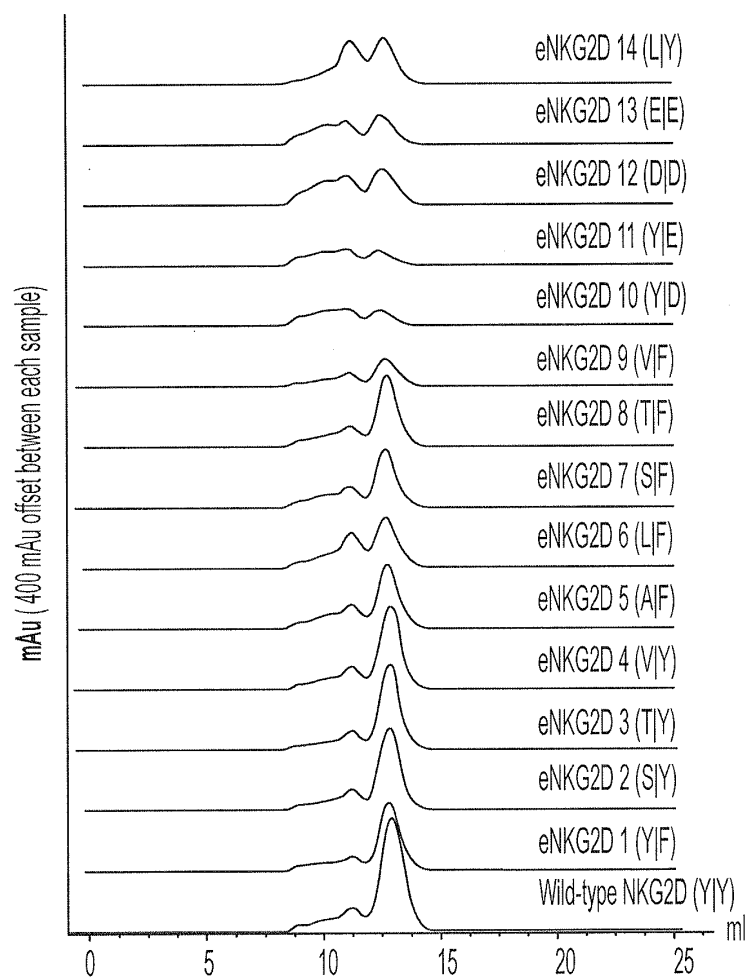
Figure 3



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Figure 4



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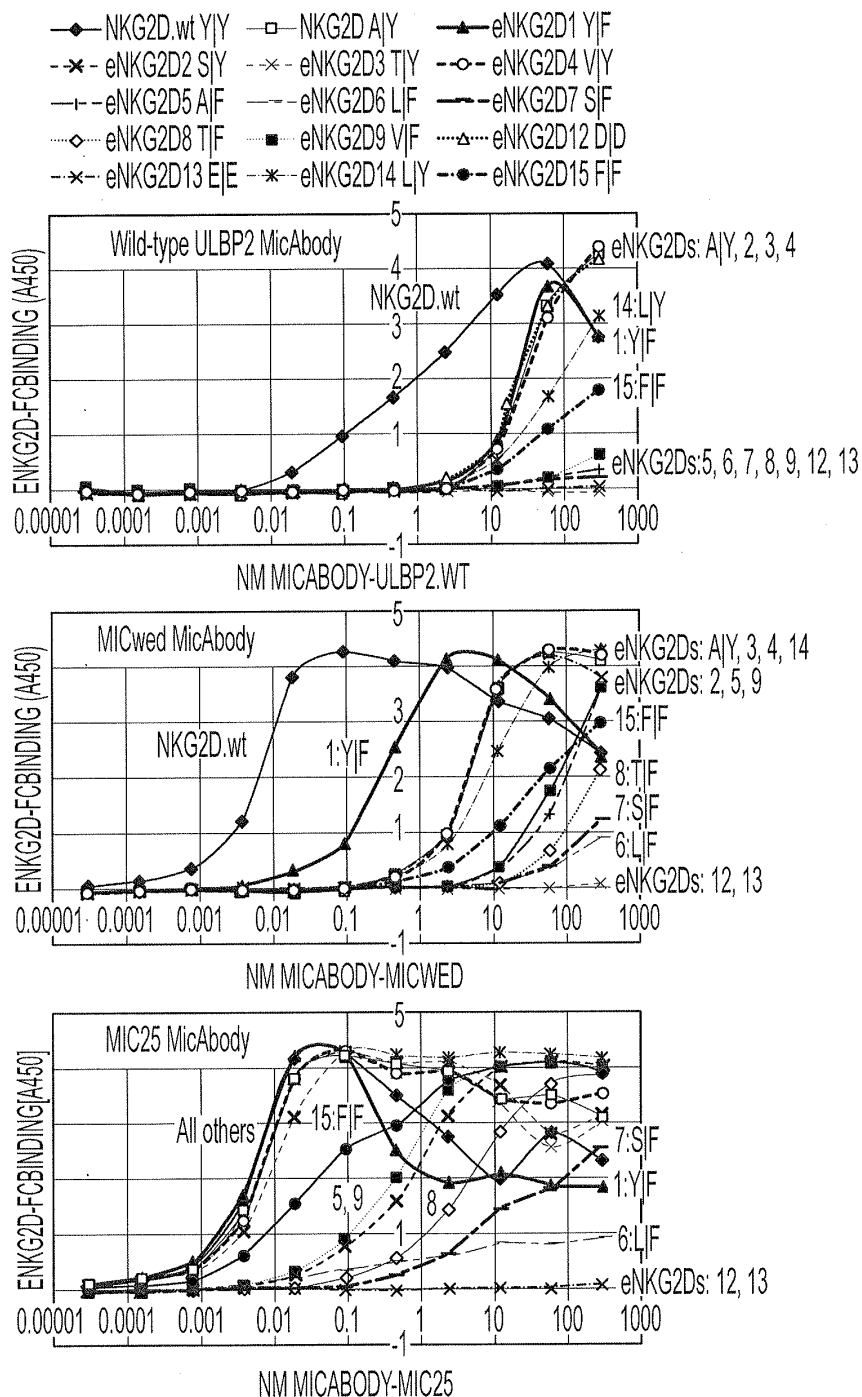
Figure 5

eNKG2D variant	Y152 substitution	Y199 substitution	MICwed MicAbody binding	MIC25 MicAbody binding
wild-type			100	100
Y152A	A		50	100
Y199A		A	nt	nt
Y152A/Y199A	A	A	nt	nt
1		F	100	100
2	S		50	100
3	T		50	100
4	V		50	100
5	A	F	0	50
6	L	F	nt	nt
7	S	F	0	50
8	T	F	0	50
9	V	F	0	50
10		D	nt	nt
11		E	nt	nt
12	D	D	nt	nt
13	E	E	nt	nt
14	L		nt	nt
15	F	F	nt	nt

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Figure 6



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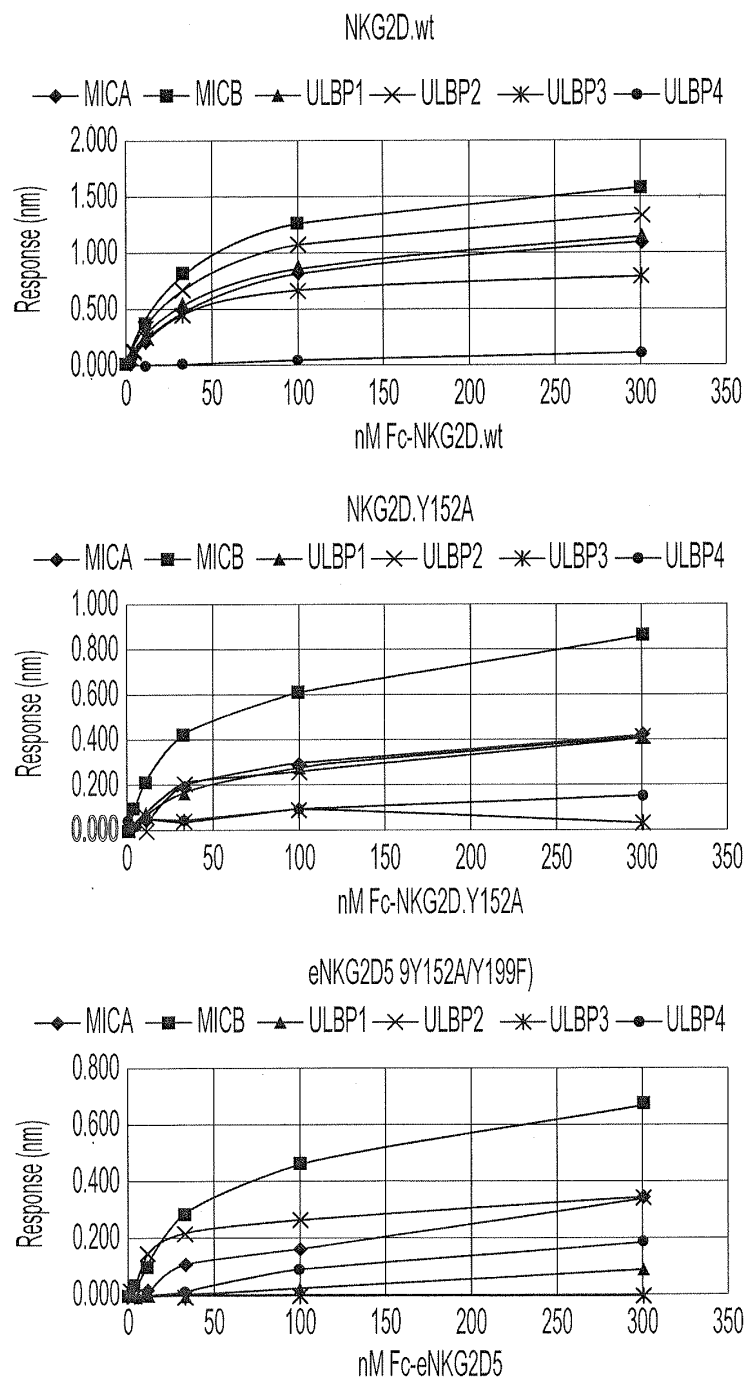
Figure 7

		MicAbody		
	Fc-eNKG2D	ULBP2.wt	MICwed	MIC25
wt	NKG2D.wt Y Y	1.41	0.0067	~0.0039
Y152	eNKG2D A Y	27.86	4.30	0.0057
	eNKG2D2 S Y	34.78	4.16	0.0056
	eNKG2D3 T Y	31.14	4.33	0.0056
	eNKG2D4 V Y	35.78	4.84	~0.0043
	eNKG2D14 L Y	87.63	9.39	0.010
Y199	eNKG2D1 Y F	23.08	0.32	0.0048
	eNKG2D10 Y D	nt	nt	nt
	eNKG2D11 Y E	nt	nt	nt
Y152 Y199	eNKG2D5 A F	nb	280.5	0.79
	eNKG2D6 L F	nb	nb	0.37
	eNKG2D7 S F	nb	347.3	20.94
	eNKG2D8 T F	nb	570.6	4.51
	eNKG2D9 V F	nb	90.0	0.43
	eNKG2D15 F F	57.05	31.3	0.046
	eNKG2D12 D D	nb	nb	nb
	eNKG2D13 E E	nb	nb	nb

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Figure 8



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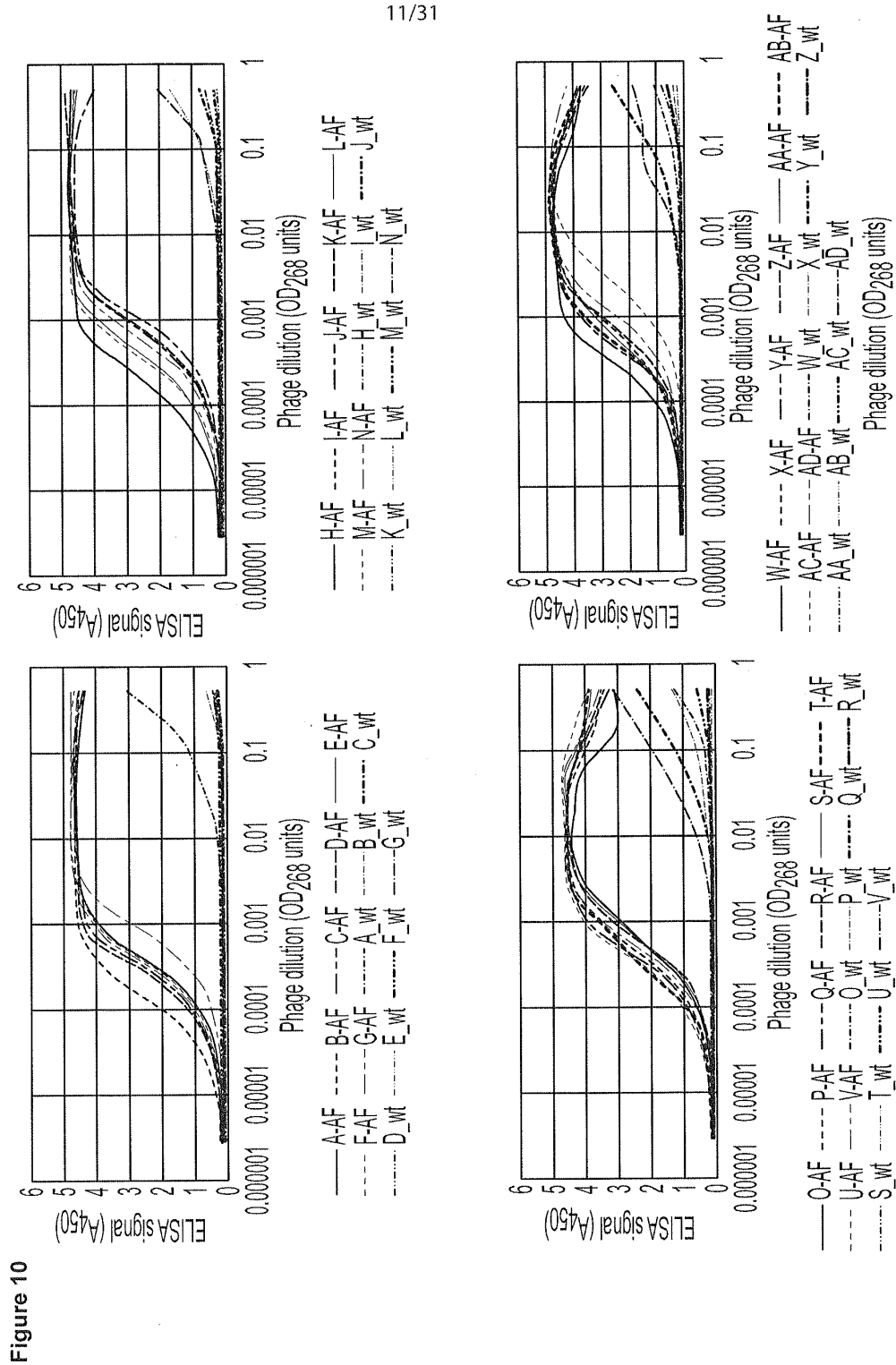
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Figure 9

Phage clone frequency (of the subset examined by spot ELISA)	ULBP2 residue							
	8	80	154	155	156	157	158	159
	C	R	M	S	F	H	Y	F
83	S	W	T	T	F	W	Q	I
9	S	W	T	M	L	R	Q	W
6	S	W	T	L	L	W	G	W
4	S	W	T	I	L	W	Q	T
4	S	W	T	M	L	R	Q	F
3	S	W	T	L	L	W	Q	A
3	S	W	T	L	L	W	S	W
2	S	W	T	L	L	W	Q	W
2	S	W	T	M	F	W	S	W
2	S	W	T	M	L	W	K	W
2	S	W	T	S	L	W	S	W
2	S	W	T	T	L	W	Q	V
2	S	W	T	V	L	W	Q	A
2	S	W	T	V	L	W	S	A
1	S	W	T	E	L	W	R	T
1	S	W	T	G	L	W	H	A
1	S	W	T	H	L	W	G	W
1	S	W	T	H	L	W	K	F
1	S	W	T	I	L	W	S	T
1	S	W	T	L	F	S	W	Y
1	S	W	T	L	L	P	A	W
1	S	W	T	L	L	R	Q	F
1	S	W	T	L	L	W	A	A
1	S	W	T	L	L	W	H	A
1	S	W	T	L	M	S	W	W
1	S	W	T	L	M	W	Q	W
1	S	W	T	M	F	R	Q	W
1	S	W	T	M	F	R	Q	Y
1	S	W	T	M	F	W	Q	W
1	S	W	T	M	I	Y	S	W
1	S	W	T	M	L	A	H	W

Phage clone frequency (of the subset examined by spot ELISA)	ULBP2 residue							
	8	80	154	155	156	157	158	159
	C	R	M	S	F	H	Y	F
1	S	W	T	M	L	F	Q	W
1	S	W	T	M	L	K	Q	W
1	S	W	T	M	L	M	Q	W
1	S	W	T	M	L	P	Y	W
1	S	W	T	M	L	R	Q	S
1	S	W	T	M	L	R	Q	Y
1	S	W	T	M	L	T	Q	W
1	S	W	T	M	L	W	H	A
1	S	W	T	M	L	W	N	W
1	S	W	T	M	L	W	S	A
1	S	W	T	M	L	W	W	S
1	S	W	T	M	M	R	Q	W
1	S	W	T	N	I	W	Q	Y
1	S	W	T	N	L	W	N	V
1	S	W	T	N	L	W	Q	S
1	S	W	T	N	L	W	S	A
1	S	W	T	N	L	W	S	Y
1	S	W	T	N	M	W	G	W
1	S	W	T	S	L	C	W	Y
1	S	W	T	S	L	W	G	A
1	S	W	T	S	L	W	G	I
1	S	W	T	S	L	W	Q	S
1	S	W	T	S	L	W	Q	V
1	S	W	T	S	L	W	Q	Y
1	S	W	T	S	L	W	S	A
1	S	W	T	T	F	W	G	T
1	S	W	T	T	F	W	Q	M
1	S	W	T	T	L	W	P	S
1	S	W	T	T	L	W	S	S
1	S	W	T	T	M	W	Q	V
1	S	W	T	V	L	W	Q	M

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Figure 11

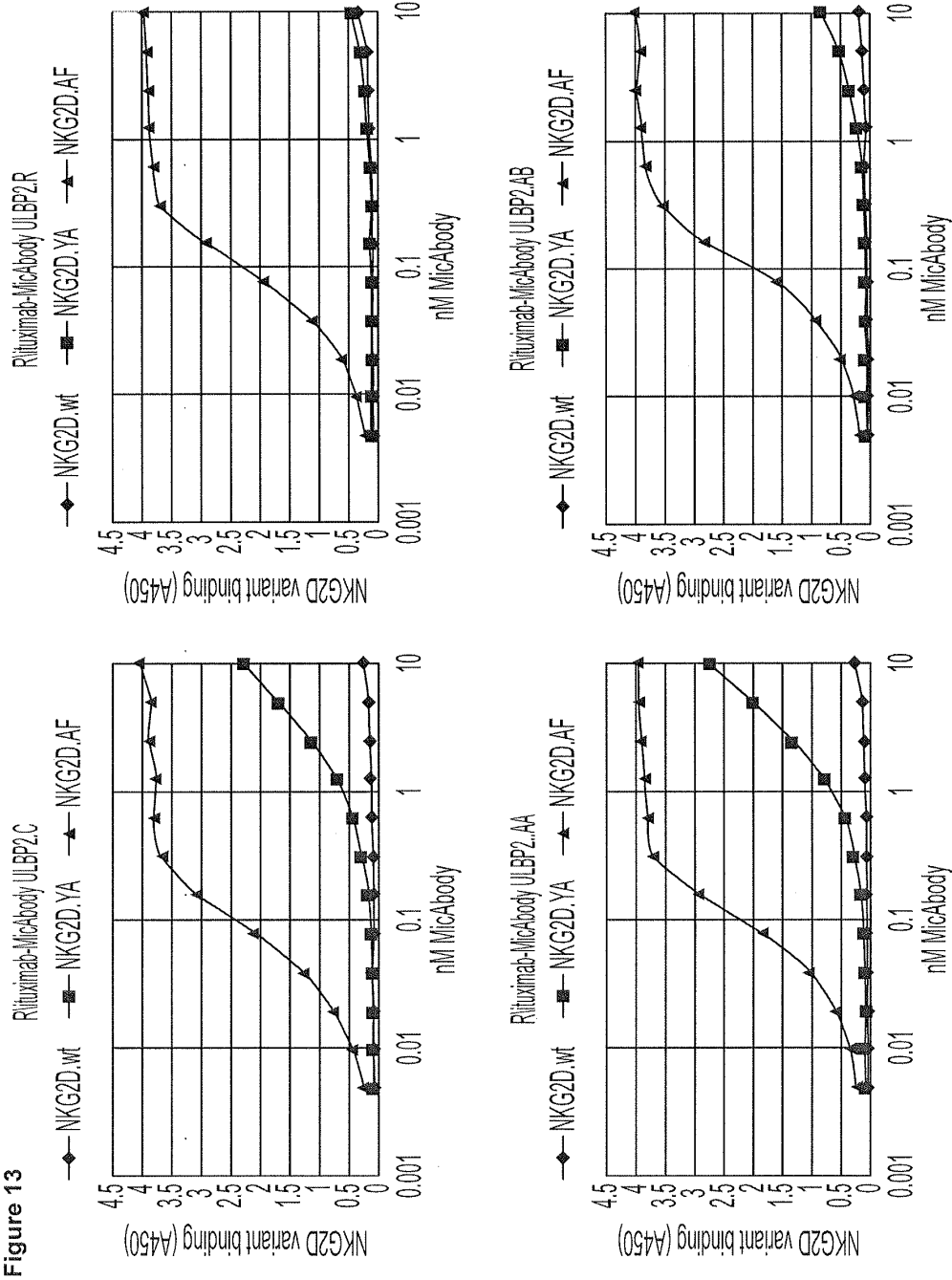
ULBP2 variant	ULBP2 residue								ELISA EC50s - Rituximab-MicAbody (light-chain ULBP2 fusion) binding to NKG2D.wt or NKG2D.AF			
	8	80	154	155	156	157	158	159	wt EC50 nM	AF EC50 nM	wt/AF EC50 ratio	AF/wt EC50 ratio
	C	R	M	S	F	H	Y	F				
A	S	W	T	T	F	W	Q	I	28.95	0.061	471.12	0.00212
B	S	W	T	M	L	R	Q	W	34.18	0.025	1373.49	0.00073
C	S	W	T	I	L	W	Q	T	129.83	0.029	4414.23	0.00023
D	S	W	T	L	L	W	Q	A	10.02	0.020	489.80	0.00204
E	S	W	T	L	L	W	S	W	51.45	0.031	1650.77	0.00061
F	S	W	T	V	L	W	Q	A	37.58	0.023	1639.79	0.00061
G	S	W	T	V	L	W	S	A	40.54	0.024	1664.58	0.00060
I	S	W	T	N	I	W	Q	Y	1.04	0.010	99.11	0.01009
J	S	W	T	H	L	W	G	W	5.77	0.062	93.61	0.01068
L	S	W	T	L	F	W	Q	S	25.33	0.053	479.31	0.00209
O	S	W	T	S	L	W	Q	S	17.04	0.026	652.71	0.00153
P	S	W	T	M	L	R	Q	F	2.37	0.069	34.45	0.02903
R	S	W	T	L	L	W	G	W	104.45	0.031	3398.27	0.00029
T	S	W	T	L	L	W	Q	W	4.37	0.029	151.52	0.00660
U	S	W	T	M	L	W	K	W	19.58	0.033	595.39	0.00168
W	S	W	T	M	F	R	Q	W	27.09	0.020	1322.15	0.00076
Y	S	W	T	S	L	W	S	W	83.49	0.090	927.71	0.00108
Z	S	W	T	N	L	W	S	A	98.80	0.025	3892.50	0.00026
AA	S	W	T	M	F	W	S	W	654.83	0.033	20092.30	0.00005
AB	S	W	T	L	M	W	Q	W	389.34	0.036	10801.65	0.00009
AD	S	W	T	T	L	W	Q	V	57.33	0.038	1504.95	0.00066

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Figure 12

M154	S155	F156	H157	Y158	F159
T	M	L	E	L	W
	K	M	T	V	I
	W		S	I	
	L		Q	T	
	T		Y		
			R		



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Figure 14

		MHC I HLA supertype representative											
	Pos	A0101	A0201	A0301	A2402	A2601	B0702	B0801	B1501	B2705	B3901	B4001	B5801
ULBP2 wild-type	0	48	75	75	23	95	35	49	38	36	55	28	50
	1	14	34	46	55	6.5	12	5.5	3.5	20	0.7	0.15	29
	2	34	60	95	80	36	47	46	90	60	24	55	75
	3	16	44	40	13	11	25	16	9.5	32	22	17	49
	4	29	70	70	85	6.5	40	70	75	29	22	48	65
	5	4	21	0.7	12	2.5	17	46	0.5	8.5	19	18	0.08
	6	1.7	13	9.5	0.7	1.9	6	6.5	0.6	24	14	9	0.9
	7	8	11	21	10	23	19	14	34	29	20	25	8.5
	8	4	0.6	4.5	2.5	4	4.5	0.8	0.7	9.5	2.5	6	4
	9	5.5	21	13	19	9	29	7	6	29	14	15	6.5
	10	34	55	42	19	22	46	70	55	55	20	20	47
	11	12	39	36	40	10	43	70	65	55	3	17	42
	12	13	37	42	0.4	28	24	39	24	16	1.6	9.5	26
	13	15	50	70	14	35	49	24	17	75	24	38	21
	14	14	16	30	8.5	4.5	22	37	8.5	21	6	21	0.15
	15	14	0.8	18	18	15	12	12	8	13	11	12	28
ULBP2C	0	48	75	75	23	95	35	49	38	36	55	28	50
	1	26	50	70	85	26	36	28	24	39	6	1.9	45
	2	17	37	65	20	28	29	21	60	34	1.9	22	28
	3	19	41	50	37	26	24	9.5	31	43	10	11	45
	4	26	39	60	21	10	32	55	70	29	7	60	7.5
	5	27	20	8	50	11	17	80	19	16	25	18	5
	6	12	4	26	19	20	24	45	55	28	24	21	5.5
	7	19	34	70	28	41	27	70	55	49	34	36	12
	8	7	8.5	14	18	0.8	4.5	30	2	9.5	3	11	1
	9	39	40	23	29	16	24	30	32	55	15	55	70
	10	34	14	17	37	29	29	75	27	40	34	25	24
	11	24	75	75	16	33	70	60	44	47	70	27	43
	12	6.5	19	65	8.5	19	25	22	7	5.5	0.9	2.5	20
	13	9.5	70	65	70	27	42	33	18	70	24	44	11
	14	22	35	32	8.5	7	21	55	14	31	8.5	2.9	0.3
	15	14	0.8	18	18	15	12	12	8	13	11	12	28
ULBP2R	0	48	75	75	23	95	35	49	38	36	55	28	50
	1	26	50	70	85	26	36	28	24	39	6	1.9	45
	2	14	35	60	28	20	21	12	55	35	0.6	14	34
	3	15	36	42	29	20	27	13	34	34	15	15	42
	4	24	40	60	14	9	30	49	70	27	8.5	55	4
	5	31	16	12	40	20	23	75	19	20	34	25	5
	6	8	17	17	3	8.5	23	48	24	20	15	20	0.05
	7	24	37	55	60	20	27	70	65	47	60	34	20
	8	8	4.5	18	6.5	4	5.5	19	6.5	5.5	1.2	6	1.7
	9	19	13	15	16	19	27	37	30	42	14	38	60
	10	28	8.5	16	31	21	29	70	27	24	17	35	27
	11	32	65	65	14	36	65	65	60	55	65	25	46
	12	8	26	70	16	46	19	23	21	14	10	17	14
	13	47	75	85	28	65	60	55	22	60	55	43	26
	14	10	27	38	9.5	6.5	20	35	8.5	20	8	19	0.175
	15	14	0.8	18	18	15	12	12	8	13	11	12	28

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Figure 14 continued

ULBP2AA	0	48	75	75	23	95	35	49	33	38	55	28	50
	1	26	50	70	85	26	36	28	24	39	6	1.9	45
	2	15	41	65	38	8	18	15	44	34	1.6	20	27
	3	16	44	45	14	12	29	25	17	32	33	20	50
	4	24	42	60	19	7	32	49	65	35	9	55	6
	5	25	9.5	10	42	11	21	85	34	18	32	26	5
	6	6.5	18	14	0.4	9	13	12	6.5	21	8.5	17	0.04
	7	12	29	43	43	25	31	43	55	42	50	32	20
	8	3	0.5	7.5	1.5	0.5	3	5.5	0.9	5	0.4	5	0.5
	9	11	12	7.5	8.5	17	26	28	9.5	26	6	21	33
	10	23	30	29	9	21	38	85	55	35	13	23	20
	11	22	42	55	13	18	65	70	47	42	27	14	24
	12	3	15	60	11	19	16	24	10	10	5.5	9.5	3
	13	40	75	85	16	65	45	45	18	65	37	48	29
	14	10	27	38	9.5	6.5	20	35	8.5	20	8	19	0.175
	15	14	0.8	18	18	15	12	12	8	13	11	12	28
ULBP2AB	0	48	75	75	23	95	35	49	38	36	55	28	50
	1	26	50	70	85	26	36	28	24	39	6	1.9	45
	2	14	35	60	28	20	21	12	55	35	0.6	14	34
	3	18	40	45	40	12	22	17	26	34	24	22	35
	4	30	41	70	19	11	31	55	70	28	8.5	55	7.5
	5	28	21	7	60	9.5	14	80	23	19	24	16	5.5
	6	8	20	18	1.4	8	21	48	23	26	13	23	0.01
	7	19	36	65	36	31	29	60	55	43	47	35	17
	8	5	1.8	16	6	3.5	8.5	9.5	7.5	6	2	12	0.6
	9	14	13	13	9.5	12	20	13	18	32	11	35	55
	10	23	11	17	20	28	31	55	15	14	12	27	14
	11	34	50	55	11	19	60	70	46	40	30	16	45
	12	7.5	5	55	6.5	20	19	20	3	1.8	0.8	2.5	22
	13	35	80	85	21	60	55	45	20	60	38	48	29
	14	10	27	38	9.5	6.5	20	35	8.5	20	8	19	
	15	14	0.8	18	18	15	12	12	8	13	11	12	28

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Figure 15

	pos	HLA-DP										HLA-DQ									
		DPA10103-DPB10301	DPA10103-DPB10401	DPA10103-DPB10402	DPA10103-DPB10601	DPA10201-DPB10101	DPA10201-DPB10501	DPA10201-DPB10401	DPA10301-DPB10402	DPA10103-DPB10201	DQA10101-DQB10501	DQA10102-DQB10501	DQA10102-DQB10502	DQA10102-DQB10602	DQA10103-DQB10603	DQA10104-DQB10503	DQA10201-DQB10202	DQA10201-DQB10301	DQA10201-DQB10303	DQA10201-DQB10402	DQA10301-DQB10301
ULBP2_wt	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				
	8																				
	9		WB							WB											WB
	10		WB		WB					WB						WB			WB	WB	
	11		WB		WB					WB						SB			WB	WB	
	12		WB		WB					WB			WB			SB			WB	WB	
	13		WB							WB	WB		WB			SB			WB	WB	
	14		WB	WB						WB	WB					SB			WB	WB	
	15			WB						WB	WB					SB			WB		
	16			WB							WB										
	17			WB							WB										
	18			WB																	
	19										WB										
	20			WB							SB		WB			WB					
	21			SB						WB	SB		WB			WB					
	22			SB						WB	SB		WB			SB					
	1																				
	2																				

SUBSTITUTE SHEET (RULE 26)

Figure 15 continued

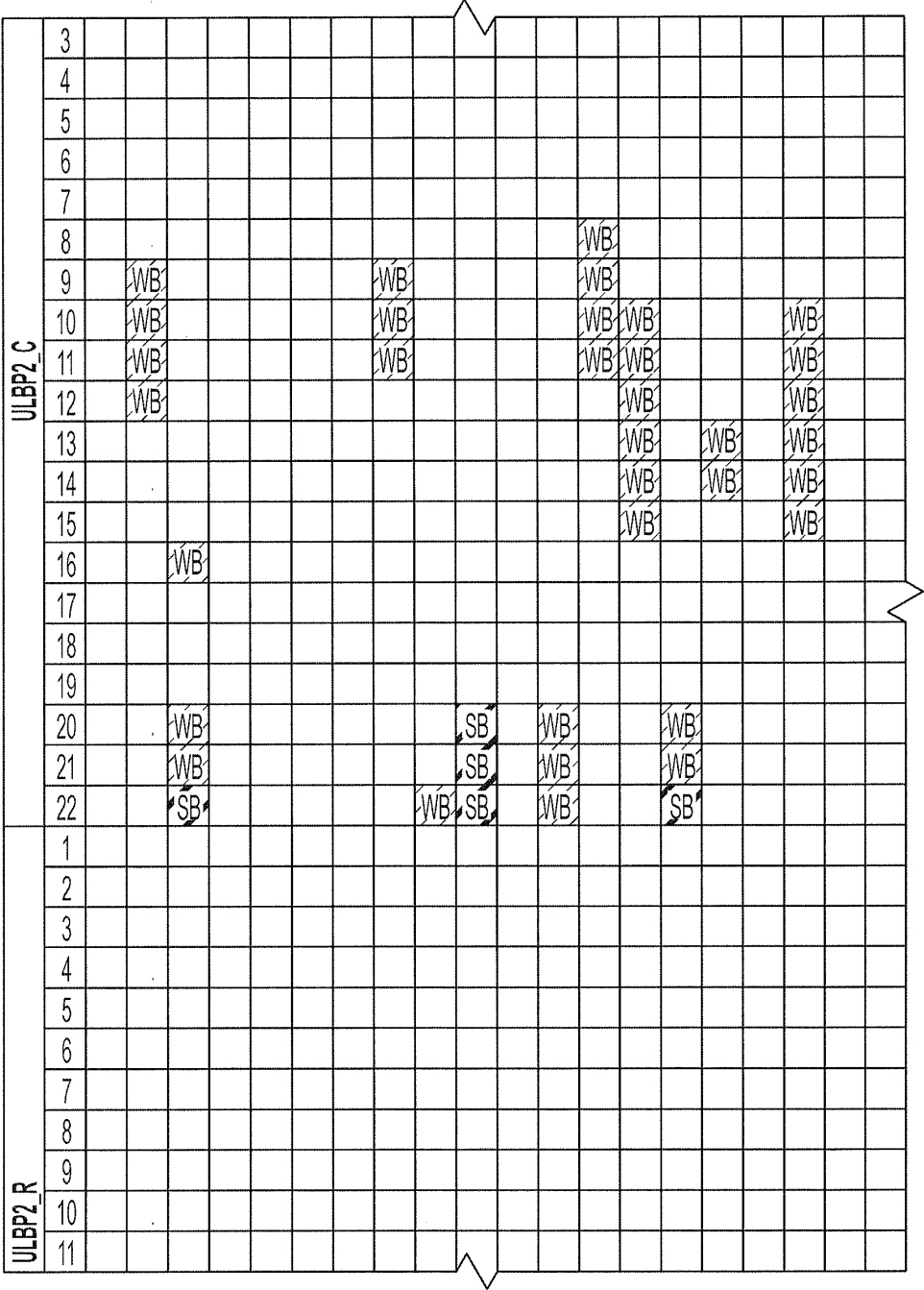


Figure 15 continued

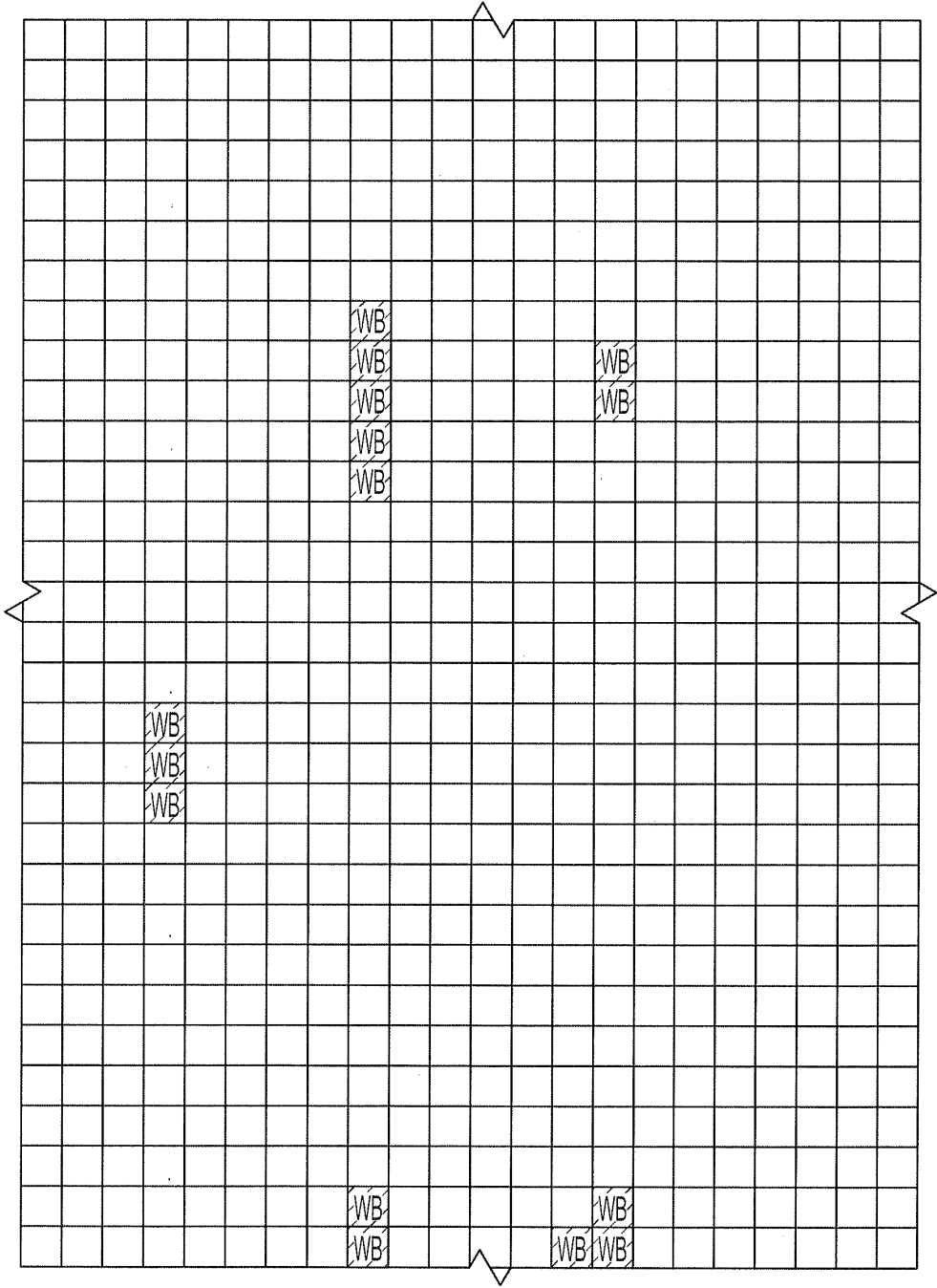
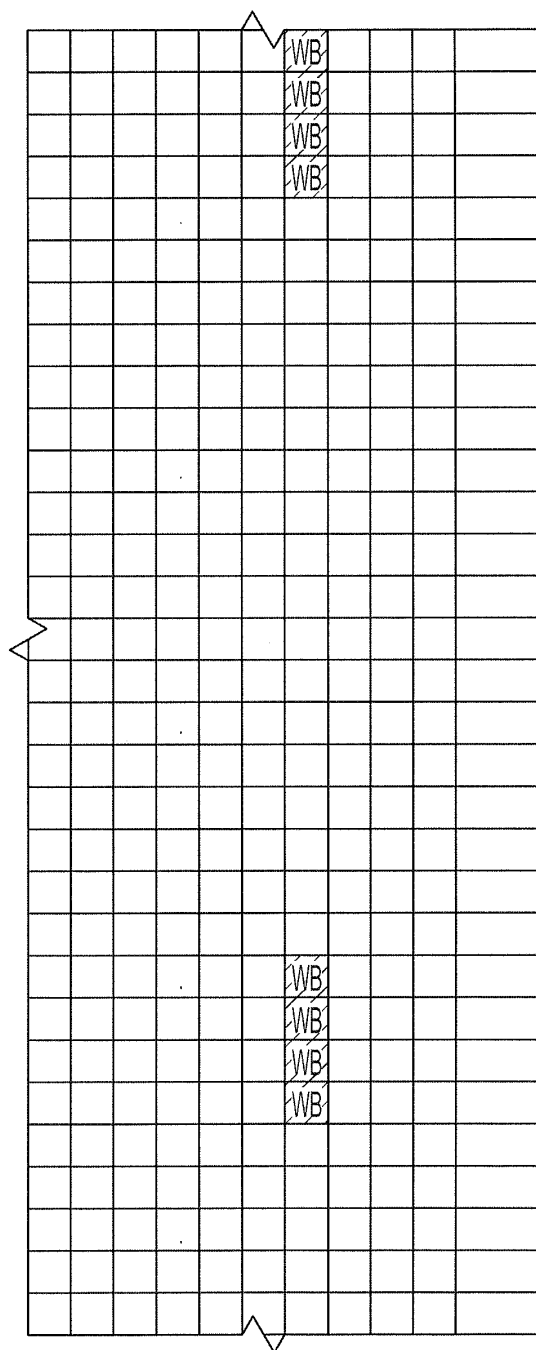


Figure 15 continued

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SUBSTITUTE SHEET (RULE 26)

Figure 15 continued

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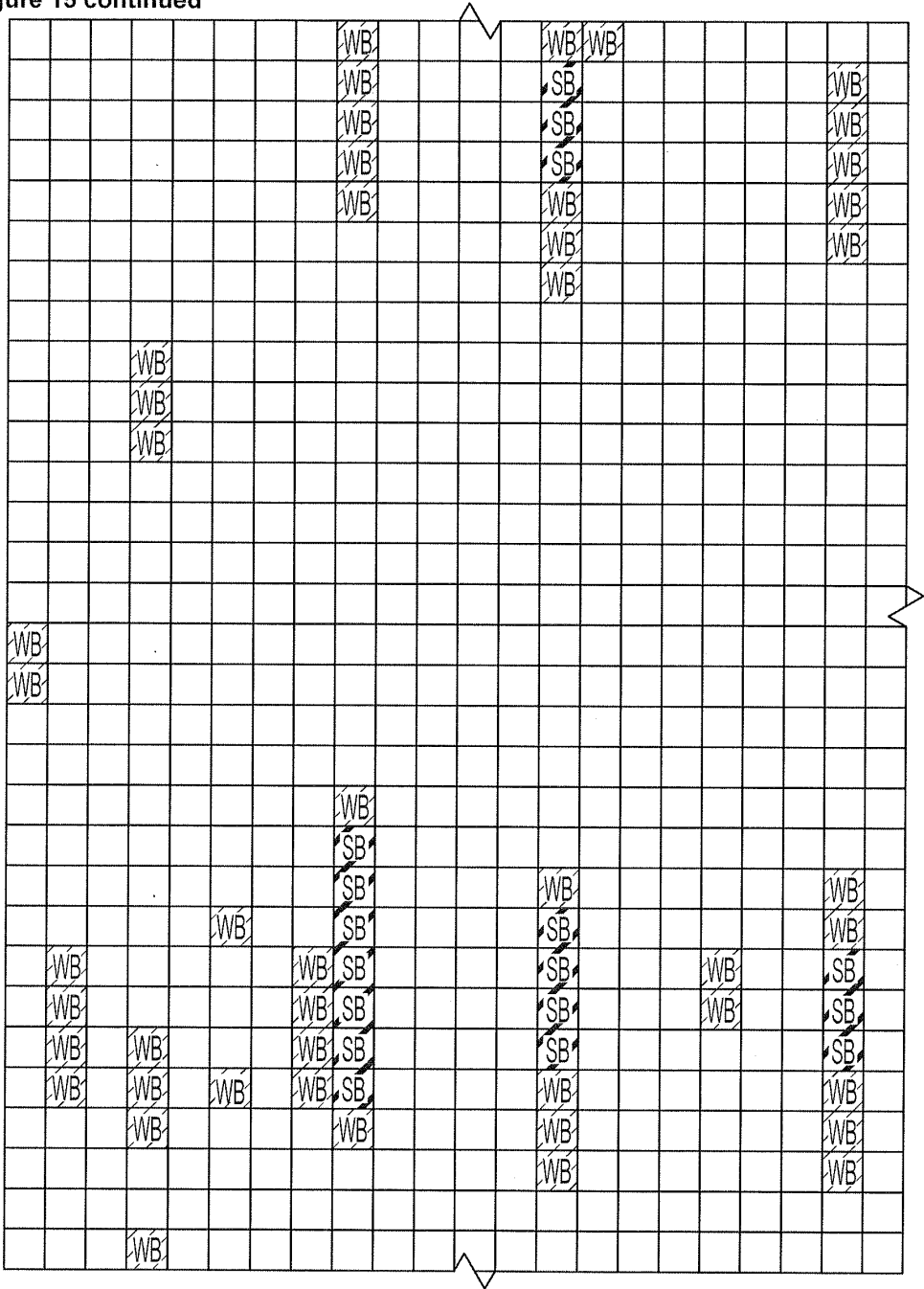


Figure 15 continued

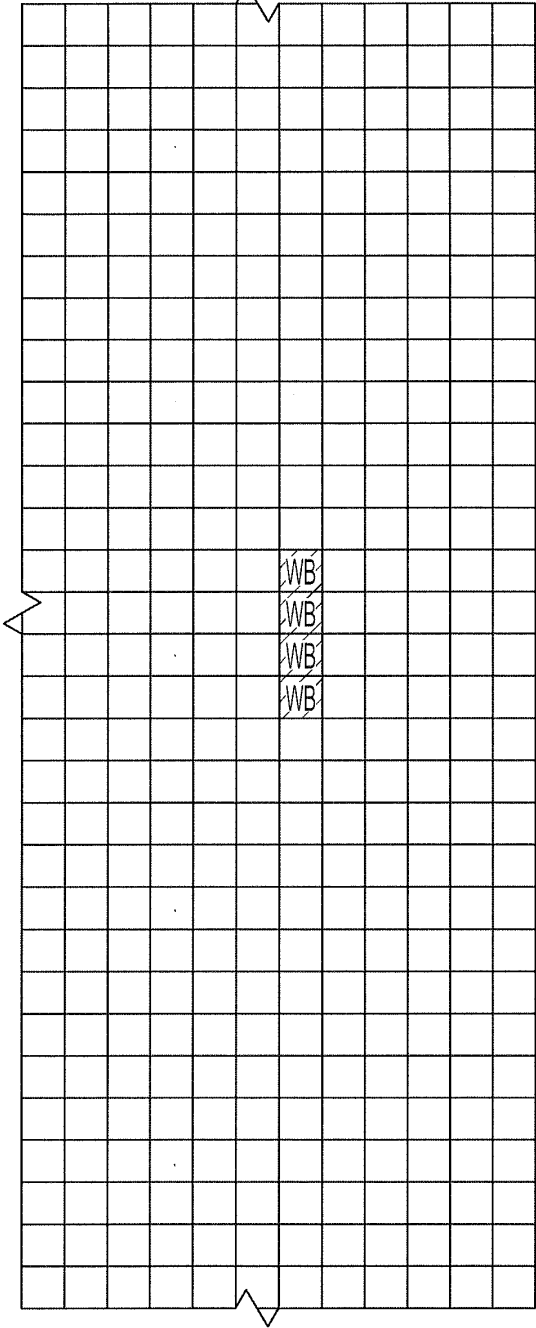
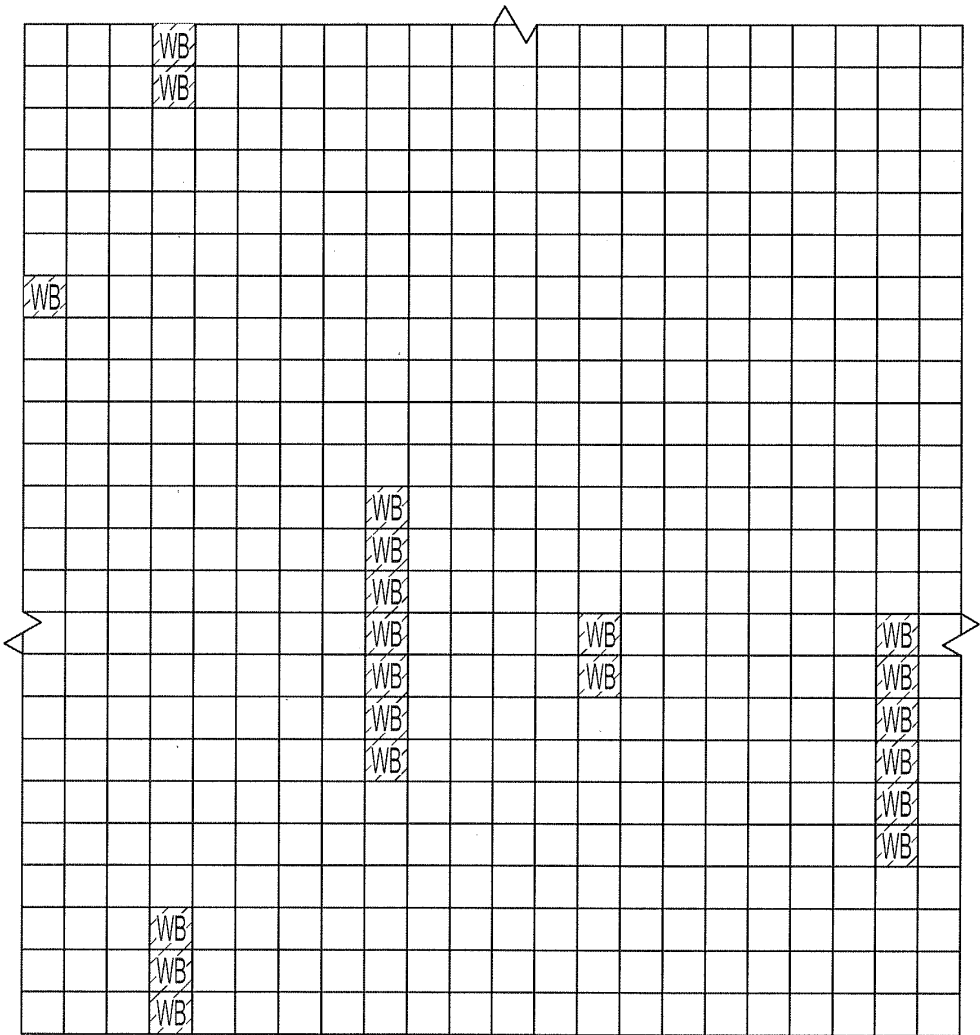


Figure 15 continued

ULBP2 AB	21		WB				WB	SB	WB	WB				
	22		SB				WB	SB	WB		SB			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	9								WB					
	10								WB					
	11								WB					
	12													
	13													
	14													
	15							WB						
	16		WB					WB						
	17		WB					WB						
	18													
	19							WB						
	20		WB					SB	WB		WB			
	21		WB					WB	SB	WB		WB		
	22		SB					WB	SB	WB		SB		

Figure 15 continued



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Figure 15 continued

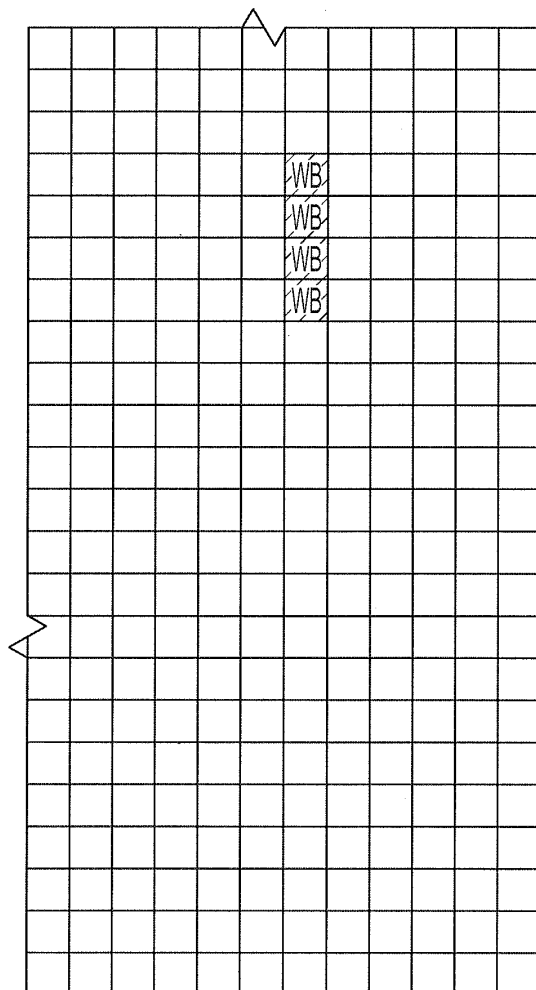


Figure 16A

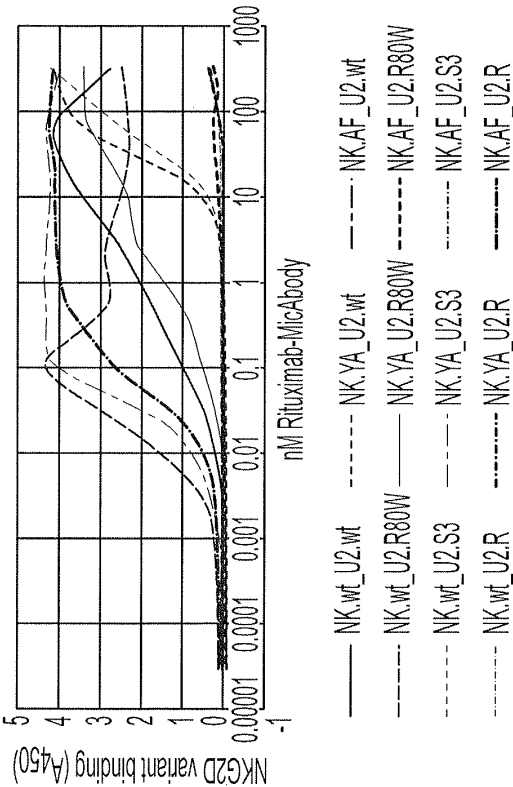


Figure 16B

Ligand	Receptor		
	NKG2D.wt	NKG2D.YA	NKG2D.AF
ULBP2.wt	1.02	27.9	nd
ULBP2.R80W	0.0173	2.18	14.1
ULBP2.S3	52.7	0.0298	nd
ULBP2.R	nd	nd	0.0619

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Figure 17A

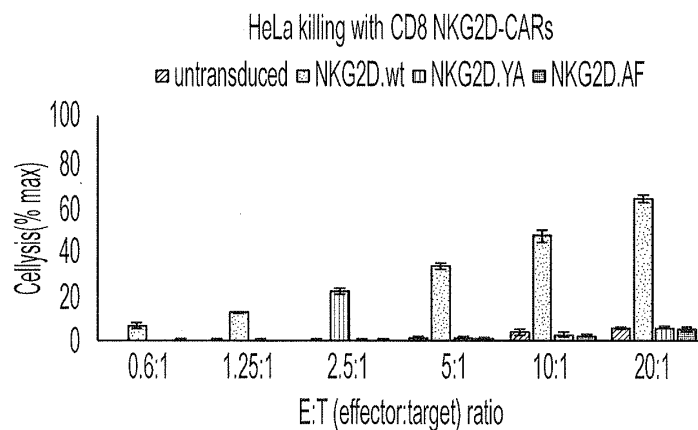


Figure 17B

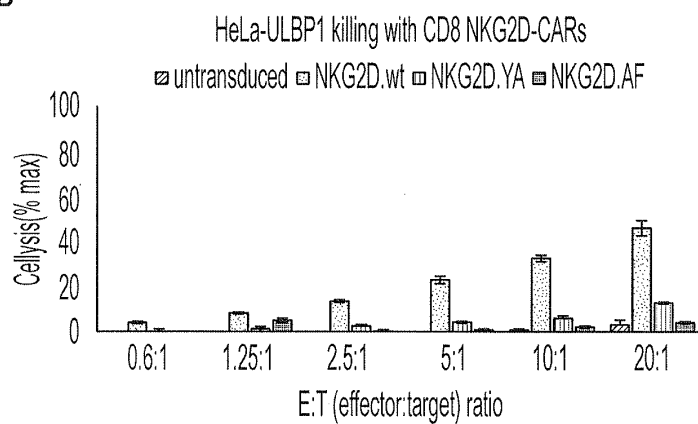
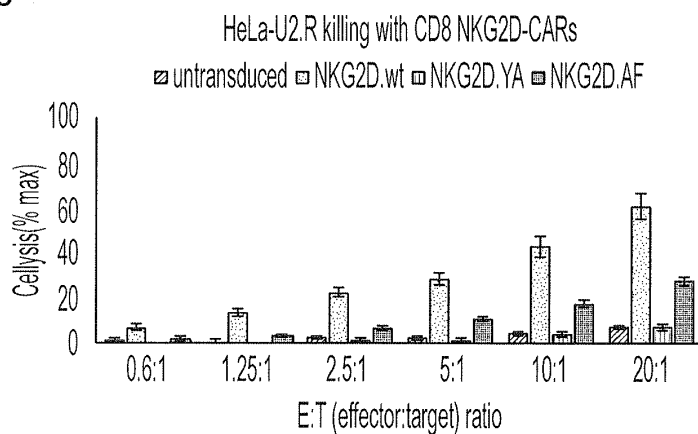


Figure 17C



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Figure 18A

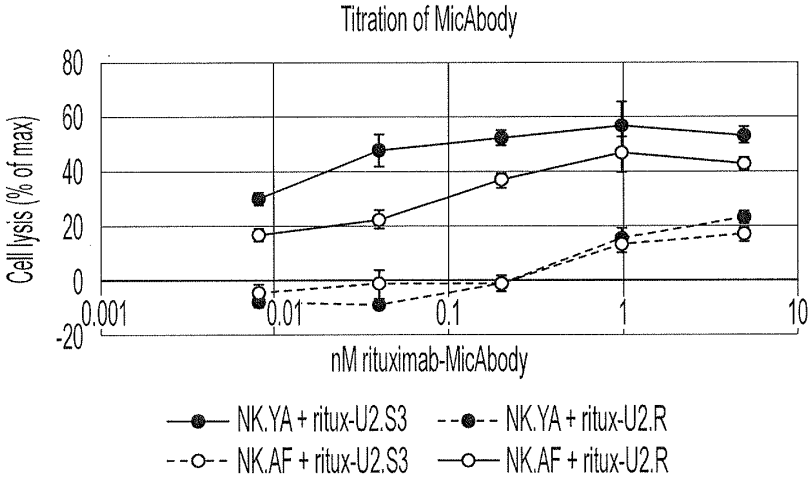


Figure 18B

