

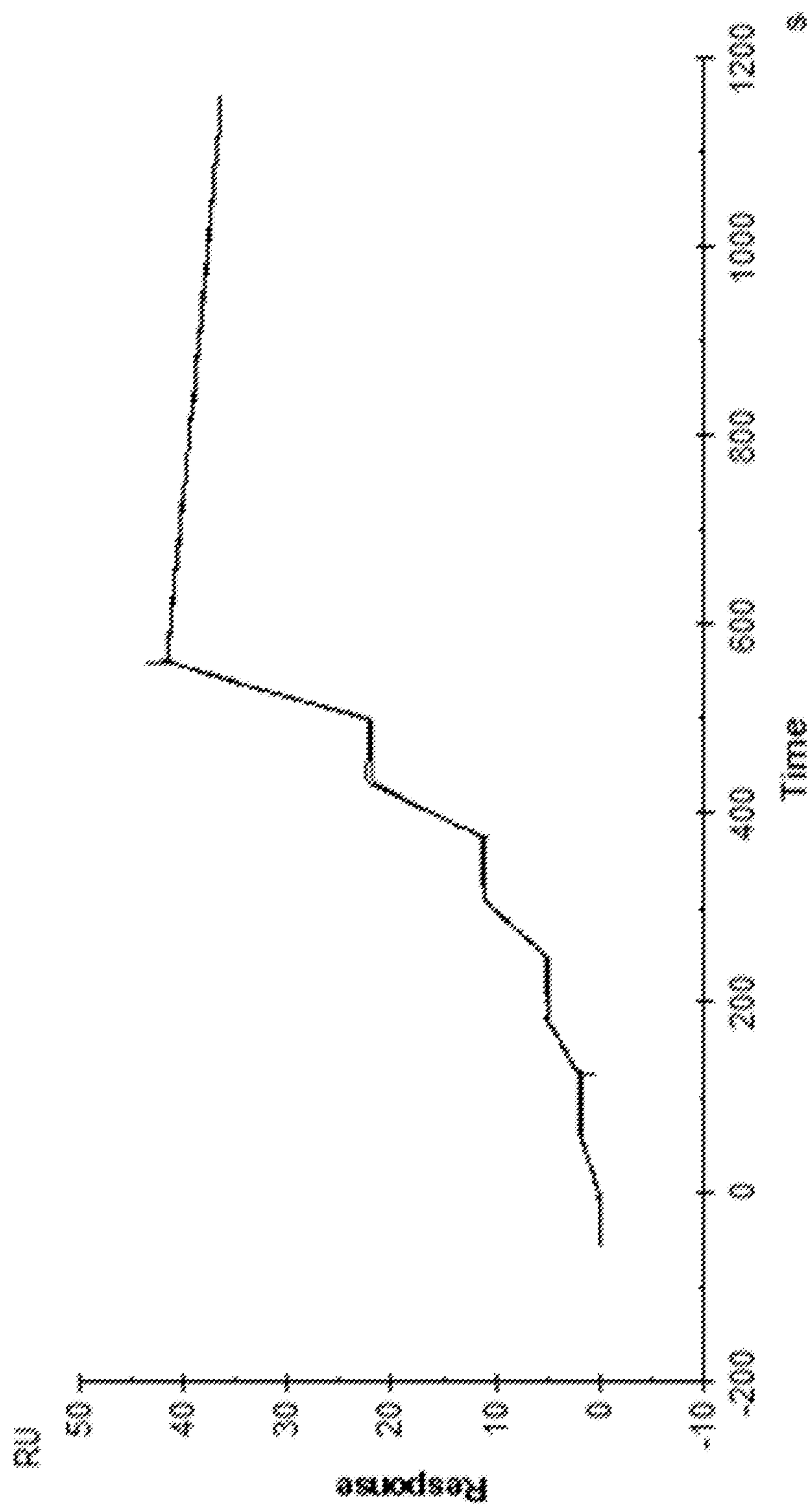


Figure 1

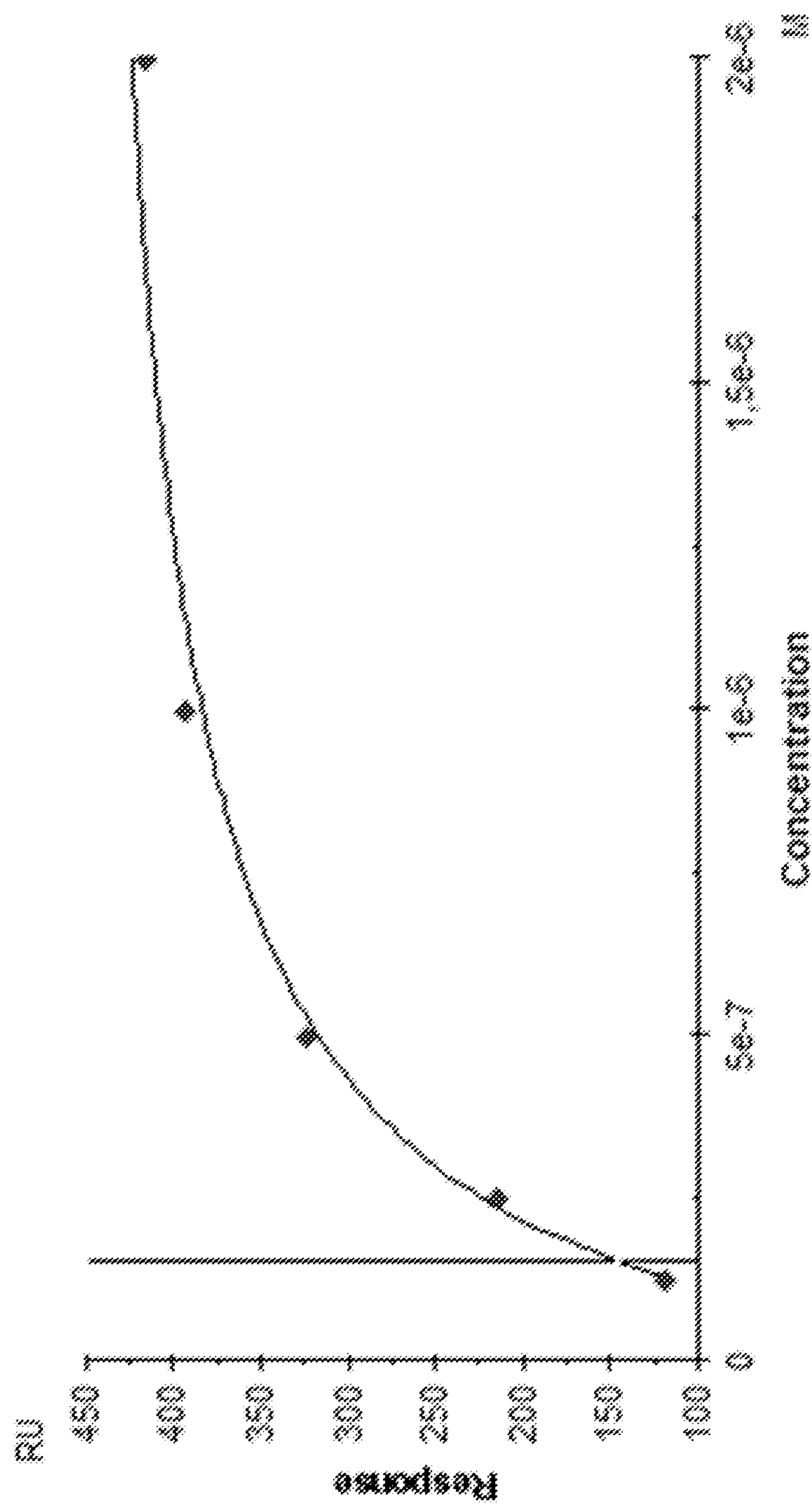


Figure 2

ELISA on purified AS20 scFv

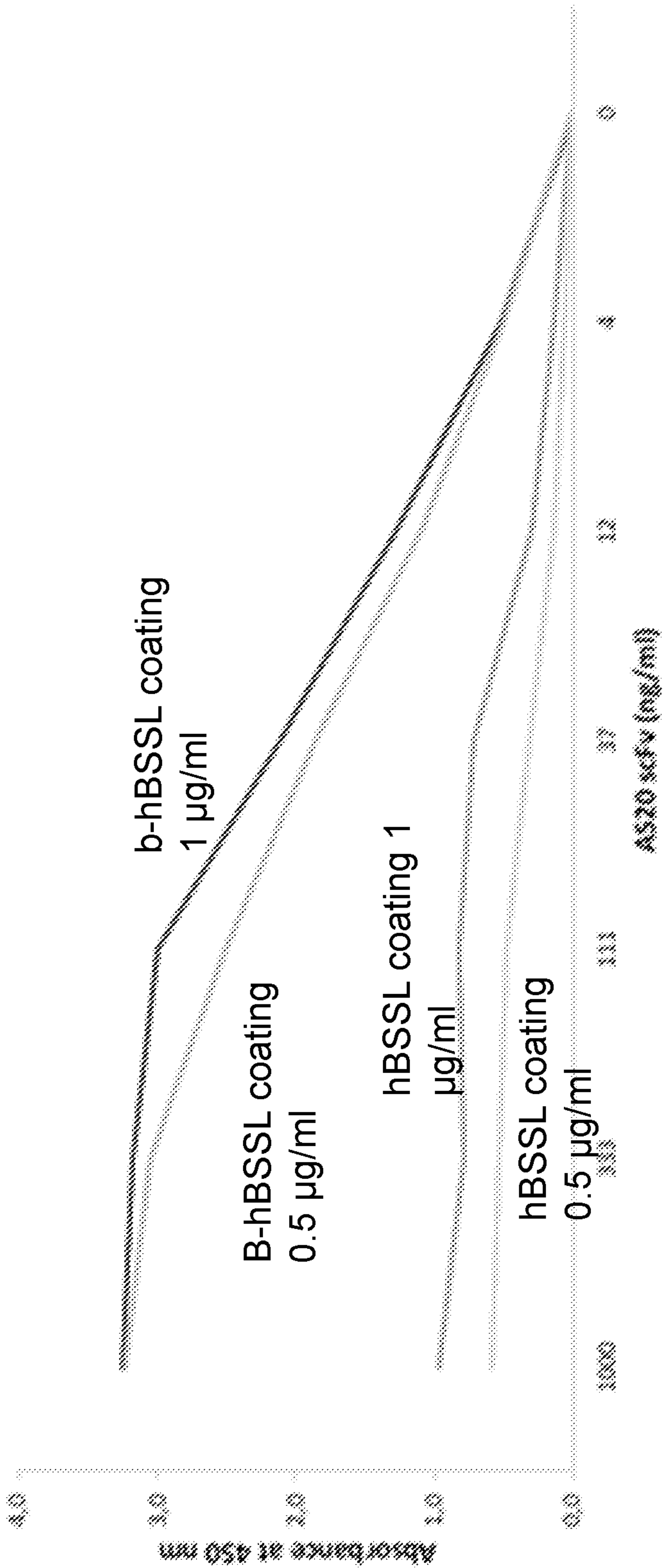


Figure 3a

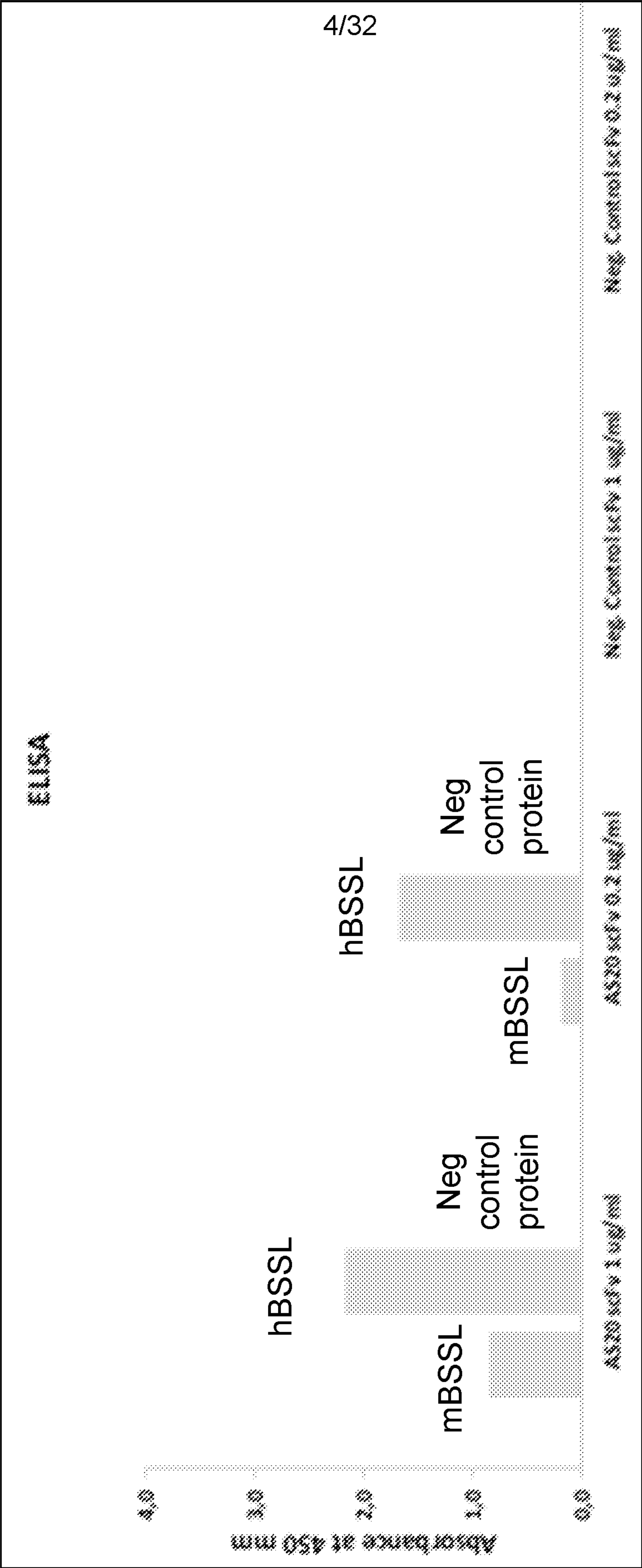


Figure 3b

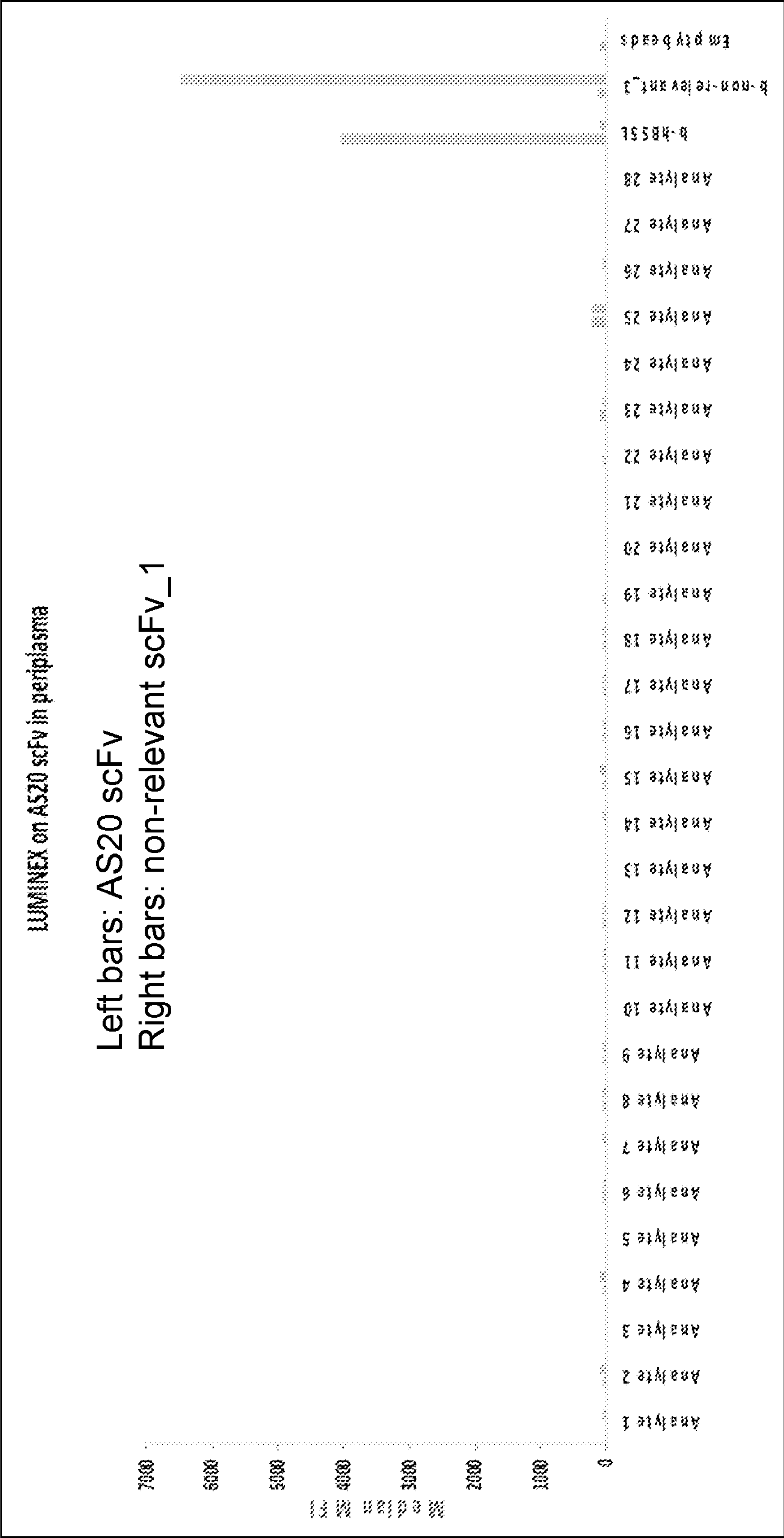


Figure 4

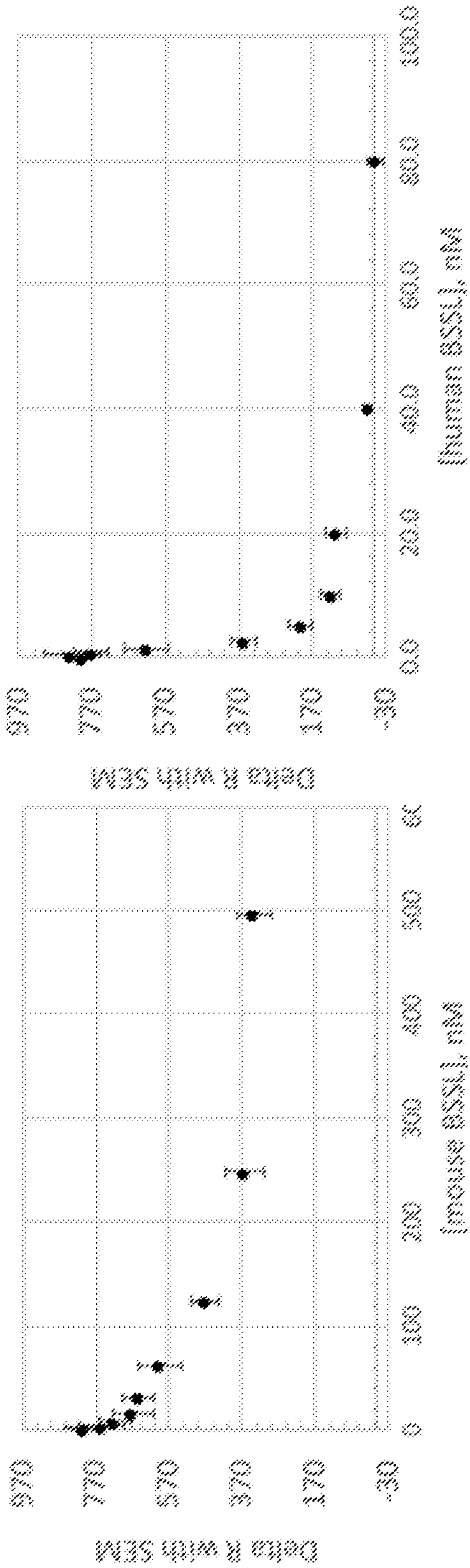


Figure 5

HCVR

IGOT numbering	HCVR1	HCVR2
AS20	Q V Q L Q Q P G A E L V K P S A S V K M S G C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G	Q V Q L Q Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G
IGHV1-48IGHJ1.4	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G
CDR graft clone	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G
AS20 humanization library scaffold	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G
AS20 humanization library diversity	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y N M H W V K Q T P G Q S L E W I G V I Y P G N Q D T S Y N Q K F E K G

HCVR3

IGOT numbering	HCVR3
AS20	K A T L T A D K S S T A Y M C L S S L T S S E D S A V Y Y C A R D Y Y G S S P L C Y W G Q G T T L T Y S S S
IGHV1-48IGHJ1.4	R V T M T R D T S T S T V Y M E L S S L R S S E D T A V Y Y C A R D Y Y G S S P L C Y W G Q G T T L T Y S S S
CDR graft clone	R V T M T R D T S T S T V Y M E L S S L R S S E D T A V Y Y C A R D Y Y G S S P L C Y W G Q G T T L T Y S S S
AS20 humanization library scaffold	R V T M T R D T S T S T V Y M E L S S L R S S E D T A V Y Y C A R D Y Y G S S P L C Y W G Q G T T L T Y S S S
AS20 humanization library diversity	R V T M T R D T S T S T V Y M E L S S L R S S E D T A V Y Y C A R D Y Y G S S P L C Y W G Q G T T L T Y S S S

LCVR

IGOT numbering	LCVR1	LCVR2
AS20	Q I V L T Q S P A V M S A S P G E K V T T C S A S S S I S Y M H W Y Q Q K P G T S P R R W I Y D T S S X Z A S S S	Q I V L T Q S P A V M S A S P G E K V T T C S A S S S I S Y M H W Y Q Q K P G T S P R R W I Y D T S S X Z A S S S
IGHV1-39IGHJ2	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S
CDR graft clone	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S
AS20 humanization library scaffold	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S
AS20 humanization library diversity	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S	D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S I S Y L N W Y Q Q K P G K A P K L L I Y A S S S A S S S

LCVR3

IGOT numbering	LCVR3
AS20	V P A R F S S G S S G T S Y S L T I S S M E A E D A A T Y Y C H Q R S S Y P T F E G G S T K L E L K
IGHV1-39IGHJ2	V P S R F S S G S S G T D E T I T I S S L Q P E D F A T Y Y C H Q R S S Y P T F E G G S T K L E L K
CDR graft clone	V P S R F S S G S S G T D E T I T I S S L Q P E D F A T Y Y C H Q R S S Y P T F E G G S T K L E L K
AS20 humanization library scaffold	V P S R F S S G S S G T D E T I T I S S L Q P E D F A T Y Y C H Q R S S Y P T F E G G S T K L E L K
AS20 humanization library diversity	V P S R F S S G S S G T D E T I T I S S L Q P E D F A T Y Y C H Q R S S Y P T F E G G S T K L E L K

Figure 6

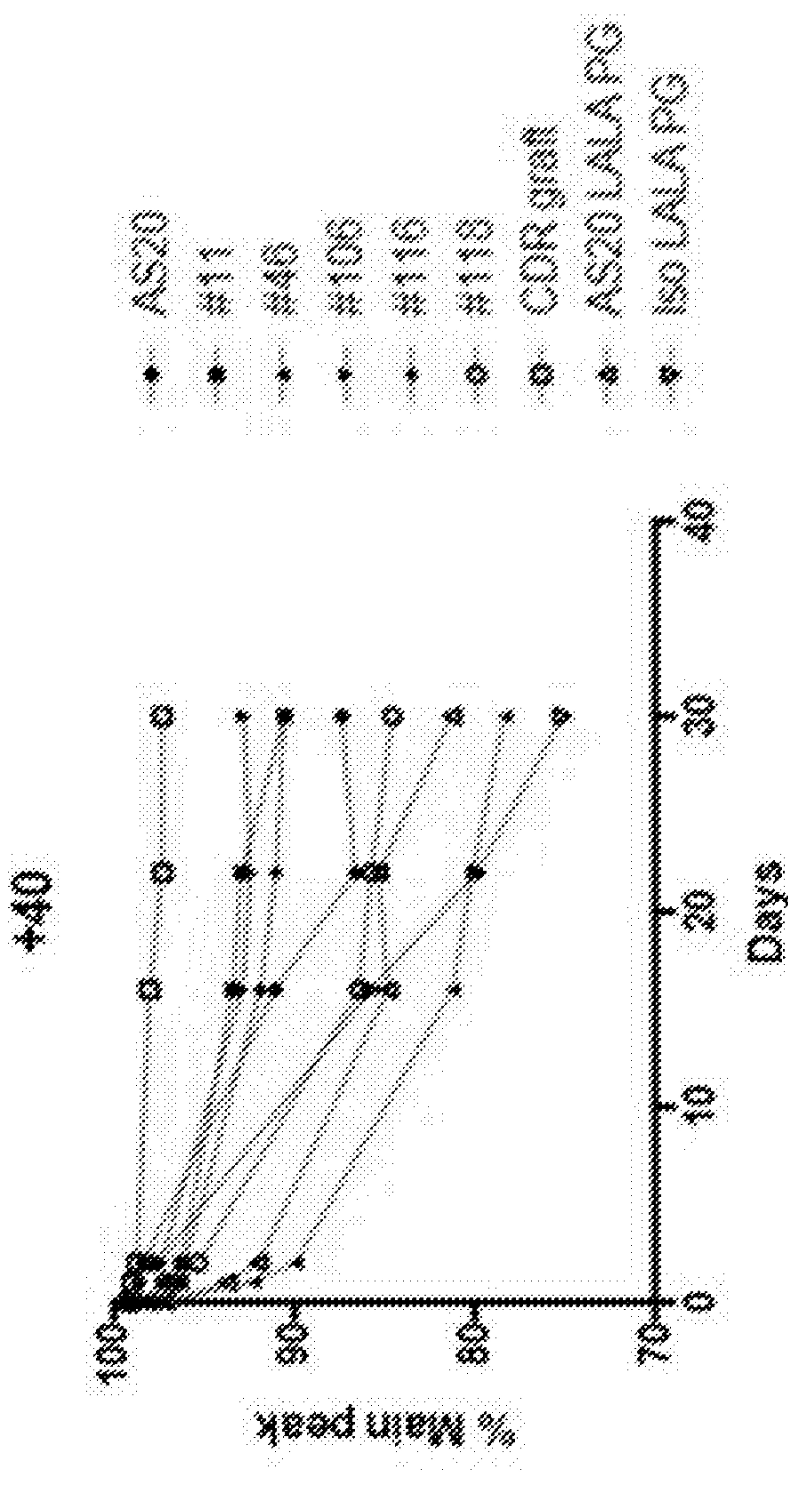


Figure 7A

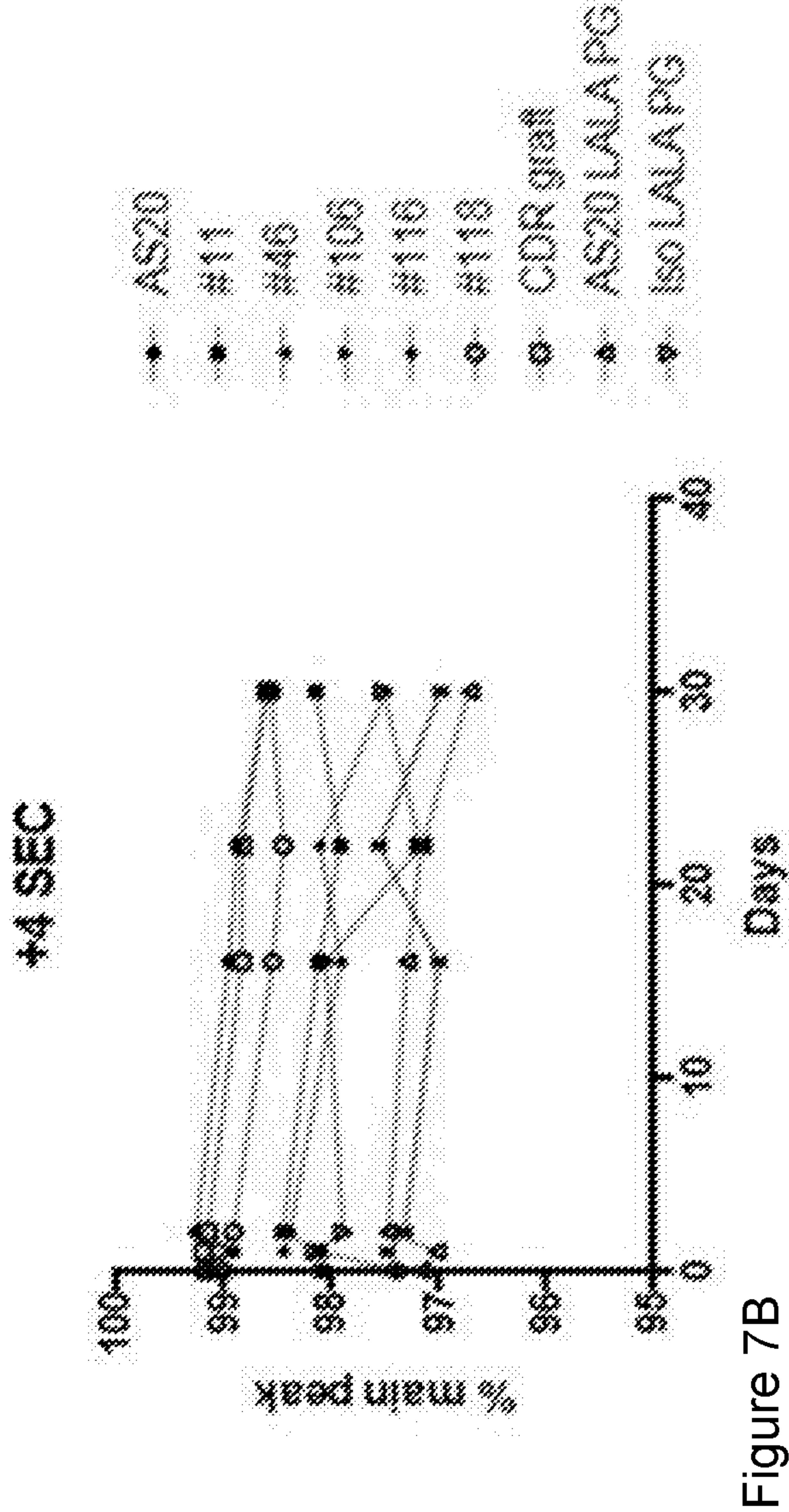


Figure 7B

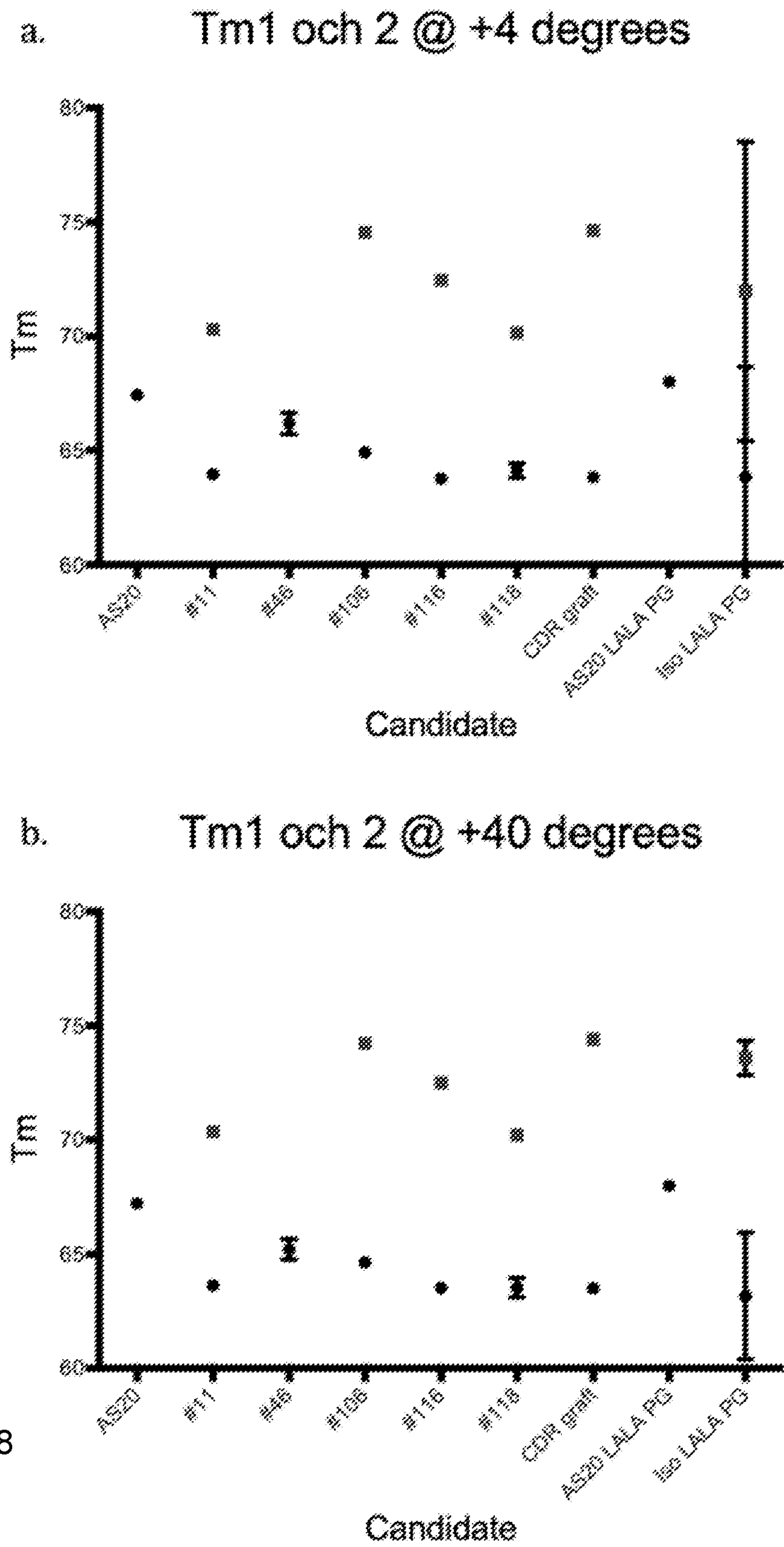


Figure 8

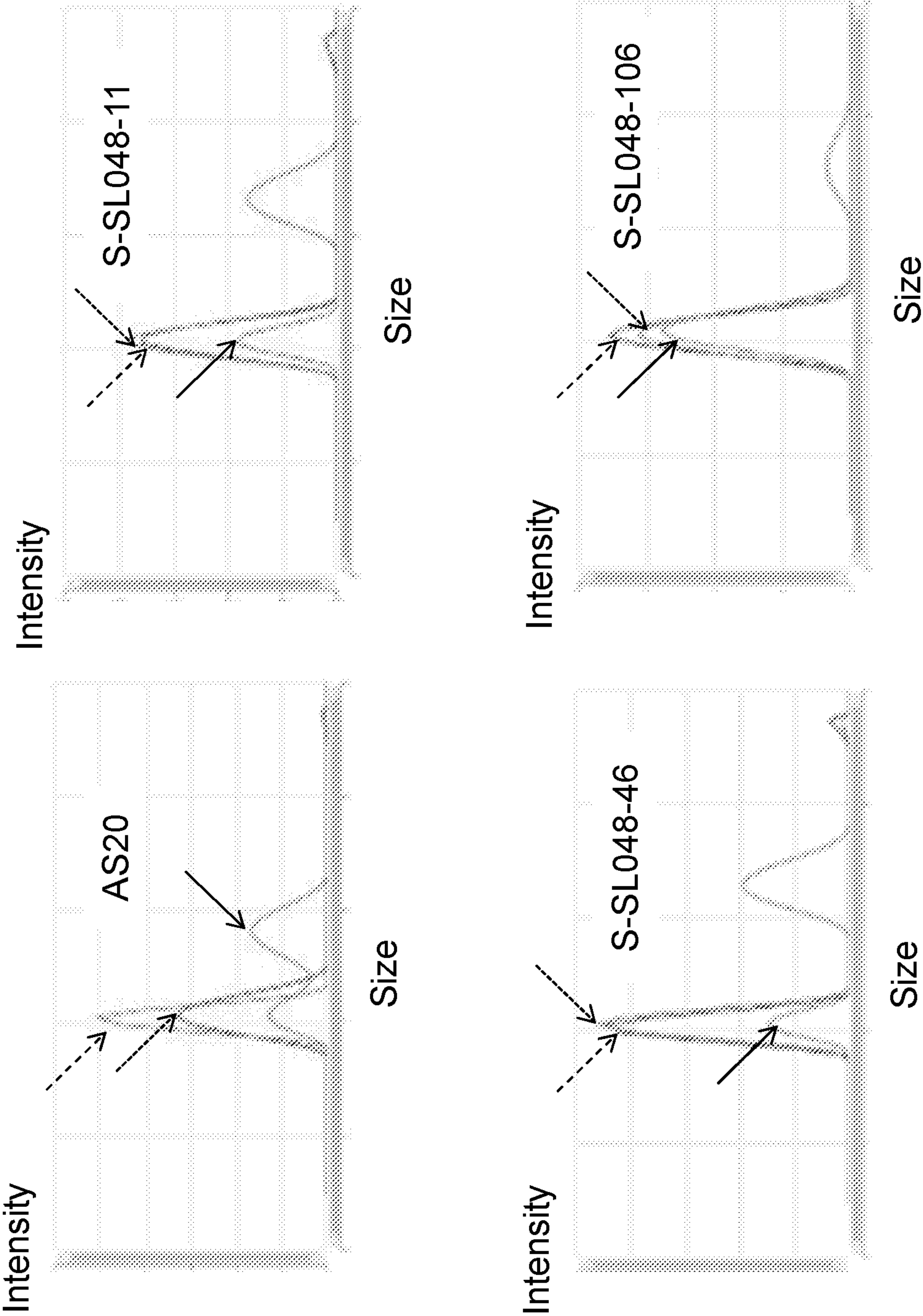


Figure 9

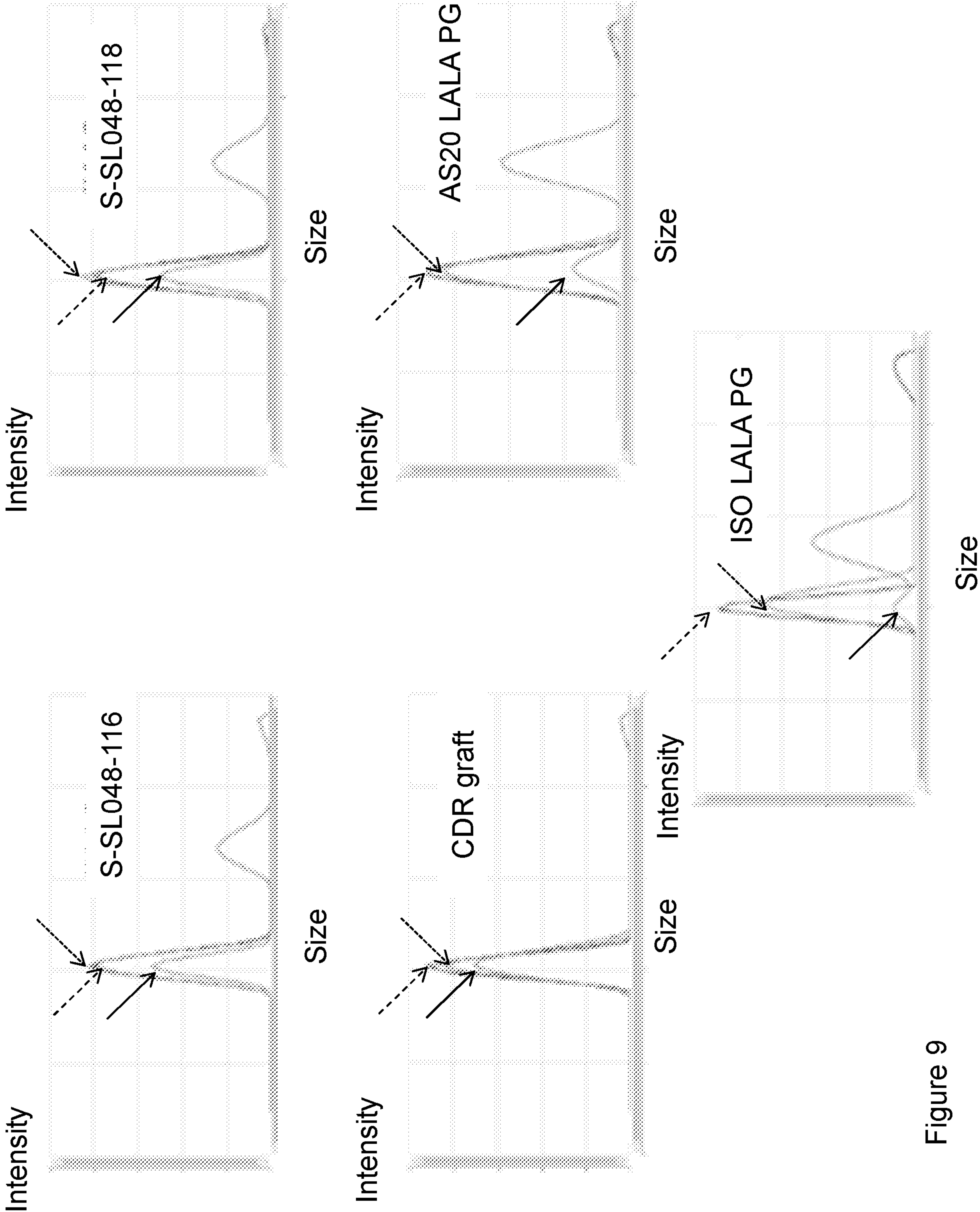


Figure 9

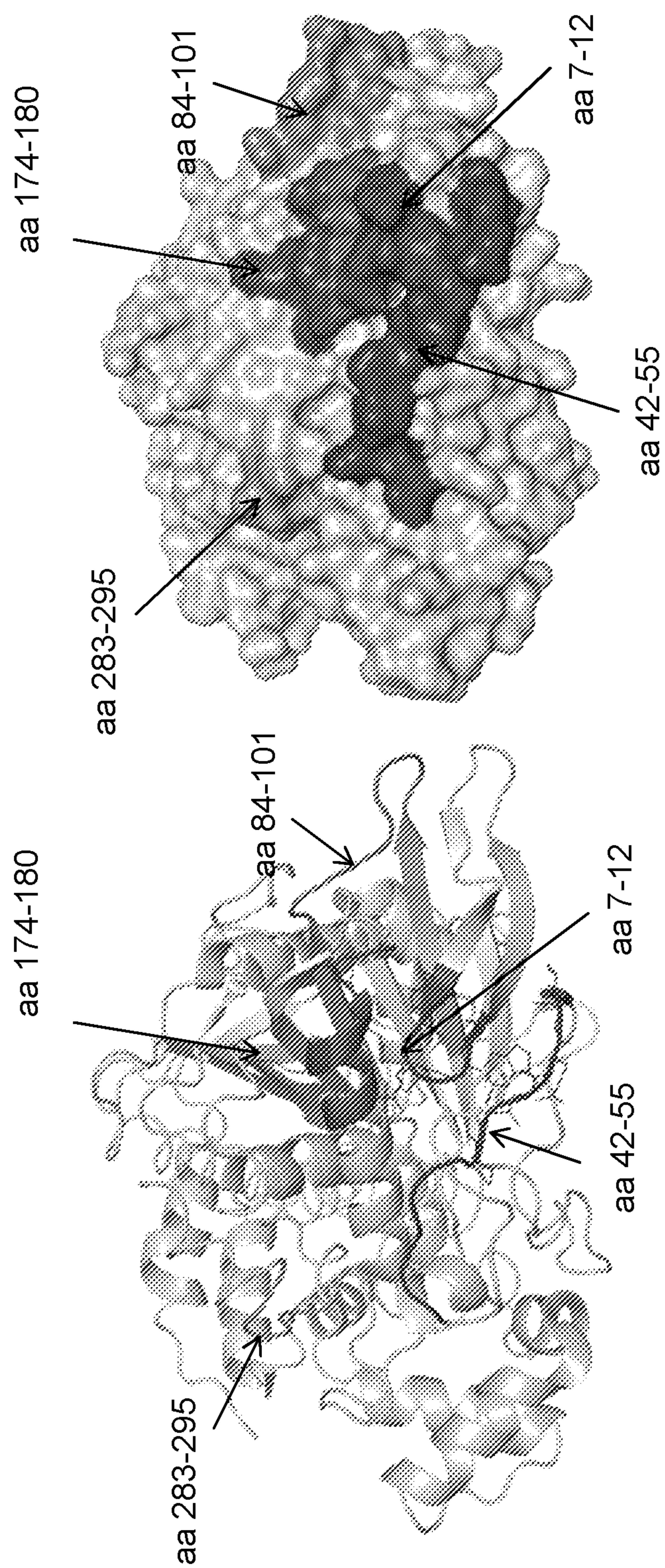


Figure 10

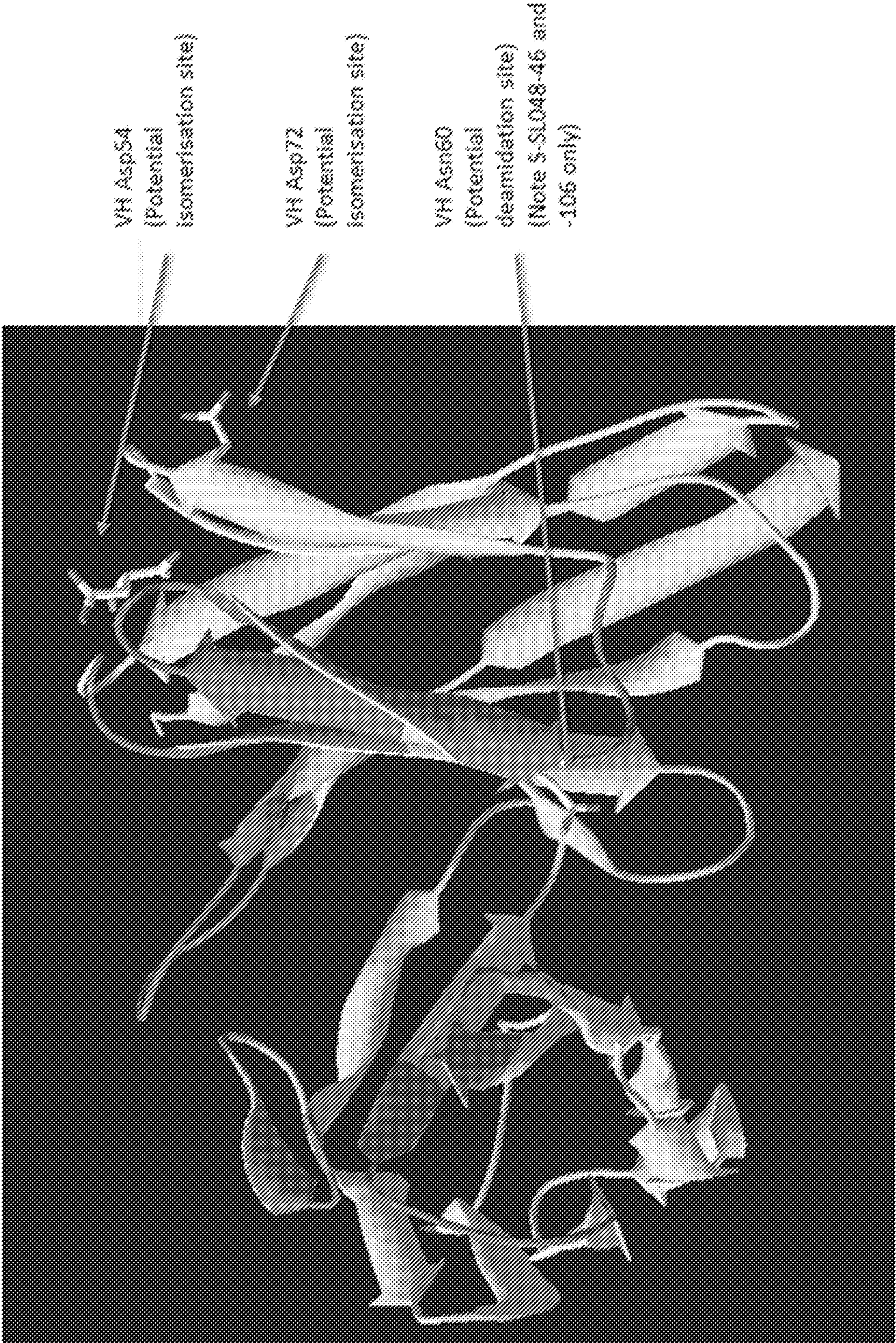


Figure 11

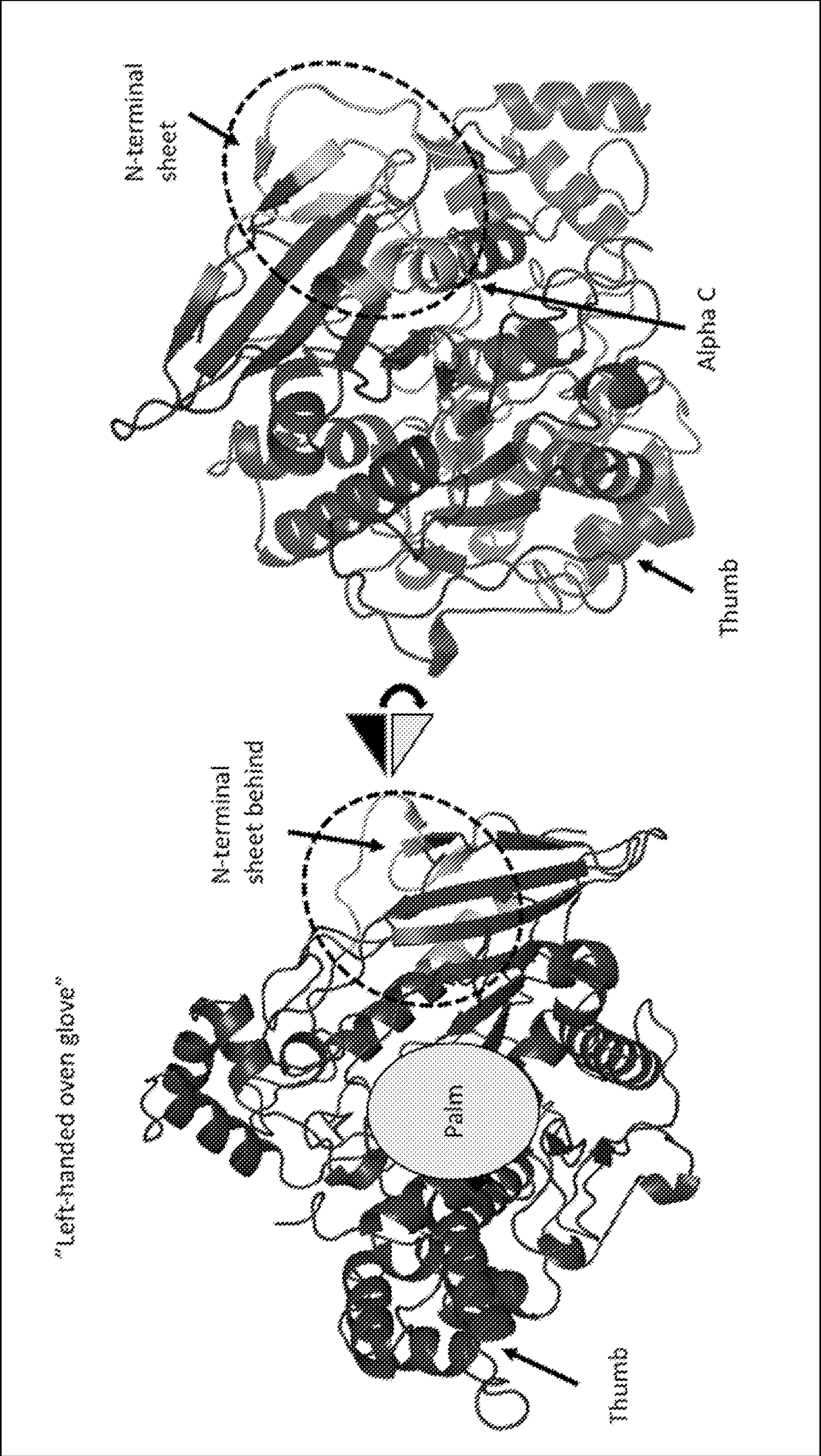


Figure 12

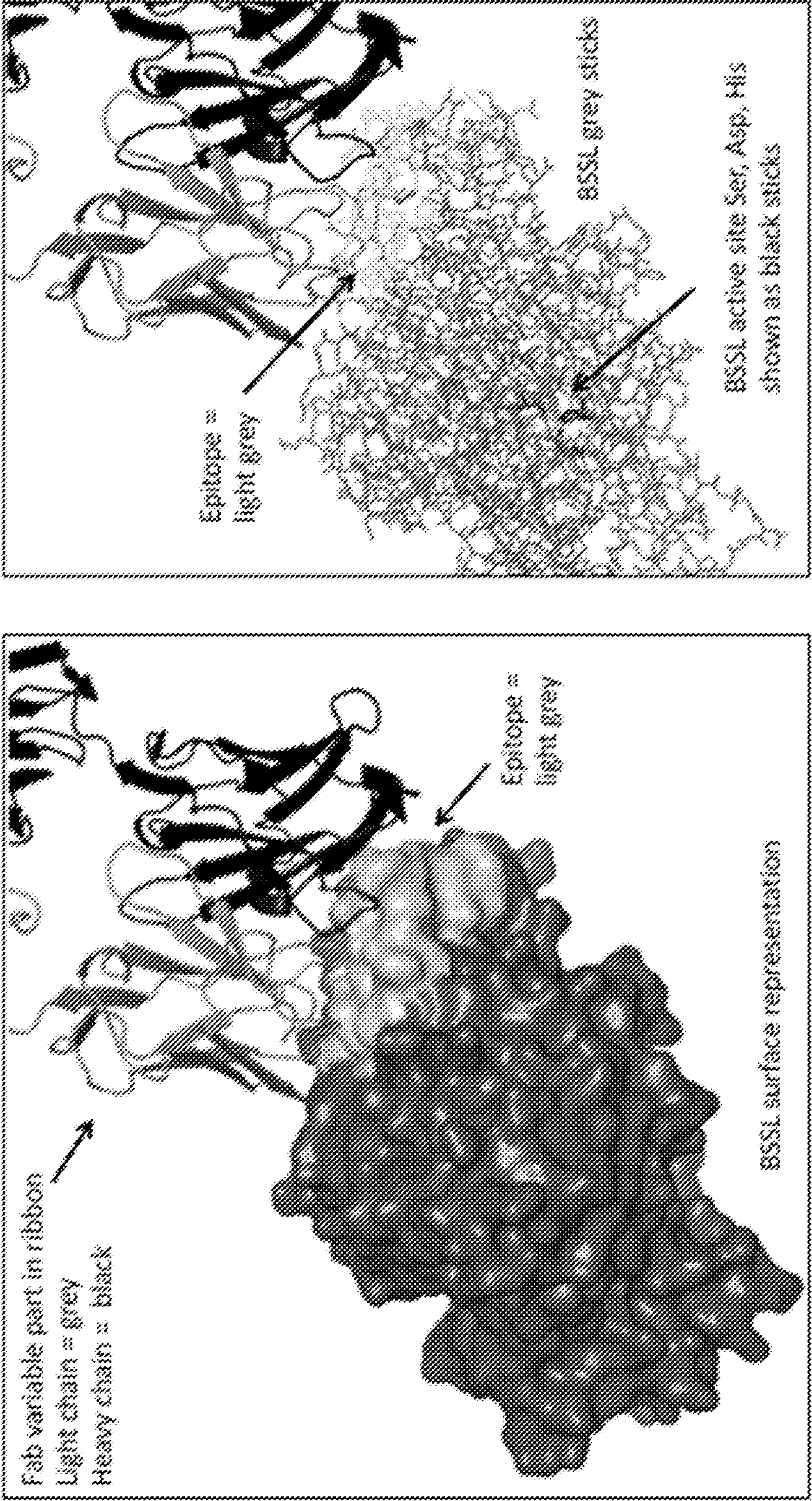


Figure 13

16/32

	FW regions	H1	CDRH2										CDRH3		CDRL1				CDRL2				CDRL3				
			38	39	40	41	42	43	44	45	46	47	57-107		24	27	30	40	56	57	60	69	125	127	128	131	132
AS20	mouse	N	I	V	Y	G	N	D	N	K			DYGGSSPLG		S	S	M	H	D	T	K	A	H	R	S	Y	P
AS20 CDR graft	human	N	I	V	Y	G	N	D	N	K			DYGGSSPLG		S	S	M	H	D	T	K	A	H	R	S	Y	P
Humanization scaffold	human	Y	M	I	N	S	G	S	A	Q			DYGGSSPLG		R	Q	L	N	A	A	S	Q					
S-SL048-10	human	N	I	V	N	S	D	Y	A	Q			DYGGSSPLG		R	P	L	N	A	T	R	P	Q	R	S	S	P
S-SL048-11	human	N	M	V	Y	G	D	A	A	K			DYGGSSPLG		S	P	M	N	A	T	S	A	H	R	S	Y	P
S-SL048-12	human	N	M	V	N	S	D	A	T	Q			DYGGSSPLG		S	S	M	H	A	A	R	A	H	R	S	T	S
S-SL048-13	human	N	I	V	N	S	D	A	T	Q			DYGGSSPLG		S	P	L	N	A	T	S	A	Q	R	S	S	P
S-SL048-14	human	N	M	V	N	G	D	A	A	K			DYGGSSPLG		R	P	L	N	A	T	R	P	Q	R	S	S	P
S-SL048-17	human	N	M	V	N	G	D	Y	T	K			DYGGSSPLG		R	Q	L	N	A	A	K	P	Q	R	S	S	P
S-SL048-18	human	N	M	V	N	S	D	A	T	K			DYGGSSPLG		R	S	L	N	A	T	R	Q	H	R	S	T	P
S-SL048-38	human	N	M	V	N	G	D	A	A	Q			DYGGSSPLG		R	P	L	N	A	T	S	A	Q	R	S	S	P
S-SL048-40	human	N	I	V	N	S	D	A	A	Q			DYGGSSPLG		R	S	L	N	A	A	R	P	Q	R	S	S	P
S-SL048-41	human	N	I	V	Y	G	D	A	A	K			DYGGSSPLG		R	S	M	N	A	T	R	P	H	R	S	S	P
S-SL048-43	human	N	M	V	N	G	D	A	T	Q			DYGGSSPLG		R	P	M	N	A	A	S	P	Q	R	S	S	P
S-SL048-45	human	N	M	V	N	S	D	A	T	Q			DYGGSSPLG		R	Q	L	H	A	A	R	A	H	R	S	T	P
S-SL048-46	human	N	M	V	N	G	D	A	N	Q			DYGGSSPLG		R	S	L	N	A	A	S	A	H	R	S	S	P
S-SL048-47	human	N	I	V	N	G	G	Y	T	Q			DYGGSSPLG		S	P	M	N	A	T	R	Q	Q	R	S	T	P
S-SL048-48	human	N	I	V	N	G	D	A	A	Q			DYGGSSPLG		R	P	M	N	A	T	R	Q	H	R	S	S	P
S-SL048-65	human	N	I	V	N	G	D	A	A	K			DYGGSSPLG		S	Q	L	N	D	A	S	P	H	R	S	S	P
S-SL048-66	human	N	M	V	N	G	D	A	N	K			DYGGSSPLG		S	Q	M	N	D	A	S	A	H	R	S	T	P
S-SL048-74	human	N	I	V	N	G	D	A	A	Q			DYGGSSPLG		R	Q	L	N	A	A	S	A	H	R	S	S	P
S-SL048-75	human	N	M	V	Y	G	G	Y	A	K			DYGGSSPLG		R	Q	L	N	A	T	N	Q	H	R	S	S	P
S-SL048-79	human	N	M	V	N	S	D	A	A	Q			DYGGSSPLG		S	Q	L	N	A	A	R	P	H	R	S	Y	P
S-SL048-81	human	N	I	V	N	G	D	A	A	Q			DYGGSSPLG		R	S	M	H	A	A	S	Q	H	R	S	S	P
S-SL048-86	human	N	M	V	N	G	D	A	A	Q			DYGGSSPLG		R	Q	L	H	A	T	S	Q	H	R	S	Y	P
S-SL048-89	human	N	M	V	N	S	S	S	T	Q			DYGGSSPLG		R	P	L	N	A	T	R	P	Q	R	S	N	P
S-SL048-103	human	N	M	V	N	G	D	A	A	K			DYGGSSPLG		R	S	M	H	A	T	S	A	H	R	S	S	P
S-SL048-104	human	N	M	V	N	G	D	A	D	K			DYGGSSPLG		S	S	L	H	A	A	R	A	H	R	S	T	P
S-SL048-105	human	N	M	V	N	S	D	A	A	Q			DYGGSSPLG		S	Q	L	H	A	A	K	P	H	R	S	Y	P
S-SL048-106	human	N	I	V	Y	G	D	S	N	Q			DYGGSSPLG		S	S	M	N	A	T	K	P	H	R	S	T	P
S-SL048-107	human	N	M	V	N	G	D	A	N	Q			DYGGSSPLG		R	P	L	N	A	T	S	P	Q	R	S	S	P
S-SL048-108	human	N	I	V	N	S	D	A	A	K			DYGGSSPLG		S	P	L	N	A	T	S	A	Q	R	S	S	P
S-SL048-109	human	N	M	V	N	S	D	A	A	K			DYGGSSPLG		R	P	L	N	A	T	R	A	Q	R	S	S	P
S-SL048-110	human	N	M	V	N	G	D	A	N	K			DYGGSSPLG		S	Q	L	H	A	A	R	A	H	R	S	T	P
S-SL048-112	human	N	I	I	Y	G	D	A	N	K			DYGGSSPLG		R	S	M	H	A	T	R	A	H	R	S	T	P
S-SL048-115	human	N	M	V	N	S	D	A	A	K			DYGGSSPLG		R	Q	M	H	A	A	K	P	H	R	S	S	P
S-SL048-116	human	N	M	V	N	G	D	A	A	K			DYGGSSPLG		R	P	M	N	A	T	S	A	H	R	S	S	P
S-SL048-118	human	N	M	V	N	G	D	A	A	K			DYGGSSPLG		S	P	M	N	A	T	S	P	H	R	S	S	P
S-SL048-125	human	N	I	V	N	G	D	A	A	Q			DYGGSSPLG		R	S	L	H	A	A	R	P	H	R	S	Y	P
S-SL048-131	human	N	I	V	N	G	D	A	A	K			DYGGSSPLG		R	P	L	N	A	A	K	A	H	R	S	S	P
S-SL048-134	human	N	M	V	N	S	D	A	A	K			DYGGSSPLG		R	P	L	N	A	T	K	P	Q	R	S	Y	P

Positions/regions marked in dark gray corresponds to diversity as found in the original AS20 clone

Positions/regions marked in light gray corresponds to diversity as found in the human germline gene used as scaffold for library generation

Positions marked in white corresponds to amino acid residues not found in AS20 or human germline gene used as scaffold for library generation

Figure 14

	CDRH1 (KABAT)																			
	CDRH1 (IMGT)																			
IMGT numbering	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AS20 VH	Q	V	Q	L	Q	Q	R	Q	A	S	E	L	V	K	R	Q	A	S	V	R
IGHV1-46*01/IGHJ-4*01	Q	V	Q	L	Q	Q	S	Q	A	S	E	L	V	K	R	Q	A	S	V	R
CDR graft clone	Q	V	Q	L	Q	Q	S	Q	A	S	E	L	V	K	R	Q	A	S	V	R
AS20 humanization library scaffold	Q	V	Q	L	Q	Q	S	Q	A	S	E	L	V	K	R	Q	A	S	V	R
AS20 humanization library diversity	Q	V	Q	L	Q	Q	S	Q	A	S	E	L	V	K	R	Q	A	S	V	R

	CDRH2 (KABAT)																			
	CDRH2 (IMGT)																			
IMGT numbering	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
AS20 VH	N	V	K	Q	T	R	Q	Q	Q	L	R	N	I	Q	V	I	I	R	S	Q
IGHV1-46*01/IGHJ-4*01	N	V	R	Q	A	R	Q	Q	Q	L	R	N	I	Q	V	I	I	R	S	Q
CDR graft clone	N	V	R	Q	A	R	Q	Q	Q	L	R	N	I	Q	V	I	I	R	S	Q
AS20 humanization library scaffold	N	V	R	Q	A	R	Q	Q	Q	L	R	N	I	Q	V	I	I	R	S	Q
AS20 humanization library diversity	N	V	R	Q	A	R	Q	Q	Q	L	R	N	I	Q	V	I	I	R	S	Q

	CDRH3 (KABAT)																			
	CDRH3 (IMGT)																			
IMGT numbering	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
AS20 VH	K	A	T	L	T	A	D	K	S	S	T	A	Y	M	Q	L	S	S	L	T
IGHV1-46*01/IGHJ-4*01	R	V	T	M	T	R	D	T	S	T	S	T	V	Y	M	E	L	S	S	L
CDR graft clone	R	V	T	M	T	R	D	T	S	T	S	T	V	Y	M	E	L	S	S	L
AS20 humanization library scaffold	R	V	T	M	T	R	D	T	S	T	S	T	V	Y	M	E	L	S	S	L
AS20 humanization library diversity	R	V	T	M	T	R	D	T	S	T	S	T	V	Y	M	E	L	S	S	L

	CDRH3 (KABAT)																			
	CDRH3 (IMGT)																			
IMGT numbering	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124
AS20 VH	A	R	D	Y	Y	G	S	S	S	P	L	G	Y	W	G	Q	G	T	T	L
IGHV1-46*01/IGHJ-4*01	A	R											Y	W	G	Q	G	T	L	Y
CDR graft clone	A	R	D	Y	Y	G	S	S	S	P	L	G	Y	W	G	Q	G	T	L	Y
AS20 humanization library scaffold	A	R	D	Y	Y	G	S	S	S	P	L	G	Y	W	G	Q	G	T	L	Y
AS20 humanization library diversity	A	R	D	Y	Y	G	S	S	S	P	L	G	Y	W	G	Q	G	T	L	Y

Figure 15A

	Target positions VH	Designed* diversity
1	38	N, <u>Y</u>
2	53	I, <u>M</u>
3	55	<u>V</u> , <u>I</u>
4	57	Y, <u>N</u>
5	59	<u>G</u> , <u>S</u>
6	62	<u>N</u> , <u>G</u> , <u>S</u> , D**
7	64	D, <u>S</u> , A, Y**
8	68	N, <u>A</u> , T, D**
9	72	K, <u>Q</u>

*Bold amino acids are those found in AS20, in underlined those in human germline
**binary diversity cannot be accomplished with normal NNS oligoes

Figure 15B

IMGT numbering	CDRL1 (KABAT)																			
	CDRL1 (IMGT)																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AS20 VL	Q	L	V	L	S	Q	S	S	A	V	N	S	N	S	P	R	E	N	N	N
IGKV1-39/IGKJ2	D	L	Q	N	L	Q	S	S	S	S	L	S	A	S	V	Q	Q	N	V	N
CD8 graft clone	D	L	Q	N	L	Q	S	S	S	S	L	S	A	S	V	Q	Q	N	V	N
AS20 humanization library scaffold	D	L	Q	N	L	Q	S	S	S	S	L	S	A	S	V	Q	Q	N	V	N
AS20 humanization library diversity	D	L	Q	N	L	Q	S	S	S	S	L	S	A	S	V	Q	Q	N	V	N

IMGT numbering	CDRL2 (KABAT)																			
	CDRL2 (IMGT)																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
AS20 VL	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
IGKV1-39/IGKJ2	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
CD8 graft clone	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library scaffold	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library diversity	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N

IMGT numbering	CDRL3 (KABAT)																			
	CDRL3 (IMGT)																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
AS20 VL	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
IGKV1-39/IGKJ2	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
CD8 graft clone	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library scaffold	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library diversity	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N

IMGT numbering	CDRL3 (KABAT)																			
	CDRL3 (IMGT)																			
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
AS20 VL	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
IGKV1-39/IGKJ2	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
CD8 graft clone	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library scaffold	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library diversity	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N

IMGT numbering	CDRL3 (KABAT)																			
	CDRL3 (IMGT)																			
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
AS20 VL	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
IGKV1-39/IGKJ2	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
CD8 graft clone	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library scaffold	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library diversity	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N

IMGT numbering	CDRL3 (KABAT)																			
	CDRL3 (IMGT)																			
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
AS20 VL	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
IGKV1-39/IGKJ2	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
CD8 graft clone	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library scaffold	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N
AS20 humanization library diversity	N	L	Q	Q	Q	X	P	Q	S	P	Q	S	S	S	N	N	N	N	N	N

Figure 16A

Target positions		Designed* diversity
1 2 3 4 5 6 7 8 9 10 11 12 13	VL	
	24	S, <u>R</u>
	27	S, <u>Q</u> , P, STOP**
	39	M, <u>L</u>
	40	H, <u>N</u>
	56	D, <u>A</u>
	57	T, <u>A</u>
	66	K, <u>S</u> , N, R**
	68	A, <u>Q</u> , P, E**
	105	H, <u>Q</u>
	107	R, <u>S</u>
	108	Y, <u>S</u>
	115***	Y, T, N, S
	116***	P, H, L, Y, S, F

VH-VL library size:
4096 x 393 216 = 1.6 x 10⁹ variants

**Bold amino acids are those found in AS20, in underlined those in human germline*
***binary diversity cannot be accomplished with normal NNS oligoes*
**** functional position, include amino acid normally found in IMGt analysis of L3s of length 8*

Figure 16B

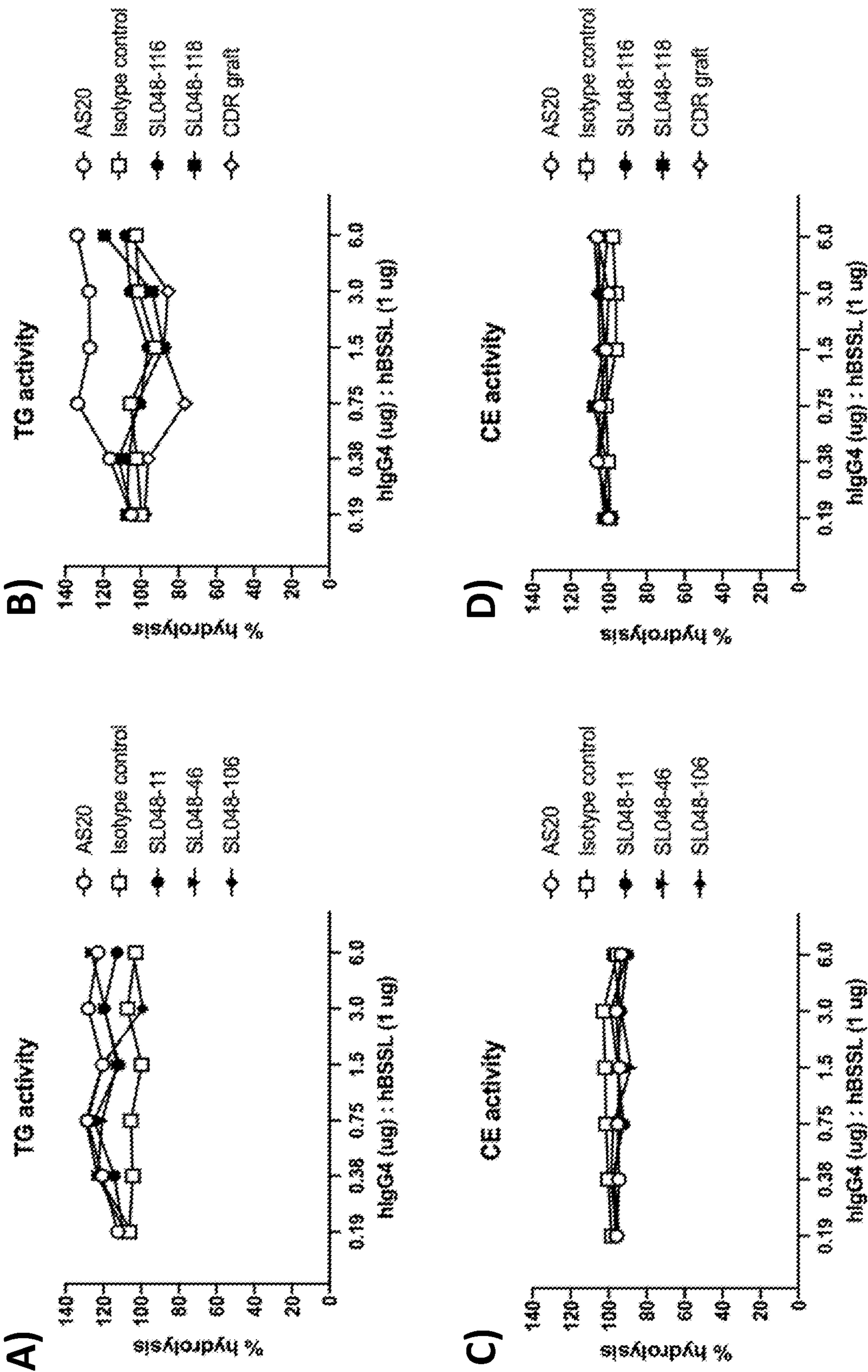


Figure 17

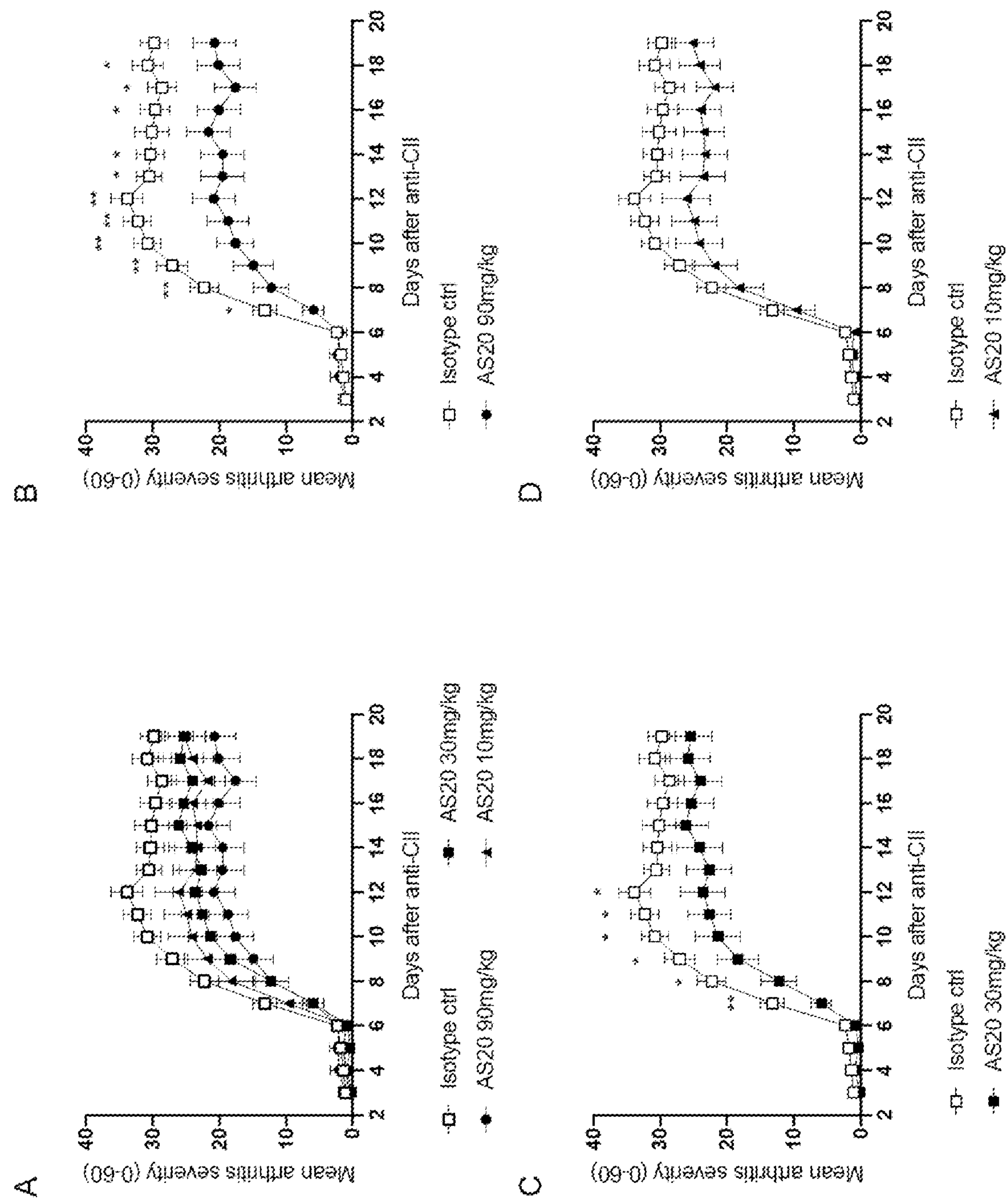


Figure 18

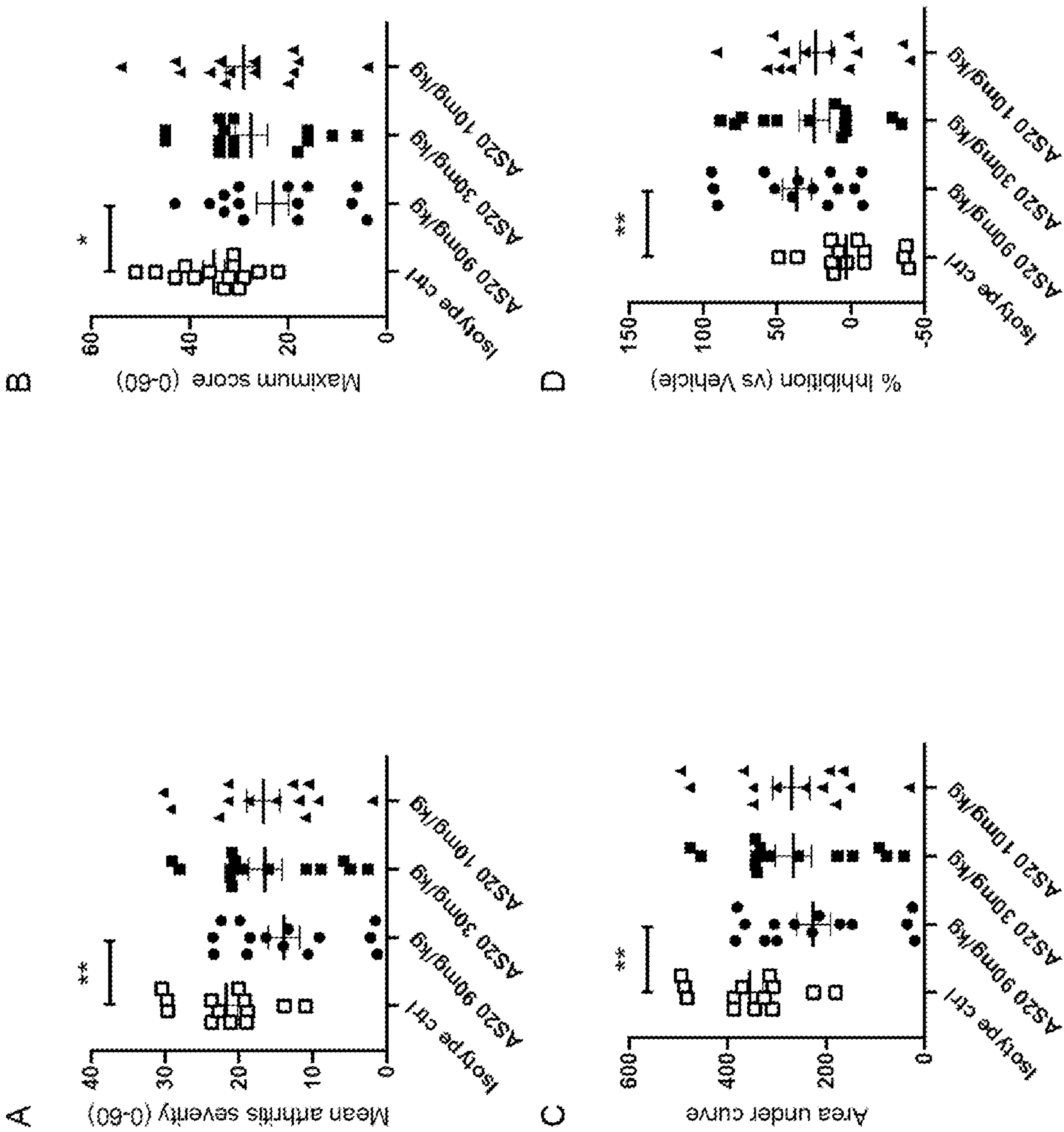


Figure 19

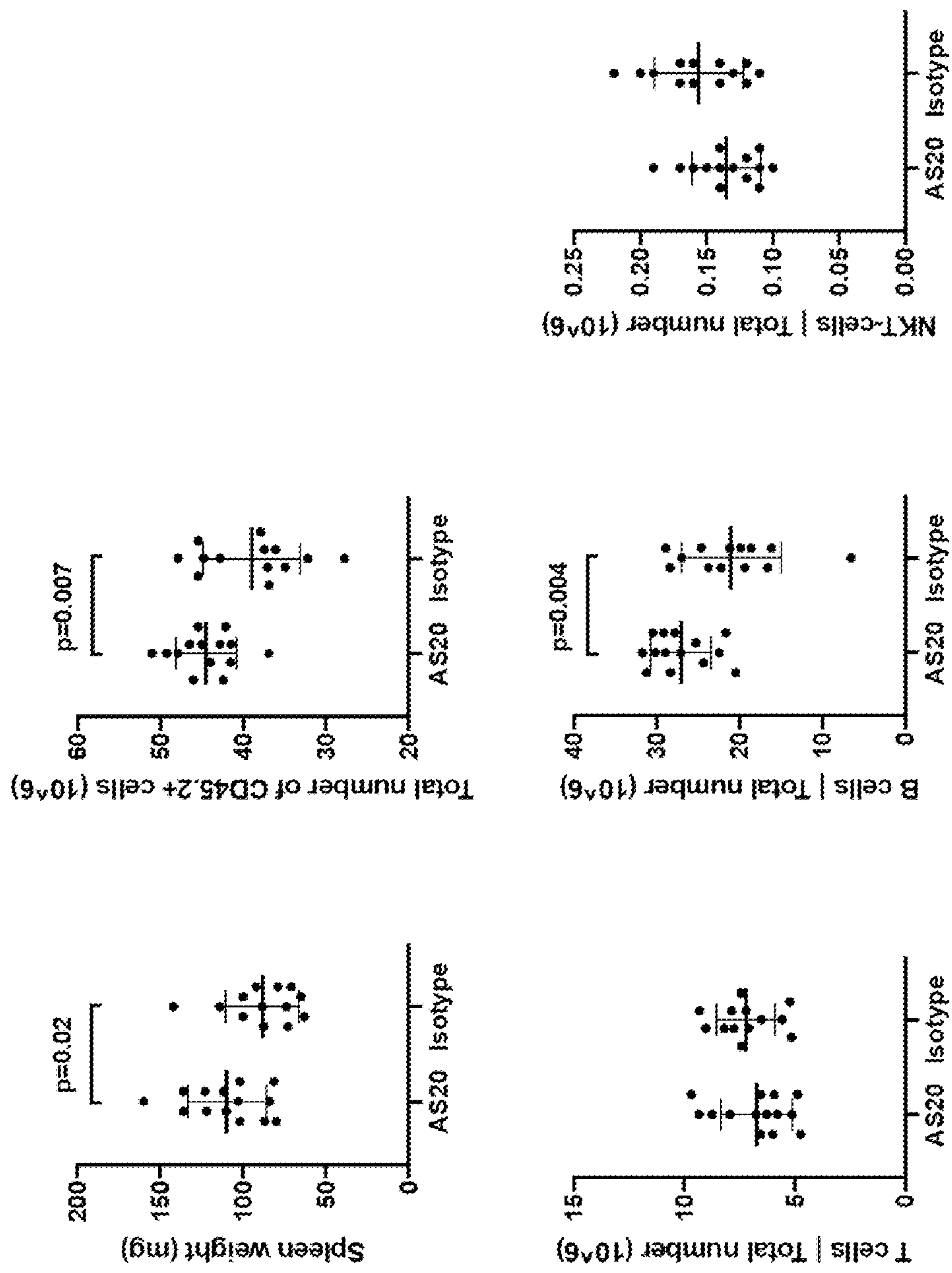


Figure 20

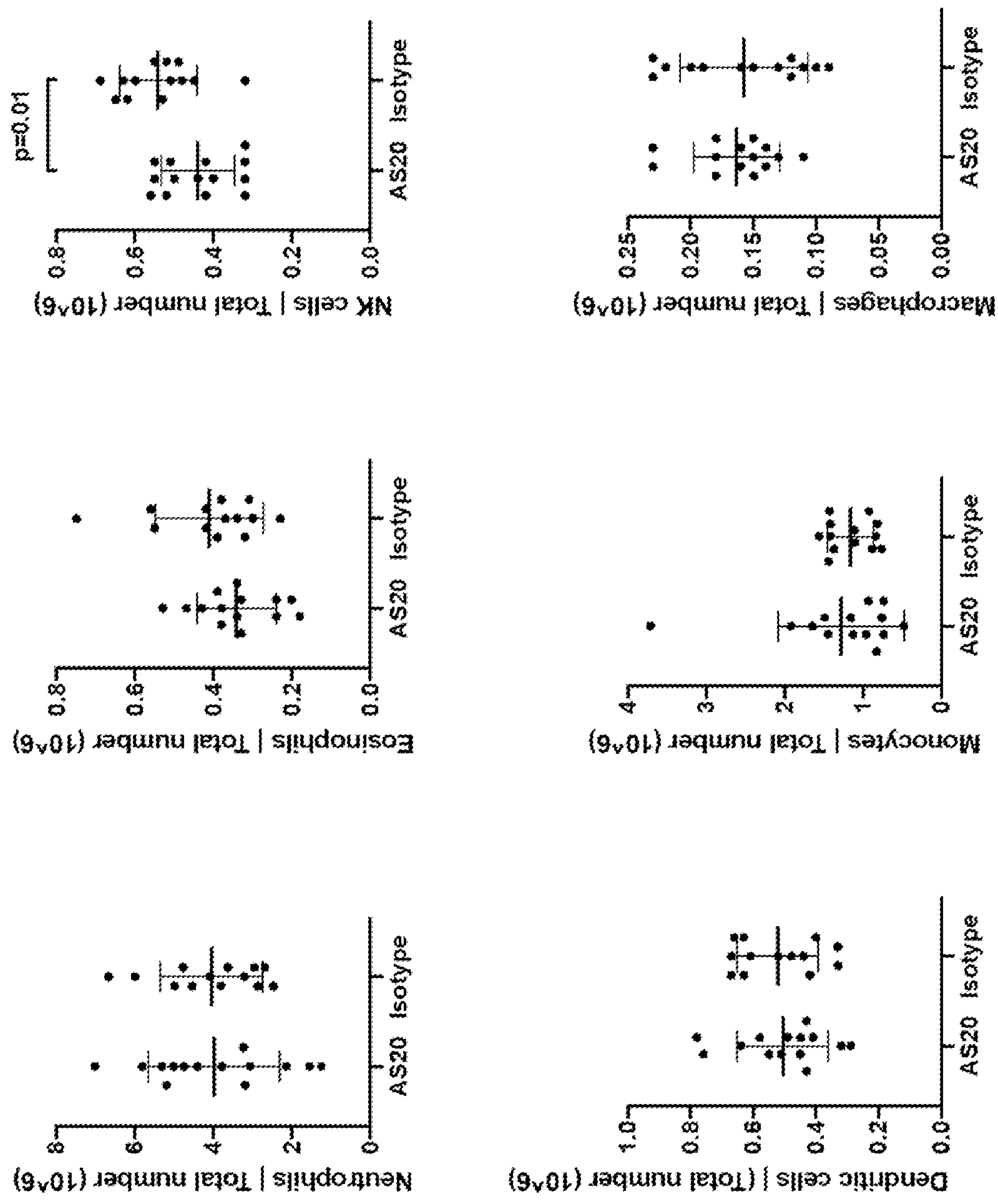


Figure 20

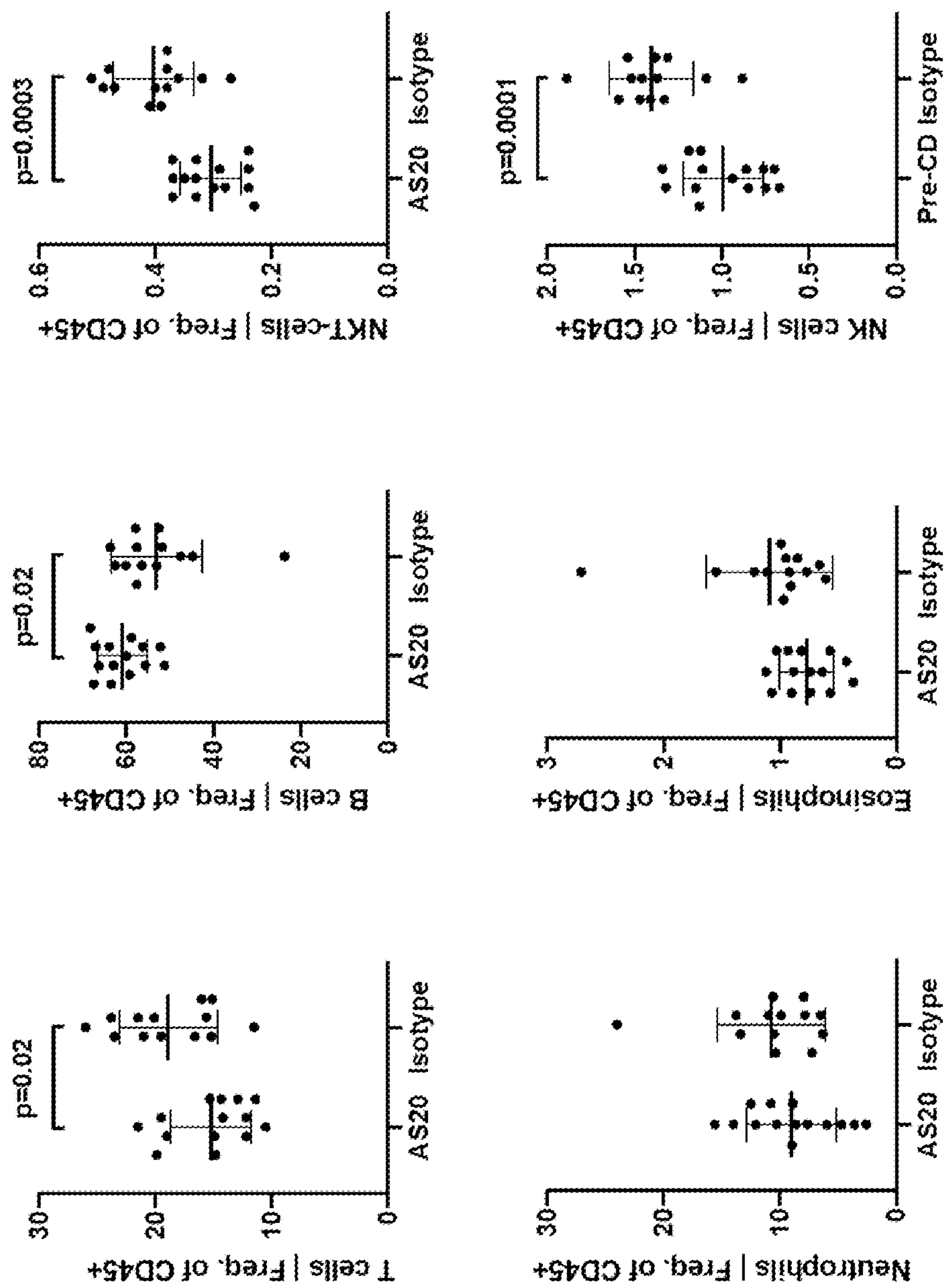


Figure 21

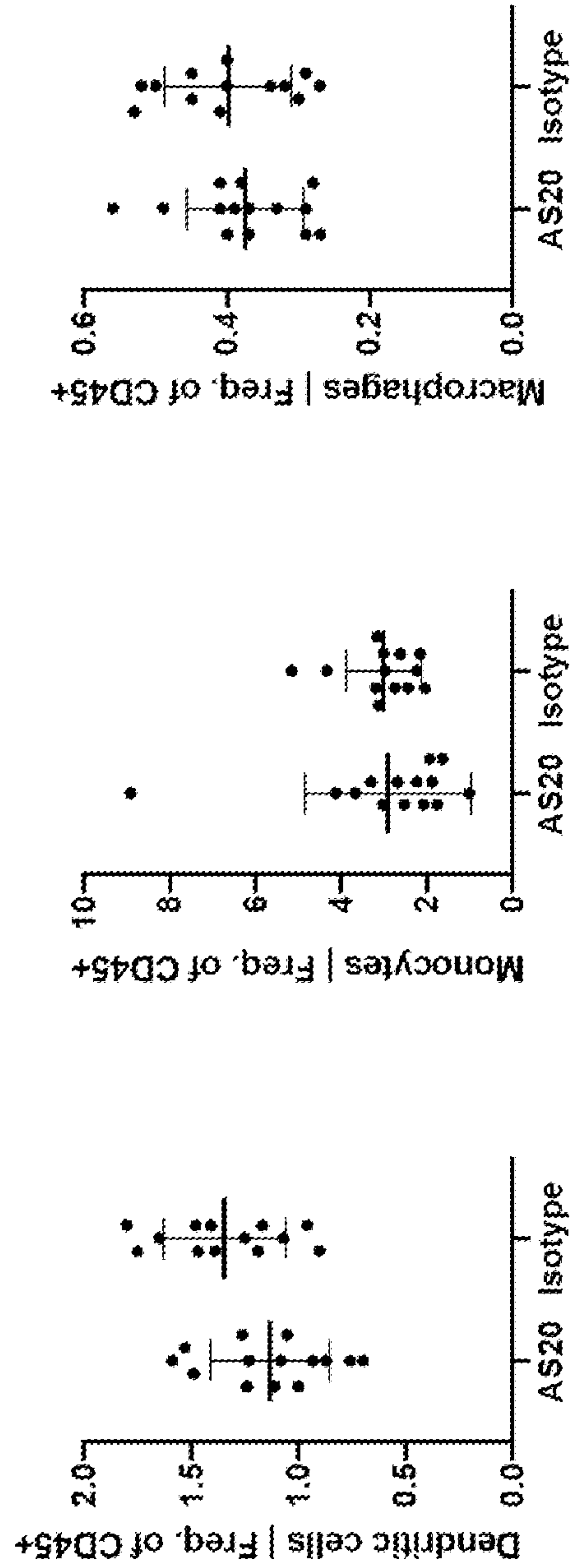


Figure 21

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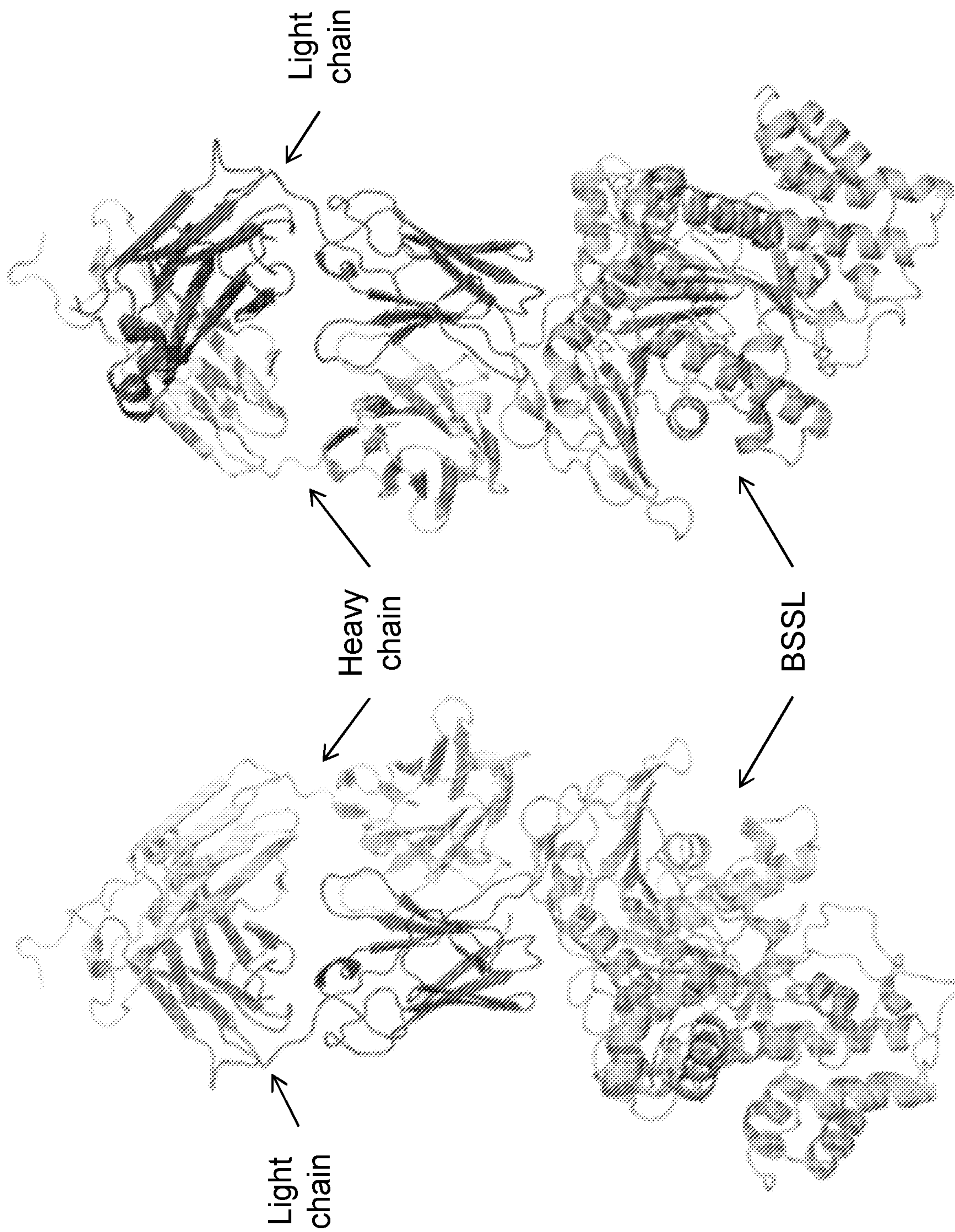


Figure 22

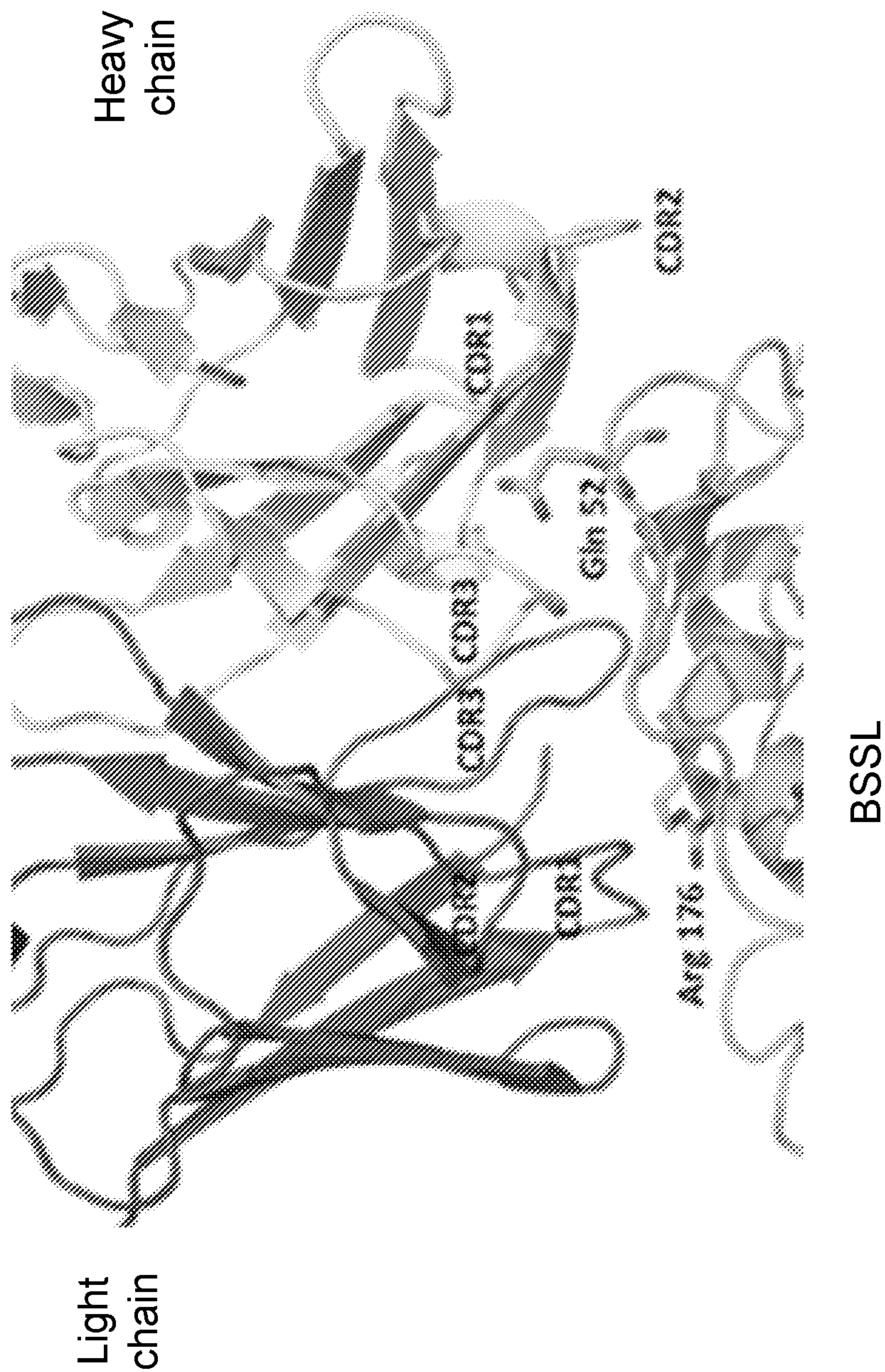


Figure 23

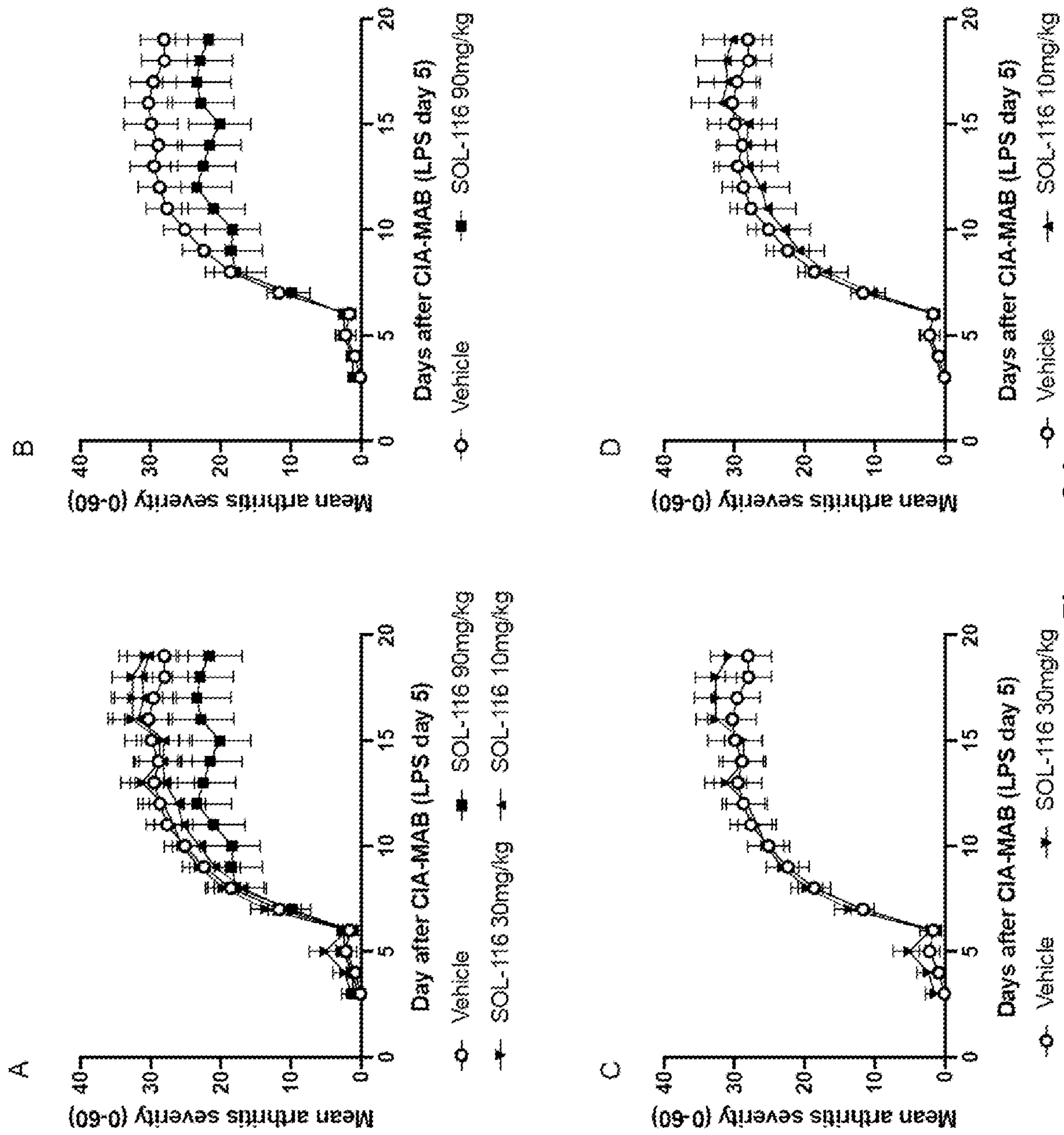


Figure 24

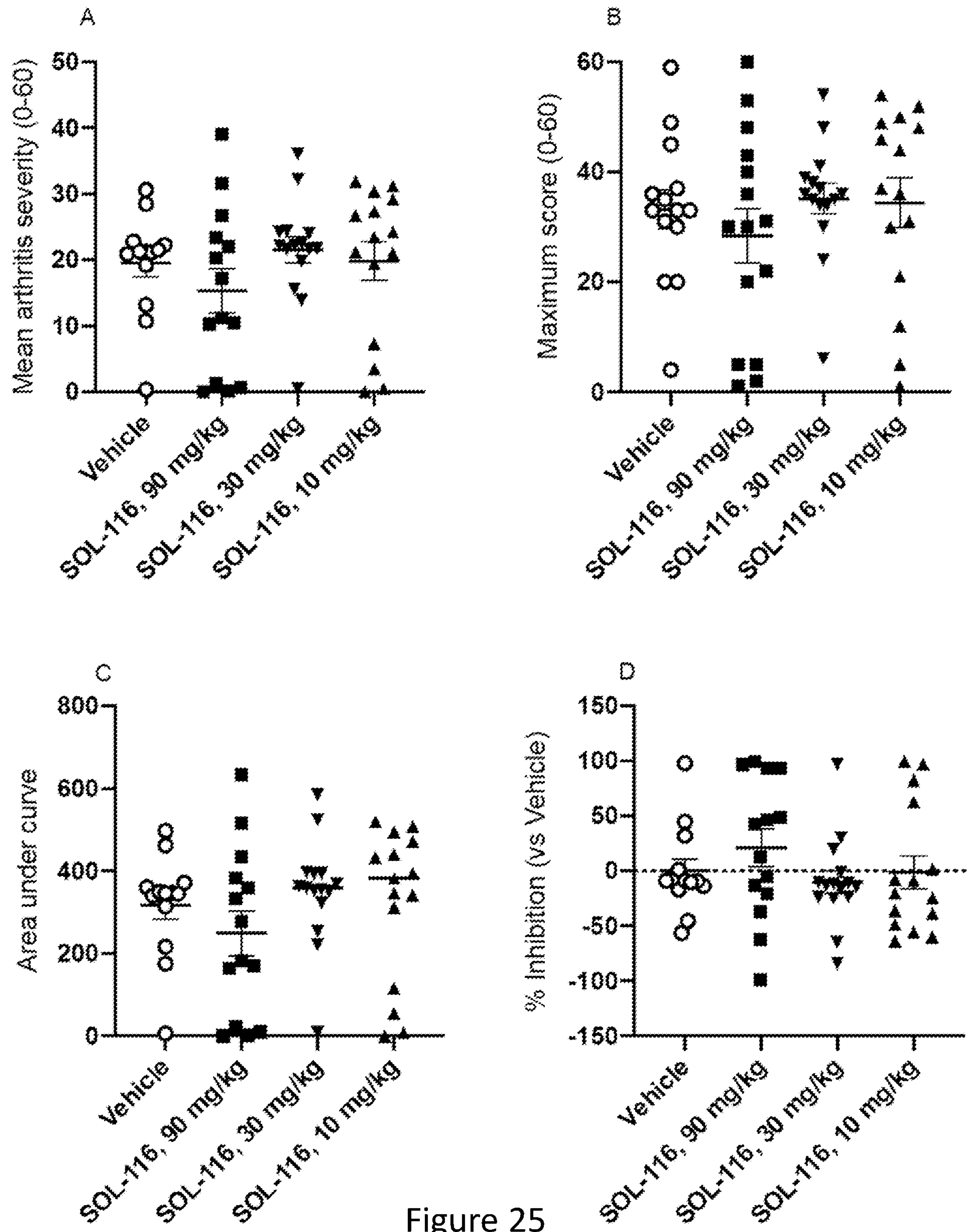


Figure 25

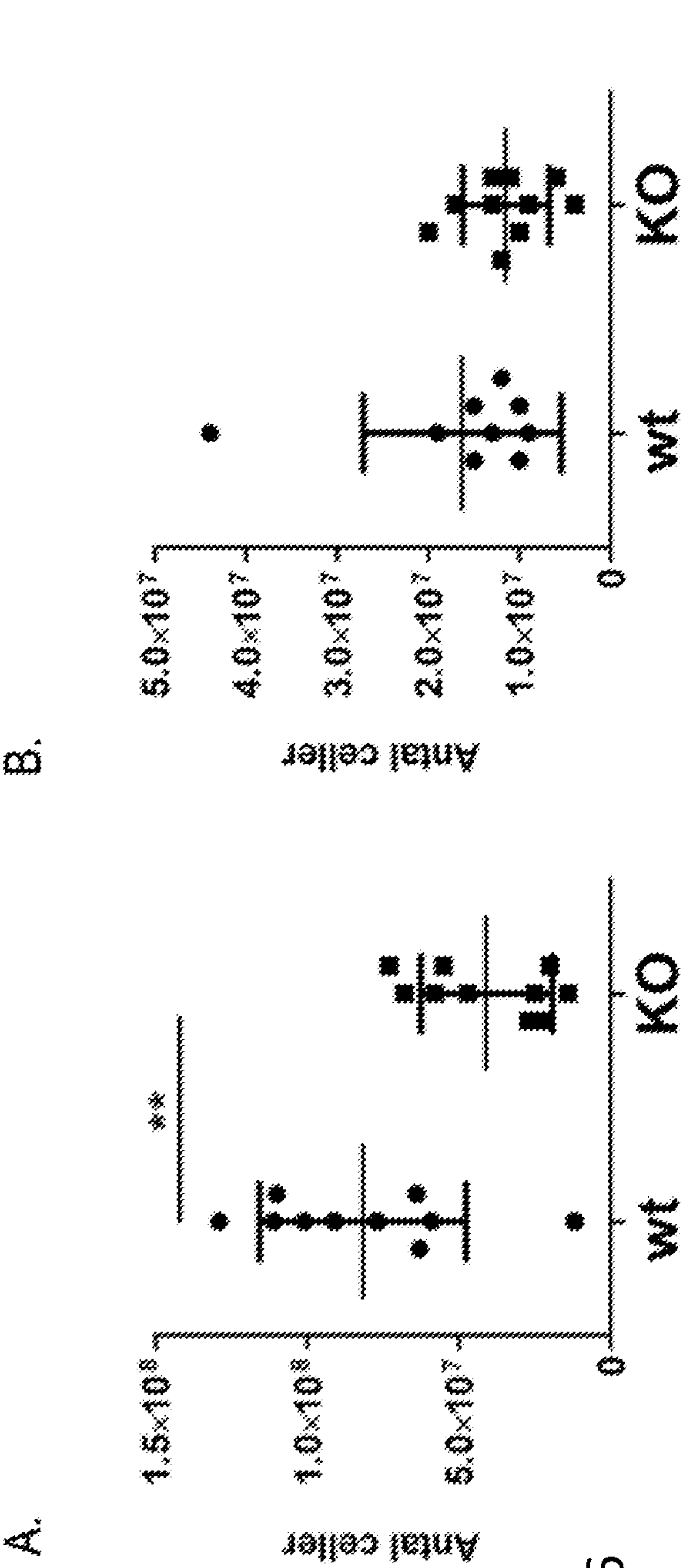


Figure 26

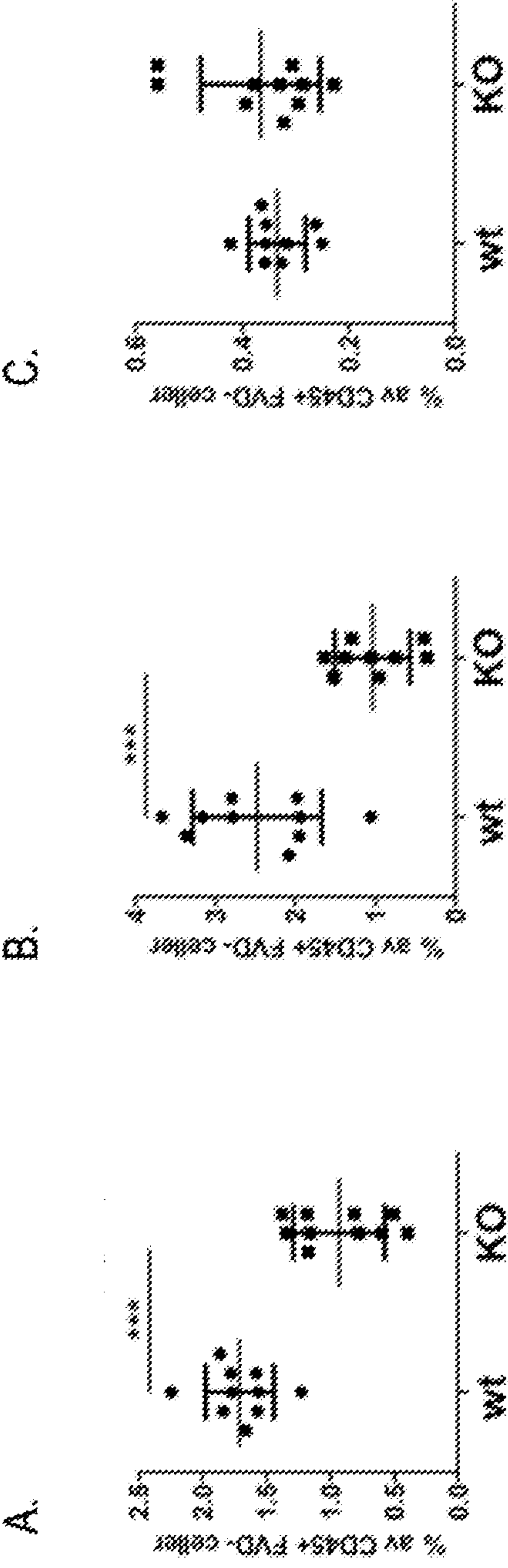


Figure 27