

Supporting Information

Antibodies with weakly basic isoelectric points minimize trade-offs between formulation and physiological colloidal properties

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A

hOKT3 V_H

QVQLVQSGSELKPKGASVKMSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKFKDRATLTDDKSTSTAYMQLSSLRSEDTA
VYYCARYYDDHYSLDYWGQGLTVTVSS

hOKT3 V_L

QIVLTQSPATLSLSPGERATMSCSASSSVSYMNWYQQKPKGKAPKRWIYDTSKLAGVPSRFRGSGSGTDYTLTISSLQPEDFATYYCQQWSSN
PFTFGGGTKVEIK

Trastuzumab V_H

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTA
VYYCSRWGGDGFYAMDYWGQGLTVTVSS

Trastuzumab V_L

DIQMTQSPSSLSASVGRVTITCRASQDVNTAVAWYQQKPKGKAPKLLIYSASFLYSGVPSRFRGSGSGTDFTLTISLQPEDFATYYCQQHYTT
PPTFGQGTKVEIK

Siltuximab V_H

EVQLVESGGKLLKPGGSLKLSAASGFTFSSFAMSWFRQSPKRELEWVAEISSGGSYTYYPDTVTGRFTISRDNKNTLYLEMSSLRSEDTAMYY
CARGLWGYALDYWGQGTSVTVSS

Siltuximab V_L

QIVLIQSPAIMSASPGEKVTMTCSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAATYYCQQWSGYPY
TFGGGKLEIK

Lebrikizumab V_H

QVQLVQSGSELKPKGASVKMSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKFKDRATLTDDKSTSTAYMQLSSLRSEDTAV
YYCARRRPGQGYFDFWGQGLTVTVSS

Lebrikizumab V_L

DIVMTQSPDLSVSLGERATINCRASKSVDSYGNFSFMHWYQQKPGQPPELLIYLASNLESGVPDRFSGSGSGTDFTLTISLQAEDVAVYY
CQQNNEDPRTFGGGTKVEIK

B

IgG1 C_H1-hinge-Fc

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSQVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDK
RVEPKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS
FFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKLSLSLSPG

IgG1 C_L (kappa)

RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSKADYEEKHKVYACEVTHQGL
SSPVTKSFNRGEC

Figure S1 . Summary of the antibody amino acid sequences used in this study. (A) The four clinical-stage antibodies (Fv) and (B) the heavy (IgG1) and light (IgK) constant regions.

HCDR3 sequence of variants

- RRPGQGYFDF
- RQRFPPYFDY
- YDHYSGSSDY
- YDGIYGELDF
- YYDDHYSLDY
- HRSGYFSMDY
- Parent HCDR3

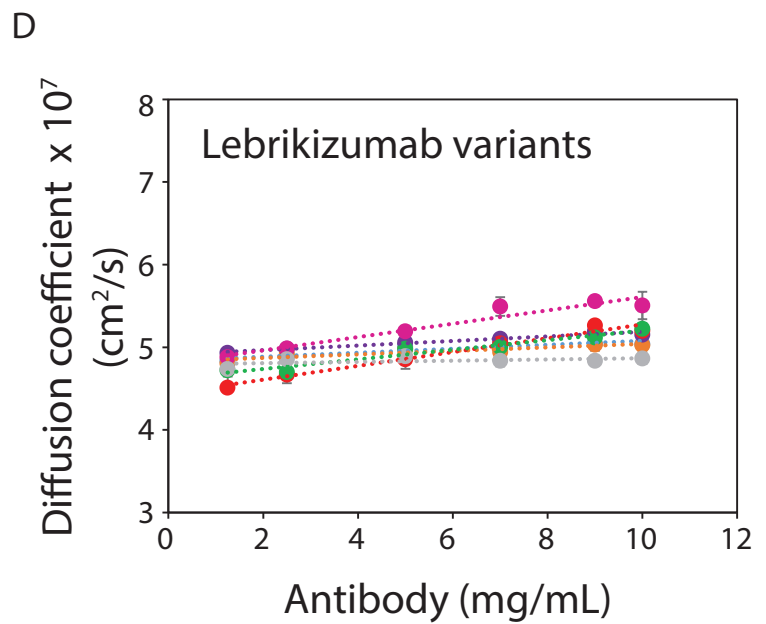
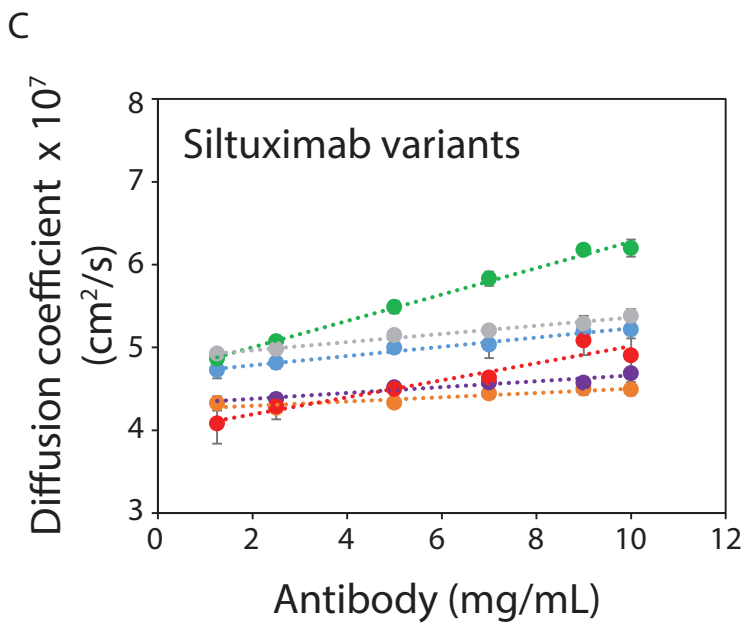
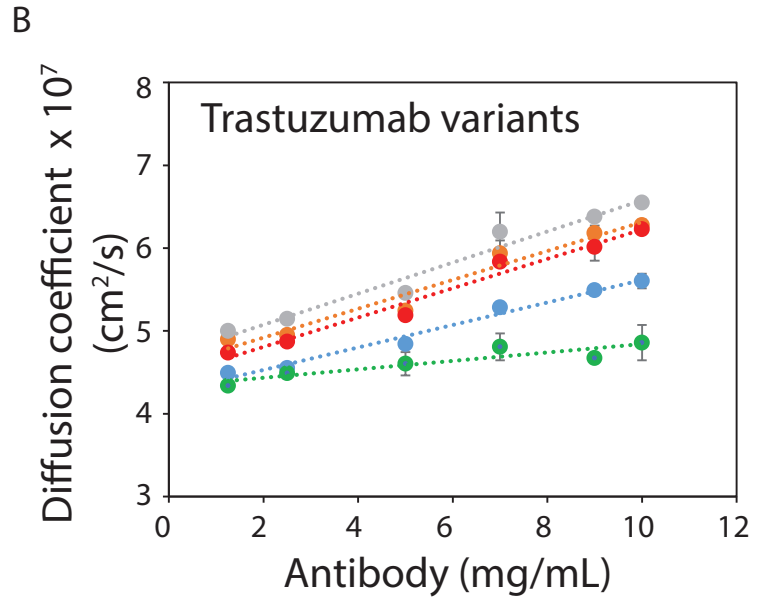
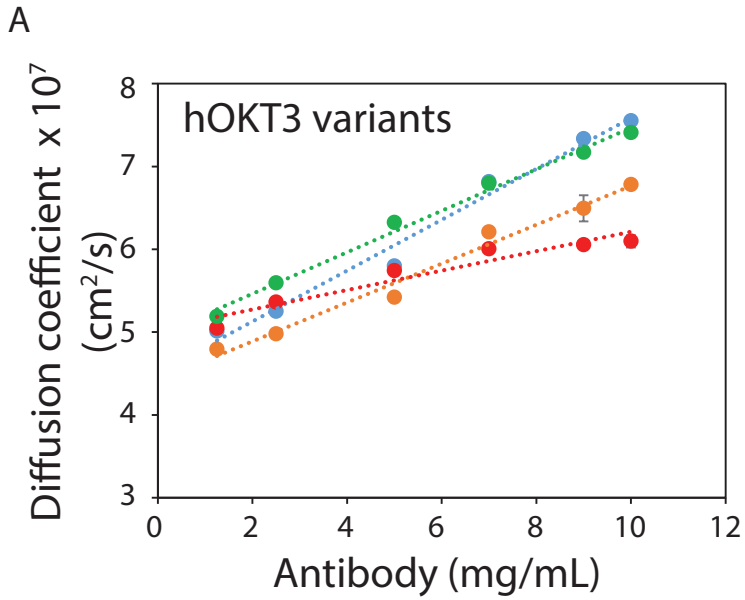


Figure S2. Measurements of diffusion interaction parameters (k_D) by light scattering. The diffusion coefficients measured were plotted as a function of concentration to calculate k_D from the slopes. The HCDR3 sequences of variants are noted in the legend. The values reported are averages for three independent experiments and the error bars are standard deviations.

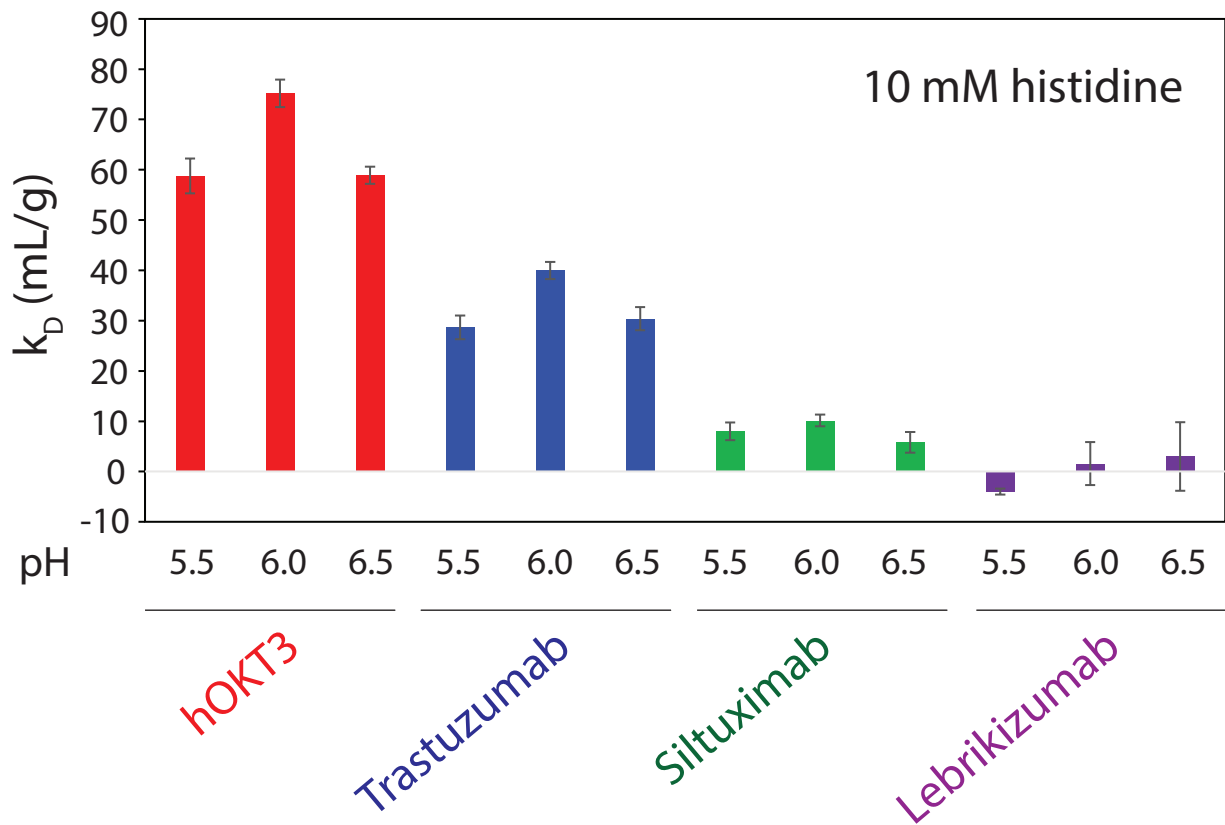


Figure S3. Evaluation of mAb self-interactions as a function of pH in formulations with 10 mM histidine. The mAbs were evaluated using dynamic light scattering to measure diffusion interaction parameters (k_D). The reported values are averages of three independent experiments and the errors are standard deviations.

- ▲ HCDR3 net charge ≥ 0
- HCDR3 net charge < 0

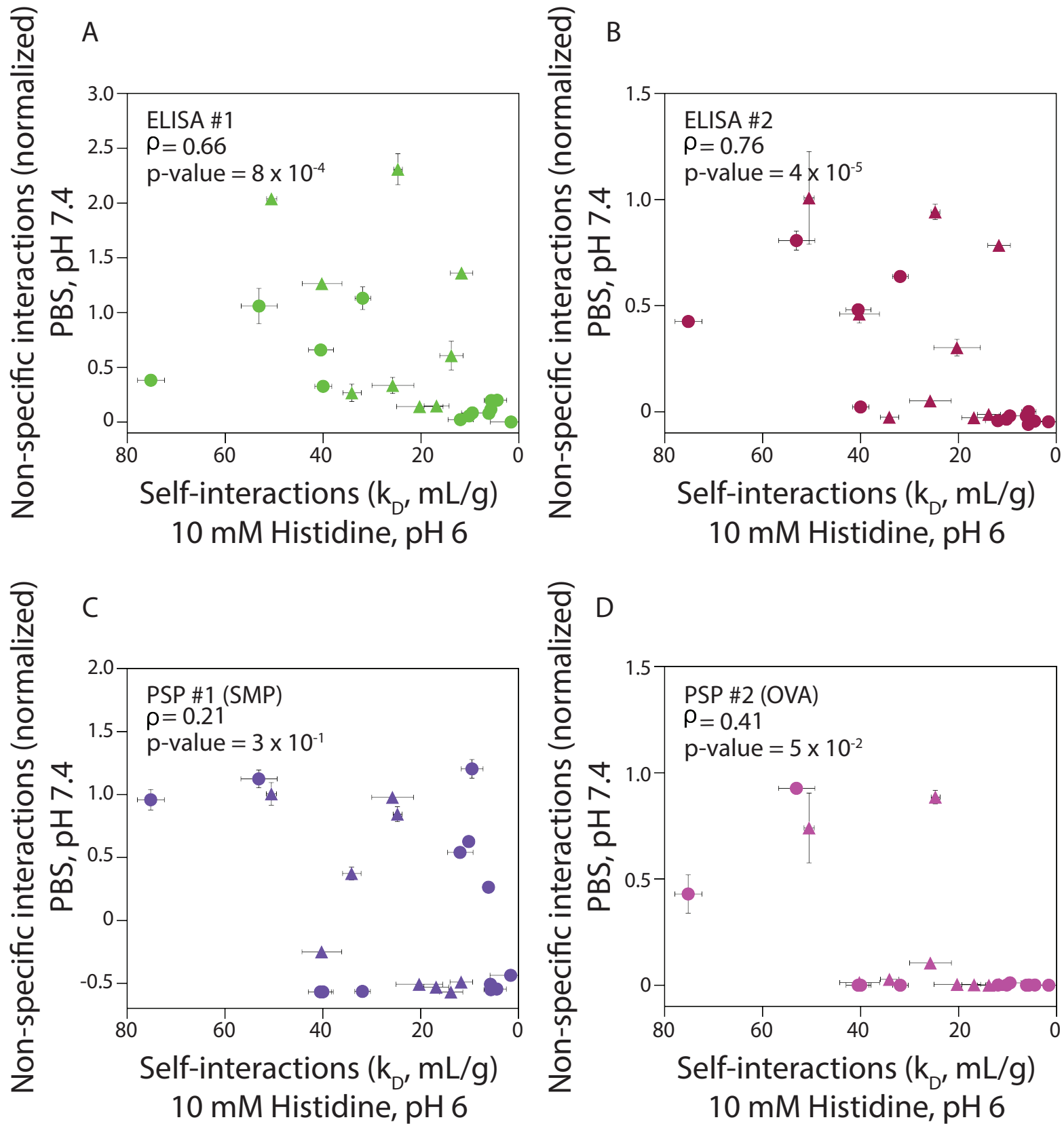


Figure S4. Comparison of colloidal interactions in physiological (PBS, pH 7.4) and formulation (10mM histidine, pH 6) conditions .

Antibody Variant	Analytical size exclusion chromatography					
	Average			STD		
	%LMWS	% Monomer	%HMWS	%LMWS	% Monomer	%HMWS
hOKT3-HCDR3-1 (Parent)	0.04	97.68	2.28	0.06	0.29	0.26
hOKT3-HCDR3-2	0.05	97.11	2.44	0.06	0.19	0.59
hOKT3-HCDR3-3	0.06	98.60	1.35	0.06	0.04	0.01
hOKT3-HCDR3-4	0.05	98.65	1.30	0.04	0.05	0.02
hOKT3-HCDR3-5	0.57	96.06	3.37	0.88	0.32	0.57
hOKT3-HCDR3-6	2.60	97.36	0.05	0.34	0.32	0.03
hOKT3-HCDR3-7	0.47	99.42	0.10	0.49	0.48	0.10
hOKT3-HCDR3-8	2.63	95.82	1.54	0.41	0.57	0.19
hOKT3-HCDR3-9	5.60	93.66	0.74	0.60	0.69	0.09
hOKT3-HCDR3-10	1.47	96.77	1.77	1.26	1.74	0.53
Trastuzumab-HCDR3-1	1.15	92.91	5.95	0.60	1.09	0.48
Trastuzumab-HCDR3-2	1.11	95.80	3.09	0.21	0.15	0.15
Trastuzumab-HCDR3-3	0.77	96.82	2.41	0.27	0.33	0.07
Trastuzumab-HCDR3-4 (Parent)	0.91	96.76	2.34	0.25	0.23	0.13
Trastuzumab-HCDR3-5	0.80	95.46	3.74	0.10	0.16	0.07
Trastuzumab-HCDR3-6	0.82	97.35	1.82	0.29	0.50	0.21
Trastuzumab-HCDR3-7	0.85	98.00	1.15	0.20	0.23	0.23
Trastuzumab-HCDR3-8	0.70	99.02	0.28	0.19	0.17	0.06
Trastuzumab-HCDR3-9	1.17	98.06	0.77	0.28	0.33	0.05
Trastuzumab-HCDR3-10	1.90	91.88	6.23	0.42	0.53	0.11
Lebrikizumab-HCDR3-1*	0.05	86.34	13.62	0.02	0.13	0.13
Lebrikizumab-HCDR3-2	0.06	92.05	7.89	0.04	1.24	1.25
Lebrikizumab-HCDR3-3	0.06	91.41	8.52	0.06	0.09	0.13
Lebrikizumab-HCDR3-4	0.02	99.60	0.37	0.04	0.20	0.20
Lebrikizumab-HCDR3-5	0.08	90.43	9.50	0.08	0.48	0.41
Lebrikizumab-HCDR3-6	0.00	96.58	3.42	0.01	0.16	0.16
Lebrikizumab-HCDR3-7	0.00	99.30	0.70	0.00	0.09	0.09
Lebrikizumab-HCDR3-8	0.04	94.76	5.20	0.02	0.33	0.32
Lebrikizumab-HCDR3-9*	0.11	66.16	33.73	0.11	0.24	0.34
Lebrikizumab-HCDR3-10	0.03	91.28	8.69	0.01	0.15	0.15
Lebrikizumab (Parent)	0.01	99.55	0.45	0.01	0.10	0.10
Siltuximab-HCDR3-1	0.00	99.50	0.91	0.00	0.57	0.00
Siltuximab-HCDR3-2	0.06	98.24	1.69	0.06	0.03	0.03
Siltuximab-HCDR3-3	0.01	99.28	0.71	0.01	0.04	0.03
Siltuximab-HCDR3-4	0.00	99.51	0.49	0.00	0.01	0.01
Siltuximab-HCDR3-5	0.01	99.47	0.52	0.01	0.08	0.10
Siltuximab-HCDR3-6	0.00	90.38	9.63	0.00	0.12	0.12
Siltuximab-HCDR3-7	0.01	98.68	1.32	0.01	0.04	0.02
Siltuximab-HCDR3-8	0.00	98.69	1.31	0.00	0.06	0.06
Siltuximab-HCDR3-9	0.00	98.53	1.47	0.00	0.04	0.04
Siltuximab-HCDR3-10	0.04	98.81	1.20	0.04	0.04	0.08
Siltuximab (Parent)	0.05	99.66	0.30	0.04	0.06	0.03

Table S1. Protein A purified antibody variants were analyzed for purity by analytical size exclusion chromatography. The reported values, which are averages from three independent experiments, are % low molecular weight species (LMWS), % monomer and % high molecular weight species (HMWS). Variants with less than 90% monomer (*) were subjected to preparative size exclusion chromatography to remove aggregates.

Antibody Variant	Thermal Stability Analysis
	Fab, T _m (°C)
hOKT3-HCDR3-1 (Parent)	80.40
hOKT3-HCDR3-2	75.50
hOKT3-HCDR3-3	67.40
hOKT3-HCDR3-4	74.20
hOKT3-HCDR3-5	73.10
hOKT3-HCDR3-6	73.80
hOKT3-HCDR3-7	73.50
hOKT3-HCDR3-8	73.50
hOKT3-HCDR3-9	67.50
hOKT3-HCDR3-10	67.30
Trastuzumab-HCDR3-1	77.60
Trastuzumab-HCDR3-2	76.10
Trastuzumab-HCDR3-3	75.00
Trastuzumab-HCDR3-4 (Parent)	79.70
Trastuzumab-HCDR3-5	66.50
Trastuzumab-HCDR3-6	79.40
Trastuzumab-HCDR3-7	79.90
Trastuzumab-HCDR3-8	76.40
Trastuzumab-HCDR3-9	76.90
Trastuzumab-HCDR3-10	81.00
Lebrikizumab-HCDR3-1	63.80
Lebrikizumab-HCDR3-2	64.50
Lebrikizumab-HCDR3-3	61.90
Lebrikizumab-HCDR3-4	70.90
Lebrikizumab-HCDR3-5	61.10
Lebrikizumab-HCDR3-6	66.40
Lebrikizumab-HCDR3-7	68.90
Lebrikizumab-HCDR3-8	63.80
Lebrikizumab-HCDR3-9	58.80
Lebrikizumab-HCDR3-10	57.70
Lebrikizumab (Parent)	71.60
Siltuximab-HCDR3-1	67.80
Siltuximab-HCDR3-2	65.10
Siltuximab-HCDR3-3	69.60
Siltuximab-HCDR3-4	69.70
Siltuximab-HCDR3-5	69.20
Siltuximab-HCDR3-6	70.40
Siltuximab-HCDR3-7	71.20
Siltuximab-HCDR3-8	73.70
Siltuximab-HCDR3-9	74.20
Siltuximab-HCDR3-10	77.30
Siltuximab (Parent)	71.20

Table S2. Thermal melting temperature (midpoint of unfolding of Fab, T_m) of the Protein A purified antibody variants determined by dynamic scanning fluorimetry.