

Supporting Information for

Fine epitope mapping of the CD19 extracellular domain promotes design

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Other supplementary materials for this manuscript include the following:

CSV and TSV processed datasets available at Figshare

Excel File S1: SSM primers, and selection per-mutation z-score and feature data

Note S1: Full-length DNA and amino acid sequences of CD19 variants. *BbvCI* site in green. Five N-linked glycosylation sites at N67, N106, N119, N162, and N246 were mutated to glutamine (purple color) in the No_N-Linked sequences.

>CD19.1 DNA

CCCGAGGAACCTCTAGTGGTAAGGTGGAAGAGGGAGATGAGGCTTGGCTCCCTGCCTCAAGGGGA
CCTCCGATGGCCCCACTCAGCAGCTGACCTGGTCTCGGGAGTCCCCGCTTAAGCCCTCCTAAAAGTG
AGTTTCGGGTGCCAGGACTGGCGTCCACGTGAGGCCAACGCCGTCTCTGTATCTCTAACGTC
TCTCAACAGATGGGGGCTTCTACCTGTGCCAGCCGGGGCCCCCTCTGAGAAAGGCCTGGCAGCCTGG
CTGGACAGTCATGTGGAGGGCAGCGGGAGCTGTTCCGGTGGAAATGTTCCGGACCTAGGTGGCTGG
GCTGTGGCCTGAAGAACAGGTCTCAGAGGGCCCCAGCTCCCCTCCGGAAAGCTCATGAGCCCCAAG
CTGTATGTGGGCAAAGACCAGCCTGAGATCTGGAGGGAGAGCCTCCGTGTCTCCACCGAGGG
CAGCCTGAACCAGAG **CCTCAGC**AGGGACATGACTGTGGCCCCCTGGCTCCACACTCTGGCTGTCTGT
GGGTACCCCTGACTCTGTGTCCAGGGCCCCCTCCTGGACCCATGTGCACCCCAAGGGCCTAAG
TCATTGCTGAGCCTAGAGCTGAAGGACGATGCCCGGCCAGAGATATGTGGTAACTGGTACTGGCT
GTTTCTGCCTCGGGCACAGCTCAAGACGCTGGAAAGTATTATTGCCACCGTGGCACCTGACCATGT
CATTCCACCTGGAGGTCAAAGCTCGGCCAGTCTGCTCACACTAAACTAAGAACTGGTGGCTGGAAG

>CD19.1 Translated

PEEPLVVKEEGDEAWLPCLKGTSQQLTWSRESPLKFLKVSFGVPGLVHVRPNA VSLVISNVSQQ
MGGFYLCQPGPPSEKAQPGWTVNVEGSGELFRWNVSDLGGLGCGLKRSSEGPSSPGKLMSPKLYVW
AKDRPEIWEGEPPCLPPRDSLNS**S**LQSRDMTVAPGSTLWLSCGVPPDSVRGPLSWTHVHPKGPKSLLSLELK
DDRPARDMWVTGTRLFLPRATAQDAGKYYCHRGNLMSFHLEVKARPVSAHTKLRGGWK

>CD19.1-No_N-Linked

CCCGAGGAACCTCTAGTGGTAAGGTGGAAGAGGGAGATGAGGCTTGGCTCCCTGCCTCAAGGGGA
CCTCCGATGGCCCCACTCAGCAGCTGACCTGGTCTCGGGAGTCCCCGCTTAAGCCCTCCTAAAAGTG
AGTTTCGGGTGCCAGGACTGGCGTCCACGTGAGGCCAACGCCGTCTCTGTATCTCTAACGTC
TCTCAACAGATGGGGGCTTCTACCTGTGCCAGCCGGGGCCCCCTCTGAGAAAGGCCTGGCAGCCTGG
CTGGACAGTCATGTGGAGGGCAGCGGGAGCTGTTCCGGTGGCAGGTTCCGGACCTAGGTGGCTGG
GCTGTGGCCTGAAGCAGAGGTCTCAGAGGGCCCCAGCTCCCCTCCGGAAAGCTCATGAGCCCCAAG
CTGTATGTGGGCAAAGACCAGCCTGAGATCTGGAGGGAGAGCCTCCGTGTCTCCACCGAGGG
CAGCCTGCAGCAGAGCCTCAGCAGGGACATGACTGTGGCCCCCTGGCTCCACACTCTGGCTGTCTGT
GGGTACCCCTGACTCTGTGTCCAGGGCCCCCTCCTGGACCCATGTGCACCCCAAGGGCCTAAG
TCATTGCTGAGCCTAGAGCTGAAGGACGATGCCCGGCCAGAGATATGTGGTAACTGGTACTGGCT
GTTTCTGCCTCGGGCACAGCTCAAGACGCTGGAAAGTATTATTGCCACCGTGGCACCTGACCATGT
CATTCCACCTGGAGGTCAAAGCTCGGCCAGTCTGCTCACACTAAACTAAGAACTGGTGGCTGGAAG

>CD19.1-No_N-Linked_Translated

PEEPLVVKEEGDEAWLPCLKGTSQQLTWSRESPLKFLKVSFGVPGLVHVRPNA VSLVIS**Q**VSQQQ
MGGFYLCQPGPPSEKAQPGWTVNVEGSGELFRW**Q**VSSDLGGLGCGLK**Q**RSSEGPSSPGKLMSPKLYVW
AKDRPEIWEGEPPCLPPRDSL**Q**LSLQSRDMTVAPGSTLWLSCGVPPDSVRGPLSWTHVHPKGPKSLLSLELK
DDRPARDMWVTGTRLFLPRATAQDAGKYYCHRG**Q**LMSFHLEVKARPVSAHTKLRGGWK

Note S2: The CD19 linear epitope tag and the Fn_{EGFR}-CD19 linear epitope tag fusion DNA and translated sequence. The base plasmid is pCT-40. The fusion protein is Fn variant 6.2.6 prime.

Genetic elements in order:

- 1) *PstI* (no color, bold, underlined)
- 2) PAS40 linker (Purple)
- 3) (G₄S)₃ linker (Green)
- 4) *NheI* (Yellow, bold, underlined)
- 5) *BamHI* (Red, bold, underlined)
- 6) Linear epitope tag (No Color)
- 7) GS spacer between the epitope tag and the myc tag (Green)
- 8) MYC Tag (Blue)
- 9) Double Stop (no color)

>CD19 Linear Epitope Tag DNA

```
CTGCAGGCTAGTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCAAGCTCC  

TGCTGCCTCTCCAGCTGCACCTGCTCCAGCTTCTCCAGCAGCTCCTGCACCTAGTGCTCCTGCTGGGGG  

TGGAGGCTCTGGCGAGGTGGGTCTGGTGGGGCGGATCTGCTAGCGGATCCAAGCTGTATGTGTGG  

GCCAAAGACCCGCTGAGATCTGGGAGGGAGAGCCTCCGGCTCTGAACAAAAGCTTATTCTGAAG  

AGGACTTGTAAAG
```

>CD19 Linear Epitope Tag Translated

```
LQASASPAAPAPASPAAPAPSAPAASPAAPAPASPAAPAPSAPAGGGGSGGGGSGGGGSASGSKLYVWAK  

DRPEIWEGEPPGSEQKLISEEDL**
```

Genetic elements in order:

- 1) *PstI* (no color, bold, underlined)
- 2) PAS40 linker (Purple)
- 3) (G₄S)₃ linker (Green)
- 4) *NheI* (Yellow, bold, underlined)
- 5) Fn_{EGFR} (No Color)
- 6) *BamHI* (Red, bold, underlined)
- 7) Linear epitope tag (No Color)
- 8) GS spacer between the epitope tag and the myc tag (Green)
- 9) MYC Tag (Blue)
- 10) Double Stop (no color)

>Fn_{EGFR} - CD19 Linear Epitope Tag DNA

```
CTGCAGGCTAGTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCAAGCTCC  

TGCTGCCTCTCCAGCTGCACCTGCTCCAGCTTCTCCAGCAGCTCCTGCACCTAGTGCTCCTGCTGGGGG  

TGGAGGCTCTGGCGAGGTGGGTCTGGTGGGGCGGATCTGCTAGCGTTCCGATTTCCGAGGGAC  

CTGGAGGTTGTTGCTGCACCCCCACCAGCCTACTGATCAGCTGGTCGACTACGCTGTGACTTATTAC  

AGGATCACCTACGGAGAACAGGAGGAATAGCCCTGTCAGGAGTTCACTGTGCTGGTGGATCTC  

CACTGCTACCATCAGCGGCCTAACACCTGGAGTTGATTATACCATCACTGTGATGCTGCACTGACAA  

CTCTCGTTGGCCTTTCGCTCTACTCCAATTCCACTAATTACCGAACAGAAATTGACAAACCACCCCA  

GGGATCCAAGCTGTATGTGTGGCCAAAGACCCGCTGAGATCTGGAGGGAGAGCCTCCGGCTCT  

GAACAAAAGCTTATTCTGAAGAGGACTTGTAAAG
```

> Fn_{EGFR} - CD19 Linear Epitope Tag Translated

```
LQASASPAAPAPASPAAPAPSAPAASPAAPAPASPAAPAPSAPAGGGGSGGGGSGGGGSASVSDVPRDLEV  

VAATPTSLLISWFDYAVTYYRITYGETGNNSPVQEFTVPGWISTATISGLKPGVDYTITVYAVTDNSRWPFR  

STPISTNYRTEIDKPPQGSKLYVWAKDRPEIWEGEPPGSEQKLISEEDL**
```

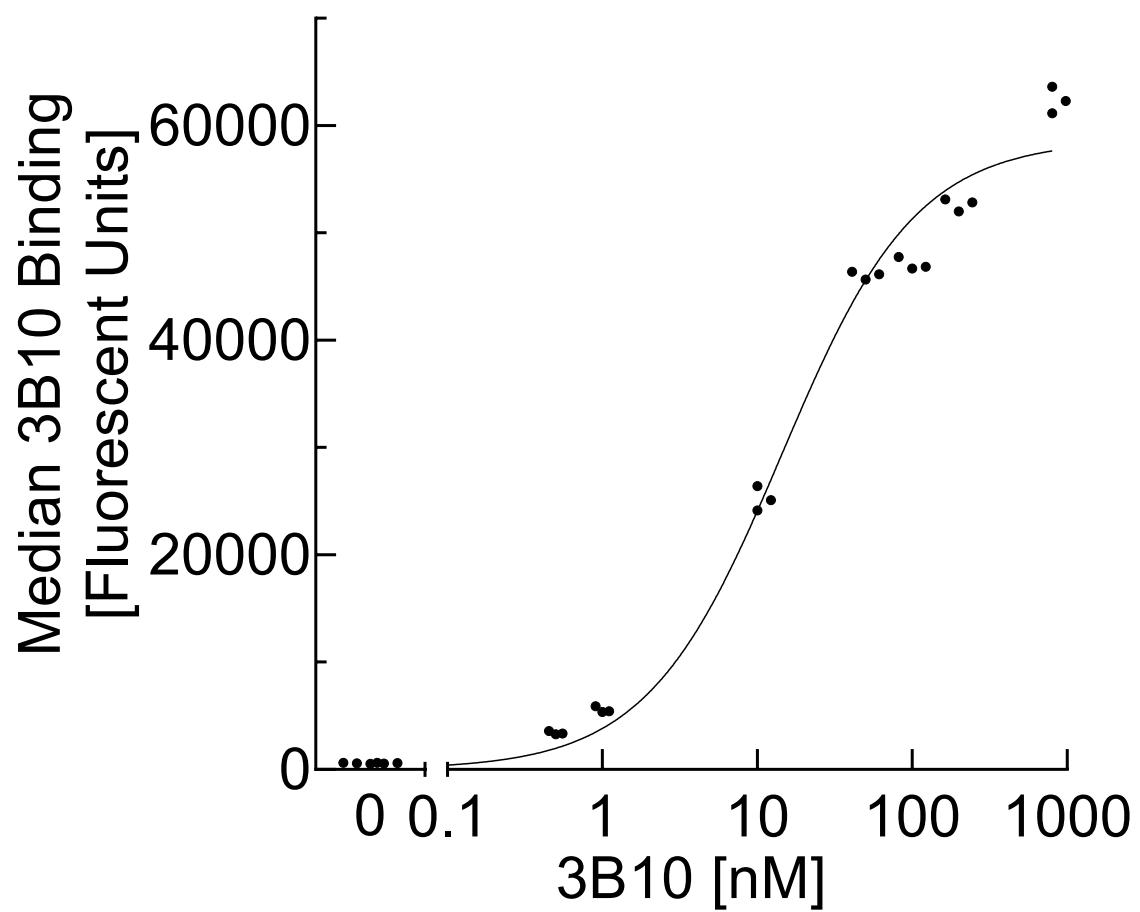


Figure S1: Affinity titration of 3B10 on yeast surface displayed CD19.1. The median binding fluorescence of samples are fitted with a one site – specific binding equation: $K_d = 14.4 \text{ nM}$ (95% CI = 11.4 – 18.0 nM, $N \geq 3$ on different days).

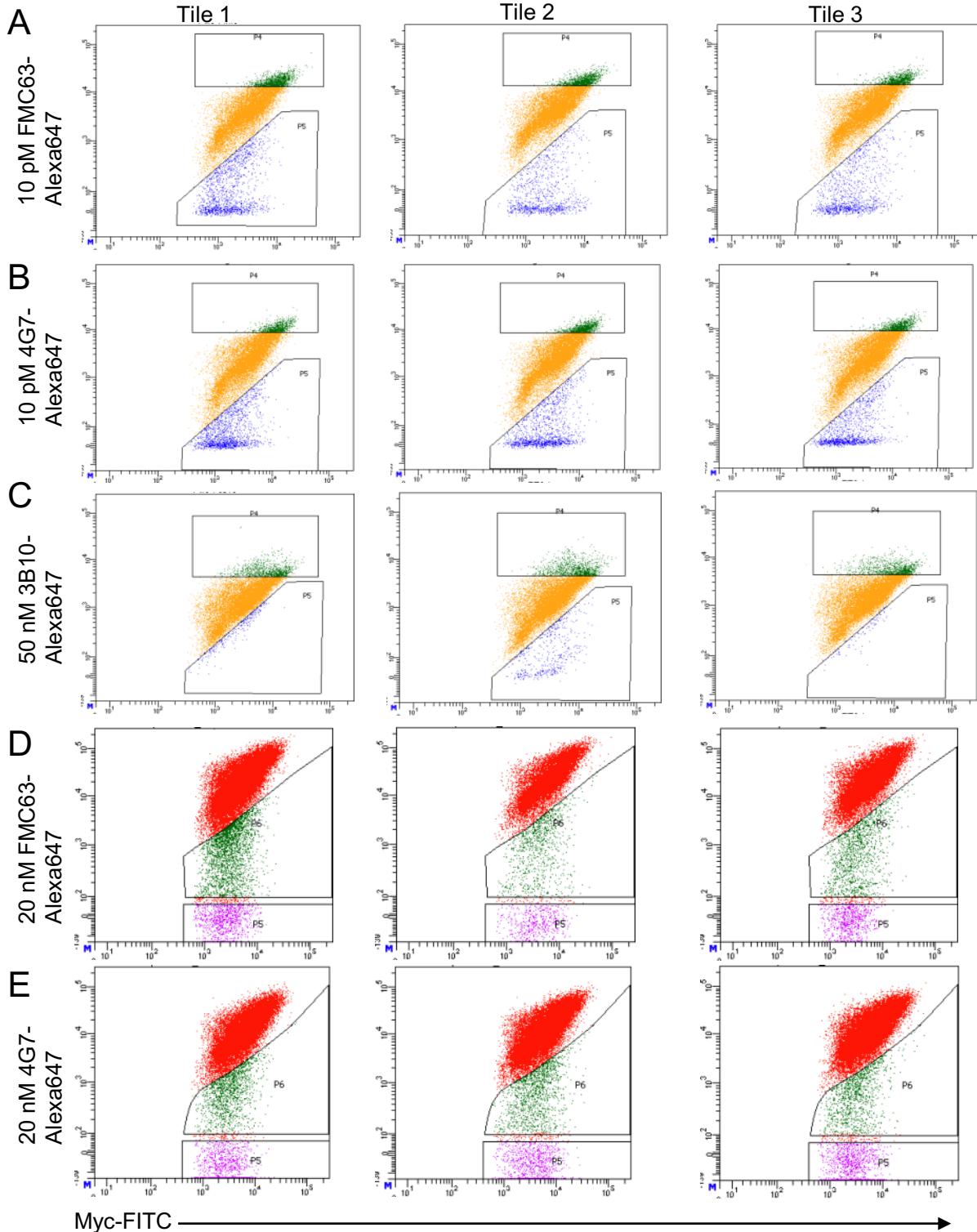


Figure S2: Sort gates of epitope mapping experiments. Yeast displaying the library were labelled with their respective primary anti-CD19 antibody. They were then labelled with goat anti-mouse Alexa647 and goat anti-Myc FITC to measure binding and full-length protein display respectively. The reference population was separately collected off of scatter, and the full display population separately off of Myc⁺ cells.

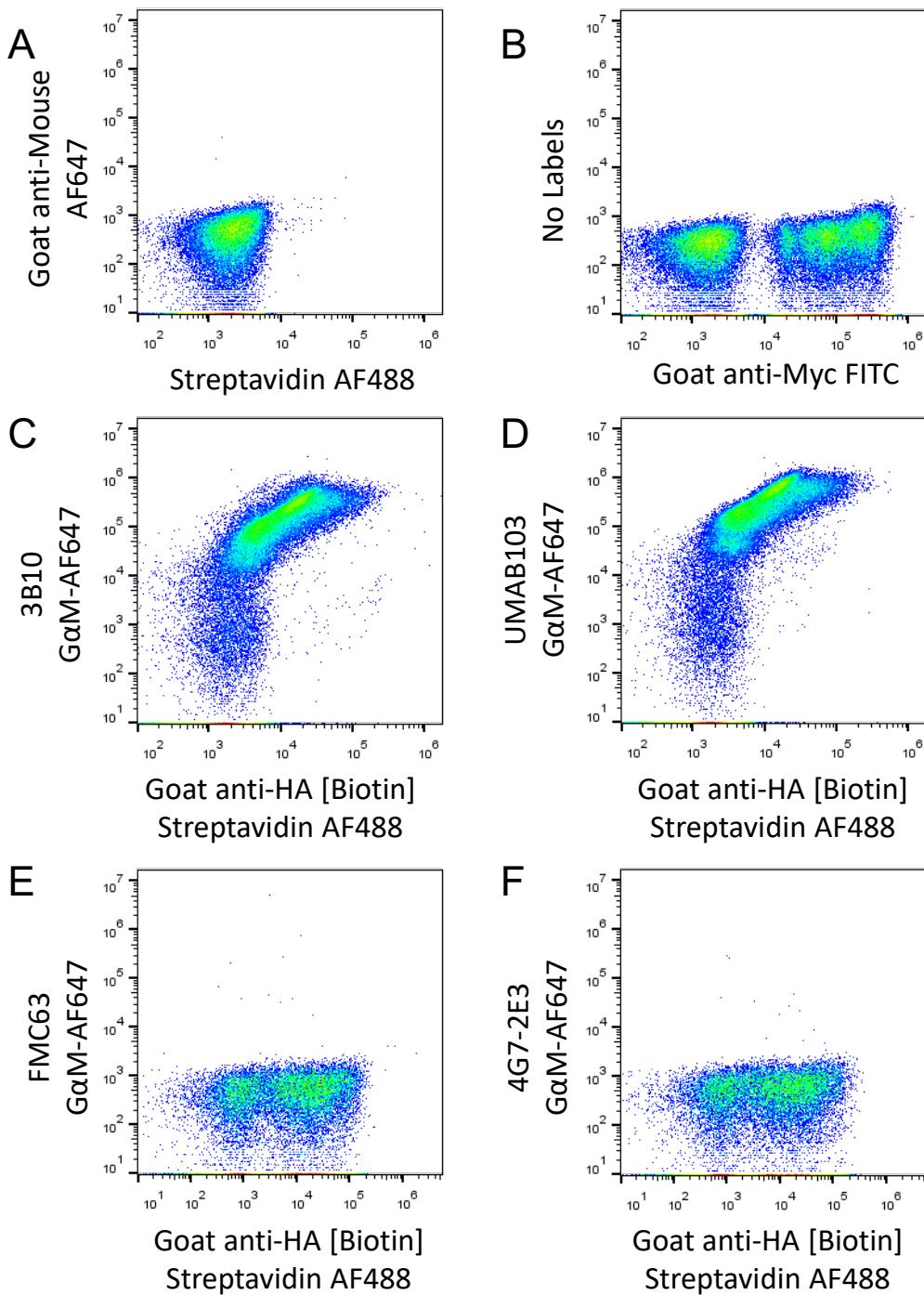


Figure S3: Antibody binding against yeast displaying the linear epitope tag (KLYVWAKDRPEIWEGEPP). Yeast were induced to display the linear epitope tag and labeled with the following reagents: A) Negative control of secondary antibodies goat anti-mouse AF647 and streptavidin AF488; B) goat anti-Myc FITC (control to test for full-length display); C-F) goat anti-HA biotin followed by streptavidin-AlexaFluor488 to assess display; C and D) mouse monoclonal antibodies 3B10 and UMAB103 at 50 nM; E and F) mouse monoclonal FMC63 and 4G7 at 100 nM.

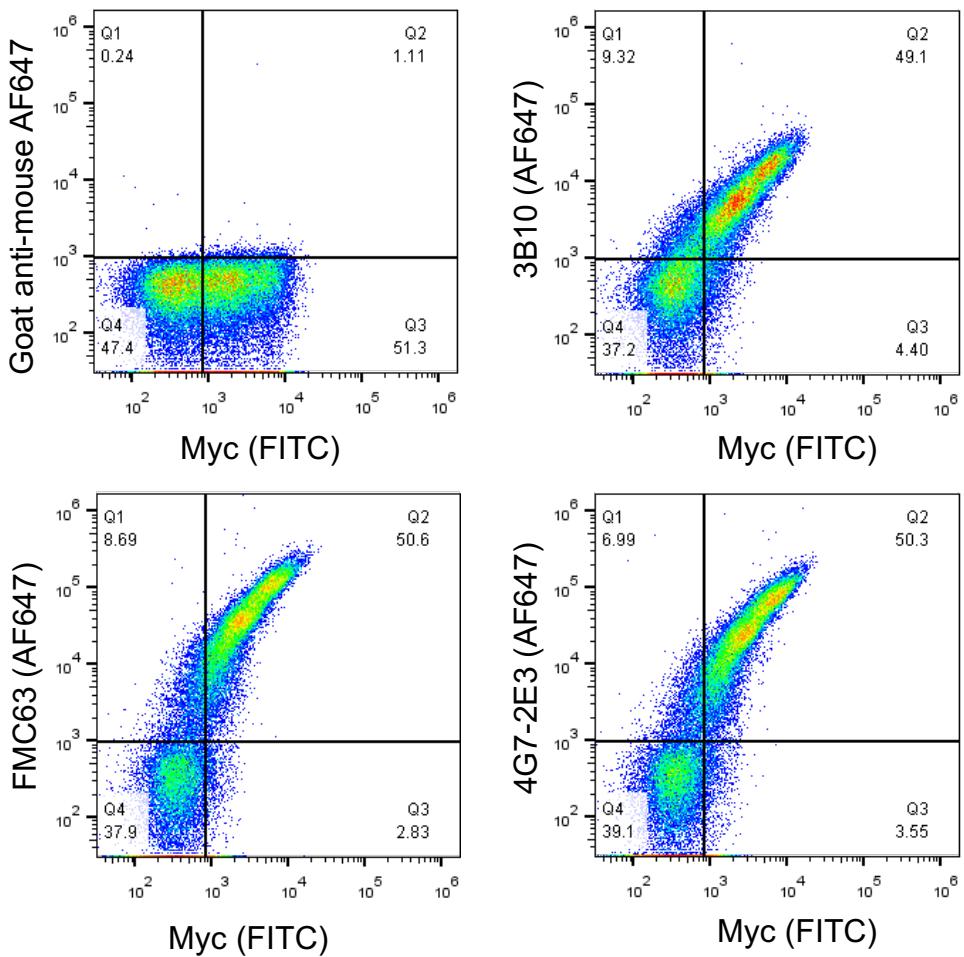


Figure S4: Removal of N-linked glycosylation sites from the CD19.1 scaffold. Yeast were induced to display CD19 with all N-linked glycosylation sites mutated. Yeast were labeled with anti-Myc-FITC and 10 nM of the indicated antibody followed by goat anti-mouse AlexaFluor647.

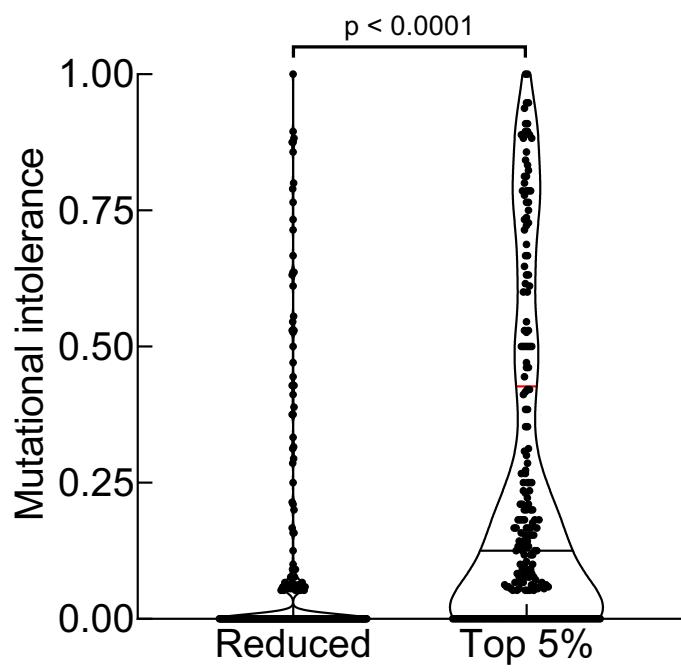


Figure S5: Mutational intolerance of sites found in the FMC63 reduced binding and top 5% approaches. A two-tailed Mann-Whitney test was performed to assess the two distributions. The median is colored black and quartiles red within each violin.

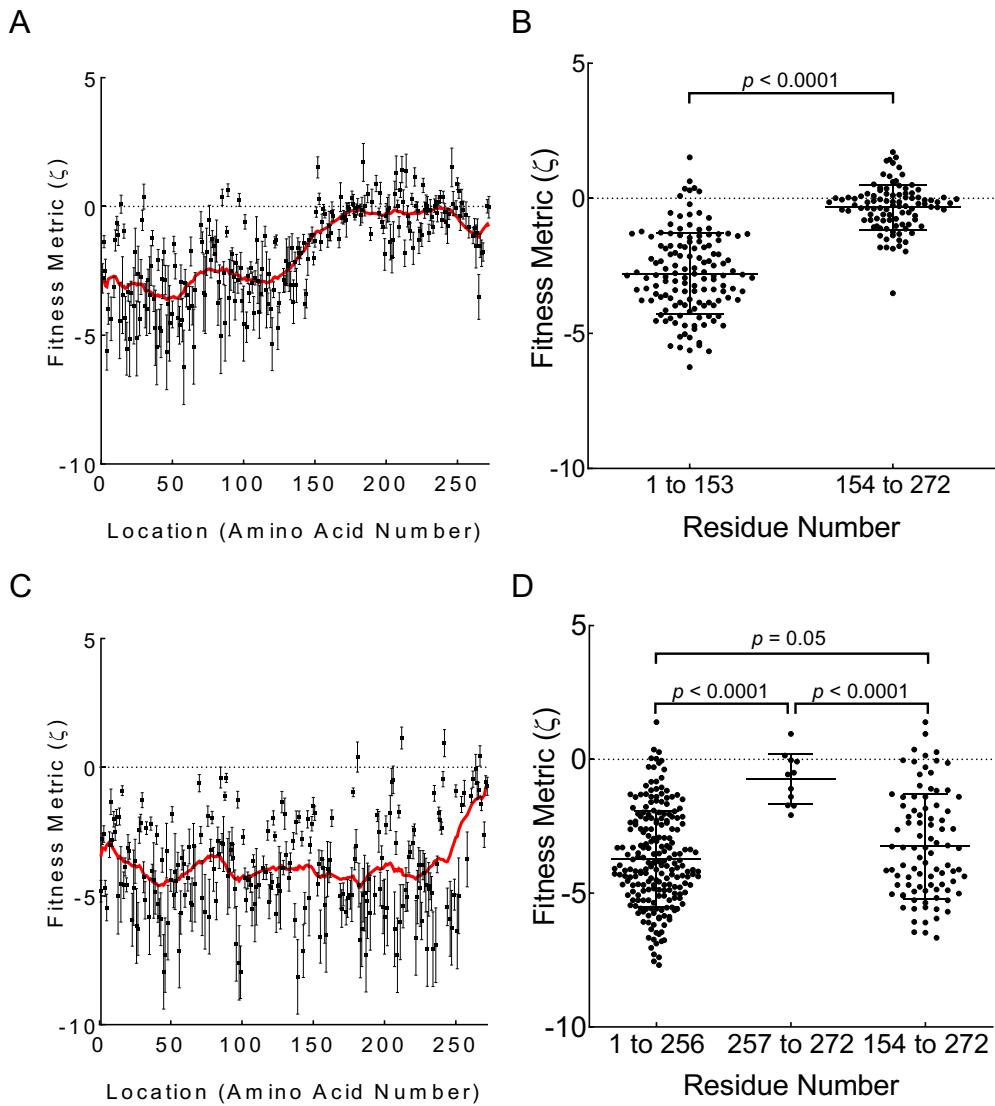


Figure S6: Fitness metrics of nonsense mutations versus position in CD19.1 epitope screens.
The fitness metrics from the top 5% of binding signal square gate are plotted for (A,B) 3B10 and (C,D) 4G7. For (A,C) a locally weighted scatterplot smoothed curve (red) is added to each figure to aid the eye, and error bars are plotted to 1 SD. For 4G7, residue 276 is the first loop C-terminal residue based on the solved structure. An unpaired t-test with Welch's correction was performed for all datasets. Mean and 1 SD are plotted.

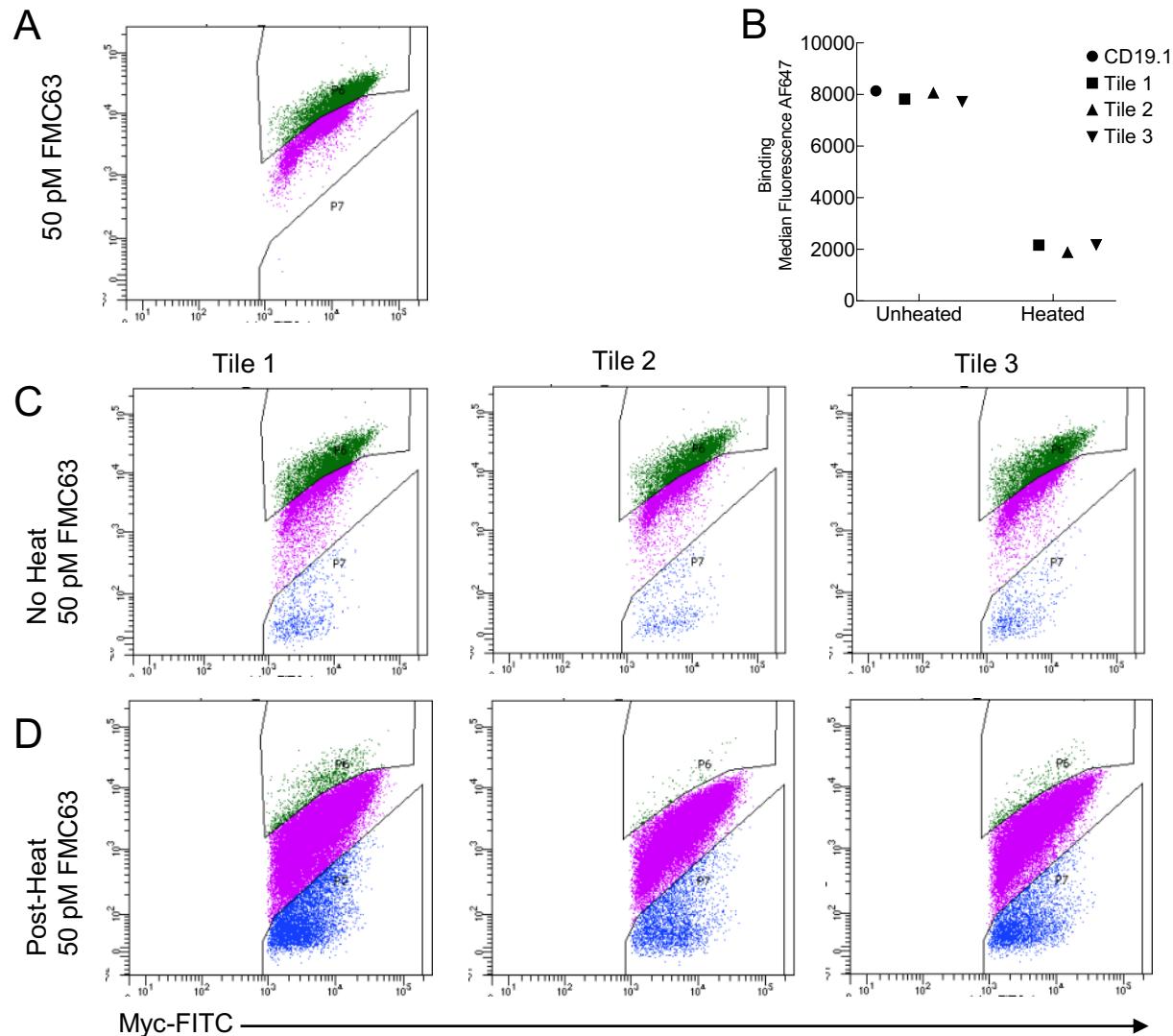


Figure S7: FMC63 post-melt sort strategy. (A) CD19.1 labelled at 50 pM FMC63 with no heat treatment. (B) Median binding signal of CD19.1 and SSM libraries of unheated and heated samples. (C) CD19.1 single-site saturation libraries labelled at 50 pM FMC63 with no heat treatment. (D) Yeast displaying the CD19.1 single-site saturation library were heated at 80°C for 30 minutes. They were then labelled at 50 pM with FMC63, and then cells in gate P6 were collected and sequenced. Cells were also labelled with 20 nM PE and were rejected if they were PE⁺ as a control for non-specific binding. Sort statistics in **Table S1**.

Table S1: Sorting statistics. Tile 1 is 1P to 92W, tile 2 is 93T to 180S, and tile 3 is 181C to 272K.

Tile	Population	Codons	Theoretical DNA Library Size	Cells Collected	Fold Coverage	Cells Able to be Collected
FMC63 at 0.01 nM						
1	Reference	92	2,944	300,000	101.9	382,516
	Top 5%			211,454	71.8	12,854,520
	Reduced			494,738	168.0	12,854,520
	Display			300,000	101.9	711,866
2	Reference	88	2,768	300,000	108.4	384,792
	Top 5%			233,928	84.5	10,422,650
	Reduced			378,865	136.9	10,422,650
	Display			300,000	108.4	736,996
3	Reference	92	2,944	300,000	101.9	370,476
	Top 5%			210,986	71.7	12,564,370
	Reduced			423,434	143.8	12,564,370
	Display			300,000	101.9	687,261
4G7 at 0.01 nM						
1	Reference	92	2,944	300,000	101.9	359,136
	Top 5%			303,048	102.9	13,727,281
	Reduced			484,348	164.5	13,727,281
	Display			300,000	101.9	711,201
2	Reference	88	2,768	300,000	108.4	378,841
	Top 5%			231,610	83.7	8,621,900
	Reduced			338,993	122.5	8,621,900
	Display			300,000	108.4	689,046
3	Reference	92	2,944	300,000	101.9	357,911
	Top 5%			290,088	98.5	5,893,195
	Reduced			364,898	123.9	5,893,195
	Display			300,000	101.9	602,596
3B10 at 50 nM						
1	Reference	92	2,944	300,000	101.9	364,141
	Top 5%			303,318	103.0	15,295,385
	Reduced			55,767	18.9	15,295,385
	Display			300,000	101.9	741,793
2	Reference	88	2,768	300,000	108.4	378,631
	Top 5%			338,920	122.4	16,840,181
	Reduced			214,681	77.6	16,840,181
	Display			300,000	108.4	673,646
3	Reference	92	2,944	300,000	101.9	388,221
	Top 5%			325,891	110.7	13,250,056
	Reduced			15,055	5.1	13,250,056
	Display			300,000	101.9	668,606
Reference for FMC63 and 4G7 at 20 nM						
1	Reference	92	2,944	505,997	171.9	
2	Reference	88	2,768	536,479	193.8	
3	Reference	92	2,944	635,608	215.9	
FMC63 at 20 nM						
1	Reduced	92	2,944	455,369	154.7	2,861,895
	Myc Only			162,453	55.2	2,861,895
2	Reduced	88	2,768	260,276	94.0	3,393,545
	Myc Only			164,643	59.5	3,393,545
3	Reduced	92	2,944	286,129	97.2	5,209,800
	Myc Only			179,024	60.8	5,209,800
4G7 at 20 nM						
1	Reduced	92	2,944	352,661	119.8	3,876,622
	Myc Only			180,707	61.4	3,876,622
2	Reduced	88	2,768	256,293	92.6	3,909,340
	Myc Only			173,387	62.6	3,909,340
3	Reduced	92	2,944	209,300	71.1	1,430,745
	Myc Only			183,890	62.5	1,430,745
Post-heat treatment with 0.05 nM FMC63						
1	Gate P6	92	2,944	133,367	45.3	2,984,109
2	Gate P6	88	2,768	32,833	11.7	1,986,707
3	Gate P6	92	2,944	97,255	34.0	2,732,172

Table S2: Deep sequencing statistics for the epitope mapping selections.

Selection	Tile	Population	Synonymous Reads		Nonsynonymous Reads		Nonsynon. codon coverage (fold)		Nonsynon. AA Coverage (%)
			Ref	Sel	Ref	Sel	Ref	Sel	
FMC63 (0.01 nM)	1	Top 5%	250,827	122,826	141,081	106,314	87.9	43.1	86.1
		Reduced	141,081	32,886	250,555	207,513	87.9	72.8	83.6
		Display	141,081	98,704	250,860	168,933	88.0	59.2	86.7
	2	Top 5%	76,133	111,897	172,999	172,612	63.4	63.3	89.5
		Reduced	76,133	35,428	172,115	207,384	63.1	76.0	80.0
		Display	76,133	86,367	172,837	168,207	63.4	61.7	87.6
	3	Top 5%	126,341	143,712	167,608	121,293	58.8	42.5	83.2
		Reduced	126,341	37,700	167,255	266,515	58.6	93.4	80.5
		Display	126,341	108,413	167,675	143,919	58.8	50.5	84.1
4G7 (0.01 nM)	1	Top 5%	92,113	117,955	160,998	128,354	56.5	45.0	85.1
		Reduced	92,113	23,702	160,653	162,187	56.3	56.9	82.2
		Display	92,113	105,487	161,186	173,029	56.5	60.7	87.0
	2	Top 5%	40,259	127,903	79,146	199,191	29.0	73.0	85.5
		Reduced	40,259	35,129	77,921	265,302	28.6	97.3	72.6
		Display	40,259	76,252	79,203	153,175	29.0	56.1	85.1
	3	Top 5%	115,724	149,667	147,170	125,948	51.6	44.2	82.9
		Reduced	115,724	25,244	146,672	214,564	51.4	75.2	79.9
		Display	115,724	125,109	147,266	164,843	51.6	57.8	84.2
3B10 (50 nM)	1	Top 5%	77,030	55,833	137,152	136,761	48.1	48.0	84.0
		Reduced	77,030	91,964	136,937	96,029	48.0	33.7	82.7
		Display	77,030	100,947	137,386	174,968	48.2	61.3	86.1
	2	Top 5%	144,023	59,013	291,716	171,725	106.9	62.9	90.6
		Reduced	144,023	5,291	291,622	167,967	106.9	61.6	89.7
		Display	144,023	90,720	291,799	177,081	107.0	64.9	91.3
	3	Top 5%	88,132	86,195	117,415	169,739	41.2	59.5	82.5
		Reduced	88,132	98,584	116,852	111,952	41.0	39.3	78.0
		Display	88,132	102,716	117,425	129,828	41.2	45.5	82.6
FMC63 (20 nM)	1	Reduced	102,850	36,726	182,450	239,746	64.0	84.1	83.3
		Myc Only	102,850	33,328	182,427	280,587	64.0	98.4	83.0
	2	Reduced	113,572	25,263	246,231	319,782	90.3	117.2	88.8
		Myc Only	113,572	6,607	246,192	259,847	90.2	95.3	88.5
	3	Reduced	127,435	40,410	182,360	310,800	63.9	109.0	82.4
		Myc Only	127,435	106,990	182,603	195,395	64.0	68.5	83.6
4G7 (20 nM)	1	Reduced	102,850	39,229	182,459	236,303	64.0	82.9	83.3
		Myc Only	102,850	32,811	182,430	189,520	64.0	66.5	83.0
	2	Reduced	113,572	18,707	246,229	254,799	90.3	93.4	88.9
		Myc Only	113,572	22,293	246,219	242,968	90.3	89.1	88.7
	3	Reduced	127,435	26,574	182,296	213,406	63.9	74.8	81.8
		Myc Only	127,435	34,944	182,290	252,637	63.9	88.6	81.5
FMC63 Melt	1	Gate P6	97,919	95,692	249,310	110,467	87.4	38.7	88.5
	2	Gate P6	144,624	128,789	340,164	171,303	124.7	62.8	92.6
	3	Gate P6	177,797	132,923	281,363	144,763	98.7	50.8	88.2

Table S3: Standard deviation of synonymous wild-type mutations at different depths of coverage.

Selection	Tile	Population	WT Enrichment	All Counts	N	≥ 12 Counts	N
FMC63 (0.01 nM)	1	Top 5%	0.3912	0.7784	276	0.6993	170
		Reduced	-1.4616	1.607	189	1.5252	149
		Display	0.0563	0.808	261	0.6595	169
	2	Top 5%	0.3993	1.0023	243	0.8674	129
		Reduced	-1.1458	1.7496	191	1.603	119
		Display	0.1819	1.108	230	0.8972	129
	3	Top 5%	0.3517	0.7693	282	0.6184	186
		Reduced	-1.8598	1.5357	205	1.5044	170
		Display	0.0128	0.8615	267	0.6731	186
4G7 (0.01 nM)	1	Top 5%	0.4160	0.6788	283	0.5249	173
		Reduced	-1.5639	1.5413	200	1.4201	164
		Display	0.0781	0.651	260	0.5882	173
	2	Top 5%	0.2239	0.9398	229	0.8797	117
		Reduced	-1.6062	1.487	189	1.414	105
		Display	-0.0130	0.8259	220	0.7229	117
	3	Top 5%	0.3122	0.7093	291	0.5859	170
		Reduced	-2.1225	1.6109	198	1.5175	149
		Display	-0.0224	0.7633	254	0.7233	169
3B10 (50 nM)	1	Top 5%	-0.3024	0.8792	232	0.7184	139
		Reduced	0.4408	0.8533	247	0.8101	138
		Display	0.0341	0.7992	248	0.6578	138
	2	Top 5%	-0.3479	0.8848	244	0.6926	185
		Reduced	-3.4420	1.4865	153	1.1406	141
		Display	0.0592	0.7225	287	0.6063	185
	3	Top 5%	-0.3451	0.7188	248	0.5167	158
		Reduced	0.1273	1.0664	258	1.1687	155
		Display	0.0488	0.7461	256	0.6078	158
FMC63 (20 nM)	1	Reduced	-1.5554	1.5281	198	1.4406	159
		Myc Only	-1.9718	1.7424	197	1.714	159
	2	Reduced	-2.2147	1.7173	186	1.6269	150
		Myc Only	-3.7888	1.8334	119	1.5725	107
	3	Reduced	-1.9492	1.5725	211	1.5246	170
		Myc Only	-0.2431	0.7903	288	0.7049	188
4G7 (20 nM)	1	Reduced	-1.4768	1.5506	217	1.4744	165
		Myc Only	-1.4827	1.7576	212	1.7011	161
	2	Reduced	-2.2961	1.7150	185	1.4487	152
		Myc Only	-2.0686	1.8904	175	1.7204	143
	3	Reduced	-2.0015	1.6385	211	1.5358	173
		Myc Only	-1.9369	1.6208	212	1.5996	172
FMC63 Melt	1	Gate P6	0.7427	0.9983	267	1.023	157
	2	Gate P6	0.5337	1.9714	240	2.0768	166
	3	Gate P6	0.2728	1.6467	292	1.8498	190

Table S4: Reagents table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat anti-Myc FITC conjugated antibody	Bethyl Laboratories Inc	Cat# A190-104F, RRID:AB_66866
FMC63	MilliporeSigma	Cat# MAB1794, RRID:AB_2244297
FMC63-PE	MilliporeSigma	Cat#MAB1794H, RRID:AB_94155
4G7-2E3	R&D Systems	Cat# MAB4867, RRID:AB_2072941
3B10	Novus	Cat# NBP2-46116 RRID:AB_2802147
UMAB103	OriGene	Cat# UM570071 RRID:AB_2802148
Goat anti-Mouse (H+L) Alexa Fluor 647	ThermoFisher	Cat# A21235 RRID:AB_2535804
Rabbit Anti-6X His tag Polyclonal Antibody, Fluorescein Conjugated	Abcam	Cat# ab1206, RRID:AB_298815
Anti-His antibody, PE conjugated	R&D Systems	Cat#IC050P, RRID:AB_357260
Purified anti-c-Myc Antibody	BioLegend	Cat# 626802, RRID:AB_2148451
Streptavidin Alexa Fluor 488	ThermoFisher	Cat# S32354 RRID:AB_2315383
Goat anti-HA [Biotin]	GenScript	Cat# A00203-100
anti-C-Myc Antibody (Chicken) - FITC Conjugated	IMMUNOLOGY CONSULTANTS LABORATORY, INC	Cat# CMYC-45F
Bacterial and Virus Strains		
High-efficiency competent DH5α	NEB	Cat# C2987H
XL1-Blue electroporation-competent cells	Agilent	Cat# 200228
Chemicals, Peptides, and Recombinant Proteins		
Human EGFR	Sino Biological Inc.	Cat# 10001-H08H
Deposited Data		
Deep mutational scanning data of w/t CD19 ECD	Klesmith et al., 2019	Figshare: https://doi.org/10.6084/m9.figshare.8291759.v1
Raw Illumina Sequencing	This paper	Figshare: 10.6084/m9.figshare.8298161
Processed Illumina sequencing to p-mutant z-score	This paper	Figshare: 10.6084/m9.figshare.8298161
Solved w/t CD19 ECD co-crystal structure with B43	Teplyakov et al., 2018	PDB: 6AL5
Experimental Models: Cell Lines		
SKOV3/luc	Cell Biolabs	Cat#AKR-232
Experimental Models: Organisms/Strains		
<i>Saccharomyces cerevisiae</i> : Meyen ex E.C. Hansen EBY100	ATCC	ATCC: MYA-4941

Oligonucleotides		
Tile 1 FWD: TTTCCCTACACGACGCTTCCGATCTGGCGGATCTGCTAGC	IDT	N/A
Tile 1 REV: GTTCAGACGTGTGCTCTCCGATCTGCCCTCACATTGACTGT	IDT	N/A
Tile 2 FWD: TTTCCCTACACGACGCTTCCGATCTGCAGCCTGGCTGG	IDT	N/A
Tile 2 REV: GTTCAGACGTGTGCTCTCCGATCTAGGGGGTACC CCACA	IDT	N/A
Tile 3 FWD: TTTCCCTACACGACGCTTCCGATCTCCACACTCTGGCTGTCC	IDT	N/A
Tile 3 REV: GTTCAGACGTGTGCTCTCCGATCTATAAGCTTTGTTCGGATCC	IDT	N/A
Primers for SSM library, see Excel File 1	This paper	N/A
Recombinant DNA		
pCT-40	Stern et al., 2016	N/A
CD19.1-SSM Libraries	This paper	N/A
CD19.1-Aglyco	This paper	N/A
3B10-Tag	This paper	N/A
FnEGFR-3B10-Tag	This paper	N/A
Software and Algorithms		
PACT: Protein Analysis and Classifier Toolkit	Klesmith and Hackel, 2018	2018.6 https://github.com/jKlesmith/PACT/
Python Scripts	This paper	https://github.com/JKlesmith/CD19_Epitope_Mapping
Python	Python Software Foundation	3.7.1
NumPy	NumPy developers	1.16.4
SciPy	SciPy developers	1.2.1
Prism	GraphPad	8.0.1
FlowJo	FlowJo LLC	10.0.7r2
FACSDiva	BD	8.0.1
PyMOL	Schrodinger	1.7.0.1 and 2
Other		
Rosetta modelled w/t CD19 structure based off of PDB: 6AL5	Klesmith et al., 2019	N/A

Table S5: Z-scores of amino acid encoding mutations enriched in the FMC63 thermal stability screen. The processed dataset was required to have at least 12 read counts in the reference sequenced population and encode amino acids. The w/t z-scores originate from (Klesmith et al., 2019) NaN = mutations not passing quality filters.

Resi.	Mut.	Stability	FMC	4G7	FMC63	4G7 10	FMC63	4G7 20	FMC63	4G7 20
			w/t	w/t	10 pM	pM red.	20 nM	nM red.	20 nM	nM Myc
18	E	2.20	-1.37	0.14	-3.42	-3.08	-4.01	-4.01	-3.44	-3.39
21	I	2.07	-1.17	0.30	-2.93	NaN	-2.10	-2.13	-1.76	-1.74
110	K	2.16	NaN	NaN	-1.38	NaN	-1.67	-1.42	-0.51	-1.43
112	Y	2.51	-2.23	-2.23	NaN	NaN	-1.79	-1.54	-0.62	-1.53
130	H	2.49	NaN	NaN	NaN	NaN	-1.21	-0.96	-0.08	-1.00
140	H	2.14	NaN	NaN	1.62	4.32	0.77	3.13	-0.25	1.30
141	M	2.01	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
145	I	2.28	NaN	NaN	-2.09	3.58	-2.15	3.49	-0.96	3.20
147	Q	2.24	NaN	NaN	NaN	NaN	-1.57	-0.39	-0.41	-0.80
147	R	2.12	4.26	-1.26	-2.12	NaN	-1.29	1.11	-0.16	-1.08
159	P	2.73	-0.63	-1.89	-0.97	-2.28	-1.47	-1.22	-0.33	-1.24
160	L	2.18	NaN	NaN	NaN	-2.28	-1.95	-1.70	-0.77	-1.68
179	I	2.43	NaN	NaN	NaN	-1.27	NaN	NaN	NaN	NaN
192	W	2.05	4.39	5.06	-2.09	-2.51	-2.54	-2.06	-0.68	-2.33
216	H	2.17	NaN	NaN	-2.50	-1.87	-1.30	-0.88	-0.16	-1.13
219	I	2.50	NaN	NaN	NaN	-1.71	-1.30	-0.88	-1.43	-1.13
223	M	2.43	0.00	0.00	-2.28	-1.40	NaN	NaN	2.69	NaN
227	I	2.25	NaN	NaN	NaN	NaN	-0.61	-1.08	-3.85	-0.46
235	F	2.48	NaN	NaN	NaN	-1.40	-2.15	-1.70	-1.86	-1.96
243	M	2.01	1.62	NaN	-2.23	NaN	-1.37	-0.94	0.78	-1.20
253	F	2.07	NaN	NaN	0.64	0.66	0.50	1.23	-0.57	-1.90
260	Y	2.02	-3.00	-1.89	NaN	NaN	-2.04	-1.58	-0.88	-1.85
264	K	2.91	7.43	8.36	-3.23	-1.77	-2.64	-2.16	-2.65	-2.43
264	E	2.28	5.42	6.26	-2.40	-2.49	-3.02	-2.53	-0.76	-2.80
264	N	2.23	7.23	6.76	-0.62	-0.50	0.32	-0.68	0.54	-0.05
264	R	2.17	5.85	6.23	-3.55	-3.39	-2.11	-3.37	-1.37	-3.65
269	R	2.09	2.74	1.90	-2.40	-2.27	-1.87	-1.79	-0.69	-1.07
270	P	2.06	NaN	NaN	-2.09	-1.44	-2.40	-1.93	-1.27	-2.20