Supplementary Information

Detection of a Large Antigen through the Masking and Exposure of Fragment of Split Luciferase

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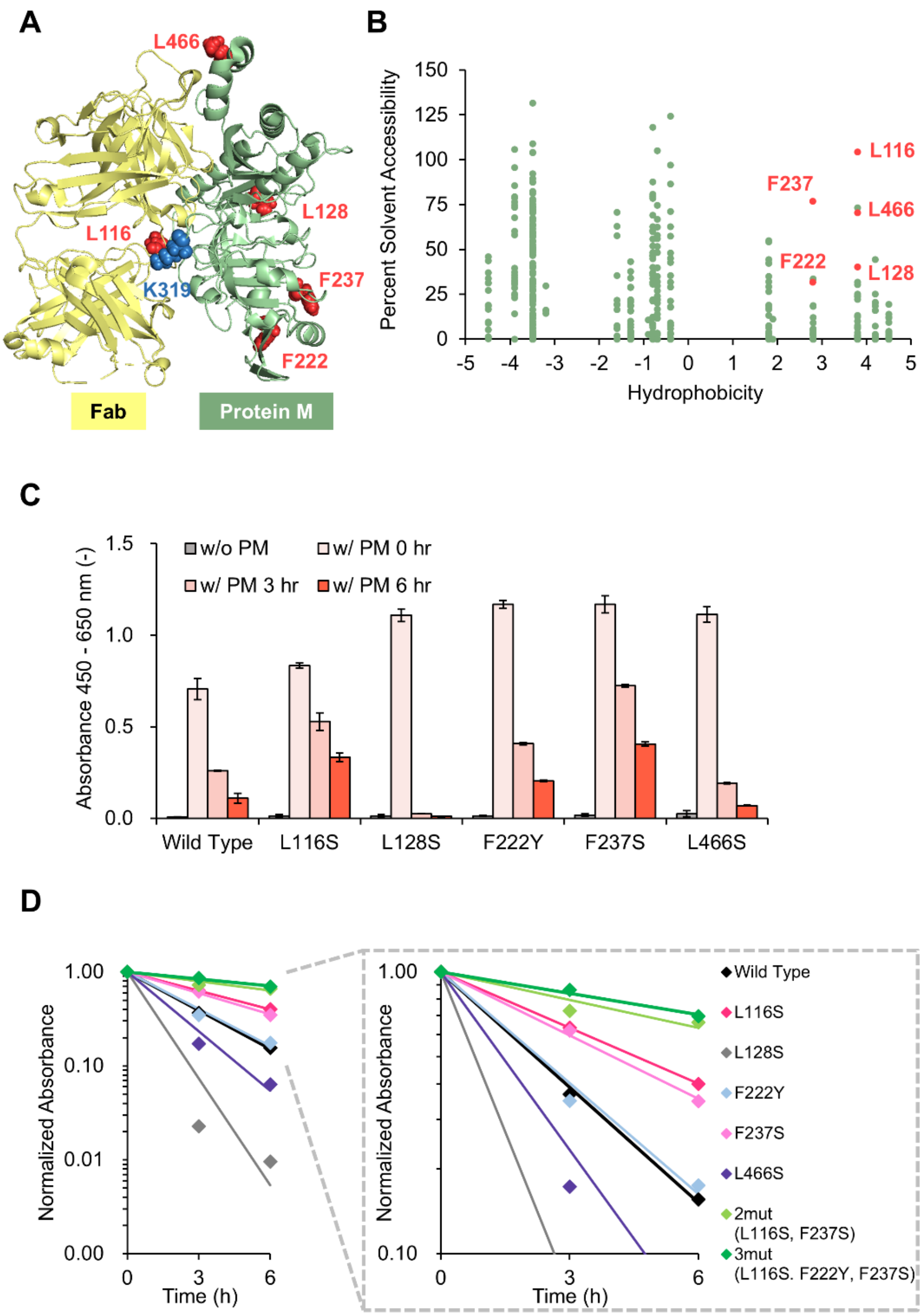

Figure S1. (A) Structure of PM bound Fab (PDB: 4NZR) with K319 shown as blue sphere and L116, L128, F222, F237, L466 shown as red sphere. (B) Dot plot of hydrophobicity and percent solvent accessibility for each amino acid residue on PM. The five residues selected for high hydrophobicity and solvent accessibility are highlighted with red dots. (C) Evaluation of binding activity of the wild-type PM and its mutants to polyclonal human IgGs after incubation at 42 °C for 0, 3, and 6 hours by ELISA. (n = 3). The mutants, L116S, F222Y, and F237S had improved thermostability. (D) Time-dependent reduction of the binding activity in wild type PM and its mutants including 2mut (L116S, F237S), 3mut (L116S, F222Y, F237S), with exposure to 42 °C. (n = 1)

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   1  ATG   AGC   GAT   AAA   ATT   ATT   CAC   CTG   ACT   GAC   GAC   AGT   TTT   GAC   ACG   GAT   GTA   CTC   AAA   GCG   GAC   GGG   GCG   ATC   CTC     25
       M     S     D     K     I     I     H     L     T     D     D     S     F     D     T     D     V     L     K     A     D     G     A     I     L
  26  GTC   GAT   TTC   TGG   GCA   GAG   TGG   TCC   GGA   CCG   TCC   AAA   ATG   ATC   GCC   CCG   ATT   CTG   GAT   GAA   ATC   GCT   GAC   GAA   TAT     50
       V     D     F     W     A     E     W     S     G     P     S     K     M     I     A     P     I     L     D     E     I     A     D     E     Y
  51  CAG   GGC   AAA   CTG   ACC   GTT   GCA   AAA   CTG   AAC   ATC   GAT   CAA   AAC   CCT   GGC   ACT   GCG   CCG   AAA   TAT   GGC   ATC   CGT   GGT     75
       Q     G     K     L     T     V     A     K     L     N     I     D     Q     N     P     G     T     A     P     K     Y     G     I     R     G
  76  ATC   CCG   ACT   CTG   CTG   CTG   TTC   AAA   AAC   GGT   GAA   GTG   GCG   GCA   ACC   AAA   GTG   GGT   GCA   CTG   TCT   AAA   GGT   CAG   TTG    100
       I     P     T     L     L     L     F     K     N     G     E     V     A     A     T     K     V     G     A     L     S     K     G     Q     L
 101  AAA   GAG   TTC   CTC   GAC   GCT   AAC   CTG   GCC   GGT   TCT   GGT   TCT   GGC   CAT   ATG   CAC   CAT   CAT   CAT   CAT   CAT   TCT   TCT   GGT    125
       K     E     F     L     D     A     N     L     A     G     S     G     S     G     H     M     H     H     H     H     H     H     S     S     G
 126  CTG   GTG   CCA   CGC   GGT   TCT   GGT   ATG   AAA   GAA   ACC   GCT   GCT   GCT   AAA   TTC   GAA   CGC   CAG   CAC   ATG   GAC   AGC   CCA   GAT    150
       L     V     P     R     G     S     G     M     K     E     T     A     A     A     K     F     E     R     Q     H     M     D     S     P     D
 151  CTG   TGG   AGC   CAT   CCC   CAG   TTC   GAG   AAA   GGC   GGT   GGA   TCT   GGT   GGC   GGT   TCG   GGA   GGT   TCA   GCC   TGG   TCG   CAT   CCG    175
       L     W     S     H     P     Q     F     E     K     G     G     G     S     G     G     G     S     G     G     S     A     W     S     H     P
 176  CAG   TTT   GAG   AAA   GGT   ACC   GAC   GAC   GAC   GAC   AAG   GCC   ATG   GGT   AAT   GAT   GGT   AGC   TAT   CAG   AGC   GAA   ATT   GAT   CTG    200
       Q     F     E     K     G     T     D     D     D     D     K     A     M     G    N78     D     G     S     Y     Q     S     E     I     D     L
 201  AGC   GGT   GGT   GCA   AAT   TTT   CGT   GAA   AAA   TTT   CGC   AAC   TTT   GCC   AAC   GAA   CTG   AGC   GAA   GCA   ATT   ACC   AAT   AGC   CCG    225
       S     G     G     A     N     F     R     E     K     F     R     N     F     A     N     E     L     S     E     A     I     T     N     S     P
 226  AAA   GGT   TCG   GAT   CGT   CCG   GTT   CCG   AAA   ACC   GAA   ATT   TCA   GGT   CTG   ATT   AAA   ACG   GGT   GAT   AAC   TTT   ATT   ACC   CCG    250
       K     G   L116S   D     R     P     V     P     K     T     E     I     S     G     L     I     K     T     G     D     N     F     I     T     P
 251  TCC   TTT   AAA   GCC   GGT   TAT   TAC   GAT   CAT   GTT   GCA   TCA   GAT   GGT   AGC   CTG   CTG   AGC   TAT   TAT   CAG   AGC   ACC   GAA   TAT    275
       S     F     K     A     G     Y     Y     D     H     V     A     S     D     G     S     L     L     S     Y     Y     Q     S     T     E     Y
 276  TTC   AAT   AAT   CGT   GTG   CTG   ATG   CCG   ATT   CTG   CAG   ACC   ACC   AAT   GGC   ACC   CTG   ATG   GCA   AAT   AAT   CGC   GGT   TAT   GAT    300
       F     N     N     R     V     L     M     P     I     L     Q     T     T     N     G     T     L     M     A     N     N     R     G     Y     D
 301  GAT   GTT   TTT   CGT   CAG   GTG   CCG   AGC   TTT   AGC   GGT   TGG   AGC   AAT   ACC   AAA   GCA   ACC   ACC   GTT   AGC   ACC   AGC   AAT   AAT    325
       D     V     F     R     Q     V     P     S     F     S     G     W     S     N     T     K     A     T     T     V     S     T     S     N     N
 326  CTG   ACC   TAT   GAT   AAA   TGG   ACC   TAC   TAT   GCA   GCA   AAA   GGT   AGT   CCG   CTG   TAT   GAT   AGT   TAT   CCG   AAC   CAC   TCT   TTT    350
       L     T     Y     D     K     W     T     Y   F222Y   A     A     K     G     S     P     L     Y     D     S     Y     P     N     H   F237S   F
 351  GAG   GAC   GTT   AAA   ACC   CTG   GCA   ATT   GAT   GCC   AAA   GAT   ATT   AGC   GCA   CTG   AAA   ACC   ACC   ATT   GAT   AGC   GAA   AAA   CCG    375
       E     D     V     K     T     L     A     I     D     A     K     D     I     S     A     L     K     T     T     I     D     S     E     K     P
 376  ACC   TAT   CTG   ATT   ATT   CGT   GGT   CTG   AGT   GGT   AAT   GGT   AGT   CAG   CTG   AAT   GAA   CTG   CAG   CTG   CCG   GAA   AGC   GTT   AAA    400
       T     Y     L     I     I     R     G     L     S     G     N     G     S     Q     L     N     E     L     Q     L     P     E     S     V     K
 401  AAA   GTT   AGC   CTG   TAT   GGT   GAT   TAT   ACG   GGT   GTT   AAT   GTG   GCC   AAA   CAA   ATT   TTT   GCC   AAT   GTG   GTC   GAA   CTG   GAA    425
       K     V     S     L     Y     G     D     Y     T     G     V     N     V     A     K     Q     I     F     A     N     V     V     E     L     E
 426  TTT   TAC   AGC   ACC   AGT   TAG   GCA   AAC   AGC   TTT   GGT   TTT   AAT   CCG   CTG   GTT   CTG   GGT   AGC   AAA   ACC   AAT   GTT   ATT   TAT    450
       F     Y     S     T     S   K319*   A     N     S     F     G     F     N     P     L     V     L     G     S     K     T     N     V     I     Y
 451  GAC   CTG   TTT   GCC   AGC   AAA   CCG   TTT   ACC   CAT   ATC   GAT   CTG   ACC   CAG   GTT   ACC   CTG   CAG   AAT   AGC   GAT   AAT   AGC   GCC    475
       D     L     F     A     S     K     P     F     T     H     I     D     L     T     Q     V     T     L     Q     N     S     D     N     S     A
 476  ATT   GAT   GCA   AAC   AAA   CTG   AAA   CAG   GCA   GTT   GGC   GAT   ATT   TAC   AAC   TAT   CGT   CGT   TTT   GAA   CGT   CAG   TTC   CAG   GGT    500
       I     D     A     N     K     L     K     Q     A     V     G     D     I     Y     N     Y     R     R     F     E     R     Q     F     Q     G
 501  TAT   TTT   GCC   GGT   GGT   TAT   ATC   GAT   AAA   TAT   CTG   GTG   AAA   AAC   GTG   AAC   ACC   AAC   AAA   GAT   AGT   GAT   GAT   GAT   CTG    525
       Y     F     A     G     G     Y     I     D     K     Y     L     V     K     N     V     N     T     N     K     D     S     D     D     D     L
 526  GTT   TAT   CGC   AGC   CTG   AAA   GAA   CTG   AAT   CTG   CAT   CTG   GAA   GAG   GCA   TAT   CGT   GAA   GGT   GAT   AAT   ACC   TAT   TAT   CGT    550
       V     Y     R     S     L     K     E     L     N     L     H     L     E     E     A     Y     R     E     G     D     N     T     Y     Y     R
 551  GTG   AAC   GAG   AAC   TAT   TAT   CCC   GGG   GCA   AGC   ATT   TAT   GAA   AAT   GAA   CGT   GCA   AGC   CGT   GAT   AGC   GAG   TTT   CAG   AAT    575
       V     N     E     N     Y     Y     P     G     A     S     I     Y     E     N     E     R     A     S     R     D     S     E     F     Q     N
 576  GAA   ATC   CTG   AAA   CGT   GCG   GCC   TCA   GGT   GGA   GGG   AGC   GGT   GGA   GGC   TGC   GGC   GGC   GGA   AGT   GGC   GGT   GGA   TCC   CTC    600
       E     I     L     K    R468   A     A     S     G     G     G     S     G     G     G     C     G     G     G     S     G     G     G     S     L
 601  GAG   CAC   CAC   CAC   CAC   CAC   CAC   TGA                                                                                                      608
       E     H     H     H     H     H     H     *
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Figure S2. The DNA and amino acid sequences of the PM3mut (319 Amber) protein are presented. The thioredoxin tag is shown in blue, while the PM fragment, from N38 to R468 is shown in green. Mutations for thermostability and incorporation of unnatural amino acid are shown in red and blue, respectively.

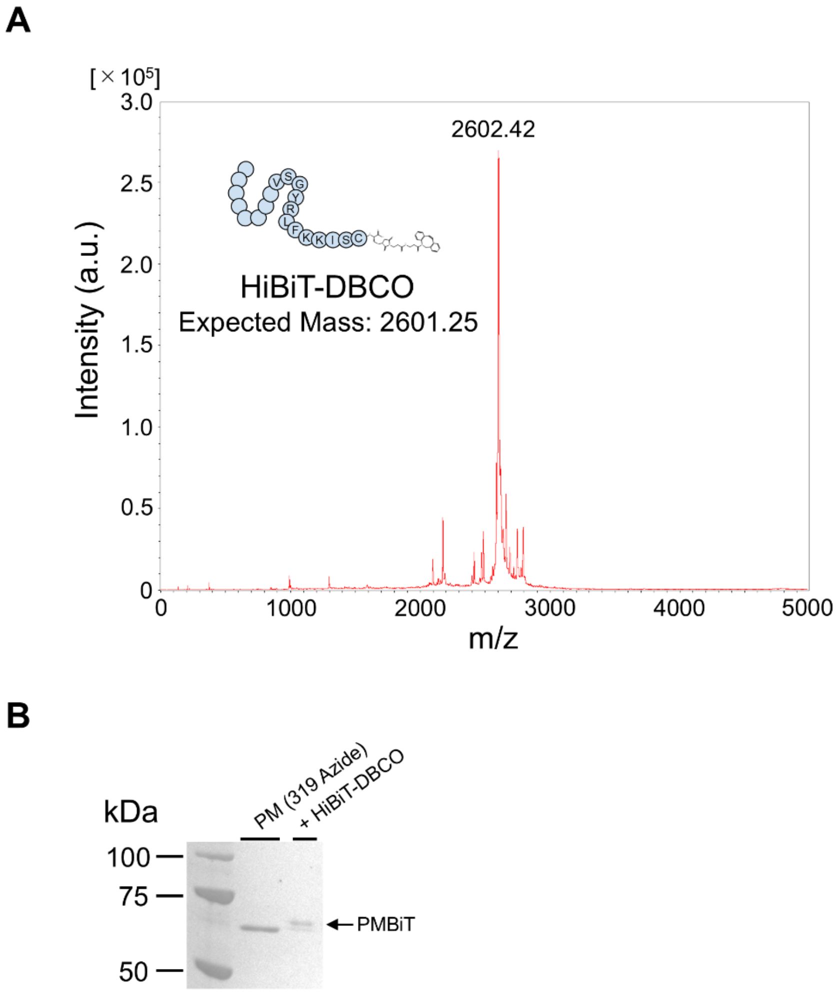

Figure S3. Preparation of HiBiT-DBCO and PMBiT. (A) Mass spectra of HiBiT-DBCO. (B) SDS-PAGE analysis of purified PM (319 Azide) with and without the conjugation of HiBiT-DBCO.

Table S1. DNA sequence of primers used for this study

Mutation	Primer name	Nucleotide Sequence (5'-3')
L116S	Hphi1_Back (L2S)	CCAATAGCCCGAAAGGTTCGGATCGTCCGGTTCCG
	Hphi1_For	ACCTTTCGGGCTATTGGTAATTG
F222Y	Hphi4_Back (F2Y)	GATAAATGGACCTACTATGCAGCAAAAGGTAGTC
	Hphi4_For	GTAGGTCCATTTATCATAGGTCAG
F237S	Hphi6_Back (F2S)	GTTATCCGAACCACTCTTTTGAGGACGTTAAAAC
	Hphi6_For	GTGGTTCGGATAACTATCATAC
K319Amb	PM (K319Amb)_back	AGCACCAGTTAGGCAAACAGCTTTGG
	PM (K319Amb)_for	GTTTGCCTAACTGGTGCTGTAAAATTCCAG
	T7Prom_primer	TAATACGACTCACTATAGGG
	T7Term_primer	ATGCTAGTTATTGCTCAGCGG

Table S2. Steric clash from superimposing antigen-bound Fab selected from PDB with PM-bound Fab.

No.	**PDB ID**	**Antigen Size (kDa)**	**Total number of Clashes**	**Number of Clashes in Region 1**	**Number of Clashes in Region 2**	**Number of Clashes in Region 3**
1	5Y9F	55.9	37	0	0	37
2	5VCN	25	0	0	0	0
3	3TT3	57.87	2860	619	388	930
4	3NCY	93.88	1049	220	8	763
5	6WIK	63.67	533	0	0	483
6	2ZUQ	20.09	0	0	0	0
7	7SBD	14.63	1233	3	153	880
8	2JEL	9.12	7	0	0	7
9	3HFM	14.31	957	8	1	876
10	8HPK	45.76	585	0	0	440
11	5EZO	39.27	1084	76	34	863
12	8DCM	79.96	1073	114	0	597
13	8DCN	15.24	76	0	0	76
14	4BZ2	11.27	36	0	0	36
15	1RJL	10.03	2	0	0	0
16	2J88	40.85	0	0	0	0
17	6M58	66.48	470	2	0	462
18	1TQB	12.19	1076	24	90	823
19	3OPZ	71.31	403	0	0	296
20	3EOB	20.65	2543	544	507	642
21	8T9Z	51.7	693	77	95	77
22	5VJO	14.41	623	0	0	573
23	6XQW	11.17	532	0	0	532
24	8J7E	11.77	0	0	0	0
25	8Q6K	16	0	0	0	0
26	1IQD	17.73	1050	47	3	835
27	6PHF	20.2	1586	276	92	796
28	8DFG	11.74	303	0	0	225
29	4NNP	32.84	15	0	0	15
30	7REW	12.29	1121	97	42	873
31	7BSC	20.46	834	6	10	646

32	5USH	27.93	810	12	0	733
33	6VYH	63.67	482	0	0	455
34	5JQ6	35.78	0	0	0	0
35	7LF8	15.38	0	0	0	0
36	7STR	14.29	140	0	0	140
37	6VLW	21.85	0	0	0	0
38	7R9D	13.14	113	0	0	113
39	3NPS	26.43	1671	351	321	877
40	5EPM	3.98	306	0	0	302
41	6XZW	26.86	851	0	7	721
42	7JIE	34.01	141	36	0	11
43	7TUG	44.7	1711	249	253	377
44	4F3F	8.04	18	0	0	18
45	3GI8	48.24	502	3	5	493
46	6CEZ	1.92	0	0	0	0
47	7OH0	50.43	440	0	25	307
48	6XR0	80.23	787	22	0	711
49	6GV1	44.33	0	0	0	0
50	4M5Z	24.86	342	0	0	338
51	8TLM	75.94	146	0	0	146
52	2YPV	26.81	210	0	0	210
53	6OE4	57.89	1594	383	274	677
54	7FCI	38.5	563	0	91	472
55	6XPY	32.59	2080	370	378	975
56	1OSP	27.64	1097	1	0	963
57	6MTO	18.21	22	0	0	22
58	7UVS	22.67	906	172	169	563
59	7URQ	25.92	598	29	142	422
60	7LR3	13.85	130	0	0	130
61	6AYZ	17.41	751	6	1	713
62	4OT1	14.42	0	0	0	0
63	7N0V	20.72	77	0	0	77
64	7O9S	40.31	0	0	0	0
65	3W9E	34.1	1225	103	37	896
66	4IJ3	26.96	0	0	0	0
67	7VNG	16.18	12	0	0	12

68	7W71	9.76	618	0	0	515
69	4KUC	29.92	0	0	0	0
70	5VIC	10.78	26	0	0	26
71	5VQM	64.47	35	0	0	31
72	6RPS	31.57	812	2	0	808
73	6VY5	48.84	391	0	0	391
74	3PNW	8.97	0	0	0	0
75	6D9W	45.23	31	0	0	31
76	6MHR	15.66	0	0	0	0
77	6R8X	26.83	907	48	63	730
78	3ZKM	42.09	124	0	0	40
79	3TJE	17.47	0	0	0	0
80	6ADA	50.34	46	0	0	46
81	6OCB	30.36	2675	581	613	855
82	4FG6	49.57	53	0	0	53
83	7R58	22.55	2635	719	424	607
84	7URX	30.62	364	0	0	331
85	5MEV	18.2	388	0	17	371
86	3O0R	68.53	1774	387	176	944
87	6QB6	18.2	145	0	25	120
88	5YWY	37.04	863	142	59	662
89	5BK1	44.12	581	0	0	562
90	8D1T	40.11	280	3	0	268
91	4XHJ	107.85	4316	706	545	923
92	7SO7	62.59	2051	514	279	829
93	3LD8	39.38	0	0	0	0
94	5D96	26.98	702	0	0	586
95	8D2S	56.65	259	0	0	149
96	7CJ2	40.5	2920	661	523	579
97	7NKS	40.31	465	0	93	337
98	8D48	139.69	0	0	0	0
99	6I04	62.34	1182	192	101	769
100	8IVX	65.89	264	39	91	91