Suppl. Table SI. Sequences and AutoMatch scores of Tbab1 proteins. The mutation sites suggested by AutoMatch are colored in red.

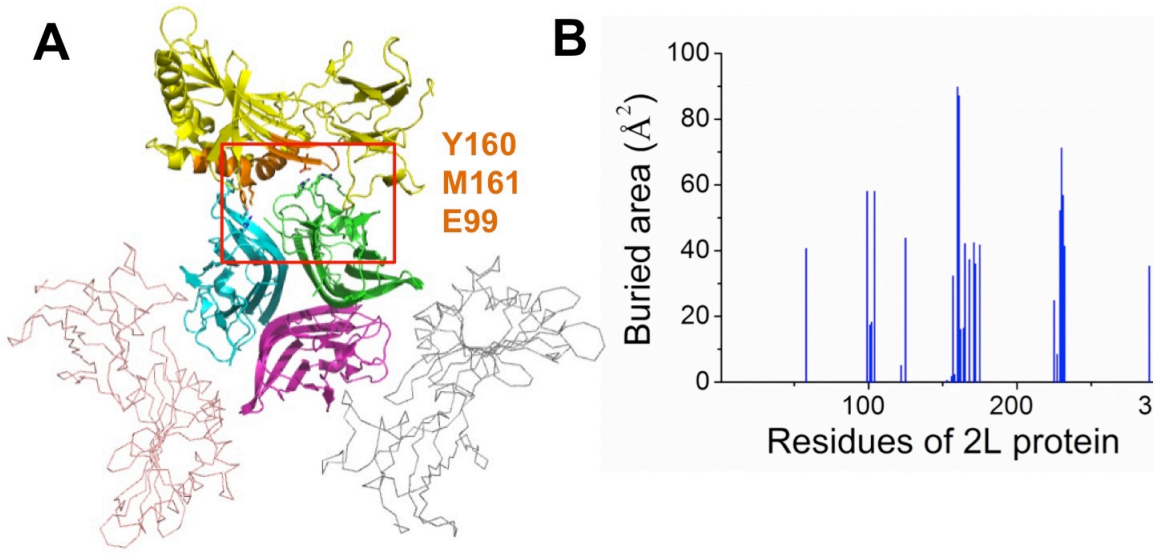
	Sequence	Interaction Score	Conformat -ion Score	Combined score
DS119	GSG QVRTIWV GG TPEELKKLKEEAKKA NIRVTFWGD			
Tbab1-1	GSG QVRTIWV GG TPEELEKLKEEAKMY NIRVTFWGD	-24.1	4.5	-23.0
Tbab1-2	GSG QVRTIWV GE TPEELMYLKEEAKKA NIRVTFWGD	-23.74	4.3	-22.6
Tbab1-3	GSG QVRTIWV GG TPEELKKLKYEAMKA NIEVTFWGD	-23.6	4.5	-22.5
Tbab1-4	GSG QVRTIWV GG YPEMLKKLKEEAKKA NIRVTFWGD	-21.3	4.2	-20.2
Tbab1-5	GSG QVRTIWV GG TPEELMYLKEEAKKA NIRVTFWED	-22.0	7.3	-20.2
Tbab1-6	GSG QVRTIWV GG TPEELYKLMEEAKKA NIEVTFWGD	-20.6	4.2	-19.5
Tbab1-7	GSG QVRTIWV GY TMEELKKLKEEAKKA NIRVTFWGD	-20.4	8.9	-19.2
Tbab1-8	GSG QVRTIWV GG TPEELKKLKYEAKMA EIRVTFWGD	-20.0	5.8	-18.9
Tbab1-9	GSG QVRTIWV GY MPEELEKLKEEAKKA NIRVTFWGD	-20.2	5.1	-18.7

Suppl. Table SII. TNFα-Tbab interaction rate constants measured by SPR.

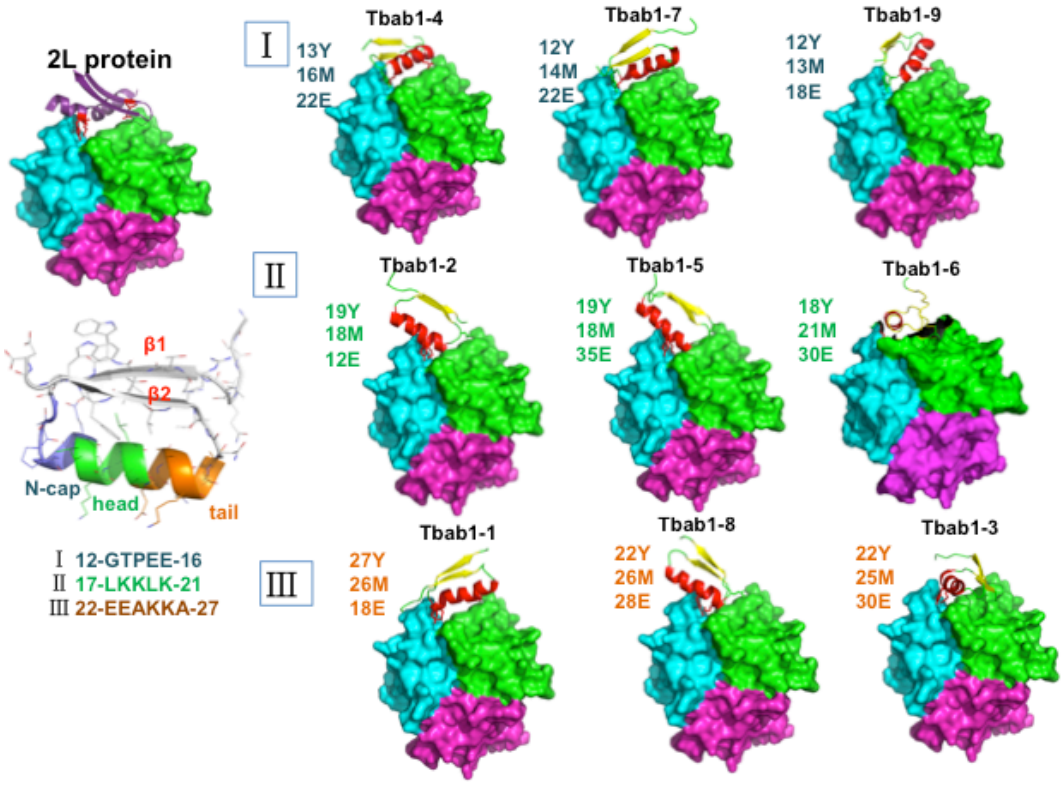
	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})
Tbab1-1	$1.03\times10^{3} \pm 0.22\times10^{3}$	$6.42\times10^{-3} \pm 0.32\times10^{-3}$
Tbab1-4	$2.42\times10^{3} \pm 0.04\times10^{3}$	$7.70\times10^{-3} \pm 0.21\times10^{-3}$
Tbab1-7	$4.34\times10^{3} \pm 0.10\times10^{3}$	$3.70\times10^{-3} \pm 0.05\times10^{-3}$
Tbab1-8	$2.66\times10^{3} \pm 0.22\times10^{3}$	$2.56\times10^{-3} \pm 0.06\times10^{-3}$
Tbab1-9	$1.12\times10^{3} \pm 0.14\times10^{3}$	$4.30\times10^{-3} \pm 0.11\times10^{-3}$
Tbab2-4	$3.14\times10^{3} \pm 0.05\times10^{3}$	$1.14\times10^{-3} \pm 0.08\times10^{-3}$

Suppl. Table SIII. Size exclusion profile for Tbab proteins.

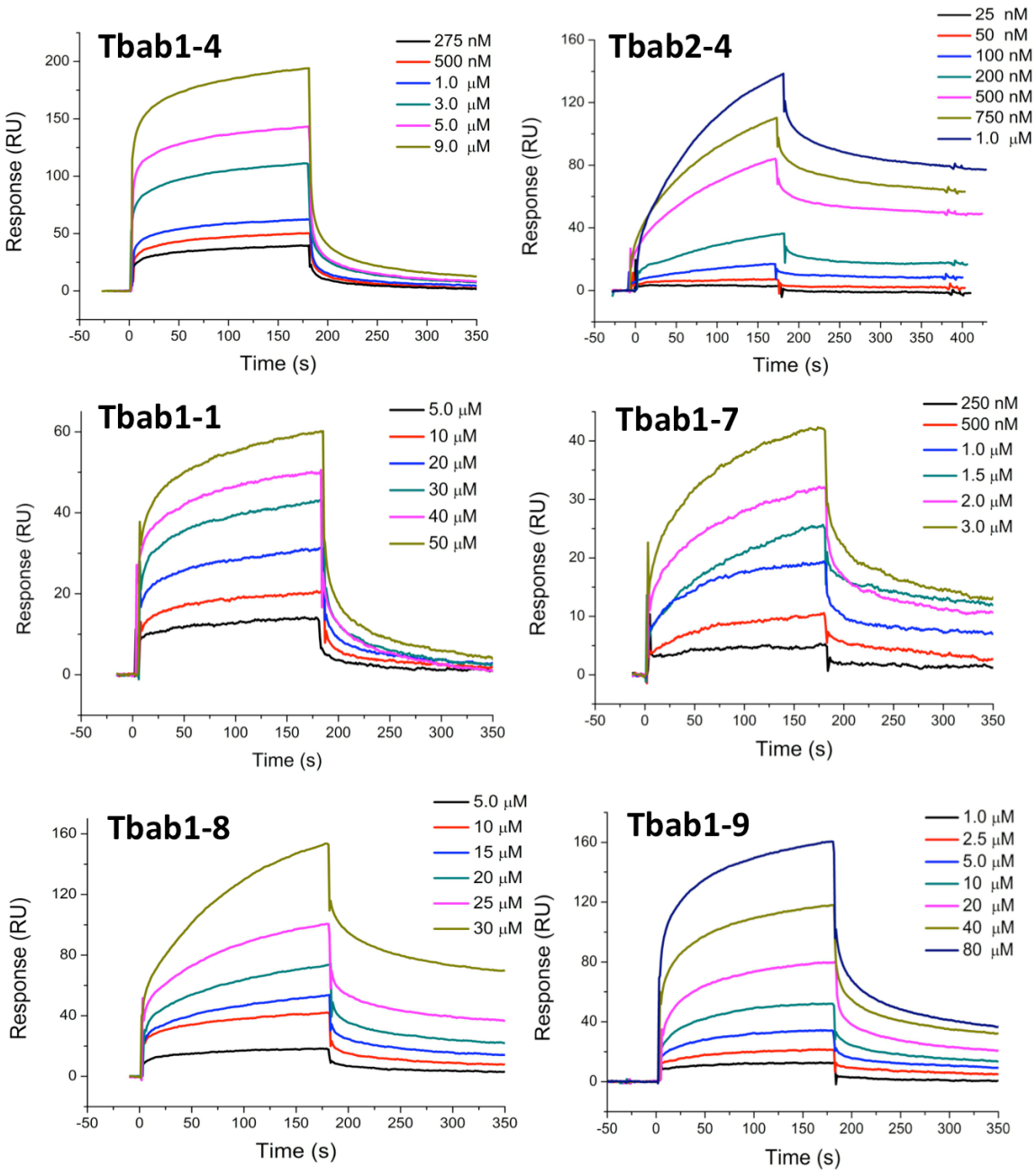
	pI	Molecular Weight (Dalton)	Apparent Molecular Weight (Dalton)	Aggregation State
Tbab1-1	5.20	4125.6	3700.2	0.89
Tbab1-2	5.20	4139.7	n.a.	n.a.
Tbab1-3	6.36	4039.6	4637.4	1.15
Tbab1-4	9.70	4093.7	4153.7	1.02
Tbab1-5	5.11	4040.5	8704.6	2.15
Tbab1-6	4.82	4139.7	8860.0, 7924.7	2.14, 1.91
Tbab1-7	9.31	4169.8	4752.0, 3774.3	1.14, 0.91
Tbab1-8	8.38	4081.7	3855.6	0.94
Tbab1-9	6.36	4166.7	4755.5, 3800.6	1.14, 0.91
Tbab2-4	9.82	4120.7	4155.8	1.01



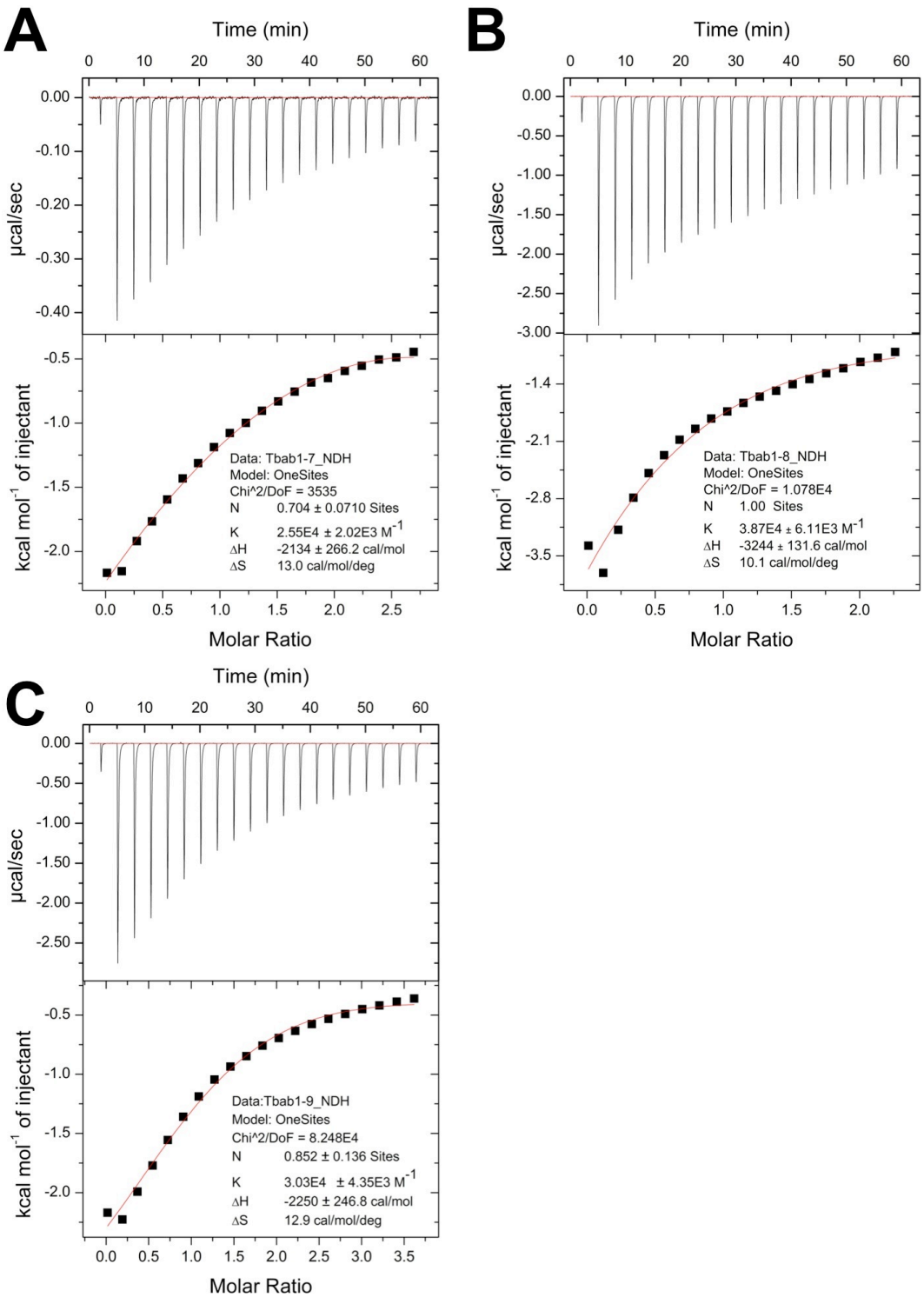
Suppl. Figure S1. Identification of key residues through calculation of buried area in TNFα-2L complex structure.



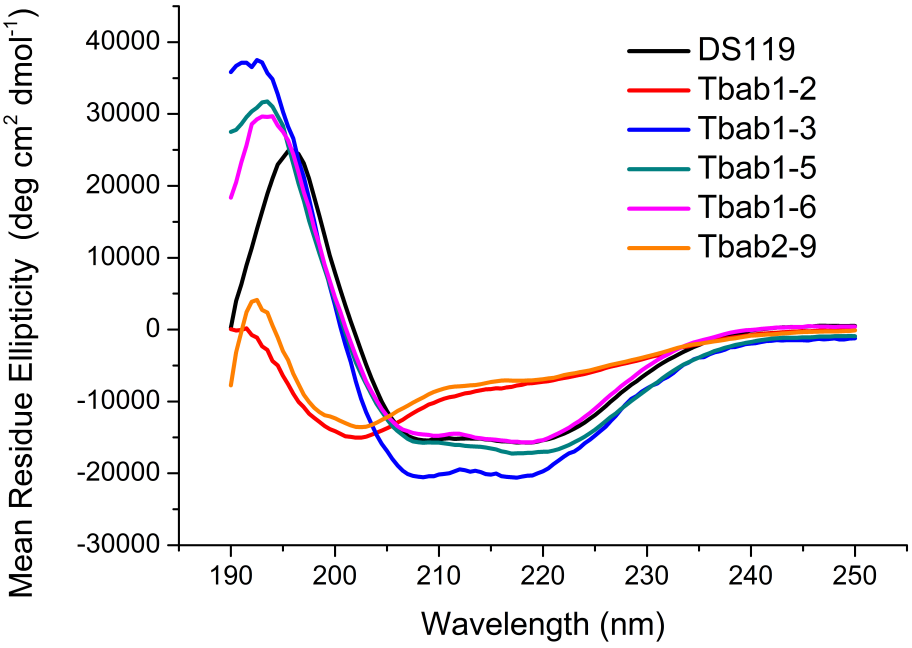
Suppl. Figure S2. Classification of Tbab1 proteins based on the binding orientations.



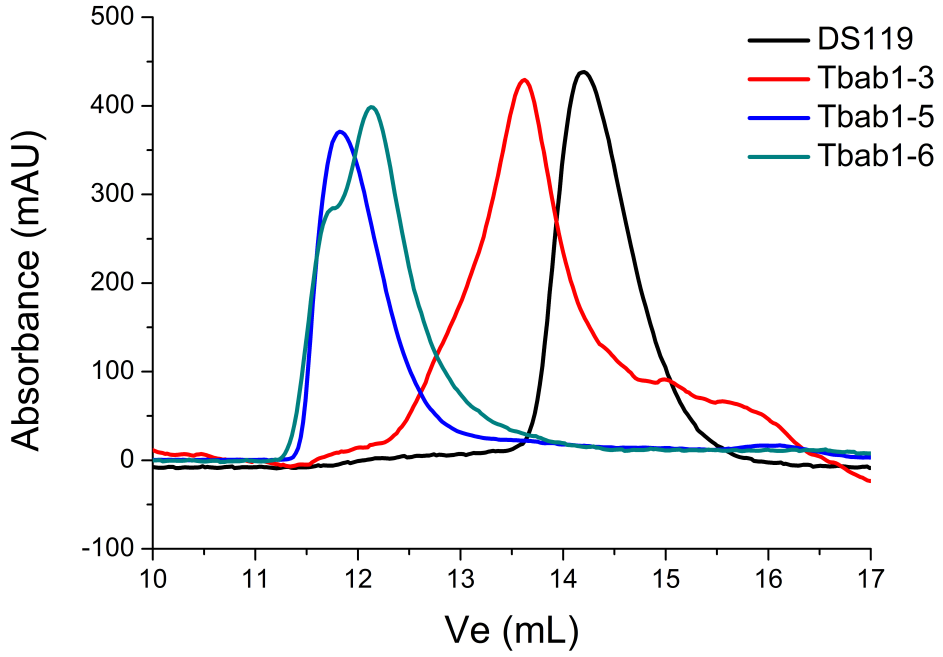
Suppl. Figure S3. SPR binding curves of Tbab proteins.



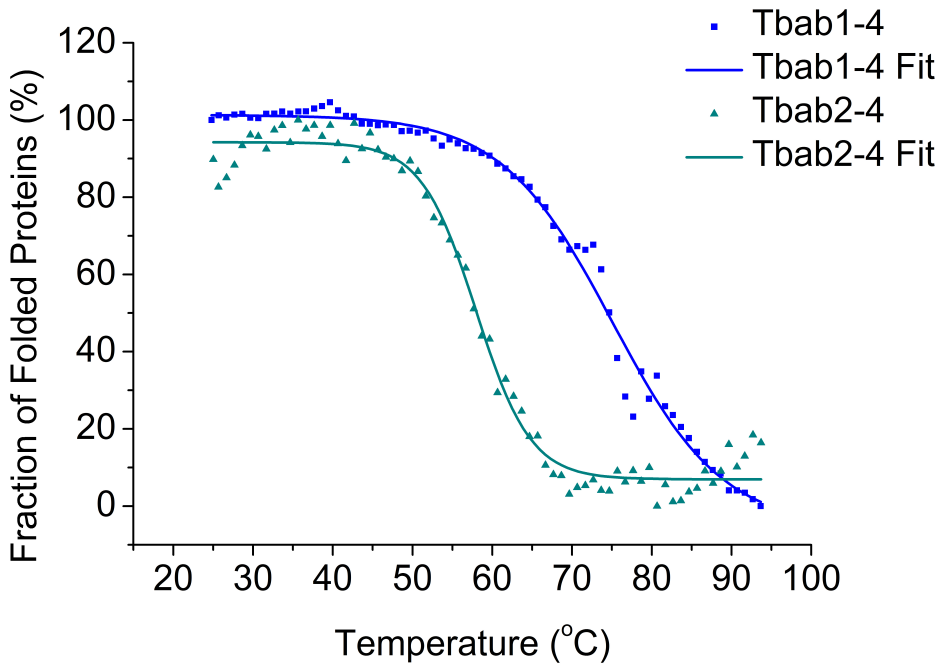
Suppl. Figure S4. Binding affinities of Tbab1-7, Tbab1-8 and Tbab1-9 to TNFα measured by ITC.



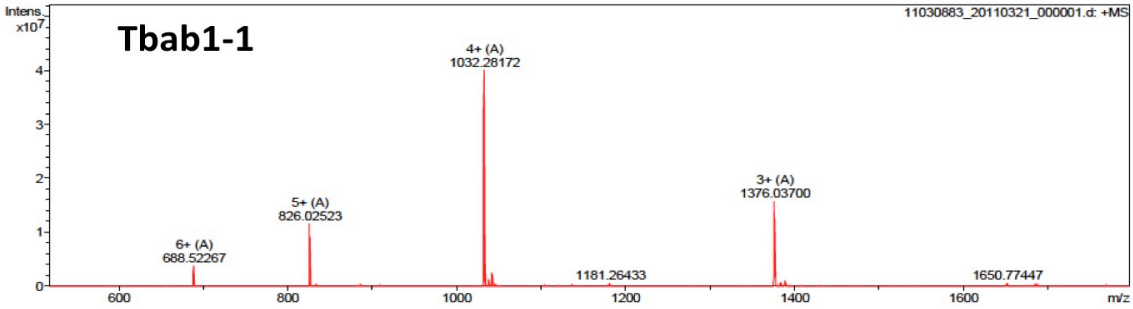
Suppl. Figure S5. CD spectra of Tbab proteins that have no binding affinities to TNFα. They have good secondary structures except for Tbab1-2 and Tbab2-9, which are unstructured proteins.

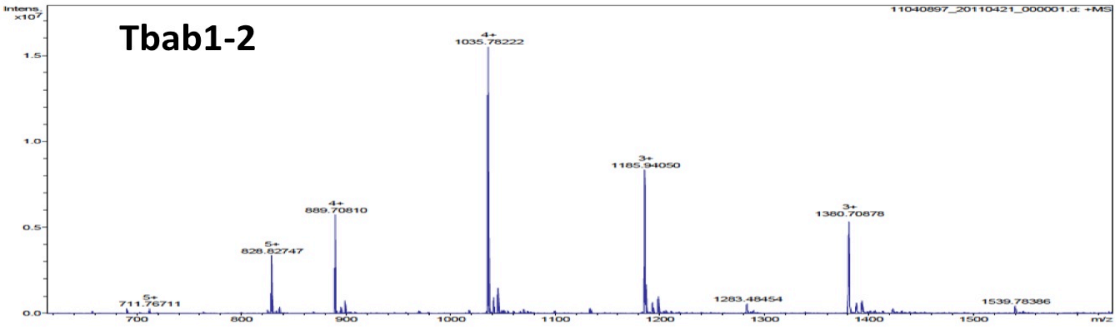


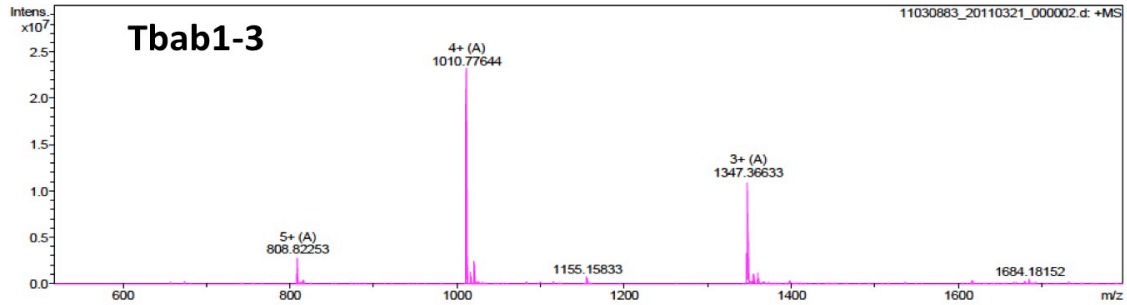
Suppl. Figure S6. Size exclusion chromatography of Tbab proteins that have no binding affinities to TNFα. Tbab1-3, 1-5 and 1-6 have good secondary strucutre (Figure S4) but they are dimers or exhibit exchange between monomers and dimers. Tbab1-2 and Tbab2-9 are unstructured proteins and not tested.

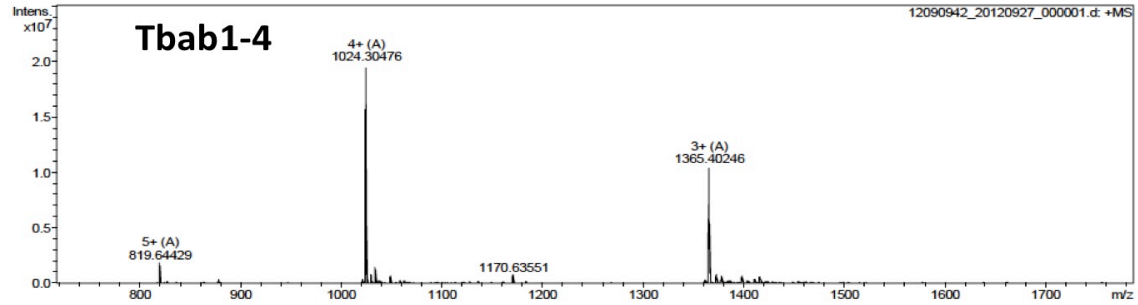


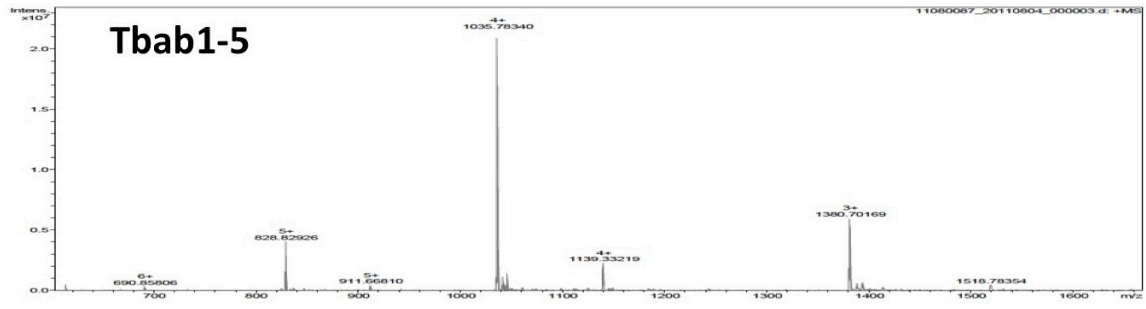
Suppl. Figure S7. Thermal denaturation curves of Tbab1-4 and Tbab2-4. The CD signal at 222 nm is monitored as the proteins unfold. The curves are fitted to a two-state protein folding model and the resulting melting temperatures for Tbab1-4 and Tbab2-4 are 85℃ and 55℃, respectively.

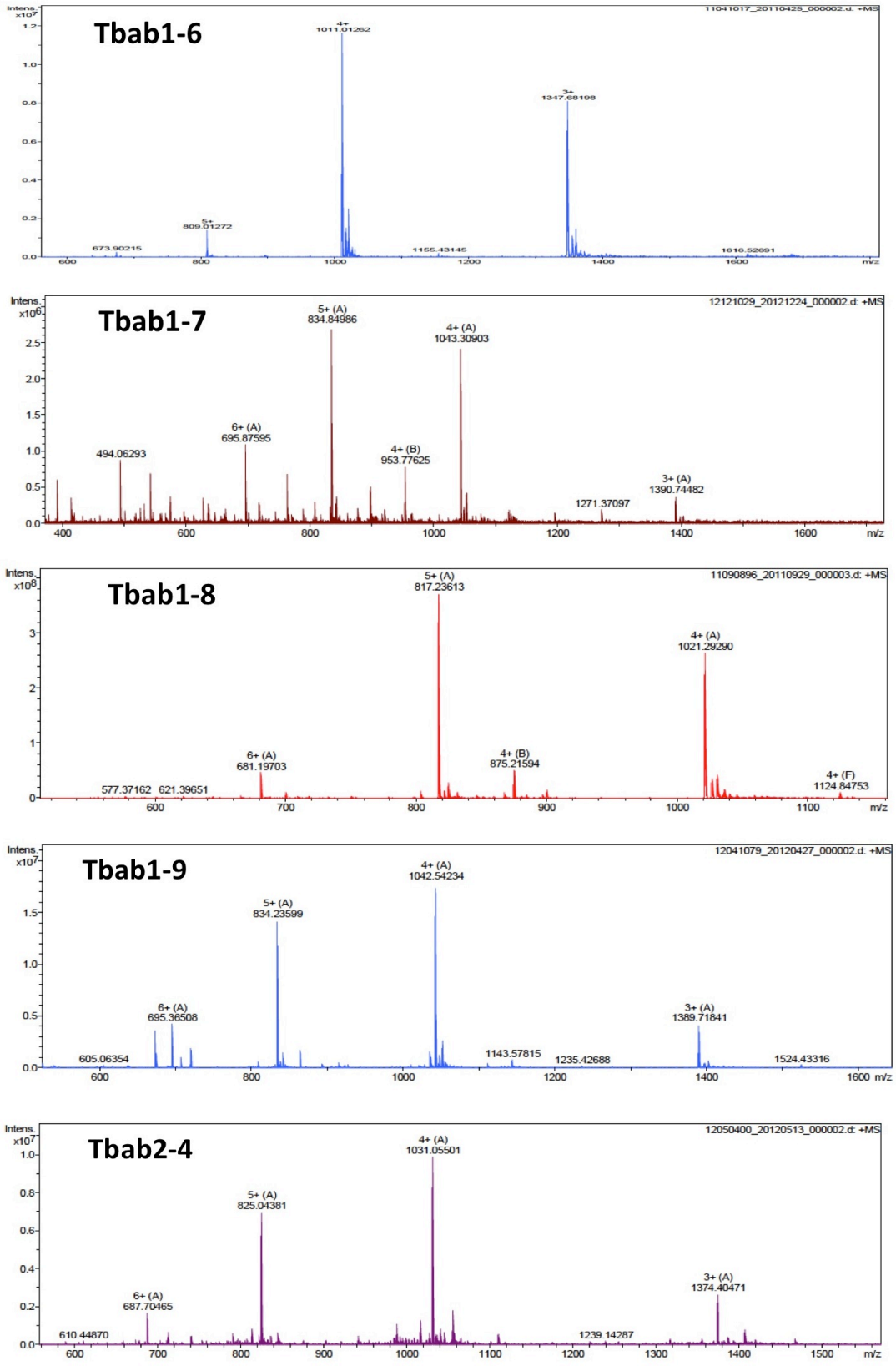

Tbab1-1
11030883_20110321_000001.d: +MS
Intens.
x10^7
4+ (A)
1032.28172
5+ (A)
826.02523
6+ (A)
688.52267
3+ (A)
1376.03700
1181.26433
1650.77447
m/z


Tbab1-2
11040897_20110421_000001.d: +MS
Intens.
x10^7
4+
1035.78222
3+
1185.94050
4+
889.70810
3+
1380.70878
5+
828.82747
5+
711.76711
1283.48454
1539.78386
m/z


Tbab1-3
11030883_20110321_000002.d: +MS
Intens.
x10^7
4+ (A)
1010.77644
3+ (A)
1347.36633
5+ (A)
808.82253
1155.15833
1684.18152
m/z


Tbab1-4
12090942_20120927_000001.d: +MS
Intens.
x10^7
4+ (A)
1024.30476
3+ (A)
1365.40246
5+ (A)
819.64429
1170.63551
m/z


Tbab1-5
11080087_20110804_000003.d: +MS
Intens.
x10^7
4+
1035.78340
3+
1380.70169
5+
828.82926
4+
1139.33219
6+
690.85506
5+
911.66810
1518.78354
m/z



Suppl. Figure S8. Mass spectrometry images of Tbab proteins.