Supplementary Materials

Table S1. Retention volume of scFv clones in the gel filtration chromatography.

scFv clones	Retention volume (mL)
AGE73scFv	12.8
73MuL-S30P	12.8
73MuL-S56P	13.0
73MuL-V94A	14.4

Table S2. Mutated residues in the selected clones by bio-panning from the second and third library.

Second library			
Mutations in VL	Number of clone	Mutations in VL	Number of clone
V94A	5	S56P	1
K39R	2	V58A	1
M78V	2	S65G	1
T22A	1	Y71N	1
C23R	1	E81G	1
S27L	1	D82G	1
S27aG	1	A84G	1
V28R	1	S91G	1
S30P	1	T97S	1
S43P	1	G100E	1
S52Y	1		

Third library	
Mutations in VH	Number of clone
D100hG	4
F40L	1
Y47H	1
L53F	1
D100hV	1
D100hG	4

Table S3. The population of open and closed states of scFvs.

scFv clones	Distance between two peaks (nm)	Percentage	State
AGE73scFv	2.8 ± 0.3	7.0	Closed
	4.7 ± 0.7	93.0	Open
AGE73scFv + CBZ-GA-pyridine	2.8 ± 0.4	59.1	Closed
	4.2 ± 0.5	40.9	Open
73MuL-V94A	2.7 ± 0.5	65.0	Closed
	4.3 ± 0.5	35.0	Open
73MuL-V94A + CBZ-GA-pyridine	2.6 ± 0.4	63.8	Closed
	4.1 ± 0.5	36.2	Open
73MuH19	2.4 ± 0.7	81.3	Closed
	4.4 ± 0.5	18.7	Open
73MuH19 + CBZ-GA-pyridine	1.9 ± 0.3	39.3	Closed
	2.8 ± 0.9	60.7	Closed

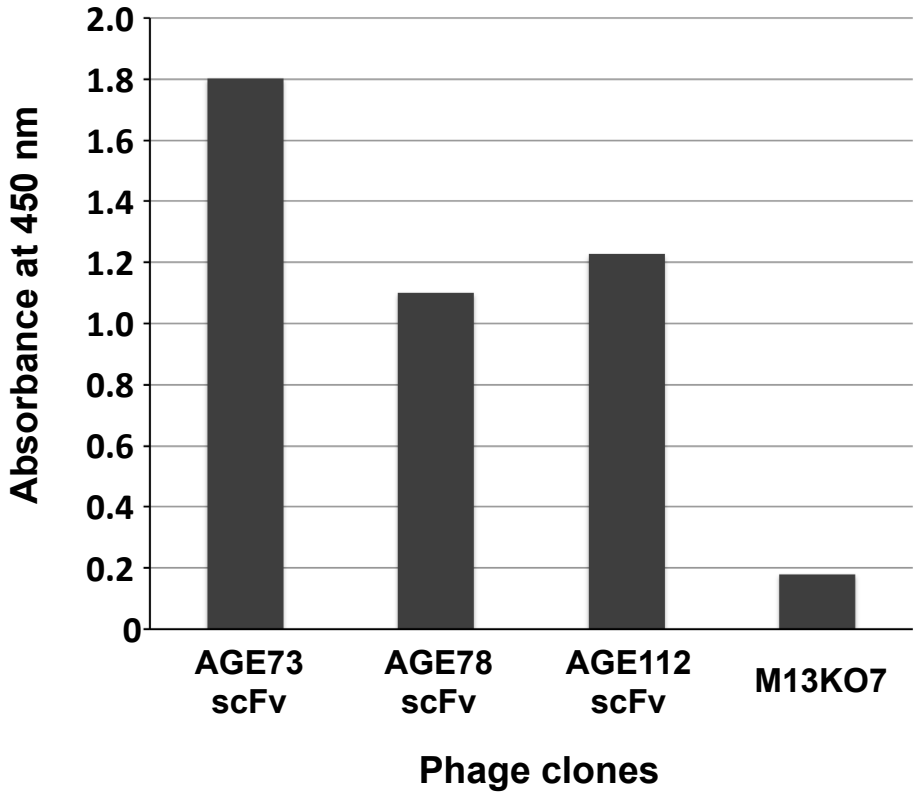


Figure S1. Binding of scFv-display phage clones from GA-KLH immunized mouse antibody library in phage ELISA to GA-BSA. M13KO7 helper phage was used as a control sample.

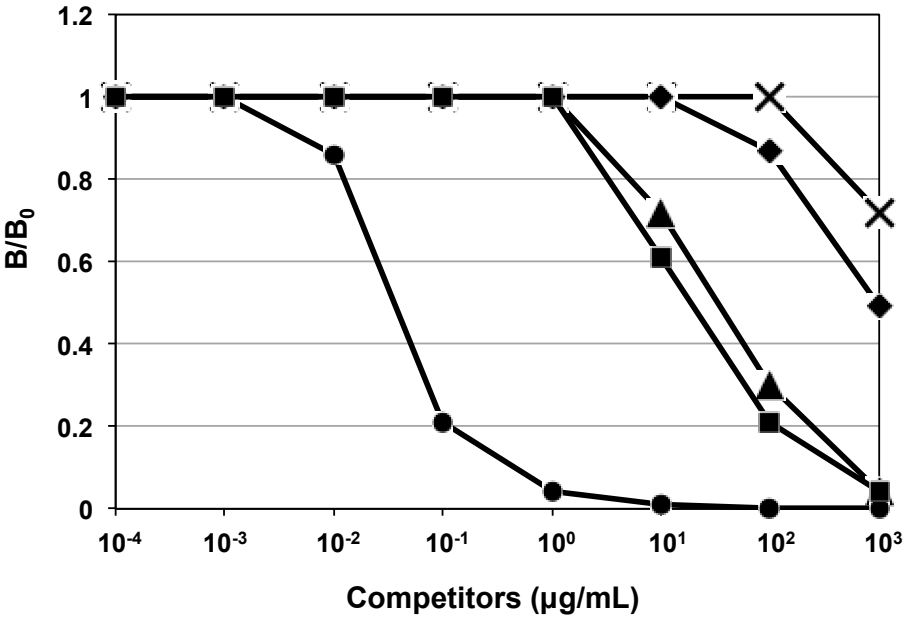


Figure S2. Immunoreactivity of AGE73scFv. GCA-BSA, Glc-BSA, MGO-BSA and GO-BSA were prepared in the similar way as described previously [21]. Competitive ELISAs were performed using the similar procedures described previously [21]. Reactivity of AGE73scFv to GA-BSA was analyzed using several AGE-modified BSAs as competitors. AGE-modified BSAs utilized are as follows: GA-BSA (circle), GCA-BSA (square), Glc-BSA (triangle), MGO-BSA (diamond) and GO-BSA (cross).

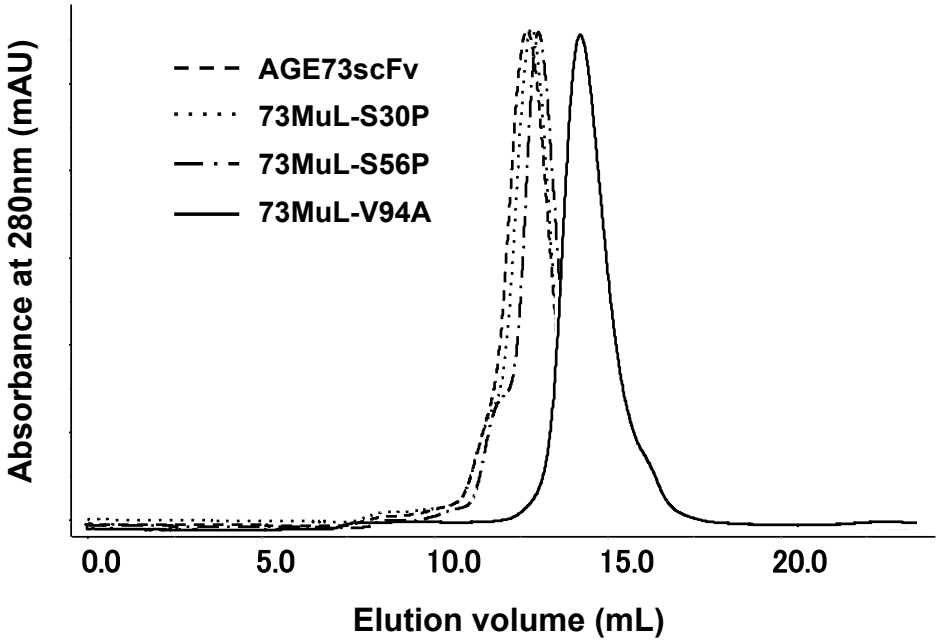
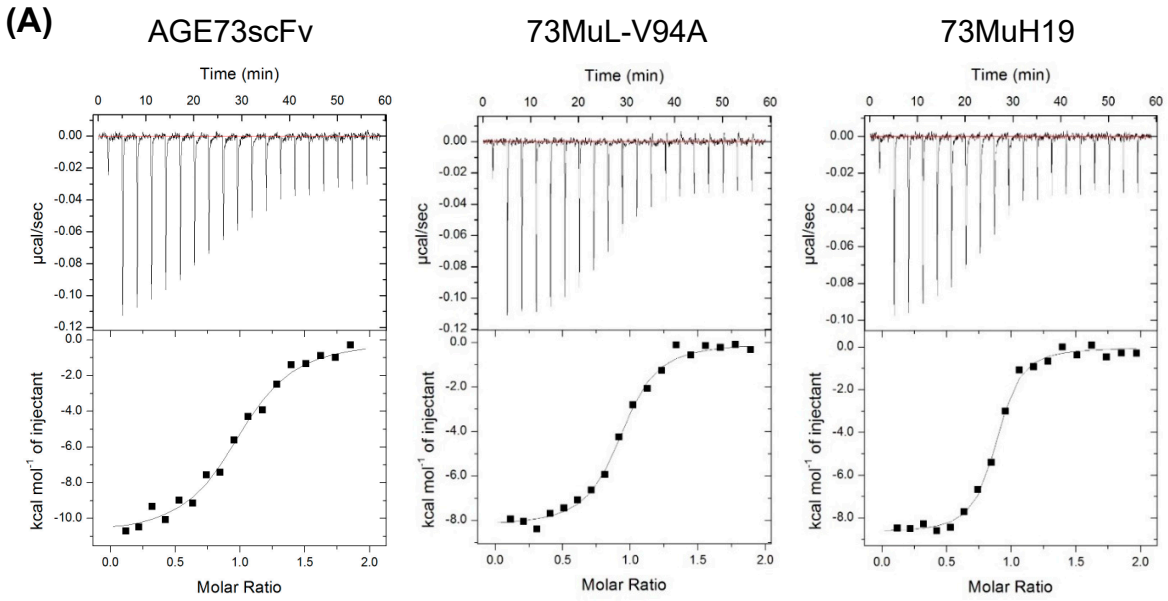


Figure S3. Gel filtration chromatography. The lines represent AGE73scFv (dashed line), 73MuL-S30P (dotted line), 73MuL-S56P (dashed and dotted line) and 73MuL-V94A (solid line), respectively.



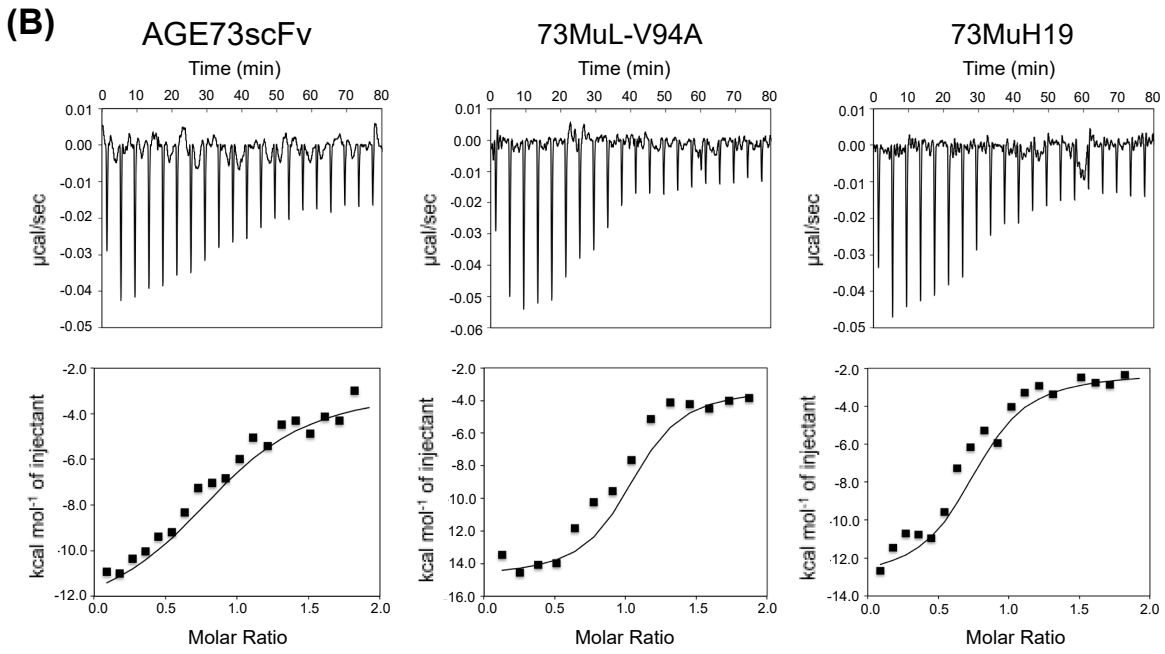


Figure S4. Titration calorimetry of the interactions between scFv and CBZ-GA-pyridine at 25 °C (A) and 37 °C (B). Top, typical calorimetric titration of scFv (5 µM) with CBZ-GA-pyridine (50 µM) in HBS-EP buffer; bottom, interaction plot of the data calculated from the raw data. The solid line corresponds to the best fitting curve using the one set of sites fitting model.

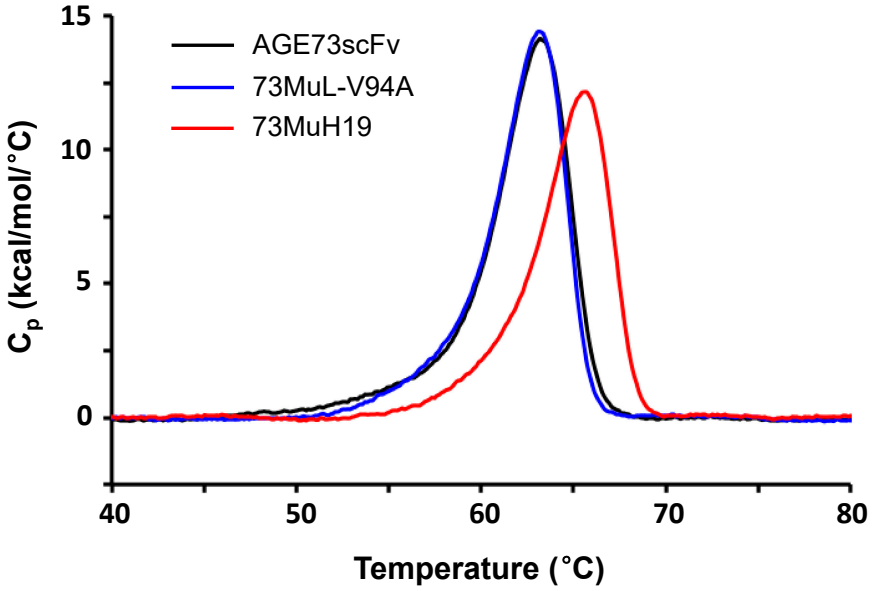


Figure S5. DSC thermogram showing T_m values of AGE73scFv (black), 73MuL-V94A (blue), and 73MuH19 (red). The scFv concentration was 0.5 mg/ml in PBS. The melting temperatures (T_m) were calculated by the non-two-state model.

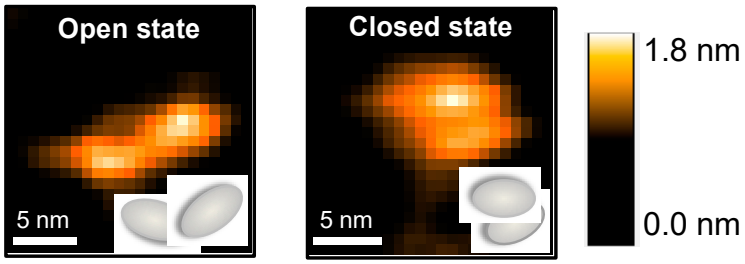


Figure S6. Representative snap shots of HS-AFM in the open (left panel) and closed (right panel) states of the scFv.

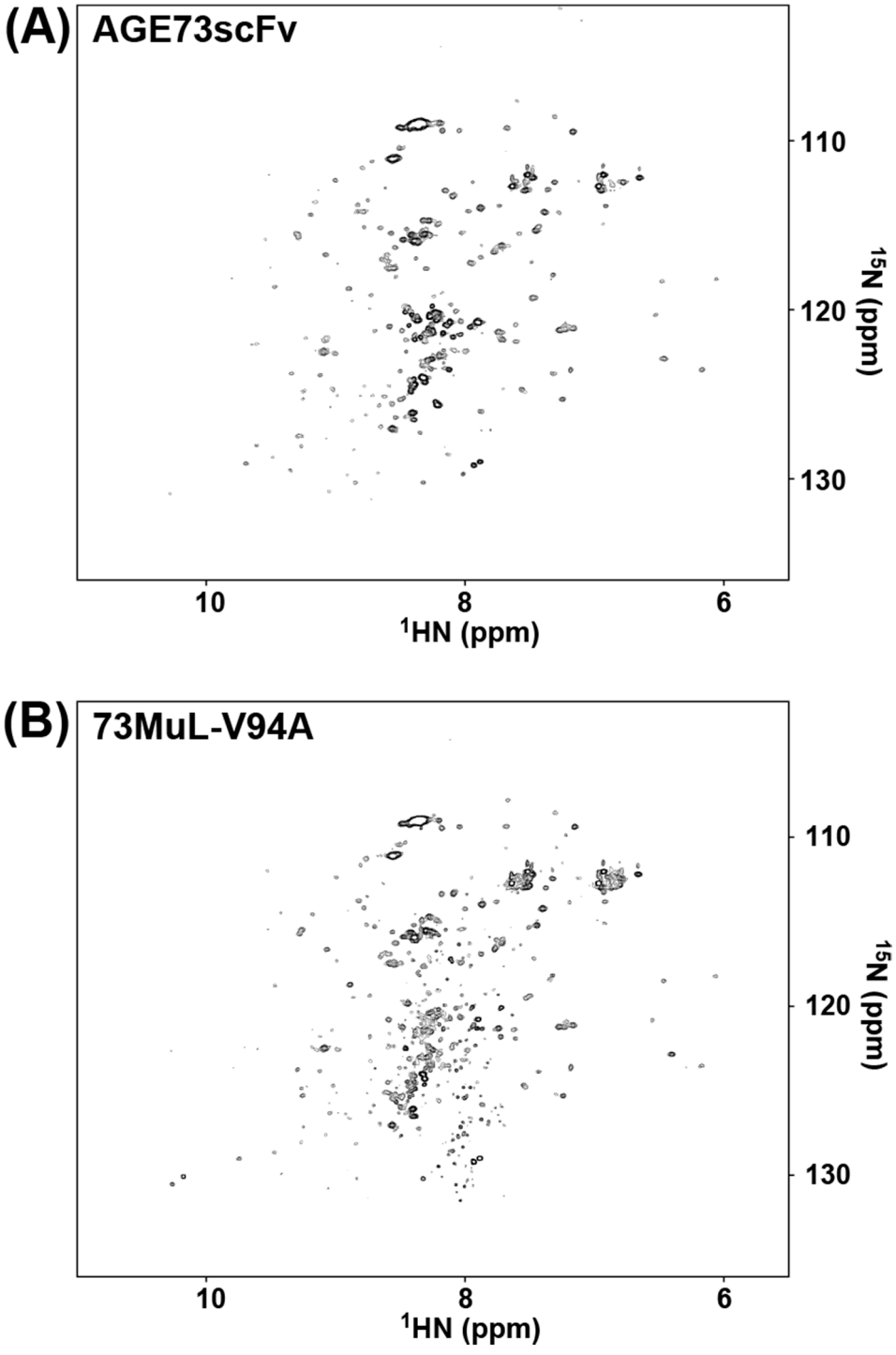


Figure S7. The ^{1}H-^{15}N HSQC spectra of AGE73scFv (A) and 73MuL-V94A (B) in the antigen free state at 37 °C.

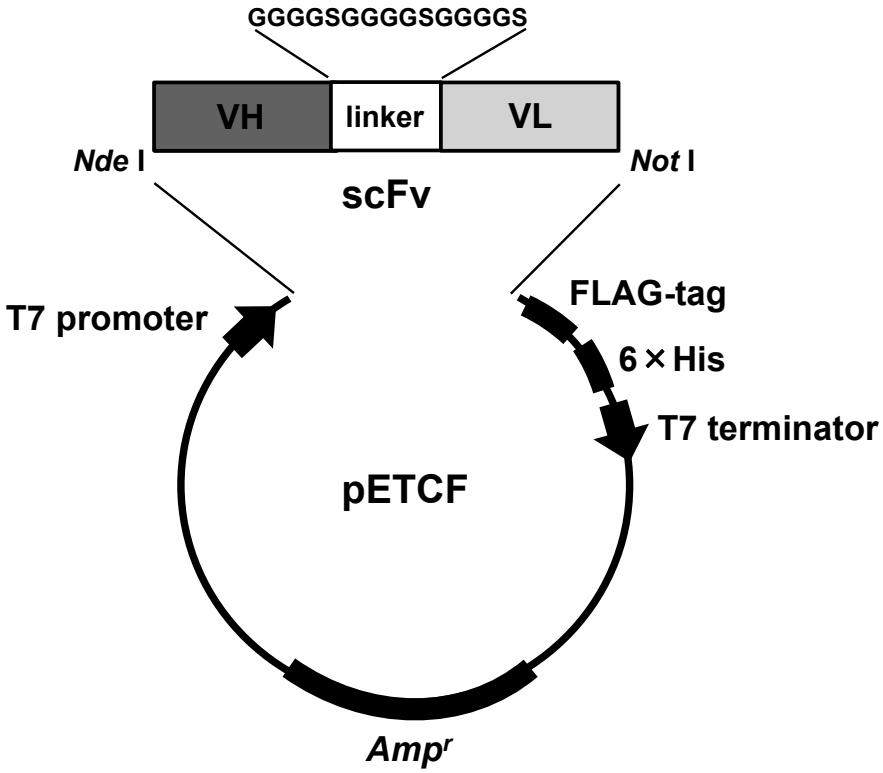


Figure S8. Expression vector of the single-chain Fv antibodies specific for GA-pyridine.