Figure S1. Pre-mutated and mutated amino acid sequences of human anti-TSLP-single-chain antibody variable fragment. M1, amino acid W mutated to R; M2, amino acid W mutated to K; M4, amino acid W mutated to L, M5, amino acid W mutated to E. The red boxes indicate the sites of the mutation.

Figure S2. Amplification of scFv and signal peptide (sp)-scFv-Fc by PCR. (A) Amplification of scFv by PCR (84, sequence prior to mutation; M4, mutated sequence). (B) Amplification of sp-scFv-Fc by PCR. scFv, single-chain antibody variable fragment.
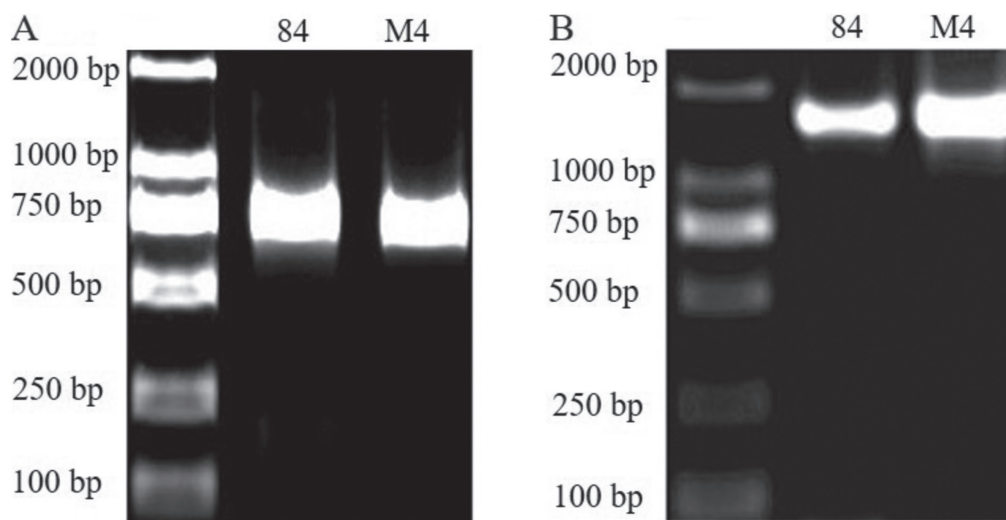
Figure S3. Construction of the PMH3^{EN}-sp-scFv-Fc-84/M4 recombinant plasmid. (A) The pcDNA3.1 vector was digested with *Hind*III and *Not*I, the products of sp-scFv-Fc-84 and M4 were detected by agarose gel electrophoresis. The mutated M4 gene were obtained from 84 gene. Each lane represents a different clone. (B) The sp-scFv-Fc-84 (pre-mutated sequence) and sp-scFV-Fc-M4 (mutated sequence) in pcDNA3.1 were amplified by PCR, digested with *Hind*III and *Not*I, and ligated using T4 ligase into PMH3^{EN} (digested with *Hind*III and *Not*I) to generate the PMH3^{EN}-sp-scFv-Fc recombinant vector. The scFv-84 and scFv-M4 products were detected by agarose gel electrophoresis. scFv, single-chain antibody variable fragment.
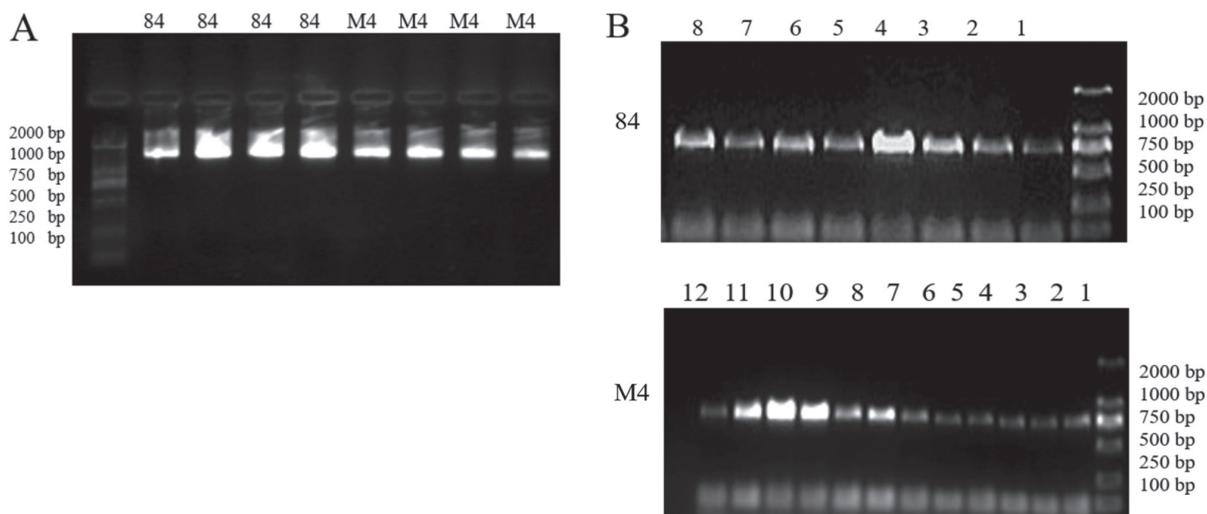
Table SI. Amino acids mutated to improve affinity of the single-chain antibody variable fragment.

Amino acid	Mutated amino acid	Mutated base sequence
M1, TRP (109)	ARG	CGU/CGC/CGA/CGG
M2, TRP (109)	LYS	AAA/AAG
M3, TRP (109)	TYR	UAU/UAC
M4, TRP (109)	LEU	CUU/CUC/CUA/CUG
M5, TRP (109)	GLU	GAA/GAG

109 is the amino acid number in the protein sequence and TRP is 109th amino acid in variable the region.

Table SII. Design of primers for single amino acid mutation of scFv.

Primer name	Primer sequence
M1	
scFv84MR1-TSLP	5'-TCAAAAGCCCGTTCCCCTCTCGCACAG-3'
scFv84MF1-TSLP	5'-GGAACGGGCTTTTGATATCTGGGGCCA-3'
M2	
scFv84MR2-TSLP	5'-ATCAAAAGCCTTTTCCCCTCTCGCACAG-3'
scFv84MF2-TSLP	5'-GGAAAAGGCTTTTGATATCTGGGGCCA-3'
M3	
scFv84MR3-TSLP	5'-TCAAAAGCGTATTCCCCTCTCGCACAG-3'
scFv84MF3-TSLP	5'-GGAATACGCTTTTGATATCTGGGGCCA-3'
M4	
scFv84MR4-TSLP	5'-TCAAAAGCCAGTTCCCCTCTCGCACAG-3'
scFv84MF4-TSLP	5'-GGAACTGGCTTTTGATATCTGGGGCCA-3'
M5	
scFv84MR5-TSLP	5'-TCAAAAGCCTCTTCCCCTCTCGCACAG-3'
scFv84MF5-TSLP	5'-GGAAGAGGCTTTTGATATCTGGGGCCA-3'
scFv84	
scFv84F-TSLP	5'-CATGCCATGGCCGGCCCAGCCGGCCC-3'
KM168	5'-CTGAGTAGAAGAACTCAAACTA-3'

F, forward; R, reverse; scFv, single-chain antibody variable fragment; TSLP, thymic stromal lymphopoietin; KM168, reverse primer of vector *pLZ16*.