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.

Range 1: 1 to 248 Graphics	Vext Match 🔺 Previo	Range 1: 1 to 2	248 Graphics	🔻 Next Match 🔺 Previous Match
Score Expect Method Identities	Positives Gaps	Score	Expect Method	Identities Positives Gaps
496 bits(1278) 0.0 Compositional matrix adjust. 247/248(99%) 2	247/248(99%) 0/248(0%)	497 bits(1279)) 0.0 Compositional matrix adjust.	247/248(99%) 247/248(99%) 0/248(0%)
Query 1 ***********************************	60	Query 1 ****	*****	****************** 60
Sbjot 1 ***********************************	60	Sbjot 1 ****	*****	******************* 60
Query 61 ***********************************	120	Query 61 ****	*****	WARDINGQGINVIV 120
Sbjot 61 ***********************************	120	Sbjet 61 ****	******	KAPDINGQGINVIV 120
Query 121 SSGGGGSGGGGGGGGGS*********************	180	Query 121 SSGG	905506065660651++++++++++++++++++++++++++	************** 180
Sbjot 121 SSGGGSGGGGSGGGGS	180	Sbjet 121 SSGG	700506065666651 266566665666651	***************************************
Query 181 **********************************	240	Query 181 ****	*****	***************************************
Sbjot 181 **********************************	240	Sbjet 181 ****	*****	***************************************
Query 241 LEIKRAAA 248		Query 241 LEIK	TRAAA 248	
Sbjot 241 LEIKRAAA 248 M1		Sbjet 241 LEIK	ikaaa Ikaaa 248	M2
Range 1: 1 to 248 Graphics	🔻 Next Match 🔺 Previo	Range 1: 1 to 2	248 Graphics	Vext Match 🔺 Previous Match
Range 1: 1 to 248 Graphics Score Expect Method Identities Po	Vext Match A Previo Ositives Gaps	Range 1: 1 to 2 Score	248 <u>Graphics</u> Expect Method 1	Next Match Previous Match Identities Positives Gaps
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Expect Method Identities Pr 497 bits(1279) 0.0 Compositional matrix adjust. 247/248(99%) 24 Query 1 ************************************	Vext Match A Previo vositives Gaps 47/248(99%) 0/248(0%)	Range 1: 1 to 2 Score 497 bits(1280) Query 1 *****	248 Graphics Expect Method 1 0.0 Compositional matrix adjust. 2	Vext Match & Previous Match Identities Positives Gaps 247/248(99%) 247/248(99%) 0/248(0%)
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Range 1: 1 to 248 Graphics Score Expect Method Identities Prevent Method 497 bits(1279) 0.0 Compositional matrix adjust. 247/248(99%) 24 Query 1 ************************************	▼ Next Match ▲ Previo Jositives Gaps 47/248(99%) 0/248(0%) 0 0 0 0 0 0 0 0 00 0 00 0 00 0 00 0 00 0 00 0	Range 1: 1 to 7 Score 497 bits(1280) Query 1 \$bjot 1 ##### Query 61 ##### Query 121 \$Sbjot 12 \$Sbjot 13 ##### Query 14 \$Sbjot 13 ##### Query 14 \$Sbjot 13 \$Sbjot 14 \$Sbjot 15 \$Sbjot 16 \$Sbjot 121 \$SS99 \$Sbjot 121	248 <u>Graphics</u> Expect Method I 0.0 Compositional matrix adjust. 2	Vext Match Previous Match Identities Positives Gaps 247/248(99%) 247/248(99%) 0/248(0%) ********** 60 ********** 60 ********** 120 ************ 180
Range 1: 1 to 248 Graphics Score Expect Method Identities P4 497 bits(1279) 0.0 Compositional matrix adjust. 247/248(99%) 24 Query 1 ************************************	▼ Next Match ▲ Previo vositives Gaps 47/248(99%) 0/248(0%) 0 0	Range 1: 1 to 7 Score 497 bits(1280) Query 1 \$bjot 1 ##### Query 61 \$bjot 61 ##### Query 121 \$SS99 \$bjot 121 \$SS99 \$bjot 121 \$SS99 \$bjot 131	248 Graphics Expect Method I 0.0 Compositional matrix adjust. 2	V Next Match Previous Match Identities Positives Gaps 247/248(99%) 247/248(99%) 0/248(0%) *********** 60 60 ************ 60 7 ************ 80 7 ************** 80 7 ************ 120 7 ******************* 180 ************************* ************************** 240
Range 1: 1 to 248 Graphics Score Expect Method Identities P. 497 bits(1279) 0.0 Compositional matrix adjust. 247/248(99%) 24 Query 1 ************************************	▼ Next Match ▲ Previo Iositives Gaps 47/248(99%) 0/248(0%) 0 0	Range 1: 1 to 7 Score 497 bits(1280) Query 1 Sbjet 1 ****** Query 61 ****** Query 121 Sbjet 61 ****** Query 121 Sbjet 121 SS09 Query 181 ******	248 <u>Graphics</u> Expect Method I 1.0.0 Compositional matrix adjust. 2	Next Match Previous Match Identities Positives Gaps 247/248(99%) 247/248(99%) 0/248(0%) *********** 60 *********** 60 *********** 60 *********** 100 ********************* 180 **************************** 240
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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*.