Supplementary Material

Materials and methods

PCR amplification and construction of expression vector

To obtain the anti-CIT scFv fragments, the hybridoma cells (about 2×10^6) secreting IgG antibody against CIT were used to isolate the total RNA using Trizol method. First-strand cDNA was synthesized by reverse transcription PCR (RT-PCR) with reverse transcriptase and random hexadeoxyribo-nucleotide primers, using the isolated mRNA as a template. The coding sequences for the variable regions of the heavy chain (V_H) and light chain (V_L) were then amplified from the first-strand cDNA through primary PCR amplification as described *(25)*. A specific linker DNA fragment encoding a short flexible peptide (Gly4Ser)3 was used to assemble scFv gene fragments by SOE-PCR (molecular ratio of V_L to V_H to linker DNA is 3: 3: 1). The assembled scFv fragment was digested with restriction endonuclease *Eco*R I and *Hind* III and cloned into the same digested pBD-*his6-mbp-linker* expression vector, and then the ligated product was transformed into *E. coli* BL21 (DE3) by electroporation. The resulted recombinant vectors were identified by PCR and DNA sequence.

Expression and purification of wild type scFv

To obtain the soluble scFv antibody, the culture was incubated in 100 mL fresh LB-medium containing 50 μg/mL kanamycin and grown at 37℃ with shaking until OD_{600}=0.6~0.8, and the target protein was expressed with IPTG (0.5 mM) induction. The expressed product form the strain pBD-*his6-mbp-linker -scFv/*BL21 was defined as MBP-Linker-scFv. Subsequently, the expressed protein was purified by Ni^{2+} affinity

chromatography. At last, the expressed and purified proteins were visualized by SDS-PAGE using 12% polyacrylamide gels, and the concentration of protein was detected by Nano-Drop 2000/2000c spectrophotometer for further study.

Results

ScFv assembling and expression

Total RNA extracted from hybridoma cells were used as the template to synthesis the first chain cDNA by RT-PCR, and the V_H and V_L genes of IgG were amplified, respectively. As shown in **Fig. S1**, the length of amplified V_H and V_L DNA fragments were 372 bp and 321 bp, respectively (**Fig. S1A**), and a scFv band (about 750 bp) were assembled and amplified successfully by SOE-PCR (**Fig. S1B**). To obtain the soluble scFv protein, the resulted scFv fragment was digested and cloned into the expression vector pBD-his6-*mbp-linker* to form MBP-linker-scFv fusion protein. After being expressed successfully, the target MBP-linker-scFv was purified and displayed a single band with an expected molecular size of approximately 70 kDa on SDS-PAGE (**Fig. S1C**).

Legends

Table S1 Primers used for Ala and Lys mutaion in HCDR3 and LFR1.

Target gene and primer	DNA sequence(5'-3')
H-P/A-F	TTACTGTGCAAGAGAGGGGGGATGGTTACTAC
H-P/A-R	CCTCTCTTGCACAGTAATAGACCGCAGAGTCCTCA
L-T/A-F	AGCAATCGCGTCTGCATCTCCAGGGGAGAAGG
L-T/A-R	ATGCAGACGCGATTGCTGGAGACTGGGTGAGC
H-P/K-F	ACTGTAAAAGAGAGGGGGGATGGTTACTACGTT
H-P/K-R	CCCCCTCTCTTTTACAGTAATAGACCGCAGAGTCCTCA
L-T/K-F	CAGCAATCAAGTCTGCATCTCCAGGGGAGAAG
L-T/K-R	TGCAGACTTGATTGCTGGAGACTGGGTGAGCT

Fig. S1 Expression and purification of wild type scFv. **A**: PCR products of V_H and V_L DNA fragment. Lane M: the marker DL-2000, Lane 1: PCR products of V_H gene, Lane 2: PCR products of V_L gene. **B**: the assembling scFv fragment. Lane M: the marker DL-2000, Lane 1-2: PCR products of the assembling scFv. **C**: SDS-PAGE analysis of the soluble scFv antibodies. Lane M: low molecular weight protein marker; Lane 1: negative control (empty vector), Lane 2: the expressed product of MBP-linker-scFv; Lane 3-4: the purified MBP-linker-scFv by Ni^{2+} affinity chromatography.

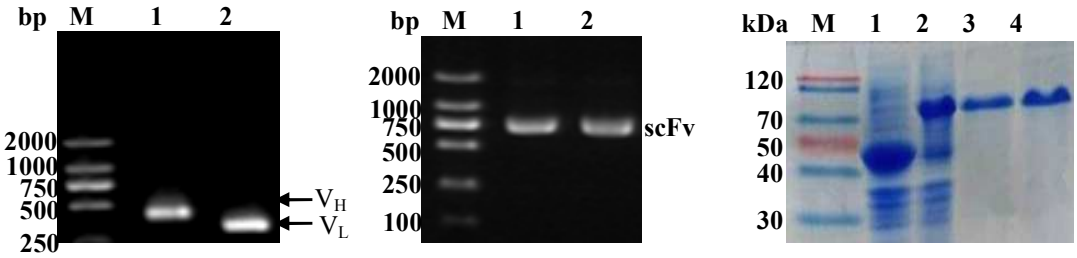

Fig. S1