

Optimized Expression and Characterization of a Novel Fully Human Bispecific Single-Chain Diabody Targeting Vascular Endothelial Growth Factor165 and Programmed Death-1 in *Pichia pastoris* and Evaluation of Antitumor Activity In Vivo

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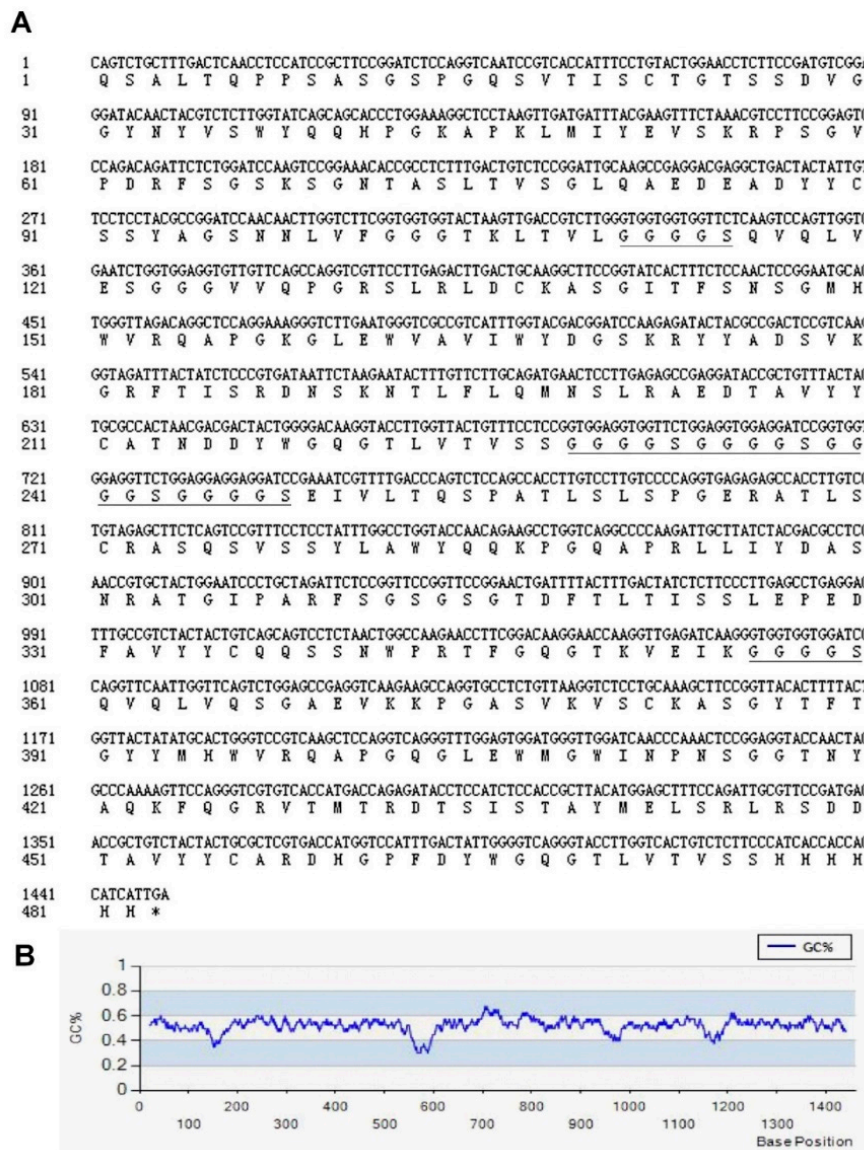


Figure S1. Optimization of recombinant BsDb gene fragment. (A) The amino acid sequence and optimized nucleotide sequence of this BsDb. Underline: (G4S)_n linker, *: stop codon. (B) G+C content analysis of this BsDb gene fragment after optimization. GC% was approximately 52.09%.

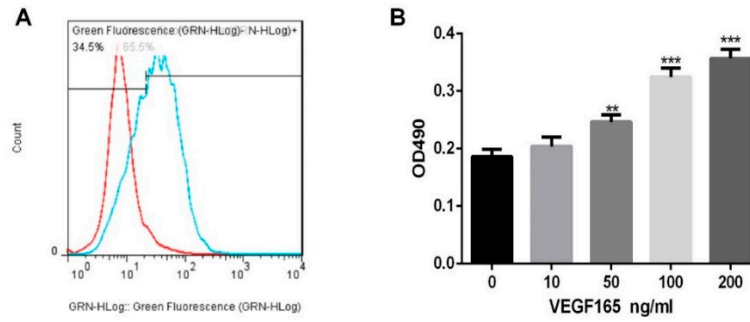


Figure S2. Identification of primary HUVECs. (A) VEGFR2 expression on primary HUVECs was detected using mouse anti-human VEGFR2 antibody (Sino Biological Inc., Beijing, China) and FITC-conjugated Secondary antibody (CW biotech, Beijing, China) by FCM. FCM analysis indicated VEGFR2 expressed on primary HUVECs with high levels. (B) VEGF165-induced proliferation of primary HUVECs was detected by MTT assay. The experiment was done in triplicate, and the mean values \pm SD were presented. * $p < 0.05$, ** $p < 0.01$, two-tailed students t-test, versus control group.

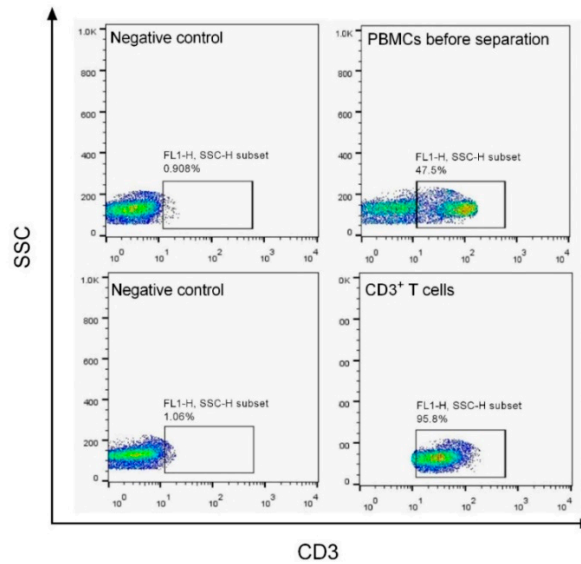


Figure S3. Analysis of T cells isolated from PBMCs. T cells were isolated from PBMCs using CD3 microbeads (Miltenyi, Cologne, Germany) by MACS. Purity of T cells was analyzed by FCM using a FITC-conjugated CD3 antibody (Sino Biological Inc., Beijing, China). Purity of T cells before and after separation were approximately 47.5% and 95.8%, respectively.

Table S1. Primers for PCR amplification of recombinant BsDb and colony PCR.

primer 1	5'-GGAATTCAGTCTGCTTTGACTCAA-3'
primer 2	5'-GCTCTAGATCAAT GATGGTGGTGGTGA-3'
AOX1-F	5'-GACTGGTTCCAATTGACAAGC-3'
AOX1-R	5'-GCAAATGGCATTCTGACATCC-3'

Underline: restriction sites.