

Supplementary Material for

Establishment of Nectin-4 VHH-based CAR-T cells and evaluation to specific killing of bladder cancer cells *in vitro*

Fig. S1 Double digestion of plasmids pCDH-VHH-CAR-GFP and pCDH-scFv-CAR-GFP.

Fig. S2 The expression of the surface antigen Nectin-4 was detected by flow cytometry in different cancer cells. SW780, RT4, 5637, human cerebral astrocytoma cells U87-MG, human gastric cancer cells HGC-27, human breast cancer cells MB231, human colon cancer cells SW620, human ovarian cancer cells ES-2, human ovarian cancer cells SKOV-3, and human osteosarcoma cells HOS.

Figure. S3 Gating information of figure 2B

Fig. S4 Generation of VHH-CAR^{Anti-Nectin4}(HA tag)-T cells. (A) Schematic diagram of VHH-CAR Anti-Nectin4 (HA tag), including the CD8 signal peptide (SP), VHH, HA tag, CD8 α hinge, CD28TM, CD28, and CD3 ζ domain. (B) The positive rates of GFP and HA in the T cells detected by FCM.

Fig. S5 The expression of Nectin-4 on the tumor cells surface detected by FCM. SW780, RT4 and U87-MG cells were staining with or without anti-Nectin-4 antibody, then the expression level of Nectin4 on the surface of cancer cells were detected by FCM

Table S1 The titer of Lentivirus

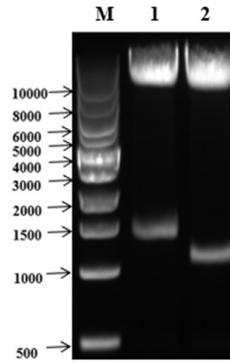


Fig. S1 Double digestion of plasmids pCDH-VHH-CAR-GFP and pCDH-scFv-CAR-GFP LaneM: 1000 bp marker, Lane1: Digestion products of pCDH-VHH-CAR-GFP, Lane2: Digestion products of pCDH-scFv-CAR-GFP

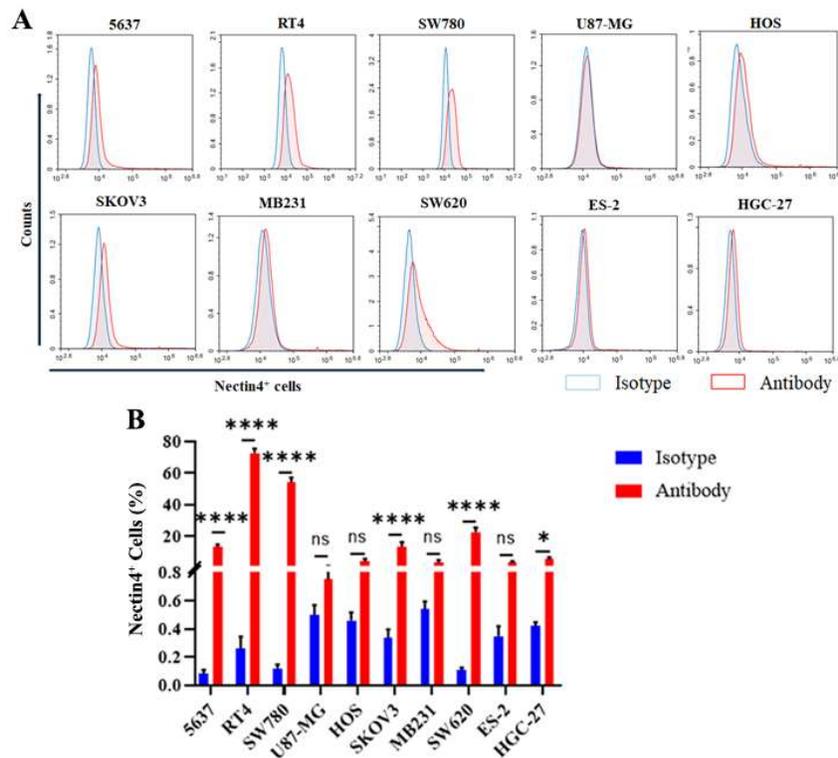


Fig. S2 The expression of Nectin-4 on the tumor cells surface were detected by FCM in different cancer cells. (B) The proportion of Nectin-4 of different cancer cells. (mean \pm SEM; ns, not significant, * $p < 0.05$, **** $p < 0.0001$)

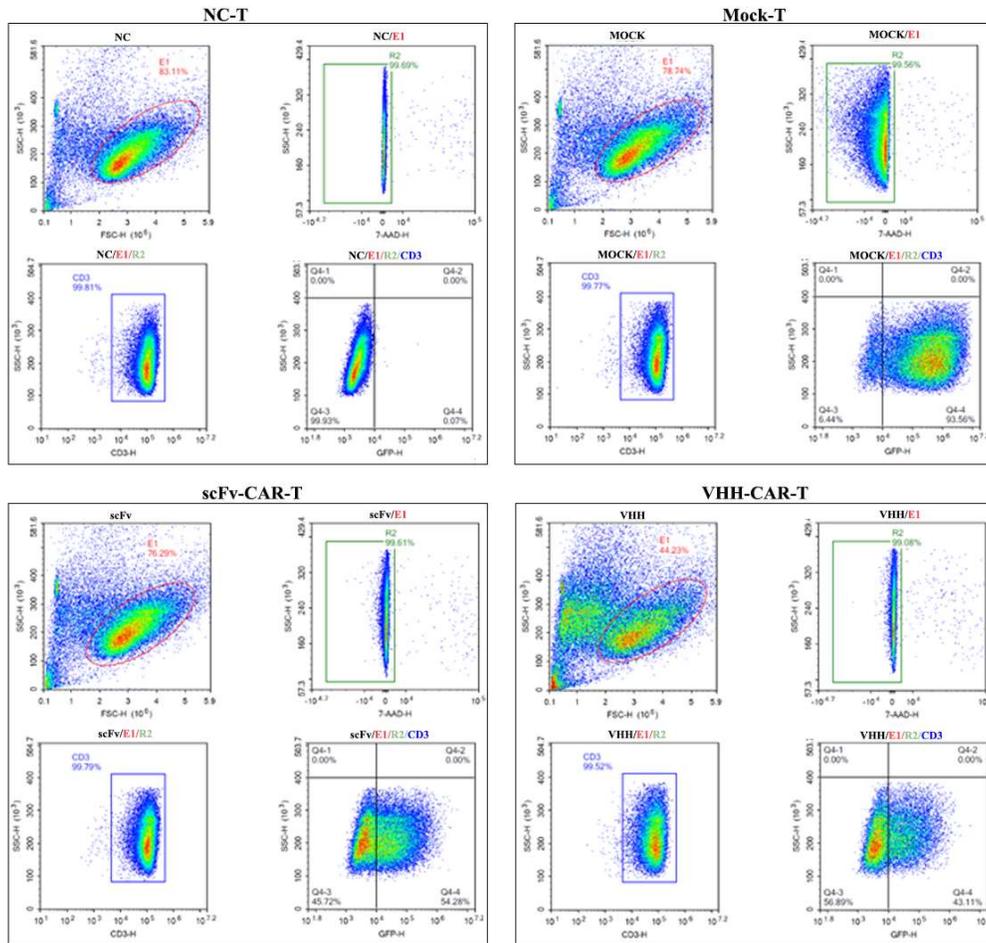


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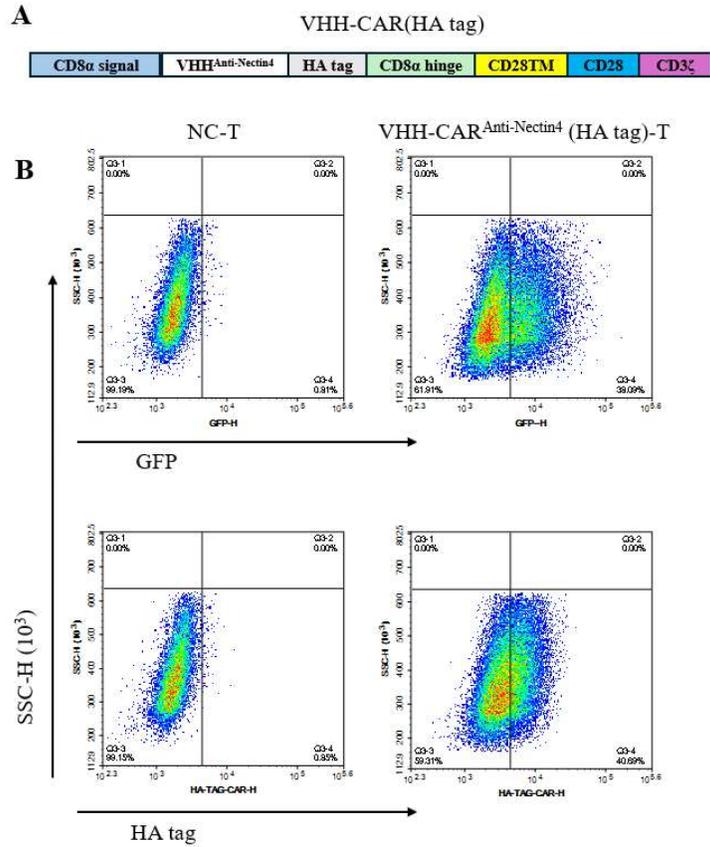


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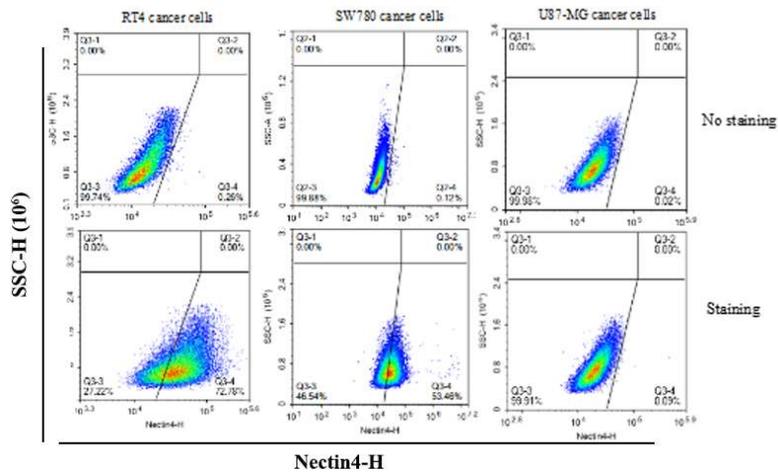


Fig. S5 The expression of Nectin-4 on the tumor cells surface detected by FCM.

Table S1 The titer of Lentivirus

Group	virus titer (TU/mL)
Mock	6.43×10^9
scFv-CAR	4.10×10^9
VHH-CAR	1.62×10^9