Supporting Information for

Original article

A novel shark VNAR antibody-based immunotoxin targeting TROP-2 for cancer therapy

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Running title: A novel shark VNAR antibody-based immunotoxin targeting TROP-2 for cancer therapy

Table S1. The binding avidity of VNARs against the TROP2 ECD. The K_D values were measured on Octet using one concentration (100 nmol/L) of the VNAR protein.

Sample ID	Conc. (nmol/L)	Response	K_D (mol/L)	K_D Error	K_{on} (1/Ms)	k_{on} Error	K_{dis} (1/s)	k_{dis} Error	RMax	R_{Max} error	K_{obs} (1/s)
5G8	100	0.1295	8.73E-09	6.45E-10	6.57E+04	2.87E+03	5.74E-04	3.42E-05	0.2496	0.008	7.15E-03
4G3	100	0.1548	1.27E-07	1.77E-08	2.45E+04	3.40E+03	3.10E-03	4.95E-05	0.7896	0.0986	5.55E-03
5C10	100	0.1008	1.51E-09	8.41E-10	6.86E+04	4.96E+03	1.04E-04	5.72E-05	0.1972	0.0103	6.96E-03
5E9	100	0.021	2.30E-09	2.59E-09	3.09E+07	3.47E+07	7.09E-02	5.65E-03	0.0197	0.0006	3.16E+00
4B6	100	0.3787	5.23E-08	2.02E-09	4.14E+04	1.54E+03	2.16E-03	2.08E-05	1.1084	0.0345	6.30E-03
5D2	100	0.5851	2.59E-08	6.80E-10	8.72E+04	2.06E+03	2.25E-03	2.60E-05	1.0135	0.0165	1.10E-02

Table S2. List of the new interacting residue pairs established at the TROP-2-ECD interface upon VNAR-5G8 anchoring.

No.	Residue Pair	$\Delta\Delta G$ (kcal/mol)
1	R52|E59	–10.20 ± 4.22
2	R56|E98	–11.23 ± 1.89
3	D65|S90	–3.58 ± 1.06
4	T70|T92	–2.66 ± 0.38
5	S71|T92	–3.29 ± 0.65
6	L74|W91	–2.04 ± 0.37
7	P110|L97	–3.33 ± 0.44
8	P110|W91	–2.84 ± 0.36
9	P110|P96	–2.03 ± 0.30
10	E111|P96	–2.44 ± 0.48
11	K212|N93	–2.78 ± 1.37
12	G215|N93	–2.39 ± 0.88

Only the residue pairs having the difference in pairwise binding energies ($\Delta\Delta G$) lower than 2.0 kcal/mol are listed.

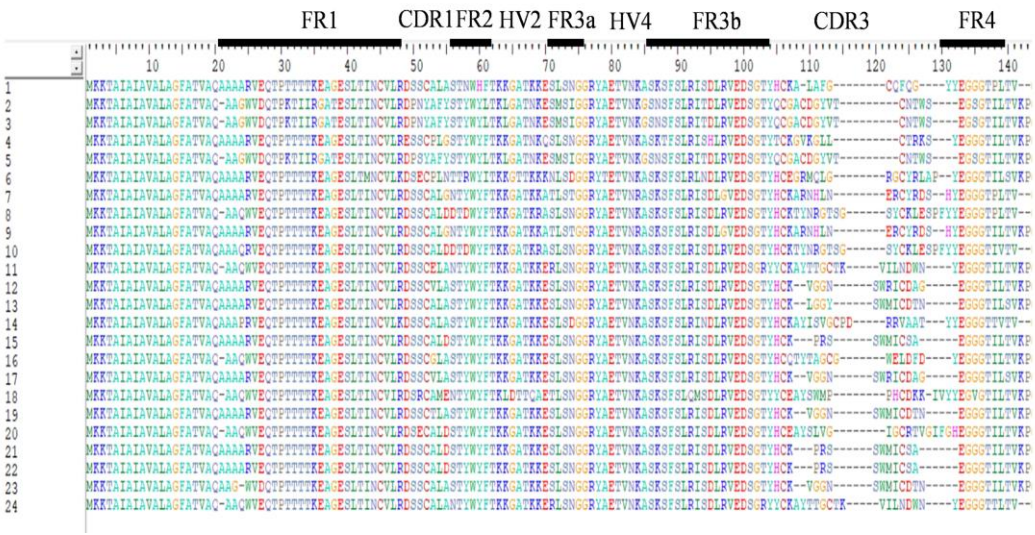

Figure S1 Sequencing results of 24 monoclonal clones selected from the phage display library. The sequence structure of VNAR mainly includes three parts: framework region, hypervariable region and complementarity determining region. The framework region consists of FR1, FR2, FR3a, FR3b and FR4; the hypervariable region consists of HV2 and HV4; the complementarity determining region only has CDR1 and CDR3. Due to somatic mutations, shark VNAR does not have the CDR2 region.

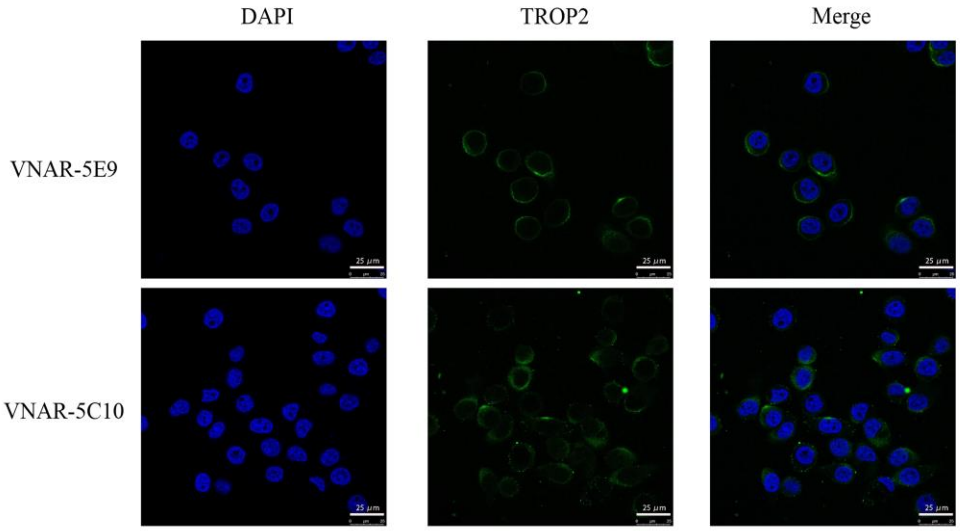

Figure S2 Confocal immunofluorescence images of MDA-MB-468 cells stained by the VNAR 5E9, and 5C10. After VNAR-5E9 and VNAR-5E10 were combined with MDA-MB-468 respectively, TROP2 (green) and cell nuclei (blue) localized by VNAR in cells were determined by immunofluorescence staining. The experiment was performed three times. (Scale bar = 25 μm).

>VNAR-5G8
QWVEQTPTTTTKEAGESLTINCVLRDSSCPLASTYWYFTKKGATKKESL
SNGGRYAETVNKASKSFSLRISDLRVEDSGTYHCKAVNSWTNCAPLERY
YEGGGTILSVKPAA

Figure S3 Amino acid sequence of VNAR-5G8.

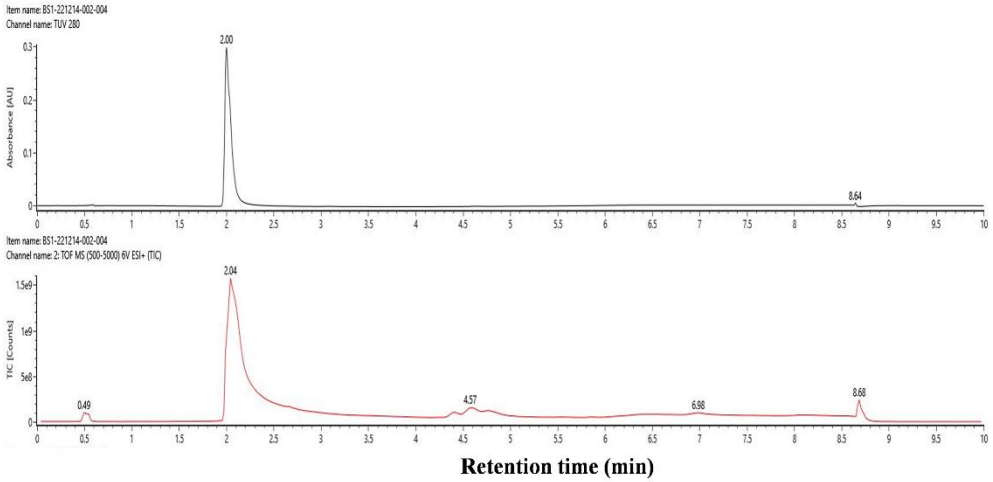

Figure S4 UV and TIC plots of VNAR 5G8. The UV spectrum shows that the VNAR-5G8 protein can present a single peak shape and has high purity; the TIC spectrum also shows a single peak shape of the VNAR-5G8 protein. Although trace amounts of impurities are also detected, they do not affect the functional experiments of the protein.

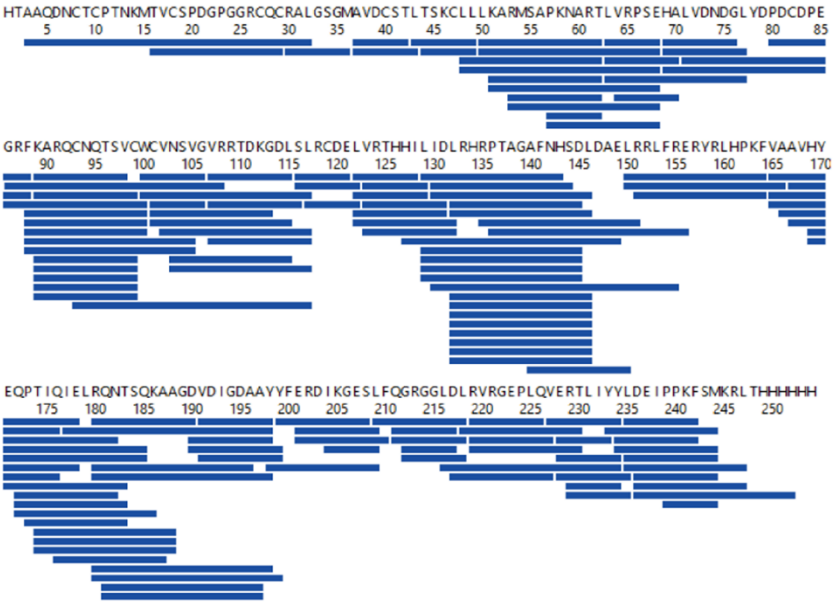

Figure S5 The peptic fragments coverage map of TROP-2 showed the quality of the HDX-MS data. The HDX-MS spectrum shows that after the TROP2-ECD protein is digested by protease, peptide fragments in the protein can be detected, and the peptide coverage rate reaches 98%.

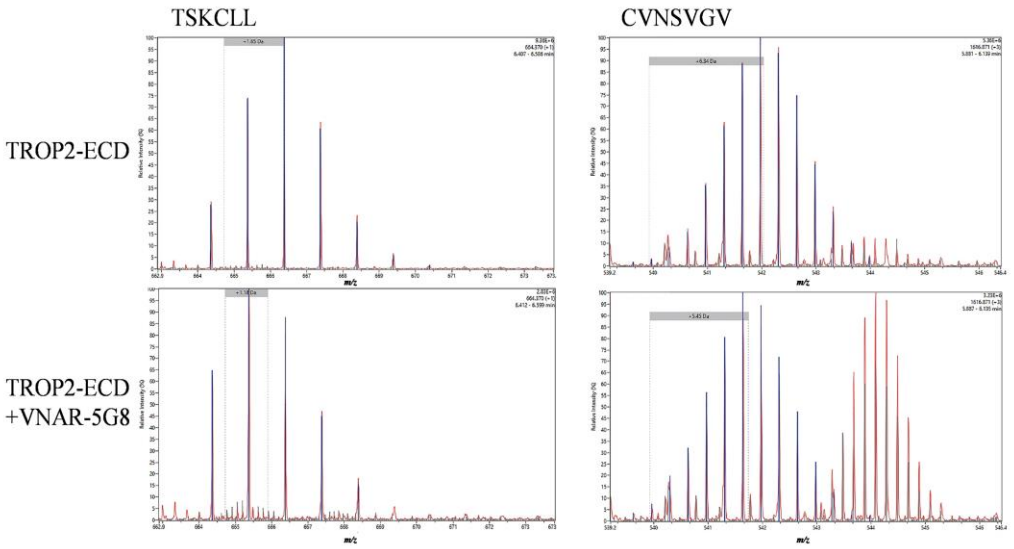

Figure S6 Two representative peptides in the regions with the most significant difference in relative fractional uptake of deuterium between TROP-2 and TROP-2+5G8. There are obvious differences in the hydrogen-deuterium exchange signals of the two peptides of TSKCLL and CVNSVGV in the TROP2-ECD protein.

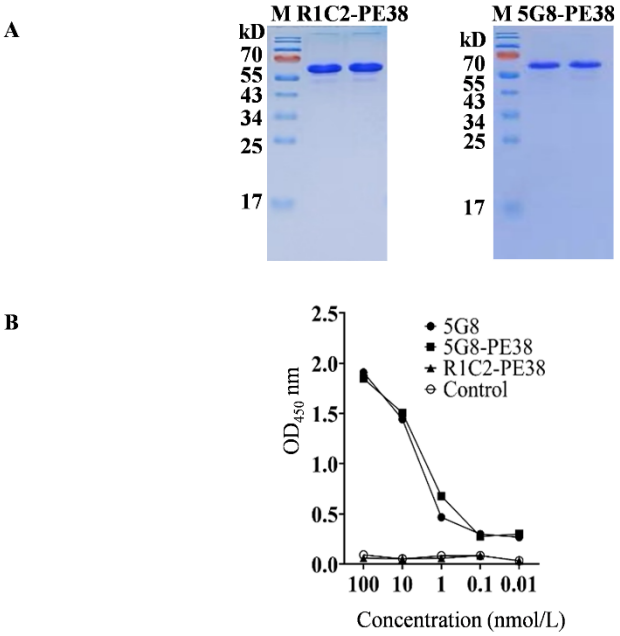

Figure S7 Generation and characterization of 5G8-PE38 with affinities for TROP2. (A) SDS-PAGE analysis of the constructed bands of VNAR-PE38 fused toxin. (B) Concentration-dependent binding activities of 5G8-PE38 to TROP2-ECD, as determined by ELISA (mean ± SD, n = 3 in all data).

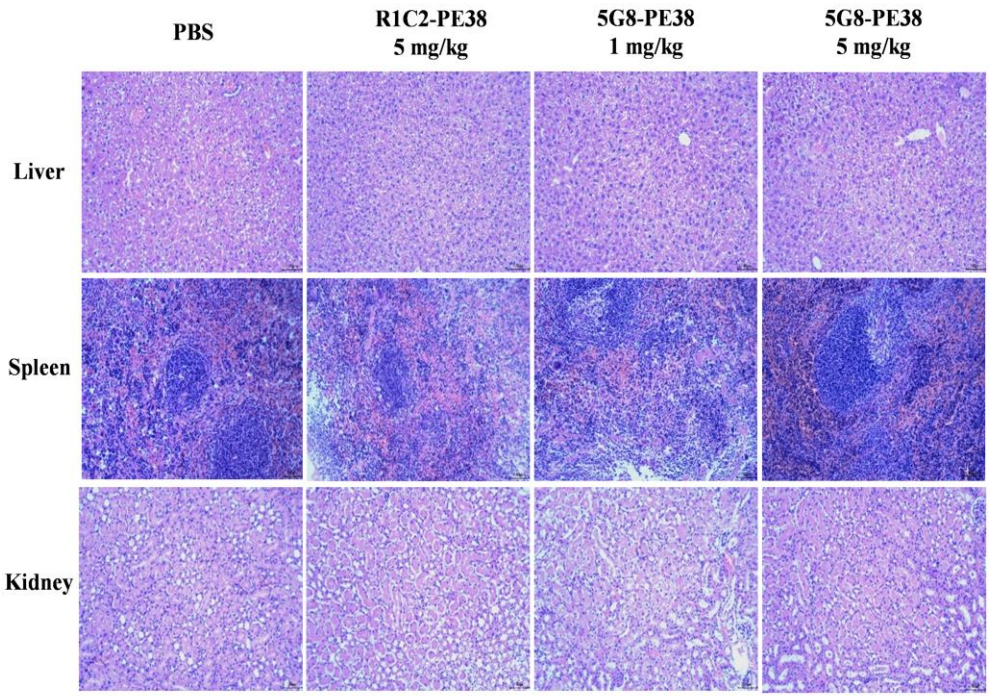

Figure S8 *In vivo* safety profile of 5G8-PE38. H&E staining analysis of tissue sections of major organs (liver, spleen and kidney) of tumor model nude mice, there are not obvious pathological damage was found in the liver, spleen, and kidneys of nude mice (scale bar = 100 μm).