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Supplemental information

Structural insights into the *cis* and *trans* assembly of human trophoblast cell surface antigen 2

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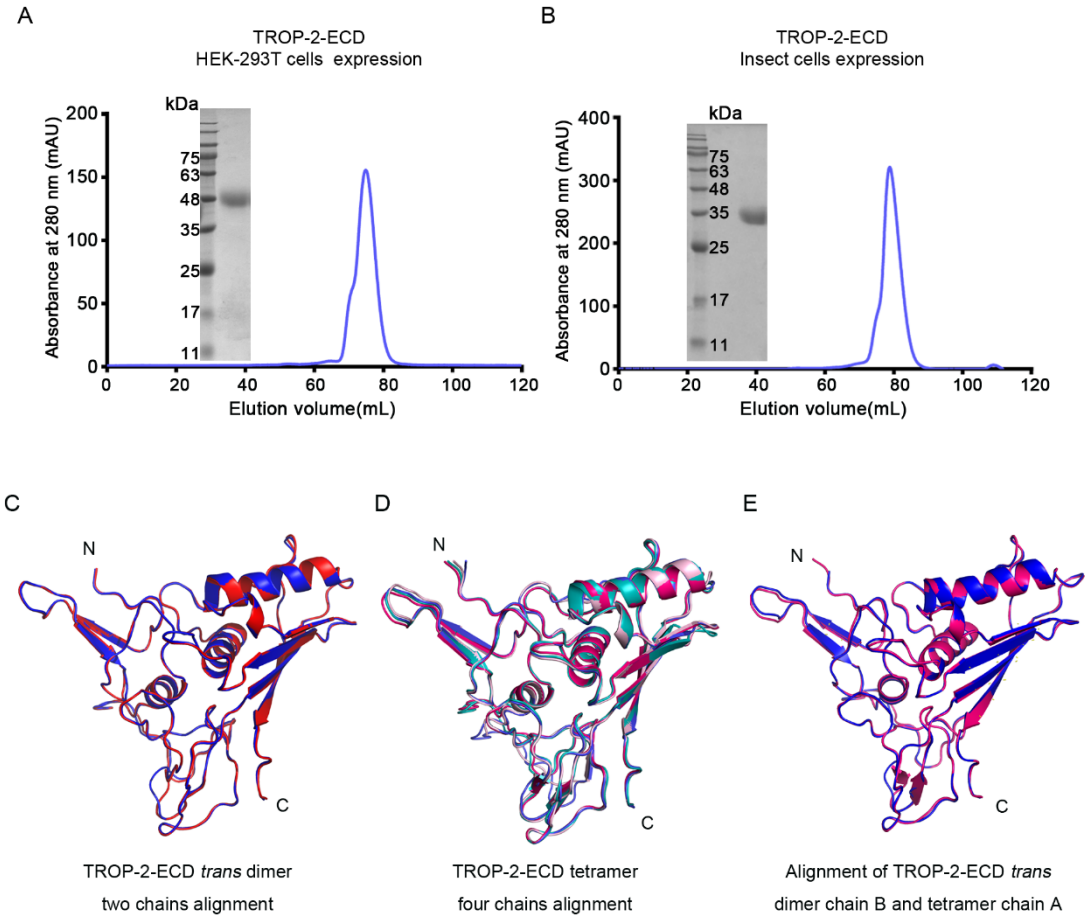

Figure S1. Biophysical characterization of the TROP-2-ECD and comparison of the structural differences of the TROP-2-ECD subunits, related to Figures 1, 2 and 3

(A-B) Size exclusion analysis of TROP-2-ECD proteins with Hiload Superdex 200 16/600 pg column. The 280-nm absorbance curve and the SDS-PAGE migration profile of the pooled sample are shown. (A) TROP-2-ECD proteins expressed from 293T cells. (B) TROP-2-ECD proteins expressed from insect cells. (C) Structural alignment of two protomers in TROP-2-ECD *trans*-dimer, with RMSD at 0.202. (D) Structural alignment of the four protomers in TROP-2-ECD tetramer, with RMSD between any of the two protomers ranged from 0.255 to 0.390. (E) Structural alignment between one subunit (chain B) of *trans*-dimer and one unit (chain A) of the tetramer, with RMSD at 0.232.

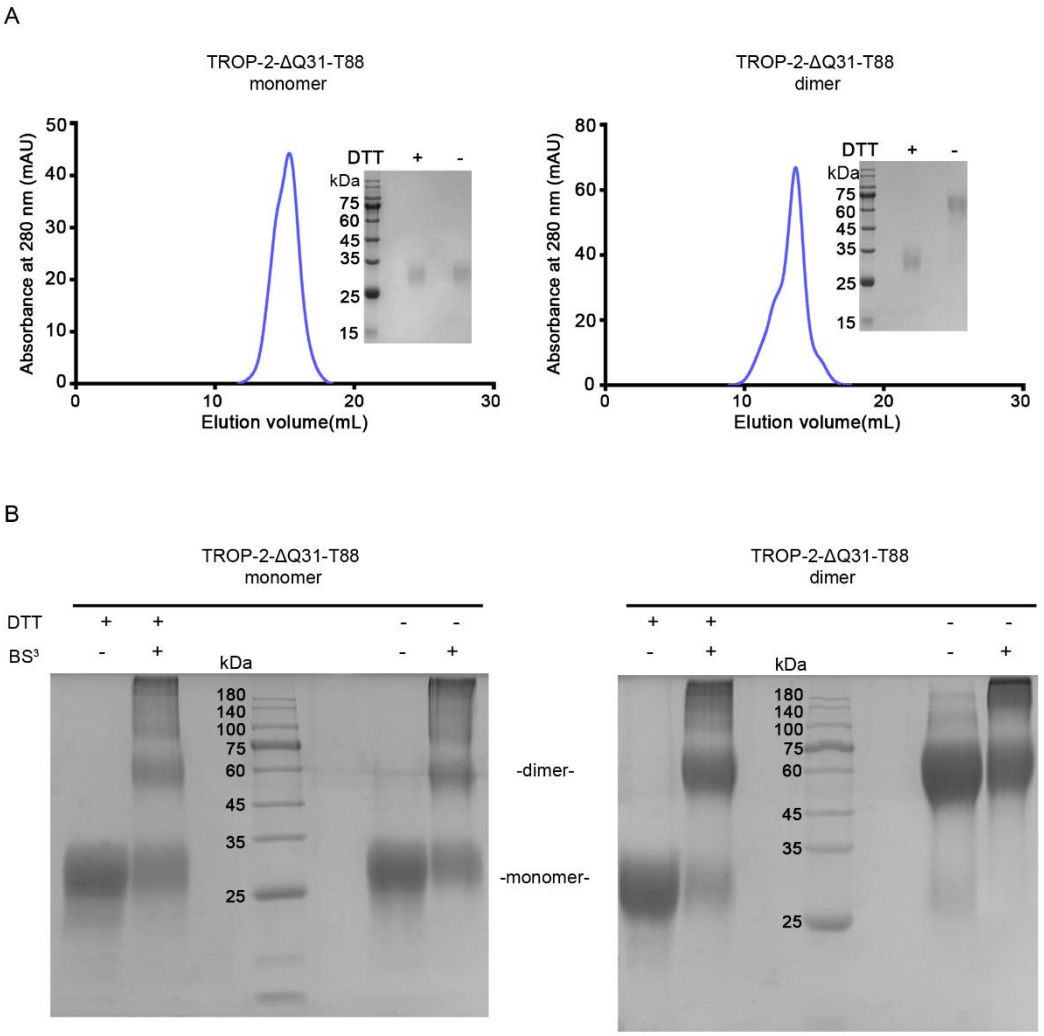

Figure S2. Preparation of monomeric and dimeric TROP-2-ΔQ31-T88 proteins and cross-linking analysis, related to Figure 1.

(A) Size exclusion analysis of monomeric (left) or dimeric TROP-2-ΔQ31-T88 proteins (right) with Superdex 200 Increase 10/300 GL column. The 280-nm absorbance curve and the SDS-PAGE migration profile of the pooled sample are shown. (B) Coomassie Blue staining SDS-PAGE analysis of cross-linked states of monomeric (left) or dimeric TROP-2-ΔQ31-T88 proteins (right) expressed from HEK-293T cells under reducing (with DTT) or non-reducing (without DTT) conditions. Both TROP-2-ΔQ31-T88 monomer and dimer can be cross-linked by the addition of BS^3 (bis (sulfosuccinimidyl) suberate) and presented as higher levels of oligomers.

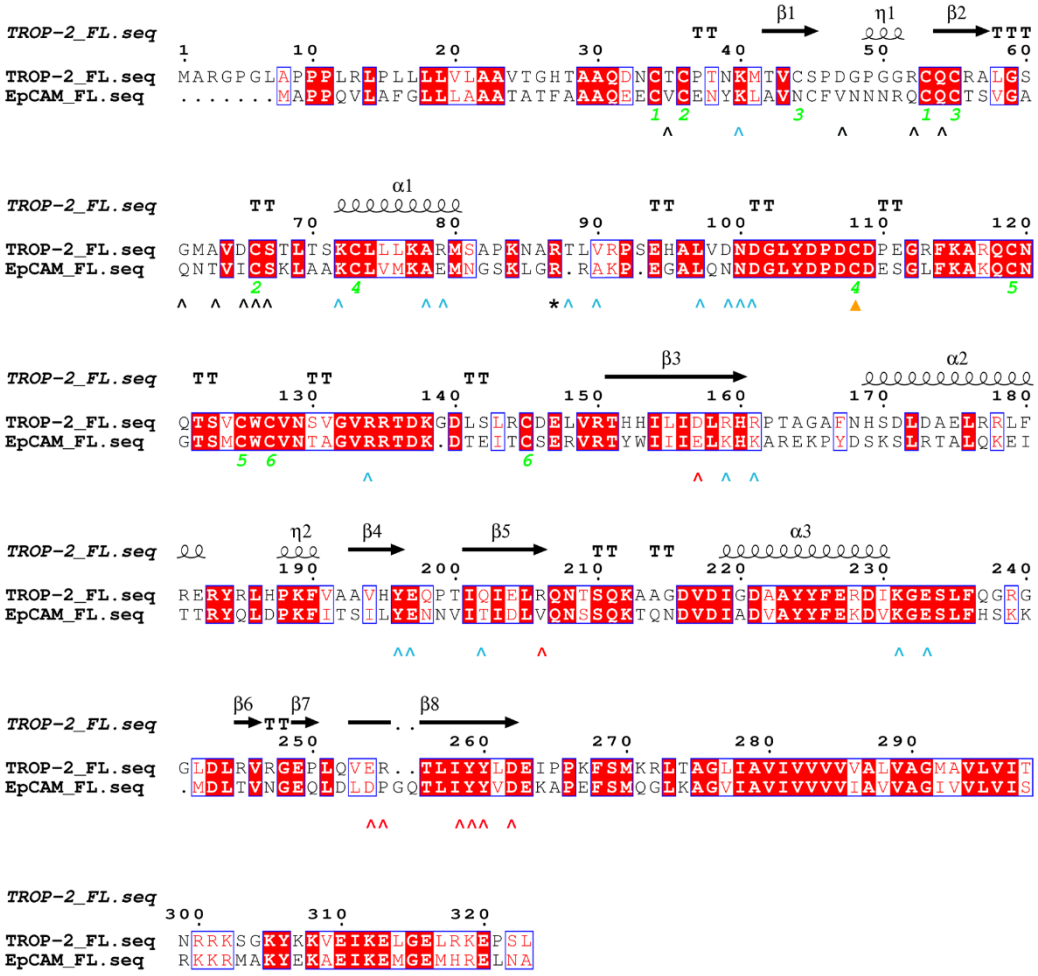

Figure S3. Sequence alignment of TROP-2 and EpCAM, related to Figure 1.

The alignment is numbered concerning the TROP-2 sequence, and the secondary structure of TROP-2 is shown above the alignment. The red, blue, and black arrows below the sequence represent hydrogen bond interactions in *trans*-, *cis*-dimer, and *cis*-tetramerization interface mediated by the N-terminal CRD respectively.

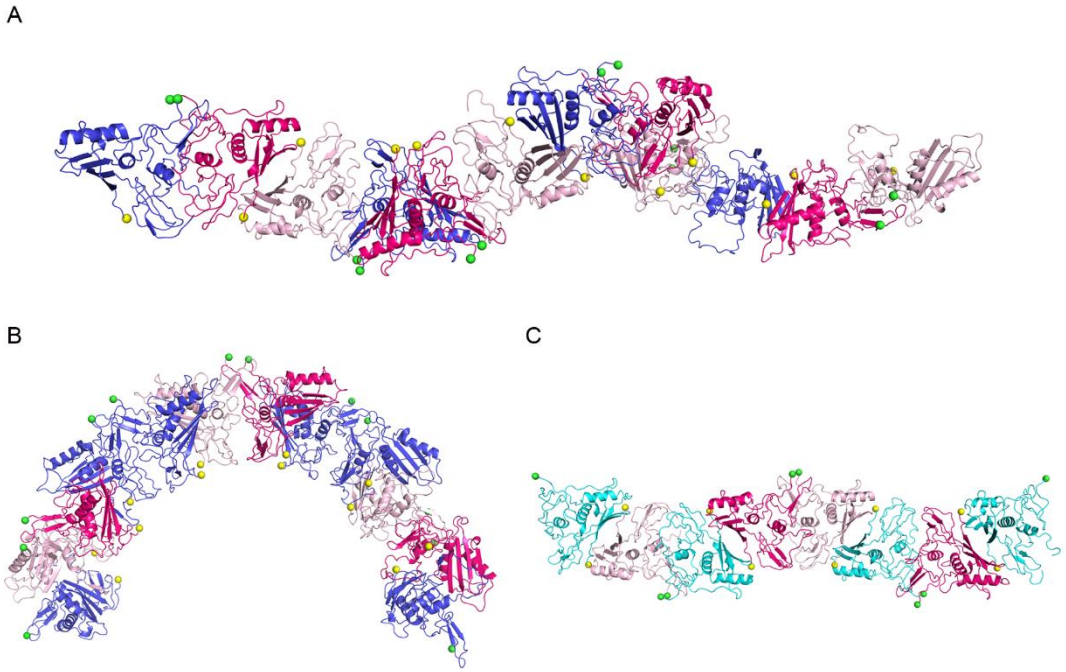

Figure S4. Varied types of intercellular TROP-2 assembly model, related to Figure 3.
(A) The connection of TROP-2-ECD *cis*- and *trans*-dimers mediated by CRD domain. (B) The oligomer all composed of *cis*-dimers of TROP-2-ECD. (C) The oligomer is composed of TROP-2-ECD *trans*-dimers. All the green spheres represent N-termini, and the yellow spheres represent C-termini.

Figure S5. Sequence alignment of human and mouse TROP-2, related to Figure 5.
The alignment is numbered concerning the TROP-2 sequence. The residues in the blue rectangular box indicate the two loops in CPD domain, Q237-Q252 for mRCPD and T209-G215 for mACPD, that have been replaced to define the binding region of sacituzumab.

Table S1. Crystallographic data collection and refinement statistics, related to Figures 2 and 3.

	TROP-2-ECD *trans*-assembly	TROP-2-ECD *cis*-assembly
Data collection		
Space group	P 1 21 1	P 43 2 2
Wavelength (Å)	0.97853	0.97890
Unit cell dimensions		
a, b, c (Å)	51.54, 126.88, 54.07	145.14, 145.14, 217.72
α, β, γ (°)	90.00, 118.39, 90.00	90.00, 90.00, 90.00
Resolution (Å)	45.34-3.19	50-3.20
No. reflections	7911	38952
R_{merge}	0.179/0.900	0.193/0.851
I/σ	8.832 (1.668)*	13.677 (2.821)
Completeness (%)	77.42	99.5
Redundancy	6.2 (6.3)	11.6 (12.2)
Refinement		
Resolution (Å)	45.34-3.19	49.95-3.20
R_{work} / R_{free}	0.239/0.284	0.241/0.276
No. atoms	3690	7399
Protein	3000	7399
Ligands	0	0
Water	0	0
R.m.s. deviations		
Bond lengths (Å)	0.003	0.003
Bond angles (°)	0.689	0.807
Ramachandran plot		
Favored (%)	92.74	98.39
Allowed (%)	6.84	1.61
Outliers (%)	0.43	0.00

*Values in parentheses are for highest-resolution shell.

Table S2. Residues contributed to *trans*-dimer interaction of TROP-2-ECD, related to Figure 2.

Chain A	Chain B	Contacts
E197	D101	15 (3)[b]
Q198	D101	2[a]
L261	H153, E204, L261, R206	12, 3, 1, 5
Y259	L155, D157, Q202, L257, Y259	5, 10 (1), 6, 1, 15 (1)
L257	D157, R255, L257, Y259	1, 1, 1, 1
F268	H169, D173, V194, H195	12, 5, 5, 5
P265	A292	5
P266	A292, H195	5, 5
D101	E197, Q198	11 (3), 3 (1),
R134	E197	1
Y260	E204	1
H152	R260	2 (1)
R255	L257	1
L155	Y259	5
Q202	Y259	6
D157	Y259	7 (1)
E204	Y260, L261	1, 3,
H153	L261	13
A192	P265, P266	5, 1
A193	P266	5
H195	P266, F268	4, 3
D173	F268	1
H169	F268	13
V194	F268	4

[a]Numbers represent the number of atom-to-atom contacts between residues of different chains of TROP-2-ECD, which were analyzed by the contact program in CCP4 suite (the distance cutoff is 4.5 Å).

[b]Numbers in the parentheses represent the number of potential hydrogen bonds (the distance cutoff is 3.5 Å).

Table S3. Residues contributed to *cis*-dimer interaction of TROP-2-ECD, related Figure 2 and 3.

Chain A	Chain B	Contacts
K40	E254, R161	13[a], 3
M41	R255	2
L58	R159	1
K72	D99	1
R79	R255, L257	7 (1)[b], 1
M80	L257	4
S81	R255	1
P83	T200	1
A86	E197	5
R87	E197	4
T88	E197, H195, Y196	11 (2), 5, 3 (1)
L89	Q202, E197, T200, H195, Y196	15, 1, 1, 1, 1
V90	Q202, E204, L155, H195	13 (2), 6, 4, 4
R91	L155	2
P92	L155, Y259	2, 12
S93	L261	6
H95	L261, P265, P266	3, 3, 3
A96	P261, Y260, Y259	5, 7, 1
L97	Y260, Y259, I258, N129, V133, D262	17 (2), 2, 1, 1, 2, 1
V98	Y260, I258, V133, Y259	1, 4, 3, 5
D99	V133, Y260, G102, D101, K72	7, 11 (1), 4, 4, 3 (1)
N100	D101, G102, D99	17 (1), 5, 1
D101	R134	4 (1)
L103	L257	1
N129	L97	1
V133	L97, D99	6, 1
R134	L97	4
R135	L97	1
L155	P92, V90	5, 2
R159	P83, S81, R79, A78	1, 5, 5 (1), 3 (1)
R161	K40, L58, M41	7 (1), 1, 1
H195	L81, V90	3, 3
Y196	T88, L89	3, 2
E197	L89, T88, R87	6, 13, 4
T200	L89, P83	6, 2
Q202	L89, V90	15, 15 (1)
V204	V90	2

E227	D99	1
K231	K231, D101	5, 7
E233	R254, R255, K231, T256	6, 7, 3 (1), 8
G238	R161	1
R239	R161	5
G240	R261	5
Q252	E233	6
V253	E233	5
E254	R79, M41	6 (1), 4
R255	R79, M80, P83, A82	3, 2, 1, 2
T256	D99	1
L257	D99, N100	2, 2
I258	D99, L97, V98	14 (1), 1, 2
Y259	L97, A96, P92, V98	6, 1, 14, 6
Y260	L97, H95, A96, D99	14 (2), 1, 3,10 (1)
L261	S93, H95, A96	2, 3, 2
D262	L97	1
P265	H95	5
P266	L97, H95	3, 2

[a]Numbers represent the number of atom-to-atom contacts between residues of different chains of TROP-2-ECD, which were analyzed by the contact program in CCP4 suite (the distance cutoff is 4.5 Å).

[b]Numbers in the parentheses represent the number of potential hydrogen bonds (the distance cutoff is 3.5 Å).

Table S4. Residues contributed to *cis*-tetramer interaction of TROP-2-ECD, related to Figure 3.

Chain C	Chain A	Contacts
N33	R52	1[a]
C34	R52	1
T35	R52, P49	5 (1)[b], 1
C36	R52	2
P37	P49, R52	4, 1
D47	D65, S67	3, 3 (1)
G48	S67	4
P49	S67, P37, C66	5, 2, 6
R52	C53, T35, C34, N33	10, 9 (1), 1, 1
C53	R52	8
Q54	V64, D65, C66, S67	3, 17 (1), 6 (1), 2
R56	A63	2
G59	P110	4
S60	P110, M62,	8, 3
G61	P110, M62, A63	7, 6, 4 (1)
M62	M62, G61, A63, S60	14, 6, 6, 2
A63	G61, M62, A63, C55, R56	4, 6, 9, 1, 2
V64	Q54	2
D65	Q54, D47	5 (1), 1
C66	Q54, P49, R52	6, 7, 2
S67	D47, P49, G48, Q54	3 (1), 3, 4, 1
P110	G59, S60, G61	4, 6, 1
T122	R144	1
L141	L141	2
R144	T122	1

[a]Numbers represent the number of atom-to-atom contacts between residues of different chains of TROP-2-ECD, which were analyzed by the contact program in CCP4 suite (the distance cutoff is 4.5 Å).

[b]Numbers in the parentheses represent the number of potential hydrogen bonds (the distance cutoff is 3.5 Å).