

Supplementary Materials: Stabilization of the CD81 Large Extracellular Loop with *De Novo* Disulfide Bonds Improves Its Amenability for Peptide Grafting

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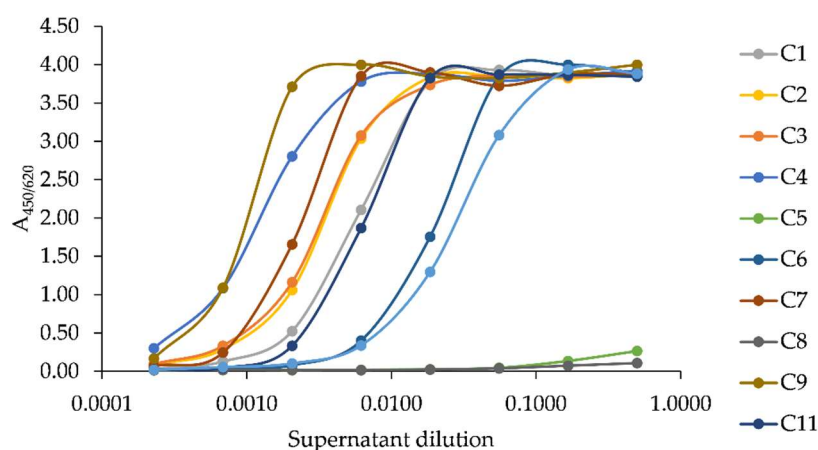


Figure S1. ELISA testing the reactivity of *de novo* disulfide bond endowed hCD81 LEL mutants with structure-reporter antibody M38.

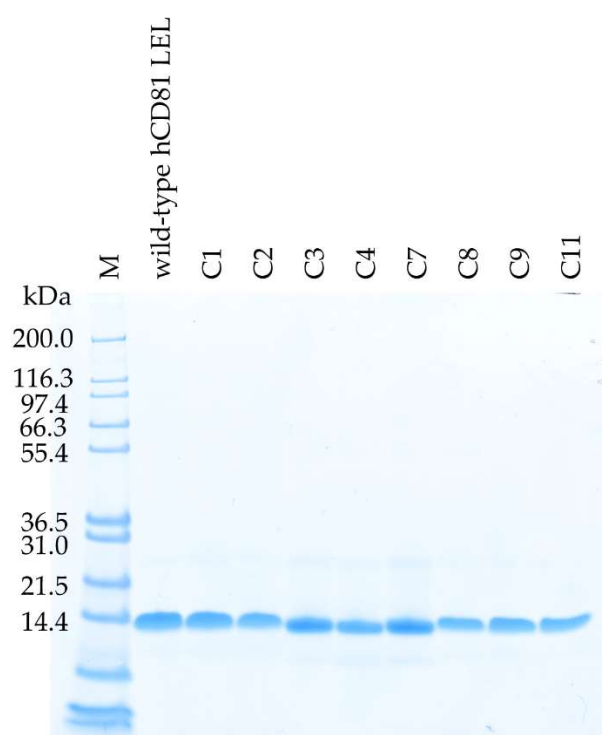


Figure S2. SDS-PAGE of purified hCD81 LEL variants with *de novo* disulfide bonds.

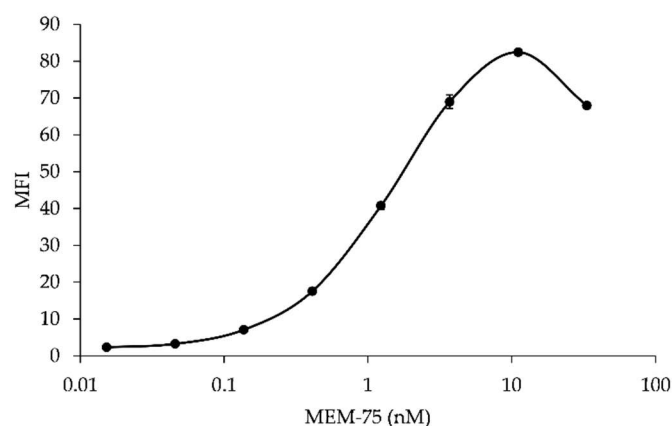


Figure S3. Titration of binding of anti-hTfr antibody MEM-75 to the surface of SK-BR3 cells.

Table S1. Amino acid sequences of hCD81 LEL and peptide-grafted variants. First and last residue pertaining to hCD81 LEL sequence are in bold, native cysteine residues are in orange, the sequence of peptide graft in in blue and novel cysteine residues are in green, his-tag sequence is underlined.

hCD81 LEL Mutant	Amino Acid Sequence
wild-type	<u>HHHHHHHH</u> GGSTGENLYFQGASFVNKDQIAKDVKQFYDQALQQAVV DDDANNAKAVVKTFFHETLD CC GSSTLTALTTSVLKNNL C PSGSNIISNL FKED CH QKIDDLFSGK
hCD81 LEL_Tfr1	<u>HHHHHHHH</u> GGSTGENLYFQGASFVNKDQIAKDVKQFYDQALQQAVV DDDANNAKAVVKTFFHETLD CC GSSTLTALTTSVLKNNL C PSGSN HAIY PRHSSG CH QKIDDLFSGK
hCD81 LEL_Tfr2	<u>HHHHHHHH</u> GGSTGENLYFQGASFVNKDQIAKDVKQFYDQALQQAVV GSSHAIYPRHSSG ANNAKAVVKTFFHETLD CC GSSTLTALTTSVLKNNL C PSGSNIISNLFKED CH QKIDDLFSGK
hCD81 LEL_Tfr2_C4	<u>HHHHHHHH</u> GGSTGENLYFQGASFVNKDQIAKDVKQFYDQALQQ CVV GSSHAIYPRHSSG ANNA CA VVKTFFHETLD CC GSSTLTALTTSVLKNNL C PSGSNIISNLFKED CH QKIDDLFSGK

Table S2. Oligonucleotides used for the construction of *de novo* disulfide bond stabilized mutants.

hCD81 LEL Variant	Substitution	Oligonucleotide Sequence
C1	Ala134Cys	CAGGCCCTACAGCAGTGCCTGGTGGATGATGAC GTCATCATCCACCACGCACTGCTGTAGGGCCTG
	Ala143 Cys	GATGATGCCAACAACACTGCAAGGCTGTGGTGAAG CTTACCACAGCCTTGCAGTTGTTGGCATCATC
C2	Ala130Cys	CAGTTCTATGACCAGTGTCTACAGCAGGCCGTG CACGGCCTGCTGTAGACACTGGTCATAGAAGT
	Val146Cys	AACAACGCCAAGGCTTGTGTGAAGACCTTCCAC GTGGAAGGTCTTCACACAAGCCTTGGCGTTGTT
C3	Gln133Cys	GACCAGGCCCTACAGTGCCTGGTGGATGATG CATCATCCACCACGGCGCACTGTAGGGCCTGGTC
	Ala143Cys	GATGATGCCAACAACACTGCAAGGCTGTGGTGAAG CTTACCACAGCCTTGCAGTTGTTGGCATCATC
C4	Ala134Cys	CAGGCCCTACAGCAGTGCCTGGTGGATGATGAC GTCATCATCCACCACGCACTGCTGTAGGGCCTG
	Lys144Cys	GACGCCAACAACGCCTGTGCTGTGGTGAAGACC GGTCTTACCACAGCACAGGCGTTGTTGGCGTC
C5	Leu154Cys	GACCTTCCACGAGACGTGTGACTGCTGTGGCTC GAGCCACAGCAGTCACACGTCTCGTGAAGGTC
	Lys193Cys	GAGGACTGCCACCAGTGTATCGATGACCTCTTC GAAGAGGTCATCGATACACTGGTGGCAGTCTTC
C6	Ala120Cys	CAACAAGGACCAGATCTGTAAGGATGTGAAGCAG CTGCTTCACATCCTTACAGATCTGGTCTTGTG
	Phe198Cys	AAGATCGATGACCTCTGTTCCGGGAAGTGATGAG CTCATCACTTCCCGGAACAGAGGTCATCGATCTT
C7	Ala130Cys	CAGTTCTATGACCAGTGTCTACAGCAGGCCGTG CACGGCCTGCTGTAGACACTGGTCATAGAAGT
	Ala143Cys	GATGATGCCAACAACACTGCAAGGCTGTGGTGAAG CTTACCACAGCCTTGCAGTTGTTGGCATCATC
C8	Leu131Cys	TTCTATGACCAGGCTGCCAGCAGGCCGTGGTG CACCACGGCCTGCTGGCAGGCTGGTCATAGAA
	Leu165Cys	AGCACACTGACTGCTGTACCACCTCAGTGCTC GAGCACTGAGGTGGTACAAGCAGTCAGTGTGCT
C9	Val135Cys	GCCCTACAGCAGGCCCTGTGTGGATGATGACGCC GGCGTCATCATCCACACAGGCCTGCTGTAGGGC
	Ser168Cys	GCTTTGACCACCTGTGTGCTCAAGAACAAT ATTGTICTTGAGCACACAGGTGGTCAAAGC
C10	Val169Cys	TTGACCACCTCAGTGTGCAAGAACAATTTGTGTC GACACAAATTGTTCTTGCACACTGAGGTGGTCAA
	Leu174Cys	GTGCTCAAGAACAATTGTTGCCCTCGGGCAGC GCTGCCCGAGGGACAACAATTGTTCTTGAGCAC
C11	Ser160Cys	GACTGCTGTGGCTCCTGTACACTGACTGCTTTG CAAAGCAGTCAGTGTACAGGAGCCACAGCAGTC
	Asp189Cys	AACCTCTCAAGGAGTGTGCCACCAGAAGATC GATCTTCTGGTGGCAACACTCCTTGAAGAGGTT