frontiers

Supplementary Material

Oligomannose-rich membranes of dying intestinal epithelial cells promote host colonization by adherent-invasive *E. coli*

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

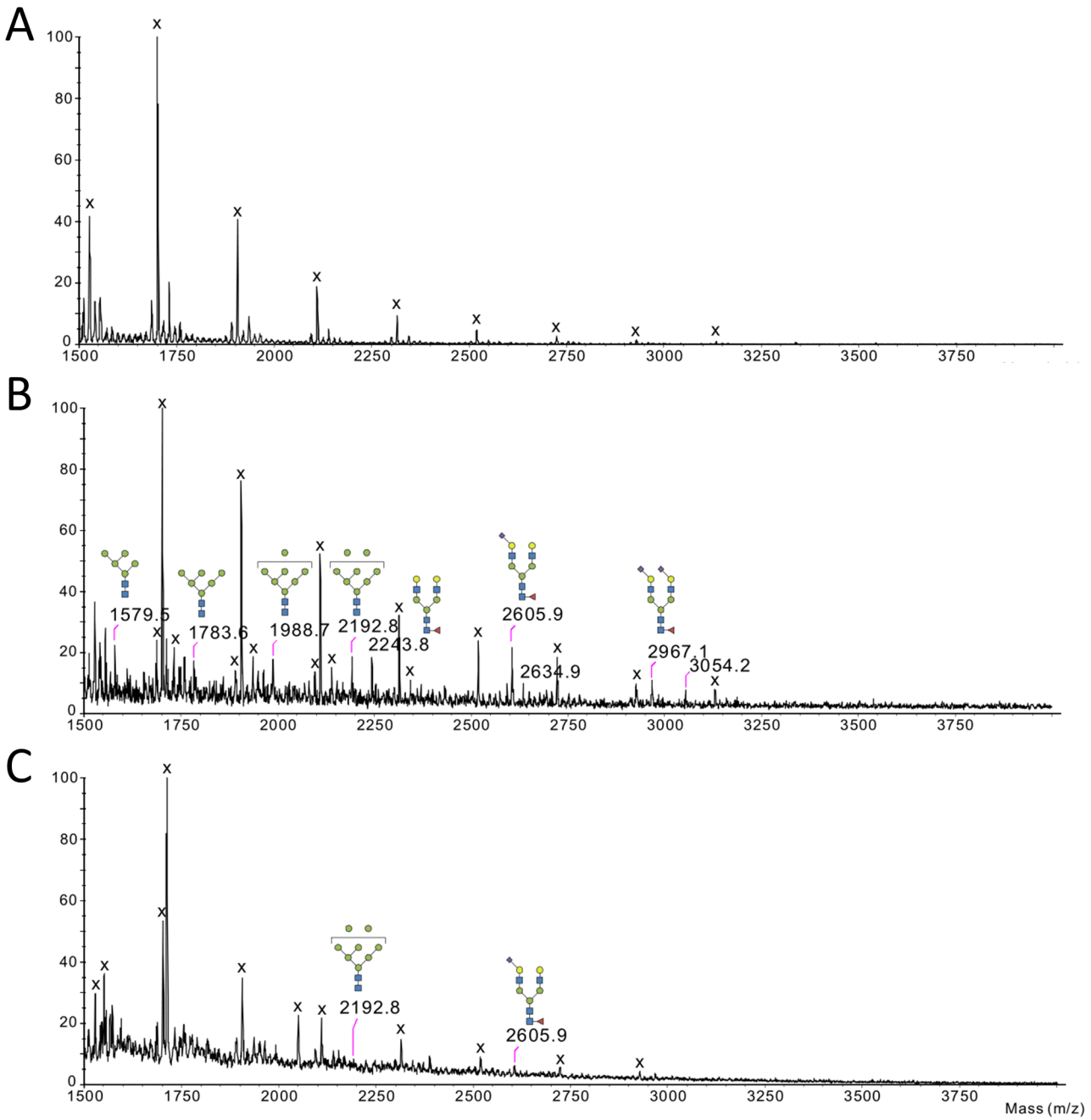


Figure S1. MALDI MS/MS analysis of vesicles of HeLa cells: untreated **(A)**, treated with UV-B as positive inducer of apoptosis **(**90 sec UV-B irradiation followed by 4 h incubation**) (B)** or FimH lectin (10 FimH-MP per cell followed by 4 h incubation) **(C)**. No glycans were detected in the membranous vesicles of untreated cells. Polyhexose contamination is indicated with "x".

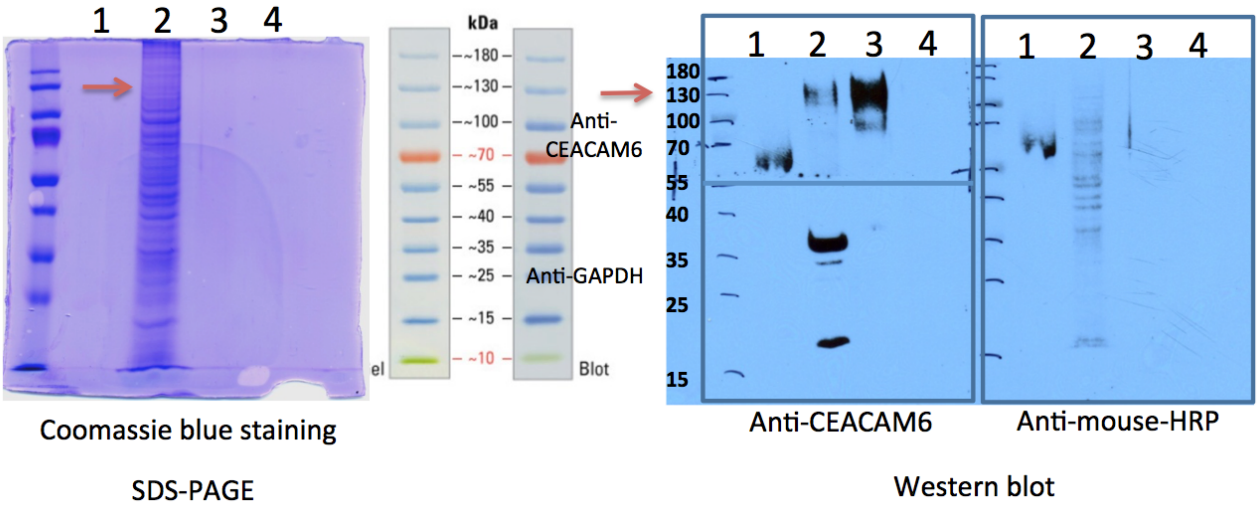
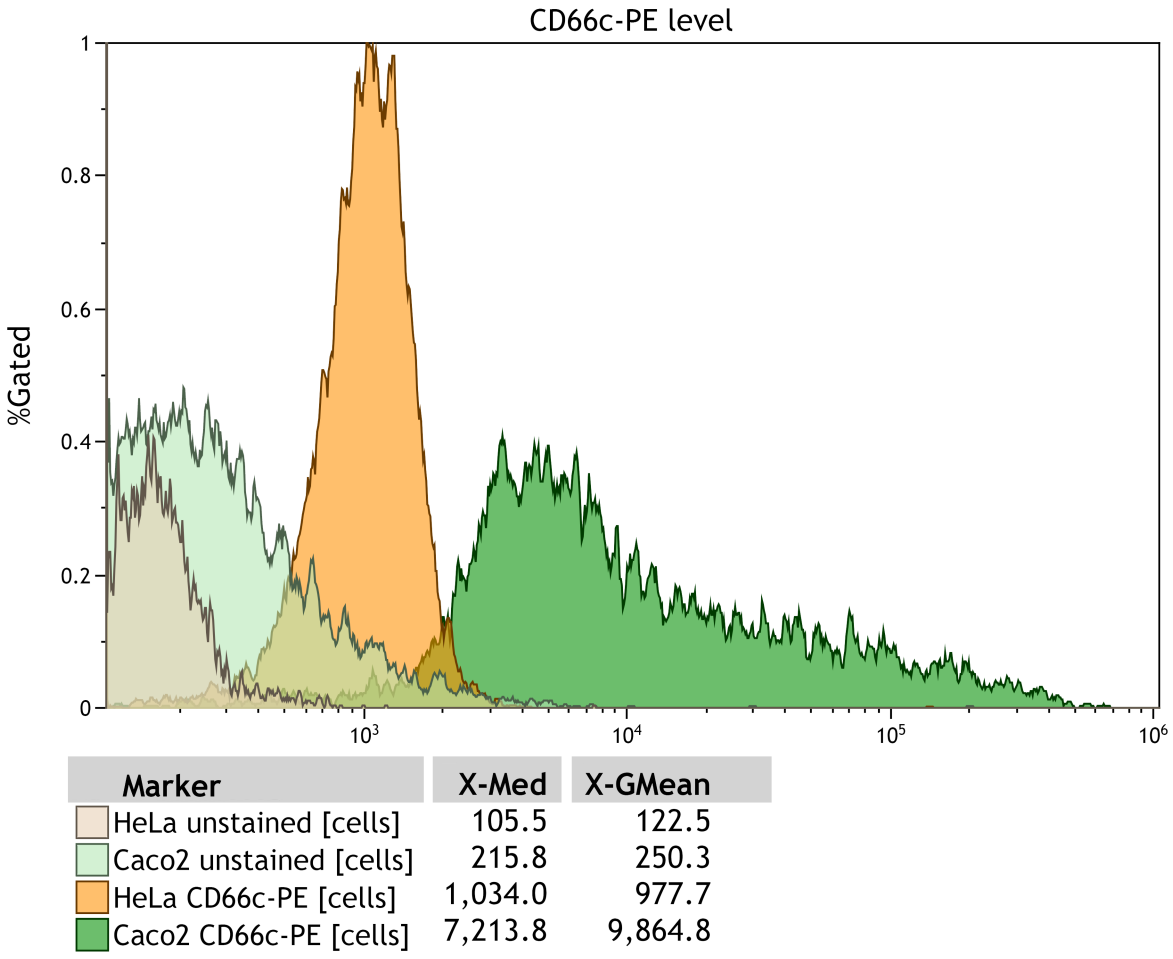


Figure S2. Coomassie blue staining and Western blot of CEACAM6 purification by immunoprecipitation:

1. Flow-through to verify antibody 9A6 (Aldevron) coupling
2. Flow-through to verify that CEACAM6 was retained by the column
3. Eluate
4. 2nd elution (to ensure complete CEACAM6 elution)



Marker	X-Med	X-GMean
HeLa unstained [cells]	105.5	122.5
Caco2 unstained [cells]	215.8	250.3
HeLa CD66c-PE [cells]	1,034.0	977.7
Caco2 CD66c-PE [cells]	7,213.8	9,864.8

Figure S3. Binding of anti-CD66c-PE antibodies (aimed to detect CEACAM6) to HeLa and Caco-2 cells:
Flow cytometric measurements with anti-CD66c-PE (anti-CEACAM6 antibody fluorescently labelled with phycoertyhrin) show an about 10-fold lower detection of CEACAM6 on HeLa *versus* Caco-2 cells.

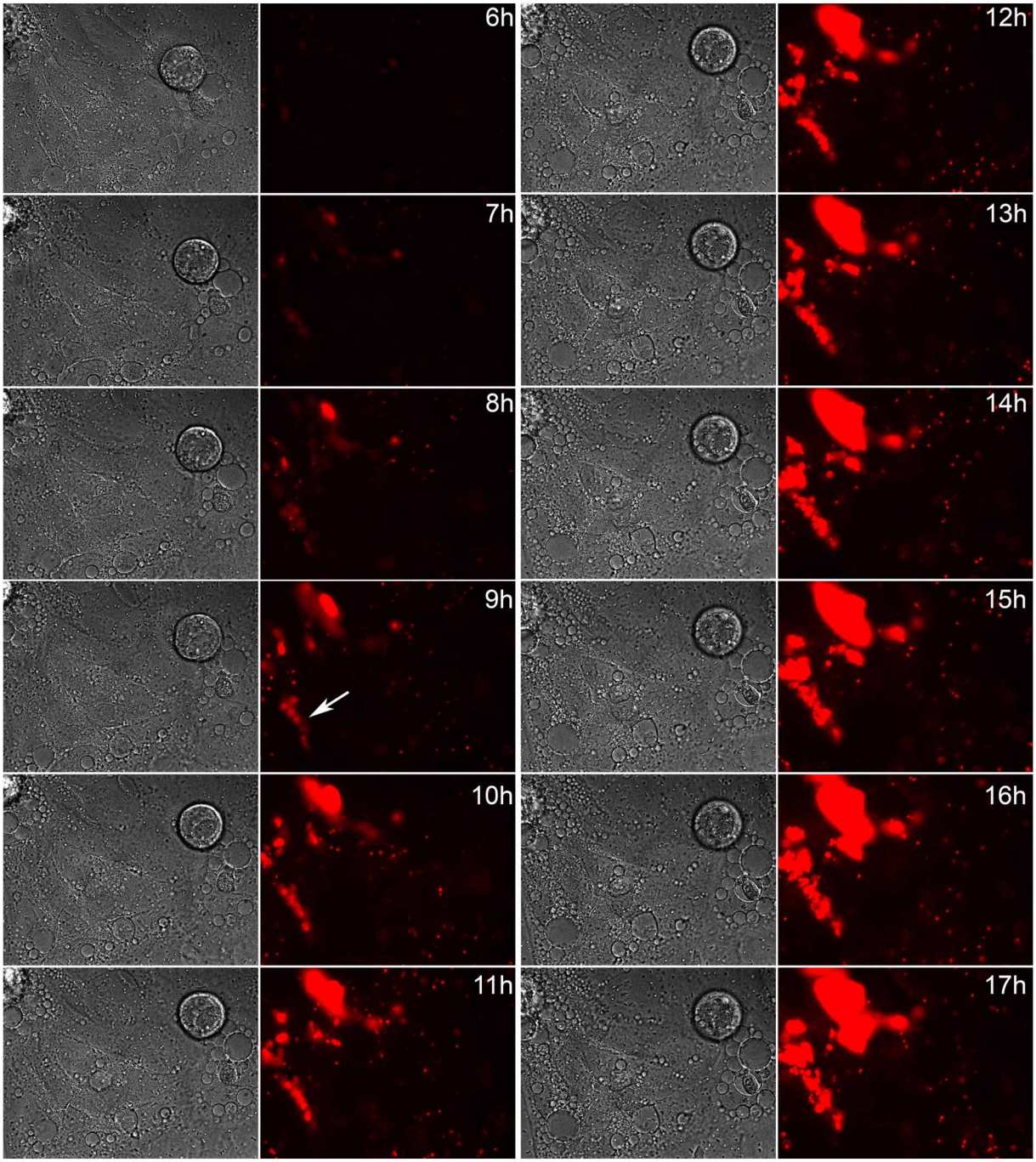


Figure S4A. Propagation of pathogenic bacteria in the intercellular spaces 6 to 17 h after infection: co-culture of the human epithelial cell line Caco-2 with the bacterial strain LF82. LF82 expresses the NIR fluorescent protein TurboFP635 (red).

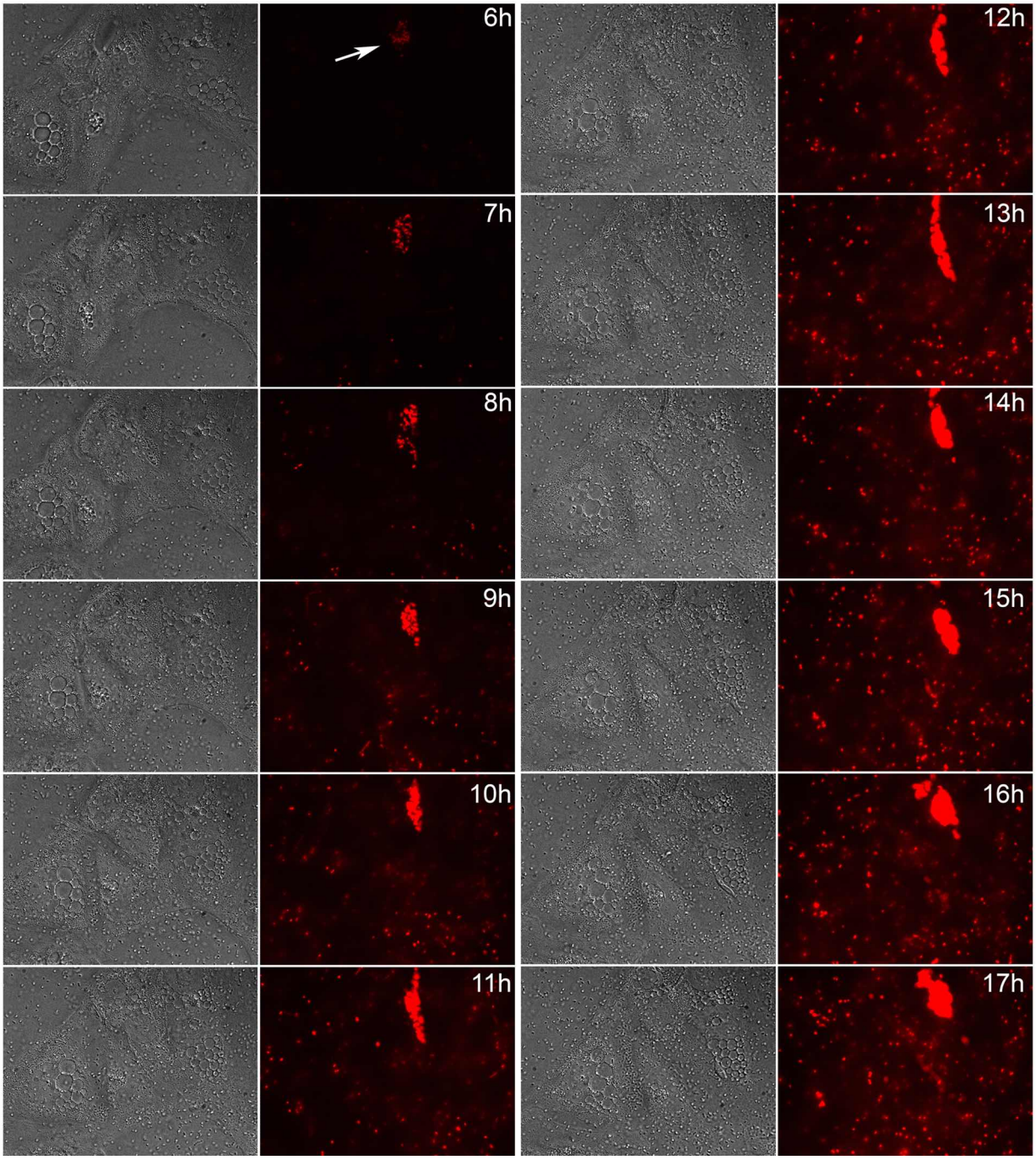


Figure S4B. The formation of intracellular bacterial communities at 6 to 17 h after initial infection: co-culture of the human epithelial cell line Caco-2 with the bacterial strain LF82. LF82 expresses the NIR fluorescent protein TurboFP635 (red).

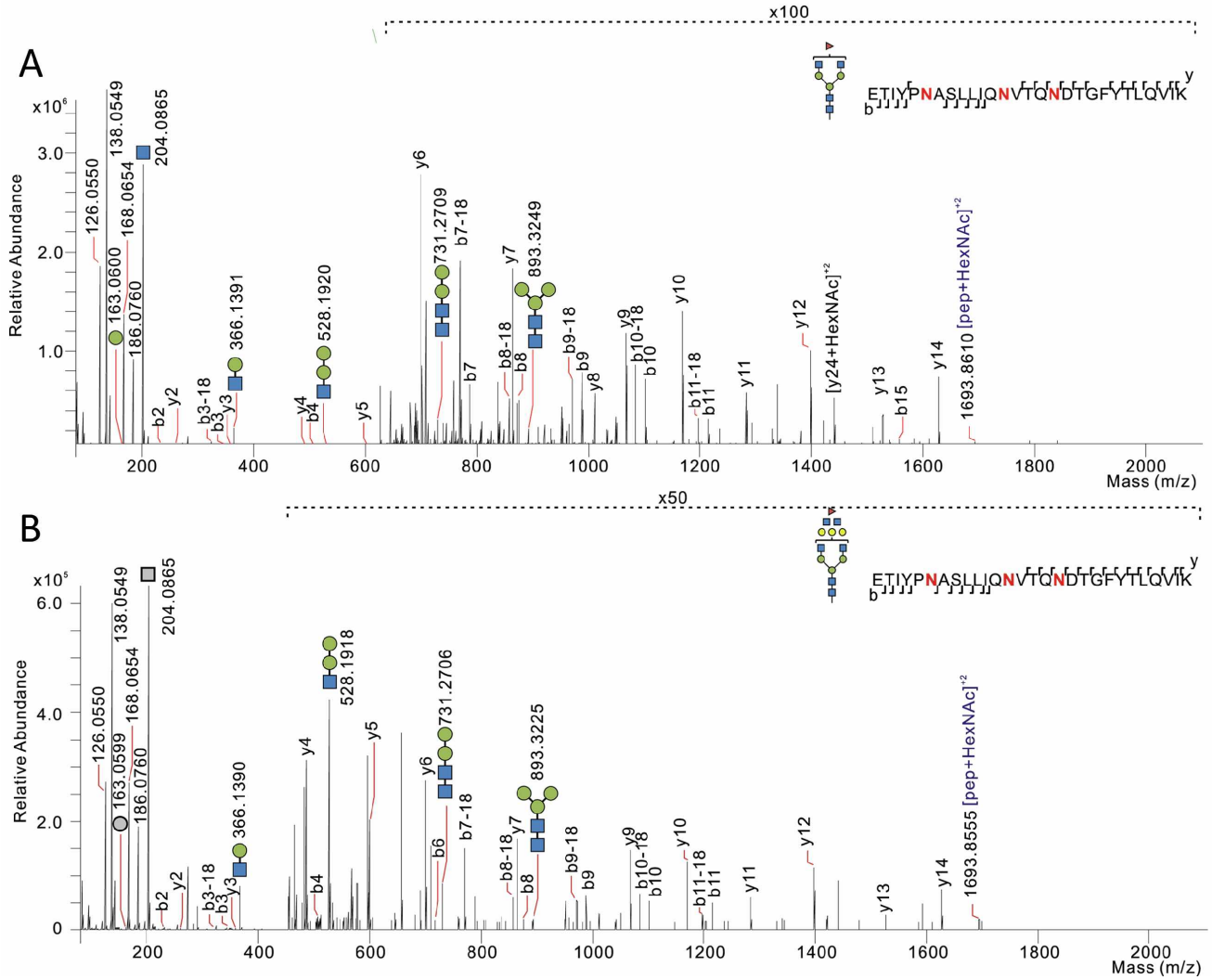


Figure S5. Glycosylation analyses of recombinant human CEACAM6 (rhCEACAM6) protein: HCD-MS/MS spectra of ETIYPNASLLIQNVTQNDTGFYTLQVIK modified with **(A)** GlcNAc4Man3Fuc1 or **(B)** GlcNAc6Man3Hex3Fuc1. The potential *N*-glycosylation sites are highlighted in bold red (positions Asn104, Asn111 and Asn115).

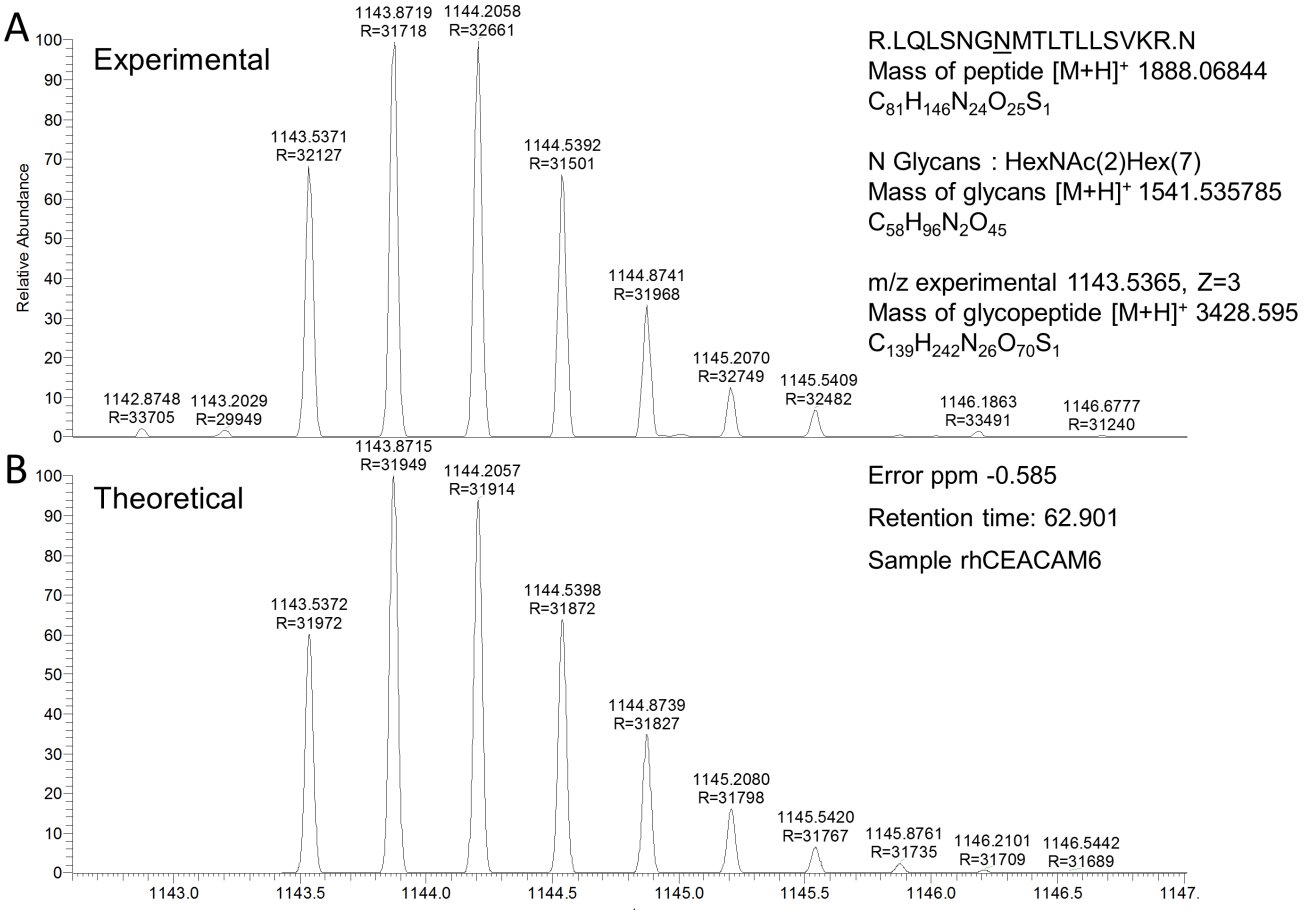


Figure S6 Isotopic profile of recombinant human rhCEACAM6 glycopeptide identified by LC-MSMS orbitrap Q-Exactive plus: **(A)** Glycopeptide at m/z 1143.5365, Z=3 identified ($[M+H]^+$ 3428.595) at retention time 62.90 min includes the amino acid sequence R.LQLSNGNMTLTLLSVKR.N ($[M+H]^+$ 1888.06844, $C_{81}H_{146}N_{24}O_{25}S_1$) and *N*-glycans HexNAc(2)Hex(7) ($[M+H]^+$ 1541.535785, $C_{58}H_{96}N_2O_{45}$). **(B)** Theoretical profile of glycopeptide at m/z 1143.53716, Z=3, $C_{139}H_{242}N_{26}O_{70}S_1$. The error is -0.585 ppm between experimental and theoretical.

1.2 Supplementary Tables

Table S1. Comparison of the highest Byonic scores in different CEACAM6 samples: rhCEACAM6 and CEACAM6 isolated from non-infected cells (control) and after infection with the bacterial strains LF82 or K12.

Protein Name = >sp|P40199|CEAM6_HUMAN Carcinoembryonic antigen-related cell adhesion molecule 6 OS=Homo sapiens GN=CEACAM6 PE=1 SV=3, # AAs 344								
Sample condition Name of Data	**|LogProb|**	**Best |Log Prob|**	**Best Score**	**#Spectra**	**# Unique Peptides**	**# Modified Peptides**	**% Coverage**	**Intensity**
In-Gel Control CTRL_CEACAM6	160.67	15.35	916	86	42	37	50.3	8.46E+07
In-Gel LF82 LF82_CEACAM6	196.5	13.28	935.3	105	59	54	41.6	1.16E+08
In-Gel K12 K12_CEACAM6	187.27	14.51	833.5	73	41	39	23.8	8.33E+07
eFASP recombinant rh_CEACAM6	2713.72	19.78	1375.6	1664	460	442	90.1	1.19E+10