MATERIALS AND METHODS

Bacterial strain and infection

Neisseria meningitidis (Nm) 2C4.3 strain, (formerly clone 12) that is a piliated capsulated Opa- Opc- variant of the serogroup C meningococcal clinical isolate 8013 and its isogenic non-piliated PilE defective mutant and that of non-adherent PilV defective mutant were described before (1). Bacterial strains were stored frozen at -80°C and routinely grown at 37°C under 5% CO2 on GC agar plates (Difco) containing Kellogg's supplements. Kanamycin was added when required at a concentration of 100 µg/mL.

The day of infection, a suspension of the bacteria from an overnight culture on GCB agar plate was adjusted to OD600=0.05 and incubated for 2h at 37°C in a pre-warmed cell culture medium. Cells were infected with bacteria at a multiplicity of infection (MOI) of 100 bacteria per cell (OD= 0.1) for 30 minutes, washed twice to remove non-adherent bacteria and infection was allowed to proceed for various periods of time.

Cell lines, plasmids and transfection

Cell lines

Human Cerebral Microvascular Endothelial Cells (hCMEC/D3) and Rat Brain Endothelial cells clone 4 (RBE4) are fully differentiated brain endothelial cell line derived from human and rat brain capillaries, respectively, engineered in our laboratory, and which recapitulate the major phenotypic features of the blood-brain barrier (2, 3). hCMEC/D3 were grown onto cultrex rat collagen type I-coated dishes (R&D) in Endothelial Cell Basal Medium-2 (Lonza) supplemented with 5% of FCS, 1.4 µM hydrocortisone (Lonza), 5 µg/mL ascorbic acid (Lonza), 1 ng/mL b-FGF (Lonza), at 37°C in 5% CO2. RBE4 were grown onto type I collagen-coated dishes in -medium/Ham's F-10 (1:1; Life Technologies), supplemented with 10% FBS, 1 ng/mL basic fibroblast growth factor (Sigma-Aldrich) and 300 µg/mL G418 (Sigma-Aldrich).

BEND3 are endothelioma derived from mice brain cortex purchased from ATCC® (CRL-2299™). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% FBS.

HBMECs, a human bone marrow capillary endothelial cell line provided by Dr B. Weksler (4), were grown in DMEM Glucose 4.5g/L + Glutamax (Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum FBS, 7.5 µg/mL endothelial cell growth supplement (Sigma-Aldrich), 7 UI heparin (Sigma-Aldrich) and 10 mM Hepes.

Primary Human Dermal Microvascular Endothelial Cells (HDMEC) isolated from the dermis of juvenile foreskin and adult skin (different locations) and their specific endothelial cell growth medium were purchased from PromoCell.

CHO-K1 cells stably expressing CD147 (CHO-hCD147) or the empty vector (CHO-pcDNA) as control were kindly provided by Dr Bukrinsky (the George Washington University, Washington, DC, USA) (5). CHO cells were cultured in DMEM/Nut-F12 (1/1, Life Technologies) supplemented with 5% FBS.

Plasmids

The HA tag was introduced by PCR into pcDNA3-CD147 vector using the Phusion site directed mutagenesis kit (Finnzyme) as described (6). Deletion of the CD147 D1 and D2 domain and Asp to Ala mutations in the 3 different glycosylation sites were generated by PCR on the previous construction. All the constructs were confirmed by sequencing analysis. The primer sequences used in this work are provided in supplementary Table S4.

Transfection

HBMECs were transfected using the Amaxa Nucleofactor Kit V (Amaxa Biosystem) according to the manufacturer's instructions. Briefly, for optimal transfection of HBMECs, 4.10^6 cells were suspended in 100 µL of solution V provided by Amaxa in the presence of 4 µg DNA, and subjected to electroporation using the program U15. Cells were then plated at the same density as cultures that

had reached confluency on Permanox coverslips (Costar) in complete medium for 24 hours before infection and fixation for confocal immunofluorescence analysis.

Pull Down assays and immunoblotting

For pull down assays, transfected cells were lyzed in lysis buffer containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl 10% Glycerol, 1 mM CaCl2, 1 mM MgCl2, 5 mM NaF, 1% Triton. Cell lysates, or purified recombinant proteins, were incubated with agarose bound WGA lectin beads (Clinisciences, AL-1023S- 2) for 1 hour at 4°C. After 3 washes, beads were resuspended in Laemmli buffer and the presence of the proteins of interest was assessed by immunoblot analysis. Quantitative analyses were conducted on 3 independent experiments using ImageJ software.

Confocal immunofluorescence microscopy

Cells were grown to confluence on Permanox coverslips (Thermo Fischer Scientific). After the indicated transfection, treatment and/or infection, cells were fixed and labeled with the appropriate antibodies. Image acquisitions were performed with a DMI6000 microscope (Leica, 63x). Image analysis were done with ImageJ software (NIH). Quantitative analysis of protein recruitment under bacterial colonies was determined as the proportion of colonies showing underlying proteins of interest. At least 30 colonies were observed per coverslip. Each experiment was repeated at least 3 times. The antibody dilutions/concentrations used in this work are provided in supplementary Table S5.

Adhesion assays on living cells and immobilized recombinant proteins

Meningococcal adhesion on hCMEC/D3 cells was assayed under static conditions, as previously described (7). When indicated, cells were preincubated with 20 µg/mL soluble lectin (Vector laboratories) or chitotriose (TCI-Europe). To inhibit protein sialylation, cells were treated for 3 weeks with the sialyltransferase inhibitor 3Fax-Peracetyl Neu5Ac (3Fax-P-Neu5a).

Meningococcal adhesion on cells was quantified as previously described, following a 30-min infection

in static conditions by plating dilutions on GCB agar (7). The number of colony forming units (CFU) was compared to that of the control strain in the same experiment. Experiments were performed at least three times in duplicate.

To perform adhesion assays on defucosylated cells, cells were treated for 1h with 0.08 u/mL α-L-fucosidase (F5884, SIGMA) or vehicle as control and washed gently with medium, before infection. As fucosidase treatment induced cell detachment, cells were fixed and labeled with antibodies targeting bacteria, CD147 and rhodamin-phalloidin to stain actin. Image acquisitions were performed with a DMI6000 microscope (Leica, 63x). Quantitative analysis were done with ImageJ software (NIH) and are presented as colonisation index corresponding to the area occupied by the fluorescently labelled bacteria in relation to cell area delineated by actin staining (n= 20 to 30 fields from 3 independent experiments).

For adhesion assays on immobilized recombinant proteins, experiments were performed as previously described (1). CD147-Fc and ALCAM-Fc were immobilized on Lab-Tek Chamber Slides 8 wells (#177455). When indicated, slides were preincubated with 20 µg/mL soluble lectin. Briefly, slides were coated with poly-l-lysine (0.5 µg/mL for 5 min RT), washed with PBS, cross-linked with glutaraldehyde (0.5% for 15 min RT), washed, incubated with anti-Fc (1 µg/well) in assay buffer (PBS with 3% BSA) for 1 h RT, washed in assay buffer and incubated overnight at 4 °C with 1 µg/well of recombinant protein at 4 °C overnight. Slides were washed in assay buffer for 1h RT, before infection for 30 min with meningococcal suspension of OD600 0.1. Slides were then washed three times with PBS, fixed with PFA 4% for 10 min and washed three times with PBS. Bacteria were labeled and visualized with a Leica DMI6000 microscope using a 63× oil-immersion objective. The number of adherent bacteria per field was quantified for 30 fields using ImageJ software. To verify that the quantity of coated proteins was equal in each assay, the coated proteins were collected in Laemmli buffer in a control well and revealed by SDS-Page and immunoblot.

Protein treatments

To perform enzymatic deglycosylation, 5 µg of CD147-Fc were incubated for 2h at 37°C with 1 µL of Rapid PNGase F (New England Biolabs, P0710S) in 20 µL (final volume) of PNGase F buffer (20 mM TRIS-HCl pH 8.0, 150 mM NaCl) before immobilization on different slides (1 µg/slide). To perform enzymatic desialylation, 5µg of CD147-Fc were incubated for 2h at 37°C with 1µL of α2-3,6,8,9 Neuraminidase A (New England Biolabs, P0722S) in 10 µL (final volume) of α2-3,6,8,9 Neuraminidase A buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 50 mM NaCl). To perform enzymatic sialylation, 3µg of CD147-Fc or ALCAM-Fc were incubated for 2h at 37°C with 1 µL of sialyltransferase (Sigma-Aldrich-Merck, S1951-1UN) in 30 µL (final volume) of α-2,3-Sialyltransferase buffer (100 mM Tris-HCl buffer, pH 8.0, containing 1 mM CMP-Neu- 5-Ac (Sigma-Aldrich- Merck # C8271). To perform enzymatic defucosylation, 3 µg of CD147-Fc were incubated for 2h at 37°C with 2 µL α-1-2,3,4,6-L-Fucosidase (Megazyme, E-FUCHS) in 20 µL (final volume) of α-1- 2,3,4,6-L- Fucosidase buffer (100mM sodium acetate pH 4.0). As control for each treatment, 3 to 5 µg of recombinant proteins were similarly incubated in the corresponding enzymatic buffer alone, before immobilization on slides (1 µg/slide). For each experiment, one slide was lysed with lysis buffer and submitted to western blot analysis using anti-Fc antibody to verify the immobilization efficacy of the different modified recombinant proteins.

N-glycan analysis by mass spectrometry

N-glycans were released by peptide-N-glycosidase F according to the manufacturer's instructions (New England biolabs). Samples were purified using extract-clean SPE C18 columns (Grace Davison Discovery Sciences) activated with methanol and equilibrated in water. The flow through was lyophilised and the samples were permethylated for 2 hours in 200 µL DMSO, 10 mg NaOH and 300 µL ICH_3 under argon and strong shaking. The reaction was stopped with 1mL of 5% acetic acid and permethylated N-glycans were purified on a column Oasis HBL 6cc 200mg (Waters) activated in methanol and equilibrated in water. The column was washed with 5 % methanol and the sampes were eluted in 100 % methanol. For enzymatic defucosylation, disulfide bridges were reduced by 10

mM DTT in 0.1M NH_4HCO_3 at 56°C for 45 minutes and alkylated by 55mM iodacetamide in 0.1M NH_4HCO_3 for 30 minutes in the dark. After dialysis against water using Slide-A-lyzer mini dialysis devices, samples were lyophilised and defucosylated enzymatically before PNGAse F treatment. Permethylated N-glycans were analysed in the positive ion mode by MALDI-TOF-MS using an Ultraflex II (Bruker Daltonics). A total of 5 000 shots were accumulated per spectrum.

Statistical analysis

Data were examined for significance using Prism GraphPad software. One-way ANOVA (Bonferroni's multiple camparison) were performed for multiple comparisons. Quantification of adhesion on cells was performed at least 2 times in triplicate. Quantification of adhesion to immobilized recombinant protein was performed from at least 3 independent experiments.

SI APPENDIX REFERENCES

1. Bernard SC*, et al.* (2014) Pathogenic Neisseria meningitidis utilizes CD147 for vascular colonization. *Nature medicine* 20(7):725-731.
2. Durieu-Trautmann O*, et al.* (1993) Immortalized rat brain microvessel endothelial cells: II--Pharmacological characterization. *Adv Exp Med Biol* 331:205-210.
3. Weksler BB*, et al.* (2005) Blood-brain barrier-specific properties of a human adult brain endothelial cell line. *Faseb J* 19(13):1872-1874.
4. Schweitzer KM*, et al.* (1997) Characterization of a newly established human bone marrow endothelial cell line: distinct adhesive properties for hematopoietic progenitors compared with human umbilical vein endothelial cells. *Lab Invest* 76(1):25-36.
5. Pushkarsky T*, et al.* (2001) CD147 facilitates HIV-1 infection by interacting with virus-associated cyclophilin A. *Proceedings of the National Academy of Sciences of the United States of America* 98(11):6360-6365.
6. Maissa N*, et al.* (2017) Strength of Neisseria meningitidis binding to endothelial cells requires highly-ordered CD147/beta2-adrenoceptor clusters assembled by alpha-actinin-4. *Nature communications* 8:15764.
7. Coureuil M*, et al.* (2010) Meningococcus Hijacks a beta2-adrenoceptor/beta-Arrestin pathway to cross brain microvasculature endothelium. *Cell* 143(7):1149-1160.

Supplementary Figure S1

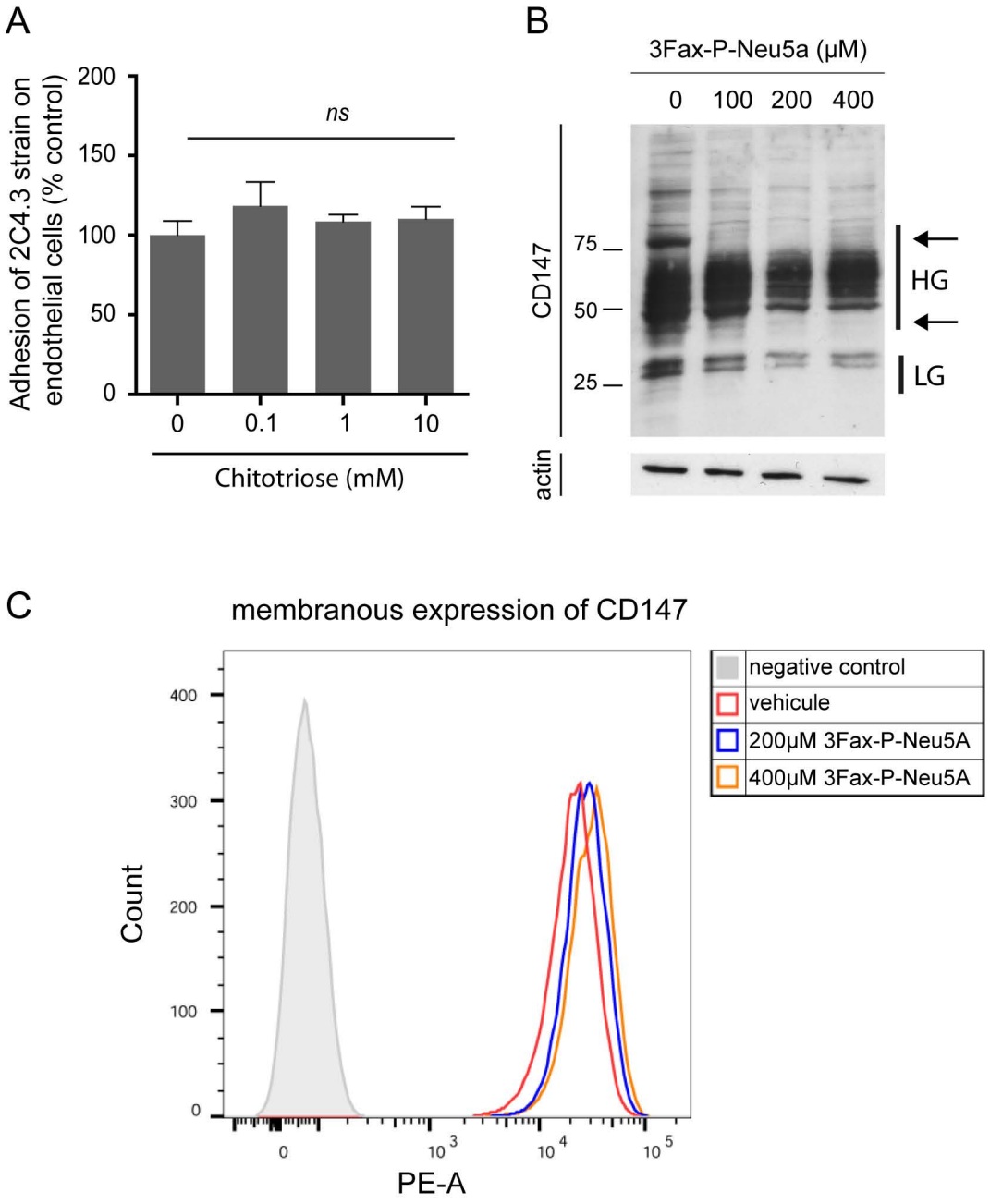


Figure S1

(A) Competing with GlcNAc does not affect bacterial adhesion. HCMEC/D3 cells were infected for 30 min in the presence of chitotriose, before determining CFU. Bars represent the percentage of bacterial adhesion in relation to the control condition (in the absence of chitotriose); ns: non significant; One-way ANOVA.

(B) Whole protein extracts were processed for SDS-Page and immunoblot analysis using anti-CD147 antibody. Brackets point to the highly glycosylated (HG) and the low glycosylated (LG) forms of CD147. Arrows point at the glycosylated forms of CD147 that disappear upon 3Fax-P-Neu5a treatment.

(C) HCMEC/D3 cells were treated for 3 weeks with varying concentrations of the sialyl transferase inhibitor 3Fax-P-Neu5Aa, or vehicle as a control, and the membranous expression of CD147 was assessed by flow cytometry using anti-hCD147 antibody (clone MEM-M6/6 from AbD Serotec).

Supplementary Figure S2

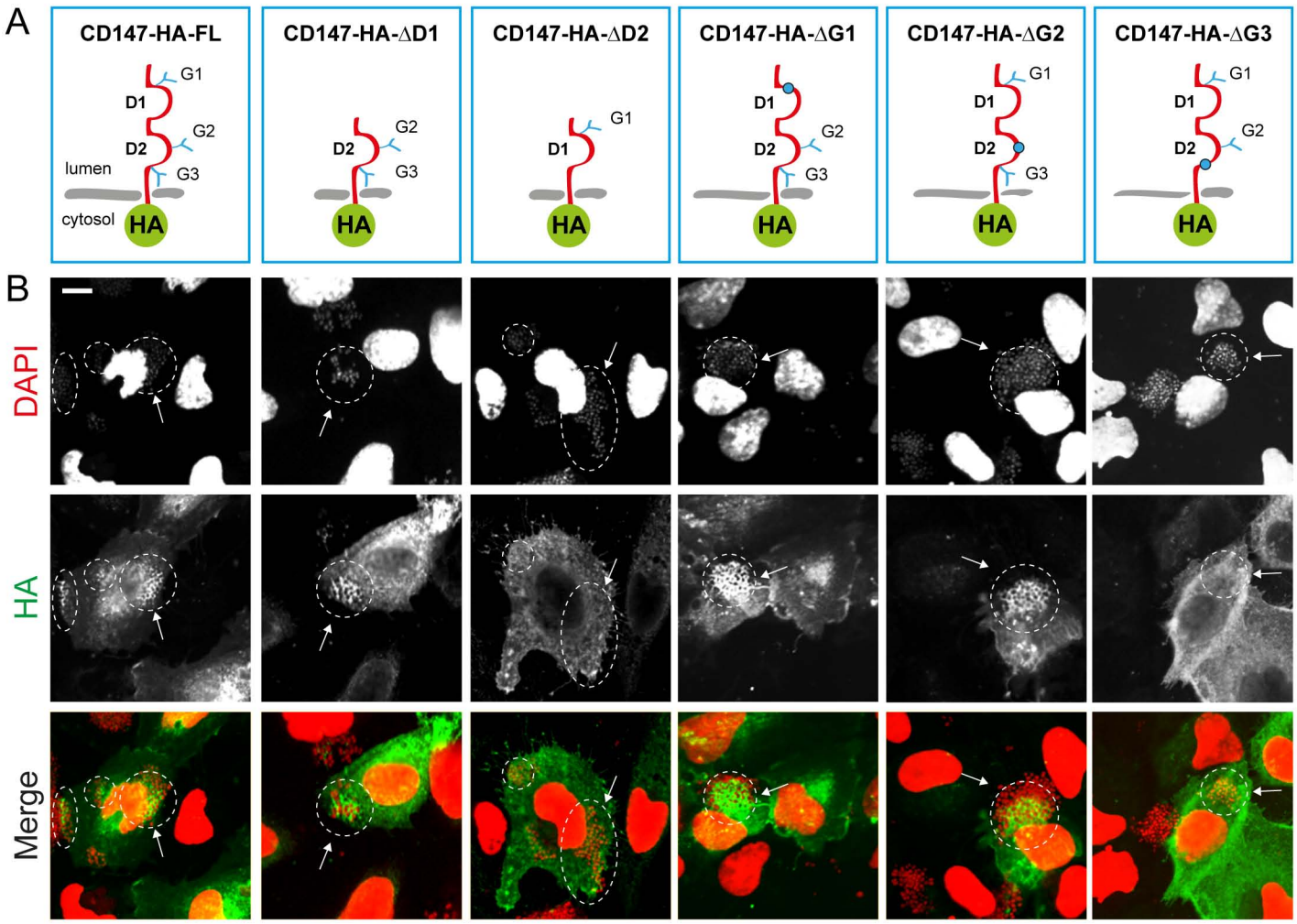


Figure S2:

(A) Schematic representation of the different engineered HA-tagged human CD147 constructs: full length (CD147-HA-FL), deleted of the distal Ig domain 1 (CD147-HA-ΔD1), deleted of the membrane proximal Ig domain 2 (CD147-HA-ΔD2), or lacking one of the three glycosylation sites by replacement of Asparagine 44, 152 or 186 to Alanine (CD147-HA-ΔG1, ΔG2 or ΔG3, respectively).

(B) HBMECs were transfected with the different constructs, infected with N. meningitidis 2C4.3 for 2h and processed for immunofluorescence staining with DAPI and anti-HA antibody before analysis by confocal microscopy. Scale bar: 10µm.

Supplementary Figure S3

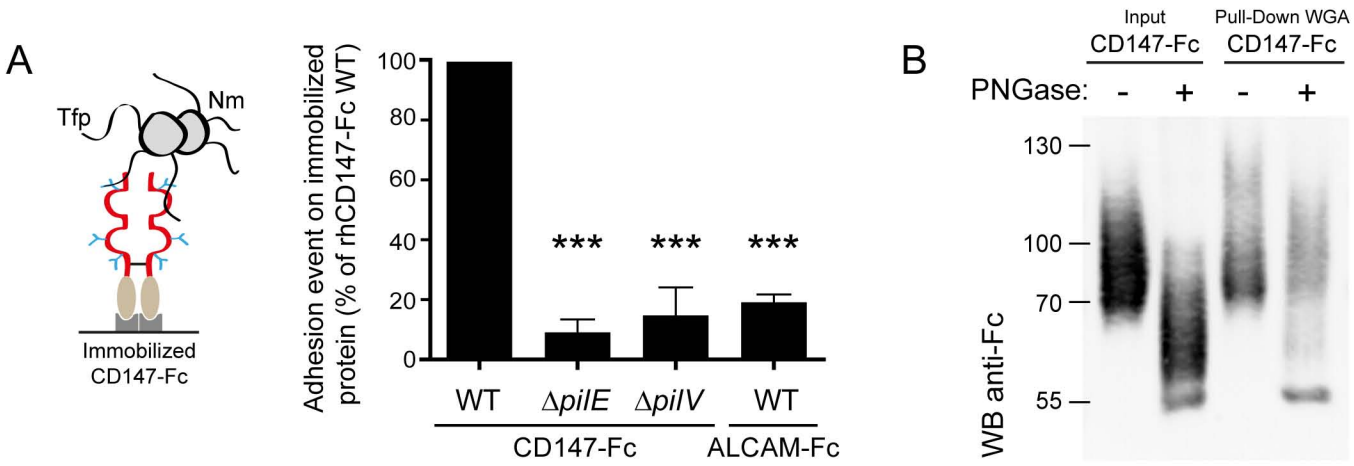


Figure S3:

(A) Adhesion of *N. meningitidis* 2C4.3 wild-type (WT) or ΔpilE and ΔpilV mutant derivatives to immobilized recombinant human CD147-Fc, or to immobilized recombinant human ALCAM–Fc after 1 hour of infection. Bars represent the percentage of adhesion events in relation to the adhesion of the WT strain on immobilized CD147-Fc *** : $p<0.001$; One-way ANOVA.

(B) CD147-Fc recombinant proteins were deglycosylated using PNGase or left in PNGase buffer alone as control before SDS-Page and analyse by western blot.

Supplementary Figure S4

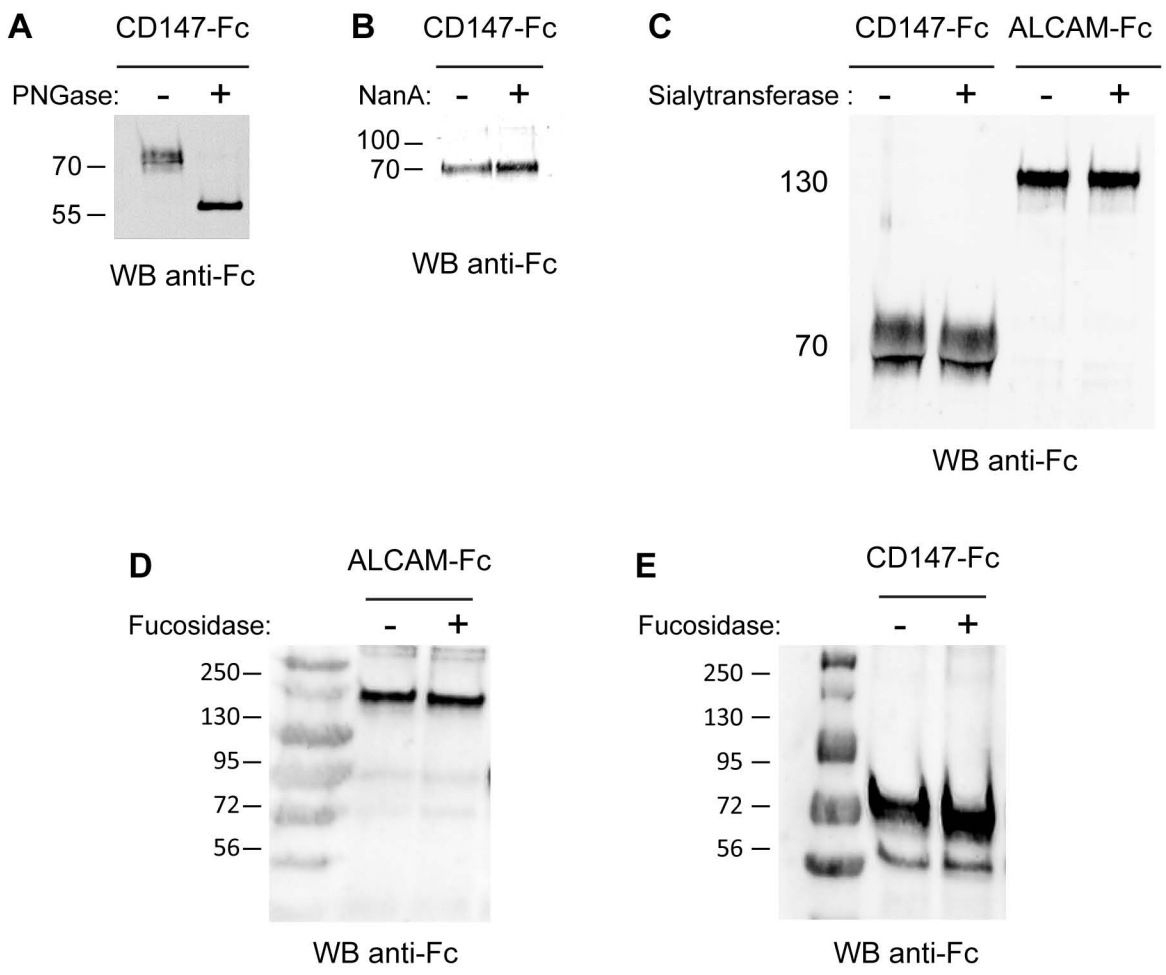


Figure S4:

Native and enzymaticcaly modified recombinants CD147-Fc and ALCAM-Fc were similarly immobilized on slides. CD147-Fc or ALCAM-Fc recombinant proteins were either deglycosylated using PNGase (A), desialylated using NanA (B), sialylated using sialyltransferase (C), or defucosylated using α-L-fucosidase (D,E) and immobilized on slides. To verify, in each assay, that native and ensymatically modified proteins were similarly immobilized on slides, proteins were collected in Laemmli buffer from a control well and analyzed be western blot using anti-Fc antibody (Jackson). Representative immunoblots are presented.

Supplementary Figure S5

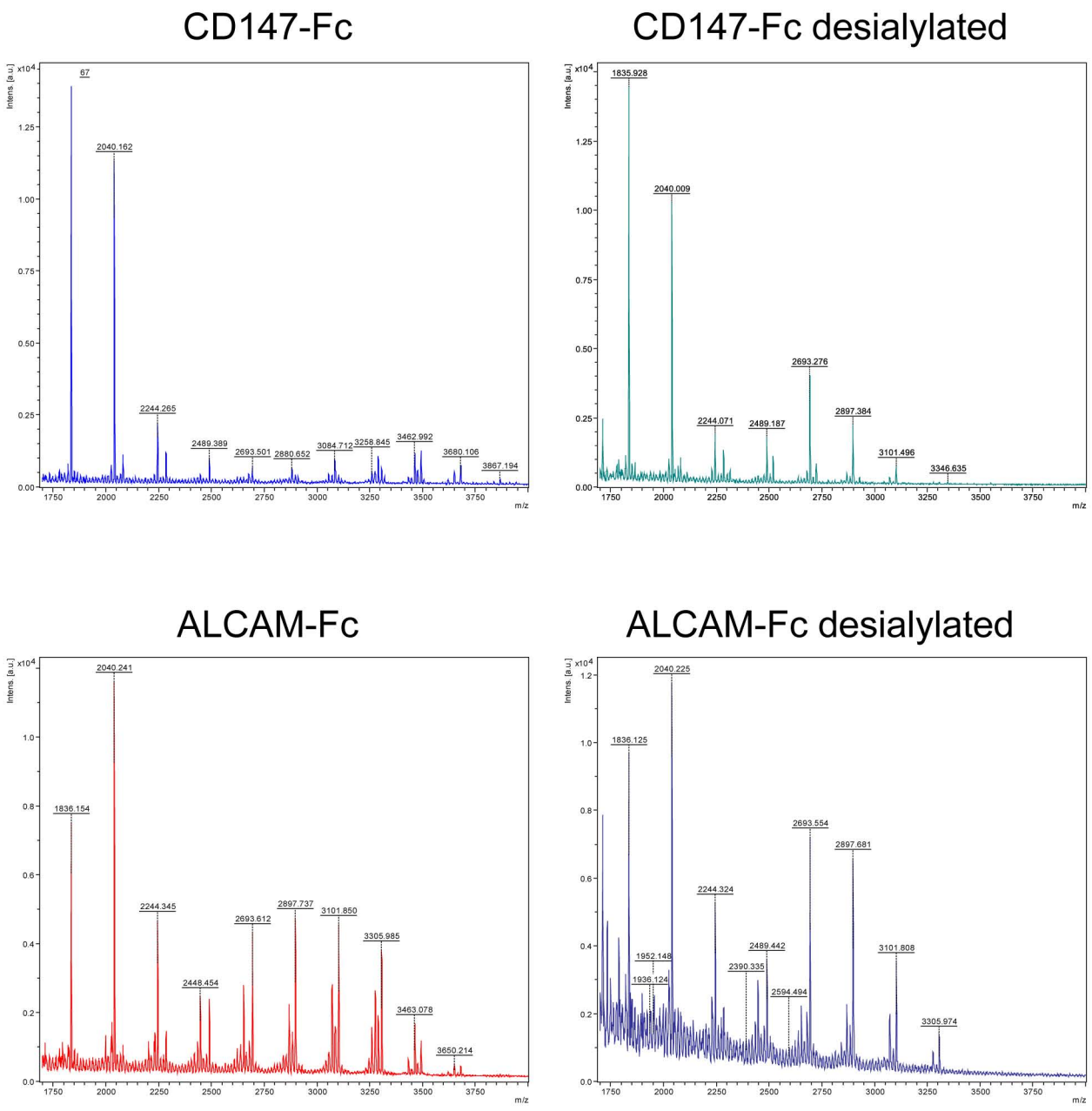


Figure S5:

Laser desorption ionization mass spectrum of desialylated and native recombinant CD147-Fc and ALCAM-Fc. Representative spectra out of 3 independent experiments.

Supplementary figure S6

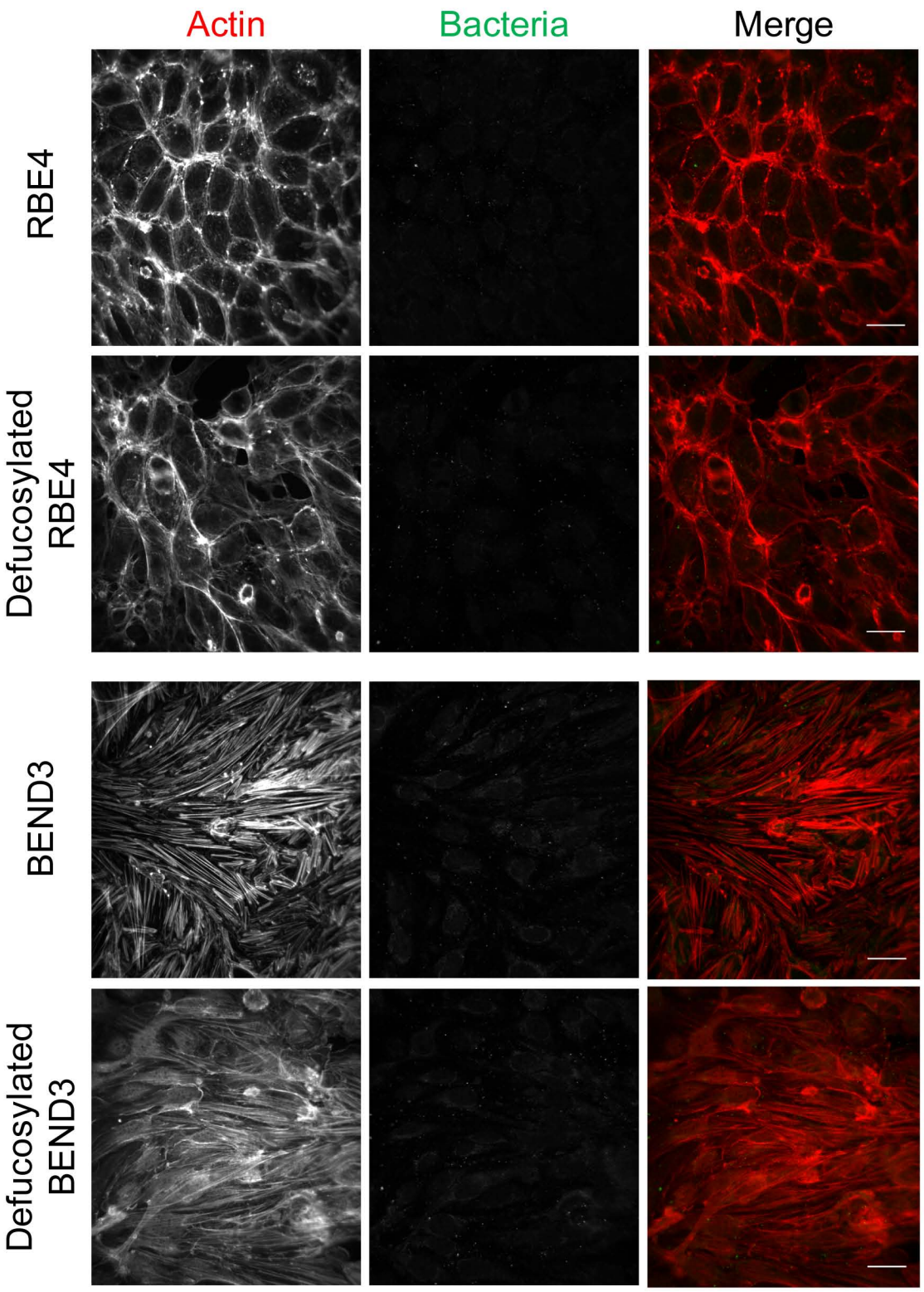


Figure S6:

Brain endothelial cells from mouse (BEND3) and rat (RBE4) origin were treated for 1h with 0.08U/mL human α-L-fucosidase or vehicle as control. Cells were then gently washed and infected by *N. meningitidis* 2C4.3 for 1h. Cells were fixed and stained for cellular actin (phalloïdin) and bacteria (anti-2C4.3) and analysed by confocal microscopy. The images are representative of two independent experiments performed in triplicate (scale bars, 10 µm).

Supplementary figure S7

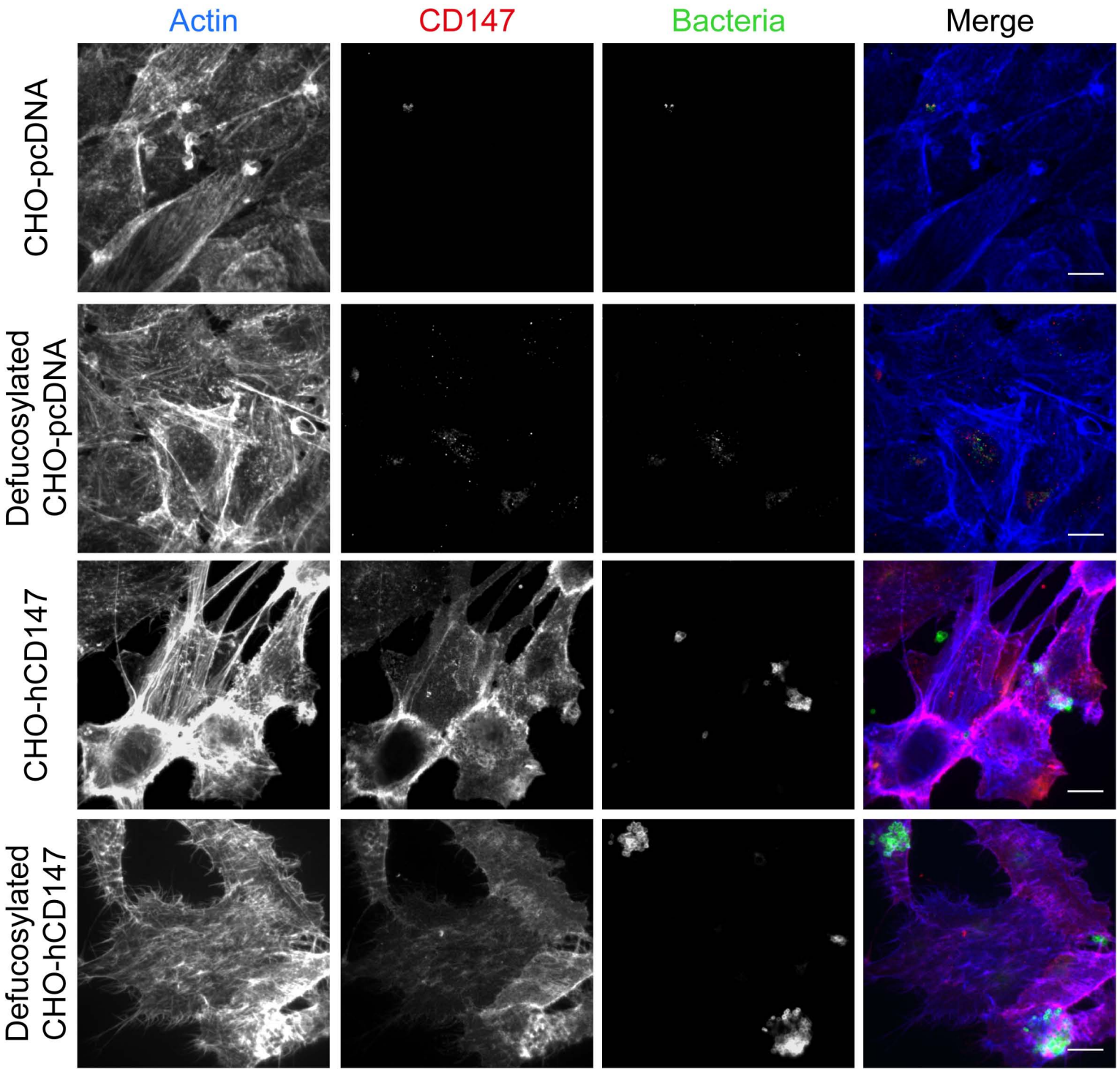


Figure S7:

CHO-pcDNA and CHO-hCD147 cells were treated for 1h with 0.08U/mL human α-L-fucosidase or vehicle as control. Cells were then gently washed and infected by *N. meningitidis* 2C4.3 for 1h. Cells were fixed and stained for cellular actin (phalloïdin), human Cd147 and bacteria (anti-2C4.3) and analysed by confocal microscopy. The images are representative of three independent experiments performed in triplicate (scale bars, 10µm).

Table S1

Inhibitory effect of lectin (20µg/ml) on meningococcal adhesion, determined on the basis of observation.

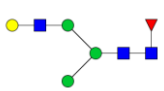
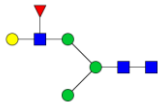
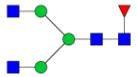
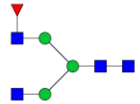
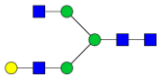
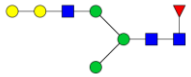
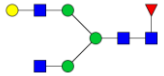
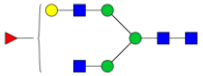
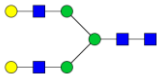
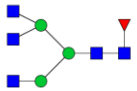
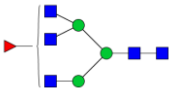
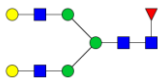
Lectins name	Target	Abbreviations	Inhibition of adhesion [a]
Concanavalin A	D-mannose or D-glucose	ConA	-
Glycine max (soybean) agglutinin	α/β N-acetylgalactosamine	SBA	-
Triticum vulgaris (wheat germ) agglutinin	sialic acid and poly N-acetylglucosamine	WGA	++
Dolichos biflorus agglutinin	α-N-acetylgalactosamine	DBA	-
Ulex europaeus agglutinin 1	α-L-Fucose ; anti-H blood group specificity	UEA 1	-
Ricinus communis agglutinin	β-D-galactosyl residues	RCA_{120}	-
Arachis hypogaea (peanut) agglutinin	galactosyl (β-1,3) N-acetylgalactosamine	PNA	-
Griffonia simplicifolia lectin I	terminal α-D-galactose	GSL I	-
Pisum sativum agglutinin	terminal α-D-mannose	PSA	-
Lens culinaris agglutinin	α-D-mannosyl and α-D-glucosyl residues	LCA	-
Phaseolus vulgaris Erythroagglutinin	red cell agglutination	PHA-E	-
Phaseolus vulgaris Leucoagglutinin	lymphocyte agglutination	PHA-l	-
Sophora japonica agglutinin	β-N-acetylgalactosamine	SJA	-
Succinylated wheat germ agglutinin	N-acetylglucosamine oligomers	sWGA	-
Griffonia simplicifolia lectin II	α/β N-acetylglucosamine	GSL II	-
Datura Stramonium lectin	(β-1,4) linked N-acetyl-D-glucosamine oligomers	DSL	-
Erythrina cristagalli lectin	D-galactose and D-galactosides	ECL	-
Lycopersicon esculentum (tomato) lectin	N-acetyl-β-D-glucosamine and N-acetyl-D-lactosamine oligomers	LEL	+
Solanum tuberosum lectin	N-acetyl-β-D-glucosamine and N-acetyl-D-lactosamine oligomers	STL	+
Vicia villosa agglutinin	N-acetyl-D-galactosamine	VVA	-
Maackia amurensis lectin	Sialic acid (α2-3) gal (β-1,4) glcNAc	MAL I / MAA	-
Maackia amurensis lectin II	Sialic acid (α2-3) N-acetyl-galactosamine	MAL II / MAH	-
Sambucus nigra bark lectin	Sialic acid (α2-6) gal (β-1,4) glcNAc	SNA	-

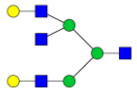
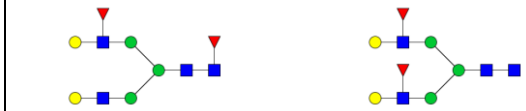
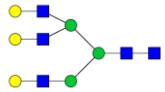
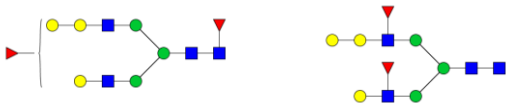
(a) ++ : strong inhibitory effect ; + : inhibitory effect ; - : no effect.

Sources: www.vectorslabs.com and www.sigmaaldrich.com

Table S2: Matrix-assisted laser desorption/ionization mass spectrometry analysis of recombinant CD147-Fc and ALCAM-Fc

Structure of untreated and desialylated recombinant CD147-Fc and ALCAM-Fc and defucosylated ALCAM-Fc. Expressed as percentage of the total ion population.

Mass	Core fucosylated	Fucosylated on GlcNAc	No Fucose	CD147-Fc	CD147-Fc desialylated	ALCAM-Fc	ALCAM-Fc desialylated	ALCAM-Fc defucosylated
1794				0%	0%	1.9%	0%	0%
1835				35.9	37.9	12.7	14.9	12.9
1865				0.9	1.7	0	0	0
1998				0	0	1.8	0	5.6
2040				28	26	19.5	21.4	13.4
2070				0.7	1.3	0	2.7	0
2081				2.1	1.9	1.2	0	0
2244				5	3.9	7.1	8.4	8.9

2285		2.1	2.6	1.8	3	2.6
2315		0	1.2	0	0	0
2418		0.7	0	1.2	2.1	0
2448		1	0.8	3.6	4.7	5.8
2489		2.1	3.5	2.8	5.3	5.5
2519		0	2	0	0	0
2622		0	0	1.4	1.9	0
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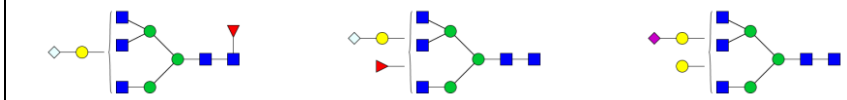
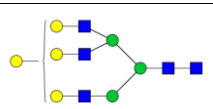
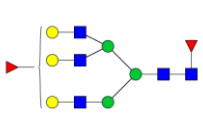
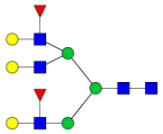
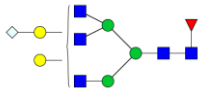
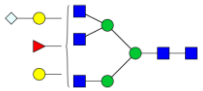
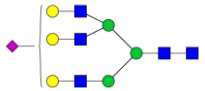
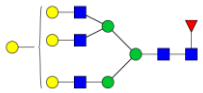
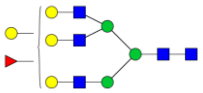
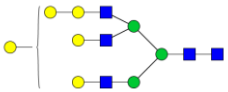
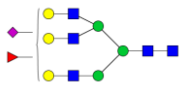
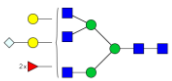
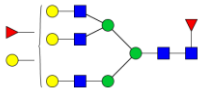
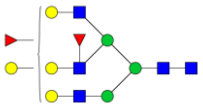
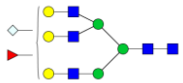
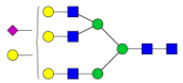
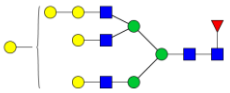
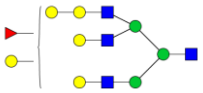
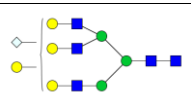
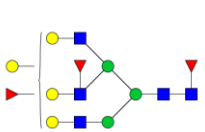
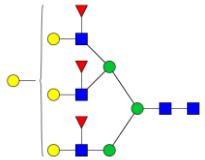
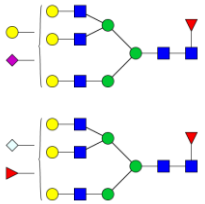
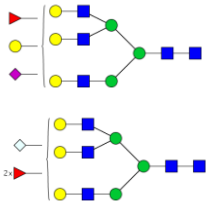
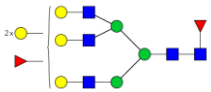
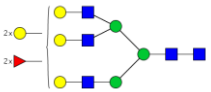
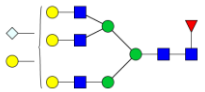
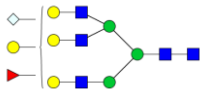
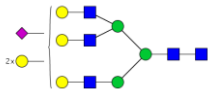
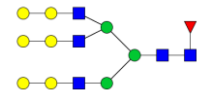
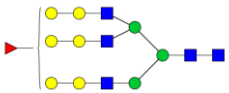
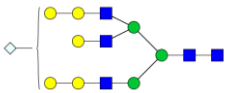
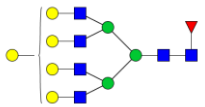
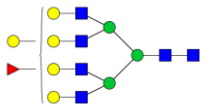
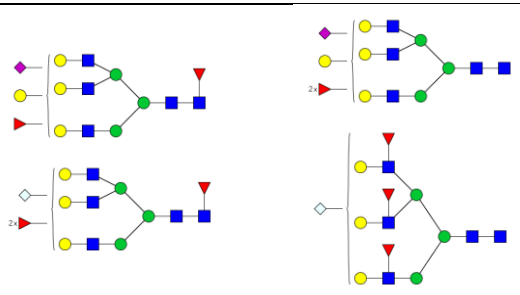
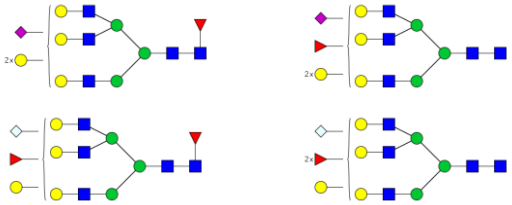
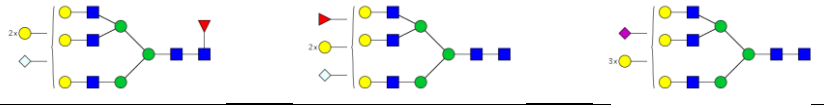
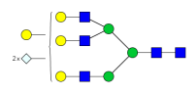
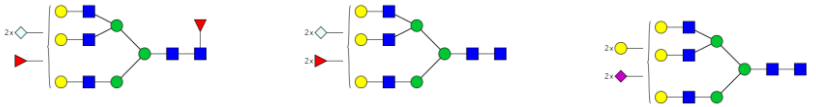
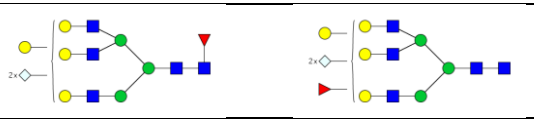
2676		0.8	0	0	0	0
2693		1.3	7.5	5.7	9.9	9.2

2723		0	1.4	0	1.4	0
2867	 	0	0.9	2.8	3	0
2880	  	1.1	0	1.2	0	0
2897	 	0.8	3.9	6	8.7	10.9
2927		0	0.7	0	0	0
3054	   	0.7	0	0	0	0
3071	 	0.7	0.6	3.4	2.3	0
3084	  	**1.5**	**0**	**0**	**0**	**1.7**
3101	 	0.8	1.3	5.4	4.3	8.8

3114		0.5	0	0	0	0
3245	 	0	0	0.8	0	0
3258	 	1	0	1.6	0	1.1
3275	 	0	0	2.8	1.1	0
3288	  	1.7	0	1.8	0	2
3305	 	1	0	4.4	1.5	5.8
3318		0.6	0	0	0	0
3346	 	0	0.3	0	0	0

3432		0.5	0	0.8	0	0
3462		1.9	0	1.8	0	0.8
3475		0.8	0	0.5	0	0
3492		1.8	0	1.2	0	1
3505		0.5	0	0	0	0
3619		0.4	0	0	0	0
3649		0.9	0	0.5	0	0

3679			1.1	0	0.5	0	0

Glycan symbols and colour coding as recommended by the Consortium for Functional Glycomics: ■ GlcNAc; ● Man; ○ Gal; ▲ Fuc; ◆ Neu5Ac; ◇ Neu5Gc

Table S3: Amino-acid sequence alignment of the human, rat, mouse and chinese hamster CD147/Basigin homologues

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SP|P35613|BASI_HUMAN      MAAALFVLLGFALLGTHGASGAAGFVQAPLSQQRWVGGSVELHCEAVGSPVPEIQWWFEG 60
SP|P26453|BASI_RAT        MAAALLLALAFTFLSGQGACAAAGFLKAPMSQEQWAGGSVVLHCEAVGSPMPEIQWWFEG 60
SP|P18572|BASI_MOUSE      MAAALLLALAFTLLSGQGACAAAGFLKAPLSQERWAGGSVVLHCEAVGSPIPEIQWWFEG 60
SP|Q99PA3|BASI_HAMSTER    RAAALLLALGFALLCGQGACAAA------------------------------------

SP|P35613|BASI_HUMAN      QGPNDTCSQLWDGARLDRVHIHATYHQHAASTISIDTLVEEDTGTYECRASNDPDRNHLT 120
SP|P26453|BASI_RAT        NEPNDSCSQLWDGARLDRVHIHATYRQHAASTLSVDGLAAEDTGTYECRASSDPDRNHLT 120
SP|P18572|BASI_MOUSE      NAPNDSCSQLWDGARLDRVHIHAAYRQHAASSLSVDGLTAEDTGTYECRASSDPDRNHLT 120
SP|Q99PA3|BASI_HAMSTER    ------------------------------------------------------------

SP|P35613|BASI_HUMAN      RAPRVKWVRAQAVVLVLEPGTVFTTVEDLGSKILLTCSLNDSATEVTGHRWLKGGVVLKE 180
SP|P26453|BASI_RAT        RPPRVKWVRAQASVVVLEPGTIVTSVQEVDSKTQLTCFLNSSGIDIVGHRWMRGGKVLQE 180
SP|P18572|BASI_MOUSE      RPPRVKWVRAQASVVVLEPGTIQTSVQEVNSKTQLTCSLNSSGVDIVGHRWMRGGKVLQE 180
SP|Q99PA3|BASI_HAMSTER    -----------------AAGTIQTSVNDVGSKTHLTCSLNSSGVDIIGHRWMRGGKILQE 89
                                            **: *:*:::.**  *** **.*. :: ****::** :*:*

SP|P35613|BASI_HUMAN      DALPGQKTEFKVDSDDQWGEYSCVFLPEPMGTANIQLHGPPRVKAVKSSEHINEGETAML 240
SP|P26453|BASI_RAT        DTLPDLQMKYTVDADDRSGEYSCIFLPEPVGRGNINVEGPPRIKVGKKSEHASEGEFVKL 240
SP|P18572|BASI_MOUSE      DTLPDLHTKYIVDADDRSGEYSCIFLPEPVGRSEINVEGPPRIKVGKKSEHSSEGELAKL 240
SP|Q99PA3|BASI_HAMSTER    DTLPDLQTQYTVDIDDRSGDYACIFLPEPVGRSNIVVEGPPRIKVGKKSEHSSEGENVRL 149
                          *:**. : :: ** **: *:*:*:*****:* .:* :.****:*. *.*** .*** . *

SP|P35613|BASI_HUMAN      VCKSE-SVPPVTDWAWYKITDSEDKALMN---GSESRFFVSSSQGRSELHIENLNMEADP 296
SP|P26453|BASI_RAT        ICKSEASHPPVDEWVWFKTSDTGDQ-TISNGTEANSKYVIISTPELSELIISDLDMNVDP 299
SP|P18572|BASI_MOUSE      VCKSDASYPPITDWFWFKTSDTGEEEAITNSTEANGKYVVVSTPEKSQLTISNLDVNVDP 300
SP|Q99PA3|BASI_HAMSTER    ICKSESSHPPVTEWSWFKTSDSGDQLIT-N--SSESKYVVISTADRSELTISNLDINSDP 206
                          :***: * **: :* *:* :*: ::        ::.::.: *:   *:* *.:*::: **

SP|P35613|BASI_HUMAN      GQYRCNGTSSKGSDQAIITLRVRSHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLD 356
SP|P26453|BASI_RAT        GTYVCNATNSQGSARETISLRVRSRLAALWPFLGIVAEVLVLVTIIFIYEKRRKPDQTLD 359
SP|P18572|BASI_MOUSE      GTYVCNATNAQGTTRETISLRVRSRMAALWPFLGIVAEVLVLVTIIFIYEKRRKPDQTLD 360
SP|Q99PA3|BASI_HAMSTER    GTYMCNATNTQGSVQEIMTLRVRSRLAALWPFLGIVAEVLVLVTIIFIYEKRRKPDQTLD 266
                          * * **.*.::*: :  ::*****::*******************************::.**

SP|P35613|BASI_HUMAN      DDDAGSAPLKSSGQHQNDKGKNVRQRNSS 385
SP|P26453|BASI_RAT        EDDPGAAPLKGSGSHLNDKDKNVRQRNAT 388
SP|P18572|BASI_MOUSE      EDDPGAAPLKGSGTHMNDKDKNVRQRNAT 389
SP|Q99PA3|BASI_HAMSTER    EDDPGAAPLKGSGHHMNDKDKNVRQRNAT 295
                          :** *:****.** * ***.*******::
```

C Cysteine engaged in disulfide bonds

N N-glycosylation sites

* conserved residue

. conservation between groups of weakly similar properties

: conservation between groups of strongly similar properties

Table S4: Primers used in this study

To introduce the HA tag into pcDNA3-CD147 vector:
CD147-HA Forward: 5' GTTCCAGATTACGCTTGAGGCAGGTGGCCCGAGGACG 3'
CD147-HA Reverse: 5' ATCGTATGGGTAGGAAGAGTTCCTCTGGCGG 3'
To delete the D1 domain of CD147:
CD147-del-D1 Forward: 5' CACGGGCCTCCCAGAGTGAAGGCTGTG 3'
CD147-del-D1 Reverse: 5' CCCGGAGGCTCCGTGGGTGCCCAGCAGC 3'
To delete the D2 domain of CD147:
CD147-del-D2 Forward: 5' CTCCGCGTGCGCAGCCACCTGGCCG 3'
CD147-del-D2 Reverse: 5' TCTGGGAGGCCCGTGGAGCTGGATGTTGG 3'
To delete glycosylation site 1 of CD147 domain 1:
D1-del-N1 Forward: 5' GCCGACAGCGCCACAGAGGTC 3'
D1-delN1 Reverse: 5' CAAGGAGCAGGTGAGGAG 3'
To delete glycosylation site 1 of domain 2:
D2-del-N1 Forward: 5'GCCGGCTCCGAGAGCAGGTTC 3'
D2-del-N1 Reverse: 5' CATGAGGGCCTTGTCCTC 3'
To delete glycosylation site2 of domain 2:
D2-del-N2 Forward: 5' GCCGGCACCAGCTCCAAGGGC 3'
D2-del-N2 Reverse: 5' GCACCGGTACTGGCCGGG 3'

Table S5: Antibodies used in this study

Target	*Reference*	*Provider*	*Condition of use*
Primary Antibodies			
hCD147 (Basigin)	MEM-M6/1	AbD Serotec	IF= 1/100
hCD147 (Basigin)	MEM-M6/6	AbD Serotec	IF= 1/100
HA	12CA5	Roche	IF= 1/100
hIgG Fc	109-005-098	Jackson	WB=1/100
Clathrin	610500	BD transduction Lab	WB 1/1000
Ezrin	Serum	Dr Paul Mangeat	IF= 1/1000
N. meningitidis 2C4.3	Serum	Dr Xavier Nassif	IF= 1/1000
DAPI	D9542	Sigma Aldrich	300nM
Rhodamine-phalloidin	P1951	Sigma Aldrich	IF= 1/200
Secondary antibodies			
Goat Anti-Mouse CY3	115-165-146	Jackson ImmunoResearch	IF= 1/500
Goat Anti-Mouse CY5	115-175-146	Jackson ImmunoResearch	IF= 1/500
Goat Anti-Rabbit CY3	111-165-144	Jackson ImmunoResearch	IF= 1/500
Goat Anti-Rabbit CY5	111-175-144	Jackson ImmunoResearch	IF= 1/500