MATERIALS AND METHODS

Reagents

Cell culture

The immortalized human brain microvessel endothelial cell line hCMEC/D3 which retains the main characteristics of primary brain endothelial cells, has been previously described (*S1*). hCMEC/D3 were grown at a density of 25 000 cells per cm² in flasks coated with 5 μ g/cm² rat tail collagen type I (BD Bioscience) in EBM-2 medium (Lonza) supplemented with 5% Fetal Calf Serum, 1% Penicillin-Streptomycin, 1.4 μ M hydrocortisone, 5 μ g.ml⁻¹ ascorbic acid, 10mM HEPES and 1ng.ml⁻¹ bFGF at 37°C in a humidified incubator in 5% CO2.

For permeability studies, these cells were seeded at a density of 50 000 cells per cm² on 0.4 μ m pore size Millicell® (Millipore) inserts pre-coated with rat tail collagen type I (thick coating modified from Bornstein and Murray (*S2*) for 12-15 days). The medium is changed every 3-4 days and 24h prior to the experiment; fresh medium is supplemented with Simvastatin 1nM.

The ability of the bacteria to traverse the monolayers of hCMEC/D3 cells was tested using cells grown on Millicell® inserts of $0.4\mu m$ (Fig. 4A, 4B) or $3\mu m$ pore size (Fig. 4C, 4D).

Plasmids

Plasmids coding for Eps15 dominant negative DIII-GFP, EH29-GFP and the control counterpart D3D2-GFP were generously provided by Dr. Alexandre Benmerah (*S3*) (Institut Cochin, CNRS (UMR 8104), Paris, France). Plasmids encoding par6-YFP, par6DN-myc (Par6 102-371), par3-myc and par3DN-myc (Par3 1-373) were generously provided by Dr. Ian Macara (*S4*) (University of Virginia at Charlottesville, Center for Cell Signaling: Department of Microbiology, USA). Par6DN-myc is able to bind Cdc42 but not PKCζ. Par3DN-myc is able to interact with Par6 but not with the downstream target. Plasmids encoding N17Cdc42-myc, N17Cdc42-GFP, N17Rac1-myc and N17Rac1-GFP were used in cotransfection assays (*S5*). Cells were transfected using AMAXA BIOSYSTEMS Nucleofactor Kit for HUVEC (Amaxa Biosystems), according to the manufacturer's protocol.

VE-cadherin shRNA expression vector (Clone ID: NM_001795) and TurboGFPTM shRNA Control Vector were purchased from Sigma-Aldrich. Puromycin (Sigma-Aldrich). Selection was introduced 1 day after transfections at 4μ g/ml. Selected clones were chosen for homogeneity of VE-cadherin knockdown by immunofluorescence assays and assessed by immunoblotting.

siRNA experiments

SiRNA were transfected using HUVEC cells AMAXA BIOSYSTEMS Nucleofactor Kit according to the manufacturer's protocol. Three days after transfection, the efficacy of knockdown was assessed by immunoblotting and immunofluescence assays. Infections with N. meningitidis were performed three days after transfection. p120-catenin siRNA 5'-GCCAGAGGUGGUUCGGAUA-3' (S6),Par6 siRNA 5'-5'-UACUUGGCUAUACGGAUGUU-3' *(S7)*, Cdc42 siRNA 5'-AAGUGGGUGCCUGAGAUAACU-3' non-targeting siRNA (S8).Α GATAAGGTGCTGCGTGGACCC-3' (Eurogentec) was used as control.

To silence Par-3, a pool of four siRNA duplexes (ON-Targetplus SMARTpool M-015602-00, Dharmacon) was used. The siCONTROL nontargeting siRNA (Dharmacon) was used as control.

Antibodies

The following polyclonal antibodies (pAb) and monoclonal antibodies (mAb) were used for immunofluoresence labelling or immunoblotting: anti-VE-cadherin rabbit pAb (Bender Medsystems), anti-VE-cadherin mouse mAb clone TEA1/31 (Beckman Coulter, France), anti-PAN-cadherin rabbit pAb (Cell Signaling Technology), anti-p120-catenin mouse mAb (BD Transduction Laboratories) and rabbit pAb (H-90), anti- β -catenin mouse mAb (both Santa Cruz Biotechnology, Inc), anti-ZO1 rabbit pAb, anti-Claudin-5 mouse mAb and anti-ZO2 mouse mAb (Zymed), anti-JAM-A goat pAb (R&D Systems), anti-ezrin rabbit pAb was generously provided by Dr. P. Mangeat (CNRS, UMR5539. Montpellier, France), anti-actin rabbit mAb (Sigma-Aldrich), anti-Par6 rabbit pAb (AbCam), anti ASIP/Par3 rabbit pAb (Millipore), anti-Cdc42 rabbit pAb (Cell Signaling), anti-cortactin mouse mAb clone 4F11 (Upstate), Arp2/3 complex was detected using the antibody raised against p16ARC: mouse mAb clone 323H3 (Synaptic Systems) generously provided by Dr. P. Chavrier (Membrane and Cytoskeleton Dynamics, CNRS, UMR144. Paris, France).

Drugs

Cycloheximide (CHX) was purchased from Sigma-Aldrich; Myristoylated Pseudosubstrate inhibitor for PKC ζ and PKC η were purchased from Biosource International; The Src kinase inhibitor PP2 was purchased from Calbiochem.

Bacterial strains and infection

Cells were infected with 2C43, a piliated capsulated Opa– variant of the serogroup C meningococcal strain 8013 (*S9, S10*). Non encapsulated derivatives of this strain were engineered by introduction of a *siaD* mutation. An isogenic non piliated and piliated Opa+ strain of this non encapsulated derivative of 2C43 was constructed, and designated SiaD Opa+ Δ pilE and SiaD Opa+, respectively. An isogenic non piliated derivative of 2C43 was constructed, and designated Δ pilE, An isogenic *pilT*- derivative of 2C43 was constructed using a previously described *pilT* mutated allele (*S11*) and designated Δ pilT. Briefly, the day before the infection the hCMEC/D3 culture medium was replaced with a serum-free medium (starvation medium: EBM2, 0.1% Bovine Serum Albumin). The day of infection, bacteria from an overnight culture were adjusted to OD600=0.05 and incubated for 2 hours at 37°C in prewarmed starvation medium. Bacteria were added to cells at a MOI of 100, allowed to adhere for 30 minutes. Unbound bacteria were harvested and infection was allowed to proceed for 2h. When needed, inhibitors and drugs were added to starvation medium 1h before infection.

Gentamicin protection assay

Cells grown on coverslips were infected at a MOI of 100. Bacteria were allowed to adhere for 30 minutes. Unbound bacteria were harvested and infection was allowed to proceed for 2h. Then cells were washed six times with phosphate-buffered saline (PBS) to remove nonadherent bacteria and incubated with fresh medium containing gentamicin (100 μ g/ml) at 37°C. After 30 min of incubation, the medium was removed; the cells washed again 4 times with PBS, harvested and vortexed in order to dissociate the

bacteria. Bacteria were enumerated by plating dilutions onto GCB agar. This usually corresponds to the number of internalized bacteria. In addition, the total number of cell-associated colony forming units (CFU) was determined after extensive washing of the cell monolayer followed by scraping the cells from the dish before plating dilutions. This number represents both adhesive and internalized bacteria. The number of internalized bacteria in relation to the number of adherent bacteria was determined. To determine if the above ratio of gentamicin protected bacteria had some relevance, a control was performed with the same number of bacteria but without cells.

Potassium depletion and cytosol acidification assay

Potassium depletion experiments were adapted from Puri *et al* (*S12*). Briefly, cells were rinsed with K+ free buffer (140mM NaCl, 20mM Hepes, 1mM CaCl2, 1 mM MgCl2, 1mg/ml D-glucose, pH 7.4) and then incubated in hypotonic buffer (K+ free buffer diluted 1:1 with distilled water) for 5 min. Cells were then washed three times in K+ free buffer and incubated for 20 min at 37°C in K+ free buffer. In order to have proper bacterial growth during the potassium depletion assay, starvation medium was added to K+ free buffer at the ratio 1:5 during meningococcal infection. Cytosol acidification experiments were adapted from Hansen *et al* (*S13*). Briefly, before infection cells were incubated in starvation medium containing 10mM acetic acid for 5 min and rinsed in starvation medium. After the first 30 min of infection cells were washed and incubated again in starvation medium. Infection was carried out for 2h in starvation medium.

Permeability studies

Permeability to Lucifer Yellow

In order to address changes in endothelial monolayer permeability, a small hydrophilic fluorescent molecule, Lucifer Yellow (LY) was used as a marker of tight junction integrity.

Permeability to LY was assessed on fully polarized hCMEC/D3 monolayer grown on $0.4\mu m$ pore size MilliCell® insert. Monolayers were infected at a MOI of 100, allowed to adhere for 30 minutes after which unbound bacteria were harvested and infection was allowed to proceed for 4h. Non infected monolayers were used as control. PKC ζ and η pseudosubstrate inhibitor were added to cell 1h before infection.

After 4h of infection, LY (50µM) was added to the cells on the insert, which is also the luminal compartment (or donor chamber), and the amount of LY found in the abluminal compartment was quantified. Incubations were performed at 37°C, 95% humidity and 5% C02 in transport buffer (EBM-2 medium (Lonza) plus 10mM HEPES and 1mM Sodium pyruvate) for 30 minutes. Endothelial permeability was calculated from the clearance rate and the surface area as described by Siflinger-Birnboim *et al.* (*S14, S15*). This allowed the determination of a concentration-independent permeability value. First the clearance was determined (clearance (mL) = X/Cd). Where X is the amount of LY (in mol) in the receptor chamber and Cd (mol.mL⁻¹) is the concentration in the donor chamber. During the 30min experiment, the clearance volume increases linearly with time. The average volume cleared is plotted versus time, and the slope estimated by linear regression analysis. The slope of the curve for the coated insert without cells: PSf.

Thus 1/PSt - 1/PSf = 1/PSe, where PSe is the permeability of the endothelial monolayer per unit of time (ml.min⁻¹). The PSe values were divided by the surface area (cm²) of the porous membrane. The resulting value is the endothelial permeability coefficient (Pe, in cm².min⁻¹). LY permeability values were expressed as fold increase compared to a control well of non infected and non treated cells. Following permeability studies, the LY mass balance was checked, and consistently found to be close to 100%. Each point was the mean value from six different inserts obtained from 3 series of independent experiments.

Because LY is described as a fluid-phase endocytic marker, the LY permeability coefficient was checked at 37°C and at 4°C, the latter temperature representing a condition in which endocytosis is inhibited. Values were identical, thus excluding that the LY crossed the monolayer using the endocytic pathway.

Permeability to N. meningitidis

HCMEC/D3 cells were grown on 3.0µm pore size MilliCell® insert and infected at a MOI of 10. Four hours after infection, the unit was placed in a well with fresh medium. The number of bacteria present in the abluminal compartment after 30 minutes was then determined. Simultaneously the same numbers of bacteria that are present in the luminal compartment of the infected wells were placed in a MilliCell® insert which does not have a cellular monolayer. We then determined the number of CFU in the abluminal compartment of the insert containing a monolayer of endothelial cells (CFU_{cell}) and of an insert which does not contain an endothelial cell monolayer (CFU_{insert}). We then determined the ratio (CFU_{cell} / CFU_{insert}). We refer to this result as diffusion of *N. meningitidis*. Results in figure 4D are expressed as fold increase of the diffusion of the tested strain as compared to the piliated capsulated strain through a non treated monolayer.

In order to estimate surface and number of macroscopic cell-cell junction opening, MilliCell® insert were then fixed in 4% PFA and immunostained for VE-cadherin and actin. Several baso-lateral cross-sections of each insert were acquired using a Leica SP5 confocal microscope. We determined the surface and number of gaps within the monolayer using the Leica software: *Leica Application Suite-AF (LAS-AF)*. Data are expressed as the number of gaps per $4.10^5 \mu m^2$ of hCMEC/D3 monolayer surface. The size of the gaps was determined in terms of the size of a *N. meningitidis* diplococcus. DAPI staining allowed us to estimate the apparent surface of a diploccocus at $1.7 \mu m^2$. Thus 1.7 to 3.4 μm^2 corresponds to 1 to 2 diplococci; 3.4 to 7.6 μm^2 : 2 to 4 diplococci; 7.6 to 15 μm^2 : 4 to 8 diplococci; 15 to 30 μm^2 : 8 to 16 diplococci; and >30 μm^2 : more than 16 diplococci.

Immunofluescence and analysis of protein recruitment under N. meningitis colonies

HCMEC/D3 cells were grown to confluence on 12-mm diameter glass coverslips coated with 5 μ g/cm² of rat tail collagen type I. Immunofluorescence assays were performed 2h post-infection. Cells were fixed in PFA 4% in PBS for 20 min and permeabilized for 5 min with PBS containing 0.1% Triton X-100. Cells were incubated with primary antibody diluted 1:50, 1:100 or 1:200 in PBS containing 0.3% serum albumin bovin for 1h. Alexa conjugated phalloidin and DAPI (0.5 μ g/ml) were added to Alexa conjugated secondary antibody (Invitrogen, France) diluted 1:200 in PBS containing 0.3% bovin serum albumin for 1h. After several washes, coverslips were mounted in mowiol. Image

acquisition was performed on laser-scanning confocal microscopes (Zeiss LSM 510 Pascal and Leica SP5). Images were collected and processed using LSM Image Browser (Zeiss) and Leica Application Suite AF lite (Leica) software, respectively. Z-axis reconstructions were obtained from stacks of 0.12µm interval images. 3D reconstructions of stack of 0.12µm were obtained using IMARIS 6.1.5 software.

<u>Recruitment efficiency</u> was estimated by determining the presence of the immunostained, tagged protein or Alexa coupled phalloidin-staining in at least 50 colonies per coverslip. Each experiment was repeated at least 3 times in duplicate. Percentage of recruitment was expressed as mean percentage of recruiting colonies \pm SEM. Data was consequently tested for significance using Student's *t*-test.

<u>Relocalization of membrane bound VE-cadherin</u> was followed by pulse-chase assay using a TEA1/31 mouse monoclonal antibody able to interact with the extracelluar domain of VE-cadherin. Cells grown on coverslips were incubated 15 min at room temperature with 0.01μ g/ml of antibody. For cell monolayers grown on MilliCell® insert, antibody was added only to the abluminal compartment. Cells were washed several times before infection and infected as described above. Cells were then fixed with PFA 4% and the TEA1/31 antibody was detected by a secondary antibody coupled to Alexa 488. Results obtained for cell grown on coverslips or on MilliCell® inserts were similar.

Immunoblots

HCMEC/D3 cells were washed with cold PBS and lysed in modified RIPA buffer (50mM Tris pH 7.5, 2mM EDTA, 150mM NaCl, 0.1 % (w/v) SDS, 0.5 % (w/v) Na deoxycholate, 2mM orthovanadate, 5mM NaF, 1mM PMSF, 10 μ g/ml aprotinin, 10 μ g/ml leupeptin, 10 μ g/ml pepstatin). Whole cell lysates were then boiled and analyzed by SDS-PAGE.



N. meningitidis recruitment of the ectopic junction-like domain is type IV pili dependent. (A) Adhesion of the non piliated Opa+ non capsulated strain (SiaD Opa+ Δ pilE) does not induce the formation of cortical plaque and the recruitment of junctional proteins VE-cadherin, p120-catenin, ZO-1 and ZO-2. On the other hand, an isogenic piliated (P+) Opa+ non encapsulated strain (SiaD Opa+) had a phenotype similar to that of the wild type encapsulated piliated strain, i.e. 40% of the colonies recruit the above junctional proteins. Arrows indicate the location of bacterial colonies. Scale bars: 10 µm. (B) Adhesion of the SiaD Opa+ Δ pilE strain does not induce recruitment of over-expressed par6-YFP and par3-myc. On the other hand an isogenic P+ Opa+ non encapsulated (SiaD Opa+) strain had a phenotype similar to that of the wild type capsulated piliated strain, i.e. 40% of the bacterial colonies recruit the above polarity proteins, thus ruling out the possibility that the polysacharidic capsule has an effect by itself on the signaling. Arrows indicate the location of bacterial colonies. Scale bars: 10 µm. (C) VE-cadherin, p120-catenin, β-catenin and ZO-1 are strictly localized at cell-cell junctions of non infected cells. Scale bars: 20 µm. (D, E) Adhesion of the *pilT*- strains (Δ pilT), which is deficient in twitching motility, had a phenotype similar to that of the wild type strain. (D) The *pilT*- strain recruits VE-cadherin, p120-catenin and actin. Arrows indicate the location of bacterial colonies. Scale bars: 10 µm. (E) 40% of the colonies recruit the above junctional proteins. Data are expressed as mean +/- SEM.



Figure S2

The Cdc42-Par3/Par6/PKCζ pathway controls the recruitment of N. meningitidis induced ectopic junction-like domains. (A) HCMEC/D3 cells were transfected with tagged dominant negative mutants of Cdc42 or Rac1 (N17Cdc42 and N17Rac1). The latter was used as a control. To assess the ability of the transfected cells to recruit Par6 and Par3, the cells were cotransfected with par6-YFP or par3-myc, respectively. N17Cdc42 expression reduced the recruitment of par6-YFP and par3-myc by 3 and 5 fold, respectively. * t test (p<0.001). (B) Cells were transfected with dominant negative mutants of Cdc42 or Rac1 (N17Cdc42 and N17Rac1). The recruitment of VE-cadherin, p120-catenin and actin was determined by immunofluorescence assay. N17Cdc42 expression reduced the recruitment of VE-cadherin, p120-catenin and actin by 3.4 fold, 3 fold and 3.7 fold, respectively. * t test (p<0.001). It should be pointed out that the inhibition of the GTPase Rac1 did not inhibit the recruitment of components of the polarity complex and of the junctional proteins. (C, D) HCMEC/D3 cells were transfected with the dominant negative forms of Par6 and Par3, par6DN and par3DN, respectively. Control cells were transfected with par6-YFP or par3-myc. The recruitment of VE-cadherin, p120-catenin and actin was determined by immunofluorescence assay. (C) Compared to the control, par6DN expression reduced the recruitment of VE-cadherin, p120-catenin and actin by 2 fold, 2.5 fold and 2.4 fold, respectively. * t test (p<0.01). (D) Compared to the control, par3DN expression reduced the recruitment of VE-cadherin by 2.8 fold. * t test (p<0.01). (E) HCMEC/D3 cells were co-transfected with (i) par3-myc and, par6-YFP or Par6DN, (ii) par6-YFP and, par3-myc or Par3DN. Par6DN reduced par3-myc recruitment by 3.5 fold. * t test (p<0.001). Data are expressed as mean +/- SEM.



Figure S3

The Cdc42, Par6 and Par3 differentially control the recruitment of VE-cadherin, p120-catenin and actin honeycombs. Knockdown of Cdc42 and Par6 was performed using specific siRNA duplex (Cdc42 siRNA and Par6 siRNA, respectively). Knockdown of Par3 was performed using a pool of four siRNA duplexes (Par3 siRNA). Knockdown cells were infected and fixed 2h post infection. (A) The efficacy of Cdc42, Par6 and Par3 silencing was determined by immunoblots using total cell lysates. (B) Knockdown of Cdc42 and Par6 inhibit VE-cadherin and actin recruitment under *N. meningitidis* colonies. Knockdown of Par3 inhibit VE-cadherin recruitment but not that of actin recruitment. VE-cadherin was revealed by immuno-fluorescence assay (green). Actin filaments were revealed using Alexa 546 phalloïdine (red). Arrows indicate the location of bacterial colonies. Scale bar: 10µm.



P120-catenin is key in the recruitment of both actin and AJ proteins. (A) The efficacy of the VE-cadherin silencing (VEC shRNA) was determined by immunoblot using total cell lysates. GFP shRNA was used as control. (B) Silencing of p120-catenin (p120-siRNA) was determined by immunoblot on total cell lysates. A scramble siRNA duplex was used as control. (C) Silencing of p120-catenin inhibited cortactin recruitment under N. meningitidis colonies (left panel) as well as recruitment of p16-ARC (a member of the Arp2/3 complex) (right panel). Recruitment of cortactin and p16-ARC was determined by immunofluorescence assay. Areas outlined in white indicate the presence of a N. meningitidis colony. Scale bars: 10µm. It should be pointed out that cortactin and p16-ARC systematically colocalized with actin under N. meningitidis colonies. (D) HCMEC/D3 cells were incubated with 10µM of the Src kinase inhibitor PP2. Two hours after infection, p120-cateninin was recruited underneath the microcolonies while VE-cadherin and actin are not. Arrows indicate the location of bacterial colonies. Scale bars: 10µm (E) HCMEC/D3 cells transfected with a Cdc42 siRNA or control siRNA were incubated with 10µM PP2 or left untreated. Knockdown of Cdc42 in addition to the Src kinase inhibitor PP2 reduced VE-cadherin, p120-catenin and actin recruitment by 4.5 fold, 3 fold and 3.5 fold, respectively. Results are identical to that obtained when Cdc42 alone was inhibited. On the other hand, inhibition of the Src Kinase alone with PP2 reduced VE-cadherin and actin recruitment by 4 fold and 2.5 fold, respectively, but not that of p120catenin. These data are consistent with the hypothesis that following recruitment of p120-catenin underneath colonies by a Cdc42 pathway, actin and VE-cadherin are recruited following cortactin phosphorylation by the Src kinase. The Src kinase has been previously shown to be activated following the phosphorylation of the ErbB2 transmembrane receptor induced by its homodimerisation. The activation of the Src kinase is responsible for the phosphorylation of the cortactin (S16).



VE-cadherin targeting required endocytosis of clathrin coated pits. (A) The recruited VE-cadherin is not synthesized *de novo* but is recruited from an existing pool. HCMEC/D3 cells were treated with the inhibitor of protein biosynthesis cycloheximide (50μ g/ml CHX). The percentage of *N. meningitidis* colonies recruiting VE-cadherin, p120-catenin and actin were identical to that of non treated monolayers. (B, C) Clathrin coated pit formation is required for VE-cadherin recruitment beneath *N. meningitidis* colonies. Clathrin coated pit formation was inhibited (i) by performing potassium depletion and cytosol acidification (shown in panel B) (*S12, S13*) and (ii) by transfecting a GFP-Eps15 dominant negative mutant (DIII), a GFP plasmid (D3D2) (*S3*) was used as a control (shown in panel C). The percentage of *N. meningitidis* colonies that recruits VE-cadherin, p120-catenin and actin was determined. Recruitment of VE-cadherin was reduced by potassium depletion and cytosol acidification (2.4 fold) and by the transfection of the GFP-Eps15 dominant negative mutant (3 fold). * *t* test (*p*<0.001). Data are expressed as mean +/- SEM.



Figure S6

Tagged VE-cadherin is recruited from cell-cell junction. (A, B) Membrane bound VE-cadherin is recruited beneath *N. meningitidis* colonies. HCMEC/D3 cells grown on coverslips were pre-incubated with 0.01µg/ml of mouse monoclonal VE-cadherin antibody clone TEA1/31. TEA1/31 bound VE-cadherin was detected post infection by an Alexa conjugated antibody raised against mouse IgG. TEA1/31 bound VE-cadherin (green) was recruited beneath *N. meningitidis* colonies visualized by DAPI staining (blue).

(A, B) Stack of 0.12µm interval images was acquired using a Leica SP5 confocal microscope. Scale bar: 10µm.

(A) Left panel: apical x,y cross section. This panel shows relocalisation of TEA1/31 bound VE-cadherin at the apical surface of cells. White asterisks indicate apical localization of tagged VE-cadherin. Middle panel: baso-lateral x,y cross section. This panel shows a dramatic reduction of TEA1/31 bound VE-cadherin staining at cell-cell contact. The cells are designated 1 though 6. The cell 1 carries the bacterial microcolony and the juxtaposing cells are cell 2 though 6. This reduction is concomitant with apical localization of TEA1/31 bound VE-cadherin. Right panel: merge of the baso-lateral cross section (VE-cadherin: green ; DAPI staining: blue).

(B) 3D reconstruction of an intercellular junction corresponding to the areas outlined in yellow shown on A (left panel). Numbering of cells is identical to that in A.

Middle panel (B.b) Staining of the junctional TEA1/31 bound VE-cadherin is outlined in blue. Staining of apical relocalization of TEA1/31 bound VE-cadherin is included within the blue dotted lined.

Top and botton panel (B.a and B.c) B.c is identical to B.a but shows in addition the DAPI staining. Both panels correspond to a rotation of the 3D reconstruction according to the yellow axis annotated "view 1" in B.b. B.a and B.c show a clear reduction of junctional TEA1/31 bound VE-cadherin staining at cell-cell contact between cell 1, which is infected, and cell 4, whereas the staining between cell 4 and 3 on one hand, and 4 and 5 on the other hand is conserved. These images clearly show interruption of the junctional staining in infected cells, where the VE-cadherin staining is relocated to the apical surface of the cell underneath the bacteria visualized by DAPI staining. Each square of the grid is 5µm x 5µm. (see also Movie S2).



Figure S7

Schematic representation of the signaling pathways activated by *N. meningitidis* Tfp dependent adhesion. Type-IV pili initiate the interaction of virulent capsulated *Neisseria meningitidis* with human endothelial cells by interacting with a yet non identified cellular receptor. This interaction induces the recruitment of ezrin, the clustering of transmembrane proteins (such as ErbB2, CD44 and ICAM-1) (*S16, S17*) and leads to Cdc42 activation and recruitment of the polarity complex Par6/PKC ζ . Activation of the polarity complex promotes recruitment of p120-catenin which is necessary for cortactin and Arp2/3 recruitment under *N. meningitidis* colonies. In the mean time, Src tyrosine kinase, activated by the homodimerisation of ErbB2 in the cortical plaque (S17), promotes cortactin phosphorylation and actin polymerization. In addition following the recruitment of p120-catenin, junctional proteins are actively recruited. Targeting of VE-cadherin requires also Par3 recruitment by the Par6/PKC ζ complex. Ultimately, recruitment of p120-catenin and junctional proteins favor cell-cell junctional leakage.

Movie S1 Formation of the *N. meningitidis* microcolony onto the apical surface of endothelial cells. Endothelial cells grown on a glass slide were placed under a fluorescence microscope at 37° C and 5% CO2. Bacteria growing on the top of the cells were followed by the use of a time-lapse video microscopy. Real-time duration is 200 min.

Movie S2

3D reconstruction from a stack of 0.12μ m interval images, acquired using a Leica SP5 confocal microscope. TEA1/31 bound VE-cadherin (green) is relocated from cell-cell junctions underneath the *N. meningitidis* colony.

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