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Vitronectin Binds to a Specific Stretch within the Head Region of Yersinia Adhesin A and Thereby Modulates Yersinia enterocolitica Host Interaction

Mühlenkamp, Melanie C.; Hallström, Teresia; Autenrieth, Ingo B.; Bohn, Erwin; Linke, Dirk; Rinker, Janina; Riesbeck, Kristian; Singh, Birendra; Leo, Jack C.; Hammerschmidt, Sven; Zipfel, Peter F.; Schütz, Monika S.

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PO Box 117
221 00 Lund
+46 46-222 00 00

1 **Vitronectin binds to a specific stretch within the head region of *Yersinia***
2 **adhesin A and thereby modulates *Yersinia enterocolitica* host interaction**

3 Running title: YadA-mediated interaction of *Ye* with vitronectin

4

5 Melanie C. Mühlenkamp*, Teresia Hallström†, Ingo B. Autenrieth*, Erwin Bohn*, Dirk
6 Linke‡, Janina Rinker*, Kristian Riesbeck§, Birendra Singh§, Jack C. Leo‡, Sven
7 Hammerschmidt¶, Peter F. Zipfel†|| and Monika S. Schütz*

8

9 *) Institute for Medical Microbiology and Hygiene, University Hospital Tübingen, Elfriede-
10 Aulhorn-Str. 6, 72076 Tübingen, Germany

11 †) Department of Infection Biology, Leibniz Institute for Natural Product Research and
12 Infection Biology, Hans Knöll Institute, Jena, Germany

13 ‡) Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo,
14 Norway

15 §) Clinical Microbiology, Dept. of Translational Medicine, Lund University, Jan
16 Waldenströms gata 59, SE-205 02 Malmö, Sweden

17 ¶) Department Genetics of Microorganisms, Interfaculty Institute for Genetics and Functional
18 Genomics, Ernst-Moritz-Arndt-Universität, Greifswald, Greifswald, Germany

19 ||) Friedrich Schiller University Jena

20

21 Corresponding author: Dr. Monika Schütz, Institute for Medical Microbiology and Hygiene,
22 University Hospital Tübingen, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany, email:
23 monika.schuetz@med.uni-tuebingen.de, phone: +49-7071-2981527 fax: +49-7071-294972

24

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26 **ABSTRACT**

27 Complement resistance is an important virulence trait of *Yersinia enterocolitica* (*Ye*). The
28 predominant virulence factor expressed by *Ye* is the *Yersinia* adhesin A (YadA), which
29 enables bacterial attachment to host cells and extracellular matrix (ECM) and additionally
30 allows acquisition of soluble serum factors. The serum glycoprotein vitronectin (Vn) acts as
31 an inhibitory regulator of the terminal complement complex (TCC) by inhibiting the lytic pore
32 formation. Here, we show YadA-mediated direct interaction of *Ye* with Vn and investigated
33 the role of this Vn-binding during mouse infection *in vivo*. Using different *Yersinia* strains,
34 we identified a short stretch in the YadA head domain of *Ye* O:9 E40, similar to the “uptake
35 region” of *Y. pseudotuberculosis* (*Yps*) YPIII YadA, as crucial for efficient Vn binding. Using
36 recombinant fragments of Vn, we found the C-terminal part of Vn, including the heparin-
37 binding domain (HBD) 3, to be responsible for binding to YadA. Moreover, we found that Vn
38 bound to the bacterial surface is still functionally active and thus inhibits C5b-9 formation. In
39 a mouse infection model, we demonstrate that Vn protects *Ye* from complement-mediated
40 lysis and thus improved bacterial survival. Taken together, these findings show that YadA-
41 mediated Vn binding influences *Ye* pathogenesis.

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49 INTRODUCTION

50 *Yersinia enterocolitica* (*Ye*) and *Yersinia pseudotuberculosis* (*Yps*) are enteropathogens
51 causing enteric and systemic diseases [1,2]. Besides the chromosomally encoded adhesins
52 Invasin and Ail [3-5], the trimeric autotransporter adhesin (TAA) YadA is the decisive factor
53 that determines pathogenicity of *Ye* [6]. YadA forms rigid fibrous structures which protrude ~
54 23 nm from the cell surface [7,8] and mediates adhesion to ECM proteins such as collagen,
55 fibronectin and laminin, and to complement factors [9]. Being the prototype of the TAA
56 family of proteins, YadA is characterized by a modular composition of several domains: the
57 extracellularly located N-terminal head domain is followed by a connector element (also
58 called the neck region) leading into a coiled-coil stalk. The stalk is connected to the C-
59 terminal translocator or membrane anchor domain, consisting of 4 β -strands per monomer [9].
60 To form a functional adhesin on the bacterial surface, three YadA monomers trimerize and
61 form the pore of the translocator domain, which is inserted into the outer membrane [10]. The
62 translocator enables the transport of the passenger domains onto the bacterial surface, where
63 they also form obligate trimers [9].

64 YadA knockout strains of *Ye* are avirulent and do not cause infection in a mouse infection
65 model [11-13]. This striking effect has been attributed mainly to the reduced efficiency of
66 effector protein (Yop) delivery by a dedicated type three secretion system (T3SS), which
67 requires proper adhesion to host cells and loss of adherence results in the inability to resist
68 phagocytosis [14,15]. However, in *Yps*, which is more closely related to *Yersinia pestis*, YadA
69 is dispensable for virulence and Yop injection [16]. YadA of *Yps* and *Ye* do not only differ in
70 their role during infection, but also in sequence and binding repertoire of host ECM proteins
71 and cellular receptors. YadA of *Yps* carries an additional stretch within its head region that
72 enables entry into host cells [17]. This important stretch is absent in YadA of several *Ye*

73 serotypes and strains. Moreover, the binding capacities of YadA differ between *Ye*, which
74 binds collagen and laminin, and *Yps*, which binds fibronectin [18].

75 By interacting with several complement factors, serum resistance is an important virulence
76 trait of *Ye*. It has been shown that factor H, C4b-binding protein (C4BP) and C3 bind to the
77 YadA stalk domain and thus inhibit complement killing [19,20]. Recently, we demonstrated a
78 novel mechanism that contributes to serum resistance in *Ye* O:8 WA-314 and amended the
79 current model of direct factor H-binding to YadA^{0:3} and YadA^{0:9}. We have shown that *Ye*
80 binds C3b or iC3b and thereby attracts high amounts of factor H to the bacterial surface [21].
81 This is different to the direct binding of factor H, which was shown earlier [19,20,22].
82 Importantly, by binding these complement regulatory factors, *Ye* is able to interfere with
83 complement activity by inhibiting complement-mediated killing at an early stage of the
84 cascade.

85 The human glycoprotein Vn is synthesized in the liver and secreted into plasma [23], where it
86 is present as a monomer (65 and 75 kDa) at high concentrations (200 – 400 µg/mL) [24]. Vn
87 also exists as an extravascular cell-bound multimeric form in several tissues, and Vn mRNA
88 can be detected in high concentrations in the liver, brain, heart, and adipose tissue but is rare
89 or absent in the kidney and spleen [25]. It comprises an N-terminal somatomedin-binding
90 domain (SMB) consisting of 43 amino acid (aa) residues, followed by the host cell integrin
91 receptor-binding motif RGD (Arg-Gly-Asp). In addition to four hemopexin-like domains with
92 unknown function, Vn also contains 3 HBDs which span aa 82-137 (HBD-1), aa 175-219
93 (HBD-2) and aa 348-361 (HBD-3) [26,27]. Vn is an important regulator of complement
94 activity at the level of TCC formation, a component of the ECM and also fulfills functions in
95 cell migration and tissue repair [27].

96 At the level of TCC formation, Vn regulates complement activity by directly binding to the
97 protein complex C5b-7 or to C9 [28]. The exact mode of regulation is not fully understood. It

98 has been postulated, however, that Vn binds the nascent precursor complex C5b-7, resulting
99 in a Vn-C5b-7 complex that is unable to insert into the cell membrane [27,28]. Vn can also
100 directly bind C9 and thereby inhibit C9 polymerization. This binding takes places through
101 HBD-3, whereas the binding site for the nascent C5b-7 is still unknown [27-29].

102 A wide variety of bacteria bind Vn via various surface proteins. The respiratory pathogens
103 *Moraxella catarrhalis* (*Mc*) and *Haemophilus influenzae* (*Hi*) as well as the urogenital
104 pathogen *H. ducreyi* express proteins belonging to the TAA family. These proteins are the
105 ubiquitous surface protein A2 (UspA2) of *Mc*, the *Haemophilus* surface fibrils (Hsf) and the
106 *Haemophilus* adhesin (Hia) of *Hi* or the *H. ducreyi* serum resistance protein A (DsrA) [9,30-
107 36]. In the invasive bacterial pathogen *Neisseria meningitidis* the three proteins Opc, Opa and
108 Msf interact with Vn [37-40]. However, to date no enteropathogenic bacteria have been
109 described to use Vn to escape complement-mediated attack and thus mediate serum
110 resistance.

111 *Ye* has evolved a multitude of mechanisms in order to evade the host immune system.
112 Amongst these, serum resistance is of uttermost importance. The significance of the
113 complement regulator Vn in complement evasion and modulation of host cell interaction with
114 bacterial and fungal pathogens has recently been recognized [27,30-32,37,39-44]. *Ye* is able to
115 bind several regulators of complement activity; however, the role of Vn in the *Ye*-host cell
116 interaction and pathogenicity has not yet been addressed in detail, but it was shown in
117 previous studies that YadA from *Ye* O:8 does not bind Vn under stringent assay conditions
118 [45]. In this study, we systematically investigated i) Vn binding of different *Ye* strains, ii)
119 which components of *Ye* might enable this binding and iii) how this interaction modulates *Ye*
120 serum resistance, host cell interaction and overall pathogenicity. Importantly, we were able to
121 demonstrate a novel mechanism facilitating *Ye* serum resistance mediated by the surface
122 adhesin YadA binding to Vn. We found that subtle differences within the YadA head domain

123 of different *Yersinia* strains determine the efficacy of the Vn binding. An additional stretch in
124 *Ye* YadA^{O:9}, which is similar to the “uptake region” of *Yps* YadA^{YPIII} [18], was identified as a
125 crucial region for high affinity binding of Vn. Moreover, we located HBD-3 within Vn as the
126 YadA-binding site. Notably, bound Vn is active on the bacterial surface and protects bacteria
127 from complement-mediated lysis by inhibition of C9 polymerization. This mechanism allows
128 enhanced survival of *Ye* O:9 E40 during early phase of a mouse infection *in vivo*.

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143 MATERIALS AND METHODS

144 *Mice*

145 C57BL/6 wild-type mice were purchased from Harlan Winkelmann (Horst, Netherlands).
146 B6.129S2(D2)-*Vtn*^{tm1Dgi}/J mice (<http://jaxmice.jax.org/strain/004371.html>) with a C57BL/6
147 background were purchased from Jackson Laboratories (Bar Harbor, USA). All mice were
148 bred under specific pathogen-free conditions in individually ventilated cages with access to
149 water and food *ad libitum*. Experiments were performed with six to eight-week-old female
150 mice according to German law with permission of the Regierungspräsidium Tübingen
151 (permission number H4/15).

152 *Plasmids*

153 Plasmids used in this study are listed in Table 1. [46,47]

154 *Bacterial strains and culture conditions*

155 All *Yersinia* strains were cultivated in lysogeny broth (LB) medium with supplements
156 (antibiotics as indicated in Table 2) overnight at 27°C. To promote YadA expression, a 1:20
157 dilution of the overnight culture was made with fresh medium and incubated for 3h at 37°C.
158 *Moraxella* strains were grown overnight at 37°C in brain-heart infusion (BHI) medium. All
159 bacteria were washed twice with PBS and subsequently the optical density at 600 nm was
160 determined. The number of bacteria used for the individual experimental set ups are indicated
161 in the respective sections. All bacterial strains used in this study are listed in Table 2. [48-53]

162 *Serum*

163 Normal human serum (NHS) was collected from at least 4 healthy volunteers and pooled.
164 Aliquots were stored at -80°C and thawed only once. Heat inactivated serum (HIS) was
165 generated by incubating at 56°C for 30 min immediately before use.

166 *Antibodies*

167 Antibodies used in this study are listed in Table 3.

168 *Purified proteins used in this study*

169 Purified monomeric and multimeric Vn was purchased from BD Bioscience (Heidelberg, DE)
170 and Millipore (Schwalbach, DE), respectively. Vn fragments were expressed and purified as
171 previously described [35,54].

172 *Binding assay with serum or purified proteins analysed by flow cytometry*

173 To analyse binding of purified Vn or Vn and factor H from HIS, a total of 1×10^7 bacteria per
174 assay were incubated with 5-50 % HIS (as indicated in figures and/or figure legends) or
175 purified Vn (1-10 $\mu\text{g/ml}$) diluted with PBS (Life Technologies, Darmstadt, DE) in a total
176 volume of 100 μl for 30 min at 37°C. As an internal control, each strain was also treated with
177 PBS only. Recombinant Vn fragments were used at 4 $\mu\text{g/ml}$. After washing with 1 % BSA in
178 PBS (washing buffer), bacteria were spun down and the pellet was resuspended in 200 μl 4 %
179 paraformaldehyde (PFA) in PBS for 1 h at room temperature. Bacteria were washed once
180 again and finally incubated with primary polyclonal antibodies (pAb) directed against Vn or
181 factor H overnight at 4°C. The next day, bacteria were washed once and incubated with
182 suitable secondary antibodies for 1 h at room temperature. After a final washing step bacteria
183 were transferred to FACS tubes and analysed with a Fortessa LSR II instrument. Data analysis
184 was carried out using WinMDI version 2.8. The PBS only control was used to determine
185 background staining using the same primary and secondary antibodies as for all other
186 samples. Values obtained for the control samples were subtracted from the values obtained for
187 the corresponding samples that were incubated in serum or purified Vn. All flow cytometry
188 figures show background subtracted values.

189

190 *Detection of Vn-binding or YadA expression by Western blot*

191 To analyse Vn binding by immunoblotting, 5×10^8 bacteria (bacterial numbers were
192 determined photometrically by measuring the optical density at 600 nm; a volume
193 corresponding to the desired number of bacteria was harvested by centrifugation and the
194 bacterial pellets were then used to carry out the assay) were incubated in 100 μ l 50 % HIS
195 diluted in PBS as described above. Thereafter, bacteria were washed twice with washing
196 buffer, once with PBS and finally resuspended in 50 μ l deionized water. For detection of
197 YadA, bacteria were simply washed after harvest. After addition of 25 μ l 4x Laemmli buffer
198 (Bio-Rad Laboratories, München, DE), samples were boiled for 5 min at 95°C and separated
199 in a 10 % acrylamide SDS gel (Bio-Rad Laboratories, München, DE). Each lane was loaded
200 with an equal number of bacteria. After blotting, membranes were blocked with 3 % BSA, 5
201 % milk powder in TBS for 1 h at room temperature. Membranes were then incubated with
202 desired antibodies (a complete list of antibodies and working dilutions is given in Table 3) for
203 1 h at room temperature or at 4°C overnight, washed with 0.1 % TBS-T and then incubated
204 with the suitable secondary antibody (Table 3). Fluorescence signals were recorded using a
205 LICOR Odyssey imaging system.

206 *Detection of Vn-binding by Blot Overlay Assay*

207 Bacterial lysates were prepared as described above, separated by SDS-PAGE and blotted.
208 After blocking with 5 % milk, 3 % BSA in PBS for 3 h at room temperature, the membrane
209 was incubated with 7 μ g/ml purified monomeric Vn in 3 % BSA in PBS-T overnight at 4°C.
210 After washing with 0.1% TBST, Vn was detected with rabbit anti-Vn pAb and a secondary
211 DyLight 680-conjugated goat-anti-rabbit pAb. Fluorescence signals were recorded using a
212 LICOR Odyssey imaging system.

213

214 *Purification of DNA from Yersinia colonies*

215 *Yersinia* strains were streaked on LB agar plates without antibiotics. Next day a single colony
216 was used for DNA extraction using the Qiagen QIAmp DNA Mini kit according to the
217 manufacturers protocol. DNA was finally eluted in 100 µl ultrapure water.

218 *PCR amplification of the YadA head region*

219 To test *Yersinia* YadA for the presence of the additional stretch (enabling recruitment of Vn)
220 within its head region, we used the primers YadA_Seroseq_435F (5'-gatcagtgtctctgcggcat-3')
221 and YadA_Seroseq_435R (5'-gccccataagtaactgccga-3') that bind to highly conserved regions
222 up- and downstream of the uptake region (see **Fig. S1**). According to the sequence alignment
223 the PCR reaction should yield a fragment of 442 bp with *Ye* O:9 E40 or 451 bp with *Yps*
224 YPIII (both harboring the uptake region of ~ 90 bp) or 337 bp with *Ye* O:8 WA-314, 346 bp
225 with *Ye* O:3 6471/76 and *Ye* O:5,27 (all three lacking the uptake region) and thus allow us to
226 discriminate between YadA with and without “uptake region”. 1 µl of DNA was used as
227 template. The PCR program we used was the following: 2 min 95°C (initial denaturation), 30
228 sec 95°C→1 min 55°C→ 30 sec 68°C (repeated 29x), 5 min 72°C (final extension) and
229 cooling at 4°C until further processing.

230 *Separation of PCR products by capillary gelelectrophoresis*

231 To determine the size of PCR products they were analysed using a QIAxcel capillary
232 gelelectrophoresis system according to manufacturer's protocol.

233 *DNA sequencing*

234 PCR products were purified using the Promega Wizard® SV Gel and PCR Clean-Up System
235 according to the manufacturer's protocol. Subsequently Sanger sequencing was performed by
236 GATC using the same primers as for the PCR reaction.

237 *Heparin inhibition assay*

238 Sterile glass coverslips were coated with purified Vn (10 µg/ml) at 4°C overnight and air-
239 dried. Coverslips were then placed in a 24-well plate and either incubated with PBS or 100
240 µM Heparin in PBS. 5×10^7 bacteria (*Ye* O:9 E40 pBla EGFP) were added to each well, spun
241 down for 5 min at 300 g and incubated for 1 hour at 37°C in humidified atmosphere.
242 Afterwards, the supernatant was removed, cells were washed two times and finally fixed by
243 the addition of 4 % PFA in PBS. After washing, slides were mounted in Mowiol and
244 micrograph pictures were acquired using a ZEISS LSM 510. To quantify adhesion, the
245 number of bacteria for a given field of view (representative for the entire coverslip) was
246 counted.

247 *Analysis of C5b-9 deposition by flow cytometry*

248 To analyse whether bound Vn was functionally active, bacteria were incubated with Vn (10-
249 50 µg/ml) or C4BP (10-50 µg/ml) for 30 min at 37°C. After washing, bacteria were incubated
250 with C5b-6 (1 µg/ml) and C7 (1 µg/ml) for 10 min and thereafter C8 (0.4 µg/ml) and C9 (1
251 µg/ml) were added for 30 min at 37°C. All complement components except for Vn were from
252 Complement Technology (Tyler, TX, USA). Deposited C5b-9 was detected by mouse anti-
253 human C5b-9 mAb followed by Alexa fluor®-647-conjugated goat anti-mouse pAb. After
254 two additional washes, bacteria were analysed by flow cytometry (EPICS XL-MCL; Coulter,
255 Hialeah, FL). All incubations were kept in a final volume of 100 µl 1 % BSA in PBS and
256 washes were performed with the same buffer. Primary and secondary pAb were added
257 separately as negative controls for each strain analysed.

258 *In vitro serum killing assay*

259 To analyse the susceptibility of *Ye* and *Yps* against complement-mediated killing in human
260 serum, 5×10^6 bacteria were incubated in 100 µl 20 % NHS or HIS for 30 min at 37°C.

261 Complement activity was stopped by adding 100 μ l BHI medium and placing the samples for
262 5 min on ice. Afterwards, a serial dilution of the samples were prepared, plated on selective
263 agar plates and incubated at 27°C for 48 h. The colony forming units (CFU) were determined.
264 The serum bactericidal effect was calculated as the survival percentage, taking the bacterial
265 counts obtained with bacteria incubated in HIS as 100 %.

266 *In vivo serum killing assay*

267 To analyse lytic activity of serum complement against *Ye* in C57BL/6 and B6.129S2(D2)-
268 *Vtn^{tm1Dgi}/J* mice, animals were infected intravenously with 1×10^7 bacteria. After 30 min, mice
269 were sacrificed by CO₂ asphyxiation and blood was withdrawn from the heart. Heparin (100
270 μ l at 100 μ g/ml) (Sigma-Aldrich, Steinheim, DE) was mixed with the blood to avoid
271 coagulation. Serial dilutions of the samples were plated on selective agar and incubated at
272 27°C for 48 h. The CFU were determined by counting the colonies.

273 *Bioinformatics and statistical analysis*

274 The GI numbers or the references of the sequences used in this work are listed in Table 2.
275 Alignments were produced with Kalign or Muscle, and further edited manually [55,56]. Data
276 are means \pm SD and were analysed with the Student t test, or with one-way ANOVA for
277 multiple comparison as described in the figure legends. GraphPad Prism 6.0 was used to
278 analyse the data (GraphPad Software, La Jolla, CA). Differences were considered significant
279 when $p \leq 0.05$. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

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284 RESULTS

285 *Yersinia enterocolitica* O:9 E40 efficiently binds Vn

286 Vn plays an important role in the complement resistance of *e.g.* *Mc*, *Hi* and *Streptococcus*
287 *pneumoniae* [32,34,44,54,57]. In order to test if *Ye* is able to bind Vn and if there are
288 differences in the binding capacity of various *Ye* strains and serotypes, we incubated a set of
289 strains in 50 % HIS, washed the cells and detected Vn bound to bacteria by immunostaining
290 with antibodies directed against Vn and subsequent flow cytometry analysis (**Fig. 1A**). Upon
291 incubation with HIS we found very diverse binding properties of *Ye* strains compared to *Mc*
292 RH4 and *Yps* YPIII. *Mc* RH4 served as a positive control [30,34], whereas *Yps* YPIII was
293 used as an additional comparator. It has been recognized earlier that *Yps* YPIII YadA differs
294 from YadA sequences of other strains and that this difference coincides with a change in
295 preferred ECM binding partners and this might possibly also affect interaction with Vn (*Yps*
296 YPIII YadA preferentially binds to Fibronectin instead of collagen and laminin as observed
297 with *Ye*) [18]. *Ye* O:9 E40 was able to bind exceptionally high amounts of Vn which led to a
298 mean fluorescence intensity ~ 2.8 times higher compared to that measured with *Mc* RH4
299 (133.9 ± 33.9 vs. 47.4 ± 19.6). Also *Yps* bound Vn, but at concentrations comparable to that
300 of the *Mc* RH4 positive control (56.7 ± 11.0 vs. 47.4 ± 19.6). *Ye* O:8 WA-314 and *Ye* O:3
301 6471/176 also bound Vn, though to a lesser extent compared to *Mc* RH4 (~ 54.1 or 70.7 % of
302 *Mc* RH4 signal). *Ye* O:8 8081 bound only residual amounts of Vn (6.7 ± 0.6). Interestingly,
303 the binding of Vn to *Ye* O:9 E40 depended on the presence of the plasmid of *Yersinia*
304 virulence (pYV) and was dose-dependent (**Fig. 1C and D**). In a plasmid-deficient strain (*Ye*
305 O:9 E40 Δ pYV) Vn binding was almost abolished (6.2 ± 2.8). To test if the strain-specific
306 binding-pattern of Vn (O:9 E40 > YPIII > RH4 > O:3 > O:8 WA-314 > O:9 E40 Δ pYV = O:8
307 8081) is exclusive compared to other serum factors, we also tested the binding of factor H
308 (**Fig. 1B**). Factor H has been shown to interact with several discontinuous stretches within the

309 stalk domain of YadA [20-22,58]. Our data corroborate previous findings that binding of
310 factor H by *Yersinia* strains relies on the presence of YadA, but in contrast to Vn, there is no
311 significant difference in binding efficiency in the various serotypes tested. This indicates
312 different mechanisms of binding of Vn and factor H. Taken together, we found that *Ye* O:9
313 E40 is able to bind high amounts of serum-derived as well as purified Vn but only in the
314 presence of the pYV plasmid, in a dose-dependent manner. In contrast, *Ye* O:8 WA-314, 8081
315 and *Ye* O:3 6471/76 are weak Vn binders, although they also carry the pYV plasmid. This
316 partially substantiates earlier findings that YadA-dependent Vn binding is at least weak if not
317 non-existent for *Ye* O:8 WA-314 in whole-cell adhesion assays under specific flow conditions
318 [45].

319 *Binding of Vn is YadA-dependent*

320 In order to assess whether YadA is the determinant for binding of Vn to *Yersinia* we used
321 flow cytometry to compare Vn binding in a *Ye* O:9 E40 wildtype strain (WT), a mutant
322 deficient for YadA (Δ YadA), a mutant deficient for the chromosome-encoded adhesin Invasin
323 (Δ Inv), the corresponding double mutant (Δ Inv Δ YadA; $\Delta\Delta$), and again the cured strain
324 lacking the pYV plasmid (Δ pYV) (**Fig. 2A left panel**). We used *Mc* RH4 as positive control
325 and a *Mc* Δ UspA2H [32] knockout strain as a negative control (**Fig. 2A right panel**). Our
326 data indicate that the presence of YadA, but not of Inv, is decisive for binding of Vn to *Ye* O:9
327 E40. Thus, in contrast to *Ye* O:9 E40 wildtype or Δ Inv, Vn did not bind to Δ YadA, the
328 Δ Inv Δ YadA double mutant or the pYV-cured strain. We could corroborate these findings by
329 blot overlay assays and Western blot (**Fig. 2B, C**). Analysis of the influence of YadA and
330 more specifically a distinct region within YadA of *Yps* for Vn binding revealed that also in
331 *Yps*, YadA is the Vn binding determinant (**Fig. 2A middle panel**). Moreover, the deletion of
332 30 aa (Δ 53-83) corresponding to the “uptake region” in the head domain of *Yps* YadA^{YPIII}
333 abolishes Vn binding (**Fig. 2A middle panel**). Thus, our data demonstrate that YadA is

334 essential for mediating Vn binding in *Ye* and that a stretch of 31aa within the head region of
335 YadA^{YPIII} is decisive for binding of Vn in *Yps*.

336 *A specific stretch within YadA discerns Vn-low-binding from Vn-high-binding*

337 We found that *Ye* expressing YadA derived from the O:8 WA-314 strain is a relatively weak
338 binder compared to *Ye* O:9 E40 (**Fig. 1A**). Therefore, we aimed to determine if also other
339 strains carry the uptake region and also what actually discerns YadA^{O:9 E40} from YadA^{O:8} and
340 if this difference might be causative for the discriminative Vn binding behavior. The head
341 domain of YadA^{YPIII} contains a stretch of sequence (“uptake region”), which is crucial for cell
342 adhesion and efficient internalization of *Yersinia* via YadA [18]. This motif is absent in YadA
343 of *Ye* O:8 but present in the *Ye* O:9 E40 strain (aa 56-88) (**Fig. 3A**). It is rich in prolines and
344 charged residues, suggesting an undefined loop structure (**Fig. 3B**), inserted in a shorter loop
345 that is not resolved in the crystal structure of the *Ye* O:3 YadA head (PDB: 1P9H) [59].

346 To investigate whether this motif is present exclusively in *Ye* O:9 E40 or can be found also in
347 other *Yersinia* strains and especially strains isolated from clinical specimens we carried out
348 PCRs. We designed primers binding to rather conserved regions within the YadA sequence
349 flanking that part of the head domain which comprises the uptake region (**Fig. S1**). The size
350 of the PCR products allowed us to easily detect the presence of the uptake region. Predicted
351 lengths of the YadA head fragments were: 346 bp (*Ye* O:3), 337 bp (*Ye* O:8), 346 bp (*Ye*
352 O:5,27), 451 bp (*Ye* O:9) and 442 bp (*Yps* YPIII). Strikingly, the additional stretch present in
353 YadA of *Ye* O:9 E40 and *Yps* YPIII was present in all tested clinical isolates of serotype O:9,
354 but absent in all other strains (belonging to the indicted serotypes; **Fig. 3C**) we tested. *Ye* O:9
355 E40 Δ YadA and water control were included as negative controls (**Fig. 3C**). All strains
356 depicted in (C) were also tested for Vn binding. Cell-surface associated Vn after incubation in
357 HIS was quantified by flow cytometry (**Fig. 3D**). Whereas all strains belonging to serotype
358 O:9 (No. 08-14) and possessing the uptake region within YadA bound Vn in comparable high

359 amounts as *Ye* E40 O:9, *Ye* strains of serotype O:3 (No. 01, 02, 03), O:8 (No. 04) and O:5,27
360 (No. 06, 07) turned out to be rather weak binders. Thus we assume that the presence of the
361 uptake region is the major determinant that allows binding of Vn and (at least in the strains we
362 have tested) is present exclusively in YadA of *Ye* strains of serotype O:9.

363 To test this hypothesis, we generated a YadA hybrid where we replaced the N-terminus of the
364 head domain of YadA^{O:8} by that of YadA^{O:9 E40} (including the uptake region) and a YadA^{O:9}
365 ^{E40} deletion mutant lacking the uptake region (aa 56-88) (**Fig. 4A**). We then compared Vn
366 binding by flow cytometry. The strains *Ye* O:9 Δ YadA expressing YadA^{O:8 WA-314} or YadA^{O:9}
367 ^{E40} were also included in this analysis. In addition, we used *Ye* O:8 WA-314, *Ye* O:9 E40 and
368 *Ye* O:9 E40 Δ YadA as controls (**Fig. 4B**). Ectopic expression of YadA^{O:9 E40} was able to
369 rescue Vn-binding of *Ye* O:9 E40 Δ YadA. This was also true for the O:9/O:8 hybrid YadA.
370 Additionally, deletion of the “uptake region” from YadA^{O:9} led to significantly reduced Vn
371 binding (**Fig. 4B**). Our data show that the “uptake region” of YadA^{O:9 E40} significantly
372 enhances recruitment of Vn. Of note, a sequence alignment of YadA from different *Yersinia*
373 strains revealed also insertions in the stalk regions of *Ye* YadA^{O:9 E40} and YadA^{O:3 6471/76} that
374 are not found in YadA^{YPIII} and YadA^{O:8 WA-314} (**Fig. S2**). However, these regions show no clear
375 association with Vn or factor H binding (**Fig. 1A**). Finally, we wanted to assess whether
376 cofactors expressed by *Yersinia* are necessary or if YadA containing the “uptake region”
377 alone is sufficient to mediate efficient binding of Vn. We tested Vn binding of *E. coli* omp2
378 [60] which ectopically expressed the YadA version described above (**Fig. S3**). We found that
379 expression of YadA^{O:9} or the hybrid YadA^{O:9/O:8} is sufficient to mediate binding of Vn. Thus
380 we conclude that the decisive factor for Vn binding is YadA comprising the “uptake region”.

381 *Vn* interacts with YadA via its C-terminal heparin-binding-domain 3 (HBD-3)

382 Previous work with *Mc* and *Hi* revealed HBD-3 as the decisive part of Vn for interaction with
383 UspA2 or Hsf [34,35]. Therefore, we wanted to know if this domain might also mediate the

384 interaction of Vn with YadA. In order to test this we first analysed whether Heparin might
385 block the binding of *Ye* to Vn by occupying the HBDs. This would be a clear indicator for the
386 involvement of one of the HBDs in the interaction with YadA. Coverslips were coated with
387 Vn and afterwards incubated with *Ye* O:9 E40 (expressing enhanced green fluorescent protein
388 (eGFP) for easier detection of binding) either in the presence or absence of Heparin.
389 Thereafter coverslips were washed, fixed, mounted and analysed by fluorescence microscopy
390 (**Fig. 5A**). Our results demonstrate that, in the presence of Heparin, binding of bacteria to Vn-
391 coated coverslips is significantly reduced. Therefore, we conclude that at least one of the
392 HBDs is involved in mediating the binding of Vn to YadA^{O:9 E40}.

393 To locate the sites within Vn that actually determine YadA binding, we used a set of
394 recombinant Vn fragments (**Fig. 5B**). These fragments essentially comprise C-terminally
395 truncated Vn molecules as well as deletion mutants lacking parts of HBD-3 (comprising aa
396 348-361) or adjacent regions. All fragments were tested for appropriate quality (**Fig. S4**). Our
397 binding assay (**Fig. 5C**) demonstrates that the fragments Vn 80-396, 80-379, 80-373 and 80-
398 363 are efficiently bound by *Ye* O:9 E40. However, further C-terminal truncation comprising
399 either parts or the entire HBD-3 (80-353, 80-339) led to a reduction of binding. Fragments
400 lacking the entire HBD-3 plus the adjacent N-terminal region (80-330, 80-229) bound only
401 weakly to *Ye* O:9 E40 (**Fig. 5C**). Thus, we assume that not only HBD-3 but also the adjacent
402 N-and especially the C-terminal ~10-20 aa are important for a stable interaction of Vn with *Ye*
403 O:9 E40. These findings are in agreement with the fact that also a Vn molecule lacking the C-
404 terminal part of HBD-3 plus the adjacent C-terminal region (Δ 352-374) is impaired in binding
405 to *Ye* O:9 E40, whereas deletion of either only part of HBD-3 (Δ 352-362) or the adjacent C-
406 terminal region only (Δ 362-374) does not significantly influence binding. In conclusion, aa
407 331-363 are decisive for stable interaction of Vn with *Ye* O:9 E40.

408

409 *Vn is functionally active and inhibits the terminal pathway when bound to the surface of Ye*

410 Besides modulating the adhesive properties of pathogens, Vn regulates the terminal
411 complement pathway and blocks TCC formation. In order to test if Vn bound to *Ye* is
412 functionally active and inhibits the terminal complement pathway, we assayed C5b-9
413 deposition in the presence of Vn bound to intact bacteria. To this end, *Ye* O:9 E40 was
414 preincubated with Vn or C4BP followed by addition of C5b-6, C7, C8 and C9. C5b-9
415 deposition was determined by using an anti-C5b-9 mAb and flow cytometry.

416 We clearly demonstrate that Vn bound to the surface of *Ye* O:9 E40 was functionally active
417 and inhibited C5b-9 deposition in a dose-dependent manner (**Fig. 6A, B**). Vn (50 µg/ml)
418 inhibited C5b-9 deposition by 61 %. C4BP, the C3 convertase inhibitor of the classical/lectin
419 pathways, did not influence the C5b-9 deposition and thus the terminal pathway. From this we
420 conclude that Vn when bound to intact *Ye* is functionally active and inhibits the terminal
421 complement pathway and C5b-9 deposition.

422 *Binding of Vn decreases the susceptibility to complement mediated killing by human serum*

423 YadA-mediated serum resistance is an important virulence trait of *Ye* [21,61,62]. To analyse
424 the importance of Vn binding for preventing complement mediated killing, we performed
425 serum killing assays. We incubated *Ye* O:9 E40, the corresponding YadA-deficient mutant
426 (Δ YadA) and *Ye* O:8 WA-314 in normal human serum (**Fig. 7A**, “control strains”). Their
427 survival was calculated as the survival percentage, taking the bacterial counts obtained with
428 samples incubated in HIS as 100 %. Our data show that *Ye* O:9 E40 – a strong Vn binder - is
429 resistant to complement-mediated killing (% survival in NHS compared to HIS 119.1 ± 40.39)
430 whereas the *Ye* O:9 E40 Δ YadA mutant strain was highly susceptible for killing by the
431 complement system (16.74 ± 9.83). Compared to *Ye* O:9 E40, the weak Vn binder *Ye* O:8
432 WA-314 was significantly more susceptible to complement-mediated killing (39.21 ± 7.11)

433 compared to *Ye* O:9 E40 (**Fig. 7A**). Furthermore, we also tested *Ye* O:9 E40 $\Delta\Delta$ expressing
434 either $YadA^{O:9}$, $YadA^{O:9/O:8}$, $YadA^{O:9 \Delta uptake region}$ or $YadA^{O:9}$ for serum resistance. We found
435 that the expression of $YadA^{O:9}$ (103.6 ± 3.42) and also of the O:9/O:8 hybrid $YadA$ ($105.7 \pm$
436 $27,56$) conferred serum resistance comparable to that of the *Ye* O:9 E40 wildtype strain. In
437 contrast the serum survival was significantly reduced upon expression of $YadA^{O:8}$ ($48.55 \pm$
438 9.36). Compared to all these strains, a strain expressing the $YadA$ O:9 lacking the “uptake
439 region” showed the greatest sensitivity towards serum treatment (16.8 ± 7.57). These data
440 clearly indicate that the $YadA$ -dependent binding of Vn plays an important role in preventing
441 the lysis of *Ye* by the complement system.

442 *Mice deficient for Vn expression eliminate Ye more rapidly in short-term systemic infection*

443 It is known that $YadA$ is decisive for survival of *Ye* upon contact with serum [6,12]. This is
444 one reason why $YadA$ -deficient strains of *Ye* are avirulent in the mouse model [12]. However,
445 the contribution of $YadA$ -dependent recruitment of Vn to survival of *Ye* in a mouse model has
446 not been addressed so far. In order to test if the presence of Vn has an influence on the
447 survival of *Ye in vivo* we infected $Vn^{-/-}$ and wildtype mice with *Ye* O:9 E40, sacrificed mice
448 30 min post-infection and determined the bacterial burden in the blood. We found that the
449 bacterial load in the blood was significantly reduced (\log_{10} CFU per g blood = 2.7 ± 0.8) for
450 the $Vn^{-/-}$ mice compared to wildtype mice (\log_{10} CFU per g blood = 4.2 ± 1.0) (**Fig. 7B**). In
451 line with the reduction of C5b-9 deposition on *Ye* by Vn, these data would suggest that Vn
452 protects *Ye* from early killing in the blood stream.

453 Compared to $YadA$ of *Ye* O:9 E40, the $YadA$ of *Ye* O:8 WA-314 shows low Vn binding
454 capacity. Therefore, we hypothesized that due to this low Vn binding capacity and in contrast
455 to our findings with *Ye* O:9 E40, the availability of Vn should only marginally impact the
456 outcome of an early bloodstream infection with the *Ye* O:8 WA-314 strain. However, since
457 the *Ye* O:9 and O:8 strains exhibit additional differences with regards to sequence and also

458 virulence mechanisms [63-66], this experiment may not solve the question whether the uptake
459 region actually contributes to better clearance of infection by mediating more efficient
460 binding of Vn *specifically*. Therefore, we used a slightly different approach. To clearly assess
461 the role of the uptake region and to exclude that other differences between the *Ye* O:8 and the
462 *Ye* O:9 strain tamper the result of our experiments we infected mice with *Ye* harboring
463 pYadA^{O:9/8} hybrid or pYadA^{O:8} in the same strain background (*Ye* O:9 E40 $\Delta\Delta$). The basic
464 sequence of the YadA of these strains is identical with exception of the part encoding the
465 uptake region. Surprisingly, the infection of C57BL/6 wildtype or Vn^{-/-} mice with *Ye* O:9 E40
466 $\Delta\Delta$ + pASK-IBA4c_yadA^{O:8} led to a small but significant difference in bacterial counts (**Fig.**
467 **S5A**; $5,9 \pm 0,3$ log₁₀ CFU per g blood in wildtype mice vs. $6,4 \pm 0,3$ in Vn^{-/-} mice). As
468 observed previously with *Ye* O:9 E40, also infection with *Ye* O:9 E40 $\Delta\Delta$ harboring pASK-
469 IBA4c_yadA^{O:9/O:8} hybrid revealed a significantly reduced bacterial load in the blood for
470 the Vn^{-/-} mice (log₁₀ CFU per g blood = $4,9 \pm 0,2$) compared to wildtype mice (log₁₀ CFU per
471 g blood = $5,5 \pm 0,2$) (**Fig. S5B**). This leads to the assumption that the binding of Vn to
472 different regions of YadA may have various implications on YadA function. While binding of
473 Vn to the uptake region seems to increase virulence, binding of Vn to other regions of YadA
474 might also reduce virulence.

475 **DISCUSSION**

476 Complement inhibitor recruitment by bacterial cell surface proteins and adhesins is an
477 important virulence mechanism used by many pathogens. Accordingly, several complement
478 regulators (factor H, Factor H like protein-1, C4BP) and complement proteins (C3b, iC3b)
479 have been identified that interact with the Gram-negative enteropathogen *Ye* [19-22,61,62,67].
480 Here, we describe a novel mechanism that contributes to *Ye* complement resistance and
481 overall virulence of *Ye*. We show that the TAA YadA of different *Yersinia* species binds Vn
482 and we demonstrate that especially a part of the YadA head domain of YadA^{O:9 E40} comprising

483 aa 56-88 binds Vn with high efficiency. Recruitment of Vn to YadA led to reduced surface
484 formation and deposition of C5b-9 (TCC) and thus enhanced complement resistance.
485 Moreover, *Ye* O:9 E40 was completely resistant to complement mediated killing in human
486 serum in contrast to the YadA-deficient strain. In addition, it turned out that in comparison to
487 *Ye* O:8 WA-314, *Ye* O:9 E40 is significantly more serum resistant. Using Vn-deficient mice
488 we were also able to demonstrate reduced survival of *Ye* O:9 E40 in the absence of Vn in an
489 *in vivo* serum killing assay. Thus, binding of Vn to the surface of *Ye* has great impact on the
490 interaction of *Ye* with the host.

491 In our experiments, we found that different strains of *Ye* and *Yps* bind Vn in a YadA-
492 dependent manner although different *Yersinia* strains exhibited divergent Vn binding
493 capacities. Previous studies with different *Mc* wildtype strains show that *Mc* also binds Vn
494 with different affinities via UspA2 [30]. The N-terminus of the UspA2 head domain sequence
495 displays two different conserved regions that may explain these Vn-binding differences [68].
496 Furthermore, we show for the first time that *Ye* strains of serotype O:9 - unlike all other *Ye*
497 strains we tested - exhibit an additional stretch in their YadA head domain. These strains, and
498 to a lesser extent *Yps* YPIII, showed high-affinity binding to Vn while other tested *Ye* strains
499 showed only low-affinity binding. Unfortunately, we were not able to correlate the ability to
500 bind Vn and the pathogenic potential of clinical isolates due to the low frequency of *Ye*
501 infection (and thus available isolates) and the fact that systemic infection with *Ye* happens
502 only on rare occasions. The stretch in YadA^{O:9} is highly similar to the uptake region described
503 for *Yps* YPIII [18], which is important for the ability of YadA to promote invasion of *Ye* into
504 host cells. *Yps* binds preferentially to fibronectin, but has low affinity for laminin or collagen
505 type I, which is in contrast to the ECM protein binding capacity of *Ye* which preferentially
506 associates with collagen type I and laminin. This indicates that the uptake region may
507 modulate the overall affinity to different ECM proteins. Sequence comparison of YadA^{O:9} E40

508 also revealed additional amino acid stretches in the YadA stalk domain lacking in some other
509 *Ye* strains. However, comparison of the Vn binding capacity of different *Ye* and *Yps* strains
510 show no clear indication that this region may also contribute to the differences in Vn binding
511 since YadA^{O:3 6471/76} has the same insertion in the stalk region. In contrast to Vn binding, the
512 interaction with factor H, which was shown to bind to the stalk region of YadA in *Ye* and *Yps*
513 strains, revealed no differences [20]. This indicates that the presence or absence of the uptake
514 region modulates affinity to Vn.

515 The site of interaction between *Mc* and Vn was mapped to the N-terminal residues 30-177
516 within UspA2 [34]. This region is located in the head domain of UspA2, which is similar with
517 YadA^{O:9 E40}. Our data show that subtle differences within the YadA protein sequence can
518 significantly influence the protein interaction repertoire of *Ye*. The recruitment of such
519 proteins to the surface of *Ye* may exert a significant influence on serum resistance and host
520 cell interaction.

521 Localization of the Vn binding domain within the YadA protein is a crucial step when
522 analyzing the function of YadA in complement evasion. In contrast to complement regulators
523 factor H or the complement component C3, which bind to the stalk domain of YadA [20], we
524 found that Vn is bound via the YadA head domain. In *Ye*, the neutrophil binding domain is
525 located at the N-terminal part of YadA, whereas the collagen binding domain is located at the
526 central and C-terminal part of the YadA head domain [59,69-72]. The inhibition of Vn
527 binding with heparin was already shown for *Mc* and *Hi*. In both species the interaction of Vn
528 with UspA2 or Hsf was assigned to the HBD-3 [34,35]. In contrast, for *Ye* O:9 E40 not only
529 the HBD-3 but also the adjacent N- and C-terminal portion of Vn are decisive for the efficient
530 interaction with YadA. We conclude that complement evasion of *Ye* is not limited to
531 interactions mediated by the stalk domain, but can involve the head domain of YadA,

532 depending on the strain in question. Furthermore, the “uptake region” in *Ye* O:9 seems to
533 provide a binding domain for Vn which strongly amplifies binding of Vn.

534 Previous studies showed that recruitment of Vn by *Mc* or *Hi* inhibits C5b-9 formation to block
535 pore formation [27]. However, analyzing the TCC formation in *Ye* with purified complement
536 proteins (C5b-6, C7, C8 and C9) we showed that bound Vn inhibits the deposition of C5b-9
537 on the bacterial surface. Consequently, these data show that Vn bound to the bacterial surface
538 via YadA is functionally active and inhibits the terminal pathway and thus contributes to
539 complement resistance. Indeed, in *in vitro* serum killing assays we showed that *Ye* O:9 E40 is
540 the strain that sustains treatment with serum most efficiently compared to *Ye* O:8 and *Yps*
541 YPIII. In contrast, a YadA-deficient strain of *Ye* O:9 E40 was susceptible to serum killing.
542 Thus YadA-mediated binding of Vn in *Ye* O:9 E40 is decisive for survival of serum treatment
543 *in vitro*. The situation is different in *Ye* O:8 WA-314. This strain is much more sensitive to
544 serum treatment compared with *Ye* O:9. We know that in *Ye* O:8 serum resistance is mediated
545 by YadA-dependent recruitment of C3b/iC3b, factor H and C4BP [21,33]. As all these factors
546 bind to YadA and at least for C4BP the binding site(s) within YadA is unknown, there might
547 be competition for binding sites and this might lead to binding of low levels of Vn. Still,
548 binding of all the other negative regulators of complement can mediate serum resistance to a
549 certain extent. A decisive role of YadA for serum resistance of *Yps* YPIII is rather unlikely as
550 it has been shown that *Yps* serum resistance occurs independently of the presence of a
551 virulence plasmid (that encodes YadA; [73]). Known mechanisms involved in serum
552 resistance of *Yps* are binding of C4BP and factor H via Ail [74,75]. Nevertheless, we have
553 shown that also *Yps* binds Vn via YadA. We think that in this case the recruitment of Vn has a
554 function different from mediating serum resistance and speculate that it might be involved e.g.
555 in modulation of host cell targeting [66] and interaction [24].

556 Consequently, this should also improve survival of *Ye in vivo*. Indeed, short-term infection of
557 Vn-deficient mice with *Ye* O:9 E40 revealed that Vn protects *Ye* from being killed by the
558 immune system. A short-term infection of mice was used to avoid (as far as possible), the
559 action of other virulence mechanisms such as those provided by the T3SS. According to *ex*
560 *vivo* measurements the injection of Yops should efficiently show its action at later time points.
561 Therefore, the short-term mouse experiments should reflect predominantly the impact of Vn
562 on complement killing as the complement system is activated within seconds after infection.
563 Thus, the mouse infection experiments provide evidence that the inhibition of the TCC
564 formation by Vn via binding to YadA indeed has biological relevance. These findings clearly
565 demonstrate the importance of Vn binding to the “uptake region” for the pathogenicity of *Ye*.
566 However, binding of Vn may also counteract YadA-mediated virulence, which is indicated by
567 the slightly increased bacterial load after infection of Vn-deficient mice with *Ye* O:9 E40 $\Delta\Delta$
568 expressing YadAO:8. We assume that the weak binding of Vn outside of the uptake region
569 might interfere with binding of other factors to YadA which are critical for YadA as a
570 virulence factor. From an evolutionary point of view the acquirement of the uptake region
571 converts Vn from a factor protecting against infection into a factor mediating immune
572 evasion.

573 Although persons lacking terminal complement components are known to be more susceptible
574 to *Neisseria meningitidis* [76] but not especially to *Ye* infections, Vn binding is one important
575 mechanism contributing to the overall serum resistance of *Ye*. *Ye* YadA interacts with a
576 multitude of complement regulatory factors (C4bp, C3b, iC3b and factor H), that all
577 contribute to serum resistance of *Ye* in a true infection situation. These interactions in sum
578 finally determine the success of *Ye* within the host.

579 Taken together, our data add a novel mechanism how YadA mediates immune evasion. By
580 binding the HBD-3 domain of Vn, YadA containing the “uptake region” mediates efficient

581 inhibition of TCC formation and thus contributes to complement resistance and better survival
582 of *Ye*. YadA is a multifunctional protein mediating complement resistance and also adhesion,
583 which in turn is critical for subsequent injection of Yops into the host cells via the T3SS.
584 Beyond bacteriolysis mediated by the assembly of the TCC, the even more important effect of
585 Vn may be to modulate the interaction of *Ye* with immune cells [66] and further studies will
586 now address how Vn may influence adhesion, invasion and Yop injection during mouse
587 infection.

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814 **FIGURE LEGENDS**

815 **Fig. 1 Vn is efficiently bound by *Ye* O:9 E40 and *Yps*.** (A) Several strains of *Ye* (serotype
816 O:9 with (O:9 E40) and without virulence plasmid (O:9 E40 Δ pYV), serotype O:3 (O:3
817 6471/76) and serotype O:8 (O:8 8081; O:8 WA-314) and one *Yps* (*Yps* YPIII) wildtype strain
818 were incubated with heat-inactivated whole human serum, washed and subsequently analysed
819 for the presence of Vn on the bacterial surface by flow cytometry. *Mc* (*Mc* RH4), which is
820 known to bind Vn and *Yps* which we supposed to also bind Vn (see main text) were included
821 as a positive control for Vn binding. *Ye* O:9 E40 which was cured from the virulence plasmid
822 (plasmid of *Yersinia* virulence; pYV) that encodes for the *Ye* T3SS, effector proteins and
823 *YadA* was included as negative control as we surmised that Vn-binding is pYV-dependent.
824 *YadA* protein levels were analysed by western blot analysis in whole cell lysates and are
825 shown below the bar chart (one representative western blot is shown). RNA polymerase
826 protein was used as loading control. *YadA*^{O:3 6471/76} has a calculated molecular weight of
827 approximately 141 kDa (455 aa), *YadA*^{O:8 8081} of 132 kDa (422 aa), *YadA*^{O:8 WA-314} of 132
828 kDa (422 aa), *YadA*^{O:9 E40} of 153 kDa (487 aa), *YadA*^{YPIII} of 135 kDa (434 aa) and *UspA2H*
829 of about 272 kDa (876 aa). (B) To test if strain-specific differences in binding of Vn are
830 exclusive, we compared Vn binding levels to that of Factor H. In contrast to Vn, Factor H is
831 bound in comparable amounts by all *Yersinia* strains tested, except for the negative control
832 strain (O:9 E40 Δ pYV). The protein levels of *YadA* and the RNA polymerase as a loading
833 control were analysed by western blot analysis in whole cell lysates and are shown below the
834 bar chart (one representative western blot is shown). (C) Binding of serum-derived Vn to *Ye*
835 O:9 E40 is dose dependent. *Ye* O:9 E40 and the pYV-cured version thereof were incubated
836 with increasing serum concentrations. Afterwards cell-surface associated Vn was quantified
837 by flow cytometry. (D) *Ye* O:9 E40 and the pYV-cured version thereof were incubated with
838 increasing amounts of purified Vn. Afterwards cell-surface associated Vn was quantified by

839 flow cytometry. Binding of purified Vn to *Ye* O:9 E40 is dose dependent. Data are means \pm
840 SD of at least four (A, B, C, D) individual experiments. (A, B) The main p values were
841 determined by one-way ANOVA (A, B: $p < 0.0001$). Multiple comparisons were performed
842 by one-way ANOVA with a Dunnett's multiple comparisons test and the p values are
843 indicated with asterisks. (C, D) The p values were determined by student t test. The error bars
844 denote the SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

845 **Fig. 2 Vn binding to *Ye* is YadA-dependent. (A, left panel)** A *Ye* O:9 E40 wildtype strain
846 (WT) or strains carrying individual deletions for the adhesins Invasin (Δ Inv) or YadA
847 (Δ YadA) and a respective double knockout strain ($\Delta\Delta$) as well as a virulence plasmid-cured
848 strain (Δ pYV) were incubated with serum, washed and Vn binding was quantified by flow
849 cytometry. **(A, middle panel)** *Yps* YPIII wildtype (WT) and corresponding strains lacking
850 expression of the surface adhesin YadA (Δ YadA) or expressing a YadA version lacking part
851 of the head domain (Δ 53-83) were included as controls. **(A, right panel)** A *Mc* wildtype strain
852 (WT) known to bind Vn via the surface adhesin UspA2 and a corresponding strain lacking
853 expression of UspA2 (Δ UspA2) were included as positive and negative controls. YadA
854 protein levels were analysed by western blot analysis in whole cell lysates and are shown
855 below the bar chart (one representative blot is shown). **(B)** A selection of the strains used in
856 (A) was tested for Vn binding in a blot overlay assay. Vn and YadA were detected on the
857 identical blot with specific antibodies and differently labeled secondary antibodies (emission
858 max. at 680 and 800 nm, respectively) simultaneously. Vn is bound only in presence of YadA
859 (*Ye*) or UspA2, respectively. **(C)** In a direct binding assay, essentially performed as in (A), Vn
860 can be detected in the expected molecular weight (65 and 75 kDa) by western blot only in
861 those *Ye* strains expressing YadA. Data are means \pm SD of at least four individual
862 experiments (A), or one representative experiment out of three is shown (B, C). The main p
863 value was determined by one-way ANOVA (A: $p < 0.0001$). Multiple comparisons were

864 performed by one-way ANOVA with a Dunnett's multiple comparisons test and the p values
865 are indicated with asterisks. The error bars denote the SD. * p < 0.05, ** p < 0.01, *** p <
866 0.001, **** p > 0.0001.

867 **Fig. 3 A specific region in the YadA head domain is decisive for efficient binding of Vn.**

868 **(A)** Alignment of the head of various YadA variants. White letters on gray background: signal
869 peptide, black letters on gray background: the canonical "SVAIG" head repeats of YadA,
870 italics: the neck region that links the head to the coiled-coil stalk of YadA. The originally
871 proposed insertion of *Yps* by Heise & Dersch [18] is displayed in bold, and is slightly shifted
872 towards the N-terminus of YadA. The dashed line on top shows the corrected position of the
873 insertion, based on improved alignments and on the structure of the YadA head from *Ye* O:3,
874 where the short insertion is not resolved (underlined region). This, and the unusually high
875 number of prolines in this region suggests that it is not structured. The long version of
876 insertion carries a strongly positive net charge (+5 for *Yps* YPIII, +4 for the *Ye* O:9 E40),
877 which probably plays a role for the binding to fibronectin and Vn. **(B)** Schematic view of the
878 differences in the YadA heads. The *Yps* YPIII and *Ye* O:9 E40 variants have long insertions in
879 a unstructured loop region close to the N-terminus of the head. **(C)** PCR products comprising
880 the YadA head region of *Ye* O:8 WA-314, *Ye* O:9 E40 with and without YadA, *Yps* YPIII,
881 and clinical isolates derived from fecal samples (*Ye* O:3, No. 01-03; *Ye* O:8, No. 04; *Ye*
882 O:5,27, No. 06-07 and *Ye* O:9, No. 08-12) or blood (No. 13) were separated by capillary
883 gelelectrophoresis. Predicted length of PCR products was: *Ye* O:3 346 bp; *Ye* O:8 337 bp; *Ye*
884 O:9 451 bp; *Ye* O:5,27 346 bp; *Yps* YPIII 442 bp. Water control and a YadA-deficient strain
885 were included as negative controls. **(D)** The strains shown in (C) were tested for Vn binding.
886 Cell-surface associated Vn after incubation in HIS was quantified by flow cytometry. One
887 representative experiment out of three is shown (D).

888 **Fig. 4 The uptake region is decisive for YadA-mediated Vn binding.** Schematic
889 representation of different YadA versions that were expressed from a plasmid in *Ye* O:9 E40
890 and analysed for Vn binding capacity. The YadA versions tested comprise YadA^{O:8}, YadA^{O:9},
891 a YadA^{O:9/O:8} hybrid consisting of the O:9 head domain fused to the corresponding *Ye* O:8
892 head/stalk and membrane anchor domain (see Material and Methods for details) and *Ye*
893 YadA^{O:9} with the uptake region deleted (Δ uptake region). **(B)** Flow cytometry analysis of Vn
894 binding to different *Ye* strains carrying plasmids for inducible expression of the YadA
895 versions depicted in (A). As control strains we used *Ye* strains expressing wildtype YadA
896 from the endogenous pYV plasmid (*Ye* O:9 = positive control, *Ye* O:8) and a *Ye* O:9 E40
897 YadA-deficient strain (*Ye* O:9 Δ YadA = negative control). YadA protein levels were analysed
898 by western blot analysis in whole cell lysates and are shown below the bar chart. Data are
899 means \pm SD of at least three individual experiments (B, flow cytometry) or one representative
900 experiment out of three is shown (B, western blot). The main p value was determined by one-
901 way ANOVA (B, flow cytometry: $p < 0.0001$). Multiple comparisons were performed by one-
902 way ANOVA with a Dunnett's multiple comparisons test and the p values are indicated with
903 asterisks. The error bars denote the SD. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p >$
904 0.0001 .

905 **Fig. 5 Vn interacts with YadA via its C-terminal heparin-binding-domain 3 (HBD-3).**
906 **(A)** Adhesion of *Ye* to Vn-coated coverslips can be blocked by heparin. **(B)** Schematic
907 representation of Vn, the C-terminally truncated Vn-molecules [54] and the Vn molecules
908 carrying deletions within and adjacent to the Heparin-binding-domain 3 (HBD-3) [35] that
909 were used for a direct binding assay. **(C)** Western blot of a binding assay of *Ye* O:9 E40 with
910 full length Vn and all fragments depicted in (B). Vn fragments appear in green, YadA, which
911 was detected simultaneously, appears in yellow bands (trimer runs at ~ 200 kDa). **(D)** Flow
912 cytometry analysis of Vn binding to *Ye* O:9 E40 with full length Vn and all fragments

913 depicted in (B). Data are means \pm SD of at least three individual experiments (A, D), or one
914 representative experiment out of three is shown (C). The p value for the comparison of –
915 Heparin and +Heparin was determined by Student's t test. The main p value was determined
916 by one-way ANOVA (D: $p < 0.0001$). Multiple comparisons were performed by one-way
917 ANOVA with a Dunnett's multiple comparisons test and the p value is indicated with
918 asterisks. The error bars denote the SD. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p >$
919 0.0001 .

920 **Fig. 6 Vn is functionally active and inhibits the terminal pathway when bound to the**
921 **surface of *Ye*.** (A) Histogram overlay of flow cytometry analyses of TCC formation (detected
922 by formation of the neopeptide C5b-9) on the surface of *Ye* O:9 E40 wildtype after
923 preincubation of bacteria with PBS or different concentrations of Vn in PBS (10, 25, 50
924 $\mu\text{g/ml}$). Preincubation with Vn reduces the amount of TCC that is formed. (B) Bar chart
925 depicting C5b-9 deposition as percent of the amount of C5b-9 that was formed on the surface
926 of bacteria preincubated with PBS only compared to bacteria preincubated with either Vn or
927 C4BP at different concentrations (10, 25, 50 $\mu\text{g/ml}$). Vn, but not C4BP is able to reduce
928 formation of C5b-9. Ab control indicates background signal that was obtained using
929 secondary antibody only for detection. Data are means \pm SD of at least three individual
930 experiments. The main p value was determined by one-way ANOVA (B: $p < 0.001$). Multiple
931 comparisons were performed by one-way ANOVA with a Dunnett's multiple comparisons
932 test and the p value is indicated with asterisks. The error bars denote the SD. * $p < 0.05$; ** p
933 < 0.01 , *** $p < 0.001$.

934 **Fig. 7 *Ye* O:9 E40 is resistant to complement mediated killing *in vitro* and in an *in vivo***
935 **serum killing assay, *Ye* is more efficiently eliminated in the absence of Vn.** (A) *In vitro*
936 serum killing assay using *Ye* O:9 E40, *Ye* O:9 E40 ΔYadA , *Ye* O:8 WA-314, *Yps* YPII, *Ye* O:9
937 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:8, *Ye* O:9 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:9, *Ye* O:9 E40

938 $\Delta\Delta$ + pASK-IBA4C_yadAO:9/O:8 hybrid, *Ye* O:9 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:9
939 Δ uptake region. The serum bactericidal effect was calculated as the survival percentage. **(B)**
940 Wildtype and *Vn*^{-/-} mice were infected intravenously with 1×10^7 *Ye* O:9 E40 for 30 min.
941 After that, mice were killed, blood was withdrawn and plated on selective agar plates. CFU
942 was determined by counting colonies the next day and is shown as log₁₀ CFU per g blood.
943 Data are means \pm SD of at least three individual experiments (A). The main p value was
944 determined by one-way ANOVA (A, $p < 0.0001$). Multiple comparisons were performed by
945 one-way ANOVA with a Dunnett's multiple comparisons test and the p values are indicated
946 with asterisks. The p value for the comparison of C57BL/6 and *Vn*^{-/-} mice was determined by
947 Student t test (B). The horizontal lines denote the mean, the error bars the SD. * $p < 0.05$; **
948 $p < 0.01$, *** $p < 0.001$, **** $p > 0.0001$, , n=6

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Table 1. Plasmids used in this study.

Plasmid name	Description	Resistance	Reference
pBla	expression of YopE aa1-53- β -lactamase hybrid protein under control of the YopE promoter	Kanamycin	(46)
pACYC184 EGFP	EGFP expressed under control of a constitutive tac/lac promoter	Chloramphenicol	(47)
pASK-IBA4C_yadAO:8	<i>yadA</i> from <i>Ye</i> O:8 WA-314 cloned into pASK-IBA4C; expression under control of an anhydrotetracycline inducible promoter	Chloramphenicol	this study
pASK-IBA4C_yadAO:9	<i>yadA</i> from <i>Ye</i> O:9 E40 cloned into pASK-IBA4C; expression under control of an anhydrotetracycline inducible promoter	Chloramphenicol	this study
pASK-IBA4C_yadAO:9/O:8 hybrid	Plasmid for inducible expression of a hybrid protein consisting of the N-terminal aa 1-89 of <i>yadA</i> from <i>Ye</i> O:9 E40 fused to aa 55-422 of <i>yadA</i> from <i>Ye</i> O:8 WA-314; expression under control of an anhydrotetracycline inducible promoter	Chloramphenicol	this study
pASK-IBA4C_yadAO:9 Δuptake region	Plasmid for inducible expression of <i>yadA</i> from <i>Ye</i> O:9 E40 lacking aa 60-86 comprising the uptake region; expression under control of an anhydrotetracycline inducible promoter	Chloramphenicol	this study

Table 2. Bacterial strains used in this study.

Bacterial strain	Description	Resistance	Reference
<i>Ye</i> O:3 6471/76	Serotype O:3, fecal isolate, wildtype	-	(48) GI:48607
<i>Ye</i> O:8 8081	Serotype O:8, fecal isolate, wildtype	-	(49) GI:122815846
<i>Ye</i> O:8 WA-314 YadA wt	coding sequence of YadA WA-314 O:8 was reinserted into a YadA0 strain	Nal, Kan, Spec	(12) GI:310923211
<i>Ye</i> O:9 E40 pBla	<i>Ye</i> O:9 E40 Δ sd transformed with pMK-Bla	Nal, Kan, Ars	(46) GI:972903261
<i>Ye</i> O:9 E40 Δ pYV pBla	<i>Ye</i> O:9 E40 Δ sd without virulence plasmid transformed with pMK-Bla	Nal, Kan	(47)
<i>Ye</i> O:9 E40 Δ Inv pBla	inv mutant strain obtained by recombinational integration of suicide plasmid pMS154 into E40 Δ sd, transformed with pMK-Bla	Nal, Kan, Ars, Tet	(47)
<i>Ye</i> O:9 E40 Δ YadA pBla	pYV- Δ sd strain was transformed with pLJM4029 (YadA-) and with pMK-Bla	Nal, Kan, Ars, Strep	(47)
<i>Ye</i> O:9 E40 Δ Inv Δ YadA pBla	pYV- Δ sd Δ inv strain was transformed with pLJM4029 (YadA-) and with pMK-Bla	Nal, Kan, Ars, Tet, Strep	(47)
<i>Ye</i> O:9 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:8	<i>Ye</i> O:9 E40 Δ sd lacking expression of both YadA and Invasin transformed with pASK-IBA4C_yadAO:8	Nal, Kan, Ars, Strep, Cm	this study
<i>Ye</i> O:9 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:9	<i>Ye</i> O:9 E40 Δ sd lacking expression of both YadA and Invasin transformed with pASK-IBA4C_yadAO:9	Nal, Kan, Ars, Strep, Cm	this study
<i>Ye</i> O:9 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:9/O:8 hybrid	<i>Ye</i> O:9 E40 Δ sd lacking expression of both YadA and Invasin transformed with pASK-IBA4C_yadAO:9/O:8 hybrid	Nal, Kan, Ars, Strep, Cm	this study
<i>Ye</i> O:9 E40 $\Delta\Delta$ + pASK-IBA4C_yadAO:9 Δ uptake region	<i>Ye</i> O:9 E40 Δ sd lacking expression of both YadA and Invasin transformed with pASK-IBA4C_yadAO:9 Δ uptake region	Nal, Kan, Ars, Strep, Cm	this study
<i>Ye</i> O:9 E40 pBla eGFP	<i>Ye</i> O:9 E40 pBla transformed with pACYC184 EGFP	Nal, Kan, Ars, Cm	(47)
<i>Ye</i> O:3 01	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:3 02	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:3 03	Clinical isolate derived from swine (tongue)	-	this study
<i>Ye</i> O:8 04	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:5,27 06	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:5,27 07	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:9 08	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:9 09	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:9 10	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:9 11	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:9 12	Clinical isolate derived from fecal sample	-	this study
<i>Ye</i> O:9 13	Clinical isolate derived from blood sample	-	(51)
<i>Ye</i> O:9 14	Clinical isolate derived from fecal sample	-	this study
<i>Yps</i> YPIII	<i>Yersinia pseudotuberculosis</i> wildtype strain, pIB1	-	(45)
<i>Yps</i> YP46 pIB1	yadA Δ 53-83	Kan, Amp	(18)
<i>Yps</i> YP47 pIB1	yadA-	Kan	(17)
<i>Ec</i> omp2 + pASK-IBA4C	<i>Ec</i> BL21 lacking expression of ompF transformed with pASK-IBA4C	Cm	this study
<i>Ec</i> omp2 + pASK-IBA4C_yadAO:8	<i>Ec</i> BL21 lacking expression of ompF transformed with pASK-IBA4C_yadAO:8	Cm	this study
<i>Ec</i> omp2 + pASK-	<i>Ec</i> BL21 lacking expression of ompF transformed	Cm	this study

IBA4C_yadAO:9	with pASK-IBA4C_yadAO:9		
<i>Ec omp2</i> + pASK-IBA4C_yadAO:9/O:8 hybrid	<i>Ec</i> BL21 lacking expression of ompF transformed with pASK-IBA4C_yadAO:9/O:8 hybrid	Cm	this study
<i>Ec omp2</i> + pASK-IBA4C_yadAO:9 Δuptake region	<i>Ec</i> BL21 lacking expression of ompF transformed with pASK-IBA4C_yadAO:9 Δ uptake region	Cm	this study
<i>Mc</i> RH4 WT	<i>Moraxella catarrhalis</i> wildtype strain	-	(52)
<i>Mc</i> RH4 ΔUspA2H	<i>Moraxella catarrhalis</i> lacking expression of UspA2H	Zeo	(53)

Amp: ampicillin, Ars: arsenite, Cm: chloramphenicol, Kan: kanamycin, Nal: nalidixic acid, Spec:

spectinomycin, Strep: streptomycin, Tet: tetracycline, Zeo: zeocine.

Table 3. Antibodies used in this study.

Antibody	Conjugate	Clone	Manufacturer	Working dilutions
Primary antibodies				
Goat anti factor H	-	polyclonal	Complement Technology	1:100
Rabbit anti Vn	-	polyclonal	Complement Technology	FACS 1:100; WB 1:1000
Rabbit anti Ye Yada	-	polyclonal	Lab antibody; I. Autenrieth	1:200
Rabbit anti Yps Yada	-	polyclonal	Lab antibody; P. Dersch	1:200
Sheep anti Vn	-	polyclonal	AbD Serotech	1:100
Mouse anti human C5b-9	-	aE11	Dako	1:1000
Mouse anti β subunit of <i>E.coli</i> RNA-Polymerase	-	8RB13	NeoClone Biotechnology	1:2000
Secondary antibodies				
Donkey anti rabbit	APC		Jackson ImmunoResearch	1:200
Goat anti rabbit	DyLight 800		Thermo Scientific	1:10000
Goat anti rabbit	DyLight 680		Thermo Scientific	1:10000
Goat anti mouse	DyLight 680		Thermo Scientific	1:10000
Rabbit anti sheep	DyLight 800		Thermo Scientific	1:10000
Rabbit anti goat	Alexa-488		Jackson ImmunoResearch	1:200
Goat anti mouse	Alexa-647	polyclonal	Jackson ImmunoResearch	1:2500

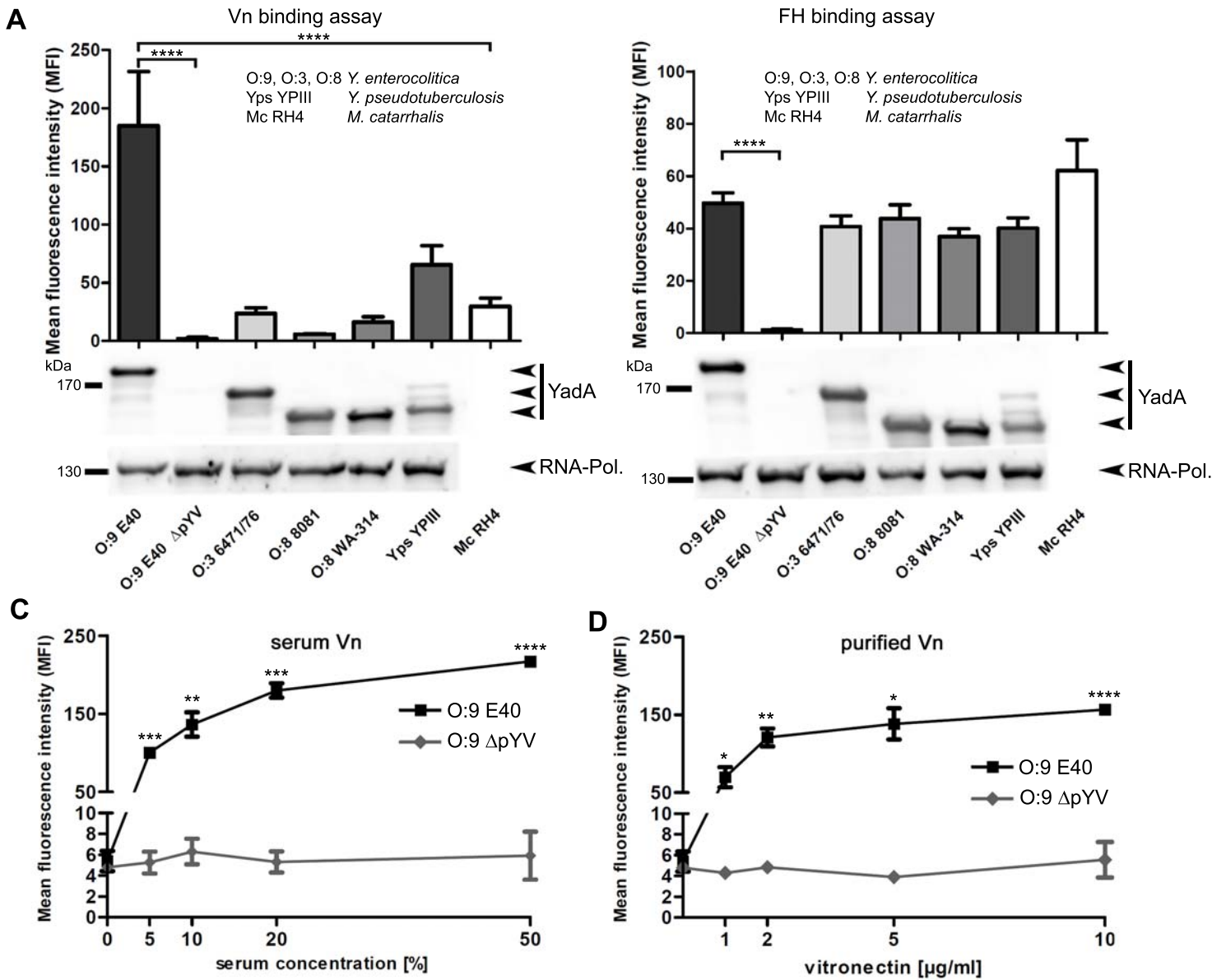


Figure 1, Mühlenkamp et al.

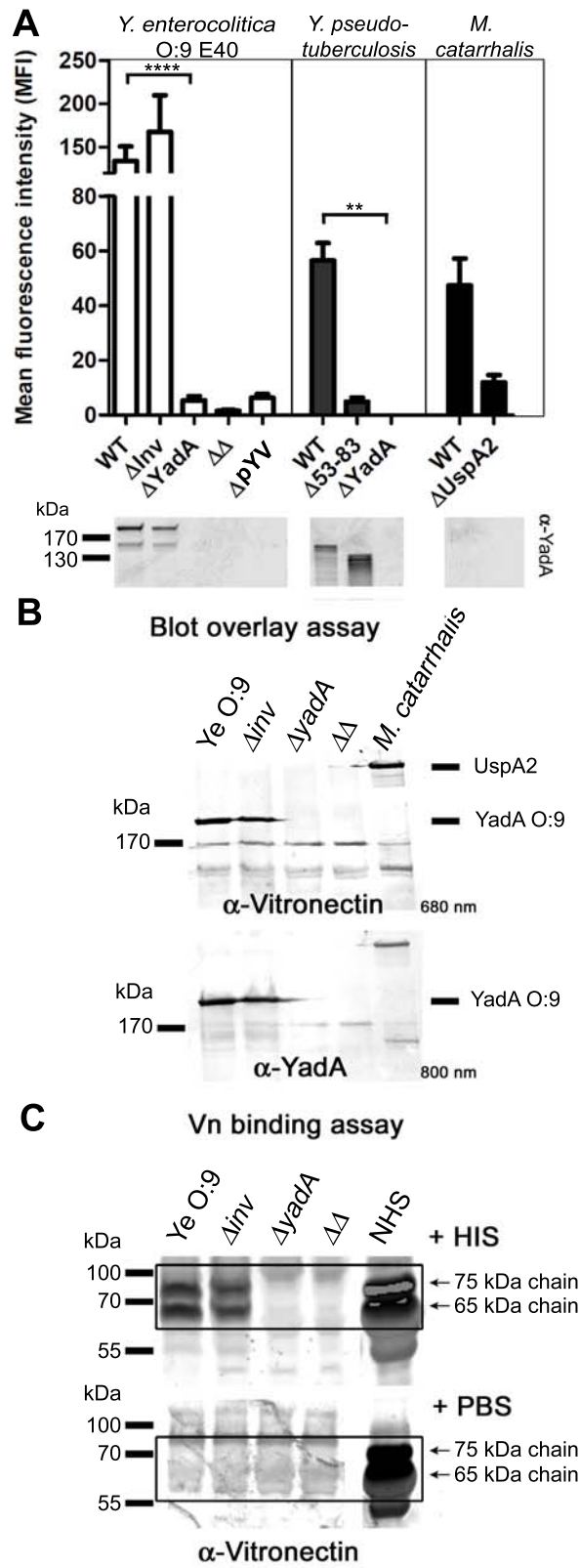


Figure 2, Mühlkamp et al.

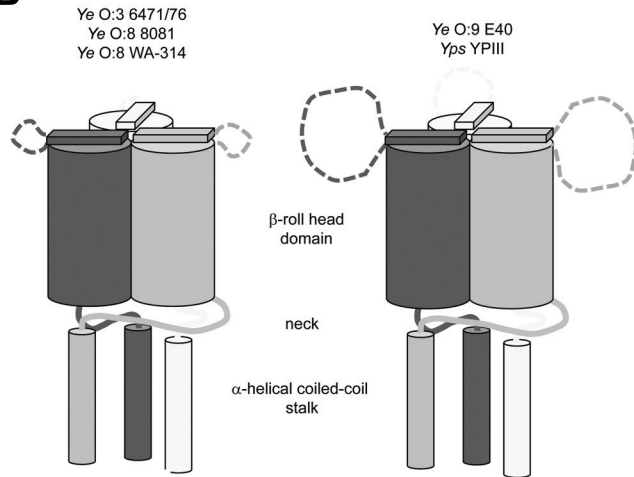
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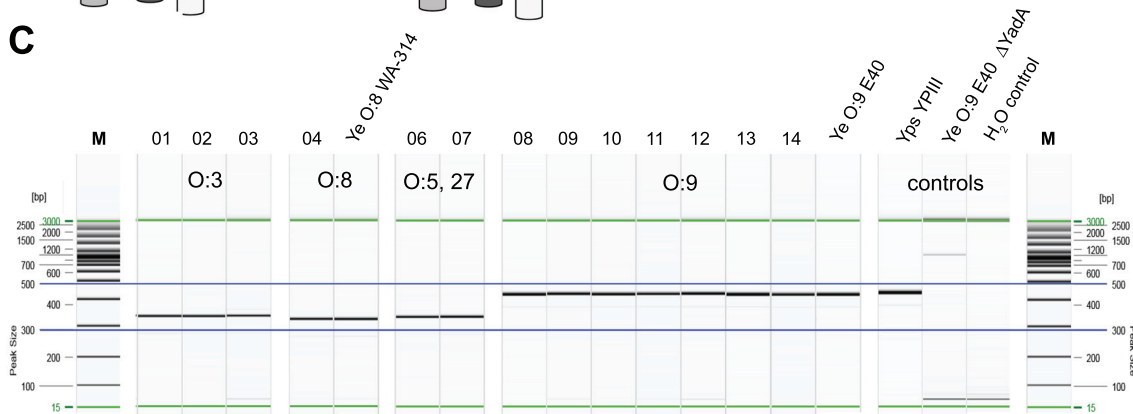
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Ye O:9 E40    MTKDKFKISYSAALISALFSSPYAFI E---DRDGIPLSAVQISPNADPELGVGLYPARPILRPENPKLPPEKPGSRLERSRLHLAESILPRVPGAGGLNASAKGIHSTIAIGA 110
Ye O:8 WA-314 MTKDKFKISYSAALISALFSSPYAFI N----NDEV-HFTAVQISPNDDSHVMIFQPE-----VRAPGGTNALAKGTHSTIAVGA 83
Ye O:8 8081   MTKDKFKISYSAALISALFSSPYAFI N----NDEV-HFTAVQISPNADPDDSHVMIFQPA-----AEALGGTNALAKSIHSTIAVGA 83
Ye O:3 6471/76 MTKDKFKISYSAALISALFSSPYAFI D---DYDGIPLNTAVQISPNADPALGLEYPVRPP-----VPGAGGLNASAKGIHSTIAIGA 85

Yps YPIII      TAEAAKAAVAVGSGSIATGVNSVAIGPLSKALGDSAVTYGASSTAQKDGVAIGARASASDTGVAVGFNSKVDQNSVAIGHSSHVAADHGYSTIAIGDHSKTDRENSVSI GHE 226
Ye O:9 E40    TAEAAKEAAVAVGAGTIATGVNSVAIGPLSKALGDSAVTYGAASTAQKDGVAIGARASTSDTGAVGFNSKADAKNSVAIGHSSHVAVDHDYSTIAIGDRSKTDRENSVSI GHE 223
Ye O:8 WA-314 SAEAAEAAVAVGAGSIATGVNSVAIGPLSKALGDSAVTYGAGSTAQKDGVAIGARASTSDTGAVGFNSKVDKNSVAIGHSSHVVVDHDYSTIAIGDRSKTDRENSVSI GHE 196
Ye O:8 8081   SAEAAKAAVAVGAGSIATGVNSVAIGPLSKALGDSAVTYGASSTAQKDGVAIGARASTSDTGAVGFNSKVDKNSVAIGHSSHVAVDHDYSTIAIGDRSKTDRENSVSI GHE 196
Ye O:3 6471/76 TAEAAKAAVAVGAGSIATGVNSVAIGPLSKALGDSAVTYGAASTAQKDGVAIGARASTSDTGAVGFNSKADAKNSVAIGHSSHVAANHGYSTIAIGDRSKTDRENSVSI GHE 198
  
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B



C



D

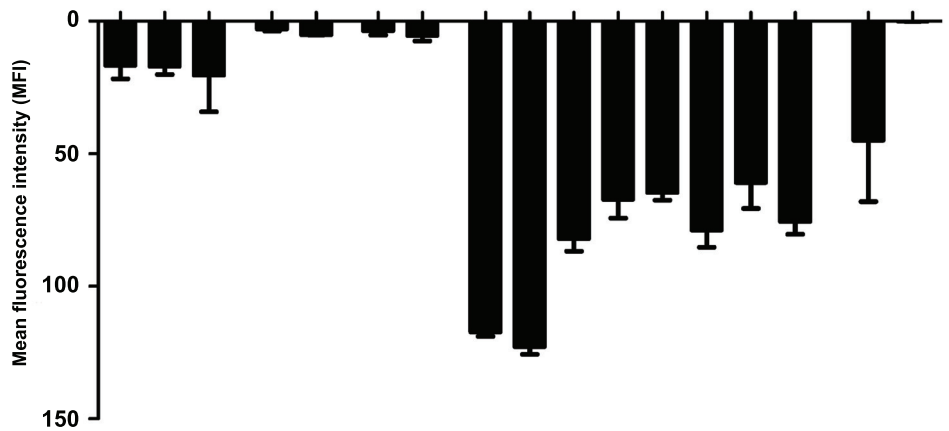


Figure 3, Mühlenkamp et al.

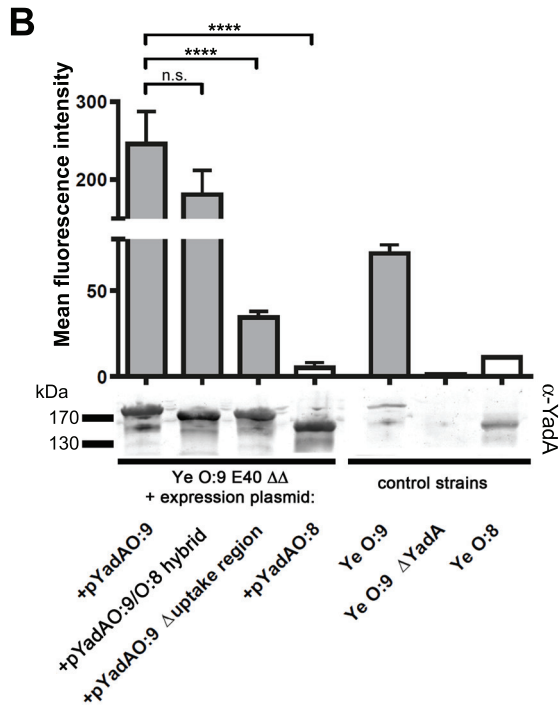
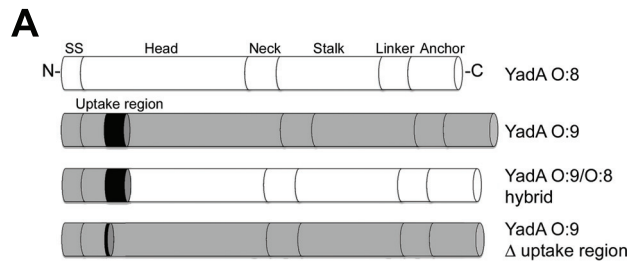


Figure 4, Mühlenkamp et al.

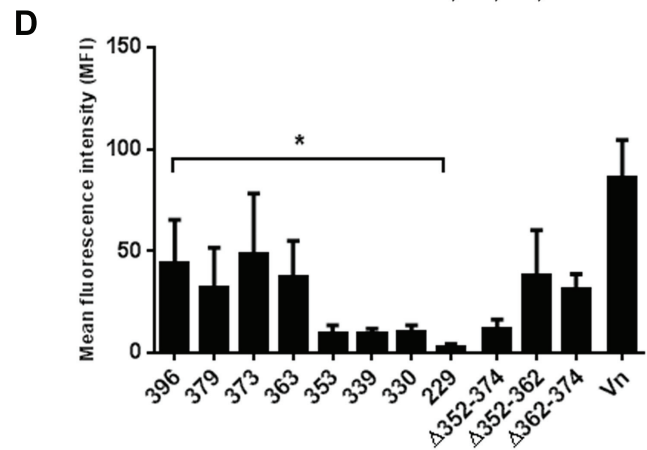
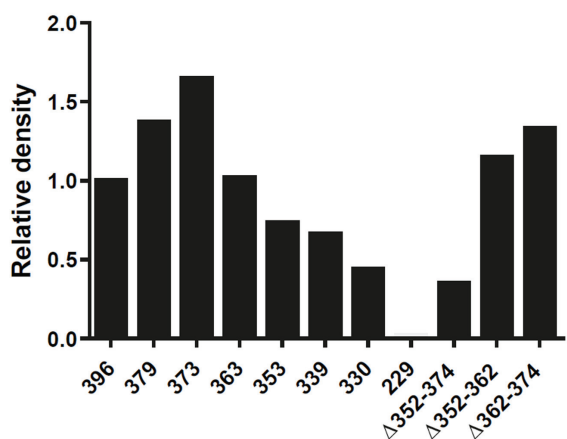
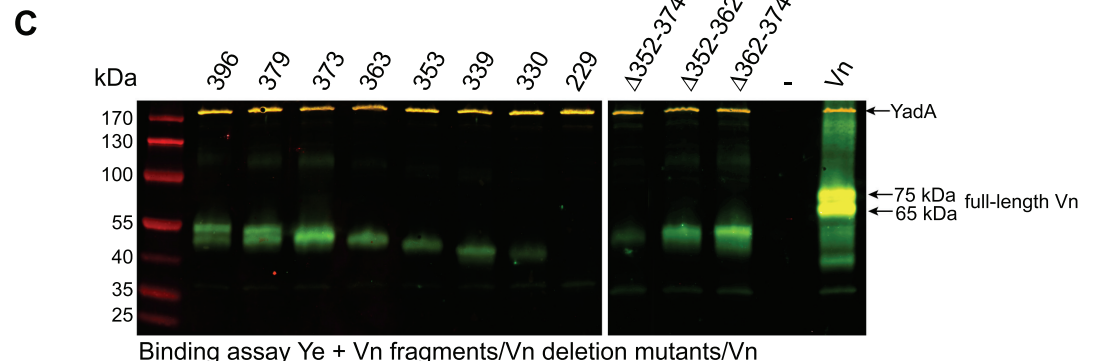
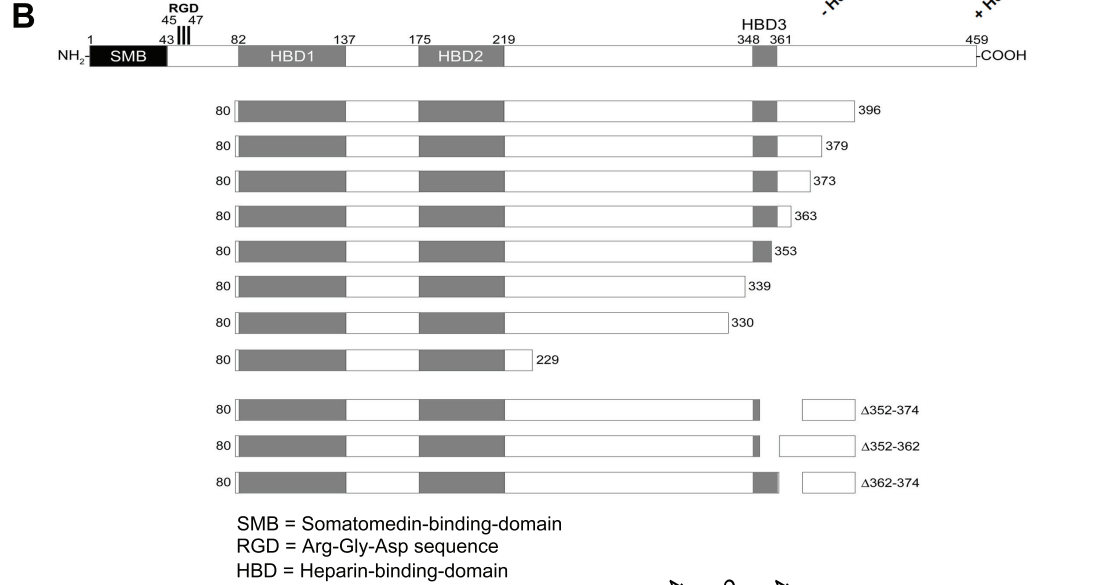
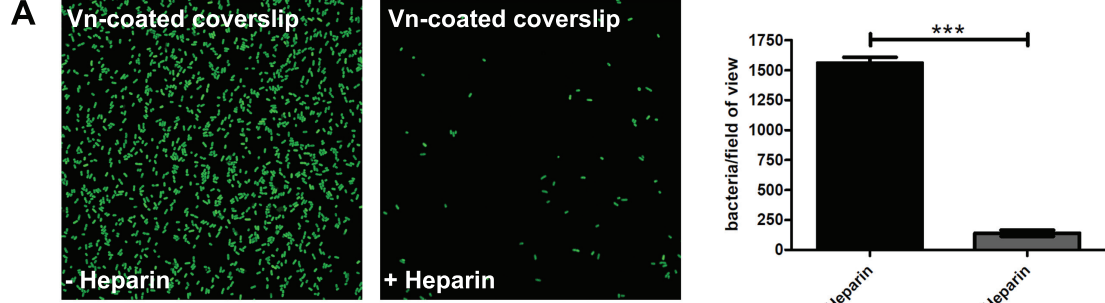


Figure 5, Mühlkamp et al.

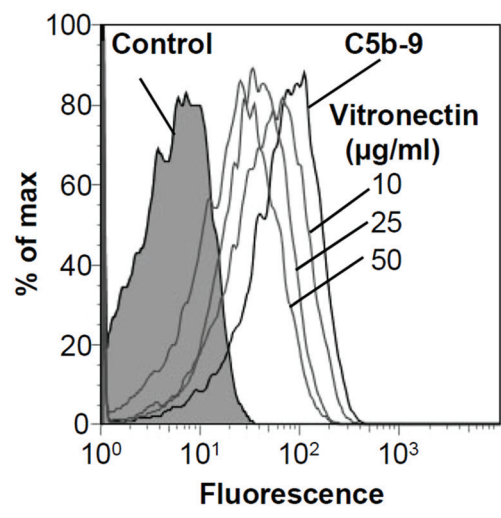
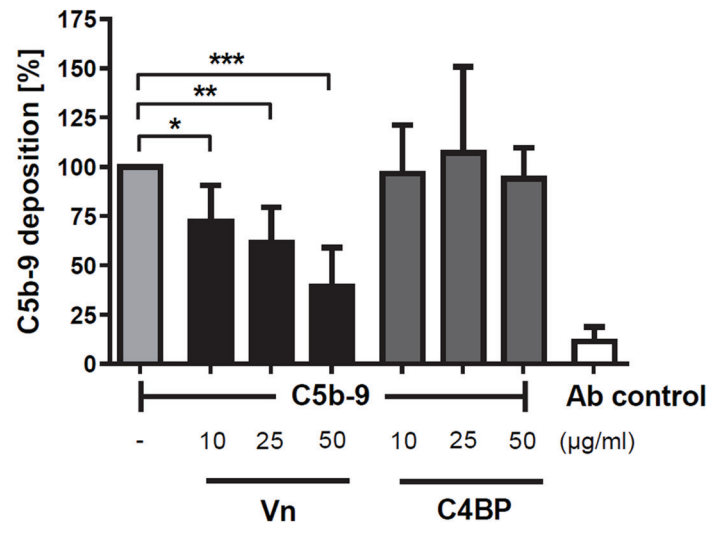
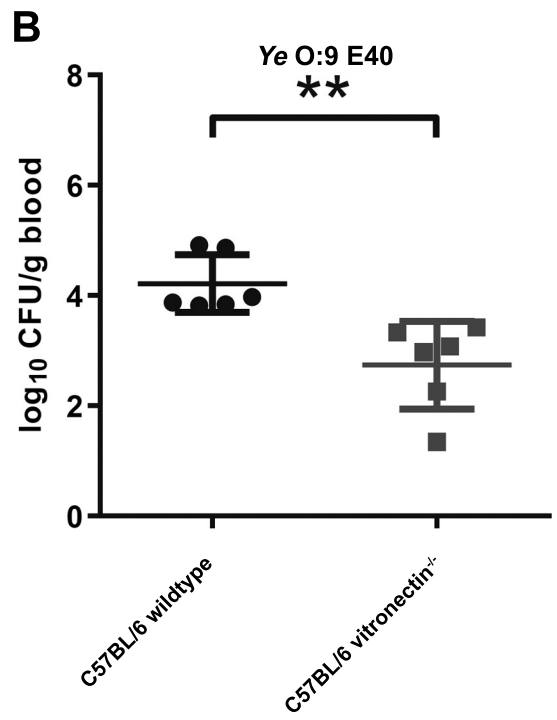
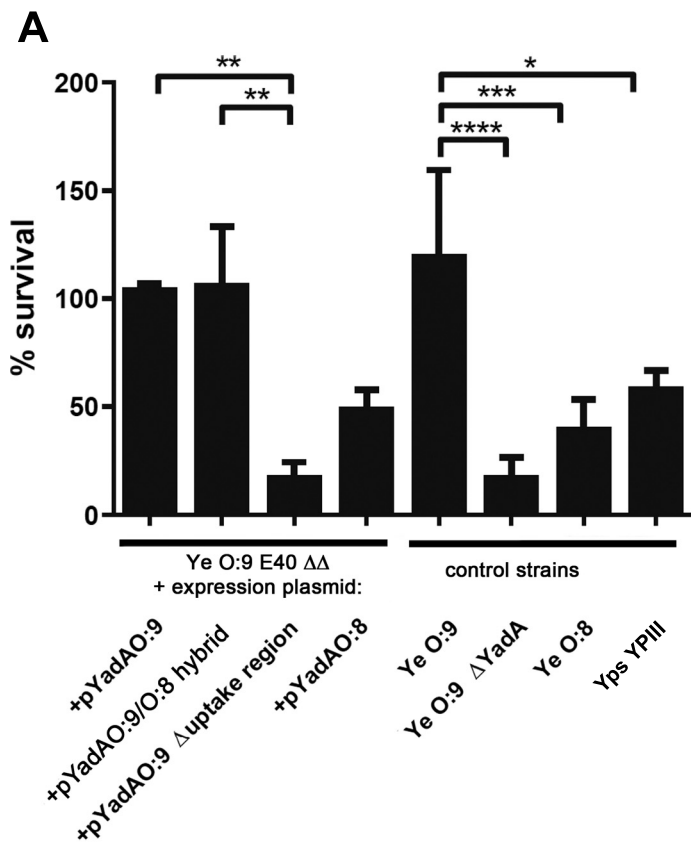
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Figure 6, Mühlenkamp et al.



revised Figure 7, Mühlenkamp et al.

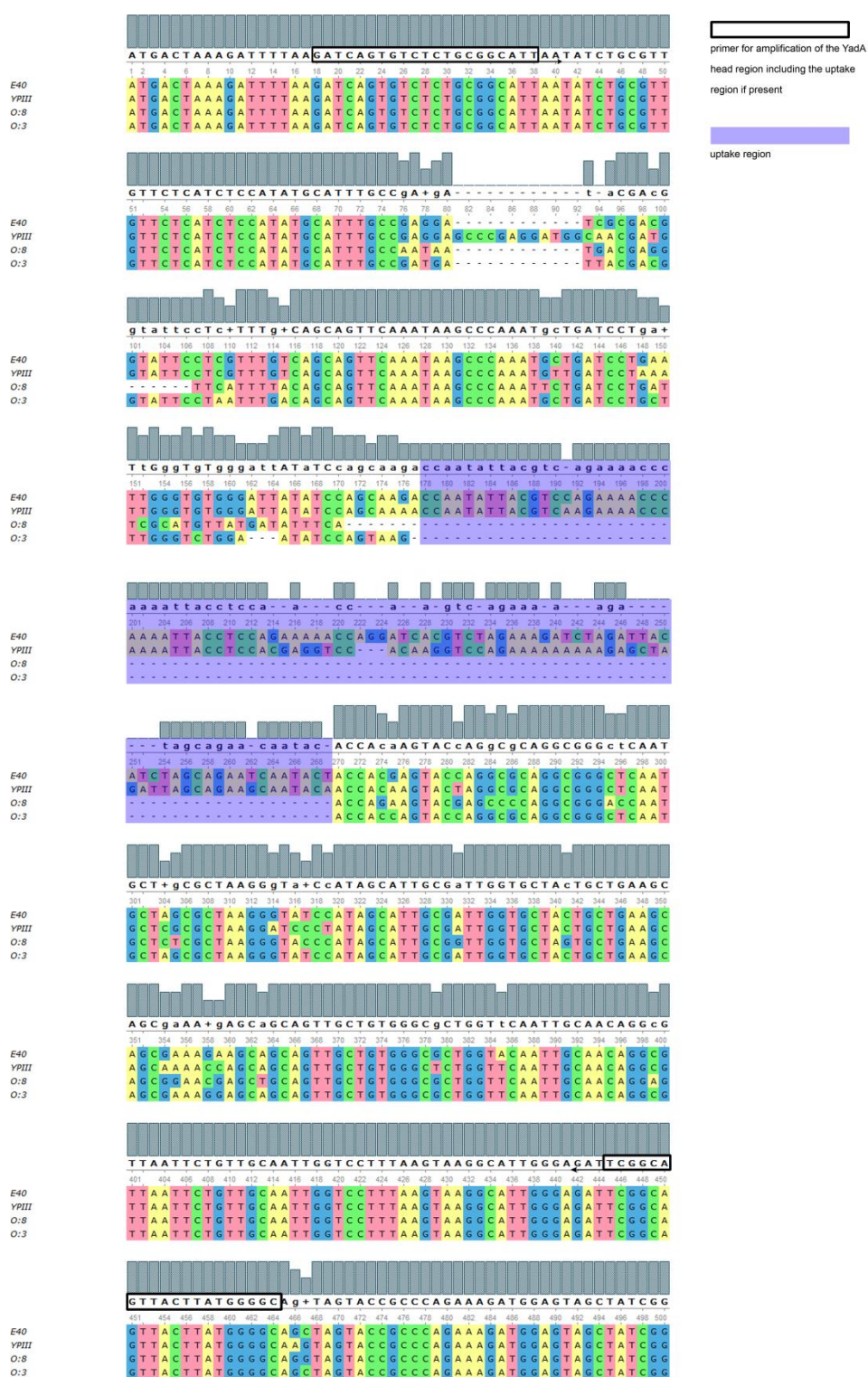


Fig. S1 DNA sequence alignment of YadA from *Ye* strains of different serotypes. DNA alignment of YadA of *Ye* O:9 E40 (E40), O:8 WA-314 (O:8), O:3 6471/76 (O:3) and *Yps* YPIII (YPIII). Alanine is given in yellow, tyrosine in red, guanine in blue and cytosine in green. Amino acid conservation among the different *Yersinia* strains is shown in the grey bars above. The “uptake region” is highlighted in lilac. Primers that were chosen for PCR amplification of the YadA head region are indicated by black boxes.


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YPS YPIII      MTKDFKISVSAALISALFSSPYAFAEPEPEDGNDGIPRLSAVQISPNDPKLGVGLYPAKPILRQENPKLPPRGPQGP-EKKRRARLAEATQPQVLGAGGLNARAKDPYSIAIGATAEAAKPAAVAVGSGSIATGVNSVAIGPLSKALGDSA
YE 09 E40      MTKDFKISVSAALISALFSSPYAFAE----DRDGI PRLSAVQISPNDPELGVGLYPARPILRPENPKLPPEKPGSRLESLRLHLAESILPRVPGAGGLNASAKGIHSIAIGATAEAAKEAAVAVGAGTIATGVNSVAIGPLSKALGDSA
YE 08 WA-314   MTKDFKISVSAALISALFSSPYAFAN-----NDEV-HFTAVQISPNSDPDSHVMI FQPE-----VRAPGGTNALAKGTHSIAVGASAEAAERA AVAVGAGSIATGVNSVAIGPLSKALGDSA
YE 08 8081     MTKDFKISVSAALISALFSSPYAFAN-----NDEV-HFTAVQISPNDPDSHVVI FQPA-----AEALGGTNALAKSIHSIAVGASAEAAKQA AVAVGAGSIATGVNSVAIGPLSKALGDSA
YE 03 6471/76  MTKDFKISVSAALISALFSSPYAFAD----DYDGI PNLTAVQISPNDPALGLEYPVRPP-----VPGAGGLNASAKGIHSIAIGATAEAAKGA AVAVGAGSIATGVNSVAIGPLSKALGDSA

YPS YPIII      VTYGASSTAQKDGVAIGARASASDTGVAVGFNSKVDAQNSVAIGHSSHVAADHGY SIAIGDHSKTDRENSVSI GHESLNRQLTHLAAGTEDTDAVNVAQLKKE-----AETLE
YE 09 E40      VTYGAASTAQKDGVAIGARASTSDTGVAVGFNSKADAKNSVAIGHSSHVAVVDHDSIAIGDRSKTDRENSVSI GHESLNRQLTHLAAGTKD TDAVNVAQLKKEIEKTQENTNKSAELLANANAYADNKSSSVLGIANNYTD SKSAETLE
YE 08 WA-314   VTYGAGSTAQKDGVAIGARASTSDTGVAVGFNSKVDAKNSVAIGHSSHVVVDHDSIAIGDRSKTDRKNSVSI GHESLNRQLTHLAAGTKD TDAVNVAQLKKEIEKTQENANKKSAE-----VLGIANNYTD SKSAETLE
YE 08 8081     VTYGAASTAQKDGVAIGARASTSDTGVAVGFNSKVDAKNSVAIGHSSHVAVVDHDSIAIGDRSKTDRKNSVSI GHESLNRQLTHLAAGTKD TDAVNVAQLKKEIEKTQVNANKKSAE-----VLGIANNYTD SKSAETLE
YE 03 6471/76  VTYGAASTAQKDGVAIGARASTSDTGVAVGFNSKADAKNSVAIGHSSHVAANHGY SIAIGDRSKTDRENSVSI GHESLNRQLTHLAAGTKD TDAVNVAQLKKEIEKTQENTNKSAELLANANAYADNKSSSVLGIANNYTD SKSAETLE

YPS YPIII      NARKEFLAQSNDVLDAAK-----KHSNSVARTTLETAEEHANKKSAELVSAKVYADSNSSHTLKTANSYTDVTVSSSTKKAISESNQYTDHKFSQLDNRLDKLDRVDKGLASSAALNSLFQPYGVGKVNFTAGVGGYR
YE 09 E40      NARKEFAQSKDVLNMAKAHSNSVARTTLETAEEHANSVARTTLETAEEHANKKSAELASANVYADSKSSHTLKTANSYTDVTVSNSTKKAIRESNQYTDHKFRQLDNRLDKLDRVDKGLASSAALNSLFQPYGVGKVNFTAGVGGYR
YE 08 WA-314   NARKEAFDLSNDALDMAK-----KHSNSVARTTLETAEEHTNKSAETLASANVYADSKSSHTLKTANSYTDVTVSNSTKKAIRESNQYTDHKFHQLDNRLDKLDRVDKGLASSAALNSLFQPYGVGKVNFTAGVGGYR
YE 08 8081     NARKEAFDLSNDALDMAK-----KHSNSVARTTLETAEEHTNKSAETLARANVYADSKSSHTLQTANSYTDVTVSNSTKKAIRESNQYTDHKFRQLDNRLDKLDRVDKGLASSAALNSLFQPYGVGKVNFTAGVGGYR
YE 03 6471/76  NARKEFAQSKDVLNMAKAHSNSVARTTLETAEEHANSVARTTLETAEEHANKKSAELASANVYADSKSSHTLKTANSYTDVTVSNSTKKAIRESNQYTDHKFRQLDNRLDKLDRVDKGLASSAALNSLFQPYGVGKVNFTAGVGGYR

YPS YPIII      SSQALAIGSGYRVNESVALKAGVAYAGSSDVMYNASFNIEW
YE 09 E40      SSQALAIGSGYRVNENVALKAGVAYAGSSDVMYNASFNIEW
YE 08 WA-314   SSQALAIGSGYRVNESVALKAGVAYAGSSDVMYNASFNIEW
YE 08 8081     SSQALAIGSGYRVNESVALKAGVAYAGSSDVMYNASFNIEW
YE 03 6471/76  SSQALAIGSGYRVNENVALKAGVAYAGSSDVMYNASFNIEW

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BLUE: SIGNAL PEPTIDE
 GREEN: NECK
 RED: CANONICAL YADA HEAD REPEATS
 ORANGE: MEMBRANE ANCHOR

Fig. S2 Alignment of YadA sequences. Kalign/Muscle alignment of YadA of *Yps* YPIII, *Ye* O:9 E40, *Ye* O:8 WA-314, *Ye* O:8 8081 and *Ye* O:3 6471/76. The signal peptide is given in blue, the neck region is given in green and the membrane anchor domain is given in yellow. Canonical YadA head repeats are highlighted in red.

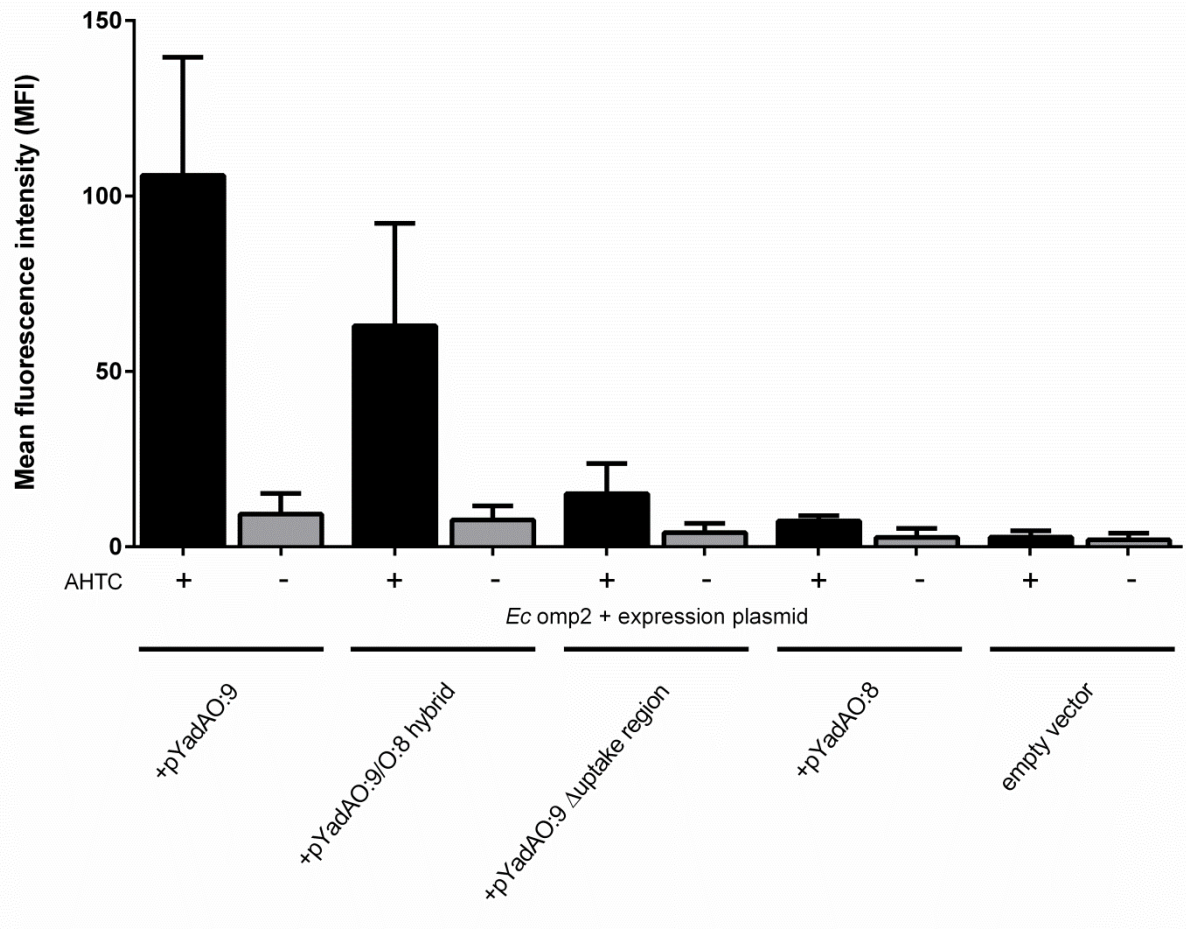


Fig. S3 Vn binding assay analysed by flow cytometry. YadA constructs were expressed in *E.coli* omp2 (Prilipov et al., 1998) and Vn binding from serum was quantified by staining with antibodies directed against Vn and flow cytometry. YadA that contains the “uptake region” (i.e. YadA O:9 and the hybrid YadAO:9/O:8) mediates efficient binding of Vn to the surface of *E.coli* (- = not induced; + = induced with anhydrotetracycline (AHTC) 1:10000). Data are means \pm SD of three individual experiments.

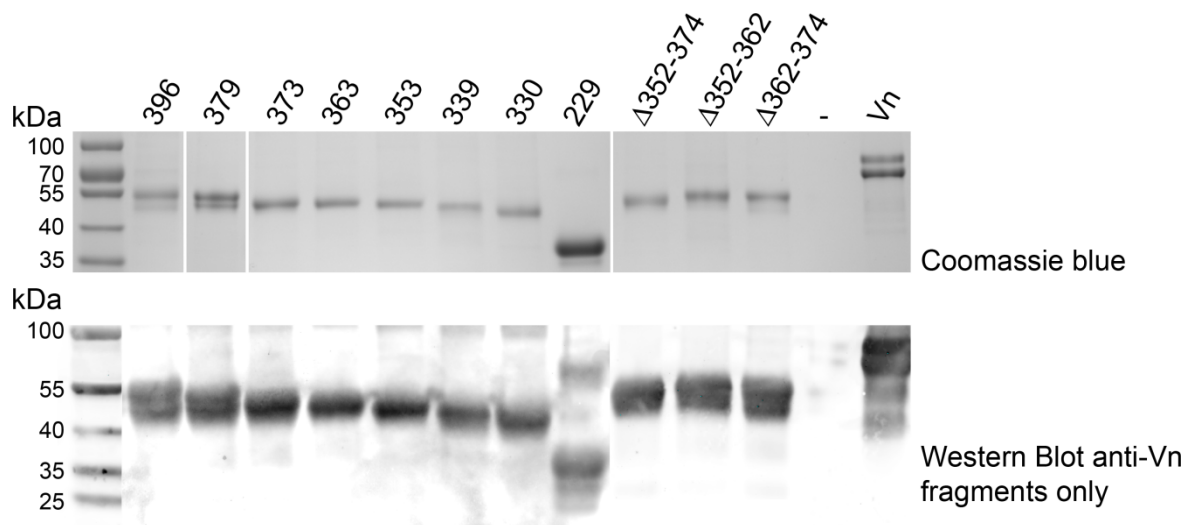


Fig. S4 Quality control of Vn fragments. Integrity and amount of purified Vn fragments was tested by a Coomassie gel and a Western Blot. All fragments have the expected size and concentration and can be detected with the used anti-Vn antibody in comparable sensitivity. One representative experiment out of three is shown.

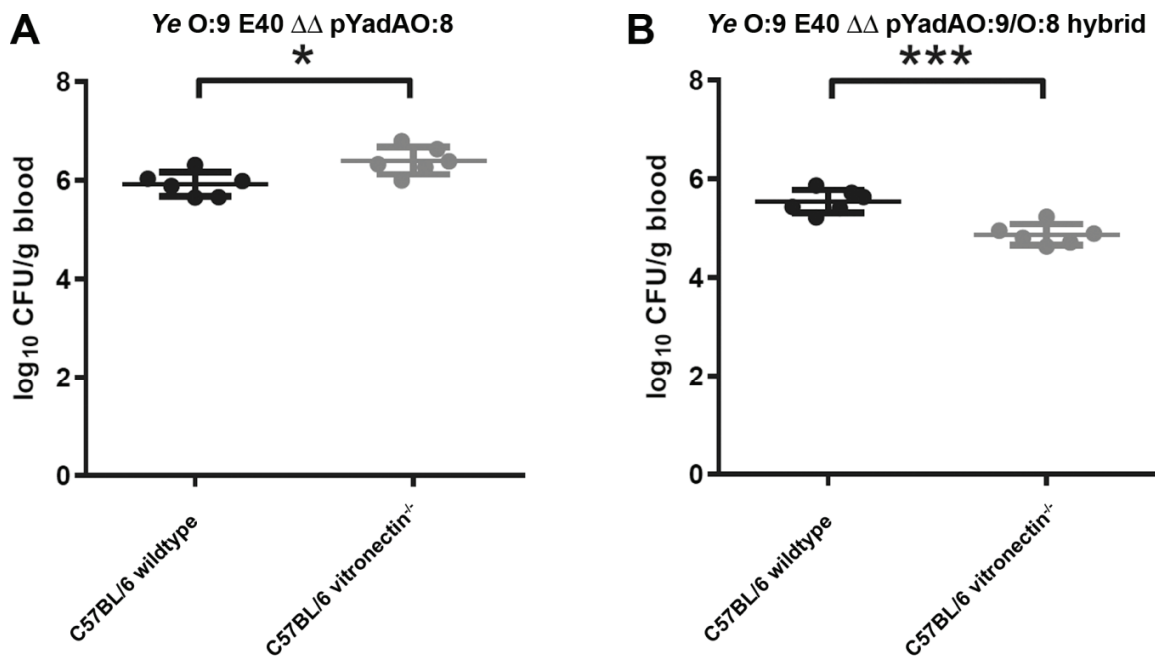


Fig. S5 Comparison of *Ye* O:9 E40 expressing YadAO:8 or the YadAO:9/O:8 hybrid protein in an *in vivo* serum killing assay. Wildtype and Vn^{-/-} mice were infected intravenously with 1×10^7 *Ye* O:9 E40 for 30 min. After that, mice were killed, blood was withdrawn and plated on selective agar plates. The bacterial load was determined by counting colonies the next day and is shown as log₁₀ CFU per g blood. The p value for the comparison of C57BL/6 and Vitronectin^{-/-} mice was determined by Student t test (B). The horizontal lines denote the mean, the error bars the SD. * p < 0.05; ** p < 0.01, *** p < 0.001, **** p < 0.0001, , n=6.