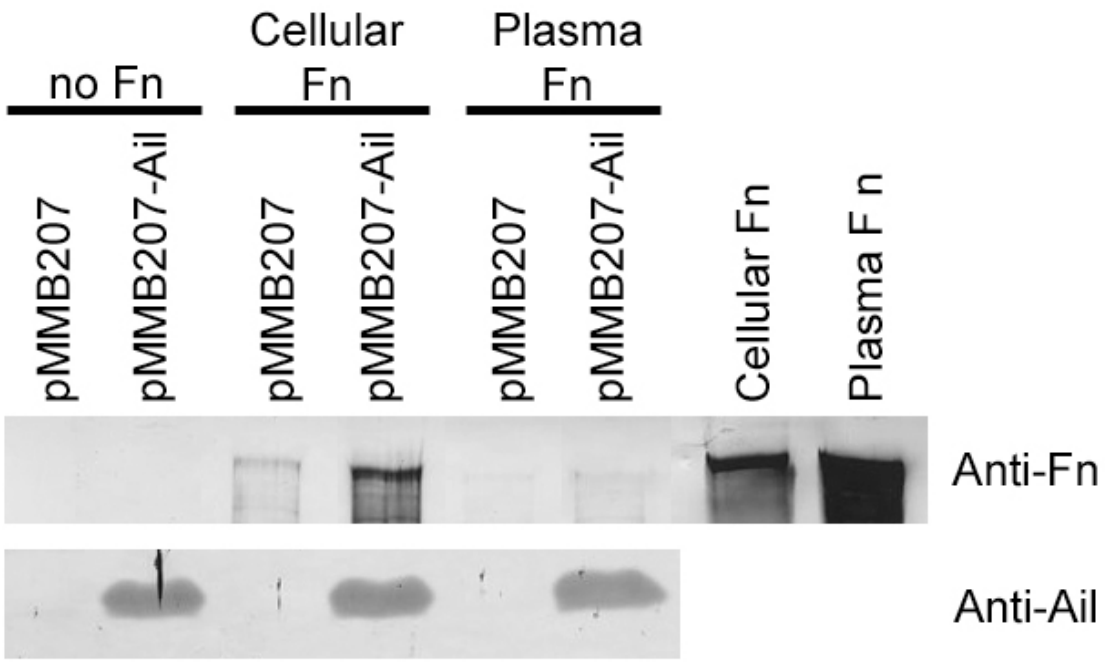


Figure S1: Ail binds cellular Fn in solution much more efficiently than plasma Fn. $3.6x10^7$ cells of *E. coli* strain AAEC185 expressing empty vector (pMMB207) or Ail, were grown overnight at 28°C with 100μM IPTG, washed and pelleted bacteria were resuspended with 20μg/ml cellular or plasma Fn in 200μl PBS + 0.4% BSA solution at 37°C for 1 h with rolling. Bacteria were pelleted to pull down bound Fn for 5 min at 13K rpm in a microcentrifuge. Bacteria were washed once with 1ml PBS and respun prior to boiling in SDS sample buffer and loading on a 7.5% SDS-PAGE gel (for anti-Fn) and 15% SDS-PAGE for anti-Ail). Fn at 10μg/ml was also run on the gel as a control for total Fn available for binding. Western blotting with an anti-Fn polyclonal antibody (1:1000) or anti-Ail polyclonal antibody (1:500) followed by goat anti-rabbit-AP conjugated 2° antibody allowed detection of bacterial-bound Fn and Ail expressed in *E. coli*, respectively.

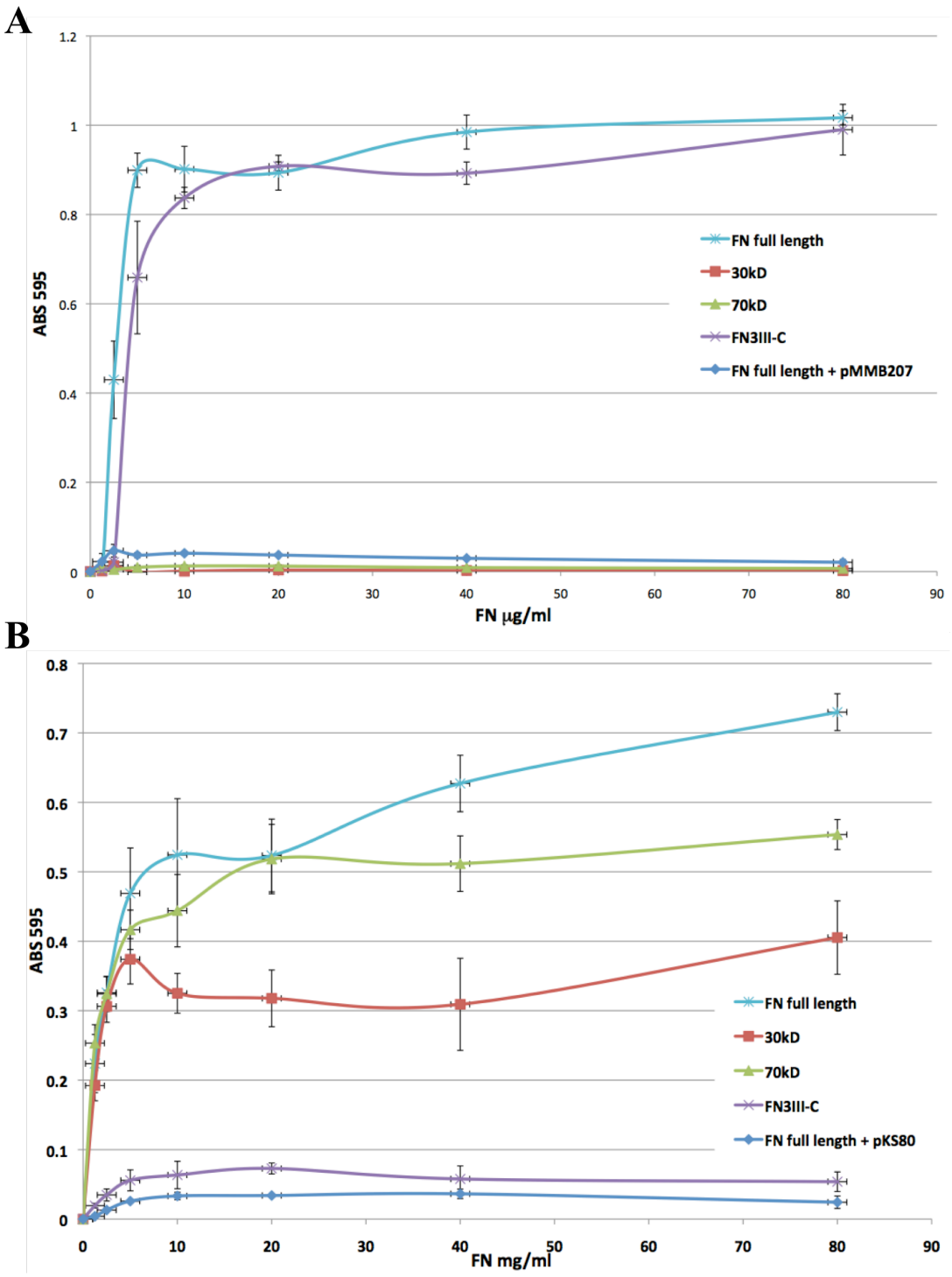


Fig. S2: Ail and FnBP bind FN fragments in a dose-dependent manner. *E. coli* expressing Ail or containing empty vector (pMMB207) were allowed to bind to increasing concentrations of full length plasma FN or various proteolytic or recombinant fragments of FN immobilized on 96-well microtiter plates (A). *L. lactis* expressing *Staphylococcus aureus* FnBPA or empty vector (pKS80) were allowed to bind to the same increasing concentrations of full length FN or fragments (B).

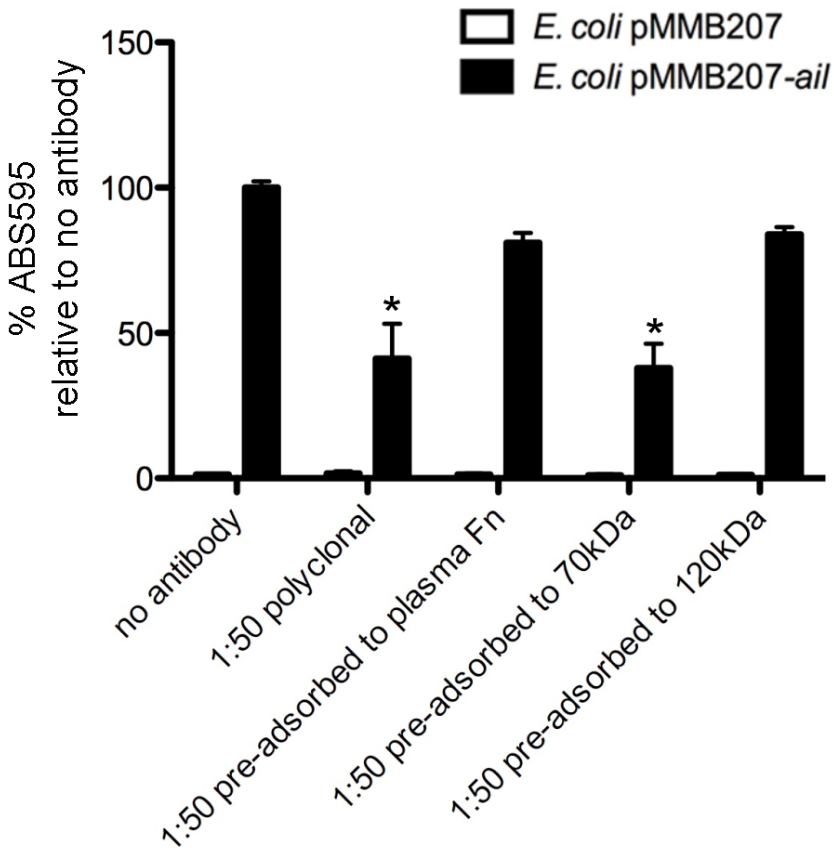


Figure S3: Polyclonal anti-Fn antibody pre-adsorbed to the 120-kDa fragment can no longer inhibit Ail binding. Microtiter wells were coated with plasma Fn (10μg/ml). The polyclonal antibody and pre-adsorbed antibodies were added at a 1:50 dilution for 1 h at 37°C prior to addition of *E. coli* AAEC185 derivatives expressing empty vector and Ail. Bacteria were allowed to bind for an additional 1 h at 37°C. Bound bacteria were stained with 0.01% crystal violet, washed and the cells were solubilized and read at A_{595}. Shown are the results of three experiments in triplicate. * $p<0.0005$.

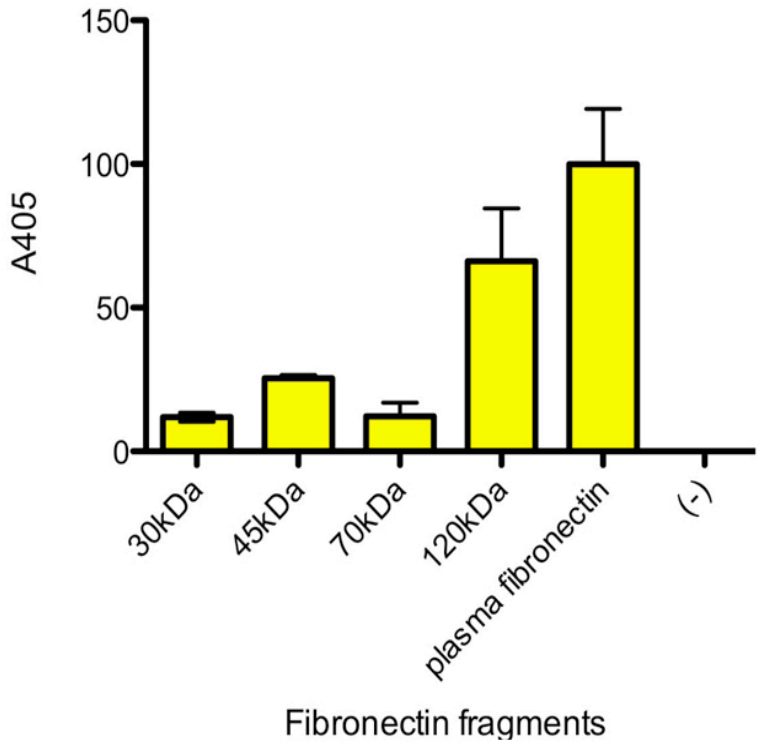
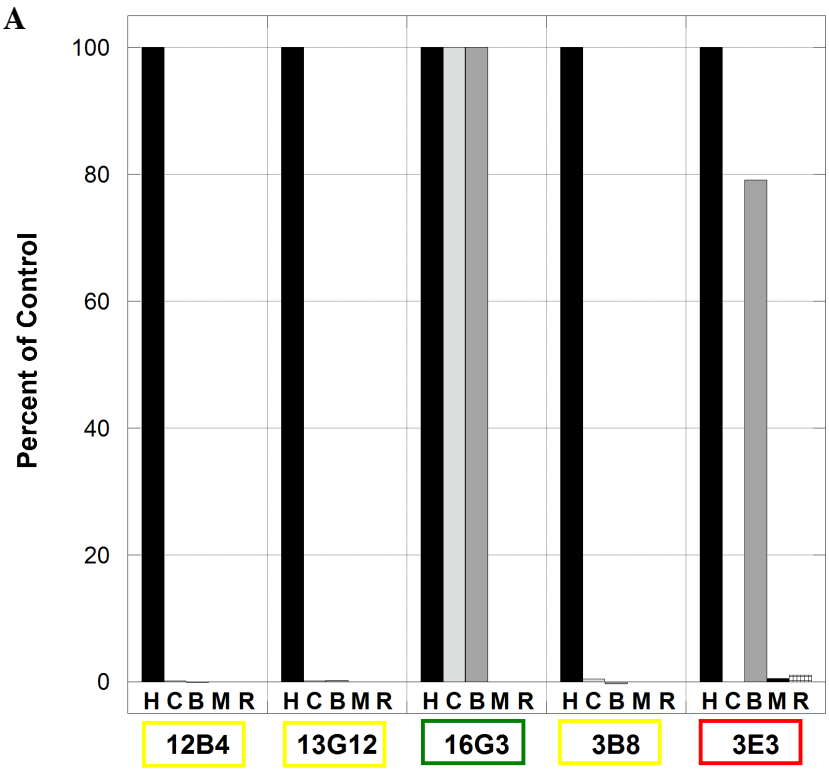


Figure S4: Polyclonal anti-Fn antibody recognizes epitopes throughout Fn. To examine the epitopes recognized by the polyclonal antibody, we performed a modified ELISA assay. Plasma Fn and fibronectin fragments were coated onto microtiter wells and the polyclonal anti-Fn antibody was added. The polyclonal antibody recognized full-length plasma Fn and this signal was normalized to 100%. The polyclonal antibody recognized the 120-kDa fragment, although the signal was less than full length Fn, as expected. The 30-kDa, 45-kDa, and 70-kDa fragments were also recognized but to a lower level. This might be expected, as these Fn fragments are shorter than the 120-kDa or full-length Fn derivatives. Without any Fn coated onto well, the polyclonal antibody gave no signal.



B

^{9}FNIII

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 1448                                                                                                1537
 Hu   GLDSPTGIDF SDITANSFTV HWIAPRATIT GYRIRHHPEH FSGRPREDRV PHSRNSITLT NLTPGTEYVV SIVALNGREE SPLLIGQQST
 Bo   ALDSPSGIDF SDITANSFTV HWIAPRATIT GYRIRHHPEN MGGRPREDRV PPSRNSITLT NLNPGTEYVV SIVALNSKEE SLPLVGQQST
Rat   GLDSPTGFDS SDVTANSFTV HWVAPRAPIT GYIIRHHAEH SAGRPRQDRV PPSRNSITLT NLNPGTEYIV TIIAVNGREE SPPLIGQQST
 Ch   GLDSPTGLDF SDITANSFTV HWIAPRATIT GYKIRHHPEH GVGRPKEDRV PPSRNSITLT NLLPGTEYVV SIIAVNGREE SVPLVGQQTT
 Mu   GLDSPTGFDS SDITANSFTV HWVAPRAPIT GYIIRHHAEH SVGRPRQDRV PPSRNSITLT NLNPGTEYVV SIIAVNGREE SPPLIGQQAT
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^{10}FNIII

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 1538                                                                                                1631
 Hu   VSDVPRDLEV VAATPTSLLI SWDAPAVTVR YYRITYGETG GNSPVQEFTV PGSKSTATIS GLKPGVDYTI TVYAVTGRGD SPASSKPISI NYRT
 Bo   VSDVPRDLEV IAATPTSLLI SWDAPAVTVR YYRITYGETG GSSPVQEFTV PGSKSTATIS GLKPGVDYTI TVYAVTGRGD SPASSKPVSI NYRT
Rat   VSDVPRDLEV IASTPTSLLI SWEPPAVSVR YYRITYGETG GNSPVQEFTV PGSKSTATIN NIKPGADYTI TLYAVTGRGD SPASSKPVSI NYQT
 Ch   VSDVPRDLEV NPTSPTSLEI SWDAPAVTVR YYRITYGETG GSSPVQEFTV PGTMSRATIT GLKPGVDYTI TVYAVTGRGD SPASSKPVTV TYKT
 Mu   VSDIPRDLEV IASTPTSLLI SWEPPAVSVR YYRITYGETG GNSPVQEFTV PGSKSTATIN NIKPGADYTI TLYAVTGRGD SPASSKPVSI NYKT
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Figure S5: Reactivity of various anti-Fn monoclonal antibodies with a variety of Fn molecules from different species. mAbs were allowed to bind mouse, human, bovine, rat or chicken Fn immobilized on microtiter plates. Binding of each antibody was then revealed using anti-mouse or anti-rat secondary antibodies conjugated to alkaline phosphatase. Reactivity to human Fn was set to 100% for normalization (A). To adjust for variation in antigen coating between species, each mAb was further normalized to a species-specific positive control. Potential binding sites based on reactivity are highlighted in yellow for antibodies 12B4, 13G12 and 3B8 in ^{9}FNIII, green for antibody 16G3 in ^{10}FNIII and red for 3E3 in ^{10}FNIII (B). While V1609 is a potential recognition residue for mAb 16G3 in ^{10}FNIII, it is not exposed on the surface, based on the crystal structure of Fn (1). Hu=human Bo=bovine, Ch=chicken and Mu=murine.

Table S1: Strains and plasmids used in this study

Strains	Features	Source
E. coli		
AAEC185	*supE44 hsdR17 mcrA mcrB endA1 thi-1* Δ*fimB-fimH* Δ*recA*	(2)
Rosetta(DE3)pLysS	F^- *ompT* $hsdS_B(R_B^- m_B^-)$ *gal dcm* λ(DE3 *[lacI lacUV5*-T7 *gene1 ind1 sam7 nin5*]) pLysSRARE (Cam^R)	Lab strain (Novagen)
Y. pestis		
KIM5 D27	Δ*pgm*, $pCD1^+$, $pPCP1^+$, $pMT1^+$	(3)
KIM5 D27 Δ*pla*	Δ*pgm* Δ*pla* $pCD1^+$ $pPCP1^+$ $pMT1^+$	this study
KIM5 D27 Δ*ail* Δ*pla*	Δ*pgm* Δ*ail* Δ*pla* $pCD1^+$, $pPCP1^+$, $pMT1^+$	this study
L. lactis		
SP18	wild-type non-adherent *L. lactis* MG1363 + pKS80	(4)
SP9	MG1363 + pRM9 (pFnBP)	(4)
Plasmids		
pMMMB207	Cm-resistant expression plasmid	(5)
pMMB207-*ail*	*ail*-expressing plasmid	(6)
pKS80	Erythromycin-resistant expression plasmid	(4)
pFnBP	pRM9, FnBPA-expressing plasmid	(4)
pET30b+TEV	pET30b+ His-tagging vector with a TEV protease site replacing the enterokinase cleavage site	this study

References for Supplemental Materials:

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