SUPPORTING INFORMATION

The invasin D protein from *Yersinia pseudotuberculosis* selectively binds the Fab region of host antibodies and affects colonization of the intestine

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Running title: InvD selectively binds the Fab region of antibodies

The supporting information contains:

-one supplemental table
-five supplemental figures
-supporting references

SUPPORTING TABLES

Table S1

Strain or Plasmid or Primer	Description	Source or reference
Bacterial Strains		
E. coli K12		
DH101β	F$^-$*endA1 recA1 galE15 galK16 nupG rpsL* Δ*lacX74*Φ80*lacZ*ΔM15*araD139*Δ*(ara,leu)7697 mcrA* Δ*(mrr-hsdRMS-mcrBC)*λ$^-$	Invitrogen
DH5α	F-Φ80*lacZ*ΔM15 Δ(*lacZYA-arg*F) U169 *recA1endA1hsdR17* (rk-, mk+) *phoAsupE44*λ - *thi*-1 gyrA96 *relA1*	Invitrogen
S17-1 λpir	*recA thi pro hsdR*$^-$M$^+$(RP4-2 Tc::Mu-Km::Tn7), *λpir*	(1)
Rosetta2 DE3	F- *ompT hsd*S_B (r_B- m_B-) *gal dcm* (DE3) pRARE2 (Cam^R)	Invitrogen
Y. pseudotuberculosis		
YPIII	pIB1, wild type	(2)
YP197 (Δ*invD*)	*YPIII invD::kan*	This study
Plasmids		
pPS001	pET28derived, InvD_P1737-1976,Kan^R,N-His6	This study
pPS003	pCOLA Duet, InvD G1640-1976, Kan^R,N-His6	This study
pPS004	pCOLA Duet, InvD P1838-1976, Kan^R,N-His6	This study
pPS005	pCOLA Duet, InvD G1640-1976, Kan^R,N-Strep	This study
pPS029	pCOLA Duet, InvD G1640-1839, Kan^R,N-Strep	This study
pPS019	pCOLA Duet, InvDG1640-1976,Kan^R, N-His6_TEV_3xflag	This study
pPS020	pCOLA Duet, InvD G1640-1839,Kan^R, N-His6_TEV_3xflag	This study
pPS021	pCOLA Duet, InvA P500-I985,Kan^R, N-His6_TEV_3xflag	This study
pAKH3	*sacB*$^+$, *Amp*^{R}	(3)
pFU54	*luxCDABE*, ori SC101*, Amp^R	(4)

pFU58	*gfpmut3.1*, ori29807, Amp^R	(4)
pFU189	*luxCDABE*, ori ColE1, Cm^R	(5)
pFU217	*invA-luxCDABE*, ori ColE1, Cm^R	(5)
pFU228	*gapDH*-dsred2, ori ColE1, Cm^R	(4)
pKD4	Kanamycin cassette template, Kan^R	(6)
pCP20	*flp*, ori $SC101_{ts}$, Amp^R	(6)
pRG01	pFU58, *invD-gfpmut3.1*, ori29807, Amp^R	This study
pRG03	pFU54, *invD-luxCDABE*, oriSC101*, Amp^R	This study
pRG05	pRG03, *invD-luxCDABE*, ori ColE1, Amp^R	This study
pRG09	pAKH3, *invD*::*kn*, $sacB^+$, Amp^R	This study
Primers		
III790	CCGGGGGATCCCCTATCCCCACATC CAAAATCAGAGATTT	
III791	CCGGGGTCGACTTAATATACGCTCAT AGATAACGAACACCC	
III792	CCGGGGAGCTCCCTATCCCCACATC CAAAATCAGAGA	
III797	GCTCATAGATAACGAAACAACCTTCTT TA	
III793	CCGGGGAGCTCACATCTCGTCCACTT CGCAAATGAG	
III795	CCGGGGAGCTCGTTAGTAGTTAATGC GGCTCTGTCGCA	
I661	GTGTAGGCTGGAGCTGCTTC	
I662	CATATGAATATCCTCCTTAGTTCC	
InvD_G1640_n_f	AAGAATGCGGCCGCGGCAACCTGAG CACCACGAAC	
InvD_N1976_P_r	TTTTCTGCAG TTA GTTAGATCCGG	
ΔAD-InvD_G1839_P_r	TTTTCTGCAGTTACCCCGGCGTTATTGT CACCATC	
InvA_P500_notI_f	AAGAATGCGGCCGCCCTCAGTTGACAT TAACGGCGGCC	
InvA_I985_pstI_r	TTTTCTGCAGTTATATTGACAGCGCACA GAGCGG	

* reduced copy number variant (1-2 copies/cell)

SUPPORTING FIGURES

FIGURE S1

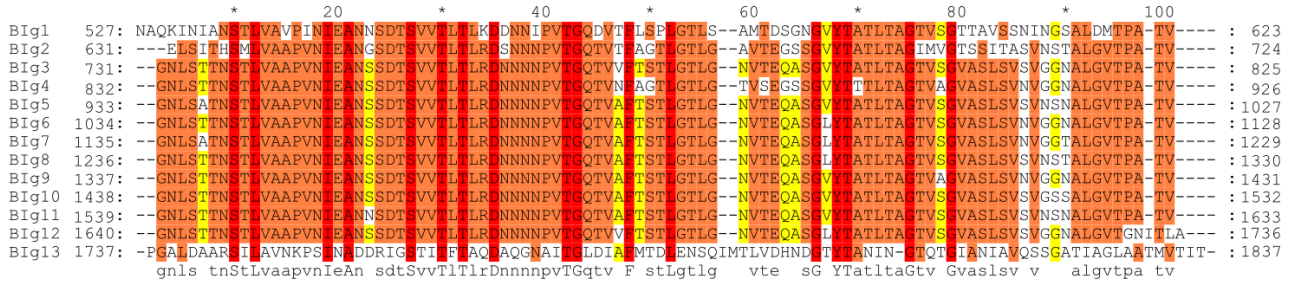


Fig. S1. Details on sequence similarity of InvD. Sequence alignment of BIg1-13 domains of InvD (100% conserved amino acids are marked in red, 80 % in orange and 60 % in yellow). Alignment is generated with Genedoc (7).

FIGURE S2

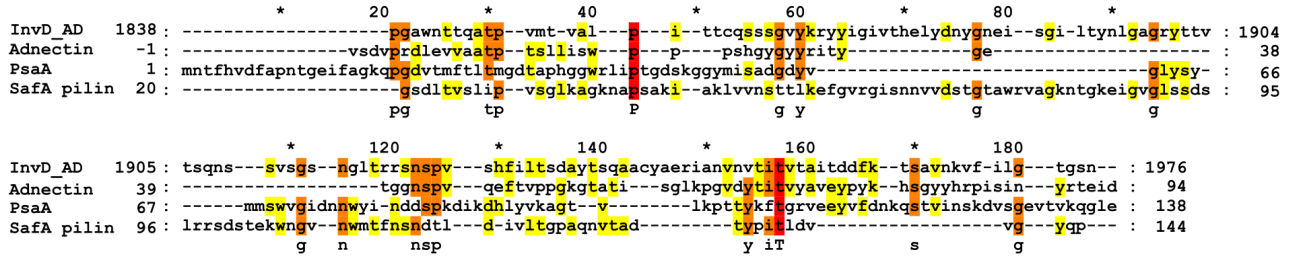


Fig. S2. Similarity to InvD-AD. Sequence alignment of InvD-AD with Adnectin (4OV6:G), PsaA (4F8O:A) and SafA pilin (2CNY:A). (100% conserved amino acids are marked in red, 80% in orange and 60% in yellow). Alignment is generated with Genedoc (7).

FIGURE S3

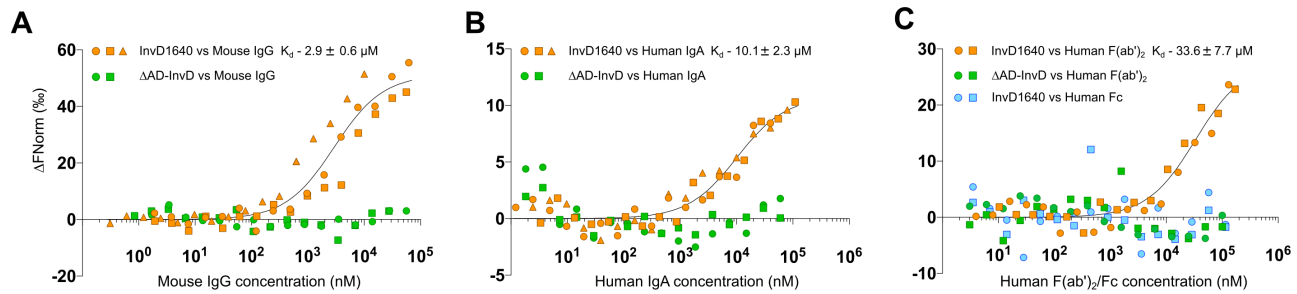


Fig. S3. Analysis of InvD-immunoglobulin interaction. Binding affinities of InvD1640 with mouse IgG (A), human IgA (B) and human F(ab')$_2$ or Fc (C) were determined by Microscale Thermophoresis. ΔAD-InvD (lacking the adhesion domain) was used as a negative control. Individual measurements performed with different concentrations of antibodies are shown. As the data does not reach saturation, the affinity constant (K_d) could not be precisely quantified, but the data allowed for an estimation of the approximate K_d using a global fit in the software DYNAFIT (8).

FIGURE S4

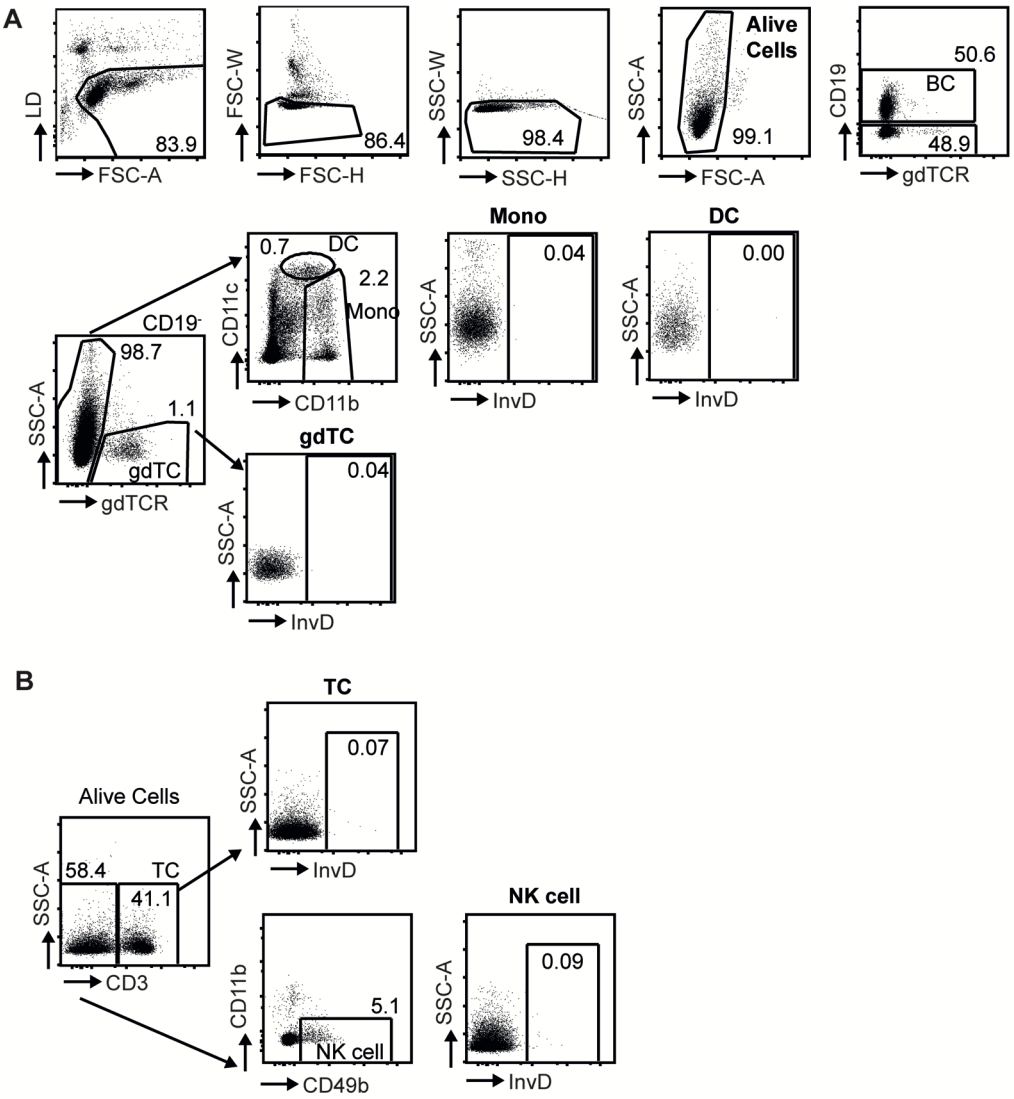
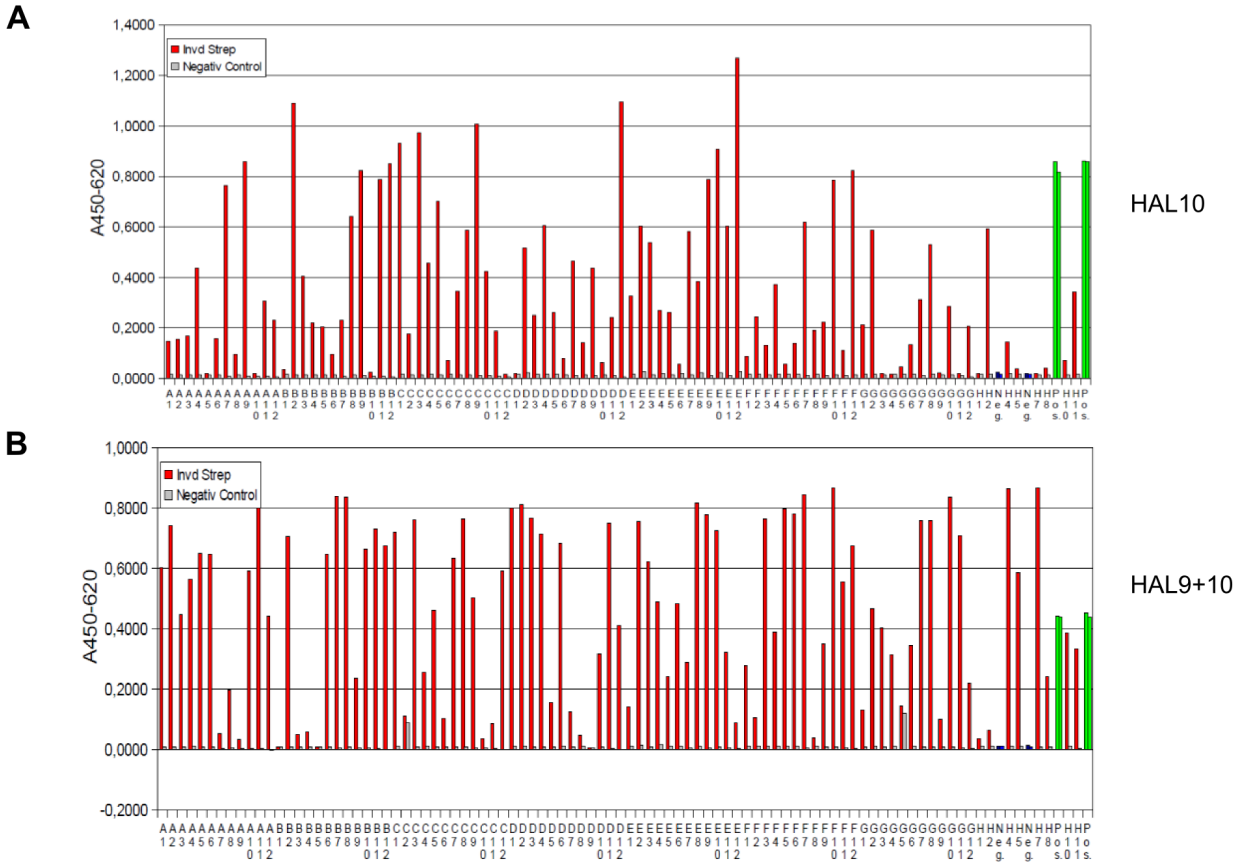


Fig. S4. Gating strategy for the identification of different hematopoietic cell subsets. Single cell suspensions from spleens were analyzed using flow cytometry. Numbers indicate frequency of parental population. All gatings on specific hematopoietic cell populations rely on previous exclusion of dead cells and duplets.

(A) Gating strategy for the identification of B cells ($CD19^+$), γδT cells ($CD19^-\gamma\delta TCR^+$), dendritic cells (DC, $CD19^-\gamma\delta TCR^-CD11b^{med}CD11c^+$) and monocytes (Mono, $CD19^-\gamma\delta TCR^-CD11c^-/CD11c^{low}CD11b^+$).

(B) Gating strategy for the identification of T cells (TC, $CD3^+$) and NK cells ($CD3^-CD49b^+$).

FIGURE S5


A
Invd Strep
Negativ Control
A450-620
HAL10
B
Invd Strep
Negativ Control
A450-620
HAL9+10

C

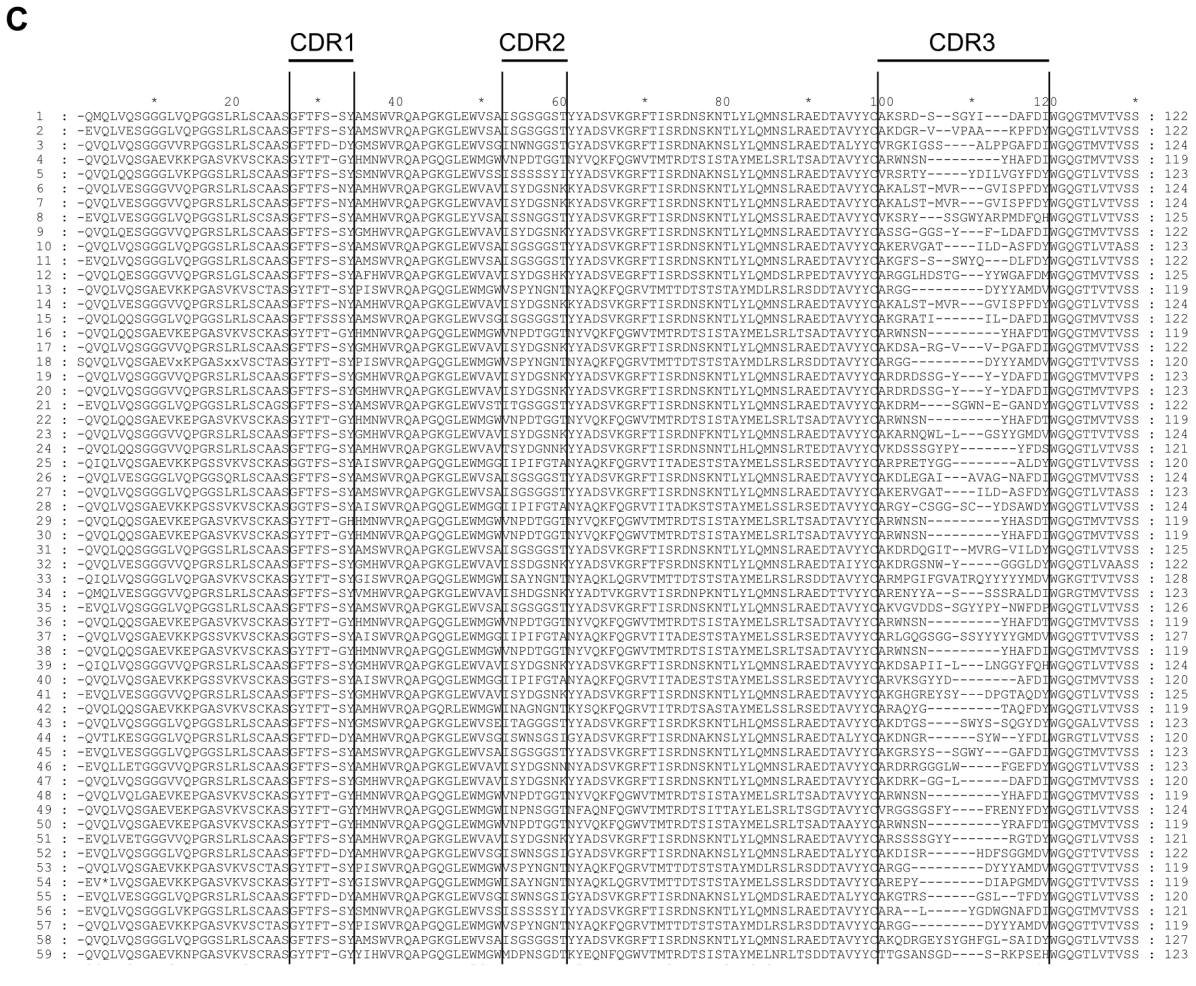

CDR1
CDR2
CDR3

Fig. S5. Identification and alignment of positive clones obtained from phage display panning. (A-B) Binding assessment of soluble scFv to InvD1640 by ELISA. Binding of soluble scFv from (A) HAL10 and (B) HAL9+10 library to InvD1640 (Invd strep) and ΔAD-InvD (Neg. control) was analyzed by microtiter plate ELISA. Green bar represents the positive control (lysozyme + anti-lysozyme scFv), blue bar represents the background signal of the system (streptavidin + *E. coli* supernatant + components of detection system). (C) Alignment of 59 sequenced VH fragments (from HAL 9+10 library) indicates broad diversity in CDR3 region.

SUPPORTING REFERENCES

1. Simon, R., Priefer, U., and Puhler, A. (1983) A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. *Nat Biotech*. **1**, 784–791

2. Bölin, I., Norlander, L., and Wolf-Watz, H. (1982) Temperature-inducible outer membrane protein of Yersinia pseudotuberculosis and Yersinia enterocolitica is associated with the virulence plasmid. *Infect. Immun*. **37**, 506–12

3. Nagel, G., Lahrz, a., and Dersch, P. (2001) Environmental control of invasin expression in Yersinia pseudotuberculosis is mediated by regulation of RovA, a transcriptional activator of the SlyA/Hor family. *Mol. Microbiol*. **41**, 1249–1269

4. Uliczka, F., Pisano, F., Kochut, A., Opitz, W., Herbst, K., Stolz, T., and Dersch, P. (2011) Monitoring of gene expression in bacteria during infections using an adaptable set of bioluminescent, fluorescent and colorigenic fusion vectors. *PLoS One*. **6**, e20425

5. Pisano, F., Kochut, A., Uliczka, F., Geyer, R., Stolz, T., Thiermann, T., Rohde, M., and Dersch, P. (2012) In vivo-induced InvA-like autotransporters Ifp and InvC of Yersinia pseudotuberculosis promote interactions with intestinal epithelial cells and contribute to virulence. *Infect. Immun*. **80**, 1050–64

6. Datsenko, K. A., and Wanner, B. L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A*. **97**, 6640–6645

7. Nicholas, K. B., Nicholas, H. B. J., and Deerfield, D. W. I. (1996) GeneDoc: Analysis and visualization of genetic variation, EMBNEW. **4**, 14

8. Kuzmic, P. (1996) Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV proteinase. *Anal. Biochem*. **237**, 260–273