SUPPLEMENT TO

FOLD AND FUNCTION OF THE INLB B-REPEAT

Maria Ebbes[1], Willem M. Bleymüller[1], Mihaela Cernescu[2], Rolf Nölker[1], Bernd Brutschy[2], Hartmut H. Niemann[1]

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Supplementary Table S1.
X-ray data collection statistics

Protein	B-repeat native	$InlB_{392}$
Data collection		
Space group	$P2_12_12_1$	$P3_2$ 2 1
Unit cell axes (Å)	28.61/ 58.34/ 158.07	126.40/ 126.40/ 107.86
Wavelength (Å)	0.81/ 0.95/ 1.9	0.98
Resolution range (Å)	20-1.30 (1.33-1.30)	15-3.2 (3.28-3.20)
Unique reflections	66287	16581
I/σ	31.81 (2.98)	18.33 (2.89)
Completeness (%)	99.9 (99.3)	98.7 (100)
Redundancy	22.69	14.7
R_{meas} (%)	6.6 (71.9)	13.1 (54.9)

Supplementary Table S2.
X-ray data collection and phasing

Protein	B-repeat SeMet		
Data collection			
Wavelength (Å)	inflection	peak	high remote
	0.97838	0.97776	0.95370
Resolution range (Å)	20-2.0 (2.05-2.0)		
Unique reflections	33,433	33,456	34,128
I/σ	12.47 (2.16)	12.67 (2.19)	11.60 (2.16)
Completeness (%)	93.3 (64.1)	93.4 (64.2)	95.3 (72.6)
Redundancy	3.33	3.33	3.39
R merge (%)	9.8 (44.0)	9.7 (43.1)	11.1 (51.6)
Phasing			
Correlation coefficient			
All/ Weak	49.4/ 32.9		

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Supplementary Table S3.
Refinement Statistics of $InlB_{392}$

Resolution range (Å)	15.0-3.20 (3.28-3.20)
R_{cryst}	19.7 (29.6)
R_{free}	22.5 (33.1)
No. of reflections	
Working set	15752 (1105)
Test set	829 (58)
No. of atoms	
Protein	2271
Solvent/ion	0/3
R.m.s. deviation	
from ideal geometry	
Bond Lengths (Å)	0.023
Bond Angles (°)	2.037
Ramachandran plot:	
Favored (%)	86.8
Disallowed (%)	1.4

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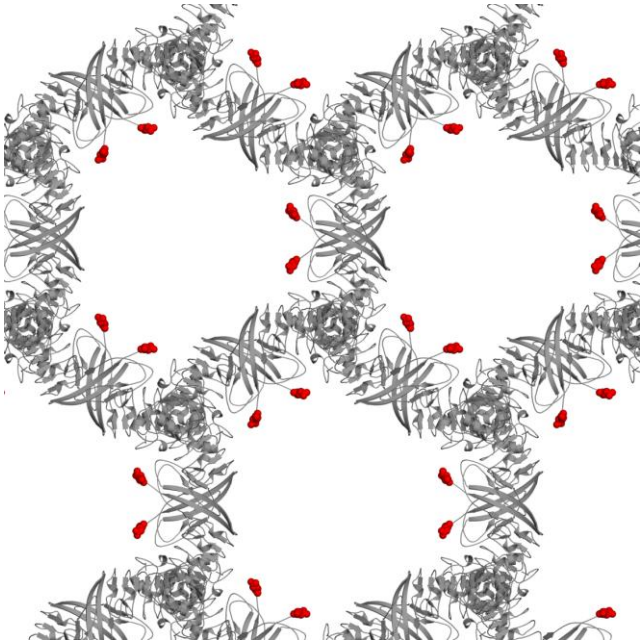

SUPPLEMENTARY FIGURE S1. **Crystal packing of InlB$_{392}$**. The crystals of InlB$_{392}$ are loosely packed with a solvent content of 80%. Crystal contacts are formed by the InlB interanlin domain (gray). No electron density is visible for the B-repeat, which probably dangles freely in the large solvent channels. The last C-terminal residue of the internalin domain that is visible in the electron density is shown as red spheres and indicates the position where the B-repeat starts.

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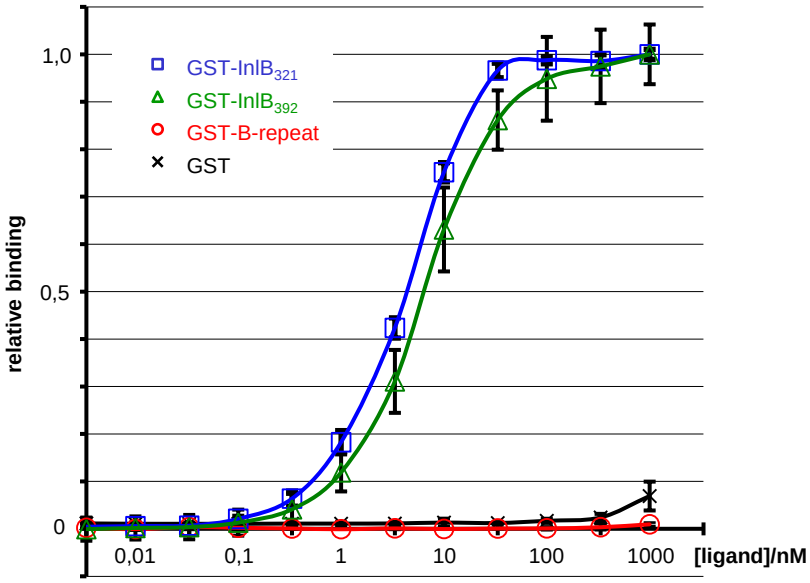

SUPPLEMENTARY FIGURE S2. **The B-repeat does not interact with the Met ectodomain in a solid-phase binding assay (ELISA)**. The complete Met ectodomain (Met_{928}) was immobilized on ELISA plates and incubated with increasing concentrations of a GST-B-repeat, GST-$InlB_{321}$ and GST-$InlB_{392}$ fusion proteins. Binding was detected with an anti-GST antibody coupled to horse-radish peroxidase. GST alone served as negative control.

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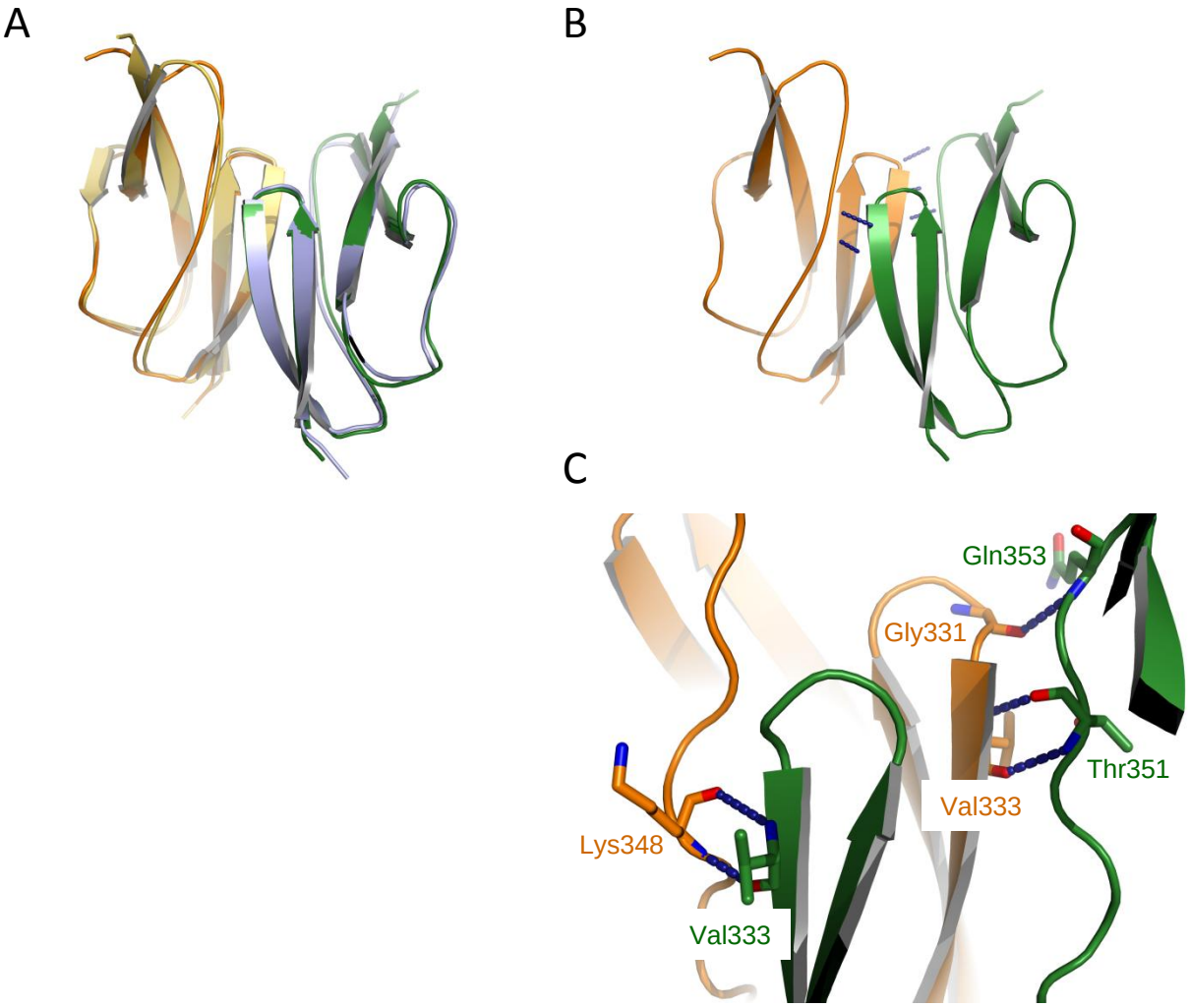

SUPPLEMENTARY FIGURE S3. **Crystal packing of the InlB B-repeat.** *A*, Monomers A and B (green and orange) and monomers C and D (lightblue and yellow) pack into the same dimeric arrangement. *B*, The dimer is not 2-fold symmetric as can be seen from the different hydrogend bonds (blue dashed lines) formed between strand β2 of one monomer and the extended loop connecting strands β2 and β3 from the other monomer. *C*, Detailed view of the hydrogend bonds formed between chain A and chain B.

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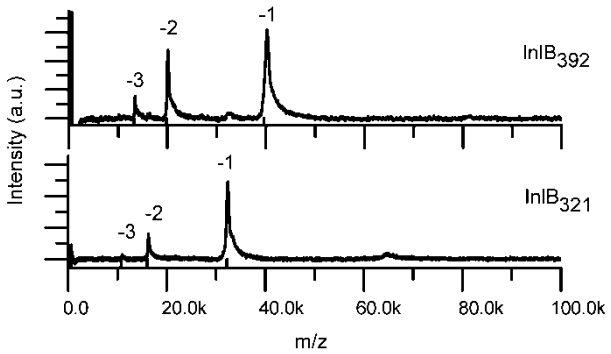

SUPPLEMENTARY FIGURE S4. **LILBID control measurement.** Mass spectra of $InlB_{321}$ and $InlB_{392}$ in 20 mM ammonium acetate and 50 mM ammonium acetate, respectively. The monomer peaks appear at three different overall charge states (the black sticks indicate the theoretical mass/charge positions).

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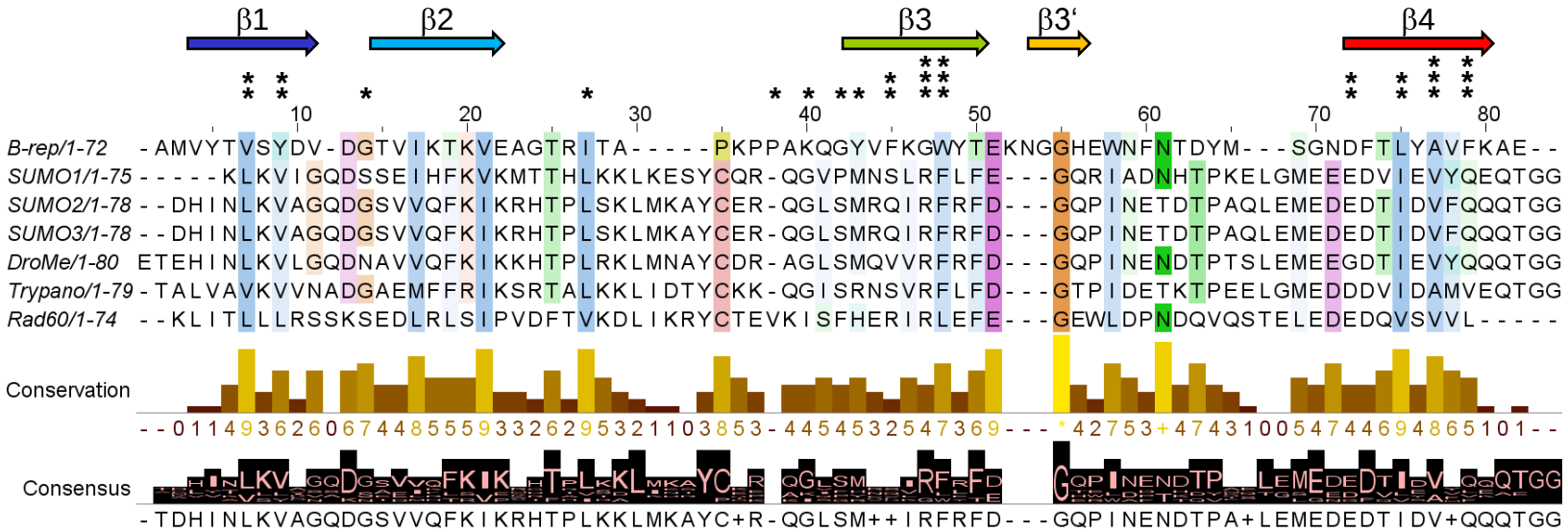

SUPPLEMENTARY FIGURE S5. **Structure based multiple sequence alignment of the InlB B-repeat and six representative structures from the Pfam Rad60-SLD („Ubiquitin-2 like Rad60 SUMO-like) family (PF11976).**

•SUMO1: human SUMO1 (PDB ID 2io2)
•SUMO2: human SUMO2 (PDB ID 2io0)
•SUMO3: human SUMO3 (PDB ID 2io1)
•DroMe: SUMO3 from *Drosophila melanogaster* (PDB ID 2k1f)
•Trypano: SUMO from *Trypanosoma brucei* (PDB ID 2k8h)
•Rad60: DNA repair protein rad60 from *Schizosaccharomyces pombe* (PDB ID 3goe)

Secondary structure of the B-repeat (chain D) is indicated above the sequence. The coloring of β-strands is the same as in Fig 2. Residues important for the fold of the B-repeat are marked with asterisks according to their conservation score as reported by Jalview (see Fig. 2). Conservation score 5 & 6: (*); 7 & 8: (**), 9 & +: (***). The sequence alignment was colored in Jalview according to sequence conservation with the Clustalx color scheme and a Conservation Color Increment of 30.

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Supplementary Table S4: Pairwise structural comparison of chain D of the B-repeat with PDB entries 2kt7, 2kvz and 3lyy and with the N- and C-terminal domains (B1 and B2) of PDB entry 3i57 using the DaliLite server.

Protein	PDB	Z	rmsd	Lali	%ID
mucus binding protein repeat (Mub-R5) N-terminal domain (B1) *Lactobacillus reuteri*	3i57N	5.6	2.4	61	11
mucus binding protein repeat (Mub-R5) C-terminal domain (B2) *Lactobacillus reuteri*	3i57C	3.6	2.1	54	17
putative peptidoglycan bound protein lmo0835 residues 161-235 *Listeria monocytogenes*	2kvz	5.2	2.5	58	17
putative peptidoglycan bound protein lmo0835 residues 34-128 *Listeria monocytogenes*	2kt7	3.3	2.7	57	9
adhesion protein PEPE_0118 *Pediococcus pentosaceus*	3lyy	4.4	2.8	58	17

Supplementary Table S5: Results from a Dali search with chain D of the B-repeat against the complete PDB. The top result is given for a small ubiquitin like modifier (SUMO), ubiquitin, the protein Mth1743 from *Methanobacterium thermoautotrophicum* and for the immunoglobulin-binding proteins protein G and protein L from *Streptococcus sp*. and *Peptostreptococcus magnus*, respectively.

Protein	PDB	Z	rmsd	Lali	%ID
SUMO3	2io1	6.4	2.5	63	14
ubiquitin	3nob	5.0	3.1	61	15
Mth1743 (protein of unknown function) *Methanobacterium thermoautotrophicum*	1ryj	4.5	2.9	59	10
protein L *Peptostreptococcus magnus*	1kh0	3.7	3.2	52	10
protein G *Streptococcus sp*	1mi0	2.5	3.0	45	11

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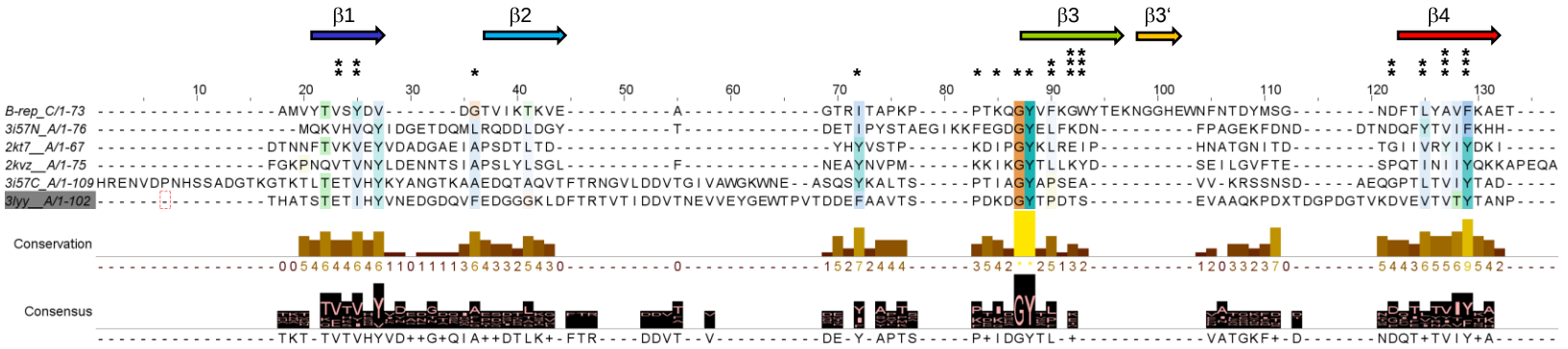

SUPPLEMENTARY FIGURE S6. **Structure based multiple sequence alignment of the InlB B-repeat and structurally similar bacterial domains.** Sequence similarity is highest in strand β4, in the turn leading into strand β3 and in strand β1. The highly conserved GW signature motif of the B-repeat/Flg_new domains and strand β3‘ are missing in the other bacterial domains.

•B-rep: InlB B-repeat
•3i57N: B1 domain of repeat 5 of mucus binding protein (Mub-R5) from *Lactobacillus reuteri* (PDB ID 3i57)
•2kt7: residues 34-128 of lmo0835, a putative peptidoglycan bound protein from *Listeria monocytogenes* (PDB ID 2kt7)
•*2*kvz*:* residues 161-235 of lmo0835, a putative peptidoglycan bound protein from *Listeria monocytogenes* (PDB ID 2kvz)
•3i57C: B2 domain of repeat 5 of mucus binding protein (Mub-R5) from *Lactobacillus reuteri* (PDB ID 3i57)
•3lyy: adhesion protein PEPE_0118 from *Pediococcus pentosaceus* (PDB ID 3lyy)

Secondary structure of the B-repeat (chain D) is indicated above the sequence. The coloring of β-strands is the same as in Fig 2. Residues important for the fold of the B-repeat are marked with asterisks according to their conservation score as reported by Jalview (see Fig. 2). Conservation score 5 & 6: (*); 7 & 8: (**), 9 & +: (***). The sequence alignment was colored in Jalview according to sequence conservation with the Clustalx color scheme and a Conservation Color Increment of 30.

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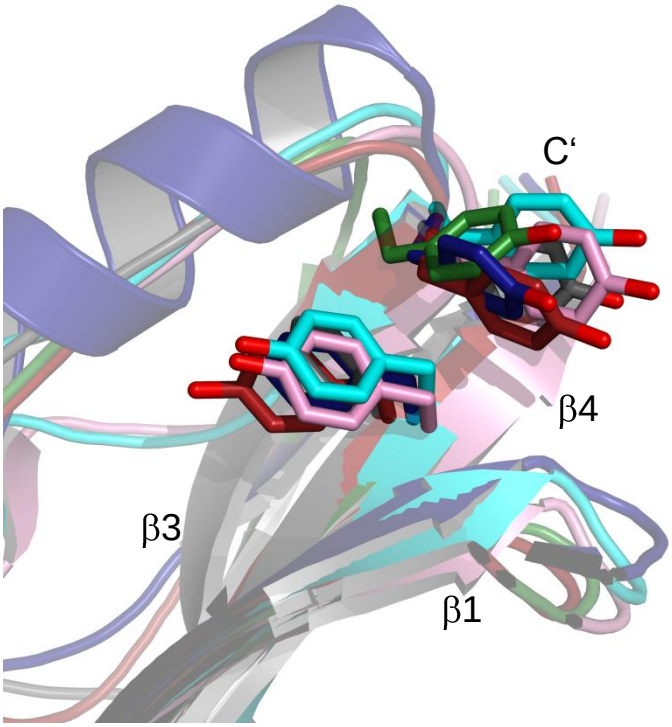

SUPPLEMENTARY FIGURE S7. **Overlay of aromatic residues in the B-repeat and structurally similar bacterial domains.** Color coding is the same as in Fig. 7. Cartoon representation of the InlB B-repeat (gray), the B1 (dark blue) and B2 (cyan) domains of repeat 5 of mucus binding protein (Mub-R5) from *Lactobacillus reuteri* (PDB ID 3i57), residues 34-128 (red, PDB ID 2kt7) and residues 161-235 (green, PDB ID 2kvz) of lmo0835, a putative peptidoglycan bound protein from *Listeria monocytogenes*, and of the adhesion protein PEPE_0118 from *Pediococcus pentosaceus* (pink, PDB ID 3lyy). The residues shown in stick representation are the tyrosine from the GY motif at the start of strand β3 and the aromatic residue at the end of strand β4 (phenylalanine in the B-repeat and the B1 domain of Mub-R5 and tyrosine in the others).