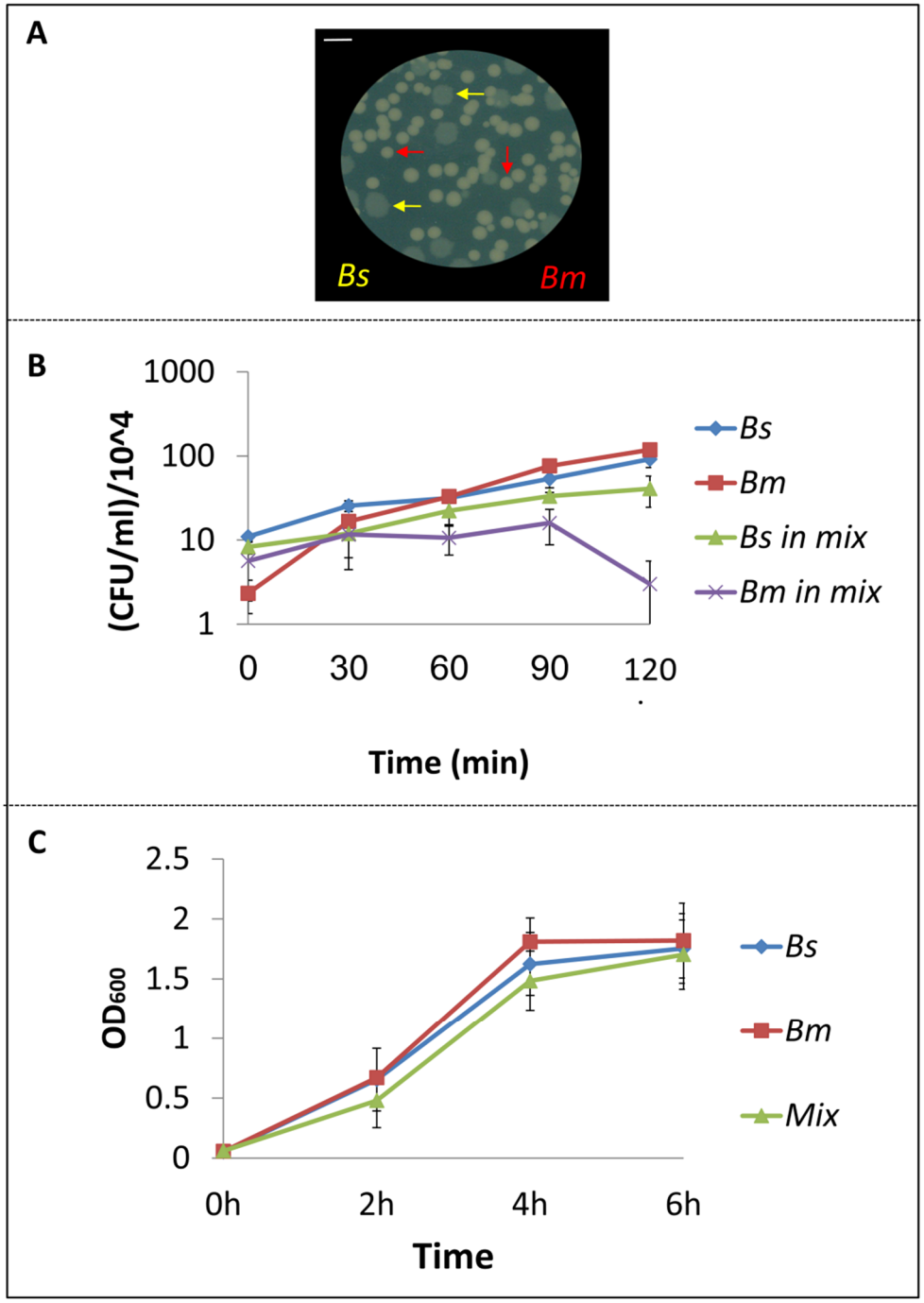
Description of Supplementary Files

File name: Supplementary Information
Description: Supplementary figures, supplementary tables and supplementary references.

File name: Peer review file



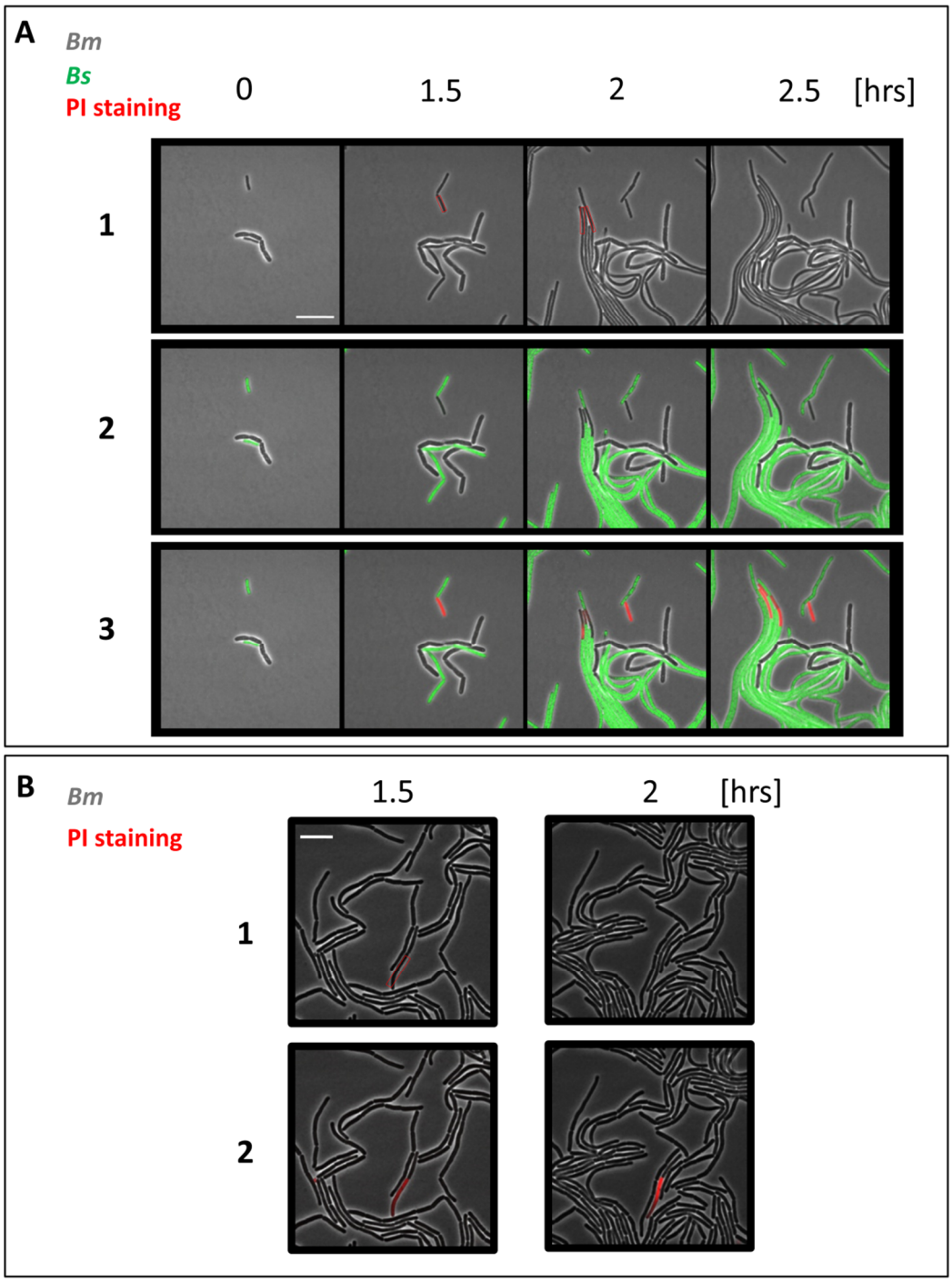
Supplementary Figure 1: Growth characteristics of *Bs* and *Bm*

(A) *Bs* (PY79) and *Bm* (OS2) were grown separately to OD_{600} 0.5, diluted to 10^{-3}, then mixed and immediately plated on LB plate. Cells were incubated ON at 37°C. Shown is

an image of a plate containing a mixture of *Bs* (yellow arrows) and *Bm* (red arrows) colonies. Scale bar represents 1 cm.

(B) *Bs* (PY79) and *Bm* (OS2) were grown separately or in a mixture in LB medium at 37°C. Cells were plated for CFUs at the indicated time points. *Bs* and *Bm* were differentiated by colony morphology as seen in (A). Each point represents an average value and SD of three independent experiments.

(C) *Bs* (PY79) and *Bm* (OS2) were grown separately or in a mixture in LB medium at 37°C. Cell density (OD_{600}) was examined at the indicated time points. Each point represents an average value and SD of three independent experiments.



Supplementary Figure 2: *Bs* toxicity does not perturb *Bm* membrane integrity

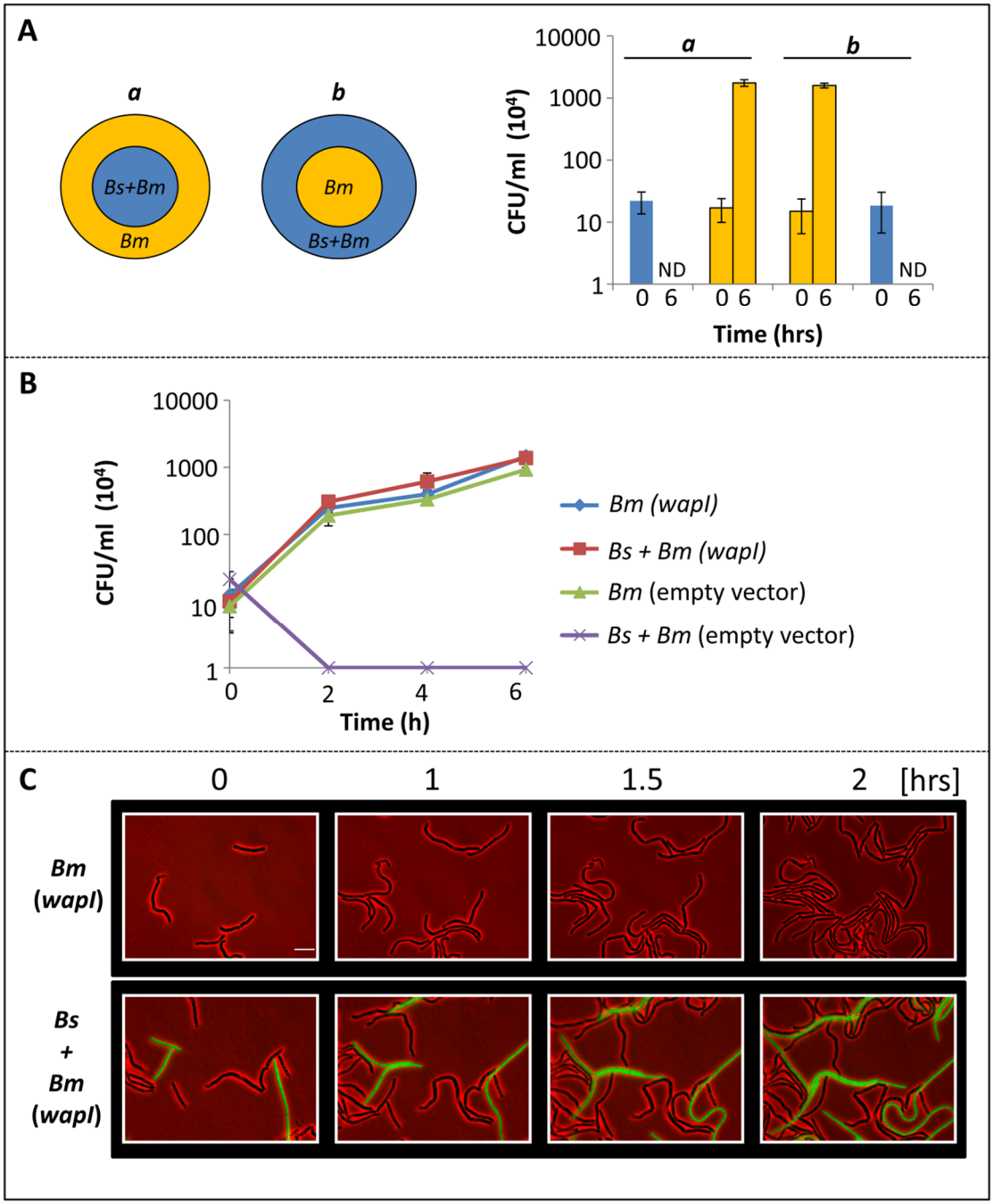
Cells were spotted at low density on solid LB supplemented with propidium iodide (PI) (2 μM) and incubated at 37°C in a temperature controlled chamber and imaged by confocal microscopy. As a positive control, specific cells (marked regions) were irradiated using the 405 nm laser beam at full power (5 mW) and were repeatedly

bleached (50 iterations) in order to observe loss of membrane integrity and PI penetration.

(A) Shown are images of a mixture of *Bs* (AR16: *amyE::*P_{rrnE}-*gfp*) and *Bm* (OS2) taken at the indicated time points of (1) phase contrast with red squares indicating the irradiated regions, (2) overlay fluorescence images of GFP (green) and phase contrast (grey), (3) and overlay fluorescence images of GFP (green), PI (red) and phase contrast (grey).

(B) Shown are images of *Bm* (OS2) taken at the indicated time points of (1) phase contrast with red squares indicating the irradiated regions, and (2) overlay fluorescence images of PI (red) and phase contrast (grey).

Bm exhibits no fluorescence (black), live *Bs* express GFP (green) and irradiated cells are susceptible to PI staining (red). Scales represent 5 μm.



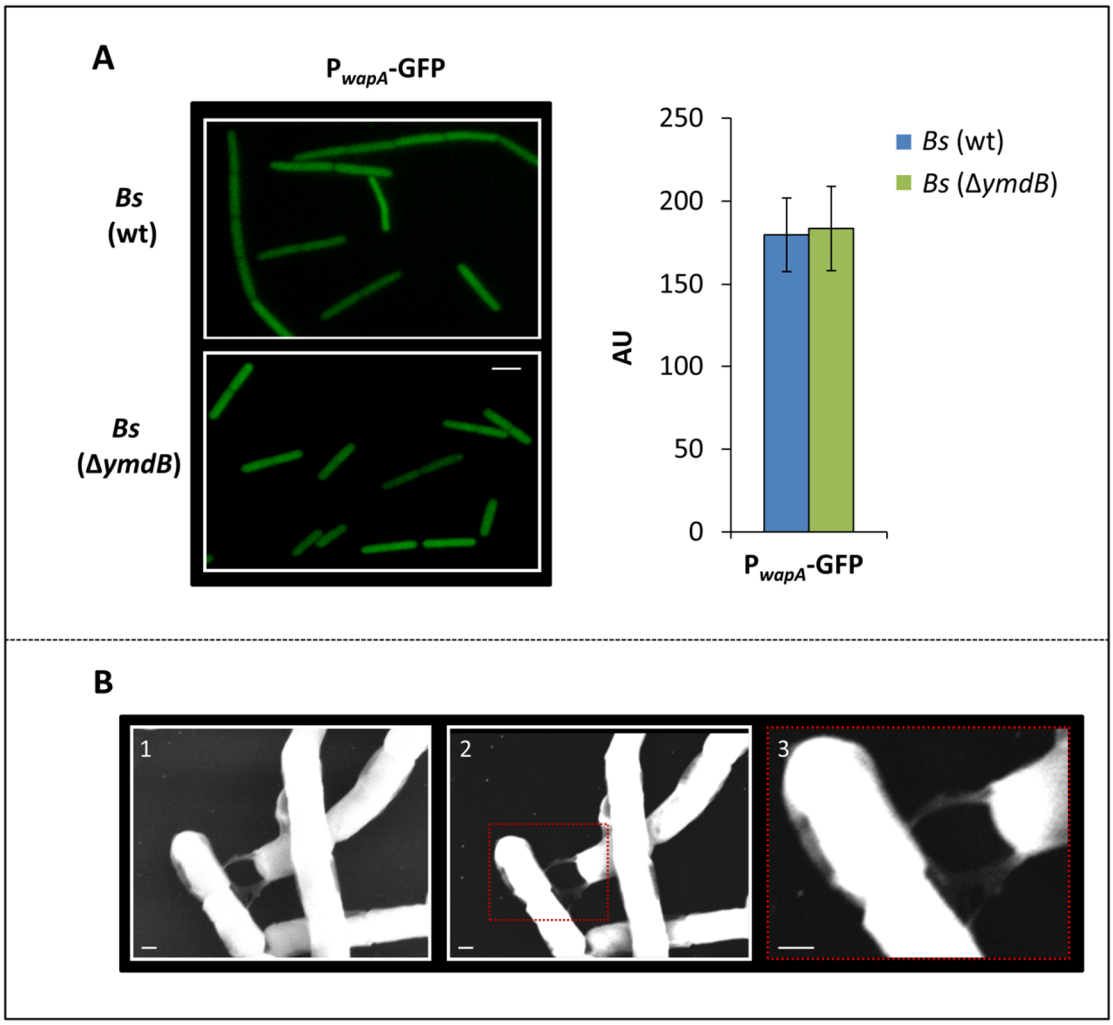
Supplementary Figure 3: WapI from *Bs* confers immunity to *Bm*

(A) Illustration of a Transwell chamber with mono-culture of *Bm* (OS2) (yellow) and co-culture of *Bm* (OS2) and *Bs* (PY79) (blue). Histogram indicates the growth of *Bm* cells alone or with *Bs* at t_0 and after 6 hrs. Each bar represents an average value and SD of three independent experiments. ND-not detected.

(B) *Bm* (pSSBm85-*wapI*) harboring a plasmid containing *wapI* from *Bs*, and *Bm* (pSSBm85) harboring an empty vector were grown separately or in a mixture with *Bs*

(PY79) at 37°C in LB containing xylose to induce *wapI* expression. Cells were plated for CFUs at the indicated time points. Each point represents an average value and SD of three independent experiments.

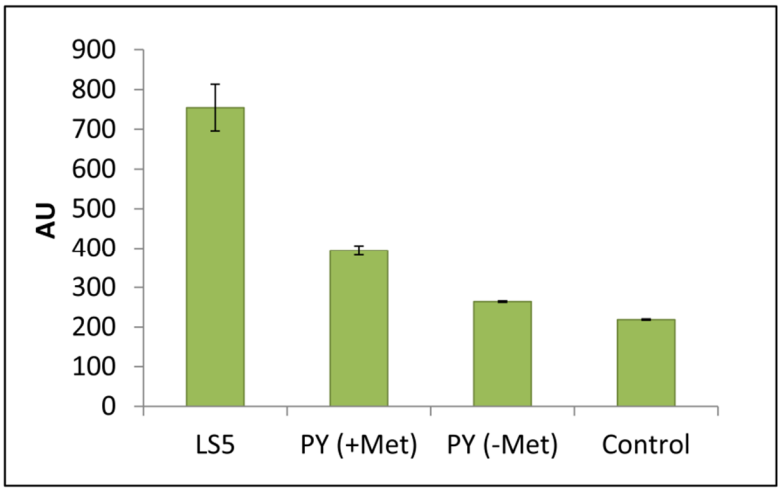
(C) Representative time lapse microscopy images of *Bm* (pSSBm85-*wapI*) (upper panel) and a mixture of *Bm* (pSSBm85-*wapI*) and *Bs* (AR16: *amyE::*P_{rrnE}-*gfp*) (lower panel). Shown are overlay fluorescence from GFP (green) and phase contrast (red) images, captured at the indicated time points. *Bm* cells are shown in black while *Bs* cells are shown in green. Scale bar represents 10 µm.



Supplementary Figure 4: Evidence that YmdB affects WapA delivery

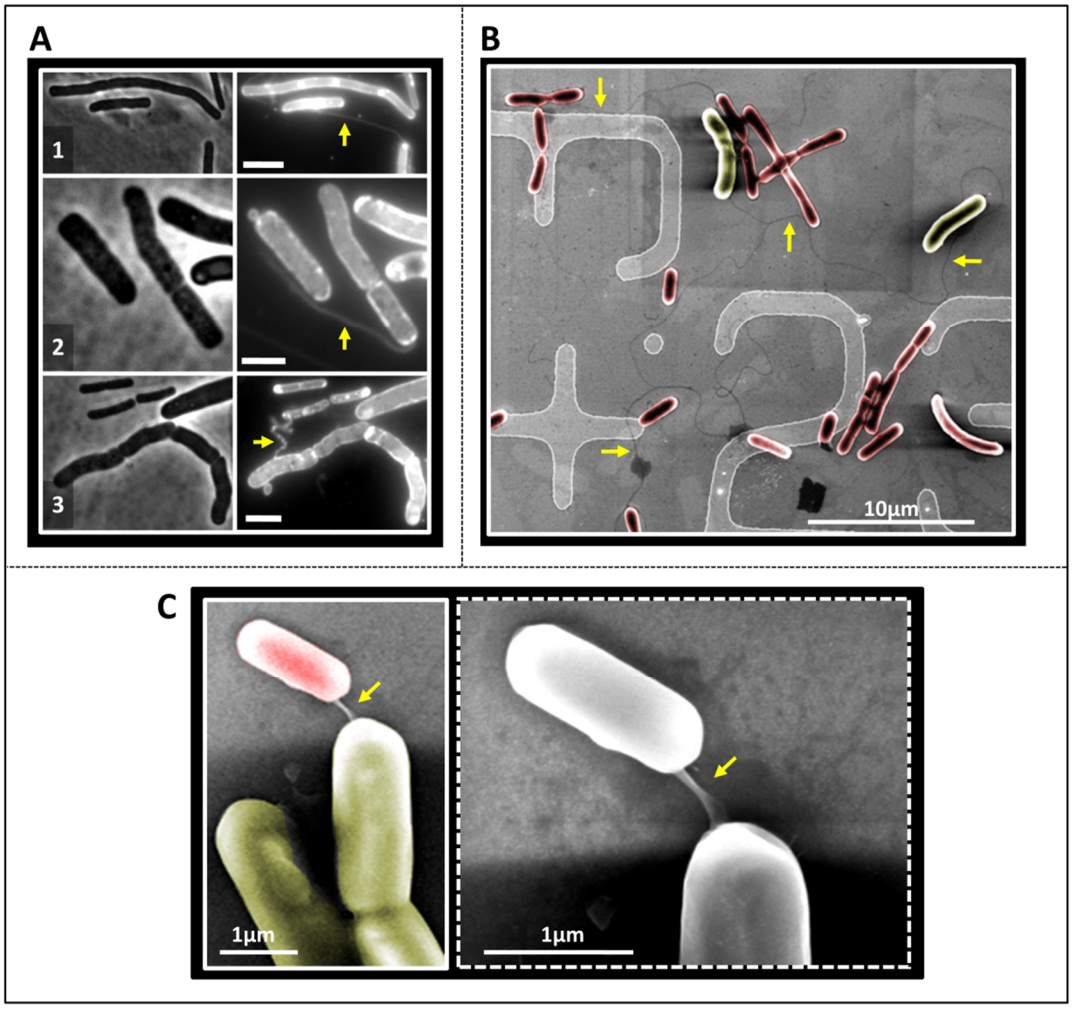
(A) Representative fluorescence microscopy images of *Bs* (wt) and *Bs* (*ΔymdB*) harboring *amyE::*P$_{wapA}$*-gfp* (left panels), and their average fluorescence in arbitrary units (AU) (right panels). Shown are average values and SD of three independent experiments (n≥ 100 cells). Scale bar represents 5 µm.

(B) *Bs* (GB168: *ΔymdB, Δhag, amyE*::P$_{hyper\text{-}spank}$*-ymdB*) cells, lacking the HA tag, were spotted onto EM grids. Cells were subjected to immuno-gold HR-SEM using primary antibodies against HA and secondary gold-conjugated antibodies. Samples were not coated before observation. Shown are cells connected by intercellular nanotubes. No gold signal (white dots) was obtained from the cells' surface or nanotubes. Some non-specific background gold signal was observed. HR-SEM images were acquired using TLD-SE (1) and vCD (low-kV high-contrast detector) (2). An enlargement of the boxed region in (2) is shown in (3). Scale bar represents 200 nm.



Supplementary Figure 5: Characterization of AHA uptake by *Bs*

Bs (PY79) cells were grown over night in S7 medium in the presence or the absence of Met (50 µg/ml). In addition, *Bs* (LS5: *ΔmetE*) cells were grown in the presence of Met (50 µg/ml). Cells were then washed in PBSx1 and resuspended in S7 supplemented with AHA (1 mM). Cells were incubated for additional 2 hrs, underwent click reaction and visualized by fluorescent microscopy. Shown is quantification of the fluorescent signal emitted from the cells. Control bar represents PY79 cells grown under similar conditions without undergoing the final click reaction. Each bar represents an average value and SD from at least 100 cells.



Supplementary Figure 6: Visualizing intercellular nanotubes linking the two *Bacilli*

(A) *Bs* (PY79) and *Bm* (OS2) cells were spotted at low density onto an ITO-coated cover slip, covered with dialysis membrane and incubated in LB medium supplemented with a fluorescent membrane dye. Shown are phase contrast (left panels) and examples of typical nanotubes observed by fluorescent microscopy (right panels) of (1) *Bs*, (2) *Bm* and (3) interspecies nanotubes. Of note, *Bs* is significantly smaller than *Bm.* Arrows point to nanotube structures. Scale bars represent 2 µm.

(B-C) *Bs* (GB168: *ΔymdB, Δhag, amyE*::P$_{hyper\text{-}spank}$-*ymdB*) and *Bm* (OS2) cells were spotted at low density onto an ITO cover slip, covered with a dialysis membrane, and incubated in LB medium for 1 hr. Cells were then fixed and visualized by HR-SEM without coating. Shown is an HR-SEM image of (B) a nanotube network formed between *Bs* (red) and *Bm* (yellow) (x10,000), and (C) Left: a short nanotube linking two

adjacent cells *Bs* (red) and *Bm* (yellow) (x50,000). Right: is higher resolution (x100,000) of the tube region shown in the left panel. Arrows point to nanotube structures.

Supplementary Table 1: Investigating the effect of *Bs* mutants on *Bm* growth inhibition

Strain name	Mutant gene(s)	Gene function	Reference
GD215	Δ*hag*	Flagellum subunit	[1]
SG157	Δ*tasA*	Production of an ECM protein	[2,3]
GD278	Δ*tasA,* Δ*hag*		[4]
SB781	Δ*spo0A*	Master regulator of sporulation, affects biofilm formation.	[5]
OS16	Δ*wapA*	Toxin that was found to cleave tRNases, perturbing translation.	[6]
GD413	Δ*sinR*	Transcriptional regulator, repressor of many biofilm formation genes.	[7]
GD420	Δ*sinI*	Antagonist of SinR	[7]
GM3	Δ*yvfA*	Antagonist of SinR	[8]
GM5	Δ*veg*	Antagonist of SinR	[9]
GM9	Δ*ylbF*	Antagonist of SinR	[10]
GB61	Δ*ymdB*	Phosphodiesterase, affects biofilm formation	[11]
GM8	Δ*yqeK*	Predicted hydrolase involved in multicellularity	[10]
GM10	Δ*yhxB*	Alpha-phosphoglucomutase, involved in multicellularity	[10]
GD15	Δ*yveQ*	Biofilm formation: exopolysaccharide synthesis	[5]
SG33	Δ*yveR*	Biofilm formation: exopolysaccharide synthesis	[5]
GD8	Δ*ywpE*	A putative sortase	[4]
GD41	Δ*yhcS*	A putative sortase	[4]
GD43	Δ*ywpE,* Δ*yhcS*		[4]
GD71	Δ*ywnE*	Membrane metabolism: cardiolipin synthase	[12]
GD73	Δ*ywjE*	Membrane metabolism: cardiolipin synthase	[12]
GD84	Δ*ywiE*	Membrane metabolism: lipid synthase, involved in cardiolipin synthesis	[12]
GD86	Δ*ypbR*	Membrane metabolism: a putative GTP-binding dynamin like protein	[12]
GD87	Δ*ypfP*	Membrane metabolism: glycosyltransferase	[13]
GD88	Δ*pgsA*	Membrane metabolism: lipid synthase, involved in synthesis of phosphatidylglycerol (Hashimoto et al., 2009)	[14]
GD89	Δ*yerQ*	Membrane metabolism: diacylglycerol kinase, required for lipoteichoic acid production	[15]
GD91	Δ*yfiX*	Membrane metabolism: a putative membrane protein, involved in membrane lipid synthesis	[4]
GD70	Δ*psd*	Membrane metabolism: involved in synthesis of the membrane lipid phosphatidylethanolamine	[16]
IB97	Δ*sigD*	Sigma factor, required for motility, chemotaxis and autolysis.	[17,18]

Supplementary Table 2: Bacterial strains and plasmids

Species	Strain name	Genotype	Description
Bm	OS2	Wild type	Soil isolate
Bm	*Bm*-pSSBm85	pSSBm85 *tet*^{R}	OS2 was transformed with pSSBm85 [19] harboring *tet*^{R} gene.
Bm	*Bm*-pSSBm85-*wapI*	pSSBm85 *tet*^{R}, P_{xyl}-*wapI*	OS2 was transformed with pSSBm85-*wapI* harboring *tet*^{R} gene.
Bs	PY79	Wild type	[20]
Bs	AR16	*amyE::*P_{rrnE}*-gfp-spec*	[21]
Bs	LS5	*metE::erm*	[22]
Bs	GB168	*ymdB::tet, amyE::*P_{hyper}*-spank-ymdB- spec, hag::erm*	[23]
Bs	GB41	*ΔymdB::spec*	[23]
Bs	GB61	*ΔymdB::tet*	[23]
Bs	BDR2642	*sacA::*P_{veg}*-mCherry*	A gift from D. Rudner (Harvard U)
Bs	OS16	*ΔwapA:: spec*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *wapA::spec*-P1-P4
Bs	OS21	*ΔmetE::erm ΔymdB::spec*	LS5 was transformed with genomic DNA of strain GB41
Bs	OS23	*ΔymdB::tet, sacA::*P_{veg}*-mCherry*	GB61 was transformed with genomic DNA of strain BDR2642
Bs	OS24	*sacA::*P_{veg}*-mCherry, ΔwapA::spec*	BDR2642 was transformed with genomic DNA of strain OS16
Bs	OS25	*ΔmetE::erm, ΔwapA::spec*	LS5 was transformed with genomic DNA of strain OS16
Bs	SH3	*amyE::*P_{wapA}*-gfp-spec*	PY 79 was transformed with pSH2
Bs	SH4	*ΔymdB::tet amyE::*P_{wapA}*-gfp-spec*	GB61 was transformed with genomic DNA of strain SH3
Bs	SH25	*wapA*-2×HA*:: cm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *wapA*-2×HA-P1-P6
Bs	SH29	*wapA*-2×HA*:: cm ymdB::tet, amyE::*P_{hyper}*-spank-ymdB- spec, hag::erm*	GB168 was transformed with genomic DNA of strain SH25
Plasmids			
pSSBm85	Generously provided by Rebekka Biedendieck (Technische Universität Braunschweig) [19].		
pSSBm85-*wapI*	*wapI* from *Bs* was amplified from PY79 genomic DNA using primers 3216 wapI-BglII and 3217 wapI-SphI. The amplified DNA was digested with *Bgl*II and *Sph*I and was cloned into pSSBm85 digested with the same enzymes.		
pSH2	*wapA* promoter region was amplified from PY79 genomic DNA using primers PwapA-F-BamHI and PwapA-R-HindIII. The amplified DNA was digested with *BamH*I and *Hind*III and was cloned into pMM47 [24], containing the *gfp* ORF and the *spec* gene, digested with the same enzymes.		

Supplementary Table 3: List of primers used in this study

Primer name	**Primer sequence (5' - 3')**
16S rRNA- 7F	AAGAGTTGATCATGGCTCA
16S rRNA- 1511R	GGTTACCTTGTTACGACTT
wapA::spec - P1	CAGAGCATACCAATCTGCC
wapA::spec - P2	CTGAGCGAGGGAGCAGAATTCCTC TCTCCTTTTGTAATAAAAGTAATA
wapA::spec - P3	GTTGACCAGTGCTCCCTGTAAGGTTAAGCGAGGGGGG
wapA::spec - P4	CGGGTGTTGGCTGGTATTG
3216 *wapI*-BglII	AAACCCAGATCTATGGCCAAAATAAAAGATGATTGTATAGAAC
3217 *wapI*-SphI	ACCTAGGCATGCAGGAAGTTTAGAAGGATTTTTTGTATTTTC
P*wapA*-F-BamHI	AAACCCGGATCC GTAAAAAGGCCTATGCGG
P*wapA*-R-HindIII	AAACCCAAGCTT CATTTCCTCTCTCCTTTTGTAATA
wapA-2×HA - P1	AAGGCGAAGACAAGCCATTC
wapA-2×HA - P2	CATAGGGATAGCCAGCGTAATCTGGAACATCATATGGGTAACG CCTTCTCTTAGATTTATTTCC
wapA-2×HA - P3	TTACGCTGGCTATCCCTATGACGTCCCGGACTACGCATAATAA GGTTAAGCGAGGGGGG
wapA-2×HA - P4	CTGAGCGAGGGAGCAGAACCCATTTTTATCCAATGCAGA
wapA-2×HA - P5	GTTGACCAGTGCTCCCTGATGGGGCATTTATTGATGGA
wapA-2×HA - P6	CGTCTTCTACTTCGGGGTCA
qRT-PCR primers	
wapA F	CCAGCCGATGTACTAGCAAAATC
wapA R	GCTTCCTCAGTTTGTTCTTTCTGAAG
rpsE F	GCGTCGTATTGACCCAAGC
rpsE R	TACCAGTACCGAATCCTACG
16S rRNA F	CTCGTGTCGTGAGATGTTGG
16S rRNA R	GTTTCGCTGCCCTTTGTTCT

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