Table S1. Strains used in this study.

Strain	Description[a]	Reference
E. coli		
DH5α	F$^-$, Δ(*lacZYA-argF)U169 thi-1 hsdR17 gyrA96 recA1 endA1 supE44 relA1 phoA Φ80 dlacZΔM15*	Hanahan, 1983
SM10λpir	*thi thr leu fhuA lacY supE recA*::RP4-2-Tc::Mu *λpir* R6K Kan^R	Miller and Mekalanos, 1988
B. pertussis		
B213	Sm^R derivative of Tohama I	King et al., 2001
B213 Δ*mlaF*	Δ*mlaF::gm* mutant of B213, Gm^R	This study
B213 Δ*pldA*	Δ*pldA::gm* mutant of B213, Gm^R	This study
B213 Δ*mlaF* Δ*pldA*	Δ*pldA::gm* mutant of B213 Δ*mlaF*, Gm^R	This study
B213 Δ*pqiAB*	Δ*pqiAB::gm* mutant of B213, Gm^R	This study
B. bronchiseptica		
BB-D09-SR	Sm^R derivative of BB-D09	de Jonge et al., 2021
BB-D09-SR Δ*mlaF*	Δ*mlaF::gm* mutant of BB-D09-SR, Gm^R	This study

[a]Sm^R, streptomycin resistant; Gm^R, gentamicin resistant; Kan^R, kanamycin resistant

Table S2. Primers used in this study.

Name	Sequence[a]	Restriction site
mlaF_up_Fw	AACTACGATGCGCAGAAAGACC	
mlaF_up_Rev	AAGTCGACAGCTATTGCGGGTATGAACG	SalI
mlaF_down_Fw	AAGTCGACACAACAAGAAGGACGCAAGC	SalI
mlaF_down_Rev	AATCGTGACATGATGAATCCGTA	
pldA_up_Fw	ATCGCCGATACCCTGGTC	
pldA_up_Rev	GCGCGCGTCGACTCGAGCCGGTAGGAAATG	SalI
pldA_down_Fw	GCGCGCGTCGACCTGGACATGAACGGCTACCT	SalI
pldA_down_Rev	CGGCATGATCTGGTAAAATAA	
pqiAB_up_Fw	GAGGCCATCACCAAGCAG	
pqiAB_up_Rev	TAGCCTCAGGTTGGCCAGCACGAAGAC	Eco81I
pqiAB_down_Fw	TAGCCTGAGGCGTTGTTGAAAGGGC	Eco81I
pqiAB_down_Rev	CGGTGGAACCGTATCCTAAAG	
Gm_Fw_S	GCGCGCGTCGACGACGCACACCGTGGAAA	SalI
Gm_Rev_S	GCGCGCGTCGACGCGGCGTTGTGACAATTT	SalI
Gm_Fw_E	GCGCGCCCTGAGGGACGCACACCGTGGAAA	Eco81I
Gm_Rev_E	GCGCGCCCTCAGGGCGGCGTTGTGACAATTT	Eco81I

[a]Recognition sites for the restriction enzymes indicated in the last column are underlined

Table S3. Plasmids used in this study.

Plasmid	Description[a]	Reference
pCRII	TA cloning vector, Amp^R, Kan^R	Invitrogen
pYRC	pBBR1MCS-5, *lacI*, Gm^R	Arts et al., 2007
pKAS32	Allelic-exchange suicide vector, Amp^R	Skorupski and Taylor, 1996
pKAS32-mlaF$_{KO}$	pKAS32 carrying an *mlaF* knockout construct, Amp^R, Gm^R	This study
pKAS32-pldA$_{KO}$	pKAS32 carrying a *pldA* knockout construct, Amp^R, Gm^R	This study
pKAS32-pqiAB$_{KO}$	pKAS32 carrying a *pqiAB* knockout construct, Amp^R, Gm^R	This study

[a]Amp^R, ampicillin resistant; Kan^R, kanamycin resistant; Gm^R, gentamicin resistant

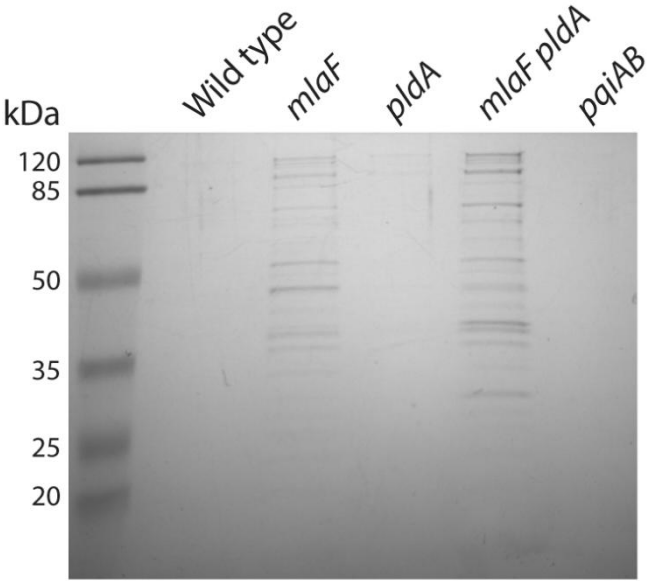

Fig. S1. OMV release by *B. pertussis* strain B213 and its mutant derivatives after growth in conical tubes. Bacteria were grown for 24 h in Verwey medium, and OMVs were isolated from equal amounts of cells, based on OD_{600}. OMVs were analyzed by SDS-PAGE, and the proteins were stained with the Bradford reagent. Molecular weight markers are indicated on the left.

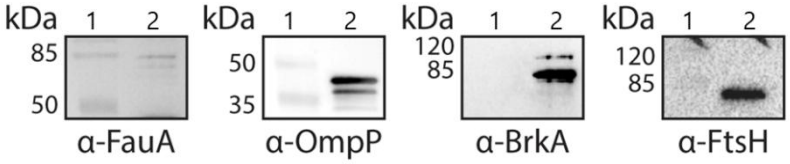

Fig. S2. Detection of various proteins in whole-cell lysates. Whole-cell lysates of wild-type *B. pertussis* strain B213 were analyzed by Western blotting using antisera directed against FauA (78 kDa), OmpP (39 kDa), BrkA (73 kDa), and FtsH (69 kDa) as indicated below the different panels. Lanes 1 contain molecular weight standard proteins, the molecular masses of which are shown at the left. Lanes 2 contain the whole-cell lysates.

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