Supplementary Information

The small GTPase MglA together with the TPR domain protein SgmX stimulate type IV pili formation in *M. xanthus*

Anna Potapova, Luís Antonío Menezes Carreira & Lotte Søgaard-Andersen

Corresponding author: Lotte Søgaard-Andersen

E-mail: sogaard@mpi-marburg.mpg.de

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Supplementary Materials & Methods

Cell growth and construction of strains. All in-frame deletions and plasmid integrations were verified by PCR. Plasmids were propagated in *Escherichia coli* Mach1 [Δ*recA1398 endA1 tonA φ80ΔlacZΔM15 ΔlacX74 hsdR(rK− mK+)]. *E. coli* cells were grown in LB or on 1.5% agar plates containing LB at 37°C with added antibiotics if appropriate (1). All DNA fragments generated by PCR were verified by sequencing.

Motility assays and determination of reversal frequency. Motility assays were done according to (2). Briefly, in population-based assays, *M. xanthus* cells from exponentially growing cultures were harvested at 4000 g for 10 min at room temperature (RT) and resuspended in 1% CTT to a calculated density of 7×10⁹ cells/ml. 5 µl aliquots of cell suspensions were placed on 0.5% agar plates supplemented with 0.5% CTT for T4P-dependent motility and 1.5% agar plates supplemented with 0.5% CTT for gliding motility (3) and incubated at 32°C. After 24 hrs, colony edges were visualized using a Leica M205FA stereomicroscope and imaged using a Hamamatsu ORCA-flash V2 Digital CMOS camera (Hamamatsu Photonics). For higher magnifications of cells at colony edges on 1.5% agar, cells were visualized using a Leica DM6000B microscope and imaged using a Cascade II 1024 EMCCD camera (Photometrics). To track individual cells moving by T4P-dependent motility, 5 µl of exponentially growing cultures were spotted into a 24-well polystyrene plate (Falcon). After 10 min at RT, cells were covered with 500 µl of 1% methylcellulose in MMC buffer (10 mM MOPS, 4 mM MgSO₄, 2 mM CaCl₂, pH 7.6), and incubated at RT for 30 min. Subsequently, cells were visualized for 10 min at 20 s intervals at RT using a Leica DMI8 inverted microscope and imaged using a Leica DFC9000 GT camera. Individual cells were tracked using Metamorph 7.5 (Molecular Devices) and ImageJ 1.52b (4). 20 cells were tracked per strain per biological replicate. For each cell, the distance moved per 20 s interval was determined and the mean speed calculated as µm/min. For reversals, 50 cells were tracked as described and the number of reversals per cell per 10 min determined manually. To calculate mean squared displacement (MSD) of cells, the distance of a cell to its original position at t=0 was determined for every time interval of a time-lapse series, squared and plotted as function of time. Cells were followed for 10 min and recorded every 20 s. For each strain 50 cells were followed.

Bacterial two-hybrid assay. Plasmids listed in Table S2 were transformed pairwise into chemically competent *E. coli* BTH101 cells [F-cya-99 araD139 galE15 galK16 rpsL1 (Str⁻) hsdR2 mcrA1 mcrB1] (Euromedex, France), and cells plated on LB plates containing 50 µg/ml ampicillin, 30 µg/ml kanamycin, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and 40 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). Plates were incubated at 30°C for 48 hrs, and then randomly selected individual colonies were suspended in 50 µl LB, and 5 µl were spotted onto fresh LB ampicillin/kanamycin/IPTG/X-gal plates and incubated for 48 hrs at 30°C and imaged using an Epson Perfection V700 Photo scanner.
Transmission electron microscopy. Transmission electron microscopy was used to visualize T4P as described (5). Briefly, 50 µl of exponentially growing *M. xanthus* cells were placed on Parafilm. A small piece of carbon-coated mica was dipped into the drop for 2 min, allowing cells to adsorb to the surface, excess liquid was soaked off, the film was placed briefly on a drop of distilled water, excess liquid was soaked off again, and the film transferred on a drop of 2% uranyl acetate (wt/vol) for 3-4 seconds and blotted dry. Transmission electron microscopy was performed on a JEOL JEM-1400 electron microscope at calibrated magnifications.

T4P shear-off assay. T4P were sheared off *M. xanthus* cells as described (6) with minor modifications. Briefly, cells were grown on 1% CTT/1.5% agar plates at 32°C for 3 days, gently harvested and resuspended in resuspension buffer (100 mM Tris-HCl pH 7.6, 100 mM NaCl). Cells were harvested for 2 min at 13,000 g at RT, resuspended in 200 µl SDS lysis buffer (10% (v/v) glycerol, 60 mM Tris-HCl pH 6.8, 5 mM EDTA, 2% (w/v) SDS, 100 mM DTT, 0.005% bromphenol blue) and immediately denatured at 95°C for 10 min. These samples represent whole cell lysate fractions. To shear-off T4P, cells were vortexed for 10 min and then harvested by centrifugation for 20 min at 13,000 g at 4°C. The supernatant was removed and kept on ice; cells were resuspended in resuspension buffer, vortexed for 5 min and harvested as described. The supernatant removed and combined with the previous supernatant. The combined supernatant was centrifuged twice for 10 min at 13,000 g at 4°C. Subsequently, T4P in the final supernatant were precipitated ON at 4°C by addition of PEG6000 and MgCl₂ to final concentrations of 2% (w/v) and 0.1 M, respectively. Whole cell fraction and sheared fraction were analyzed by immunoblot using α-PilA antibodies (7) and α-PilC antibodies (8) as a loading control.

Immunoblot analysis. Immunoblot analysis was done as described (1). Rabbit polyclonal α-PilA (7), α-PilQ, α-PilC and α-PilM (8), α-TsaP (9), α-PilO, α-PilP and α-PilN (10), α-PilB and α-PilT (5) antibodies were used together with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Sigma) as secondary antibody. Mouse α-GFP antibodies (Sigma) were used together with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin G (GE Healthcare) as secondary antibody. Blots were developed using Luminata Crescendo or Forte Western HRP Substrate (Millipore) and visualized using a LAS-4000 luminescent image analyzer (Fujifilm). Proteins were separated by SDS-PAGE as described (1).

Pull-down experiments. In experiments involving MglA-His₆, the protein (final concentration: 2.1 µM) was preloaded with GTP or GDP (final concentration: 104 µM) for 30 min at RT in buffer 1 (50 mM Tris pH 7.5, 150 mM NaCl, 5% glycerol, 5 mM MgCl₂). To test for direct interactions between MglA-His₆ and SgmX-Strep, the two proteins were incubated in buffer 1 for 120 min at 4°C in a total volume of 500 µl (final concentrations: 2.0 µM and 1.8 µM, respectively) in the presence of the relevant nucleotide (final concentration: 100 µM). Then 100 µl of a Ni²⁺-NTA-agarose resin previously equilibrated in buffer 1 supplemented with the relevant nucleotide were added for 120 min. The resin was washed four times with 1 ml buffer 1 containing the relevant nucleotide at 100 µM.
Proteins were eluted in 100 µl buffer 1 supplemented with 400 mM imidazole. Fractions were analyzed by SDS-PAGE as described (1).

**Protein purification.** All proteins were expressed in *E. coli* BL21 (DE3) (B F<sup>ompT</sup> gal dcm lon hsdS<sub>8(r<sub>8</sub>m<sub>8</sub>)</sub> λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB<sup>kat</sup>λ<sup>12</sup>]) at 18°C or 30°C. To purify His<sub>6</sub>-tagged proteins, Ni-NTA affinity purification was used. Briefly, cells were washed in wash buffer A (50 mM Tris pH 7.5, 150 mM NaCl, 10 mM imidazole, 5% glycerol, 5 mM MgCl<sub>2</sub>) and resuspended in lysis buffer A (50 ml of wash buffer A supplemented with EDTA-free protease inhibitors (Complete Protease Inhibitor Cocktail Tablet EDTA-free (Roche))). Cells were lysed by French press and cell debris removed by centrifugation (48,000 g, 4°C, 30 min). The cleared cell lysate was pre-mixed for 1 hr with 1 ml Ni<sup>2+</sup>-NTA-agarose preloaded with NiSO<sub>4</sub> as described by the manufacturer and pre-equilibrated in wash buffer A and sequentially loaded on a gravity column. The column was washed with 50 column volumes of column wash buffer (50 mM Tris pH 7.5, 150 mM NaCl, 10 mM imidazole, 5 mM MgCl<sub>2</sub>). Proteins were eluted with elution buffer A (50 mM Tris pH 7.5, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 500 mM imidazole) using a linear imidazole gradient from 50-500 mM. Fractions containing purified His<sub>6</sub>-tagged proteins were combined and loaded onto a HiLoad 16/600 Superdex 200 pg (GE Healthcare) gel filtration column that was equilibrated with buffer 1 supplemented with 5% glycerol. Fractions containing His<sub>6</sub>-tagged proteins were pooled, frozen in liquid nitrogen and stored at -80°C. To purify SgmX-Strep, biotin affinity purification was used. Briefly, cells were washed in wash buffer B (100 mM Tris pH 7.5, 150 mM NaCl, 5 mM MgCl<sub>2</sub>) and resuspended in lysis buffer B (30 ml of wash buffer D supplemented with protease inhibitors (Complete Protease Inhibitor Cocktail Tablet (Roche))). Cells were lysed were pre-mixed for 1 h with 1 ml Strep-Tactin®XT Superflow® (IBA-lifesciences), preequilibrated with wash buffer D and sequentially loaded on a gravity column. The column was washed with 50 column volumes of wash buffer D. Protein was eluted with elution buffer D (150 mM Tris pH 7.5, 150 mM NaCl, 2.5 mM Dethiobiotin). Elution fractions containing SgmX-Strep were loaded onto a HiLoad 16/600 Superdex 200 pg (GE Healthcare) gel filtration column that was equilibrated with buffer D supplemented with 5% glycerol. Fractions with SgmX-Strep were pooled, frozen in liquid nitrogen and stored at -80°C.

**Bioinformatics.** BlastP (11) and Pfam v31.0 (pfam.xfam.org) (12) were used to identify protein domains. TMHMM v2.0 (13) and SignalP v.4.1 (14) were used to test for transmembrane domains and signal sequences with default gathering thresholds, respectively. % identity/similarity between homologous proteins were calculated using EMBOSS Needle software (15) (pairwise sequence alignment). The KEGG SSDB (Sequence Similarity DataBase) database (16) was used to identify protein homologs.

**Construction of plasmids:** pLC51 (plasmid for generation of in-frame deletion of *sgmX*): up- (5766 A/5766 B) and downstream fragments (5766 C/5766 D) were amplified from genomic DNA of *M. xanthus* DK1622. Subsequently, the AB and CD fragments were used as template for overlapping PCR (5766 A/5766 D) to generate the AD fragment. AD fragment was digested with HindIII+EcoRI, cloned in pBJ114 and sequenced.
pIB123 (plasmid for generation of in-frame deletion of pilT): up- (oPilT-A/oPilT-B) and downstream fragments (oPilT-C/oPilT-D) were amplified from genomic DNA of M. xanthus DK1622. Subsequently, the AB and CD fragments were used as template for overlapping PCR (oPilT-A/oPilT-D) to generate the AD fragment. AD fragment was digested with HindIII+EcoRI, cloned in pBJ114 and sequenced.

pAP12 (plasmid for expression of Pnat pilB-mCherry from the attB site): Pnat pilB (PilBnat fw / PilB rev) fragment was amplified from genomic DNA of M. xanthus DK1622 and digested with NdeI +BamHI. Subsequently, fragment was cloned in pNG062 carrying C-terminal mCherry and sequenced.

pAP16 (plasmid for expression of Pnat mCherry-pilM from the attB site): Pnat (PilMnat fw / PilM rev) and pilM (PilM fw/PilM rev) fragments were amplified from genomic DNA of M. xanthus DK1622 and digested with NdeI+BglII and XbaI+HindIII, respectively. Subsequently, fragments were cloned in pNG063 carrying N-terminal mCherry and sequenced.

pAP19 (plasmid for generation of in-frame deletion of frzE): up- (frzE A/frzE B) and downstream fragments (frzE C/ frzE D) were amplified from genomic DNA of M. xanthus DK1622. Subsequently, the AB and CD fragments were used as template for overlapping PCR (frzE A/ frzE D) to generate the AD fragment. AD fragment was digested with HindIII+EcoRI, cloned in pBJ114 and sequenced.

pAP34 (plasmid for expression of Pnat sgmX from the attB site): Pnat sgmX fragment (PnatsgmX fw/ PnatsgmX rev) was amplified from genomic DNA of M. xanthus, digested with HindIII+EcoRI, cloned in pSWU30 and sequenced.

pAP35 (plasmid for sgmX replacement by sgmX-mVenus at native site): up- (SgmX-mvA/SgmX-mvB) and downstream fragments (SgmX-mvC/SgmX-mvD) were amplified from genomic DNA of M. xanthus DK1622. A fragment containing mVenus was amplified from plasmid pLC20 carrying mglA-mVenus (Venus/Cherry Fw / Venus/Cherry Rv). Subsequently, AB and mVenus fragments were used as template for overlapping PCR (SgmX-mvA/Venus/Cherry Rv) to generate the AB-mVenus fragment. AB-mVenus and CD fragments were digested with HindIII+Xbal and Xbal+EcoRI, respectively. Fragments were cloned in pBJ114 and sequenced.

pAP37 (plasmid for pilQ replacement by pilQ-sfgfp at native site): up- (PilQ-sfGFP A/ PilQ-sfGFP B) and downstream fragments (PilQ-sfGFP C/ PilQ-sfGFP D) were amplified from genomic DNA of M. xanthus DK1622. A fragment containing sfGfp was amplified from plasmid pSC101 (10) (sfGFP Fw / sfGFP Rev). Subsequently, AB and sfGfp fragments were used as template for overlapping PCR (PilQ-sfGFP A/sfGFP Rev) to generate the AB-sfGFP fragment. AB-sfGFP and CD fragments were digested with HindIII+Xbal and Xbal+EcoRI, respectively. Fragments were cloned in pBJ114 and sequenced.

pAP39 (plasmid for overexpression of SgmX-Strep): sgmX strep fragment was amplified (SgmX-Strep fw/SgmX-Strep rev) from genomic DNA of M. xanthus DK1622; Strep tag
was introduced using SgmX-Strep rev primes. Fragment was digested with XbaI+HindIII, cloned in pMAT135 (derivative of pET45-b+ lacking protein tag) and sequenced.

pAP87 (plasmid for expression of Pnat mCherry-pilT from the attB site): Pnat (PilTnat fw / PilTnat rev) and pilT (PilT fw/PilT rev) fragments were amplified from genomic DNA of M. xanthus DK1622 and digested with NdeI+BglII and XbaI+HindIII, respectively. Subsequently, fragments were cloned in pNG063 carrying N-terminal mCherry and sequenced.

pAP88 (plasmid for mglB replacement by mglB^{A64R G68R}-mCherry at native site): up-(MglB^{AGR}-mChA / MglB^{AGR}-mChB) and downstream fragments (MglB^{AGR}-mChC/ MglB^{AGR} mChD) were amplified from genomic DNA of M. xanthus SA3954. A fragment containing mCherry was amplified from plasmid pDK145 carrying mglB-mCherry (Venus/Cherry Fw / Venus/Cherry Rev). Subsequently, AB and mCherry fragments were used as template for overlapping PCR (MglB^{AGR}-mChA/Venus/Cherry Rev) to generate the AB-mCherry fragment. AB-mCherry and CD fragments were digested with HindIII+XbaI and XbaI+EcoRI, respectively. Fragments were cloned in pBJ114 and sequenced.

pDK56 (C-terminal fusion of mglA with T25 fragment of B. pertussis adenylate cyclase) and pDK76 (N-terminal fusion of mglA with T18 fragment of B. pertussis adenylate cyclase): mglA (BTHMglAfw/BTHMglArvstop for pDK56 and BTHMglAfw/BTHMglArv for pDK76) was amplified from genomic DNA of M. xanthus DK1622. Both fragments were digested with XbaI+EcoRI, cloned into pKT25 and pUT18 vectors and sequenced.

pDK55 (C-terminal fusion of mglB with T25 fragment of B. pertussis adenylate cyclase) and pDK77 (N-terminal fusion of mglB with T18 fragment of B. pertussis adenylate cyclase): mglB (BTHMglBfw/BTHMglBrvstop for pDK55 and BTHMglBfw/BTHMglBrv for pDK77) was amplified from genomic DNA of M. xanthus DK1622. Both fragments were digested with XbaI+EcoRI, cloned into pKT25 and pUT18 vectors and sequenced.

pSC57, pSC59 (C- and N-terminal fusions of pilB with T25 fragment of B. pertussis adenylate cyclase, respectively) and pSC69, pSC70 (C- and N-terminal fusions of pilB with T18 fragment of B. pertussis adenylate cyclase, respectively): pilB (opiB-pKT25fw/opiB-pKT25rv for pSC57/pSC70 and opiB-pKT25fw/opiB-pKNT25rv for pSC59/pSC69) was amplified from genomic DNA of M. xanthus DK1622. Both fragments were digested with BamHI+EcoRI, cloned into pKT25 and pUT18 vectors and sequenced.

pSC58, pSC60 (C- and N-terminal fusions of pilT with T25 fragment of B. pertussis adenylate cyclase, respectively) and pSC71, pSC72 (C- and N-terminal fusions of pilT with T18 fragment of B. pertussis adenylate cyclase, respectively): pilT (opiIT-pKT25fw/opiIT-pKT25rv for pSC58/pSC72 and opiIT-pKT25fw/opiIT-pKNT25rv for pSC60/pSC71) was amplified from genomic DNA of M. xanthus DK1622. Both fragments were digested with BamHI+EcoRI, cloned into pKT25, pKNT25, pUT18 and pUT18C vectors in proper combinations and sequenced.

pSC73, pSC74 (C- and N-terminal fusions of pilM with T25 fragment of B. pertussis adenylate cyclase, respectively) and pSC61, pSC62 (C- and N-terminal fusions of pilM with T18 fragment of B. pertussis adenylate cyclase, respectively): pilM (opiIM-pKT25fw/opiIM-pKT25rv for pSC61/pSC62 and opiIM-pKT25fw/opiIM-pKNT25rv for pSC73/pSC74) was amplified from genomic DNA of M. xanthus DK1622. Both fragments were digested with BamHI+EcoRI, cloned into pKT25, pKNT25, pUT18 and pUT18C vectors in proper combinations and sequenced.
with T18 fragment of *B. pertussis* adenylate cyclase, respectively): *pilM* (opilM-pUT18fw/opilM-pUT18Crv for pSC73/pSC62 and opilM-pUT18fw/opilM-pUT18Crv for pSC74/pSC62) was amplified from genomic DNA of *M. xanthus* DK1622. Both fragments were digested with BamHI+EcoRI, cloned into pKT25, pKNT25, pUT18 and pUT18C vectors in proper combinations and sequenced.
Figure S1. MglA is essential for T4P-dependent motility.

(A) Schematic of T4PM with the 10 core proteins. Proteins labelled with a single letter have the Pil prefix. Bent arrows indicate incorporation at and removal from the pilus base of PilA during extension and retraction, respectively. OM and IM, outer and inner membrane, respectively.

(B) MglA is essential for T4P-dependent motility. Strains were incubated at 32°C for 24h on 0.5% agar, 0.5% CTT. Scale bar, 1.0 mm.

(C) Speed of single cells moving by T4P-dependent motility. Histogram shows mean values, error bars indicate standard deviation (s.d.). Fraction of motile cells is indicated in white ± s.d. in the individual columns. For the WT, ΔpilA and ΔaglQ strains the cells analyzed are the same as in Fig. 5B. N = 20 cells for each strain from three independent experiments. NA, not applicable.
Figure S2. Accumulation and localization of MglA-mVenus variants, MglB-mCherry variants and RomR-mCherry.

(A-C) Immunoblot analysis of protein accumulation. Total cell lysates were loaded from the same number of cells per lane. Analyzed proteins are indicated on the right and molecular size markers on the left. Calculated molecular mass of monomers of the analyzed proteins is indicated on the right. In C, all samples were analyzed on the same blot and lanes were removed for presentation purposes.

(D) Localization of MglA-mVenus variants, MglB-mCherry variants and RomR-mCherry. Cells were treated, imaged and analyzed as in Fig. 3B. N= 150 cells per strain. Scale bars, 5 µm. For MglA-mVenus in WT, the data are the same as in Fig. 6C. For MglB-mCherry and RomR-mCherry in WT, the data are the same as in Fig. S10C.
Figure S3. T4P are active at both poles in bipolarly piliated cells
(A, B) Speed and reversals of single cells moving by T4P-dependent motility. Strains
indicated in blue lack FrzE. In A, histogram shows mean values, error bars indicate s.d. and fraction of motile cells is indicated in white ± s.d. N = 20 cells for each strain from three independent experiments. For calculating the speed, only time intervals in which cells displayed movement were included. In the boxplots in B, boxes enclose the 25th and 75th percentile with the black line representing the mean, whiskers indicate the 10th and 90th percentile, and circles outliers. N=50 cells for each strain. * P ≤ 0.01, two-sided Student’s t-test. NS, not significant.

(C) Quantification of cell displacement by MSD analysis. Cells were followed for 10 min with frames captured every 20 s; N=50 cells for each strain.
Figure S4. Analysis of mCherry-PilM and PilQ-sfGFP functionality and accumulation.
(A) Pnat mCherry-PilM complements T4P-dependent motility defect of ΔpilM mutant. Strains were incubated at 32°C for 24h on 0.5% agar, 0.5% CTT. Scale bar, 0.5 mm. mCherry-pilM was expressed from the native promoter in a single copy from the attB site.
(B) Genetic map of the pilM locus. Numbers below indicate distance in bp between start and stop codons of flanking genes. The arrow above indicated the native pilM promoter used for ectopic expression of mCherry-pilM. MXAN locus tags are indicated.
(C) Immunoblot analysis of mCherry-PilM accumulation. Total cell lysates were loaded from the same number of cells per lane. Calculated molecular mass of monomeric mCherry-PilM is indicated on the right and a molecular size marker on the left.
(D) PilQ-sfGFP supports T4P-dependent motility. pilQ-sfGFP was expressed from the native site. Strains were treated as in (A). Scale bar, 0.5 mm.
(E) Immunoblot analysis of PilQ-sfGFP accumulation. Total cell lysates were loaded from the same number of cells per lane. Molecular size markers are indicated on the left. Calculated molecular mass of monomeric PilQ-sfGFP is indicated on the right and a molecular size marker on the left. All samples were analyzed on the same blot but lanes were removed for presentation purposes.
**Figure S5. Localization of PilQ-sfGFP by epi-fluorescence microscopy.**
Cells were treated, imaged and analyzed as in Fig. 3B. The data for WT are the same as in Fig. 7. N = 150 cells for each strain. Scale bars, 5 μm.
Figure S6. MglA does not detectably interact with PilB, - T or -M in a bacterial two hybrid assay.

Full-length MglA, MglB, PilB, PilT and PilM were fused to variants of the *Bordetella pertussis* adenylate cyclase and co-expressed as indicated in *E. coli* BTH101 (17). In constructs labelled 18-P and P-18 the fusions were created in the plasmids pUT18C and pUT18, respectively. In constructs labelled 25-P and P-25 the fusions were created in the plasmids pKT25 and pKNT25, respectively. In the positive control (+, upper right panel), pKT25-zip and pUT18C-zip were co-transformed. In the negative control (-, upper right panel), empty vectors encoding T25 and T18 fragments were co-transformed. In the lower panel, each MglA, MglB, PilB, PilT and PilM construct was co-expressed with pKT25-zip or pUT18C-zip to rule out possible false positive interactions. An interaction is indicated by a blue color and no interaction by a white color.
Figure S7. Analysis of the *sgmX* locus.

(A) The *sgmX* locus is largely conserved in Cystobacterineae. Distance between start and stop codons of genes in the *sgmX* locus in *M. xanthus* are indicated above. Homologous genes are indicated in the same color. %identity/%similarity are indicated for individual proteins. The *Bd2492* locus of *Bdellovibrio bacteriovorus* is included for comparison (18). Functional annotation of flanking genes is included. Together MXAN_5764 and MXAN_5763 are predicted to make up a TamA/TamB system for efficient secretion of autotransporters (18, 19). Orientation of arrows indicates direction of transcription. Cross indicates that the corresponding gene is lacking. Two parallel, slanted lines indicate the presence of two unrelated genes in *Vulgatibacter incomptus.*
(B) Genetic map of the \textit{sgmX} locus. Numbers below indicate distance in bp between start and stop codons of flanking genes. The arrow above indicated the native \textit{sgmX} promoter used for ectopic expression of \textit{sgmX}. MXAN locus tags are indicated.
Figure S8. SDS-PAGE analysis of purified MglA-His₆ and SgmX-Strep. 15 ng of the indicated proteins were separated by SDS-PAGE and gels stained with Coomassie Brilliant Blue. Calculated molecular mass of the monomer of each protein is indicated on the right. Molecular markers sizes are indicated on the left. Note that SgmX-Strep separates as a protein with a size of ~233 kDa by SDS-PAGE suggesting that it is a heat- and detergent-stable dimer.
Figure S9. Accumulation of SgmX-mVenus and MglA-mVenus variants.
(A-C) Immunoblot analysis of protein accumulation. Total cell lysates were loaded from the same number of cells per lane. Analyzed proteins are indicated on the right together with the calculated molecular mass of the monomeric proteins. Molecular size markers are indicated on the left. Note that SgmX-mVenus separates as a protein with a size of ~282 kDa suggesting that it forms a heat- and detergent-stable dimer (see also Fig. S8).
Figure S10. Accumulation and localization of MglB-mCherry and RomR-mCherry. (A, B) Immunoblot analysis of protein accumulation. Total cell lysates were loaded from the same number of cells per lane. Analyzed proteins are indicated on the right together with the calculated molecular mass of the monomeric proteins. (C) Localization of MglB-mCherry and RomR-mCherry in the absence of SgmX by epi-fluorescence microscopy. Cells were treated, imaged and analyzed as in Fig. 3B. The data for the WT strains are the same as in Fig. S2D. Scale bars, 5 µm.
Figure S11. Accumulation of 10 core T4PM proteins in the absence of SgmX
Immunoblot analysis of accumulation of core T4PM proteins. Samples were prepared and analyzed as in Fig. 3A. For each protein, samples are from the same blot and lanes were removed for presentation purposes.
Figure S12. Analysis of PilB-mCherry and mCherry-PilT functionality and accumulation.
(A) PilB-mCherry complements T4P-dependent motility defect of a ∆pilB mutant. Strains were incubated at 32°C for 24h on 0.5% agar, 0.5% CTT. Scale bars, 0.5 mm. pilB-mCherry was ectopically expressed from the native promoter in a single copy from the attB site.
(B) Genetic map of the pilB locus. Numbers below indicate distance in bp between start and stop codons of flanking genes. The arrow above indicated the native pilB promoter used for ectopic expression of pilB-mCherry. MXAN locus tags are indicated.
(C) Immunoblot analysis of PilB-mCherry accumulation. Total cell lysates were loaded from the same number of cells per lane. Calculated molecular mass of monomeric PilB-mCherry is indicated on the right and a molecular size markers are on the left. All cell
lysates were analyzed on the same blot and lanes were removed for presentation purposes.

(D) mCherry-PilT complements T4P-dependent motility defect of a ΔpilT mutant. Strains were incubated at 32°C for 24h on 0.5% agar, 0.5% CTT. Scale bar, 0.5 mm. mCherry-pilT was ectopically expressed from the native promoter in a single copy from the attB site.

(E) Genetic map of the pilT locus. Numbers below indicate distance in bp between start and stop codons of flanking genes. The arrow above indicated the native pilT promoter used for ectopic expression of mCherry-pilT. MXAN locus tags are indicated.

(F) Immunoblot analysis of mCherry-PilT accumulation. Total cell lysates were loaded from the same number of cells per lane. Calculated molecular mass of monomeric mCherry-PilT is indicated on the right and a molecular size markers are on the left. All cell lysates were analyzed on the same blot and lanes were removed for presentation purposes.
Figure S13. Localization of PilB-mCherry and mCherry-PilT.
Cells were treated, imaged and analyzed as in Fig. 3B. N = 150 cells for each strain. Scale bars, 5 µm. The data for WT are the same as in Fig. 7.
Table S1. *M. xanthus* strains used in this work

| Strain   | Genotype${}^1,2$ | Source or reference |
|----------|------------------|---------------------|
| DK1622   | Wild-type        | (20)                |
| DK10410  | ΔpilA            | (21)                |
| DK10416  | ΔpilB            | (21)                |
| DK10417  | ΔpilC            | (21)                |
| DK8615   | ΔpilQ            | (22)                |
| SA3001   | ΔpilO            | (10)                |
| SA3002   | ΔpilM            | (23)                |
| SA3005   | ΔpilP            | (10)                |
| SA3044   | ΔpilN            | (10)                |
| SA6011   | ΔtsaP            | (9)                 |
| SA4420   | ΔmglA            | (24)                |
| SA3387   | ΔmglB            | (24)                |
| SA3300   | ΔromR            | (25)                |
| DK10409  | ΔpilT            | (21)                |
| SA3011   | ΔmglA ΔpilT      | (26)                |
| SA3833   | mglA$^{Q82A}$    | (25)                |
| SA3954   | mglB$^{A64R \ G68R}$ | (27)             |
| SA5293   | ΔaglQ            | (28)                |
| SA7101   | ΔmglB ΔaglQ      | This work           |
| SA3936   | ΔmglB ΔromR      | (25)                |
| SA8476   | ΔmglB ΔromR ΔaglQ| This work           |
| SA10743  | ΔmglB ΔromR ΔaglQ ΔfrzE | This work         |
| SA7107   | mglA$^{Q82A}$ ΔaglQ | This work           |
| SA7124   | mglB$^{A64R \ G68R}$ ΔaglQ | This work         |
| SA7110   | ΔromR ΔaglQ      | (2)                 |
| SA7135   | ΔaglQ ΔfrzE      | (2)                 |
| SA7136   | ΔmglB ΔaglQ ΔfrzE| This work           |
| SA7148   | mglA$^{Q82A}$ ΔaglQ ΔfrzE | This work         |
| SA8447   | mglB$^{A64R \ G68R}$ ΔaglQ ΔfrzE | This work         |
| SA7147   | ΔromR ΔaglQ ΔfrzE| This work           |
| SA7125   | ΔpilB attB::Pnat pilB-mCherry (pAP12) | This work |   |
| SA7145   | ΔpilB ΔmglA attB::Pnat pilB-mCherry (pAP12) | This work |   |
| SA8403   | ΔpilB ΔmglB attB::Pnat pilB-mCherry (pAP12) | This work |   |
| SA7146   | ΔpilB ΔmglA$^{Q82A}$ attB::Pnat pilB-mCherry (pAP12) | This work |   |
| SA7170   | ΔpilB ΔsgmX attB::Pnat pilB-mCherry (pAP12) | This work |   |
| SA8497   | ΔpilB ΔsgmX ΔmglA attB::Pnat pilB-mCherry (pAP12) | This work |   |
| SA8465   | ΔpilT attB::Pnat mCherry-pilT (pAP87) | This work |   |
| SA8466   | ΔpilT ΔmglA attB::Pnat mCherry-pilT (pAP87) | This work |   |
| SA8467   | ΔpilT ΔmglB attB::Pnat mCherry-pilT (pAP87) | This work |   |
| SA8468   | ΔpilT ΔmglA$^{Q82A}$ attB::Pnat mCherry-pilT (pAP87) | This work |   |
| SA8469   | ΔpilT ΔsgmX attB::Pnat mCherry-pilT (pAP87) | This work |   |
| SA8498   | ΔpilT ΔsgmX ΔmglA attB::Pnat mCherry-pilT (pAP87) | This work |   |
| SA7130   | ΔpilM attB::Pnat mCherry-pilM (pAP16) | This work |   |
| SA8461   | ΔpilM ΔmglA attB::Pnat mCherry-pilM (pAP16) | This work |   |
| SA8462   | ΔpilM ΔmglB attB::Pnat mCherry-pilM (pAP16) | This work |   |
| SA8409   | ΔpilM ΔmglA$^{Q82A}$ attB::Pnat mCherry-pilM (pAP16) | This work |   |
| SA7174   | ΔpilM ΔromR attB::Pnat mCherry-pilM (pAP16) | This work |   |
| SA7171   | ΔpilM ΔsgmX attB::Pnat mCherry-pilM (pAP16) | This work |   |
| SA7192   | pilQ-sfGFP        | This work           |
| SA7193   | ΔmglA pilQ-sfGFP  | This work           |
|   |   |   |
|---|---|---|
| SA7164 | ΔsgmX | This work |
| SA7175 | ΔsgmX attB::Pnat sgmX (pAP34) | This work |
| SA7168 | ΔpilT ΔsgmX | This work |
| SA8416 | ΔmglA ΔsgmX | This work |
| SA8417 | mglA<sup>Q82A</sup> ΔsgmX | This work |
| SA8458 | ΔsgmX pilQ-sfGFP | This work |
| SA7195 | sgmX-mVenus | This work |
| SA7196 | ΔmglA sgmX-mVenus | This work |
| SA8410 | mglA<sup>Q82A</sup> sgmX-mVenus | This work |
| SA8448 | ΔmglB sgmX-mVenus | This work |
| SA8442 | ΔromR sgmX-mVenus | This work |
| SA8185 | mglA-mVenus | (2) |
| SA9845 | ΔsgmX mglA-mVenus | This work |
| SA8455 | mglB<sup>G68R A64R</sup> mglA-mVenus | This work |
| SA8183 | mglA<sup>Q82A</sup>-mVenus | (2) |
| SA3963 | mglB-mCherry | (25) |
| SA9958 | ΔsgmX mglB-mCherry | This work |
| SA10812 | mglA<sup>Q82A</sup> mglB-mCherry | This work |
| SA8471 | mglB<sup>G68R A64R</sup>-mCherry | This work |
| SA7507 | romR-mCherry | (2) |
| SA9912 | ΔsgmX romR-mCherry | This work |
| SA9853 | mglA<sup>Q82A</sup> romR-mCherry | This work |
| SA10014 | mglB<sup>G68R A64R</sup> romR-mCherry | This work |

<sup>1</sup> Plasmids listed in parentheses were integrated in a single copy at the Mx8 attB site.

<sup>2</sup> P<sub>nat</sub> indicates that the relevant gene is expressed from the native promoter.
| Plasmid       | Description                                                                 | Reference                        |
|--------------|-----------------------------------------------------------------------------|----------------------------------|
| pSWU30       | Tet<sup>R</sup>, att<sup>P</sup>                                            | (6)                              |
| pBJ114       | Kan<sup>R</sup>, galK                                                       | (29)                             |
| pSWU19       | Kan<sup>R</sup>, att<sup>P</sup>                                            | (30)                             |
| pNG062       | pSWU19 derived vector for the generation of C-terminal mCherry fusions      | N. Gómez-Santos                  |
| pNG063       | pSWU19 derived vector for the generation of N-terminal mCherry fusions      | N. Gómez-Santos                  |
| pMAT135      | pET45b+ derived vector for overexpression of proteins                       | A. Treuner-Lange                 |
| pTM1         | Overexpression of MglA-His<sub>5</sub>                                       |                                  |
| pKT25        | Vector for bacterial two hybrid system                                       | Euromedex                        |
| pKNT25       | Vector for bacterial two hybrid system                                       | Euromedex                        |
| pUT18        | Vector for bacterial two hybrid system                                       | Euromedex                        |
| pUT18C       | Vector for bacterial two hybrid system                                       | Euromedex                        |
| pKT25-zip    | pKT25 fused to leucine zipper of GCN4 yeast protein                          | Euromedex                        |
| pUT18C-zip   | pUT18C fused to leucine zipper of GCN4 yeast protein                          | Euromedex                        |
| pDK56        | MglA (pKT25)                                                                | This work                        |
| pDK70        | MglA (pKNT25)                                                               | (32)                             |
| pDK76        | MglA (pUT18)                                                                | This work                        |
| pDK75        | MglA (pUT18C)                                                               | (32)                             |
| pDK55        | MglB (pKT25)                                                                | This work                        |
| pDK71        | MglB (pKNT25)                                                               | (32)                             |
| pDK77        | MglB (pUT18)                                                                | This work                        |
| pDK74        | MglB (pUT18C)                                                               | (32)                             |
| pSC57        | PilB (pKT25)                                                                | This work                        |
| pSC59        | PilB (pKNT25)                                                               | This work                        |
| pSC69        | PilB (pUT18)                                                                | This work                        |
| pSC70        | PilB (pUT18C)                                                               | This work                        |
| pSC58        | PilT (pKT25)                                                                | This work                        |
| pSC60        | PilT (pKNT25)                                                               | This work                        |
| pSC71        | PilT (pUT18)                                                                | This work                        |
| pSC72        | PilT (pUT18C)                                                               | This work                        |
| pSC73        | PilM (pKT25)                                                                | This work                        |
| pSC74        | PilM (pKNT25)                                                               | This work                        |
| pSC61        | PilM (pUT18)                                                                | This work                        |
| pSC62        | PilM (pUT18C)                                                               | This work                        |
| pLC20        | pBJ114; for native site replacement of mglA by mglA-mVenus                  | (2)                              |
| pLC44        | pBJ114; for native site replacement of mglA by mglA<sup>032A</sup>-mVenus   | (2)                              |
| pDK145       | pBJ114; for native site replacement of mglB by mglB-mCherry                 | (25)                             |
| pLC32        | pBJ114; for native site replacement of romR by romR-mCherry                 | (2)                              |
| pLC51        | pBJ114; for generation of in-frame deletion of sgmX                          | This work                        |
| pBJ1ΔagQ     | pBJ114; for generation of in-frame deletion of aglQ                          | (33)                             |
| pES2         | pBJ114; for generation of in-frame deletion of mglA                          | (24)                             |
| pTS08        | pBJ114; for native site replacement of mglA by mglA<sup>032A</sup>           | (25)                             |
| pSL16        | pBJ114; for generation of in-frame deletion of mglA                          | (27)                             |
| pSL37        | pBJ114; for generation of in-frame deletion of romR                          | (25)                             |
| pIB123       | pBJ114; for generation of in-frame deletion of pilT                          | This work                        |
| pAP12        | pNG062; Pil<sub>nat</sub>B-mCherry                                           | This work                        |
| pAP16 | pNG063; P\text{nat} m\text{Cherry}-pilM | This work |
| pAP19 | pBJ114; for generation of in-frame deletion of frzE | This work |
| pAP34 | pSWU30; P\text{nat} sgmX | This work |
| pAP35 | pBJ114; for native site replacement of sgmX by sgmX-m\text{Venus} | This work |
| pAP37 | pBJ114; for native site replacement of pilQ by pilQ-sfGFP | This work |
| pAP39 | pMAT135; overexpression SgmX-Strep | This work |
| pAP87 | pNG063; P\text{nat} m\text{Cherry}-pilT | This work |
| pAP88 | pBJ114; for native site replacement of mglB by mglB\text{A64R}G68R-m\text{Cherry} | This work |
| Primer | Sequence | Used to construct plasmid |
|--------|----------|---------------------------|
| 5766 A | ATCGAAGCTTCGAGCTGAGTGCTCGGT | pLC51 |
| 5766 B | GCGCGCCTTAAACAGATCATCGAAGCC | |
| 5766 C | GATCTTGGTAAAGCGCCGCAAGGTCCG | |
| 5766 D | ATCGGAATTCGAGCTGAGTGCTCGGT | |
| oPilT-A | ATCGAATTCGAGCGTGACGCCGAAGC | |
| oPilT-B | ACTCGTCCAGCGAAGCTGCGGCGGAGA | |
| oPilT-C | GCGAAGCTGACTCGTCCATGCAGGTCG | |
| oPilT-D | ATCAAGCTTCTCTGGTCTGACGCAACC | |
| PilB<sub>nat</sub> fw | ATCCATATGAGGCGCAGCTGAGTGCTCGGT | pIB123 |
| PilB<sub>nat</sub> rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilB | GCCTGCGAGCTGAGTGCTCGGT | |
| PilB rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilM<sub>nat</sub> fw | ATCCATATGCTCAGAAGCTGCGGCGGAGA | |
| PilM<sub>nat</sub> rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilM | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilM rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| frzE A | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| frzE B | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| frzE C | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| frzE D | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| P<sub>nat</sub>sgmX fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| P<sub>nat</sub>sgmX rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| SgmX-mvA | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| SgmX-mvB | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| SgmX-mvC | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| SgmX-mvD | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| Venus/Cherry Fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| Venus/Cherry Rv | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilQ-sfGFP A | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilQ-sfGFP B | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| sfGFP Fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| sfGFP Rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilQ-sfGFP C | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilQ-sfGFP D | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| SgmX-Strep fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| SgmX-Strep rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilT<sub>nat</sub> fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilT<sub>nat</sub> rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilT fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| PilT rev | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| MglB<sup>GR-mChA</sup> | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| MglB<sup>GR-mChB</sup> | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| MglB<sup>GR-mChC</sup> | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| MglB<sup>GR-mChD</sup> | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| BTHMglAf fw | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| BTHMglArv | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |
| BTHMglArvstop | ATCAAGCTTCTGAGCTGAGTGCTCGGT | |

Table S3. Primers used in this work
| Gene         | Forward Sequence               | Reverse Sequence                     | Plasmids         |
|-------------|--------------------------------|--------------------------------------|------------------|
| BTHMglBfw   | ATCGGTCTAGAGATGGGCACGCACTGGTGATG |                                      | pDK55, pDK77     |
| BTHMglBrv   | ATCGGGAATTCGACTCGCTGAAGGTTGTCGATATCG |                                      |                  |
| BTHMglBrvstop| ATCGGGAATTTCGAATTACTCGCTGAAGGTTGTCGATATCG |                                      |                  |
| opilB-pKT25fw| ATCGGGGATCCATCGGCTCGACTCGGT |                                      | pSC57, pSC59, pSC69, pSC70 |
| opilB-pKT25rv| ATCGGGAATTCCCTAGAAGCGGTCCGGGGCG |                                      |                  |
| opilB-pKNT25rv| ATCGGGAATTCGGGAAAGCGGTCCGGGGCGGGGCT |                                      |                  |
| opilT-pKT25fw| ATCGGGGATCCCGTGGCCAACCTGCACCAG |                                      | pSC58, pSC60, pSC71, pSC72 |
| opilT-pKT25rv| ATCGGGAATTCCCTAGAAGCGGTCCGGGGCG |                                      |                  |
| opilT-pKNT25rv| ATCGGGAATTCGGGAAAGCGGTCCGGGGCGGGGCT |                                      |                  |
| opilM-pUT18fw| ATCGGGGATCCCATGGGAGCTCAGGTCCGAG |                                      | pSC61, pSC62, pSC73, pSC74 |
| opilM-pUT18rv| ATCGGGAATTCGGGAGCTCAGGTCCGAGCTCCGAG |                                      |                  |
| opilM-pUT18Crv| ATCGGGAATTTCGAAGCCTTTGTCGCCG |                                      |                  |
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