SUPPLEMENTARY INFORMATION

Outside-in assembly pathway of the type IV pili system in *Myxococcus xanthus*

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Inventory

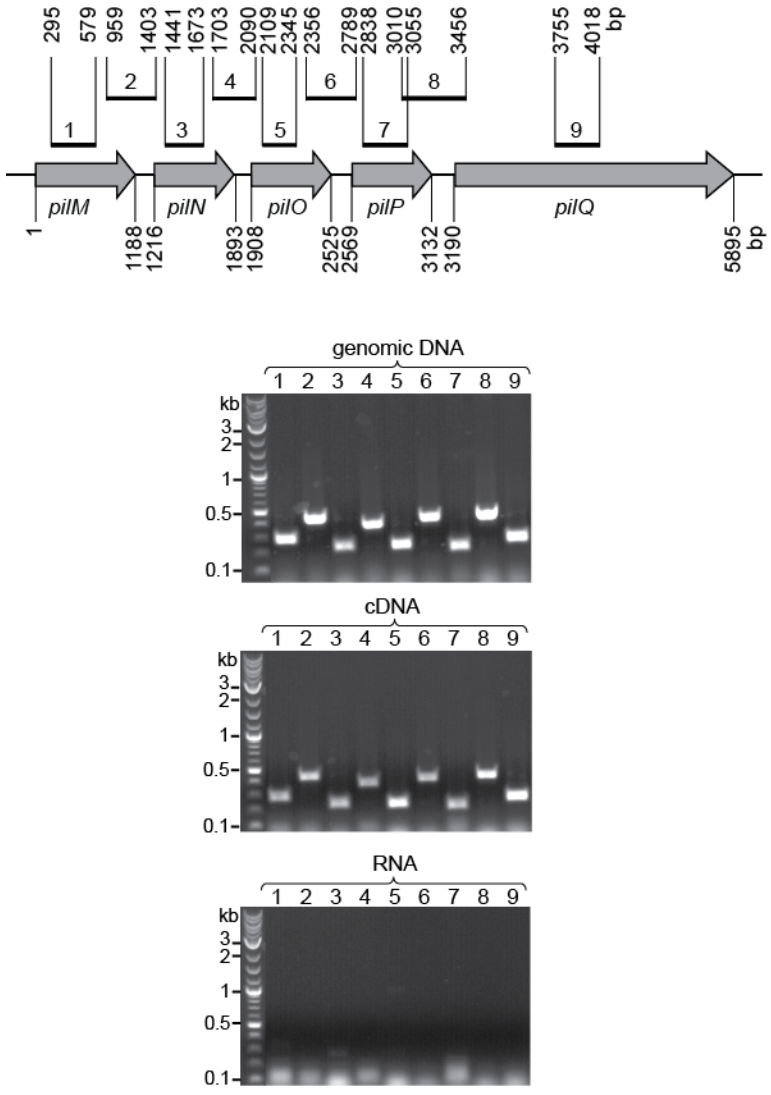

Figure S1. *pilMNOPQ* are part of an operon.

Arrows indicate the direction of transcription of the *pilMNOPQ* genes. All coordinates are relative to the start codon in *pilM*. Numbers below the genes indicate start and stop codons of genes. The lines labeled 1 to 9 show intragenic (uneven numbers) and intergenic regions (even numbers) with the numbers indicating the coordinates of the fragments amplified by PCR. The PCR products amplified using genomic DNA, cDNA and RNA as templates were separated on a 1% agarose gel. Numbers above the individual lanes correspond to the numbers of the different PCR products. Molecular size markers in kb are shown on the left.

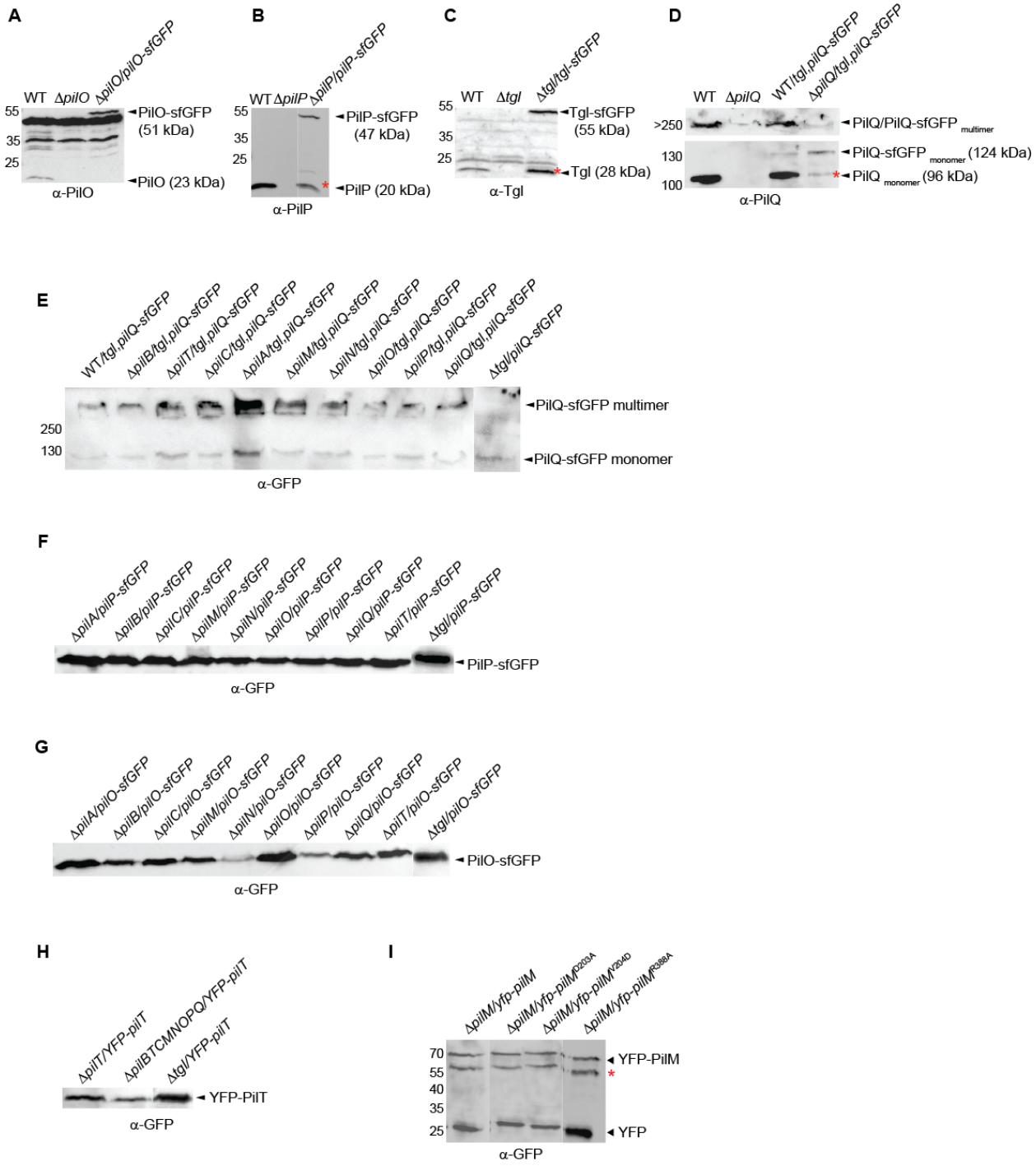

Figure S2. Immunoblot analysis of the accumulation of fluorescently tagged proteins.

(**A-I**) Total cell lysates from exponentially growing cultures of the indicated genotypes were separated by SDS–PAGE (protein from 7 $\times 10^7$ cells loaded per lane), and analyzed by

immunoblotting using specific antibodies as indicated. Relevant proteins and their calculated molecular masses are indicated on the right and molecular size markers in kDa on the left. * indicates degradation products of the fluorescently tagged protein.

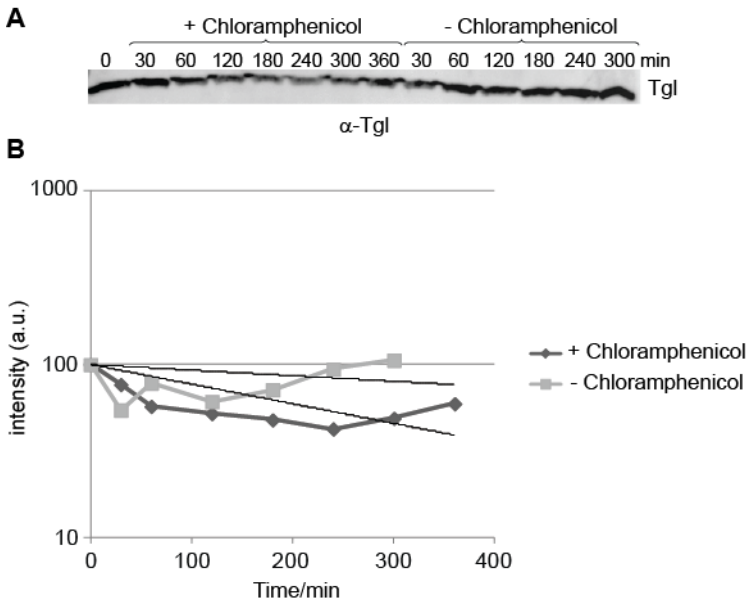

Figure S3. Determination of $T_{1/2}$ of Tgl.

(**A**) DK1622 WT cells were grown in 1.0% CTT medium. At t= 0 min, the culture was split in two and chloramphenicol added to one at a concentration of 25 µg/ml, which inhibits protein synthesis under these conditions (6). At the indicated time-points, samples were harvested. Protein from 7×10^7 cells were loaded per lane, separated by SDS-PAGE and Tgl levels determined by immunoblotting using α-Tgl antibodies.

(**B**) Quantitative analysis of Tgl accumulation. The intensities of the bands corresponding to Tgl in the immunoblot in (A) were quantified. All intensities are relative to t= 0 min and plotted as a function of time. Lines represent best fit curves.

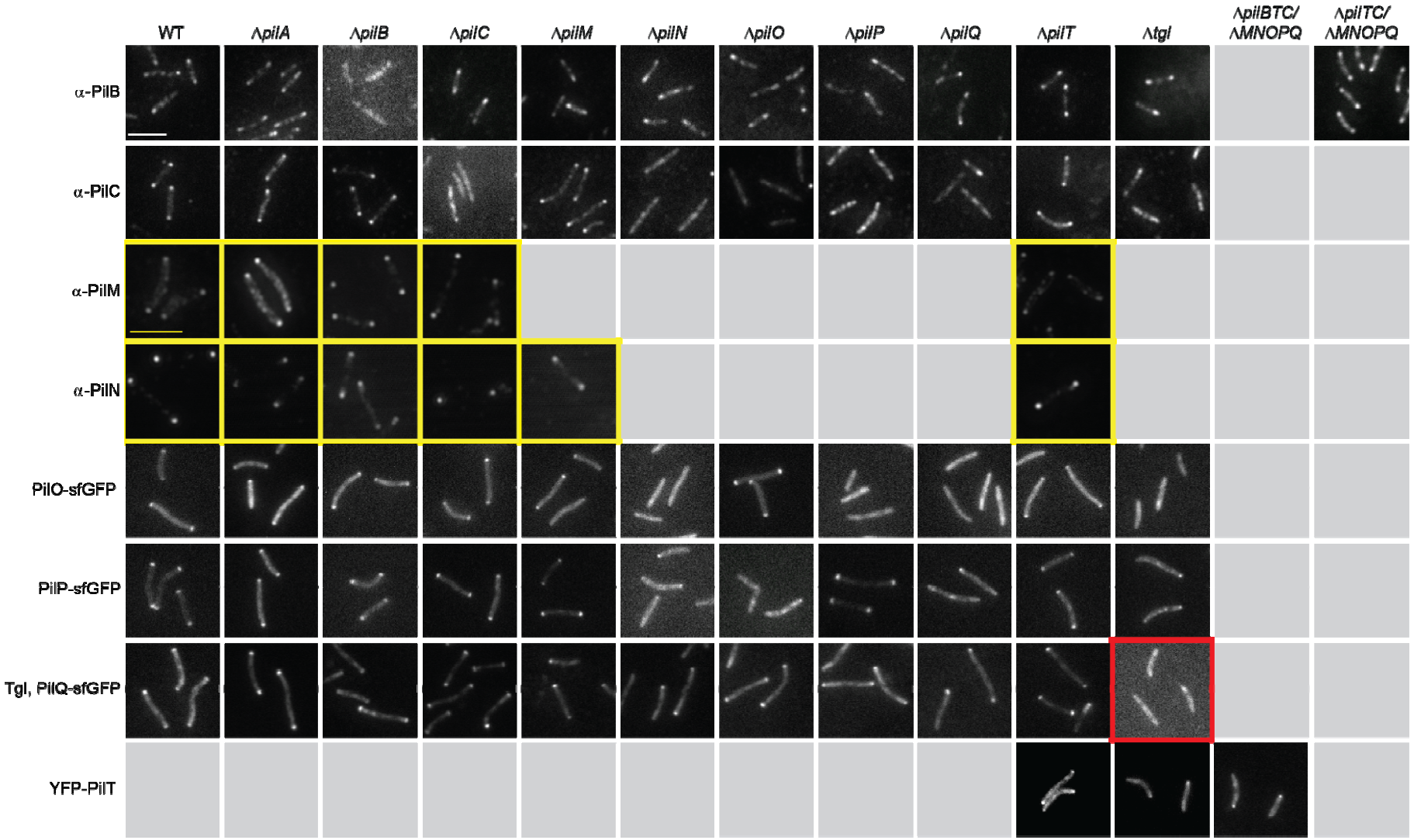
WT
ΔpilA
ΔpilB
ΔpilC
ΔpilM
ΔpilN
ΔpilO
ΔpilP
ΔpilQ
ΔpilT
Δtgl
ΔpilBTC/ ΔMNOPQ
ΔpilTC/ ΔMNOPQ
α-PilB
α-PilC
α-PilM
α-PilN
PilO-sfGFP
PilP-sfGFP
Tgl, PilQ-sfGFP
YFP-PilT

Figure S4. Localization of T4PS proteins.

Cells from exponentially growing cultures of the indicated genotypes and containing the indicated fusion proteins were transferred to a 1.5% agar pad on a microscope slide and imaged by fluorescence microscopy in the case of the fluorescent fusion proteins of PilT, PilO, PilP and PilQ. For native PilB, PilC, PilM and PilN, cells were fixed and imaged by immunofluorescence microscopy using specific antibodies. In the case of Tgl, PilQ-sfGFP, all strains contained extra Tgl. with the exception of the Δ*tgl* strain, which did not contain Tgl (highlighted in red). Scale bars 5 μm. Notice different scale bars in images highlighted in yellow.

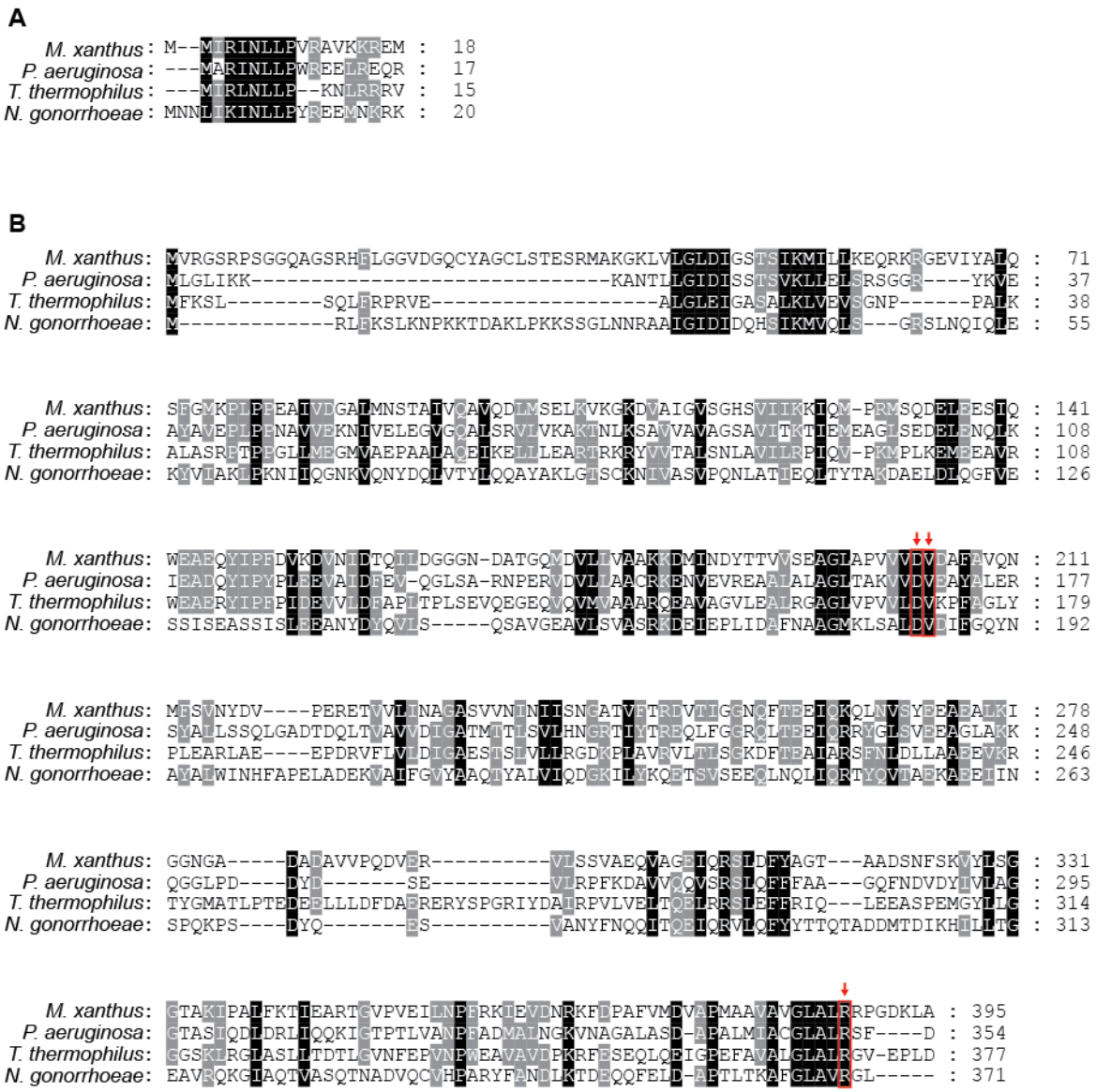

Figure S5. Comparison of N-terminus of PilN and PilM to selected PilN and PilM proteins.

(**A**) Alignment of N-termini of PilN proteins from *M. xanthus*, *P. aeruginosa*, *T. thermophilus* and *N. gonorrhoea*. The indicated 15 residues of *T. thermophilus* PilN were included in the PilM structure (5). Residues are shaded according to conservation. Residues indicated white on black are 100% conserved and residues indicated white on grey are 75% conserved.

(**B**) Alignment of PilM proteins from *M. xanthus*, *P. aeruginosa*, *T. thermophilus* and *N. gonorrhoea*. Conservation is indicated as in (A). Residues marked red interact with the N-terminal PilN peptide in the PilM structure and were substituted in the PilM variants analyzed.

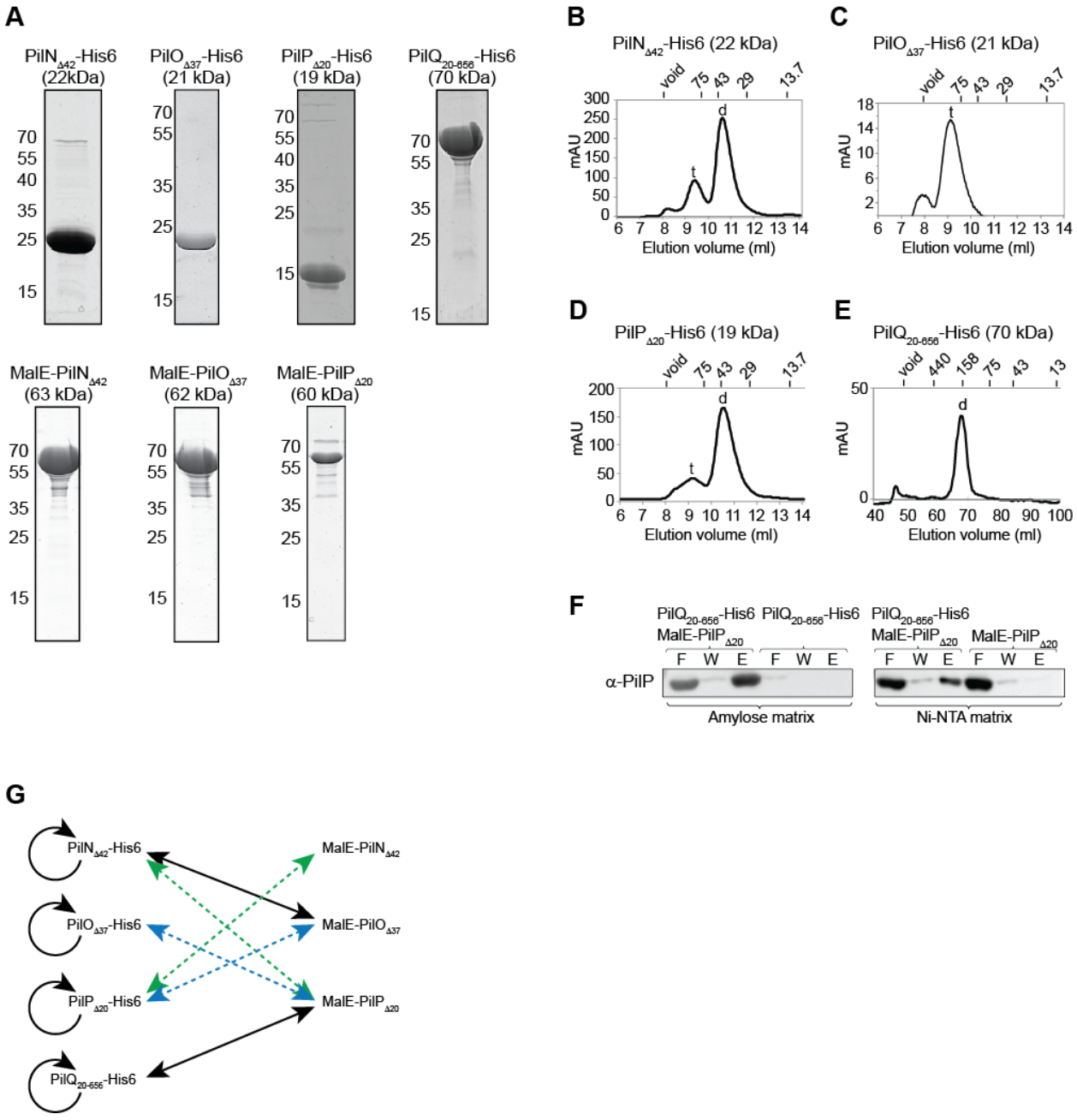

Figure S6. *In vitro* analysis of the periplasmic parts of PilN, PilO, PilP and PilQ.

(**A**) SDS–PAGE analysis of the indicated purified proteins after staining with Coomassie G-250. Positions of molecular markers are indicated on the left in kDa. Calculated molecular masses are shown in parentheses.

(**B-E**) Periplasmic domains of PilN, PilO and PilP form dimers and tetramers in solution, while PilQ forms dimers. The diagrams show the elution profiles of the indicated His6-tagged proteins from size-exclusion chromatography experiments (Superdex 75 10/300 GL for PilNΔ42-His6, PilOΔ37-His6, PilPΔ20-His6 and HiLoad 16/60 Superdex 200 prep grade for PilQ20-656-His6). Molecular size markers are indicated at the top in kDa. Peaks marked “t” and “d” correspond to the size of a tetramer and dimer, respectively.

(**F**) PilP and PilQ interact directly. Samples from the experiment shown in Fig. 5B were stained with PilP-specific antibodies by immunoblotting. Shown are relevant sections of the immunoblot of the flowthrough (F), wash (W) and elution (E) fractions with MalE-PilP$_{\Delta 20}$ (60 kDa) and PilQ$_{20\text{-}656}$-His6 (69 kDa) from Ni-NTA or amylase matrices.

(**G**) Diagram of proteins tested *in vitro* for direct interactions. Black lines indicate combinations of one or two proteins in which interactions were observed; green stippled lines indicate the two combinations of PilN and PilP tested for direct interactions with negative results; blue stippled lines indicate the two combinations of PilO and PilP tested for direct interactions with negative results.

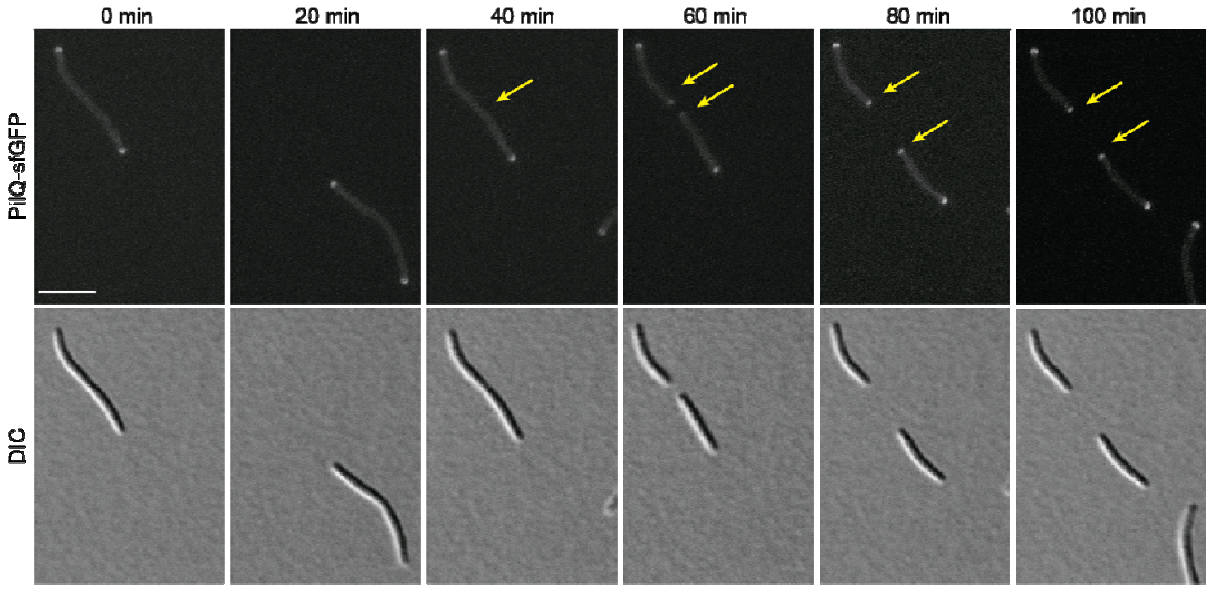

Figure S7. PilQ localizes polarly late during or immediately after cell division. Cells were treated as in Fig. 7, and imaged by fluorescence and DIC microscopy at 20 min intervals. Arrows indicate newly formed polar clusters. Strain used, SA6060 (Δ*aglZ*/*tgl, pilQ-sfGFP*,). Scale bar 5 µm.

Supplemental Materials and Methods

Antibody generation. To generate α-PilN antibodies, a PilN variant was purified in which the 42 N-terminal residues were deleted and a C-terminal His6-tag was added ($PilN_{\Delta 42}$-His6). For α-PilP antibodies, a variant $PilP_{\Delta 55}$-His6 was used. For overexpression and purification of $PilN_{\Delta 42}$-His6 and $PilP_{\Delta 55}$-His6, the plasmids pSC5 and pSC7 were separately transformed into *E. coli* Rosetta 2 [F-*ompT hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm p*RARE2] (Novagen). Both proteins were purified under native conditions on a Ni^{2+}-NTA-agarose column using native lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 10 mM mercaptoethanol, 10% glycerol; pH 8). Bound protein was eluted with native lysis buffer containing 50-250 mM imidazole. Size-exclusion chromatography (HiLoad Superdex 200 prep grade for PilN; HiLoad Superdex 75 prep grade for PilP) was used to further purify both proteins. To generate α-PilO antibodies, $PilO_{\Delta 37}$-His6 (pSC6) was overexpressed in Rosetta 2 and purified under denaturing conditions (100 mM NaH_2PO_4, 10 mM Tris-HCl, 8 M urea; pH 8) using a 1 ml Bio-scale mini profanity IMAC cartridge (BioRad) charged with Ni^{2+} ions and 10 ml Bio-scale mini Bio-Gel P-6 desalting cartridge (Profinia, BioRad). For α-Tgl antibodies, $Tgl_{\Delta 55}$-His6 (pIB48) was purified from Rosetta 2 under denaturing conditions on a Ni^{2+}-NTA-agarose column using the same buffer as for PilO purification. The elution fractions for PilO and Tgl were dialyzed against the same buffer containing 5 M urea. All four purified proteins were used to immunize rabbits using standard procedures (7).

Size-exclusion chromatography. Purified proteins were applied to HiLoad 16/60 Superdex 200 prep grade (SD200) or Superdex 75 10/300GL (SD75) size exclusion columns equilibrated with lysis buffer. The SD200 column was calibrated with ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa) and ribonuclease (13.7 kDa) (GE Healthcare) using the same buffer and conditions. For the SD75 conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and apronitin (6.5 kDa) were used for calibration. Blue dextran (GE Healthcare) was used to determine the void volume of the columns.

Determination of $T_{1/2}$ of Tgl. DK1622 WT cells were grown in 1.0% CTT medium. At t= 0 min, the culture was split in two and chloramphenicol added to one at a concentration of 25 μg/ml, which inhibits protein synthesis under these conditions (6). At relevant time-points, samples were harvested. Protein from 7×10^7 cells were separated by SDS-PAGE and Tgl levels determined by immunoblotting using α-Tgl antibodies. The intensities of the bands

corresponding to Tgl in the immunoblot were quantified using the program Multi Gauge V3.1 (Fujifilm). All intensities were normalized to t= 0 min and plotted as a function of time.

Construction of plasmids. To construct **pSC5** for overexpression of PilNΔ42-His_6, a truncated version of *pilN* was amplified with opilN-3 and opilN-4, cut with BamHI and HindIII and ligated into the expression vector pET24b$^+$.

For overexpression of PilOΔ37-His_6 a truncated version of *pilO* was amplified using opilO-3 and opilO-4. The fragment was cut with BamHI and HindIII and ligated into the expression vector pET24b$^+$ resulting in **pSC6**.

To construct **pSC7** for overexpression of PilPΔ55-His_6, a truncated version of *pilP* was amplified with the primers opilP-3 and opilP-4. The gene product was cut with BamHI and HindIII and fused into pET24b$^+$.

For overexpression of TglΔ55-His_6, the truncated gene was amplified with otgl-8 and otgl-9, digested with BamHI and HindIII and ligated into the pET24b$^+$ vector resulting in **pIB48.**

To create **pSC43** for overexpression of PilPΔ20-His_6, a truncated version of *pilP* was amplified with the primers opilP-8 and opilP-9. The fragment was digested with BamHI and HindIII and fused into pET24b$^+$.

For overexpression of the periplasmic part of PilQ, **pSC108** was constructed. A part of the gene coding for aminoacids 20-656 was amplified using the primers opilQ-17 and opilQ-18 carrying the EcoRI and HindIII restriction sites. After digestion, the gene product was fused into the pET24b$^+$.

To express MalE-PilNΔ42 the truncated gene was amplified with opilN-8 and opilN-9, digested with HindIII and EcoRI and cloned into the pMal-c2x expression vector (**pSC44**).

For overexpression of MalE-PilOΔ37 the truncated gene was amplified with opilO-8 and opilO-9, cut with HindIII and EcoRI and cloned into the pMal-c2x expression vector giving rise to **pSC45**.

For overexpression of MalE-PilPΔ20 the truncated gene was amplified with opilP-10n and opilP-11n, cut with HindIII and EcoRI and cloned into the pMal-c2x expression vector giving rise to **pSC46**.

Complementation constructs for *pilM, pilN, pilO, pilP, pilQ* and *tgl* were constructed by amplifying the respective genes using the primers: opilM-3 and opilM-4 (**pSC2**), opilN-5 and opilN-6 (**pSC37**), opilO-5 and opilO-6 (**pSC38**), opilP-5 and opilP-6 (**pSC39**), opilQ-8 and opilQ-9 (**pIB55**) and otgl-10 and otgl-12 (**pIB54**). All gene products were digested with XbaI and HindIII and cloned into the pSW105 vector.

To construct **pSC101**, the gene *sfGFP* was amplified from the vector pET21-sfGFP using the primers osfGFP-1 and osfGFP-2. The osfGFP-2 primer contained a linker (ctggagggcccggcgggcctg) which resembles the linker recommended by (2) and was optimized in terms of codon usage for *M. xanthus*. The PCR product was digested with BamHI and HindIII and cloned into the vector pSWU30.

To localize PilP, we constructed the plasmid **pSC102**. We amplified the *pilA* promoter with the full-length *pilP* gene from the complementation plasmid pSC39 using the primers oPpilAforw and opilP-12. After digestion with EcoRI and BamHI, the gene product was cloned into the plasmid pSC101 carrying the *sfGFP* gene.

Similarly, the genes *tgl* and *pilO* were amplified with the *pilA* promoter from the complementation plasmids pIB54 (oPpilAforw and otgl-13) and pSC38 (oPpilAforw and opilO-10) and were cloned into the pSC101, giving rise to the plasmids **pSC104 (Tgl-sfGFP)** and **pSC106 (PilO-sfGFP)**.

The first localization construct for PilQ called **pSC110** was cloned similarly amplifying *pilQ* from pIB55 using oPpilAforw and opilQ-15.

The second localization construct **pSC120** carried the genes for *tgl* and *pilQ* under the *pilA* promoter and fused C-terminally to *sfGFP*. *pilQ* was amplified with the upstream intergenic region between *pilP* and *pilQ* (57 bp) using the primers opilQ-21 and opilQ-15 carrying the restriction sites for HindIII and KpnI. *tgl* was amplified using otgl-10 and otgl-12 carrying XbaI and HindIII. Both gene products were digested and cloned simultaneously in the vector pSC110 from which the *pilQ* gene has previously been removed with HindIII and XbaI.

Site-directed mutagenesis of *pilM* (**pSC97, 98, 56**) was performed using the QuikChange II XL site directed mutagenesis protocol as described by the manufacturer (Stratagene). The mutations were introduced in the plasmid **pSC11** carrying the gene *pilM* which was cut from pSC2 with XbaI and HindIII and fused into the plasmid pSK$^-$. After introducing the mutations with the primer pairs opilM-34 and opilM-35 (R388A), opilM-43 and opilM-44 (D203A) and opilM-45 and opilM-46 (V204D), *pilM* was cut out by XbaI and HindIII and ligated into pSC8.

For the in-frame deletion of *pilN* (full-length 678 bp, deletion from 49-662 bp), the up- and downstream regions of the gene were amplified using the primer pairs opilN-A and opilN-B-Xba and opilN-C-Xba and opilN-D. Both fragments were digested with EcoRI and XbaI and HindIII and XbaI, respectively and cloned simultaneously into the vector pBJ113 giving rise to **pIB59**.

The deletion constructs for *pilO* (full-length 618 bp, deletion from 25-570 bp; **pIB18,** *pilP* (full-length 564 bp, deletion from 70-516 bp; **pIB21**) and *tgl* (full-length 762 bp, deletion from 31-732

bp; **pSC125**) were cloned similarly using the primers named opilO-A/B/C/D, opilP-A/B/C/D and otgl-A/B/C/D, respectively. Due to overlapping regions of the primers B and C, the gene products of the primer pairs A/B and C/D were fused in a third PCR using the primers A/D. After digestion with HindIII and EcoRI, the products were fused into the vector pBJ114 or pBJ113 (compare Table S1). To delete multiple genes, as it was done for *pilMNOPQ* (deletion from position 49 bp in *pilM* to position 2676 bp in *pilQ*; **pSC89**), *pilBTC* (deletion from position 25 bp in *pilB* to position 1230 bp in *pilC*; **pSC83**) or *pilTC* (deletion from position 91 bp in *pilT* to position 1230 bp in *pilC,* pSC84*)* the up- and downstream fragments were amplified with the primers opilM-A and opilM-Q-B and opilM-Q-C and opilM-Q-D for pSC89, with opilB-A, opilB-B and opilC-C_{CS}, opilC-D in the case of pSC83 and using opilT-A, opilT-B NheI, opilC-C_{CS} and opilC-D for pSC84 and subsequently cloned into the vector pBJ114 as described above.

213 **Table S1.** Plasmids used in this work.

Plasmid	Relevant characteristics	Reference
pSW105	Vector with Mx8 *attP* site and *pilA* promoter, Km^R	S. Weiss (pers. communication)
pBJ114	Vector for in-frame deletion constructs, Km^R	(4)
pBJ113	Vector for in-frame deletion constructs, Km^R	(4)
pET45b$^+$	Expression vector, T7 promoter, N-term. His_6-tag, Amp^R	Merck Millipore
pSC8	pSWU30 - P_{pilA}-*yfp-pilM;* Tet^R	(1)
pIB75	pSWU30 - P_{pilA}-*yfp-pilT;* Tet^R	(1)
pMal-c2x	Expression vector, *tac* promoter, C-term. MBP-tag, Amp^R	New England Biolabs
pET24 b$^+$	Expression vector, T7 promoter, C-term. His_6-tag, Km^R	Merck Millipore
pBluescript II SK- (pSK$^-$)	cloning vector, Amp^R	Fermentas
pSWU30	Vector with Mx8 *attP* site, Tet^R	(3)
pET-21-sfGFP	pET-21 - sfGFP, Amp^R	(2)
pSC2	pSW105 - *pilM;* Km^R	This work
pIB18	pBJ114 - *pilO* in-frame deletion, Km^R	This work
pIB21	pBJ113 - *pilP* in-frame deletion, Km^R	This work
pIB59	pBJ113 - *pilN* in-frame deletion, Km^R	This work
pSC37	pSW105 - *pilN,* Km^R	This work
pSC38	pSW105 - *pilO,* Km^R	This work
pSC39	pSW105 - *pilP,* Km^R	This work
pIB54	pSW105 - *tgl,* Km^R	This work
pIB55	pSW105 - *pilQ,* Km^R	This work
pSC101	pSWU30 - sfGFP, Tet^R	This work

pSC102	pSC101 - P_{pilA}-*pilP*, Tet^R	This work
pSC104	pSC101 - P_{pilA}-*tgl*, Tet^R	This work
pSC106	pSC101 - P_{pilA}-*pilO*, Tet^R	This work
pSC110	pSC101 - P_{pilA}-*pilQ*, Tet^R	This work
pSC120	pSC101 - P_{pilA}-*tgl*-*pilQ*, Tet^R	This work
pSC11	pSK^- - *pilM*, Amp^R	This work
pSC56	pSC8 - $pilM^{R388A}$, Tet^R	This work
pSC97	pSC8 - $pilM^{D203A}$, Tet^R	This work
pSC98	pSC8 - $pilM^{V204D}$, Tet^R	This work
pSC5	pET24 b^+ - $pilN_{\Delta 42}$, Km^R	This work
pSC6	pET24 b^+ - $pilO_{\Delta 37}$, Km^R	This work
pSC7	pET24 b^+ - $pilP_{\Delta 55}$, Km^R	This work
pSC43	pET24 b^+ - $pilP_{\Delta 20}$, Km^R	This work
pIB48	pET24 b^+ - $tgl_{\Delta 55}$, Km^R	This work
pSC44	pMal-c2x - $pilN_{\Delta 42}$, Amp^R	This work
pSC108	pET24b^+ - $pilQ_{20\text{-}656}$, Km^R	This work
pSC45	pMal-c2x - $pilO_{\Delta 37}$, Amp^R	This work
pSC46	pMal-c2x - $pilP_{\Delta 20}$, Amp^R	This work
pSC83	pBJ114 - *pilBTC* in-frame deletion, Km^R	This work
pSC84	pBJ114 - *pilTC* in-frame deletion, Km^R	This work
pSC89	pBJ114 - *pilMNOPQ* in-frame deletion, Km^R	This work
pSC125	pBJ114 - *tgl* in-frame deletion, Km^R	This work

214

215

Table S2. Primers used in this work

Name	Nucleotide sequence (5'-3')[1]
opilM-3	atcgg**aagctt**TCAGGCCAGCTTGTCGCC
opilM-4	atcgg**tctaga**GTGGTGCGAGGCTCCCGT
opilN-A	atcgg**aagctt**TCATCTCCAACGGCGCGA
opilN-B-Xba	atcgg**tctaga**ACGCTTCTTCACCGCCCG
opilN-C-Xba	atcgg**tctaga**CAACTACGCCATCTGACA
opilN-D	atcgg**gaattc**GTTCTTGAGTTTGGCCGA
opilO-A	atcgg**aagctt**GTCGCTTCAACCAAGGCG
opilO-B	ggccttctgGAATTGATCCAGGTACTT
opilO-C	gatcaattcCAGAAGGCCGCTGCGTCG
opilO-D	atcgg**gaattc**GAACACCTCTGTCACCGT
opilP-A	atcgg**aagctt**GAAATCGGTTGGGCGGTG
opilP-B	gttgtaggcCGGCTCCTCGCACGCAGC
opilP-C	gctgcgtgcCAGGACCCCGCCTACAAC
opilP-D	atcgg**gaattc**CGACGCGGTCATGCGTTC
opilN-5	atcgg**tctaga**atgATGATTCGCATCAAC
opilN-6	atcgg**aagctt**TCAGATGGCGTAGTTGGA
opilO-5	atcgg**tctaga**atgGACAAGTACCTGGAT
opilO-6	atcgg**aagctt**CTATTTCTTCGAGTTCGA
opilP-3	atc**ggatcc**catgTACAGCTATGTGTACAAC
opilP-4	atc**aagctt**CTCTCCGTAGTTCCTGCC
opilP-5	atcgg**tctaga**ATGAAGACGTTCAAGGCC
opilP-6	atcgg**aagctt**CTACTCTCCGTAGTTCCT
oTgl-10	atcgg**tctaga**ATGTTCCGCCTTTCCACC
oTgl-12	atcgg**aagctt**CTACTAGAGCTTTTCCAGCAG
opilQ-9	atcggtctagaATGCTCGAGGAGAGCGCT

opilQ-8	atcgg**aagctt**TTACAGAGTCTGCGCAAT
osfGFP-1	atcgg**aagctt**ttaTTTGTAGAGCTCATCCAT
osfGFP-2	atcgg**ggatcc**ctggagggcccggcgggcctgATGAGCAAAGGAGAAGAACT
oPpilAforw	atcgg**gaattc**GCGGCGTTGAACGAGGGG
opilO-10	atcgg**ggatcc**TTTCTTCGAGTTCGAGTT
opilQ-15	atcgg**ggtacc**CAGAGTCTGCGCAATGGT
opilQ-21	atcgg**aagctt**CGCCCTCCAAGGCGCTCC
otgl-10	atcgg**tctaga**ATGTTCCGCCTTTCCACC
otgl-12	atcgg**aagctt**CTACTAGAGCTTTTCCAGCAG
opilM-34	CGTGGGACTGGCGCTGGCGCGCCCGGGCGACAAG
opilM-35	CTTGTCGCCCGGGCGCGCCAGCGCCAGTCCCACG
opilM-43	CCCGGTGGTGGTGGCCGTGGATGCCTTCG
opilM-44	CGAAGGCATCCACGGCCACCACCACCGGG
opilM-45	GTGGTGGTGGACGACGATGCCTTCGCCG
opilM-46	CGGCGAAGGCATCGTCGTCCACCACCAC
opilN-3	atc**ggatcc**catgGACCGACAGAGCGAGCTT
opilN-4	atc**aagctt**GATGGCGTAGTTGGAAGT
opilO-3	atc**ggatcc**catgCCCACGGAGGAAGAAATC
opilO-4	atc**aagctt**TTTCTTCGAGTTCGAGTT
opilP-8	atcgg**ggatcc**catgGAGGAGCCGCCGGCTCCT
opilP-9	atcgg**aagctt**CTCTCCGTAGTTCCTGCC
opilN-8	atcgg**gaattc**atgGACCGACAGAGCGAG
opilN-9	atcgg**aagctt**TCAGATGGCGTAGTTGGA
opilO-8	atcgg**gaattc**atgCCCACGGAGGAAGAA
opilO-9	atcgg**aagctt**CTATTTCTTCGAGTTCGA
opilP-10n	atcgg**gaattc**atgGAGGAGCCGCCGGCTCCT
opilP-11n	atcgg**aagctt**CTACTCTCCGTAGTTCCT
opilQ-17	atcgg**aagctt**CAGCGACCGGCTAAAGGA

opilQ-18	atcgg**gaattc**catgGTCGTTCTTGTGGGCGCC
opilP-12	atcgg**ggatcc**CTCTCCGTAGTTCCTGCC
otgl-13	atcgg**ggatcc**GAGCTTTTCCAGCAGCCT
opilT-A	atc**gaattc**CTCGCCCAGCGTCTGGCG
opilT-B NheI	atc**gctagc**GCGAAGCTGCGGCGGAGA
opilB-A	atcgg**gaattc**TGCCATCCGCGTCCAACTG
opilB-B	atcgg**gctagc**CAGTTCACCGAGTCGACC
opilC-C CS	atcgg**gctagc**TCGCTTGCCGGCGCCATC
opilC-D	atc**aagctt**AGCACCCGCGTGGGCGGC
opilM-A	atcgg**aagctt**GGGCTCACCGCAGAGGCC
opilM-Q-B	ctgccggttGTGCCTGGAGCCCGCCTG
opilM-Q-C	tccaggcacAACCGGCAGACCATTGCG
opilQ-D	atcgg**gaattc**CTCGGTCGACTTGCCGTT
otgl-A	atcgg**aagctt**TACCGCGGGCTGCCCGCC
otgl-B	ccggcagtcCGAACAGGACGCGGTGGA
otgl-C	tcctgttcgGACTGCCGGAGGCTGCTG
otgl-D	atcgg**gaattc**ACGAGCCGGTCGGACTCG
otgl-8	atcgg**ggatcc**atgCAGGTGTCGCTGAAGAAC
otgl-9	atcgg**aagctt**GAGCTTTTCCAGCAGCCT
opilM-6	CAGGACCTGATGTCCGAGCTGAA
opilM-7	GGAGACCACCGTGGTGTAGTCGT
opilM-8	CCGACTCGAACTTCAGCAAGGTC
opilN-5-o	GCAATCCTCGCCTTGGTTGAAGC
opilN-6-o	AACACCCGGAAGGCCGAAGTGGA
opilN-7-o	TTGGGTGTCCACACCACACCGTT
opilN-8-o	GGGACAGCAAGACAGCACGAGTC
opilO-5-o	ACTCTTGTCCGCCAGTTCCAGGT

opilO-6-o	AATGAGCGTCGGCGCGAGATGGA
opilO-7-o	GAGCGCAATCTCGTGGTAGTTGC
opilO-8-o	AGATGGCCAACATGCGCCGCATC
opilP-5-o	TCGATCGGACTCCGGAAAGGGTC
opilP-6-o	CCTCTGCTCGTTCGACTTGGATC
opilP-7-o	CCGGATTCTTGATGATCTCTCCG
opilP-8-o	GTGACAGAGGTGTTCTCCGGCAA
opilQ-12	AGAGCCCTCGTGGTGTCCCTTGA
opilQ-13	ACGTGGTGGCTGCCGAGGCTGAT
opilQ-14	CCAGCACCAGGCGAACCTTGTCT

[1] Sequences in bold display restriction sites used for cloning. Sequences in upper case indicate sequences complementary to the respective genes. Sequences in lower case show added sequences required for cloning. Underlined nucleotides were substituted during site-directed mutagenesis.

Supplemental References

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