

JC1	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
SK-93-1035	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
DGI2	MCSLLKKIVFSALLAAGPATSFAAQRVP <small>T</small> TENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
PID18	MCSLLKKIVFSALLAAGPATSFAAQRVP <small>T</small> TENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
PID2059	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
MS11	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
PID1	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
PID332	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
NCCP11945	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
FA19	MCSLLKKIVFSALLAAGPATSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	61
MC	...LLKKIVFSALLVAGP <small>T</small> TSFAAQRVPATENTPHVVSISKRNLSSRIAIEGGRISSWKFME	58
JC1	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
SK-93-1035	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
DGI2	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGNNIVIDVAGANRRE	122
PID18	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGNNIVIDVAGANRRE	122
PID2059	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
MS11	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
PID1	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
PID332	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
NCCP11945	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
FA19	GDLELQKDTTTGQLFVRSLTSNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRRE	122
MC	GDLELQKDTTTGQLFVRSLTSSPTNLFVVSSEEGKTYLLVLKPTSKQGDNIVIDVAGANR <small>E</small>	119
JC1	AAVLASQTGPMPVTMNSTEYVRAIKKMMTSMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
SK-93-1035	AAVLASQTGPMPVTMNSTEYVRAIKKMMTSMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
DGI2	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
PID18	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
PID2059	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
MS11	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
PID1	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
PID332	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
NCCP11945	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
FA19	AAVLASQTGPMPVTMNSTEYVRAIKKMMASMMRGTTGDMGINHSHEYQTVPPLWKTTLFIQN	183
MC	AAVLASRTGPMPVTMNSTEYVRTIKKMMTSMMRGTTGDMGINHSHEYQTIPWKNTLFIQN	180
JC1	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
SK-93-1035	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
DGI2	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
PID18	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
PID2059	GSYTAADMQGFSTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
MS11	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
PID1	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
PID332	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
NCCP11945	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
FA19	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRPGG	244
MC	GSYTAADMQGLSFTLTNLGTQLEIREQEFYRQGVLAVALAVRKQILQPGEITDVFIIISRLGG	241

Figure S1. Alignment of the amino acid sequence of TraK from eight sequenced *Neisseria gonorrhoeae* strains carrying the GGI and two *N. gonorrhoeae* strains (JC1 and PID2059) for which we sequenced the *traK* locus. A representative *N. meningitidis* strain (MC: α275) is included for reference. In some strains of *N. meningitidis*, including α275, an insertion sequence is present at the start of the *traK* gene (1). Non-conserved amino acids are highlighted in gray.

DGI2	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
FA19	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
MS11	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
NCCP11945	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
PID1	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
PID18	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
PID332	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
SK-93-1035	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRCPNARNSSDDPYCESISSNYKASVAGV	60
MC	MMRLKALSITAAILILSGCSTLTMSGIGGSEKFRC SNAWNSDDPYCESISSNYKASVAGV	60
DGI2	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
FA19	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
MS11	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
NCCP11945	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
PID1	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
PID18	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
PID332	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
SK-93-1035	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
MC	LKDGMKRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
DGI2	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
FA19	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
MS11	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
NCCP11945	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
PID1	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
PID18	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
PID332	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
SK-93-1035	YVVLNQGDWLIAHNGQQIINEYRPIRLLGDGAKTSGSSVETSPNSNNTNDRIPDVGVNL	180
MC	YVVLNQGDWLIAHN QQQIIDEYRPIRLLGNGAKTS DSTVDPSS TNSNNTNDRIPDVGVNL	180
DGI2	HLOETSGIPGTVR 193	
FA19	HLOETSGIPGTVR 193	
MS11	HLOETSGIPGTVR 193	
NCCP11945	HLOETSGIPGTVR 193	
PID1	HLOETSGIPGTVR 193	
PID18	HLOETSGIPGTVR 193	
PID332	HLOETSGIPGTVR 193	
SK-93-1035	HLOETSGIPGTVR 193	
MC	N LQETSGIPGT TR 193	

Figure S2. Alignment of the amino acid sequence of TraV from eight sequenced *Neisseria gonorrhoeae* strains carrying the GGI. A representative *N. meningitidis* strain (MC: a275) is included for reference. Non-conserved amino acids are highlighted in gray.

DGI2	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
NCCP11945	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
PID18	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
SK-93-1035	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
PID1	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
PID332	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
MS11	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
FA19	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILRTDFTSPQAGI	70
MC	MRVKVNKFKLSAARKRQLAIAGMVAGSMAAIVGSAIYVIEGNKKKSAEVTOVQNKRQILKTDFDTSPQAGI	70
DGI2	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
NCCP11945	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
PID18	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
SK-93-1035	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
PID1	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
PID332	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
MS11	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
FA19	TDNSLWMTTESSKIEYANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGRLPPAP	140
MC	TDNSLWMTTESSKIEDANRKISELETMVQELKEKENSSNPDTSKGLGPDPGLGKPPAISGIGDNGOLPPAP	140
DGI2	PAGTLPGAPPADRPPIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
NCCP11945	PAGTLPGAPPADRPPIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
PID18	PAGTLPGAPPADRPPIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
SK-93-1035	PAGALPGAPPADRSIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
PID1	PAGTLPGAPPADRSIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
PID332	PAGTLPGAPPADRSIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
MS11	PAGTLPGAPPADRSIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
FA19	PAGTLPGAPPADRSIERKIVSGSMSEAELOPGTTGSVNTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
MC	PAGTLPGVPPADRSIERKIVSGSMSEAELOPGTTGAVENTGNPNENVRVSPQLKEAEWVKSCTPRMNIEV	210
DGI2	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
NCCP11945	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
PID18	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
SK-93-1035	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
PID1	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
PID332	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
MS11	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
FA19	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
MC	VEDGGKVVVSQGKFRARDSYIPSGTFFRSVLLGGVDAPTGGEAQNAQASPHPVLMRVTDAQLPNRFKYNFR	280
DGI2	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
NCCP11945	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
PID18	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
SK-93-1035	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
PID1	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
PID332	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
MS11	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
FA19	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
MC	ECFVTGQAYGDISSERAYIRLQLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTQGQOLLANALMSG	350
DGI2	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
NCCP11945	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
PID18	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
SK-93-1035	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
PID1	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
PID332	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
MS11	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
FA19	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
MC	VISGMGKGVSEAFKVTNNATAFGSTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVFPVVEVNAGRQ	420
DGI2	VDVVLTQGIEIDTGETK	437
NCCP11945	VDVVLTQGIEIDTGETK	437
PID18	VDVVLTQGIEIDTGETK	437
SK-93-1035	VDVVLTQGIEIDTGETK	437
PID1	VDVVLTQGIEIDTGETK	437
PID332	VDVVLTQGIEIDTGETK	437
MS11	VDVVLTQGIEIDTGETK	437
FA19	VDVVLTQGIEIDTGETK	437
MC	VDVVLTQGIEIDTGETK	437

Figure S3. Alignment of the amino acid sequence of TraB from eight sequenced *Neisseria gonorrhoeae* strains carrying the GGI. A representative *N. meningitidis* strain (MC: α275) is included for reference. Non-conserved amino acids are highlighted in gray.

Plasmid	Diagram of <i>maLE-traK</i> fusions	TraK amino acids	MAb recognition		
			2E5	3H4	3B8
pMR12	CA S X	L44-G244	+	+	+
pCK3		L44-I113, G231-G244	-	-	+
pCK4		G231-G244	-	-	-
pCK5	A S X	D114-G244	+	+	-
pMR81	A	D114-I146	-	-	N.D.
pMR82	A	D114-D190	-	-	N.D.
pMR84	S X	S152-G244	+	+	N.D.
pMR85	S X	S185-G244	-	-	N.D.
pMR92		S152-G193	-	-	N.D.

100 bp

Figure S4. Characterization of monoclonal antibodies for TraK. Epitopes for three monoclonal antibodies (2E5, 3H4, and 3B8) were determined using MBP-TraK fusions. A series of plasmids was constructed in which different lengths of *traK* were fused to *maLE*. The MBP-TraK fusion proteins were expressed in *E. coli*, and Western blots were performed on *E. coli* lysates with each monoclonal antibody. In the plasmid diagrams, the broken black rectangle represents *maLE*, while the white rectangle indicates the portion of the *traK* sequence expressed in each fusion. Several restriction sites in TraK are indicated for reference: Clal (C), AgeI (A), StuI (S), XmaI (X). The TraK amino acids expressed from each fusion are also indicated. The reactivity of the monoclonal antibodies to each fusion protein was determined by immunoblot. +, detection in immunoblot; -, no detection in immunoblot; N.D., reactivity not determined.

A

TraK _{GGI}	MCSLLKKIVFSALLAAGPATSFA-----	23	60
TraK _F	MRKNNTAIIFGSL--FFSCSVMA-----	21	60
VirB9 _{Ti}	MTRKALFI--LA C LFAAATGAEAEDTPMAGKLDPRMRYLAYNPQVVRNSTAVGATLVVT	58	60
TraO _N	MKKLLLLSAVVLSQLGAATNVMALEVGRNSPYDYRIKSVVVNPVNWKIDAVAGVATHIV	60	60
TraK _{GGI}	-----AQRPATENTPHVVISKRNLNSRIAIEGGRISWKFMEGDLLELQKDTTTGQLF	76	120
TraK _F	-ANGTLAPTVPMVNGQASIAISNTSPNLFTVPGDRIIAVNLDGALTNEQTASGGVV	80	120
VirB9 _{Ti}	FATNETVTSVAVSNSKDLAALPRGNYLFFKASQVLTPQPVIVLTASDSGM-RRYVFSI	117	120
TraO _N	VAPDETYITHAFGDSESRTFAHKMNHFVVKPKQAMSDTNLVIT-----DKRTYNIVL	113	120
TraK _{GGI}	VRSLT-----SNPTNLFVISEEGKTYLLVLKPTSKQGDNIVIDVAGANRREAAVLASQ	129	180
TraK _F	VATVN-----KKPFTFILETERGLNLSIQAVPREGAGRTIQLVSDLRGTGEA-----	128	180
VirB9 _{Ti}	KTLSHLDKEQPDLYS-----VQFAYPADDAAARRREAQQRAVVDRLHA	161	180
TraO _N	HFGEETKKNADGTVSKSFIETPWAVRHAVLQLTYEYPFEQQEKAKSAADKKRITQKLQ	173	180
TraK _{GGI}	TGPMPVTMNSTEYVRAIKMMMASMMRGTTGDMGINHSHEYQTVPLWKDTLFIQNGSYTAA	189	240
TraK _F	----GAWETSTPYESLLVTISQAVRGKGKLPAGWYQVPVTKETLQAPAGLSSVADAVWTGN	184	240
VirB9 _{Ti}	EAQYQRKAEDLLDQPVTALEGATDSNWHYVAQGD---RSLLPLEVFDNGFTTVFHFGNVR	218	240
TraO _N	TA-----FAGAKNYQYVMSEQPEMRSIQPVHVWDNYRFTRFEFPANAE	216	240
TraK _{GGI}	DMQGLSFTLTNLGKQLEIREQEYRQGVLAVALRKQILQ--PGEITDVFIISRPGG---	244	300
TraK _F	HLKMVRFAVENKTL SALNIRESDFWQPGTRAVMFMSQ PASQQLLAGARMMDVYVIRDGEVN--	242	300
VirB9 _{Ti}	I PSIYTINPDGKEAVANYSVKGSD---VEISSVSRGWRLRDGHTVIL C IWNAAAYDPVGQRP	275	300
TraO _N	LPQVYIMISASGKETLPNSHVVGENRNIIIEVETVAKERIRLGDKVVGVRNNNFAPGRGAV	276	300
TraK _{GGI}	-----	244	318
TraK _F	-----	242	318
VirB9 _{Ti}	QTGTVRPDVKRVLKGAKG	293	318
TraO _N	ATGTASPDVRRVQIGEDN	294	318

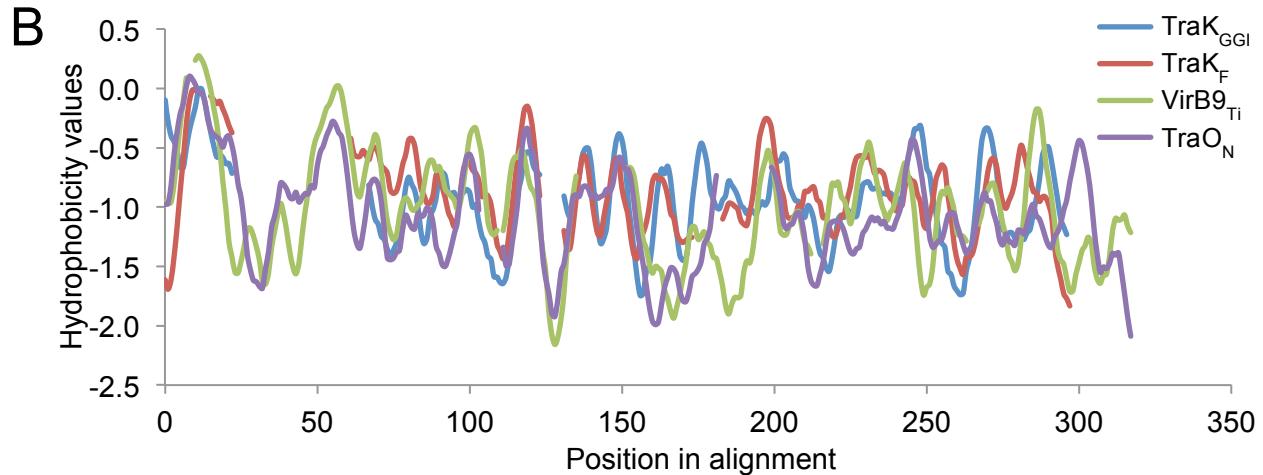


Figure S5. Alignment of TraK_{GGI} homologs. **(A)** Alignment of gonococcal TraK (TraK_{GGI}, NCBI Accession No. AAW83065) with homologs from F plasmid (TraK_F, NCBI Accession No. BAA97947), pKM101 (TraO_N, NCBI Accession No. AAA86458), and *A. tumefaciens* (VirB9 encoded by pTi15955, NCBI Accession No. CAA29979) using AlignMe (Alignment of Membrane Proteins) (2, 3). Identical residues are highlighted in gray. The arrow indicates the predicted Sec-dependent signal peptide cleavage site (SignalP) (4). Cysteines are in red, with the VirB9_{Ti} cysteine that contributes to the disulphide bridge with VirB7_{Ti} also boxed. The first column of numbers on the right is the amino acid number in each protein. The second column of numbers is the position in the overall alignment. **(B)** Hydrophobicity plot of the alignment (2, 3).

A

	*					
TraV _{GGI}	MMRLKALSITAAILILSGCSTL-----	TMSGIGGSEKFRCPNARNSSDPYCESISS	51	60		
TraV _F	MKQTSFFIPLLGTLLLYGCAGTSTEFECNATTSDTCMTMEQANEKAKKLERSSEAKPVAA		60	60		
VirB7 _{Ti}	-----MKYCLLCLVVALSGCQTNDTIASCKGPIFPLNVGRWQPTPSDLQLRNSGGRYDGA-		55	60		
TraN _N	-----MRSLLLMGVLLISACSSGHKPPPEPDWSNTVPVNKTIPV-----DTQGGRNES-		48	60		
TraV _{GGI}	NY-----KASVAGVLKDGMKRQVGQPYTDQSVRTILMLTQAYSSGT-----		91	120		
TraV _F	SLPRLAEGNFRTMPVQTVTATTPSGSRPAVTAHPEQKLLAPRPLFTAAREVKTVVPVSSV		120	120		
VirB7 _{Ti}	-----		55	120		
TraN _N	-----		48	120		
TraV _{GGI}	-----PVRSQSEIARIWVAPYLDTDGDLNDQSFTYVVLNQGDWLIAHNGQQIINEYRP		144	180		
TraV _F	TPVTPPRPLRTGEQTAALWIAPYIDNQDVYHQPVSSVFFVIKPSAWGKPRIN-----		171	180		
VirB7 _{Ti}	-----		55	180		
TraN _N	-----		48	180		
TraV _{GGI}	IRLLGDGAKTSGSSVETSPTNSNNDRIPDVGVLNLHQETSGIPGTVR		193	229		
TraV _F	-----		171	229		
VirB7 _{Ti}	-----		55	229		
TraN _N	-----		48	229		

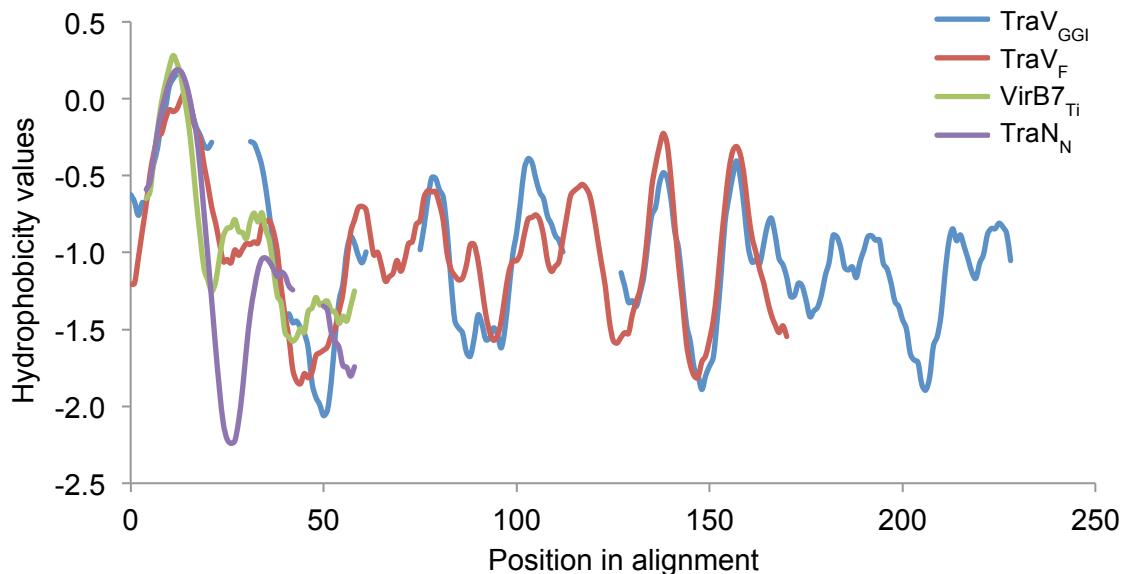
B

Figure S6. Alignment of TraV_{GGI} homologs. **(A)** Alignment of gonococcal TraV (TraV_{GGI}, NCBI Accession No. AAW83068) with homologs from F plasmid (TraV_F, NCBI Accession No. BAA97952), pKM101 (TraN_N, NCBI Accession No. AAA86456), and *A. tumefaciens* (VirB7 encoded by pTi15955, NCBI Accession No. CAC15164) using AlignMe (Alignment of Membrane Proteins) (2, 3). Identical residues are highlighted in gray. Cysteines are shown in red, with the N-terminal lipidated cysteines indicated by an asterisk. Cys24 in VirB7_{Ti}, which contributes to the disulphide bridge with VirB9, is boxed (5). The first set of numbers on the right is the amino acid number in each protein. The second set of numbers of the position in the overall alignment. **(B)** Hydrophobicity plot of the alignment generated using AlignMe (2, 3).

A

TraB _{GGI}	-----MRVKVNKFKLS-----AARKRQLAIAGMVAGSMAAIVGSAIYV	38	60
TraB _F	-----MASINTIVKRKQYLWLGIVVVGTTASAIGGALYL	33	60
VirB10 _{Ti}	-----MNNDSQQAAHEVDASGSLVSDKHRRRLSGSQKLIVGGVVLALSLSIWLG-	50	60
TraF _N	MARKSVDVDQELDENTGDGEFESERGGFNGSNRRSAPRMKA <u>F</u> VILMALLALVFIGITVMG	60	60
TraB _{GGI}	I-----EGNKKKSAEVTVQVNKRQILRTDFTSPQAGITDNSLWMNTTESSKIEYANRKISE	93	120
TraB _F	-SDVDMMSGNGETVAEQEPVPDMTGVVDTTFDDKVRQHATTEMQVTAAMQKQYEEIRREL	92	120
VirB10 _{Ti}	-----	50	120
TraF _N	-----	60	120
TraB _{GGI}	LETMV <u>Q</u> ELKEKENSSNPDTSKGLGP <u>D</u> GLGKPPAISGIGDN <u>R</u> LPAPPAGTL <u>P</u> NGAPPAD	153	180
TraB _F	DVLNK <u>Q</u> RGDDQRRIE <u>K</u> LQDNAALAEQV <u>K</u> ALGANPVTAT <u>G</u> E <u>P</u> V <u>P</u> QMPASPPGPE <u>E</u> PQPG	152	180
VirB10 _{Ti}	-----GRQKKVNDNASPSTLIAANTKPFHPAPIEVPPDTPAVQEAVQPTVPQP	98	180
TraF _N	KIRAPAKAEADKDGGKA <u>Q</u> Q <u>A</u> NTLPNYSFN <u>S</u> DPDVNK <u>P</u> ATAQNSPTDARAVQAAA <u>Q</u> ADA	120	180
TraB _{GGI}	RSIERKIVSGSMS--EAELQPGTTGSVNTGNPNENVRVSPQLKEAEWVKS <u>K</u> TPRMNIEVV	211	240
TraB _F	NTPVSFPPQGSVAVPPPTAFYPGNGVTPPPQV <u>T</u> QSV <u>V</u> PNRI <u>Q</u> RKV <u>F</u> TRN-----	203	240
VirB10 _{Ti}	-----PRGE <u>P</u> ER <u>H</u> E <u>P</u> RP <u>E</u> ETPI <u>F</u> AYSS <u>G</u> D <u>Q</u> GVSKRAS <u>Q</u> GD <u>M</u> GR <u>R</u> QED <u>D</u> KRD <u>D</u> NSLPNGEV	152	240
TraF _N	GSSNTGARTSN <u>K</u> RE <u>P</u> SP <u>E</u> ELAM <u>Q</u> RRL <u>G</u> GE <u>L</u> A <u>Q</u> T <u>N</u> QA <u>A</u> TSNS <u>P</u> GA <u>O</u> -----PQDNETS	173	240
TraB _{GGI}	EDGGKVVVS <u>Q</u> GKFRARD <u>S</u> YIP <u>S</u> GTFFRSVLLGGVDAPT <u>G</u> GEAQ <u>N</u> ASPHV <u>L</u> MRV <u>T</u> DFA <u>Q</u> L	271	300
TraB _F	-----EGKQGPSLP <u>I</u> PSGSFA <u>K</u> AML <u>I</u> E <u>G</u> ADA-NASV <u>T</u> GNE <u>S</u> TPV <u>M</u> QL <u>R</u> IT <u>G</u> L <u>V</u> E <u>M</u>	253	300
VirB10 _{Ti}	SGEND <u>L</u> SIRMK <u>P</u> TEL <u>Q</u> PSRAT <u>L</u> LPHPD <u>M</u> V <u>T</u> Q <u>G</u> T <u>I</u> I <u>P</u> C <u>I</u> LO <u>Q</u> TA <u>I</u> D <u>T</u> N <u>LA</u> GYVK <u>C</u> VL <u>P</u> QDI	212	300
TraF _N	EGSSALAKNLTP <u>A</u> RL <u>K</u> ASRAGV <u>M</u> PSLTV <u>P</u> K <u>G</u> KMIPCGTG <u>T</u> EL <u>D</u> TTVPG <u>Q</u> VS <u>C</u> RV <u>S</u> QDV	233	300
 			*
TraB _{GGI}	PNRF <u>K</u> YN <u>F</u> REC <u>V</u> TF <u>G</u> QAY <u>G</u> DI <u>S</u> SERAY <u>I</u> RL <u>Q</u> NL <u>S</u> CV <u>G</u> T <u>D</u> GRA <u>I</u> D <u>M</u> P <u>V</u> --K <u>G</u> Y <u>V</u> A <u>G</u> E <u>D</u> G <u>K</u> T	329	360
TraB _F	PNS <u>K</u> TY <u>D</u> AT <u>G</u> CF <u>V</u> GLE <u>A</u> WG <u>D</u> V <u>S</u> S <u>E</u> RA <u>I</u> V <u>R</u> TR <u>N</u> I <u>S</u> CL-KDG <u>K</u> TID <u>M</u> P <u>I</u> --KG <u>H</u> V <u>S</u> FR-G <u>K</u> N	309	360
VirB10 _{Ti}	R <u>G</u> T <u>T</u> NN <u>I</u> V <u>L</u> LDRG <u>T</u> TV <u>V</u> GE <u>I</u> Q <u>R</u> GL <u>Q</u> Q <u>D</u> ER <u>V</u> F <u>V</u> L <u>W</u> D <u>R</u> A-ETPD <u>H</u> AMISLT <u>S</u> PS <u>A</u> DEL <u>G</u> RP	271	360
TraF _N	YSAD <u>G</u> LV <u>R</u> L <u>I</u> D <u>K</u> GS <u>W</u> VD <u>G</u> Q <u>I</u> T <u>GG</u> I <u>K</u> D <u>Q</u> AR <u>V</u> F <u>V</u> L <u>W</u> ER <u>I</u> R <u>N</u> D <u>Q</u> D <u>G</u> T <u>I</u> V <u>N</u> I <u>D</u> S <u>A</u> G <u>T</u> N <u>S</u> LG <u>S</u> A	293	360
 			*
TraB _{GGI}	* * α2		α3
TraB _F	GVRGNLVT <u>K</u> Q <u>G</u> QL <u>LA</u> N <u>AL</u> MSG <u>V</u> IS <u>GM</u> KG <u>V</u> SEA <u>F</u> K <u>V</u> N <u>N</u> T <u>AF</u> G <u>S</u> TT <u>S</u> I <u>R</u> G <u>S</u> D <u>Q</u> Y <u>R</u> AGIAS	389	420
VirB10 _{Ti}	GI <u>K</u> GE <u>V</u> VM <u>R</u> NG <u>K</u> IL <u>GW</u> A <u>W</u> G <u>A</u> G <u>F</u> V <u>D</u> G <u>I</u> Q <u>G</u> ME <u>R</u> AS <u>Q</u> PA--V <u>GL</u> G <u>A</u> T <u>A</u> Y <u>G</u> AG <u>D</u> V <u>L</u> K <u>M</u> G <u>I</u> GG	367	420
TraF _N	GLPGSV <u>D</u> SH <u>W</u> Q <u>R</u> F <u>S</u> G <u>A</u> M <u>L</u> SA <u>V</u> Q <u>G</u> A <u>F</u> Q <u>A</u> AS--TY <u>AG</u> S <u>SS</u> GGG--MS <u>F</u> N <u>S</u> F <u>Q</u> NN <u>G</u> E <u>Q</u> TT <u>E</u>	326	420
 			*
TraB _{GGI}	GIP <u>G</u> Q <u>V</u> DA <u>H</u> W <u>E</u> R <u>L</u> AG <u>A</u> IM <u>I</u> SL <u>F</u> S <u>D</u> TL <u>T</u> AL <u>V</u> --N <u>Q</u> T <u>Q</u> S <u>N</u> --I <u>Q</u> Y <u>N</u> ST <u>E</u> N <u>S</u> GG <u>Q</u> LAS	346	420
 			α3
TraB _{GGI}	G <u>I</u> GG <u>A</u> AD <u>R</u> L <u>A</u> E <u>Y</u> I <u>K</u> L <u>A</u> D <u>K</u> V <u>F</u> P <u>V</u> V <u>E</u> N <u>A</u> G <u>R</u> Q <u>V</u> D <u>V</u> V <u>L</u> T <u>Q</u> G <u>I</u> E <u>I</u> D <u>T</u> G <u>E</u> K-----	437	480
TraB _F	G <u>A</u> SK <u>A</u> Q <u>T</u> L <u>D</u> Y <u>I</u> K <u>R</u> A <u>E</u> Q <u>Y</u> H <u>P</u> V <u>I</u> P <u>I</u> G <u>A</u> G <u>N</u> E <u>V</u> V <u>F</u> Q <u>D</u> G <u>F</u> Q <u>L</u> K <u>T</u> V <u>E</u> M <u>A</u> L <u>E</u> R <u>T</u> Q <u>S</u> R <u>A</u> E <u>E</u> D	427	480
VirB10 _{Ti}	TALKAT <u>I</u> IP <u>T</u> L <u>K</u> N <u>Q</u> GT <u>D</u> TV <u>S</u> I <u>F</u> V <u>A</u> R <u>D</u> L <u>D</u> FF <u>G</u> V <u>Y</u> Q <u>L</u> R <u>L</u> T <u>G</u> G <u>A</u> R <u>G</u> R <u>N</u> R <u>R</u> S-----	377	480
TraF _N	E <u>A</u> LR <u>S</u> Y <u>M</u> S <u>I</u> P <u>P</u> T <u>L</u> D <u>Q</u> Q <u>G</u> DA <u>S</u> I <u>F</u> V <u>A</u> R <u>D</u> L <u>D</u> F <u>G</u> V <u>Y</u> T <u>L</u> A <u>D</u> N-----	386	480
TraB _{GGI}	-----	437	528
TraB _F	NPESPVPVPPSAESH <u>L</u> NG <u>F</u> NTD <u>Q</u> ML <u>K</u> QL <u>G</u> N <u>L</u> NP <u>Q</u> Q <u>F</u> MSG <u>S</u> Q <u>GG</u> ND <u>G</u> K	475	528
VirB10 _{Ti}	-----	377	528
TraF _N	-----	386	528

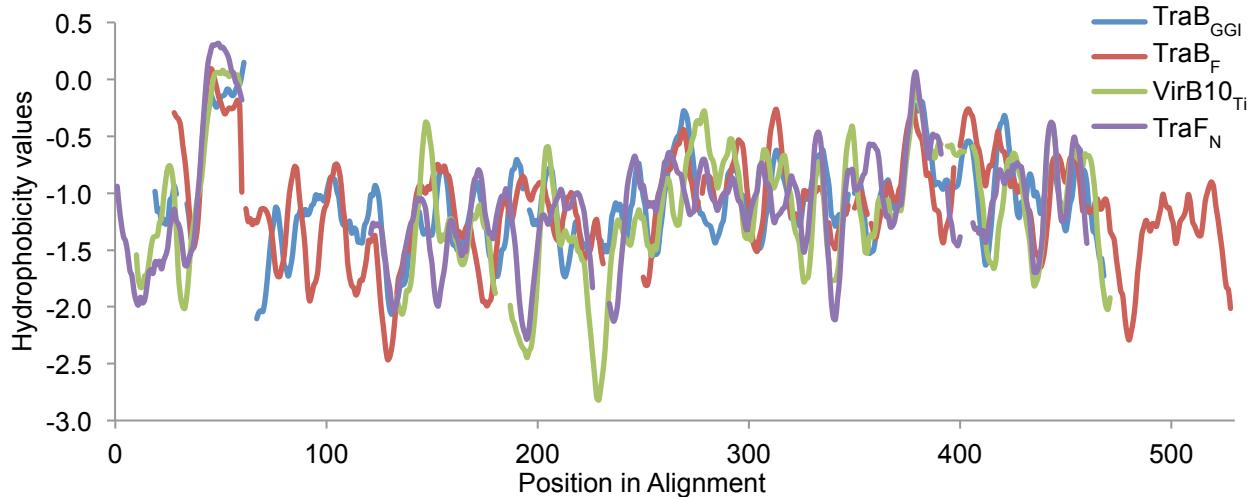
B

Figure S7. (A) Alignment of gonococcal TraB (TraB_{GGI}, NCBI Accession No. AAW83066) with homologs from F plasmid (TraB_F, NCBI Accession No. BAA97948), pKM101 (TraF_N, NCBI Accession No. AAA86459), and *A. tumefaciens* (VirB10 encoded by pTi15955, NCBI Accession No. CAA29981) using AlignMe (Alignment of Membrane Proteins) (2, 3). Identical residues are highlighted in gray. The alpha helix predicted to span the inner membrane ($\alpha 1$) is labeled and underlined (predicted using the TMHMM Server v. 2.0) (6). The highly conserved GxxGxxG motif involved in channel gating in *A. tumefaciens* is indicated with asterisks (7). The alpha helices that form the outer membrane pore ($\alpha 2$ and $\alpha 3$) in the TraF_N crystal structure are indicated by boxes (8). The predicted alpha helical regions in VirB10_{Ti}, TraB_{GGI}, and TraB_F are underlined (Phyre2) (9). The alignment was generated by manually diving the amino acid sequences into two regions (after the $\alpha 1$ helix) and aligning the two regions separately. **(B)** Hydrophobicity plot of the alignment generated using AlignMe (2, 3).

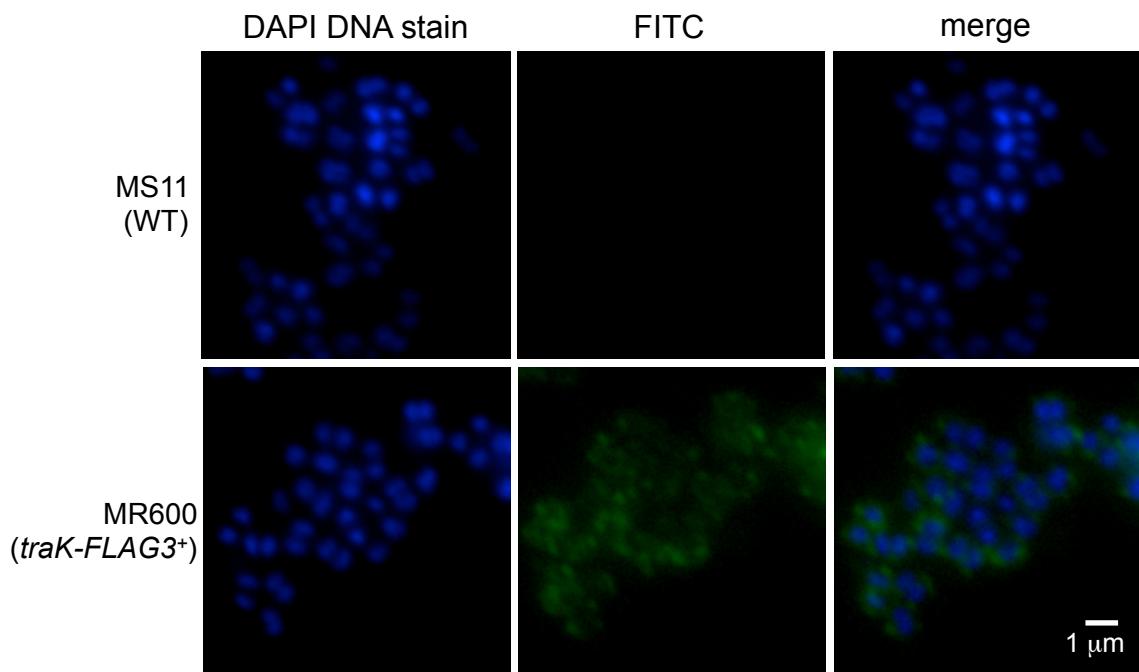


Figure S8. Immunofluorescence microscopy for TraK-FLAG3 in MS11 (WT) and MR600 (*traK-FLAG3*⁺). The DAPI DNA stain was used to counterstain the cells. A FITC-conjugated secondary antibody was used to detect TraK-FLAG3.

References

1. **Woodhams KL, Benet ZL, Blonsky SE, Hackett KT, Dillard JP.** 2012. Prevalence and detailed mapping of the gonococcal genetic island in *Neisseria meningitidis*. *J. Bacteriol.* **194**:2275-2285.
2. **Stamm M, Staritzbichler R, Khafizov K, Forrest LR.** 2013. Alignment of helical membrane protein sequences using AlignMe. *PLoS One* **8**:e57731.
3. **Khafizov K, Staritzbichler R, Stamm M, Forrest LR.** 2010. A study of the evolution of inverted-topology repeats from LeuT-fold transporters using AlignMe. *Biochemistry* **49**:10702-10713.
4. **Petersen TN, Brunak S, von Heijne G, Nielsen H.** 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat. Meth.* **8**:785-786.
5. **Spudich GM, Fernandez D, Zhou XR, Christie PJ.** 1996. Intermolecular disulfide bonds stabilize VirB7 homodimers and VirB7/VirB9 heterodimers during biogenesis of the *Agrobacterium tumefaciens* T-complex transport apparatus. *Proc. Natl. Acad. Sci. U. S. A.* **93**:7512-7517.
6. **Jakubowski SJ, Kerr JE, Garza I, Krishnamoorthy V, Bayliss R, Waksman G, Christie PJ.** 2009. *Agrobacterium* VirB10 domain requirements for type IV secretion and T pilus biogenesis. *Mol. Microbiol.* **71**:779-794.
7. **Banta LM, Kerr JE, Cascales E, Giuliano ME, Bailey ME, McKay C, Chandran V, Waksman G, Christie PJ.** 2011. An *Agrobacterium* VirB10 mutation conferring a type IV secretion system gating defect. *J. Bacteriol.* **193**:2566-2574.
8. **Chandran V, Fronzes R, Duquerroy S, Cronin N, Navaza J, Waksman G.** 2009. Structure of the outer membrane complex of a type IV secretion system. *Nature* **462**:1011-1015.

9. **Kelley LA, Sternberg MJ.** 2009. Protein structure prediction on the Web: a case study using the Phyre server. *Nat. Protoc.* **4**:363-371.