Supplemental Data

Fig S1. Acetylation patterns of eight gonococcal strains. Ngo cells were grown in supplemented GCB to OD_{600} = 0.4 and lysed in SDS-PAGE buffer. Whole cell lysates of strains 1291, PID2, FA1090, MS11, 4505, 8551, WS-1, and 1342 (lanes 1-8, respectively) were separated on an acrylamide gel and immunoblotted with an antibody specific to acetylated lysine (mAb Ac-K-103).

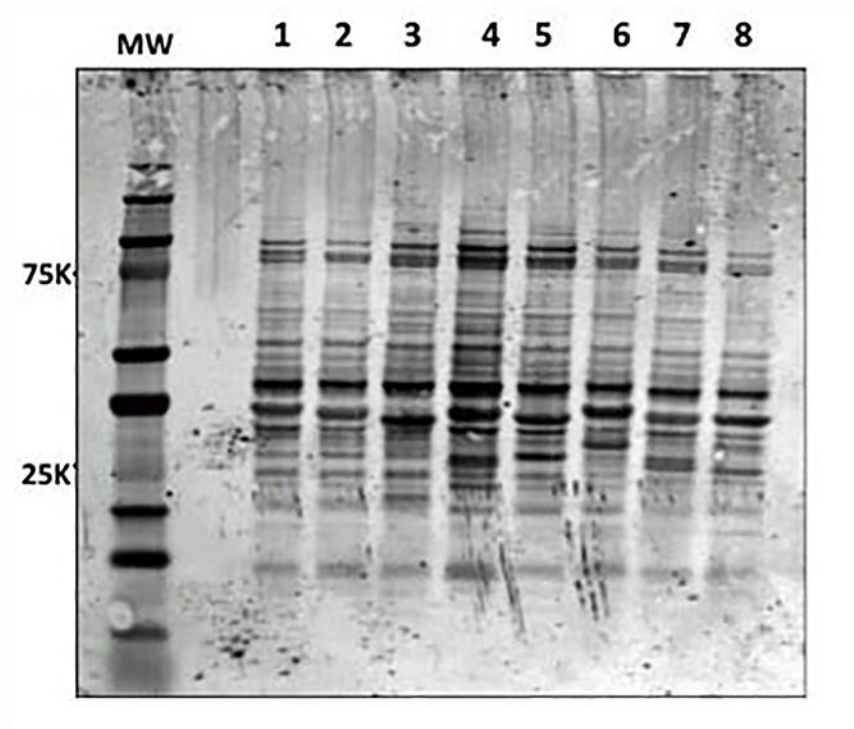

Fig S2. Translated *pilT* and *pilU* suppressor mutations in *pilT*$_{K117Q}$ transformants. (A) Alignment of wt PilT and PilT$_{\Delta 108\text{-}111,K117Q}$ (mutated_T) sequences; K117, red; Walker box A motif, magenta; Walker box B motif, cyan; other acetylatable lysines, yellow. (B) Predicted structure of PilT$_{\Delta 108\text{-}111,K117Q;}$ Q117, red; Walker box A motif, magenta; Walker box B motif, cyan. Arrowhead indicates the position of Q117. (C) Alignment of wt PilU and PilU (truncated_U) in one *pilT*$_{K117Q}$ transformant; Walker box A motif, magenta; Walker box B motif, cyan; acetylatable lysines, yellow.

A

```
mutated_T       MQITDLLAFGAKNKASDLHLSSGISPMIRVHGDMRRINLPEMSAEEVGNMVTSVMNDHQR 60
PilT_Ngo        MQITDLLAFGAKNKASDLHLSSGISPMIRVHGDMRRINLPEMSAEEVGNMVTSVMNDHQR 60
                ************************************************************

mutated_T       KIYQQNLEVDFSFELPNVARFRVNAFNTGRGPAAVFRTIPSTVLSLE----PSIFQQIAE 116
PilT_Ngo        KIYQQNLEVDFSFELPNVARFRVNAFNTGRGPAAVFRTIPSTVLSLEELKAPSIFQKIAE 120
                ************************************************    *****:***

mutated_T       SPRGMVLVTGPTGSGKSTTLAAMINYINETQPAHILTIEDPIEFVHQSKKSLINQRELHQ 176
PilT_Ngo        SPRGMVLVTGPTGSGKSTTLAAMINYINETQPAHILTIEDPIEFVHQSKKSLINQRELHQ 180
                ************************************************************

mutated_T       HTLSFANALSSALREDPDVILVGEMRDPETIGLALTAAETGHLVFGTLHTTGAAKTVDRI 236
PilT_Ngo        HTLSFANALSSALREDPDVILVGEMRDPETIGLALTAAETGHLVFGTLHTTGAAKTVDRI 240
                ************************************************************

mutated_T       VDVFPAGEKEMVRSMLSESLTAVISQNLLKTHDGNGRVASHEILIANPAVRNLIRENKIT 296
PilT_Ngo        VDVFPAGEKEMVRSMLSESLTAVISQNLLKTHDGNGRVASHEILIANPAVRNLIRENKIT 300
                ************************************************************

mutated_T       QINSVLQTGQASGMQTMDQSLQSLVRQGLIAPEAARRRAQNSESMSF 343
PilT_Ngo        QINSVLQTGQASGMQTMDQSLQSLVRQGLIAPEAARRRAQNSESMSF 347
                ***********************************************
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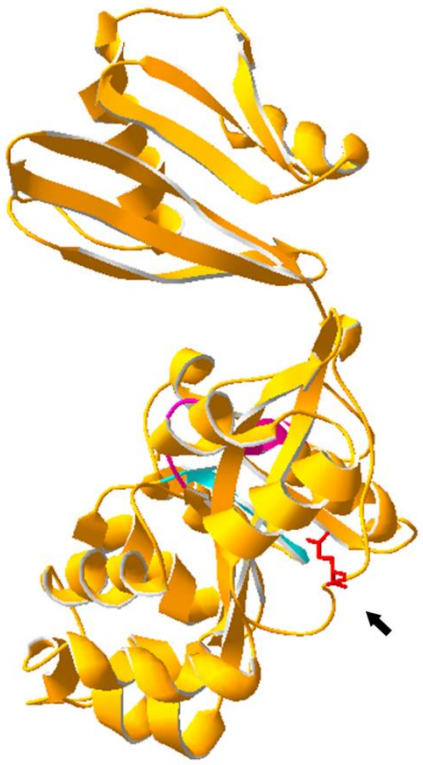
B



C

```
truncated_U     MNTDNLHDILDETVQVYSQKKQSRSETPAEIGTHFHPLLDRLCETAEAQNASDILISKGF 60
PilU_Ngo        MNTDNLHDILDETVQVYSQKKQSRSETPAEIGTHFHPLLDRLCETAEAQNASDILISKGF 60
                ************************************************************

truncated_U     PPSLKINSALTPQPQKALTGEETAAIAASTMNAEQSEIFRRDGEINYSVQSRSGTRYRAN 120
PilU_Ngo        PPSLKINSALTPQPQKALTGEETAAIAASTMNAEQSEIFRRDGEINYSVQSRSGTRYRAN 120
                ************************************************************

truncated_U     AYHSQGSAGLVLRRINHVIPQMRELGLPEKLKDLAVAPRGLLIIVGPTGSGKSTTMATML 180
PilU_Ngo        AYHSQGSAGLVLRRINHVIPQMRELGLPEKLKDLAVAPRGLLIIVGPTGSGKSTTMATML 180
                ************************************************************

truncated_U     EHRNKTLAR---------PYRYHRR---------------------------PD------ 198
PilU_Ngo        EHRNKTLPGHIVTIEDPIEFIYKPRRCIFTQREIGVDTINWQTAVQNAMRQSPDVVCIGE 240
                *******.           : *: *                            **

truncated_U     ------------------------------------------------------------
PilU_Ngo        VRSRESMEYAMQLAQTGHLCIFTLHANTAPQSLERILNFYPKEQHNQILIDIALNLTGII 300

truncated_U     ------------------------------------------------------------
PilU_Ngo        CQRLALKKDKTGRTAVVDLLINTPAIQDFILKGDLMNISKIMETAKTDGMQTMDQNLFEL 360

truncated_U     ------------------------------------------------
PilU_Ngo        YRHGIISYEEALRQSVSANNLRLHIQLHKEGKTPELLYDRVNGLNLIS 408
```

Fig S3. PilE levels in wt, Δ*pilT*, and i*pilE*. Cells were grown in the absence of IPTG and whole cell lysates were separated by 15% SDS-PAGE and immunoblotted with a monoclonal antibody to PilE (SM1).

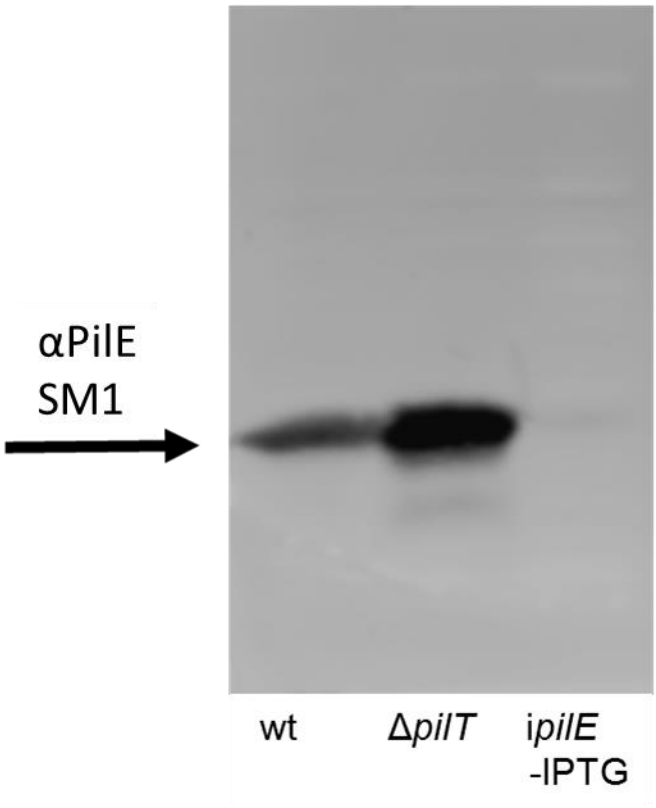


Fig S4. Translated *pilU* suppressor mutations in a piliated i*pilE pilT*$_{K117Q}$ clone. Walker box A motif, magenta; Walker box B motif, cyan; missense mutation, green; acetylatable lysines, yellow.

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PilU_Ngo        MNTDNLHDILDETVQVYSQKKQSRSETPAEIGAHFHPLLDRLCETAEAQNASDILISKGF
PilU_trunc      MNTDNLHDILDETVQVYSQKKQSRSETPAEIGAHFHPLLDRLCETAEAQNASDILISKGF
                ************************************************************

PilU_Ngo        PPSLKINSALTPQPQKALTGEETAAIAASTMNAEQSEIFRRDGEINYSVQSRSGTRYRAN
PilU_trunc      PPSLKINSALTPQPQKALTGEETAAIAASTMNAEQSEIFRRDGEINYSVQSRSGTRYRAN
                ************************************************************

PilU_Ngo        AYYSQGSAGLVLRRINHVIPQMRELGLPEKLKDLAVAPRGLLIIVGPTGSGKSTTMATML
PilU_trunc      AYHSQGSAGLVLRRINHVIPQMRELGLPEKLKDLAVAPRGLLIIVGPTGSGKSTTMATML
                **:********************************************************

PilU_Ngo        EHRNKTLPGHIVTIEDPIEFIYKPRRCIFTQREIGVDTINWQTAVQNAMRQSPDVVCIGE
PilU_trunc      EHRNKTLPGHIVTIEDPIEFIYKPRRCIFTQREIGVDTINWQTAVQNAMRQSPDVVCIGE
                ************************************************************

PilU_Ngo        VRSRESMEYAMQLA-QTGHLCIFTLHANTAPQ--SLERIL-NFYPKEQHNQILIDIALNL
PilU_trunc      VRSRESMEYAMQLRPKPGHICMFLRSPANTGAAVASERILQLSYP---------------
                ********** *  : ** * *     .:    : ****   **

PilU_Ngo        TGIICQRLALKKDKTGRTAVVDLLINTPAIQDFILKGDLMNISKIMETAKTDGMQTMDQN
PilU_trunc      ------------------------------------------------------------


PilU_Ngo        LFELYRHGIISYEEALRQSVSANNLRLHIQLHKEGKTPELLYDRVNGLNLIS
PilU_trunc      -----------------------------------------------------
```

Fig S5. Detection of H.8 and GAPDH in cytosolic/periplasmic and inner/outer membrane fractions isolated from Ngo wt, Δ*pilT*, Δ*pilE*, i*pilE* (-IPTG), i*pilE* *pilT*$_{K117Q}$, and i*pilE* *pilT*$_{K117R}$. Fractions were separated by 15% SDS-PAGE and immunoblotted with a monoclonal antibody to H.8 and a polyclonal antibody to GAPDH to assess fraction purity.

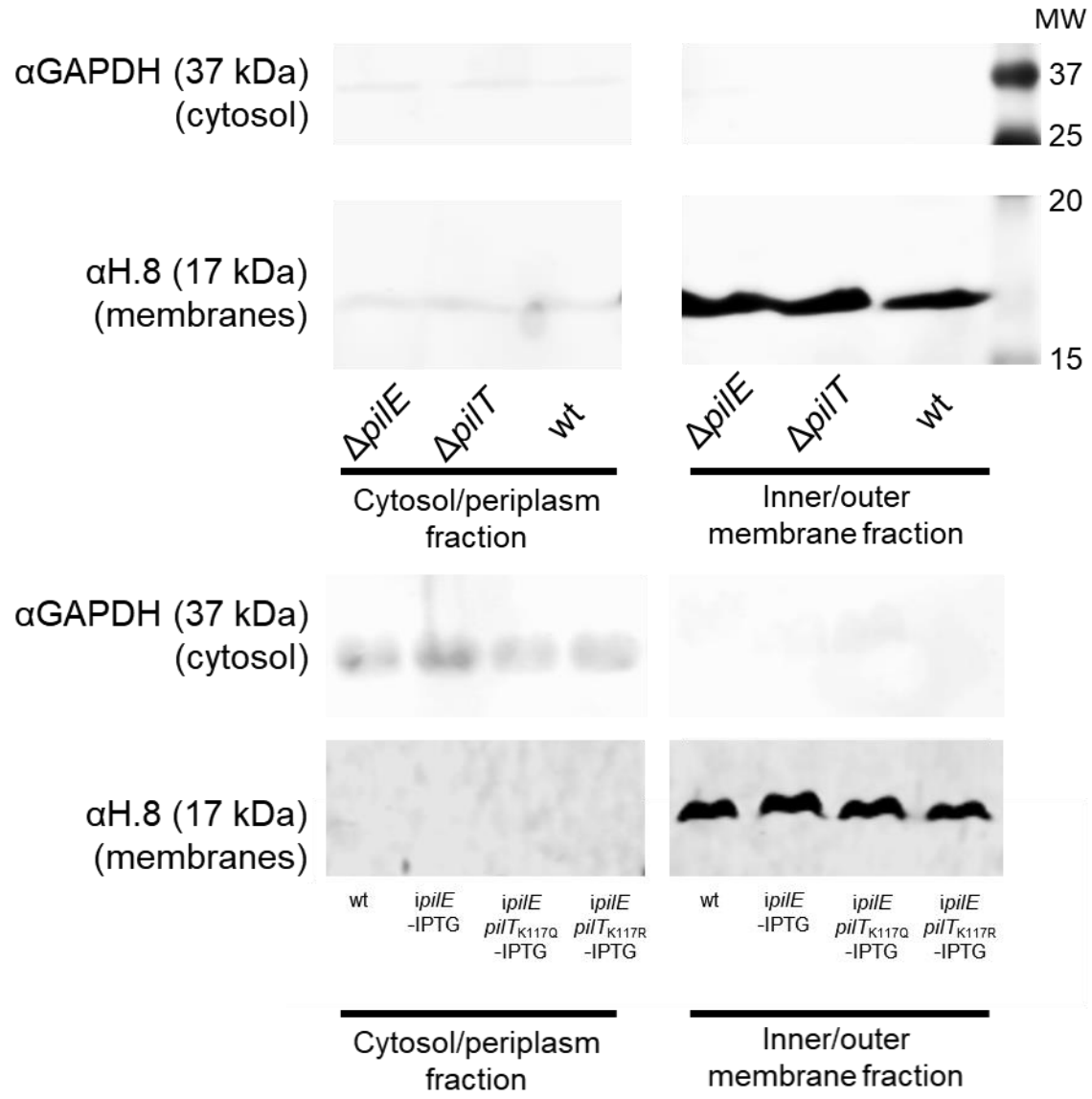
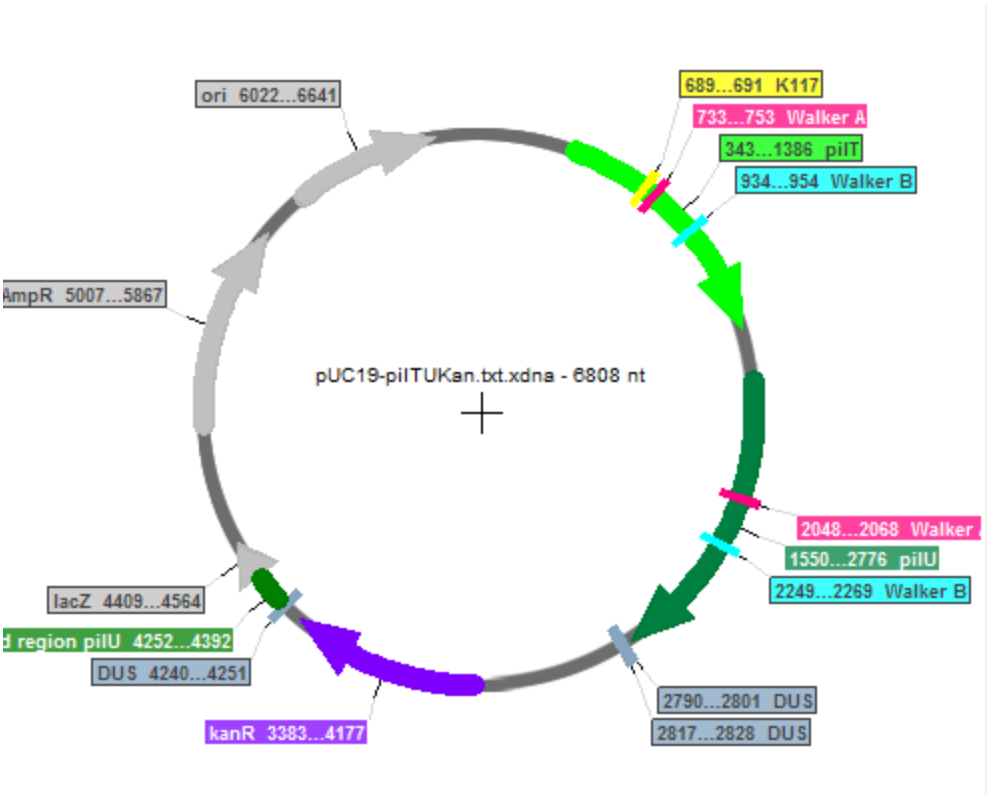


Fig S6. Plasmid map of *pilT* mutagenesis construct



Strain	Transformation frequency*
wt	6.89 x 10^{-4} (± 2.63)
Δ*pilT*	< 6.16 x 10^{-6} **
i*pilE* + IPTG	2.01 x 10^{-4} (± 0.62)
i*pilE* K117Q + IPTG	4.35 x 10^{-4} (± 0.96)
i*pilE* K117R + IPTG	6.81 x 10^{-4} (± 0.96)

Table S1. Transformation frequency of i*pilE pilT*$_{K117Q}$ and *ipilE pilT*$_{K117R}$ strains.
*Transformation frequency = Rif^R cfu/total input cfu/mg Rif^R genomic DNA. Values are the average of 3 independent experiments ± SEM; Student's two-tailed t-test. **Limit of detection.

Table S2. Primers used in this study

Primer pair	Use	Sequence
AH64F	Amplification of Ngo *pilT* for cloning into pET28a	agtcaggctagcATGCAGATTACCGACTTACTCGC
AH64R		ctgactggatccTTCCTGTTCGGAAGGGTATG
K117QF	*pilT* mutagenesis	AGAATTGAAAGCCCCGAGCATTTTCCAA**CA** **A**ATCGCAGAATCGCCGCGCGGCATGGT
K117QR		ACCATGCCGCGCGGCGATTCTGCGAT**TTG**TT GGAAAATGCTCGGGGCTTTCAATTCT
K117RF	*pilT* mutagenesis	AGAATTGAAAGCCCCGAGCATTTTCCAA**CG** **T**ATCGCAGAATCGCCGCGCGGCATGGT
K117RR		ACCATGCCGCGCGGCGATTCTGCGAT**ACG**TT GGAAAATGCTCGGGGCTTTCAATTCT
AH 106F	Amplification of *pilTU*	TCAGGGCGGTATAATCAAGG
AH 106R		tgatga**ggatcc**GGAAGCGAGGTAATGAGCAG
AH107F	Amplification of *kan*	tgatga**ggatcc**GAGTCAGTGAGCGAGGAAGC
AH107R		ttcagacggcatGAAATCTCGTGATGGCAGGT
AH108	3' *pilU* UTR sequence used to promote recombination of the construct with the endogenous *pilTU* locus	AGGCCGCCGGCGATGATGCCGAGTACGAAG GGCATGGCGAGCTTGGGTTCGCCTAGCTGCC AGACGATGGAGGCGGCGGTAAAGACACTGG CGAAAACGGttcagacggcatGAAATCTCGTGATG GCAGGT

Table S3. Plasmids used in this study

Plasmid	Use
pET28a-*pilT*	Vector used to overexpress His_6-PilT
pUC19-*pilT*$_{K117Q}$,*pilU-kan*	Vector used to transform K117Q into Ngo
pUC19-*pilT*$_{K117R}$,*pilU*-kan	Vector used to transform K117R into Ngo