**Table S1. Designations of Inc proteins mentioned in this study.** Listed are CTL and CT locus tag designations (referring to genes in *C. trachomatis* L2/434/Bu and *C. trachomatis* D/UW-3/Cx, respectively) of all Incs mentioned. For CpoS, designations of additional orthologs mentioned in the study are listed as well.

| Inc protein | *Chlamydia* strain | Locus tag | Other designation used in this study for simplicity |
|---|---|---|---|
| CpoS | *C. trachomatis* L2/434/Bu | CTL0481 | CpoS(L2) |
| | *C. trachomatis* D/UW-3/Cx | CT229 | CpoS(D) |
| | *C. trachomatis* A/HAR-13 | CTA0251 | CpoS(A) |
| | *C. trachomatis* E/Bour | BOUR00241 | CpoS(E) |
| | *C. muridarum* Nigg | TC0500 | CpoS(Cm) |
| | *C. suis* MD56 | Q499_0250 | CpoS(Cs) |
| IPAM | *C. trachomatis* L2/434/Bu | CTL0476 | IPAM(L2) |
| | *C. trachomatis* D/UW-3/Cx | CT223 | IPAM(D) |
| IncA | *C. trachomatis* L2/434/Bu | CTL0374 | IncA(L2) |
| | *C. trachomatis* D/UW-3/Cx | CT119 | IncA(D) – *term not used explicitly in this study* |
| IncB | *C. trachomatis* L2/434/Bu | CTL0484 | IncB(L2) – *term not used explicitly in this study* |
| | *C. trachomatis* D/UW-3/Cx | CT232 | IncB(D) – *term not used explicitly in this study* |
| IncD | *C. trachomatis* L2/434/Bu | CTL0370 | IncD(L2) |
| | *C. trachomatis* D/UW-3/Cx | CT115 | IncD(D) |
| unnamed | *C. trachomatis* L2/434/Bu | CTL0475 | |
| | *C. trachomatis* D/UW-3/Cx | CT222 | |

**Table S2. *Chlamydia* strains used in this study.**

| Strain name | Short name | Description | CpoS variant expressed |
|---|---|---|---|
| CTL2 | CTL2 | *C. trachomatis* strain L2/434/Bu | Endogenous CpoS(L2) |
| CTL2-*cpoS*::*cat* | CTL2-*cpoS*::*cat* | Derived from CTL2, *cat* insertion in *cpoS* | CpoS(L2) truncated after aa 92 |
| CTL2-*ipaM*::*bla* | CTL2-*ipaM*::*bla* | Derived from CTL2, *bla* insertion in *ipaM* | Endogenous CpoS(L2) |
| CTL2-*ipaM*::*bla*-*cpoS*::*cat* | CTL2-*ipaM*::*bla*-*cpoS*::*cat* | Derived from CTL2, *bla* insertion in *ipaM, cat* insertion in *cpoS* | CpoS(L2) truncated after aa 92 |
| CTL2-*cpoS*::*cat* / pCpoS(L2)-FLAG | pCpoS(L2) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain L2/434/Bu (CTL0481) | Plasmid-expressed CpoS(L2)-FLAG |
| CTL2-*cpoS*::*cat* / pCpoS(A)-FLAG | pCpoS(A) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain A/HAR-13 (CTA0251) | Plasmid-expressed CpoS(A)-FLAG |
| CTL2-*cpoS*::*cat* / pCpoS(D)-FLAG | pCpoS(D) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain D/UW-3/CX (CT229) | Plasmid-expressed CpoS(D)-FLAG |
| CTL2-*cpoS*::*cat* / pCpoS(E)-FLAG | pCpoS(E) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain E/Bour (BOUR00241) | Plasmid-expressed CpoS(E)-FLAG |
| CTL2-*cpoS*::*cat* / pCpoS(Cm)-FLAG | pCpoS(Cm) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. muridarum* strain Nigg (TC0500) | Plasmid-expressed CpoS(Cm)-FLAG |
| CTL2-*cpoS*::*cat* / pCpoS(L2Δ60)-FLAG | pCpoS(L2Δ60) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain L2/434/Bu (CTL0481) with a 60 aa C-terminal deletion | Plasmid-expressed CpoS(L2Δ60)-FLAG |
| CTL2-*cpoS*::*cat* / pCpoS(L2-CC1*)-FLAG | pCpoS(L2-CC1*) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain L2/434/Bu (CTL0481) with two point mutations in CC1 (I99P + L113P) | Plasmid-expressed CpoS(L2-CC1*)-FLAG (=CpoS(L2-I99P-L113P)-FLAG) |
| CTL2-*cpoS*::*cat* / pCpoS(L2-CC2*)-FLAG | pCpoS(L2-CC2*) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain L2/434/Bu (CTL0481) with two point mutations in CC2 (N181P + L188P) | Plasmid-expressed CpoS(L2-CC2*)-FLAG (=CpoS(L2-N181P-L188P)-FLAG) |
| CTL2-*cpoS*::*cat* / pCpoS(L2-CC1/2*)-FLAG | pCpoS(L2-CC1/2*) | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of FLAG-tagged CpoS from *C. trachomatis* strain L2/434/Bu (CTL0481) with two point mutations in CC1 (I99P + L113P) and two point mutations in CC2 (N181P + L188P) | Plasmid-expressed CpoS(L2-CC1/2*)-FLAG (=CpoS(L2-I99P-L113P-N181P-L188P)-FLAG) |

**Table S2 (continued). *Chlamydia* strains used in this study.**

| Strain name | Short name | Description | CpoS variant expressed |
|---|---|---|---|
| CTL2 / pIPAM(L2)-MYC | pIPAM(L2)-MYC | Derived from CTL2, transformed with plasmid enabling expression of MYC-tagged IPAM from *C. trachomatis* strain L2/434/Bu (CTL0476) | Endogenous CpoS(L2) |
| CTL2 / pIncA(L2)-MYC | pIncA(L2)-MYC | Derived from CTL2, transformed with plasmid enabling expression of MYC-tagged IncA from *C. trachomatis* strain L2/434/Bu (CTL0374) | Endogenous CpoS(L2) |
| CTL2 / pIncD(L2)-MYC | pIncD(L2)-MYC | Derived from CTL2, transformed with plasmid enabling expression of MYC-tagged IncD from *C. trachomatis* strain L2/434/Bu (CTL0370) | Endogenous CpoS(L2) |
| CTL2 / p2TK2-SW2-mCherry | n/a | Derived from CTL2, transformed with plasmid enabling expression of mCherry | Endogenous CpoS(L2) |
| CTL2-*cpoS*::*cat* / p2TK2-SW2-mCherry | n/a | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of mCherry | CpoS(L2) truncated after aa 92 |
| CTL2 / pTL2-tetO-IncB-GFP11x7-FLAG | CTL2/pIncB-GFP11 | Derived from CTL2, transformed with plasmid enabling expression of IncB tagged with seven repeats of GFP11 and a FLAG tag | Endogenous CpoS(L2) |
| CTL2-*cpoS*::*cat* / pTL2-tetO-IncB-GFP11x7- FLAG | CTL2-*cpoS*::*cat*/pIncB-GFP11 | Derived from CTL2-*cpoS*::*cat*, transformed with plasmid enabling expression of IncB tagged with seven repeats of GFP11 and a FLAG tag | CpoS(L2) truncated after aa 92 |

**Table S3. Primers used in this study.**

| Purpose | Primer name | Primer sequence (5'→ 3') |
|---|---|---|
| Confirmation of cloned EGFP-Rab constructs | pEGFPC1for | GATCACTCTCGGCATGGAC |
| Retargeting of vector pDFTT3 for disruption of CTL0476 | IBS-CTL0476 | AAAAAAGCTTATAATTATCCTTACTTCTCTTCTTTGTGCGCCCAGATAGGGTG |
| | EBS1d-CTL0476 | CAGATTGTACAAATGTGGTGATAACAGATAAGTCTTCTTTTATAACTTACCTTTCTTTGT |
| | EBS2-CTL0476 | TGAACGCAAGTTTCTAATTTCGGTTAGAAGTCGATAGAGGAAAGTGTCT |
| Verification of intron insertion into CTL0476 | CTL0476_seqF | GACTTGCACACTTAGTATCC |
| | CTL0476_seqR | GGTGGTGCTGTTGTATTGG |
| Verification of intron insertion into CTL0481 | CTL0481_seqF | CTCTGCTGTTAATTGACTGC |
| | CTL0481_seqR | GCTACTCTTTTCGCAAAAGG |
| Amplification of CpoS orthologs and variants for insertion into p2TK2-SW2 | CTL0481_F [a] | GGTACCGGTCTGAGAGGGGGTATATCTTTTG |
| | CTL0481_R [b] | GCAGGTACCTTTTTTACGACGGGATGCCTG |
| | CTL0481Δ60_R [c] | GCAGGTACCTGTTGCGG CATGACGCTTAC |
| | CTA0251_R [d] | GCAGGTACCTTTTTTACGATGGGATGCCTG |
| | TC0500_F [e] | GGTACCGGTTAGAGAGTCTTTTGGGACTAAATG |
| | TC0500_R [f] | GCAGGTACCCTTCTTCTTAGCTTTAACACA |
| Amplification of IPAM-MYC for insertion into p2TK2-SW2 | CTL0476-MYC_F | CTCGTCCACGGTCACCGGTGTTCGATTTTAAGATTCTTAGTGCAATG |
| | CTL0476-MYC_R | GCAGCTAGCTTACAGATCCTCTTCTGAGATGAGTTTTTGTTCCACCCGAGAGCCGTAATTGAAATC |
| Amplification of IncA-MYC for insertion into p2TK2-SW2 | CTL0374-MYC_F | CTAGGTGCCACGGTACCGGTATCGTTAATCCAGTCAACAATATTAAT |
| | CTL0374-MYC_R | TTAGCTAGCTTACAGATCCTCTTCTGAGATGAGTTTTTGTTCGGAGCTTTTTGTAGAGGGTGATGC |
| Amplification of IncD-MYC for insertion into p2TK2-SW2 | CTL0370-MYC_F | CTAAGTTCCACGGTACCGGTGCTATCTCTTGTTGCTCCGAGTTAAAC |
| | CTL0370-MYC_R | TTAGCTAGCTTACAGATCCTCTTCTGAGATGAGTTTTTGTTCGCTCGCCCCTTTTTTACTCACCGA |
| Amplification of OCRL-HA for insertion into pcDNA3.1 | OCRL_F | ATAGGATCCATGGAGCCGCCGCTCCCGGT |
| | OCRL_R | ATAGCGGCCGCTTAAGCGTAATCTGGAACATCGTATGGGTAGTCTTCTTCGCTCCCAAGCA |
| Amplification of ACAP2-HA for insertion into pcDNA3.1 | ACAP2_F | GCCGCGGATCCATGAAGATGACTGTGGATTTCG |
| | ACAP2_R | ATAGCGGCCGCTTAAGCGTAATCTGGAACATCGTATGGGTAGAATTTCTGTGAATCTTGCT |

[a] Forward primer used for amplification of CTL0481 and CTL0481Δ60 (*C. trachomatis* L2/434/Bu), CTA0251 (*C. trachomatis* A/HAR-13), CT229 (*C. trachomatis* D/UW-3/CX), and BOUR00241 (*C. trachomatis* E/Bour). [b] Reverse primer used for amplification of CTL0481(*C. trachomatis* L2/434/Bu), CT229 (*C. trachomatis* D/UW-3/CX), and BOUR00241 (*C. trachomatis* E/Bour). [c] Reverse primer used for amplification of CTL0481Δ60 (*C. trachomatis* L2/434/Bu). [d] Reverse primer used for amplification of CTA0251 (*C. trachomatis* A/HAR-13). [e] Forward primer used for amplification of TC0500 (*C. muridarum* Nigg). [f] Reverse primer used for amplification of TC0500 (*C. muridarum* Nigg).

**Table S4. siRNAs used in this study.**

| Reagent | Vendor | Catalogue Number |
|---|---|---|
| siGENOME siRNA SMARTpool targeting human STING | Dharmacon | M-024333-00-0005 |
| siGENOME siRNA SMARTpool targeting human Rab1A | Dharmacon | M-008283-01-0005 |
| siGENOME siRNA SMARTpool targeting human Rab1B | Dharmacon | M-008958-01-0005 |
| siGENOME siRNA SMARTpool targeting human Rab2A | Dharmacon | M-010533-01-0005 |
| siGENOME siRNA SMARTpool targeting human Rab2B | Dharmacon | M-010370-00-0005 |
| siGENOME siRNA SMARTpool targeting human Rab4A | Dharmacon | M-008539-01-0005 |
| siGENOME siRNA SMARTpool targeting human Rab5A | Dharmacon | M-004009-00-0005 |
| siGENOME siRNA SMARTpool targeting human Rab8A | Dharmacon | M-003905-00-0005 |
| siGENOME siRNA SMARTpool targeting human Rab8B | Dharmacon | M-008744-01-0005 |
| siGENOME siRNA SMARTpool targeting human Rab35 | Dharmacon | M-009781-00-0005 |
| siGENOME siRNA SMARTpool targeting human OCRL | Dharmacon | M-010026-01-0005 |
| siGENOME siRNA SMARTpool targeting human ACAP2 | Dharmacon | M-012811-00-0005 |
| siGENOME siRNA SMARTpool targeting human MICAL1 | Dharmacon | M-010192-00-0005 |
| siGENOME siRNA SMARTpool targeting human MICALL1 | Dharmacon | M-015102-01-0005 |
| siGENOME siRNA SMARTpool targeting human FSCN1 | Dharmacon | M-019576-01-0005 |
| siGENOME siRNA SMARTpool targeting human RUSC2 | Dharmacon | M-026133-01-0005 |

**Table S5. Primary antibodies used in this study.**

| Reagent | Source | Dilution used in microscopy | Dilution used in western blot |
|---|---|---|---|
| mouse-anti-FLAG | Merck (F1804) | 1:250-1:500 | |
| mouse-anti-FLAG | Merck (F3165) | 1:250-1:500 | 1:1000 |
| rabbit-anti-FLAG | Merck (F7425) | | 1:1000-1:5000 |
| rabbit-anti-FLAG | Cell signaling (14793) | | 1:1000 |
| rabbit-anti-MYC | Cell Signaling (2278) | 1:500 | 1:1000 |
| rat-anti-HA | Merck (11867423001) | 1:500 | |
| rabbit-anti-GFP | Cell Signaling (2956) | | 1:1000 |
| mouse-anti-β-actin | Cell Signaling (3700) | | 1:2000-1:5000 |
| mouse-anti-β-actin (HRP-labeled) | Abcam (ab49900) | | 1:50,000 |
| mouse-anti-α-tubulin | Merck (T5168) | 1:250-1:1000 | |
| rabbit-anti-Src | Cell Signaling (2123) | | 1:1000 |
| rabbit-anti-phospho(Tyr416)-Src | Cell Signaling (6943) | | 1:1000 |
| mouse-anti-phospho(Tyr416)-Src | Merck (05-677) | 1:100 | |
| rabbit-anti-CERT | Proteintech (15191-1-AP) | 1:1000 | |
| rabbit-anti-STING | Cell Signaling (13647) | | 1:1000 |
| rabbit-anti-phospho(Ser366)-STING | Cell Signaling (19781) | | 1:1000 |
| rabbit-anti-IRF3 | Cell Signaling (11904) | | 1:1000 |
| rabbit-anti-phospho(Ser386)-IRF3 | Cell Signaling (37829) | | 1:1000 |
| rabbit-anti-Rab1A | Cell Signaling (13075) | | 1:1000 |
| rabbit-anti-Rab1B | Abcam (ab116336) | | 1:1000 |
| rabbit-anti-Rab2A | Proteintech (15420-1-AP) | | 1:1000 |
| rabbit-anti-Rab2B | Proteintech (11756-1-AP) | | 1:1000 |
| rabbit-anti-Rab4A | Proteintech (10347-1-AP) | | 1:1000 |
| rabbit-anti-Rab5A | Cell Signaling (46449) | | 1:1000 |
| rabbit-anti-Rab8A | Cell Signaling (6975) | | 1:1000 |
| rabbit-anti-Rab35 | Proteintech (11329-2-AP) | | 1:1000 |
| rabbit-anti-OCRL | Proteintech (17695-1-AP) | | 1:1000 |
| rabbit-anti-ACAP2 | Proteintech (14029-1-AP) | | 1:10000 |
| rabbit-anti-MICAL1 | Proteintech (14818-1-AP) | | 1:1000 |
| rabbit-anti-MICALL1 | Proteinatlas (HPA043480-25) | | 1:1000 |
| rabbit-anti-FSCN1 | Proteintech (14384-1-AP) | | 1:1000 |
| rabbit-anti-RUSC2 | ProSci (155-6659-0.02) | | 1:1000 |
| rabbit-anti-Slc1 | (1) | 1:400 | 1:1000 |
| rabbit-anti-CpoS | (2) | | 1:200 |

**Table S5 (continued). Primary antibodies used in this study.**

| Reagent | Source | Dilution used in microscopy | Dilution used in western blot |
|---|---|---|---|
| rabbit-anti-IncA | (3) | | 1:1000 |
| rabbit-anti-CT222/CTL0475 | gift from Ted Hackstadt (RML/NIAID, (4)) | 1:100 | 1:1000 |
| rabbit-anti-IPAM | gift from Daniel Rockey (Oregon State University, (5)) | 1:100-1:200 | |
| mouse-anti-IPAM | gift from Daniel Rockey (Oregon State University, (6)) | 1:1000 | 1:1000 |

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