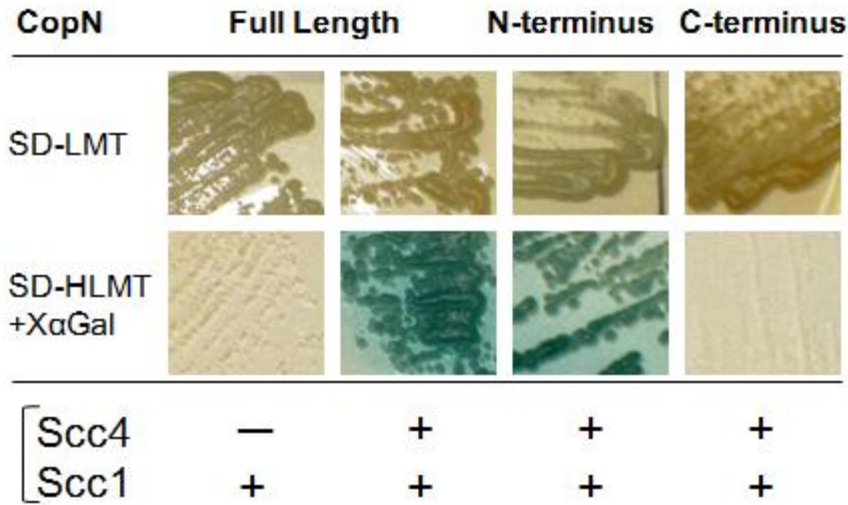


Figure S1: The N- (aa, 1-201) and C- (aa, 202-422) terminus as well as full length CopN from *C. trachomatis*, serovar D, were cloned into pGADT7 plasmid. The chlamydial T3S chaperones Scc4 and Scc1 were cloned into the pBRIDGE™ plasmid. Both plasmids were transferred into the yeast host and grown on synthetic amino acid dropout media (SD) lacking leucine, methionine and tryptophan (top row). Growth indicates the presence of both plasmids. The bottom row demonstrates the transformed *Saccharomyces cerevisiae*, Y2HGold , growing on SD media lacking leucine, methionine, tryptophan, histidine and supplemented with x-Alpha-Gal substrate . Blue colonies indicate an interaction between the protein produced from the pGADT7 plasmid and the proteins produced by the pBRIDGE™ plasmid (bottom row).

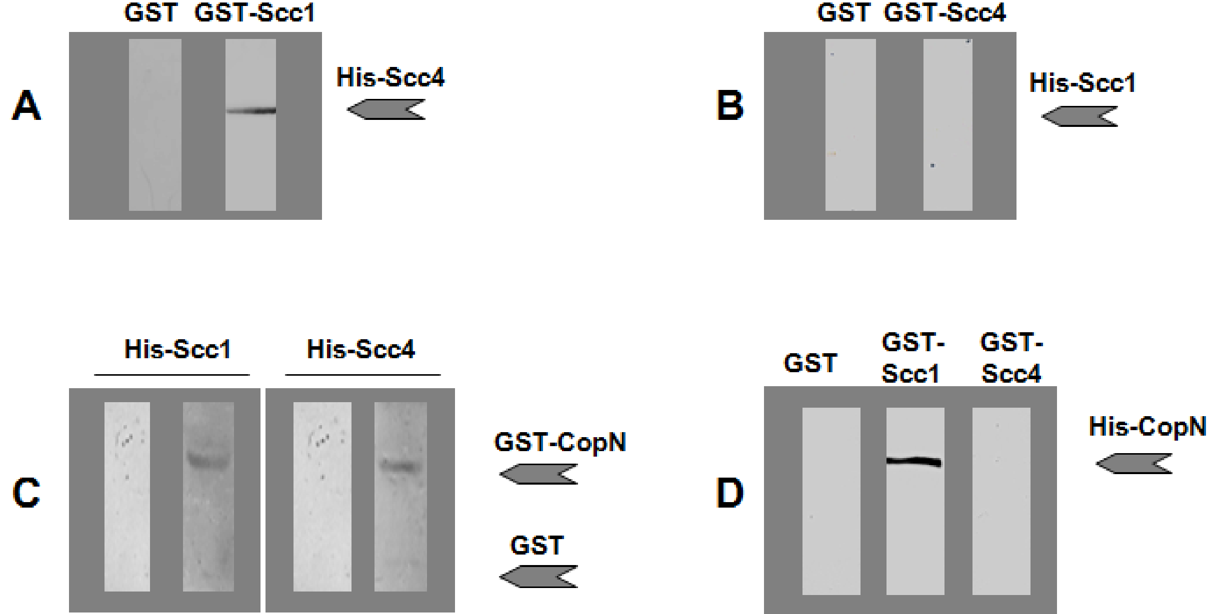


Figure S2: Pull-down assays showing the protein-protein interaction for GST- and 6x-His tagged recombinant Scc1, Scc4 and CopN. Glutathione agarose beads loaded with recombinant GST protein (Panels A, B and D) and recombinant GST protein alone (Panel C) served as controls for non-specific binding. Anti-6x-His-Scc4 (Panel A), anti-6x-His-Scc1 (Panel B), anti-GST- CopN (Panel C) and anti-6x-His-CopN (Panel D) mouse polyclonal sera were used to detect the target proteins.

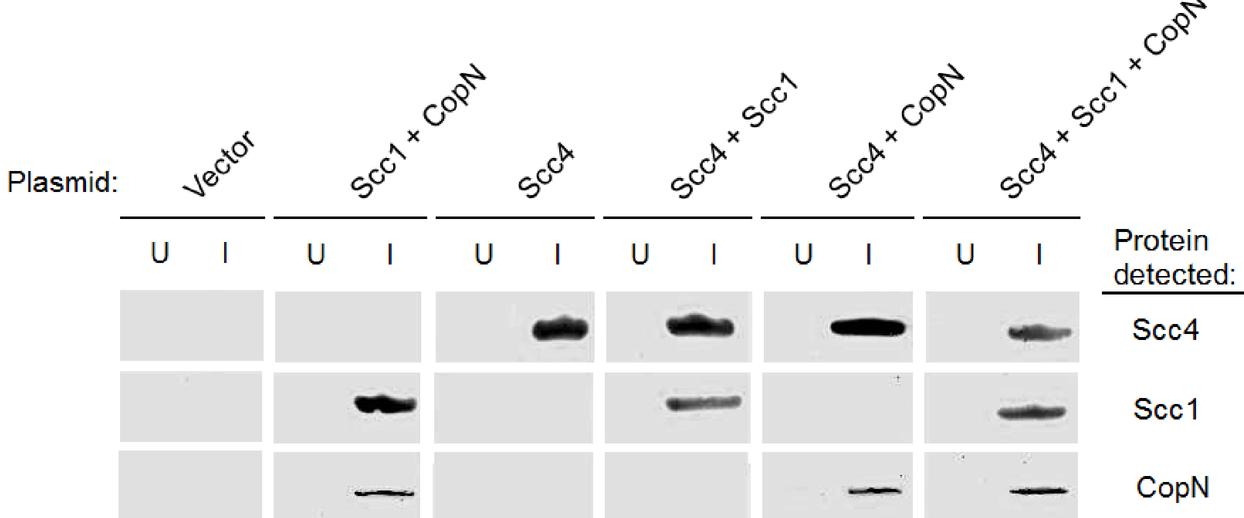
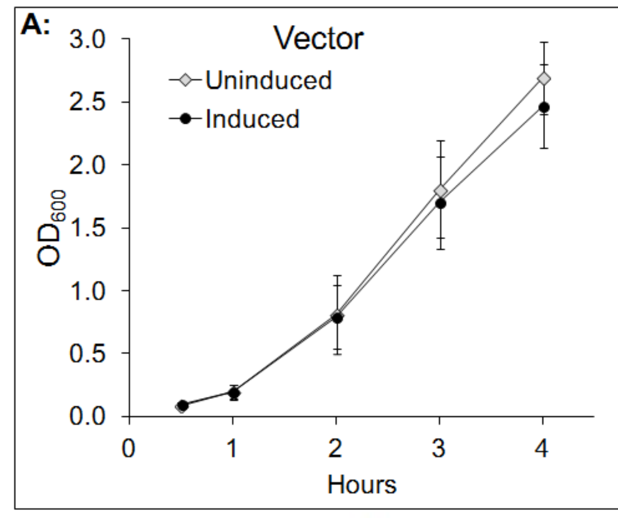
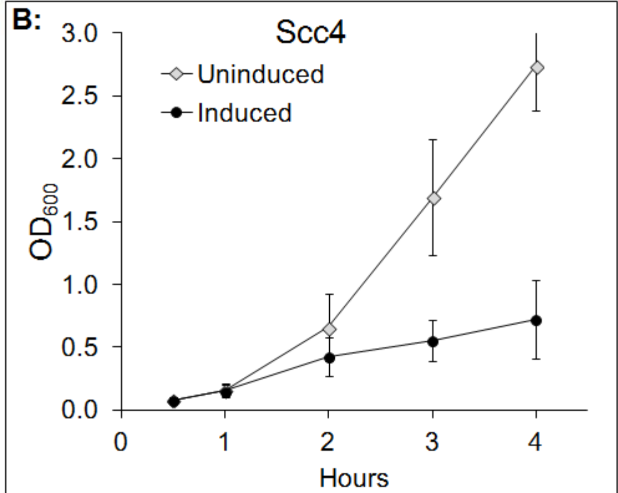
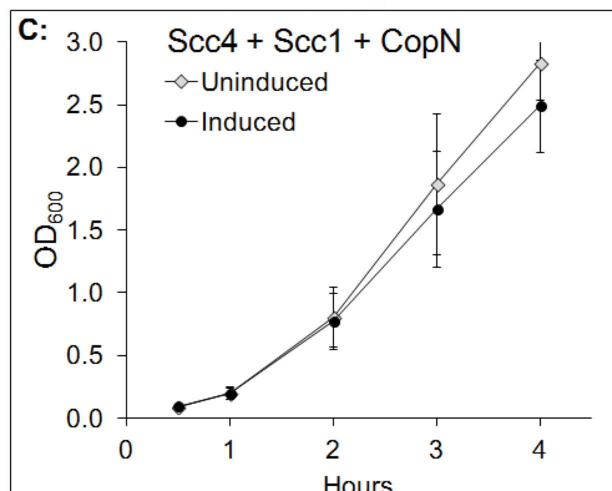
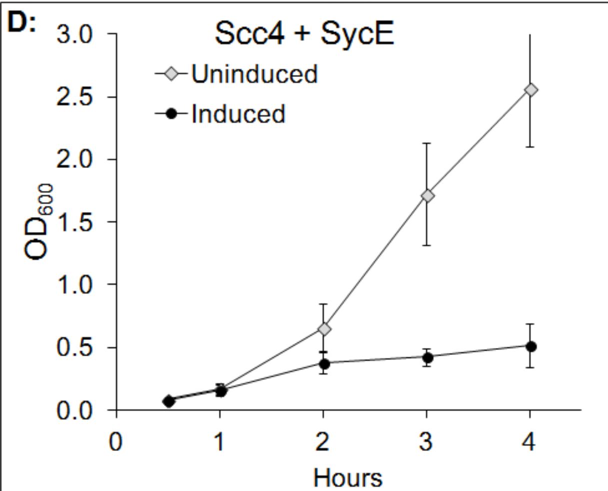


Figure S3: Western blots verifying expression in *E. coli* of Scc4, Scc1, and CopN after 4 h of induction, in various combinations, from an expression plasmid. The top row was probed with mouse polyclonal anti-His-Scc4, the middle row with mouse polyclonal anti-His-Scc1 and the bottom row with mouse polyclonal anti-His-CopN. (U) uninduced, (I) induced with 0.1 mM IPTG.


A:
Vector
Uninduced
Induced
OD600
Hours
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4


B:
Scc4
Uninduced
Induced
OD600
Hours


C:
Scc4 + Scc1 + CopN
Uninduced
Induced
OD600
Hours


D:
Scc4 + SycE
Uninduced
Induced
OD600
Hours

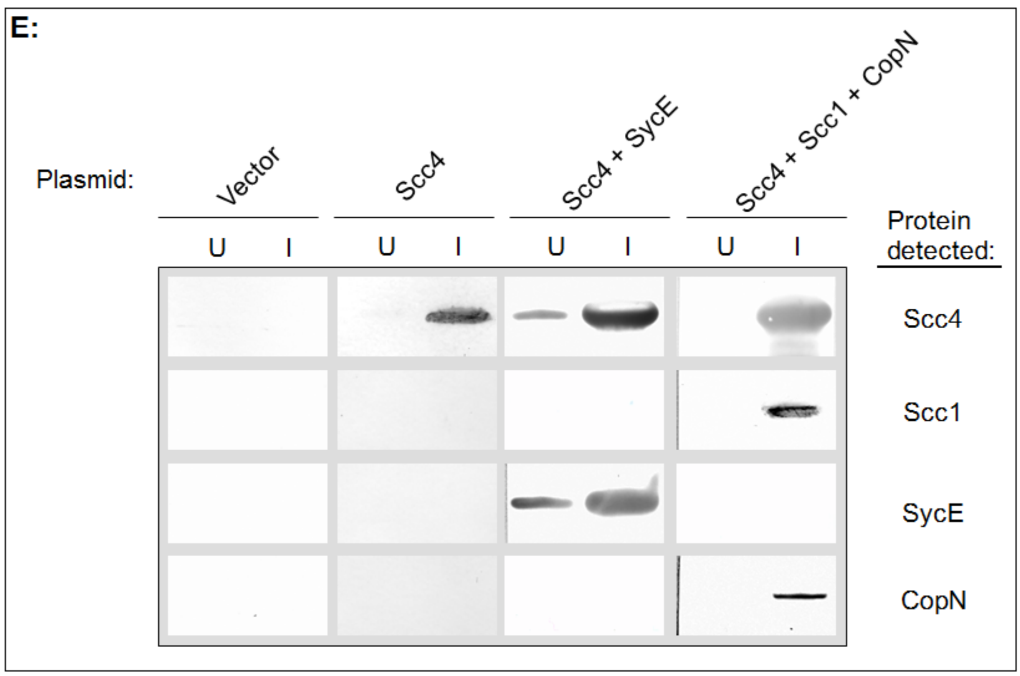

E:
Plasmid:
Vector
Scc4
Scc4 + SycE
Scc4 + Scc1 + CopN
U
I
Protein detected:
Scc4
Scc1
SycE
CopN

Figure S4: Control experiments examining whether the growth inhibition of *C. trachomatis* Scc4 could be reversed by co-expression with the non-interacting protein *Yersinia* SycE. Each graph shows the growth curve in the absence (Uninduced) or presence of IPTG (Induced), with the x-axis showing time after addition of IPTG to the induced sample. (A) Empty vector plasmid, (B) Scc4 alone, showing growth defect, (C) Scc4, Scc1, and CopN, showing antagonism of Scc4 growth defect, and (D) Scc4 and *Yersinia* SycE. (E) Western blots verifying expression of Scc4, Scc1, CopN, and *Yersinia* SycE from *E. coli* growth curves after 4 h of induction. The top row was probed with mouse polyclonal anti-His-Scc4, the second row with mouse polyclonal anti-His-Scc1, the third row with rabbit polyclonal anti-SycE, and the bottom row with mouse polyclonal anti-His-CopN. (U) uninduced, (I) induced with 0.1 mM IPTG.

Supplemental Table 1: Cloning vectors and recombinant plasmids used in this study.

Plasmid	Properties	Source
pET45b+	Ap^r; expression vector for creation of N-terminal His_6-containing recombinant proteins	Novagen
pET45b+ CT088	Ap^r; PCR-amplified *C. trachomatis scc1* (CT088; 441 nt) fused in frame with an N-terminal His_6-tag	This study
pET45b+ CT089	Ap^r; PCR-amplified *C. trachomatis copN* (CopN; 1,263 nt) fused in frame with an N-terminal His_6-tag	This study
pET45b+ CT663	Ap^r; PCR-amplified *C. trachomatis scc4* (CT663; 402 nt) fused in frame with an N-terminal His_6-tag	This study
pGADT7	Ap^r; *LEU2*; cloning vector for expression of a cloned "prey" protein that is fused to GAL4 activation domain (AΔ)	Clontech
pBridge	Kan^r; *TRP1*; cloning vector for expression of a two cloned "bait" proteins. Main bait protein cloned in MCSI and fused to GAL-4 DNA-Binding Domain (BD) and "bridge protein" that cloned in MCSII, conditionally expressed in methionine depleted media	Clontech
pGADT7-CT089-4	Ap^r; *LEU2*; PCR-amplified *C. trachomatis copN* (CT089; 1266 nt) in pGADT7 vector N-terminal AD is fused in frame with full length CopN	This study
pGADT7N-CT089-2	Ap^r; *LEU2*; PCR-amplified *C. trachomatis copN* (CT089; 1-603 nt, coding for N-terminus) in pGADT7 vector N-terminal AD is fused in frame with N-terminus of CopN	This study
HCpGADT7C-CT089	Ap^r; *LEU2*; PCR-amplified *C. trachomatis copN* (CT089; 604-1266 nt, coding for C-terminus) in pGADT7 vector N-terminal AD is fused in frame with C-terminus of CopN	This study
pBridgeMSC1-CT088-3	Kan^r; *TRP1*; PCR-amplified *C. trachomatis scc1* (CT088, 441nt), vector for expression of "bait" protein fused to GAL-4 DNA-Binding Domain (BD)	This study
pBridgeMSC1-CT663-2	Kan^r; *TRP1*; PCR-amplified *C. trachomatis scc4* (CT663, 402 nt), vector for expression of "bait" protein fused to GAL-4 DNA-Binding Domain (BD)	This study
pBridgeMSC1-CT862	Kan^r; *TRP1*; PCR-amplified *C. trachomatis scc3* (CT862, 597nt), vector for expression of "bait" protein fused to GAL-4 DNA-Binding Domain (BD)	This study
pBridgeMSC1-CT663-1-MCS2-CT088-2	Kan^r; *TRP1*; PCR-amplified gene coding for *C. trachomatis scc4* (CT663, 402nt) in MCS1, fused in frame with GAL-4 DNA-Binding Domain (BD) and PCR-amplified *C. trachomatis scc1* (CT088, 441nt) in MCS2 as a "bridge" protein.	This study
pBridgeMSC1-CT088-3-MCS2-CT663-1	Kan^r; *TRP1*; PCR-amplified *C. trachomatis scc1* (CT088, 441nt) in MCS1, fused in frame with GAL-4 DNA-Binding Domain (BD) and gene coding for *C. trachomatis scc4* (CT663, 402nt) in MCS2 as a "bridge" protein.	This study
pBridgeMSC1-CT663-1-MCS2-	Kan^r; *TRP1*; PCR-amplified gene coding for *scc4* (CT663, 402nt) in MCS1, fused in frame with GAL-4 DNA-Binding Domain (BD) and	This study

YersSycE-2	PCR-amplified *Yersinia pseudotuberculosis sycE* in MCS2 as a “bridge” protein.	
pBridgeMSC1-CT088-3-MCS2-YersSycE-2	Kan^r; *TRP1*; PCR-amplified gene coding for *scc4* (CT663, 402nt) in MCS1, fused in frame with GAL-4 DNA-Binding Domain (BD) and PCR-amplified *Yersinia pseudotuberculosis sycE* in MCS2 as a “bridge” protein.	This study
pST44	Ap^r; Polycistronic expression system for coexpression of multicomponent protein complexes	Selleck and Tan (1)
pMT1649	Ap^r; PCR-amplified *C. trachomatis scc4* fused in frame to pST44	This study
pMT1652	Ap^r; PCR-amplified *C. trachomatis scc4* and *scc1* fused in frame to pST44	This study
pMT1653	Ap^r; PCR-amplified *C. trachomatis scc4* and *copN* fused in frame to pST44	This study
pMT1654	Ap^r; PCR-amplified *C. trachomatis scc1* and *copN* fused in frame to pST44	This study
pMT1655	Ap^r; PCR-amplified *C. trachomatis scc4*, *scc1*, and *copN* fused in frame to pST44	This study
pMT1668	Ap^r; PCR-amplified *C. trachomatis scc4* and *Y. pseudotuberculosis sycE* fused in frame to pST44	This study

1. **Selleck, W., and S. Tan.** 2008. Recombinant protein complex expression in E. coli. Curr Protoc Protein Sci **Chapter 5:**Unit 5 21.