





b



Supplementary Figure 1. Fourier shell correlation curves for sub-tomogram averages and comparisons to other published *in situ* T3SS structures. a, Resolution estimates after applying Fourier shell correlation to independently processed half-data sets (gold-standard procedure, 0.5/0.143 criterion are both indicated. (upper graph) FSC curve for the host-free (red, 3.3 nm) and host-contact T3SS (blue, 3.8 nm) assuming 12-fold symmetry and using a mask to align the basal body and outer membrane. (middle graph) FSC curve for the host-free (red, 4.3 nm) and host-contact (blue, 4.6 nm) T3SS assuming 6-fold symmetry and using a mask to align the basal body, inner membrane, and sorting platform-ATPase in the cytosol. (lower graph) FSC curve for host-free *Salmonella* T3SS basal body assuming 12-fold symmetry. **b**, Side-by-side comparison of the T3SS from host-free *Chlamydia* to T3SS from the bacterial envelope of *Yersinia enterocolitica* (EMD-5694), *Shigella flexneri* (EMD-2667), and *Salmonella enterica* (Fig. 1d) mini-cells.

1 CdsC	921 Chlamydia N ₀ N ₁ N ₂ Secretin
YscC	$ \begin{array}{c} 1 \\ N_0 \\ N_1 \\ N_3 \\ \end{array} $ Secretin
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	1 MNIVTSNIGSKILRIIQNNKKLGLLSALVVLDAALLSVNSRSGEGLIGQSASLPNYHEAE 1
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	61 QQIAACPKNIAKNLAKKSSPGAKPTVGAPSPARPVSVKAAPAKPQAPVAQTRHFKKSHQI 1
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	121 FSPNFTQSSQQVNKLEERRRPLESRYLQGAAKQAAAAKEKKALEQEVSKQEEEASKLWEE 1
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	181 KQSYARRAVNAINFSVRKQIEEQQKTISNPGNDQTLPGKKDPHTSGEPVIQTVQDCSQDQ 1
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	241 EEEKKVIERINKRSITCODIKEVGUTVNFEDISTELLIOPVSKISGTNFVFDSNDLOF 3 YMLKKSSWILAGSLIGTPLAMANEFSASFKGTDIOESTELTVGKNEKTITOPS-VRG 6 VIRPFSIMLVFLADFKPAAAEBFSASFKGTDIOESTETVSKNENKTVIIOPS-VRG 10 VIACAAUVVVFGYSEKIPVGSGFUADDSLETPFDAMADUKEPVIVGKC-AAK 10 TLIVLFVIVNNNTDSHLEQNDIAKYAQSDTVGSFFRFSILMVFIVGKC-AAK 12 VIEGTLDISSYSMAQEDWLPIPUVVAKGESLEDLGTPGANYDATVVGDKIN 11 AMFCCSAQMAPSLEKRLGKNEMFIITSSPVRAIMDFANYSIPVFISSVN
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	299 NVTIVEHDPTSVDDLATILDOVIKMHDLKUVEOGNVLIYRNPELSKLSTVVTDGSAKDT 60 KVDVRSFDTLNEGOYYSFFLSVLEVYGFAUVEMDNGVLKVIKSKDAKTSAIPVLSGEERA 63 TITVRSYDMLNEGYYOFFLSVLEVYGFAUINMNGVLKVVRSKDAKTSAIPVLSGEERA 66 KKITCNFEFHDENALUFKISLOLGUTIVEFDGOAIVIVDAS
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	359 CEAVUVTRVFRLYSVSPSAAVGIIQP-ULSHDAIISASESTRHIIVSDIAGNIEKVRE 120 NGDEVITQVVAVKNVSVREISPLAQ-IIDHAGAGNVVHYDPANIILITGRAAVVNRLAE 123 IGDEVVTRVPJTNVAARDIAPLIRQ-INDJAGAGSVVHYDPSVLIMTGRAAVVNRLAE 106EMRAAVVSLRHVGINEFNFLKR-SGLYNKNYPLEGDNRKGFYVSGPVVDMVN 109ELISKVILLENISLNYLIQYKD-ANLYDHRYPIRGNISDKTFYISGPPALVELVAN 108EVASRLIRUQESEAAELKQAUQR-SGIWEPRFGWRPDASNRLVIVSGPPRIELVEQ 105EISRSIITPYLDIDSILKYLSDTISVKNSCNURKITFNSEVRGVECIKYITS N2/N3
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	416 LLQANDSPCTAIDMSEYDVQFNPMALUSYCQDVLGAMAEEEAFQIFIQPGTNKI 179 IIRRVDQAGD-KEIEVVELNNASAAEMURIVEALNKTDAQNTPEFLKPKFVADEETNSI 182 IVERVDNAGD-RSVVTVPLAWASAADVVKLVTELNKDTSKSATPGSMVANVVADETNAV 162 AATMMDKONDG
CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	471 FVISSPRLTAKTIQLLESIDIPEMÄHTLDDÜTSPAALGSSGAANPKSLRFFMYKLKYON 238 LISCOPKVRERLKRLIGOLDVEMÄÄKGNNRÜVYLKYÄKÄEDLVEVLKGVSENLOABRGTG 241 LVSCEPNSRORIIAMIKOLDROONTOGNTKUIYLKYÄKÄSDLVEVLTGISSTMOSEKOAA 203 VIPCIATAIERIJOGEEOPLGNIVSSEPPAMPAFSANGEKGKAANYAGGMSLOEALLONA 206 VIPCIATAIERIJOGEEOPLGNIVSSEPPAMPAFSANGEKGKAANYAGGMSLOEALLONA 207 AAPGVATVVERLLNNGTAISNROJONDOP-MPPFNITOKVSEDSNDFSFSSVTNSS 207 AAPGVATILORVISDATIQOVTVDNORIPOUATRASNOR

CdsC	531	GAAIAOAIODIGYNLYVTTAMDEDFINTLNSTOWLEVNNSTWATENOANVDKVVSTUNGT
GspD Vcholer	298	OPTTSKRN
GspD Koxyto	301	KPVAALDKNIIKAHGOUNALTUTAAPDUUNDLERVAAU
InvG	263	AAGNIK VAY PDWSLLVEGTAEOVHFIEM VKAT
MxiD	260	ILED
YSCC	247	VEAD SLNAI IVED SPERMPMY OR THAT
EscC	238	SUT SUD PRIMAWAY DREIT DI YOOM SEI
2300	200	
CdeC	591	DI PEROVERUI DI ETSI EKSUDE VIOVA ALGDEOGKUAVASGI I SNTGLTDELENOSI.P
GapD Vcholer	338	THERA OVILLE ALLAVEMAE GOGT NACYOU GSLESS SULOYOUTGAST GNUM TGLESAKD
Gapp Koyyto	341	
InvG	298	
MyiD	295	
VSCC	276	
EscC	270	
2300	210	Secretin
CdeC	651	VAPNPONT ST. PTPCOT. & CTSDMMYQSSAFEL GTTCNVL. HNGKSVL-TLCOTTSTPONG
GapD Vcholer	396	TTOTKAUVDTNINGLENETTTTTKCDVTKLAGAISSTOGAAUGTAMG DWTNTUVSNDS
GapD Koryto	398	SSLASAMSSENCTABGEVOC NWAMNUT MISSSE
InvG	333	
MyiD	330	DSFGASENMSSSASTSTIDGNKETASUMETINGKK
VECC	311	NNHOVUIKTTGJOSNIASNGALOSLIDARGLDVILDRUNLURNEG
EscC	305	
Lace	505	ZALINI WEDINGWIND AND WENNINGWIN WITH AND
CdeC	710	DTTWEENER MANDTONISER CONTROLOGY AND STORES AN
GanD Vcholer	455	SCHTLOSDOTTUMDUCED SETUCEFULDUTUCEFULCENUS CONNENDER TUDET VIEWUDOT
Gapp Koruto	436	ENDITATEOTIVAT DIVESTMENTO OF VENTOS INGS IN SEA DA TENTVER MUNICIPALINA A VILLA V
Jane Caspo Koryco	364	
Main	364	
VSCC	356	
EscC	342	KAY TISOPSTIMI. NNNOATI. NY NY TYTEV SOEKVAS
2300	344	
CdsC	770	APNNY
GspD Vcholer	515	NEGNS
Capp Venute		
USDU KOXVEO	495	NEGDSVINE IEOEVSSVADNASSTSSDLGATFNTRTVNNAVLVGSCET
InvG	495	NEGDSVLMEIEQEVSSVADAASSTSSDLGATFNTRTVNNAVLVGSCET SADCOIEMSMDIGOGUDKTPOSDTTSVDAMOSVGEVLISTIATVDECKS
InvG MxiD	495 417 417	NEGDSVLLEIEQEVSSVADAASSTSSDLGATFNTRTVNNAVLVGSGET SADCOIEMSUDIEDGNDKTPOSDTTSVDAUPEVGRTLISTIARVPHCKS SSRCOIEMSUTIEDGTGNSOSNYNYNNENTSVUPEVGRUKISTIARVPOCKS
InvG MxiD YscC	495 417 417 409	NEGDSVLHEIGOVSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADCOIEMSUDIGD-GNDKTPQSDTTSVDADPEVGRTLISTIARVEHCKS SSRCOIEMSUTIGDGTGNSQSNYNYNNENTSVLPEVGRTKISTIARVEOCKS LTOCDKSIEMSUTIGDCOKPNSSGIGGIETISRTVVDTVVAVVGRGGS
InvG MxiD YscC EscC	495 417 417 409 395	NEGDSVLMEIGOVSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADCOIEMSIDIGDCNDKTPOSDTTSVDAMPEVGEVLISTIARVEHCKS SSRCOIEMSITEDGTCNSOSNIVINNENTSVLPEVGEVKISTIARVEPOCKS LTOCDKSEISINIHIEDGNOKPNSSGIEGIPTISTAVVDVARVGHCOS LDDSSNSLTGKRREVRHLDIODCNOSTNOSNADDASSTATVONSEMTHEATLSAGEG
GSDD_KOXYEO InvG MxiD YscC EscC	495 417 417 409 395	NEGDSVLMEIBOVSSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADCQIEMSIDIGDCNDKTPQSDTTSVDAAPEVGRULISTIARVEHCKS SSRCQIEMSIDIEDCTCNSQSNYNYNNENTSVDEEVGRUKISTIARVEQCKS LTQCDKSEISINMHIEDGNQKPNSSGIECIPTISRUVVDVARVGHCQS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTAPEVQNSEMTTEATLSAGES
GSDD_KCXYCO InvG MxiD YscC EscC	495 417 417 409 395	NEGDSVLMEIGUVSSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGQIEMSMDIGDGNDKTPQSDTTSVDADEVGRTLISTIARVPHGKS SSRCQIEMSMTIBDGTGNSQSNYNYNNENTSVDPUGEVGRTKISTIARVPHGKS LTQCDKSEISMMHEDGNQKPNSSGIEGIPTISRTVVDTVARVGHGQS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTAPEVQNSEMTTEATLSAGES
GSpD_KOXYUG InvG MxiD YscC EscC CdsC	495 417 417 409 395 814	NEGDSVLHEIGUVSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADCOIEMSUDIED-GNDKTPQSDTTSVDADPEVGRTLISTIARVPHCKS SSRCOIEMSUTIEDGTCNSQSNYNYNNENTSVDPEVGRTKISTIARVPHCKS LTQCDKSEISUNHIEDGNQKPNSSGIEGTPTISRTVVDTVARVGHCQS LDDSSNSLTGKRRERVRULLDIQDCNQSTNQSNAQDASSTLPEVQNSEMTTEATLSAGES
CdsC GspD_KCKYtO MxiD YscC EscC	495 417 417 409 395 814 559	NEGDSVLBEIEQUVSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADCOIEMSIDIEDCNDKTPQSDTTSVDADPEVGRTLISTIARVPHGKS SSRCOIEMSIDIEDGTGNSQSNYNYNNENTSVLDEVGRTKISTIARVPHGKS LTQCDKSEISINLHIEDGTGNSQSNYNYNNENTSVLDEVGRTKISTIARVPOCKS LDDSSNSLTGKRRERVRLLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTEATLSAGES
GSpD_KOXYUO InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxvto	495 417 409 395 814 559 543	NEGDSVLMEIEQUYSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGOIEMSMDIGDGNDKTPQSDTTSVDADEVGRTLISTIARVPIGKS SSRCOIEMSMDIGGGNSQSNYNNNENTSVDPUGEVGRTKISTIARVPIGKS LTQCDKSEISMMHIEDGNQKPNSSGIEGIPTISRTVVDTVARVGHGQS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTEATLSAGES WMSCHIRDKLTKIVSGVPIJSSLPLFKGIFSRSIDQRQRNIMIEIKPKVTSSFEEGTA LVLGGLIDERALESESKVPILGDTPLGQLFRSTSSQVEFKNLWUPIKPTIRDGVTADG
CdsC GspD_Koxyto CdsC GspD_Vcholer GspD_Koxyto InvG	495 417 409 395 814 559 543 467	NEGDSVLHEIEQUYSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADGOIEMSUDIEDGNDKTPQSDTTSVDADEVGRTLISTIARVPECKS SSRCOIEMSUTIEDGTGNSQSNYNYNNENTSVLPEVGRTKISTIARVPECKS LTQCDKSEISUNIHIEDGNOKPNSSGIECTPTISRTVVDTVARVGEGOS LDDSSNSLTGKRRERVRLLLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTTEATLSAGES VVNGELRDKLTKIVSGVPLLSSLPLIKGLESRSIDQRORRNIMIFIKPKVISSFEEGTA LVLGGLIDERALESESKVPLLGDIPLIGQLERSTSSQVEKNLMVPIKPTIRDGVTADG VVVGCLLDKSVSDTADKVPLLGDIPLIGGLERSTSKVSKNSNVVRVPMIEPKEIVDPLTPDA
CdsC GspD_Koxyto CdsC GspD_Vcholer GspD_Koxyto InvG MxiD	495 417 409 395 814 559 543 467 469	NEGDSVLHEIEQUVSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADCOIEMSUDIED-GNDKTPQSDTTSVDADPEVGRTLISTIARVPHGKS SSRCOIEMSUTIEDGTGNSQSNYNYNNENTSVDPEVGRTKISTIARVPHGKS LTQCDKSEISUNLHIEDGNOKPNSSGIEGIPTISRTVVDTVVATVGRCOS LDDSSNSLTGKRRERVRLLDIQGNQSTNQSNAQDASSTLPEVQNSEMTTEATLSAGES WMSGHIRDKLTKIVSGVPLISSLPLIKGLFSRSIDOROTRNIMIFIKPKVISSFEEGTA LVLGGLIDERALESESKVPLLGDIPLCQLFRSTSSOVEKKNLMVFIKPTIIRDGVTADG VVVGGLLDKSVSDTADKVPLLGDIPVIGALFRSTSKKVSRNLMLFIRPVVRDRDEYRQ LLVGGYTRDANTDTVQSIPFLGKIPLIGSLFRYSSKNSNVVRVMIEPKEIVDPLIPDA
CdsC CdsC CdsC CgspD_Vcholer GspD_Koxyto InvG MxiD YscC	495 417 409 395 814 559 543 467 469 458	NEGDSVLIEICUVSSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGOIEMSIDIEDGNDKTPQSDTTSVDADEVGRTLISTIARVPEGKS SSRCOIEMSIDIEDGTGNSQSNYNNNENTSVDPUGEVGRTKISTIARVPEGKS LTQCDKSEISINIHIEDGNQKPNSSGIECIPTISRTVVDTVARVGEGOS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTTEATLSAGES VUNGCLIDERALESESKVPLLGDIPLCQUFRSTSSQVENKNLWVFIKPTIRDGVTADG VVVGGLLDKSVSDTADKVPLLGDIPVIGALFRSTSKKVSRNLMLFIRPTVIRDRDEYRQ UVVGGLLDKSVSDTADKVPLLGDIPVIGALFRSTSKKVSRNLMLFIRPTVIRDRDEYRQ LVGGYTEDANTDTVQSIFFLGKIPLIGSDFRYSSKNKSNVVRVFMIEPKEIVDPLTPDA LUGGYTEDANTDTVQSIFFLGKIPLIGSDFRYSSKNKSNVVRVFMIEPKEIVDPLTPDA LIGGYTHETNSNEIISIFPSSIFVIGNVFKYKTSNISNIVRVFLIEPKEIVDPLTPDA
CdsC GspD_Koxyto VscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	495 417 409 395 814 559 543 467 469 458 455	NEGDSVLMEIEQUVSSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADGOIEMSIDIEDGNDKTPQSDTTSVDAUPEVGRTLISTIARVPECKS SSRCOIEMSIDIEDG-GNSQSNYNYNNENTSVLPEVGRTKISTIARVPECKS LTQCDKSEISMNHEIDGNQKPNSSGIEGTPTISRTVVDTVARVGEGOS LDDSSNSLTGKRRERVRLLLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTTEATLSAGES VVVGCLIDERALESESKVPLLGDIPLIGQLFRSTSSQVERKNIMVFIKPTIIRDGVTADG UVVGCLIDESVSDTADKVPLLGDIPUIGGLFRSTSSQVERKNIMVFIKPTIIRDGVTADG LLGGYTRDANTDTVQSIPTCKLPLIGLFRSTSKVSKNKSNVRVPMIEPKEIVDPLTPDA LLIGGYTHETNSNEIISIPTLSSIPVIGNVFKYKSNISNIVRVPIICPGIEKESSYNT LIGGYTRDESVALSKVPLLGDIPUIGSLFRSTSSCVERKNIGPIICPGIEFESTYNT LIGGTYRDESVALSKVPLLGDIPUIGSLFRSTSSELTRTVRLFIIEPKIVDFLTPDA
CdsC GspD_Koxyto VscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	495 417 409 395 814 559 543 467 469 458 455	NEGDSVLHEIEQUVSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADCOIEMSLDIEDGNDKTPQSDTTSVDADEVGRTLISTIARVPHCKS SSRCOIEMSLTIEDGTGNSQSNYNYNNENTSVLPEVGRTKISTIARVPHCKS LTQCDKSEISLNHHEDGCQKPNSSGIEGTPTISRVVDTVARVGHCQS LDDSSNSLTGKRRERVRLLLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTTEATLSAGES VVJGCLIDERALESESKVPLLGDIPLLGQLFRSTSSQVEKKNLMVPIKPTIRDGVTADG VVVGCLLDKSVSDTADKVPLLGDIPLLGQLFRSTSSQVEKKNLMVPIKPTIRDRUFADG VVVGCLUKSVSDTADKVPLLGDIPLIGLFRSTSSQVEKKNLMVPIKPTIRDRUFADG LIDGSTRDAKTENSNEISIPFLSSTPVIGNVFKYKSNISNIVRVFMIEPKEIVDPLTPDA LIGGYTHETNSNEISIPFLSSTPVIGNVFKYKSNISNIVRVFMIEPKEIVDPLTPDA
GSpD_KOXYUG InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	495 417 409 395 814 559 543 467 469 458 455	NEGDSVLIEICVSSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADGOIEMSIDIEDGNDKTPQSDTTSVDADEVGRTLISTIARVPHGKS SSRCOIEMSIDIEDGTGNSQSNYNYNNENTSVDPYGRTSISTIARVPHGKS LTQCDKSEISINIHIEDGNQKPNSSGIECIPTISRTVVDTVARVGHGOS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTAPEVQNSEMTTEATLSAGES VVNGGLIDKSVSDTADKVPLGDIPLIGOLFRSTSSQVEKKNLMVFIKPTIRDGVTADG VVVGGLLDKSVSDTADKVPLGDIPLIGOLFRSTSSQVEKKNLMLFIRPTVIRDRDEYRQ MLVGGYTRDANTDTVQSIPFICKIPLIGSLFRSTSKNSNVFRVFMIEPKEIVDPLTPDA LLIGGYTHETNSNEIISIPFLSSIPVIGNVFKYKTSNISNIVRVFMIEPKEIVDPLTPDA LLIGGYTHETNSNEIISIPFLSSIPVIGNVFKYKTSNISNIVRVFIEFKEIVDPLTPDA
CdsC GspD_Koxyto MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC	495 417 409 395 814 559 543 467 469 458 455 874	NEGDSVLMEIEQUYSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGO
CdsC CdsC CdsC CdsC CdsC CdsC CdsC CdsC	495 417 409 395 814 559 543 469 458 455 874 619	NEGDSVLMEIEQUVSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGOIEMSUDIEDGNDKTPQSDTTSVDADEVGRTLISTIARVPEGKS SSRGOIEMSUTIEDGTGNSQSNYNYNNENTSVLPEVGRTKISTIARVPEGKS LTQCDKSEISMNHEIEDGNQKPNSSEIEGTPTISRTVVDTVARVGEGQS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTDPEVQNSEMTTEATLSAGES VVVGGLIDERALESESKVPLLGDIPLIGQLFRSTSSQVERKNLMVPIKPTIRDGVTADG VVVGGLIDERALESESKVPLLGDIPLIGQLFRSTSSQVERKNLMVPIKPTIRDGVTADG LUGGYTDANTDVQSIPTGKIPLIGJIFNTSKKVSKNLMVFIKPTIRDGVTADG LUGGYTDANTDVQSIPTGKIPLIGSLFRSTSKVSKNLMVFIKPTIRDRDEVR LIGGYTHETNSNEIISIPFLSSIPVIGNVFKYKTSNISNIVRVFMIEPKEIVDPLTPDA LIGGYTHETNSNEISIPFLSSIPVIGNVFKYKTSNISNIVRVFMIEPKEIVDPLTPDA LUGGFIQDKESSKDGIPLIGDIPVIGSLFRSTSKVSKNJKNLVRUFMIEPKEIVDPLTPDA LIGGYTHETNSNEISIPFLSSIPVIGNVFKYKTSNISNIVRVFMIEPKEIVDPLTPDA LUGGFIQDKESSKDGIPLLGDIPVIGSLFRSTSVKQKHSVVRLFIKATPIKSASSE LSNTEGYRYNWESERGSLEVAERHAPECQHIPKVQESNFKMLEIEA
CdsC CdsC CdsC CdsC CdsC CdsD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC CdsC CdsC CdsC InvG MxiD YscC EscC	495 417 409 395 814 559 543 467 458 455 874 603	NEGDSVLLEICVSSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADGOIEMSUDIEDGNDKTPQSDTTSVDADEVGRTLISTIARVPEGKS SSRCOIEMSUDIEDGTONSQSNYNYNNENTSVDPYGRTKISTIARVPEGKS LTQCDKSEISUNHHEDGNQKPNSSGIEGIPTISRTVVDTVARVGEGOS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTAPEVQNSEMTTEATLSAGES VVNGGLIDERALESESKVPLLGDIPLIGOLPRSTSSQVEKKNLMVFIKPTIRDGVTADG VVVGGLIDERALESESKVPLLGDIPLIGOLPRSTSSQVEKKNLMVFIKPTIRDGVTADG LVVGGLIDERALESESKVPLLGDIPLIGOLPRSTSSQVEKKNLMVFIKPTIRDGVTADG LVVGGLIDERALESESKVPLGDIPLIGOLFRSTSSKVSKNLMLFIRPTVIRDRDEYRQ MLVGGYTRDANTDTVQSIPFIGHLPIGSLFRSTSKKVSKNLMLFIRPTVIRDRDEYRQ LIGGYTRDANTDTVQSIPFIGHLPIGSLFRSTSKVSKNSNVFRVFMIEPKEIVDPLTPDA LIGGYTHETNSNEIISIPFISSIPVIGNVFKYKTSNISNIVRVEMIEPKEIVDPLTPDA LIGGYTHOLSVALSKVPLGDIPVIGSLFSSTVKQKHSVVRUPLIGAEHHL LLGGFTODKESSSKDGIPLISDIPVIGSLFSSTVKQKHSVVRUPLIKATPIKSASSE LSNTEGYRYNWESERGSLEVAPRHAPECOHIPKVQAESNFKMLEIEA ITQRKYNYIRAEQLFRAEKGLRLIDDASVPVIPKFGDDRRHSPEIOFIE-QMEAKQ- JSSGQYTAFNDAQSKQRGEENNDAMISNDLEIYF-RQTAAFRQVSAAID-AFNLGGN
CdsC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC CdsC CdsC GspD_Vcholer GspD_Vcholer GspD_Koxyto InvG	495 417 4095 395 814 5543 467 469 458 455 874 6193 527	NEGDSVLIEICUVSSVADAASSTSSDIGATFNRTVNNAVLVGSGET SADGOIEMSIDIBDGNDKTPQSDTTSVDADEVGRTLISTIARVPIGKS SSRCOIEMSIDIBD-GNSQSNYNYNNENTSVDPUGEVGRTLISTIARVPIGKS LTQCDKSEISINHEDGNQKPNSSGIEGIPTISRTVVDVVAVGHGQS LDDSSNSLTGKRRERVRULLDIQDCNQSTNQSNAQDASSTDPEVQNSEMTEATLSAGES VVNGCLIDERALESESKVPILGDTPLGQLFRSTSSQVEMKNLWVFIKPTIRDGVTADG VVGGVIDANTDTVQSIPFLGKIPLIGOLFRSTSSQVEMKNLWVFIKPTIRDGVTADG LVGGVTDANTDTVQSIPFLGKIPLIGSLFRSTSSNVSNVFWMTEPKEIVDPLTPDA DLIGGYTHENSNEISIPPLSSTPVIGNVFKYKTSNISNVVRVFMTEPKEIVDPLTPDA LIGGYTHENSNEISIPPLSSTPVIGNVFKYKTSNISNVVRVFMTEPKEIVDPLTPDA LIGGFIQDKESSKDGIPLLSDIPVIGSLFSSTVKOKHSVVRLFLFRATVRESSSS LSNTEGYRYNWESERGSLEVAPRHAPECQHIPKVQAESNFKMLEIEA ITQRKYNYIRAEQLFRAEKGLRLIDDASVPVLPKGDDRHASPEIQAFIE-QMEAKQ- SSGQYTAFNDAQSKQRGKENNDAMSNDLEIYPRQDTAAFRQVSAID-AFNLGM
CdsC CdsC CdsC CdsC CdsC CdsC CdsC CdsC	495 417 409 395 814 559 3467 469 458 455 874 603 529	NEGDSVLMEICUVSSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADGOIEMSIDIEDGNDKTPQSDTTSVDAUPEVGRTLISTIARVPEGKS SSRCOIEMSIDIEDGTGNSQSNYNYNNENTSVDPUVGRTKISTIARVPEGKS LTQCDKSEISMNHHEDGROKYNSSGIEGTPTISRTVVDTVARVGRGQS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTOPEVQNSEMTTEATLSAGES VVVGGLIDERALESESKVPLLGDTPLLGQLFRSTSSQVERKNLMVFIKPTIRDGVTADG UVVGGLIDERALESESKVPLLGDTPLLGQLFRSTSSQVERKNLMVFIKPTIRDGVTADG UVVGGLIDERALESESKVPLLGDTPLLGQLFRSTSSQVERKNLMVFIKPTIRDGVTADG UVVGGLIDERALESESKVPLLGDTPLLGQLFRSTSSQVERKNLMVFIKPTIRDGVTADG UVVGGLIDESNSSTPLGKLPLIGSLFRSTSKKVSKRLMVFIKPTIRDGVTADG UVVGGLIDESNSSTPLGKLPLIGSLFRSTSKKVSKRLMVFIKPTIRDGVTADG UVVGGLIDESVSDTADKVPLGDTPUGSLFRSTSSVERKNLMVFIKPTIRDGVTADG UVVGGLIDESVSTPLGKLPLIGSLFRSTSKKVSKRLMVFIKPTIRDGVTADG ULGGYTHDANTDTVQSIPTCKLPLIGSLFRSTSKKVSKRLMLFI ULIGGYTHENSNEIISIPTGSIPVIGSLFSTVKQKHSVVRUPMIEPKEIVDPLTPDA LIGGYTHENSNEISKVPLLGDTPVGSLFSSTVKQKHSVVRUPLIGAFHKSSSE LSNTEGYRYNWESERGSLEVAPRHAPECQHIPKVQAESNFKMLEIEA ITQRKYNYIRAEQLFRAEKGLRLDDASVPVDFKFGDDRHSPEIQAFIE-QMEAKQ SSGQYTAFNDAQSKQRGKENNDAMISNDLLEIYPRQEAIK
CdsC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC	495 417 417 395 814 559 543 467 469 8455 874 603 5279 518	NEGDSVLLEICVSSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGOIEMSUDIDGNDKTPQSDTTSVDADEVGRTLISTIARVPEGKS SSRCOIEMSUDID-GNDKTPQSDTTSVDAUEVGRTKISTIARVPEGKS LTQCDKSEISJNHHEDGNQKPNSSGIEGIPTISRTVVDTVARVGEGOS LDDSSNSLTGKRRERVRULLDIQDGNQSTNQSNAQDASSTAPEVQNSEMTTEATLSAGES VVNGGLIDERALESESKVPLGDIPLIGOLPRSTSSQVEKKNLMVFIKPTIRDGVTADG VVVGGLIDERALESESKVPLGDIPLIGOLPRSTSSQVEKKNLMVFIKPTIRDGVTADG LVVGGLIDERALESESKVPLGDIPLIGOLPRSTSSQVEKKNLMVFIKPTIRDGVTADG LVGGYTRDANTDTVQSIPFIGRIPLIGOLPRSTSKVSKNLMLFIRPTVIRDRDEYRQ MLVGGYTRDANTDTVQSIPFIGRIPLIGOLFRSTSKVSKNSNVFRVFMIEPKEIVDPLTPDA LLIGGYTHETNSNEIISIPFISSIPVIGNVFKYKTSNISNVFRVFMIEPKEIVDPLTPDA LLIGGYTHETNSNEIISIPFISSIPVIGSLFSSTVKQKHSVVRUPMIEPKEIVDPLTPDA SCOUTAFDAQSKQGKENNDAMISNDLEIYF-RQTAAFRQVSAAID-AFNLGGN SESVNNILKQSGWSGDKKQKWVVIDDRGGEAIK
CdsC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC	495 417 417 395 814 559 543 469 458 455 874 619 527 529 518	NEGDSVLMEIGUVSSVADAASSTSSDIGATFNRTVNNAVLVGSGET SADGOIEMSMDIDGNDKTPQSDTTSVDADEVGRTLISTIARVPIGKS SSRCOIEMSMDID-GNSQSNYNYNNENTSVDPUGEVGRTLISTIARVPIGKS LTQCDKSEISMMHEDGNQKPNSSGIEGIPTISRTVVDVAVGHGQS LDDSSNSLTGKRRERVRULLDIQDCNQSTNQSNAQDASSTAPEVQNSEMTTEATLSAGES VVVGGLIDERALESESKVPLLGDIPLIGQUFRSTSSQVEMKNLMVPIKPTIRDGVTADG LVCGLIDESVSDTADKVPLLGDIPVIGALFRSTSKVSSRNLMLFIRPVVTRDRDEYRQ LVGGYTEDANTDTVQSIFFLGKIPLIGSDFRYSSNKSNVVRVEMTEPKEIVDPLTPDA DLIGGYTEDSNEISIFPDSSIPVIGNVFKYKTSNISNIVRVEMTEPKEIVDPLTPDA LIGGYTEDSNEISIFPDSSIPVIGNVFKYKTSNISNIVRVEMTEPKEIVDPLTPDA LIGGYTEDSNEISIFPDSSIPVIGNVFKYKTSNISNIVRVEMTEPKEIVDPLTPDA LIGGFIQDKESSKDGIPLLSDIFVIGSLFSSTVKQKHSVVRLELIKATPIKSASSE LSNTEGYRYNWESERGSLEVAERHAPECQHIPKVQZESNFKMLEIEA ITQRKYNYIRAEQLFRAEKGLRLIDDASVPVLPKFGDDRHASPEIQAFIE-QMEAKQ- SSGQYTAFNDAQSKQRKENNDAMSNDLEIYPRQDTAAFRQVSAID-AFNLGGN SESVNNILKQSGANSGDDKLQKWVVYDDRGQEAIK
CdsC CdsC CdsC CdsC CdsC CdsC CdsC MxiD YscC EscC CdsC CdsC CdsC CdsC CdsC CdsC CdsC CdsC EscC CdsC EscC CdsC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC MxiD YscC EscC CdsC CdsC MxiD YscC EscC CdsC EscC CdsC EscC EscC CdsC EscC	495 417 417 395 814 559 543 467 458 455 874 619 3027 529 518	NEGDSVLMEICUVSSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGOIEMSMDIDGNDKTPQSDTTSVDADEVGRTLISTIARVPICKS SSRCOIEMSMDID-GNSQSNYNYNNENTSVDPUGEVGRTKISTIARVPICKS LTQCDKSEISMNHEDGROKKPNSSGIEGIPTISRTVVDVVAVVGRCGS LDDSSNSLTGKRRERVRULLDIQDCNQSTNQSNAQDASSTDPEVQNSEMTEATLSAGS VVVGCLIDERALESESKVPLLGDTPLLGQLFRSTSSQVERKNIMVFIKPTIRDGVTADG VVVGCLIDERALESESKVPLLGDTPLLGQLFRSTSSQVERKNIMVFIKPTIRDGVTADG VVVGCLIDESNSDTADKVPLLGDIPVIGALFRSTSKVSKNIMVVFVMIEPKEIVDPLTPDA DIGGYTRDANTDTVQSIPFLCKLPLIGSLFRSTSSQVERKNIMVFIKPTIRDGVTADG UVVGCLIDKSVSDTADKVPLLGDIPVIGALFRSTSKNSNVVRVFMIEPKEIVDPLTPDA DIGGYTRDANTDTVQSIPFLCKLPLIGSLFRSTSSQVERKNIMSNVRVFMIEPKEIVDPLTPDA LIGGYTRDANTDTVQSIPFLCKLPLIGSLFRSSSTVKSKNSNVFVFMIEPKEIVDPLTPDA DIGGYTRDANTDTVQSIPFLCKLPLIGSLFRSSSVKSKNSNVFFFIEFTIRDGVAFK SSRCO
CdsC CdsC CdsC CdsC CdsC CdsC CdsC CdsC	495 417 4109 395 814 559 3467 469 458 455 874 603 5229 518 921	NEGDSVLMEIGUVSSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADCO
CdsC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG Koxyto InvG Koxyto CdsC CdsC GspD_Vcholer CdsC	495 417 409 395 814 5543 467 469 874 619 527 529 518 921	NEGDSVLMEIGUVSSVADAASSTSSDIGATFNRTVNNAVLVGSGET SADGO
CdsC CdsC	495 417 419 395 814 459 5543 467 458 455 874 619 652 529 518 921 660	NEGDSVLMEICUVSSVADAASSTSSDIGATFNRTVNAVLVGSGET SADGO
CdsC CdsC CdsC CdsC CdsC CdsC CdsC CdsC	4955 4177 409395 814 559 559 543 467 469 603 527 528 455 874 669 603 527 5518 921 6660	NEGDSVLMEIECVSSVADAASSTSSDIGATFNTRTVNNAVLVGSGET SADGO
CdsC GspD_Koxyto EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD YscC EscC CdsC GspD_Vcholer GspD_Koxyto InvG MxiD	495 417 409 395 814 455 559 559 559 467 458 455 874 458 455 874 603 527 529 5118 921 660	NEGDSVLMEIGUVSSVADAASSTSSDIGATFNTRTVNAVLVGSGET SADGO
CdsC InVG InVG InVG InVG INV INVG I	495 417 409 395 814 455 954 3467 458 455 874 619 603 529 518 921 6660 578	NEGDS VLMEIEQUYSVADAASS TSSDIGATFNTTVNNAVLVGSGET SADGO IEMSMDIBD GND KTPQSDTTSVDAUPEVGTLISTIARVPIGKS SSRCO IEMSMDIBD GND KTPQSDTTSVDAUPEVGTLISTIARVPIGKS SSRCO IEMSMDIBD

Supplementary Figure 2. Domain architecture of CdsC and YscC and the corresponding multiple sequence alignment of type II and type III secretin homologues (GspD and SctC). The *Chlamydia* secretin, CdsC, contains an N-terminal domain of 250 amino acids (dark red) in addition to the classical N-domains (yellow) found in other type II or type III secretins. CdsC

also uniquely contains type-II specific insertions throughout the N-domains, in the secretin domain (orange) and in the N3 loop (red). The predicted signal-peptide is also indicated (purple). Multiple-sequence alignments were produced by ClustalW Omega and graphically illustrated with BOXSHADE. Identical residues are shaded in black and similar residues in grey. Colored annotations in the sequence alignment correspond to the domains in the secretin schematic. Accession numbers are listed for each bacterial species. CdsC, *C. trachomatis* (M9UG25); GspD, *V. cholera* (C3LSG0); GspD, *K. oxytoca* (A0A068H9C0); InvG, *S. enterica* (P35672); MxiD, *S. flexneri* (Q04641); YscC, *Y. enterocolitica* (Q93KT1); EscC, enteropathogenic *E. coli* (B7UMB3).

CdsP	1	
YSCP	1	MNKITTRSPLEPEYOPLGKPHHDLOARVDFEOALLHNKDNRHPKEEPRRPVRPHDLGKKEGOKGDGLRAH
EscP	1	
Spa32	1	MALDNINLNFSSDKOIEKCEKLSSIDNIDSLVLKKKKKVEIPEYSLIASN
SpaN/InvJ	1	MGDVSAVSSSGNILLPOODEVGGLSEALKKAVENHKTEYSGDKKDRDYGDAFVMHKETALPLLLAAW
CdsP	29	BQREVRVFSLEGRSSTRQEKADRMPGRTSSRQESSK
YSCP	71	APLAATFQPGREVGLKPQHNHQNNHDFNLSPLAEGATNRKHLYQQDSRFDDRVDSIINALMPLAPFLEGV
EscP	1	MTRVSLKRNLIEPVSFRPSD
Spa32	51	YFTIDKHFENKHDKGEIYSGIKNAFELRNERATYSDIPDS
SpaN/InvJ	68	RHGAPAKSEH NGNVSGLHHNGKSELRIAEKLLKVTAEKSVGLISAEAKVDKSAAL
CdsP	65	SSEEGAVHESTAGVSSKEEEESKGDGFFTGGNPTSG
YSCP	141	TCETGTSSESPCEPFGHDELFVQQSPIDSAQPVQLNTKPTVQSLNPAADGAEVMIWSVGRDTLASIAKNQ
EscP	21	DWPQGEESFNDIFYHIKKRSPD
Spa32	91	MAIKENILIPDQDIKAREKINIGDMRGIFSYNKSGN
SpaN/InvJ	124	LSSKNRPLESVSGKKLSADLKAVESVSEVTDNATGIS
CdsP	101	MALVETPMAVVSEAMMETSMMTWSQVDLQMVEQLVTSTVES
YSCP	211	RDSROKRLAEEPLPLHOEALPEVCPPAWSTTPDDHLVARWCATPVAEVAEKSARFPHKATVOSEOLDMTE
EscP	43	MASLWKRLFYSSGRRR
Spa32	127	ADKNFERSHUSSUNPDNLLESINRNGOIGLKNHSLS
SpaN/InvJ	161	DDNIKALPGDNKAIAGEGVRKEGAPLARDVAPARMAAANTGKPED
CdsP	142	LLVADIDGKOLVEIVLDNSNTVRAAFCG-ANTILVOTCEEISVSFSNFV-DOAOLTE
YSCP	281	LADRSOHLTDGVDSSKDTIEPPRPEELLLPREETLPEMYSLSFTAPVINPCDHLLATMRATR-LASVSEO
EscP	64	RYFEEGEHSFSILCGRLRGVULMIKCSNGIIYLSIK-ISONNRN
Spa32	163	IDKNIADIISLONGSVAKSFEDPUMNKNTADITPSMSLQ-EKSIVEN
SpaN/InvJ	206	KDHKKVKDVSQDELQPTTIAD SQLEGCDEKMPLAAQSKPMMTIFPT
CdsP	197	ATOR VOON PKOLVSLVESLKAROINITELVV-GNVAVSLPTIE-KIETPIHMIAATIRHHDO
YSCP	350	LIOTA RLAVELELRGGSSOVTOLHINLPEL-GAIMVRIAEIPGKLHVELIASRETLRILAOGSYDLLER
EscP	107	HVF YHKKDYVFDKLKEIFPDEAIEFTIEYE-N
Spa32	209	DKNVFOKNSEMTYHFKOWGAGHSVSISVESG-SFVLKPSDOFVGNKLDLILKODAEG
SpaN/InvJ	253	ADGVKGEDSSLTYRFORWGNDYSVNIOAROAGEFSLIPSNTOVEHRLHDOWONGNPO
CdsP	257	EGDOEGEGRODOHOGOHOEKKVIJJAHI
YscP	419	LORIEPTOLDFOASGDSEOESROKRHVYEEWBAEE-
EscP		
Spa32	265	NYRFDSSQHNKGNKNNSTGYNEQSBEC-
SpaN/InvJ	310	RWHLTRDDQQNPQQQHRQQSG

Supplementary Figure 3. Multiple sequence alignment of tape measure protein SctP. The tape measure protein SctP regulates the needle length in T3SS-containing bacterial pathogens. Residue length and accession numbers are listed for each bacterial species. CdsP, *C. trachomatis* (283, M9UDI3); YscP, *Y. enterocolitica* (453, Q93KT6); EscP, enteropathogenic *E. coli* (138, B7UMA4); Spa32, *S. flexneri* (292, D2AJK8); SpaN/InvJ, *S. enterica* (336, P40613). Multiple sequence alignments were produced by ClustalW Omega and graphically illustrated with BOXSHADE. Identical and similar residues are shaded in black and grey respectively.





host-free C. trachomatis T3SS



Supplementary Figure 4. Distance between bacterial outer membrane and target host

membrane. a, Histogram of length measurements for needle-mediated contact (average 33 ± 4 nm; range 22-44 nm; n =120) and non-specific adhesion (average 20 ± 3 nm; range 14-25 nm; n = 20). Measurements in both cases were recorded manually from representative tomogram slices in IMOD **b**, Dimension comparisons for host-free and host-contact *Chlamydia* T3SSs.



CdsD	601	DKSEMLYKVDALSFVKSVDDNVIDDEAVWQEMNILLSKNPEFKGISMQSPEPCIFVISCY
YSCD	194	MRLS QNFLESHGIPFRLELRSMEELRQGAEFILQRLGHGUEVSLAPQAGW QLNCE
PrgH	207	RQV ARGDYDNARVINENEENKRISIWLDTYYPQLAFYRHFD-EPRKPVFWISRQRN
MxiG	184	SVA NKTGFT CRYILVSNKEINR QQYINQRFPFINLYVLNLVSDKAELLVF SKERN
EscD	188	DLTPFFNYLKENIFYYNKIICNNO ISAINDVLTEYGYKDIITKGNKPCFFLLSCY
CdsD	661	LKTEEOAACHADYINLHFNILSLLDNKVIIESOVMKAHAGHIVOSGFANVHVSFTNEAV
YSCD	252	USERTOKOKTOSLUGAEVOGLLGVENKURTAGOORKRUDALUE OFGODSDETUNUK CELT
PraH	266	THE KEELEVISOK PALMANADE WITH MODUTAAGOAFACIK OAIPYSPRNHKC WTF
Main	244	CONTROL AND A DEPONENT AND A LINE AND A
MXIG	244	SSRJTELDK KAR I VEF STRATKATKI SDB. AKGDAKGIFTKVAVQIKEICENAKVII
ESCD	240	IPPSPRWSEVEN-GLENTGOVAGWEIHNNSN-MKINEGASEFRKNKGINIVNIFRKNDVI
CdsD	721	LT-GYINNKDADKFRTVVQELQDIAGIGAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS
CdsD YscD	721 312	LT-CYINNKDADKFRTVVQELQDIACIRAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRCQVNDEKLSSFNQLQQTFRQEFCNRPKLELVNVGGQPQHDELNEVQAIS
CdsD YscD PrgH	721 312 326	LT-CYINNKDADKFRTVVQELQDIACIEAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRCQVNDEKLSSFNQLQQTFRQEFCNRPKLELVNVGGQPQHDELNEVQAIS VIQCALDDVEILRARQFVDSYYRTMCGCYQFAIELKDDMLKCRSFQ
CdsD YscD PrgH MxiG	721 312 326 304	LT-GYINNKDADKFRTVVQELQDIAGIRAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRGOVNDEKLSSFNQLQQTFRQEFGNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILRARQFVDSYYRTWGG2YVQFAIELKDDWLKGRSFQ SVREELTDEKLELINRLISEHKNIXGCQYIEFSVLLDDDEKCKSYL
CdsD YscD PrgH MxiG EscD	721 312 326 304 304	LT-CYINNKDADKFRTVVQELQDIACICAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRCQVNDEKLSSFNQLQQTFRQEFCMPFKLELVNVGGQPQHDELNEVQAIS VIQCALDVEILRARQFVDSYYRTMGCYUQFAIELKDWLKCRSFQ SVREELTDEKLELINRLISEHKNIYCDQYIEFSVLLIDDDEKCKSYL IVACEVSQQNESKILAIINAMNKNSNVKILFONIOPYISADIEPCKIL
CdsD YscD PrgH MxiG EscD	721 312 326 304 304	LT-GYINNKDADKFRTVVQELQDIAGIFAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRGQVNDEKLSSFNQLQQTFRQEFGNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILRARQFVDSYYRTWGG2YQFAIELKDDWLKGRSFQ SVREELTDEKLELINRLISEHKNIYGDQYIEFSVLLIDDDFKCKSYL IVAGEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIFPCKILR
CdsD YscD PrgH MxiG EscD	721 312 326 304 304	LT-GYINNKDADKFRTVVQELQDIAGIGAVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRGQVNDEKLSSFNQLQQTFRQEFGNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILRARQFVDSYYRWGGYVQFAIELKDDWLKGRSFQ SVREELTDEKLELINRLISHKNIXCDQYIESVLLDDDFKCKSYL IVAGEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIPCKILR
CdsD YscD PrgH MxiG EscD CdsD	721 312 326 304 304	LT - CYINNKDADKFRTVVQELQDIACI AVKNFVVLLPAE GVIDLNMRYPGRYRVTGFS ELRCQVNDEKLSSFNQLQQTFRQEFCN PKLELVNVGGQPQHDELNEVQAIS VIQCALDDVEILRARQFVDSYYRTMGGYVQFAIELKDDWLKCRSFQ SVREELTDEKLELINRLISEHKNIYCDQYIEFSVLLIDDDKKCKSYL IVACEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADI PCKILR
CdsD YscD PrgH MxiG EscD CdsD	721 312 326 304 304 780	LT-GYINNKDADKFRTVVQELQDIAGIFAVKNFVVLLPAEGVIDLNMRYPGRYRVTGFS ELRGVNDEKLSSFNQLQQTFRQEFCNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILRARQFVDSYYRTWGGYQFAIELKDDWLKGRSFQ SVREELTDEKLELINRLISEHKNIYCDQYIEFSVLLIDDDFKCKSYL IVAGEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIFPCKILR KCGDISNVVVNGRILTRGDILDCMTVTSIQP-HCIFLEREGLKYKEYNK
CdsD YscD PrgH MxiG EscD CdsD YscD	721 312 326 304 304 780 365	LT - GYINNKDADKFRTVVQELQDIAGI AVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRGQVNDEKLSFNQLQQTFRQEFGNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILARQFVDSYYRWGGYQFAIELKDDWLKGRSFQ SVREELTDEKLELINRLISEHKNIYCDQYIEFSVLLIDDEKCKSYL IVAGEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIPCKILR KCGDISINVVVNGRILTRGDILDCMTVTSIQP-HCIFLEREGLKYKFEYNK LG
CdsD YscD PrgH MxiG EscD CdsD YscD PrgH	721 312 326 304 304 780 365 373	LT-CYINNKDADKFRTVVQELQDIAGIEAVKNFVVLLPAEGVIDLNMRYPGRYRVTGFS ELRCQVNDEKLSSFNQLQQTFRQEFCNRPKLELVNVGGQPQHDELNEVQAIS VIQCALDDVEILRARQFVDSYYRTMCGYVQFAIELKDDMLKCRSFQ SVREELTDEKLELINRLISEHKNIYCDQYIEFSVLLIDDDFKCKSYL IVACEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIPCKILR KCGDISENVVVNGRILTRGDILDCMTVTSIQP-HCIFLEREGLKYKEYNK LGXVPIVVDDNHQRYPEGAILNNCYRILAIRR-DAVIVSKGKREFVDQLNGGKPR YGAEGYKMSPGHWYFPSPL
CdsD YscD PrgH MxiG EscD CdsD YscD PrgH MxiG	721 312 326 304 304 780 365 373 351	LT-GYINNKDADKFRTVVQELQDIAGIAVKNFVVLLPAEGVIDLNMRYPGRYRVTGFS ELRGVNDEKLSSFNQLQQTFRQEFGNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILRARQFVDSYYRTWGGYVQFAIELKDDWLKGRSG SVREELTDEKLELINRLISEHKNIYGDQYIEFSVLLKDDWLKGRSYL IVAGEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIFPCKILR KCGDISENVVVNGRILTRGDILDCMTVTSIQP-HCIFLEREGLKYKFEYNK LGWPVVMDNQRYPEGAILNNCVRILAIRR-DAVIVSKGKREFVQLNGGKPR IGAEGYIKMSPGHWYFPSPL
CdsD YscD PrgH MxiG EscD CdsD YscD PrgH MxiG EscD	721 312 326 304 304 780 365 373 351 353	LT - GYINNKDADKFRTVVQELQDIAGI AVKNFVVLLPAEEGVIDLNMRYPGRYRVTGFS ELRGQVNDEKLSFNQLQQTFRQEFGNPKLELVNVGGQPQHDELNEVQAIS VIQGALDDVEILARQFVDSYYRWGGGYUEFAIELKDDDLKGRSFQ SVREELTDEKLELINRLISEHKNIYCDQYIEFSVLLIDDDEKGKSKI IVAGEVSQQNESKILAIINAMNKNSNVKILFQNIQPYISADIPCKILR KCGDISINVVVNGRILTRGDILDCMTVTSIQP-HCIFLEREGLKYKFEYNK LG

Supplementary Figure 5. Domain architecture of major inner membrane proteins CdsD and YscD and multiple sequence alignment of SctD homologues. The *Chlamydia* inner membrane protein, CdsD, contains an additional N-terminal segment of 360 residues (pink and grey) including an extra cytoplasmic FHA domain. Colored annotations in the sequence alignment correspond to the domains in the CdsD/YscD schematic. Accession numbers are listed for each bacterial species. CdsD, *C. trachomatis* (M9UG17); YscC. *Y. enterocolitica* (Q01245); PrgH, *S. enterica* (P41783). MxiG, *S. flexneri* (P0A221); EscD, enteropathogenic *E. coli* (B7UM96). Multiple sequence alignments were produced by ClustalW Omega and graphically illustrated with BOXSHADE. Identical and similar residues are shaded in black and grey respectively.



Supplementary Figure 6. Symmetry analysis of *Chlamydia* **T3SS sorting platform.** To detect symmetry in the sorting platform, a masked asymmetric average was compared to a similarly masked average that had been rotated axially by intervals corresponding to rotational symmetries from c1 to c30. The cross-correlation coefficient was plotted as a function of the corresponding symmetry. Peaks along the cross-correlation coefficient plot will be observed when a rotated average exhibits a high degree of similarity with the unrotated average, demonstrating rotational symmetry.



Supplementary Figure 7. Multi-reference alignment classification of *Chlamydia* subtomograms. Sub-tomograms were initially separated into 10 classes by multi-reference alignment. One of the 10 class averages contained mostly noise and thus has been eliminated. **a**, Central slices through 9 class averages of *Chlamydia* T3SSs. The class averages are sorted by basal body height (h), which varies from 34 to 29.5 nm. **b**, Distribution of host-free and hostcontact *Chlamydia* T3SSs across the 9 classes depicted in (a). The most populated classes are marked with an asterisk. Host-free T3SSs were mainly present in classes with elongated basal bodies (classes 1-6). Host-contact T3SSs were mainly present in classes with short, compact basal bodies (classes 7-9). **c**, Out of plane tilt of all sub-tomograms using MRA with 5 classes (left, and as shown in Fig. 3) and with 9 classes (right, as shown in (a)). There is no predominant bias in the distribution of particles into classes based on their out of plane tilt angle. Scale bar, 30 nm.