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Supplementary Information for

Chlamydia-induced curvature of the host-cell plasma membrane is required for infection

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Supplementary Methods

Plasmid constructs, cloning procedures

For heterologous secretion in *S. flexneri*, N-terminal sequences of *cpn0677* and *cpn0678* were integrated into pUC19cya using *Hind*III and *Xba*I sites. For protein expression, *cpn0677* and *cpn0678* were amplified from *C. pneumoniae* GiD genomic DNA and integrated into pFT25 to generate C-terminal 10His-Tag. Cpn678ΔAPH$_{10His}$ and Cpn678ΔPRR1$_{10His}$ were amplified by PCR from WT constructs and integrated into pFT25. GST-SNX9_pGEX5.1 was generated by cloning the SNX9 coding sequence into the *BamH*I and *Not*I sites of pGEX5X.1. GST-SNX9-SH3_pGEX was kindly provided by NM Alto. The SH3 domain of SNX9 was amplified from a WT construct and fused to GST by integration into pKM36.

CPn0678-GFP WT and deletion variants were generated by cloning of *cpn0678* into pKM55. mCherry expression constructs were generated by recloning of *Grb2*, *c-Cbl* and *Cin85* (gift from MA McNiven) into pKM95 (C-terminal mCherry), and *SNX9* and of deletion variants into pAE66 (N-terminal mCherry). SNX9 shRNA plasmids used for generation of stable cell lines were obtained by integration of SNX9 target sequences shRNA_1 (gagagtcagcatcatgtct) or shRNA_2 (taagcactttgactggttat) into *Mlu*I and *Cla*I sites of pLVTHM, respectively.

Transcript analysis

For transcriptome analyses throughout the chlamydial infection cycle (0-96 h.p.i.), a home-made microarray spotted with 300- to 500-bp PCR products covering all *C. pneumoniae* ORFs was used. Chlamydial mRNA and gDNA was isolated at 2, 6, 12 and 24 h.p.i. using an MOI of 100, and at 24, 36, 48, 72 and 96 h.p.i. using an MOI of 5. mRNA was Cy5-labeled during reverse transcription, mixed with fragmented gDNA labeled with Cy3 and hybridized to the array. Signal detection and quantification were performed using ImaGen and GeneSpring software by comparing mRNA/gDNA signal intensities of three biological replicates per time point. Differentially regulated genes are those that showed at least a 2-fold increase or decrease in signal intensity.

Quantitative real-time PCR (qRT-PCR)

HEp-2 cells were infected with *C. pneumoniae* (at MOI 100 and observed at 2-12 hpi, or at MOI 5 for analysis over the period 24-96 hpi) and nucleic acids were isolated at the indicated time points. cDNA was synthesized using pd(N)$_6$ random hexamers as primers on isolated RNA. To measure the ratio of mRNA per genome, previously isolated gDNA was used as a template in real-time PCRs following the manufacturer's instructions

(RealMasterMix SYBR ROX 2.5x, 5PRIME). ΔCT values were determined using defined amounts of the cloned plasmid pSW1. This plasmid contains a fragment of the chlamydial *ompA* gene and was used as a standard for real-time PCRs in a PCR ABI Prism 7000 (Applied Biosystems). The ratios of specific mRNAs to genomic DNA were calculated from the measured ΔCT values.

Co-immunoprecipitation

For *in vivo* co-immunoprecipitation (Co-IP) of infected cells, HEp-2 cells were cultivated to 100% confluency in 6-well plates and infected with gradient-purified *C. pneumoniae* EBs (MOI 100) by centrifugation for 20 min (25°C at 2900 rpm). After centrifugation, cells were shifted to 37°C and grown under 6% CO_2 for 15 min. Infected cells were washed three times with HBSS and lysed with Phospho-Lysis buffer (1% NP40, 1% Triton X100, 20 mM Tris, 140 mM NaCl, 2 mM EDTA, 1 mM Na_2VO_4, Merck Protease Inhibitor Cocktail). The lysate was cleared by centrifugation for 10 min at 4°C at 10,000x*g*. The supernatant was mixed with Protein G MicroBeads (Miltenyi Biotech) that had been preincubated with 2 µg antiSNX9 or anti-CPn0678 overnight at 4°C. Co-IP assays of co-transfected cells were performed using anti-GFP MicroBeads. Eluted proteins were resolved by SDS/PAGE and detected by immunoblot.

FRET-FLIM measurements and single-pixel FLIM analysis

Measurements and analysis were performed after 18 h of expression in HEp-2 cells incubated in a prewarmed environment (37°C). Time-domain FLIM was performed on a confocal laser scanning microscope (Zeiss LSM 780) additionally equipped with a single-photon counting device with picosecond time resolution (PicoQuant Hydra Harp 400). eGFP fluorescence was excited at 485 nm using a linearly polarized diode laser (LDH-D-C-485) operated at a repetition rate of 32 MHz. Excitation power was monitored and adjusted to around 1 µW at the objective (40× water immersion, Zeiss C-PlanApo, NA 1.2). The emitted light was collected and fluorescence was then detected by Tau-SPADs (PicoQuant) within a narrow segment of the eGFP emission spectrum (band-pass filter: HC520/35 AHF). Images were taken with 12.6 µs pixel time and a resolution of 0.17 µm per pixel (zoom 5, 256 × 256). A series of 80 frames was merged into one image and further analyzed. Additionally, fluorescence of mCherry was recorded by merging 10 frames excited with a DPSS-561 nm cw laser to verify acceptor presence. The instrument response function (IRF) of the setup was measured on each experimental day using a KI-quenched erithrosin B solution.

The fluorescence lifetime of eGFP was analyzed using the software tool SymPhoTime 64, version 2.3 (PicoQuant, Berlin, Germany). Due to the low excitation power used to prevent photobleaching during image acquisition, and the small pixel size adopted to increase spatial resolution, the number of photons per pixel was still low after merging of frames, ranging from 100 to a maximum of 1700 photons per pixel. An individual ROI was generated for every dataset to ensure that only pixels with a minimum of 200 photons contributed to the modeling of the average lifetime. The photon numbers within the ROI generate a histogram which is used for a single exponential fit to approximate the average eGFP lifetime with background contribution and correction for shifting of the IRF. The same model was applied to the CPn0678-GFP donor control and the CPn0678-GFP mCherry-SNX9 coexpression.

EGF uptake assay

HEp-2 cells were transiently transfected with CPn0678-GFP or GFP alone for 15 h, washed twice with PBS and placed in serum-free medium for 5 h at 37°C and 6% CO_2. EGF labeled with Alexa594 (100 ng/ml) was added for 15 min at 37°C. Cells were then washed twice with PBS and fixed with PFA for 10 min. EGF uptake was quantified by analyzing the presence of intracellular EGF vesicles in confocal images using ImageJ.

Pulldown assay

Recombinant His- or GST-tagged proteins were expressed in *E. coli* BL21 and purified according to the manufacturer's protocols for *cOmplete* His-Tag purification resin (Roche) and Pierce Glutathione Agarose (Thermo Scientific). Aliquots (200 µg) of purified protein were incubated for 2 h at 4°C with either His-Tag purification resin or Glutathione Agarose, then 200 µg of the test protein was added and incubated for an additional 2 h at 4°C or the incubation sequence was reversed. The mixture was washed four times with PBS containing 50 mM imidazole (Merck) or 50 mM TRIS/HCL pH 8 (Merck). Eluted fractions were collected by adding 100 µl of 500 mM imidazole in PBS or 50 mM TRIS/HCl and 10 mM reduced glutathione in PBS, resolved by SDS/PAGE and detected by immunoblot.

Preabsorption of polyclonal antibodies

Green fluorescent latex beads (diam. 1.036 µm, Polysciences) were coated with 40 µg/ml rCPn0677, rCPn0678 or BSA respectively. Purified antibodies were incubated with coated beads overnight at 4 C under constant agitation. The supernatant was collected after pelleting the beads by centrifugation (10 min at 17,000x*g*) and used for immunofluorescence staining.

Immunofluorescence staining

Transfected and/or infected cells were fixed at the indicated time points with 3% paraformaldehyde in PBS (PFA) for 10 min, then washed three times with PBS, and permeabilized with either 100% methanol for 10 min or with 2% saponin (Merck) in PBS for 20 min. Depending on the permeabilization protocol, primary antibodies were diluted in PBS or in 0.5% saponin solution and incubated for 30 min at 37°C. Cells were washed three times with PBS with or without 0.5% saponin and incubated with secondary antibodies (anti-rabbit/mouse Alexa488/594) for 30 min at 37°C in PBS with or without 0.5% saponin. DAPI was used to visualize DNA.

Infection experiments

HEp-2 cells either stably expressing, or transiently transfected with, the appropriate constructs were infected with *C. pneumoniae* EBs (MOI 1) by short centrifugation as described before, then shifted to 37°C for 2 h before the infection medium was replaced by fresh medium and the cells were incubated for 48 h. The number of inclusions formed was quantified by confocal imaging, using an antibody directed against the inclusion membrane protein Cpn0147 and anti-rabbit Alexa594, as described previously.

Internalization assay

HEp-2 cells stably expressing the appropriate constructs were cultivated to 70% confluency in 24-well plates on glass coverslips (Ø 1 cm^2), then infected with purified *C. pneumoniae* EBs (MOI 5) by centrifugation for 20 min (25°C at 2900 rpm). After centrifugation, cells were shifted to 37°C and grown under 6% CO_2 for 2 h, washed three times with PBS and fixed with 3% paraformaldehyde in PBS (PFA) for 10 min. Internalization rates were determined by immunostaining with anti-Chlamydia and anti-rabbit Alexa488 and DAPI. Cells were imaged by confocal microscopy and internalization ratios were determined by counting external Alexa488-positive and all DAPI-positive bacteria.

Pearson Correlation Coefficient estimation for non-random cololalization

"Pearson Correlation Coefficient" (PCC) of EGFR & Cpn0678, EGFR & SNX9 and SNX9 & CPn0678 signal pairs were estimated using the *"Fiji-plugin JaCoP".* In each image Regions Of Interest (ROIs) of 100x100 pixel were chosen representing regions of strong chlamydial infection (with Cpn), which were compared to regions with cytoplasmic background signal (w/o Cpn). Image metadata were directly read by the plugin and completed manually (e.g. numerical aberture of objective or wavelength). PCC values were estimated using "JaCoP". Simultaneously, the significance of the *"Costes*

randomization" was calculated by "JaCoP", which compares PCCs of the original data with the PCCs of data with a randomly shuffled channel using 1000 iterations. To further stress the *"Costes randomization"* we applied an 8 pixel blocksize, representing the average signal size of the EB surrounded by EGFR/ SNX9/ CPn0678 for the scrambling procedure. "Costes randomization" significance was performed on each ROI at a blocksize of 8 pixel. In total 23 of 24 ROIs chosen for "with Cpn" and 20 of 24 ROIs for "w/o Cpn" regions passed the *"Costes randomization"* test using a minimum significance level of 95 % at a blocksize of 8 pixel. ROIs below the significance level were excluded from the PCC analysis.

Supplementary Figures

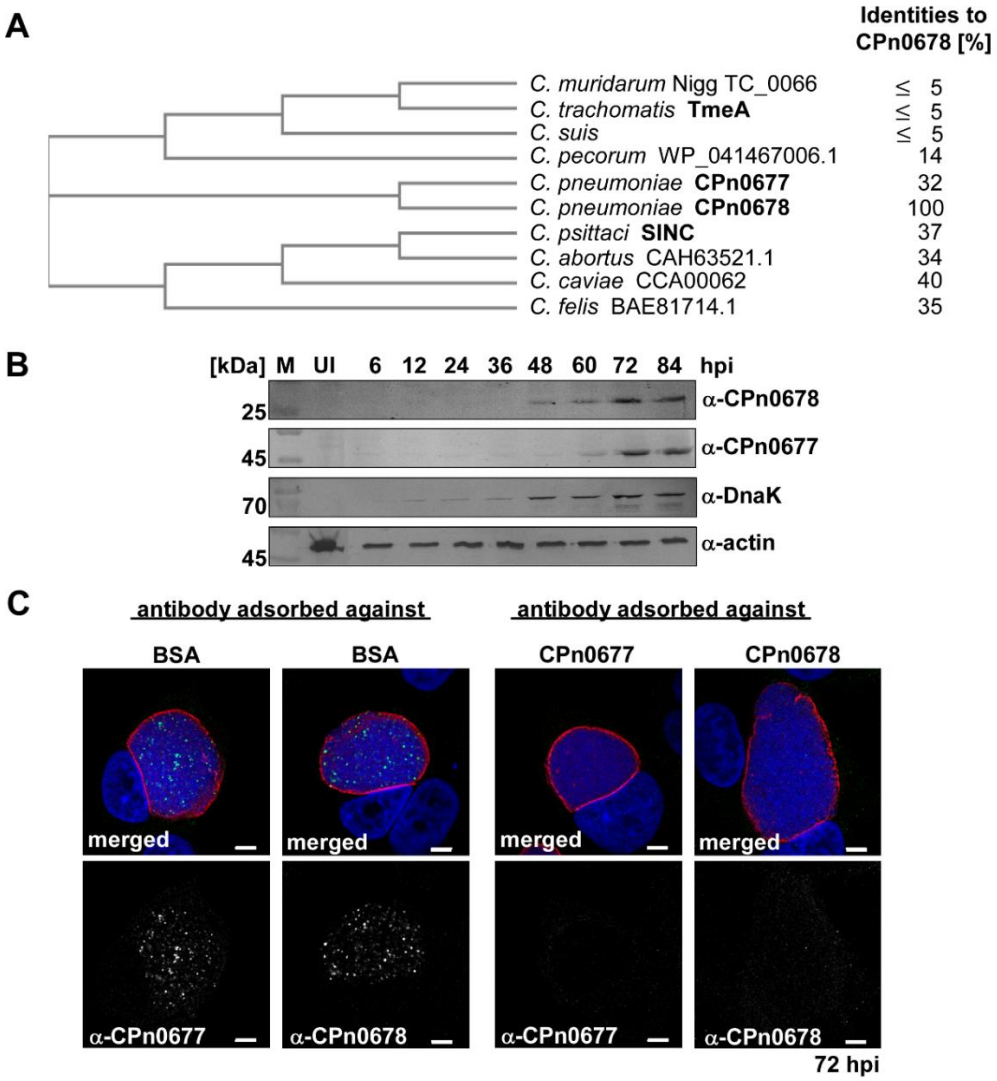

Figure S1: CPn0677 and CPn0678 are expressed late during infection.

(A) Phylogenetic analysis of CPn0678 homologs and syntenic proteins in the indicated species was performed using EMBL-EBI multiple sequence alignment and simple phylogeny tools. **(B)** Infected cells (MOI 5) were harvested at the indicated time points, and equal amounts of protein were fractionated by SDS/PAGE and probed with specific antibodies against CPn0677, CPn0678, DnaK and actin. **(C)** Confocal images of HEp-2 cells infected for 72 h, fixed with PFA, permeabilized with methanol and stained with antibodies against CPn0677 or CPn0678 which had been preabsorbed against either BSA (left panel) or recombinant CPn0677 or CPn0678 (right panel). Anti-CPn0677 and anti-CPn0678 antibodies were visualized with anti-rabbit Alexa488, and the inclusion membrane with anti-IncA antibody and anti-mouse Alexa 594. Host and bacterial DNAs were labeled with DAPI. Bar 5 µm.

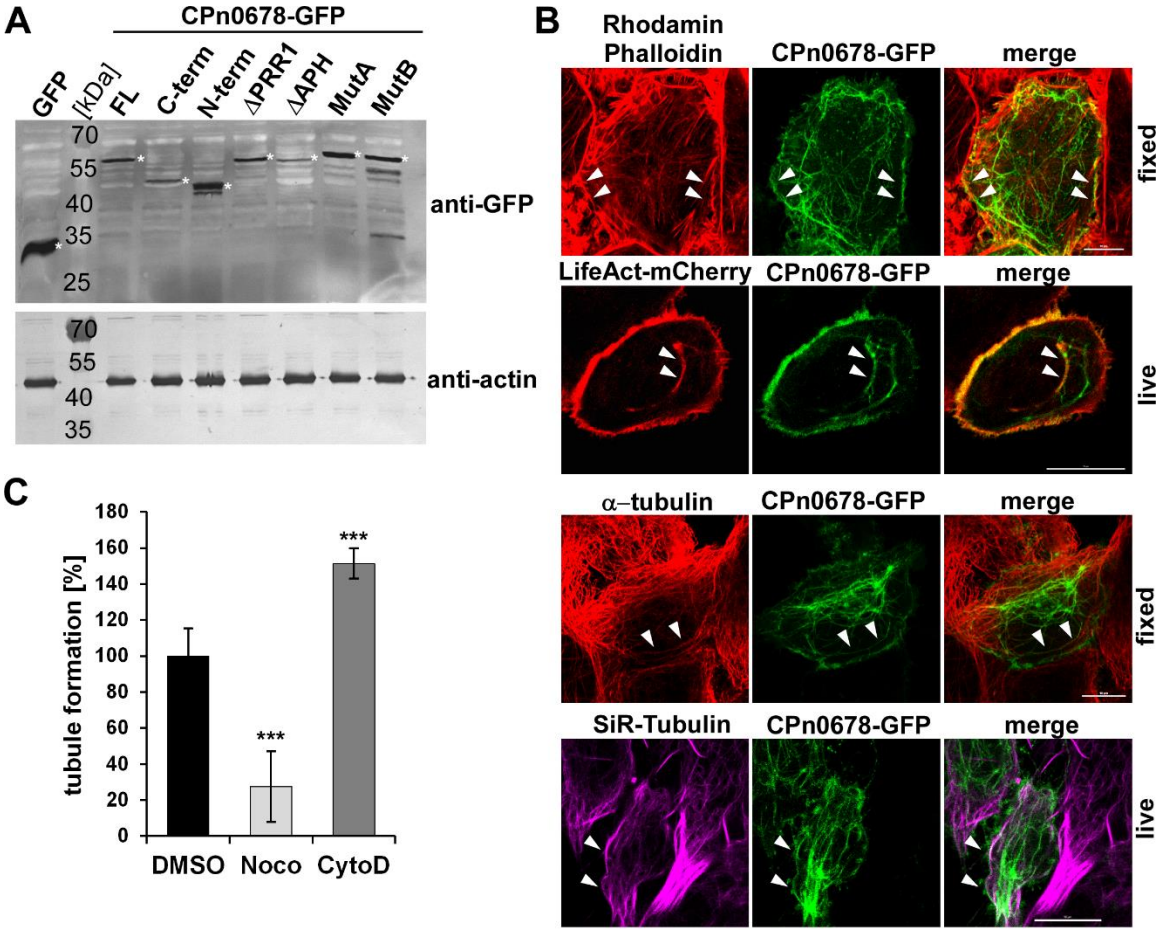

Figure S2: CPn0678-generated membrane tubules associate with actin filaments and microtubules.

(A) HEp-2 cells were transiently transfected with CPn0678-GFP or variants thereof for 24 h, lysed, fractionated by SDS/PAGE and probed with specific antibodies against GFP and actin. Expected bands are marked by white asterisks. **(B)** Confocal images of HEp-2 cells transfected for 15 h with CPn0678-GFP fixed and stained either with rhodamine phalloidin for actin, or with an anti-α-tubulin antibody and anti-mouse Alexa594, or live images of CPn0678-GFP-expressing cells cotransfected with LifeAct-mCherry or treated with SiR-Tubulin. White arrows mark sites of colocalization. Bar 10 µm. **(C)** Quantification of tubule formation in cells transfected for 15 h with CPn0678-GFP, and treated for 30 min at 37°C with DMSO Cytochalasin D (CytoD) or Nocodazole (Noco). On average, 80 cells were analyzed. Data are represented as mean ± SD (n=3). *P* value: *** ≤0.001.

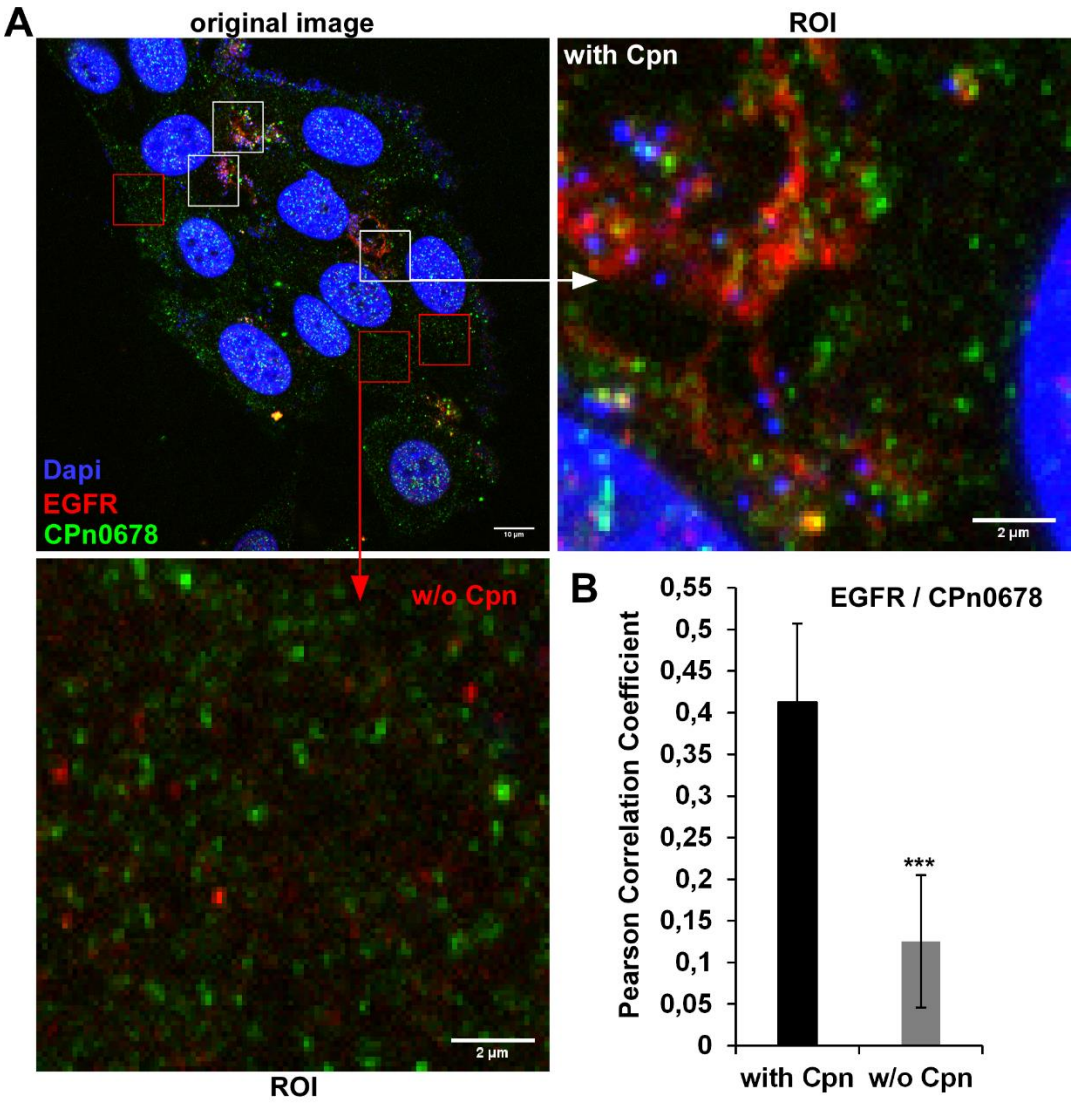
A
original image
ROI
with Cpn
Dapi
EGFR
CPn0678
w/o Cpn
2 µm
ROI
B
EGFR / CPn0678
Pearson Correlation Coefficient
0,55
0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
***
with Cpn
w/o Cpn
C
EGFR / SNX9
Pearson Correlation Coefficient
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
***
with Cpn
w/o Cpn
D
CPn0678 / SNX9
Pearson Correlation Coefficient
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
***
with Cpn
w/o Cpn

Figure S3: Pearson Correlation Coefficient estimation for non-random cololalization.

(A-D) Confocal images used for colocalization studies shown in Figs. 4A,B; 5A,B and 5D,E were subjected to the Fiji-plugin JaCoP for estimation of non-random colocalization. **(A)** ROIs of 100x100 pixel were chosen representing regions of strong chlamydial infection (with Cpn), which were compared to regions with cytoplasmic background signal (w/o Cpn). Bar 10 µm, insets 2µm. **(B-D)** PCC values of corresponding signal pairs of EGFR & Cpn0678 **(B)**, EGFR & SNX9 **(C)** and CPn0678 & SNX9 **(D)** were estimated using JaCoP. Data are represented as mean ± SD (n=3). *P* value: *** ≤0.001.

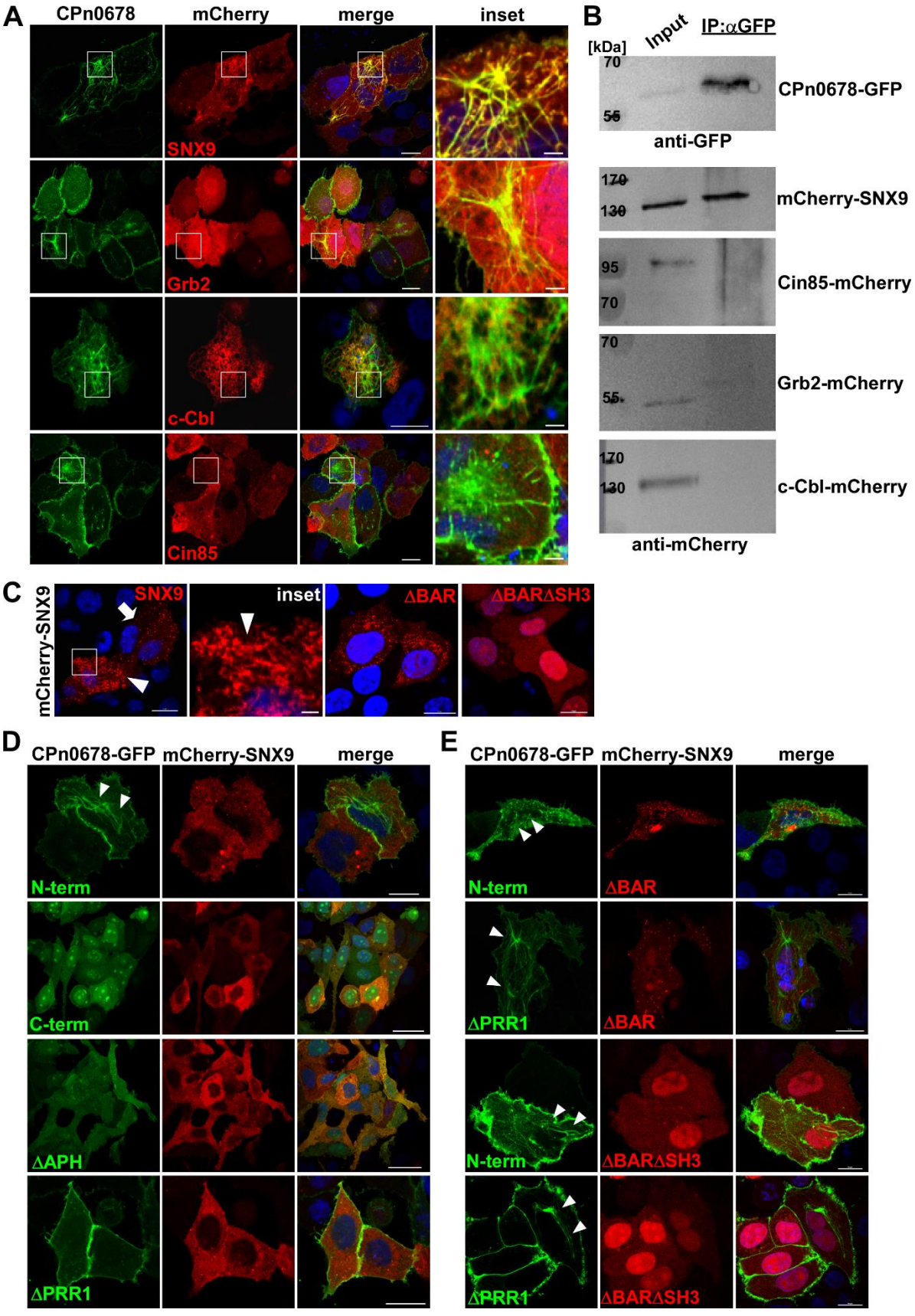
A
CPn0678
mCherry
merge
inset
SNX9
Grb2
c-Cbl
Cin85
B
[kDa]
Input
IP:αGFP
70
55
CPn0678-GFP
anti-GFP
170
130
mCherry-SNX9
95
70
Cin85-mCherry
70
55
Grb2-mCherry
170
130
c-Cbl-mCherry
anti-mCherry
C
mCherry-SNX9
SNX9
inset
ΔBAR
ΔBARΔSH3
D
CPn0678-GFP
mCherry-SNX9
merge
N-term
C-term
ΔAPH
ΔPRR1
E
CPn0678-GFP
mCherry-SNX9
merge
N-term
ΔBAR
ΔPRR1
ΔBAR
N-term
ΔBARΔSH3
ΔPRR1
ΔBARΔSH3

Figure S4: CPn0678 interacts with SNX9 via the first PRR domain.

(A) Confocal images of HEp-2 cells cotransfected with CPn0678-GFP and SNX9, Grb2, c-Cbl or Cin85, each fused to mCherry. Insets represent areas outlined in white boxes. Bar 10 µm, insets 1 µm. **(B)** Coimmunoprecipitation analysis of lysates of the HEp-2 cells shown in **(A)** using µMACS anti-GFP microbeads. Eluted samples were fractionated on SDS/PAGE and probed with antibodies against GFP and mCherry. **(C)** Confocal images of HEp-2 cells expressing mCherry-SNX9 or deletion variants (ΔBAR, ΔBARΔSH3). Inset represents the area in the white box. The white arrow marks vesicular SNX9, the white arrowhead points to the tubulation phenotype. Bar 10 µm, insets 1 µm. **(D, E)** Confocal images of HEp-2 cells cotransfected with mCherry-SNX9 **(D)** or deletion variants **(E)** in combination with various CPn0678-GFP variants. White arrowheads mark CPn0678-GFP-positive membrane tubules. Bar 10 µm.

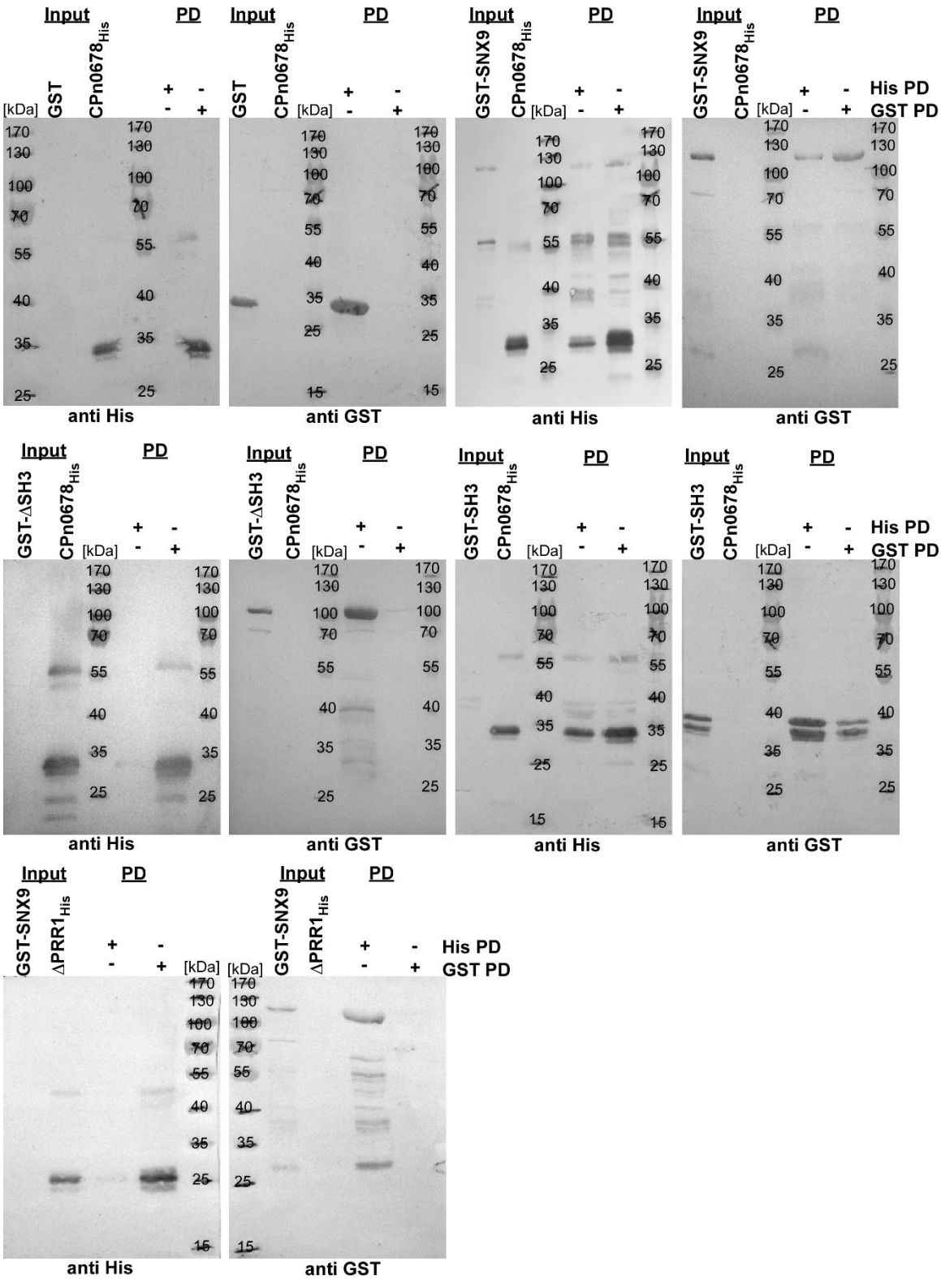
Input
PD
GST
CPn0678His
GST-SNX9
[kDa]
His PD
GST PD
anti His
anti GST
GST-ΔSH3
GST-SH3
ΔPRR1His

Figure S5: GST-SNX9 interacts with CPn0678$_{10His}$ via the first PRR domain in pulldown experiments.

Full immunoblots of pulldown experiments using purified recombinant GST or GST-SNX9 variants (ΔSH3, SH3) and CPn0678$_{10His}$ or CPn0678ΔPRR1$_{10His}$ shown in Fig.4C. Input and elution samples obtained from His-pulldowns and GST-pulldowns were fractionated by SDS/PAGE and probed with anti-GST and anti-His antibodies.

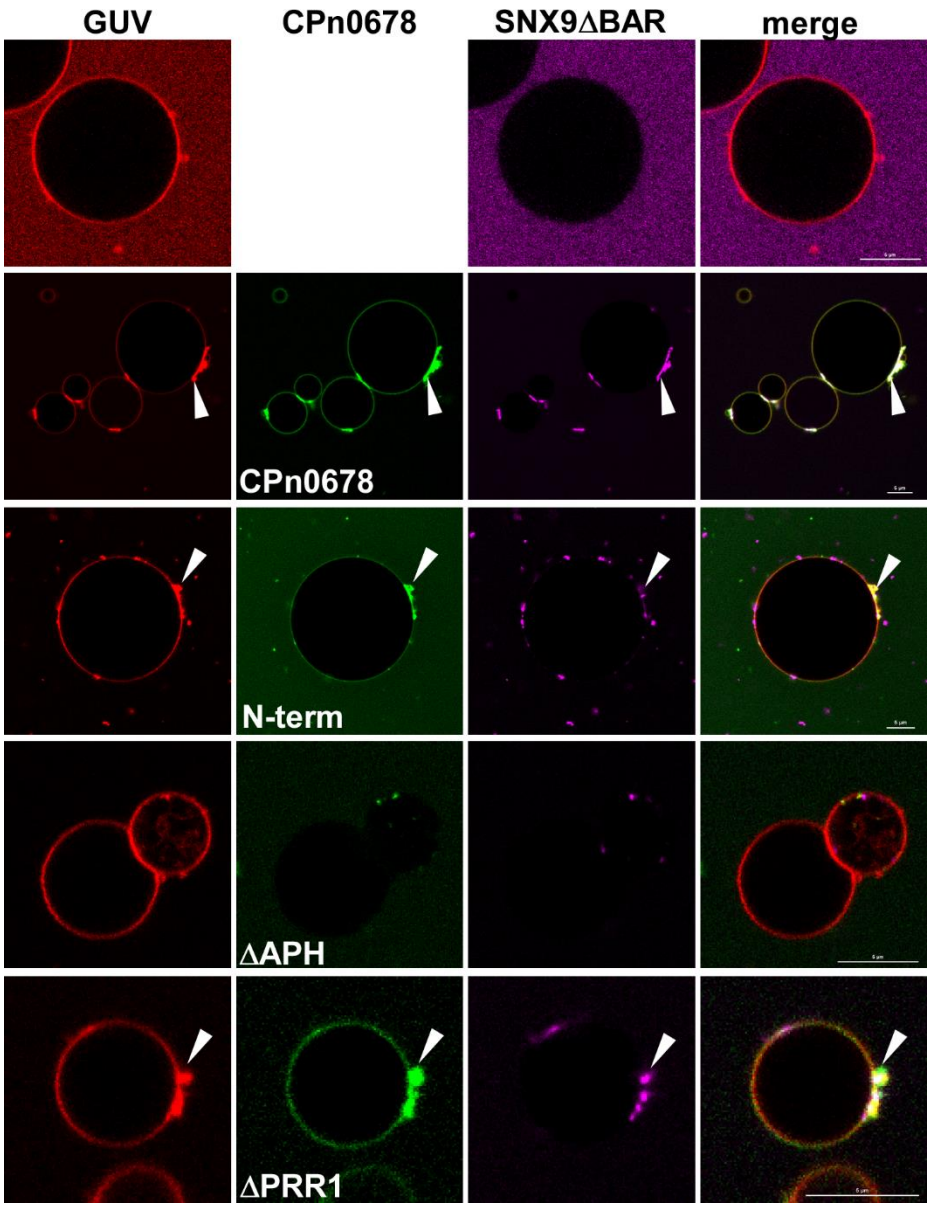

Figure S6: CPn0678 tubulates artificial GUV membranes and recruits SNX9 independently of the BAR domain.

Confocal images of GUVs containing PS and PIP4,5 stained with Texas Red, incubated for 10 min with DyLight650-labeled SNX9ΔBAR and then with FITC-labeled recombinant CPn0678 variants. White arrowheads mark recruitment of SNX9ΔBAR to CPn0678-mediated membrane tubulations. Full movies in Supplementary Information (Movies S1-14). Bar 5 µm.

Gene	Annotation	fold change 48h to 24h	fold change 72h to 24h
CPn0001	CPn0001	5,636750823	5,9693084
CPn0063	CPn0063	6,234661239	14,71591787
CPn0070	CPn0070	5,963166207	8,876614032
CPn0074	tufA	4,80222784	4,519117701
CPn0075	secE	3,816863106	3,195971074
CPn0081	rpoB	4,616851004	6,364280215
CPn0083	tal	3,267103236	3,070514282
CPn0104	CPn0104	3,121271447	3,69889154
CPn0105	CPn0105	5,299547897	7,567230545
CPn0117	trmD	3,403976579	2,653074447
CPn0121	CPn0121	3,524192589	2,82073811
CPn0134	groEL_1	7,137862952	10,12196602
CPn0135	groES	4,458671547	8,504381872
CPn0142	CPn0142	3,085759832	3,256367156
CPn0221	CPn0221	3,769521106	2,432021189
CPn0273	tdk	3,58238979	2,805801535
CPn0331	CPn0331	3,08932564	7,066483163
CPn0350	CPn0350	3,997664608	4,746521382
CPn0384	hctB	6,243293038	23,35712839
CPn0387	CesT	3,209297737	3,424596904
CPn0392	dcd	3,017808845	2,885840882
CPn0446	pmp_8	3,613773987	3,680811914
CPn0450	pmp10	4,229176216	11,55393438
CPn0453	pmp_13	3,225334003	2,415916297
CPn0498	CPn0498	3,306810145	5,673387521
CPn0502	grpE	4,959182475	6,721933579
CPn0503	dnaK	9,638993899	6,866805375
CPn0521	glyA	4,160381583	3,8166806
CPn0525	CPn0525	3,351073037	2,775699902
CPn0533	NrdR	3,023427178	2,637249774
CPn0557	omcB	24,23364768	71,65717528
CPn0558	omcA	25,71720254	75,32399083
CPn0560	gltX	4,33112995	5,394737039
CPn0564	secD/secF	3,111667379	2,737708025
CPn0572	Tarp	3,216960244	5,66072443
CPn0604	fliY	5,264478678	3,957157533
CPn0610	rho	4,268642364	2,276450897
CPn0623	CPn0623	5,803184015	2,577977638
CPn0624	gapA	4,20749384	2,722804801
CPn0637	rl14	4,371686142	7,695898741

CPn0638	rs17	3,293526802	2,614938829
CPn0639	rl29	3,053997208	2,032349506
CPn0641	rs3	4,105711779	3,373365178
CPn0642	rl22	3,318607439	2,659027068
CPn0644	rl2	3,824933838	2,270614442
CPn0646	rl4	3,975266781	2,663691329
CPn0647	rl3	3,209502269	2,056019633
CPn0650	lpxA	5,373997691	3,631535883
CPn0656	CPn0656	3,530970339	3,523790031
CPn0661	mip	2,967889366	3,017835292
CPn0677	CPn0677	4,428453467	8,292621175
CPn0678	CPn0678	3,932756299	8,753746766
CPn0684	parB	3,992034508	6,650759783
CPn0693	CPn0693	6,925530235	10,24016188
CPn0695	ompA	7,913186864	10,11095233
CPn0699	rrf	4,604749085	3,848708393
CPn0704	fliN	3,820195584	6,297715929
CPn0705	CPn0705	5,703365947	6,930167613
CPn0707	yscN	3,275211221	4,588337382
CPn0708	CPn0708	6,297047366	11,0428756
CPn0709	CPn0709	6,163549626	9,132716774
CPn0710	CPn0710	5,324950954	5,794437208
CPn0711	CPn0711	3,625958098	3,835542043
CPn0718	CPn0718	3,17039914	3,299864175
CPn0725	CPn0725	3,210708213	3,395663651
CPn0726	CPn0726	4,626968354	12,30647872
CPn0733	rs4	3,839536253	4,448298678
CPn0748	ispA	3,164654888	2,80526245
CPn0754	rs20	3,542849838	4,168256764
CPn0756	rpoD	6,399320818	7,155749341
CPn0809	CopB	5,005876779	12,24520674
CPn0810	CPn0810	5,855721004	8,739304991
CPn0854	porB	6,365874268	6,914044482
CPn0877	ybcL	5,099918455	6,025136319
CPn0884	YajC	4,268163969	3,499484265
CPn0909	rsbV_2	5,230623806	6,439035859
CPn0951	rs6	5,013305187	3,143890631
CPn0963	pmp_21	4,450203889	4,994199482
CPn0979	htrA	3,041502413	2,031007588
CPn0984	nrdA	3,145236674	3,42077434
CPn0985	nrdB	4,201575217	4,748826062

CPn0997	mesJ	6,07740541	6,282719074
CPn0998	ftsH	3,26511914	4,116033268
CPn0999	pnp	6,689058981	5,278182514
CPn1025	pgi	3,342370146	4,428105883
CPn1028	mdhC	3,589979954	3,038683291
CPn1031	arcD	5,355892407	6,353506298
CPn1032	AaxB	5,928502191	8,630076346
C. pn. specific			> 3-fold

Table S1: Transcriptional analysis of *C. pneumoniae* genes upregulated late in infection.

Gene names and Uniprot annotations (www.uniprot.org) are listed for 88 *C. pneumoniae* genes that are upregulated late in infection (≥ 3-fold) as indicated by comparative microarray studies. The Table shows the calculated fold-change for comparisons of 48 hpi to 24 hpi and 72 hpi to 24 hpi.

Movie S1 (separate file). Untreated (control) GUVs.
GUVs containing PS and PIP4,5 were stained with Texas Red, and imaged for 10 min.

Movie S2 (separate file). GUVs with CPn0678.
2 µg CPn0678 labeled with FITC was added to GUVs containing PS and PIP4,5 stained with Texas Red and imaged for 5 min in PBS.

Movie S3 (separate file). GUVs with CPn0678 N-term.
A 2-µg aliquot of CPn0678 N-term labeled with FITC was added to GUVs containing PS and PIP4,5 stained with Texas Red and imaged for 5 min in PBS.

Movie S4 (separate file). GUVs with CPn0678 ΔAPH.
A 2-µg aliquot of CPn0678 ΔAPH labeled with FITC was added to GUVs containing PS and PIP4,5 stained with Texas Red and imaged for 5 min in PBS.

Movie S5 (separate file). GUVs with CPn0678 ΔPRR1.
A 2-µg aliquot of CPn0678 ΔPRR1 labeled with FITC was added to GUVs containing PS and PIP4,5 stained with Texas Red and imaged for 5 min in PBS.

Movie S6 (separate file). GUVs with SNX9.
A 2-µg aliquot of SNX9 labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and imaged for 10 min.

Movie S7 (separate file). GUVs with SNX9 and CPn0678.
A 2-µg aliquot of SNX9 labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 labeled with FITC. GUVs were imaged for 5 min.

Movie S8 (separate file). GUVs with SNX9 and CPn0678 N-term.
A 2-µg aliquot of SNX9 labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 N-term labeled with FITC. GUVs were imaged for 5 min.

Movie S9 (separate file). GUVs with SNX9 and CPn0678 ΔAPH.

A 2-µg aliquot of SNX9 labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 ΔAPH labeled with FITC. GUVs were imaged for 5 min.

Movie S10 (separate file). GUVs with SNX9 and CPn0678 ΔPRR1.

A 2-µg aliquot of SNX9 labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 ΔPRR1 labeled with FITC. GUVs were imaged for 5 min.

Movie S11 (separate file). GUVs with SNX9ΔBAR.

A 2-µg aliquot of SNX9ΔBAR labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and imaged for 10 min.

Movie S12 (separate file). GUVs with SNX9ΔBAR and CPn0678.

A 2-µg aliquot of SNX9ΔBAR labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 labeled with FITC. GUVs were imaged for 5 min.

Movie S13 (separate file). GUVs with SNX9ΔBAR and CPn0678 N-term.

A 2-µg aliquot of SNX9ΔBAR labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 N-term labeled with FITC. GUVs were imaged for 5 min.

Movie S14 (separate file). GUVs with SNX9ΔBAR and CPn0678 ΔAPH.

A 2-µg aliquot of SNX9ΔBAR labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 ΔAPH labeled with FITC. GUVs were imaged for 5 min.

Movie S15 (separate file). GUVs with SNX9ΔBAR and CPn0678 ΔPRR1.

A 2-µg aliquot of SNX9ΔBAR labeled with DyLight was added to GUVs containing PS and PIP4,5 stained with Texas Red and incubated for 10 min prior to addition of 2 µg CPn0678 ΔPRR1 labeled with FITC. GUVs were imaged for 5 min