- Supplementary Information -

Structure of the Human Signal Peptidase Complex Reveals the Determinants for Signal Peptide Cleavage

Liaci et al. 2020

Content:

- Supplementary Text
- Supplementary Figures 1-17
- Supplementary Tables 1-3
- Legends for Supplementary Movies 1-3

Supplementary Text

Native mass spectrometry of SPC paralogs

Upon gas-phase activation, SPC dimers, overall, exhibit comparable dissociation stabilities, whereby SPC-A exhibits a slightly more stable dimer with ~45 % of total ion intensity corresponding to the ejected subunits compared to ~65 % for SPC-C at the same dissociation conditions. Additionally, SEC11A has a more stable conformation within the dimeric complex compared to SEC11C, which is estimated based on the intensity-weighted retained charge of an ejected subunit. Here, in the case of SPC-A, SEC11A and SPC22/23 retain ~32% and ~53% of their original precursor charges, respectively, upon dissociation; in the case of SPC-C, SEC11C and SPC22/23 retain ~44% and ~36% of their original precursor charges, respectively. The higher average charge of dissociated SEC11C is indicative of a more extended conformation, *i.e.* a higher degree of unfolding (98, 99), compared to SEC11A.

Supplementary Figures

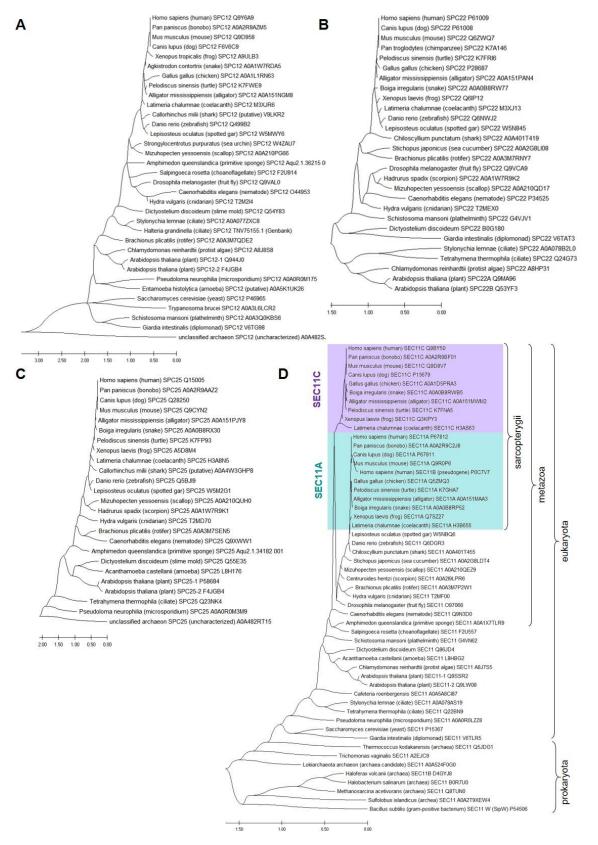


Figure S1. | **Phylogeny of SPC subunits.** (**A**) Phylogenetic tree of SPC12. (**B**) Phylogenetic tree of SPC22/23. (**C**) Phylogenetic tree of SPC25. (**D**) Phylogenetic tree of ER-type SPases. SEC11A sequences are highlighted in teal, SEC11C in purple. SEC11C proteins form a distinct clade. The earliest known ancestor with two SEC11 paralogs is the 'living fossil' coelacanth *Latimeria chalumnae*. The evolutionary distances (x-axis) are in the units of the number of amino acid substitutions per site.

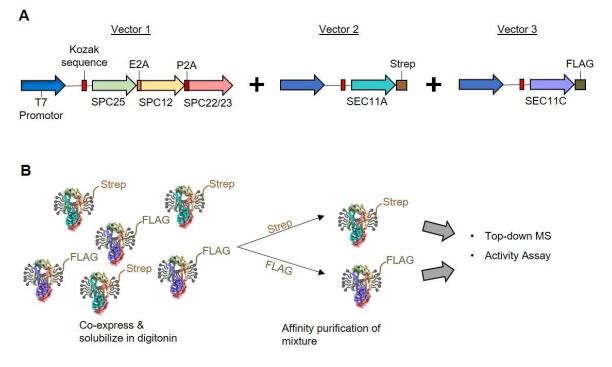


Figure S2 | Expression constructs and purification workflow for SPC composition experiments. (A) Expression constructs used for the analysis of SPC composition. (B) Description of the purification workflow. Accessory subunits were co-expressed in HEK 293 cells with SEC11A-Strep and SEC11C-FLAG in a vector mass ratio of 4:1:1 and solubilized in digitonin. The batch was purified with both Strep and FLAG affinity resin, and the respective eluates were analyzed for composition and *in vitro* activity.

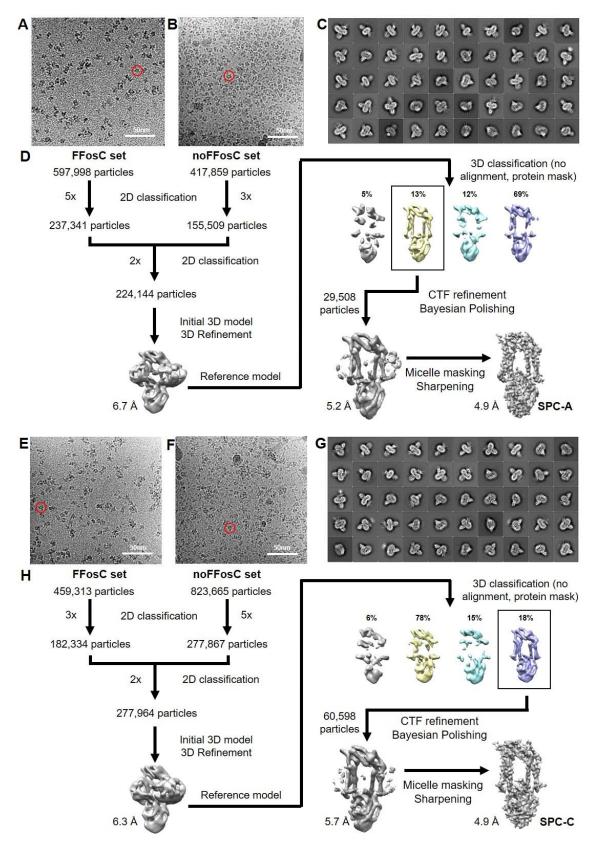


Figure S3 | Single Particle Data Processing Workflow. (A) Representative micrograph of SPC-A collected with 1.5 mM FFosC at 2.6 μ m defocus. Exemplary particle highlighted by a red circle. (B) Representative micrograph of SPC-A collected without FFosC at 2.9 μ m defocus. Protein particles are interspersed with empty amphipols. (C) Top 50 2D classes for the SPC-A dataset. (D) Processing workflow for the SPC-A dataset. (E) Representative micrograph of SPC-C collected with 1.5 mM FFosC at 2.6 μ m defocus. (F) Representative micrograph of SPC-C collected with 1.5 mM FFosC at 2.6 μ m defocus. (F) Representative micrograph of SPC-C collected with 0.5 mM FFosC at 2.6 μ m defocus. (H) Processing workflow for the SPC-C dataset.

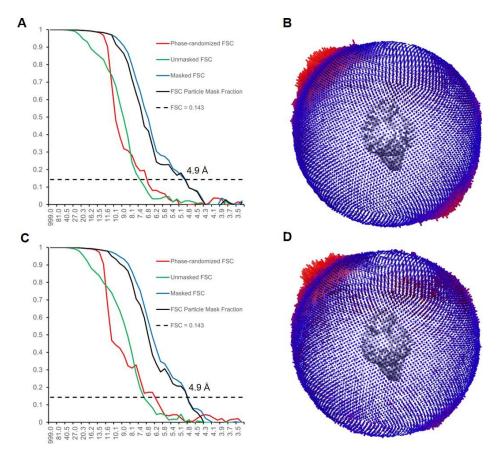


Figure S4 | Cryo-EM map quality metrics. (A) FSC curve of SPC-A as reported by Relion. (**B**) Angular distribution of particles within the SPC-A dataset (**C**) FSC curve of SPC-C as reported by Relion. (**D**) Angular distribution of particles within the SPC-C dataset.

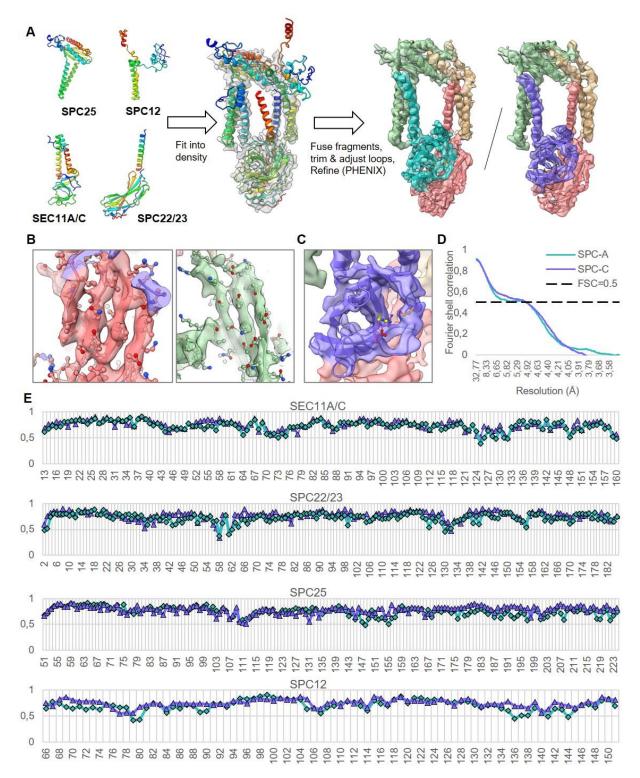


Figure S5 | SPC model building. (A) Initial structures of SPC subunits were calculated using trRosetta and fulllength sequences (UniProtKb IDs Q15005, Q9Y6A9, P61009, P67812, Q9BY50). These models were fitted into the observed density maps. Flexible regions were trimmed and adjusted using all-atom refinement in Coot 0.9 with tight geometry restraints. The models were refined against the density maps using PHENIX real space refine. If necessary, rotamer outliers and regions with poor density fit were adjusted in Coot and subjected again to PHENIX real space refine. Individual subunits are colored according to their residue number (from blue to red), and the subunits of assembled SPC-A and SPC-C are colored as in **Fig. 1**. (**B**) Representative map features of SPC-C. Beta sheets and large, well-ordered side chains can be distinguished. (**C**) Map-to-model fit of the luminal domain of SPC-C. (**D**) Map-to-model FSC curves for SPC-A and SPC-C as determined by PHENIX Mtriage. (**E**) Per-residue real space correlation as reported by PHENIX real space refine. Teal diamonds = SPC-A subunits; purple triangles= SPC-C subunits.

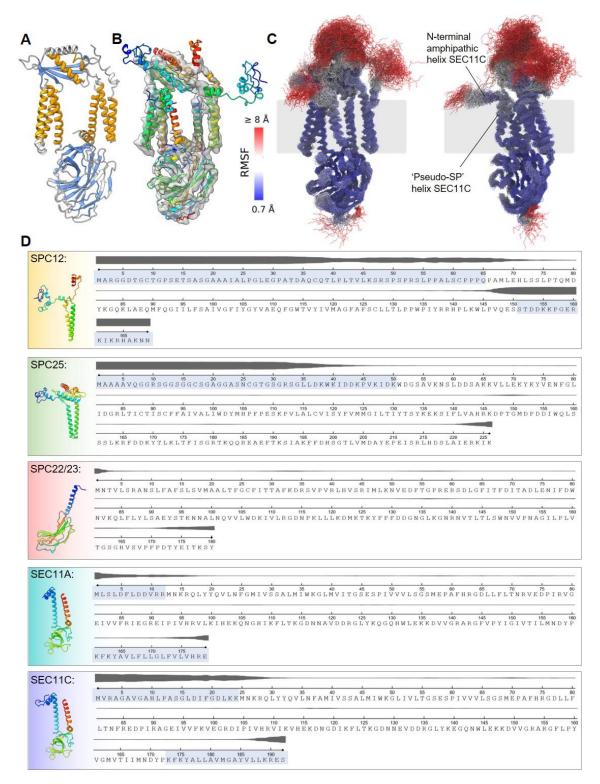


Figure S6 | **Secondary structure and disorder predictions.** (**A**) Predicted secondary structure elements (orange = alpha helices; blue = beta strands) are mapped onto the observed SPC-C model. (**B**) Areas without ordered density are depicted. (**C**) Flexibility of the SPC-C model in atomistic MD simulations. Two views of representative aligned snapshots obtained from a 150 ns MD simulation of SPC-C embedded in POPC are shown. The color scale represents the backbone RMSF in relation to the average structure of the simulations. Termini of SEC11 and the membrane (grey transparent box) are indicated. Except for the experimentally unresolved parts, the overall organization of the SPC complex was maintained. Some small relaxation of TM helices is observed which is consistent with the different environment of experiments (amphipol micelle) and simulations (phospholipid bilayers). (**D**) Disorder predictions for each subunit are shown as grey lines. The thicker the line, the higher the disorder probability. Highlighted sequence stretches are not resolved in the atomic models. Full-length trRosetta models (colored as in Fig. S5) are shown.

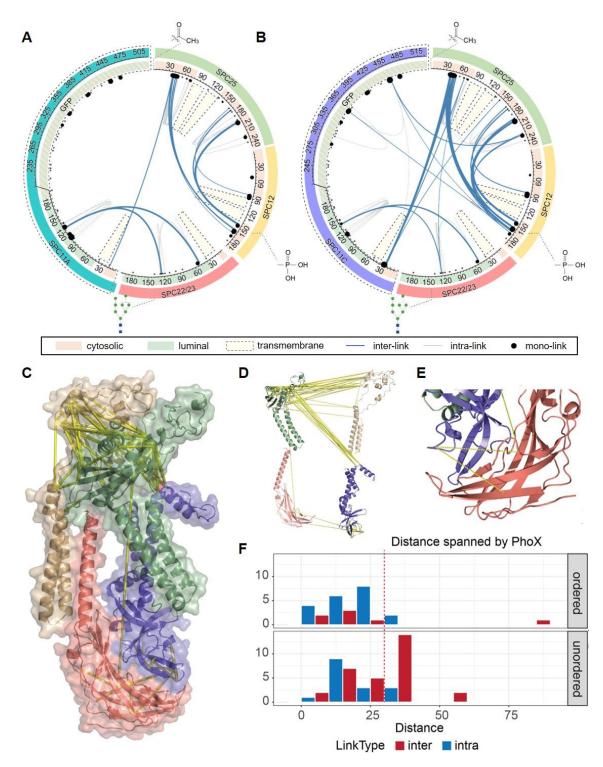


Figure S7 | **XL-MS of SPC-A and SPC-C.** Circos representation of the detected cross-links for SPC-A and SPC-C demonstrates excellent overlap in the structure. As anticipated, no cross-links are detected inside the transmembrane domains. In addition, very few cross-links are detected to the exposed GFP tag, indicating this protein region remains out of distance from the complex. (**A**) Detected cross-links for SPC-A. (**B**) Detected cross-links for SPC-C. (**C**) The identified cross-links cluster in the exposed regions of the complex with a heavy preference for the cytosol-exposed part. (**D**) The organization of the individual subunits in the complex is readily revealed by the identified cross-links. (**E**) The SPC22/23-SEC11A/C interface, as revealed by native mass spectrometry, is highly stable. Relatively few cross-links are located here due to the low flexibility and lower abundance of lysines. (**F**) The measured distances in ordered parts of the complex reveal that the intra-links are well within the distance constraint determined for the PhoX crosslinker (24) (indicated by a red dashed line). The distances for the disordered regions are an estimation based on the structural model predicted by trRosetta. These regions were not detected in the EM density and likely represent a high degree of error.

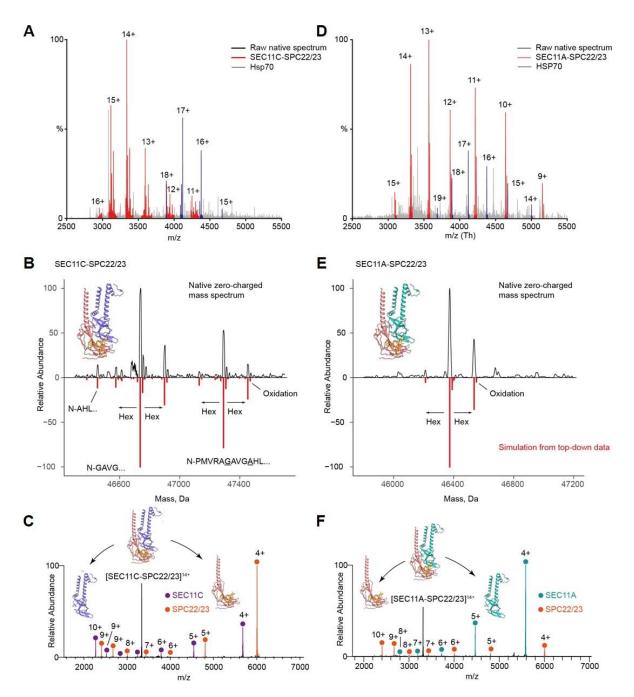


Figure S8 | Native MS analysis of SPC22/23-SEC11 subcomplexes in SPC-A and SPC-C. Samples were prepared in 0.15 M ammonium acetate containing 0.01% β -DDM. (A) Raw native MS spectrum demonstrating the SPC22/23-SEC11A dimer as the primary species in the SPC-A sample. The peripheral subunits SPC12 and SPC25 are likely lost when the samples are buffer exchanged. (B) Identification of SEC11C-SPC22/23 in the deconvoluted zero-charged spectrum by comparison with simulated mass spectrum from top-down MS. Primary PTMs contributing to microheterogeneity are annotated in the simulated spectrum. (C) Gas-phase activation of the most abundant 14+ charge state of SEC11C-SPC22/23 dimer confirms the oligomeric state and identity of non-covalently attached subunits. (D-F), Same as A-C but for the SPC-A complex.

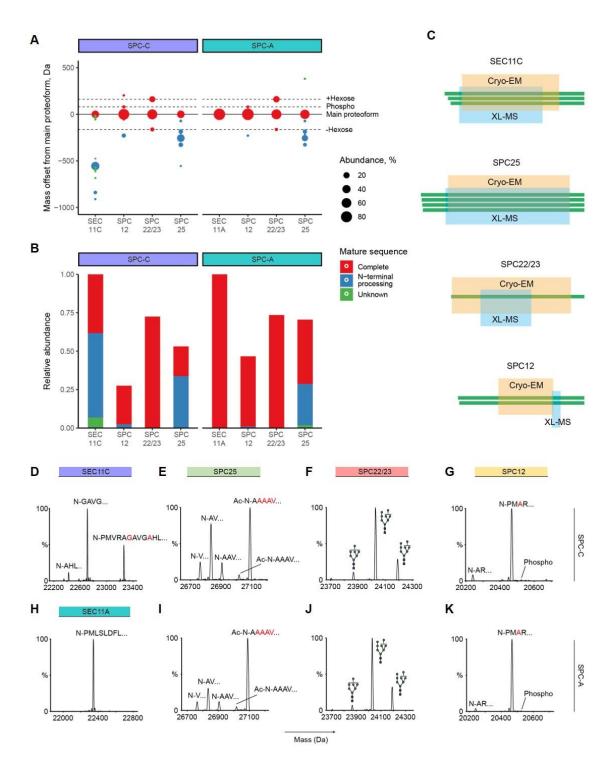


Figure S9 | Top-down mass spectrometry reveals mature sequences and SPC proteoforms. (A) Proteoform mass offsets determined for SPC subunits. Each offset is defined by the difference between a mass of a proteoform and the mass of the primary proteoform. The most prominent PTMs are highlighted with dashed lines. Main proteoforms are highlighted by a solid line. Apart from SEC11C and the N-glycosylated form of SPC22/23, the main proteoforms of all SPC subunits are unmodified. Circle size represents the fractional abundance of a proteoform per subunit. (B) Abundances of SPC subunits demonstrating the contribution of complete sequences, sequences with N-terminal processing, and undetermined sequence processing. (C) Sequences of distinct SPC proteoforms in the context of sequence coverages achieved with cryo-EM (orange) and XL-MS (blue). (D-K) Mass profiles displaying the primary proteoforms of SPC subunits for SPC-C (D-G) and SPC-A (H-K) complexes. For subunits with N-terminal sequence processing, a few N-terminal amino acids are displayed above the corresponding peaks. SPC25 was identified in N-acetylated and in N-terminally truncated, non-acetylated forms. For SPC22/23, the identified N-glycans are indicated above the corresponding proteoform peaks. A low-stoichiometric phosphorylated form of SPC12 is annotated in (G,K).

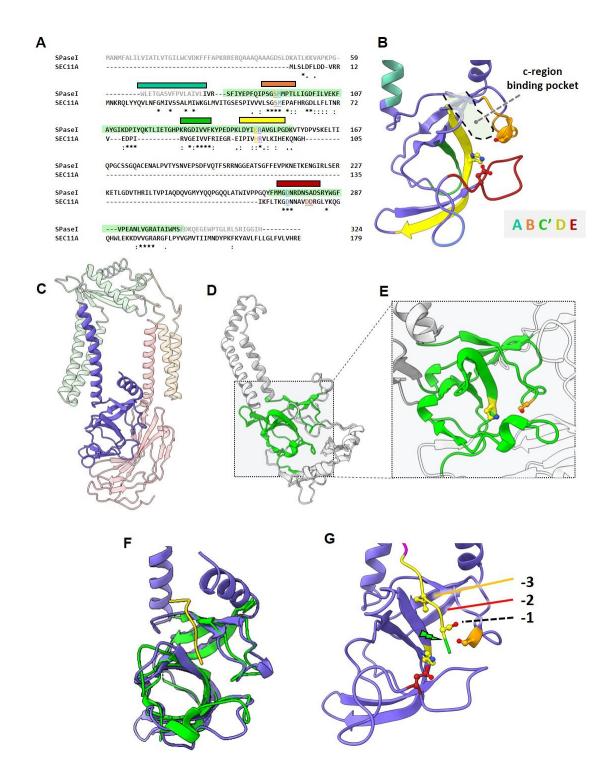


Figure S10 | Comparison of human SEC11 and *E.coli* **SPase I reveals the c-region binding pocket**. (A) Pairwise sequence alignment of SEC11A (UniProtKb ID P67812) and *E.coli* SPase I (UniProtKb ID P00803). Conserved boxes are highlighted by color (A = turquoise, B = orange; C' = green; D = yellow; E =brown). Sequence of the SPase I core highlighted in green. (B) The conserved boxes map to the transmembrane segment and SP binding pocket of SEC11. (C) Atomic model of SPC-C, colored as in **Fig. 1**. (D) trRosetta model of full-length *E.coli* SPase I. The catalytic domain is highlighted in green. (E) Zoom- in into the SP binding pocket of *E.coli* SPase I. The catalytic Ser-Lys dyad is highlighted. (F) Superposition of the luminal SEC11C portion with *E.coli* SPase I complexed with the lipopeptide inhibitor arylomycin (PDB-ID 1T7D, inhibitor in yellow). (G) Based on F, the c-region of bovine pre-prolactin was tentatively placed into the SEC11C binding pocket. In this hypothetic model, as in the bacterial enzyme, the inhibitor is forced into a beta strand conformation. The -1 and -3 positions point towards the shallow, hydrophobic pocket. The scissile bond (indicated with a green arrow) is located close to the catalytic residues.

Species/Abbry													1111111	1111.	TTITT						•
1. Homo_sapiens_(human)_SEC11C_Q98Y50				# V	RAGA	VGABL	PASGI	D	FODLK	K 12 N H	ROLYN	OVENT	AMIVS	ALMIW	GLIVE	T a S E S	PVVVL	SOSM	PAFHR	DLLF	TNFREDP
2. Pan_paniscus_(bonobo)_SEC11C_A0A2R98F01				M V	RAGA	VGANI	PASSI	0	IFGDLK	X H N F	ROLYN	QVLNI	AUIVS	ALMIW	GLIVL	TOSES	PIVVVL	SOSM	PAEHRS	DLLF	LTNFREDP
3. Canis_lupus_(dog)_SEC11C_P13679				M.V.	RAGA	VGTH	PASOL	0	FODLR	KH N P	ROLYN	AVLNT	AHIVS	ALMIW	GLIVE	TGSES	PIVVVL	SOSM	PAFHR	DLLF	LTNFREDP
4. Mus_musculus_(mouse)_SEC11C_Q9D8V7				MV	RAGA	VOTEL	PTSSI	D	FODLR	KH N H	ROLMO	OVENT	ANIVS	ALMIW	GLIVI	TOSES	PIVVVL	S Q S M B	PAFHRO	DILF	LTNFREDP
5. Gallus_gallus_(chicken)_SEC11C_A0A1D5PRA3									LIGDLR	R M N H	ROLVI	QVLNI	AHIVS	ALMIW	GLIVI	GSES	PIVVVL	5 G 5 M 5	PAFHRO	DLLT	LTNIHDDP
5. Aligator_mississippiensis_(aligator)_SEC11C_A0A151MWM2							1	D	LFGDLR	RN N.H	ROLYN	QVLN P	FANI VS	SALMIW	GLIVV	TOSES	PIVVVL	5 0 5 M	PAFHR	DLLF	LTNFQDDP
7. Bolga_irregularis_(snake)_SEC11C_A0A0B8RWB5								D	FGDLR	R U N H	ROLYS	QVLNI	AWIVS	SALMIW	COL (V)	T G S E S	PIVVVL	SOSM	PAFHR	DLLF	LTNFRDDP
8. Pelodiscus_sinensis_(turtle)_SEC11C_K7FNA5								D	FGDLR	R.H N.M	ROLVY	OVLN7	FAMINS	SALMIW	GLIVI	T G S 📕 S	PIVVVL	SGSM	PAFHRS	DLLF	LTNFQEDP
9. Xenopus_laevis_(frog)_SEC11C_Q3KPY3								D	LFGDLR	RH N H	ROLYN	OVLNE	AMIVS	SALMIW	6 L I V V	TOSES	PIVVVL	SOSME	PAFHRO	DLLF	LTNFGEDP
10. Latimeria_chalumnae_(coelacanth)_SEC11C_H3AS63								N	LLGDIR	RL N M	ROLVA	4 1.1 1	AMMVS	SALMIW	GLIVE	TESES	PVVVL	SGSM	PVFHR	DLLLL	LTNYQEDP
11. Homo_sapiens_(human)_SEC11A_P67812							W L S I	D	FLDDVR	R M N H	ROLYS	OVI.N.	GUIVS	SALMIW	GLUVI	TGSES	PVVVL	S 0 5 11 8	PAFHR	DLLF	LTNRVEDP
12. Pan_paniscus_(bonobo)_SEC11A_A0A2R9C2J8							#LSI		FLODVR	R M N M	ROLYS	OVENT	GNIVS	SALMIW	GLMVI	13525	PIVVVL	5 0 5 M 8	PAFHR	DLLF	LTNRVEDP
13. Canis_lupus_(dog)_SEC11A_P67811		* * * * *					W L S 1	D	FLODVR	R.M N.H	ROLYN	OVLN	GUIVS	SALMIW	GLMVI	GSES	PIVVVL	SGSM	PAFHR	DLLF	LTNRVEDP
14. Mus_musculus_(mouse)_SEC11A_Q9R0P6							NLSI	. D	FLOOVR	R M N H	ROLYS	QVI,NF	GHIVS	5 ALMIW	GLMVI	T G S E S	PIVVVL	5 0 5 M	PAFHRO	DULF	LTNRVEDP
15. Galus_galus_(chicken)_SEC11A_Q5ZMQ3							WISI	D	FLDDVR	R.12 N H	ROLM	QVLNT	GIIIVS	SALMIW	C G L M V V	T G S E S	PIVVVL	SGSM	PAFHR	DLLT	LTNRIEDP
16. Aligator_mississippiensis_(aligator)_SEC11A_A0A151MAA3							• • # L S L	D	FLODVR	R M N H	RQLVY	QVLNI	GIIIVS	SALMIW	GLNVV	GSES		SOSNE	PAFHR	DLLF	LTNRIEDP
17. Bolga_irregularis_(snake)_SEC11A_A0A0B8RP52								D	FLODVR	R N N H	ROLYN	VLN7	0 11 1 V S	SALMIW	GLMVA	T G S E S	PIVVVL	SOSM	PAFAR	DLLF	LTNRIEEP
18. Pelodiscus_sinensis_(turtle)_SEC11A_K7GHA7		****		* * * *									• # V S	SALMIW	GLUVV	GSES	PIVVVL	SGSM	PAFHRC	DLLT	LTNRIEDP
19. Xenopus_laevis_(frog)_SEC11A_Q7SZ27							· · NMSN	D	FLOOVR	R M N H	ROLYS	OVLN7	GHIVS	ALMIW	GLMVI	T G S E S	PIVVVL	SOSNE	PAFHR	DLLF	LTNRVDDP
20. Lepisosteus_oculatus_(spotted_gar)_W5NBQ6						/	KHLSI	D	LDDVR	R H N H	ROLYS	OVINI	GIIIVS	ALMIW	GLMVV	GSES	PIVVVL	SGSM	PAFHRS	DLLF	LTNRVEDP
21. Latimeria_chalumnae_(coelacanth)_SEC11A_H38655							ULSI	D	FLODVR	R M + + N H	ROLYN	OVLNT	GUIVS	ALMIW	GLMVV	T G S E S		SGSM	PAFHRE	DLLF	LTNRVEDP
22. Danio_rerio_(zebrafish)_SEC11_Q6DGR3							H L S L	0	FLODVR	RUNH	ROLVI	OVENT	GIIIVS	SALMIW	C G L II V V	T G S E S		SOSM	PALHR	DLLF	LTNRVEDP
23. Chiloscyllum_punctatum_(shark)_SEC11_A0A401T455							· · ##\$1	0	FLNDVR	R.N N.H	ROLYN	QVENT	GUIVS	SALMIW	GLMVL	GSES	Contract of the	SGSN	PAFHR	DLLT	LTNRIEDP
24. Centruroides_hentzi_(scorpion)_SEC11_A0A2I9LPR6							· · · · W [0	FLODVR	RM NK	ROLIN	AVENT	GUIVS	ALMIW	GL NVV	GSES	100000000	5 0 S M 8	PAFHRO	DLLF	TNHREDP
25. Drosophila_melanogaster_(fruit_fly)_SEC11_097066		* * * * *			5	- MGV #	SMCO	D E	MLGDFN	HM NH	ROSLY	VLSI	AHIVS	ALMIW	GLUVV	GSES		SGSM	PAFHRC	OLL!	TNYKEEP
26. Mizuhopecten_yessoensis_(scallop)_SEC11_A0A210QEZ9					11.5	KEKIIS	SGNFGT	E	LODVR	R M N M	ROLYN	VLNY	GIIIVS	SALMIW	GLUVV	GSES		SGSM	PAFHRO	DLLF	TNYGEEP
27. Stichopus_japonicus_(sea_cucumber)_SEC11_A0A2G8LDT4							- MASI	D	ELDEVR	R 2 N B	ROLEN	VLNI	GHIVS	SALMIW	GLUVV	TAS S	VVVL	SOSM	PAFYRE	DULF	TNYGDOP
 Amphimedon_queenslandica_(primitive_sponge)_SEC11_ADA1X7TL Amphimedon_queenslandica_(primitive_sponge)_SEC11_ADA1X7TL 	LR							W	TLGDLK	KLNR	R G L Y	PLSI	UNIVS	ALMIW	G N N V V	I G S S	VVVL		APPRE	U L	THYEEDP
29. Csenorhabditis_elegans_(nematode)_SEC11_Q9N3D0							- HKFL	POVA	TSEIR	WHN	C C C C	GLN	AUVVS	ALMIW	GHUVI	GSDS	VVVL		A DYRK	C.L.	NDLEDP
30. Schistosoma_mansoni_(plathelminth)_SEC11_G4VN62 31. Brachionus_plicatilis_(rotifer)_SEC11_A0A3M7P2W1					1107	1	GLIDI	· · · · ·	FOORK	R II N F		YLT	ANVVA	ALMIW	LL VIII		D I WWWL		A F H R	A L Y	THEFT
									N IN DCO				de VS	ALMIN	GLUVV		VVVL				HHERCH
32. Dictyostelium_diacoideum_SEC11_Q86JD4 33. Hydra_vulgaria_(cnidarian)_SEC11_T2MF00							LI LI D	0.1.5	WIDEUP	D.U. P.F	-	a vinit	CH HA	ALMIN	aller		PUVVL				THOORY
		CONTI						DIAD	W DEVR	AH TH		Y 1. 6	0 1 1 2 2	ALMIN							THEREER
34. Salpingpeca_rosetta_(choanoflagellate)_SEC11_F2U557 35. Acanthamoeba_castellanii (amoeba)_SEC11_L8HBG2		1 2011	AVAI	apap	d 2 H M	11400	E Y I L G M		HT GELS				CAL YY	ALMIN	GL M V V	TOSES					THURSDA
35. Acanthamoeba_castellanis_amoeba_scc11_conbuz 36. Cafeteria roenbergensis SEC11_A0A5A8Cl87									I DEL D	RER.			01145	ALMIN		GSES			20505		
37. Saccharomyces_cerevisiae_(yeast)_SEC11_P15367									-LUSLA	U U							- WWWI				
38. Arabidopsis_thalana_(plant)_SEC11-1_Q9SSR2										5 0				10. Ma	a such						HUCKS .
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39. Arabidopsis_thalana_(plant)_SEC11-2_Q9LW08												-									
40. Stylonychia_lemnae_(ciliate)_SEC11_A0A078AS19											Dr										
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40. Sytonycha, jemnas.(cilalla), SEC11_AGA07AAS19 14. Tetrahymena, jihermophila, (cilallae), SEC11_G22080 22. Peeudosiuma_neurophila_(microspondium)_SEC11_AGA0R0LZ28	۵ 				H • • •			K	TIKEIA ELAQLK	SN K NL S DD	RKIL	Q F I TA	AYS <mark>I</mark> M	SALMIW STYMIW	AIGLE	N N D S	P VVVL	T G S M I	PGFKR	DILE	L
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40. Stylonycha, jemna, (cikala), SEC11, AGA07AS19 41. Tertahymmen, Jacobs, SEC11, AG20H9 42. Pesudokoma, neurophila, (mcrospondum), SEC11, AGA0R0LZZS 5pecies/Abbry 2 4. Nom_sapiens, (human), SEC11C, QB9Y50		• • • • • • • • •	X VE G X VE G X VE G		H VHRV VHRV		V L S I V K D Q F L S A K D N - G K D N - G	50 K F	T I K E J A E L A Q L K • • • • • L T K G O N L T K G O N L T K G O N	SN K / N L SN BDD *	R K L	KEGON KEGON	L L L L L L L L L L L L L L L L L L L		A IGLE				POFKR VALLAV	NGAY NGAY NGAY	LKPKE
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Figure S11 | Residue conservation inSEC11 among eukaryotes. Conserved boxes are highlighted as in **Fig. S10**. Catalytic Ser, His and the candidate Asp residues are highlighted in black boxes. Grey = additional region conserved in eukaryotes involved in binding pocket formation; purple = bowsprit helix, grey/magenta/cyan = segments of the pseudo-SP helix.

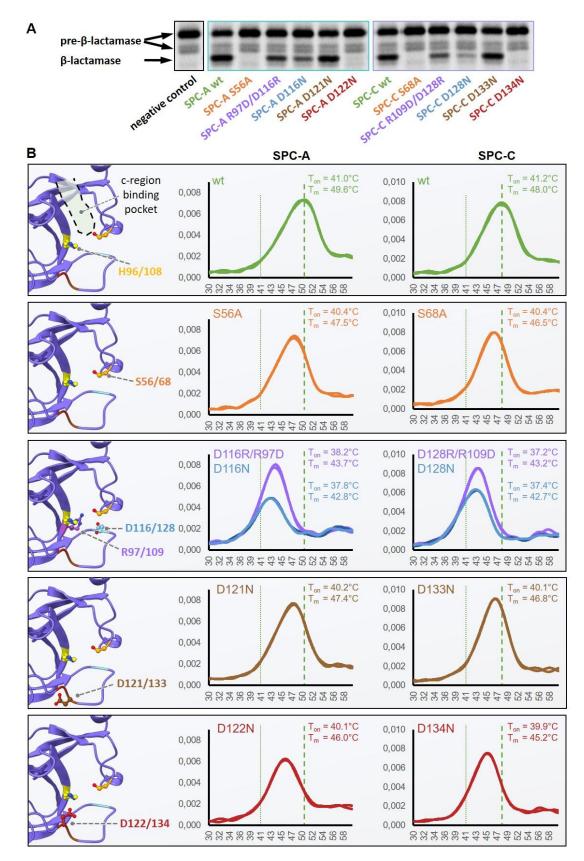


Figure S12 | **SPC** is a serine protease with a catalytic triad. (A) In vitro cleavage assay showing the processing of radiolabeled pre- β -lactamase in digitonin by SPC-A and SPC-C mutants. Negative control = no SPC added. (B) nano-differential scanning fluorimetry showing how the respective mutations affect protein stability. Left panel: Location of the respective residue (coloring as in Fig. 2). Middle and right panel: The first derivative of the fluorescence ratio at 350 nm and 330 nm (Y axis) is plotted against the temperature in °C (x axis). Melting onset (T_{on}) and melting temperature (T_m) are listed for each mutant. The melting profiles were determined as duplicates.

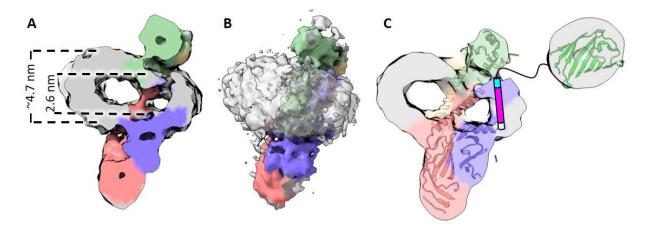


Figure S13 | Membrane thinning in digitonin. (A) Clipped view of SPC-C in digitonin at 12 Å resolution. The observed membrane thinning effect is akin to **Fig. 3A**. Coloring according to **Fig 3**, digitonin micelle depicted in grey. (**B**) Full view of SPC-C reconstituted in digitonin. (**C**) GFP (green cartoon) fused to the C-terminus of SEC11C is located at the cytosolic face of the particle, indicating that the C-terminus of SEC11C contains a transmembrane segment, termed here the pseudo-SP helix (indicated in grey/magenta/cyan).

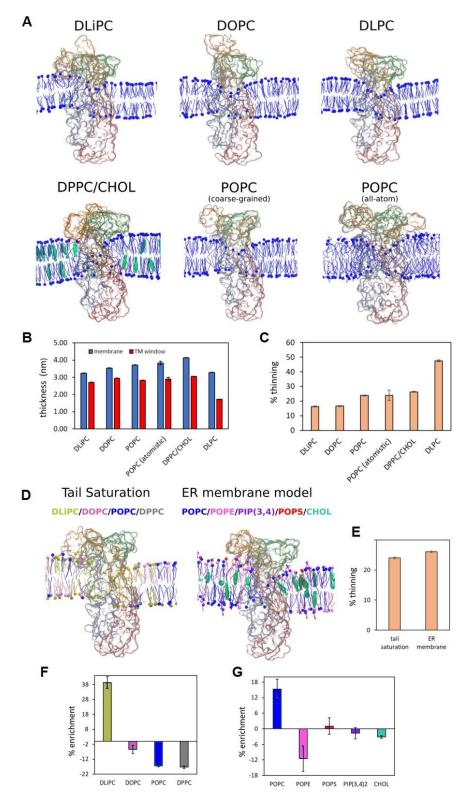


Figure S14 | Lipid enrichment and membrane thinning induced by the TM window of SPC. (A-C) Thinning induced in simple lipid bilayers. (**A**) Representative snapshots of coarse-grained MD simulations with SPC embedded in DLiPC, DOPC, DLPC, POPC and DPPC/CHOL (abbreviations see Table S3). For POPC, the results obtained with atomistic simulations are also shown. Coordinates were smoothed by averaging the coordinates of 4 neighbor frames of the trajectory. Only a slice of the bilayer around the TM window of SPC is highlighted. Blue = Phospholipids; cyan = cholesterol. SPC is represented by a transparent surface, with each protein chain identified by the same colors used in **Fig. 3** of the main text. (**B**) Bilayer thickness of the bulk membrane (without SPC, blue) and in the TM window (red) obtained in the MD simulations. (**C**) Percentage of thinning induced by SPC in different membrane environments. (**D**-**G**) Thinning in complex membranes. (**D**) Representative snapshots of coarse-grained MD simulations of SPC embedded in two complex membrane environment: (i) in the left panel, a mixture of PC

lipids with different level of tail saturations; (ii) in the right panel, an endoplasmic reticulum (ER) membrane model. For more details about lipid ratio, see **Table S3**. (**E**) Percentage of thinning induced by SPC in the complex lipid mixtures. (**F**) and (**G**) Percentage of lipid enrichment in the TM window region in relation to the lipid ratio of bulk membrane. Negative values indicate depletion of lipids. Results are displayed for the mixture of PC lipids with different level of tail saturations (**F**) and for the ER membrane model (**G**).

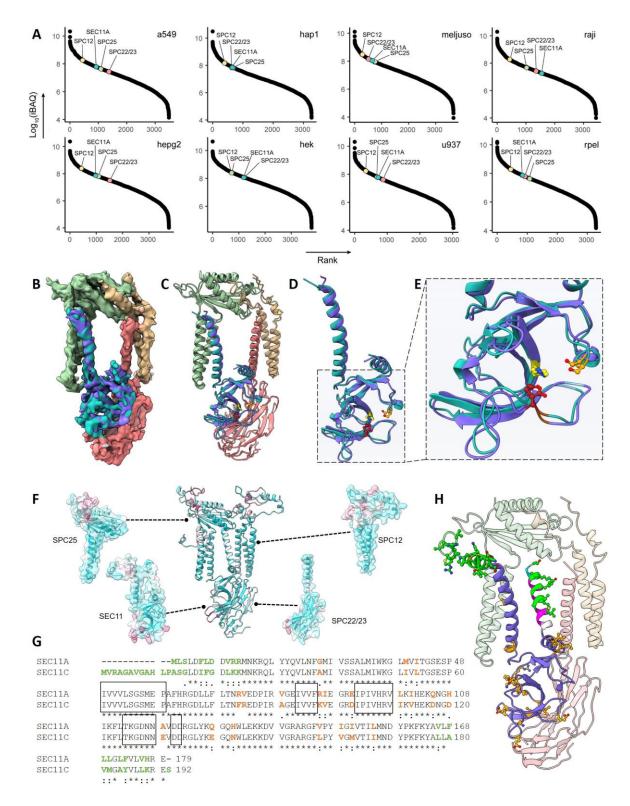
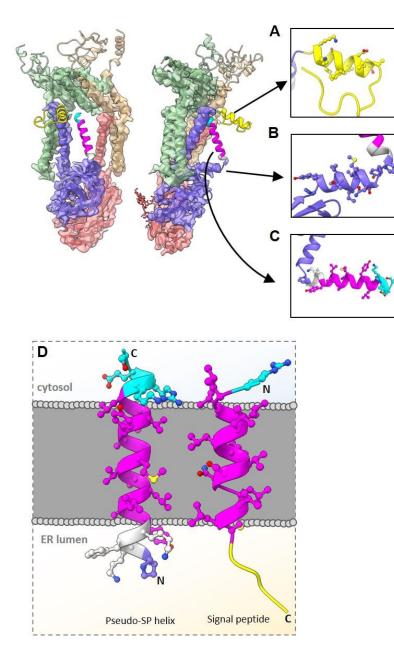


Figure S15 | **Differences between SEC11A and SEC11C**. (**A**) iBAQ intensities of SPC proteins determined for eight different cell lines are plotted by rank. SPC12, SPC25, SPC22/23, and SEC11A are marked; SEC11C was not detected in any of the tested cell lines. (**B**) Superposition of density maps for SPC-A and SPC-C (colored according to **Fig. 1**). (**C**) Superposition of atomic models for SPC-A and SPC-C. (**D**-**E**), Superposition of atomic models for SEC11A (teal) and SEC11C (purple). (**F**) Conservation mapped onto the SPC structure (teal = conserved, red = variable). Contact points between subunits as well as the SP binding groove are highlighted in black boxes. Variable residues that are resolved in the maps are highlighted in orange, variable residues in flexible regions are highlighted in green. (**H**) differences between SEC11A and SEC11C mapped onto the SPC-C structure, colored as in **F**.



N-terminal amphipathic helix

- Short amphipathic helix
- Unordered
- First 12 AA processed (specific to SEC11C)

'Bowsprit helix'

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_ _

- Short amphipathic helix Stiff & ordered Half inserted into membrane

'Pseudo-TM helix' (C-terminus)

- Unordered
- Short hydrophobic segment (13 AA) Connected to figurehead through
- conserved proline

Figure S16 | Unresolved regions of SEC11A/C. Unresolved regions of the SPC are shown as predicted by trRosetta. (A-C) Zoom-in and characteristics of terminal SEC11 helices. (D) comparison of the pseudo-SP helix of SEC11C to pre-prolactin SP (PDB ID 3JC2).

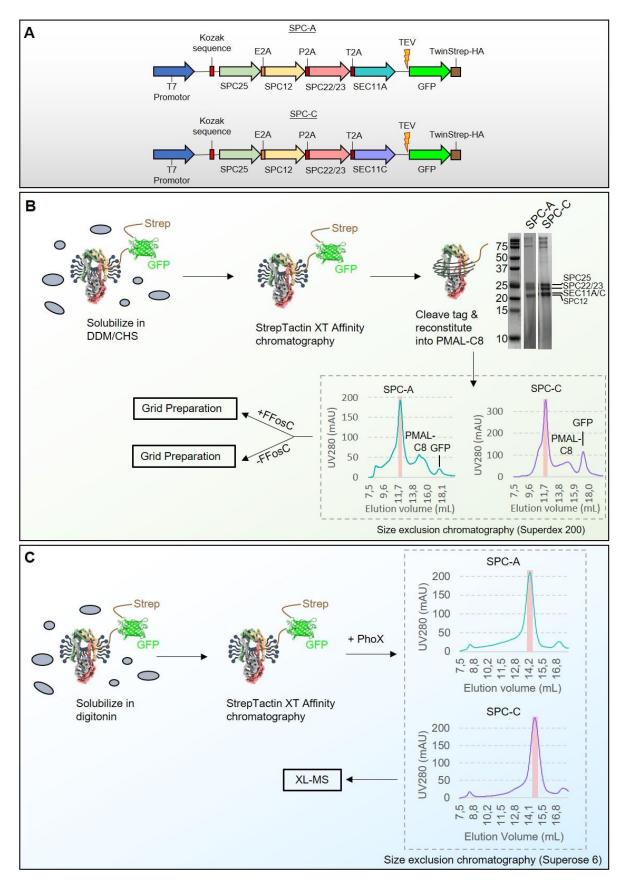


Figure S17 | Purification of human SPC for cryo-EM and XL-MS. (A) Expression constructs used for SPC purification. (**B**) Purification for cryo-EM. FFosC = fluorinated Fos-choline. (**C**) Purification for XL-MS. Workflows were analogous for SPC-A (teal) and SPC-C (purple). SEC11A/C are indicated in grey in panels B and C, the other subunits are colored according to a. Monodisperse SEC peaks used for analysis are highlighted in red. PhoX = crosslinker.

Supplementary Tables

Table S1 | Data collection and refinement statistics

	SPC-A	SPC-C
Data collection and processing		
Microscope	Talos Arctica	Talos Arctica
Camera	K2 summit	K2 summit
Magnification	165,000	165,000
Voltage (kV)	200	200
Electron exposure (e ⁻ /Ų)	60	60
Defocus range	0.5-4.0	0.5-4.0
Pixel Spacing (Å)	0.81	0.81
Symmetry imposed	C1	C1
Final Nr. particle images	29,508	60,598
Map Resolution	4.9	4.9
FSC Threshold	0.143	0.143
Map sharpening B factor (Å ²)	-180	-180
Model validation		
MolProbity Score	1.7	1.5
Clashscore	4.5	3.9
Rotamer Outliers (%)	0.2	0.0
Ramachandran plot		
Favored	91.9	95.0
Allowed	100.0	100.0
Outliers	0.0	0.0
Real-space correlation	0.68	0.73
Mean model B factor (Å ²)	144	231

Table S2 | Mass Spectrometry data availability

	Associated files	Experi ment type	Pa- nels	Comments
Fig. S7	20191026_F1_Ag5_Steig002_SA_SPC_C12_KonstrA_E (.raw/.txt) 20191026_F1_Ag5_Steig002_SA_SPC_D12_KonstrA_E (.raw / .txt) 20191030_F1_Ag5_Steig002_SA_SPC_C11_KonstrB_E (.raw / .txt) 20191030_F1_Ag5_Steig002_SA_SPC_C12D12_KonstrB_E(.raw/.txt)	XL-MS	all	Crosslinks on SPC complex after SEC purification
Fig. S15	20200714_OR16_UM7_Tamar002_SA_HEK_TRYPSIN_115min.raw 20200714_OR16_UM7_Tamar002_SA_HEK_TRYPSIN_115min.raw 20200714_OR16_UM7_Tamar002_SA_HEK_TRYPSIN_115min_2.ra w 20200723_OR16_UM7_Tamar002_SA_A549_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_A549_115min_2.raw 20200723_OR16_UM7_Tamar002_SA_A549_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_A549_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_HAP1_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_HAP1_115min_2.raw 20200723_OR16_UM7_Tamar002_SA_HAP1_115min_2.raw 20200723_OR16_UM7_Tamar002_SA_HAP1_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_HEPG2_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_HEPG2_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_HEPG2_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_MELJUSO_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_MELJUSO_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_MELJUSO_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_1.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RAJI_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw 20200723_OR16_UM7_Tamar002_SA_RPEL_115min_3.raw	Shot- gun proteo mics	a	SPC subunits detected in different cell lines with bottom-up LC- MS/MS
Fig. S8	20200723_OR16_UM7_Tamar002_SA_U937_115min_3.raw evidence.txt Tamar002_UHMR_spcA.raw Tamar002_UHMR_spcA_HCD125.raw Tamar002_UHMR_spcA_HCD150.raw Tamar002_UHMR_spcC.raw Tamar002_UHMR_spcC.raw Tamar002_UHMR_spcC_HCD125.raw	Native MS	all	Native MS data for SPC-A and SPC-C, and HCD spectra of z=14+ of SPC22/23- SEC11A and SPC22/23 SEC140
Fig. 1	Tamar002_UHMR_spcC_HCD150.raw Tamar002_SA_SPC_A_STREP_LOHI_ETD.raw Tamar002_SA_SPC_A_STREP_LOHI_ETD.xlsx Tamar002_SA_SPC_A_STREP_LO_FMS.csv Tamar002_SA_SPC_A_STREP_LO_FMS.raw Tamar002_SA_SPC_C_FLAG_LOHI_ETD.raw Tamar002_SA_SPC_C_FLAG_LOHI_ETD.xlsx Tamar002_SA_SPC_C_FLAG_LOHI_ETD.raw Tamar002_SA_SPC_C_FLAG_LOHI_ETD.xlsx Tamar002_SA_SPC_C_FLAG_LOFMS.csv Tamar002_SA_SPC_C_FLAG_LO_FMS.csv	TD- MS	b, c	SPC22/23-SEC11C. Quantification of SPC proteins in FLAG and STREP pull-downs
Fig. S9	Tamar002_SA_SPC_A_LOHI_ETD.raw Tamar002_SA_SPC_A_LOHI_ETD.raw Tamar002_SA_SPC_A_LO_FMS.csv Tamar002_SA_SPC_C_LOHI_ETD.raw Tamar002_SA_SPC_C_LOHI_ETD.raw Tamar002_SA_SPC_C_LOHI_ETD.raw Tamar002_SA_SPC_C_LOHI_ETD.raw Tamar002_SA_SPC_C_LOHI_ETD.raw Tamar002_SA_SPC_C_LOHI_ETD.raw Tamar002_SA_SPC_C_LOFMS.csv Tamar002_SA_SPC_C_LO_FMS.raw	TD- MS	a-k	Overview of proteoforms detected in top-down and intact mass MS of SPC-A and SPC-C complexes

 Table S3 | Summary of the MD simulations performed in this study.
 All simulations were performed with and without SPC embedded in the membranes.

Resolution	Lipid Composition*	Duration
Coarse-Grained	DLPC	20 µs
(Martini 3)	DLiPC	20 µs
	DOPC	20 µs
	POPC	20 µs
	DPPC, CHOL (0.70:0.30)	20 µs
	DLIPC, DOPC, POPC, DPPC	20 µs
	(0.25: 0.25: 0.25: 0.25)	-
	Endoplasmic reticulum membrane model	20 µs
	POPC, POPE, POPS, PI(3,4)P2, CHOL	
	(0.442:0.255:0.0425:0.1105:0:15)	
All-Atom (CHARMM 36m)	POPC	2x 150 ns

*Abbreviations: DLPC=1,2-dilauroyl-sn-glycero-3-phosphocholine; DLiPC=1,2-dilinoleoyl-sn-glycero-3-phosphocholine; DOPC=1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPC=-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; CHOL=cholesterol; POPE= 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; POPS=1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine; PI(3,4)P2=phosphatidylinositol 3,4-bisphosphate

Supplementary Movies

Movie S1 | Structures of SPC-A and SPC-C. The movie compares the density maps obtained for SPC-C and SPC-A (colored as in **Fig. 2**) and depicts the map-to-model fit of both atomic models. The models are superposed, and the catalytic residues are highlighted as ball-and-stick models. N-glycosylation at Asp141 of SPC22/23 is also depicted, although not visible at the given threshold.

Movie S2 | Membrane thinning by SPC-C. The map-to model fit of SPC-C is shown (colored as in **Fig. 2**). The signal peptide of bovine pre-prolactin (represented as ribbons, colored as in **Fig. 4A**) was placed into the putative SP binding pocket using *Coot*. The -1 and -3 positions of the SP c-region are highlighted as ball-and-stick models as in **Fig. S10G**. The Gaussian-filtered, experimentally determined density for the PMAL-C8 micelle is shown at different threshold levels in order to depict the membrane thinning within the TM window. The thickness of the micelle in the putative h-region binding pocket is consistent with the length of the SP h-region.

Movie S3 | A glimpse of the membrane thinning induced by SPC-C in an ER membrane model during 2µs of a coarse-grained MD simulation. Coordinates were smoothed by averaging the coordinates of 4 neighbor frames of the trajectory. Only a slice of the membrane around the TM window of SPC is highlighted. Lipids are shown with different colors: POPC (blue), POPS (red), POPE (pink), PI(3,4)2 (purple) and cholesterol (green). SPC-C is represented by a transparent surface, with each protein chain identified by the same colors used in **Fig. 3**.