Supplementary Material for

Multibudded tubules formed by COPII on artificial liposomes

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Supplementary Figures

Figure S1

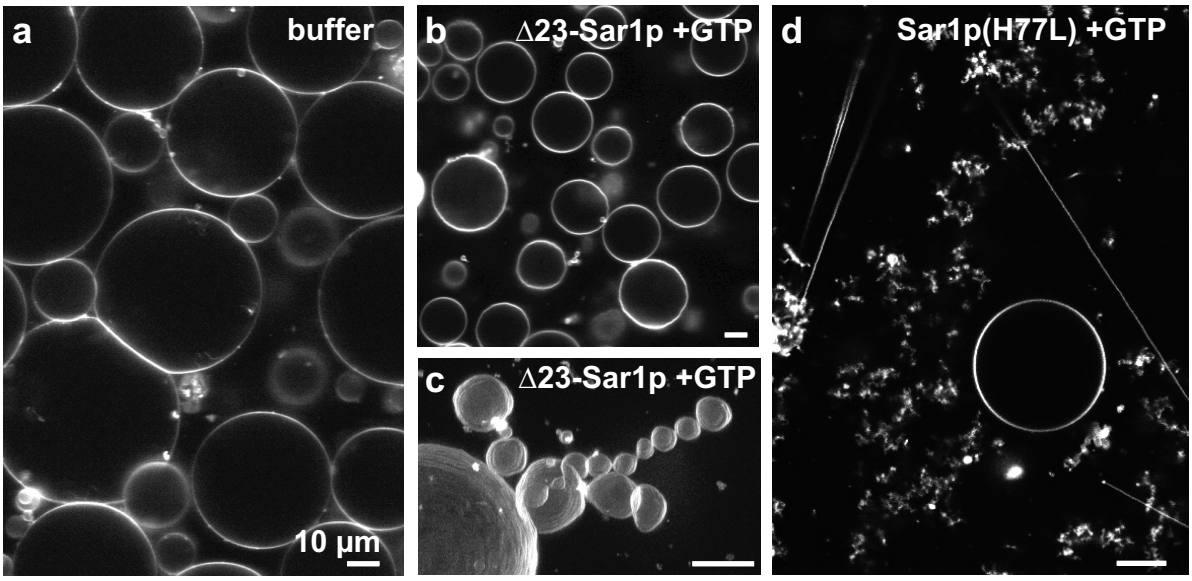
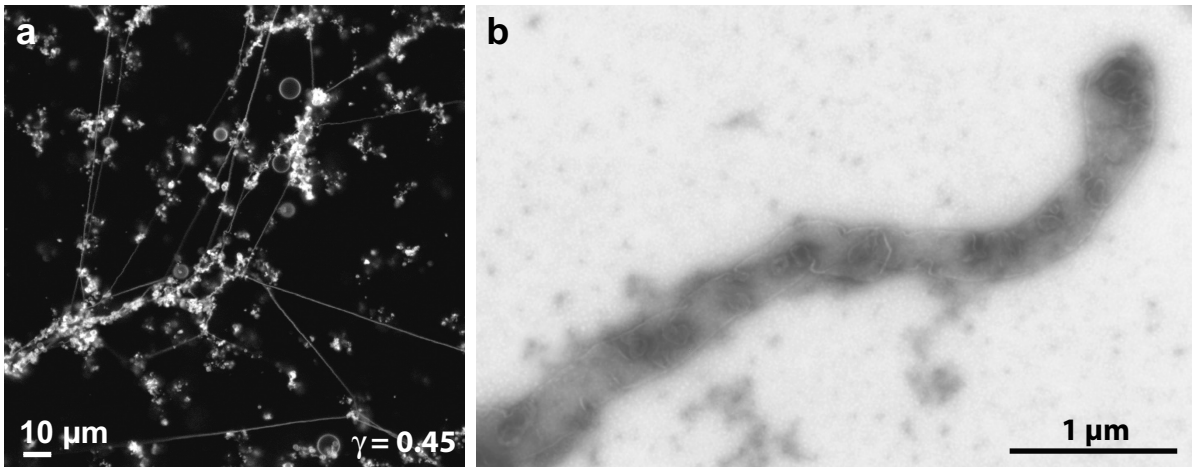


Figure S2



Supplementary Figure Legends

Figure S1
Confocal microscopy of fluorescently labeled GUV membranes. Scale bars represent 10 µm.
(a) 'Buffer only' control: GUVs mixed with HKM buffer in the absence of Sar1p remained mostly intact.
(b, c) Δ23-Sar1p control: GUVs remained mostly unaffected when incubated with a truncated Sar1p which lacks the N-terminal amphipathic helix[1] [5 µM Δ23-Sar1p, 1 µM Sec12ΔCp, 1 mM GTP_r].
(c) A few pearling vesicles (displayed as a projection) were observed in control experiments with Δ23-Sar1p and GTP. Pearling vesicles were also observed in control experiments with wild-type Sar1p and GDP (Fig. 1a inset). The pearling vesicles were relatively large and not uniform in size. Note that the pearling of tubes has been previously observed after the addition of simple, amphiphilic polymers[2]. The pearling vesicles of the 'Major-Minor' lipid mixture appeared to remain connected.
(d) In the presence of GTP_r , Sar1p(H77L) formed straight rigid tubes with the same appearance as those formed by wild-type Sar1p (Fig. 1e) [10 µM Sar1p(H77L), 1 µM Sec12ΔCp, 1 mM GTP_r].

Figure S2
Aggregation of membranes with Sar1p and Sec23/24p in the absence of Sec13/31p [2 µM Sar1p, 320 nM Sec23/24p, 1 µM Sec12ΔCp, 1 mM GMP-PNP].
(a) Overview by confocal microscopy. Networks of tubules connected clusters of 'sticky' liposome material. Intensities are displayed on a nonlinear intensity scale to highlight the tubules.
(b) Negatively stained and dried tubule from the same reaction in electron microscopy. The crinkled appearance contrasted with the tubules in Fig. 3 j,k. In this incubation the membranes appear deformable, which is in agreement with the presumed lack of a complete and rigid protein scaffold when Sec13/31p is omitted.

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1. Lee,M.C. *et al.* Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. *Cell* **122**, 605-617 (2005).

2. Tsafrir,I. *et al.* Pearling instabilities of membrane tubes with anchored polymers. *Phys. Rev. Lett.* **86**, 1138-1141 (2001).

Supplementary Movie Legends

Movie S1
Fast confocal imaging timeseries. A GUV was tubulated by GTP-activated Sar1p, leading to its explosion. Despite thorough mixing of proteins and GUVs, tubulation was initiated locally. The membrane tension increased, the GUV exploded and formed a dense ball of soft, wiggling tubules. The video corresponds to the still images in Fig. 1 b, c and d, but with a nonlinear intensity scale.

Movie S2
Confocal imaging timeseries. Extensions formed from GUVs by COPII with non-hydrolysable GMP-PNP were straight and rigid.

Movie S3
Confocal imaging timeseries. Extensions formed by COPII with the Sar1p(H77L) mutant and regular GTP were similarly straight and rigid.