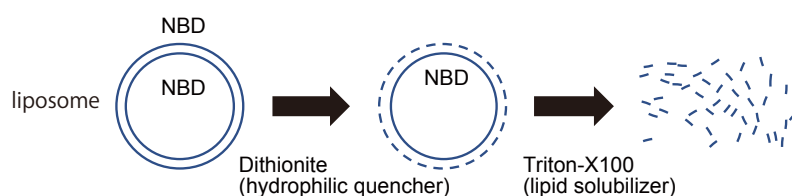


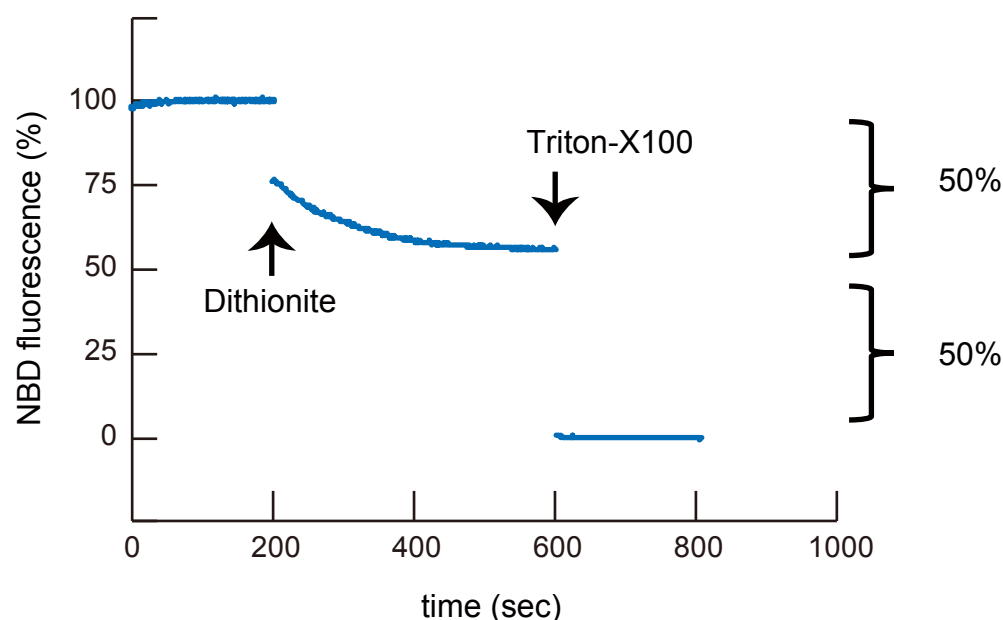
Figure S1

A



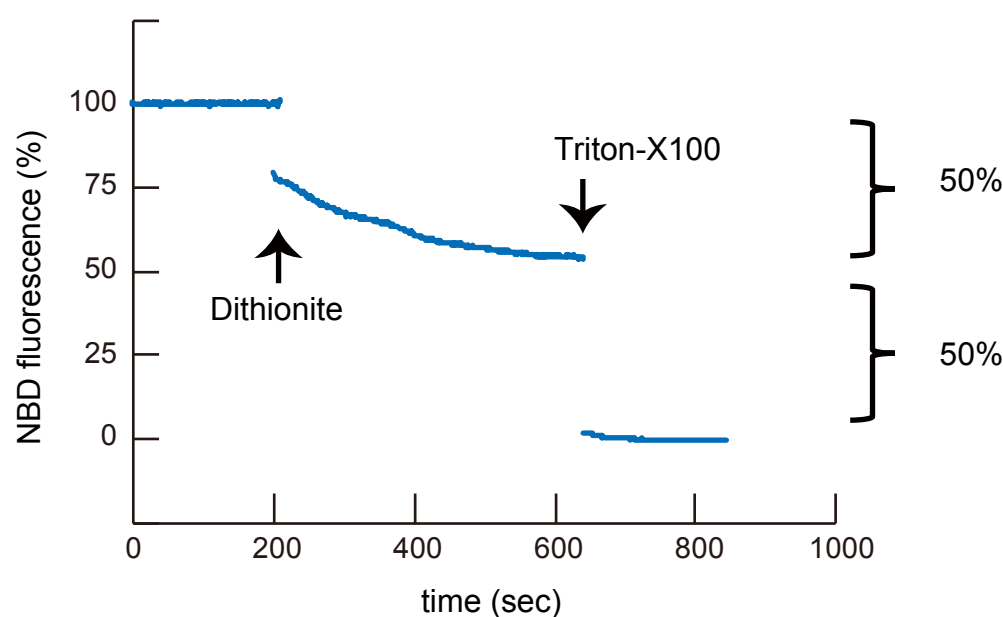
B

POPC/POPS/cholesterol/NBD-DOPE (head)



C

POPC/POPS/NBD-DOPE (head)

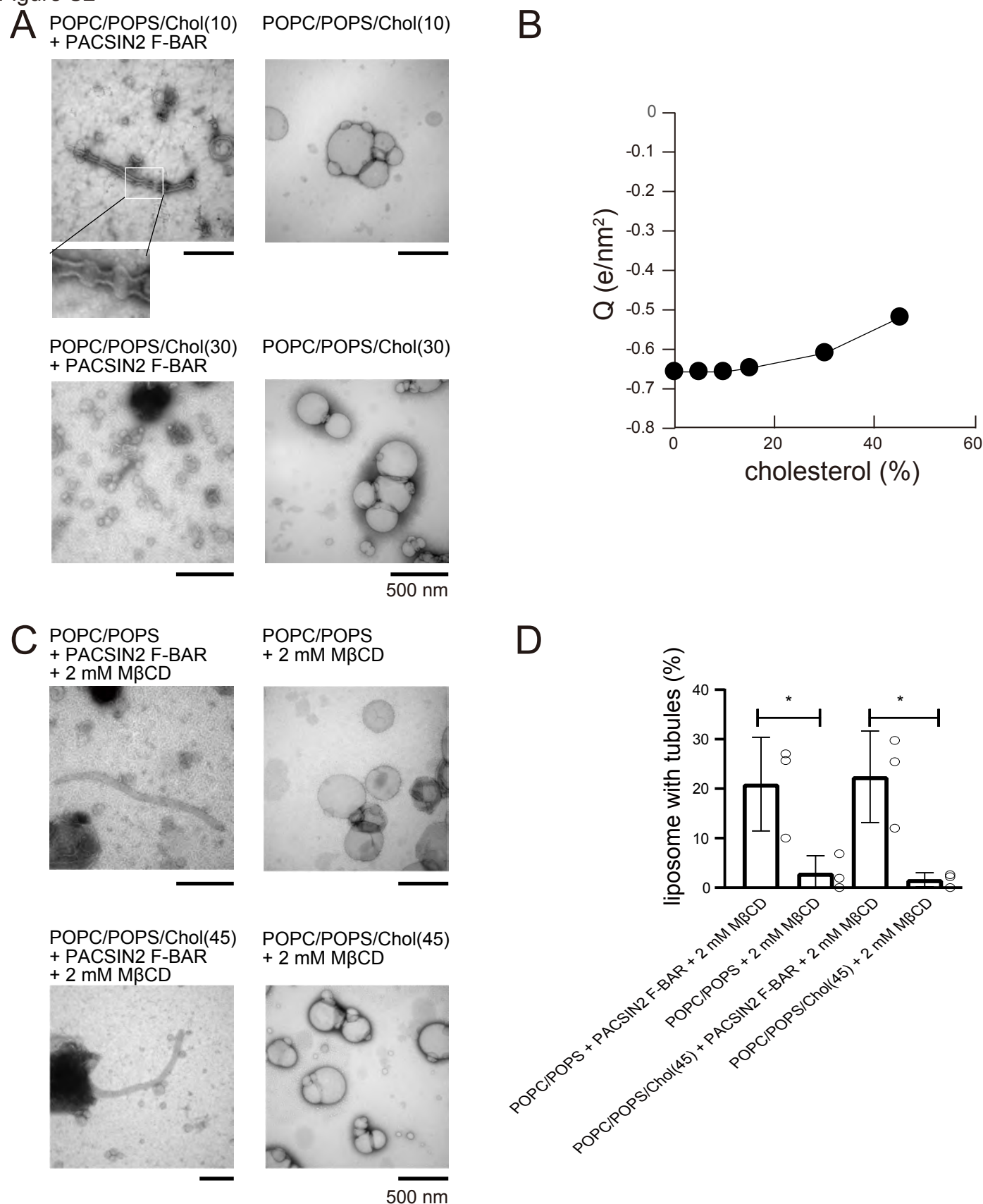


**Figure S1. The estimation of lamellarity of liposomal membranes by quenching.**

(A) The schematic illustration of the quenching of NBD-labelled lipids in liposome. Both outer and inner leaflets of liposomal membranes contained NBD-labelled DOPE. The hydrophilic quencher Dithionite is impermeable to membrane. Dithionite quenched the NBD at the head of DOPE at the outer leaflet of liposome in the absence of Triton-X 100. After the quenching of NBD on the outer leaflet, the Triton-X 100 was added to solubilize the liposomes, which enabled Dithionite to quench NBD that was located at the inner leaflet of liposome.

(B and C) The time-course of NBD quenching. The liposomes composed of POPC/POPS/cholesterol/NBD-DOPE (33 / 22 / 45 / 1) (B) and POPC/POPS/NBD-DOPE (60 / 40 / 1) (C) were quenched by Dithionite, then followed by the addition of Triton-X 100.

Figure S2

**Figure S2. The cholesterol dependency of tubule formation by PACSIN2 F-BAR domain in vitro.**

(A) Electron microscopic images of negatively stained liposomes. The liposomes of POPC/POPS/Chol(10) and POPC/POPS/Chol(30) in the presence or absence of PACSIN2 F-BAR domain (5  $\mu$ M). Liposome concentration, 0.125, 0.1375, and 0.1625  $\mu$ g/ $\mu$ l, respectively, to have the same amount of POPS.

(B) Molecular dynamics simulation for the estimation of model membrane parameters. The charge density (electron/nm<sup>2</sup>) was plotted against the percentage of cholesterol.

(C) Electron microscopic images of negatively stained liposomes. The liposomes of POPC/POPS and POPC/POPS/Chol(45) in the presence or absence of PACSIN2 F-BAR domain (5  $\mu$ M) in the presence of 2 mM M $\beta$ CD. Liposome concentration, 0.125  $\mu$ g/ $\mu$ l.

(D) The quantification of the liposomes with tubules in (A). N= 3. \* shows the statistical significance ( $p < 0.05$  by a one-way ANOVA followed by Tukey's post hoc test).