

Supplemental Figures

Fig. S1 Lack of GST-MFGE8-C2 labeling in *cho1Δ* yeast

The labeling of GST-MFGE8-C2 is not detected in the plasma membrane (A, Pp), endoplasmic reticulum (ER) (yellow, Eo and pink, Pi in B), nuclear (C), or vacuolar (D) membranes of *cho1Δ* yeast that lacks phosphatidylserine (PtdSer) synthesis. Eo: E-face of the outer side of the ER membrane, Pp: P-face of the plasma membrane, Pi: P-face of the inner side of the ER membrane (bar, 200 nm).

Fig. S2 The gold labeling densities of GST-MFGE8-C2 increased concomitantly with the concentration of phosphatidylserine (PtdSer) in liposomes

The labeling of GST-MFGE8-C2 is shown in the unilamellar liposomes containing 0.1–5 mol% of PtdSre (PS) by the quick-freeze and freeze-fracture labeling method. The experiment was repeated three times and the number of colloidal gold particles per μm^2 was measured in more than 80 randomly selected liposomes. Values for all concentrations were statistically different among the different conditions (for example, 1 versus 2 mol%; mean $\pm$ SEM; Student's *t*-test; $P < 0.05$) (bar, 100 nm).

Fig. S3 The distributions of phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) in the plasma membrane of HFF-1 and yeast cells

Replicas of HFF-1 and budding yeast cells are labeled with GST-MFGE8-C2 (Aa, Ca) and biotin-duramycin (Ab, Cb). Data were presented as the mean $\pm$ SEM of three independent experiments ($n > 30$). (B and D) Gold labeling densities of PtdSer (a) and PtdEtn (b). Both PtdSer and PtdEtn were predominantly located in the cytoplasmic

leaflet of the plasma membranes of the HFF-1 cells as well as the yeast cells (bar, 200 nm in (A) and 1 μ m in (C)).

Fig. S4 The distribution of phosphatidylserine (PtdSer) in the cortical endoplasmic reticulum membrane of wild-type (WT) yeast cells

PtdSer labeling is observed in the cytoplasmic leaflets of both the outer (Po, blue in B) and inner (Pi, pink in A) membranes in the cortical endoplasmic reticulum of WT yeast. Eo: E-face of the outer side of the ER membrane, Ep: E-face of the plasma membrane, Pp: P-face of the plasma membrane, Pi: P-face of the inner side of the ER membrane (bar, 200 nm).