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Supplemental Information

Membrane-Spanning Sequences in Endoplasmic Reticulum Proteins Promote Phospholipid Flip-Flop

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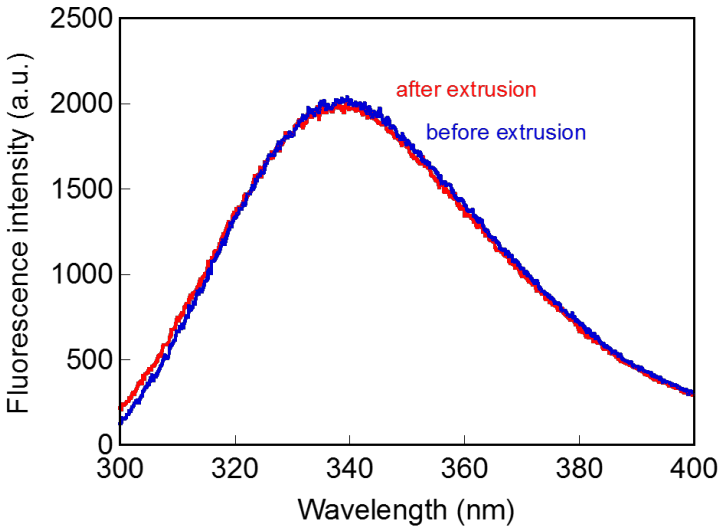

FIGURE S1 Trp fluorescence spectra of EDEM1 before and after extrusion of vesicles. EDEM1/lipid mixture (0.2 mol% EDEM1) was hydrated with a HEPES-NaOH buffer. The suspension was either directly solubilized by adding heptaethylene glycol monododecyl ether (HED) or extruded to form LUVs as described in MATERIALS AND METHODS section in the main text, and then solubilized with HED. Fluorescence spectra of the solubilized samples were recorded at phospholipid concentration of 500 μM in the presence of 1 v/v% HED.

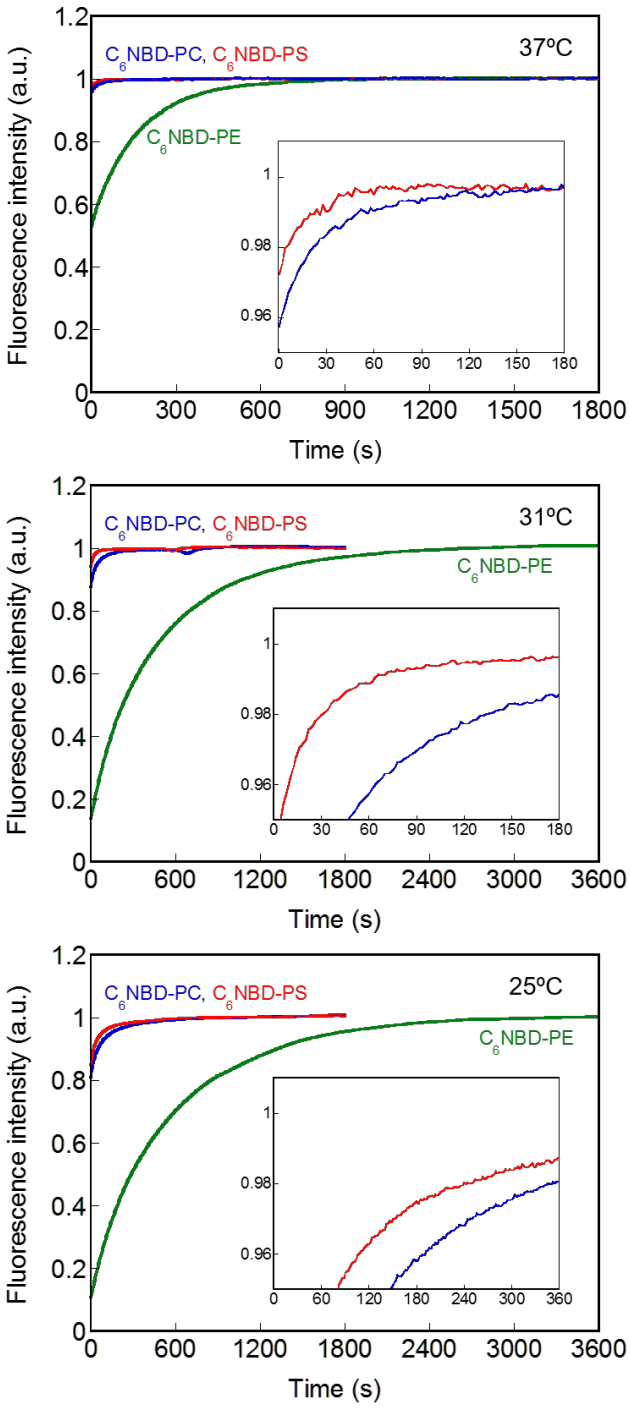

FIGURE S2 Incorporation of C_6NBD-PC, -PE, and -PS into LUVs at 25, 31, 37°C. Fluorescence intensity was monitored over time after fluorescent lipids were added, and the data were normalized to the intensity detected at the end of the measurement periods (t = 1800 or 3600 s). Insets are expanded traces of the initial time period.

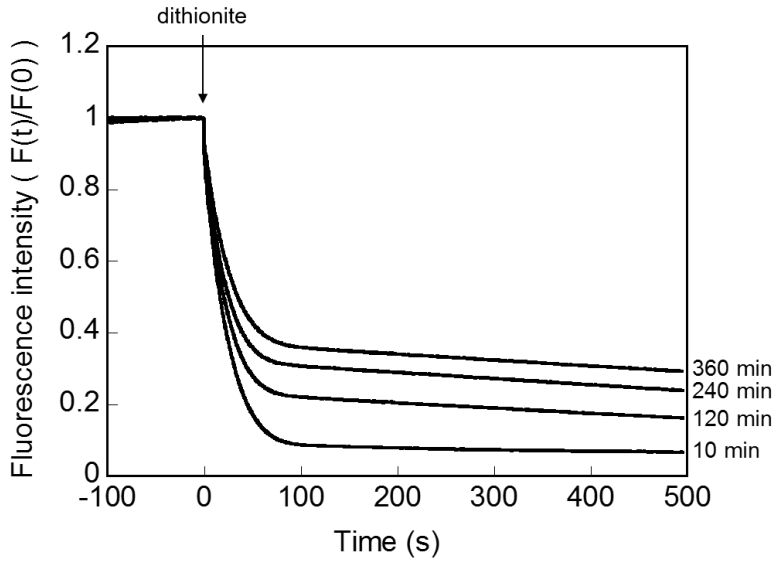

FIGURE S3 Representative dithionite-quenching profiles of C_6NBD-PC fluorescence following incubation with LUVs containing 0.025 mol% EDEM1 for 10, 120, 240, or 360 min at 37°C. The fluorescence intensity ($F(t)$) was monitored as a function of time after dithionite was added and was normalized to the intensity measured at t = 0. The profiles were fitted using the following equation:

$$F(t)/F(0) = (1-\Phi_{\text{inner}})\exp(-k_q t) + \Phi_{\text{inner}}\exp(-k_{\text{flop}} t)$$

where Φ_{inner}, k_q, and k_{flop} are fitting parameters; k_q and k_{flop} are the rate constant of the dithionite quenching of fluorescent lipids in the outer leaflet and the flop rate constant, respectively; and Φ_{inner} is the mole fraction of the fluorescent lipids in the inner leaflet.

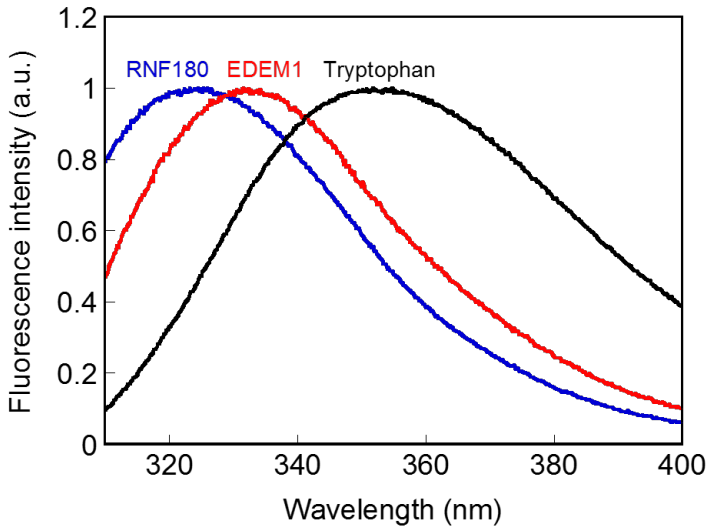

FIGURE S4 Trp fluorescence spectra of RNF180 and EDEM1 in LUVs, and free tryptophan in aqueous solution. The peptide/lipid ratio was 0.1 mol%.

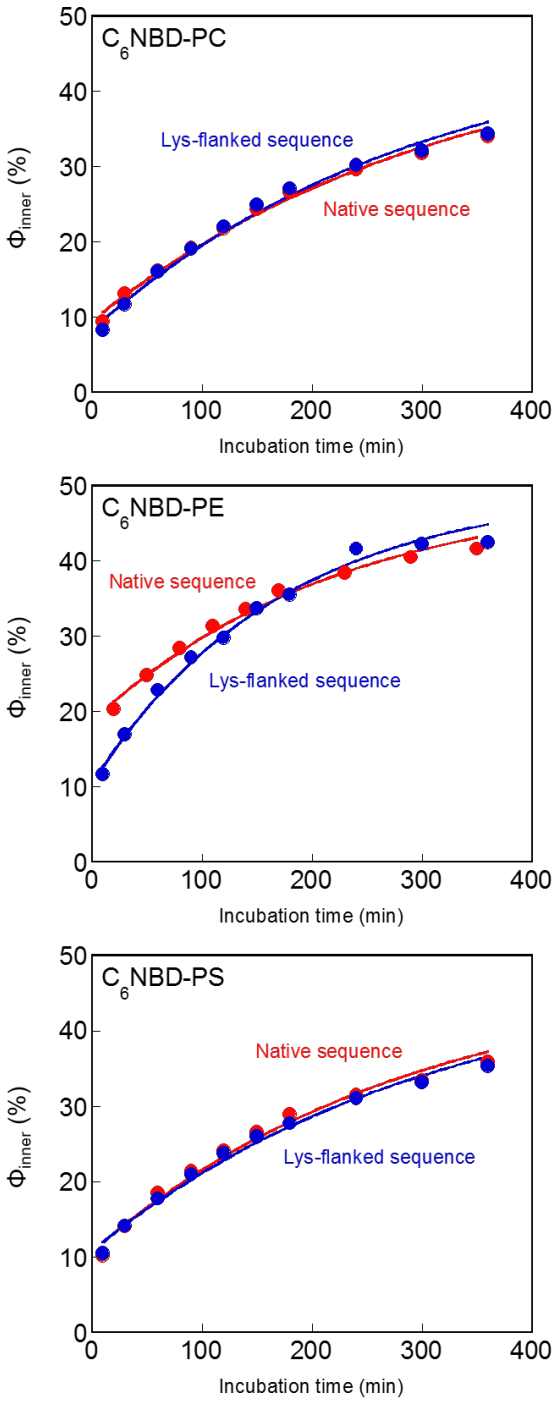

FIGURE S5 Flip assays for C_6NBD-PC, -PE, and -PS in LUVs containing 0.1 mol% ORP5 (Lys-flanked sequence) or ORP5 native sequence peptide at 37°C.

Lys-flanked sequence: Ac-KKSPRSWFLLCVFLACQLFINHILKKK-NH_2

Native sequence: Ac-APTPGLLQSPRSWFLLCVFLACQLFINHILK-NH_2

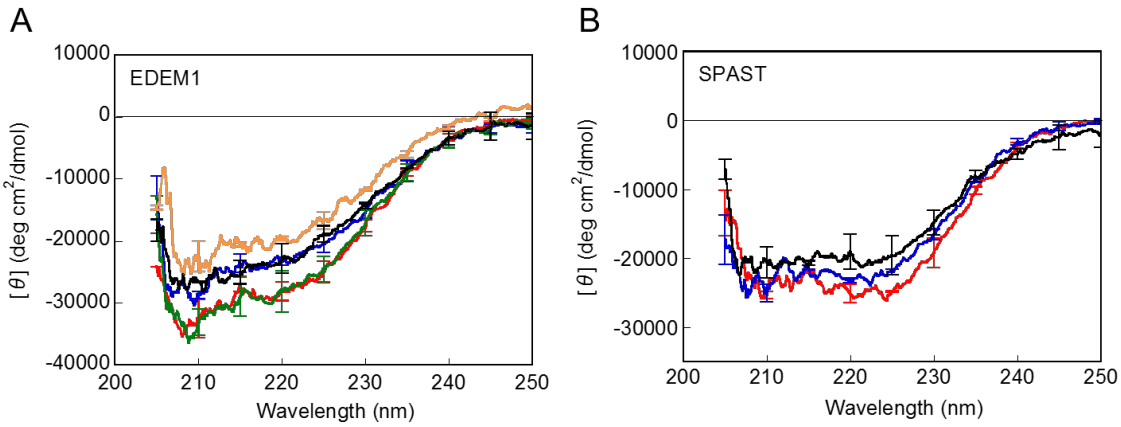

FIGURE S6 CD spectra of EDEM1, SPAST, and their mutant peptides in LUVs. The peptide/lipid ratio was 0.2 mol%. (*A*) EDEM1 WT (*black*), R10A (*red*), H14A (*blue*), or R10E (*green*) in the absence of cholesterol, or EDEM1 WT in the presence of 20 mol% cholesterol (*orange*). (*B*) SPAST WT (*black*), R13A (*red*), or H18A (*blue*). Error bars represent SDs from 2 experiments.

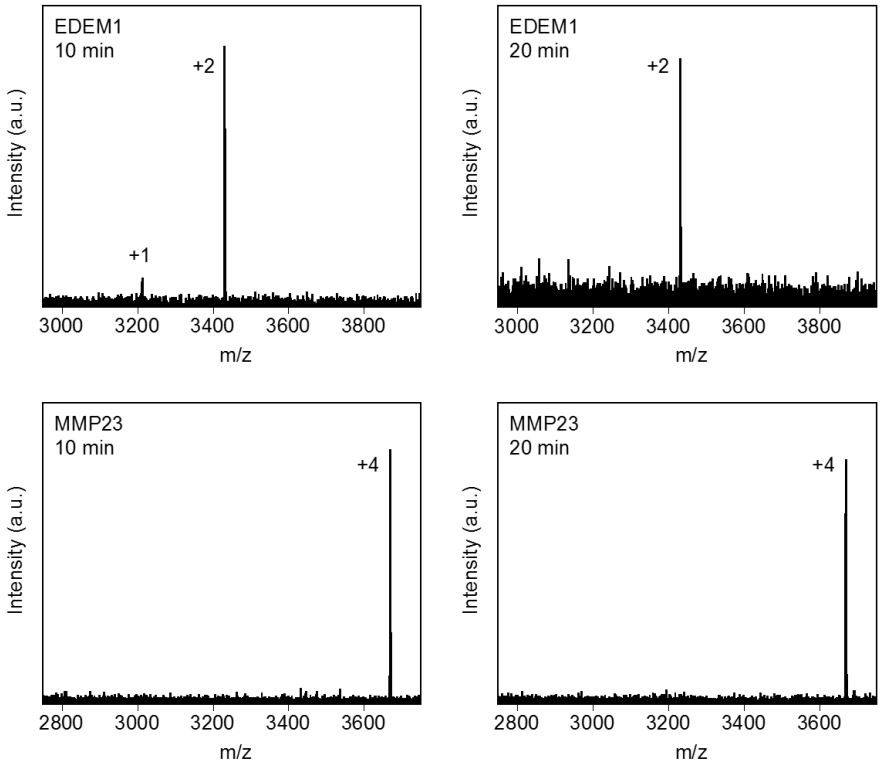

FIGURE S7 Evaluation of peptide topology in an artificial membrane by mass spectrometry. To POPC/cholesterol (4:1 molar ratio) LUVs (4.5 mM) with 0.1 mol% of peptide (EDEM1 [upper figures] or MMP23 [lower figures]) in Tricine-NaOH buffer, 250 mM $mPEG_4$-NHS was added to obtain a final reagent/peptide ratio of 500:1. After a 10- (left) or 20-min (right) incubation at 25°C, the reaction was quenched by adding a 10-fold larger amount of NH_4HCO_3 than that of $mPEG_4$-NHS. Changes in the molecular mass of the peptides were analyzed using MALDI-TOF-MS. The mass spectra of the EDEM1 and MMP23 peptides with chol-20% LUVs after 10 and 20 min incubation with $mPEG_4$-NHS are shown. The values +1, +2, and +4 represents the *m*/*z* ratios of the peptides combined with 1, 2, and 4 molecules of $mPEG_4$-NHS, respectively.

TABLE S1 Flip rate constant of C_6NBD-PC, C_6NBD-PE, and C_6NBD-PS in LUVs with/without synthesized peptides at 37°C

	k_{flip} ($\times 10^{-4}$ min^{-1})		
Peptide (mol%)	C_6NBD-PC	C_6NBD-PE	C_6NBD-PS
No peptide	4.36 ± 0.45	3.27 ± 0.25	4.06 ± 0.77
ATF6	3.79 ± 0.87	6.82 ± 2.18	3.28 ± 1.27
EDEM1	124	–	–
EDEM1 (0.06)	79.9	–	–
EDEM1 (0.025)	21.2 ± 1.2	72.2 ± 13.1	72.2 ± 26.8
EDEM1 (0.0175)	15.9	55.4	42.7
EDEM1 (0.01)	8.38	30.7	20.6
FACL4	7.17 ± 3.46	19.1 ± 8.5	4.68 ± 1.41
JP3	2.16 ± 0.21	4.47 ± 0.15	2.11 ± 0.41
JP4	11.3 ± 0.6	34.3 ± 4.9	12.2 ± 3.14
ORP5	19.8 ± 4.2	30.0 ± 1.2	21.7 ± 7.7
P4HTM	10.0 ± 0.4	31.2 ± 1.8	6.47 ± 0.43
RNF180	32.4 ± 6.6	30.9 ± 5.8	13.0 ± 2.0
SPAST	23.2	55.5	37.4
SPAST (0.05)	10.7 ± 2.3	37.6 ± 5.7	18.6 ± 2.6
TMED7	9.36 ± 2.48	40.5 ± 7.7	5.61 ± 1.61
UBE2J2	2.59 ± 0.70	5.11 ± 0.77	2.06 ± 0.55

The SDs shown are representative of 2 independent experiments. The peptide/lipid ratios are 0.1 mol%, unless otherwise indicated. Plots of these data are shown in Fig. 3 in the main text.

TABLE S2 Flip rate constant of C_6NBD-PC, C_6NBD-PE, and C_6NBD-PS in LUVs with mutant peptides of SPAST and EDEM1 at 37°C

	k_{flip} ($\times 10^{-4}$ min^{-1})		
Peptide (mol%)	C_6NBD-PC	C_6NBD-PE	C_6NBD-PS
SPAST R13A (0.1)	3.82	4.53	3.80
SPAST R13A (0.05)	3.95	3.84	3.79
SPAST H18A (0.1)	6.17	37.4	13.6
SPAST H18A (0.05)	5.29	14.4	6.19
EDEM1 R10A (0.1)	4.18	5.10	3.98
EDEM1 R10A (0.025)	4.43	3.27	3.63
EDEM1 H14A (0.025)	7.43	29.3	13.4
EDEM1 H14A (0.0175)	–	21.3	–
EDEM1 H14A (0.01)	4.34	11.8	4.94
EDEM1 R10E (0.1)	3.97	–	3.25
EDEM1 R10E (0.04)	–	9.57	–
EDEM1 R10E (0.025)	4.25	9.73	4.02
EDEM1 R10A (0.025) + EDEM1 H14A (0.025)	7.01 ± 0.66	30.3 ± 3.7	13.5 ± 0.3

The SDs shown are representative of 2 independent experiments. Plots of these data are shown in Fig. 4 in the main text.

TABLE S3 Flip rate constant of C_6NBD-PC, C_6NBD-PE, and C_6NBD-PS in LUVs with/without 0.025 mol% EDEM1 at 25, 31, or 37°C

		k_{flip} ($\times 10^{-4}$ min^{-1})		
Peptide (mol%)	Temperature (ºC)	C_6NBD-PC	C_6NBD-PE	C_6NBD-PS
No peptide	37	4.36 ± 0.45	3.27 ± 0.25	4.06 ± 0.77
	31	1.61	0.908	1.26
	25	1.16	0.778	1.14
EDEM1 (0.025)	37	21.2 ± 1.2	72.2 ± 13.1	72.2 ± 26.8
	31	13.4	38.5	48.6
	25	7.82	21.2	29.7

The SDs shown are representative of 2 independent experiments.

TABLE S4 Flip rate constant of C_6NBD-PC, C_6NBD-PE, and C_6NBD-PS in LUVs containing 0–0.025 mol% EDEM1 in the presence of 5 or 20 mol% cholesterol at 37°C

		k_{flip} ($\times 10^{-4}$ min^{-1})		
Peptide (mol%)	Cholesterol (mol%)	C_6NBD-PC	C_6NBD-PE	C_6NBD-PS
No peptide	20	3.22 ± 0.13	2.05 ± 0.32	3.04 ± 0.38
EDEM1 (0.025)	5	22.2	71.1	83.8
	20	6.53	17.8	21.5
EDEM1 (0.015)	20	4.66	11.0	11.6

The SDs shown are representative of 2 independent experiments. Plots of these data are shown in Fig. 6 in the main text.