Miyata et al., http://www.jcb.org/cgi/content/full/jcb.201601082/DC1

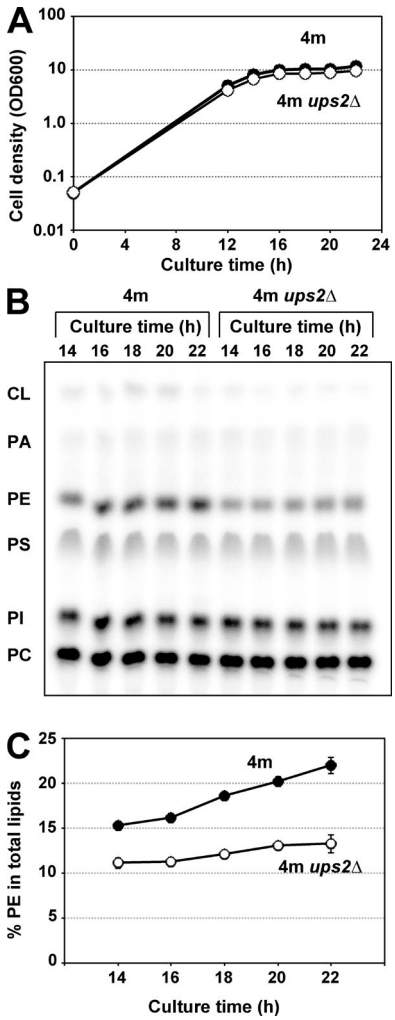

Figure S1. **Ups2 enhances PE accumulation in the post–log phase in a synthetic medium.** (A) 4m and 4m *ups2Δ* cells (OD_{600}, 0.05) were cultured in SCD supplemented with 3 mM choline for the indicated periods. At each time point, the cell density was analyzed by measuring OD_{600}. Closed circle, 4m cells; open circle, 4m *ups2Δ* cells. (B and C) 4m and 4m *ups2Δ* cells (OD_{600}, 0.05) were cultured in SCD supplemented with 3 mM choline in the presence of [^{32}P]Pi for the indicated periods. At each time point, total cellular phospholipids were extracted, separated by TLC, and then analyzed by an imaging analyzer. (C) The percentages of PE relative to total phospholipids. The mean values for two independent experiments and deviations are shown. Closed circle, 4m cells; open circle, 4m *ups2Δ* cells.

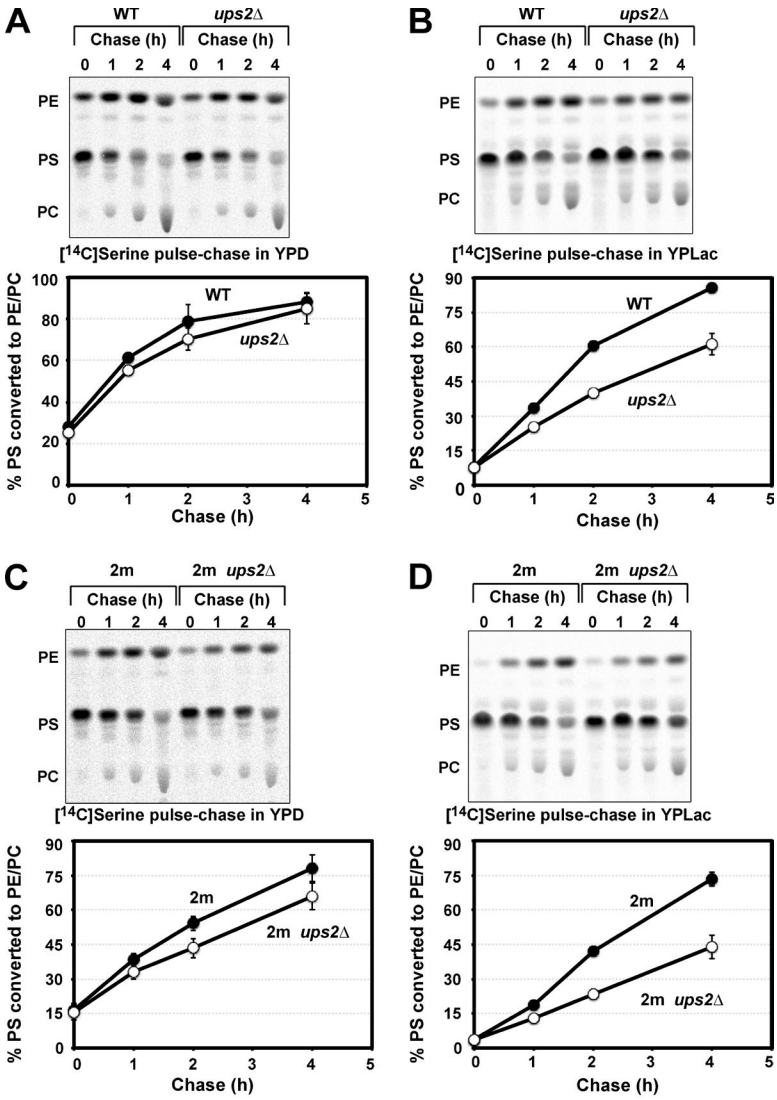

Figure S2. **Ups2 is involved in PS conversion to PE in wild-type and 2m cells under nonfermentable conditions.** (A) PS conversion to PE and PC in wild-type and *ups2Δ* cells growing in YPD was analyzed as in Fig. 5. The mean values for two independent experiments and deviations are shown. (B) PS conversion to PE and PC in wild-type and *ups2Δ* cells growing in YPLac was analyzed as in Fig. 5. The mean values for two independent experiments and deviations are shown. (C) PS conversion to PE and PC in 2m and 2m *ups2Δ* cells growing in YPD was analyzed as in Fig. 5. The mean values for two independent experiments and deviation are shown. (D) PS conversion to PE and PC in 2m and 2m *ups2Δ* cells growing in YPLac was analyzed as in Fig. 5. The mean values for two independent experiments and deviations are shown.

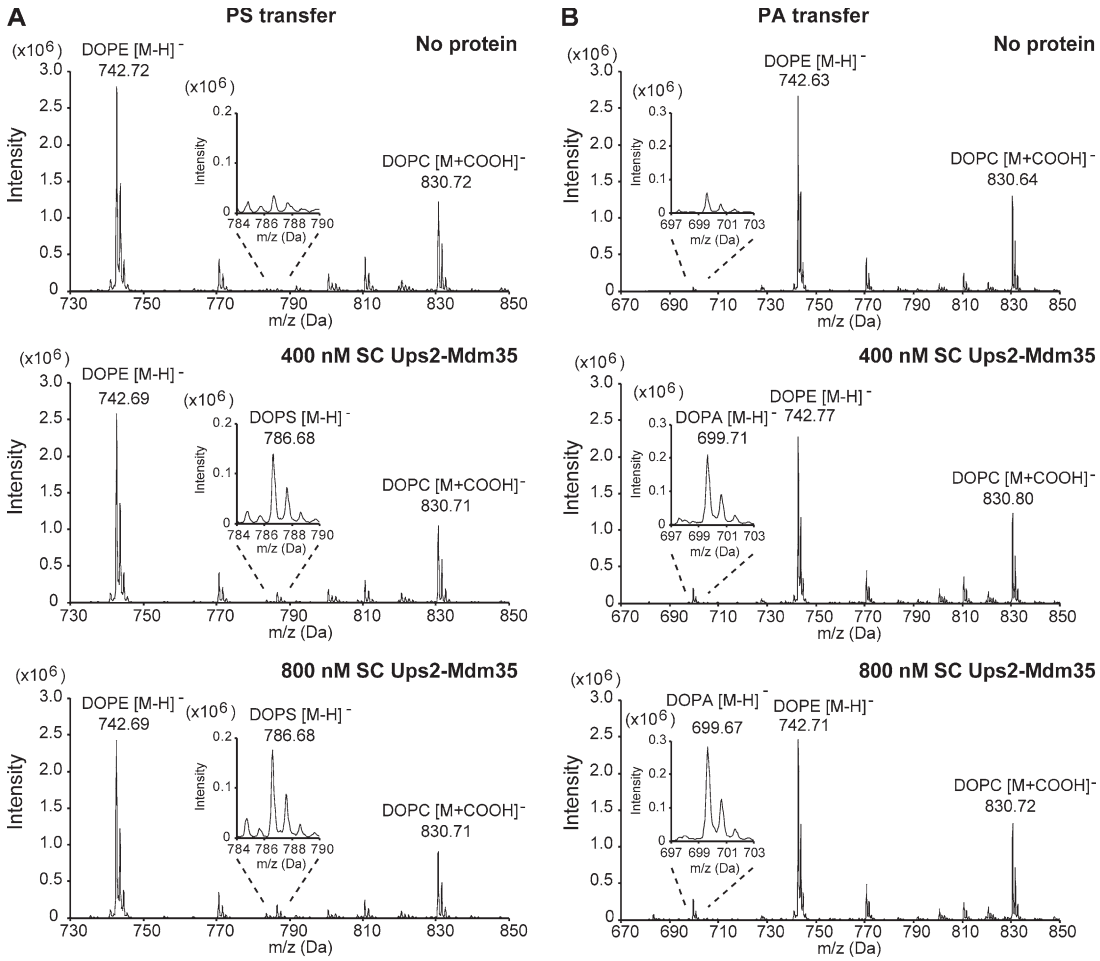

Figure S3. **Mass spectrometry–based phospholipid transfer assay.** (A and B) SC Ups2–Mdm35 was incubated with heavy donor liposomes (200 µM; DOPC/DOPE/DOPS/18:1 Liss-Rhod-PE = 50/29.9/20/0.1) or (200 µM; DOPC/DOPE/DOPA/18:1 Liss-Rhod-PE = 50/29.9/20/0.1) containing 10% sucrose and light acceptor liposomes (200 µM; DOPC/DOPE/18:1 CL = 50/40/10) at 25°C for 10 min. Liposomes were separated by sucrose density-gradient ultracentrifugation. Phospholipids in the acceptor liposomes were analyzed by mass spectrometry. DOPE, DOPS, and DOPA in acceptor liposomes were detected as singly charged ions and indicated in the charts. DOPC in acceptor liposomes was detected as a formate adduct ion and indicated in the charts.

Table S1. **Yeast strains used in this study**

Strain name	Genetic background	Mating type	Genotype	Source
TKY705 (*Wild Type*)	W303	a	*ura3-52 trp1Δ2 leu2-3_112 his3-11 ade2-1 CAN1*	Kuroda et al., 2011
OKY7063 (*ups2Δ*)	W303	a	TKY705, *ups2Δ::CgLEU2*	This study
OKY6003 (*psd1Δ*)	W303	a	TKY705, *psd1Δ::natMX4 can1-100*	This study
OKY6030 (*psd1Δ ups2Δ*)	W303	a	TKY705, *psd1Δ::natMX4 ups2Δ::hphNT1 can1-100*	This study
OKY7049 (2m or *psd2Δ dpl1Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1*	This study
OKY7055 (2m *ups2Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 ups2Δ::CgLEU2*	This study
OKY7081 (4m or *psd2Δ dpl1Δ cho2Δ opi3Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 cho2Δ::hphNT1 opi3Δ::natNT2*	This study
OKY7084 (4m *ups2Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 cho2Δ::hphNT1 opi3Δ::natNT2 ups2Δ::CgLEU2*	This study
OKY7112 (4m *mdm35Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 cho2Δ::hphNT1 opi3Δ::natNT2 mdm35Δ::CgLEU2*	This study
OKY7111 (4m *UPS2↑ MDM35↑*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 cho2Δ::hphNT1 opi3Δ::CgHIS3 GPDpr-UPS2::kanMX4 GPDpr-MDM35::natNT2*	This study
OKY7103 (4m *ups1Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 cho2Δ::hphNT1 opi3Δ::natNT2 ups1Δ::CgHIS3*	This study
OKY7104 (4m *ups1Δ ups2Δ*)	W303	a	TKY705, *psd2Δ::CgURA3 dpl1Δ::CgTRP1 cho2Δ::hphNT1 opi3Δ::natNT2 ups1Δ::CgHIS3 ups2Δ::CgLEU2*	This study
OKY7068 (*UPS1-HA*)	W303	a	TKY705, *UPS1-HA::kanMx4*	This study
OKY7069 (*UPS2-HA*)	W303	a	TKY705, *UPS2-HA::kanMx4*	This study

GPD, glyceraldehyde-3-phosphate dehydrogenase.

Table S2. **PCR templates and primers used for gene manipulation**

Gene	Template plasmid	Source	Primers (5′ to 3′)
ups2Δ::CgLEU2	pCg*LEU2-NT1*	This study	1: TCAGACTAAGATAAAATAATCGAGAATAATTAAAAGACGATAATGCGTACGCTGCAGGTCGAC 2: AAGTAGTATGCAGTGCCATGCGGGATCAAGGAATTTGTATCTCTAATCGATGAATTCGAGCTCG
ups2Δ::hphNT1	pFA6a-*hphNT1*[a]	EUROSCARF	1: TCAGACTAAGATAAAATAATCGAGAATAATTAAAAGACGATAATGCGTACGCTGCAGGTCGAC 2: AAGTAGTATGCAGTGCCATGCGGGATCAAGGAATTTGTATCTCTAATCGATGAATTCGAGCTCG
psd1Δ::natMX4	p4339[b]	Gift from C. Boone[c]	1: TTGGTCGTTATTTTTTGAAGAAGAAGGAAAAGCAAAGCCAGCATGACATGGAGGCCCAGAATA CCCT 2: TATATACAGCAAAATAAATGCTAACTTTACATATGATTGCTTTCACAGTATAGCGACCAGCATTCAC
psd2Δ::CgURA3	pCg*URA3-NT2*	This study	1: TGGTAAAGAATCCTCGATTTTCAGGAGCATCCAACGACGAAGATGCGTACGCTGCAGGTCGAC 2: TTTTTCCATTTTGGTAACCACTAACTACAGCCAATTTTTCGGCGGCTTCAATCGATGAATTCGAGCTCG
dpl1Δ::CgTRP1	pCg*TRP1*-TB	This study	1: AAGTAGGCTAGCTTCTGTAAAGGGATTTTTCCATCTAATACAATGCGTACGCTGCAGGTCGAC 2: CTCTCGTTCTTTAAATTATGTATGAGATTTGATTCTATATAGCTAATCGATGAATTCGAGCTCG
cho2Δ::hphNT1	pFA6a-*hphNT1*[a]	EUROSCARF	1: CGAGTGATTTTCTTAGTGACAAAGCTTTTTCTTCATCTGTAGATGCGTACGCTGCAGGTCGAC 2: ATCCTAGTACTTTTTAAATATATATACTCAAAAAAAAAAAACTCAATCGATGAATTCGAGCTCG
opi3Δ::natNT2	pFA6a-*natNT2*[a]	EUROSCARF	1: TAAACAGCAATTGAAGACAACAAGAATAGCGCAAGTCAAGCGATGCGTACGCTGCAGGTCGAC 2: GCATAGGCTTCTAACATTATAGAATATATAGAAATAGAGCACTTAATCGATGAATTCGAGCTCG
opi3Δ::CgHIS3	p*CgHIS3*-TB	This study	1: TAAACAGCAATTGAAGACAACAAGAATAGCGCAAGTCAAGCGATGCGTACGCTGCAGGTCGAC 2: GCATAGGCTTCTAACATTATAGAATATATAGAAATAGAGCACTTAATCGATGAATTCGAGCTCG
mdm35Δ::CgLEU2	pCg*LEU2-NT1*	This study	1: GTGTTTTAACTTGAATTACAATAACAATAATACCAGTTTTATATGCGTACGCTGCAGGTCGAC 2: TTACATGTTGAATAATGCACATTCTGTGCTAAAATATATACTTCAATCGATGAATTCGAGCTCG
UPS1Δ::CgHIS3	p*CgHIS3*-TB	This study	1: TCTGGCTTCTGAGACGGCGGTAAGATATCCTTAAGAGTTGCAATGCGTACGCTGCAGGTCGAC 2: CTCGCCCATGGTGATATCTTTAAAGATCTTTAAATGGGAACATCAATCGATGAATTCGAGCTCG
GPDpr-UPS2::kanMx4	pYM-N14[a]	EUROSCARF	1: TCAGACTAAGATAAAATAATCGAGAATAATTAAAAGACGATAATGCGTACGCTGCAGGTCGAC 2: CTGGTCCCATGGATAGTTGAAATCGTAACTGTTTTGAAACAATTTCATCGATGAATTCTCTGTCG
GPDpr-MDM35::natNT2	pYM-N15[a]	EUROSCARF	1: GTGTTTTAACTTGAATTACAATAACAATAATACCAGTTTTATATGCGTACGCTGCAGGTCGAC 2: CAGGTCAGTGCATTCAGGCGCAAAACTAGCTGACATTATATTCCCCATCGATGAATTCTCTGTCG
UPS1-HA::kanMX4	pYM45[a]	EUROSCARF	1: TCTGGCTTCTGAGACGGCGGTAAGATATCCTTAAGAGTTGCAATGCGTACGCTGCAGGTCGAC 2: GCATTTGTTATCCAAAAACTCGAAGAGGCGAGAAATCCTCAGTTTCGTACGCTGCAGGTCGAC
UPS2-HA::kanMX4	pYM45[a]	EUROSCARF	1: AAGTAGTATGCAGTGCCATGCGGGATCAAGGAATTTGTATCTCTAATCGATGAATTCGAGCTCG 2: CAGCAAAATATTGACTTGTTTAGAGACGCATACAACCACGAAAATCGTACGCTGCAGGTCGAC

[a]Janke et al., 2004.
[b]Tong and Boone, 2006.
[c]University of Toronto, Toronto, Ontario, Canada.

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