Supporting Information

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SI Text

CHY56, which contains *VPS33-LINKER-TEVSITE-GST*, was constructed by transforming BJ3505 *VPS33-GFP*, which was made as described (1), with DNA PCR-amplified from CSY14 (2) chromosomal DNA using primer set 1 (Table S1). VPS33-LINKER-TEVSITE-GST DNA was PCR-amplified from CHY56 chromosomal DNA using primer set 2, digested with SpeI and SalI, gel-purified (QIAquick Gel Extraction Kit; Qiagen), and ligated into the same sites in p403GAL1 (2) to create p403GAL1-VPS33-LINKER-TEVSITE-GST. pFA6a-kanMX6-PGAL1-MBP was created by digestion of pFA6a-kanMX6-PGAL1-GST (3) with PacI and AscI to remove GST, followed by the insertion of an MBP gene that was PCR-amplified from pMBP-Parallel (4) using primer set 3. CHY69 was created in a manner similar to CHY31 (2) except that the *VPS33* under GAL1 promoter control was from p403GAL1-VPS33-LINKER-TEVSITE-GST, and the GAL1 promoter upstream of *VPS39* was amplified from pFA6a-kanMX6-PGAL-MBP in a two-round PCR using primer set 4 then primer set 5, creating a gene that encodes N-terminally MBP-tagged Vps39p under control of the GAL1 promoter.

A 600-mL culture of CHY69 cells was grown at 30 °C for 24 h in CSM –trp –ura –his –leu + 200 μg/mL G418 and 100 μg/mL nourseothricin (Werner Bioagents) with 2% D-galactose and 1 mg/mL monosodium glutamate in place of $(NH_4)_2SO_4$, then used to start a 10-L culture in YP + 2% D-galactose medium that was grown overnight at 30 °C. Dextrose was added to a final concentration of 2% and growth was continued for 2 h. Cells were harvested, washed with RB400 [20 mM NaHepes (pH 7.4), 10% glycerol, 400 mM NaCl], resuspended in a minimal volume of RB400 with PMSF added at a final concentration of 1 mM, frozen dropwise in liquid N_2, and broken in a Waring blender while suspended in liquid N_2. Lysate was thawed on ice, diluted to 180 mL with RB400, and 20 mL of 10% Triton X-100 in RB400 was added. After 1 h on ice, the lysate was centrifuged for 1 h at 40,000 rpm in a Beckman type 45 Ti rotor at 4 °C, and the lysate supernatant was added to 20 mL of Glutathione Sepharose 4B and nutated at 4 °C for 1 h. The resin was washed four times in two 50-mL conical tubes with 40 mL of 20 mM NaHepes (pH 7.4), 400 mM NaCl, 10% glycerol, 0.5% Triton X-100, 5 mM β-mercaptoethanol per tube, then four times with 40 mL of low-Triton buffer (the same buffer but with only 0.004% Triton X-100) per tube, poured into a column, and eluted with 200 mM NaHepes (pH 7.4), 400 mM NaCl, 10% glycerol, 0.004% Triton X-100, 50 mM glutathione, 5 mM β-mercaptoethanol. Fractions containing protein (by Bradford assay) were pooled and concentrated (Amicon Ultra-15 with a 100-kDa cutoff; Millipore) to $\approx$400 μL, and TEV protease was added at a 1:1 molar ratio to Vps33-GST, which was estimated to comprise half of the protein mass in the purified material. After overnight cleavage at 4 °C, the reaction was applied to a Superdex 200 10/300 GL column (GE) equilibrated in low-Triton buffer. The column was developed with low-Triton buffer at 0.25 mL/min, and 0.5-mL fractions were collected. Fractions containing the most concentrated HOPS complex, as assessed by SDS/PAGE and Coomassie Brilliant Blue R-250 staining (two or three fractions), were pooled and frozen in aliquots in liquid N_2. Low-Triton buffer was used as the HOPS buffer.

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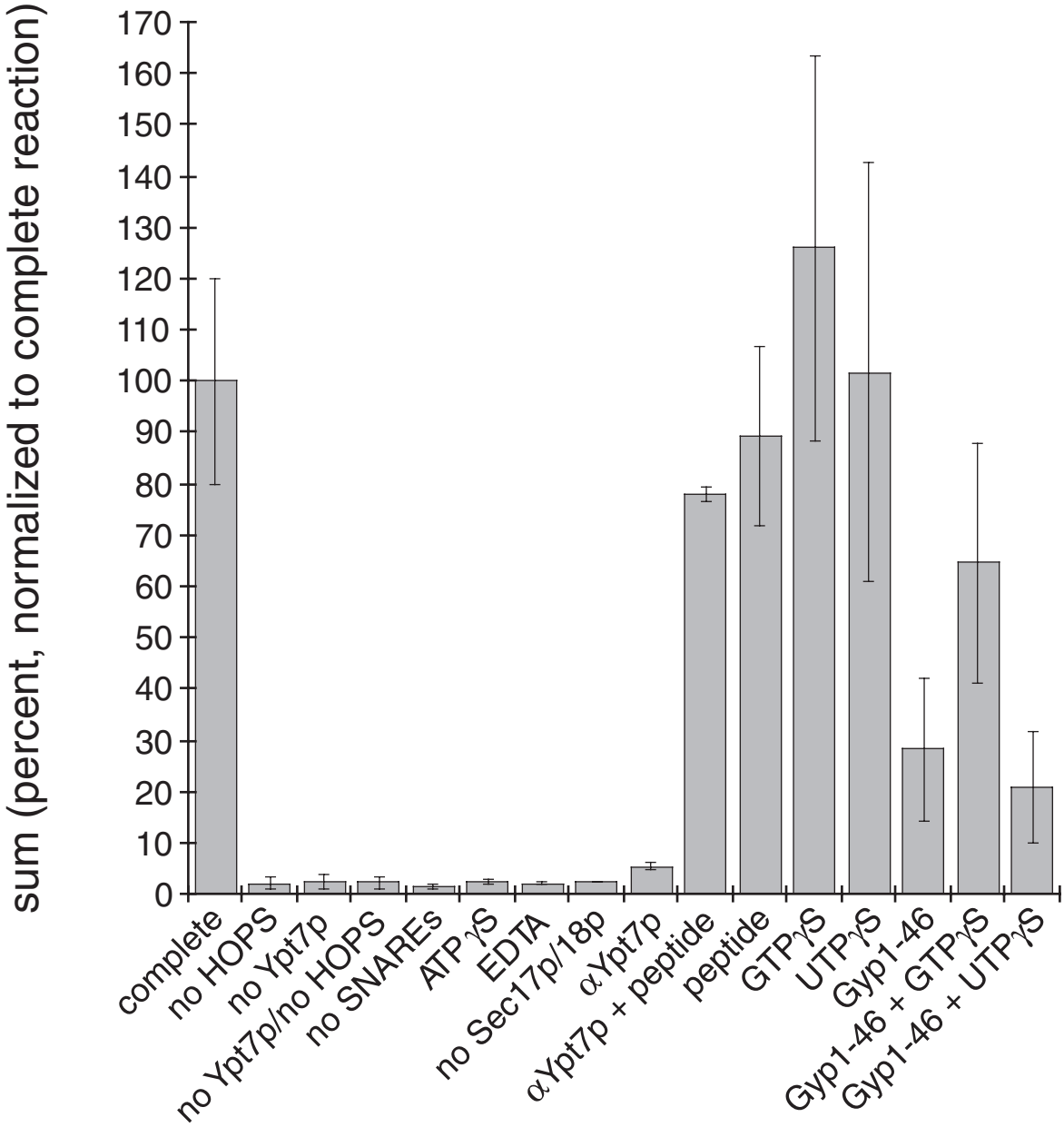


Fig. S1. Representation of proteoliposome dequenching as the normalized sum of the dequenching values for 0–45 min (see *Methods*). Note that the average values for the Gyp1–46-treated and Gyp1–46 + GTPγS-treated conditions are significantly different by one-way ANOVA ($P = 0.02252$).

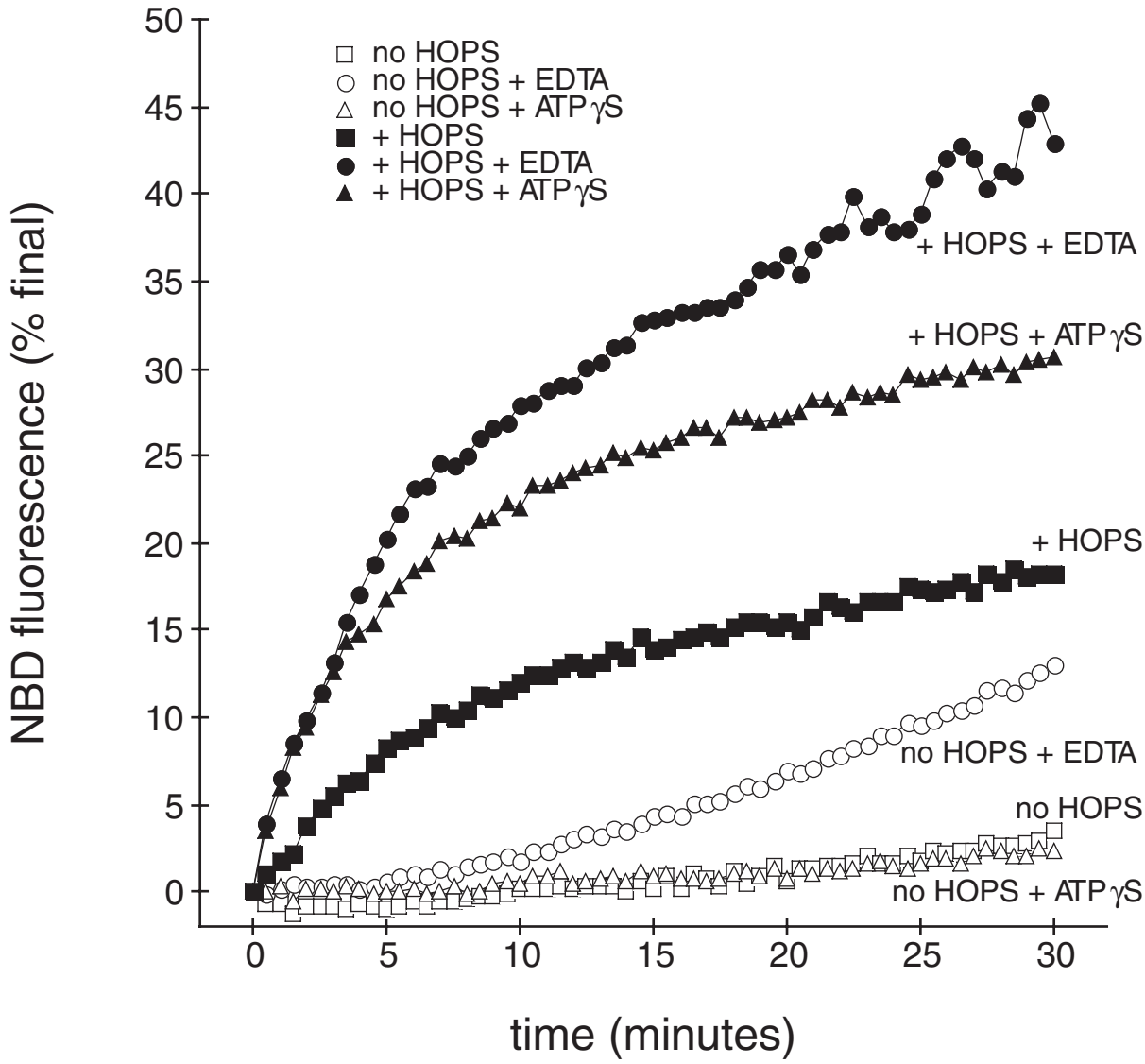


Fig. S2. Neither EDTA nor ATPγS inhibits HOPS complex-stimulated proteoliposome lipid mixing. Standard-method acceptor proteoliposomes (0.4 mM) bearing Vam3p and Vti1p were mixed with donor proteoliposomes (0.05 mM) bearing Nyv1p (5), Vam7p (500 nM), GST-tagged HOPS complex (2) (146 nM) or its buffer, and ATPγS (1 mM), EDTA (10 mM), or no addition, as indicated. All reactions were in RB150 + 5 mM $MgCl_2$ and were incubated at 27 °C for 45 min. Fluorescence intensity (λ_{ex} 460 nm/λ_{em} 538 nm) was recorded every 30 s, and dequenching was calculated as described (5).

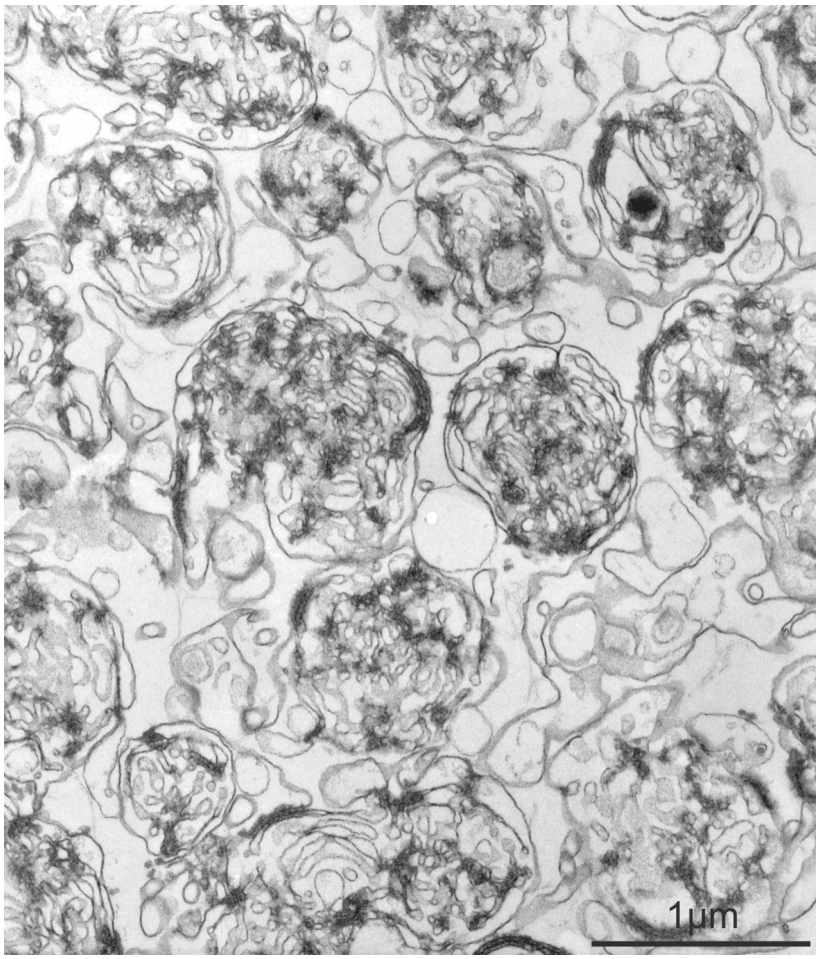


Fig. S3. Low-magnification electron micrograph of the products of a fusion reaction, with HOPS complex, after 45 min. The sample was prepared as described in the legend to Fig. 2. (Scale bar, 1 μm.)

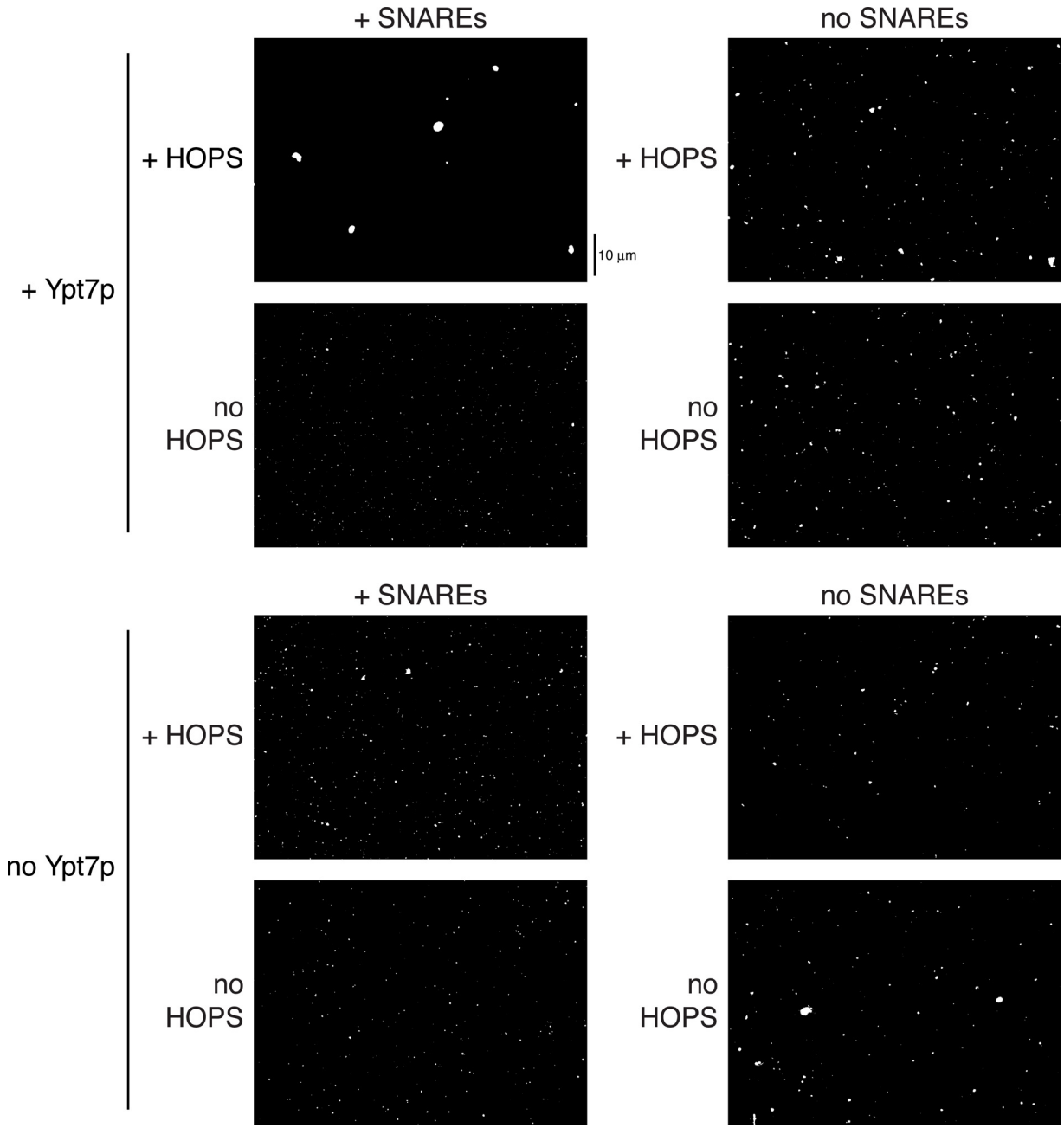
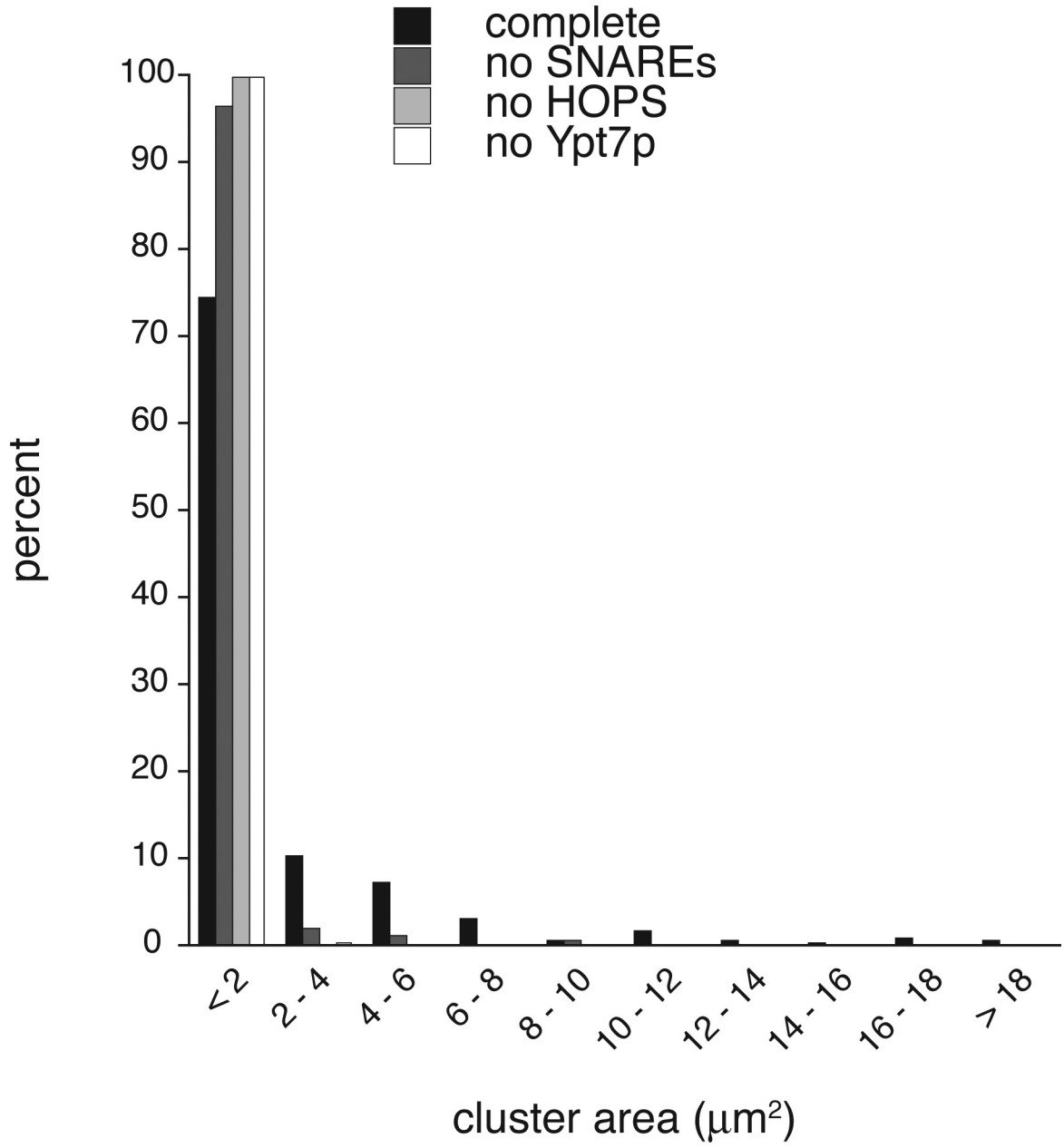


Fig. S4. (*A*) Representative images from proteoliposome fusion reactions, thresholded at 50 by using Adobe Photoshop. (*B*) Histogram analysis of selected cluster area distributions shown in Fig. 4*A*. The vertical axis is the percentage of the total number of clusters in each distribution, because each distribution has a different number of clusters. (*C*) HOPS complex increases the mean fluorescence intensity of proteoliposome clusters in a Ypt7p- and SNARE-dependent fashion. Mean intensities of clusters used for size distributions shown in Fig. 4*A* were calculated by using ImageJ (National Institutes of Health). A probability distribution plot of each cluster's mean intensity is shown. The minimum intensity value is 50 because a threshold of 50 was used for size calculation. The distribution for the + Ypt7p + SNARE + HOPS reaction (■) is significantly different ($P < 0.0001$) from all of the other distributions by the Wilcoxon-Mann–Whitney test. The distributions for the two reactions using proteoliposomes without SNAREs (triangles) are not significantly different ($P = 0.296$) by the same test. (*D*) Median cluster areas for distributions shown in Fig. 4*A*. *P* values for pairs of distributions that are significantly different by the Wilcoxon-Mann–Whitney test are indicated. All other pairs of distributions are not significantly different. (*E*) Sec17p and Sec18p are required for proteoliposome clustering. A cumulative distribution plot showing proteoliposome cluster sizes is shown. All reactions used proteoliposomes with SNAREs and Ypt7p, with HOPS (squares) or HOPS buffer (circles), and Sec17p and Sec18p (filled symbols) or Sec17p and Sec18p buffers (open symbols). Reactions were performed and analyzed as described in Fig. 5. The distribution for the reaction with HOPS, Sec17p, and Sec18p is significantly different ($P < 0.0001$) from all other distributions by the Wilcoxon-Mann–Whitney test. Because of a high fluorescence background, of unknown cause, in the absence of Sec17p and Sec18p, a threshold of 100 was used for size measurement of all of the clusters depicted in this plot. (*F*) ATPγS blocks HOPS complex-dependent proteoliposome clustering. Reactions were performed and analyzed as described in Fig. 5, except that ATPγS (Mg^{2+} salt) was used in place of ATP (Mg^{2+} salt) in two reactions, and all proteoliposomes contained Ypt7p. Distributions of proteoliposome cluster size are shown. The distribution for the reaction with HOPS complex and ATP was significantly different ($P < 0.0001$) from all other distributions by the Wilcoxon-Mann–Whitney test.



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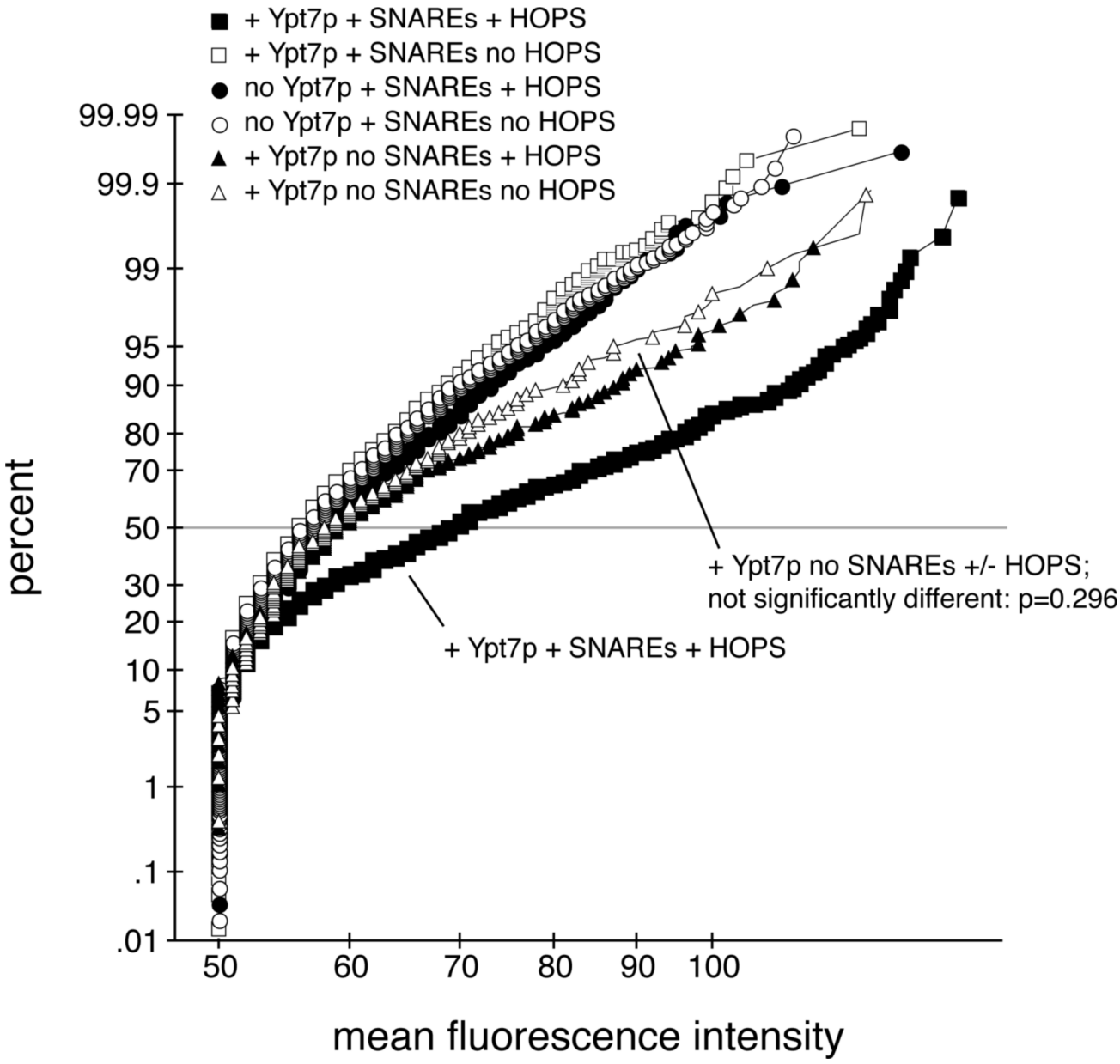

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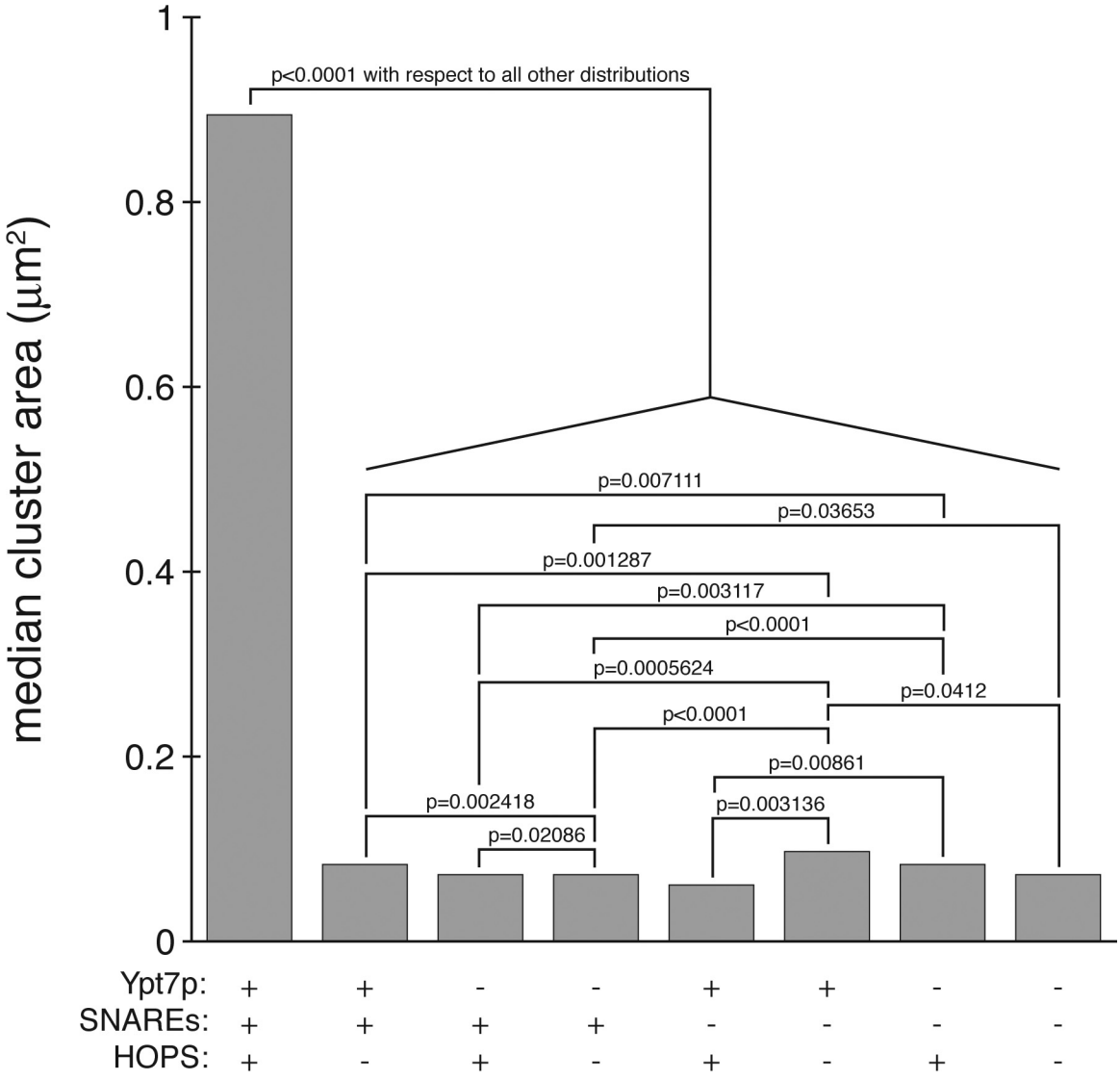

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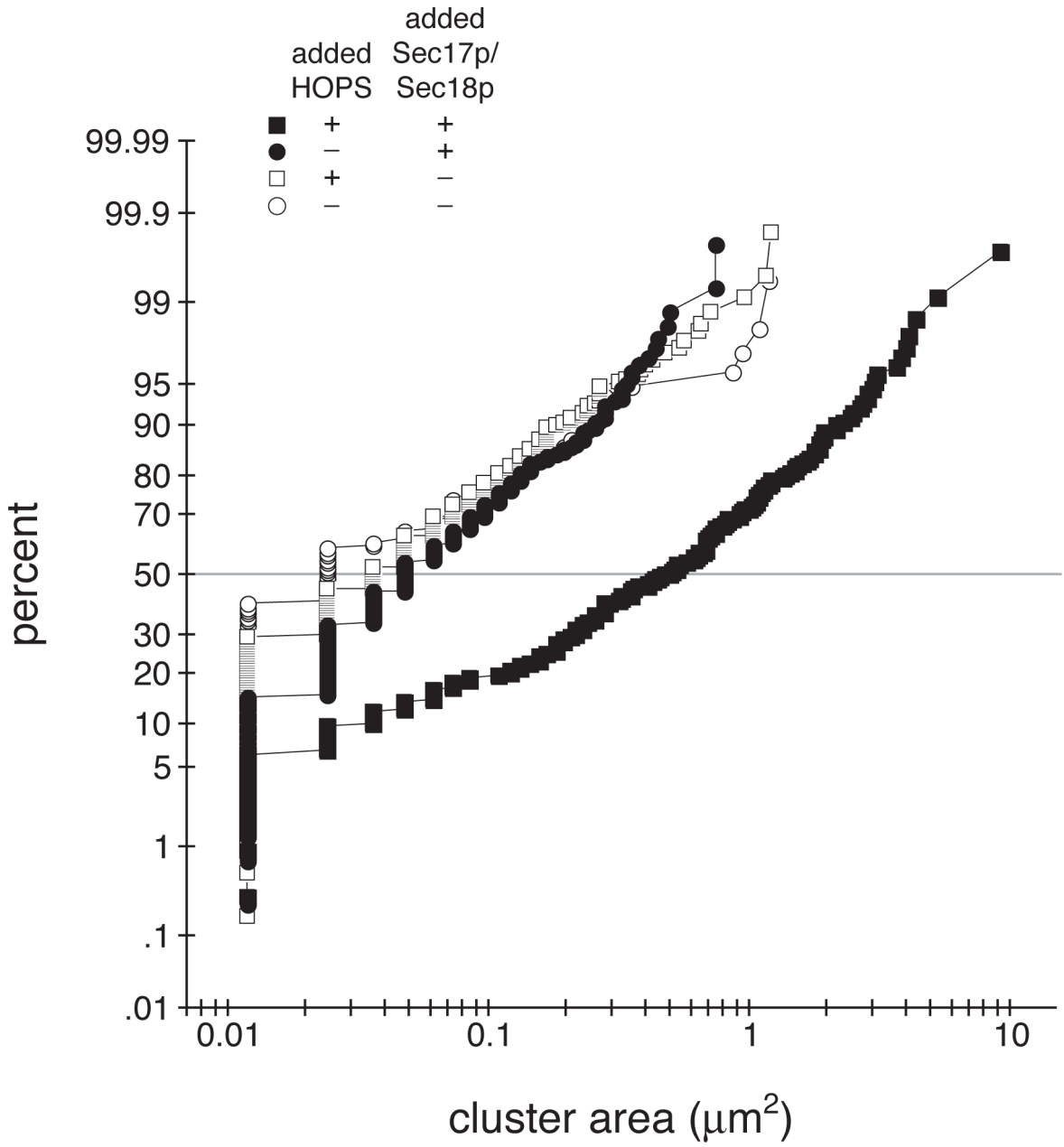

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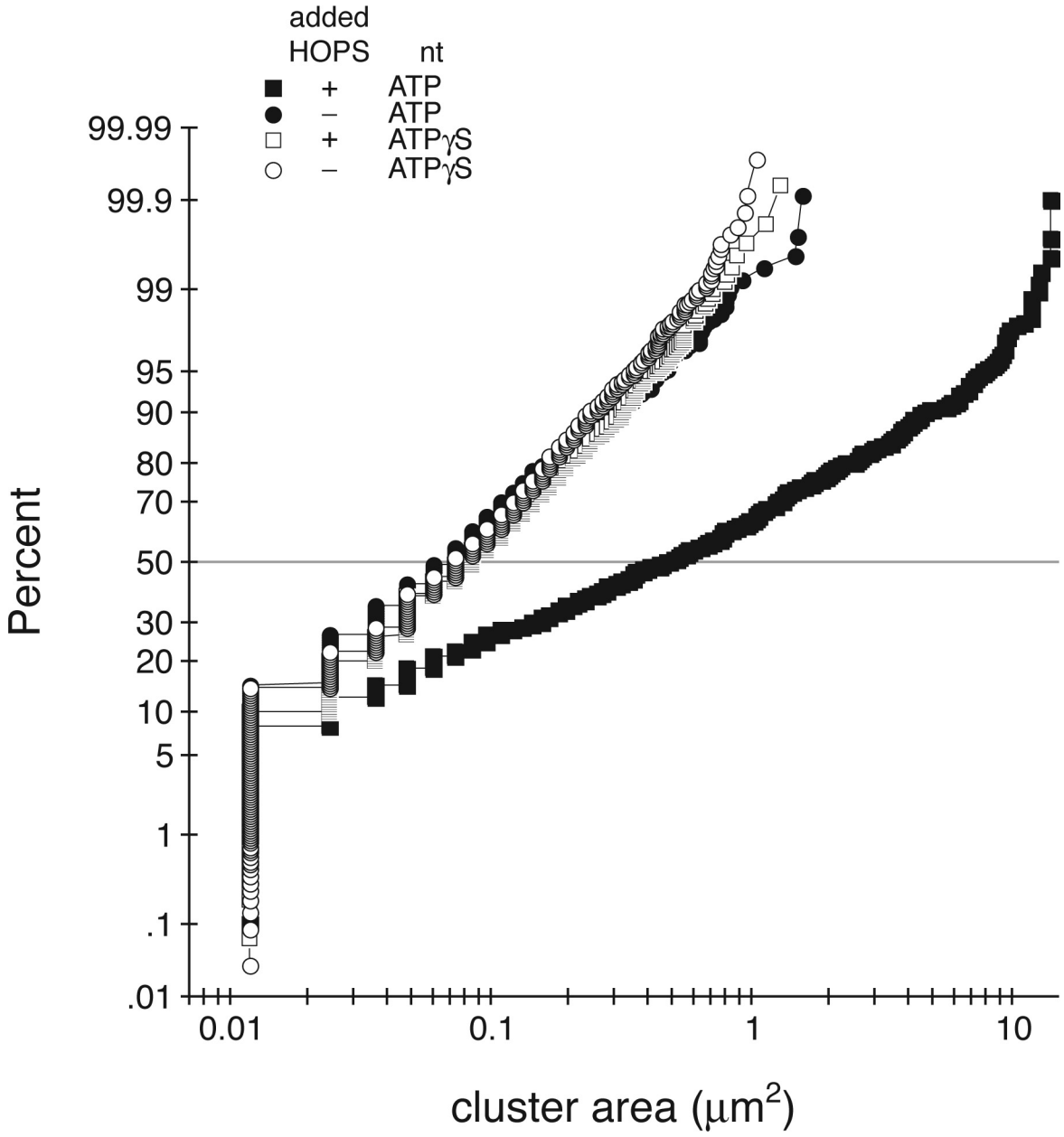

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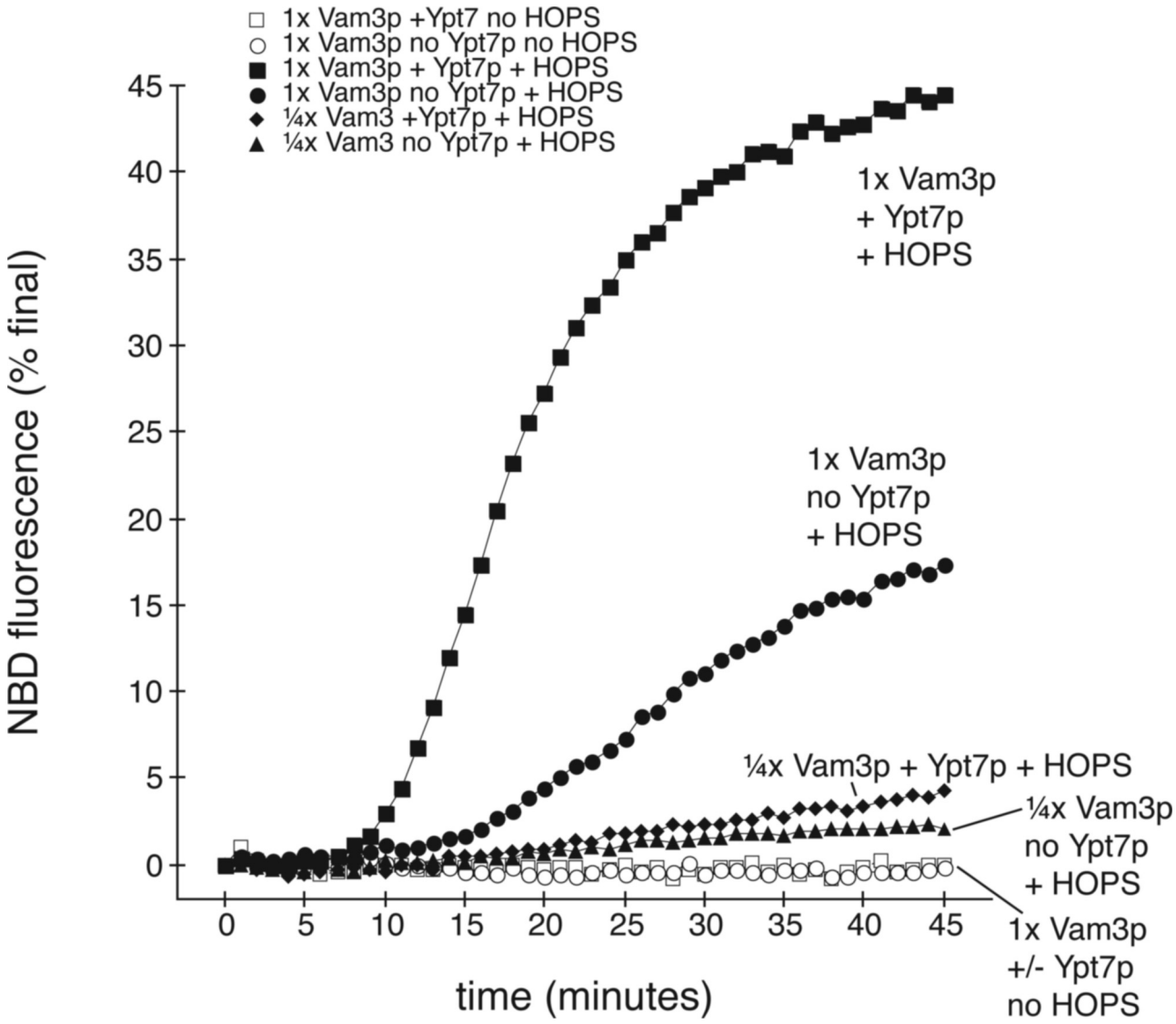
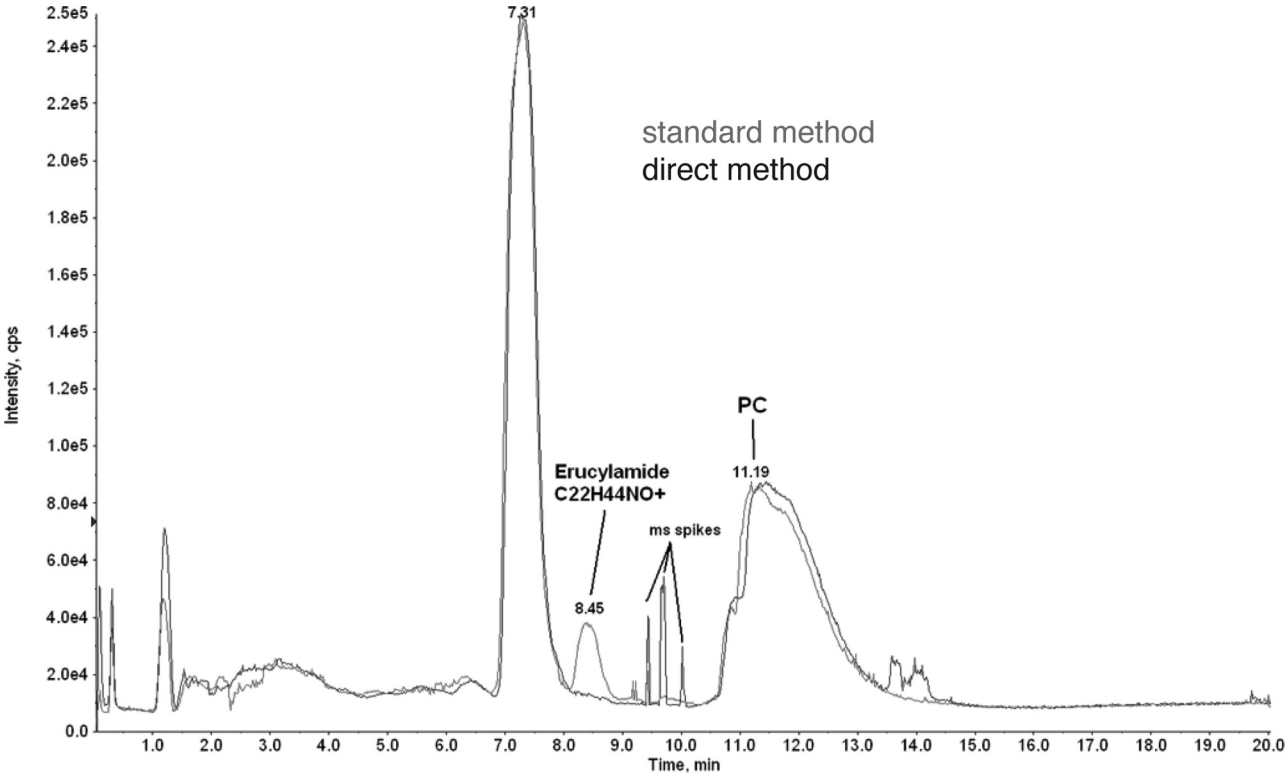


Fig. S5. (*A*) Lower Vam3p levels do not cause Ypt7p dependence for fusion. Standard-method proteoliposomes were prepared with standard levels of Vam3p and with ¼ the normal amount of Vam3p. SDS/PAGE and Coomasssie Brilliant Blue staining confirmed that the second set of proteoliposomes contained approximately ¼ the amount of Vam3p as the first set. Proteoliposome fusion reactions were performed as described (5), using proteoliposomes and with GST-tagged HOPS complex (2) or HOPS buffer as indicated above. (*B*) Lipid compositions of standard-method and direct-method proteoliposomes, assessed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) in positive-ion mode. The number over each peak is the retention time in min. The peak at 7.31 min has not been identified, but is unchanged between standard-method and direct-method liposomes. Lipids from standard-method and direct-method proteoliposomes were extracted by the Bligh-Dyer method (6), then analyzed by LC-ESI-MS in positive-ion mode as described (7). (*C*) Lipid compositions of standard-method and direct-method proteoliposomes, assessed by LC-ESI-MS in negative-ion mode. The number over each peak is the retention time in min. Lipids were extracted by the Bligh-Dyer method (6), then analyzed by LC-ESI-MS in negative-ion mode as described (8). (*D*) Erucylamide does not cause bypass of the Ypt7p requirement for fusion. Proteoliposome fusion reactions using direct-method proteoliposomes were performed as described (see *Methods*), with 22.5 μM erucylamide (Sigma) added as a DMSO solution (1 μL; filled symbols). Reactions without erucylamide (open symbols) received 1 μL of DMSO. (*E*) Cardiolipin levels influence the level of stimulation of fusion by Ypt7p. Standard-method proteoliposomes were made with 1.6% or 0.8% cardiolipin (denoted 1x CL or ½ CL, respectively), and with or without Ypt7p, as described (5). Fusion assays were performed as described (5), with (filled symbols) or without (open symbols) GST-tagged HOPS complex, using proteoliposomes as indicated above.



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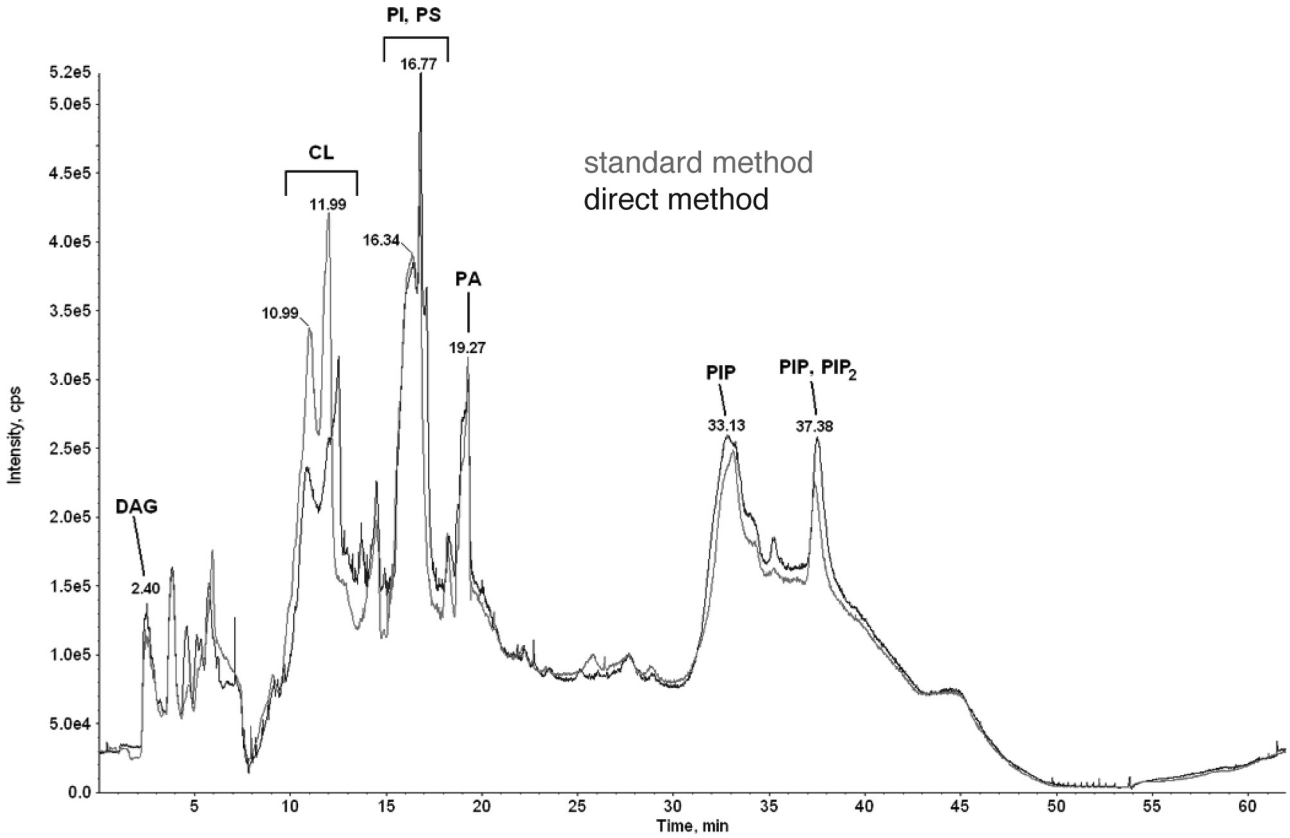

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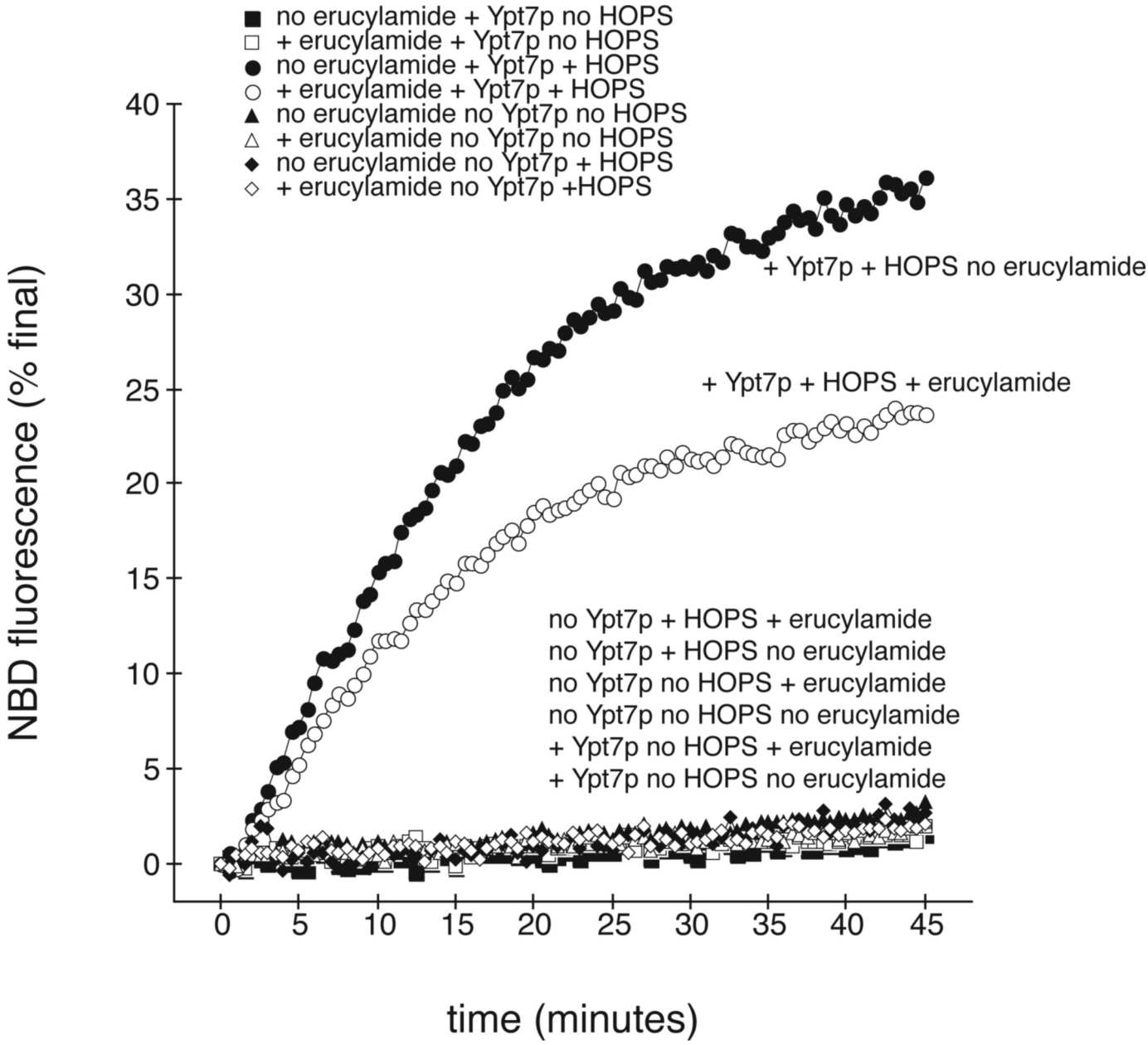

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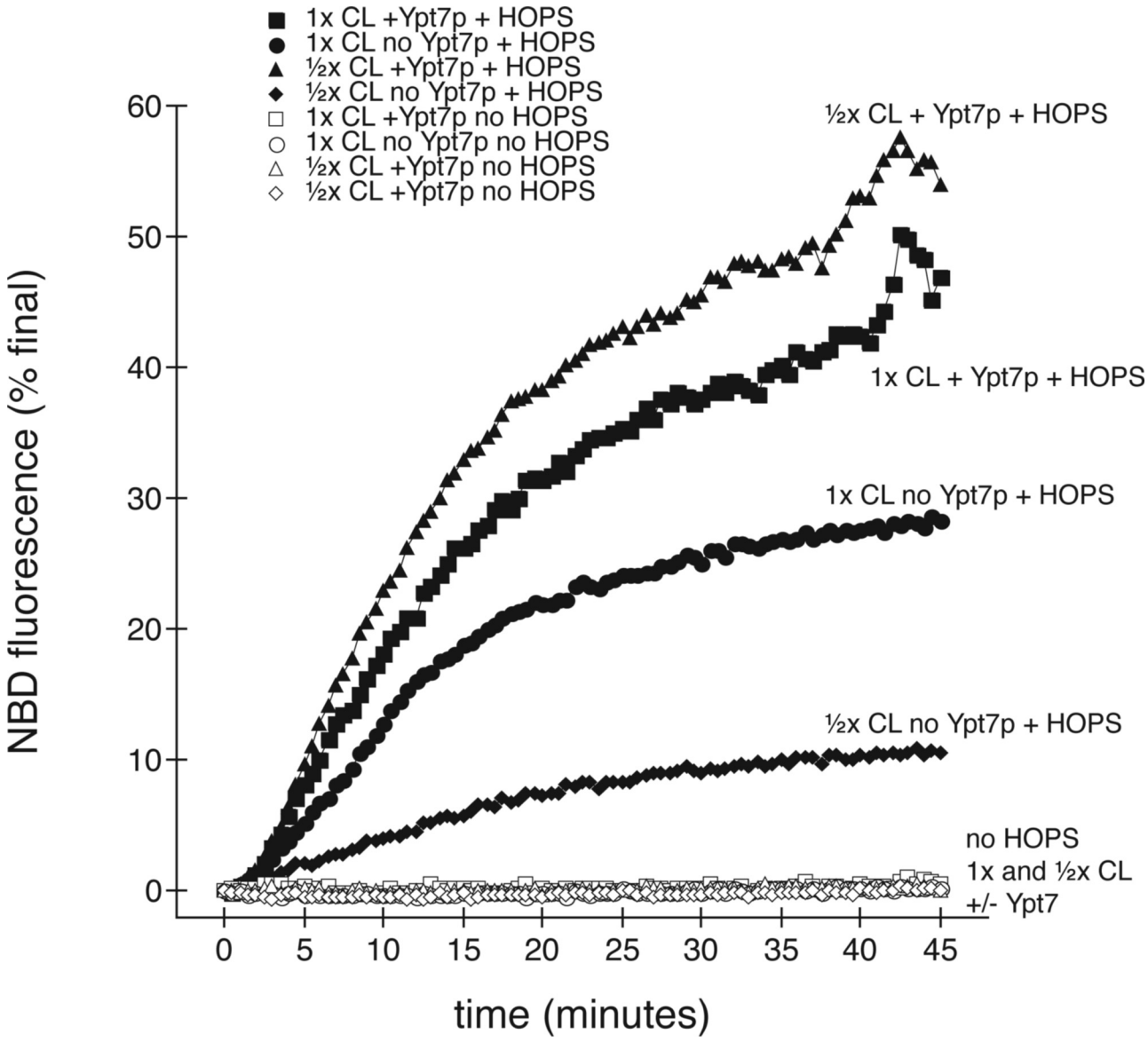

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Table S1. Primers used for plasmid and strain construction

Set	Primer	Sequence
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	b	ATCTGTTGGCACAACATTGC
2	a	GCGCGTCGACTTATCAACGCGGAACCAGATCCG
	b	GCGCACTAGTATGAATAGATTTTGGAATACTAAG
3	a	GCGCttaattaaTATGAAAATCGAAGAAGGTAAACTG
	b	GCGCggcgcgccTCACGAGCTCGAATTAGTCTGC
4	a	GATCAGCAAAAACCCTTCAAAATATCAATTTATACCAAAAATTAAGAATTCGAGCTCGTTTAAAC
	b	GAGCTCTTAAACCTGGACCACCTTGAAAATACAAATTTTCACCTGGACCCGAGCTCGAATTAGTCTGCG
5	a	CGGCTGTTCAATTATCTTTTCAACCGGACGGGTCTTATATTGATCAGCAAAAACCCTTCAAAATATC
	b	AGTGGGCAAAATCGCAGTGATATCCGATGATTTCAGCGAGTGTAGCTTTTGAGCTCTTAAACCTGGACCAC