Calcium-dependent and -independent lipid transfer mediated by tricalbins in yeast

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This SI file contains: Supplemental Figures 1 to 6.

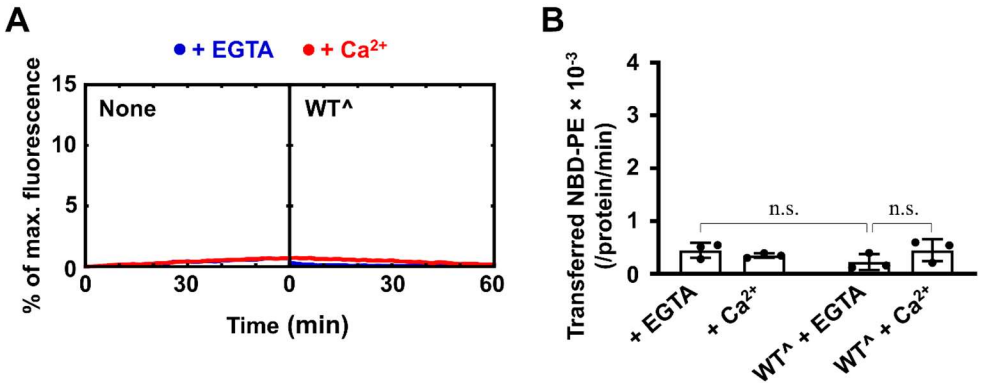

Figure S1. The lipid transfer activity of Tcb3 requires acceptor liposomes. (A) Lipid transfer of the donor liposomes in the absence or presence of 1 μM Tcb3. The FRET-based lipid transfer assays were performed as described in Fig 1C, except the acceptor liposomes were omitted. The lipid transfer reactions included 0.1 mM EGTA or $CaCl_2$. **(B)** Initial lipid transfer rates of NBD-labeled PE in the reactions shown in A. Error bars indicate standard deviation. Data are presented as mean ± SD (n = 3 independent replicates). P values were calculated using two-way ANOVA with Tukey's multiple comparisons test. n.s., $P > 0.05$. F (1, 8) = 0.4947, P = 0.5018 for protein factor and F (1, 8) = 0.5345, P = 0.4856 for Ca^{2+} factor. The interaction between the two factors is F (1, 8) = 3.515, P = 0.0977.

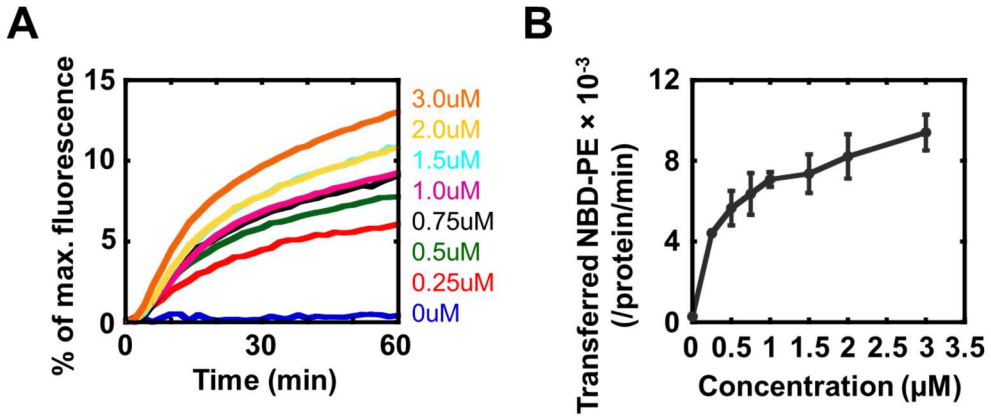

Figure S2. Dose dependence of Tcb3 activity in the Ca^{2+}-dependent lipid transfer reaction. **(A)** Lipid transfer of the protein-free liposomes in the absence or presence of Tcb3 at the indicated concentrations. The lipid transfer reactions included 0.1 mM $CaCl_2$. **(B)** Initial lipid transfer rates of NBD-labeled PE in the reactions shown in A. Error bars indicate standard deviation. Data are presented as mean ± SD (n = 3 independent replicates).

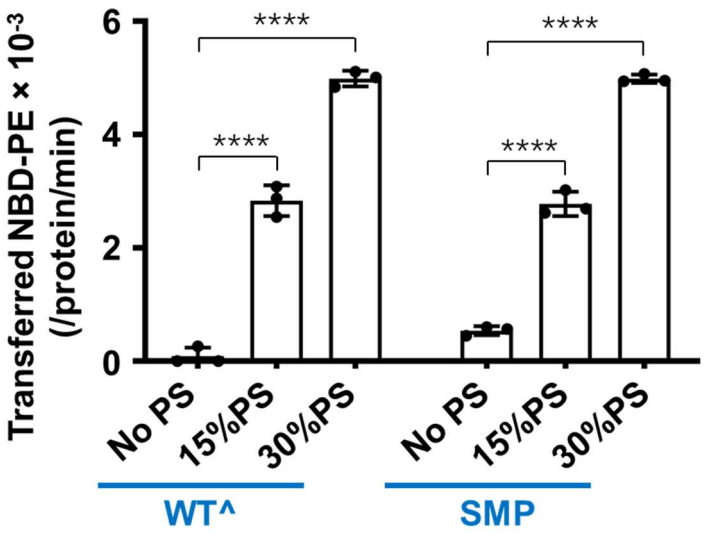

Figure S3. The lipid transfer activity of the SMP domain depends on the PS. (A) The fluorescent lipid transfer assays were performed as described in Fig. 2A. PS was reconstituted into the liposomes at the indicated concentrations. The reactions included 0.1 mM EGTA. Initial lipid transfer rates of NBD-labeled PE in the presence of 1 μM Tcb3 (WT^ or the SMP domain) were shown. The error bar represents the standard deviation. Data are presented as mean ± SD (n = 3 independent replicates). P values were calculated using two-way ANOVA with Tukey's multiple comparisons test. ****, $p < 0.0001$. $F (2, 12) = 1135$, $P < 0.0001$ for lipid factor and $F (1, 12) = 2.717$, $P = 0.1252$ for protein factor. A significant interaction between the two factors is $F (2, 12) = 4.019$, $P = 0.0461$.

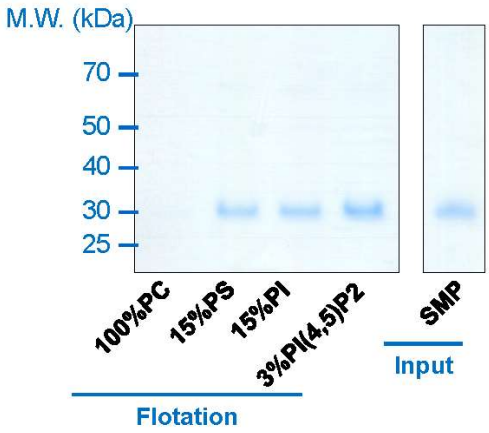

Figure S4. The SMP domain binds to negatively charged liposomes. Left: Coomassie blue-stained SDS-PAGE gel showing the binding of SMP domain to protein-free liposomes in the presence of 0.1 mM EGTA. The liposomes were prepared with PC and the indicated percentage of PS, PI, or PI(4,5)P2. Right: Coomassie blue-stained gel showing the recombinant Tcb3 SMP domain protein. The amount of SMP protein was loaded as 20% of the total input.

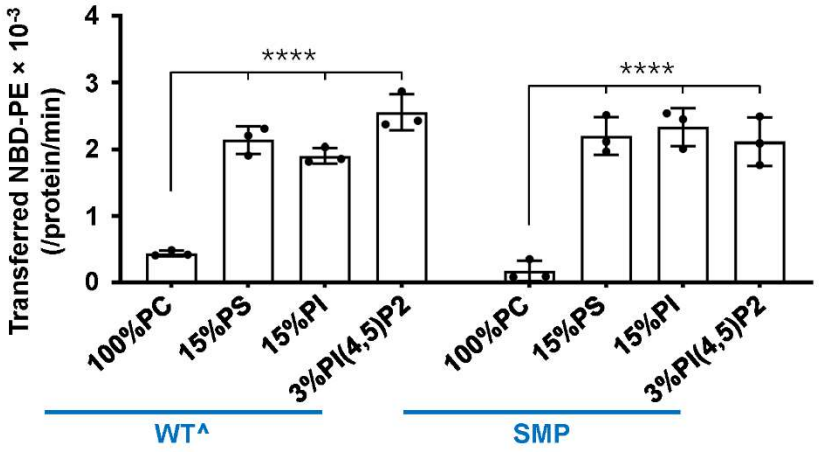

Figure S5. The lipid transfer activity of the SMP domain with different species of negatively charged lipids. (A) The FRET-based lipid transfer assays were performed as described in Fig 1C. PS, PI, or PI(4,5)P2 was reconstituted into the liposomes at the indicated concentrations. The reactions included 0.1 mM EGTA. Initial lipid transfer rates of NBD-labeled PE in the presence of 1 μM Tcb3 (WT^ or the SMP domain) were shown. Error bar represents the standard deviation. Data are presented as mean ± SD (n = 3 independent replicates). P values were calculated using two-way ANOVA with Tukey's multiple comparisons test. ****, $p < 0.0001$. $F (3, 16) = 99.07$, $P < 0.0001$ for lipid factor and $F (1, 16) = 0.3060$, $P = 0.5878$ for protein factor. A significant interaction between the two factors is $F (3, 16) = 3.957$, $P = 0.0275$.

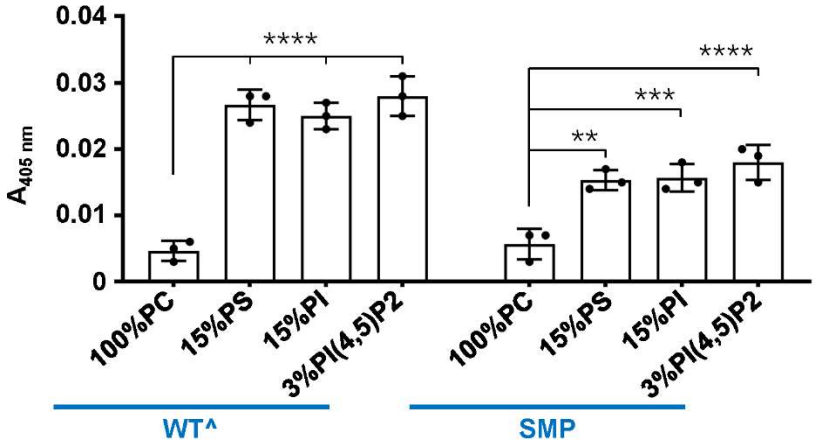

Figure S6. Effect of negatively charged lipids on the membrane tethering activity of the SMP domain. The turbidity of the protein-free liposomes with the indicated percentage of lipids in the presence of 1 μM Tcb3 (WT^ or the SMP domain) was shown. The reactions included 0.1 mM EGTA. Error bars indicate standard deviation. Data are presented as mean ± SD (n = 3 independent replicates). P values were calculated using two-way ANOVA with Tukey's multiple comparisons test. **, p = 0.0014. ***, p = 0.0010. ****, p < 0.0001. F (3, 16) = 81.71, P < 0.0001 lipid factor and F (1, 16) = 66.56, P < 0.0001 for protein factor. A significant interaction between the two factors is F (3, 16) = 9.734, P = 0.0007.