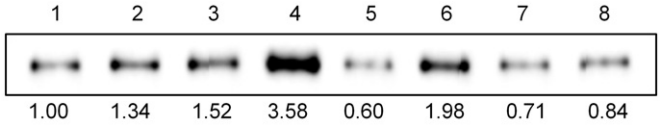
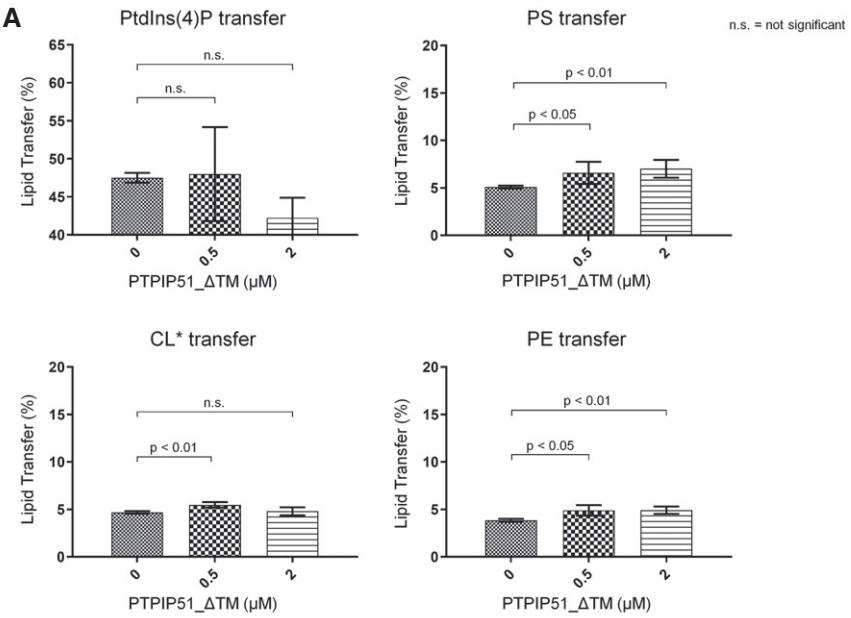
Expanded View Figures



1: 100% POPC
2: 50% POPC, 50% POPE
3: 50% POPC, 40% POPE, 10% CL mix
4: 50% POPC, 30% POPE, 20% CL mix
5: 50% POPC, 40% POPE, 10% PtdIns(4)P
6: 50% POPC, 30% POPE, 20% PtdIns(4)P
7: 50% POPC, 40% POPE, 10% POPS
8: 50% POPC, 30% POPE, 20% POPS

Figure EV1. Liposome precipitation-based phospholipid-binding assay.

Results of the liposome precipitation assay of PTPIP51 with liposomes containing a CL mixture, PtdIns(4)P, and PS. The relative band intensities on Western blots were quantified using ImageJ (National Institutes of Health, USA).



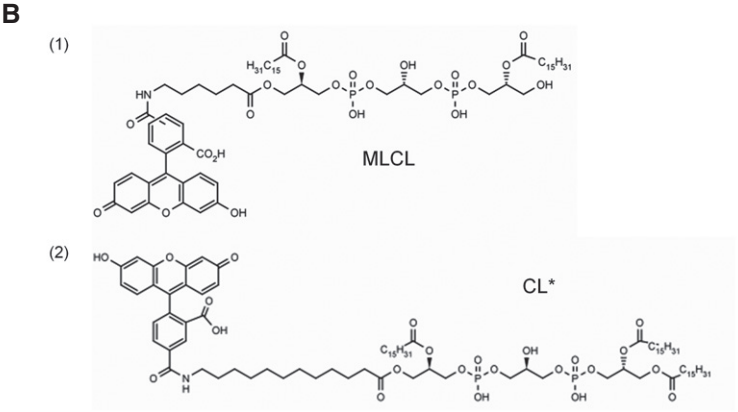


Figure EV2. Streptavidin–biotin bead-based phospholipid transfer assay.

A Results of the *in vitro* phospholipid transfer assay with PTPIP51 and various phospholipids. The percentages of lipid transfer were calculated with the following formula: $100\times F_{acceptor}/(F_{acceptor}+F_{donor})$. The data are presented as the mean ± SD of technical quadruplicate experiments from one representative experiment ($n = 2$), and *P*-values calculated using Student's *t*-test are shown.

B Two kinds of fluorescent CLs were used in this assay. The fluorescent MLCL in Fig 3 has the chemical structure shown in (1), and fluorescent CL* has the chemical structure shown in (2).

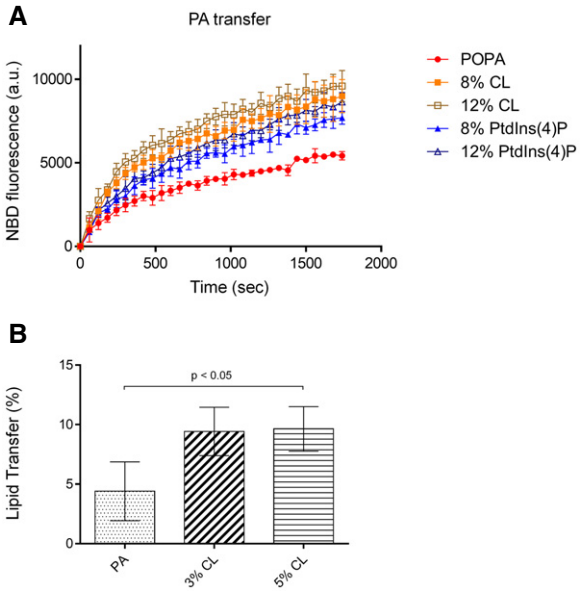


Figure EV3. Lipid transfer assays in the presence of other PTPIP51-interacting phospholipids.

Results of phospholipid transfer assays using PA-containing liposomes supplemented with a CL mixture or PtdIns(4)P. Liposomes with different contents were tested according to the type of transfer assay.

A FRET-based phospholipid transfer assay: acceptor liposomes (POPC:POPE:POPA = 50:40:10 (%)) and donor liposomes (POPC:POPE:Rhod-PE:NBD-PA = 50:40:2:8, POPC:POPE:Rhod-PE:NBD-PA/CL = 50:32:2:8:8 and 50:28:2:8:12, and POPC:POPE:Rhod-PE:NBD-PA/PtdIns(4)P = 50:32:2:8:8 and 50:28:2:8:12 (%)).

B Bead pulldown-based phospholipid transfer assay: acceptor liposomes (100% POPC) and donor liposomes (POPC/biotinyl cap-PE:TopFluor TMA-PA = 97:2:1, POPC:biotinyl cap-PE:TopFluor TMA-PA:CL = 94:2:1:3 and 92:2:1:5 (%)).

Data information: All data are presented as the mean ± SD of technical quadruplicate experiments from one representative experiment (n = 2). The P-value was calculated using one-way ANOVA.

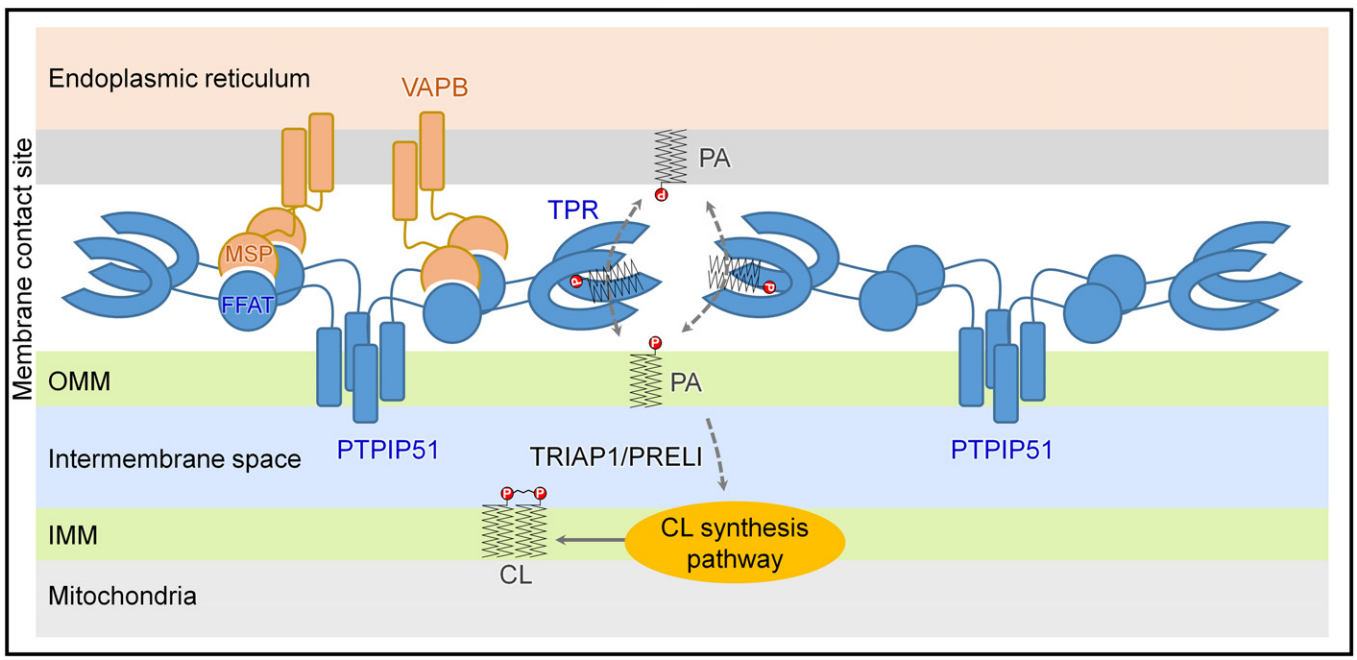


Figure EV4. Model of the PTPIP51–VAPB complex at MAMs and its phospholipid transfer function.

The interaction between tetrameric PTPIP51 and dimeric VAPB mediated by the FFAT-like motif and MSP domain of each protein is shown. The PA transfer function of the TPR domain of PTPIP51 is also presented.