

Figure S5 Validation of the membrane binding experiments by Beclin 1 ECD. (A) The liposomes used in our binding experiments exhibit normal appearance as judged by electron microscopic analysis. Shown here are liposomes containing 50 percent PC and 50 percent specific phospholipid as indicated. Liposomes of other composition are similarly well formed. (B) The C2 domain of a milk-fat globule protein MFG-E8 exhibits a strong preference towards PS-enriched liposomes. The C2 domain of MFG-E8 (residues 306-426), expressed and purified to homogeneity, was examined for binding to liposomes with variable components. In contrast to Beclin 1 ECD, strong preference was observed for PS-enriched liposomes. This result is fully consistent with previous reports [2, 3] and validates our liposome binding assays.

References

- 1. Macedo-Ribeiro S, Bode W, Huber R, *et al.* Crystal structures of the membrane-binding C2 domain of human coagulation factor V. *Nature* 1999; **402**: 434-439.
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- 3. Nakatani H, Aoki N, Nakagawa Y, *et al.* Weaning-induced expression of a milk-fat globule protein, MFG-E8, in mouse mammary glands, as demonstrated by the analyses of its mRNA, protein and phosphatidylserine-binding activity. *Biochem J* 2006; **395**: 21-30.