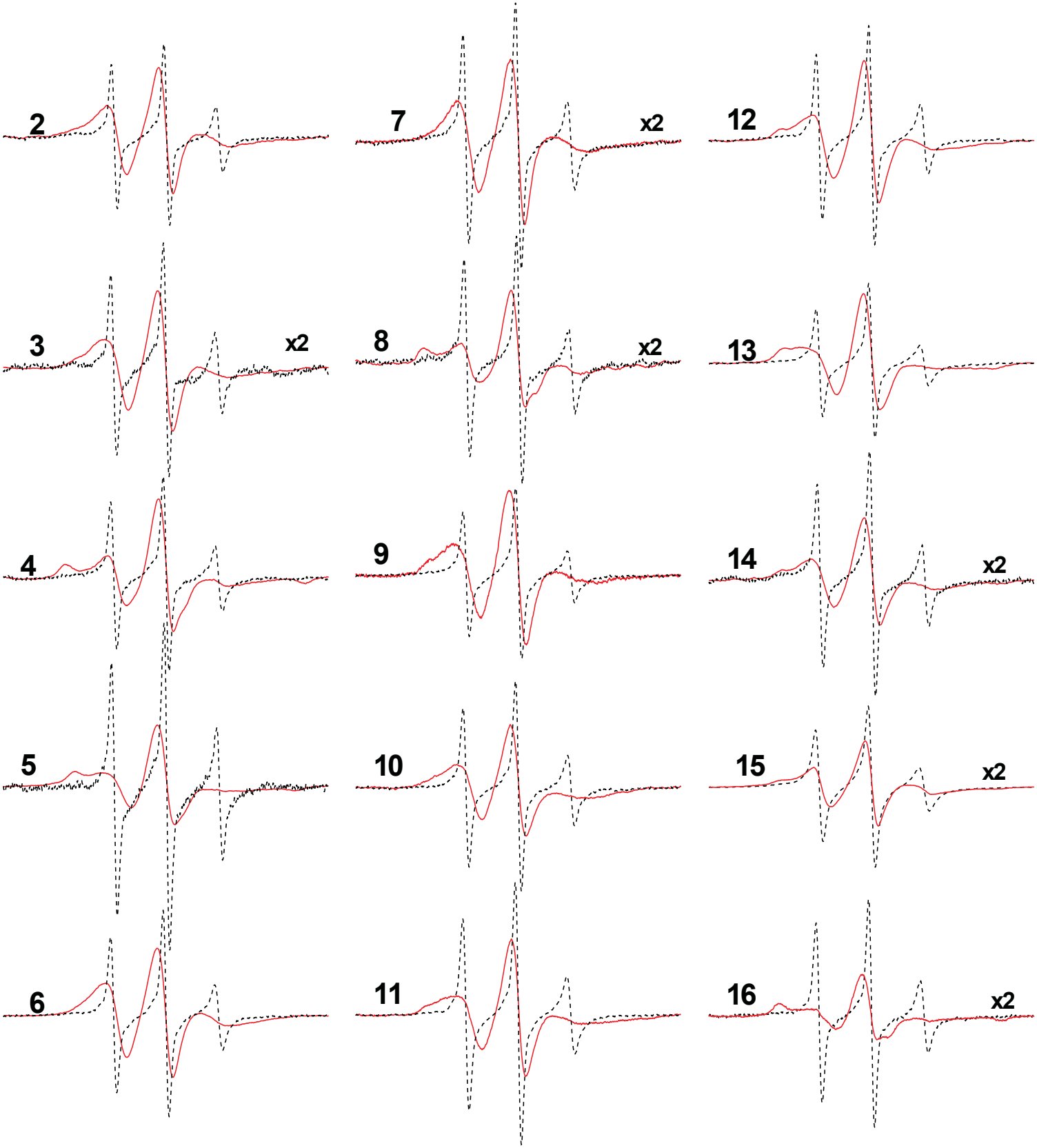
Supplementary Figure 1

EPR Spectra



EPR spectra of R1-labeled endophilin A1 N-BAR derivatives in the absence (black dashed line) and presence of membranes (solid red line). Spectra were normalized to the same number of spins. Spectra of membrane-bound samples were magnified by a factor of 2.5 and spectra of the samples in solution were magnified by the amount indicated on the right.

For labelling a 5x molar excess of spin-label MTSL was incubated with each protein for 30min at room temperature and excess removed by a PD-10 column. This results in a near-quantative labelling.

Supplementary Figure 2

Sequence alignments and 3D models of C. elegans endophilin A and human endophilin B2 N-BAR domains

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r-EndoA1     msvaglkkqfhkatqkvsekvggaEGTKLDDDFKEMERKVDVTSRAVMEI   50
C.ele-EndoA  MSLSGLRKQFNKANQYLSETMGAAEPTKLDDVFNEMEKNVDTTYNLITDL
h-EndoB2     KLASDAGIFFTRAVQFTEEKFGQAEKTELDAHFENLLARADSTKNWTEKI

r-EndoA1     MTKTIEYLQPNPASRAKlsmintmskirgqekgpgyPQAEALLAEAMLKF  100
C.ele-EndoA  VAGTNEYLQPNPATRAKMATQVALSKVRGTTKTSPYPQTEGMLADVMQKY
h-EndoB2     LRQTEVLLQPNPSARVEEFLYEKLDRKVPSRVTN-----GELLAQYMADA

r-EndoA1     GRELGDDCNFGPALGEVGEAMRELSEVKDSLDMEVKQNFIDPLQNLHDKD  150
C.ele-EndoA  GQQLGDNSDLGKSLNDAAETYRQMADIKYQMEDNVKQNFLDPLTHLQNNE
h-EndoB2     ASELGPTTPYGKTLIKVAEAEKQLGAAERDFIHTASISFLTPLRNFLEGD

r-EndoA1     LREIQHHLKKLEGRRLDFDYKKKRQGKIPDEELRQALEKFDESKE--IAESS  200
C.ele-EndoA  LKDVNHHRTKLKGRRLDYDCKKRQQR--RDDEMIQAEEKLEESKR--LAEMS
h-EndoB2     WKTISKERRLLQNRRLDLDACKARLKKAKAAEAKATTVPDFQETRPRNYILS

r-EndoA1     MFNLLEMDIEQVSQLSALVQAQLEYHKQAVQILQQVTVRLEERIRQA     247
C.ele-EndoA  MFNVLSNDVEQISQLRALIEAQLDFHRQTAQCLENLQQQLGHRIKDA
h-EndoB2     ASASALWNDEVDKAEQELRVAQTEFDRQAEVTRLLLEGISSTHVNH-
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conserved negative residues

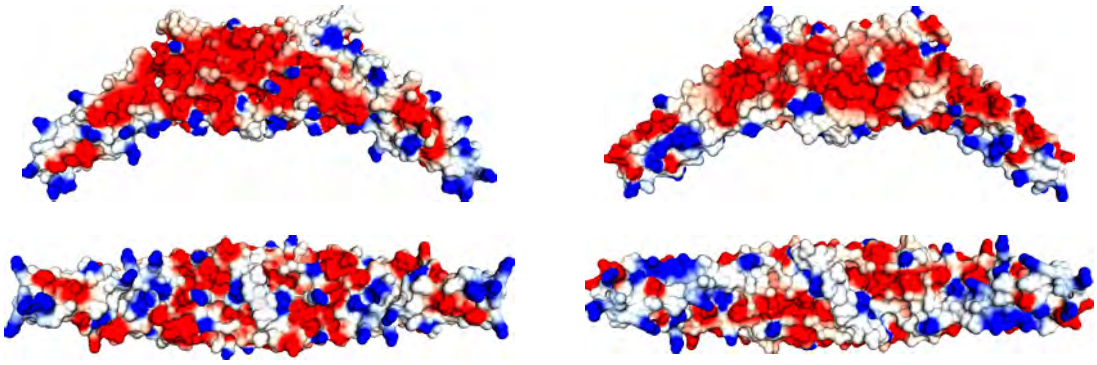
motif breaking helix 1

conserved hydrophobic residues

conserved positive residues

human endophilin B2
side and concave face

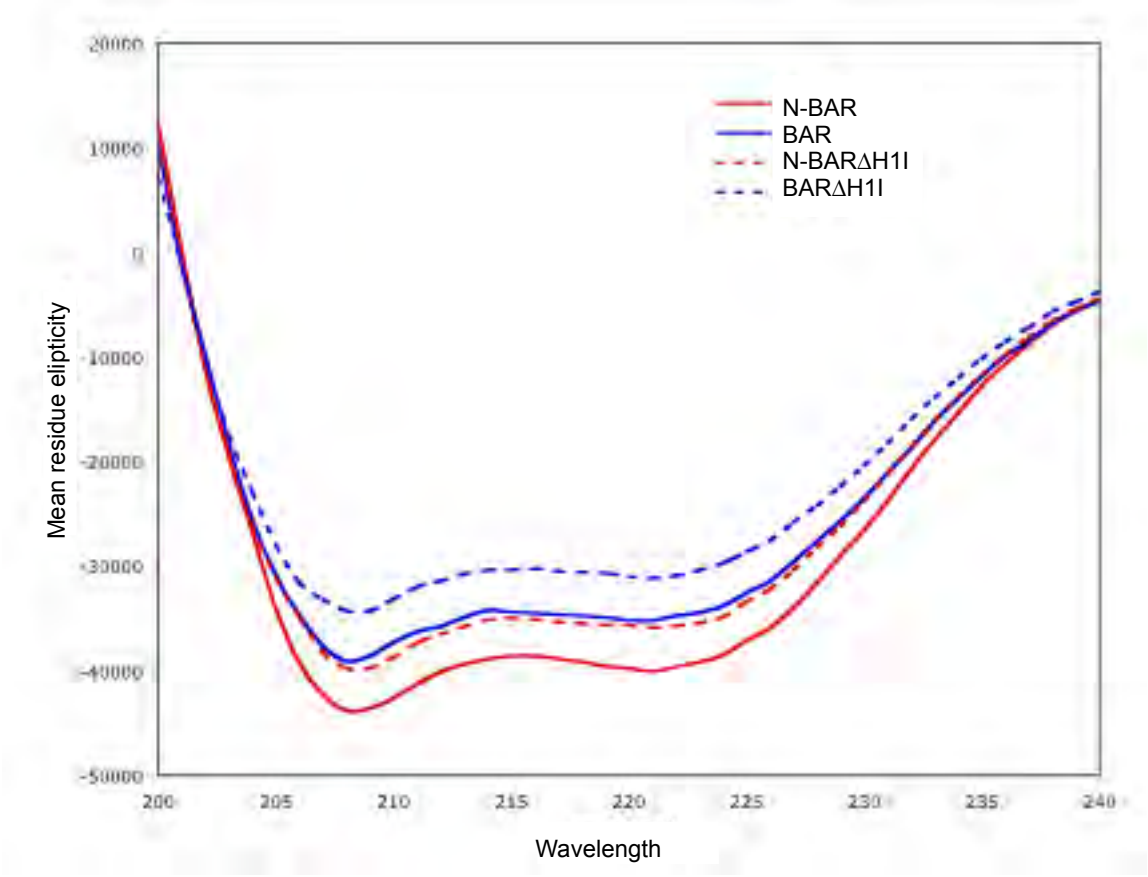
C. elegans endophilin A
side and concave face



Alignments between rat endophilin A1 and other endophilins with highlighting of conserved charged and hydrophobic residues and the QPNP sequence which breaks helix 1. The models are constructed according to the alignments and indicate that the negative surface charge distribution is conserved amongst the endophilins. The positive charge density on the concave face is widely distributed rather than the clustering on the ends of the BAR as seen with endophilin A1.

Supplementary Figure 3

Circular dichroism of endophilin constructs

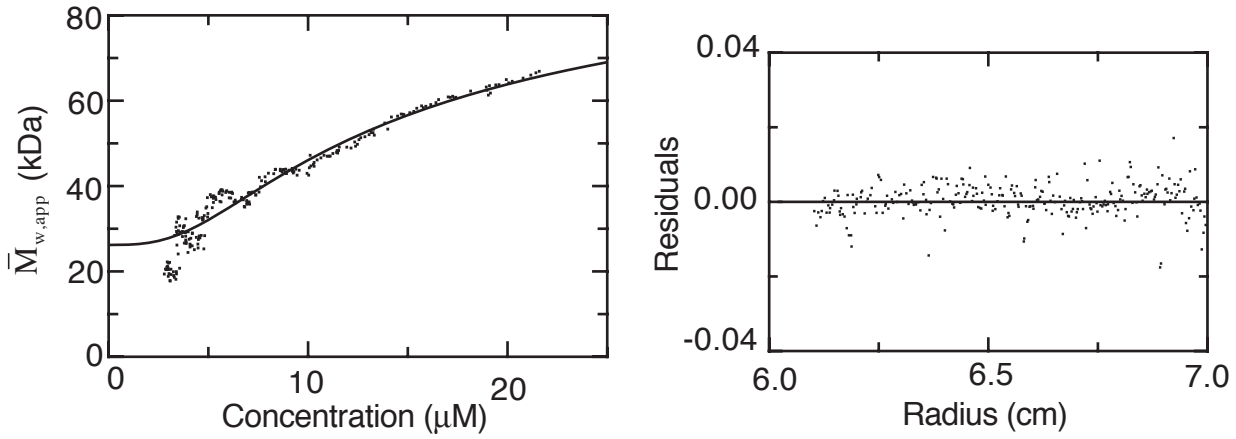


CD of proteins obtained using a Jasco J-810 spectropolarimeter.
Proteins were diluted to 0.2 mg/ml in 5 mM Hepes, 30 mM NaCl, 0.4 mM DTT. Readings were performed at 20 °C using an average of 5 scans with buffer subtracted. Mean Residue Ellipticity was calculated using DICHROWEB (BBSRC).

DICHROWEB is an online server for protein secondary structure analyses from circular dichroism spectroscopic data
Whitmore, L and Wallace, B.A. (2004) Nucleic Acids Research 32:W668-673

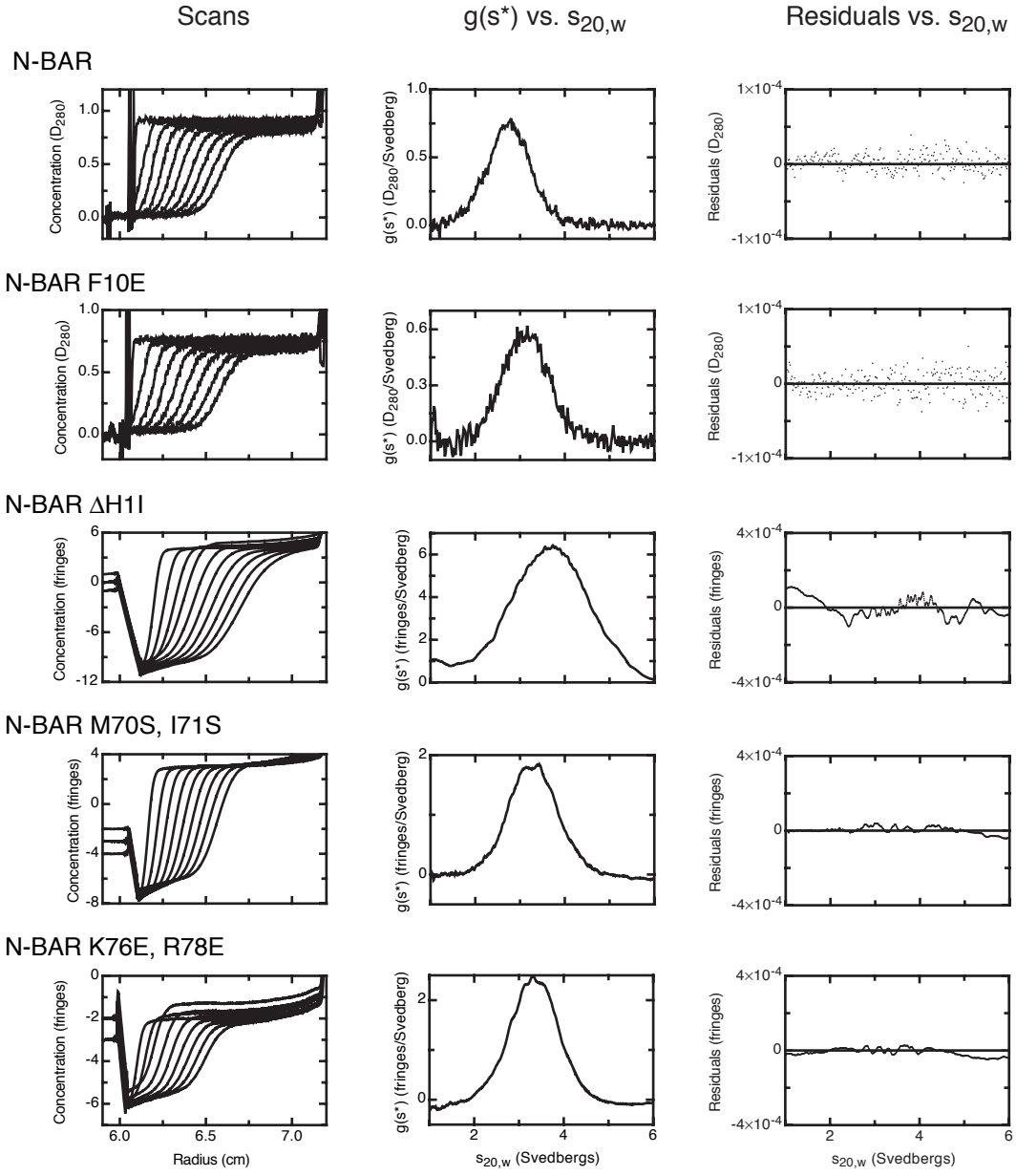
Supplementary Figure 4

A Analytical ultracentrifugation on rat endophilin A1



Equilibrium sedimentation of full-length endophilin shows the formation of higher order oligomers. The data are fitted with a monomer/dimer/tetramer equilibrium. For the fit the monomer is predicted to be 26.2kDa (but it should be 39.9kDa). The errors in the Kd are also very large and thus we conclude that there is a higher order oligomerisation occuring in the cell.

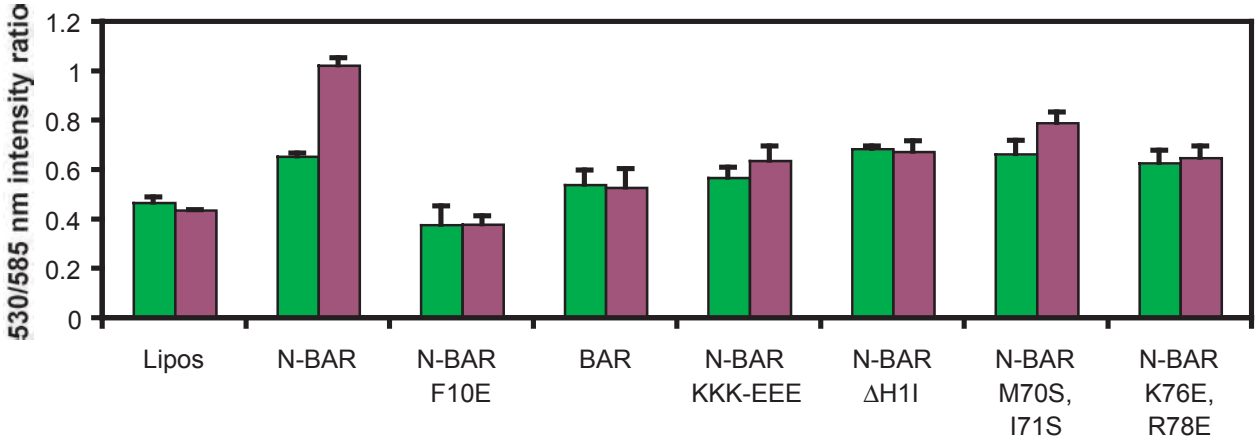
B Analytical ultracentrifugation on endophilin N-BAR mutants



Velocity sedimentation on endophilin N-BAR domain and mutants (the data is summarised in a table in Figure 6 and further details are found in supplementary methods). The scans plot shows every 10th trace. The method used for the fitting has been previously described (Gallop et al. Nature 438: 675-678 2005). Except for N-BAR ΔH1I, the sedimentation fits as a dimer. For the helix1 insert excision, it can be seen that the peak in g(s*) is broader and this fits as a monomer.

Supplementary Figure 5
FRET assay for membrane fusion

Bar chart showing mixed and uniform liposome emission maxima intensity ratios



There is fusion of liposomes when endophilin N-BAR and the M70S, I71S mutant are added, shown by the decrease in FRET on dilution of the two fluorophores and increase in the ratio of donor and acceptor emission maxima (purple bars). In the control condition (uniform liposomes) there is also a change on addition of protein, shown by the green bars with the proteins compared with liposomes alone. The experimental condition (purple bars) is with mixed liposomes. Errors are s.e.m. Values shown are for 55 μM protein.