Supporting Information

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SI Materials and Methods

Rat Endophilin A1 Protein Purification and Spin Labeling. Cysteine and S75D mutations were introduced using a QuikChange Site-Directed Mutagenesis kit (Stratagene). All DNA constructs were examined for accuracy by DNA sequencing. Recombinant rat endophilin A1 was expressed and purified as previously described (1, 2). In short, proteins were expressed in BL21 cells for 16 h at 18 °C. Lysed cells were centrifuged at 40,000 × *g* for 20 min at 4 °C, and the supernatant was bound to glutathione beads for 1 h at 4 °C. The beads were washed with an excess of 20 mM Hepes·HCl, pH 7.4, 150 mM NaCl, 1 mM DTT, and 1 mM EDTA. The GST tag was cleaved by incubation with thrombin protease (Sigma-Aldrich) for 16 h at 4 °C. Cleaved proteins were further purified by anion exchange chromatography and Superdex 200 gel filtration. Purified protein was reacted with 8× molar excess of the spin label (1-oxy-2,2,5,5-tetramethyl-*d*-pyrroline-3-methyl)-methanethiosulfonate. Excess spin label was removed by using PD10 columns (GE Healthcare). Protein concentrations were quantified by using UV absorbance at 280 nm and an ε of 5,240 M^{-1} cm^{-1}.

Lipid and Tube Sample Preparation. Optimized conditions for tube formation were found by using lipid vesicles composed of 1,2-Dioleoyl-sn-Glycero-3-[Phospho-*rac*-(1-glycerol)] sodium salt and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine combined in a 2:1 mass ratio, respectively (Avanti Polar Lipids). The chloroform mixture was dried by a constant stream of N_2 and desiccated overnight. The lipid film was then resuspended into buffer (20 mM Hepes, pH 7.4) to a final concentration of 4 mg/mL. Each sample was prepared with 100 μg of lipid mixture and 50 μg of spin-labeled protein in ∼50 μL total volume (1:70 protein:lipid molar ratio) and allowed to react for 20 min at room temperature. Dilution of spin-labeled endophilin A1 to 25% during experiments that tested for spin–spin coupling was done through the addition of threefold excess WT endophilin A1 with its single cysteine mutated to serine (C108S) and threefold excess lipid mixture. This mutation does not alter the ability of endophilin to tubulate vesicles, as evidenced by negative-stain transmission EM (1). The tube-bound and unbound proteins were separated by centrifugation at 16,000 × *g* for 15 min at 37 °C, removing the supernatant, and resuspending the pellet with 6 μL of buffer (20 mM Hepes, pH 7.4, 150 mM NaCl). Tube formation was confirmed for each sample through negative-stain EM. Aliquots (4 μL) of each sample were loaded onto a Formvar Copper Film (Electron Microscopy Sciences), stained with 1% uranyl acetate for 60–90 s, and visualized by using a JEOL JEM-1400 transmission electron microscope. WT and S75D mutant samples were done side-by-side using the conditions described earlier.

Titration Assay for Detecting Membrane Binding. Lipid titration assays were performed by using the conditions described earlier or with a previously used lipid composition of a 5:2:1:1 molar ratio of L-α-phosphatidylcholine, L-α-phosphatidylethanolamine, L-α-phosphatidylserine, and cholesterol (3). The latter composition was rehydrated in buffer (20 mM Hepes, pH 7.4, 150 mM NaCl), made into small unilamellar vesicles by using a tip sonicator, and centrifuged for 10 min at 16,000 × *g* to remove metallic debris. For both lipid compositions, increasing amounts of lipid were incubated with 20 μM spin-labeled endophilin derivatives and monitored by continuous-wave (CW) electron paramagnetic resonance (EPR). All samples were measured by using the same number of scans on a Bruker EMX spectrometer fitted with a Bruker ER4119HS resonator (12.7 mW power), and amplitudes were obtained from the signal measured by WinAcquisit (Bruker Biospin).

CW EPR Continuous Wave and Power Saturation Measurements. EPR spectra as well as power saturation experiments were obtained by using a Bruker EMX spectrometer fitted with a dielectric resonator. Spectra were obtained at 1.85 mW incident microwave power. Power saturation measurements were typically performed by using a range of 1.26–63.46 mW incident microwave power. In the case of high NiEDDA accessibilities, experiments were performed by using a range of 1.26–100.58 mW incident microwave power. Scan width is 150 G. O_2 and NiEDDA accessibilities were obtained by power saturation as previously described (4). Briefly, accessibility to O_2 was measured with samples equilibrated with air, and accessibility to NiEDDA by adding 10 mM NiEDDA and flushing the O_2 out of the sample by applying a constant stream of N_2. The depth parameter Φ was calculated by using the relationship $\Phi = \ln[\Pi(O_2)/\Pi(\text{NiEDDA})]$. Φ Values less than −1.5 represent bulk solvent exposure and lie outside the range of calibration (Fig. S1). Calibration of immersion depth in relation to accessibility was measured by using 1-palmitoyl-2-stearoyl-(n-DOXYL)-*sn*-glycero-3-phosphocholine and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho(tempo)choline (Avanti Polar Lipids). The following linear relation was determined between depth and Φ for the nitroxide moiety: $[\text{Å}] = 7.86\Phi - 2.83$ (Fig. S1). Depth values reported for vesicle-bound endophilin were obtained by using a previously reported depth calibration for this system (2).

Pulse EPR and Distance Analysis. For intermolecular distance measurements between single cysteine mutants in the N terminus (H0) of endophilin A1, samples were prepared as described earlier and with 100% of the protein spin-labeled to enhance signals from neighboring dimers. Intramolecular distance measurements between single cysteine spin-labeled mutants in the insert region of endophilin A1 (between subunits of the dimer) were prepared as described earlier, but were constituted with twofold excess of cysless endophilin A1 (C108S) to minimize intermolecular signals. Centrifuged sample pellets were resuspended in cryoprotectant buffer containing 20–25% (wt/vol) sucrose before loading into a quartz capillary and flash-freezing. All samples were flash-frozen, and data were acquired at 78 K. A Bruker Elexsys E580 X-band pulse EPR spectrometer fitted with a 3-mm split ring (MS-3) resonator, a temperature controller (ITC503S; Oxford Instruments), and a continuous-flow helium cryostat (CF935; Oxford instruments) was used for all four-pulse double electron-electron resonance (DEER) experiments. The data were fitted by using Tikhonov regularization (5) as implemented in the DEERAnalysis 2011 package (6). The background contribution from nonspecific interaction was subtracted by using a fit dimensionality model.

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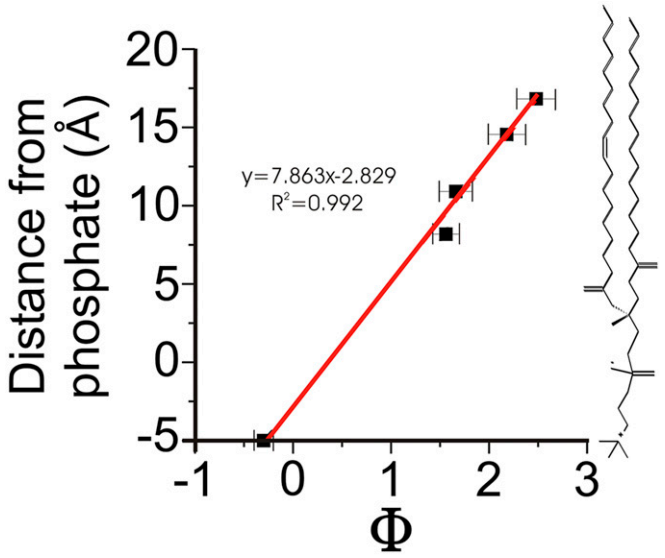

Fig. S1. Depth calibration using spin-labeled lipids. Accessibility to O_2 and NiEDDA of spin-labeled lipids under endophilin A1-induced tubulating conditions (*Materials and Methods*) are summarized by $\Phi = \ln(\Pi O_2/\Pi NiEDDA)$. Doxyl-labels (black squares) were used for positions 5, 7, 10, and 12 along the acyl chain. TempoPC was used to introduce a label in the headgroup region (substituting an *N*-methyl group on the choline headgroup). Spin-label immersion depths were taken from previous studies (1–3). The points were fit by using the following equation, with d representing distance from the phosphate group: $d = (7.863\Phi - 2.829)$ Å; ($R^2 = 0.992$). A generic phospholipid structure is plotted alongside the plot for scale. Positive values indicate deeper immersion depths.

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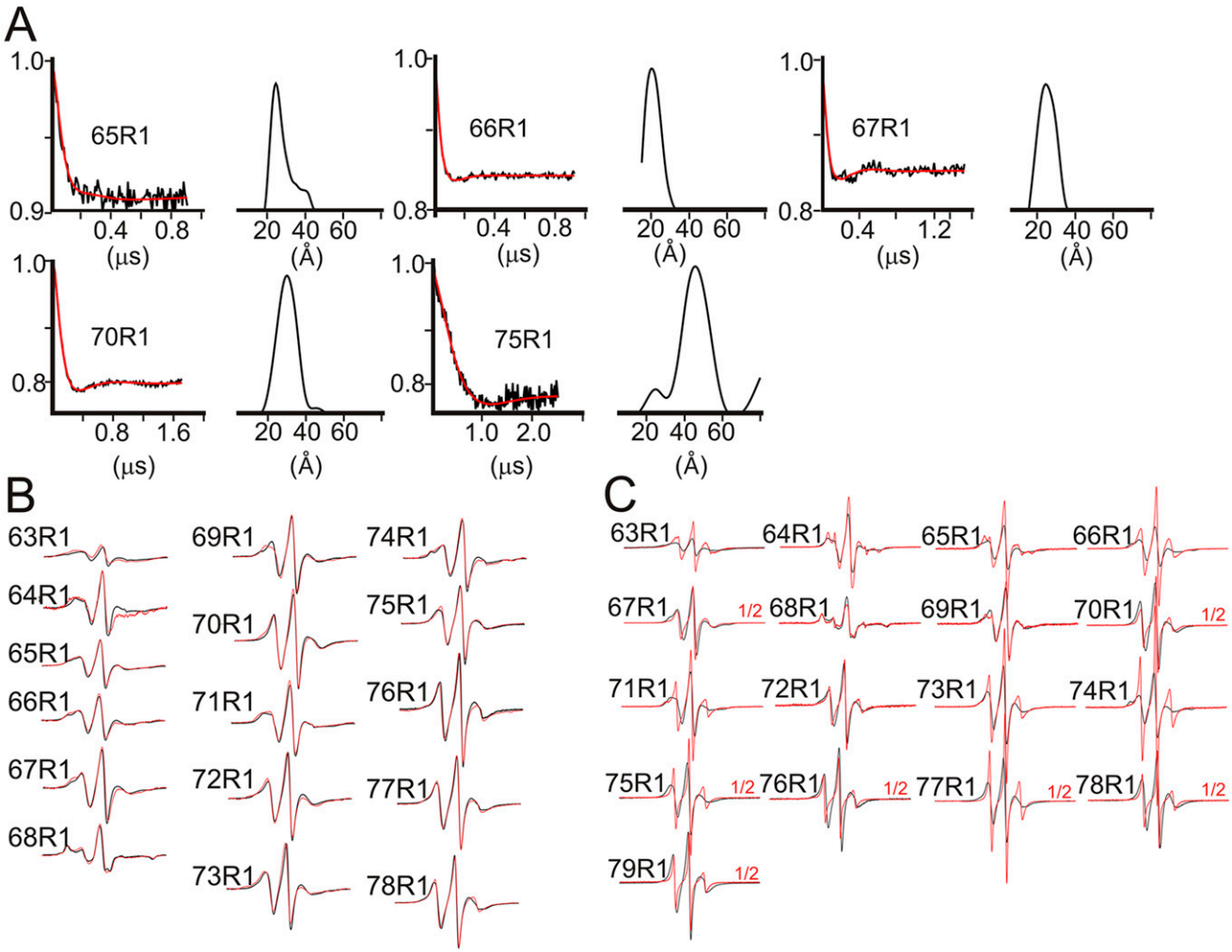

Fig. S2. CW EPR and DEER measurements of endophilin A1 insert region. (*A*) Baseline subtracted time-evolution data (*Left,* black) from DEER experiments of spin-labeled endophilin A1 derivatives bound to tubes. Labeled derivatives were diluted with nonparamagnetically labeled derivatives to reduce intermolecular signals. Data were subjected to Tikhonov regularization (*Left*, red), resulting in the corresponding distance distribution (*Right*). (*B*) CW EPR spectra of rat endophilin A1 spin labeled (R1) at the indicated positions bound to tubes (black) or small and highly curved vesicles (red). Similar but not identical spectra were obtained in both cases. Scan width is 100 G. (*C*) CW EPR spectra of rat endophilin A1 derivatives spin-labeled at the indicated positions in solution (red) and bound to tubes (black). The EPR spectra of certain indicated derivatives in solution (red) are shown at half amplitude for space considerations. Scan width is 150 G.

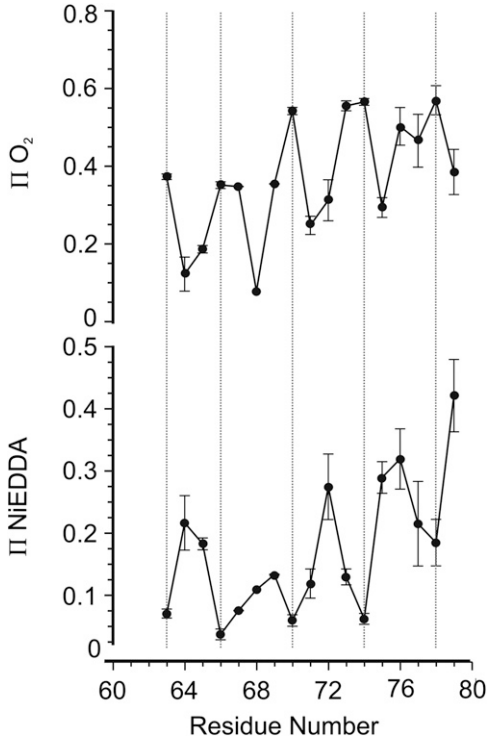

Fig. S3. O_2 and NiEDDA accessibilities for insert region residues. Accessibility to paramagnetic colliders O_2 (ΠO_2) and NiEDDA (ΠNiEDDA) of the indicated spin-labeled sites from tube-bound endophilin A1. A pronounced out-of-phase periodicity is observed, which is typically observed for membrane-exposed amphipathic α-helices (1).

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