Schulz et al., http://www.jcb.org/cgi/content/full/jcb.200905007/DC1

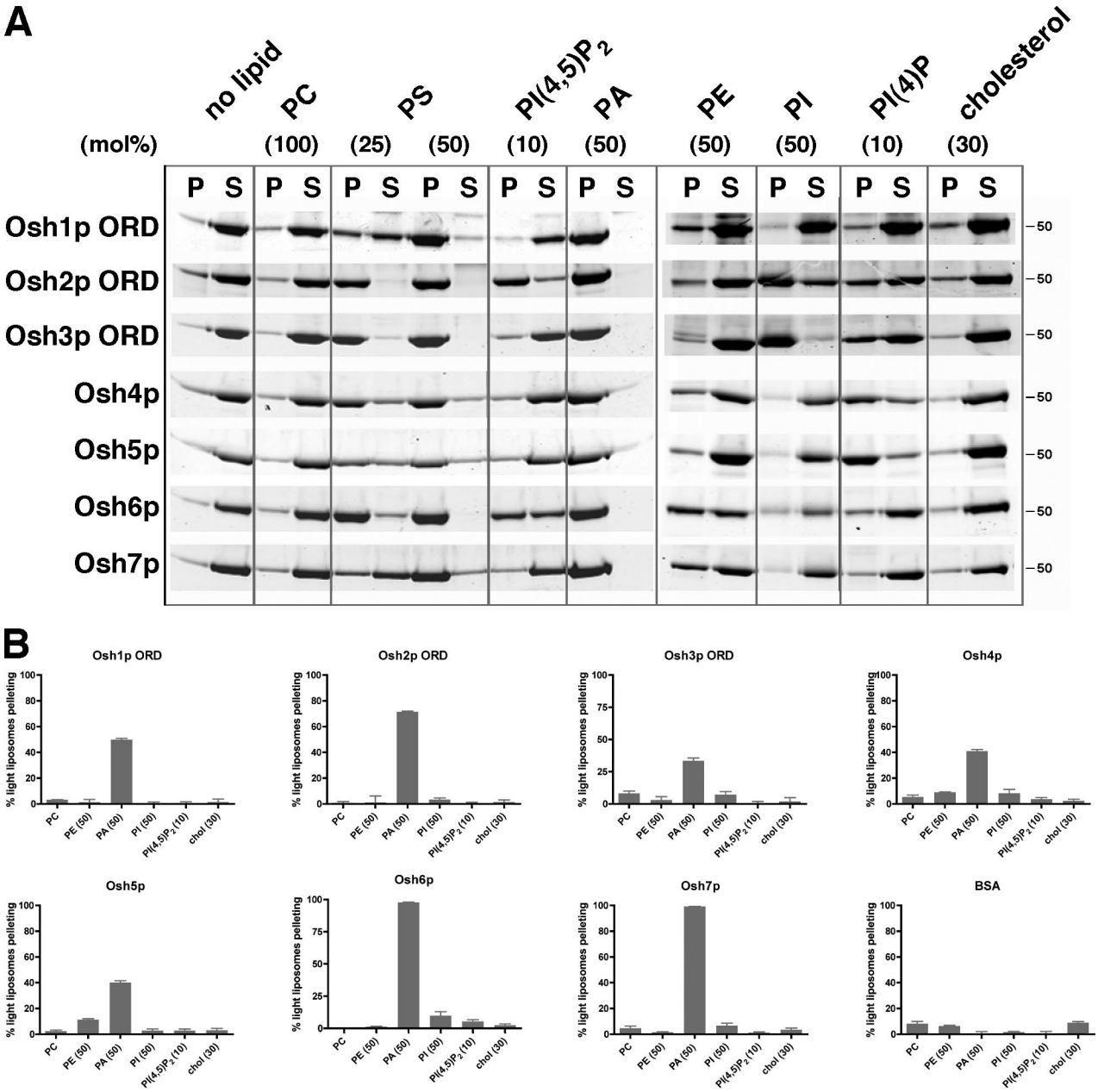

Figure S1. **Yeast ORPs bind to liposomes containing acidic phospholipids.** (A) 950 µM sucrose-loaded liposomes consisting of PC and the indicated percentage of a lipid were mixed with 120 pmol of the indicated Osh protein in a total volume of 100 µl. (left) No-lipid controls are shown. After incubation for 30 min at 30°C, vesicles were pelleted at 16,000 *g* for 10 min, and 90 µl supernatant was removed. The protein in the pellet (P) and supernatant (S) fractions were concentrated by cold acetone precipitation and separated by SDS-PAGE. Gels were stained with Coomassie blue. Gray lines indicate that intervening lanes have been spliced out. (B) Tethering assays were measured by centrifugation as in Fig. 2 A using liposomes of various compositions and the indicated proteins. Error bars indicate mean ± SEM (n = 3).

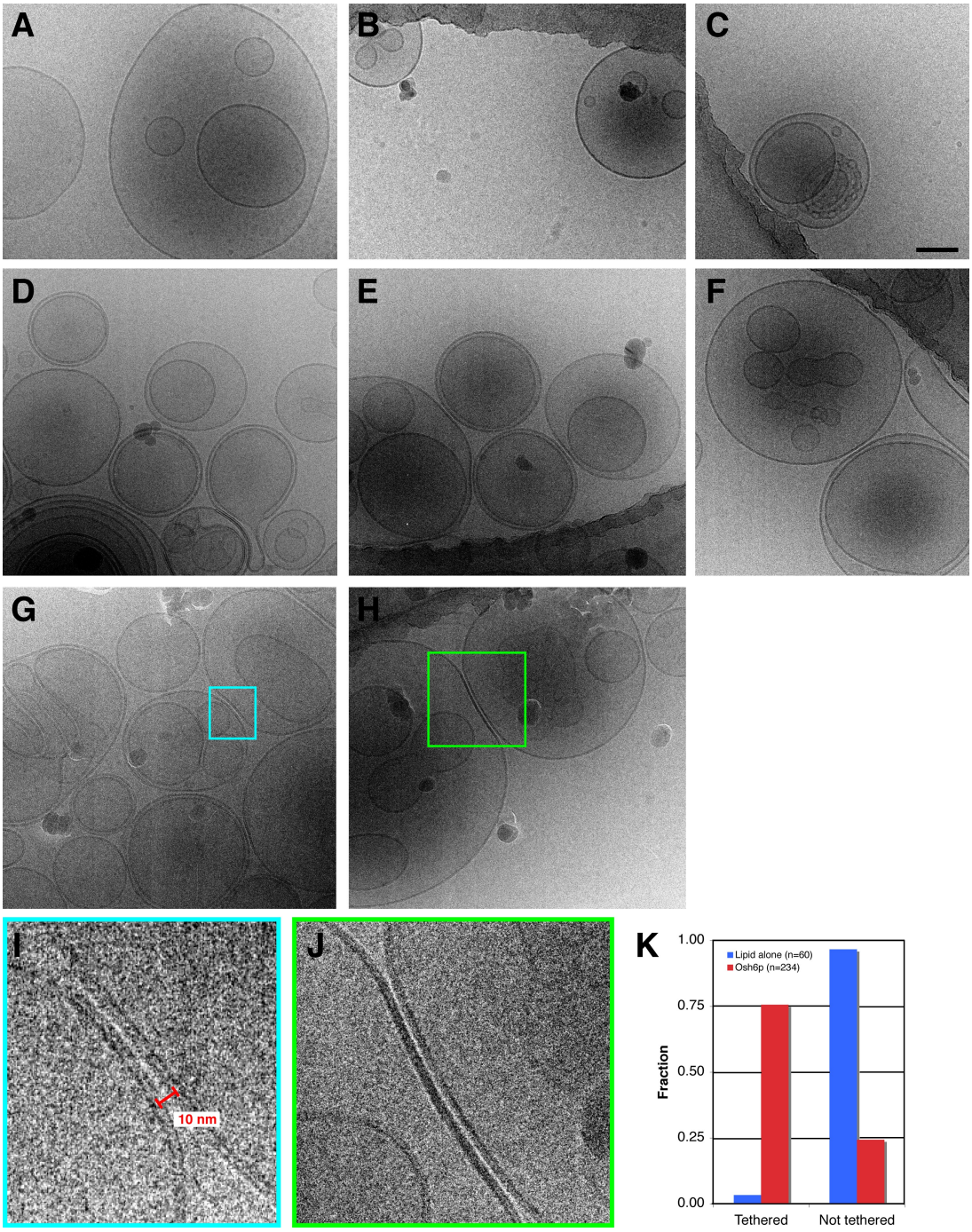

Figure S2. **Aggregation of liposomes by Osh6p and Osh4p visualized with cryo-EM.** Samples were prepared with liposome concentrations between 0.1 and 1.0 mM and protein concentrations between 0.1 and 1.0 µM. (A–H) Images were taken either without protein (A–C), with Osh4p (D–F), or with Osh6p (G and H). (I and J) Magnifications of the boxed regions in G and H, respectively, are shown to highlight the proximity of the neighboring membranes. The Osh6p sample was used to quantify the amount of tethering (fraction of total liposomes) shown in K. A tethered liposome is defined as having at least one other liposome within 10 nm of its membrane (although there were usually several liposomes tethered in the Osh4 and Osh6 samples, leading to the clustering observed in D–H). Bar, 100 nm.

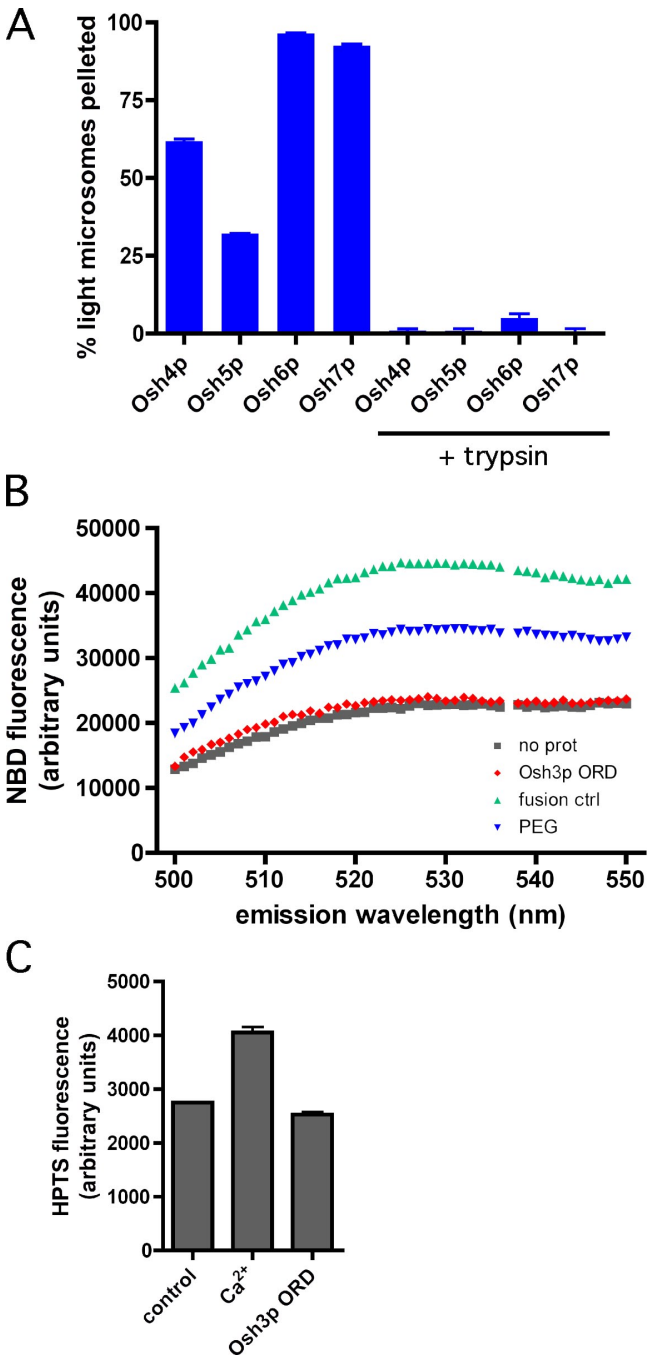

Figure S3. **Osh-mediated tethering is reversible.** (A) Tethering sedimentation assays were performed as described in Fig. 1 B. After the initial incubation period, mixtures were treated with trypsin at a ratio of 1:4 trypsin to Osh protein for 1 h at RT. Liposomes were pelleted by centrifugation, and tethering was quantified as described in Materials and methods. (B) Lipid-mixing assay was measured by FRET between the donor/acceptor pair NBD/Rho. All vesicles contained 50% DOPS. As described in Materials and methods, NBD-PS/Rho-PE–labeled vesicles (1 mol% each) were mixed with an equal amount of unlabeled vesicles and either 40 pmol Osh protein, buffer (negative control), or an equal volume of 50% PEG (25% PEG final) to induce fusion as a positive control. Fusion is measured as a dequenching of NBD fluorescence at an emission wavelength of 530 nm (467 nm excitation), as the concentration of the fluorophores decrease within the membrane (decreasing the chance of collision between NBD and its quencher, Rho). A full fusion control (liposomes containing 0.5 mol% each of NBD-PS and Rho-PE) was also included to determine the expected fluorescence increase if total lipid mixing took place. (C) Content-mixing assay using the fluorophore HPTS and its quencher, DPX. 100% DOPS vesicles loaded with HPTS and DPX in the aqueous interior were incubated with a ninefold concentration of unlabeled DOPS vesicles plus either buffer (control), 5 mM Ca^{2+} (positive fusion control), or 40 pmol Osh protein. All reaction mixtures also contained 50 mM DPX to quench the signal from any HPTS leaking out of the vesicle interior. Fluorescence was monitored at excitation and emission wavelengths of 460 nm and 520 nm, respectively.

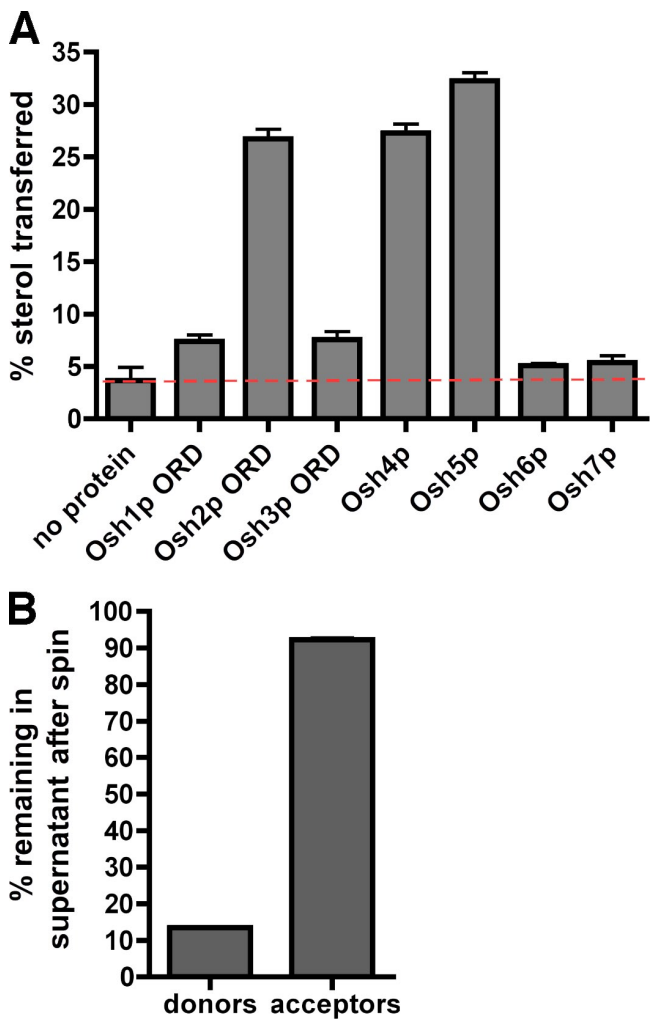

Figure S4. **Cholesterol transport by yeast ORPs.** (A) Cholesterol transport assays were performed using liposomes containing [^{14}C]cholesterol and 20 pmol of the indicated Osh protein. The amount of transfer in no-protein control reactions was subtracted to give the total amount of cholesterol transferred by ORP (n = 4). (B) The percentage of donor and acceptor vesicles that pellet after centrifugation at 16,000 g for 10 min (n = 3). Error bars indicate mean ± SEM.

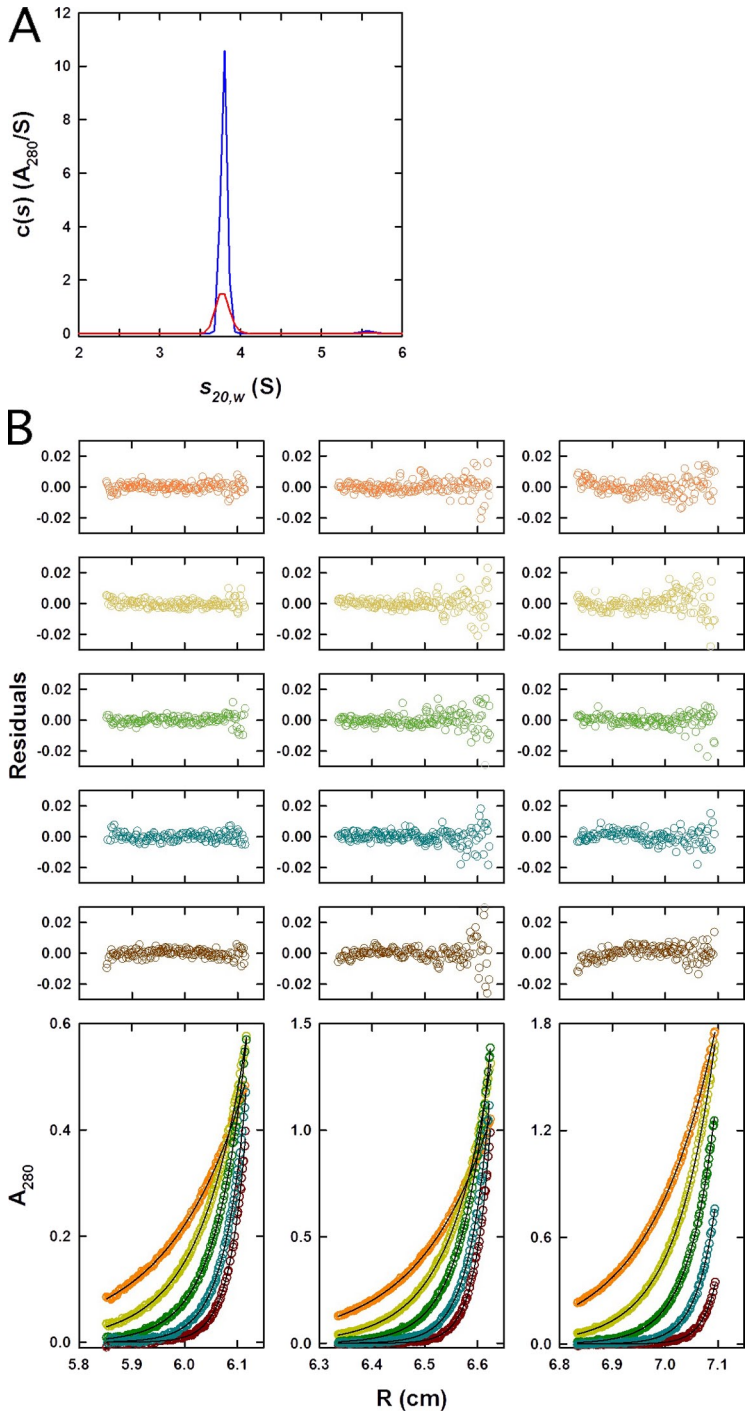

Figure S5. **Osh4p is a monodisperse monomer.** (A) The c(*s*) distributions obtained for Osh4p at 4.0 (red line) and 12.1 μM (blue line) based on sedimentation velocity absorbance data collected at 50 krpm and 20°C. In all cases, data were consistent with the presence of a single species having a mean $s_{20,w}$ of 3.77 ± 0.02 S and an estimated mass of 48.3 ± 0.9 kD, which is indicative of an Osh4p monomer (calculated mass of 49.492 kD). (B) Sedimentation equilibrium profiles for Osh4p at 20°C plotted as a distribution of the absorbance at 280 nm versus radius at equilibrium. Data were collected at 14 (orange), 18 (yellow), 22 (green), 26 (cyan), and 30 (brown) krpm and loading A_{280} of 0.30 (left), 0.53 (middle), and 1.10 (right). The best-fit analysis in terms of a single ideal solute, shown by the solid lines, returns a molecular mass of 49.7 ± 0.9 kD, which is consistent with an Osh4p monomer (*n* = 1.00 ± 0.02). The corresponding residuals to this fit are shown in the plots above.