

Figure S1. OCs are apparently inaccessible to GFP-tagged integral membrane proteins. Confocal images of COS7 cells co-expressing DiHcRED-MAL (red) and GFP-Pendrin, MAL-YFP, VSVG-GFP, GFP-tagged Influenza hemaglutinine (HA) (green); bars = 10 μ m.

Movie 1. FRAP within and out of an ordered cluster (OC). A square was photobleached within an OC (right), over the PM (left) in a COS7 cell expressing DiHcRED-MAL.

Movie. 2. OC dynamics in the presence of cyclohexamide. Time lapse images (inverted) of a COS7 cell expressing DiHcRED-MAL were captured at 15 s intervals for 3 h.

Movie 3. OCs are comprised of crosslinked upper and lower PM. Time-lapse images of living COS7 cells co-expressing DiHcRED-MAL (red) and a cytosolic GFP (green) were captured using a confocal microscope after addition of 40 nM latrunculin B.

Movie 4. Effect of exogenously added ceramides on ordered clusters. Addition of ceramide-BSA complexes to living COS7 cells expressing DiHcRED-MAL. Confocal images of living COS7 cells incubated and imaged for 1-2 hours at 60 s intervals in the presence of C₂₄-Cer (left) C₁₂-Cer-BSA complex (middle) or defatted BSA (BSA, right). Black arrowheads point to OCs.

Movie 5. Cholesterol effect on DiHcRED-MAL OCs. Living cells were imaged as described in Methods 20 hours after transfection with DiHcRED-MAL. Cholesterol-saturated M β CD was added (10 mM final concentration) and images were captured at 30-second intervals for 90 minutes.

