Supplementary Information 5

Materials and Methods

Lipids

PIP_2 and PI were obtained from Avanti Polar Lipids (Alabama, USA), except for lipid overlays where PIP_2 was purchased from Sigma (USA). Phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC) were purchased from Sigma (USA). All other phosphoinositides were from Echelon Research Laboratories (Utah, USA).

PIP_2 overlays

PIP_2 in TBS-OG (pH 7.4, 25 mM TRIS, 140 mM NaCl , 7 mM KCl and 2 µM octylglycoside) was spotted on a nitrocellulose membrane (Hybond C extra, Amersham Biosciences, UK). After blocking in 3% milk TBS-OG, the membrane was incubated with 5 µg/ml purified Myc-Syntenin-2 or GST-Myc. Binding was visualized by enhanced chemoluminescence after incubation with anti-Myc antibodies (9E10, Santa Cruz biotechnology, CA, USA) and a horseradish peroxidase coupled secondary antibody (Amersham Biosciences, UK).

Preparation of vesicles

Phospholipids mixed in the appropriate molar ratios were dried under a stream of N2. Vesicles were prepared by rehydration of dried lipids in HBS buffer. Lipids were vortexed and put in a 40°C water bath for 30 minutes. The lipid suspensions were freeze-thawed 7 times, sonicated for 2 minutes at 40°C and extruded 10 times through a 100 nm pore size polycarbonate filter in an AVANTI mini-extruder (Avanti, Alabama, USA).

Surface Plasmon Resonance (SPR) experiments

Surface plasmon resonance was measured using a Biacore 2000 instrument. The L1 chip was cleaned by a 4 min injection with 1% octylglycoside and by a 4 min injection with 0.5% SDS at a flow rate of 5 µl/min. Before injection of the lipid vesicles, each flow cell was conditioned with 1% octylglycoside for 1 min at a flow rate of 10µl/min. The L1 chip was coated with lipid vesicles at a concentration of 1.5 mM for 11 min at a flow rate of 5 µl/min. The immobilized vesicles were washed with 10 mM NaOH at 100 µl/min flow rate for 12 sec to remove unattached vesicles. Purified proteins were diluted in HBS running buffer to the concentrations indicated and perfused at a flow rate of 30µl/min. The proteins were allowed to associate for 4 minutes and then allowed to dissociate for at least 4 minutes. The sensor surface was regenerated by short pulses of 10 mM NaOH. The lipid layers were removed by injecting 1% octylglycoside.