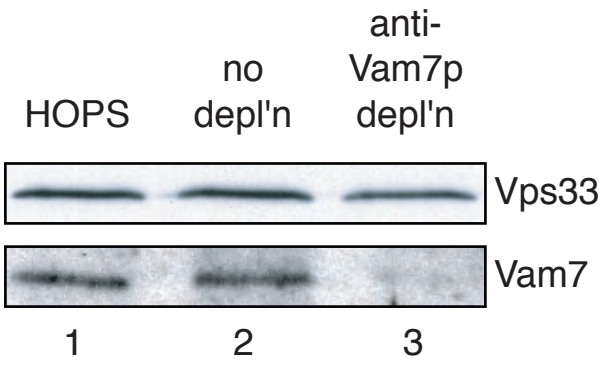
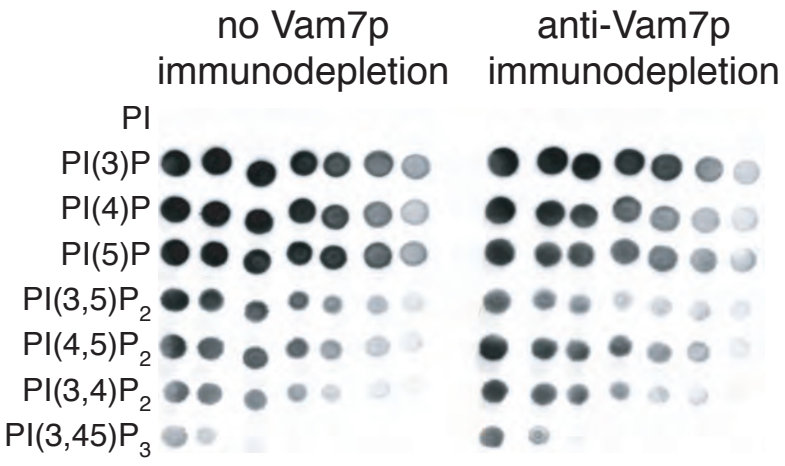
Figure S3





probe: anti-Vps33

Figure S3 Effect of immunodepletion of Vam7p from HOPS complex on HOPS-phosphoinositide interactions. Vam7p was immunodepleted by incubating pure HOPS with anti-Vam7p antibodies (1 μg) and protein A Sepharose CL-4B (GE; 10 μl). Immunodepleted HOPS complex, and HOPS complex incubated with protein A Sepharose CL-4B alone, were incubated with PIP Arrays (Echelon), washed and probed for Vps33p as described in Figure 6A. Top panel, SDS-PAGE/immunoblot analysis of immunodepletion. Lane 1, starting material; lane 2, mock-immunodepleted material; lane 3, material immunodepleted with anti-Vam7p. Bottom panel, PIP arrays probed for Vps33p. Immunodepletion removed almost all of the trace amounts of Vam7p present in the pure HOPS complex (top panel), but the interaction between the HOPS complex and PI(3)P was not diminished (bottom panel). HOPS complex in the immunodepleted sample did bind PI(4)P, PI(5)P and PI(3,5)P_2 at slightly lower levels than HOPS complex in the non-immunodepleted sample, but since the PX domain of Vam7p has no affinity for these phosphoinositides (Cheever et al., 2001) this effect was not caused by lack of Vam7p. The slightly reduced concentration of HOPS complex in the immunodepleted sample relative to the mock-immunodepleted sample (top panel) may be responsible for the lowered binding of HOPS to PI(4)P, PI(5)P and PI(3,5)P_2.