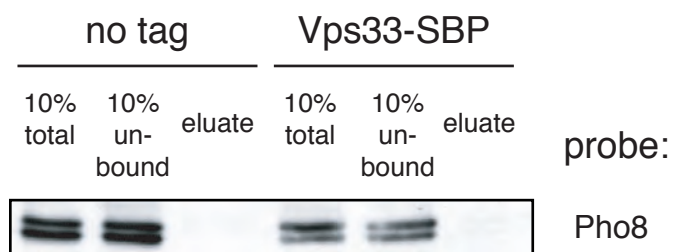


A



B

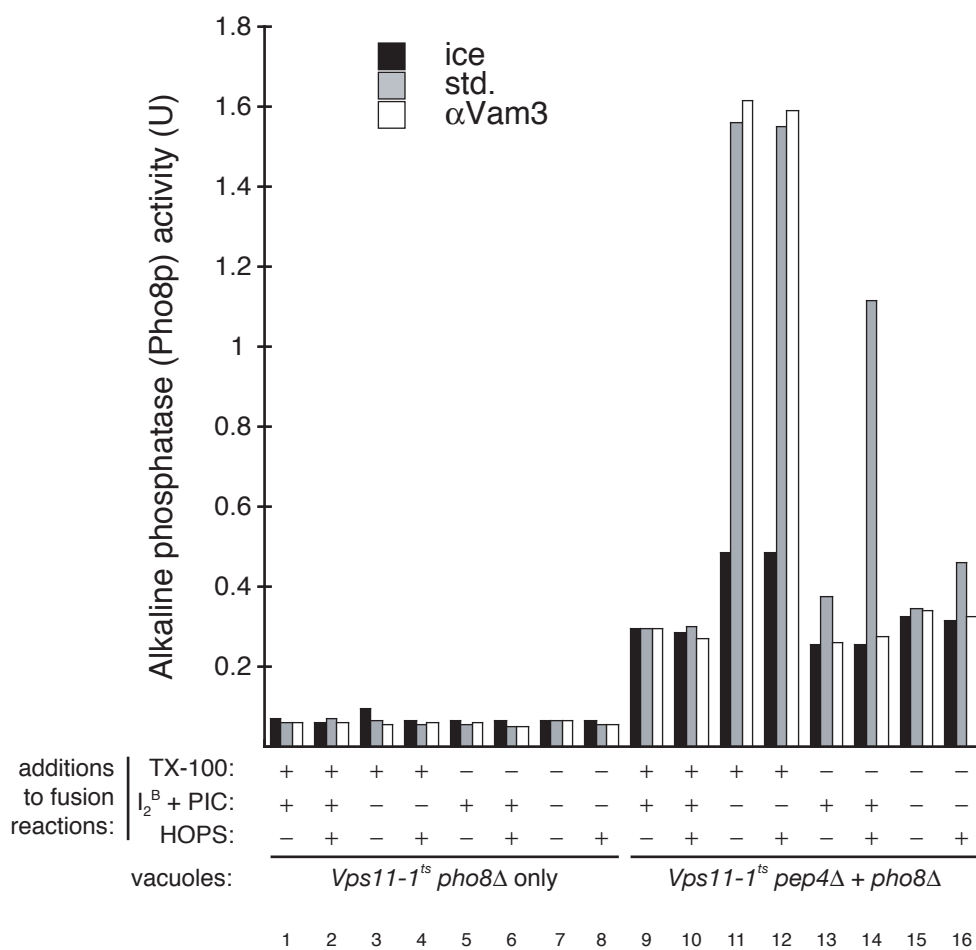


Figure S1 Pure HOPS complex in BSA does not contain detectable Pho8p protein or activity. **(A)** Lysates from BJ2168 and CSY12 vacuoles, and unbound and bound material from streptavidin affinity chromatography performed on each lysate, were subjected to SDS-PAGE and immunoblotting for Pho8p. **(B)** Vacuole fusion reactions containing only CSY10 vacuoles (*pho8Δ*), or CSY9 (*pep4Δ*) and CSY10 vacuoles, were supplemented with the indicated reagents, incubated at 27°C for 90 min., and developed. No Pho8p activity was detected in the absence of CSY9 vacuoles (sets 1-9), and no increase in Pho8p activity was seen when pure HOPS complex was added to reactions in the presence of Triton X-100 (to lyse vacuoles and permit fusion-independent, i.e. anti-Vam3 resistant, activation of alkaline phosphatase) without protease inhibitor cocktail and I₂^B (compare sets 11 and 12).