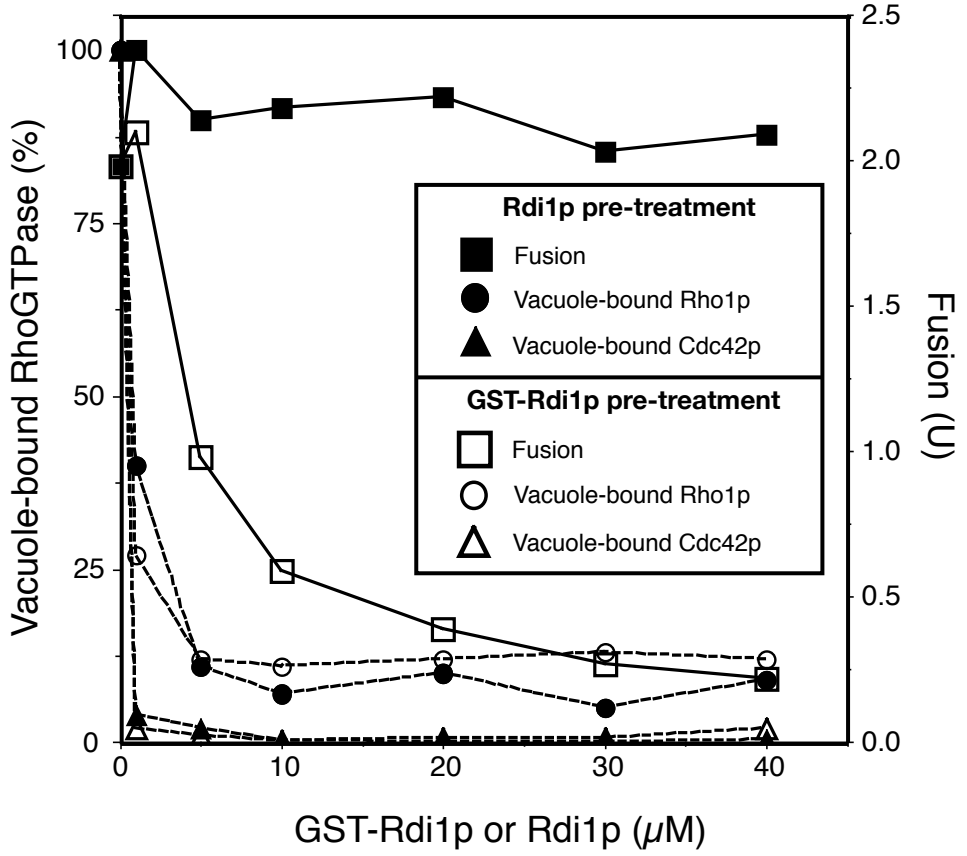
Supplementary Figure S2



Supplementary Figure S2. GST-Rdi1p does not inhibit fusion via RhoGTPase extraction.

Two sets of standard 30 μl vacuole fusion reactions lacking ATP were incubated on ice with GST-Rdi1p (open symbols) or untagged Rdi1p (closed symbols) for 25 min., then diluted with 60 μl buffer (20 mM PIPES.KOH, pH 6.8, 200 mM sorbitol, 125 mM KCl, 5 mM $MgCl_2$) and reisolated via centrifugation (8,000g, 4 min, 4 °C). Supernatants were aspirated. For fusion analysis, one set of vacuole pellets was gently resuspended in this buffer, and coenzyme A, IB2, and ATP were added to standard final concentrations (30 μl final volume; see Materials and Methods). Fusion reactions were incubated at 27 °C for 90 min and assayed for Pho8p activity (squares, solid lines). The remaining vacuole pellets were resuspended in 30 μl buffer, and mixed with SDS-loading buffer. Equivalent fractions were resolved by SDS-PAGE and transferred to nitrocellulose. Membranes were probed with antibodies to Pho8p, Rho1p, and Cdc42p. Quantification of vacuole-bound Rho1p (circles) and Cdc42p (triangles) was achieved with ImageQuantNT v5.1 and Personal Densitometer SI scanner (Molecular Devices).