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Invertase secretion assay

Cell cultures of W3031A, JMY128, JMY304, JMY307, and JMY309 were grown to 0.5 OD_{600} units/ml in synthetic complete media containing 2% galactose at 30°C. 1 OD_{600} unit of each cell culture was harvested and washed once with 10 mM NaN3, once with 0.5M NaOAc, pH 5.0, and resuspended in 1 ml of the same buffer. Glucose produced by external invertase was generated in a reaction containing 25 µl of the cells (0.025 OD_{600} units) in 0.5 M NaOAc, pH 5.0, and 25 µl of 0.5 M sucrose. The cells and substrate were incubated at 30°C for 5 min, and the reaction was stopped by adding 100 µl of 0.5 M sodium phosphate buffer, pH 7.4, followed by immediate heating at 100°C for 5 min. The amount of glucose generated by invertase was determined by a coupled glucose oxidase peroxidase reaction using Amplex red as a fluorogenic substrate (Amplex red glucose/Glucose Oxidase Kit; Invitrogen).

Halo secretion assay

Cell cultures of JMY204, JMY307, and JMY309 were grown at 30°C overnight in synthetic complete media with 2% galactose, whereas the MATa tester strain RC634 (MATa sst1-3 rme1 ade2 his6 met1 ura1 can1 cyh2) was grown in YRPG (2% raffinose and 2% galactose) media. Each strain was back diluted to ~0.5 OD_{600} in the appropriate media and allowed to grow for 2 h. The cultures were then washed three times with water and resuspended in water to a final volume of 0.5 OD_{600}/ml. A 1:10 dilution of RC634 in water was made, and 500 µl of sample were spread onto a YPRG plate and allowed to completely dry. 10 µl of JMY204, JMY307, and JMY309 (0.005 OD_{600} of cells) were spotted onto the MATa tester lawn and grown for 2 d at 30°C to test for α-factor secretion. The halo represents a zone of cell cycle arrest in the MATa lawn as a result of α-factor secretion from the MATα strains. The size of the halo is a qualitative measure of secretion.

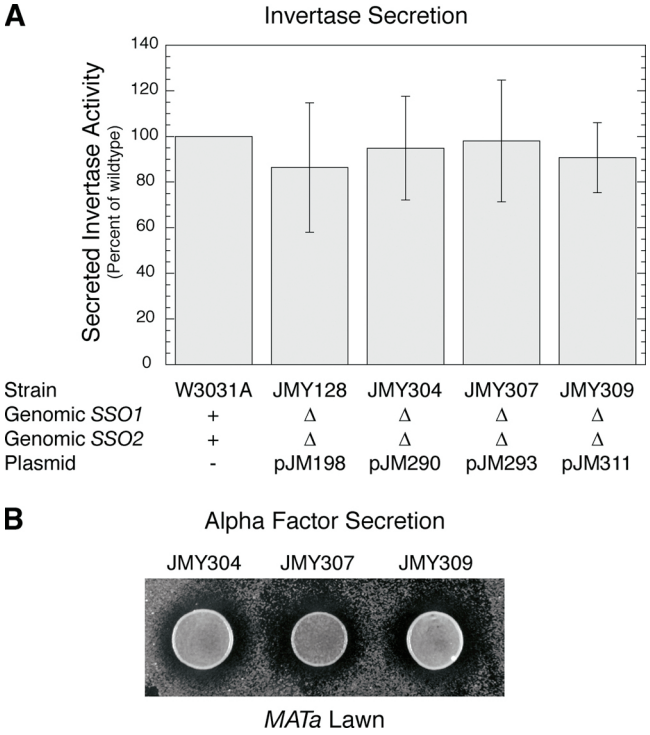

Figure S1. **Strains expressing only the tandem t-SNAREs have similar levels of secretion as wild-type cells.** (A) External invertase activity. Cell cultures of W3031A, JMY128, JMY304, JMY307, and JMY309 were grown in synthetic complete media containing 2% galactose at 30°C, and external invertase activity was measured. Levels of external invertase were determined for each strain and compared with the W3031A wild-type strain. Three independent samples from each strain were measured twice in the coupled assay. The data in A represents an average of three independent sets of cultures. The error bars are standard errors of the mean. (B) α-Factor secretion. 10 µl of washed cells [JMY304, 307, and 309] grown to mid-log phase were spotted onto a YPRG plate containing a lawn of *MATa* tester cells [RC634]. The cells were grown at 30°C for 2 d. Each of the tandem t-SNARE strains appears to secrete equal amounts of α factor as determined by the size of the halo around the cells.