Supplemental Material

Error in kinetics of buoyant mass change. Two sources of error need to be accounted for in order to compare the buoyant mass data from kinetic experiments to those from population measurements. The first is a sampling error due to the fact that kinetic data are acquired from a small number of spores that may not represent the population accurately. This error can be decreased by trapping additional spores, as a collection of multiple spores is more likely to represent the true mean of the population. The standard error for this can be calculated from the population data using the first term under the radical in Eq. (S1) where μ_S and μ_D represent the mean values for the buoyant mass of spores in the sucrose solution and D_2O, respectively, σ_D represents the standard deviation of the latter, and n is the number of spores in the resonator.

$$SE_{(m,t)} = \sqrt{\frac{\left(\frac{\sigma_D}{\mu_D}(\mu_D - \mu_S)\right)^2}{n} + \left(\frac{\mathrm{Adev}(t)}{n}\right)^2} \qquad \text{Eq. (S1)}$$

The second source of error for kinetic measurements (Adev(t) in equation S1) arises due to the inherent stability of the resonator. The baseline mass values for these measurements are acquired from a blank fluid switch in an empty resonator at the beginning of an experiment. Any subsequent change in resonator frequency is assumed to be due to the mass of spores, even if it was due to drift via other sources of noise. The stability of a resonator can be represented by calculating the Allan deviation, the variation of the frequency signal at different time scales. The Allan deviation for the resonator system used herein is plotted in Fig S1. A fit to the second arm of this plot is used to estimate the stability error at a given time since the acquisition of the baseline measurements. This error can be decreased by running experiments over time-scales where drift is minimal, or by increasing the stability of the resonator, for example with thermal

control or other sources of noise reduction. Adev(t) is the value of this fit for the time-since-baseline (t) at which each fluid switch was carried out.

Estimating sucrose content of spores after the fluid switch. To quantify what (if any) portion of the sucrose may remain in the spore requires a more thorough analysis. The population experiments were performed in 97.5% D_2O (and a sucrose solution at equivalent density) as a result of the volume of spore suspension (in water) that was added to D_2O. In contrast, the kinetic experiments switch the spores into pure D_2O from a solution of sucrose that is equivalent to that density. We know the density response of the spore to each molecule (the slope of a line between the mass in water and the mass in said molecule) and can scale the buoyant mass for the populations to what they would be in the denser solutions. We subtract the scaled mean in sucrose (109.7 fg) from that in pure D_2O (124.9 fg) and calculate a standard error. This yields a buoyant mass difference of 16.2 ± 0.9 fg. Comparing this value with that obtained from our kinetic measurements suggests that 1.7 ± 1.1 fg of sucrose per spore remain at the conclusion of these switches "relative to the population measurements. If this is the case, this reserve of sucrose must have a rate of exit slower than the 10 sec measured here, but faster than the 30-60 min over which population measurements are acquired.

There may be differences between the population and kinetic experiments that are not accounted for by these analyses. As an alternative way to quantify whether sucrose remains in the spore during our standard fluid exchange, we perform the exchange in reverse. Spores that are initially suspended in D_2O are exposed to a sucrose solution for ~9 sec before the buoyant mass change is determined and they are switched back to D_2O. We reason that since these spores are only briefly in the sucrose solution, any sucrose that cannot exit some portion of the spore within 9 sec for the standard assay also cannot enter this portion in 9 sec for the reverse assay. Accordingly, the

magnitude of the buoyant mass change would be greater than in the standard reaction because the loss of D_2O mass will not be offset by the gain of some portion of the sucrose mass that can occur on a longer timescale. The red dots in Fig. 3c represent the absolute value of the mass lost from this exchange: 18.9 ±0.3 fg. Compared to the standard exchange, this suggests that an additional 1 ± 0.7 fg of sucrose enters the spore at a time beyond 9 sec. Taken together, our kinetic measurements show that the vast majority of the sucrose has left the spore before (or concurrent with) the mass increase observed in Fig. 3a, but suggest that between 5% ± 3% and 8% ± 5% of the sucrose that enters the spore may remain in the spore to leave at a lower rate.

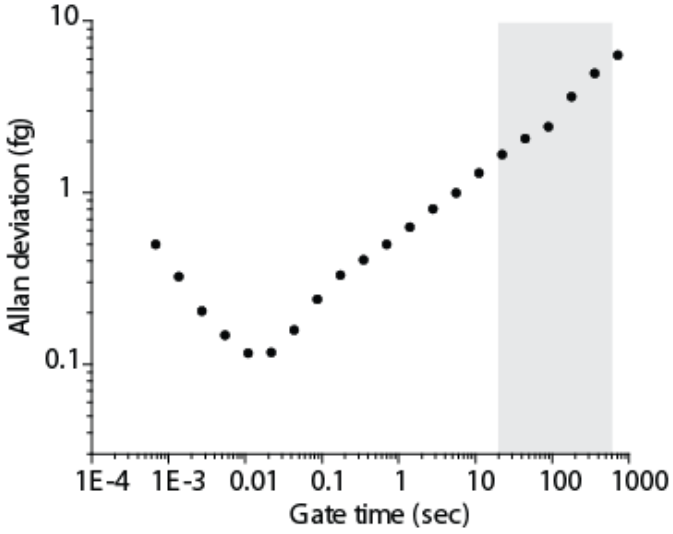

Fig S1. The Allan deviation for the resonator system used herein is shown, with resonator frequency converted to mass based on sensitivity at the cantilever tip, where spores are trapped.. A fit to the second arm of this plot is used to estimate the variation at the time of each fluid switch, Adev(t). The switches for the data shown in Fig 3c take place between 20 and 600 sec (shaded) after the reference switch.

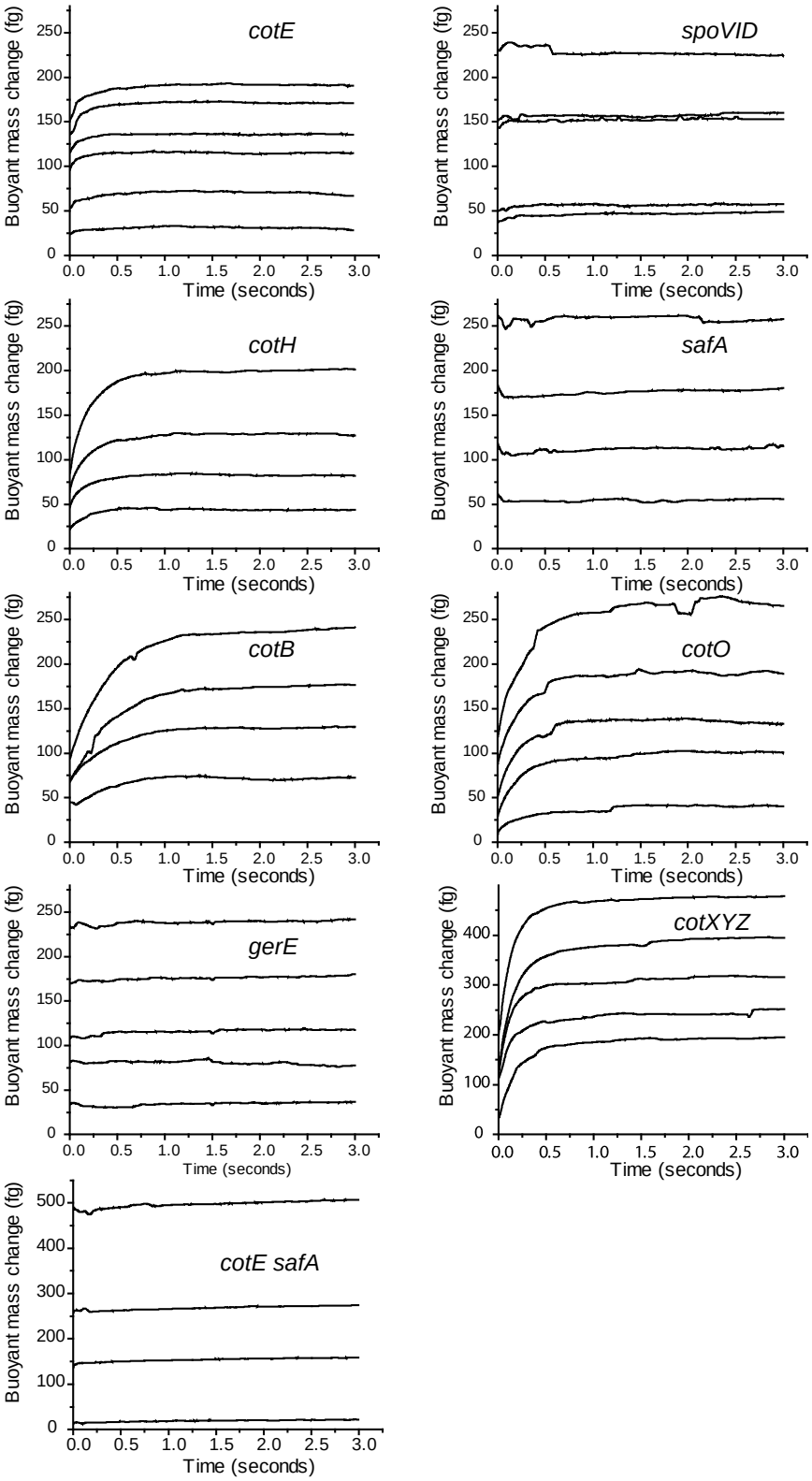

Fig S2. The buoyant mass change resulting from replacement of spore H_2O with D_2O is plotted for coat-mutant spores as indicated.