SUPPLEMENTAL TABLE

Table S1. Primers Used in this Study

Name	DNA Sequence (5' to 3')
prTorAHis$_6$C-F	GCCAAAACAGCCAAGCTTGCATGCCTCCAGTTAGCAATGATGATGATGAT GATGCTCGAGTTTGTATAGTTCATCCATG
prTorAHis$_6$C-R	CATGGATGAACTATACAAACTCGAGCATCATCATCATCATTGCTAAC TGCAGGCATGCAACTTGGCTGTTTTGGC
prTorA-ScII-F	GAAGAGTTCTTCTCCTTTGCTCCGCGGCGCTTGCGGCGCAGTCGCACG
prTorA-ScII-R	CGTGCGACTGCGCCGCAAGCGCCGCGGAGCAAAGGAGAAGAACTCTTC
prSufI-sp-F	CCCGCCCCGCGGCGGTTGCTGTTGCCCGGCTGCGCTGGCCTTCAGGGGAA CAGCGCCTGC
prSufI-sp-R	CCCGCCCCGCGGCGGTTGCTGTTGCCCGGCTGCGCTGGCCTTCAGGGGAA CAGCGCCTGC

SUPPLEMENTAL FIGURES

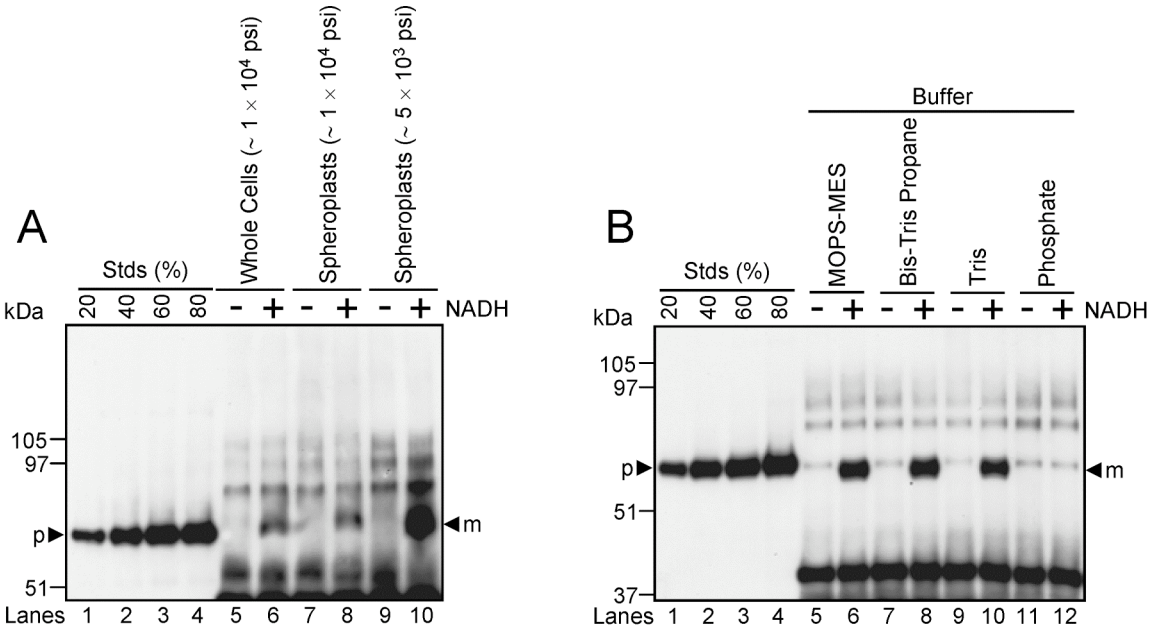

Figure S1. Efficiency of the Transport Assay – Effect of IMV Formation Method and Transport Buffer on pre-SufI Transport Efficiency. Features of our transport assay that may explain the high transport efficiencies obtained are: 1) the use of pre-SufI as a precursor substrate; 2) the formation of spheroplasts before French press treatment; 3) the use of low pressure for IMV formation, and a single pass through the French press cell; and 4) the use of organic buffers for the translocation reactions. The natural substrate pre-SufI was clearly transported more efficiently than the commonly used spTorA-GFP chimera (compare Figs. 1G and 2A). The use of low pressure for IMV formation was essential (Fig. S1A). Due to the low pressure used, IMV yield was low unless spheroplasts were used (Fig. S1A). Phosphate buffer did not support in vitro translocation reactions with NADH as the energy source (Fig. S1B).
(A) Effect of IMV formation method on pre-SufI transport efficiency. The highest pre-SufI transport efficiencies were observed when IMVs were made from spheroplasts at low pressure (*lanes 9-10*). Lower transport efficiencies were observed when IMVs were made from intact cells (*lanes 5-6*) or from spheroplasts at high pressure (*lanes 7-8*).
(B) Effect of buffer on pre-SufI transport efficiency. Phosphate buffer did not support transport. All buffers were used at 50 mM, pH 7.

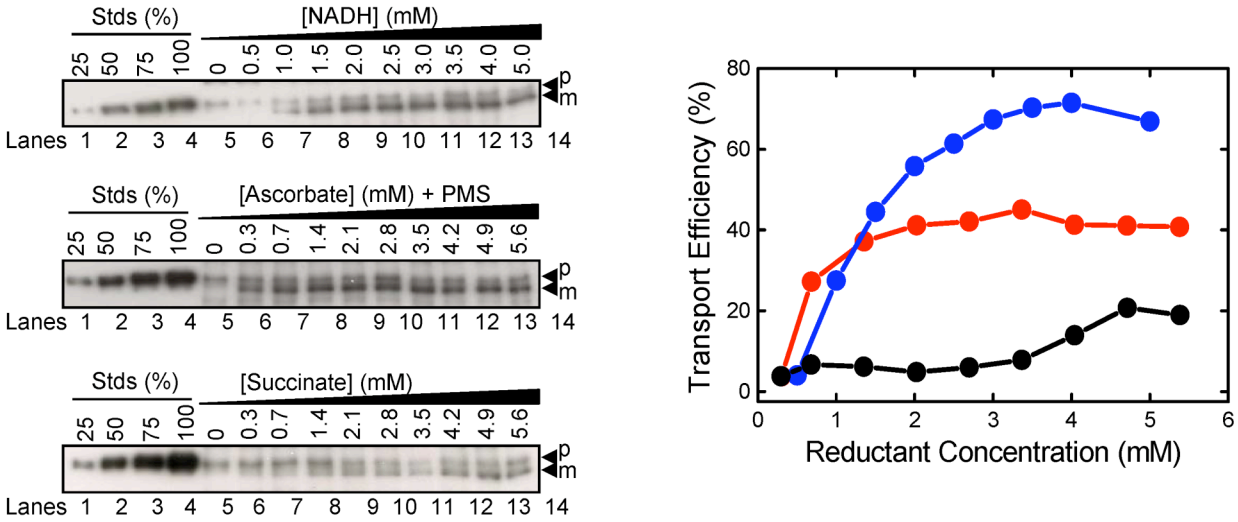

Figure S2. Transport Efficiency Dependence on Energy Source. These anti-SufI immunoblots show pre-SufI transport assays performed under standard conditions (Fig. 1C) when IMVs were energized by different concentrations of succinate *(black)*, ascorbate and 0.3 mM PMS *(red)*, and NADH *(blue)*. For this figure only, the Buffer B (Materials and Methods) used to make IMVs did not contain KCl. Under the tested conditions, energization with 4 mM NADH resulted in the best SufI transport efficiencies (72%).

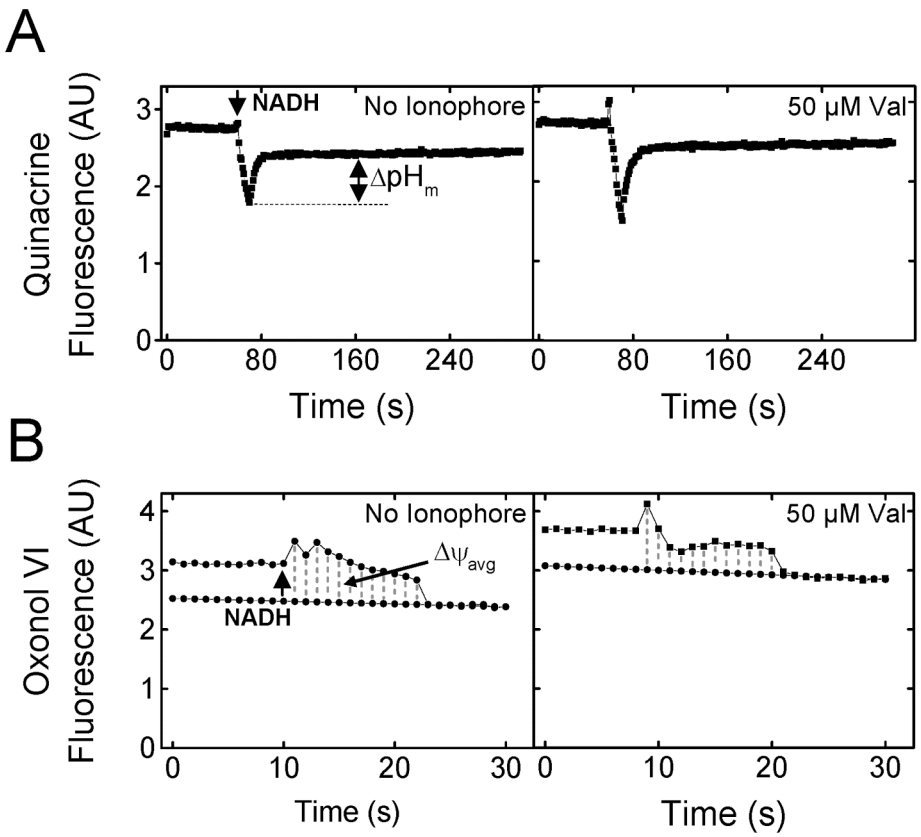

Figure S3. Effect of High Valinomycin Concentration on ΔpH and Δψ. Under our conditions, even high concentrations of valinomycin could not completely collapse the Δψ (Fig. 5D and this figure). This could be a consequence of a Donnan equilibrium contributing to the overall Δψ. The K^+ concentration in the IMV lumen was low (~1 mM). In contrast, the external K^+ concentration in our transport assays was 50 mM. This K^+ concentration difference can support a Donnan potential of ~100 mV (positive inside).

(A) The presence of a ΔpH determined as in Fig. 5 in the presence and absence of 50 µM valinomycin, as indicated.

(B) The presence of a Δψ determined as in Fig. 5 in the presence and absence of 50 µM valinomycin, as indicated.