Supporting Information

Zhou and Graham 10.1073/pnas.0904293106

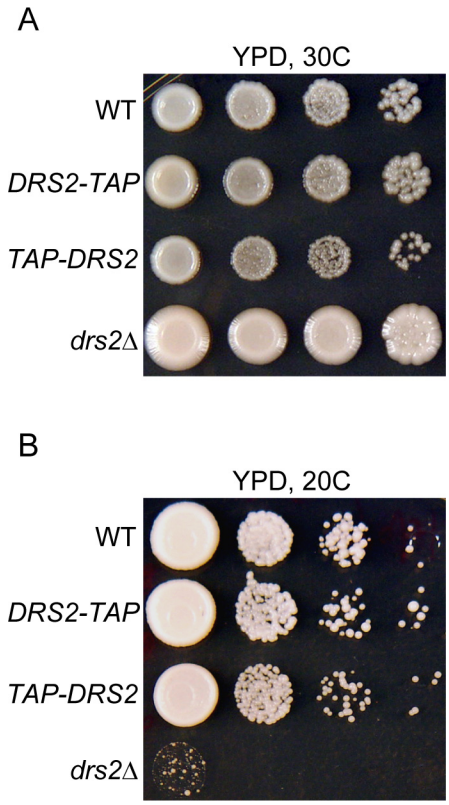

Fig. S1. Growth phenotype of yeast strains WT (*DRS2*; *atp2*Δ), *DRS2-TAP* (*DRS2*::*TAP*; *atp2*Δ), *TAP-DRS2* (*TAP*::*DRS2*; *atp2*Δ), and *drs2*Δ (*drs2*Δ; *ATP2*) at 30 °C (*A*) and 20 °C (*B*). Note that the *drs2*Δ strain grew better than others at 30 °C because of its intact *ATP2* gene (mitochondrial F1-ATPase), but it exhibited a growth defect at 20 °C.

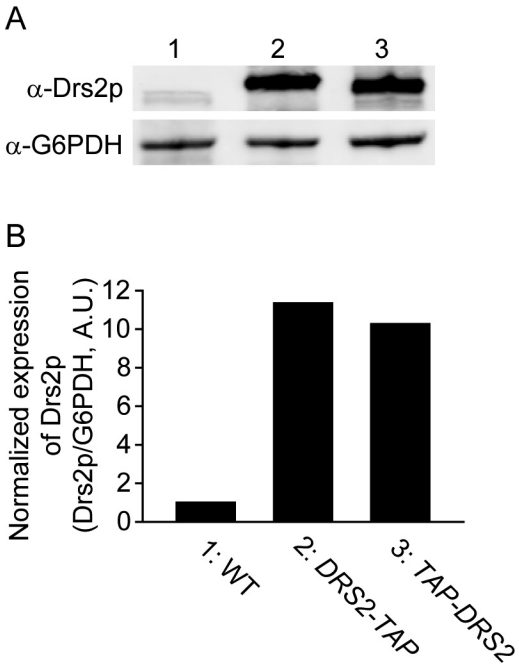

Fig. S2. Overexpression of *DRS2-TAP* and *TAP-DRS2* in yeast. (*A*) Yeast BY4742 (WT, lane 1), XZY38b (*DRS2-TAP*, lane 2), and XZY60m (*TAP-DRS2*, lane 3) were grown in rich medium and harvested in mid-log phase (0.4–0.6 OD_{600}/mL). The cell lysates were subjected to SDS/PAGE and Western blotting. Primary antibodies were used at 1:2000 (anti-Drs2p) and 1:10000 (anti-G6PDH). Blotting for G6PDH served as a loading control. (*B*) Quantification of Drs2p overexpression in (*A*). Drs2p values were corrected for the loading control and defined as (Drs2p signal)/(G6PDH signal). The WT sample was defined as 1 arbitrary unit (A.U.).

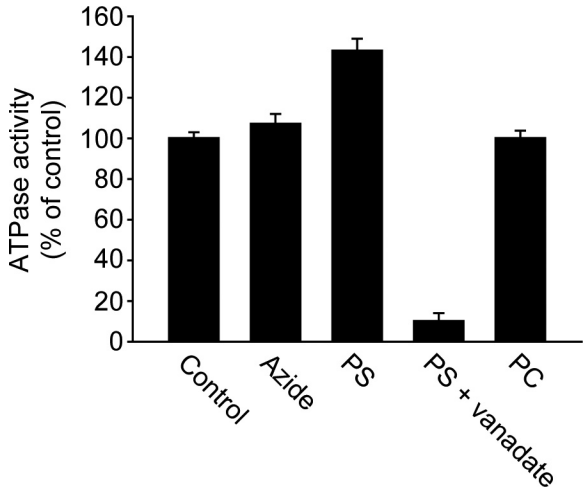

Fig. S3. ATPase activity of purified TAP-Drs2p. ATP hydrolysis was assayed as described in *Materials and Methods* in the presence of azide (1 mM), PS (POPS, 400 μM), PS (POPS, 400 μM plus vanadate (orthovanadate, 80 μM), or PC (POPC, 400 μM). Phospholipids were incubated with TAP-Drs2p for 10 min at room temperature before the addition of ATP.

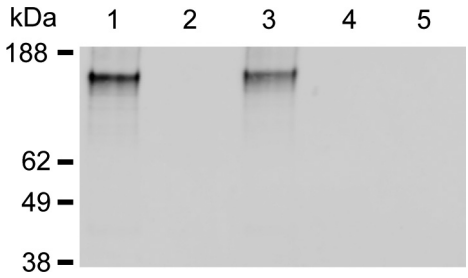

Fig. S4. Protease protection assay of TAP-Drs2p proteoliposomes using trypsin. Proteoliposomes were incubated with 0.001% (wt/vol) trypsin at 37 °C for 5 min, and trypsin digestion was stopped by addition of trypsin inhibitors (5 mM benzamidine hydrochloride, 2.5 μM aprotinin, 40 μM leupeptin, and 10 mM PMSF) before mixing with SDS/PAGE sample buffer. Drs2p was detected by Western blotting as described in Fig. S2 using a primary antibody that recognizes the ATPase domain of Drs2p. Lane 1, proteoliposomes alone; lane 2, proteoliposomes incubated with trypsin before addition of trypsin inhibitors; lane 3, proteoliposomes incubated with trypsin in the presence of trypsin inhibitors; lane 4, proteoliposomes incubated with trypsin in the presence of 0.1% Triton X-100; Lane 5, trypsin only.

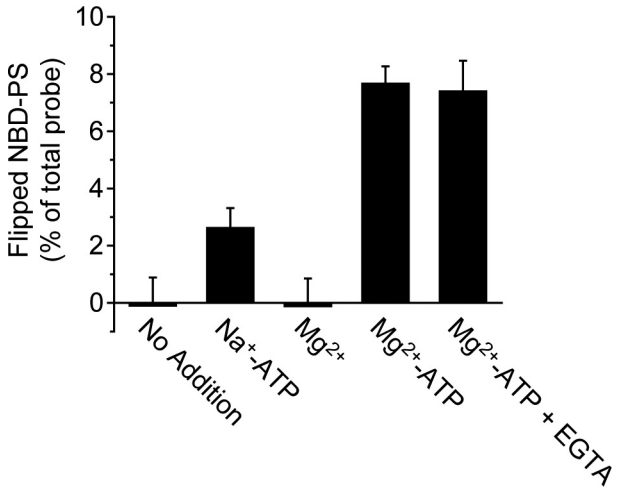

Fig. S5. Flippase assay with TAP-Drs2p proteoliposomes containing NBD-PS. Proteoliposomes were incubated with no addition (proteoliposomes alone), Na^{+}-ATP (5 mM), Mg^{2+} (5 mM), Mg^{2+}-ATP (5 mM), or Mg^{2+}-ATP (5 mM) plus EGTA (1 mM) at 37 °C for 30 min, and NBD-PS flipping was measured as described in *Materials and Methods*. Results were averaged from 4–6 independent experiments.

Table S1. Proteins identified in Drs2p-TAP preparations

Protein name	Peptides recovered
Drs2p	177
Cdc50p	17
Vtc4p	16
Ssa1p	11
Atp1p*	10
Atp2p†	7
Pma1p	7
Vtc3p	6
Rpl4ap‡	5
Rtn1p	4
Tdh3p	4
Vtc2p	4
Dnf1p	3
Por1p	3
Sac1p	3
Cdc19p	2
Eno1p	2
Hsp26p	2
Kar2p	2
Rcy1p	2
Ssa2p	2
Tdh1p	2
Tub2p	2
Tef1p	1

*α subunit of F1-ATPase.
†β subunit of F1-ATPase.
‡Several additional ribosomal proteins detected by mass spectrometry are not listed.

Table S2. Secondary structure prediction from circular dichroism spectra (200–240 nm) of Drs2p by the K2D2 web server (www.ogic.ca/projects/k2d2)

%	TAP-Drs2p	Drs2p-TAP
α helix	31.63	47.81
β strand	13.51	10.21
Other	54.86	41.98