Figure S 1.

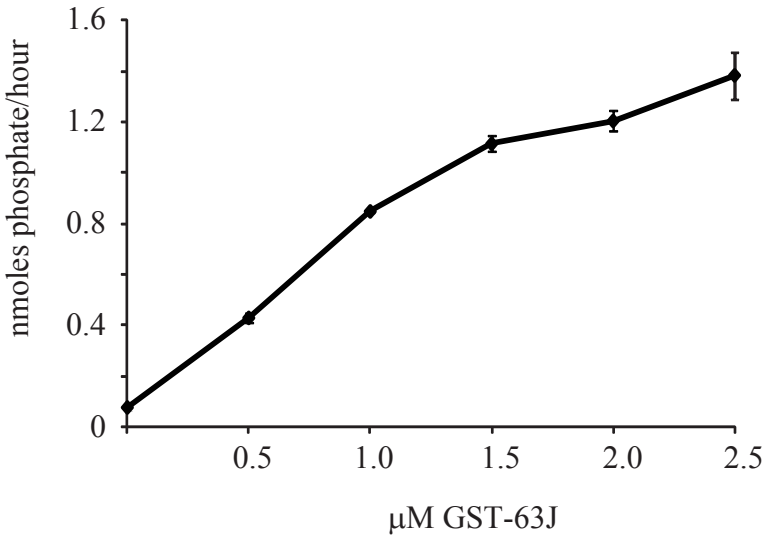


Figure S2.

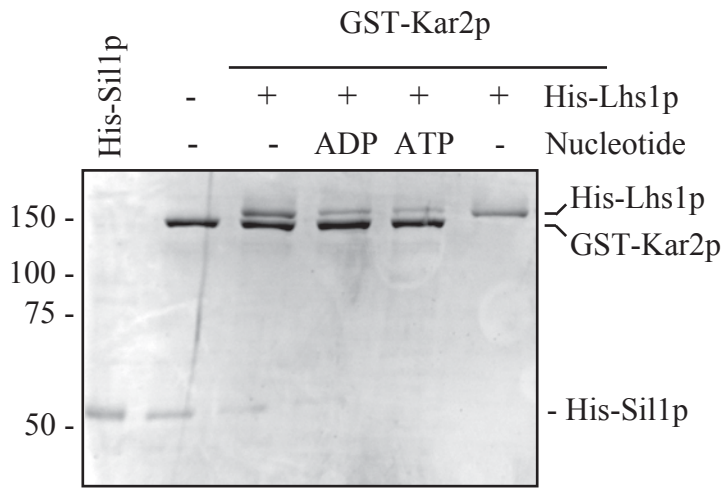
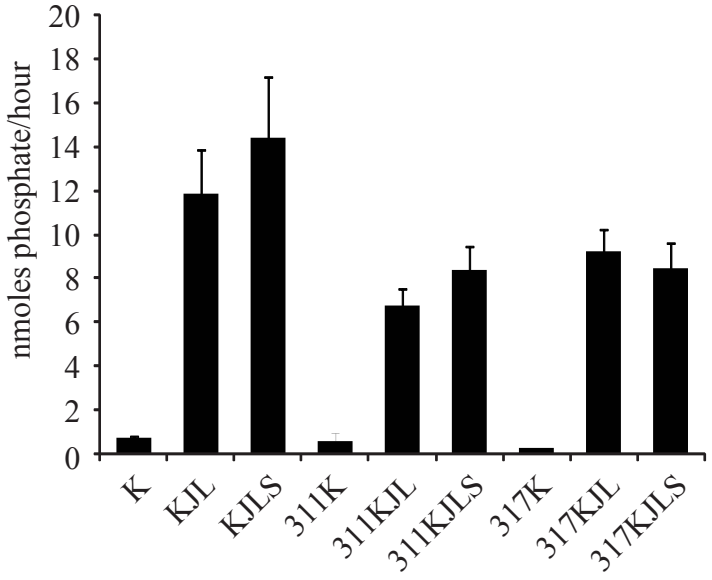

GST-Kar2p
His-Sil1p
- + + + + His-Lhs1p
- - ADP ATP - Nucleotide
150 -
100 -
75 -
50 -
His-Lhs1p
GST-Kar2p
- His-Sil1p

Figure S3


nmoles phosphate/hour
20
18
16
14
12
10
8
6
4
2
0
K
KJL
KJLS
311K
311KJL
311KJLS
317K
317KJL
317KJLS

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. GST-63J stimulated Kar2p activity. The ability of GST-63J to stimulate the steady state ATPase activity of Kar2p was analysed by incubating 1µM Kar2p with increasing amounts of GST-63 J (as annotated) in the presence of 2mM ATP. The amount of phosphate released was measured by a colorimetric assay at A_{640nm} and calculated using a phosphate standard curve.

Fig. S2. The binding of Sil1p and Lhs1p to Kar2p appears to be mutually exclusive. The binding of Sil1p to Kar2p was analyzed in the presence of 5-fold excess of Lhs1p to see if the NEFs bind exclusively to Kar2p. GST-Kar2p was bound to glutathione agarose followed by incubation with His-Sil1p +/- an excess of Lhs1p. NEF binding was analyzed by coomassie staining.

Fig. S3. The addition of both Sil1p and Lhs1p fails to stimulate the ATPase activity of Kar2p above that seen in the presence of Lhs1p alone. The steady state ATPase activity of Kar2p (K) and the Kar2p mutants (311K and 317K) was analyzed in the presence of GST-63J (J) plus Lhs1p (L) or both Lhs1p and Sil1p (LS) together and the amount of free phosphate was calculated as described previously.