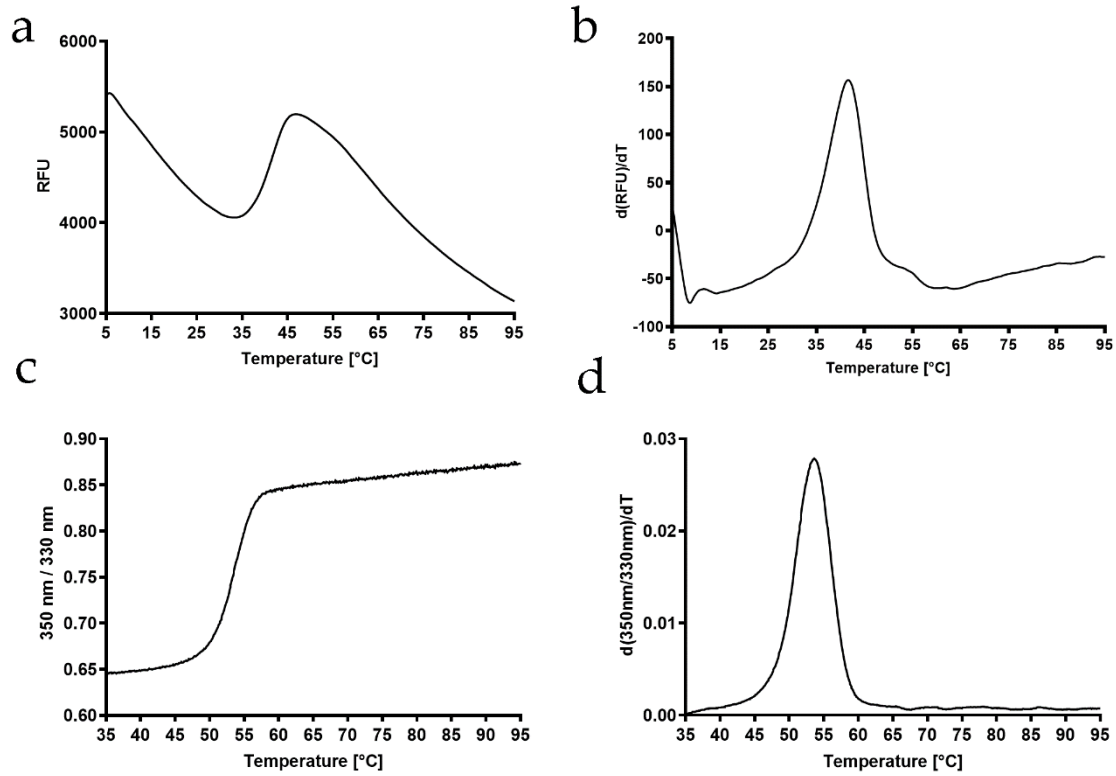




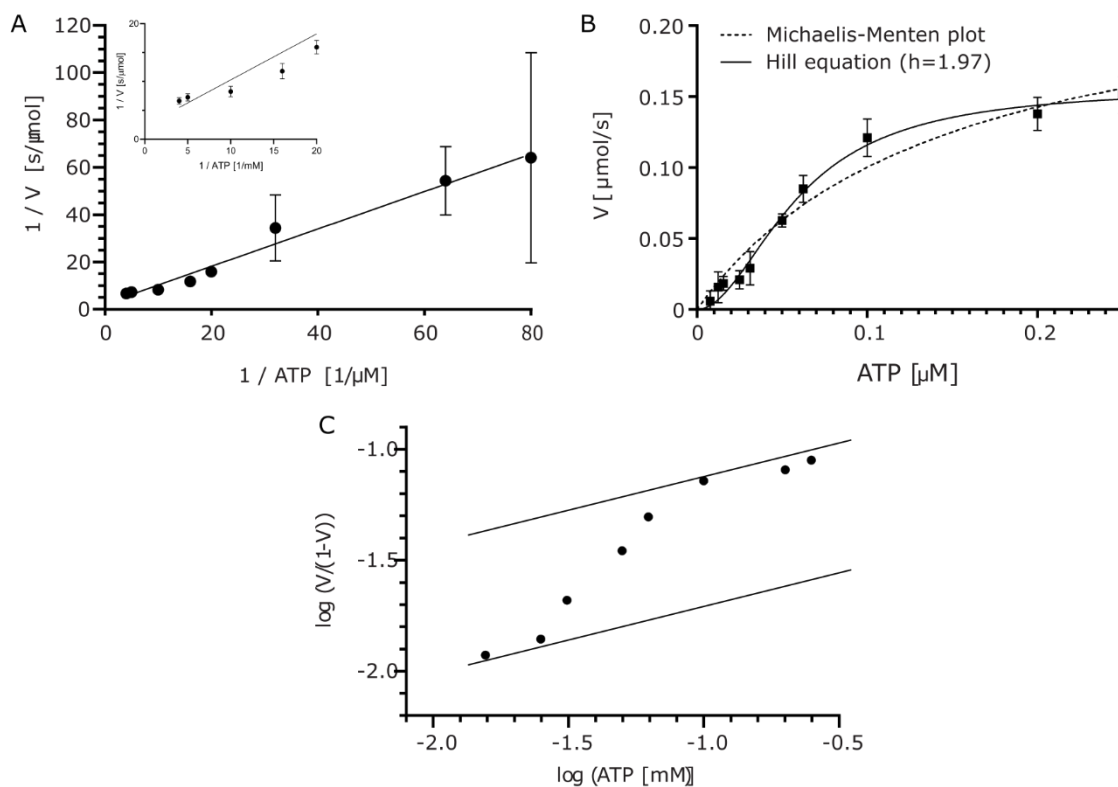
1 Supplemental Information

2 Supplemental Figures



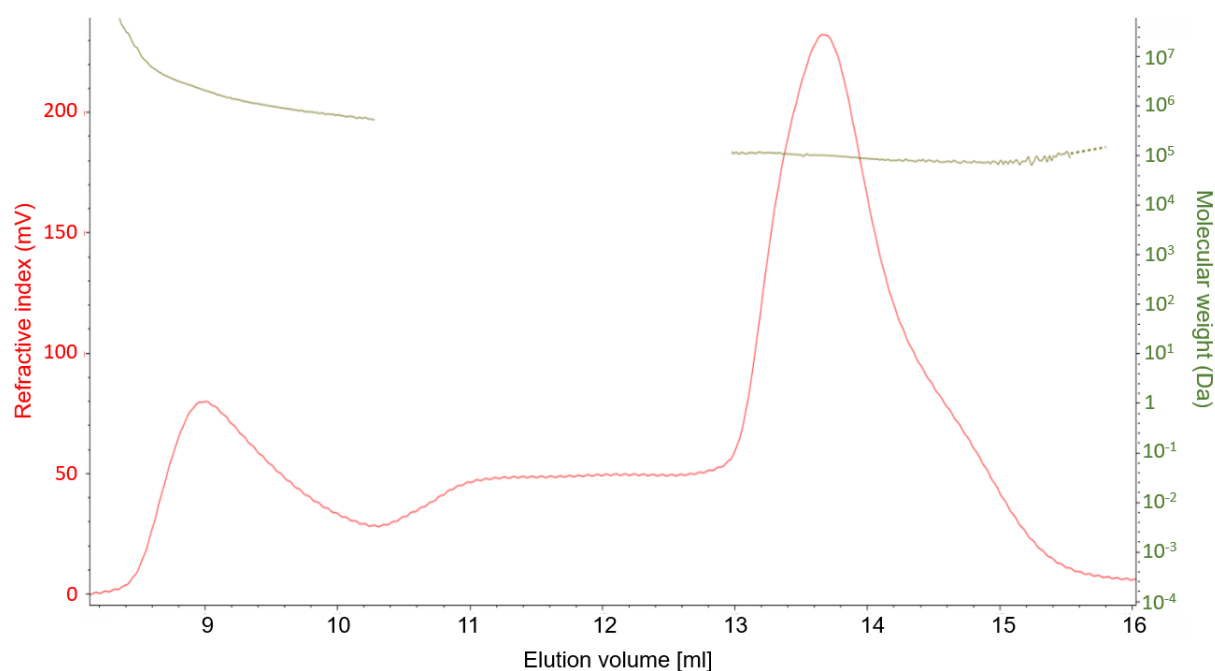
3  
4 **Figure S1.** Stability studies of *KIGlk1* protein using Thermal Shift Assay (panels **a** and **b**) and  
5 Tycho (panels **c** and **d**). Panels (**a**) and (**c**) represent the raw fluorescence data, panels (**b**) and (**d**)  
6 show the corresponding first derivative.

7



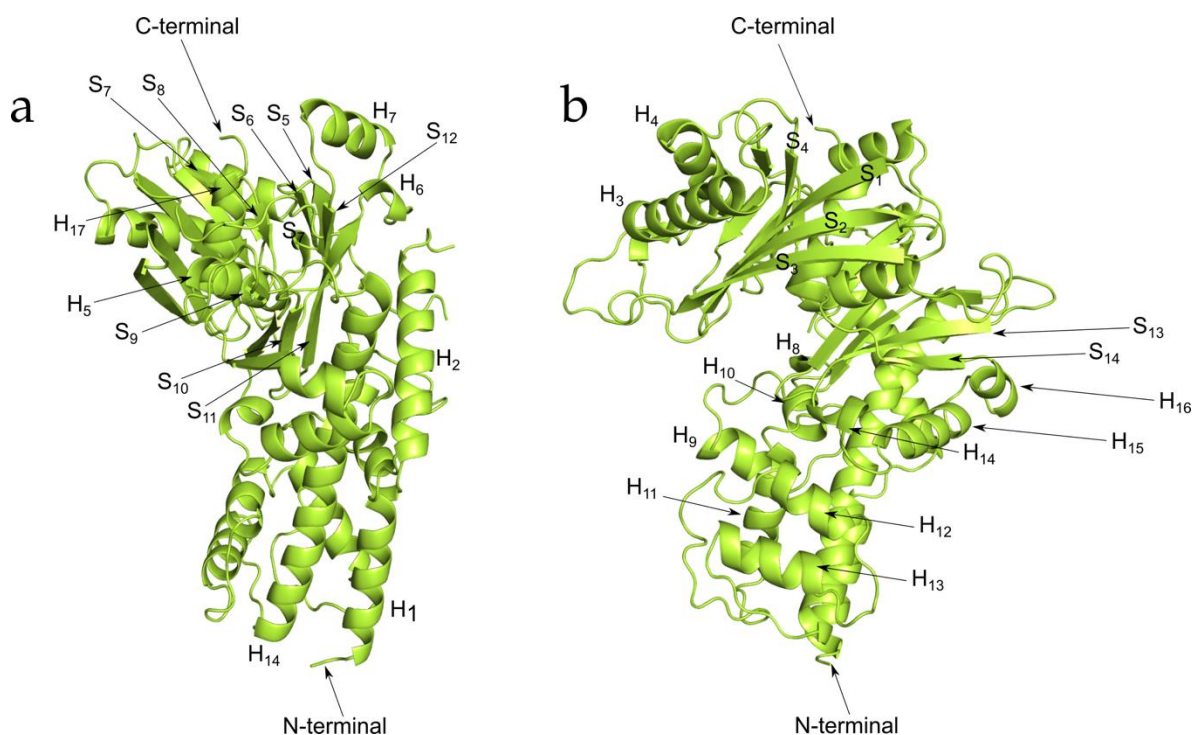
8

9 **Figure S2.** Kinetic analysis of *KIGlk1* activity. (a) Lineweaver-Burk linearization (reciprocal plot).  
 10 Datapoints for low ATP concentrations obey linear trend and were considered for fitting of a  
 11 linear model (solid line,  $R^2=0.98$ ).  $V_{max}$  was determined from the Y-intercept, X-intercept is equal  
 12 to  $-1/K_m$ . Error bars correspond to SD,  $N=3$ . (b) Comparative fit of classical Michaelis-Menten  
 13 plot ( $R^2=0.96$ ) and a Hill plot ( $R^2=0.99$ ,  $h=1.97$ ). (c) Hill Plot. Error bars correspond to SD,  $N=3$ .



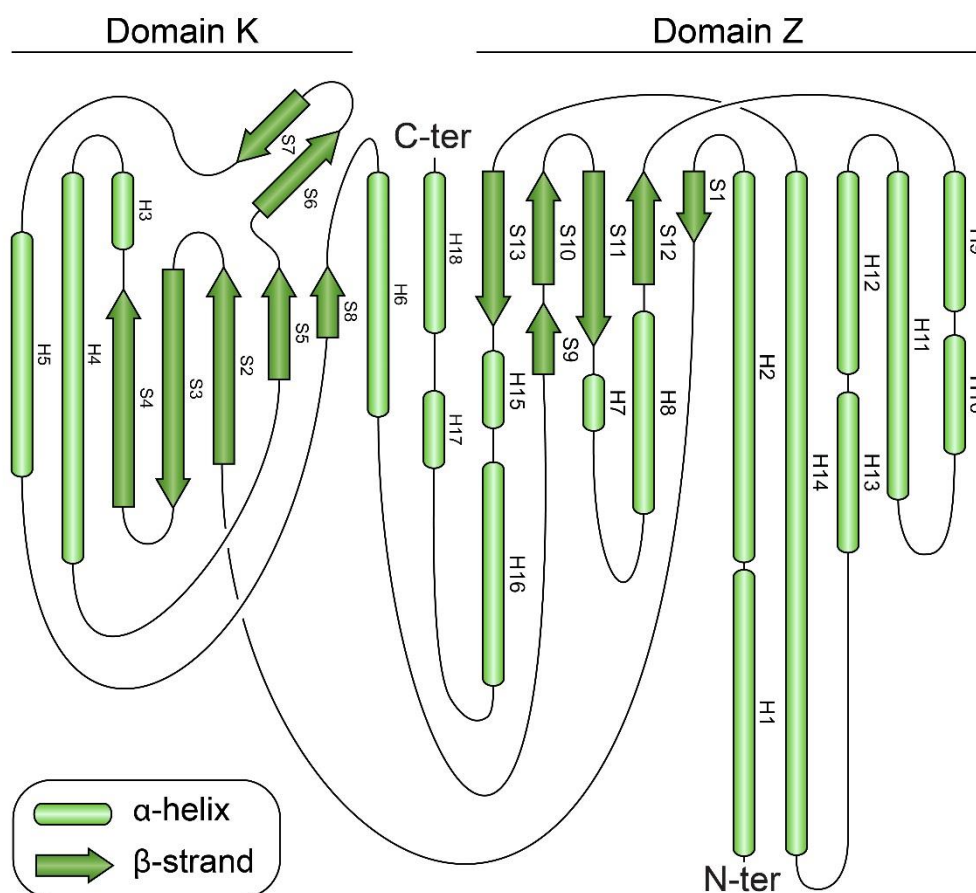
14

15 **Figure S3.** Analysis of RALS/LALS distribution for *KIGlk1*. Elution profile indicates formation of a  
 16 *KIGlk1* dimer in the solution with calculated molecular weight of 100 kDa (second peak). Refractive  
 17 index (mV) and corresponding calculated molecular weight (Da) are represented as a red and green  
 18 line, respectively.



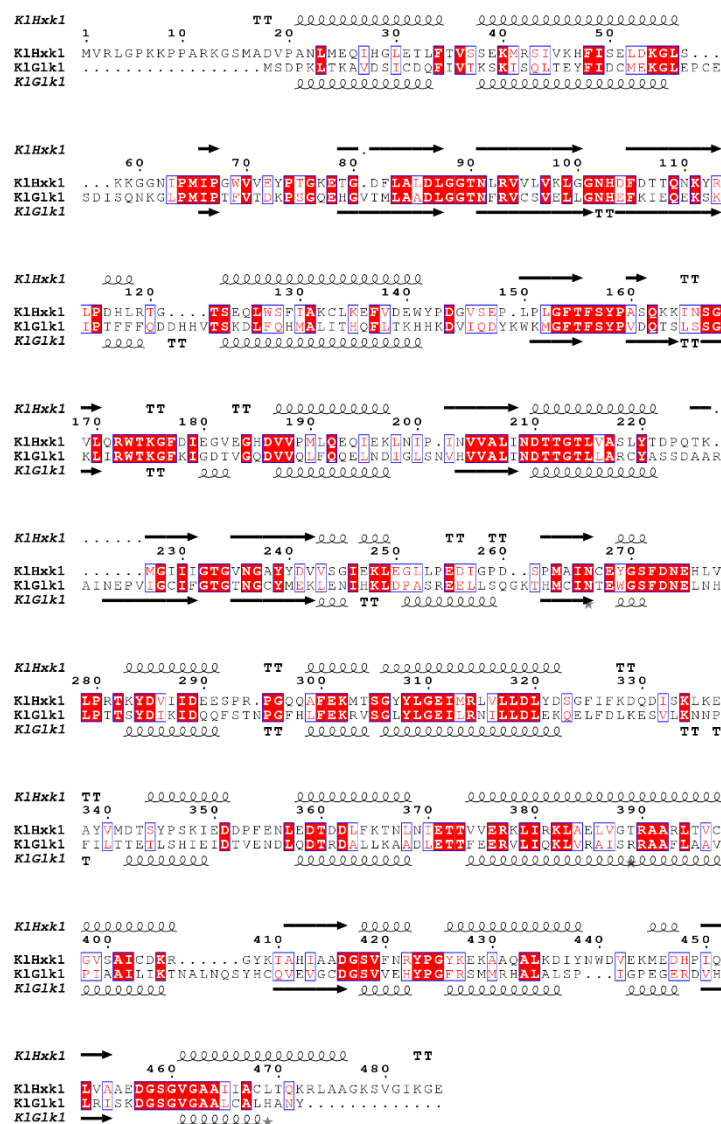
19

20 **Figure S4.** Complete topology of *KIGlk1* protein. Side view (a) and front view (b) of *KIGlk1*. H  
 21 indicates helices, S indicates strands.



22

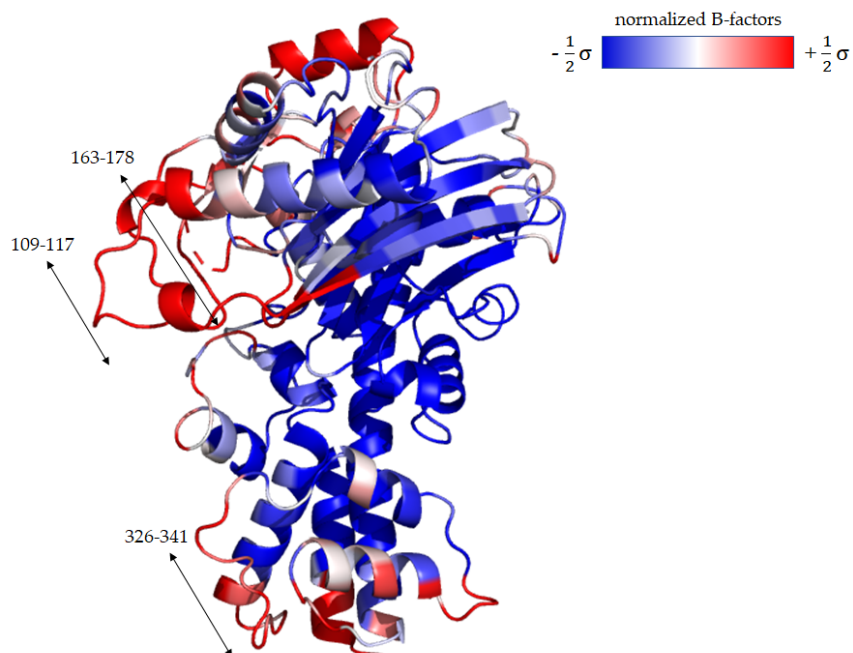
23 **Figure S5.** 2D topology map of *KIGlk1* monomer.



24  
25

26  
27  
28

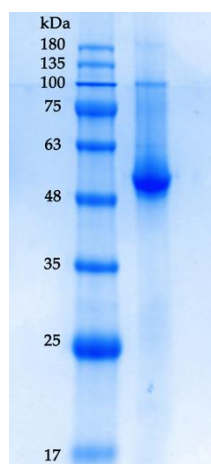
**Figure S6.** Amino acid sequence alignment of *KlG1k1* and *KlHxk1* glucose kinases from *Kluyveromyces lactis*. Amino acid sequence identity between *KlG1k1* and *KlHxk1* is 37%. Sequence Secondary structures for both proteins are indicated.



29

30 Figure S7. Coloured representation of normalized B-factors in *KIGlk1* protein structure. Red parts  
31 represent high, white middle, blue low B factors.

32



33

34 Figure S8. SDS-PAGE gel showing purity of the *KIGlk1* after final step of purification.

35