

# Complex stability and dynamic subunit interchange modulates the disparate activities of the yeast moonlighting proteins Hal3 and Vhs3

J. Albert Abrie<sup>1</sup>, Cristina Molero<sup>2</sup>, Joaquín Ariño<sup>2</sup> and Erick Strauss<sup>1\*</sup>

<sup>1</sup>Department of Biochemistry, Stellenbosch University, South Africa

<sup>2</sup>Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Spain

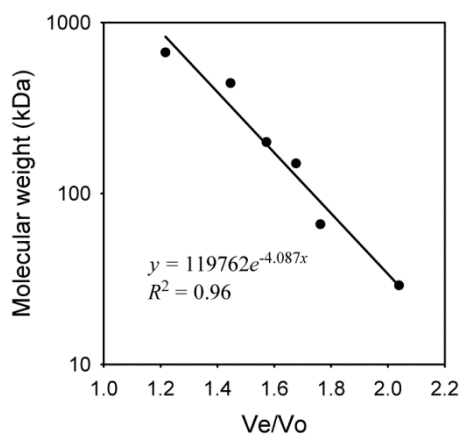
## SUPPLEMENTARY INFORMATION

### Supplementary Table S1: List of oligonucleotides

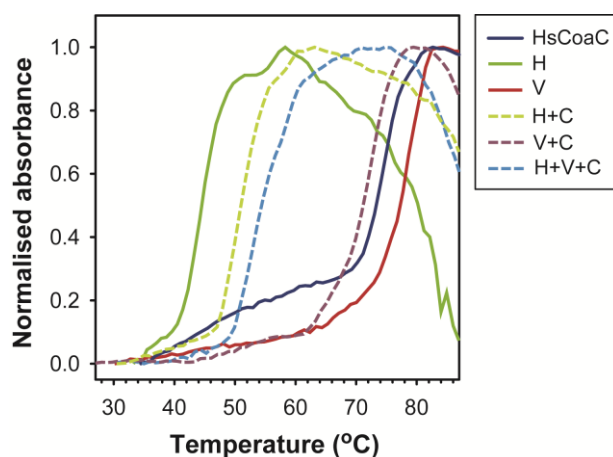
Oligonucleotide name	Utilization	Sequence
5'-Vhs3CoaC	Construction of pET28a-Vhs3-PD	5'-GGATCCAAGGCTTCATATGGATGACGGCAAGC-3'
3'-Vhs3CoaC	Construction of pET28a-Vhs3-PD	5'-CATCCTCTTCTTCCTCGAGTCACGCAGAAACATCG-3'
5'-Ykl088wCoaC	Construction of pET28a-Cab3-PD	5'-GAGTTCCTTTTCAGCATATGGACGACAAGAAATTC-3'
3'-Ykl088wCoaC	Construction of pET28a-Cab3-PD	5'-CTTGCTCTTTATCTCGAGTTTCTCAGTCCCTAGCTTTC-3'
pDuet_PPZ1 <sup>Cter</sup> -5a	Construction of pET-Duet1 Ppz1 <sup>Cter</sup> _Hal3 (MCS 1)	5'-TTGGCGCGCCTGATAATGCAATCTTCCGGAA-3'
pDuet_PPZ1_T1-3	Construction of pET-Duet1 Ppz1 <sup>Cter</sup> _Hal3 (MCS 1)	5'-GGGAAGCTTCAATACACACGCGAAG-3'
pDuet_Hal3_5	Construction of pET-Duet1 Ppz1 <sup>Cter</sup> _Hal3 (MCS 2)	5'-GCAAGATCTGACTGCCGTCGCCTCTACT-3
pDuet_Hal3_3	Construction of pET-Duet1 Ppz1 <sup>Cter</sup> _Hal3 (MCS 2)	5'-CCGCTCGAGTTATTGATGCTTATCTATT-3

### Supplementary Table S2: List of plasmids

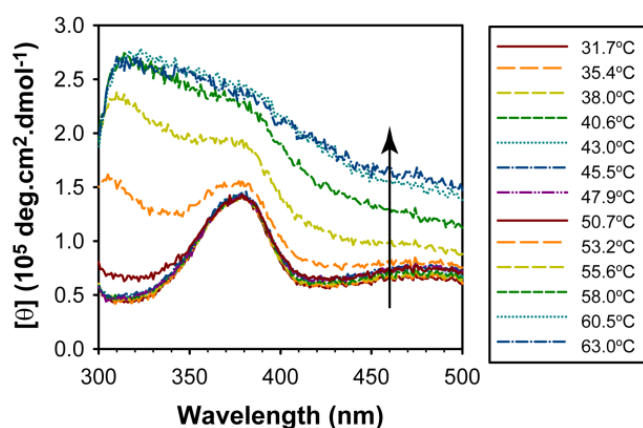
Plasmid	Utilization	Origin/ Reference
pENTR4T-Hal3 <sup>PD</sup>	Construction of pETDuet-1_Hal3 <sup>PD</sup> _Cab3 <sup>PD</sup>	This work
pENTR4T-Vhs3 <sup>PD</sup>	Construction of pETDuet-1_Vhs3 <sup>PD</sup> _Cab3 <sup>PD</sup>	This work
pET28a-Cab3 <sup>PD</sup>	Attempted expression of 6xHis-Cab3 <sup>PD</sup>	This work
pET28a-Hal3 <sup>PD</sup>	Expression of 6xHis-Hal3 <sup>PD</sup>	Ref. 1
pET28a-Vhs3 <sup>PD</sup>	Expression of 6xHis-Vhs3 <sup>PD</sup>	This work
pET28a-Vhs3 <sup>PD_H459A</sup>	Expression of 6xHis-Vhs3 <sup>PD_H459A</sup>	This work
pETDuet-1_Cab3 <sup>PD</sup> (MCS2)	Construction of pETDuet-1 plasmids	This work
pETDuet-1_Hal3 <sup>PD</sup> _Cab3 <sup>PD</sup>	Co-expression of 6xHis-Hal3 <sup>PD</sup> and Cab3 <sup>PD</sup>	This work
pETDuet-1_Hal3 <sup>PD_H378A</sup> _Cab3 <sup>PD</sup>	Expression of 6xHis-Hal3 <sup>PD_H378A</sup> and Cab3 <sup>PD</sup>	This work
pETDuet-1_Vhs3 <sup>PD</sup> _Cab3 <sup>PD</sup>	Co-expression of 6xHis-Vhs3 <sup>PD</sup> and Cab3 <sup>PD</sup>	This work
pPROEX-Hta-HsCoaC	Expression of 6xHis-HsCoaC	Ref. 2
pGEX6P1-Hal3	Expression of GST-Hal3/Construction of pET28a-Hal3 <sup>PD</sup>	Ref. 3
pGEX6P1-Vhs3	Construction of pET28a-Vhs3 <sup>PD</sup>	Ref. 3
pGEX6P1-Ykl088w	Construction of pET28a-Cab3 <sup>PD</sup>	Ref. 4
pGEX6P1-Hal3_H378A	Construction of pETDuet-1_Hal3 <sup>PD_H378A</sup> _Cab3 <sup>PD</sup>	Ref. 4
pGEX6P1-Vhs3_H459A	Construction of pET28a-Vhs3 <sup>PD_H459A</sup>	Ref. 4
pGEX6P1-Ppz1 <sup>Cter</sup>	Expression of GST-Ppz1( $\Delta$ 1-344)	Ref. 3
pETDuet-1_Ppz1 <sup>Cter</sup> _Hal3	Co-expression of 6xHis-Ppz1 <sup>Cter</sup> and Hal3	This work



**Supplementary Fig. S1. SEC calibration curve.** Calibration curve used to determine the size of the protein and complex peaks in the SEC elution profiles shown in Figure 4b–f.



**Supplementary Fig. S2. Heat-induced changes in the PD proteins' absorbance at 222 nm.** Normalised absorbance data collected simultaneously with the circular dichroism data shown in Figure 6a.



**Supplementary Fig. S3. Typical changes in the visible CD spectra of the PD proteins observed as a function of temperature.** CD spectra of the Hal3<sup>PD</sup>/Vhs3<sup>PD</sup>/Cab3<sup>PD</sup> complex obtained at increasing temperatures (as indicated by the arrow), with the legend next to the figure indicating the temperature at which the respective spectra were obtained.

## References

- 1 Abrie, J. A., González, A., Strauss, E & Ariño, J. Functional mapping of the disparate activities of the yeast moonlighting protein Hal3. *Biochem. J.* **442**, 357-368 (2012).
- 2 Daugherty, M., Polanuyer, B., Farrell, M., Scholle, M., Lykidis, A., de Crecy-Lagard, V. & Osterman, A. Complete reconstitution of the human coenzyme A biosynthetic pathway via comparative genomics. *J. Biol. Chem.* **277**, 21431-21431 (2002).
- 3 Ruiz, A., Munoz, I., Serrano, R., Gonzalez, A., Simon, E. & Arino, J. Functional Characterization of the *Saccharomyces cerevisiae* VHS3 Gene. *J. Biol. Chem.* **279**, 34421-34421 (2004).
- 4 Ruiz, A., Gonzalez, A., Munoz, I., Serrano, R., Abrie, J. A., Strauss, E. & Arino, J. Moonlighting proteins Hal3 and Vhs3 form a heteromeric PPCDC with Ykl088w in yeast CoA biosynthesis. *Nat. Chem. Biol.* **5**, 920-928 (2009).