

**AMB Express**

**Balanced trafficking between the ER and the Golgi apparatus increases protein secretion in yeast**

*Jichen Bao<sup>1,2</sup>, Mingtao Huang<sup>1,2</sup>, Dina Petranovic<sup>1,2</sup> and Jens Nielsen<sup>1,2,3,#</sup>*

<sup>1</sup>Department of Biology and Biological Engineering, Chalmers University of Technology, SE41296 Gothenburg, Sweden

<sup>2</sup>Novo Nordisk Foundation Center for Biosustainability, Chalmers University of Technology, SE41296 Gothenburg, Sweden

<sup>3</sup>Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK2970 Hørsholm, Denmark

<sup>#</sup>To whom correspondence should be addressed.

Email: nielsenj@chalmers.se

Tel: +46 31 772 38 04

Fax: +46 31 772 38 01

Table S1 Primers used in this study.

Name	Sequence (5' to 3')
GCS1-up-100-F	gaaccaaattgcacaactatatcaagaatg
GCS1-up-100-R-amds	ctgcgcacgtcaagactgtcaaggagggtattctgggcctccatgtcgctctataatccgcgataaaa ttgc
amdS-F	gacatggaggcccagaatac
REC-amdSYM-R(gcs1)	atatagttgtcaatttggttcttgacaaaaactggcgttgcttgcgggtaaccgcgacagtatag cgaccagcattcacatac
REC-P-TEF1-F(GCS1)	tcgcgcggttaccgcaagcaacgccagttttgtcaagaaccaaattgcacaactatatatagcttc aaaatgtttctactcctttttactcttc
gcs1-TEF1-R	gcaaaagacgcctgcgggtatctgggtccactttccaatctgacattttgtaaacttagattagattg ctatgctttcttctaataagc
GCS1-F	atgtcagattggaagtggaccagatacccg
GCS1-MID-R	ccttttgaaaattggcaccattactag
GLO3-up-120-F	cgtaaacacgatttagctggc
GLO3-up-120-R-amds	ctgcgcacgtcaagactgtcaaggagggtattctgggcctccatgtctgtcgctatgtgtatactaaat tgctc
REC-amdSYM-R(glo3)	aagaatagttgtttcttttttttttttttttcgaaactttaccgtgtcgtcgaaaatcagtatagcgacc agcattcacatac
REC-P-TEF1-F(glo3)	attttcgacgacaggtaaagtttcgaaaaaaaaaaaaaaaaaagaaaaaactattcttatagctt caaatgtttctactcctttttactcttc
glo3-TEF1-R	gagtagtctgttccgtggcaaatgtttctcctcatcgttactcatTTTGTTaaacttagattagattgc tatgctttcttctaataagc
GLO3-F	atgagtaacgatgaaggagaaacattgccacggaacagactactc
GLO3-MID-R	cttgaccgttcaagactaattctg

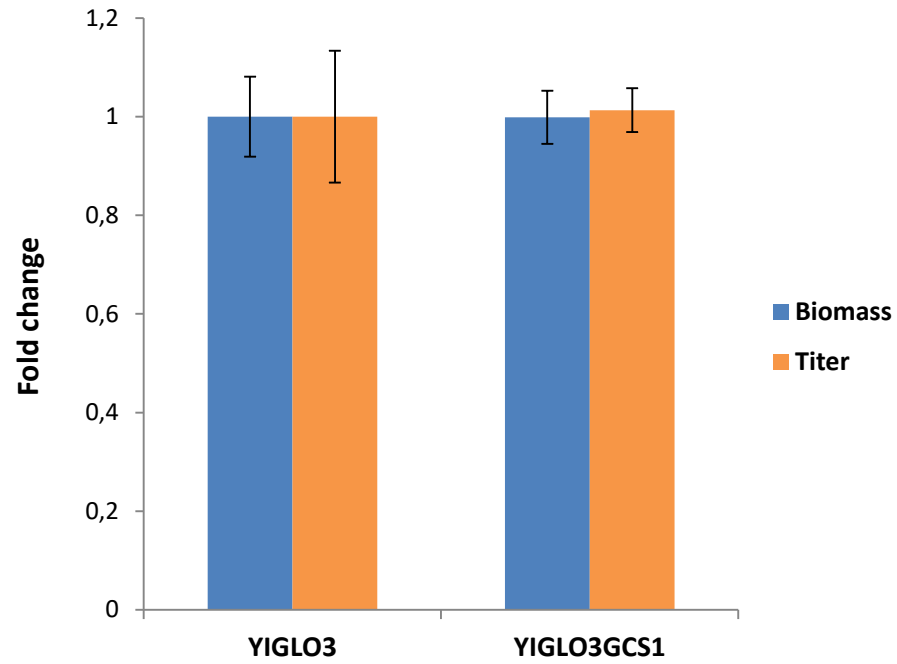


Fig. S1 Co-overexpression of *GCS1* and *GLO3* in YIGS16 does not further increase the protein secretion. Measurements are reported as the average value  $\pm$  standard deviation from independent quintuplicates.

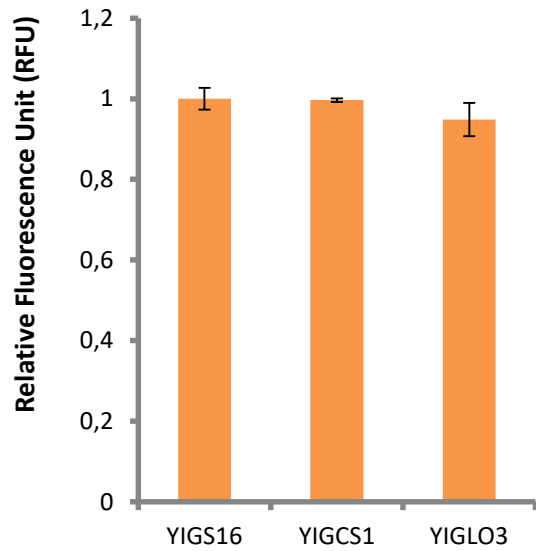


Fig. S2 Intracellular ROS levels of strains YIGS16, YIGCS1 and YIGLO3 using dihydrorhodamine 123. Measurements are reported as the average value  $\pm$  standard deviation from independent triplicates.

## Intracellular $\alpha$ -amylase per cell

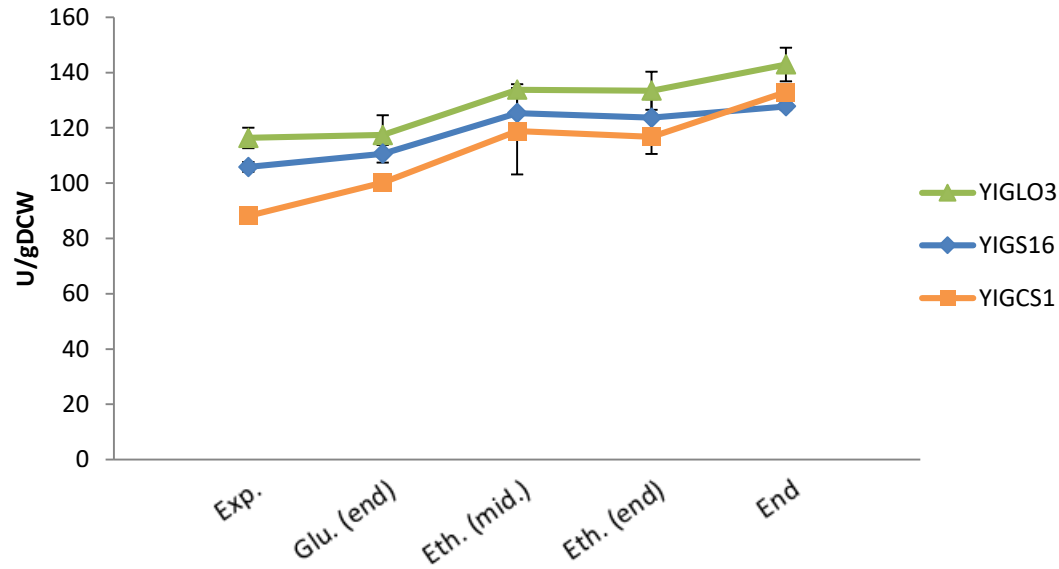


Fig. S3 The intracellular  $\alpha$ -amylase per cell of the three strains. Measurements are reported as the average value  $\pm$  standard deviation from independent triplicates.

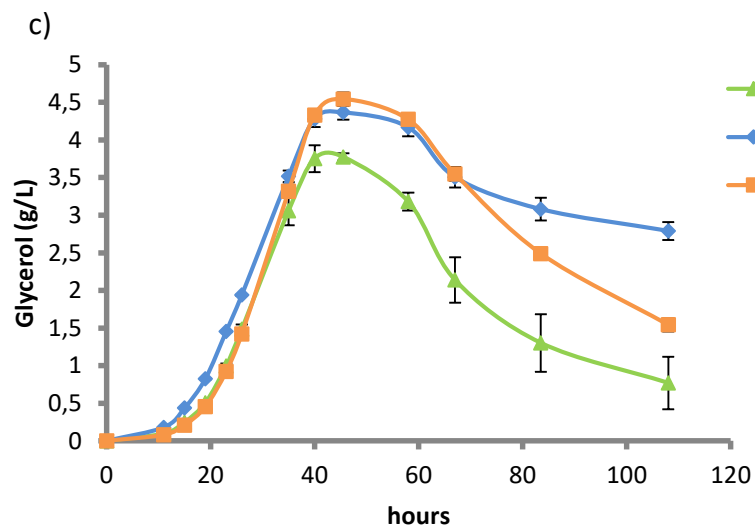
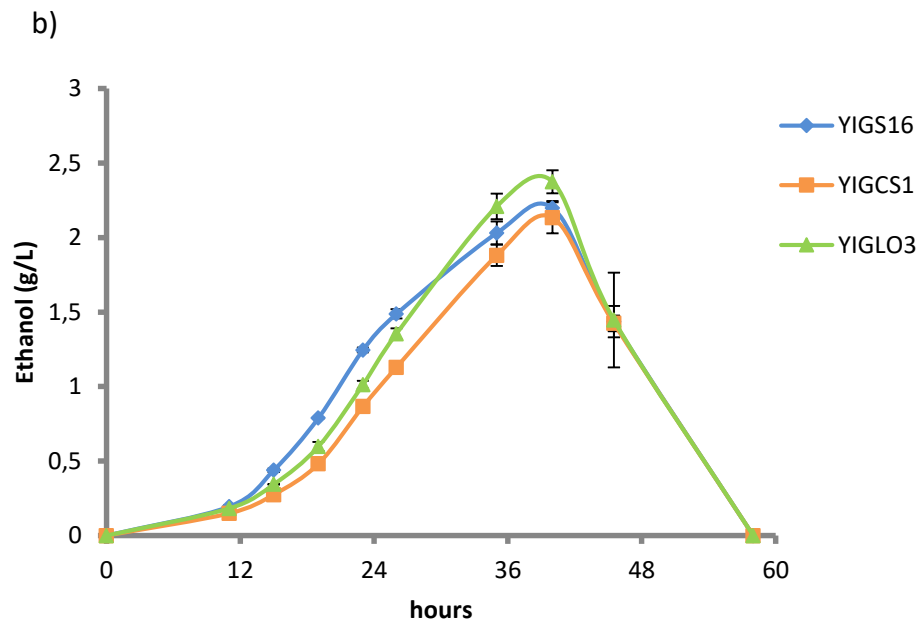
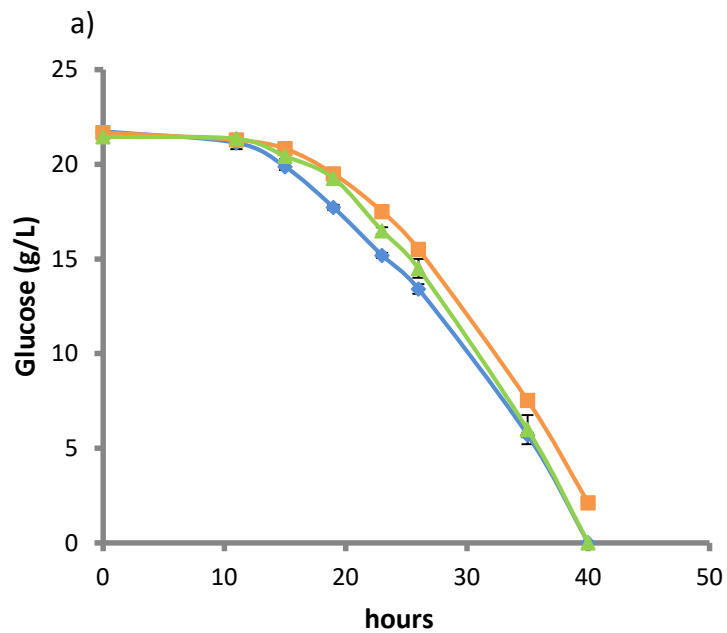


Fig. S4 a) the glucose consumption curves, b) the ethanol curves and c) the glycerol curves of the three strains. Measurements are reported as the average value  $\pm$  standard deviation from independent triplicates.