Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Data

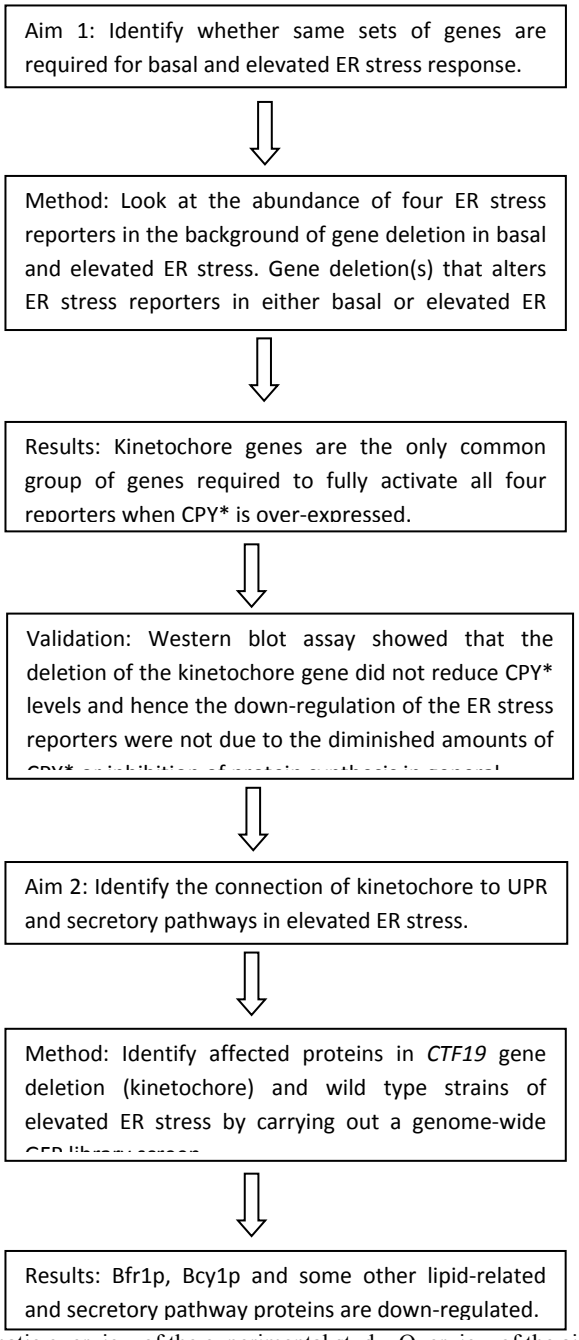

Fig. S1. A schematic overview of the experimental study. Overview of the aim, method and results of this study.

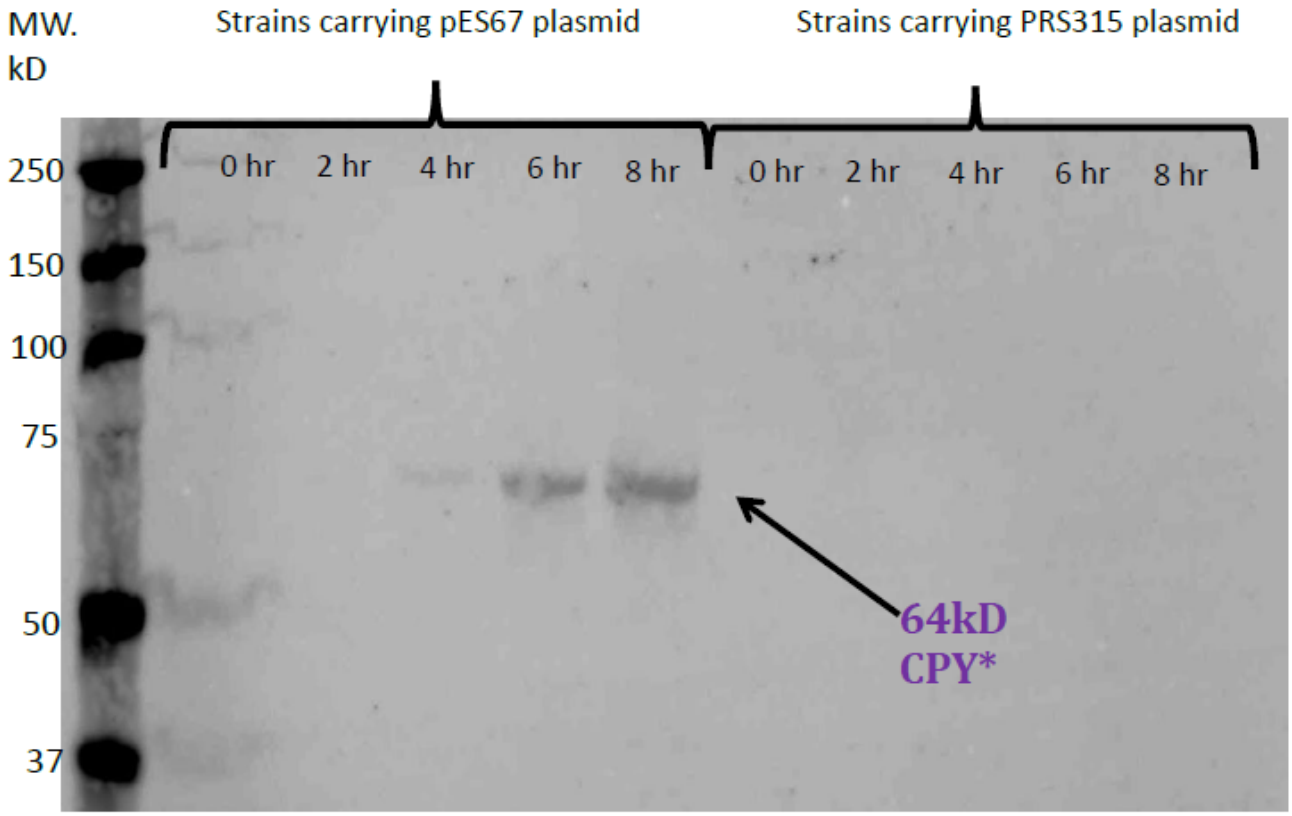

Fig. S2. Western blot analysis of CPY* at different hours of galactose induction. Two yeast strains carrying either the pES67 plasmid (CPY* over-expression) or the PRS315 plasmid (control) were grown in galactose for 0, 2, 4, 6, 8 hours. At 4 hours, a 64kDa band was detected, indicating the abundance level of CPY* protein. The band continues to increase at 6 and 8 hours. The strain that carries the PRS315 plasmid did not have any detectable CPY* band. The HA-tagged CPY* proteins were detected by utilising a primary HA-tagged antibody that was tagged with a secondary goat-raised anti-rabbit labelled with Cy5 antibody.

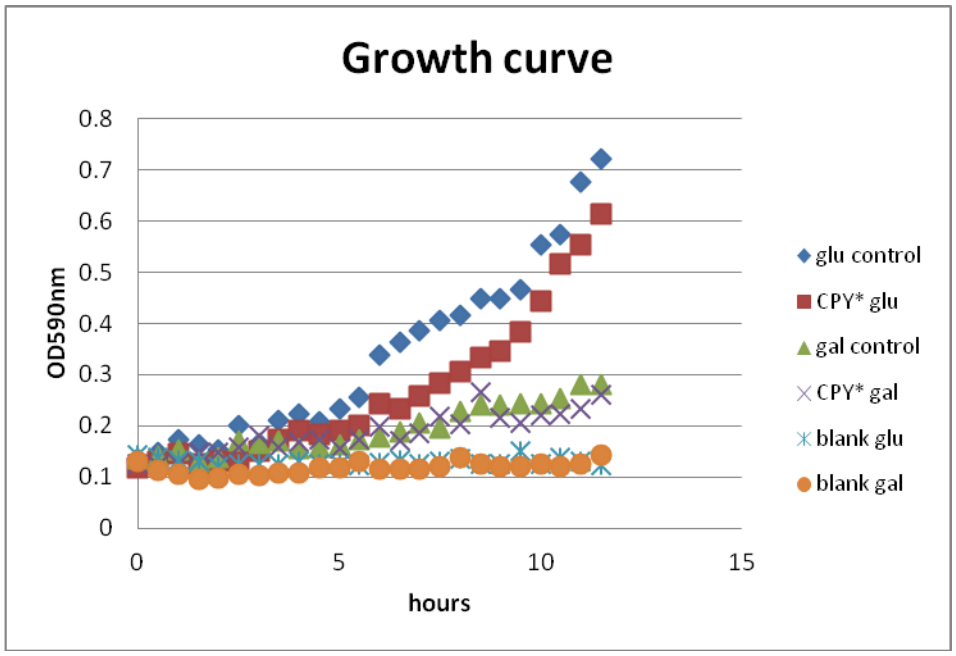

Fig S3. Growth curves of yeast strains with and without CPY* plasmid grown in galactose and glucose media. Both strains with and without CPY* plasmid grown in 2% galactose show similar growth curves pattern at all times (upto 12 hours). There were slight variation in the growth rate between both strains when grown in glucose media probably due to some experimental variations as CPY* was not expressed in glucose media.

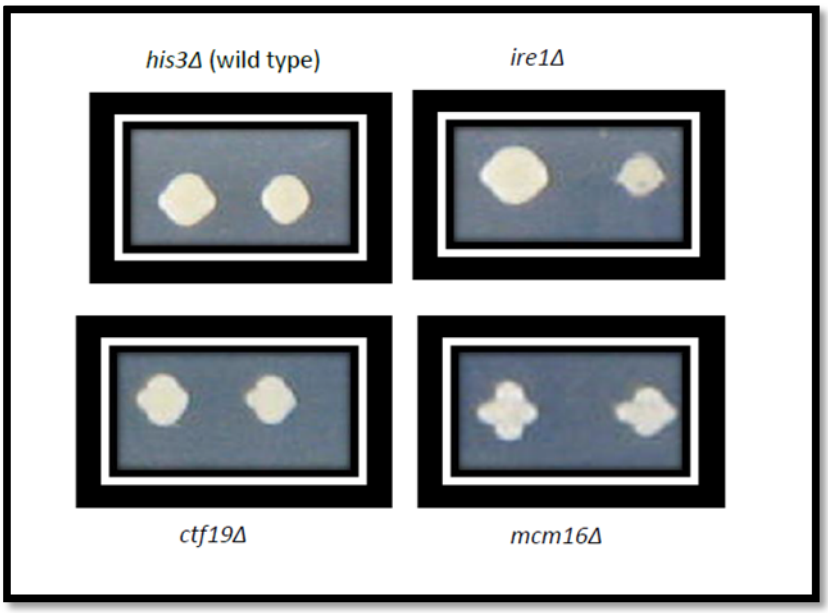

Fig S4. Colony sizes of gene deletion yeast strains with (right colony) and without (left colony) CPY* plasmid in galactose media. Colony sizes were compared between the control (left colony) and CPY* over-expressed (right colony) strains of several candidate gene deletions such as *IRE1*, *CTF19* and *MCM16* grown in an overnight galactose agar media. Growth of yeast strains with CPY* over-expression in the background of *CTF19* or *MCM16* gene deletion were not affected compared to their respective control strains. *IRE1* deletion shows that growth was affected (smaller colony size) when CPY* was over-expressed in the absence of *IRE1* compared to the left colony (*IRE1* deletion with control plasmid).

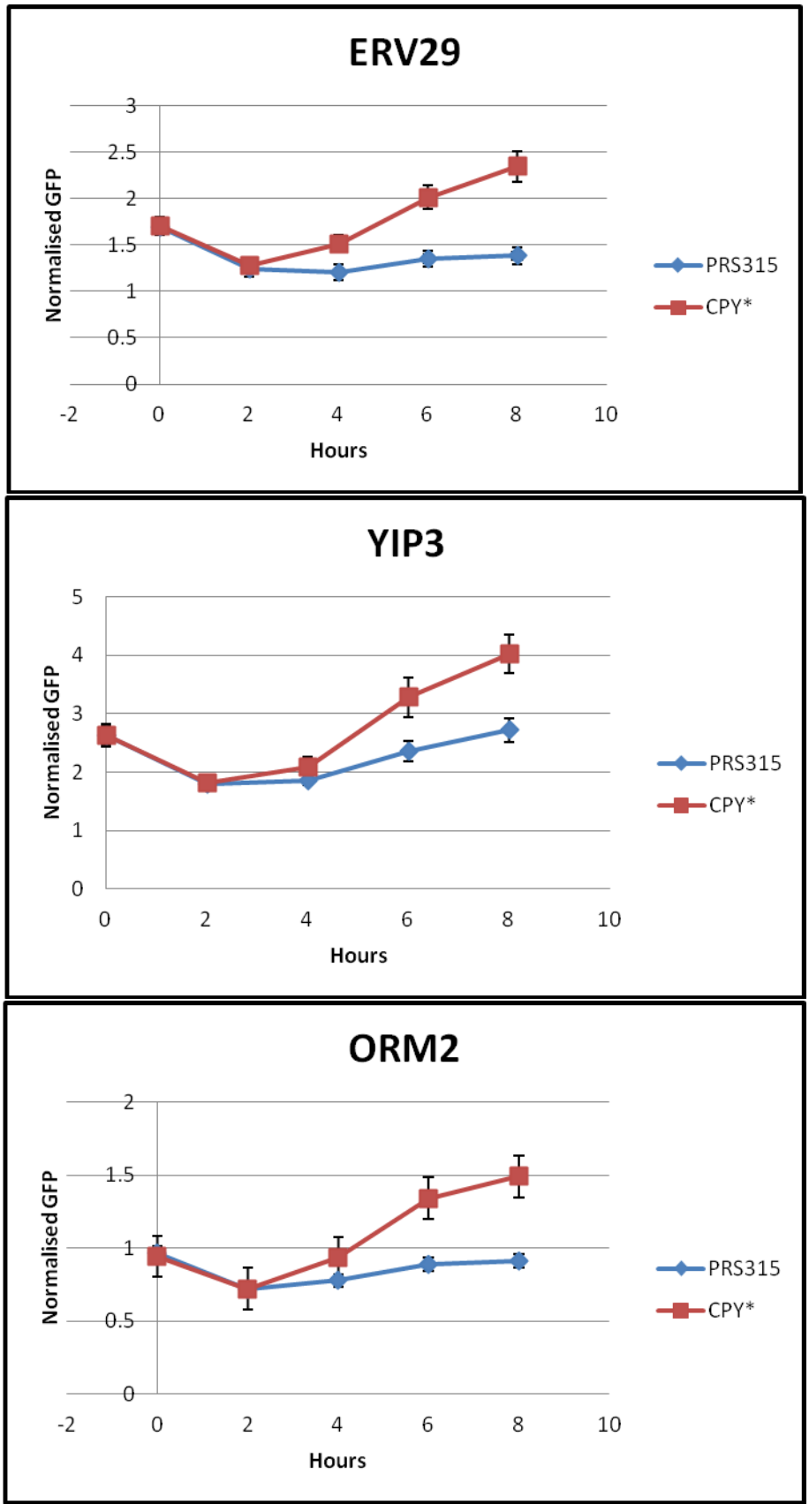

Fig. S5. Normalised GFP expression levels of ER stress reporters at different time intervals of galactose induction. All three reporters with CPY* plasmid show increased expression from 4 hours onwards. Each values represent mean of 48 independent replicates ± SD. The normalised GFP was the normalised value of GFP/RFP.

Table S1. Genome-wide deletions that uniquely affect reporters at basal levels of ER stress.

Categories	**UPRE**	**ERV29**	**YIP3**	**ORM2**
Cell Cycle	**BFA1, DMA1**, PPH21, **YCK2**			
Cell Wall	**CWH41**, NAB6, **ROT2**			
Chromosome segregation	HCM1, NKP2, **YBP2**			
Cytoskeleton	BEM4, **WHI2**, YBR266C			
DNA repair				
Lipid metabolism	**OPI3, FAT1, MUQ1, PSD2, SPF1**			
Mitochondria	**CSF1,** EMI5, TCM62, **YMC2**		FMP18	
RNA/mRNA processing	CBC2, TGS1, DBP3			
Protein processing	**ALG6, ALG8, ALG9, BUL1, ECM39, HRD1, LHS1, MDM39, OST3, PMT2, RHK1, SCJ1, SEC66, SPC2, UBR2, VID28, YCL045C, YDR221W**, YKL206C, **YMR247C**, YOR322C, **YUR1**	**HRD1**	**HRT3**	
Protein trafficking/transport	ARF1**, BST1, ERP1, ERV29**, MOG1, **PEX5, PIL1, RMD7, SEC22**, SXM1, **YIP3**			
Ribosome/translation	**BUD21, CGR1**, DBP7, GCN3, MBA1, RPE1, RPL35B, RPL37B, RPP1A, RPS9B, **YJL123C**	**LEO1, RXT2**		
Signaling	BMH1, **DCR2, NBP2, RHO5, YNL305C**			
Transcription	CST6, **EAF3,** HAA1, HAP2, **RPN4**, **SET2, SNF6, STB5**, SWD3			**TOS9**
Others	ACH1, ACN9, BNA1, CCC2**, CPA1, CTT1, ESC2,** FUN34, GDS1, **GLG1,** HMO1, **HSD1**, **ICE2**, IPK1, OSH3, **PGM2**, SPP1, **STE24**, TPS1**, UBC8, YMR163C, YOR021C**	CDC73		
Unknown	**ENT4**, IES5, **PIN2**, **SHE10,** YEL033W, **YFL006W, YGL235W, YGL261C, YHL029C, YHR151C, *YNL011C*, ZRG17**	***YNL011C***		***YNL011C***
Dubious	**YBL083C, YDR360W, YDR537C, YGL042C**, YHR049C-A, YKL097C, YLR374C, **YMR135W-A, YNR025C, YOR015W**, YOR135C, **YOR333C**			

In **BOLD** indicates that the deletion caused up-regulation of the reporters, non-bold indicates down-regulation of the reporters, while italics hits indicate that the hits are shared between three or more reporter. Underlined indicates hits that are also found in Jonikas et al. (2009).

BiNGO GO categories of hits for all GFP-tagged reporters

Besides grouping the hits into intuitive general functional categories for the ease of discussion in this study (Table 1 and S1), for completeness, enrichment of functional categories based on objective GO BiNGO [17] categorisation in Cytoscape [18] were also carried out to look for significant functional enrichments of the hits in each reporter assays. All CPY* over-expression reporter assays show enrichment of genes that are involved in sister chromatid cohesion.

Table S2. GO categories from hits of UPRE basal levels ER stress assay.

P-value	Fold enrichment	GO categories	Genes
4.73E-07	23.54	dolichol-linked oligosaccharide biosynthetic process	ALG3|ALG6|ALG8|ALG9|ALG12
5.86E-07	7.32	protein glycosylation	OST3|YUR1|CWH41|PMT2|ALG3|ALG6|ALG8|ALG9|ALG12|GTB1

The basal levels ER stress UPRE assay revealed enrichment of genes that are involved in dolichol-linked oligosaccharide and glycosylation processes**.**

Table S3. GO categories from hits of UPRE CPY* over-expression assay.

P-Value	Fold enrichment	GO categories	Genes
4.26E-21	52.22	mitotic sister chromatid cohesion	MRC1|IML3|CTF3|DCC1|CSM3|CTF4|MCM16|MCM21|MCM22|CTF19|TOF1|CHL4|CTF8
3.11E-06	35.05	galactose metabolic process	GAL3|GAL4|GAL10|GAL7

The elevated levels of ER stress of the UPRE-GFP assay revealed enrichment of genes that are involved in mitotic sister chromatid cohesion and galactose metabolic processes**.**

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Table S4. GO categories from hits of Erv29p basal levels ER stress assay.

P-value	Fold enrichment	GO categories	Genes
1.23E-03	37.08	regulation of transcription from RNA polymerase I promoter	LEO1|CDC73
2.19E-03	10.99	regulation of organelle organization	COX14|LEO1|CDC73

The basal levels ER stress Erv29p assay revealed enrichment of genes that are involved in transcription regulation and organelle organisation.

Table S5. GO categories from hits of Erv29p CPY* treated assay.

P-value	Fold enrichment	GO categories	Genes
2.13E-03	26.05	regulation of histone acetylation	CHD1|SET2
3.50E-03	20.84	proton-transporting ATP synthase complex biogenesis	ATP18|ATP23
2.23E-10	19.54	mitotic sister chromatid cohesion	CTF19|CTF18|IML3|DCC1|MCM16|TOF1|CHL4|CTF8|MCM21
2.46E-04	12.26	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	CBC2|NAM7|NMD2|NMD4
2.62E-03	10.42	carbon catabolite activation of transcription from RNA polymerase II promoter	HAP3|HAP5|GAL4

The elevated levels ER stress Erv29p assay revealed enrichment of genes that are involved in histone regulation, ATP synthase, mitotic sister chromatid cohesion, mRNA process and transcription**.**

Table S6. GO categories from hits of Yip3p basal levels ER stress assay.

P-value	Fold Enrichment	GO categories	Genes
4.97E-06	32.40	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RPS6B|RPS11B|RPS23A|SLX9

The basal levels ER stress Yip3p assay revealed enrichment of genes that are involved in maturation of rRNA transcript.

Table S7. GO categories from hits of Yip3p CPY* treated assay.

P-value	Fold Enrichment	GO categories	Genes
5.73E-06	55.09	negative regulation of G2/M transition of mitotic cell cycle	MRC1|CSM3|TOF1
4.58E-09	18.36	mitotic sister chromatid cohesion	MRC1|CTF18|CTF3|CSM3|MCM16|TOF1|CHL4|MCM21
3.14E-04	11.60	DNA integrity checkpoint	MRC1|CSM3|TOF1|BMH1

The elevated levels ER stress Yip3p assay revealed enrichment of genes that are involved in negative regulation of G2/M mitotic cell cycle, mitotic sister chromatid cohesion and DNA integrity checkpoint.

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Table S8. GO categories from hits of Orm2p CPY* treated assay.

P-value	Fold Enrichment	GO categories	Genes
1.81E-06	80.33	attachment of spindle microtubules to kinetochore	CTF19|IRC15|BUB1
1.48E-03	32.13	negative regulation of DNA-dependent DNA replication	MRC1|CHD1
1.48E-03	32.13	protein insertion into ER membrane	GET2|GET1
5.75E-08	13.97	sister chromatid segregation	MRC1|CTF19|IML3|IRC15|DCC1|MCM16|BUB1|CHL4

Note that there were no enrichments for the hits obtained from Orm2p basal levels ER stress assay. However the elevated levels ER stress of Orm2p assay revealed enrichment of genes that are involved in attachment of spindle microtubule to kinetochores, DNA replication, protein insertion into the ER and sister chromatid segregation.

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Table S9. GFP protein hits that were affected by CPY* over-expression in *ctf19Δ* strains.

Categories	**0 HRs**	**8 HRs**
cell cycle		**BFR1**
cell wall		**MCD4, PMI40, YPS1**
cytoskeleton		SAC6
ion transport	**ZRT1**	**FET3,** CUP5
lipid metabolism	CPR1, GRE2, POT1, SIP18, FMP45, YNL194C	**ACB**1, AYR1, **ERG13, ERG3, FAS1, HNM1, INO1, ITR1, ORM2, OPI3,** SIP18, FMP45, YNL194C
mitochondria	ALD5, ALD6, MMF1, OM45	
protein folding	CPR6, SSE2	HSP104, **KAR2, SSE1**
protein modification	UBC4	ALG9, **SEC53, STE24, ADD37, YOR138C**
protein trafficking/transport	**VPS21, YPT7**	**ERP1, ERP2, ERV29, SEC31,** SNA3, **YIP3, YPT7**, NCE102, NCE103
signalling	BCY1, BMH1, RDI1, RHO5, SKM1	BCY1, **SCP160,** SKM1
Translation	DBP1, FUN12, MES1, RPL18B, **RPL21A,** RPL22A, RPL24B, RPL26A, **RPL2A**, RPL33B, **RPL34A,** RPL42B, RPL43B, RPL5, RPP1B, RPS19A, RPS27B, TIF1, YEF3, TMA19, RRT8	**EFT1,** IFM1**, NEW1, NOP58, RPL2A, RPL34A, TIF4631**
transcription	**RPO26**	**HTZ1, TFB1**
others	AHP1, ACS1, **ADH4**, ARO8, CPA2, CUP1-1, CUP1-2, CYS3, ECM4, GLO1, PGI1, SAM1	AHP1, **ADH4**, ADH5, ADH6, ARG4, ARG8, CPA2, CUP1-1, DIP5, **GAL3**, HIS4, HIS7, **HXK2**, ILV1, LEU1, OAC1, PAI3, RTN2, **SOL3**, **TAL1**
unknown	FSH1, HUA2, RTC3, YKL071W, YRO2	SSP120, TMT1, **FMP16, YNR034W-A,** YRO2

General functional categories of hits that show differences in intensity or localisation between *his3Δ* and *ctf19*Δ strains at 0 and 8 hours of ER stress induction. Underlined indicates hits that were observed in both 0hrs and 8hrs. In BOLD indicates that the proteins were down-regulated.