nature portfolio

https://doi.org/10.1038/s41586-023-06239-6

Supplementary information

Structural basis of mitochondrial protein import by the TIM23 complex

In the format provided by the authors and unedited

SUPPLEMENTARY DISCUSSION

Our study proposes a model that is notably different from some frequently proposed models regarding the stoichiometry of the complex and the function of the Tim23 subunit. Below we discuss possible sources of these different results and interpretations. We also discuss limitations of our study.

Copy numbers of Tim23/17

The stoichiometry of the Tim23/17 subunits has long been debated. While several past biochemical studies have suggested that the TIM23 complex contains multiple copies of Tim23 and/or Tim17 (Alder et al., 2008; Bauer et al., 1996; Demishtein-Zohary et al., 2015; Gomkale et al., 2021; Meinecke et al., 2006), some other studies have observed the apparent 1:1 stoichiometry of Tim23 and Tim17 (Moro et al., 1999; Ryan et al., 1998). In addition, the dynamics of subunit assembly have remained unclear. Tim23 dimers, detected by chemical crosslinking, were first proposed to monomerize upon substrate engagement (Bauer et al., 1996). However, more recently, Tim23-Tim23 crosslinking was shown to increase in the presence of a laterally sorted translocation substrate or when non-essential Tim21 is overexpressed but was not detectable in the non-translocating state (Popov-Čeleketić et al., 2008). Also, effects of Tim50 on putative Tim23 dimerization have been debated (Meinecke et al., 2006; Tamura et al., 2009).

Our cryo-EM analysis of the endogenous core complex indicates that the stable structure of Tim23 and Tim17 is a 1:1 heterodimer in a back-to-back arrangement, at least in the idle state (Extended Data Fig. 3). Given the new data showing that Mgr2 is positioned at the lateral opening of Tim17 (present study; Ieva et al., 2014), we consider it unlikely that an additional copy of Tim23 or Tim17 binds to the lateral opening of Tim17. Furthermore, without drastic conformational changes, the current structures of Tim17 and Tim23 seem incompatible with the formation of a channel through face-to-face docking of two copies of Tim17/23. We have also exercised structural predictions of 2:1, 1:2, and 2:2 ratios of Tim23 and Tim17 using AlphaFold2. Regardless of the ratio, they all invariably produced a 1:1 heterodimer in the back-to-back arrangement as in our cryo-EM structure without any pore formation, whereas the extra copy of Tim23 or Tim17 resulted in inconsistent positions.

While previous co-immunoprecipitation results support the idea that additional copies of Tim17 and/or Tim23 exist in the TIM23 complex, their stoichiometry remains unclear. In the study by Gomkale et al. (Gomkale et al., 2021), immunoprecipitation of ALFA-tagged Tim23 and Tim17 copurified untagged Tim23 and Tim17 respectively, but the amounts seemed noticeably substoichiometric to the ALFA-tagged protein when their relative amounts in ‘total’ and ‘elution’ samples are compared. This was also the case for the experiment performed for Tim23 in Tamura et al. (Tamura et al., 2009). The exact nature of these small amounts of additional Tim23/17 copies that can be copurified remains unclear. The TIM23 complex might transiently form a larger complex, since mitochondrial protein import typically involves coupled translocation processes by the TOM and TIM23 complexes. For example, it is conceivable that

a single dimeric TOM complex may engage with two copies of the TIM23 complex simultaneously during substrate translocation. It is also possible that some populations of Tim23 and Tim17 proteins assemble into homodimers or form other transient interactions. Previous chemical crosslinking observations might represent these forms (Bauer et al., 1996; Demishtein-Zohary et al., 2015; Popov-Čeleketić et al., 2008; Tamura et al., 2009). Clarification of these issues will require additional functional and structural studies.

Channel formation by Tim23

It has long been thought that the Tim23 subunit forms an aqueous channel. Main supporting data for this model were electrophysiological observations of cation-selective currents by Tim23, which could be further modified by addition of presequence peptides. However, a majority of such experiments were conducted in planar bilayers using refolded Tim23 proteins that were first expressed in *E. coli* as inclusion bodies (Meinecke et al., 2006; Truscott et al., 2001; Zhou et al., 2021). Also, these experiments did not use any other essential membrane components, such as Tim17. It is unclear what structures such denatured Tim23 proteins fold into under these conditions. Recently, an NMR study has used a similar refolding approach in an attempt to determine the structure of the Tim23 protein (Zhou et al., 2021; PDB ID 7CLV). Interestingly, the study found that Tim23 forms a homodimer with a channel-like structure containing a larger cavity within the membrane between the two molecules of Tim23. However, all transmembrane helices of Tim23 in this structure exhibit abnormally loose arrangements without being packed against each other like typical membrane proteins. The structure also has no resemblance to our cryo-EM structure of Tim23, cryo-EM structures of homologous human and yeast Tim22 (Qi et al., 2021; Zhang et al., 2021), or AlphaFold2 predictions of Tim23 (for yeast Tim23, https://alphafold.ebi.ac.uk/entry/P32897), all of which showed the same overall fold. Thus, in our view, this NMR structure does not represent a physiologically relevant form of Tim23.

It is also difficult to rule out the possibility that effects of presequence peptides observed in electrophysiological experiments are due to their interactions with the membrane or with the denatured proteins, as presequences are known to form an amphipathic helix. Some other electrophysiological experiments were performed by isolating inner mitochondrial membranes and reconstituting them into proteoliposomes using a drying and rehydrating method (Lohret et al., 1997; Martinez-Caballero et al., 2007). It is unclear whether TIM23 maintains its intact structure during this harsh treatment and whether observed currents were generated truly by TIM23. Because the proteoliposomes contain hundreds of other mitochondrial proteins and manipulation of TIM23 subunit levels can affect the mitochondrial proteome, it may be possible that observed currents were derived from other proteins. It is important to note that there have been no electrophysiological studies on TIM23 performed with a gold standard method like patch clamping of a native membrane with careful controls using pharmacological channel blockers. Without comparison with such data, it is difficult to interpret the physiological relevance of observed channel activities.

Unlike our model, a recent biochemical study has suggested that Mgr2 associates directly with the Tim23 subunit, based on an in-vitro pulldown assay (Matta et al., 2020). However, this

assay also used a Tim23 protein refolded from inclusion bodies and GST-tagged Mgr2 separately purified from *E. coli*, which were mixed in a solution containing Triton X-100. Thus the folding states of these proteins may not represent their native structures.

Limitations of our study

Although the laterally open cavity of Tim17 that can be enclosed by Mgr2 association provides a structurally plausible model for polypeptide translocation, several mechanistic aspects remain yet to be understood. Currently, it is not understood how presequences would initially engage with the Tim17 cavity and whether Mgr2 plays a role in this step. It is also unclear how exactly hydrophilic polypeptides move across the Tim17 cavity without Mgr2 and what the functions of Mgr2 are during protein translocation. Mgr2 may help minimize proton leakage, or prevent misfolding or mis-sorting of client polypeptides in transit, but further structural and functional studies will be necessary to investigate such hypotheses. Our proposed model does not require major conformational changes in Tim17 or Tim23 to explain the protein translocation function of the complex. Nevertheless, we cannot rule out the possibility that the current structure of the core TIM23 complex represents an idle state and that the membrane domain of the translocase may undergo certain conformational changes during the protein translocation cycle. Lastly, many questions remain open regarding the structure and function of other subunits, such as Tim50, Tim21, and the PAM (presequence translocase-associated motor) complex, and whether any further structural rearrangements in the complex occur with the association of these proteins.

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Supplementary Table 1. List of yeast strains

Name	Genotype/Description	Reference
BY4741	*MAT***a** *his3-1, leu2-0, met15-0, ura3-0*	Horizon Discovery
yMLT62	*MAT***a** *leu2-0*::*pACT1-GEV*::*HIS3, rps9Δ, mek1Δ, his3-1, met15-0, ura3-0*	A gift from J. Thorner
R1158	BY4741 *URA*::P_{CMV}-*tTA*	Horizon Discovery
W303-1A	*MAT***a** *leu2*-3/112, *ura3*-1, *trp*1-1, *his3*-11/15, *ade*2-1, *can*1-100	A gift from J. Thorner
ySS150	W303-1A *URA3*::P_{CMV}-*tTA*	This study
ySS078	yMLT62 *TIM17-3C-Spot*::*HphMX*	ED Fig. 1b, 3 b and c
ySS025	yMLT62 *ura3-0*::pSS011(Tim23/17-Spot/50/44/21)::*URA3 HO*::pSS015(Pam16/17/18/Mgr2)::*LEU2*	ED Fig. 1c
ySS055	yMLT62 *ura3-0*::pSS077(Tim23/17-Spot/44[–GS])::*URA3 HO*::pSS082(Pam16/18)::*LEU2*	ED Fig. 2
ySS121	BY4741 *TIM50-HA*::*NatMX TIM23-Myc*::*HphMX*	ED Fig. 6h
ySS171	W303-1A *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX*	Fig. 3c, and ED Fig. 8a, b, and d
ySS176	yMLT62 *TIM17-3C-Spot*::*HphMX ura3-0*::pSS219(*pALD6*-Tim44 (–GS)-*tENO1*)::*URA3*	ED Fig. 3a-c
ySS183	yMLT62 *TIM17-3C-Spot*::*HphMX tim21Δ::LEU2*	ED Fig. 3b and c
ySS184	yMLT62 *TIM17-3C-Spot*::*HphMX ura3-0*::pSS219(*pALD6*-Tim44 (–GS)-*tENO1*)::*URA3 tim21Δ::LEU2*	ED Fig. 3b-d
ySS199	W303-1A *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX mgr2Δ::URA3*	Fig. 3e, ED Fig. 8b-d, 9e-h
ySS204	W303-1A *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX mgr2Δ::URA3 HO::pSS254(pMGR2-Strep-Mgr2-tMGR2)::KanMX*	ED Fig. 8 b and c
ySS205	W303-1A *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX mgr2Δ::URA3 HO::pSS241(pALD6-Strep-Mgr2-tMGR2)::KanMX*	Fig. 3 c and g. ED Fig. 8b-i, 9j
ySS207	W303-1A *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX mgr2Δ::URA3 HO::pSS239(pMGR2-Mgr2-tMGR2)::KanMX*	ED Fig. 8b
yYC17a	R1158 P_{TIM17}*::KanMX-tetO7-*P_{CYC1}	Fig. 2f, ED Fig. 6a-g
yYC17b	ySS150 P_{TIM17}*::KanMX-tetO7-*P_{CYC1}	Fig. 3d, ED Fig. 9a-d
yYC23a	R1158 P_{TIM23}*::KanMX-tetO7-*P_{CYC1}	ED Fig. 6m
yYC23b	ySS150 P_{TIM23}*::KanMX-tetO7-*P_{CYC1}	Fig. 3d, ED Fig. 9a,b
yYC02	BY4741 *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX*	Fig. 2h, ED Fig. 6k
yYC03	BY4741 *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX HO*::*pTIM17*(WT)-Spot::*LEU2*	Fig. 2h, ED Fig. 6k
yYC04	BY4741 *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX HO*::*tim17*(D17N/E126Q)-*Spot*::*LEU2*	Fig. 2h, ED Fig. 6k
yYC05	BY4741 *TIM17-HA*::*NatMX TIM23-Myc*::*HphMX HO*::*tim17*(D76N/E126Q)-*Spot*::*LEU2*	Fig. 2h, ED Fig. 6k

Supplementary Table 2. List of plasmids

Name	Description	Reference
pYTK001 to pYTK096	Original MoClo YTK plasmids	Ref. 51
pYTK-e105	*HO* integration vector containing a *KanMX* marker. Assembled from pYTK094, pYTK008, pYTK047, pYTK073, pYTK077, pYTK088, and pYTK090.	This study
pYTK-e106	*HO* integration vector containing a *LEU2* marker. Assembled from pYTK094, pYTK008, pYTK047, pYTK073, pYTK075, pYTK088, and pYTK090.	Lab collection
pYTK-e112	CEN/ARS plasmid containing a *LEU2* marker. Assembled from pYTK084, pYTK008, pYTK047, pYTK073, pYTK075, and pYTK081.	Lab collection
pYTK-e113	CEN/ARS plasmid containing a *HIS3* marker. Assembled from pYTK084, pYTK008, pYTK047, pYTK073, pYTK076, and pYTK081.	This study
pYTK-e115	CEN/ARS plasmid containing a *NatMX* marker. Assembled from pYTK084, pYTK008, pYTK047, pYTK073, pYTK078, and pYTK081.	This study
pYTK-e122	2μ plasmid containing a *LEU2* marker. Assembled from pYTK084, pYTK008, pYTK047, pYTK073, pYTK075, and pYTK082.	This study
pYTK-e201	MoClo YTK part (type 4a) for 3C-2xSpot (Amino acid sequence: GSASGTLEVLFQGPTASGPDRVRA-VSHWSSGGGSGGGSTPDRVRAVSHWSS*; also see Supplementary Table 4)	This study
pYTK-e203	MoClo YTK part (type 4a) for 7xHis (Amino acid sequence: SGHHHHHHH*; also see Supplementary Table 4)	This study
pYTK001-Tim23	MoClo YTK part (type 3) for Tim23 (also see Supplementary Table 4)	This study
pYTK001-Tim17	MoClo YTK part (type 3) for Tim17 (also see Supplementary Table 4)	This study
pYTK001-Tim50	MoClo YTK part (type 3) for Tim50 (also see Supplementary Table 4)	This study
pYTK001-Tim44	MoClo YTK part (type 3) for Tim44 (also see Supplementary Table 4)	This study
pYTK001-Tim44 (–GS)	MoClo YTK part (type 3) for Tim44 (contains a stop codon after the last amino acid; thus, a GlySer linker is absent at the C-terminus) (also see Supplementary Table 4)	This study
pYTK001-Tim21	MoClo YTK part (type 3) for Tim21 (also see Supplementary Table 4)	This study
pYTK001-Pam16	MoClo YTK part (type 3) for Pam16 (also see Supplementary Table 4)	This study
pYTK001-Pam17	MoClo YTK part (type 3) for Pam17 (also see Supplementary Table 4)	This study
pYTK001-Pam18	MoClo YTK part (type 3) for Pam18 (also see Supplementary Table 4)	This study
pYTK001-Mgr2	MoClo YTK part (type 3) for Mgr2 (also see Supplementary Table 4)	This study
pYTK001-Strep-Mgr2-*tMGR2*	MoClo YTK part (type 3) for Strep-Mgr2-*tMGR2* (also see Supplementary Table 4)	This study
pYTK095-Tim23	MoClo YTK expression cassette for *pGAL1*-Tim23. Assembled from pYTK095, pYTK002, pYTK030, pYTK001-Tim23, pYTK051, and pYTK067.	This study
pYTK095-Tim23-Spot	MoClo YTK expression cassette for *pGAL1*-Tim23-Spot. Assembled from pYTK095, pYTK002, pYTK030, pYTK001-Tim23, pYTK-e201, pYTK061, and pYTK067.	This study
pYTK095-Tim17	MoClo YTK expression cassette for *pGAL1*-Tim17. Assembled from pYTK095, pYTK003, pYTK030, pYTK001-Tim17, pYTK051, and pYTK068.	This study
pYTK095-Tim17-Spot	MoClo YTK expression cassette for *pGAL1*-Tim17-Spot. Assembled from pYTK095, pYTK003, pYTK030, pYTK001-Tim17, pYTK-e201, pYTK061, and pYTK068.	This study

pYTK095-Tim50	MoClo YTK expression cassette for *pGAL1*-Tim50. Assembled from pYTK095, pYTK004, pYTK030, pYTK001-Tim50, pYTK051, and pYTK069.	This study
pYTK095-Tim44	MoClo YTK expression cassette for *pGAL1*-Tim44. Assembled from pYTK095, pYTK005, pYTK030, pYTK001-Tim44, pYTK051, and pYTK070.	This study
pYTK095-Tim44 (–GS)	MoClo YTK expression cassette for *pGAL1*-Tim44 (contains a stop codon after the last amino acid; thus, a GlySer linker is absent at the C-terminus).	This study
pYTK095-Tim21	MoClo YTK expression cassette for *pGAL1*-Tim21. Assembled from pYTK095, pYTK006, pYTK030, pYTK001-Tim21, pYTK051, and pYTK071.	This study
pYTK095-Pam16	MoClo YTK expression cassette for *pGAL1*-Pam16. Assembled from pYTK095, pYTK002, pYTK030, pYTK001-Pam16, pYTK052, and pYTK067.	This study
pYTK095-Pam17	MoClo YTK expression cassette for *pGAL1*-Pam17. Assembled from pYTK095, pYTK003, pYTK030, pYTK001-Pam17, pYTK052, and pYTK068.	This study
pYTK095-Pam18	MoClo YTK expression cassette for *pGAL1*-Pam18. Assembled from pYTK095, pYTK004, pYTK030, pYTK001-Pam18, pYTK052, and pYTK069.	This study
pYTK095-Mgr2	MoClo YTK expression cassette for *pGAL1*-Mgr2. Assembled from pYTK095, pYTK005, pYTK030, pYTK001-Mgr2, pYTK052, and pYTK070.	This study
pYTK095 L2-RE	MoClo YTK filler cassette (ConL2-Spacer-ConRE). Assembled from pYTK095, pYTK004, pYTK048, and pYTK072.	This study
pYTK095 L3-RE	MoClo YTK filler cassette (ConL3-Spacer-ConRE). Assembled from pYTK095, pYTK005, pYTK048, and pYTK072.	This study
pYTK095 L4-RE	MoClo YTK filler cassette (ConL4-Spacer-ConRE). Assembled from pYTK095, pYTK006, pYTK048, and pYTK072.	This study
pYTK095 L5-RE	MoClo YTK filler cassette (ConL5-Spacer-ConRE). Assembled from pYTK095, pYTK007, pYTK048, and pYTK072.	This study
pYTK095 LS-R1	MoClo YTK filler cassette (ConLS-Spacer-ConR1). Assembled from pYTK095, pYTK002, pYTK048, and pYTK067.	This study
pSS011	pYTK096 (Tim23, Tim17-Spot, Tim50, Tim44, Tim21). Assembled from pYTK096, pYTK095-Tim23, pYTK095-Tim17-Spot, pYTK095-Tim50, pYTK095-Tim44, pYTK095-Tim21, and pYTK095-L5-RE.	ED Fig. 1c
pSS015	pYTK-e106 (Pam16, Pam17, Pam18, Mgr2). Assembled from pYTK-e106, pYTK095-Pam16, pYTK095-Pam17, pYTK095-Pam18, pYTK095-Mgr2, and pYTK095 L4-RE.	ED Fig. 1c
pSS077	pYTK096 (Tim23, Tim17-Spot, Tim44 (–GS)). Assembled from pYTK096, pYTK095-Tim23, pYTK095-Tim17-Spot, pYTK095 L2-R3 (spacer), pYTK095-Tim44 (–GS), and pYTK095 L4-RE.	ED Fig. 2
pSS082	pYTK-e106 (Pam16, Pam18). Assembled from pYTK-e106, pYTK095-Pam16, pYTK095 L1-R2 (spacer), pYTK095-Pam18, and pYTK095 L3-RE.	ED Fig. 2
pSS122	pYTK-e112 *pTIM17*-Tim17-Spot (also see Supplementary Table 4)	ED Fig. 6h
pSS219	pYTK096 *pALD6*-Tim44 (–GS)-*tENO1*. Assembled from pYTK096, pYTK018, pYTK001-Tim44 (–GS), and pYTK051	ED Fig. 3a-d
pSS239	pYTK-e105 *pMGR2*-Mgr2-*tMGR2* (also see Supplementary Table 4)	ED Fig. 8b
pSS241	pYTK-e105 *pALD6*-Strep-Mgr2-*tMGR2*. Assembled from pYTK-e105, pYTK018, and pYTK001-Strep-Mgr2-*tMGR2*	Fig. 3g, ED Figs. 8b-d, 9j
pSS254	pYTK-e105 *pMGR2*-Strep-Mgr2-*tMGR2* (also see Supplementary Table 4)	ED Fig. 8 b and c
pSS277	pYTK-e113 *pALD6*-Strep-Mgr2-*tMGR2*	Fig. 3e, ED Fig. 9e-h
pSS070	pETDuet-1 6xHis-3C-Tim44-CTD (residues 210–431) (also see Supplementary Table 4)	This study

SNRtRNA/pBpaRS TRP	Plasmid to incorporate Bpa into an amber codon	Ref. 65
pYC17a	pYTK-e115 *pTIM17*-Tim17-HA (also see Supplementary Table 4)	Fig. 2f, ED Fig. 6a-c
pYC17b	pYTK-e122 *pDDI2*-Tim17-HA (also see Supplementary Table 4)	ED Fig. 6d-g
pYC23	pYTK-e115 *pTIM23*-Tim23-HA (also see Supplementary Table 4)	ED Fig. 6m
pYC002	pET32a Cyb2Δ-DHFR (also see Supplementary Table 4)	Fig. 2h, ED Fig. 6i-k
pYC003	pYTK-e113 *pTIM17*-Tim17-Spot	Fig. 3d, ED Fig. 9a-d
pYC008	pYTK-e123 p*TIM23*-Tim23-Spot (also see Supplementary Table 4)	Fig. 3d, ED Fig. 9a,b
pYC005	pYTK-e112 *pGAL1*-Grx5-S80-sfGFP (also see Supplementary Table 4)	Fig. 3c-e, g, ED Fig. 8d-i, and 9a-h, j
pYC006	pYTK-e112 *pGAL1*-Grx5-S99(TM)-sfGFP (also see Supplementary Table 4)	ED Fig. 8d
pYC007	pYTK-e112 *pGAL1*-Grx5-S80-sfGFP-2xTEV-ALFA (also see Supplementary Table 4)	ED Fig. 8a

Supplementary Table 3. List of primers

Name	Sequence (including notes)
EP_442	GATGCACTAAGAGGCAAACATGAC (To confirm chromosomal integration at *TIM23* locus)
EP_443	GGACGGCTCTGACAGTTTCG (To confirm chromosomal integration at *TIM23* locus)
EP_446	GTTGGAGGCATACAAGGAACAG (To confirm chromosomal integration at *TIM17* locus)
EP_447	GCACTAGCTTTTGGCTTGTTG (To confirm chromosomal integration at *TIM17* locus)
EP_450	AGAATACAGCAGGAGCAAATGG (To confirm chromosomal integration at *TIM50* locus)
EP_451	GCATCAGATCATTAGGTGTGTCTACATC (To confirm chromosomal integration at *TIM50* locus)
SS_1696	cctcttctactctctttgcctgtacatactacacgttatagcgttaacaaaagcagatagGGCGTTAGTATCGAATCG (To replace the endogenous *TIM23* promoter with the TRE promoter. Uppercase for sequence specific to TRE-kanMX and lower case for sequences homologous to yeast chromosomal sequences directly before the starting codon of Tim23)
SS_1697	ttggccgcccacggcagcattcgcatcatcggtaggtgtcttatctccaaaaagccacgacatGGATCCCCCGAATTG (To replace the endogenous *TIM23* promoter with the TRE promoter. Uppercase for sequence specific to TRE-kanMX and lower case for sequences homologous to the N-terminus of Tim23)
SS_1698	GGTTATTGCATTCGCC (To confirm chromosomal integration of the TRE cassette into the *TIM23* locus)
SS_1699	CAGGACCTGATATTATGTTATTG (To confirm chromosomal integration of the TRE cassette into the *TIM23* locus)
SS_1700	ccaaatccagaggataaaagcactattctcatcaaaagatggaaaagctgtgaaagagtcGGCGTTAGTATCGAATCG (To replace the endogenous *TIM17* promoter with the TRE promoter. Uppercase for sequence specific to TRE-kanMX and lower case for sequences homologous to yeast chromosomal sequences directly before the starting codon of Tim17)
SS_1701	agcaccaccgaaatcatttagtatgactataggacatggatctctcgaatgatcggctgacatGGATCCCCCGAATTG (To replace the endogenous *TIM17* promoter with the TRE promoter. Uppercase for sequence specific to TRE-kanMX and lower case for sequences homologous to the N-terminus of Tim17)
SS_1702	ACTGCTATTGTTCAACAAAG (To confirm chromosomal integration of the TRE cassette into the *TIM17* locus)
SS_1703	GCTCACCTAATGGCG (To confirm chromosomal integration of the TRE cassette into the *TIM17* locus)
SS_1942	CTCGGTCCTCTCGCT (To confirm chromosomal deletion of *TIM21*)
SS_2337	gcatcgtctcatcggtctCATATGCTGAAATACAAACCT (To clone Cyb2Δ-sfGFP into pYTK001)
SS_2341	atgccgtctcaggtctcaggatccGCCCTTGTATAACTCGT (To clone Cyb2Δ-sfGFP into pYTK001)
SS_2455	gaaagaattcGATTCACAATGTCTACCTGTA (To amplify a DNA segment expressing Mgr2 and the endogenous *MGR2* promoter with a 5' *Eco*RI site)
SS_2456	gaaactgcagtgcggatccATTGTCTATTATATGCTTTGGTTC (To amplify a DNA segment expressing Mgr2 and the endogenous *MGR2* promoter with a 3' *Pst*I site)
SS_2578	ataaaggatacagaaagcagtgaaaagatgttcagctctactaggtcaaacctgagggcgCTGTGGATAACCGTAGTCG (To replace the endogenous *TIM21* gene with a selection marker from MoClo YTK. Uppercase for sequence specific to selection marker and lower case for sequences homologous to *TIM21*)

SS_2579	tgtaaggtccaacacgtataacaggtgatacacatagaaagacacgtggaaataacagtcGGGCGTTTTTTATTGGTC (To replace the endogenous *TIM21* gene with a selection marker from MoClo YTK. Uppercase for sequence specific to selection marker and lower case for sequences homologous to *TIM21*)
SS_2580	GGTGTACATTATATGCGTCATGTCT (To confirm chromosomal deletion of *TIM21*)
YC_1760	gaaagaattcCTCCAGCATTATAAAGC (To amplify a DNA segment expressing Tim17 with a 5' *Eco*RI site)
YC_1761	gaaaggatccAGTTTCTGCACTAGC (To amplify the expression cassette of Tim17 with a 3' *Bam*HI site)
YC_1762	gaaagaattcATTGAAAAAAAGAGAAAATACTG (To amplify a DNA segment expressing Tim23 with a 5' *Eco*RI site)
YC_1763	gaaaggatccGCCATCGAAAACAATAG (To amplify a DNA segment expressing Tim23 with a 3' *Bam*HI site)
YC_1981	gaaagaattcCAGCCCACATACTAC (To amplify a DNA segment of the *DDI2* promoter with a 5' *Eco*RI site)
YC_1982	gaaaggtaccGATTGATTCTTTTGAAGAGAAG (To amplify a DNA segment of the *DDI2* promoter with a 3' *KpnI* site)
YC_2425	agaccaacattaccaaacagactccactacttttccataagaaggaagcacagaacaccaCTGTGGATAACCGTAGT CG (To replace the endogenous *MGR2* gene with a selection marker from MoClo YTK. Uppercase for sequence specific to selection marker and lower case for sequences homologous to *MGR2*)
YC_2426	ggaagcgtaaatatatgcaaaatttccccctcagtccttacgtataccgtatttggcacgGGGCGTTTTTTATTGGTC (To replace the endogenous *MGR2* gene with a selection marker from MoClo YTK. Uppercase for sequence specific to selection marker and lower case for sequences homologous to *MGR2*)
YC_2427	GCAGATAAGTAACAATGTTTAAG (To confirm chromosomal deletion of *MGR2*)
YC_2521	CCAAATTACTACAACTTCGTAATTCGAG (To confirm chromosomal deletion of *MGR2*)

Supplementary Table 4. DNA sequences

(Note, sequences are inserts only; the plasmid backbones are not included.)

Name	DNA sequence	Reference
pYTK-e201 (backbone: pYTK-001)	GGTCTCAatccgctagtggtaccctggaggtgttatttcagggcccgactgctagcggccctgatagagttagagcagtct cacattggtcttctggtggaggttctggcggtggttcaactccagatagagtacgtgctgtttctcactggagttcttaatggctGA GACC (Uppercase, *Bsa*I sites)	This study
pYTK-e203 (backbone: pYTK-001)	GGTCTCAatccggtcaccatcaccaccatcatcactaatggctGAGACC (Uppercase, *Bsa*I sites)	This study
TIM17-3C-Spot (PCR product to introduce a Spot-tag to chromosomal *TIM17*)	GATATGCTGCTTGGCAAGCCAAACCTATGGCTCCTCCTTTGCCCGAAGCACCTTCCT CTCAACCTCTGCAAGCTgctagtggtaccctggaggtgttatttcagggcccgactgctagcggccctgatagagt tagagcagtctcacattggtcttctggtggaggttctggcggtggttcaactccagatagagtacgtgctgtttctcactggagttc ttgataaggcgcgccacttctaaataagcgaatttcttatgatttatgatttttattattaaataagttataaaaaaaaataagtgtata caaatttaaagtgactcttaggttttaaaacgaaaattcttattcttgagtaactctttcctgtaggtcaggttgctttctcaggtata gtatgaggtcgctcttattgaccacacctctaccggcagatccgctagggataacagggtaatatagatctgtttagcttgcctc gtccccgccgggtcacccggccagcgacatggaggcccagaataccctccttgacagtcttgacgtgcgcagctcagggg catgatgtgactgtcgcccgtacatttagcccatacatccccatgtataatcatttgcatccatacattttgatggccgcacggcg cgaagcaaaaattacggctcctcgctgcggacctgcgagcagggaaacgctcccctcacagacgcgttgaattgtcccca cgccgcgcccctgtagagaaatataaaaggttaggatttgccactgaggttcttctttcatatacttccttttaaaatcttgctagg atacagttctcacatcacatccgaacataaacaacc**atg**ggtaaaaagcctgaactcaccgcgacgtctgtcgagaagtttc tgatcgaaaagttcgacagcgtctccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtagga gggcgtggatatgtcctgcgggtaaatagctgcgccgatggtttctacaaagatcgttatgtttatcggcactttgcatcggccg cgctcccgattccggaagtgcttgacattggggaattcagcgagagcctgacctattgcatctcccgccgtgcacagggtgtc acgttgcaagacctgcctgaaaccgaactgcccgctgttctgcagccggtcgcggaggccatggatgcgatcgctgcggcc gatcttagccagacgagcgggttcggcccattcggaccgcaaggaatcggtcaatacactacatggcgtgatttcatatgcg cgattgctgatccccatgtgtatcactggcaaactgtgatggacgacaccgtcagtgcgtccgtcgcgcaggctctcgatgag ctgatgctttgggccgaggactgccccgaagtccggcacctcgtgcacgcggatttcggctccaacaatgtcctgacggaca atggccgcataacagcggtcattgactggagcgaggcgatgttcggggattcccaatacgaggtcgccaacatcttcttctgg aggccgtggttggcttgtatggagcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgcggctc cgggcgtatatgctccgcattggtcttgaccaactctatcagagcttggttgacggcaatttcgatgatgcagcttgggcgcag ggtcgatgcgacgcaatcgtccgatccggagccgggactgtcgggcgtacacaaatcgcccgcagaagcgcggccgtct ggaccgatggctgtgtagaagtactcgccgatagtggaaaccgacgccccagcactcgtccgagggcaaaggaa**taa**tc agtactgacaataaaaagattcttgttttcaagaacttgtcatttgtatagttttttatattgtagttgttctattttaatcaaatgttagc gtgatttatatttttttttcgcctcgacatcatctgcccagatgcgaagttaagtgcgcagaaagtaatatcatgcgtcaatcgtatgt gaatgctggtcgctatactgctgtcgattcgatactaacgccgccatccagtgtcgaaaacgagctccaattcatcgatgatat cagatccactagtggcctatgcggGACGTATCGCACTAGCCTCCTTTACGTTTTTACTTTATTTC AGCCTTTTATTTCAAGATTACCAACCATTTCTC (Uppercase, homology arms; bold/underlined, start and stop codons of hygromycin B phosphotransferase)	This study
TIM17-HA (PCR product to introduce an HA-tag to chromosomal *TIM17*)	GATATGCTGCTTGGCAAGCCAAACCTATGGCTCCTCCTTTGCCCGAAGCACCTTCCT CTCAACCTCTGCAAGCTgctggaggggctaccacggctagtggtaccggcgaaaatttatactttcaaggtactg ctagcgggggcggttcttatccctatgacgtcccggactatgcaggatcctatccatatgacgttccagattacgcttaaggcg cgccacttctaaataagcgaatttcttatgatttatgatttttattattaaataagttataaaaaaaaataagtgtatacaaatttaaa gtgactcttaggttttaaaacgaaaattcttattcttgagtaactctttcctgtaggtcaggttgctttctcaggtatagtatgaggtcg ctcttattgaccacacctctaccggcagatccgctagggataacagggtaatatagatctgtttagcttgcctcgtccccgccgg gtcacccggccagcgacatggaggcccagaataccctccttgacagtcttgacgtgcgcagctcaggggcatgatgtgact gtcgcccgtacatttagcccatacatccccatgtataatcatttgcatccatacattttgatggccgcacggcgcgaagcaaaa attacggctcctcgctgcagacctgcgagcagggaaacgctcccctcacagacgcgttgaattgtccccacgccgcgcccc tgtagagaaatataaaaggttaggatttgccactgaggttcttctttcatatacttccttttaaaatcttgctaggatacagttctcac atcacatccgaacataaacaacc**atg**gtaccactcttgacgacacggcttaccggtaccgcaccagtgtcccgggggac gccgaggccatcgaggcactggatgggtccttcaccaccgacaccgtcttccgcgtcaccgccaccggggacggcttcac cctgcgggaggtgccggtggacccgcccctgaccaaggtgttccccgacgacgaatcggacgacgaatcggacgacgg ggaggacggcgacccggactcccggacgttcgtcgcgtacggggacgacggcgacctggcgggcttcgtggtcgtctcgt actccggctggaaccgccggctgaccgtcgaggacatcgaggtcgccccggagcaccgggggcacggggtcgggcgc gcgttgatggggctcgcgacggagttcgcccgcgagcggggcgccgggcacctctggctggaggtcaccaacgtcaacg caccggcgatccacgcgtaccggcggatggggttcaccctctgcggcctggacaccgccctgtacgacggcaccgcctcg gacggcgagcaggcgctctacatgagcatgccctgcccc**taa**tcagtactgacaataaaaagattcttgttttcaagaacttg tcatttgtatagttttttatattgtagttgttctattttaatcaaatgttagcgtgatttatatttttttcgcctcgacatcatctgcccagat	This study

	gcgaagttaagtgcgcagaaagtaatatcatgcgtcaatcgtatgtgaatgctggtcgctatactgctgtcgattcgatactaac gccgccatccagtgtcgaaaacgagctcgaattcatcgatgatatcagatccactagtggcctatgcggccGACGTATC GCACTAGCCTCCTTTACGTTTTTACTTTATTTCAGCCTTTTATTTCAAGATTACCAACC ATTTCTC (Uppercase, homology arms; bold/underlined, start and stop codons of nourseothricin N-acetyl transferase)	
TIM23-myc (PCR product to introduce a myc-tag to chromosomal *TIM23*)	GGTTTGAAACCCATGGGTTATTCCTCGGCAATGGTGGCCGCTGCGTGCGCCGTCTG GTGTAGTGTCAAGAAAAGACTACTTGAAAAAgctagtggtaccctggaggtgttatttcagggcccgact gctagcggcgaacaaaagttgatttctgaagaagatttgaacggtgaacaaaagctaatctccgaggaagacttgtgataa ggcgcgccacttctaaataagcgaatttcttatgatttatgatttttattattaaataagttataaaaaaaataagtgtatacaaattt taaagtgactcttaggttttaaaacgaaaattcttattcttgagtaactctttcctgtaggtcaggttgctttctcaggtatagtatgag gtcgctcttattgaccacacctctaccggcagatccgctagggataacagggtaatatagatctgtttagcttgcctcgtccccg ccgggtcacccggccagcgacatggaggcccagaataccctccttgacagtcttgacgtgcgcagctcaggggcatgatgt gactgtcgcccgtacatttagcccatacatccccatgtataatcatttgcatccatacattttgatggccgcacggcgcgaagca aaaattacggctcctcgctgcggacctgcgagcagggaaacgctcccctcacagacgcgttgaattgtccccacgccgcg cccctgtagagaaatataaaaggttaggatttgccactgaggttcttctttcatatacttccttttaaaatcttgctaggatacagttc tcacatcacatccgaacataaacaacc**atg**ggtaaaaagcctgaactcaccgcgacgtctgtcgagaagtttctgatcgaa aagttcgacagcgtctccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggagggcgtg gatatgtcctgcgggtaaatagctgcgccgatggtttctacaaagatcgttatgtttatcggcactttgcatcggccgcgctcccg attccggaagtgcttgacattggggaattcagcgagagcctgacctattgcatctcccgccgtgcacagggtgtcacgttgca agacctgcctgaaaccgaactgcccgctgttctgcagccggtcgcggaggccatggatgcgatcgctgcggccgatcttag ccagacgagcgggttcggcccattcggaccgcaaggaatcggtcaatacactacatggcgtgatttcatatgcgcgattgct gatccccatgtgtatcactggcaaactgtgatggacgacaccgtcagtgcgtccgtcgcgaggctctcgatgagctgatgct ttgggccgaggactgccccgaagtccggcacctcgtgcacgcggatttcggctccaacaatgtcctgacggacaatggccg cataacagcggtcattgactggagcgaggcgatgttcggggattcccaatacgaggtcgccaacatcttcttctggaggccgt ggttggcttgtatggagcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgcggctccgggcgt atatgctccgcattggtcttgaccaactctatcagagcttggttgacggcaatttcgatgatgcagcttgggcgcagggtcgatg cgacgcaatcgtccgatccggagccgggactgtcgggcgtacacaaatcgcccgcagaagcgcggccgtctggaccgat ggctgtgtagaagtactcgccgatagtggaaaccgacgccccagcactcgtccgagggcaaaggaa**taa**tcagtactga caataaaaagattcttgttttcaagaacttgtcatttgtatagttttttatattgtagttgttctattttaatcaaatgttagcgtgatttata ttttttttcgcctcgacatcatctgcccagatgcgaagttaagtgcgcagaaagtaatatcatgcgtcaatcgtatgtgaatgctg gtcgctatactgctgtcgattcgatactaacgccgccatccagtgtcgaaaacgagctccaattcatcgatgatatcagatcca ctagtggcctatgcggACTGATGGCGCTTGTATATAGCATTTGAAAAATAATAGTACGTAACG CAGAAAACAAACCAATGAA (Uppercase, homology arms; bold/underlined, start and stop codons of hygromycin B phosphotransferase)	This study
TIM50-HA (PCR product to introduce an HA-tag to chromosomal *TIM50*)	TATTTGAAGAGGAAAAGAAAAAGAAGAAGATTGCTGAATCCAAAgctggaggggctaccacg gctagtggtaccggcgaaaatttatactttcaaggtactgctagcgggggcggttcttatccctatgacgtcccggactatgca ggatcctatccatatgacgttccagattacgcttaaggcgcgccacttctaaataagcgaatttcttatgatttatgatttttattatta ataaagttataaaaaaaaataagtgtatacaaattttaaagtgactcttaggttttaaaacgaaaattcttattcttgagtaactctttt cctgtaggtcaggttgctttctcaggtatagtatgaggtcgctcttattgaccacacctctaccggcagatccgctagggataac agggtaatatagatctgtttagcttgcctcgtccccgccgggtcacccggccagcgacatggaggcccagaataccctccttg acagtcttgacgtgcgcagctcaggggcatgatgtgactgtcgcccgtacatttagcccatacatccccatgtataatcatttgc atccatacattttgatggccgcacggcgcgaagcaaaaattacggctcctcgctgcagacctgcgagcagggaaacgctc ccctcacagacgcgttgaattgtccccacgccgcgcccctgtagagaaatataaaaggttaggatttgccactgaggttcttct ttcatatacttccttttaaaatcttgctaggatacagttctcacatcacatccgaacataaacaacc**atg**ggtaccactcttgacg acacggcttaccggtaccgcaccagtgtcccgggggacgccgaggccatcgaggcactggatgggtccttcaccaccga caccgtcttccgcgtcaccgccaccggggacggcttcaccctgcgggaggtgccggtggacccgcccctgaccaaggtgtt ccccgacgacgaatcggacgacgaatcggacgacggggaggacggcgacccggactcccggacgttcgtcgcgtacg gggacgacggcgacctggcgggcttcgtggtcgtctcgtactccggctggaaccgccggctgaccgtcgaggacatcgag gtcgccccggagcaccgggggcacggggtcgggcgcgcgttgatggggctcgcgacggagttcgcccgcgagcgggg cgccgggcacctctggctggaggtcaccaacgtcaacgcaccggcgatccacgcgtaccggcggatggggttcaccctct gcggcctggacaccgccctgtacgacggcaccgcctcggacggcgagcaggcgctctacatgagcatgccctgcccc**ta** **a**tcagtactgacaataaaaagattcttgttttcaagaacttgtcatttgtatagttttttatattgtagttgttctattttaatcaaatgtta gcgtgatttatatttttttttcgcctcgacatcatctgcccagatgcgaagttaagtgcgcagaaagtaatatcatgcgtcaatcgta tgtgaatgctggtcgctatactgctgtcgattcgatactaacgccgccatccagtgtcgaaaacgagctcgaattcatcgatgat atcagatccactagtggcctatgcggccCACACCCTCATTTTGTTACTGTCATGTGAATAAAACTTA TGTAT (Uppercase, homology arms; bold/underlined, start and stop codons of nourseothricin N-acetyl transferase)	This study

pYTK001-Tim23	GGTCTCatatgtcgtggctttttggagataagacacctaccgatgatgcgaatgctgccgtgggcggccaagatacaacc aagcctaaggaactatcgttgaagcagagtttaggtttcgagccaaacatcaataacataatatcaggtcctggtggaatgc atgtcgacaccgctaggctgcatccttggctggtctagacaagggtgtggagtatttagatctggaagaagaacaactatcct cgttagaaggctcacagggtctgatcccttcccgtgggtggaccgatgacctatgttacggtaccggtgccgtctacctgctgg gacttggtatcggagggttttctggtatgatgcagggtctacagaatattccgcccaatagtcccggaaaattgcaattgaaca ccgtcctgaatcacattactaagagaggtcccttcttaggtaataatgcggggattctcgcgttgagctacaatatcatcaattct acaatagatgcactaagaggcaaacatgacaccgcgggctccattggcgctggggccctcacgggcgctttgttcaagtctt caaaaggtttgaaacccatgggttattcctcggcaatggtggccgctgcgtgcgccgtctggtgtagtgtcaagaaaagacta cttgaaaaaggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Tim17	GGTCTCatatgtcagccgatcattcgagagatccatgtcctatagtcatactaaatgatttcggtggtgcttttgccatgggtg ccattggtggtgttgtttggcatgggattaaaggttttagaaattcgccattaggtgagcgtggttcaggagctatgagcgccatt aaagcgcgtgctcccgtactgggtggtaattttggtgtgtggggtggtttatttttcgacttttgattgcgctgtgaaggccgttagaa agagagaggacccatggaatgctatcattgcagggtttttcactggtggcgctttagctgtaagaggtggttggaggcataca aggaacagttcgatcacgtgtgcttgtttgttgggtgtgattgaaggtgtgggactaatgtttcaaagatatgctgcttggcaagc caaacctatggctcctcctttgcccgaagcaccttcctctcaacctctgcaagctggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Tim50	GGTCTCatatgctgtccattttaagaaattcggtgagactaaattcaagggctcttagggttgtgccatctgccgccaacac cttaacatcggttcaagcatccagaagactttaacaagttattcaagtttttacaaaaagaaacaaaagacgacaagccta aatccattcttacagatgatatgctgttcaaggccggtgttgacgtcgatgagaagggccaaggcaaaaacgaggaaacat caggagaaggaggagaggacaagaatgaaccttcttccaaaagtgaaaaatctagaagaaaaagacaaacttctacag atattaaaagagaaaagtatgctaactggttttacatttttcgttgtctgcgttgacaggtactgcaatctacatggcaagggatt gggagcctcaagagtctgaagaattgaagaaagacatcgataatggctacactttatcacttatgtataaaagattcaaggc caggttcaactcaatgttcacctacttccaagagccacctttccctgatttactacctccaccaccaccaccgtaccaaag gccattaactcttgttatcacattggaagatttttggttcattctgagtggtcacaaaagcatggttggagaacggccaaaaga cctggtgctgactacttcttgggttacctatcgcagtattacgaaattgttttgttttcatccaactatatgatgtactctgacaaaatc gctgaaaaattagatccaatccatgcattcgtatcttataatttgttcaaggaacactgtgtttacaaagacggtgtgcacattaa ggatctgtcaaaattgaatagagatttgagtaaagtaatcattattgacactgaccctaacagttacaaattgcaacctgaaaa tgctattccaatggagccatggaatggtgaagctgatgacaaattagtaagattgattccatttttggagtaccttgccactcaac aaaccaaggatgttagaccaatcttgaacagctttgaagacaagaagaacctagcagaagaatttgatcatcgtgtgaaaa aattgaaggataaattttacggagatcataaatctggtggcaactgggcaatgacggcactaggtctaggaaattccctggg cggcagcaccaagttcccgctcgatttgattcatgaagaaggacaaaagaactatttaatgttcatgaagatgattgaggaa gaaaaggaaaaaattagaatacagcaggagcaaatgggcgggcaaacatttacgctgaaagactatgttgaaggtaact tgccttcgccagaagaacaaatgaaaatacaattggagaagcagaaggaggtagacgccttatttgaagaggaaaagaa aaagaagaagattgctgaatccaaaggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Tim44	GGTCTCatatgcacagatccacttttatcaggacgtccggcacgagctctaggacactaaccgcaaggtacagatcgc agtacacaggattactcgttgcccgagtattattctccacctctacgacccgtgcgcaaggtggaaaccctcgatcaccactc cagattttccgcgatacattcaagaaggaatgggagaagtctcaggaactacaggagaacataaagacgctgcaagatg cttcgggaaagttaggcgagtctgaggcctacaaaaaggctagggaagcatatttgaaagctcagagaggctccacaatt gtgggtaaaactctgaaaaagaccggtgaaaccatggaacatatagccactaaggcctgggagtccgaactcggtaaga acacaagaaaggcggctgccgccacggcgaagaagctggatgagagttttgagccagtgagacagacgaagatttaca aggaagtctcagaagtcattgacgatggggagagttcccgatacggtgggtttatcacgaaagagcaaaggagacttaaa cgtgagagagatctggcctctgggaaaagacacagggcagtaaagagcaatgaagatgcaggaacagcagtggttgcg acaaatatcgagtctaaagaatcgtttggtaagaaagtggaggatttcaaggagaaaaccgtggttggccgttctatacaatc tttaaagaacaaattgtgggatgaaagtgaaaaccccttaattgttgtcatgaggaaaataaccaacaaagtgggcggtttctt tgcagaaacagaatcctcccgtgtttacagtcaatttaagctaatggacccaacctttcgaacgaaagcttcaccagacactt aagagaatacattgttcccgagattctcgaagcgtatgtgaaaggcgatgtcaaagttctcaaaaaatggttcagcgaggcg ccattcaatgtttacgccgcccaacagaaaatcttcaaagaacaggatgtttacgccgatggccgtatcctagatatcagggg cgttgaaatagtgagtgcaaagttattggctccccaagacatcccagtcttggtggtcgggtgtagagcacaagaaatcaac ctttacaggaaaaagaaaactggcgagattgcggctggtgacgaagctaatatcttgatgagctcttatgccatggttttcacc agagatccagagcaaatcgacgatgacgaaacagaagggtggaagatcttggagtttgtgcgcgggggttctagacaatt caccggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Tim44 (–GS)	GGTCTCatatgcacagatccacttttatcaggacgtccggcacgagctctaggacactaaccgcaaggtacagatcgc agtacacaggattactcgttgcccgagtattattctccacctctacgacccgtgcgcaaggtggaaaccctcgatcaccactc cagattttccgcgatacattcaagaaggaatgggagaagtctcaggaactacaggagaacataaagacgctgcaagatg cttcgggaaagttaggcgagtctgaggcctacaaaaaggctagggaagcatatttgaaagctcagagaggctccacaatt gtgggtaaaactctgaaaaagaccggtgaaaccatggaacatatagccactaaggcctgggagtccgaactcggtaaga acacaagaaaggcggctgccgccacggcgaagaagctggatgagagttttgagccagtgagacagacgaagatttaca	This study

	aggaagtctcagaagtcattgacgatggggagagttcccgatacggtgggtttatcacgaaagagcaaaggagacttaaa cgtgagagagatctggcctctgggaaaagacacagggcagtaaagagcaatgaagatgcaggaacagcagtggttgcg acaaatatcgagtctaaagaatcgtttggtaagaaagtggaggatttcaaggagaaaaccgtggttggccgttctatacaatc tttaaagaacaaattgtgggatgaaagtgaaaaccccttaattgttgtcatgaggaaaataaccaacaaagtgggcggtttctt tgcagaaacagaatcctcccgtgtttacagtcaatttaagctaatggacccaacctttcgaacgaaagcttcaccagacactt aagagaatacattgttcccgagattctcgaagcgtatgtgaaaggcgatgtcaaagttctcaaaaaatggttcagcgaggcg ccattcaatgtttacgccgcccaacagaaaatcttcaaagaacaggatgtttacgccgatggccgtatcctagatatcagggg cgttgaaatagtgagtgcaaagttattggctccccaagacatcccagtcttggtggtcgggtgtagagcacaagaaatcaac ctttacaggaaaaagaaaactggcgagattgcggctggtgacgaagctaatatcttgatgagctcttatgccatggttttcacc agagatccagagcaaatcgacgatgacgaaacagaagggtggaagatcttggagtttgtgcgcgggggttctagacaatt cacctaaggatcctGAGACC	
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pYTK001-Pam16	GGTCTCatatggctcacagggctttcatacaggttataatcacaggaactcaagtttttggaaaagcattcgccgaggcgt atagacaagcggcttcacaatcagtgaaacaaggtgctacaaatgcatcaagaaggggaacaggaaaaggcgaatatg gtggtattacgttggatgagagttgtaaaatttaaatattgaagaatccaagggcgatttaaacatggacaagattaataaca ggtttaactatctatttgaggttaacgataaagaaaaaggtggaagcttctacttacagagcaaagtttatcgagcagcagaa aggttaaaatgggaactggctcagagagaaaaaaatgcgaaggcgaaagcaggggacgcttcaacagcgaaacctcc tccgaattcaacaaattcatctggtgcagataatagtgcaagcagcaatcagggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Pam17	GGTCTCatatgtttaccagtgccattagattgtcatcgcaaagactgttcgctagtcaaccttctgtcaccgctgcggcattg cgctcgactgctacaaccttacccttaagatcatattctcagcccgcatcccttcaagactccagtatcttgacatggtctgatttttt tcaaattgaggaaacagcagcgtagaatcaatgttggttcttcgctgtttactgctcttttgggctgtaacgtttcatgggcttacctt tccacaatggaaatagacccgactcaaatgctattcggattcgacccattaactgtaatttcagctgggataatagcctctggt gcactaggctacttgttgggtccgatagttggttcgcaagttttcaaactttcccataaccaacaattggcacagttcaacaaca aaaataaagagtttctaaaacatatcatcaataacagggtcgatgcctcttctcaaagtttcagtaatcctgttccagattattac ggtgaaaagataggttccttaaaggaatataagcaatggttaagagattgtcacgcttacgcaaagaaagccaaagaatttt tgggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Pam18	GGTCTCatatgagttctcaaagtaatactggtaattctattgaggcaccacaactacccattcctggtcaaactaatggctct gcgaacgttactgttgatggagctggtgttaatgtcggtatccagaatggttcgcagggtcaaaagaccggaatggacctttat tttgatcaagctttgaactacatgggagaacatcctgtgataacaggttttggggccttttaactttatattttacagccggtgcat ataaatcaatatcgaagggacttaacggtggaaaatccactactgccttcttgaaaggcggatttgacccgaaaatgaattct aaagaggctctacagattttgaatttgacagaaaatacattgactaaaaaaaagttgaaagaggttcataggaaaattatgtt agctaatcatcctgacaaaggtggttctccatttttggccactaagataaacgaagctaaggacttttggaaaaaaggggtat tagcaaaggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Mgr2	GGTCTCatatgcctcctcttccacaaaattatgcgcaacagcagccttcgaattgggacaaattcaaaatggggttgatga tgggtactaccgtcggtgtctgcacaggcatcctatttggtggatttgccatcgcaactcaaggcccaggtcctgatggtgtagtt agaacactagggaaatacattgctggttcagcgggtacctttgggctatttatgtccatcgggtctataatcagaagtgatagtg aaagtagtccaatgtcccatcctaacctgaacctacagcaacaggcaagactggaaatgtggaagcttcgtgccaaatacg gtatacgtaaggacggatcctGAGACC (Uppercase, *Bsa*I sites)	This study
pYTK001-Strep-Mgr2-*tMGR2*	GGTCTCatatgtggtcccaccctcagttcgaaaagcctcctcttccacaaaattatgcgcaacagcagccttcgaattggg acaaattcaaaatggggttgatgatgggtactaccgtcggtgtctgcacaggcatcctatttggtggatttgccatcgcaactca aggcccaggtcctgatggtgtagttagaacactagggaaatacattgctggttcagcgggtacctttgggctatttatgtccatc gggtctataatcagaagtgatagtgaaagtagtccaatgtcccatcctaacctgaacctacagcaacaggcaagactggaa atgtggaagcttcgtgccaaatacggtatacgtaaggactgaggggaaattttgcatatatttacgcttccccatcgttttcttaa acattgttacttatctgcttatttttatttaagatatctatctacctgtttataactctctcaataccatgtatttaagttcatcacgacgag gcttatttacttaatatatttgtatacgtgggaaatgtctaatatgtataccggtttgcatcttatttgggtggggtcactaacggcaa gactcataaaataaagtattcgctaagcactctgttatatttgtaaactttgagaaaggaaatgggaaccaaagcatataatag acaatgctgtGAGACC	

	(Uppercase, *Bsa*I sites)	
pSS122 (P_{TIM17}-Tim17-Spot)	GAATTCctccagcattataaagcatatctaacaataccattcgggttatactgaatagcccacgcacgggttcggggttgt aacgattatatgcatttcataacatgccactgtcagtggcgccacggaaggatagcgggactttatcaaatcattaatttctgtg cataaagtgtatagtatattcatatagatatgtatatatatcattgaatacagaaatcttcgggtgtatacaacgaacattcatcgg acgccttttttactgctattgttcaacaaagtgggtaacccggcattcttgcgccaaagtttccatttttatgccaaatccagaggat aaaagcactattctcatcaaaagatggaaaagctgtgaaagagtcaagttcagaagaaagcgttaagaaaaatagagta cacgggagcgtt**atg**tcagccgatcattcgagagatccatgtcctatagtcatactaaatgatttcggtggtgcttttgccatggg tgccattggtggtgttgtttggcatgggattaaaggttttagaaattcgccattaggtgagcgtggttcaggagctatgagcgcca ttaaagcgcgtgctcccgtactgggtggtaattttggtgtgtggggtggtttattttcgacttttgattgcgctgtgaaggccgttaga aagagagaggacccatggaatgctatcattgcagggtttttcactggtggcgctttagctgtaagaggtggttggaggcatac aaggaacagttcgatcacgtgtgcttgtttgttgggtgtgattgaaggtgtgggactaatgtttcaaagatatgctgcttggcaag ccaaacctatggctcctcctttgcccgaagcaccttcctctcaacctctgcaagctactagtcctgatagagttagagcagtctc acattggtcttctggtggaggttctggcggtggttcaactccagatagagtacgtgctgtttctcactggagttct**tag**gcatgtag acattatatgagccatttttttcatcgttgttggaagtacccttattcggcatgtttttgttacataaatgacgtatcgcactagcctcct ttacgtttttactttatttcagccttttatttcaagattaccaaccatttctctcaaccatgtacatattatatattgaaaaagtaccatac ttcatctctgaagagaaaatatcaacaagccaaaagctagtgcagaaactGGATCCgcaCTGCAG (Uppercase, *Eco*RI, *Bam*HI, *Pst*I sites; bold/underlined, start and stop codons)	This study
pSS239 (P_{MGR2}-Mgr2-T_{MGR2})	GAATTCgattcacaatgtctacctgtagattgctgtgttcaatcggaaactggaaattctttcttaacattatgagccaaccac ttttattttctttttcaaaactcccgaacaagaccaacattaccaaacagactccactacttttccataagaaggaagcacagaa caccacacaagatcatatagcacaaatagcgcaacagcttcaagtccaatattaagatata**atg**cctcctcttccacaaaatt atgcgcaacagcagccttcgaattgggacaaattcaaaatggggttgatgatgggtactaccgtcggtgtctgcacaggcat cctatttggtggatttgccatcgcaactcaaggcccaggtcctgatggtgtagttagaacactagggaaatacattgctggttca gcgggtacctttgggctatttatgtccatcgggtctataatcagaagtgatagtgaaagtagtccaatgtcccatcctaacctga acctacagcaacaggcaagactggaaatgtggaagcttcgtgccaaatacggtatacgtaaggac**tga**gggggaaatttt gcatatatttacgcttccccatcgttttcttaaacattgttacttatctgcttatttttatttaagatatctatctacctgtttataactctctca ataccatgtatttaagttcatcacgacgaggcttatttacttaatatatttgtatacgtgggaaatgtctaatatgtataccggtttgc atcttattttgggtggggtcactaacggcaagactcataaaataaagtattcgctaagcactctgttatatttgtaaactttgagaa aggaaatgggaaccaaagcatataatagacaatGGATCCgcaCTGCAG (Uppercase, *Eco*RI, *Bam*HI, *Pst*I sites; bold/underlined, start and stop codons)	This study
pSS254 (P_{MGR2}-Strep-Mgr2- T_{MGR2})	GAATTCgattcacaatgtctacctgtagattgctgtgttcaatcggaaactggaaattctttcttaacattatgagccaaccac ttttattttctttttcaaaactcccgaacaagaccaacattaccaaacagactccactacttttccataagaaggaagcacagaa caccacacaagatcatatagcacaaatagcgcaacagcttcaagtccaatattaagatata**atg**tggtcccaccctcagttc gaaaagcctcctcttccacaaaattatgcgcaacagcagccttcgaattgggacaaattcaaaatggggttgatgatgggta ctaccgtcggtgtctgcacaggcatcctatttggtggatttgccatcgcaactcaaggcccaggtcctgatggtgtagttagaac actagggaaatacattgctggttcagcgggtacctttgggctatttatgtccatcgggtctataatcagaagtgatagtgaaagt agtccaatgtcccatcctaacctgaacctacagcaacaggcaagactggaaatgtggaagcttcgtgccaaatacggtata cgtaaggac**tga**gggggaaattttgcatatatttacgcttccccatcgttttcttaaacattgttacttatctgcttatttttatttaagat atctatctacctgtttataactctctcaataccatgtatttaagttcatcacgacgaggcttatttacttaatatatttgtatacgtgggа aatgtctaatatgtataccggtttgcatcttattttgggtggggtcactaacggcaagactcataaaataaagtattcgctaagca ctctgttatatttgtaaacttgagaaaggaaatgggaaccaaagcatataatagacaatGGATCCgcaCTGCAG (Uppercase, *Eco*RI, *Bam*HI, *Pst*I sites; bold/underlined, start and stop codons)	This study
pSS070 (pETDuet-1 6xHis-3C-Tim44CTD)	GGATCCgctggaagtgctgtttcagggcccgacaaatatcgagtctaaagaatcgtttggtaagaaagtggaggatttca aggagaaaaccgtggttggccgttctatacaatctttaaagaacaaattgtgggatgaaagtgaaaacccttaattgttgtca tgaggaaaataaccaacaaagtgggcggtttctttgcagaaacagaatcctcccgtgtttacagtcaatttaagctaatggac ccaacctttttcgaacgaaagcttcaccagacacttaagagaatacattgttcccgagattctcgaagcgtatgtgaaaggcg atgtcaaagttctcaaaaaatggttcagcgaggcgccattcaatgtttacgccgcccaacagaaaatcttcaaagaacagg atgtttacgccgatggccgtatcctagatatcaggggcgttgaaatagtgagtgcaaagttattggctccccaagacatccca gtcttggtggtcgggtgtagagcacaagaaatcaacctttacaggaaaaagaaaactggcgagattgcggctggtgacga agctaatatcttgatgagctcttatgccatggttttcaccagagatccagagcaaatcgacgatgacgaaacagaagggtgg aagatcttggagtttgtgcgcgggggttctagacaattcacc**taa**CTCGAG (Uppercase, *Bam*HI and *Xho*I sites; bold/underlined, stop codon)	This study
pYC17a (pYTK-e115 *TIM17*-HA)	GAATTCctccagcattataaagcatatctaacaataccattcgggttatactgaatagcccacgcacgggttcggggttgt aacgattatatgcatttcataacatgccactgtcagtggcgccacggaaggatagcgggactttatcaaatcattaatttctgtg cataaagtgtatagtatattcatatagatatgtatatatatcattgaatacagaaatcttcgggtgtatacaacgaacattcatcgg acgccttttttactgctattgttcaacaaagtgggtaacccggcattcttgcgccaaagtttccatttttatgccaaatccagaggat aaaagcactattctcatcaaaagatggaaaagctgtgaaagagtcaagttcagaagaaagcgttaagaaaaatagagta cacgggagcgtt**atg**tcagccgatcattcgagagatccatgtcctatagtcatactaaatgatttcggtggtgcttttgccatggg tgccattggtggtgttgtttggcatgggattaaaggttttagaaattcgccattaggtgagcgtggttcaggagctatgagcgcca ttaaagcgcgtgctcccgtactgggtggtaattttggtgtgtggggtggtttattttcgacttttgattgcgctgtgaaggccgttaga aagagagaggacccatggaatgctatcattgcagggtttttcactggtggcgctttagctgtaagaggtggttggaggcatac	Fig. 2f, ED Fig. 6a-c

	aaggaacagttcgatcacgtgtgcttgtttgttgggtgtgattgaaggtgtgggactaatgtttcaaagatatgctgcttggcaag ccaaacctatggctcctcctttgcccgaagcaccttcctctcaacctctgcaagctactagttatccctatgacgtcccggactat gcaggatcctatccatatgacgttccagattacgct**tag**gcatgtagacattatatgagccatttttcatcgttgttggaagtacc cttattcggcatgttttttgttacataaatgacgtatcgcactagcctcctttacgtttttacttttatttcagccttttatttcaagattacca accatttctctcaaccatgtacatattatatattgaaaaagtaccatacttcatctctgaagagaaaatatcaacaagccaaaa gctagtgcagaaactGGATCCgcaCTGCAG (Uppercase, *Eco*RI, *Bam*HI and *Pst*I sites; bold/underlined, start and stop codons of Tim17-HA)	
pYC17b (pYTK-e122 P_{DDI2}-TIM17-HA)	GAATTCcagcccacatactacttttttccttttgttttttttttttatatttcaaaggttaaactcgcttagactatgtctataatataaa aaaaaaatagctctattccgtttctttttattctatttgatatttccatcacacttttcatcttaatcacggatgtatactgataatagggttg actgcgcctgtacggattacagtgccctcttcaattggaaaatccaagctttcaagatgggtaactgttattcaaaggatcctct aagataaaacacagatcgacagatccgagagttggcttctgtgccttgggctcaaattcctttcccacctcatgcaaattgatttt tctgactccaaaaaaagacagagccctgcgatagttcccgaatgttgtaacatcaaagccaagcactcctttatagaagtcg catgaacgttgaatactagcagctggtgaaactacagggtctaaactaactagtatccatatctttttgagagcattgaaagtat acggagtacaagctgggttagaaggaatttttatcttaacagcaatgaaaatcaactttctagactgaatccctcaagaaaatt gcaaaagactaactgatactggtttaaaagagaaagatgtcaaatatgcggagttataccatcaaacaactttggacggcc ccgaaacaaatgtccgcaaaaaagatcttattaaagtgcatggacactatcatttctataatacaaaatactccaccgcaca atagtttgtcgggaagtcatcaatcaatcttgtacgagctttacaaataacttttttaggatcggtcccctcataaaattatatataa atgggttagtttccttcttcttctgttaacatgaagttgcttcgtactgtttttttgccttgcttctcttcaaaagaatcaatcggtacc**atg**t cagccgatcattcgagagatccatgtcctatagtcatactaaatgatttcggtggtgcttttgccatgggtgccattggtggtgttgt ttggcatgggattaaaggttttagaaattcgccattaggtgagcgtggttcaggagctatgagcgccattaaagcgcgtgctcc cgtactgggtggtaattttggtgtgtggggtggtttattttcgacttttgattgcgctgtgaaggccgttagaaagagaggaggccc atggaatgctatcattgcagggtttttcactggtggcgctttagctgtaagaggtggttggaggcatacaaggaacagttcgatc acgtgtgcttgtttgttgggtgtgattgaaggtgtgggactaatgtttcaaagatatgctgcttggcaagccaaacctatggctcct ccttgcccgaagcaccttcctctcaacctctgcaagctactagttatccctatgacgtcccggactatgcaggatcctatccata tgacgttccagattacgct**tag**gcatgtagacattatatgagccatttttcatcgttgttggaagtacccttattcggcatgttttttgt tacataaatgacgtatcgcactagcctcctttacgtttttacttttatttcagccttttatttcaagattaccaaccatttctctcaaccat gtacatattatatattgaaaaagtaccatacttcatctctgaagagaaaatatcaacaagccaaaagctagtgcagaaactG GATCCgcaCTGCAG (Uppercase, *Eco*RI, *Bam*HI and *Pst*I sites; bold/underlined: start and stop codons of Tim17-HA)	ED Fig. 6d-g
pYC23 (pYTK-e115 *TIM23*-HA)	GAATTCattgaaaaaaagagaaaatactgaaaaaaaaaagacaccgacaaaaaaaagagagaaaggaaccttta cgtagagcaaaagggaaacatttgttgcgtttaattaatagaaataataggttattgcattcgccctccattgcagaaaaaaaa aaaaaaaagaccattttcctcttctactctctttgcctgtacatactacacgttatagcgttaacaaaagcagatagaaaaaaa aaaaataaccaagataatataggtatacttgtttaacagatcacacacaatc**atg**tcgtggctttttggagataagacacctac cgatgatgcgaatgctgccgtgggcggccaagatacaaccaagcctaaggaactatcgttgaagcagagtttaggtttcga gccaaacatcaataacataatatcaggtcctggtggaatgcatgtcgacaccgctaggctgcatccttttggctggtctagaca agggtgtggagtatttagatctggaagaagaacaactatcctcgttagaaggctcacagggtctgatcccttcccgtgggtgg accgatgacctatgttacggtaccggtgccgtctacctgctgggacttggtatcggagggttttctggtatgatgcagggtctgc agaatattccgcccaatagtcccggaaaattgcaattgaacaccgtcctgaatcacattactaagagaggtcccttcttaggta ataatgcggggattctcgcgttgagctacaatatcatcaattctacaatagatgcactaagaggcaaacatgacaccgcggg ctccattggcgctggggccctcacgggcgctttgttcaagtcttcaaaaggtttgaaacccatgggttattcctcggcaatggtg gccgctgcgtgccgccgtctggtgtagtgtcaagaaaagactacttgaaaaaactagttatccctatgacgtcccggactatgc aggatcctatccatatgacgttccagattacgct**tag**gcaacacaagaacctactctctctctttctctctctctccctcttc gcttttcccccatgcactgatggcgcttgtatatagcatttgaaaaataatagtacgtaacgcagaaaacaaaccaatgaaag tagaaacccggagaaaagatctaaaaaaacaaaaaaaaaagatggaaagagcgcatgtatgtgtagatatgtacatata tacaactacttgtatgttatttgccttttgtactgttaagctattgttttcgatggcGGATCCgcaCTGCAG (Uppercase, *Eco*RI, *Bam*HI and *Pst*I sites; bold/underlined: start and stop codons of Tim23-HA)	ED Fig. 6m
pYC002 (pET32a-Cyb2Δ-DHFR)	CAT**ATG**ctgaaatacaaaccttactgaaaatctcgaagaactctgaggctgctatcctgcgcgcgtctaagactcgcttg aacacaatccgcgcgtacggttctaccgttccaaaatccaagtcgttcgaacaagactcaagttccgtggcgtatctgaactg gcataatggccaaatcgacaacgagccgaaactggatatgaataaacaaaagatttcgcccgctgaagttgccaagcata acaagcccgatgattcgtgggttgtgatcaatggttacgtatacgacttaacgcgtttcctgccaaatcatccaggtgggcagg atgttatcaagtttaacgccgggaaagatgtcactgctatttttgaaccactgcacgctcctaatgtcatcgataagtatattgctc ccgaaaaaaaattgggtcccctgcaaggatccggcggtaccgttcgtccattgaactgcatcgtcgccgtgtcccaaaatat ggggattggcaagaacggtgaccttccctggcctccgctccgcaacgagttcaagtacttccaacgtatgaccacaacctctt cagtggaaggtaaacagaatctggtgattatgggtcgcaaaacctggttctccattcctgagaagaatcgtcctttaaaggac cgtattaatattgttctcagtcgtgaactcaaagaaccaccacgtggagctcattttcttgccaaaagtttggatgatgccttacgt cttattgaacaaccggaattggcaagtaaagtagacatggtttggattgtcggaggcagttctgtttaccaggaagccatgaat caaccaggccacctccgcctctttgtgacacgcatcatgcaggaatttgaaagtgacacgtttttcccagaaattgatttgggg	Fig. 2h, ED Fig. 6i-k

	aaatataaacttctcccagaatacccaggcgtcctctctgaggtccaggaggaaaaaggcatcaagtataagtttgaagtct acgagaagaaagacCTCGAGcaccaccaccaccaccac**tga** (Uppercase, *Nde*I and *Xho*I sites; bold/underlined: start and stop codons)	
pYC008 (pYTK-e123 *pTIM23*-Tim23- Spot	GAATTCattgaaaaaaagagaaaatactgaaaaaaaaaagacaccgacaaaaaaaagagagaaaggaaccttta cgtagagcaaaagggaaacatttgttgcgtttaattaatagaaataataggttattgcattcgccctccattgcagaaaaaaaa aaaaaaaagaccatttttcctcttctactctctttgcctgtacatactacacgttatagcgttaacaaaagcagatagaaaaaaa aaaaataaccaagataatatagtatacttgtttaacagatcacacacaatc**atg**tcgtggctttttggagataagacacctac cgatgatgcgaatgctgccgtgggcggccaagatacaaccaagcctaaggaactatcgttgaagcagagtttaggtttcga gccaaacatcaataacataatatcaggtcctggtggaatgcatgtcgacaccgctaggctgcatcctttggctggtctagaca agggtgtggagtatttagatctggaagaagaacaactatcctcgttagaaggctcacagggtctgatcccttcccgtgggtgg accgatgacctatgttacggtaccggtgccgtctacctgctgggacttggtatcggaggtttttctggtatgatgcagggtctgc aaaatattccgcccaatagtcccggaaaattgcaattgaacaccgtcctgaatcacattactaagagaggtcccttcttaggta ataatgcggggattctcgcgttgagctacaatatcatcaattctacaatagatgcactaagaggcaaacatgacaccgcggg ctccattggcgctggggccctcacgggcgctttgttcaagtcttcaaaaggtttgaaacccatgggttattcctcggcaatggtg gccgctgcgtgcgccgtctggtgtagtgtcaagaaaagactacttgaaaaaactagtcctgatagagttagagcagtctcac attggtcttctggtggaggttctggcggtggttcaactccagatagagtacgtgctgtttctcactggagttct**tag**gcaacacaa gaacctactctctctctctttctctctctctcctcttcgcttttcccccatgcactgatggcgcttgtatatagcatttgaaaaata atagtacgtaacgcagaaaacaaaccaatgaaagtagaaacccggagaaaagatctaaaaaaacaaaaaaaaaaga tggaaagagcgcatgtatgtgtagatatgtacatatatacaactacttgtatgttatttgccttttgtactgttaagctattgttttcgat ggcggatccgcaCTGCAG (Uppercase, *Eco*RI and *Pst*I sites; bold/underlined: start and stop codons of Tim23-Spot)	Fig. 3d, ED Fig. 9a,b
pYC005 (pYTK-e112 *pGAL*1-Grx5-S80- sfGFP)	**ATG**TTTCTCCCAAAATTCAATCCCATAAGGTCATTTTCCCCCATCCTCCGGGCTAAGA CTCTTCTTCGTTACCAAAATCGGATGTATTTGAGCACAGAGATAAGAAAAGCTATTGA AGATGCCATCGAATCGGCTCCAGTGGTTCTTTTCATGAAAGGTACTCCTGAATTTCC CAAGTGTGGATTTTCAAGAGCAACCATTGGATTATTAGGAAATCAAGGCGTTGACCC GGCCAAATTTGCGGCTTATAATGTTtagaagacccagagctacgtgaaggtatcaaagagttttcagaat ggccaactattccacagttatatgtaaacaaagaattcattggtggatgtgatgttattacaagtatggcacgctctggtgaattg gccgatttgctagaagaggcacaggcattggtacctggcggatcaggaggttcttcaggtagttacggttctaccgttccaaa atccaagtcgttcgaacaagactcaagttccgtggcgtatctgaactggcataatggccaaatcgacaacgagccgaaact ggatatgaataaacaaaagatttcgcccgctgaagttgccaagcataacaagcccgatgattcgtgggttgtgatcaatggtt acgtatacgactctggatccggcggtaccTCCAAGGGAGAGGAGTTGTTCACTGGAGTTGTACCT ATCTTGGTCGAGTTAGACGGCGACGTCAACGGACACAAGTTTAGTGTAAGAGGTGA GGGAGAGGGTGACGCTACCAATGGAAAGTTAACTTTAAAGTTCATATCCACTACTGG CAAGTTACCTGTCCCTTGGCCTACTTTAGTGACTACTTTGACATACGGAGTACAAATG TTTAGTAGATATCCTGACCACATGAAGAGACATGACTTCTTCAAGTCCGCTATGCCA GAGGGATACGTCCAAGAGAGAACGATCTCTTTCAAGGACGACGGTACTTATAAGACT AGAGCTGAGGTTAAGTTTGAGGGAGACACTTTAGTCAATAGGATCGAGTTGAAGGG GATTGACTTTAAGGAAGACGGAAACATTTTGGGTCACAAGTTGGAGTATAATTTTAAC AGTCATAATGTCTATATTACGGCTGACAAGCAGAAGAATGGCATAAAGGCAAACTTC AAGATTAGACACAACGTGGAAGACGGTTCCGTGCAATTGGCGGACCATTACCAACA AAATACTCCAATCGGGGACGGTCCAGTCTTGTTGCCTGACAACCATTATCTTTCTAC CCAATCAGTGCTATCAAAGGACCCTAATGAGAAGAGGGACCACATGGTCCTATTAGA GTTCGTGACAGCTGCTGGAATTACTCATGGAATGGACGAGTTATACAAGGGCggatcc ggttctagattggaagaggaattgagaaggagattaactgag**taa** (Uppercase, Grx5 and sfGFP; bold/underlined: start and stop codons)	Fig. 3c-e, ED Fig. 8d-i, and 9a-h,j
pYC006 (pYTK-e112 *pGAL*1-Grx5- S99(TM)-sfGFP)	**atg**tttctcccaaaattcaatcccataaggtcattttccccatcctccgggctaagactcttcttcgttaccaaaatcggatgtatt tgagcacagagataagaaaagctattgaagatgccatcgaatcggctccagtggttcttttcatgaaaggtactcctgaatttc ccaagtgtggattttcaagagcaaccattggattattaggaaatcaaggcgttgacccggccaaatttgcggcttataatgtttt agaagacccagagctacgtgaaggtatcaaagagttttcagaatggccaactattccacagttatatgtaaacaaagaattc attggtggatgtgatgttattacaagtatggcacgctctggtgaattggccgatttgctagaagaggcacaggcattggtacctg gcggatcaggaggttcttcaggtagttacggttctaccgttccaaaatccaagtcgttcgaacaagactcaAGAATTTGT ACTCAATCTTGGACTGCTTTGAGAGTTGGTGCTATTTTGGCTGCAACTagttccgtggcgtat ctgaactggcataatggccaaatcgacaacgagccgaaactggatatgaataaacaaaagatttcgcccgctgaagttgc caagcataacaagcccgatgattcgtgggttgtgatcaatggttacgtatacgactctggatccggcggtacctccaaggga gaggagttgttcactggagttgtacctatcttggtcgagttagacggcgacgtcaacggacacaagtttagtgtaagaggtga gggagagggtgacgctaccaatggaaagttaactttaaagttcatatccactactggcaagttacctgtcccttggcctacttta gtgactactttgacatacggagtacaaatgtttagtagatatcctgaccacatgaagagacatgacttcttcaagtccgctatgc cagagggatacgtccaagagagaacgatctctttcaaggacgacggtacttataagactagagctgaggttaagtttgagg gagacacttagtcaataggatcgagttgaaggggattgactttaaggaagacggaaacattttgggtcacaagttggagtat aattttaacagtcataatgtctatattacggctgacaagcagaagaatggcataaaggcaaacttcaagattagacacaacg tggaagacggttccgtgcaattggcggaccattaccaacaaaatactccaatcggggacggtccagtcttgttgcctgacaa	ED Fig. 8d

	ccattatctttctacccaatcagtgctatcaaaggaccctaatgagaagagggaccacatggtcctattagagttcgtgacagc tgctggaattactcatggaatggacgagttatacaagggcggatccggttctagattggaagaggaattgagaaggagatta actgag**<u>taa</u>** (Uppercase, sorting signal with IC mutation; bold/underlined: start and stop codons)	
pYC007 (pYTK-e112 *pGAL*1-Grx5-S80- sfGFP-2xTEV- ALFA)	**<u>atg</u>**tttctcccaaaattcaatcccataaggtcattttcccccatcctccgggctaagactcttcttcgttaccaaaatcggatgtatt tgagcacagagataagaaaagctattgaagatgccatcgaatcggctccagtggttcttttcatgaaaggtactcctgaatttc ccaagtgtggattttcaagagcaaccattggattattaggaaatcaaggcgttgacccggccaaatttgcggcttataatgtttt agaagacccagagctacgtgaaggtatcaaagagttttcagaatggccaactattccacagttatatgtaaacaaagaattc attggtggatgtgatgttattacaagtatggcacgctctggtgaattggccgatttgctagaagaggcacaggcattggtacctg gcggatcaggaggttcttcaggtagttacggttctaccgttccaaaatccaagtcgttcgaacaagactcaagttccgtggcgt atctgaactggcataatggccaaatcgacaacgagccgaaactggatatgaataaacaaaagatttcgcccgctgaagttg ccaagcataacaagcccgatgattcgtgggttgtgatcaatggttacgtatacgactctggatccggcggtacctccaaggg agaggagttgttcactggagttgtacctatcttggtcgagttagacggcgacgtcaacggacacaagtttagtgtaagaggtg agggagagggtgacgctaccaatggaaagttaactttaaagttcatatccactactggcaagttacctgtcccttggcctacttt agtgactactttgacatacggagtacaaatgtttagtagatatcctgaccacatgaagagacatgacttcttcaagtccgctatg ccagagggatacgtccaagagagaacgatctctttcaaggacgacggtacttataagactagagctgaggttaagtttgag ggagacactttagtcaataggatcgagttgaaggggattgactttaaggaagacggaaacattttgggtcacaagttggagt ataattttaacagtcataatgtctatattacggctgacaagcagaagaatggcataaaggcaaacttcaagattagacacaa cgtggaagacggttccgtgcaattggcggaccattaccaacaaaatactccaatcggggacggtccagtcttgttgcctgac aaccattatctttctacccaatcagtgctatcaaaggaccctaatgagaagagggaccacatggtcctattagagttcgtgac agctgctggaattactcatggaatggacgagttatacaagggcggatccggcGAAAATTTATACTTTCAAGGT ggttctggaagtggtGAAAACTTGTATTTCCAAGGaggctcaagattggaagaggaattgagaaggagattaa ctgag**<u>taa</u>** (Uppercase, TEV cleavage sites; bold/underlined: start and stop codons)	ED Fig. 8a