**Supplemental material**

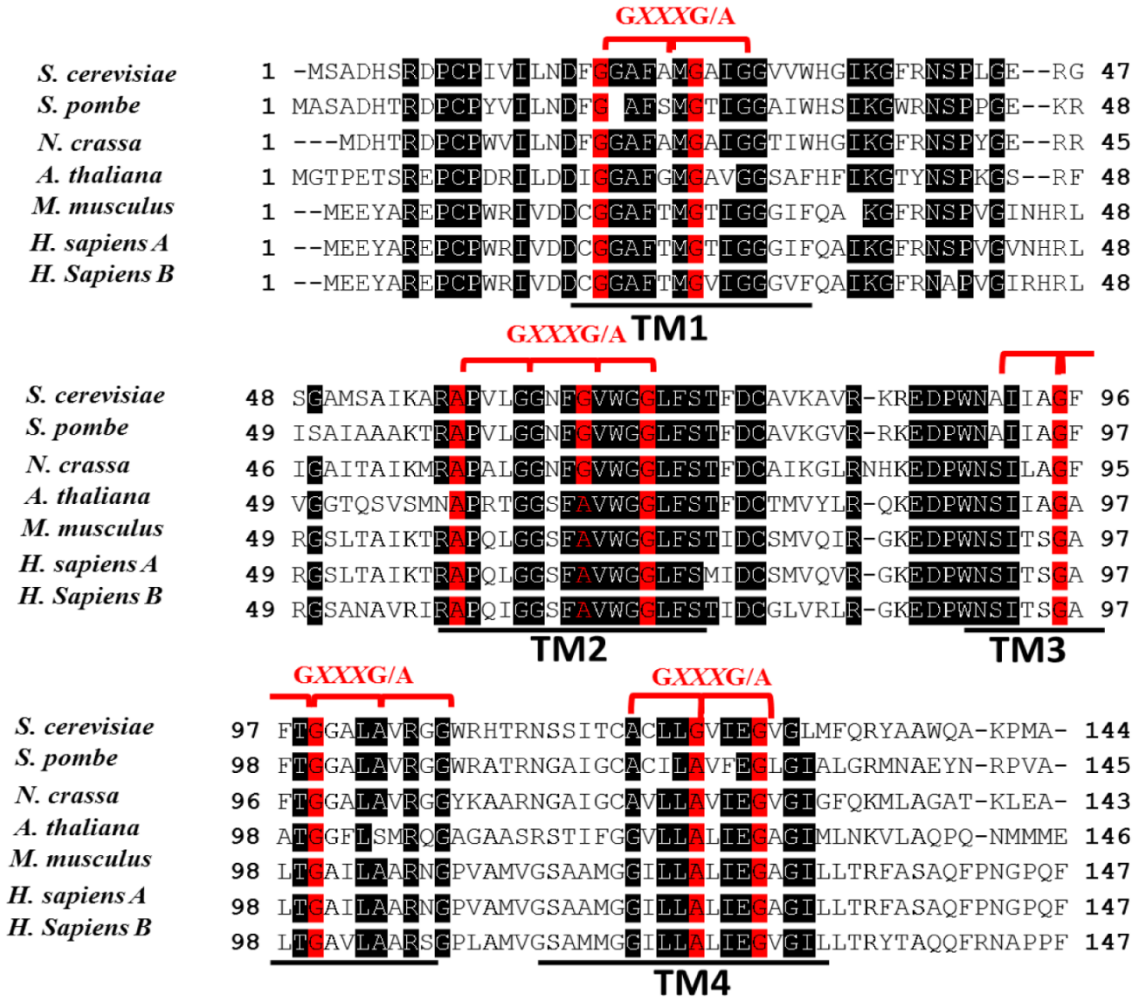


**Fig. S1** Sequence alignment of Tim17 protein from different species. (A) Orthologous Tim17 sequences from different species including *S. cerevisiae*, *N. crassa*, *S. pombe*, *A. thaliana*, *M. musculus* and *H. sapiens* were aligned using ClustalW2 online tool. The position of transmembrane helices TM1-TM4 has been indicated. The sequence corresponding to the G/AXXXG/A motif and the position of the conditional mutants isolated are highlighted in red colour.

**Table S1:** Yeast strains used in this study.

| Strain | Genotype | Source |
|---|---|---|
| TIM17-316 | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS316-*Tim17*] | (Schilke et al., 2012) |
| TIM17-314 | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-*Tim17*] | This study |
| TIM17 | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-*Tim17*]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G19A}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G19A}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{A23L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{A23L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G25L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G25L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G19A/A23L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G19A/A23L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G29L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G29L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{A58L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{A58L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G62L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G62L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G66L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G66L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G70L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G70L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{A58L/G70L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{A58L/G70L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{A91L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{A91L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G95L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G95L}$]-*Ttim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G99L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G99L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{A103L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{A103L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G107L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G107L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G123L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G123L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G127L}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS314-$Tim17_{G127L}$]-*Tim23*-FLAG::*kanMX4* | This study |
| $Tim17_{G123L/G127L\text{-}TEF}$ | *MATa ade2 his3 ura3 leu2 trp1 can1 Tim17Δ*::*CgHIS3* [pRS414-$Tim17_{G123L/127L}$] -*Tim23*-FLAG::*kanMX4* | This study |