SUPPLEMENTARY MATERIAL

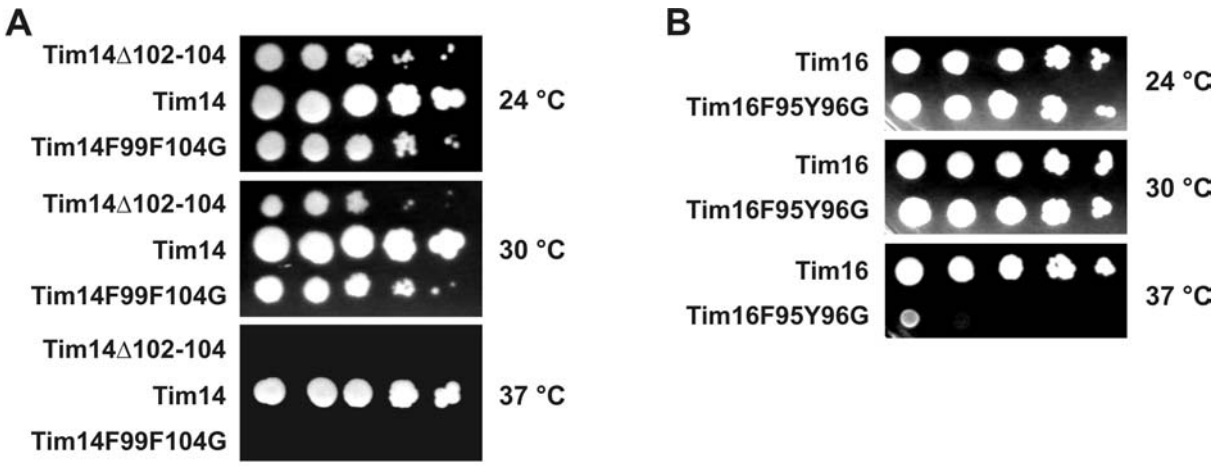
Detailed description of Tim14-Tim16 contacts

Part I

Two major areas of interactions connect Tim14 and Tim16. First, the N-terminal arm of Tim14 embraces helix III of Tim16 and, in addition, makes contacts with helices I and II. These contacts are both polar and hydrophobic. Flanking residues of the Tim14 arm, Phe99 and Phe104, protrude into two distinct hydrophobic pockets formed predominantly by Tim16. They fix Tim14 by formation of a clamp. The side chain of Phe99 of Tim14 is stabilized by van der Waals interactions with the conserved residues Phe84, Phe95, Asn98 and Tyr102 of Tim16, whose side chains form a hydrophobic pocket. The second hydrophobic pocket is formed by both Tim14 and Tim16. Side chains of Tim16 residues Tyr96, Lys100 (aliphatic part), Leu97 and Ile61 as well as residues Phe149, Lys153 (aliphatic part) and Leu150 of Tim14 make up the pocket. Furthermore, the carbonyl oxygen of Phe99 and backbone nitrogen of Phe104 in Tim14 are stabilized by formation of strong hydrogen bonds with side chains of Arg103 and Glu57 in Tim16. The side chain of Tim14Leu100 forms van der Waals interactions with Phe95 of Tim16 while its backbone nitrogen and carbonyl oxygen are hydrogen bridged to side chains of Ser99 and Arg103 in Tim16. The peptide backbones of Lys101 and Gly102 of Tim14 are involved in the formation of a hydrogen bonding network with Lys100 of Tim16, including water molecules. Interestingly, their side chains are not in contact with Tim16 although these two residues are conserved in their primary sequence among different species. Gly103 of Tim14, also a conserved residue, is not involved in protein-protein interactions, but a larger side chain at this site would cause a steric clash with helix I of Tim16.

Part II

The second interface of Tim14 and Tim16 is a continuation of the hydrophobic groove formed around Phe104 of Tim14 and is responsible for the back-to-back arrangement of the two subunits. Numerous contacts exist which involve helices I, II and III of both subunits, as well as the loops between helices II and III. In particular, Phe149 and Leu150 of the N-terminal part of helix III of Tim14 interact with hydrophobic parts of Ile61, Leu83 and Val101 of Tim16. On the other hand, Tyr96 and Leu97 of the N-terminal part of helix III of Tim16 form van der Waals bonds to Ile116, Ile136 and Ile154, thus stabilizing helices I, II and III of Tim14. Hydroxyl group in the side chain of Tyr96 in Tim16 and the carboxyl group in the side chain of Glu112 in Tim14 make the strongest hydrogen bond visible in the complex.



Mokranjac et al Supplementary Figure 1

Supplementary Figure 1. (A) Tenfold serial dilutions of *Δtim14* cells expressing indicated genes were spotted on rich medium containing glucose and incubated at the indicated temperatures for 3 days. (B) Tenfold serial dilutions of *Δtim16* cells expressing indicated genes were spotted on rich medium containing glucose and incubated at the indicated temperatures for 3 days.