Supplemental Data **Naoki Shigi**

Supplementary Experimental procedures

Primers

1. For the construction of expression vectors

ttuB-F; 5'-gcatggtacc tctagaggag gaccatatga gggtcgttct gcgcctgcc -3'
ttuB-R; 5'-tgataggaatt ctaccctccc gagatggcgg agaggacc -3'
ttc1133-F; 5'-gcatggtacc tctagaggag gaccatatgg cgcctccttc ggtgccc -3'
ttc1133-R; 5'-actatgaatt cctagagcac caccagggcc ac -3'
The restriction enzyme sites are *underlined*.

2. For the construction of the co-expression vector

Hind-insert-F; 5'-cgggtaccga gctcgaattc ctgcagaagc ttgtaatcat gtcatagctg tttcc -3'
Hind-insert-R; 5'-ggaaacagct atgacatgat tacaagcttc tgcaggaatt cgagctcggt acccg-3'
ttuB-F and ttuB-R; (same as in 1)
ttuA-F; 5'-gcatgaattc tctagaggag gaccatatgg tctgcaaggt ctgcgggc-3'
ttuA-R; 5'-tgataaagct tttaaccggc acggaggggc ttc-3'
The restriction enzyme sites are *underlined*.

3. For the construction of the *ΔttuB-ttuA* strain

ttuBA-5'-F; 5'-cctggggaag aagctcaacc-3'
ttuBA-5'-R; 5'-gtaccatatc cgccgtcata tcacctcacc ccgctagtgt agc-3'
ttuBA-3'-F; 5'-gttaatcatg ttggttacgt aagcccgagg tgtgccgcc-3'
ttuBA-3'-R; 5'-tggttcggca ccaggtccac-3'
htk-F; 5'-tgatatgacg gcggatatgg tac-3'
htk-R; 5'-cgtaaccaac atgattaac-3'
The sequences complementary to the *htk* gene cassette are *underlined*.

4. For the construction of the *Δttc1133* strain

ttc1133-5'-F; 5'-agtagtcccc gcccttgtcc -3'
ttc1133-5'-R; 5'-gtaccatatc cgccgtcata tcacgggtct cctccagaag gcc -3'
ttc1133-3'-F; 5'-gttaatcatg ttggttacgc ttcggcacgg acggggtcc -3'
ttc1133-3'-R; 5'-ttgccgctcg gcgaggtctc-3'
htk-F and htk-R; (same as in 3)
The sequences complementary to the *htk* gene cassette are *underlined*.

Legends to Supplementary Figures

Supplementary Fig. S1 Ubls and E1-like proteins in three kingdoms of life

A. Sequence alignment of Ubls. Ubls from *Haloferax volcanii* (Hv), *Thermus thermophilus* (Tt), *Escherichia coli* (Ec), and *Saccharomyces cerevisiae* (Sc) are aligned. TtuB is the Ubl investigated in this study. MoaD (M), MoaD (W), and ThiS are sulfur carriers for the biosynthesis of molybdenum cofactor, tungsten cofactor, and thiamin, respectively. Ub and Rub1 are protein modifiers. Urm1, SAMP1, and SAMP2 function as protein modifiers and sulfur carriers (for detail see the Introduction and the Discussion). For TtMoaDs, only the N-terminal Ubl domains are aligned. The conserved C-terminal GG motif is indicated below the alignment. **B**. Structures of TtuB (homology model (1)) and Yeast ubiquitin (PDBid: 3CMM). C-terminal glycines are indicated. **C.** Sequence alignment of E1-like proteins. Uba1, Uba4, TtuC, UbaA, MoeB, and ThiF are E1/E1-like enzymes for Ub, Urm1, Tt Ubls including TtTtuB, SAMPs, MoaD, and ThiS, respectively. Whole sequences are aligned for prokaryotic family members. For yeast Uba1, only the adenylation domain (AD) (414-601 and 861-928) is aligned. The two parts are separated by '/'. For yeast Uba4, the N-terminal AD (35-316) is aligned. The P-loop, catalytic Cys, and CXXC motifs are indicated below the alignment.

Supplementary Fig. S2 TtuB forms protein conjugates in *T. thermophilus* cells (related to Fig. 1)

A. Growth curves in rich medium (*left panel*) and in minimal medium (MM, *right panel*) are shown. **B.** (*Upper panels*) Western blot analysis of cell extracts (22 μg) from *T. thermophilus* against a TtuB antibody. Extracts were prepared from cells in the culture conditions and times indicated above the *panels*. Recombinant TtuB (6 ng) was loaded *in lanes 5* and *14*. (*Lower panels*) Ponceau stained membranes show equal amounts of protein loaded.

Supplementary Fig. S3 Characterization of the JAMM domain protein Ttc1133 (related to Fig. 5)

A. Sequence alignment of JAMM domain containing proteins. Whole sequences of JAMM domain proteins from prokaryotes (*first 4 rows*) and the N-terminal JAMM domains from ScRpn11 (1–175) and ScCsn5 (45–231) are also aligned. QbsD functions in siderophore synthesis (2) and Mec^{+} is involved in cysteine synthesis (3). Af2198 was examined by structural analysis (4). Rpn11 on the lid of the 26S-proteasome removes polyUb chains from proteasome targeted proteins (5). The Csn5 subunit of the COP9 signalosome cleaves the Ubl Nedd8 from SCF ubiquitin ligase (6). The active site residues are indicated below the alignment (E47, H101, S102, H103, S111, and D114 for Ttc1133). Pf: *Pseudomonas fluorescens*, Mt: *Mycobacterium tuberculosis*, Ttc: *Thermus thermophilus*, Af: *Archaeoglobus fulgidus*, and Sc; *Saccharomyces cerevisiae*. **B**. Growth phenotypes of wild-type, *Δttc1133*, *ΔttuA*, and *ΔttuC* strains. Cells were cultured in rich medium (30 μg/ml kanamycin was added to the mutant strains) at 70°C overnight. Diluted cultures (A_{600} = 0.1) and serial dilutions of these (10^{-1}, 10^{-2}, and 10^{-3}) were spotted onto rich medium plates and incubated at 60°C for 22 h, or at 70°C, 75°C, 80°C, or 82°C for 14 h. The *Δttc1133* strain showed normal growth phenotype, in contrast to the temperature sensitive phenotypes of *ΔttuA* and *ΔttuC* strains (1).

Supplementary Fig. S4 Steady-state level of TtuC in *ttuB* mutants

The amount of TtuC was measured by immunoblotting with an anti-TtuC antibody. The band intensities were quantified and presented in the *right panels* (n=4). **A**. Comparison between wild-type and *ΔttuB* strains. The difference in the amounts of TtuC was minimal. **B**. Comparison between *ΔttuB* strains transformed with pWUR-ttuB-WT and pWUR-ttuB-*Δ*G, respectively. The amount of TtuC was unchanged.

Supplementary References

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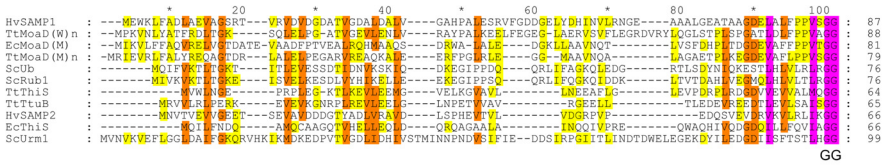
A



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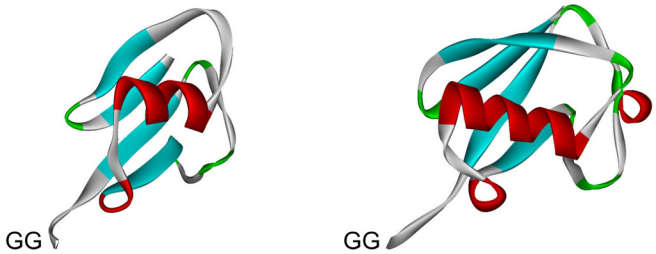

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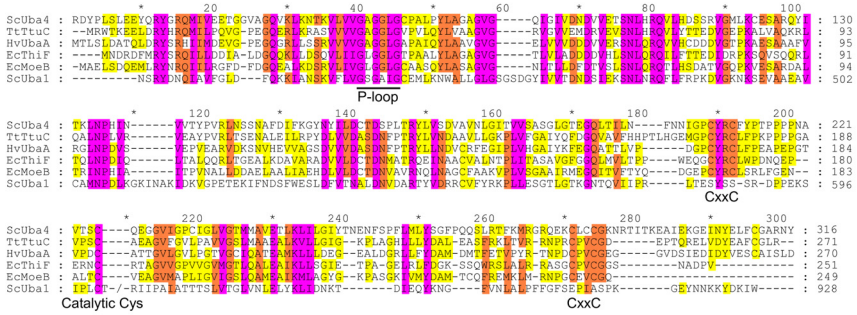
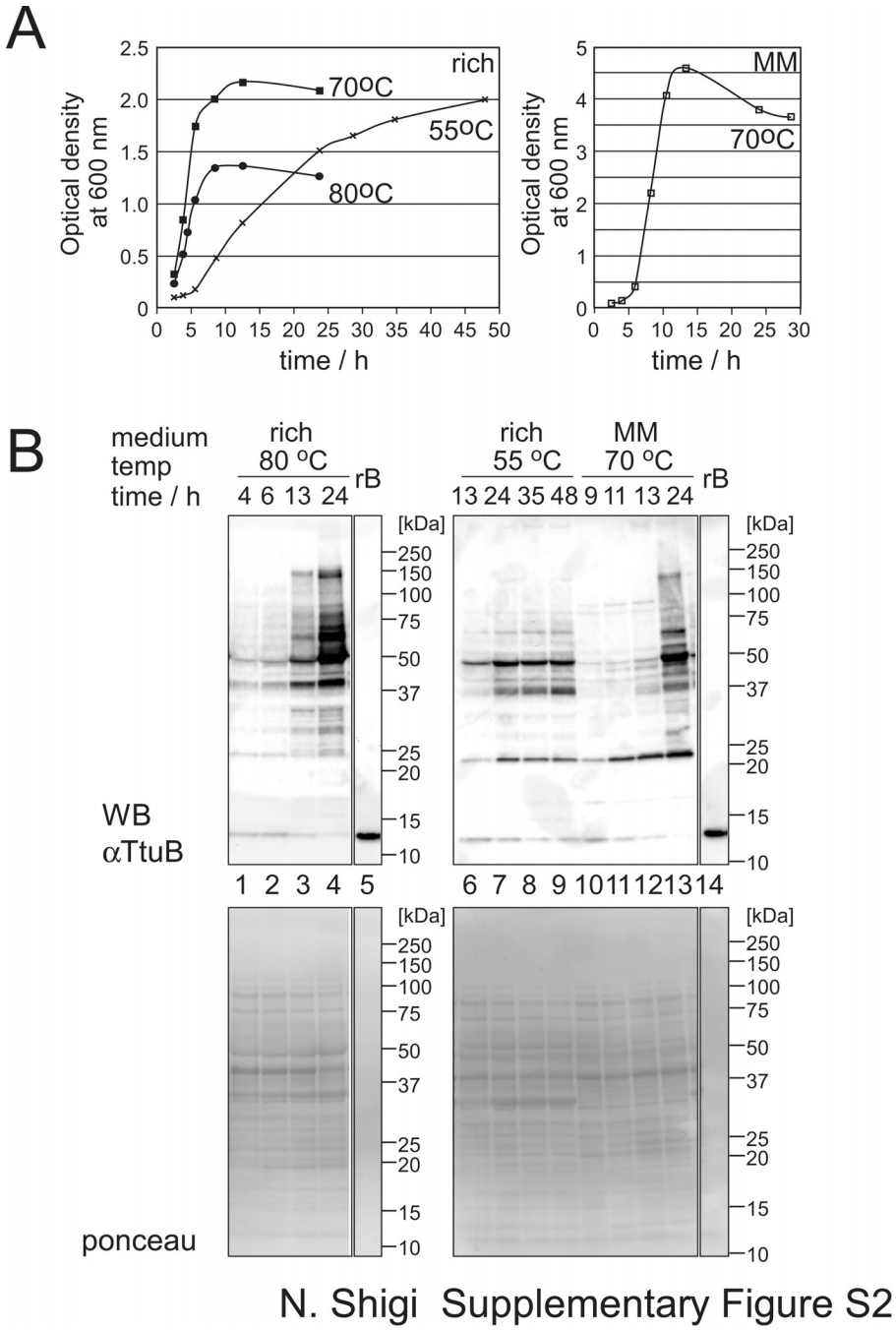
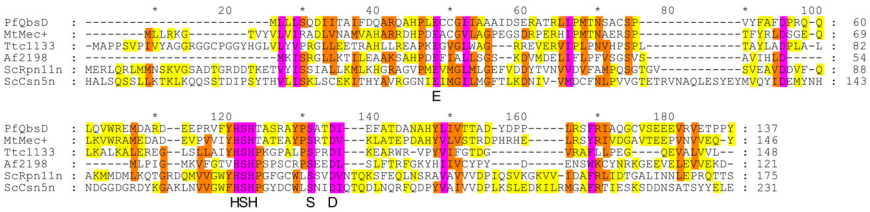


N. Shigi Supplementary Figure S1



N. Shigi Supplementary Figure S2

A



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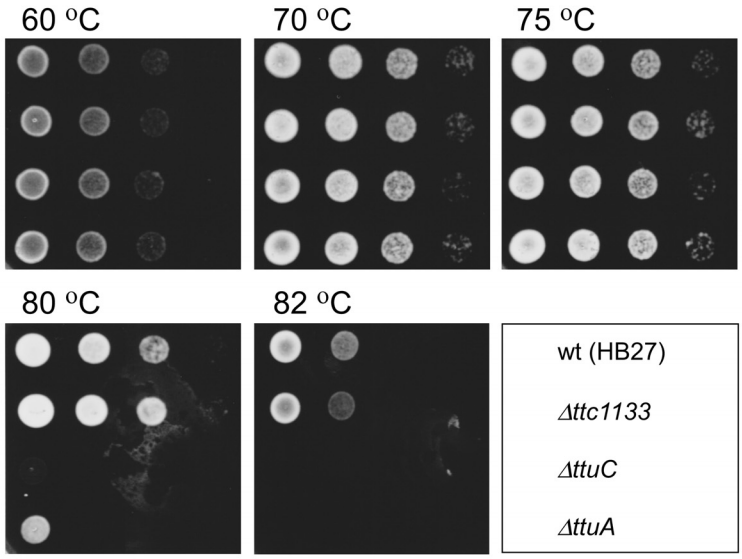
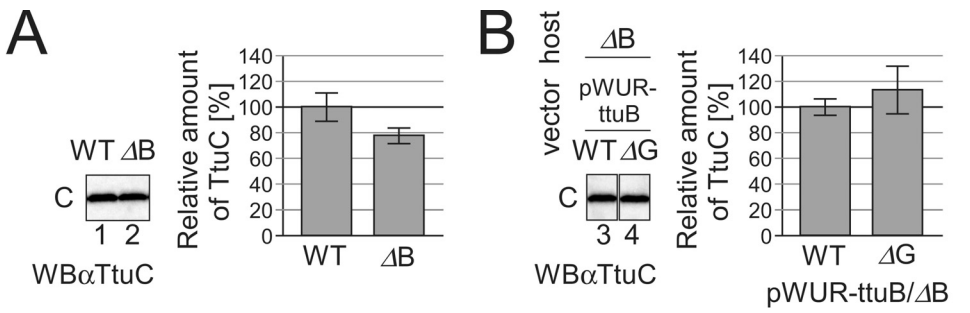


N. Shigi Supplementary Figure S3



N. Shigi Supplementary Figure S4