

## Supplementary data

### **Crystal structure of *Saccharomyces cerevisiae* mitochondrial GatFAB reveals a novel subunit assembly in tRNA-dependent amidotransferases**

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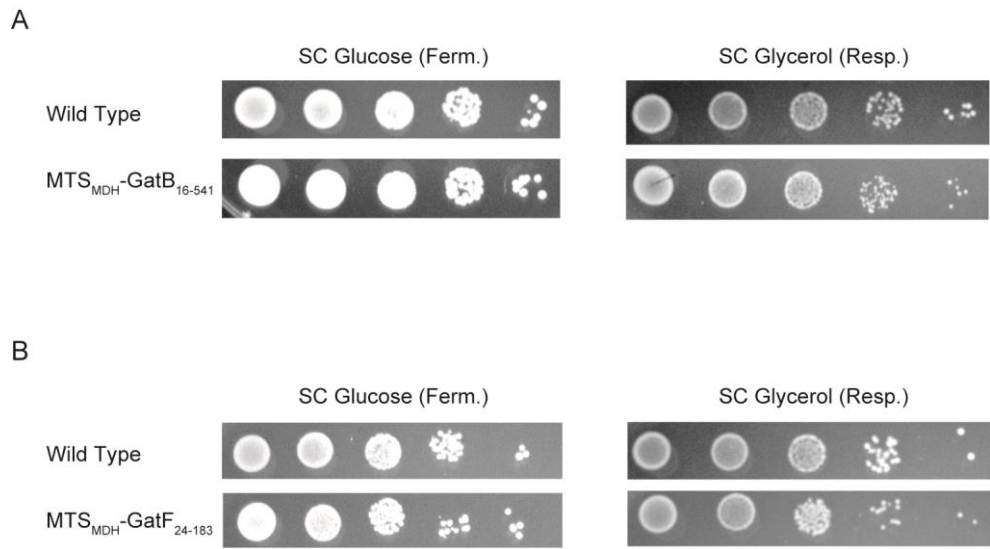
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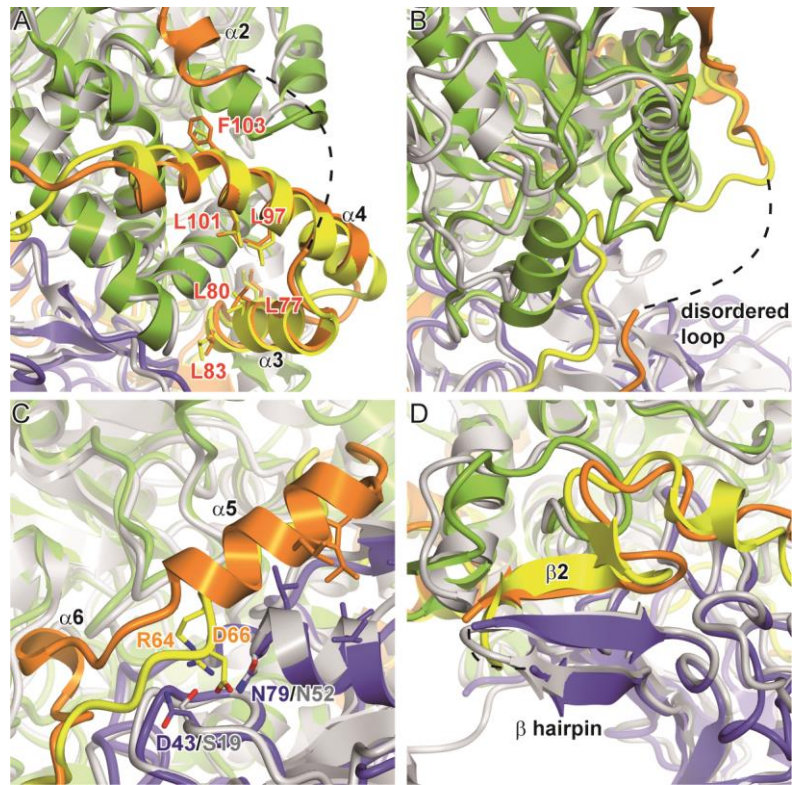
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**Figure S1. Yeast complementation of the RS453 wild type strain and the GatB<sub>16-541</sub> (A) and GatF<sub>24-183</sub> (B) mutants fused to MTS of MDH.**



**Figure S2. Interactions between GatAB and the GatC-like portion of GatF.**

The crystal structure of *S. aureus* GatCAB is superposed onto that of GatFAB. GatFAB is colored as in Figure 1. *S. aureus* GatAB and GatC are colored gray and purple, respectively. (A) The conserved helical bundle. The conserved hydrophobic residues between GatF and GatC are shown as yellow and purple sticks, respectively. (B) The disordered loop followed by the helical bundle. (C) The insertion helices  $\alpha 5$  and  $\alpha 6$ . The residues involving hydrophobic interactions for anchoring  $\alpha 5$  helix are shown as yellow and green sticks. The conserved residues forming a hydrogen bond network in bacterial GatC are shown as purple, green and gray sticks. (D) The C-terminal  $\beta 2$  strand forming an antiparallel  $\beta$ -sheet with a  $\beta$  hairpin of GatB.

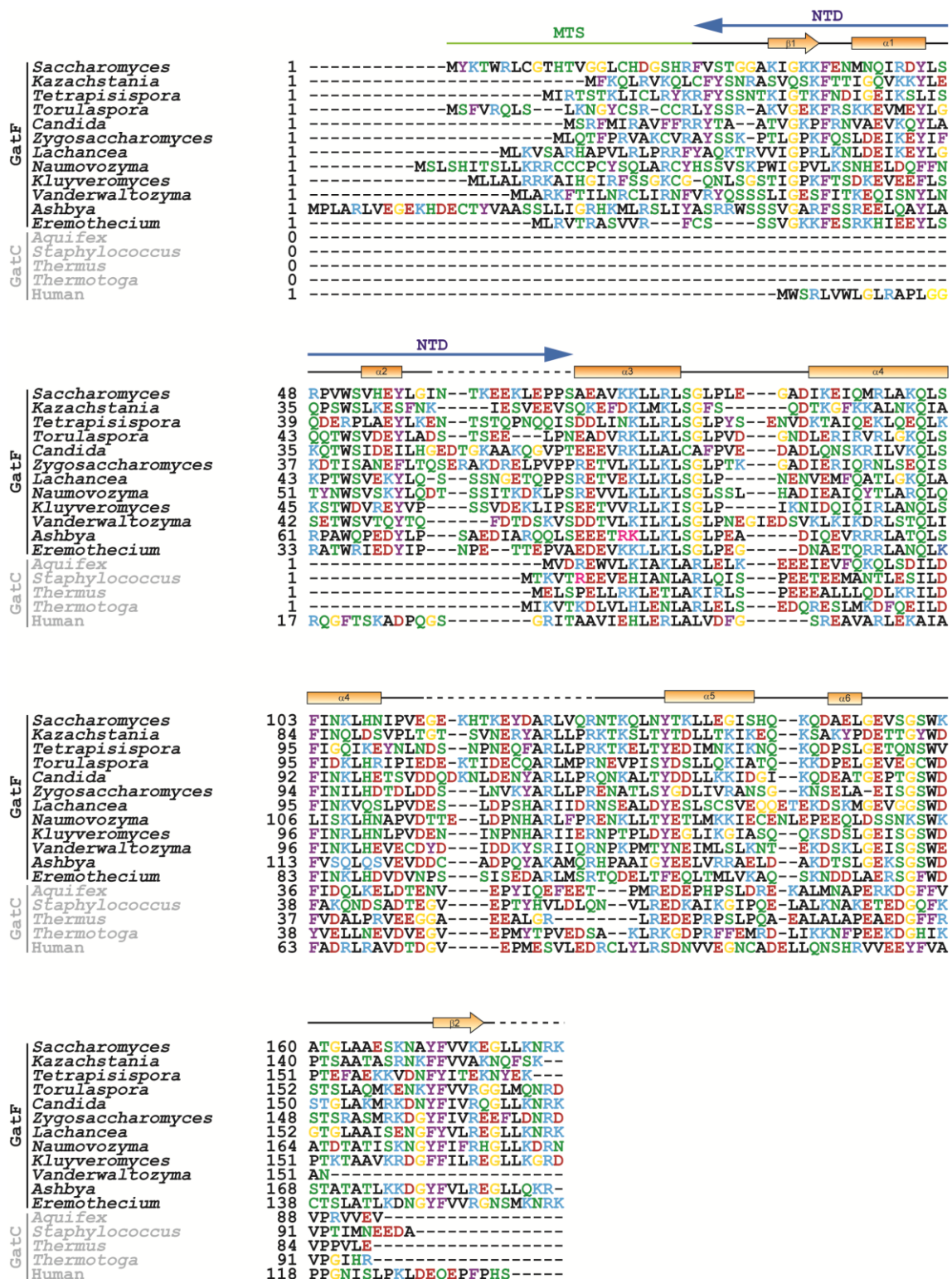
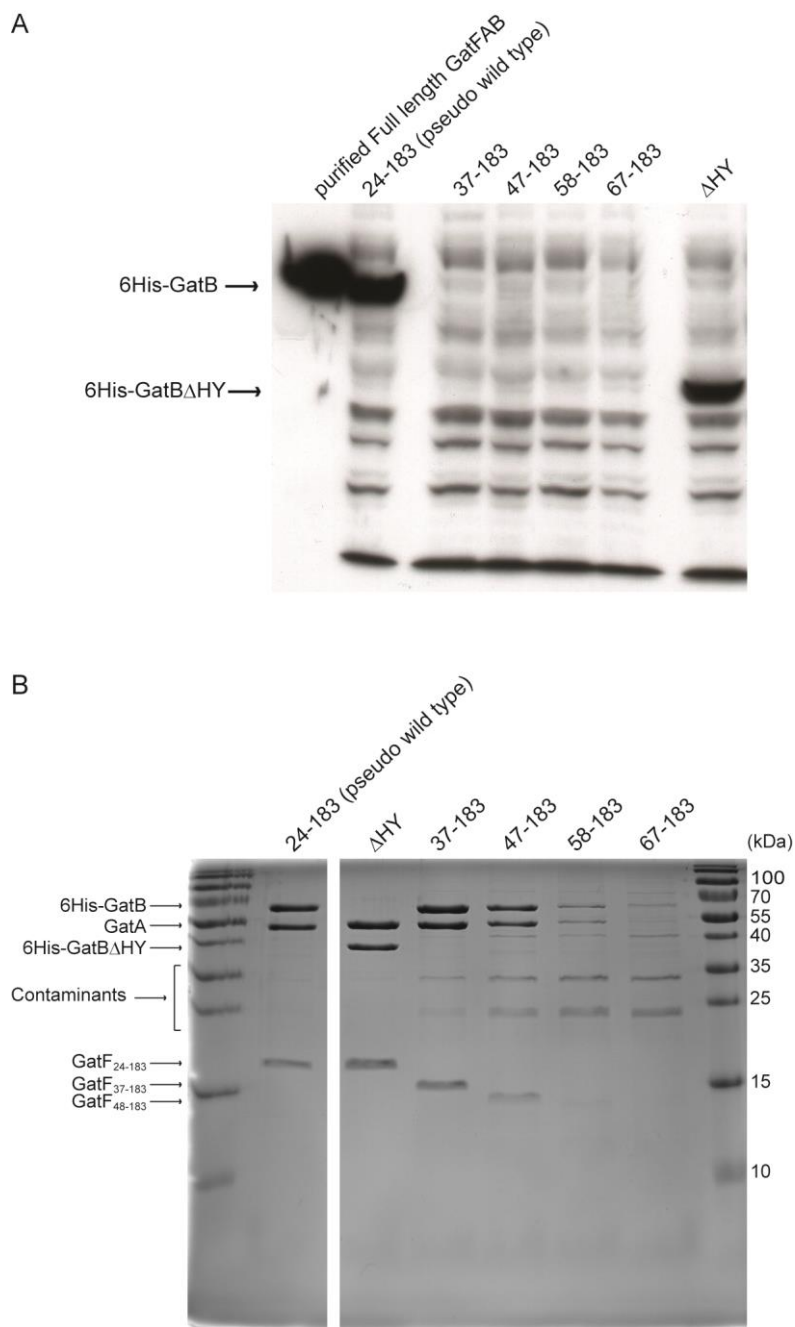


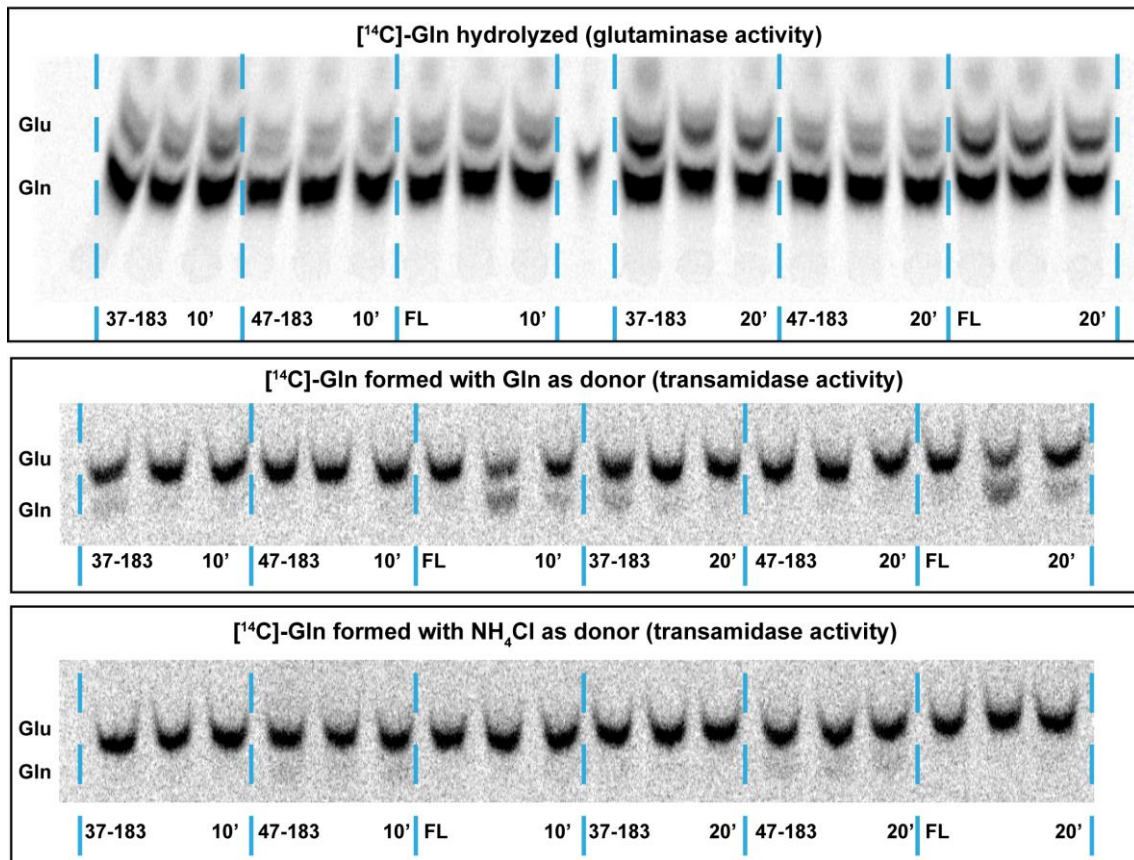
Figure S3. Sequence alignment of *S. cerevisiae* GatF with GatF and GatC.

The MTS, NTD and secondary structure of *S. cerevisiae* GatF are illustrated.



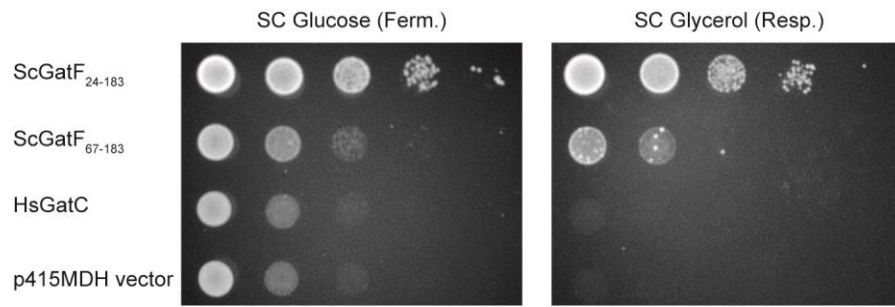
**Figure S4. Expression and purification of the GatFAB mutants.**

(A) Western blot quantification of the expression levels of GatFAB mutants in *E. coli* total protein extract by anti-His tag antibody. 6His tag is fused to the C-terminus of GatB in each construction. (B) Coomassie blue-stained protein gel of the GatFAB mutants, purified by MagneHis (Promega).



**Figure S5. Thin-layer chromatography activity assays for GatF NTD deletion mutants.**

Three reactions were performed for each of the three activities tested: Glutamine hydrolysis, transamidation with Gln donor and transamidation with NH<sub>4</sub>Cl donor. Time points were taken at 10 and 20 minutes. Relative activities were obtained by dividing the value of each of three points for a mutant at a given time for a given reaction (e.g., glutaminase, 37-183, 10') by the average value of the three corresponding full length enzyme measurements (e.g., glutaminase, FL, 10'). Relative activities at 10' and 20' were combined and the averages of those six relative activities for each of the two mutants in the three conditions tested are presented in Figure 5B. The standard deviation of each group of six time points was used for the error bars.



**Figure S6. Yeast complementation of the NTD-deprived GatF mutant (GatF<sub>67-183</sub>) and human GatC.**