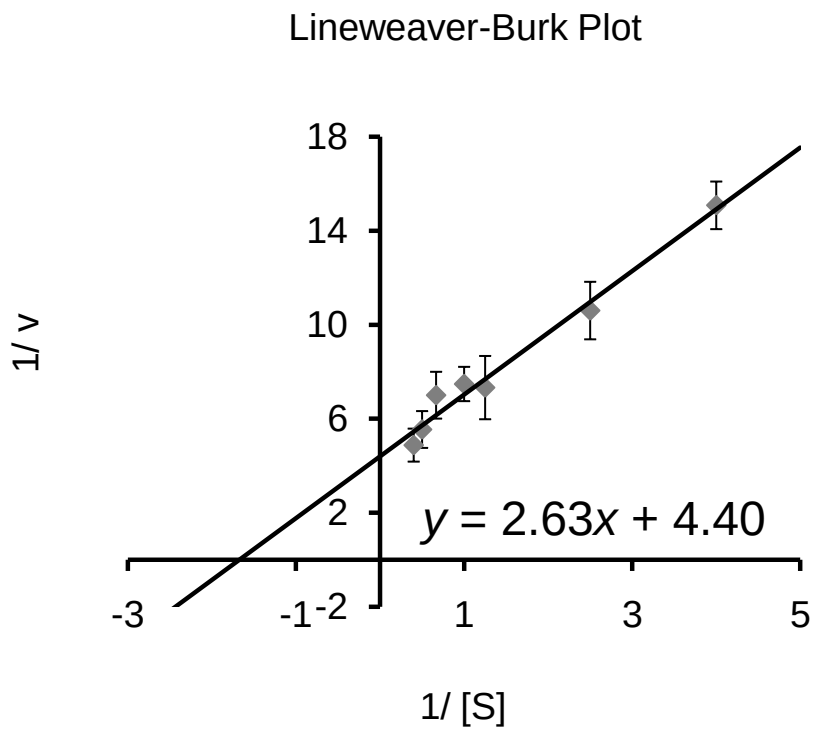
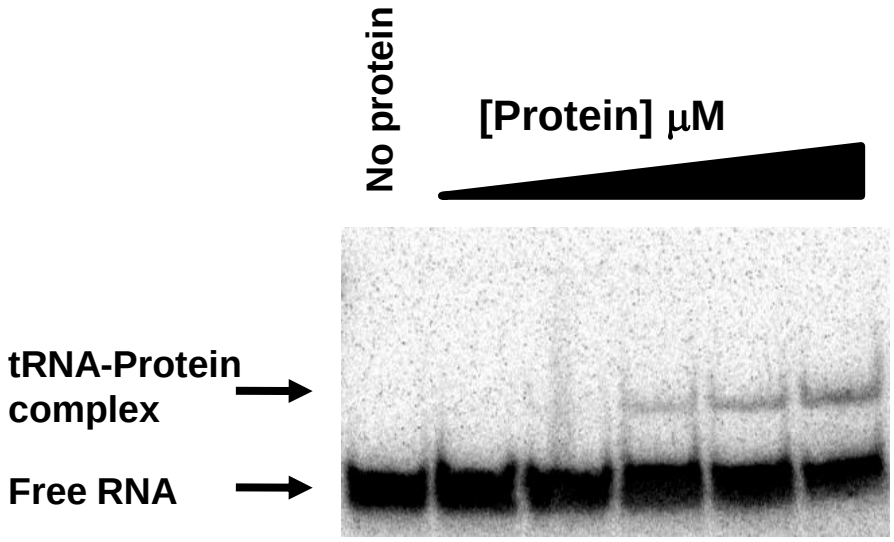
Supplementary Figure Legends

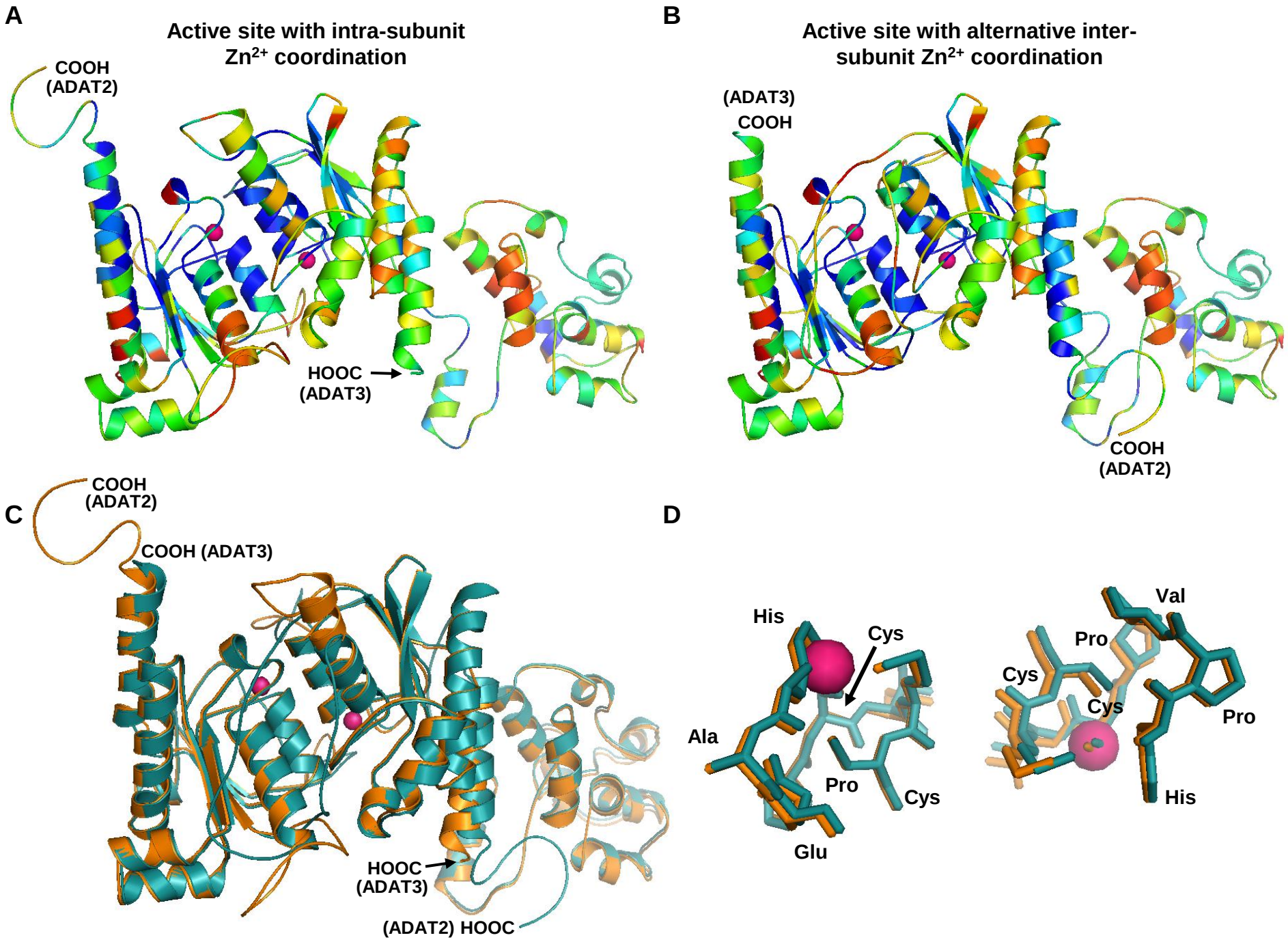
Supplementary Figure 1 Lineweaver-Burk plot for the kinetic constants of the wild type *Tb*ADAT2/3 heterodimer. The Km is calculated in micromolar (μM) while the velocity is calculated in pmol per minute (pmol/min). The equation for the line is shown along the x-axis ($y = 2.63x + 4.40$).

Supplementary Figure 2 *Tb*ADAT2 binds tRNA with a much lower affinity than the wild type *Tb*ADAT2/3 heterodimer. 6xHis-tagged *Tb*ADAT2 in the absence of ADAT3 was over-expressed in *E. coli*s and purified via Ni^{2+} affinity. Purified protein was stored as aliquots at -80^{o}C in ADAT2/3 storage buffer (50 mM Hepes pH 8, 100 mM KCl, 1mM $MgCl_2$, 0.1 mM EDTA, and 2 mM DTT) with 20% glycerol. Increasing concentrations of ADAT2 (0 to 2.5 μM) was incubated with end radiolabeled G_{34} containing transcript $tRNA^{Val}$. The "no protein" lane is a mock reaction in which no protein was added to the RNA; this serves as a marker for the migration of free tRNA in the gel. Arrows indicate the free tRNA and the protein-tRNA complex.

Supplementary Figure 3 Structural model supports the possibility of an alternative inter-subunit zinc coordination in *Tb*ADAT2/3. Using the ADATa (TadA) homodimer (co-crystallized with tRNA anticodon loop) from *Staphylococcus aureus* (2B3J in the Protein Data Bank) as a modeling template, two ADAT2/3 heterodimer models were built using the FRankenstein's Monster method. (*A*) The model in this panel represents the heterodimer most like the ADATa homodimer in that each subunit individually coordinates Zn^{2+} (intra-subunit coordination). (*B*) This panel represents an alternative "swapped" model, which was built by exchanging parts of ADAT2 and ADAT3 containing the HXE and CXXC motifs; this model supports the possibility of inter-subunit Zn-coordination. The models are colored by conservation: blue is invariant, red is highly variable, yellow and green are intermediate. Magenta spheres represent Zn^{2+}. The C-terminal ends (COOH) are label for each subunit. (*C*) Superposition of the two models in (*A*) and (*B*) reveals only minor structural difference between the two models. The intra-subunit model (*A*) is shown in orange while the inter-subunit "swapped" model (*B*) is shown in teal. Also highlighted are the C-termini of each protein subunit (denoted by COOH). (*D*) Superposition of the active sites from the two different models shows the active sites are nearly identical. The figure is colored as in (*C*).


Lineweaver-Burk Plot
18
14
10
6
2
-2
1/ v
y = 2.63x + 4.40
-3
-1
1
3
5
1/ [S]


No protein
[Protein] μM
tRNA-Protein complex
Free RNA


A
Active site with intra-subunit Zn2+ coordination
COOH (ADAT2)
HOOC (ADAT3)
B
Active site with alternative inter-subunit Zn2+ coordination
(ADAT3) COOH
COOH (ADAT2)
C
COOH (ADAT2)
COOH (ADAT3)
HOOC (ADAT3)
(ADAT2) HOOC
D
His
Cys
Ala
Pro
Cys
Glu
Val
Pro
Cys
Cys
Pro
His