Supplementary data

Deciphering the influence of the [4Fe-4S] cluster of tRNA thiolation enzymes on tRNA binding

Supplementary Figures

Figure S1. Secondary structure of the tRNA and mini-RNA transcripts used to study their binding to MmTtuI and EcTtcA. The boxed areas indicate the conserved regions between the full-length tRNA and the mini-RNA. (A) MmtRNA^{Lys} and its truncated form, TLYS41A. Uridine 8, the target base of MmTtuI, is highlighted in red. (B) EctRNA$^{Arg\ (ICG)}$and its mini-RNA loop. Cytidine 32, the target base of EcTtcA, is highlighted in red.

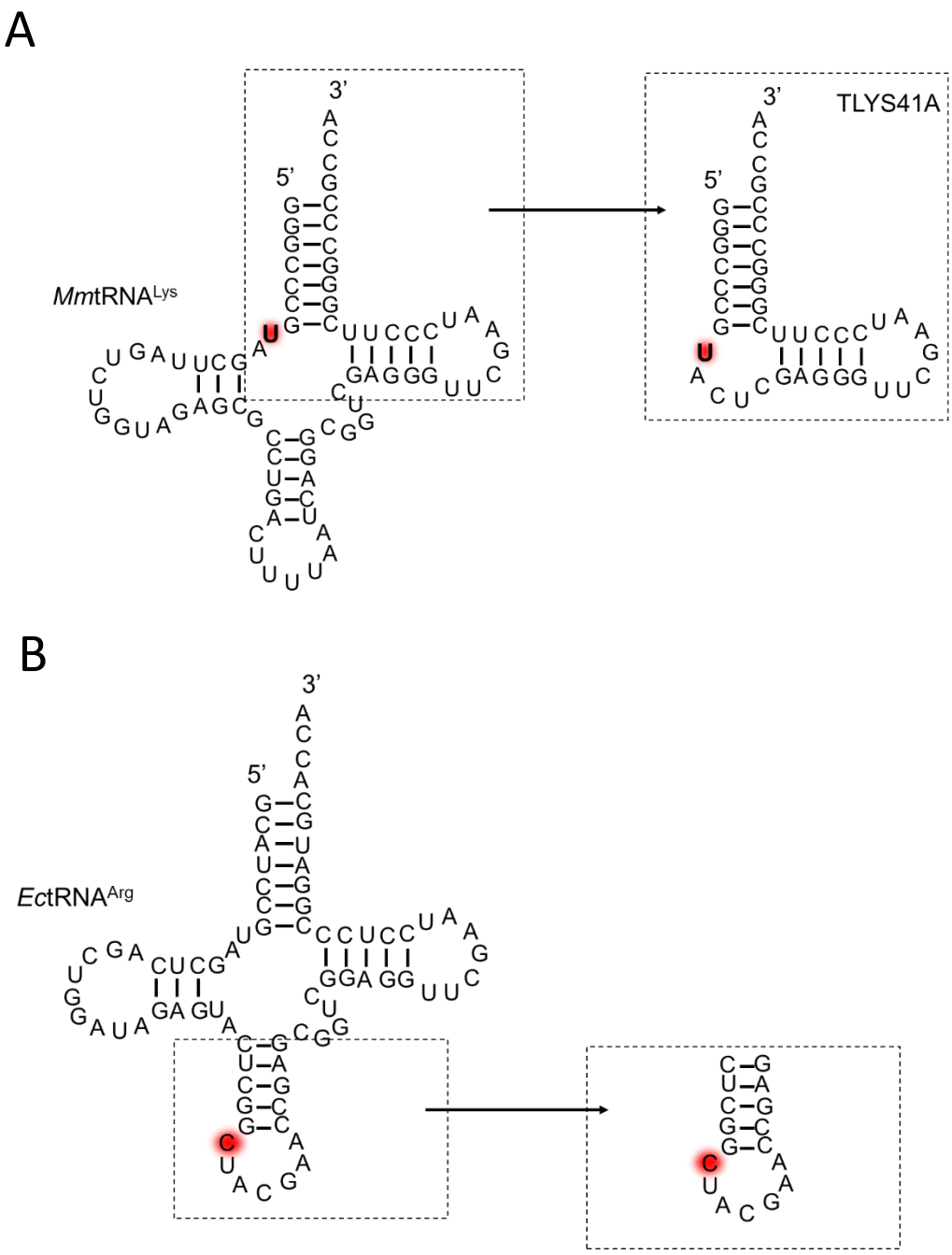


Figure S2. Monitoring binding of MmtRNA^{Lys} to apo-MmTtuI by fluorescence. (A) Fluorescence spectrum obtained at λ_{ex} = 295 nm of apo-MmTtuI (0.5 µM) in the presence of various concentrations of tRNA (0 µM in green, 0.25 to 2.5 µM in black and 3 µM in blue). (B) Variation of fluorescence of apo-MmTtuI at 355 nm as a function of tRNA concentration. (C) Fluorescence spectrum obtained at λ_{ex} = 295 nm of apo-MmTtuI (0.5 µM) in the presence of 3 mM AMP-CPP after the addition of various concentrations of tRNA (0 µM in red, 0.25 to 2.5 µM in black and 3 µM in violet). (D) Variation of fluorescence at 355 nm of apo-MmTtuI in the presence of 3 mM AMP-CPP as a function of tRNA concentration.

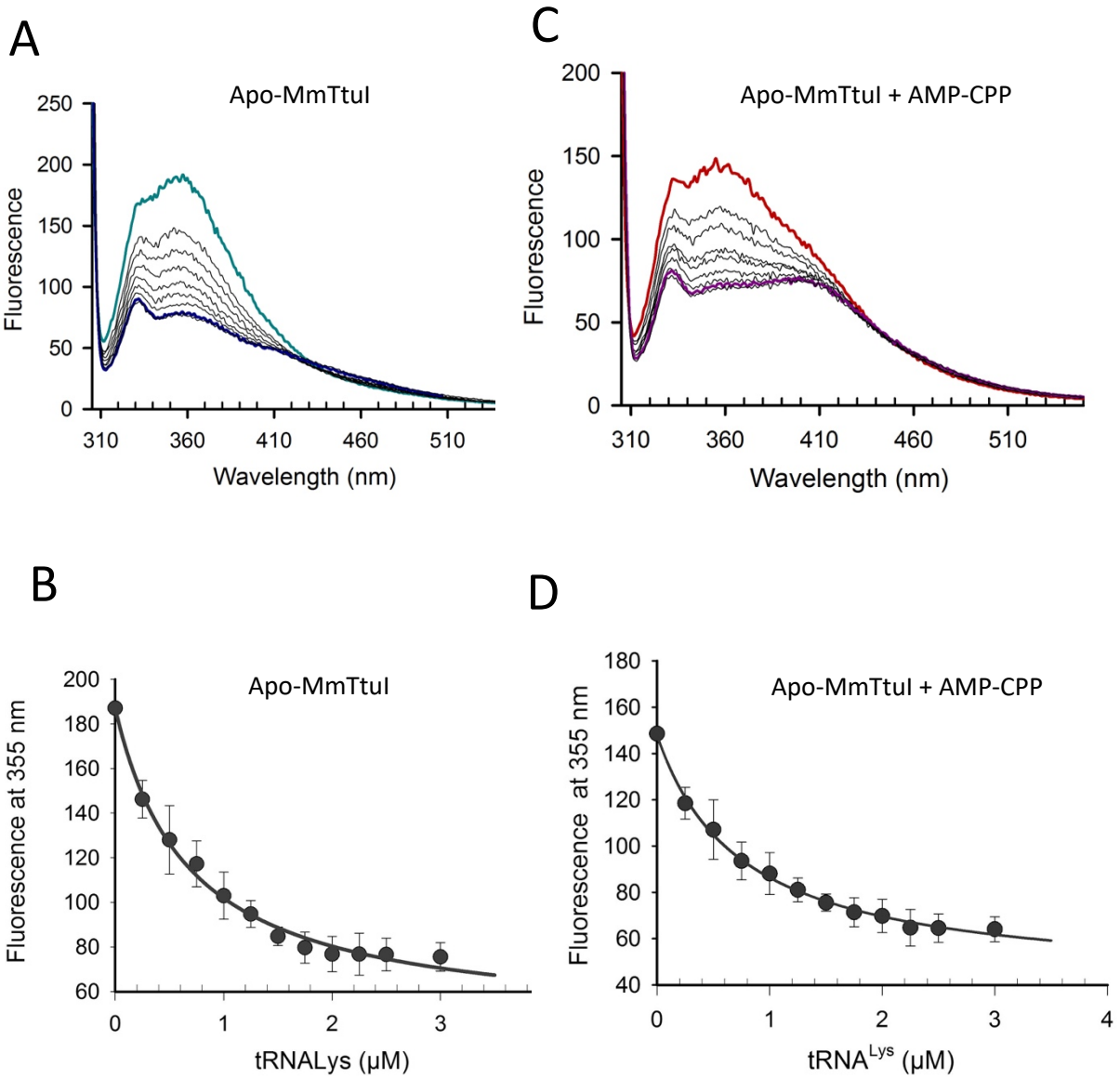


Figure S3. Effect of $MgCl_2$ on RNA binding by apo-MmTtuI. The variation of fluorescence of apo-MmTtuI as a function of RNA concentration was monitored in the absence (black) or presence (gray) of 3 mM $MgCl_2$. (A) Titration of apo-MmTtuI by TLYS41A. (B) Titration of apo-MmTtuI by $MmtRNA^{Lys}$.

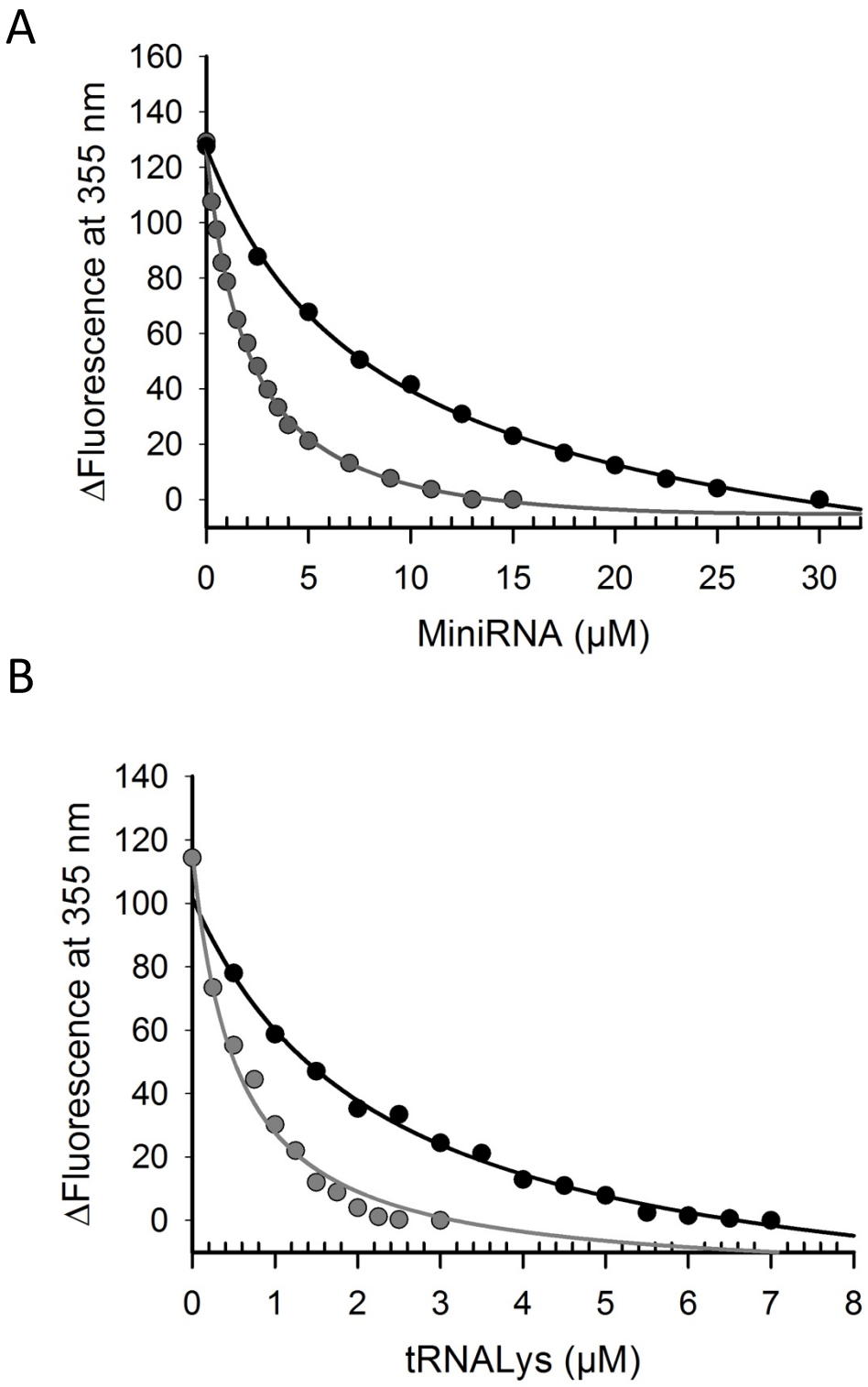
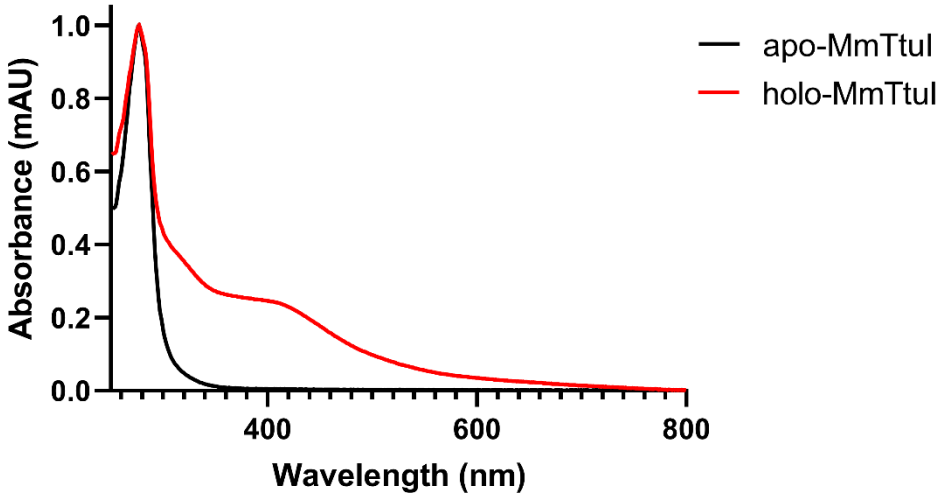


Figure S4. UV-visible spectra of MmTtuI and EcTtcA before and after cluster reconstitution. (A) MmTtuI. (B) EcTtcA.

A



B

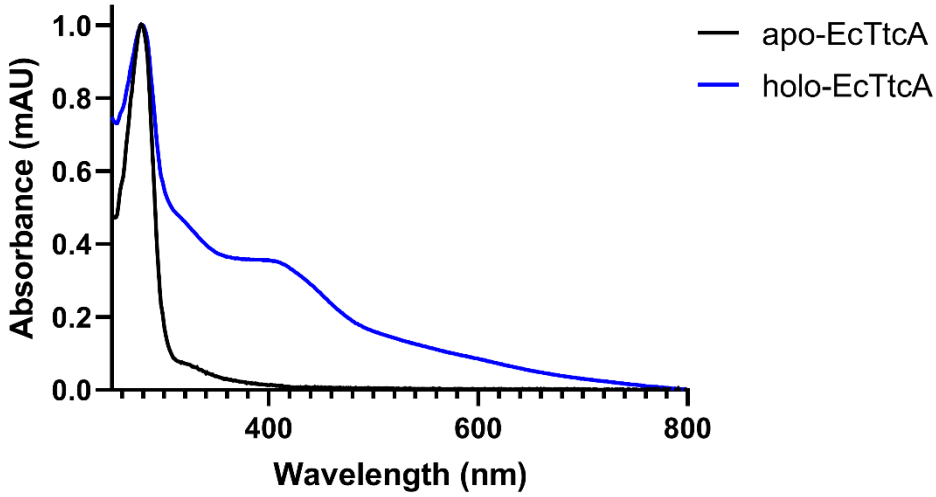
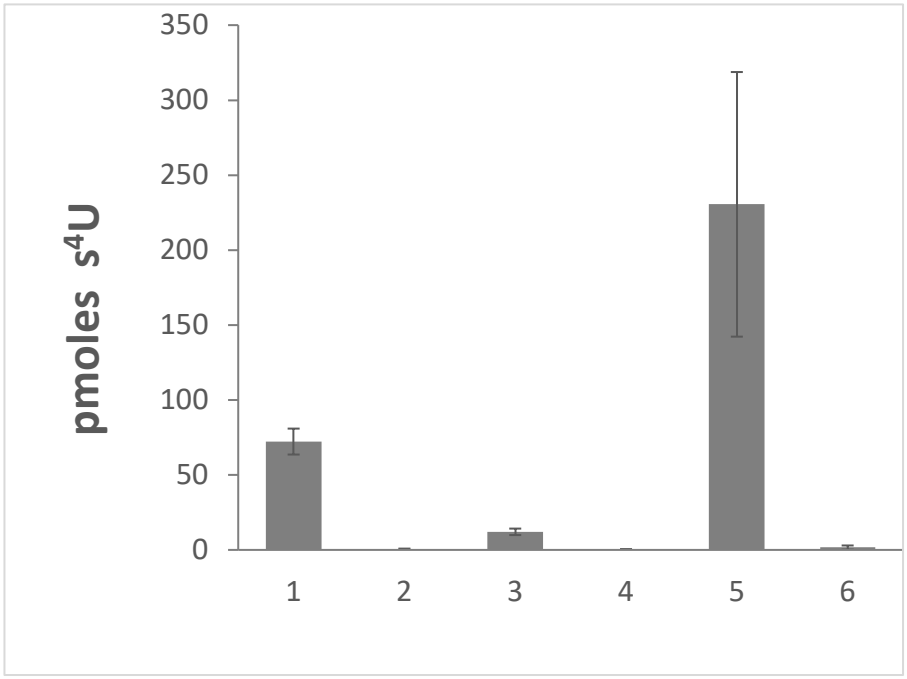


Figure S5. Catalytic activity of holo-MmTtuI and holo-EcTtcA. holo-enzyme (5 µM) was incubated for 1h at 37°C with tRNA transcript or mini-RNA (15 µM), in the presence of 2.5 mM MgCl2, 0.25 mM ATP and 1 mM Na_2S as the sulfur source. Formation of s^4U- and s^2C-tRNA by MmTtuI and EcTtcA, respectively, was monitored by HPLC-coupled MS/MS after tRNA digestion. The data shown are mean values based on 3 different experiments. (A) holo-MmTtuI + $MmtRNA^{Lys}$ (1), $MmtRNA^{Lys}$ alone (2), holo-MmTtuI + TLYS41A (3), TLYS41A alone (4), holo-MmTtuI + 2AP9-$MmtRNA^{Lys}$ (5), 2AP9-$MmtRNA^{Lys}$ alone (6). (B) holo-EcTtcA + $EctRNA^{Arg}$ (1), $EctRNA^{Arg}$ alone (2), holo-EcTtcA + mini-RNA^{Arg} (3), mini-RNA^{Arg} alone (4).

A



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