Supplementary Data

Refinement of AptTFIIB-4

The method we used to stitch multiple elements together in a composite RNA molecule requires the identification of a double stranded stem in each element that can be used as the point of integration (26). For AptTFIIB-4, we identified such a stem by creating a series of deletion constructs. By testing their binding activity and compare their predicted secondary structure, we were able to propose the model depicted in Figure 1A. Here we provide more data regarding several critical and representative deletion constructs.

First, we deleted 28 nucleotides from the 5' end to form the construct B4-Del5 [5'-GGGAGCUAAUGUAGGAUGCUGGGGUAGUCCAGCCCUAGAAUAAGCGCUAGUACUACAAGCUUCUGGAGCUCGGU-3']. Second, we deleted 13 nucleotides from the 3' end to form the construct B4-Del3 [5'-GGGAGAAUUCAACUGCCAUCUAGGCAAAGAGCUAAUGUAGGAUGCUGGGGUAGUCCAGCCCUAGAAUAAGCGCUAGUACUACAAGCU-3']. Both deletions left a putative stem intact and, as shown in Figure S1, fully retained their binding activity. Next we deleted both the 5' and the 3' segment to form the MiniB4 construct [5'-GGGAGCUAAUGUAGGAUGCUGGGG UAGUCCAGCCCUAGAAUAAGCGCUAGUACUACAAGCU-3']. This construct was still active as measured by electrophoretic mobility shift assay (EMSA), as shown in Figure S1.

The EMSA was performed with ^{32}P labeled RNA probes prepared using the MAXIscript T7 *in vitro* transcription kit (Ambion) and [α-^{32}P]CTP (GE Healthcare). The binding buffer contained 50 mM Tris-Cl [pH 7.6], 200 mM potassium acetate, 5 mM $MgCl_2$, and 2.5 mM dithiothreitol. The binding mix was run on a 2.5% agarose gel in ¼ TBE buffer (22.25 mM Tris base, 22.25 mM borate, 0.5 mM EDTA) at 4ºC.

We used the program *mfold* (15) to predict secondary structures for these three constructs and the full-length aptamer. As shown in Figure S1B, these all share the same three-way junction, which connects two stem-loops and the stem we identified as the point of integration with the rest of the taRNA molecule.

RNA expression and ribozyme function in yeast

After transformation, it was critical to confirm that the taRNA and the control constructs, both positive and negative, were expressed at a similar level, so their activity could be compared. It was also important to confirm the functionality of some designed features such as the ribozyme activity. For this type of conformation we used RT-PCR assays. In Figure S2A, we indicate the annealing sites of three primers on the RNA transcript. Their sequences are as follows: U1 [5'-CTGTGGTGCTCGCGGCTGGGAACGAAA-3']; U2 [5'-GCGACGAAACCTCGAGTCATACTCG-3']; D [5'-CGGCAGTACTCCGGAACGCTGGTGGA-3']. Different aptamers or aptamer derivatives were inserted at a point between U2 and D. The cleavage site of the 5' hammerhead ribozyme was located between U1 and U2.

For each RT-PCR reaction 100 ng total RNA was used. Reverse transcription was performed at 45ºC for 20min, followed by 20 PCR cycles. Each RT-PCR was paired with a control reaction (NRT) in which the reverse transcriptase was omitted. As shown in Figure S2B, when the primer pair U2 and D was used, a band of similar intensity was observed for taRNA and its control constructs, and no DNA contamination was detected in this assay. In contrast, when the primer pair U1 and D was used, no RT-PCR product was detected for the taRNA (Figure S2C), indicating that cleavage of the ribozyme had occurred.

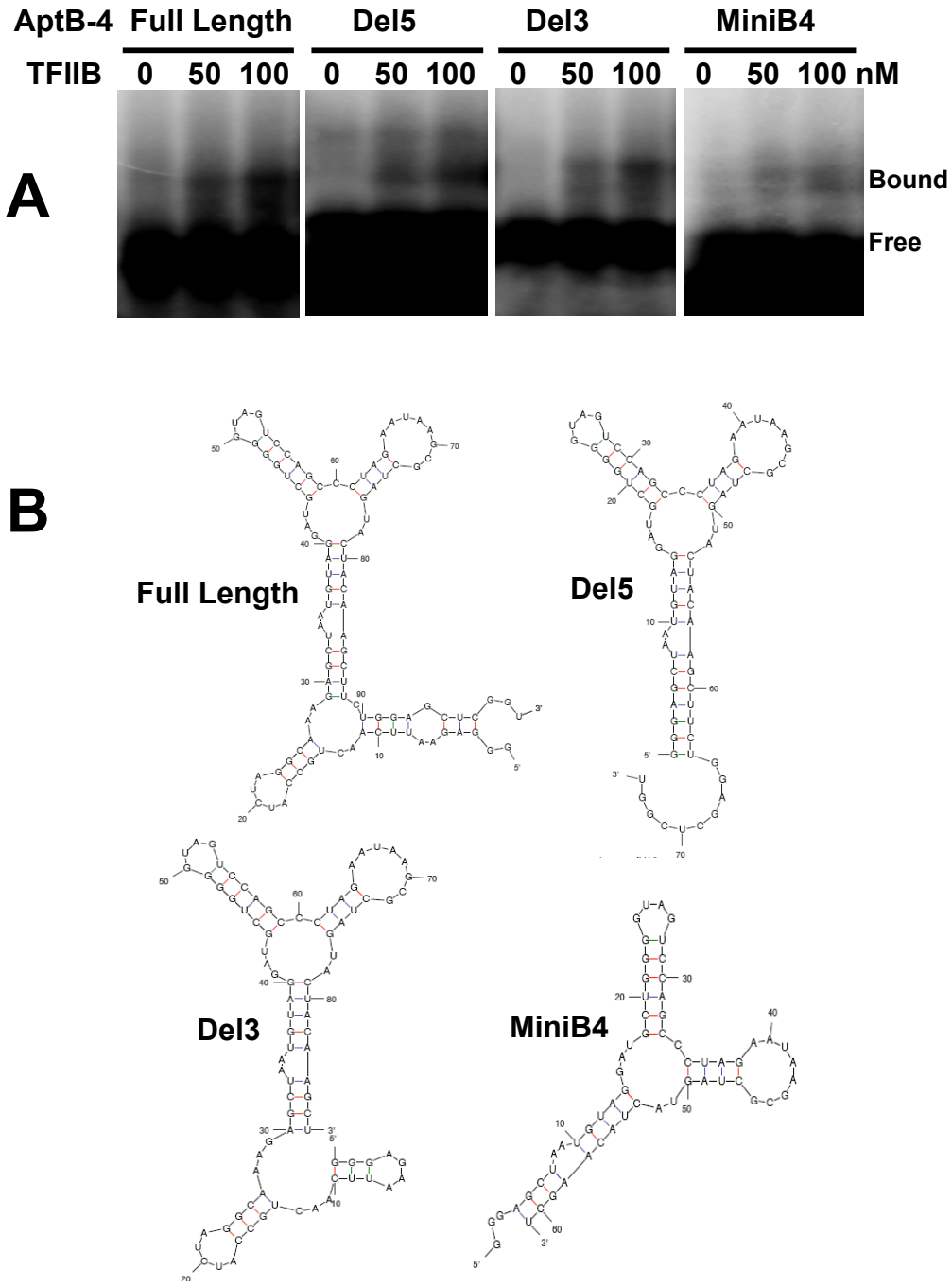

Figure S1. Refinement of AptTFIIB-4. (A) Activity of deletion constructs as measured by EMSA. (B) Predicted secondary structures for these constructs.

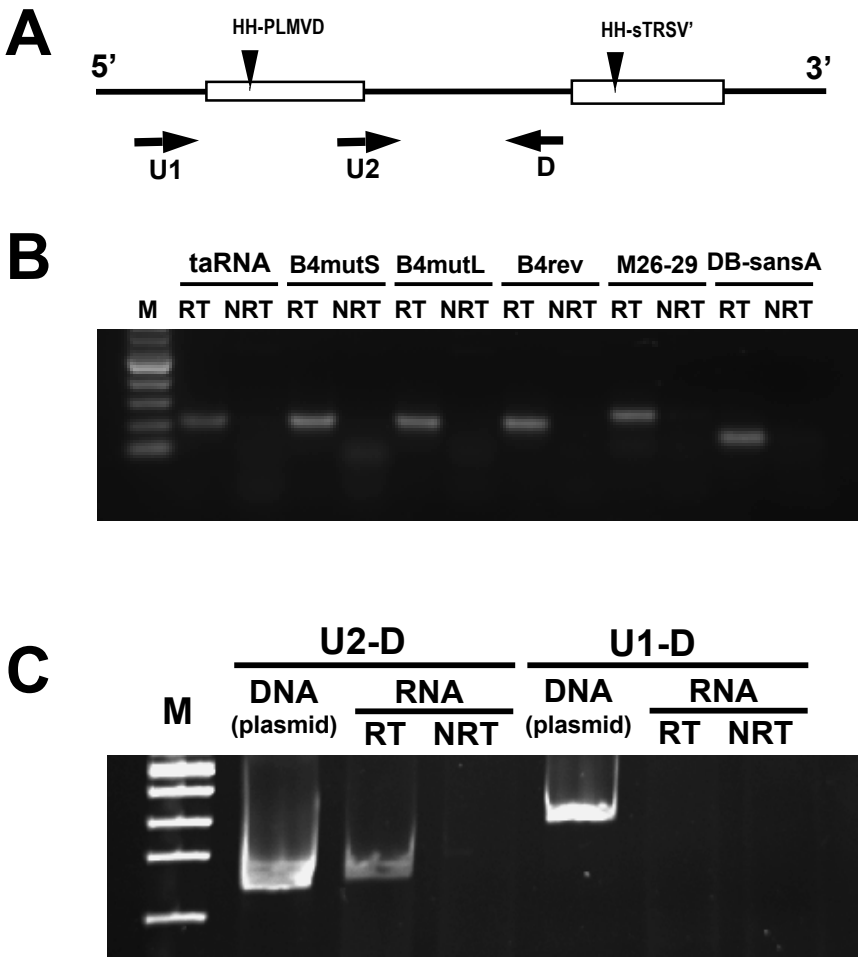

Figure S2. RNA expression and ribozyme function in yeast. (A) Schematic diagram of the RNA transcript with primer annealing sites indicated. (B) RT-PCR assay using the U2-D primer pair revealing similar level of expression for different constructs. NRT: no reverse transcriptase added. (C) Failure of U1-D primer pair to amplify the RNA transcript, indicating cleavage of the ribozyme. The taRNA transcript was used for the assay presented here. M: molecular weight marker.