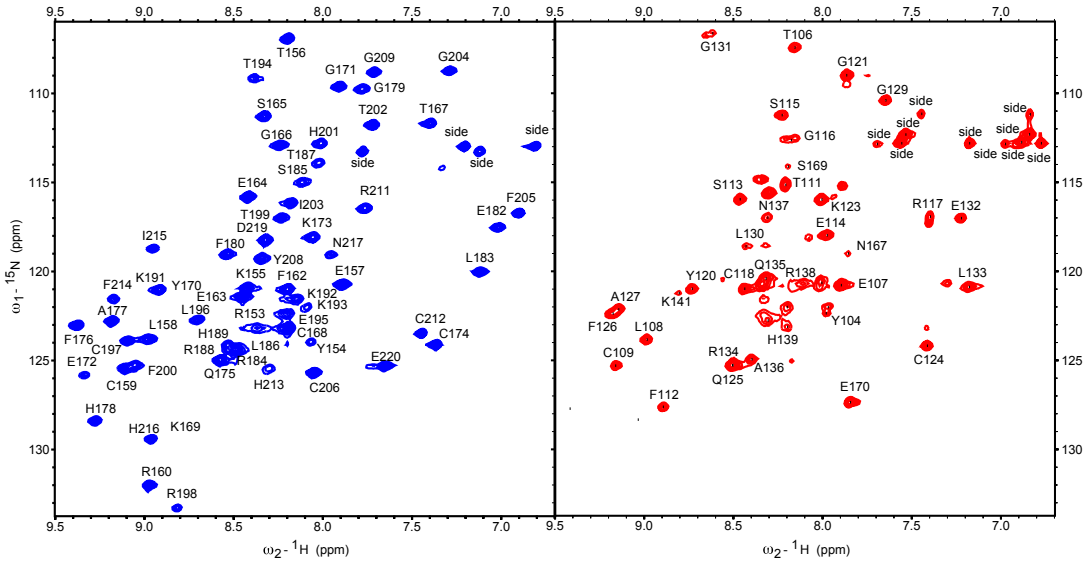
Three residues make an evolutionary switch for folding and RNA-destabilizing activity in the TTP family of proteins

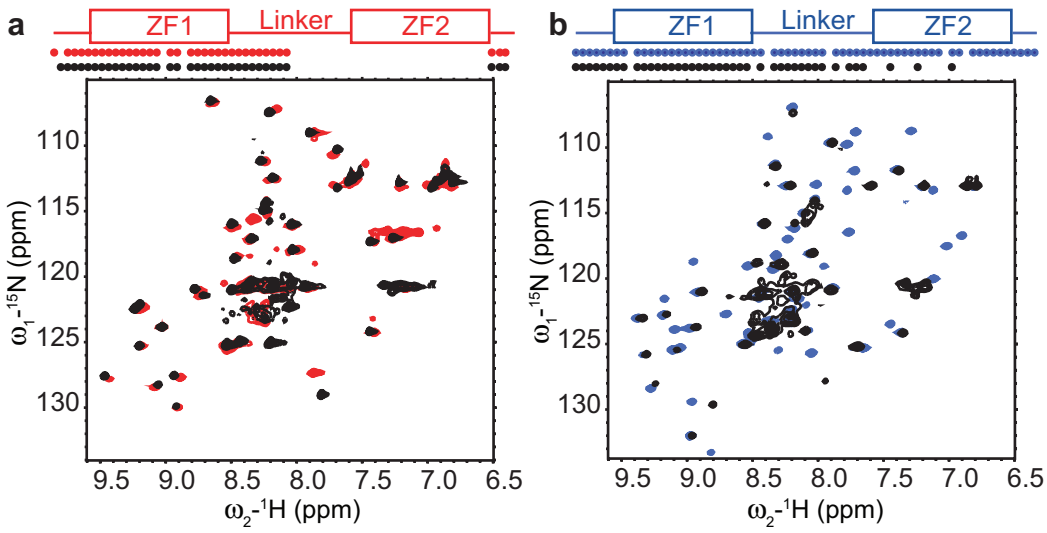
Laura M. Deveau and Francesca Massi*

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605

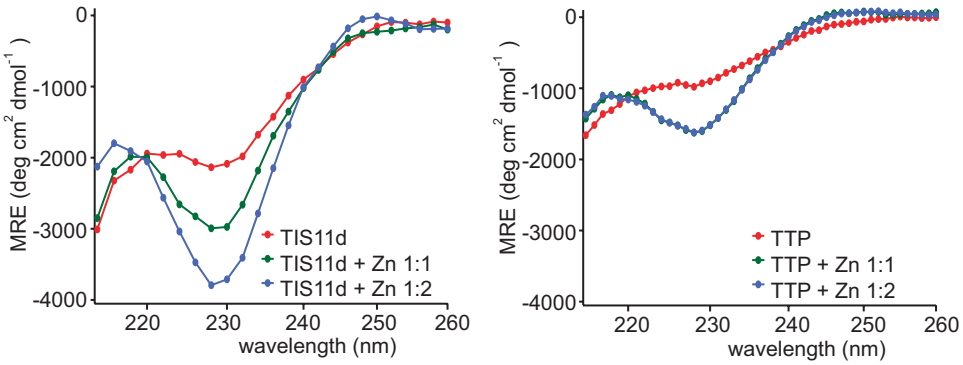
SUPPLEMENTARY INFORMATION



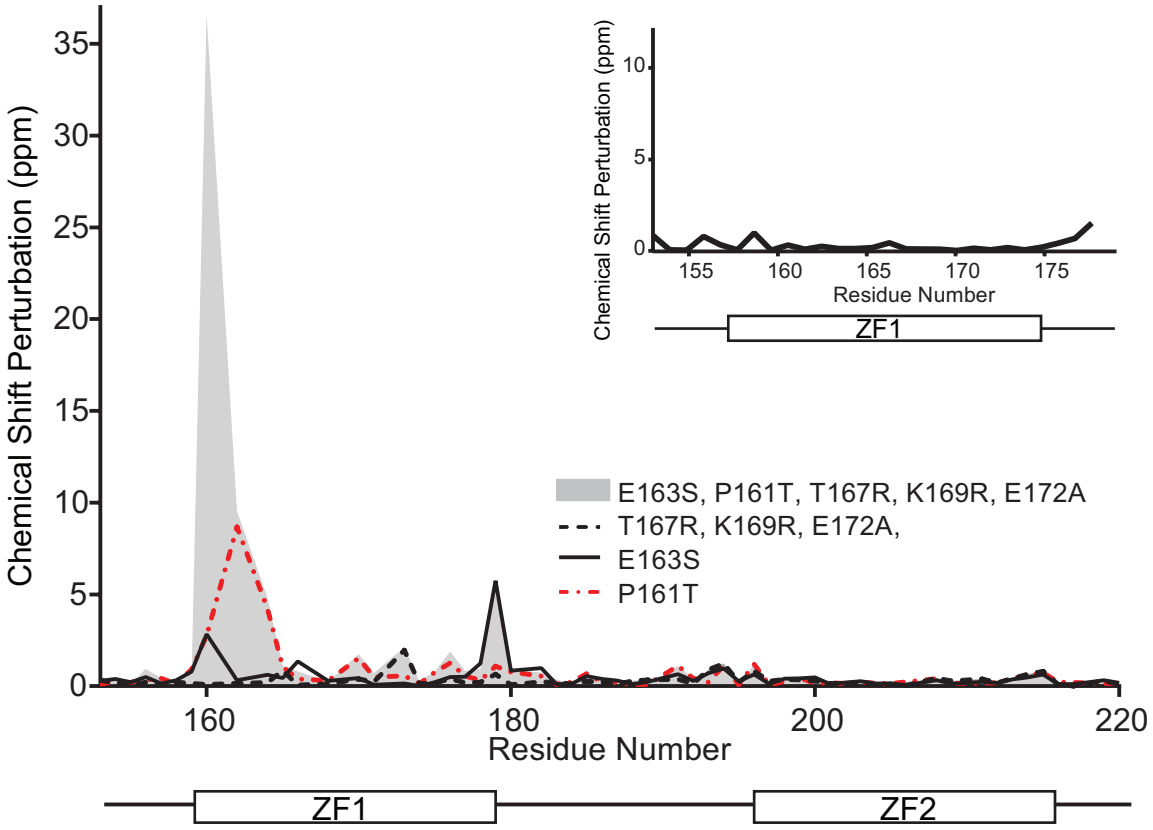
Supplementary Figure 1. ^{15}N-^{1}H 2D HSQC spectra of TIS11d and TTP. ^{15}N-^{1}H HSQC spectra of TIS11d and TTP are shown in blue and red, respectively. The resonance assignments are indicated on each spectrum.



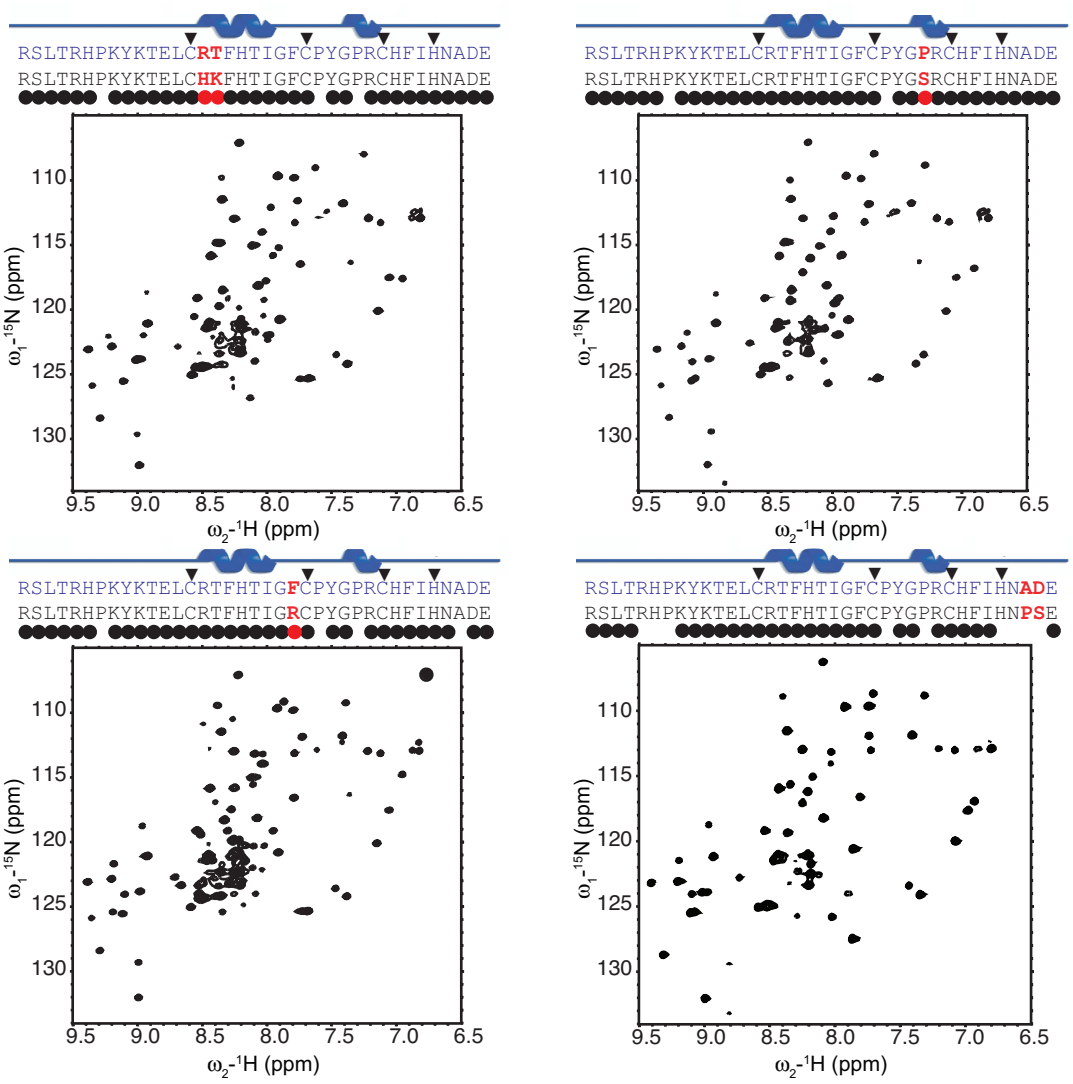
Supplementary Figure 2. ^{15}N-^{1}H HSQC spectra of TTP, C162S TTP mutant, TIS11d and C212S TIS11d mutant. a) Overlay of ^{15}N-^{1}H HSQC spectra of TTP (red) and C162S TTP mutant (black), whose C-terminal ZF cannot fold due to a missing Zn-coordinating Cys residue. b) Overlay of ^{15}N-^{1}H HSQC spectra of TIS11d (blue) and C212S TIS11d mutant (black), whose C-terminal ZF cannot fold due to a missing Zn-coordinating Cys residue shows that only the N-terminal ZF of the mutant protein adopts a stable fold. On top, a schematic representation of the TZF domain shows the zinc fingers as rectangles and the linker as line. The dots indicate residues along the sequence with a cross-peak in the ^{15}N-^{1}H HSQC spectrum.



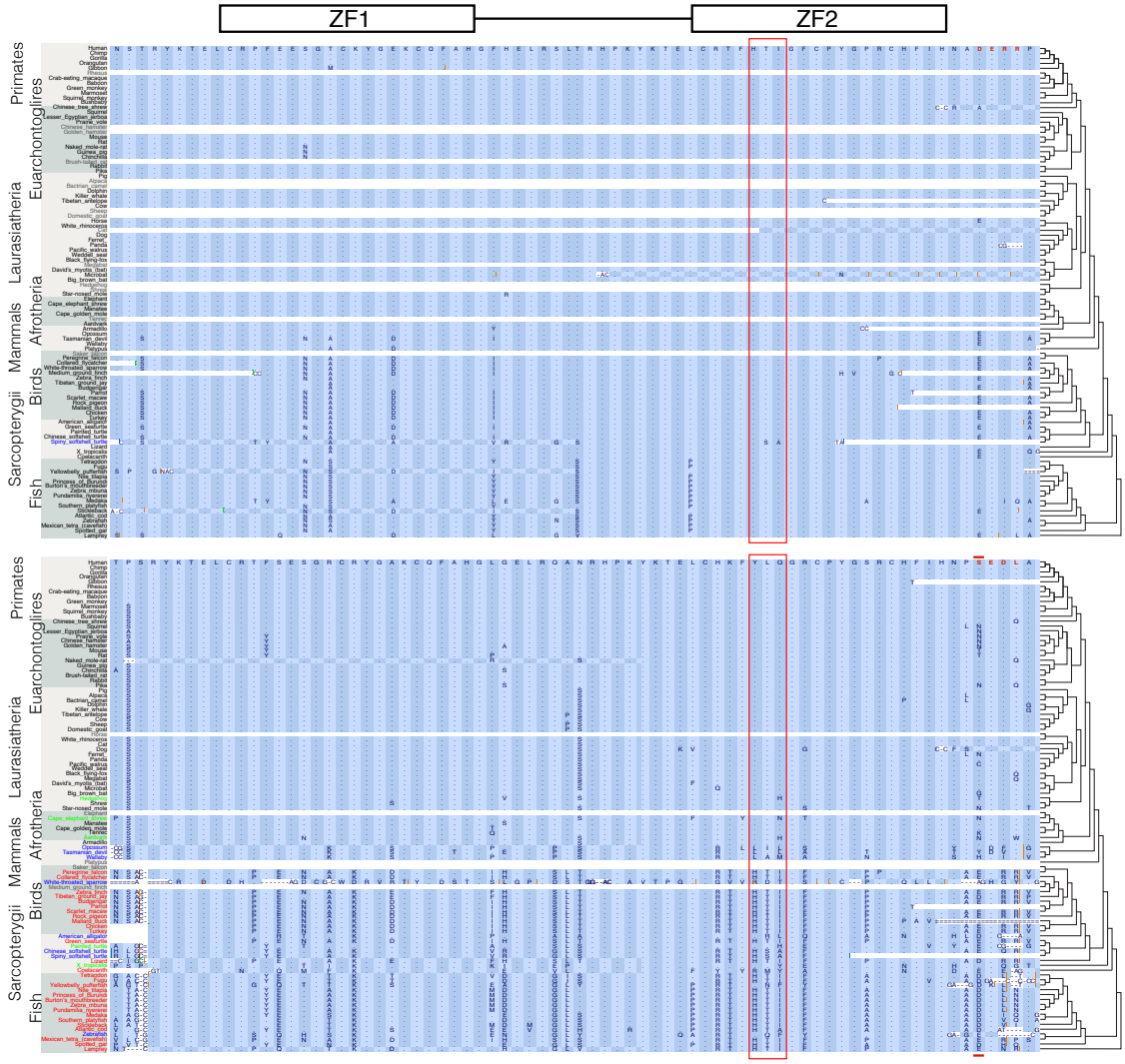
Supplementary Figure 3. CD spectra of TIS11d and TTP at different concentration of Zn^{2+}. Far-UV circular dichroism (CD) spectra of TIS11d (left) and TTP (right) collected with different amounts of Zn^{2+}. The three spectra are collected at different values of the concentration ratio [$Zn2^{+}$]/[TZF]: 0 (red), 1 (green) and 2 (blue).



Supplementary Figure 4. Chemical shift perturbation of TIS11d mutant proteins. The chemical shift difference between WT TIS11d and different mutant proteins containing one or more single point mutations in ZF1 is plotted as a function of residue number. All mutations transform WT residues in TIS11d to the corresponding residue in TTP. The largest differences in chemical shifts are observed upon mutation of P161 to the corresponding residue in TTP (T111). The inset shows the chemical shift difference observed between TTP and the mutant protein of TIS11d where we mutated all the residues of TIS11d ZF1 to those found in TTP (P161T, E163S, T167R, K169R, E172A). A schematic representation of the TZF domain that shows the zinc fingers as rectangles and the linker as line is depicted below the x-axis.



Supplementary Figure 5. ^{15}N-^{1}H HSQC spectra of TIS11d mutants. The figure shows ^{15}N-^{1}H HSQC spectra of the TIS11d mutants designed to identify the residues important for stabilizing the structure of ZF2. On top of each spectrum the sequence of ZF2 the particular mutant protein is shown in black. Point mutations to the ZF2 are highlighted in red. The amino acid sequence of WT TIS11d is shown for reference in blue. The residues that have a cross-peak in the HSQC are represented by black dots. Black triangles represent the CCCH zinc coordinating residues.



Supplementary Figure 6. Sequence alignment of the TZF domain of TIS11d and TTP. Sequence alignment of the TZF domain of TIS11d (top) and TTP (bottom) taken from 100 vertebrate species. The alignment was generated from the Genome browser (http://genome.ucsc.edu/)[1-3]. The three residues located at the C-terminal half of the α-helix of ZF2, that alone determine the structure or lack of structure of ZF2, are highlighted by a red box. Additional residues flanking the TZF domain that are important for stabilizing TIS11d ZF2 are shown in red. A schematic representation of the TZF domain depicting the zinc fingers as rectangles and the linker as line is shown on top.

REFERENCES

(1) Blanchette, M., Kent, W. J., Riemer, C., Elnitski, L., Smit, A. F. A., Roskin, K. M., Baertsch, R., Rosenbloom, K., Clawson, H., Green, E. D., Haussler, D., and Miller, W. (2004) Aligning multiple genomic sequences with the threaded blockset aligner. *Genome Res. 14*, 708–715.
(2) Hubisz, M. J., Pollard, K. S., and Siepel, A. (2011) PHAST and RPHAST: phylogenetic analysis with space/time models. *Brief. Bioinformatics 12*, 41–51.
(3) Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., and Haussler, D. (2002) The human genome browser at UCSC. *Genome Res. 12*, 996–1006.