

## Supplemental Materials:

### An Expanded Class of Histidine-Accepting tRNA-like Structures

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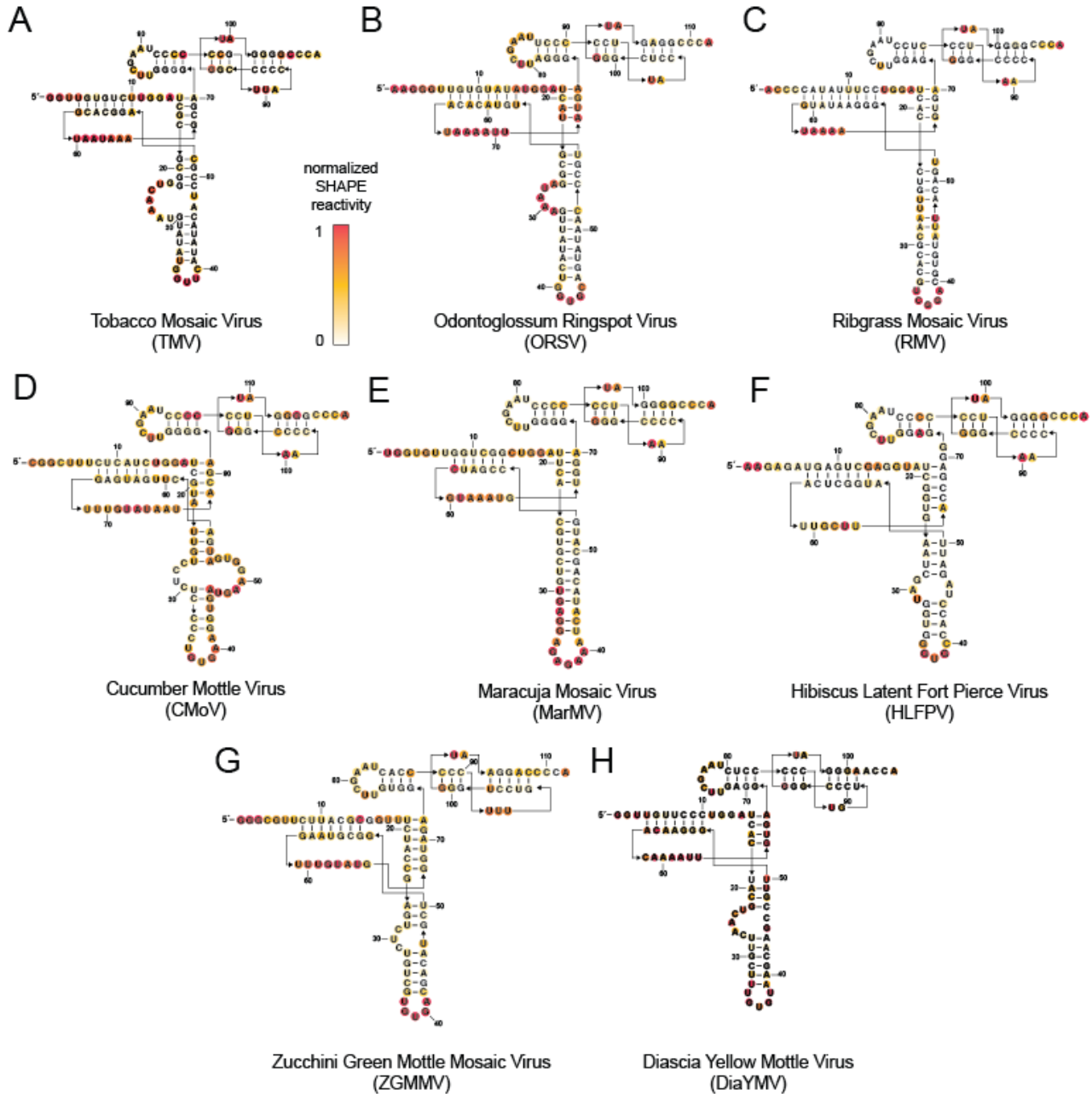
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**Figure S1:** Chemical probing data for diverse TLS<sup>His</sup>

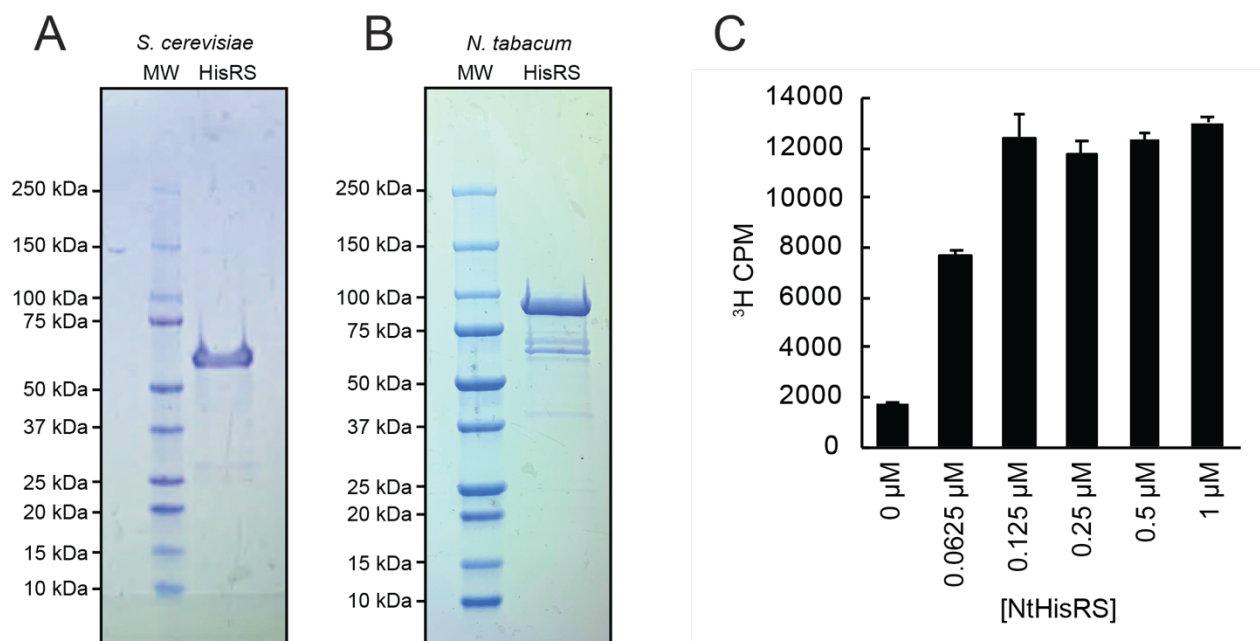
**Figure S2:** SDS-Page of purified HisRS from and *S. cerevisiae* *N. tabacum*

**Figure S3:** Additional D-loop/T-loop mutant <sup>3</sup>H-incorporation assays

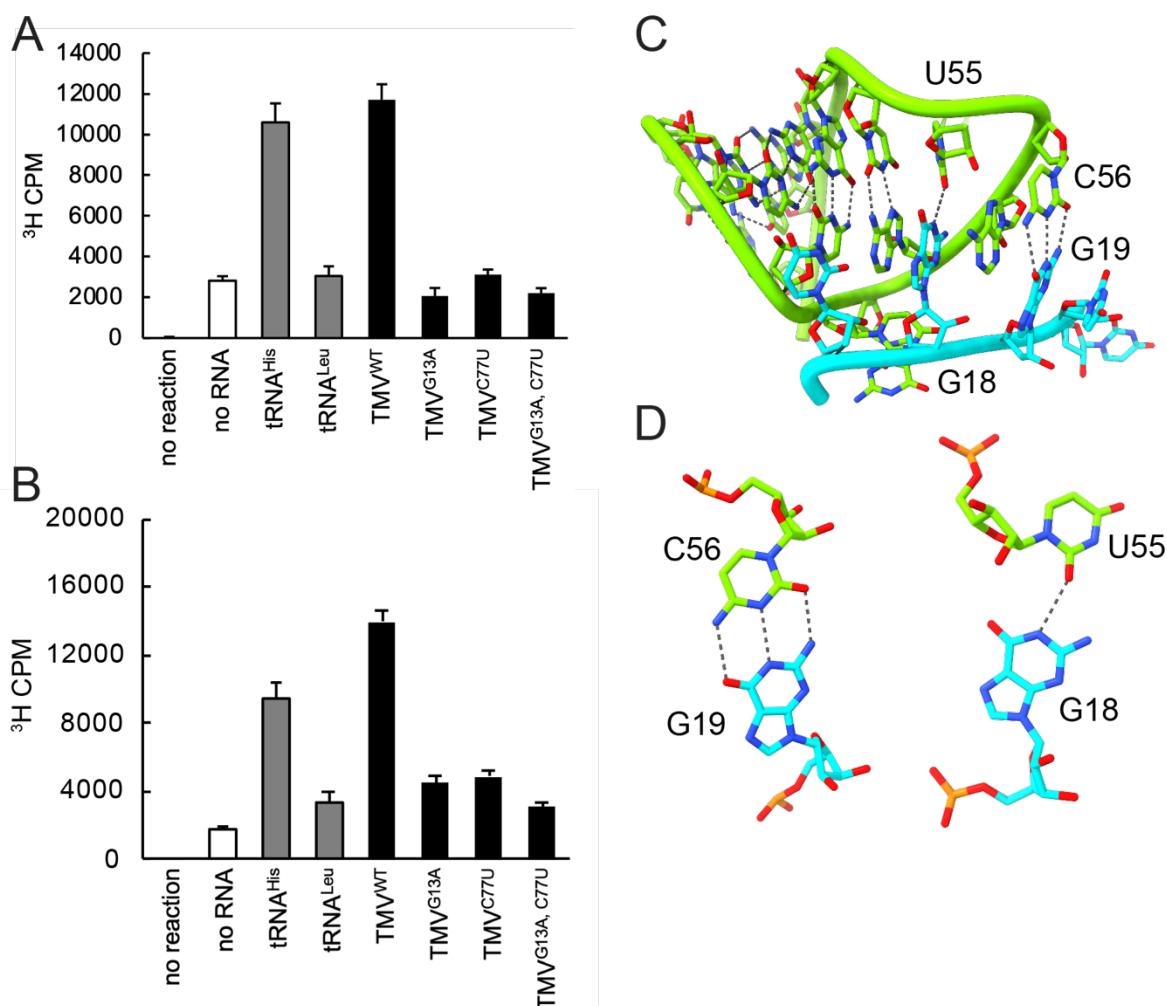
**Figure S4:** Chemical probing data for D-loop/T-loop mimic mutants



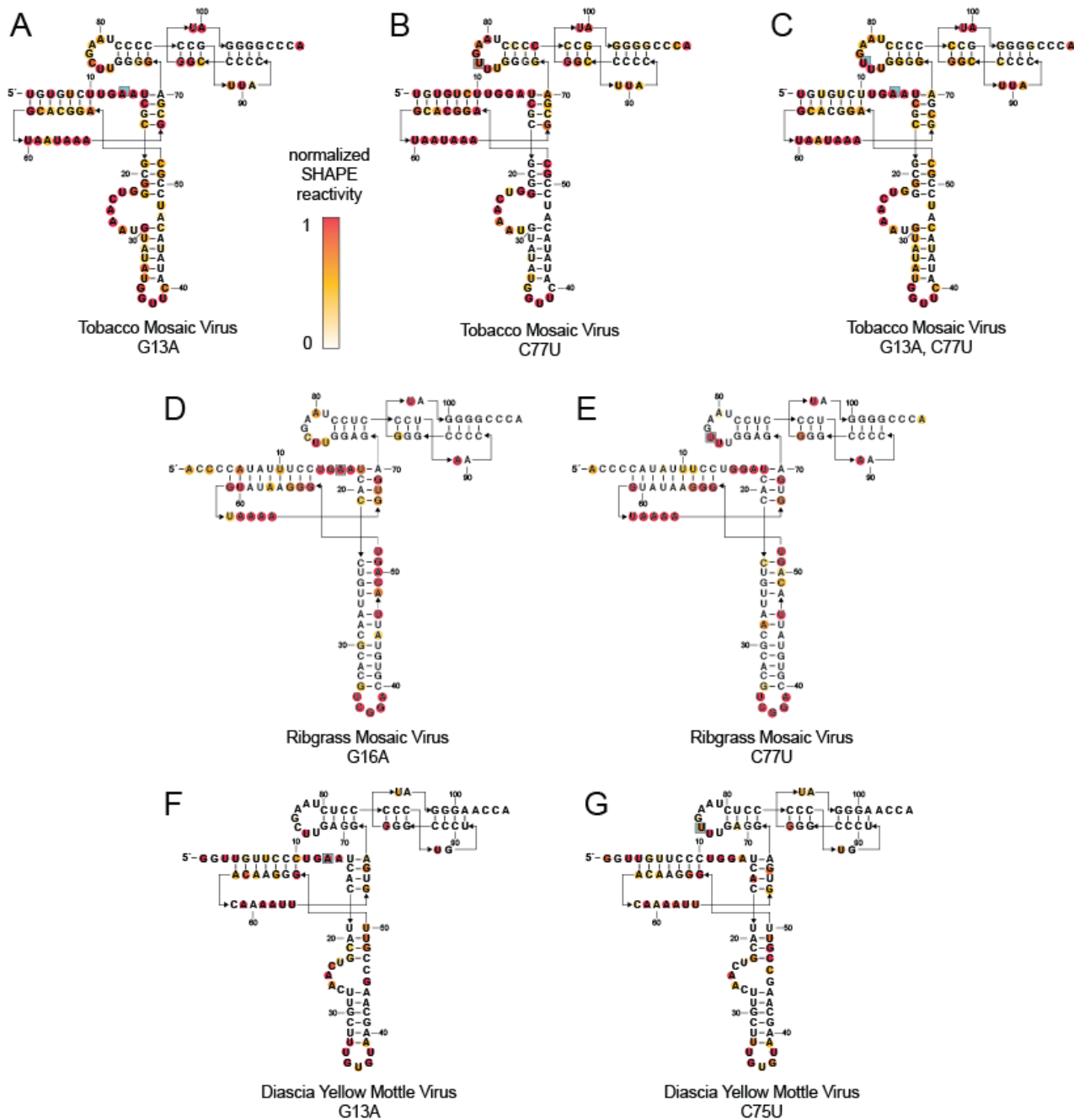
**Supplemental Figure S1. Chemical probing data for diverse TLS<sup>His</sup>.** A-H) Chemical probing of all representative TLS<sup>His</sup> RNAs using the SHAPE reagent NMIA: Tobacco Mosaic virus (A), Odontoglossum Ringspot Virus (B), Ribgrass Mosaic Virus (C), Cucumber Mottle Virus (D), Maracuja Mosaic Virus (E), Hibiscus Latent Fort Pierce Virus (F), Zucchini Green Mottle Mosaic Virus (G), and Diascia Yellow Mottle Virus (H). Reactivity was background subtracted and normalized to flanking 5' and 3' normalization hairpins (not depicted, see Supplemental File 2 for sequence details). Coloring represents degree of normalized modification according to the inserted legend.



**Supplemental Figure S2. SDS-Page of purified HisRS from *S. cerevisiae* and *N. tabacum*. A-B)** SDS-PAGE gels demonstrating the purity of (A) *S. cerevisiae* HisRS and (B) *N. tabacum* HisRS (NtHisRS). **C)**  $^3\text{H}$ -L-histidine incorporation of ORSV by varying NtHisRS concentration. Histidylation of each RNA, as measured by covalent incorporation of  $^3\text{H}$ -L-histidine by *N. tabacum* histidine tRNA-synthetase, is reported in CPM. [RNA] was  $\sim 0.1 \mu\text{M}$ . Reactions were incubated for one hour and performed in triplicate. Error bars represent one standard error from the mean.



**Supplemental Figure S3. Additional D-loop/T-loop mutant <sup>3</sup>H-incorporation assays. A-B)** <sup>3</sup>H-L-histidine incorporation of four TMV TLS<sup>His</sup> RNAs: TMV<sup>WT</sup>, TMV<sup>G13A</sup>, TMV<sup>C77U</sup>, and TMV<sup>G13A, C77U</sup>. Histidylation of each RNA, as measured by covalent incorporation of <sup>3</sup>H-L-histidine by (A) *S. cerevisiae* histidine tRNA-synthetase and (B) *N. tabacum* histidine tRNA-synthetase, is reported in CPM. Each reaction was performed in triplicate. Error bars represent one standard error from the mean. **C)** D-loop (cyan)/T-loop (green) interaction of *E. coli* tRNA<sup>His</sup> (PDB: 4RDX). **D)** Specifics of the long-range interaction between the D-loop and T-loop of *E. coli* tRNA<sup>His</sup> demonstrate the Watson-Crick compensatory mutations in panel A and B are unlikely to restore the canonical interaction in this region of TLS<sup>His</sup>.



**Supplemental Figure S4. Chemical probing data for D-loop/T-loop mimic mutants. A-G)** Chemical probing of all mutant D-loop/T-loop mimic TLS<sup>His</sup> RNAs using the SHAPE reagent NMIA: Tobacco Mosaic virus G13A (A), Tobacco Mosaic virus C77U (B), Tobacco Mosaic virus G13A, C77U (C), Ribgrass Mosaic Virus G16A (D), Ribgrass Mosaic Virus C77U (E), Diascia Yellow Mottle Virus G13A (F), and Diascia Yellow Mottle Virus C75U (G). Reactivity was background subtracted and normalized to flanking 5' and 3' normalization hairpins (not depicted, see Supplemental File 2 for sequence details).