Supporting Information

Variant Bacterial Riboswitches Associated with Nucleotide Hydrolase Genes Sense Nucleoside Diphosphates

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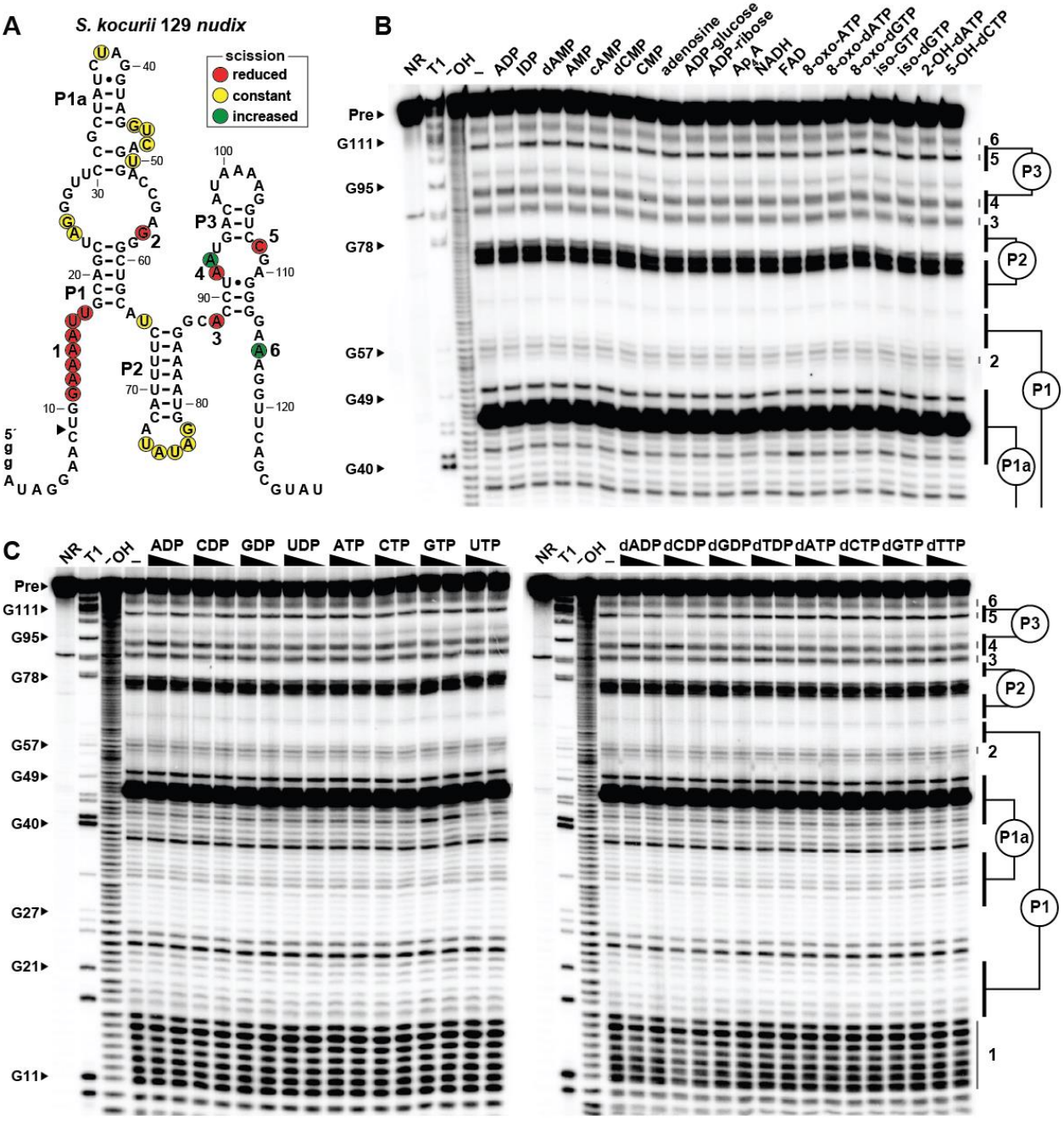

Figure S1. Nucleoside diphosphates are bound by another representative *ykkC* subtype 2c RNA. (A) Sequence and secondary structure of the 129 nucleotide RNA derived from a *nudix* gene of *S. kocurii*. (B) Polyacrylamide gel electrophoresis (PAGE) analysis of the spontaneous RNA cleavage products generated during in-line probing of 5′-^{32}P-labeled 129 *nudix* RNA. In-line probing experiments contained either no ligand (–) or 1 mM of the compound indicated. Regions that correspond to the base-paired P1, P2, and P3 structures are identified. (C) PAGE analysis of

the spontaneous RNA cleavage products generated during in-line probing of 5′-^{32}P-labeled 129 *nudix* RNA. In-line probing experiments contained either no ligand (–), 1 mM, or 100 µM of the nucleoside di- or triphosphate indicated. Additional annotations are described in the legend to **Figure 2**.

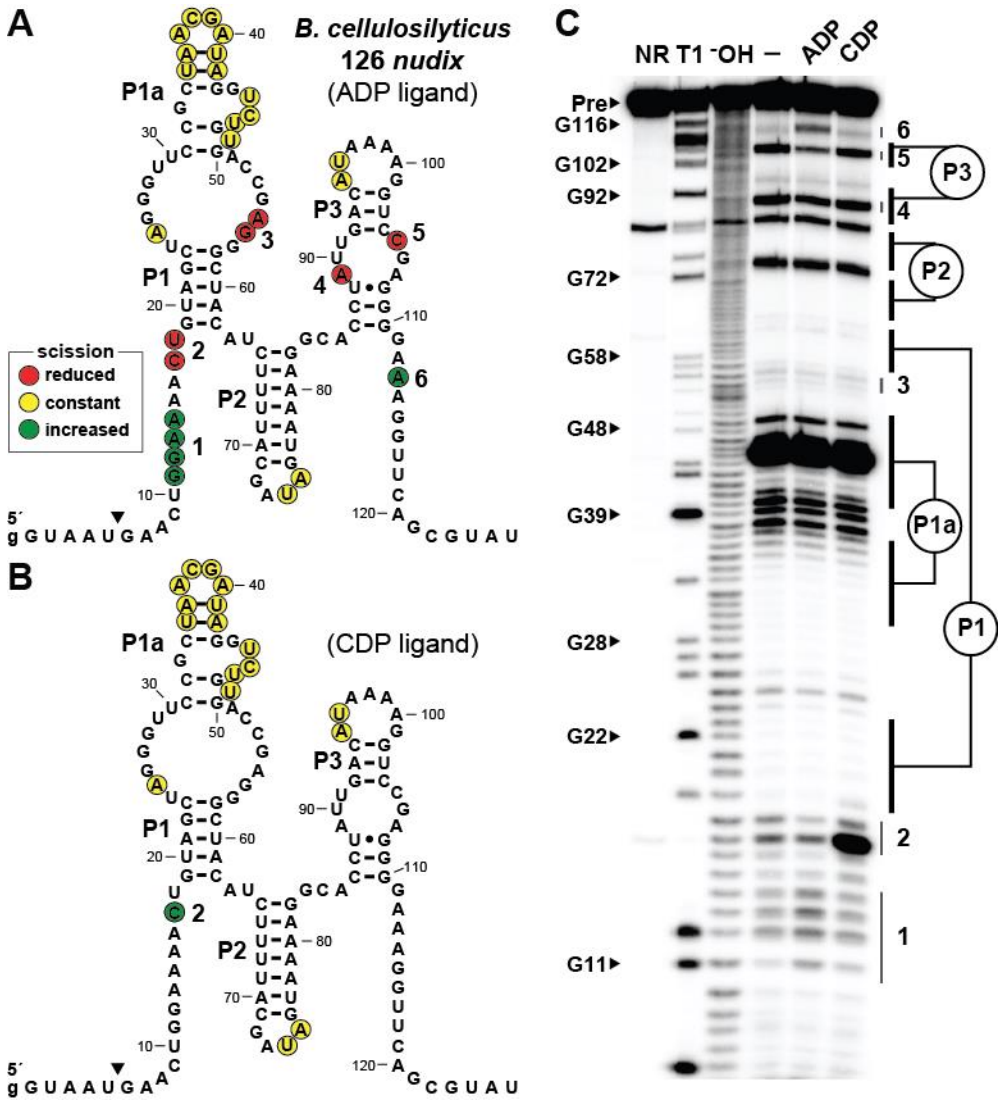

Figure S2. The *ykkC* subtype 2c RNA 126 *nudix* responds differently to ADP and CDP. (A) Sequence and secondary structure of the 126 nucleotide RNA derived from a *nudix* gene form *B. cellulosilyticus*. In-line probing data used to infer structural modulation with ADP were derived from the in-line probing reactions depicted in C. Other annotations are as described for **Figure 2A**. (B) Sequence and secondary structure model depicted in A, but representing the in-line probing data for CDP. Note that CDP is added to the in-line probing assay depicted in C at a concentration below its K_D value (**Figure 4C**), and thus the extent of structural modulation is likely to be poorer than if it were saturated with ligand. Annotations are as described for A. (C) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 126 *nudix* RNA, as depicted in panels A and B, in the presence of ADP or CDP. In-line probing experiments contained either no

ligand (–) or 2 mM of the compound indicated. Annotations are described in the legend to **Figure 2B**.

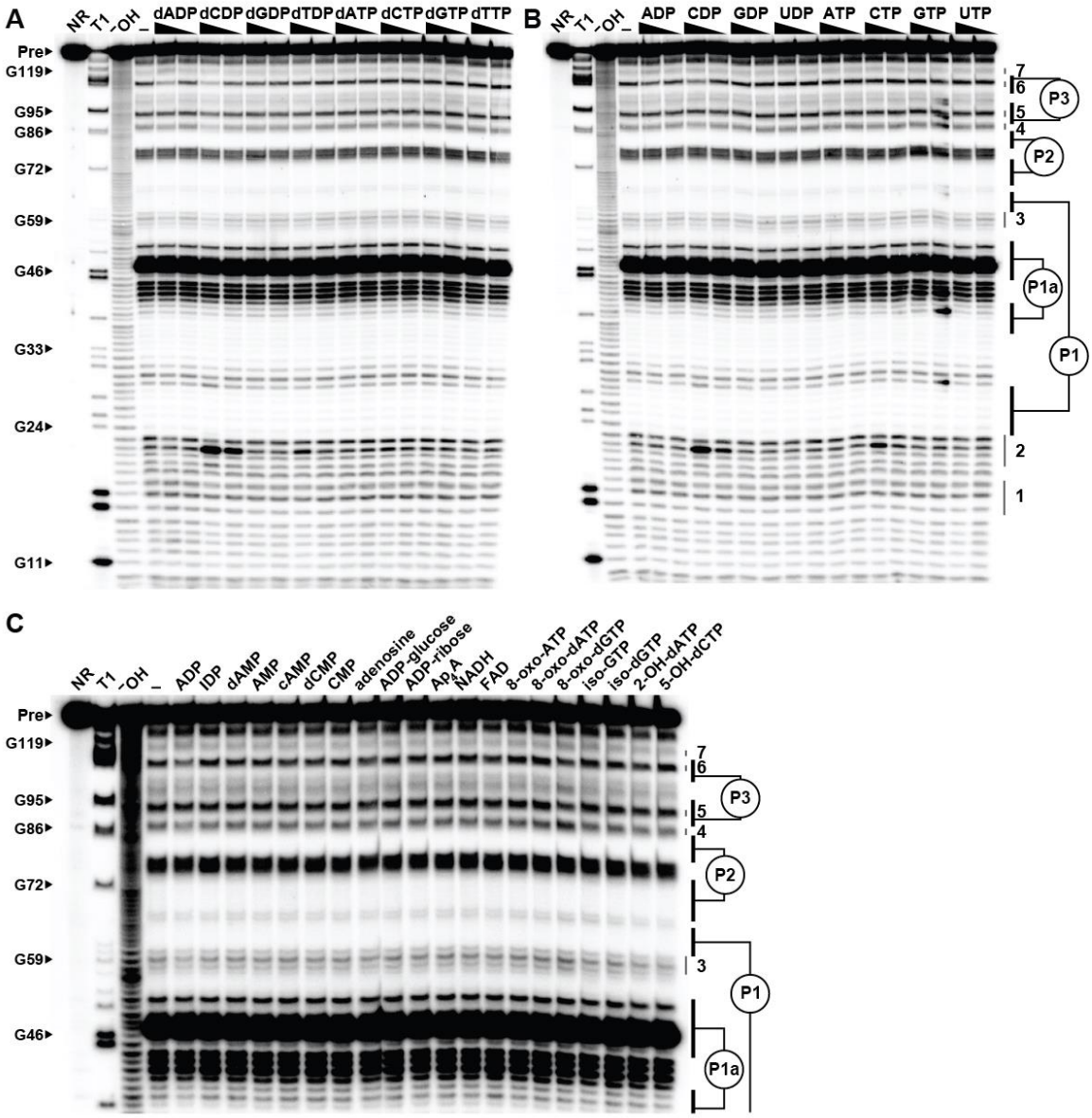

Figure S3. Nucleoside diphosphate binding by the 130 *had* RNA from *L. mesenteroides*. (A, B) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 130 *had* RNA (**Figure 4B**) in the absence of ligand (–), or in the presence of either 1 mM or 100 μM of the nucleoside di- or triphosphate indicated. Annotations are described in the legend to **Figure 2B**. (C) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 130 *had* RNA in the absence of ligand (–) or in the presence of 1 mM of the compound indicated. Annotations are described in the legend to **Figure 2B**.

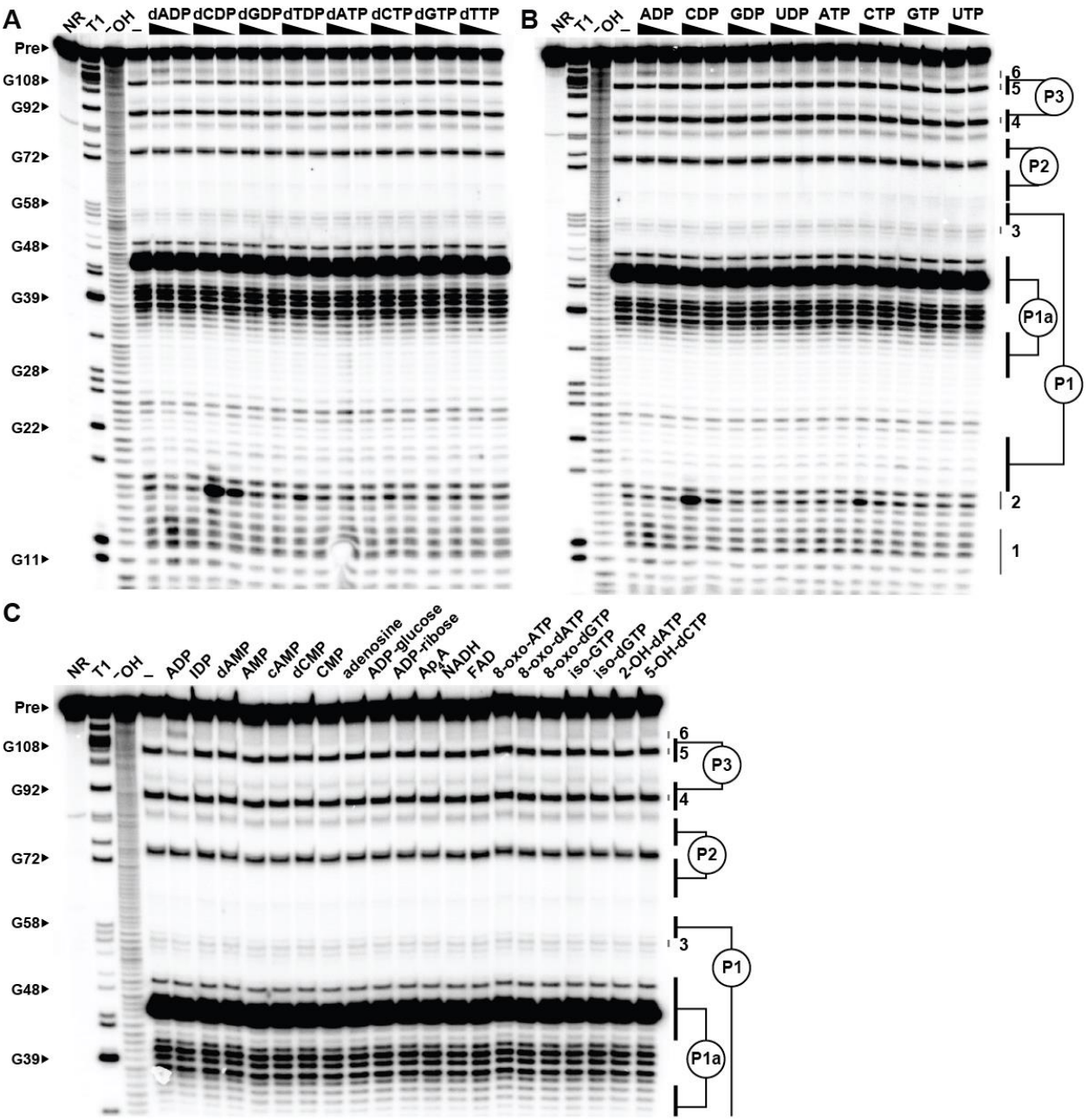

Figure S4. Ligand binding by the 126 *nudix* RNA from *B. cellulosilyticus*. (A, B) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 126 *nudix* RNA (**Figure S1**) in the absence of ligand (–), or in the presence of 1 mM or 100 μM of the nucleoside di- or triphosphate compound indicated. Annotations are described in the legend to **Figure 2B**. (C) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 126 *nudix* RNA in the presence of either no ligand (–) or 1 mM of the compound indicated. Annotations are described in the legend to **Figure 2B**.

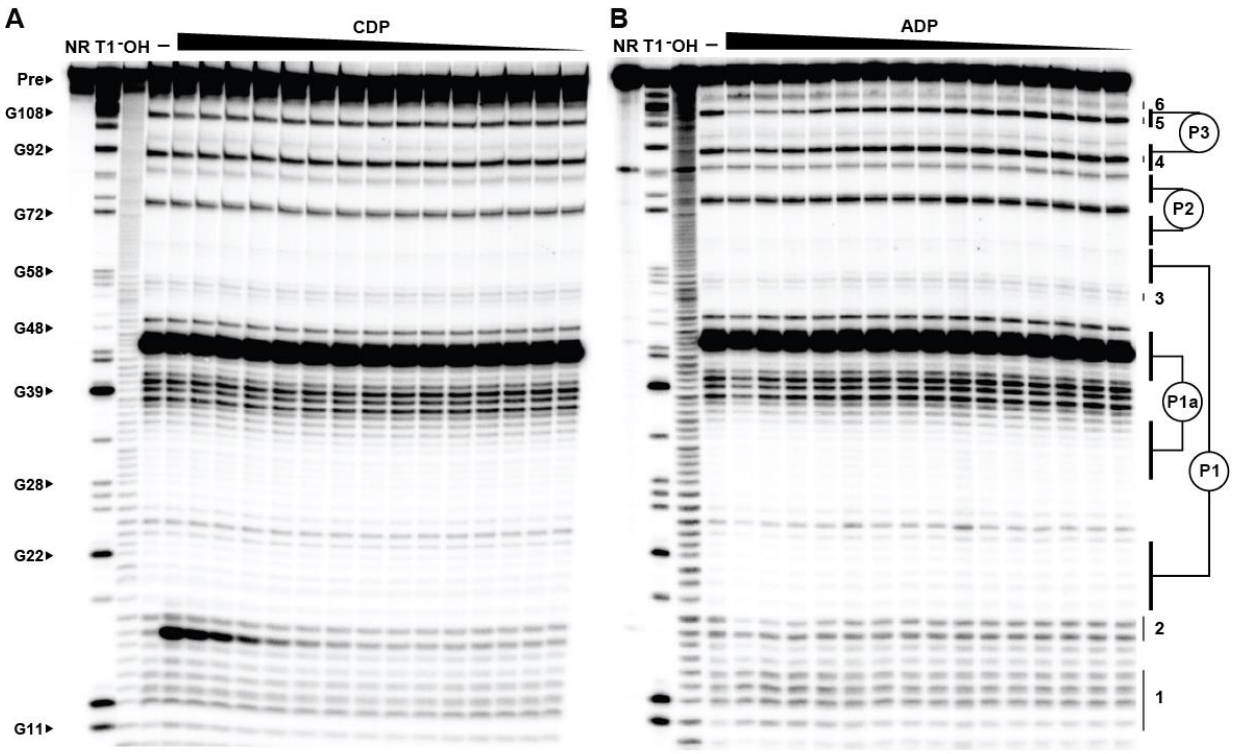

Figure S5. Nucleoside diphosphate binding by the 126 *nudix* RNA from *B. cellulosilyticus*. PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 126 *nudix* RNA (**Figure 4A**) in the presence of increasing CDP (A) or ADP (B) concentrations. In-line probing experiments contained either no ligand (–) or ligand ranging from 5 M to 100 nM. Annotations are described in the legend to **Figure 2B**.

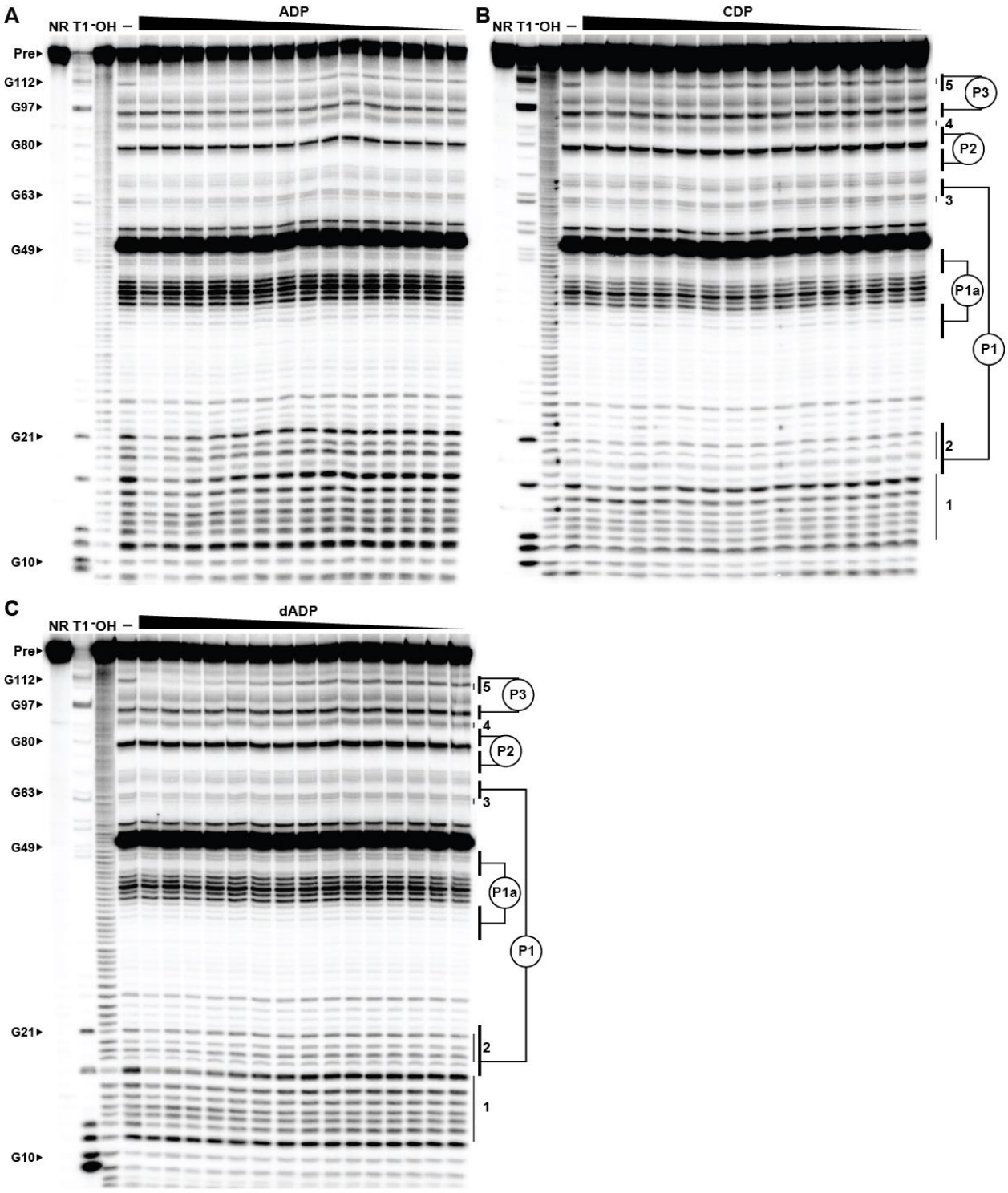

Figure S6. Nucleoside diphosphate binding by the 131 *nudix* RNA from *C. botulinum*. (A, B, C) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 126 *nudix* RNA (**Figure 3A**) in the presence of various concentrations of ADP, CDP, or dADP, respectively. In-line probing

experiments contained either no ligand (–) or ligand ranging from 5 M to 100 nM. Annotations are described in the legend to **Figure 2B**.

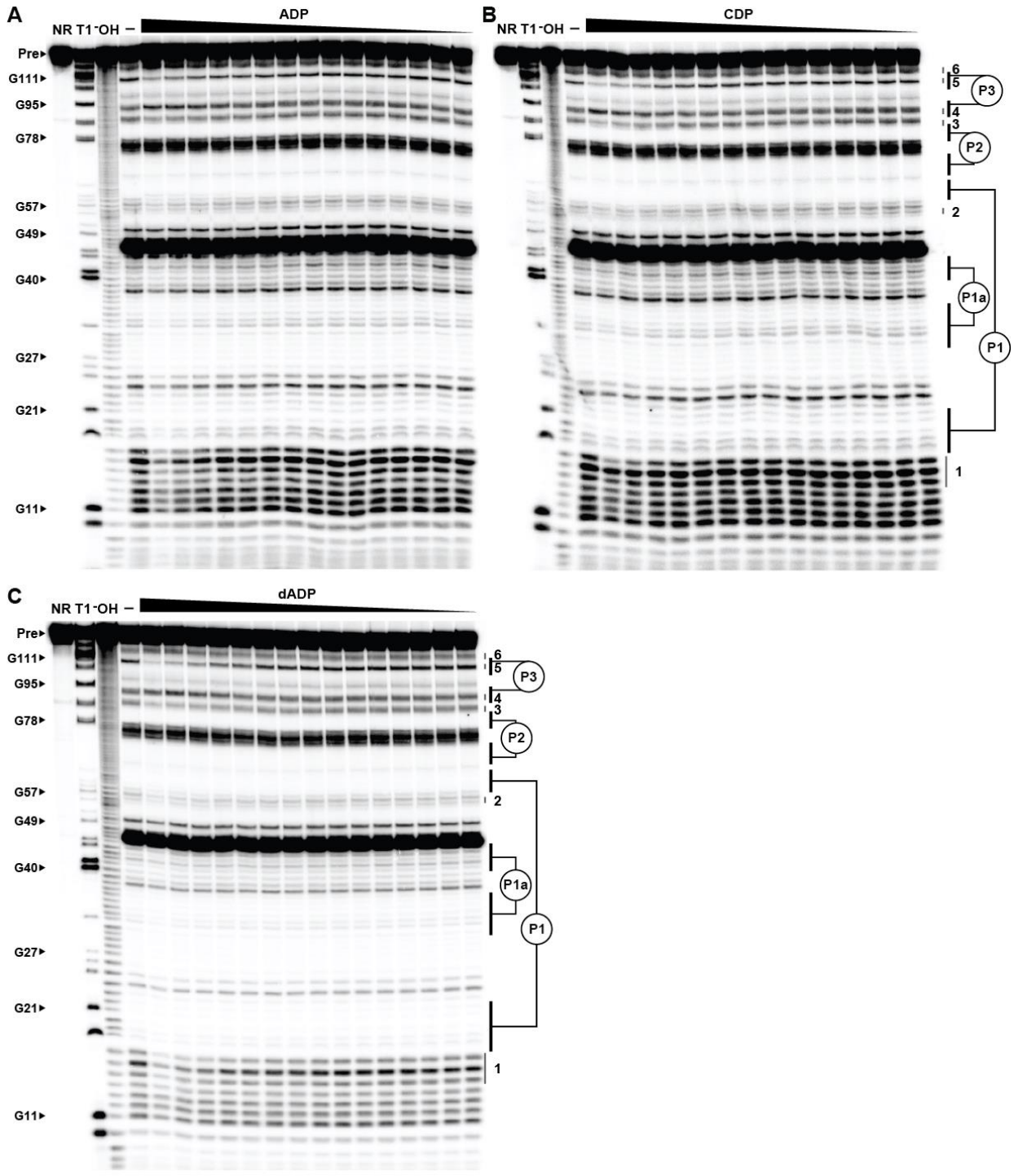

Figure S7. Nucleoside diphosphate binding by the 129 *nudix* RNA from *S. kocurii*. (A, B, C) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 129 *nudix* RNA (**Figure 2A**) in the presence of various concentrations ADP, CDP, or dADP, respectively. In-line probing

experiments contained either no ligand (–) or each compound ranging from 5 mM to 100 nM. Annotations are described in the legend to **Figure 2B**.

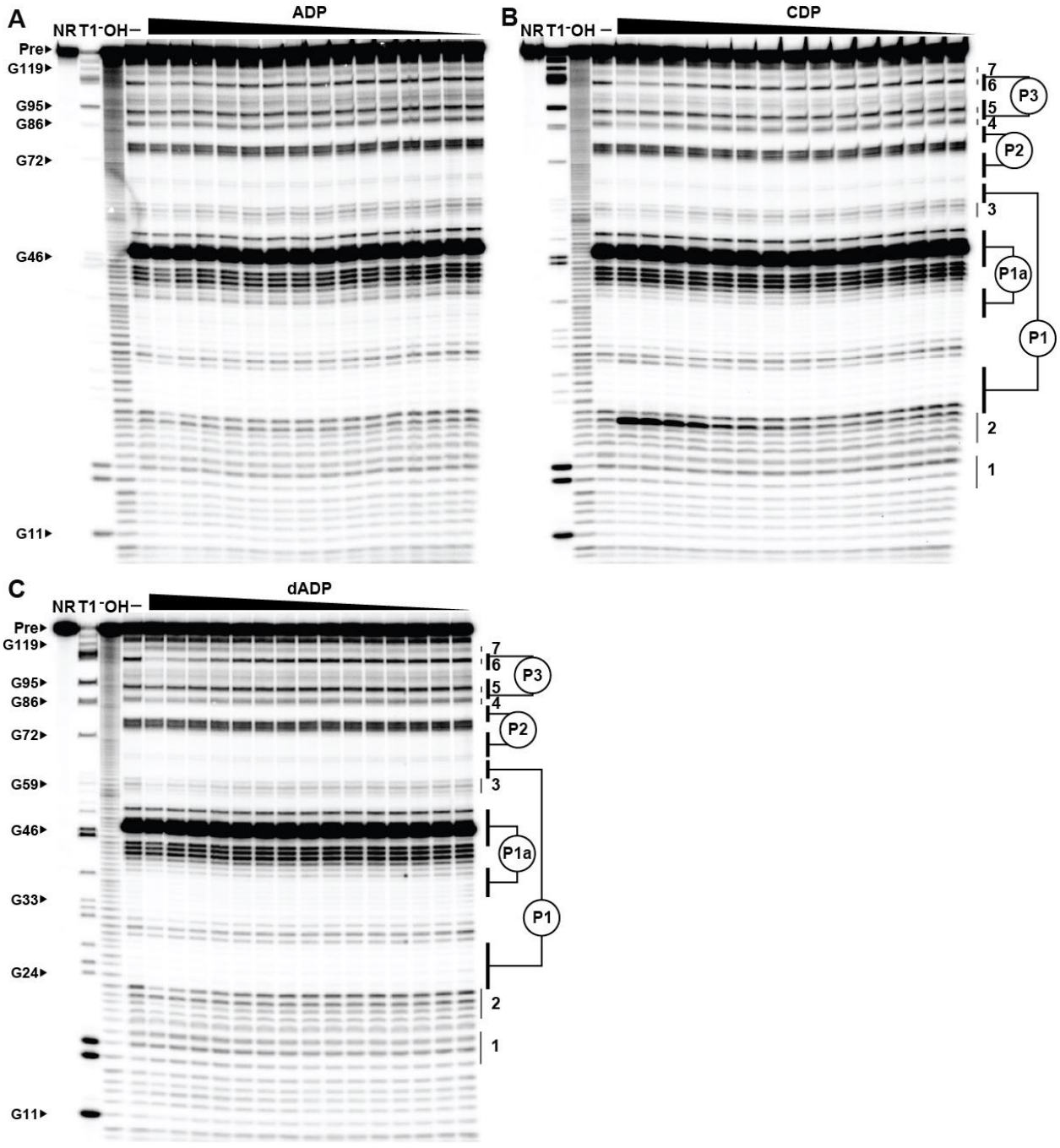

Figure S8. Nucleoside diphosphate binding by the 130 *had* RNA from *L. mesenteroides*. (A, B, C) PAGE analysis of the products of in-line probing of 5′-^{32}P-labeled 130 *had* RNA (**Figure 4B**) in the presence of various concentrations of ADP, CDP, or dADP, respectively. In-line probing experiments contained either no ligand (–) or each compound ranging from 5 mM to 100 nM. Annotations are described in the legend to **Figure 2B**.

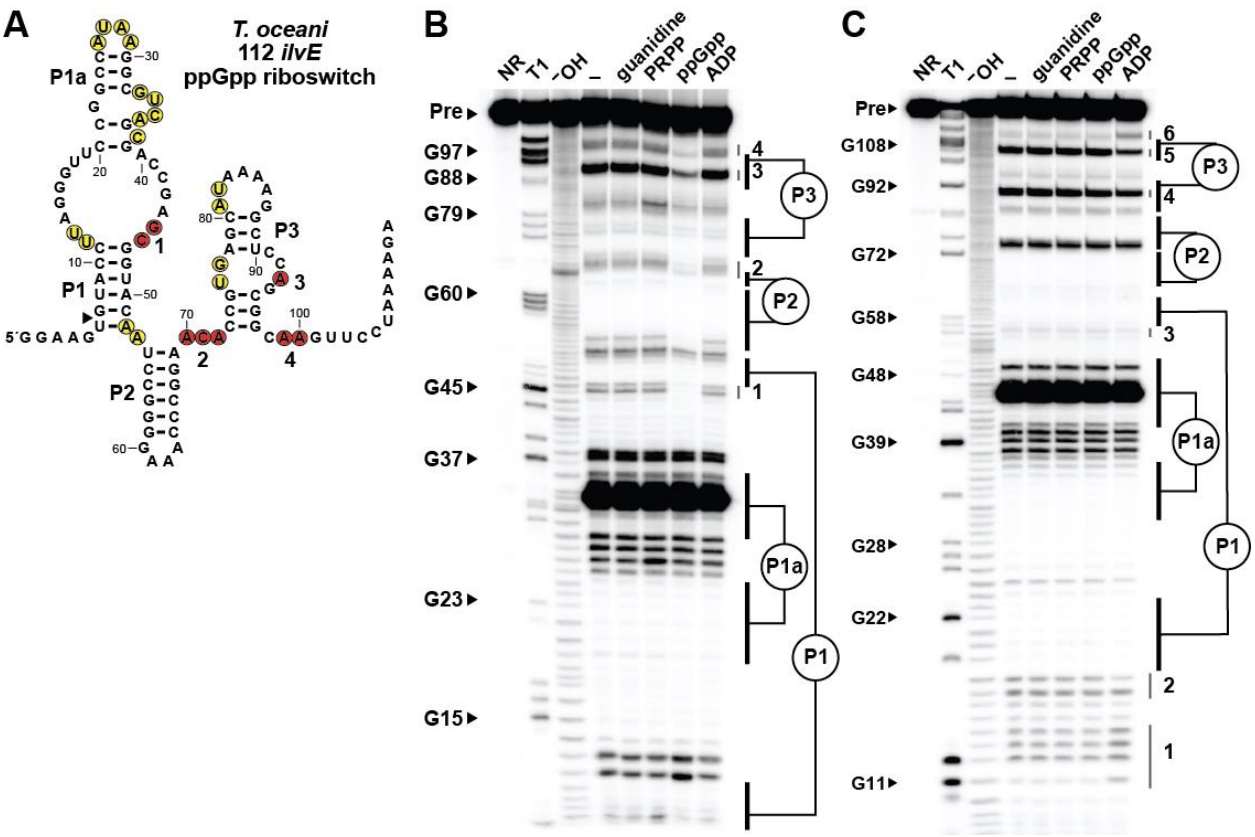

Figure S9. Nucleoside diphosphates are selectively recognized by only subtype 2c *ykkC* RNAs. (A) Sequence and secondary structure of the 112 nucleotide ppGpp riboswitch RNA derived from the *ilvE* gene of *T. oceani*. Data collected in B were used to determine regions of constant or reduced scission upon the addition of ppGpp to in-line probing reactions. (B) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 112 *ilvE* as depicted in A in the presence of ligands for previously-validated riboswitches represented by *ykkC* motif RNAs. (C) PAGE analysis of the products of in-line probing of 5′ ^{32}P-labeled 126 *nudix* as depicted in **Figure 4A** in the presence of ligands for previously-validated riboswitches represented by *ykkC* motif RNAs. In-line probing experiments contained either no ligand (–) or 1 mM of the compound indicated. Annotations are described in the legend to **Figure 2B**.

Table S1. Sequences of Synthetic DNAs used in this Study.

Name	Sequence	Annotation
HS005	TAATACGACTCACTATAGGTATTGAACTGGGACTAGATAGCTAGGGTTCCGCCTTAATATATTAAGGGTTTGTGACCAAGCGCTATAGCTCTTGTTTTG	Forward template for transcription of the WT, M2 and M3 *C. botulinum* 131 *nudix* RNAs
MES140	AACACGCTGAACCTTCCCCCTCGGGCATTTTATTCCAATAGGTGCCTCTTGCAAAACAAGAGCTATAGCGC	Reverse template for transcription of the WT *C. botulinum* 131 *nudix* RNA
HS006	TAATACGACTCACTATAGGATAGGAACTGGAAAATTGCAGCTAGGGTTCCGCTATCTAGGTAGGTCAGTGACCGAGGGCTGCATCTTTTAC	Forward template for transcription of the WT *S. kocurii* 129 *nudix* RNA
MES142	ATACGCTGAACCTTTCCCCTCGGACCTTTTATGTCATTAGGTGCCTTTTACCTATATGTAAAAGATGCAGCCCTCGG	Reverse template for transcription of the WT *S. kocurii* 129 *nudix* RNA
HS015	TAATACGACTCACTATAGGTAATGAACTGGAAAACTGTAGCTAGGGTTCCGCTAACGATAGGTCTGTGACCGAGGGCTACAT	Forward template for transcription of the WT *B. cellulosilyticus* 126 *nudix* RNA
HS016	ATACGCTGAACCTTTCCCCTCGGACCTTTTATGTCAATAGGTGCCTTTTACTATCGTAAAAGATGTAGCCCTCGGTCACAGACCT	Reverse template for transcription of the WT *B. cellulosilyticus* 126 *nudix* RNA
MES289	TAATACGACTCACTATAGGAATTACAAACGAACTGGAAAACTGGAGCTAGGGTTCCGCAATATGGTCAGTGACCGAG	Forward template for transcription of the WT *L. mesenteroides* 130 *had* RNA

	GGCTCCATC	
MES266	AAAACGCTAAACCTTTCCCCTCGGATTTTTTATATCAATAGGTGCCTTTTGAATATTTCAAAAGATGGAGCCCTCGGTCACTG	Reverse template for transcription of the WT *L. mesenteroides* 130 *had* RNA
MES137	TAATACGACTCACTATAGGAAGTGTACCTTAGGGTTCCGGCCATAAGGCGTCAGCGACCGAGCGGTACAATCCGGGG	Forward template for transcription of the WT *T. oceanii* 112 *ilvE* RNA (ppGpp aptamer)
MES138	TCTTTTAGGAACTTGCCGCTGGAGCCTTTTATGCTCACGGTGTTCCGGGTTTCCCCGGATTGTACCGCTCGG	Reverse template for transcription of the WT *T. oceanii* 112 *ilvE* RNA (ppGpp aptamer)
HS017	TAATACGACTCACTATAGGTATTGAACTGGGACTAGATAGCTAGGGTTCCGCCTTAATATATTAAGGGTTTGTGACCAAACGCTATAGCTCTTGTTTTG	Forward template for transcription of the M1 *C. botulinum* 131 *nudix* RNA
MES224	AACACGCTGAACCTTCCCCCTCGGGCATTTTATTCCAATAGGTGCCTCTTGCAAAACAAGAGCTATAGCGT	Reverse template for transcription of the M1 *C. botulinum* 131 *nudix* RNA
MES225	AACACGCTGAACCTTCCCCCTCGGGCATTTTATTCCAATAGGCGCCTCTTGCAAAACAAGAGCTATAGCGC	Reverse template for transcription of the M2 *C. botulinum* 131 *nudix* RNA
MES226	AACACGCTGAACCTTCCCCCGCGGGCATTTTATTCCAATAGGTGCCTCTTGCAAAACAAGAGCTATAGCGC	Reverse template for transcription of the M3 *C. botulinum* 131 *nudix* RNA