

Toxin ( <i>H. canis</i> )	<input type="checkbox"/> tr V8CEW3 V8CEW3_9HELI	- M I R K I D I K N T F K K D Y R K V L K Q G W S E C D I D E V I K Q L A D D D I L D P A L K D H P L I G D Y K D F R E C	60
Toxin ( <i>H. macacae</i> )	<input type="checkbox"/> tr V8CCL2 V8CCL2_9HELI	- - M R E I H F Q N T Y L K D F K L V K K Q G W D L E K I K E V V I T L Q T C D I L E P K L K D H A L L G E Y K D F R E C	59
Toxin ( <i>H. canis</i> )	<input type="checkbox"/> tr V8CKN3 V8CKN3_9HELI	- M K Y T I T Y T K A F K K D F K K L S P K D Q E - - H T L E I L T R L A N G E S L E P K Y K D H A L K G E Y A N C R D C	58
Toxin ( <i>H. sp. MIT 99-10781</i> )	<input type="checkbox"/> tr A0A3D8HR83 A0A3D8HR83_9HELI	- M E Y E I R I S K E Y K K N Y K K L T L Y E K D - - L V D E I V C R L S K N E I L E K K Y K D H K L K G E F K D Y R E C	58
Toxin ( <i>H. fennelliae</i> )	<input type="checkbox"/> tr T1D439 T1D439_9HELI	M S K Y Q I R V S N S Y K N Q R K K I K K D D L A - - L I D E A V T K L A N G E K L E P K Y K D H K L K G K Y K D F K E C	59
Toxin ( <i>H. apodemus</i> )	<input type="checkbox"/> tr A0A4U8UFD3 A0A4U8UFD3_9HELI	- - M Y Q I D F T S A F K K E Y K K F V K L G K A - K A I D T L I Q S L A N G E N L D Q K Y K D H A L K G E Y K G F R E C	58
Toxin ( <i>H. cinaedi</i> )	<input type="checkbox"/> tr I7GZJ3 I7GZJ3_9HELI	- - M Y K V H L T K S F K K D A K A L S Q A D R I - - L S M Q I I D T L A K G E K L E S K H K D H A L S G N L Q G F R E C	57
Toxin ( <i>H. sp. MIT 99-10781</i> )	<input type="checkbox"/> tr A0A3D8HJY9 A0A3D8HJY9_9HELI	M A K Y S I I Y F K S F V K D Y K K L N A K E Q A - - L V D E V I T S L A N D E N L P A K Y K D H Q L K G E L K D F R E C	59
Toxin ( <i>H. bilis</i> )	<input type="checkbox"/> tr C3XFM1 C3XFM1_9HELI	M A K Y T I L Y H R K F V K D Y E K L S N N D R L - - L V D E V I E K L A N G E I L D Q K Y K D H K L K G N L K D F R E C	59
Toxin ( <i>H. enhydrae</i> )	<input type="checkbox"/> tr A0A1B1U4G8 A0A1B1U4G8_9HELI	- - M M K I D F S K S F K K S Y K K L S K K D K E - - L F S K V S L K L A N D E I L E P K Y R D H A L K G D Y V G F R E C	57
Toxin ( <i>H. saguini</i> )	<input type="checkbox"/> tr A0A099B628 A0A099B628_9HELI	M A K Y K I S Y R K A F R K N F K K L S K E N K N - - L A N E V I N K I A N G E V L D S S Y N N H K L K G D L Q D F M E C	59
HP0892 ( <i>H. pylori</i> )	<input type="checkbox"/> tr O25552 O25552_HELPY	- - M L T I E T S K K F D K D L K I L V K N G F D L K L L Y K V V G N L A T E Q P L A P K Y K D H P L K G G L K D F R E C	59
HP0894 ( <i>H. pylori</i> & <i>H. pylori</i> SS1)	<input type="checkbox"/> tr O25554 O25554_HELPY	- - M L K L N L K K S F Q K D F D K L L L N G F D D S V L N E V I L T L R K K E P L D P Q F Q D H A L K G K W K P F R E C	59

Toxin ( <i>H. canis</i> )	<input type="checkbox"/> tr V8CEW3 V8CEW3_9HELI	H V K G D L V I V Y K R S A E I - - L S L Y R I G R H Q D I F K G Y	92
Toxin ( <i>H. macacae</i> )	<input type="checkbox"/> tr V8CCL2 V8CCL2_9HELI	H I F G D L V I V Y K R D K Q T - - L T L Y R I G R H Q D L F K N Y	91
Toxin ( <i>H. canis</i> )	<input type="checkbox"/> tr V8CKN3 V8CKN3_9HELI	H I R P D L V L I Y R Y N D D F L E L V A M R I S S H S E V F - - -	89
Toxin ( <i>H. sp. MIT 99-10781</i> )	<input type="checkbox"/> tr A0A3D8HR83 A0A3D8HR83_9HELI	H I K P D L L L V Y Q K Q D N I L V L T C I N V G S H S E L F - - -	89
Toxin ( <i>H. fennelliae</i> )	<input type="checkbox"/> tr T1D439 T1D439_9HELI	H I K P D L L L V Y R I I D D V L E L Y L A Q V G S H S E L F - - -	90
Toxin ( <i>H. apodemus</i> )	<input type="checkbox"/> tr A0A4U8UFD3 A0A4U8UFD3_9HELI	H I E P D L L L I Y K K Y E D I L L L S C V R L G S H T H L F K K Q	92
Toxin ( <i>H. cinaedi</i> )	<input type="checkbox"/> tr I7GZJ3 I7GZJ3_9HELI	H I K P D L L L I Y E L C D D I L Q L N A L R V G S H S K L F K K -	90
Toxin ( <i>H. sp. MIT 99-10781</i> )	<input type="checkbox"/> tr A0A3D8HJY9 A0A3D8HJY9_9HELI	H V K P D L L L I Y E K E E N I L L S I A R V G S H S Q L F K K -	92
Toxin ( <i>H. bilis</i> )	<input type="checkbox"/> tr C3XFM1 C3XFM1_9HELI	H V K P D L L L M Y E I Q E N I L H L N L M R V G S H S D L F K K -	92
Toxin ( <i>H. enhydrae</i> )	<input type="checkbox"/> tr A0A1B1U4G8 A0A1B1U4G8_9HELI	H L K P D L L L I Y K K Q D D K I L Y C L D I G S H S E L F - - -	88
Toxin ( <i>H. saguini</i> )	<input type="checkbox"/> tr A0A099B628 A0A099B628_9HELI	H I K P D L L L I Y K K F D D I L V L C V N I G S H S D L F - - -	90
HP0892 ( <i>H. pylori</i> )	<input type="checkbox"/> tr O25552 O25552_HELPY	H L K P D L L L V Y Q I K K Q E N T L F L V R L G S H S E L F - - -	90
HP0894 ( <i>H. pylori</i> & <i>H. pylori</i> SS1)	<input type="checkbox"/> tr O25554 O25554_HELPY	H I K P D V L L V Y L V K D D - - E L I L L R L G S H S E L F - - -	88

**Supplementary Figure S1.** HP0894 homologues from 13 species of *Helicobacter* are compared using the multiple sequence alignment program, CLUSTAL (Sievers F, Higgins DG. The Clustal Omega Multiple Alignment Package. *Methods Mol Biol.* 2021;2231:3-16). The stars represent the conserved His residues that play a critical role in metal binding. The blue color represents the highly conserved residues between proteins. The sequence identities of 12 proteins compared to HP0894 were above 40%.

(A)

Organism	Blast Name	Score	Number of Hits	Description
<a href="#">Helicobacter</a>	<a href="#">e-proteobacteria</a>		<a href="#">218</a>	
• <a href="#">Helicobacter pylori</a>	<a href="#">e-proteobacteria</a>		<a href="#">200</a>	
• • <a href="#">Helicobacter pylori 26695</a>	<a href="#">e-proteobacteria</a>	174	<a href="#">8</a>	<a href="#">Helicobacter pylori 26695 hits</a>
• • <a href="#">Helicobacter pylori Rif1</a>	<a href="#">e-proteobacteria</a>	173	<a href="#">1</a>	<a href="#">Helicobacter pylori Rif1 hits</a>
• • <a href="#">Helicobacter pylori Rif2</a>	<a href="#">e-proteobacteria</a>	173	<a href="#">1</a>	<a href="#">Helicobacter pylori Rif2 hits</a>
• • <a href="#">Helicobacter pylori 26695-1</a>	<a href="#">e-proteobacteria</a>	173	<a href="#">1</a>	<a href="#">Helicobacter pylori 26695-1 hits</a>
• • <a href="#">Helicobacter pylori Hp P-16</a>	<a href="#">e-proteobacteria</a>	171	<a href="#">1</a>	<a href="#">Helicobacter pylori Hp P-16 hits</a>
• • <a href="#">Helicobacter pylori Cuz20</a>	<a href="#">e-proteobacteria</a>	170	<a href="#">1</a>	<a href="#">Helicobacter pylori Cuz20 hits</a>
• • <a href="#">Helicobacter pylori Hp H-45</a>	<a href="#">e-proteobacteria</a>	170	<a href="#">2</a>	<a href="#">Helicobacter pylori Hp H-45 hits</a>
• • <a href="#">Helicobacter pylori HPAG1</a>	<a href="#">e-proteobacteria</a>	169	<a href="#">1</a>	<a href="#">Helicobacter pylori HPAG1 hits</a>
• • <a href="#">Helicobacter pylori Shi112</a>	<a href="#">e-proteobacteria</a>	168	<a href="#">1</a>	<a href="#">Helicobacter pylori Shi112 hits</a>
• • <a href="#">Helicobacter pylori B38</a>	<a href="#">e-proteobacteria</a>	167	<a href="#">1</a>	<a href="#">Helicobacter pylori B38 hits</a>
• <a href="#">Helicobacter pylori</a>	<a href="#">e-proteobacteria</a>	173	<a href="#">200</a>	<a href="#">Helicobacter pylori hits</a>



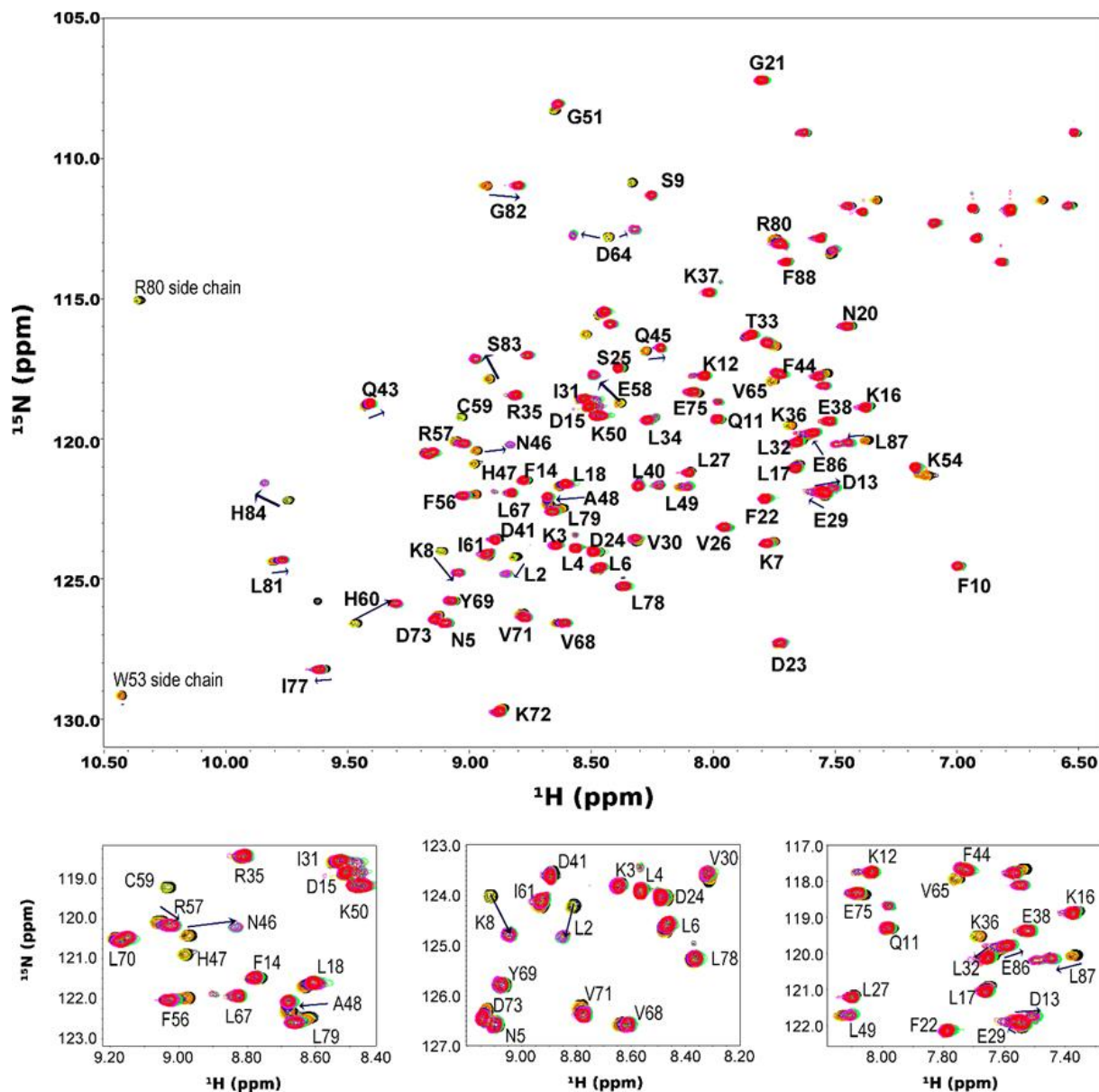
(B)

NCBI Multiple Sequence Alignment Viewer, Version 1.25.0

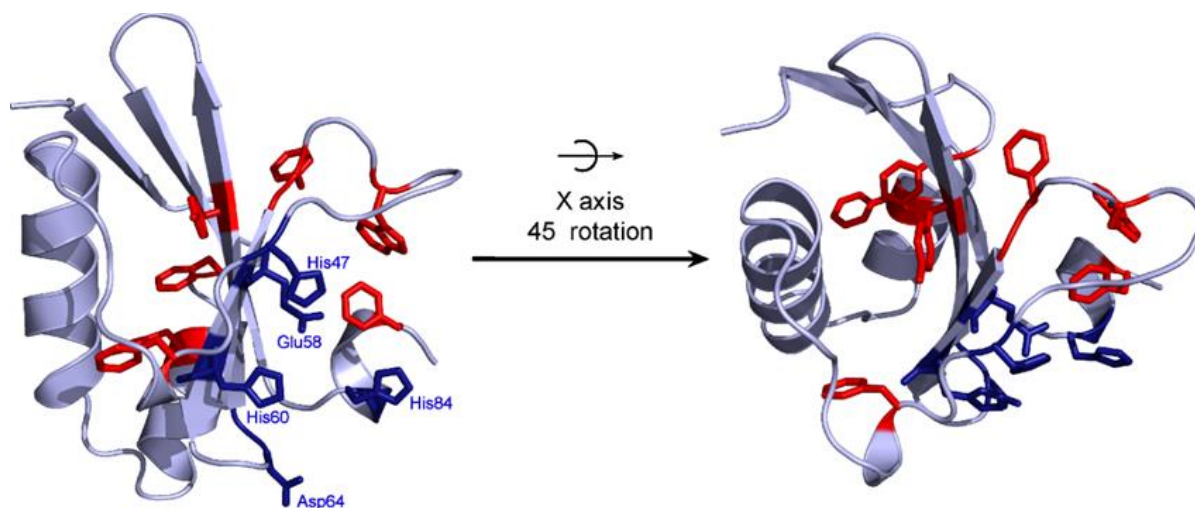
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4LTT_A	(+)	1	88	Helicobacter pylori 26695
WP_000916169.1	(+)	1	88	Helicobacter pylori
WRG10393.1	(+)	1	88	Helicobacter pylori
WOZ18620.1	(+)	1	88	Helicobacter pylori
4LSY_A	(+)	4	91	Helicobacter pylori 26695
WP_000916166.1	(+)	1	88	Helicobacter pylori
WP_097568989.1	(+)	1	88	Helicobacter pylori
WP_000916165.1	(+)	1	88	Helicobacter pylori
WP_120845582.1	(+)	1	88	Helicobacter pylori
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WP_181331616.1	(+)	1	88	Helicobacter pylori

**Supplementary Figure S2.** Sequence homology of HP0894 among *Helicobacter pylori* strains. (A) Blast sequence alignment shows 218 homologues of HP0894. (B) The example of the sequence alignment. The first 'query\_8782175' is HP0894. The conserved amino acids are represented by the symbol, dot. The color scheme depends on the amino acid types.

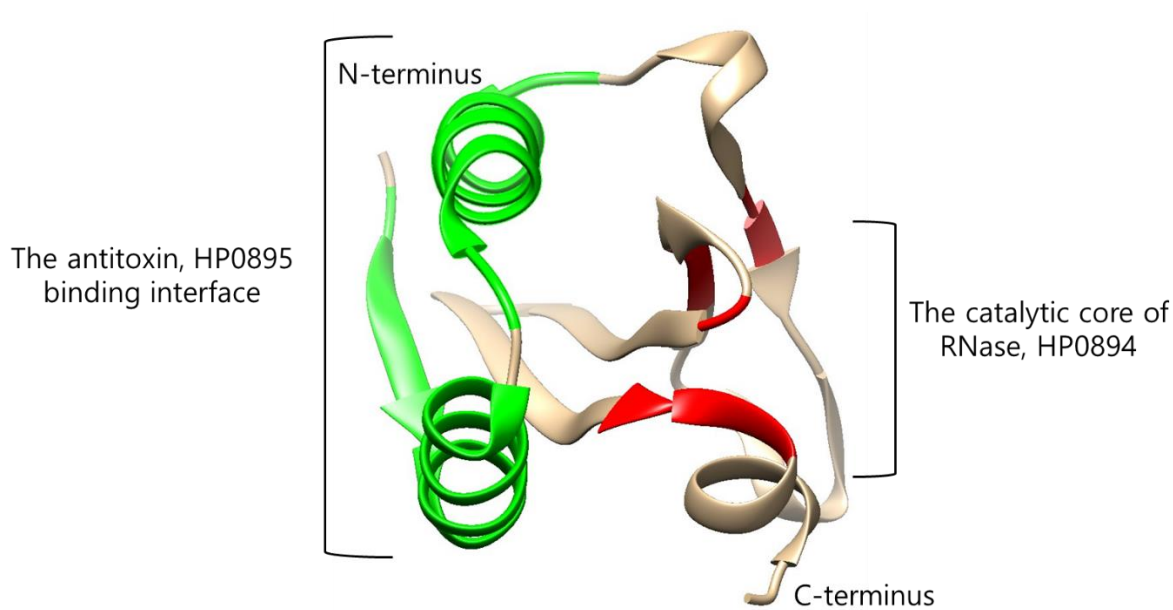




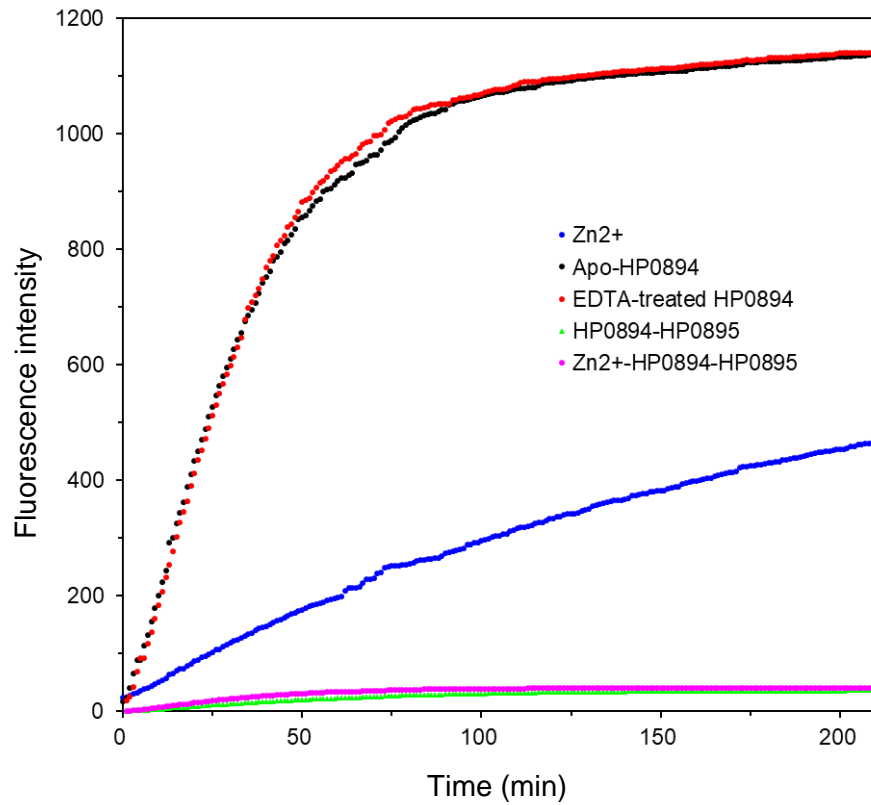
**Supplementary Figure S3** NMR titration of HP0894 with increasing concentration of zinc ions. A series of two-dimensional  $[^1\text{H}-^{15}\text{N}]$  HSQC spectra of  $150\ \mu\text{M}$  U- $^{15}\text{N}$  HP0894 alone (black) and upon addition of 1:0.5 molar ratio of  $\text{ZnCl}_2$  (lime green), 1:1 ratio (orange), 1:1.5 ratio (blue), 1:2 (green), 1:2.5 ratio (purple), and 1:3 ratio (red) are illustrated. The overlapping regions in the HSQC spectra are illustrated in the inset for clarity, as well as the side chain of aromatic amino acids.



**Supplementary Figure S4.** Effect of zinc on aromatic residues of HP0894. The residues comprising the binding pocket of HP0894 is illustrated in blue. The effect of binding of zinc ions with HP0894 on aromatic residues as seen by near-UV region in circular dichroism is mapped into the structure of HP0894 and illustrated in red.



**Supplementary Figure S5.** The binding regions of HP0894. The antitoxin protein, HP0895 binds through mainly the N-terminal helices (Leu2, Leu4–Asn20, Phe22–Glu38, and Leu40, green) while the  $\text{Zn}^{2+}$  ion binds the surface of  $\beta$ -sheet (red) that localizes opposite of the N-terminal helices.



**Supplementary Figure S6.** Comparison of RNase activity of the HP0894 samples. Apo-HP0894 (black), 2 mM EDTA-added HP0894 (red), Zn<sup>2+</sup>-added HP0894 (blue), 20  $\mu$ M (more than two equivalent of HP0894) HP0895-added HP0894 (green), and 2 mM Zn<sup>2+</sup>-HP0894-20  $\mu$ M HP0895 (pink) are shown. The concentration of HP0894 was 8  $\mu$ M. The inhibitory effect of the antitoxin for the RNase activity of HP0894 was very strong and did not show no significant difference depending on the presence of Zn<sup>2+</sup>.