

## Supplemental Material

### **(p)ppGpp-dependent regulation of the nucleotide hydrolase PpnN confers complement resistance in *Salmonella enterica* serovar Typhimurium**

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Running title: PpnN confers complement resistance

1 **Figure S1. RelA-SpoT is required for growth in minimal medium.** Growth curves of  
2 wild type *S. Typhimurium*,  $\Delta relA$ ,  $\Delta relA \Delta spoT$ , and  $\Delta ppnN$  in (A) LB and (B) M9-  
3 glucose media. Data are the means  $\pm$  SEM (error bars) of three independent experiments.

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5 **Figure S2. PpnN from *E. coli* str. K-12 and *S. Typhimurium* str. SL1344 share**  
6 **significant amino acid sequence homology.** (A) PpnN has two domains of unknown  
7 function, DUF4478 and DUF3412, and a lysine decarboxylase (LDC)-like domain. The  
8 LDC-like domain contains a conserved “PGG<sub>x</sub>GT<sub>xx</sub>E” motif characteristic of the Lonely  
9 Guy (LOG) protein family. (B) Amino acid sequence alignment of PpnN from *E. coli* str.  
10 K-12 and *S. Typhimurium* str. SL1344. Protein sequences share 94% pairwise identity in  
11 amino acid sequences. DUF4478, DUF3412, and LDC-like domains are highlighted. The  
12 conserved “PGG<sub>x</sub>GT<sub>xx</sub>E” motif found in LOG homologues is underlined in red. Asterisks  
13 indicate the amino acid residues involved in (p)ppGpp binding.

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15 **Figure S3. PpnN from *E. coli* str. K-12 is a pyrimidine/purine nucleosidase.** PpnN  
16 hydrolyzes uridine 5'-monophosphate (UMP) to uracil and ribose 5'-phosphate. Heat-  
17 inactivation of PpnN abrogates protein function. Data are representative of two replicates.

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19 **Figure S4. PpnN from *S. Typhimurium* str. SL1344 is a pyrimidine/purine**  
20 **nucleosidase.** PpnN hydrolyzes guanosine 5'-monophosphate (GMP) to guanine and  
21 ribose 5'-phosphate. Introduction of an E264Q mutation in PpnN abrogates protein  
22 function. Data are representative of two replicates.

23 **Figure S5. PpnN does not contribute to the protein composition of the outer**  
24 **membrane.** Outer membrane protein composition of the  $\Delta ppnN$  mutant is similar to wild  
25 type *S. Typhimurium* str. SL1344, whereas, the  $\Delta relA \Delta spoT$  mutant shows significant  
26 differences. Data are representative of two replicates.

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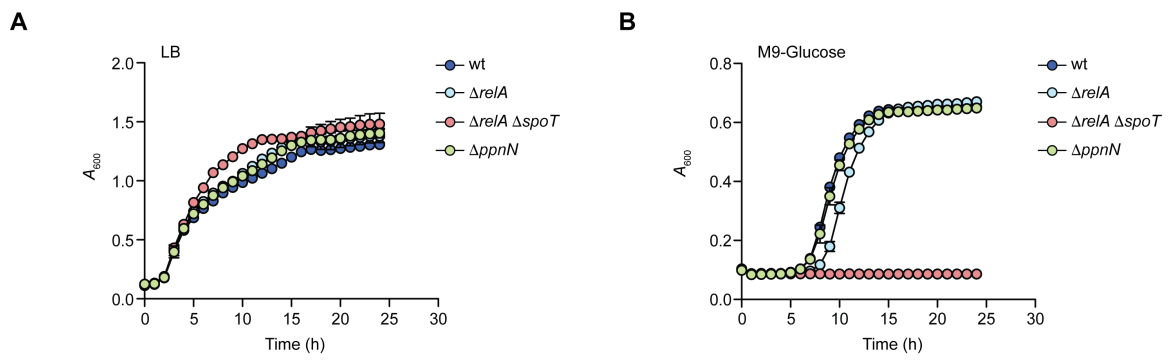


Figure S1

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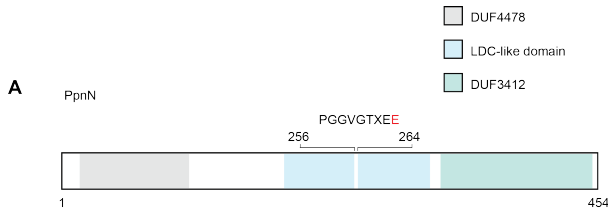
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**B**

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| <i>E. coli</i>        | 1   | M I T H I S P L G S M D M L S Q L E V D M L K R T A S S D L Y Q L F R N C S L A V L N S G S L T D N S K E L L S R F E N F D I N V L R R E R G |
| <i>S. Typhimurium</i> | 1   | M I T H I S P L G S M D M L S Q L E V D M L K R T A S S D L Y Q L F R N C S L A V L N S G S L T D N S K E L L S R F E N F D I N V L R R E R G |
| <i>E. coli</i>        | 72  | V K L E L I N P P E E A F V D G R I I R A L Q A N L F A V L R D I L F V Y G Q I H N T V R F P N L N L D N S V H I T N L V F S I L R N A R A L |
| <i>S. Typhimurium</i> | 72  | V K L E L I N P P E A F V D G R I I R A L Q A N L F A V L R D I L F V N G Q I H N A G R F Q H L D L E S S T H I T N L V F S I L R N A R A L   |
| <i>E. coli</i>        | 143 | H V G E A P N M V V C W G G H S I N E N E Y L Y A R R V G N Q L G L R E L N I C T G C G P G A M E A P M K G A A V G H A Q Q R Y K D S R F I G |
| <i>S. Typhimurium</i> | 143 | H V G E A P N M V V C W G G H S I N E N E Y L Y A R R V G T Q L G L R E L N I C T G C G P G A M E A P M K G A A V G H A Q Q R Y K D S R F I G |
| <i>E. coli</i>        | 214 | M T E P S I I A A E P P N P L V N E L I I M P D I E K R L E A F V R I A H G I I I F P G G V G T A E E L L Y L L G I L M N P A N K D Q V L P L |
| <i>S. Typhimurium</i> | 214 | M T E P S I I A A E P P N P L V N E L I I M P D I E K R L E A F V R I A H G I I I F P G G V G T A E E L L Y L L G I L M N P A N K N Q V L P L |
| <i>E. coli</i>        | 285 | I L T G P K E S A D Y F R V L D E F V V H T L G E N A R R H Y R I I I D D A A E V A R L M K K S M P L V K E N R R D T G D A Y S F N W S M R I |
| <i>S. Typhimurium</i> | 285 | I L T G P K E S A D Y F R V L D E F I T H T L G E A A R R H Y R I I I D D A A E V A R L M K K A M P Q V K E N R R D T G D A Y S F N W S M R I |
| <i>E. coli</i>        | 356 | A P D L Q M P F E P S H E N M A N L K L Y P D Q P V E V L A A D L R R A F S G I V A G N V K E V G I R A I E E F G P Y K I N G D K E I M R R M |
| <i>S. Typhimurium</i> | 356 | A P D L Q V P F E P S H E N M A N L K L Y P D Q P V E I L A A D L R R A F S G I V A G N V K E V G I R A I E A N G P Y K I H G D R E M M R R M |
| <i>E. coli</i>        | 427 | D D L L Q G F V A Q H R M K L P G S A Y I P C Y E I C T   |
| <i>S. Typhimurium</i> | 427 | D D L L Q G F V A Q H R M K L P G S A Y I P C Y E I C A   |

Legend:  
 □ DUF4478   □ LDC-like domain   □ DUF3412

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Figure S2

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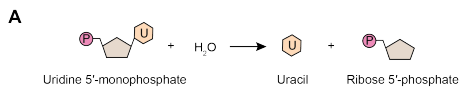
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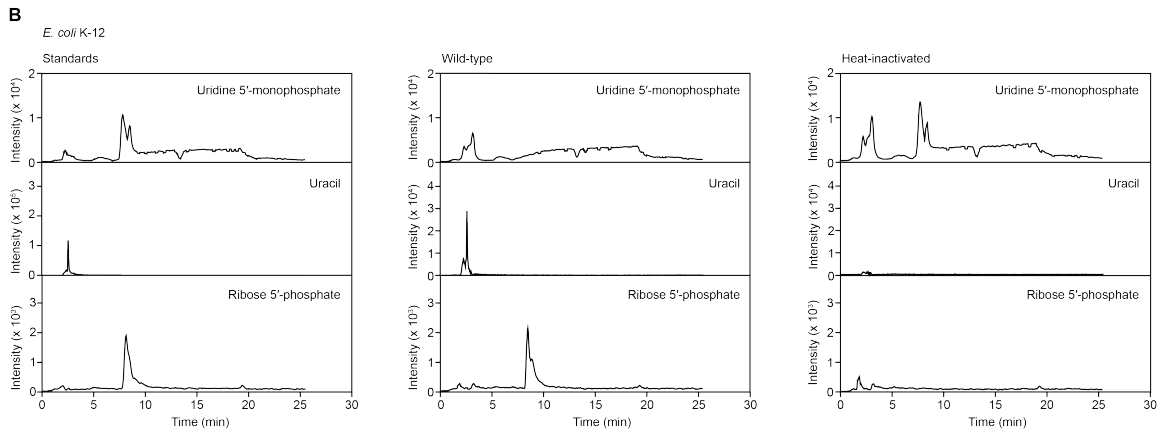
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Figure S3

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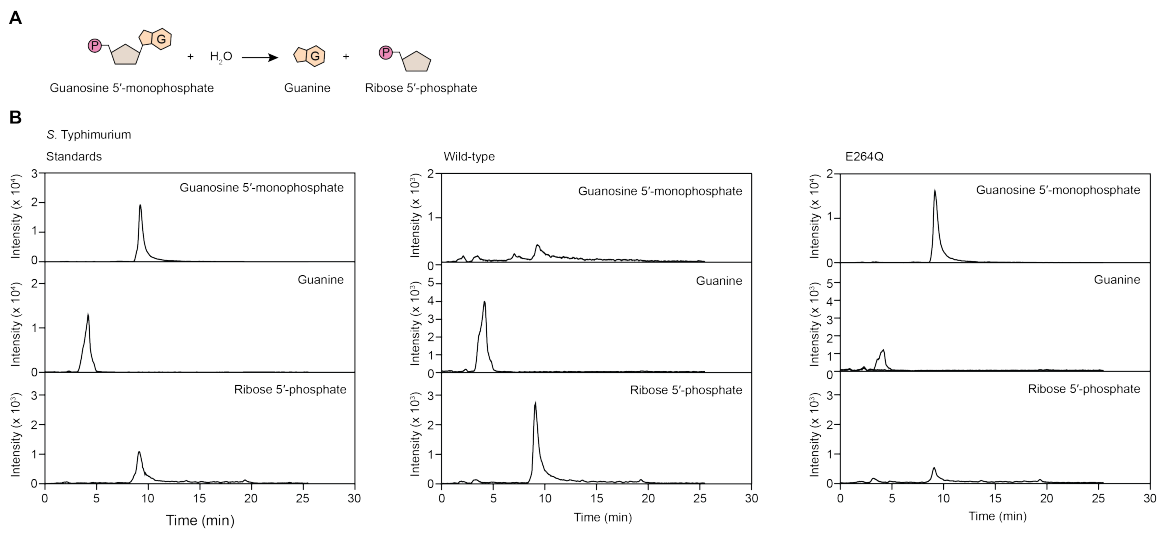
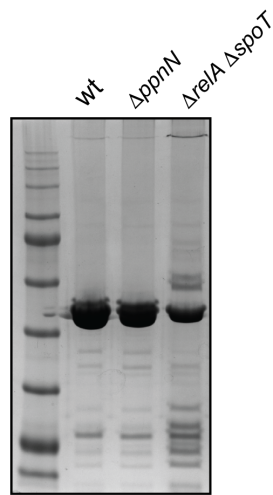


Figure S4



**Figure S5**