**Supplementary Data Liang et al**

**Methods**

Oligonucleotides used in the study are listed in Table S1. Polyphosphate extraction in Figure S2 was carried out using a GENECLEAN™ kit (MP Biomedicals Europe) as described [1]. Briefly, 4 M guanidine isothiocyanate (GITC)–50 mM Tris-HCl, pH 7.0 (GITC lysis buffer), prewarmed to 95°C was used to lyse pelleted cells, then 10% sodium dodecyl sulphate (SDS), 95% ethanol, and Glassmilk was added to adsorb polyphosphate, washing with New Wash buffer. Polyphosphate was eluted from the pellet by adding 50 l of 50 mM Tris-HCl (pH 8.0) at 95°C for 2 min, recovery of polyphosphate was completed with two additional elutions.

Polyphosphate chain length in polyphosphate extracts (Fig S2) was visualised in polyacrylamide gels by 4’,6-diamidino-2-phenylindol (DAPI) negative staining [2]. Gels were agitated for 30 min in 2 mg/mL DAPI in fixative at room temperature. Gels were then exposed to 365 nm light via a UV transilluminator for 2 – 20 min to induce specific photobleaching of polyphosphate bound to DAPI.

**References**

1. Ault-Riche D, Fraley CD, Tzeng C-M, Kornberg A. Novel Assay Reveals Multiple Pathways Regulating Stress-Induced Accumulations of Inorganic Polyphosphate in Escherichia coli. J Bacteriol. 1998;180:1841-1847.

2. Serafim LS, Lemos PC, Levantesi C, Tandoi V, Santos H, Reis MAM. Methods for detection and visualization of intracellular polymers stored by polyphosphate-accumulating microorganisms. Journal of Microbiological Methods. 2002;51:1-18.

**Oligonucleotides used in this study**

Table S1

|  |  |
| --- | --- |
| Name | Sequence (5’-3’) |
| PPK1-F | AGTGAGCTCATGGGTCAGGAAAAGCTATACATCGAAAAAGAACTC |
| PPK1-R | AATAAAGCTTTTATTCAGGTTGTTCGAG |
| PPX-F | GGATCCAAATGGAAGGACGTTTCCGT |
| PPX-R | GAATTCCCCGCAAAGTATTAAGCGG |
| ACYCDuetUP1 | GGATCTCGACGCTCTCCCT |
| DuetDOWN1 | GATTATGCGGCCGTGTACAA |
| DuetUP2Primer | TTGTACACGGCCGCATAATC |
| T7 Terminator Primer. | GCTAGTTATTGCTCAGCGG |

Fig. S1



Fig. S2



**Figure Legends**

**Figure S1 Vector Cloning Strategy**

**Figure S2 Size distribution of whole cell extracted polyphosphate.** DAPI

PAGE gel of polyphosphate extractions from *E. coli* quantified in Figure 3.

Polyphosphate associated with DAPI is bleached by prior UV exposure and

appears as a dark vertical band. High molecular weight shadows below the

inoculation well correspond to lipid (extracts treated with micrococcal nuclease to

remove DNA). Lane 1, 5 Sodium phosphate glass Type 45 (Sigma)

Lane 2,3,4 Time zero extractions. Lane 6,7,8 time 24 hours Lane 9,10,11 time 48

hours. Lane 2,6,9 *E.coli* BL21 (DE3) control. Lane 3,7,10 *E. coli* BL21 (DE3)

pML1 (*p18ppk1*). Lane 4,8,11 E.coli BL21 (DE3) pML1 (*p18ppk1*)

pLysSPduABJKNU .