



MG1655, ppGpp° ( $\Delta relA \Delta spoT$ ),  $\Delta dksA$ , and ppGpp+ ( $\Delta relA spoT203$ ) strains were transformed with the indicated transcriptional fusions. The culture were grown overnight at 30°C in LB supplemented with kanamycin. The values show the mean ratio of GFP fluorescence over optical density at 600 nm, in arbitrary units (A.U.). The values are the mean of 6 replicas. The error bars show the standard error of the mean. ns : non-significant, \* : p < 0.05, \*\*\*\*: p < 0.0001 in a two-way ANOVA statistical analysis.



**Figure S2. Accumulation of Psd-3Flag proenzyme.** MG1655 *E. coli* wild type strain was co-transformed by pBAD24 or pBAD-*rpoE* plasmids and pACYC-psd3Flag or pACYC-psd(S254A)3Flag plasmids. Cultures were grown in LB supplemented with ampicillin until  $OD_{600nm}=0.7$  then plasmid expression was induced with 0.5% arabinose for 2 hours. Proteins were separated by 12% SDS-PAGE and detected by Western-Blot using anti-Flag monoclonal antibody.



Figure S3. Growth of the regulation mutant strains. MG1655, Psd\_mutP $\sigma$ E, Psd\_mutCpxR, and Psd\_mutCpxRmutP $\sigma$ E strains were grown in 96-well plates in 150  $\mu$ l LB at 37°C in a TECAN M200 microplate reader. Lines represent the mean of 6 replicas for each strain.



Figure S4. Quantification of the Psd $\alpha$ -3Flag bands of the Western blots of Figures 5 and 6. One-way ordinary ANOVA statistical tests were performed. However, note that these quantifications were performed on only 2 replicas for panels B and C, which relativizes the scope of the statistic analyses on this kind of data.

## Table S1 : Oligonucleotides

Lab code	5' - 3' sequence	Name
Ebm435	ccg <b>ctcgag</b> TCGGCTGTATCACTTCCCGC	Prom psd FW
Ebm436	cg <b>agatct</b> GCGAGTAAGCCATAGTTTCGGC	Prom psd RV
Ebm446	TCTACGACTTCCGGCTGTGC	DOWN psd RV
Ebm448	CAGAAAAGTGATAATCAGGCGCACGTCAGCGTTTCCTTTGATGGAC ATATGAATATCCTCCTTAG	Psd-3Flag RV
Ebm472	GAACACGACGCCAGCCCATTGGTTGACGACAAAAAAGACCAGGTCA TTCCAACTACTGCTAGC	psd-3Flag FW
Ebm488	AGAACGCTGACGTCTGCGGGCAAAGGTCGTCAGGCGGGAAGTTTGT CCATGGAAAAGAGAAG	MscM-SPA FW
Ebm489	ATCAGTTTTGTTTGTGAGCCGGATTGGTTCATCCGGCACACAAACC ATATGAATATCCTCCTTAG	MscM-SPA RV
Ebm968	ttg <b>ctcgag</b> AAGCAGCTCCAGCCTACACG	RV P1pKD13
Ebm981	acc <b>gaattc</b> atgGTGAAAAAAGCGATAGTGAC	nlpE ORF FW
Ebm982	ttg <b>ctcgag</b> ttaCTGCCCCAAACTACTGCAATC	nlpE ORF RV
Ebm1023	cg <b>ggatcc</b> TCGGTATCGTGTTTGCAATCGC	Psd PsigE RV
Ebm1762	cg <b>agatct</b> AAGCGGTGCATGAGCGTACC	UP psd FW
Ebm1763	ccg <b>ctcgag</b> ttaGACCTGGTCTTTTTTGTCGTCAAC	psd ORF RV
Ebm1777	GGAAAGCATGGCGCAGGTccAgACGCGTAAAAACTTTTCTG	psd mutCpxR FW
Ebm1778	CAGAAAAGTTTTTACGCGTcTggACCTGCGCCATGCTTTCC	psd mutCpxR RV
Ebm1785	TTACTCTGATGGGATGT <b>G</b> ATAATCGGGCCGAAGTCGATAC	nlpE N22D FW
Ebm1786	GTATCGACTTCGGCCCGATTAT <b>C</b> ACATCCCATCAGAGTAA	nlpE N22D RV
Ebm1808	caaatcactcagggctttgtAgaGttccaTGACTATTTAGGTCTG	psd mutP $\sigma^{E}$ FW
Ebm1809	CAGACCTAAATAGTCAtggaaCtcTacaaagccctgagtgatttg	psd mutP $\sigma^{E}$ RV
Ebm1911	GTCGCTTTAAACTCGGcgCCACCGTTATCAACCTG	psd S254A FW
Ebm1912	CAGGTTGATAACGGTGGcgCCGAGTTTAAAGCGAC	psd S254A RV
Ebm2079	GAAAACGACGGTTCTGTGGC	UP mscM FW