SUPPLEMENTARY MATERIAL

A single-cell view of the BtsSR/YpdAB pyruvate sensing network in *Escherichia coli* and its biological relevance

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Running Head: Phenotypic heterogeneity in *E. coli*

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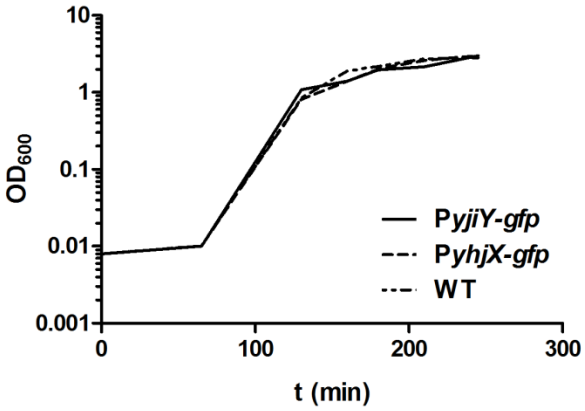

FIG S1 Growth of reporter strains. *E. coli* cells expressing *gfp* under the control of P_{yhjX} or P_{yjiY} and the MG1655 strain (WT, without promoter-*gfp* fusion) were grown in LB medium.

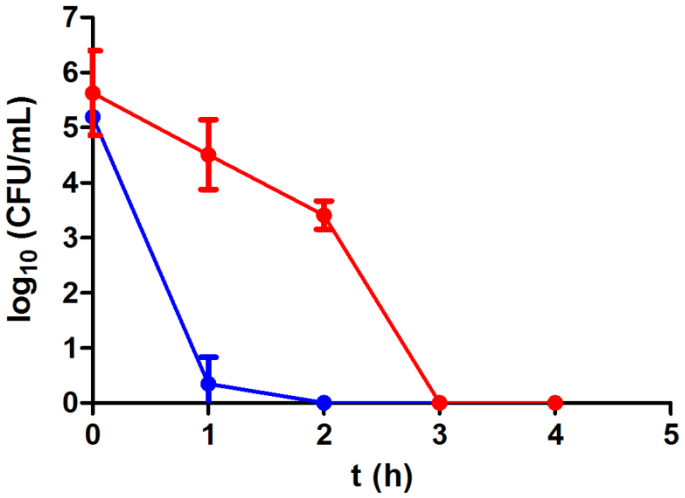

FIG S2 Determination of the minimum duration of killing (MDK) after ofloxacin treatment. *E. coli* cells of either WT (blue line) or mutant Δ*btsSRypdAB* (red line) were grown in LB-medium. At the post-exponential growth phase cells were challenged with ofloxacin (5 μg/ml). Samples were taken and analyzed for colony forming units (CFUs). The MDK_{99} value was taken as the time needed to kill 99% of the initial population. Experiments were performed three independent times and error bars indicate the standard deviations of the means.

	- INDUCER		+ INDUCER	
STRAINS	**Cells (%)**		**Cells (%)**	
	OFF	**ON**	**OFF**	**ON**
WT GFP (IPTG)	**92.7**	**7.3**	**3.1**	**96.9**
btsSRypdAB GFP (IPTG)	**97.8**	**2.2**	**51.0**	**49.0**
WT GFP-DppA (Arabinose)	**94.9**	**5.1**	**24.4**	**75.6**
btsSRypdAB GFP-DppA (Arabinose)	**98.1**	**1.9**	**99.4**	**0.6**
WT LysP-mCherry (Arabinose)	**97.7**	**2.3**	**33.4**	**66.6**
btsSRypdAB LysP-mCherry (Arabinose)	**98.2**	**1.8**	**98.5**	**1.5**

TABLE S1 The BtsSR/YpdAB network promotes overproduction of proteins. *E. coli* cells of either WT or the *btsSRypdAB* mutant harboring an overproduction vector with IPTG inducible promoter for the overproduction of GFP; an arabinose inducible promoter for the overproduction of DppA-GFP and an arabinose inducible promoter for the overproduction of LysP-mCherry were grown in LB medium. Samples were taken before (- inducer) and after (+ inducer) the addition of the inducer. Flow cytometry was used to count fluorescent cells (maximum of 2000

events), and the percentages of OFF (non-fluorescent cells) and ON cells (fluorescent cells) were calculated from the raw data. Experiments were performed three independent times and standard deviations were below 10%.