Putrescine reduces antibiotic-induced oxidative stress as a mechanism of modulation of antibiotic resistance in *Burkholderia cenocepacia*

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SUPPLEMENTARY FIGURES

FIG S1 Comparison of the autofluorescence of cells relative to fluorescence signals of fluorescent probes detecting reactive oxygen species in *B. cenocepacia* K56-2. (a) Emission signal following treatment with HPF without correction for autofluorescence background; (b) Autofluorescence of cells at the same inoculum size and under the same conditions used for HPF assay; (c) Emission signal following treatment with DCF without correction for autofluorescence background; (d) Autofluorescence of cells at the same inoculum size and under the same conditions used for DCF assay. n=3 from one representative experiment.



FIG S2 The relative growth of cells in the luminescence expression assay for *oxyR* expression in the wild type (OME56) compared to putrescine synthesis mutants ($\Delta BCAL2641$ background, OME57; and $\Delta BCAM1111-1112$ background, OME58) at 3 h shown in Figure 3A. Results are shown as percentage of OD₆₀₀ relative to the control (untreated K56-2 background). The mean OD₆₀₀ of the control is 0.1663. * p<0.05, ** p<0.01 and *** p<0.001.



FIG S3 The relative growth of cells in the luminescence expression assay for the different putrescine synthesizing enzymes in response to 500 µg/ml PmB at 3 h shown in Figure 4. Results are shown as percentage of OD₆₀₀ relative to the control (untreated K56-2 background). (a) Expression of BCAL2641 in the wild type (OME50) and $\Delta BCAM1111-1112$ (OME51) backgrounds. n=6 from 2 different clones. The mean OD₆₀₀ of the control is 0.1422. (b) Expression of BCAM1111 in the wild type (OME52) and $\Delta BCAL2641$ (OME53) backgrounds. n= 6 from 2 different clones. The mean OD₆₀₀ of the control is 0.1422. (b) Expression of BCAM1111 in the wild type (OME52) and $\Delta BCAL2641$ (OME53) backgrounds. n= 6 from 2 different clones. The mean OD₆₀₀ of the control is 0.1523. (c) Expression of BCAM1112 in the wild type (OME54) and $\Delta BCAL2641$ (OME55) backgrounds. n= 7 from 2 different clones. The mean OD₆₀₀ of the control is 0.1017. * p<0.05, ** p<0.01 and *** p<0.001.



FIG S4 Effect of different bactericidal antibiotics on superoxide radical at different concentrations determined using DCF. n=6 from 2 independent experiments.



FIG S5 The relative growth of cells in the luminescence expression assay for BCAL2641, (in OME50), *oxyR* (in OME56), and BCAM1111 (in OME52) in response to different bactericidal antibiotics at 3 h shown in Figure 6. Results are shown as percentage of OD₆₀₀ relative to the control (untreated K56-2 background). n= a minimum of 6 from at least 2 different clones. The mean OD₆₀₀ of the control is 0.1943 for BCAL2641; 0.1816 for OxyR and 0.2166 for BCAM1111. * p<0.05, ** p<0.01 and *** p<0.001.