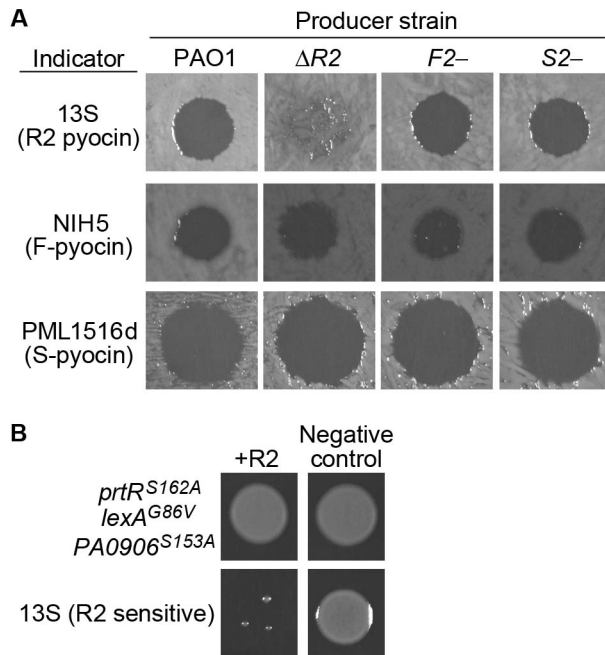
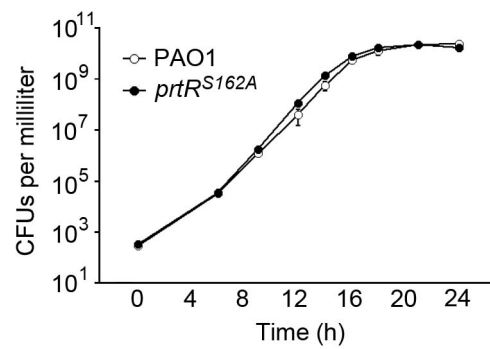


Supplementary Material

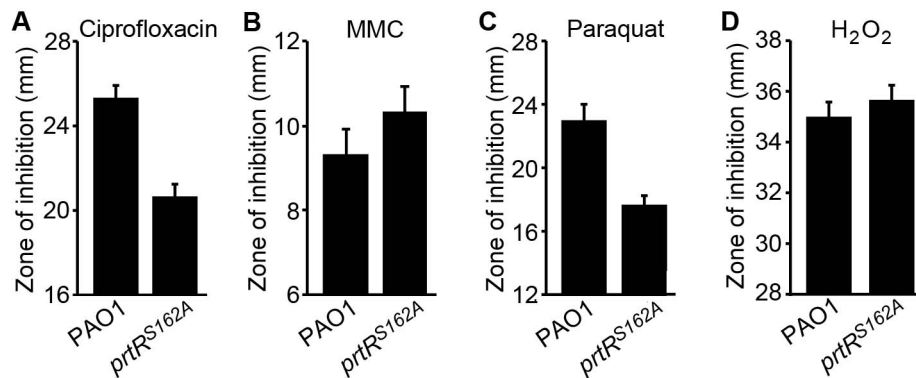


Supplementary Figure S1. Pyocin sensitivity of indicator strains and the triple *PA0906*^{S153A} *lexA*^{G86V} *prtR*^{S162A} mutant. (A) Production of pyocin by wild type and indicated pyocin mutants. Filtered supernatant from cultures treated with MMC (3 μg/ml) was spotted on lawns of an indicator strain. Clearing of the bacterial lawn is indicative of pyocin-mediated death. Note that strain 13S is a specific indicator of R2 pyocin activity. PML1516d and NIH5 are sensitive to more than one pyocin produced by PAO1. (B) Strain *PA0906*^{S153A} *lexA*^{G86V} *prtR*^{S162A} is resistant to R2 pyocin. *PA0906*^{S153A} *lexA*^{G86V} *prtR*^{S162A} cells were mixed 1:1 (V/V) with supernatant containing pyocin produced by MMC-treated PAO1 (+R2) and $\Delta R2$ (negative control) cultures. The mixture was then spotted agar plates. Confluent growth of the spot after overnight incubation indicates resistance to pyocin.

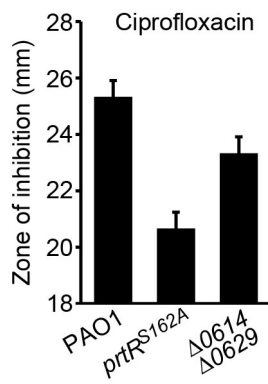


Supplementary Figure S2. Growth curves for the parental PAO1 and *prtR*^{S162A} strains.

Cultures were inoculated with cells derived from an agar plate and incubated in a baffled flask on a platform shaker. Each data point represents the average of three biological replicates. Error bars indicate SD.



Supplementary Figure S3. Autoproteolytic activity of PrtR reduces resistance to agents that cause or induce DNA damage. **(A)** Zone of growth inhibition of indicated strains around a filter disk on which 5 µg of ciprofloxacin was applied. Error bars indicate SD. **(B)** Zone of growth inhibition of indicated strains around a filter disk on which 20 µg of mitomycin C was applied. Error bars indicate SD. **(C)** Zone of growth inhibition of indicated strains around a filter disk on which 10 µl of 100 mM paraquat was applied. Error bars indicate SD. **(D)** Zone of growth inhibition of indicated strains around a filter disk on which 10 µl of 6% H₂O₂ solution was applied. Error bars indicate SD.



Supplementary Figure S4. Ciprofloxacin resistance of strains $\Delta 0614 \Delta 0629$. (A) Zone of growth inhibition of indicated strains around a filter disk on which 5 μg of ciprofloxacin was applied. Error bars indicate SD.

Table S1. Minimal inhibitory concentration of several antibiotics for *prtR*^{S162A}, *PA0906*^{S153A}, and PAO1 strains.

| | Ciprofloxacin | Tobramycin | Gentamycin | Kanamycin | Carbenicillin | Tetracycline |
|--------------------------------|---------------|------------|------------|-----------|---------------|--------------|
| PAO1 | 0.75 µg/ml | 0.25 µg/ml | 0.25 µg/ml | 64 µg/ml | 16 µg/ml | 10 µg/ml |
| <i>prtR</i> ^{S162A} | 2.0 µg/ml | 0.5 µg/ml | 0.5 µg/ml | 128 µg/ml | 16 µg/ml | 10 µg/ml |
| <i>PA0906</i> ^{S153A} | 0.75 µg/ml | 0.5 µg/ml | 0.5 µg/ml | 128 µg/ml | 16 µg/ml | 10 µg/ml |