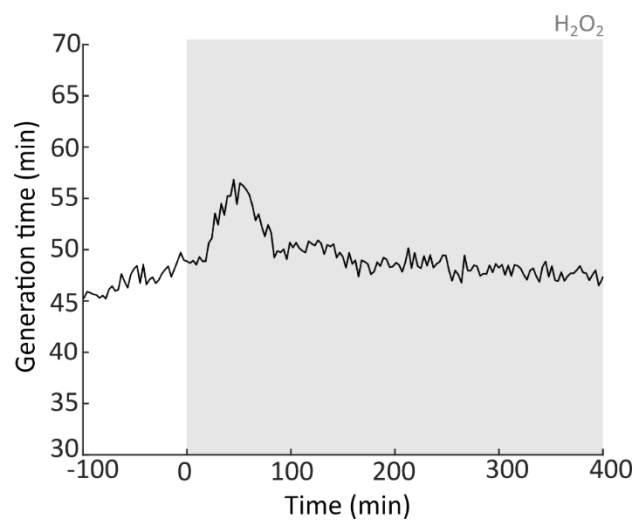


APPENDIX

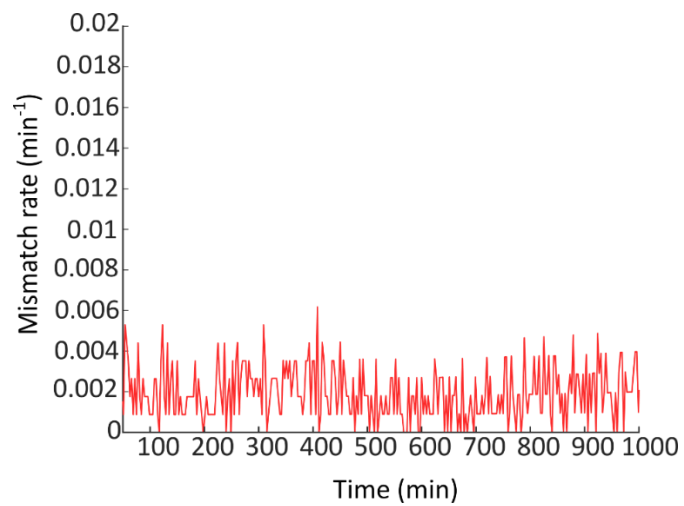
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Appendix Figure S1 - Transient increase of the generation time in WT cells treated with H₂O₂.

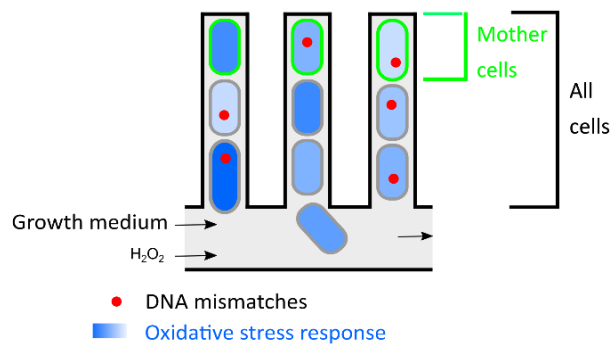
Generation time of WT cells treated with 100 μ M of H₂O₂ (6562 cells, 8 biological replicates).



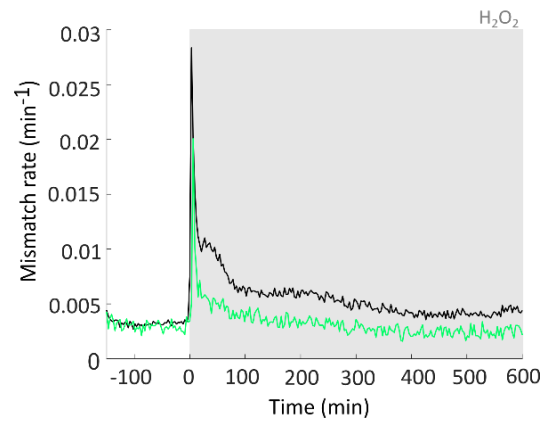
Appendix Figure S2 - Mismatch rate is stable over time in untreated cells.

Rate of DNA mismatches per cell per minute in untreated cells (651 cells, 1 biological replicate).

A



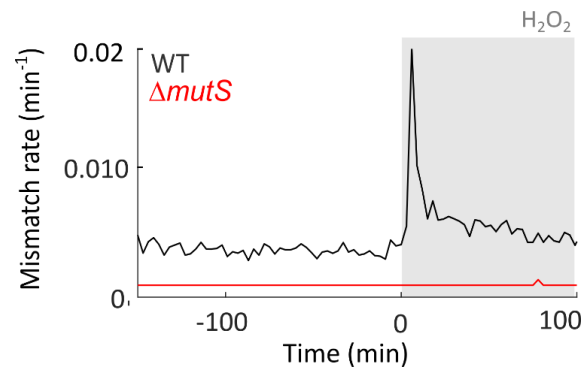
B



Appendix Figure S3 - All cells in the microfluidic growth trenches experience a burst of mutagenesis.

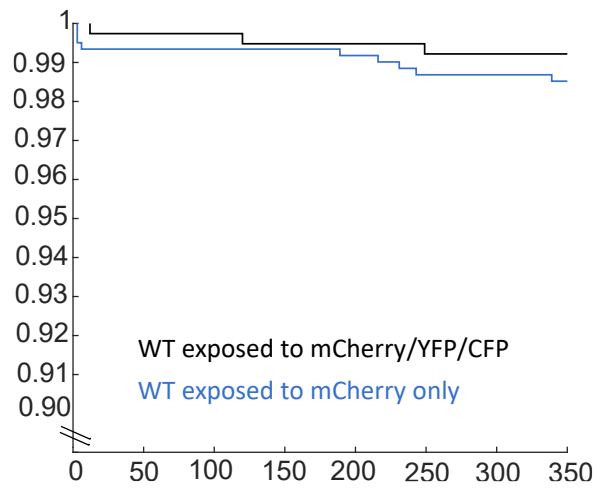
A Throughout the study, we focused our analysis on the mother cells that can be continuously observed at the closed end of each growth trench over multiple generations.

B Rate of DNA mismatches per cell per minute for all cells in the trenches with 100 μ M H_2O_2 treatment (35082 cells, 8 biological replicates).



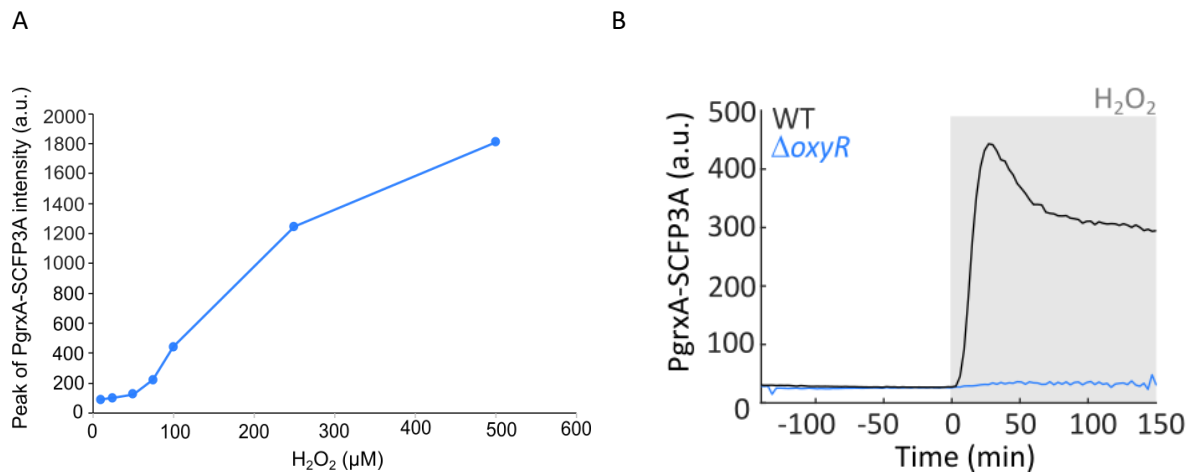
Appendix Figure S4 - No MutL-mYPet foci are detected in a $\Delta mutS$ deletion strain.

Rate of DNA mismatches per cell per minute of a $\Delta mutS$ mutant strain with 100 μ M H₂O₂ treatment (551 cells, 1 biological replicate).



Appendix Figure S5 - YFP and CFP excitation lights do not induce toxicity

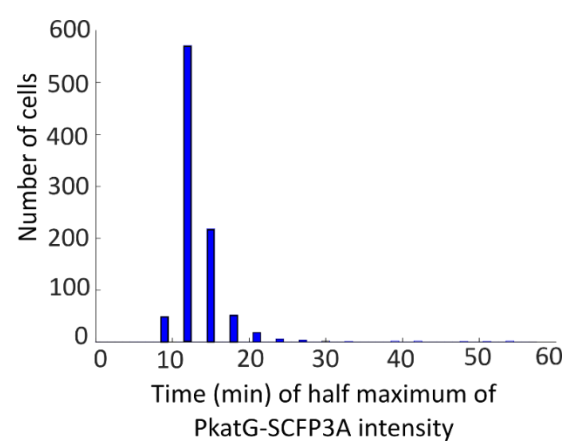
Fraction of cells surviving without treatment for WT cells exposed to CFP, YFP and mCherry light (black, 551 cells, 1 biological replicate) and WT cells exposed to mCherry light only (dark blue, 608 cells, 1 biological replicate).



Appendix Figure S6 - PGrxA expression is dose dependent and is not induced in $\Delta oxyR$ mutant cells.

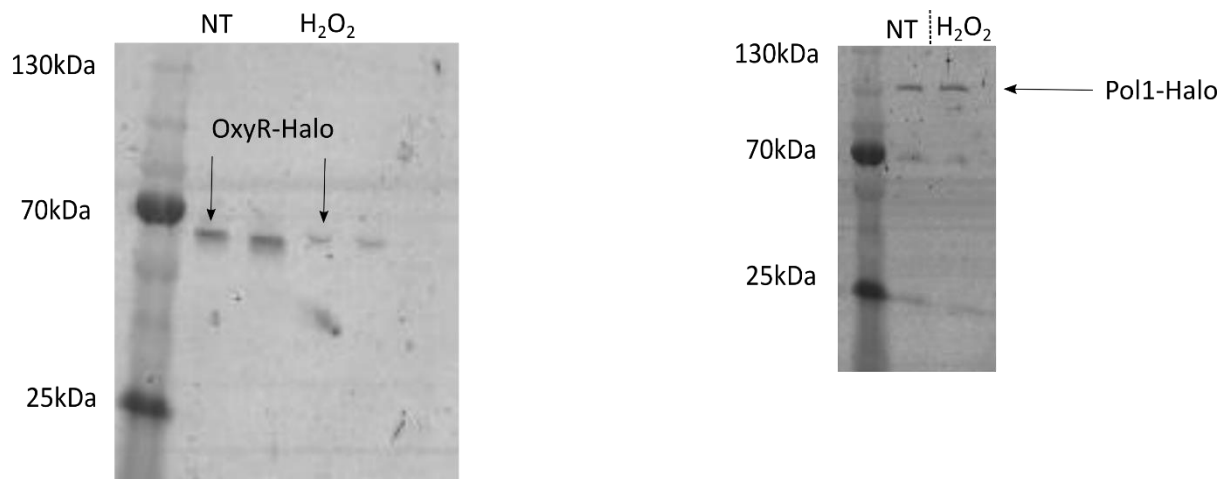
A Peak of PgrxA-SCFP3A reporter intensity in response to different doses of H₂O₂ (10 μM (651 cells, 1 biological replicate), 25 μM (1281 cells, 2 biological replicates), 50 μM (465 cells, 1 biological replicate), 75 μM (466 cells, 1 biological replicate), 100 μM (1848 cells, 2 biological replicates), 250 μM (331 cells, 1 biological replicate), 500 μM (764 cells, 1 biological replicate)).

B PgrxA-SCFP3A expression stays at a basal level in $\Delta oxyR$ cells treated with 100 μM H₂O₂ (728 cells, 3 biological replicates), compared to WT (black).



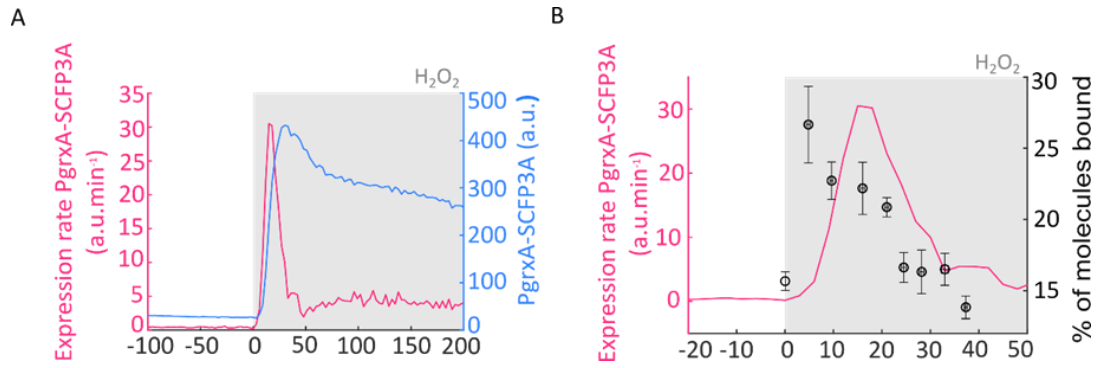
Appendix Figure S7 - KatG expression is induced shortly after the start of the treatment.

Histogram of the lag time distribution for PkatG-SCFP3A to reach half-maximal intensity after start of 100 μM H₂O₂ treatment (988 cells, 1 biological replicates).



Appendix Figure S8 - In-gel fluorescence confirms stability of OxyR-Halo and Pol1-Halo fusions.

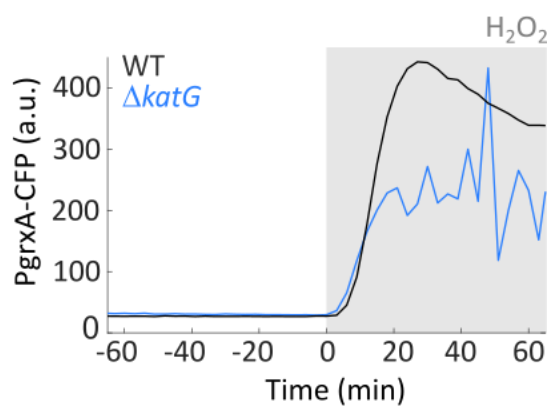
Strains expressing OxyR-Halo and Pol1-Halo were labelled with TMR dye as for microscopy experiments. Cells were lysed and analysed via SDS-PAGE without treatment (NT) and 30 min after treatment with 100 μ M H₂O₂. For OxyR-Halo fusion, one band is detected on the gel corresponding to the expected size of OxyR (34.4 kDa) plus the HaloTag (33 kDa). For Pol1-Halo fusion, one band is detected on the gel corresponding to the expected size of Pol1 (94.08 kDa) plus the HaloTag (33 kDa). The 2 unlabelled lanes in the OxyR-Halo gel are not relevant to this work.



Appendix Figure S9 - Expression rate and intensity of PgrxA-mSCFP3 reporter.

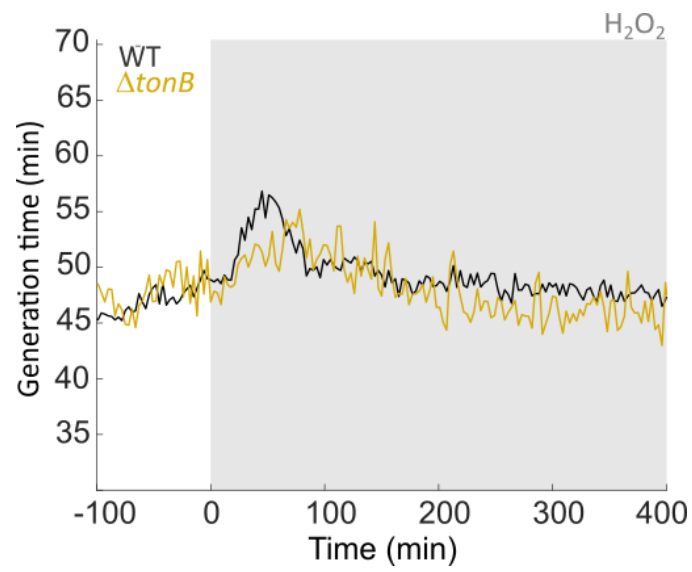
A Expression rate of PgrxA-SCFP3A (pink, 878 cells, 1 biological replicate) and PgrxA-SCFP3A intensity (blue, 878 cells, 1 biological replicate) with 100 μ M H_2O_2 treatment.

B Expression rate of PgrxA-SCFP3A (pink, 878 cells, 1 biological replicate) and percentage of bound OxyR-Halo molecules (black, from Fig. 3D).



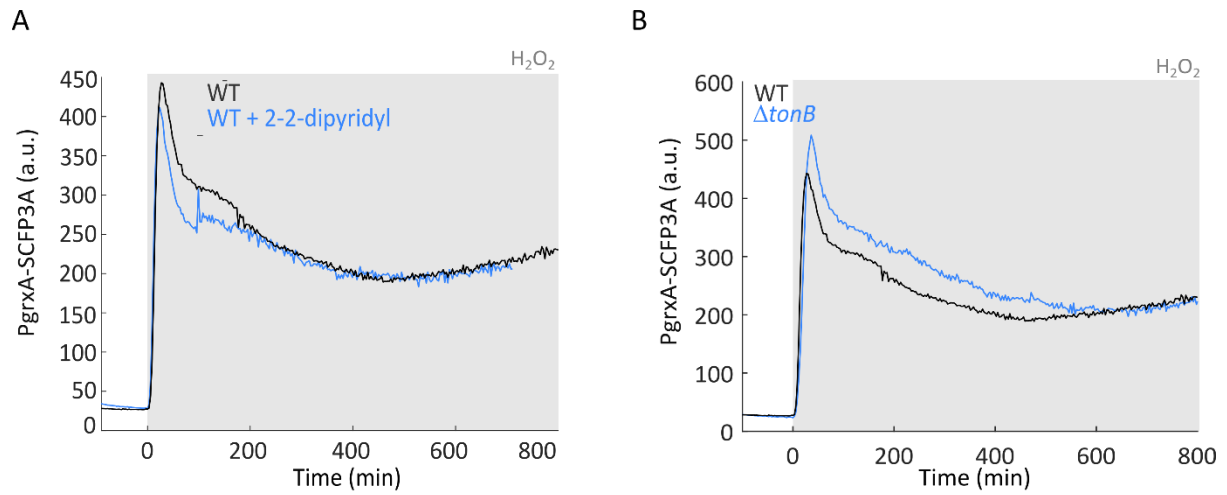
Appendix Figure S10 - PGrxA expression in $\Delta katG$ mutant cells.

PgrxA-SCFP3A expression in $\Delta katG$ cells treated with 100 μM H_2O_2 (1083 cells, 1 biological replicate), compared to WT (black).



Appendix Figure S11 - Effect of iron limitation on cell cycle duration with H_2O_2 treatment.

Generation time of WT (black) and $\Delta tonB$ (yellow, 1753 cells, 3 biological replicates) mutant cells with 100 μM H_2O_2 treatment.

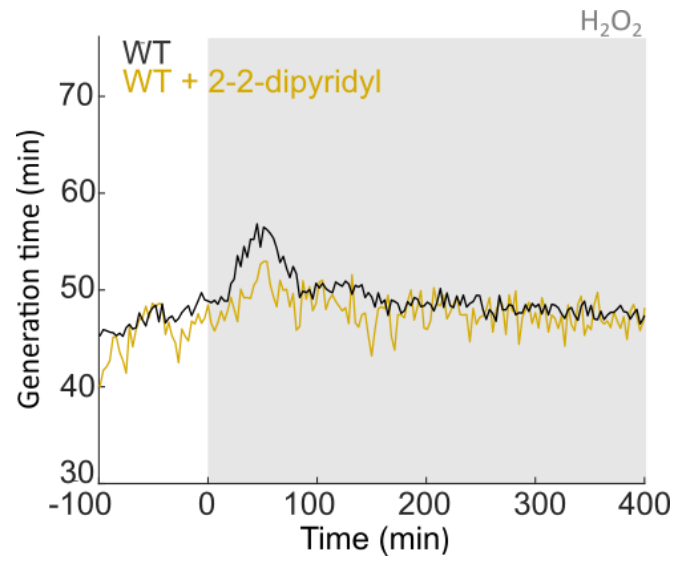


Appendix Figure S12 - Effects of perturbed iron homeostasis on OxyR response expression with 100 μM H_2O_2 treatment.

A PgrxA-SCFP3A intensity of cells in presence of DP (2270 cells, 3 experiments (biological replicates).

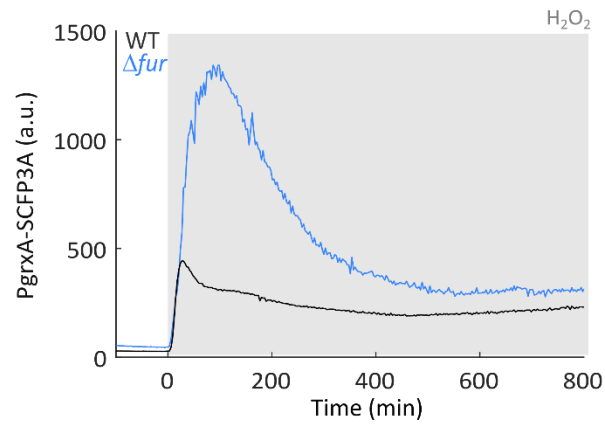
B PgrxA-SCFP3A intensity of $\Delta tonB$ mutant (573 cells, 2 biological replicates).

WT is shown in black for comparison (2355 cells, 2 biological replicates).



Appendix Figure S13 Effect of DP on cell cycle duration with H_2O_2 treatment.

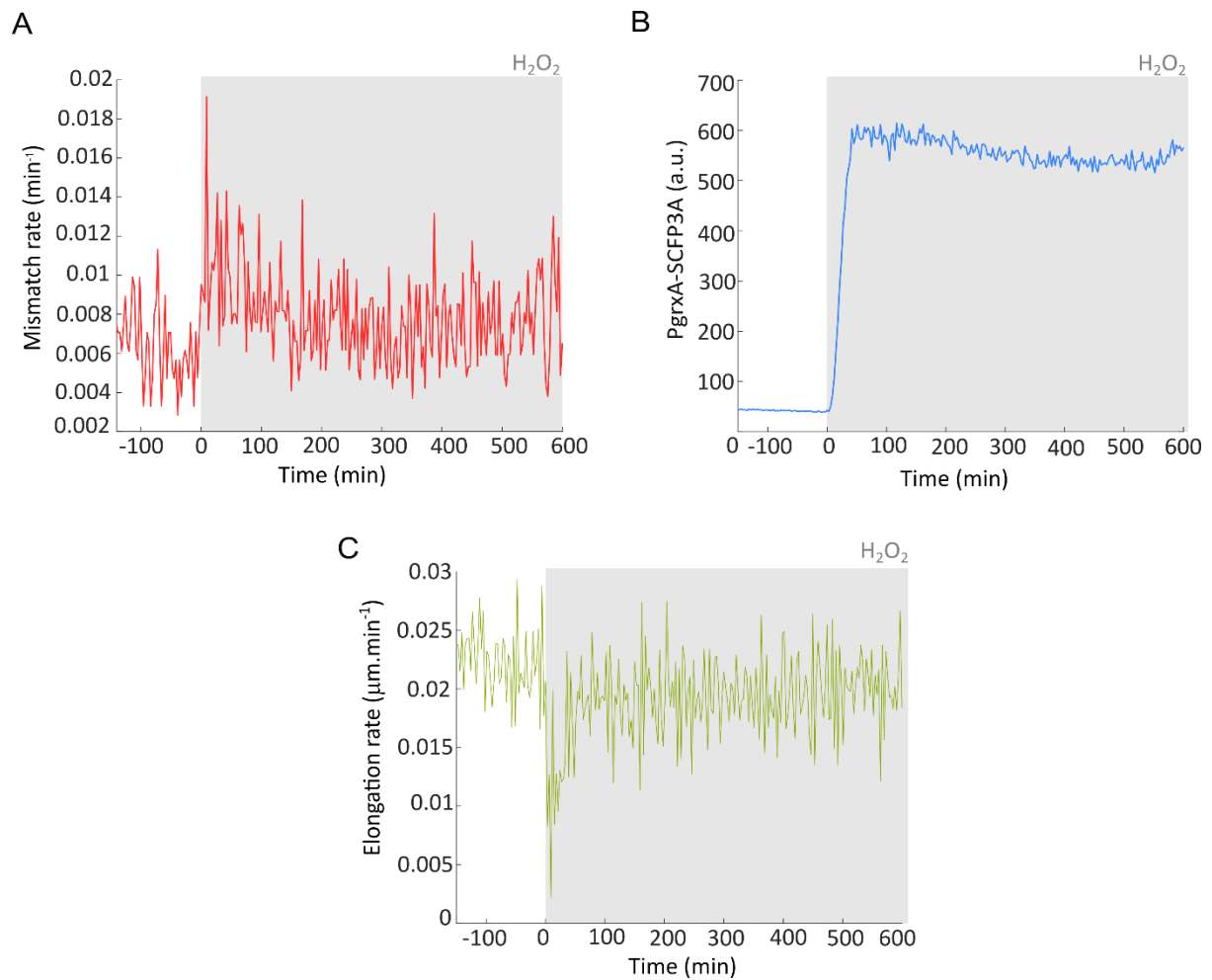
Generation time of WT cells with 100 μM H_2O_2 treatment in medium supplemented with 50 μM 2-2-dipyridyl (yellow, 2010 cells, 3 experiments biological replicates) or without supplement (black).



Appendix Figure S14 - Effects of *fur* deletion on OxyR response expression with 100 μM H_2O_2 treatment.

PgrxA-SCFP3A intensity of Δfur mutant (520 cells, 1 biological replicate).

WT is shown in black for comparison (2355 cells, 2 biological replicates).



Appendix Figure S15 - Similar mismatch rate and adaptation dynamics are observed in *E. coli* strain MG1655 compared to AB1157.

A Rate of DNA mismatches per cell per minute before and during constant treatment with 100 μM H_2O_2 (710 cells, 2 biological replicates).

B PgrxA-SCFP3A expression before and during constant treatment with 100 μM H_2O_2 (710 cells, 2 biological replicates).

C Cell elongation rate before and during constant treatment with 100 μM H_2O_2 (672 cells, 2 biological replicates).