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1 **Single-cell analysis of the Dps response to oxidative stress**

2

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10 **Running title:** Single-cell analysis of Dps response

11 **Abstract**

12

13 Microorganisms have developed an elaborate spectrum of mechanisms to respond and
14 adapt to environmental stress conditions. Among these is the expression of *dps*, coding for
15 the DNA-binding protein from starved cells. Dps becomes the dominant nucleoid-organizing
16 protein in stationary-phase *Escherichia coli* cells and is required for robust survival under
17 stress conditions including carbon or nitrogen starvation, oxidative stress, metal exposure,
18 and irradiation. To study the complex regulation of Dps in *E. coli*, we utilized time-lapse
19 fluorescence microscopy imaging to examine the kinetics, input-encoding, and variability of
20 the Dps response in single cells. In the presence of an oxidative stressor, we observed a
21 single pulse of activation of Dps production. Increased concentrations of H₂O₂ led to
22 increased intensity and duration of the pulse. While lower concentrations of H₂O₂ robustly
23 activated the Dps response with little effect on growth rate, higher concentrations of H₂O₂
24 resulted in dramatically slower and highly variable growth rates. Comparison of cells within
25 the same concentration of H₂O₂ revealed that increased levels of Dps expression did not
26 confer a growth advantage, indicating that recovery from stress may rely primarily upon
27 variation in the amount of damage caused to individual cells.

28 **Importance**

29

30 We show for the first time the response of the DNA-binding protein from starved cells (Dps)
31 to oxidative stress in single cells of *E. coli*. Through time-lapse fluorescence microscopy, a
32 single pulse of Dps production is observed in cells exposed to H₂O₂, with a duration and
33 intensity of the induction proportional to the concentration of the applied stress. A more
34 intense Dps expression did not provide a growth benefit to the bacteria, suggesting that

35 healing from oxidative stress may largely depend upon the amount of damage in each
36 individual cell.

37 **Introduction**

38

39 Bacteria encounter many stresses during their development, and they need to be able to
40 adapt quickly to the environment to survive. Bacterial response mechanisms frequently
41 involve specific sets of genes activated to help the cell adapt to the stress. Alternative sigma
42 factors, of which *Escherichia coli* has seven, are a frequent regulatory mechanism (1). While
43 housekeeping genes expressed during exponential growth are controlled by the
44 transcription factor σ^{70} (2, 3), alternative sigma factors act as transcription initiation factors
45 to control the activation of specialized regulons during specific growth or stress conditions
46 (4). The general stress response sigma factor σ^S activates the transcription of more than 70
47 genes, conferring resistance to carbon/phosphate/nitrogen starvation, heat shock, high/low
48 pH, UV-radiation, and oxidative stress, among others (5, 6).

49 Microorganisms living in an aerobic environment unavoidably encounter oxidative stress as a
50 by-product of their aerobic metabolism (7). The resultant formation of reactive oxygen
51 species (ROS) can lead to the damage of cellular components including membranes, DNA,
52 and proteins (8). As an adaptation to this condition, bacteria produce enzymes such as
53 superoxide dismutases and reductases to scavenge these toxic components (9). Additionally,
54 cells also face external sources of oxidative stress: macrophages produce superoxide and
55 nitric oxide to kill invading bacteria (10); following perception of pathogens, plants also
56 induce the synthesis of organic peroxides (11); certain communities of microorganisms

57 excrete ROS to inhibit the growth of their competitors (12); and exposure to environmental
58 redox cycling compounds can cause damaging intracellular redox reactions (13).

59 In this challenging environment, bacteria have developed refined molecular mechanisms of
60 defense. The DNA-binding protein from starved cells (Dps) plays a crucial role during stress
61 exposure. *Escherichia coli dps* mutants experience a severe reduction in survival when
62 exposed to any of several different stressors including oxidative stress, heat shock, metal
63 exposure, UV and gamma irradiation, or extreme pH (14-16). Additionally, Dps was shown to
64 protect cells against DNA strand breakage (17). In *E.coli*, the protective effect of Dps is
65 attributed to its dual biochemical functions. Dps has the ability to bind DNA and form Dps-
66 DNA crystals, which may provide mechanical shielding against damaging agents (14, 18, 19).
67 The ferroxidase activity of Dps may also contribute significantly to its protective abilities.
68 Hydroxyl radicals can be formed intracellularly through chemical reaction between ferrous
69 iron and H₂O₂, either internally generated or derived from the environment. Dps catalyzes
70 the oxidation of ferrous iron, preferring H₂O₂ as a reactant rather than O₂, thereby
71 preventing the formation of hydroxyl radicals (20). Dps oligomers are composed of 12
72 identical monomers, each one folded into a compact four-helix bundle (21), surrounding a
73 central cavity that can store up to 500 iron atoms (22). The DNA-binding and ferroxidase
74 activities of Dps are biochemically separable, but they both contribute to maintain DNA
75 integrity and cellular viability (23).

76 Intracellular Dps levels are controlled by a complex regulatory network. During the transition
77 from exponential to stationary phase, the number of Dps molecules within a single *E. coli*
78 bacterium increases from approximately 6000 to 180,000, whereby it becomes the most
79 abundant DNA-binding protein (24). *dps* is transcribed from a single promoter recognized by

80 either the σ^{70} (housekeeping) or σ^S (stationary phase) sigma factor in response to different
81 growth and environmental conditions (25-27). In exponential growth, *dps* can be activated in
82 an OxyR-dependent manner by treatment of the cells with H₂O₂, recruiting σ^{70} to initiate
83 transcription. During stationary phase or carbon starvation, σ^S controls *dps* expression (25).
84 When bacteria are growing exponentially and not exposed to stress, the *dps* promoter is
85 downregulated by two nucleoid-binding proteins: Fis and H-NS (26, 28).

86 Despite the knowledge acquired in recent years, the behavior of the Dps response is not
87 understood at the single-cell level. Upon exposure to oxidative stress, each cell that sustains
88 oxidative damage will require sufficient upregulation of enzymes that can counteract the
89 damage in order to maintain its health. However, the high-resolution fluctuations of Dps
90 production levels over time and the intensity and duration of Dps production during the Dps
91 response are still unknown at the single-cell level as well as in bulk cultures. Very little is
92 known also about the variability of the Dps stress response in individual cells and its effect
93 on cellular growth rate, which could play a crucial role in the ability of a bacterial population
94 to maintain competitive advantage in adverse environmental conditions. In addition, it is
95 unknown how the dynamics of Dps production are affected when the concentration of
96 stressor is varied, a question that is central to the ability of a cell to respond appropriately to
97 changes in its environment. Clear insights into these biological processes require recently
98 developed single-cell technologies to overcome the limitations of bulk experiments, allowing
99 for quantification of the cell-to-cell variability in a population as well as characterization of
100 the dynamics of stress responses (29-35).

101 In this work, we examined the kinetics and variability of activation of Dps production at the
102 single-cell level upon exposure to different levels of oxidative stress. We observed one single

103 pulse of Dps production, with an intensity and duration proportional to the concentration of
104 H₂O₂ applied, until the highest concentration of H₂O₂ resulted in saturation of the intensity
105 but not the duration of Dps production. Cell growth was not linearly correlated with the
106 H₂O₂ concentration, such that low concentrations resulted in robust Dps production but only
107 a minor decrease in initial growth rate. Higher concentrations of H₂O₂ were associated with
108 major reductions in growth rate, accompanied by dramatically increased variation. A
109 comparison of bacteria that were exposed to the same concentration of stressor revealed
110 that higher levels of Dps production were associated with similar or slower growth
111 compared to cells with lower Dps production. This behavior was perhaps due to variation in
112 the amount of damage experienced by individual cells that drove both reduced growth and
113 increased Dps production.

114 **Materials and Methods**

115 *dps-mCherry* strain construction

116

117 The *E. coli dps-mCherry* strain was created from the *E. coli* K-12 strain W3110 (CGSC# 4474)
118 by replacement of the genomic *dps* gene by a counter-selectable *cat-sacB* cassette (23) and
119 subsequent replacement with a *dps-mCherry* cassette.

120 The *dps-mCherry* cassette was created using an adapted version of the Gibson DNA assembly
121 protocol (36) and introduced into the pBAD33 plasmid to create the pM1 plasmid. The
122 backbone plasmid pBAD33 (37) was amplified using PCR to create compatible ends for
123 recombination with the *dps-mCherry* cassette. The following primers were used: forward
124 MDM1 5'-GATCCCCGGGTACCGAGCTC-3' and reverse MDM2 5'-CAAGCTTGGCTGTTTTGGCG-
125 3'. The *mCherry* gene was amplified using PCR from the plasmid pROD22 (38) to introduce

126 the *dps* ribosome binding site (RBS) sequence immediately upstream of the *mCherry* gene.
127 The following primers were used (the sequence of the RBS is underlined): forward MDM3 5'-
128 CATCAAAGAGGATATGAAATTATGGCTATCATTAAGAGTTC-3' and reverse MDM4 5'-
129 TTA^{CTTGTACAGCTCGTCCATGC}-3'. This RBS-*mCherry* PCR product was further amplified to
130 introduce an upstream flanking sequence homologous to the *dps* gene and a 30-bp
131 downstream flanking sequence homologous to the pBAD33 plasmid. The following primers
132 were used (the sequences of the homologous regions are underlined): forward MDM5 5'-
133 GTTTATCGAGTCTAACATCGAATAACATCAAGAGGATATGAAATTATG-3' and reverse MDM6 5'-
134 TTCTCTCATCCGCCAAAACAGCCAAGCTTGTTACTTGTACAGCTCGTCC-3'. The *dps* gene was
135 amplified from the pET17b-*dps* plasmid (23) to introduce a 30-bp upstream flanking
136 sequence homologous to the plasmid pBAD33 and a downstream flanking sequence
137 homologous to the RBS-*mCherry* gene. The following primers were used (the sequences of
138 the homologous regions are underlined): forward MDM7 5'-
139 TAGCGAATTCGAGCTCGGTACCCGGGGATCATGAGTACCGCTAAATTAGT-3' and reverse MDM8
140 5'-CATAATTCATATCCTCTTGATGTTATTCGATGTTAGACTCGATAAAC-3'.

141 The three fragments were DpnI (New England Biolabs (NEB))-digested at 37°C for 1 hour and
142 purified with Wizard® SV Gel and PCR Clean-Up System (Promega), then assembled using
143 Gibson DNA assembly (36). The assembly reaction was prepared by combining 15 µL of
144 Gibson assembly master mix (320 µL of 5X ISO buffer [0.5 M Tris-HCl (Sigma) pH 7.5, 50 mM
145 MgCl₂, 4 mM dNTP (Invitrogen) mix (equal concentration of the four nucleotides), 50 mM
146 DTT (Sigma), 25% w/v PEG-8000 (Sigma), 5 mM NAD (NEB)], 0.64 µL of 10 U µL⁻¹ T5
147 exonuclease (Epicentre), 20 µL of 2 U µL⁻¹ Phusion polymerase (Finnzymes), 160 µL of 40 U
148 µL⁻¹ Taq ligase (NEB), dH₂O to 1.2 ml), 100 ng of linearized vector backbone, and 100 ng of
149 each assembly fragment in a total volume of 20 µL. The reaction was incubated at 50°C for

150 60 min. Electrocompetent *E. coli* W3110 cells were transformed with 5 μ L of the assembly
151 reaction using electroporation. The positive colonies carrying the chloramphenicol resistance
152 gene from the pBAD33 plasmid were identified, and the accuracy of the sequence was
153 checked with sequencing analysis.

154 The *dps-mCherry* cassette was amplified from the pM1 plasmid using PCR to introduce 50-bp
155 flanks homologous to the chromosomal *dps* flanks. The following primers were used (the
156 sequences of the homologous regions are underlined): forward MDM9 5'-

157 TACTTAATCTCGTTAATTACTGGGACATAACATCAAGAGGATATGAAATTATGAGTACCGCTAAATTA

158 G-3' and reverse MDM10 5'-

159 AGGAAGCCGCTTTTATCGGGTACTAAAGTTCTGCACCATCAGCGATGGATTTACTTGTACAGCTCGTC

160 CA-3'. The fragment was DpnI-digested and purified, then introduced with electroporation
161 into a W3110 *dps::cat-sacB* strain (23). Homologous recombination was allowed to occur for
162 3 hours in LB medium at 37°C while shaking at 250 rpm, and cells were plated on NaCl-free
163 LB 10% sucrose agar (counterselective for *sacB*). Plates were incubated overnight at 30°C.

164 Healthy-looking colonies were re-streaked on LB agar containing 25 μ g mL⁻¹
165 chloramphenicol. Colonies that did not grow on chloramphenicol were screened using
166 colony PCR, and gene replacement was verified by sequence analysis. The *mCherry*
167 expression was confirmed with fluorescence-activated cell sorting (FACS) (data not shown).

168 **Growth conditions for microscopy**

169

170 For the single-cell microscopy experiments, one colony of *dps-mCherry* was inoculated
171 overnight into Hi-Def Azure medium (3H500, Teknova) supplemented with 0.2% glucose and
172 grown overnight at 37°C. This preculture was diluted 1:100 and grown for around 2 hours at

173 37°C until early exponential phase (O.D.₆₀₀ 0.2-0.3). The culture was diluted to O.D.₆₀₀=0.005
174 for seeding onto the agarose pad.

175 **Agarose pad preparation**

176

177 Agarose pads were prepared with a modified version of the protocol in (39). The pads were
178 prepared freshly for each experiment. 2% (w/v) low-melt Agarose LE (V3125, Promega) was
179 added to 5 mL of Hi-Def Azure medium and dissolved by microwaving. After the agarose
180 solution had cooled, H₂O₂ was added. Agarose pads were formed immediately thereafter.
181 Cover glass slides of 20 mm² (631-0122, VWR) were placed on Parafilm M® (Bemis Company,
182 Inc.), and 900 µL of agarose were pipetted onto each. Immediately after pipetting, a second
183 cover glass was placed on top of the agarose. The pads were allowed to solidify for 45–60
184 min at room temperature while covered with a lid to prevent edge evaporation. When the
185 agarose was solidified, it was cut into pads of 0.5 x 0.5 cm. 2 µL of bacterial culture diluted to
186 O.D.₆₀₀ 0.005 was seeded onto individual agarose pads. The culture was allowed to
187 evaporate and absorb into the agarose for about 10 min at room temperature. When the
188 surface appeared to be dry, the pad was flipped with a scalpel onto a 4-well slide-base tissue
189 culture chamber (Starstedt). The chamber was closed with a lid and sealed with Parafilm M®
190 to avoid evaporation during the imaging. The cells were able to grow in a monolayer due to
191 their placement between the glass bottom of the chamber and the agarose pad on top.

192 The variability of H₂O₂ distribution in the pads was determined using rhodamine as a
193 fluorescent reporter. During the preparation of the agarose pads, dihydrorhodamine 123
194 (D1054, Sigma-Aldrich) was added to a final concentration of 20 µM after the agarose
195 solution had cooled, and the pads were formed immediately thereafter. The pads were

196 scanned using a Typhoon Trio (Amersham Biosciences), and the images were analyzed using
197 ImageJ software (40). The fluorescence intensity values of 80 different pixels in 2 different
198 pads were averaged, and the standard deviation was calculated, showing an upper limit of
199 variability of 12.9%. We expect a lower variability for the H₂O₂ molecule than for rhodamine,
200 since the diffusion coefficient of H₂O₂ is 1 order of magnitude larger than that of rhodamine:
201 $1.305 \pm 0.83 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (41) and $4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ respectively (42).

202 **Fluorescence microscopy**

203

204 Microcolonies on agarose pads were imaged by time-lapse fluorescence microscopy using an
205 inverted microscope (Olympus IX81), an AMH-200 lamp (Andor), and a Cy3 filter cube
206 (4040C). Images were acquired with Luca R EMCCD camera (Andor). Andor iQ software was
207 used to control the microscope and to perform automatic imaging acquisition. Experiments
208 were performed at 37°C using an incubation chamber (H201-T, Okolab) to allow precise
209 temperature control. Phase contrast images (500 ms exposure time, 3 images +/- 0.2 μm
210 from the focus) and fluorescence images (100 ms exposure time) were recorded every 5 min,
211 for 3-4 hours.

212 **Data analysis**

213

214 Images were analyzed using a custom Matlab program (43) based on the Schnitzcells
215 program (39). Data analysis consisted of three steps: segmentation, tracking, and extraction
216 of cell parameters. Each phase contrast image (average of three) was segmented: the
217 background was separated from the cells, and clumps of cells were cut based on concavity
218 and phase contrast maxima. Then the outline of each individual cell was detected. Cell

219 edges were determined using Laplacian of Gaussian filter. The segmentation of the cells was
220 checked and corrected manually when necessary. Next, tracking was performed, in which
221 cell lineages were traced by a tracking algorithm that searches for nearby cells in successive
222 frames. Lastly, cell length was extracted from segment properties, and growth rate was
223 determined from exponential fits of lengths-in-time. Individual cell fluorescence was
224 extracted from fluorescent images using segmentation obtained from phase contrast images
225 (for more details on analysis see Supplemental material). For each microcolony, the
226 fluorescence intensity curves were fitted with the best-fitting polynomial (degree 5), and the
227 maximum of this function was considered to be the maximum fluorescence intensity. Dps
228 production activity is defined as the rate of mCherry protein production (33, 35, 39, 44). The
229 duration of Dps production was calculated as time from the beginning of the exposure to
230 H₂O₂. Between 11 and 19 colonies for each stress condition were analyzed for a total of 75
231 colonies.

232 **Results**

233 **Construction of a reporter strain for Dps production**

234

235 To explore Dps production dynamics, we constructed a reporter strain of *E. coli* (named
236 “*dps-mCherry*”), with the *mCherry* gene introduced as a reporter for Dps production. The
237 two genes are both present in the *dps* promoter, with *mCherry* immediately downstream of
238 *dps*. A ribosome binding site (RBS) sequence identical to that of the *dps* RBS was placed
239 upstream of the *mCherry* reporter gene (Fig. S1). This construct allowed the detection and
240 the quantification of Dps production activity in single cells through monitoring of the
241 collective fluorescence emitted by the fluorescent proteins. In order to characterize the

242 health of the *dps-mCherry* strain, we compared its growth with the wild-type parental strain
243 in the presence of H₂O₂ concentrations between 0 and 10 mM. Both the strains showed a
244 similar growth response (Fig. S2). The growth kinetics were comparable at concentrations of
245 H₂O₂ up to 1 mM, showing similar robust exponential-phase kinetics and final optical
246 densities. At higher concentrations of H₂O₂, both strains showed growth inhibition. Thus, the
247 engineered *dps-mCherry* strain exhibits similar growth response to H₂O₂ as the wild-type
248 strain.

249 To verify Dps production, both strains were exposed to 0, 0.5, and 1 mM H₂O₂, and Dps
250 protein levels were analyzed through Western blotting. An increase in Dps concentration,
251 proportional to the stressor concentration, was detected in both strains in the presence of
252 H₂O₂ (Fig. S3).

253 An engineered strain carrying a chimeric version of *dps*, fused C-terminally to the *mCherry*
254 gene as translational reporter, was also constructed. Cells expressing this protein showed a
255 non-homogeneous distribution of fluorescence throughout the cell volume, with visible
256 puncta of more intense fluorescence (data not shown). Previous work has similarly shown
257 that fusion of Dps with the GFP protein resulted in aggregation of this fusion protein in *E. coli*
258 cells (45). This strain was therefore excluded from further experimentation.

259

260 **Dps production dynamics during oxidative stress**

261

262 Cells exposed to concentrations of H₂O₂ between 0 and 100 μM were analyzed using
263 quantitative time-lapse fluorescence microscopy to detect Dps protein production, defined
264 as the rate of mCherry protein production (33, 35, 39, 44), in each individual cell over time.

265 The *E. coli* cells were grown in rich defined medium to early exponential phase, then

266 transferred to an agarose pad in which H₂O₂ was incorporated, to begin the application of
267 oxidative stressors. Individual cells grew and divided over time to give rise to a microcolony.
268 A difference in growth and fluorescence could be observed in cells not exposed to any
269 stressor compared to those in the presence of different concentrations of H₂O₂. In the
270 colonies without applied stress, we observed that the fluorescence of each cell is
271 indistinguishable from background during the entire duration of the measurement (Fig. S4
272 A). In the presence of H₂O₂, we detected a fluorescent signal that was roughly proportional
273 to the amount of applied stress (Fig. S4 B-E). We observed a general trend for the intensity
274 of the fluorescence signal over time: the intensity increased during the initial period of the
275 measurement and then decreased thereafter. Reduced growth was apparent at higher
276 concentrations of H₂O₂, and at 100 μM H₂O₂ there was a near-complete inhibition of cell
277 division (Fig. S4 D-E).

278 The data was analyzed using modified Schnitzcells software (39) to extract the fluorescence
279 intensity within single cells as mean fluorescence per unit area (43). In the absence of
280 oxidative stress, the fluorescence intensity of each individual cell present within a
281 microcolony over time was very low (Fig. 1 A). Exposure to hydrogen peroxide induced a
282 single pulse of fluorescence that started shortly after the cell progenitor of the colony first
283 experienced the stress, in every individual cell analyzed (100%) (Fig. 1 B-E). The pulse was
284 highly synchronized between the individual cells within each microcolony population
285 throughout the duration of the imaging. The variability of fluorescence signal among cells
286 within a colony at each time point was evaluated by calculation of the coefficient of variation
287 (CV) as the ratio of the standard deviation to the mean. As the single cells divided to form a
288 small microcolony over the course of the experiment, the CV remained low, between 0.0
289 and 0.25, for colonies exposed to 0, 50, or 100 μM H₂O₂. For cells exposed to 10 or 30 μM

290 H₂O₂, the CV increased steadily over time to reach values around 0.5 (Fig. 1 F). This increase
291 in CV over time might be due to an asymmetric division of oxidative components or mCherry
292 molecules among individual bacteria as the cell population increases through cell division.

293

294 In order to compare fluorescent responses between microcolonies, we calculated the
295 average of the fluorescence values of all cells within a microcolony, at each time point
296 measured (Fig. 2). Every colony grown in the absence of stressor showed a low average
297 fluorescence signal that decreased slightly over the duration of the imaging (Fig. 2 A). The
298 colonies exposed to 10, 30, or 50 μ M H₂O₂ showed a similar fluorescence profile: a large
299 transient increase in fluorescence over time that took the form of one major peak. Colonies
300 exposed to the same amount of hydrogen peroxide showed varying peak amplitudes and
301 durations (Fig. 2 B-D). In contrast, at 100 μ M H₂O₂ no peak of fluorescence was detected.
302 Instead, the average fluorescence signal in each colony rose to a plateau, over a variable
303 period of time (Fig. 2 E). Calculation of the average fluorescence profile over time for all
304 colonies within each experimental condition revealed that increasing concentrations of H₂O₂
305 resulted in both an increase of the intensity and the duration of fluorescence signal. The
306 standard deviations associated with certain conditions showed a large overlap, especially
307 between 50 μ M and 100 μ M (Fig. 2 F). The variability of the average fluorescence signal
308 among different microcolonies in the same stress condition was evaluated by calculation of
309 the coefficient of variation at each time point. The CV values observed at 0, 30, and 100 μ M
310 H₂O₂ remained around 0.3, while at 10 and 50 μ M H₂O₂ the CV values were higher, reaching
311 a maximum value of around 0.6 at 10 μ M and 0.9 at 50 μ M H₂O₂ before decreasing again
312 (Fig. 2 G).

313

314 To assess whether the observed dynamics of Dps induction were an artifact of the
315 experimental procedure, several control experiments were performed. To determine the
316 consequences of the light exposure on the mCherry protein during the time-lapse
317 fluorescence microscopy process, a photobleaching test was performed on a strain of *E. coli*
318 with constitutive *mCherry* expression (Supporting information). We observed an average of
319 about 20% decrease in the fluorescence signal due to the cumulative photobleaching effect
320 of our image acquisition process on a single cell (Fig. S5). To test the effect of imaging on the
321 cellular fluorescence, images of the *dps-mCherry* strain in the presence of 30, 50, or 100 μM
322 H_2O_2 were acquired every 30 minutes. These fluorescence curves showed a shape similar to
323 those obtained from image acquisition every 5 minutes (Fig. S6, Fig. 2) demonstrating that
324 that the imaging process does not significantly affect the measured cellular behavior.
325 Similarly shaped peaks of fluorescence were observed both in the agarose pad system and in
326 a microfluidics device (46) in which H_2O_2 was constantly applied to the cells over the
327 duration of the imaging (Fig. S7), indicating that the shape of the fluorescence curve is not
328 due to degradation of H_2O_2 over time. The stability of mCherry signal in the presence of the
329 oxidating effect of 50 and 100 μM H_2O_2 was also investigated, showing no statistically
330 significant difference in mCherry degradation or loss of fluorescence intensity due to
331 oxidation (Fig. S8).

332 **Correlations between oxidative stressor concentration and the intensity** 333 **and duration of Dps production**

334

335 For a quantitative analysis of Dps induction in the presence of oxidative stress, we analyzed
336 the intensity and the length of the fluorescence peak. For each microcolony, the curve
337 representing the average fluorescence intensity among its constituent cells was fitted with a

338 polynomial function in order to extract both the maximum value of the fluorescence and the
339 time point at which it was reached. These values were calculated from averaged microcolony
340 fluorescence values since individual cells within each microcolony showed low variability in
341 fluorescence at timepoints before the peak of fluorescence was reached (Fig. 1F). Calculation
342 of the average maximum fluorescence values of colonies exposed to the same amount of
343 H₂O₂ revealed that higher concentrations of stressor were correlated with higher peak
344 amplitude for H₂O₂ concentrations between 0 μM and 50 μM (Fig. 3 A). No increase in
345 average maximum fluorescence value was observed when the H₂O₂ concentration was
346 increased from 50 μM to 100 μM (Fig. 3 A). The variability in the maximum fluorescence
347 intensity among different colonies in the presence of the same concentration of stressor was
348 evaluated by calculation of the coefficient of variation. These values ranged between 0.23
349 and 0.47, with the maximum variability observed at 10 μM H₂O₂ (Fig. 3 B). No overall trend
350 was seen between the coefficient of variation and the maximum fluorescence values over
351 the various concentrations of H₂O₂ (Fig. 3 C). No significant differences were observed in the
352 distribution of maximum fluorescence values when microcolonies were grown on the same
353 agarose pad versus different agarose pads.

354 The average time at which the maximum fluorescence signal was observed for microcolonies
355 in each experimental condition increased steadily with the amount of H₂O₂ applied to the
356 culture (Fig. 4 A). The coefficient of variation for the time of maximum fluorescence
357 intensity was calculated between different microcolonies in the same stress condition and
358 ranged between 0.10 and 0.29, lower than the variability observed for the strength of the
359 induction (Fig. 4 B). No relationship was observed between the coefficient of variation values
360 and the time to the maximum fluorescence, over the concentrations of H₂O₂ (Fig. 4 C). Taken
361 together, our data indicate that an increase in hydrogen peroxide concentration led to an

362 increase of Dps production activity. In addition, the duration of the protein synthesis also
363 increased with the concentration of the stressor.

364 Correlation analyses were performed on the extracted values for the maximum fluorescence
365 intensity and the duration of the increase in fluorescence for individual microcolonies. When
366 comparing all the stress conditions simultaneously, the Pearson correlation coefficient (R)
367 between the time to reach the fluorescence peak and its intensity was 0.80 with a p value <
368 0.0001 (Fig. 5 A). Fluorescence peaks that were higher in amplitude were therefore strongly
369 correlated with a longer period of Dps production. While this strongly positive correlation
370 was observed through analyzing the pooled data, the data for each individual H₂O₂
371 concentration considered separately showed a weaker positive correlation, ranging from
372 0.23 to 0.82 with an average of 0.49 (Fig. 5 A).

373 **Effects of oxidative stress on cellular growth**

374

375 To analyze the effect of oxidative stress on cellular morphology, the parameter of cell length
376 was calculated as the length of the axis between the two poles of a cell (43). We compared
377 the average length of all cells within each microcolony over time for all the microcolonies
378 analyzed (Fig. S9). If 0 μM or 10 μM H₂O₂ was applied, we observed the trend that the cell
379 length slightly decreased over time, declining from an average of 5.5 μm down to 3.5 μm
380 (Fig. 6, Fig. S9 A-B). Application of higher H₂O₂ concentrations of 30 μM or 50 μM resulted in
381 little increase in the average cell length, but a higher proportion of elongated cells, reaching
382 a length of up to 12.5 μm (Fig. 6, Fig. S9 C-D). The highest concentration of H₂O₂ applied, 100
383 μM, caused a complete halt of cell growth and division; each cell remained at the same
384 length throughout the course of the experiment. (Fig. 6, Fig. S9 E). The standard deviation

385 for average cell length per microcolony overlapped greatly between conditions, such that
386 the amount of stressor applied is a poor predictor of cell length (Fig. 6). We observed that
387 the variability of cell length increased over time for colonies exposed to 0-50 μM H_2O_2 , rising
388 from near-zero at the start of imaging to 0.6, but remained close to zero for 100 μM H_2O_2
389 (Fig S9 F).

390 We also evaluated cellular growth rate over time upon exposure to oxidative stress. The
391 instantaneous growth rate, μ , was calculated by fitting the cell length over time to an
392 exponential function (43). Cell width was not seen to vary significantly during the
393 experiments. We calculated the average instantaneous growth rate of all the cells within a
394 microcolony at each point in time (Fig. 7). The cells exposed to either 0 μM or 10 μM
395 hydrogen peroxide showed a similar, slightly increasing growth rate over time, ranging
396 between 1.1 and 1.8 μh^{-1} (Fig. 7 A-B). Each further increase of the stressor concentration led
397 to a reduction of cell growth. We observed that at 30 μM H_2O_2 the colonies grew only
398 moderately during the initial part of the experiment, with an average starting growth rate of
399 approximately 0.6 μh^{-1} , but showed a complete recovery of growth over several hours (Fig.
400 7 C). At 50 μM concentration of hydrogen peroxide, the growth was severely affected. Initial
401 growth rates of 0.2- 0.3 μh^{-1} increased slowly over time but only partially recovered over the
402 course of imaging (Fig. 7 D). When the hydrogen peroxide was increased to 100 μM , cellular
403 growth was completely stalled during the entire duration of the imaging (Fig. 7 E). Overall,
404 increasing concentrations of H_2O_2 resulted in a greater initial decrease in cell growth and
405 increasingly impaired recovery of cell growth over time (Fig. 7 F).

406 Analysis of the average growth rate per microcolony over the duration of the experiment
407 revealed that concentrations of H_2O_2 up to 30 μM had moderate effects on the average

408 growth rate, producing a decrease from $1.9 \mu \text{ h}^{-1}$ at $0 \mu \text{M}$ to $1.4 \mu \text{ h}^{-1}$ at $30 \mu \text{M}$. Strong
409 reduction of growth was observed at exposure to $50 \mu \text{M H}_2\text{O}_2$ with an average of $0.6 \mu \text{ h}^{-1}$,
410 and at $100 \mu \text{M}$ cell growth was negligible, with an average growth rate of $0.01 \mu \text{ h}^{-1}$ (Fig. 8 A).
411 The coefficients of variation for the average growth rates were low for the $0\text{-}30 \mu \text{M H}_2\text{O}_2$
412 conditions, ranging from $0.09\text{-}0.24$, while the variation for $50 \mu \text{M H}_2\text{O}_2$ was extremely high at
413 0.75 (Fig. 8 B). For $100 \mu \text{M H}_2\text{O}_2$ the coefficient of variation could not be accurately
414 calculated because the mean value of the growth rate was close to zero for most
415 microcolonies. Higher H_2O_2 concentrations were correlated with higher coefficient of
416 variation values (Fig. 8 C). Thus, an increase in H_2O_2 concentration was strongly correlated
417 with both a decrease in growth rate and an increase in growth rate variability, primarily for
418 the higher concentrations of stressor.

419 To analyze the relationship between Dps induction parameters and cellular growth, we
420 determined Pearson correlation coefficients between the average growth rate within
421 microcolonies and the intensity and the duration of induction peaks. Between the average
422 growth rate and amplitude of Dps induction for all stress conditions compared
423 simultaneously, we observed a strong negative correlation ($R = -0.71$) with a p value < 0.0001
424 (Fig. 5 B). Interestingly, the correlation coefficients calculated within each stress condition
425 were dramatically weaker, with an average of 0.03 , and not significantly correlated.
426 Similarly, the correlation coefficient comparing the growth rate and the time to reach the
427 maximum fluorescence was strongly negative when calculated over all conditions ($R = -0.85$)
428 with a p value < 0.0001 , but much weaker within each individual condition (average $R = -$
429 0.18) (Fig. 5 C). Lower average growth rate was therefore seen to be strongly associated with
430 both higher Dps production and a longer induction time over a range of H_2O_2 concentrations.

431 We further analyzed the relationship between the mean fluorescence signal per colony and
432 the mean growth rate per colony over time, identifying three response categories. The first
433 category consisted of colonies that showed a constant high growth rate with a small-
434 amplitude pulse or decrease over time of the fluorescence signal (Fig. 9A). In the second
435 category, the colonies exhibited a steady increase of growth rate over time, starting around
436 $0.5 \mu \text{ h}^{-1}$ and reaching values around $1.8\text{-}2 \mu \text{ h}^{-1}$. The fluorescence signal initially increased,
437 reached its peak value, and then decreased again (Fig. 9B). The third category contained
438 colonies in which the growth rate remained constantly low while the fluorescence signal
439 increased robustly over time (Fig. 9C).

440 Increasing concentrations of H_2O_2 resulted in microcolony growth that was increasingly likely
441 to exhibit a higher-numbered category of response, associated with increasingly impaired
442 growth rate. All (100%) of the colonies grown without H_2O_2 showed Group I-type response
443 (Fig. 9 D). Of the colonies grown in $10 \mu\text{M H}_2\text{O}_2$, an intermediate behavior was seen in which
444 37% showed Group I response and 63% showed Group II response (Fig. 9 D). All (100%) of
445 the colonies exposed to $30 \mu\text{M H}_2\text{O}_2$ exhibited Group II response (Fig. 9 D). Of the colonies
446 grown in $50 \mu\text{M H}_2\text{O}_2$, an intermediate behavior was again seen in which 79% showed Group
447 II behavior and 21% showed Group III response (Fig. 9 D). Finally, all (100%) of the colonies at
448 $100 \mu\text{M H}_2\text{O}_2$ showed Group III response (Fig. 9 D). The presence of these three distinct
449 patterns may suggest a threshold model in which increasing stressor levels cause a
450 recoverable reduction in growth rate at a lower threshold concentrations or a long-term halt
451 in growth rate at a higher threshold concentration, due to still-uncharacterized internal
452 regulatory processes.

453 Discussion

454

455 In this study we have investigated for the first time the Dps stress response at the single-cell
456 level. When exposed to H₂O₂, *E. coli* cells exhibit a single pulse of activation of Dps
457 production. Higher concentrations of H₂O₂ induce an increase in both the intensity and
458 duration of the activation pulse. The correlation between cellular growth and stressor
459 intensity is quite non-linear. Low H₂O₂ concentrations initiate a robust Dps response but
460 have little effect on cellular growth, while higher concentrations of H₂O₂ slow down the
461 growth rate dramatically and cause high variability. Cells exposed to the same H₂O₂
462 concentration do not receive a growth advantage in case of higher Dps induction. The
463 recovery from stress may thus rely more upon the degree of damage generated in individual
464 cells than to the strong induction of specific stress response proteins.

465 Stressor intensity predicts pulse amplitude and duration but not growth 466 rate variability

467

468 The single pulse of induction of Dps production likely arises from more general features of
469 the oxidative stress-induced response in *E. coli*. In the presence of H₂O₂, *dps* activation is
470 regulated by the OxyR protein (25), a key regulator of the adaptive response to oxidative
471 stress (47, 48). During exponential growth, H₂O₂ converts OxyR protein to an oxidized active
472 form that recruits δ^{70} -RNA polymerase to initiate *dps* transcription (25). In *E. coli* cells
473 treated with 200 μ M H₂O₂, OxyR was fully converted to its oxidized form within 30 seconds
474 of exposure to the stressor. Thereafter, OxyR reverted back to its reduced form with a half-
475 life of \sim 5 minutes, and no oxidized OxyR was detected after 10 minutes (49). This transient
476 activation response provides a potential window for *dps* transcription lasting only on the

477 order of minutes. Specific analysis of *dps* transcription kinetics in cells exposed to 10 μ M
478 H₂O₂ revealed *dps* induction to be active for a limited period of time as well. Maximum levels
479 of *dps* transcript were detected at 1 minute after exposure, followed by a steady decrease
480 until returning to background levels by 20 minutes post exposure (50). Following the
481 decrease of OxyR activity, transcriptional repression of *dps* occurs via the formation of an
482 unproductive complex between the nucleoid-associated protein Fis and δ^{70} on the *dps*
483 promoter (26), that may provide stringent downregulation of *dps* transcription at the end of
484 its pulse of activation. The initial increase in observed Dps reporter signal intensity is thus
485 likely due to the transient burst of *dps* transcriptional activity. Thereafter, the decrease in
486 signal intensity likely derives from a combination of transcriptional repression of the *dps*
487 promoter and an increase in cellular growth rate that dilutes the reporter protein (Fig. 7),
488 with a minor contribution from photobleaching of the reporter protein (Fig. S5). The absence
489 of a decrease in signal intensity at the highest concentration of H₂O₂ (Fig. 2E,F) can be
490 explained by the near-zero cellular growth rate in this condition (Fig. 7E,F) that results in a
491 lack of dilution of the reporter protein.

492 We observe a correlation between the amount of stress applied to the cells and the peak
493 intensity of the Dps response, which saturates at the highest concentrations of stressor (Fig.
494 3). A correlation between the magnitude of the stress and the duration of the Dps response
495 is also indicated by our observations (Fig. 4), so that stronger stresses are associated with
496 both longer and stronger Dps production. The lack of increase in the peak intensity of the
497 Dps response between our highest two concentrations of H₂O₂ (Fig. 3A) seems to indicate a
498 saturation of the Dps production mechanism, perhaps due to the limited number of OxyR
499 regulatory molecules present in the cells. The speed of the initial increase of protein
500 production was seen to be similar under all conditions, such that stronger Dps responses are

501 achieved by modulation of the duration of production. However, not all bacterial stress
502 response genes show a similar pattern of expression. In *Bacillus subtilis*, the addition of
503 increasing concentrations of stressors results in production of the general stress response
504 factor σ^B with either an increase in peak amplitude but no alteration in the duration of the
505 response (35) or an increase in the frequency of pulses of induction, accompanied by only
506 weak changes in pulse amplitude and duration (33). In contrast, the highly modulated
507 duration of the Dps response under varying intensities of oxidative stress may be a strategy
508 to allow for an extended period of repair under conditions of more extensive damage.

509 Over a range of H₂O₂ concentrations, lower average growth rate is strongly correlated with
510 both stronger Dps production and a longer induction time (Fig. 5). Interestingly, cells
511 exposed to the same concentration of H₂O₂ do not receive a growth advantage from
512 increased levels of Dps production but instead exhibit similar or slower growth, even in the
513 50 μ M H₂O₂ condition where Dps production levels varied by up to 4-fold. This observation
514 indicates that the kinetics of recovery from stress are not dictated by the magnitude of
515 induction of specific stress response enzymes. Rather, we propose that individual cells may
516 vary significantly in their amount of oxidative damage, such that cells sustaining more
517 damage both have slower growth and induce a larger stress response. The development of
518 real-time *in vivo* markers of oxidative damage will be quite interesting for study of the
519 relationship between damage and stress response induction.

520 Analysis of the stress conditions separately reveals that a low dose of H₂O₂ does not result in
521 a major reduction in cell growth rate, although the Dps enzyme is already produced (Fig. 1,
522 7). When the H₂O₂ concentration reaches a critical level, the bacteria exhibit extremely high
523 variability in growth rate. This variability does not correlate with either the intensity or the

524 variability of Dps production (Fig. 3, 8). Noise in metabolic gene expression has been shown
525 to affect the growth stability of a cell under conditions of active metabolism (32). While
526 metabolic reactions are crucial to synthesize enzymes and molecules necessary for cell
527 development, stress response processes are responsible for maintaining the stability of
528 cellular equilibrium under disruptive conditions. The observed variation in cell growth during
529 exposure to high levels of oxidative stress might be linked to increased stochastic noise of
530 one or more essential metabolic pathways under these conditions.

531

532 **Cell-to-cell variability in Dps production is greater between microcolonies**

533

534 The Dps response to oxidative stress shows some features of excitable dynamics, a class of
535 transient cellular differentiation in which cells probabilistically enter into an ON state and
536 return to the initial OFF state after a certain stereotypical period of time (51). Within the
537 resolution of our experiments, we detect a single burst of protein production that rapidly
538 activates a temporary stress-response state (Fig. 1, 2). Unlike a true excitable noise-triggered
539 system, the return to an OFF state is not stereotypical in the case of Dps production. Instead,
540 the return to the initial state occurs after a variable period of time that partially depends on
541 growth kinetics. Additionally, we do not observe probabilistic entry into the ON state.
542 Instead, every cell that was exposed to hydrogen peroxide was seen to initiate Dps
543 production, and the kinetics and amplitude of the stress response were synchronized over
544 each microcolony throughout the duration of imaging.

545 Cells lacking the *dps* gene are more sensitive to oxidative stress, showing dramatically
546 reduced viability and elevated DNA damage (16, 18, 23). Because the Dps protein is a key

547 protector in stress survival, especially during the initial stage of the exposure, the non-
548 probabilistic initiation of Dps production allows all affected cells to respond to the oxidative
549 damage. The similar kinetics of the Dps response among individual cells within microcolonies
550 is likely a consequence of the majority of the active Dps production taking place in the
551 single-cell stage, before the founding cell has undergone cell division. Once an oxidatively
552 damaged cell resumes growth, the profile of the response is primarily reflective of dilution
553 only, which seems to exhibit low variation (Fig. 1).

554 The profile of the Dps response showed greater variability between different microcolonies
555 exposed to the same amount of stress than among different cells within microcolonies (Fig.
556 1, 2). While some of this variability may originate from non-homogeneous distribution of
557 hydrogen peroxide in the environment, a relatively moderate amount of site-to-site
558 variability was observed on the agarose pads. The variability between microcolonies was
559 seen to be dramatically higher for stressor concentrations in which the microcolonies were
560 seen to fall into either of two different patterns of growth and expression behavior rather
561 than only one (Fig. 2, 9). Most of the variability observed in the Dps responses is likely due to
562 differences between the progenitor cells of each individual colony. Non-genetic cell-to-cell
563 heterogeneity within a clonal population is common to many biological processes (34) and
564 can arise from a broad range of phenomena including noise in gene expression or
565 intracellular protein concentration, stochastic biochemical interactions, or non-synchronicity
566 in cell cycle stage (30, 52-54). A genome-wide survey of phenotypic noise over
567 approximately 75% of *E. coli* promoters found that stress-response genes such as *dps* exhibit
568 particularly variable expression during non-stressful growth (54). On top of this baseline
569 variability, we find that the variability in Dps production activity can increase more than
570 three-fold between non-stress and high-stress conditions (Fig. 2). Whether this dramatic

571 increase in variability under stress or upregulation is a common feature of all bacterial genes
572 or is limited to certain functional classes will require further investigation.

573

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724 **Figure legends**

725

726 **Fig. 1.** Exposure to H₂O₂ induces a single pulse of Dps production activity synchronized over
727 the individual cells within each microcolony. A-E) Examples of fluorescence intensity over
728 time in individual cells in a microcolony exposed to different concentrations of H₂O₂. Each
729 line represents a single cell. F) The average coefficient of variation (CV) over time of the
730 fluorescence intensity among all the cells exposed to the same stress condition, for varying
731 concentrations of H₂O₂.

732 **Fig. 2.** The Dps response per microcolony exhibits variation in peak amplitude and duration.
733 A-E) The average fluorescence signal over time of microcolonies exposed to different
734 concentrations of H₂O₂. Each line represents the average fluorescence intensity of all cells
735 within one microcolony. F) The average fluorescence signal over time of all the colonies
736 exposed to the same stress condition. The shaded area represents the standard deviation. G)
737 The coefficient of variation (CV) over time of the average fluorescence signals of all the
738 microcolonies exposed to the same stress condition, for varying concentrations of H₂O₂.

739 **Fig. 3.** Dps induction intensity increases with exposure to higher concentrations of H₂O₂. A)
740 The average maximum values of the fluorescence signal for each microcolony, for each
741 concentration of H₂O₂. The error bars represent the standard deviation. The letters
742 represent the statistical significance: samples labeled with different letters are statistically
743 different (ANOVA test, p <0.05). B) The maximum values of the fluorescence signal for each
744 microcolony, and the coefficient of variation (CV) of the maximum fluorescence intensity
745 among microcolonies, for each H₂O₂ concentration. C) Scatter plot of the coefficient of

746 variation vs. the average maximum fluorescence value for each concentration of H₂O₂. R
747 represents the Pearson correlation coefficient.

748

749 **Fig. 4.** The duration of Dps induction increases with exposure to higher concentrations of
750 H₂O₂. A) The average time at which the maximum value of the fluorescence signal was
751 observed for each microcolony, for each concentration of H₂O₂. The error bars represent the
752 standard deviation. The letters represent the statistical significance: samples labeled with
753 different letters are statistically different (ANOVA test, $p < 0.05$). B) The time to the maximum
754 value of the fluorescence signal for each microcolony, and the coefficient of variation (CV) of
755 the time to the maximum fluorescence intensity among microcolonies, for each H₂O₂
756 concentration. C) Scatter plot of the coefficient of variation vs. the time to the average
757 maximum fluorescence value for each concentration of H₂O₂. R represents the Pearson
758 correlation coefficient.

759

760 **Fig. 5.** Correlations between growth rate, intensity, and duration of Dps induction. A) Scatter
761 plot of the average maximum fluorescence intensity vs. the average time to the maximum
762 fluorescence for individual microcolonies. B) Scatter plot of the average growth rate per
763 colony vs. the average maximum fluorescence intensity for individual microcolonies. C)
764 Scatter plot of the average growth rate per colony vs. the average time to the maximum
765 fluorescence intensity for individual microcolonies. Each dot represents a single microcolony.
766 Microcolonies exposed to the same H₂O₂ concentration are represented in the same color.
767 The R value in the top right corner of each graph represents the Pearson correlation
768 coefficient over all the data. The R below the label for each concentration of H₂O₂ represents
769 the R value calculated over all microcolonies in each stress condition. * = $p < 0.05$.

770 **Fig. 6.** Effects of oxidative stress on cellular length. A) Distribution of the length of all cells in
771 a microcolony, averaged over all timepoints within each experiment, for different
772 concentrations of H₂O₂. The top and bottom of the vertical bars represent the maximum and
773 the minimum length values, respectively; the top and bottom of the rectangular box
774 represent the 75th and the 25th percentile; the horizontal line within the box is the median;
775 and the square in the box is the mean value. The letters represent the statistical significance:
776 samples with different letters are statistically different (ANOVA test, p <0.05). B) The average
777 length of all cells within the microcolonies over time, for varying concentrations of H₂O₂. The
778 shaded areas represent the standard deviation.

779 **Fig. 7.** Oxidative stress suppresses initial rates of cellular growth. A-E) The average
780 instantaneous growth rate of all the cells within a colony over time, for different
781 concentrations of H₂O₂. Each line represents the average growth rate of all cells within one
782 microcolony. F) The average growth rate over time of all the colonies in the presence of the
783 same concentration of H₂O₂. The shaded areas represent the standard deviation.

784 **Fig. 8.** Variation in microcolony growth rate increases at lower growth rates. A) The average
785 growth rate per microcolony, averaged over all cells over all timepoints for each
786 microcolony, for each concentration of H₂O₂. The error bars represent the standard
787 deviation. The letters represent the statistical significance: samples labeled with different
788 letters are statistically different (ANOVA test, p <0.05). B) The average growth rate for each
789 microcolony, and the coefficient of variation (CV) of growth rate among microcolonies, for
790 each H₂O₂ concentration. Two of the microcolonies exposed to 50 μM H₂O₂ and ten of the
791 microcolonies exposed to 100 μM H₂O₂ are not visible in the plot because their average

792 growth rate is 0. C) Scatter plot of the coefficient of variation vs. the average growth rate for
793 each concentration of H₂O₂. R represents the correlation coefficient. *= p <0.05.

794

795 **Fig. 9.** Microcolonies exhibited three categories of Dps response. A-C) Scatter plots of the
796 mean fluorescence signal and the mean growth rate per microcolony, over time, for example
797 microcolonies in each category. Each dot represents the average fluorescence and the
798 average growth rate of a single microcolony at a specific timepoint, colored to indicate the
799 timepoint. A) Category I: a constant high growth rate associated with a slightly decreasing,
800 low fluorescence signal. B) Category II: a steady increase in growth rate over time associated
801 with a pulse of fluorescence signal. C) Category III: a robust increase in fluorescence signal
802 over time associated with a constant low growth rate. D) The percentage of microcolonies in
803 each response category, for each concentration of H₂O₂. The letters represent the statistical
804 significance: samples in each group labeled with different letters are statistically different
805 (two-sample *t*-test; p <0.05).

Fig. 1

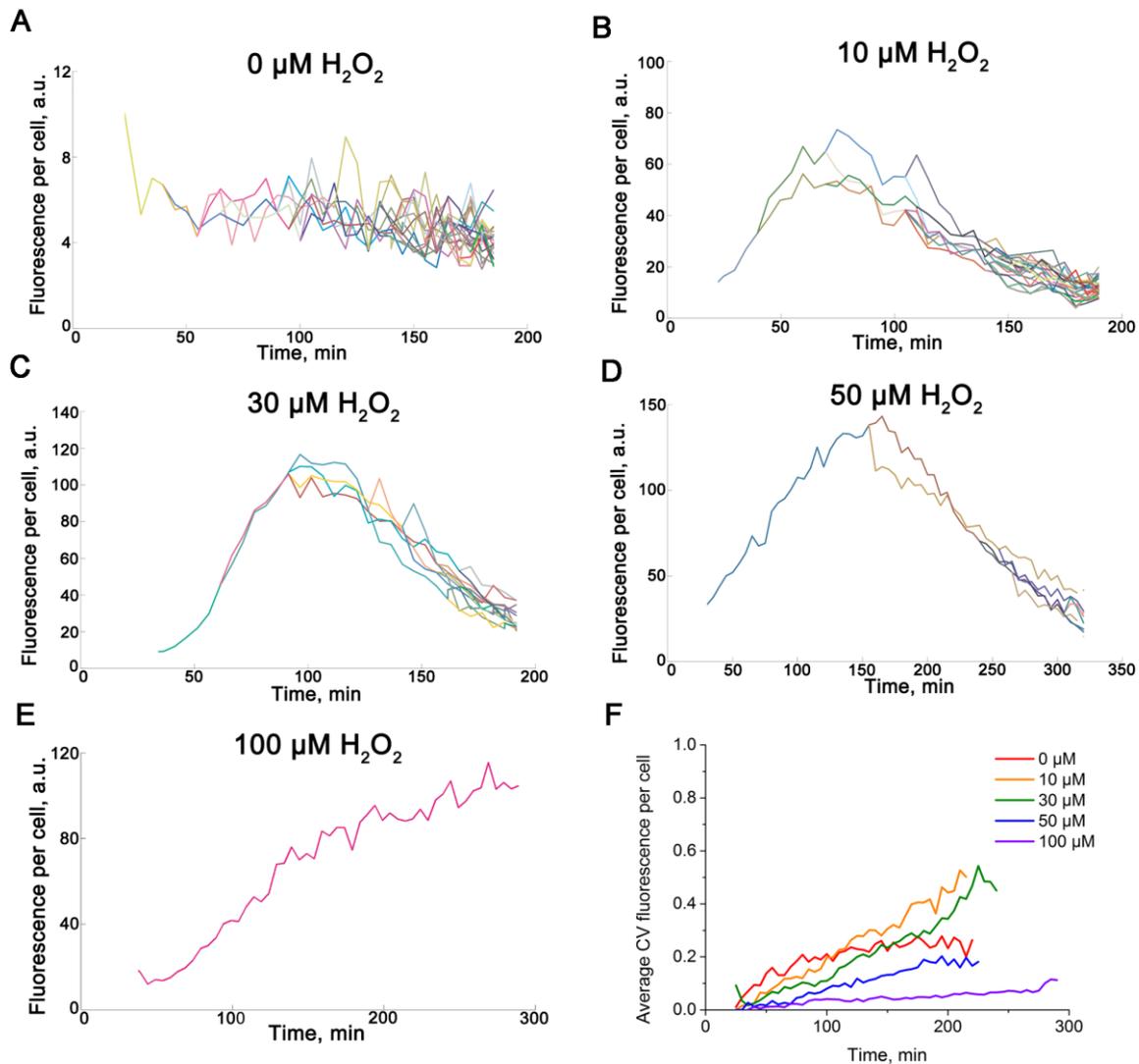


Fig. 1. Exposure to H_2O_2 induces a single pulse of Dps production activity synchronized over the individual cells within each microcolony. A-E) Examples of fluorescence intensity over time in individual cells in a microcolony exposed to different concentrations of H_2O_2 . Each line represents a single cell. F) The average coefficient of variation (CV) over time of the fluorescence intensity among all the cells exposed to the same stress condition, for varying concentrations of H_2O_2 .

Fig. 2

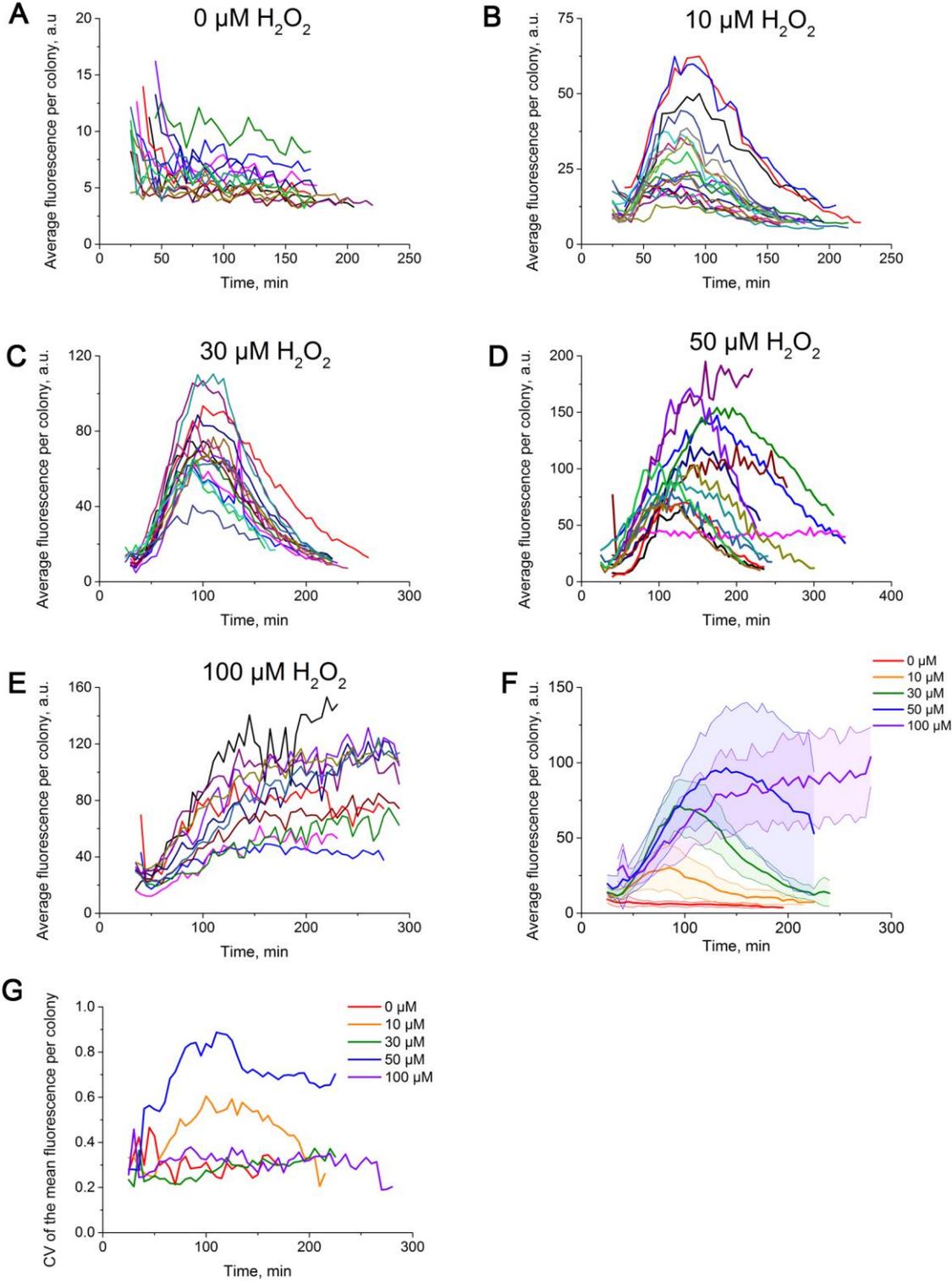


Fig. 2. The Dps response per microcolony exhibits variation in peak amplitude and duration. A-E) The average fluorescence signal over time of microcolonies exposed to different

concentrations of H_2O_2 . Each line represents the average fluorescence intensity of all cells within one microcolony. F) The average fluorescence signal over time of all the colonies exposed to the same stress condition. The shaded area represents the standard deviation. G) The coefficient of variation (CV) over time of the average fluorescence signals of all the microcolonies exposed to the same stress condition, for varying concentrations of H_2O_2 .

Fig. 3

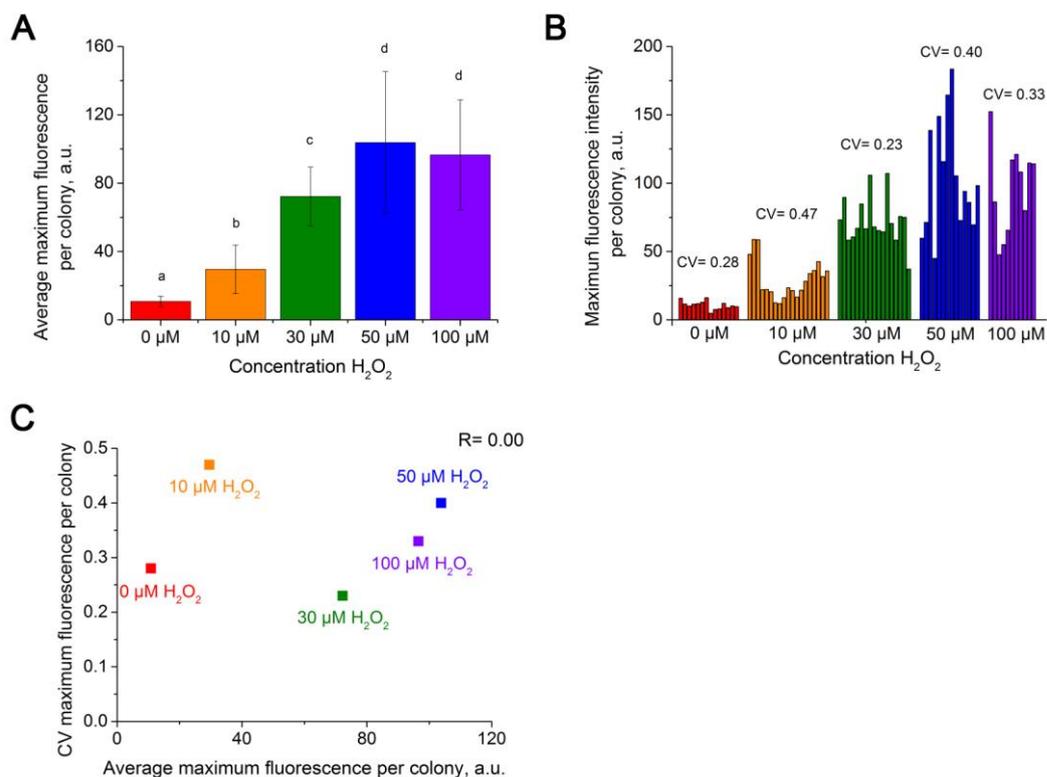


Fig. 3. Dps induction intensity increases with exposure to higher concentrations of H_2O_2 . A) The average maximum values of the fluorescence signal for each microcolony, for each concentration of H_2O_2 . The error bars represent the standard deviation. The letters represent the statistical significance: samples labeled with different letters are statistically different (ANOVA test, $p < 0.05$). B) The maximum values of the fluorescence signal for each microcolony, and the coefficient of variation (CV) of the maximum fluorescence intensity among microcolonies, for each H_2O_2 concentration. C) Scatter plot of the coefficient of variation vs. the average maximum fluorescence value for each concentration of H_2O_2 . R represents the Pearson correlation coefficient.

Fig. 4

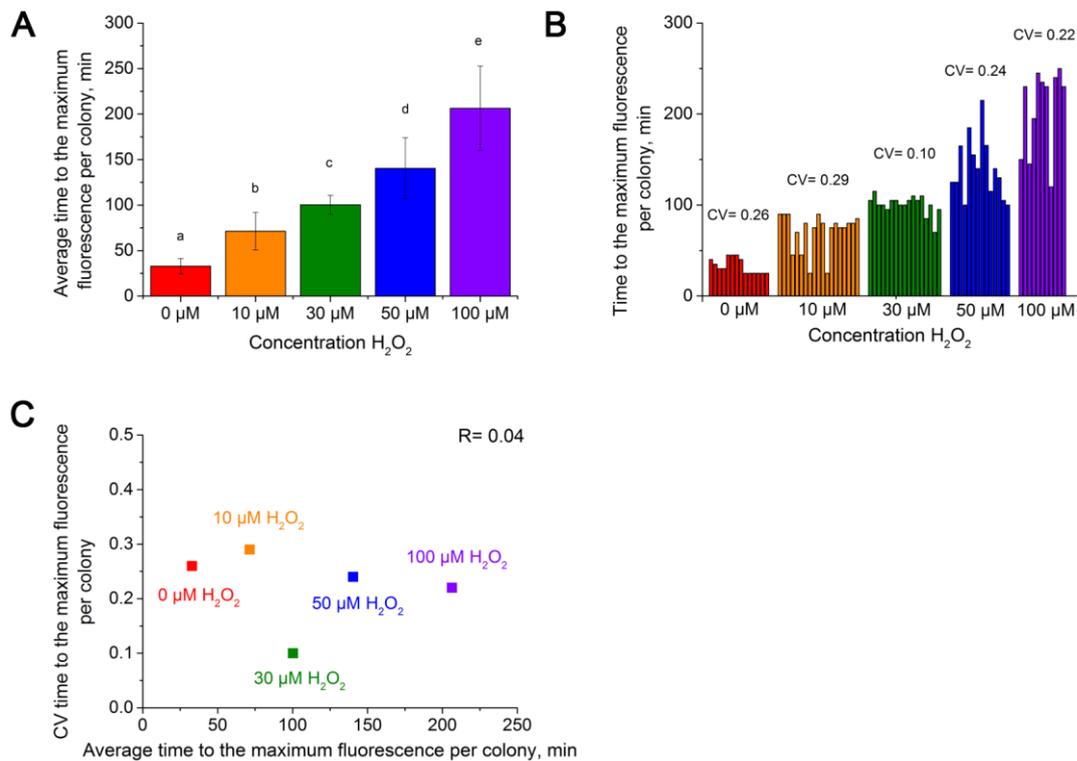


Fig. 4. The duration of Dps induction increases with exposure to higher concentrations of H₂O₂. A) The average time at which the maximum value of the fluorescence signal was observed for each microcolony, for each concentration of H₂O₂. The error bars represent the standard deviation. The letters represent the statistical significance: samples labeled with different letters are statistically different (ANOVA test, $p < 0.05$). B) The time to the maximum value of the fluorescence signal for each microcolony, and the coefficient of variation (CV) of the time to the maximum fluorescence intensity among microcolonies, for each H₂O₂ concentration. C) Scatter plot of the coefficient of variation vs. the time to the average maximum fluorescence value for each concentration of H₂O₂. R represents the Pearson correlation coefficient.

Fig. 5

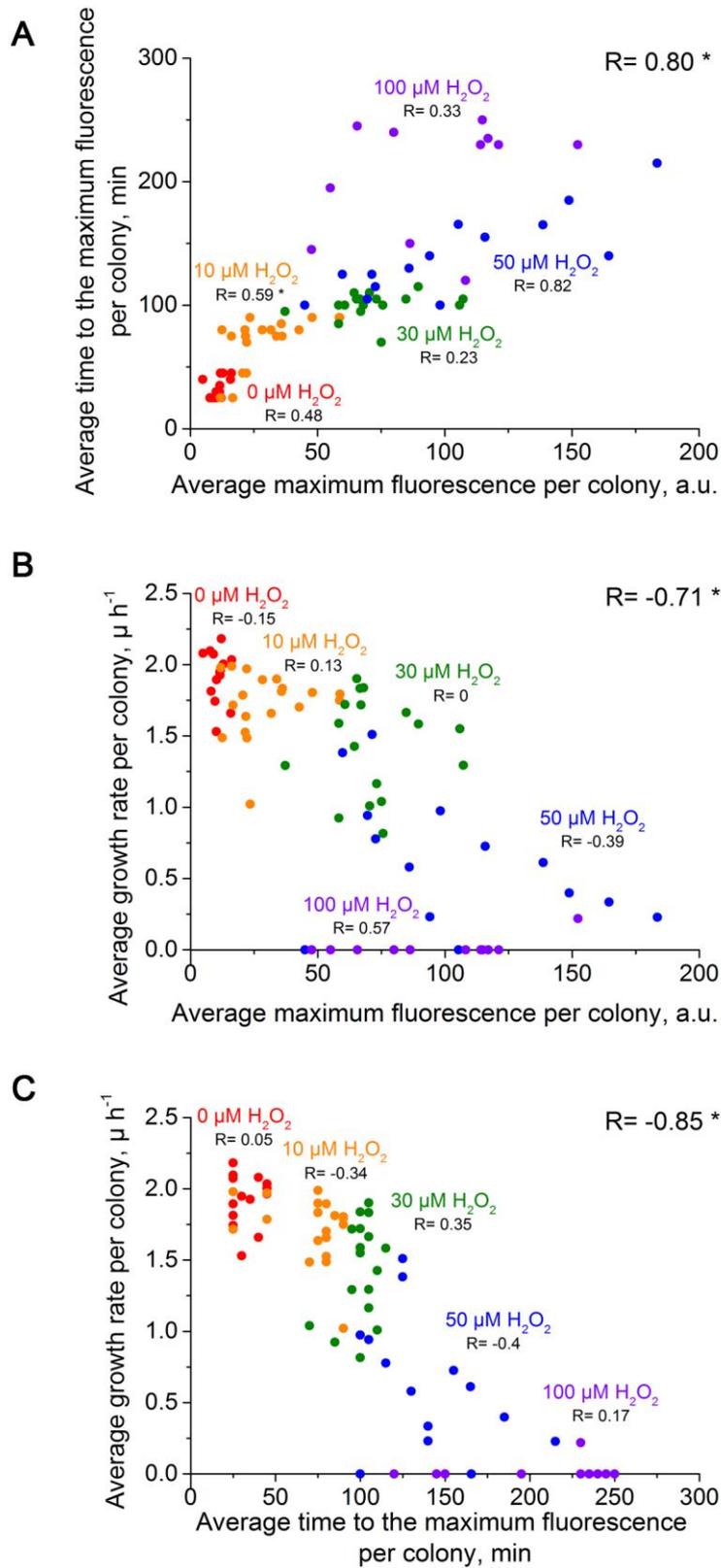


Fig. 5. Correlations between growth rate, intensity, and duration of Dps induction. A) Scatter plot of the average maximum fluorescence intensity vs. the average time to the maximum

fluorescence for individual microcolonies. B) Scatter plot of the average growth rate per colony vs. the average maximum fluorescence intensity for individual microcolonies. C) Scatter plot of the average growth rate per colony vs. the average time to the maximum fluorescence intensity for individual microcolonies. Each dot represents a single microcolony. Microcolonies exposed to the same H_2O_2 concentration are represented in the same color. The R value in the top right corner of each graph represents the Pearson correlation coefficient over all the data. The R below the label for each concentration of H_2O_2 represents the R value calculated over all microcolonies in each stress condition. * = $p < 0.05$.

Fig. 6

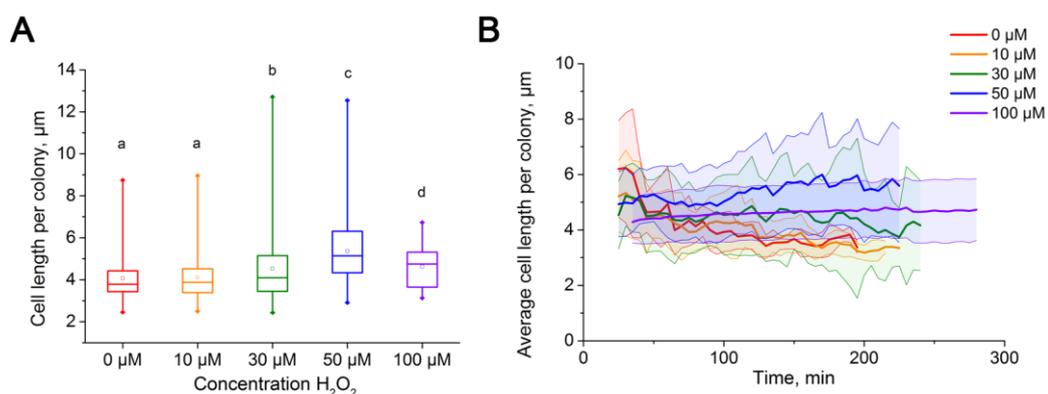


Fig. 6. Effects of oxidative stress on cellular length. A) Distribution of the length of all cells in a microcolony, averaged over all timepoints within each experiment, for different concentrations of H_2O_2 . The top and bottom of the vertical bars represent the maximum and the minimum length values, respectively; the top and bottom of the rectangular box represent the 75th and the 25th percentile; the horizontal line within the box is the median; and the square in the box is the mean value. The letters represent the statistical significance: samples with different letters are statistically different (ANOVA test, $p < 0.05$). B) The average length of all cells within the microcolonies over time, for varying concentrations of H_2O_2 . The shaded areas represent the standard deviation.

Fig. 7

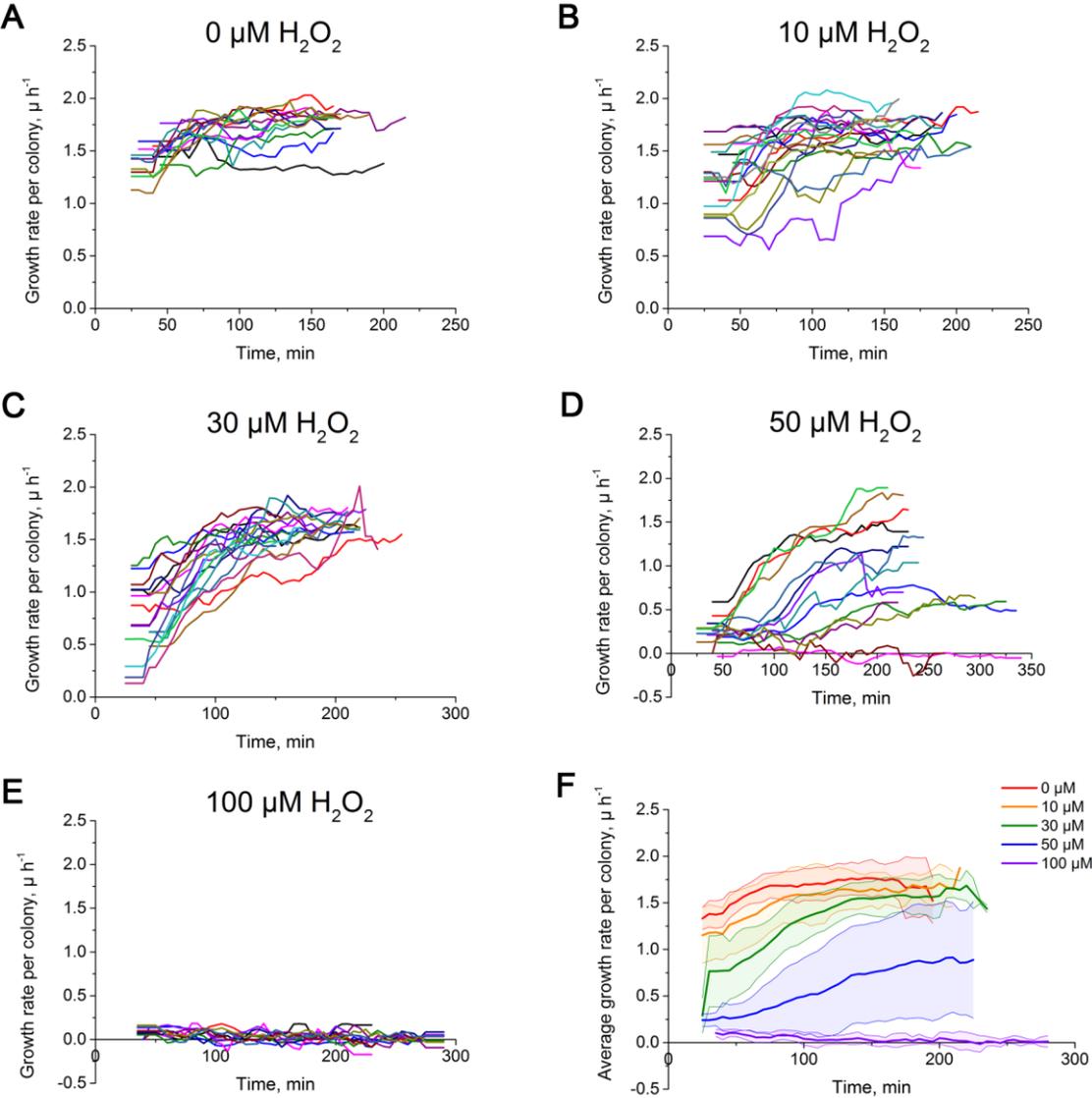


Fig. 7. Oxidative stress suppresses initial rates of cellular growth. A-E) The average instantaneous growth rate of all the cells within a colony over time, for different concentrations of H_2O_2 . Each line represents the average growth rate of all cells within one microcolony. F) The average growth rate over time of all the colonies in the presence of the same concentration of H_2O_2 . The shaded areas represent the standard deviation.

Fig. 8

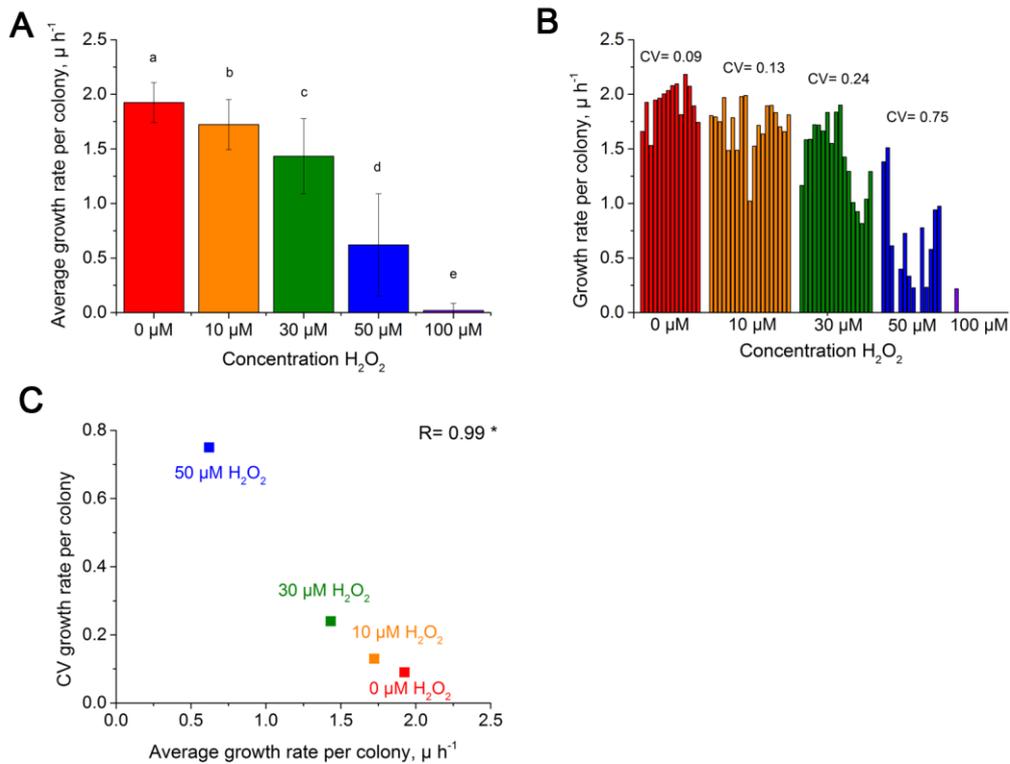


Fig. 8. Variation in microcolony growth rate increases at lower growth rates. A) The average growth rate per microcolony, averaged over all cells over all timepoints for each microcolony, for each concentration of H_2O_2 . The error bars represent the standard deviation. The letters represent the statistical significance: samples labeled with different letters are statistically different (ANOVA test, $p < 0.05$). B) The average growth rate for each microcolony, and the coefficient of variation (CV) of growth rate among microcolonies, for each H_2O_2 concentration. Two of the microcolonies exposed to $50 \mu M H_2O_2$ and ten of the microcolonies exposed to $100 \mu M H_2O_2$ are not visible in the plot because their average growth rate is 0. C) Scatter plot of the coefficient of variation vs. the average growth rate for each concentration of H_2O_2 . R represents the correlation coefficient. * = $p < 0.05$.

Fig. 9

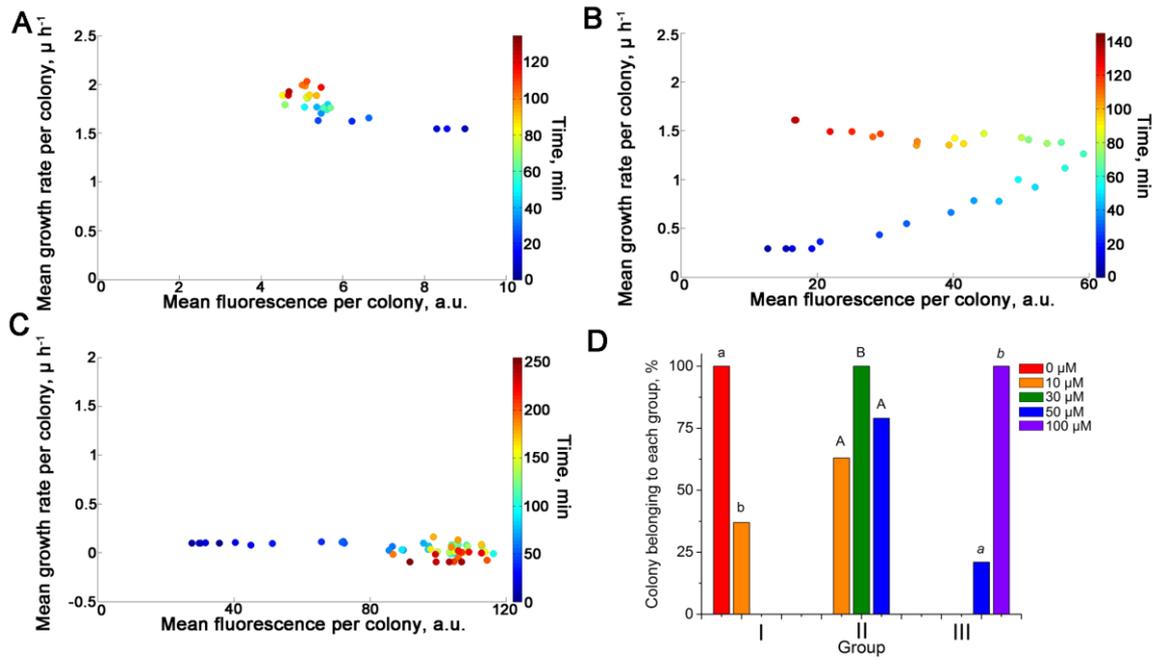


Fig. 9. Microcolonies exhibited three categories of Dps response. A-C) Scatter plots of the mean fluorescence signal and the mean growth rate per microcolony, over time, for example microcolonies in each category. Each dot represents the average fluorescence and the average growth rate of a single microcolony at a specific timepoint, colored to indicate the timepoint. A) Category I: a constant high growth rate associated with a low fluorescence signal. B) Category II: a steady increase in growth rate over time associated with a pulse of fluorescence signal. C) Category III: a robust increase in fluorescence signal over time associated with a constant low growth rate. D) The percentage of microcolonies in each response category, for each concentration of H₂O₂. The letters represent the statistical significance: samples in each group labeled with different letters are statistically different (two-sample *t*-test, $p < 0.05$).