

# 1 *Escherichia coli* can survive stress by noisy growth modulation

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7 **Gene expression can be noisy<sup>1-3</sup>, as can the growth of single cells<sup>4,5</sup>. Such cell-to-cell variation has**  
8 **been implicated in survival strategies for bacterial populations<sup>6-8</sup>. However, it remains unclear**  
9 **how single cells couple gene expression with growth to implement these survival strategies. Here**  
10 **we show how noisy expression of a key stress response regulator, *rpoS*<sup>9</sup>, allows *E. coli* to modulate**  
11 **its growth dynamics to survive future adverse environments. First, we demonstrate that *rpoS* has**  
12 **a long-tailed distribution of expression in an unstressed population of cells. We next reveal how a**  
13 **dynamic positive feedback loop between *rpoS* and growth rate produces multi-generation *rpoS***  
14 **pulses, which are responsible for the *rpoS* heterogeneity. We do so experimentally with single-cell,**  
15 **time-lapse microscopy<sup>10</sup> and microfluidics<sup>11</sup> and theoretically with a stochastic model<sup>12,13</sup>. Finally,**  
16 **we demonstrate the function of the coupling of heterogeneous *rpoS* activity and growth. It**  
17 **enables *E. coli* to survive oxidative attack by causing prolonged periods of slow growth. This**  
18 **dynamic phenotype is captured by the *rpoS*-growth feedback model. Our synthesis of noisy gene**  
19 **expression, growth, and survival paves the way for further exploration of functional phenotypic**  
20 **variability.**

21 *E. coli* respond to stress by expressing a host of protective genes. Global stress response is  
22 controlled, in large part, by *rpoS*, which is an alternative sigma factor<sup>9</sup>. Sigma factors are a  
23 component of the RNA polymerase holoenzyme that recognise and bind to the promoter region of  
24 genes<sup>14</sup>. The housekeeping sigma factor,  $\sigma^{70}$ , promotes the transcription of genes responsible for  
25 growth, for instance ribosomal genes<sup>15</sup>. On the other hand, *rpoS* up-regulates stress response genes<sup>9</sup>  
26 (Fig. 1a). It is strongly up-regulated in the transition from exponential to stationary phase when cells  
27 are starved for resources<sup>16</sup>. Populations in exponential phase have been shown to express small  
28 amounts of functional *rpoS*<sup>17,18</sup>. However, these studies were of bulk cultures, which can mask single  
29 cell phenotypes.

30 We therefore first asked the question: How is this small *rpoS* expression in exponential phase  
31 distributed amongst single cells? It could be that all cells have basal levels of *rpoS* or some cells could  
32 express the majority of the *rpoS*. To answer this question we grew cells in bulk culture into  
33 exponential phase and examined aliquots of the culture with single cell resolution under a  
34 microscope<sup>10</sup> (see Fig. 1b, and Methods). As a proxy for *rpoS* we used a transcriptional reporter with  
35 a promoter from an *rpoS*-responsive gene fused to GFP:  $P_{bolA}$ -GFP<sup>15,19</sup>. By computing histograms of  
36 mean *rpoS* level per cell we discovered that *rpoS* is heterogeneously distributed amongst single cells  
37 (Fig. 1c). To test our conclusion we carried out the same liquid culture assay on an *rpoS*-knockout  
38 ( $\Delta rpoS$ , Fig. 1c). The characteristic long tail of the heterogeneous WT distribution vanished in the  
39 knockout strain, with gene expression levels near background. We found similar behaviour when  
40 alternative reporters for *rpoS* were tested (Sup. Fig. 1)<sup>15</sup>. To test whether the long-tail was specific to  
41 *rpoS*, we examined  $\sigma^{70}$  reporters. The distributions of  $\sigma^{70}$  levels in WT populations had less

42 pronounced long-tails due to the higher abundance of  $\sigma^{70}$  in cells and did not change significantly in  
43  $\Delta rpoS$  (Sup. Fig. 2)<sup>15</sup>.

44 We next investigated the mechanism by which the *rpoS* distribution is produced. Reasoning that  
45 the distribution is due to a dynamic equilibrium, not a fixed subpopulation, we tracked single cells  
46 over multiple generations using time-lapse microscopy<sup>10</sup> and the Mother Machine microfluidic  
47 device<sup>11</sup> (Fig. 2a, Methods). Indeed, we found rich dynamic *rpoS* activity (see Methods). Some cell  
48 lineages have high *rpoS* activity pulses lasting multiple generations while others have very small  
49 pulses (Fig. 2b, Methods). We found a long-tailed distribution of pulse heights supporting the idea  
50 that the long-tailed liquid culture distribution is generated by cells pulsing *rpoS* on to different levels  
51 (Fig. 2c). We chromosomally integrated the *P<sub>bolA</sub>-GFP* reporter and found a similar consistency  
52 between bulk culture and microfluidic experiments suggesting the dynamics did not arise due to  
53 plasmid segregation noise (Sup. Fig. 3a-c). However, the fluorescence signal was very dim, thus we  
54 proceeded with the plasmid-based reporter.

55 We further observed rich dynamics in the growth rate of single cells (Fig. 2b, Methods). The  
56 sample lineages illustrate that cell growth slows down when *rpoS* activity is high. This relationship  
57 was quantified as a large negative value near zero time-shift in the cross-correlation of growth rate  
58 and *rpoS* activity (Fig. 2d, Sup. Fig. 3e, Methods). The strong anti-correlation suggested that growth  
59 rate should also be widely distributed, which is what we observed (Fig. 2e, Sup. Fig. 3d, 4b).  
60 However, the  $\Delta rpoS$  strain also had a wide growth rate distribution suggesting growth rate is  
61 intrinsically heterogeneous<sup>4</sup> (Fig. 2e). Furthermore,  $\sigma^{70}$  activity was positively correlated with growth  
62 rate suggesting it is related to this intrinsic variability (Sup. Fig. 4a).

63 We propose a coupled molecular and physiological model to explain our observations. First, we  
64 propose the intrinsic variability in growth rate arises due to stochastic molecular reactions that  
65 promote growth. Second, *rpoS* molecules repress growth and growth dilutes *rpoS*. This results in the  
66 anti-correlation between growth rate and *rpoS*.

67 To test our proposal we constructed a mathematical model. For simplicity, we chose to model  
68 two molecular species, growth factor ( $\gamma$ ) and *rpoS* ( $r$ ). We used a stochastic Gillespie simulation for  
69 the reactions. Both were assumed to be produced by zeroth order reactions and degraded by first  
70 order reactions (Fig. 2f, see Methods for details). The reactions occurred in a cell, which grew at  
71 deterministic time intervals. As the cell volume increased molecule concentration was diluted. The  
72 growth rate at each deterministic time step explicitly depended on the most recent  $\gamma$  and *rpoS*  
73 concentration via the product of Hill functions (Fig. 2f). The Hill function for  $\gamma$  rose with  
74 concentration while that for *rpoS* decreased. This captured the promoting and repressing effects on  
75 growth rate of the two kinds of molecules, respectively.

76 This coupled molecular and physiological simulation can be summarized as a mutual inhibition  
77 feedback between *rpoS* and growth rate<sup>20</sup> (Fig. 2f). Using a coarse-grained exploration of the  
78 parameter space we found parameters for the stochastic simulation and Hill functions which  
79 reproduced the *WT* and *rpoS*-knockout experimental growth distributions (Fig. 2i) as well as the  
80 population growth rate. With these parameters set, the model then produced a long-tailed  
81 distribution of *rpoS* pulse heights, which decreased in prominence when the negative *rpoS* feedback  
82 on growth rate was removed *in silico* (Fig. 2g). The model also captured the rich single-cell *rpoS* and  
83 growth dynamics observed (Fig. 2j), as well as the anti-correlation between growth rate and *rpoS*  
84 (Fig. 2h).

85 We tested our understanding of the feedback model by perturbing population growth rate. As  
86 population growth rate is reduced, *rpoS* levels should increase due to decreased dilution (Fig. 3a).

87 We reduced population growth rate by reducing culture temperature, using reduced quality media,  
88 or combinations of the two (see Sup. Tab. 2) and imaged single cells from bulk cultures (see  
89 Methods). Indeed, *rpoS* levels increased with decreasing population growth rate (Fig. 3b).

90 The ability of *rpoS* to reduce growth rate could decrease with population growth rate due to  
91 globally reduced rates of transcription<sup>21,22</sup>. On the other hand, *rpoS* efficacy could remain constant,  
92 or even increase, allowing *rpoS* to control a greater portion of transcription. We used the model to  
93 distinguish between these possibilities. We modelled a reduction in population growth rate by  
94 decreasing  $g_{max}$  (see Methods). The effect of *rpoS* on growth rate could scale with this maximum  
95 growth rate, reflecting a constant *rpoS* efficacy, or remain fixed, reflecting an attenuated *rpoS*  
96 efficacy. We modelled the former by keeping  $f$  constant in the *rpoS* Hill function as  $g_{max}$  was varied.  
97 The latter was done by keeping the product  $f \cdot g_{max}$  constant, thereby flattening the repressive Hill  
98 function (Fig. 3c, Methods).

99 Comparing the theory to experiments, we found *rpoS* efficacy reduced with population growth  
100 rate. Using the Mother Machine assay and reduced culture temperatures we experimentally  
101 observed a convergence of the growth rate distributions of *WT* and  $\Delta rpoS$  populations (Fig. 3e, Sup.  
102 Fig. 5b). We found that the constant efficacy model overestimated the effect of *rpoS* on single-cell  
103 growth rate as population growth rate was reduced (Fig. 3d, e Sup. Fig. 5a, b), whereas the reduced  
104 *rpoS* efficacy model faithfully represented reality (Fig. 3e, f, Sup. Fig. 5b, c). Additionally, the reduced  
105 efficacy model captured the increasing levels of *rpoS* at reduced population growth rates (Fig. 3b).

106 The *rpoS* regulon allows cells to survive a variety of environmental stresses, for instance  
107 oxidative stress<sup>9,17,23</sup>. To test the function of heterogeneous *rpoS* expression, we assayed the survival  
108 of exponential phase cells against hydrogen peroxide ( $H_2O_2$ ). We used a short, intense pulse of stress  
109 to study the effect of *rpoS* already present in the bacteria, as opposed to the well-studied stress-  
110 induced *rpoS* response<sup>17</sup>. Using the Mother Machine we allowed cells to grow in fresh media, briefly  
111 switched to media containing  $H_2O_2$ , and then back to fresh media (Fig. 4a, see Methods for details).  
112 The population of cells that survived the stress upregulated *rpoS* approximately three hours prior to  
113 the stress (Fig. 4b). Consistent with literature<sup>17</sup>, *rpoS* knockout populations had a reduced survival  
114 fraction compared to *WT* (Fig. 4f).

115 Intriguingly, the surviving population also had reduced growth rate prior to the stress (Fig. 4c).  
116 Using the Receiver Operating Characteristic (ROC) curve we found that both *rpoS* activity and growth  
117 rate immediately preceding stress application are strong predictors of survival (Fig. 4d and  
118 Methods). This suggested two alternative hypotheses; either *rpoS* directly causes the survival  
119 phenotype, or it acts by first reducing growth rate, which in turn allows cells to survive the stress  
120 (Fig. 4e).

121 To distinguish between the two hypotheses, we noted the fraction of cells growing slower than  
122 the optimal threshold for survival increased for both *WT* and  $\Delta rpoS$  populations as population  
123 growth rate decreased (Fig. 4d, Methods, Sup. Fig. 5b). If *rpoS* directly caused survival, then the  
124 difference in survival fraction between *WT* and  $\Delta rpoS$  populations should increase at reduced  
125 temperature due to the increased *rpoS* present in *WT* cells (Fig. 3b). On the other hand, if growth  
126 rate was causing survival, the difference should decrease. We tested this experimentally by a bulk  
127 culture Colony Forming Units (CFU) stress assay (see Methods) and found the latter (Fig. 4f).  
128 Furthermore, we observed *rpoS*-knockout cells that survived in the Mother Machine assay at 37°C  
129 also down-regulated growth prior to stress (Sup. Fig. 6). This prompted the question: What is the  
130 role of *rpoS* at fast population growth rates?

131 To answer this question we analysed periods when cells were growing slower than the optimal  
132 threshold for survival (Fig. 4g). The role of *rpoS* is to prolong the duration of these slow growth  
133 events. We observed this as a higher frequency of long duration slow growth events in *WT*  
134 compared to *ΔrpoS* (Fig. 4h, Sup. Fig. 3f). The frequency with which cells attempt to grow slowly for  
135 any duration is similar for *WT* and *ΔrpoS* populations (Sup. Fig. 7a, 3g). The *rpoS*-growth feedback  
136 model captures this dynamic *rpoS* phenotype (Fig. 4i, Sup. Fig. 7b).

137 Slow growth mediated by *rpoS* has been implicated in the closely related persistence  
138 phenomenon<sup>24</sup>. Persister cells are slow growing cells in a clonal population of otherwise fast growing  
139 cells that can survive transient antibiotic treatment<sup>6,7</sup>. However, sudden downshifts in nutrient  
140 quality can generate nearly homogenous persister populations that are characterised by  
141 upregulation of the *rpoS* regulon<sup>24</sup>. We wondered if the heterogeneously generated surviving cells  
142 we observed were in fact persisters. There are several key differences. First, cells surviving oxidative  
143 stress were able to grow ~30x faster than persisters<sup>7,24</sup>. These survivors also occurred several orders  
144 of magnitude more frequently<sup>7</sup>. Finally, the small molecule ppGpp has been implicated in the  
145 production of heterogeneous persister cells<sup>25</sup>. We found that cells devoid of the ppGpp synthase,  
146 *relA*<sup>26</sup>, exhibited similar long-tailed *rpoS* distributions as wild type cells (Supp. Fig. 8), further  
147 distinguishing *rpoS* survivors from persisters.

148 Despite these differences, the two phenomena may be connected. Exposure to antibiotics can  
149 enhance subsequent survival against acid stress, a response mediated by *rpoS*<sup>27</sup>. Perhaps *E. coli*  
150 experienced antibiotic stress simultaneously with harsh environments in its evolutionary history.  
151 Cells able to coordinate persistence with the *rpoS* survival strategy revealed here would out-  
152 compete uncoordinated cells.

153 We combined theory and experiments to reveal how mutual inhibition of *rpoS* and growth can  
154 generate a rich, dynamic phenotype. Our coupled stochastic molecular and cell growth model  
155 provides a platform to explore more detailed mechanistic models. We have also demonstrated how  
156 the predictions of such a theory can be fruitfully compared to quantitative single-cell data. The  
157 active degradation of *rpoS* by proteases<sup>9</sup> and the promotion of anti- $\sigma^{70}$ , *rsd*, by *rpoS*<sup>28</sup> are two  
158 mechanisms that could provide greater agreement between theory and experiments. We therefore  
159 anticipate more functional phenotypic variability will be revealed by this approach.

## 160 **Methods**

### 161 **Strains and growth conditions**

#### 162 *Media*

163 M9 (1xM9 Salts, 2mM MgSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub>; 5xM9 Salts 34g/L Na<sub>2</sub>HPO<sub>4</sub>, 15g/L KH<sub>2</sub>PO<sub>4</sub>, 2.5 g/L NaCl, 5 g/L  
164 NH<sub>4</sub>Cl) supplemented with 0.2% Casamino acids and 0.4% glucose as carbon source. Media for Mother  
165 Machine experiments was also supplemented with 0.2 mg/mL Bovine Serum Albumin (BSA). For growth rate  
166 perturbation experiments glucose was replaced with 0.4% mannose and Casamino acids with 1 mM thiamine  
167 (see Sup. Tab. 2 for further details).

#### 168 *Reporter plasmid*

169 Reporter plasmids were sourced from the Alon library<sup>19</sup> using standard procedures and Qiagen Miniprep kits.  
170 Strains were transformed with the appropriate reporter plasmids by using a variant of the Top10 Chemical  
171 Competence protocol (OpenWetWare) followed by standard transformation by heat shock. Either an overnight  
172 culture or cells taken directly from glycerol stocks were grown up to exponential phase in LB. The cells were  
173 washed and concentrated in pre-chilled CCMB80 buffer 2-3 times (CCMB80: 10mM KOAc, 80 mM CaCl<sub>2</sub>·2H<sub>2</sub>O,

174 20 mM MnCl<sub>2</sub>·4H<sub>2</sub>O, 10 mM MgCl<sub>2</sub>·6H<sub>2</sub>O, 10% glycerol, adjusted to pH 6.4 with HCl). Next the plasmid was  
175 added to the cells and the mixture incubated on ice for 20-30 minutes. After a 1 minute 42°C heat shock, cells  
176 were allowed to recover in 1 mL LB at 37°C for 1 hour before plating on LB agar plates with 25ug/mL  
177 Kanamycin selection overnight. See Sup. Tab. 1 for list of strains.

178 *Knockout construction*

179 Knockouts strains were sourced from the Keio collection<sup>29</sup>. The knockout site with Kan<sup>r</sup> was amplified by PCR  
180 and used to perform knockouts in the MG1655 *E. coli* strain. Knockouts were carried out by the commercial  
181 Red/ET Recombination system (Gene Bridges, Germany) following the recommended protocol. However,  
182 instead of electroporation for transforming with the Red/ET recombination plasmid and FLPe flipase plasmid  
183 we used chemical transformation. The transformation was as above except the recovery was carried out at  
184 30°C and 1,000 rpm in a benchtop shaker and plates incubated at 30°C as the plasmid replication ceases at  
185 37°C. Knockouts were verified by colony PCR and sequencing.

186 *Chromosomal integration of reporter*

187 Knockins were performed as above for knockouts with the Red/ET recombination system (Gene Bridges). The  
188 integrated DNA was amplified off the reporter plasmid. The reporter plasmids were sequenced and used as  
189 references for the integration.

190 *Mother Machine microfluidic device*

191 The Mother Machine microfluidics device has been described previously<sup>11</sup>. Briefly, it consists of a feed trench  
192 (~50 μm x 100 μm x 30 mm) with many channels (~1.4 μm x 1.4 μm x 25 μm) attached perpendicular to the  
193 trench. These channels hold the cells and media is supplied to the cells via the trench. We used an epoxy  
194 master mould to fabricate our devices, which was a kind gift of Suckjoon Jun. The devices were fabricated by  
195 casting Sylgard 184 polydimethylsiloxane (PDMS) (Dow Corning, USA) with a ratio of 10:1 base to curing agent  
196 onto the master mould and cured overnight at 65°C. The chips were then cut out and plasma bonded (Femto  
197 Plasma System, Diener, Germany) to a glass bottom dish (HBSt-5040, Wilco Wells, Netherlands). To strengthen  
198 the bonding the chips were incubated for approximately ten min at 65°C. The chips were passivated with 20  
199 mg/ml Bovine Serum Albumin (BSA) for approximately one hour at 37°C prior to cell loading.

200 **Data acquisition**

201 *Bulk culture snaps*

202 We used the imaging protocol described previously<sup>10</sup> with minor modifications. Cells were grown from glycerol  
203 stocks in M9 at 37°C to late exponential phase and then diluted back into M9 to an OD of 0.01. After re-  
204 growing for approximately 2 hours 20 minutes, up to early exponential phase (OD~0.15), 0.3 μL of the cell  
205 culture was spotted onto pads of 1.5% low-melting agarose in Phosphate-Buffered Saline (PBS). Cells were  
206 imaged expediently, typically within ~20 minutes of leaving the incubator.

207 *Population growth rate perturbation*

208 Cells were grown from glycerol stocks using the modified media and temperature into exponential phase.  
209 Optical density measurements were taken after cells were diluted and grown up to exponential phase for  
210 imaging.

211 *Mother Machine movies*

212 Cells were grown from glycerol stocks as above. They were concentrated by centrifugation (4,000 rpm for 10  
213 min) and injected into the Mother Machine devices. A second centrifugation step for 5 min at 4,000 rpm using  
214 a spin coater (Polos Spin150i, SPS, Netherlands) forced cells into the channels. Cells were allowed to settle in  
215 the device while being supplied with fresh media for ~2 hours prior to beginning acquisition. Media was  
216 supplied at a flowrate of 1 ml/h by either a Fluigent pressure pump (MFCS-EZ, Fluigent, France) with an M-  
217 Flow sensor (Fluigent, France) or a syringe pump (Fusion 100, Chemyx, USA).

218 **Microscopy**

219 We used a widefield microscope with epifluorescence and phase contrast imaging modes (Nikon Ti-eclipse,  
220 Nikon, UK) equipped with the Nikon Perfect Focus (PFS) Unit. Illumination for the epifluorescence was  
221 provided by a white light LED source (SOLA SE Light Engine or Spectra X Light Engine, Lumencor, USA),  
222 transmitted by a liquid light guide (Lumencor, USA), through a fluorescence filter cube (49002-ET-EGFP,  
223 excitation: ET470/40x, dichroic: T495LP, emitter: ET525/50m, Chroma, USA), and a CFI Plan Apochromat 100x  
224 oil immersion objective (NA 1.45, Nikon). Phase contrast illumination was provided by a 100 W lamp via a  
225 condenser unit (Nikon). Images were acquired on a CoolSNAP HQ<sup>2</sup> camera (Photometrics, USA). The sample  
226 was held in motorized stages (Nikon). The sample was incubated along with much of the microscope body  
227 using a temperature controlled, heated chamber (Solent Scientific, UK). The microscope was controlled with  
228 MetaMorph software (version 7.8.10.0, Molecular Devices, USA). Fluorescent beads (TetraSpeck microspheres,  
229 0.5  $\mu$ m, Molecular Probes, USA) were imaged as a calibration standard.

230 **Quantifying gene expression and growth rate**

231 *Bulk culture single-cell gene expression*

232 A custom MATLAB (Mathworks, USA) script based on the published Schnitzcells software was used for image  
233 analysis<sup>10</sup>. The microscope was calibrated for each experiment with fluorescent beads to mitigate the effect of  
234 non-uniform sample illumination and daily variations in the apparatus. Cells were taken from a field of view  
235 computed from the beads to be within 80% of maximum intensity. Cells were segmented in the phase contrast  
236 channel. The mean fluorescence was then the corresponding pixels in the GFP channel normalized to cell area.  
237 A threshold was applied to exclude debris and substrate autofluorescence was subtracted from the mean cell  
238 fluorescence. Finally, the cell fluorescence was normalized by the fluorescence of the top 2% of fluorescent  
239 beads.

240 *Growth perturbation experiments*

241 Gene expression was computed as above. Growth rate was calculated by fitting an exponential curve to the  
242 OD measurements.

243 *Mother Machine movies*

244 Cell segmentation was done on the phase contrast channel using MATLAB (Mathworks, USA) scripts. The  
245 mother cell – the cell that remained at the end of growth channels farthest from the feed trench – was  
246 isolated and tracked. The image analysis was robust most of the time, but failed intermittently. Thus, every  
247 frame used in subsequent analysis was manually checked, and corrected if necessary. Growth was assumed to  
248 be exponential for each cell<sup>30</sup>, *i.e.*  $dl/dt = g*l$ , where  $l$  is cell length and  $g$  the growth rate. We thus computed  
249 growth rate as the difference in cell length between consecutive frames normalized by the first length. We  
250 note that the Mother Machine technique over-represents slow growing cells compared to bulk culture since  
251 the slow growing cells do not have to compete with fast cells in the Mother Machine. The population growth  
252 rate of mother cells was computed as  $g_{pop} = \ln(2)/t_D$  where  $t_D$  was found by numerically solving:

$$\frac{P_{final}}{P_{initial}} = 2 = \sum_i n_i 2^{t_D/c_i}$$

253 where  $P_x$  are number of cells,  $n_i$  are the fraction of cells growing with cell cycle time  $c_i$ .

254 *Promoter (*rpoS*) Activity*

255 Gene expression level was calculated as above. Calibration to beads was done using only the top 2%  
256 normalization – no cells were excluded due to position in the field of view. Promoter activity ( $A$ ) was computed  
257 as the component of the time-derivative of the expression corrected for by growth rate and bleaching<sup>1</sup>:

$$A = l m \left( \frac{1}{l} \frac{dl}{dt} + p \right) + l \frac{dm}{dt}$$

258 where  $l$  is cell length as above,  $m$  is mean fluorescence, and  $p$  is an adjustable parameter accounting for  
259 photobleaching of GFP. We set  $p = 0.1$ .

260 *Cross-correlation*

261 The normalized cross-correlation between growth rate and promoter activity was computed as follows:

$$\tilde{c}_{g-A}(\Delta t) = \sum_{t \in \text{all time}} \frac{(g(t + \Delta t) - \bar{g})}{c_{g-g}(0)} \frac{(A(t) - \bar{A})}{c_{A-A}(0)}$$

262 where  $g$  is growth rate,  $A$  is promoter activity,  $\Delta t$ , is the time difference between the two signals, overbars  
263 indicate averages over time, and  $c$  is the auto-correlation:

$$c_{a-a}(\Delta t) = \sum_{t \in \text{all time}} (a(t + \Delta t) - \bar{a}) (a(t) - \bar{a})$$

264 where  $a$  is either promoter activity or growth rate.

265 **Survival assay**

266 *Mother Machine assay*

267 Cells were loaded into the Mother Machine as above. Cells were allowed to grow in fresh media for 10 hours,  
268 then exposed to 35 mM H<sub>2</sub>O<sub>2</sub> for 35 minutes and then supplied with fresh media again for at least 12 hours.  
269 The media was switched with a Fluitgent 2-switch or M-switch (Fluitgent, France). Two 35 minute pulses of 3 to  
270 12 mM propidium iodide were supplied with the second round of fresh media and the cells were imaged in the  
271 RFP channel to observe DNA chelation of dead cells. This approach was not robust for identifying survivors and  
272 dead cells. Thus the movies for each mother cell were manually curated to determine survival using solely the  
273 phase contrast channel. If the cell began growing post-H<sub>2</sub>O<sub>2</sub> treatment and before the movie ended, it was  
274 counted as a survivor. Ambiguous cases were excluded from the tally (WT, 14% of cells excluded, *ΔrpoS*, 5%),  
275 however including these cells in the survival fraction calculation did not change the results.

276 *Receiver Operating Characteristic (ROC) curve*

277 A ROC curve measures how well a binary classifier performs as the threshold of the classifier is varied. We used  
278 growth rate and *rpoS* activity to classify the survival of cells in the Mother Machine survival assay. The True  
279 Positive Rate (TPR) as a function of the threshold was computed as:

$$TPR(\text{threshold}) = \frac{\# \text{ surviving cells past threshold}}{\text{Total} \# \text{ alive cells}}$$

280 Similarly, the False Positive Rate (FPR) was computed as:

$$FPR(\text{threshold}) = \frac{\# \text{ non-surviving cells past threshold}}{\text{Total} \# \text{ non-surviving cells}}$$

281 When growth rate was used as the classifier, cells passed the threshold if their growth rate was below the  
282 tested value; while for *rpoS* activity if it was above. The TPR was plotted against the FPR to generate the ROC  
283 curve. The optimal threshold was computed by finding the threshold that resulted in the maximum difference  
284 between the TPR and FPR. The Area Under the Curve (AUC), computed by numerical integration of the ROC  
285 curve, is a measure of the quality of the classifier. A perfect classifier has AUC = 1, while one that is no better  
286 than random guessing has AUC = 0.5.

287 *Bulk culture Colony Forming Units (CFU) assay*

288 Cells were grown into exponential phase from glycerol stocks at either 37°C or 28°C and diluted into 10 mL  
289 fresh media. They were grown into exponential phase again and aliquoted into 2 mL cultures. These aliquots

290 were exposed to either water or 26 mM H<sub>2</sub>O<sub>2</sub> and incubated for a further 20 minutes. Cultures were then  
291 serially diluted in M9 and plated on LB agar plates. The colonies on the plates were counted after an overnight  
292 incubation at 37°C to determine the Colony Forming Units (CFU). Survival fraction was computed as cells/mL  
293 from the stress condition divided by the cells/mL from the water condition. Averages were taken over all  
294 plates that were in the dynamic range of the assay (30 to 300 colonies per plate).

295 **Stochastic simulation coupled to single cell growth model**

296 We modelled a single cell growing as a function of molecular reactions occurring inside it. A single lineage was  
297 followed, *i.e.* only one daughter cell was followed at each cell division. To model growth, we assumed rod-  
298 shaped cells with fixed radius and modelled growing cells by the changing length at a fixed, deterministic time  
299 interval,  $\Delta t$ :

$$\Delta l_i = g_{i-1} \cdot \Delta t \cdot l_{i-1}$$

300 where  $g_i$  and  $l_i$  are the growth rate and cell length at the  $i^{th}$  time point, respectively. Cell division was assumed  
301 to follow the adder rule<sup>30</sup>:

$$l_i = \begin{cases} l_{i-1} + \Delta l_i, & \sum_{\substack{\text{last division} \\ \text{otherwise}}}^i \Delta l_k < \Delta L \\ (l_{i-1} + \Delta l_i)/2, & \text{otherwise} \end{cases}$$

302 where  $\Delta L$  is a fixed length the cell must add before it can divide. The numbers of molecules in the cell were  
303 determined by a standard Gillespie stochastic simulation algorithm<sup>12</sup> that ran between the deterministic steps  
304 of the growth model. Two molecular species *rpoS*,  $r$ , and growth factor,  $\gamma$ , were modelled. They were  
305 generated with zeroth order constitutive production and first order degradation reactions:



306 where  $k_{xp}$  are the production propensities and  $k_{xd}$  are the degradation propensities for species  $x$ . The reaction  
307 propensities in the Gillespie algorithm do not change with cell volume since the reactions are zeroth and first  
308 order<sup>13</sup>. At division the number of molecules were simply divided in half and rounded to the closest integer  
309 lower than the quotient:

$$species_i = \lfloor species_{i-1}/2 \rfloor$$

310 The concentration of the molecular species was the number of species divided by cell length (volume):

$$[species_i] = \frac{species_i}{l_{i-1}}$$

311 Growth rate was a function of the concentration of the two molecular species generated most recently by the  
312 Gillespie algorithm:

$$g_i = g_{\max} \cdot \left( \frac{1}{1 + \left( \frac{h_{\gamma}}{[r_i]} \right)^{n_{\gamma}}} \right) \cdot \left( \frac{1 - f}{1 + \left( \frac{h_r}{[r_i]} \right)^{n_r}} + f \right)$$

313 where  $g_{\max}$  is the maximum growth rate;  $f$  represents the lowest growth rate can be reduced to in the limit of  
314 infinite *rpoS* concentration;  $h_{\gamma}$  and  $h_r$  are the values of growth factor and *rpoS* leading to half-maximal growth,  
315 respectively; and  $n_{\gamma}$ ,  $n_r$  are the Hill coefficients. Growth factor was considered a downstream target of  $\sigma^{70}$  so  $n_{\gamma}$

316 was positive, while  $n_r$  was chosen to be negative to capture the repressive effect of *rpoS* on growth. Growth  
317 perturbation simulations were implemented by varying  $g_{max}$ , while all other parameters were kept constant.  
318 However, in the reduced *rpoS* efficacy model the parameter  $f$  was increased to keep the product  $f \cdot g_{max}$   
319 constant. See Sup. Table 3 for parameter values used and Sup. Mat. for the pseudo code of the algorithm.

320 **Code availability**

321 Code used for simulations and for analysis of data reported in this study is available upon request from the  
322 corresponding author.

323 **Data availability**

324 Data that support the findings reported in this study are available upon request from the corresponding  
325 author.

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390

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399 **Author contributions**

400 OP, AP, JCWL conceived and designed the study, analysed and interpreted the data, and wrote the article.  
401 OP and MJ performed the experiments. CS developed microfluidics apparatus. OP and DG  
402 constructed strains. DG provided technical assistance and advice. All authors provided input into the  
403 manuscript.

404 **Competing interests**

405 The authors declare no competing financial interests.

## 406 Figure legends:

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407 **Figure 1 | The stress response master regulator, *rpoS*, is heterogeneously expressed in unstressed**  
408 **cells.** **a**, Schematic of the role of sigma factors  $\sigma^{70}$  and *rpoS* in promoting growth and activation of  
409 the stress response regulon, respectively. Also illustrated is the *rpoS* reporter, a transcriptional  
410 fusion to a stress response promoter. **b**, Representative phase contrast and fluorescence composite  
411 image of *rpoS* reporter,  $P_{bolA}$ -GFP, in *WT*; channel ranges chosen for display. **c**, Histograms of mean  
412 *rpoS* per cell (line: mean, shaded region:  $\pm$  std dev) in *WT* (10 biological replicates, 4,037 cells, mean  
413 = 0.21, CV = 0.51) and  $\Delta rpoS$  (9 bio. reps., 4,069 cells, mean = 0.11, CV = 0.27) strains. The long tail of  
414 high *rpoS* levels present in the *WT* is absent in the knockout.

415 **Figure 2 | Growth-*rpoS* mutual inhibition produces multi-generation *rpoS* pulses and**  
416 **heterogeneous *rpoS* expression.** **a**, Sample montage of a mother cell (orange outline) in the Mother  
417 Machine pulsing on *rpoS* and reducing growth rate (1 frame/10 minutes). Phase contrast and  
418 fluorescence channel ranges chosen for display. **b**, Sample time traces of *rpoS* activity and growth  
419 rate for four mother cells. Grey vertical lines indicate cell divisions. **c**, Histogram of *rpoS* activity pulse  
420 height (3,608 pulses). **d**, Cross-correlation between growth rate and *rpoS* activity. **e**, Histogram of  
421 growth rate at one frame from all movies for *WT* and  $\Delta rpoS$ . In (c-e) the mean  $\pm$  std dev is plotted  
422 with the line and the shaded region, respectively for *WT* (11 technical replicates drawn from 7  
423 biological replicates, 563 mother cells) and  $\Delta rpoS$  (10 tech. reps. drawn from 6 bio. rep., 279 mother  
424 cells). **f**, Schematic illustration of mathematical model. Stochastic molecular reactions occur in a  
425 growing cell. The reactions are simulated with the Gillespie algorithm, while cell growth happens at  
426 deterministic time steps. Growth at each time step is dependent on molecular concentration via Hill  
427 functions. The result is a mutual inhibition between growth rate and *rpoS* concentration. **g-j** Analysis  
428 from 1,000 simulations run for 500 hours; only the last 250 hours are used to capture steady-state  
429 behaviour. **g**, Histograms of simulated *rpoS* concentration with and without feedback of *rpoS* on  
430 growth rate (88,865 and 133,126 pulses, respectively). **h**, Cross-correlation between simulated  
431 growth rate and *rpoS* concentration. **i**, Histograms of growth rate sampled at 24 hour intervals over  
432 all 1,000 simulations to mitigate effects of correlations. **j**, Sample time traces of simulated *rpoS*  
433 concentration and growth rate for four cells. Grey vertical lines indicate cell divisions.

434 **Figure 3 | *rpoS* levels increase, but are less potent, at reduced population growth rate.** **a**,  
435 Schematic illustrating effect of reduced population growth rate. *rpoS* is concentrated due to lower  
436 dilution by growth rate. However, its effect on growth rate could diminish at low population growth  
437 rate. **b**, Median *rpoS* levels in liquid culture ( $\pm$  std dev, mean growth rate  $\pm$  std dev, at least two  
438 biological replicates, see Sup. Tab. 2 for details) and scaled *rpoS* concentration from simulations as  
439 functions of population growth rate. Dashed lines are exponential fits. Scaling factor (0.29) was  
440 found by minimizing root-mean-square error between the fits over the range of observed growth  
441 rates  $\pm$  20% (0.29 to 1.6/hr). **c**, Hill functions of growth rate as functions of *rpoS* concentration used  
442 in simulations. Fast population growth corresponds to simulation matching experimentally observed  
443 growth rate at 37°C (Fig. 2e, i). The constant and reduced efficacy models behave differently in the  
444 large *rpoS* concentration limit as population growth rate is reduced. **d-f**, Growth rate histograms for  
445 *WT* and  $\Delta rpoS$ . **e**, Cells grown at reduced temperature, 28°C, in the Mother Machine (mean  $\pm$  std  
446 dev, *WT*, 4 technical replicates drawn from 3 biological replicates, 84 mother cells;  $\Delta rpoS$ , 4 tech.

447 reps. drawn from 2 bio. rep., 85 mother cells). Simulation results at corresponding population  
448 growth rate with constant *rpoS* efficacy (**d**) and reduced *rpoS* efficacy (**f**) (100 simulations for 19  
449 values of  $g_{max}$ , sampled every 24 hours, in the final 250 hours of 500 hour simulations).

450 **Figure 4 | *rpoS* enables survival of stress by prolonging duration of slow growing state.** **a**,  
451 Schematic of the stress assay and sample montages of surviving (top) and non-surviving (bottom)  
452 mother cell. Mother cell outlined in orange; 1 frame/10 minutes; phase contrast and fluorescence  
453 channel ranges identical for both montages and chosen for display. Cells were grown for 10 hours in  
454 fresh media, followed by a 35 minute application of  $H_2O_2$  stress, and fresh media once again. **b**,  
455 Median value of *rpoS* activity distributions for time points prior to stress application ( $t = 0$ ), sorted  
456 according to survival (line and shaded area are mean  $\pm$  std dev, 7 technical replicates drawn from 4  
457 biological replicates; 72 surviving cells, 212 non-surviving, 284 total mother cells). **c**, Same as (**b**) for  
458 growth rate. **d**, Receiver Operating Characteristic curve for growth rate (black) and *rpoS* activity  
459 (green) from time point preceding stress application. Grey dashed line is True Positive Rate = False  
460 Positive Rate. Circles represent locations of optimal thresholds (0.65/hr for growth rate, 0.020 AU for  
461 *rpoS* activity). Area Under the Curve (AUC) is 0.90 for growth rate and 0.86 for *rpoS* activity. **e**,  
462 Schematic illustrating alternative mechanisms of stress survival. High *rpoS* activity could directly  
463 allow cells to survive or it might first reduce growth rate, which in turn allows survival. **f**, Fraction of  
464 cells surviving stress in the Mother Machine assay (mean  $\pm$  min/max, *WT*: 7 tech. reps., represented  
465 as circles, drawn from 4 bio. reps., 1,087 cells,  $\Delta rpoS$ : 5 tech. reps. drawn from 3 bio. reps., 996 cells)  
466 and bulk Colony Forming Units assay at two temperatures (mean  $\pm$  min/max; at least two biological  
467 replicates for bulk culture assays, represented as circles). **g**, Illustration of a low growth event based  
468 on the ROC curve optimal threshold (0.65/hr) (**d**). **h**, Cumulative distribution of duration of low  
469 growth events in *WT* and  $\Delta rpoS$  populations (line and shaded area are mean  $\pm$  std dev, *WT*, 11 tech.  
470 reps. drawn from 7 bio. reps., 563 mother cells, 941 events;  $\Delta rpoS$ , 10 tech. reps. drawn from 6 bio.  
471 rep., 279 mother cells, 391 events). **i**, Same as (**h**) from simulations (1,000 simulations run for 500  
472 hours, only the final 250 hours were used; *WT*, 75,787 events and  $\Delta rpoS$ , 49,114 events).

473 **Supplementary Figure 1 | Alternative *rpoS* reporters have long-tailed distributions of *rpoS* levels;**  
474 **the long tails vanish in the *rpoS*-knockout.** **a**, Transcriptional fusion of *P<sub>blc</sub>-GFP* in *WT* (6 biological  
475 replicates, 2,509 cells, mean = 0.050 AU, CV = 0.46) and  $\Delta rpoS$  (4 bio. reps., 1,190 cells, mean =  
476 0.025 AU, CV = 0.21). **b**, Similarly for *P<sub>poxB</sub>-GFP* (*WT*: 5 bio. reps., 1,087 cells, mean = 0.12 AU, CV =  
477 0.59;  $\Delta rpoS$ : 7 bio. reps., 1,463 cells, mean = 0.023 AU, CV = 0.17). Lines and shaded region are mean  
478  $\pm$  std dev, respectively.

479 **Supplementary Figure 2 | Reporters of  $\sigma^{70}$  have distributions with lower coefficients of variation**  
480 **than *rpoS* reporters and distributions that are similar in *WT* and  $\Delta rpoS$ .** **a**, Transcriptional fusion of  
481 *P<sub>rpsL</sub>-GFP* in *WT* (5 bio. reps., 1,576 cells, mean = 2.1 AU, CV = 0.25) and  $\Delta rpoS$  (3 bio. reps., 647 cells,  
482 mean = 1.7 AU, CV = 0.25). **b**, Similarly for *P<sub>lacI</sub>-GFP* in *WT* (3 bio. reps., 503 cells, mean = 0.14 AU, CV  
483 = 0.31) and  $\Delta rpoS$  (3 bio. reps., 497 cells, mean = 0.12 AU, CV = 0.34). Lines and shaded region are  
484 mean  $\pm$  std dev, respectively.

485 **Supplementary Figure 3 | Long-tailed *rpoS* distribution is not due to plasmid segregation effect,**  
486 **nor are the growth effects due to plasmid toxicity.** Using chromosomally integrated *P<sub>bolA</sub>-GFP* in *WT*:  
487 **a**, Sample time traces of *rpoS* activity and growth rate for four mother cells. Grey vertical lines  
488 indicate cell divisions. **b**, Distribution of *rpoS* level from bulk liquid culture (3 biological replicates,

489 465 cells, mean = 0.045 AU, CV = 0.23). **c**, Pulse height distribution in Mother Machine experiments  
490 (1,438 peaks). **d**, Growth rate histogram. **e**, Cross-correlation between growth rate and *rpoS* activity.  
491 **f**, Duration distribution of low growth events. **g**, Distribution of frequency of entering low growth  
492 event. In **c-g**, 4 technical replicates drawn from 2 bio. reps., 143 mother cells were used. The plasmid  
493 data in **d-g** is reproduced from elsewhere in this work for ease of comparison. Lines and shaded  
494 region are mean  $\pm$  std dev, respectively.

495 **Supplementary Figure 4 | Constitutive,  $\sigma^{70}$ , reporter is positively correlated with growth and high**

496 **GFP expression does not affect growth rate distribution.** Using  $P_{rpsL}$ -GFP in WT and  $\Delta rpoS$ . **a**, Cross-  
497 correlation between growth rate and  $\sigma^{70}$  activity in WT cells. **b**, Growth rate histogram for WT and  
498  $\Delta rpoS$ . WT: 2 biological replicates, 86 mother cells;  $\Delta rpoS$ : 2 biological replicates, 81 mother cells.  
499 Lines and shaded region are mean  $\pm$  std dev, respectively.

500 **Supplementary Figure 5 | The influence of *rpoS* on growth is attenuated as population growth rate**

501 **decreases.** **a**, Growth rate histograms for simulated WT and  $\Delta rpoS$  at three population growth rates  
502 achieved by keeping  $f$  constant as  $g_{max}$  was reduced. Dashed black lines correspond to optimal  
503 survival threshold of 0.65/hr (Fig. 4d). Insets: Hill functions of growth rate vs *rpoS* concentration. **b**,  
504 Experimental growth rate histograms for WT and  $\Delta rpoS$  grown at three temperatures (mean  $\pm$  std  
505 dev, 28°C and 37°C reproduced from main text; 33°C WT, 5 technical replicates drawn from 3  
506 biological replicates, 72 mother cells;  $\Delta rpoS$ , 6 tech. reps. drawn from 3 bio. rep., 137 mother cells).  
507 **c**, Growth rate histograms for simulated WT and  $\Delta rpoS$  with  $f^*g_{max}$  constant as  $g_{max}$  was reduced.  
508 Insets: same as **(a)**.  $g_{max}$  values for the simulations were chosen such that population growth rates  
509 matched the experimentally observed population growth rates, see Methods for details. For **(a)** and  
510 **(c)** 100 simulations were used for each condition, sampled every 24 hours, in the final 250 hours of  
511 500 hour simulations.

512 **Supplementary Figure 6 | Slow growing  $\Delta rpoS$  cells survive oxidative stress.** Cells were treated as  
513 in Fig. 3. **a**, Median value of growth rate distributions for time points prior to stress application ( $t =$   
514 0), sorted according to survival (mean  $\pm$  std dev, 5 technical replicates drawn from 3 biological  
515 replicates, 41 surviving cells, 128 non-surviving cells, 169 total mother cells). **b**, Receiver Operating  
516 Characteristic curve for growth rate (optimal threshold is 0.70/hr, Area Under Curve is 0.74).

517 **Supplementary Figure 7 | Frequency of slow growth initiation similar between WT and  $\Delta rpoS$ .** **a**,  
518 Experimental distributions of frequency of entering low growth event for WT and  $\Delta rpoS$  (mean  $\pm$  std  
519 dev; WT, 11 technical replicates drawn from 7 biological replicates, 563 mother cells, 821 events;  
520  $\Delta rpoS$ , 10 tech. reps. drawn from 6 bio. rep., 279 mother cells, 342 events). **b**, Same as **(a)** for  
521 simulations (1,000 simulations run for 500 hours, only the final 250 hours were used; WT, 75,628  
522 events and  $\Delta rpoS$ , 49,041 events).

523 **Supplementary Figure 8 | ppGpp does not affect long-tailed *rpoS* expression.** **a**, WT strain used in  
524 this work, MG1655, and  $\Delta rpoS::kan$  harbouring reporter with kanamycin resistance replaced with  
525 spectinomycin resistance ( $P_{bolA}$ -GFP::spec). WT (2 biological replicates, 696 cells, mean = 0.18 AU, CV  
526 = 0.38) and  $\Delta rpoS$  (2 bio. reps., 1,244 cells, mean = 0.12 AU, CV = 0.18). **b**, The same in the WT strain  
527 of the Keio collection<sup>29</sup>, BW25113. WT (2 bio. reps., 739 cells, mean = 0.20 AU, CV = 0.27) and  $\Delta rpoS$   
528 (2 bio. reps., 651 cells, mean = 0.13 AU, CV = 0.19). **c**, Two isolates from the Keio collection of  $\Delta relA$ ,  
529 from plates 53 and 54 with WT from **(b)**:  $\Delta relA$ , 53 (2 bio. reps., 898 cells, mean = 0.19 AU, CV =

530 0.29) and *ΔrelA*, 54 (2 bio. reps., 543 cells, mean = 0.16 AU, CV = 0.29). Lines and shaded region are  
531 mean  $\pm$  std dev, respectively.

# 532 Supplementary Material

533 **Table S.1 Strain list**

	Strain name	Genotype	Construction procedure	Source
1a	MG1655 seq (CGSC#6300)	K12 with <i>F</i> - $\lambda$ - <i>rph-1</i>	WT strain of reporter library <sup>19</sup>	Yale CGSC
1b	MG1655 (CGSC#7740)	K12 with <i>F</i> - $\lambda$ - <i>rph-1</i>	WT strain of reporter library <sup>19</sup>	Yale CGSC
2	63Dr	Same as 1a with $\Delta rpoS::Kan^r$	Used a PCR product from Keio collection $\Delta rpoS$ strain <sup>29</sup>	This work
3a	63DrF-	Same as 2, markerless	FLPe recombinase	This work
3b	MGDrF-	Same as 1b with $\Delta rpoS$	Used a PCR product from Keio collection $\Delta rpoS$ strain <sup>29</sup> and FLPe recombinase	This work
4	MGChrRep	Same as MG1655 with chromosomally integrated reporter::kan	Used Red/ET system and PCR product amplified from reporter plasmid <sup>19</sup>	This work
4	BW25113	Same as MG1655 with <i>rrnB3</i> $\Delta lacZ4787$ <i>hsdR514</i> $\Delta(araBAD)567$ $\Delta(rhaBAD)568$ and $\Delta crl$ $\Delta valX$ <i>mhpC365991</i>	WT strain of Keio collection <sup>29</sup>	Yale CGSC
5	KDr	Same as BW25113 with $\Delta rpoS::Kanr$	-	From Keio collection <sup>29</sup>
6a	DrelA53	Same as BW25113 with $\Delta relA::Kanr$	-	From Keio collection <sup>29</sup> (plate 53)
6b	DrelA54	Same as BW25113 with $\Delta relA::Kanr$	-	From Keio collection <sup>29</sup> (plate 54)

534 **Table S.2 Growth conditions and population growth rates**

Growth condition*	WT		$\Delta rpoS$	
	Biological replicates; number of cells	Growth rate (1/hr), mean $\pm$ std dev	Biological replicates; number of cells	Growth rate (1/hr), mean $\pm$ std dev
0.4% glucose, 0.2% casamino acids (37°C)	4; 711	1.42 $\pm$ 0.07	3; 427	1.42 $\pm$ 0.08
0.4% glucose, 0.2% casamino acids (33°C)	2; 547	0.98 $\pm$ 0	2; 510	1.02 $\pm$ 0
0.4% glucose, 0.2% casamino acids (28°C)	2; 747	0.55 $\pm$ 0.02	2; 601	0.59 $\pm$ 0.01
0.4% mannose, 0.2% casamino acids (37°C)	3; 720	1.20 $\pm$ 0.04	2; 453	1.23 $\pm$ 0
0.4% mannose, 0.2% casamino acids (33°C)	2; 346	0.84 $\pm$ 0	2; 511	0.85 $\pm$ 0.02
0.4% mannose, 0.2% casamino acids (28°C)	2; 604	0.48 $\pm$ 0.02	2; 595	0.51 $\pm$ 0.01
0.4% glucose, 1 mM thiamine (37°C)	2; 896	0.74 $\pm$ 0.04	2; 536	0.74 $\pm$ 0.02
0.4% mannose, 1 mM thiamine (37°C)	3; 2,719	0.49 $\pm$ 0.02	3; 2,298	0.52 $\pm$ 0.03

535 \*M9 supplemented with the following and grown at (temperature).

536 **Model Details**

537 *Pseudo code*

```

538 for k = 1 to number of simulations
539     %initializing first step
540
541     for t = 1 to Number of time steps
542         %first run the rpoS Gillespie:
543
544         while till accumulated Gillespie time does not exceeds growth clock
545             %Perform standard Gillespie algorithm
546         end
547
548         %compute concentration of the molecules
549
550         %Update growth rate using Hill function
551
552         %adder rule:
553         if added length > adder value
554             %divide cell and molecules in half
555         else
556             %increase cell length
557         end
558
559         %store values with sampling resolution
560         if mod(t,storestep) == 1
561             %store simulation step
562         end
563     end
564
565 end

```

566 **Table S.3 Model Parameters**

Parameter	Value in model	Value in physical units	Description
<i>Gillespie</i>			
$k_{\gamma p}$	2.2	$13 \text{ hr}^{-1}$	$\gamma$ zeroth order production rate constant
$k_{\gamma d}$	0.2	$1.2 \text{ hr}^{-1} \gamma^{-1}$	$\gamma$ first order degradation rate constant
$k_{rp}$	0.3	$1.8 \text{ hr}^{-1}$	<i>rpoS</i> zeroth order production rate constant
$k_{rd}$	0.01	$0.06 \text{ hr}^{-1} r^{-1}$	<i>rpoS</i> first order degradation rate constant
$\gamma_{init}$	1	1 molecule	Initial value of $\gamma$
$r_{init}$	1	1 molecule	Initial value of <i>rpos</i>
<i>Growth</i>			
$\Delta L$	1	$2 \mu\text{m}$	Length cell must grow before dividing
$l_i$	1	$2 \mu\text{m}$	Initial cell length
<i>Coupling growth and Gillespie models</i>			
$g_{max}$	1.2	$7.2 \text{ hr}^{-1} (0.7 \text{ hr}^{-1})$	Maximum (average) growth rate achievable by cell
$h_{\gamma}$	17	17 molecules/cell	Half-maximum value for $\gamma$ -growth Hill function
$n_{\gamma}$	1	-	Hill coefficient for $\gamma$ -growth Hill function
$h_r$	2	2 molecules/cell	Half-maximum value for <i>rpoS</i> -growth Hill function
$n_r$	-4	-	Hill coefficient for <i>rpoS</i> -growth Hill function
$f$	0.25	-	Minimum value <i>rpoS</i> can reduce growth rate by
<i>Technical parameters</i>			
	100 or 1,000	-	Number of simulations
	3000	500 hrs	Number of time steps
	0.005	3 s	Simulation time resolution
	$1/0.005 = 200$	10 min	Sampling resolution

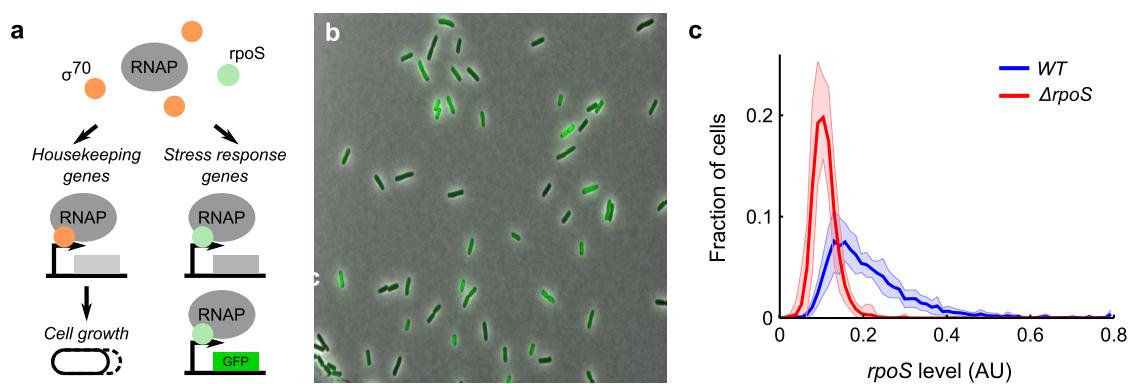


Figure 1

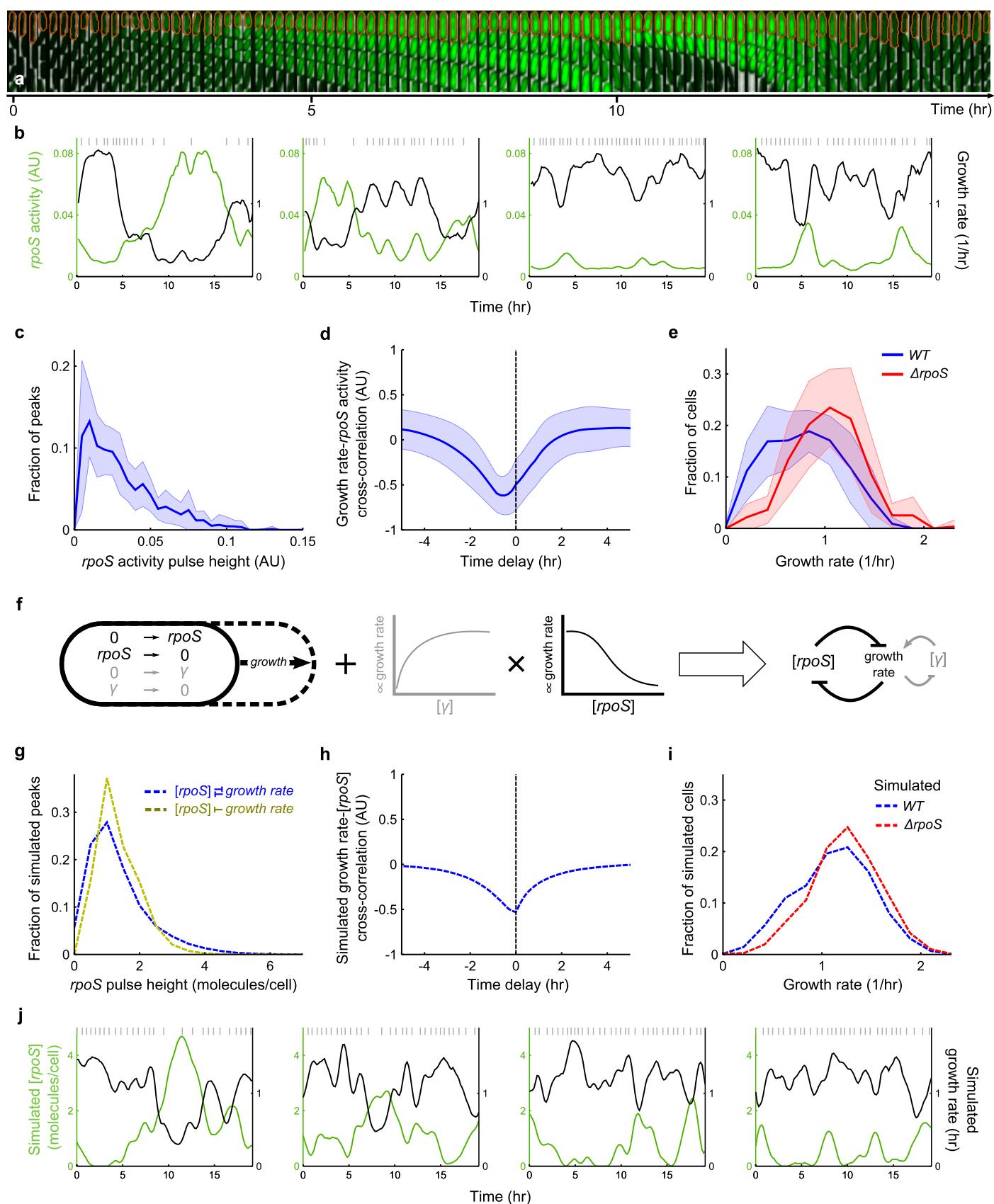


Figure 2

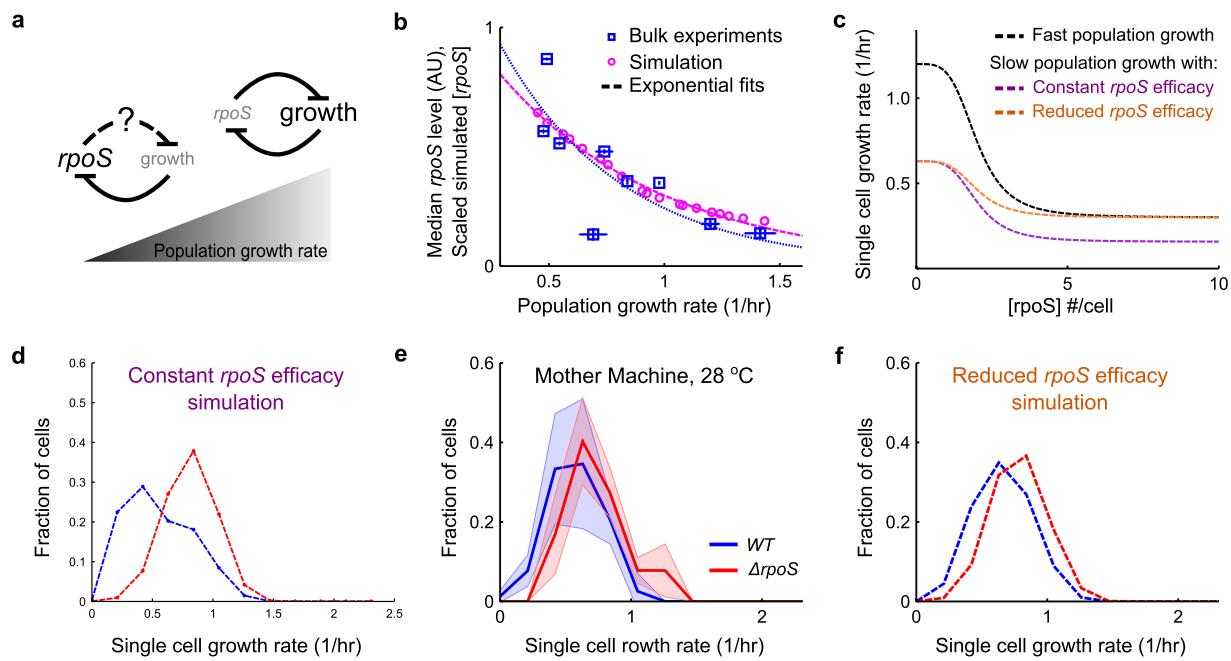


Figure 3

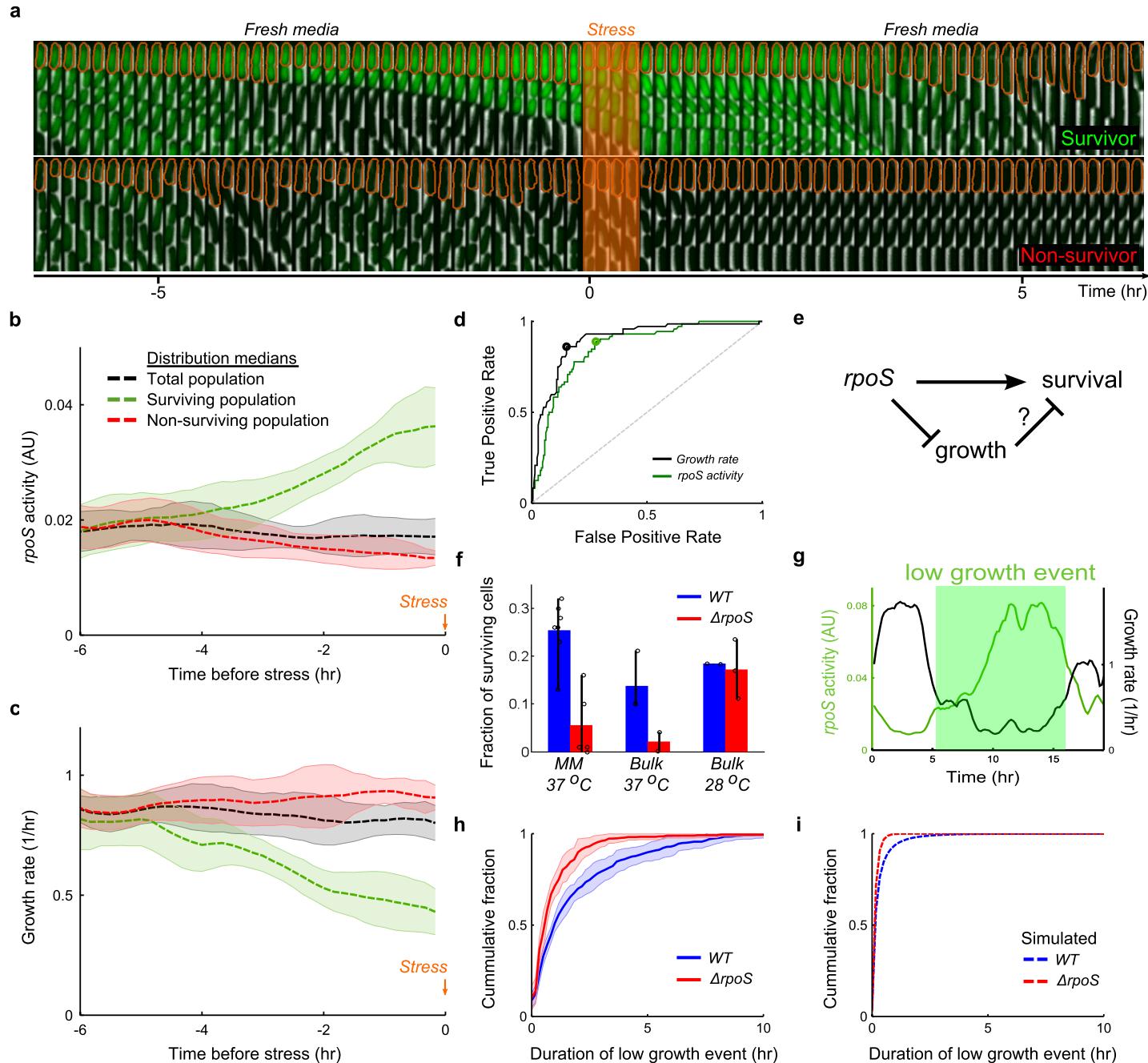
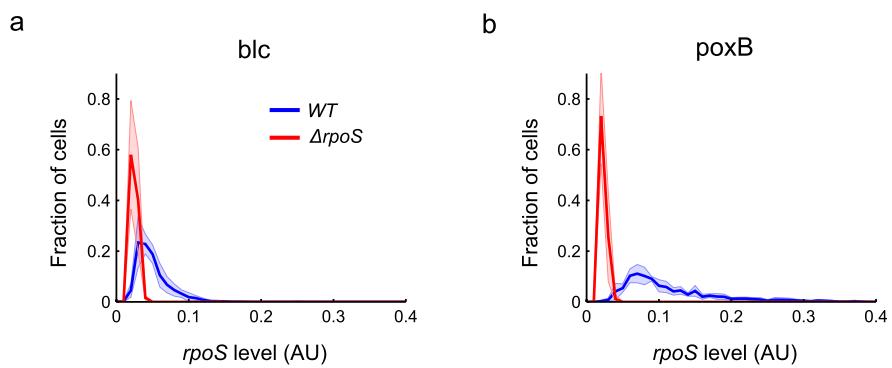


Figure 4



**Figure S.1**

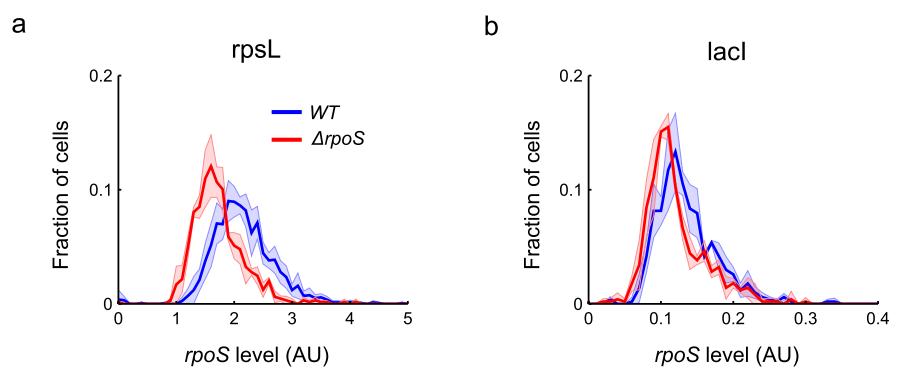


Figure S.2

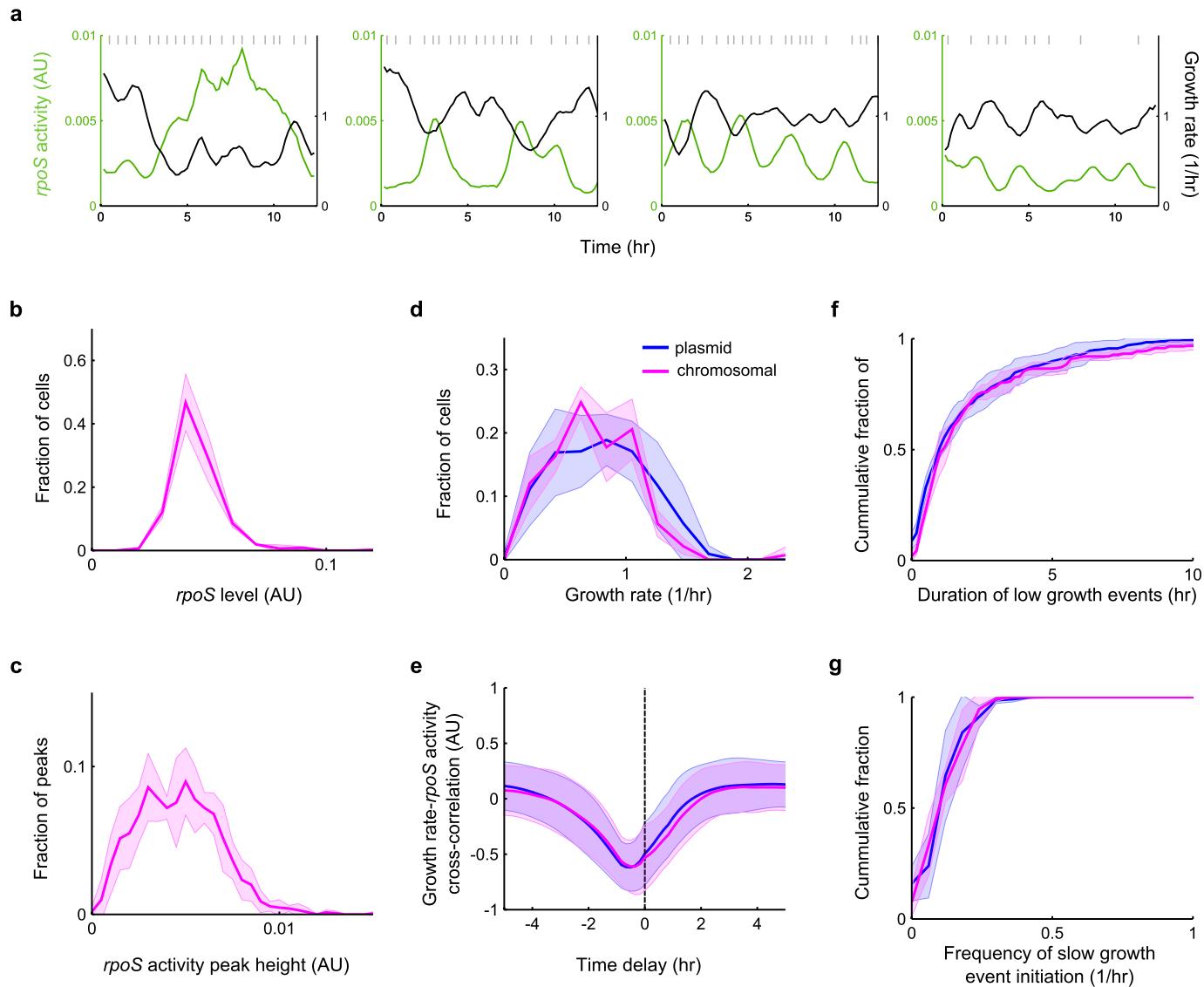


Figure S.3

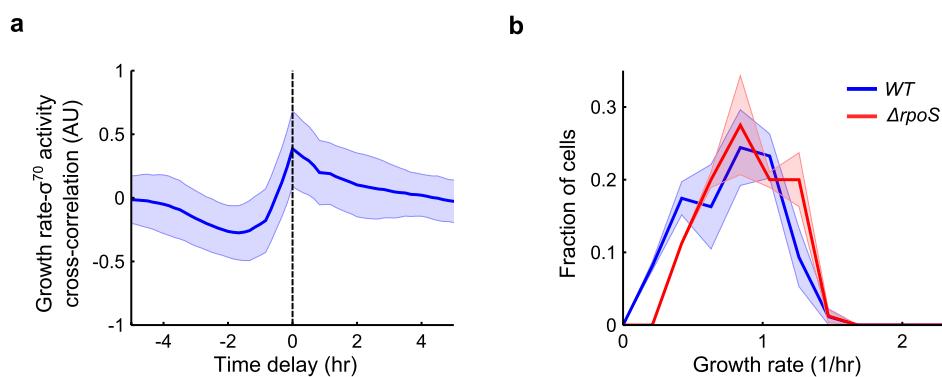


Figure S.4

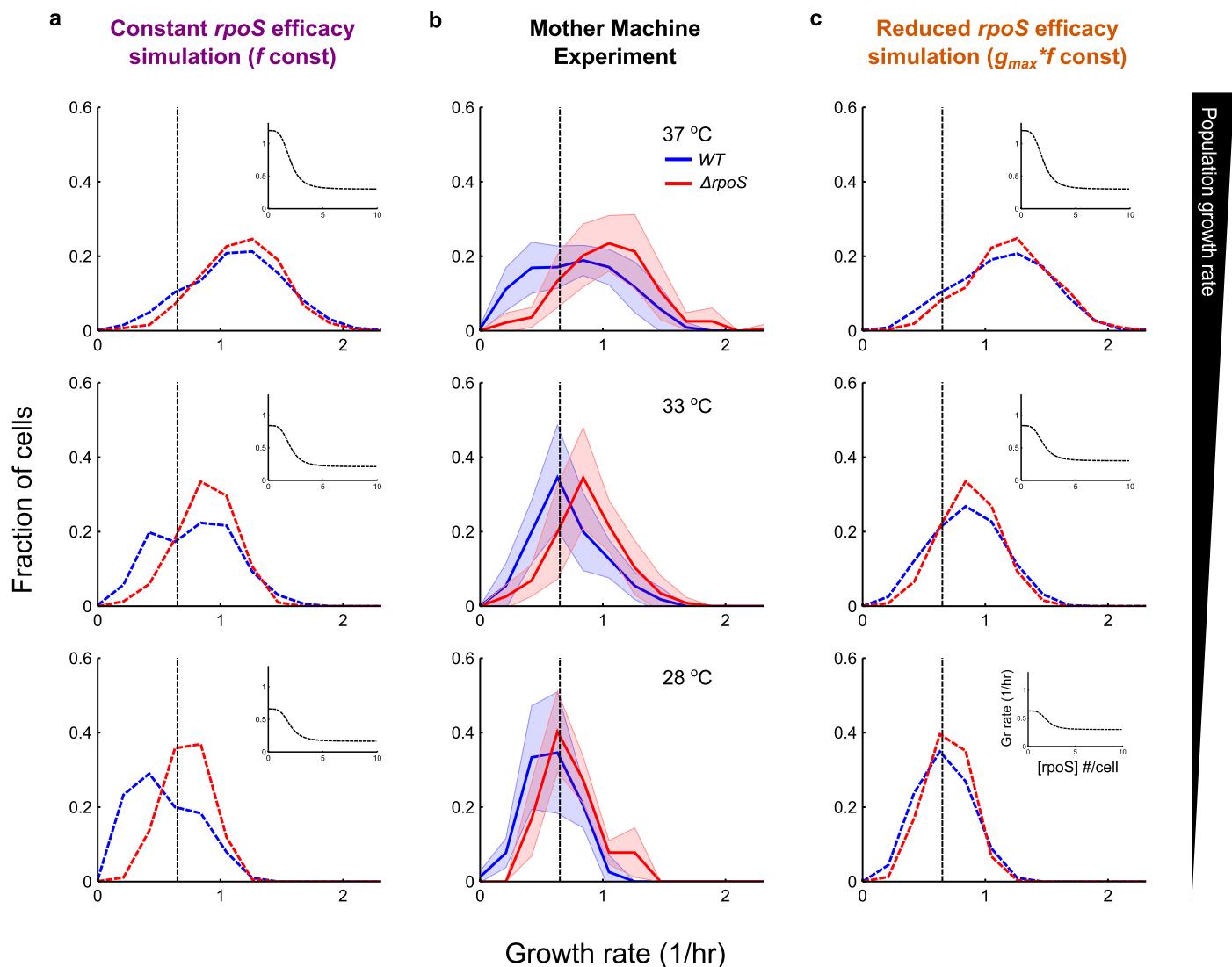


Figure S.5

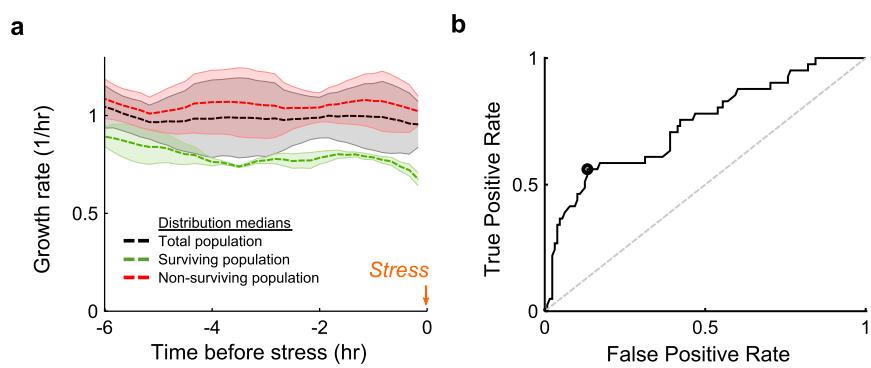


Figure S.6

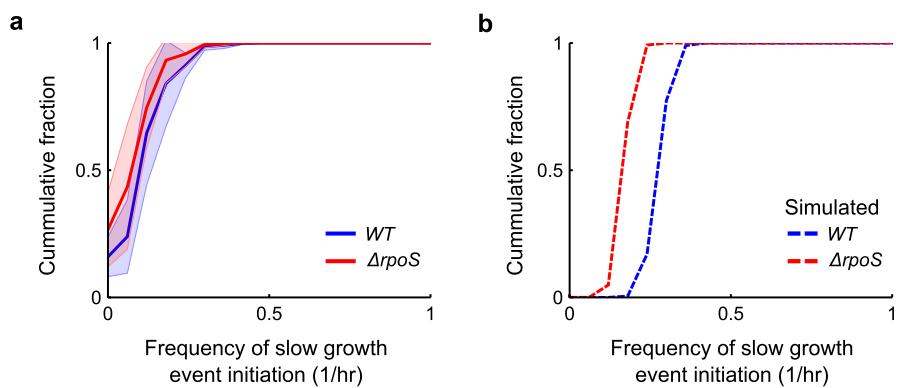


Figure S.7

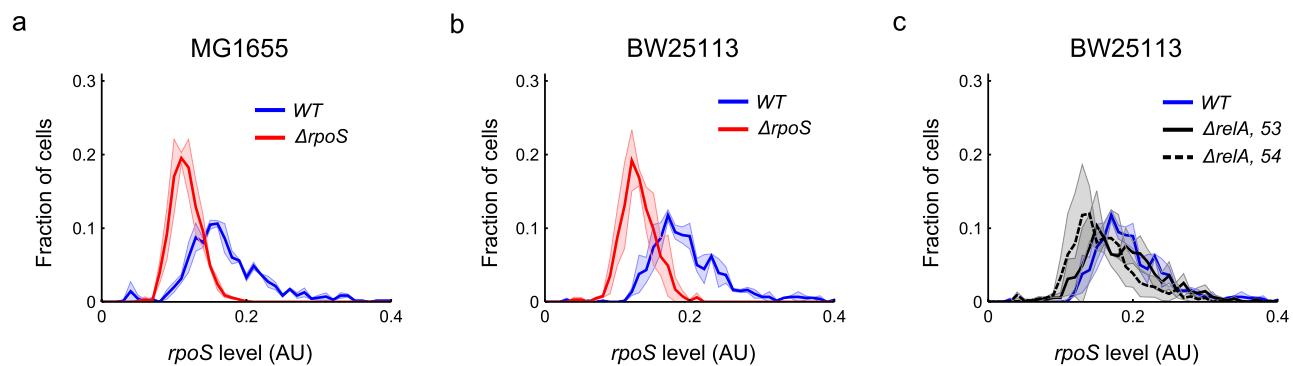


Figure S.8