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Supplementary Materials for

Single-cell imaging and characterization of *Escherichia coli* persister cells to ofloxacin in exponential cultures

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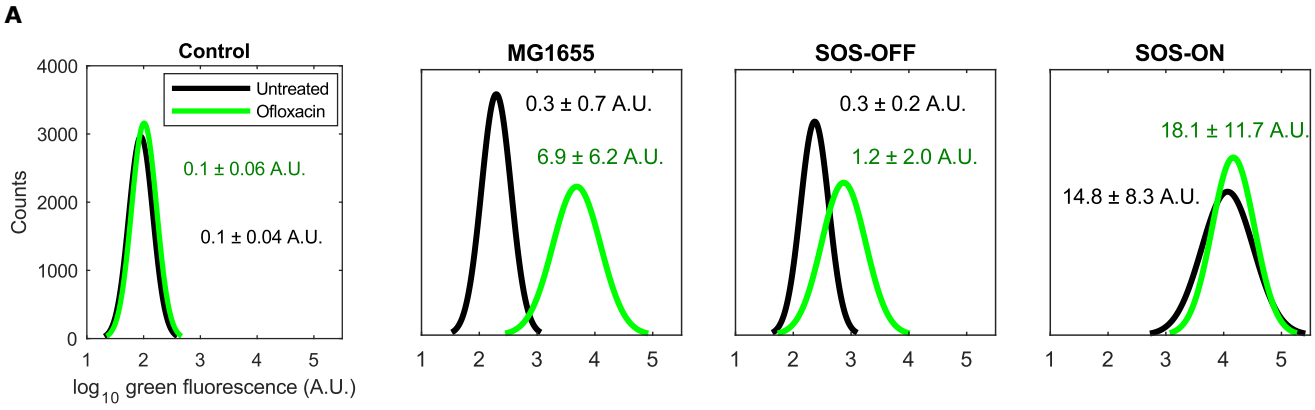
The PDF file includes:

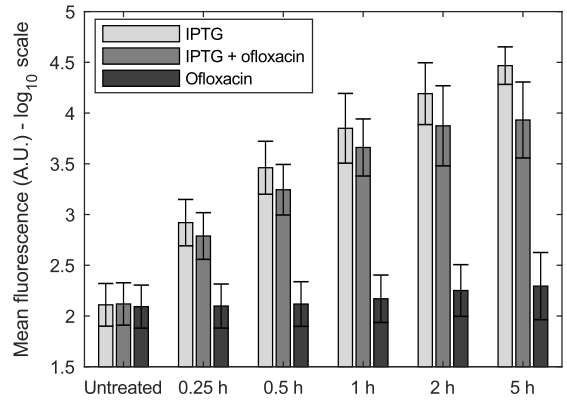
Other Supplementary Material for this manuscript includes the following:

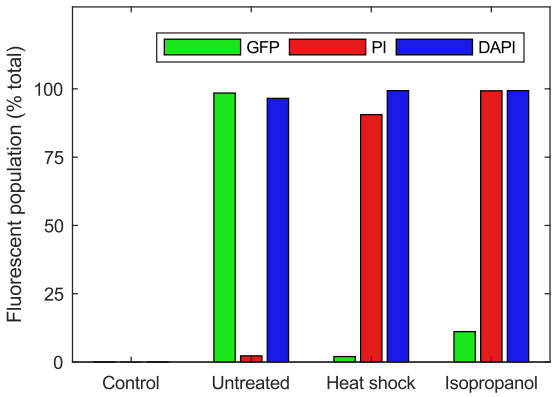
(available at advances.sciencemag.org/cgi/content/full/5/6/eaav9462/DC1)

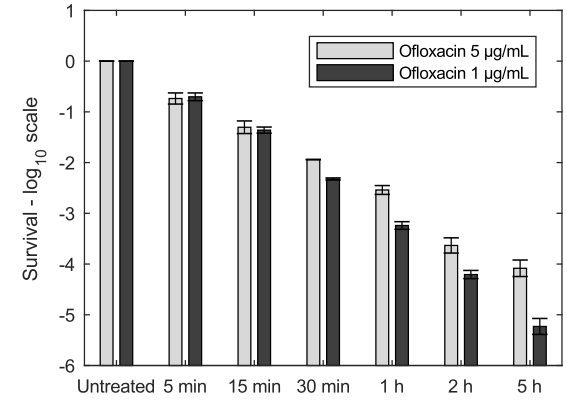
Table S1. Bacterial strains and plasmids used in this work.

Strains/plasmids	Genotype/description	Reference
MG1655	*E. coli* K-12 F^-, □-, *ilvG*, *rfb-50*, *rph1*	(*56*)
MG1655 *ΔsulA*	MG1655 *ΔsulA*	This work
MG1655 *lexA3*	MG1655 *lexA3 malB::*Tn9	This work
MG1655 *lexA51 ΔsulA*	MG1655 *lexA51 malB::*Tn9 *ΔsulA*	This work
MG1655 *ΔtisAB*	MG1655 *ΔtisAB*	This work
MG1655 *hupA::gfp*	MG1655 *hupA::gfp*	(*65*)
pUA66 *psulA::gfp*	pUA66 derivative containing the *gfpmut2* gene under control of the *sulA* promoter	Our lab
DJ624	MG1655 *lacX74 mal::lacI*^{q}	D. Jin (NCI, NIH, USA)
fluorescence-based two color system	pET-GFP plasmid contains the *gfp* gene under the control of an IPTG-inducible promoter pC17-Crimson plasmid contains E2-Crimson gene under the control of a canonical constitutive promoter	(*28*)
pKD4	Template plasmid for the amplification of the kanamycin cassette	(*59*)
pKD46	Thermosensitive plasmid encoding for the λ Red recombinase system	(*59*)
pCP20	Thermosensitive helper plasmid encoding the FLP recombinase.	(*59*)


A
Control
MG1655
SOS-OFF
SOS-ON
Untreated
Ofloxacin
0.1 ± 0.06 A.U.
0.1 ± 0.04 A.U.
0.3 ± 0.7 A.U.
6.9 ± 6.2 A.U.
0.3 ± 0.2 A.U.
1.2 ± 2.0 A.U.
18.1 ± 11.7 A.U.
14.8 ± 8.3 A.U.
Counts
log10 green fluorescence (A.U.)


B
IPTG
IPTG + ofloxacin
Ofloxacin
Mean fluorescence (A.U.) - log10 scale
Untreated
0.25 h
0.5 h
1 h
2 h
5 h


C
GFP
PI
DAPI
Fluorescent population (% total)
Control
Untreated
Heat shock
Isopropanol


D
Ofloxacin 5 µg/mL
Ofloxacin 1 µg/mL
Survival - log10 scale
Untreated
5 min
15 min
30 min
1 h
2 h
5 h

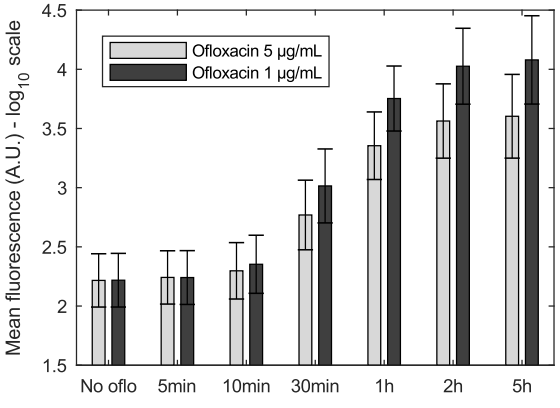
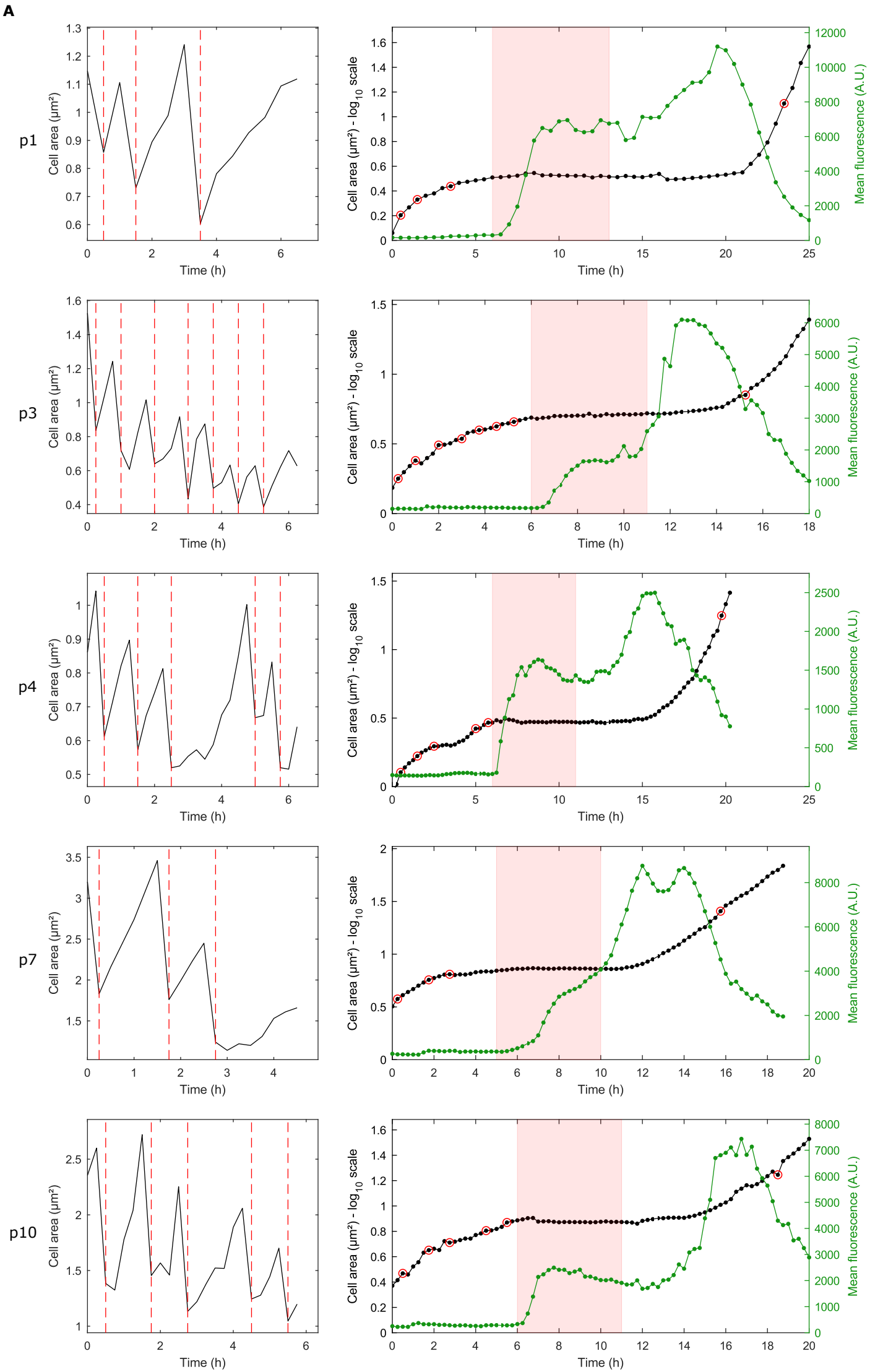
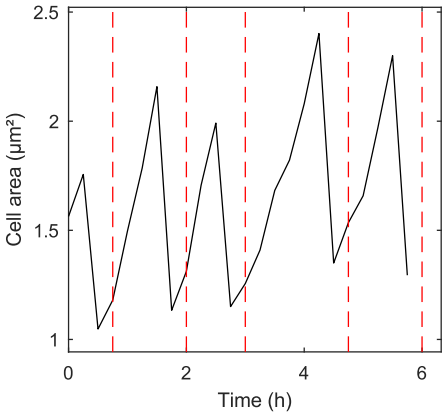
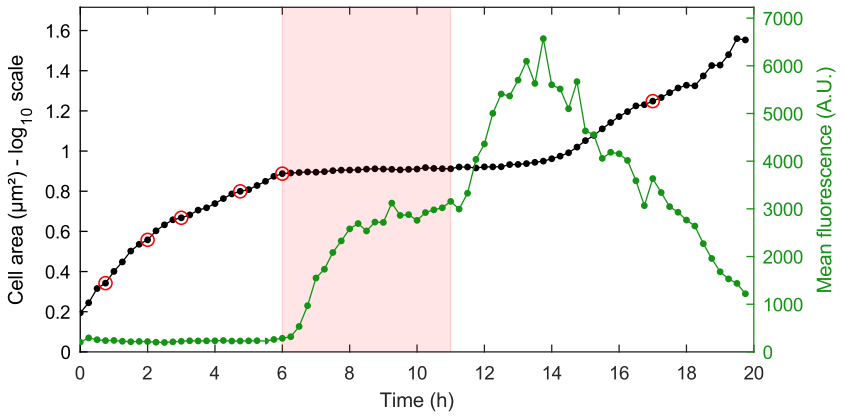

E
Ofloxacin 5 µg/mL
Ofloxacin 1 µg/mL
Mean fluorescence (A.U.) - log10 scale
No oflo
5min
10min
30min
1h
2h
5h

Fig. S1. The level of induction of the SOS reporter is likely to reflect the extent of DNA damage. (A) Heterogeneous induction of SOS before and after 5 h of ofloxacin treatment in the wild-type strain, SOS OFF and SOS ON mutants. Plots of green fluorescence of the MG1655 strain containing the promoter-less plasmid and of the MG1655 strain and isogenic LexA3 and LexA51 ΔSulA mutants containing the *psulA::gfp* reporter plasmid. Cultures were grown to mid-exponential phase (OD 0.3) in MOPS-based medium supplemented with 0.4% glucose and then challenged with ofloxacin (5 µg/ml final concentration) for 5h. Fluorescence was measured before (gray histograms) and after (green histograms) ofloxacin treatment. 225,000 events were acquired out of 3 independent experiments for every strain and condition. (B) Cells still undergo transcription and translation of *gfp* during the first few hours of ofloxacin treatment. Fluorescence of DJ624 cells carrying the Crimson and GFP reporter plasmids was measured as a function of time as indicated. IPTG (1 mM) and/or ofloxacin (5 µg/ml final concentration) were added at time 0 and both green and red fluorescence were monitored. Values are mean values and error bars are standard deviations measured on a sample of 75,000 cells. (C) The bulk of the population does not stain as 'DEAD' after 5h of ofloxacin treatment. MG1655 cells carrying the *psulA::gfp* reporter plasmid were treated with ofloxacin (5 µg/ml final concentration) and samples were withdrawn before (indicated as control) and after 5 h of ofloxacin treatment, treated for 1h (room T° indicated as native, 72°C indicated as heat shock or isopropyl alcohol 70% at room T° indicated as isopropanol on the graph), stained with PI (10 µg/mL) and DAPI (10 µg/mL) for 20 minutes at room T° and analyzed by flow cytometry. Green, red and blue bars represent the fraction of highly fluorescent cells for GFP, PI and DAPI respectively, as described in the materials and methods section. Analyses were performed on 75,000 events.
(D) Loss of viability upon ofloxacin exposure. MG1655 cells carrying the *psulA::gfp* reporter plasmid were treated with ofloxacin (5 µg/ml final concentration). Cultures were sampled at indicated time-points and plated on LB-agar. CFU/ml were measured and normalized by the CFU/ml at time 0. Values are means of 3 independent replicates and error bars are standard deviations. (E) Induction of the SOS response is anti-correlated with cell survival during ofloxacin treatment. Samples collected from (D) were further analyzed by flow cytometry in order to monitor the induction of the SOS response. Values are mean values and error bars are standard deviations measured on a sample of 75,000 cells.


A
p1
p3
p4
p7
p10
Cell area (µm²)
Time (h)
Cell area (µm²) - log10 scale
Mean fluorescence (A.U.)

p14





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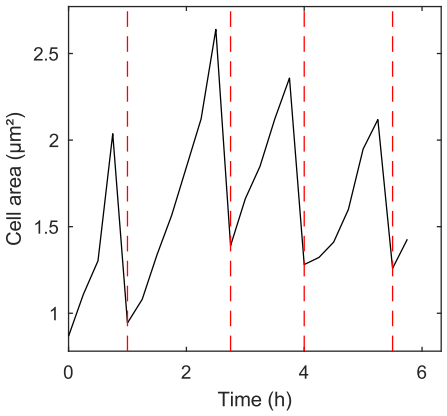

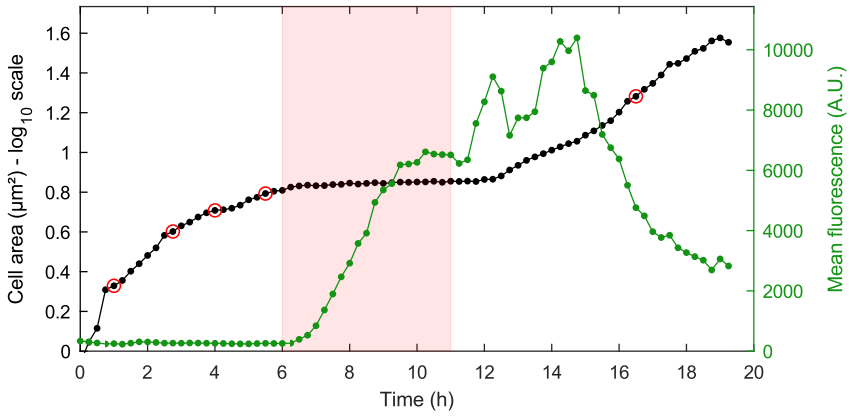

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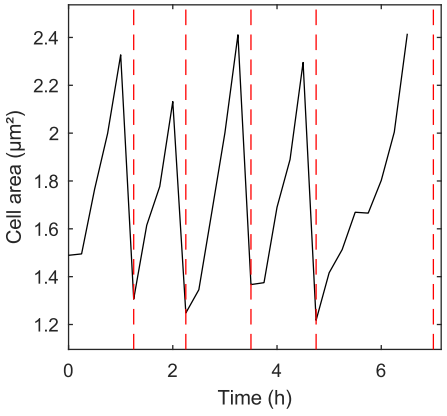

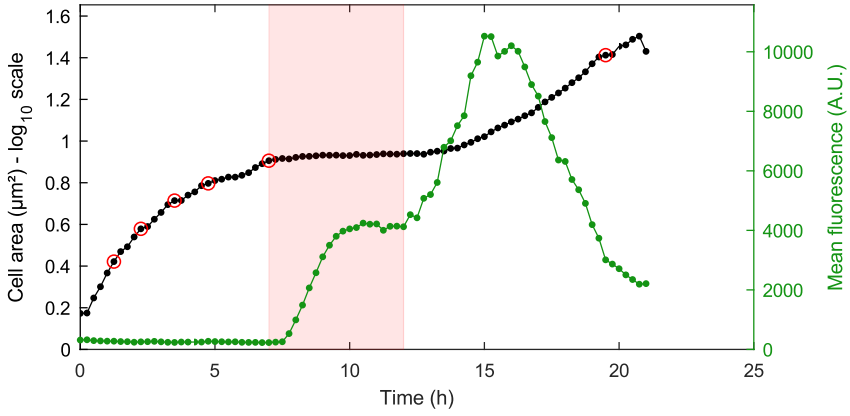

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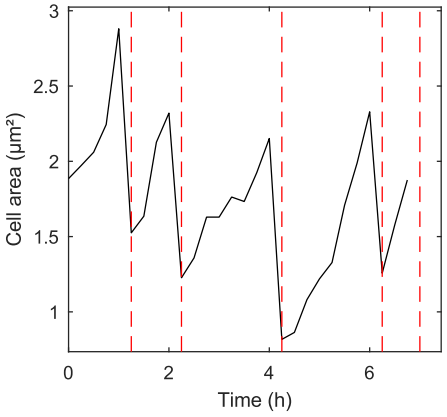

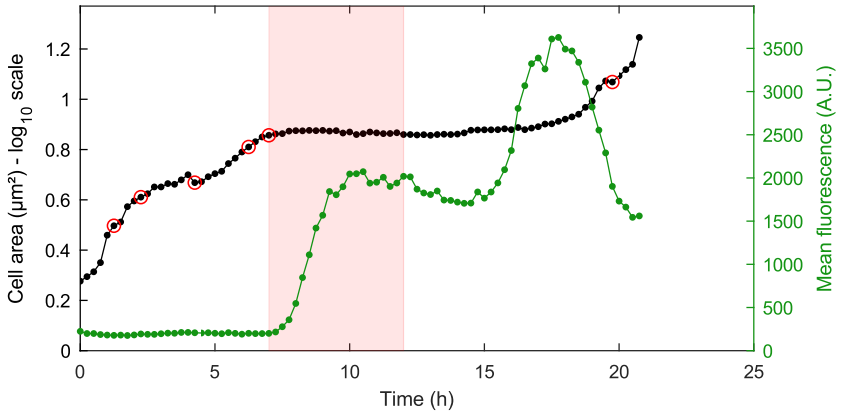

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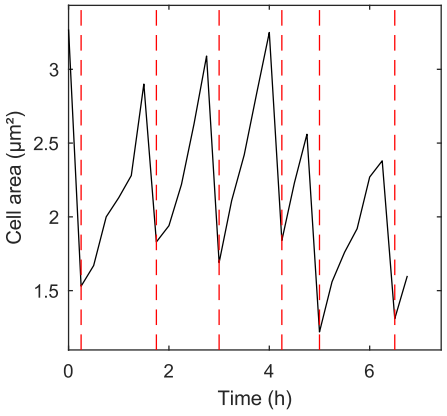
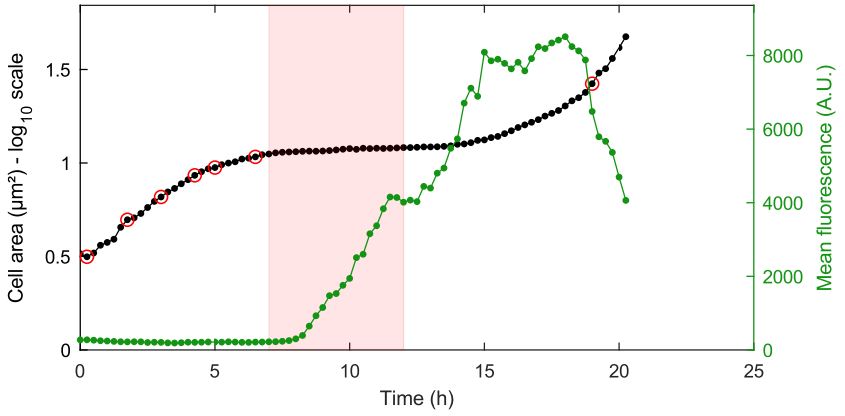
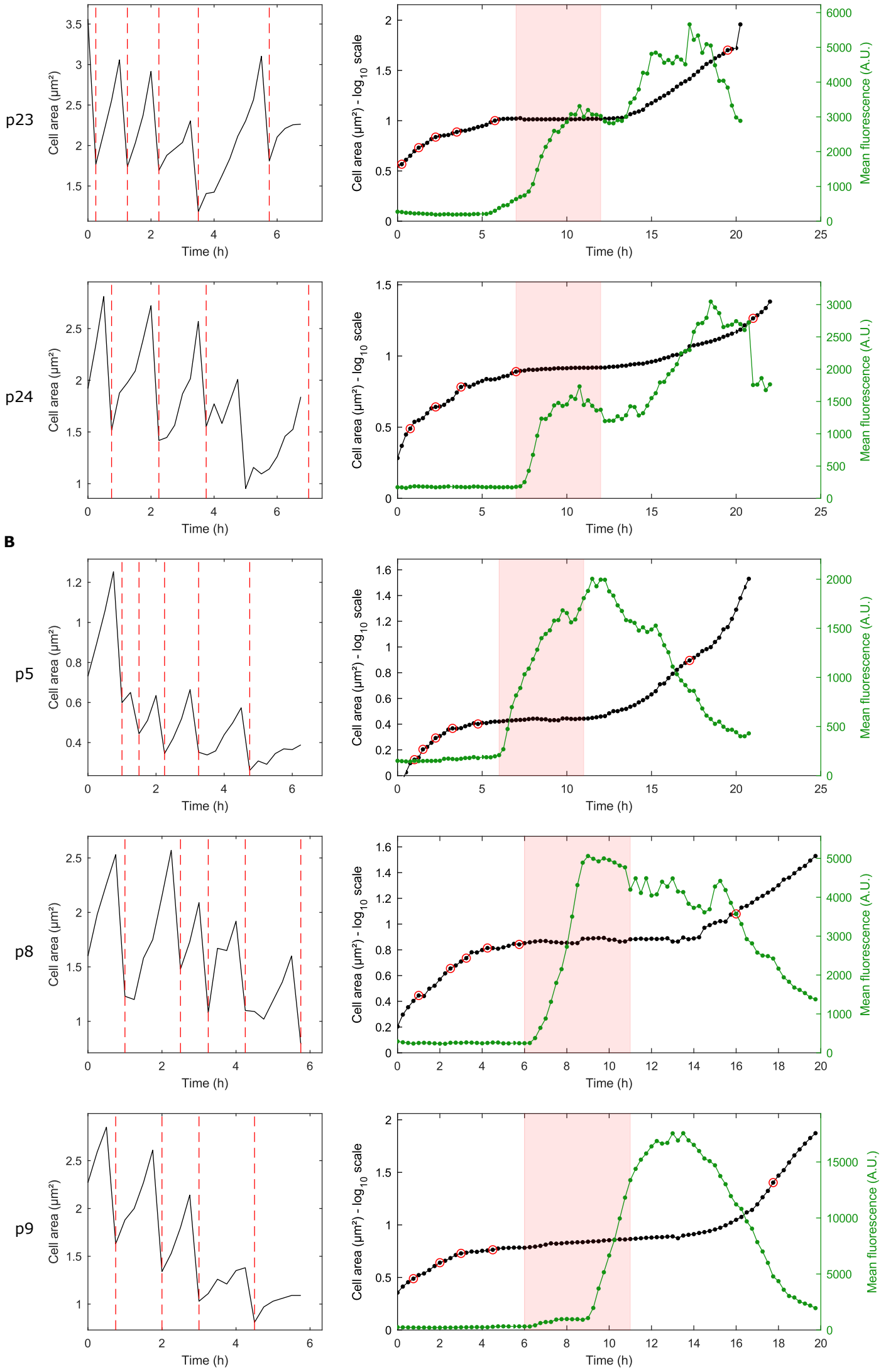
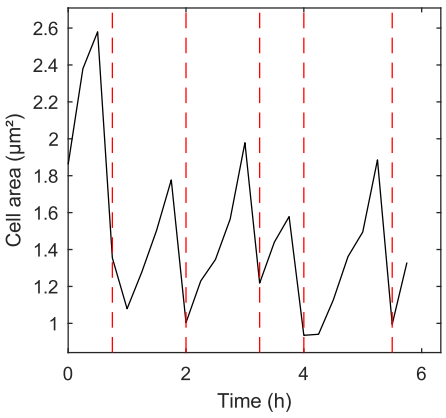
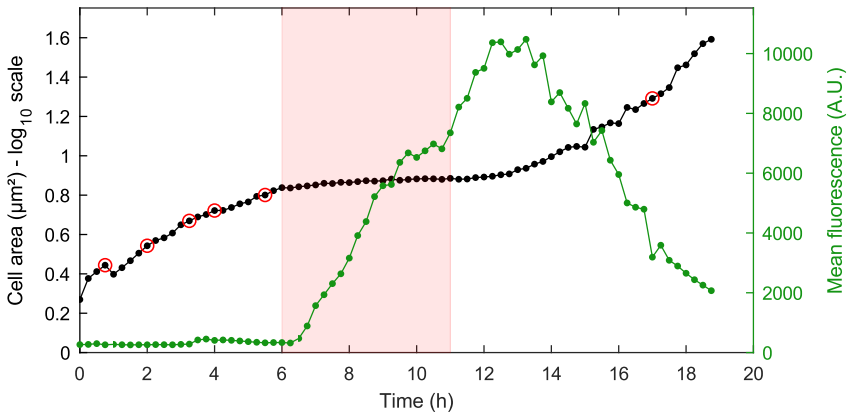





p23
p24
B
p5
p8
p9
Cell area (µm²)
Time (h)
Cell area (µm²) - log10 scale
Mean fluorescence (A.U.)

p11





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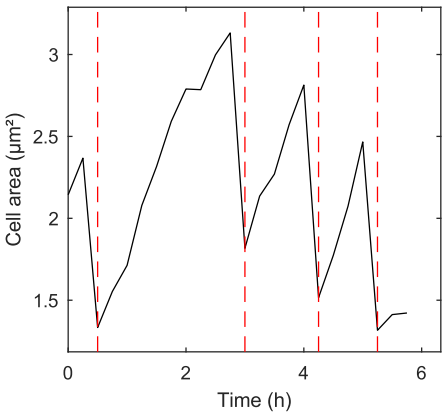

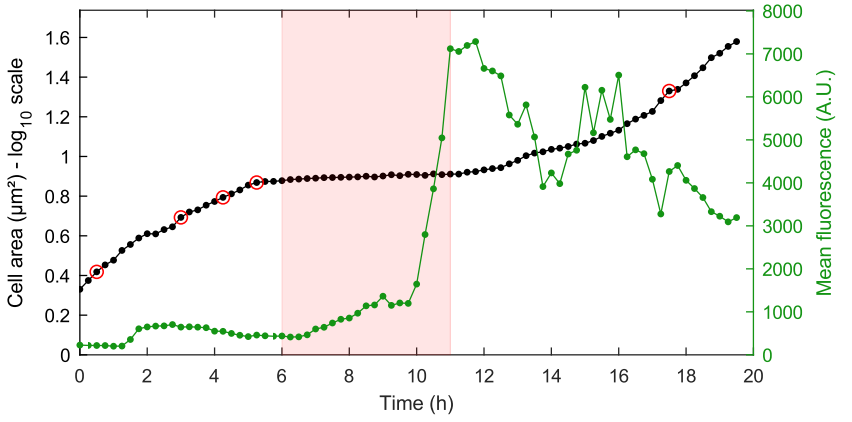

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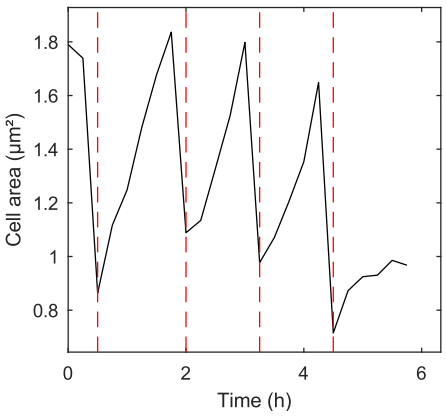

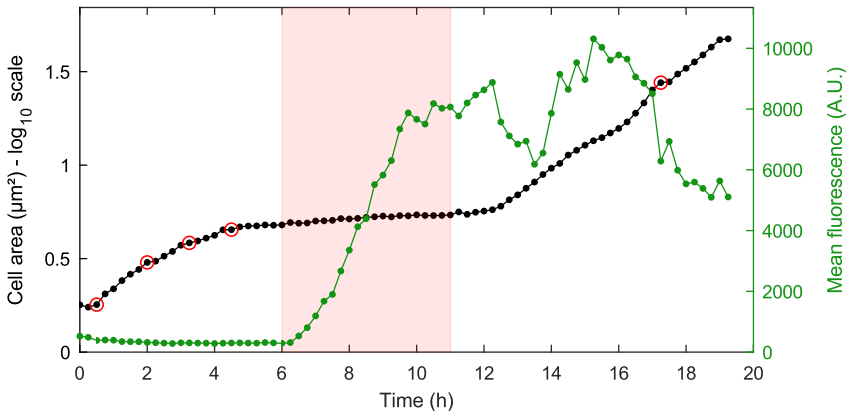

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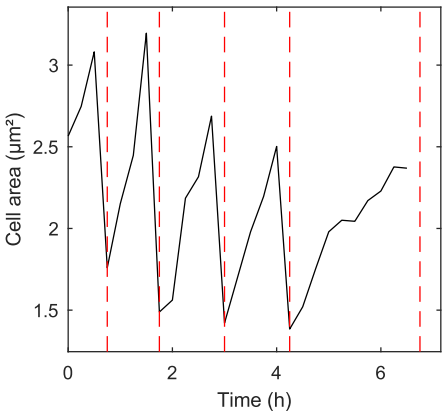

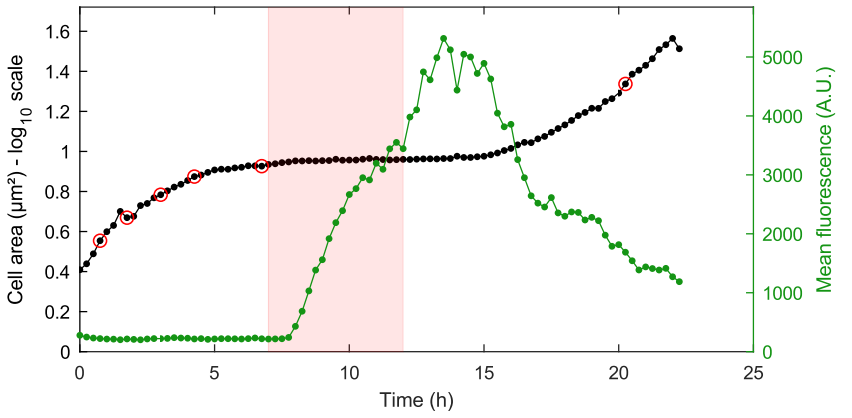

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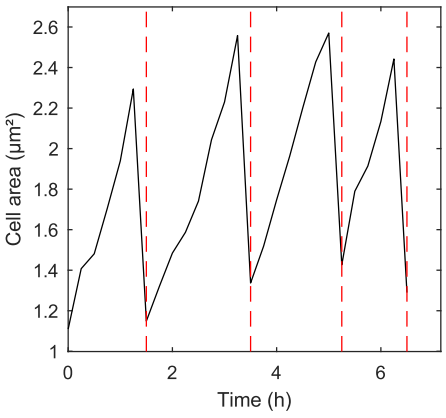
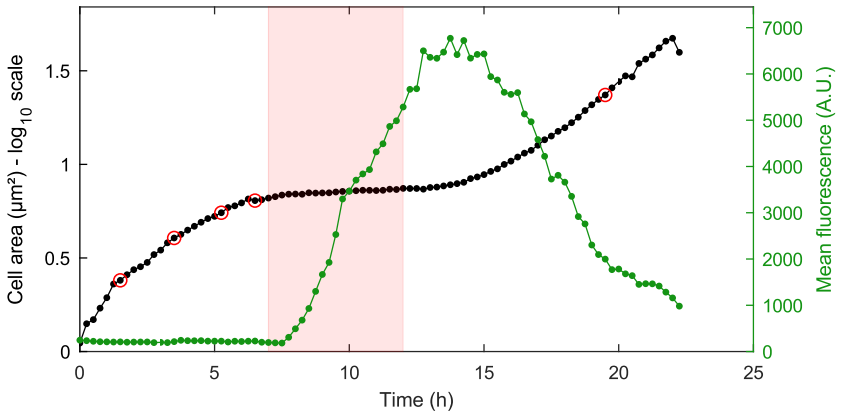
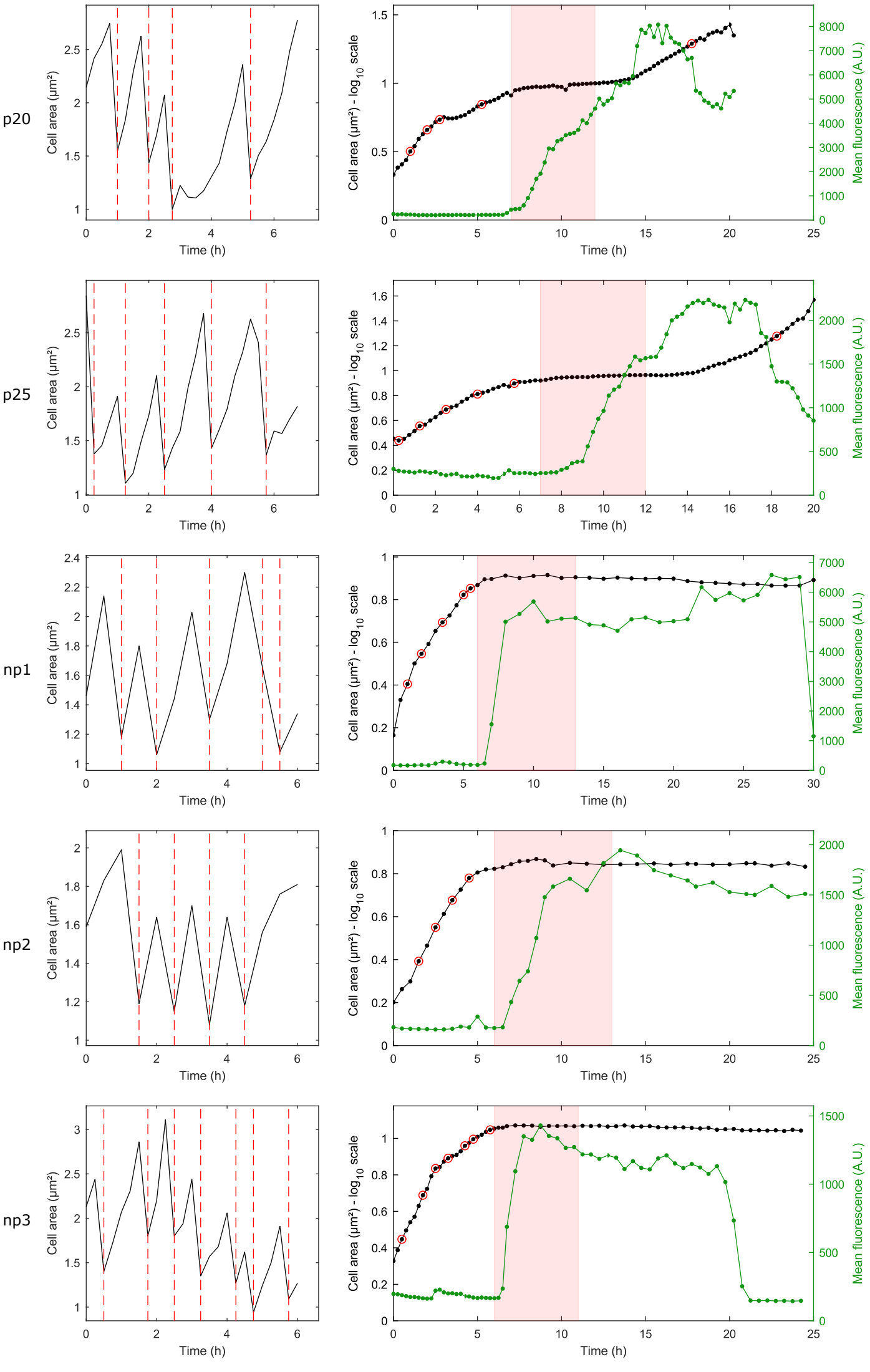
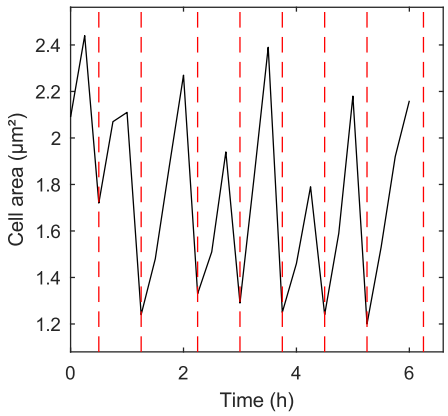
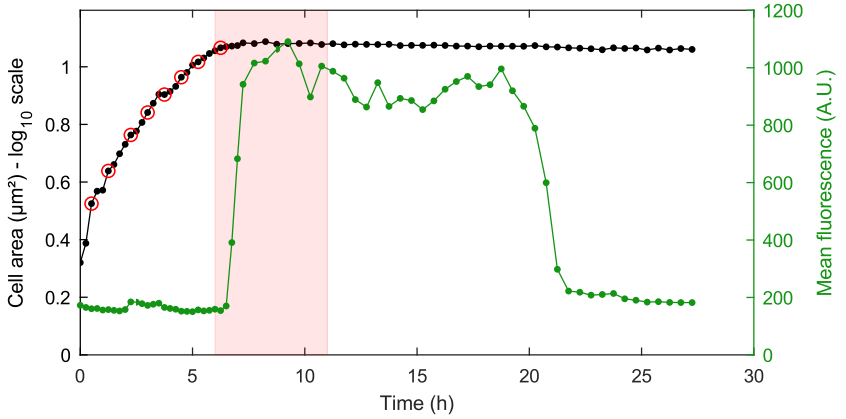





p20
p25
c
np1
np2
np3
Cell area (µm²)
Time (h)
Cell area (µm²) - log10 scale
Mean fluorescence (A.U.)

np4





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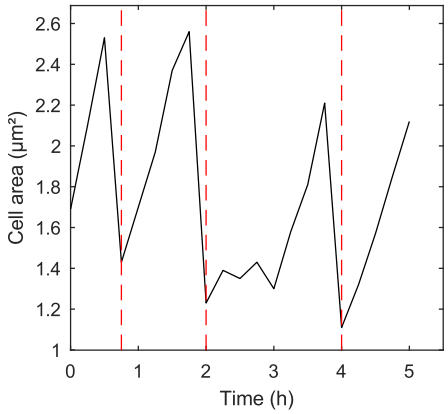

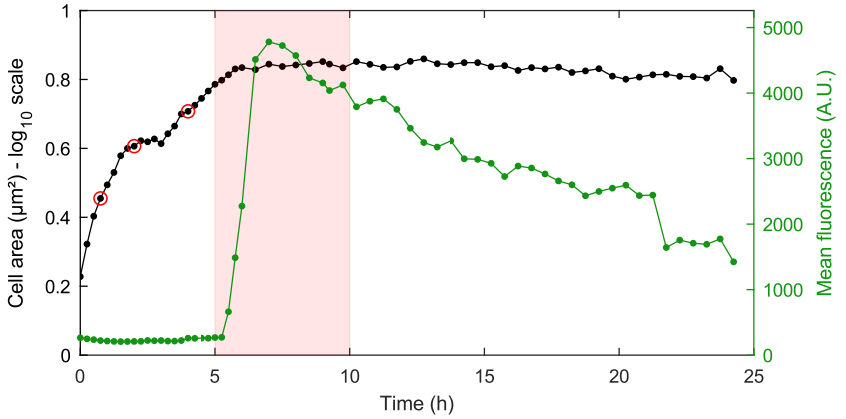

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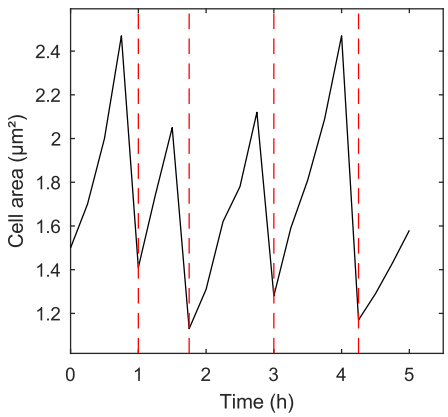

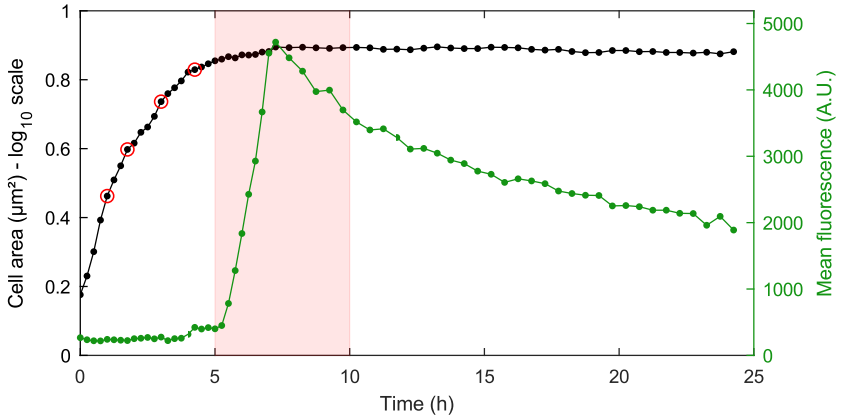

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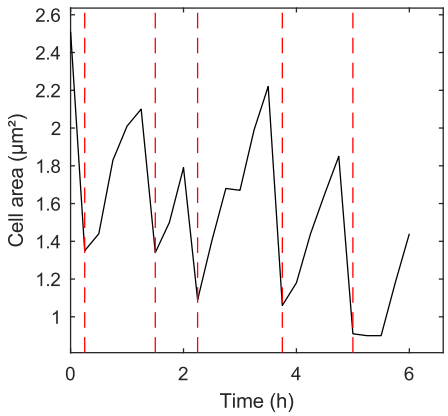

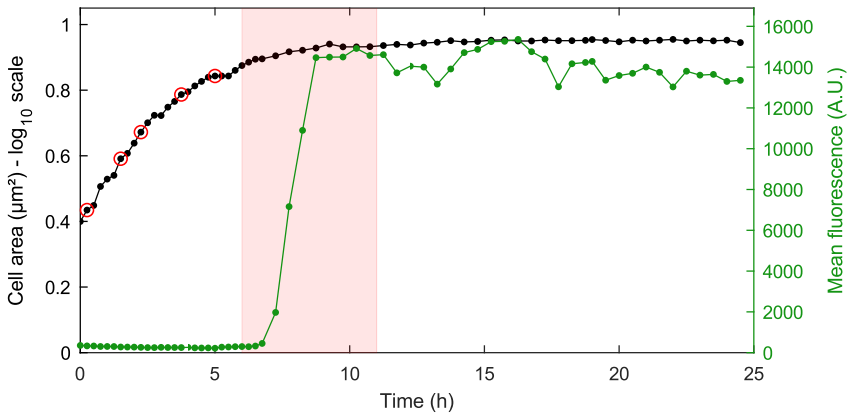

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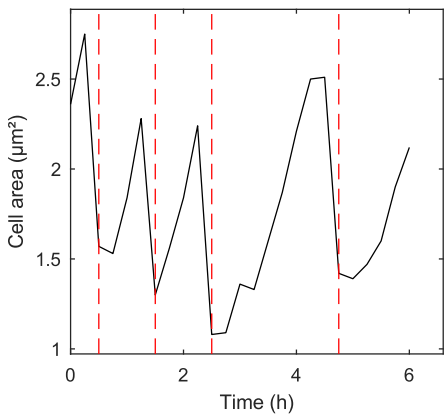

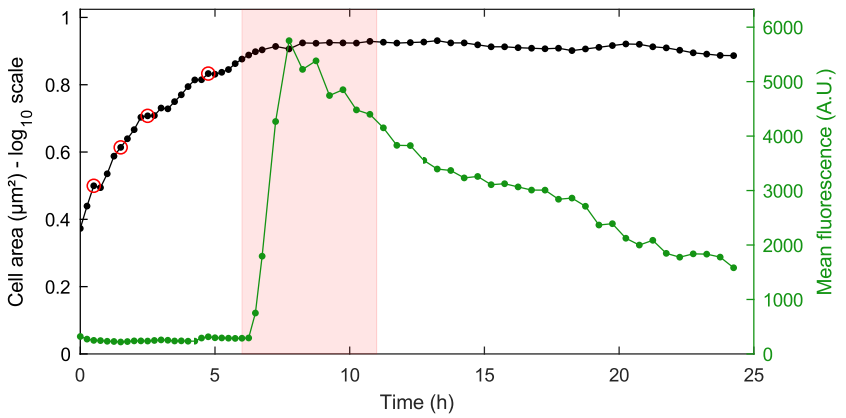

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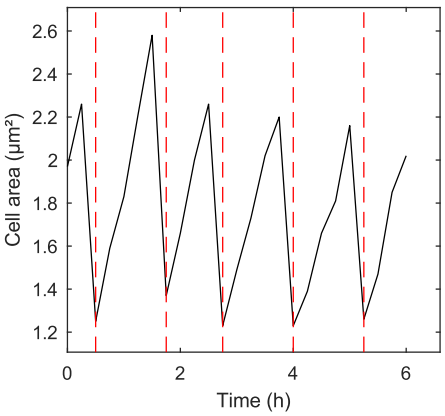

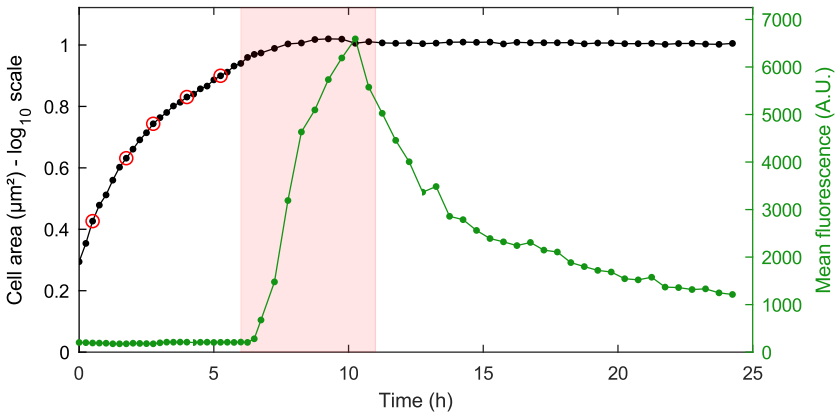

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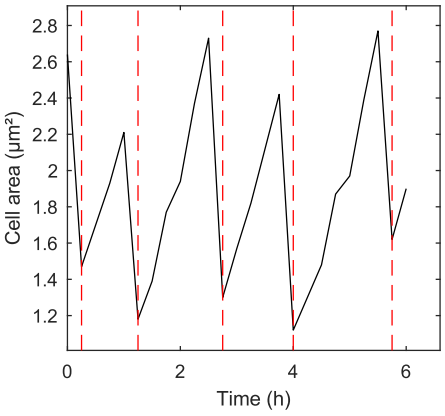

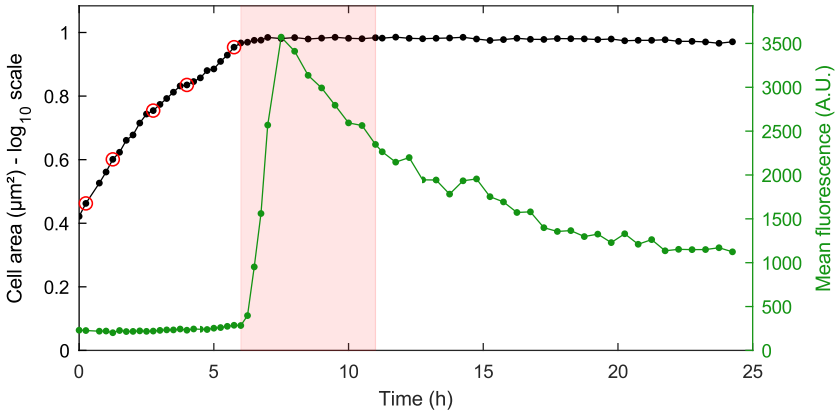

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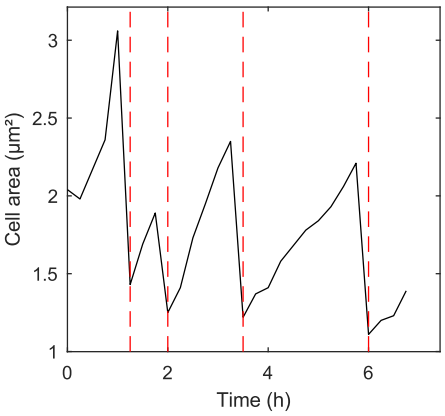

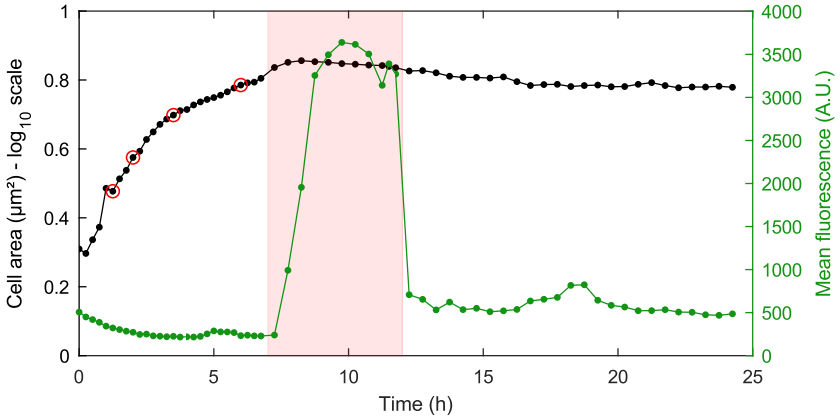

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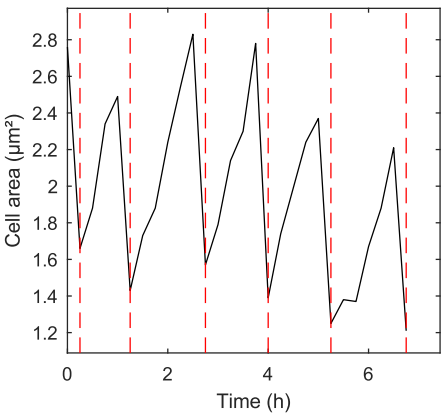

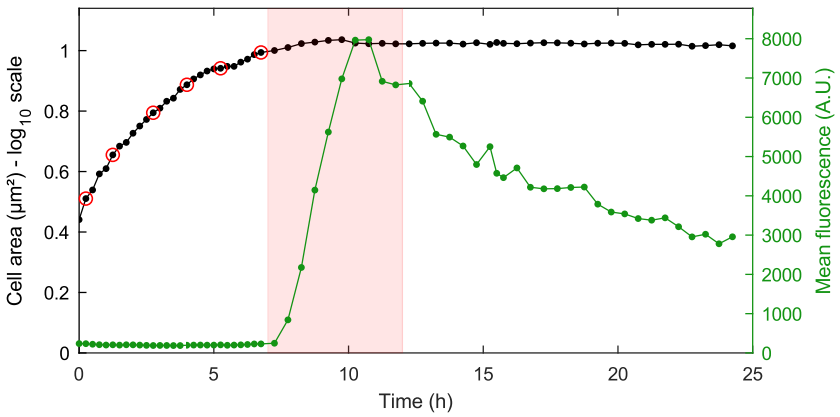

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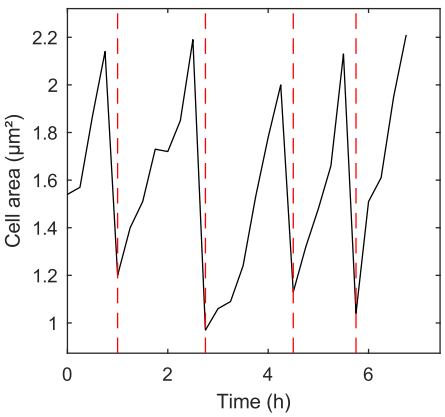

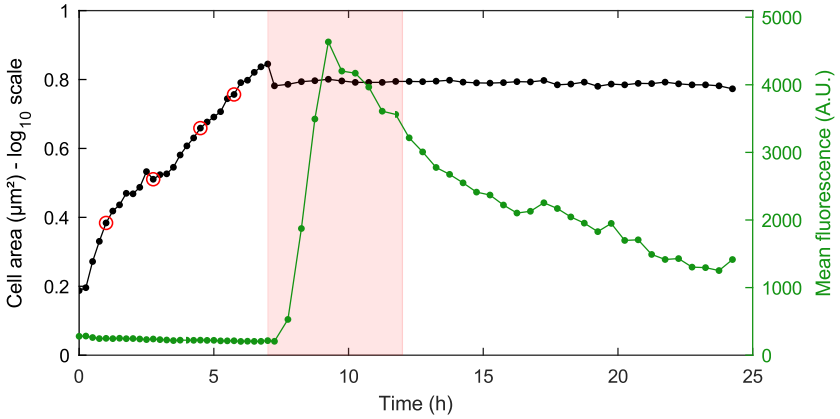

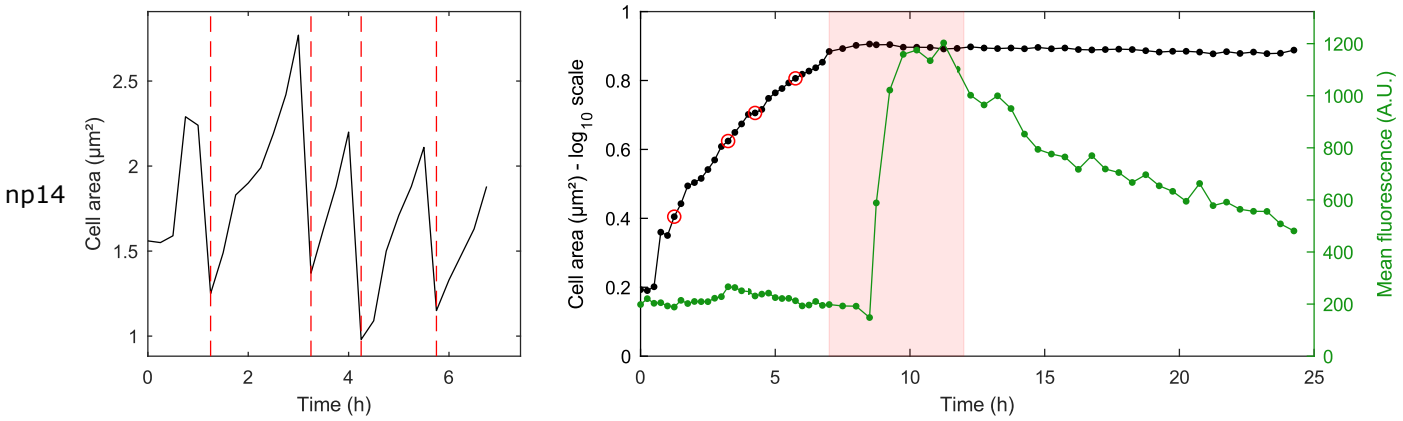

Fig. S2. Growth and fluorescence profiles of 23 persister and 14 nonpersister cells before, during, and after ofloxacin treatment. Single-cell area (saw-tooth growth profile), cumulated single-cell area (linear growth profile) and mean fluorescence (fluorescence par µm²) are displayed for each of the 23 persister cells (A – B) and each of the 14 non-persister cells (C) as a function of time. In the saw-tooth growth profiles, dotted lines indicate cell division events during the growth phase. Red circles indicate cell division events before addition of ofloxacin (5 µg/ml) and the first cell division event at recovery. Red shaded area indicates the ofloxacin treatment duration. (A) Persister cells in which the SOS response plateau during the ofloxacin treatment. (B) Persister cells in which the SOS response gradually increase during the ofloxacin treatment.

Movie S1. Single-cell imaging of 10 representative ofloxacin persister cells in an active growth state before the antibiotic treatment.
Movie S2. Spontaneous induction of the SOS response does not lead to persistence.
Movie S3. Single-cell imaging and monitoring of a slow-growing ofloxacin persister cell.
Movie S4. Single-cell imaging and monitoring of a fast-growing ofloxacin persister cell.
Movie S5. Persister cells elongation does not rely on the SOS response.
Movie S6. DNA localization and dynamics in ofloxacin persister cells.