Supplemental Materials

Molecular Biology of the Cell

Raghunathan *et al*.

Supplementary result

Relation between birth length, length added and length removed during division in damage-induced filaments

In general, for an adder system (Si et al., 2019; Wallden et al., 2016; Campos et al., 2014), a very specific relation must hold between the length growth law and the division time's dependence on birth length. If one knows how the length of a cell grows with time, then one can derive this relation. Here, we do this for the particular case where cell lengths grow exponentially with a fixed rate.

Let $l(t)$ be the length of a cell at time t, and let $t_{div}(l_{birth})$ be the time of next division of a cell that has length l_{birth} when it is created. Here, we assume that t_{div} depends *only* on the birth length of a cell (or rather that all other parameters that may affect t_{div}, such as medium composition, are kept fixed). Then, for an adder system it must be that

$$l(t_{div}(l_{birth}))-l_{birth}=\text{constant}. \qquad (1)$$

In fact, this constant must be equal to the length of the wild type cell, l_{WT}, so that when the birth length is l_{WT}, the length added before the cell divides is exactly l_{WT}.

If cell lengths grow exponentially in time at a fixed rate γ, i.e.,

$$l(t_2)=l(t_1)\exp(\gamma\,(t_2-t_1)), \qquad (2)$$

then combining equations (1) and (2) gives a relation that must be satisfied for an adder with exponentially growing cells.

$$t_{div}(l_{birth})= \ln(1+l_{WT}/l_{birth})/\,\gamma. \qquad (3)$$

Matlab code for producing the plots in Figure S2

```
% matrix d(:,4) is read from each xlsx file
% d(:,1) = time (min)
% d(:,2) = length (um)
% d(:,3) = length of one of the two cells created upon division (only on rows right after
%where division indicator=1)
%       (other cell length can be computed by subtracting this from previous length in
%column 2; note - shorter of the two is by definition the daughter cell, the longer is the
%mother cell)
% d(:,4) = division indicator (0 or 1)

clear all;
f=dir('M*'); % change to 'F*' for ceph cells, no change for MMC cells but make sure xlsx
```

```
%files for wild-type cells are not in the same folder
n=1;
j=1;

for fi=1:length(f)

clear d;d=xlsread(f(fi).name);

tdivnum=find(d(:,4)==1);
birthtime=tdivnum(1)+1;
for i=2:length(tdivnum) % start looking at data from first division onwards
if (tdivnum(i)>birthtime) % no growth curve if divisions at successive time points
  % calc growth rate from fit to exponential
x=d(birthtime:tdivnum(i),1);
y=log(d(birthtime:tdivnum(i),2)); SStotal = (length(y)-1) * var(y);
rsq(n) = 1 - SSresid/SStotal;
numdat(n)=length(x);
n=n+1;
 end

 % calc birth length and div time and length added/removed
 q(j,1)=d(birthtime-1,2)-d(birthtime,3);
 q(j,2)=d(tdivnum(i),1)-d(birthtime-1,1);
 q(j,3)=d(tdivnum(i),2)-d(birthtime-1,2)+d(birthtime,3);
 q(j,4)=min(d(tdivnum(i)+1,3),d(tdivnum(i),2)-d(tdivnum(i)+1,3));
 q(j,5)=max(d(tdivnum(i)+1,3),d(tdivnum(i),2)-d(tdivnum(i)+1,3));
 j=j+1;

 % update birthtime
 birthtime=tdivnum(i)+1;
end

% now n-1=number of growth curves=length(p), p(:,1)=fitted slope of log growth curve,
%p(:,2)=fitted intercept of log growth curve
% rsq(:)=R-squared of the fit, numdat(:)=how many data points where in the
%corresponding growth curve
% and j-1=length(q)=total number of divisions (can be more than number of growth
%curves because sometimes a division happens immediately after another
% q(:,1)=length at birth, q(:,2)=division time
% q(:,3)=length added, q(:,4)=length removed=daughter length just after division,
%q(:,5)=mother cell length just after division
% q(:,1:3) calculations correct for the fact that there is time delay (typically 2min)
%between data points during which time cells lengthen
% the formula to get q(:,3) above gives negative values for a few cases in MMC cells -
```

```
%this is because sometimes the filament divides in two places simultaneously producing
%two daughter cells
end
figure;
fplot(@(x) x,[min(q(:,4)) max(q(:,4))],'r','LineWidth',2);
hold on;plot(q(:,5),q(:,4),'k.','MarkerSize',13);
xlabel('Mother cell length (\mum)');ylabel('Daughter cell length (\mum)');
title('WT cells');

figure(2);
subplot(2,3,1); fplot(@(x) median(p(:,1)),[0 length(p)],'r','LineWidth',2);
hold on;scatter([1:length(p)],p(:,1),numdat,1-[rsq' rsq' rsq'],'filled');
set(gca,'YLim',[0 0.06]);
ylabel('Fitted growth rates (1/min)');xlabel('Cell index');title('WT cells');

figure;
plot(q(:,2),q(:,3),'ro','LineWidth',1);hold on;plot(q(:,2),q(:,4),'b.','MarkerSize',13);
set(gca,'YLim',[0 max(max(q(:,3)),max(q(:,4)))]); % making the lower limit of the Y-axis
zero hides the divisions where length added comes out to be negative (see above)
%legend('Length added','Length removed');
xlabel('Division time (min)');ylabel({'Length added (red) and';'removed (blue) in
\mum'});title('WT cells');

figure(2);
subplot(2,3,4);plot(q(:,1),q(:,3),'ro','LineWidth',1);hold
on;plot(q(:,1),q(:,4),'b.','MarkerSize',13);
set(gca,'YLim',[0 max(max(q(:,3)),max(q(:,4)))]);
%legend('Length added','Length removed');
xlabel('Birth length (\mum)');ylabel({'Length added (red) and';'removed (blue) in
\mum'});title('WT cells');

figure;
fplot(@(x) log(1+median(q(:,4))./x)/median(p(:,1)),[0 max(q(:,1))],'r','LineWidth',2);hold
on;
plot(q(:,1),q(:,2),'k.','MarkerSize',13);
set(gca,'YLim',[0 max(q(:,2))]);
xlabel('Birth length (\mum)');ylabel('Division time (min)');title('WT cells');

```

Supplementary figure legends

Figure S1: Asymmetric cell division in DNA-damage induced filaments A. Cell length of L_D and short daughter S_D generated from a DNA damage-induced filament during recovery in M9-Cas. Each grey dot represents a single division event. The red line plots the expected values if all cells were dividing at their mid-point (n = 402). B. Distribution of S_D cell lengths for MMC or cephalexin treated filaments. Wild type cell length distribution (asynchronous) is also plotted (n = 1110 (wild type), 531 (MMC), 201 (cephalexin)). C. As (A) for cephalexin-treated filaments. (n = 201) D. Distribution of relative position of division for filament lengths between 12-40 µm after 30, 60 or 90 min of MMC induction. (n = 142 (30 min), 518 (60 min), 95 (90 min) E-F. Location of division as a function of cell length during recovery from 30 or 90 min DNA damage treatment respectively. (n = 144 (30 min), 120 (90 min)). G. As (A) for 90 min of damage treatment (n = 149). H-I. As (E) for recovery in M9-Cas and cephalexin treatment respectively. (n = 402 and 201) J. Percentage of cells carrying ≤2 or >2 RecA-mCherry foci for L_D and S_D during recovery time-lapse from MMC is plotted. (n = 200). K. As a control for (J) percentage of cells carrying ≤2 or >2 RecA-mCherry foci binned by cell length after treatment with cephalexin for 1 hr is plotted. Cephalexin causes cellular elongation without DNA damage. An increase in length does not result in an increase in foci number, indicating that L_D retain damage foci, while S_D inherit damage-free chromosomes (n = 100) L. CFU/ ml is plotted for no damage control (grey), and DNA damage recovery after 30 (dashed and dotted), 60 (dashed) or 90 (dotted) min of damage exposure. OD_{600} of the culture is normalized to 0.05 while plating for each time point (n = 3 independent repeats, mean and std. dev is shown). M. Cell length distribution during DNA damage recovery time-course for 30 min, 60 min and 90 min durations of damage treatment respectively. Individual dots represent length of a single cell. Black line represents median (n = 30 min (1688, 1540, 1049, 969, 875, 974 , 979, 907, 603, 660, 1107, 1079, 1578, 1265), 60 min (1110, 1403, 778, 742, 914, 924, 1348, 1056, 1539, 1487, 930, 969, 1398, 1064), 90 min (866, 936, 386, 615, 289, 467, 571, 422, 790, 666, 514, 545, 1007, 551)).

Figure S2: Division dynamics of damage-induced filaments. A-B. Length vs time curves of the cells are fitted to an exponential function, and the resulting fitted growth rates are plotted as dots. The colour of the dot represents the R^2 value of the fit (black=1; white=0; closer to 1 is a better fit). The size of the dot represents the number of time points in each length vs time curve (the more points, the more reliable the fit). The red line marks the median fitted growth rate. (n = 211 and 492, for MMC-treated and wild type cells respectively). X-axis reflects number of divisions. C. Time between divisions as a function of cell length at birth for wild type cells. Red line shows the relation that would be necessary for the system to be an adder (equation (3) in supplementary results), given that cells are growing exponentially with the rates given in Fig. S2A-B (n = 107). D. Oufti is used to generate segmented profiles of phase contrast images of cells. These are then used to identify potential sites of constriction and detect division events (automated). Phase profile for cephalexin-treated cell before and after division is plotted. Constriction is marked with an * and divisions are identified by Oufti.

Figure S3: Role of Min system in division positioning A. (top) FtsZ-GFP localizations across increasing cell length represented as a kymograph (n = 150). (bottom) Number of FtsZ-GFP localizations against cell length for damage-induced filaments (n = 150). B. (left) As an illustrative example, localization of MinD-GFP in a single MMC or cephalexin treated filament is shown. (right) Number of Min localizations as a function of increasing cell length (n = MMC (266), cephalexin (467)). C. Heat map of transcript levels (from RNA-seq) of genes involved in cell division during a damage recovery time-course. As a control, genes induced under the SOS response are also highlighted (bold). Log_2-fold change normalized to control without damage is plotted. D. Number of constrictions per cell during DNA damage recovery for wild type, *slmA* and *sulA* cells and for cells during cephalexin recovery (n = 140 (wild type), 81 (Δ*slmA*), 69 (Δ*sulA*), 47 (cephalexin)). E. Relative position of division plotted as a function of filament length at division for Δ*minCDE* (n = 186). F. Representative time-lapse montage of division in Δ*minCDE* cells during damage recovery. Asterix marks site of division.

Figure S4: Impact of chromosome and terminus segregation on division regulation A. Representative time-lapse montage of anucleate division in cells during recovery. Grey – phase, red – HupA-mCherry (chromosome); scale bar - 5 μm; time in min. Fluorescence intensity traces for the cell in montage is provided below. Division sites are marked with '*'. B. Representative time-lapse montage of FtsZ-GFP localization during damage recovery time-lapse. Grey – phase, red – HupA-mCherry (chromosome), green – FtsZ-GFP. C. Position of least intensity of HupA fluorescence (gaps between chromosomes) plotted against the position of the FtsZ-ring at the time of cell division. Positions are relative to cell length (n = 145). D. Segmentation profile of phase image is plotted in black for a single cell. Time-lapse frames before a constriction (a.) and up to one frame before a division (c.) are shown. Cell length (0) indicates the location at which a constriction is identified and 2 μm ± this location is plotted. Along with phase profile, fluorescence profile of chromosome (red) and *terminus* (green) is shown. Single terminus focus splitting into two is highlighted with an asterix. E. Representative time-lapse montage of *terminus* localization during damage recovery time-lapse. White asterisks highlight *terminus* location prior to division. red – HupA-mCherry (chromosome), green – ParBpMT1-GFP, *parS*pMT1 (at *ter)*. F. CFU/ ml is plotted for no damage control (grey) and DNA damage recover for wild type (dotted) and Δ*matP* (dashed) cells. OD_{600} of the culture is normalized to 0.05 while plating for each time point (three independent repeats). G. CFU/ml is plotted without damage for wild type and Δ*matP*. No significant difference was observed (unpaired T-test; three independent repeats).

Figure S5: Asymmetric chromosome segregation and cell division in DNA damage-induced *Escherichia coli* filaments

Min oscillations dictate division site positioning. Chromosome segregation additionally influences the timing of division during nucleated cell divisions. Short daughter cells of wild type size tend to be devoid of damage and grow and divide as wild type. For details, see main text.

Table S1: Strains and plasmids used in this study

Name	Background	Description	Source/ construction	Experiment
JJC5789	MG1655	MG1655 *lac::P_{recA}-recA-mCherry (chl)*	(Vickridge et al., 2017)	Fig. S1J-K
SJ1737	MG1655	MG1655 low motile *hupA-mruby2 (chl)(FRT); P1 parS @ 33.7' (kan); pALA2705 (carb) with gfp-parB*	(Youngren et al., 2014)	Source for NAB214
NP127	MG1655	MG1655 *zapA-gfp hupA-mCherry (Chl)(FRT)*	(Buss et al., 2017)	Fig. 4E
NAB94(SR)	BW25113	BW25113 *ΔminCDE (kan)*	CGSC stock centre	Source for NAB332
SS6282	MG1655	MG1655 *hupA-mCherry(kan)(FRT); P_{sulA}-gfp*	(Marceau et al., 2011)	Source for NAB227
NAB95(SR)	MG1655	MG1655 *P_{ftsZ}-ftsZ-gfp (chl)*	(Jena et al., 2020)	Source for NAB98
RSB156	MG1655	MG1655 *ΔslmA (tet)*	CGSC stock centre	Source for NAB331
NAB96(SR)	MG1655	MG1655 *ΔsulA (kan)*	CGSC stock centre	Source for NAB329
NAB98	MG1655	MG1655 *hupA-mCherry(kan)(FRT); P_{ftsZ}-ftsZ-gfp (chl)*	This study *hupA-mCherry was transduced into MG1655. Kanamycin cassette was flipped out using the FRT sites flanking the antibiotic (NAB227). Following this, P_{ftsZ}-ftsZ-gfp (chl) was transduced into this strain. Verified via microscopy*	Fig. 4E, G. S3. A and S4. B-C
NAB99(SR)	MG1655	MG1655 *ΔmatP (kan)*	*ΔmatP (kan) from CGSC stock centre in MG1655 background. Verified by PCR*	Fig. 4F and S4. F-G
NAB214	MG1655	MG1655 *hupA-mCherry(kan)(FRT); P1 parS @ 33.7' (kan); pALA2705 (amp) with gfp-parB*	This study *P1 parS @ 33.7' (kan) was transduced into MG1655 hupA-mCherry (FRT) (NAB227); pALA2705 (amp) was transformed into this. Verified by microscopy*	Fig. 4E and S4. D-E

NAB227	MG1655	MG1655 *hupA-mCherry*	This study *hupA-mCherry (kan) was transduced into MG1655. Kanamycin cassette was flipped out using the FRT sites flanking the antibiotic. Verified by microscopy*	Figures pertaining to wild type recovery and chromosome dynamics (HupA)
NAB325	MG1655	MG1655 pNIH299-3 P_{lac} *egfp-minD minE-mCherry (amp)*	This study *pNIH299-3 was transformed into MG1655. Verified by microscopy*	Fig. S3B
NAB329	MG1655	MG1655 *ΔsulA (kan);hupA-mCherry;* P_{ftsZ}*-ftsZ-gfp (chl)*	This study *ΔsulA was transduced into NAB98. Strain was verified by PCR*	Fig. 3C, S3D and 4F
NAB331	MG1655	MG1655 *ΔslmA (tet); hupA-mCherry;* P_{ftsZ}*-ftsZ-gfp (chl)*	This study *ΔslmA was transduced into NAB98. Verified by PCR*	Fig. 3B, S3D and 4F
NAB332	MG1655	MG1655 *ΔminCDE (kan) hupA-mCherry;* P_{ftsZ}*-ftsZ-gfp (chl)*	This study *ΔminCDE was transduced into NAB98. Verified by PCR*	Fig. 3D, S3E-F
NAB341	MG1655	MG1655 *hupA-GFP; pRHmcherryFtsN*	This study *pRHmcherryFtsN was transformed into NAB98. Verified by microscopy*	Fig. 4E
pALA2705	Plasmid in DH5α	P_{trc90}*-gfp-parB (amp)*	(Youngren et al., 2014)	Source for NAB214
pRHmCherryFtsN	Plasmid in DH5α	P_{rha}*-mCherry-ftsN (amp)*	(Söderström et al., 2018)	Source for NAB341
NIH299-3	Plasmid in DH5α	P_{lac} *egfp-minD minE-mCherry (amp)*	*Plasmid was made by Genscript. Briefly, EcoR1-mGFPmut3.1-XbaI and BamHI-mCherry-HindIII fragments were synthesized by Genscript. pYLS68 (Shih et al., 2002) carrying YFP-minD-minE-CFP was first digested with EcoR1-XbaI to excise YFP and mGFP was ligated into the same. The resultant vector was digested with BamHI-HindIII to excise CFP and mCherry fragment was ligated into the same.*	Source for NAB325

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Table S2: Oligonucleotides used in this study

Name	Sequence	Description
SR_Oligo_221	GCGAGGCTCTTTCCGAA	*sulA* upstream forward
SR_Oligo_223	ACCGCTTCAGACAAGCCTC	*sulA* downstream reverse
SR_Oligo_258	CGGTCATAGCGTGGGTGCCGCC	*slmA* upstream forward
SR_Oligo_259	ATGGCGGAACATCTGGCGGCGGTTAAGG	*slmA* downstream reverse
SR_Oligo_264	GTCTTCGGAACATCATCGCGCGCTGGC	*minCDE* upstream forward
SR_Oligo_265	TGGGAACAGCCTTAAGGTGTAAAGGGGGAGG	*minCDE* downstream reverse
SR_Oligo_291	CCTGGCATGGGCGTTAAAGC	*matP* upstream forward
SR_Oligo_292	ACGTTGTAACTCAGCCGCAGG	*matP* downstream reverse

Supplementary references

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Figure S1

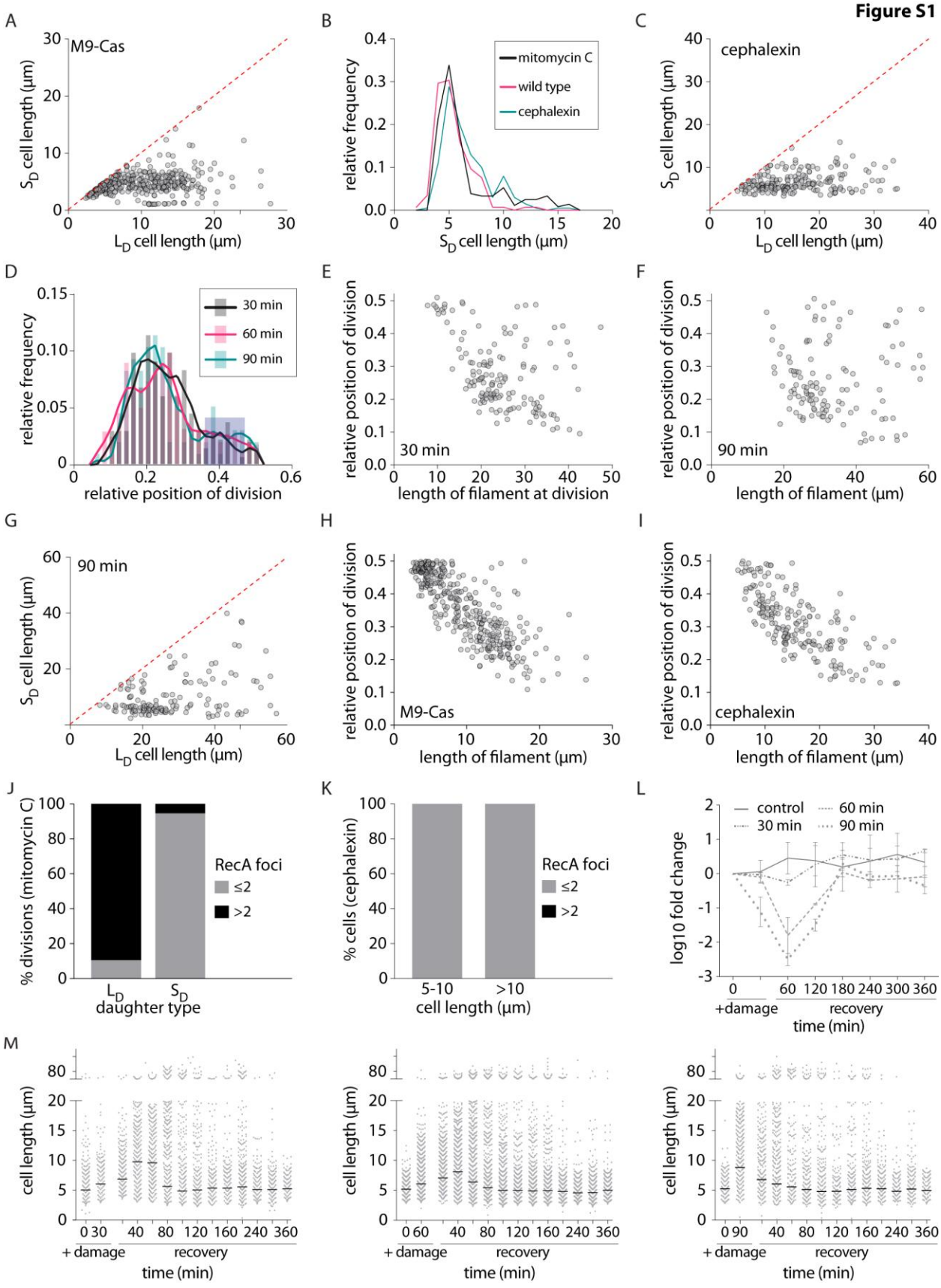

A
M9-Cas
S_D cell length (μm)
L_D cell length (μm)
B
mitomycin C
wild type
cephalexin
relative frequency
S_D cell length (μm)
C
cephalexin
S_D cell length (μm)
L_D cell length (μm)
D
30 min
60 min
90 min
relative frequency
relative position of division
E
30 min
relative position of division
length of filament at division
F
90 min
relative position of division
length of filament (μm)
G
90 min
S_D cell length (μm)
L_D cell length (μm)
H
M9-Cas
relative position of division
length of filament (μm)
I
cephalexin
relative position of division
length of filament (μm)
J
% divisions (mitomycin C)
RecA foci
≤2
>2
L_D
S_D
daughter type
K
% cells (cephalexin)
RecA foci
≤2
>2
5-10
>10
cell length (μm)
L
control
30 min
60 min
90 min
log10 fold change
+damage
recovery
time (min)
M
cell length (μm)
+ damage
recovery
time (min)

Figure S2

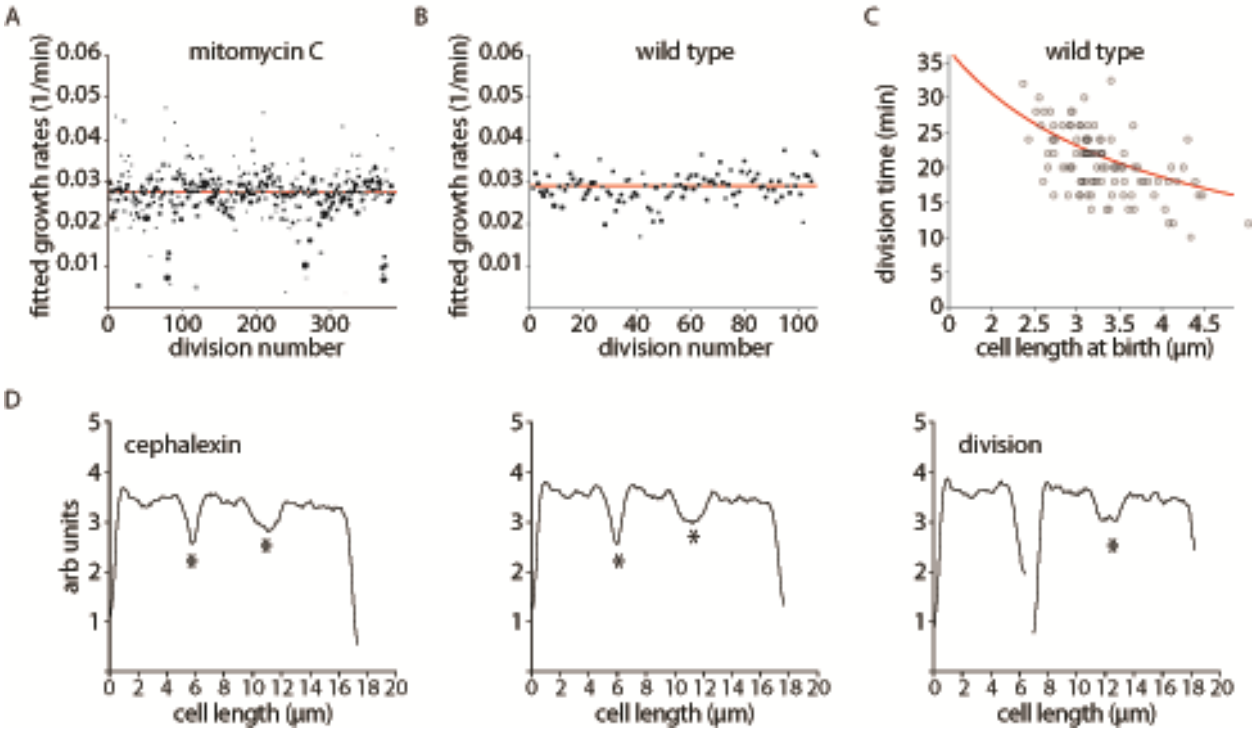
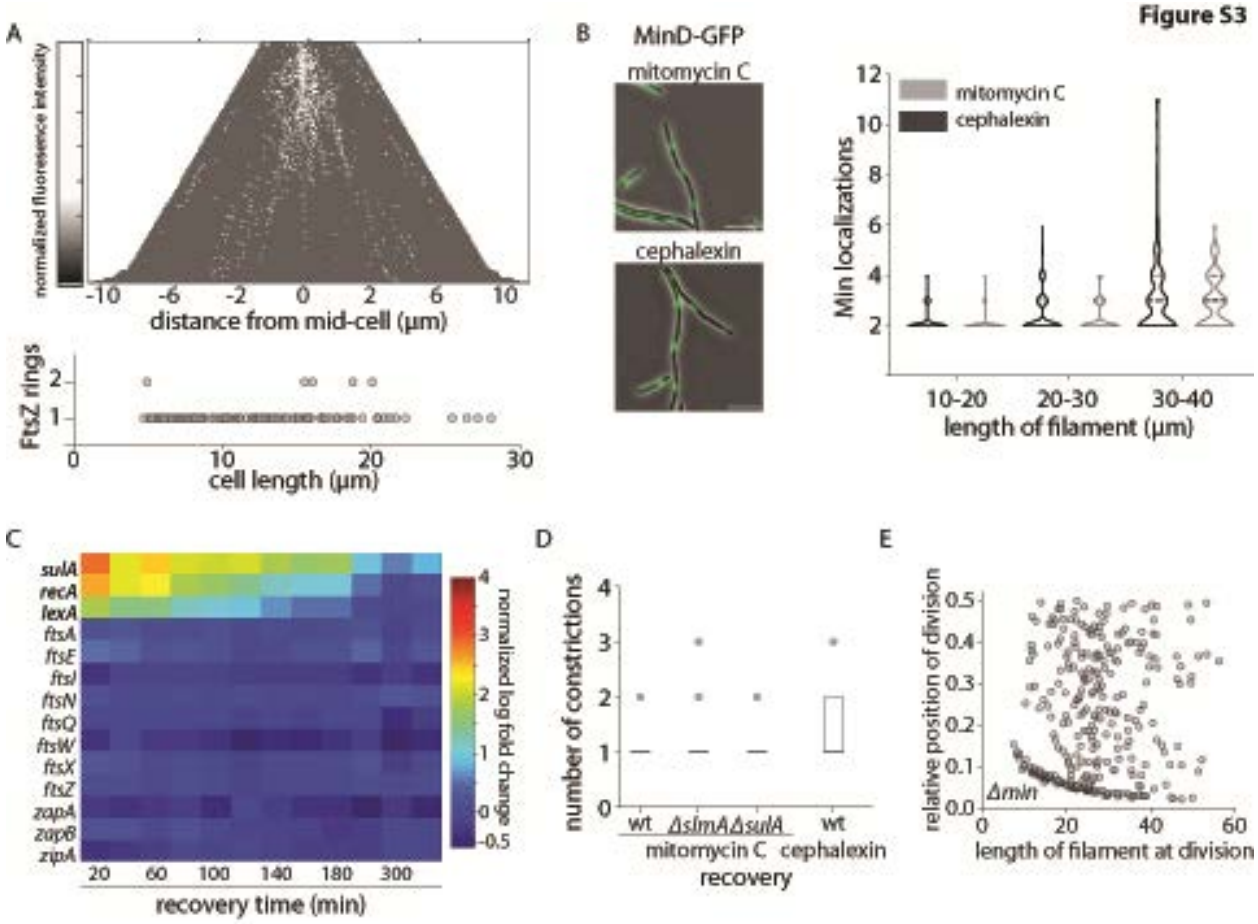

A
mitomycin C
fitted growth rates (1/min)
division number
B
wild type
fitted growth rates (1/min)
division number
C
wild type
division time (min)
cell length at birth (μm)
D
cephalexin
division
arb units
cell length (μm)

Figure S3


A
normalized fluorescence intensity
-10 -6 -2 0 2 6 10
distance from mid-cell (μm)
FtsZ rings
2
1
0 10 20 30
cell length (μm)
B
MinD-GFP
mitomycin C
cephalexin
Min localizations
12
10
8
6
4
2
mitomycin C
cephalexin
10-20 20-30 30-40
length of filament (μm)
C
sulA
recA
lexA
ftsA
ftsE
ftsI
ftsN
ftsQ
ftsW
ftsX
ftsZ
zapA
zapB
zipA
20 60 100 140 180 300
recovery time (min)
normalized log fold change
4
3
2
1
0
-0.5
D
number of constrictions
4
3
2
1
0
wt ΔslmAΔsulA wt
mitomycin C cephalexin
recovery
E
relative position of division
0.5
0.4
0.3
0.2
0.1
0.0
ΔminE
0 20 40 60
length of filament at division


F
ΔminCDE
32
38
44
50
56
62
68
74

Figure S4

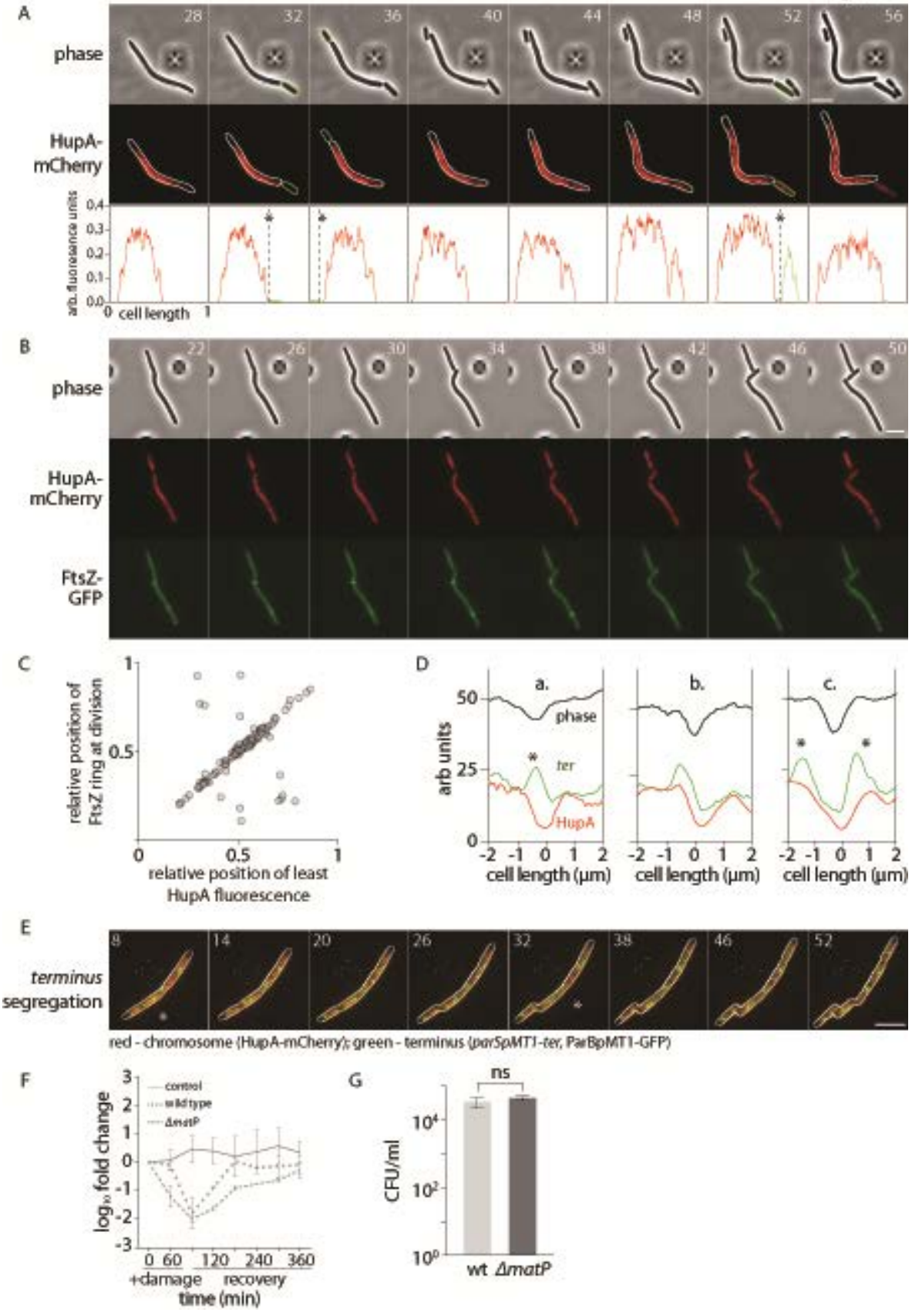
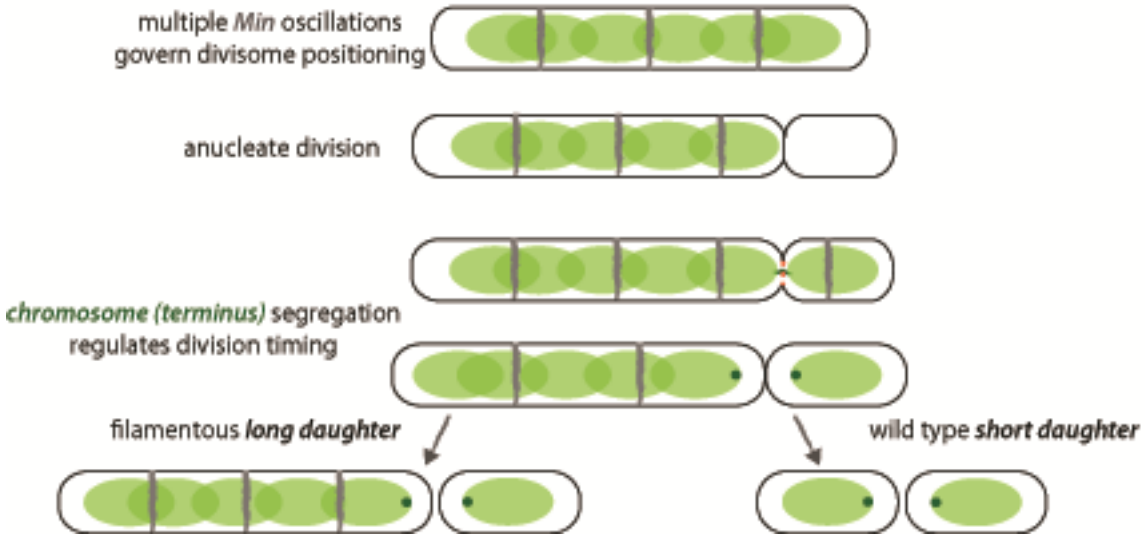

A
phase
HupA-mCherry
arb. fluorescence units
cell length
B
phase
HupA-mCherry
FtsZ-GFP
C
relative position of FtsZ ring at division
relative position of least HupA fluorescence
D
a.
b.
c.
phase
ter
HupA
arb units
cell length (μm)
E
terminus segregation
red - chromosome (HupA-mCherry); green - terminus (parSpMT1-ter, ParBpMT1-GFP)
F
control
wild type
ΔmatP
log10 fold change
+damage
recovery
time (min)
G
ns
CFU/ml
wt
ΔmatP

Figure S5


multiple *Min* oscillations
govern divisome positioning
anucleate division
chromosome (terminus) segregation
regulates division timing
filamentous ***long daughter***
wild type ***short daughter***