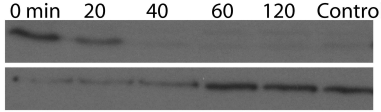
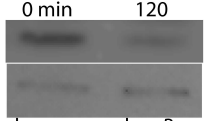
A. Western Blot

MukE Depletion

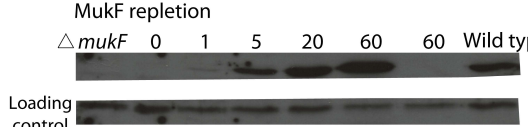


plasmid *sspB*



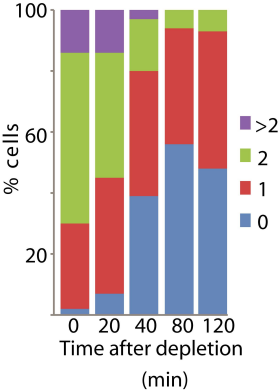
chromosomal *sspB*

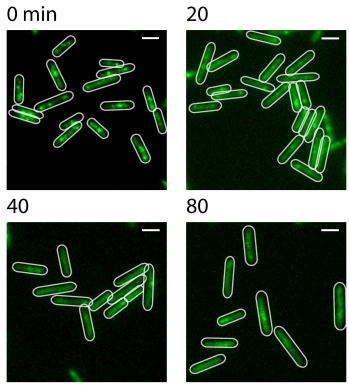
MukF repletion



no induction

B. Loss of MukB foci during MukE depletion





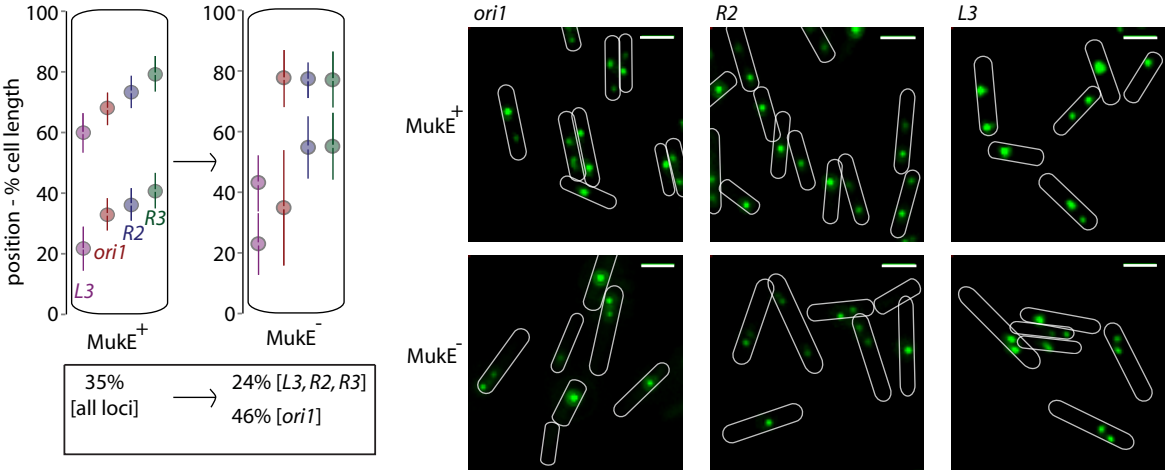
MukB: green

Fig. S1: Characterization of the MukE depletion and MukF repletion system:

A. Western Blot: Depletion of MukE in steady state cells in LB was observed by Western blot before (0) and 20, 40, 80 and 120 min after arabinose addition (for SspB expression from plasmid (strain Ab19 + pAb41) or chromosome (strain Ab86)). Weak band at 120 min can be attributed to non-specific interaction of the anti-Myc antibody with control sample (Control) with no c-Myc tag. Repletion of MukF in steady state cells was observed by Western Blot before (0) and 1, 5, 20 and 60 minutes after arabinose addition (strain Ab174). As a control cells deleted for MukF (Δ*mukF*) (strain Ab188) and cells not induced for MukF repletion (60 no induction) were also tested. Wild type protein levels are reached between 5 and 20 min of arabinose addition (See Wild type lane).

B. Loss of MukB foci was followed 20, 40, 80 and 120 min after chromosomal SspB expression in steady state cells (strain Ab86). % of cells with 0, 1, 2 or >2 foci per cell is plotted. Snapshot images of cells with labeled MukB (green) are shown on the right. Cell outlines are shown in white. n≥200. White bar in micrographs is 2μm here and elsewhere.

A. MukE depletion results in mispositioning of sister loci in steady state cells

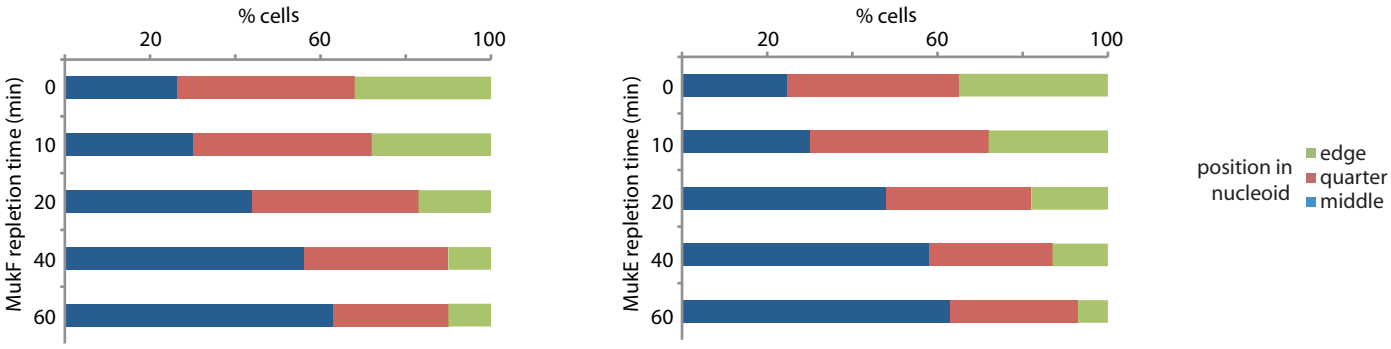


Fig_S2

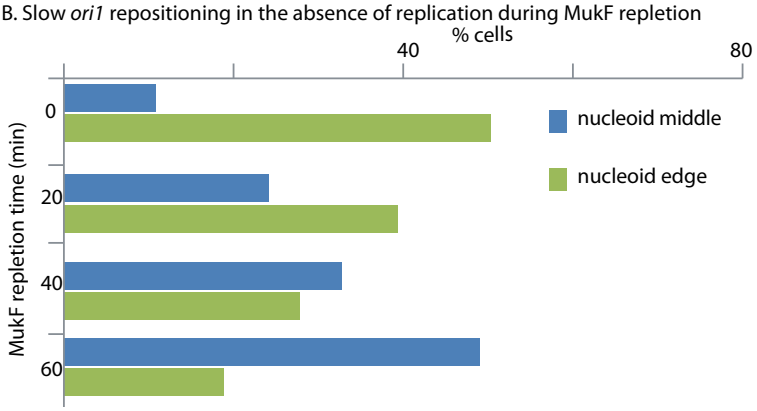
Fig. S2: MukE depletion changes *ori1* and *L3-R2-R3* position in steady state cells.

A. Relative positions of sister *ori1* (red), *R2* (blue), *L3* (purple) or *R3* (green) loci is estimated as % of cell length before and after 2 h of MukE depletion in steady state cells (left panel) (strains Ab62 and Ab82 respectively). Mean positions (circles) and standard deviations (vertical lines) are plotted in the schematics of *E. coli* cells, while the sister locus distances (SLD) are shown in the panel below. Snapshot images of cells with *ori1, R2,* or *R3* before and after depletion (labeled green) are shown on the right. Cell outlines are shown in white. n≥300.

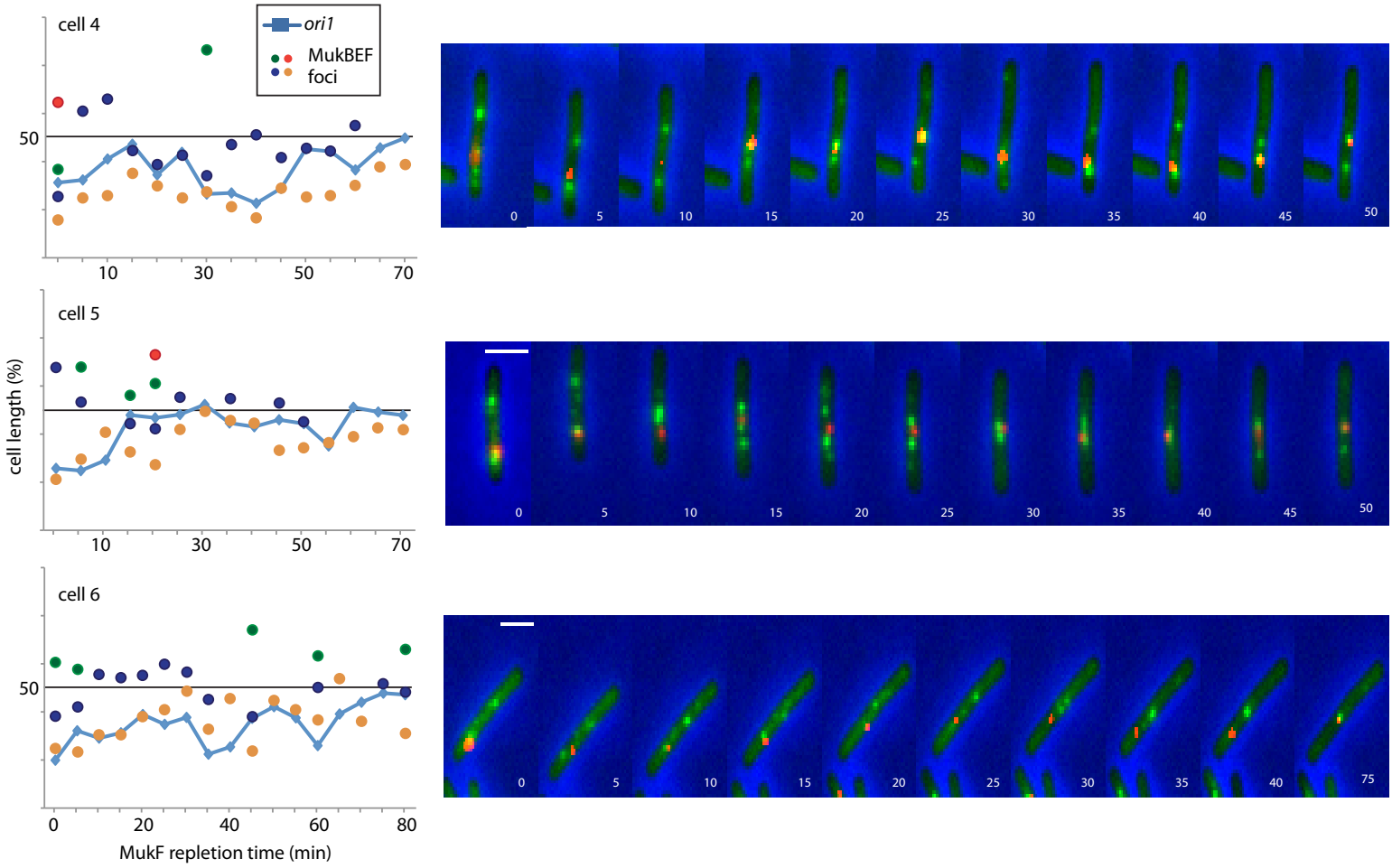
A. MukF and MukE repletion results in slow repositioning of *ori1* in steady state cells



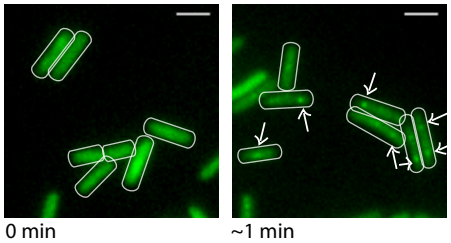
B. Slow *ori1* repositioning in the absence of replication during MukF repletion



C. MukBEF focus dynamics and *ori1* repositioning during MukF repletion in the absence of replication



D. MukBEF focus appearance is independent of cell size



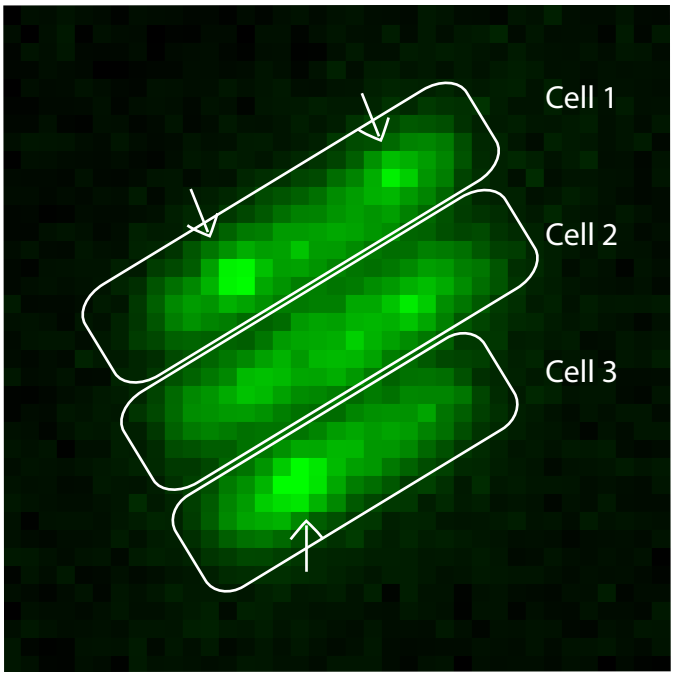
~1 min after induction of MukF repletion

cell size (μm)	% cells lacking foci	% cells with foci
2.2-2.8	28	72
2.9-3.6	28	72
>3.7	26	74

Fig_S3

Fig. S3: *ori1* repositioning during MukF or MukE repletion is slow

A. MukF and MukE repletion results in repositioning of *ori1* in steady state cells. Relative position of non-duplicated (single) *ori1* loci is estimated as % of nucleoid length before and after 1 h of MukF (left) or MukE (right) repletion in steady state cells and results are plotted as in Fig. 1B. n≥200
B. MukF-repletion as a function of time in non-replicating cells (strain Ab174). Snapshots were taken at the indicated times (0, 20, 40 and 60 min) during MukF repletion and proportions of cells with *ori1* at the indicated positions (middle or edge of the nucleoid) are shown. n≥300.
C. Time-lapse of MukB foci dynamics and *ori1* repositioning during MukF repletion in non-replicating cells (strain Ab174). Time-lapse traces of *ori1* (blue lines) as well as the position of MukB foci (circles) for 3 representative cells are shown (cell 4, 5 and 6). Numbers represent time (in min). *ori1:* red; MukB: green.
D. Snapshot images of cells with MukB-GFP (green) before (0) and ~1 min after induction of MukF repletion in steady state cells (strain Ab234). White arrows indicate position of MukBEF foci. Transcription is blocked at each time-point by the addition of rifampicin. Cells are classified based on size and % of cells with or without MukBEF foci after induction of MukF repletion are shown in the panel on the right.



MukB focus: green

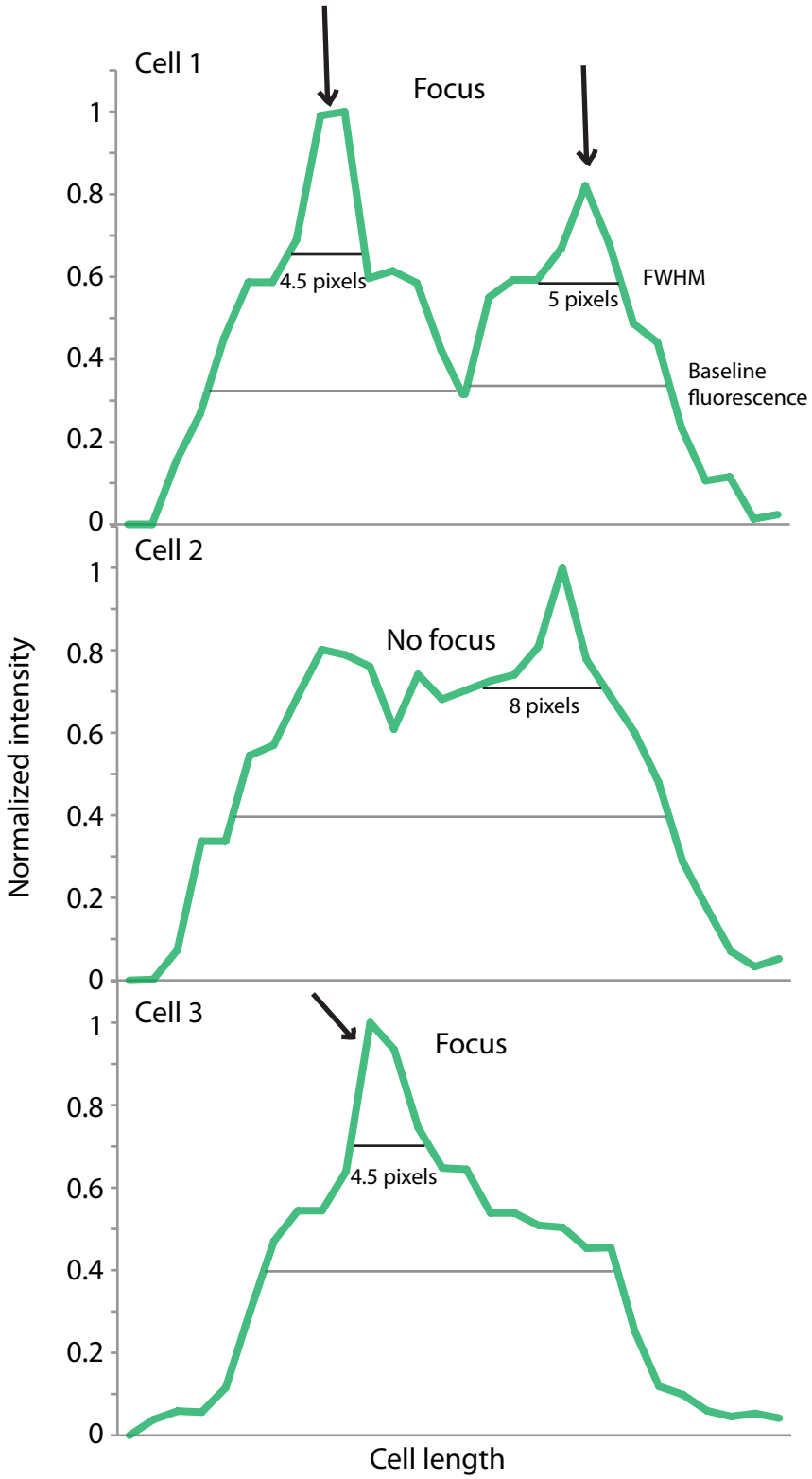


Fig. S4: Defining a MukB focus

Three representative cells with MukB fluorescence in green (left) are shown 1min after MukF repletion in steady state cells (strain Ab234). Line profiles of MukBfluorescence are shown on the right. Normalized fluorescence intensity is plotted on the Y-axis. While Cell1 and Cell3 were considered to have foci, Cell2 was considered not to have any focus. Background fluorescence is marked with a black line across the line profiles.