Electronic Supplementary Material (ESI) for Faraday Discussions.

SUPPLEMENTARY INFORMATION

A study of SeqA subcellular localization in *Escherichia coli* using Photo-Activated Localization Microscopy

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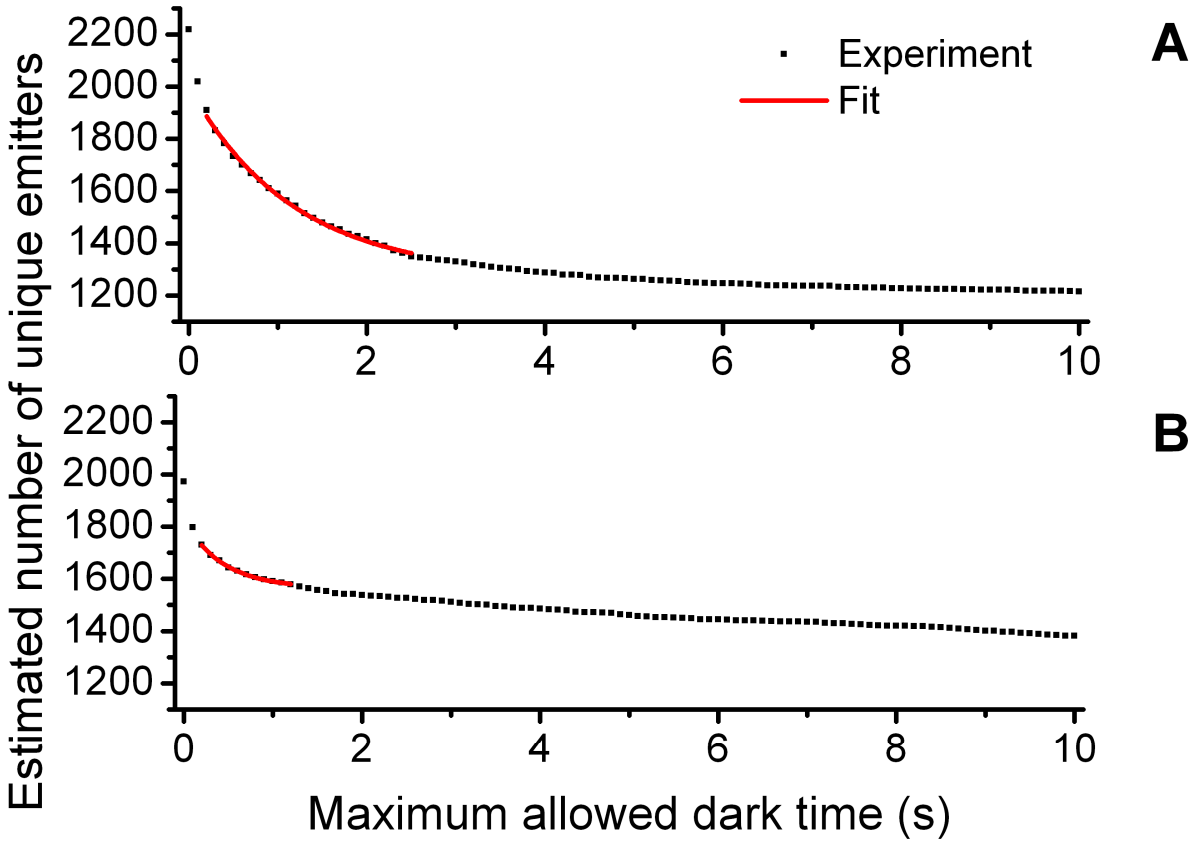
Supplementary figures



SFigure 1. Comparison of the estimated number of unique emitters as a function of maximum allowed dark time for mEos3.2 and PAmCherry for two data sets with a similar number of emitters.

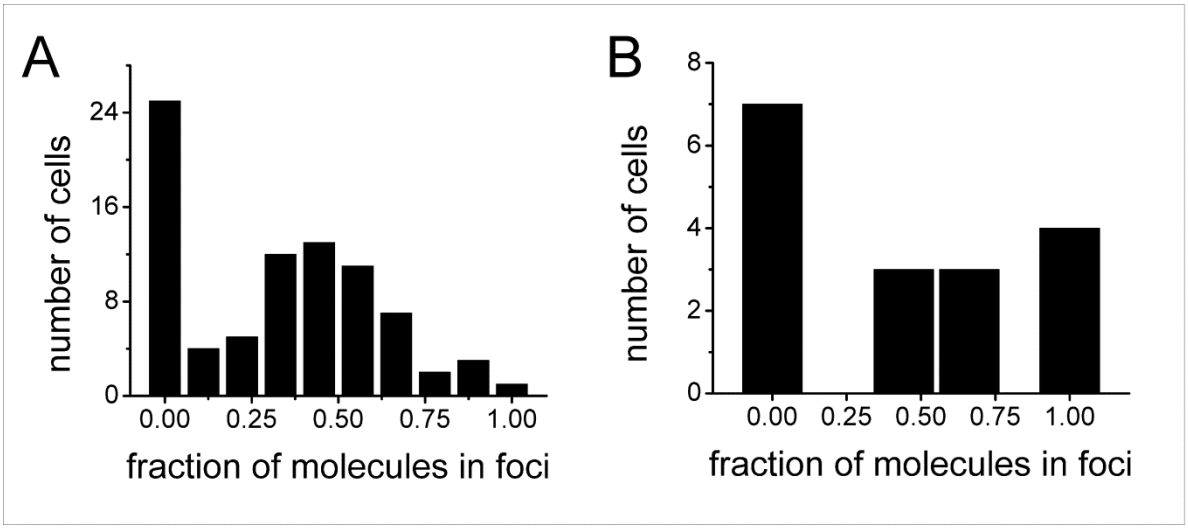
Fluorescent proteins going into transient dark states are observed multiple times, which can lead to overestimation of the numbers of SeqA-mEos3.2/PamCherry molecules. To correct for that, as explained in detail in Experimental procedures, the estimated number of molecules is plotted as a function of maximum allowed dark time for mEos3.2 and PAmCherry for two data sets with a similar number of emitters (black points) and the exponential regime of the curve is fitted as depicted by the red line, based on Annibale[1]. A: mEos3.2 – displays more blinking than PAmCherry and stays longer in the dark state, as seen by the higher amplitude and half-time of the exponential curve fit. B: PAmCherry – only a small fraction of molecules blinks, the half time of blinking is not long. Acquisition time 100 ms per frame.

Stable 1 Comparison of blinking properties of mEos3.2 and PAmCherry.

Property	mEos3.2	PAmCherry
ratio of unique emitters after consolidation of emitters in subsequent frames	50-60%	>60%
ratio of unique emitters after consolidation of emitters taking blinking into account	30%	50%
ratio of double counts due to blinking to the real number of unique emitters	50%	10-15%
half-lifetime of the exponential curve fit	1.69 +/- 0.08 seconds	0.62 +/- 0.04 seconds
compensated molecules*	4%	1,5%

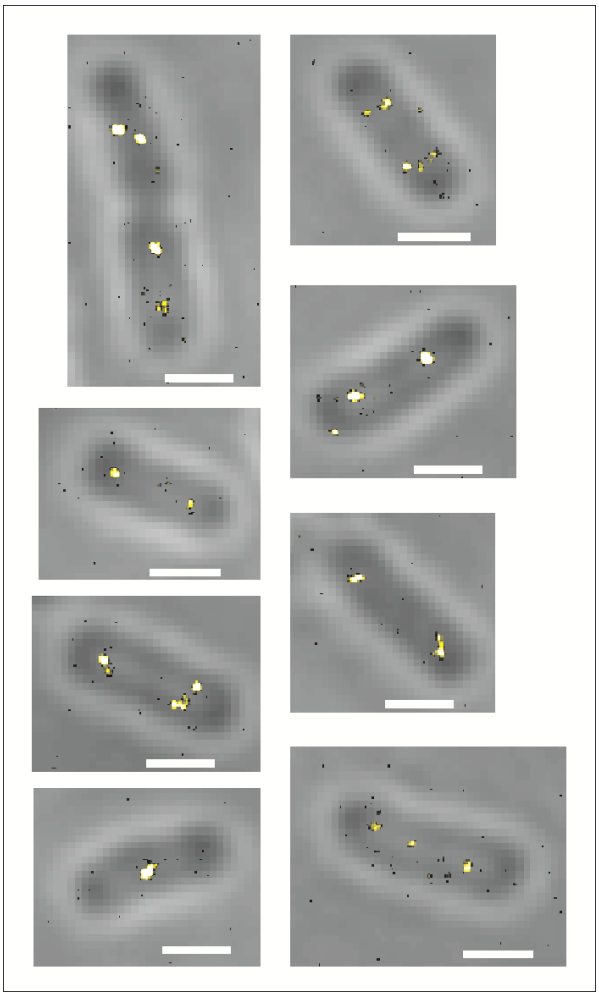
*Compensated molecules is the sum of "double counts" and "missed counts" and is calculated from the exponential and the linear fit.

The results of the consolidation of identical emitters analysis from the data set in SFigure 1 based on fitting as introduced by Annibale[1], see Experimental procedures section for details.



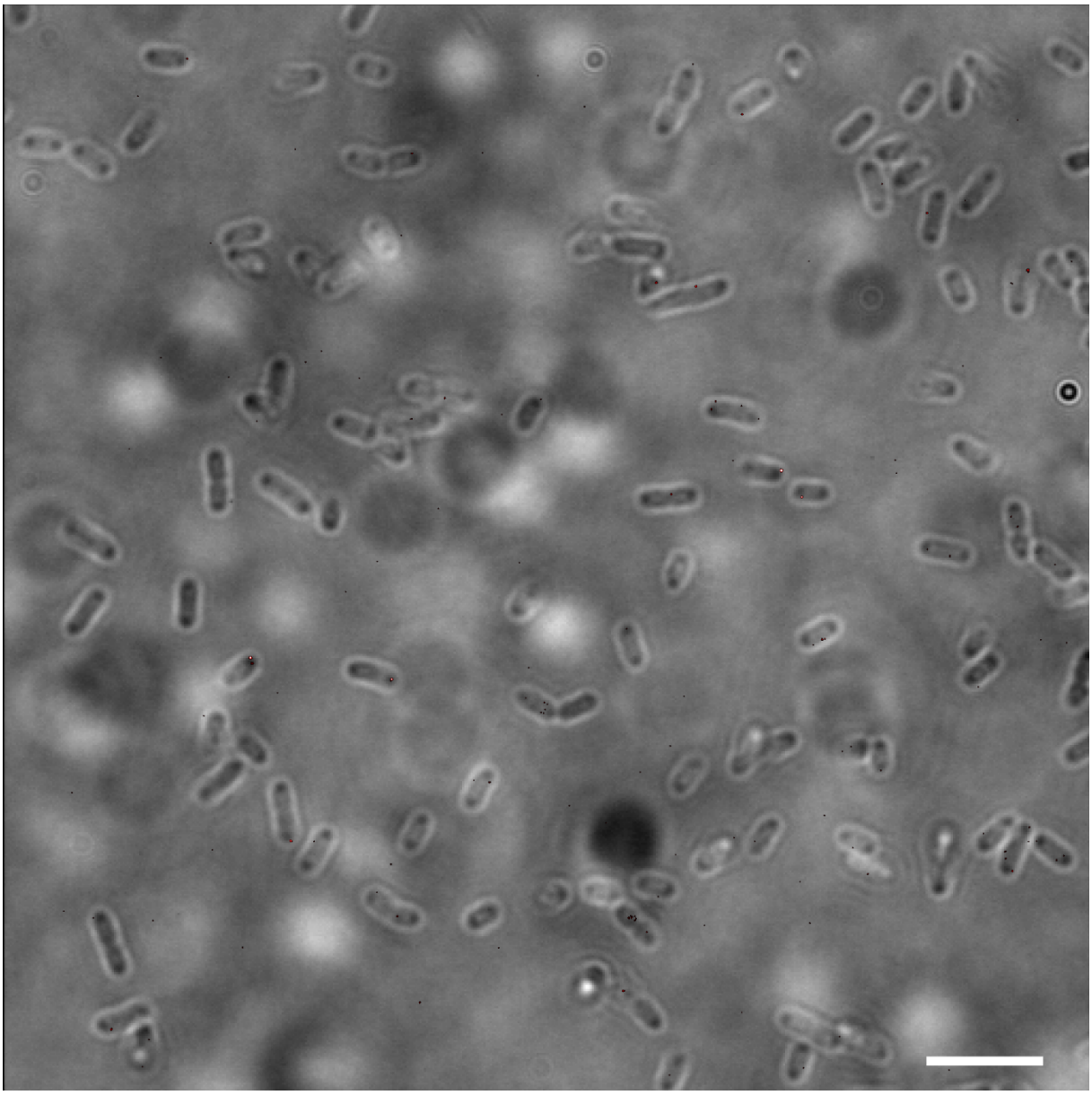
SFigure 2. Not all SeqA-PamCherry molecules are located in foci.

Histograms displaying the amount of SeqA-PAmCherry molecules that are localized into foci in *E.coli* cells expressing the protein from the genome for two selected datasets: (A) synchronized cells grown at 37°C (n= 82 cells) and (B) non-synchronized cells grown at 37°C (n=17 cells). The amounts of molecules were quantified from PALM images for each cell and for each focus as described (see main text) and divided to obtain the ratio.



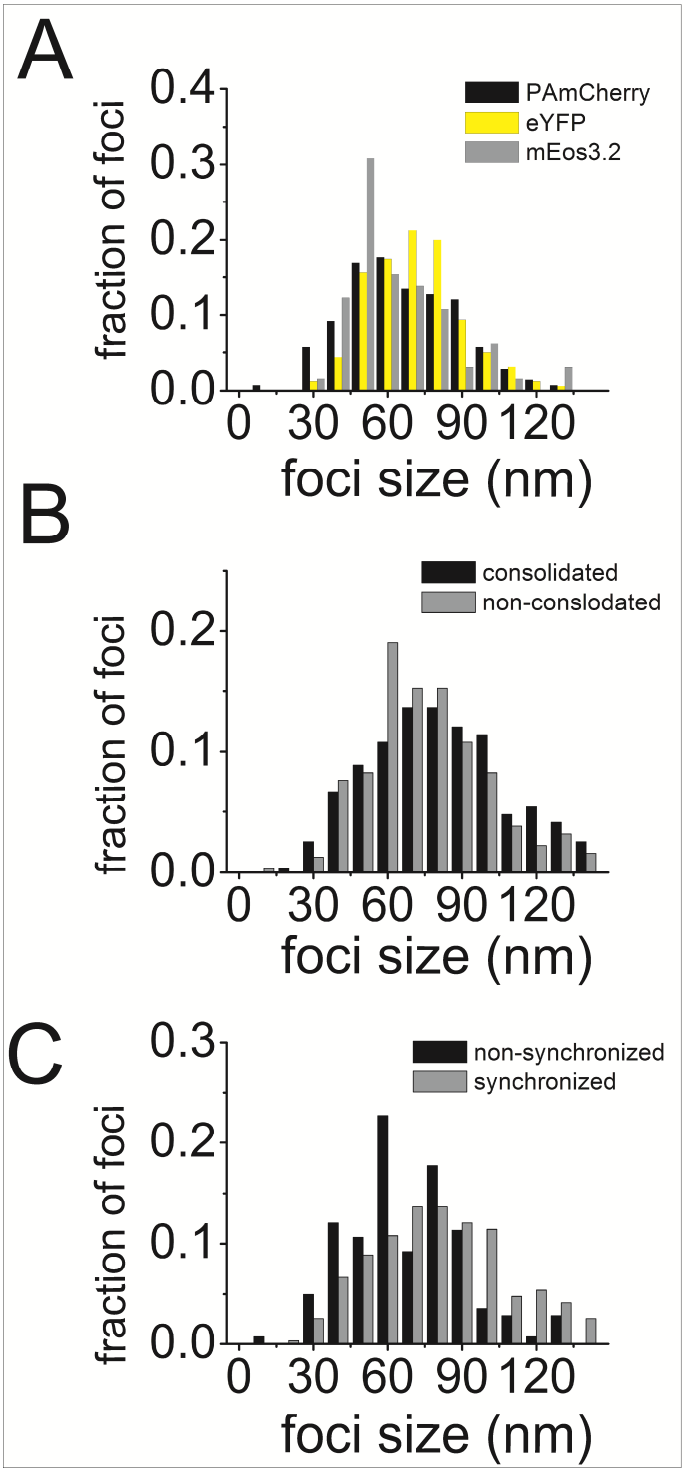
SFigure 3. PALM images of *Escherichia coli* expressing SeqA-eYFP from the genome.

Examples of images of *E.coli* SeqA-eYFP genomic knock-in cells grown at 37^{o}C in LB medium. The cells were harvested in late exponential phase and not synchronized. Overlay of transmittance images with PALM reconstructions; the localized SeqA-eYFP molecules are false-colored yellow. Scale bar 1 µm.



SFigure 4. PALM reconstruction of *Escherichia coli* cells not expressing fluorescent proteins.

Overlay of a transmittance image with a PALM reconstruction of wild-type *E.coli* cells (MG1655) not expressing FPs. The PALM reconstruction was created using the same settings as used for the PAmCherry datasets. Only sporadic localizations are observed. Out of 120 imaged cells, 86 cells did not have any localizations within the cells' boundaries and on average there was 1,5 localizations per cell. The cells that did show localizations upon PALM reconstruction had typically 1-5 localizations per cell.



SFigure 5. Comparison of the SeqA foci sizes obtained with different fluorescent protein tags, analysis methods and physiological conditions in *Escherichia coli*.

Histograms displaying foci sizes obtained using the L-clustering analysis (see main text for details) in *E.coli* cells genomically expressing SeqA-FPs grown in LB at 37^{o}C. A: comparison of foci sizes for SeqA tagged with different fluorescent proteins (non-synchronized cell cultures). B: comparison of SeqA-PAmCherry foci sizes obtained for data sets, where the identical emitters were consolidated or non-consolidated. C: comparison of SeqA-PAmCherry foci sizes obtained for synchronized cells (data from all time points combined) with non-synchronized cells.

STable 2. SeqA-PAmCherry foci sizes in synchronized *Escherichia coli* cells grown at 37°C.

Time (min)	Average (nm)	Median (nm)	Nr of cells	Nr of foci
0	75.2	73.8	29	95
7	67	73	13	51
13	67	64.2	16	75
18	95.2	85.6	15	44
24	90.1	83.5	14	54
Total	**76.9**	**72.8**	**87**	**315**

STable 3. SeqA-PAmCherry foci sizes in synchronized *Escherichia coli* cells grown at 30°C.

Time (min)	Average (nm)	Median (nm)	Nr of cells	Nr of foci
0	57.7	53.5	13	40
10	67.1	*63.7*	33	93
20	59.5	53.5	27	82
30	74.5	72.4	24	73
40	75.8	72.3	27	64
50	68.3	62.6	19	38
60	63.1	63.7	31	66
Total	**66.6**	**62**	**174**	**456**

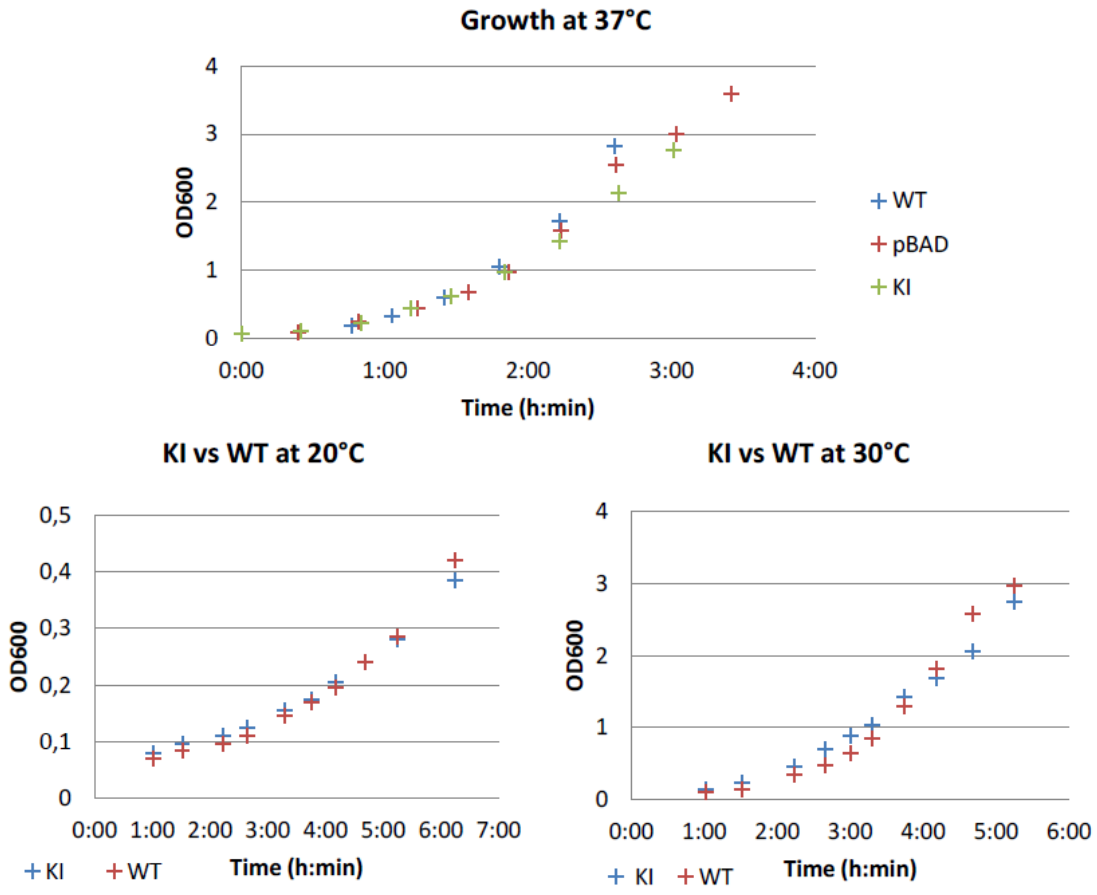
STable 4. Comparison of the SeqA-FP foci sizes in *Escherichia coli* cells obtained for different fluorescent proteins. *

SeqA fusion to	Average (nm)	Median (nm)	Nr of cells	Nr of foci
PAmCherry	61.9	59.9	30	141
eYFP	64.8	63.1	67	160
mEos3.2	62.4	61	20	65

*Cells grown at 37°C in LB and not synchronized. Because consolidation of identical emitters for eYFP was not performed, the table compares non-consolidated datasets.

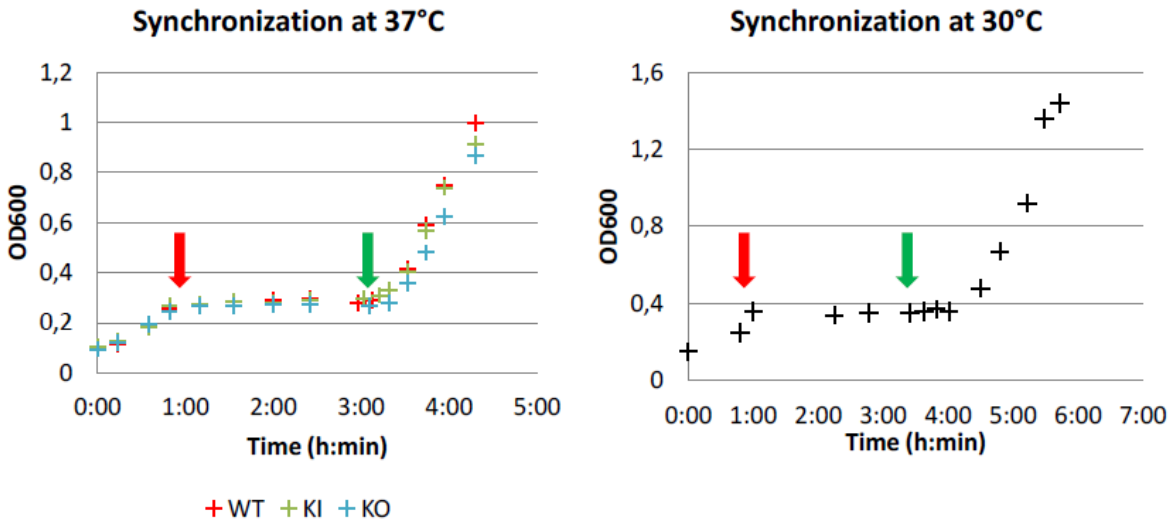
STable 5 . Comparison of the SeqA-FP foci sizes in *Escherichia coli* cells obtained for synchronized and non-synchronized cells and in data sets corrected or not for the multiple counting of identical emitters (consolidated vs. non-consolidated).

SeqA-PAmCherry	Average (nm)	Median (nm)	Nr of cells	Nr of foci
Non-synchronized, Consolidated	61.9	59.9	30	141
Synchronized, consolidated	76.9	72.3	87	315
Synchronized, Non-consolidated	71.8	62.2	87	315



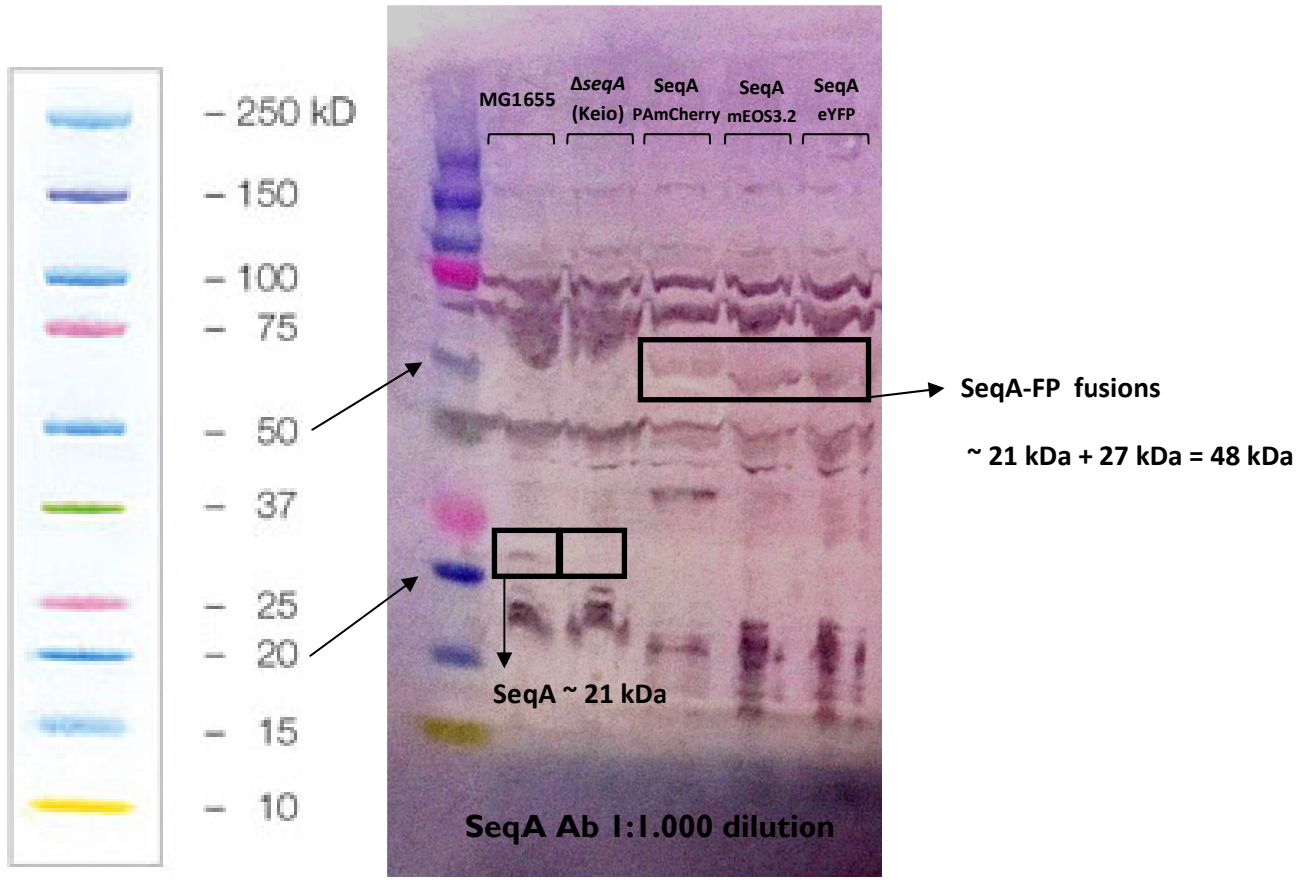
SFigure 6. The genomic introduction of SeqA-PAmCherry does not affect the of growth of *Escherichia coli.*

Optical densities of *E.coli* cultures grown in LB at 37°C (top), 20°C (bottom left) and 30°C (bottom right). The increase of optical density of cultures resulting from cell growth is similar for wild-type cells (*E.coli* MG1655) and cells where the SeqA-PamCherry was introduced into genome or overexpressed from the plasmid (pBAD-SeqA-PamCherry).



SFigure 7. Changes in the growth rate of *Escherichia coli* during and after synchronization experiments.

Optical densities of *E.coli* cultures grown in LB at 37°C (left) and 30°C (right). At an OD_{600} ~0.3 the cell cycle synchronization agent serine hydroxymate (SHX) is introduced (red arrow) and the cultures' optical densities are not increasing. More than 90 minutes after introduction of SHX, the agent is removed (green arrows) and the cells resume growth – optical densities of cultures increase. Left panel displays the changes of optical densities resulting from cell growth and synchronization at 37°C for *E.coli* MG1655 (WT, red crosses), the SeqA-PamCherry genomic knock in (KI, green crosses) and a strain where SeqA was deleted (KO, blue crosses); the changes in cell growth in the course of this experiment are similar for the different strains tested. Right panel displays the changes of optical density of the culture of the SeqA-PamCherry genomic knock-in at 30°C.



SFigure 8. Western blot with anti-SeqA antibody of the *Echerichia coli* strains used in the study.

Comparison of expression levels of SeqA between wild-type *E.coli* (MG1655, lane 2), the SeqA knock-out (Keio collection BW25113 *ΔseqA,* lane 3) and the genomic knock-ins used in this study (SeqA-PAmCherry, SeqA-mEos3.2 and SeqA-eYFP, lanes 3,4 and 5, respectively). Lane 1 is the pre-stained marker. The Western blot has shown a significant amount of non-specific bands, yet it is possible to observe a band at 21 kDa for the wild-type *E.coli* that corresponds to the expected molecular weight of SeqA; the band is absent in the *ΔseqA* strain. The SeqA-FP genomic knock-ins all show a band at approximately 50 kDa which corresponds to expected molecular weight of the SeqA-FP proteins; this band is not present in the wild-type nor the *ΔseqA* strains.

Supplementary References

1. Annibale, P., Vanni, S., Scarselli, M., Rothlisberger, U. & Radenovic, A. Quantitative photo activated localization microscopy: unraveling the effects of photoblinking. *PLoS ONE* **6**, e22678 (2011).