Descriptions of Supplementary Videos 1–8

Supplementary Video 1

ClpP-Venus YFP forms bright fluorescent foci in live *E. coli* cells. The ClpP-Venus YFP foci are not present in all cells, the foci localize preferentially to the cell poles and mid-cell region and show binary segregation at cell division. Cells without a focus have a cytoplasmic YFP signal (not visible in this movie) and usually form a YFP focus in the next cell cycles. A few ClpP-Venus YFP foci appear blurred because they are in different focal planes (z-stacks are required to detect all foci in a micro-colony, data not shown). Some cells have small foci, which are very faint and barely visible in the movie. The YFP images of this movie were acquired with 2 × 2 binning (effective pixel size is 129 nm). The cell boundary (red) was determined by segmenting the respective phase images. The scale bar (white) corresponds to 1 μm. The movie is part of a dual-color experiment; see **Supplementary Video 2** for the corresponding RFP movie.

Supplementary Video 2

Degradation of an mCherry-ssrA reporter in the ClpP-Venus YFP strain shows that the ClpP-Venus YFP foci generate post-division cell-to-cell variability. The synthesis of mCherry-ssrA was pulse-induced before the cells were monitored. The RFP images were subjected to a "per-frame auto-scaling" to better display the post-division variability between daughter cells. The foci in the RFP image are not due to spectral bleedthrough from the ClpP-Venus YFP foci but probably represent immortal mCherry molecules bound to the ClpP-FP foci (**Supplementary Fig. 1**). The RFP images of this movie were acquired with 2 × 2 binning (effective pixel size is 129 nm). The cell boundary (red) was determined by segmenting the respective phase images. The scale bar (white) corresponds to 1 μm. The movie is part of a dual-color experiment; see **Supplementary Video 1** for the corresponding YFP movie.

Supplementary Video 3

Degradation of the mCherry-ssrA reporter displays very little post-division variability in the wildtype strain (protease is not tagged with an FP). The mCherry-ssrA reporter was pulse-induced before imaging. mCherry-ssrA is specifically degraded by ClpXP and ClpAP. The RFP images were per-frame auto-scaled to better illustrate the low degree of variability after cell division. The YFP images of this movie were acquired with 2 × 2 binning (129 nm effective pixel size). The cell boundary (red) was determined by segmenting the respective phase images. The scale bar (white) corresponds to 1μm.

Supplementary Videos 4–7

HILO microscopy of cells expressing ClpA-mGFPmut3, ClpP-mGFPmut3 and ClpX-mGFPmut3 (**Supplementary Videos 4**–**6**, respectively). Cells have ~50 particles, which are not localized in a foci but in fact move rapidly and seem to sample the entire cell. mGFPmut3 expressed alone (i.e., not fused to another protein) from the P_{clpX} promoter at the endogenous locus displays a uniform cytoplasmic distribution (**Supplementary Video 7**). HILO imaging was performed as described (see **Online Methods**). The scale bar (white) is 1 μm. The images of the movie sequences were subjected to a quantitative grayscale scaling (left) and to a "per-frame auto-scaling" (right) to better display the particle movement despite fast photo-bleaching (see **Online Methods**).

Supplementary Video 8

Time-lapse microscopy of *E. coli* strains with the Hfq-mGFPmut3, PepP-mGFPmut3, IbpA-mGFPmut3, MviM-mGFPmut3, and FruK-mGFPmut3 fusions, respectively. The fusions are constructed at the endogenous gene loci. Cells were grown to exponential phase in imaging medium and micro-colony growth was filmed on an agar pad with exposures every 10 min. Cell growth and imaging was performed at 30 ˚C. GFP images with 1 sec exposure time were acquired every 10 min. The GFP images of the movies were acquired with no binning (effective pixel size is 64.5 nm). The scale bar (white) corresponds to 1 μm. The GFP images of the movie sequences were subjected to a quantitative grayscale scaling.