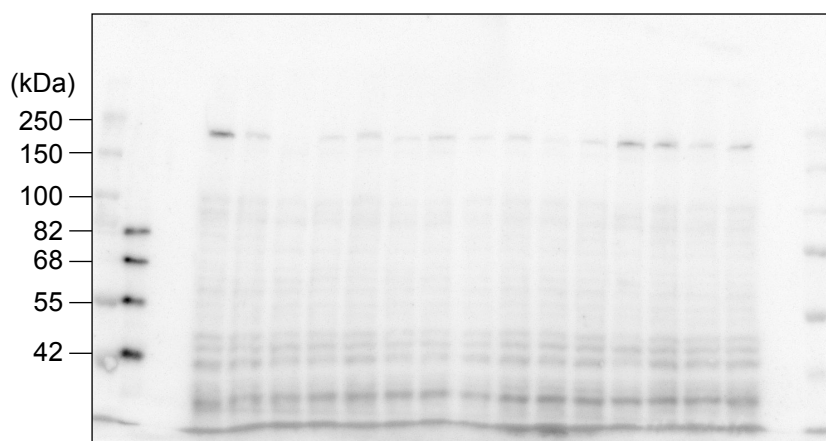
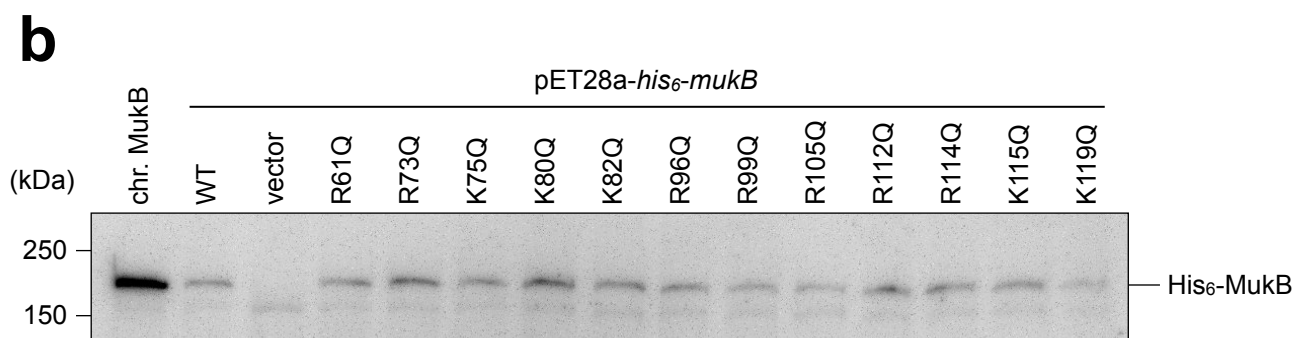
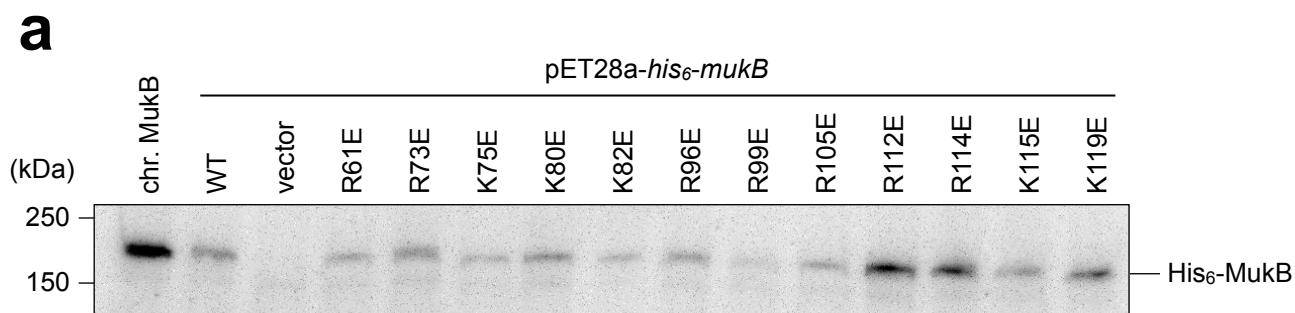


Supplementary Table 1

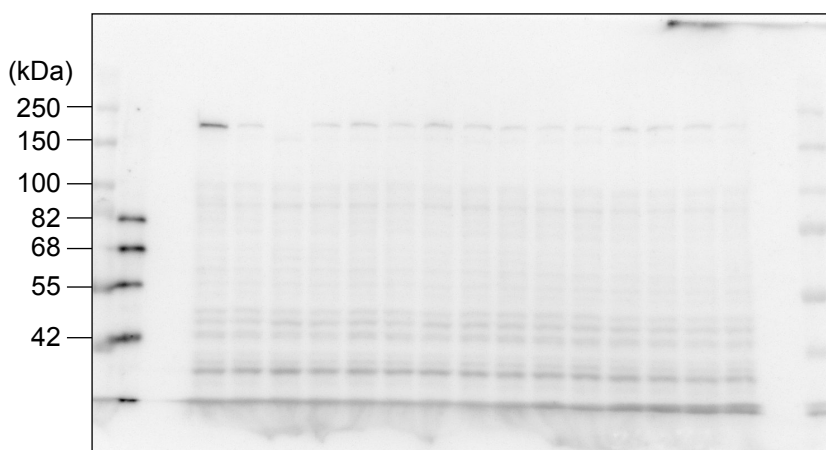
Strain	Relevant Genotype	Source
MC1061	WT	Lab stock
YAN4081	MC1061 $\Delta mukB::cat$	This study

Supplementary Table 2

Plasmid	Vector	Coding Gene
pET28a	pET28a	empty
pHis6-MUKB	pET28a	<i>his₆-mukB</i>
pHis6-MUKB ^{R61E}	pET28a	<i>his₆-mukB^{R61E}</i>
pHis6-MUKB ^{R73E}	pET28a	<i>his₆-mukB^{R73E}</i>
pHis6-MUKB ^{K75E}	pET28a	<i>his₆-mukB^{K75E}</i>
pHis6-MUKB ^{K80E}	pET28a	<i>his₆-mukB^{K80E}</i>
pHis6-MUKB ^{R96E}	pET28a	<i>his₆-mukB^{R96E}</i>
pHis6-MUKB ^{R99E}	pET28a	<i>his₆-mukB^{R99E}</i>
pHis6-MUKB ^{R105E}	pET28a	<i>his₆-mukB^{R105E}</i>
pHis6-MUKB ^{R112E}	pET28a	<i>his₆-mukB^{R112E}</i>
pHis6-MUKB ^{R114E}	pET28a	<i>his₆-mukB^{R114E}</i>
pHis6-MUKB ^{K115E}	pET28a	<i>his₆-mukB^{K115E}</i>
pHis6-MUKB ^{K119E}	pET28a	<i>his₆-mukB^{K119E}</i>
pKAK6	pET28a	<i>his₆-mukB^{R61Q}</i>
pKAK7	pET28a	<i>his₆-mukB^{R73Q}</i>
pKAK8	pET28a	<i>his₆-mukB^{K75Q}</i>
pKAK9	pET28a	<i>his₆-mukB^{K80Q}</i>
pKAK10	pET28a	<i>his₆-mukB^{R96Q}</i>
pKAK11	pET28a	<i>his₆-mukB^{R99Q}</i>
pKAK12	pET28a	<i>his₆-mukB^{R105Q}</i>
pKAK13	pET28a	<i>his₆-mukB^{R112Q}</i>
pKAK14	pET28a	<i>his₆-mukB^{R114Q}</i>
pKAK15	pET28a	<i>his₆-mukB^{K115Q}</i>
pKAK16	pET28a	<i>his₆-mukB^{K119Q}</i>
pKAK199	pET28a	<i>his₆-mukB^{K82E}</i>
pKAK200	pET28a	<i>his₆-mukB^{K82Q}</i>
pKAK151	pET28a	<i>his₆-mukB^{K75D}</i>
pKAK152	pET28a	<i>his₆-mukB^{K75A}</i>
pKAK153	pET28a	<i>his₆-mukB^{K75F}</i>
pKAK154	pET28a	<i>his₆-mukB^{K75L}</i>
pKAK155	pET28a	<i>his₆-mukB^{K75R}</i>
pKAK156	pET28a	<i>his₆-mukB^{R112D}</i>
pKAK157	pET28a	<i>his₆-mukB^{R112A}</i>
pKAK158	pET28a	<i>his₆-mukB^{R112F}</i>
pKAK159	pET28a	<i>his₆-mukB^{R112L}</i>
pKAK160	pET28a	<i>his₆-mukB^{R112K}</i>



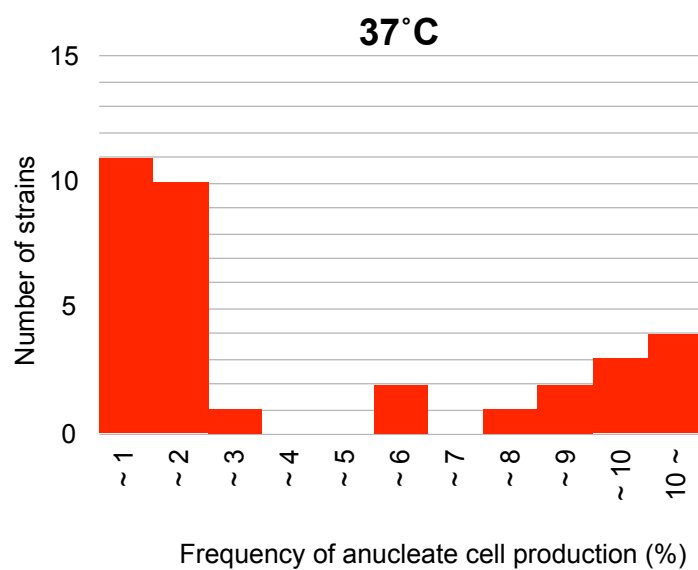
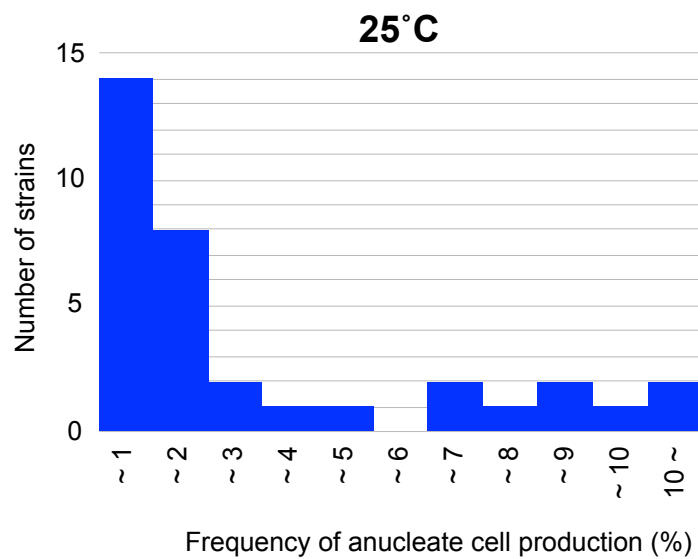
The unedited original data of panel (a)



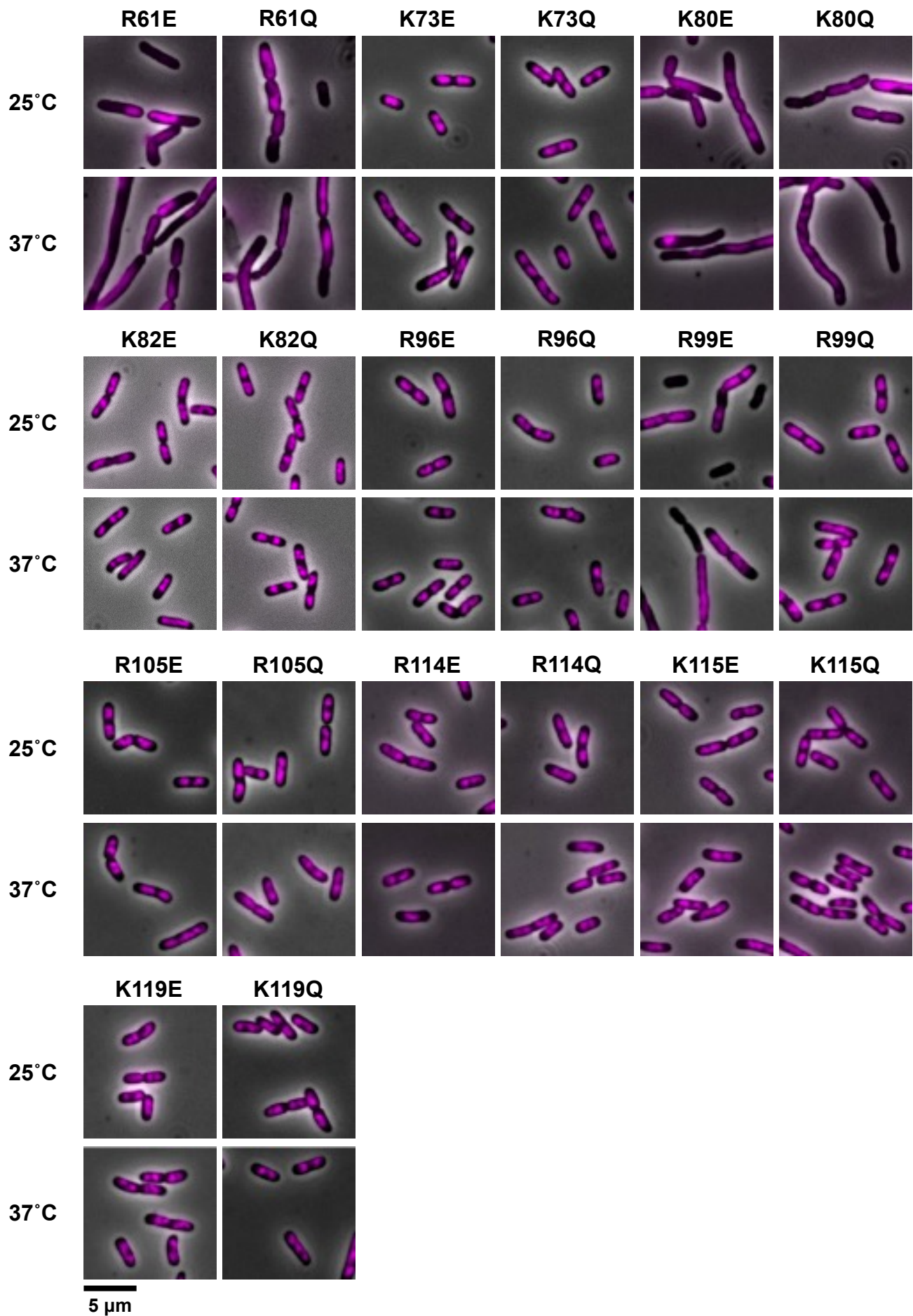
The unedited original data of panel (b)

Supplementary Figure 1. Accumulation level of leaky expressed head domain mutants., related to Figure 2
 YAN4081 (*DmukB::cat*) strains harboring the plasmid encoding the indicated His₆-MukB mutant were grown at 25°C and the accumulation level of His₆-MukB protein were detected by immunoblotting analysis by using anti-MukB antibody. The sample of MC1061, *mukB*⁺ strain, was loaded as it represents chromosomal expression level of MukB in wild type cell. The unedited gel images are also shown.

Supplementary Figure 1

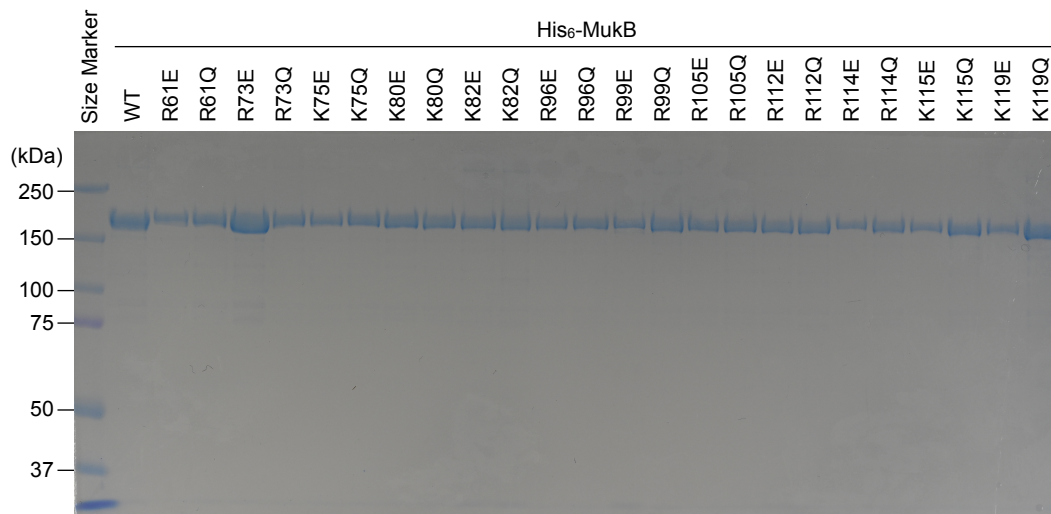


Supplementary Figure 2. Histogram of frequencies of the anucleate cell production., related to Table 1. Frequencies of anucleate cell production in Table 1 are represented as a histogram.



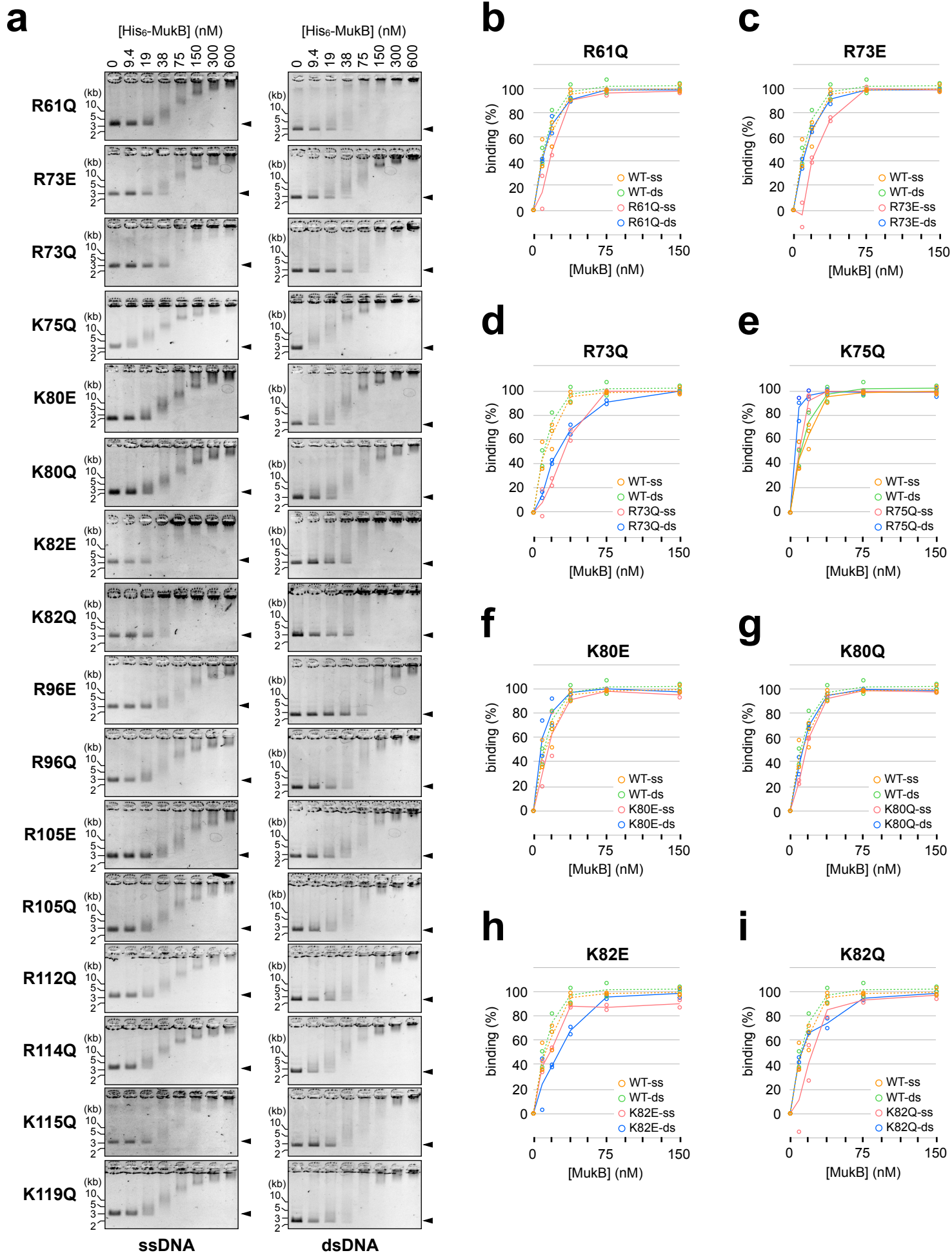
Supplementary Figure 3. Nucleoid segregation in cells expressing head domain mutants., related to Figure 3
 In addition to the strains shown in Figure 3, a series of YAN4081 (*DmukB::cat*) strains harboring the plasmid encoding the indicated His₆-MukB mutant is depicted as merged images of phase-contrast images and DAPI-stained fluorescent images (pseudo color in magenta). The images of cells cultured at 25°C are displayed in the upper panels, and images at 37°C are shown in the lower panels. The scale bar indicates 5 µm. Also see Figure 3.

Supplementary Figure 3

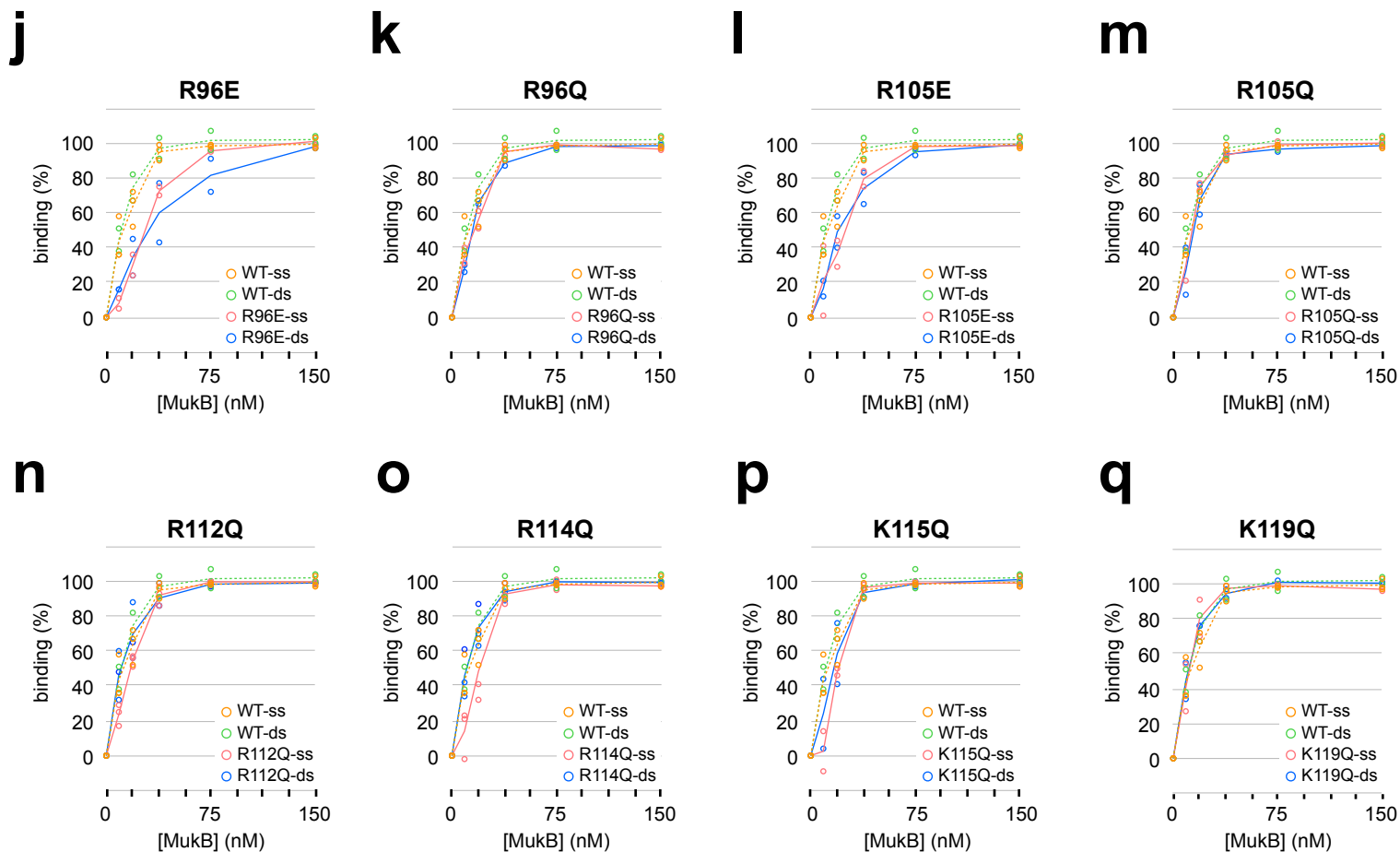


This is the unedited original data.

Supplementary Figure 4. Integrity of the purified proteins., related to Figure 4 and 5
 6 pmol of purified proteins dissolved in 1x SDS sample buffer were analyzed by 8% Laemmli gel and visualized by CBB staining.



Supplementary Figure 5



Supplementary Figure 5. The ssDNA-binding and dsDNA-binding activities of the MukB head domain mutants., related to Figure 4.

(a) Gel images of the electromobility shift assay (EMSA). The amount of purified His₆-MukB mutant proteins in each reaction is indicated in the top row. The left and the right panels show EMSA with cssDNA and cccDNA, respectively. Arrows indicate the positions of DNA to which the MukB protein does not bind. (b-q) Kinetics of the ssDNA-binding and dsDNA-binding activities of the MukB head domain mutants were investigated. A single amino acid substitution of the MukB head domain mutants is indicated at the top of each graph. The intensities of the DNA bands to which the MukB protein does not bind in the EMSA gels in (a) are graphically shown according to inputs of the MukB protein, ranging from 0 to 150 nM. The DNA-binding activities of the wild-type MukB protein are shown as the common results for each panel. The mean of the two or three independent experiments are graphically indicated as lines with individual plots shown as open circles. The DNA-binding activities of the wild-type MukB protein are displayed for each reaction. Also see Figure 4.

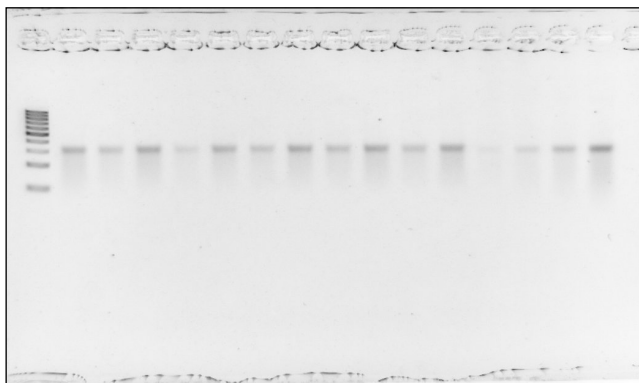


Supplementary Figure 6



Supplementary Figure 6. The unedited gel images of Figure 4 and Supplementary Figure 5.

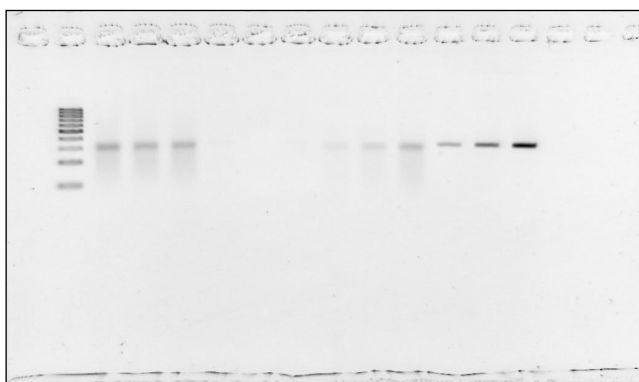
Supplementary Figure 6



The original data of Figure 5a



The original data of Figure 5b



The original data of Figure 5c

Supplementary Figure 7. The unedited gel images of Figure 5, related to Figure 5.