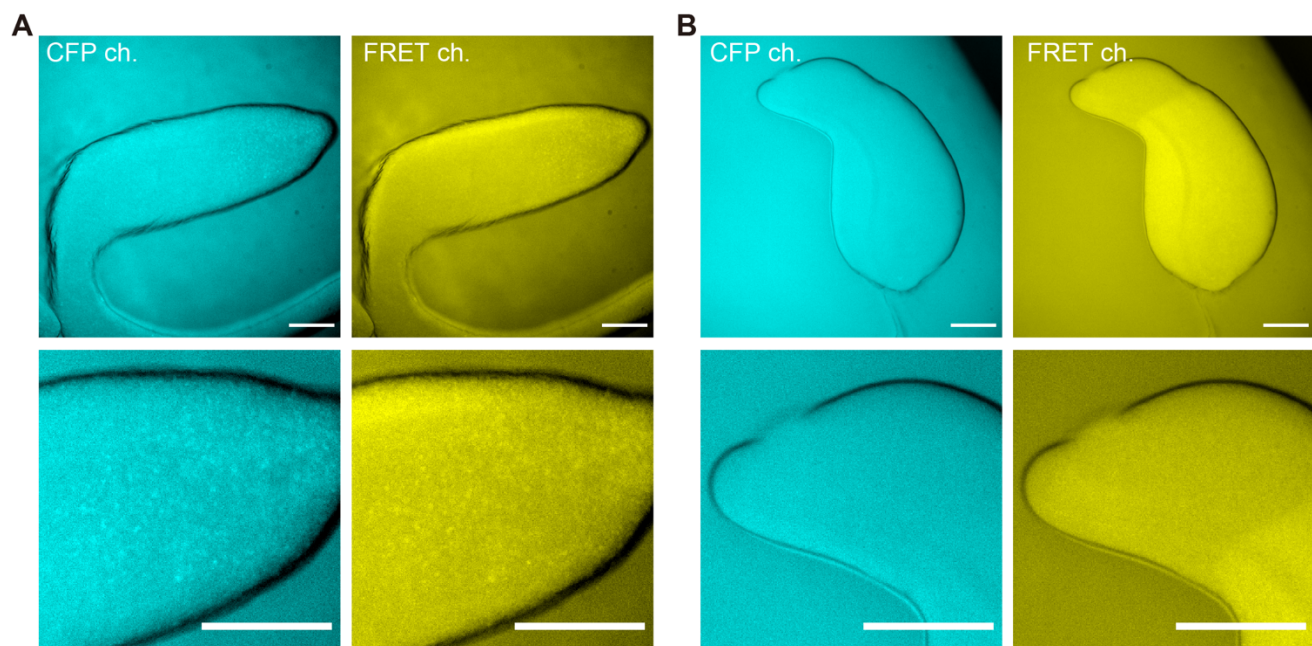


## Supplementary Materials

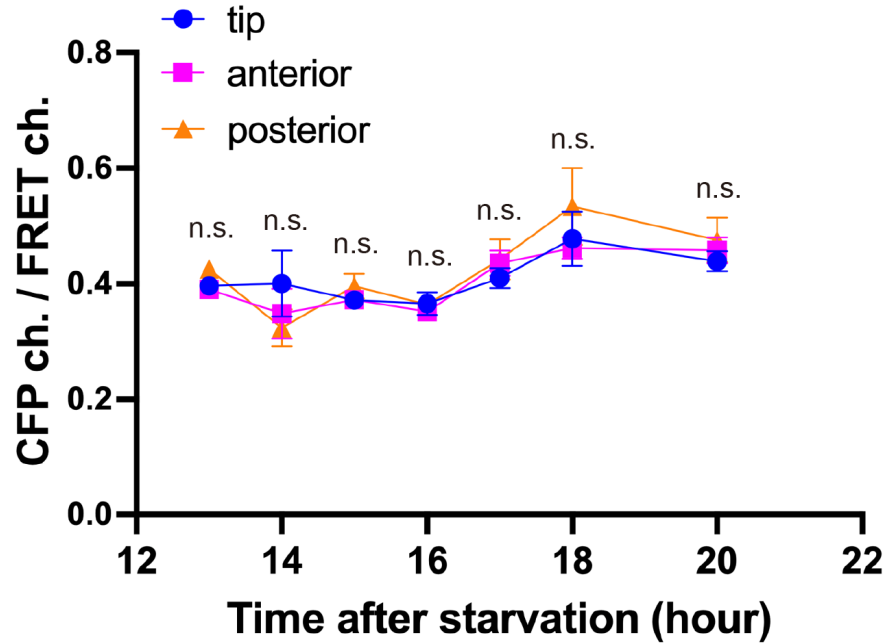
### Visualization of c-di-GMP signal in *Dictyostelium* multicellular body

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**Supplementary Figure S1.** Representative fluorescence images of autofluorescence in *Dictyostelium* multicellular body are shown. **(A)** Fluorescence images of autofluorescence of a multicellular body grown in HL5 medium including glucose. The lower panels show the enlargement of upper images. **(B)** Fluorescence images of autofluorescence of a multicellular body grown in low fluorescence medium. The lower panels show the enlargement of upper images. Scale bars, 100  $\mu\text{m}$ .



**Supplementary Figure S2.** Timelapse measurement of the autofluorescence in AX2 during development. Ratio values of CFP/FRET channels for each region within the multicellular body at each time point after starvation. Mean  $\pm$  standard errors are shown ( $n = 9$ ). Data were assessed with ANOVA with Tukey's multiple comparisons test; n.s., no significant difference.

### Supplementary Table S1

*Dictyostelium discoideum* strains used in this study.

Strain name	Characteristics	Background	Source or reference
AX2	wild type	AX2	Lab stock
<i>dgcA</i> <sup>-</sup>	<i>dgcA</i> <sup>-</sup> (bsR)	AX2	(Chen and Schaap, 2012) NBRP-nenkin (ID: S90402)
mYpet-YcgR-mCypet /AX2	(neoR)	AX2	This study
mYpet-YcgR(R118A)-mCypet /AX2	(neoR)	AX2	This study
mYpet-YcgR-mCypet / <i>dgcA</i> <sup>-</sup>	(neoR)	AX2	This study

### Supplementary Table S2

Plasmids used in this study.

Plasmid name	Characteristics	Backbone	Source or reference
pMMB67EH-Spy	mYpet-YcgR-mCypet	pMMB67EH	(Christen et al., 2010) Addgene (#90102)
pDM304/mYpet-YcgR-mCypet	mYpet-YcgR-mCypet	pDM304 (neoR)	This study
pDM304/mYpet-YcgR(R118A)-mCypet	mYpet-YcgR(R118A)-mCypet	pDM304 (neoR)	This study