# Supporting Information

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### SI Methods

**Gene Constructs.** Two cDNA pools kindly provided by T. Sato were used to obtain zebrafish homologs of Cdt1 (zCdt1) and geminin (zGem). They were synthesized using total RNA isolated from zebrafish embryos at 24–48 hpf. Full-length or partial zCdt1 (GenBank: XM_690072) and zGem (GenBank: NM_200086) cDNA was amplified using primers containing 5′-XhoI and 3′-XbaI sites, and digested products were cloned in-frame into the XhoI/XbaI sites of pcDNA3/mKO2 and pcDNA3/mAG (1), respectively. The resulting constructs were pcDNA/mKO2-zCdt1(1/X) and pcDNA/mAG-zGem(1/X), where X denotes the C-terminal amino acid position of zCdt1 or zGem. cDNA encoding mKO2-zCdt1(1/X), mAG-zGem(1/X), and mAG-hGem(1/60) was amplified using appropriate restriction enzyme sites and digested products were cloned into the BamHI/ClaI sites of pT2KXIGΔin (2, 3) for expression in fish cells. mKO2-hCdt1(30/120) or mAG-hGem(1/110) cDNA was cloned into a fish expression vector carrying the hspa8 promoter (H.S., an unpublished construct; ZFIN, ENSDARG00000068992).

**Imaging of GEM-81 Cells.** A permanent cell line (GEM-81) established from spontaneous goldfish erythrophoromas was obtained from the RIKEN Bio Resource Center (RCB1174). Cells were grown at 27 °C on a 35-mm glass-bottom dish in L-15 (GIBCO) supplemented with 20% FBS. Cells were transiently electroporated with cDNA using Nucleofector solution V (Amaxa, program X-13) and subjected to long-term time-lapse imaging using an inverted microscope (Olympus IX71) with a standard 75-W Xenon lamp, a 20× objective lens (N.A. 0.4), a CCD camera (Olympus, DP30), and two filter cubes: one with excitation (BP520–540HQ) and emission (BP555–600HQ) filters for observing mKO2 fluorescence, and the other with excitation (470DF35) and emission (510WB40) filters for observing mAG fluorescence. Cell morphology was monitored by differential interference contrast (DIC) to follow cell division. Image acquisition and analysis were performed using MetaMorph software (Molecular Devices).

**Zebrafish.** Zebrafish were maintained under standard conditions in the Center for Zebrafish Research at Okazaki Institute for Integrative Bioscience or Riken Brain Science Institute according to established protocols. Zebrafish embryos were allowed to develop at 28 °C and were staged according to Kimmel et al. (4).

**Generation of Transgenic Lines.** The gene for the $G_1$ or $S/G_2/M$ marker was cloned into a plasmid containing the zebrafish Tol2 transposable element (pT2KXIGΔin) (2, 3). This construct was injected along with in vitro-transcribed Tol2 transposase mRNA into wild-type embryos at the 1–4 cell stage, as described previously (5). Embryos were screened under a fluorescence dissecting stereomicroscope for bright fluorescence.

**Primary Cultured Cells from Cecyil.** Dechorionated embryos at 14–20 hpf were mechanically dissociated into cell clumps, which were incubated in a plastic dish (FALCON) in L-15 (GIBCO) supplemented with 20% FBS, 10 units/mL penicillin, and 10 μg/mL streptomycin. Fresh medium was added repeatedly until cells attached to and spread over the dish. Well-dissociated cells were subjected to long-term, time-lapse imaging using an FV300 (Olympus) confocal inverted microscope system equipped with 488 nm (argon) and 543 nm (He/Ne) laser lines. Image acquisition and analysis were performed using MetaMorph or ImageJ software. Cells grown on a plastic dish at 60% confluence were treated with 10 μM EdU for 5 min at room temperature. After fixation with 4% PFA for 10 min at room temperature, cells were treated with reagents from the Click-iT™EdU Alexa Fluor imaging kit (Molecular Probes) containing Alexa647-azide. Image acquisition was performed using an FV1000 (Olympus) confocal microscope system equipped with 473 nm, 559 nm, and 633 nm laser lines.


1. Sakaue-Sawano A, et al. (2008) Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* 132:487–498.
2. Urasaki A, Morva G, Kawakami K (2006) Functional dissection of the Tol2 transposable element identified the minimal *cis*-sequence and a highly repetitive sequence in the subterminal region essential for transposition. *Genetics* 174:639–649.
3. Kawakami K (2004) Transgenesis and gene trap methods in zebrafish by using the Tol2 transposable element. *Methods Cell Biol* 77:201–222.
4. Kimmel CB, et al. (1995) Stages of embryonic development of the zebrafish. *Dev Dyn* 203:253–310.
5. Higashijima S, et al. (1997) High-frequency generation of transgenic zebrafish which reliably express GFP in whole muscles or the whole body by using promoters of zebrafish origin. *Dev Biol* 192:289–299.

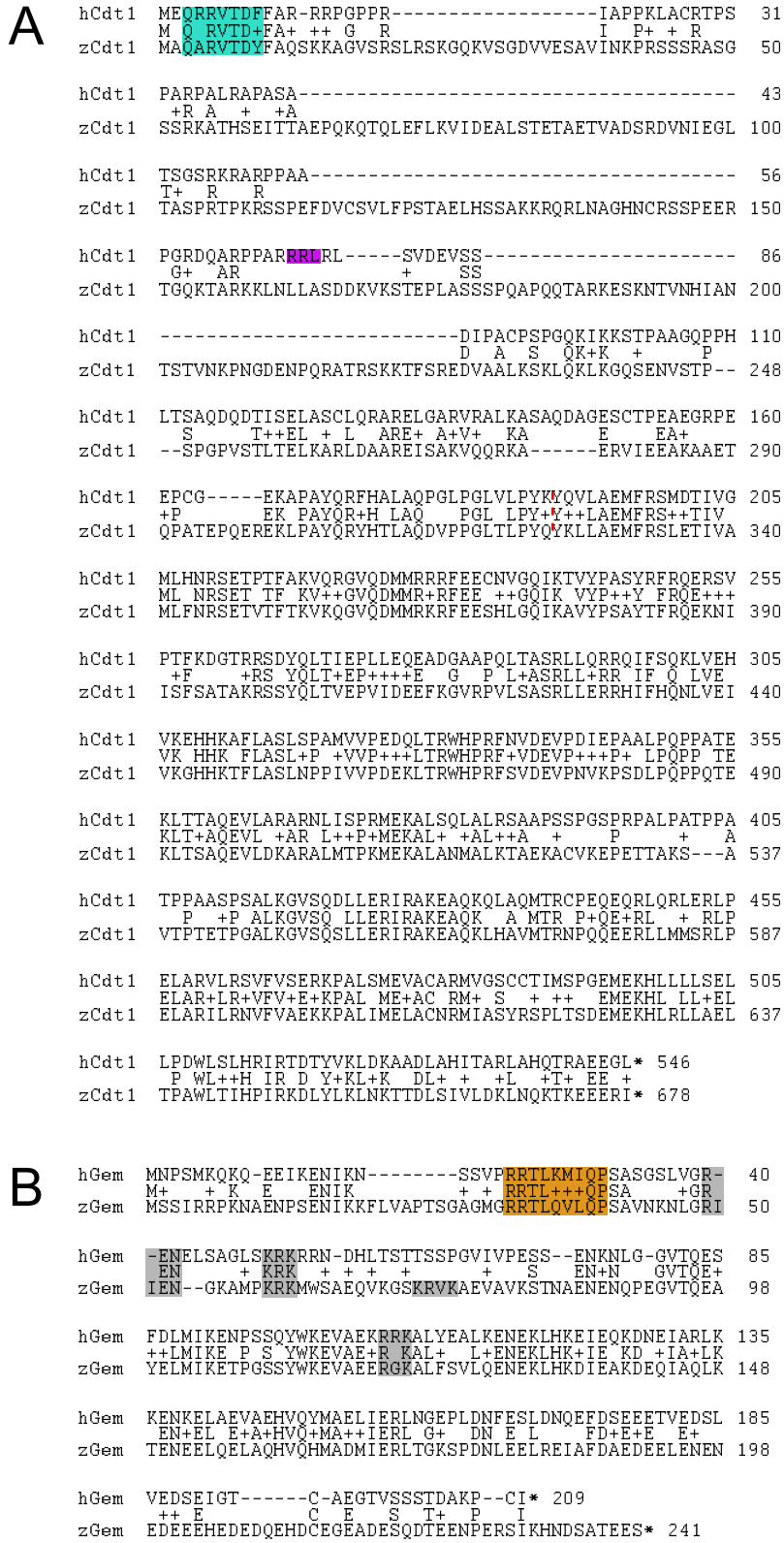



**Fig. S1.** Comparison of the amino acid sequences of the zebrafish Cdt1 and geminin with their human homologs. Identical amino acids are indicated in the middle. Conserved substitutions are indicated by +. (*A*) Amino acid alignment between hCdt1 (human Cdt1) and zCdt1 (zebrafish Cdt1). Cyan box, PIP box or QXRVTDF motif (amino acids 3–9); violet box, Cy motif (amino acids 68–70 of hCdt1). (*B*) Amino acid alignment between hGem (human geminin) and zGem (zebrafish geminin). Orange box, D (destruction) box; black box, NLS.

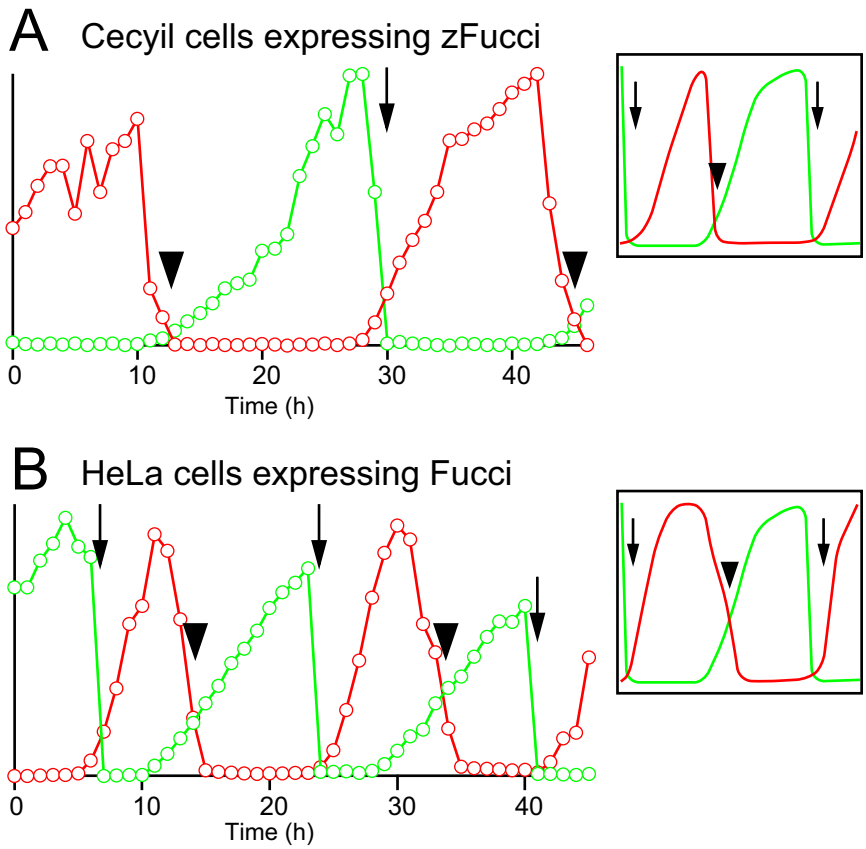



**Fig. S2.** Comparison of temporal profiles between Fucci and zFucci. (*A*) Typical time course of zFucci fluorescence intensity (mKO2-zCdt1(1/190) and mAG-zGem(1/100)) with a schematic diagram (*Inset*). Arrows, cell division. Arrowheads, $G_1$/S transition. (*B*) Typical time course of Fucci fluorescence intensity (mKO2-hCdt1(30/120) and mAG-hGem(1/110)) with a schematic diagram (*Inset*). Arrows, cell division. Arrowheads, $G_1$/S transition. Fish cells were highly mobile, making it difficult to track both $G_1$/S (orange to green) and M/$G_1$ (green to orange) transitions.

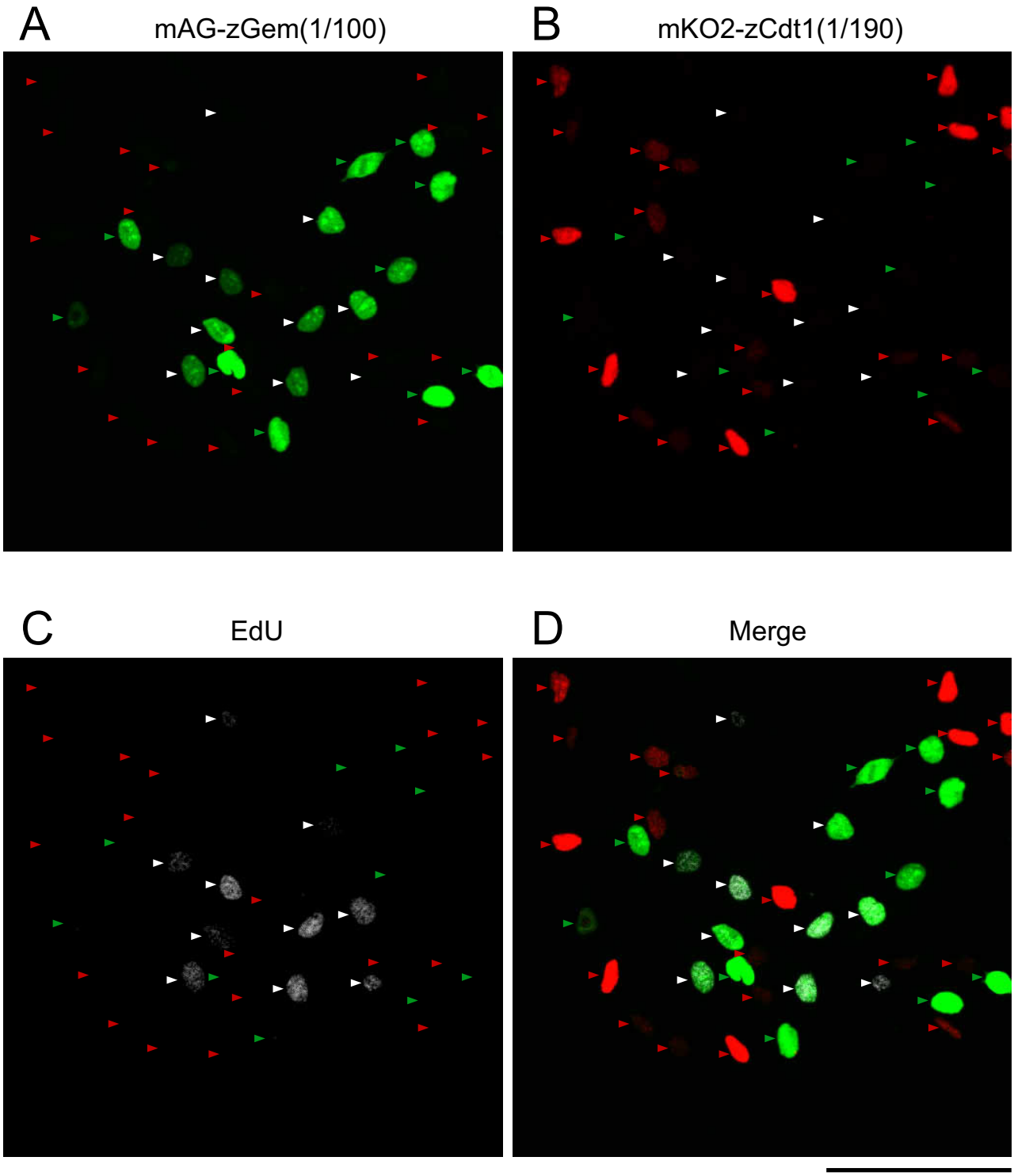



**Fig. S3.** Characterization of zFucci for cell cycle progression. (*A–D*) A wide field image of Cecyil cells expressing zFucci: mKO2-zCdt1(1/190) and mAG-zGem(1/100) and detection of incorporated EdU. Red, green, and white arrows indicate $G_1$, S, and $G_2$ phases, respectively. (Scale bar, 100 $\mu$m.)

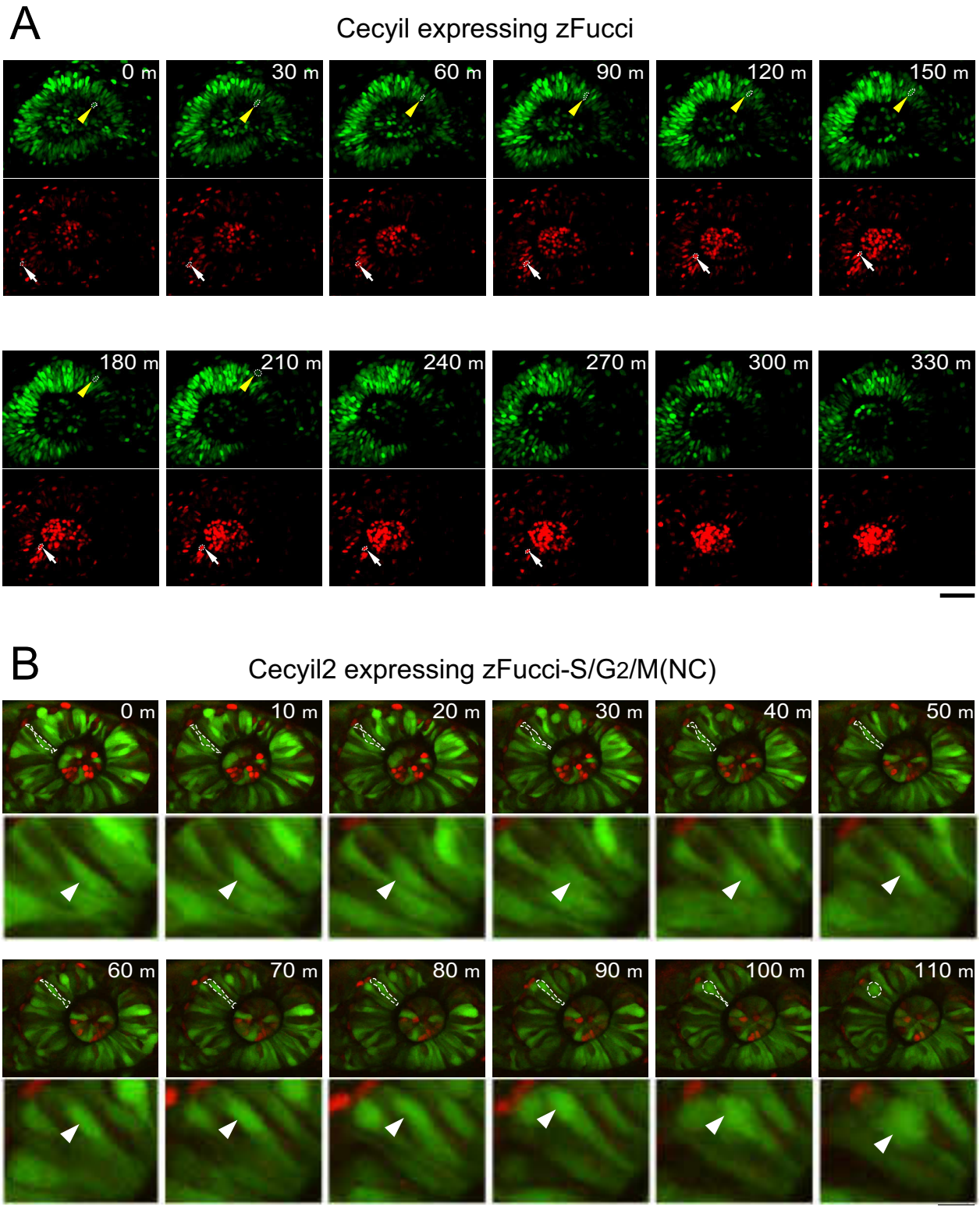
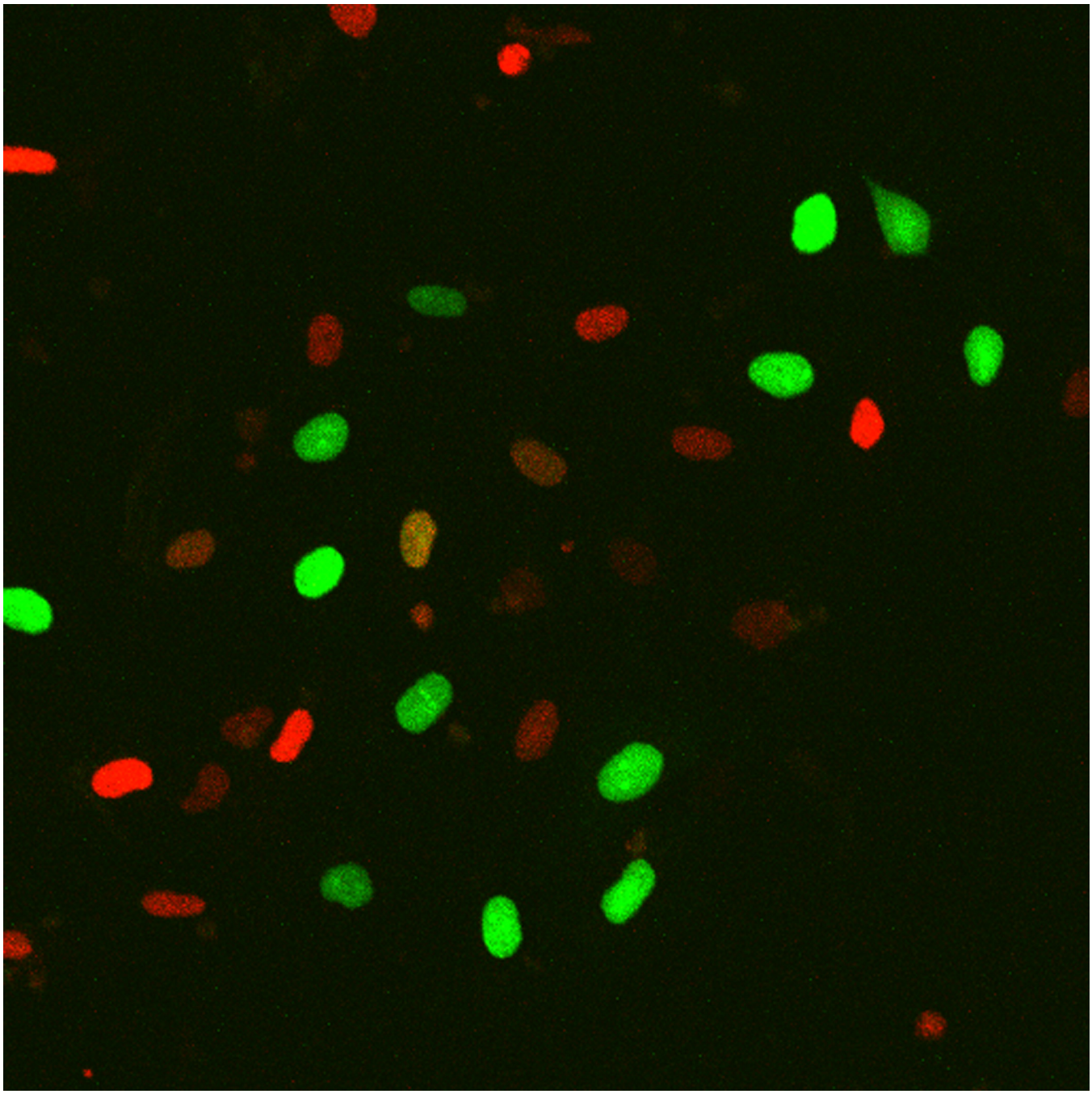
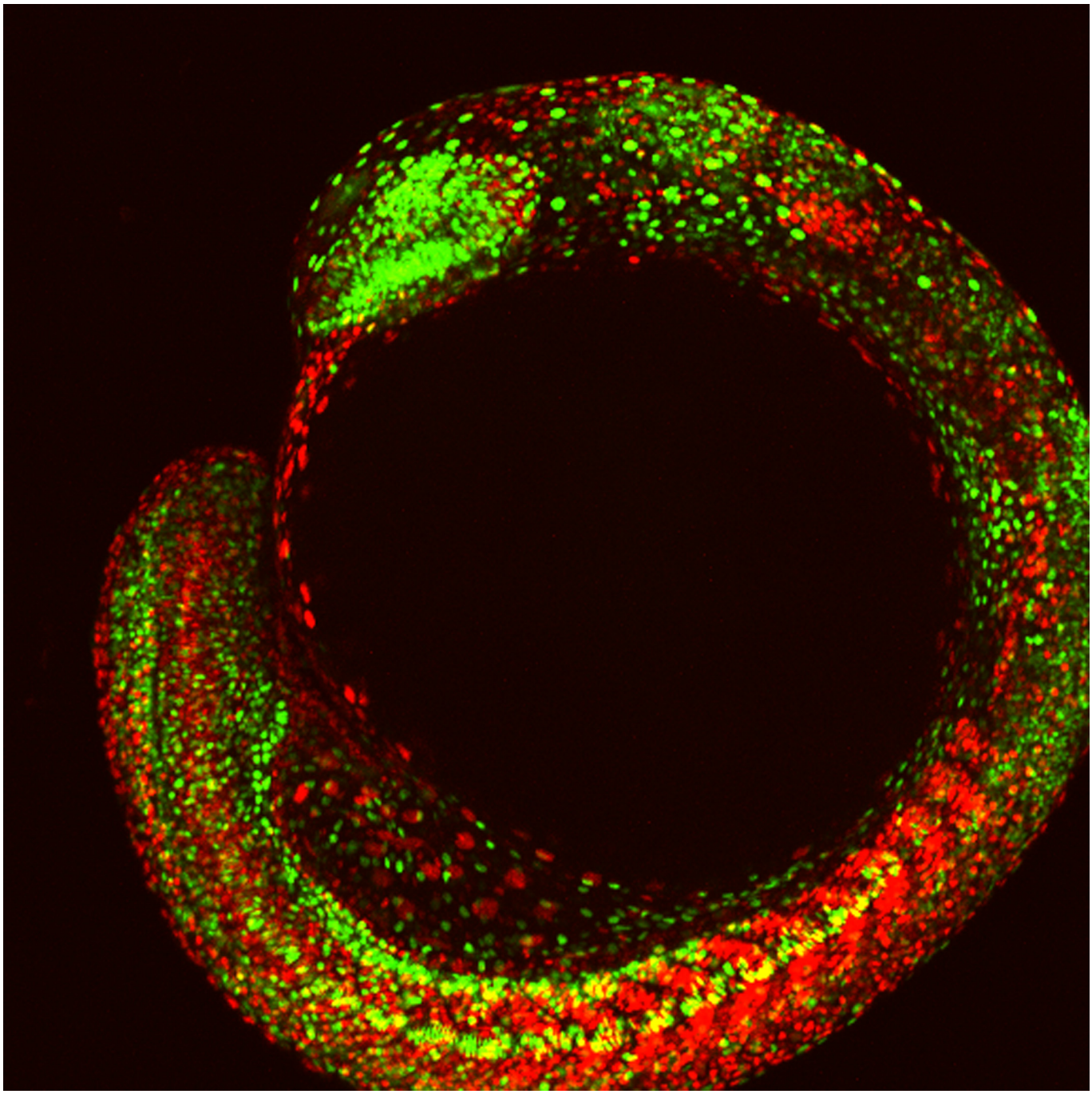



**Fig. S4.** Interkinetic nuclear migration in the retina at early stages. An embryo at 22 hpf was anesthetized with 0.00168% Tricaine and embedded in 0.3% agar on a culture dish with the eye facing up. Fluorescence images were collected using an Olympus FV1000 upright confocal microscope to visualize interkinetic nuclear migration, namely, the apical-basal movement of nuclei in phase with the cell cycle. The developing lens at the center of the eye showed intense orange signal, suggesting early differentiation of the lens. (*A*) Fluorescence images of an eye from a Cecyil embryo expressing zFucci: mAG-zGem(1/100) (*Top*) and mKO2-zCdt1(1/190) (*Bottom*). Each nucleus in the sheet emitted either green or orange fluorescence. (Scale bar, 50 $\mu$m.) (*B*) Fluorescence images of an eye from a Cecyil2 embryo expressing zFucci-S/$G_2$/M(NC): mAG-hGem(1/60) and mKO2-zCdt1(1/190) (merged). (Scale bar, 50 $\mu$m.)



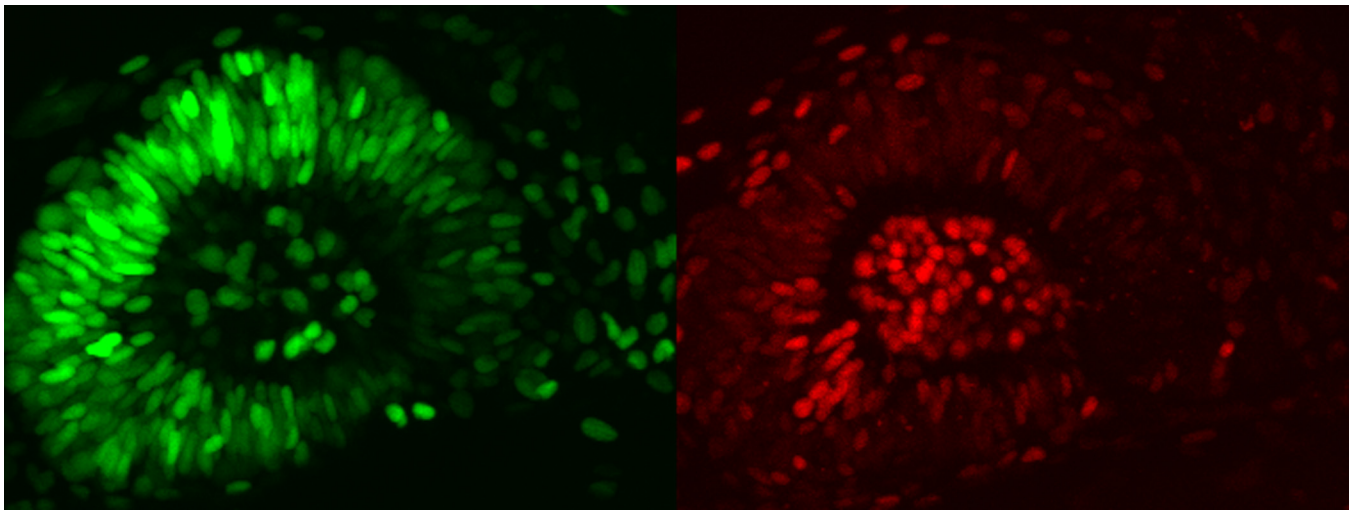
**Movie S1.** Time-lapse imaging of cells prepared from Cecyil embryos. Cells were grown on a glass-bottom dish, and time-lapse imaging was performed with an FV300 (Olympus). Images were acquired every 3 min. Playback speed is 11,118 × real time. Total imaging time, 52.5 h.

Movie S1



**Movie S2.** Time-lapse 3D imaging of a Cecyil embryo expressing zFucci. Three-dimensional images were obtained every 5 min; each z-stack image was created by compiling 40 confocal images. Playback speed is 5,400 × real time. Total imaging time, 15 h. Because Fucci's signals result from accumulation of mKO2 or mAG in $G_1$ or $S/G_2/M$ phases, respectively, the signal intensity depends on the length of each cell-cycle phase. Whereas the duration of $S/G_2/M$ phase is relatively constant, that of $G_1$ phase varies substantially. Thus, whereas the green fluorescence is constant throughout embryogenesis, the orange fluorescence is very weak at early stages because of the rapid cell cycling, but is very strong in later stages, particularly in differentiated cells. A drastic change in green-to-orange color balance was also noticeable during segmentation, as shown in Fig. 2 and Movie S2.

Movie S2



**Movie S3.** Time-lapse 3D imaging of a Cecyil retina expressing zFucci. Three-dimensional images were obtained every 10 min; each z-stack image was created by compiling 32 confocal images. Playback speed is 1,650 × real time. Total imaging time, 5.5 h.

Movie S3

**Table S1. Construction of a $G_1$ marker for fish cells**

| | Performance in GEM-81 cells | | Performance in transgenic zebrafish | | | |
|---|---|---|---|---|---|---|
| Construct | Localization | ON/OFF regulation | TG lineTg (promoter:gene)[allele name] | Fluoresence signal (~) | ON/OFF regulation | |
| mKO2-hCdt1(30/120) | Nuc | X | Tg(hspa8:mKO2-hCdt1(30/120))[rw0401a*] | 50%epiboly | ND | |
| | | | Tg(hspa8:mKO2-hCdt1(30/120))[rw0401b*] | 50%epiboly | X | |
| | | | Tg(hspa8:mKO2-hCdt1(30/120))[rw0401c*] | 50%epiboly | X | |
| mKO2-zCdt1(1/100) | Nuc+Cyt | X | | | | |
| mKO2-zCdt1(1/120) | Nuc+Cyt | X | | | | |
| nKO2-zCdt1(1/138) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/138))[rw0402a*] | 50%epiboly | X[a] | Bright |
| | | | Tg(EF1α:mKO2-zCdt1(1/138))[rw0402b*] | 50%epiboly | X[a] | |
| mKO2-zCdt1(1/156) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/156))[rw0403a*] | 50%epiboly | ND | |
| mKO2-zCdt1(1/177) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/177))[rw0404a] | 50%epiboly | ND | |
| | | | Tg(EF1α:mKO2-zCdt1(1/177))[rw0404b*] | 50%epiboly | O | |
| | | | Tg(EF1α:mKO2-zCdt1(1/177))[rw0404c] | 50%epiboly | ND | |
| mKO2-zCdt1(1/190) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/190))[rw0405a] | 50%epiboly | ND | |
| | | | Tg(EF1α:mKO2-zCdt1(1/190))[rw0405b**] | 50%epiboly | O | Bright; cross-bred with Tg(EF1α:mAG-zGem(1/100))[rw0410 h]: Cecyil |
| | | | Tg(EF1α:mKO2-zCdt1(1/190))[rw0405c] | 50%epiboly | ND | |
| | | | Tg(EF1α:mKO2-zCdt1(1/190))[rw0405d**] | 50%epiboly | O | Bright; cross-bred with Tg(EF1α:mAG-hGem(1/60))[rw0412a]: Cecyil2 |
| mKO2-zCdt1(1/217) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/217))[rw0406a*] | | ND | |
| | | | Tg(EF1α:mKO2-zCdt1(1/217))[rw0406b*] | | ND | |
| mKO2-zCdt1(1/236) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/236))[rw0407a*] | 10 somite | O | |
| mKO2-zCdt1(1/250) | Nuc | O | Tg(EF1α:mKO2-zCdt1(1/250))[rw0408a*] | | ND | |

Nuc, nucleus; Cyt, cytoplasm. TG (transgenic) lines with asterisks are being maintained (*) and are available (**). ND, not determined. X[a], fluorescence fluctuation was not clear. O, pass; X, failure.

**Table S2. Construction of S/$G_2$/M marker for fish cells**

| | Performance in GEM-81 cells | | Performance in transgenic zebrafish | | | |
|---|---|---|---|---|---|---|
| Construct | Localization | ON/OFF regulation | TG line Tg(promoter:gene)$^{\text{allele name}}$ | Fluoresence signal (~) | ON/OFF regulation | |
| mAG-hGem(1/110) | Nuc | O | Tg(hspa8:mAG-hGem(1/110))$^{rw0409a*}$ | 50% epiboly | O | |
| | | | Tg(hspa8:mAG-hGem(1/110))$^{rw0409b*}$ | 50% epiboly | ND | |
| | | | Tg(hspa8:mAG-hGem(1/110))$^{rw0409c*}$ | 50% epiboly | O | Bright |
| | | | Tg(hspa8:mAG-hGem(1/110))$^{rw0409d}$ | 50% epiboly | O | Bright |
| mAG-zGem(1/100) | Nuc | O | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410a}$ | 50% epiboly | ND | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410b}$ | 50% epiboly | ND | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410c}$ | 50% epiboly | ND | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410d}$ | 50% epiboly | O | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410e}$ | 50% epiboly | ND | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410f}$ | 50% epiboly | ND | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410\ g*}$ | 50% epiboly | O | Bright |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/100))$^{rw0410\ h**}$ | 50% epiboly | O | Bright; cross-bred with Tg(EF1$\alpha$:mKO2-zCdt1(1/190))$^{rw0405b}$: Cecyil |
| mAG-zGem(1/120) | Nuc | O | Tg(EF1$\alpha$:mAG-zGem(1/120))$^{rw0411a}$ | 50% epiboly | ND | |
| | | | Tg(EF1$\alpha$:mAG-zGem(1/120))$^{rw0411b}$ | 50% epiboly | O | |
| mAG-hGem(1/60) | Nuc+Cyt | O | Tg(EF1$\alpha$:mAG-hGem(1/60))$^{rw0412a**}$ | 50% epiboly | O | Bright; cross-bred with Tg(EF1$\alpha$:mKO2-zCdt1(1/190))$^{rw0405d}$: Cecyil2 |
| | | | Tg(EF1$\alpha$:mAG-hGem(1/60))$^{rw0412b}$ | 50% epiboly | O | |

Nuc, nucleus; Cyt, cytoplasm. TG lines with asterisks are being maintained (*) and are available (**). ND, not determined. O, pass; X, failure.