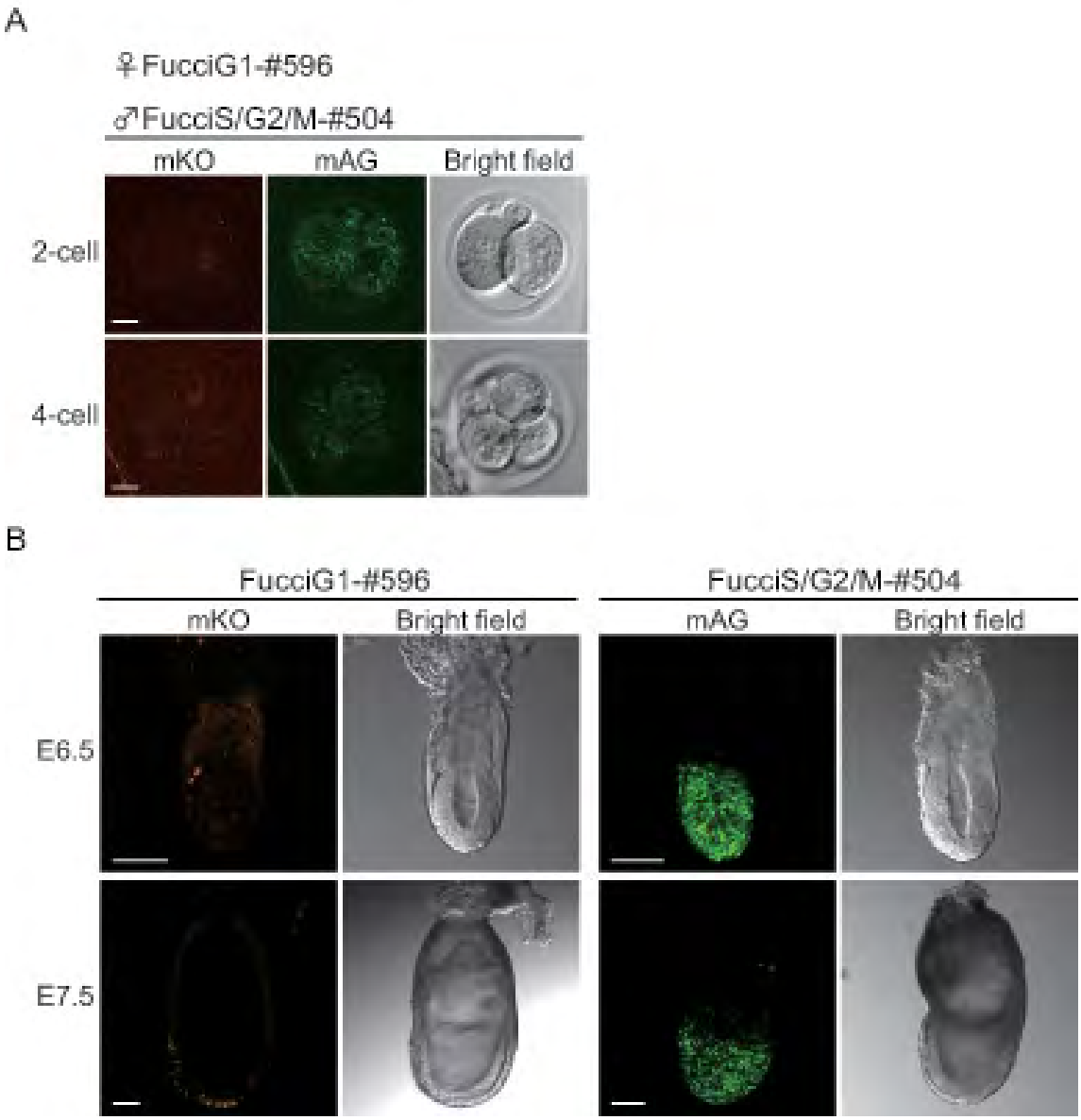

Fig. S1. Fucci signals in *CAG-Fucci* transgenic lines. (**A**) Images of two-cell and four-cell double hemizygous embryos obtained by the cross between FucciG$_1$ (*CAG-mKO2-hCdt1(30/120))#596* female and FucciS/G$_2$/M (*CAG-mAG-hGm(1/110))#504* male previously generated (Sakaue-Sawano et al., 2008). Scale bar: 20 μm. (**B**) Images of E6.5 and E7.5 hemizygous embryos obtained by the cross between wild-type female and *CAG-mKO2-hCdt1(30/120)#596* or *CAG-mAG-hGme#504* male, respectively. Scale bar: 100 μm. mKO and mAG represent mKusabira-Orange2-hCdt1(30/120) and mAzami-Green-hGem signals, respectively.

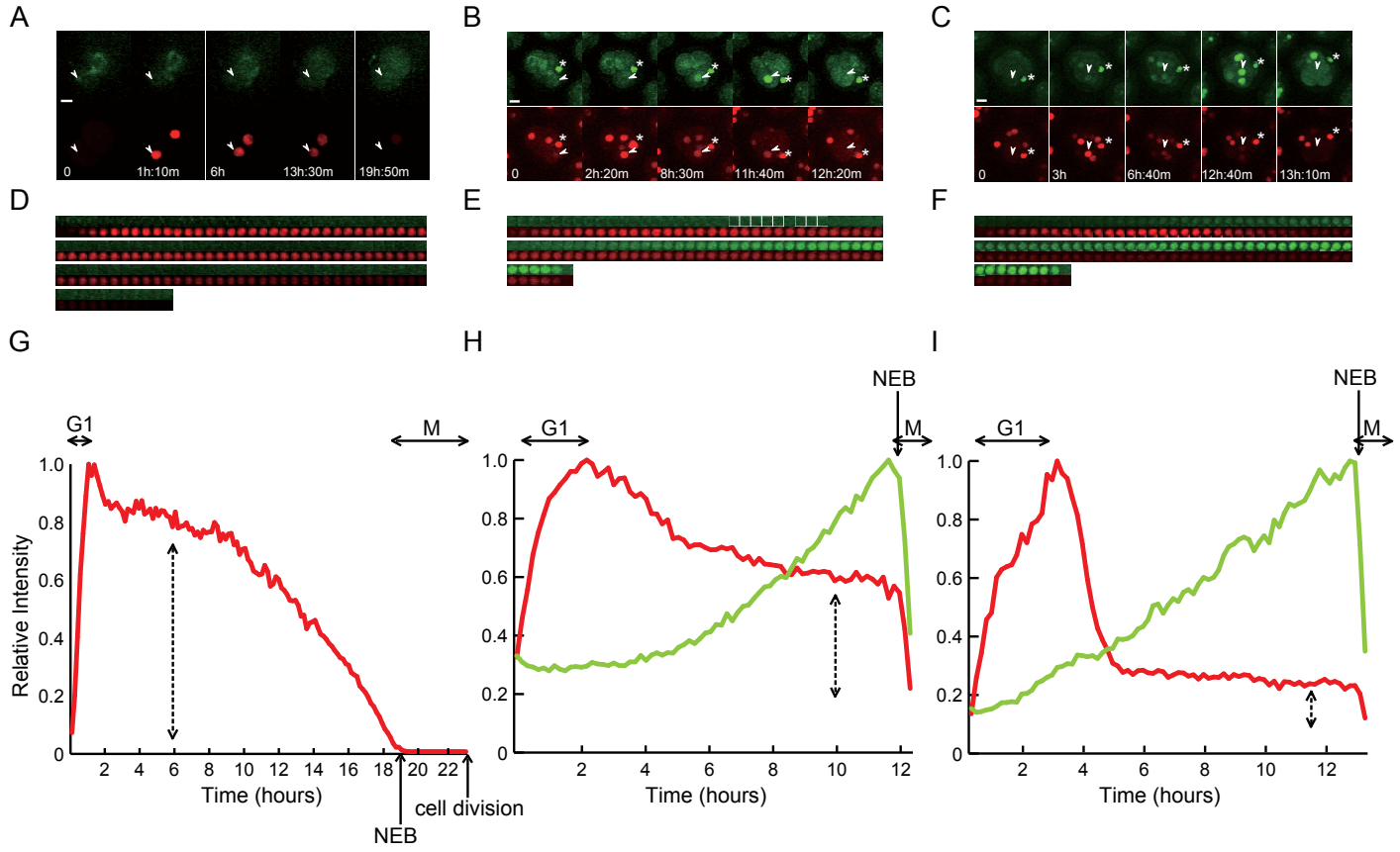

Fig. S2. Fucci2 signals in *R26p-Fucci2* from two-cell to eight-cell stages. (**A-C**) Snapshot images of time-lapse observations from the two-cell to eight-cell stages in hemizygous embryos obtained by crossing a *R26p-Fucci2* female with a wild-type male. White arrowheads indicate nuclei that are tracked. Scale bar: 20 μm. Asterisks indicate polar body. (**D-F**) Higher magnification images of nuclei indicated by arrowheads in A-C, respectively, at 10-minute intervals. The tracked nuclei are those indicated by white arrowheads in A-C. (**G-I**) Changes in signal intensities of Fucci2 probes. mCherry-hCdt1(30/120) and mVenus-hGem(1/110) signals in the nuclei indicated by white arrowheads in A-C are given by red and green lines, respectively. The peak intensities of mCherry and mVenus during the observation periods are each set to 1.0, and each intensity at each time point is given as the relative value to them. The mCherry-hCdt1(30/120) signal peak intensity at the four-cell and eight-cell stages was at the same level as that at the 16-cell stage (Fig. 1E) and that at the two-cell stage it was about 50-fold greater than at the 16-cell stage. mCherry signals at S/G_2/M phases indicated by double-arrowed lines may represent leftovers of maternal mCherry-hCdt1(30/120). mVenus-hGem(1/110) signal was absent at the one-cell and two-cell stage; its peak intensity at the four-cell stage was about half of that at the 16-cell stage and at the 8-cell stage it was comparable with that at the 16-cell stage. In A. the green fluorescence is absent in polar body, but in B,C it is present (asterisks). This green fluorescence is actually mCherry-hCdt1(30/120) red fluorescence by the 488 nm excitation light for a green fluorescent protein (Shioi et al., 2011); a large amount of maternal mCherry-hCdt1(30/120) may be present in polar body. A was detected using a band-pass filter (Olympus, 495-540 nm) for mVenus and a long-pass filter (575nm) for mCherry, whreas B and C were detected with a triple-band filter (YOKOGAWA, transmission: 495-555 nm, 575-635 nm and 665-775 nm).

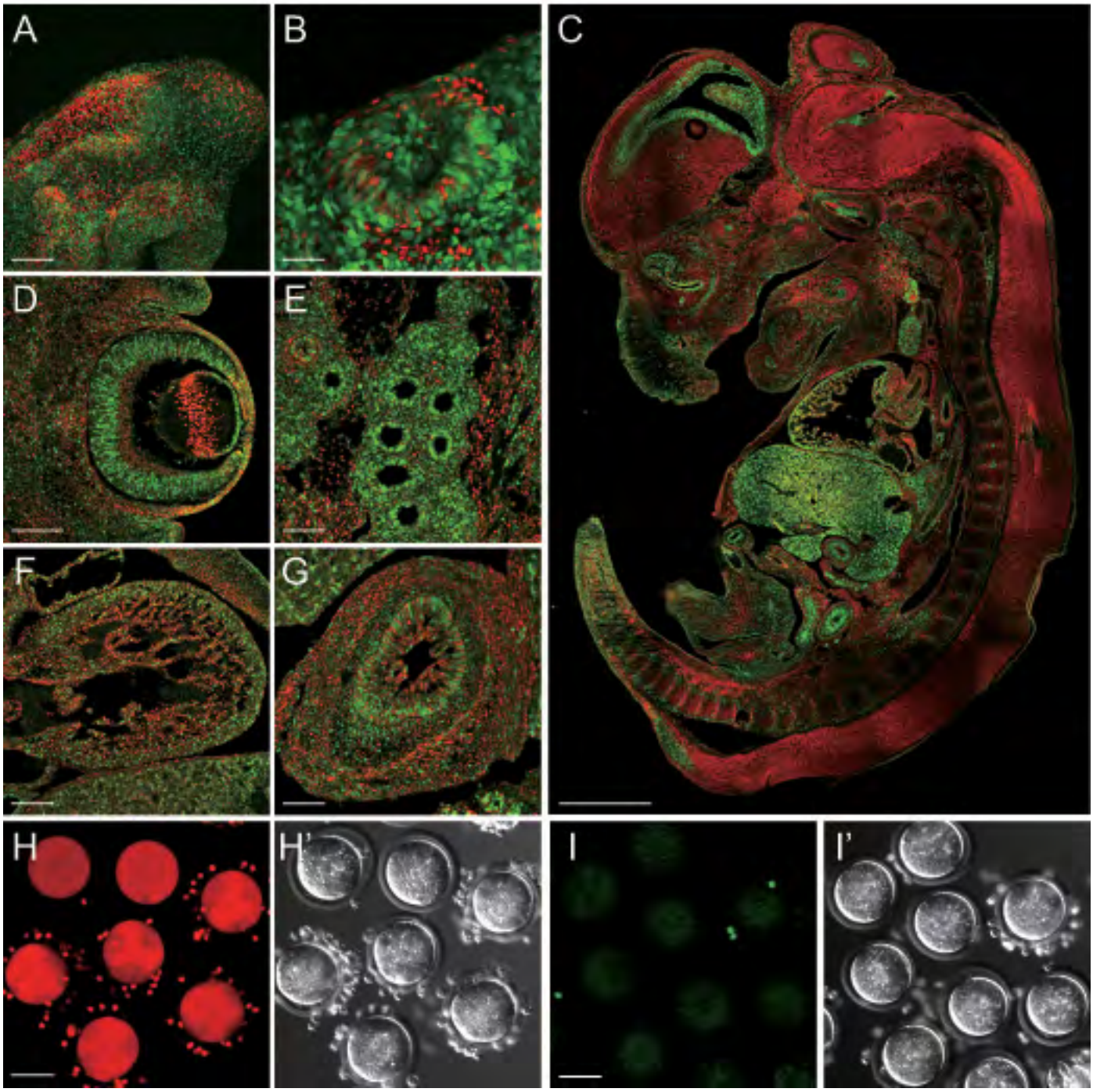

Fig. S3 Fucci2 signals in E9.5 and E13.5 *R26p-Fucci2* embryos and in *R26-mCherry-hCdt1(30/120)* and *R26-mVenus-hGem(1/110)* oocytes. (**A**,**B**) Higher magnification images of Fucci2 signals in E9.5 *R26p-Fucci2* embryos; (A) surface ectoderm at rhombencephalic region and (B) otic pit. (**C**) Fucci2 signals in an sagittal frozen section of an E13.5 *R26p-Fucci2* embryo; and (**D-G**) higher magnification images. (D) Eye, (E) lung, (F) heart and (G) stomach. (H) mCherry-hCdt1(30/120) signal in *R26-mCherry-hCdt1(30/120)* oocytes and (I) mVenus-hGem(1/110) signal in *R26-mVenus-hGem(1/110)* oocytes. (H′,I′) Bright-field views of H,I, respectively. Scale bars: in A, 200 μm; in B, 100 μm; in C, 1 mm; in D-G, 100 μm; in I,J, 50 μm.

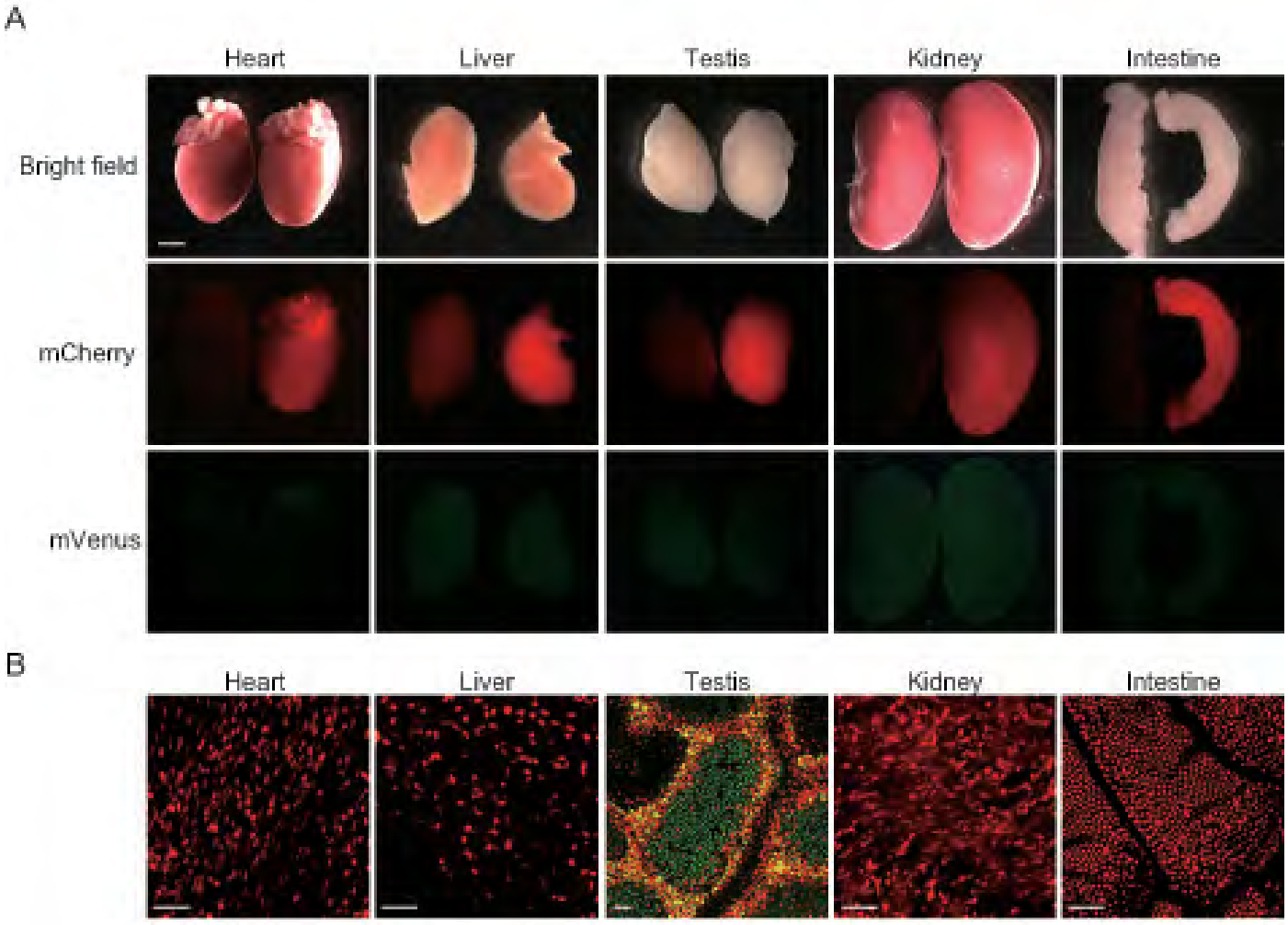

Fig. S4 Fucci2 signals in adult *R26p-Fucci2* organs. Images of live organs: heart, liver, testis, kidney and intestine. (**A**) Stereoscopic microscope images; (**B**) confocal microscope images. mCherry and mVenus represent red mCherry-hCdt1(30/120) and green mVenus-hGem(1/110) signals, respectively. Green, red and yellow signals were found in testis; in other tissues most of the cells exhibited red signals. Scale bars: 2 μm in A; 50 μm in B.

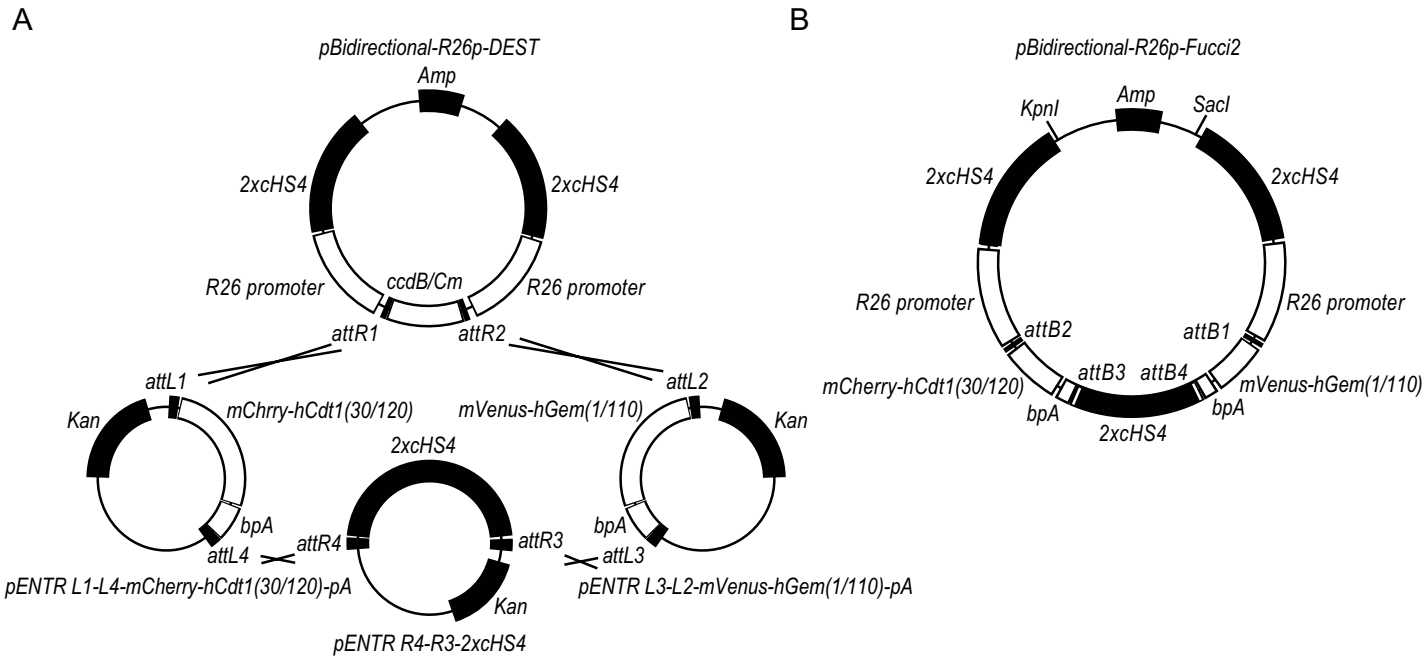

Fig. S5 Construction of *R26p-Fucci2* transgene by gateway technology. (**A**) *mCherry-hCdt1(30/120)* and *mVenus-hGem(1/110)* cDNAs were cloned into the *pENTR L1-L4* and *pENTR L3-L2* vector with the bovine growth hormone polyadenylation sequence, respectively. *2xcHS4* was cloned into the *pENTR R4-R3* vector. The *pBidirectional-R26p-DEST* is a destination vector to generate *pBidirectional-R26p Fucci2*; this has two *R26* promoters with two *2xcHS4* on opposite sides. The DNA fragments in the *pENTR* vectors were recloned into the *pBidirectional-R26p-DEST* vector by LR recombination reaction that generates *attB* sites. (**B**) The *pBidirectional-R26p-Fucci2* vector. *attL*, *attR* and *attB,* λ phase recombination sites; *ccdB*, negative selection gene in bacteria; *bpA*, bovine growth hormone polyadenylation sequence; *Kan*, kanamycin resistance gene; *Amp*, ampicillin resistance gene; *Cm*, chloramphenicol resistance gene; *cHS4*, chicken hypersensitive site 4.