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SUPPLEMENTARY MATERIAL

corresponding to:

Live imaging reveals spatial separation of parental chromatin until the four-cell stage in *Caenorhabditis elegans* embryos

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SUPPL. TABLE S1

CELL CYCLE LENGTH IN CONTROL DENDRA2-H2B EMBRYOS AND IN DENDRA2-H2B EMBRYOS IN WHICH CHROMATIN WAS PHOTOCONVERTED

Embryo	PC	Timing of division				
		AB	P1	Aba/p	EMS	P2
1	no	14.0	18.0	32.5	34.0	36.5
2*	no	12.2	15.8	28.0	31.0	34.0
3	no	13.0	15.0	26.0	28.0	32.0
4	no	13.5	15.0	28.0	30.0	34.0
5	no	13.5	15.0	27.0	29.5	32.5
6	no	13.0	15.0	27.0	30.0	34.0
7	no	13.0	15.0	27.0	29.0	32.0
8	no	13.0	15.0	25.0	29.0	32.0
	AV	**13.1**	**15.5**	**27.6**	**30.1**	**33.4**
	SD	**0.4**	**0.7**	**1.5**	**1.2**	**1.3**
9	yes	12.0	14.0	24.0	28.0	30.0
10	yes	12.5	14.5	30.0	32.0	
11	yes	14.0	16.0	28.0	31.0	34.0
12	yes	14.0	16.0	27.0	30.0	35.0
13	yes	14.0	16.0	28.0	31.0	34.5
14	yes	12.0	14.0	26.0	28.0	32.0
15	yes	16.0	18.0	32.0	34.0	40.0
16	yes	16.0	18.0	31.0	35.0	38.0
17	yes	15.0	18.0	31.0	34.0	37.0
18	yes	14.0	16.0	29.0	32.0	36.0
19	yes	14.0	16.0	30.0	32.0	36.0
	AV	**14.0**	**16.0**	**28.7**	**31.5**	**35.3**
	SD	**1.0**	**1.1**	**1.9**	**1.8**	**2.2**

When indicated, the chromatin in one of the pronuclei was photoconverted (PC) using the 405 nm laser line as described in the Materials and Methods section. The subsequent development was imaged at intervals of 1-2 minutes. Movie number 2 (*) was acquired using DIC illumination, all others using fluorescence imaging. The time at which the metaphase plate was observed in the 1-cell embryo was set at 0.0. The time to the next metaphase was measured for each of the indicated blastomeres from the imaging data. No significant differences were found between irradiated (bottom) and control (top) embryos. AV, average; SD, standard deviation.

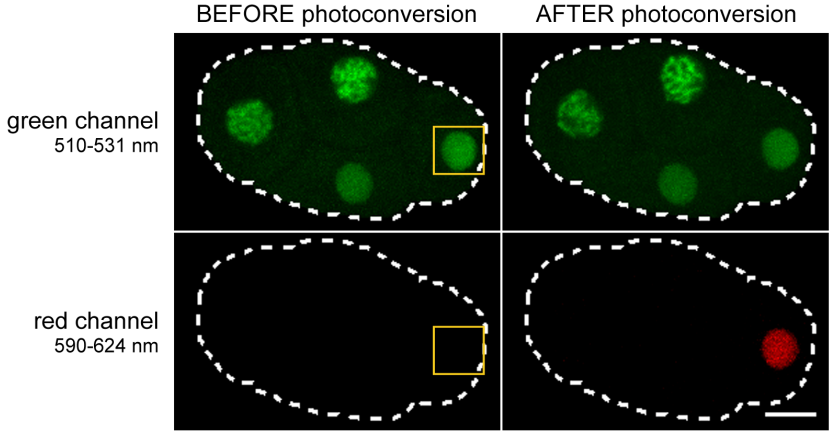

Fig. S1. Photoconversion of Dendra2-labeled chromatin in a *C. elegans* embryo. *The P2 nucleus of a living 4-cell embryo expressing Dendra2-H2B (strain JBL1) was illuminated with 405 nm light. Left and right panels display embryo before and after photoconversion, respectively. The fluorescence signal at λ = 510 nm-531 nm (green channel) is shown on top. The fluorescence signal at λ = 590 nm-624 nm (red channel) is shown at bottom. The region targeted for photoconversion comprised only the P2 nucleus (boxed). Note the appearance of a strong signal in the red channel in the P2 nucleus after photoconversion. Shown are maximum projections of image stacks corresponding to an embryonic cross-section of 10 μm. The contour of the embryo is depicted by a dotted line. Scale bar, 5 μm.*

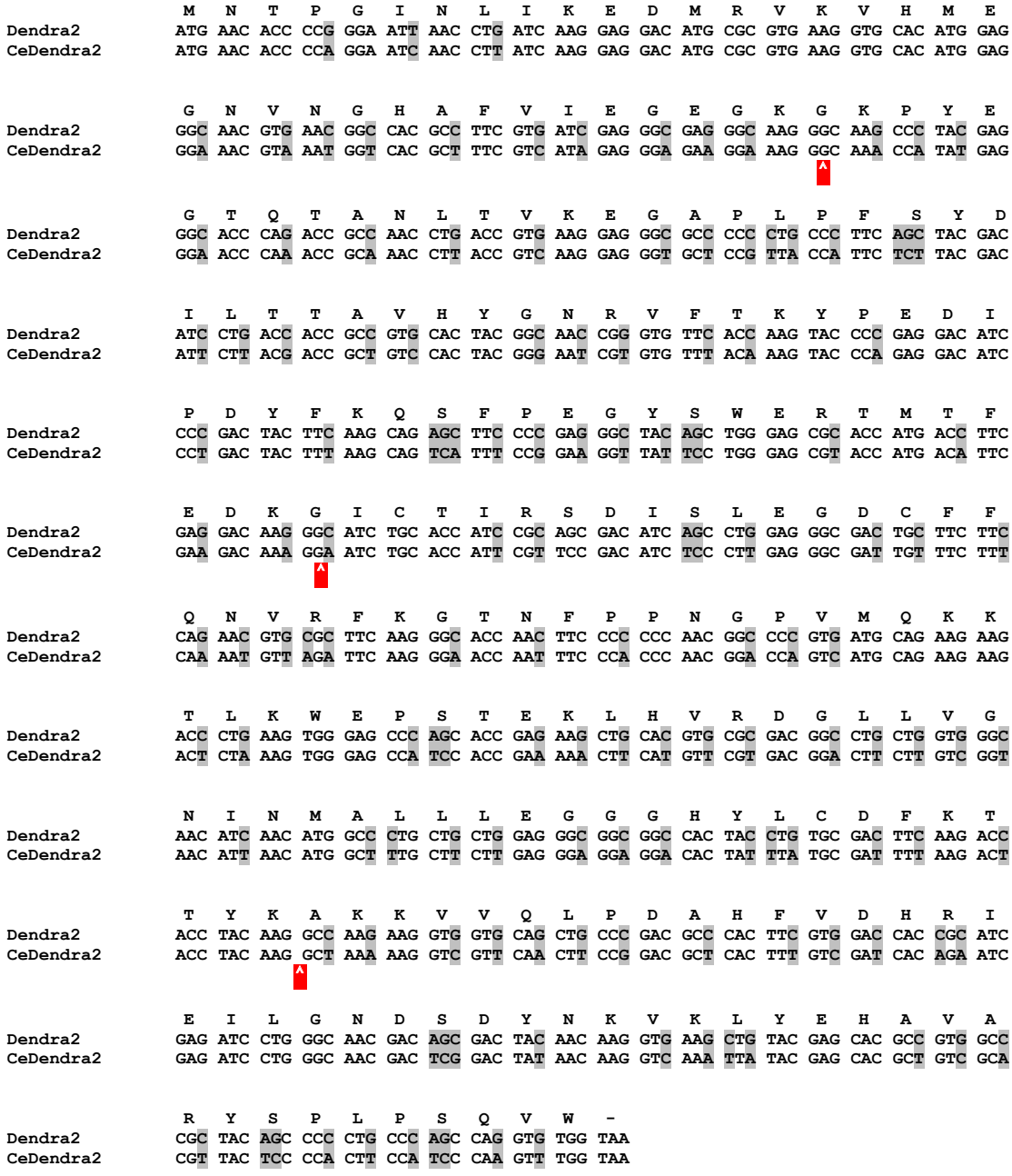

Fig. S2. Alignment of the Dendra2 coding sequence and the Dendra2 coding sequence optimized for expression in *C. elegans*. *The identity is 79% (125/230 codons were optimized). Nucleotide changes are highlighted in gray. The percentage of AT is 53% in the optimized sequence (up from 37% in the starting sequence). The positions of the 3 short introns that were inserted in the expression cassette are indicated by arrowheads. The predicted protein sequence is shown.*

Video S1. Expression of Dendra2-H2B in the *C. elegans* strain JBL1 visualized by single plane illumination microscopy. *The Dendra2-H2B signal is shown in green. The autofluorescence signal from gut granules (obtained through excitation with green light) was pseudo-colored in red and overlaid on the Dendra2-H2B signal. The fusion protein is expressed in the gonads and in the embryos inside the uterus. Note the more intense expression of the transgene starting in pachytene nuclei. Dendra2-H2B is expressed in spermatozoa in this strain.*

Video S2. Spatial distribution of parental chromatin in the early *C. elegans* embryo. *The male pronucleus was photoconverted in the early zygote of the Dendra2-H2B-expressing strain (JBL1), after which imaging of the chromatin from the photoconverted pronucleus (red) and from the other pronucleus (green) was performed for 22 minutes, almost until the 4-cell stage. Shown are maximum projections of image stacks (~30 optical sections, ~15 μm in total thickness) acquired at 30-second intervals. In order to account for the variations in signal intensity that result from varying levels of chromatin condensation during the cell cycle, the brightness was adjusted independently for each image. The P0, AB and P1 nuclei are labeled. The contour of the embryo is depicted by a dotted line. The breakdown of the pronuclear envelopes occured at time 0:00. Note the clear segregation of the photoconverted chromatin in the 2-cell embryo. Note also that since the division of the AB blastomere occurs along the z-axis of the imaging volume, the separation of paternally- and maternally-derived chromatin in the daughter nuclei is not faithfully represented on the maximum z-projections that are shown here (time points 13:00 to 15:00). At the beginning of the movie (time points 0:00 to 2:30), the arrows point to the photoconverted chromatin in order to highlight the 180° rotation of the nascent nuclei. Time is indicated in minutes:seconds. Scale bar, 5 μm.*

Video S3. Rotational movement of cytoplasmic P granules during division of the *C. elegans* zygote. *A worm strain expressing Dendra2-H2B and GFP-labeled PGL-1, a marker of P granules, was imaged at 10-second intervals starting at zygotic metaphase. The Dendra2-H2B signal, which is much weaker than the GFP-PGL-1 one, was mostly detected during mitosis (the first 2.5 minutes). The asymmetric cytokinetic furrow (arrowhead) is clearly visible starting at time point 1:40. Three individual P granules are color-labeled to visualize their position throughout the movie. These underwent a rotational movement during cytokinesis, as did the second polar body (arrow), which ended up being closer to the objective as a result (and therefore displayed a stronger signal at later time points). Time is indicated in minutes:seconds. Scale bar, 5 μm.*