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Supplementary Information

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Cell-cycle dependent organization and dynamics of Polymerase I in live human cells

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10 **Supplementary Table 1: sgRNA sequences targeted *POLR1A* gene**

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	Sequence (5′ ⇒ 3′)
sgRNA #1	(forward) CACCGTTCAGCCGAATACATCCCGA
	(reverse) AAACTCGGGATGTATTCGGCTGAAC
sgRNA #2	(forward) CACCGCCGCCGCCAGGGCATGTTCT
	(reverse) AAACAGAACATGCCCTGGCGGCGGC
sgRNA #3	(forward) CACCGTCGGCTGAAGAGCTCAAGTA
	(reverse) AAACTACTTGAGCTCTTCAGCCGAC
sgRNA #4	(forward) CACCGGTCGGGTAGCGTGCCCAGCC
	(reverse) AAACGGCTGGGCACGCTACCCGACC
sgRNA #5	(forward) CACCGCATTTCCTTCGGGATGTATT
	(reverse) AAACAATACATCCCGAAGGAAATGC

12   * Red : BbsI restricted DNA overhang sequences

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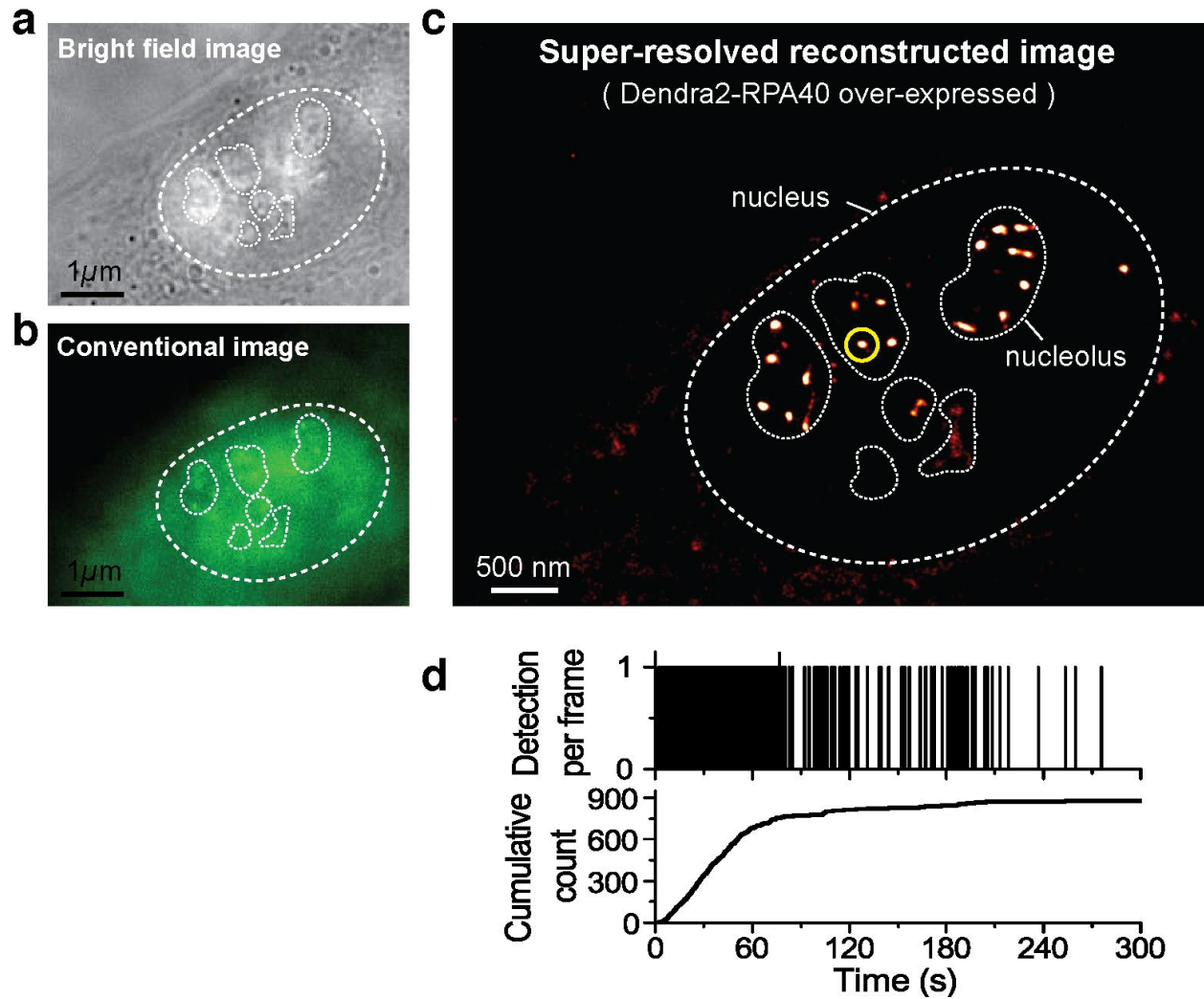19 **Supplementary Table 2: Repair template for Dendra2 gene knock-in**

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Homology left arm (5′ UTR of *POLR1A*)	TTGTACCGTCGACAGCCAGCGAACAGCAGATACAACCGCCATCTTTGATTTCTCTG CAGAGCATGTTGGGAAAGCGTGCCTCGACCTTCAGGAAGGTGGGGCGCAGTGGG CGGGGAGCAGGGAACAGCTCCGCTATTGGCTGAAGCGGAAGAAGACGAAAGCAA TCATAAAATGGGAGGTTGCAAGCTCATGGTTTGAAAGACTTCGTCACGGAAGCTAA AAGCTCTATACACCCGATTTGCCTCGGAGGAATTTTCCTAAATGATTATTTTGATGT CTTATATATCTTTGATTGTTTTCAAAACAAAAGAGCGAGCAGAGAGTCGTACAACTA TTTGTTCCCCCCCCATGTAGAAGTGATCTCATCCACGTAAATGTCGTTCCTGCGAC CGCTTCCGCGCGCAAGCGCACGTTGAATCGCGTGGTGACTCCGGGCTTGAGGTTG AATTAAGAATAGTCAGGTGGTGAGTGGAACGTCTCTTGGGGTGTCGGAATTCAAAA
Dendra2 sequence	CGGACCTGGAGG<mark style="background-color:#00ff00">ATG</mark>AACACCCCGGGAATTAACCTGATCAAGGAGGACATGCGCG TGAAGGTGCACATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCG AGGGCAAGGGCAAGCCCTACGAGGGCACCCAGACCGCCAACCTGACCGTGAAGG AGGGCGCCCCCCTGCCCTTCAGCTACGACATCCTGACCACCGCCGTGCACTACGG CAACCGGGTGTTCACCAAGTACCCCGAGGACATCCCCGACTACTTCAAGCAGAGC TTCCCCGAGGGCTACAGCTGGGAGCGCACCATGACCTTCGAGGACAAGGGCATCT GCACCATCCGCAGCGACATCAGCCTGGAGGGCGACTGCTTCTTCCAGAACGTGCG CTTCAAGGGCACCAACTTCCCCCCCCAACGGCCCCGTGATGCAGAAGAAGACCCTG AAGTGGGAGCCCAGCACCGAGAAGCTGCACGTGCGCGACGGCCTGCTGGTGGGC AACATCAACATGGCCCTGCTGCTGGAGGGCGGCGGCCACTACCTGTGCGACTTCA AGACCACCTACAAGGCCAAGAAGGTGGTGCAGCTGCCCGACGCCCACTTCGTGGA CCACCGCATCGAGATCCTGGGCAACGACAGCGACTACAACAAGGTGAAGCTGTAC
Homology right arm (*POLR1A* gene sequence)	GAGCACGCCGTGGCCCGCTACAGCCCCCTGCCCAGCCAGGTGTGG<mark style="background-color:#ffff00">ATG</mark>TTGATCT C**A**AAGAACATGCCCTGGCGGCG**A**CTGCAGGGCATTTC**A**TTCGGGATGTATTC**A**GC TGA**G**GAGCTCAAGTAAGGAGTTGGTCGGGTAGCGTG**T**CCAGCCTGGATCTTGAGG TCGGCGCCCTAAACTACGATTCCCAGAAGCGCTTTTGCCCAGTCTATCCTCTTGAG TCCAGGCTTGTCTGGCGCTTGTCCTGCTGGGAGCCGTAGTTCTGGGAACGT TCCTGGGCCGGCGTGGACTGCCTGGATAAGAGGCCGGTGGTGCTGGGGGAAAGA GTTTGCGCTGAGGTAGGGGAATATAAGGGTGGGGAGGGTCCTGGTCTGCAGCGTT ATCATCTGCCAGTCTCCTCGTTAACTAACAAGCATTTGTGGAATTAACCATAGTGTA CCTGACATGGTTAATTAGGCACGTAGTAAGTTTCCTCTTGTTTGTAAAATGATTGTC CTTCAAGTGTCAGTTCAGGCGTTAACTTCTCTTTGCTAGGACCCTGCTATAAGCTTT ATTGAT

21 * <mark style="background-color:#00ff00">Green</mark> : start site of Dendra2   * <mark style="background-color:#ffff00">Yellow</mark> : start site of *POLR1A*   * **Red bolded letters** : silent mutation

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a Bright field image

1μm

b Conventional image

1μm

c Super-resolved reconstructed image
(Dendra2-RPA40 over-expressed)

nucleus

nucleolus

500 nm

d Detection per frame

Cumulative count

Time (s)

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Supplementary Figure 1. RNA Polymerase I clusters in a Dendra2-RPA40 overexpression system

As a pilot experiment to verify the results of our CRISPR knockin, we expressed a Dendra2 cloned RPA40 (AddGene, Plasmid #17658) vector in a wild type U2OS cell line. (**a**) A bright field image of a Dendra2-RPA40 over-expressed cell. (**b**) A conventional image shows pre-converted Dendra2 signals in the cell. (**c**) A super-resolution reconstructed image showing bright Dendra2-Pol I clustered spots in nucleoli. (**d**) A representative tcPALM plot of a Pol I clustered locus (yellow circle in c) shows a stable cluster from the start of image acquisition.

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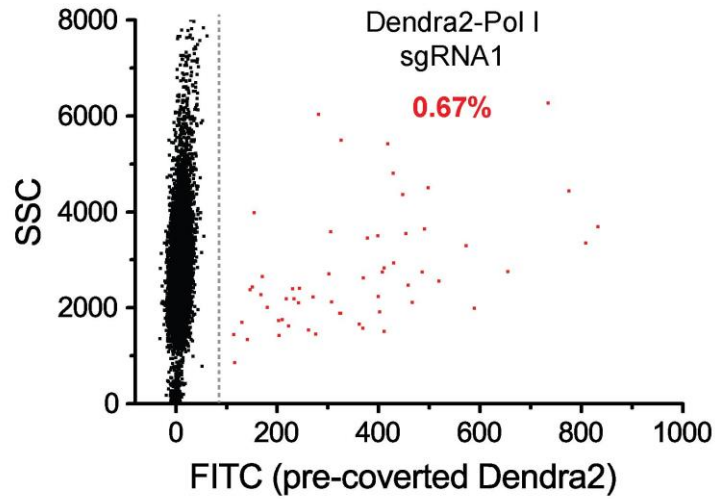
Supplementary Figure 2. Fluorescence activated cell sorting (FACS) dot plots

Cells transfected with sgRNA#1-Cas9 construct along with Dendra2 repair template were sorted using FACS to identify fluorescent cells. A dot plot shows measurements of side scatter (SSC) and fluorescence detection of pre-converted Dendra2 through FITC filter (488-nm excitation) for 10,794 cells. We set a sorting threshold on the maximum intensity detected in wild type cells to isolate successfully transfected cells. 0.67% of the fluorescent cells displaying intensities above the threshold were collected for live cell imaging.
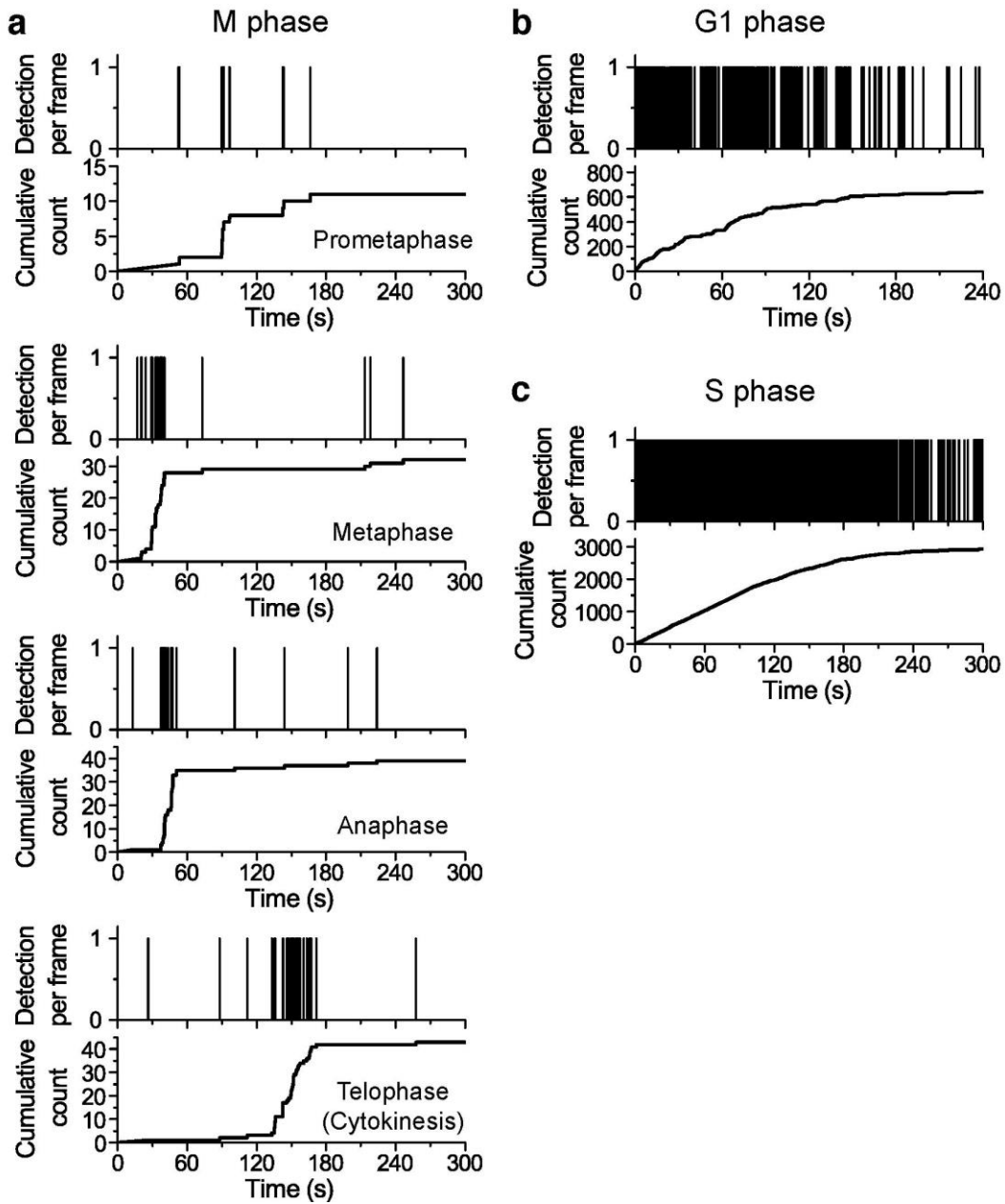
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45 **Supplementary Figure 3. Example traces for Dendra2-Pol I in M-, G1- and S-phase**

46 (**a**) Dendra2-Pol I traces in various stages of M-phase reveal transient clusters. (**b**) A G1-phase trace shows

47 a stable cluster. (**c**) An S-phase trace shows a signal of stable cluster. S-phase clusters display more

48 polymerase detections than G1 clusters. This higher detection count corresponds to more robust rRNA

49 transcription.