Supplementary Figure Legends

Figure S1. Depletion of Orc2 also results in non-lethal cell cycle block in untransformed cells, but cancer cell arrest and death.

(A) Indicated cell types were transfected with 100 nM luciferase (siLuc)- or Orc2 (siOrc2)-targeted siRNA for 72h. Transfected cells were fixed and stained with propidium iodide (PI), followed by FACS analysis of >10,000 cells per condition. Sub-G_1 populations were calculated based upon all counts per sample; G_1/S/G_2/M populations were calculated from non-sub-G_1 counts. As mentioned in Figure 1, G_1 contains G_1 and G_1/S cells and G_2/M contains late S and G_2/M cells. (B) siRNA-treated cells were lysed and subjected to immunoblotting analysis with anti-Orc2 or anti-GAPDH (loading control) antibody.

Figure S2. Serum deprivation-synchronization of RPE1 cells.

(A) RPE1 cells were incubated in serum-free DMEM:F-12 (1:1) supplemented with 1.2 g/L sodium bicarbonate for 72-96 h. Cells were washed with and released into media supplemented with 10% FBS for 14, 16, 18, or 22 h before harvest by trypsinization. Cells were fixed, stained with PI, and subjected to FACS analysis. Individual histogram plots based on DNA content were generated based on 10,000 cells. Below the FACS profiles, serum deprivation-synchronized cells at indicated timepoints were lysed and cell lysates were subjected to immunoblotting analysis with anti-Cdc6 antibody. (B) Serum deprivation-synchronized, coverslip-grown RPE1 cells were stimulated by addition of serum as in (A), and at indicated timepoints, subsequently pulse-labeled with 20 μM 5-bromo-2'deoxyuridine (BrdU) for 15 min. Coverslips were fixed and immunostained with anti-BrdU antibody and 4,6-diamidino-2-phenylindole (DAPI) for DNA.

Figure S3A. Determination of cell cycle distribution of siLuc-treated cells by MCM2/BrdU immunofluorescence analysis.

Asynchronous coverslip-grown RPE1 cells were treated with 100 nM siLuc for 60 h, and subsequently pulse-labeled with 20 μM BrdU for 15 min. Then, cells were washed, CSK extracted, fixed and immunostained with indicated antibodies and DAPI (DNA). Representative nuclei from each cell cycle phase are shown. Average relative DAPI intensities of nuclei were also extrapolated by ImageJ (NIH). Percentages of cells at different stages of the cell cycle were determined based on manual cell counts of >150 cells from two independent experiments.

Figure S3B. Determination of cell cycle distribution of siCdc6-treated cells by MCM2/BrdU immunofluorescence analysis.

Asynchronous coverslip-grown RPE1 cells were treated with 100 nM siCdc6 for 60 h, and subsequently pulse-labeled with 20 μM BrdU for 15 min. Then, cells were washed, CSK extracted, fixed and immunostained as in S3A. Representative nuclei from each cell cycle phase are shown. Average relative DAPI intensities of nuclei were also extrapolated by ImageJ (NIH). Percentages of cells in different stages of the cell cycle were calculated based on manual cell counts of >150 cells from two independent experiments.

Figure S3C. Determination of cell cycle distribution of siLuc and siATR co-treated cells by MCM2/BrdU immunofluorescence analysis.

Asynchronous coverslip-grown RPE1 cells were treated with 100 nM siLuc for 12 h and then further co-treated with 100 nM siATR for an additional 48 h. Cells were then pulse-labeled with 20 μM BrdU for 15 min, washed, CSK extracted, fixed and immunostained as in S3A. Representative nuclei from each cell cycle phase are shown. Percentages of cells in different stages of the cell cycle were calculated based on manual cell counts of >150 cells from two independent experiments.

Figure S3D. Determination of cell cycle distribution of siCdc6 and siATR co-treated cells by MCM2/BrdU immunofluorescence analysis.

Asynchronous coverslip-grown RPE1 cells were treated with 100 nM siCdc6 for 12 h and then further co-treated with 100 nM siATR for an additional 48 h. Cells were then pulse-labeled with 20 μM BrdU for 15 min, washed, CSK extracted, fixed and immunostained as in S3A. Representative nuclei from each cell cycle phase are shown. Percentages of cells in different stages of the cell cycle were calculated based on manual cell counts of >150 cells from two independent experiments.

Figure S3E. Determination of cell cycle distribution of siLuc and caffeine co-treated cells by MCM2/BrdU immunofluorescence analysis.

Asynchronous coverslip-grown RPE1 cells were treated with 100 nM siLuc for 48 h and further incubated for 12 h in the presence of 2.5 mM caffeine. Cells were then pulse-labeled with 20 μM BrdU for 15 min, washed, CSK extracted, fixed and immunostained as in S3A. Representative nuclei from each cell cycle phase are shown. Percentages of cells in different stages of the cell cycle were calculated based on manual cell counts of >150 cells from two independent experiments.

Figure S3F. Determination of cell cycle distribution of siCdc6 and caffeine co-treated cells by MCM2/BrdU immunofluorescence analysis.

Asynchronous coverslip-grown RPE1 cells were treated with 100 nM siCdc6 for 48 h and further incubated for 12 h in the presence of 2.5 mM caffeine. Cells were then pulse-labeled with 20 μM BrdU for 15 min, washed, CSK extracted, fixed and immunostained as in S3A. Representative nuclei from each cell cycle phase are shown. Percentages of cells in different stages of the cell cycle were calculated based on manual cell counts of >150 cells from two independent experiments.

Figure S4. Representative photos of dual-labeled DNA fibers from RPE1 cells

(A) Shown are schematic of different replication structures (new firing origins or progressing forks) and representation of different fork progression rates. CldU or IdU pulse-labeling is represented by green or red lines, respectively. (B) representative photos of dual-labeled DNA fibers analyzed in Figure 5 from asynchronous RPE1 cells treated with siLuc alone, siCdc6 alone, siLuc + siATR, or siCdc6 + siATR as indicated are shown. Coverslip-grown siRNA(s) treated RPE1 cells were labeled for 10 min with 10 μM CldU, and then washed and labeled with 10 μM IdU for 10 additional min. DNA fibers were generated and stained with anti-CldU (green) and anti-IdU (red) antibodies. (C) Asynchronous RPE1 cells were co-treated with siCdc6 and siATR, and subsequently labeled for 1 h with 10 μM CldU, and then washed and labeled with 10 μM IdU for one additional h. DNA fibers were generated and stained as in (B). A representative field from each condition is shown as indicated.

Figure S5. Representative photos of dual-labeled DNA fibers from HeLa cells

(A) Shown are representative photos of dual-labeled DNA fibers analyzed in Figure 6 from asynchronous HeLa cells treated with siLuc alone, siCdc6 alone, siLuc + siATR, or siCdc6 + siATR as indicated. Coverslip-grown siRNA(s) treated HeLa cells were labeled for 10 min with 10 μM CldU, and then washed and labeled with 10 μM IdU for 10 additional min. DNA fibers were generated and stained with anti-CldU (green) and anti-IdU (red) antibodies. (B) Asynchronous HeLa cells were co-treated with siCdc6 and siATR, and subsequently labeled for 1 h with 10 μM CldU, and then washed and labeled with 10 μM IdU for one additional h. DNA fibers were generated and stained as in (A). A representative field from each condition is shown as indicated.

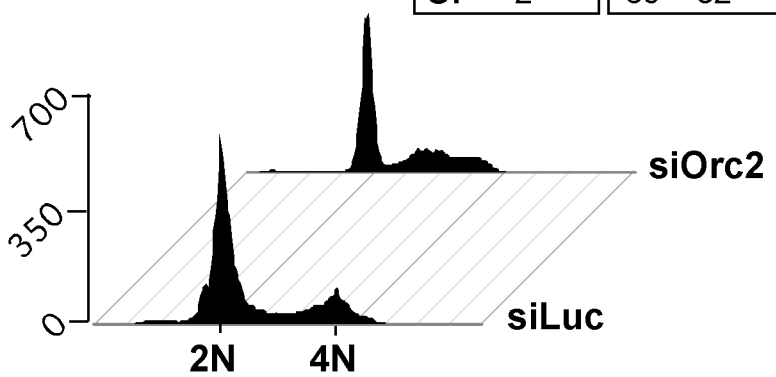
S6. Quantitative analysis of HP1α or Me-K9-H3 immuostaining signals in siLuc or siCdc6 treated RPE1, HeLa or HCT116 cells.

(A) Large representative fields of asynchronously growing RPE1, HeLa or HCT116 cells treated with indicated siRNA for 48 h are shown. (B) Integrated immunostaining signal intensity of HP1α or Me-K9-H3 per nuclei was quantitated using ImageJ (NIH) and sorted by percentage into three bins based on arbitrary signal intensity units (AU; +: 1-19,999 AU; ++: 20,000-39,999 AU; +++: >40,000 AU).

A

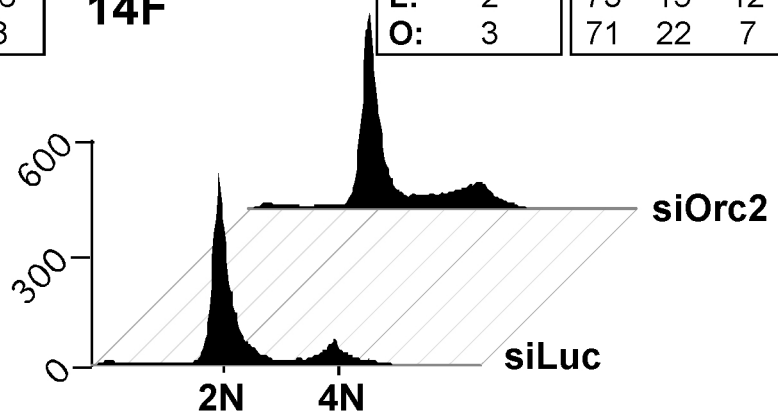
RPE1

	% of total Sub-G1	% of viable G1	S	G2/M
L:	3	59	25	16
O:	2	60	32	8



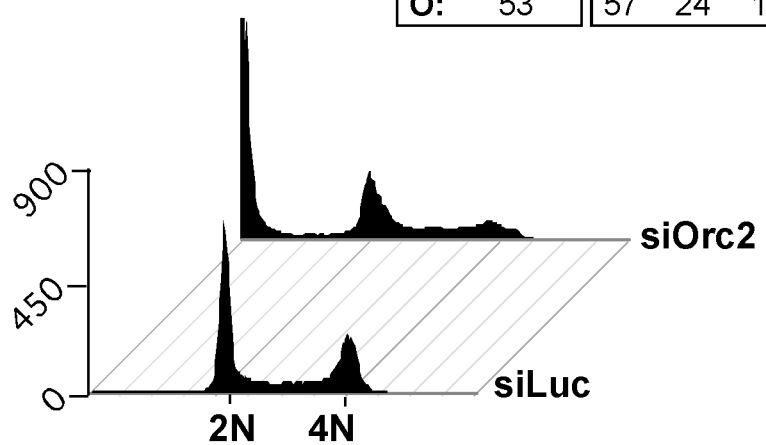
14F

	% of total Sub-G1	% of viable G1	S	G2/M
L:	2	73	15	12
O:	3	71	22	7



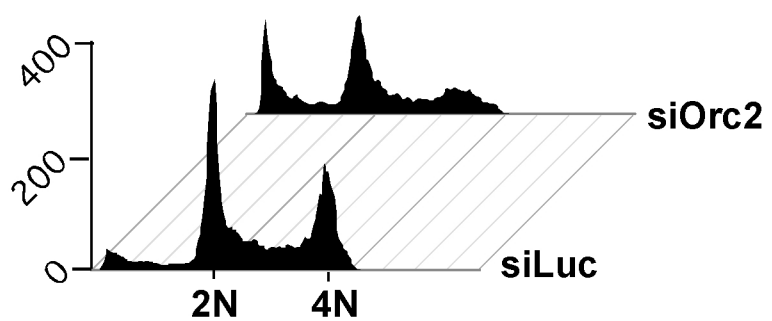
HeLa

	% of total Sub-G1	% of viable G1	S	G2/M
L:	3	47	23	30
O:	53	57	24	19



HCT116

	% of total Sub-G1	% of viable G1	S	G2/M
L:	9	42	23	35
O:	30	57	16	27



B

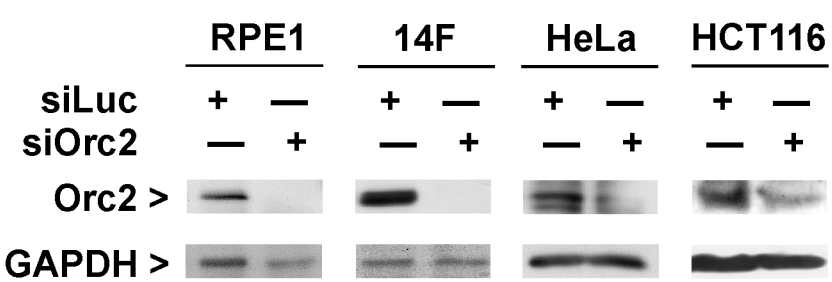


Figure S1. Lau et al, 2009

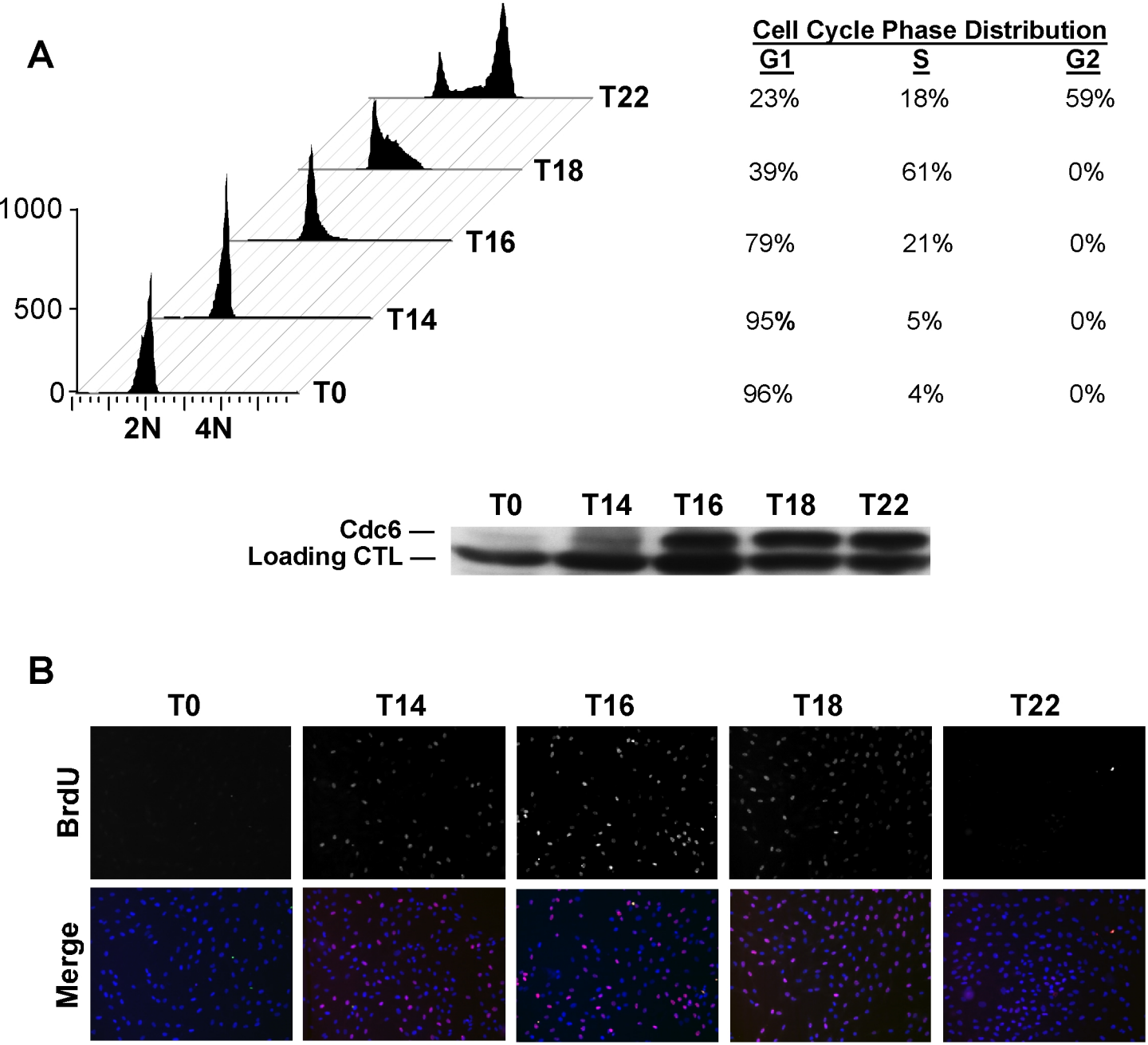


Figure S2. Lau et al, 2009

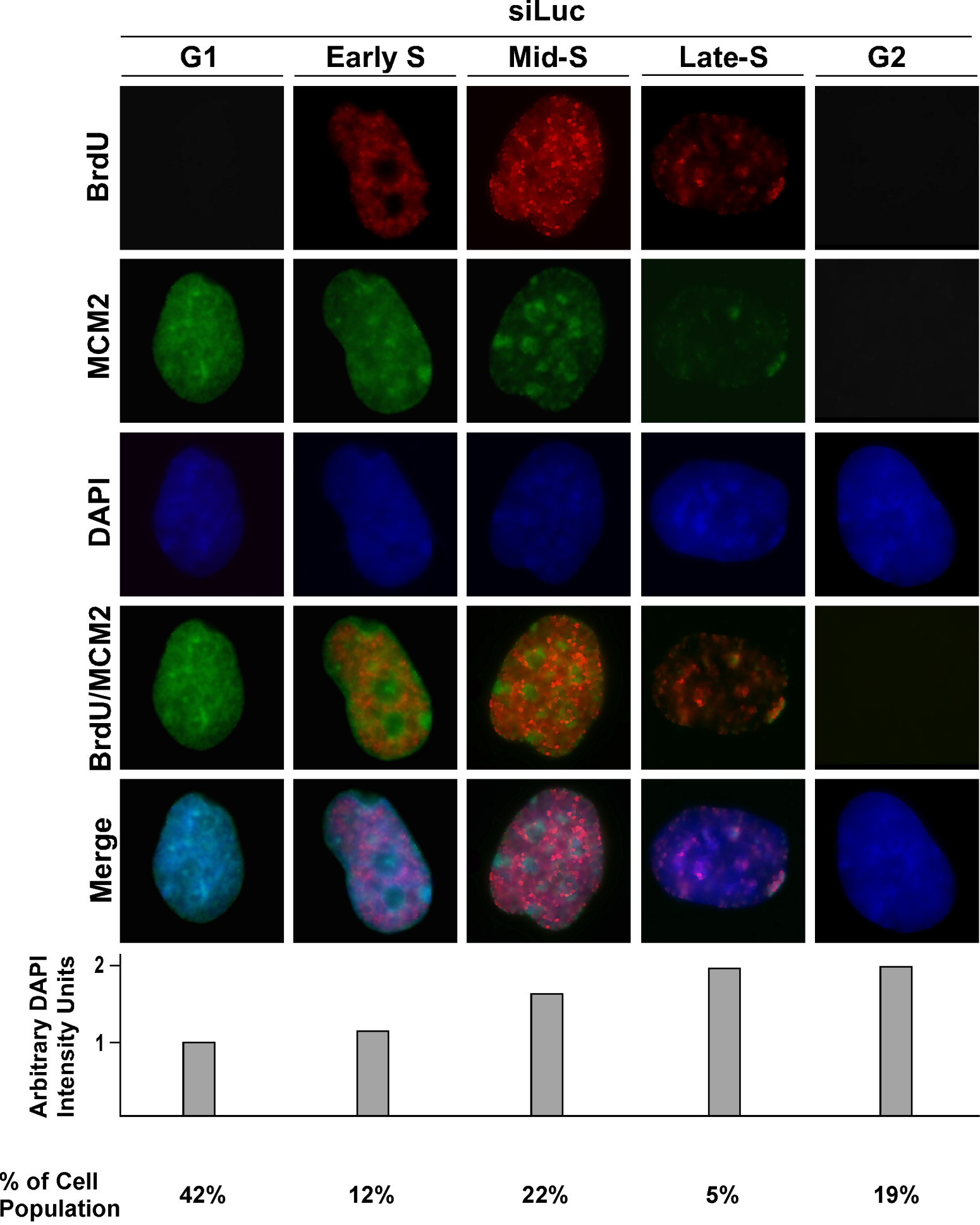


Figure S3A, Lau et al, 2009

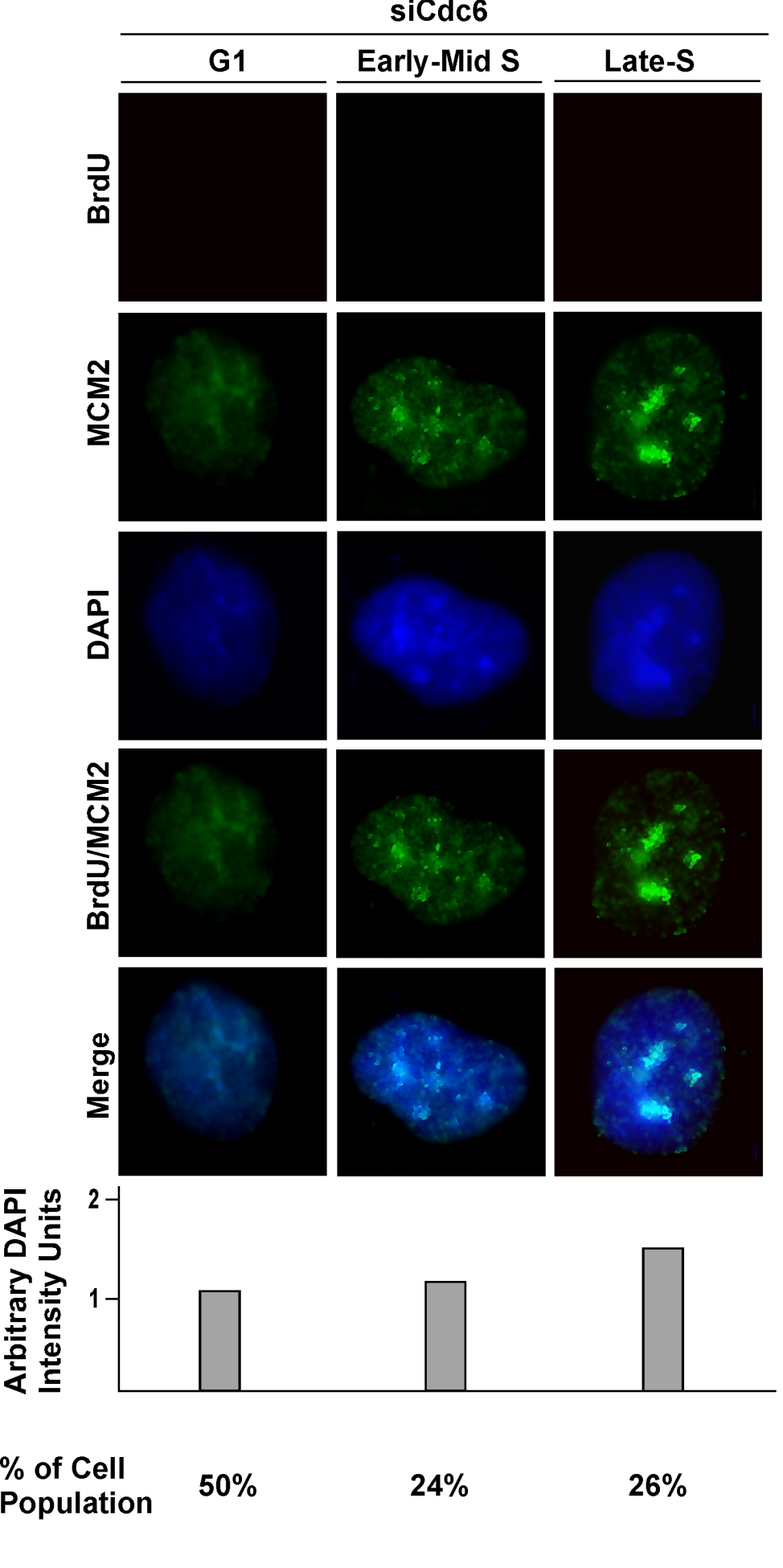
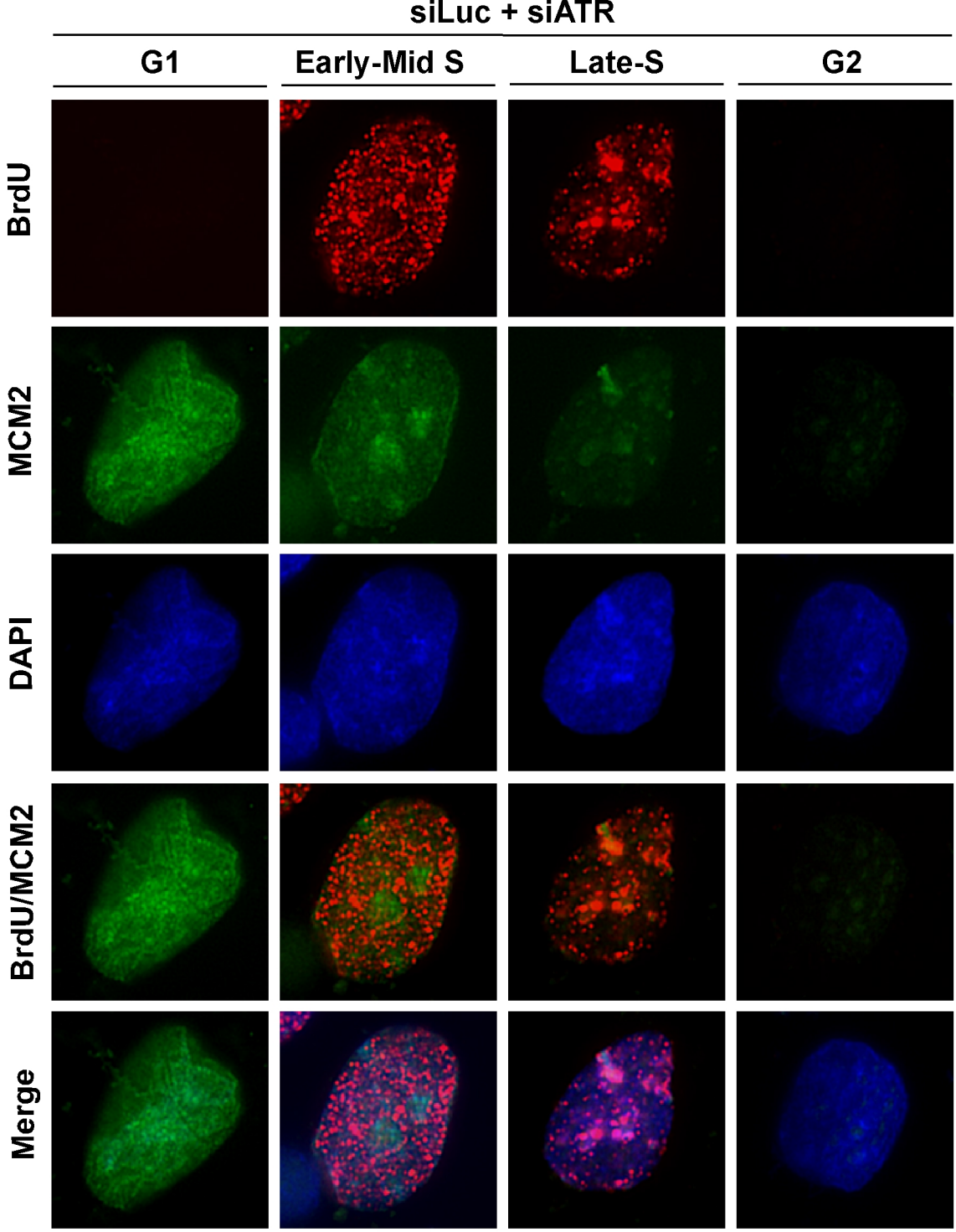


Figure S3B, Lau et al, 2009



% of Cell Population	41%	21%	14%	24%

Figure S3C, Lau et al, 2009

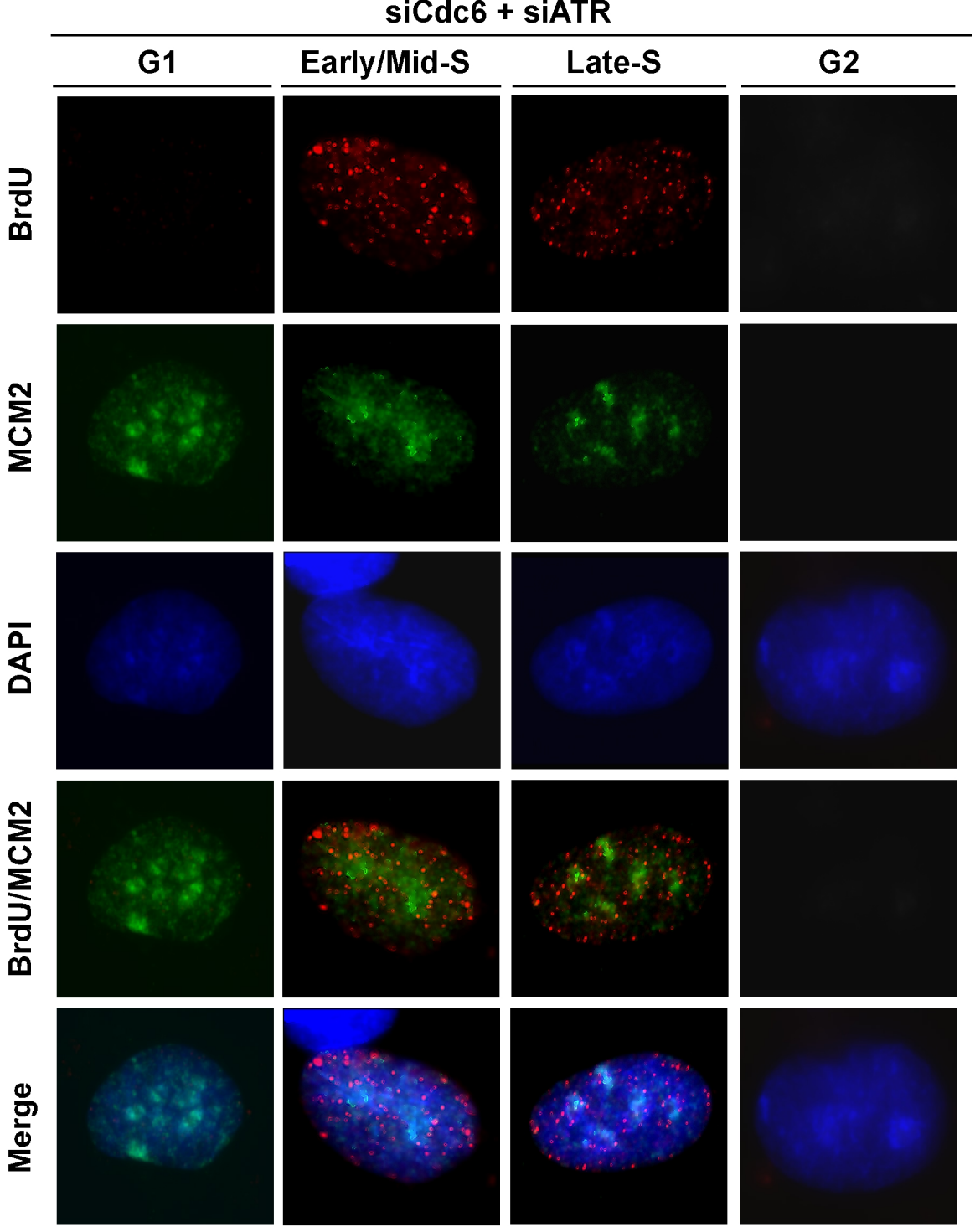


Figure S3D, Lau et al, 2009

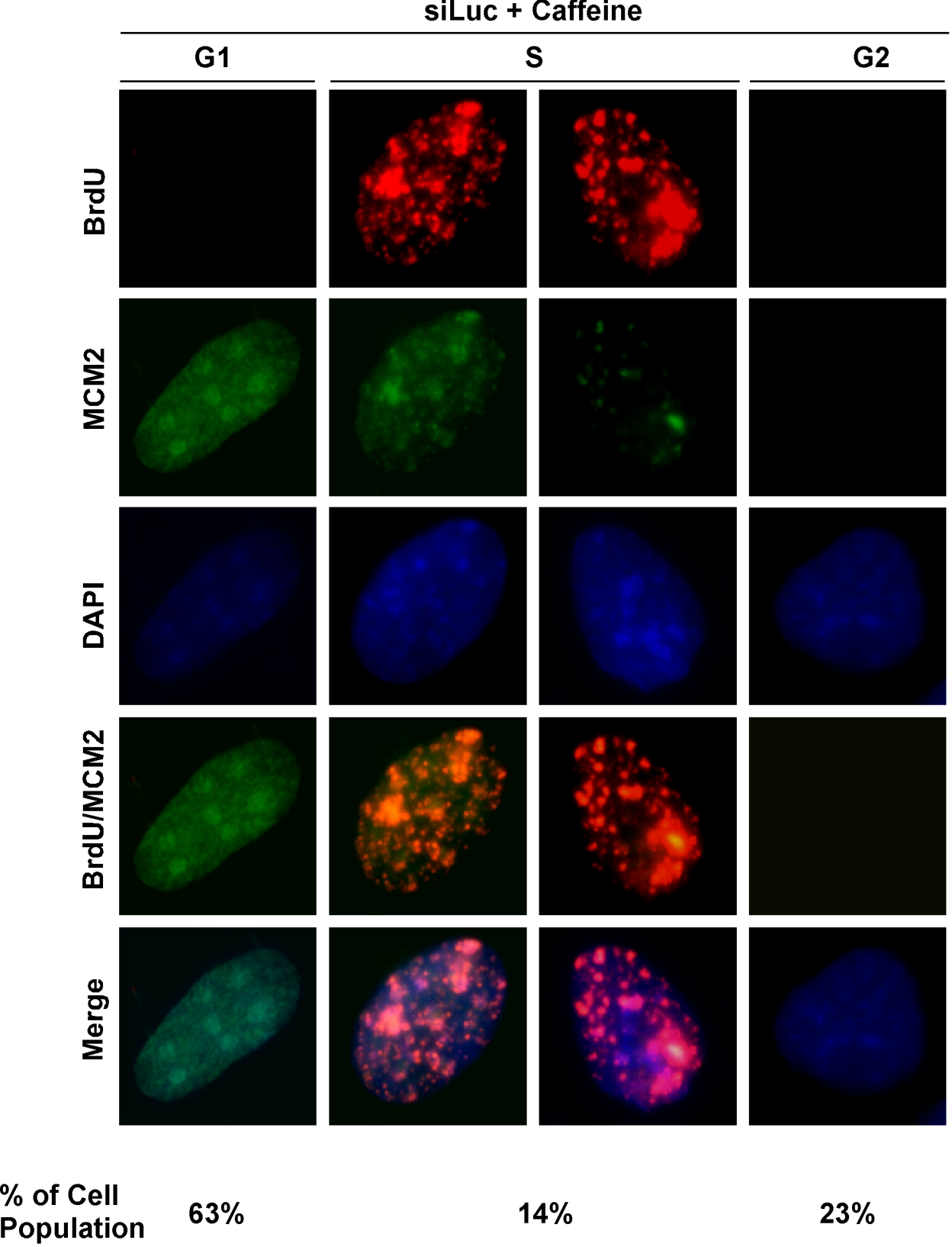


Figure S3E, Lau et al, 2009

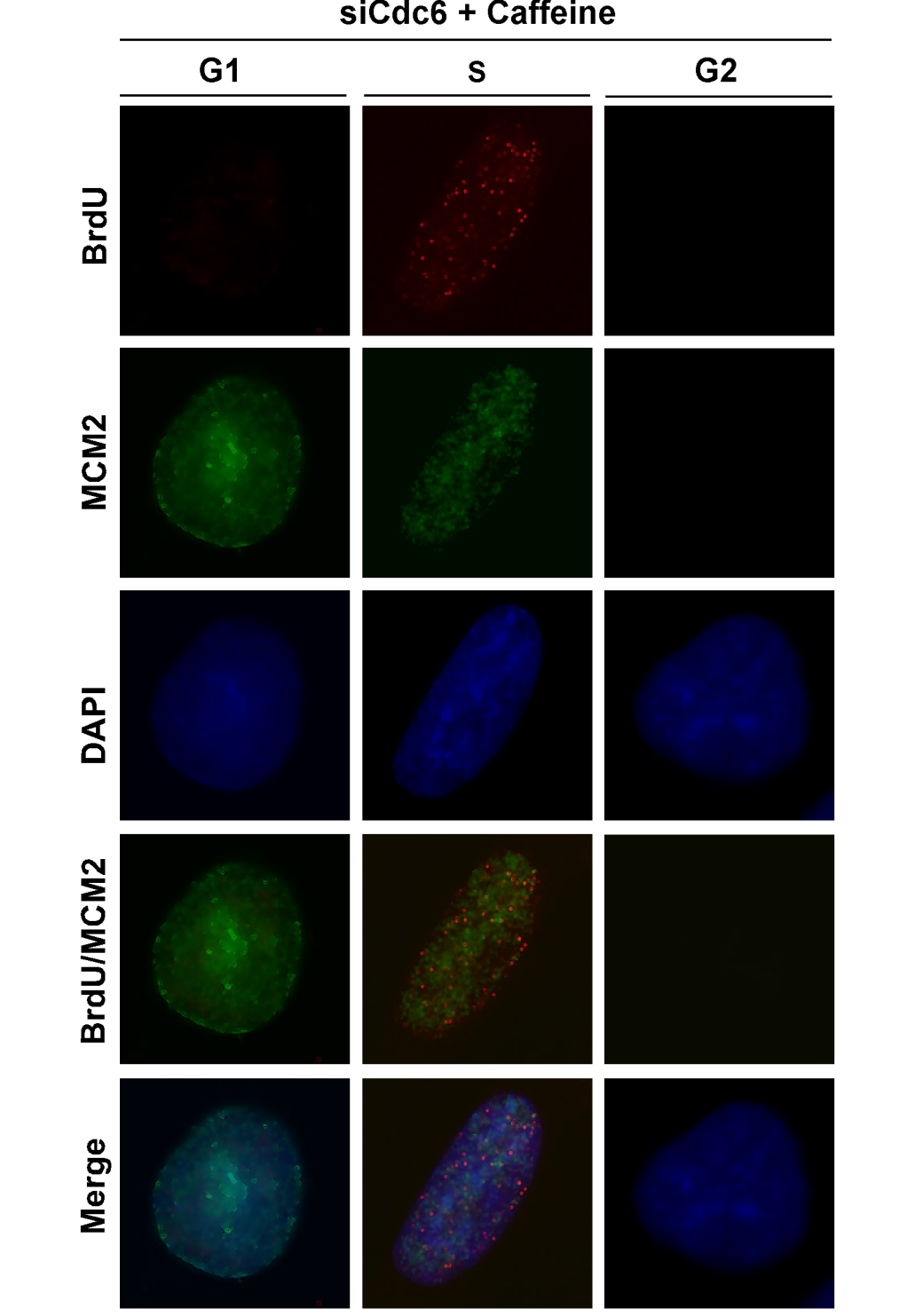


Figure S3F, Lau et al, 2009

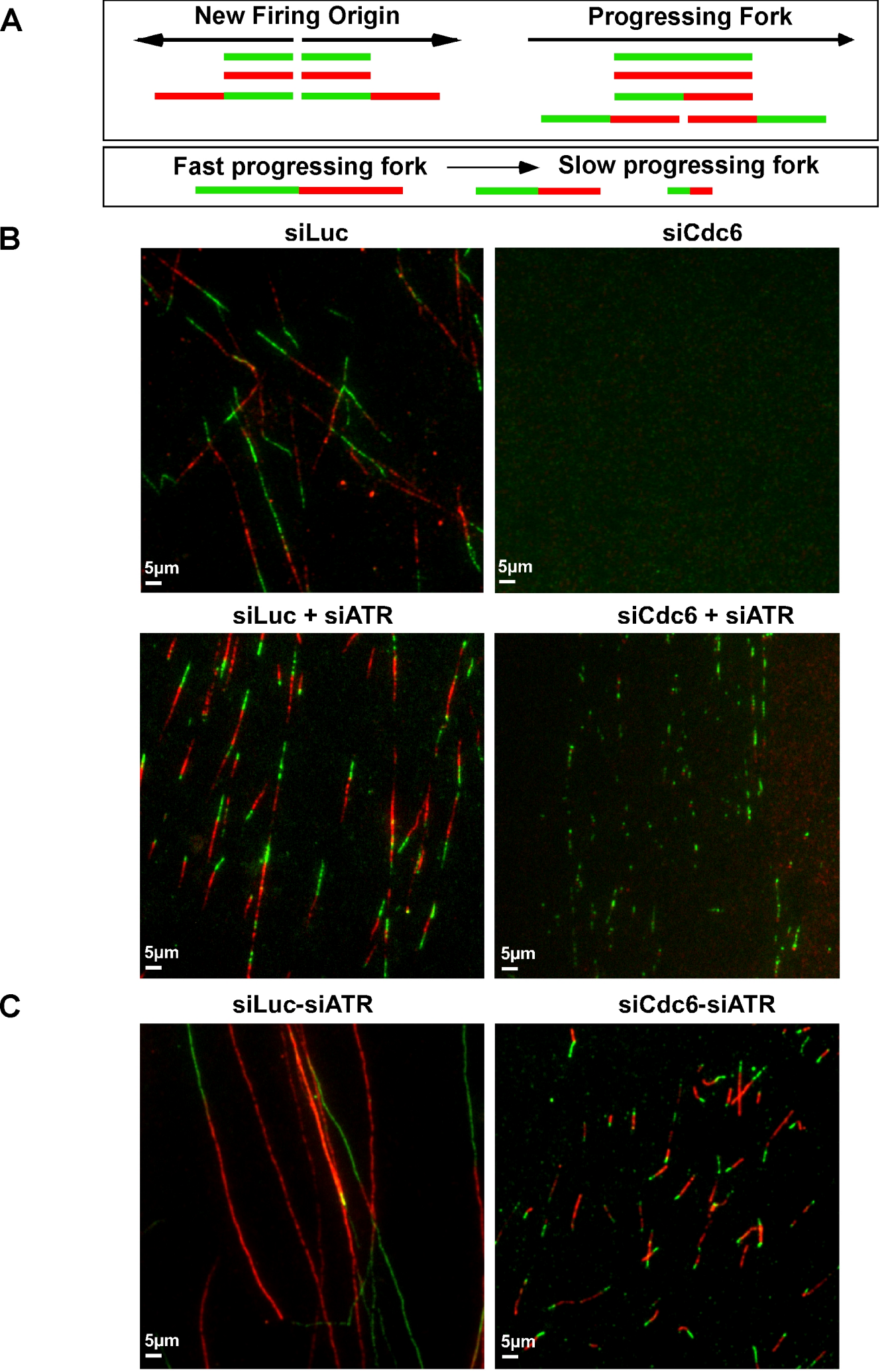


Figure S4. Lau et al, 2009

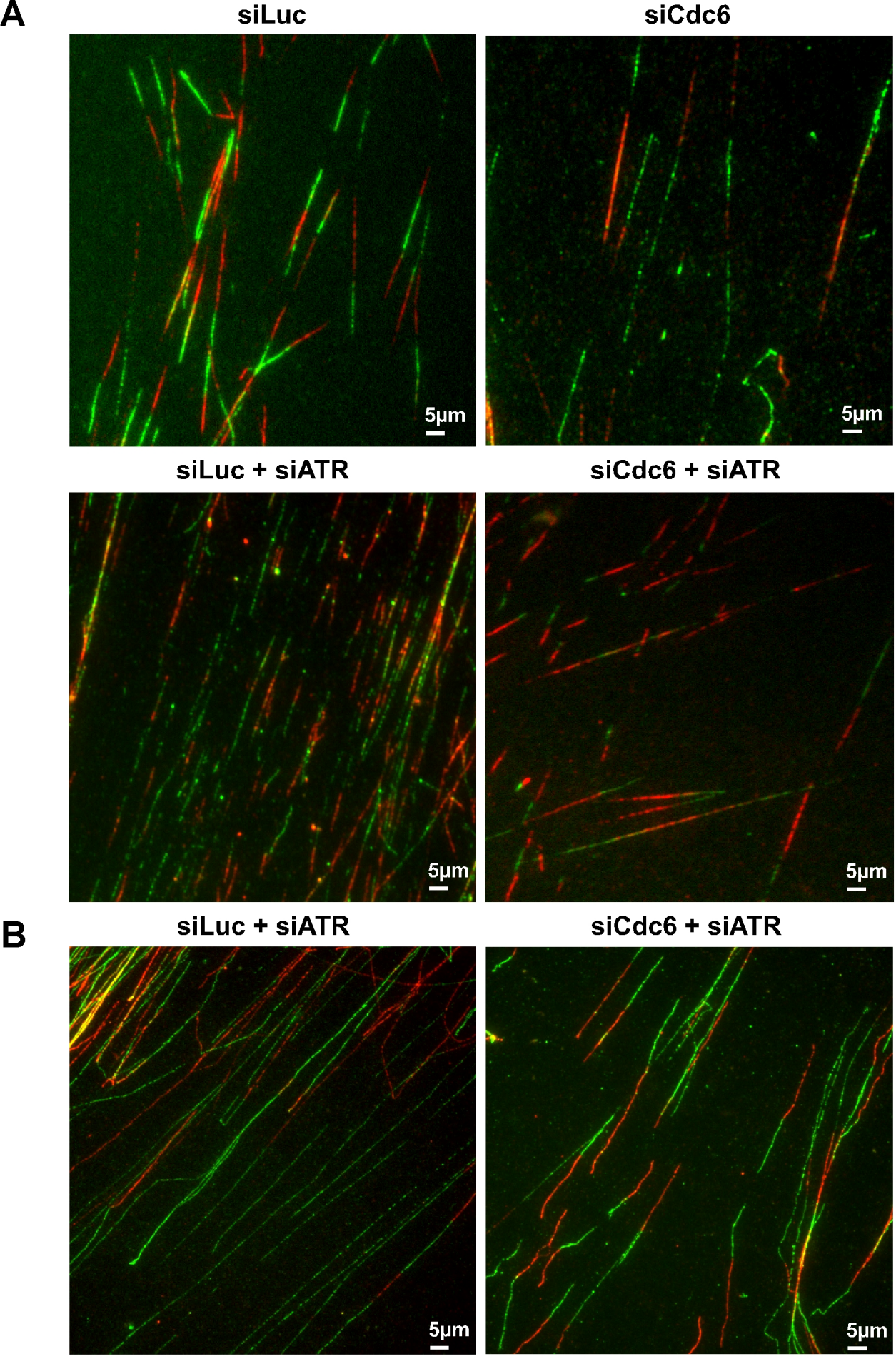
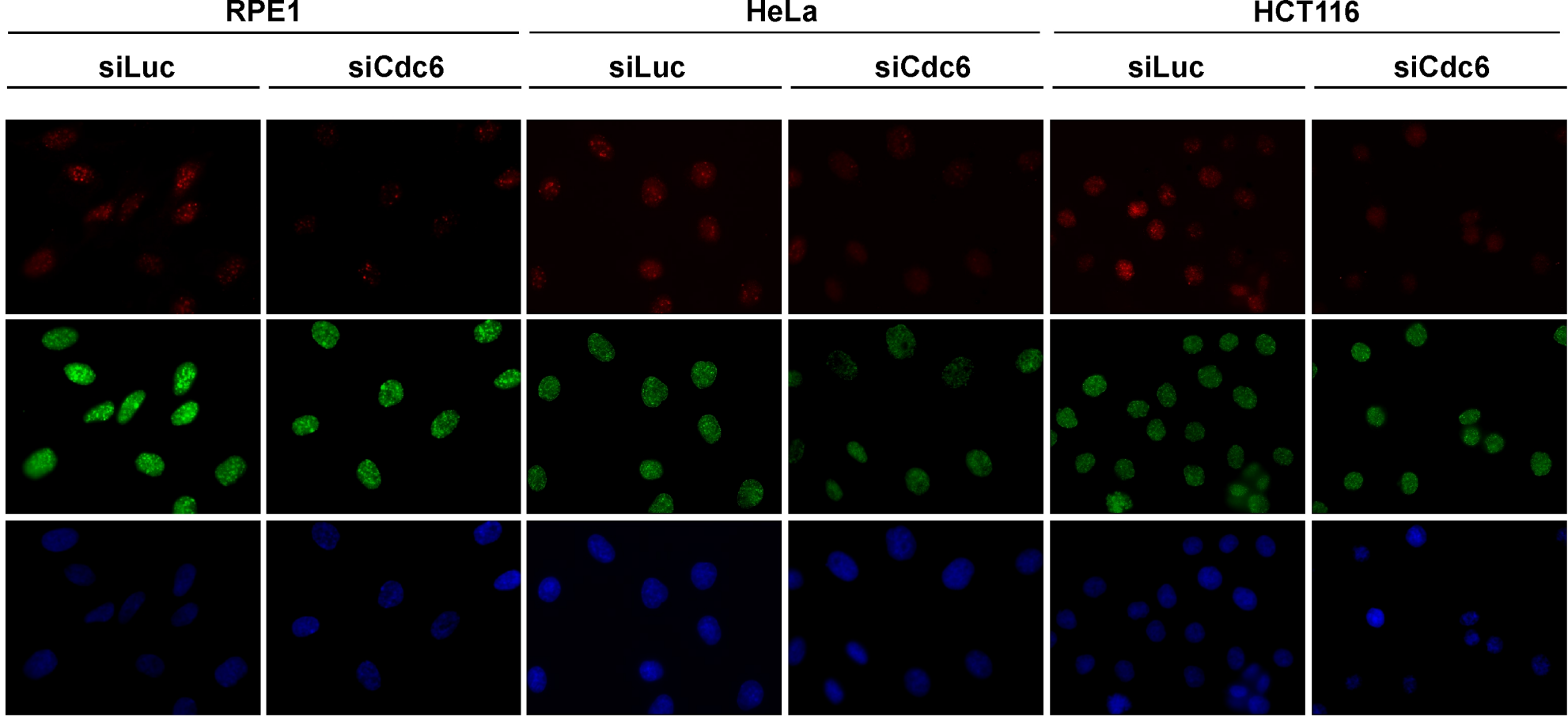


Figure S5. Lau et al, 2009

A



B

		RPE			HeLa			HCT116		
		+	++	+++	+	++	+++	+	++	+++
HP1α	L	0	10	90	15	48	37	13	25	62
	6	28	47	25	45	43	12	67	31	2
MeK9 H3	L	0	0	100	0	40	60	2	28	69
	6	0	0	100	16	47	37	0	40	60

Figure S6, Lau et al, 2009