**SUPPLEMENTARY TABLES AND FIGURES**

**Supplementary file 1a: Cell line characteristics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Species** | **Type** | **Genome size ( Gbp)** | **Reference** |
| HeLa K | Homo sapiens | Cervical adenocarcinoma |  9.682±0.002  | [(Erfle et al., 2007)](https://sciwheel.com/work/citation?ids=1001802&pre=&suf=&sa=0&dbf=0) |
| HeLa K GFP-PCNA | Homo sapiens | Cervical adenocarcinoma |  9.682±0.002 |  [(Chagin et al., 2016)](https://sciwheel.com/work/citation?ids=2069726&pre=&suf=&sa=0&dbf=0) |
| HeLa K GFP-RPA34 | Homo sapiens | Cervical adenocarcinoma |  9.682±0.002 | This study |
| HeLa K FRTLacZ | Homo sapiens | Cervical adenocarcinoma |  9.682±0.002 | [(Chagin et al., 2016)](https://sciwheel.com/work/citation?ids=2069726&pre=&suf=&sa=0&dbf=0) |
| IMR90 | Homo sapiens | Fibroblasts from lung tissue | 6.37 | [(Nichols et al., 1977)](https://sciwheel.com/work/citation?ids=8508791&pre=&suf=&sa=0&dbf=0) |

**Supplementary file 1b: Plasmid characteristics**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **pc number** | **Fluorophore** | **Protein of Interest** | **Promoter** | **References** |
| pmiRFP670-PCNA | 3385 | miRFP670 | Human PCNA | CMV |  [(Rausch et al., 2021)](https://sciwheel.com/work/citation?ids=11830976&pre=&suf=&sa=0&dbf=0) |
| pFRT-B-GRPA | 1232 | GFP | Human RPA34 | EF1⍺ |  This study |
|  pFRT-B-GPCNA | 1274 | GFP | Human PCNA | EF1⍺ |  [(Chagin et al., 2016)](https://sciwheel.com/work/citation?ids=2069726&pre=&suf=&sa=0&dbf=0)  |

\* pc: plasmid collection.

**Supplementary file 1c: Nucleotide and chemical characteristics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Application** | **Detection** | **Cat #** | **Company** |
| Cy3-dUTP | Replication labeling(Labeling of nascent DNA) | - | ENZ-42501 | Enzo life sciences, Farmingdale, NY, USA |
| Aphidicolin | Replisome disruption by polymerase inhibition | - | A0781-1MG | Sigma-Aldrich, StLouis, MO, USA |
| 5-ethynyl-2’-deoxyuridine (EdU) | Labeling of nascent DNAin pulse (chase) experiments | ClickIT chemistry | E10415 | Thermo FisherScientific, Waltham,MA, USA |

**Supplementary file 1d: Primary and secondary antibody characteristics**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Reactivity** | **Host** | **Clonality** | **Dilution** | **Application** | **Cat / Clone#** | **Company / References** |
| anti RPA34 | Mouse | Monoclonal | 1:2 | IF, WB | 9H8H4**#** | Gift from Mark Kenny/J. Hurwitz[(Kenny et al., 1990)](https://sciwheel.com/work/citation?ids=1287800&pre=&suf=&sa=0) |
| anti RPA70A | Mouse | Monoclonal | 1:2 | IF, WB | 7G9E3**#** | Gift from Mark Kenny/J. Hurwitz[(Kenny et al., 1990)](https://sciwheel.com/work/citation?ids=1287800&pre=&suf=&sa=0) |
| Anti MCM2 | Rabbit | Monoclonal | 1:5000 IF, 1:10000 WB | IF, WB | ab 108935/ EPR4120 | Abcam, Cambridge, United Kingdom |
| anti MCM2pS108 | Rabbit | Monoclonal | 1:1000 | IF,WB | 3267-1 | Epitomics,Burlingame​, CA, United States |
| anti pol Alpha | Mouse | Monoclonal | Undiluted | IF,WB | SJK-287-38**#** | ATCC[(Tanaka et al., 1982)](https://sciwheel.com/work/citation?ids=2084999&pre=&suf=&sa=0) |
| anti pol Delta | Mouse | Monoclonal | 1:500 | IF,WB | 610972 | BD biosciences,New Jersey, USA[(Li et al., 2016)](https://sciwheel.com/work/citation?ids=3106104&pre=&suf=&sa=0) |
| anti pol Epsilon | Rabbit | Polyclonal | 1:500 | IF, WB |  GTX132100 | GeneTex, Irvine, California, United States |
| anti PCNA | Mouse | Monoclonal | 1:100 | IF\*,WB | M0879 / PC10**#** | Dako,Hamburg,Germany[(Waseem and Lane, 1990)](https://sciwheel.com/work/citation?ids=2085678&pre=&suf=&sa=0) |
| anti Histone H3 | Rat | Monoclonal | 1:250 | WB | 61647/1C8B2# | Active Motif,California, USA |
| anti MacroH2A1 | Rabbit | Polyclonal | 1:1000 | WB | 07-219 | Active Motif,California, USA |
| anti GFP | Rat | Monoclonal | 1:1000 | WB | 3H9**#** | Chromotek 3H9, Planegg-Martinsried, Germany |
| Anti tubulin alpha | Mouse | Monoclonal | 1:5000 | WB | clone DM1A#/ T9026 | Sigma, Missouri, United States |
| anti-mouseIgG Cy3 | Donkey | Polyclonal | 1:800 | IF (fluorescentsecondary) | 715-165-151 | The JacksonLaboratory, BarHarbor, ME, USA |
| anti-rabbitIgG Cy3 | Donkey | Polyclonal | 1:800 | IF (fluorescentsecondary) |  711-165-152 | The JacksonLaboratory, BarHarbor, ME, USA |
| anti-mouseIgG Cy5 | Donkey | Polyclonal | 1:800 | IF (fluorescentsecondary) | 715-175-150 | The JacksonLaboratory, BarHarbor, ME, USA |
| anti-rabbitIgG Cy5 | Donkey | Polyclonal | 1:800 | IF (fluorescentsecondary) | 711-175-152 | The JacksonLaboratory, BarHarbor, ME, USA |
| anti-mouseIgG AF488 | Goat | Polyclonal | 1:800 | IF (fluorescentsecondary) | 2120125 | Invitrogen,Waltham, Massachusetts, USA |
| anti-rabbitIgG AF488 | Donkey | Polyclonal | 1:800 | IF (fluorescentsecondary) | A11034 | Invitrogen,Waltham, Massachusetts, USA |
| anti rat IgG HRP | Goat | Polyclonal | 1:5000 | WB (HRP secondary) | 112-035-068 | The JacksonLaboratory, BarHarbor, ME, USA |
| anti mouse IgG HRP | Sheep | Polyclonal | 1:5000 | WB (HRP secondary) | NA931 | Amersham pharmacia, Amersham, United Kingdom |

**\***Methanol treatment required #Clone number

**Supplementary file 1e: Imaging systems characteristics**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Microscope/****Company** | **Lasers/lamps** | **Filters (ex. &****em. [nm])\*** | **Objectives/****lenses** | **Detection****system** | **Incubation****system** | **Application** |
| Ultra-ViewVoXspinning diskmicroscope/PerkinElmer LifeSciences,UK | solid statediode lasers (405 nm,488 nm, 561 nm,640 nm) | 405/488/568/640\*\*405: 415–475488: 505–549561: 580–650640: 664–754 | oil immersion60x Plan-Apochromat (NA 1.45) | cooled 14-bitHamamatsu®C9100-50EMCCD | closed live-cell microscopy chamber(ACU control,Olympus) fortime-lapsemicroscopy | time-lapsemicroscopy& confocal z-stack imaging |
| Widefield microscopeAxiovert200 /Zeiss,Germany | HBO100 mercurylamp | 488: 473-491 &506-534561: 550-580 &590-650640: 590-650& 663-738 | oil immersion 63x Plan-Apochromat(NA 1.4) | 12-bitAxioCam mRM | - | Multi channel wide-field imaging  |
| Leica SP5 II confocal microscope /Wetzlar, Germany | 405 nm diode 488 nm Argon, 561 nm DPSS, 633 nm HeNe  | AOBS beam splitter | HCX PL APO 63x / 1.4-0.6 oil lambda blue & HCX PL APO 100x (NA 1.44) oil Corr CS | 2 HyD Hybrid Detectors |  - | confocal z- stack imaging |
| Amersham AI600 imager | Chemiluminescence, UV transillumination | - | large aperture FUJINONTM f/0.85 43 mm | 16-bit Peltier cooled Fujifilm Super CCD | - | Western blots and DNA agarose gels |
| Operetta high throughput imaging/ PerkinElmer Life Sciences, UK | Xenon fiber-optic light source, 300 W, 360 – 640 nm continuous spectrum LED light source for transmission mode | ex:360/400, 460/490, 560/580em: 410/480, 500/550, 560/630 | 20x or 40x air (0.45 NA and 0.95 NA) long WD\*\*\* |  14 bit Jenoptik firecamj203 Sony Chip ICX285 cooled 20°C below environment | - | high throughput, high content imaging and image analysis |
| Nikon TiE2 inverted with crest spinning disk unit/ Nikon, Japan | SPECTRA X light engine395/25 nm with 295 mW440/20 nm with 256 mW470/24 nm with 196 mW510/25 nm with 62 mW540/30 nm with 231 mW550/15 nm with 260 mW575/25 nm with 310 mW | LED-DA/FI/TR/Cy5-4X-B Quadbandpassex:390/18,475/35, 535/50em:460/60,530/43,580LP | 40x air (0.95 NA) & 250 µm WD\*\*\* | Cooled Nikon Qi2 camera and 16.25 megapixel sCMOS sensor. readout noise is: 2.2. electron | - | high throughput, high content imaging and image analysis |

\* ex.: excitation & em.: emission, \*\* dichroic specification, \*\*\* WD: working distance.

**Supplementary file 1f: Data description for DNA quantification**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Name** | **Cell stage** | **DAPI SUM** | **Correction factor (C)** | **Corrected genome size (GSxC) Gbp** |
| Cell1\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 6.55E+08 | 1.05 | 10.185  |
| Cell2\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 8.49E+08 | 1.05 | 10.185  |
| Cell3\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 9.68E+08 | 1.05 | 10.185  |
| Cell4\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.14E+09 | 1.25 | 12.125 |
| Cell5\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.15E+09 | 1.77 | 17.169 |
| Cell6\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 8.29E+08 | 1.05 | 10.185 |
| Cell7\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 9.49E+08 | 1.05 | 10.185 |
| Cell8\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.01E+09 | 1.25 | 12.125 |
| Cell9\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.17E+09 | 1.25 | 12.125 |
| Cell10\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.00E+09 | 1.25 | 12.125 |
| Cell11\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.34E+09 | 1.77 | 17.169 |
| Cell12\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.06E+09 | 1.25 | 12.125 |
| Cell13\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.49E+09 | 1.77 | 17.169 |
| Cell14\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 8.71E+08 | 1.05 | 10.185 |
| Cell15\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.18E+09 | 1.77 | 17.169 |
| Cell16\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.48E+09 | 1.77 | 17.169 |
| Cell17\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.09E+09 | 1.77 | 17.169 |
| Cell18\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.07E+09 | 1.77 | 17.169 |
| Cell19\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.17E+09 | 1.77 | 17.169 |
| Cell20\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.09E+09 | 1.25 | 12.125 |
| Cell21\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.02E+09 | 1.77 | 17.169 |
| Cell22\_crop\_DAPI\_Cy3dUTP\_HeLa | SL | 1.02E+09 | 1.77 | 17.169 |
| Cell23\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 9.33E+08 | 1.05 | 10.185 |
| Cell24\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 8.90E+08 | 1.05 | 10.185 |
| Cell25\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 7.67E+08 | 1.05 | 10.185 |
| Cell26\_crop\_DAPI\_Cy3dUTP\_HeLa | SE | 8.39E+08 | 1.05 | 10.185 |
| Cell27\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.04E+09 | 1.25 | 12.125 |
| Cell28\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.01E+09 | 1.25 | 12.125 |
| Cell29\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.05E+09 | 1.25 | 12.125 |
| Cell30\_crop\_DAPI\_Cy3dUTP\_HeLa | SM | 1.02E+09 | 1.25 | 12.125 |
| Cell1\_DAPI\_Cy3dUTP\_IMR90 | - | 1.08E+09 | - | 6.37 |
| Cell2\_DAPI\_Cy3dUTP\_IMR90 | - | 5.04E+08 | - | 6.37 |
| Cell3\_DAPI\_Cy3dUTP\_IMR90 | - | 1.09E+09 | - | 6.37 |
| Cell4\_DAPI\_Cy3dUTP\_IMR90 | - | 8.72E+08 | - | 6.37 |
| Cell5\_DAPI\_Cy3dUTP\_IMR90 | - | 1.08E+09 | - | 6.37 |
| Cell6\_DAPI\_Cy3dUTP\_IMR90 | - | 5.39E+08 | - | 6.37 |
| Cell7\_DAPI\_Cy3dUTP\_IMR90 | - | 6.33E+08 | - | 6.37 |
| Cell8\_DAPI\_Cy3dUTP\_IMR90 | - | 5.52E+08 | - | 6.37 |
| Cell9\_DAPI\_Cy3dUTP\_IMR90 | - | 6.20E+08 | - | 6.37 |
| Cell10\_DAPI\_Cy3dUTP\_IMR90 | - | 6.68E+08 | - | 6.37 |
| Cell13\_DAPI\_Cy3dUTP\_IMR90 | - | 7.43E+08 | - | 6.37 |
| Cell14\_DAPI\_Cy3dUTP\_IMR90 | - | 6.53E+08 | - | 6.37 |
| Cell15\_DAPI\_Cy3dUTP\_IMR90 | - | 7.25E+08 | - | 6.37 |
| Cell16\_DAPI\_Cy3dUTP\_IMR90 | - | 4.41E+08 | - | 6.37 |
| Cell17\_DAPI\_Cy3dUTP\_IMR90 | - | 1.29E+09 | - | 6.37 |
| Cell18\_DAPI\_Cy3dUTP\_IMR90 | - | 7.51E+08 | - | 6.37 |

**Supplementary file 1g: Data description for 2D confocal fixed images**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  **Name** | **Cell stage** | **Time (s)** | **Channels\*** | **Pixel size (nm)** | **frame rate (ms)** |
| Cell1\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell2\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell3\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell4\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell5\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell6\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell7\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell8\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell9\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell10\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell11\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell12\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell13\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell14\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell15\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell16\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell17\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell18\_HeLa\_fixedcells.tif | G2 | 40 | 3 | 120 | 500 |
| Cell19\_HeLa\_fixedcells.tif | G1 | 40 | 3 | 120 | 500 |
| Cell20\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell21\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell22\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell23\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell24\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell25\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell26\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell27\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell28\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell29\_HeLa\_fixedcells.tif | SM | 40 | 3 | 120 | 500 |
| Cell30\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell31\_HeLa\_fixedcells.tif | SL | 40 | 3 | 120 | 500 |
| Cell32\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell33\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell34\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell35\_HeLa\_fixedcells.tif | SE | 40 | 3 | 120 | 500 |
| Cell1\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell2\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell3\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell4\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell5\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell6\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell7\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell8\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell9\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell10\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell11\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell12\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell13\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell14\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell15\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell16\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell17\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |
| Cell18\_IMR90\_fixed.tif | - | 40 | 3 | 120 | 500 |

**Supplementary file 1h: Data description for 2D confocal live images**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Cell stage** | **Time****(s)** | **Exposure time****(ms)** | **Channels\*** | **Frame rate****(ms)** |
| n001\_G\_Aph\_4\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n001\_G\_Aph\_4\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n001\_G\_Aph\_4\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n001\_G\_Aph\_4\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n002\_G\_Aph\_4\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n002\_G\_Aph\_4\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n002\_G\_Aph\_4\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n002\_G\_Aph\_4\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n003\_G\_Aph\_5\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n003\_G\_Aph\_5\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n003\_G\_Aph\_5\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n003\_G\_Aph\_5\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n004\_G\_Aph\_5\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n004\_G\_Aph\_5\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n004\_G\_Aph\_5\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n004\_G\_Aph\_5\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n005\_G\_Aph\_5\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n005\_G\_Aph\_5\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n005\_G\_Aph\_5\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n005\_G\_Aph\_5\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n006\_G\_Aph\_6\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n006\_G\_Aph\_6\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n006\_G\_Aph\_6\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n006\_G\_Aph\_6\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n007\_G\_Aph\_10\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n007\_G\_Aph\_10\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n007\_G\_Aph\_10\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n007\_G\_Aph\_10\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n008\_G\_Aph\_10\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n008\_G\_Aph\_10\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n008\_G\_Aph\_10\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n008\_G\_Aph\_10\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n009\_G\_Aph\_10\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n009\_G\_Aph\_10\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n009\_G\_Aph\_10\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n009\_G\_Aph\_10\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n0012\_G\_Aph\_11\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n0012\_G\_Aph\_11\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n0012\_G\_Aph\_11\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n0012\_G\_Aph\_11\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n0013\_G\_Aph\_11\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n0013\_G\_Aph\_11\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n0013\_G\_Aph\_11\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n0013\_G\_Aph\_11\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n0015\_G\_Aph\_15\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n0015\_G\_Aph\_15\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n0015\_G\_Aph\_15\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n0015\_G\_Aph\_15\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n0017\_G\_Aph\_16\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n0017\_G\_Aph\_16\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n0017\_G\_Aph\_16\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n0017\_G\_Aph\_16\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n0018\_G\_Aph\_18\_AT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n0018\_G\_Aph\_18\_AT\_c02.tif | G1 | - | 500 | 3 | - |
| n0018\_G\_Aph\_18\_BT\_c01.tif | G1 | 40 | 300 | 1 | 500 |
| n0018\_G\_Aph\_18\_BT\_c02.tif | G1 | - | 500 | 3 | - |
| n0020\_G\_Aph\_21\_AT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n0020\_G\_Aph\_21\_AT\_c02.tif | G2 | - | 500 | 3 | - |
| n0020\_G\_Aph\_21\_BT\_c01.tif | G2 | 40 | 300 | 1 | 500 |
| n0020\_G\_Aph\_21\_BT\_c02.tif | G2 | - | 500 | 3 | - |
| n001\_S\_Aph\_1\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n001\_S\_Aph\_1\_AT\_c02.tif | S | - | 500 | 3 | - |
| n001\_S\_Aph\_1\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n001\_S\_Aph\_1\_BT\_c02.tif | S | - | 500 | 3 | - |
| n002\_S\_Aph\_1\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n002\_S\_Aph\_1\_AT\_c02.tif | S | - | 500 | 3 | - |
| n002\_S\_Aph\_1\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n002\_S\_Aph\_1\_BT\_c02.tif | S | - | 500 | 3 | - |
| n004\_S\_Aph\_1\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n004\_S\_Aph\_1\_AT\_c02.tif | S | - | 500 | 3 | - |
| n004\_S\_Aph\_1\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n004\_S\_Aph\_1\_BT\_c02.tif | S | - | 500 | 3 | - |
| n006\_S\_Aph\_2\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n006\_S\_Aph\_2\_AT\_c02.tif | S | - | 500 | 3 | - |
| n006\_S\_Aph\_2\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n006\_S\_Aph\_2\_BT\_c02.tif | S | - | 500 | 3 | - |
| n008\_S\_Aph\_4\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n008\_S\_Aph\_4\_AT\_c02.tif | S | - | 500 | 3 | - |
| n008\_S\_Aph\_4\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n008\_S\_Aph\_4\_BT\_c02.tif | S | - | 500 | 3 | - |
| n009\_S\_Aph\_5\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n009\_S\_Aph\_5\_AT\_c02.tif | S | - | 500 | 3 | - |
| n009\_S\_Aph\_5\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n009\_S\_Aph\_5\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0012\_S\_Aph\_6\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0012\_S\_Aph\_6\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0012\_S\_Aph\_6\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0012\_S\_Aph\_6\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0013\_S\_Aph\_7\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0013\_S\_Aph\_7\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0013\_S\_Aph\_7\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0013\_S\_Aph\_7\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0014\_S\_Aph\_7\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0014\_S\_Aph\_7\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0014\_S\_Aph\_7\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0014\_S\_Aph\_7\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0015\_S\_Aph\_8\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0015\_S\_Aph\_8\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0015\_S\_Aph\_8\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0015\_S\_Aph\_8\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0016\_S\_Aph\_8\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0016\_S\_Aph\_8\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0016\_S\_Aph\_8\_BT\_c01.tif | S | - | 500 | 3 | 500 |
| n0016\_S\_Aph\_8\_BT\_c02.tif | S | 40 | 300 | 1 | - |
| n0017\_S\_Aph\_8\_AT\_c01.tif | S | - | 500 | 3 | 500 |
| n0017\_S\_Aph\_8\_AT\_c02.tif | S | 40 | 300 | 1 | - |
| n0017\_S\_Aph\_8\_BT\_c01.tif | S | - | 500 | 3 | 500 |
| n0017\_S\_Aph\_8\_BT\_c02.tif | S | 40 | 300 | 1 | - |
| n0018\_S\_Aph\_8\_AT\_c01.tif | S | - | 500 | 3 | 500 |
| n0018\_S\_Aph\_8\_AT\_c02.tif | S | 40 | 300 | 1 | - |
| n0018\_S\_Aph\_8\_BT\_c01.tif | S | - | 500 | 3 | 500 |
| n0018\_S\_Aph\_8\_BT\_c02.tif | S | 40 | 300 | 1 | - |
| n0019\_S\_Aph\_9\_AT\_c01.tif | S | - | 500 | 3 | 500 |
| n0019\_S\_Aph\_9\_AT\_c02.tif | S | 40 | 300 | 1 | - |
| n0019\_S\_Aph\_9\_BT\_c01.tif | S | - | 500 | 3 | 500 |
| n0019\_S\_Aph\_9\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0020\_S\_Aph\_9\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0020\_S\_Aph\_9\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0020\_S\_Aph\_9\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0020\_S\_Aph\_9\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0021\_S\_Aph\_1\_0\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0021\_S\_Aph\_1\_0\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0021\_S\_Aph\_1\_0\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0021\_S\_Aph\_1\_0\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0022\_S\_Aph\_1\_2\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0022\_S\_Aph\_1\_2\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0022\_S\_Aph\_1\_2\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0022\_S\_Aph\_1\_2\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0023\_S\_Aph\_1\_3\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0023\_S\_Aph\_1\_3\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0023\_S\_Aph\_1\_3\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0023\_S\_Aph\_1\_3\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0024\_S\_Aph\_1\_4\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0024\_S\_Aph\_1\_4\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0024\_S\_Aph\_1\_4\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0024\_S\_Aph\_1\_4\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0025\_S\_Aph\_1\_7\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0025\_S\_Aph\_1\_7\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0025\_S\_Aph\_1\_7\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0025\_S\_Aph\_1\_7\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0026\_S\_Aph\_1\_7\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0026\_S\_Aph\_1\_7\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0026\_S\_Aph\_1\_7\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0026\_S\_Aph\_1\_7\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0027\_S\_Aph\_21\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0027\_S\_Aph\_21\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0027\_S\_Aph\_21\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0027\_S\_Aph\_21\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0028\_S\_Aph\_21\_AT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0028\_S\_Aph\_21\_AT\_c02.tif | S | - | 500 | 3 | - |
| n0028\_S\_Aph\_21\_BT\_c01.tif | S | 40 | 300 | 1 | 500 |
| n0028\_S\_Aph\_21\_BT\_c02.tif | S | - | 500 | 3 | - |
| n0001\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0002\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0003\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0004\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0005\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0006\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0007\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0008\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0009\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0010\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0011\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0012\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0013\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0014\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0015\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0016\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0017\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0018\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0019\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0020\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0021\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0022\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0023\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0024\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0025\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0026\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0027\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0028\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0029\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0030\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0031\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0032\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n0033\_coloc\_c02.tif | S | 40 | 300 | 2 | 500 |
| n001\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n002\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n003\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n004\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n005\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n006\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n007\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n008\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n009\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n010\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n011\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n012\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n013\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n014\_G1\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G1 | 40 | 300 | 2 | 500 |
| n001\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n002\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n003\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n004\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n005\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n006\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n007\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n008\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n009\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n010\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n011\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n012\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n013\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n014\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n015\_G2\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | G2 | 40 | 300 | 2 | 500 |
| n001\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n002\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n003\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n004\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n005\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n006\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n007\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n008\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n009\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n010\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n011\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n012\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n0013\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |
| n0014\_S\_IMR90\_PCNA\_Cy3dUTP\_60x.tif | S | 40 | 300 | 2 | 500 |

**Supplementary file 1i: Software and macros**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Version** | **Website** | **Company/University** | **Application** |
| Volocity | 6.3 | - | PerkinElmer, USA | Acquiring live cell time lapses |
| ImageJ | 1.53c | <https://imagej.nih.gov/ij/> | Wayne Rasband, National Institutes of Health, USA | Image processing and image analysis |
| RStudio | 1.1.447-1.2.5033 | <https://rstudio.com/> | RStudio | Statistical analysis and plotting |
| Harmony | 3.5.1 | <https://www.perkinelmer.com/product/harmony-4-8-office-hh17000001> |  PerkinElmer, USA | High content microscopy imaging and analysis |
| KNIME Analytics Platform | 3.5.2 | https://www.knime.com/knime-analytics-platform | KNIME AG, Switzerland | High content microscopy image processing and analysis |
| Adobe Illustrator CS6 | 16 | <https://www.adobe.com/> | Adobe, USA | Graphical sketch and figures arrangement |

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