Supplementary table S1a: Minimum Information about a Flow Cytometry Experiment (MIFlowCyt)

Requirement	Please Include Requested Information
1.1. Purpose	Sort 200 000 cells per phase for RNA sequencing and 500 000 cells for immunoblotting from HeLa S. FUCCI cell line.
1.2. Keywords	Cell cycle, circadian molecular clock, FUCCI
1.3. Experiment variables	Cell cycle-sorted HeLa cell lines using FUCCI reporters.
1.4. Organization name and address	Department of Cytokinetics, Institute of Biophysics CAS, v.v.i., Královopolská 135, 612 65 Brno, Czech Republic
1.5. Primary contact name and email address	Michael Andäng, michael.andang@ki.se, Department of Physiology and Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden. Central European Institute of Technology, Masaryk University, 625 00 Brno, Czech Republic.
1.6. Date or time period of experiment	18.6.2010 – 20.12.2012
1.7. Conclusions	200 000 cells per phase were sorted for subsequent analyses into 200 µl of RLT lysis buffer with 1% 2-mercaptoethanol for the RNA sequencing, and 500 000 cells per phase for immunoblotting into 100 µl of lysis buffer.
1.8. Quality control measures	Cell aggregates and debris were excluded from the analysis based on FCS-A vs. FCS-H, and FSC-A vs. SSC-A a dual-parameter dot plots. For each phase post-sorting control was done.
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	Cells were grown to sub-confluency, collected by trypsinisation, washed with full DMEM/McCoy's with 10% serum, 5% Glutamax and 100U/ml penicillin-streptomycin, spinned down by centrifugation at 200 g for 5 minutes and resuspended in culture media before sorting.
2.1.1.2. Biological sample source description	HeLa.S FUCCI human cervical carcinoma cell line – source: Riken Cell Bank.
2.1.1.3. Biological sample source organism description	Human.
2.1.2.2. Environmental sample location	-
2.3. Sample treatment description	Samples were not treated.

<table>
<tr><td>2.4. Fluorescence reagent(s) description</td><td>
<table>
<tr><th>Marker</th><th>Laser</th><th>Manufacturer</th><th>Power</th><th>Detector</th><th>Dichroic Mirror // Band Pass Filter (nm)</th><th>Instalation date</th><th>Instrument</th></tr>
<tr><td>mAG-hGem</td><td>488</td><td>Coherent Sapphire</td><td>100 mW</td><td>F - Blue Octagon</td><td>505LP // 525/50</td><td>2009</td><td>FACSAria II Sorp</td></tr>
<tr><td>mKO2-hCdt1</td><td>488</td><td>Coherent Sapphire</td><td>100 mW</td><td>E - Blue Octagon</td><td>550LP // 575/25</td><td>2009</td><td>FACSAria II Sorp</td></tr>
</table>
</td></tr>
<tr><td>3.1. Instrument manufacturer</td><td>BD Biosciences, San Jose, CA 95131 USA</td></tr>
<tr><td>3.2. Instrument model</td><td>BD FACSAria™ II Cell Sorter (ver. Sorp 4L), Serial number: PY500001</td></tr>
<tr><td>3.3. Instrument configuration and settings</td><td>488nm laser excitation:</td></tr>
<tr><td>4.1. List-mode data files</td><td>FCS data files can be obtained by contacting Michael Andäng after this work has been published.</td></tr>
<tr><td>4.2. Compensation description</td><td>Manual compensation was applied based on quantification of medians of positive and negative populations on both markers hGem-mAG (detector F) vs. hCdt1-mKO2 (detector E).</td></tr>
<tr><td>4.3. Data transformation details</td><td>FACSDiva (Version 6.1.3; BD Biosciences) and FlowJo software (Version 10.0.7, Tree Star, Ashland, OR, USA) was used for data visualization.</td></tr>
<tr><td>4.4.1. Gate description</td><td>Single cells: FSC-A vs. FSC-H gate to define single cells from doublets.
Debris exclusion: FSC-A vs. SSC-A gate to define compact population of cells without debris.
G1: hGem-mAG (detector F) vs. hCdt1-mKO2 (detector E) gate to define G1 cell cycle phase.
S: hGem-mAG (detector F) vs. hCdt1-mKO2 (detector E) gate to define G1/S cell cycle phase
G2: hGem-mAG (detector F) vs. hCdt1-mKO2 (detector E) gate to define S/G2/M cell cycle phase</td></tr>
<tr><td>4.4.2. Gate statistics</td><td>Please see the next section.</td></tr>
<tr><td>4.4.3. Gate boundaries</td><td></td></tr>
</table>

Supplementary table S1b: Minimum Information about a Flow Cytometry Experiment (MIFlowCyt)

<table>
<tr><th>Requirement</th><th>Please Include Requested Information</th></tr>
<tr><td>1.1. Purpose</td><td>Sort 200 000 cells per phase for RNA sequencing of U2OS FUCCI cell line.</td></tr>
<tr><td>1.2. Keywords</td><td>Cell cycle, FUCCI</td></tr>
<tr><td>1.3. Experiment variables</td><td>Cell cycle-sorted U2OS cell line using FUCCI reporters.</td></tr>
<tr><td>1.4. Organization name and address</td><td>Karolinska Institutet CMM

Flow Cytometry facility L8:03, 17176 Stockholm, Sweden</td></tr>
<tr><td>1.5. Primary contact name and email address</td><td>Mikael Altun, Mikael.altun@ki.se

Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden.</td></tr>
<tr><td>1.6. Date or time period of experiment</td><td>7.10.2013 – 28.1.2014</td></tr>
<tr><td>1.7. Conclusions</td><td>200 000 cells per phase were sorted for subsequent analyses into 200 µl of Qiazol Lysis Reagent (Qiazol)</td></tr>
<tr><td>1.8. Quality control measures</td><td>Cell aggregates and debris were excluded from the analysis based on FSC-A vs SSC-A and FSC-A vs FSC-W</td></tr>
<tr><td>2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description</td><td>Cells were grown to sub-confluency, collected by trypsinisation, washed with full McCoy's with 10% serum, 5% Glutamax and 100U/ml penicillin-streptomycin, spun down by centrifugation at 500 g for 5 minutes and resuspended in culture media before sorting.</td></tr>
<tr><td>2.1.1.2. Biological sample source description</td><td>U2OS FUCCI human osteosarcoma cell line – source: Gift from Dr. Masai at the Tokyo Metropolitan Institute of Medical Science, Japan.</td></tr>
<tr><td>2.1.1.3. Biological sample source organism description</td><td>Human.</td></tr>
<tr><td>2.1.2.2. Environmental sample location</td><td>-</td></tr>
<tr><td>2.3. Sample treatment description</td><td>Samples were not treated.</td></tr>
<tr><td>2.4. Fluorescence reagent(s) description</td><td>
<table>
<tr><th>Marker</th><th>Laser</th><th>Manufacturer</th><th>Power</th><th>Detector</th><th>Dichroic Mirror // Band Pass Filter (nm)</th><th>Instalation date</th><th>Instrument</th></tr>
<tr><td>mAG-hGem</td><td>488</td><td>Coherent</td><td>150mW</td><td>3</td><td>555DLP//530/40</td><td>01/2002</td><td>MoFlo legacy</td></tr>
</table>
</td></tr>
</table>

	mKO2-hCdt1	561	Melles Griot	35mW	6	605DLP//585/29	01/2002	MoFlo Legacy

3.1. Instrument manufacturer	Beckman Coulter, USA
3.2. Instrument model	MoFlo Legacy
3.3. Instrument configuration and settings	488nm laser excitation: 530/40 (detector 3) 561nm laser excitation: 585/29 (detector 6)
4.1. List-mode data files	-
4.2. Compensation description	Manual compensation was applied based on quantification of medians of positive and negative populations on both markers hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6).
4.3. Data transformation details	Summit (V4.3.02) was used for data visualization.
4.4.1. Gate description	Single cells: FSC-A vs. FSC-W gate to define single cells from doublets. Debris exclusion: FSC-A vs. SSC-A gate to define compact population of cells without debris. G1: hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6) gate to define G1 cell cycle phase. S: hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6) gate to define G1/S cell cycle phase G2: hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6) gate to define S/G2/M cell cycle phase
4.4.2. Gate statistics	Please see the next section.
4.4.3. Gate boundaries	