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	Department of Cytokinetics, Institute of Biophysics CAS, v.v.i., Královopolská 135, 612 65 Brno, Czech
and address	Republic
1.5. Primary contact	Michael Andäng, <u>michael.andang@ki.se</u> ,
address	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 μ l of RLT lysis buffer with 1% 2-mercaptoethanol for the RNA sequencing, and 500 000 cells per phase for immunoblotting into 100 μ l 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.

2.4. Fluorescence reagent(s) description	Marker	Laser	Manufacturer	Power	Detector	Dichroic Mirror // Band Pass Filter (nm)	Instalation date	Instrument	
	mAG-hGem	488	Coherent Sapphire	100 mW	F - Blue Octagon	505LP // 525/50	2009	FACSAria II Sorp	
	mKO2-hCdt1	488	Coherent Sapphire	100 mW	E - Blue Octagon	550LP // 575/25	2009	FACSAria II Sorp	
3.1. Instrument manufacturer	BD Biosciences, San Jose, CA 95131 USA								
3.2. Instrument model	BD FACSAria™ II Cell Sorter (ver. Sorp 4L), Serial number: PY500001								
3.3. Instrument configuration and settings	488nm laser excitation:								
4.1. List-mode data files	FCS data file	s can b	e obtained by cont	acting M	ichael Andäng a	after this work has b	peen publis	hed.	
4.2. Compensation description	Manual com on both mar	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).							
4.3. Data transformation details	FACSDiva (Version 6.1.3; BD Biosciences) and FlowJo software (Version 10.0.7, Tree Star, Ashland, OR, USA) was used for data visualization.								
4.4.1. Gate description	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								
4.4.2. Gate statistics	Please see th	ne next	section.						
4.4.3. Gate boundaries	Brede calls Brede								

Requirement	Please Include Requested Information								
1.1. Purpose	Sort 200 000 cells per phase for RNA sequencing of U2OS FUCCI cell line.								
1.2. Keywords	Cell cycle, FL	Cell cycle, FUCCI							
1.3.	Cell cycle-sorted U2OS cell line using FUCCI reporters.								
Experiment									
variables									
1.4.	Karolinska Institutet CMM								
Organization									
name and	Flow Cytometry facility L8:03, 17176 Stockholm, Sweden								
address									
1.5. Primary	Mikael Altun, Mikael.altun@ki.se								
contact name									
and email	Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden.								
address	7.40.2042								
1.6. Date or	7.10.2013 -	28.1.20)14						
time period of									
	200,000,colla	norn	accountry control fo		want analyzas i	nto 200 ul of Oiozol		ant (Oiszal)	
1.7.	200 000 cens	s per p		n subset	quent analyses i		LYSIS REAR	ent (Qiazoi)	
1.8 Quality	Cell aggregat	es and	l debris were evclu	hed from	the analysis ha	used on ESC-A vs SSC	-A and ESC	-A vs FSC-W	
control	Cell aggregat		ruebris were exclud		i the analysis ba	13eu 011 1 3C-A V3 33C		-A V315C-W	
measures									
2.1.1.1.	Cells were gr	own to	o sub-confluency, c	ollected	by trypsinisatio	n, washed with full	McCov's wi	th 10% serum.	
(2.1.2.1	5% Glutamax	c and 1	.00U/ml penicillin-s	treptom	vcin, spun dowr	n by centrifugation a	at 500 g for	5 minutes and	
2.1.3.1.)	resuspended	resuspended in culture media before sorting.							
Sample									
description									
2.1.1.2.	U2OS FUCCI human osteosarcoma cell line – source: Gift from Dr. Masai at the Tokyo Metropolitan Institute								
Biological	of Medical Science, Japan.								
sample source									
description									
2.1.1.3.	Human.	Human.							
Biological									
sample source									
organism									
description									
2.1.2.2.	-								
Environmental									
sample									
location									
2.3. Sample	Samples wer	e not t	reated.						
treatment									
description									
2.4.						Dichroic Mirror //	Instalation		
Fluorescence	Marker	Laser	Manufacturer	Power	Detector	Band Pass Filter (nm)	date	Instrument	
reagent(s)	mAGhCorr	400	Cohoront	150	2		01/2002	MaElalazaari	
description	mad-ndem	400	Conerent	1301110	5	5550LP//550/40	01/2002	world legacy	

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

	mKO2-hCdt1	561	Melles Griot	35mW	6	605DLP//585/29	01/2002	MoFlo Legacy		
3.1.	Beckman Coulter, USA									
Instrument										
		McEle Legacy								
J.Z.	WOI TO Legac	INIOFIO Legacy								
model										
3.3.	488nm laser	488nm laser excitation: 530/40 (detector 3)								
Instrument	561nm laser	561nm laser excitation: 585/29 (detector 6)								
configuration										
and settings										
4.1. List-mode	-	-								
data files							<u> </u>			
4.2.	Manual com	Manual compensation was applied based on quantification of medians of positive and negative populations								
description	on both markers hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6).									
4 3 Data	Summit (V/4 2 02) was used for data visualization									
transformation	Summe (V4.	5.02, 1		Suunzuun	511.					
details										
4.4.1. Gate	Single cells: I	SC-A \	s. FSC-W gate to de	efine sing	gle cells from do	oublets.				
description	Debris exclus	sion: F	SC-A vs. SSC-A gate	to define	e compact popu	lation of cells witho	out debris.			
	G1: hGem-m	AG (de	etector 3) vs. hCdt1	-mKO2 (detector 6) gate	to define G1 cell cy	cle phase.			
	S: hGem-mA	S: hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6) gate to define G1/S cell cycle phase								
	G2: hGem-m	G2: hGem-mAG (detector 3) vs. hCdt1-mKO2 (detector 6) gate to define S/G2/M cell cycle phase								
4.4.2. Gate	Please see the next section.									
statistics										
4.4.3. Gate	256	625-23.								
boundaries	192-		10 ⁴ R	4						
	5 128- 8 128-		10 ³ - R2	1-1 1/4 1/4						
	64-									
	0 64 128 FS Lin	92 256	102		R6					
	R3		101-							
	5 128-			199 1 1	<u> </u>					
	64-		10 ⁰ 10 ⁹ 10 ¹	102 103	104					
			FL 1	rea coub						
	0 64 128 Pulse Width	92 256								