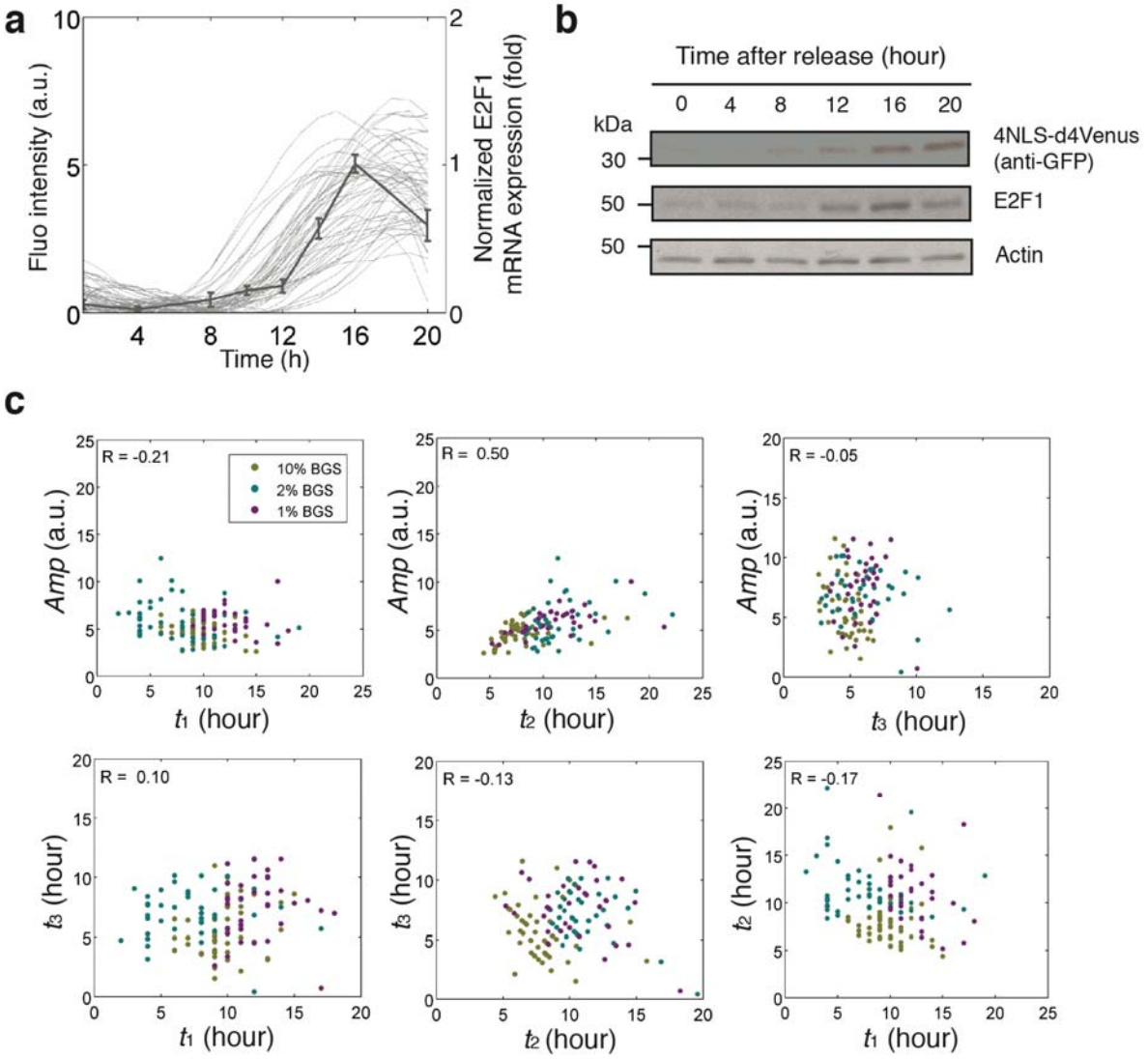
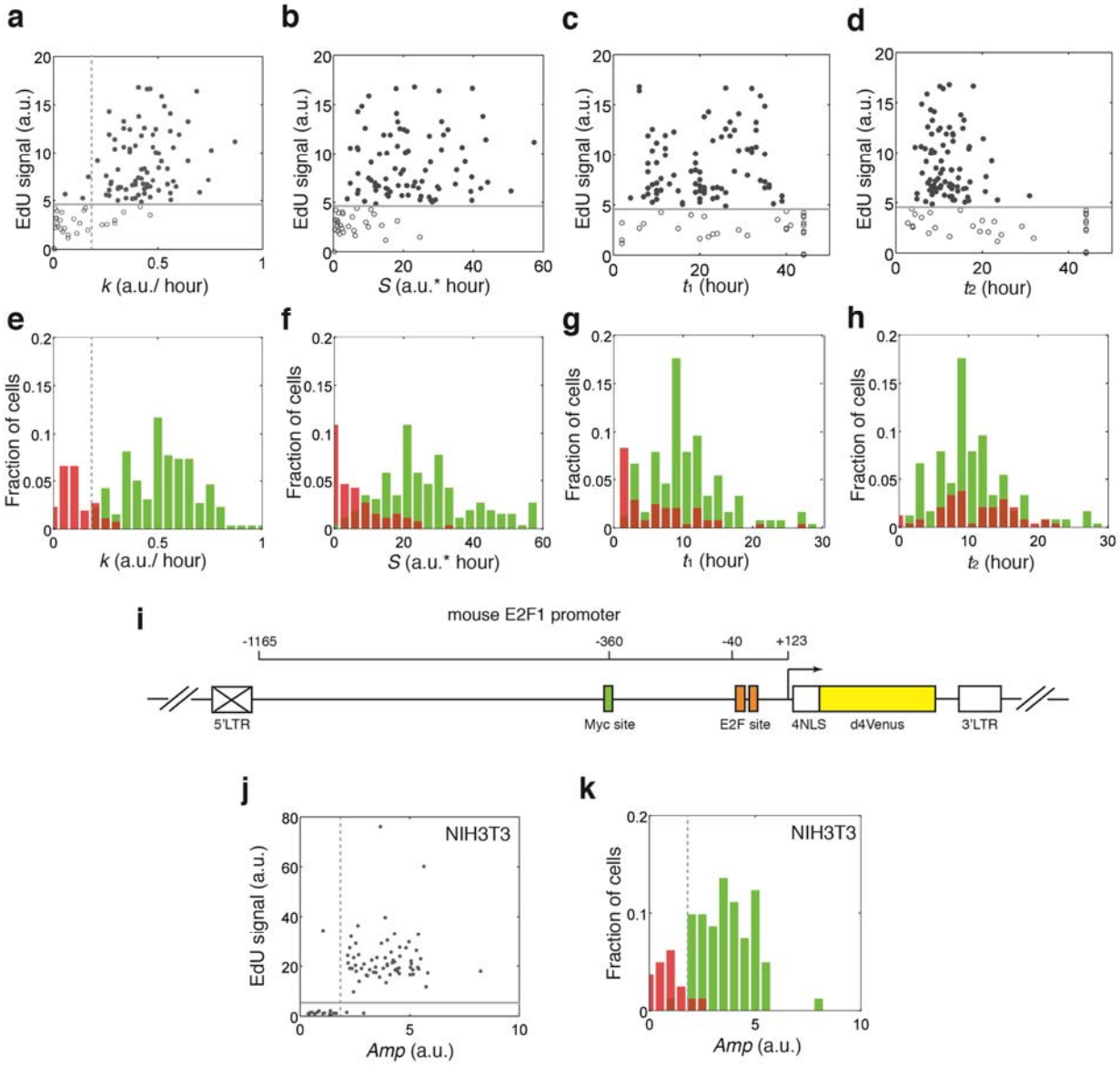
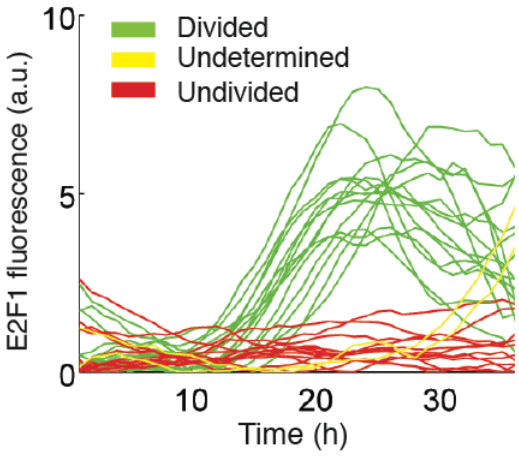


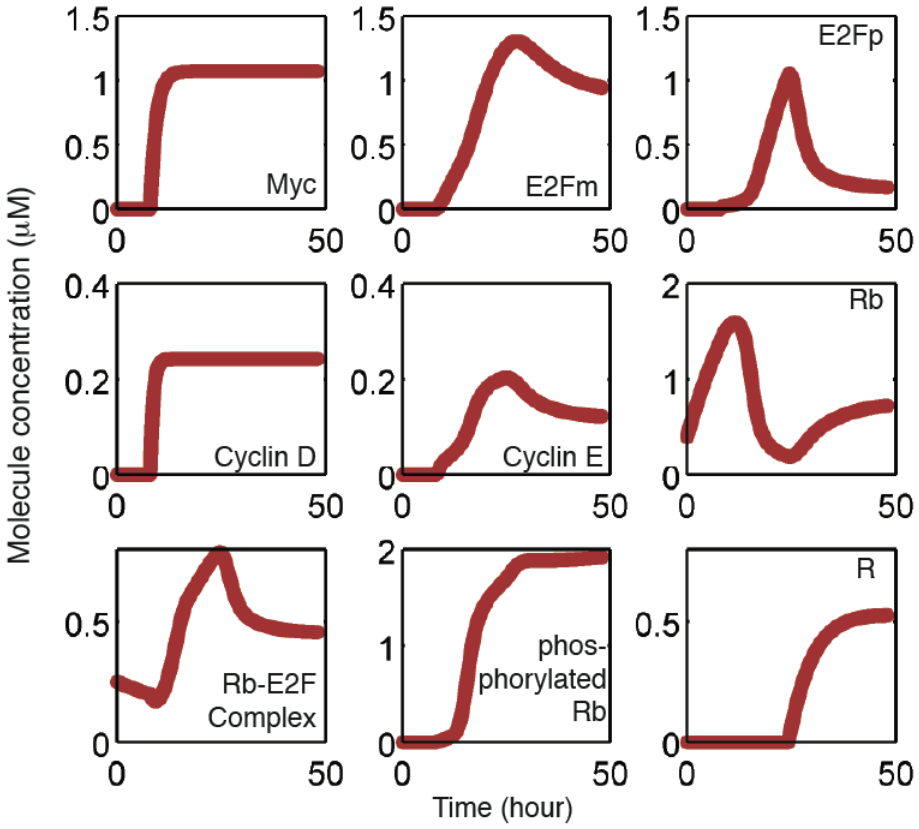
Supplementary Figure 1. Confirmation of REF52-hE2F1p::4NLS-d4Venus reporter cells and characterization of E2F dynamics. (**a**) Alignment of E2F dynamics trajectories to endogenous E2F1 mRNA expression. Gray curves indicates 53 smoothened E2F dynamics trajectories measured after REF52-hE2F1p::4NLS-d4Venus cells were released from serum starvation after the addition of 10% BGS. The black curve indicates endogenous E2F1 mRNA expression (normalized to the peak value) measured by qRT-PCR at different time points. (**b**) Western blot of Venus and endogenous E2F1 protein expression following serum stimulation in a single cell clone of REF52-hE2F1p::4NLS-d4Venus cells integrated with the reporter. See Supplementary Fig. 9 for the full blot. (**c**) Scatter plots shows the pairwise correlation of basic E2F1 dynamics properties. Data points under different serum levels are mixed in plotting and labeled with different colors.



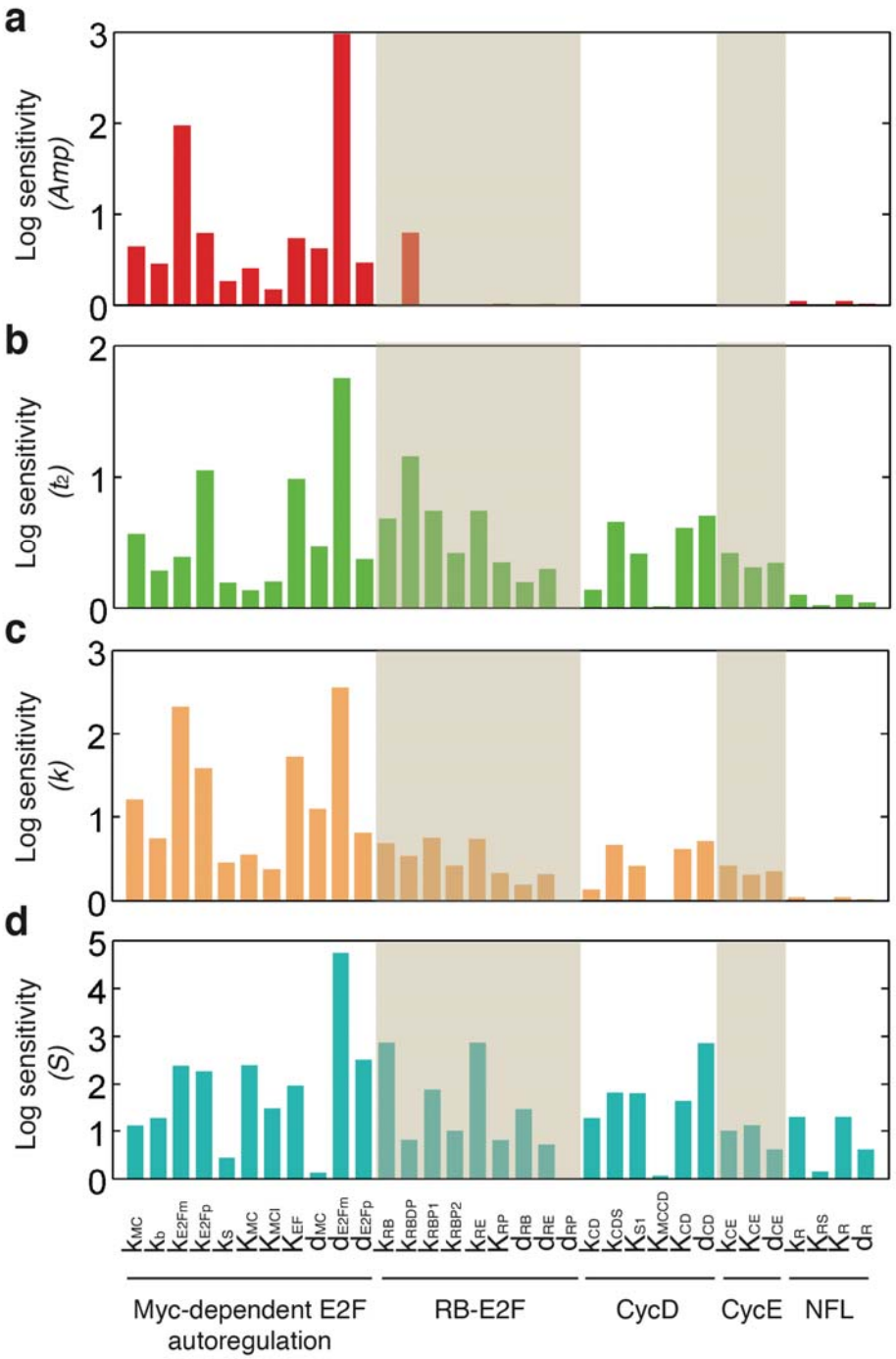
Supplementary Figure 2. *Amp* correlates with cell cycle entry and predicts cell division. (**a-d**). Scatter plot based on cell fate (red, undivided; green divided) as determined by k, S, t_1 or t_2. Solid lines indicate the boundary of signal between EdU-positive and -negative cells and dash lines indicate an apparent threshold. (**e-h**). Histogram based on cell fate (red, undivided; green divided) as determined by k, S, t_1 or t_2. (**i**) mE2F1p::4NLS-d4Venus reporter construct. (**j**) and (**k**). NIH3T3-mE2F1p::4NLS-d4Venus cells were released from serum starvation by adding 0.5% BGS and 20 μM EdU. E2F dynamics were measured for 48 hours before cell fixation and EdU staining. Similar analyses as described in Figure 3D and 3E based on NIH3T3 cells stably integrated with pQCXIP-mE2F1p::d4V reporter.



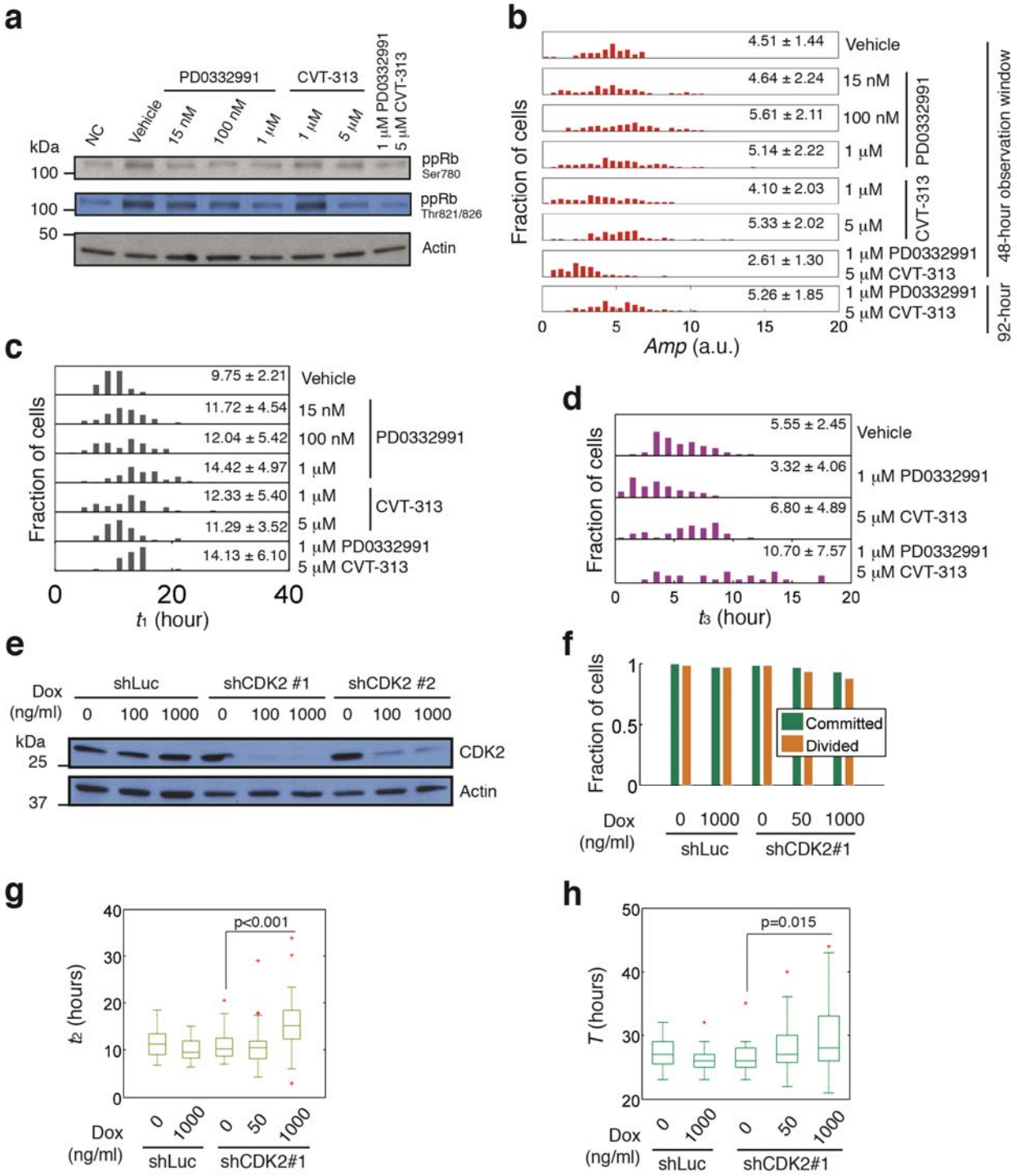
Supplementary Figure 3. Representative E2F trajectories in different sub-groups. Divided cells (green curves) show significant E2F increase while undivided cells (red curves) show little increase in E2F *Amp*. In addition, a small proportion of cells (< 5%, yellow curves) show E2F increase starting at the end of the observation window but have not divided during the observation window. This sub-group of cells was omitted from the entire population subjected to statistical analysis of the relationship between *Amp* and cell fate.



Supplementary Figure 4. Temporal trajectories of all the factors in Myc/Rb/E2F network by simulation. Time-course simulation was performed based on the model mentioned (Table S1-4) by using a stepwise updating algorithm. The initial molecule concentration for the 9 variables (Supplementary Table 1) was set as ([*Myc*], [*E2Fm*], [*E2Fp*], [*CD*], [*CE*], [*RB*], [*RE*], [*RP*], [*R*]) = (0, 0, 0, 0, 0, 0.4, 0.25, 0, 0) μM. As mentioned in Methods, Myc and Cyclin D were constrained to be zero during first 8 hours whereas R was constrained to be zero until E2Fp concentration reached a threshold of 0.4 μM. The interval for variable value update is 6 seconds. Simulation was performed with a relatively high serum input for 48 hours and the dynamic trajectory of each variable was plotted above.

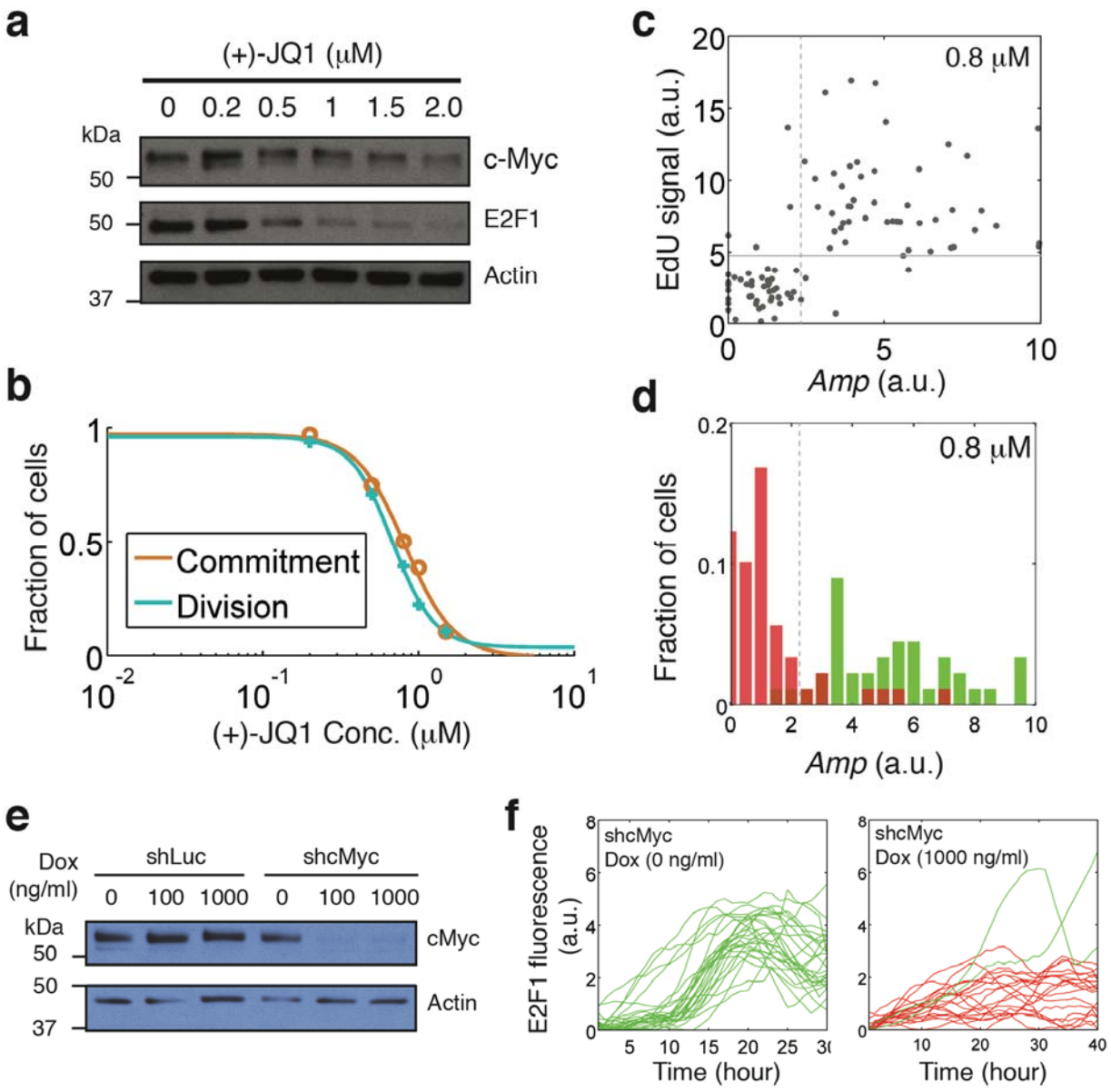


Supplementary Figure 5. Sensitivity analysis of *Amp*, t_2, *k* and *S* to different model parameters. a-d. Sensitivity analysis was done by performing simulation using the full model after varying each parameter value within 0.1 and 10-fold. Log sensitivity for each metric-parameter pair was derived by calculating |*dlog*(*metric*)/*dlog*(*parameter*)|. Results were plotted in for each metric (*Amp*, t_2, *k* and *S*). According to their definitions in the full model, 33 parameters (see Supplementary Table 4) were divided into five functional groups corresponding to five modules: Myc-dependent E2F autoregulation, RB-E2F, CycD, CycE and Negative feedback loop (NFL).

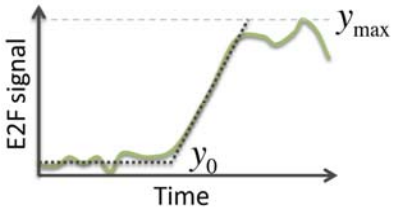


Supplementary Figure 6. Perturbation of Cyclin D/E predominantly affects cell cycle timing rather than commitment decision. (**a**) Rb phosphorylation (at Ser-780 or Thr-821/826 sites) variation detected by western blot in different cases of cyclins perturbation. Actin was used as a loading control. (**b-d**) *Amp* (**b**), t_1 (**c**) or t_3 (**d**) variation in different cases of cyclins

perturbation. Mean ± s.d. is shown on top left of each panel. (**e**) Efficacy of CDK2 knockdown by two different shRNAs as determined by western blot. Sorted cells with shCDK2-inducible system were cultured in medium with 10% FBS and doxycycline at indicated doses for 48 hours and then collected for western blot. (**f**) Proportion of committed/divided cells under different conditions during a 48-hour observation window. Fate of REF52-hE2F1p::4NLS-d4Venus cells transduced with shCDK2#1 was followed in single-cell analysis. (**g**) and (**h**). Box plot of t_2 (**g**) and T (**h**) under different conditions. A rank-sum test was used to obtain p values. Red crosses indicate outliers. See Supplementary Fig. 9 for the photos of full blots.

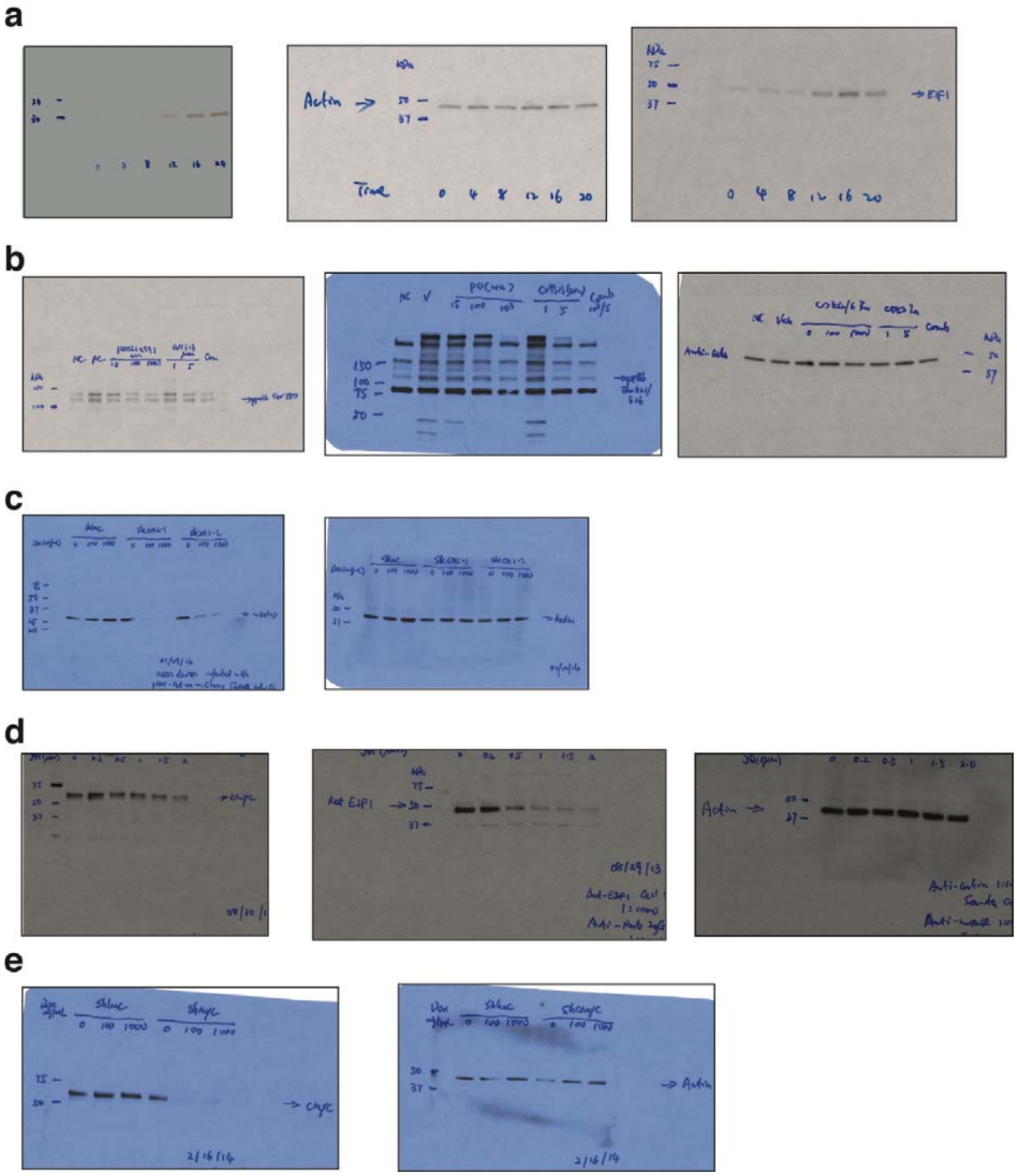


Supplementary Figure 7. Control of commitment into cell cycle entry by Myc. (**a**) Validation of (+)-JQ1 effect on endogenous c-Myc and E2F1 level under different concentrations. REF52-hE2F1p::4NLS-dsVenus reporter cells were released from serum-starvation by adding 10% BGS and different concentrations of (+)-JQ1. Samples were collected at 14 hours after release. (**b**) Dose-response curves indicate the proportion of divided/committed cells under different (+)-JQ1 concentrations. (**c**) Scatter plot of commitment as determined by *Amp* at 0.8 μM (+)-JQ1. (**d**) Histogram of cell division as determined by *Amp* at 0.8 μM (+)-JQ1. (**e**) Confirmation of shRNA knockdown effect on cMyc in REF52-hE2F1p::4NLS-d4Venus cells with tet-inducible shcMyc system at different doxycycline concentrations. (**f**) E2F dynamics trajectories at doxycycline concentration of 0 or 1000 ng/ml. Committed/divided cells were plotted in green color while uncommitted/undivided ones were plotted in red. See Supplementary Fig. 9 for the photos of full blots.



$$y = \begin{cases} y_0 & (0 \le t \le t_1) \\ \dfrac{y_{max} - y_0}{t_2}(t - t_1) & (t_1 \le t \le t_2) \end{cases}$$

Supplementary Figure 8. Analysis of E2F dynamics trajectories. Each smoothed and pre-processed E2F trajectory was then fit to a two-phase regression model to automatically derive optimal values for t_1 and t_2. Thus, the problem equals to search for $\arg\{t_1, t_2\}$ that gives $\min\left\{\sum_{0 \le t \le t_2} [y_{E2F}(t) - y(t)]^2\right\}$. Relevant parameters represent: t_1, initial delay; t_2, activation time; y_0, E2F basal level (the average of fluorescence values of the initial four time points); y_{max}, E2F peak level (maximum fluorescence value of each trajectory); $y_{E2F}(t)$, E2F signal in dynamic trajectory at the moment t.



Supplementary Figure 9. Full blots relative to figures of the main manuscript. (**a**) Full blot of Supplementary Fig. 1b. (**b**) Full blot of Supplementary Fig. 6a. (**c**) Full blot of Supplementary Fig. 6e. (**d**) Full blot of Supplementary Fig. 7a. (**e**) Full blot of Supplementary Fig. 7e.

Supplementary Table 1. Variable definitions used for Myc/Rb/E2F network simulation analysis

S	Serum and mitogenic stimultion
MYC	Myc
E2Fm	E2F mRNA
E2Fp	Free E2F protein (not binding to Rb)
CD	Cyclin D
CE	Cyclin E
RB	Rb pocket protein
RE	Rb-E2F complex
RP	Phosphorylated RB
R	Repressor mediating E2F negative feedback loop (*e.g.* Cyclin A and Skp2)

Supplementary Table 2. Description of reaction terms

Reaction	Term	Description
$\phi \xrightarrow{S} \mathrm{MYC}$	$k_{\mathrm{MC}} \cdot \frac{[S]}{K_{\mathrm{S}}+[S]}$	Serum-dependent Myc production
$\mathrm{MYC} \rightarrow \phi$	$d_{\mathrm{MC}} \cdot [MYC]$	MYC decay
$\phi \xrightarrow{\mathrm{MYC}} \mathrm{E2Fm}$	$k_{\mathrm{b}} \cdot \frac{[MYC]}{K_{\mathrm{MCI}}+[MYC]}$	E2Fm synthesis regulated by Myc alone
$\phi \xrightarrow{\mathrm{MYC\&E2F}} \mathrm{E2Fm}$	$k_{\mathrm{E2Fm}} \cdot \frac{[MYC]}{K_{\mathrm{MC}}+[MYC]} \cdot \frac{[E2Fp]}{K_{\mathrm{EF}}+[E2Fp]}$	E2Fm synthesis regulated by Myc/E2F cooperation
$\mathrm{E2Fm} \rightarrow \phi$	$d_{\mathrm{E2Fm}} \cdot [E2Fm]$	E2Fm decay
$\mathrm{E2Fm} \rightarrow \mathrm{E2Fp}$	$k_{\mathrm{E2Fp}} \cdot [E2Fm]$	E2Fp production through translation
$\mathrm{E2Fp+RB} \rightarrow \mathrm{RE}$	$k_{\mathrm{RE}} \cdot [RB] \cdot [E2Fp]$	Rb-E2F complex formation
$\mathrm{E2Fp} \xrightarrow{R} \phi$	$\frac{K_{\mathrm{R}}+[R]}{K_{\mathrm{R}}} \cdot d_{\mathrm{E2Fp}} \cdot [E2Fp]$	R-regulated E2Fp decay
$\phi \xrightarrow{\mathrm{MYC}} \mathrm{CD}$	$k_{\mathrm{CD}} \cdot \frac{[MYC]}{K_{\mathrm{MCCD}}+[MYC]}$	Myc-dependent Cyclin D production
$\phi \xrightarrow{S} \mathrm{CD}$	$k_{\mathrm{CDS}} \cdot \frac{[S]}{K_{\mathrm{S}}+[S]}$	Serum-dependent Cyclin D production
$\mathrm{CD} \rightarrow \phi$	$d_{\mathrm{CD}} \cdot [CD]$	Cyclin D decay
$\phi \xrightarrow{\mathrm{E2F}} \mathrm{CE}$	$k_{\mathrm{CE}} \cdot \frac{[E2Fp]}{K_{\mathrm{EF}}+[E2Fp]}$	E2F-dependent Cyclin E production
$\mathrm{CE} \rightarrow \phi$	$d_{\mathrm{CE}} \cdot [CE]$	Cyclin E decay
$\phi \rightarrow \mathrm{RB}$	k_{RB}	Constitutive Rb synthesis
$\mathrm{RP} \rightarrow \mathrm{RB}$	$k_{\mathrm{RBDP}} \cdot \frac{[RP]}{K_{\mathrm{RP}}+[RP]}$	Rb production through de-phosphorylation of RP
$\mathrm{RB} \xrightarrow{\mathrm{CD}} \mathrm{RP}$	$k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RB]}{K_{\mathrm{CD}}+[RB]}$	Cyclin D-dependent phosphorylation of Rb

$\mathrm{RB} \xrightarrow{\mathrm{CE}} \mathrm{RP}$	$k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RB]}{K_{\mathrm{CE}} + [RB]}$	Cyclin E-dependent phosphorylation of Rb
$\mathrm{RE} \xrightarrow{\mathrm{CD}} \mathrm{E2Fp}$	$k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RE]}{K_{\mathrm{CD}} + [RE]}$	E2Fp release due to Cyclin D-dependent phosphorylation of RE
$\mathrm{RE} \xrightarrow{\mathrm{CE}} \mathrm{E2Fp}$	$k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RE]}{K_{\mathrm{CE}} + [RE]}$	E2Fp release due to Cyclin E-dependent phosphorylation of RE
$\mathrm{RB} \rightarrow \phi$	$d_{\mathrm{RB}} \cdot [RB]$	Rb decay
$\mathrm{RP} \rightarrow \phi$	$d_{\mathrm{RP}} \cdot [RP]$	RP decay
$\mathrm{RE} \rightarrow \phi$	$d_{\mathrm{RE}} \cdot [RE]\, k_{\mathrm{R}} \cdot \frac{[E2Fp]}{K_{\mathrm{R}} + [E2Fp]}$	RE decay
$\phi \xrightarrow{\mathrm{E2F}} \mathrm{R}$	$k_{\mathrm{R}} \cdot \frac{[E2Fp]}{K_{\mathrm{R}} + [E2Fp]}$	E2F-dependent R transcription
$\mathrm{R} \rightarrow \phi$	$d_{\mathrm{R}} \cdot [R]$	R decay

Supplementary Table 3. Equations for the ODE model of Myc/Rb/E2F network

$\frac{d[MYC]}{dt} = k_{\mathrm{MC}} \cdot \frac{[S]}{K_{\mathrm{S}} + [S]} - d_{\mathrm{MC}} \cdot [MYC]$
$\frac{d[E2Fm]}{dt} = k_{\mathrm{b}} \cdot \frac{[MYC]}{K_{\mathrm{MCI}} + [MYC]} + k_{\mathrm{E2Fm}} \cdot \frac{[MYC]}{K_{\mathrm{MC}} + [MYC]} \cdot \frac{[E2Fp]}{k_{\mathrm{EF}} + [E2Fp]} - d_{\mathrm{E2Fm}} \cdot [E2Fm]$
$\frac{d[E2Fp]}{dt} = k_{\mathrm{E2Fp}} \cdot [E2Fm] + k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RE]}{K_{\mathrm{CD}} + [RE]} + k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RE]}{K_{\mathrm{CD}} + [RE]} + k_{\mathrm{RE}} \cdot [RB] \cdot [E2Fp]$ $- \frac{K_{\mathrm{R}} + [R]}{K_{\mathrm{R}}} \cdot d_{\mathrm{E2Fp}} \cdot [E2Fp]$
$\frac{d[CD]}{dt} = k_{\mathrm{CD}} \cdot \frac{[MYC]}{K_{\mathrm{MCCD}} + [MYC]} + k_{\mathrm{CDS}} \cdot \frac{[S]}{K_{\mathrm{S}} + [S]} - d_{\mathrm{CD}} \cdot [CD]$
$\frac{d[CD]}{dt} = k_{\mathrm{CE}} \cdot \frac{[E2Fp]}{K_{\mathrm{EF}} + [E2Fp]} - d_{\mathrm{CE}} \cdot [CE]$
$\frac{d[RB]}{dt} = k_{\mathrm{RB}} + k_{\mathrm{RBDP}} \cdot \frac{[RP]}{K_{\mathrm{RP}} + [RP]} + k_{\mathrm{RE}} \cdot [RB] \cdot [E2Fp] + k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RB]}{K_{\mathrm{CD}} + [RB]} + k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RB]}{K_{\mathrm{CE}} + [RB]} - d_{\mathrm{RB}} \cdot [RB]$
$\frac{d[RP]}{dt} = k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RB]}{K_{\mathrm{CD}} + [RB]} + k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RB]}{K_{\mathrm{CE}} + [RB]} + k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RE]}{K_{\mathrm{CD}} + [RE]} + k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RE]}{K_{\mathrm{CE}} + [RE]} - k_{\mathrm{RBDP}} \cdot \frac{[RP]}{K_{\mathrm{RP}} + [RP]}$ $- d_{\mathrm{RP}} \cdot [RP]$
$\frac{d[RE]}{dt} = k_{\mathrm{RE}} \cdot [RB] \cdot [E2Fp] - k_{\mathrm{RBP1}} \cdot \frac{[CD] \cdot [RE]}{K_{\mathrm{CD}} + [RE]} - k_{\mathrm{RBP2}} \cdot \frac{[CE] \cdot [RE]}{K_{\mathrm{CE}} + [RE]} - d_{\mathrm{RE}} \cdot [RE]$
$\frac{d[R]}{dt} = k_{\mathrm{R}} \cdot \frac{[E2Fp]}{K_{\mathrm{RS}} + [E2Fp]} - d_{\mathrm{R}} \cdot [R]$

Supplementary Table 4. Values of model parameters

Parameter	Base Value	Description
k_{MC}	1 μM/h	MYC synthesis rate (by serum)
d_{MC}	0.7 /h	MYC decay constant
k_b	0.15 μM/h	E2Fm synthesis rate (by Myc alone)
k_{CD}	0.03 μM/h	Cyclin D synthesis rate (by Myc)
k_{CDS}	0.45 μM/h	Cyclin D synthesis rate (by serum)
d_{CD}	1.5 /h	Cyclin D decay constant
k_{RBP1}	18 /h	RB phosphorylation rate (by Cyclin D/CDK4/6)
k_{E2Fm}	0.40 μM/h	E2Fm synthesis rate (Myc/E2F co-regulation)
d_{E2Fm}	0.25 /h	E2Fm decay constant
k_{E2Fp}	0.40 /h	E2Fp translation rate
d_{E2Fp}	0.35 /h	E2Fp decay constant
k_{RBP2}	18 /h	Rb phosphorylation rate (by Cyclin E/CDK2)
k_{CE}	0.35 μM/h	Cyclin E synthesis rate (by E2Fp)
d_{CE}	1.5 /h	Cyclin E decay constant
k_R	0.10 μM/h	Repressor synthesis rate
d_R	0.10/h	Repressor decay constant
k_{RB}	0.18 μM/h	Rb synthesis rate
d_{RB}	0.06 /h	Rb decay constant
k_{RE}	18 /(μM*h)	Rb-E2F complex formation rate
d_{RE}	0.03 /h	Rb-E2F complex decay constant

k_{RBDP}	5 μM/h	RP dephosphorylation rate
d_{RP}	0.06 /h	RP decay constant
K_S	0.5	Half-maximal serum concentration
K_{MC}	0.15 μM	Half-maximal Myc (E2Fm autoregulation)
K_{MCI}	2.5 μM	Half-maximal Myc (E2Fp-independent E2Fm regulation)
K_{MCCD}	0.15 μM	Half-maximal Myc (Cyclin D synthesis)
K_{EF}	0.15 μM	Half-maximal E2Fp (E2F autoregulation)
K_{CD}	0.92 μM	Half-maximal Cyclin D (Rb phosphorylation)
K_{CE}	0.92 μM	Half-maximal Cyclin E (Rb phosphorylation)
K_{RP}	0.01 μM	Michaelis-Menten constant (Rb dephosphorylation)
K_R	0.10 μM	Half-maximal R for E2Fm repression
K_{RS}	0.15 μM	Half-maximal E2Fp (R synthesis)

Supplementary Table 5. shRNA sequences

shRNA	Hairpin Sequence
shCDK2#1	5'-CCGG-TACTTCTATGCCTGATTATAA-CTCGAG-TTATAATCAGGCATAGAAGTA-TTTTTG-3'
shCDK2#2	5'-CCGG-TTCTTCCAGGATGTGACTAAA-CTCGAG-TTTAGTCACATCCTGGAAGAA-TTTTTG- 3'
shcMyc	5'-CCGG-TCTACTCACCAGCACAATTAT-CTCGAG-ATAATTGTGCTGGTGAGTAGA-TTTTTG-3'