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Supplemental information

CycleFlow simultaneously quantifies cell-cycle phase lengths and quiescence *in vivo*

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Challenge	Addressed by
Cells labeled at any stage of S phase.	Subphases track progress within cycle phases.
Variable cycle progression speed.	Distributions for phase durations.
Ambiguity between G_0 and G_1.	Model states are unambiguous cycle phases, not gates.
EdU is consumed gradually.	Decrease in labeling efficiency is fitted from data.
DNA content reflects cycle phase with limited accuracy.	Cells at boundaries of DNA staining gates assigned to cycle phases via model fit to data.

Table S1 | Model features introduced to address the main challenges in the interpretation of thymidine analog labeling, Related to STAR Methods.

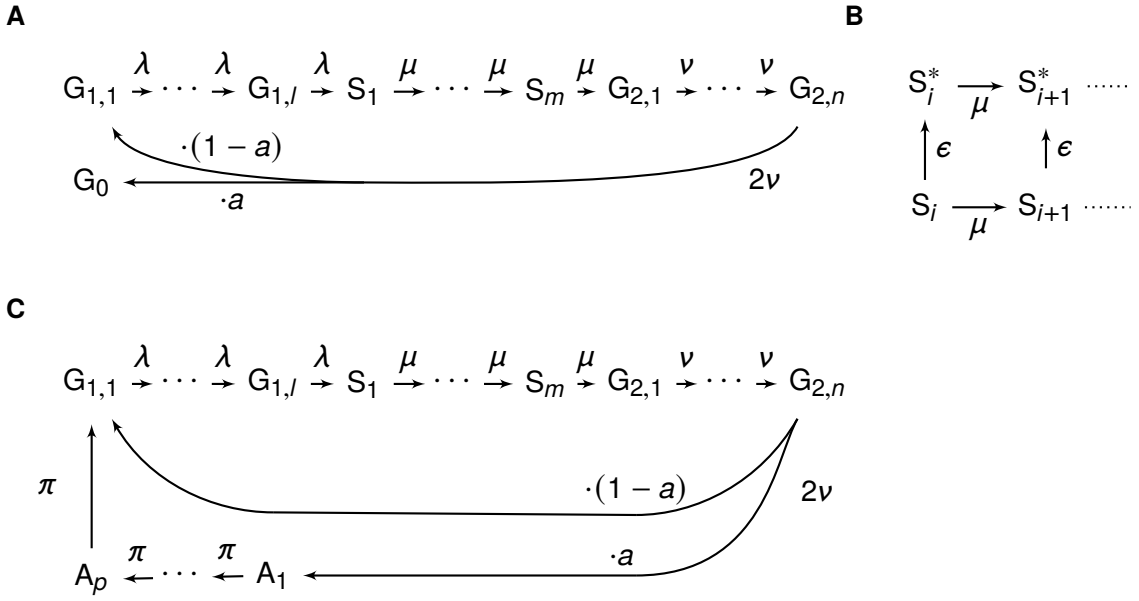

Figure S1 | Dynamical models, Related to STAR Methods. **A** Cells progress through the subphases of G_1, S and G_2 phase. The average and variability of the time to complete each cycle phase is governed by the progression rate and substep number. At the end of the cycle, cells divide. With probability a, daughter cells arrest and enter state G_0; otherwise they continue the cycle. From any cycle state, cell may be lost at rate δ (not shown). **B** Cells in subphase S_i can acquire the label to end up in state S^*i and transition to the next subphase. **C** Extended cell cycle model, not used in CycleFlow. Upon division, cells enter arrest with probability a. The arrested phase A is subdivided into subphases $A_1, \ldots, A_p$, after which cells return into the cycle at $G_{1,1}$. The remaining transitions are as in **A**.

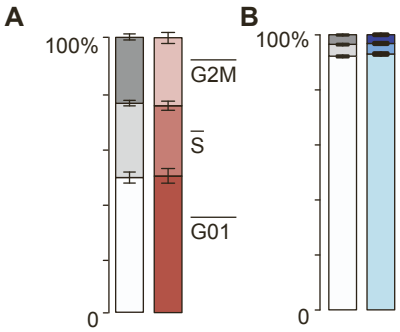

Figure S2 | Measured and inferred steady-state cell-cycle fractions based on DNA content, Related to Figure 2. **A** TET21N, experimental data averaged over all time points (left diagram, error bars indicate standard error of the mean) vs Model fit (right diagram, error bars indicate 90% credible interval) **B** same as **A** for DP thymocytes

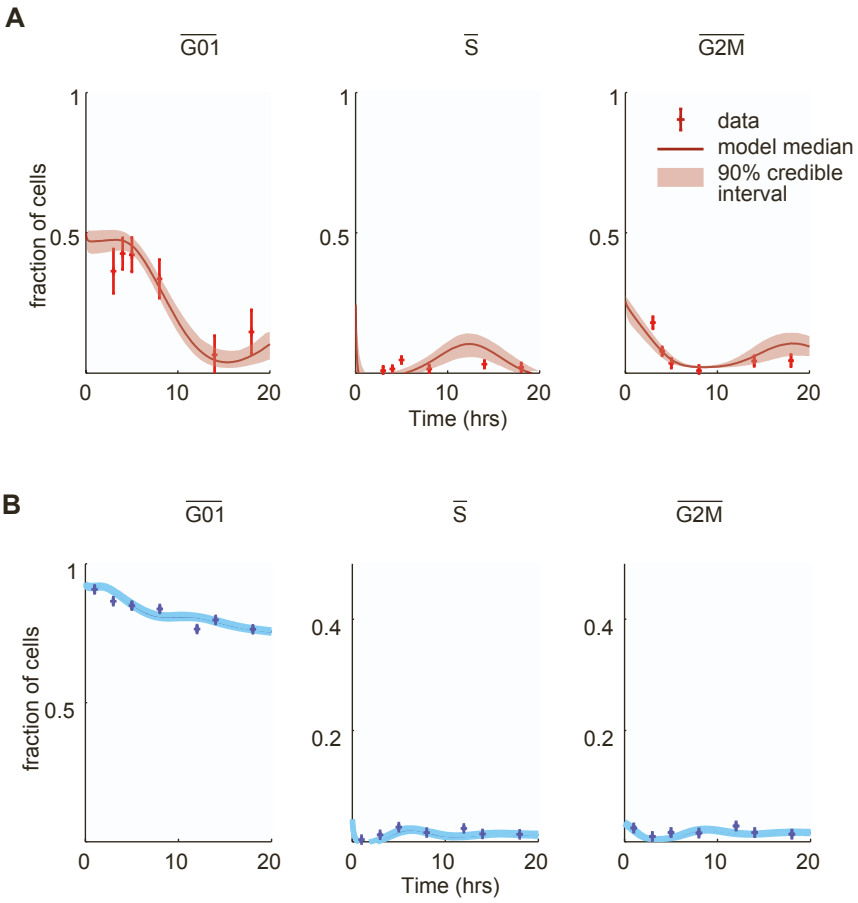

Figure S3| Time courses of EdU-unlabeled cells in $\overline{G01}$, $\overline{S}$, and $\overline{G2M}$ gates compared between experimental data and model prediction, Related to Figure 2. **A** TET21N and **B** DP cells (error bars, pooled SEM; n = 3 to 6 per time point) and model fit. Population sizes are given as fractions of total cells.

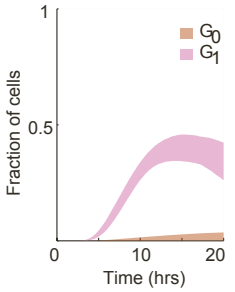

Figure S4| Inferred time course of EdU-labeled G_0 and G_1 TET21N cells in the $\overline{G01}$ gate, Related to Figure 2. Population sizes are given as fractions of total cells.

Parameter	Description	Unit	Prior range
λ	G_1 subphase progress rate	h^{-1}	$[0.01, 6]$
μ	S subphase progress rate	h^{-1}	$[1, 5]$
ν	G_2 subphase progress rate	h^{-1}	$[1, 10]$
a	Cycle arrest probability		$[0, 1]$
τ_E	EdU degradation time	h	$[0.5, 5]$
ϵ_0	initial labeling rate	h^{-1}	$[1, 15]$
l	G_1 subphase number		$[2, 30]$
m	S subphase number		15, fixed
n	G_2 subphase number		15, fixed
$m_{\overline{01}}$	S subphases assigned to $\overline{\mathsf{G01}}$		$[0, 5]$
$m_{\overline{2}}$	S subphases assigned to $\overline{\mathsf{G2M}}$		$[0, 5]$

Table S2 | Model parameters with allowed ranges, Related to STAR Methods.

Parameter	Description	Unit	TET21N	DP
λ	G_1 subphase progress rate	h^{-1}	$3.6(1.7, 4.9)$	$0.49(0.4, 0.6)$
μ	S subphase progress rate	h^{-1}	$2.3(1.8, 2.9)$	$3.7(3.1, 4.2)$
ν	G_2 subphase progress rate	h^{-1}	$2.8(2.6, 3.2)$	$7.5(6.9, 8.8)$
a	Cycle arrest probability		$0.016(0.002, 0.046)$	$0.42(0.415, 0.43)$
τ_E	EdU degradation time	h	$2.6(2, 4)$	$1(0.8, 1.3)$
ϵ_0	initial labeling rate	h^{-1}	$8.6(3, 14.3)$	$5(2.2, 14)$
l	G_1 subphase number		$24(11, 30)$	$2(2, 2)$
$m_{\overline{01}}$	S subphases assigned to $\overline{\mathsf{G01}}$		$2(0, 3)$	$4(3, 5)$
$m_{\overline{2}}$	S subphases assigned to $\overline{\mathsf{G2M}}$		$1(0, 3)$	$0(0, 1)$
l/λ	G_1 duration	h	$6.6(5.5, 7.9)$	$4(3.3, 4.9)$
m/μ	S duration	h	$6.4(5.2, 8.2)$	$4(3.5, 4.8)$
n/ν	G_2 duration	h	$5.3(4.6, 5.8)$	$2(1.7, 2.1)$
$2a$	G_0 fraction		$0.03(0, 0.09)$	$0.84(0.83, 0.86)$

Table S3 | Model parameters medians and 90% credible intervals, Related to STAR Methods.