

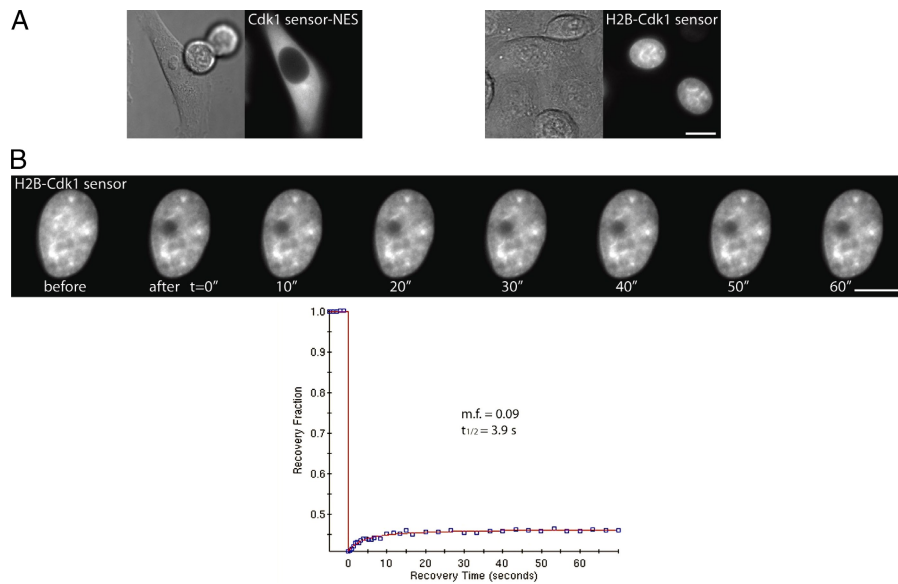
Gavet and Pines, <http://www.jcb.org/cgi/content/full/jcb.200909144/DC1>

Figure S1. **The nuclear targeted FRET sensor is stably associated with chromatin.** (A) Fluorescence and DIC images of cells expressing either the cytoplasmic (NES) or nuclear targeted (histone H2B) cyclin B1-Cdk1 FRET sensor. (B) FRAP experiment showing the stable association of H2B-cyclin B1-Cdk1 FRET sensor with chromatin ($n = 6$). Images were taken before and immediately after bleaching at the indicated times. Fluorescence images (top) and quantification of the fluorescence recovery in the bleached area (bottom) are shown with the calculated mobile fraction (mf) and half-life of recovery. Bars, 10 μ m.

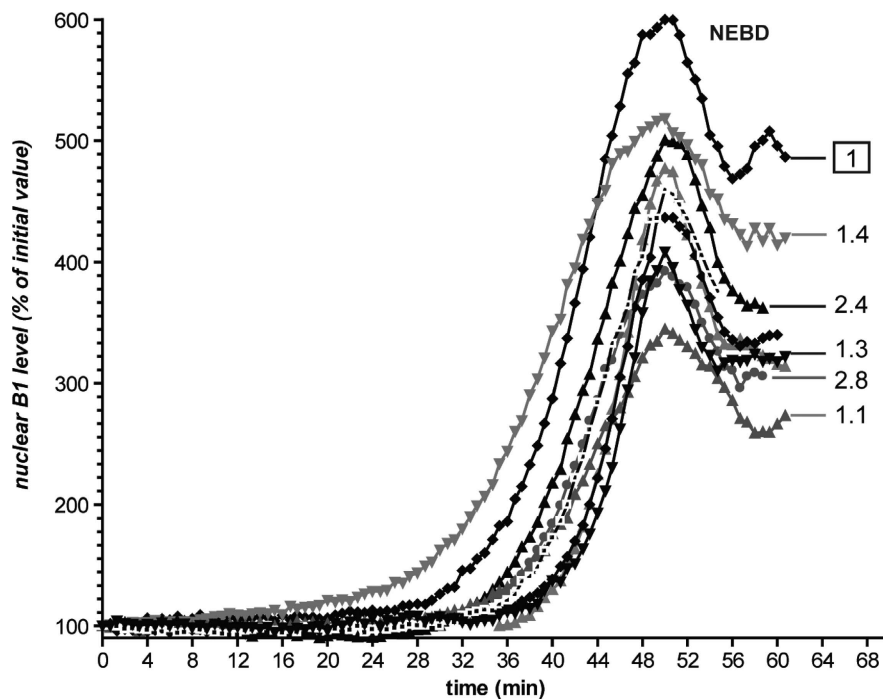


Figure S2. **Kinetics of cyclin B1 nuclear accumulation is independent of the expression level.** The nuclear accumulation of cyclin B1-GFP was quantified on optical sections in different cells entering prophase ($n = 9$). To quantify the expression level of cyclin B1, the mean GFP signal in a region of the cytoplasm was determined for each cell in G2 (at $t = 0$) and normalized to the lowest expressing cell. For clarity, only six values are displayed on the right. For clarity, only six values are displayed on the right, and the values for only six cells are plotted. Note that the timing and rate of cyclin B1 nuclear accumulation are independent of the expression level.

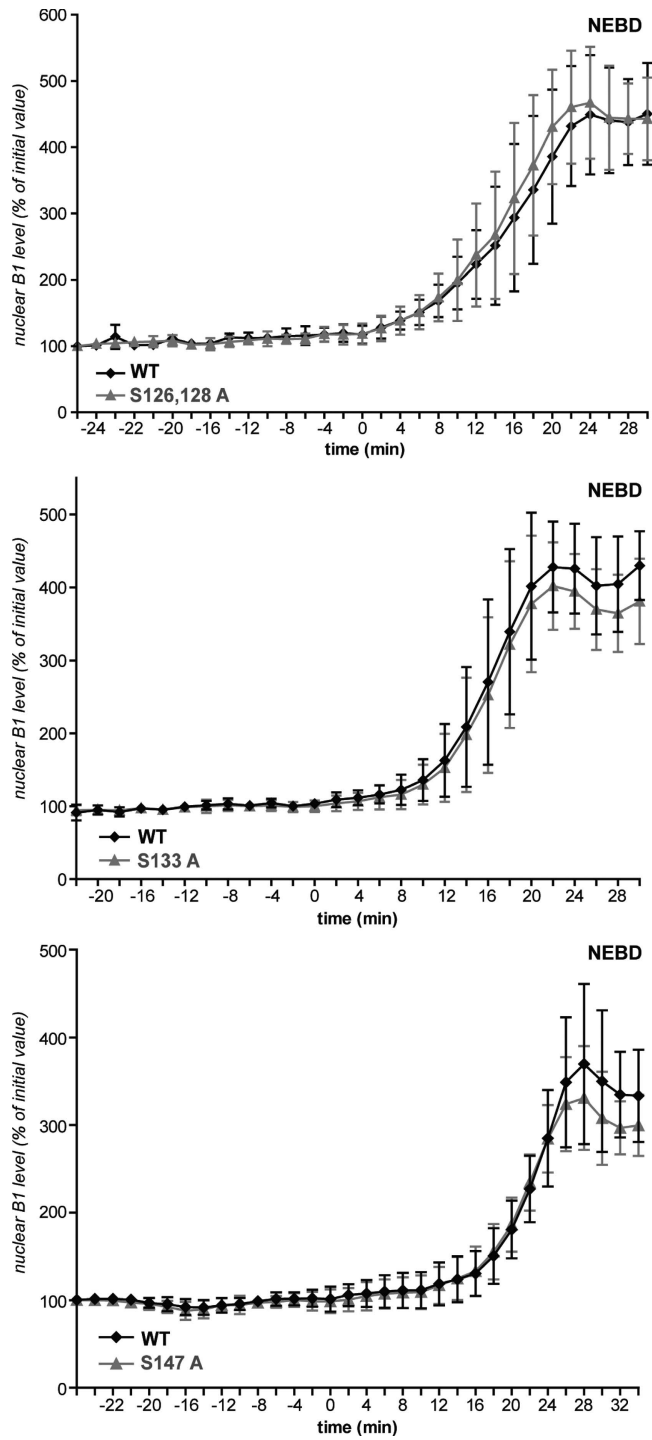


Figure S3. **Nuclear accumulation of cyclin B1 in prophase is independent of its phosphorylation on Ser126, 128, 133, and 147.** Nuclear accumulation of cyclin B1 was assayed in cells coexpressing wild-type (WT) cyclin B1-mCherry and one of the following mutants: Ser126- and 128A-, Ser133A-, or Ser147A-cyclin B1-GFP. Mean curves \pm SEM of quantifications in different cells are displayed for each experiment ($n = 4, 4,$ and $6,$ respectively).

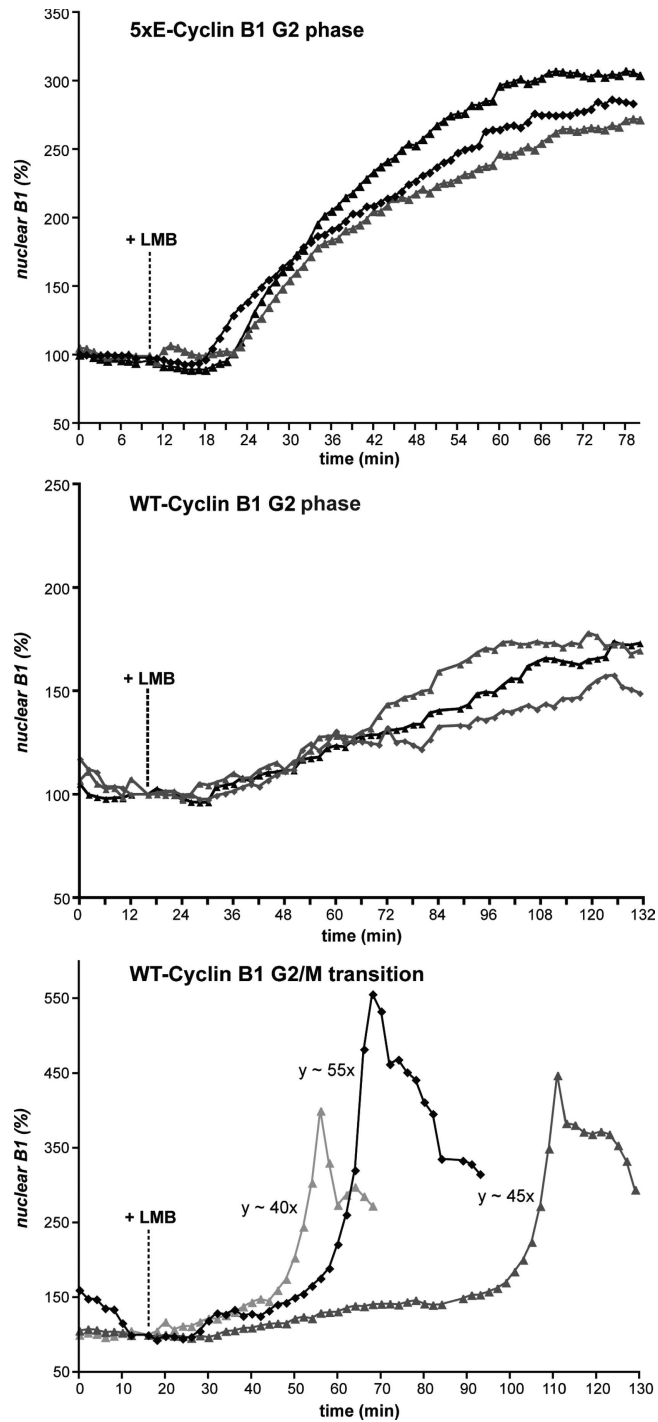


Figure S4. **Cyclin B1 nuclear import rate increases significantly at mitotic entry.** Cells expressing 5xE (top) or wild-type (WT; middle and bottom) cyclin B1 were recorded, and the nuclear import was quantified after adding 20 nM LMB to cells in G2 phase. Note the sudden increase of the wild-type cyclin B1 import rate when cells enter in mitosis. The results from three different cells are shown for each experiment.

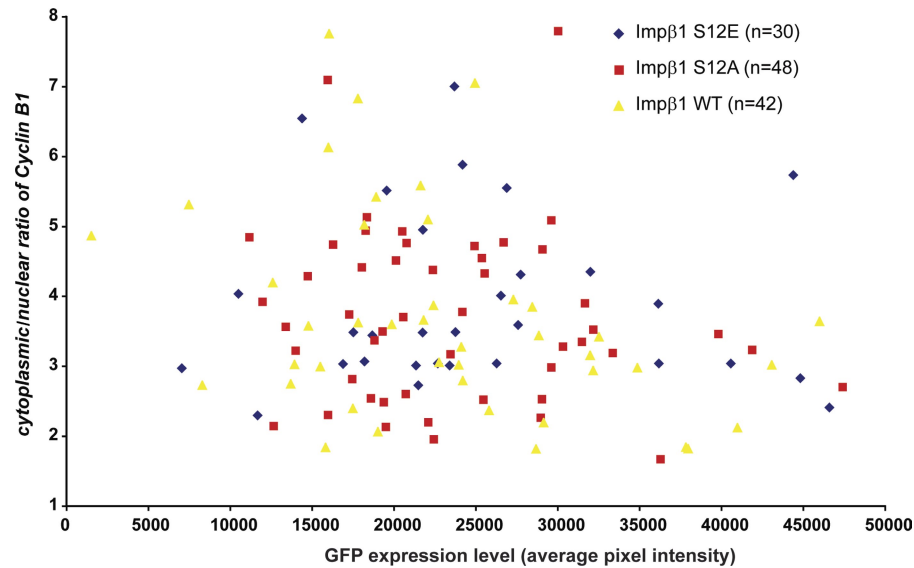
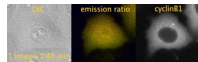
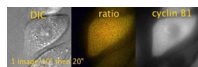


Figure S5. **The nuclear/cytoplasmic distribution of cyclin B1 in interphase is not affected by overexpression of wild-type or phosphorylation mutants of importin β 1.** Cells coexpressing wild-type (WT) cyclin B1–mCherry and untagged wild-type, Ser12A, or Ser12E mutants of importin β 1 (imp β 1) expressed from a vector with GFP under the control of an IRES (p-importin β 1–IRES2–GFP vectors) were assayed. The nuclear/cytoplasmic ratio of cyclin B1 and the expression level of GFP were quantified on optical sections of asynchronous interphase cells and are plotted against each other. The number of cells analyzed in each condition is indicated.



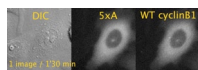
Video 1. **HeLa cell expressing FRET sensor and cyclin B1 entering mitosis.** HeLa cells cotransfected with the FRET sensor and cyclin B1–mCherry constructs were recorded using a microscope (Deltavision; Applied Precision) with a frequency of one frame every 1 min 40 s. Intensity-modulated display of the emission ratio and cyclin B1 level are displayed at middle and right, respectively.



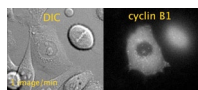
Video 2. **HeLa cell expressing FRET sensor and cyclin B1 treated with a Cdk inhibitor during prophase.** HeLa cells cotransfected with the FRET sensor and cyclin B1–mCherry were recorded using a microscope (Deltavision; Applied Precision) with a frequency of one frame every 10 s and then every 20 s (at $t \approx 14$ min). 300 nM Cdk1/2 inhibitor III was added in prophase at $t \approx 4$ min. Intensity-modulated display of the emission ratio and cyclin B1 level are displayed at middle and right, respectively.



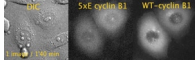
Video 3. **HeLa cell expressing FRET sensor targeted to both the nucleus and the cytoplasm undergoing mitosis.** HeLa cells cotransfected with the cytoplasmic and nuclear targeted FRET sensor were recorded using a microscope (Deltavision; Applied Precision) with a frequency of one frame every 1 min 40 s. The acceptor emission (inverted greyscale; middle) shows the distribution of the sensors, and the emission ratio (intensity-modulated display; right) shows the activity of cyclin B1–Cdk1.



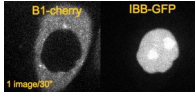
Video 4. **HeLa cell expressing the 5xA Ala mutant and wild-type cyclin B1 entering mitosis.** HeLa cells cotransfected with 5xA–cyclin B1–GFP (middle) and wild-type (WT) cyclin B1–mCherry (right) were recorded using a microscope (Deltavision; Applied Precision) with a frequency of one frame every 1 min 30 s.



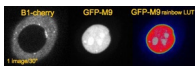
Video 5. **HeLa cell expressing wild-type cyclin B1 entering mitosis and treated with the Plk inhibitor BI 2536.** HeLa cells transfected with wild-type cyclin B1–mCherry were recorded using a microscope (Deltavision; Applied Precision) with a frequency of one frame per minute. 100 nM of the Plk inhibitor BI 2536 was added at $t \approx 10$ min.



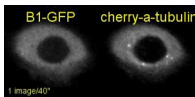
Video 6. **HeLa cell expressing the 5xE glutamic acid mutant and wild-type cyclin B1 entering mitosis and treated with a Cdk inhibitor.** HeLa cells cotransfected with 5xE cyclin B1-GFP (middle) and wild-type (WT) cyclin B1-mCherry (right) were recorded using a microscope (Deltavision; Applied Precision) with a frequency of one frame every 1 min 40 s. 300 nM Cdk inhibitor III was added at $t \approx 10$ min.



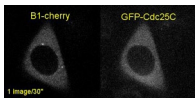
Video 7. **HeLa cell expressing wild-type cyclin B1-mCherry and IBB-GFP entering mitosis.** HeLa cells cotransfected with wild-type cyclin B1-mCherry (left) and IBB-GFP (right) were recorded using a spinning-disk microscope (Intelligent Imaging Innovations) with a frequency of one frame every 30 s.



Video 8. **HeLa cell expressing wild-type cyclin B1-mCherry and GFP-M9 entering mitosis.** HeLa cells cotransfected with wild-type cyclin B1-mCherry (left) and GFP-M9 (middle and right) were recorded using a spinning-disk microscope with a frequency of one frame every 30 s.



Video 9. **HeLa cell expressing wild-type cyclin B1-GFP and mCherry-α-tubulin entering mitosis.** HeLa cells cotransfected with wild-type cyclin B1-GFP (left) and mCherry-α-tubulin (right) were recorded using a spinning-disk microscope with a frequency of one frame every 40 s.



Video 10. **HeLa cell expressing wild-type cyclin B1-mCherry and GFP-Cdc25C entering mitosis.** HeLa cells cotransfected with the wild-type cyclin B1-mCherry (left) and GFP-Cdc25C (right) were recorded using a spinning-disk microscope with a frequency of one frame every 30 s.

Supplemental data

Ypet Polo box domain flexible linker phosphorylation site cerulean Flag tag

Purple indicates a restriction site.

Cyclin B1–Cdk1 FRET sensor:

GCTAGCGCTACCGGTATGGTGAGCAAAGGCGAAGAGCTGTTACCCGGCGTGGTGCC
CATCTTGGTGGAGCTGGACGGCGACGTGAACGGCCACAAGTTCAGCGTGAGCGGCG
AGGGCGAGGGCGACGCCACCTACGGCAAGCTGACCCTGAAGCTGCTGTGCACCACC
GGCAAGCTGCCCGTGCCCTGGCCCACCCTGGTGACCACCCTGGGCTACGGCGTGCAG
TGCTTCGCCCCGTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGAGCGCCATG
CCCGAGGGCTACGTGCAGGAGCGGACCATCTTCTTCAAGGACGACGGCAACTACAA
GACCCGGGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGGATCGAGCTGA
AGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGCCACAAGCTGGAGTACAAC
TACAACAGCCACAACGTGTACATCACCGCCGACAAGCAGAAGAACGGCATCAAGGC
CAACTTCAAGATCCGGCACAACATCGAGGACGGCGGCGTGCAGCTGGCCGACCACT
ACCAGCAGAACACCCCATCGGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACC
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CAACCACTACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCG

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Cyclin B1–Cdk1 FRET sensor **NES**:

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CAAGCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCG
TGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGGCGGCGGCTTG
CCCCCTTGAGCGCTTGACCTTGGCTAAGGATCC

H2B flexible linker cyclin B1–Cdk1 FRET sensor:

GCTAGCATGCCAGAGCCAGCGAAGTCTGCTCCCGCCCCGAAAAAGGGCTCCAA
GAAGGCGGTGACTAAGGCGCAGAAGAAAGGCGGCAAGAAGCGCAAGCGCAGC
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TGACACCGGCATTTCTGTCGAAGGCCATGGGCATCATGAATTCGTTTGTGAACG
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Cyclin B1–**mCherry**:

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mCherry- α -tubulin:

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pIBB-GFP:

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GCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGC
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pEGFP-M9:

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CAAGCTGCCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC
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**GCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGGCGACGGCCCC
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