

Figure S1. Related to Figure 1:

Ade4 maintains a constant concentration, independent of size or cell cycle.

(A) *cdc2-ts* cells expressing Ade4-GFP (yFS982) were shifted to the restrictive temperature of 35°C and sampled at 0, 2, 4 and 6 hours. Ade4-GFP signal and cell length were measured microscopically in individual cells. The concentration of Ade4 was calculated as the mean cellular fluorescence. The mean signals from 25-cell bins are shown in large opaque symbols with error bars depicting standard deviation.

(B) The same cells (yFS982) were grown at the permissive temperature of 25°C. Cells were imaged and analyzed in (A). The lack of change in the Ade4-GFP signal as cells grow from 7 to 15 μm shows that Ade4 expression does not vary with cell-cycle progression.

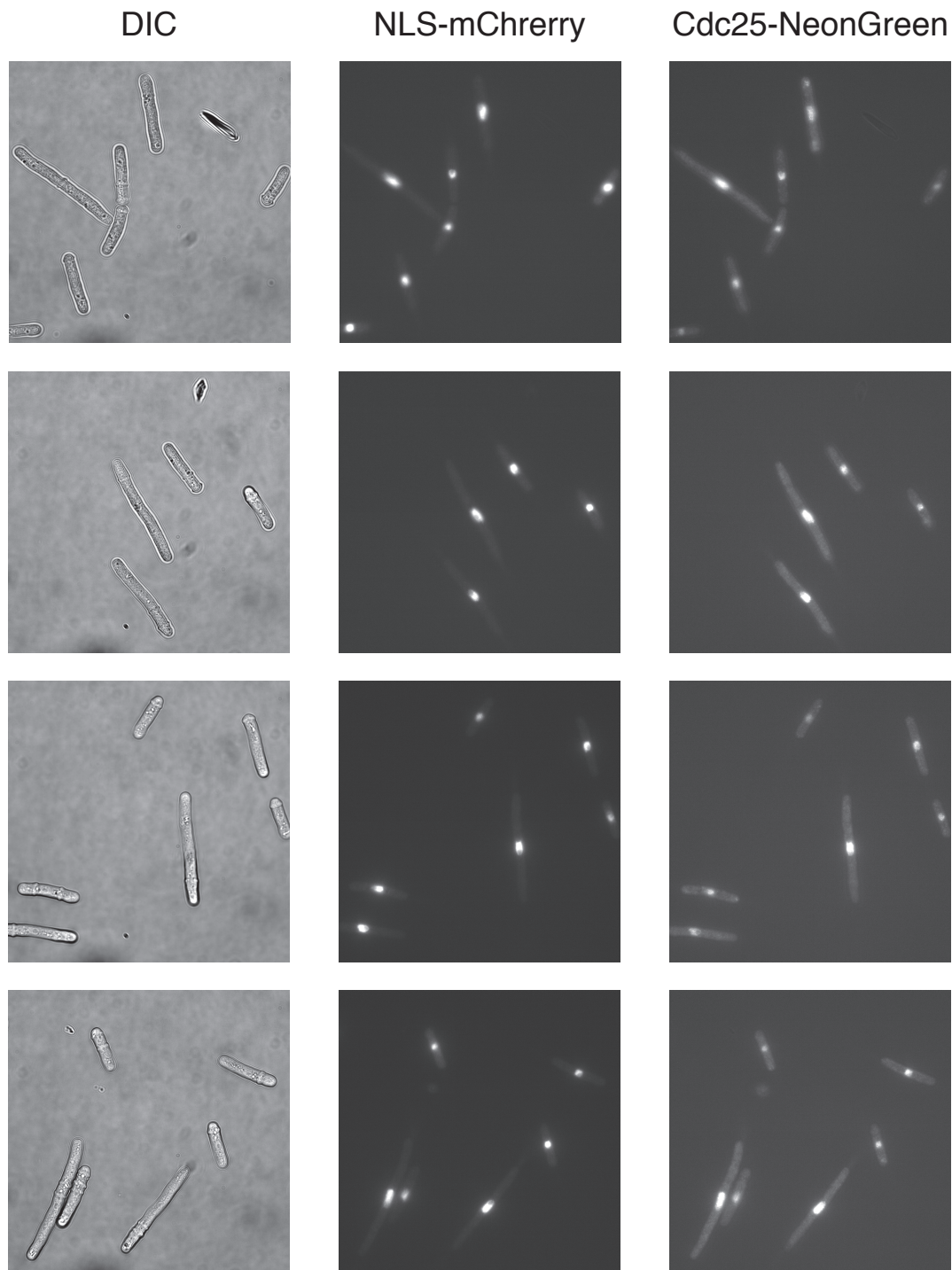


Figure S2. Related to Figure 1:

Images of fluorescent cells quantitated in Figure 1D.

cdc2-ts cells expressing Cdc25-NeonGreen and GST-NLS-mCherry (yFS978) were shifted to the restrictive temperature of 35°C for 2, 4 or 6 hours, mixed and imaged by DIC and wide-field epifluorescence microscopy. To examine if nuclear localization changes with size, we measured the ratio of nuclear to cytoplasmic signal. There is only a modest difference between the ratio in the smallest 20% of cells (2.88 ± 0.34) and the largest 20% of cells (3.31 ± 0.27) suggesting that the degree of nuclear localization is largely size independent..

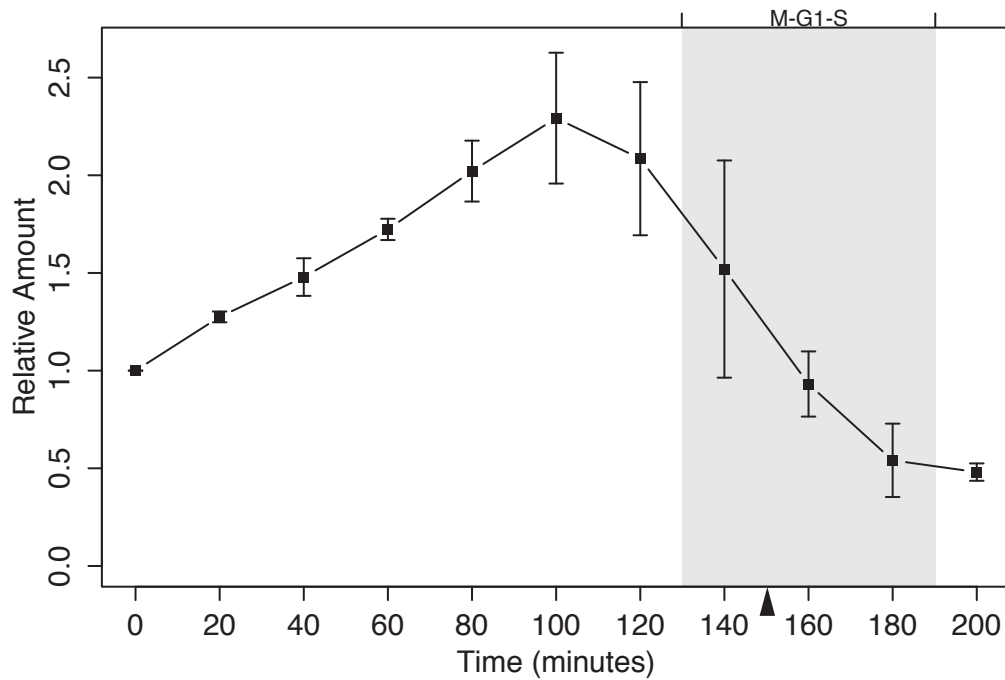
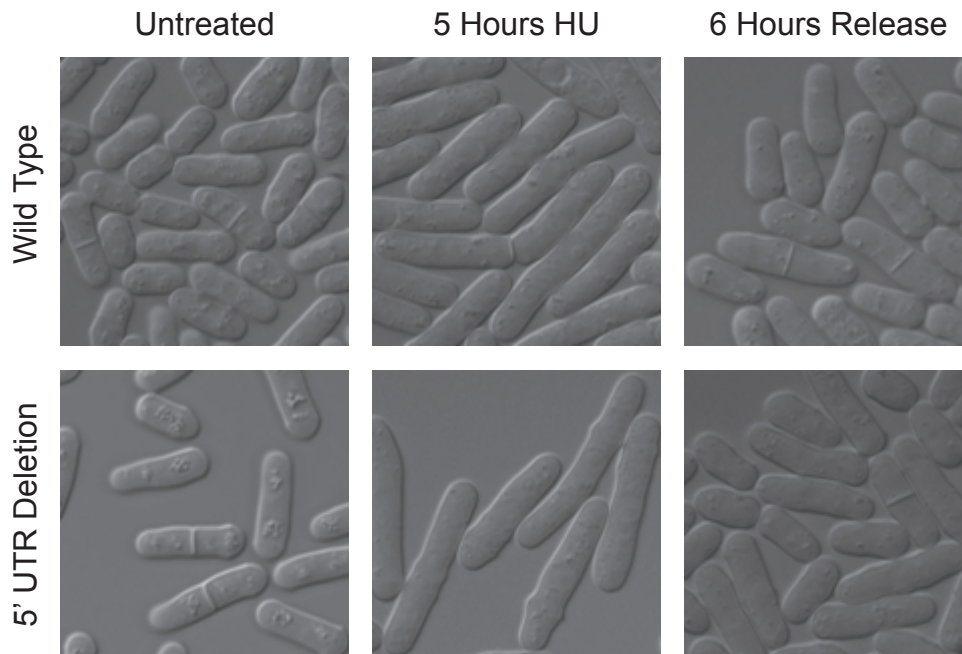
A**B**

Figure S3. Related to Figure 1:

Size-dependent expression of Cdc25 does not require its 5' UTR.

(A). Cells deleted for the *cdc25* 5'-UTR and tagged with luciferase (yFS885) were analyzed as in Figure 1A.

(B). Wild-type (yFS105) and cells deleted for the *cdc25* 5'-UTR and tagged with luciferase (yFS885) were arrested for 5 hours in 10 mM HU and released for 6 hours. Both strains rapidly return to their normal cell size after G2 elongation, demonstrating size homeostasis.

Table S1. Oligonucleotides used for PCR (Related to STAR Methods)

DK6	TTGGCCAAAGTGTGTTAGCTTCCCCAGACGTTAATGATTCTCCTACTGCCATGCATTCCCTCTCTACAC TTAGAAGATTTTCGGATCCCCGGGTTAATTAA
DK7	AGAAAAAAGTGGTTAGAAAAGTTGAATATATAAGAGTATACTTCAGGCTAGGTAAAGTATTGAGT CAGCCTAAAATCAGAATTCGAGCTCGTTTAAAC
DK26	TTGCAGAAGATGAGGAACGTGAAGCTCCCGAAGACATTTCTCTCCATAACACACATTCAGATGTTACT TTTGATTTTGTTCGGATCCCCGGGTTAATTAA
DK27	GATTAGAGCATCAATCTAGACAAAGTAAATGGAGGATTGGTTATTATAATAAAGCACTAAGCATTGA ATAAATTGGGGAAGAATTCGAGCTCGTTTAAAC
NR186	CCATAATTTATGAAGGTATTCATGGATCTTCTTCTAACCCCCAGGGTGATCAAATGATGGAAGATTGG CAGGTGAATGTTTCGGATCCCCGGGTTAATTAA
NR187	GCTAAACAGATTTTGGAAAGCCATTCCCTTATTTGCAATTCGCGAGTAATAAACATTGAGAACAAGAG TCTCTAAAAGGTGAATTCGAGCTCGTTTAAAC
MM6	AGAAAAAAGTGGTTAGAAAAGTTGAATATATAAGAGTATACTTCAGGCTAGGTAAAGTATTGAGT CAGCCTAAAATCACGCACTTAACCTTCGCATCTG
MM41	TTGGCCAAAGTGTGTTAGCTTCCCCAGACGTTAATGATTCTCCTACTGCCATGCATTCCCTCTCTACAC TTAGAAGATTTTCGGATCCCCGGGTTAATTAACAT
MO294	GATTAGAGCATCAATCTAGACAAAGTAAATGGAGGATTGGTTATTATAATAAAGCACTAAGCATTGA ATAAATTGGGGAATTATTCTTTGCCCTCGGACGAGT
MO296	TTGCAGAAGATGAGGAACGTGAAGCTCCCGAAGACATTTCTCTCCATAACACACATTCAGATGTTACT TTTGATTTTGTAGTAAAGGAGAAGAACTTTTCACTG
cdc25f	ATGACCTGCACCAAGGCTAT
cdc25r	TCATTAACGTCTGGGGAAGC
wee1f	GATGAGGTTTGTGGGTTGA
wee1r	CATTCACCTGCCAATCTTCC
srp7f	GTGCATGTTTCGGTGGTCTCG
srp7r	AAGACCCGGTAGTGATGTGC

Table S2. Oligonucleotides used for FISH (Related to STAR Methods)

<p>rpb1 FISH Probe Array</p>	<p>gtgaaaactgaatcccgctc,cttcgcaacgctcattgaac,tattggcggaggaggattacta,gcatactgctcattaagacc,ttccatc caccgaaatacta,attgtccatatacgtggcaa,ttttgtaaagcctgaggctg,tacttttaagaggctgcca,gatcaccggtaatc acagta,tggaacaccgagttcatcta,ctctcctgtatcacgaatga,cattttgtgaagggatggct,cgagtttcttctgactgagg, acaatctgcttaggtacat,attcattacagcgttgcgag,gcttatcgtcgtctctaatt,gtctctcgttgattgagaa,cgacgcaa gcagacatttga,tacgtttgccttcaacgatt,cacgagattctggagagtcg,ttgaggagttaaaccctta,gcagatcaatcaa accctc,aacaagacgcctttggatgt,aaccattacatcctccatag,actcctatcctccattaat,gttctcaatagagttctcca,g catcacctttggggaaaat,tcggtaaaagatcgggtgggt,ttccattattaccggtttta,accaaggtaacgttcttag,gaatttcg gttcagaggta,taagcttctgcatatcc,tacggatcatcatcgcaatg,ctctcaagcatatgaccttc,acgagtaatgttcgg cacac,gtaagatttatgccgtctgt,gtaaatgccatgacgggtaa,agcagcatccataaggattt,ccagaatcaaccattggtga, tggactagtataaccagggg,tgaagtaagtccataccag,ggaatatcccggagatgatg,tcgatggcatataagcaggt,tagg agggactagttggaga,aatatgatgggcttgtggga,agagggactagtcgggtgaat,gagggactagttggagagta,tagggga atacaggggactc</p>
<p>cdc25 FISH Probe Array</p>	<p>gccagatagagtgttgtaa,cagcaggacgcaatacattc,ctcttgatttgcattgcgat,gtgtttggatttcgggaaga,gatcca ctaattgtgaggca,gctgtcgaacaagttttcc,cagcagctaaggaagatgca,gctttcatcgatagcatgt,aagagcgtacg acgaggagt,gagaggggtttctacagtac,tccgggagaggtgaaacaat,ctcggtaaagcgtcattcg,ggaatacttcgatgc aggtt,tagtgctggaaacacgtggg,tgctttaggcttaaagctgt,cgcttttggtggtttaa,acgttaggacgaaggttagga,g ttcctgatgatcgagaac,gaactcgatctggatcga,cagaagttcccttcttttg,taagtcaaatggcgggtagc,ctgacta caggtacgggata,caagtaagctggtcgtgttg,cttgccatcgtaaatgtat,agttcaacagagctttctgg,aatagaggcagc gtcttcaa,gcaccaaaatcactgttggg,gggacctaaacttcaagcta,tcttcttgcatagcgaatgc,gggtatcttctcatcat a,aacatgctttgggtacgac,ttgaaaagccctagtctgt,aacgcacacaagatcttggc,cactttctttggtcgattgt,cacgg cgaagcaaggaagag,attcgtttcaatgagtcctc,agaccgagcaatggttcttg,ccacaatagcttcttcgta,cccgatgagta agaggttga,gcgcactatggtcacaatga,agtgcaatgccaatgaggt,gactattcattcgtctgtcg,acgacttgaaccacca tga,ttgggtcacaacggttttg,atcgttcatcggaacgtagt,cttggtgcaggtcataacat,aacgtctggggaagctaaca,tgc atggcagtaggagaatc</p>