Figure S1. Representative combed molecules.

Examples of combing data quantitated in A) Figure 1B, B) Figure 2A and C) Figure 2B. The FISH probes are in red and the BrdU incorporation in green. Background was removed to clarify the signal.

Figure S2. Expression levels of *dfp1* alleles.

A) Protein levels measured by Western blot. Cells were elutriation synchronized and harvested in S phase as determined by septation index and flow cytometry. 150 μg of whole cell lysate was separated by SDS-PAGE on a 10% gel, transferred to a PVDF membrane and visualized using anti-Dpf1 antibodies as previously described (Takeda et al., 1999). The bands representing Dpf1 and the Dpf1-2xGFP fusion are indicated; asterisks indicate non-specific bands. The membrane was reprobed with anti-tubulin antibodies. When normalized to the tubulin control, the adh1-expressed Dfp1 is approximately 3-fold more abundant that the wild-type Dfp1 and the Dfp1-2xGFP is approximately equal.

B) Protein activity measured by *in vitro* kinase assay. Cells were elutriation synchronized and harvested in S phase as determined by septation index and flow cytometry. IP kinase assay was performed as described, using polyclonal anti-Dfp1 antibodies and myelin basic protein as substrate (Takeda et al., 1999). Lanes 1 and 2 are wild type (yFS240) cells; lane 3 is *adh1:dfp1* (yFS458) cells. Lane 1 is a mock IP, using no antibody; lanes 2 and 3 are Dpf1 IPs. Quantitation of activity is shown below the figure in arbitrary units with the background in Lane 1 subtracted.

Figure S3. Over-expression of Dfp1 does not activate or inhibit the replication checkpoint.

A) Dfp1 over-expressing cells are not sensitive to chronic exposure to HU. Wild-type (yFS240), *adh1:dfp1* (yFS458) and *cds1::ura4* (yFS199) cells were grown to mid-log, 10-fold serially diluted, spotted onto YES plates containing 0, 1 or 3 mM HU and grown for 5 days.

B) Dfp1 over-expressing cells are not sensitive to acute exposure to HU. Wild-type (yFS240), *adh1:dfp1* (yFS458) and *cds1::ura4* (yFS199) cells were grown to mid-log, transferred to YES

containing 10 mM HU, grown for the indicated time, plated on YES, grown for 5 days and counted. Data points represent mean +/- s.e.m.; n = 4.

C) Dfp1 over-expressing cells activate Cds1 normally in response to HU. Wild-type (yFS240), *adh1:dfp1* (yFS458) and *cds1::ura4* (yFS199) cells were grown to mid-log, transferred to YES containing 10 mM HU for 4 hours and harvested. Cds1 was immunoprecipitated from 10 OD pellets and assayed by *in vitro* kinase assay using myelin basic protein as a substrate (Lindsay et al., 1998). Quantitation is mean ± SEM; n is 3 or 4.

Figure S4. Genome-wide transcript levels of in cells with Gal4-Dfp1 tethered at *AT3003*.
Relative transcript levels in wild-type and *5xGal4 UAS:AT3003 Gal4-Dfp1* (yFS459) cells were determined by competitive hybridization of labeled cDNA to an microarray containing probes for all 5004 pombe annotated ORFs as described (Oliva et al., 2005). Wild-type (yFS105) cDNA was used as a reference in both cases to control for dye bias. The figures show relative difference in transcript levels between the two strains (Log 2) versus chromosome position. Relative p-values are shown by circle size; a circle of p = 0.01 is indicated on Chromosome 2. 98% (5282/5414) probes showed less than a two-fold difference between the two strains. The location of the Gal4 UAS site on Chromosome 3 is indicated; the bar shows the 50 kb surrounding the sites. All array data will be available at ArrayExpress (www.ebi.ac.uk/arrayexpress).

Table S1 Oligonucleotides

P96	CTTTTGTTTGGATACGGCATCATGTTTCGGGAGTACTATATTCGCAGATCGAGGAGC TAGAGGACATCTTCCTAGGTTCATACGGGATCCTCTAGAGTC
P97	TTGGATAGAAAGAGCTTTTCGTGAAATTTTGTGCTTCTGTTGGATAAATTGTTCTTT TTCTCAAGTTAATCATATAATTCGAGCTCGTTTAAACTGGA
P112	GAAGCTTCGTACGGTCGACTAGGTGGCATGAACCTAGGAAGATGTCCTCTA
P113	TAAAGATGTTAATTAACCCGGGATCTGGCCTTAAGGGACGTTGAAC
P127	ACGCATGCTTAAACTTTCGTATTCGCTACAGTGTTACAGTGTCTGTTTAAGCTTGTT GTTTTTGCTAACTATAAATCGACGGATCCCCGGGTTAATTAA
P128	TATTTTTTTTGAACGCATATGATAGAATCTCAGTATCCTTACATAAAAGCGAAATAT GTTTACAACTAACATACCCGATGAATTCGAGCTCGTTTAAAC
P129	CGATGAATTCGAGCTCGTTTAAAC
P138	TAAAGTGAAAGCTTTGCCGCCCCTTCCGTCGTCTGGTACACGCTTCTTTGCTTTAAA AAGAATGAGTGGTCTTATATATACGACGGATCCCCGGGTTAATTAA
P139	CGAATAGGGTAGTAGAAAGACAGAACGAAGCAAATTTTTTCGGCAAATGCTTAGA ATTCACTTCAAAGGCAACGTTTGTTACGATGAATTCGAGCTCGTTTAAAC
P156	ATTAGCTCGTACGGAATGAAGCTACTGTCTTCTATCGAA
P157	TAATCGTCCTAGGTTCATACCGGTACCCGATACAGTCAACTGTCTTTGACC
P166	GAGAATTAATCCCAAGCTAGGCTCTCATTAAGAGGAAAAATCAAATACACTAATTT AGTAAGGTGGCTGTACCACATGAAGCTACTGTCTTCTATCGAA
P167	CCTTTATTAAGCAAGAATCATTTTGCTCAGCCAGGTAGTAATTCATAAGCAACGAT GACTAACTTCCCCAATAAACGATGAATTCGAGCTCGTTTAAAC
P213	GAAGGTTTGCCGAGAATAACGATAATTTTAAAGACCTTGATGAACTGTTTGCCCTT GTTCAACGTCCCTTAAGGCCAGATCGGATCCCCGGGTTAATTAA
P216	AACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTAC ACATGGCATGGATGAACTATACAAAGGAGACGCTGCGGCCGCACGGATCCCCGGG TTAATTAA

Figure S1

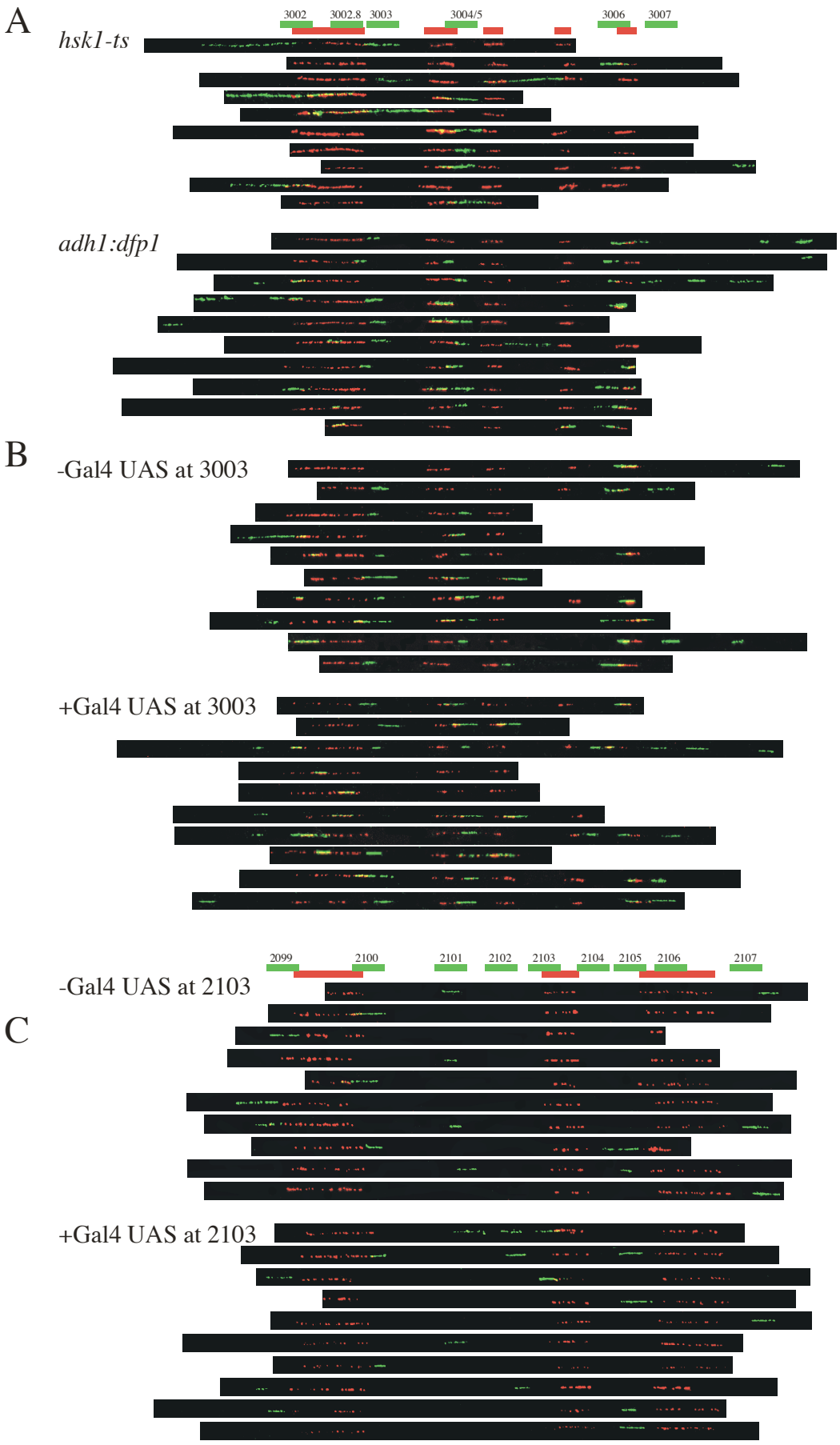

A
hsk1-ts
3002
3002.8
3003
3004/5
3006
3007
adh1:dfp1
B
-Gal4 UAS at 3003
+Gal4 UAS at 3003
C
-Gal4 UAS at 2103
2099
2100
2101
2102
2103
2104
2105
2106
2107
+Gal4 UAS at 2103

Figure S2

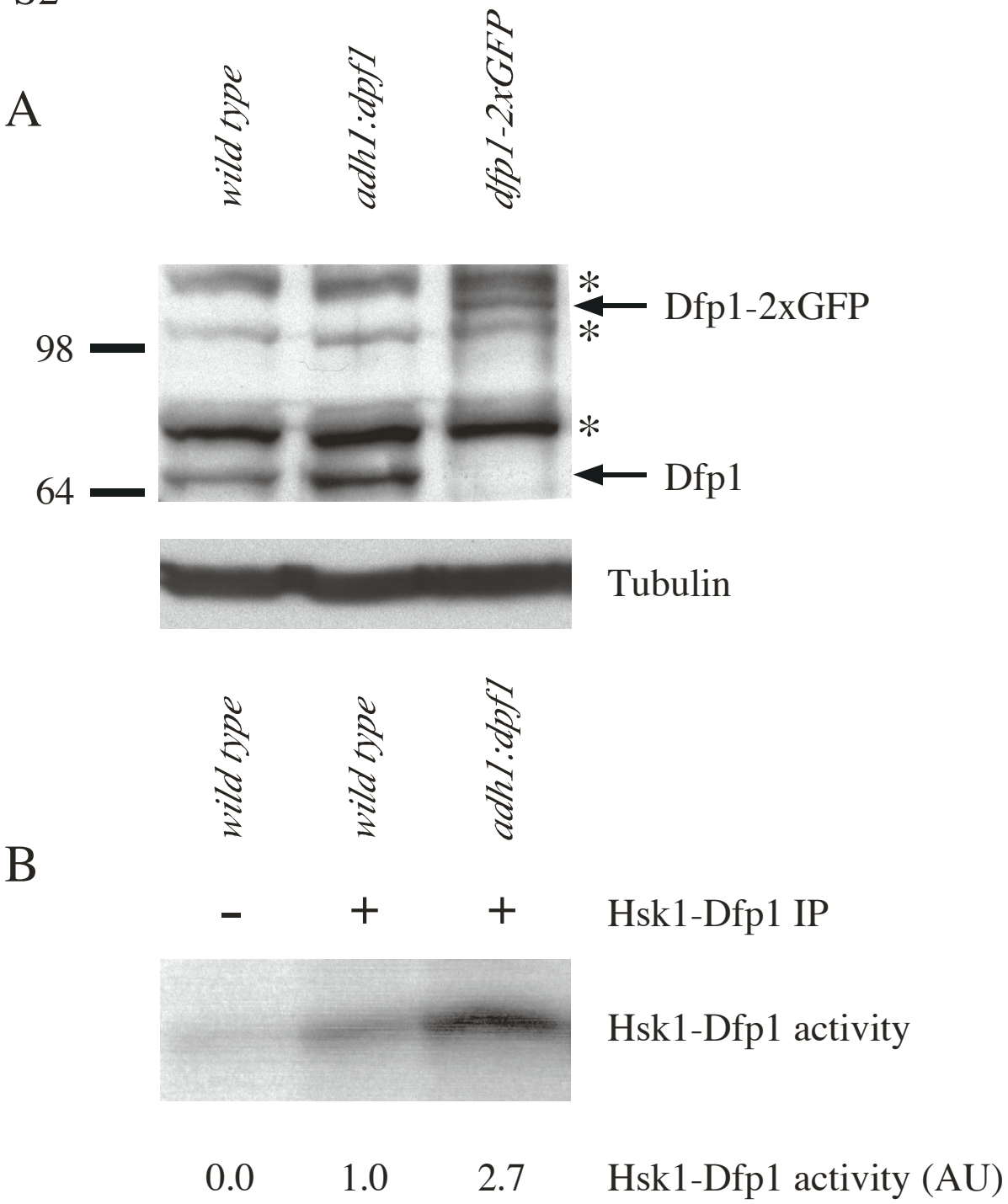

A
wild type
adh1:dpf1
dfp1-2xGFP
98
64
Dfp1-2xGFP
Dfp1
Tubulin
B
wild type
wild type
adh1:dpf1
-
+
+
Hsk1-Dfp1 IP
Hsk1-Dfp1 activity
0.0
1.0
2.7
Hsk1-Dfp1 activity (AU)

Figure S3

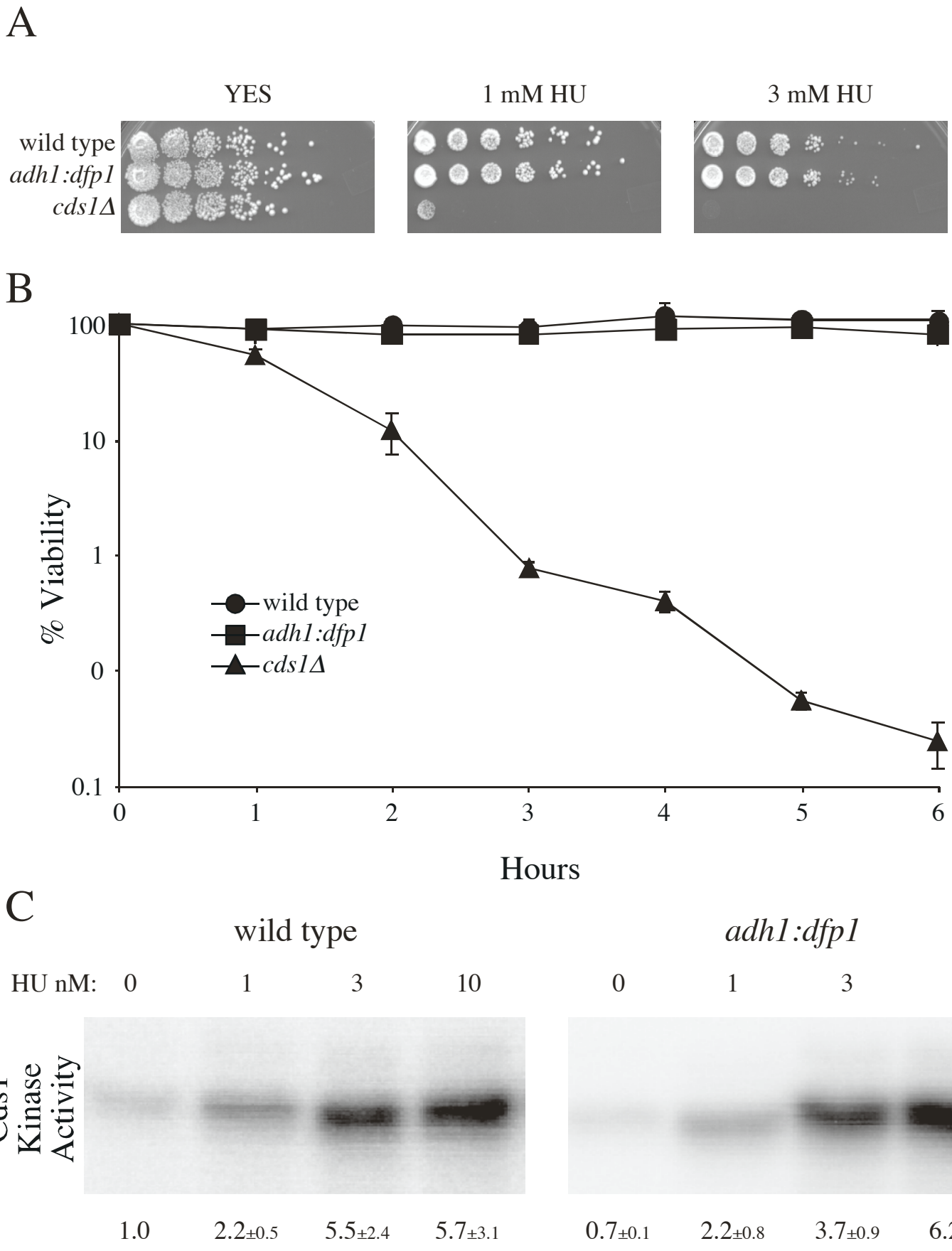
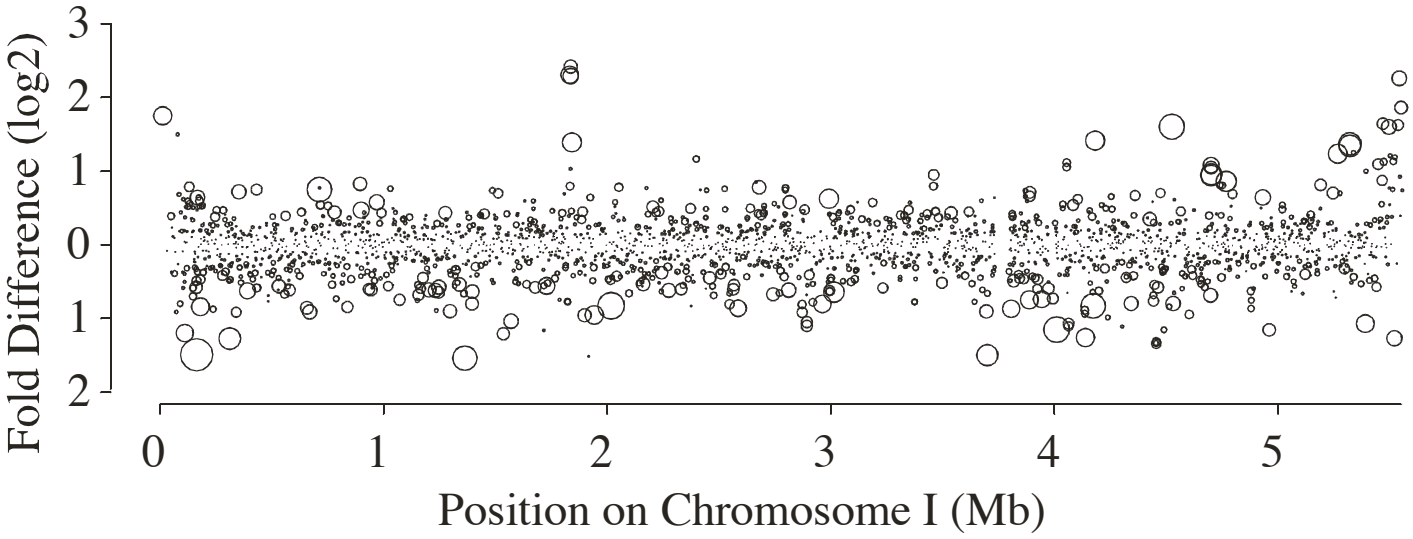
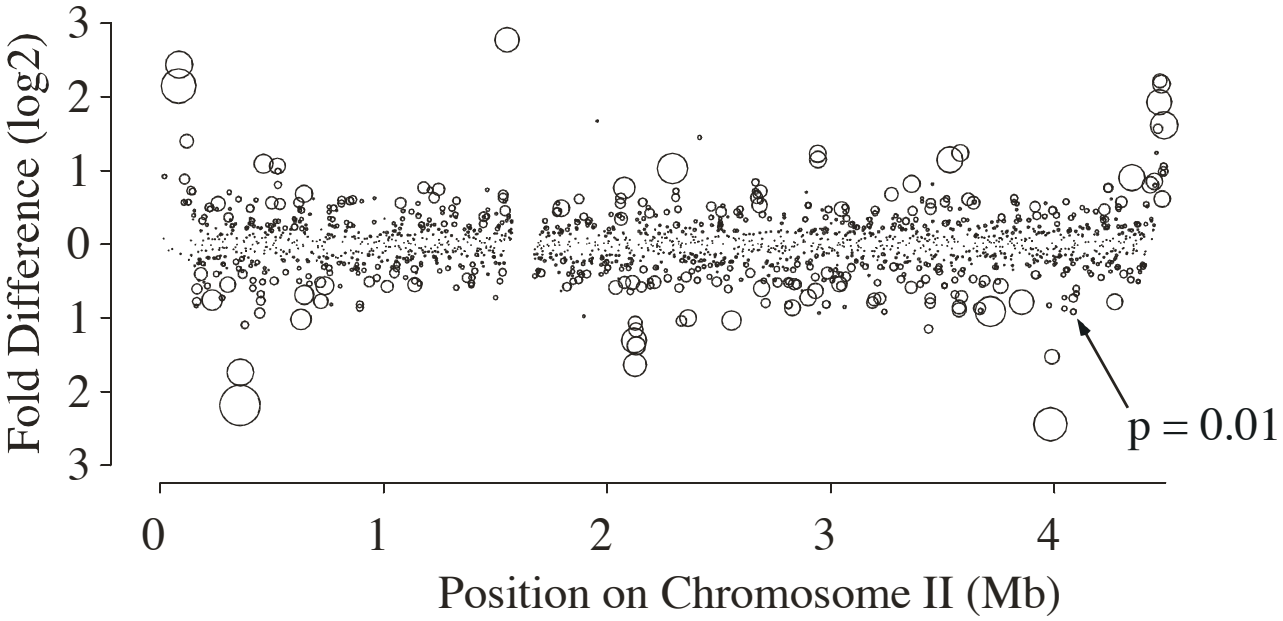
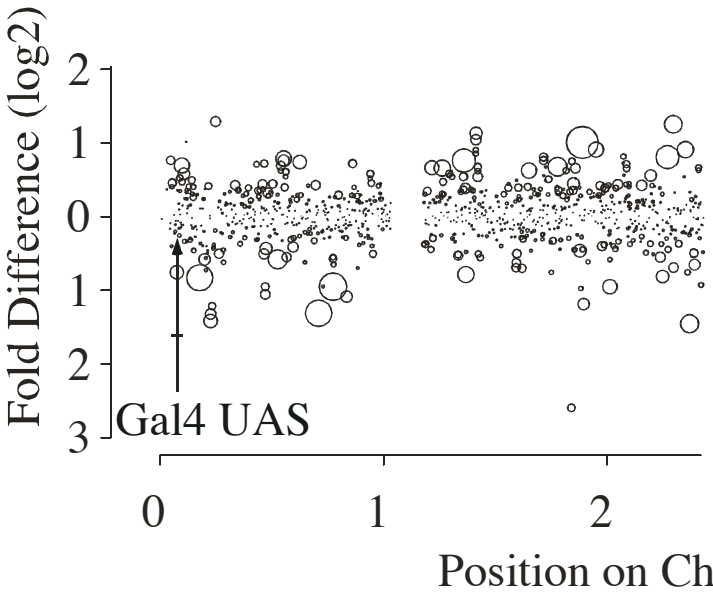

A
YES
1 mM HU
3 mM HU
wild type
adh1:dfp1
cds1Δ
B
100
10
1
0
0.1
% Viability
wild type
adh1:dfp1
cds1Δ
0
1
2
3
4
5
6
Hours
C
wild type
adh1:dfp1
HU nM: 0 1 3 10
0 1 3 10
Cds1 Kinase Activity
1.0
2.2±0.5
5.5±2.4
5.7±3.1
0.7±0.1
2.2±0.8
3.7±0.9
6.2±2.5

Figure S4


Fold Difference (log2)
3
2
1
0
1
2
0
1
2
3
4
5
Position on Chromosome I (Mb)


Fold Difference (log2)
3
2
1
0
1
2
3
p = 0.01
0
1
2
3
4
Position on Chromosome II (Mb)


Fold Difference (log2)
2
1
0
1
2
3
Gal4 UAS
0
1
2
Position on Chromosome III (Mb)