Dataset 1

The attached Excel file lists G1/S genes selected from the genome-wide RNA microarrays based upon their induction at 30 or 45 min after the release from mating pheromone and repression at 60 min. The genes induced in response to different genotoxic agents, Rad53-dependent and the Nrm1-regulated are indicated. *RNR1* does not appear in this list because it falls just below the required p-value due to an inconsistency in its behavior in duplicate experiments.

Table S1

Enrichment for DNA binding motifs (Mbp1, Swi4, Swi6) within the 1000 bp upstream of the transcription start site:

DNA binding motif	Our study	Spellman et al.
Mbp1	7.70E-07	3.18E-06
Swi4	1.50E-06	0.18
Swi6	2.70E-10	1.07E-05

Enrichment for GO-Slim terms:

GO term	Our study	Spellman et al.
Cell cycle	5.20E-10	0.69
DNA metabolic process	0.00168	0.035
Stress response	0.04	0.304

Table S1: Table showing the enrichment for the specified categories of the unique G1/S genes for each study: ours (147 genes), Spellman et al. (103 genes; (Spellman et al., 1998)). DNA binding motifs for Mbp1, Swi4 and Swi6 as defined by ChIP-chip data (Iyer et al., 2001).

Figure S1

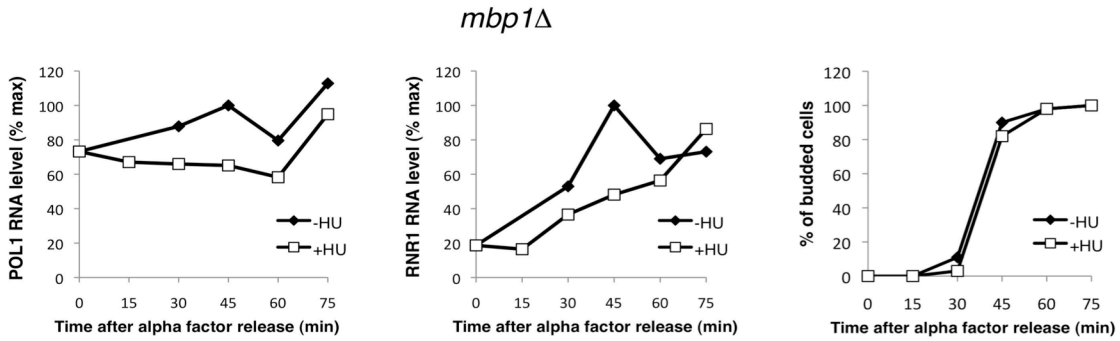

Figure S1: MBF is required for the MBF targets induction in response to DNA replication stress.

*mbp1*Δ cells were grown in rich medium, synchronized during G1 phase with α-factor and released into media with or without 0.2 M HU. MBF target genes, *POL1* (left) and *RNR1* (right), RNA for each time point are shown as percentage of the maximal value in untreated cells. Expression of the MBF target genes is the same either cells are treated with HU or left untreated. Given that MBF acts as a transcriptional repressor, in both cases, transcription is constitutively active. Budding index is shown as a marker of cell cycle progression.

Figure S2

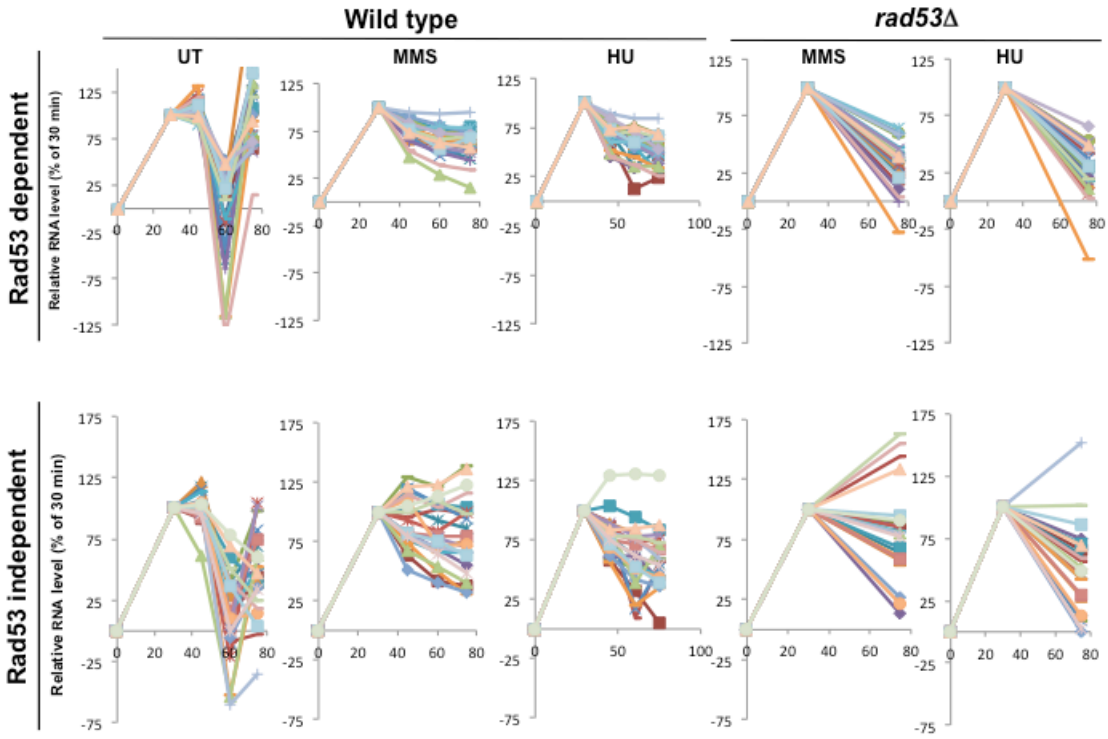

Figure S2: Expression pattern for some of the G1/S genes belonging to the cell cycle or DNA metabolic process GO Slim categories that are induced by MMS. The expression levels of 27 Rad53-dependent genes and 23 Rad53-independent genes in wild type and *rad53*Δ mutant cells are shown. Cells are either untreated or treated with HU or MMS as described previously. Values are shown as percentage of maximal expression (30 min).

Figure S3

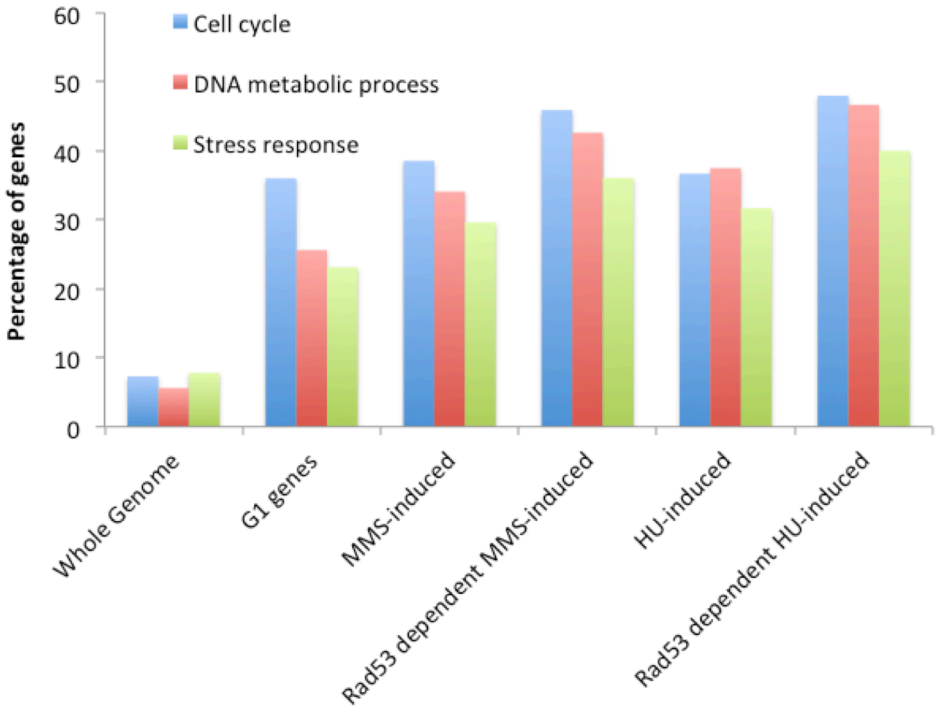

Figure S3: Based upon GO Slim terms, more than one third of the genes that we classified as G1/S genes are annotated as cell cycle-related and approximately one fourth as involved in DNA metabolic processes. This represents a considerable enrichment compared to the whole genome where those classes represent 7 % and 5.6 %, respectively. Both HU- and MMS-induced G1/S genes are slightly enriched for those GO categories over all G1/S genes and among those that are Rad53-dependent, the fraction falling under those GO terms is even greater.

Figure S4

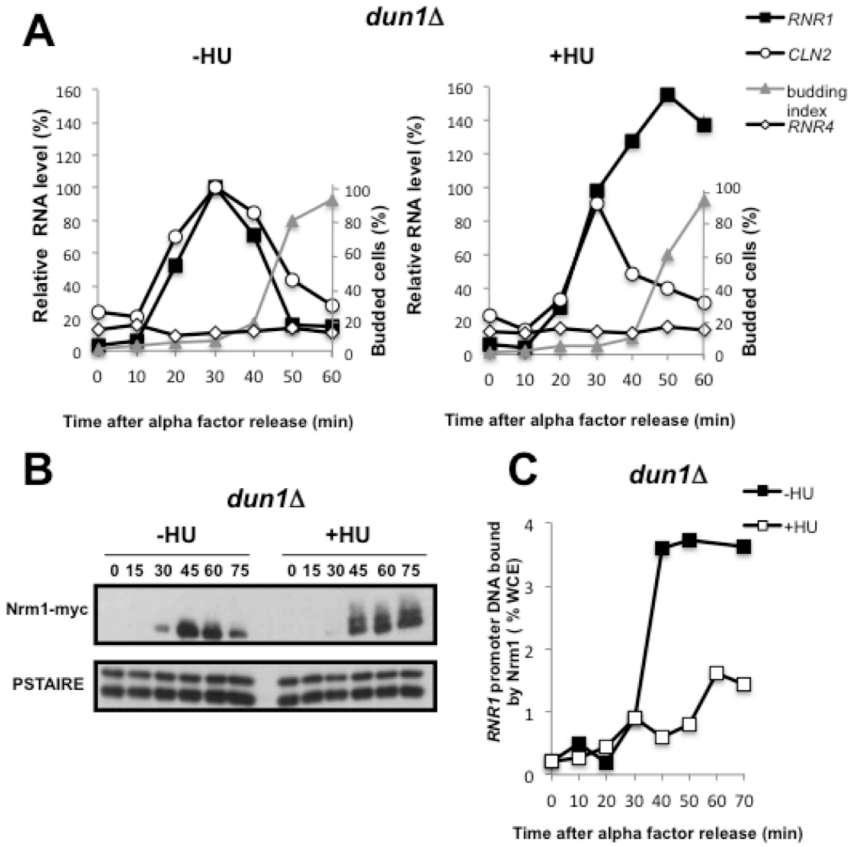

Figure S4: Dun1 is dispensable for the regulation of MBF-dependent transcription and Nrm1 inhibition.
(A) *Dun1 is not required for MBF-regulated gene expression in response to DNA replication stress*. *dun1*Δ cells were grown in rich medium, synchronized during G1 phase with α-factor and released into media without (left) or with (right) 0.2 M HU. *CLN2*, *RNR1* and *RNR4* RNA for each timepoint are shown as percentage of the maximal value in untreated cells. Expression of the MBF target gene *RNR1* is induced by HU in a *dun1*Δ mutant, much as it is in a wild type strain, whereas induction of *RNR4* is abrogated
(B) *Nrm1 phosphorylation in response to DNA replication stress is Dun1-independent*. Nrm1-Myc protein was visualized by immunoblot in cells from the same timecourse as in (A). Anti-PSTAIRE is shown as a loading control.
(C) *Inactivation of Nrm1 binding to MBF-regulated promoters by replication stress is Dun1-independent*. Nrm1-myc binding to *RNR1* promoter DNA was detected by ChIP in cells from the same timecourse as in (A). Values are shown as percentage of whole cell extract (WCE). Nrm1 phosphorylation, as judged by mobility (B), and its release from the *RNR1* promoter (C) occur normally in the *dun1*Δ mutant.

Supplemental Material and Methods

List of yeast strains used in this study.

Yeast Strain	Genotype	Source
CWY231	Mat*a ade1 leu2-3,112 his2 trp1-1 ura3*Δ*ns bar1*Δ	15Daub; (Hadwiger and Reed, 1988)
CWY1559	CWY231 *NRM1*-13xmyc::*KAN*^{r}	(de Bruin et al., 2006)
CWY2045	CWY231 *NRM1*-13xmyc::*URA3 sml1*::*TRP1 rad53*::*HIS3*	This study
CWY2043	CWY231 *NRM1*-13xmyc::*URA3 sml1*::*TRP1 dun1*::*KAN*^{r}	This study
CWY1881	CWY231 *DUN1*-TAP::*URA3*	This study
CWY2009	Mat*a his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0 *bar1*::*KAN*^{r}	BY4741; (Winzeler et al., 1999)
CWY2079	CWY2009 *rad53*::*HYG sml1*::*KAN*^{r} *bar1*::*LEU2*	This study
CWY2203	CWY2009 *TRP1*:GAL-*SIC1*ΔP(*URA3*)	This study
CWY2199	CWY2009 *TRP1*:GAL-*SIC1*ΔP(*URA3*) *nrm1*::*KAN*^{r}	This study
CWY2308	CWY231 *nrm1*::*TRP1 URA3*:Nrm1-4A-13xmyc(KAN^r)	This study
DSY910	CWY231 *mbp*1::URA3	(Stuart and Wittenberg, 1995)

Supplemental References

Hadwiger, J.A., and Reed, S.I. (1988). Invariant phosphorylation of the Saccharomyces cerevisiae Cdc28 protein kinase. Mol Cell Biol *8*, 2976-2979.

Stuart, D., and Wittenberg, C. (1995). CLN3, not positive feedback, determines the timing of CLN2 transcription in cycling cells. Genes Dev *9*, 2780-2794.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H.*, et al.* (1999). Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science *285*, 901-906.