Genetics: Published Articles Ahead of Print, published on September 15, 2004 as 10.1534/genetics.104.028613

X-ray survival characteristics and genetic analysis for nine *Saccharomyces* deletion mutants that affect radiation sensitivity.

John C. Game, Marsha S. Williamson and Clelia Baccari

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720.

Running Head:

X-ray survival in nine yeast mutants

Keywords:

Saccharomyces

DNA repair

DOT1

Radiation sensitivity

SAGA

Corresponding author:

John C. Game,

Donner Laboratory Room 326, Lawrence Berkeley National Laboratory,

I Cyclotron Road, Berkeley, CA 94720

Phone: 510 486 5651

Fax: 510 486 6488

Email: jcgame@lbl.gov

ABSTRACT

The availability of a genome-wide set of Saccharomyces deletion mutants provides a chance to identify all the yeast genes involved in DNA repair. Using X-rays, we are screening these mutants to identify additional genes that show increased sensitivity to the lethal effects of ionizing radiation. For each mutant identified as sensitive, we are confirming that the sensitivity phenotype co-segregates with the deletion allele and are obtaining multipoint survival-versus-dose assays in at least two haploid and one homozygous diploid strains. We present data for deletion mutants involving the genes *DOT1*, *MDM20*, *NAT3*, *SPT7*, *SPT20*, *GCN5*, *HFI1*, *DCC1* and *VID21/EAF1*, and discuss their potential roles in repair. Eight of these genes have a clear radiation-sensitive phenotype when deleted, but the ninth, *GCN5*, has at most a borderline phenotype. None of the deletions confer substantial sensitivity to ultra-violet radiation, although one or two may confer marginal sensitivity. The *DOT1* gene is of interest because its only known function is to methylate one lysine residue in the core of the histone H3 protein. We find that histone H3 mutants (supplied by K. Struhl) in which this residue is replaced by other amino-acids are also X-ray sensitive, seeming to confirm that methylation of the lysine-79 residue is required for effective repair of radiation damage.

Yeast mutants initially isolated on the basis of sensitivity to ionizing radiation (IR) have proved invaluable for understanding many aspects of DNA transactions in eukaryotes. Despite this, pathways and mechanisms of cellular recovery from ionizing radiation (IR) damage are less well understood than many other aspects of DNA repair. To fully understand IR repair, it is important to identify all the genes involved. The availability of a genome-wide set of deletion mutants (WINZELER *et al.* 1999) now makes it possible to identify virtually all the non-essential genes in *Saccharomyces* that play a role in conferring resistance to ionizing radiation. We and others have begun a systematic search for such genes that were missed in classical screens of mutagenized cells. These screens, summarized by Game and Mortimer (1974), were apparently very effective in identifying mutants that confer a high degree of sensitivity. Few or no new genes have been found in the last thirty years whose mutants confer the extreme IR sensitivity of deletion alleles in the *RAD51* epistasis group. However, classical screens were less effective at identifying mutants with moderate IR-sensitivity, and a significant number of such mutants remain to be characterized (BENNETT *et al.* 2001; GAME *et al.* 2003). Mutants that combine a growth defect with modest radiation-sensitivity can be especially hard to identify in replica-plating screens, since they will form small colonies whose sensitivity is hard to identify compared to poor growth on the

control plate. In addition, assigning novel mutants in unknown genes to specific loci can be laborious. However, identifying a modest IR-sensitive phenotype regardless of growth rate is not difficult when pure cultures of known mutants are tested for X-ray sensitivity individually. Even modestly sensitive mutants are important in understanding repair, because they can uncover unrecognized or redundant pathways and mechanisms. Double or multiple mutant combinations may serve to expose the full role of such pathways.

We have been using the genome-wide deletion set (WINZELER *et al.* 1999) to identify new genes or open reading frame (ORFs) whose deletion alleles confer increased sensitivity to killing by X-rays. Some preliminary information is already available about the IR sensitivity of many of the catalogued yeast deletion mutants. Bennett *et al*. (2001) screened 3,670 diploid deletion strains in plate assays and identified 107 as potentially IR-sensitive. However, follow-up tests were not done and the list includes some false positives where factors other than the deletion allele contributed to IR-sensitivity (GAME *et al.* 2003). In addition, more than a thousand new deletion strains have become available since the work of Bennett *et al*. (2001). Further information is available from a screen developed in the laboratories of J. Martin Brown and R.W. Davis at Stanford (see BIRRELL *et al.* 2001; GAME *et al.* 2003). The screen utilizes a pool of nearly all the deletion

mutants combined into one culture. It involves ranking the relative change in abundance, as judged by ratios of hybridization signals of unique barcode sequences to a microarray plate, of each mutant after irradiation and brief growth of the pool, compared to the starting abundance for that mutant. Mutants with enhanced sensitivity show less relative abundance in the pool after irradiation. Using this methodology more than 4,600 mutants were ranked in order of relative abundance in a pool of mutants 18 hours after IR treatment compared to un-irradiated controls (GAME *et al.* 2003). However, approximately 9% of deletion mutants in this ranking were not adequately monitored for IR sensitivity using this method because they were insufficiently abundant in the pool prior to irradiation to allow adequate assessment of radiation effects (see GAME *et al.* 2003).

We have been directly assaying IR sensitivity in some of the 1,000 or so mutants that were not in the collection surveyed by Bennett *et al.* (2001), focusing especially on those that also could not be adequately ranked by the pool method (BIRRELL *et al.* 2001; GAME *et al.* 2003). In addition, we have used quantitative survival assays in combination with genetic analysis to more rigorously test and characterize X-ray sensitivity in several other mutants that were identified as potentially sensitive either in the pool assay or by Bennett *et al.* (2001), or were identified by us as interesting based on relationships to other known mutants.

Secondary factors unrelated to the created deletions can sometimes be responsible for IR sensitivity in mutant strains from the genomic library (GAME *et al.* 2003). These can include independent mutations, polyploidy, and in diploids, homozygosity for the Mating Type locus. Hence it is necessary to verify that the phenotype of each newly identified IR-sensitive mutant is truly conferred by the deletion. In addition, it is important to obtain survival curves for mutants initially identified on the basis of qualitative tests.

In this report, we present survival characteristics for nine deletion mutants, eight of which we have confirmed genetically to confer X-ray sensitivity. The ninth, *gcn5Δ*, may confer marginal sensitivity. Six of these mutants, involving deletions of the genes *MDM20*, *DOT1*, *SPT7*, *SPT20*, *HFI1* and *GCN5*, were initially identified as sensitive in spot tests in our own laboratory. We chose the remaining three, deleted for the *NAT3*, *VID21* and *DCC1* genes, based on previously reported sensitivity in spot tests (BENNETT *et al.* 2001). We discuss the possible significance of these genes in DNA repair, and also report difficulties in confirming the radiation sensitivity of two additional mutants. We also tested the mutants for cross-sensitivity to ultraviolet radiation, and we present data suggesting either no sensitivity or at most minor UV-sensitivity.

MATERIALS AND METHODS

Yeast strains: We obtained a library of ~ 4,700 individual haploid deletion strains in the alpha Mating Type (background strain BY4742) from Research Genetics, Huntsville, AL (now Invitrogen Life Technologies). These deletion strains can also be obtained from EUROSCARF (Frankfurt, Germany). Genotypes of the parental yeast strain BY4742 and construction of the deletion strains have been described (BRACHMANN *et al.* 1998; WINZELER *et al.* 1999). Information is also available at the Saccharomyces Genome Deletion Project website, http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html. The prototrophic wild-type strains X2180-1A and X2180-1B were available to us from R.K. Mortimer. They are haploid spore-clones derived from diploid X2180, which was identified by Mortimer as a spontaneous diploid *MATa/MAT*α derivative of haploid strain S288C (see MORTIMER and JOHNSTON 1986). They are thus isogenic in background to the strains used to generate the deletions, which were derived from S288C by a series of transformations and subsequent background-isogenic crosses (BRACHMANN *et al.* 1998).

Genetic methods and media: Genetic methods including tetrad dissection were as described (SHERMAN *et al*. 1982). Cultures were incubated at 30° unless a temperature-conditional phenotype was segregating, in which case 25° was used.

Rich media (YPD) and supplemented minimal media were prepared as described (SHERMAN *et al.* 1982). To make inositol-less medium, we used yeast nitrogen base (YNB) without inositol, obtained from Q-biogene, in place of regular YNB. To induce meiosis, we incubated cultures for four or more days, usually at 30°, on solid Fogel's sporulation medium. This contains 9.65 g potassium acetate, 1g glucose, 2.5 g yeast extract (Difco) and 2% agar made up to one liter in water and autoclaved. To score geneticin-resistance, we used YPD plates supplemented with geneticin (obtained from Sigma) added from filter-sterilized solution shortly before pouring plates, to give a final concentration of 150 μg/ml.

Spot-testing the mutants for X-ray sensitivity: We retrieved mutants from cold storage and grew up cultures. We arrayed fresh cultures in thin patches of twenty per plate on YPD medium and replica-stamped each plate immediately to six more YPD plates. We irradiated two plates from each set with 78 kilorads (7.8 Gray) and two more with 156 kilorads (15.6 Gray) of X-rays. We incubated one plate from each pair at 25° and one at 37°, together with the un-irradiated plates. We monitored each set at one day and two days after irradiation for evidence of sensitivity in any mutant, as evidenced by less than average growth or survival compared to the un-irradiated plate at the same temperature.

X-ray treatments: For all X-ray exposures, we used a Machlett OEG 60 X-ray tube with a beryllium window and a Spellman power supply operated at 30 kilovolts and 15 milliamps to deliver a dose-rate of 130 rads/second of "soft" X-rays. For survival curves, unless otherwise noted, log-phase cells from overnight liquid YPD cultures grown at 30°C were diluted appropriately in fresh YPD and grown for several more hours with vigorous shaking. When a cell density of $1x10^{7}$ to $2.5x10^{7}$ cells/ml was reached, as determined with a hemocytometer, serial dilutions were made in distilled water cooled in ice. Cells plated from the appropriate dilution were irradiated at room temperature on YPD plates, to yield (ideally) about 200 surviving colonies/plate, with two plates per dose. Cultures were inspected for clumpiness. If clumps were observed, cultures were sonicated briefly to disperse them. Colonies were counted after incubation for 5 to 6 days at 30°C unless otherwise noted.

Ultraviolet Radiation treatments: Cells were prepared in log phase for UV survival curves as outlined above for X-rays. They were irradiated on YPD plates using a shielded apparatus containing five General Electric G8T5 tubes giving most of their radiation at 254 nm. Plates were incubated in the dark and colonies counted as for X-ray curves.

RESULTS and DISCUSSION

Spot-testing individual mutants: To identify additional yeast genes that might be involved in IR repair, we began by spot-testing 357 mutants, selected by criteria described earlier, for sensitivity to X-rays at 37°C and at 25°C using two doses as described. We also noted temperature effects on growth in the absence of radiation. It is important to assess IR-sensitivity at more than one temperature, even in deletion mutants, since some yeast proteins play a role in repair that is temperature-dependent (LOVETT and MORTIMER 1987).

Of the 357 mutant cultures initially tested, 25 were chosen for re-testing from those that appeared the most convincing candidates for X-ray sensitivity at one or both temperatures in the first test. After these 25 were re-tested in similar plate tests, three mutants, the *dot1*, *mdm20* and *spt20* deletion strains, were chosen for further study. Three more mutants, those carrying the *spt7*, *hfi1* and *gcn5* deletions, were also chosen for study, based on their known relationship (GRANT *et al.* 1997; HORIUCHI *et al.* 1997) to the identified s*pt20* mutant. Two of these, the s*pt7* and *hfi1* deletion strains, showed sensitivity in spot tests. The third, the *gcn5* deletion, showed no clear sensitivity in spot tests, but showed a mild sensitivity (less than

the other mutants) in survival curves. These three mutants were also amongst the approximately 1,000 deletions absent from the initial set. One was also absent from the pool of mutants studied in the Brown laboratory. The other two showed a borderline low score or a borderline low signal in the pool assay but were not selected in our initial list of 357 cultures.

In addition to the above six mutants, we chose to study five mutants reported by Bennett *et al*. (2001) to be sensitive to Cesium-137 gamma rays. These were the deletions *of NAT3*, *VID21* (ORF YDR359C), *DCC1*, *HTL1* and *DEF1* (*VID31*). We chose the five for which the authors presented the most convincing observations of sensitivity in a diploid strain and in both haploid parents. We confirmed that the deletion library strains listed as *MAT*α haploids containing each of these five deletions were X-ray sensitive in spot tests.

Genetic analysis of mutants: As shown previously (GAME *et al.* 2003), some mutant strains from the genomic library contain secondary genetic changes in addition to the created deletion. To determine if the deletion by itself is both necessary and sufficient to confer X-ray sensitivity, we crossed each deletion mutant with a wild-type *MATa* strain. We determined whether the X-ray sensitive phenotype co-segregated with the deletion in meiotic tetrads from these crosses,

using resistance to geneticin conferred by the *KanMX4* marker to score the deletion allele. These crosses also served to generate new spore-clones carrying the deletions in each mating type and with different combinations of auxotrophic markers. We used these spore-clones to determine the consistency of the survival characteristics in two or more haploid strains for each mutant, as shown in Figures 1 through 7, and to construct our own homozygous diploids for testing, as shown in Figures 8 and 9.

Table 1 shows spore-viability data for each of the 11 deletion mutants we chose for further study, crossed with our *MATa* wild-type strain, g1201-4C. This strain is isogenic in background with the strains used for constructing the deletion library. It was made by crossing the deletions strains carrying multiple auxotrophic markers with the prototrophic strain X2180-1B, which shares the same strain background (see Materials and Methods). Crosses generated haploid spores in each mating type carrying only the *lys2Δ* marker (from BY4742) or the *met15Δ* marker (from BY4741). These single-auxotroph strains can be mated with the deletion mutants created in the BY4741 or BY4742 strains respectively, and the resulting diploids can be selected on minimal medium. These diploids are heterozygous for four auxotrophic mutations in addition to the deletion of interest.

In tetrad analysis these segregating markers, along with Mating Type, help to identify polyploid or aneuploid cells and false tetrads.

Table 1 reveals that in 9 of 11 deletion strains analyzed, meiotic spore viability from the heterozygous diploids was high, and the deletion alleles as scored by geneticin resistance segregated 2+:2- per tetrad, as expected. In eight mutants, the geneticin-resistance phenotype co-segregated with an X-ray sensitive phenotype that could be readily observed on replica plates given 156 kilorads of radiation. Fifteen or more tetrads with four viable spores were monitored for co-segregation for each mutant. In the case of the *spt7* deletion, a second cross was needed, since poor spore viability led to only three tetrads with four live spore-clones in the first cross. Since these three tetrads and other partial tetrads showed convincing segregation for X-ray sensitivity, we hypothesized that the poor viability was unrelated to the deletion. A back-cross to wild-type using an *spt7Δ* spore-clone from one of these tetrads confirmed this by giving high spore-viability, and also confirmed the co-segregation relationship, as shown in Table 1. The tetrad data in Table 1 show that an unlinked secondary marker is not responsible for the X-ray sensitivity in these 8 mutants, and the high spore viability and 2+:2- segregation of recessive markers from each parent effectively rules out polyploidy. The possibility of a secondary mutation closely linked to the deletion itself is not

excluded, but in *Saccharomyces* the probability of such close linkage of mutations by chance is less than 1%. We conclude that at least for this genetic background, the deletion allele is both necessary and sufficient to confer the observed X-ray sensitivity in each of the first eight mutants in Table 1. For a ninth mutant, the *gcn5* deletion, a minimal X-ray sensitivity that was apparent in survival curves (Figure 3) was insufficient to permit reliable scoring of this phenotype in replica-plated tetrad sets, as discussed below.

Two mutants, those with deletions in the *HTL1* and *DEF1* genes, failed to behave in the same way as those discussed above. Spore viability from the crosses with wild-type was poor in each case (see Table 1). In addition there was wide variation in the size of the germinating spore colonies, with many weak spore-clones that could not be reliably scored for IR-sensitivity. Aneuploidy or polyploidy is a likely possibility in these crosses, especially in the case of *htl1Δ*, which is itself known to lead to increased frequencies of spontaneously polyploidized cells (LANZUOLO *et al.* 2001). Since polyploidy by itself is well known to affect IR-sensitivity (MORTIMER 1958; LASKOWSKI 1960), it is difficult to assess without reconstructing the strains whether these deletion mutations also confer sensitivity. We have not pursued the *htl1Δ* and *def1Δ* mutants further.

X-ray survival curves: We performed X-ray survival curves for at least two haploids and one homozygous mutant diploid strain for the nine out of eleven deletion mutants in Table 1 that showed 2+:2- segregation for geneticin resistance when crossed to wild-type. The results are shown in Figures 1 through 9. Additional whole or partial survival assays for many of the mutants (not shown) served to confirm these data. We prefer to present individual survival assays instead of averaging measurements at each dose from separate curves, in part because dose-points within a curve are related based on serial dilutions and are not independent measurements. In addition, their accuracy in different curves will vary according to colony count. Hence, taking mean values may be misleading. We include two haploid wild-type strains with a genetic background isogenic to the mutants. These are g1201-4C and MW5067-1C, each derived from crosses as described above. These strains were isolated as fresh spore-clones from crosses with high viability and from tetrads with four viable spores, hence the presence of any gross chromosomal abnormalities that could have accrued in the parent strain over time is unlikely. The wild-type curves shown are representative of several done during the course of this project. A survival curve (not shown) for strain BY4742, the *MAT*α haploid wild-type from the deletion collection, was almost identical to that of MW5067-1C. The diploid shown, B4743, is the wild-type from the deletion collection.

It can be seen that eight of the mutant strains are significantly more X-ray sensitive than wild-type in both haploid and diploid configurations. The ninth mutant, deleted for *GCN5*, appears to show marginal sensitivity. None of these mutants show such strong sensitivity as the major recombinational repair mutants. These are represented by the *rad51null* haploid and diploid strains shown in the figures for comparison. Results and discussion for each mutant are detailed below.

***DOT1*: Histone methylation is involved in IR-repair:** We find that haploid and homozygous diploid strains deleted for the *DOT1* gene (SINGER *et al*. 1998) show significant X-ray sensitivity (see Figure 1 and Figure 8). It can be seen that the *dot1Δ* haploid survival curves are about equal to that of a deletion mutant of the *RAD5* gene. This typifies mid-range sensitivity that is substantially less than that of the *rad51Δ* mutant (Figure 1). Interestingly, the homozygous *dot1Δ/dot1Δ* mutant diploid (Figure 8) is comparatively less sensitive compared to the wild-type diploid than the *dot1Δ* haploid strains are to haploid wild types (Figure 1). This could imply a role in repair of recessive lethal damage, such as base damage rather than double-strand breaks (DSBs), which are thought to be dominant lethal lesions in the absence of repair (reviewed in GAME 1983). Alternatively, *DOT1* could mediate DSB repair that primarily involves sister-chromatids rather than

homologous chromosomes. The *DOT1* gene is highly conserved throughout eukaryotes (FENG *et al.* 2002) and its product has a single known function, the methylation of histone H3 protein at one residue, lysine-79, in the core of the protein (NG *et al.* 2002; FENG *et al.* 2002; VAN LEEUWEN *et al.* 2002). This methylation is required for DNA silencing near telomeres and elsewhere (NG *et al.* 2002; NG *et al.* 2003), and *dot1* mutants were initially isolated and named based on loss of this function (disruptor of telomeric silencing) (SINGER *et al.* 1998). *DOT1* is important in differentiating heterochromatin from euchromatin (NG *et al.* 2003). To determine whether the *DOT1* gene functions in repair via its known activity in histone H3 lysine-79 methylation or via some other unidentified function, we obtained three mutants from Dr. Kevin Struhl in which the lysine-79 residue in histone H3 is replaced by a different amino acid (alanine, proline and glutamine repsectively) that is not a substrate for methylation (NG *et al.* 2002). In these strains, the altered histone H3 gene is placed on a CEN plasmid and the two chromosomal genes for histone H3 are deleted. We expected that such mutants would be similar in IR-sensitivity to *dot1null* mutants even in a *DOT1* wild-type background if lysine-79 methylation is required for normal DNA repair.

We found that all three of these mutant strains showed X-ray sensitivity in spot tests that is similar to that of the *dot1Δ* mutant. Figure 1 shows a survival

curve for a mutant in which lysine-79 is replaced by glutamine. In the same Figure, it can be seen that no IR-sensitivity is present in a strain (UCC1111) in which a wild-type histone H3 gene is provided on a plasmid to cover the chromosomally deleted histone H3 genes. This confirms that the mutant alleles themselves, rather than the location of the histone H3 gene on a plasmid, are responsible for the IR-sensitivity of the H3 lysine-79 replacement mutants. In comparing the *hht2-K79Q* mutant with the *dot1Δ* mutant, we are comparing the effect of histone H3 containing a glutamine at residue 79 versus histone H3 containing an unmethylated lysine at residue 79. While these altered histone proteins may not be exactly equivalent, the fact that both strains are significantly sensitive to ionizing radiation provides strong evidence of a repair function for lysine methylation in the core of histone H3. We are currently determining which repair pathways are affected in the *dot1* mutant by constructing and studying *dot1 rad* double mutant strains carrying blocks in each of the currently known IR-repair mechanisms.

***SPT7, SPT20, HFI1 and GCN5*: Components of the SAGA complex:** Figures 2 and 9 show that deletions of the *SPT7* and *SPT20* genes each confer modest but consistent IR sensitivity in haploids and detectable sensitivity in diploids. The *SPT* genes in yeast were identified by WINSTON *et al.* (1984) based on a mutant

phenotype that involves suppression of the effects of *T*y elements inserted into the promoters of other yeast genes. *SPT7* and *SPT20* code for two components of the yeast Spt-Ada-Gcn5-acetyltransferase (SAGA) complex (GRANT *et al.* 1997). SAGA is a conserved multi-protein complex involved in normal transcription in yeast. Loss of the complex results in altered mRNA levels for about 10% of the genes in Saccharomyces (reviewed in WU and WINSTON 2002). Its components include the catalytic subunit Gcn5p, which is a histone acetyltransferase (BROWNELL *et al.* 1996). The histone acetylation activity of Gcn5P is modulated by the associated adaptor proteins Ada2p and Ada3p (MARCUS *et al.* 1994; HORIUCHI *et al.* 1995) and these three proteins also occur as parts of a second complex called ADA ((EBERHARTER *et al.* 1999). In addition, the SAGA complex interacts with TATA box binding protein (TBP) and, in the classical form of SAGA that contains the Spt8 protein, inhibits its interaction with the TATA box at some promoters (STERNER *et al.* 1999; BELOTSERKOVSKAYA *et al.* 2000). The Spt7 and Spt20 proteins, together with the product of the *HFI1* gene, (also known as *ADA1* **(**HORIUCHI *et al.* 1997) and originally identified as *SUP110* (BROWN 1994)), are each believed to be essential for the structural integrity of the SAGA complex, which does not form if any of them are absent (STERNER *et al.* 1999). SAGA includes additional known proteins and can also exist in one or more alternate forms (WU and WINSTON 2002; STERNER and BERGER 2000).

Because of the functional relationship of *GCN5* and *HFI1* with the *SPT7* and *SPT20* genes, we also undertook genetic crosses to wild-type with the *gcn5* and *hfi1* deletion mutants and performed survival assays of haploid and diploid strains, as with the other mutants. As shown in Figure 3, there is unequivocal X-ray sensitivity for the *hfi1* deletion strains. The haploid *hfi1* survival curves in Figure 3 show a slightly greater sensitivity than the *gcn5* curves in the same figure or the *spt7Δ* and *spt20Δ* strains in Figure 2. The homozygous diploid *hfi1Δ/hfi1Δ* strain is very close in survival to the *spt7Δ/spt7Δ* and *spt20Δ/spt20Δ* diploids (Figure 9). We found that the IR sensitivity phenotype of the *hfi1Δ* mutant could be readily scored in irradiated replica-plate assays and co-segregates with the deletion allele. We also confirmed that the *hfi1* deletion strain is auxotrophic for inositol, as expected for this mutant (HORIUCHI *et al.* 1997), and that inositol auxotrophy co-segregates with X-ray sensitivity and the *KanMX4* marker. While this work was in progress, a new mutant (*srm12*) that was identified in a screen for decreased spontaneous mutagenesis to the mitochondrial rho$^-$ state was shown to be a nonsense allele of the *HFI1* gene, and was also shown to confer IR-sensitivity (KOLTOVAYA *et al.* 2003). A diploid homozygous for this mutation showed sensitivity to Co^{60} gamma rays (KOLTOVAYA *et al.* 2003) that was comparable to

our observations (Figure 9) for the *hfi1Δ* homozygous mutant diploid treated with X-rays.

In contrast to *hfi1Δ*, the *gcn5* deletion confers at most marginal IR sensitivity. The strains whose survival is shown in Figures 3 and 9 were consistently more sensitive than wild-type, but the phenotype is sufficiently mild that we were unable to demonstrate that X-ray sensitivity co-segregated with the *gcn5* deletion using replica-plates of spore-clones from meiotic tetrads. We then took 20 spore-clones from five meiotic tetrads and measured survival at a single dose (122.9 kr/12.29 Gy) by counting plated colonies, as in our survival curve assays. We found a small difference between mutant and wild type average survival, but this was largely masked by variation between the individual measurements. The finding that the *gcn5Δ* mutant is scarcely IR-sensitive and certainly less sensitive than the other SAGA mutants we studied may implicate functions other than histone acetylation in mediating the repair functions of this complex. A role for the complex in the transcriptional activation of one or more repair proteins could explain the observation that mutants in genes required for its structural integrity are more sensitive than a mutant deleted only for the histone acetylation activity. Analysis of sensitivity in strains lacking other components of

SAGA and its alternate and related complexes will clarify which of their activities is involved in recovery from IR.

***NAT3* and *MDM20*: Subunits of the Nat3B protein:** Survival curves for two haploid strains containing the deletion of *NAT3* and two strains deleted for *MDM20* are shown in Figure 4. Curves for diploid strains homozygous for each of these deletions are shown in Figure 8. The *nat3* and *mdm20* deletions confer comparable sensitivity that is greater than that of the other mutants described here or by GAME et al. (2003). While this work was in progress, it was shown that *NAT3* and *MDM20* code for subunits of a single protein, NatB N^{α}-terminal acetyltransferase (POLEVODA *et al.* 2003; see also SINGER and SHAW 2003). Hence, the similarity in their IR-sensitive phenotypes is to be expected. The *NAT3* gene was previously identified as the probable catalytic subunit of this protein (POLEVODA *et al.* 1999), which is one of three known enzyme-complexes that between them acetylate the N-termini of a large number of yeast proteins. Specifically, the NatB protein acetylates yeast proteins with N-termini consisting of Met-Glu or Met-Asp, and sub-classes of proteins with Met-Asn or Met-Met, and partially acetylates some proteins with other termini (see POLEVODA and SHERMAN 2003).

Previously reported phenotypes for both *nat3* and *mdm20* deletion strains include increased sensitivity in spot tests to several chemicals that lead to DNA damage, including camptothecin, bleomycin, hydroxyurea and caffeine (POLEVODA *et al.* 2003), an inability to grow at 37°, reduced growth on nonfermentable carbon sources, diminished mating efficiency, and other effects confirmed and reviewed by POLEVODA *et al.* (2003). In our hands, at 30° radiation-sensitivity convincingly co-segregated 2+: 2- with the deletion alleles, for both *mdm20* and for *nat3* deletion strains when crossed with wild-type (see Table 1). Each mutant also showed a temperature-conditional growth phenotype. However, as reported elsewhere (SINGER and SHAW 2003), this phenotype was more pronounced in *mdm20Δ* strains, which showed essentially no growth at 37°, than in *nat3Δ* strains, which we found to show limited but real growth at 37° over a period of two or more days.

In the cross of the original temperature-sensitive *mdm20Δ* mutant to our wild-type strain g1201-4C, a fraction of the tetrads showed a digenic segregation for temperature-sensitive growth, such that one or in some cases both the spore-clones containing the *mdm20Δ::KanMX4* allele showed wild-type growth at 37°C. None of the *MDM20+* spores were inviable at 37°C, indicating that the deletion was necessary but not always sufficient to confer the temperature-conditional

growth phenotype in this cross, and that an unlinked suppressor of the conditional lethality was also segregating in some tetrads. We mated *mdm20Δ* spore-clones that were able to grow at 37° with those that were inviable at this temperature. The resulting diploids were viable at 37°, indicating that the suppressor phenotype is dominant. However, radiation-sensitivity was apparently unaffected by this suppressor, since *mdm20Δ* spore-clones that were viable at 37° remained IR-sensitive both at this temperature and at 30°C. Survival curves (at 30°) of a temperature-conditional *mdm20Δ* strain (g1229-1D) and one able to grow at 37° (g1229-9B) were equivalent (Figure 4).

We observed a similar phenomenon with *nat3Δ* strains. In this case, about half the *nat3Δ* spores showed a growth defect at 37° that was more pronounced than that of the initial *nat3Δ* mutant culture, although still not as strong as that seen in *mdm20Δ* strains. We inferred that a weak suppressor of the temperature conditional *nat3Δ* phenotype was present in our starting culture. We tested whether this influenced IR sensitivity by comparing survival of a strongly temperature-conditional spore-clone with that of a weakly temperature-conditional one. We also tested the effect of temperature on IR sensitivity in each strain by incubating parallel sets of plates at 25°, 30° and 37° immediately after irradiation. (Growth prior to irradiation in this experiment was at 25°). Even the strain with the

stronger temperature-sensitive growth defect was able to form small but countable colonies at 37°. The results are presented in Figure 5, where it can be seen that there is a small effect of temperature on survival in both strains, but no clear difference between the strains. There is also a minor difference in the survival curves at two temperatures in the wild-type strain (Figure 5). Although this is less than that seen in the two *nat3Δ* strains, it seems likely that as with *mdm20Δ*, the IR-sensitive phenotype and the temperature sensitive growth defect in the *nat3* deletion are conferred through separate mechanisms.

These observations are consistent with findings of SINGER *et al.* (2000), who identified and studied nine dominant suppressor mutations of the *mdm20* deletion. They found that all nine of these suppressors represent mis-sense alleles in the structural genes for actin or tropomyosin (*ACT1* and *TPM1* respectively). They argued from this and other evidence that many of the phenotypes of the *mdm20* deletion mutant arise from its destabilization of actin-tropomyosin interactions, causing partial loss of function. It has been shown independently that the NatB complex is required for acetylation of both actin (POLEVODA *et al.* 1999) and tropomyosin (SINGER and SHAW 2003), supporting this hypothesis. However, POLEVODA *et al.* (2003) point out that several of the many other potential target proteins for the NatB acetyltransferase are involved in repair. In addition, as in the

case of the *mdm20* deletion (SINGER *et al.* 2000), they identified suppressors of the *nat3Δ* temperature-sensitive phenotype that represented alterations in the *ACT1* and *TPM1* genes (POLEVODA *et al.* 2003). In spot-tests, these suppressors did not suppress the sensitivity of *nat3Δ* mutants to hydroxyurea and camptothecin, although the *TPM1* (but not *ACT1*) mutants did partially suppress sensitivity of *nat3Δ* strains to bleomycin (POLEVODA *et al.* 2003). These authors concluded that the increased sensitivity of *nat3Δ* and *mdm20Δ* strains to DNA damaging agents in spot-tests may arise from effects of the mutations on other target proteins. If so, this could explain our observation that suppressors of the temperature-conditional phenotype of the *mdm20Δ* and *nat3Δ* mutants fail to affect their radiation-sensitive phenotypes. Additional studies of mis-sense mutants involving potential NatB target enzymes that cannot be acetylated, and of *mdm20* or *nat3* double mutant combinations with these and other mutants should clarify the cause of the IR-sensitivity conferred by these deletions.

ORF YDR359C *(VID21/EAF1)*: The name *VID21* was standardized for ORF YDR359C by the Saccharomyces Genome Database in August 2003. The name represents a suggested function in ***v***acuolar ***i***mport and ***d***egradation, proposed earlier for twenty other genes numbered *VID1* to *VID20* (HOFFMAN and CHIANG 1996). Very recently YDR359C has also been shown to be the ORF for an

independently named gene, *EAF1*, identified from a protein (Esa1-associated factor-1) that is a member of the NuA4 histone acetyltransferase complex (DOYON *et al.* 2004). Strains deleted for *VID21* were reported by Bennett *et al.* (2001) to be IR-sensitive. We observed significant IR-sensitivity in the BY4742 *MAT*α strain deleted for ORF YDR359C (*VID21/EAF1*), and confirmed that an IR sensitive phenotype co-segregates with the deletion allele (see Table 1). X-ray survival curves for two haploid and one homozygous diploid *vid21*Δ*::KanMX4* mutant strains are shown in Figures 6 and 8, respectively. It can be seen that the deletion confers significant X-ray sensitivity in both the haploids and in the homozygous diploid strain. The mutant effect is only slightly less than that shown by the *mdm20* and *nat3* deletion strains in Figure 4.

The recent finding that *VID21/EAF1* encodes a member of the NuA4 HAT complex (DOYON *et al.* 2004) will help to clarify its role in repair. The catalytic unit of this complex is the essential gene *ESA1*, whose product acetylates *in vivo* up to four lysine residues in the tail region of histone H4 and probably some sites on other histone proteins to a lesser degree (ALLARD *et al.* 1999; STERNER and BERGER 2000). Mis-sense mutants that are viable but partially defective in the activity of Esa1 protein, and histone H4 mutants in which the target lysine residues are altered to non-acetylated amino acids, have both been shown to confer

sensitivity to MMS and camptothecin (BIRD *et al.* 2002). Another mutant involving the NuA4 HAT complex, *yng2null*, has also shown to be sensitive to MMS and camptothecin, and to show a reduction in the repair of MMS-induced DSBs in an assay using pulsed-field gel electrophoresis (CHOY and KRON 2002). It has been suggested (BIRD *et al.* 2002) that some NuA4 complex mutants may affect replication-coupled repair, based on their increased sensitivity to camptothecin, which is a topoisomerase I poison known to lead to DSBs during replication (D'ARPA *et al.* 1990).

DCC1*:** The *DCC1* gene is represented by ORF YCL016C. Its gene product was identified as a protein that binds to others involved in sister-chromatid cohesion, and the gene was named *DCC1* based on its mutant phenotype of ***defective sister ***c***hromatid ***c***ohesion (MAYER *et al.* 2001). A human homolog, hDCC1, has been cloned and characterized (MERKLE *et al.* 2003). Strains from the genomic deletion library that were deleted for YCL016C (*DCC1*) were reported as IR-sensitive in spot-tests by Bennett *et al*. (2001), and we verified that this phenotype is conferred by the deletion (see Table 1). Detailed X-ray survival curves (Figure 7) confirm the IR sensitivity, with two haploid spore-cones showing excellent agreement. A diploid homozygous for the *dcc1Δ::KanMX4* deletion, constructed by mating two spore-clones, also shows substantial X-ray sensitivity (see Figure 8).

It is likely that recombinational repair involving sister chromatids could be defective in *dcc1* deletion strains given the known role of *DCC1* in enabling sister-chromatid cohesion (MAYER *et al.* 2001). Recombinational repair using sister-chromatids as templates is believed to be a major route for repair of IR-induced DSBs in wild-type haploid yeast cells (see KUPIEC 2000; GAME 2000 for reviews). In addition, work with several organisms including Saccharomyces (SJOGREN and NASMYTH 2001), Schizosaccharomyces (HARTSUIKER *et al.* 2001) and Coprinus (CUMMINGS *et al.* 2002) has shown that mutants directly affected in sister-chromatid cohesion are often sensitive to radiation and compromised in recombinational repair (reviewed by STRUNNIKOV and JESSBERGER 1999). Dcc1p forms part of an "alternative" replication factor C (RFC) complex (MAYER *et al.* 2001) that has recently been shown to load the sliding-clamp proliferating cell nuclear antigen (PCNA) on to DNA (BERMUDEZ *et al.* 2003). The same clamp-protein (PCNA) is also loaded on to DNA by the related RFC1 complex, which contains several proteins in common but lacks Dcc1p (CULLMANN *et al.* 1995; reviewed in WAGA and STILLMAN 1998). It has been proposed that the Dcc1p-containing complex loads PCNA on to DNA at certain sites, and that this effects a change in the replication-polymerase machinery to enable cohesin proteins to be laid down at these sites, leading to sister-chromatid cohesion (MAYER *et al.* 2001).

In addition, modified forms of PCNA are major players in controlling post-replication/translesion synthesis repair. Modification of PCNA is mediated by the *RAD6*, *RAD18*, *RAD5* and *UBC13* gene products in a complex way to provide ubiquitinated and SUMO-ylated forms of this protein, and these modifications are thought to control its roles in different repair mechanisms (reviewed in MATUNIS 2002; see also HOEGE *et al.* 2002; STELTER and ULRICH 2003).

There are six other proteins in the same heptameric alternative RFC complex that includes the Dcc1 protein. These consist of four RFC proteins that are essential and occur in the other RFC complexes, plus the products of the *CTF8* and *CTF18* genes (HANNA *et al.* 2001; MAYER *et al.* 2001; NAIKI *et al.* 2001). We have not yet tested strains deleted for either *CTF8* or *CTF18*, but note that Bennett *et al*. (2001) have reported that strains deleted for *CTF8* showed IR-sensitivity in spot tests. *CTF18* was initially identified and named *CHL12* based on a screen for mutants with increased chromosome-loss (KOUPRINA *et al.* 1993). The deletion mutant was found to show elevated spontaneous mitotic recombination, slow growth, and cold-sensitive phenotypes (KOUPRINA *et al.* 1994).

Testing the mutants for cross-sensitivity to ultra-violet radiation: Yeast mutants that are sensitive to IR frequently show cross-sensitivity to ultraviolet

radiation (UV). To determine if the IR sensitive mutants discussed above also confer UV-sensitivity, we obtained UV survival curves for at least one strain carrying each mutant. Results are shown in Figure 10, which includes a wild-type strain and a highly UV-sensitive *rad14null* excision repair mutant as controls. It can be seen that none of the nine other mutants confer high UV sensitivity, and all except possibly the *mdm20Δ* and *nat3Δ* strains fall within or very close to the wild-type range. In the case of *nat3Δ* and *mdm20Δ*, we also studied meiotic tetrads from heterozygous diploids to test for segregation of a UV-sensitive phenotype. On UV-irradiated replica plates, we could not observe any clear segregation for a sensitivity phenotype among spore-clones from five and 13 complete tetrads segregating for *mdm20Δ* and *nat3Δ* respectively. The weaker growth of the mutant spore clones tended to complicate the interpretation, but we inferred that the difference in sensitivity between wild-type and mutant spore-clones was at most minor.

GENERAL DISCUSSION

We have documented here an IR-sensitive phenotype for haploid and homozygous diploid yeast mutants involving deletions of the *DOT1*, *SPT7*, *SPT20*, *HFI1*, *MDM20*, *NAT3*, *VID21(EAF1)* and *DCC1* genes. We have found at most a

borderline X-ray sensitivity in mutants deleted for another gene, *GCN5*. In each case except *GCN5*, we have demonstrated that IR-sensitivity co-segregates with the deletion allele in meiotic tetrads. To confirm that a deletion mutation confers IR-sensitivity, we consider it essential to obtain quantitative survival data and also to demonstrate that the phenotype is conferred by the deletion itself. With this in mind, we confirmed that the *nat3Δ*, *vid21Δ* and *dcc1Δ* mutations do confer IR sensitivity, following a previous report (BENNETT *et al.* 2001) that identified strains containing these deletions as sensitive in qualitative tests. Strains reported as sensitive that carried deletions in *htl1Δ* or *def1Δ/vid31Δ* (BENNETT *et al.* 2001) gave very poor spore viability in our crosses of the *MAT*α haploids to wild-type. We did not confirm that sensitivity is conferred by the deletion rather than another change such as altered ploidy, although both factors could have contributed to the sensitive phenotype. Apart from these mutants, we focused our initial screen on 357 mutants based on other criteria described earlier. Finally, we screened deletions involving three additional genes because their products formed complexes with the product of the *SPT20* gene whose deletion we identified as IR-sensitive.

The results reported here complement the earlier identification of the *RAD61* gene, which resulted from information from the pool assay (BIRRELL *et al.* 2001; GAME *et*

al. 2003) and has recently been reported to show a defect in sister-chromatid cohesion (WARREN *et al.* 2004). We are continuing to characterize both the *rad61* deletion and the mutants reported on here in terms of epistasis relationships and phenotypes involving mutation and recombination. No inferences can be drawn about the overall frequency of genes involved in IR-sensitivity from this work, because we used non-random criteria in choosing the initial set to study.

We find that none of the mutants reported on here confers substantial cross-sensitivity to UV radiation. This may argue against a significant role in post-replication repair (PRR) for these mutants. While not all known PRR mutants are significantly radiation sensitive, those that do confer IR sensitivity, such as *rad6null*, *rad18null* and *rad5null*, usually also confer substantial UV-sensitivity. In contrast, mutants in recombinational repair usually confer only minor UV-sensitivity (see GAME, 1983).

We searched the online datasets at the Saccharomyces Genome Database to determine if the wild-type genes whose mutants we studied are induced by radiation. We found no evidence in the literature for a strong and consistent induction of any of these genes by IR. This is not necessarily surprising in view of reports that there is a poor correlation between Saccharomyces genes that are

induced by toxic agents and genes responsible for resisting those agents (BEGLEY *et al.* 2002; BIRRELL *et al.* 2002).

Of the 9 mutants we report on here perhaps the most interesting is the *dot1* deletion, which specifically eliminates the methylation of the lysine-79 residue in the core of the histone H3 protein (NG *et al.* 2002; FENG *et al.* 2002; VAN LEEUWEN *et al.* 2002). Histone H3 mutants in which this target lysine is replaced by other amino-acids show nearly equivalent IR-sensitivity (see Figure 1). This provides strong evidence that this methylation, which cannot occur on the substituted amino acids, is responsible for the role of the highly conserved *DOT1* gene in IR-resistance. It further links histone modification to DNA repair, and provides an opportunity to use epistasis analysis and additional characterization to identify the repair pathways involved.

In addition to histone H3 lysine-79 methylation, we have shown that five genes involved in histone acetylation and transcriptional regulation (HAT) complexes play some role in IR-resistance. Four of these, *SPT7*, *SPT20*, *HFI1* and *GCN5*, encode components of the SAGA protein complex, while *VID21/EAF1* encodes a protein in the separate NuA4 HAT complex. Given the milder phenotype in *gcn5Δ* compared to the other HAT mutants, it is plausible that the IR sensitivity seen in

these different HAT complex mutants could be mediated indirectly by effects on transcriptional activation, which could influence the activity levels of other repair genes, rather than via histone acetylation *per se*. This is also possible in the case of the *dot1null* mutant, but perhaps less likely given the greater degree of IR-sensitivity and the known role of *DOT1* in the control of gene silencing and chromatin structure. Two other genes amongst the nine, *NAT3* and *MDM20*, are also involved in acetylation, although there are multiple target protein(s) for the NatB acetylase that they encode (POLEVODA and SHERMAN 2003). In contrast, it seems probable that the deletion of *DCC1* leads to X-ray sensitivity through disruption of sister-chromatid cohesion, since the gene product has a known function in ensuring this process (MAYER *et al.* 2001), and it is known that mutants affected in sister-chromatid cohesion can be sensitive to radiation and compromised in recombinational repair (reviewed by STRUNNIKOV and JESSBERGER 1999).

The diverse roles of the genes studied here confirm that much remains to be learned about recovery from IR damage. None of them has a product that is known to interact directly with DNA. Genes whose products modify other proteins that themselves may interact with DNA are emerging as a significant class of all those involved in IR-recovery. Such genes include the well-known *RAD5*, *RAD6*,

and *RAD18* loci (HOEGE *et al.* 2002; STELTER and ULRICH 2003) as well as some of those more recently identified. Since some protein-modifying enzymes such as the Nat3 complex have multiple targets, knowledge of their enzymatic function does not necessarily clarify their role in repair, or even which repair mode they are involved in. However, double-mutant analysis can take advantage of the many Saccharomyces genes already associated with identified repair pathways. This should determine which if any of the currently known pathways require the functions recently identified as relevant.

We thank J. Martin Brown and James A. Brown at Stanford for generously sharing strains and information. We thank Kevin Struhl for providing mutants strains with substitutions at the K79 residue of the histone H3 protein. This work was supported by NIH grant GM5997901 to JCG.

LITERATURE CITED

ALLARD, S., R. T. UTLEY, J. SAVARD, A. CLARKE, P. GRANT *et al.*, 1999 NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. Embo J **18:** 5108-5119.

BEGLEY, T. J., A. S. ROSENBACH, T. IDEKER and L. D. SAMSON, 2002 Damage recovery pathways in Saccharomyces cerevisiae revealed by genomic phenotyping and interactome mapping. Mol Cancer Res **1:** 103-112.

BELOTSERKOVSKAYA, R., D. E. STERNER, M. DENG, M. H. SAYRE, P. M. LIEBERMAN *et al.*, 2000 Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. Mol Cell Biol **20:** 634-647.

BENNETT, C. B., L. K. LEWIS, G. KARTHIKEYAN, K. S. LOBACHEV, Y. H. JIN *et al.*, 2001 Genes required for ionizing radiation resistance in yeast. Nat Genet **29:** 426-434.

BERMUDEZ, V. P., Y. MANIWA, I. TAPPIN, K. OZATO, K. YOKOMORI *et al.*, 2003 The alternative Ctf18-Dcc1-Ctf8-replication factor C complex required for sister chromatid cohesion loads proliferating cell nuclear antigen onto DNA. Proc Natl Acad Sci U S A **100:** 10237-10242.

BIRD, A. W., D. Y. YU, M. G. PRAY-GRANT, Q. QIU, K. E. HARMON *et al.*, 2002 Acetylation of histone H4 by Esa1 is required for DNA double-strand break

repair. Nature **419:** 411-415.

BIRRELL, G. W., J. A. BROWN, H. I. WU, G. GIAEVER, A. M. CHU *et al.*, 2002 Transcriptional response of Saccharomyces cerevisiae to DNA-damaging agents does not identify the genes that protect against these agents. Proc Natl Acad Sci U S A **99:** 8778-8783.

BIRRELL, G. W., G. GIAEVER, A. M. CHU, R. W. DAVIS and J. M. BROWN, 2001 A genome-wide screen in Saccharomyces cerevisiae for genes affecting UV radiation sensitivity. Proc Natl Acad Sci U S A **98:** 12608-12613.

BRACHMANN, C. B., A. DAVIES, G. J. COST, E. CAPUTO, J. LI *et al.*, 1998 Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115-132.

BROWN, N. G., 1994 Interactions among Pet54, Pet122 and Pet494: three nuclearly encoded translational activators of the mitochondrial COX3 gene in *Saccharomyces cerevisiae* and the search for other translational components that interact with them. Ph.D. thesis, Cornell University Library.

BROWNELL, J. E., J. ZHOU, T. RANALLI, R. KOBAYASHI, D. G. EDMONDSON *et al.*, 1996 Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell **84:** 843-851.

CHOY, J. S., and S. J. KRON, 2002 NuA4 subunit Yng2 function in intra-S-phase

DNA damage response. Mol Cell Biol **22:** 8215-8225.

CULLMANN, G., K. FIEN, R. KOBAYASHI and B. STILLMAN, 1995 Characterization of the five replication factor C genes of Saccharomyces cerevisiae. Mol Cell Biol **15:** 4661-4671.

CUMMINGS, W. J., S. T. MERINO, K. G. YOUNG, L. LI, C. W. JOHNSON *et al.*, 2002 The Coprinus cinereus adherin Rad9 functions in Mre11-dependent DNA repair, meiotic sister-chromatid cohesion, and meiotic homolog pairing. Proc Natl Acad Sci U S A **99:** 14958-14963.

D'ARPA, P., C. BEARDMORE and L. F. LIU, 1990 Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. Cancer Res **50:** 6919-6924.

DOYON, Y., W. SELLECK, W.S. LANE, S. TAN and J. CÔTÉ, 2004 Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to human. Mol Cell Biol **24:** 1884-96.

EBERHARTER, A., D. E. STERNER, D. SCHIELTZ, A. HASSAN, J. R. YATES, 3RD *et al.*, 1999 The ADA complex is a distinct histone acetyltransferase complex in Saccharomyces cerevisiae. Mol Cell Biol **19:** 6621-6631.

FENG, Q., H. WANG, H. H. NG, H. ERDJUMENT-BROMAGE, P. TEMPST *et al.*, 2002 Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol **12:** 1052-1058.

GAME, J. C., 1983 Radiation-sensitive mutants and repair in yeast. In: Yeast Genetics, Fundamental and Applied Aspects, Eds. Spencer, J.F.T., D. Spencer and A.R.W. Smith. Springer-Verlag, New York, pp. 109-137.

GAME, J. C., 2000 The Saccharomyces repair genes at the end of the century. Mutat Res **451:** 277-293.

GAME, J. C., and R. K. MORTIMER, 1974 A genetic study of x-ray sensitive mutants in yeast. Mutat Res **24:** 281-292.

GAME, J. C., G. W. BIRRELL, J. A. BROWN, T. SHIBATA, C. BACCARI *et al.*, 2003 Use of a genome-wide approach to identify new genes that control resistance of Saccharomyces cerevisiae to ionizing radiation. Radiat Res **160:** 14-24.

GRANT, P. A., L. DUGGAN, J. COTE, S. M. ROBERTS, J. E. BROWNELL *et al.*, 1997 Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev **11:** 1640-1650.

HANNA, J. S., E. S. KROLL, V. LUNDBLAD and F. A. SPENCER, 2001 Saccharomyces cerevisiae CTF18 and CTF4 are required for sister chromatid cohesion. Mol Cell Biol **21:** 3144-3158.

HARTSUIKER, E., E. VAESSEN, A. M. CARR and J. KOHLI, 2001 Fission yeast Rad50 stimulates sister chromatid recombination and links cohesion with repair. Embo J **20:** 6660-6671.

HOEGE, C., B. PFANDER, G. L. MOLDOVAN, G. PYROWOLAKIS and S. JENTSCH, 2002 RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature **419:** 135-141.

HOFFMAN, M., and H. L. CHIANG, 1996 Isolation of degradation-deficient mutants defective in the targeting of fructose-1,6-bisphosphatase into the vacuole for degradation in Saccharomyces cerevisiae. Genetics **143:** 1555-1566.

HORIUCHI, J., N. SILVERMAN, B. PINA, G. A. MARCUS and L. GUARENTE, 1997 ADA1, a novel component of the ADA/GCN5 complex, has broader effects than GCN5, ADA2, or ADA3. Mol Cell Biol **17:** 3220-3228.

HORIUCHI, J., N. SILVERMAN, G. A. MARCUS and L. GUARENTE, 1995 ADA3, a putative transcriptional adaptor, consists of two separable domains and interacts with ADA2 and GCN5 in a trimeric complex. Mol Cell Biol **15:** 1203-1209.

KOLTOVAYA, N. A., A. S. GUERASIMOVA, I. A. TCHEKHOUTA and A. B. DEVIN, 2003 NET1 and HFI1 genes of yeast mediate both chromosome maintenance and mitochondrial rho(-) mutagenesis. Yeast **20:** 955-971.

KOUPRINA, N., A. TSOULADZE, M. KORYABIN, P. HIETER, F. SPENCER *et al.*, 1993 Identification and genetic mapping of CHL genes controlling mitotic chromosome transmission in yeast. Yeast **9:** 11-19.

KOUPRINA, N., E. KROLL, A. KIRILLOV, V. BANNIKOV, V. ZAKHARYEV *et al.*, 1994

CHL12, a gene essential for the fidelity of chromosome transmission in the yeast Saccharomyces cerevisiae. Genetics **138:** 1067-1079.

KUPIEC, M., 2000 Damage-induced recombination in the yeast Saccharomyces cerevisiae. Mutat Res **451:** 91-105.

LANZUOLO, C., S. EDERLE, A. POLLICE, F. RUSSO, A. STORLAZZI *et al.*, 2001 The HTL1 gene (YCR020W-b) of Saccharomyces cerevisiae is necessary for growth at 37 degrees C, and for the conservation of chromosome stability and fertility. Yeast **18:** 1317-1330.

LASKOWSKI, W., 1960 Inaktivierungsversuche mit homozygoten hefestammen verschiedenen ploidiegrades. Z Naturforsch **15b**: 495-506.

LOVETT, S. T., and R. K. MORTIMER, 1987 Characterization of null mutants of the RAD55 gene of Saccharomyces cerevisiae: effects of temperature, osmotic strength and mating type. Genetics **116:** 547-553.

MARCUS, G. A., N. SILVERMAN, S. L. BERGER, J. HORIUCHI and L. GUARENTE, 1994 Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. Embo J **13:** 4807-4815.

MATUNIS, M. J., 2002 On the road to repair: PCNA encounters SUMO and ubiquitin modifications. Mol Cell **10:** 441-442.

MAYER, M. L., S. P. GYGI, R. AEBERSOLD and P. HIETER, 2001 Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister

chromatid cohesion in S. cerevisiae. Mol Cell **7:** 959-970.

MERKLE, C. J., L. M. KARNITZ, J. T. HENRY-SANCHEZ and J. CHEN, 2003 Cloning and characterization of hCTF18, hCTF8, and hDCC1. Human homologs of a Saccharomyces cerevisiae complex involved in sister chromatid cohesion establishment. J Biol Chem **278:** 30051-30056.

MORTIMER, R. K, 1958 Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of Saccharomyces cerevisiae. Radiat Res **9:** 312-326.

MORTIMER, R. K., and J. R. JOHNSTON, 1986 Genealogy of principal strains of the yeast genetic stock center. Genetics **113:** 35-43.

NAIKI, T., T. KONDO, D. NAKADA, K. MATSUMOTO and K. SUGIMOTO, 2001 Chl12 (Ctf18) forms a novel replication factor C-related complex and functions redundantly with Rad24 in the DNA replication checkpoint pathway. Mol Cell Biol **21:** 5838-5845.

NG, H. H., D. N. CICCONE, K. B. MORSHEAD, M. A. OETTINGER and K. STRUHL, 2003 Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. Proc Natl Acad Sci U S A **100:** 1820-1825.

NG, H. H., Q. FENG, H. WANG, H. ERDJUMENT-BROMAGE, P. TEMPST *et al.*, 2002 Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev **16:**

1518-1527.

POLEVODA, B., and F. SHERMAN, 2003 N-terminal acetyltransferases and sequence requirements for N-terminal acetylation of eukaryotic proteins. J Mol Biol **325:** 595-622.

POLEVODA, B., J. NORBECK, H. TAKAKURA, A. BLOMBERG and F. SHERMAN, 1999 Identification and specificities of N-terminal acetyltransferases from Saccharomyces cerevisiae. Embo J **18:** 6155-6168.

POLEVODA, B., T. S. CARDILLO, T. C. DOYLE, G. S. BEDI and F. SHERMAN, 2003 Nat3p and Mdm20p are required for function of yeast NatB Nalpha-terminal acetyltransferase and of actin and tropomyosin. J Biol Chem **278:** 30686-30697.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1982 Methods in yeast genetics. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

SINGER, J. M., and J. M. SHAW, 2003 Mdm20 protein functions with Nat3 protein to acetylate Tpm1 protein and regulate tropomyosin-actin interactions in budding yeast. Proc Natl Acad Sci U S A **100:** 7644-7649.

SINGER, J. M., G. J. HERMANN and J. M. SHAW, 2000 Suppressors of mdm20 in yeast identify new alleles of ACT1 and TPM1 predicted to enhance actin-tropomyosin interactions. Genetics **156:** 523-534.

SINGER, M. S., A. KAHANA, A. J. WOLF, L. L. MEISINGER, S. E. PETERSON *et al.*, 1998

Identification of high-copy disruptors of telomeric silencing in Saccharomyces cerevisiae. Genetics **150:** 613-632.

SJOGREN, C., and K. NASMYTH, 2001 Sister chromatid cohesion is required for postreplicative double-strand break repair in Saccharomyces cerevisiae. Curr Biol **11:** 991-995.

STELTER, P., and H. D. ULRICH, 2003 Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature **425:** 188-191.

STERNER, D. E., and S. L. BERGER, 2000 Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev **64:** 435-459.

STERNER, D. E., P. A. GRANT, S. M. ROBERTS, L. J. DUGGAN, R. BELOTSERKOVSKAYA *et al.*, 1999 Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol Cell Biol **19:** 86-98.

STRUNNIKOV, A. V., and R. JESSBERGER, 1999 Structural maintenance of chromosomes (SMC) proteins: conserved molecular properties for multiple biological functions. Eur J Biochem **263:** 6-13.

VAN LEEUWEN, F., P. R. GAFKEN and D. E. GOTTSCHLING, 2002 Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell **109:** 745-756.

WAGA, S., and B. STILLMAN, 1998 The DNA replication fork in eukaryotic cells. Annu Rev Biochem **67:** 721-751.

WARREN, C. D., D. M. ECKLEY, M. S. LEE, J. S. HANNA, A. HUGHES *et al.*, 2004 S-phase checkpoint genes safeguard high-fidelity sister chromatid cohesion. Mol Biol Cell **15:** 1724-1735.

WINSTON, F., D. T. CHALEFF, B. VALENT and G. R. FINK, 1984 Mutations affecting Ty-mediated expression of the HIS4 gene of Saccharomyces cerevisiae. Genetics **107:** 179-197.

WINZELER, E. A., D. D. SHOEMAKER, A. ASTROMOFF, H. LIANG, K. ANDERSON *et al.*, 1999 Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science **285:** 901-906.

WU, P. Y., and F. WINSTON, 2002 Analysis of Spt7 function in the Saccharomyces cerevisiae SAGA coactivator complex. Mol Cell Biol **22:** 5367-5379.

TABLE 1.

Spore viability and available co-segregation data for nine deletion-mutant heterozygous diploids.

Gene name	Systematic name	% spore viability	# tetrads obtained with 4 live spore-clones	# tetrads showing 2+:2- co-segregation for geneticin resistance and IR-sensitivity.
DOT1	YDR440W	95.8	21	21
*SPT7**	YBR081C	97.9	22	22
SPT20	YOL148C	85.0	31	31
HFI1 (ADA1)	YPL254W	95.5	18	18
MDM20	YOL076W	97.5	17	17
NAT3	YPR131C	94.2	20	19**
VID21	YDR359C	98.4	15	15
DCC1	YCL016C	97.4	18	18
GCN5	YGR252W	90.6	31	See Text
HTL1	YCR020W-B	38.9	0	0
DEF1 (VID31)	YKL054C	40.3	2	0

*The data for *SPT7* are from a secondary cross using an *spt7Δ* spore-clone back-crossed to wild-type, see text.
** In one tetrad, all four spores-clones were geneticin-resistant and X-ray sensitive. This could have arisen from a diploid cell that had become homozygous for the deletion. Two of these spore-clones appeared less IR-sensitive than the other two, for unknown reasons, but were clearly more sensitive than wild-type.

FIGURE LEGENDS

FIGURE 1. Survival versus X-ray dose for two haploid *dot1* deletion strains and an *hht2-K79Q* mutant haploid. This mutant (from K. Struhl) is chromosomally deleted for both of the genes that encode histone H3 (*HHT1* and *HHT2*) and both of the histone H4 genes (*HHF1* and *HHF2*) that are linked to them. It carries a CEN plasmid bearing a wild-type *HHF1* gene and a mutant allele of *HHT2* (*hht2-K79Q*) encoding a glutamine substitution at the lysine-79 position. Controls shown are UCC1111, which carries wild-type *HHT1* and *HHF1* genes on a CEN plasmid to cover the chromosomally deleted copies; and standard wild-type, *rad51Δ* and *rad5Δ* haploids in the deletion library background.

FIGURE 2. Survival versus X-ray dose for two *spt7* and two *spt20* haploid deletion strains. A wild-type and a *rad51Δ* haploid are included for comparison.

FIGURE 3. Survival versus X-ray dose for two *gcn5* and two *hfi1* haploid deletion strains. A wild-type and a *rad51Δ* haploid are included for comparison.

FIGURE 4. Survival versus X-ray dose for two *mdm20* and two *nat3* haploid deletion strains. g1229-1D *mdm20Δ* is unable to grow at 37° and g1229-9B

mdm20Δ spore clone can grow at 37°, see text. A wild-type and a *rad51Δ* haploid are included for comparison.

FIGURE 5. Survival versus X-ray dose for *nat3Δ* and wild-type strains pre-grown at 25° and incubated at 25° or 37° after X-ray treatment. Survival data for a *rad51null* haploid pre-grown and incubated at 30° is included for comparison. An experiment (not shown) in which *nat3Δ* strains were pre-grown at 25° and incubated after X-rays at 30° gave survival curves equivalent to those shown in Figure 4

FIGURE 6. Survival versus X-ray dose for two haploid *vid21* (ORF YDR359C) deletion strains. A wild-type and a *rad51Δ* haploid are included for comparison.

FIGURE 7. Survival versus X-ray dose for two haploid *dcc1* deletion strains. A wild-type and a *rad51Δ* haploid are included for comparison.

FIGURE 8. Survival versus X-ray dose for diploids homozygous for *dot1Δ*, *mdm20Δ*, *nat3Δ*, *vid21Δ*, and *dcc1Δ* mutations. Survival data for a wild-type and a *rad51null* diploid are included for comparison.

FIGURE 9. Survival versus X-ray dose for diploids homozygous for *gcn5Δ, hfi1Δ, spt7Δ and spt20Δ* mutations. Survival data for a wild-type and a *rad51null* diploid are included for comparison.

FIGURE 10. Survival versus ultra-violet radiation dose for ten haploid deletion mutant strains compared with wild-type. A *rad14null* mutant, defective in nucleotide excision repair, is included for comparison and shows the high sensitivity expected for such strains.

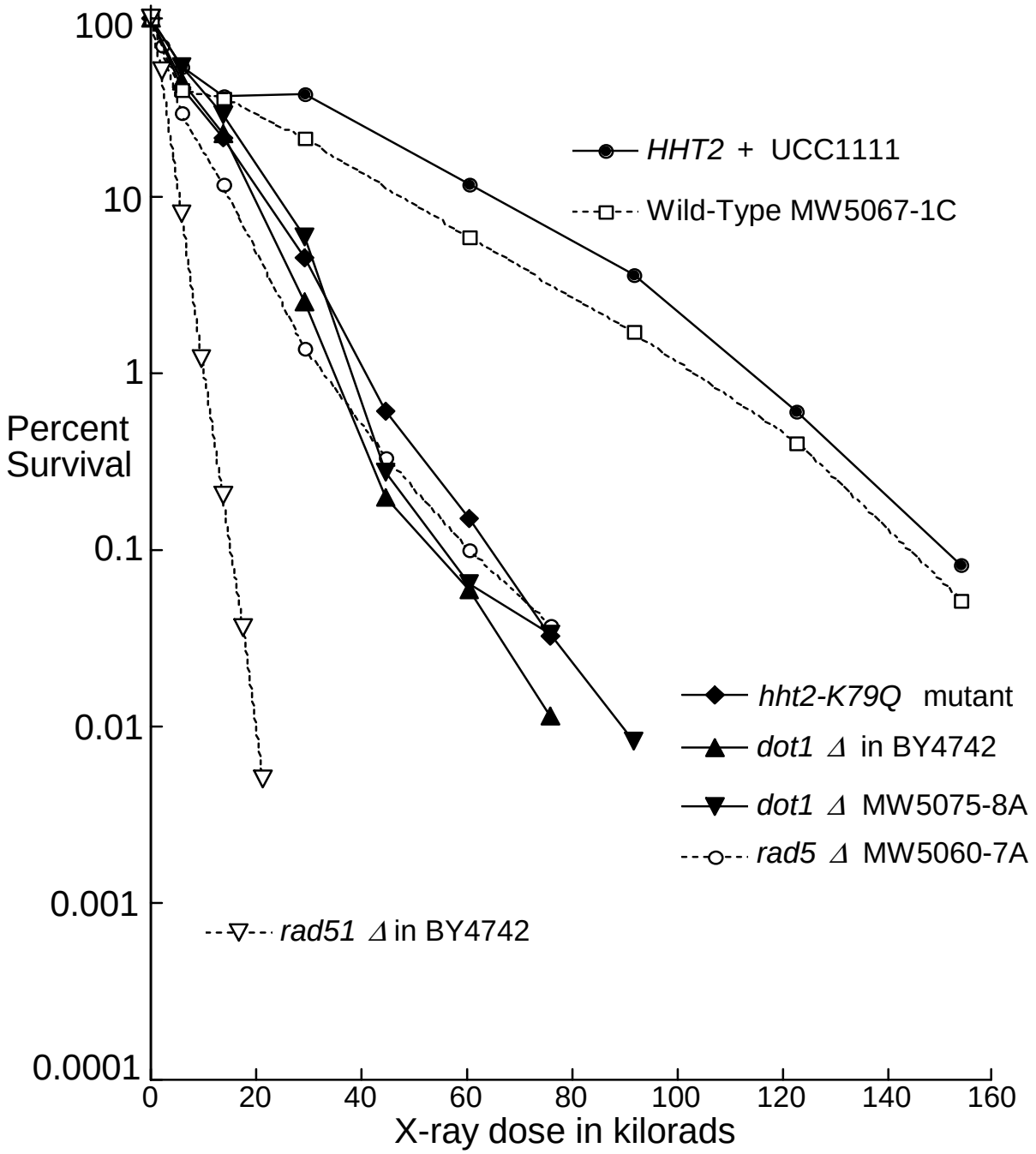
100
10
1
0.1
0.01
0.001
0.0001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
HHT2 + UCC1111
Wild-Type MW5067-1C
hht2-K79Q mutant
dot1 Δ in BY4742
dot1 Δ MW5075-8A
rad5 Δ MW5060-7A
rad51 Δ in BY4742

Figure 1

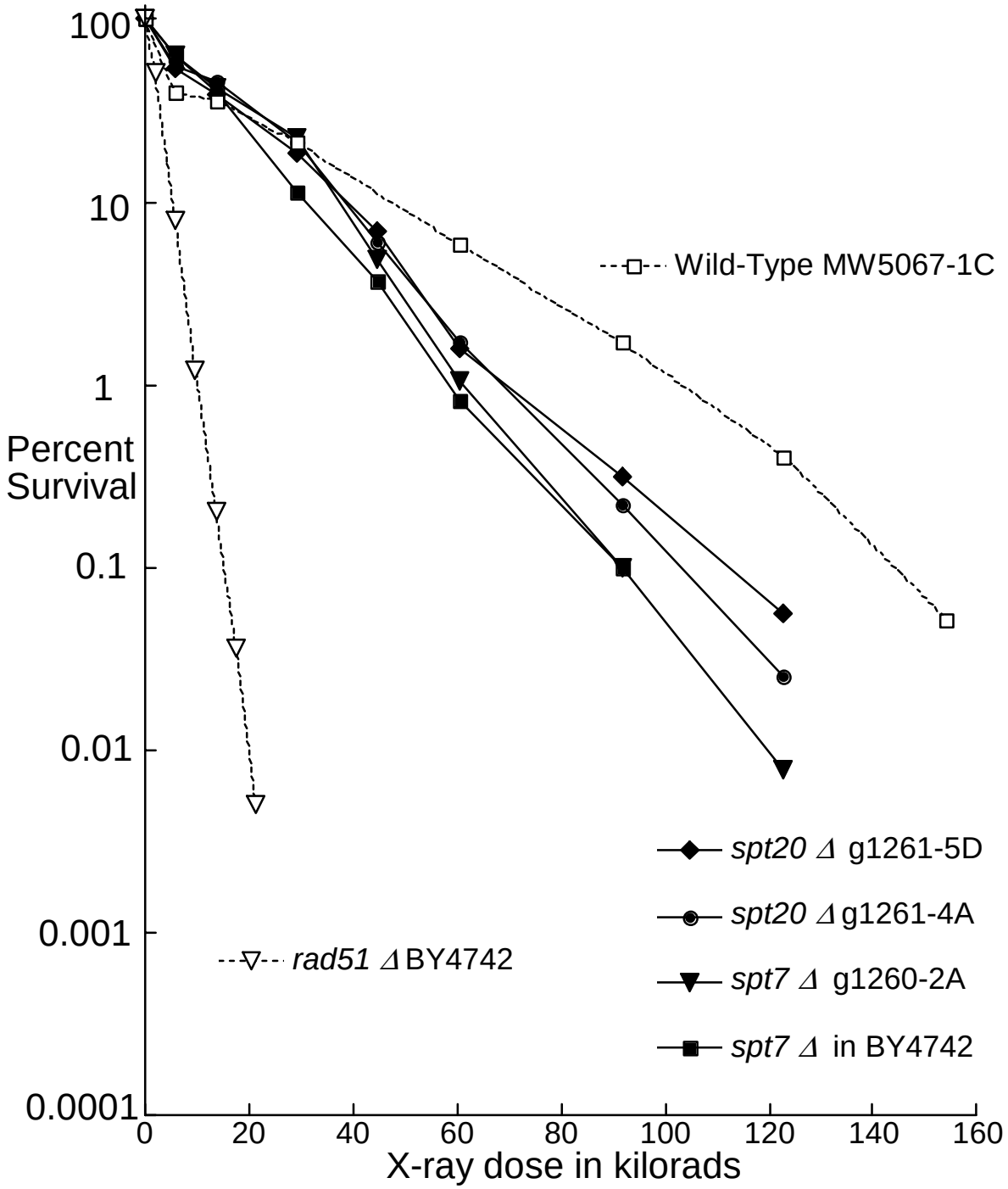
100
10
1
0.1
0.01
0.001
0.0001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
Wild-Type MW5067-1C
spt20 Δ g1261-5D
spt20 Δ g1261-4A
spt7 Δ g1260-2A
spt7 Δ in BY4742
rad51 Δ BY4742

Figure 2

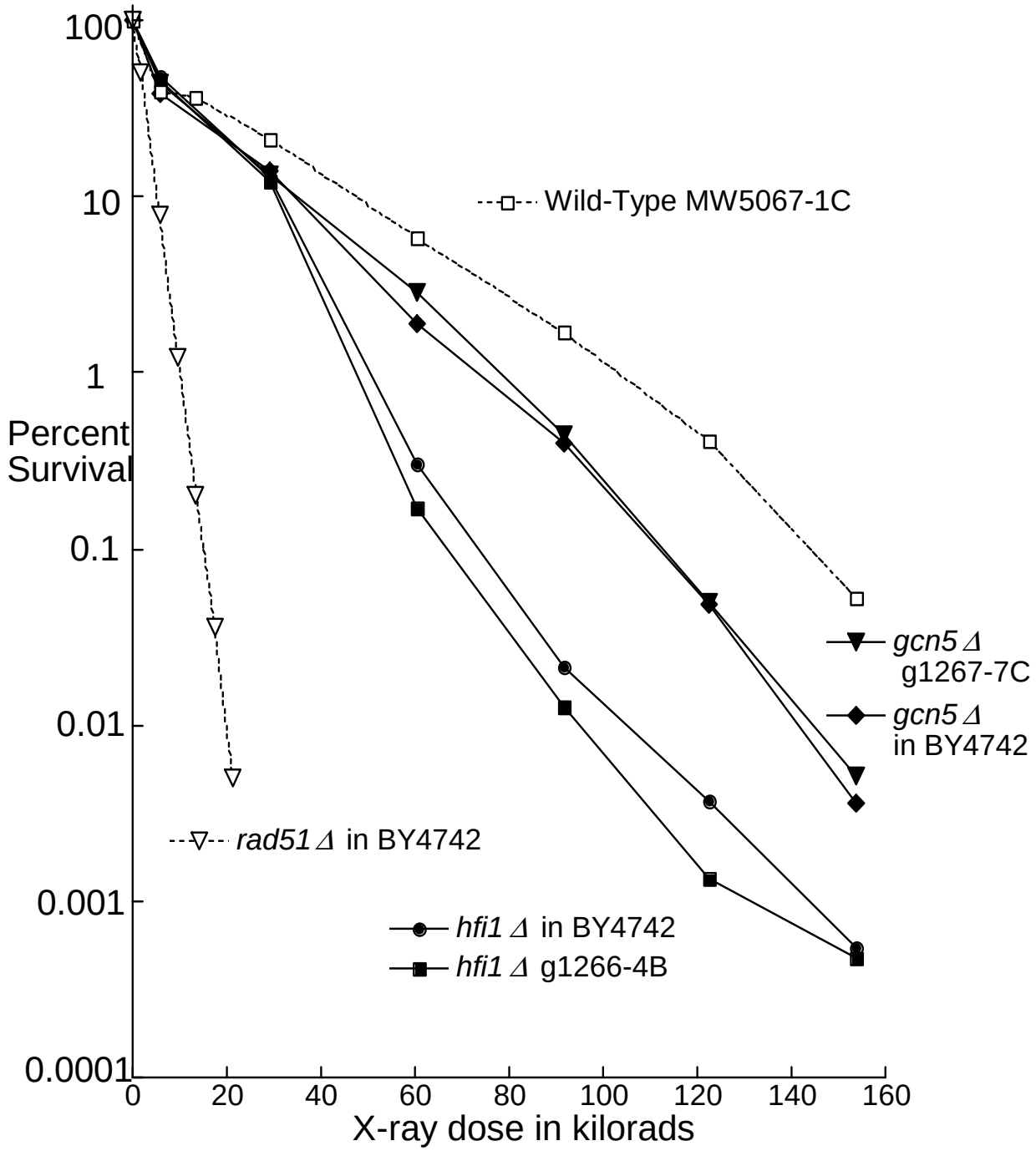
100
10
1
0.1
0.01
0.001
0.0001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
Wild-Type MW5067-1C
gcn5Δ g1267-7C
gcn5Δ in BY4742
rad51Δ in BY4742
hfi1Δ in BY4742
hfi1Δ g1266-4B

Figure 3

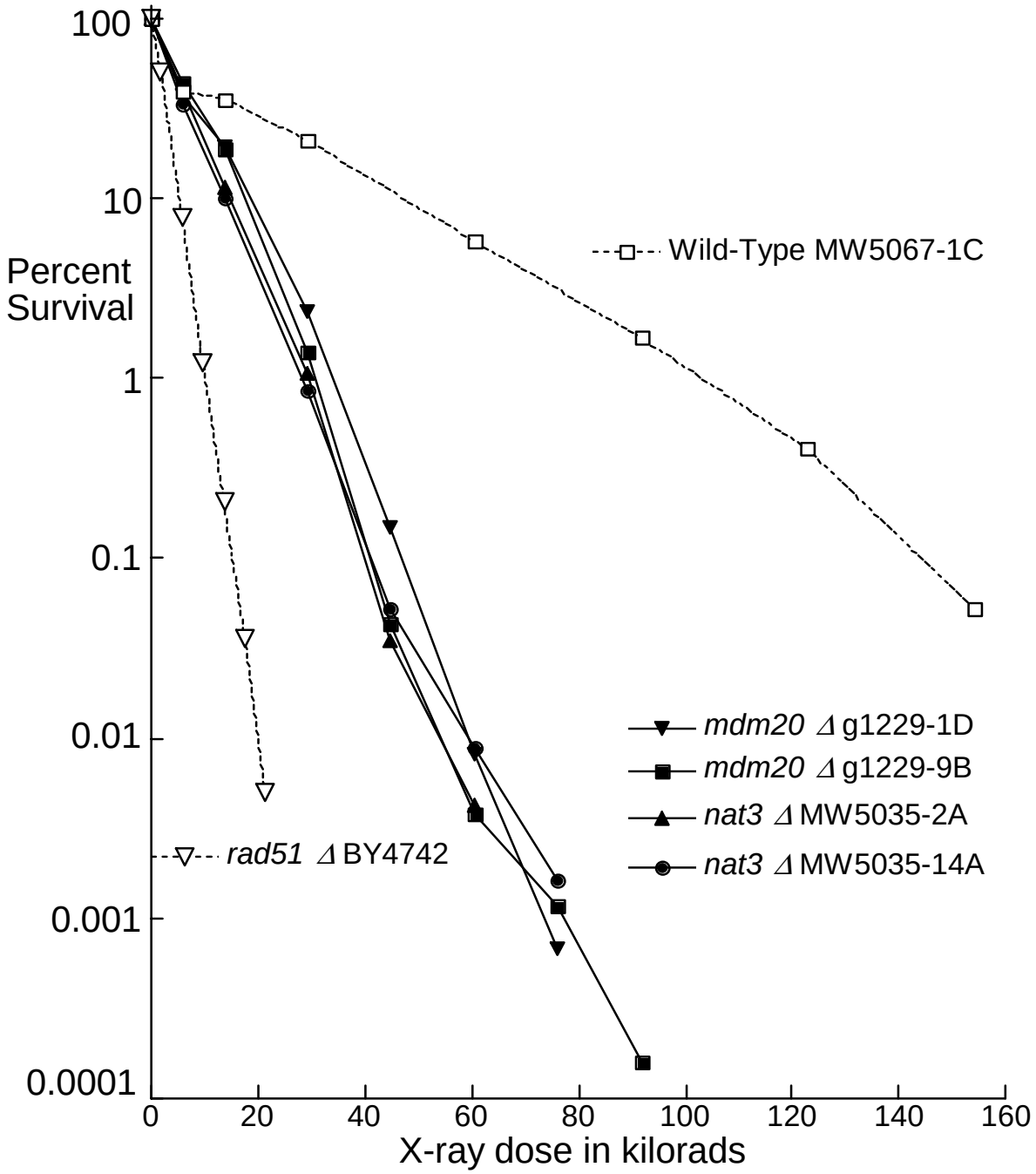
100
10
1
0.1
0.01
0.001
0.0001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
Wild-Type MW5067-1C
mdm20 Δ g1229-1D
mdm20 Δ g1229-9B
nat3 Δ MW5035-2A
nat3 Δ MW5035-14A
rad51 Δ BY4742

Figure 4

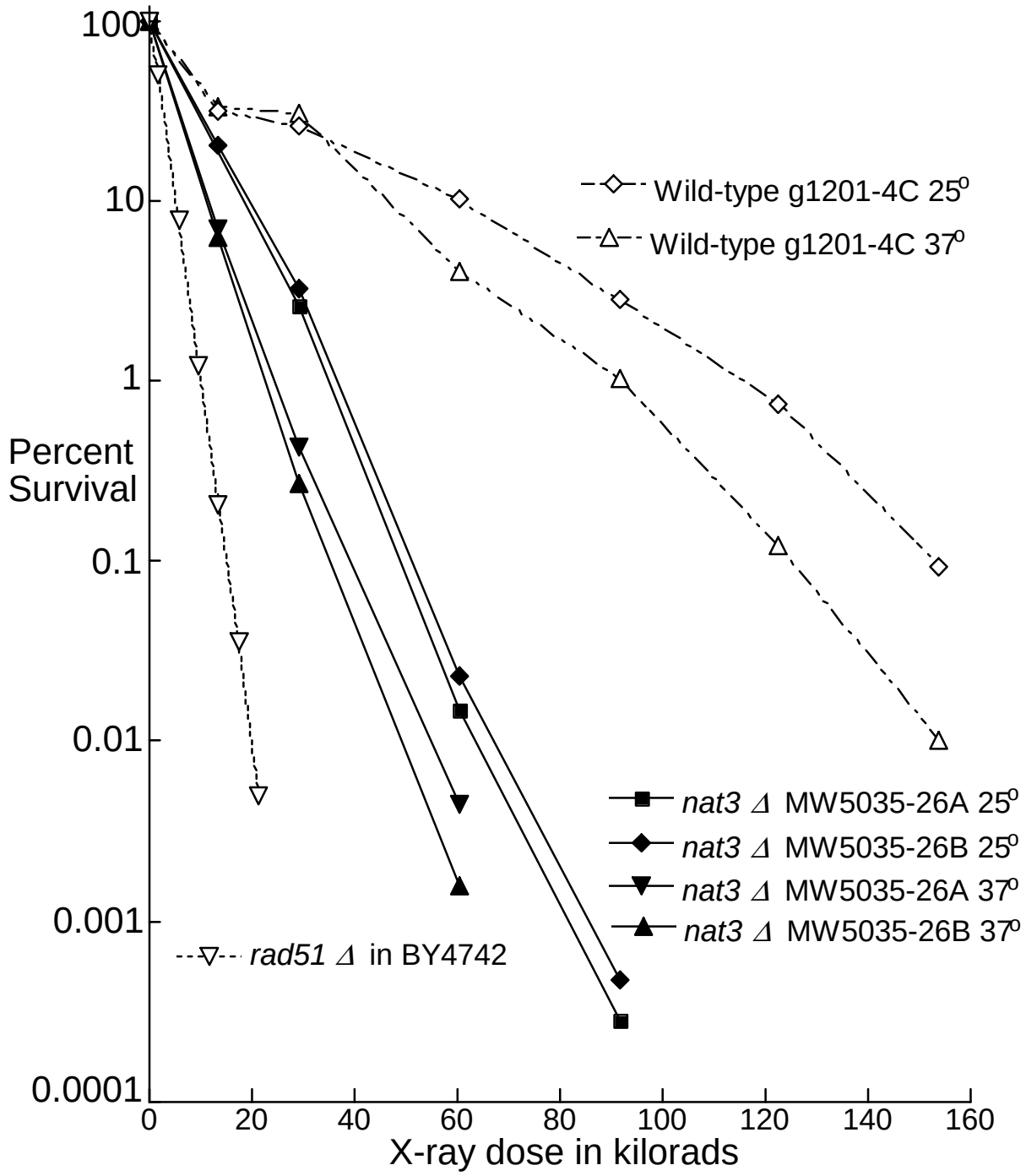
100
10
1
0.1
0.01
0.001
0.0001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
Wild-type g1201-4C 25°
Wild-type g1201-4C 37°
nat3 Δ MW5035-26A 25°
nat3 Δ MW5035-26B 25°
nat3 Δ MW5035-26A 37°
nat3 Δ MW5035-26B 37°
rad51 Δ in BY4742

Figure 5

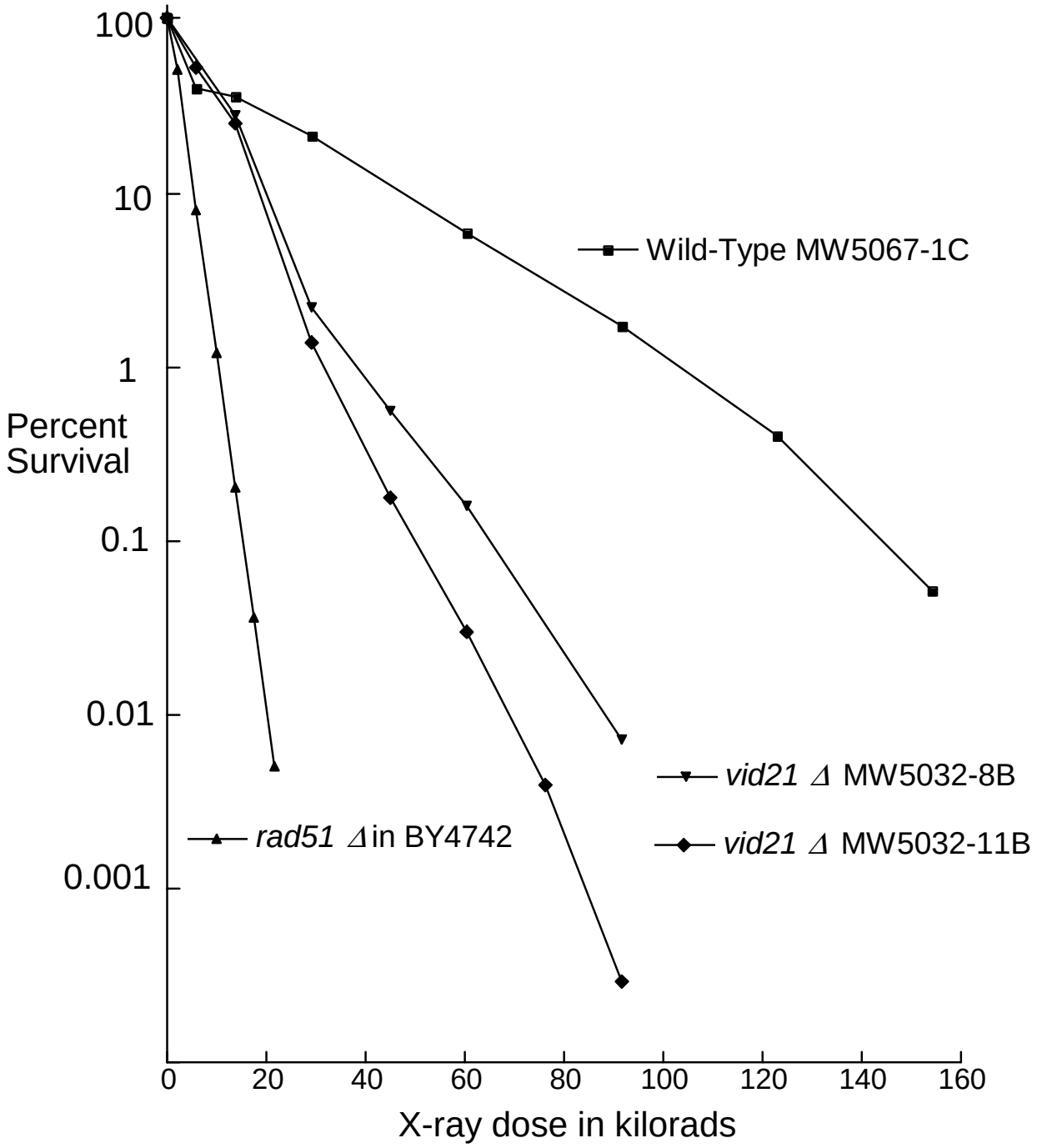
100
10
1
0.1
0.01
0.001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
Wild-Type MW5067-1C
vid21 Δ MW5032-8B
vid21 Δ MW5032-11B
rad51 Δ in BY4742

Figure 6

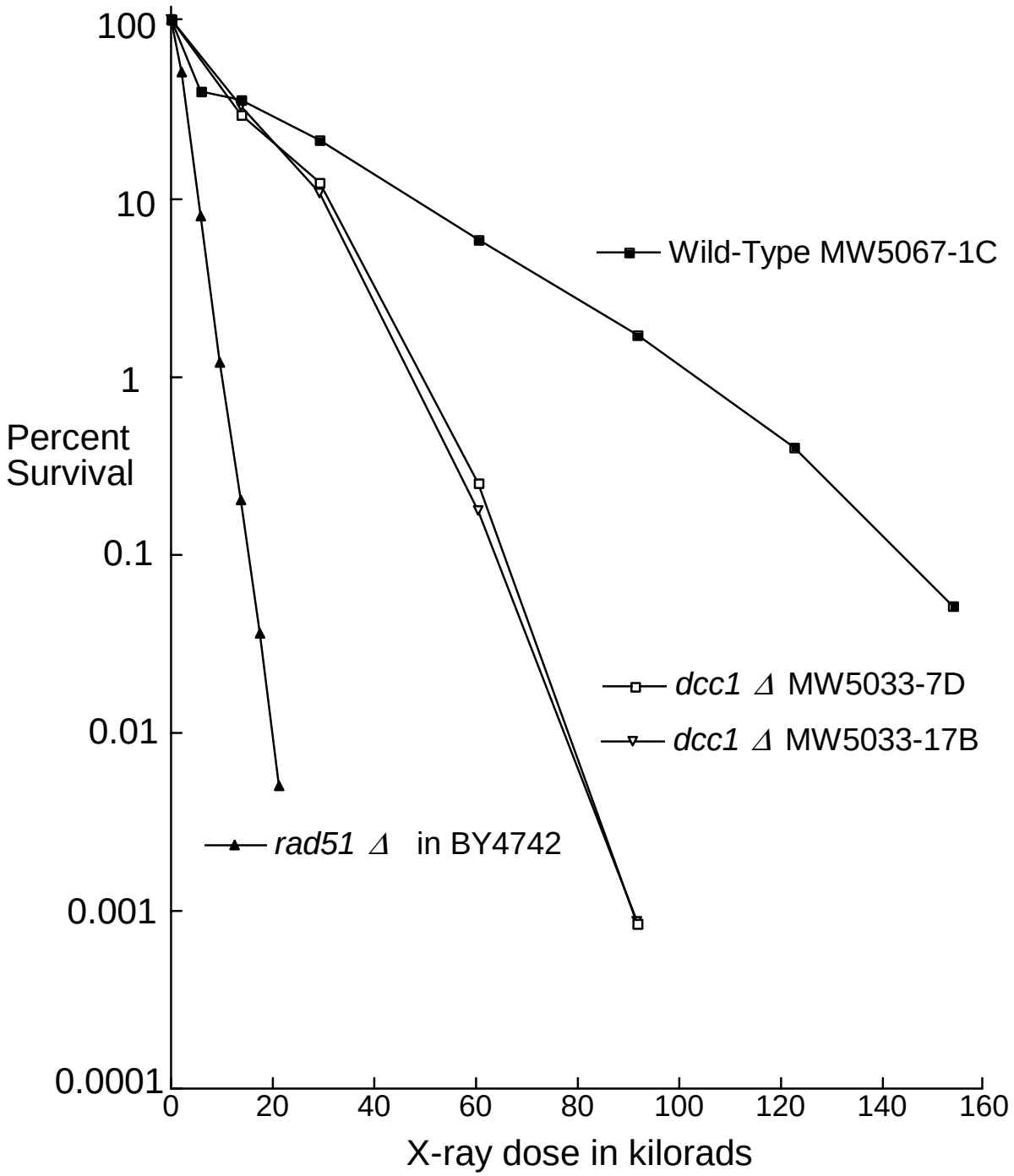
100
10
1
0.1
0.01
0.001
0.0001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
Wild-Type MW5067-1C
dcc1 Δ MW5033-7D
dcc1 Δ MW5033-17B
rad51 Δ in BY4742

Figure 7

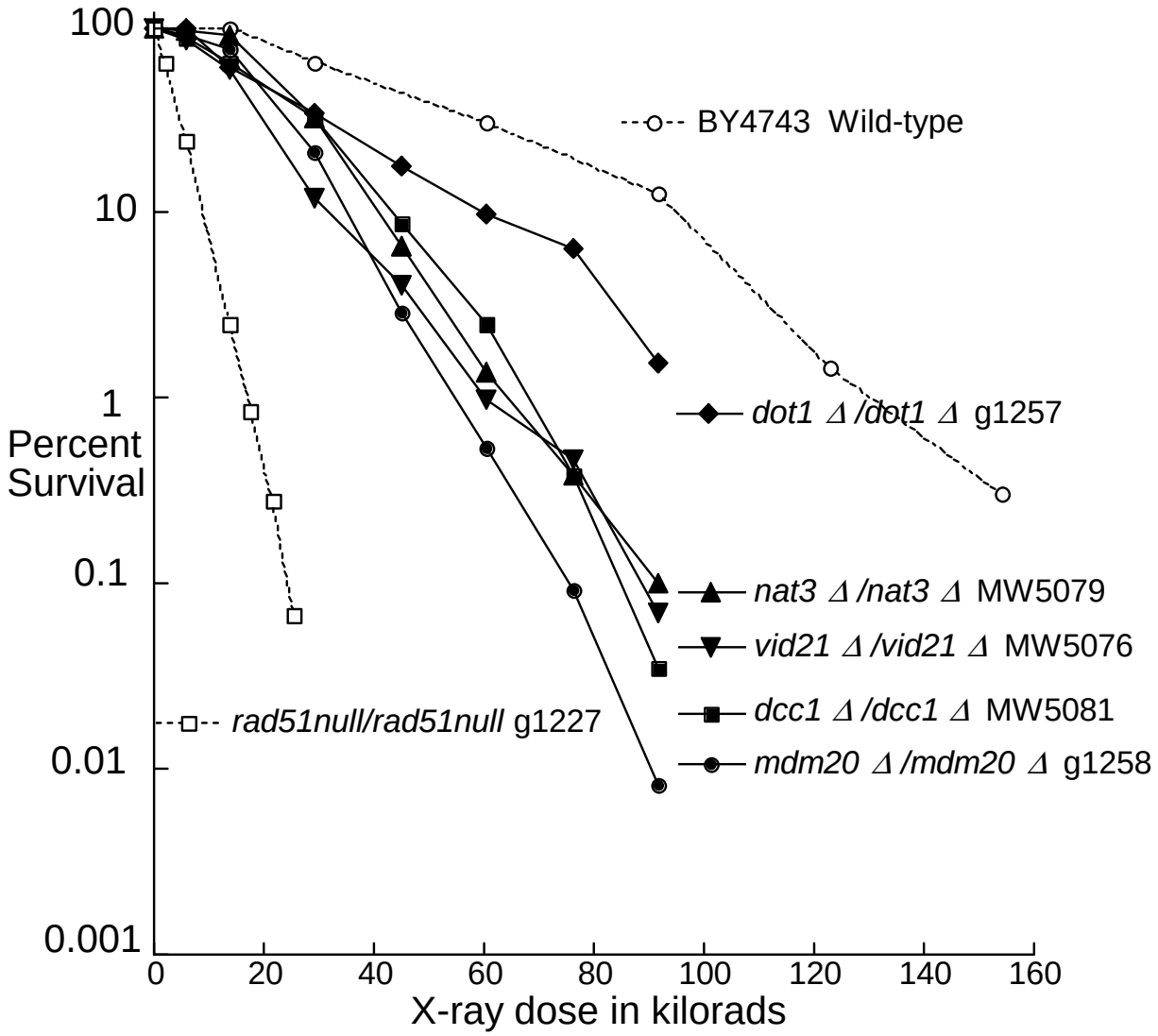
100
10
1
0.1
0.01
0.001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
BY4743 Wild-type
dot1 Δ /dot1 Δ g1257
nat3 Δ /nat3 Δ MW5079
vid21 Δ /vid21 Δ MW5076
dcc1 Δ /dcc1 Δ MW5081
mdm20 Δ /mdm20 Δ g1258
rad51null/rad51null g1227

Figure 8

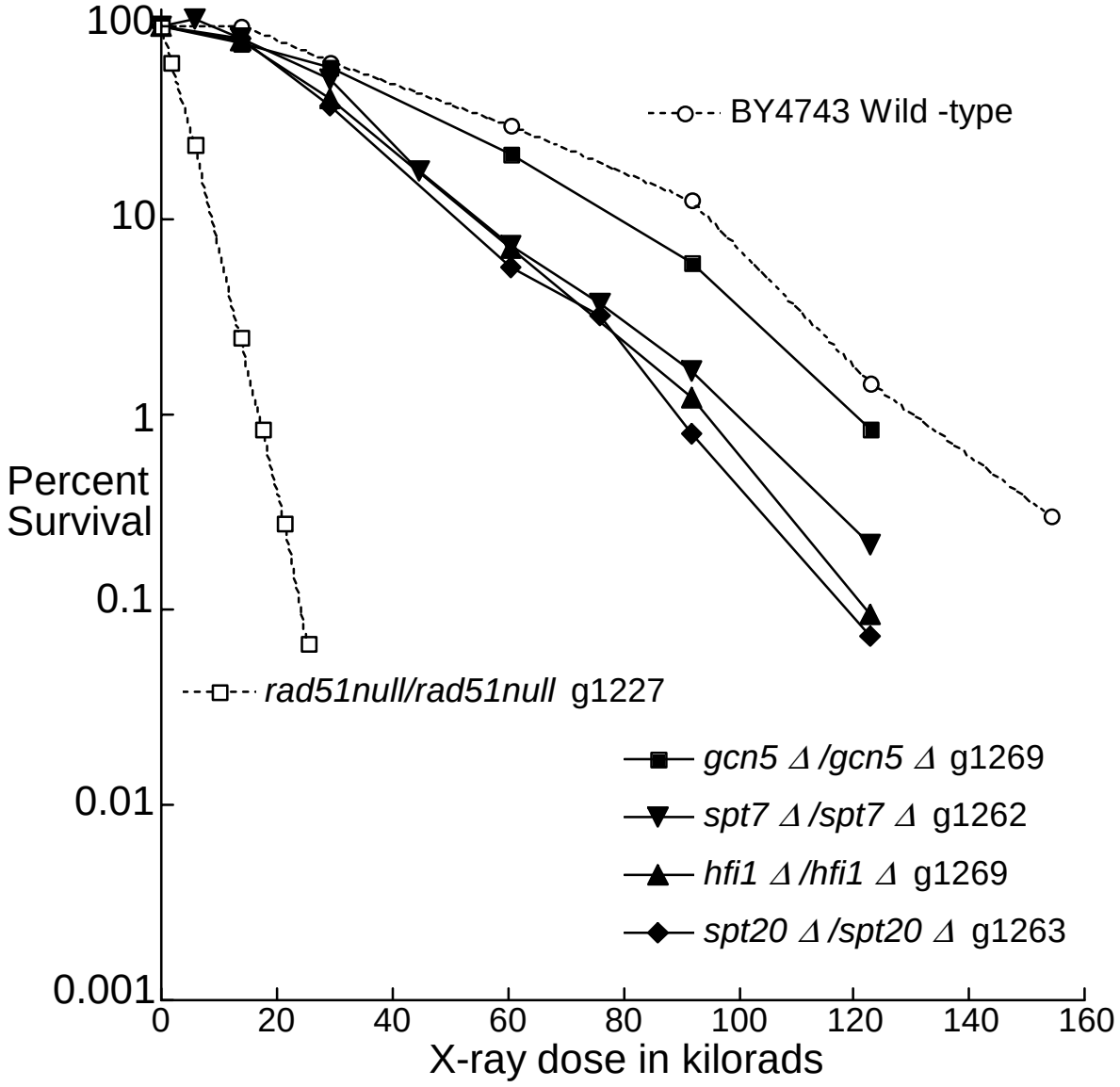
100
10
1
0.1
0.01
0.001
Percent Survival
0
20
40
60
80
100
120
140
160
X-ray dose in kilorads
BY4743 Wild -type
rad51null/rad51null g1227
gcn5 Δ /gcn5 Δ g1269
spt7 Δ /spt7 Δ g1262
hfi1 Δ /hfi1 Δ g1269
spt20 Δ /spt20 Δ g1263

Figure 9

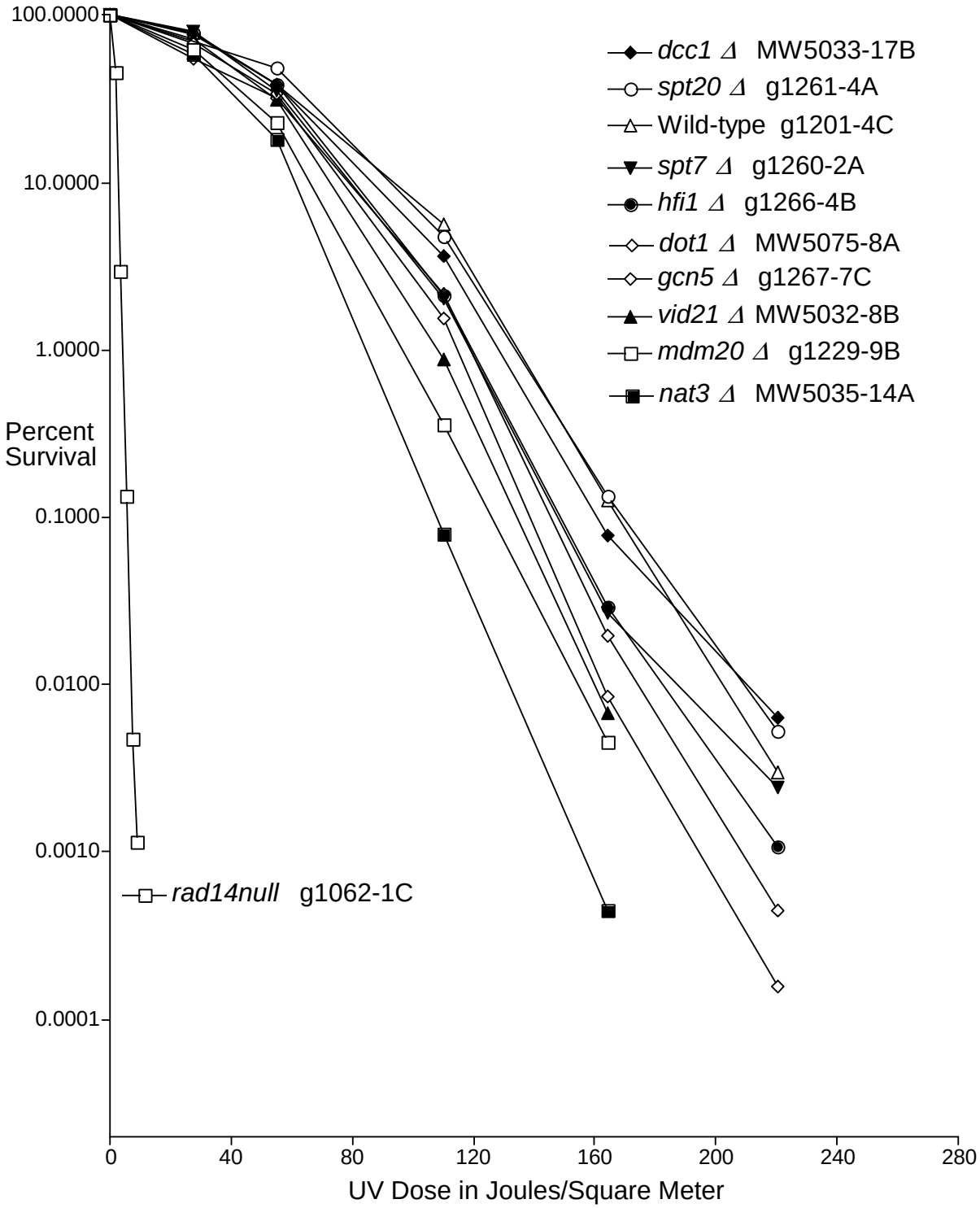
100.0000
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
Percent Survival
0
40
80
120
160
200
240
280
UV Dose in Joules/Square Meter
dcc1 Δ MW5033-17B
spt20 Δ g1261-4A
Wild-type g1201-4C
spt7 Δ g1260-2A
hfi1 Δ g1266-4B
dot1 Δ MW5075-8A
gcn5 Δ g1267-7C
vid21 Δ MW5032-8B
mdm20 Δ g1229-9B
nat3 Δ MW5035-14A
rad14null g1062-1C

Figure 10