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Supplemental Data

Differential Regulation of the Cellular Response to DNA Double-Strand Breaks in G1

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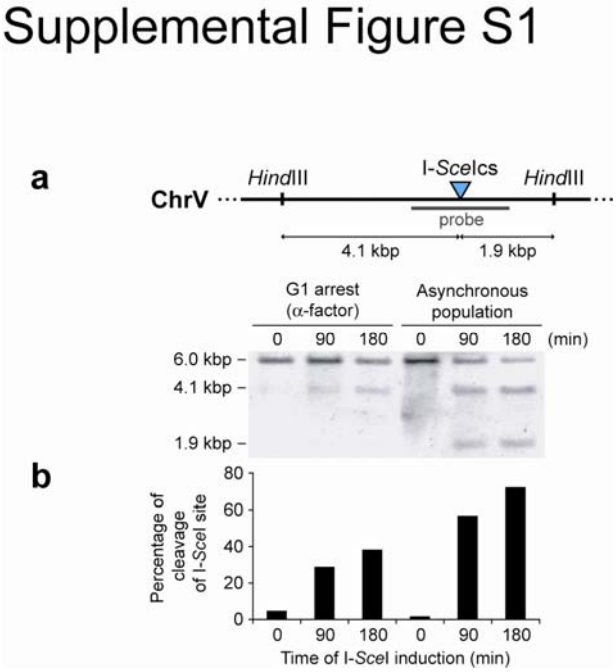

Figure S1. Quantitation of I-SceI cutting efficiency

(a) Genomic blot analysis of I-SceI cutting. The I-SceI endonuclease was induced in cells arrested by α-factor in G1 or in asynchronously growing cells (strain W4365-5B).

(b) Quantitation of genomic blot. Based on image densitometry using Openlab (Improvision), the I-SceI endonuclease cleaves approximately twice as efficient (70%) in asynchronously growing cells than in G1-arrested cells.

Rothstein Supplemental Figure S2

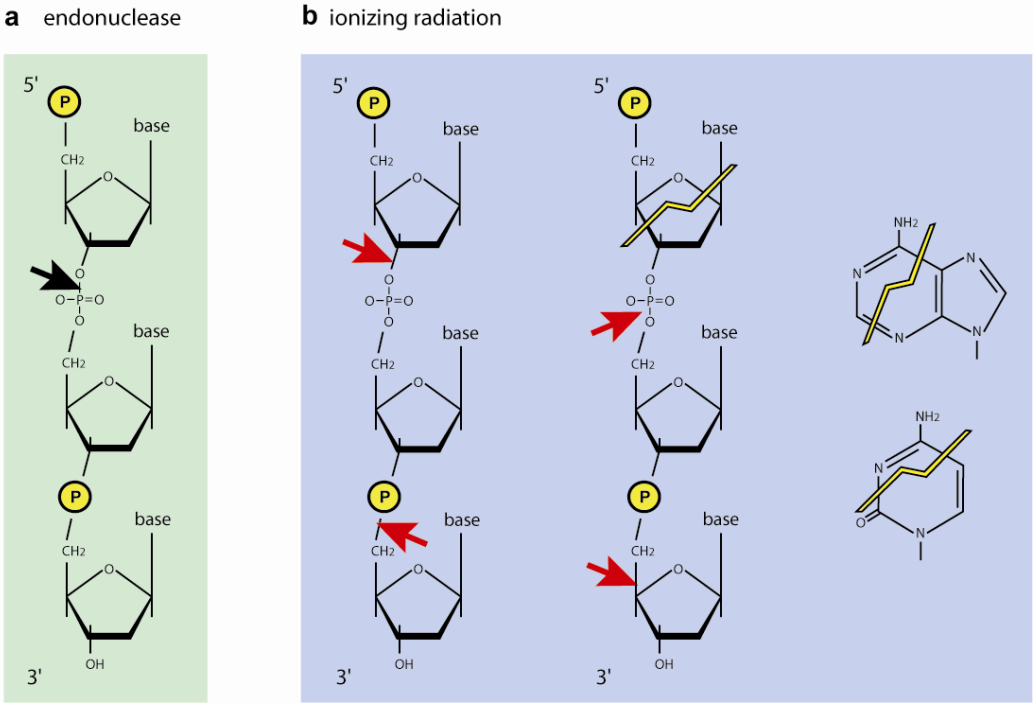

Figure S2. Points of damage on the DNA chain caused by I-SceI and IR

(a) Panel (a) shows the cleavage point on the phosphate backbone where I-SceI and other endonucleases cleave.

(b) Panel (b) enumerates the multiple sites along the DNA chain where IR may break the DNA. Black arrows show points where the DNA may be repaired by NHEJ while red arrows indicate breaks in the DNA chain that require HR.

Rothstein Supplemental Figure S3

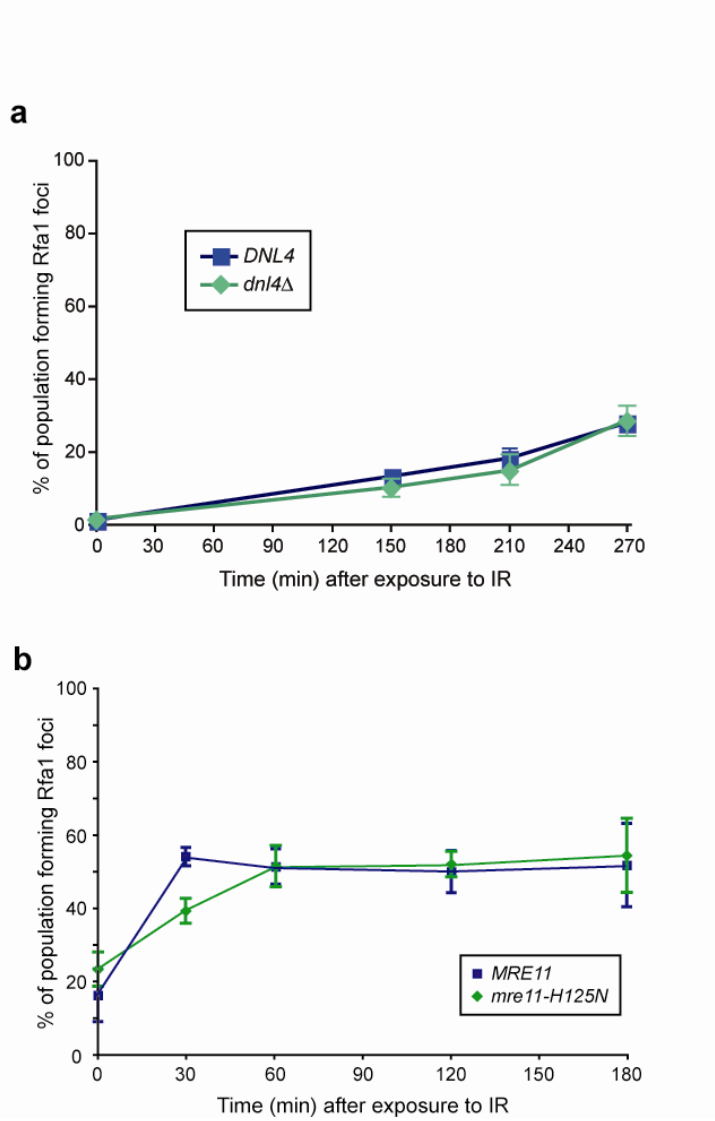

Figure S3. Regulation of Rfa1 focus formation

(a) Rfa1 focus formation in *dnl4*Δ cells. Deletion of Dnl4 does not lead to increased Rfa1 focus formation in G1 cells.

(b) Rfa1 focus formation in *mre11-H125N* cells. Cells were arrested in G1 then exposed to IR. The abrogation of Mre11 nuclease activity, exhibited in the *mre11-H125N* mutant strain (W6028-1B), has no significant effect on Rfa1 focus formation, except at the 30-minute time point, where *mre11-H125N* cells show a small but significant decrease (paired t-test, P-value < 0.05).

Supplemental figure S4

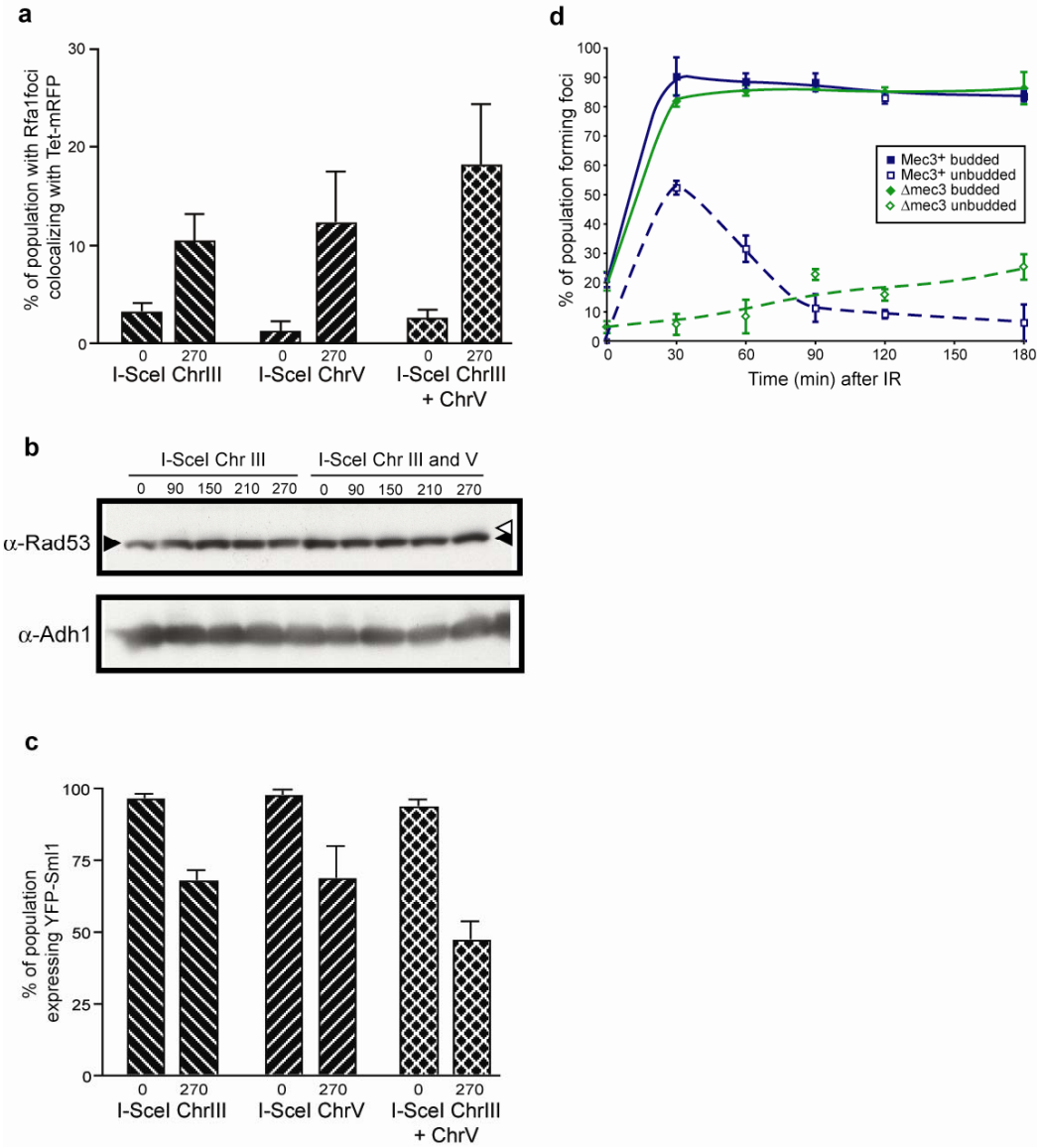

Figure S4. The DNA damage response is not significantly elevated in cells with two vs. one I-SceI cut site in G1.

(a) Rfa1 focus formation in response to one or two I-SceI cuts in G1. Cells containing two vs. one I-SceI cut sites in G1 do not induce significantly higher levels of Rfa1 foci after addition of galactose (15-20% in response to one DSB vs. ~20-25% in cells with two I-SceI cut sites).

(b) Rad53 phosphorylation in response to one or two I-SceI cuts in G1. Rad53 phosphorylation is not detected in G1 cells in response to galactose induction of the I-SceI enzyme, regardless of whether they contain either one or two I-SceI cut sites. (c) Sml1 degradation in response to one or two I-SceI cuts in G1. Cells were analyzed for the expression of YFP-Sml1 after induction of I-SceI. Cells containing two I-SceI cut sites (W7630-19C) have a higher percentage of cells that have degraded YFP-Sml1 after galactose induction than cells with only one I-SceI cut site (W7542-11D and W7630-2A). (d) Ddc2 focus formation in asynchronous *mec3*Δ cells. After exposure to 40 Gy IR, budded *mec3*Δ cells form Ddc2 foci, while the unbudded G1 population does not.

Table S1

Strains

W3483-10A	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 MRE11-YFP RAD5*
W3775-12C	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 RFA1-YFP RAD5*
W3749-14C	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 RAD52-YFP RAD5*
W3792-4B	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 DDC2-YFP RAD5*
W3923-12B	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 DDC1-YFP RAD5*
W4362-1C	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD5 MRE11-YFP ura3::3xURA3-TetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W4363-4B	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD5 MRE11-YFP ura3::3xURA3-TetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W4364-9B	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD5 DDC2-YFP ura3::3xURA3-TetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W4365-5B	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD5 RAD52-YFP ura3::3xURA3-TetOx112 I-SceI-cs(ura3-1) TetI-mRFP1(iYGL119W)*
W4965-8B	*MAT***a** *ADE2 trp1-1 LYS2 RAD52-YFP RAD5*
W4688-11D	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD5 DDC1-YFP ura3::3xURA3-TetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W5071-5D	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 RFA1-YFP RAD5 sae2::KanMX*
W5358-9A	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 DDC2-YFP mec3::URA3 RAD5*
W5713-16D	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD52-CFP RFA1-YFP RAD5 yku70Δ ura3::3xURA3-tetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W5713-18A	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 RAD52-CFP RFA1-YFP RAD5 ura3::3xURA3-tetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W5793-10B	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 RFA1-YFP RAD5 mec3::URA3*
W5872-3C	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 rfa1-t11 DDC1-YFP*
W5873-9B	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 rfa1-t11 DDC2-YFP*

W6028-1B	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 mre11-H125N RFA1-YFP RAD5*
W7542-11D	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 lys2*Δ *YFP-SML1 RFA1-CFP RAD5 ura3::3xURA3-tetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W)*
W7630-2A	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 LYS2 YFP-SML1 RFA1-CFP RAD5 TetR-mRFP1(iYGL119W) I-SceI-cs(iYCL054W) URA3::tetOx224(iYCL055W)*
W7630-19C	*MAT***a** *ade2-1 bar1::LEU2 trp1-1 lys2*Δ *YFP-SML1 RFA1-CFP RAD5 ura3::3xURA3-tetOx112 I-SceI-cs(ura3-1) TetR-mRFP1(iYGL119W) I-SceI-cs(iYCL054W) URA3::tetOx224(iYCL055W)*
W7832-1A	*MAT***a** *ADE2 bar1::LEU2 trp1-1 LYS2 mec3::KanMX DDC2-YFP cdc28-as1::URA3*
W7832-2A	*MAT***a** *ADE2 trp1-1 LYS2 DDC2-YFP cdc28-as1::URA3*
W7848-7A	*MAT***a** *mec3::KanMX rfa1-t11 cdc28-as1 DDC2-YFP*
W7848-9B	*MAT***a** *rfa1-t11 cdc28-as1 DDC2-YFP*
W8127-21B	*MAT***a** *ade2-1 LYS2 leu2::LEU2-GAL-hENT1 trp1::TRP1-GAL-dNK cdc21::KanMX RFA1-CFP*