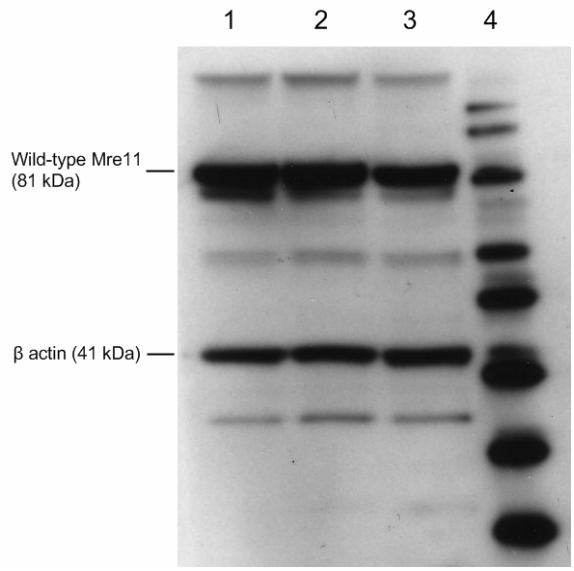
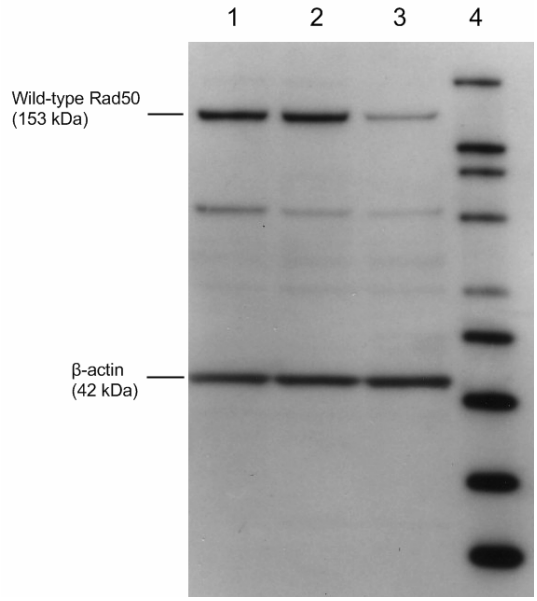
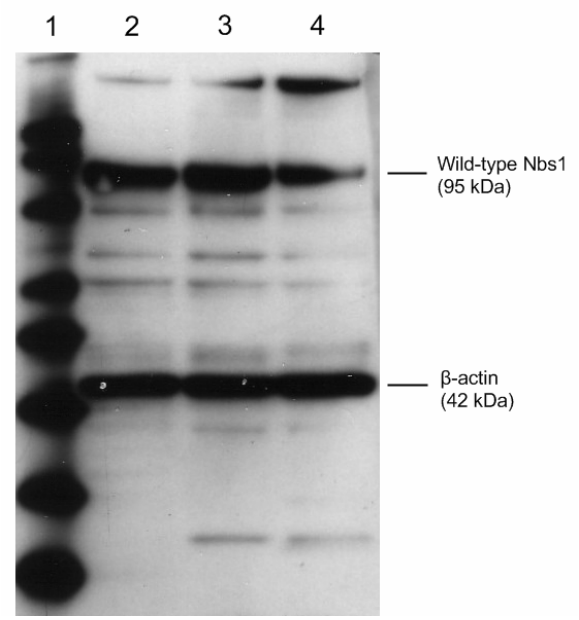


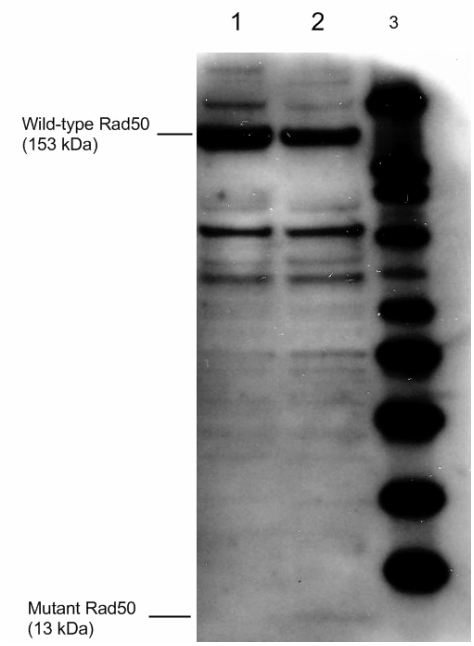
Supplemental Figure 1. Endogenous expression of Mre11 in uninfected JHU029 and JHU012 cells, as well as JHU012 cells infected with Ad-Rad50. Lane 1: JHU029 cells; Lane 2: JHU012 cells; Lane 3: JHU012 cells infected with Ad-Rad50; and Lane 4: MagicMark Western Protein Standard (Invitrogen, Cat. #LC5602): from bottom to top - 20, 30, 40, 50, 60, 80, 100, 120 kDa.



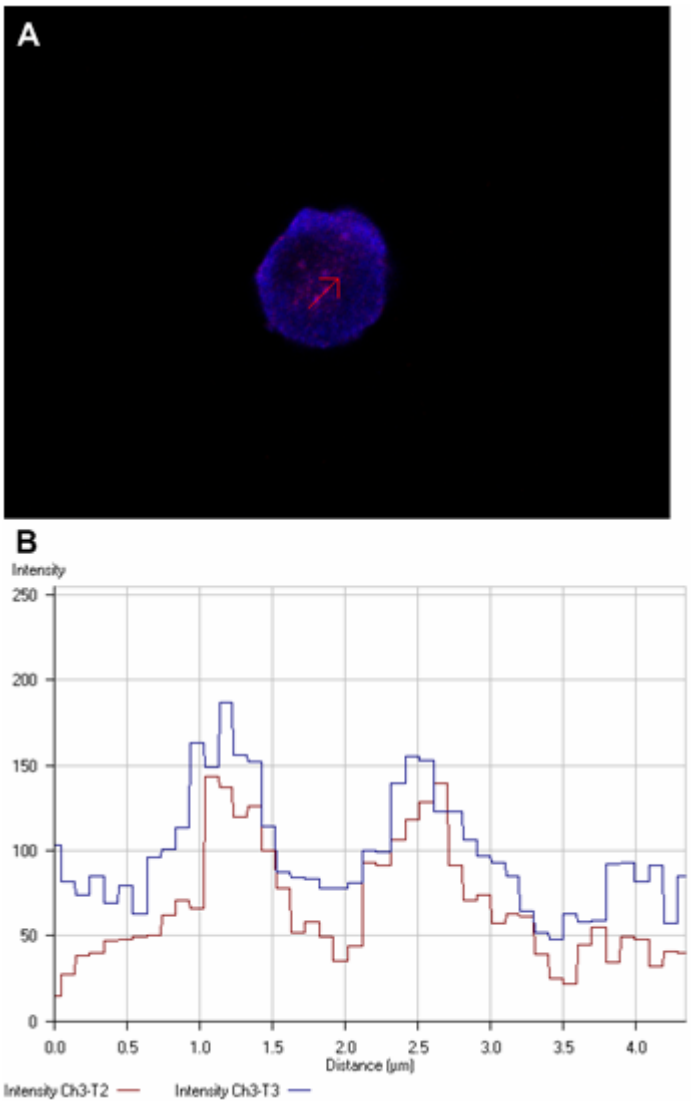
Supplemental Figure 2. Endogenous expression of Rad50 in uninfected JHU029 and JHU012 cells, as well as JHU012 cells infected with Ad-Rad50. Lane 1: JHU029 cells; Lane 2: JHU012 cells; Lane 3: JHU012 cells infected with Ad-Rad50; and Lane 4: MagicMark Western Protein Standard: From bottom to top - 20, 30, 40, 50, 60, 80, 100, 120, 220 kDa.



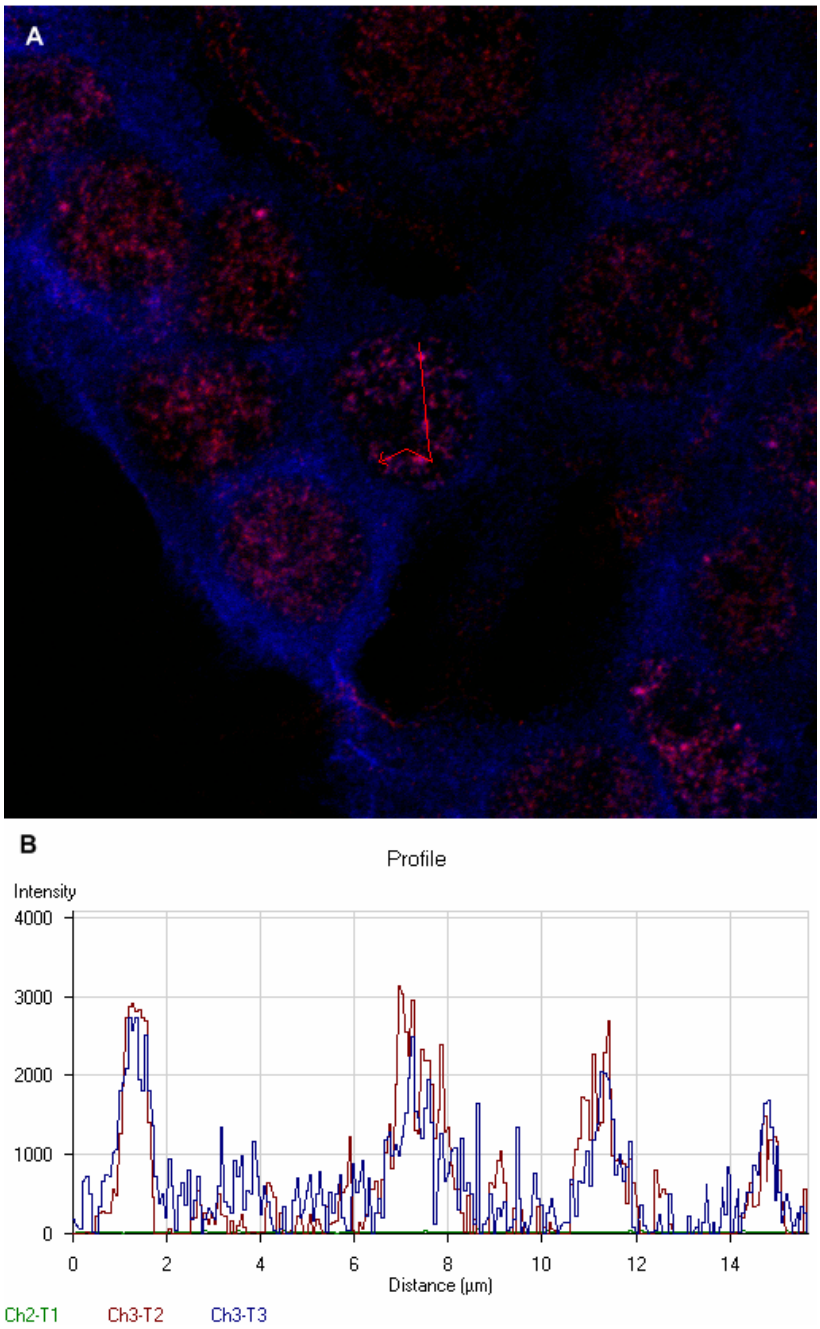
Supplemental Figure 3. Endogenous expression of Nbs1 in uninfected JHU029 and JHU012 cells, as well as JHU012 cells infected with Ad-Rad50. Lane 1: MagicMark Western Protein Standard: From bottom to top - 20, 30, 40, 50, 60, 80, 100, 120, 220 kDa; Lane 2: JHU029 cells; Lane 3: JHU012 cells; and Lane 4: JHU012 cells infected with Ad-Rad50.



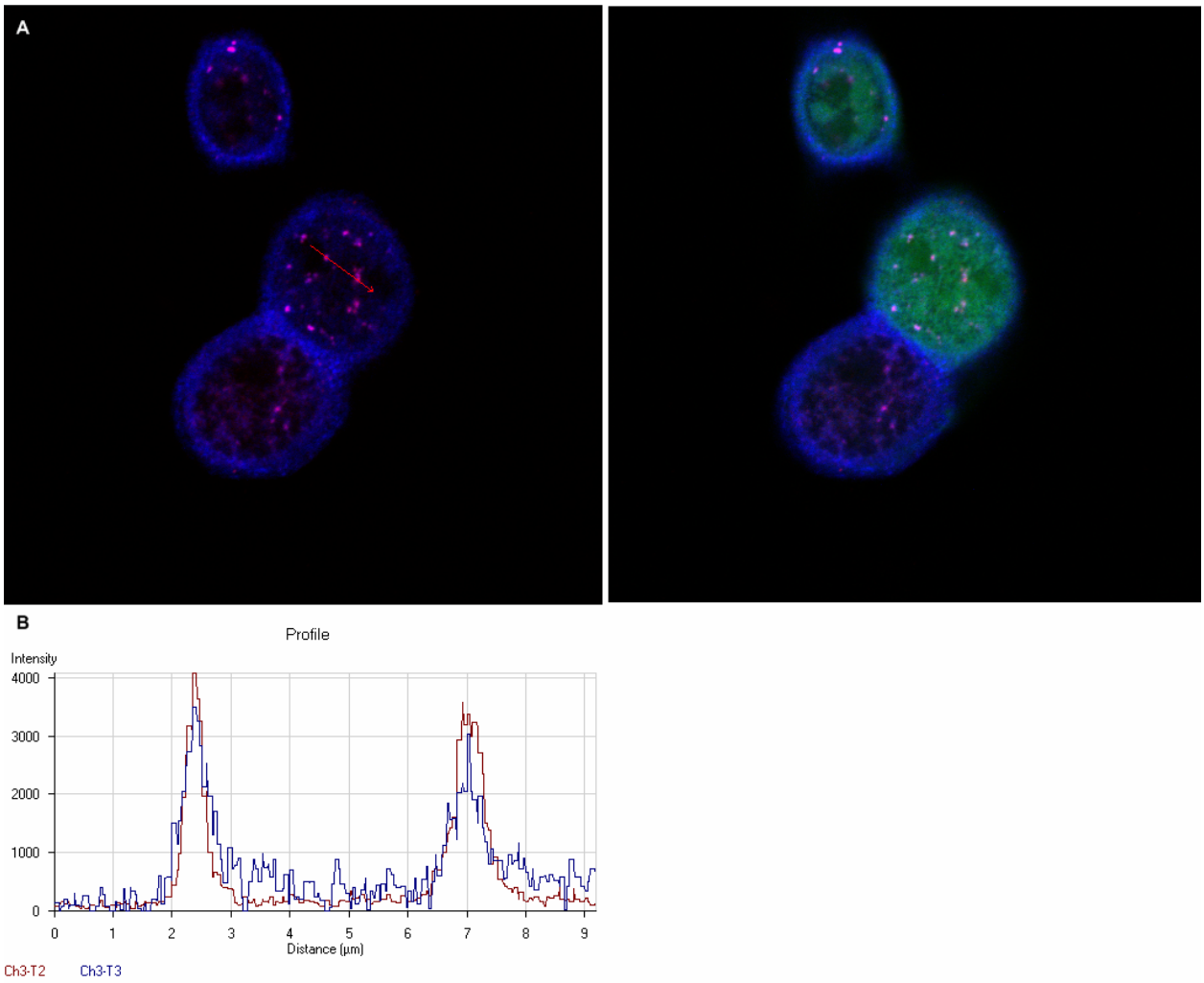
Supplemental Figure 4. Lane 1: JHU012 cells with no treatment; Lane 2: JHU012 cells infected with Ad-Rad50; and Lane 3: MagicMark Western Protein Standard: From bottom to top-20, 30, 40, 50, 60, 80, 100, 120, 220 kDa.



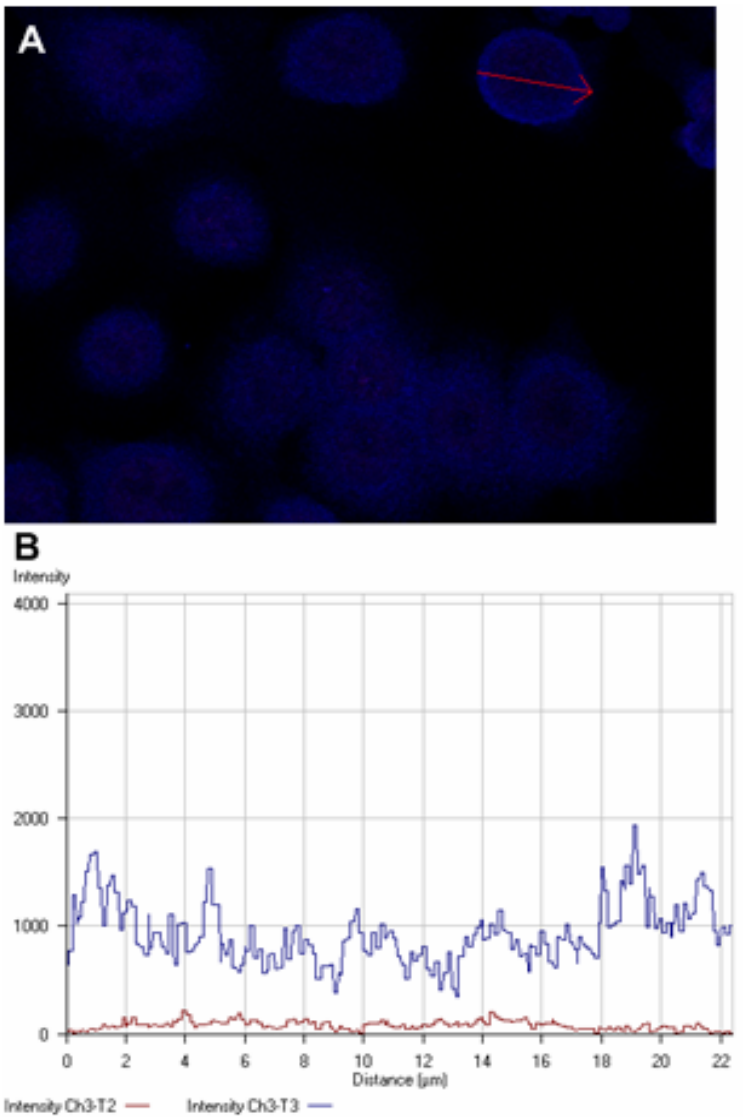
Supplemental Figure 5. (A) A single nucleus from a control JHU012 cell in which wild-type Rad50 and wild-type Mre11 proteins have been labeled with two fluorochromes that have been excited and viewed with a laser scanning confocal microscope at 63x magnification. Nuclear foci are evident. (B) Analysis of two nuclear foci reveals a close spatial relationship of the wild-type Rad50 (blue) and wild-type Mre11 (red) which is consistent with physical interaction of these two proteins.



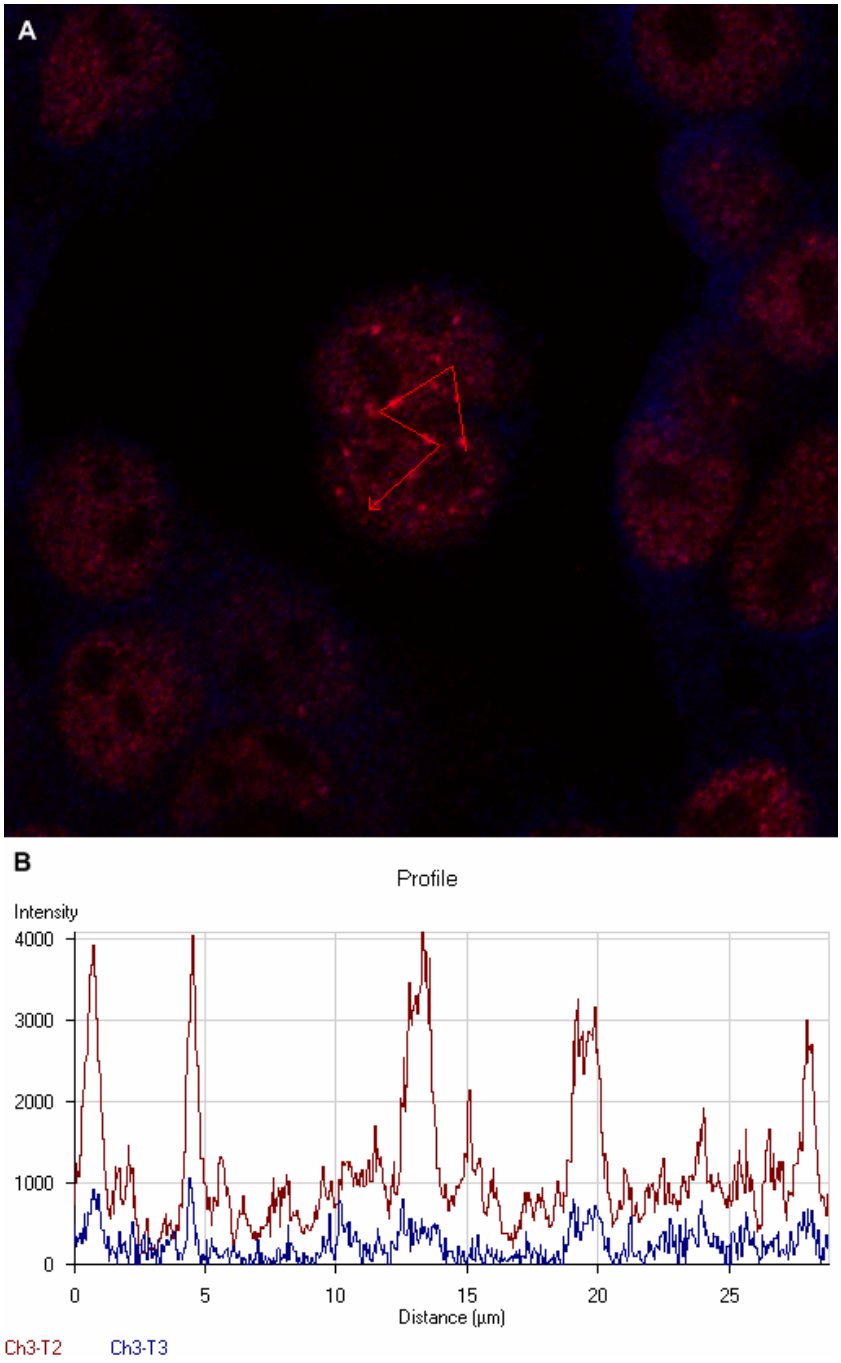
Supplemental Figure 6. (A) Nuclei from cisplatin-treated JHU012 cells in which wild-type Rad50 and wild-type Mre11 proteins have been labeled with two fluorochromes that have been excited and viewed with a laser scanning confocal microscope at 63x magnification. The DNA damage resulting from cisplatin resistance has induced an obvious increase in nuclear foci compared to controls (see Supplemental Figure 6). (B) Analysis of four nuclear foci reveals a close spatial relationship of the wild-type Rad50 (red) and wild-type Mre11 (blue) which suggests binding between these proteins.



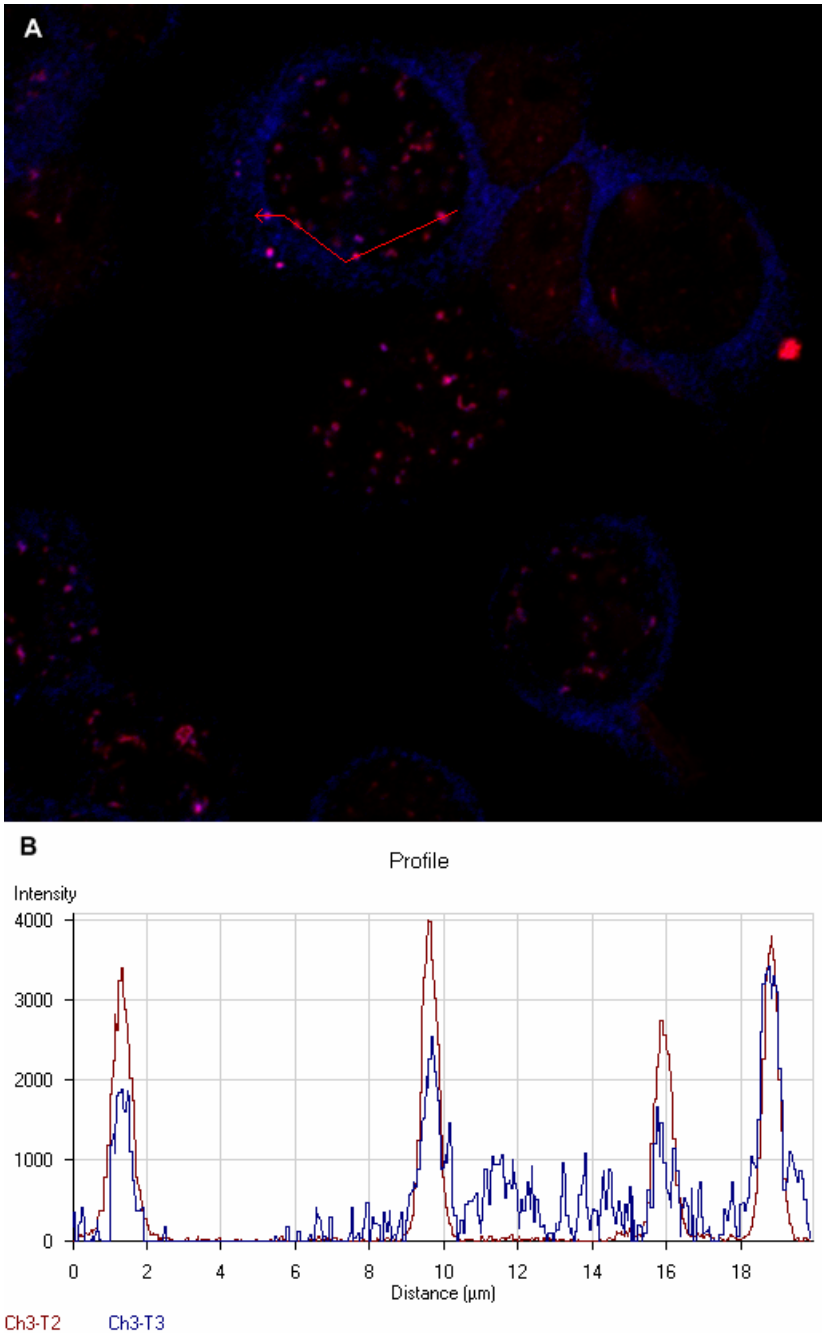
Supplemental Figure 7. (A) Three nuclei from Ad-Rad50 infected JHU012 cells in which wild-type Rad50 and wild-type Mre11 proteins have been labeled with two fluorochromes that have been excited and viewed with a laser scanning confocal microscope at 63x magnification. The nuclear foci representing MRN complex assembly at sites of DNA damage are clearly evident. Note the panel on the right which demonstrates the same cells following excitation and detection of the intra-nuclear GFP signal. This provided further confirmation that the nucleus under analysis had been infected with the Ad-Rad50 virus which includes a GFP marker. Co-localization analysis was only undertaken in those cells in which positive GFP expression and, hence, Ad-Rad50 infection was clearly evident. For simplicity, the GFP channel is not shown in the figures below. (B) Analysis of two nuclear foci reveals a close spatial relationship of the wild-type Rad50 (red) and wild-type Mre11 (blue) which is consistent with physical interaction of these two proteins.



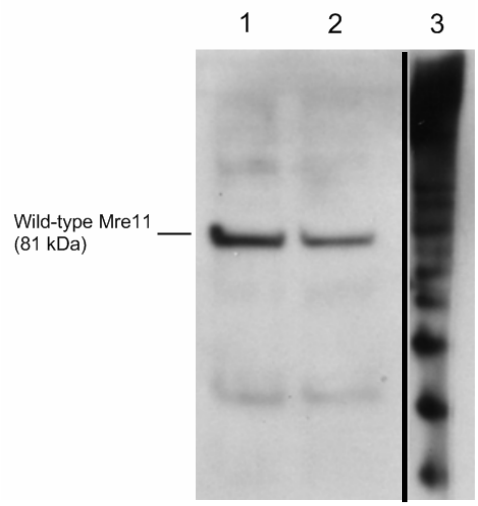
Supplemental Figure 8. (A) Multiple nuclei from a control JHU012 cell in which wild-type Rad50 and mutant Rad50 proteins have been labeled with two fluorochromes that have been excited and viewed with a laser scanning confocal microscope at 63x magnification. (B) Analysis of the nuclear signals indicates the presence of only the wild-type Rad50 protein (blue), with no signal arising from mutant Rad50 (red) evident above background noise. This is expected as these cells have not been infected with Ad-Rad50.



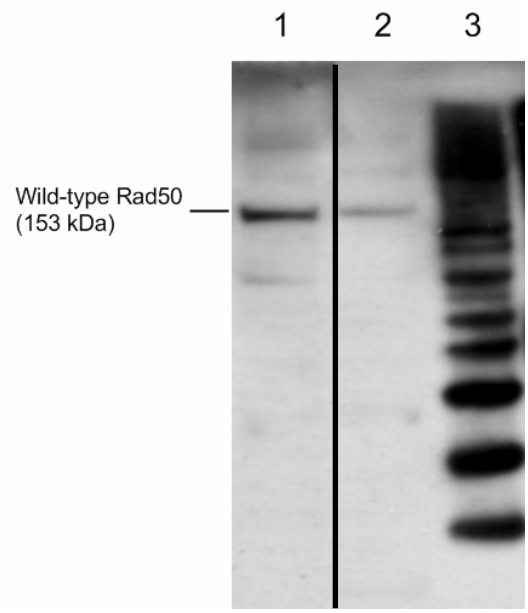
Supplemental Figure 9. (A) Multiple nuclei from cisplatin-treated JHU012 cells in which wild-type Rad50 and mutant Rad50 proteins have been labeled with two fluorochromes that have been excited and viewed with a laser scanning confocal microscope at 63x magnification. (B) Analysis of multiple foci indicates the presence of only the wild-type Rad50 protein (red) with markedly lower levels of mutant Rad50 (blue) since these cells have not been infected with Ad-Rad50.



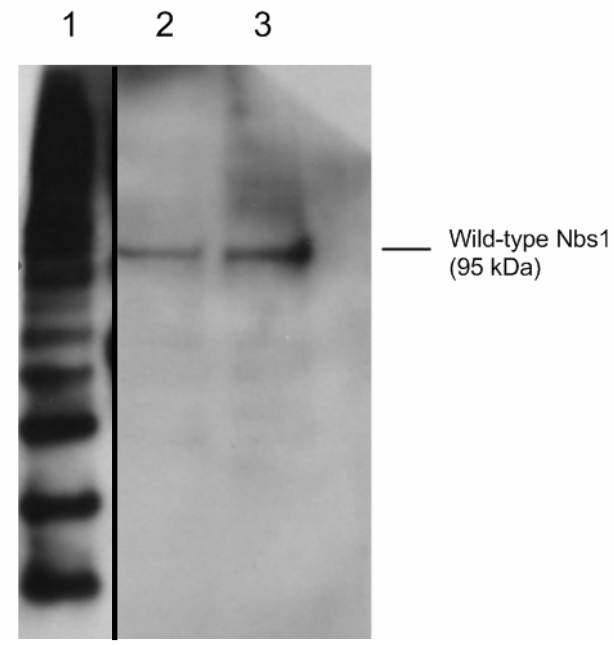
Supplemental Figure 10. (A) Multiple nuclei from Ad-Rad50 infected JHU012 cells in which wild-type Rad50 and mutant Rad50 proteins have been labeled with two fluorochromes that have been excited and viewed with a laser scanning confocal microscope at 63x magnification. (B) Analysis of multiple foci indicates co-localization of the wild-type (red) and mutant Rad50 (blue) proteins. The remarkably close spatial location of these two signals is highly suggestive of a physical binding interaction between mutant Rad50 and wild-type Rad50. This suggests that the dimerization function of the Rad50 hook is intact in the mutant Rad50 construct.



Supplemental Figure 11. Western blot - Mre11 probe representative gel. Lane 1: JHU012 cells with no treatment; Lane 2: JHU012 cells infected with Ad-Rad50; Lane 3: MagicMark Western Protein Standard: From bottom to top-20, 30, 40, 50, 60, 80, 100, and 120 kDa. Lanes were run on the same gel but were non-contiguous as indicated by the black line.



Supplemental Figure 12. Western blot - Rad50 probe representative gel. Lane 1: JHU012 cells with no treatment; Lane 2: JHU012 cells infected with Ad-Rad50; Lane 3: MagicMark Western Protein Standard: From bottom to top - 20, 30, 40, 50, 60, 80, 100, 120, 220 kDa. Lanes were run on the same gel but were non-contiguous as indicated by the black line.



Supplemental Figure 13. Western blot - Nbs1 probe representative gel. Lane 1: MagicMark Western Protein Standard: From bottom to top-20, 30, 40, 50, 60, 80, 100 kDa; Lane 2: JHU012 cells infected with Ad-Rad50; Lane 3: JHU012 cells with no treatment. Lanes were run on the same gel but were non-contiguous as indicated by the black line.