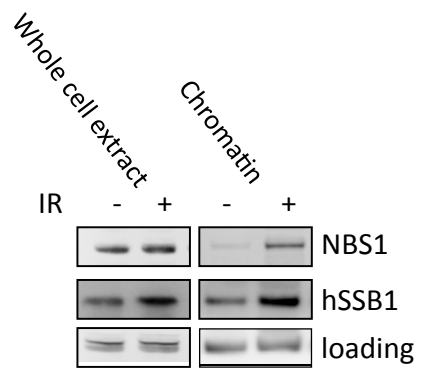
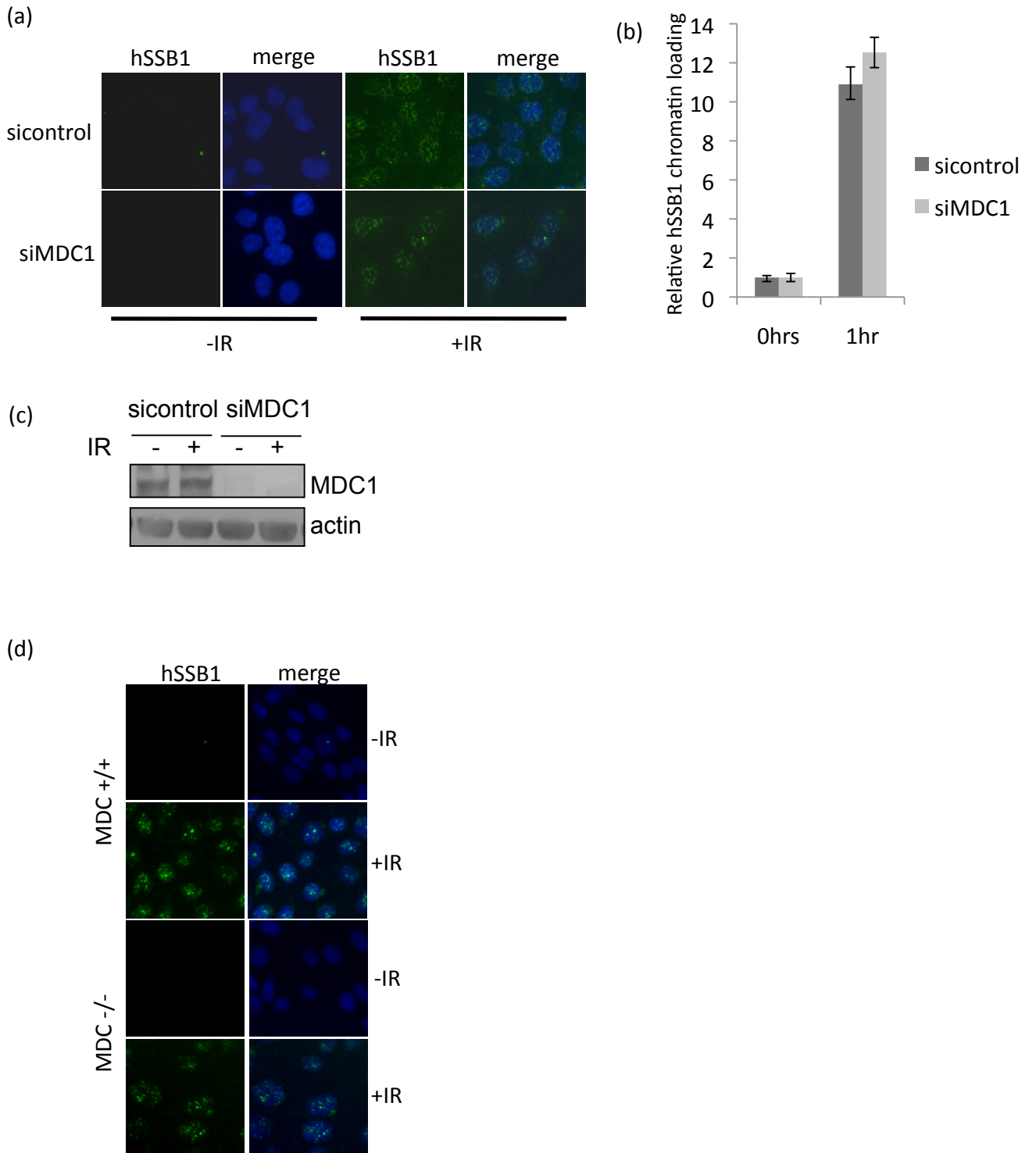


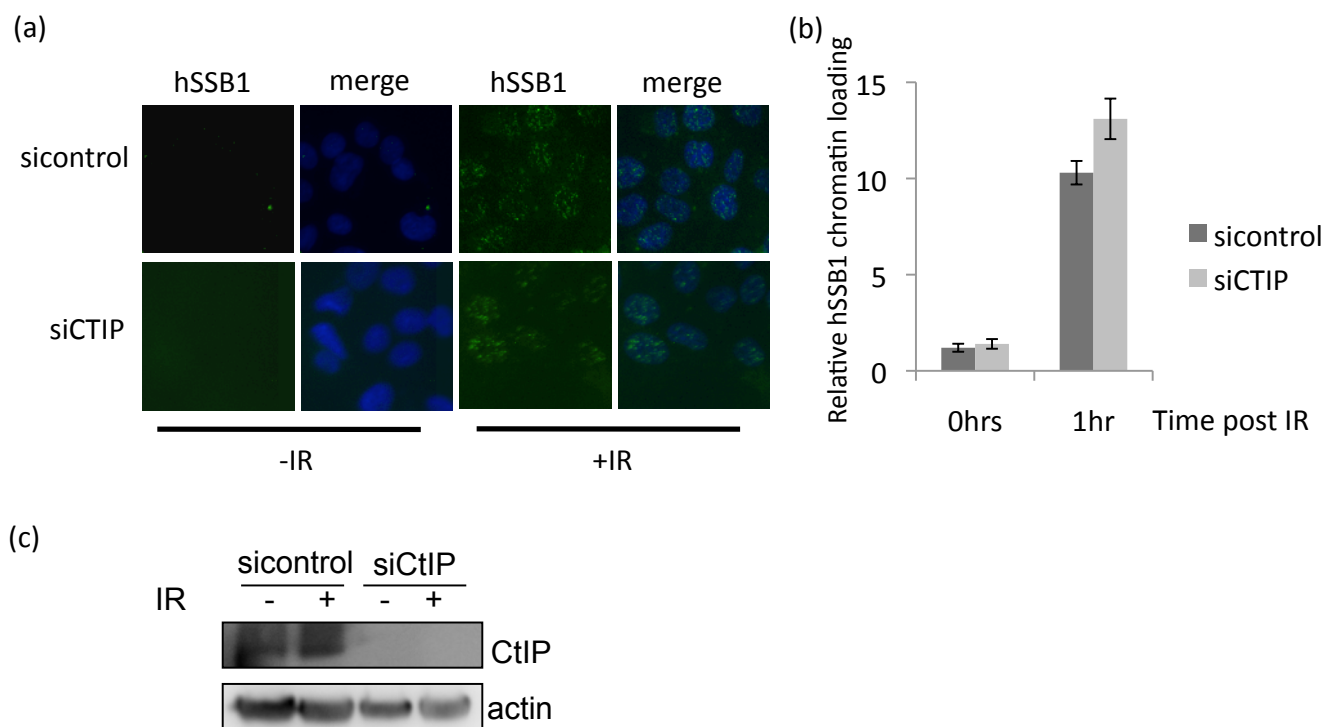
Supplementary Figure 1



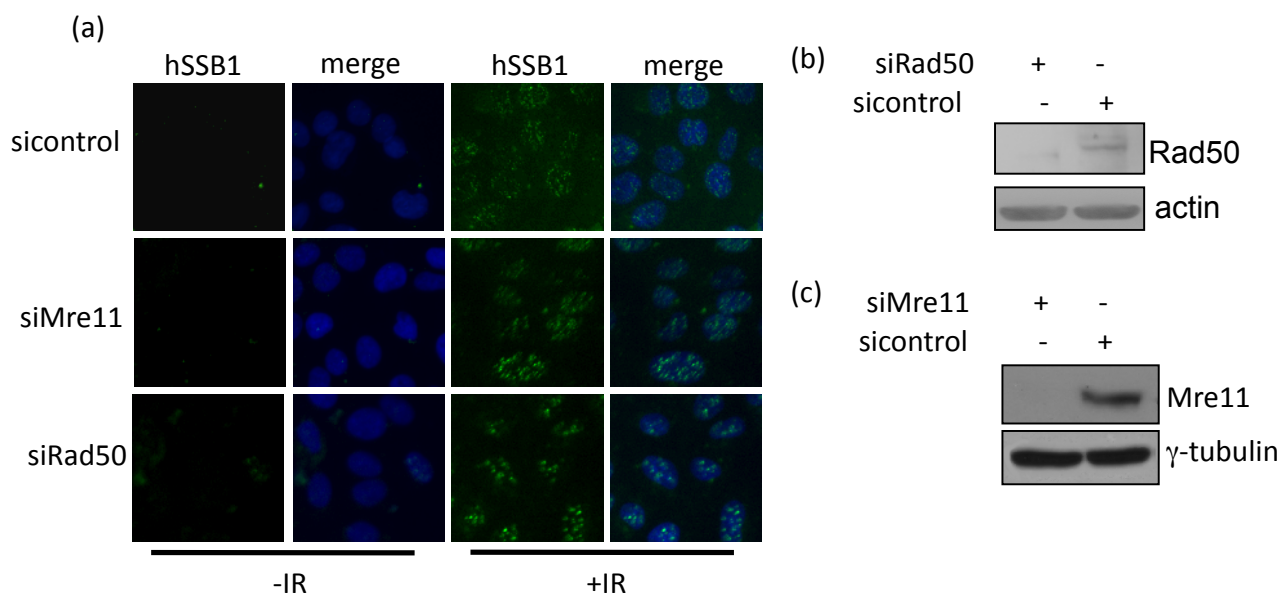
Supplementary Figure 2



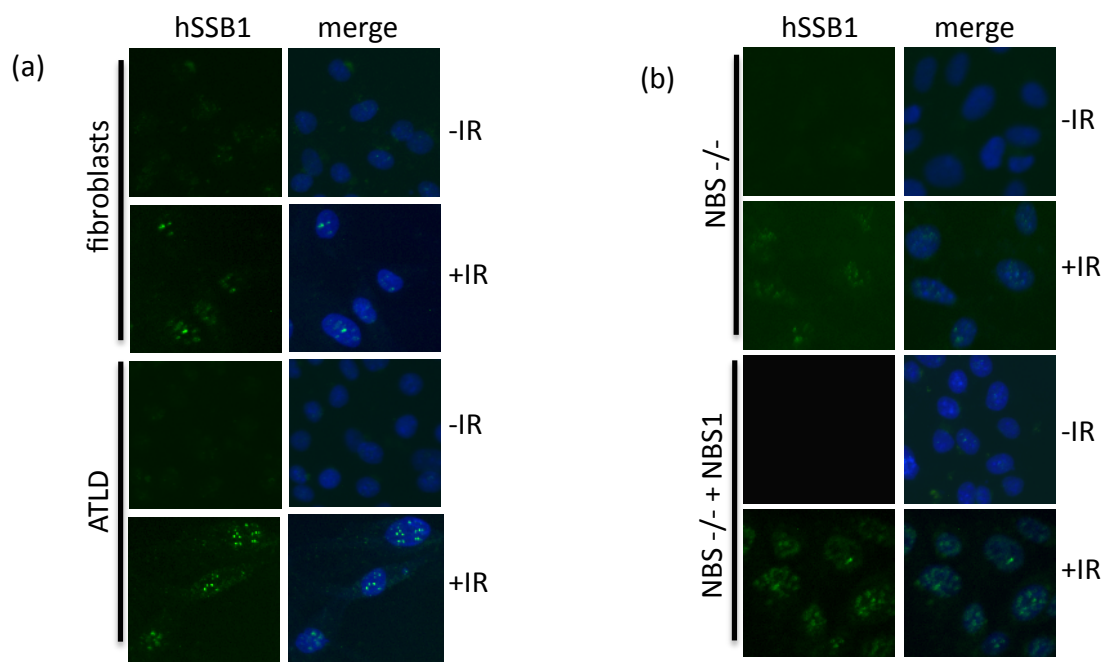
Supplementary Figure 3



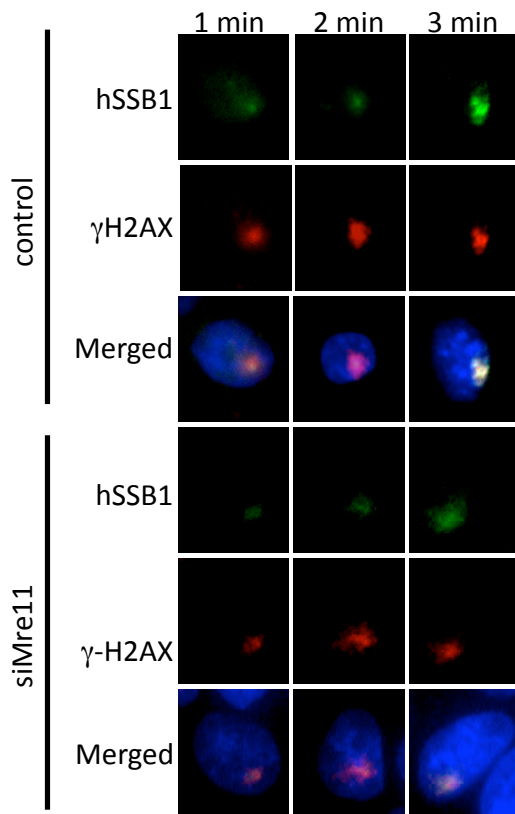
Supplementary Figure 4



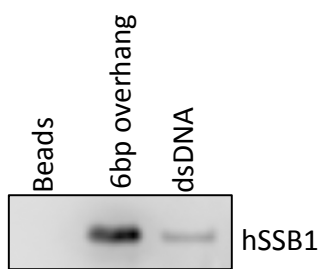
Supplementary Figure 5



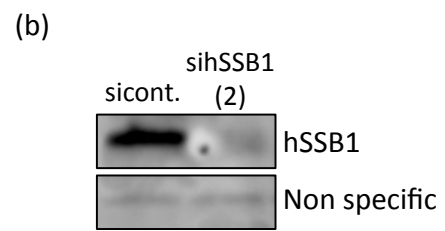
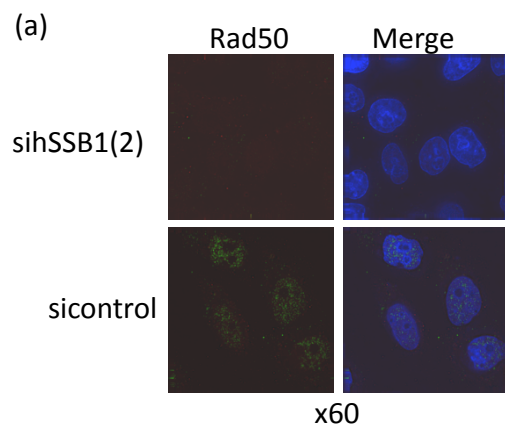
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Chromatin fractionation. hSSB1 and NBS1 loading onto chromatin following IR treatment, of U2OS cells.

Supplementary Figure 2. MDC1 is not required for hSSB1 foci formation or chromatin loading. (a) U2OS cells depleted of MDC1 by siRNA (48 hours) and stained for hSSB1 as indicated. (b) Quantification of chromatin loading using in-nuclear-western. (c) Immunoblot demonstrating effective depletion of MDC1 with target siRNA. (d) MDC1 deficient and wild type fibroblasts immuno-stained for hSSB1 as indicated. All images were taken on an x20 objective using an In-cell-2000 microscope (GE). +IR cells were irradiated with 2Gy IR and allowed to recover for 30 minutes. Error bars were calculated from the standard deviation.

Supplementary Figure 3. CtIP is not required for hSSB1 foci formation or chromatin loading. (a) U2OS cells depleted of CtIP by siRNA (48 hours) and stained for hSSB1 as indicated. (b) Quantification of chromatin loading using in-nuclear-western. (c) Immunoblot demonstrating effective depletion of MDC1 with target siRNA. All images were taken on an x20 objective using an In-cell-2000 microscope (GE). +IR cells were irradiated with 2Gy IR and allowed to recover for 30 minutes. Error bars were calculated from the standard deviation.

Supplementary Figure 4. MRN is not required for IR induced hSSB1 foci formation or chromatin loading. (a) U2OS cells were depleted of Mre11 or Rad50 by specific siRNA as indicated. Cells were immuno-stained 48hrs after for hSSB1 as indicated. +IR cells were then irradiated with 2Gy IR and allowed to recover for 30 minutes. All images were taken on an x20 objective using an In-cell-2000 microscope (GE). (b,c) Immunoblots demonstrating effective depletion of Rad50 and Mre11 by siRNA.

Supplementary Figure 5. MRN is not required for IR induced hSSB1 foci formation. (a,b) Deficiency of NBS1 and Mre11 does not impair hSSB1 foci formation. NBS1-deficient fibroblasts (ILB1) transfected with retroviral vector alone or full-length NBS1 cDNA and Mre11-deficient (ATLD) and control fibroblasts, were immunostained with anti-hSSB1 antibody 30 min after irradiation (6 Gy) or mock treated. Cells were treated with IR and allowed to recover for 1 hr prior to NP40 extraction, fixation and staining. All images were taken on an x20 objective using an In-cell-2000 microscope (GE).

Supplementary Figure 6. Mre11 is not required for the recruitment of hSSB1 to soft x-ray induced DSBs. MCF7 cells were treated with Mre11 specific siRNA 48 hrs prior to exposure to soft x-rays. Cells were fixed at indicated time points and immunostained as labeled.

Supplementary Figure 7. hSSB1 can bind to a 6bp DNA overhang. Immunoblot of a DNA pulldown assay demonstrating hSSB1 can bind to a 6bp overhang or a 34bp duplex DNA substrate.

Supplementary Figure 8. Defective IR-induced Rad50 foci formation in hSSB1- depleted cells. (a) MCF7 cells were depleted of hSSB1 using a specific siRNA (sihSSB1(2)). Rad50 foci formation was severely impaired. Images were captured on a Deltavision PDV, with a x60 objective. (b) Immunoblot showing levels of hSSB1 depletion using sihSSB1(2).