Supplementary Materials

Figure S1

The purification of TIP60 comlex .

(A) Immunoblot analysis using anti-NBS1, anti-MDC1, anti-TRRAP antibodies for TIP60 complex immunoaffinity purified from the nuclear soluble fraction of HeLa cells treated without (-) or with (+) 12 Gy IR, followed by a 5-min recovery. (B) Status of acetylation of K5-H2AX and Nterminal H4 in TIP60 or TIPM reconstituted cells where TIP60 were knocked down.

Figure S2.

TIP60 is required for accumulation of NBS1 on DNA damage sites.

(A) Example of quantification after micro-irradiation. Fluorescent signals were measured from the region from (i) micro-irradiated, (ii) un-irradiated nucleus or (ii) background. Values derived from (i)-(iii) or (i)-(ii) were stated as a fluorescent intensities from damaged or non-damaged area and plotted on the graphs. (B) Immunfluorescence analysis 1 hr after micro-irradiation using anti-γ-H2AX, anti-NBS1 or anti-MDC1 in Mock (n=11 for anti-NBS1; n=10 for anti-MDC1) or shTIP60 (n=9 for anti-NBS1; n=13 for anti-MDC1) RNA expressing MEFs. Graphs for quantified fluorescent intensities were shown. (C) Immunfluorescence analysis 4 hr after micro-irradiation, using anti-γ-H2AX, anti-NBS1 or anti-MDC1 in Mock (n=7 for anti-NBS1; n=16 for anti-MDC1) or shTIP60 (n=11 for anti-NBS1; n=10 for anti-MDC1) RNA expressing MEFs. The images (left) and the quantifications (right) were shown. (D) Immunfluorescence analysis 1 hr after micro-irradiation, using anti-γ-H2AX, anti-NBS1 or anti-MDC1 in eTIP60 (n=12 for anti-NBS1, n=7 for anti-MDC1) or eTIPM (n=12 for anti-NBS1, n=7 for anti-MDC1) reconstituted MEFs where

endogenous TIP60 was knocked down. Cell images (left) and quantifications (right) were shown. (E), (F) Immunofluorecence analysis using anti-γ-H2AX, anti-NBS1 or anti-MDC1 after IR exposure in Mock (left panel) or shTIP60 (right panel) RNA expressing cells were shown in (E). Percentages of the cells that presented more than 10 foci were plotted on the graph in (F). (G) 2 hrs after 2 Gys of IR exposure in Mock or shTIP60 RNA expressing MEFs. Percentages of the cells that presented more than 10 foci were plotted on the graph. (H) 2 hrs after 2 Gys of IR exposure in eTIP60 or eTIPM2 reconstituted MEFs where TIP60 were knocked down. Percentages of the cells that presented more than 10 foci were plotted on the graph. Graphs were shown with standard deviation. Bar 10μm.

Figure S3.

K5 acetylation of H2AX is required for accumulation of NBS1 on DNA damage sites.

(A) Localization of WT, K5R or S139A mutant versions of GFP tagged H2AXs on the chromatin. WT or mutant H2AX were reconstituted in H2AX KO MEFs and cells presented mitotic chromosomes were shown. GFP expressing MEF were shown as a control. (B) Immunofluorescence analysis were performed in MEF, H2AX KO MEF or H2AX KO MEF reconstituted by H2AX S139A. Anti-NBS1 antibodies were used. Left; Cells were fixed at 15 minutes after micro-irradiation. Right; 16 hrs after 8Gy of IR. (C) Immunfluorescence analysis after 1 hr after micro-irradiation using anti-γ-H2AX and anti-NBS1 (upper panel, H2AX WT; n=17, H2AX K5R; n=12), or anti-γ-H2AX and anti-MDC1 (lower panel, H2AX WT; n=9, H2AX K5R; n=9) in H2AX KO MEF cells that is reconstituted by H2AX WT or H2AX K5R. Images were shown in the left panel and quantifications were

shown in the right. (D) H2AX KO MEF cells that is reconstituted by H2AX WT, H2AX K5R or H2AX S139A were subjected to Immunofluorecence analysis using anti-γ-H2AX, anti-NBS1 or anti-MDC1 at 2 hrs after 2 Gys of IR exposure. Percentages of the cells that presented more than 10 foci were plotted on the graphs. Graphs were shown with standard deviation. Bar 10μm.

Figure S4.

The intensity of NBS1-GFP prior to micro-irradiation.

Prior to FRAP analysis using NBS1-GFP expressing cells, we measured the fluorecent intensities of NBS1-GFP. 2μm x 5μm square from the nucleus (ROI; region of interest) were selected, and the fluorecent intensities subtracted by background signal were measured using BZII analysis software (KEYENCE). Average intensities from all the cells used in the analysis (eTIP60 and eTIPM reonstituted cells, and H2AX, H2AX K5R and H2AX S139A reconstituted cells) were shown with standard deviation.

Table S1

The list of identified proteins from H2AX complex after DNA damage.

Table S2

The list of identified proteins from TIP60 complex after DNA damage.