Supporting Information

Kumar et al. 10.1073/pnas.0914242107

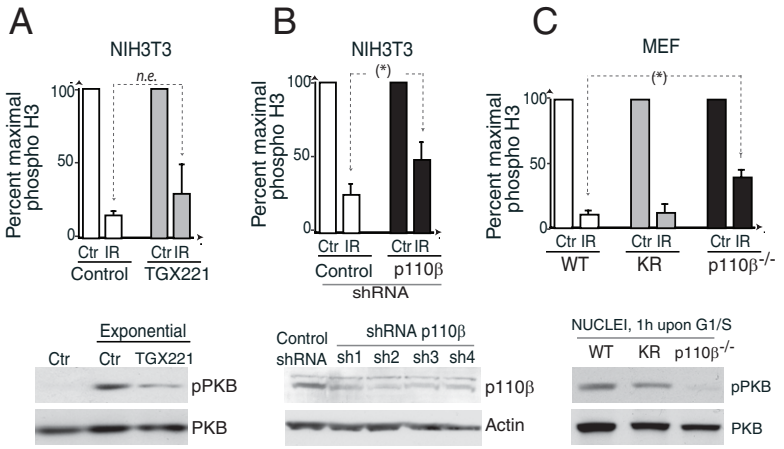


Fig. S1. p110β deletion impairs G2/M checkpoint. (*A*) NIH 3T3 cells were arrested at the G1/S border by double-Thi block and released. At 5 h after release, cells were treated with TGX221 (30 μM, 1 h), and at 6 h samples were irradiated (IR, 5 Gy). The proportion of mitotic cells was analyzed by p-histone $H3^+$ staining at 12 h after release. The graph shows the fraction (mean ± SD, $n = 3$) of p-histone $H3^+$ cells in each condition compared to maximum (~45% p-$H3^+$ cells in nonirradiated controls, considered 100). To control inhibitor activity, we examined extracts from exponentially growing NIH 3T3 cells alone or preincubated with TGX2221 (30 μM, 1 h) by Western blot using phospho-PKB Ab. (*B*) NIH 3T3 cells transfected with p110β shRNA (shRNA4, 48 h) or (*C*) $p110\beta^{-/-}$ immortalized murine embryonic fibroblasts (MEF), some reconstituted with WT or kinase-dead (KR) p110β, were treated as in *A*. Maximum p-histone $H3^+$ cells was ~15% in control NIH 3T3 G2/M cells and ~10% in control G2/M MEF. In *B*, to control shRNA efficiency, extracts from NIH 3T3 transfected with four different sets of p110β shRNA (48 h) were examined by Western blot. All four p110β shRNAs induced a similar defect in G2 arrest. In *C*, to control PI3K pathway status in MEF, we examined extracts from S phase cells by Western blot using phospho-PKB. *, $P < 0.05$.

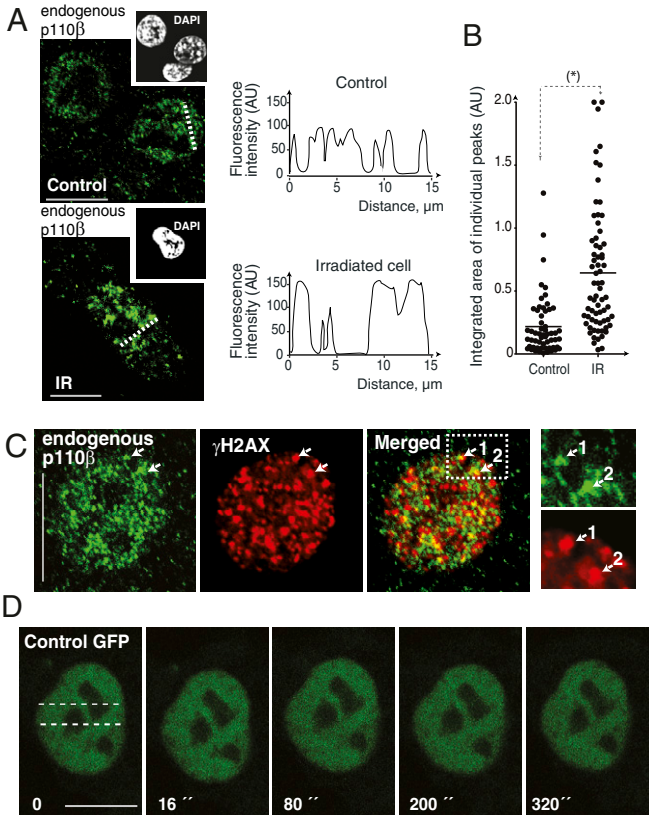


Fig. S2. p110β localizes in DSB foci. (*A*) NIH 3T3 cells were irradiated (IR, 5 Gy) and processed after 1 h for immunofluorescence (IF) using anti-p110β Ab. Insets show DAPI staining. Graphs show IF intensity along the depicted line in arbitrary units (AU) in a representative cell ($n = 50$). (*B*) Graph illustrates the integrated area of each individual peak obtained in the IF-signal profiles performed as in *A*. (*), $P < 0.05$. The graph shows that the p110β-IF integrated signal in focus of IR cells is greater. (*C*) Irradiated NIH 3T3 cells (IR, 5 Gy) were processed for indirect IF upon IR (15 min), using anti-γH2AX (red) and -p110β (green) Ab. Insets show 20× magnifications. Arrows indicate some of the foci in which γH2AX and p110β colocalize. (*D*) NIH 3T3 cells expressing GFP were microirradiated with an UV laser. We examined real-time GFP translocation to the DNA damage region. Dotted lines indicate laser paths. (Scale bars *A* and *D*, 15 μm.)

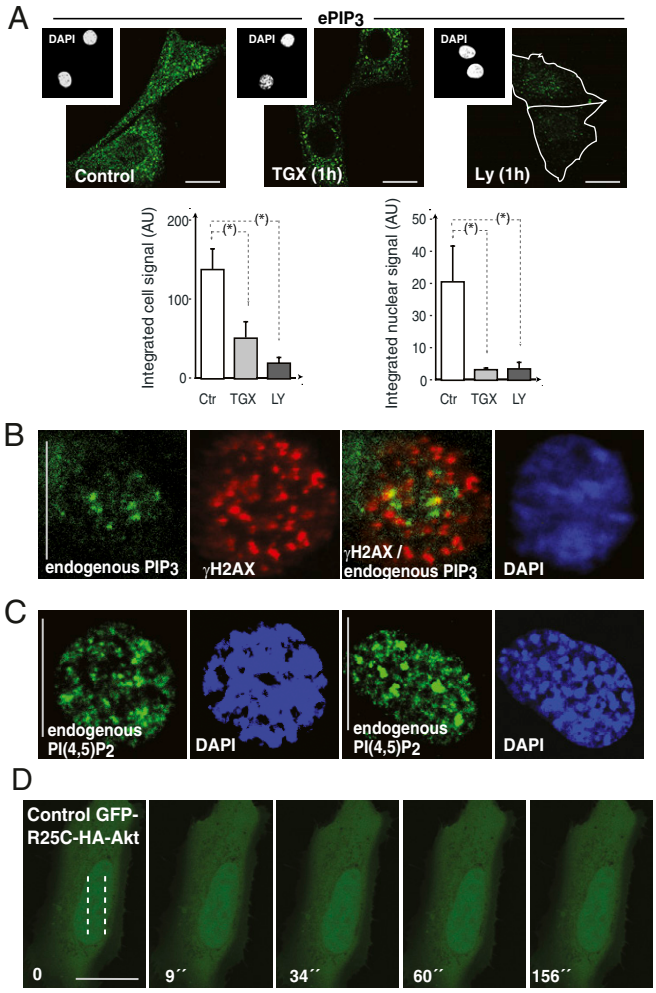


Fig. S3. Endogenous PIP_3 localizes in DSB foci. (*A*) Exponentially grown NIH 3T3 alone or pretreated (1 h) with TGX221 or Ly294002 were stained by IF using anti-PIP_3 Ab. Insets show DAPI staining. The graphs illustrate the integrated signal from the entire cell or from the nucleus (indicated). (*), $P < 0.05$. (*B*) NIH 3T3 cells were irradiated (IR, 5 Gy); after 30 min, PIP_3 (green), γH2AX (red), and DNA (DAPI) were examined by IF. (*C*) NIH 3T3 cells were irradiated (IR, 5 Gy); after 30 min, PIP_2 (green) and DNA (DAPI) were examined by IF. (*D*) NIH 3T3 cells expressing the R25C mutant of the Akt PH domain were microirradiated with an UV laser and we examined real-time translocation of the probe to the laser path (dotted lines). (Scale bars in *A*–*D*, 15 μm.)

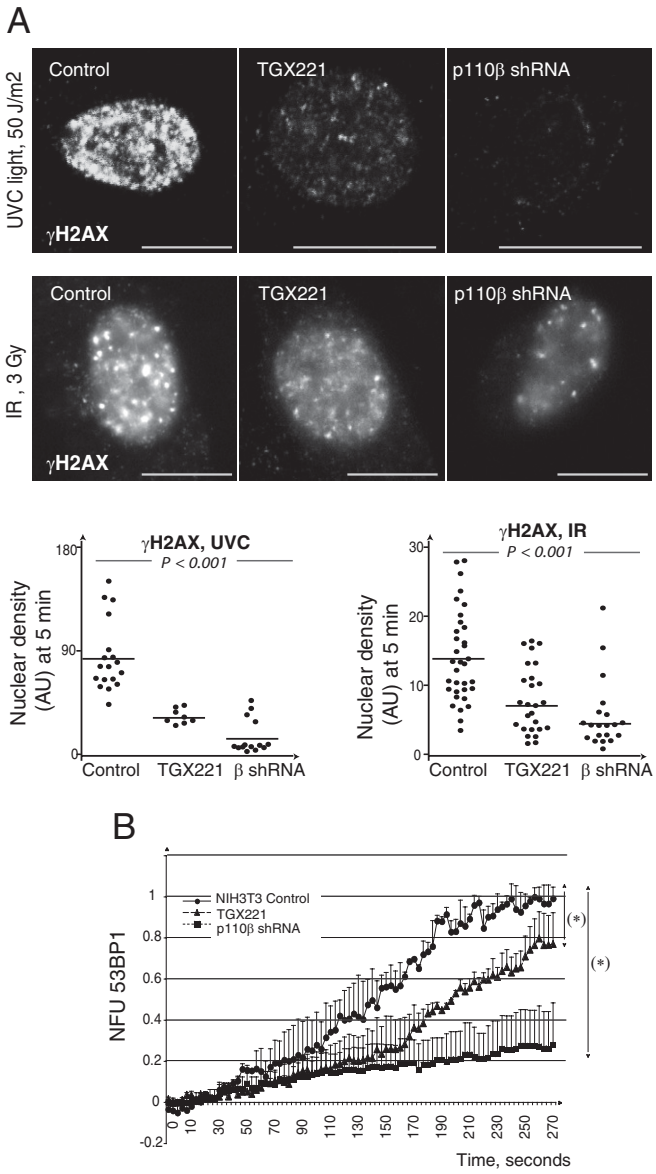


Fig. S4. Defective phospho-γH2AX and 53BP1 focus assembly in irradiated p110β-deficient cells. (*A*) NIH 3T3 cells were transfected with p110β or control shRNA (48 h); the latter cells were treated with TGX221 (30 μM) or DMSO (4 h). Cells were exposed to IR or UVC, and processed (5 min later) for indirect IF using antiphospho-γH2AX Ab. The graphs represent the integrated nuclear fluorescence intensity for a representative set of cells (n = 20) of > 100 examined. (Scale bar = 15 μm.) Unpaired χ^2 P values indicated. (B) NIH 3T3 cells were transfected with p110β or control shRNA (48 h) in combination with GFP-53BP1 (24 h); other cells were transfected with GFP-53BP1 (24 h) and treated with TGX221 (4 h). Cells were microirradiated with an UV laser. Normalized fluorescence units (NFU) along laser tracks was calculated and plotted as a function of time. The assembly curves represent mean ± SD (n = 6). (*), $P < 0.05$.

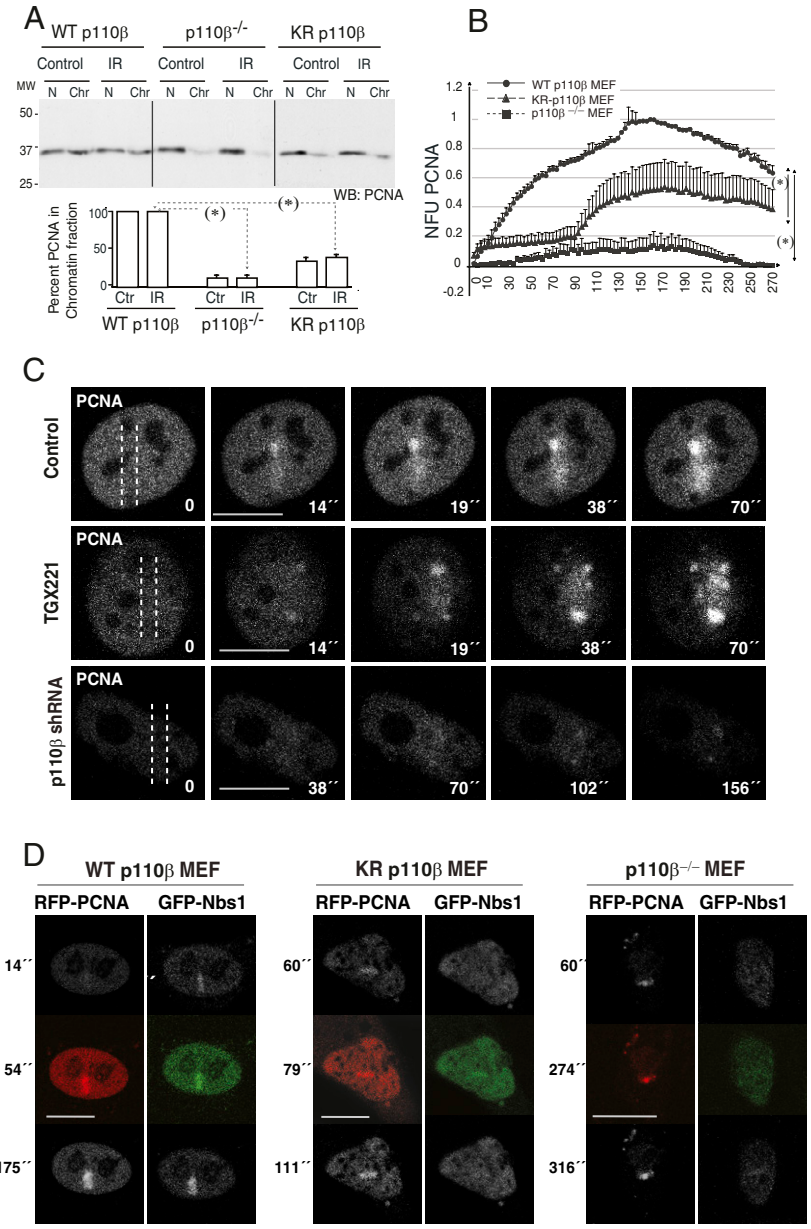


Fig. S5. p110β regulates proliferating cell nuclear antigen (PCNA) recruitment to damaged DNA. (*A*) p110β$^{-/-}$ immortalized MEF alone or reconstituted with WT or KR-p110β were irradiated (IR, 5 Gy) and collected after 1 h. Cells were fractionated into cytosol/cytoskeleton, nuclear (N) and chromatin (Chr) fractions, and the last two fractions were examined by Western blot using anti-PCNA Ab. The graph shows the percentage of chromatin-bound PCNA compared to that in the nuclear soluble fraction (considered 100%; mean ± SD, $n = 3$). (*), $P < 0.05$. (*B* and *C*) NIH 3T3 cells transfected (24 h) with RFP-PCNA were microirradiated and recorded. The RFP-PCNA concentration at double-strand break sites was determined by confocal microscopy live imaging. Dotted lines in the first image frame indicate the laser paths across the nucleus. Normalized fluorescence units along laser tracks was calculated and plotted as a function of time. Assembly curves represent the mean ± SD ($n = 6$) (*), $P < 0.05$. (*B*). (*D*) p110β$^{-/-}$ immortalized MEF alone or reconstituted with WT or KR-p110β were cotransfected with RFP-PCNA and GFP-Nbs1 (24 h). Cells were microirradiated with an UV laser. We examined real-time RFP-PCNA and GFP-Nbs1 translocation to the DNA damage region. The figures shows the RFP-PCNA and GFP-Nbs1 signals at three recording times of representative cells (indicated). (Scale bars *C* and *D*, 15 μm.)

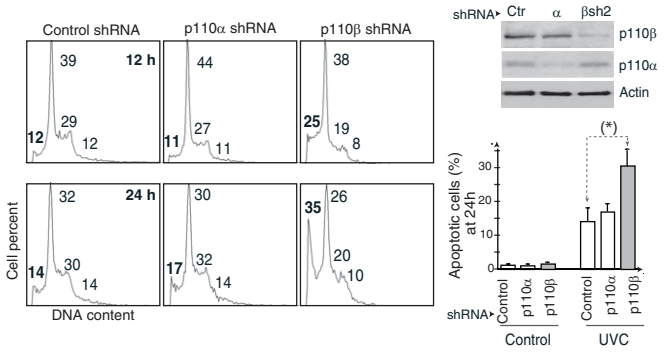
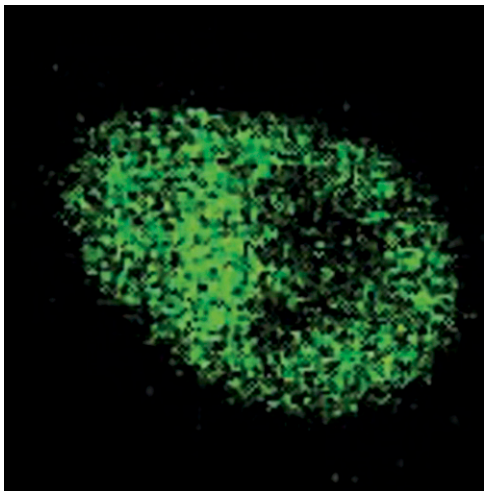
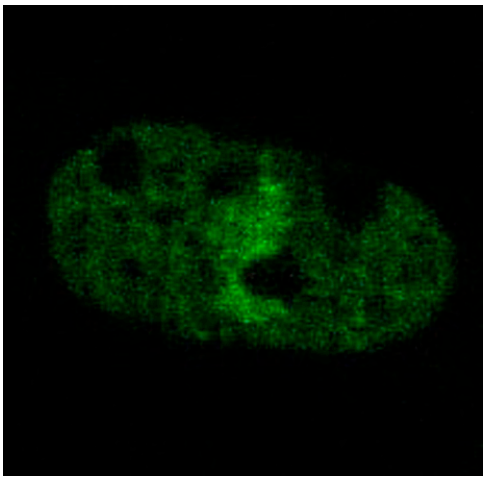


Fig. S6. p110β deletion induces radiation sensitivity. NIH 3T3 cells were transfected with p110β or p110α-specific shRNA (48 h). p110α and p110β expression levels were examined by Western blot. Cell-cycle profiles in control or p110α- and p110β-shRNA-transfected cells were examined by flow cytometry at different times post-UVC exposure (80 J/m^2). The percentage of cells with sub-G1, G0/G1, S, or G2/M DNA are indicated. The graph shows the percentage of cells with a sub-G1 DNA content 24h upon IR (mean ± SD, n = 5). (*), $P < 0.05$.



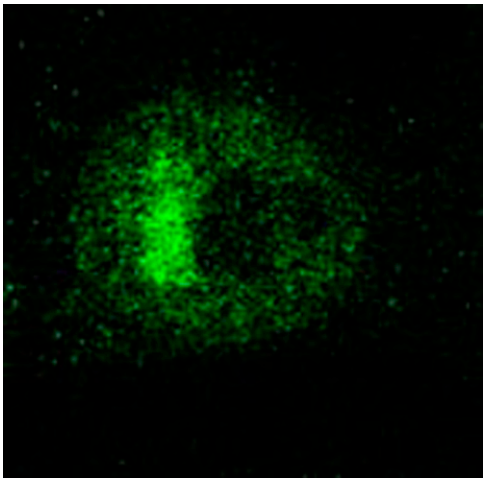
Movie S1. A representative NIH 3T3 cell expressing GFP-p110β; the video includes 89 frames taken every 3.2 s after laser irradiation.

Movie S1



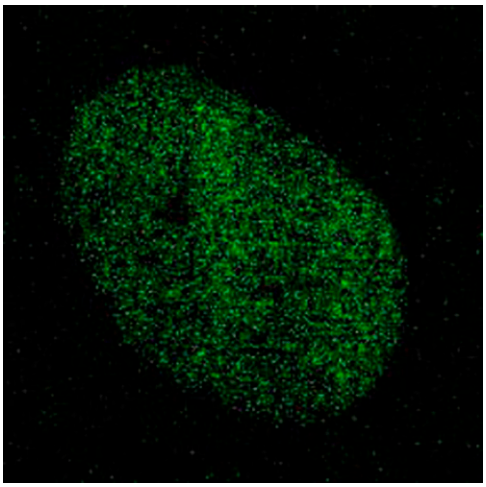
Movie S2. Three concatenated movies of NIH 3T3 cells expressing GFP-53BP1: a representative laser-irradiated NIH 3T3 cell (first cell), a NIH 3T3 cell pretreated with TGX221 (second cell), and a NIH 3T3 cell transfected (48 h before irradiation) with p110β shRNA (third cell). For recording, frames were taken every 3.2 s after laser irradiation.

Movie S2



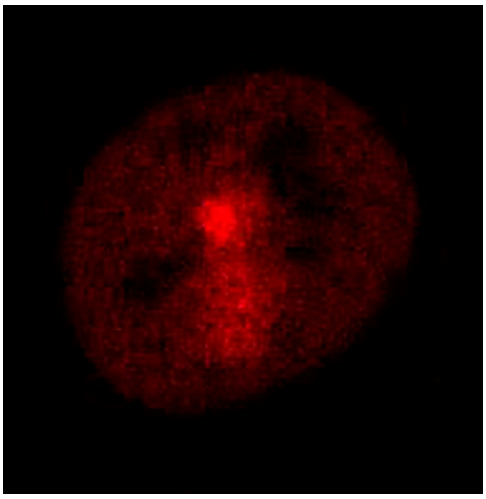
Movie S3. Concatenated movies of representative laser-irradiated MEF expressing GFP-Nbs1 recorded every 3.2 s. The movie includes a representative MEF expressing WT-p110β (first), a KR-p110β reconstituted p110$\beta^{-/-}$ MEF (second), and a p110$\beta^{-/-}$ MEF (third).

Movie S3



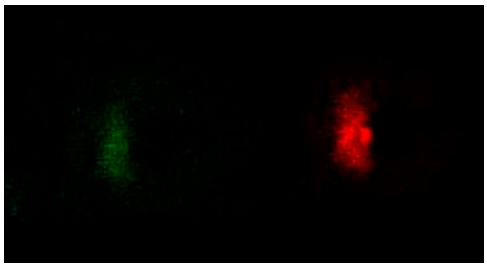
Movie S4. Concatenated movies of representative U2OS cells expressing WT or mutated GFP-hNbs1 upon laser irradiation (recorded every 3.2 s). Cells were transfected with WT-GFP-hNbs1 (first), GFP-A_4^{653}-hNbs1 (second), or GFP-A_3^{670}-hNbs1 (third).

Movie S4



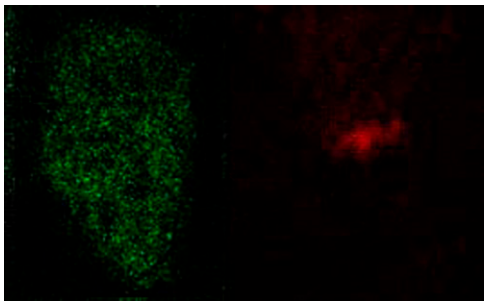
Movie S5. Representative RFC-PCNA-expressing NIH 3T3 cells microirradiated with an UV laser and recorded (first cell), or pretreated with TGX221 (1 h) before irradiation (second cell), or transfected (48 h before irradiation) with p110β shRNA (third cell, concatenated movies). The videos include ~50 frames taken every 3.2 s after irradiation.

Movie S5



Movie S6. With Movie S7, a simultaneous analysis of GFP-Nbs1 (green) and RFP-PCNA (red) concentration at laser irradiated tracks in p110$\beta^{-/-}$ MEF reconstituted with WT-p110β (first cell) or KR-p110β (second cell, concatenated movies). Video starts immediately after laser irradiation; frames were recorded every 3.2 seconds. The video was mounted with half of the Nbs1-GFP frames (every 6.4 s) and half of the RFP-PCNA frames (every 6.4 s).

Movie S6



Movie S7. A typical p110$\beta^{-/-}$ MEF examined similarly to that shown in Movie S6. Video starts immediately after laser irradiation; frames were recorded every 3.2 seconds. The video was mounted with half of the Nbs1-GFP frames (every 6.4 s) and half of the RFP-PCNA frames (every 6.4 s).

Movie S7