

Figure S1. (A) HeLa cells transfected with siRNAs control or targeting ATM were exposed to 200 ng/ml NCS for 15 min and stained 3 h later with the antibody to Ser1981-phosphorylated ATM. Three confocal images are shown for both cellular backgrounds. Scale bar, 10 μ M. (B) Immunofluorescences were performed from control and INT6 RNAi HeLa cells treated with NCS as above and stained with antibodies to Ser1981-phosphorylated ATM and to γ -H2AX. Representative confocal images are shown. The merged red and green channels show colocalization in yellow. Scale bar, 10 μ M. (C) Human fibroblasts immortalized with the telomerase catalytic subunit were transfected for 72 h with siRNAs control or targeting INT6 and were treated with 100 ng/ml NCS for 1 h followed by a 2-h recovery. Cells were fixed and stained for ATM phosphorylated on Ser 1981 and γ -H2AX. Representative confocal planes are shown. The merged red and green channels show colocalization in yellow. Scale bar, 10 μ M. (D) U2OS cells were treated with control or INT6 siRNAs for 72 h and microirradiated with a laser beam. Cells were fixed 10 min later, immunostained with antibodies against ATM phosphorylated on Ser 1981 and 53BP1, and counterstained for the nucleus with DAPI.

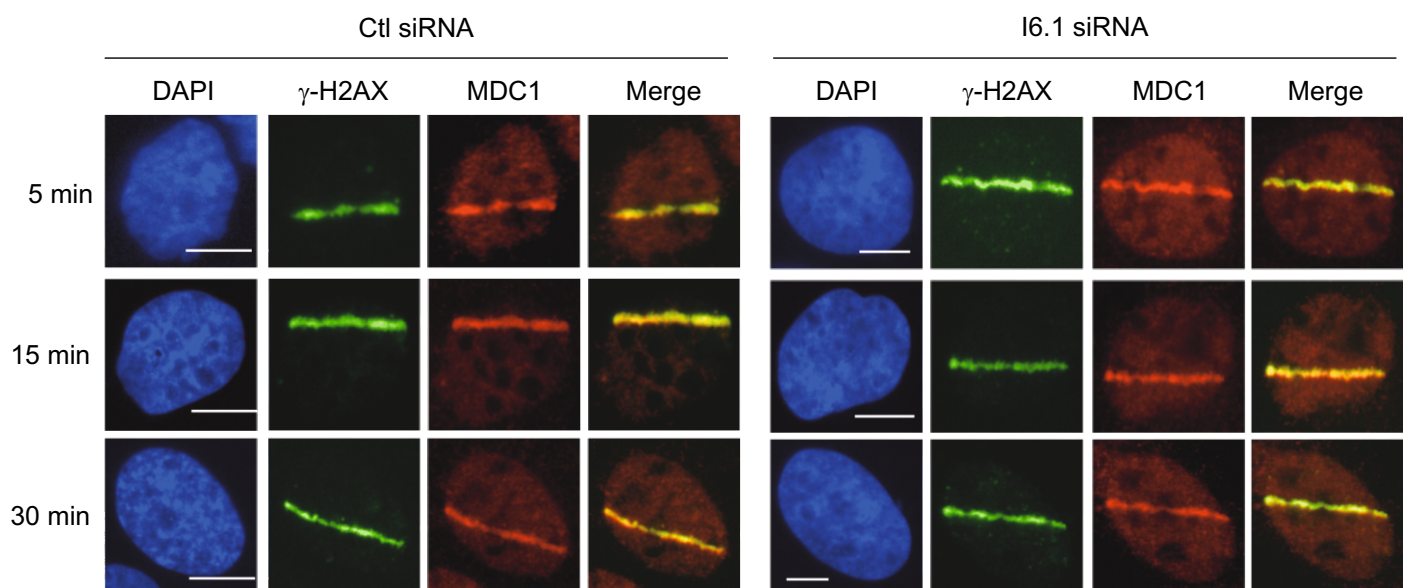


Figure S2. INT6 is not essential for the recruitment of γ -H2AX and MDC1 at laser-induced DSBs. U2OS cells were treated with control or INT6 siRNAs and micro-irradiated with a laser beam. Cells were fixed at the indicated times, immunostained with antibodies against γ -H2AX and MDC1, and counterstained for the nucleus with DAPI. The merged red and green channels show co-localization in yellow. Scale bar, 10 μ M.

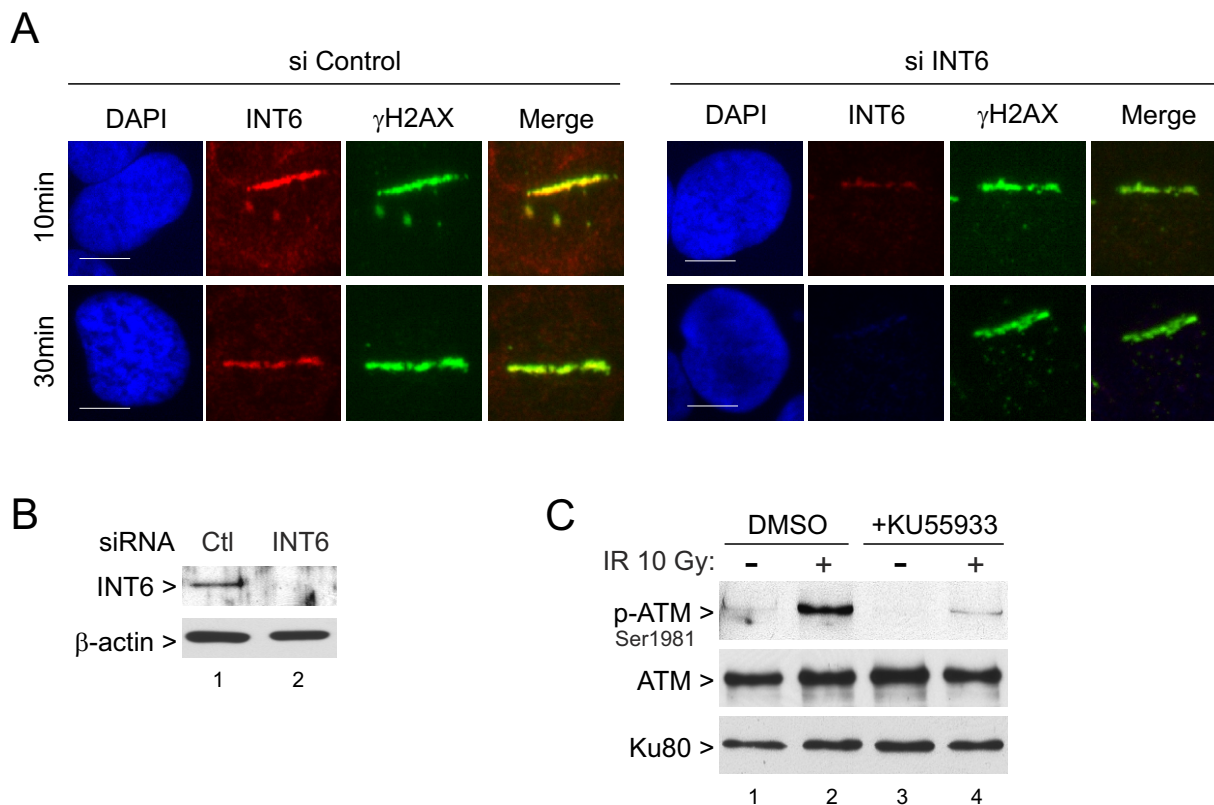


Figure S3. (A) U2OS cells were treated with control or INT6 siRNAs for 72 hrs and micro-irradiated with a laser beam. Cells were fixed at the indicated times, immunostained with antibodies to INT6 and to γ -H2AX, and counterstained with DAPI. The merged red and green channels show co-localization in yellow. Bar, 10 μ M. (B) Efficiency of the INT6 depletion was controlled by performing an immunoblot with an antibody against INT6. The β -actin panel serves as loading control. (C) U2OS cells were treated with control vehicle (DMSO) or with 5 μ M KU55933 prior exposure or not to 10 Gy of irradiation. Inhibition of ATM kinase activity by KU55933 was controlled by immunoblotting the corresponding cell extracts with an antibody specific of ATM autophosphorylated on Ser1981 and with an antibody to ATM. The Ku80 panel serves as loading control.

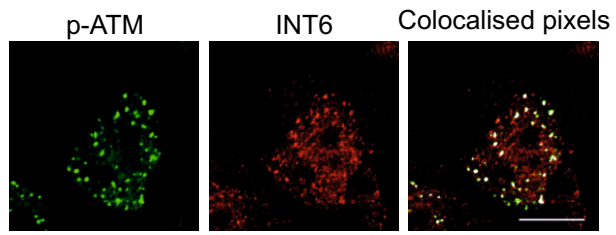


Figure S4. HeLa cells were treated with NCS as in Fig. 4A and stained with antibodies against autophosphorylated ATM and INT6. Microscopic analysis was as in Fig. 4A. Scale bar, 10 μ M.

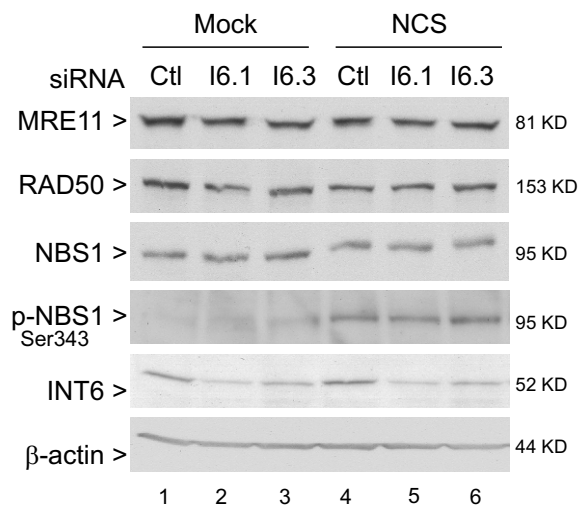


Figure S5. Cells transfected with control or INT6 specific siRNAs were untreated or treated with 200 ng/ml NCS for 15 min and were collected 3 h later. Immunoblots were probed with antibodies to MRE11, RAD50, NBS1 total or phosphorylated on Ser343, INT6, and β-actin.

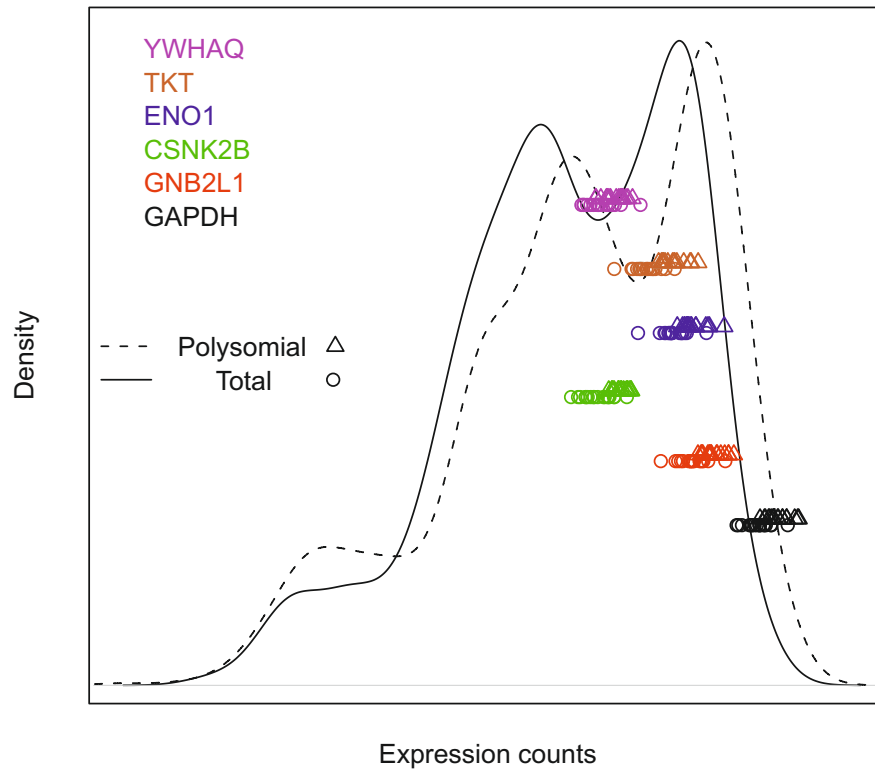


Figure S6. Distribution of expression values for all transcripts measured (lines) and for transcripts selected for the normalization process (dots). Solid line: total RNAs, dashed line: polysomal RNAs. The following genes, *CSNK2B*, *ENO1*, *GAPDH*, *GNB2L1*, *TKT*, and *YWHAQ*, were included in the NanoString nCounter assays as “control” genes because their corresponding expression values were observed to be relatively stable in total RNA samples isolated from HeLa cells (Dr P. Descombes, personal communication). To check if this was also true using RNAs engaged in translation, expression values obtained from total and polysome-bound RNAs were compared. The dispersion of expression values of these control genes was similar across polysomal RNA samples and across total RNA samples.

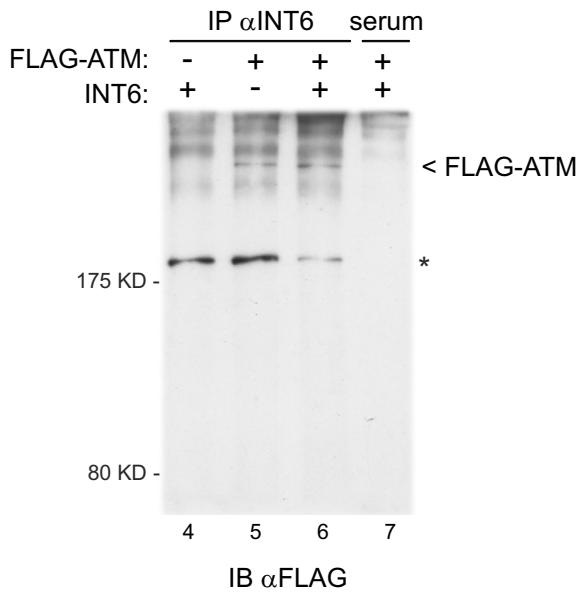
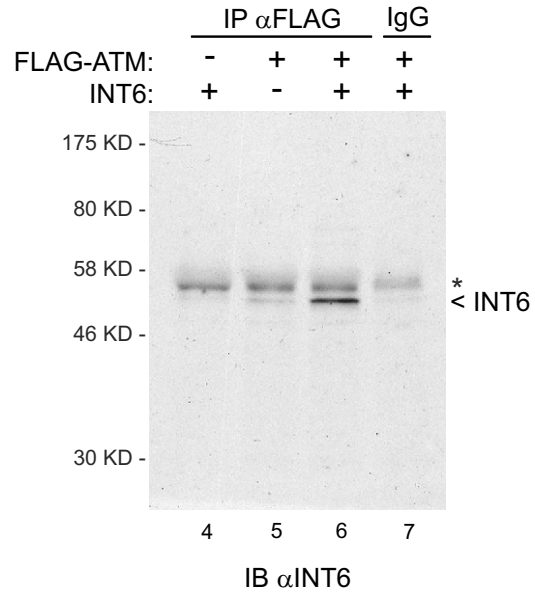
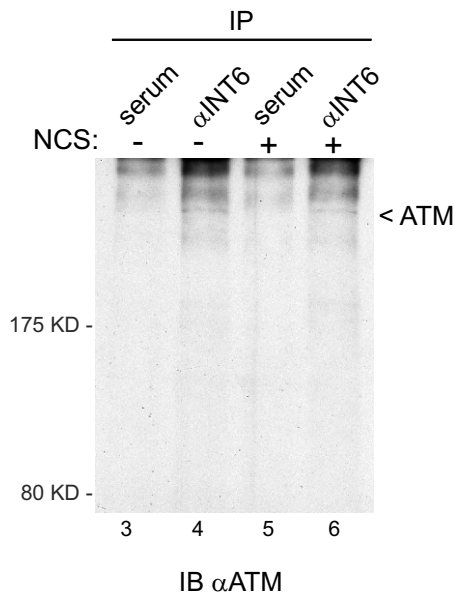
A**Figure 5A****B****Figure 5B****C****Figure 5C**

Figure S7. Full scans of immunoblots shown in Figure 5A-C. Asterisk on panel A marks a non-specific band that crossreacts with the antibody to FLAG epitope. This band was observed in the absence of exogenous FLAG-ATM protein (lane 4). In addition, such a band was not detected when the INT6 immunoprecipitates were probed using the antibody to ATM (see panel C). Asterisk on panel B marks the immunoglobulin heavy chain.