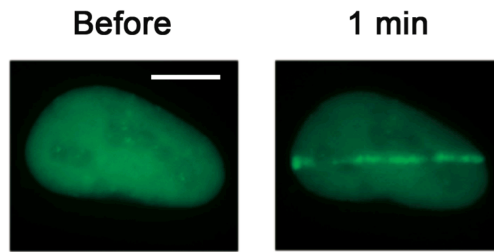
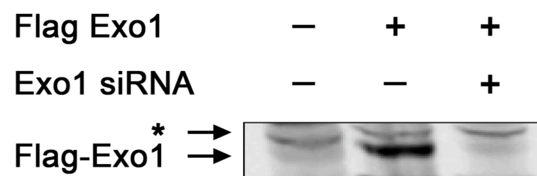
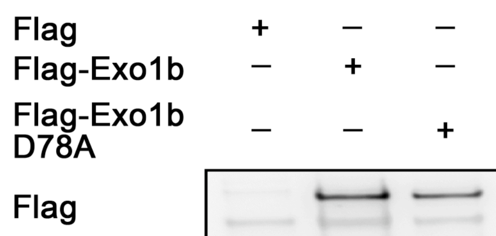


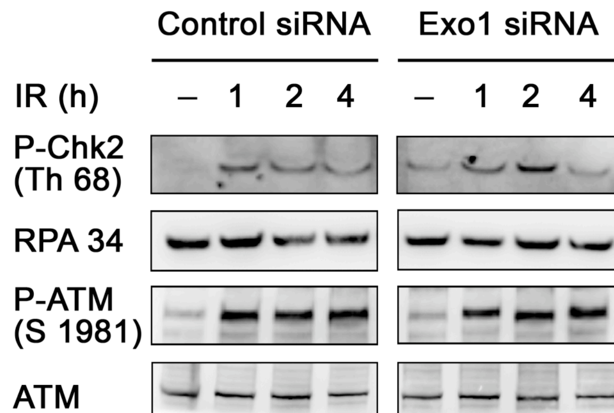
S1. A U2OS cell expressing GFP-Exo1 showing accumulation of Exo1 at the site of laser damage. The scale bar represents 5 μm.



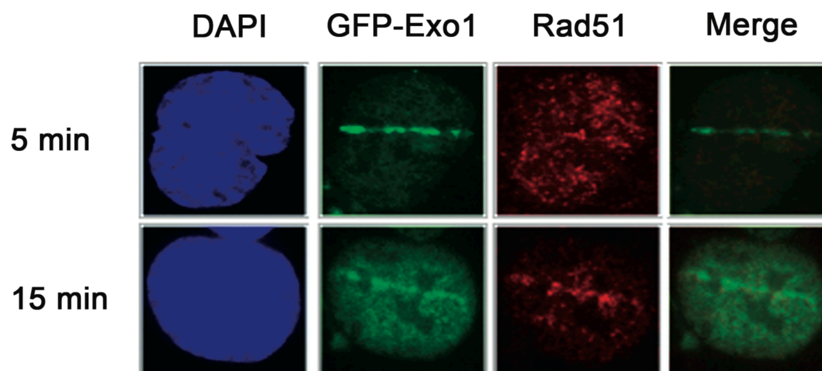
S2. SiRNA-mediated knockdown of Exo1 in HeLa cells. Due to the low levels of endogenous Exo1 and unavailability of high affinity antibodies, verification of Exo1 siRNA depletion was carried out in cells expressing a Flag-Exo1b construct. Twenty-four hours after transfection with Flag-Exo1, HeLa cells were transfected with Control or Exo1 siRNA. Immunoblotting was carried out with anti-Flag antibodies. * Non-specific band.



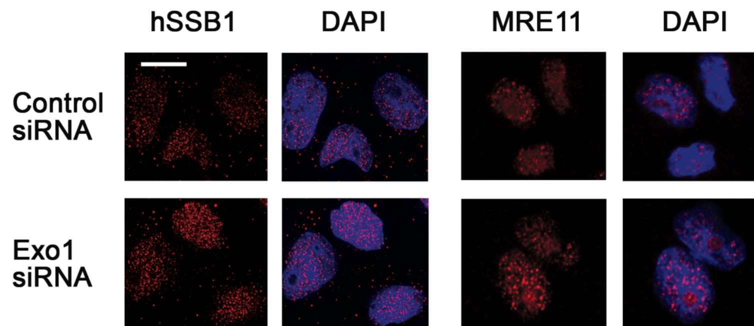
S3. Expression of Exo1 constructs. HeLa cells were transfected with Flag-Exo1 and nuclease mutant (D78A). Lysates were taken and immunoprecipitated with M2 beads. Immunoblotting was carried out with the indicated antibodies.



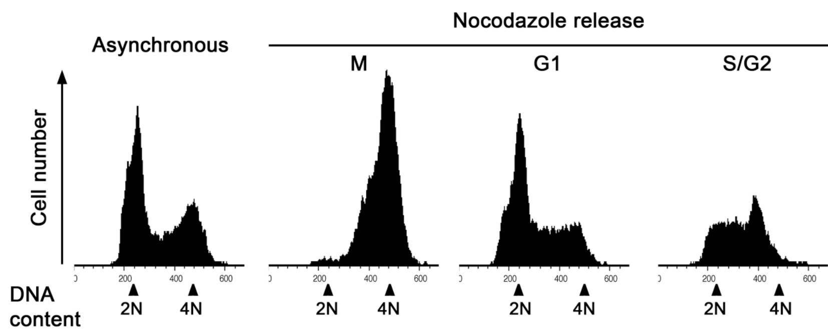
S4. Exo1-deficient cells have normal cell signalling after DNA damage. HeLa cells were transfected with control or Exo1 siRNA and treated with 6 Gy IR. Cell extracts were immunoblotted with the indicated antibodies at the timepoints indicated.



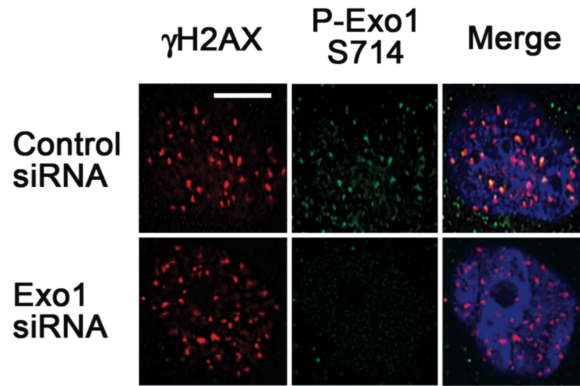
S5. Exo1 is recruited to laser tracks before Rad51. U2OS cells expressing GFP-Exo1b were micro-irradiated and fixed at the indicated timepoints. Immunostaining was carried out with anti-Rad51 antibodies.



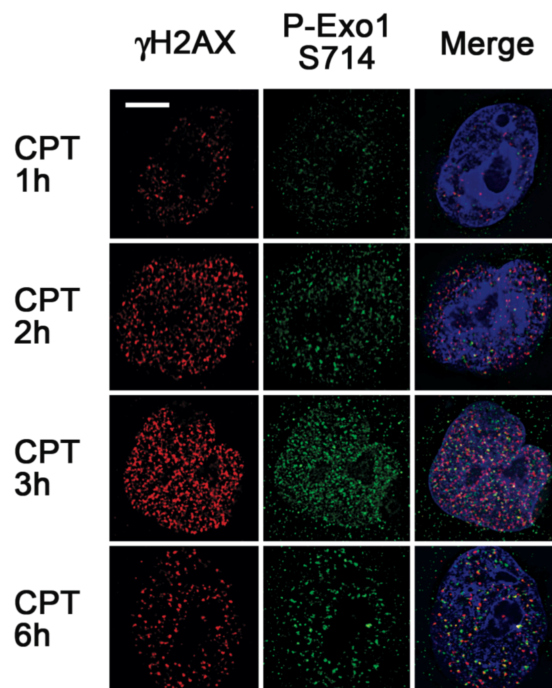
S6. hSSB1 and Mre11 are still recruited to foci in Exo1-deficient cells. HeLa cells were treated with 6 Gy IR and fixed after 1h. Immunostaining was carried out using hSSB1 and Mre11 antibodies. The scale bar represents 10 μm.



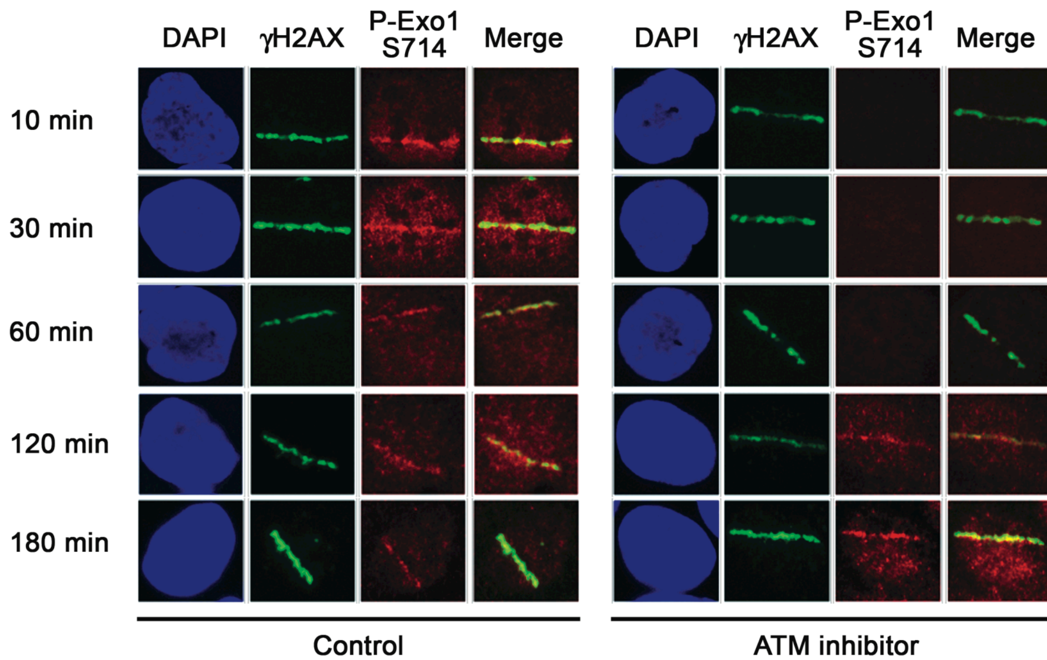
S7. Synchronisation of HeLa cells expressing Flag-Exo1b. HeLa cells were synchronized by the addition Nocodazole for 14 h. Mitotic cells were shaken off and released into fresh media. Cells were collected after 1 h (predominantly M cells), 5 h (predominantly G1 cells) and 14 h (predominantly S/G2 cells) and fixed in 70% ice-cold ethanol. Cells were stained with Propidium iodide (PI) and analysed via FACS using standard methods.



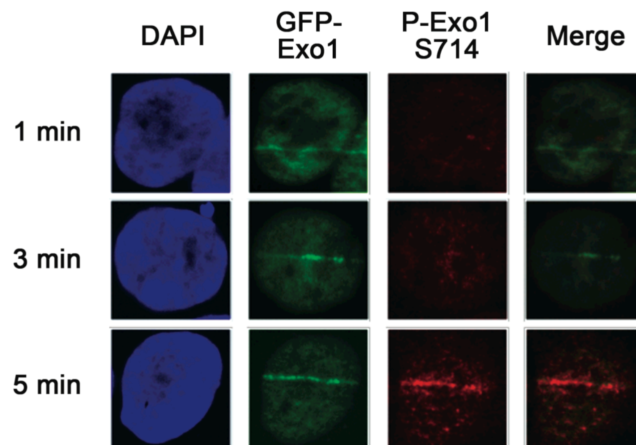
S8. Specificity of the Exo1 P-714 antibody. HeLa cells were transfected with control or Exo1 siRNA and treated with 6 Gy IR. Cells were fixed 1 h after treatment and immunoflourescence was carried out with the indicated antibodies. The scale bar represents 5 μm.



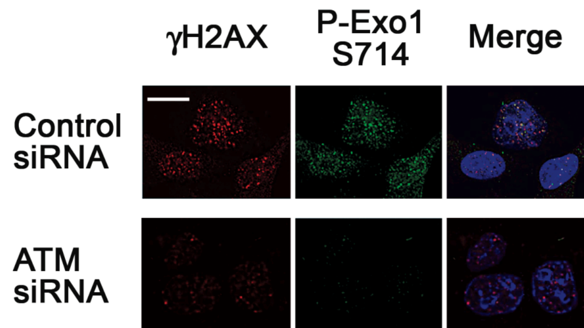
S9. Exo1 P-714 foci after CPT treatment. HeLa cells were transfected with control or Exo1 siRNA and treated with 1μM CPT for 1 h. Cells were washed with PBS to remove CPT and were fixed at the indicated times after treatment. Immunoflourescence was carried out with the indicated antibodies. The scale bar represents 5 μm.



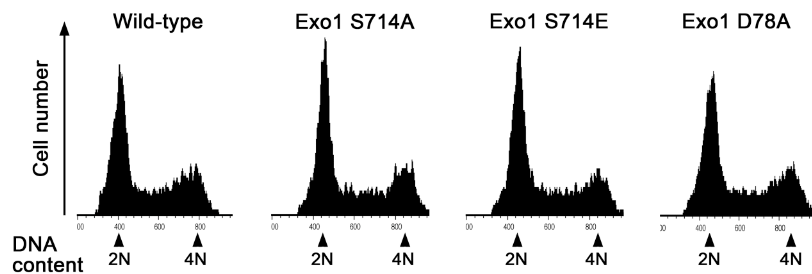
S10. Exo1 phosphorylation at laser tracks is dependent upon ATM. U2OS cells were treated or mock treated with the ATM inhibitor Ku55933 1 hour prior to micro-irradiation and fixed at the indicated timepoints. Immunostaining was carried out with anti-P-Exo1 S714 and γH2AX antibodies.



S11. Exo1 is phosphorylated after recruitment to laser tracks. U2OS cells expressing GFP-Exo1b were micro-irradiated and fixed at the indicated timepoints. Immunostaining was carried out with anti-P-Exo1 S714 antibodies.



S12. IR-induced Exo1 S714 phosphorylation is ATM dependent. HeLa cells were pre-transfected with control, ATM or ATR siRNA. 48 h after transfection cells were treated with 6 Gy IR and fixed after 1 h. Immunostaining was carried out using the indicated antibodies. The scale bar represents 10 μm.



S13. Expression of Exo1 mutant constructs does not affect the cell cycle phase distribution. HeLa cells were transfected with Flag-Exo1, D78A, S714A or S714E. 24 h after transfection cells were fixed in 70% ice-cold ethanol. Cells were stained with PI and analysed via FACS.