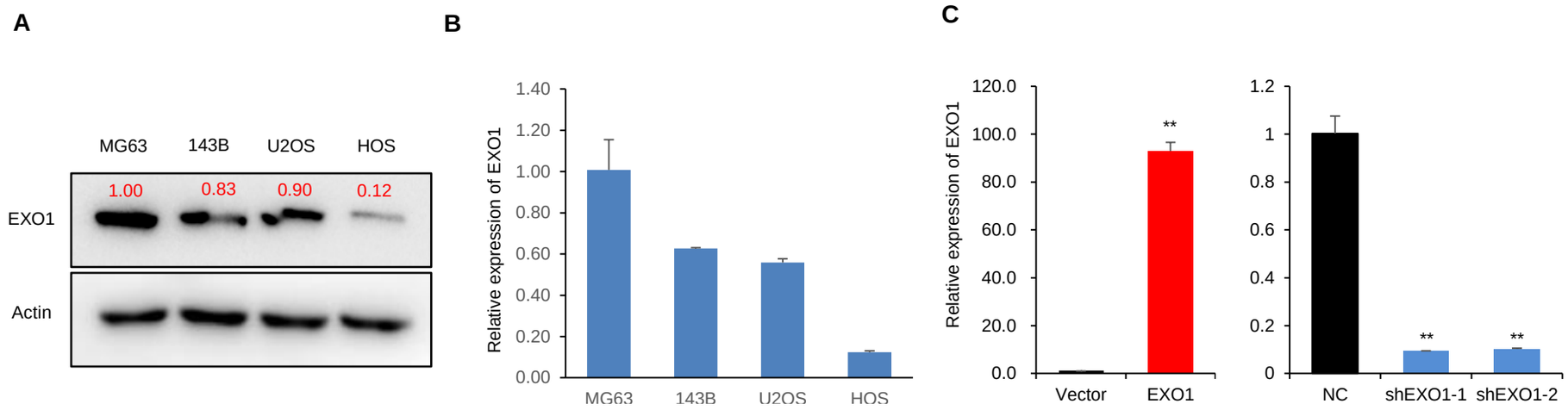
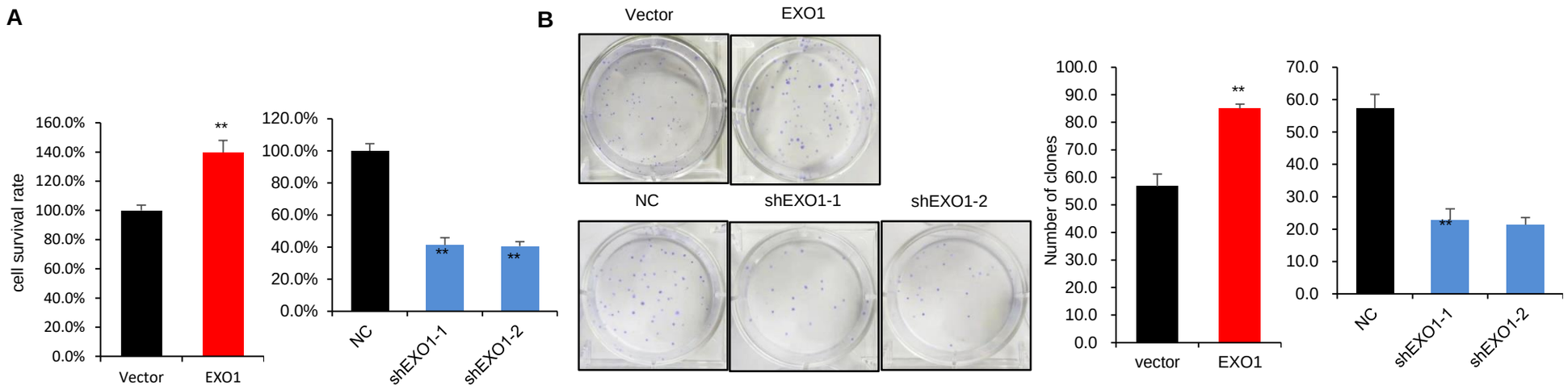
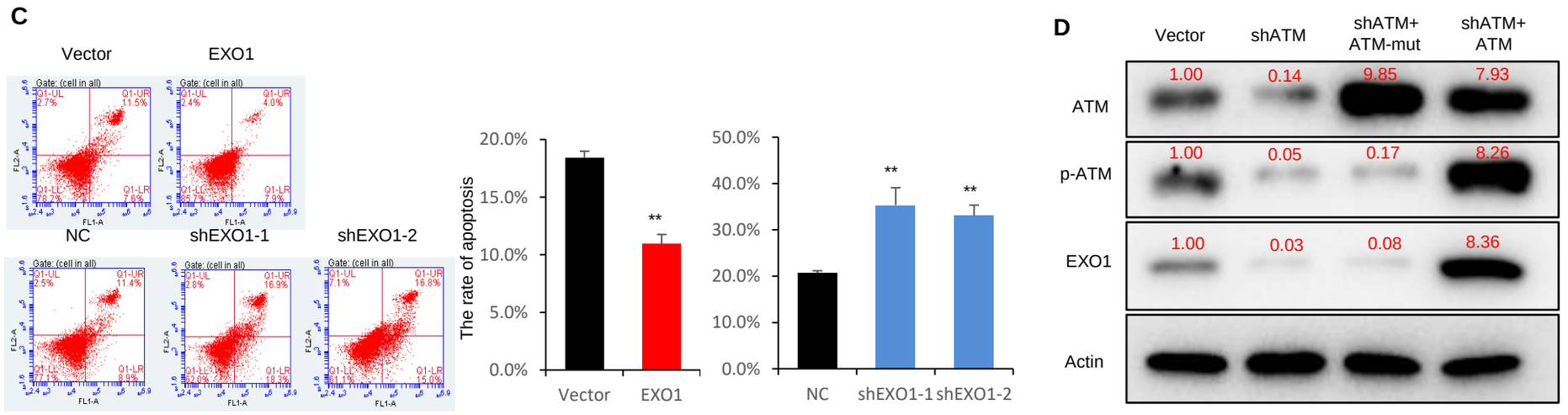
Figure S1



Supplementary Figure S1. EXO1 expression in OS cells and the effect of IR on the OS cells (A) The protein level of OS cell lines (MG63, 143B, U2OS, HOS) was tested by western blotting. (B) The mRNA level of OS cell lines was tested by qRT-PCR. (C) Overexpression and knockdown of the exonuclease 1 (*EXO1*) gene in U2OS cells on the mRNA level were confirmed using qRT-PCR. (D) Overexpression and knockdown of *EXO1* in U2OS cells on the protein level were confirmed using western blotting. (E) After differential dose irradiation, osteosarcoma (OS) cell survival was detected using the CCK-8 assay. (F) After differential dose irradiation, OS cell clone formation was evaluated using the Clonogenic assay.

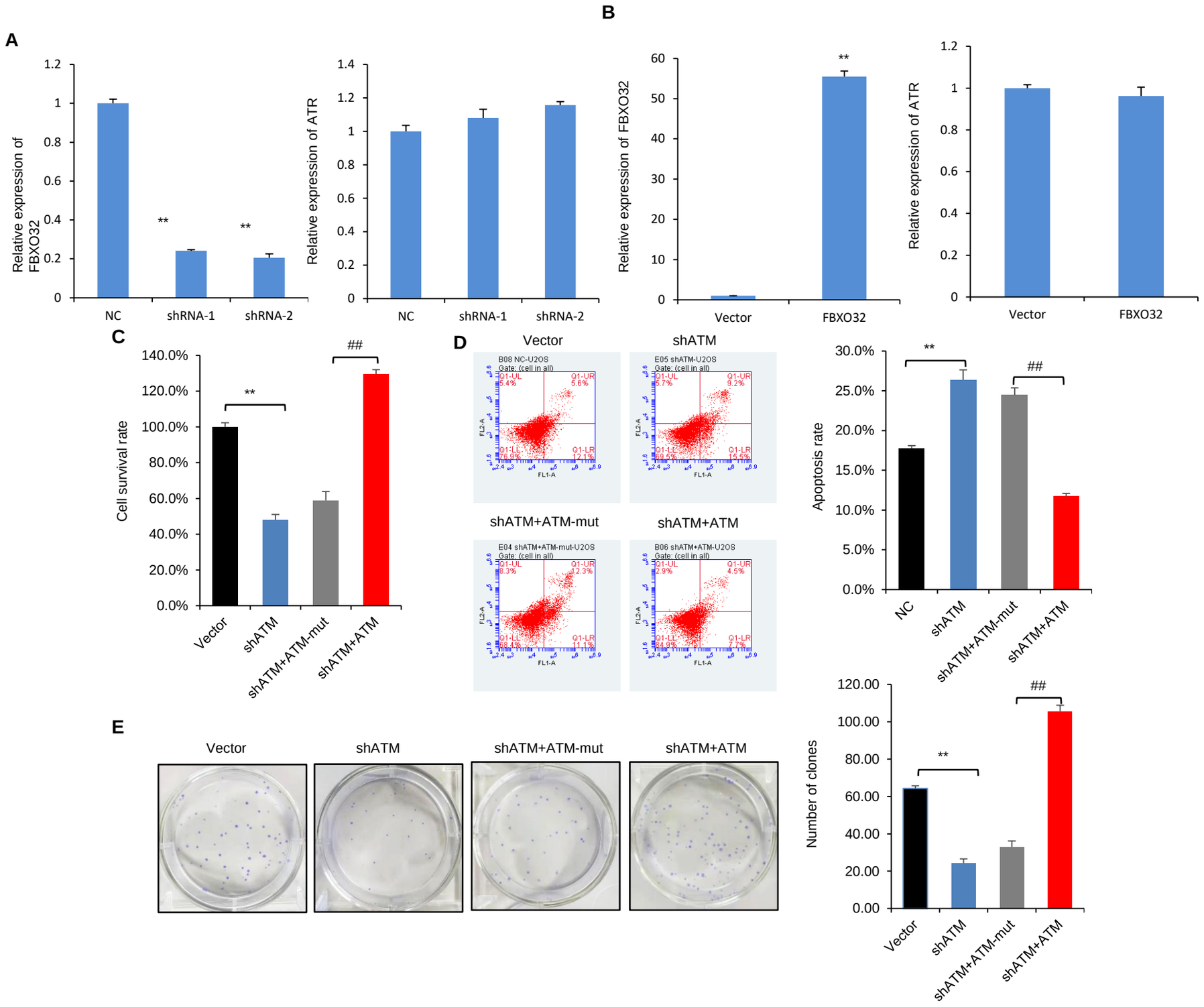
Figure S2





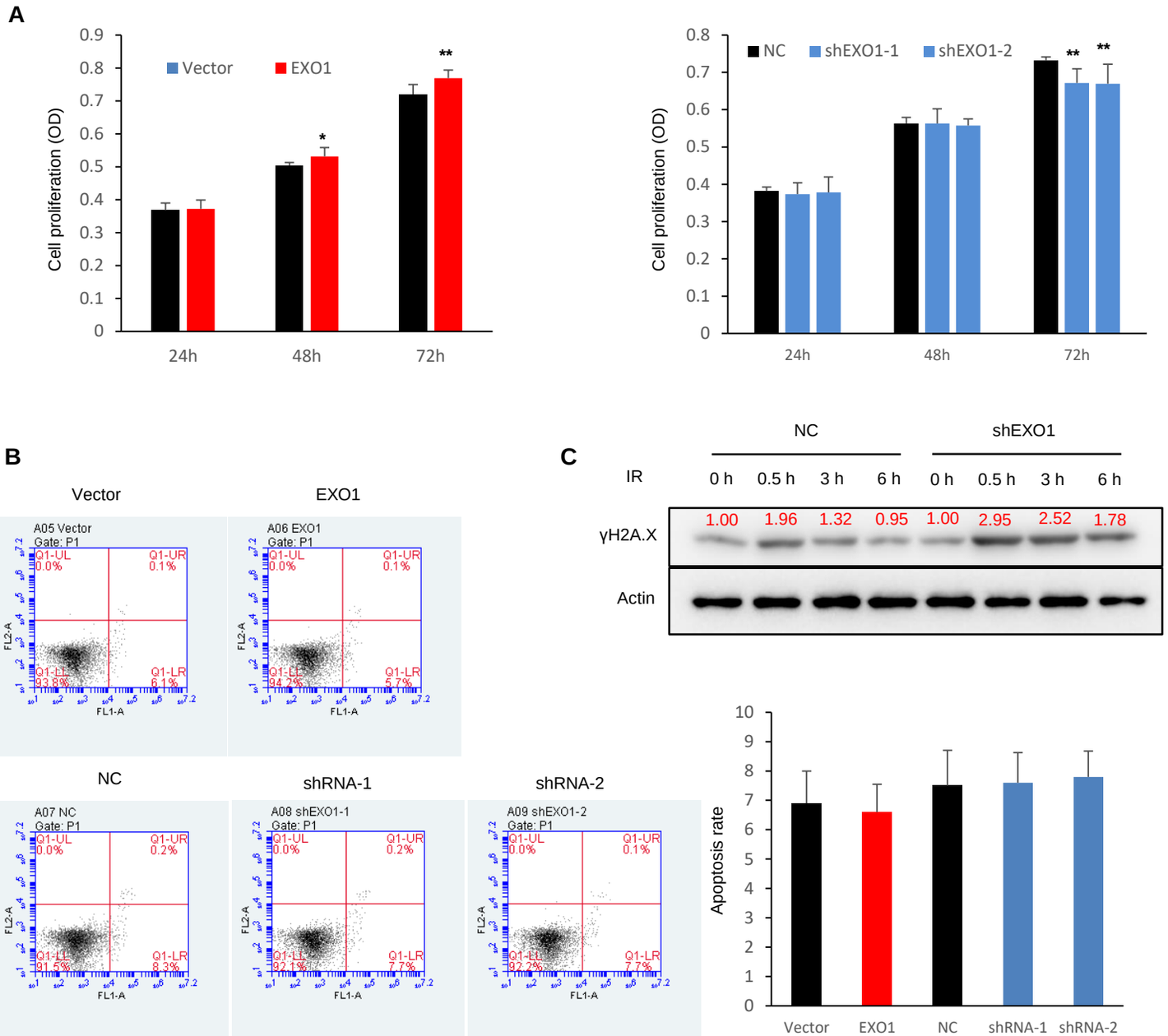
Supplementary Figure S2. EXO1 suppresses the sensitivity of U2OS cells to IR (A) CCK-8 assay shows that *EXO1* promoted U2OS cells survival after 4 Gy irradiation. (B) Clonogenic assay shows that *EXO1* promoted clone formation after 4 Gy irradiation. (C) Apoptosis assays demonstrate that *EXO1* inhibited cell apoptosis after 4 Gy irradiation. NC, negative control vs. shEXO1 (*EXO1* knockdown); empty vector vs. oe-*EXO1* (overexpressed *EXO1*); Gys (irradiation) vs. CK (blank control); ** $P < 0.01$. qRT-PCR, CCK-8 assay and Clonogenic were repeated three times. (D) Western blotting assays showed that expression levels of phosphorylated (p)-ATM and EXO1 were significantly reduced in the ATM mutation group. ATM overexpression markedly increased EXO1 expression in U2OS cells. NC (normal control) vs. shATM (ATM knockdown); empty vector vs. ATM (overexpressed ATM).

Figure S3



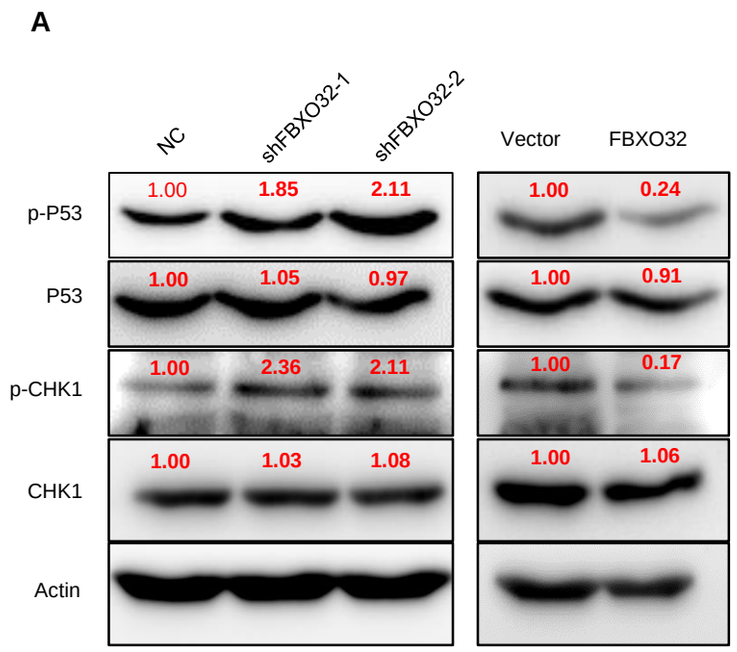
Supplementary Figure S3. FBXO32/ATR/ATM axis control the sensitivity of OS cells to IR (A) The effect of FBXO32 knockdown was validated by qPCR, and reduced expression of FBXO32 had no effect on the mRNA leve of ATR (B) The effect of FBXO32 overexpression was validated by qPCR, and high expression of FBXO32 had no effect on the mRNA leve of ATR. (C) CCK-8 assays show that ATM, but not ATM-mut, promotes cell survival under conditions of 4 Gy irradiation. (D)Apoptosis assays show that if ATM cannot be phosphorylated and activated, it will not prevent OS cell apoptosis under irradiation. Empty vector vs. shATM (ATM knockdown). (E) Clonogenic assay demonstrates that compared with ATM overexpression, ATM-mut cannot rescue the clone formation ability of osteosarcoma (OS) cells. ** $P < 0.01$; shATM+ATM-mut vs. shATM+oeATM (overexpressed ATM); ## $P < 0.01$. CCK-8 assay, Clonogenic and apoptosis assays were repeated three times.

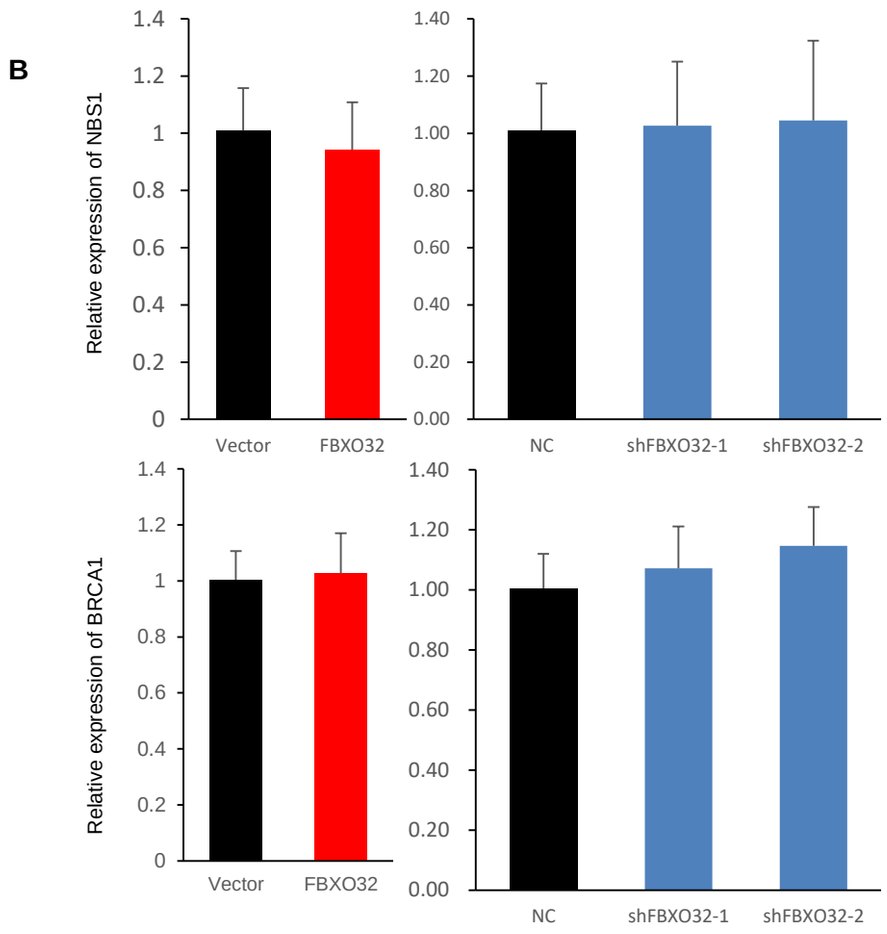
Figure S4

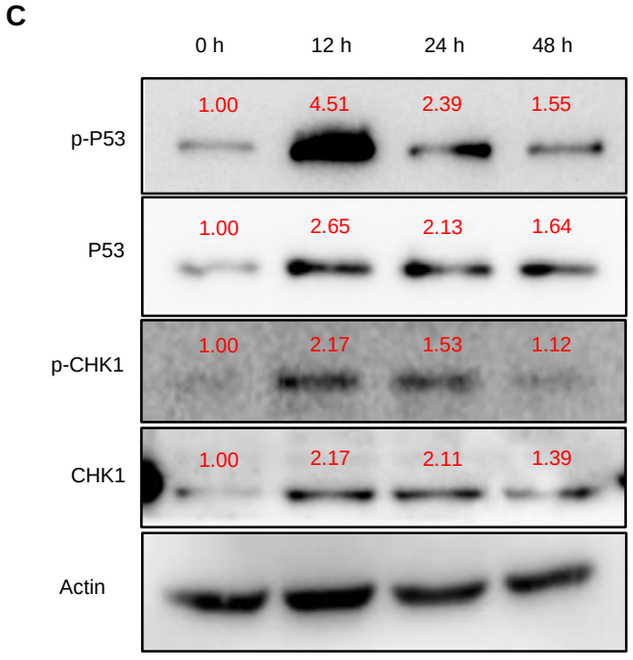


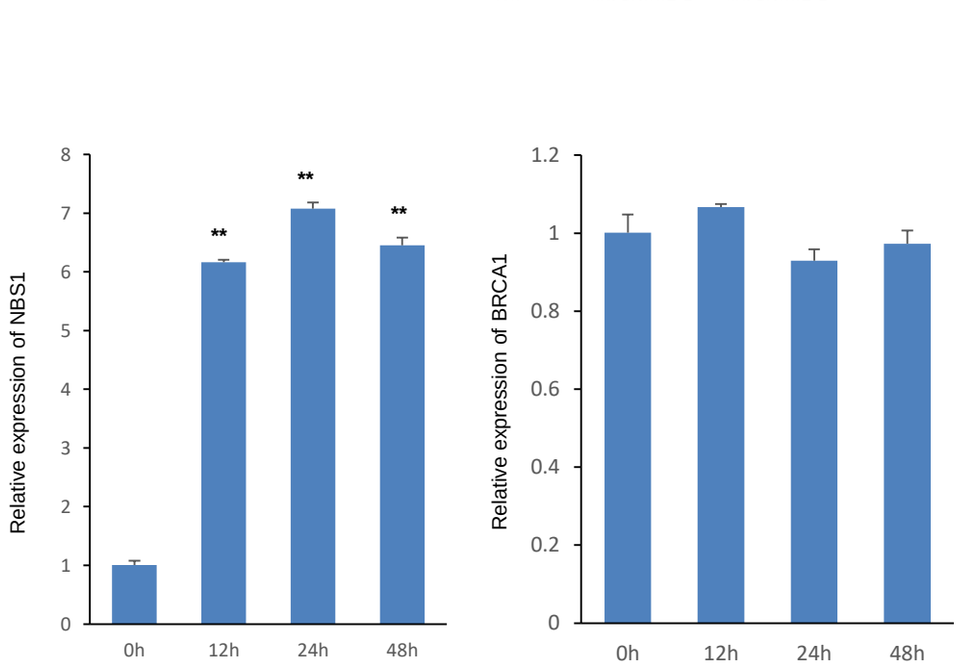
Supplementary Figure S4. Effect of EXO1 on OS cells function (A) CCK-8 assays showed that EXO1 affected MG63 cell proliferation. (B) Flow cytometry experiments showed that EXO1 did not affect MG63 cell apoptosis. (C) Western blotting was used to detect the expression of γH2A.X at different time after IR (4 Gy). CCK-8 assay and apoptosis assays were repeated three times. *$P < 0.05$, ** $P < 0.01$.

Figure S5









Supplementary Figure S5. The effects of FBXO32 on checkpoint proteins (A) Western blotting assays showed FBXO32 significantly affected p-P53 and p-CHK1 expression, and had no effect on the protein leve of P53 and CHK1. (B) qRT-PCR was used to assess the effect of FBXO32 on the relative expression of NBS1 and BRCA1 mRNA levels. (C-D) Western blotting and qRT-PCR were used to detect the expression of P53, CHK1, p-P53 and p-CHK1 at different time after IR (4 Gy). * $P < 0.05$, ** $P < 0.01$.