Figure S4

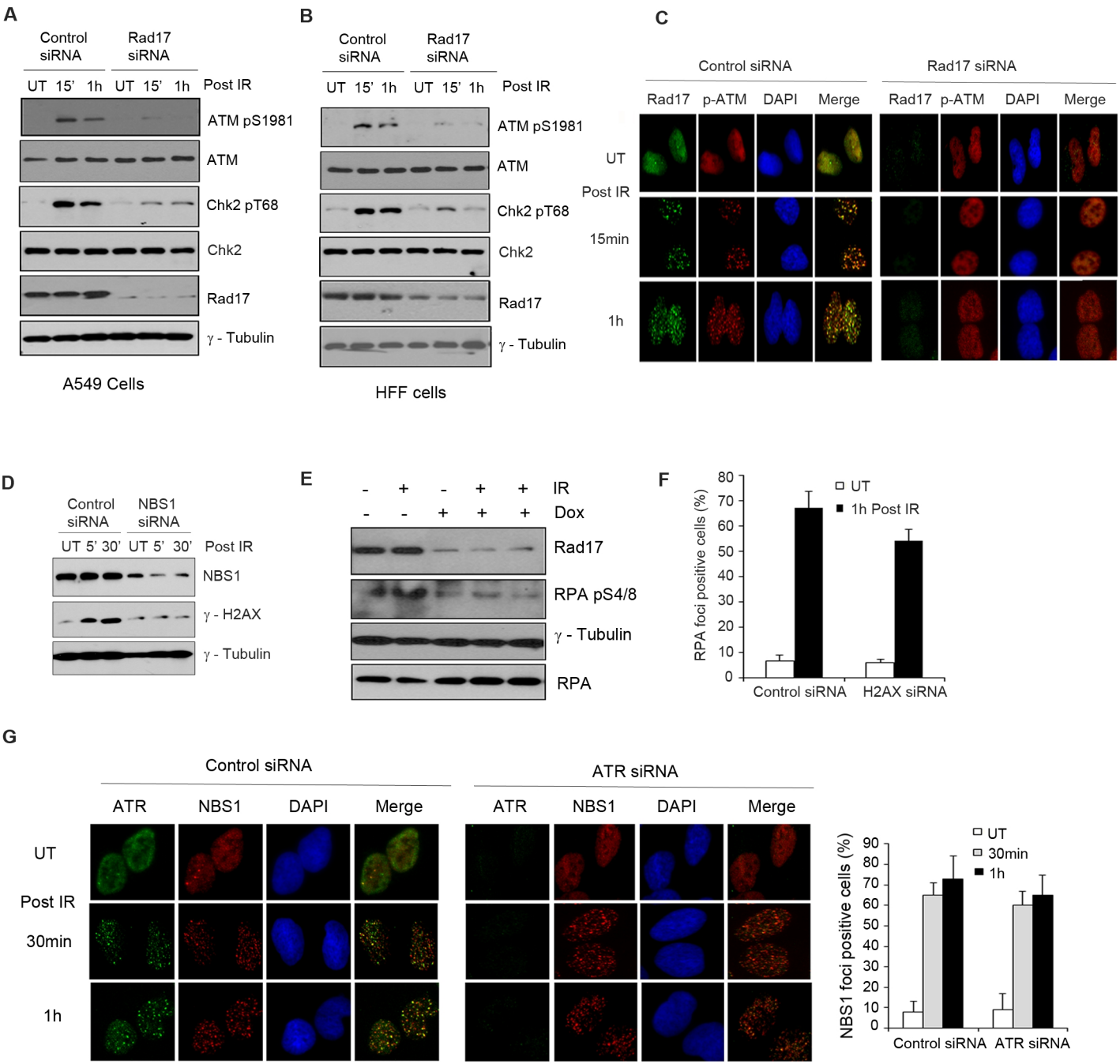
A
Control siRNA
Rad17 siRNA
UT 15' 1h UT 15' 1h Post IR
ATM pS1981
ATM
Chk2 pT68
Chk2
Rad17
γ - Tubulin
A549 Cells
B
Control siRNA
Rad17 siRNA
UT 15' 1h UT 15' 1h Post IR
ATM pS1981
ATM
Chk2 pT68
Chk2
Rad17
γ - Tubulin
HFF cells
C
Control siRNA
Rad17 siRNA
Rad17 p-ATM DAPI Merge
Rad17 p-ATM DAPI Merge
UT
Post IR
15min
1h
D
Control siRNA
NBS1 siRNA
UT 5' 30' UT 5' 30' Post IR
NBS1
γ - H2AX
γ - Tubulin
E
IR
Dox
Rad17
RPA pS4/8
γ - Tubulin
RPA
F
UT
1h Post IR
RPA foci positive cells (%)
Control siRNA
H2AX siRNA
G
Control siRNA
ATR siRNA
ATR NBS1 DAPI Merge
ATR NBS1 DAPI Merge
UT
Post IR
30min
1h
UT
30min
1h
NBS1 foci positive cells (%)
Control siRNA
ATR siRNA

Figure S4 Rad17 regulates ATM activation and DNA end resection following DSB damage. (**A** and **B**) Rad17 promotes ATM activation. A549 cells (**A**) or HFF cells (**B**) transfected with the indicated siRNA were either left untreated or exposed to 5Gy of IR. Western blot was performed. (**C**) U2OS cells transfected with indicated siRNA were either untreated or exposed to 5Gy of IR. Cells were fixed and stained with anti-Rad17 and anti-p-ATM. (**D**) U2OS cells transfected with indicated siRNA were either untreated or exposed to 5Gy of IR. Cell lysates were prepared at indicated times and Western blot was performed as indicated. (**E**) Rad17 is essential for RPA phosphorylation at Ser4/8 sites. Stable inducible Rad17 knockdown cells (U2OS) treated with or without Dox for two days were either left untreated or exposed to 5 Gy of IR. Cell lysates were prepared 1 hour after IR and Western blot was performed. (**F**) H2AX depletion has no significant effect on RPA foci formation. U2OS cells transfected with indicated siRNA were either untreated or exposed to 5Gy of IR. Cells were fixed and stained with anti-H2AX and anti-RPA. The percentage of RPA foci positive cells was plotted (mean ± SD, n=3). (**G**) ATR Knockdown has no significant effect on NBS1 recruitment to DSBs. U2OS cells transfected with indicated siRNA were either untreated or exposed to 5Gy of IR. Cells were fixed and stained with anti-ATR and anti-NBS1. The percentages of NBS1 foci positive cells were plotted (mean ± SD, n=3).