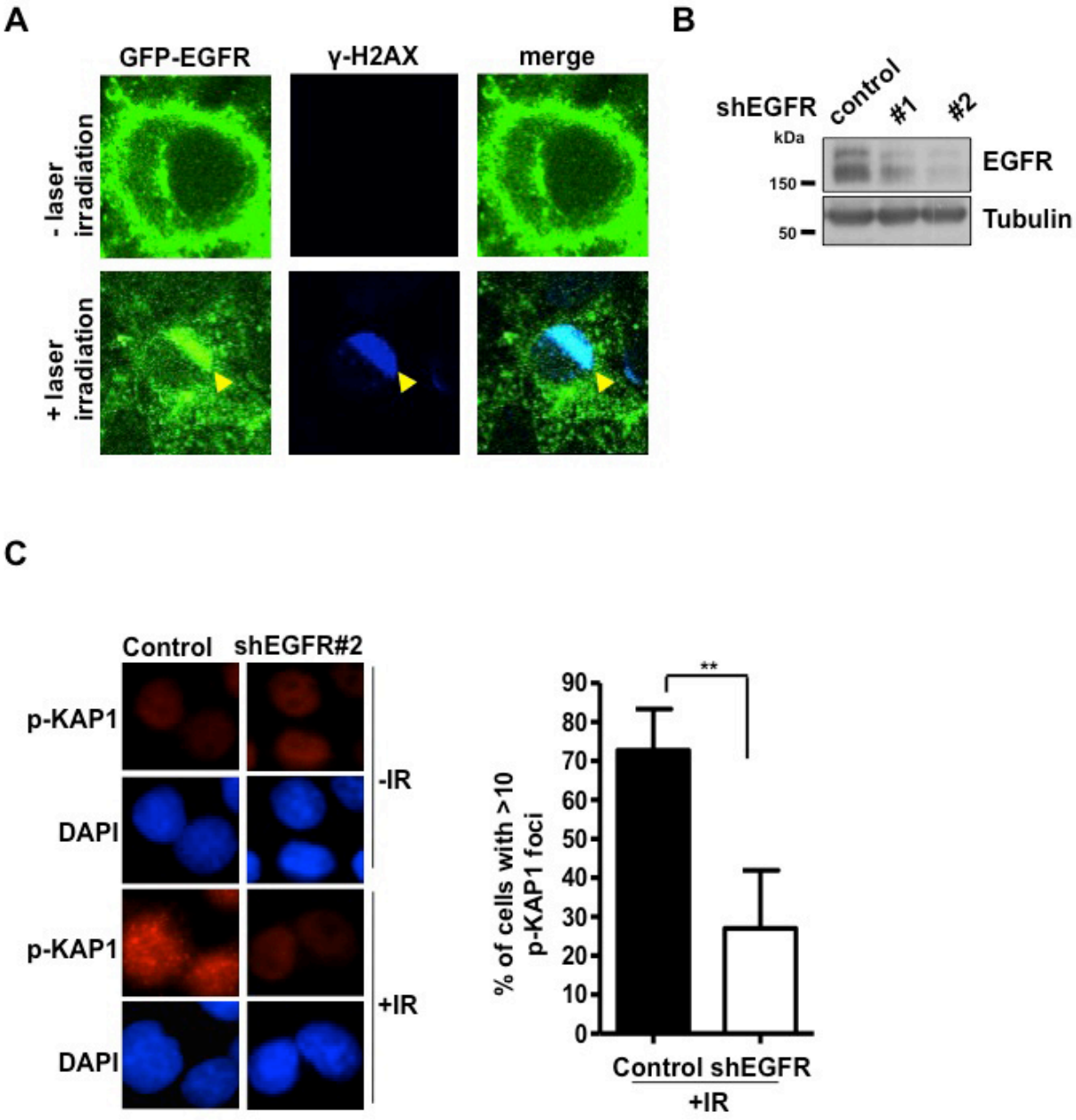
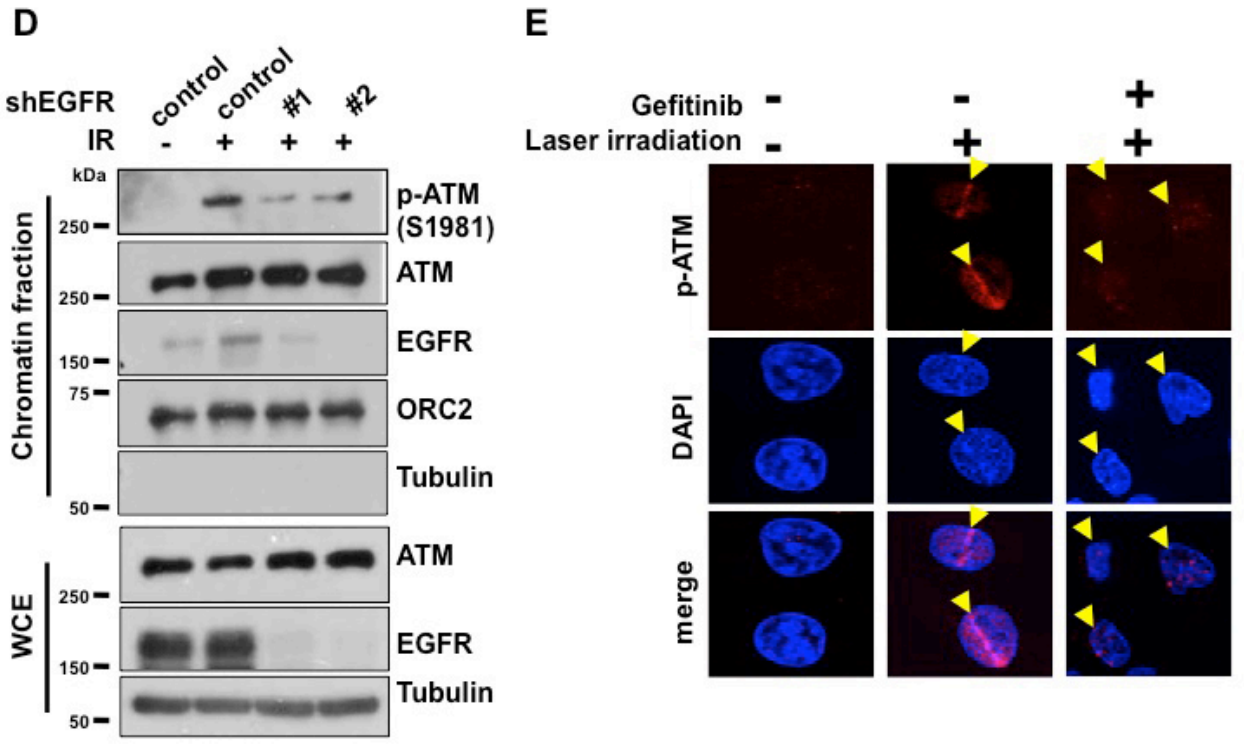

A
GFP-EGFR
γ-H2AX
merge
- laser irradiation
+ laser irradiation
B
shEGFR
control
#1
#2
kDa
150
50
EGFR
Tubulin
C
Control
shEGFR#2
p-KAP1
DAPI
-IR
+IR
% of cells with >10 p-KAP1 foci
**
Control shEGFR
+IR



Supplementary information, Figure S3. EGFR co-localizes with γ-H2AX at laser microirradiation-induced DSBs and is required for ATM S1981 autophosphorylation upon IR stimulation. **(A)** GFP-EGFR transfected U2OS cells are irradiated with 405 nm for 100 ms. Fifteen minutes after irradiation, cells are fixed and stained with indicated antibody. Yellow arrowheads, laser microirradiation-induced DSBs. **(B)** Western blot analysis of control or two different EGFR-knockdown U2OS cells. **(C)** Immunofluorescent (IF) staining of irradiation-induced foci (IRIF) in control or EGFR-depleted (shEGFR) HeLa cells with the indicated antibodies. DAPI: 4,6-diamidio-2-phenylindole. Quantitation of p-KAP1 IRIF is presented as mean ±SD. n = 106. *p < 0.05. **(D)** Chromatin enriched fractionation and WCE of control or EGFR-knockdown HeLa cells stimulated with or without IR. Control: shRNA vector control. #1 and #2 indicate two different clones of shEGFR. WCE: whole cell extract. ORC2: origin replication complex 2, serving as a chromatin fraction marker. Tubulin: cytosolic marker. **(E)**

U2OS cells are treated with or without 10 μM Gefitinib for 16 h and irradiated with 405 nm for 100 ms. 15 min after irradiation, cells are fixed and stained with indicated antibody. DAPI: 4,6-diamidio-2-phenylindole. Yellow arrowheads, laser microirradiation-induced DSBs.