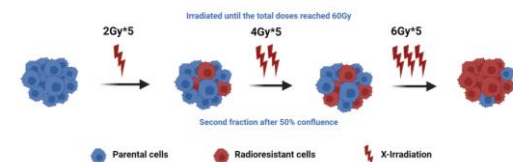
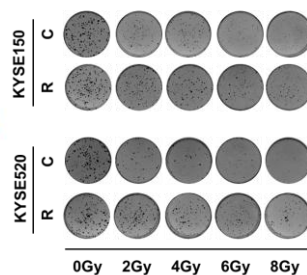


Supple Figure 1.

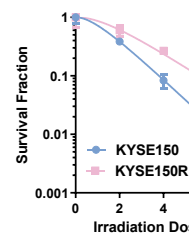
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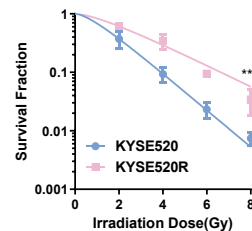
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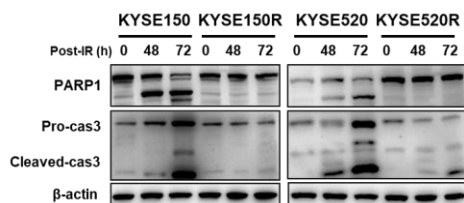
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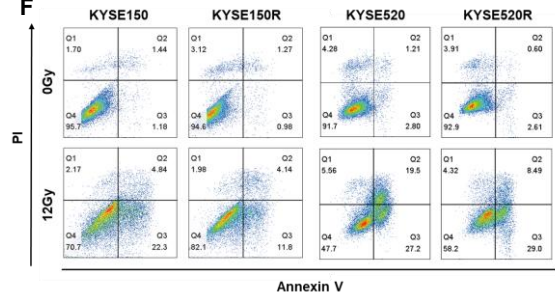
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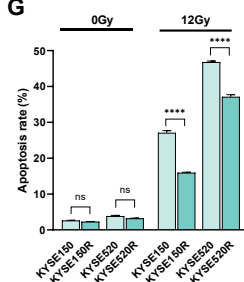
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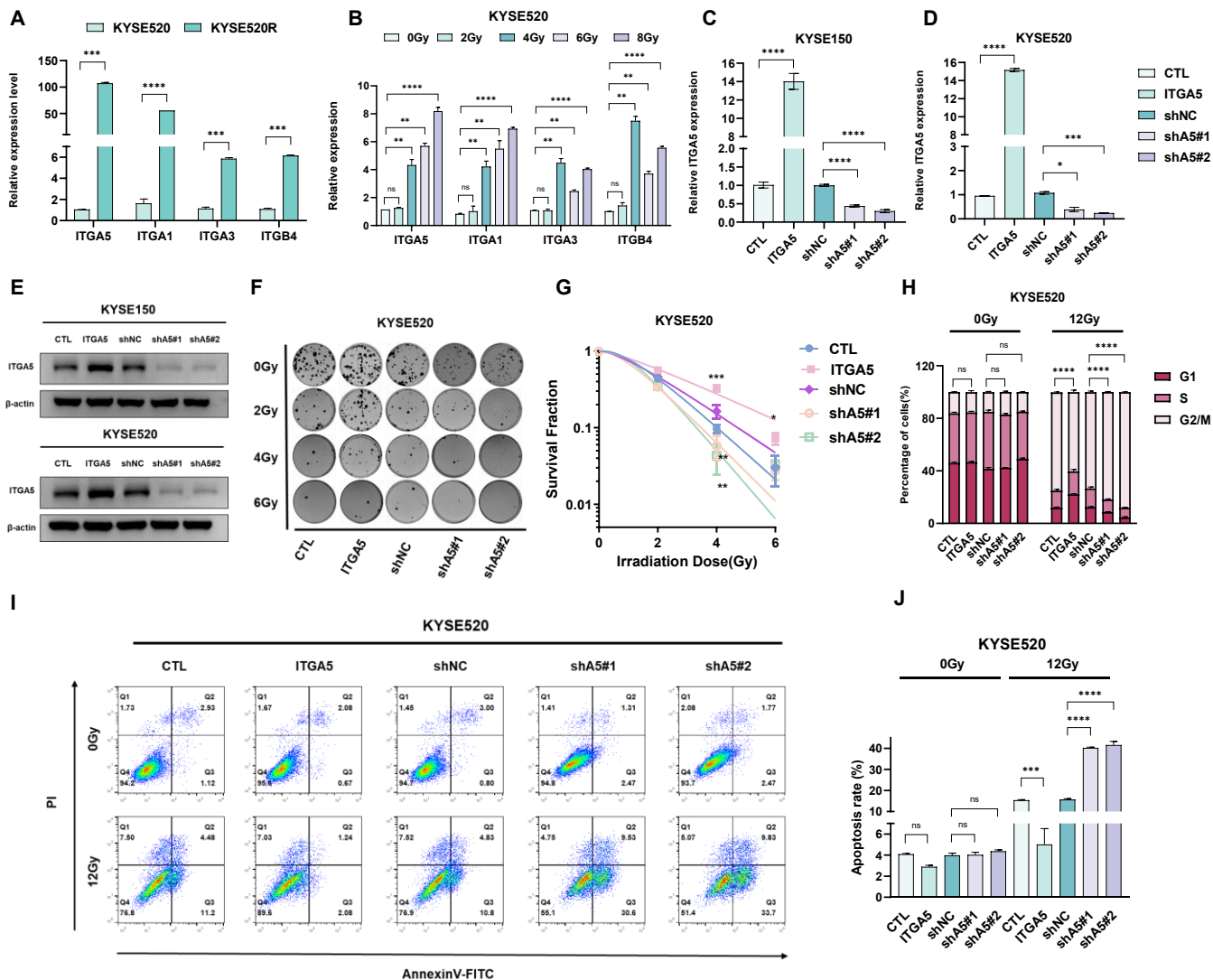
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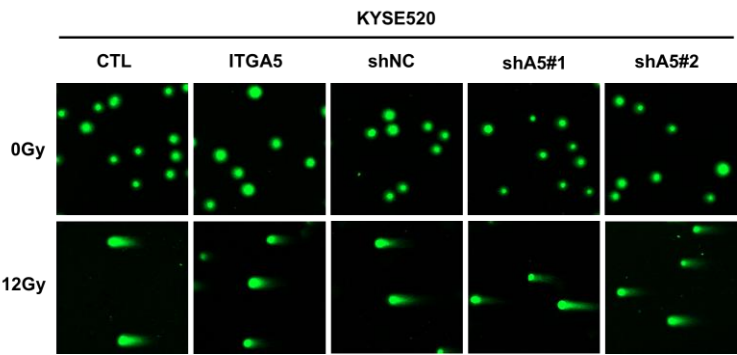


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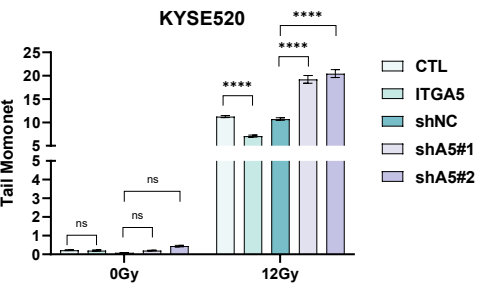


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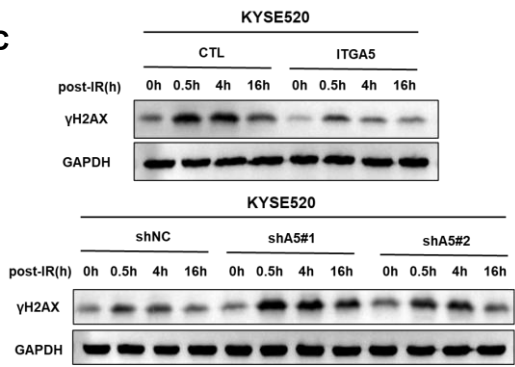
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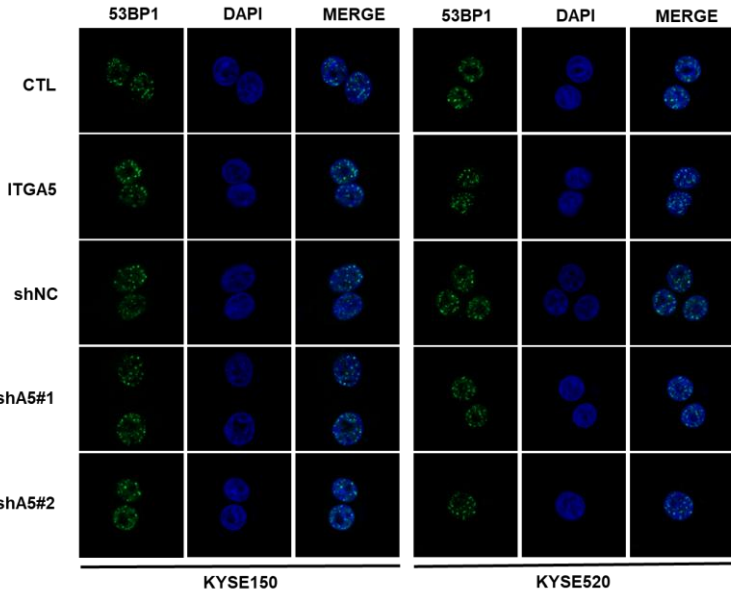
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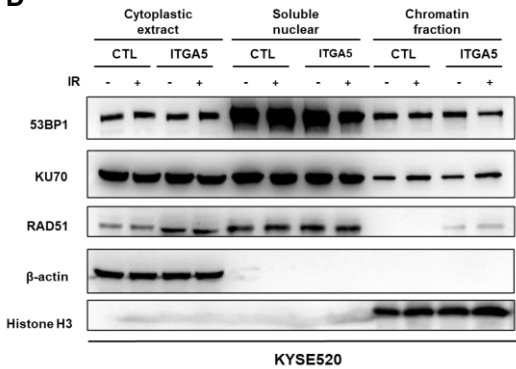
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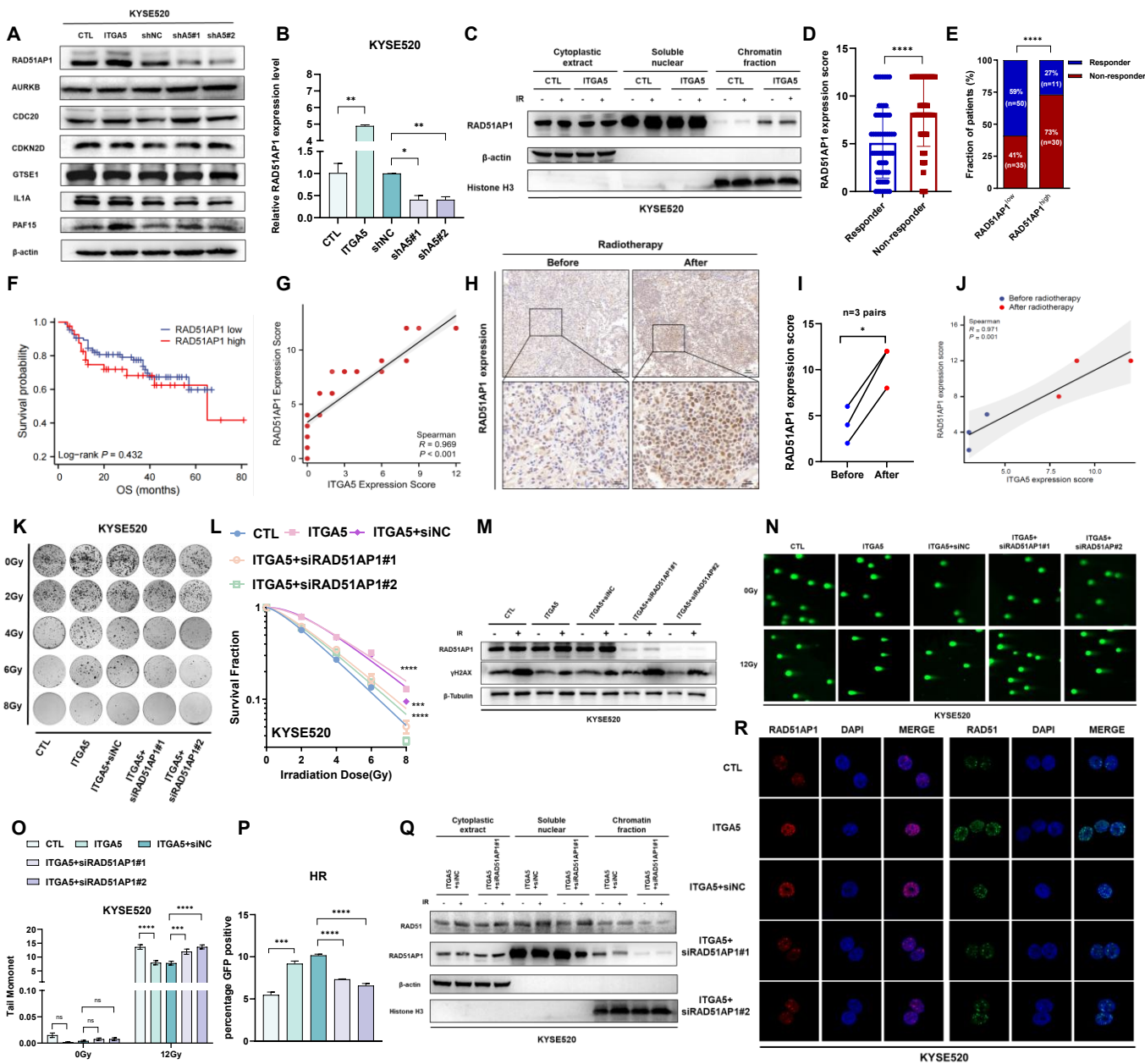
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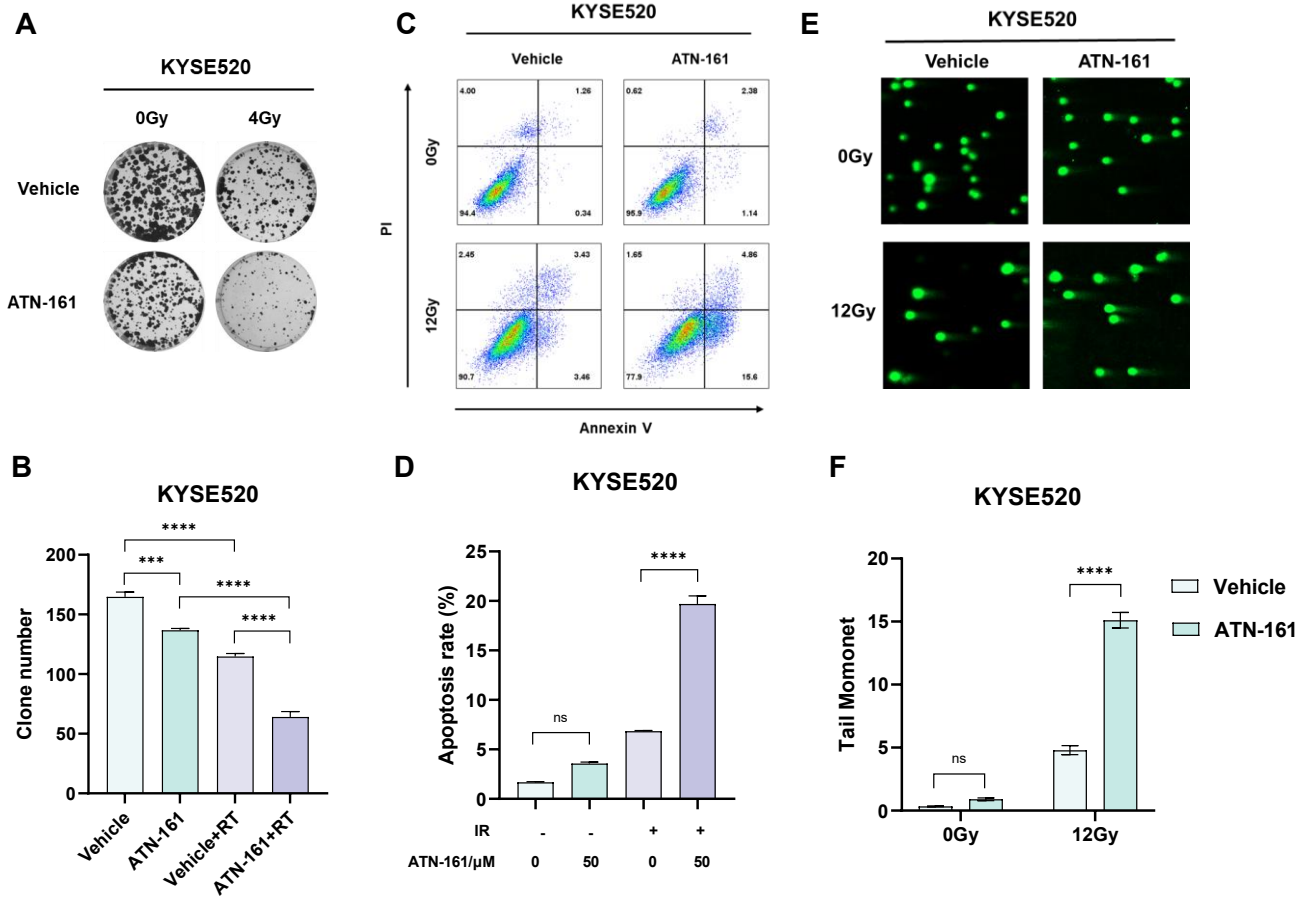
D



Supple Figure 4.



Supple Figure 5.



Supplementary Table**Supplementary Table 1. The sequences of siRNAs**

Name	Sequences
siFAK#1-F	GGGCAUCAUUCAGAAGAUATT
siFAK#1-R	UAUCUUCUGAAUGAUGCCCTT
siFAK#2-F	GAACCUCGCAGUCAUUUAUTT
siFAK#2-R	AUAAAUGACUGCGAGGUUCTT
siRAD51AP1#1-F	CAGCACCAAAGGAGUAAAATT
siRAD51AP1#1-R	GUGCAGAACUCUCAAGAUATT
siRAD51AP1#2-F	UAUCUUGAGAGUUCUGCACTT
siRAD51AP1#2-R	UAUCUUGAGAGUUCUGCACTT
siMYC#1-F	GGAACUAUGACCUCGACUA
siMYC#1-R	UAGUCGAGGUCAUAGUCC
siMYC#2-F	CCAAGGUAGUUAUCCUAA
siMYC#2-R	UUAAGGAUAACUACCUUGG

Supplementary Table 2. The sequences of used primers

Primer name	Sequences
ITGA5-F	GGCTTCAACTTAGACGCGGAG
ITGA5-R	TGGCTGGTATTAGCCTTGGGT
ITGA1-F	CTGGACATAGTCATAGTGCTGGA
ITGA1-R	ACCTGTGTCTGTTTAGGACCA
ITGA3-F	TGTGGCTTGGAGTGACTGTG
ITGA3-R	TCATTGCCTCGCACGTAGC
ITGB4-F	GCAGCTTCCAAATCACAGAGG
ITGB4-R	CCAGATCATCGGACATGGAGTT
RAD51AP1-F	TGGTGGTGTTC AAGGGAAAAG
RAD51AP1-R	AGGTGCAAAGTCTGGTTCAGT
MYC-F	GGCTCCTGGCAAAAGGTCA
MYC-R	CTGCGTAGTTGTGCTGATGT
RAD51AP1-F (ChIP)	GTCCAAGTGCCCCTATGTCC
RAD51AP1-R (ChIP)	GCTTTGTCCCTGCACTTTCG
β -actin-F	CATGTACGTTGCTATCCAGGC
β -actin-R	CTCCTTAATGTCACGCACGAT

Supplementary Table 3. The information of used antibodies

Antibody name	Catalog	Company
PARP1	113371-1-AP	Proteintech
Caspase-3	9662	Cell SignalingTechnology
Cleaved Caspase-3	9661	Cell SignalingTechnology
β -actin	4967	Cell SignalingTechnology
ITGA5	ab150361	Abcam
γ H2AX	ab2893	Abcam
GAPDH	2118	Cell SignalingTechnology
β -Tubulin	ab6046	Abcam
MRE11	ab208020	Abcam
NBS1	14956	Cell SignalingTechnology
RAD51	ab133534	Abcam
53BP1	ab175933	Abcam
KU70	ab92450	Abcam
KU80	ab80592	Abcam
Anti-rabbit IgG, HRP-linked	7074	Cell SignalingTechnology
Anti-mouse IgG, HRP-linked	7076	Cell SignalingTechnology
AF488-labeled Goat Anti-Rabbit IgG (H+L)	A0423	Beyotime
AF555-labeled Donkey Anti-Rabbit IgG (H+L)	A0453	Beyotime

Supplementary Figure Legends

S1. Construction and validation of radioresistant ESCC cell lines. (A) Schematic diagram of ESCC radioresistant cell line construction. (B) Evaluation of the effect of different radiotherapy doses on the cell clonogenic ability. (C-D) Dose-survival curves and radiobiological parameters for KYSE150R and KYSE520R cells. (E) WB analysis of cleaved caspase-3 and PARP1 at 0, 48, and 72 hours post-irradiation. (F) Apoptosis analysis by Annexin V/PI double staining with or without irradiation. (G) Quantitative analysis of radiation-induced apoptosis rates. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

S2. ITGA5 promoted ESCC radioresistance in vitro and vivo. (A) qPCR analysis of integrin family gene expression profiles in KYSE520R versus KYSE520 cells. (B) Dose-dependent effects of irradiation on the mRNA expression of integrin family genes in KYSE520 cells. (C-E) Validation of ITGA5 overexpression and knockdown efficiency in KYSE150 and KYSE520 cell lines. (F) Colony formation ability of ITGA5-overexpressing and -knockdown KYSE520 cells following irradiation at varying doses. (G) Survival fraction curve of KYSE520 cells. (H-I) Cell cycle distribution and apoptosis analysis of KYSE520 cells after irradiation. (J) Statistical analysis of apoptosis ratio of KYSE520 cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

S3. ITGA5 promoted ESCC radioresistance by promoting DNA damage repair through the homologous recombination repair pathway. (A) Comet assay assessing radiation-induced DNA DSBs in KYSE520 cells. (B) Quantitative analysis of comet tail moments in irradiated KYSE520 cells. (C) Time-course WB analysis of γ H2AX activation in irradiated KYSE520 cells. (D) Subcellular fractionation analysis of HR/NHEJ repair protein chromatin recruitment in ITGA5-overexpressing KYSE520 cells. (E) IF analysis of 53BP1 foci formation in KYSE150 and KYSE520 cells after irradiation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

S4. RAD51AP1 was essential for ITGA5-mediated DNA damage repair and radioresistance in ESCC. (A) WB validation of differentially expressed proteins following ITGA5 overexpression or knockdown. (B) qPCR analysis of RAD51AP1 mRNA expression levels under ITGA5 overexpression and knockdown conditions. (C) Chromatin recruitment of RAD51AP1 in ITGA5-overexpressing KYSE520 cells post-irradiation. (D) RAD51AP1 expression patterns in radiosensitive versus radioresistant ESCC patients. (E) Radiotherapy response rates stratified by RAD51AP1 expression levels. (F) Association between RAD51AP1 expression and OS in ESCC patients. (G) Correlation analysis of ITGA5 and RAD51AP1 expression in ESCC patients. (H-I) Representative IHC image and quantitative analysis of RAD51AP1 expression pre- and post-radiotherapy. (J) Correlation between ITGA5 and RAD51AP1 expression before and after radiotherapy. (K-L) Colony formation assay of RAD51AP1-knockdown KYSE520 cells with ITGA5 overexpression. (M) WB detection of the activation levels of γ H2AX in ITGA5-overexpressing and RAD51AP1-knockdown cells. (N) Comet assay of ITGA5-overexpressing and RAD51AP1-knockdown KYSE520 cells. (O) Quantitative analysis of comet tail distance. (P) Effect of knockdown of RAD51AP1 expression on HR repair efficiency of 293T cells overexpressing ITGA5. (Q)

Recruitment of RAD51AP1 on chromatin after knockdown of RAD51AP1 expression in KYSE520 cells overexpressing ITGA5. (R) Effect of knockdown of RDA51AP1 expression in overexpressing ITGA5 KYSE520 cells on the formation of RAD51 and RAD51AP1 foci in cells. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $***P < 0.0001$.

S5. ITGA5 inhibition sensitized ESCC to radiotherapy. (A-B) Representative images and quantitative statistical map of the clone formation ability of KYSE520 cells after blocking ITGA5 with ATN-161. (C-D) Images and statistical map of apoptosis assay of KYSE520 cells after treatment with ATN-161 combined with irradiation. (E) Comet assay to evaluate the DNA damage level of KYSE520 cells after receiving ATN-161 and/or irradiation treatment. (F) Quantitative statistical map of cell tail distance. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $***P < 0.0001$.