

## IL-6/STAT3/TWIST inhibition reverses ionizing radiation-induced EMT and radioresistance in esophageal squamous carcinoma

### Supplementary Materials

**Supplementary Table 1: The sequences of sh-Twist**

No.	sh-Twist Sequence* (Sense strand)	Target Sequence
Sh-Twist-775	5'-GAAGCTGAGCAAGATTCAGACCTTCAA GAGAGGTCTGAATCTTGCTCAGCTTTTTTTTG-3'	5'-AAGCTGAGCAAGATTCAGACC-3'
Sh-Twist--812	5'-GGTACATCGACTTCCTCTACCTTCAAGAGA GGTAGAGGAAGTCGATGTACCTTTTTTTG-3'	5'-GGTACATCGACTTCCTCTACC-3'
Sh-Twist -864	5'-GATGGCAAGCTGCAGCTATGTTTCAAGAGAAC ATAGCTGCAGCTTGCCATCTTTTTTTG-3'	5'-GATGGCAAGCTGCAGCTATGT-3'
Sh-Twist -996	5'-GACCTAGATGTCATTGTTTCCTTCAAGAGAGG AAACAATGACATCTAGGTCTTTTTTTG-3'	5'-GACCTAGATGTCATTGTTTCC-3'

\*shRNA was designed according to twist mRNA uploaded in GenBank (NM\_000474). 5'-TTCAAGAGA-3' (in bold) was designed as loop structure.

**Supplementary Table 2: The sequence of primers**

Gene	The sequence of primers
E-Cadherin	Forward primer(5'-3'): TTGCTACTGGAACAGGGACACT Reverse primer(5'-3'): GGAGATGTATTGGGAGGAAGGTC
N-Cadherin	Forward primer(5'-3'): CATCCTGCTTATCCTTGTGCTG Reverse primer(5'-3'): CTGGTCTTCTTCTCCTCCACCTT
Vimentin	Forward primer(5'-3'): AAGGAGGAAATGGCTCGTCAC Reverse primer(5'-3'): CTCAGGTTTCAGGGAGGAAAAGT
IL-6	Forward primer(5'-3'):GGGAACGAAAGAGAAGCTCT Reverse primer(5'-3'):ACCAGAAGAAGGAATGCCCA
Stat3	Forward primer(5'-3'): GAGGCCAACATACATGCCTTC Reverse primer(5'-3'): GCTCCCGCTCCTTACTGATAAAG
Twist	Forward primer(5'-3'): AGCAAGATTCAGACCCTCAAGC Forward primer(5'-3'): AGCAAGATTCAGACCCTCAAGC
GAPDH	Forward primer(5'-3'): TGA CTTCAACAGCGACACCCA Reverse primer(5'-3'): CACCCTGTTGCTGTAGCCAAA

**Supplementary Table 3: Concentration of IL-6 (pg/ml) detected by ELISA**

dose	24 h	48 h
0 Gy	209.19 ± 8.24	209.19 ± 8.24
4 Gy	305.29 ± 18.32*	344.14 ± 25.66*
8 Gy	266.42 ± 11.18*	285.22 ± 8.48*

The data represent the mean ± SEM of three independent experiments. \* means significant difference  $P < 0.05$  compared with the value of cells under 0 Gy IR.

**Supplementary Table 4: The radiobiology parameters of cells detected by colony-forming assay**

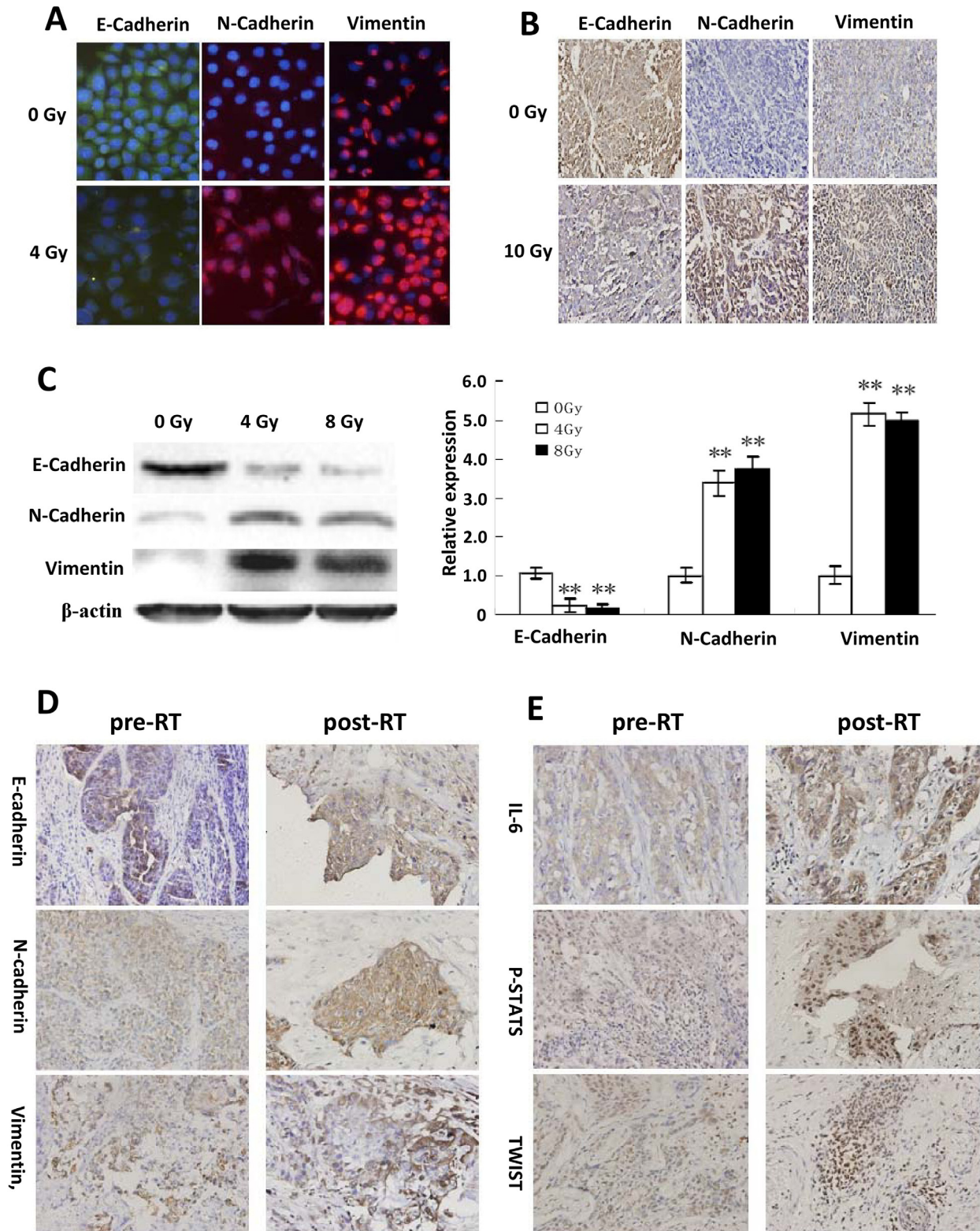
Group	SF2	D0 (Gy)	Dq (Gy)	N	SERD0	SERDq
Eca109	0.74 ± 0.01	3.14 ± 0.17	1.87 ± 0.14	1.81 ± 0.38	1	1
Eca109+4 Gy	0.81 ± 0.02*	3.48 ± 0.22*	2.49 ± 0.25*	2.04 ± 0.69*	0.89 ± 0.01*	0.75 ± 0.03*
Eca109R	0.89 ± 0.01*	3.92 ± 0.35*	3.59 ± 0.27**	2.50 ± 0.82**	0.80 ± 0.10**	0.52 ± 0.06**

The data represent the mean ± SEM of three independent experiments. \* and \*\* on behalf of significant difference  $P < 0.05$  and  $P < 0.01$  respectively compared with Eca109 group.

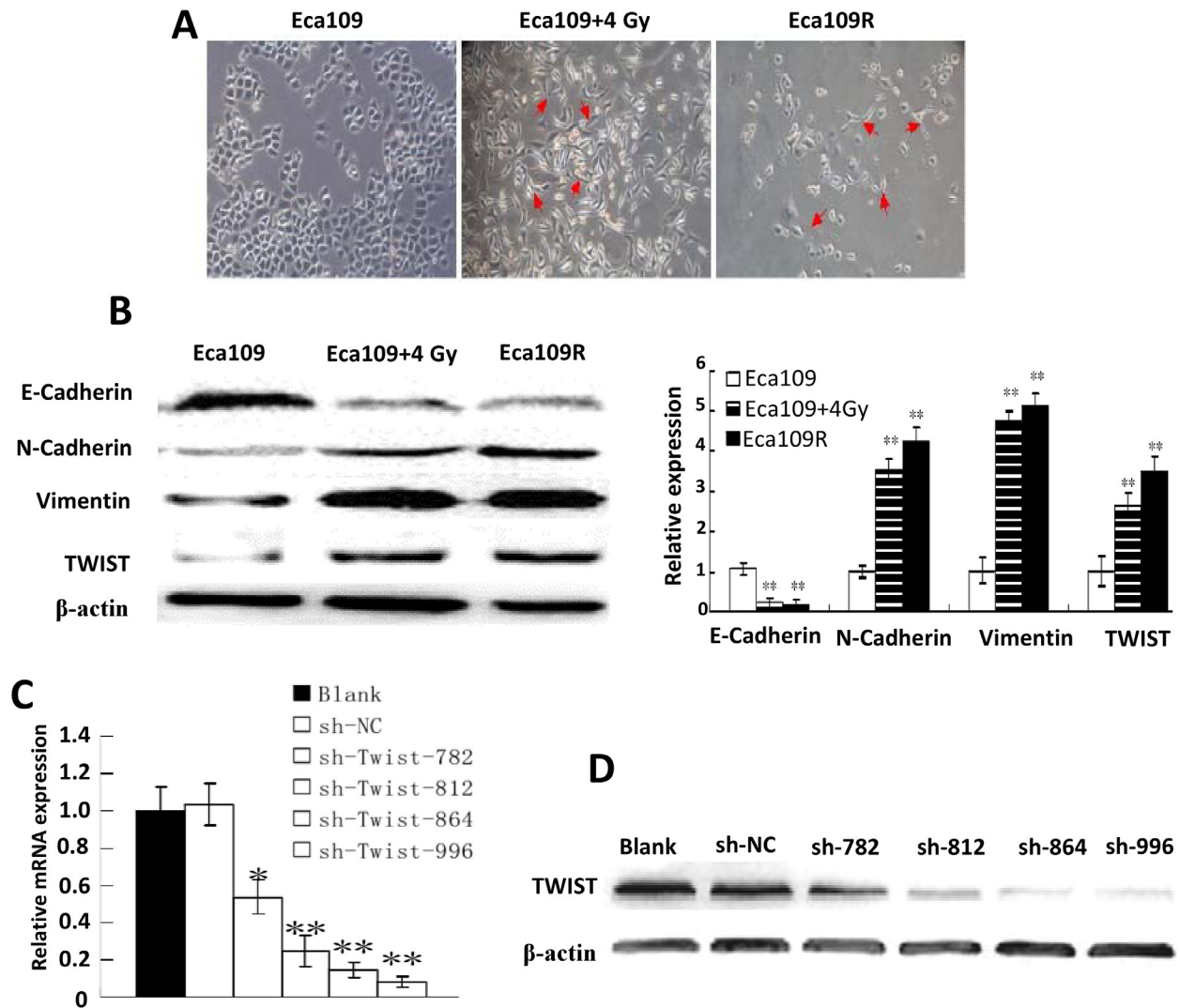
**Supplementary Table 5: Sensitizing irradiation detected by colony-forming assay**

Group	SF2	D0 (Gy)	Dq (Gy)	N	SERD0	SERDq
Eca109R/sh-NC	0.89 ± 0.01	3.92 ± 0.35	3.59 ± 0.27	2.50 ± 0.82	1	1
Eca109R/sh-Twist	0.71 ± 0.03*	3.20 ± 0.24*	1.53 ± 0.31**	1.61 ± 0.47**	1.22 ± 0.17*	2.36 ± 0.16**

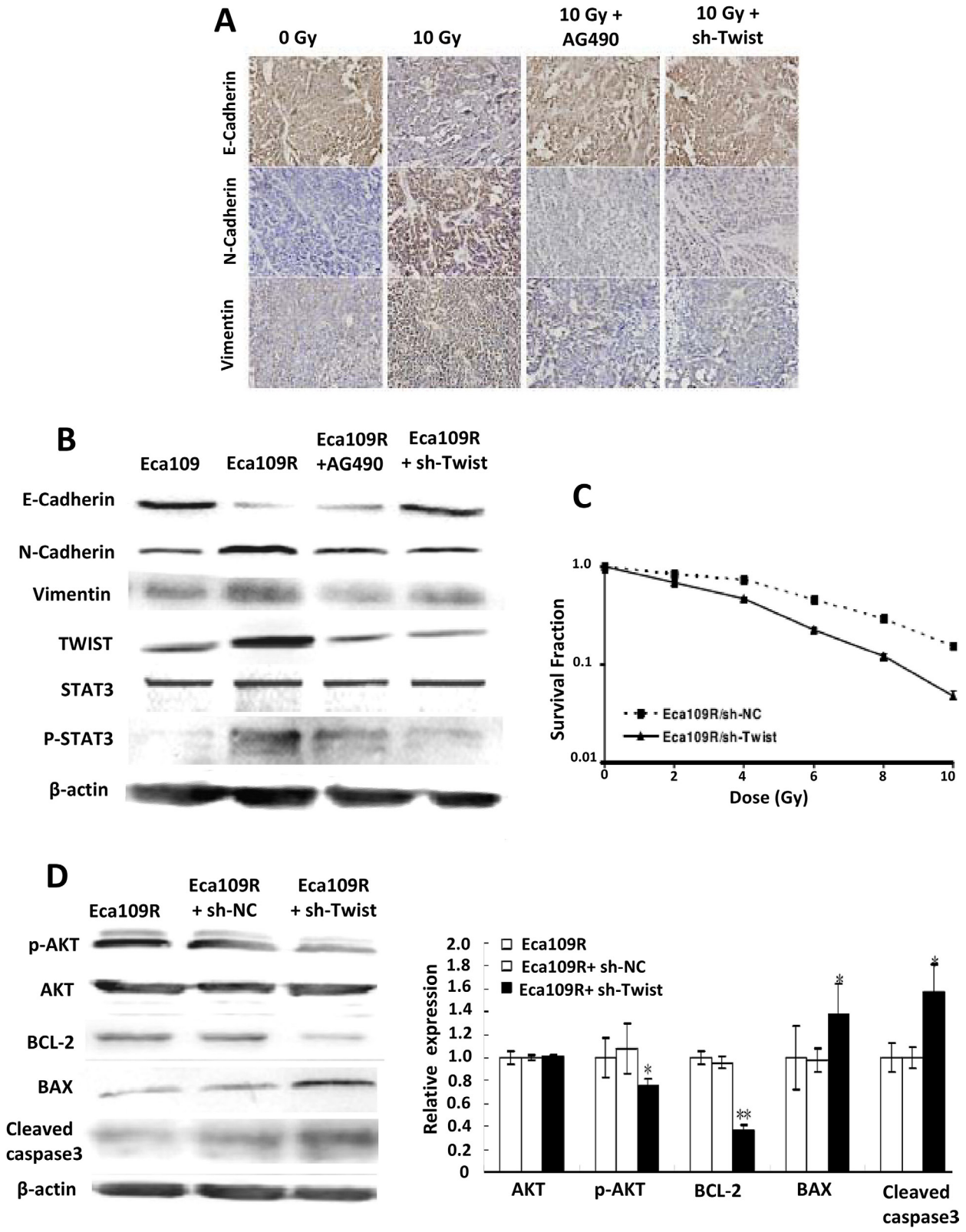
The data represent the mean ± SEM of three independent experiments. \* and \*\* on behalf of significant difference  $P < 0.05$  and  $P < 0.01$  respectively compared with Eca109R/sh-NC group.



**Supplementary Figure 1:** (A) Immunofluorescence detection of the EMT biomarkers expression. (B) Immunohistochemistry detection of the EMT biomarkers expression in xenograft tumors. (C) Western-blotting detection of the EMT biomarkers expression (left) and their quantitative analysis (right). (D) Immunohistochemistry detection of the EMT biomarkers expression in ESC patients. (E) Immunohistochemistry detection of the IL-6/STAT3/TWIST pathway activation in ESC patients.



**Supplementary Figure 2:** (A) Representative morphological changes in Eca109R and Eca109 + 4 Gy cells; (B) Western-blotting detection of the EMT biomarkers expression in Eca109R and Eca109 + 4 Gy cells (left) and their quantitative analysis (right). (C) Quantitative PCR detection of the silenced twist mRNA induced by the four shRNA. (D) Western-blotting detection of TWIST expression to verify the efficacy of the four shRNA.



**Supplementary Figure 3:** (A) Immunofluorescence detection of the EMT biomarkers expression in xenografted tumors exposed to IR, AG490 or sh-Twist. (B) Western-blotting detection of the EMT biomarkers change and IL-6 pathway inhibition under AG490 or sh-Twist treatment. (C) Eca109R cells' reversed radioresistance detected by colony-forming assay. (D) Western-blotting detection of apoptosis related proteins and AKT activation (left), and their quantitative analysis (right).