

## **Additional file 1**

### **SOX17 overexpression sensitizes chemoradiation response in esophageal cancer by transcriptional down-regulation of DNA repair and damage response genes**

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**Table S1.** The primers used in the current study.

| Gene                              | Primer               | 5'→3' Sequences                                                      | Application <sup>a</sup> | PCR size (bp)   | T <sub>m</sub> (°C) |
|-----------------------------------|----------------------|----------------------------------------------------------------------|--------------------------|-----------------|---------------------|
| <i>SOX17</i> gene_<br>(+131~+169) | Biotin label-Forward | 5'-GGG ACA CCG CTG ATC GTT TAG GGT TTT GGG TAA GTT GTA GAT TAG AT-3' | Pyrosequencing - PCR     | 172             | 56-                 |
|                                   | Reverse              | 5'-ACC TAC CCC CCT CCC CTC AA-3'                                     |                          |                 |                     |
|                                   | Sequencing           | 5'-TCC CCT CAA CCC TCC-3'                                            | Pyrosequencing           | -- <sup>b</sup> | -- <sup>b</sup>     |
| <i>SOX17</i> mRNA                 | Forward              | 5'-ACG CTT TCA TGG TGT GGG CTA AG-3'                                 | RT-qPCR                  | 113             | 60                  |
|                                   | Reverse              | 5'-GTC AGC GCC TTC CAC GAC TTG-3'                                    |                          |                 |                     |
| <i>BRCA1</i> mRNA                 | Forward              | 5'-TTG CAG TGT GGG AGA TCA AG-3'                                     | RT-qPCR                  | 168             | 60                  |
|                                   | Reverse              | 5'-CGC TTC TCA GTG GTG TTC AA-3'                                     |                          |                 |                     |
| <i>BRCA2</i> mRNA                 | Forward              | 5'-ACC CAG CTT ACC TTG AGG GTT ATT T-3'                              | RT-qPCR                  | 185             | 60                  |
|                                   | Reverse              | 5'-AAT ACG CAA CTT CCA CAC GGT TG -3'                                |                          |                 |                     |
| <i>RAD51</i> mRNA                 | Forward              | 5'-ATG GTC TCT CTG GCA GTG ATG T -3'                                 | RT-qPCR                  | 124             | 60                  |
|                                   | Reverse              | 5'-AGC AGT GCA TAC CTA GAT TCT ACC A -3'                             |                          |                 |                     |
| <i>KU80</i> mRNA                  | Forward              | 5'-CCC CAA TTC AGC AGC ATA TT-3'                                     | RT-qPCR                  | 157             | 60                  |
|                                   | Reverse              | 5'-CCT TCA GCC AGA CTG GAG AC-3'                                     |                          |                 |                     |
| <i>DNAPK</i> mRNA                 | Forward              | 5'-CAT GGA AGA AGA TCC CCA GA -3'                                    | RT-qPCR                  | 155             | 60                  |
|                                   | Reverse              | 5'-TGG GCA CAC CAC TTT AAC AA -3'                                    |                          |                 |                     |

|                                      |         |                                   |           |     |    |
|--------------------------------------|---------|-----------------------------------|-----------|-----|----|
| <i>NFAT5</i> mRNA                    | Forward | 5'-TGA GGG AAA GGA GCT GAA GA -3' | RT-qPCR   | 165 | 55 |
|                                      | Reverse | 5'-CCC ACA AAC ACT TGC AAC AC-3'' |           |     |    |
| <i>p21</i> mRNA                      | Forward | 5'-TCA CCG AGA CAC CAC TGG AG-3'  | RT-qPCR   | 300 | 60 |
|                                      | Reverse | 5'-TGG AGT GGT AGA AAT CTG TC-3'  |           |     |    |
| <i>REV3L</i> mRNA                    | Forward | 5'-TTC CGA AAA GCA AGA AGG AA-3'  | RT-qPCR   | 149 | 60 |
|                                      | Reverse | 5'-TGT GGT TCA ACA CCT TCC AA-3'  |           |     |    |
| <i>SIRT1</i> mRNA                    | Forward | 5'-GCA GAT TAG TAG GCG GCT TG-3'  | RT-qPCR   | 152 | 60 |
|                                      | Reverse | 5'-TCT GGC ATG TCC CAC TAT CA-3'  |           |     |    |
| <i><math>\beta</math>-actin</i> mRNA | Forward | 5'-GGC GGC ACC ACC ATG TAC CCT-3' | RT-qPCR   | 180 | 60 |
|                                      | Reverse | 5'-AGG GGC CGG ACT CGT CAT ACT-3' |           |     |    |
| <i>BRCA1</i>                         | Forward | 5'-CCG CTC TGG TAT TGG ATG TT-3'  | ChIP-qPCR | 234 | 60 |
|                                      | Reverse | 5'-CGA AGG TCA GAA TCG CTA CC-3'  |           |     |    |
| <i>BRCA2</i>                         | Forward | 5'-TTG TAA GAT CGG CTC GCT TT-3'  | ChIP-qPCR | 127 | 60 |
|                                      | Reverse | 5'-TTT GCT CCA GCT CAT GTT TG-3'  |           |     |    |
| <i>RAD51</i>                         | Forward | 5'-CCG TAC GCT AGC TCC ATT TC-3'  | ChIP-qPCR | 175 | 60 |
|                                      | Reverse | 5'-AGC GCT CTT GTG GTT TGT TT-3'  |           |     |    |
| <i>KU80</i>                          | Forward | 5'-AAG CTG GAG CCA CTG CTA AC-3'  | ChIP-qPCR | 155 | 60 |
|                                      | Reverse | 5'-TTA GAG CCA ACT GGG GAA GA-3'  |           |     |    |
| <i>NFAT5</i>                         | Forward | 5'-TTC AGC CTC CAA AGG TAG GA-3'  | ChIP-qPCR | 153 | 60 |
|                                      | Reverse | 5'-CTT TGG GGT TTG CAT AGC AG-3'  |           |     |    |

|              |         |                                  |           |     |    |
|--------------|---------|----------------------------------|-----------|-----|----|
| <i>p21</i>   | Forward | 5'-TGC CAC TGC TGA CTT TGT CT-3' | ChIP-qPCR | 176 | 60 |
|              | Reverse | 5'-ATA GGG GCA GTC AGC TTT CA-3' |           |     |    |
| <i>REV3L</i> | Forward | 5'-GAA CTT GAA CCC ACG CTT TC-3' | ChIP-qPCR | 177 | 60 |
|              | Reverse | 5'-TCC TCT CTG ACT TGG CCA CT-3' |           |     |    |
| <i>SIRT1</i> | Forward | 5'-GGT GTG AGG AGA GTG GGAAA-3'  | ChIP-qPCR | 167 | 60 |
|              | Reverse | 5'-GTA CAC CTG GCC TGC CTT AG-3' |           |     |    |

<sup>a</sup> RT-qPCR: Quantitative reverse transcription-polymerase chain reaction; ChIP-qPCR: chromatin-immunoprecipitation-polymerase chain reaction.

<sup>b</sup> --: Not applicable.

**Table S2.** Antibodies and their reaction conditions.

| Target               | KD  | Raised In | Application <sup>a</sup> | Dilution | Source     | Catalog No. |
|----------------------|-----|-----------|--------------------------|----------|------------|-------------|
| β-actin              | 42  | Mouse     | Western blot             | 1:5000   | GeneTex    | GTX26267    |
| BRCA1                | 220 | Mouse     | IHC                      | 1:1000   | Santa Cruz | Sc-642      |
|                      |     |           | Western blot             | 1:1000   |            |             |
| RAD51                | 37  | Rabbit    | Western blot             | 1:3000   | Abcam      | Ab133534    |
| KU80                 | 80  | Mouse     | Western blot             | 1:1000   | Neomarkers | MS-332-P-1  |
| DNAPK                | 450 | Rabbit    | Western blot             | 1:1000   | Calbiochem | PC127       |
| GAPDH                | 37  | Mouse     | Western blot             | 1:2000   | Santa Cruz | Sc-32233    |
| p21                  | 21  | Mouse     | IHC                      | 1:1200   | Santa Cruz | Sc-817      |
|                      |     | Rabbit    | Western blot             | 1:1000   | Santa Cruz | Sc-397      |
| SIRT1                | 120 | Mouse     | IHC                      | 1:1200   | Upstate    | 05-707      |
|                      |     |           | Western blot             | 1:1000   |            |             |
| SOX17<br>(clone 2F9) | 55  | Mouse     | IHC                      | 1:100    | Origene    | TA500045    |
|                      |     |           | Western blot             | 1:1000   |            |             |
| SOX17                | 55  | Mouse     | ChIP                     | 1:1000   | R&D        | AF1924      |

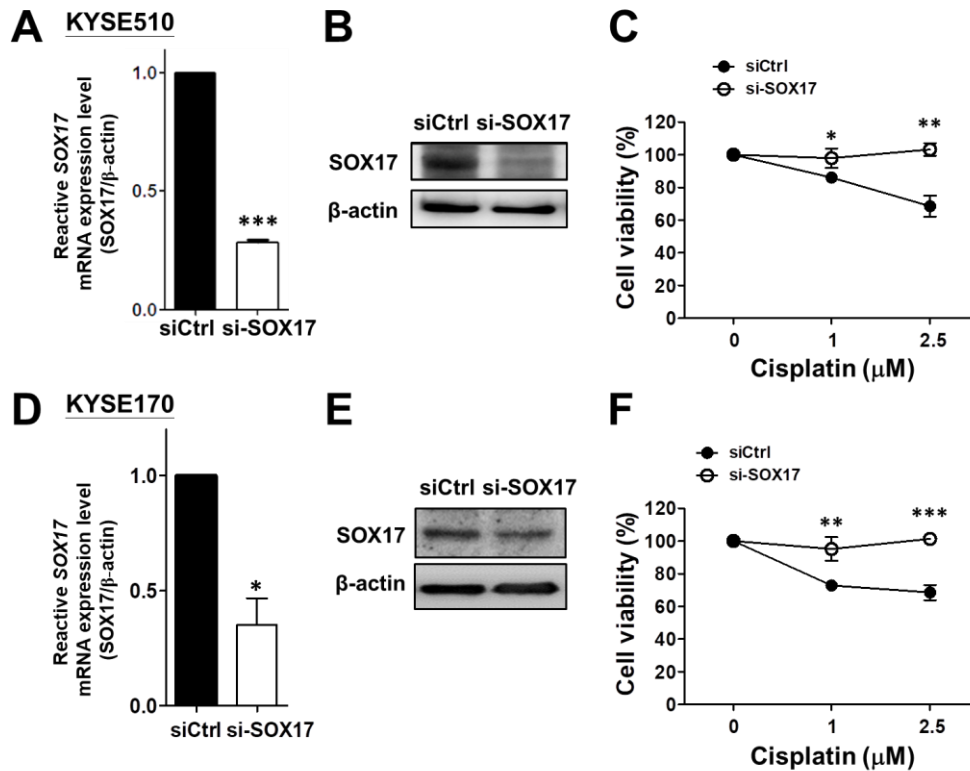
<sup>a</sup> IHC: immunohistochemistry; ChIP: chromatin-immunoprecipitation.

**Table S3.** SOX17 binding sites in promoter of DNA repair genes and putative DNA damage response genes in the current study.

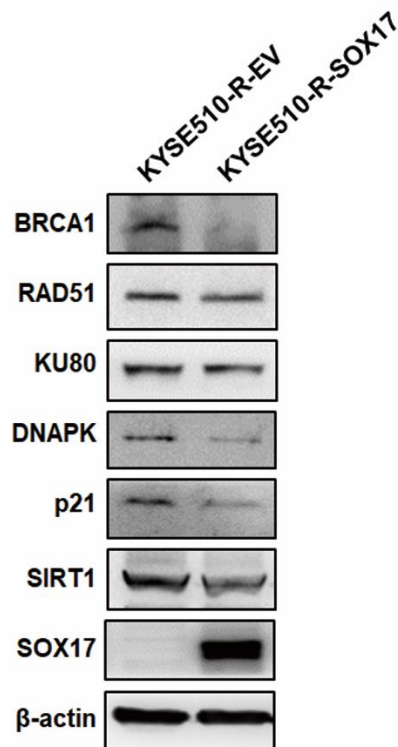
| <b>Symbol</b>            | <b>Gene Name</b>                                                                                              | <b>Function</b>                                                                                                      | <b>Number of SOX17 binding sites<sup>a</sup></b> |
|--------------------------|---------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| <i>BRCA1</i>             | <i>breast cancer 1, early onset</i>                                                                           | Homologous recombination (HR) repair pathway                                                                         | 11                                               |
| <i>BRCA2</i>             | <i>breast cancer 2, early onset</i>                                                                           | Homologous recombination (HR) repair pathway                                                                         | 8                                                |
| <i>RAD51</i>             | <i>RAD51 homolog (S. cerevisiae)</i>                                                                          | Homologous recombination (HR) repair pathway                                                                         | 4                                                |
| <i>KU80</i>              | <i>X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining)</i> | Non-homologous end joining (NHEJ) repair pathway                                                                     | 7                                                |
| <i>DNAPK</i>             | <i>protein kinase, DNA-activated, catalytic polypeptide</i>                                                   | Non-homologous end joining (NHEJ) repair pathway                                                                     | 5                                                |
| <i>p21<sup>b</sup></i>   | <i>cyclin-dependent kinase inhibitor 1A</i>                                                                   | DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest                        | 15                                               |
| <i>SIRT1<sup>b</sup></i> | <i>sirtuin 1</i>                                                                                              | Deacetylate histones and non-histone proteins to trigger cell survival in response to stresses and DNA damage        | 7                                                |
| <i>NFAT5<sup>b</sup></i> | <i>Nuclear factor of activated T-cells 5, tonicity-responsive</i>                                             | Transcription regulation, response to hypertonic stress and facilitate cell proliferation and cell cycle progression | 9                                                |
| <i>REV3L<sup>b</sup></i> | <i>REV3-like, polymerase (DNA directed), zeta, catalytic subunit</i>                                          | Error-prone translesion DNA synthesis, and trigger cell defense and survival response to stress and DNA damage       | 7                                                |

<sup>a</sup> Number of SOX17-SRY elements (5'-(A/T)(A/T)CAA(A/T)G-3') in promoter region (-1000 ~ +1) were predicted by PROMO software. The genomic map of putative SOX17-SRY binding sites is shown in [Figure 4](#).

## Supplementary figure and figure legend



**Figure S1** Knockdown of SOX17 sustained the cell viability upon cisplatin treatment in the KYSE510 and KYSE170 cells. (A, D) RT-qPCR showed low *SOX17* mRNA expression upon si-knockdown of SOX17 (si-SOX17) in KYSE510 cells (A) or KYSE170 cells (D). (B, E) Western blots confirmed low SOX17 protein expression in si-SOX17 KYSE510 cells (B) or KYSE170 cells (E).  $\beta$ -actin was used as an internal control. (C, F) Knockdown of SOX17 sustained cell viability measured by MTT assay in si-SOX17 KYSE510 cells (C) or KYSE170 cells (F). The relative cell viability was normalized to si-control (siCtrl) group. Data represent mean  $\pm$  SD from three independent experiments. *P*-values were determined by two-tailed Student's *t*-test. \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.



**Figure S2** Overexpression of SOX17 in KYSE510-R-SOX17 cells reduced the protein expression level of DNA repair and damage-responsive genes analyzed as compared to KYSE510-R-EV cells.  $\beta$ -actin was used as an internal control for western blots.