The E3 Ligase TRIM4 Facilitates SET Ubiquitin-Mediated Degradation to Enhance ER-α Action in Breast Cancer

Dianwen Han, Lijuan Wang, Li Long, Peng Su, Dan Luo, Hanwen Zhang, Zheng Li, Bing Chen, Wenjing Zhao, Ning Zhang, Xiaolong Wang, Yiran Liang, Yaming Li, Guohong Hu and Qifeng Yang*
Supplementary Materials for

The E3 ligase TRIM4 facilitates SET ubiquitin-mediated degradation to enhance ER-α action in breast cancer

Dianwen Han¹, †, Lijuan Wang², †, Li Long¹, ³, Peng Su⁴, Dan Luo¹, Hanwen Zhang¹, Zheng Li¹,
Bing Chen², Wenjing Zhao², Ning Zhang¹, Xiaolong Wang¹, Yiran Liang¹, Yaming Li¹,
Guohong Hu⁵ and Qifeng Yang¹, ², ⁶, *

*Address correspondence to: Qifeng Yang (qifengy_sdu@163.com).
Supplementary Text

Materials and methods

qPCR

Trizol (Invitrogen) was used to extract total RNA from cells based on provided directions, after which reverse transcriptase (Takara, Shiga, Japan) was used to prepare cDNA, and qPCR analyses were performed with a SYBR Green RT-PCR kit (Roche, Basel, Switzerland). GAPDH expression was used to normalize gene expression. All qPCR primers used in this study are listed in Supplementary Table 1.

Mass spectrometry analyses.

For mass spectrometry analysis, IP was performed with the whole cell lysates in MCF7 cells transfected with Flag-tagged TRIM4 or control plasmids. The IP proteins were resolved by SDS-PAGE, followed by Coomassie staining and the next mass spectrometry analyses were performed by Jingjie PTM BioLab (Hangzhou, China).

Immunofluorescent (IF) and confocal microscopy

IF analyses were performed as in a prior report [1]. Briefly, MCF7 cells were added onto glass coverslips in 24-well plates. Following TRIM4-GFP and SET-Myc plasmid transfection, cells were incubated for 90 min with anti-Myc without permeabilization. They were then washed two times with PBS, probed with a secondary Alexa Fluor 633-conjugated antibody for 30 min and imaged with a laser-scanning confocal microscope (Nikon confocal microscopy, Eclipse Ti A1).

Colony formation assay.

Colony forming ability were assessed using a colony formation assay which was performed as previously described [2]. The transfected cells were seeded into 6 cm plates at a density of $5 \times 10^3$ cells/well for 24 h, followed by treatment with indicated TAM for 48 h. After cultured with new
medium for 2 weeks, the cells were then washed twice with cold PBS, fixed with methanol-glacial acetic acid stationary solution (3:1), and stained with 0.5% crystal violet solution. Pictures were imaged and counted.

**Transwell.**

Transwell assay was carried out as previously described [3]. Briefly, after transfection, cells underwent a 6 h serum starvation step, $1 \times 10^5$ MCF7/TR or T47D/TR cells and $2 \times 10^5$ MCF-7 or T47D cells were suspended in 200μL serum-free medium and seeded into the inside of each transwell insert (Corning Costar, USA), while 700 μL medium containing 20% FBS was placed in the lower well. After incubation for 24-72 h, the infiltrating cells on the lower surface were fixed with methanol and stained with 0.1% crystal violet. Cell invasion assay was conducted using the same procedure as in the cell migration assay, except that the inside of each insert was coated with Matrigel (BD Biosciences, San Jose, CA, USA).

**Patient-derived organoid (PDO) culture**

After mincing, ER-α -positive breast cancer tissue was digested for 3 h using digest medium (10 mM HEPES, 2% BSA, 50 μg/mL gentamycin, 0.5 μg/mL hydrocortisone, 2 mg/mL collagenase IV (Sigma C5138), 1000 U/mL hyaluronidase (Sigma H3884), 1% penicillin/streptomycin and 10% FBS in DMEM) at 37 °C. Cells were then rinsed and suspended in 3D Matrigel with human complete organoid medium composed of advanced DMEM/F12 (Gibco 12634010) containing 10 mM HEPES (Invitrogen 15630-056), 1% penicillin/streptomycin, 1% GlutaMAX (Invitrogen 35050), 50 ng/mL human EGF (Peprotech 315-09, NJ, USA), 1 mM N-acetyl-L-cysteine (Sigma A9165), 100 ng/mL Noggin (Peprotech 250-38), 500 ng/mL R-spondin (BioLegend 783606, CA, USA), 1×N2 (Gibco 17502-048), 1×B27 (Gibco 17504-044), 10 mM Nicotinamide (Sigma N0636), 500 nM A83-01 (STEMCELL Technologies 72024, MA, USA), 10 μM SB202190
(Sigma S7067), 5 µM Y-27632 (Sigma 129830-38-2), 0.5 nM prostaglandin E2 (Selleck S3003), and 1×Primocin (InvivoGen ant-pm-1, CA, USA). Organoids were propagated by dissociating them using TrypLE (Gibco 12604021) prior to re-seeding in fresh Matrigel and culture medium. At 48 h post-seeding, TRIM4-overexpressing or control retroviruses were used to infect these cells. Puromycin (0.5 mg/mL; Sigma) was then used to select cells for 3 weeks, after which the organoids were imaged and counted via microscopy (20×; Zeiss ObserverZ1). Organoids were then treated for an additional 4 days with TAM (15 µM) and imaged at 20×magnification.

**Molecular modelling and protein-protein docking.**

The amino-acid sequences of TRIM4 (NP_148977.2) and SET (NP_001116293.1) retrieved from the NCBI database were used as targets for homology modeling using I-TASSER ([https://zhanglab.ccmb.med.umich.edu/I-TASSER/](https://zhanglab.ccmb.med.umich.edu/I-TASSER/)), and the ZDOCK (version 3.0.2) algorithm with the default parameters was used to predict the structures of the TRIM4-SET complex. The docking results were visualized by visual molecular dynamics (VMD). Discovery studio (version 4.5) was used to build the TRIM4 Cys27 models.

**References**

[1] M. Jia, D. Qin, C. Zhao, L. Chai, Z. Yu, W. Wang, L. Tong, L. Lv, Y. Wang, J. Rehwinkel, J. Yu, W. Zhao, *Nature immunology* **2020**, *21*(7), 727, [https://doi.org/10.1038/s41590-020-0699-0](https://doi.org/10.1038/s41590-020-0699-0).

[2] L. Xiang, B. He, Q. Liu, D. Hu, W. Liao, R. Li, X. Peng, Q. Wang, G. Zhao, *Oncology reports* **2020**, *44*(5), 1997, [https://doi.org/10.3892/or.2020.7765](https://doi.org/10.3892/or.2020.7765).

[3] C. Wei, X. Zhao, L. Wang, H. Zhang, *FEBS letters* **2020**, *594*(19), 3170, [https://doi.org/10.1002/1873-3468.13882](https://doi.org/10.1002/1873-3468.13882).
Supplementary Figure 1. Reduced TRIM4 expression is associated with TAM resistance and poor prognosis in breast cancer. (A) GSE129544 data-based heatmap displaying the deregulated genes between T47D and T47D/TR cells. (B-C) Overall breast cancer patient OS (B) and DS (C) as a function of TRIM4 expression levels were assessed with the Metabric database. (D-F) Luminal A breast cancer patient DS (D), OS (E) and RFS (F) as a function of TRIM4 expression levels were assessed with Metabric and KM-plotter database. (G) Correlations between TRIM4 expression and pathological type in breast cancer cohorts (Luminal: n=1661, Basal-like: n=319). (H) Correlations between TRIM4 expression and ER-α status in breast cancer cohorts (ER-α
positive: n=398, ER-α negative: n=117). Data information: data were presented as mean ± SD.

_Unpaired two-tailed Student’s t-test; ****p<0.0001._
Supplementary Figure 2. TRIM4 knockdown and overexpression efficiencies. (A-B) Efficient knockdown of TRIM4 expression in the indicated cells, as assessed via qPCR (A, B). (C-H) Efficient overexpression of TRIM4 expression in the indicated cells, as assessed via qPCR. (I) Knockdown efficiency of SET in MCF7 cells. (J) Knockdown efficiency of p53 in MCF7 cells. (K) Knockdown efficiency of PP2A in MCF7 cells. All data were representative of n=3 independent experiments. Data information: data were presented as mean ± SD. Unpaired two-tailed Student’s t-test; ***p<0.001; ****p<0.0001.
Supplementary Figure 3. Loss of TRIM4 confers *in vitro* breast cancer cell resistance to TAM. (A-D) MTT assays were used to calculate viability levels and TAM IC$_{50}$ values for TRIM4-silenced or control T47D cells (A) and TRIM4-overexpressing or control T47D/TR (B), MCF7 (C) and T47D cells (D) after treatment for 48 h with the indicated TAM doses (in μM). (E-F) Cell cycle progression was assessed via flow cytometry in TRIM4-silenced or control T47D cells (E) and in TRIM4-overexpressing or control T47D/TR cells (F) after treatment with TAM for 48 h. (G) TAM-resistance related changes in the MAPK and AKT signaling pathways were assessed in TAM-resistant cells and their parental cells via Western blotting. (H-I) TAM-resistance related changes in the MAPK and AKT signaling pathways were assessed in TRIM4-silenced or control T47D cells (H) and in TRIM4-overexpressing or control T47D/TR cells (I) via Western blotting. All data were representative of at least 3 independent experiments. Data information: data were presented as mean ± SD. *Unpaired two-tailed Student’s t-test; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*
Supplementary Figure 4. TRIM4 attenuates proliferation, metastasis and cell stemness properties of breast cancer cells. (A-B) Cell viabilities of TAM-resistant cells and their parental
cells, as assessed via MTT assay. (C-D) Cell viabilities of TRIM4-silenced or control MCF7 (C) and T47D (D) cells. (E-H) Cell viabilities of TRIM4-overexpressing or control MCF7 (E), T47D (F), MCF7/TR (G) and T47D/TR (H) cells. (I-J) Metastasis capacity of TRIM4-silenced or control MCF7 (I) and T47D (J) cells, as assessed via Transwell assay. (K-N) Metastasis capacity of TRIM4-overexpressing or control MCF7 (K), T47D (L), MCF7/TR (M) and T47D/TR (N) cells. (O-P) CD44+CD24- cell populations in the prepared TRIM4-silenced MCF7 (O) and TRIM4-overexpressing MCF7/TR (P) cell populations were assessed via flow cytometry. All data were representative of at least 3 independent experiments. Data information: data were presented as mean ± SD. Unpaired two-tailed Student’s t-test; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.
Supplementary Figure 5. Loss of TRIM4 is associated with TAM resistance in vivo. (A) Patient-derived breast tumor organoid formation following the overexpression of TRIM4 (n=4 replicate cultures). Data are medians with standard deviations. (B) TAM was used to treat TRIM4-overexpressing organoids for the indicated amount of time, with representative images being shown. Scale bars: 100μm. (C) Comparative histological and immunohistochemical images of breast cancer and derived organoid lines. Shown are representative examples of ductal carcinoma samples. Tissues generally exhibit a tumor epithelium layer surrounded by mesenchymal and inflammatory cells, whereas organoids are exclusively epithelial, with tumor cell organization being remarkably well conserved (HE). The ER and PR status of these organoid lines was similar to that of the original breast cancers from which they were derived. Scale bar,100 μm. Data
information: data were presented as mean ± SD. *Unpaired two-tailed Student’s t-test; *p<0.05; **p<0.01.
Supplementary Figure 6. TRIM4 induces ER-α upregulation and promotes ER-α-dependent transcriptional activity. (A) The mean expression of the multiple probes for each gene on the Affymetrix GeneChips was used to determine the correlation coefficient. (B-C) Scatter plots were used to demonstrate correlations between the expression of TRIM4 and that of GATA3 and FOXA1 in the TCGA database. Spearman rank correlation analyses were used to establish r-values in each group. (D, E) The expression of ESR1 was assessed via qPCR in TRIM4-overexpressing or control MDA-MB-231 (D) and MDA-MB-468 (E) cells. (F) The expression of indicated markers was assessed in TRIM4-overexpressing or control indicated cells via Western blotting. (G) TRIM4 expression levels were assessed via Western blotting after treatment with E2 for indicated amounts of time. (H) ERE-luciferase activity was assessed for TRIM4-silenced or control T47D cells with or without E2. (I) TFF1 and GREB1 expression levels were assessed via qPCR in TRIM4-silenced or control T47D cells with or without E2. (J) TFF1 and GREB1 expression levels were assessed via qPCR in TRIM4-overexpressing or control MDA-MB-231 cells with or without E2. (K) MTT assay was used to assess the viability of TRIM4-overexpression or control MDA-MB-231 cells treated with the indicated doses of E2 or ethanol (vehicle) for the indicated amount of time. All data were representative of at least 3 independent experiments. Data information: data were presented as mean ± SD. Unpaired two-tailed Student’s t-test; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; Δ, no significance.
Supplementary Figure 7. Potential ubiquitination sites in human SET. (A) LC-MS/MS analysis revealing the protein SET precipitated by anti-Flag antibodies from the lysates of MCF7
cells transfected with TRIM4-Flag or control. (B) Potential ubiquitination sites were predicted using the Protein Lysine Modification Database (8 lysine (K) residues). (C) The position of the predicted ubiquitination sites in the full-length SET amino acid sequence.
Supplementary Figure 8. SET is associated with poor prognosis and promotes TAM resistance in vitro.

(A-C) OS (A), RFS (B), and DMFS (C) in ER-α-positive breast cancer patients following TAM treatment were assessed as a function of SET expression level with the KM-plotter database. (D-
E) MTT assays were used to calculate viability levels and TAM IC_{50} values for SET-overexpressing or control MCF7/TR (D) and T47D/TR (E) cells after treatment for 48 h with the indicated TAM doses (in μM). (F) Representative images of colonies formed from sh-NC or sh-SET MCF7 cells expressing the indicated plasmids after treatment with TAM (5 μM) for 24h. (G) MTT assays were used to calculate viability levels and TAM IC_{50} values for sh-NC or sh-SET MCF7 cells transfected as indicated. All data were representative of at least 3 independent experiments. Data information: data were presented as mean ± SD. Unpaired two-tailed Student’s t-test; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; Δ, no significance.
Supplementary Figure 9. PI3K is a major target in the TRIM4-mediated inhibition of
breast cancer proliferation. (A) HEK-293T cells were transfected using the indicated plasmids, followed by CHX treatment for the indicated duration. Immunoblotting was then performed using lysates prepared from these cells, with the ImageJ software being used to quantify Myc-SET expression and β-actin being used for normalization. (B) MCF7 cells were transfected using the indicated plasmids, followed by CHX treatment for the indicated duration. Immunoblotting was then performed using lysates prepared from these cells, with the ImageJ software being used to quantify ER-α expression and β-actin being used for normalization. (C) MTT assays were used to assess the viability of TRIM4-Flag or control MCF7 cells that had been treated with the indicated doses of LY294002 or ethanol (vehicle) for the indicated amount of time. (D) MTT assays were used to calculate viability levels and TAM IC_{50} values for the groups as indicated. (E-F) HEK293T cells were transfected with Flag-TRIM4 and the indicated Myc-tagged plasmids, after which IP was performed to assess interactions between TRIM4, SET, p53, and PP2A using anti-Flag (E) or anti-Myc (F) antibodies; IB, immunoblot. All data were representative of at least 3 independent experiments. Data information: data were presented as mean ± SD. Unpaired two-tailed Student’s t-test; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; Δ, no significance.
### Supplementary Table 1. Key resources used in this study

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| anti-rabbit TRIM4   | Abcam  | ab171613   |
| anti-rabbit Ubiquitin (K48-linked) | Abcam | ab140601   |
| anti-rabbit Ubiquitin (K63-linked) | Abcam | ab179434   |
| anti-mouse PR       | Santa Cruz Biotechnology | sc-166169 |
| anti-mouse HER-2    | Santa Cruz Biotechnology | sc-33684  |
| anti-mouse SET      | Santa Cruz Biotechnology | sc-133138 |
| Anti-mouse Ubiquitin | Santa Cruz Biotechnology | sc-8017   |
| anti-rabbit Flag    | Sigma-Aldrich | F2555      |
| anti-rabbit HA      | Sigma-Aldrich | H6908      |
| anti-mouse β-actin  | Proteintech | 66009-1-Ig |
| anti-Ki-67          | Proteintech | 27309-1-AP |
| anti-rabbit Myc     | Bethyl Laboratories | A190-105A |
| anti-rabbit AKT     | Cell Signaling Technology | #4691     |
| anti-rabbit p-AKT   | Cell Signaling Technology | #4060     |
| anti-rabbit JNK     | Cell Signaling Technology | #9252     |
| anti-rabbit p-JNK   | Cell Signaling Technology | #9251     |
| anti-rabbit ERK     | Cell Signaling Technology | #4695     |
| anti-rabbit p-ERK   | Cell Signaling Technology | #4370     |
| anti-rabbit p38     | Cell Signaling Technology | #8690     |
| anti-rabbit p-p38   | Cell Signaling Technology | #4511     |
| anti-rabbit ER-α    | Cell Signaling Technology | #8644     |
| anti-mouse IgG      | Cell Signaling Technology | #7076     |
| anti-rabbit IgG     | Cell Signaling Technology | #7074     |
| Anti-P53            | Santa Cruz Biotechnology | sc-126    |
| Anti-PP2A           | Abcam  | ab32104    |
| **Reagents**        |        |            |
| Tamoxifen           | Sigma-Aldrich | T5648     |
| MG132               | Sigma-Aldrich | 474790    |
| β-Estradiol         | Sigma-Aldrich | E2758     |
| Chloroquine         | Selleck  | NSC-187208 |
| Cycloheximide       | Selleck  | NSC-185    |
| 3-Methyladenine     | Selleck  | 5142-23-4  |
| Ly294002            | Selleck  | S1105      |
| **Critical Commercial Assays** |        |            |
| BCA Protein Assay Kit | Thermo Scientific | 71285-3   |
| Cell lysis buffer for Western and IP | Beyotime Biotechnology | P0013    |
| KOD-Plus-Mutagenesis kit | Toyobo | SMK-101    |
| Oligonucleotides | F | R |
|-----------------|---|---|
| TRIM4           | AAGGTAAGAAGACAGTGTGCCA | CAGGGAGGCTATCAAGGGGC |
| TRIM16          | TGACACCAGAAGAGTGAAGGC | TATTTGCGCTGAACAACGGC |
| TRIM59          | TTGTACACTGCCTGGAACAC | TCCTATCGCCTTGATGTC |
| TRIM66          | GCCCTCTGTGCTACTTACTC | GCTGGTGTGGGTTACTTCT |
| TRIM62          | TACTGGGAGGTGTGTTGTGTC | CGTCCGGTGGTGAAGAGAC |
| TRIM8           | CCTATCTGCTGCACGTATT | GTTGTATAGCCCTGGTTC |
| TRIM25          | GCCTGCTGGAGCATAAG | TTGCAGTCATGCACATCC |
| TRIM29          | ATGCTTGTTGTGTCATT | GCATTCCTCCTTACAGCAT |
| TRIM47          | GTCCAAAGTCCTGAGCC | GCTACGGCTGCACTCTTG |
| TRIM27          | TTGGGAAAGGAATCAAG | ATTCCTCGGAAAGAGCCTC |
| TRIM15          | TCCCTGAAGGTTGGTCCAT |
| Gene   | Forward Primer (5’-3’)                          | Reverse Primer (5’-3’)                        |
|--------|------------------------------------------------|-----------------------------------------------|
| TRIM26 | R CAGGATCTTGCCCGAGGATT                       | F AAGTTTGTCGAGCGACAC                         |
|        |                                              | R CTCAGGTGTTTCAGGATTTTT                      |
| TRIM36 | F CGTCGGTCCCTCAGGATTTTT                      | R GTGGCAAGTTTCGTCGTCTTCC                     |
|        |                                              | F CTGCTTGGMAGTTTCCTTGAC                     |
| TRIM56 |                                              | R GTGGATGGTTSCGTACTGAG                      |
| TRIM41 | F AGCTCTTCTGCGAGGTAGAC                       | R CTCTTCAGCCCAGAAACCCTG                     |
| TFF1   | F AATAAGGGCTGCTGTTTCG                        | R ACTCCTTCTCTGAGGAGGAC                      |
| GAPDH  | F GGAGCGAGATCCCTCCAAAAT                      | R GGCTGTTGTCATACTTCTCATGG                    |
| PGR    | F GTCAGTGGGCAAGATGCTGTA                      | R AGCCCTTCCAAAGGAATTGT                      |
| GREB1  | F ATTCCACGACCCCCAATCTG                      | R GTCATCAGAATGTCAGTAAACCAG                  |
| SET    | F CATCTTCGAAGTCCACCGAAATC                   | R TGCAATCAGAATGTCAGTAAACCAG                 |
| ERα    | F CCTGATGATTGCTCCTGCTG                      | R GCCACACAAACTCCTCTCC                      |

**Generating mutant plasmid**

| Mutation | Forward Primer (5’-3’)                          | Reverse Primer (5’-3’)                        |
|----------|------------------------------------------------|-----------------------------------------------|
| Myc-SET-K15R | AGACCAAGACCCAACCCTCCTGCTCTCGG                    | R CTTTCTTTTGAGCCGGAGTGGAGAC                  |
| Myc-SET-K68R | AGAGTGAACAGAATATAAACAAAC                        | R CAAAATCTCTCTACTGGCTTTTCA                   |
| Myc-SET-K150R | AGAGTTCTCTCCAAAAGATTTTCATC                     | R ATTTTCAAAGTAAAGATTTCATCA                   |
| Myc-SET-K154R | AGAGAATTTCATCTGAATGAGAGT                      | R GGAAGAAACCTTTAATTTTCAAAGTTAA              |
| Myc-SET-K167R | AGGTCCACCGAAATCAATGGAATAT                   | R CGAAGATGGATCAACCACCTCCTCATTC              |
| Myc-SET-K172R | AGATGGAAATCTGGAGAAGATTTGA                     | R GATTTCCGGTGACCTCGAAGATGGGA                |
| Myc-SET ΔC | CCCAAGCTTATGGCCCTTAAACGCCAGT                 | R CCGCTCGAGTTATTGGCCGGAGTTTTTGTATATTTTCT   |
| Myc-SET ΔN | CCCAAGCTTATGCGATTTTTTTCAGAGAGGTGTC          | R CCGCTCGAGTATGCTTCTCCAGTCTCCTCC            |
| Flag-TRIM4 C27S | AGCGGCCACAAACTTCTGCAGCCCGGCT             | R CTCGATGACACCCCGGTCAGTGTCGAAAA             |
| Flag-TRIM4 ΔRING | CACCCATCGCGCGCGCCGCG                  | R GGTCACACTCTCTCTGAGATGTCCTCA               |
| Flag-TRIM4 ΔB-box | GACGAGGCCCTTCGAGAGCTA           |                                              |
| Flag-TRIM4 ΔCC | R | GGGGCCAGGCGCGCCGCT | F | GGGGAGAAGAGCCAGGCTCCCA |
|---------------|---|---------------------|---|------------------------|
|               | R | AACCAGGAAGTTGTGCAGCTTTGAA |
| Flag-TRIM4 ΔSPRY | F | CCAGTGACTGATAGGAATGA |
|               | R | TACAGCTTCAAGAGAATAGTTCACA |

**ShRNA sequence**

|                        |                          |                         |
|------------------------|--------------------------|-------------------------|
| human TRIM4-1          | 5′-GAAGTTGAGAGTAGAGATA-3′ |                         |
| human TRIM4-2          | 5′-GAGATTGAACAAAGAAGAA-3′ |                         |
| Sh-SET                 | 5′-CCCGACATGGATGAAGAA-3′  |                         |
| Si-P53                 | 5′-GACUCCAGUGGAUACUCUA-3′ |                         |
| Si-PP2A                | 5′-GCAAAUCAACCAGAUACAAAATT-3′ |
Supplementary Table 2.
Correlation between SET expression and clinical parameters in 116 ER-α positive breast cancer patients

| Characteristics     | SET-low (n=69) | SET-high (n=47) | p value  |
|---------------------|---------------|-----------------|----------|
| Age                 |               |                 |          |
| <59                 | 53(76.81%)    | 39(82.98%)      | 0.4208   |
| >=59                | 16(23.19%)    | 8(17.02%)       |          |
| Tumor size          |               |                 |          |
| <=T1                | 49(71.01%)    | 18(38.30%)      | <0.0001  |
| >T1                 | 14(20.29%)    | 28(59.57%)      |          |
| Unexamined          | 6(8.70%)      | 1(2.13%)        |          |
| LN metastasis       |               |                 |          |
| 0                   | 51(73.91%)    | 21(44.68%)      |          |
| 1-3                 | 14(20.29%)    | 10(21.28%)      | 0.0002   |
| >3                  | 4(5.80%)      | 16(34.04%)      |          |
| Histologic Grade    |               |                 |          |
| G1                  | 5(7.25%)      | 4(8.51%)        |          |
| G2                  | 42(60.87%)    | 29(61.70%)      | 0.2899   |
| G3                  | 6(8.70%)      | 10(21.28%)      |          |
| Unexamined          | 16(23.18%)    | 4(8.51%)        |          |
| HER-2               |               |                 |          |
| Neg                 | 53(76.80%)    | 31(65.96%)      | 0.5023   |
| Pos                 | 6(8.70%)      | 2(4.26%)        |          |
| Unexamined          | 10(14.50%)    | 14(29.79%)      |          |
| Ki-67               |               |                 |          |
| <=14%               | 27(39.13%)    | 11(23.40%)      | 0.0596   |
| >14%                | 40(57.97%)    | 36(76.60%)      |          |
| Unexamined          | 2(2.90)       | 0(%)            |          |
| PR                  |               |                 |          |
| Neg                 | 8(11.60%)     | 5(10.64%)       | 0.8727   |
| Pos                 | 61(88.40%)    | 42(89.36%)      |          |

Data are n (%)

All patients were divided into SET low (SI<=4, n=69) and SET high (SI>4, n=47) group, clinical variates included patient age, tumor size, LN metastasis, histological grade and the expression of HER-2 and Ki-67 were divided into categorical data, and the frequency was calculated. p values were determined by two-tailed Chi-square test.
### Supplementary Table 3.

**Effect of variates on OS in 116 breast cancer patients in univariate and multivariate Cox proportional hazards regression model**

| Variable                      | Univariate analysis | Multivariate analysis |
|-------------------------------|---------------------|-----------------------|
|                               | HR (95% CI)        | p         | HR (95% CI)       | p         |
| Age (>=59 vs. <59)            | 4.241(1.060-16.967) | 0.041    | 6.888(1.318-35.990) | 0.022 |
| Histologic Grade              |                     |          |                     |          |
| G2 vs. G1                     | 25863.077(0.000-2.271E+166) | 0.957    | -                   | -       |
| G3 vs. G1                     | 44996.911(0.000-3.958E+166) | 0.955    | -                   | -       |
| Unknown vs. G1                | 17768.976(0.000-1.567E+166) | 0.959    | -                   | -       |
| Tumor Size (>T1 vs. <=T1+)    | 0.568(0.115-2.816)  | 0.489    | -                   | -       |
| LN metastasis                 |                     |          |                     |          |
| 1-3 vs. 0                     | 1.012(0.105-9.737)  | 0.992    | 0.265(0.020-3.538)  | 0.315   |
| >3 vs. 0                      | 5.196(1.162-23.230) | 0.031    | 1.573(0.248-9.978)  | 0.631   |
| PR Status (pos vs. neg)       | 0.883(0.109-7.183)  | 0.908    | -                   | -       |
| HER-2 Status (pos vs. neg + unknown) | 1.984(0.243-16.171) | 0.522 | -                   | -       |
| Ki-67 Status (pos vs. neg + unknown) | 3.881(0.477-31.595) | 0.205 | -                   | -       |
| ER-α expression               |                     |          |                     |          |
| ++ vs. +                      | 25469.281(0.000-1.244E+178) | 0.960 | | |
| +++ vs. +                     | 24453.935(0.000-1.192E+178) | 0.961 | | |
| SET expression                | 2.513(0.600-10.525) | 0.207    | 0.078(0.007-0.846)  | 0.036   |
| TRIM4 Expression              | 0.094(0.012-0.761)  | 0.027    | 0.078(0.007-0.846)  | 0.036   |

Variates included patient age, tumor size, LN metastasis, histological grade and the expression of ER-α, HER-2, Ki-67, TRIM4 and SET were analyzed in univariate Cox regression model, and the p values were determined by univariate Cox regression analysis. For the multiple Cox regression analysis, histological grade, tumor size, HER-2 and Ki-67 status were excluded due to the p value larger than 0.05. The variates with a p value smaller than 0.05 in univariate analysis were included in the multiple Cox regression model, and the p values were determined by multivariate Cox regression analysis, no adjustments were made for multiple comparisons.