Figure EV1. Effect of UTX on breast tumorigenesis and on survival in breast cancer patients.

A, B Generation of UTX-knockdown or UTX-overexpressing cells. Lysates from the indicated stable UTX-knockdown (shUTX) and overexpressing cells (A) and UTX siRNA (si)-transfected cells (B) were analysed for UTX and H3K27me3 expression using immunoblotting. β-actin and histone H3 (H3) were used as loading controls for whole-cell lysate and for histone proteins, respectively.

C, D UTX retards breast tumour growth in vivo. The indicated numbers of UTX-overexpressing MDA-MB-231 (C) or UTX-knockdown SK-BR-3 (D) cells were injected into the mammary fat pads of NOD/SCID mice, and xenograft tumour growth rates were monitored at the indicated time points for approximately 5 weeks. The data shown represent the means ± SE of n = 6 mice. *P < 0.05, **P < 0.01, ***P < 0.001 versus shCON or CON at last days, two-tailed unpaired t-test.

E To determine whether UTX loss affects the neoplastic transformation of breast epithelial cells, NOD/SCID mice were injected with UTX-deficient MCF10A cells and monitored at the indicated time points for 9 weeks. The data shown represent the means ± SE of n = 6 mice. *P < 0.05, two-tailed unpaired t-test.

F A meta-analysis of the overall survival (OS), relapse-free survival (RFS) and distant metastasis-free survival (DMFS) of patients with breast cancer, as stratified according to UTX expression level using the Kaplan–Meier Plotter (http://kmplot.com/analysis). The high and low UTX expression groups were separated by median expression values of UTX that were based on the mean of all UTX probes (203990_s_at, 203991_s_at and 203992_s_at). P-value was calculated based on the log-rank test.

Source data are available online for this figure.
Figure EV2. UTX deficiency-mediated regulation of stem cell-like properties is independent of EZH2 expression.

A ChIP analysis showing the indicated histone methylation status and gene recruitment to the CDH1 gene body (left) and promoter upstream (right) in UTX-deficient MCF10A cells. IgG, control IgG for negative control. Error bars indicate the SD (n = 3 independent experiments). *P < 0.05, **P < 0.01 versus shCON; two-tailed unpaired t-test.

B To determine the effect of EZH2 on UTX deficiency-induced stemness, UTX/EZH2 double-knockdown MCF10A cells were obtained. Tet-inducible shCon (shTet) and sheZ2 (shTet-shEZH2) cell lines were treated with 1 µg/ml doxycycline (DOX) for 48 h. The CD44+/CD24-/ESA+ population in the indicated cells was measured using flow cytometry.

C, D Mammosphere formation (C) and colony-forming assays (D) were performed in UTX/EZH2 double-knockdown MCF10A cells.

E, F The protein (E) and mRNA (F) expression of the indicated genes in UTX/EZH2 double-knockdown MCF10A cells was measured using immunoblotting and qRT-PCR.

G ChIP analysis showing the recruitment of the indicated proteins and histone marks at the proximal CDH1 promoter.

Data information: The data shown in (B, C, D, F, and G) represent the means ± SD of n = 3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus shCON/shUTX; †P < 0.05 versus shUTX/shown; ns, no significance (Welch's test and Dunnett's T3 post hoc test for UTX, EZH2, vimentin, SNAIL, ZEB1 in F and for H3K27me3, SNAIL and ZEB2 in G; one-way ANOVA and Scheffe's post hoc test for others).

Source data are available online for this figure.
Figure EV3. UTX epigenetically regulates EMT-TFs gene expression.

A  Lysates of the indicated cells were analysed by immunoblotting.

B  Schematic illustration of the proximal promoter and transcription start site (TSS) regions of human SNAIL, ZEB1 and ZEB2 gene locus with adjacent E-boxes. Regions #1 and #2 indicate the target sites for ChIP analysis.

C, D  ChIP analysis showing the histone methylation status and recruitment of the indicated proteins within the indicated genomic regions of EMT-TFs in MCF10A (C) and MDA-MB-231 (D) cells. The data shown represent the means ± SD of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 versus shCON or CON; two-tailed unpaired t-test.

Source data are available online for this figure.
Figure EV4. The effect of c-Myc and LSD1 on UTX-mediated epigenetic alteration within the promoter regions of EMT-TFs.

A, B To transiently knock down c-Myc, non-targeting siRNA (CON si) or c-Myc siRNA (c-Myc si) was transfected into the indicated stable cells; the EMT-TF promoters were then analysed using a ChIP assay and the indicated antibodies.

C, D UTX-overexpressing MDA-MB-231 cells were infected with LSD1 shRNA viruses; ChIP analyses were then performed to confirm the histone methylation status and recruitment of the indicated proteins within the promoters of ZEB1 and ZEB2. The data shown represent the means ± SD of three independent experiments.

**P < 0.01, ***P < 0.001 versus CON/shCON; ††P < 0.01, †††P < 0.001 versus UTX/shCON; ns, no significance (Welch’s test and Dunnett’s T3 post hoc test for LSD1 and c-Myc in C and for LSD1 in D; one-way ANOVA and Scheffe’s post hoc test for others).

Source data are available online for this figure.