

Supplementary Information

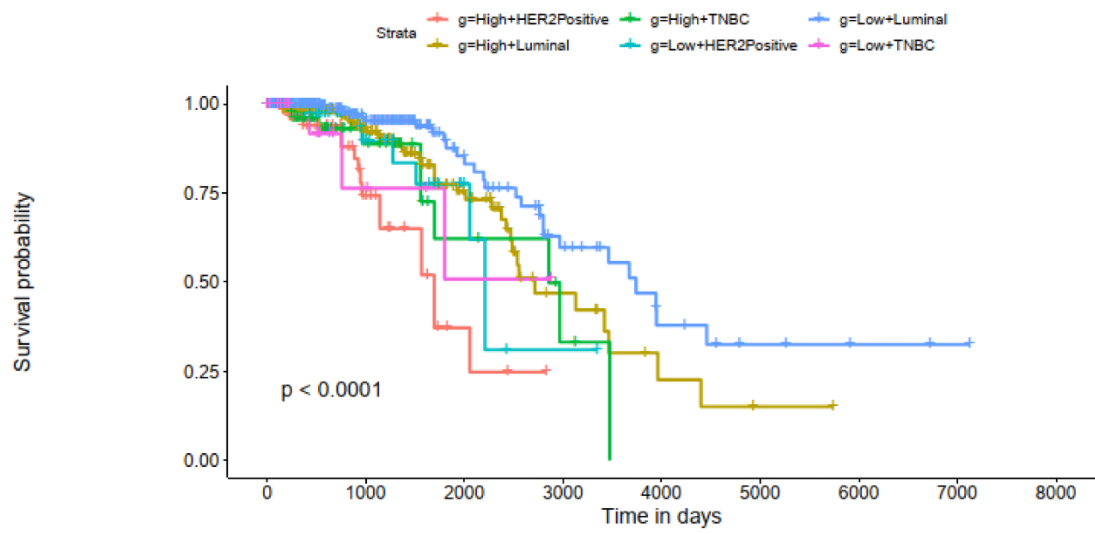
Table S1 Statistical analysis of the expression level of USP13 in normal breast tissues and three subclasses breast carcinomatous tissues in the TCGA database

Comparison	Statistical significance
Normal-vs-Luminal	1.10E-01
Normal-vs-HER2 Positive	9.26E-02
Normal-vs-TNBC	4.29E-01
Luminal-vs-HER2 Positive	4.95E-01
Luminal-vs-TNBC	1.70E-05 **
HER2 Positive-vs-TNBC	4.02E-03 **

Fig.S1 USP13 stabilizes Twist1 protein. The Kaplan–Meier curves obtained from UCSC Xena database and analyzed by the R package survival and survminer.

Fig.S2 USP13 deubiquitinates Twist1. (a) Flag-Twist1 was co-expressed with GFP-USP13 or empty vector in HEK293T cells and treated with 20 μ M MG132. The relative mRNA levels of Twist1 and USP13 were determined using qRT-PCR. (b) GFP-USP13 was expressed with increasing amounts in MDA-MB231 cells. The relative mRNA levels of Twist1 and USP13 were determined using qRT-PCR. (c) SUM159PT and MDA-MB231 cells were stably transfected with shNC control or USP13 shRNAs and the relative mRNA levels of Twist1, c-Myc and USP13 were determined using qRT-PCR. (d-g) Grayscale analysis of Fig.3a-3d revealed Twist1 relative protein levels in indicated CHX experiments. Data are means $\pm$ SD (n = 3). Two-tailed t-test was performed. * p < 0.05; ** p < 0.01. (h,i) Grayscale analysis of Fig.4a revealed Twist1 K48 or K63 ubiquitination changes in the presence or absence of GFP-USP13. Data are means $\pm$ SD (n = 3). Two-tailed t-test was performed. * p < 0.05; ** p < 0.01.

Fig.S3 USP13 promotes breast cancer cell migration, invasion and lung metastasis through Twist1. (a) SUM159PT and MDA-MB231 cells were transfected with indicated plasmids and then western blot was performed. (b) The wound healing assay in indicated breast cancer cells treated with or without 10ng/mL TGF- β 1. Scale bar = 50 μ m. (c) The protein levels of USP13 and Twist1 in indicated wound healing assays were examined by western blot. (d) The Transwell assay in indicated breast cancer cells treated with or without 10ng/mL TGF- β 1. Scale bar = 50 μ m. (e) Statistical analysis of migration and invasion cells in indicated Transwell assays. Data are means $\pm$ SD (n = 3). Two-tailed t-test was performed. * p < 0.05; ** p < 0.01. (f) Representative images showing morphology of lung tissues from the different experimental groups. (g) The protein levels of USP13 and Twist1 in indicated mouse groups were examined by western blot. (h) Lung weight of mice in different groups. Data are means $\pm$ SD (n = 3). Two-tailed t-test was performed. * p < 0.05; ** p < 0.01.



Number at risk

Strata	0	1000	2000	3000	4000	5000	6000	7000	8000
g=High+HER2Positive	79	20	3	0	0	0	0	0	0
g=High+Luminal	313	95	34	10	3	1	0	0	0
g=High+TNBC	65	21	6	2	0	0	0	0	0
g=Low+HER2Positive	73	21	5	1	0	0	0	0	0
g=Low+Luminal	278	104	37	19	8	4	2	1	0
g=Low+TNBC	22	5	2	0	0	0	0	0	0

Time in days

