Supplementary information

Acetylation of ELF5 suppresses breast cancer progression by promoting its degradation and targeting CCND1

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Supplementary Figure 1. Alignment of ELF5 sequences from different species.

Alignment of five different ELF5 vertebrate orthologs with the conserved lysine residues numbered and colored in red.

Supplementary Figure 2. Acetylation of ELF5 in HEK293T and MCF7 cells.

(a) Acetylation of exogenous ELF5. HEK293T cells were transfected with Flag-ELF5, immunoprecipitated with anti-Flag affinity gel and immunoblotted with anti-Ac Ab. (b) Acetylation of endogenous ELF5 in HEK293T cells. Cell lysis was subjected to immunoprecipitation with anti-AcK Ab or normal IgG, followed by western blot with anti-ELF5 Ab. (c-d) Acetylation of endogenous ELF5 in MCF7 cells. Cell lysis was subjected to immunoprecipitation with anti-AcK Ab or normal IgG, followed by western blot with anti-ELF5 Ab.
Supplementary Figure 3. ELF5 interacts with p300 both in vitro and in vivo.

(a) p300-mediated acetylation of exogenous ELF5. Hela cells were co-transfected with Flag-ELF5 and different HA-tagged HATs, HA-p300, HA-PCAF, HA-GCN5, or HA-Tip60, and the cell lysates were immunoprecipitated with anti-Flag affinity gel and immunoblotted with the anti-AcK Ab. (b) Exogenous ELF5 interacted with p300 in vivo. HEK293T cells expressing HA-p300 only or HA-p300 and GFP-ELF5 were prepared and subjected to affinity purification with anti-HA Ab, followed by Western blot with the indicated antibodies. (c) Exogenous ELF5 interacted with p300 in vivo. HEK293T cells transfected with Flag-ELF5 only, or with Flag-ELF5 and HA-p300 were subjected to co-immunoprecipitation with anti-Flag affinity gel followed by immunoblotting with antibodies as indicated. (d) Endogenous ELF5 interacted with p300 in vivo. T47D cells were subjected to immunoprecipitation with anti-ELF5 antibody or normal IgG followed by Western blot with the indicated antibodies. (e) ELF5 interacted with p300 in vitro. GST pull-down assays showing the interaction between GST-fusion ELF5 and endogenous p300 in T47D cells. (f) ELF5 and p300 were colocalized in the nucleus. Immunofluorescence showing the localization of ELF5 (blue) and p300(red) in the nucleus (blue) of T47D cells.
Supplementary Figure 4. Mass spectrometry analysis of the ELF5-derived peptides containing acetylated K134, K143, K197, K228, and K245.
Supplementary Figure 5. Acetylation promotes the ubiquitin-proteasome degradation of ELF5.

(a) The protein level of ELF5 influenced by p300. HEK239T cells overexpressing HA-p300 in the absence or presence of 20 μM MG132 were prepared and subjected to western blot with the indicated antibodies. (b) The protein level of ELF5 influenced by SIRT6. HEK293T cells overexpressing GFP-SIRT6 in the absence or presence of 20 μM MG132 were prepared and subjected to western blot with the indicated antibodies. (c) The ubiquitination level of ELF5 influenced by p300. Ubiquitination levels of GFP-ELF5 in HEK293T cells without or with overexpression with HA-p300. The cells were treated without or with NAM plus TSA before being harvested.
Supplementary Figure 6. ELF5 inhibit cell proliferation through CCND1.

(a) Cells that overexpressed with GFP-ELF5 WT, GFP-ELF5 6KR, GFP-ELF5-6KQ or the control vector were analyzed for CCND1 mRNA levels. (b) Western-blot detection of Cyclin D1 expression in MCF7 cells expressing a siCtrl or siCCND1. (c) Growth curves of MCF7 cells that stably overexpressed with GFP-ELF5 without or with siCCND1 as measured by CCK8 assay. Data are the means ± SDs from three determinations. *p<0.05; **p<0.01. (d) Growth curves of MCF7 cells performed a CCND1 rescue experiment overexpressing ELF5 wildtype. Data are the means ± SDs from three determinations. *p<0.05; **p<0.01. (e) Flow cytometry analysis of the cell
cycle showing the distribution of MCF7 cells stably transfected with ELF5 WT with a
Cyclin D1 rescue. (f-g) Growth curves of T47D cells expressing a shCtrl or shELF5
without or with abemaciclib (e) or ribociclib (f) treatment as measured by CCK8 assay.
*p<0.05; **p<0.01; ns, no significance.
| AAMP | ABCE1 | ABCF1 | ABCF2 | ABCF3 | ACACA | ACAD9 | ACLY |
|------|-------|-------|-------|-------|--------|--------|------|
| ACSL3 | ACTB | ADAR | ADRM1 | AGK | AGPS | AICDA | AIFM1 |
| AIM1 | AKAP8 | ALDH1A1 | ALDOA | ALEYREF | AMOT | APIB1 |
| APIM1 | AP2A1 | AP2A2 | AP2B1 | AP2M1 | AP3B1 | ARCN1 |
| ARF5 | ARMC6 | ASIC1 | ASNS | ATG3 | AUP1 | BAG6 | BAZ1B |
| BCCIP | BSN | BTAF1 | BUB3 | BUD23 | BW1 | BWZ2 | C1QBP |
| C2CD5 | CALR | CALU | CAND1 | CASC3 | CBL | CCAR2 | CCDC6 |
| CCDC9 | CCT6A | CDC16 | CDC27 | CDC40 | CDC5L | CDC73 | CDK1 |
| CDK12 | CDK9 | CENPU | CFDP1 | CHD3 | CDERP | CDMP6 | CHTF18 |
| CHTOP | CKA4 | CLINT1 | CLNS1A | CLPB | CLPX | CLU | CMC1 |
| CMC2 | CMTR1 | COPS5 | COPS6 | CPSF1 | CPSF3 | CPSF6 | CPSF7 | CSDE1 |
| CSE1L | C52N | CSTF3 | CTR9 | CTU2 | CUL1 | CUL3 | CUL4B |
| CWF19L1 | DAXX | DHR1 | DCD | DCTN2 | DD1 | DDOST | DX1 |
| DDX17 | DDX18 | DDX21 | DDX23 | DDX27 | DDX3X | DDX41 | DDX42 |
| DDX46 | DDX47 | DDX5 | DDX50 | DDX54 | DDX55 | DDX56 | DDX6 |
| DHR7 | DIXH15 | DIXH30 | DIXH36 | DIXH38 | DIXH57 | DIXH9 |
| DSS | DSK1 | DNM2 | DNMT1 | DNPEP | DOCK7 | DPM1 | DRG1 |
| DRG2 | DUS3L | EBNA1BP2 | ECP5 | EDC3 | EEA1 | EEF1B2 | EEF1D |
| EEF1G | EEF2 | EHD4 | EIF2A | EIF3A | EIF3B | EIF3C | EIF3D |
| EEF3E | EIF3F | EIF3G | EIF3H | EIF3I | EIF3J | EIF3L | EIF3M |
| ELAVL1 | ELF5 | ELPL1 | ELP2 | ELP3 | EMT1 | EMD | ENAH |
| ERI1 | ESAT2 | EWSR1 | EXOC3 | FAF1 | FAF2 | FAS | FB1L1 |
| FHL1 | FIPI1L1 | FKB1 | FLII | FLNA | FOXK1 | FOXK2 | FRAS1 |
| FRG1 | FUBP3 | FUS | FXR1 | FYCO1 | FZD6 | GBPI | GNL1 |
| G3BP2 | GANAB | GCFC2 | GCN1 | GFTPT1 | GFTPT2 | GNE | GTPBP4 |
| GLN3 | GPHN | GP3 | GRN | GRSF1 | GRWD1 | GTF21 | HDGF |
| GYS1 | HACD3 | HADHA | HADHB | HAT1 | HBS1L1 | HDA1C | HLA-A |
| HDHD5 | HELS | HES6 | HGHI | HIC2 | HK1 | HK2 | HNRNP |
| HMBG1 | HMRR | HRNRNC | HRNRPD | HRNRP | HRNRP | HRNRP | IFIT1 |
| HNRNP | HNRNPU | HSPA5 | HSPA9 | IDH3A | IDH3B | IDH3G | IPO5 |
| IFRD1 | IGBP1 | IGH1 | IK | ILF2 | ILF3 | IPO4 | KIF5B |
| IPO7 | ITGAL | IWS1 | KHSRP | KIF11 | KIF2A | KRR1 |
| KIFC1 | KLC1 | KLC2 | KLF16 | KLF4 | KPNB1 | KRI1 | LDHB |
| LAMP2 | LARP1 | LARP4 | LARP7 | LASP1 | LCK | LDIA | MAP4 |
| LEO1 | LETM1 | LGR6 | LSG1 | LUC7L | LYAR | MAGT1 | MCM3 |
| MAP7 | MASP2 | MATR3 | MAZ | MCAT | MCC2 | MCM2 | MAP1 |
| MCM4 | MCM5 | MCM6 | MCM7 | MCMBP | MEMO1 | MECPE | MPP6 |
| MFN1 | MIER1 | MLST8 | MMS19 | MOG5 | MORN1 | MOV10 | MYH10 |
| MDM3 | MRTO4 | MSH2 | MSH6 | MTA2 | MTDH | MTRX1 | NCBP1 |
| MYH9 | NAA15 | NACA | NADK | NAMPT | NASP | NAT10 | NM3D |
| NCBP3 | NCLN | NEK9 | NELFE | NEMF | NFS1 | NKRF | NOP58 |
### Supplementary Table 2. The primers for Site-directed mutagenesis

| Sense (5'-3') | Antisense (5'-3') |
|--------------|------------------|
| K130R        | gacgctgaagaagccaggccaccatacaaga | tctttgatggtggccctgctttcttcagcgtc |
| K134R        | gcaaggccacattacagaagactgctggatc | ggacacaggccaggcuugactt |
| K143R        | gattccacttctctcttcagcgtc | ggaatcagcatagtctctgatggtggccttgc |
| K197R        | aatttttcgggtggttagatcggaagccctggccagggcttccgatctaaccacccgaaaaatt |
| K228R        | ccctgagatactactacatatactgctgatttgc | cagggcttccgatctaaccacccgaaaaatt |
| K245R        | tagtgtacaaatttggaagaaatgcacacgggtg | cacccggtgtgcattcttcagcgtc |
| K130Q        | gacgctgaagaagccaggccaccatacaaga | cagggcttccgatctaaccacccgaaaaatt |
| K134Q        | gcaaggccacattacagaagactgctggatc | cagggcttccgatctaaccacccgaaaaatt |
| K143Q        | gattccacttctcttcagcgtc | ggaatcagcatagtctctgatggtggccttgc |
| K197Q        | aatttttcgggtggttagatcggaagccctggccagggcttccgatctaaccacccgaaaaatt |
| K228Q        | ccctgagatactactacatatactgctgatttgc | cagggcttccgatctaaccacccgaaaaatt |
| K245Q        | tagtgtacaaatttggaagaaatgcacacgggtg | cacccggtgtgcattcttcagcgtc |

### Supplementary Table 3. The siRNA sequence for RNA interference

| Sense (5'-3') | Antisense (5'-3') |
|--------------|------------------|
| siEP300#1    | ugcacaggccaggcuugactt | gucaaccucgcuugucuatt |
| siEP300#2    | cagacaguccugauaagg | euuaccuccagucuccuug |
| siSIRT6#1    | gauugucaccaguugagatt | ucuuaccuacgucauccu |
| siSIRT6#2    | gcucacguagacgaggucuatt | augacuccuacguacgactt |
| siCCND1#1    | ggaacaccaacagaucaacctt | guaauccugauuacuccuug |
| siCCND1#2    | gcacgccauugacaccaactt | guaauccugauuacuccuug |
| Negative control | ucuuaccgaagucagcagut | aegugacagcguacagaga |

### Supplementary Table 4. The primers for ChIP analysis

| Sense (5'-3') | Antisense (5'-3') |
|--------------|------------------|
| region 1     | gaaacactcagatttgc | gaccagtgggtcctttgcc |
| region 2     | aaggttagaaggccagcccgg | ccatactcagaacccgaga |
| region 3     | ggttgcgccgcccag | ggaagccgctcagactttgc |
Figure 4d

Figure 4e

Figure 4f

Figure 4g

Figure 4h

Figure 4i

Figure 5a

Figure 5b
