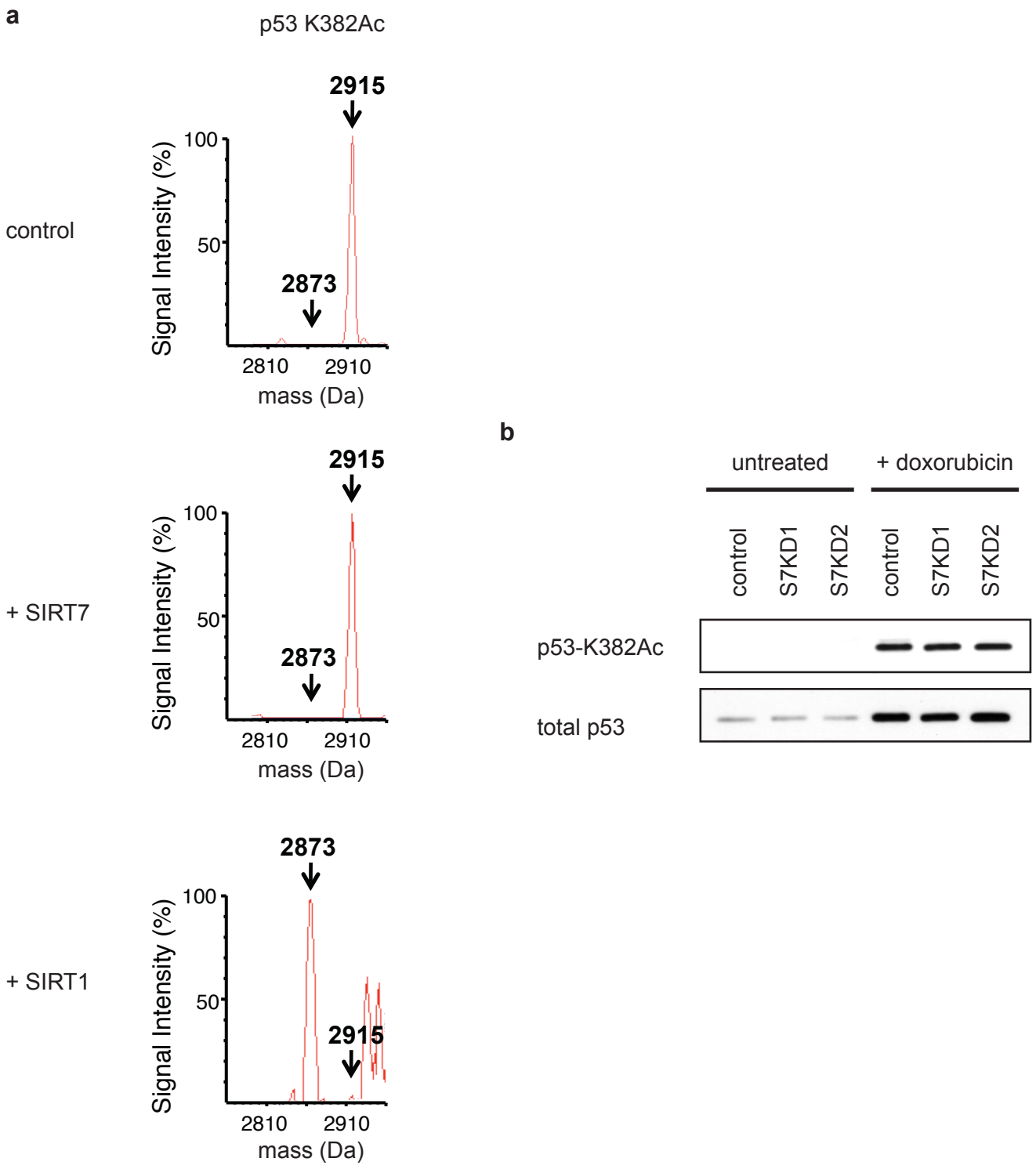
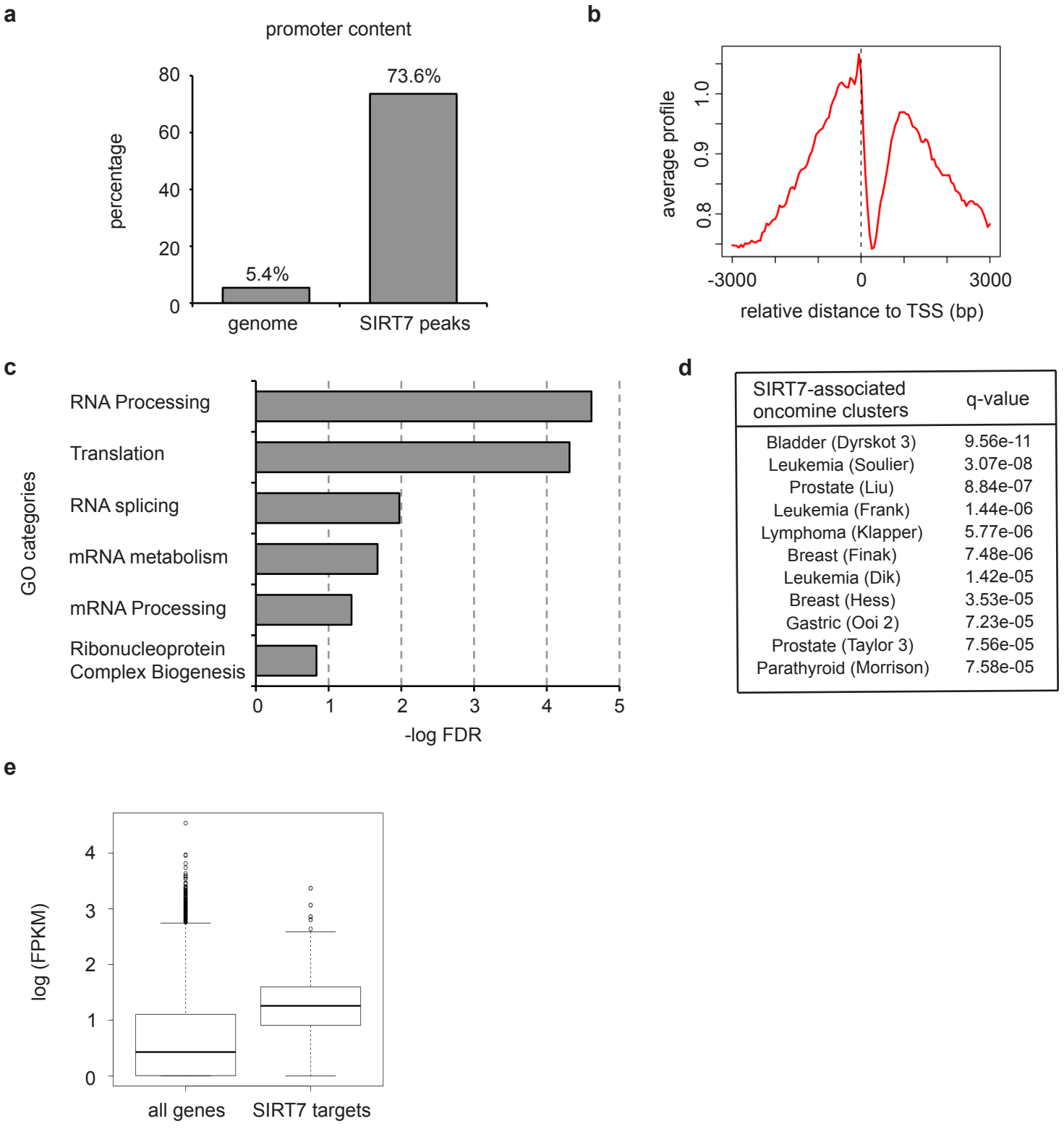
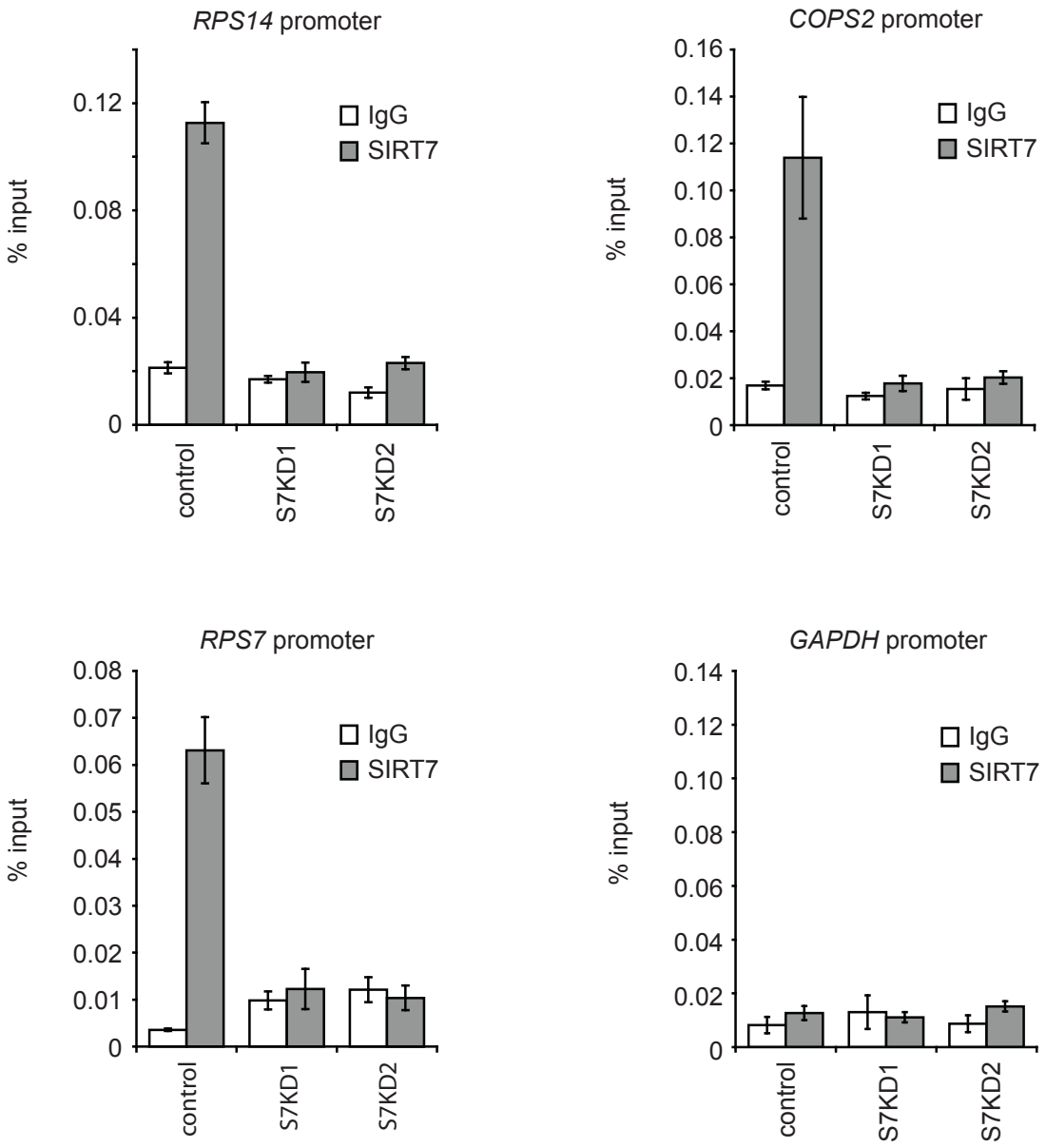


Supplementary Figure 1. SIRT7 does not deacetylate p53 *in vitro* or in cells. a, p53 K382Ac peptide was incubated either alone (control) or in the presence of recombinant SIRT7 or SIRT1 protein with NAD+, and peptide size was determined by mass spectrometry. Arrows indicate the relative positions of acetylated (2915 Da) and deacetylated (2873 Da) p53 peptides. **b,** SIRT7 does not deacetylate p53 in cells. SIRT7 was depleted from HT1080 cells using siRNAs and subsequently mock treated or treated with 1 ug/mL doxorubicin. After 24 hours, p53 was immunoprecipitated from whole cell extracts. IPs were probed with p53-K382Ac antibody and analyzed by western blot. Input samples were analyzed by western blot with p53 antibody as loading control.

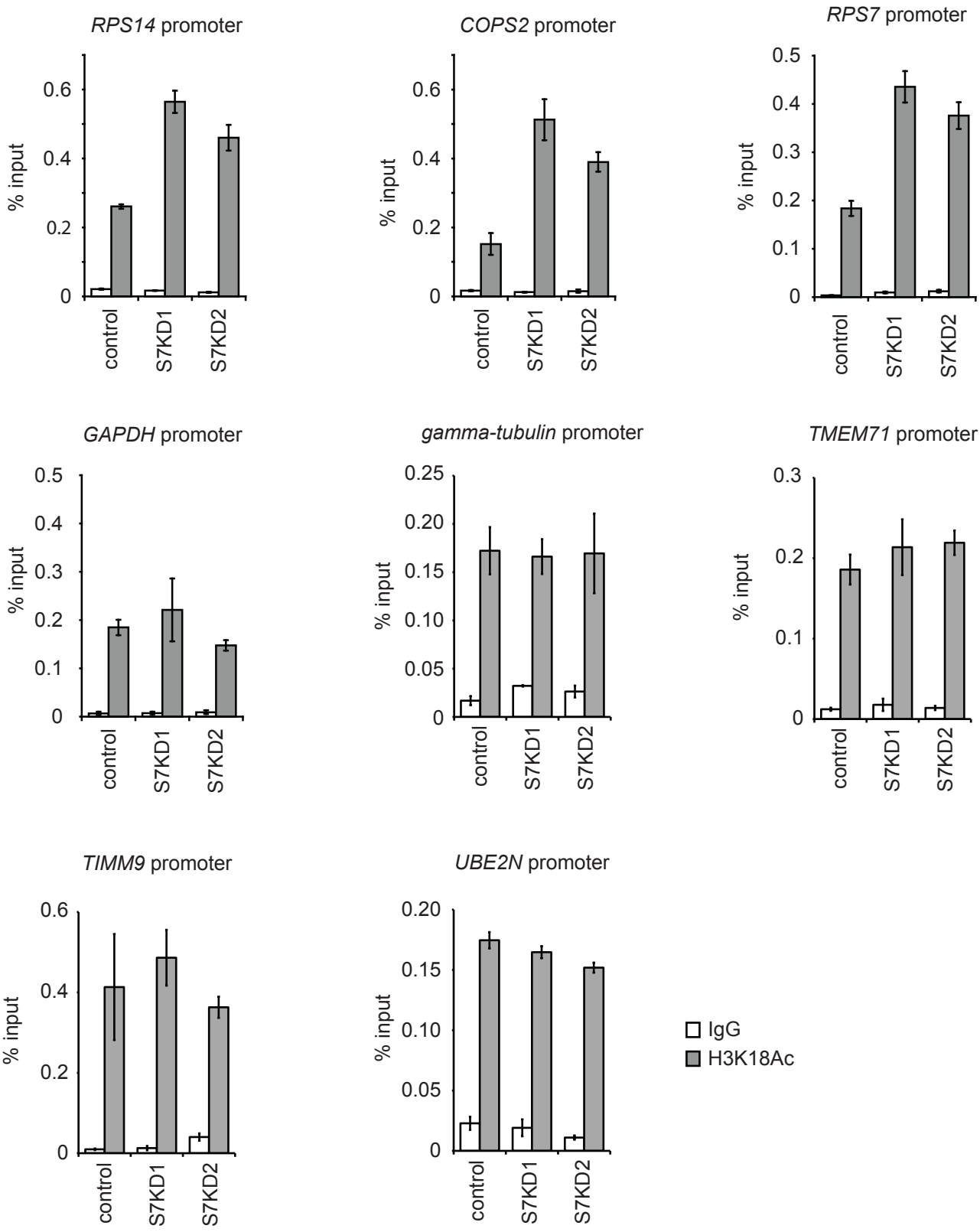


SIRT7-associated oncomine clusters	q-value
Bladder (Dyrskot 3)	9.56e-11
Leukemia (Soulier)	3.07e-08
Prostate (Liu)	8.84e-07
Leukemia (Frank)	1.44e-06
Lymphoma (Klapper)	5.77e-06
Breast (Finak)	7.48e-06
Leukemia (Dik)	1.42e-05
Breast (Hess)	3.53e-05
Gastric (Ooi 2)	7.23e-05
Prostate (Taylor 3)	7.56e-05
Parathyroid (Morrison)	7.58e-05

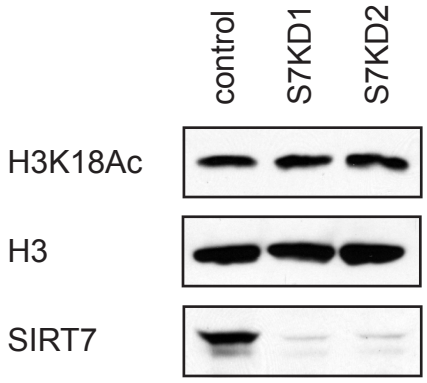
Supplementary Figure 2. Promoter enrichment and target gene analysis of SIRT7 ChIP-sequencing. a, Enrichment of SIRT7 ChIP-sequencing peaks in promoters compared to distribution over the whole genome (p-value 1.6 e-193). **b,** Average SIRT7 ChIP-sequencing signal across all transcriptional start sites (TSS). **c,** Gene ontology (GO) categories of SIRT7-occupied genes determined by ChIP-sequencing analysis. Categories were plotted and ranked based on the inverse log(10) of multiple testing corrected false discovery rate (FDR). **d,** Oncomine clusters containing signficant overlap with SIRT7 target genes as determined by ChIP-sequencing analysis, plotted and ranked based on q-value. Each cluster represents strongly misregulated genes across patients in a single cancer study. **e,** SIRT7-occupied genes are highly expressed relative to the genomic average. Box plots comparing expression of SIRT7 ChIP-seq target genes to all human genes were constructed and scaled by log(10) of fragments per kilobase per million sequence reads (FPKM).



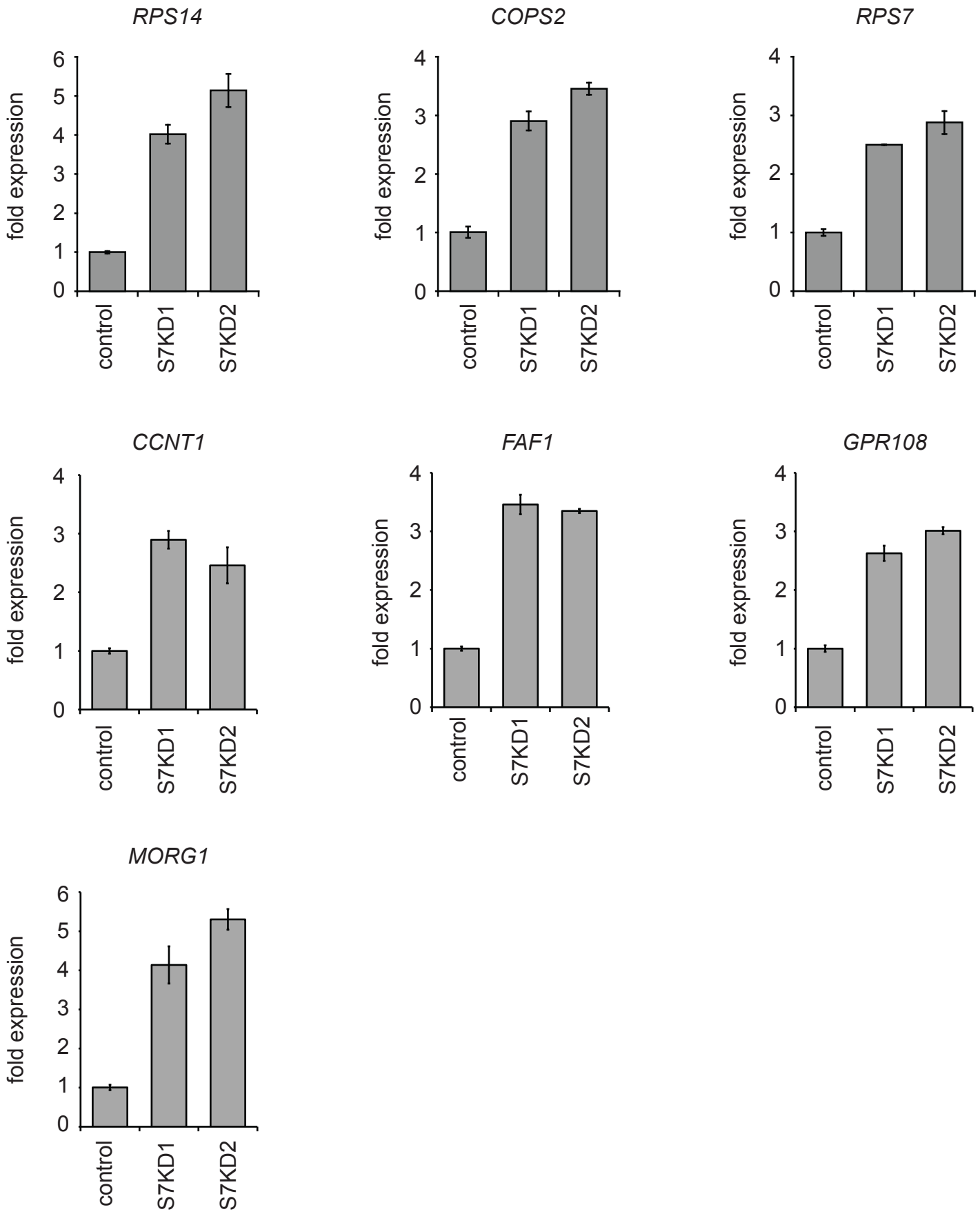
Supplementary Figure 3. SIRT7 is enriched in select gene promoters. SIRT7 occupancy in control and S7KD HT1080 cells determined by ChIP analysis (mean +/- S.E.M.). The *GAPDH* promoter was included as a negative control.



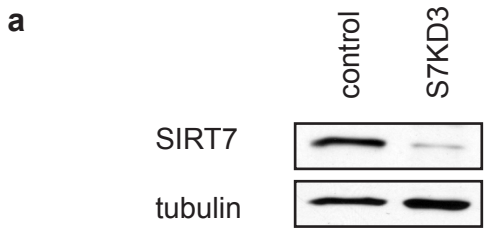
Supplementary Figure 4. SIRT7 deacetylates H3K18Ac at target promoters. Hyperacetylation of H3K18 in S7KD HT1080 cells determined by ChIP qPCR (mean +/- S.E.M.). All samples were normalized to input DNA. The *GAPDH, gamma-tubulin, TMEM71, TIMM9,* and *UBE2N* promoters were included as negative controls.

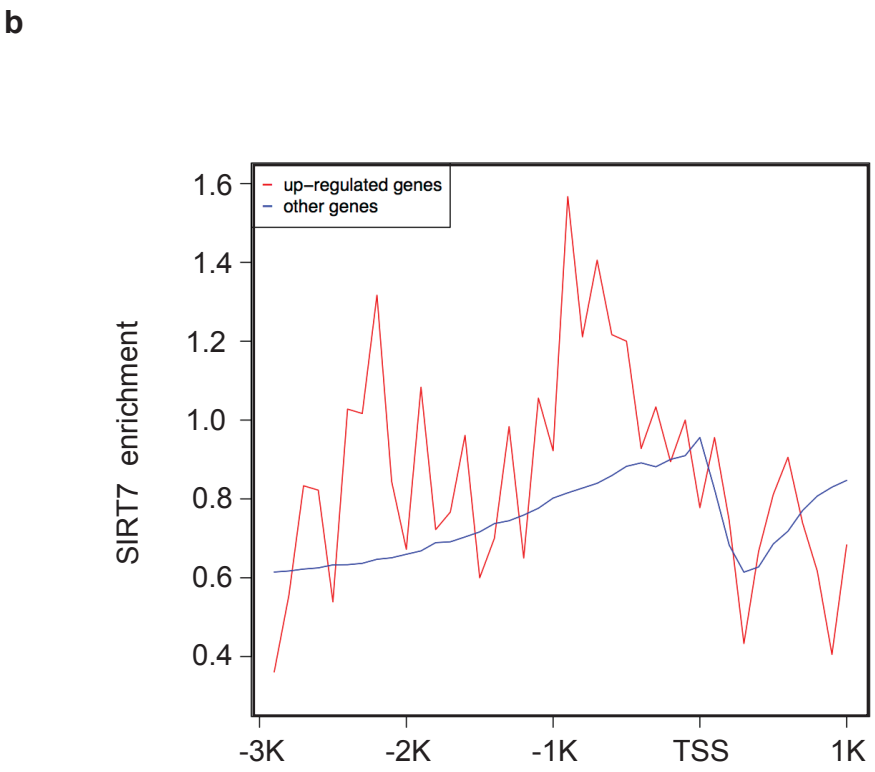


Supplementary Figure 5. SIRT7 depletion does not increase global levels of H3K18Ac. Cell extracts were prepared from control and SIRT7-depleted HT1080 cells and analyzed by western blot.

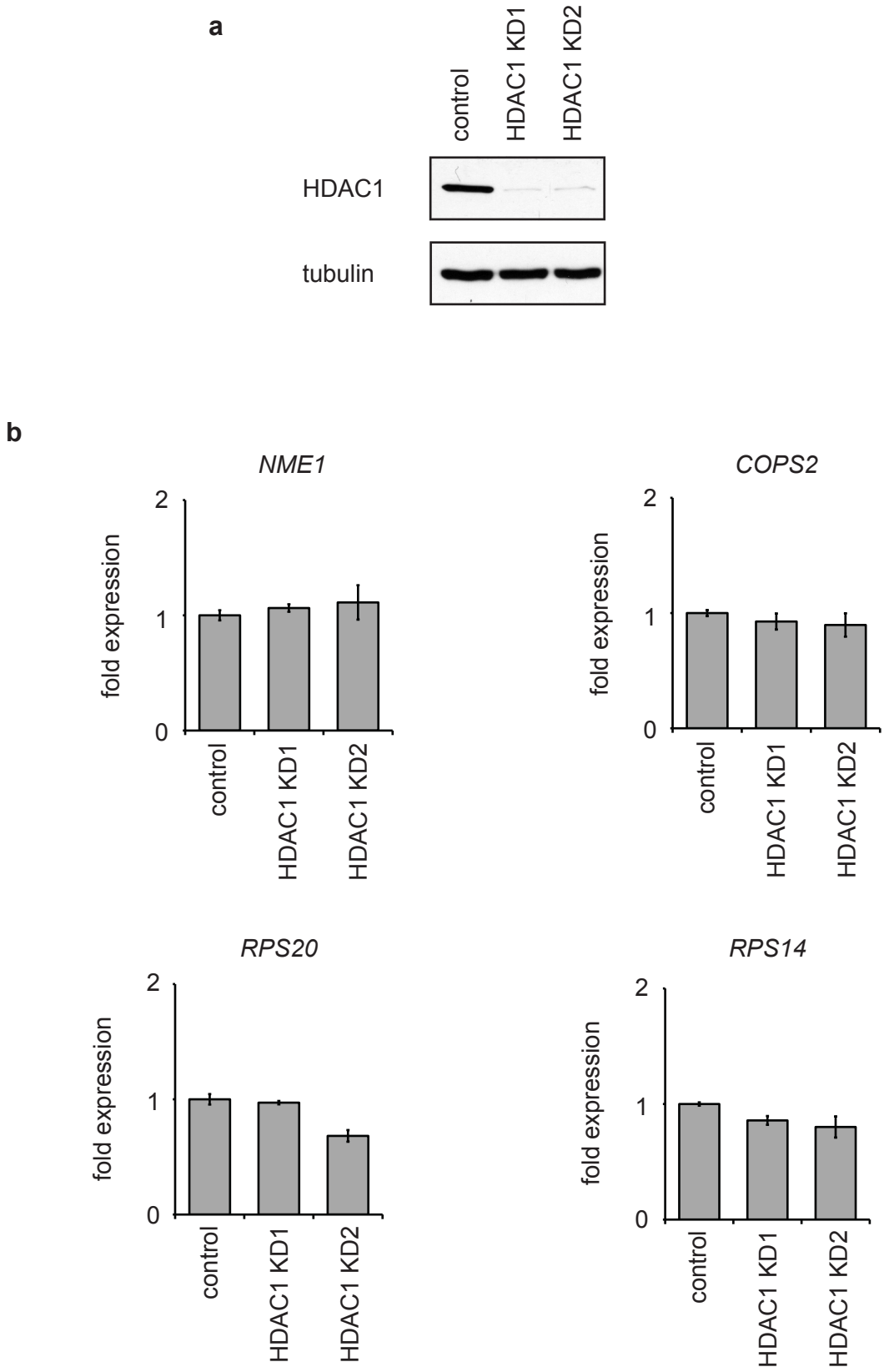


Supplementary Figure 6. SIRT7 depletion induces transcriptional activation of target genes. Increased mRNA expression (mean +/- S.E.M.) of target genes in S7KD HT1080 cells, measured by qPCR. The data are normalized to *GAPDH* mRNA levels. CCNT1: cyclin T1; FAF1: Fas-associated factor 1; GPR108: G-protein coupled receptor 108; MORG1: MAPK organizer 1.

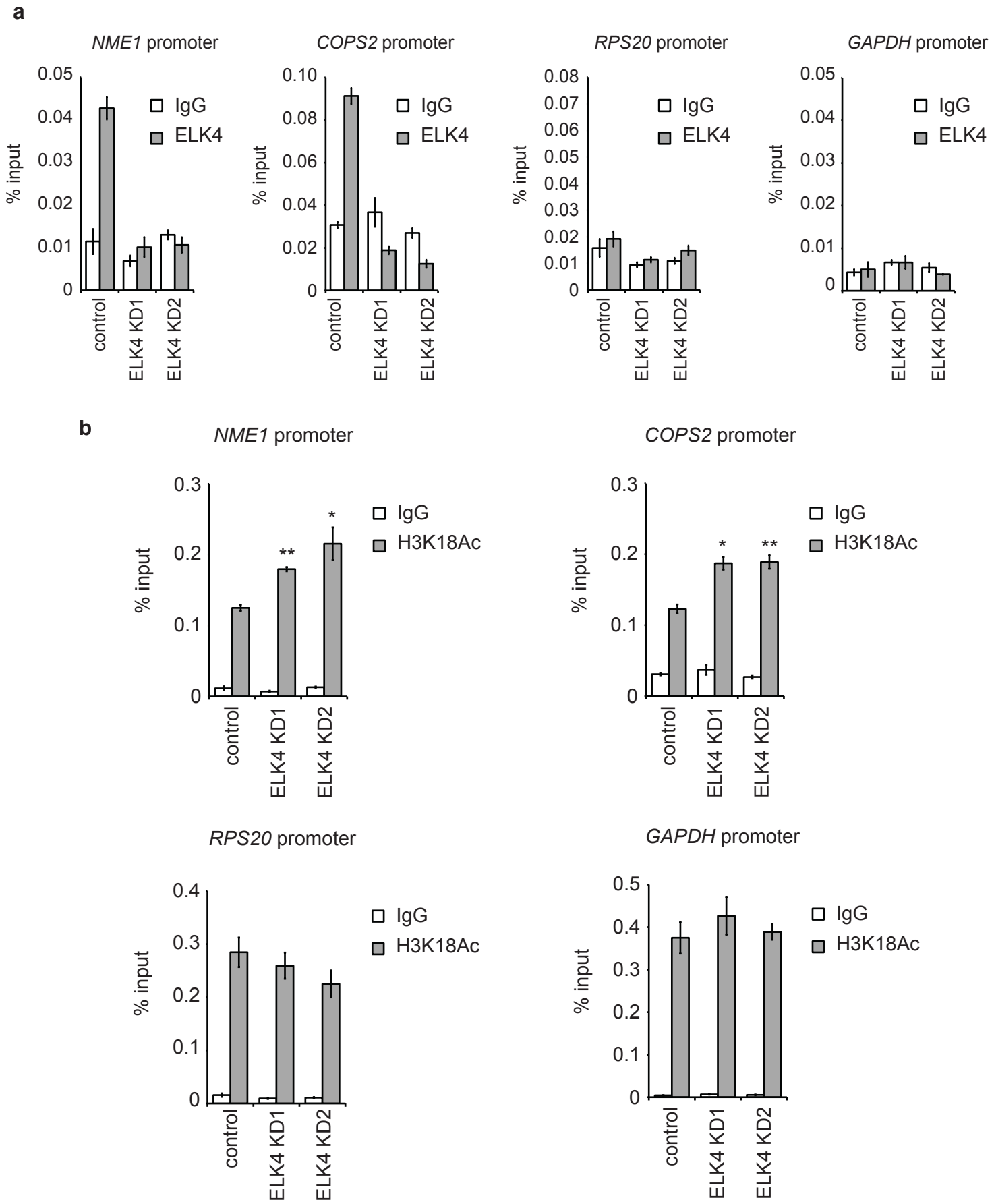




Supplementary Figure 7. SIRT7-occupied genes are up-regulated upon SIRT7 depletion. a, SIRT7 was depleted from K562 cells by lentiviral transduction. Whole cell lysates were analyzed by western blot. **b,** Average SIRT7 ChIP-seq profiles across surrounding promoter regions, from -3K upstream to 1K downstream of the transcription start sites of genes whose expression is up-regulated in S7 knockdown cells (red) compared to all other genes (blue).

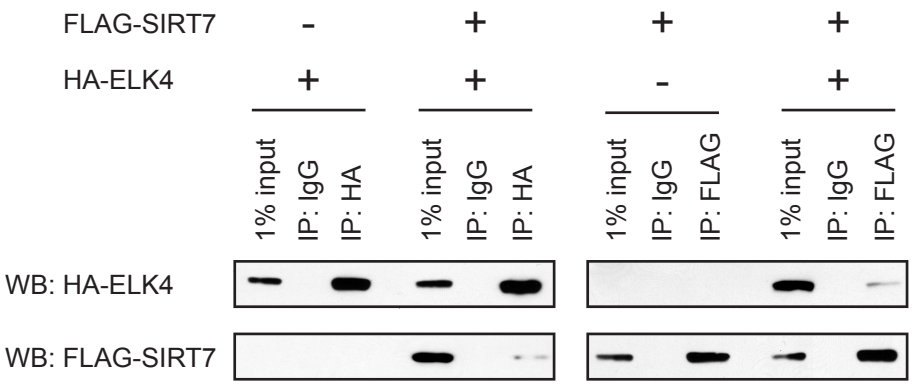


Supplementary Figure 8. HDAC1 does not regulate several SIRT7 target genes. a, HDAC1 was depleted from HT1080 cells using siRNA. Whole cell extracts were analyzed by western blot. **b,** RNA was purified from control and HDAC1-depleted HT1080 cells, and transcript levels were measured by qPCR (mean +/- S.E.M.).

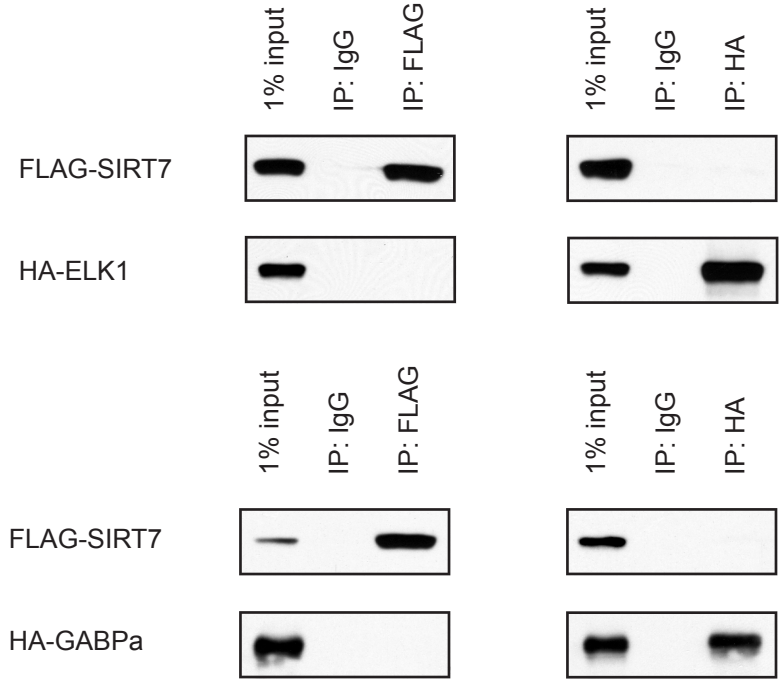


Supplementary Figure 9. ELK4 occupancy at SIRT7 target promoters. a, ChIP qPCR analysis of control or ELK4 KD HT1080 cells. ELK4 and control (IgG) IPs were measured relative to input (mean +/- S.E.M). **b,** ChIP qPCR analysis of H3K18Ac levels at target promoters. *RPS20*, which is bound by SIRT7 but not ELK4, and *GAPDH*, which is bound by neither, were included as negative controls. H3K18Ac and control (IgG) IPs were measured (mean +/- S.E.M.) relative to input (* p-value < 0.05; ** p-value < 0.01).

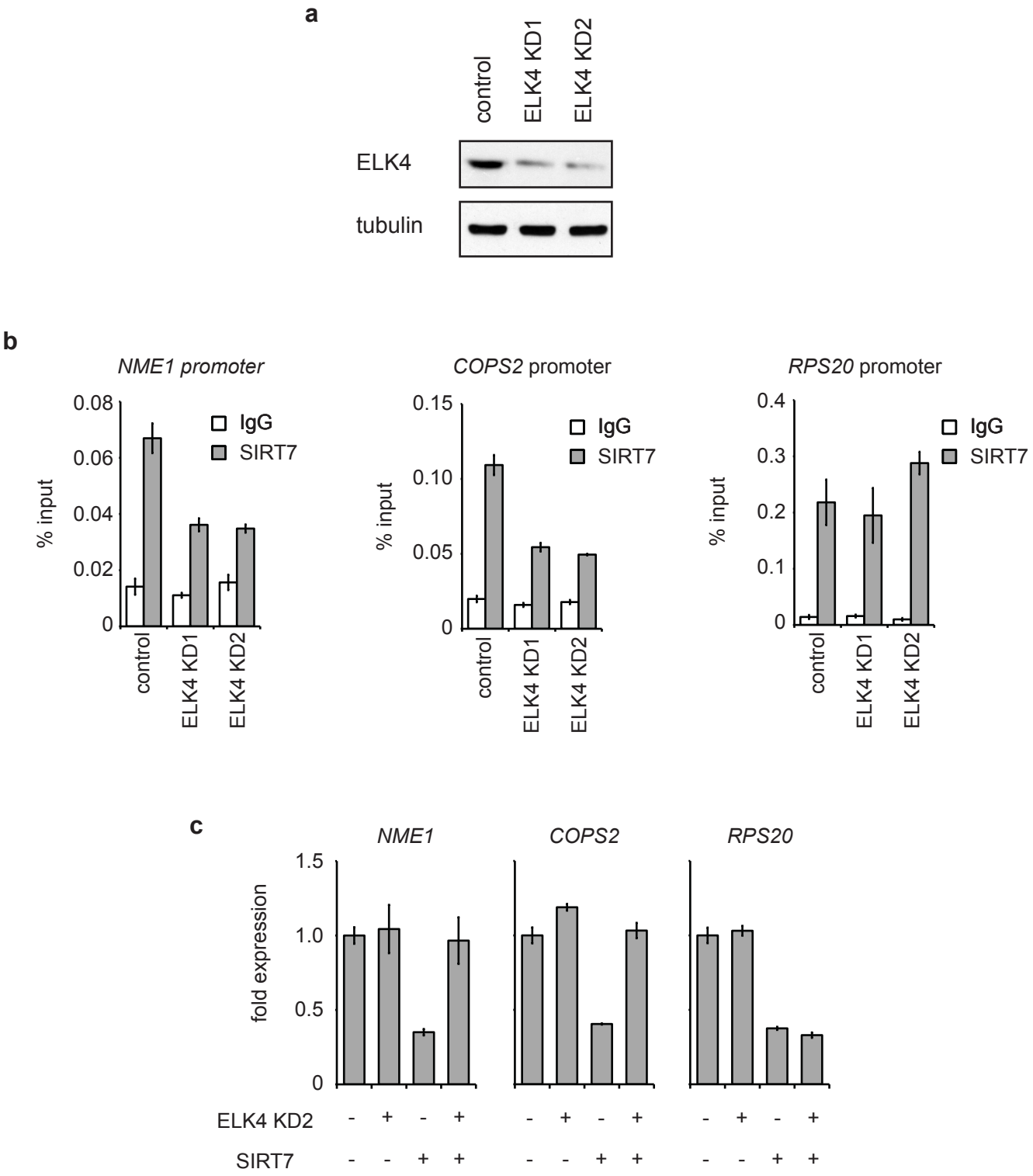
a



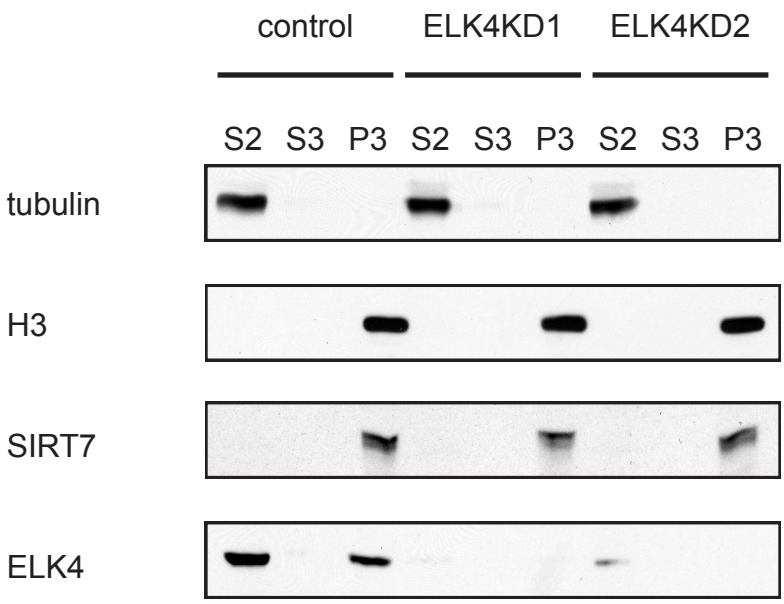
b



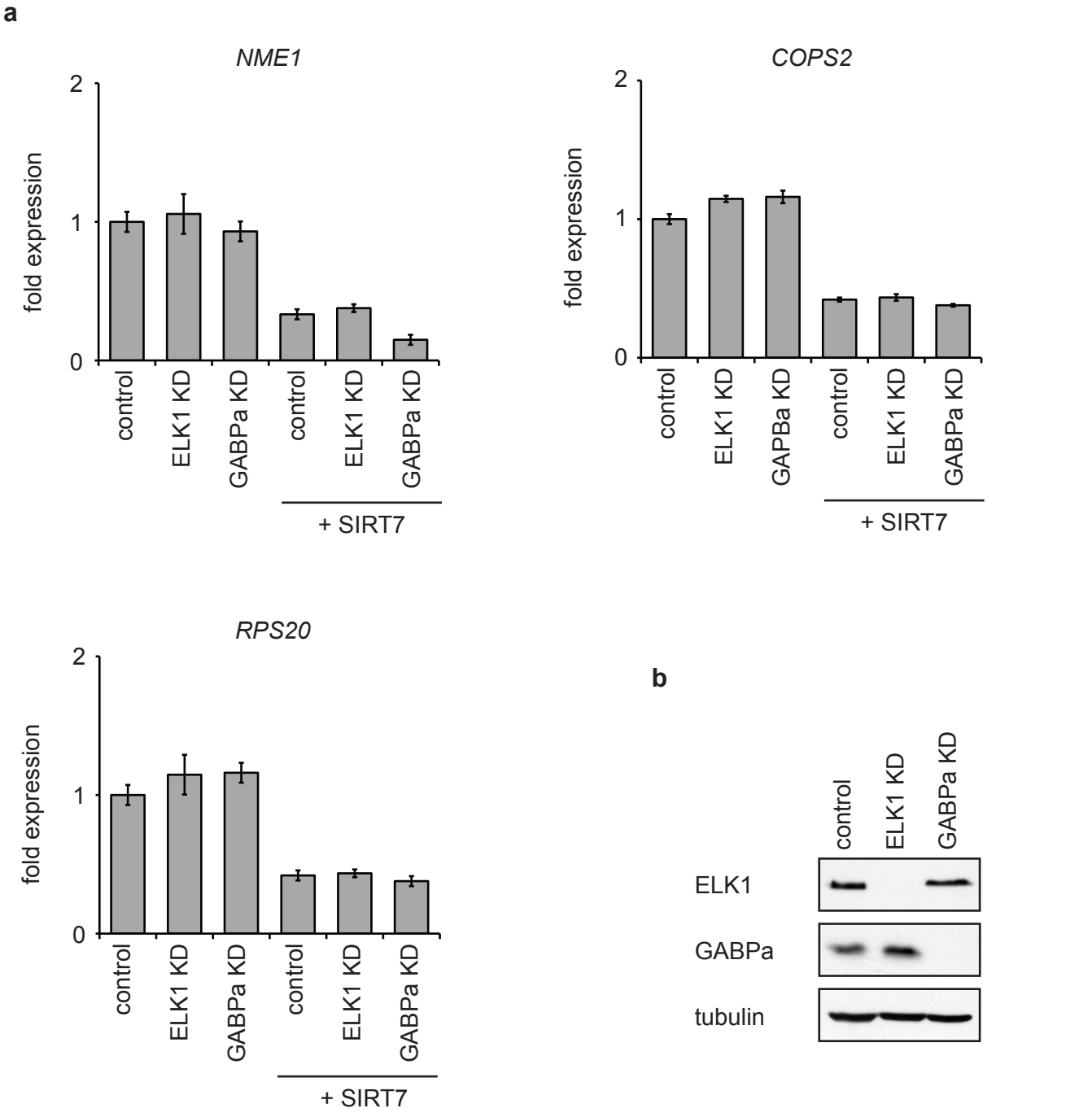
Supplementary Figure 10. SIRT7 interacts with ELK4, but not ETS proteins ELK1 or GABPa in cells. a, Western analysis showing co-immunoprecipitation (co-IP) of FLAG-tagged SIRT7 and HA-tagged ELK4 in 293T cells expressing either FLAG-SIRT7, HA-ELK4, or both. **b,** Either FLAG-SIRT7 and HA-ELK1 (top panel) or FLAG-SIRT7 and HA-GABPa (bottom panel) were co-expressed in 293T cells. Immunoprecipitations (IPs) were performed from whole cell extracts and analyzed by western blot. IgG IPs were included as negative controls.



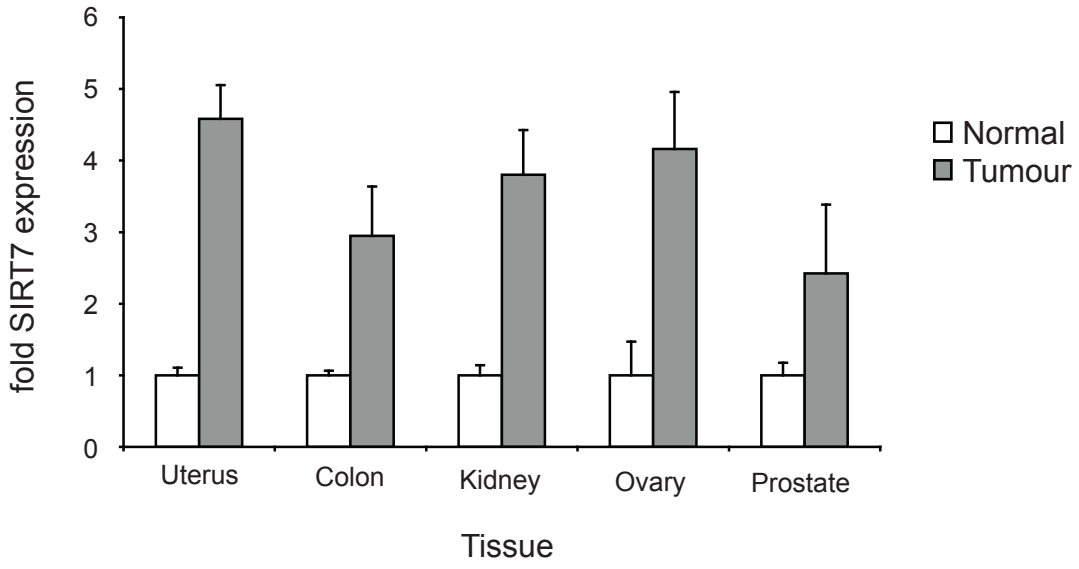
Supplementary Figure 11. ELK4 is required for full promoter occupancy and transcriptional effects of SIRT7 in DU145 prostate cancer cells. a, Western blots of extracts from DU145 cells showing knockdown of ELK4 with two independent siRNAs. **b,** ELK4 promotes SIRT7 promoter targeting in prostate cells. Control and ELK4-depleted DU145 cells were used for ChIP qPCR analysis (mean +/- S.E.M.). Negative control (IgG) and SIRT7 IPs were plotted relative to input. c, DU145 cells were treated with either control or ELK4-specific siRNAs and transfected with either empty vector or SIRT7-expressing vector. Transcript levels were quantified by qPCR (mean +/- S.E.M.). **c,** ELK4 depletion attenuates SIRT7-mediated transcriptional repression in DU145 cells, as determined by qPCR (mean +/- S.E.M.). Cells were treated with control or ELK4 siRNA, and transduced with empty or SIRT7-expressing vector as indicated.



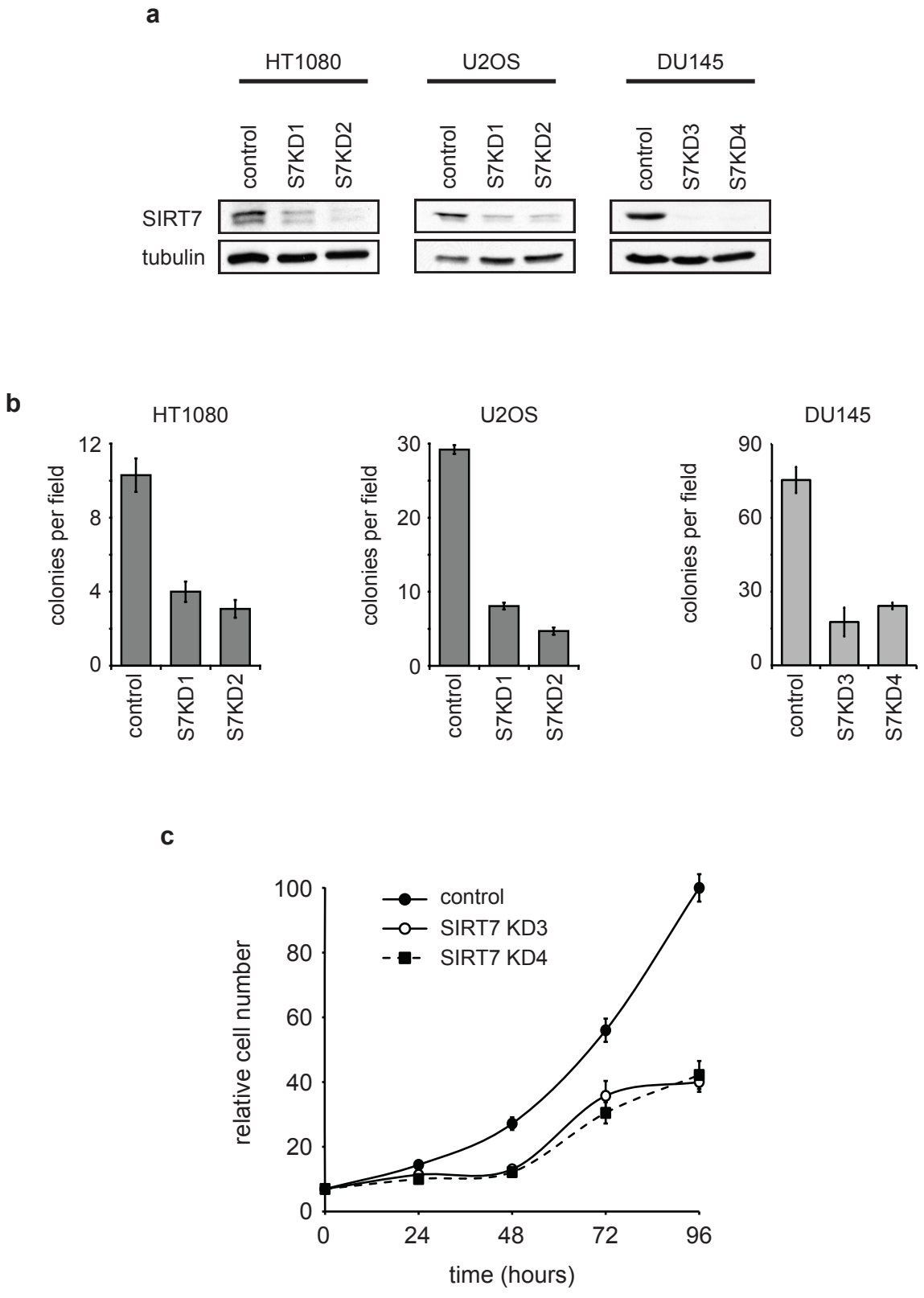
Supplementary Figure 12. ELK4 depletion does not globally alter SIRT7 chromatin association. Control and ELK4-depleted HT1080 cells were fractionated into samples enriched for cytoplasm (S2), nucleoplasm (S3), or chromatin (P3). Samples were analyzed by western blot.



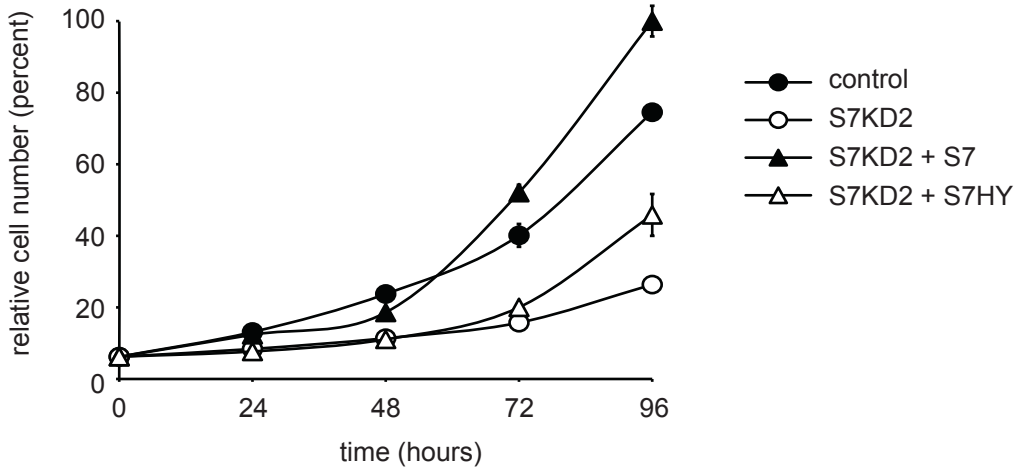
Supplementary Figure 13. ELK1 and GABPa depletion do not affect SIRT7-mediated transcriptional repression. a, HT1080 cells were treated with either control siRNA, or siRNA targeting ELK1 or GABPa. Cells were then transfected with a control vector or a vector expressing recombinant SIRT7. Transcript levels were subsequently analyzed by qPCR. **b,** Extracts from HT1080 cells treated with indicated siRNAs were analyzed by western blot.



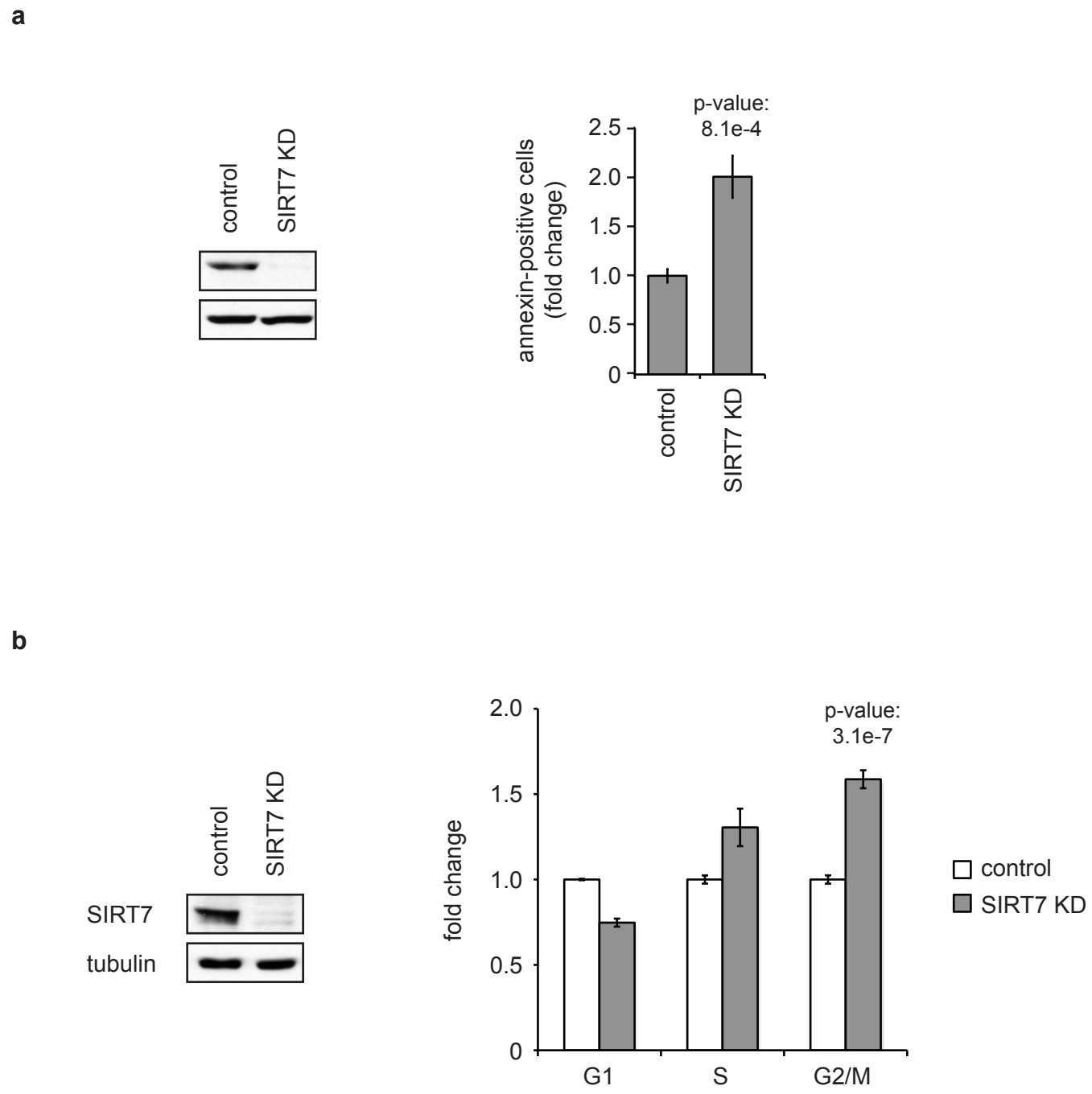
Supplementary Figure 14. SIRT7 is over-expressed in human tumour samples. Over-expression of *SIRT7* in patient-matched tumours relative to unaffected control tissue measured by quantitative RT-PCR (mean +/- S.E.M.) and normalized to *GAPDH* expression.



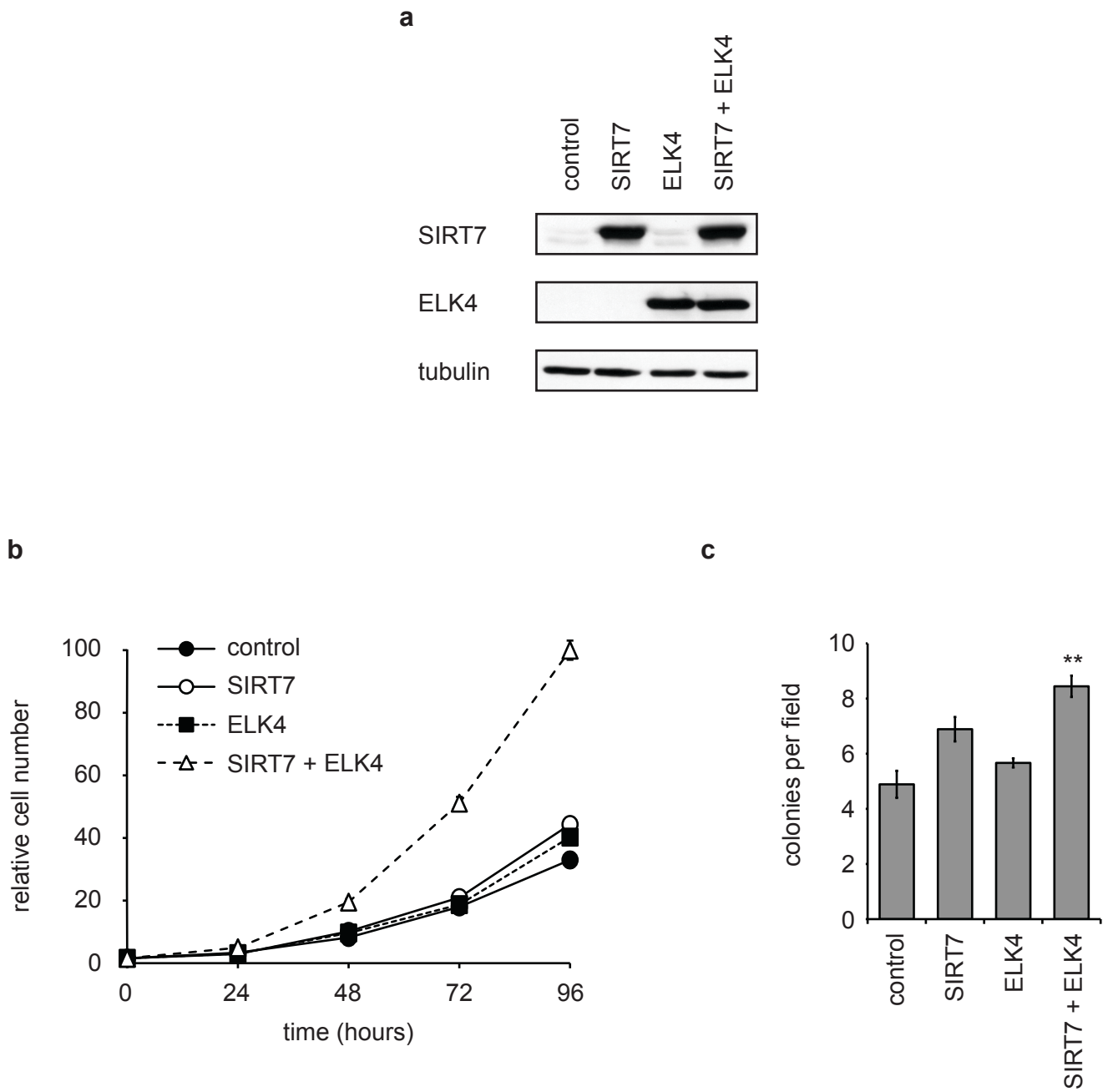
Supplementary Figure 15. SIRT7 depletion impairs cancer cell phenotypes. a, SIRT7 was depleted from HT1080, U2OS, and DU145 cells. Retroviral transduction was performed for HT1080 and U2OS, while lentiviral transduction was used for DU145. Extracts were subsequently analyzed by western blot. **b,** SIRT7 depletion impairs anchorage independent growth. Control and SIRT7 depleted cells were plated in soft-agar containing media and allowed to grow for two weeks before colonies were scored. Data represent average and S.E.M. of three independent experiments. **c,** SIRT7 regulates DU145 cell growth in low serum. SIRT7 was depleted from DU145 cells using lentiviral shRNA transduction. Cell growth in 1% new calf serum was subsequently measured over four days. Data represent averages and S.E.M. of three independent experiments.



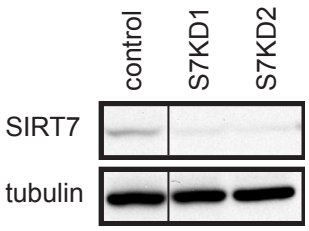
Supplementary Figure 16. SIRT7 deacetylase activity is required for proliferation in low serum. HT1080 cells were transduced with retrovirus expressing either control or SIRT7 shRNA (S7KD2), as well as empty vector, wildtype (S7), or mutant (S7HY) SIRT7 expressing vectors. Cell proliferation in 1% serum was monitored over 4 days. Data represent average and S.E.M. of three independent experiments.



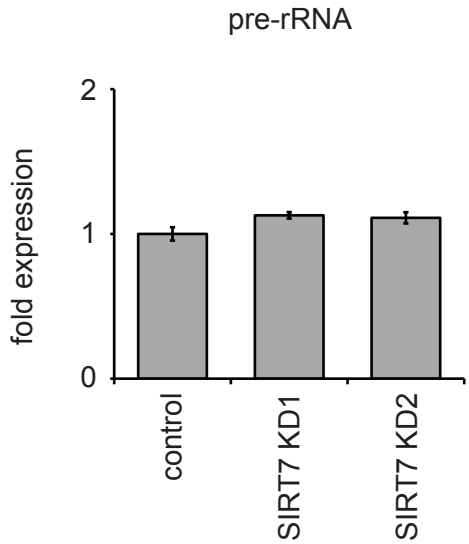
Supplementary Figure 17. SIRT7 depletion induces cell death and accumulation in G2/M phase. a, Cells were stained with annexin and propidium iodidide, then analyzed by flow cytometry (mean +/- S.E.M.). Annexin V-positive cells were plotted relative to control conditions (right panel). Whole cell extracts were analyzed by western blot (left panel). **c,** SIRT7 depletion leads to accumulation in G2/M phase (right panel). Cells were stained with propidium idodide and BrdU and cell-cycle phase was determined by flow cytometry (mean +/- S.E.M.). Each cell cycle phase was plotted relative to control cells. Whole cell extracts were analyzed by western blot (left panel).



Supplementary Figure 18. Co-expression of SIRT7 and ELK4 enhances cancer cell phenotypes. a, SIRT7 and ELK4 were stably expressed either alone or in combination in HT1080 cells. Whole cell lysates were analyzed by western blot. **b,** Cell lines were cultured in 1% serum and relative cell number was measured over four days. Data represent average and S.E.M. of three independent experiments. **c,** Indicated cell lines were plated in soft agar and colonies were scored after 2 weeks. Data represent average and S.E.M. of three independent experiments (** p-value < 0.01 relative to control).



Supplementary Figure 19. SIRT7 depletion in U251 cells. SIRT7 was depleted in U251 human glioma cells by retroviral transduction. Whole cell extracts were analyzed by western blot.



Supplementary Figure 20. SIRT7 depletion does not impair pre-ribosomal RNA synthesis in HT1080 cells. Pre-ribosomal RNA (pre-rRNA) was measured using qPCR from control and SIRT7 depleted HT1080 cells (mean +/- S.E.M.).