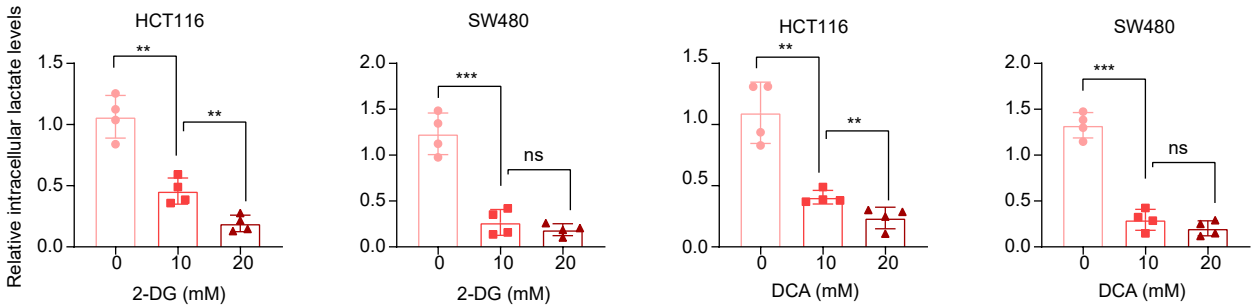
Extended Data Fig. 1

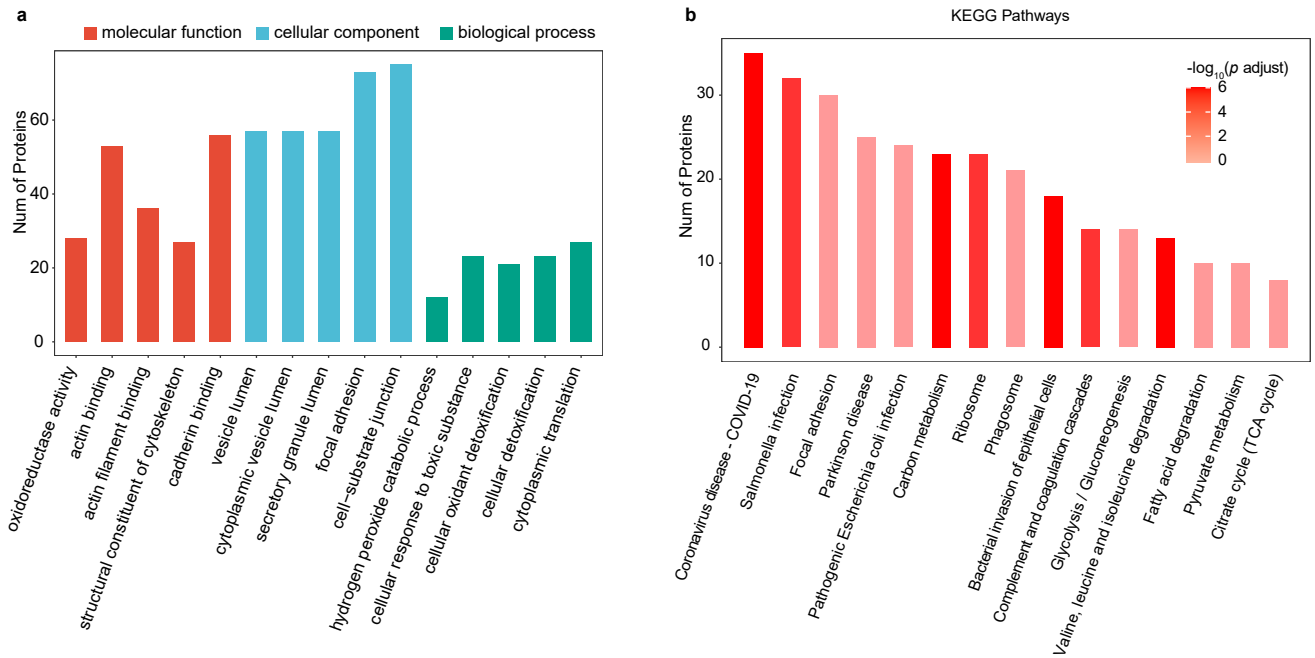
a

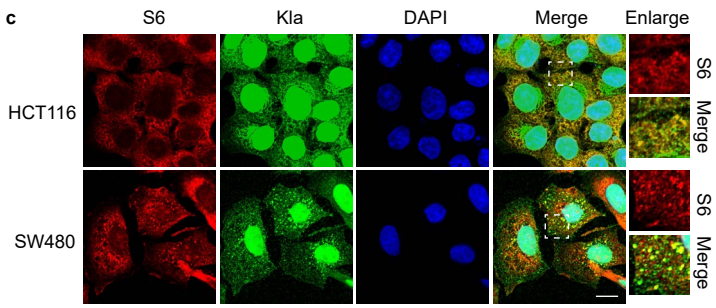


Extended Data Fig. 1

a. Intracellular lactate levels were measured from HCT116 and SW480 treating with 2-DG and DCA cultured in different concentrations of 2-DG or DCA for 24 h by a lactate colorimetric kit. The data are represented as the means ± s.d., $^{**}p < 0.01$, $^{***}p < 0.001$, unpaired, two-tailed Student's *t*-test. Representative results of n = 3 independent treated cell cultures were shown.

Extended Data Fig. 2

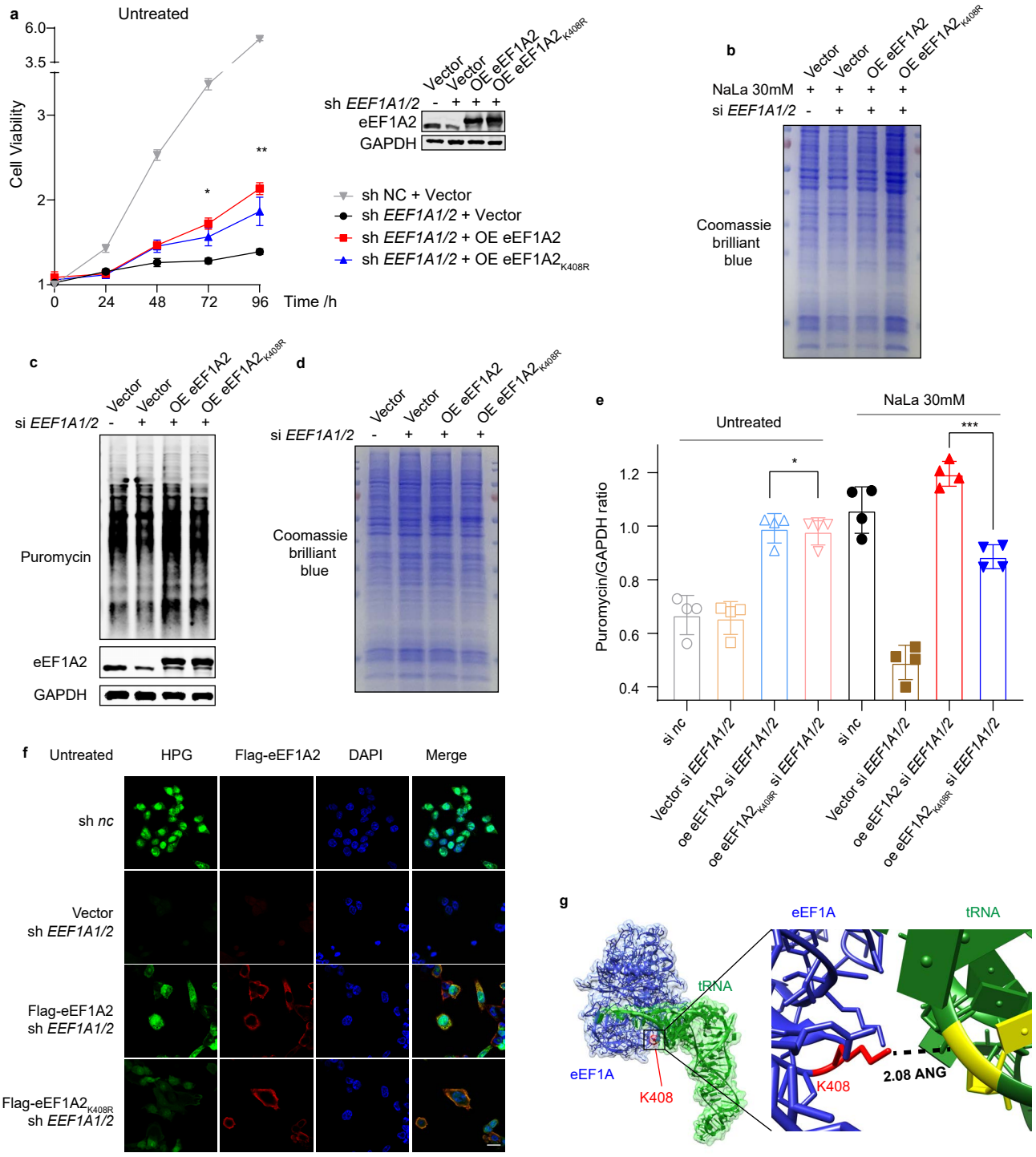




Extended Data Fig. 2

a. Gene ontology analysis associated with Kla proteins. *P* values are derived from one-sided Fisher's exact test.
b. KEGG pathway analysis of Kla proteins. *P* values are derived from empirical distribution test.
c. Representative IF staining for Kla and S6 ribosomal protein in HCT116 and SW480 cells. Scale bars, 10 μm. Representative pictures of n = 3 independent treated cell cultures were shown.

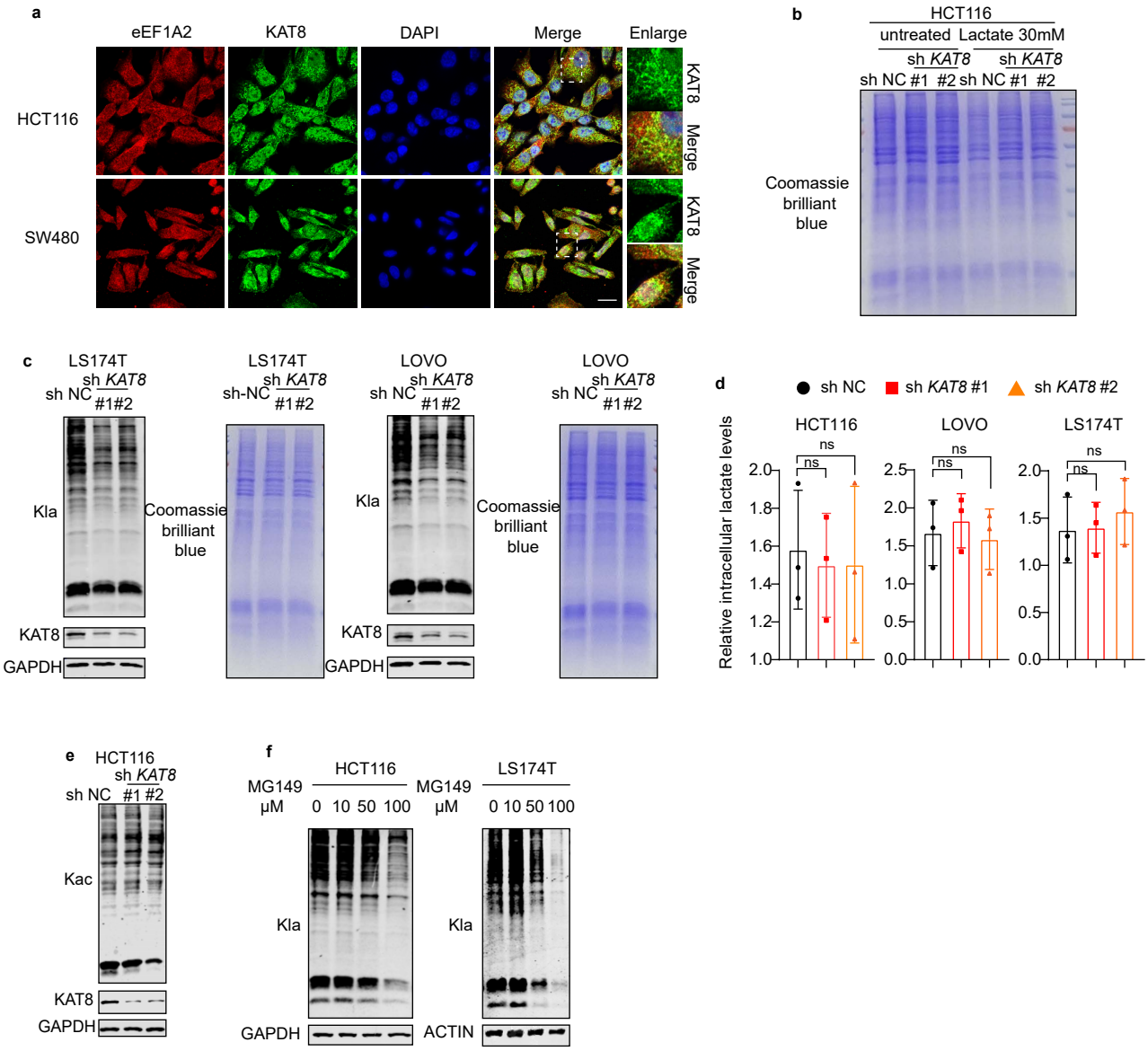
Extended Data Fig. 3



Extended Data Fig. 3

a. Cell proliferations of control or eEF1A1/2-depleted HCT116 cells complemented with WT eEF1A2 (OE eEF1A2), eEF1A2$_{K408R}$ (OE eEF1A2$_{K408R}$), or control (Vector) were measured with Incucyte cell count proliferation assay. The data are represented as the means ± s.d., *p < 0.05, **p < 0.01; unpaired, two-tailed Student's t-test. Representative results of n = 3 independent treated cell cultures were shown.
b. Coomassie stain of proteins in the SUnSET reaction in the presence of NaLa.
c. SUnSET assays under the indicated conditions reveal different levels of protein production in eEF1A1/2-depleted HCT116 cells complemented with WT eEF1A2 (OE eEF1A2), eEF1A2$_{K408R}$ (OE eEF1A2$_{K408R}$), or control (Vector). WCEs were isolated and probed the indicated antibodies. Representative pictures of n = 3 independent treated cell cultures were shown.
d. Coomassie stain of proteins in the SUnSET reaction in the absence of NaLa.
e. Mean gray value analyzed according to immunoblotting results from SUnSET assays of indicated genotype. The data are represented as the means ± s.d., *p < 0.05, ***p < 0.001; unpaired, two-tailed Student's t-test. Representative results of n = 3 independent treated cell cultures were shown.
f. HPG incorporation assay in HCT116 cells of the indicated genotype. Scale bar, 20 μm. Representative pictures of n = 3 independent treated cell cultures were shown.
g. Cryo-EM structures of eEF1A and tRNA in eukaryotic translational decoding complexes (PDB:5lzs).

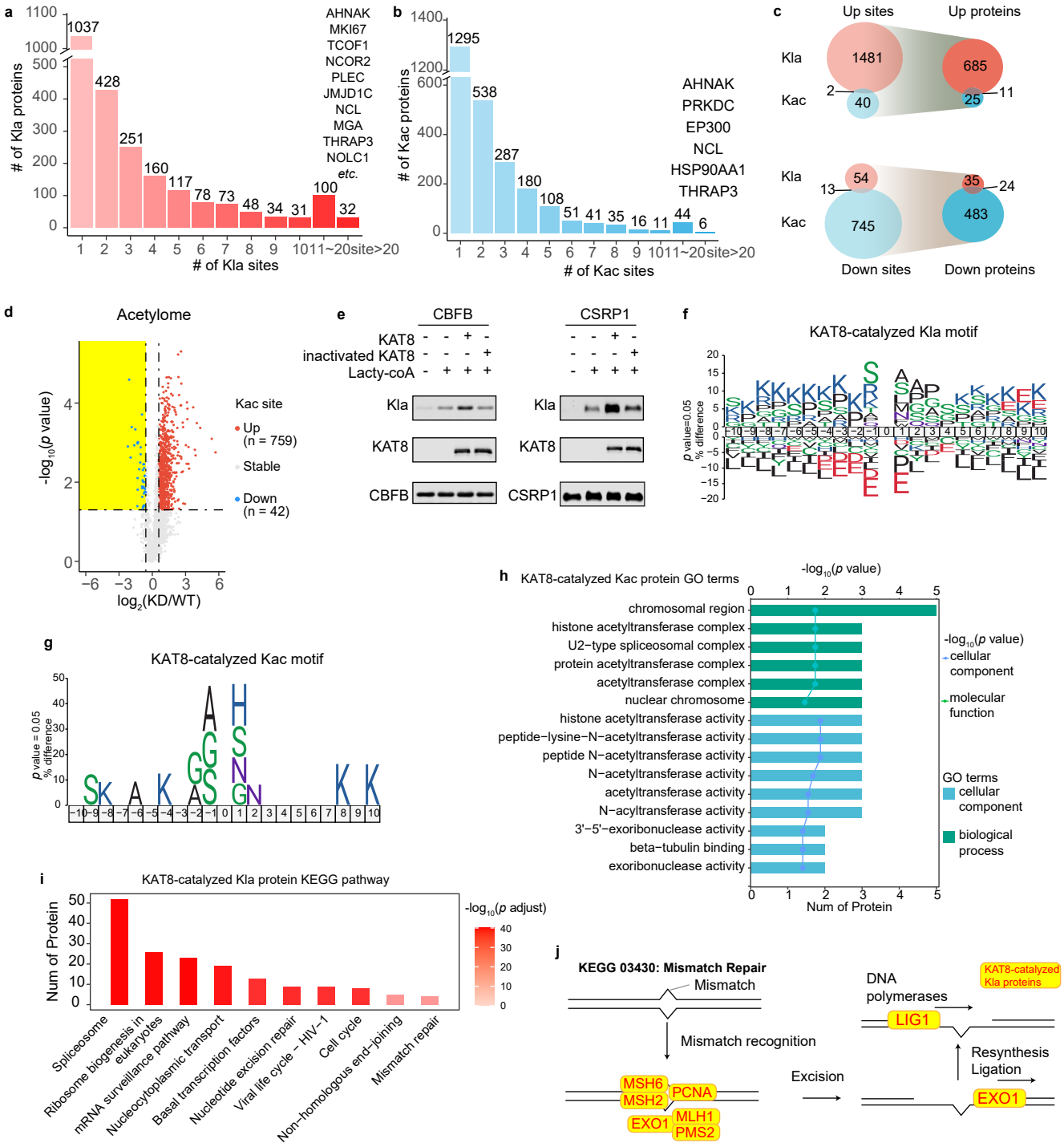
Extended Data Fig. 4



Extended Data Fig. 4

a. The level and cellular location of eEF1A2 and KAT8 were detected in HCT116 and SW480 cells were detected by immunofluorescence staining with indicated antibodies. Scale bar: 20 μm; Representative pictures of n = 3 independent treated cell cultures were shown.
b. Coomassie stain of proteins in the reaction Fig.4c.
c. Kla levels were detected in LS174T and LOVO cells transfected with two independent shRNAs targeting KAT8 or a control shRNA by immunoblotting. Representative pictures of n = 3 independent treated cell cultures were shown.
d. Intracellular lactate levels were measured from HCT116 transfected with two independent shRNAs targeting KAT8 or a control shRNA by a lactate colorimetric kit. The data are represented as the means ± s.d.; unpaired, two-tailed Student's *t*-test. Representative results of n = 3 independent treated cell cultures were shown.
e. Kac levels were detected in HCT116 cells transfected with two independent shRNAs targeting KAT8 or a control shRNA by immunoblotting. Representative pictures of n = 3 independent treated cell cultures were shown.
f. Kla levels were detected in HCT116 and LS174T cells treating with different concentrations of MG149 for 24 h by immunoblotting. Representative pictures of n = 3 independent treated cell cultures were shown.

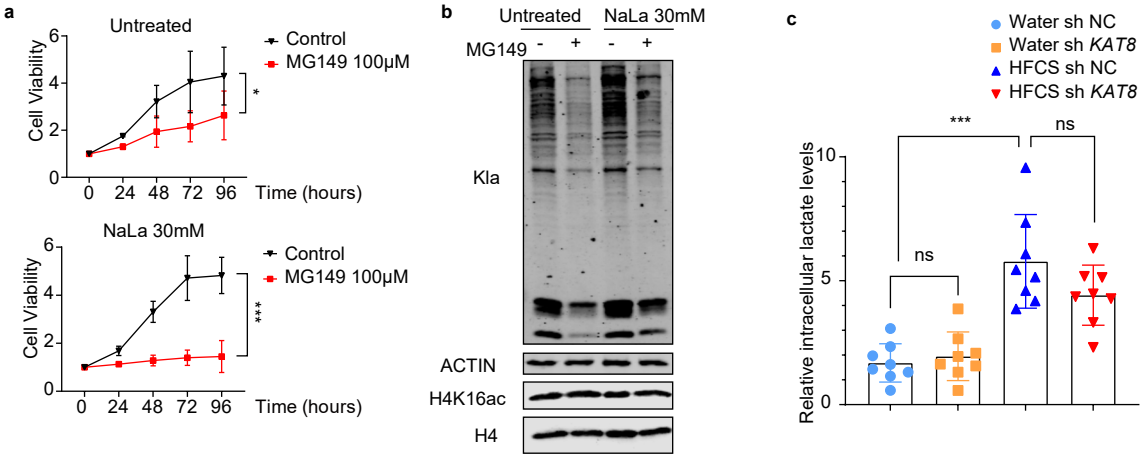
Extended Data Fig. 5



Extended Data Fig. 5

a. Histogram of the number of all Kla sites per protein. Proteins with more than 20 sites of Kla are listed above the corresponding bar.
b. Histogram of the number of all Kac sites per protein. Proteins with more than 20 sites of Kla are listed above the corresponding bar.
c. The number of up-regulated (top) and down-regulated (bottom) Kla (red) and Kac(blue) sites and proteins.
d. Volcano plot showing the global acetylome changes in cells knockdown KAT8 (KD) vs. control cells (WT). Individual sites are represented by points.
e. *In vitro* CBFB and CSRP1 lactylation assay. Purified CBFB and CSRP1 or from *E. coli* was incubated with recombinant KAT8 in the presence or absence of lactyl-CoA. CBFB and CSRP1 lactylation were analyzed by immunoblotting with indicated antibodies. Representative pictures of n = 3 independent repeats were shown.
f. Icelogo representation showing flanking sequence preferences for KAT8-catalyzed Kla sites, binomial test *p* values reported.
g. Icelogo representation showing flanking sequence preferences for KAT8-catalyzed Kac sites, binomial test *p* values reported.
h. Gene ontology analysis associated with KAT8-catalyzed Kac proteins. *P* values are derived from one-sided Fisher's exact test.
i. KEGG pathway analysis of KAT8-catalyzed Kla proteins. *P* values are derived from empirical distribution test.
j. Schematic of KAT8-catalyzed Kla proteins (yellow box and red font) and Kla proteins (yellow box) associated with mismatch repair (KEGG:03430).

Extended Data Fig. 6



Extended Data Fig. 6

a. Cell proliferations of HCT116 cells treated with MG149 in the presence or absence of 30 mM NaLa were measured with Incucyte cell count proliferation assay. The data are represented as the means ± s.d., *$p < 0.05$, ***$p < 0.001$, two-way ANOVA. Representative results of n = 3 independent treated cell cultures were shown.

b. Kla and H4K16ac levels were detected in HCT116 cells treated with MG149 in the presence or absence of 30 mM NaLa by immunoblotting. Representative pictures of n = 3 independent treated cell cultures were shown.

c. Intracellular lactate levels were measured from control or KAT8 KD HCT116 cells in high-lactate conditions or normal conditions for 24 h by a lactate colorimetric kit. The data are represented as the means ± s.d., ***$p < 0.001$; unpaired, two-tailed Student's *t*-test. Representative results of n = 3 independent treated cell cultures were shown.