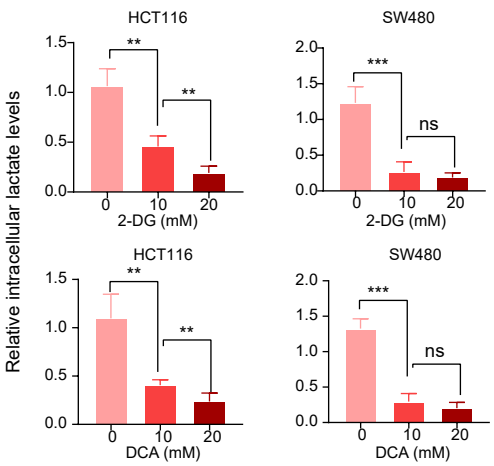
Extended Data Fig. 1

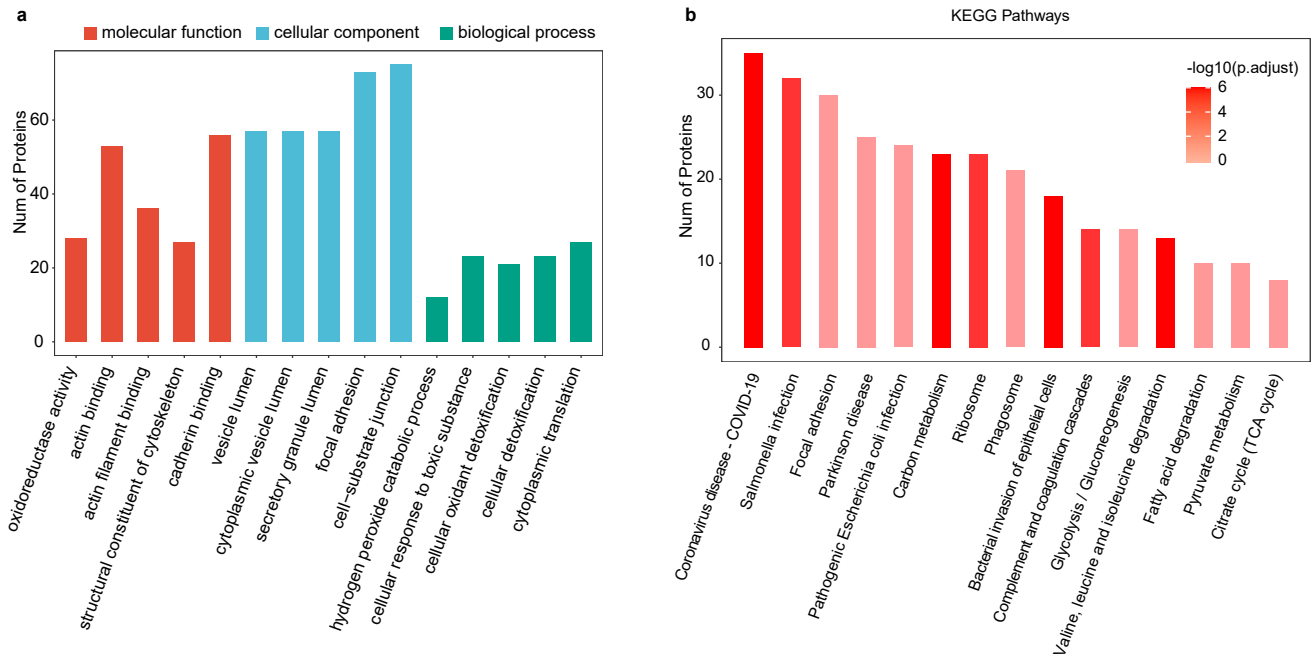
a

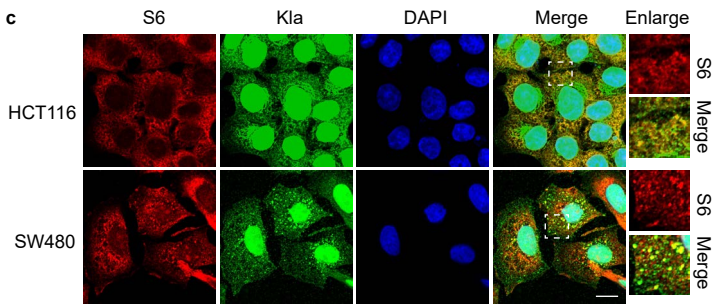


Extended Data Fig. 1

a. Intracellular lactate levels were measured from HCT116 and SW480 treating with 2DG and DCA cultured in different concentrations of 2DG or DCA for 24 h by a lactate colorimetric kit. (the data are represented as the means ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$; unpaired, two-tailed Student's t test)

Extended Data Fig. 2





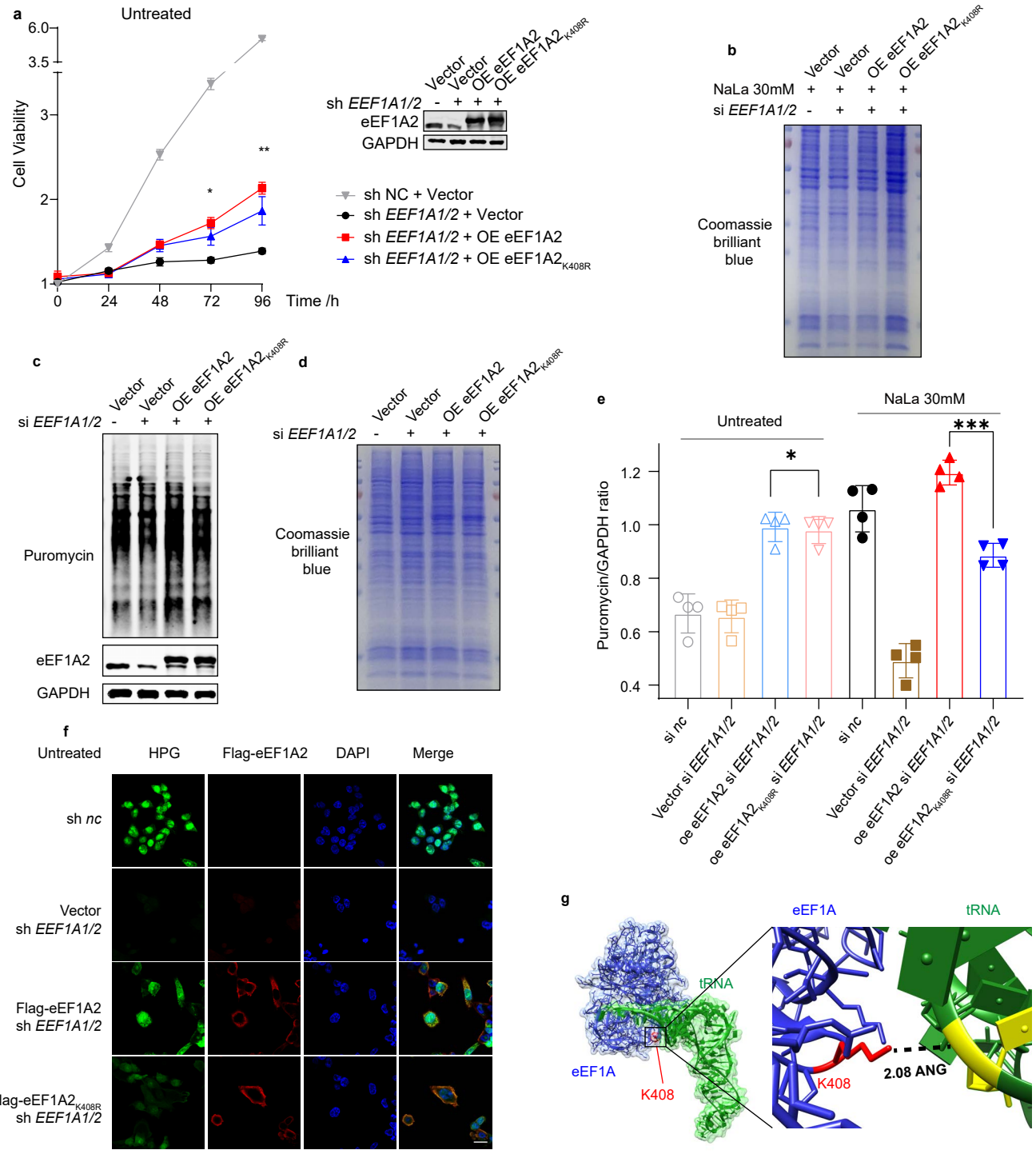
Extended Data Fig. 2

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.

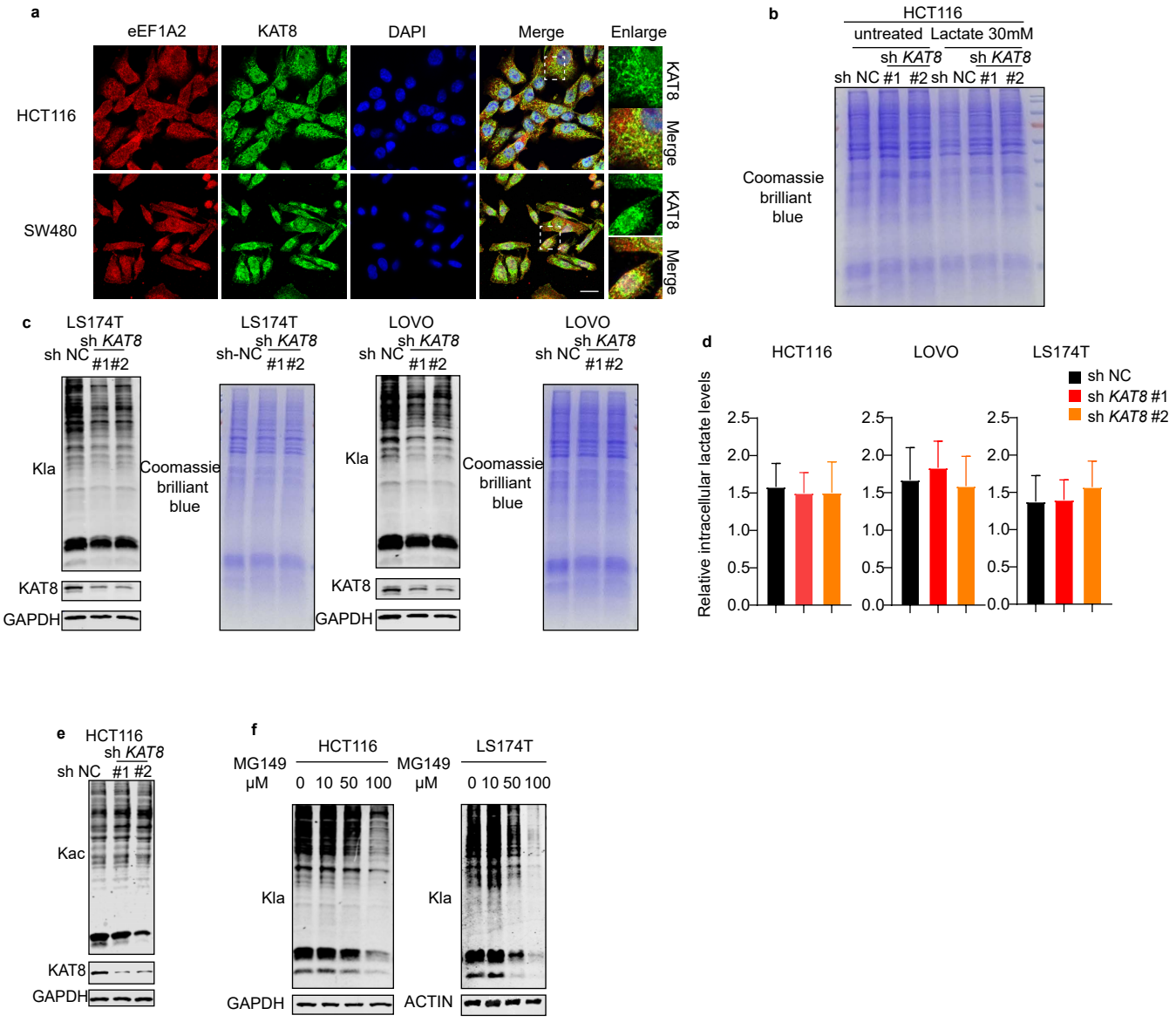
Extended Data Fig. 3



Extended Data Fig. 3

a. Cell proliferations of control or eEF1A1/2-depleted HCT116 cells complemented with WT eEF1A2, eEF1A2K408R, or control were measured with Incucyte cell count proliferation assay.
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, eEF1A2K408R, or control. WCEs were isolated and probed the indicated antibodies.
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 ± SEM, *$p < 0.05$, ***$p < 0.001$; unpaired, two-tailed Student's t test)
f. HPG incorporation assay in HCT116 cells of the indicated genotype. Scale bar, 20 μm
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: 20μm;

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 targetingKAT8 or a control shRNA by immunoblotting.

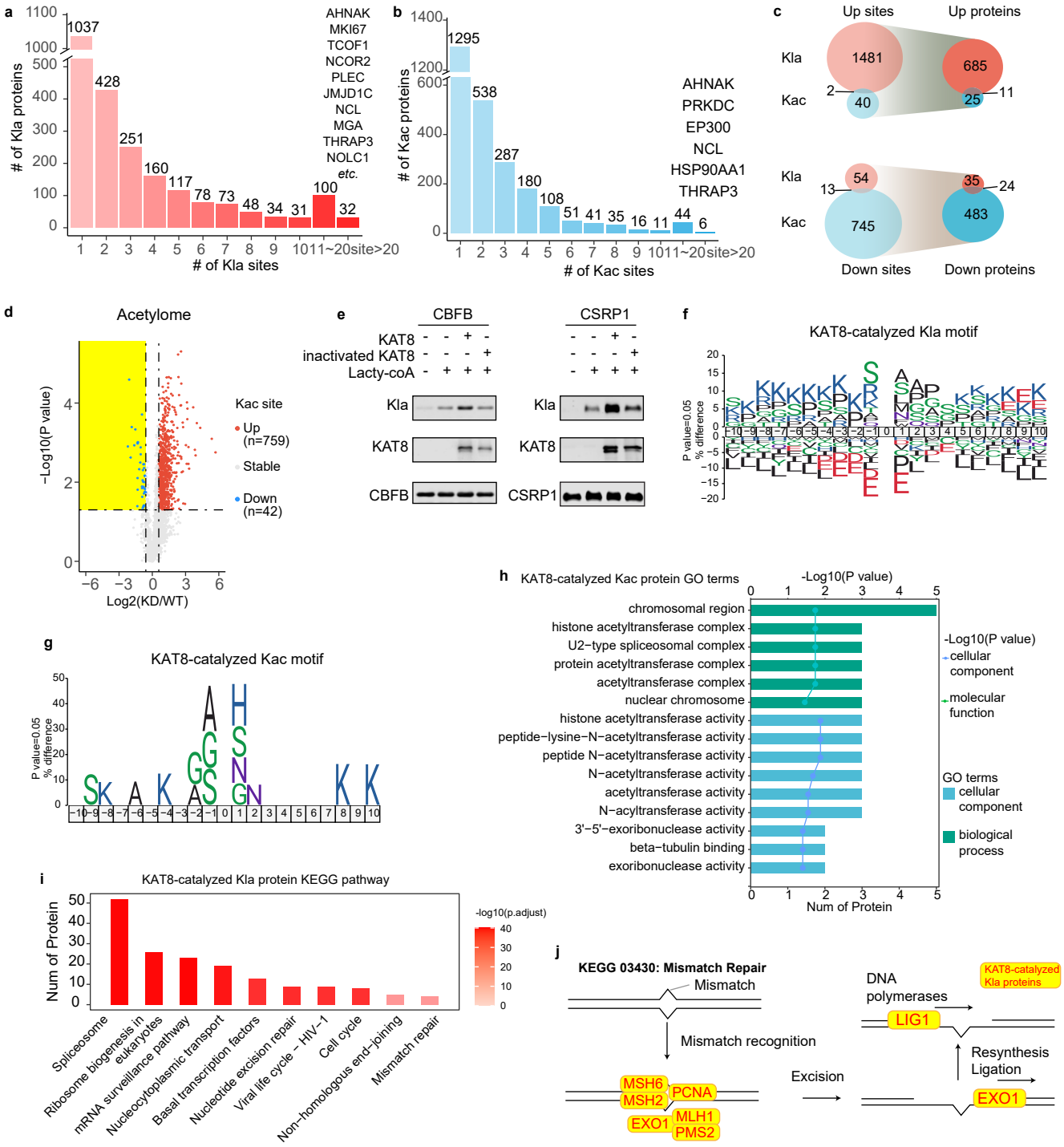
d. Intracellular lactate levels were measured from HCT116 transfected with two independent shRNAs targetingKAT8 or a control shRNA by a lactate colorimetric kit. (the data are represented as the means±SEM, $**p < 0.01$, $***p < 0.001$; unpaired, two-tailed Student's t test)

e. Kac levels were detected in HCT116 cells transfected with two independent shRNAs targetingKAT8 or a control shRNA by immunoblotting.

f. Kla levels were detected in HCT116 and LS174T cells treating with different concentrations of MG149 for 24h by immunoblotting.

g. SUnSET assays under the indicated conditions in the presence of Nala reveal different levels of protein production in eEF1A1/2-depleted HCT116 cells complemented with WT eEF1A2, eEF1A2K408R, or control. WCEs were isolated and probed the indicated antibodies. Coomassie stain of proteins in the SUnSET reaction was shown.

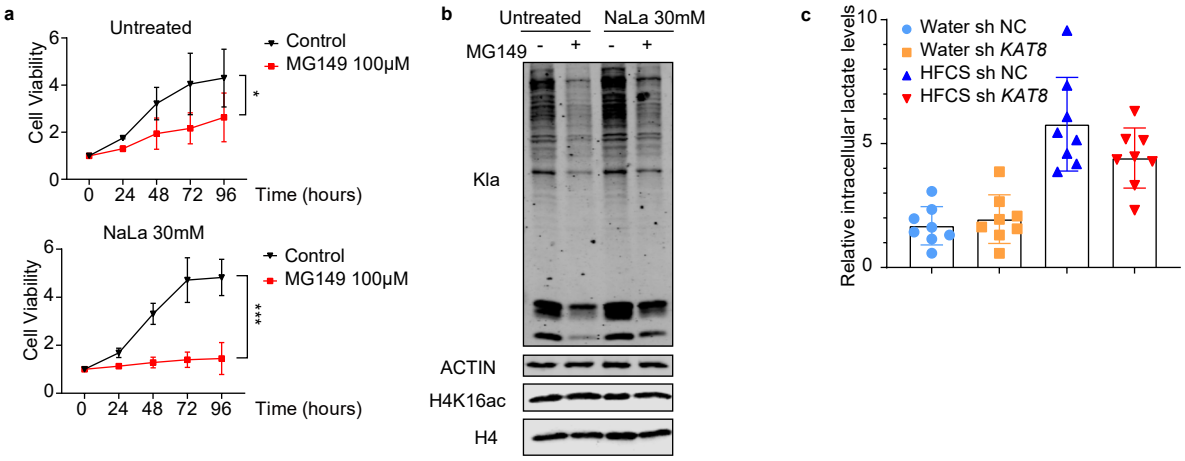
Extended Data Fig. 5



Extended Data Fig. 5

a. Volcano plot showing the global acetylome changes in cells knockdown KAT8 (KD) vs. control cells (WT). Individual sites are represented by points.
b. Histogram of the number of all Kla sites per protein. Proteins with more than 20 sites of Kla are listed above the corresponding bar.
c. Histogram of the number of all Kac sites per protein. Proteins with more than 20 sites of Kla are listed above the corresponding bar.
d. The number of up-regulated(top) and down-regulated(bottom) Kla(red) and Kac(blue) sites and proteins.
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.
f. Icelogo representation showing flanking sequence preferences for KAT8-catalyzed Kla sites.
g. Icelogo representation showing flanking sequence preferences for KAT8-catalyzed Kac sites.
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 30mM NaLa were measured with Incucyte cell count proliferation assay. (***$p < 0.001$, two-way ANOVA)
b. Kla and H4K16ac levels were detected in HCT116 cells treated with MG149 in the presence or absence of 30mM NaLa by immunoblotting.
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 ± SEM, *$p < 0.05$; unpaired, two-tailed Student's t test)