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Supplementary Materials for

The regulatory enzymes and protein substrates for the lysine β -hydroxybutyrylation pathway

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/9/eabe2771/DC1)

Tables S1 to S3

Supplementary Materials

Fig. S1. The dynamics of histone Kbhb levels in 293T cells in response to HATs overexpression. Cells were transfected with either empty vector (Control) or constructs expressing Flag-p300, Flag-CBP, Flag-GCN5 or Flag-PCAF for 48 hours. The indicated PTMs were analyzed by immunoblotting.

Fig. S2. The dynamics of histone Kbhb levels in 293T cells in response to HDACs 1-3 overexpression. Kbhb and Kac levels were detected by immunoblotting using indicated antibodies. Immunoblot of histone H3 was used as loading control.

Fig. S3. β -Hydroxybutyrate treatment increases Kbhb levels on non-histone proteins. HEK293T cells were treated for 24 hours with indicated concentrations of (R)-3-hydroxybutyric acid sodium salt. Whole cell lysates were subjected to SDS-PAGE and immunoblotted with pan anti-Kbhb (left) or pan anti-Kac (right) antibodies. Increases in Kbhb signals, but not Kac signals, were observed for non-histone proteins under the conditions tested. Data is representative of three independent experiments.

Fig. S4. Kbhb levels on NONO and NPM1 proteins were significantly elevated upon β -hydroxybutyrate treatment. HEK293T cells were transfected with Flag-NONO or Flag-SUB1, with or without β -hydroxybutyrate treatment (10mM) for 24 hours, respectively. Cell lysates were subjected to immunoprecipitation with Flag-M2 beads. Inputs and eluates were analyzed by immunoblots.

Fig. S5. Schematic of Kbhb positions on histones. Kbhb sites discovered in this study and in a previous study are indicated by red and blue diamonds, respectively.

Fig. S6. A comparison of Kbhb proteome with reported Kac, Kcr, Khib, and Ksucc proteomes. **(A)** Venn diagram shows the overlap of proteins bearing different acylations. **(B)** Venn diagram shows the overlap of different acylation sites. **(C)** Sequence motif logos show representative sequences of different acylations. **(D)** Heatmap of KEGG pathway clustering analysis shows the functional preferences of different acylations.

Table S1. Complete list of identified Kbhb sites.

Table S2. Kbhb sites located on residues that are critical for biological functions.

Table S3. KEGG pathway analysis of Kbhb proteins.

Fig. S1

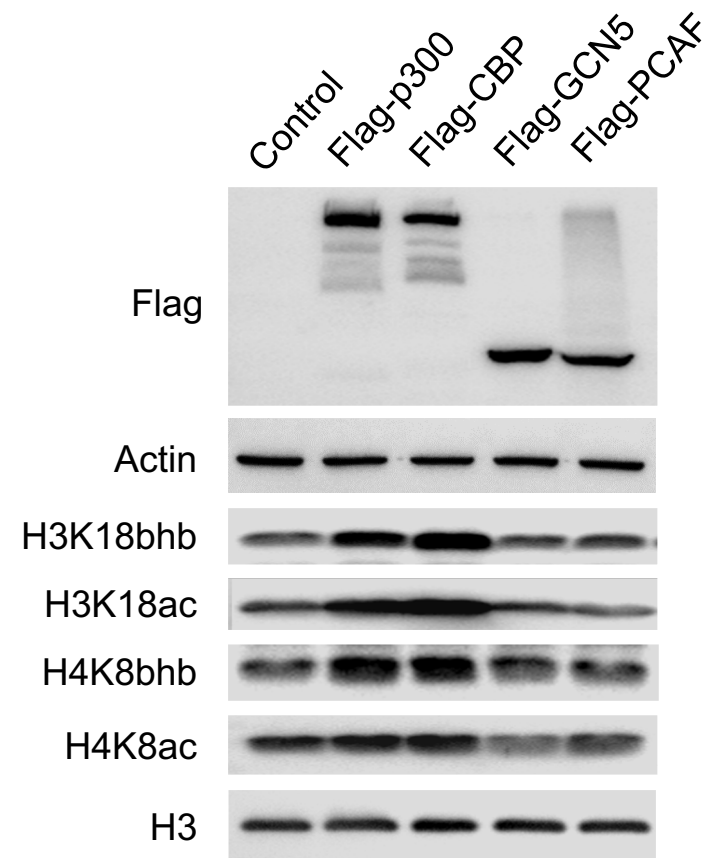


Fig. S2

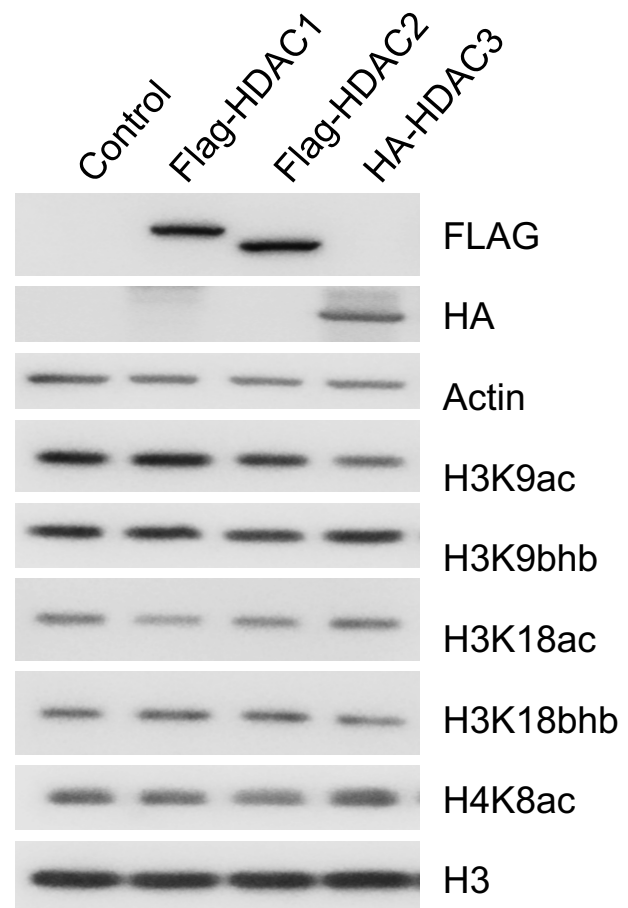


Fig. S3

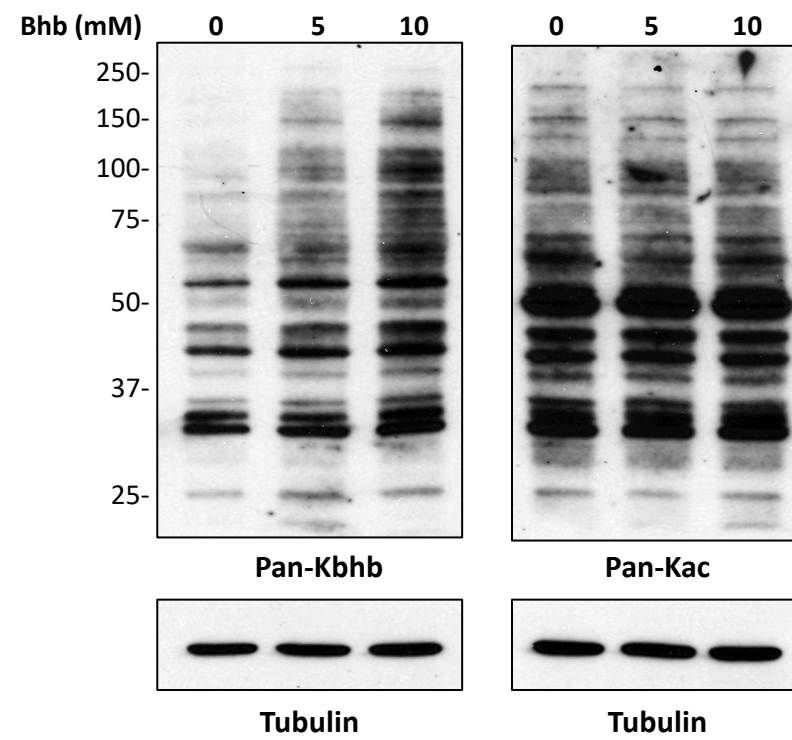


Fig. S4

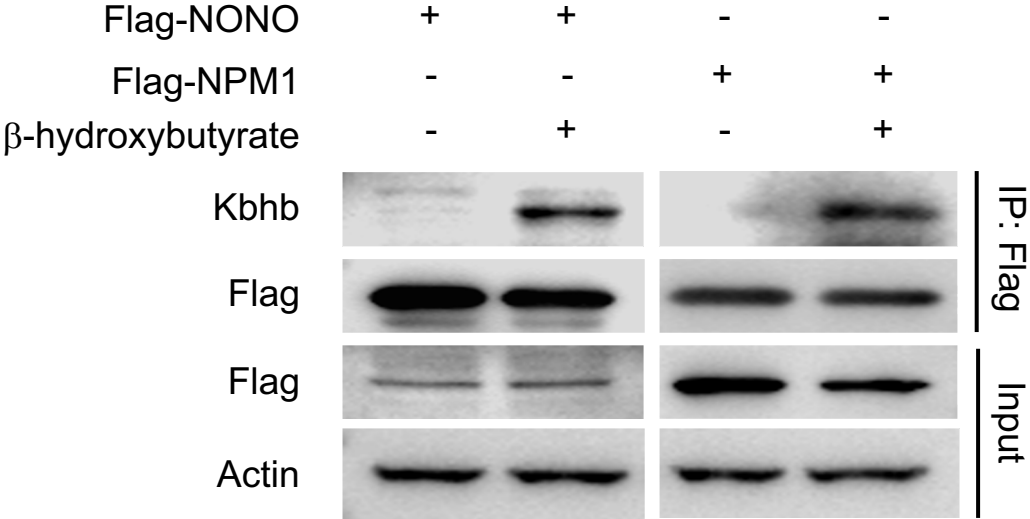


Fig. S5

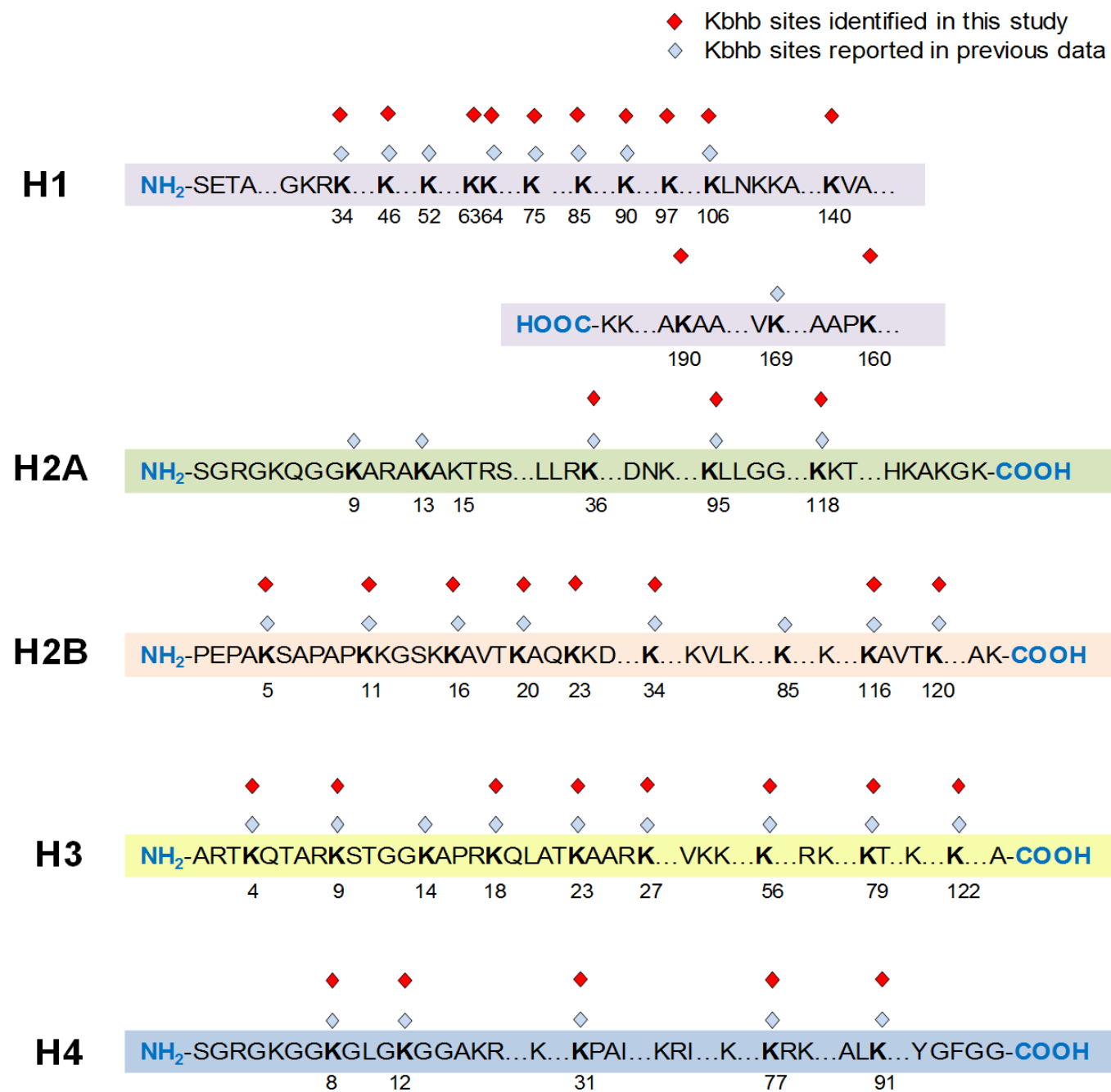


Fig. S6

