
Supplementary information

**Kinase signalling in excitatory neurons
regulates sleep quantity and depth**

In the format provided by the
authors and unedited

SI Guide

Kinase signalling in excitatory neurons regulates sleep quantity and depth

Authors

Staci J. Kim^{1*}, Noriko Hotta-Hirashima^{1*}, Fuyuki Asano^{1*}, Tomohiro Kitazono^{1*}, Kanako Iwasaki^{1*}, Shinya Nakata^{1*}, Haruna Komiya^{1*}, Nodoka Asama^{1*}, Taeko Matsuoka¹, Tomoyuki Fujiyama¹, Aya Ikkyu¹, Miyo Kakizaki¹, Satomi Kanno¹, Jinhwan Choi¹, Deependra Kumar¹, Takumi Tsukamoto¹, Asmaa Elhosainy Mohamed Mahmoud¹, Seiya Mizuno², Shinichi Miyazaki¹, Yousuke Tsuneoka³, Fumihiro Sugiyama², Satoru Takahashi², Yu Hayashi^{1,4}, Masafumi Muratani², Qinghua Liu^{1,5}, Chika Miyoshi¹, Masashi Yanagisawa^{1,6,7}✉, Hiromasa Funato^{1,3}✉

¹International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Ibaraki, 305-8575, Japan. ²Transborder Medical Research Center, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan. ³Department of Anatomy, Graduate School of Medicine, Toho University, Ota-ku, Tokyo 143-8540, Japan.

⁴Department of Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto, 606-8507, Japan. ⁵National Institute of Biological Sciences (NIBS), Beijing, 102206, China. ⁶Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas 75390, Texas, and ⁷Life Science Center for

Survival Dynamics, Tsukuba Advanced Research Alliance, University of Tsukuba,
Tsukuba, Ibaraki 305-8577, Japan. *These authors contributed equally to this work.

Corresponding authors:

Masashi Yanagisawa, MD, PhD. E-mail: yanagisawa.masa.fu@u.tsukuba.ac.jp

Hiromasa Funato, MD, PhD. E-mail funato.hiromasa.km@u.tsukuba.ac.jp

Table of content:

SI Figure 1 | Uncropped immunoblots from Main Figures. a, Immunoblots for Fig.1c from brain lysates of *Hdac4*^{SA} (+/+, n = 2; SA/+, n = 3; SA/SA, n = 2) and *Hdac5*^{SA} (+/+, n = 2; SA/+, n = 4; SA/SA, n = 2) mice. **b,** Immunoblots for Fig.2g from brain lysates of *Sik3*^{Slp} mice (+/+, n = 3; *Slp*/+, n = 3). Proteins were detected by Western blotting using the indicated antibodies. The black rectangle marks the cropped boundary.

SI Figure 2 | Uncropped gel blots from Extended Data Figures. a, Agarose gel blot for Extended Data Fig.1c of RT-PCR amplified mRNA with *Hdac4* specific primers from *Hdac4*^{Slp2} mouse tissue (+/+, n = 3; *Slp2*/+, n = 3). **b,** Agarose gel blot for Extended Data Fig.1g of RT-PCR amplified mRNA with *Hdac4* specific primers from *Hdac4*^{SA} mouse cortical tissue (+/+, n = 3; SA/+, n = 3, SA/SA, n = 3). **c,** Agarose gel blot for Extended Data Fig.1h of RT-PCR amplified mRNA with *Hdac5* specific primers from *Hdac5*^{SA} mouse cortical tissue (+/+, n = 3; SA/+, n = 3, SA/SA, n = 3). **d,** Agarose gel blot for

Extended Data Fig.3a of RT-PCR amplified mRNA from *Hdac7^{SA}* (+/+, n = 3; *SA*/+, n = 3) and *Hdac9^{SA}* mice cortical tissues (+/+, n = 3; *SA*/+, n = 3, *SA/SA*, n = 3) using target-specific primers. The black rectangle marks the cropped boundary. Hrt, heart; Ctx, cortex; Hyp hypothalamus.

SI Figure 3 | Uncropped immunoblots from Extended Data Figures. **a**, Immunoblots for Extended Data Fig.1d from brain lysates of *Hdac4^{Slp2}* mice (+/+, n = 3; *SA*/+, n = 3). **b**, Representative immunoblots for Extended Data Fig.3m from brain lysates of ad lib slept and sleep deprived (6 h) mice (*ad lib*, n = 3; sleep deprived, n = 3). **c**, Immunoblots for Extended Data Fig.3p showing decreased HDAC4 phosphorylation at serine 245 and 629 residues in HG9-91-01 treated PC12 cells. **d**, Immunoblots for Extended Data Fig.7j from brain lysates showing the SIK3 knockout by Cre recombinase in *Nestin^{Cre/+}*; *Sik3^{flox/flox}* mice (+/+, n = 2, *flox/flox*, n=2). Proteins were detected by Western blotting using the indicated antibodies. The black rectangle marks the cropped boundary. Cyto, cytoplasmic fraction; Nucl, nuclear fraction.

SI Table 1 | The number of nuclei in each cluster

The number of nuclei in each cluster (Glut, GABA, Astro, Oligo, oligo progenitor, Microglia, Vascular endothelial) of AAVSIK3 (Fig.6a) and sleep (Fig.6b), and the number of WPRE-positive cells in each cluster of AAVSIK3.

SI Table 2 | Full gene lists for each cluster for AAV-SIK3 transduction

Full gene lists with adjusted p values for each cluster (Glut, GABA, stro, Oligo, oligo progenitor, Microglia, Vascular endothelial) of the primary motor areas in comparison of transduction with AAV-SIK3 and AAV-SIK3(SLP), excluding genes detected in less than 10 % of nuclei. Genes for the glutamatergic (Fig.6d) and GABAergic (Extended Data Fig.10f) clusters were plotted as volcano plots.

SI Table 3 | Full gene lists for each cluster for sleep deprivation

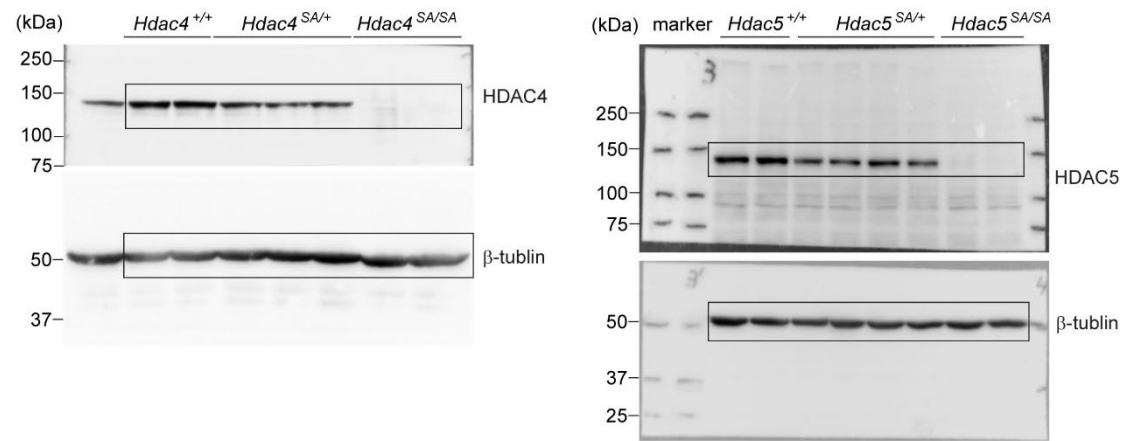
Full gene lists with adjusted p values for each cluster (Glut, GABA, Astro, Oligo, oligo progenitor, Microglia, Vascular endothelial) of the primary motor area in comparison of Sleep Deprived (SD) and ad lib sleep at ZT6, excluding genes detected in less than 10 % of nuclei. Genes for the glutamatergic (Fig.6e), GABAergic (Extended Data Fig.10g), astrocyte (Extended Data Fig.10h), oligodendrocyte (Extended Data Fig.10i), and microglia (Extended Data Fig.10j) clusters were plotted as volcano plots.

SI Table 4 | Upregulated and downregulated DEG lists for each group in the Venn diagram.

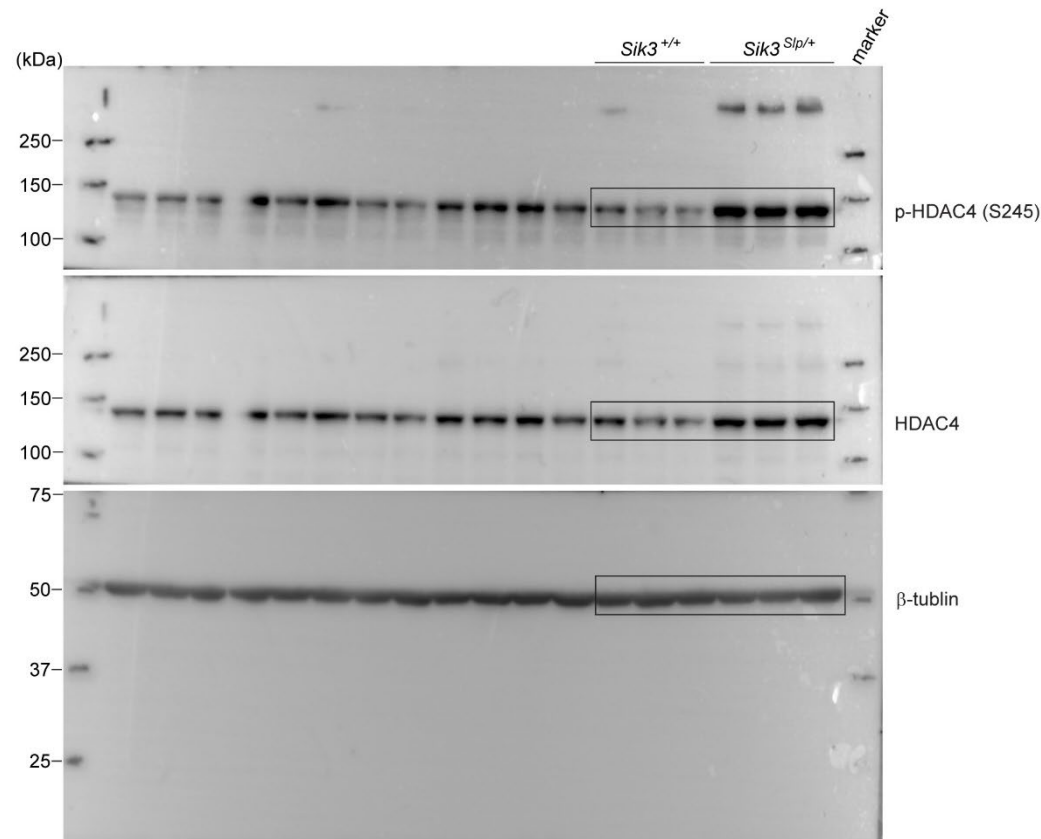
SI Table 5 | Gene Ontology analysis using DEGs common between AAVSIK3 transduction and sleep deprivation.

SI Table 6 | Summary of statistics for sleep and behavioral studies

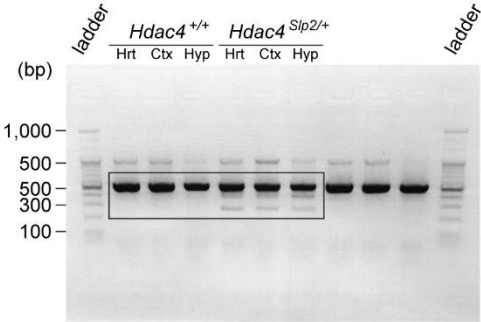
a. Figure 1c



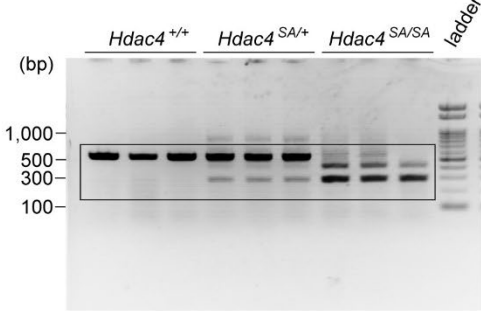
b. Figure 2g



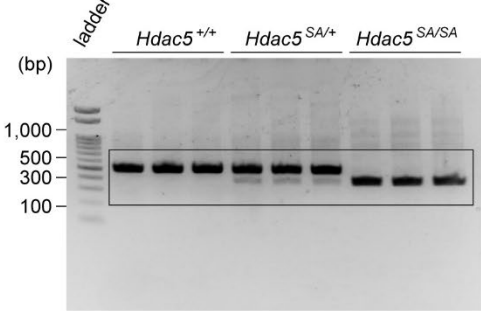
a. Extended Figure 1c



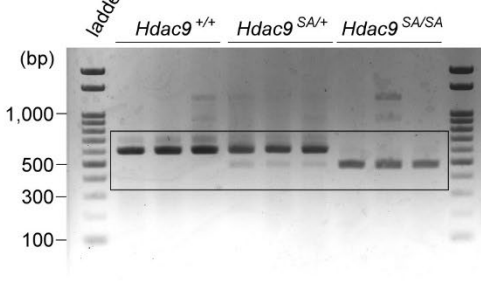
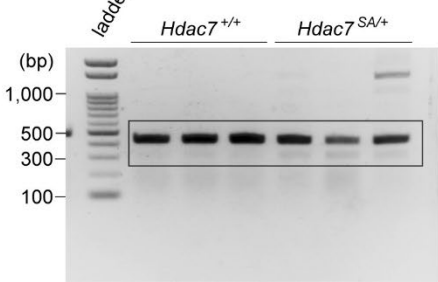
b. Extended Figure 1g



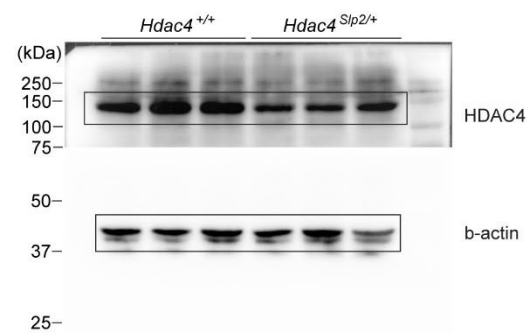
c. Extended Figure 1h



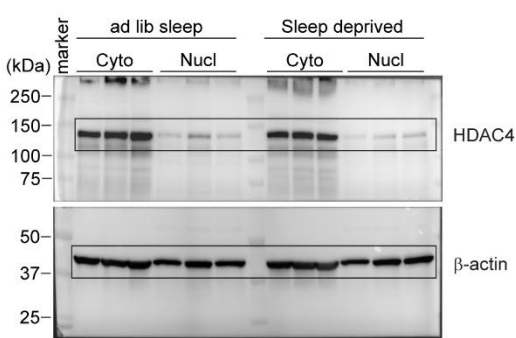
d. Extended Figure 3a



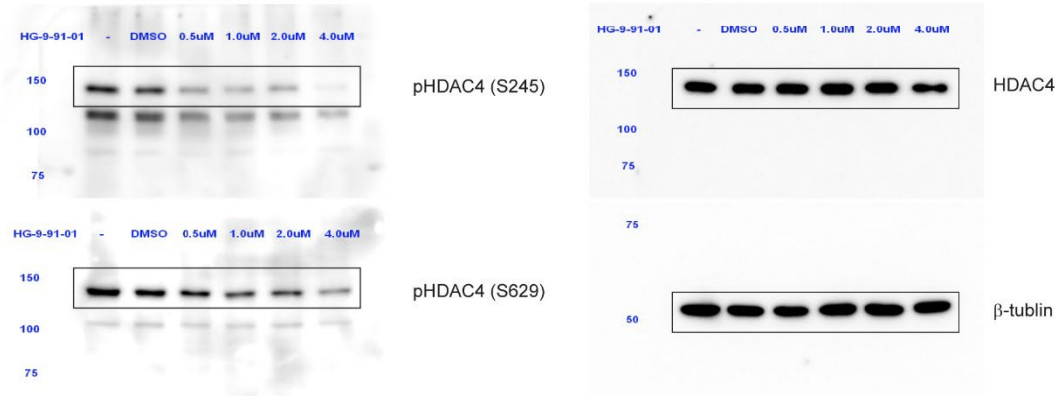
a. Extended Figure 1d



b. Extended Figure 3m



c. Extended Figure 3p



d. Extended Figure 7j

