

**Supplementary information:**

**Visualization of activity-regulated BDNF expression in the living mouse brain using non-invasive near-infrared bioluminescence imaging**

Mamoru Fukuchi<sup>1,\*</sup>, Ryohei Saito<sup>2,3</sup>, Shojiro Maki<sup>2</sup>, Nami Hagiwara<sup>1</sup>, Yumena Nakajima<sup>1</sup>, Satoru Mitazaki<sup>1</sup>, Hironori Izumi<sup>4</sup>, Hisashi Mori<sup>4</sup>

Mamoru Fukuchi, fukuchi@takasaki-u.ac.jp

Ryohei Saito, rsaito@toyaku.ac.jp

Shojiro Maki, s-maki@uec.ac.jp

Nami Hagiwara, 8.8forknight@gmail.com

Yumena Nakajima, dream.smiley2@gmail.com

Satoru Mitazaki, mitazaki@takasaki-u.ac.jp

Hironori Izumi, izuchan@med.u-toyama.ac.jp

Hisashi Mori, hmori@med.u-toyama.ac.jp

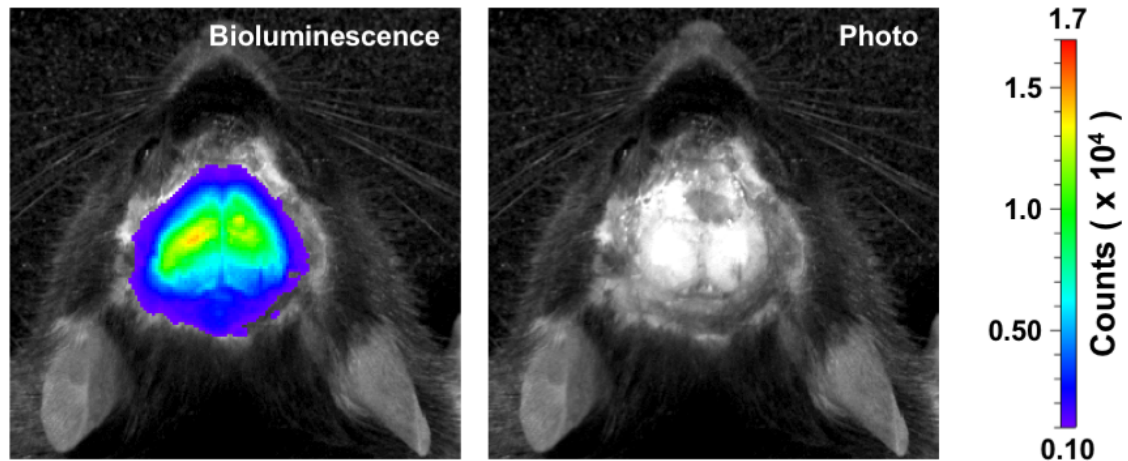
<sup>1</sup>Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki, Gunma 370-0033, Japan

<sup>2</sup>Department of Engineering Science, Graduate School of Informatics and Engineering, The University of Electro-Communications, 1-5-1 Chofugaoka, Chofu, Tokyo 182-8585, Japan

<sup>3</sup>School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

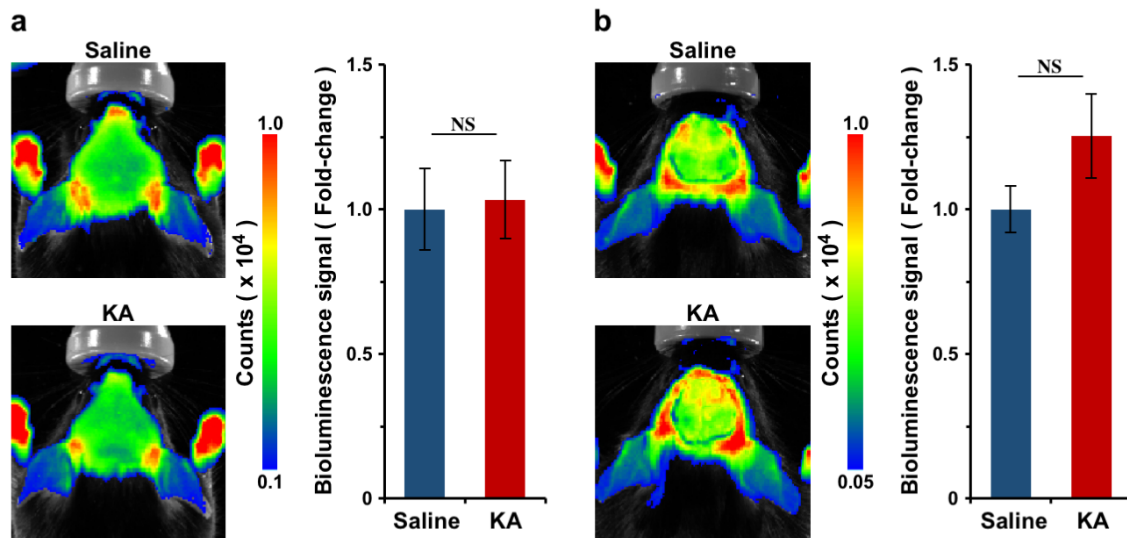
<sup>4</sup>Department of Molecular Neuroscience, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama, Toyama 930-0194, Japan

\*To whom correspondence should be addressed: Mamoru Fukuchi, Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki, Gunma 370-0033, Japan. E-mail address: [fukuchi@takasaki-u.ac.jp](mailto:fukuchi@takasaki-u.ac.jp), telephone: +81-27-352-1180, fax: +81-27-352-1118.



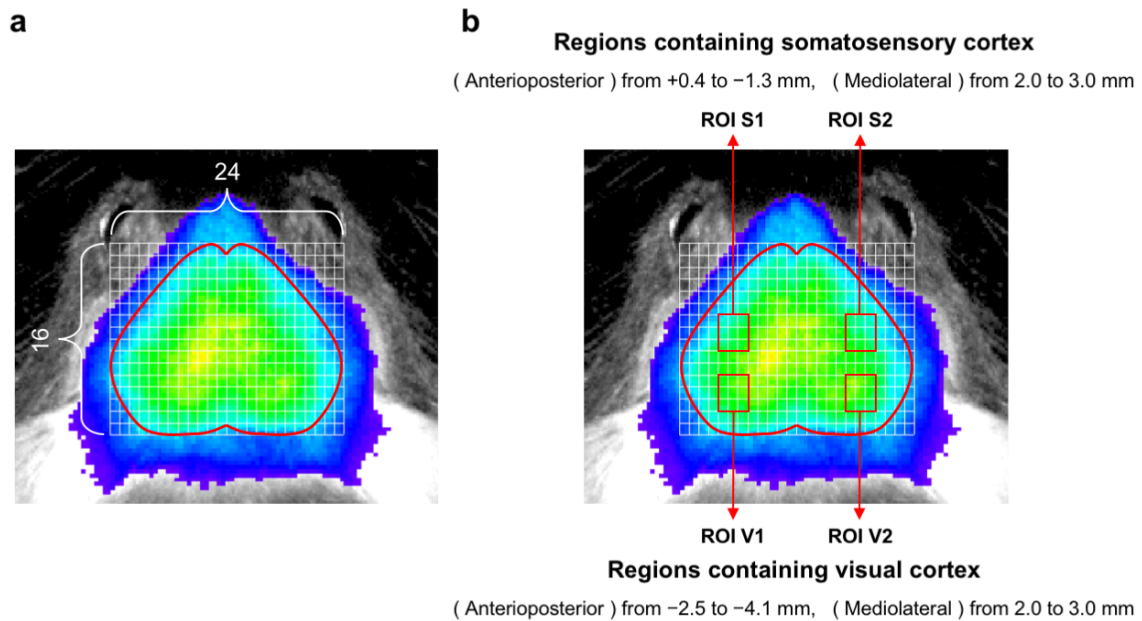
**Supplementary Figure 1. *In vivo* BLI after intracerebroventricular injection of *D*-luciferin into *Bdnf-Luc* mice.**

*Bdnf-Luc* mice were anesthetized with mixed anesthetic agents [medetomidine hydrochloride (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol tartrate (5 mg/kg)], and the skin on the top of the head was removed. Five microliters of *D*-luciferin solution (10 mg/mL) was injected intracerebroventricularly (−0.2 mm anterioposterior; +1.0 mm mediolateral; −2.5 mm dorsoventral) at a rate of 1  $\mu$ L/min using a microsyringe pump. The injection was performed under stereotaxic guidance. After the injection, *in vivo* BLI was performed. Representative images are shown and similar results were obtained from three independent experiments. Bioluminescence; counts indicated by pseudocolored images. Photo; photographs corresponding to bioluminescence images.



**Supplementary Figure 2. Detection of bioluminescence signals using *D*-luciferin 6 h after administration of saline or KA to *Bdnf-Luc* mice.**

These experiments were performed according to our previous report [16]. Briefly, saline or KA (25 mg/kg) was administered intraperitoneally to *Bdnf-Luc* mice. Six hours after the administration of saline or KA, *D*-luciferin (150 mg/kg) was injected intraperitoneally, and 10 min later, *in vivo* BLI was performed under inhalation anesthesia with 1.5% isoflurane. Data represent the mean  $\pm$  SEM of six independent experiments using the unpaired *t* test (NS; not significant). **a.** One day before *in vivo* BLI, the top of the head was shaved. **b.** One week before *in vivo* BLI, the skin of the head region was removed.



**Supplementary Figure 3. Visualization of sensory stimulation-induced *Bdnf* expression (ROI analysis).**

**a.** The area of the cerebral cortex was estimated by the bioluminescence signal image (the region surrounded by a red line), and the region was covered with  $16 \times 24$  ROIs ( $16 \times 24$  boxes shown in white line). **b.** ROIs containing visual cortex (ROI V1 and V2) or somatosensory cortex (ROI S1 and S2) were estimated by mouse brain atlas [ROI V1 and V2 correspond to the areas from approximately -2.5 mm to -4.1 mm (anterioposterior) and from approximately 2.0 mm to 3.0 mm (mediolateral). ROI S1 and S2 correspond to the areas from approximately +0.4 mm to -1.3 mm (anterioposterior) and from approximately 2.0 mm to 3.0 mm (mediolateral)].