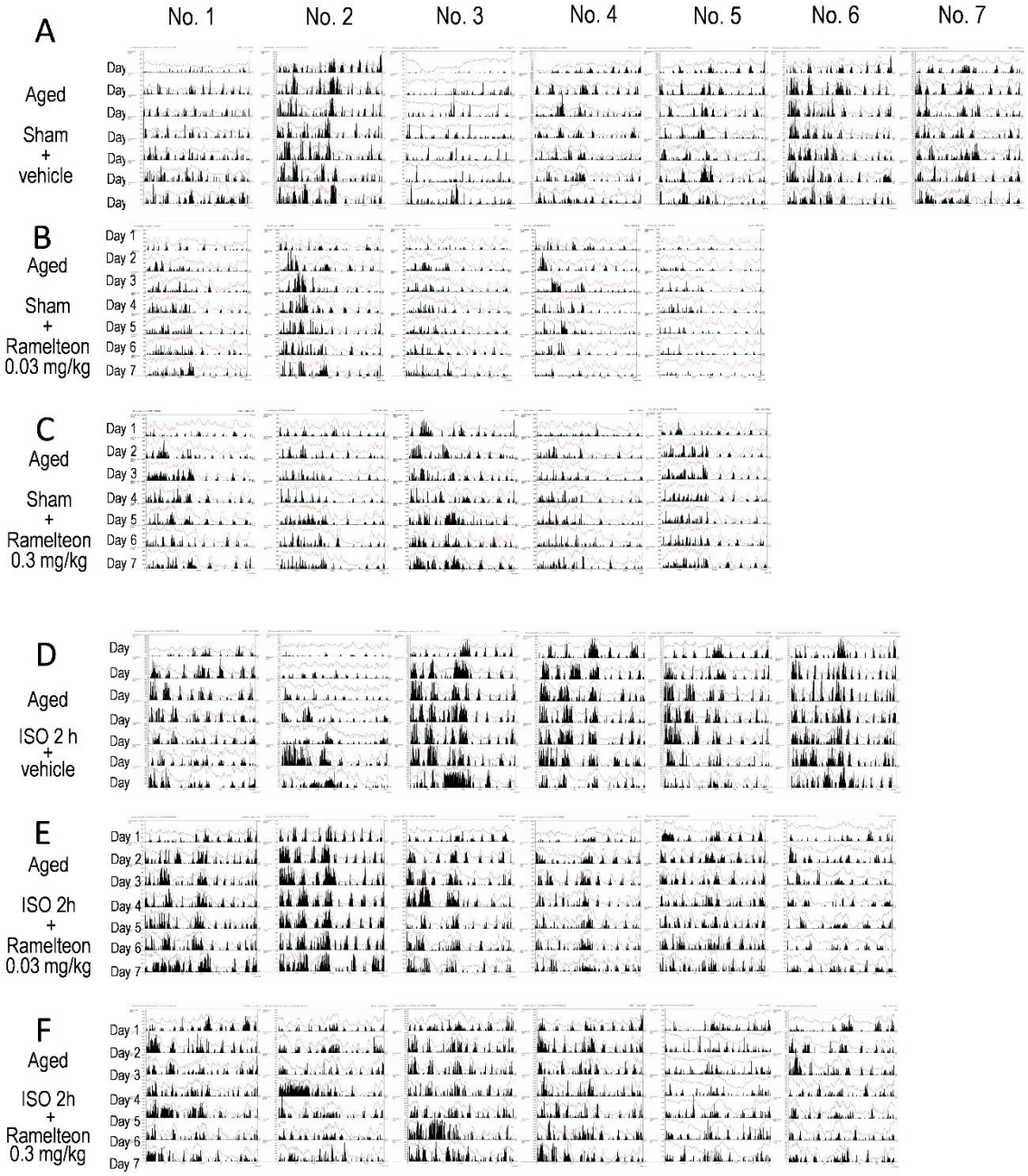
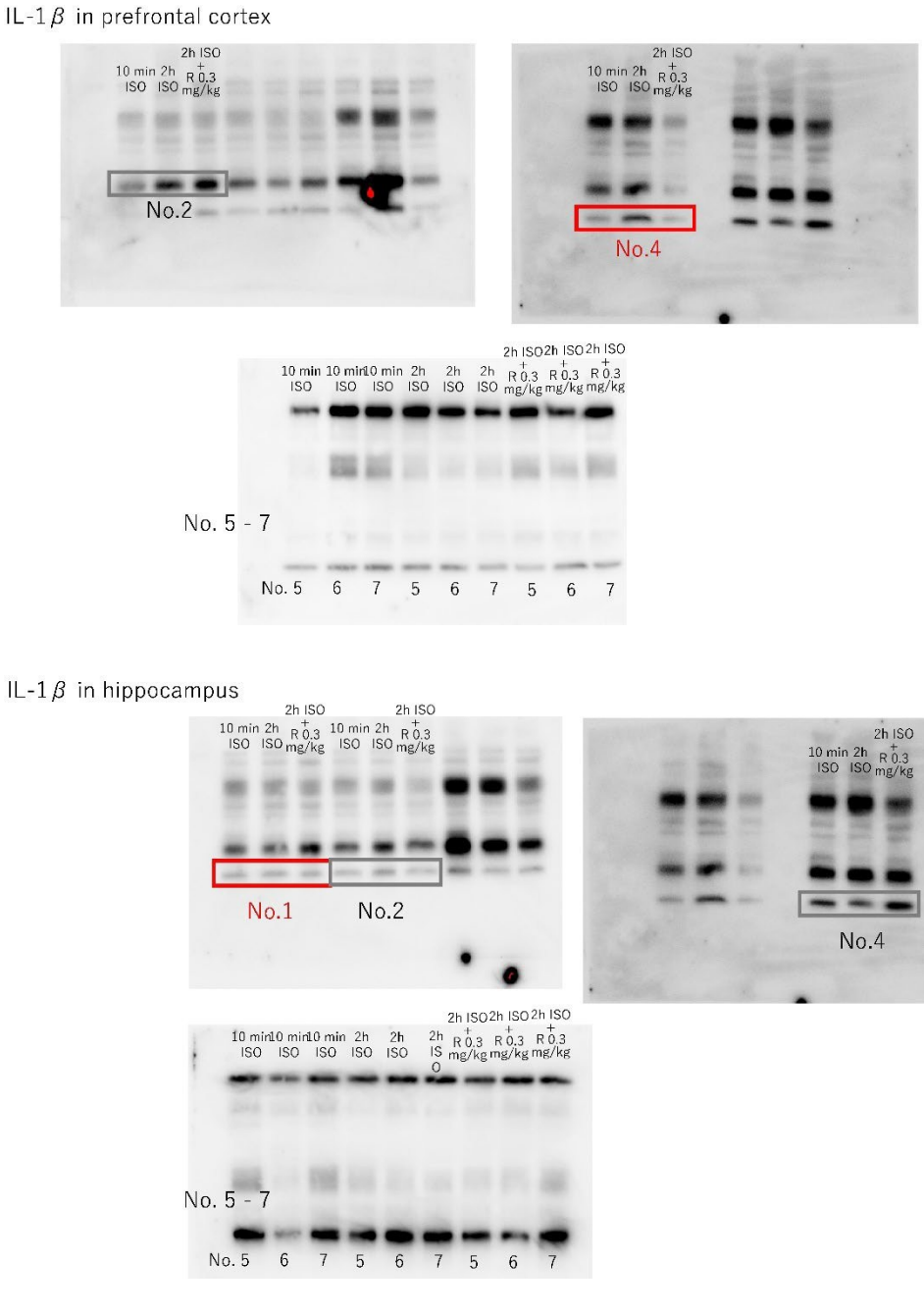
Original Research for *Journal of Integrative Neuroscience*

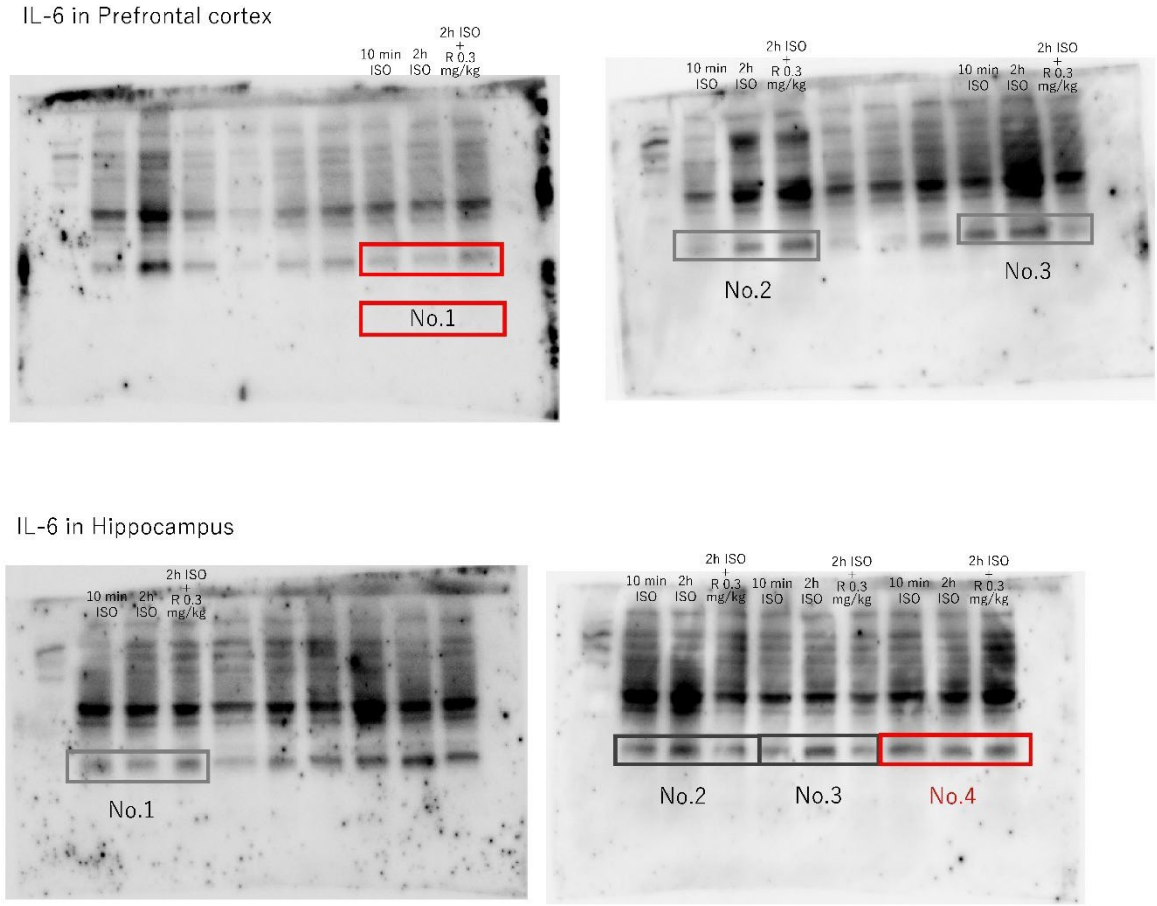
Title: Ramelteon Prevents Isoflurane Anesthesia-Induced Hyperactivity and Social Recognition Deficits in Aged C57BL/6J Mice



Supplementary Fig. 1. Actograms depicting locomotor activity from Day 1 to Day 7 in aged mice: (**A**) vehicle-treated sham-operated mice, (**B**) sham-operated mice treated with 0.03 mg/kg ramelteon, (**C**) sham-operated mice treated with 0.3 mg/kg ramelteon, (**D**) vehicle-treated mice exposed to 2-hour isoflurane, (**E**) mice exposed to 2-hour isoflurane and treated with 0.03 mg/kg ramelteon, and (**F**) mice exposed to 2-hour isoflurane and treated with 0.3 mg/kg ramelteon.

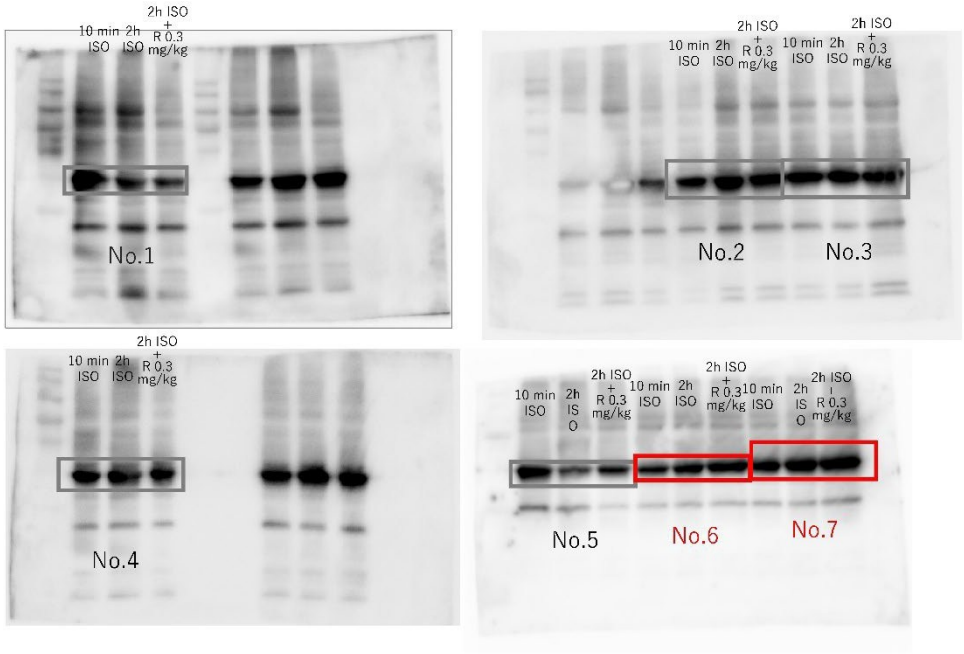


Supplementary Fig. 2. Original blots corresponding to the cropped images shown in Fig. 5A and B. Full-length membrane images for the IL-1β in prefrontal cortex (uppers) and in hippocampus (lowers) corresponding to the cropped blots presented in Fig. 5A and B. Samples were collected from the prefrontal cortex or hippocampus in sham vehicle-treated, 2-hour isoflurane anesthesia, and ramelteon 0.3 mg/kg treated mouse after the 2 hour isoflurane anesthesia mouse groups. The cropped regions shown in Fig. 5A and B are indicated by red squares in the above panels. Each 'No.' in the panel indicates the tissue number obtained from the tested mice.

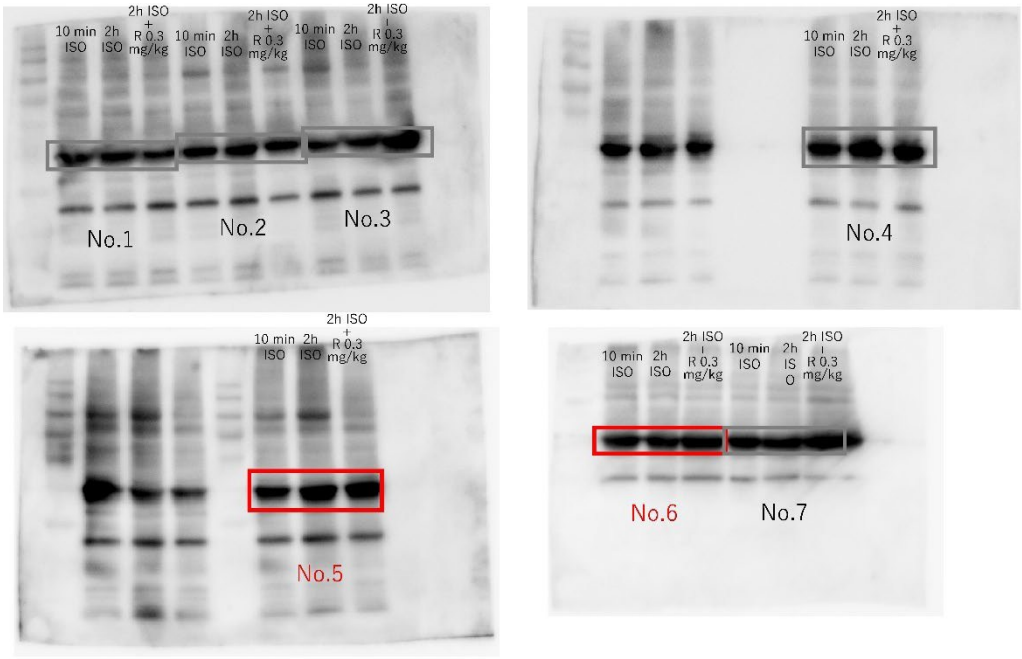


Supplementary Fig. 3. Original blots corresponding to the cropped images shown in Fig. 5A and B. Full-length membrane images for the IL-6 in prefrontal cortex (uppers) and in hippocampus (lowers) corresponding to the cropped blots presented in Fig. 5A and B. Samples were collected from the prefrontal cortex or hippocampus in sham vehicle-treated, 2-hour isoflurane anesthesia, and ramelteon 0.3 mg/kg treated mouse after the 2 hour isoflurane anesthesia mouse groups. The cropped regions shown in Fig. 5A and B are indicated by red squares in the above panels. Each 'No.' in the panel indicates the tissue number obtained from the tested mice.

β-actin in prefrontal cortex

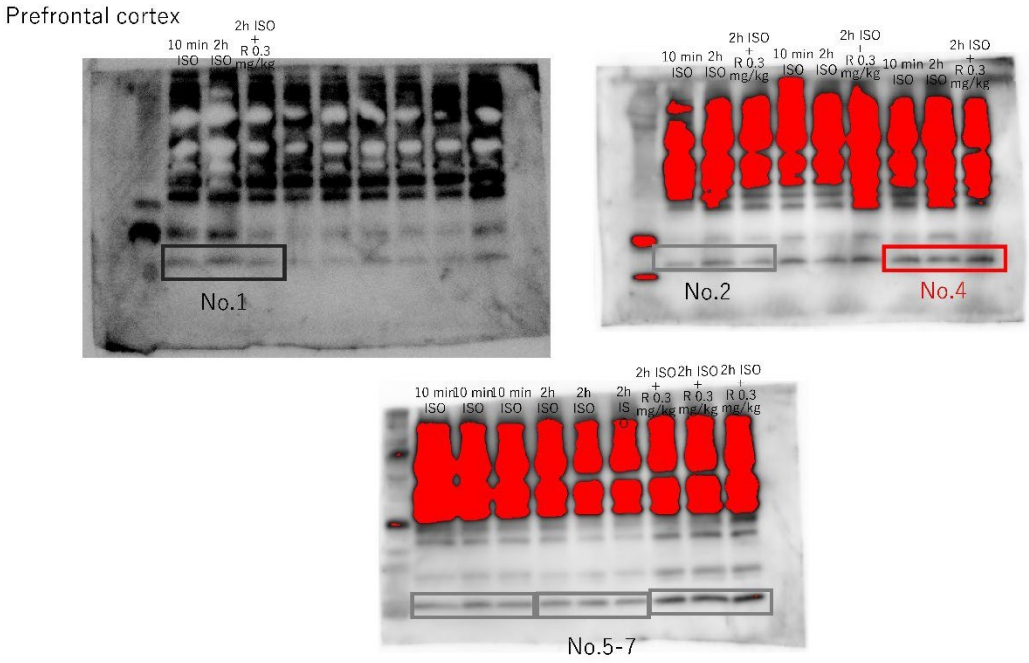


B-actin in hippocampus

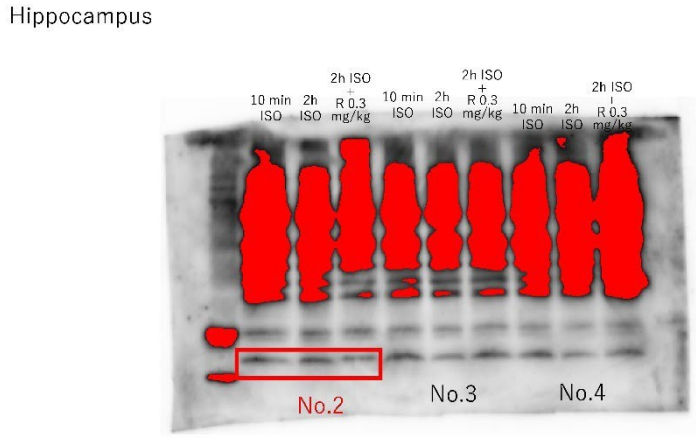


Supplementary Fig. 4. Original blots corresponding to the cropped images shown in Fig. 5G and H. Full-length membrane images for the β-actin in prefrontal cortex (uppers) and in hippocampus (lowers) corresponding to the cropped blots presented in Fig. 5G and H. Samples were collected from the prefrontal cortex or hippocampus in sham vehicle-treated, 2-hour isoflurane anesthesia, and ramelteon 0.3 mg/kg treated mouse after the 2 hour isoflurane anesthesia mouse groups. The cropped regions shown in Fig. 5G and H are indicated by red squares in the above panels. Each 'No.' in the panel indicates the tissue number obtained from the tested mice.

IBA-1 in prefrontal cortex

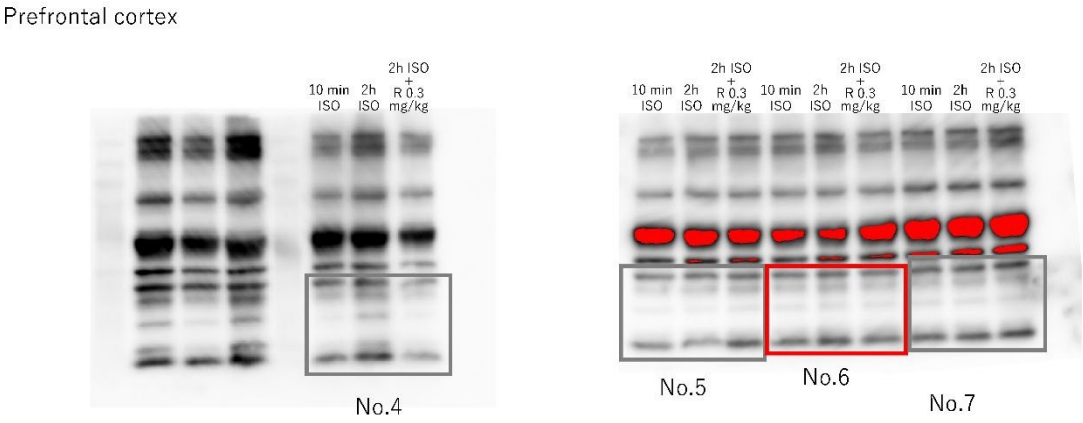


IBA-1 in hippocampus

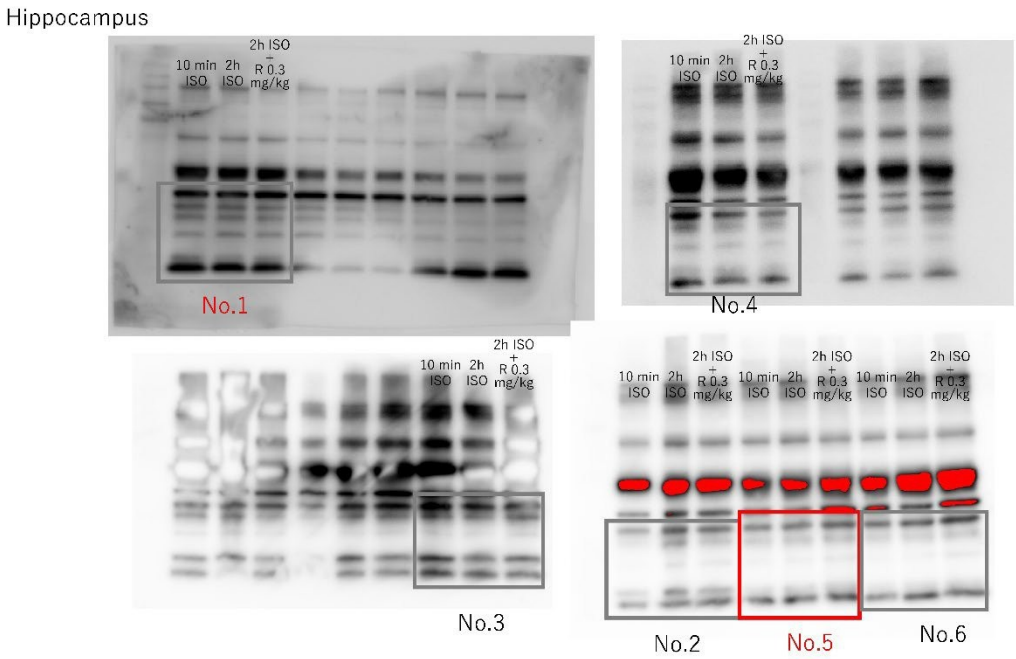


Supplementary Fig. 5. Original blots corresponding to the cropped images shown in Fig. 5G and H. Full-length membrane images for the IBA-1 in prefrontal cortex (uppers) and in hippocampus (lowers) corresponding to the cropped blots presented in Fig. 5A and B. Samples were collected from the prefrontal cortex or hippocampus in sham vehicle-treated, 2-hour isoflurane anesthesia, and ramelteon 0.3 mg/kg treated mouse after the 2 hour isoflurane anesthesia mouse groups. The cropped regions shown in Fig. 5A and B are indicated by red squares in the above panels. Each 'No.' in the panel indicates the tissue number obtained from the tested mice.

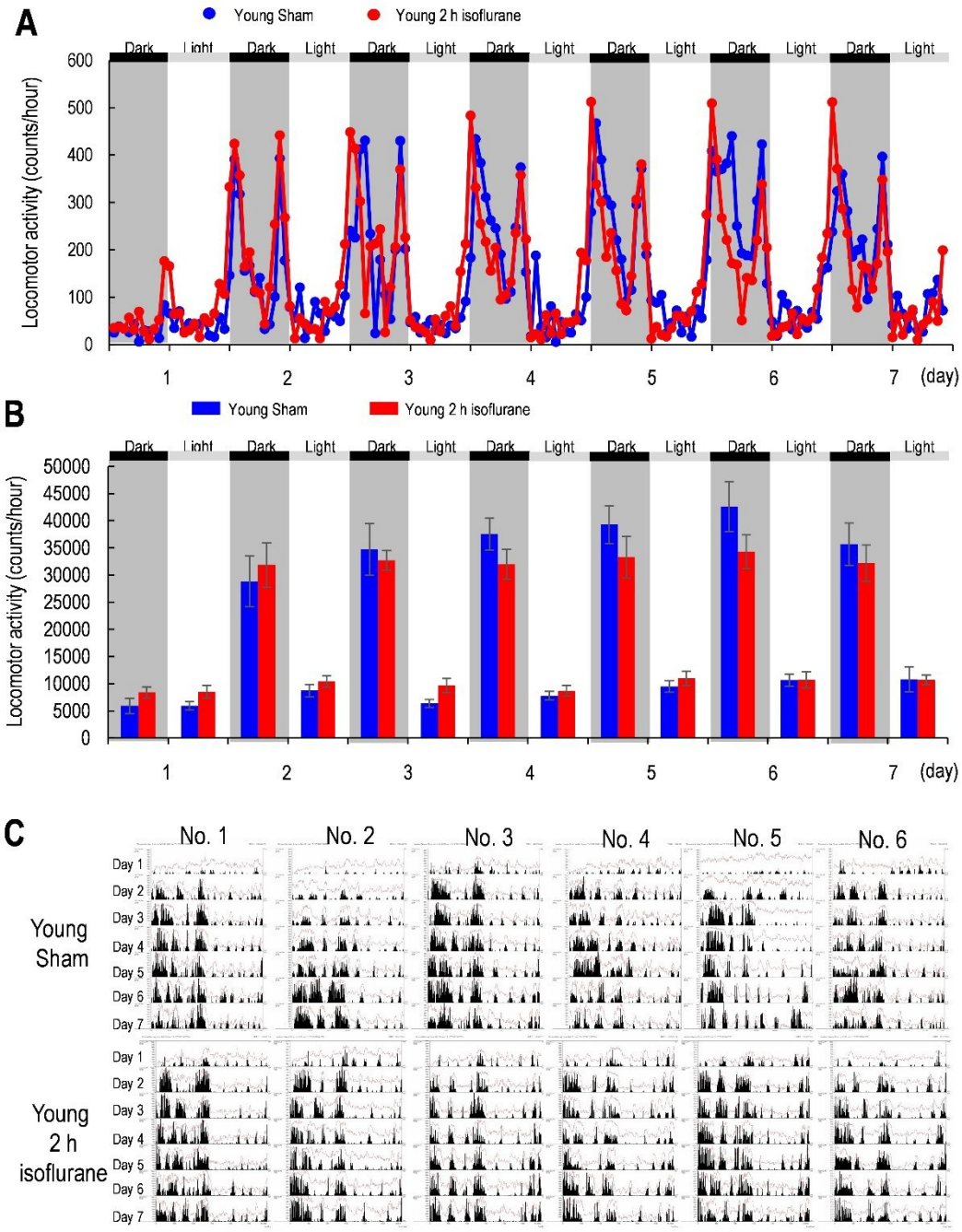
BDNF in prefrontal cortex



BDNF in hippocampus



Supplementary Fig. 6. Original blots corresponding to the cropped images shown in Fig. 5G and H. Full-length membrane images for the BDNF in prefrontal cortex (uppers) and in hippocampus (lowers) corresponding to the cropped blots presented in Fig. 5G and H. Samples were collected from the prefrontal cortex or hippocampus in sham vehicle-treated, 2-hour isoflurane anesthesia, and ramelteon 0.3 mg/kg treated mouse after the 2 hour isoflurane anesthesia mouse groups. The cropped regions shown in Fig. 5G and H are indicated by red squares in the above panels. Each 'No.' in the panel indicates the tissue number obtained from the tested mice.



Supplementary Fig. 7. Gross locomotor activity averaged in 2-hour intervals from Day 1 to Day 7 in young mice.

(**A**, **B**) Locomotor activity levels (A) and daily averaged gross locomotor activity (B) from Day 1 to Day 7 in the following groups: sham-operated mice (blue) and mice exposed to 2-hour isoflurane. Horizontal dark gray bars indicate the dark phase; horizontal light gray bars indicate the light phase. (**C**) Actograms showing locomotor activity from Day 1 to Day 7. Each panel represents the activity pattern of individual young mice: upper panels show sham-operated mice (n = 6), and lower panels show mice exposed to 2-hour isoflurane (n = 6).