



Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Miniaturized microscope with flexible light source input for neuronal imaging and manipulation in freely behaving animals



Sakthivel Srinivasan¹, Takuma Hosokawa¹, Pablo Vergara, Yoan Chérasse, Toshie Naoi, Takeshi Sakurai, Masanori Sakaguchi*

International Institute for Integrative Sleep Medicine (WPI-IIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-0006, Japan

ARTICLE INFO

Article history:

Received 19 July 2019

Accepted 22 July 2019

Available online 31 July 2019

Keywords:

Miniaturized microscope

GCaMP

Optogenetics

UCLA miniscope

Hippocampus

ABSTRACT

Simultaneous imaging and manipulation of a genetically defined neuronal population can provide a causal link between its activity and function. Here, we designed a miniaturized microscope (or 'miniscope') that allows fluorescence imaging and optogenetic manipulation at the cellular level in freely behaving animals. This miniscope has an integrated optical connector that accepts any combination of external light sources, allowing flexibility in the choice of sensors and manipulators. Moreover, due to its simple structure and use of open source software, the miniscope is easy to build and modify. Using this miniscope, we demonstrate the optogenetic silencing of hippocampal CA1 neurons using two laser light sources—one stimulating a calcium sensor (i.e., jGCaMP7c) and the other serving as an optogenetic silencer (i.e., Jaws). This new miniscope can contribute to efforts to determine causal relationships between neuronal network dynamics and animal behavior.

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1. Introduction

Fluorescence imaging employing specific sensors and a miniaturized microscope (hereafter, 'miniscope') can be used to visualize defined neuronal activity in the brains of freely moving animals [1]. Recent developments in genetically defined fluorescent sensors allow visualization of both neuronal activity and signaling status (e.g., cholinergic signaling) [2,3]. Conventional miniscopes generally consist of a minimal number of fluorescence microscope components, including a light source, dichroic mirror, focus and filter lenses, imaging sensor, and electronic circuit. This allows the entire miniscope to be attached to small animals without disturbing their daily activities while maintaining a sufficient field of view to visualize a specific brain subregion [1].

Optogenetics allows reversible manipulation of genetically defined neuronal population activity at millisecond order [4]. The key elements of optogenetic manipulation are genetically coded manipulators (i.e., opsins) and optics to deliver light to the target

neurons. The combination of a miniscope and optogenetic manipulation provides a powerful means to obtain causal evidence between neuronal activity and function [5–7]. A recent study reports the development of a miniscope with two-photon capability that enables the imaging of dendritic spines and light stimulation at a desired area in the field of view in freely behaving mice [8]. Although having significant advantages, building a two-photon miniscope requires highly sophisticated integration of optics and engineering. Therefore, it would be advantageous to modify an open source miniscope [9] to make it capable of simultaneous imaging and manipulation [5].

The light source for conventional miniscopes is a light-emitting diode (LED), which has a broader wavelength band than lasers. The LED is usually fixed to the circuit board attached to the miniscope frame, making it difficult to exchange for each needed combination of sensor (e.g., GCaMP) [3] and manipulator (e.g., Halorhodopsin) [10,11]. Therefore, providing a general port to accept external light sources could allow miniscopes to accommodate various combinations of sensors and manipulators with specific excitation and emission wavelengths. By modifying the structure of the open source UCLA miniscope [9], we designed a new miniscope with an integrated light input port. We simultaneously visualized hippocampal neuron activity with jGCaMP7c [12] and silenced this activity with Jaws opsin [13] using a combination of blue and orange lasers through the same miniscope port. These results demonstrate

Abbreviations: AAV, adeno-associated virus; CNMF-E, constrained non-negative matrix factorization adapted for endoscopic data; LED, light-emitting diode.

* Corresponding author.

E-mail address: masanori.sakaguchi@gmail.com (M. Sakaguchi).

¹ Equal contribution.

that an open source miniscope can easily be modified to enable concurrent imaging and manipulation.

2. Materials and methods

2.1. Animals

All experiments were approved by the University of Tsukuba Institutional Animal Care and Use Committee. Mice were maintained on a 12-h light/dark cycle with ad libitum access to food and water in accordance with institutional guidelines. For analgesia after surgery, mice received subcutaneous injections of ibuprofen (120 mg/kg). To prepare ibuprofen solution, 30 mg ibuprofen (T5648, Merck, USA) was dissolved in 100 μ l of 100% EtOH and added to 1000 μ l sunflower oil (Wako, Japan). The EtOH was then evaporated by centrifuge.

2.2. Virus preparation

Three adeno-associated viruses (AAVs) in serotype 10 were prepared as previously described [14]. Briefly, the AAVs of serotype rh10 for AAV-syn-FLEX-jGCaMP7c variant 1513-WPRE (Addgene #105322), AAV-CAG-FLEX-rc-[Jaws-KGC-tdTomato-ER2] (Addgene #84446), and AAV-CMV-Cre were generated by tripartite calcium phosphate transfection (AAV-rep2/caprh10 expression plasmid (Penn Vector Core), adenovirus helper plasmid (Agilent), and pAAV plasmid (described above)) into 293A cells. After 3 days in 5% CO₂ at 37 °C, 293A cells were re-suspended in artificial cerebrospinal fluid, frozen and thawed four times, and treated with benzonase nuclease (Millipore, Ref. #E1014) to degrade all forms of DNA and RNA. Cell debris was removed by centrifugation, and the virus titer in the supernatant was determined using real-time polymerase chain reaction (all exceeding 1×10^{13} viral genome/ml).

2.3. Virus injection

Virus injection was performed as previously described. Briefly, adult mice (10–20 weeks of age) were anesthetized with isoflurane and fixed in a stereotaxic frame (Stoelting, USA). AAV solution was injected into the dorsal hippocampus at anterior-posterior (AP) –2.0 mm, medial-lateral (ML) +1.5 mm, and dorsal-ventral (DV) –1.5 mm from bregma. The virus was injected in a total volume of 300 nl using a picospritzer III air pressure-based injection system (S48 Stumulator, Grass Technologies, USA) connected to a glass pipet injector. Injections were performed slowly over 15 min. After microinjection, the injector needle was left in place for another 5 min and then slowly withdrawn. Mice were allowed to recover for 2–3 weeks before lens implantation.

2.4. Lens implantation

Grin lens and baseplate implantations were performed as previously described [9]. Briefly, the bottom of the lens (1.8 mm diameter, uncoated; Edmund Optics, Germany) was implanted under anesthesia at AP –2.0 mm, ML +1.5 mm, and DV –1.5 mm from bregma. One week after lens implantation, a baseplate for the miniscope was attached. After baseplate surgery, mice were habituated to a dummy miniscope for 1–2 weeks before recording.

2.5. Miniscope construction

Construction of the UCLA miniscope was previously described [9]. We modified only the components related to the light source at the main body frame of the UCLA miniscope. The CAD files for the modified housing are available as supplemental files ([Supplemental](#)

[file 1](#)). We outsourced computer numerical control cutting using Derlin black material (Protolabs). We added a full reflective mirror ($4 \times 6 \times 1$ mm, 21004, Chroma), a drum lens for focusing light (TS N-BK7 3.2 MM #46–526 m Chroma), and an optic ferrule as a port for light sources (230- μ m bore, 1.25-mm outer diameter, 6.4-mm length; MM-FER2007CF-2300, PFP) to the modified frame. Epoxy glue (Quick Mender, Konishi) was used to fix the ferrule.

Lights from two lasers were combined using a custom-made laser combiner and transmitted using a custom-made optic patch cable (multimode, 200- μ m bore, Thorlabs). Connections between the light input port (i.e., ferrule) and the cable were bridged by a ferrule sleeve (SM-CS125S, PFP).

2.6. Imaging and manipulation

Output from blue (445 nm, custom-made) and orange (590 nm, Shanghai Laser & Optics Century, China) lasers were set 0.5–1.0 mW at the bottom of the microscope. Ca²⁺ transients were video recorded at 10 frames/s with an average exposure time of 1620 s. The laser was controlled by a program that repeated a 30-s on/30-s off cycle.

2.7. Preparation of tissue sections

After imaging, mice were perfused transcardially with phosphate-buffered saline (PBS; 0.1 M) and 4% paraformaldehyde (PFA). Brains were removed, fixed overnight in PFA, and transferred to PBS. Coronal sections (40 μ m) were cut using a vibratome (VT1200S, Leica). Sections were mounted on slides with mounting medium containing DAPI (Merck). Imaging of jGCaMP7c- and Jaws-expressing neurons were performed from sequential z-series scans with a Leica TCS SP8+ confocal microscope (Leica, Germany).

2.8. In vivo Ca²⁺ imaging and data analysis

Videos were initially processed using Mosaic Software (version 1.2, Inscopix). To reduce file size, recordings were spatially down-sampled at 10 Hz and motion-corrected. HDF5 images were developed from the raw video. Fluorescence signals from single neurons were extracted in MATLAB using constrained non-negative matrix factorization adapted for endoscopic data (CNMF-E) [15]. Seed pixels were initialized using a greedy model considering a minimum local correlation of 0.8 and a minimum peak-to-noise ratio of 8. Local background was corrected with a ring model (radius = 22). False cell detection, cell merging, and motion artifacts were verified by visual inspection of neuron shape and temporal dynamics of Ca²⁺ transients. Signals were de-convolved using the CNMF-E package (FOOPSI, AR2 model). Fluorescence trace amplitudes are reported as the standard deviation above noise.

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 7.04 for Windows (GraphPad Software, USA) or Igor Pro version 8.01B01 (Wave Metrics, USA). Type I error was set at 0.05. Shapiro-Wilk tests were performed to assess the normality of data. Other details of statistical analyses are described in the figure legends.

3. Results

By modifying the UCLA miniscope, we designed a new miniscope that permits imaging and manipulation of the same neuronal populations in freely behaving mice ([Fig. 1](#)). To allow the miniscope to accept any light source, we removed the circuit board with an

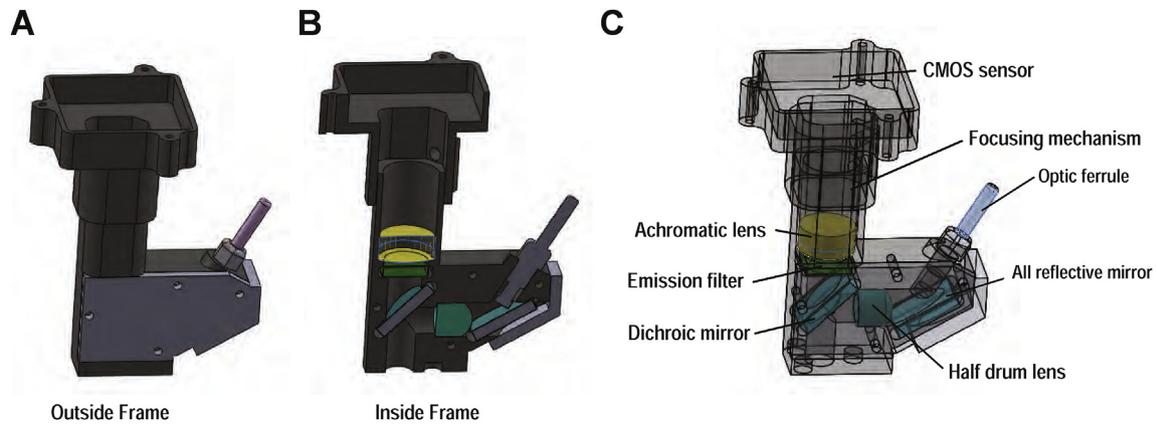


Fig. 1. Miniscope structure. (A) Outside view of frame. Instead of an LED, an optic ferrule receives light from external light sources. (B) Inside view of frame. The light path is sheathed by black Delrin material. (C) Alignment of major components.

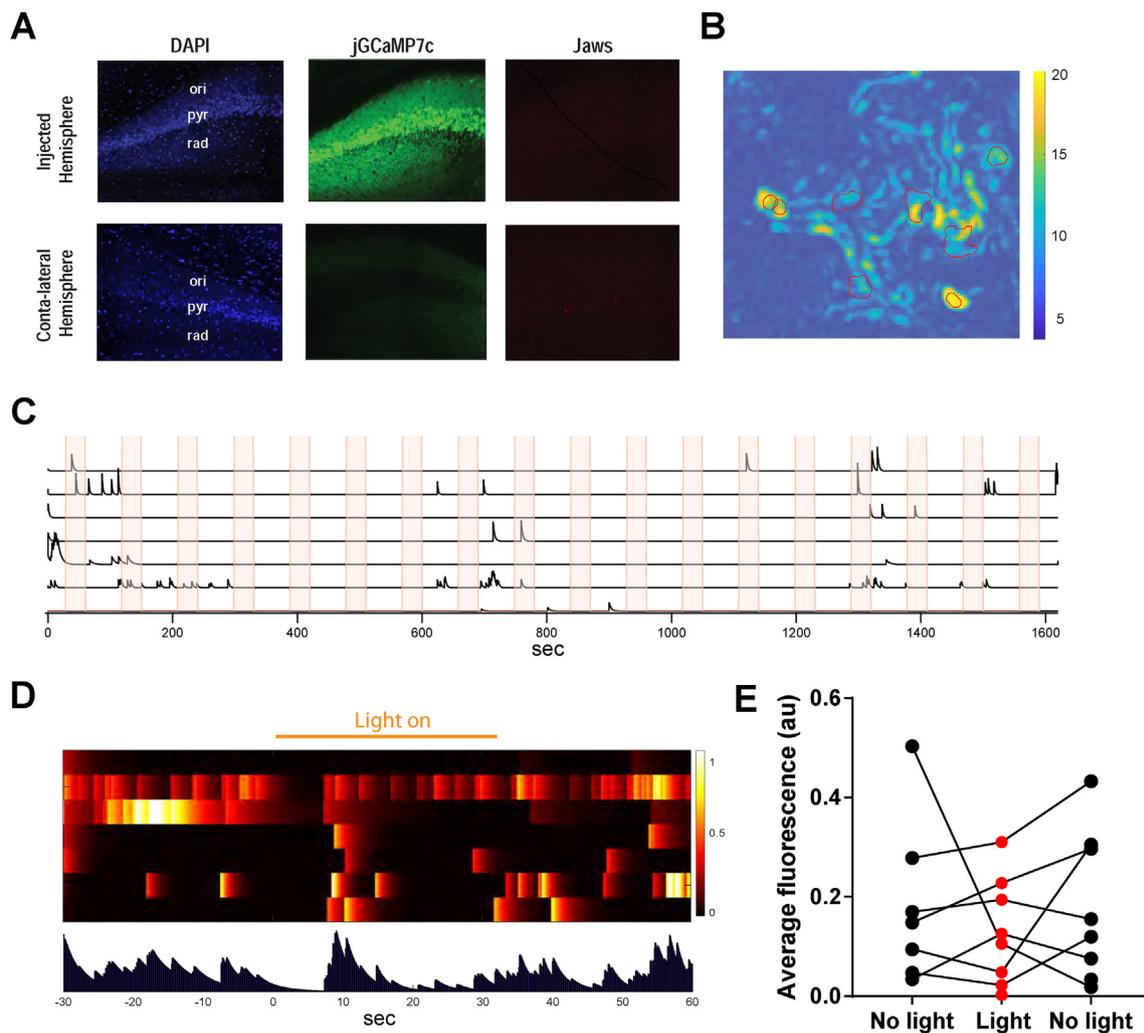


Fig. 2. Specificity of neuronal activity imaging. (A) Expression of jRCaMP7c in the injected CA1. (B) Neurons (outlined in red, $n = 7$) identified by CNMF-E algorithm. (C–D) F/F_0 traces of Ca^{2+} events in individual neurons ($n = 7$, C) and summation of Ca^{2+} fluorescence signals in individual neurons (D, top) and across all neurons (D, bottom) upon orange laser light delivery (30-s on/off cycles). (E) No change in Ca^{2+} fluorescence signal during the light on period ($n = 7$, one-way repeated measures ANOVA with Geisser-Greenhouse correction, $F(1.3, 9.1) = 0.41$, $p = 0.59$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

LED from the original UCLA miniscope and added a ferrule-based light input connector, which is widely used for conventional optogenetic experiments. To guide light to the dichroic mirror, an all-reflective mirror was inserted inside the structure. These modifications did not increase the overall weight of the miniscope (~1.9 g). However, for laser light delivery, an optic cable is now necessary in addition to the USB cable connection to the camera. The movement of mice is not limited by the addition of this optic cable if the cables are connected using coaxial slip rings for electronics and optics. The image recording capability is identical to that of the UCLA miniscope, as the modified miniscope uses the UCLA miniscope structure for imaging capability.

To demonstrate flexibility in choosing light sources for the miniscope, we performed simultaneous imaging and silencing of hippocampal CA1 neuron activity in freely behaving mice. To image neuronal activity, we utilized a genetically coded Ca^{2+} sensor, jGCaMP7c, which is excited by 450-nm blue light stimulation. A previous study shows that this sensor has very low baseline fluorescence and hence is suitable for miniscope imaging [12]. After

injection of AAV virus to express the light-sensitive protein in one hemisphere, jGCaMP7c-expressing neurons were observed in the injected CA1 (Fig. 2A). Next, we attached a Grin lens above the virus-injected CA1 and a baseplate, which are commonly used for imaging using the UCLA miniscope. After mice recovered from surgery, the modified miniscope was attached to the baseplate. As expected, stimulating jGCaMP7c by delivering blue laser light through the miniscope induced neuronal activity as visualized by Ca^{2+} events in the CA1 (Fig. 2B–C), with an observed event rate matching that previously reported (~0.02 events/s) [16]. By contrast, the delivery of orange laser light did not impact neuronal activity (Fig. 2C–E).

Next, we induced expression of Jaws opsin [13], a Cl^{-} -ion pump, which silences neuronal activity upon 590-nm orange light stimulation, together with jGCaMP7c in CA1 neurons by co-injecting the two AAV viruses into one hemisphere. The Jaws signal was diffuse, as previously described, and was observed together with the jGCaMP7c signal in the ipsilateral hemisphere but not in the contralateral hemisphere (Fig. 3A, Supplemental File 2). Next, we

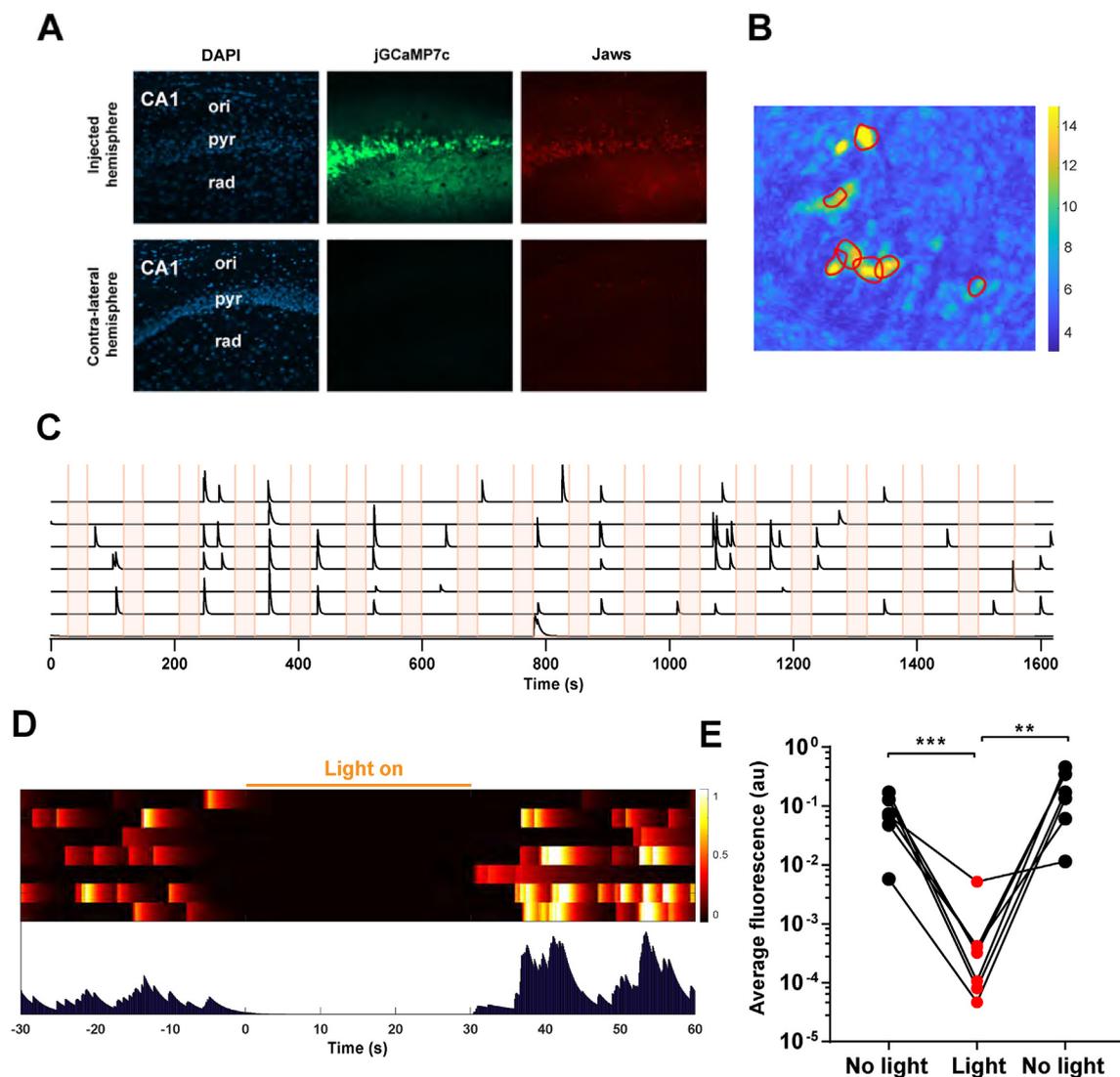


Fig. 3. Real-time simultaneous neuronal imaging and silencing in freely behaving mice. (A) Neuronal co-expression of GCaMP7c and Jaws in the injected CA1 but not the contralateral CA1. (B) Neurons (outlined in red, $n = 7$) identified by CNMF-E algorithm. (C–D) F/F_0 traces of Ca^{2+} events in individual neurons ($n = 7$, C) and summation of Ca^{2+} fluorescence signals in individual neurons (D, top) and across all neurons (D, bottom) upon orange laser light delivery (30-s on/off cycles). (E) Significant decrease in Ca^{2+} fluorescence signal during the light on period ($n = 7$, one-way repeated measures ANOVA with Geisser-Greenhouse correction, $F(1.3, 78) = 40.0$, $p = 0.0002$, Tukey's multiple comparisons test, $*p < 0.002$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

periodically delivered orange and blue laser light together to the same neurons through the miniscope, which suppressed Ca^{2+} events in those neurons (Fig. 3B–E). Importantly, orange laser light did not stimulate jGCaMP7 (i.e., no increase in Ca^{2+} events), even at the beginning of its delivery (Fig. 3C–D), indicating an absence of biological crosstalk between Jaws opsin and jGCaMP7. Furthermore, ending orange laser light delivery resulted in an increase in Ca^{2+} events in the same neurons (Fig. 3C–E), confirming the temporal specificity of the silencing. These results suggest that our modified miniscope can be used to image and manipulate the same neurons in freely behaving mice with temporal and cell-type specificity.

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.bbrc.2019.07.082>

4. Discussion

We designed a new miniscope for simultaneously imaging and manipulating the activity of a defined population of neurons in freely behaving animals. The UCLA miniscope has contributed to important discoveries in neuroscience research. However, with the addition of the capability to manipulate imaged neurons, most biological settings studied with the UCLA miniscope can now employ our new miniscope, as its modifications are simple and do not increase its weight. This modified miniscope can accept any combination of light sources, allowing freedom of choice for light or light-sensor combinations without losing its capability of being used in freely behaving animals. Thus, our miniscope can be a powerful tool for revealing causal relationships between neuronal activity and function.

Conflicts of interest

T.H. and M.S. are inventors on patent applications regarding the miniscope described here.

Acknowledgements

We thank K.G. Akers for comments on the manuscript. We also thank all WPI-IHIS members and T. Tezuka, Y. Mimura, N. Hasegawa, and E. Hasegawa for technical assistance and discussion. This work was partially supported by grants from the World Premier International Research Center Initiative from MEXT, JST CREST grant #JPMJCR1655, JSPS KAKENHI grant #16K18359, 15F15408, 26115502, and 25116530, The Takeda Science Foundation, Shimadzu Science Foundation, Kanae Foundation for the promotion of medical science, Research Foundation for Opto-Science and Technology, and Uehara Memorial Foundation to M.S. Tokyo Biochemical Research Foundation to S.S.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2019.07.082>.

Transparency document

Transparency document related to this article can be found online at <https://doi.org/10.1016/j.bbrc.2019.07.082>.

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