

Full Paper

Visualization of mechanical stress-mediated Ca^{2+} signaling in the gut using intravital imaging

Yoshiko AIHARA^{1, 2}, Yota FUKUDA^{2, 3}, Akiyoshi TAKIZAWA¹, Naomi OSAKABE³, Tomomi AIDA⁴, Kohichi TANAKA⁴, Soichiro YOSHIKAWA⁵, Hajime KARASUYAMA⁵ and Takahiro ADACHI²

¹Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe, Hyogo 657-8501, Japan

²Department of Immunology, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

³Department of Bioscience and Engineering, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, Saitama 337-5780, Japan

⁴Department of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

⁵Department of Immune Regulation, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

Received December 29, 2019; Accepted May 22, 2020; Published online in J-STAGE June 11, 2020

Mechanosensory systems have been implicated in the maintenance of gut homeostasis, but details on the related mechanisms are scarce. Recently, we generated a conditional Ca^{2+} biosensor yellow cameleon 3.60 (YC3.60)-expressing transgenic mouse model and established a five-dimensional (5D; x, y, z, time, and Ca^{2+}) intravital imaging system for investigating lymphoid tissues and enteric epithelial cell responses. To validate this gut-sensing system, we visualized responses of enteric nervous system (ENS) cells in Nestin-Cre/YC3.60^{fllox} mice with specific YC3.60 expression. The ENS, including the myenteric (Auerbach's) and submucous (Meissner's) plexuses, could be visualized without staining in this mouse line, indicating that the probe produced sufficient fluorescent intensity. Furthermore, the myenteric plexus exhibited Ca^{2+} signaling during peristalsis without stimulation. Nerve endings on the surface of enteric epithelia also exhibited Ca^{2+} signaling without stimulation. Mechanical stress induced transient salient Ca^{2+} flux in the myenteric plexus and in enteric epithelial cells in the Nestin-Cre/YC3.60 and the CAG-Cre/YC3.60 lines, respectively. Furthermore, the potential TRPM7 inhibitors were shown to attenuate mechanical stress-mediated Ca^{2+} signaling. These data indicate that the present intravital imaging system can be used to visualize mechanosensory Ca^{2+} signaling in ENS cells and enteric epithelial cells.

Key words: Ca^{2+} signaling, gut, imaging, sensing

INTRODUCTION

Ingested food and environmental components such as microorganisms are digested and recognized in the gut lumen via innate receptors, including transient receptor potential (TRP) channels [1, 2], purinergic receptors [3], fatty acid receptors [4], and taste receptors [5]. Signals received by these receptors then stimulate cytokine release, hormone secretion, and neural activities that contribute to immune responses, gastrointestinal regulation, and energy metabolism. These responses are exerted through the intestinal epithelial cells, the enteric nervous system (ENS), the central nervous system (CNS), and the gut immune

systems [6, 7]. In the intestinal tract, the immune, nervous, and endocrine systems are highly integrated, and their crosstalk is crucial for maintaining physiological activities. Indeed, the ENS and CNS influence various functions, including satiety, release of digestive enzymes, local blood flow, and immune responses. The ENS is the largest branch of the peripheral nervous system and is distributed in small ganglia, most of which are classified into two plexuses: the myenteric and submucosal plexuses. The myenteric plexus forms a continuous network from the upper esophagus to the internal anus [8]. The submucosal ganglia and connecting fiber bundles form plexuses in the small and large intestines but not in the stomach and esophagus.

*Corresponding author. Takahiro Adachi (E-mail: tadachi.imm@mri.tmd.ac.jp)
(Supplementary materials: refer to PMC <https://www.ncbi.nlm.gov/pmc/journals/2480/>)

©2020 BMFH Press



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License.
(CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

When a food bolus approaches the intestine, the gut lumen monitors the physiological state of the gut and regulates the digestive physiological responses, such as secretions of digestive fluid and gut contraction. The physical stimulus of membrane stretching is recognized by opening cation-permeable stretch-activated ion channels [9]. Such physical stimulation of the gut lumen leads to serotonin (5-hydroxytryptamine: 5-HT) secretions that activate neurons of the ENS [10, 11]. However, these processes are among the few that are induced by the entry of food in the gut. Furthermore, real-time monitoring of gut sensing is limited because of the complexity of the gut-sensing system, although intensive *in vitro* and *ex vivo* studies have been conducted.

To facilitate analyses of the dynamics and signal transduction under physiological conditions, we previously established a transgenic mouse line that conditionally expresses the Ca^{2+} biosensor yellowameleon (YC3.60) [12]. YC3.60 is a double-chromophore indicator that employs Förster/fluorescence resonance energy transfer (FRET) between cyan fluorescent protein (CFP) and a circularly permuted variant of the yellow fluorescent protein (YFP), Venus [13]. YC3.60 can be used to monitor Ca^{2+} signaling by measuring the ratio of YFP and CFP (YFP/CFP) under CFP-excited conditions. FRET-based ratiometric indicators, including YC3.60, can also be corrected for unequal sensor expression and motion-derived changes in fluorescent intensities. Therefore, ratiometric sensors such as YC3.60 are suitable for *in vivo* whole-body imaging in mice. We previously established a five-dimensional (5D; x, y, z, time, and Ca^{2+} signal) live imaging system for use in lymphoid tissues [12]. Using this system for a mouse line ubiquitously expressing YC3.60 (CAG-Cre/YC3.60), Ca^{2+} signaling was detected in enteric epithelial cells *in vivo*, and the gram-positive probiotic bacteria *Lactococcus lactis* and *Bacillus subtilis* var. *natto* were shown to stimulate epithelial cells in the small intestine [14].

To investigate the gut systems that recognize physical stimuli from food ingestion *in vivo*, we attempted to extend this intravital Ca^{2+} imaging system to the ENS using mice with specific YC3.60 expression. Neural cell-specific expression of YC3.60 showed that Ca^{2+} signaling can be monitored in ENS cells. Moreover, we analyzed mechanical stress-mediated Ca^{2+} signaling *in vivo* by comparing mice with ubiquitous YC3.60 expression in enteric epithelial cells and neural cell-specific YC3.60 expression in the ENS.

MATERIALS AND METHODS

Mice

The transgenic mouse model that conditionally expresses YC3.60 was described in our previous study [12]. In the present study, a floxed YC3.60 reporter (YC3.60^{fllox}) mouse line was crossed with a Nestin-Cre mouse line [15], resulting in neural cell-specific YC3.60 expression in Nestin-Cre/YC3.60^{fllox} mice due to the loss of the loxP-flanked neomycin cassette. YC3.60^{fllox} mice were also crossed with CAG-Cre mice that express Cre ubiquitously [16]. Mice were maintained in our animal facility under specific pathogen-free conditions in accordance with the animal care guidelines of the Tokyo Medical and Dental University. All animal procedures were approved by the Committee for Animal Care of the Tokyo Medical and Dental University (no. A2018-432).

Tissue clearing

Intestinal tissue from CAG-Cre/YC3.60^{fllox} mice was immersed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 24 hr. After washing with PBS, the fixed tissue was treated with RapiClear 1.52 reagent (SUNJin Lab, Hsinchu, Taiwan) according to the manufacturer's instructions. Images were then acquired using a Nikon A1 laser-scanning confocal microscope (Nikon, Tokyo, Japan) with a 20× objective and NIS-Elements AR software as previously described [12]. Using a dichroic mirror (DM488) and a bandpass emission filter (540/30), YFP was detected at an excitation wavelength of 488 nm. The acquired images were analyzed using Nis-Elements software (Nikon).

Intravital imaging

After anesthetizing mice, the abdomen was surgically opened. The small intestinal tract (jejunum) was then pulled out, partially immobilized on a cover glass with an adhesive containing cyanoacrylate, covered with a plastic wrap to prevent drying, and placed on a microscope stage. Alternatively, the small intestinal tract was surgically incised lengthwise, partially immobilized on a cover glass with adhesive with the luminal side down, and placed on a microscope stage. The mechanical stimulus for CAG-Cre/YC3.60^{fllox} mice was given by either flushing water between the epithelia and cover glass using a micropipette or lifting 2 mm of epithelia and placing this segment on the cover glass using a pipette tip. For Nestin-Cre/YC3.60^{fllox} mice, the mechanical stimulus given was prodding of the intestine with a pipette tip. Images were then acquired using a Nikon A1 laser-scanning confocal microscope with a 20× objective and NIS-Elements AR software as previously described [12]. Using a dichroic mirror (DM457/514) and two bandpass emission filters (482/35 for CFP and 540/30 for YFP), YFP/CFP ratios were obtained at an excitation wavelength of 458 nm. Using a different dichroic mirror (DM488), YFP images were obtained at an excitation wavelength of 488 nm. Images were acquired at approximately 2 sec/frame and were analyzed using Nis-Elements software (Nikon).

Immunohistochemistry

The duodenum, jejunum, and ileum were dissected from mice and fixed in 4% PFA, cryoprotected in 30% sucrose and embedded in Tissue-Tek O.C.T. compound (Sakura Finetek Japan, Tokyo, Japan) under liquid nitrogen vapor, and then sectioned coronally at a thickness of 4 μm . Sections were then incubated in a target retrieval solution (citrate, pH 6.0, Dako Japan, Tokyo, Japan) for 30 min at 80°C. Subsequently, sections were blocked with 5% donkey serum in PBS (blocking solution) and incubated with primary antibodies (1/5000 rabbit anti-5HT antibody, #20080, ImmunoStar, Hudson, WI, USA; 1/2000 rabbit anti-chromogranin A, #20085, Acris Antibodies, Herford, Germany; and 1/100 goat anti-TRPM7, #sc-19562, Santa Cruz Biotechnology, Dallas, TX, USA) in the blocking solution overnight at 4°C. After washing three times in PBS with Tween 20 (PBST) and once in PBS, sections were incubated with secondary antibodies (1/1,000 donkey anti-goat immunoglobulin [Ig] G conjugated with CF488A, #20016, Biotium, Fremont, CA, USA) for 30 min, washed three times in PBST and once in PBS, and incubated again with secondary antibodies (1/1,000 goat anti-rabbit conjugated with Alexa Fluor 546, #A-11035, Molecular

Probes, Eugene, OR, USA) for 30 min. Finally, sections were washed three times in PBST, mounted in FluorSave Reagent (Merck Millipore, Billerica, MA, USA), and observed using an upright fluorescence microscope (ECLIPSE Ni-U, Nikon). Images of the sections were obtained using a digital CCD camera and processed by Photoshop (version CS5; Adobe Systems).

RESULTS

Analysis of enteric nervous structure in CAG-Cre/YC3.60^{flox} and Nestin-Cre/YC3.60^{flox} mice

To analyze the enteric nervous structure more precisely, the optical transparency of gastrointestinal tissues in CAG-Cre/YC3.60 mice was increased using the mounting reagent RapiClear 1.52 [12]. Using confocal microscopy, we identified the submucous plexus, which lies in the submucosa of the intestinal wall, and the myenteric plexus, which is located between longitudinal and circular muscle layers (Fig. 1A and B and Supplementary Videos 1 and 2). Furthermore, crypts containing Paneth cells, located beneath the submucous plexus, were clearly observed. To investigate gut-sensing systems, we established a mouse model in which YC3.60 is specifically expressed in neural cells by crossing YC3.60^{flox} with Nestin-Cre mice [15]. In this transgenic mouse strain, Cre is expressed in the neuronal and glial cell precursors, and YC3.60 is expressed in most neural cells owing to the resulting genomic recombination. Consequently, the myenteric plexus of the ENS can be clearly identified in these mice using fluorescent microscopy as described previously [12]. Sufficient YC3.60 expression was confirmed in ENS cells in Nestin-Cre/YC3.60 mice. The myenteric plexus, mucous plexus, nerve fibers in the intestinal villi, and neural cells in the enteric epithelium were clearly identified (Fig. 1C).

Intravital imaging of Ca^{2+} signaling in ENS cells

To determine whether Ca^{2+} dynamics of ENS cells can be detected *in vivo*, we performed intravital imaging of intestines in Nestin-Cre/YC3.60 mice. The results revealed Ca^{2+} signaling in the myenteric plexus without exogenous stimulation (Fig. 2A and Supplementary Video 3). Furthermore, Ca^{2+} signaling was evident on the apical ends of ENS cells of the enteric epithelium, again in the absence of stimulation (Fig. 2B). In addition, the ENS cells along the lining of blood vessels also exhibited Ca^{2+} signaling (Fig. 2C and Supplementary Video 4). Thus, Ca^{2+} dynamics can be detected in ENS cells of Nestin-Cre/YC3.60 mice *in vivo*.

Intravital imaging of mechanosensory Ca^{2+} signaling in ENS cells and enteric epithelial cells

Although the effects of mechanical stimulation on gut motility have been recognized, the mechanisms of mechanical stimulation remain poorly understood. To clarify the mechanisms by which the gut responds to mechanical stress mediated by entry of ingested food and/or fluid into the intestinal tract, mechanosensory Ca^{2+} signaling was visualized *in vivo*. Water was flushed under the intestinal epithelia, and intravital imaging of the response showed that transient Ca^{2+} signaling was induced in the enteric epithelial cells of mice with ubiquitous YC3.60 expression (Fig. 3 and Supplementary Video 5). Thus, mechanical stimulation induced transient Ca^{2+} signaling. In Nestin-Cre/YC3.60^{flox} mice, the myenteric plexus also exhibited transient Ca^{2+} signaling following mechanical stimulation with ingested food and/or fluid (Fig. 4

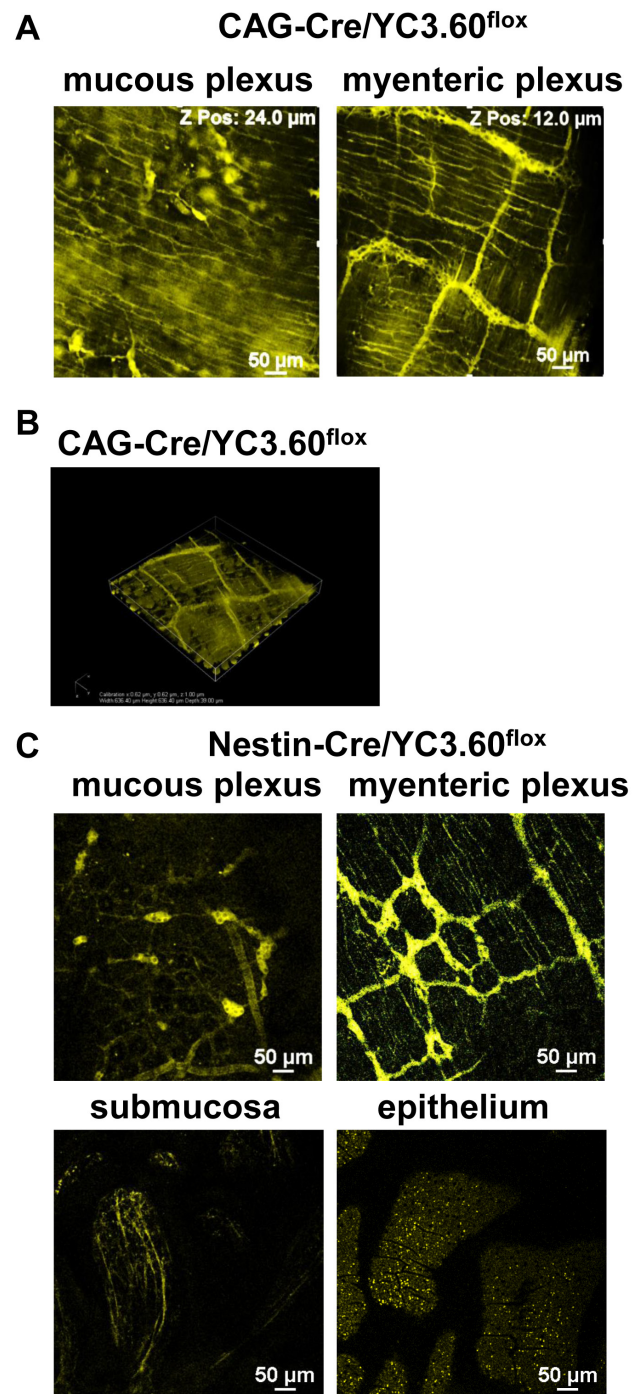


Fig. 1. Characterization of the enteric nervous system.

(A) Representative images of intestines from a CAG-Cre/YC3.60^{flox} mouse. Intestinal tissue from CAG-Cre/YC3.60^{flox} mice was treated with Rapid Clear 1.52 reagent. For Z-stack analysis of epithelial cells in the intestinal tract, imaging of small intestinal villi in the jejunum was performed using a confocal laser microscope. Yellow fluorescent protein (YFP) images (excitation at 488 nm) are shown. Z-stack images at 1-μm intervals were obtained to a depth of 39 μm. Only representative images are shown. Scale bar, 50 μm. (B) Three-dimensional (3D) structures of small intestinal epithelial cells; 3D images were generated from the Z-stack images (A) using NIS-Elements software. (C) Representative images from a Nestin-Cre/YC3.60^{flox} mouse. Intravital imaging of the myenteric and mucous plexuses, neural cells in the intestinal villi, and nerve endings in the epithelium in the jejunum are shown. Representative results of at least three mice are provided.

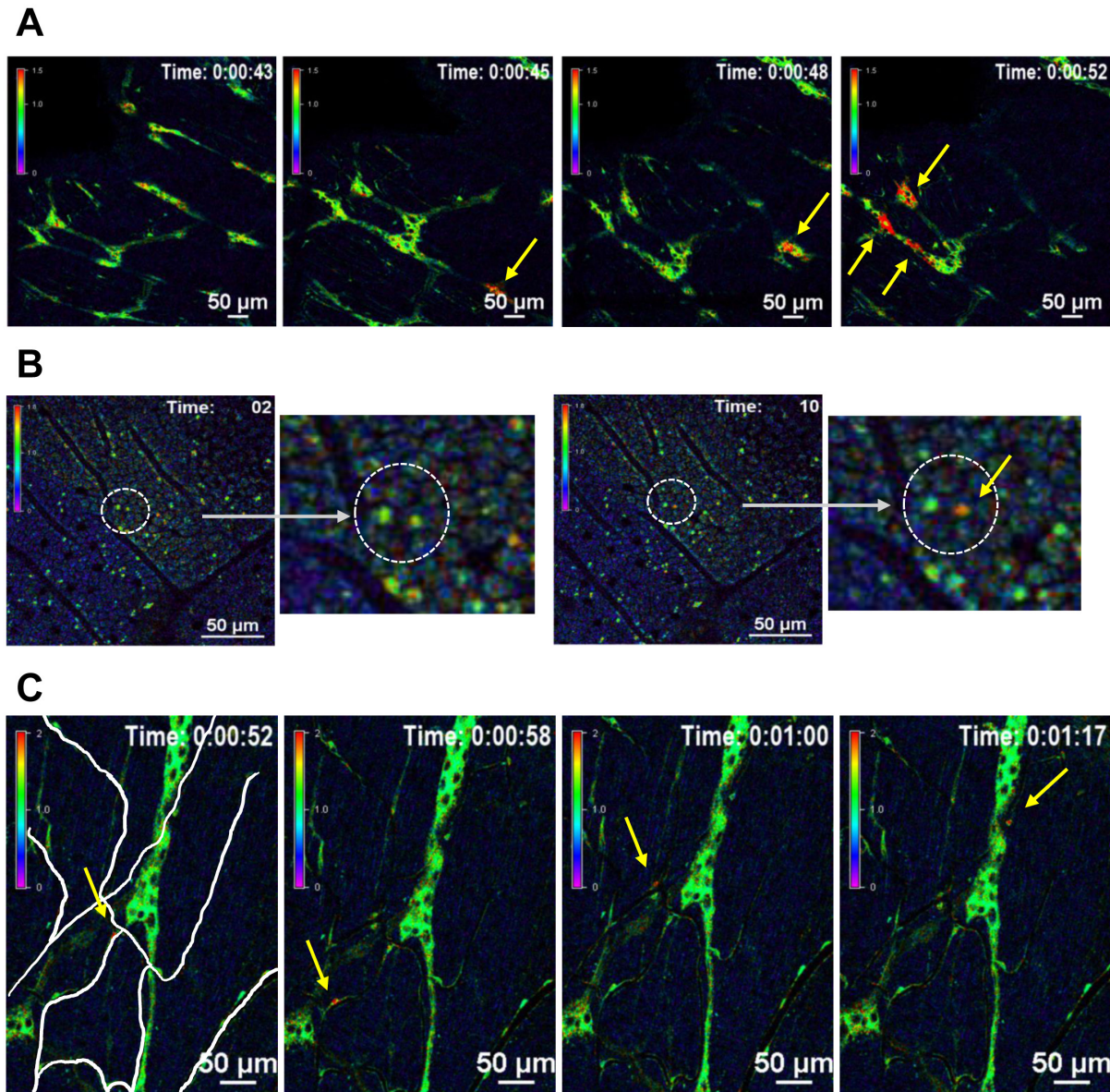


Fig. 2. Intravital imaging of Ca^{2+} signaling in ENS cells of a Nestin-Cre/YC3.60^{lox} mouse.

(A) Representative Ca^{2+} signaling images of the myenteric plexus in the jejunum from a Nestin-Cre/YC3.60^{lox} mouse. The rainbow parameter indicates relative Ca^{2+} concentrations. Fluorescence ratios of CFP to YFP (Venus) intensities (YFP/CFP) at an excitation wavelength of 458 nm are shown. Frames, 57. (B) Representative Ca^{2+} signaling images of neural cells in the enteric epithelium in the jejunum of a Nestin-Cre/YC3.60^{lox} mouse. Magnified images are also shown on the right side of each image. (C) Representative Ca^{2+} signaling images of neural cells along the lining of the blood vessel between the longitudinal and circular muscle layers in the jejunum of a Nestin-Cre/YC3.60^{lox} mouse. Frames, 49. Cells showing a high Ca^{2+} concentration are indicated by arrows. Blood streams are indicated by white lines in the leftmost image. Bars, 50 μm . Representative results of at least three mice are provided.

and [Supplementary Video 6](#)), as observed in enteric epithelial cells. These results indicate that mechanical stress induces Ca^{2+} responses in enteric epithelial cells and the myenteric plexus.

Expression of TRPM7 in intestinal epithelia

TRPM7 is a member of the melastatin subfamily of TRP channels and is known to respond to mechanical stress [17–21]. We examined the expression of TRPM7 together with serotonin (5-HT) produced by a large population of enteroendocrine cells and chromogranin A as a marker for enteroendocrine cells [22] in the intestine using immunohistochemistry. We observed cell

populations in which TRPM7 was coexpressed with chromogranin A throughout the small intestine ([Fig. 5A](#)). Double-labeling with 5-HT and TRPM7 showed that not all TRPM7-expressing cells coexpressed 5-HT ([Fig. 5B](#) and [Supplementary Fig. 1](#)). These data indicate that TRPM7 is expressed in subpopulations of enteroendocrine cells and that TRPM7⁺ cells are preferentially localized in the upper intestine ([Fig. 5C](#)).

Potential TRPM7 inhibitors suppress mechanical stress-mediated Ca^{2+} signaling in enteric epithelial cells

To determine whether TRPM7 is involved in mechanical

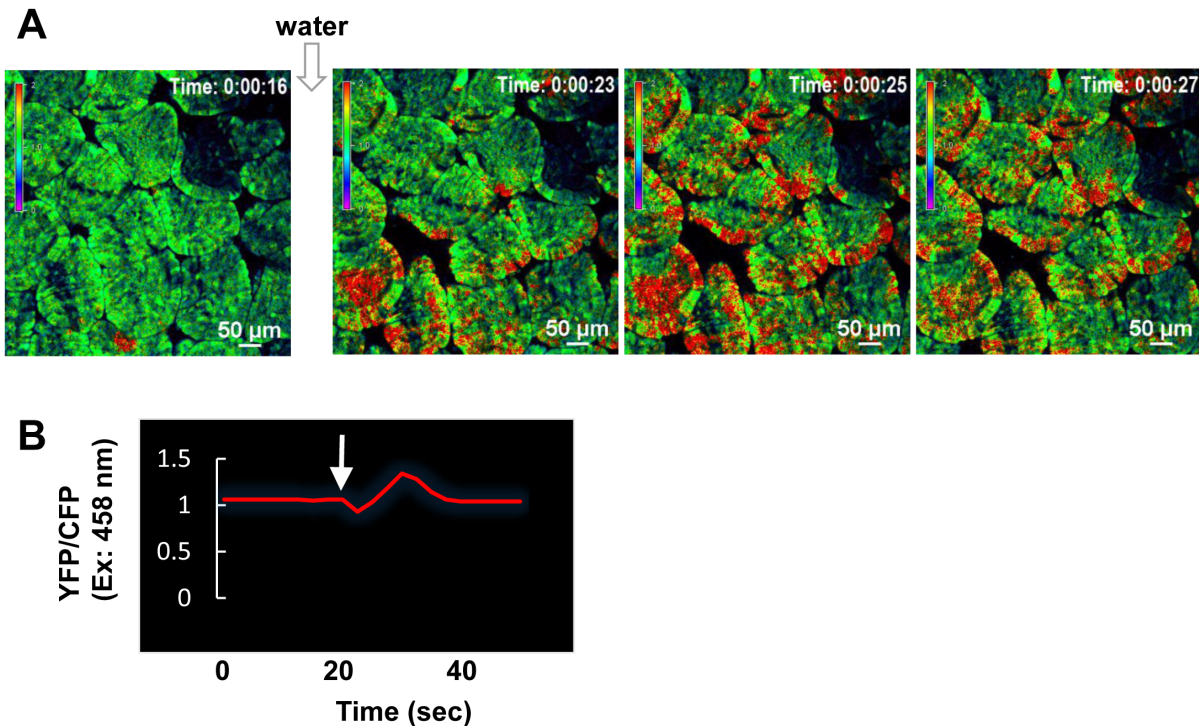


Fig. 3. Intravital imaging of Ca^{2+} signaling following mechanical stress in intestinal epithelial cells of a CAG-Cre/YC3.60^{flox} mouse.

(A) Representative Ca^{2+} signaling images of the intestinal tract of a mouse with ubiquitous YC3.60 expression. Water was flushed under the epithelium of the jejunum at the 20-sec time point. Ratiometric images (YFP/CFP ratio with excitation of 458 nm) are shown. The rainbow parameter indicates relative Ca^{2+} concentrations. Scale bar, 50 μm . (B) Time course of YFP/CFP fluorescence intensities with excitation at 458 nm. Mechanical stimulation was performed at the time point indicated by the arrow. Whole images were measured. Frames, 21. Representative results of at least 10 experiments are provided.

stress-mediated Ca^{2+} responses in intestinal epithelial cells, we performed experiments with the known lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA), which is a known inhibitor of TRPM7 [23]. We administered 10 μM NDGA to the intestinal lumen side in CAG-Cre/YC3.60 mice, and this was followed by incubation for 5 min and intravital imaging of enteric epithelial cells. Under these conditions, negligible Ca^{2+} response was observed upon mechanical stimulation (Fig. 6A and 6B and Supplementary Video 7). However, washing out of the inhibitor resulted in complete restoration of the responses of enteric epithelial cells to mechanical stimuli (Fig. 6C and 6D and Supplementary Video 8), implying that TRPM7 is involved in mechanical stress-mediated Ca^{2+} responses in enteric epithelial cells. Furthermore, although flushing water between the epithelia and cover glass induced robust Ca^{2+} signaling, water containing NDGA did not induce signaling (Fig. 7A and 7B). An additional test involving another potential TRPM7 inhibitor, 2-aminoethyl diphenyl borate (2-APB) [24], was performed to determine whether 2-APB inhibits mechanical stress-mediated Ca^{2+} signaling. Although 2-APB was less effective than NDGA, a similar result was obtained (Supplementary Fig. 2).

DISCUSSION

In this study, we visualized the ENS by analyzing neural cell-specific YC3.60 expression in Nestin-Cre/YC3.60^{flox} mice. Using the fluorescent indicator YC3.60 in intravital imaging analyses, we successfully monitored Ca^{2+} dynamics in ENS cells in the

jejunum *in vivo*. Furthermore, upon mechanical stimulation, enteric neurons and epithelial cells exhibited transient Ca^{2+} responses *in vivo*. In addition, we confirmed that the mechanical sensor TRPM7 is expressed in a subpopulation of enteroendocrine cells and showed that transient Ca^{2+} responses were inhibited by the potential TRPM7 inhibitors NDGA and 2-APB.

The mouse model validated in this study was used to demonstrate *in vivo* Ca^{2+} dynamics in the ENS, which is known to regulate gut motility and hormone secretion [25–27]. Previously, we developed an intravital imaging system for intestinal epithelial cells using CAG-Cre/YC3.60^{flox} mice [14]. The present report provides details of the application of this system in Nestin-Cre/YC3.60^{flox} mice [12] and demonstrates the visualization of Ca^{2+} dynamics in the ENS. Because the neural cells of these mice strongly expressed the Ca^{2+} biosensor YC3.60, the ENS cells of the myenteric and submucous plexuses could be clearly visualized. Furthermore, we visualized *in vivo* Ca^{2+} dynamics in ENS cells of the myenteric plexus, in nerve endings on intestinal epithelia, and in nerve fibers in intestinal villi.

We also observed mechanical stress-mediated Ca^{2+} signaling in ENS cells and epithelial cells of the intestine *in vivo*. The findings suggest that ingested food and fluid mechanically stimulate the gastrointestinal tract. Previous studies have shown that mechanical stress stimulates enteric epithelial cells to secrete serotonin, which controls gut motility [28, 29]. Furthermore, in *Drosophila*, this mechanosensor has been shown to respond to food textures [30].

TRPM7 is expressed in various tissues and clearly plays

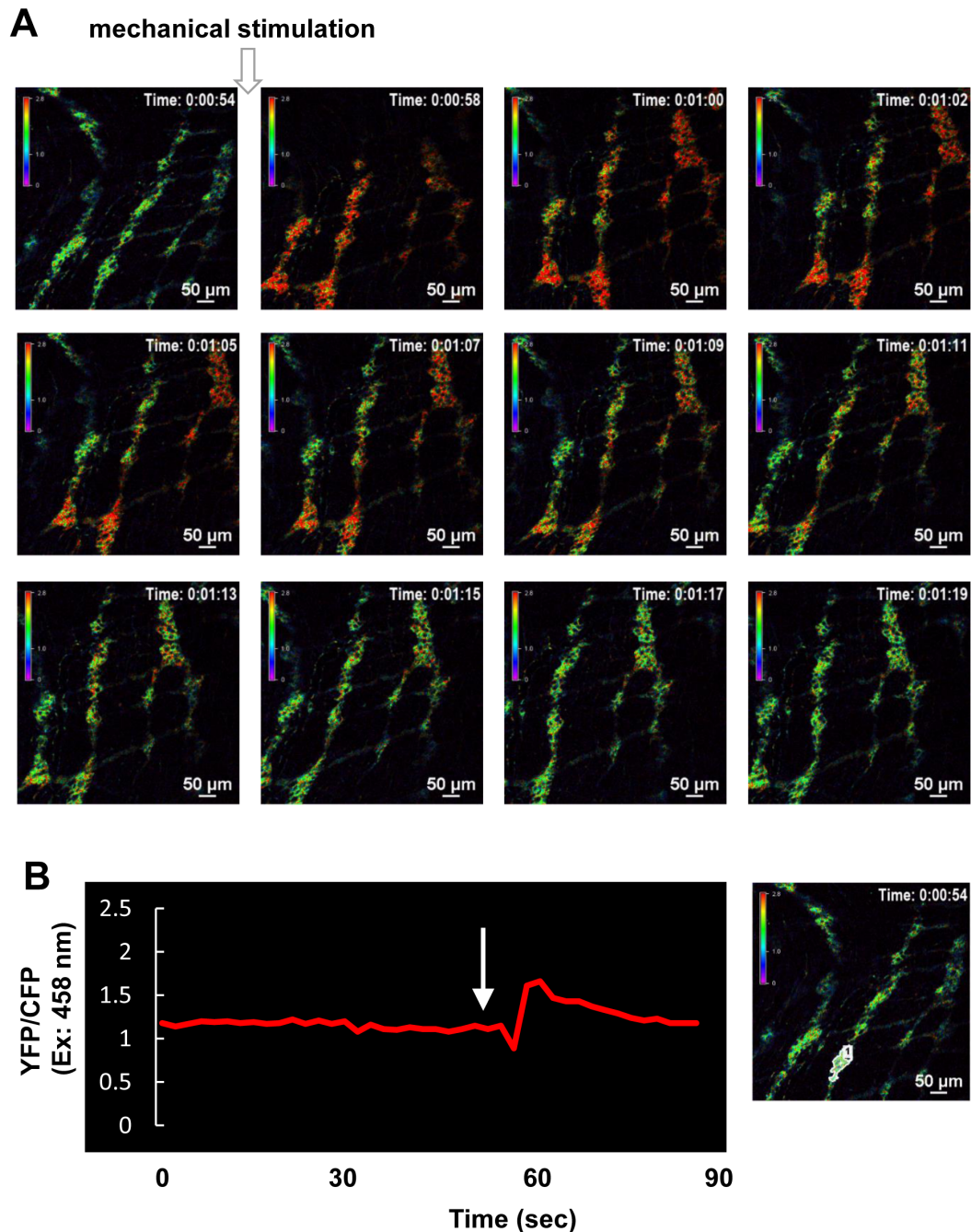


Fig. 4. Intravital imaging of Ca^{2+} signaling following mechanical stress in intestinal epithelial cells of a Nestin-Cre/YC3.60^{flox} mouse.

(A) Representative Ca^{2+} signaling images in the intestinal tract of a Nestin-Cre/YC3.60^{flox} mouse; ratiometric images (YFP/CFP ratio with excitation at 458 nm) are shown. The intestinal tract of jejunum was prodded with a pipette tip at the 55-sec time point. The rainbow parameter indicates relative Ca^{2+} concentrations. (B) Time course of YFP/CFP fluorescence intensities with excitation at 458 nm. Mechanical stimulation was performed at the time point indicated by the arrow; indicated regions were measured. Scale bar, 50 μm ; frames, 42. Representative results of at least three mice are provided.

critical biological roles, and its deficiency in mice results in embryonic lethality [31]. In the gut, TRPM7 affects the cells of Cajal, which control intestinal motility [17, 32, 33]. However, we showed that although TRPM7 is expressed in chromogranin A⁺ enteroendocrine cells, this population of cells comprises only 1–5% of all enteroendocrine cells. In addition, the proportion of TRPM7⁺ cells among 5-HT⁺ enteroendocrine cells was only 2–5%. Piezo2 mechanosensitive ion channels have been recently

identified as epithelial mechanosensors, and they are expressed by mouse and human enteroendocrine cells [28]. However, the distributions of mechanosensitive ion channels in the intestine differed from those of TRPM7. In particular, Piezo2 expression was moderately correlated with the presence of 5-HT⁺ cells, of which fewer than half expressed TRPM7. Although the TRPM7⁺ cell populations are small in the intestinal epithelia, the potential TRPM7 inhibitors NDGA [32, 33] and 2-APB clearly abrogated

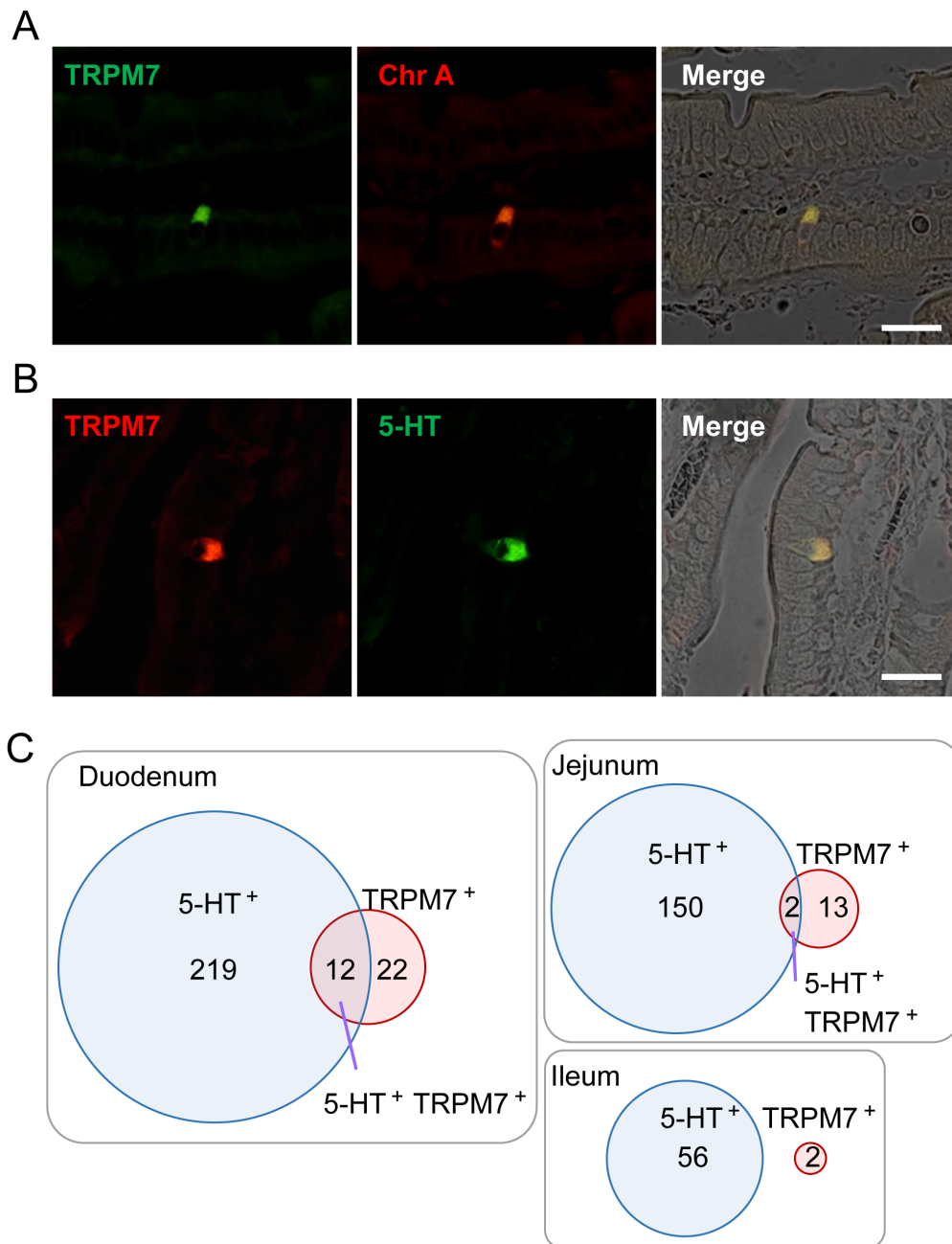


Fig. 5. TRPM7 expression in intestinal epithelial cells.

Double-labeling immunohistochemistry in mouse intestinal tissues; TRPM7 is expressed in a subset of enteroendocrine cells. (A) Fluorescent double-labeling immunohistochemistry clearly shows cellular coexpression of TRPM7 and chromogranin A⁺ (ChrA⁺). TRPM7 is expressed in a subset of ChrA⁺ cells. Merged images were processed using fluorescent and DIC images. (B) Fluorescent double-labeling immunohistochemistry with TRPM7 and 5-HT. (C) Populations of TRPM7⁺ cells among enteroendocrine cells of duodenum, jejunum, and ileum tissues; observed cell numbers are presented (sum of three sections). TRPM7 is coexpressed with 5-HT in the duodenum and jejunum but not in the ileum. Scale bars, 20 μm . Representative results of at least three mice are provided.

Ca^{2+} signaling in response to mechanical stimulation (Fig. 6 and Supplementary Fig. 1). These observations imply that TRPM7 may also be involved in Ca^{2+} signaling following mechanical stress. The intestine possibly recognizes the entry of fluid and food via mechanosensitive channels such as TRPM7 and regulates transfer, digestion, and absorption of nutrients.

Collectively, the data from our *in vivo* Ca^{2+} dynamics imaging system describe biological activities of the gut under

physiological conditions. However, further studies are required to clarify the associated neural circuits and epithelial cell functions.

ACKNOWLEDGEMENTS

We are grateful to Dr. G. Schütz (DKFZ) for providing the Nestin-Cre mice, Dr. M. Okabe (Osaka University) for providing the CAG-Cre mice, and Dr. A. Miyawaki (RIKEN) for the YC3.60

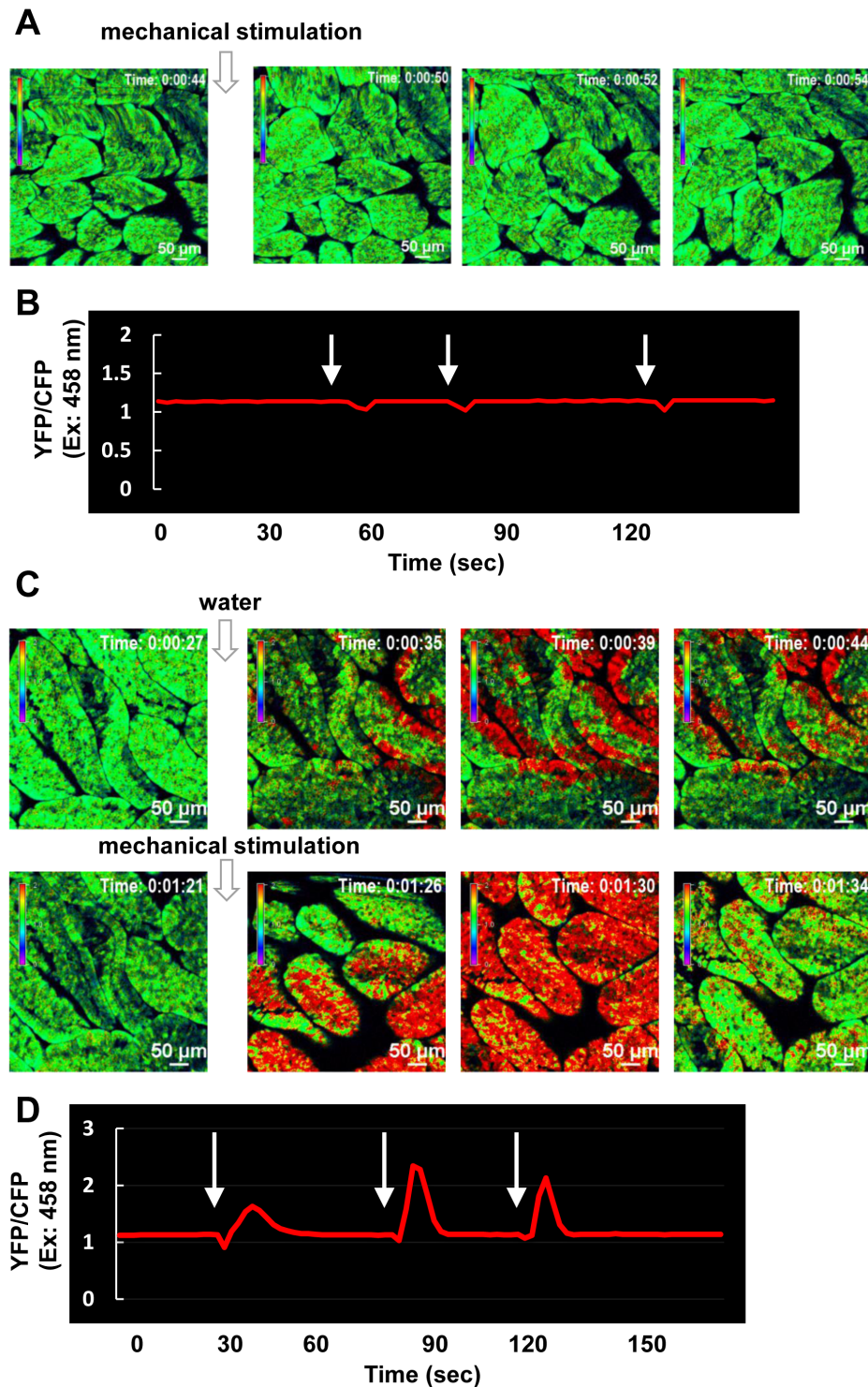


Fig. 6. Intravital imaging of Ca^{2+} signaling following mechanical stress in potential TRPM7 inhibitor (DNGA)-treated intestinal epithelial cells of a CAG-Cre/YC3.60^{fllox} mouse.

(A) Representative Ca^{2+} signaling images in the intestinal tract of a mouse with ubiquitous YC3.60 expression were generated after treatment with 10 μM NDGA. After treatment with 10 μM NDGA for 5 min, the jejunum was subjected to mechanical stimulation at the indicated time points. Ratiometric images (YFP/CFP ratio with excitation at 458 nm) are shown. The rainbow parameter indicates relative Ca^{2+} concentrations. Scale bar, 50 μm . (B) Time course of YFP/CFP fluorescence intensities with excitation at 458 nm. Mechanical stimulation was performed at the time points indicated by arrows. The whole field of each image was measured. Frames, 66. (C) Representative Ca^{2+} signaling images of the intestinal tract of a mouse with ubiquitous YC3.60 expression after washing out NDGA; the epithelium of the duodenum was flushed with water at the 30-sec time point. Then, 2-mm segments of epithelia were lifted and placed on a cover glass at the 80- and 120-sec time points. Ratiometric images (YFP/CFP ratio with excitation at 458 nm) are shown. The rainbow parameter indicates relative Ca^{2+} concentrations. Scale bar, 50 μm . (D) Time course of YFP/CFP fluorescence intensities with excitation at 458 nm. Mechanical stimulation was performed at the time points indicated by arrows. The whole field of each image was measured. Frames, 87. Representative results of two experiments are provided.

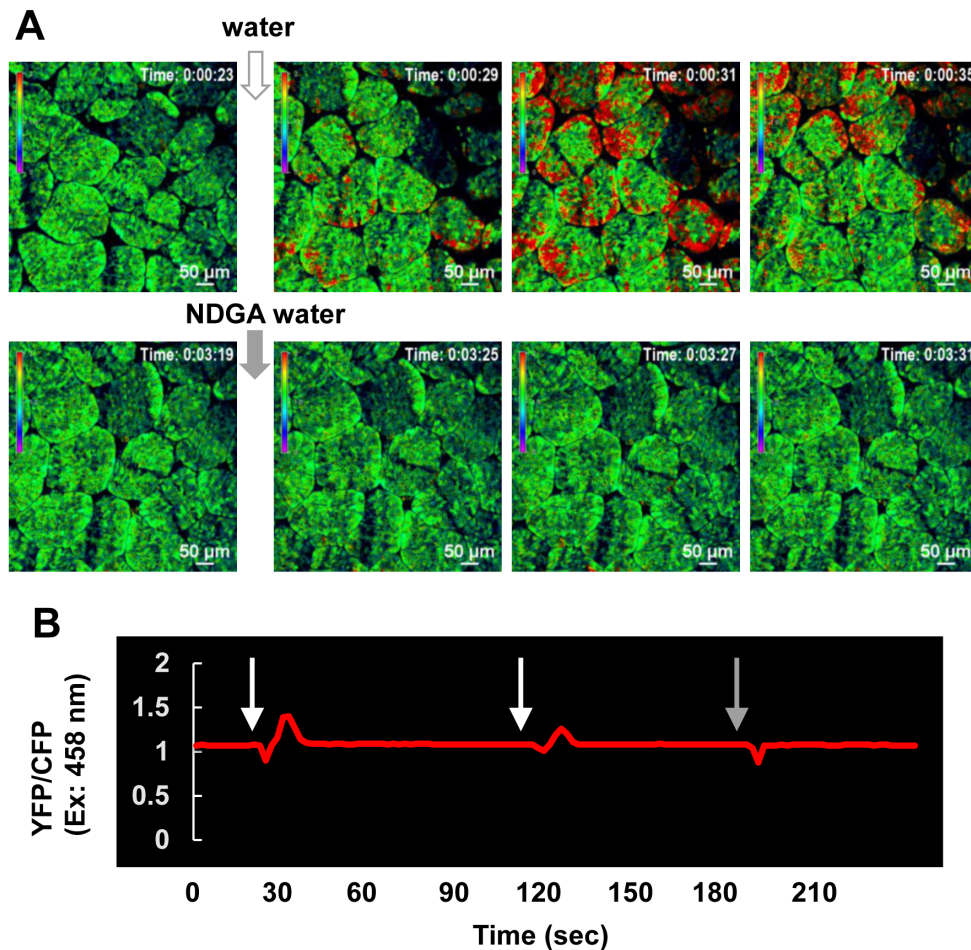


Fig. 7. Intravital imaging of Ca^{2+} signaling following mechanical stress caused by water containing NDGA in intestinal epithelial cells of a CAG-Cre/YC3.60^{fllox} mouse. (A) Representative Ca^{2+} signaling images in the intestinal tract of a mouse with ubiquitous YC3.60 expression were generated following mechanical stimulation with water at the 25- and 120-sec time points and with water containing 10 μM NDGA at the 200-sec time point. Ratiometric images (YFP/CFP ratio with excitation at 458 nm) are shown. The rainbow parameter indicates relative Ca^{2+} concentrations. Scale bar, 50 μm . (B) Time course of YFP/CFP fluorescence intensities with excitation at 458 nm. Mechanical stimulation was performed at the time points indicated by arrows. The whole field of each image was measured. Frames, 125. Representative results of two experiments are shown.

gene. This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (15K08526 to TA and 2478018 to YA), the Joint Usage/Research Program of Medical Research Institute (TMDU) (to TA, YS, HK, and NO), the Naoki Tsuchida Memorial Research Grant (TMDU) (to TA and YS), and the Yamada Research Grant (to TA).

REFERENCES

- Yu X, Yu M, Liu Y, Yu S. 2016. TRP channel functions in the gastrointestinal tract. *Semin Immunopathol* 38: 385–396. [Medline] [CrossRef]
- Balemans D, Boeckxstaens GE, Talavera K, Wouters MM. 2017. Transient receptor potential ion channel function in sensory transduction and cellular signaling cascades underlying visceral hypersensitivity. *Am J Physiol Gastrointest Liver Physiol* 312: G635–G648. [Medline] [CrossRef]
- Burnstock G. 2016. Purinergic signalling in the gut. *Adv Exp Med Biol* 891: 91–112. [Medline] [CrossRef]
- Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. 2015. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* 7: 2839–2849. [Medline] [CrossRef]
- Depoortere I. 2014. Taste receptors of the gut: emerging roles in health and disease. *Gut* 63: 179–190. [Medline] [CrossRef]
- Furness JB. 2016. Integrated neural and endocrine control of gastrointestinal function. *Adv Exp Med Biol* 891: 159–173. [Medline] [CrossRef]
- Furness JB, Rivera LR, Cho HJ, Bravo DM, Callaghan B. 2013. The gut as a sensory organ. *Nat Rev Gastroenterol Hepatol* 10: 729–740. [Medline] [CrossRef]
- Furness JB, Callaghan BP, Rivera LR, Cho HJ. 2014. The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv Exp Med Biol* 817: 39–71. [Medline] [CrossRef]
- Nilius B, Honoré E. 2012. Sensing pressure with ion channels. *Trends Neurosci* 35: 477–486. [Medline] [CrossRef]
- Bertrand PP. 2009. The cornucopia of intestinal chemosensory transduction. *Front Neurosci* 3: 48. [Medline]
- Hansen MB, Witte AB. 2008. The role of serotonin in intestinal luminal sensing and secretion. *Acta Physiol (Oxf)* 193: 311–323. [Medline] [CrossRef]
- Yoshikawa S, Usami T, Kikuta J, Ishii M, Sasano T, Sugiyama K, Furukawa T, Nakasho E, Takayanagi H, Tedder TF, Karasuyama H, Miyawaki A, Adachi T. 2016. Intravital imaging of Ca^{2+} signals in lymphocytes of Ca^{2+} biosensor transgenic mice: indication of autoimmune diseases before the pathological onset. *Sci Rep* 6: 18738. [Medline] [CrossRef]
- Miyawaki A, Griesbeck O, Heim R, Tsien RY. 1999. Dynamic and quantitative Ca^{2+} measurements using improved cameleons. *Proc Natl Acad Sci USA* 96: 2135–2140. [Medline] [CrossRef]
- Adachi T, Kakuta S, Aihara Y, Kamiya T, Watanabe Y, Osakabe N, Hazato N, Miyawaki A, Yoshikawa S, Usami T, Karasuyama H, Kimoto-Nira H, Hirayama K, Tsuji NM. 2016. Visualization of probiotic-mediated Ca^{2+} signaling in intestinal epithelial cells in vivo. *Front Immunol* 7: 601. [Medline] [CrossRef]
- Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R, Schütz G. 1999. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat Genet* 23: 99–103. [Medline] [CrossRef]

16. Matsumura H, Hasuwa H, Inoue N, Ikawa M, Okabe M. 2004. Lineage-specific cell disruption in living mice by Cre-mediated expression of diphtheria toxin A chain. *Biochem Biophys Res Commun* 321: 275–279. [[Medline](#)] [[CrossRef](#)]
17. Holzer P. 2011. TRP channels in the digestive system. *Curr Pharm Biotechnol* 12: 24–34. [[Medline](#)] [[CrossRef](#)]
18. Kim JM, Choi S, Park K. 2017. TRPM7 is involved in volume regulation in salivary glands. *J Dent Res* 96: 1044–1050. [[Medline](#)] [[CrossRef](#)]
19. Liu YS, Liu YA, Huang CJ, Yen MH, Tseng CT, Chien S, Lee OK. 2015. Mechanosensitive TRPM7 mediates shear stress and modulates osteogenic differentiation of mesenchymal stromal cells through Osterix pathway. *Sci Rep* 5: 16522. [[Medline](#)] [[CrossRef](#)]
20. Numata T, Shimizu T, Okada Y. 2007. Direct mechano-stress sensitivity of TRPM7 channel. *Cell Physiol Biochem* 19: 1–8. [[Medline](#)] [[CrossRef](#)]
21. Xiao E, Yang HQ, Gan YH, Duan DH, He LH, Guo Y, Wang SQ, Zhang Y. 2015. Brief reports: TRPM7 Senses mechanical stimulation inducing osteogenesis in human bone marrow mesenchymal stem cells. *Stem Cells* 33: 615–621. [[Medline](#)] [[CrossRef](#)]
22. Diwakarla S, Fothergill LJ, Fakhry J, Callaghan B, Furness JB. 2017. Heterogeneity of enterochromaffin cells within the gastrointestinal tract. *Neurogastroenterol Motil* 29: 29. [[Medline](#)] [[CrossRef](#)]
23. Chen HC, Xie J, Zhang Z, Su LT, Yue L, Runnels LW. 2010. Blockade of TRPM7 channel activity and cell death by inhibitors of 5-lipoxygenase. *PLoS One* 5: e111161. [[Medline](#)] [[CrossRef](#)]
24. Chokshi R, Fruasaha P, Kozak JA. 2012. 2-aminoethyl diphenyl borinate (2-APB) inhibits TRPM7 channels through an intracellular acidification mechanism. *Channels (Austin)* 6: 362–369. [[Medline](#)] [[CrossRef](#)]
25. Burnstock G. 2008. The journey to establish purinergic signalling in the gut. *Neurogastroenterol Motil* 20 Suppl 1: 8–19. [[Medline](#)] [[CrossRef](#)]
26. Vanden Berghe P. 2004. Fluorescent molecules as tools to study Ca²⁺ signaling, mitochondrial dynamics and synaptic function in enteric neurons. *Verh K Acad Geneeskd Belg* 66: 407–425. [[Medline](#)]
27. Tack J, Smith TK. 2004. Calcium imaging of gut activity. *Neurogastroenterol Motil* 16 Suppl 1: 86–95. [[Medline](#)] [[CrossRef](#)]
28. Alcaïno C, Knutson KR, Treichel AJ, Yildiz G, Strege PR, Linden DR, Li JH, Leiter AB, Szurszewski JH, Farrugia G, Beyder A. 2018. A population of gut epithelial enterochromaffin cells is mechanosensitive and requires Piezo2 to convert force into serotonin release. *Proc Natl Acad Sci USA* 115: E7632–E7641. [[Medline](#)] [[CrossRef](#)]
29. Williams EK, Chang RB, Strohlic DE, Umans BD, Lowell BB, Liberles SD. 2016. Sensory neurons that detect stretch and nutrients in the digestive system. *Cell* 166: 209–221. [[Medline](#)] [[CrossRef](#)]
30. Sánchez-Alcañiz JA, Zappia G, Marion-Poll F, Benton R. 2017. A mechanosensory receptor required for food texture detection in *Drosophila*. *Nat Commun* 8: 14192. [[Medline](#)] [[CrossRef](#)]
31. Jin J, Desai BN, Navarro B, Donovan A, Andrews NC, Clapham DE. 2008. Deletion of *Trpm7* disrupts embryonic development and thymopoiesis without altering Mg²⁺ homeostasis. *Science* 322: 756–760. [[Medline](#)] [[CrossRef](#)]
32. Kim BJ, Lee GS, Kim HW. 2013. Involvement of transient receptor potential melastatin type 7 channels on *Poncirus fructus*-induced depolarizations of pacemaker activity in interstitial cells of Cajal from murine small intestine. *Integr Med Res* 2: 62–69. [[Medline](#)] [[CrossRef](#)]
33. Kim BJ, Nam JH, Kim SJ. 2011. Effects of transient receptor potential channel blockers on pacemaker activity in interstitial cells of Cajal from mouse small intestine. *Mol Cells* 32: 153–160. [[Medline](#)] [[CrossRef](#)]