Supporting Information:

Field Potential Image Classification of Pacemaker Micro-coordination in the Intestine of the Mouse

Naoko Iwata[1], Chiho Takai[1], Naoto Mochizuki[1], Mariko Yamauchi[1],

Yoshiyuki Kasahara[2], Shinsuke Nakayama[1,*]

[1]Department of Cell Physiology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

[2]Department of Fetal and Maternal Therapeutics, Tohoku University Graduate School of Medicine, Sendai 980-8575, JAPAN

Corresponding author: Shinsuke Nakayama*

Department of Cell Physiology, Nagoya University Graduate School of Medicine,

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, JAPAN

Phone: +81 52 744 2045 Fax: +81 52 744 2048

E-mail: h44673a@nucc.cc.nagoya-u.ac.jp

ORCID: 0000-0003-4933-5429

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Supplemental Figures

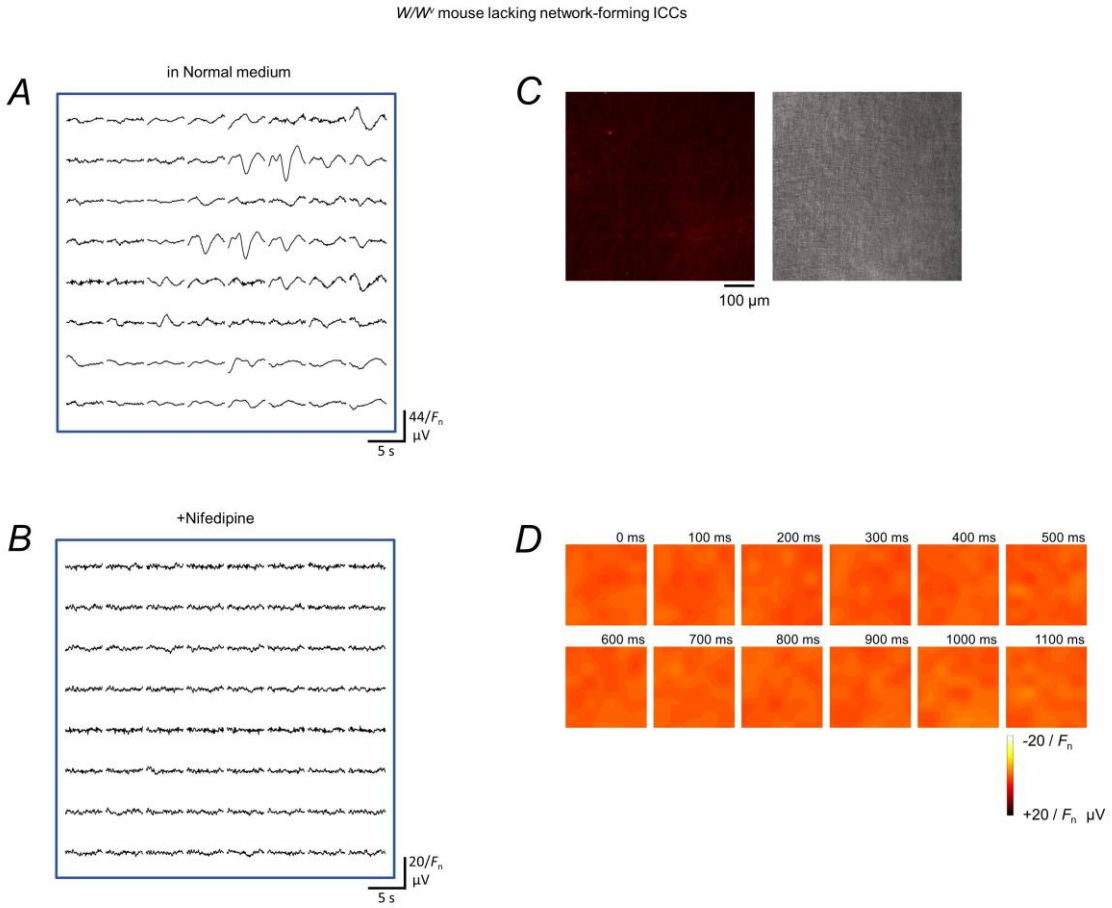

Fig. S1. Microelectrode array (MEA) recordings of spontaneous electrical activity in a sample of ileal muscle from a *W/W*^{v} mouse that lacks network-forming interstitial cells of Cajal (ICCs). After removal of the mucosa, the ileal muscle sample was mounted on an 8 × 8 MEA using a piece of dialysis membrane as shown in Fig. 1A. (**A, B**) An 8 × 8 plot of field potentials recorded under control conditions (**A**) and after exposure to the dihydropyridine Ca^{2+} channel antagonist, nifedipine (**B**). (**C**) Confocal transmitted light image and immunofluorescence image showing poor immunoreactivity for c-kit. (**D**) Field potential images showing electrical activity in the presence of nifedipine, corresponding to the MEA measurement in (**B**). In contrast to the MEA recordings made in wild-type mice, there was negligible electric activity in the ileum of *W/W*^{v} mice in the presence of nifedipine (**B**), which is known to suppress electric activity in smooth muscle but maintain pacemaker activity. These results demonstrate that network-forming ICCs were responsible for the spontaneous electric activities measured in the ileum of wild-type mice in the presence of nifedipine (i.e. the experimental conditions used to make MEA recordings in this study).

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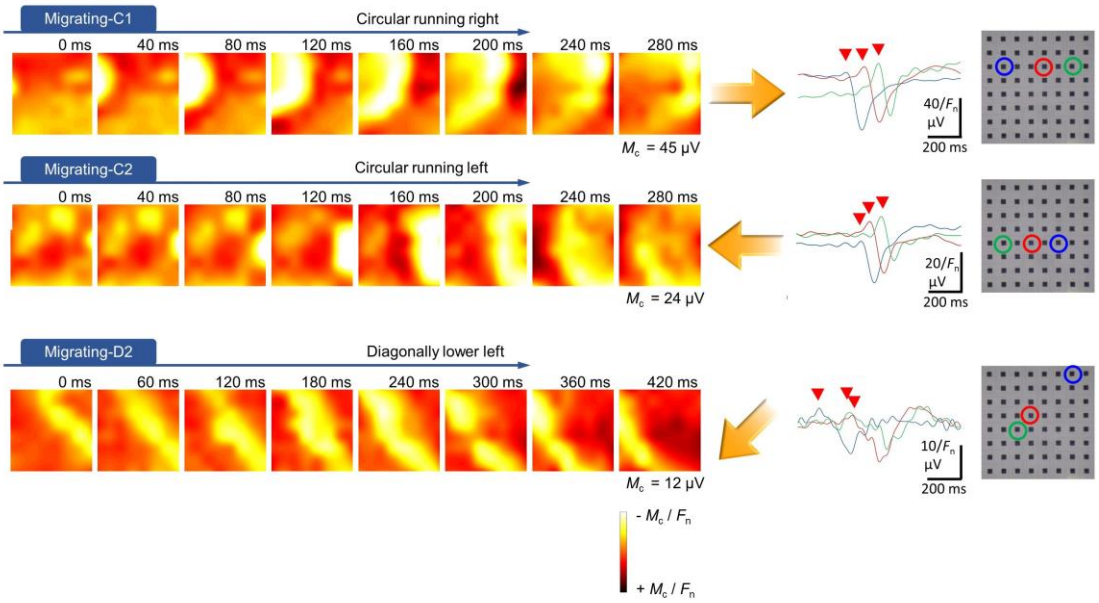

Figure S2. 'Migrating' activities propagated in circular and diagonal directions. Field potential images and representative field potential waves showing three examples of 'migrating' activity, in addition to those in Fig. 2B. Migrating-C1 and -C2: Circumferential propagation (along the circular muscle) toward the right and left, respectively. Migrating-D2: Diagonal propagation in the oro-anal direction (circumferentially toward the left). M_C: maximum potential used for color assignment. Red arrowheads indicate transient positive potentials in the 'migrating' waves. The MEs for representative field potential waves are indicated by circles of the same color on the MEA.

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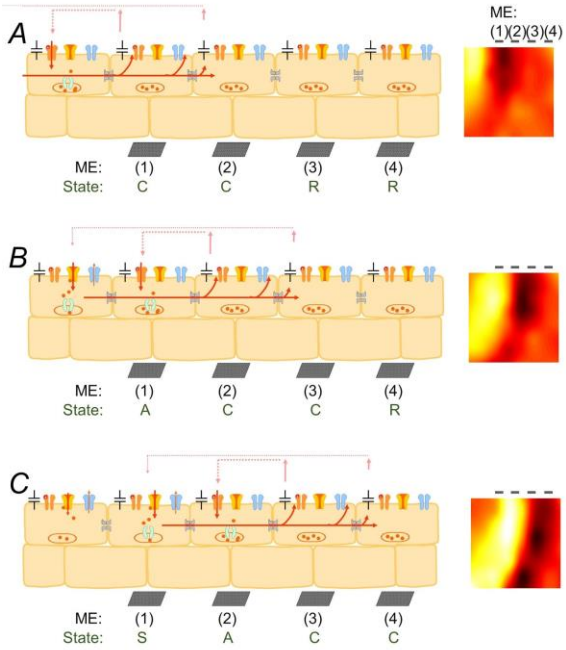

Fig. S3. Schematic illustration showing the process underlying 'migrating' activity. (**A**) Intercellular pacemaker current propagates toward the cells near ME(1) and ME(2) and charges their plasma membranes. The cells near ME(1) and ME(2) are in a charging ('C') state, and those near the other MEs are in a resting ('R') state. (**B**) Next, voltage-gated inward current (a part of pacemaker current) is activated in the cell near ME(1) so that this cell enters an activated ('A') state. Meanwhile, the intercellular pacemaker current propagates toward the cells near ME(2) and ME(3). (**C**) The cell near ME(1) enters a slow oscillation ('S') state in which transmembrane outward K^+ current and Ca^{2+}-activated inward currents are continuously activated. Intracellular Ca^{2+} release processes likely contribute to the activation of Ca^{2+}-activated inward currents (e.g., anoctamin-1 Ca^{2+}-activated Cl^- channels: the equilibrium potential of pacemaker cells ≈ -20 mV, more positive than the resting membrane potential). Meanwhile, the cell near ME(2) enters the 'A' state, and the intercellular pacemaker current further propagates toward the cells near ME(3) and ME(3). Thus, the cells near ME(1) to ME(4) form a local circuit. The intercellular pacemaker current does not propagate toward cells on the left, because the membranes of these cells are already charged. Each cell in the scheme represents a group of electrically connected pacemaker cells, adjacent interstitial cells and smooth muscle cells, and this unit thereby possesses sizeable electric capacity.

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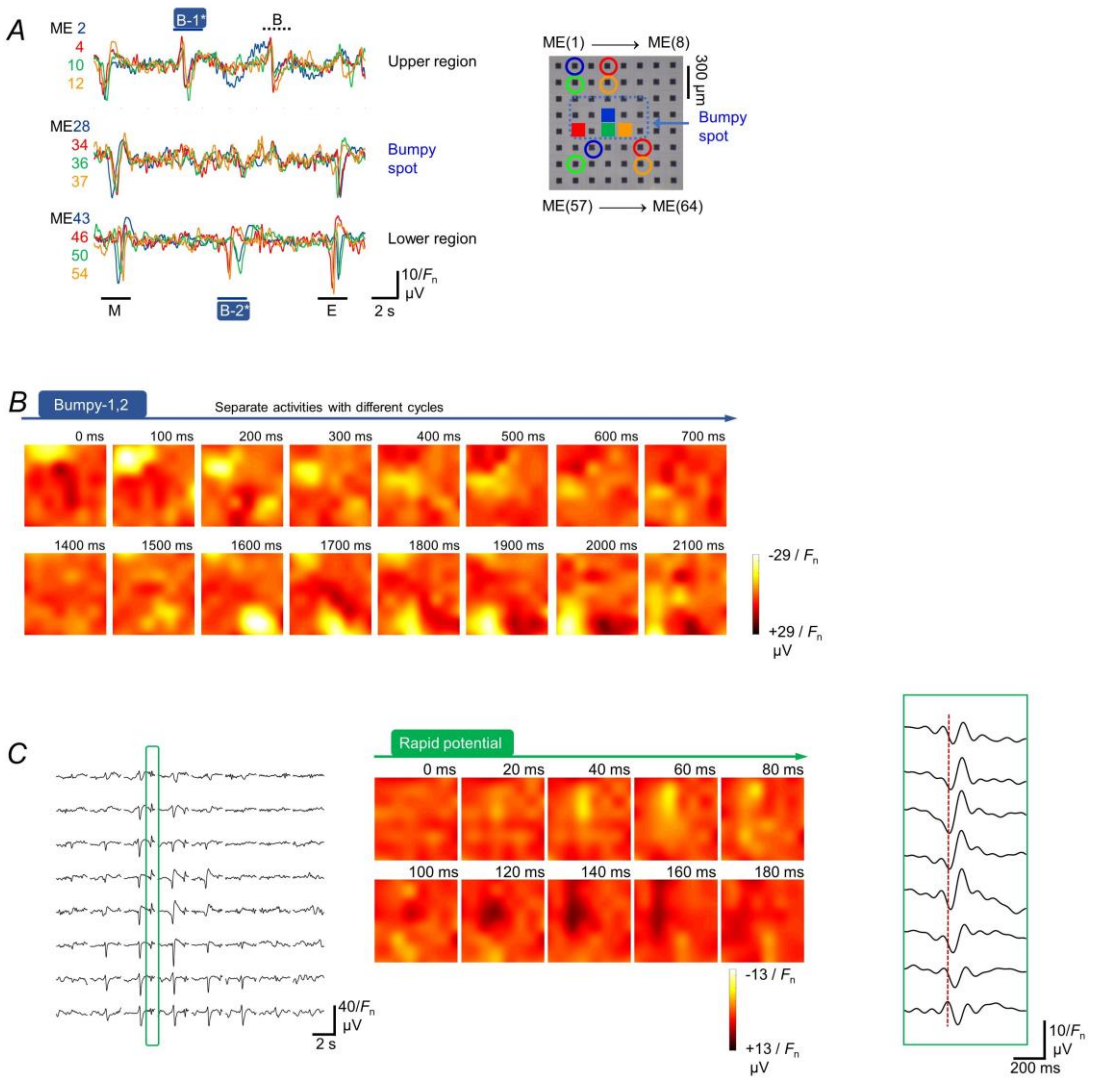

Fig. S4. Multiple pacemaker activities in the same muscle preparation occurred independently and were separated by a 'bumpy' spot, showing an interconversion of micro-coordination patterns. (**A**) Field potentials recorded from different regions of the MEA. (**B**) Field potential images showing the two 'bumpy' activities (B-1 and B-2) indicated in the field potential recorded in A. The B-1 and B-2 'bumpy' events occurred with different cycles and propagated locally in different regions (upper and lower, respectively). (**C**) Typical rapid potentials conducted longitudinally from the center region. Left panel: 8 × 8 plot of field potentials; middle and right panels: potential images and a stacked plot of rapid potentials corresponding to the period indicated by the green box in the left panel. We speculate that the repetitive rapid potentials in the center region (and associated slow potentials in upper region) destabilized the connectivity ('bumpy spot'), resulting in separated pacemaker activities.

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Supplemental Videos:

Supplemental Video SV1. Field potential video showing 'expanding' activity corresponding to the data in Fig. 1F.
SV1: Movie S1.mp4

Supplemental Video SV2. Field potential video showing 'migrating' activity corresponding to the data in Fig. 1F.
SV2: Movie S2.mp4

Supplemental Video SV3. Field potential video showing 'bumpy' activity corresponding to the data in Fig. 1F.
SV3: Movie S3.mp4

Supplemental Video SV4. Field potential video showing 'colliding' activity corresponding to the data in Fig. 1F.
SV4: Movie S4.mp4

Supplemental Video SV5. Field potential video corresponding to Fig. 2A. Three 'expanding' activities were initiated from distinct regions.
SV5: Movie S5.mp4

Supplemental Video SV6. Field potential video showing two consecutive 'migrating' activities. The 'migrating' activities propagated diagonally toward the lower-right region and longitudinally toward the upper region.
SV6: Movie S6.mp4

Supplemental Video SV7. Field potential video showing consecutive pacemaker activities in control corresponding to Fig. 4A. A variety of activities, including those of 'expanding' pattern occurred in control.
SV7: Movie S7.mp4

Supplemental Video SV8. Field potential video showing consecutive pacemaker activities in the presence of 5-HT corresponding to Fig. 4C. 'Migrating' activities alone propagated diagonally toward the lower-left region, accompanied by the increase in the frequency.
SV8: Movie S8.mp4

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