Supplementary Methods

qPCR arrays. The reverse transcription reaction for the qPCR array was incubated at 60ºC for 60 min, followed by 95ºC for 5 min to inactivate the enzyme. The reaction mix was diluted 5 times before proceeding with the qPCR reaction. The qPCR arrays used were the miScript miRNA PCR Array Human miFinder 384HC (MIHS-3001Z, Qiagen) in the 384-plate version. Each well in the plate contained a lyophilized primer for one of the 372 most common miRNAs in the miRBase database plus wells for positive and negative controls. Diluted template cDNA (100 µl) was used for each plate with the QuantiTect SYBR Green master mix and miScript Universal Primer, according to the manufacturer instructions. Data analysis was carried out using the SABioscience online tool (Supplementary Table 1). Full calculations are included in Supplementary Table 2.

RT-qPCR. After reverse transcription, 5 µl of cDNA for both sets of samples were subjected to pre-amplification using TaqMan™ PreAmp Master Mix and a primer pool containing 0.2X of each primer. All PreAmp products were then used for real-time PCR using the LightCycler 480 system with SYBR Green I Master Mix (Roche Applied Science). Hypoxanthine Phosphoribosyltransferase (HPRT) was used as the housekeeping gene for mRNA, and the small-nucleolar RNA RNU6B was used for the miRNA.

Protein extraction and western blotting. HuSMC cells were resuspended in ice-cold cell lysis buffer (0.025M Tris-HCl, 0.15M NaCl, 0.001M EDTA, 1% v/v NP-40, 5% v/v glycerol, pH=7.4) for 30 minutes over ice. After centrifugation for 30 minutes at maximum speed at 4°C, protein lysates were quantified using BCA protein assay. 30 µg of protein

lysate were loaded on Mini-PROTEAN tetra-cell (Bio-Rad) using 4-15% precast gel (Bio-Rad) and then transferred to nitrocellulose membranes. After blocking for 1 hour in 2% BSA solution, the membranes were incubated with primary antibodies, Nav1.5 (1:500, custom-made antibody by Covance [1]) and GAPDH (1:10000, Fitzgerald, MA, USA) at 4°C overnight. Finally, membranes were incubated with HRP-conjugated secondary antibody for 2 hours at room temperature and scanned using ChemiDoc Imaging systems (Bio-Rad).

Electrophysiology. The extracellular solution was (in mM): 150 Na^+, 160Cl^-, 5 K^+, 2.55 Ca^{2+}, 5.5 glucose, and 10 HEPES; pH 7.35, 300 mmol/kg osmolality. The intracellular solution was (in mM): 155 Cs^+, 125 $CH_3SO_3^-$, 35 Cl^-, 5 Na^+, 5 Mg^{2+}, 2 EGTA, and 10 HEPES; pH 7.0, 290 mmol/kg osmolality.

Traction force microscopy (TFM). Polyacrylamide substrates with shear moduli of 6.4 kPa were prepared, and fluorescent sulfate-modified latex microspheres (0.2 μm, 505/515 ex/em) (FluoSpheres, Life Technologies) were conjugated to the gel surfaces after treatment with 1 mg ml^{-1} of dopamine hydrochloride (Sigma-Aldrich) in 50 mM HEPES solution (pH 8.5). Cells were plated on the gels overnight before traction force measurements. Images of gel surface-conjugated fluorescent beads were acquired for each cell before and after cell removal using a Biotek Cytation5 microscope at 10X magnification objective.

Lentiviral transduction. The smooth muscle layer was dissected from the jejunum of 6–8 weeks old Sprague Dawley rats and washed five times with culture medium composed of M199 medium (Thermo Fisher) supplemented with 5% fetal bovine

serum (Thermo Fisher), glucose (4.5 g/l, Sigma), L-glutamine (2 mm; Thermo Fisher) and antibiotic–anti-mycotic mixture (Thermo Fisher). After equilibration at 37°C in 95% air, 5% CO_2 incubator for 3 hours, the tissues were treated with lentiviral transduction particles (Dharmacon) with a multiplicity of infection (MOI) of 5.

Contractility measurements. ImageJ software was used to determine the frequency of contractions. Movies recorded as described above were imported in ImageJ as grayscale and converted to virtual stacks. Lines across landmarks in each tissue were traced in ImageJ and used to determine the frequency at day 0 and day 5. Using the "reslice" tool, orthogonal slices were reconstructed across the stacks and contractions per minute were counted by the obtained contractility patterns.

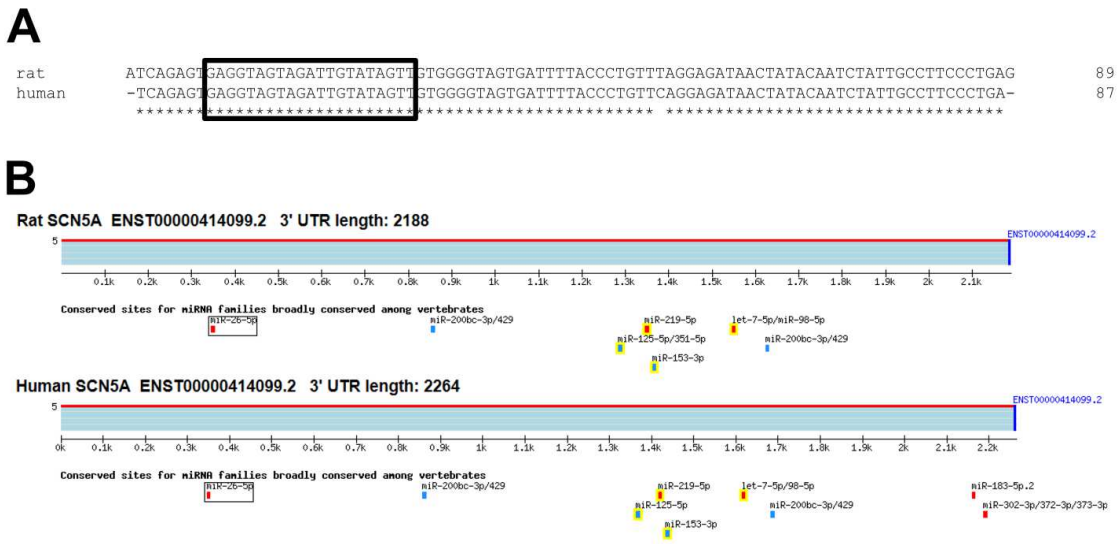
Spatial-temporal mapping. After importing each video in MATLAB (2015a, MathWorks) the contrast in each frame was enhanced using the histogram equalization function. A normalized 2D cross-correlation technique was used to track displacements of 7x5 equally spaced regions of interest (ROIs) of 201x201 pixels between an initial reference frame and every subsequent frame [2]. Each ROI was assumed not to shift more than 200 pixels in each direction during the recording. Displacements were calculated as the Euclidean distance between the reference frame and current frame at the centroid of the same ROI. The displacements over time were filtered using a lowpass Butterworth filter with a cut-off of 0.8 Hz to eliminate jumps between frames. The frequency of contraction was quantified using FFT of the displacements over time. The amplitude of contraction was measured from the displacements over time as the difference between each maximum and the local minimum within 60% of the interval determined from the fast Fourier transform. Coordination was a measure of the ratio of

displacement vectors pointing in the same direction. Data are indicated as median ± interquartile ranges (IQR), and statistics were calculated using 1way ANOVA with Dunn's multiple comparisons test.

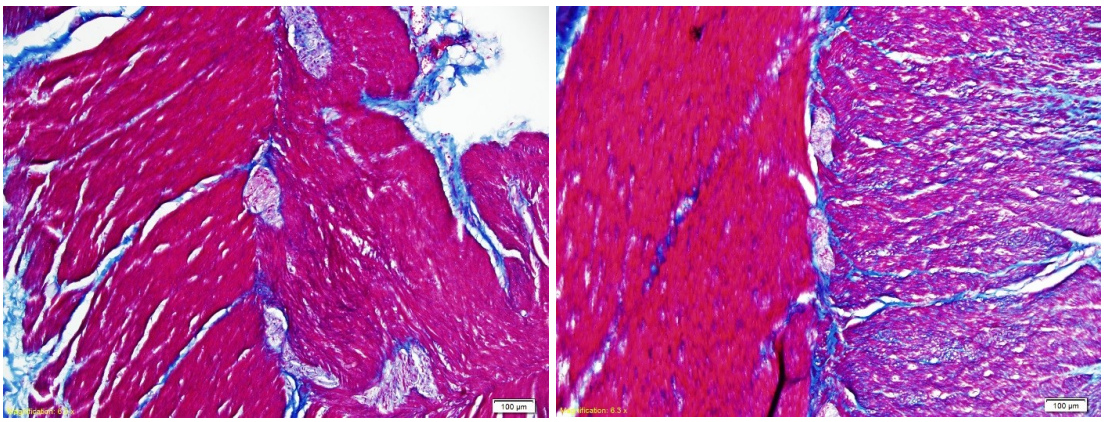
Supplementary Table 1. Links to online tools used for this study.

Online tool name	Link
SABioscience	http://pcrdataanalysis.sabiosciences.com/mirna/arrayanalysis.php
TractionsForAll	http://www.mayo.edu/research/labs/tissue-repair-mechanobiology/software
PicTar	https://pictar.mdc-berlin.de/
TargetScan	http://www.targetscan.org/vert_72/
miRBase	http://www.mirbase.org/index.shtml
miRTar-Base	http://mirtarbase.mbc.nctu.edu.tw/php/index.php

Supplementary Table 2. List of miRNAs included in the qPCR array and calculations for all miRNAs obtained using the online SABioscience tool.



Supplementary Fig.1. Human and rat let-7f have conserved sequence and target binding sites on the *SCN5A* 3'-UTR. A) Alignment of human and rat let-7f sequences show a 98.9% similarity. The seed sequence (box) is fully conserved between the two species. B) Schematic of rat (top) and human (bottom) *SCN5A* 3'UTR showing conserved binding site for let-7f between the two species.



CONTROL **STC**

Supplementary Fig.2. No difference in fibrosis in the *muscularis externa* of control versus STC patients. Fresh frozen sections were labeled with trichrome in control (left) and STC (right) muscularis. Scale bars, 100 μm.

Supplementary References

1 Koval OM, Snyder JS, Wolf RM, Pavlovicz RE, Glynn P, Curran J*, et al.* Ca2+/calmodulin-dependent protein kinase II-based regulation of voltage-gated Na+ channel in cardiac disease. Circulation 2012;**126**:2084-94.

2 Janssen PW, Lentle RG, Hulls C, Ravindran V, Amerah AM. Spatiotemporal mapping of the motility of the isolated chicken caecum. J Comp Physiol B 2009;**179**:593-604.