Supplementary Material to

"Smooth Muscle Contractility Causes the Gut to Grow Anisotropically"

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Characterization of passive mechanical properties

Tensile testing and pressurization tests were performed as previously described (38). Briefly, for tensile testing, the hindgut was pinned to the bottom of a Sylgard coated trough filled with ~100 mL DPBS (0.9 mM Ca^{2+}, 0.49 mM Mg^{2+}). The stomach of the guts was attached to a hook formed at the end of a thin glass cantilever (Figure 2a). The sensitivity (force per deflection angle) of the cantilever was determined by calibration with weights prior to the experiment. The cantilever was pulled at a constant velocity of 0.3 mm/sec to stretch the guts. The deformation of the gut tract and the deflection of the pipette were monitored with a camera and measured with ImageJ. Each gut was stretched three times, waiting 20 minutes between each stretch to ensure that the gut relaxed back to its initial length. For inflation experiments, ~1 cm long midgut segments were cannulated with a thin glass pipette; we tightened knots made out of hair at the gut-pipette junction and at the free end of the gut to allow pressure build-up inside the gut. Pressure was applied with a syringe filled with DPBS. We monitored the change in length, diameter and thickness of the gut wall; the latter could be discerned from luminal content by coloring the injected solution with a dye (methylene blue).

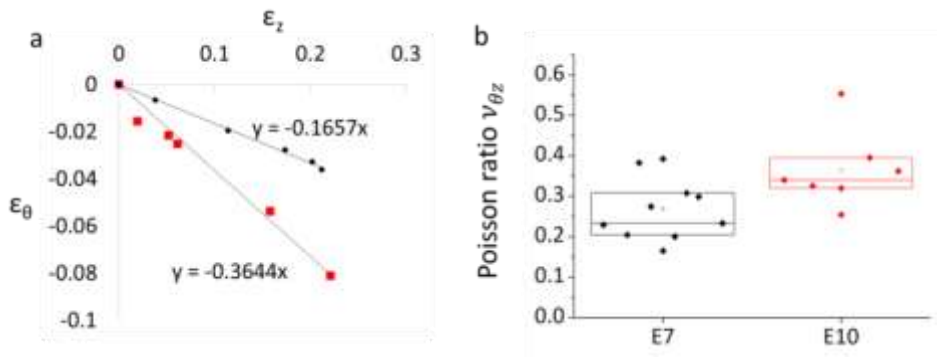
Computation of elastic moduli

We computed the longitudinal elastic modulus E_z from the slope of the stress-strain data (Fig.2b), where the longitudinal stress is $\sigma_z = f/S$ with $f = s\Delta\alpha$ the force applied by the cantilever, s the sensitivity (mN/°) of the cantilever, $\Delta\alpha$ its deflection angle and S the section of the gut. For E7 guts the lumen cross-section is negligible so that $S = \pi d^2/4$, with d the average diameter of the gut segment. For E10 guts, the lumen occupies ~5% of the gut cross-section(7) so that $S = 0.95\,\pi d^2/4$.

During phase II and III (Fig.2e) of pressurization, the gut wall thickness changes little (II) or not at all (III). In this configuration, the gut can be considered as an orthotropic, thin-walled cylinder with anisotropic mechanical properties along two directions (longitudinal and orthoradial). The governing equations are $\varepsilon_z = \frac{\sigma_z}{E_z} - \frac{\nu_{\theta z}}{E_\theta} \cdot \sigma_\theta$ (1) and $\varepsilon_\theta = -\frac{\nu_{z\theta}}{E_z} \cdot \sigma_z + \frac{\sigma_\theta}{E_\theta}$ (2), where ε_z and ε_θ are the strains, E_z and E_θ the elastic moduli and σ_z and σ_θ the stresses along the longitudinal and orthoradial directions respectively; $\nu_{\theta z}$ and $\nu_{z\theta}$ are the associated Poisson coefficient and are related by $\nu_{z\theta} = \frac{E_z}{E_\theta}\nu_{\theta z}$ for an orthotropic material. The strains $\varepsilon_z = (z - z_0)/z_0$ and $\varepsilon_\theta = (d - d_0)/d_0$ were obtained from high-magnification videos of the guts during the inflation, where z and d are the length and exterior diameter of the gut segment when a pressure p is applied, and z_0 and d_0 are the initial length and diameter at the start of phase II (or phase III) of the inflation. For a thin-walled cylinder, the orthoradial (hoop stress) and longitudinal stress are related to the pressure(41) by $\sigma_\theta = \frac{pr}{h}$ (3) and $\sigma_z = \frac{pr}{2h}$ (4). Combining (1-4), we find $E_\theta = 2E_z\left[\frac{\varepsilon_z}{\varepsilon_\theta}\left(1 - \frac{\nu_{\theta z}}{2}\right) + \nu_{\theta z}\right]$. $\frac{\varepsilon_z}{\varepsilon_\theta}$ was measured for each inflation from linear fits of ε_z vs ε_θ data (Fig.2f). The Poisson modulus of the guts $\nu_{\theta z} = \varepsilon_\theta/\varepsilon_z$ was obtained by measuring the decrease in diameter ε_θ when an applied, constant longitudinal strain ε_z was applied (Fig.S1). We found $\nu_{\theta z,E7}$ = 0.27 ± 0.08 (*n*=10) and $\nu_{\theta z,E10}$= 0.36 ± 0.09 (*n*=7).

Second Harmonic Generation Microscopy

We used a vibratome to cut 150 µm-thick transverse sections of E9 jejunum embedded in 3% agarose type VII (Sigma); longitudinal sections from the same sample were then collected. Image acquisition was performed in the IMAG'IC Facility, Institut Cochin, Paris, France. SHG images were obtained with an upright Leica SP5 microscope (Leica Microsystems Gmbh, Wetzlar, Germany) coupled to a femtosecond Ti:sapphire laser (Chameleon, Coherent, Saclay, France) tuned to a wavelength of 810 nm for all experiments. The beam was circularly polarized. We used a Leica Microsystems HCX IRAPO 25x/0.95 W objective. The SHG signal was detected in epi-collection through a 405/15-nm bandpass filter, by an NDD PMT (Leica Microsystems), with a fixed voltage supply, laser excitation power and exposure time, allowing the direct comparison of SHG signal intensities on transverse and longitudinal sections. Z-stacks of each sample were collected (total z =120 µm, step Δz=3 µm) and the maximum values of the stacks were projected. Collagen fibers contribute ~50% (15) to passive tissue mechanical properties.



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58 **Figure S1: Poisson ratio of E7 and E10 midgut. (a) Example of diameter change ε_θ as a longitudinal strain ε_z is**
59 **applied, at E7 (black) and E10 (red). (b) Measured Poisson modulus $\nu_{\theta z} = \varepsilon_\theta / \varepsilon_z$ at E7 (n=10) and E10 (n=7).**

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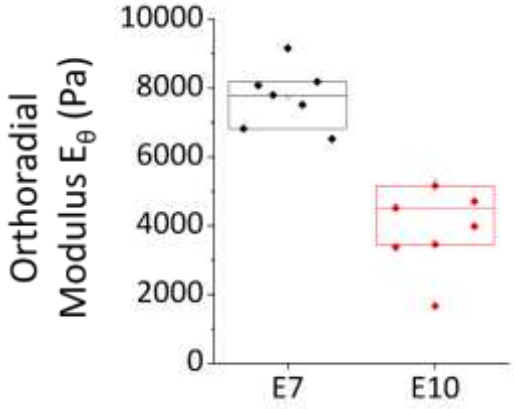

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62 **Figure S2. Orthoradial elastic modulus E_θ at E7 (n=7) and E10 (n=9), derived from phase III of pressurization.**

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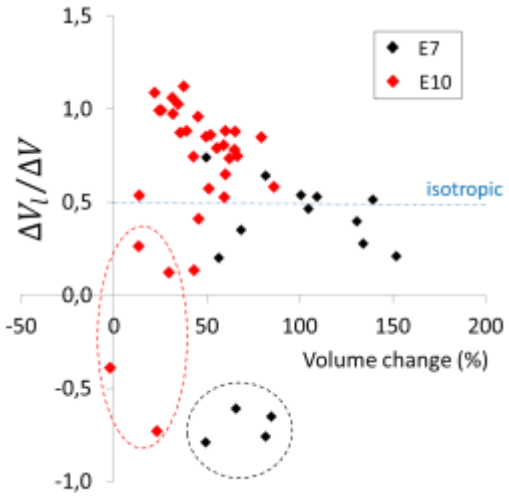

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65 **Figure S3. Relative lengthening $\Delta V_l / \Delta V$ after 2 day culture as a function of the observed volume change, for E7 and**
66 **E10 guts. Outliers in each age group (dashed ovals) exhibit a small volume change, in the lower half of the average**
67 **volume change of each age group. This indicates that the unusual behavior of these samples may arise from poor**
68 **vitality of the organ following dissection or during culture.**

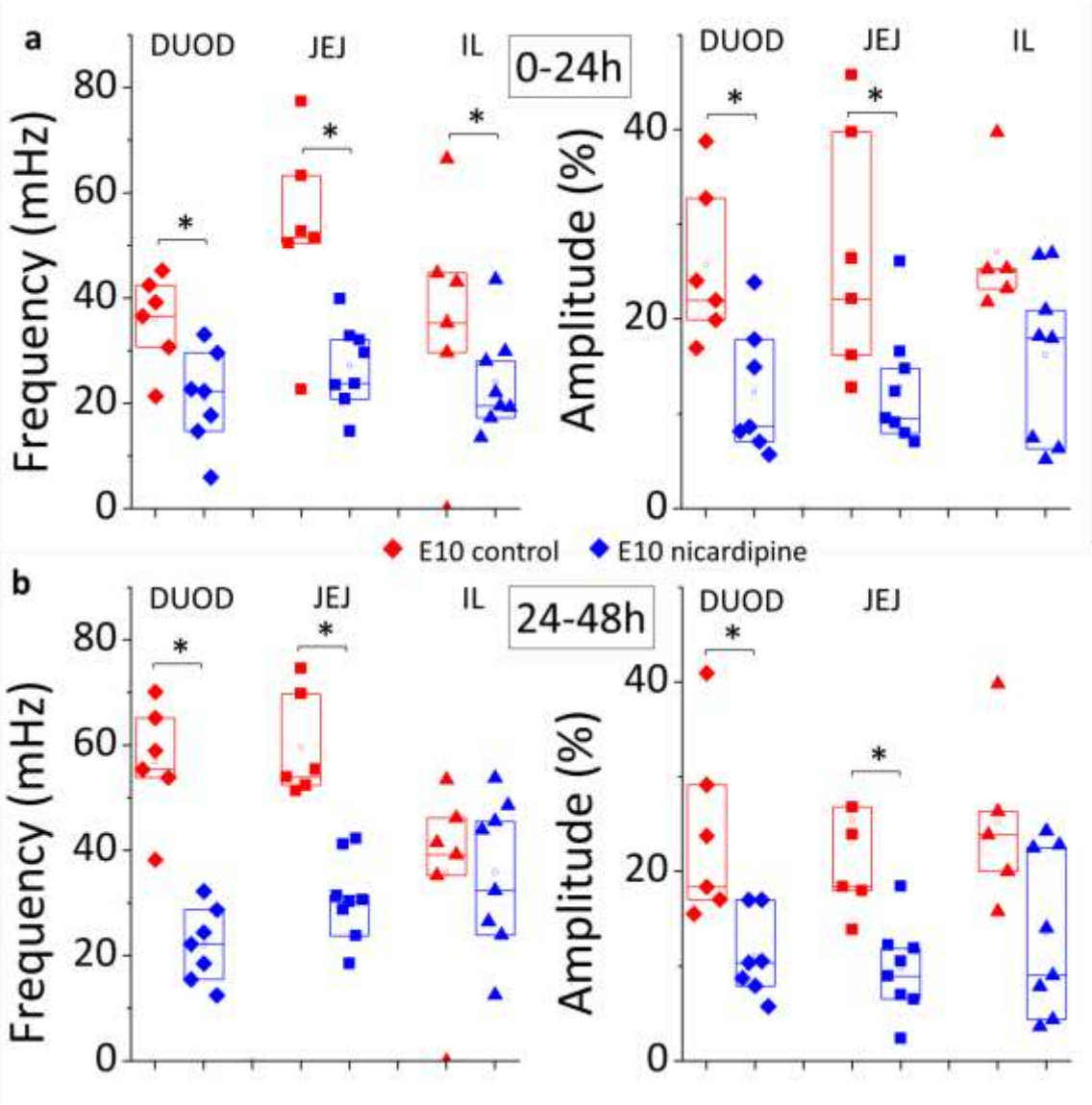

Figure S4. Nicardipine 5 µM reduces the amplitude and frequency of spontaneous contractions during 48 h culture. (a,b) Frequency and amplitude of contractions of control (red) and nicardipine (5 µM) treated guts (blue) during the first (a) and the second (b) day of culture. Each data point is a different gut, and is the mean of data sampled at 2, 8 and 22 h after application (or renewal at 24 h) of the medium with vehicle or drug. DUOD: duodenum, JEJ: jejunum, IL: ileum. *p < 0.05, Mann Whitney two-tailed test.

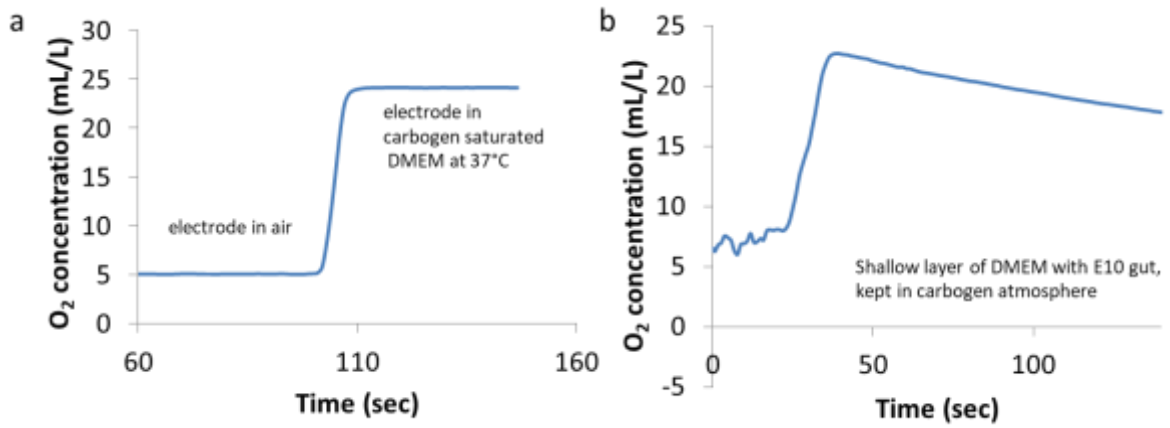

Figure S5. Clark-type electrode measurements of O_2 in (a) 40 mL DMEM at 37°C bubbled with carbogen, used for gut growth in Fig.3. (b) Shallow layer of DMEM (1 mL in 35 mm Petri dish) with E10 gut sample as used in Fig.4, after 4h culture. The electrode was quickly inserted in the medium, ~1 cm away from the gut. The oxygen concentration relaxes to lower values because the measurement is performed in air and the sample gradually equilibrates with atmospheric O_2. The maximum of the curve was measured.

Supplementary Videos

VideoS1: Compared peristaltic activity of E7 and E10 guts.

Video S2: Effect of calcium channel blocker $CoCl_2$ on E7 and E10 gut morphology and peristaltic activity.

Video S3: Pressurization of E7 and E10 guts.

VideoS4: Peristalsis of E10 guts in culture system with injected O_2, after 48h culture.

VideoS5: Time-lapse of E10 guts elongating in culture system with injected O_2, first 6 hours of culture.