Romer et al., http://www.jcb.org/cgi/content/full/jcb.201301005/DC1

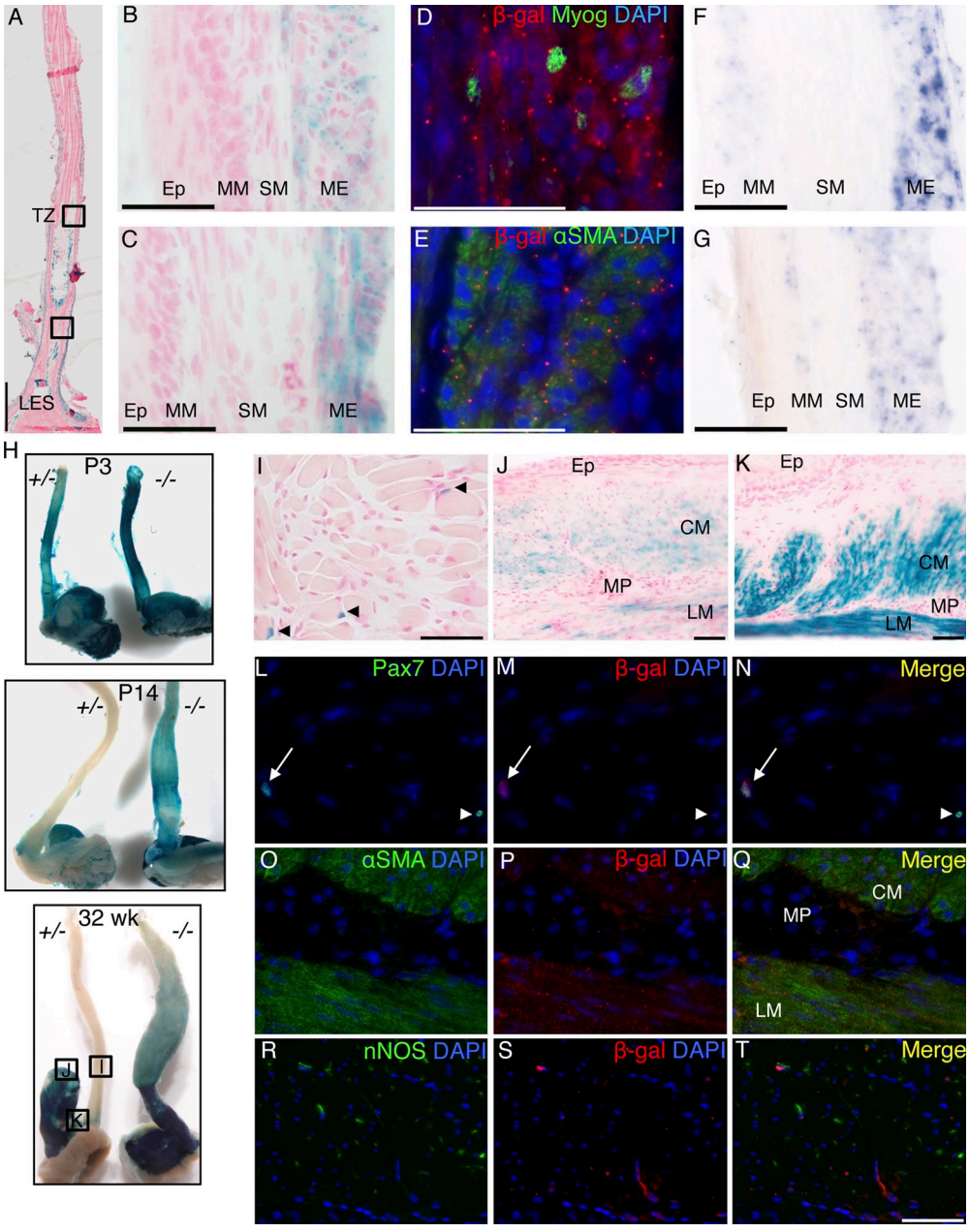


Figure S1. **Expression of *Cdo* in the postnatal esophagus.** (A) Longitudinal section of a P7 $Cdo^{+/-}$ esophagus stained for Cdo^{lacZ} (β-gal) reporter activity (blue) and with nuclear fast red. The top box denotes the transition zone (TZ) and is shown at higher magnification in B, the bottom box denotes the distal ME and is shown at higher magnification in C. Bar, 1 mm. (B and C) β-Gal is expressed in the TZ of the ME and the distal ME, but not in the epithelium (Ep) and only in rare puncta in the muscularis muscosa (MM) and submucosa (SM). (D) IFA of the TZ. β-Gal (diffuse cytoplasmic and punctate staining) is coexpressed with myogenin (nuclear) in differentiating skeletal myoblasts. (E) IFA of the distal ME. β-Gal is coexpressed with α-SMA in SMCs. (F and G) *Cdo* expression in the P7 esophagus was analyzed by thin-section mRNA in situ hybridization with an antisense riboprobe. Expression is similar to that of the *lacZ* reporter and is found in the TZ (F) and in the smooth muscle layers (G) of the distal esophagus. (H) *Cdo* expression in P3, P14, and 32-wk $Cdo^{+/-}$ and $Cdo^{-/-}$ esophagi was assessed by whole-mount β-gal activity. Boxed regions correspond to similar areas shown in indicated sections. (I) A section through the proximal esophagus stained for β-gal activity shows *Cdo* expression by satellite cells (arrowheads). (J) A section through the EGJ shows *Cdo* expression by both circular and longitudinal smooth muscle layers (CM and LM, respectively) but not the Ep or myenteric plexus (MP). (K) A section through the anterior fundus of the stomach shows strong *Cdo* expression by smooth muscle layers. (L–T) IFA of adult esophageal sections. (I–K) Satellite cells in the proximal esophagus are marked by Pax7; ∼60% of satellite cells coexpress β-gal (arrow), ∼40% do not (arrowhead). (O–Q) SMCs in the LES coexpress α-SMA and β-gal. (R–T) $nNOS^+$ myenteric inhibitory neurons do not express β-gal. Bars: (A) 1 mm; (B–G, I–T) 50 µm. Note that, as seen by others, β-gal activity was found in both a diffuse pattern and in puncta; this is often seen with relatively low levels of expression (Gerety et al., 1999; Wang et al., 2001; Rico et al., 2002).

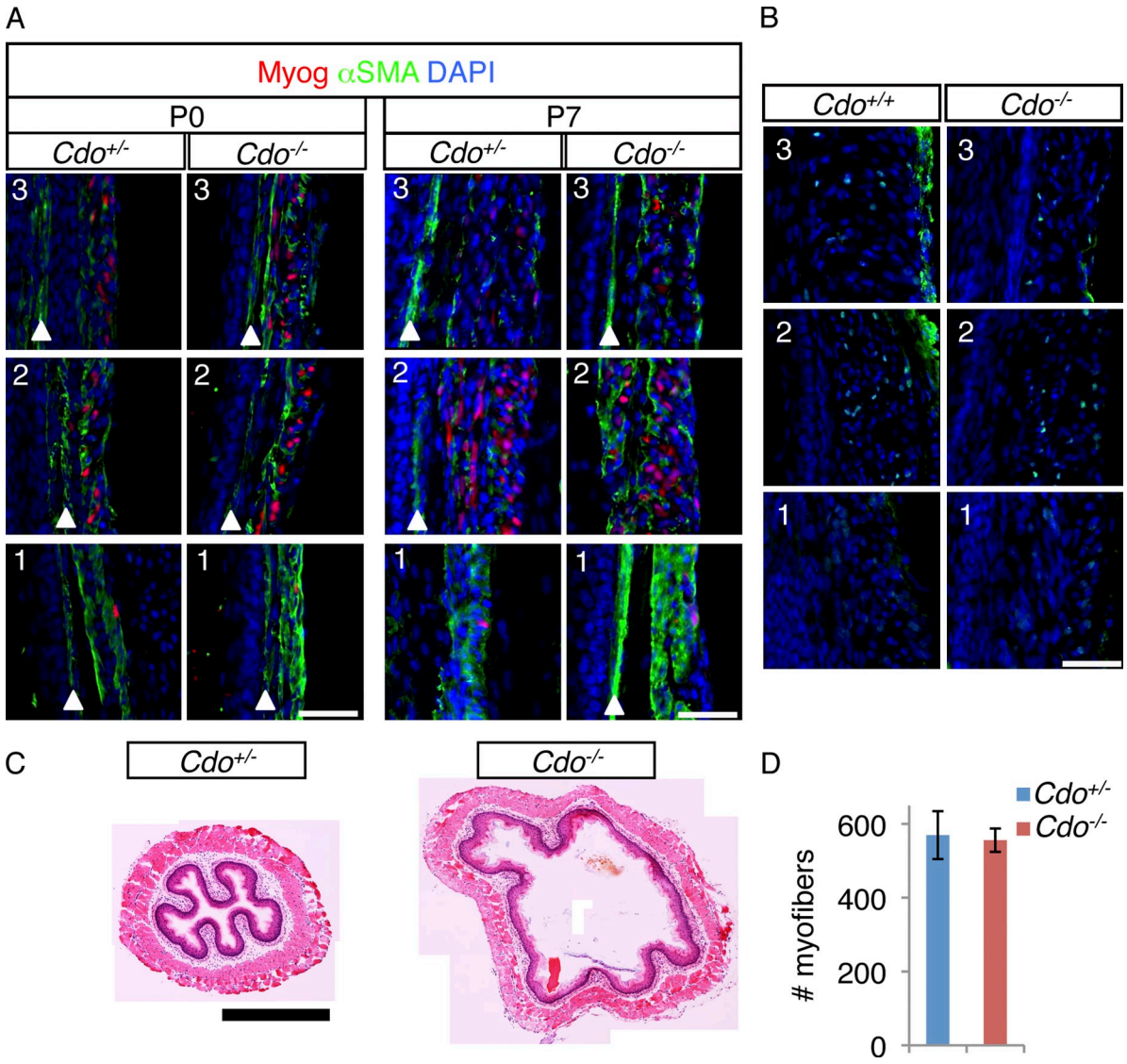


Figure S2. ***Cdo*$^{-/-}$ esophagi have normal numbers of myogenin^{+} cells, Pax7^{+} cells, and skeletal myofibers.** (A) Longitudinal sections of P0 and P7 *Cdo*$^{+/-}$ and *Cdo*$^{-/-}$ esophagi stained with antibodies to myogenin and α-SMA. (B) Longitudinal sections of P7 *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ esophagi were stained with antibodies to Pax7 (green) and with DAPI. The numbers of each panel (1, 2, 3) correspond to the equivalently numbered boxes/panels in Fig. 2. The distalmost myogenin^{+} and Pax7^{+} cells were embedded in smooth muscle (1); the majority of such cells were in the TZ mixed with dispersed SMCs (2); and the numbers of such cells diminished proximally (3). The arrowheads in A indicate the α-SMA^{+} muscularis mucosa. Bars, 50 µm. (C) Cross sections of the proximal region of adult *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ esophagi stained with H&E. Bar, 0.5 mm. (D) Quantification of skeletal myofibers in the ME of sections as in C. Values are means ± SD, *n* = 3.

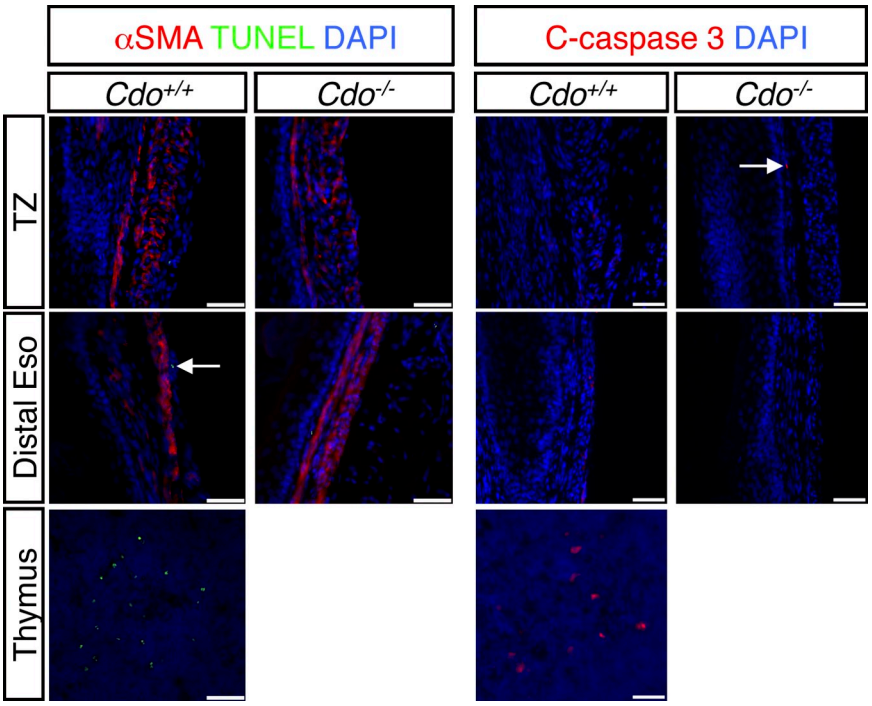


Figure S3. **Lack of apoptosis in the maturing esophageal ME.** Longitudinal sections of P7 *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ esophagi were stained for TUNEL^{+} cells, with antibodies to α-SMA and with DAPI (two left columns), or with antibodies to cleaved caspase 3 (C-caspase 3) and with DAPI (two right columns). The esophageal transition zone (TZ) and distal esophagus (Eso) were analyzed and only rare apoptotic cells outside the ME (arrows) were observed. *Cdo*$^{+/+}$ thymuses were used as a positive control and display easily detectable apoptotic cells by both TUNEL and immunofluorescence for cleaved caspase 3. Bar, 50 µm. $n = 3$.

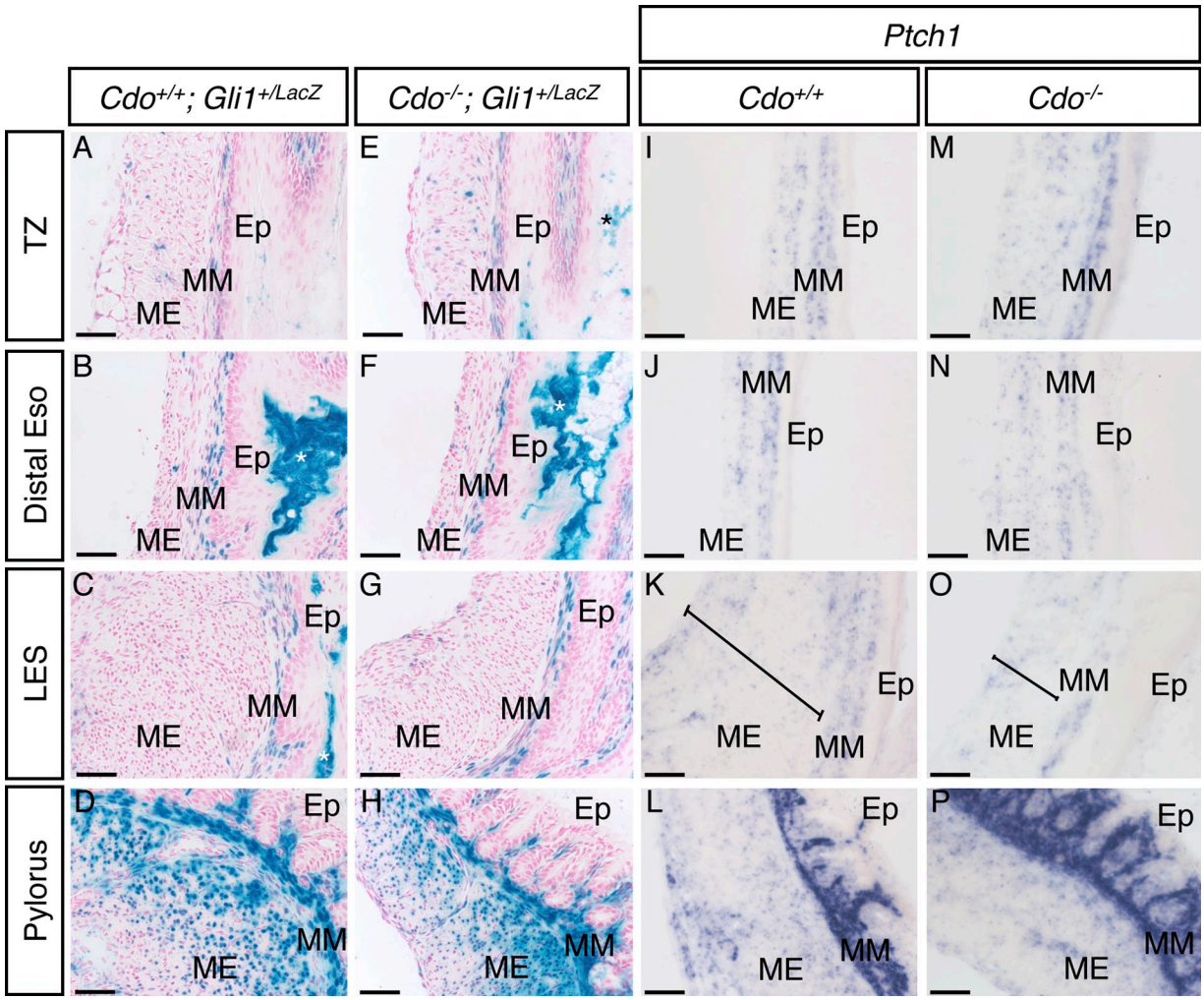


Figure S4. **Expression of Hh pathway target genes in P7 *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ esophagi.** (A–H) Longitudinal sections of P7 *Cdo*$^{+/+}$;*Gli1*$^{+/lacZ}$ and *Cdo*$^{-/-}$;*Gli1*$^{+/lacZ}$ esophagi were stained for β-gal reporter activity (blue) and with nuclear fast red. (I–P) Longitudinal sections of P7 *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ esophagi were assessed for *Ptch1* expression by in situ hybridization. The esophageal transition zone (TZ), distal esophagus (Eso), lower esophageal sphincter (LES), and pylorus were analyzed. The lines in K and O denote the width of the LES. E, epithelium; ME, muscularis externa; MM, muscularis mucosa. Asterisks in B, C, E, and F denote nonspecific staining of ingesta in the lumen. Bars, 50 µm. *n* = 4.

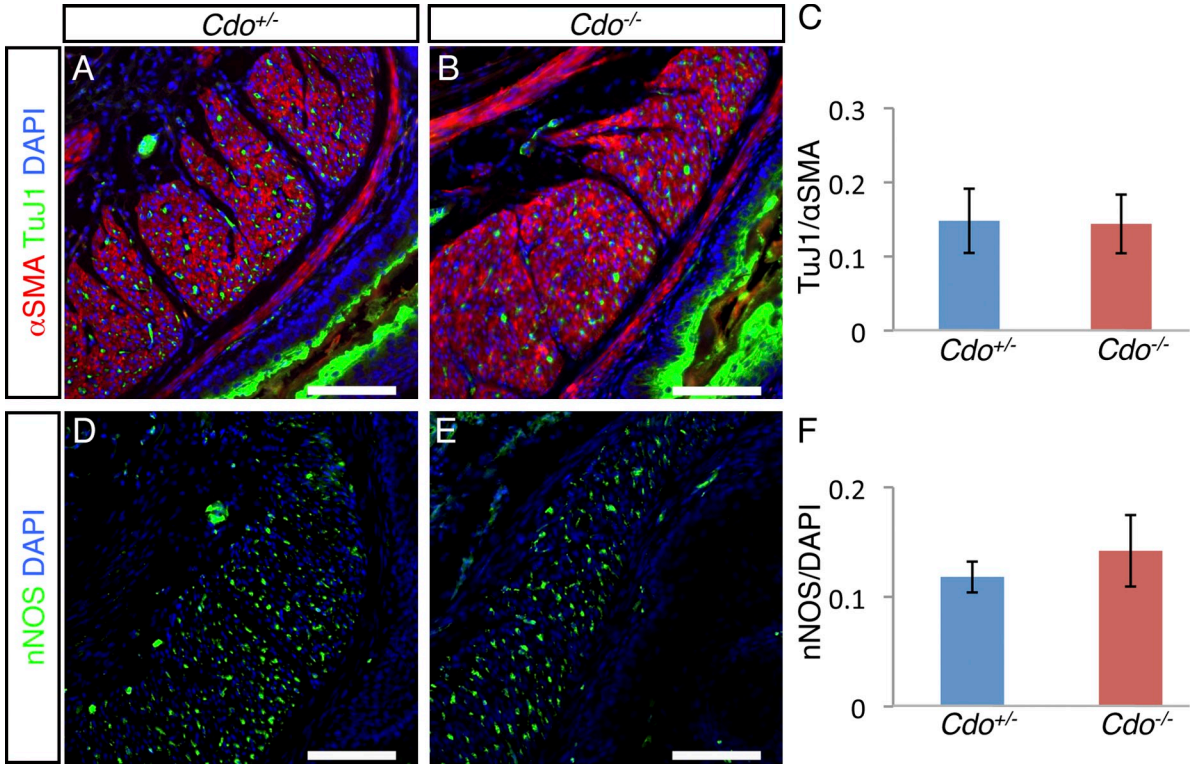
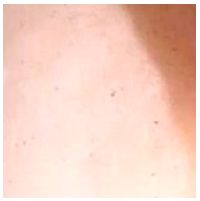
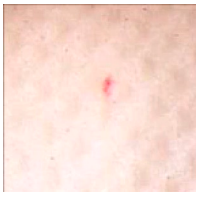


Figure S5. **Normal density of myenteric neurons in the *Cdo*$^{-/-}$ LES.** (A and B) Longitudinal sections through the LES of P14 *Cdo*$^{+/-}$ and *Cdo*$^{-/-}$ mice were stained with antibodies to β-tubulin III (TuJ1) to label neurons and to α-SMA to label SMCs, and with DAPI. (C) Quantification of the relative area of TuJ1 to α-SMA staining. (D and E) Longitudinal sections through the LES of P14 *Cdo*$^{+/-}$ and *Cdo*$^{-/-}$ mice were stained with antibodies to nNOS to label inhibitory intramural neurons and with DAPI. (F) Quantification of the relative area of nNOS to DAPI staining in the ME of the LES. Bars, 100 µm. Values in C and F are means ± SD, $n = 4$.



Video 1. **Peristaltic contractions by a *Cdo*$^{+/+}$ esophagus.** An esophagus from a *Cdo*$^{+/+}$ mouse was filmed immediately after dissection with a digital camera (model DMC-TZ4, Panasonic; 30 frames per second) and displays normal morphology and rhythmic contractions.



Video 2. **Peristaltic contractions by a *Cdo*$^{-/-}$ esophagus.** An esophagus from a *Cdo*$^{-/-}$ mouse was filmed immediately after dissection with a digital camera (model DMC-TZ4, Panasonic; 30 frames per second) and is enlarged relative to the *Cdo*$^{+/+}$ esophagus, engorged with ingesta, and displays strong, arrhythmic contractions.

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