Cdo patterns the musculature of the esophagus and is required for esophageal motility in mice

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Introduction:

Cdo is a multifunctional cell surface co-receptor that promotes Hedgehog signaling during rostroventral midline development and cadherin-mediated signaling during skeletal myogenesis. We report here novel roles for Cdo in patterning of the murine esophageal musculature and esophageal motility disorders such as achalasia.

Conclusions:

1) Cdo is required for a process of smooth muscle fascicular morphogenesis that drives formation of the mature pattern of the esophageal musculature.

2) Cdo is required to sensitize tonic smooth muscle in the LES to NO-induced relaxation; its absence results in achalasia.

Cdo$^{-/-}$ mice have megesophagus with a misspatterned ME

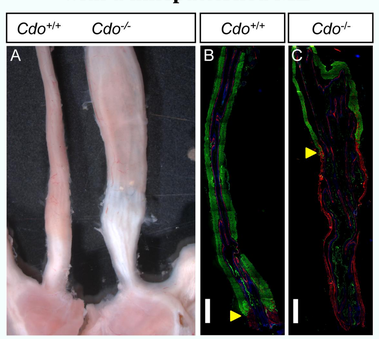

Fig. 1 **(A)** Adult *Cdo*$^{-/-}$ mice display megaesophagus. **(B-C)** Longitudinal sections of adult esophagi were stained with antibodies to αSMA (red) and SA (green) to reveal smooth and skeletal muscles, respectively. The *Cdo*$^{-/-}$ esophagus shows an aberrantly proximally located skeletal-to-smooth muscle boundry, relative to the *Cdo*$^{+/+}$ esophagus (arrowheads).

Expression of *Cdo* in the postnatal esophagus

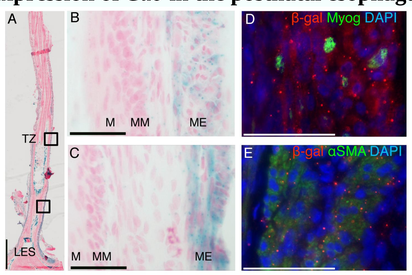

Fig. 2 **(A)** Longitudinal sections of P7 *Cdo*$^{lacZ/+}$ esophagi were stained for β-gal reporter activity (blue) and with nuclear fast red. **(B)** The transition zone (TZ). **(C)** The distal esophagus with smooth muscle in ME. **(D)** Immunofluorescence (IF) analysis of the TZ. βgal is coexpressed with myogenin (Myog) in differentiating skeletal myoblasts. **(E)** IF analysis of the distal ME. β-gal is coexpressed with αSMA in smooth muscle cells.

Defects in *Cdo*$^{-/-}$ esophagi occur postnatally

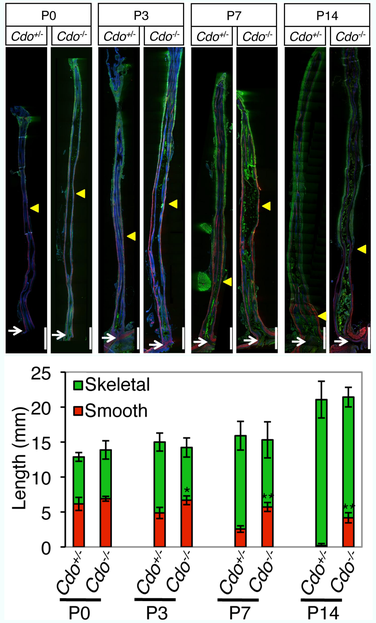

Fig. 3 Longitudinal sections of esophagi were stained as in Fig.1. The distal-most SA^{+} cell is denoted with an arrowhead; the LES is denoted by an arrow. The distance between the distal-most SA^{+} cell and the LES was measured and is represented by the red portion of the histogram bars. Note that the distance decreases progressively with age in *Cdo*$^{+/+}$ esophagi, but this fails to occur normally in *Cdo*$^{-/-}$ esophagi.

Skeletal myogenesis occurs in a transition zone in the postnatal esophagus

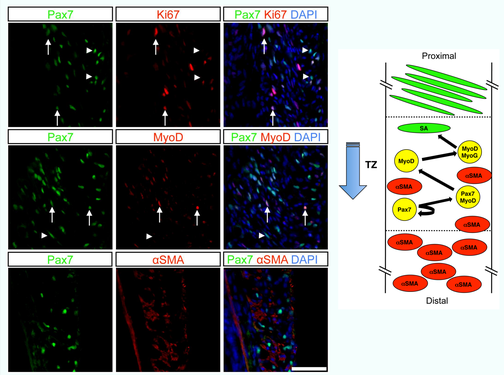

Fig. 4 Transition zone of P7 *Cdo*$^{+/+}$ esophagi stained by IF for Pax7 and to Ki67, MyoD or αSMA and with DAPI. Many Pax7^{+} cells co-expressed the proliferation marker Ki67, or the skeletal muscle determination marker MyoD but Pax7^{+} cells did not express αSMA.

Failure of skeletal myoblasts to move distally in *Cdo*$^{-/-}$ esophagus

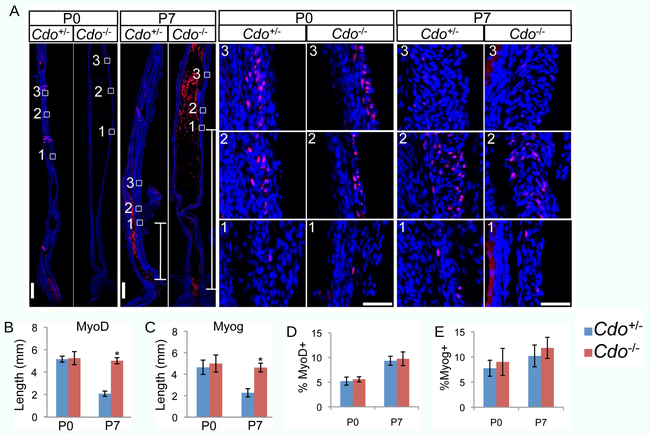

Fig. 5 **(A)** Longitudinal sections of esophagi stained for MyoD by IF (red). The boxed areas correspond to the distal most MyoD^{+} cell (Box 1), the middle of the transition zone (Box 2), and a more proximal region where, by P7, MyoD^{+} cell numbers diminish (Box 3) **(B-C)** Quantification of the distance between the distal-most MyoD^{+} **(B)** or Myog^{+} **(C)** cell and the LES. **(D-E)** Quantification of MyoD^{+} **(D)** and Myog^{+} **(E)** cells within the transition zone, measured as a percent of total DAPI^{+} cells in the ME.

Cdo is required for esophageal smooth muscle fascicles to alter their shape and orientation

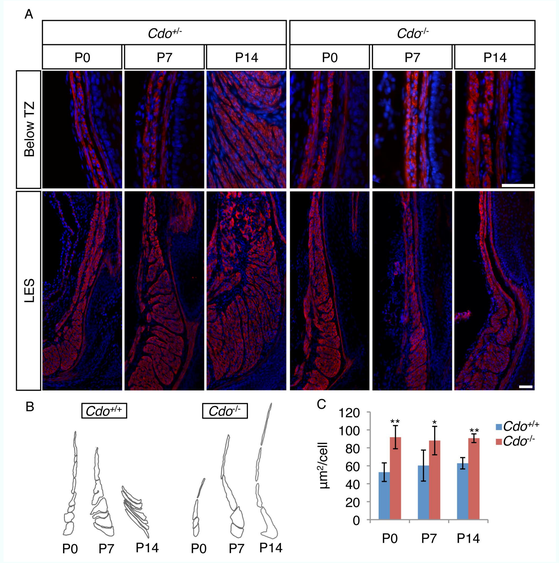

Fig. 7 **(A)** Longitudinal sections of esophagi stained by IF for αSMA (red) and DAPI. Between P0 and P14, *Cdo*$^{+/+}$ smooth muscle fascicles changed their shape and orientation in a distal-to-proximal manner; this process failed to occur properly in *Cdo*$^{-/-}$ esophagi. **(B)** Tracings of smooth muscle fascicles just proximal to the LES in P0, P7 and P14 **(C)** Quantification of the average smooth muscle cell area as assessed by morphometric analysis.

Adult *Cdo*$^{-/-}$ esophagi have mispatterned smooth muscle

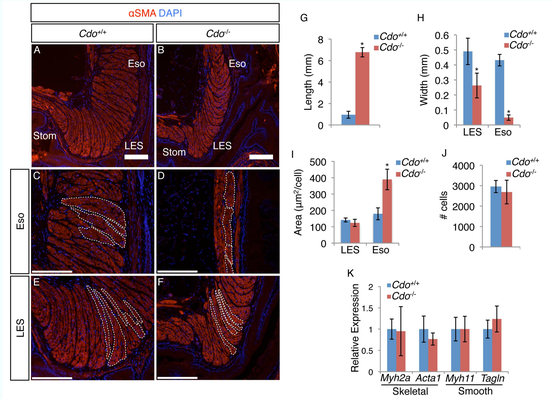

Fig. 6. (A-F) Longitudinal sections of the LES and esophageal body (Eso) stained by IF for αSMA (red) and DAPI. **(C-F)** Individual fascicles are outlined by the white dotted lines. **(G)** Length of smooth muscle segment in distal esophagus. **(H)** Width of the smooth muscle in ME at the LES and in the Eso. **(I)** Average area of smooth muscle cell in the LES and Eso as assessed by morphometric analysis. **(J)** Quantification of the total number of smooth muscle cells in the distal smooth muscle segment of the esophagus from *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ mice calculated from smooth muscle cell densities and total area measurements. **(K)** qRT-PCR analysis of expression levels of skeletal muscle-specific (*Myh2a* and *Acta1*) and smooth muscle-specific (*Myh11* and *Tagln*) genes as muscle markers normalized to expression of 36B4.

Normal density of myenteric neurons in the *Cdo*$^{-/-}$ LES

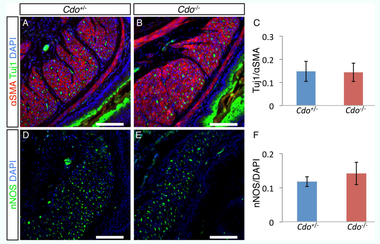

Fig. 8. **(A-B)** P14 LES stained by IF for β-tubulin III (TuJ1) to label neurons and to αSMA to label smooth muscle cells. **(C)** Quantification of the relative area of TuJ1 to αSMA staining. **(D-E)** P14 LES stained by IF for nNOS to label inhibitory intramural neurons. **(F)** Quantification of the relative area of nNOS to DAPI staining in the ME of the LES.

The *Cdo*$^{-/-}$ LES fails to relax in response to EFS or nitroprusside

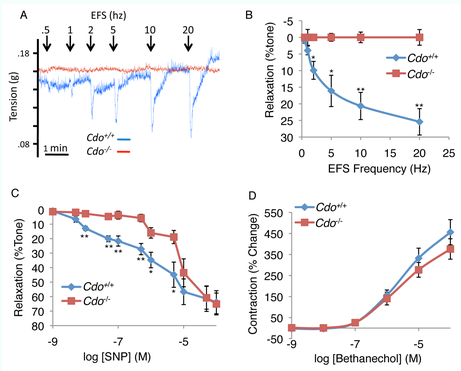

Fig. 9. **(A)** Tension recordings of dissected strips of the LES measured in an organ bath under NANC conditions in response to electric field stimulations (EFS). **(B)** Quantification of EFS frequency-dependent relaxation of LES preparations **(C-D)** Dose response curves of LES to sodium nitroprusside (SNP) **(C)** and to bethanechol **(D)**.