

Expanded View Figures

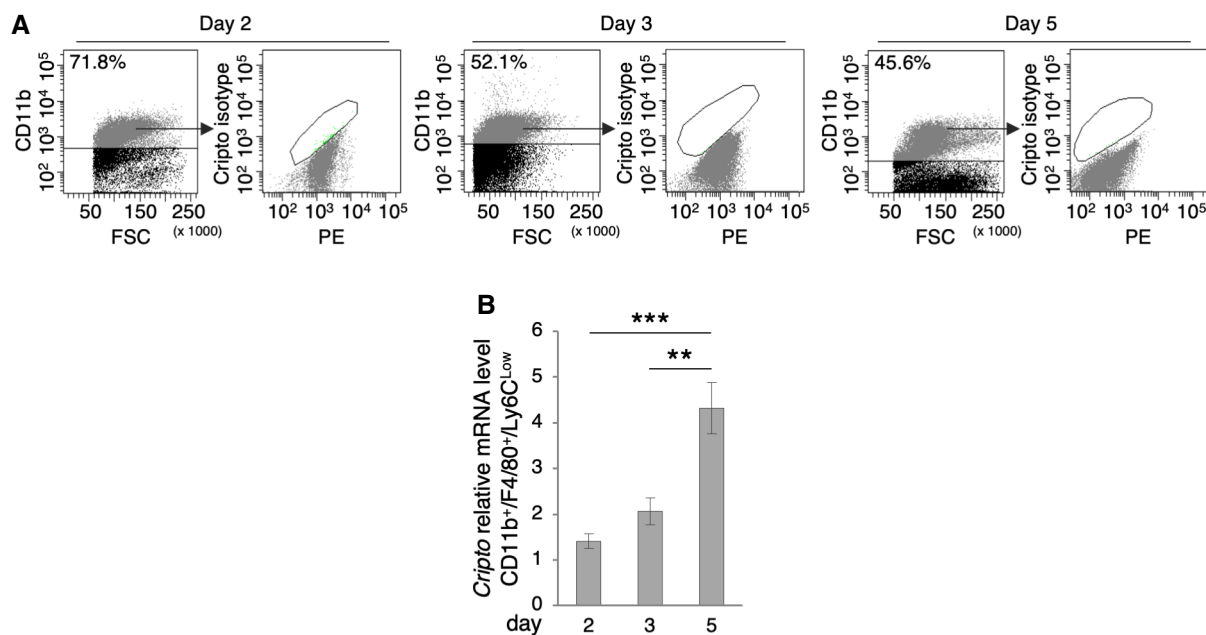


Figure EV1. Cripto isotype control and Cripto expression by qRT-PCR.

A Representative flow cytometry dot plots of Cripto isotype control in CD11b⁺ cells at days 2, 3, and 5 after injury. PE (Phycoerythrin/free channel).
 B Quantitative real-time PCR analysis of Cripto expression profile in CD11b⁺/Ly6C^{Low} MPs at days 2, 3, and 5 after injury. Relative mRNA levels were normalized to *Gapdh*. Data are mean $\pm$ SEM ($n = 6$ biological replicates; ** $P < 0.01$; *** $P < 0.001$, Student's t -test).

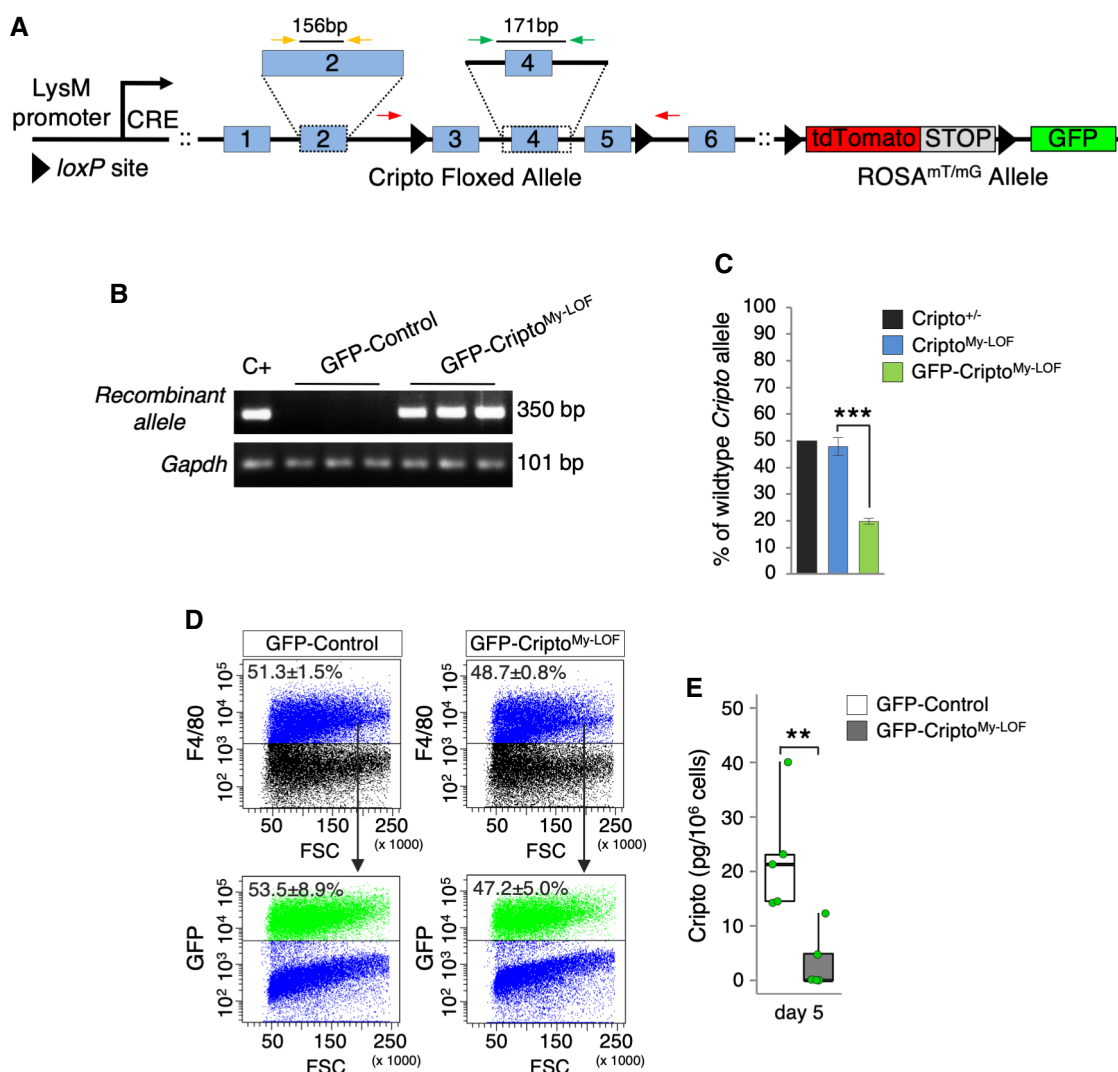


Figure EV2. Efficiency of Cripto deletion.

- A** Schematic representation of *Tg:LysMCre::R26^{mTmG}::Cripto^{fl/fl}* (GFP-*Cripto*^{My-LOF}) transgenic mice. Red arrows indicate forward and reverse primers used for PCR genotyping. Green arrows indicate forward and reverse primers used to amplify a DNA region encompassing exon 4. Yellow arrows indicate forward and reverse primers used to amplify exon 2, as reference PCR.
- B** PCR genotyping on DNA extracted from FACS-isolated GFP⁺ cells from GFP-*Cripto*^{My-LOF} and GFP-Control mice at day 2 after injury. *Gapdh* was used as control.
- C** Genomic quantitative real-time PCR (qRT-PCR) analysis of wild-type *Cripto* allele on FACS-isolated F4/80⁺ (blue bar) or GFP⁺ (green bar) MPs from *Cripto*^{My-LOF} and GFP-*Cripto*^{My-LOF} muscles, respectively, at day 2 after injury. Genomic DNA extracted from *Cripto* KO heterozygous mice (*Cripto*^{+/-}; black bar) was used as reference of 50% *Cripto* deletion. Data are expressed as percentage of wild-type allele over the reference PCR and are mean ± SEM ($n = 6$ biological replicates; *** $P < 0.000004$; Student's *t*-test).
- D** Representative flow cytometry dot plots showing the percentage of F4/80⁺ cell population and GFP⁺ cells expressing F4/80 in GFP-Control and GFP-*Cripto*^{My-LOF} muscles at day 2 after injury. Data are mean ± SEM ($n = 4$ biological replicates; $P = \text{ns}$; Student's *t*-test).
- E** ELISA-based assay of Cripto protein from total protein extracts of FACS-sorted GFP⁺ MPs from GFP-Control and GFP-*Cripto*^{My-LOF} muscles at day 5 after injury. Data are expressed as box plots displaying minimum, first quartile, median, third quartile, and maximum ($n = 5$ biological replicates; ** $P < 0.01$; Student's *t*-test).

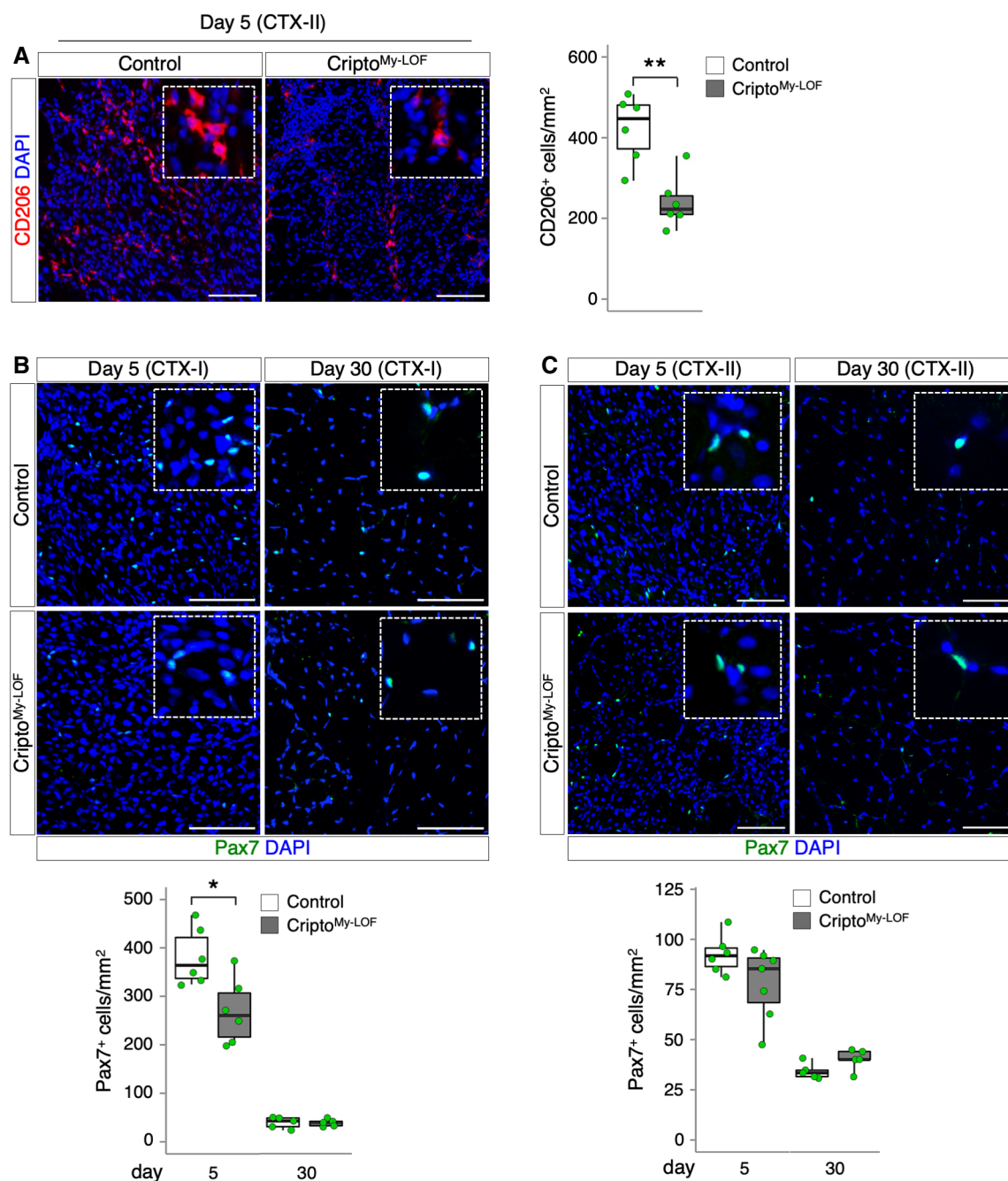


Figure EV3. Effect of Cripto^{My-LOF} on CD206⁺ MPs and Pax7⁺ satellite cells in acute injury.

- A Representative pictures of CD206 (red) immunostaining of Cripto^{My-LOF} and Control TA sections at day 5 after re-injury (left panel) and quantification of CD206⁺ cells per area (mm², right panel). Data are expressed as box plots displaying minimum, first quartile, median, third quartile, and maximum ($n = 6$ biological replicates; $**P < 0.01$; Student's t -test).
- B Representative pictures of Pax7 (green) immunostaining of Cripto^{My-LOF} and Control TA sections at days 5 (left panel) and 30 (right panel) after injury (CTX-I) and quantification of Pax7⁺ cell number per area (mm²). Data are expressed as box plots displaying minimum, first quartile, median, third quartile, and maximum ($n \geq 5$ biological replicates; $*P < 0.05$; Student's t -test).
- C Representative pictures of Pax7 (green) immunostaining of Cripto^{My-LOF} and Control TA sections at days 5 (left panel) and 30 (right panel) after re-injury (CTX-II) and quantification of Pax7⁺ cell number per area (mm²). Data are expressed as box plots displaying minimum, first quartile, median, third quartile, and maximum ($n \geq 5$ biological replicates; $P = \text{ns}$; Student's t -test). Data information: Nuclei were counterstained with DAPI (blue). Scale bar: 100 μm . Magnification of the boxes is 3.5 $\times$.

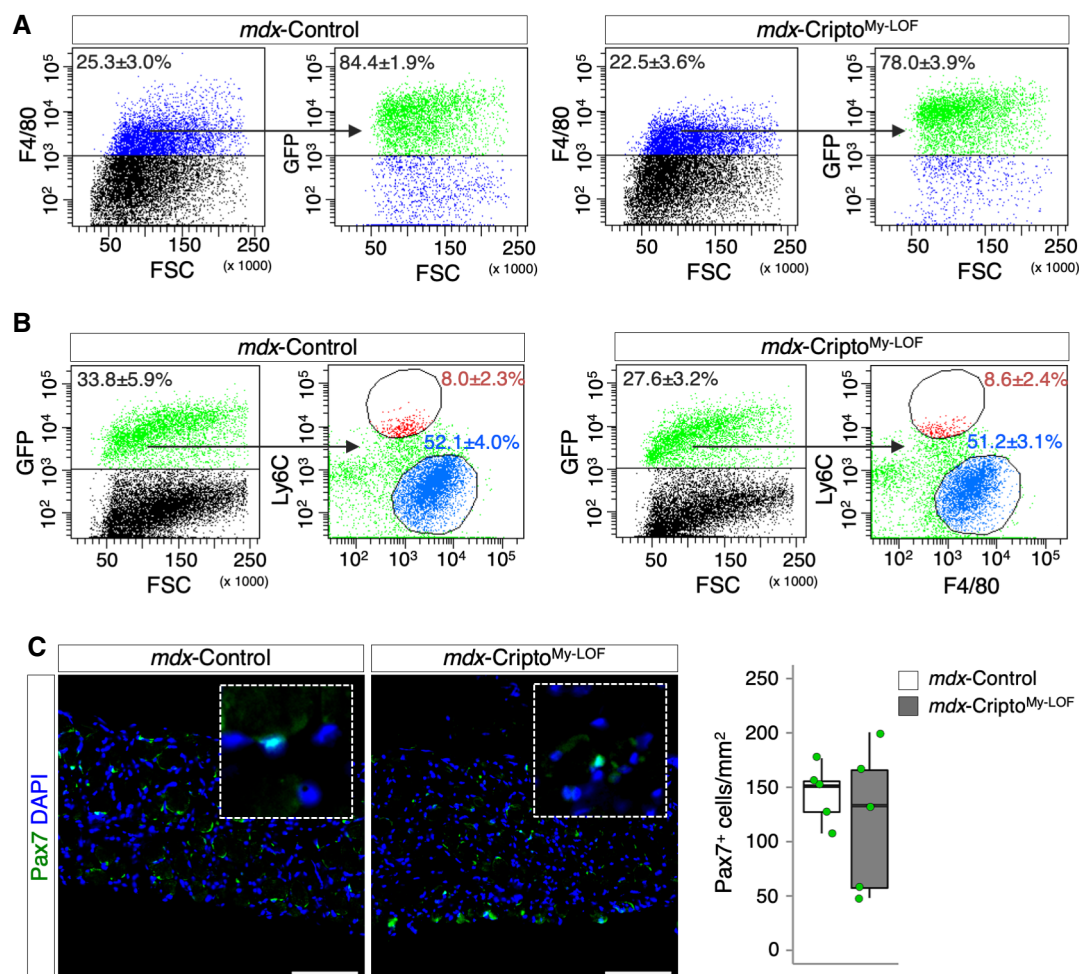


Figure EV4. Cripto^{My-LOF} does not affect Ly6C^{high/Low} distribution and satellite cells number in mdx mice.

A Representative FACS scatter plots showing the percentage of GFP⁺ gated on the F4/80⁺ cell population in *mdx-Control* and *mdx-Cripto^{My-LOF}* muscles. Data are mean ± SEM [*n* = 3 biological replicates (*mdx-Control*) and *n* = 5 biological replicates (*mdx-Cripto^{My-LOF}*); *P* = ns; Student's *t*-test].

B Representative flow cytometry dot plots showing GFP⁺ and GFP⁺/F4/80⁺/Ly6C^{Low/High} cell populations in *mdx-Control* and *mdx-Cripto^{My-LOF}* muscles. Data are mean ± SEM [*n* = 3 (*mdx-Control*) and *n* = 5 biological replicates (*mdx-Cripto^{My-LOF}*); *P* = ns; Student's *t*-test].

C Representative pictures of Pax7 (green) immunostaining of *mdx-Cripto^{My-LOF}* and *mdx-Control* diaphragm sections (left panels) and quantification of Pax7⁺ cell number per area (mm², right panels). Nuclei were counterstained with DAPI (blue). Scale bar: 100 μm. Magnification of the boxes is 3.5×. Data are expressed as box plots displaying minimum, first quartile, median, third quartile, and maximum (*n* = 5 biological replicates; *P* = ns; Student's *t*-test).

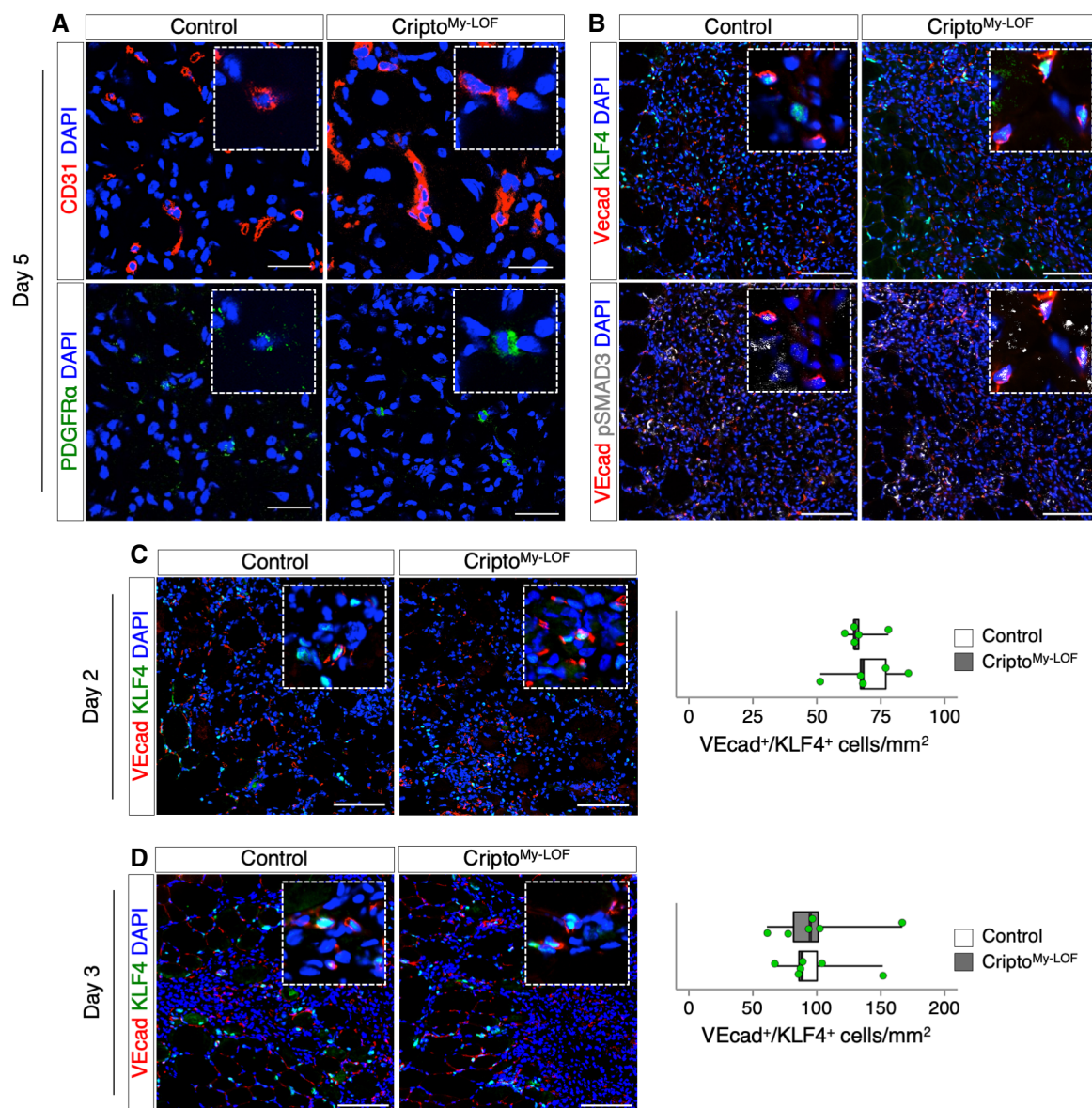


Figure EV5. Cripto^{My-LOF} affects endothelial plasticity in injured skeletal muscle.

- A Representative confocal pictures of double immunostaining with CD31 (red) and PDGFRα (green) on Control and Cripto^{My-LOF} TA sections at day 5 after injury. Split green and red channels of Fig 7E are shown.
- B Representative pictures of triple immunostaining with VEcad (red), KLF4 (green), and pSMAD3 (white) on Control and Cripto^{My-LOF} TA sections at day 5 after injury. Merged channels of VEcad/KLF4 (top panels) and VEcad/pSMAD3 of Fig 7H are shown.
- C, D Representative pictures of double immunostaining with VEcad (red) and KLF4 (green) on Control and Cripto^{My-LOF} TA sections at day 2 (C, left panels) and day 3 (D, left panels) after injury and quantification of VEcad/KLF4 double-positive cells per area (mm²) at day 2 (C, right panel) and day 3 (D, right panel).

Data information: Nuclei were counterstained with DAPI (blue). Scale bar: 100 μm. Confocal pictures (A) scale bar: 25 μm. Magnification of the boxes is 3.5×. Data are expressed as box plots displaying minimum, first quartile, median, third quartile, and maximum ($n \geq 5$ biological replicates; $P = \text{ns}$; Student's t -test).