

Appendix

Cripto shapes macrophage plasticity and restricts EndMT in injured and diseased skeletal muscle

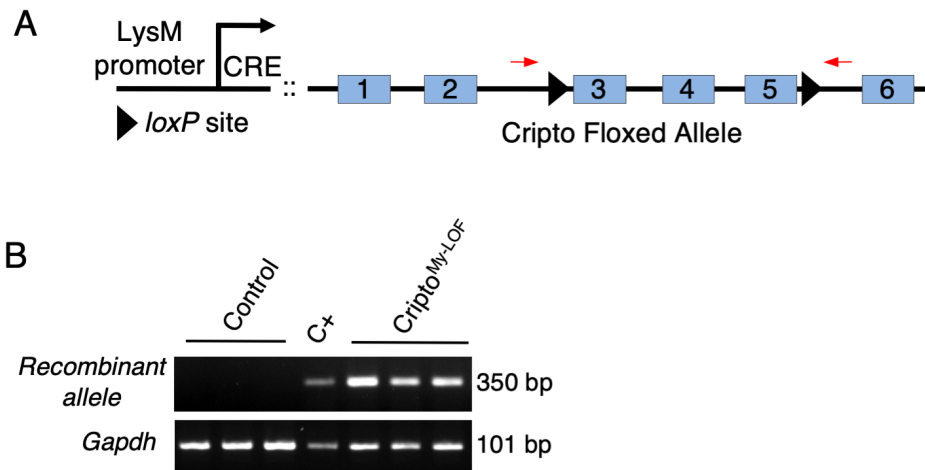
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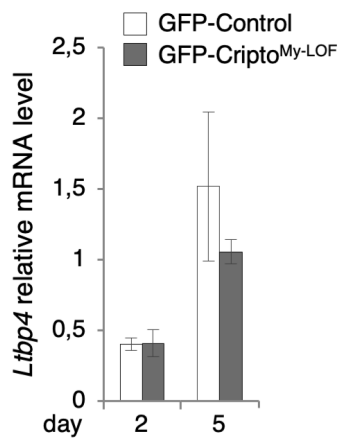
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Appendix Figure S1.

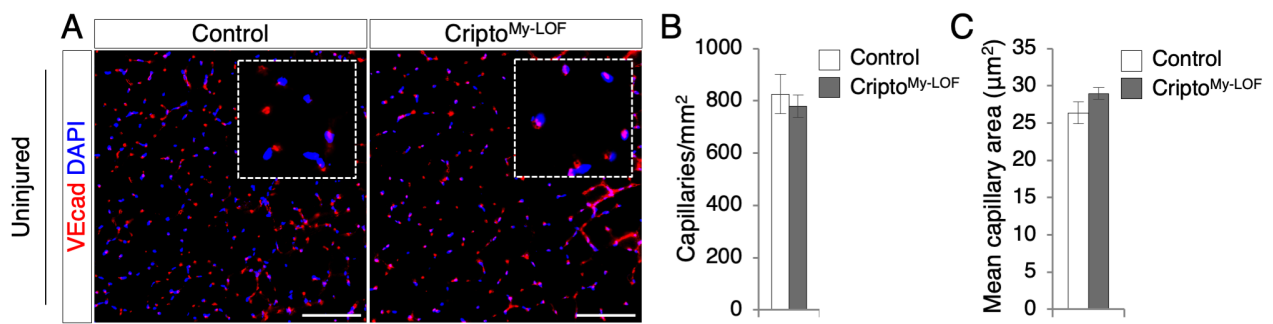
A. Schematic representation of *Tg:LysMCre::Cripto^{fl/fl}* (*Cripto^{My-LOF}*) transgenic mice. Cre is under the control of the Lysozyme M (LysM) promoter and induces the tissue-specific excision of exon 3-5 in *Cripto* floxed allele. Red arrows indicate forward and reverse primers for PCR genotyping.

B. PCR genotyping on DNA extracted from CD11b⁺ MPs FACS-isolated from *Cripto^{My-LOF}* and Control at day 2 after injury. *Gapdh* was used as control.



Appendix Figure S2.

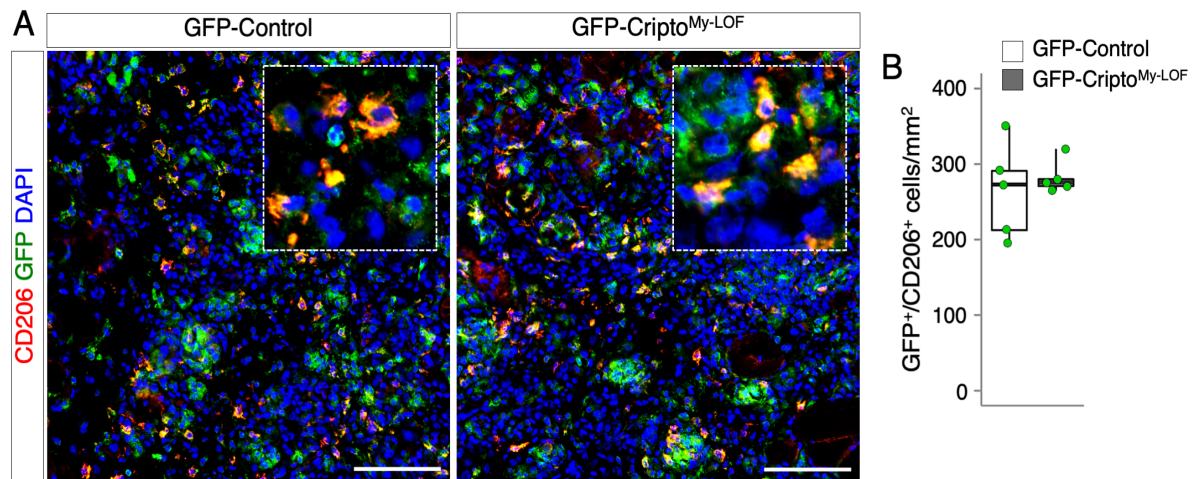
QRT-PCR analysis of *Ltbp4* in FACS-isolated GFP+ MPs from GFP-Cripto^{My-LOF} and GFP-Control muscles at day 2 and 5 after injury. Data are mean $\pm$ SEM of relative mRNA level normalized with Gapdh (n=4 biological replicates; P=ns; Student t-test).



Appendix Figure S3.

A. Representative pictures of VEcad (red) immunostaining of Cripto^{My-LOF} and control TA sections in resting conditions. Nuclei were counterstained with DAPI (blue). Scale bar is 100 μm. Magnification of the boxes is 3.5 x.

B-C. Quantification of VEcad+ capillary number (B) per area (mm²) and average of capillary cross-sectional area (μm²; C). Data are mean±SEM (n=5 biological replicates; P=ns; Student *t*-test).



Appendix Figure S4.

A. Representative pictures of double immunostaining with CD206 (red) and GFP (green) on GFP-Control and GFP-Cripto^{My-LOF} TA sections at day 3 after injury. Nuclei were counterstained with DAPI (blue). Scale bar is 100 μ m. Magnification of the boxes is 3.5 x.

B. Quantification of GFP/CD206 double positive cells per area (mm^2). Data are expressed as box plots (n=5 biological replicates; P=ns; Student *t*-test).

Appendix Table S1. The list of Primers for genotyping

Allele	Primer 1 (5'-3')	Primer 2 (5'-3')	PCR Amplification (bp)
<i>Cripto</i>	TCTGCACTGGGGCTAAACCTTATG	GCCAAGAGCCATGACAGAGATGG	380
<i>Cripto^{fl/fl}</i>	TCTGCACTGGGGCTAAACCTTATG	GCCAAGAGCCATGACAGAGATGG	580
<i>Cripto^{del}</i>	TCTGCACTGGGGCTAAACCTTATG	CATCTGGGACATGCCCACTA	350
<i>LysM</i>	CTTGGGCTGCCAGAATTTCTC	CCCAGAAATGCCAGATTACG	350
<i>LysMCre</i>	CTTGGGCTGCCAGAATTTCTC	TTACAGTCGGCCAGGCTGAC	700
<i>R26 WT</i>	AAAGTCGCTCTGAGTTGTTAT	GGAGCGGGAGAAATGGATATG	650
<i>R26^{mTmG}</i>	AAAGTCGCTCTGAGTTGTTAT	GTCGTTGGGCGGTCAG	250
<i>Cripto</i> (reference PCR)	TACACTGCTTCATCCCC	TAGCAGCCCTAAGTGTC	156
<i>Cripto</i> (undeleted allele)	CTCCTGATGCCACTTCATGC	GCCCATAAGCAACGGTTCAT	171

Appendix Table S2. The list of Primers used for qRT-PCR expression analysis

Gene	Forward (5'-3')	Reverse (5'-3')
<i>Arg1</i>	AGCTCCAAGCCAAAGTCCTTAGA	CCTCCTCGAGGCTGTCCTT
<i>Cripto</i>	GCATTTGGGACCAGAAAGAA	GCGTCCATAGAAGGAAGGAG
<i>Fizz1</i>	TCCAGCTAACTATCCCTCCACTGT	GGCCCATCTGTTTCATAGTCTTGA
<i>Gapdh</i>	TCTTCTGGGTGGCAGTGATG	TGCACCACCAACTGCTTAGC
<i>Il10</i>	ATTTGAATTCCCTGGGTGAGAAG	CACAGGGGAGAAATCGATGACA
<i>Il4ra</i>	CCTCTGTGGGCTGTCTGATTTT	GGGCTCACCCAGGACCTT
<i>Ltbp4</i>	AATTTCCCCTGCCCAGCATC	ACAAACCTGGGGATTTCGCT
<i>Mcp1</i>	CCCAATGAGTAGGCTGGAGA	GCTAAGACCTTAGGGCAGA
<i>Nos2</i>	AGCCAAGCCCTCACCTACTT	TCTCTGCCTATCCGTCTCGT
<i>Tgfβ</i>	CCCCACTGATACGCCTGAGT	AGCCCTGTATTCCGTCTCCTT
<i>Tnfa</i>	TCCCAGGTTCTCTTCAAGGGA	GGTGAGGAGCACGTAGTCGG

Appendix Table S3. The list of Antibodies and their applications

Antibody (Clone)	Species	Dilution	Company (Catalog #)	Application
CD11b-PerCP (M1/70)	Rat	1:10	R&D System (FAB1124C)	FACS
F4/80-APC (T45-2342)	Rat	1:100	BD (565853)	FACS
Ly6C-PECy7 (AL-21)	Rat	1:200	BD (560593)	FACS
Cripto-488 (237603)	Rat	1:10	R&D System (FAB1538G)	FACS
F4/80 (Cl:A3-1)	Rat	1:25	Bio-Rad (MCA497B)	IF
CD86 (GL1)	Rat	1:50	BD (550542)	IF
CD206 (MR5D3)	Rat	1:200	Bio-Rad (MCA2235)	IF
GFP	Chicken	1:1000	Abcam (ab13970)	IF
Phospho-Smad3 (Ser423/425) (C25A9)	Rabbit	1:100	Cell Signaling (9520)	IF
CD144 (11D4.1)	Rat	1:250	BD (550548)	IF
KLF4	Goat	1:250	R&D System (AF3158)	IF
TCF4 (C48H11)	Rabbit	1:100	Cell Signaling (2569)	IF
CD31 (MEC 13.3)	Rat	1:250	BD (553370)	IF
PDGF Receptor α (D13C6)	Rabbit	1:100	Cell Signaling (5241)	IF
Laminin	Rabbit	1:400	Sigma-Aldrich (L9393)	IF
Embryonic myosin heavy chain	Mouse	1:50	DSHB (F1.652)	IF
Pax7	Mouse	1:20	DSHB	IF
Alexa Fluor 488 Donkey anti-Rat IgG	Donkey	1:500	Invitrogen (A-21208)	IF
Cy3 Donkey Anti-Rat IgG	Donkey	1:500	Jackson IR (712-165-150)	IF
Alexa Fluor 647 Goat anti-Rat IgG	Goat	1:500	Invitrogen (A-21247)	IF
Alexa Fluor 488 Donkey anti-Chicken IgY	Donkey	1:500	Jackson IR (703-545-155)	IF
Alexa Fluor 647 Donkey anti-Rabbit IgG	Donkey	1:500	Invitrogen (A31573)	IF
Alexa Fluor 488 Donkey anti-Rabbit IgG	Donkey	1:500	Invitrogen (A21206)	IF
Alexa Fluor 594 Donkey anti-Rabbit IgG	Donkey	1:500	Invitrogen (A21207)	IF
Alexa Fluor 488 Donkey anti-Goat IgG	Donkey	1:500	Invitrogen (A11055)	IF
Alexa Fluor 594 Donkey anti-Mouse IgG	Donkey	1:500	Invitrogen (A21203)	IF
Biotin-SP Goat anti-Mouse IgG	Goat	1:500	Jackson IR (115-065-205)	IF

