Supplementary material for

Transcriptomic and lipidomic profiling reveals distinct bioactive lipid signatures in slow and fast muscles and highlights the role of Resolvin-D2 in fiber type determination during myogenesis.

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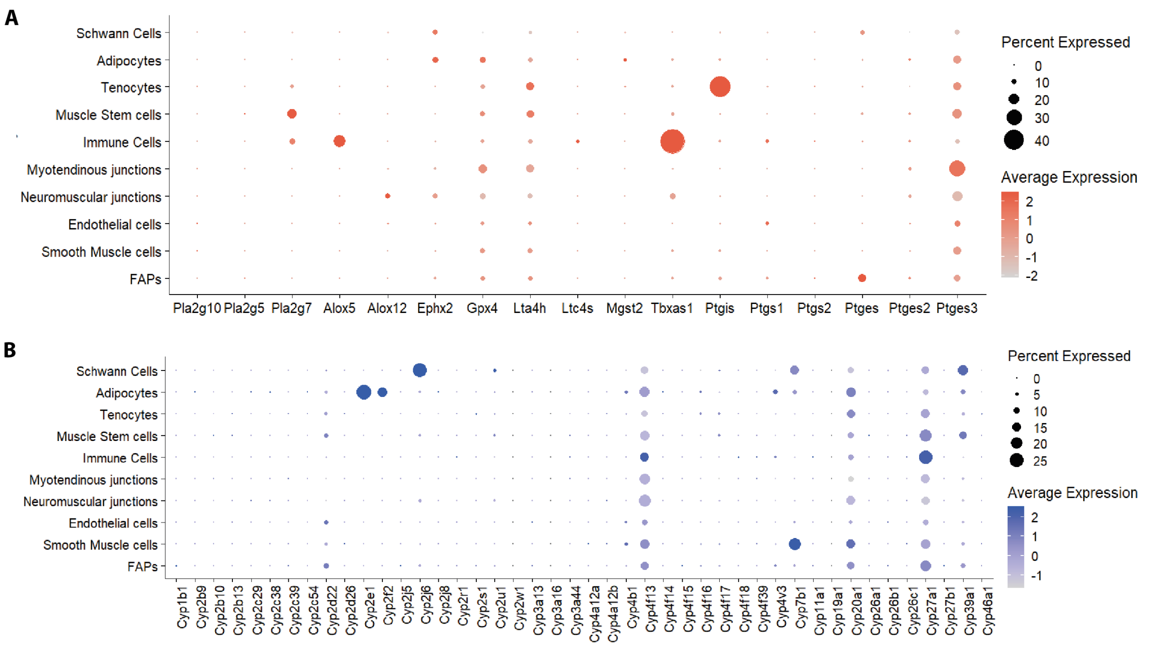
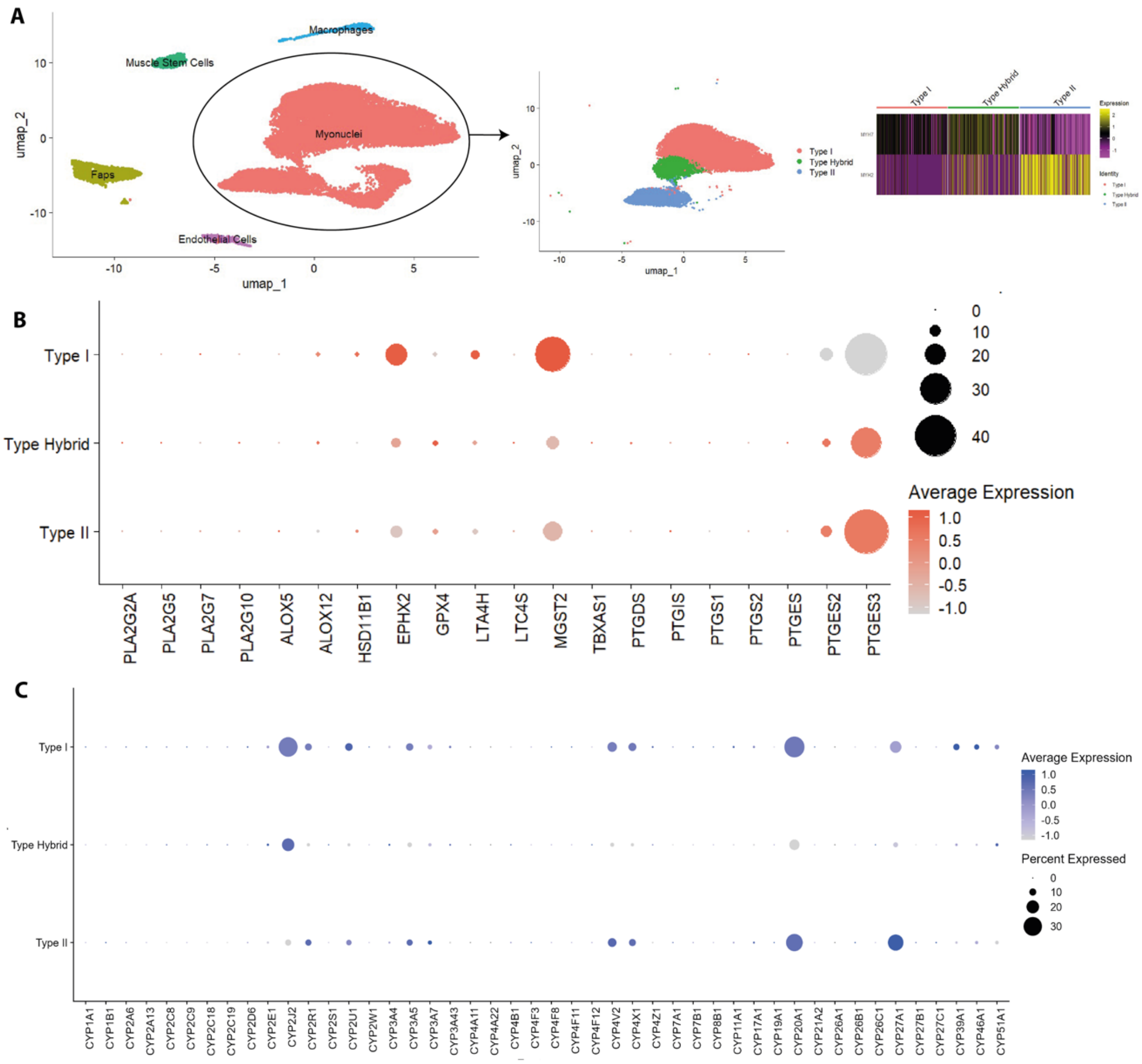
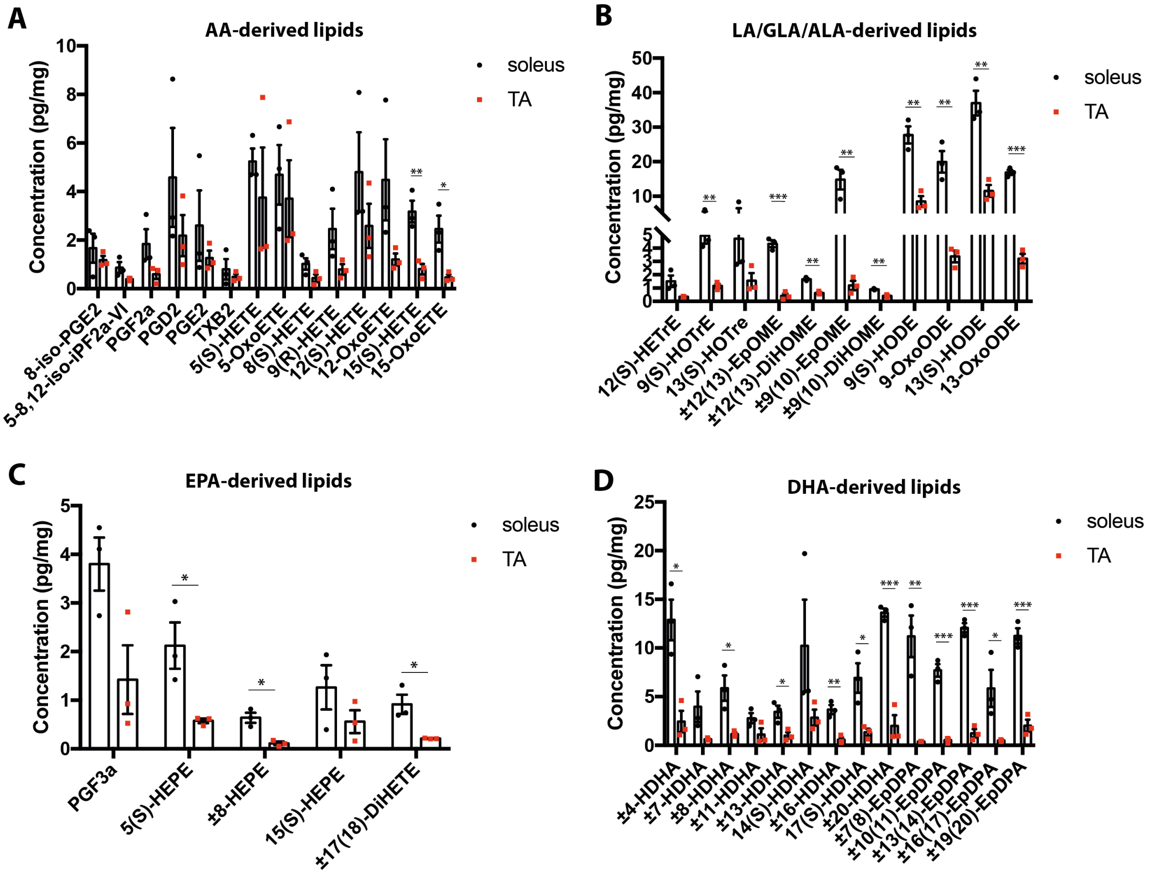


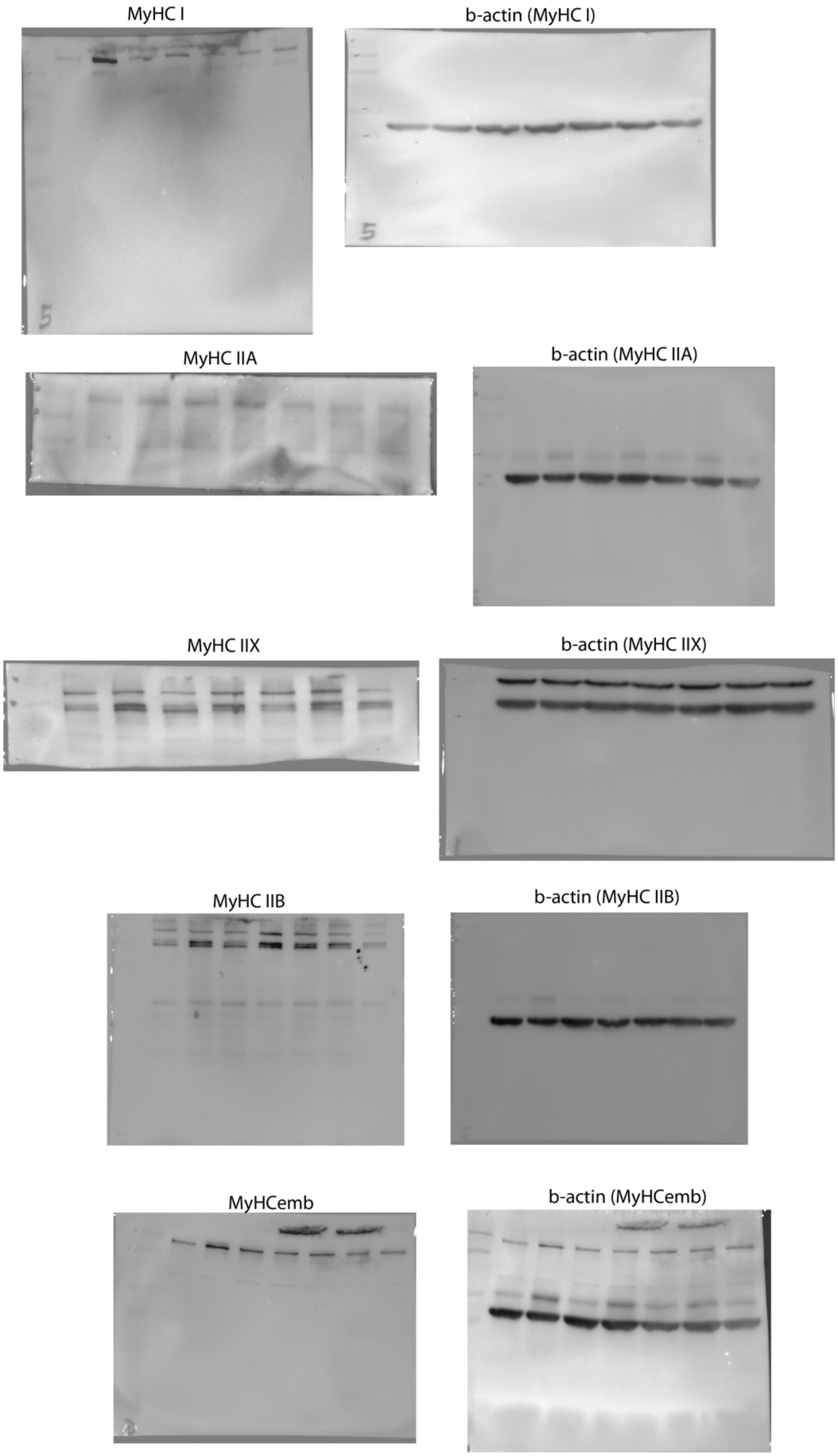
Suppl. Fig. 1: Differential expression patterns of lipid mediator enzymes in different cell types of mouse skeletal muscle. A,B) Dot plots showing the expression of different bioactive lipid biosynthesizing enzymes involved in the conversion of AA, LA, DHA, and EPA (A) as well as Cyp enzymes (B) across the different cell types: Schwann cells, adipocytes, tenocytes, muscle stem cells, immune cells, myotendinous junctions, neuromuscular junctions, endothelial cells, smooth muscle cells, and fibroadipogenic progenitors (FAPs).



Suppl. Fig. 2: Differential expression patterns of lipid mediator enzymes in slow and fast myonuclei from human muscles. A) Single nuclei RNAseq dataset of gastrocnemius muscle biopsies from human was used to study gene expression in type I, II, and hybrid fibers. **B,C**) Dot plots showing the expression of different bioactive lipid biosynthesizing enzymes involved in the conversion of AA, LA, DHA, and EPA (B) as well as Cyp enzymes (C) across the different fiber types.



Suppl. Figure 3: Detailed results showing the differential pattern of expression of bioactive lipid mediators in slow and fast-twitch muscle. Mass Spectrometry analysis was performed on slow-twitch muscle (soleus) vs fast-twitch muscle (tibialis anterior). Bar graphs showing the expression of bioactive lipids derived from **A**) Arachidonic acid (AA), **B**) linoleic acid (LA)/gamma-linoleic acid (GLA)/alpha-linolenic acid (ALA), **C**) eicosapentaenoic acid (EPA), and **D**) docosahexaenoic acid (DHA). Data are expressed as pg/mg of muscle. Statistical analysis was performed using unpaired Student's t-test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.


MyHC I

b-actin (MyHC I)

MyHC IIA

b-actin (MyHC IIA)

MyHC IIX

b-actin (MyHC IIX)

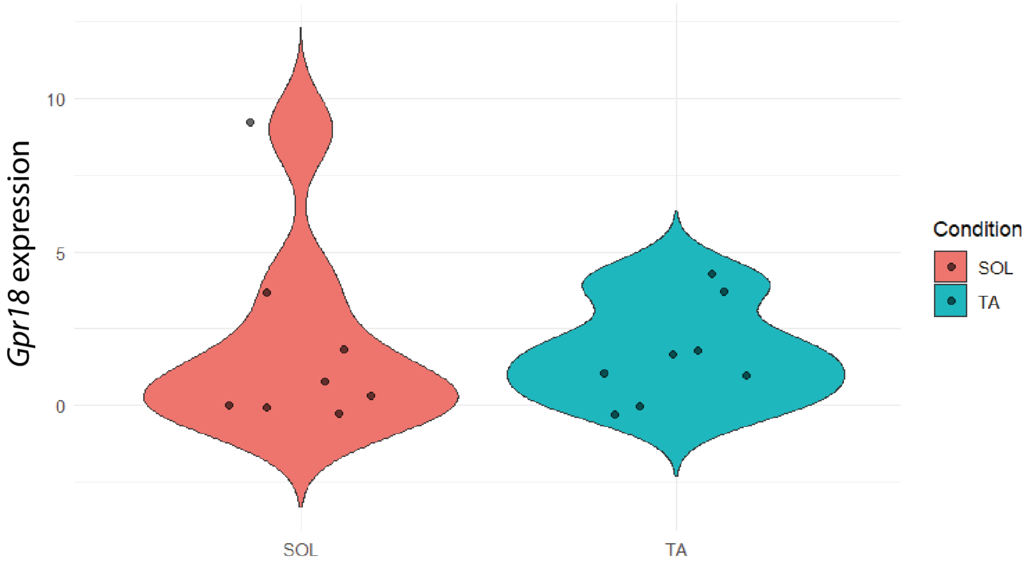
MyHC IIB

b-actin (MyHC IIB)

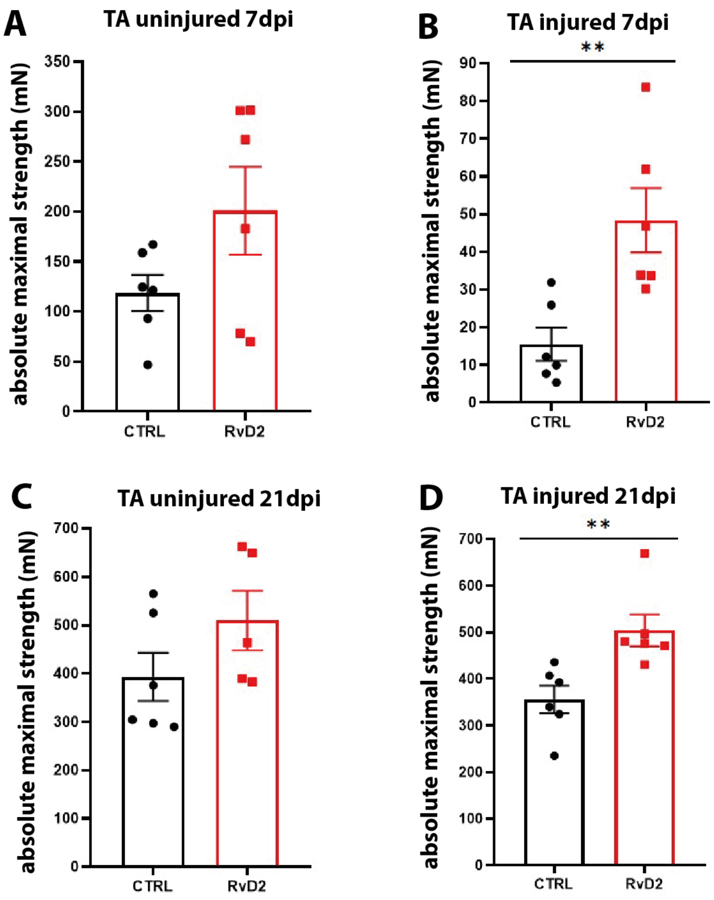
MyHCemb

b-actin (MyHCemb)

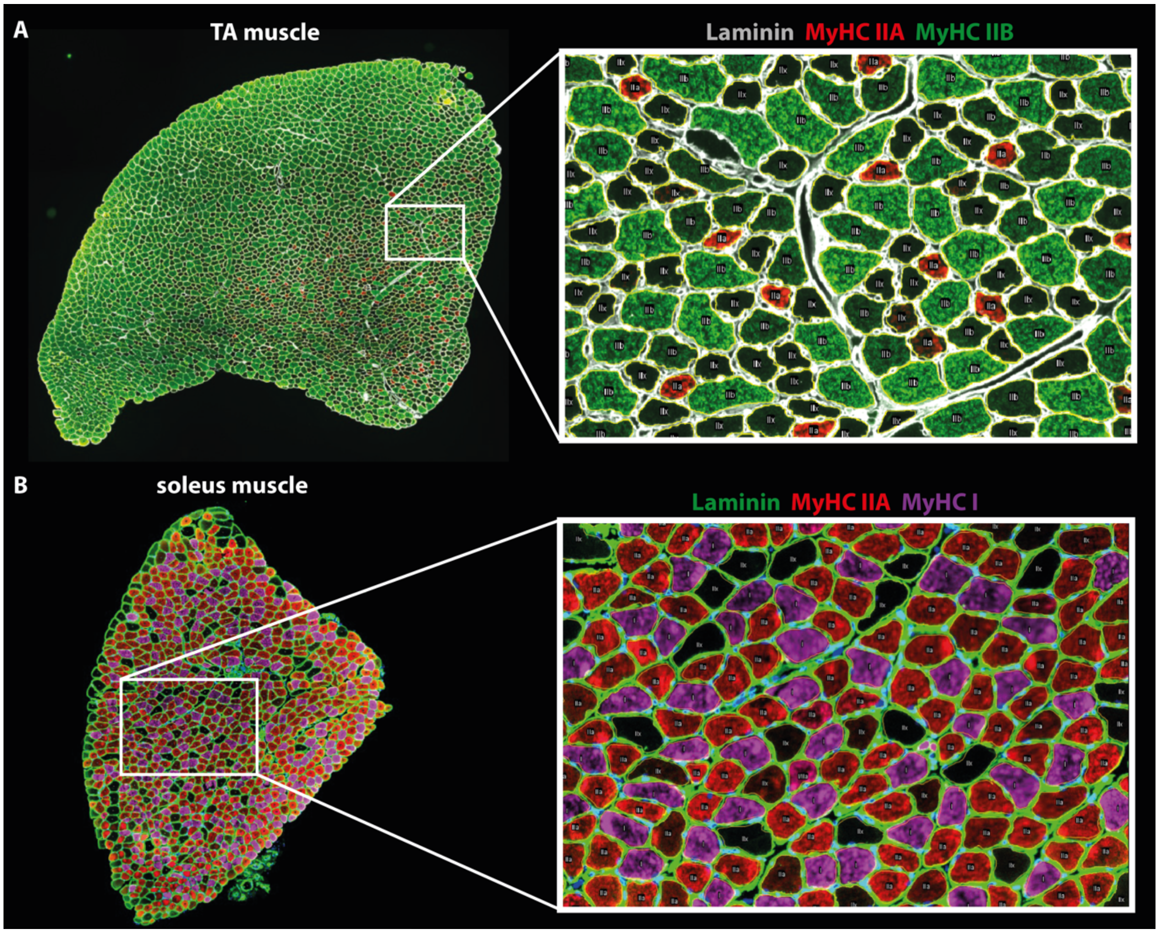
Suppl. Figure 4: Full unedited western blot.



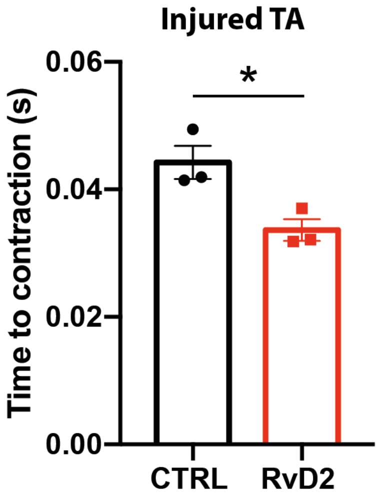
Suppl. Figure 5: *Gpr18* expression. Violin plot showing the expression of *Gpr18* in Soleus (SOL) and tibialis anterior (TA) muscle of wildtype mice.



Suppl. Figure 6: Absolute muscle strength. Maximal absolute strength (mN) of the uninjured **(A, C)** and injured TA muscle **(B, D)** at 7 **(A,B)**, and 21 **(C,D)** days post-injury (DPI).



Suppl. Fig. 7: Immunofluorescence on muscle tissue sections. **A)** Immunofluorescence staining on TA muscle section for laminin (white), MyHC IIB (green) and MyHC IIA (red). Dark fibers are type IIX. MyHC I staining was performed on another slide and was almost not expressed in TA. **B)** Immunofluorescence staining on soleus muscle section for laminin (green), MyHC I (red) and MyHC IIA (violet). Dark fibers are type IIX. MyHC IIB staining was performed on another slide and was virtually not expressed in the soleus muscle. All fibers from the whole section were analyzed to determine fiber type proportion and minimal feret diameter using the Myosight software.



Suppl. Fig. 8: Resolvin-D2 influences muscle contraction velocity. Mice were treated for 21 days post-injury with daily i.p. injection of RvD2 or vehicle. Muscle contractile properties were assessed in situ to determine the time to peak tension of injured TA muscle following twitch contraction. Data are presented as mean ± SEM and n = 3 mice per group. Data were analyzed using unpaired Student's t-test. $^*p < 0.05$.

Supplementary table 1. Multiple reaction monitoring transitions for tandem mass spectrometry optimized for each oxylipin and associated internal standard

Compound	Retention time (min)	Compound MRM transition (precursor ion → product ion)	Internal standard	Internal standard MRM transition (precursor ion → product ion)
E$_2$-Isops (15-series)				
15-E$_{2t}$-Isop	8.38	351.2 → 271.1	15-F$_{2t}$-Isop-d4	357.3 → 197.2
F$_2$-Isops (5-series)				
5-F$_{2t}$-Isop	8.03	353.0 → 115.0	5-F$_{2t}$-Isop-d11	364.2 → 115.0
5-*epi*-5-F$_{2t}$-Isop	8.19	353.0 → 115.0	5-epi-5-F$_{2t}$-Isop-d11	364.2 → 115.0
5*(RS)*-5-F$_{2c}$-IsoP	9.59	353.0 → 115.0	5*(RS)*-5-F$_{2c}$-IsoP-d11	364.2 → 115.0
F$_2$-Isops (8-series)				
8-F$_{2t}$-Isop	7.43	353.0 → 127.0	8-F$_{2t}$-Isop-d4	357.0 → 127.0
F$_2$-Isops (15-series)				
15-*epi*-15-F$_{2t}$-IsoP	7.59	353.3 → 193.2	15-F$_{2t}$-IsoP-d4	357.3 → 197.2
15-F$_{2t}$-IsoP	7.90	353.3 → 193.2	15-F$_{2t}$-IsoP-d4	357.3 → 197.2
15-*epi*-PGF$_{2\alpha}$	8.48	353.3 → 193.2	15-F$_{2t}$-IsoP-d4	357.3 → 197.2
5-*trans*-PGF$_{2\alpha}$	8.64	353.3 → 193.2	15-F$_{2t}$-IsoP-d4	357.3 → 197.2
F$_3$-Isops (5-series)				
5-F$_{3t}$-Isop	6.44	351.3 → 115.0	8-F$_{2t}$-Isop-d4	357.0 → 127.0
F$_3$-IsoPs (8-series)				
8-F$_{3t}$-Isop	6.27	351.3 → 127.0	8-F$_{2t}$-Isop-d4	357.0 → 127.0
8-*epi*-8-F$_{3t}$-Isop	6.91	351.3 → 127.0	8-F$_{2t}$-Isop-d4	357.0 → 127.0
F$_3$-Isops (15-series)				
15-F$_{3t}$-Isop	6.74	351.1 → 193.2	8-F$_{2t}$-Isop-d4	357.0 → 127.0
F$_3$-IsoPs (18-series)				
18-*epi*-18-F$_{3t}$-Isop	6.18	351.3 → 193.1	8-F$_{2t}$-Isop-d4	357.0 → 127.0
18-F$_{3t}$-Isop	6.78	351.3 → 153.0	8-F$_{2t}$-Isop-d4	357.0 → 127.0
F$_4$-NeuroPs (4-series)				
4-F$_{4t}$-NeuroP	8.81	377.2 → 101.0	5*(RS)*-5-F$_{2c}$-IsoP-d11	364.2 → 115.0
4-*epi*-4-F$_{4t}$-NeuroP	8.92	377.2 → 101.0	5*(RS)*-5-F$_{2c}$-IsoP-d11	364.2 → 115.0
F$_4$-NeuroPs (10-series)				
10-F$_{4t}$-NeuroP	8.11	377.2 → 153.0	5-epi-5-F$_{2t}$-Isop-d11	364.2 → 115.0

10-*epi*-10-F_{4t}-NeuroP	8.51	377.2 → 153.0	5-epi-5-F_{2t}-Isop-d11	364.2 → 115.0
F_4-NeuroPs (14-series)				
14-F_{4t}-NeuroP	8.29	377.2 → 205.0	5-epi-5-F_{2t}-Isop-d11	364.2 → 115.0
14-*epi*-14-F_{4t}-NeuroP	8.51	377.2 → 205.0	5-epi-5-F_{2t}-Isop-d11	364.2 → 115.0
F_4-NeuroPs (20-series)				
20-*epi*-20-F_{4t}-NeuroP	8.00	377.2 → 275.0	5-F_{2t}-Isop-d11	364.2 → 115.0
20-F_{4t}-NeuroP	8.42	377.2 → 275.0	5-epi-5-F_{2t}-Isop-d11	364.2 → 115.0
Precursors				
±17(18)-DiHETE	11.50	335.1→ 247.1	5(S)-HETE-d_8	327.2 → 116.0
±14(15)-DiHETE	11.78	335.1→ 237.1	5(S)-HETE-d_8	327.2 → 116.0
±11(12)-DiHETE	11.92	335.1→ 167.1	5(S)-HETE-d_8	327.2 → 116.0
±8(9)-DiHETE	12.14	335.1→ 185.1	5(S)-HETE-d_8	327.2 → 116.0
±5(6)-DiHETE	12.69	335.1→ 145.1	5(S)-HETE-d_8	327.2 → 116.0
±12(13)-DiHOME	12.30	313.2→ 183.2	5(S)-HETE-d_8	327.2 → 116.0
±9(10)-DiHOME	12.54	313.2→ 201.2	5(S)-HETE-d_8	327.2 → 116.0
±19(20)-DiHDPA	12.92	361.1→ 229.1	5(S)-HETE-d_8	327.2 → 116.0
±16(17)DiHDPA	13.10	361.1→ 233.1	5(S)-HETE-d_8	327.2 → 116.0
±13(14)-DiHDPA	13.20	361.1→ 193.1	5(S)-HETE-d_8	327.2 → 116.0
±10(11)-DiHDPA	13.33	361.1→ 153.1	5(S)-HETE-d_8	327.2 → 116.0
±7(8)-DiHDPA	13.64	361.1→ 127.1	5(S)-HETE-d_8	327.2 → 116.0
9*(S)*-HOTrE	13.15	293.3→ 275.2	5(S)-HETE-d_8	327.2 → 116.0
13*(S)*-HOTrE	13.29	293.3→ 195.1	5(S)-HETE-d_8	327.2 → 116.0
13*(S)*-HOTrE(*γ*)	13.41	293.3→ 193.1	5(S)-HETE-d_8	327.2 → 116.0
±18-HEPE	13.31	317.2 → 219.2	5(S)-HETE-d_8	327.2 → 116.0
15*(S)*-HEPE	13.51	317.2 → 219.2	5(S)-HETE-d_8	327.2 → 116.0
±11-HEPE	13.56	317.2 → 195.2	5(S)-HETE-d_8	327.2 → 116.0
±8-HEPE	13.62	317.2 → 155.1	5(S)-HETE-d_8	327.2 → 116.0
12*(S)*-HEPE	13.69	317.2 → 207.2	5(S)-HETE-d_8	327.2 → 116.0
±9-HEPE	13.76	317.2 → 123.1	5(S)-HETE-d_8	327.2 → 116.0
5*(S)*-HEPE	13.87	317.2 → 115.1	5(S)-HETE-d_8	327.2 → 116.0
13-OxoODE	13.99	293.1 → 113.1	5(S)-HETE-d_8	327.2 → 116.0
9-OxoODE	14.13	293.1 → 185.1	5(S)-HETE-d_8	327.2 → 116.0
13*(S)*-HODE	14.01	295.1 → 195.1	5(S)-HETE-d_8	327.2 → 116.0

9*(S)*-HODE	14.03	295.1 → 171.1	5(S)-HETE-d_8	327.2 → 116.0
±17(18)-EpETE	14.01	317.1 → 259.2	5(S)-HETE-d_8	327.2 → 116.0
±14(15)-EpETE	14.20	317.1 → 247.1	5(S)-HETE-d_8	327.2 → 116.0
±11(12)-EpETE	14.24	317.1 → 167.1	5(S)-HETE-d_8	327.2 → 116.0
±8(9)-EpETE	14.29	317.1 → 155.1	5(S)-HETE-d_8	327.2 → 116.0
±20-HDHA	14.08	343.2 → 285.2	5(S)-HETE-d_8	327.2 → 116.0
±16-HDHA	14.18	343.2 → 233.2	5(S)-HETE-d_8	327.2 → 116.0
17*(S)*-HDHA	14.20	343.2 → 201.0	5(S)-HETE-d_8	327.2 → 116.0
±13-HDHA	14.25	343.2 → 221.1	5(S)-HETE-d_8	327.2 → 116.0
±10-HDHA	14.30	343.2 → 190.1	5(S)-HETE-d_8	327.2 → 116.0
14*(S)*-HDHA	14.31	343.2 → 161.1	5(S)-HETE-d_8	327.2 → 116.0
±11-HDHA	14.40	343.2 → 165.2	5(S)-HETE-d_8	327.2 → 116.0
±7-HDHA	14.43	343.2 → 141.2	5(S)-HETE-d_8	327.2 → 116.0
±8-HDHA	14.50	343.2 → 109.1	5(S)-HETE-d_8	327.2 → 116.0
±4-HDHA	14.69	343.2 → 101.2	5(S)-HETE-d_8	327.2 → 116.0
15*(S)*-HETE	14.15	319.2 → 219.2	5(S)-HETE-d_8	327.2 → 116.0
11*(S)*-HETE	14.27	319.2 → 167.1	5(S)-HETE-d_8	327.2 → 116.0
12*(S)*-HETE	14.36	319.2 → 179.2	5(S)-HETE-d_8	327.2 → 116.0
8*(S)*-HETE	14.37	319.2 → 155.1	5(S)-HETE-d_8	327.2 → 116.0
9*(R)*-HETE	14.47	319.2 → 139.1	5(S)-HETE-d_8	327.2 → 116.0
5*(S)*-HETE	14.55	319.2 → 115.1	5(S)-HETE-d_8	327.2 → 116.0
15-OxoETE	14.13	317.1 → 113.1	5(S)-HETE-d_8	327.2 → 116.0
12-OxoETE	14.33	317.1 → 153.1	5(S)-HETE-d_8	327.2 → 116.0
5-OxoETE	14.72	317.1 → 203.1	5(S)-HETE-d_8	327.2 → 116.0
12*(S)*-HETRrE	14.63	321.1 → 180.8	5(S)-HETE-d_8	327.2 → 116.0
±12(13)-EpOME	14.59	295.1 → 195.1	5(S)-HETE-d_8	327.2 → 116.0
±9(10)-EpOME	14.65	295.1 → 171.1	5(S)-HETE-d_8	327.2 → 116.0
±19(20)-EpDPA	14.59	343.1 → 241.1	5(S)-HETE-d_8	327.2 → 116.0
±16(17)-EpDPA	14.72	343.1 → 233.1	5(S)-HETE-d_8	327.2 → 116.0
±13(14)-EpDPA	14.75	343.1 → 193.1	5(S)-HETE-d_8	327.2 → 116.0
±10(11)-EpDPA	14.77	343.1 → 153.1	5(S)-HETE-d_8	327.2 → 116.0
±7(8)-EpDPA	14.85	343.1 → 112.8	5(S)-HETE-d_8	327.2 → 116.0
Resolvins				

RvE$_1$	6.34	349.2 → 195.0	RvD$_2$-d$_5$	380.4 → 175.0
RvD$_3$	8.80	375.2 → 147.1	RvD$_2$-d$_5$	380.4 → 175.0
RvD$_2$	8.82	375.3 → 141.0	RvD$_2$-d$_5$	380.4 → 175.0
RvD$_1$	9.21	375.2 → 141.0	17(R)-RVD1-d5	380.3 → 220.3
RVD2 $_{n-3}$ DPA	9.21	377.1 → 143.0	17(R)-RVD1-d5	380.3 → 220.3
17*(R)*-RVD$_1$	9.35	375.2 → 141.0	17(R)-RVD1-d5	380.3 → 220.3
17*(R)*-RVD4, RVD$_4$	9.92	375.2 → 101.0	17(R)-RVD1-d5	380.3 → 220.3
RvD$_5$	11.30	359.2 → 199.0	17(R)-RVD1-d5	380.3 → 220.3
RVD5 $_{n-3}$ DPA	11.75	361.2 → 143.0	17(R)-RVD1-d5	380.3 → 220.3
Lipoxins				
Lipoxin B$_4$	8.68	351.2 → 221.1	Lipoxin A4-d5	356.2 → 114.9
Lipoxin A$_4$	9.16	351.2 → 114.9	Lipoxin A4-d5	356.2 → 114.9
15(R)-Lipoxin A$_4$	9.30	351.2 → 114.9	Lipoxin A4-d5	356.2 → 114.9
6(S)-Lipoxin A$_4$	9.56	351.2 → 114.9	Lipoxin A4-d5	356.2 → 114.9
Maresin				
Maresin 1	11.49	359.3 → 112.9	Leukotriene B$_4$-d$_4$	339.3 → 197.1
Maresin 2	12.40	359.0 → 173.2	Leukotriene B$_4$-d$_4$	339.3 → 197.1
Leukotrienes				
Leukotriene B$_4$	11.68	335.3 → 195.1	Leukotriene B$_4$-d$_4$	339.3 → 197.1
Protectin				
17*(R)*-PD$_1$	10.57	359.0 → 152.9	Leukotriene B$_4$-d$_4$	339.3 → 197.1
PDX	11.26	359.3 → 153.0	Leukotriene B$_4$-d$_4$	339.3 → 197.1
PD$_1$	11.39	359.3 → 153.0	Leukotriene B$_4$-d$_4$	339.3 → 197.1
Prostaglandins				
PGE$_3$	7.57	349.2 → 269.2	PGE$_2$-d$_9$	360.2 → 189.0
PGF$_3$	7.76	351.1 → 193.2	PGE$_2$-d$_9$	360.2 → 189.0
TX-B$_2$	8.03	369.3 → 195.0	TX-B$_2$-d$_4$	373.3 → 199.1
17-trans-PGF$_3$	8.09	351.1 → 193.2	PGE$_2$-d$_9$	360.2 → 189.0
PGE$_2$	8.61	351.2 → 271.1	PGE$_2$-d$_9$	360.2 → 189.0
PGD$_2$	8.77	351.2 → 271.1	PGD$_2$-d$_4$	355.1 → 193.0
PGF$_{2\alpha}$	8.80	353.3 → 193.2	PGF$_{2\alpha}$-d$_4$	357.3 → 197.2

Supplementary table 2. Lower quantification limit (LOQ) in pg/mg for each tissue.

Compound	LOQ in soleus (pg/mg)	LOQ in tibialis (pg/mg)
Derived from AA		
15-E_{2t}-Isop	2.28	1.14
5*(RS)*-5-F_{2c}-IsoP	1.67	0.83
$PGF_{2\alpha}$	1.54	0.77
PGD_2	2.41	1.21
PGE_2	1.67	0.83
TX-B_2	0.33	0.17
5*(S)*-HETE	0.33	0.17
5-OxoETE	3.33	1.67
8*(S)*-HETE	1.67	0.83
9*(R)*-HETE	1.86	0.93
12*(S)*-HETE	0.33	0.17
12-OxoETE	3.94	1.97
15*(S)*-HETE	1.81	0.90
15-OxoETE	0.92	0.46
Derived from LA, GLA, ALA		
12*(S)*-HETRrE	0.58	0.29
9*(S)*-HOTrE	0.33	0.17
13*(S)*-HOTrE	1.67	0.83
±12(13)-EpOME	0.40	0.20
±12(13)-DiHOME	0.33	0.17
±9(10)-EpOME	1.67	0.83
±9(10)-DiHOME	0.33	0.17
9*(S)*-HODE	1.67	0.83
9-OxoODE	1.67	0.83
13*(S)*-HODE	8.32	4.16
13-OxoODE	8.08	4.04
Derived from EPA		
PGF_3	7.24	3.62
5*(S)*-HEPE	1.67	0.83
±8-HEPE	1.67	0.83
15*(S)*-HEPE	3.32	1.66
±17(18)-DiHETE	1.67	0.83
Derived from DHA		
±4-HDHA	1.67	0.83
±7-HDHA	1.67	0.83
±8-HDHA	1.67	0.83
±11-HDHA	1.67	0.83

±13-HDHA	1.67	0.83
±14-HDHA	3.33	1.67
±16-HDHA	1.67	0.83
±17-HDHA	1.67	0.83
±20-HDHA	1.67	0.83
±7(8)EpDPA	5.12	2.56
±10(11)EpDPA	0.78	0.39
±13(14)EpDPA	1.67	0.83
±16(17)EpDPA	1.67	0.83
±19(20)EpDPA	6.56	3.28