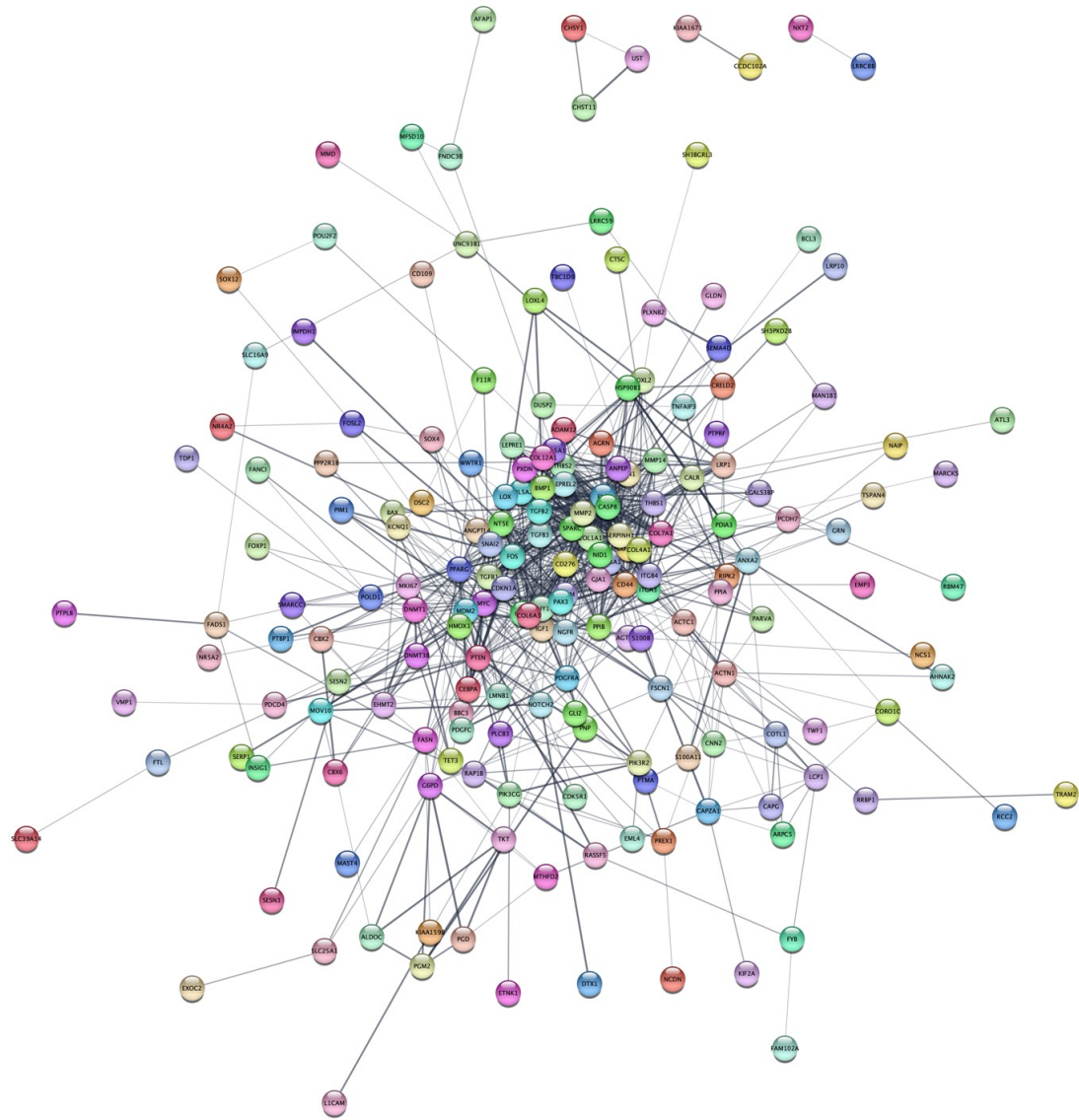


1. Supplemental Figures and Figure Legends



Number of Nodes:	235
Number of Edges:	1134
Average Node Degree:	9.65
Average Local Clustering Coefficient:	0.454
Expected Number of Edges:	513
PPI Enrichment p-value:	< 1.0e-16

Figure S1. PPI network of miR-1, miR-133a, and miR-29b targets upregulated in CLI. A PPI network of the targets of miR-1, miR-133a, and miR-29b that are upregulated in CLI gastrocnemius was constructed using STRING. Nodes represent proteins and edges represent evidence of association between nodes.

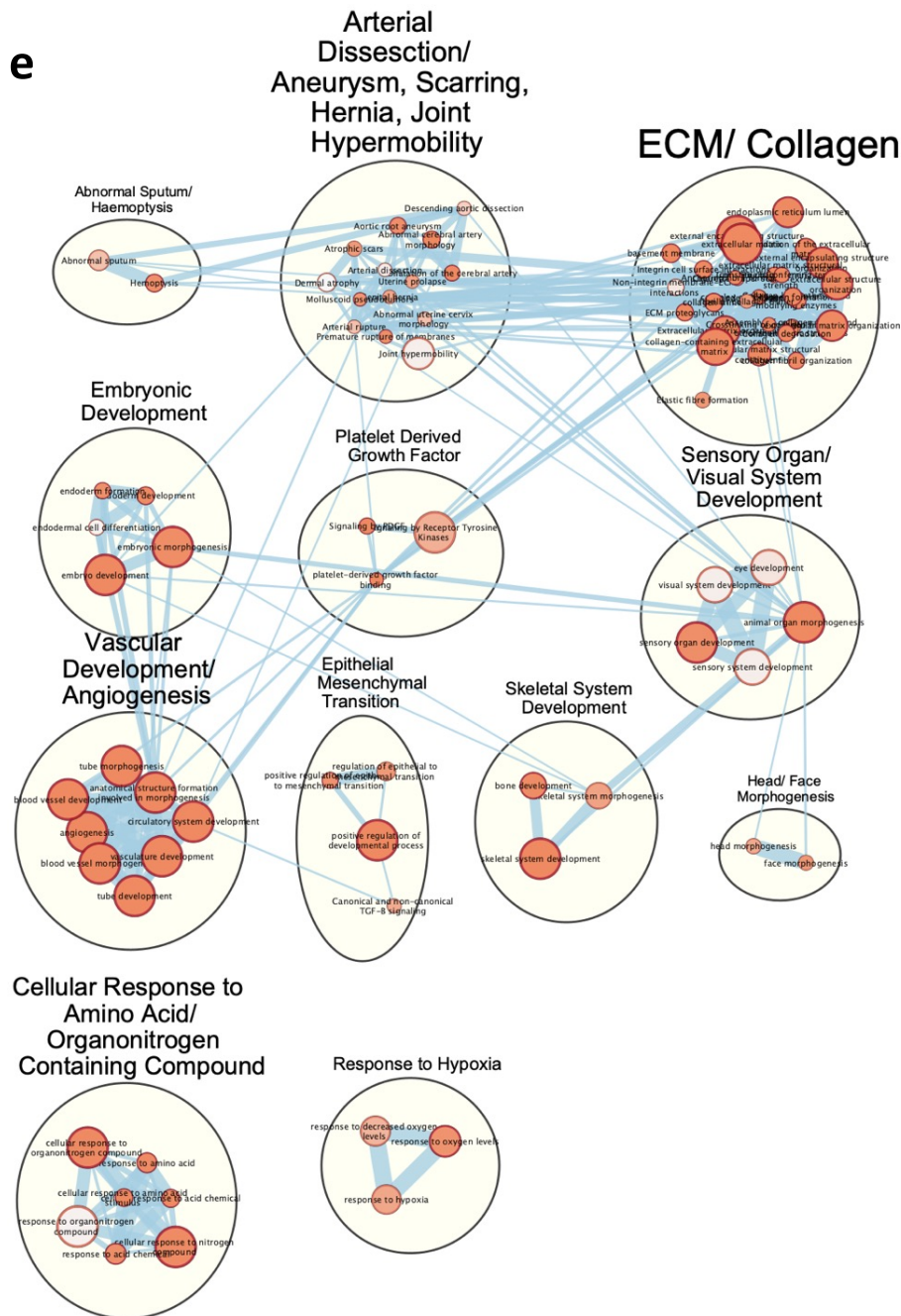


Figure S2. Enrichment Map of the targets of miR-124 (a) miR-1 (b), miR-133a (c) miR-335 (d) and miR-29b (e) which are upregulated in CLTI Muscle. Functional enrichment was performed on the targets of miR-133a that are upregulated in CLTI gastrocnemius using g:Profiler. Enrichment mapping was performed on terms with an FDR <0.05 and term size between 5-1000 using the EnrichmentMap plugin in Cytoscape. Cluster labels created using the AutoAnnotate plugin were manually edited. Nodes represent enriched pathways identified using g:Profiler. Edges represent, and are weighted by, pathway gene set overlap. Node colour is mapped to pathway enrichment significance (orange = lower Q value and white = higher Q value). Node size is mapped to gene set size.

2. Supplemental Tables and supporting information

Table S1. Top 10 miRNAs listed by smallest FDR and p-values (Note: FDR > 0.05 is considered non-significant).

A) Downregulated DEGs from CLTI vs non-PAD Controls

microRNA	p-value	FDR	Odd ratio	Number of interactions
hsa-miR-1468-5p	9.5129E-05	0.233922171	0.173308171	7
hsa-miR-6755-5p	0.001097374	0.999994696	0.343835997	11
hsa-miR-4801	0.003903698	0.999994696	0.466232962	15
hsa-miR-4731-3p	0.004305305	0.999994696	0.470990441	15
hsa-miR-640	0.004956094	0.999994696	0.576606479	25
hsa-miR-569	0.005225014	0.999994696	0.415198185	11
hsa-miR-6805-3p	0.005909226	0.999994696	0.523322712	18
hsa-miR-210-3p	0.006957766	0.999994696	0.520524195	17
hsa-miR-6852-3p	0.007274518	0.999994696	0.451960524	12
hsa-miR-4639-3p	0.007372417	0.999994696	0.580205615	23

B) Downregulated DEGs from CLTI vs IC

microRNA	p-value	FDR	Odd ratio	Number of interactions
hsa-miR-4801	0.000395	0.552	0.415	18
hsa-miR-4731-3p	0.000449	0.552	0.42	18
hsa-miR-320a	0.00203	1.00	0.696	64
hsa-miR-99a-5p	0.0026	1.00	0.507	20
hsa-miR-210-3p	0.00265	1.00	0.498	19
hsa-miR-147b	0.00389	1.00	0.267	6
hsa-miR-1468-5p	0.00729	1.00	0.259	5
hsa-let-7b-5p	0.00821	1.00	0.806	115
hsa-miR-188-5p	0.0108	1.00	0.534	16
hsa-miR-6866-3p	0.0116	1.00	0.512	14

C) Upregulated DEGs from IC vs non-PAD controls

microRNA	p-value	FDR	Odd ratio	Number of interactions
hsa-miR-572	0.000643	0.896	0.0575	3
hsa-miR-6507-3p	0.00224	0.92	0.183	5
hsa-miR-6853-5p	0.00309	0.92	0.0978	3
hsa-miR-126-5p	0.00369	0.92	0.205	5
hsa-miR-153-3p	0.00626	0.92	0.183	4
hsa-miR-335-5p	0.00803	0.92	0.667	34
hsa-miR-6775-3p	0.00916	0.92	0.144	3
hsa-miR-1908-3p	0.00923	0.92	0.0734	2
hsa-miR-6769b-3p	0.00998	0.92	0.303	6
hsa-miR-3183	0.0102	0.92	0.305	6

940 **D) Downregulated DEGs from IC vs non-PAD controls.**

microRNA	p-value	FDR	Odd ratio	Number of interactions
hsa-miR-6824-3p	0.00261	0.968	0.144	4
hsa-miR-6764-3p	0.00279	0.968	0.147	4
hsa-miR-4529-3p	0.00595	0.968	0.123	3
hsa-miR-29a-3p	0.00616	0.968	0.341	8
hsa-miR-518c-5p	0.0071	0.968	0.19	4
hsa-miR-1243	0.00799	0.968	0.137	3
hsa-miR-4643	0.01	0.968	0.304	6
hsa-miR-4774-5p	0.0117	0.968	0.158	3
hsa-miR-660-5p	0.0147	0.968	0.171	3
hsa-miR-6775-3p	0.0147	0.968	0.171	3

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Major Resources Table

To allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Mouse	Janvier Labs, France	BALB/c nude	Male

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
GSE120642	NCBI GEO	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120642

List of miRNA Primers

Description	Source / Repository	Persistent ID / URL
miR-27b-3p	Qiagen	YP00205915
cel-miR-39-3p	Qiagen	YP00203952
hsa-miR-1-3p	Qiagen	YP00204344
hsa-miR-133a-3p	Qiagen	YP00204788
hsa-miR-29b-3p	Qiagen	YP00204679
hsa-miR-124-3p	Qiagen	YP00206026
hsa-miR-335-5p	Qiagen	YP02119293

List of primer sequences for miRNA targets

Gene Name	NCBI Gene ID	Primer Sequence	PrimerBank ID
<i>S29</i>	20090	F 5' – ATGGGTCACCAGCAGCTCTA R 5' – GTATTTGCGGATCAGACCGT	N/A
<i>Gapdh</i>	14433	F 5' – AGGTCGGTGTGAACGGATTTG R 5' – TGTAGACCATGTAGTTGAGGTCA	6679937a1
<i>Col1a1</i>	12842	F 5' – GCTCCTCTTAGGGGCCACT R 5' – CCACGTCTCACCATTGGGG	34328108a1
<i>Col3a1</i>	12825	F 5' – CCTGGCTCAAATGGCTCAC R 5' – CAGGACTGCCGTTATTCCCG	22832760a1
<i>Col4a1</i>	12826	F 5' – CTGGCACAAAAGGGACGAG R 5' – ACGTGGCCGAGAATTTCCACC	33859528a1
<i>Fn1</i>	14268	F 5' – GCTCAGCAAATCGTGCAGC R 5' – CTAGGTAGGTCCGTTCCCACT	26344255a1
<i>Fbn1</i>	14118	F 5' – GGACGCCAATTTGGAGGCT R 5' – CTTTCAGCGCATCGTGTCTT	6679759a1
<i>Sparc</i>	20692	F 5' – GTGGAAATGGGAGAATTTGAGGA R 5' – CTCACACACCTTGCCATGTTT	6678077a1

<i>Mmp2</i>	17390	F 5' – CAAGTTCCCCGGCGATGTC TTCTGGTCAAGGTACCTGTC	6678902a1
<i>Mmp14</i>	17387	F 5' – CAGTATGGCTACCTACCTCCAG R 5' – GCCTTGCCTGTCACTTGTAAG	31982191a1
<i>Myh7</i>	140781	F 5' ACTGTCAACACTAAGAGGGCA R 5' - TTGGATGATTTGATCTTCCAGGG	18859641a1
<i>Acta1</i>	11459	F 5' – CCCAAAGCTAACCGGGAGAAG R 5' - GACAGCACCGCTGGATAG	133893192c1
<i>Murf1</i> (<i>Trim63</i>)	433766	F 5' – GTGTGAGGTGCCTACTTGCTC R 5' – GCTCAGTCTTCTGTCTTGA	21523717a1
<i>Atrogin1</i> (<i>Fbxo32</i>)	67731	F 5' – CAGCTTCGTGAGCGACCTC R 5' – GGCAGTCGAGAAGTCCAGTC	13385848a1
<i>Tgfb2</i>	21808	F 5' - CTTGACGTGACAGACGCT R 5' - GCAGGGGCAGTGTAACCTATT	15029686a1

ARRIVE Guidelines

The ARRIVE guidelines (<https://arriveguidelines.org/>) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigour, and reproduce the methods or findings.

Study Design

Groups	Sex	Age	Number (before experiment)	Number (after termination)	Littermates (Yes/No)	Other description
Group 1	Male	15 weeks old	3	3	No	Right limb no HLI mouse
Group 2	Male	15 weeks old	3	3	No	Right limb HLI (non- ischaemic)
Group 3	Male	15 weeks old	3	3	No	Left limb HLI (ischaemic)

Sample Size: This was a pilot study and therefore there were no a priori sample size calculations.

Inclusion Criteria: 15-week-old BALB/c nude male mice in good health status.

Exclusion Criteria: mice which lost more than 15% body weight and/or amputation of the foot after surgery would be excluded and euthanized. This was not the case for mice in our study. Adequate perfusion to the control leg is a stringent criterion for LDI scan acceptability. Animals that had >20% differences in blood perfusion between both legs before HLI surgery would be excluded.

Randomization: Mice with HLI and no-HLI entered the study at random.

Blinding: Due to the nature of this experiment, it was not possible to blind researchers during the in vivo study. However, blinding occurred at the tissue collection, where tissues were coded and the researcher performing analysis was blinded.