

## Major Resources Table

In order 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	Persistent ID / URL
Mouse	The Jackson Laboratory	C57BL/6J	M, F	<a href="https://www.jax.org/strain/000664">https://www.jax.org/strain/000664</a>
Mouse	The Jackson Laboratory	C57BL/6:129SVJ	M, F	Details below in Genetically Modified Animals

### Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent	Mouse	The Jackson Laboratory	C57BL/6:129SVJ	Myh6-merCremer	<a href="https://www.jax.org/strain/005657">https://www.jax.org/strain/005657</a>
Parent	Mouse	The Jackson Laboratory	C57BL/6:129SVJ	MADM-ML-11 <sup>GT/TG</sup>	<a href="https://www.jax.org/strain/030578">https://www.jax.org/strain/030578</a>

### Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Picro-Sirius Red	Abcam	ab150681	Details in Protocol: Picro Sirius Red Stain Kit		<a href="https://www.abcam.com/picro-sirius-red-stain-kit-connective-tissue-stain-ab150681.html">https://www.abcam.com/picro-sirius-red-stain-kit-connective-tissue-stain-ab150681.html</a>
Wheat Germ Agglutinin	Life Technologies	W11261 W32466	1:100		<a href="https://www.thermofisher.com/order/catalog/product/W11261">https://www.thermofisher.com/order/catalog/product/W11261</a>
EdU	Life Technologies	C10337	Details in Protocol: Click-IT EdU Cell Proliferation Kit for Imaging		<a href="https://www.thermofisher.com/order/catalog/product/C10337">https://www.thermofisher.com/order/catalog/product/C10337</a>
α-sarcomeric actin	Sigma	A2172	1:500		<a href="https://www.sigmaaldrich.com/US/en/product/sigma/a2172">https://www.sigmaaldrich.com/US/en/product/sigma/a2172</a>
Ki67	Abcam	ab15580	1:300		<a href="https://www.abcam.com/ki67-antibody-ab15580.html">https://www.abcam.com/ki67-antibody-ab15580.html</a>
pH3	Millipore	06-570	1:300		<a href="https://www.emdmillipore.com/US/en/product/Anti-phospho-Histone-H3-Ser10-Antibody-Mitosis-Marker,MM_NF-06-570">https://www.emdmillipore.com/US/en/product/Anti-phospho-Histone-H3-Ser10-Antibody-Mitosis-Marker,MM_NF-06-570</a>

DOI [to be added]

DAPI	Millipore	268298	1:1000		<a href="https://www.emdmillipore.com/US/en/product/DAPI-Dihydrochloride-CAS-28718-90-3-Calbiochem,EMD_BIO-268298">https://www.emdmillipore.com/US/en/product/DAPI-Dihydrochloride-CAS-28718-90-3-Calbiochem,EMD_BIO-268298</a>
CD31	R&D Systems	AF3628	1:30		<a href="https://www.rndsystems.com/products/mouse-rat-cd31-pecam-1-antibody_af3628">https://www.rndsystems.com/products/mouse-rat-cd31-pecam-1-antibody_af3628</a>
$\alpha$ -smooth muscle actin	Abcam	ab5694	1:100		<a href="https://www.abcam.com/alpha-smooth-muscle-actin-antibody-ab5694.html">https://www.abcam.com/alpha-smooth-muscle-actin-antibody-ab5694.html</a>
CD45	R&D Systems	AF114	1:100		<a href="https://www.rndsystems.com/products/mouse-cd45-antibody_af114">https://www.rndsystems.com/products/mouse-cd45-antibody_af114</a>
TUNEL	Promega	G3250	Details in Protocol: DeadEnd Fluorometric TUNEL system		<a href="https://www.promega.com/products/cell-health-assays/apoptosis-assays/deadend-fluorometric-tunel-system/?catNum=G3250">https://www.promega.com/products/cell-health-assays/apoptosis-assays/deadend-fluorometric-tunel-system/?catNum=G3250</a>

### DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
$\beta$ 2M	5'- ATGTGAGGCGGGTGGAAGTCTG, 5'- CTCGGTGACCCTGGTCTTTCTG	NIH National Library of Medicine, NCBI	<a href="https://ncbi.nlm.nih.gov/gene/">https://ncbi.nlm.nih.gov/gene/</a>  <a href="https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome">https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome</a>
Periostin (Postn)	5'- TATGCTCTGCTGCTGCTGTT, 5'- TTTCTTCCCGCAGATAGCAC	NIH National Library of Medicine, NCBI	<a href="https://ncbi.nlm.nih.gov/gene/">https://ncbi.nlm.nih.gov/gene/</a>  <a href="https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome">https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome</a>
Col1A1	5'- GCCAAGAAGACATCCCTGAA, 5'- GCCATTGTGGCAGATACAGA	NIH National Library of Medicine, NCBI	<a href="https://ncbi.nlm.nih.gov/gene/">https://ncbi.nlm.nih.gov/gene/</a>  <a href="https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome">https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome</a>
Col3A1	5'- AGAGGCTTTGATGGACGCAA, 5'- CCACCAGGACTGCCGTTATT	NIH National Library of Medicine, NCBI	<a href="https://ncbi.nlm.nih.gov/gene/">https://ncbi.nlm.nih.gov/gene/</a>  <a href="https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome">https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome</a>

### Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL

### Data & Code Availability

Description	Source / Repository	Persistent ID / URL
RNA Sequencing	GEO database (NIH NLM NCBI)	Accession number: GSE221168 <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE221168">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE221168</a>

### Other

Description	Source / Repository	Persistent ID / URL

DOI [to be added]

5-ethynyl-2'-deoxyuridine (EdU, drug administration)	Life Technologies, catalog# E10187	<a href="https://www.thermofisher.com/order/catalog/product/E10187">https://www.thermofisher.com/order/catalog/product/E10187</a>
Tamoxifen (drug administration)	Sigma	<a href="https://www.sigmaaldrich.com/US/en/substance/tamoxifen3715110540291">https://www.sigmaaldrich.com/US/en/substance/tamoxifen3715110540291</a>
Tamoxifen (TAM, for analysis in LC-MS)	Toronto Research Chemicals, catalog# T006077	<a href="https://www.trc-canada.com/product-detail/?T006077">https://www.trc-canada.com/product-detail/?T006077</a>
(Z)-4-Hydroxytamoxifen (4OHTAM, for analysis in LC-MS)	Toronto Research Chemicals, catalog# H954757	<a href="https://www.trc-canada.com/product-detail/?H954757">https://www.trc-canada.com/product-detail/?H954757</a>
miRNeasy Mini Kit	Qiagen, catalog# 217004	<a href="https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/mirna/mirneasy-kits?catno=217004">https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/mirna/mirneasy-kits?catno=217004</a>

## 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 rigor, and reproduce the methods or findings.

### Study Design

Groups	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
Group 1 (Control)	M, F	9-12 weeks old	32	32	No	Normoxia (20.9 oxygen)
Group 2	M, F	9-12 weeks old	44	44	No	Hypoxia (7% oxygen)
Group 3 (control)	M	9-12 weeks old	10	10	No	Normoxia, food restricted (20.9% oxygen)
Group 4	M	9-12 weeks old	15	10	No	Hypoxia (5% oxygen)

**Sample Size:** Please explain how the sample size was decided Please provide details of any a *prior* sample size calculation, if done.

Sample size was decided based on having a  $p \leq 0.05$  and 80% power for analysis

### Inclusion Criteria

We included male and females in our primary groups 1 and 2. Group 3 was used to eliminate diet/weight-gain as an influencing factor in cardiomyocyte proliferation. Since the results of group 3 were similar to group 1, we decided to use group 1 as the primary control group and included males and females in group 1. Group 4 was used to test if lower oxygen (5%) would increase cardiomyocyte proliferation, but when we concluded that it rather decreased proliferation, we focused on studying group 2 as the primary treatment group to induce myocyte proliferation and included males and females in group 2.

### Exclusion Criteria

Mice that died during hypoxia treatment were excluded from terminal studies and analysis. Mice only died in group 4 from severe hypoxia. We also excluded females from group 3 because the results in males showed no difference when compared to group 1. We excluded females from group 4 because the results in males showed reduced cardiomyocyte proliferation and animal survival, which was more detrimental to mice. Therefore, we focused on the analysis of group 1 vs. group 2 and included both sexes in these groups.

### Randomization

Animals were randomly divided into each group.

### Blinding

Investigators of this study were blinded to the treatment group of the animals, following tissue harvest. Investigators were unblinded after data analysis was completed.

DOI [to be added]