Fig. S1

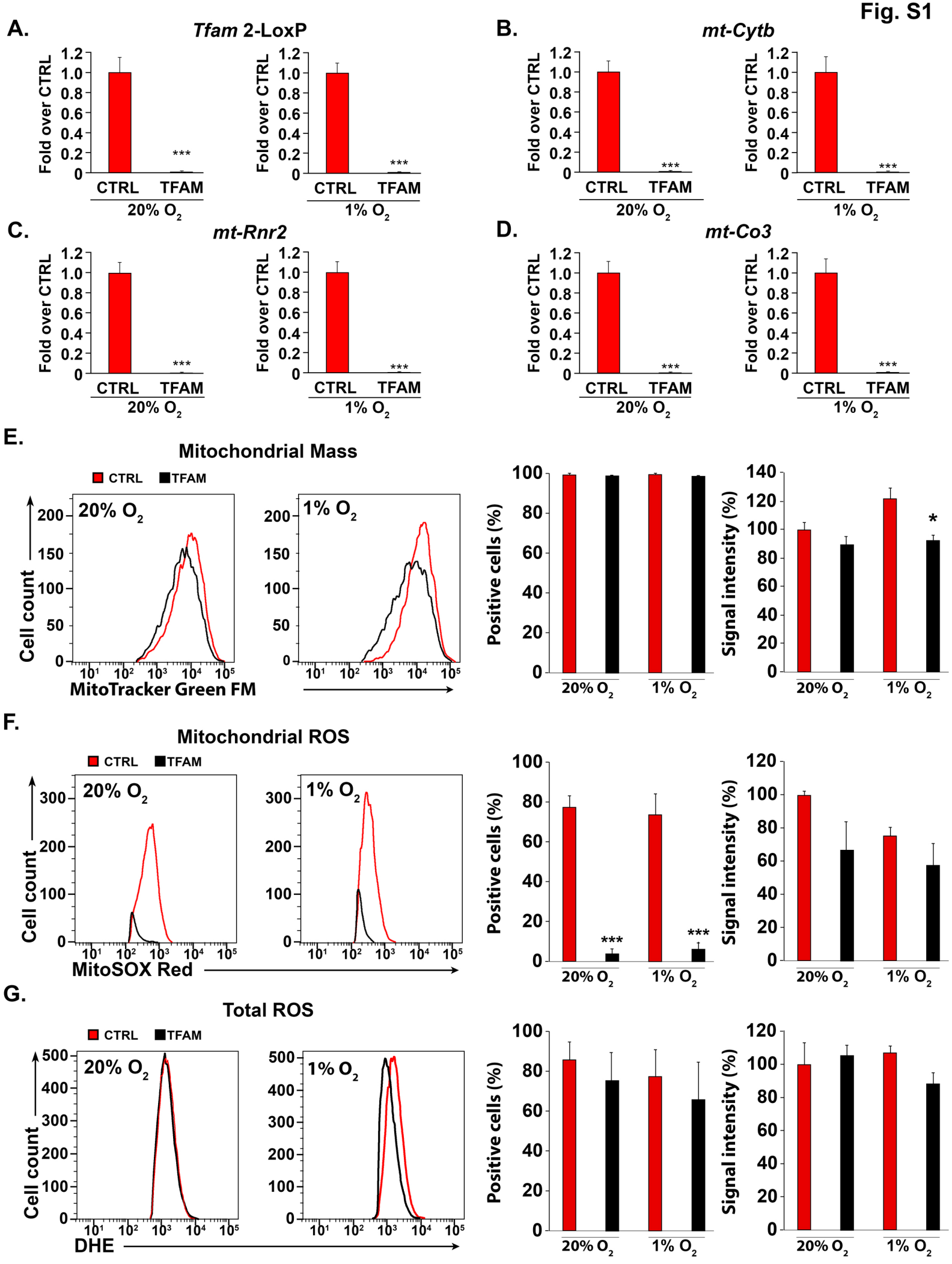

A.
Tfam 2-LoxP
B.
mt-Cytb
C.
mt-Rnr2
D.
mt-Co3
Fold over CTRL
CTRL
TFAM
20% O2
1% O2
***
E.
Mitochondrial Mass
CTRL
TFAM
Cell count
MitoTracker Green FM
Positive cells (%)
Signal intensity (%)
*
F.
Mitochondrial ROS
MitoSOX Red
G.
Total ROS
DHE

Figure S1: Characterization of chondrocytes isolated from Prx1-Cre;TFAM$^{fl/fl}$ newborn growth plates and cultured *in vitro,* Related to Figure 1.

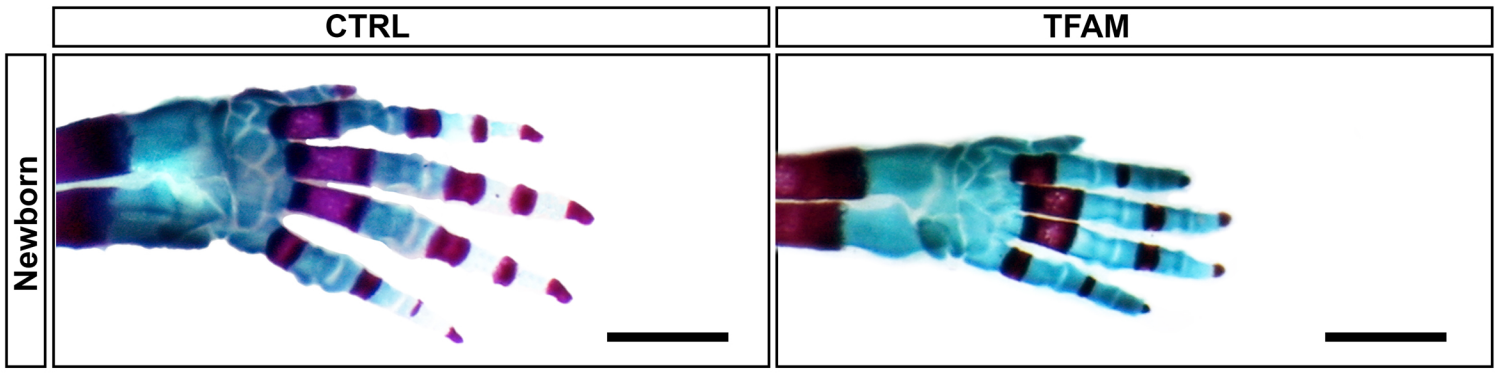
(A) 2-LoxP qPCR of genomic DNA extracted from chondrocytes isolated from newborn TFAM$^{fl/fl}$ (CTRL) or Prx1-Cre;TFAM$^{fl/fl}$ **(**TFAM) growth plates and cultured *in vitro* for 4-7 days in either 20% or 1% O_2. Data were normalized to β2-microglobulin (n=4).

(B, C, D) qPCR of mitochondrial DNA extracted from the same cells. Mitochondrial genes coding for *Cytochrome b* (*mt-Cytb*) (B), *16S rRNA* (*mt-Rnr2*) (C) and *Cytochrome c oxidase subunit 3* (*mt-Co3*) (D) were analyzed. Data were normalized to β2-microglobulin (n=4). Our data indicate that we successfully achieved homogenous *in vitro* cultures of both CTRL and TFAM cells.

(E, F, G) FACS analysis of mitochondrial mass (E), mitochondrial ROS (F) and total ROS (G) in chondrocytes isolated from CTRL or TFAM newborn growth plates and cultured as described above. A representative experiment is shown on the left; quantification of all biological replicates (n=4) is provided on the right. For the signal intensity, data are calculated as % of CTRL 20%O_2.

In all the graphs, data are presented as mean ± SEM; *p<0.05; ***p<0.01.

A.



B.

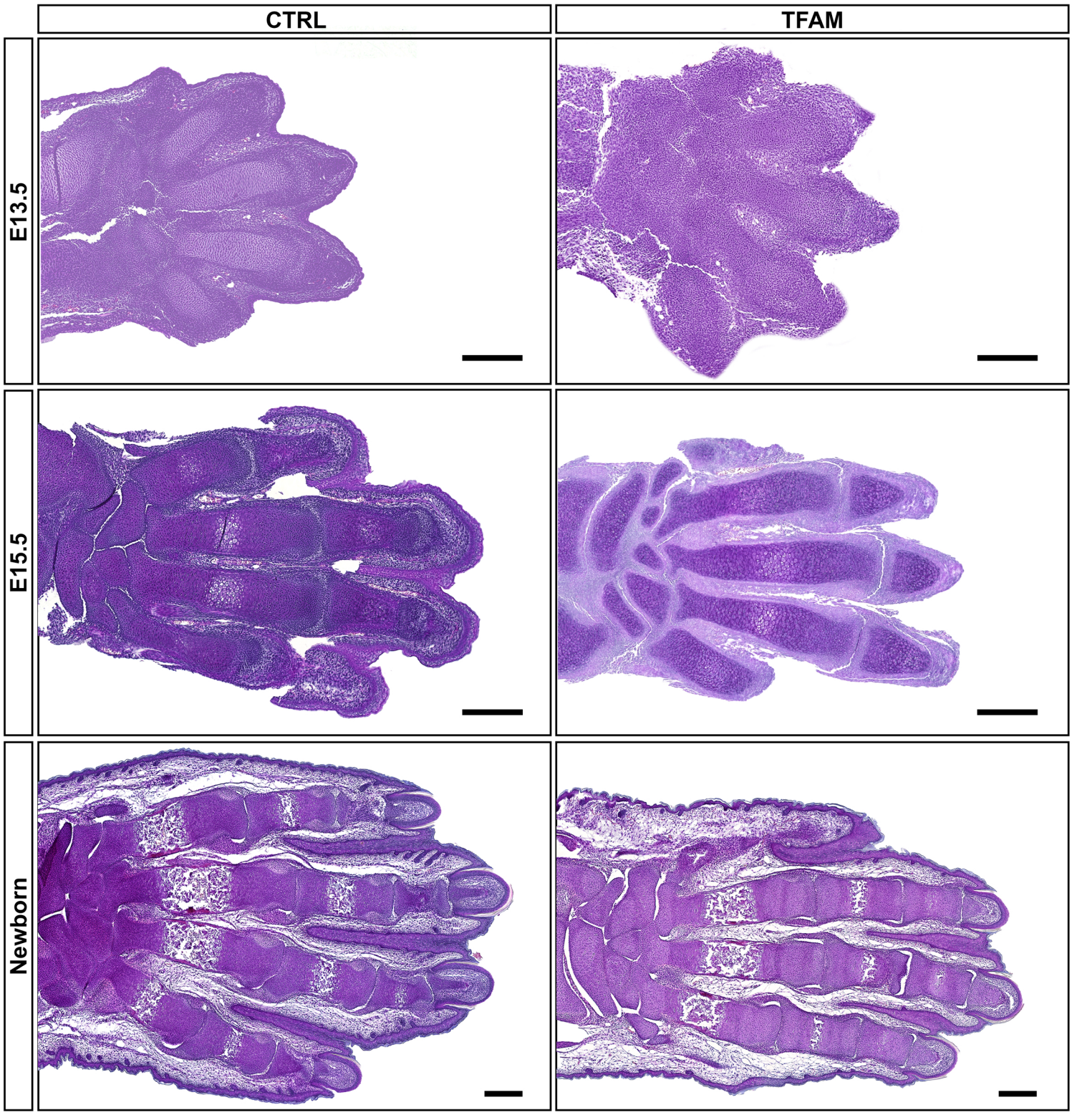
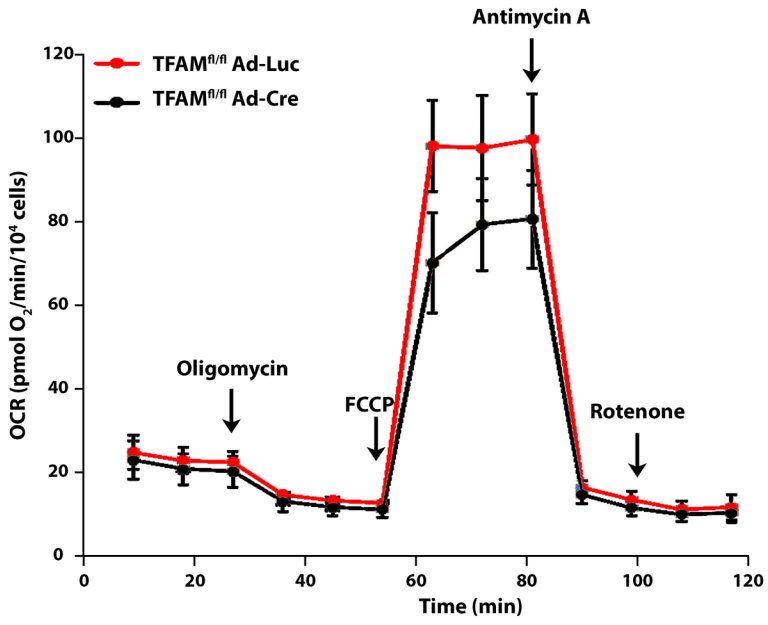


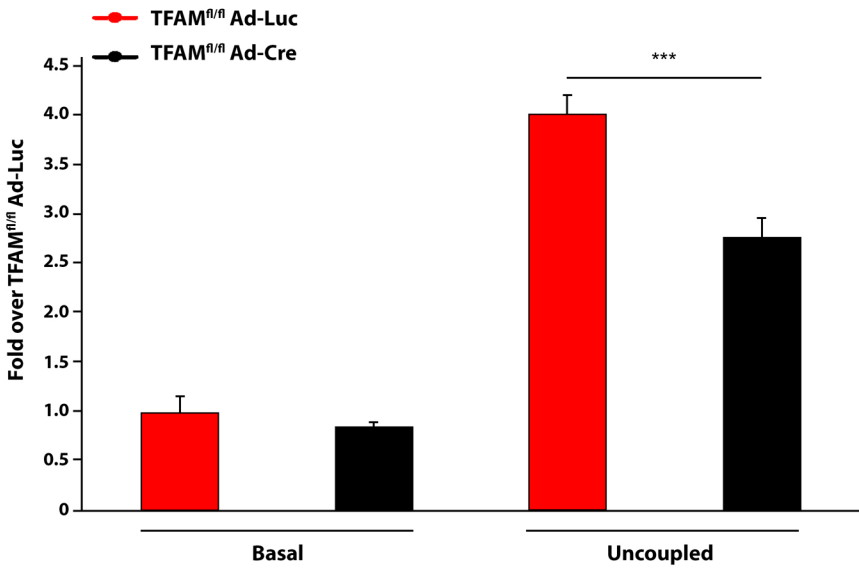
Figure S2: Normal joint formation in the autopod of mice lacking Tfam in the limb bud mesenchyme, Related to Figure 2.

(A) Whole mount Alcian Blue/Alizarin Red S staining of autopods isolated from newborn TFAM$^{fl/fl}$ **(**CTRL) or Prx1-Cre;TFAM$^{fl/fl}$ **(**TFAM) mice (n=7). Scale bars =1mm.

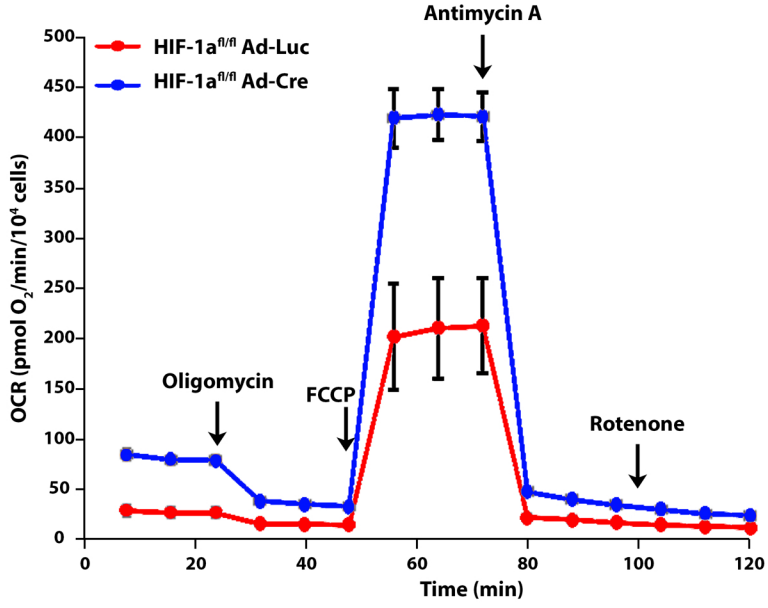
(B) H&E staining of autopods isolated from E13.5, E15.5, newborn CTRL and TFAM mice (n=3). Scale bars =250µm.

A.


Antimycin A
TFAM$^{fl/fl}$ Ad-Luc
TFAM$^{fl/fl}$ Ad-Cre
OCR (pmol O_2/min/10^4 cells)
Oligomycin
FCCP
Rotenone
Time (min)


TFAM$^{fl/fl}$ Ad-Luc
TFAM$^{fl/fl}$ Ad-Cre
***
Fold over TFAM$^{fl/fl}$ Ad-Luc
Basal
Uncoupled

B.


Antimycin A
HIF-1a$^{fl/fl}$ Ad-Luc
HIF-1a$^{fl/fl}$ Ad-Cre
OCR (pmol O_2/min/10^4 cells)
Oligomycin
FCCP
Rotenone
Time (min)

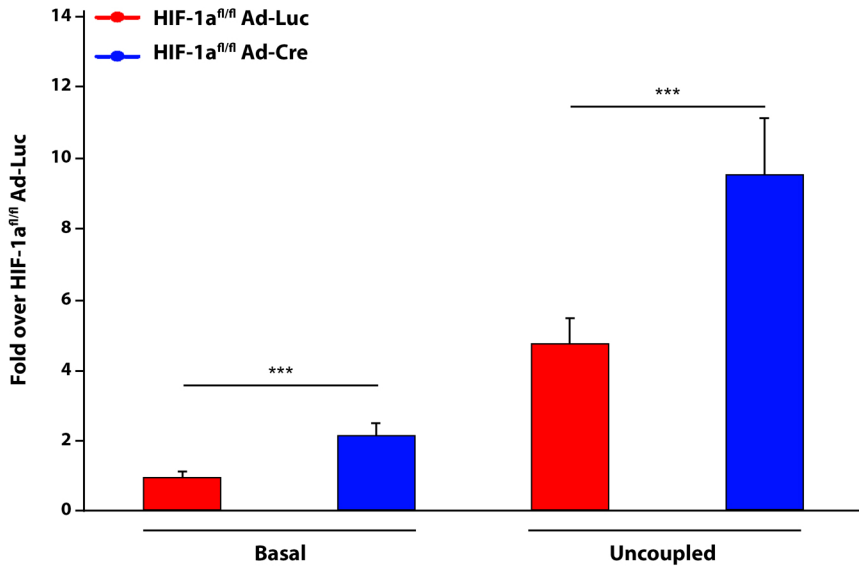
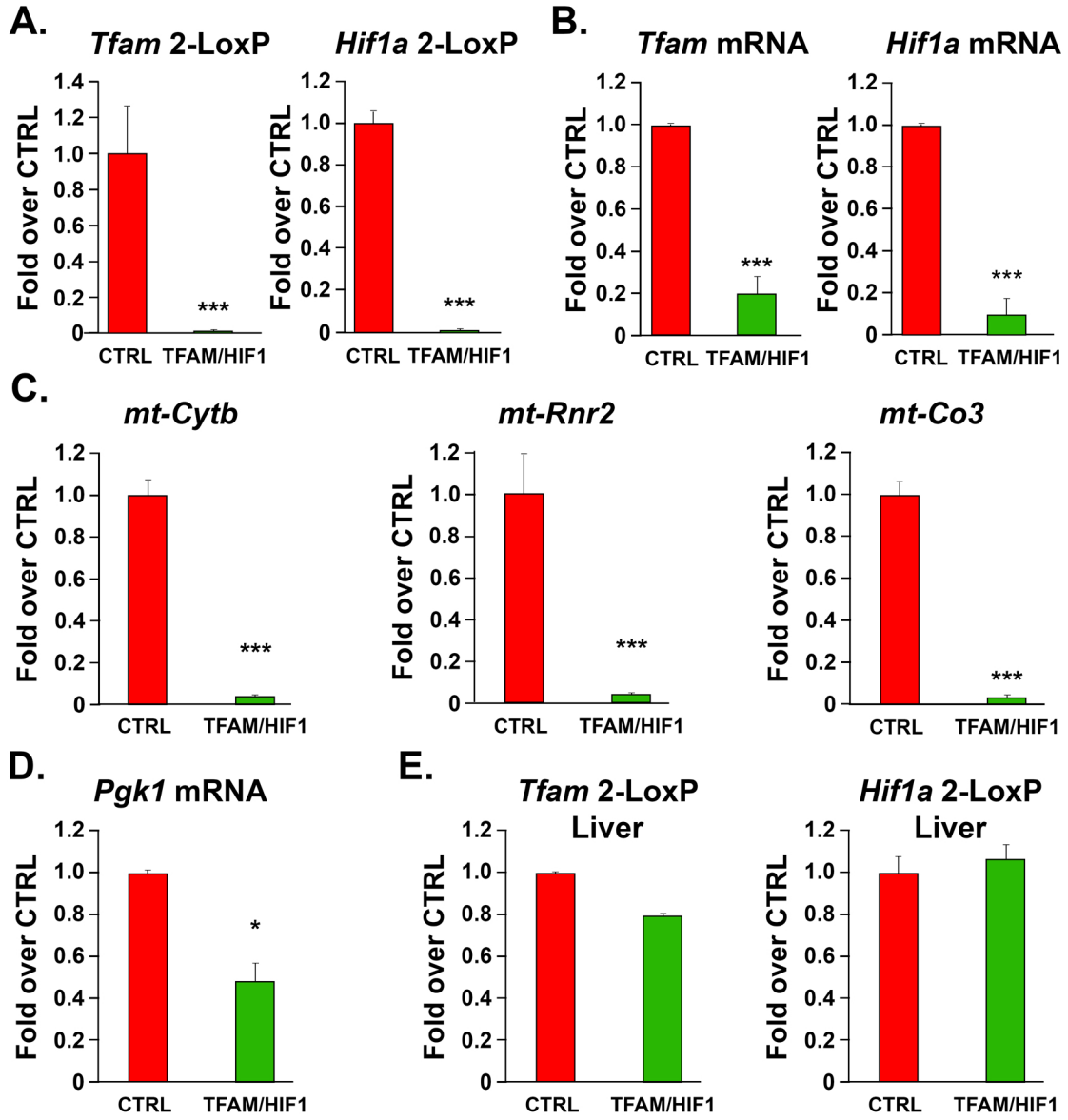

HIF-1a$^{fl/fl}$ Ad-Luc
HIF-1a$^{fl/fl}$ Ad-Cre
***
***
Fold over HIF-1a$^{fl/fl}$ Ad-Luc
Basal
Uncoupled

Figure S3: O_2 consumption rate in chondrocytes lacking Tfam or Hif1a, Related to Figures 2 and 3.

Chondrocytes were isolated from $TFAM^{fl/fl}$ (A)or $HIF\text{-}1a^{fl/fl}$ (B) mice and transduced *in vitro* with *Luciferase* Adenovirus (Ad-Luc, control) or *Cre* Adenovirus (Ad-Cre, mutant). O_2 consumption rate (OCR) was measured in control and mutant cells by Seahorse technology. A representative experiment is shown at the top and quantification of all biological replicates at the bottom (n=5). Data were calculated as fold over control at basal state. FCCP = carbonyl cyanide-p-trifluoromethoxyphenylhydrazone.

In the graph, data are presented as mean ± SEM; ***p<0.01.

Notably, loss of Hif1a increased O_2 consumption even in cells cultured in 20%O_2, which indicates that Hif1a has a biological function also in fetal growth plate chondrocytes cultured in normoxia despite its barely detectable levels of expression (**Figure S6**).


A.
Tfam 2-LoxP
Hif1a 2-LoxP
B.
Tfam mRNA
Hif1a mRNA
C.
mt-Cytb
mt-Rnr2
mt-Co3
D.
Pgk1 mRNA
E.
Tfam 2-LoxP Liver
Hif1a 2-LoxP Liver
Fold over CTRL
CTRL
TFAM/HIF1
***
*

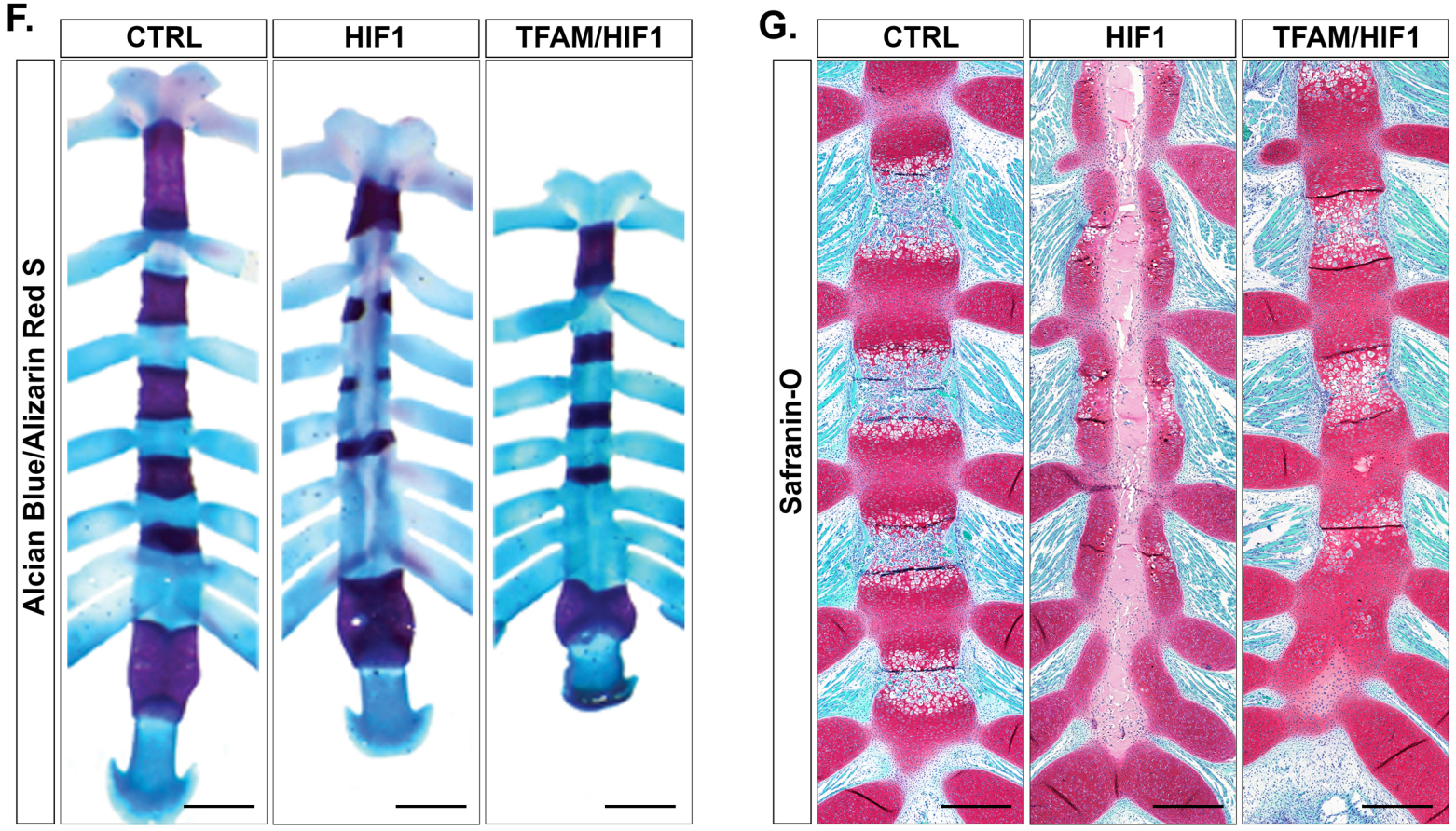

F.
CTRL
HIF1
TFAM/HIF1
Alcian Blue/Alizarin Red S
G.
Safranin-O

Figure S4: Recombination of the floxed *Tfam* locus and the floxed *Hif1a* locus in the Prx1-Cre;TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ newborn growth plate, Related to Figures 3 and 4.

(A) 2-LoxP qPCR of genomic DNA extracted from TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ (CTRL) and Prx1-Cre;TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ (TFAM/HIF1) newborn growth plates. Data were normalized to β2-microglobulin (n=5).

(B) Quantification of *Tfam* and *Hif1a* mRNAs by qRT-PCR of total RNA extracted from CTRL and TFAM/HIF1 newborn growth plates. Data were normalized to *TATA-box Binding Protein* (*TBP*) (n=3).

(C) qPCR of mitochondrial DNA extracted from newborn CTRL or TFAM/HIF1 growth plates. Mitochondrial genes coding for *Cytochrome b* (*mt-Cytb*), *16S rRNA* (*mt-Rnr2*) and *Cytochrome c oxidase subunit 3* (*mt-Co3*) were analyzed. Data were normalized to β2-microglobulin (n=5).

(D) Quantification of *phosphoglycerate-kinase 1* (*Pgk1*) mRNA, a direct downstream target of Hif1a (Schipani, et al., 2001), by qRT-PCR of total RNA extracted from newborn CTRL or TFAM/HIF1 growth plates. Data were normalized to *TBP (n=3)*.

(E) 2-LoxP qPCR for Tfam and Hif1a performed on genomic DNA extracted from CTRL and TFAM/HIF1 newborn livers. Data were normalized to β2-microglobulin (n=4).

(F) Whole-mount Alcian Blue/Alizarin Red S staining of newborn sterna isolated from TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ (CTRL), Prx1-Cre;TFAM$^{fl/+}$;HIF-1a$^{fl/fl}$ (HIF1) single mutant and Prx1-Cre;TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ (TFAM/HIF1) double mutant mice (n=4).

(G) Safranin-O staining performed on newborn CTRL, HIF1, and TFAM/HIF1 sterna (n=3). Note the severe hypocellularity in the inner core of the HIF1 sternum. The TFAM/HIF1 sternum was smaller than the CTRL sternum, but its histological

architecture was overall similar to CTRL. In regard to the Prx1-Cre transgenic mice, expression of Cre recombinase in the mesenchymal progenitors that give origin to the sternum has been previously documented (Mangiavini, et al., 2015). Scale bars=100μm.

In all the graphs, data are presented as mean ± SEM; *p<0.05, ***p<0.01.

Fig. S5

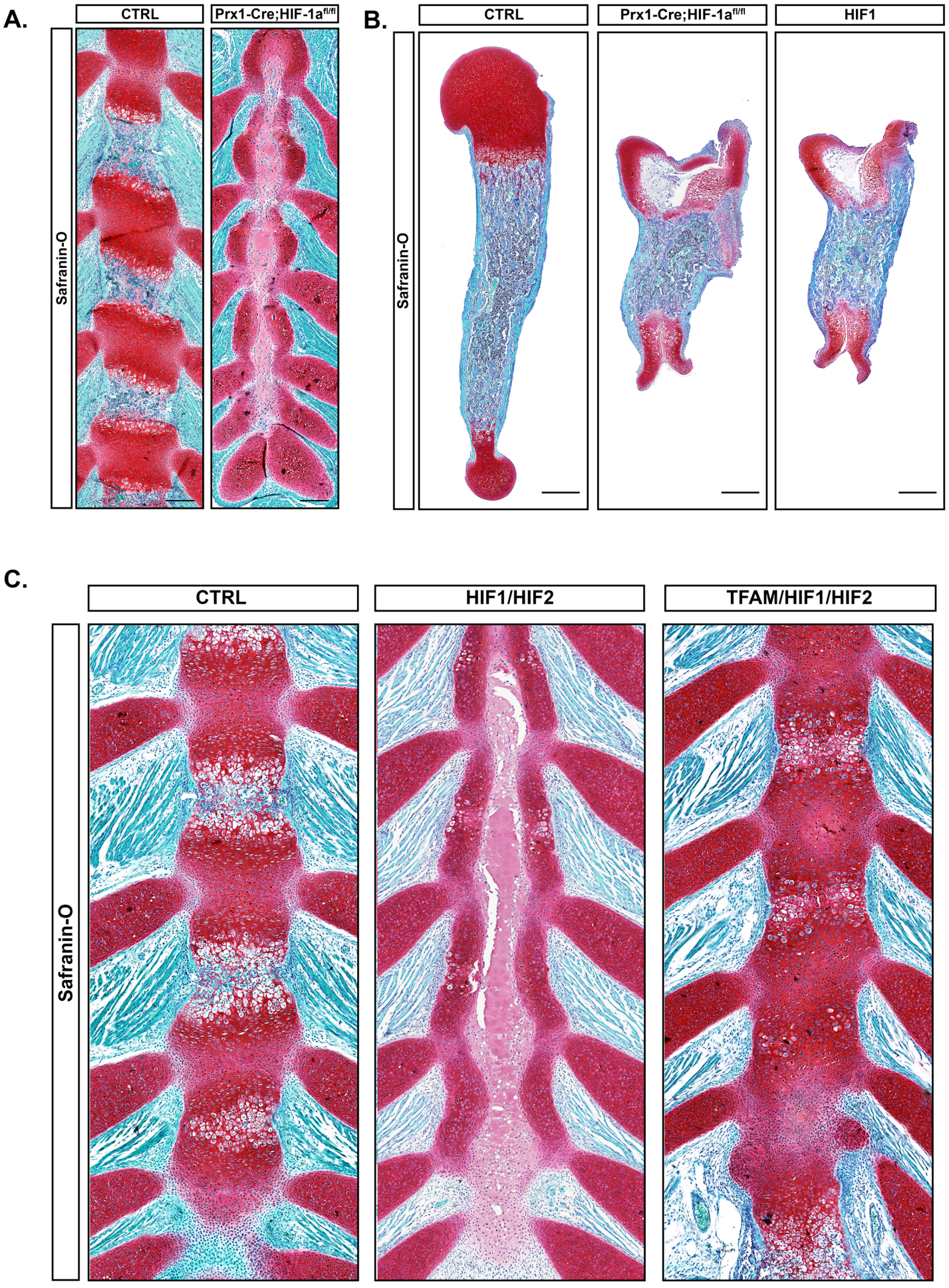

A.
CTRL
Prx1-Cre;HIF-1a^fl/fl
Safranin-O
B.
CTRL
Prx1-Cre;HIF-1a^fl/fl
HIF1
Safranin-O
C.
CTRL
HIF1/HIF2
TFAM/HIF1/HIF2
Safranin-O

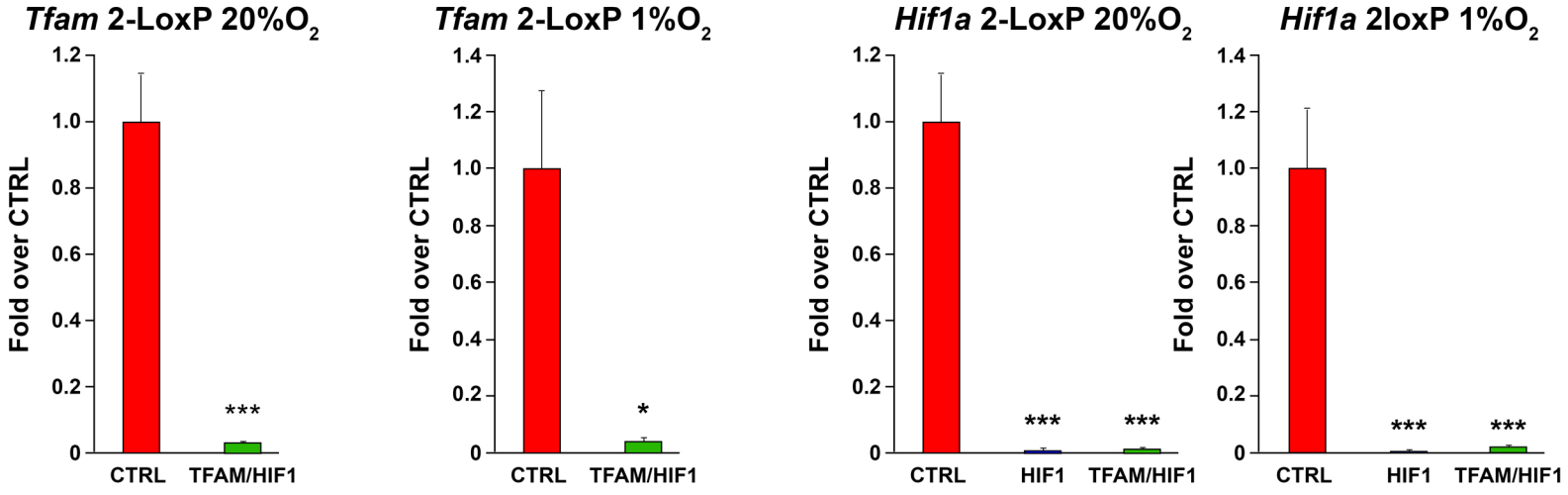
Figure S5: Loss of TFAM, Hif1a and Hif2a in the growth plate. Related to Figure 3.

(A) Safranin-O staining performed on newborn HIF-1a$^{fl/fl}$ (CTRL) and Prx1-Cre;HIF-1a$^{fl/f}$ sterna (n=3). Scale bars=200μm.

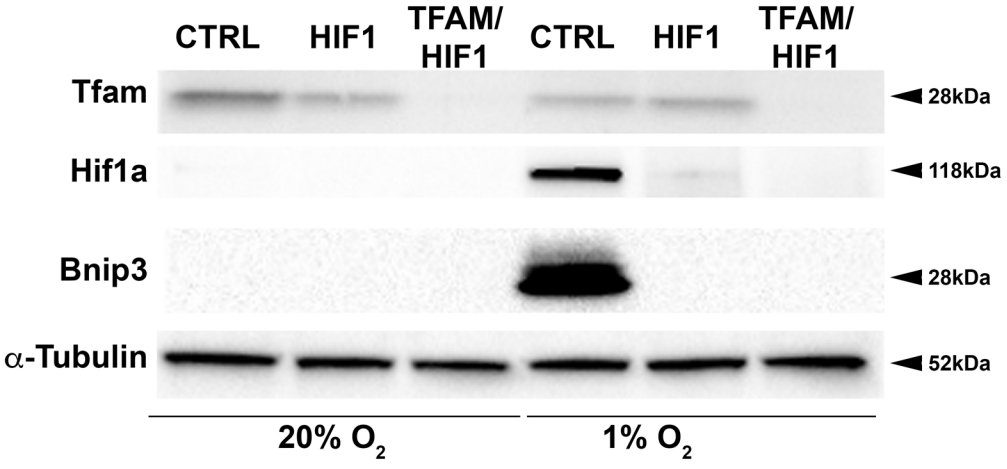
(B) Safranin-O staining performed on newborn HIF-1a$^{fl/fl}$ (CTRL), Prx1-Cre;HIF-1a$^{fl/f}$ and Prx1-Cre;TFAM$^{fl/+}$;HIF-1a$^{fl/fl}$ (HIF1) humeri (n=3). Scale bars=200μm.

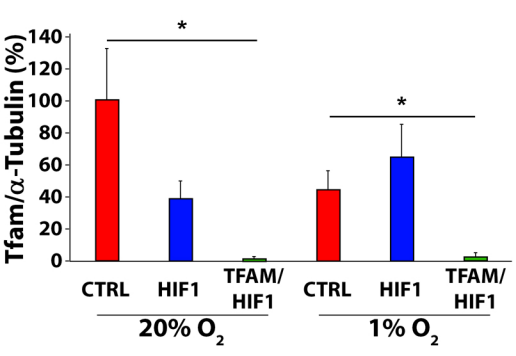
(C) Safranin-O staining performed on newborn TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$;HIF-2a$^{fl/fl}$ (CTRL) and Prx1-Cre;TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$;HIF-2a$^{fl/fl}$ (TFAM/HIF1/HIF2) sterna (n=3). Scale bars=200μm.

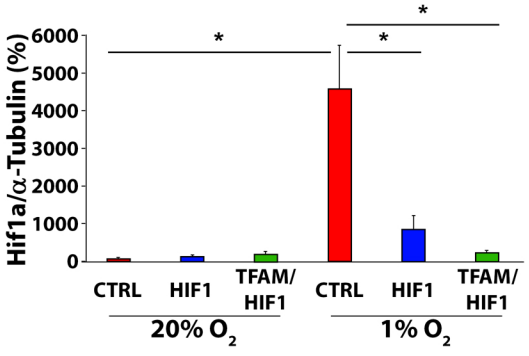
A.


Tfam 2-LoxP 20%O2
Tfam 2-LoxP 1%O2
Hif1a 2-LoxP 20%O2
Hif1a 2loxP 1%O2
Fold over CTRL
CTRL
HIF1
TFAM/HIF1
*
***

B.


CTRL
HIF1
TFAM/ HIF1
Tfam
Hif1a
Bnip3
α-Tubulin
28kDa
118kDa
52kDa
20% O2
1% O2


Tfam/α-Tubulin (%)
CTRL
HIF1
TFAM/ HIF1
20% O2
1% O2
*


Hif1a/α-Tubulin (%)
CTRL
HIF1
TFAM/ HIF1
20% O2
1% O2
*

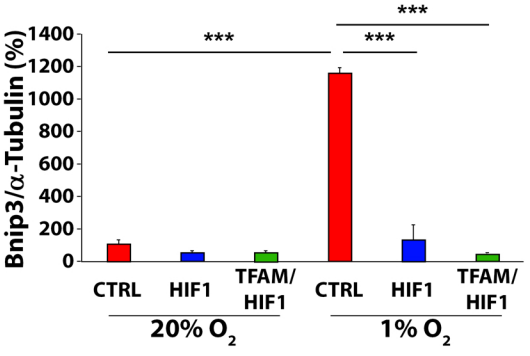

Bnip3/α-Tubulin (%)
CTRL
HIF1
TFAM/ HIF1
20% O2
1% O2
***

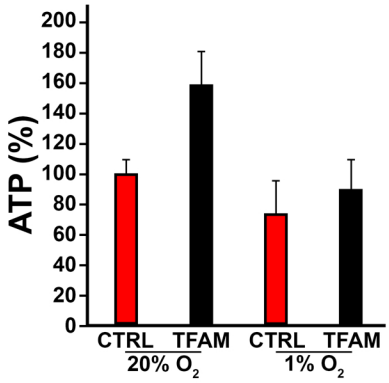
Figure S6: Recombination of the floxed *Tfam* and the floxed *Hif1a* loci in chondrocytes isolated from Prx1-Cre;TFAM$^{fl/+}$;HIF-1a$^{fl/fl}$ and Prx1-Cre;TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ newborn growth plates and cultured *in vitro*, Related to Figure 5.

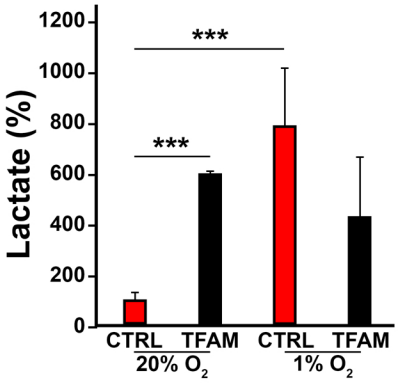
(A) 2-LoxP qPCR of genomic DNA extracted from chondrocytes isolated from TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ (CTRL), Prx1-Cre;TFAM$^{fl/+}$;HIF-1a$^{fl/fl}$ (HIF1) and Prx1-Cre;TFAM$^{fl/fl}$;HIF-1a$^{fl/fl}$ (TFAM/HIF1) newborn growth plates and cultured *in vitro* for 4-7 days in either 20% or 1% O_2. Data were normalized to β2-microglobulin (n=6).

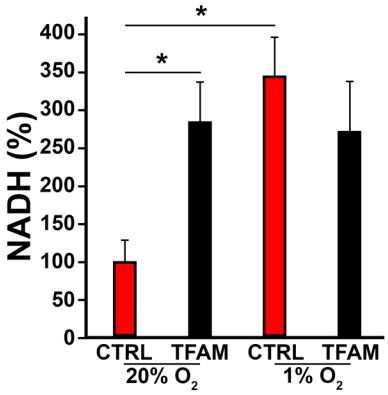
(B) Quantification of Tfam, Hif1a, and Bcl2 Interacting Protein 3 (Bnip3) by Western blot analysis of total protein lysate extracted from chondrocytes isolated from newborn CTRL, HIF1 and TFAM/HIF1 growth plates. Bnip3 is a direct downstream target of Hif1a (Zhang, et al., 2008). Representative Western blots are shown at the top and quantification of all biological replicates is provided at the bottom (n=3). Data were normalized to α-tubulin and calculated as % of the signal intensity of CTRL 20%O_2. In all the graphs, data are presented as mean ± SEM; *p<0.05, ***p<0.01.

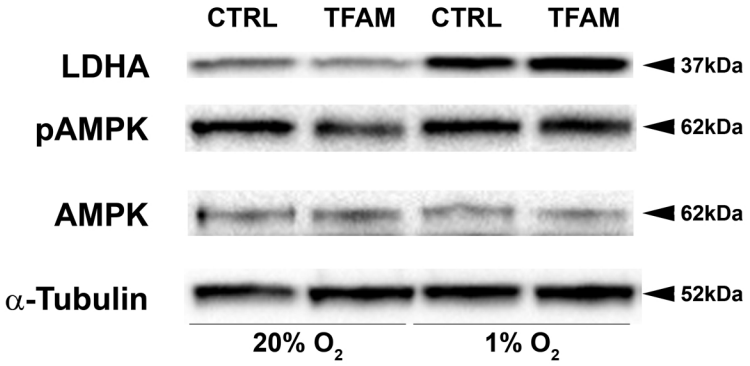
CTRL, HIF1 and TFAM/HIF1 cells were used for measurements of metabolic parameters, mitochondrial membrane potential, mitochondrial mass, total ROS and mitochondrial ROS.

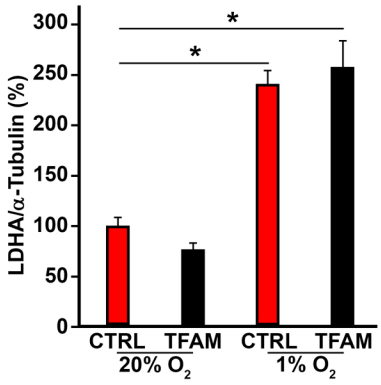
A.

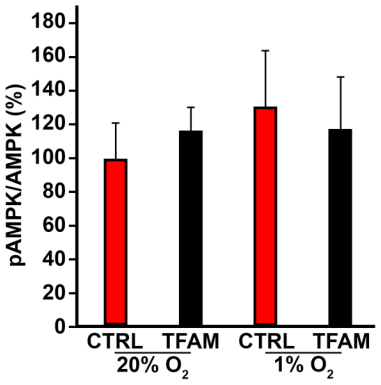

ATP (%)
200
180
160
140
120
100
80
60
40
20
0
CTRL TFAM CTRL TFAM
20% O2
1% O2


Lactate (%)
1200
1000
800
600
400
200
0
***
***
CTRL TFAM CTRL TFAM
20% O2
1% O2

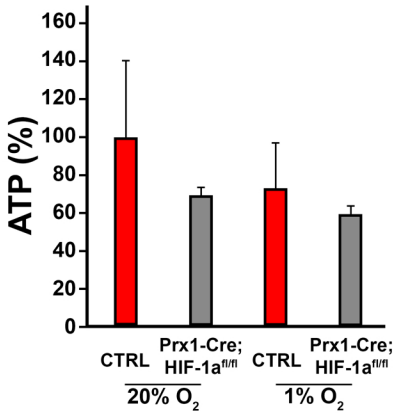

NADH (%)
400
350
300
250
200
150
100
50
0
*
*
CTRL TFAM CTRL TFAM
20% O2
1% O2


CTRL TFAM CTRL TFAM
LDHA
pAMPK
AMPK
α-Tubulin
37kDa
62kDa
62kDa
52kDa
20% O2
1% O2


LDHA/α-Tubulin (%)
300
250
200
150
100
50
0
*
*
CTRL TFAM CTRL TFAM
20% O2
1% O2


pAMPK/AMPK (%)
180
160
140
120
100
80
60
40
20
0
CTRL TFAM CTRL TFAM
20% O2
1% O2

B.


ATP (%)
160
140
120
100
80
60
40
20
0
CTRL
Prx1-Cre; HIF-1afl/fl
CTRL
Prx1-Cre; HIF-1afl/fl
20% O2
1% O2

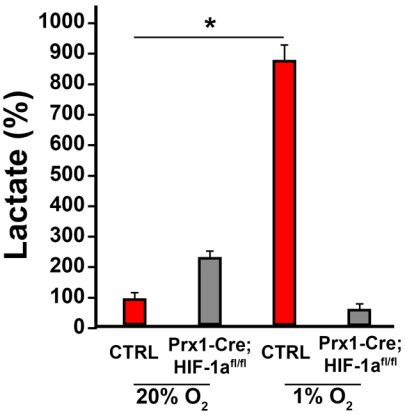

Lactate (%)
1000
900
800
700
600
500
400
300
200
100
0
*
CTRL
Prx1-Cre; HIF-1afl/fl
CTRL
Prx1-Cre; HIF-1afl/fl
20% O2
1% O2

Figure S7: Energy levels in Prx1-Cre;TFAM$^{fl/fl}$ chondrocytes, Related to Figure 5.

(A) Measurements of intracellular ATP, intracellular lactate and intracellular NADH in chondrocytes isolated from TFAM$^{fl/fl}$ (CTRL) and Prx1-Cre;TFAM$^{fl/fl}$ (TFAM) newborn growth plates and cultured *in vitro* in either 20% or 1%O_2. Data were calculated as % of CTRL 20% O_2 (n=3).

(B) Quantification of lactate dehydrogenase A (LDHA) and phospho-adenosine monophosphate activated-protein kinase (pAMPK) by Western blot analysis of total protein lysate extracted from chondrocytes isolated from CTRL and TFAM newborn growth plates. Representative Western blots are shown on the left and quantification of all biological replicates is provided on the right. Data for LDHA and pAMPK were normalized to α-Tubulin and AMPK respectively, and calculated as percentage of the signal intensity of CTRL 20%O_2 (n=3).

In all the graphs, data are presented as mean ± SEM; *p<0.05, ***p<0.01.