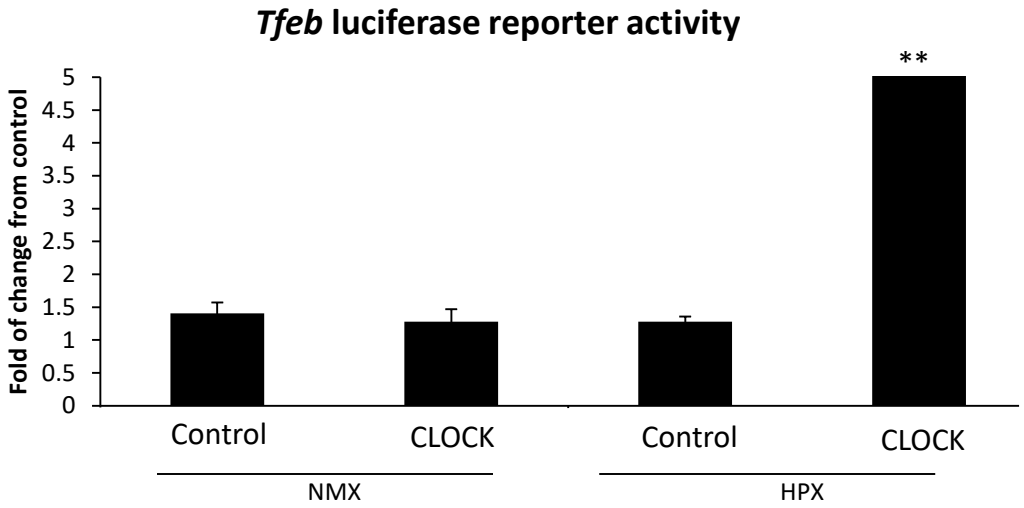

Figure S1. *Tfeb* promoter activation is dependent on CLOCK. *Tfeb* luciferase reporter activity in cardiac myocytes in the absence and presence of Ad*Clock*. Data are expressed as mean± SE. Statistical significance is indicated: **P<0.01.

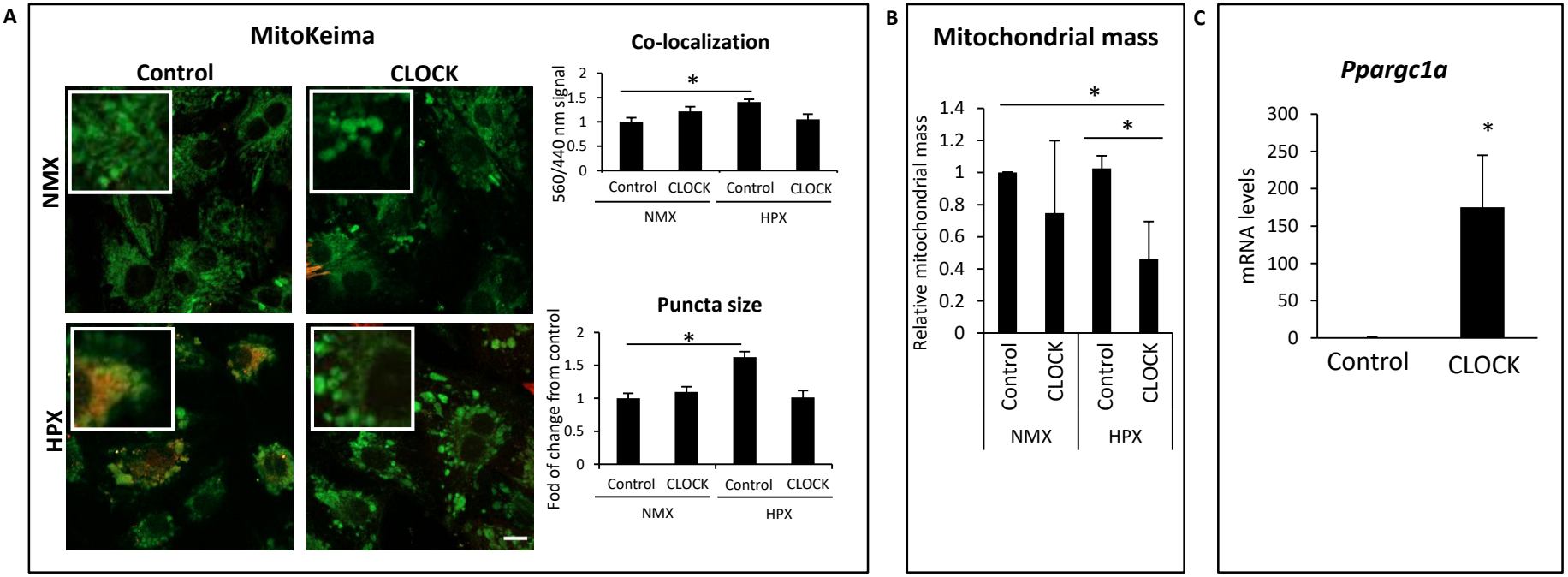

Figure S2. CLOCK restores mitochondrial autophagy and biogenesis. (**A**), Left, representative images of MitoKeima staining, as an index of mitophagy in cardiac myocytes under normoxic (NMX) and hypoxic (HPX) conditions 18 h in the absence and presence of AdCMV or Ad*Clock*, bars: 10 μm. Magnified regions are depicted by the white boxes, green fluorescent puncta demark mitochondria that are not fused with lysosomes (neutral pH), red/yellow fluorescent puncta demark mitochondria that have fused with lysosomes (acidic pH) indicative of mitophagy. Right, analysis of conditions tested on the left panel. (**B**), Mitochondrial mass in cardiac myocytes under NMX or HPX conditions, viral control AdCMV or Ad*Clock*. (**C**), mRNA levels of *PPARGC1A* in rat neonatal cardiac myocytes. Data are expressed as mean ± SEM. *p<0.05, n=3-4 independent myocyte isolations.

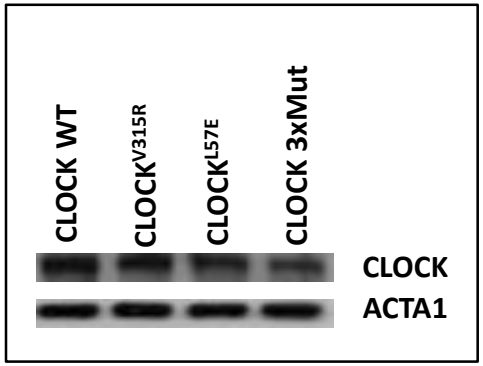

Figure S3. CLOCK protein expression levels. Top, western blot analysis of CLOCK protein expression in cells transfected with *Clock* WT or with CLOCK mutants designated as CLOCK^{V315R}, CLOCK^{L57E}, CLOCK 3xMut expression vectors; Bottom, ACTA1 expression levels for loading control.

Table S1. A partial list of genes altered in CLOCK Δ19/Δ19 hearts by transcriptome analysis.

Metabolic Process		Gene
OXPHOS	Complex I	*Ndufs1, Ndufa8, Ndufa4, Ndufb10, Ndufa3, Ndufb3, Ndufa4*
	Complex III	*Uqcrb, Uqcc2, Uqcrc2, Uqcrc1*
	Complex IV	*Cox6a2, Cox4i2, Cox4i1, Cox7b, Cox7a2l, Cox6c*
	Complex V	*Atp5e, Atp5l, Atpif1*
	OXPHOS related	*Coq5, Coq10b, Coq3, Coq9*
TCA cycle		*Acot9, Acot1, Acot2, Acot13, Acot1, Idh2, Pdp2, Sucla2*
Dynamics		*Mtfp1, Mff, Prkn*

Table S2. Table of primer sequences.

Gene	Primer A	Primer B
Atg7 (Rat)	GCAGCCAGCAAGCGAAAG	TCTCATGACAACAAAGGTGTCAAA
Clock (Mouse)	CTCCTCTAGAAGCTCACGAA	GCTCCTAACTGAGCTGAAAA
Clock⊿19 (genotyping)	AGCACCTTCCTTTGCAGTTCG	TGTGCTCAGACAGAATAAGTA
Clock WT (genotyping)	GGTCAAGGGCTACAGGTA	-
Control (genotyping)	TGGGGTAAAAAGACCTCTTGCC	-
Rpl32 (Rat+Mouse)	TAAGCGAAACTGGCGGAAAC	GCTGCTCTTTCTACGATGGCTT
Mfn2 (Rat+Mouse)	CACAGAGCTGGACATCTGGA	AGAGCCGCTCATTCACCTTA
Opa1 (Rat+Mouse)	ATACTGGGATCTGCTGTTGG	AAGTCAGGCACAATCCACTT
Sqstm1 (Mouse)	AGAAGTGGACCCATCCACAG	GAGAAACCCATGGACAGCAT
Rab7 (Mouse)	CTGCCTTCTGGTGTTTGATGT	CTGGCCTGGATGAGAAACTCG
si*Clock*	GGAGCCAUCCACCUAUGAATT	UUCAUAGGUGGAUGGCUCCTT
sc*Clock*	GGAACCUACCCAUUGCGAATT	UUCGCAAUGGGUAGGUUCCTT
Tfeb (Mouse)	GCTACATATCAGCTCCAACC	TTAGCTCTCGCTTCTGAGTC
Tfeb (Rat)	GACTCAGAAGCGAGAGCTAA	GACTTCTGCAGGTCTTTCTG
Tfam (Rat)	CCGAAGTGTTTTTCCAGCAT	GGCTGCAATTTTCCTAACCA