Supplementary data to :

Human skeletal myotubes display a cell-autonomous circadian clock implicated in basal myokine secretion

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Table of Contents

Supplementary Tables

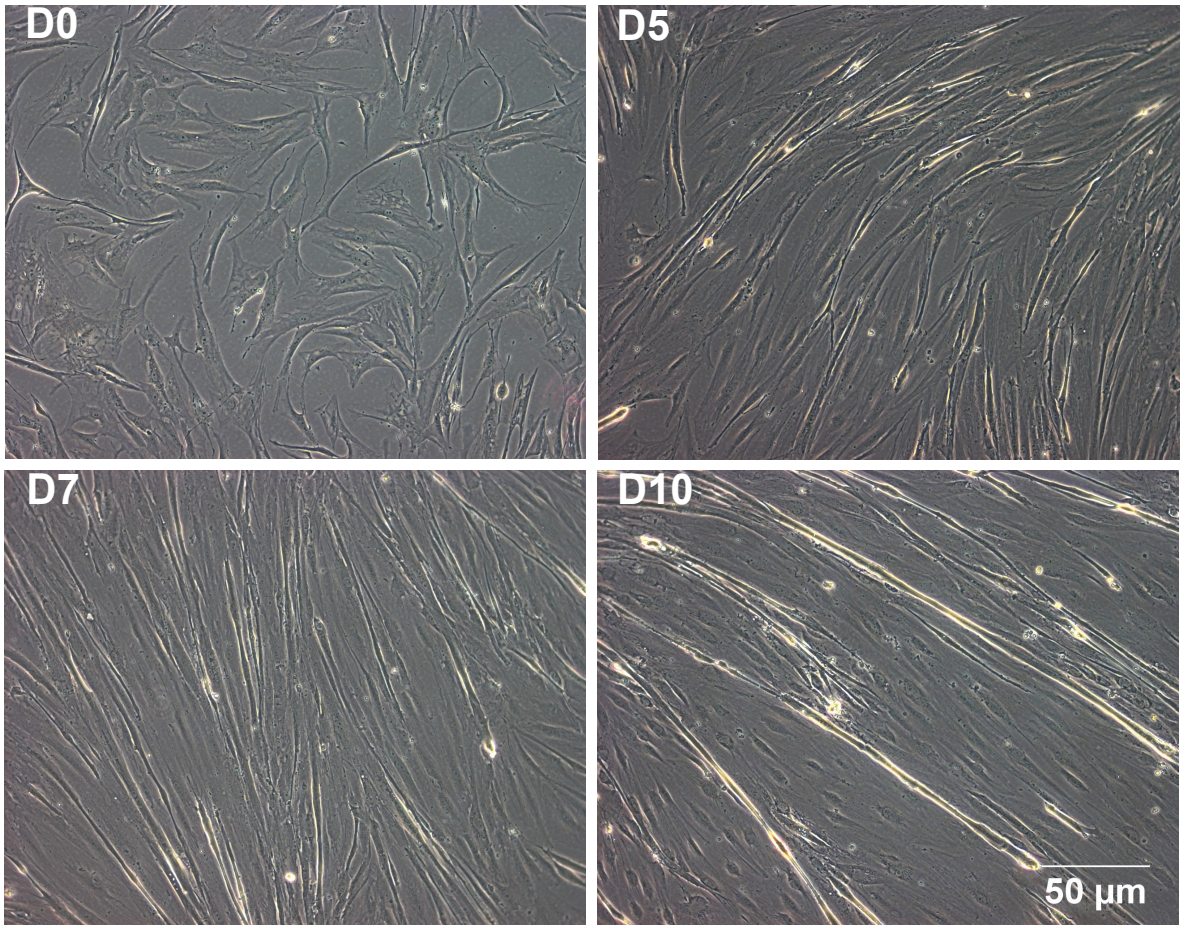
Supplementary Table 1

Sequences of human qPCR primers		
Gene name	Primer sequence	
BMAL1	Forward	5'-CCCTTGGACCAAGGAAGTAGAA-3'
	Reverse	5'-CTTCCAGGACGTTGGCTAAAAC-3'
CRY1	Forward	5'-GAGAACAGATCCCAATGGAGACTATAT-3'
	Reverse	5'-CCTCTTAGGACAGGCAAATAACG-3'
REV-ERBα	Forward	5'-GATCGTGAGTCGCGGGGTCC-3'
	Reverse	5'-TGTAGGTGATGACGCCACCTGTGT-3'
PER2	Forward	5'-CGCAGGGTGCGCTCGTTTGAA-3'
	Reverse	5'-GCTGGGCTCTGGAACGAAGCTTTCG-3'
PER3	Forward	5'-CCTGGACCCTGAACATGCA-3'
	Reverse	5'-TGTGAGCCCCACGTGTTTAA-3'
DBP	Forward	5'-TAGAAGGAGCGCCTTGAGTC-3'
	Reverse	5'-GCAACCCTCCAGTATCCAGA-3'
CLOCK	Forward	5'-CAAGCCACCGCAACAATT-3'
	Reverse	5'-GGATTCCCATGGAGCAACCTA-3'
IL6	Forward	5'-GGTACATCCTCGACGGCATCT-3'
	Reverse	5'-GTGCCTCTTTGCTGCTTTCAC-3'
9S	Forward	5'-CTCCGGAACAAACGTGAGGT-3'
	Reverse	5'-TCCAGCTTCATCTTGCCCTC-3'
GAPDH	Forward	5'-GAAGGTGAAGGTCGGAGTC-3'
	Reverse	5'-GAAGATGGTGATGGGATTTC-3'
HPRT	Forward	5'-GATTTTATCAGACTGAGGAGC-3'
	Reverse	5'-TCCAGTTAAAGTTGAGAGATC-3'

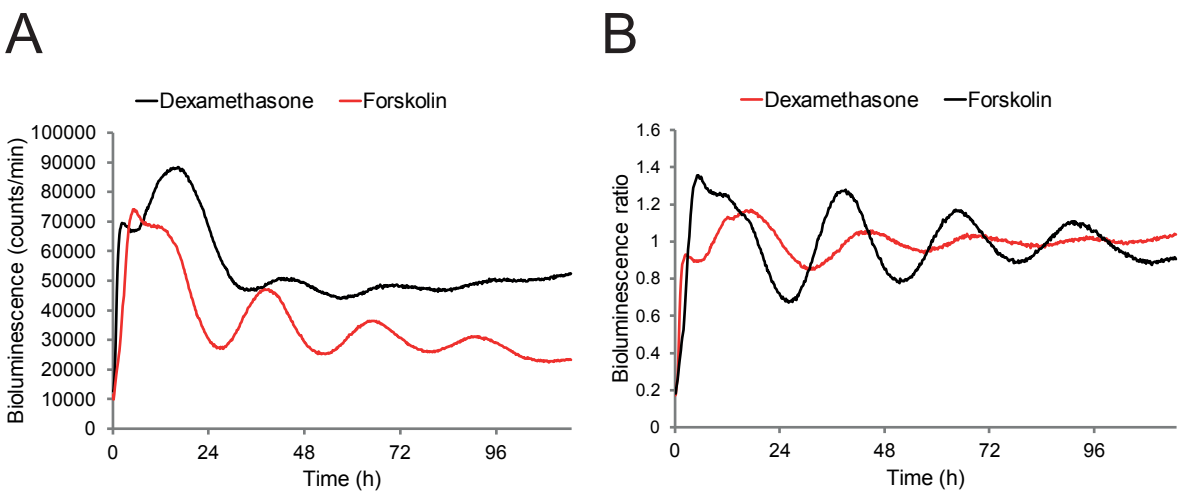
Supplementary Table 2

Amplitude of *siControl* and *siClock*-transfected samples determined by JTK_CYCLE				
	BMAL1	***REV-ERBα***	***PER3***	***DBP***
	Normalized mean amplitude (fold change)			
siControl	0.62	0.77	2.6	5.91
siClock	0.8	0.51	1.46	2.99

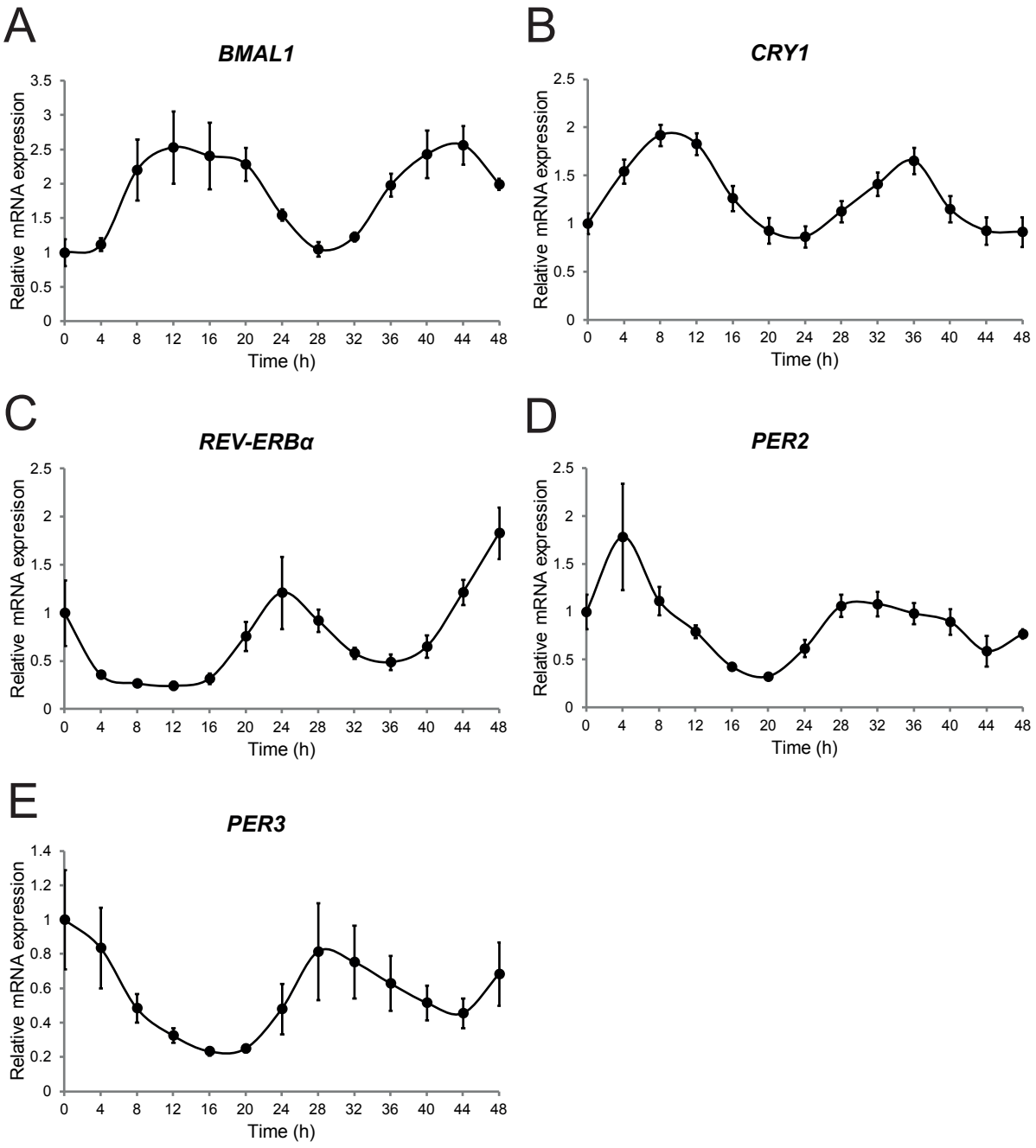
Supplementary Figures



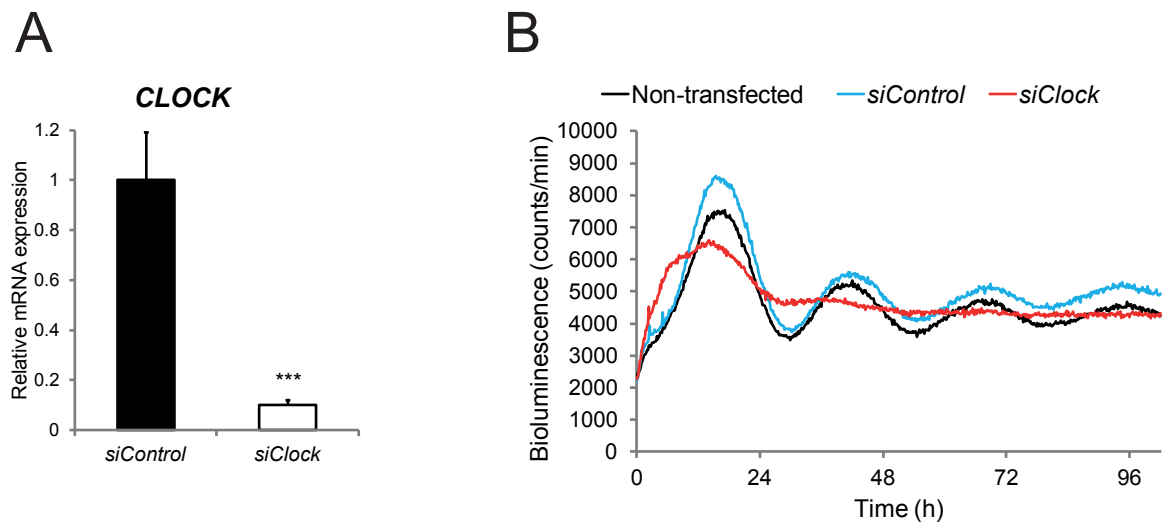
Supplementary Figure 1: Differentiation of *in vitro* cultured human skeletal myoblasts to myotubes.
Representative photos were taken with a Zeiss Axiovert 200 microscope at day 0 (D0), D5, D7, and D10 following start of the differentiation process (switch from 20% to 2% FBS-containing medium, see Material and Methods for details). D0: start of the differentiation process; D5: cells are oriented in the same direction and fusion has already begun; D7: differentiation is complete; D10: myotubes keep the differentiated status for several days.



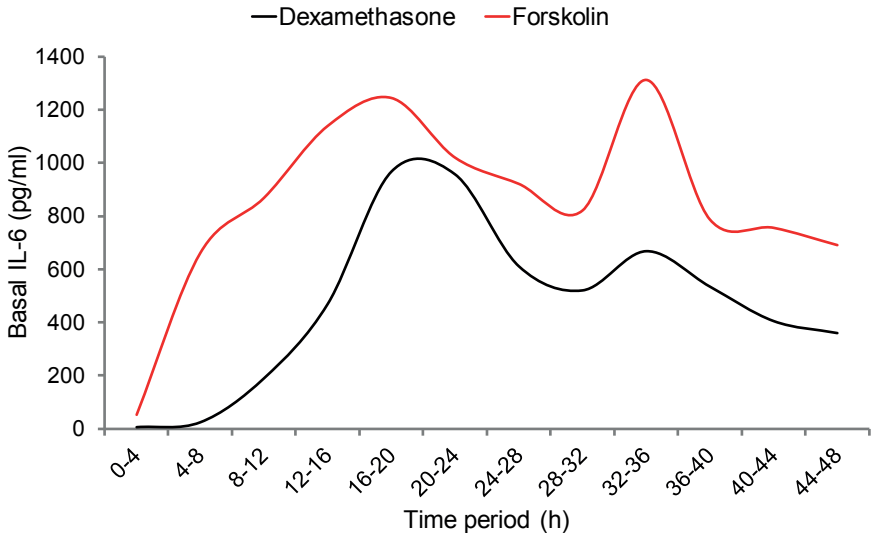
Supplementary Figure 2: *In vitro* synchronization by forskolin or dexamethasone induces pronounced circadian oscillations in human skeletal myotubes.
Bmal1-luc bioluminescence profiles were recorded from human myotubes, synchronized with a 30 min pulse of 100 nM dexamethasone (black line) or with a 1 h pulse of 10 μM forskolin (red line). Raw (A) and detrended (B) oscillation profiles are representative of 2 independent experiments (one donor each) performed in duplicates.



Supplementary Figure 3: Oscillation profiles of core clock transcripts in dexamethasone-synchronized human myotubes.
Endogenous expression of core clock transcripts was assessed in dexamethasone-synchronized myotubes, collected every 4 h during 48 h following synchronization. RT-qPCRs were performed in RNA samples extracted from human myotubes to assess (A) *BMAL1*, (B) *CRY1*, (C) *REV-ERBα*, (D) *PER2*, (E) *PER3*, and normalized to the mean of *9S-GAPDH*. Profiles are representative of $n = 4$ experiments (mean ± SEM), each performed with myotubes from one donor in duplicates for every condition.



Supplementary Figure 4: Impact of CLOCK knockdown on the oscillatory profile of dexamethasone-synchronized human myotubes.
Myoblasts were transfected with *siClock* or *siControl* and differentiated into myotubes. (A) *CLOCK* transcript expression, as assessed by RT-qPCR and normalized to the mean of *9S-GAPDH*, was reduced 90 ± 2.2% (mean ± SEM, $n = 5$) in *siClock* transfected samples compared to *siControl* counterparts (*** $p < 0.001$). (B) Representative *Bmal1-luc* oscillation profiles in *siControl*-transfected (blue line), *siClock*-transfected (red line), or non-transfected (black line) human myotubes, synchronized with dexamethasone. *Bmal1-luc* oscillation profiles were recorded in duplicates in $n = 8$ experiments (one donor per experiment).



Supplementary Figure 5: Basal IL-6 protein secretion profile after dexamethasone or forskolin synchronization.
Human myotubes, transduced with the *Bmal1-luc* lentivector, were synchronized with dexamethasone (black line) or with forskolin (red line), and perifused for 48 h with culture medium; n = 2 experiments with one donor each. The perifusion outflow medium was collected in 4 h intervals and basal IL-6 levels in the medium were assessed by ELISA (0-4 corresponds to the accumulation of IL-6 between 0 and 4 h), normalized to the total DNA content.