Supplementary Data

Supplementary Materials and Methods

Materials

Antibiotics (G418, penicillin, and streptomycin), dimethyl sulfoxide (DMSO), bilirubin, CORM, iCORM, SybrGreen Kit for mRNA analysis, anti-MHC, and anti-Myosin Skeletal Fast antibodies, Hoechst, eosin, hematoxylin, Triton X-100, glycine, diaminobenzidine (DAB Fast), and Bicinchonic Acid Protein Assay Kit were from Sigma-Aldrich. DMEM HG medium, phosphate-buffered saline (PBS) and Tris-base saline (TBS), fetal calf serum (FCS), and horse serum were from PAA Laboratories. CoPP and SnPP were from Frontier Scientific. Oligo(dT) primers, dNTP, Reporter Lysis Buffer, Luciferise Activity Assay, and CytoTox 96 Non-Radioactive Cytotoxicity Assay were from Promega. M-MLV reverse transcriptase and Fenozol Total RNA Isolation Reagent were from A&A Biotechnology. MirVana miRNA Isolation Kit, HMOX1 siRNA, pre-miRNAs, and siPORT were from Ambion. NCode miRNA First-Strand cDNA Synthesis Kit, OptiMEM medium, Alexa Fluor 546 and 488 secondary antibodies were from Invitrogen. SybrGreen Kit for miRNA was from Applied Biosystem. miRNome microRNA profiler QuantiMir™ was from System Biosciences. EnzyChrome™ Creatine Kinase Assay Kit was from BioAssays Systems. Goat serum was from GE Healthcare. Anti-PCNA antibody and mounting medium were from DAKO. Luciferin was from Caliper Life Science. Paraffin was from Thermo Scientific. Canadian balsam was from Paul Marienfeld GmbH. Primary anti-GFP and secondary IgG-HRP antibodies were from Abcam. BD™ CBA Mouse Inflammation Kit was from Becton-Dickinson. Qiazol Lysis Reagent was from Qiagen. NaOH, $FeCl_3$, paraformaldehyde, xylene, ethanol, methanol, and citrate buffer were from Polskie Odczynniki Chemiczne. Plasmid pcDNA3.1(-)-c/EBPδ was kindly provided by Dr. Peter F. Johnson (Bethesda, MD), plasmid CMV-MyoD (Addgene plasmid 8398) was kindly provided by Dr. Andrew Lassar (Boston, MA) (5), plasmid pFLAG/HA-DGCR8 (Addgene plasmid 10921) was kindly provided by Dr. Thomas Tuschl (New York, NY) (4).

Total RNA isolation

Cells were rinsed twice with ice-cold PBS, overlaid with fenozol (400 μL), scratched, transferred to the Eppendorf tubes, and mixed with chloroform (100 μL). The mixture was vortexed (30 sec), incubated on ice (20 min), and centrifuged (8000 *g*, 20 min, 4°C). Then the aqueous phase was transferred to new Eppendorf tubes, mixed with equal amount of isopropanol, incubated overnight at −20°C, and centrifuged (8000 *g*, 30 min, 4°C). Pellets were washed twice with ice-cold 70% ethanol and centrifuged (8000 *g*, 10 min, 4°C). Finally, the pellets were air-dried and resuspended in 15 μL of water. Concentration and quality of RNA was determined by measuring the absorbance at 260 nm and 280 nm.

Fragments of gastrocnemius muscles were snap-frozen in liquid nitrogen and stored at −80°C. Then they were homogenized in 1 mL of quiazol using Tissue Lyzer (Qiagen). Samples were transferred to Eppendorf tubes, supplied with 250 μL of chloroform, and treated as described above.

Reverse transcription and real time PCR analysis

RNA (1 μg) was reversely transcribed using M-MLV reverse transcriptase and oligo(dT) primers. Samples were incubated at 72°C for 10 min, cooled on ice for 1 min, and then the reaction was run at 42°C for 1 h, followed by enzyme inactivation at 95°C for 10 min. cDNA was diluted 10-fold in water and used as a template for real time PCR with SybrGreen Mix according to the following protocol: denaturation at 95°C, 10 min; 40 cycles of 95°C, 30 sec, specific primer annealing temperature (Supplementary Table S3), 60 sec; 72°C, 45 sec; final elongation at 72°C, 10 min. Gene expression was normalized to an elongation factor-2 (EF2) gene.

Analysis of miRNA profile

Total RNA including small RNA fraction was isolated with mirVana™ miRNA Isolation Kit, according to vendor's instruction. Concentration and quality of total RNA was determined by measuring the absorbance at 260 nm and 280 nm. Mature and precursor miRNAs were quantified using BioAnalyser 2100 (Agilent) by measuring the concentration of 21–23 and 60–75 nucleotide particles, respectively. Results were normalized to total RNA.

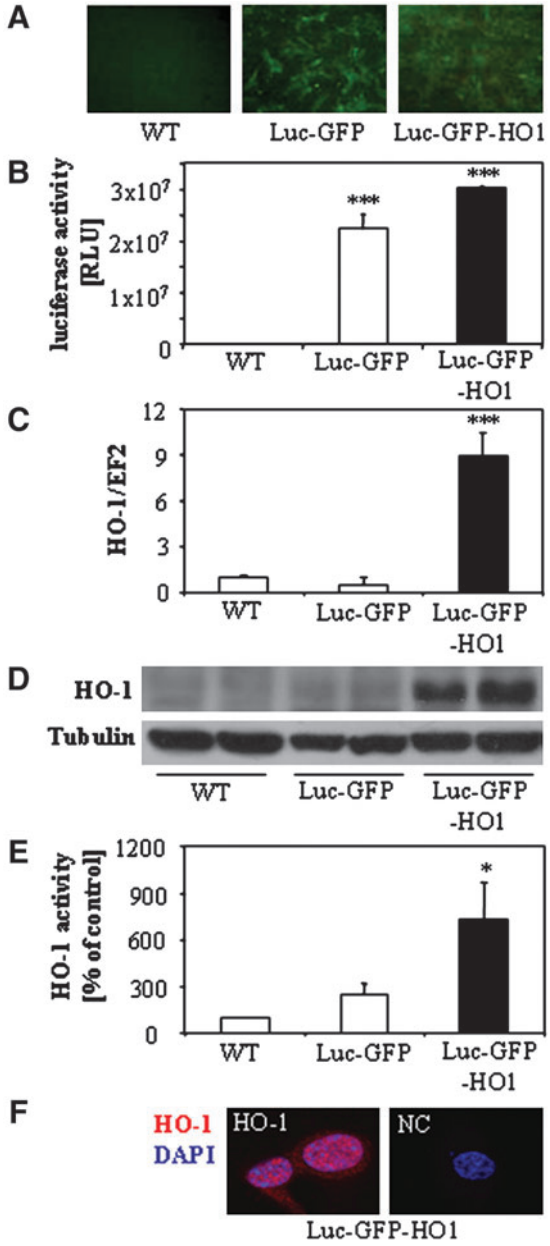
A cDNA template for analysis of miRNome was synthesized on 1 μg RNA using the NCode miRNA First-Strand cDNA Synthesis Kit following the manufacturer protocol, with exception of final volume of samples which was scaled down to 10 μL. The obtained cDNA was diluted 10-fold in water.

Analysis of microRNA transcriptome was performed using miRNome microRNA profiler QuantiMir™ kit. Real-time PCR was run in ABI 7500 HT thermal cycler with SybrGreen Mix and primers supplied by the vendor, according to the following protocol: 50°C, 2 min; 95°C, 10 min; 40 cycles of 95°C, 15 sec; 60°C, 60 sec. Gene expression was normalized to a constitutive small RNA U6.

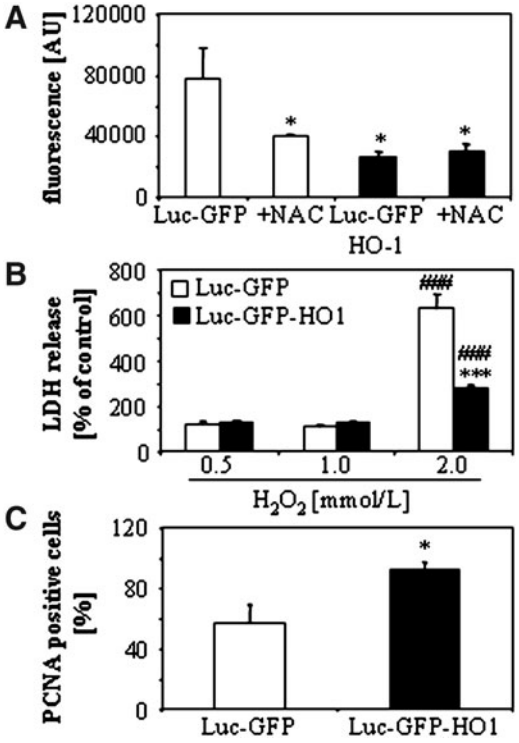
Additional real-time RT-PCR analysis was performed for some miRNAs chosen after screening of miRNA profile. Reverse transcription was performed on 1.2 μg of RNA as described earlier. cDNA was used as a template for RT-PCR run using SybrGreen Mix according to the following protocol: 50°C, 2 min; 95°C,10 min; 40 cycles of 95°C,15 sec, specific primer annealing temperature (Supplementary Table S4), 60 sec. Universal reverse primer (U6: 5′-CGCAAGGATGA CACGCAAATTC-3′) for miRNA RT-PCR was supplied by a vendor. Gene expression was normalized to a constitutive small RNA U6.

siRNA and pre-miRNA transfection

To silence HMOX1 in C2C12 Luc-GFP-HO1 cells, the siRNA sequence complementary to human HMOX1 was applied, whereas pre-miRNAs 133a, 133b, and 206 were used to overexpress specific micro-RNA. In both procedures the scrambled (sc) RNA served as a negative control. Cells were



SUPPLEMENTARY FIG. S1. Expression of transgenes in resting C2C12-Luc-GFP and C2C12-Luc-GFP-HO1 cell lines. (A) GFP fluorescence in growing cells. Fluorescence microscopy. **(B)** Luciferase activity in cell lysates. Luminometric assay. **(C)** Expression of HMOX1 mRNA measured by qRT-PCR. EF2 served as a constitutive gene. **(D)** HMOX1 protein in cell lysates detected by Western blotting. Tubulin served as a loading control. **(E)** HMOX1 activity in cell lysates measured using colorimetric method. **(F)** Cytoplasmic and nuclear localization of HMOX1 in C2C12-Luc-GFP-HO1 cells. Confocal microscopy. *Red:* HMOX1; *blue*: DAPI staining; *NC:* negative control stained with DAPI only. Representative photos. *Each bar* represents mean ± SEM of 3 experiments. $^{*}p < 0.05$, $^{***}p < 0.001$ versus wild-type (WT) cells. RLU, relative luminescence units.



SUPPLEMENTARY FIG. S2. Effect of HO-1 overexpression on C2C12 cells cultured *in vitro*. (A) Production of ROS by cells cultured in the presence or absence of an antioxidant molecule NAC (2 mmol/L). DCF oxidation assay. **(B)** Cell mortality after exposure to H_2O_2 for 24 h. LDH release assay. **(C)** Proliferation of cells after 24 h stimulation with 10% FCS. PCNA staining. *Each bar* represents mean ± SEM of 3–4 experiments. $^{*}p < 0.05$, $^{***}p < 0.001$ versus C2C12-Luc-GFP cells; $^{\#\#\#}p < 0.001$ versus cells cultured without H_2O_2. AU, arbitrary units.

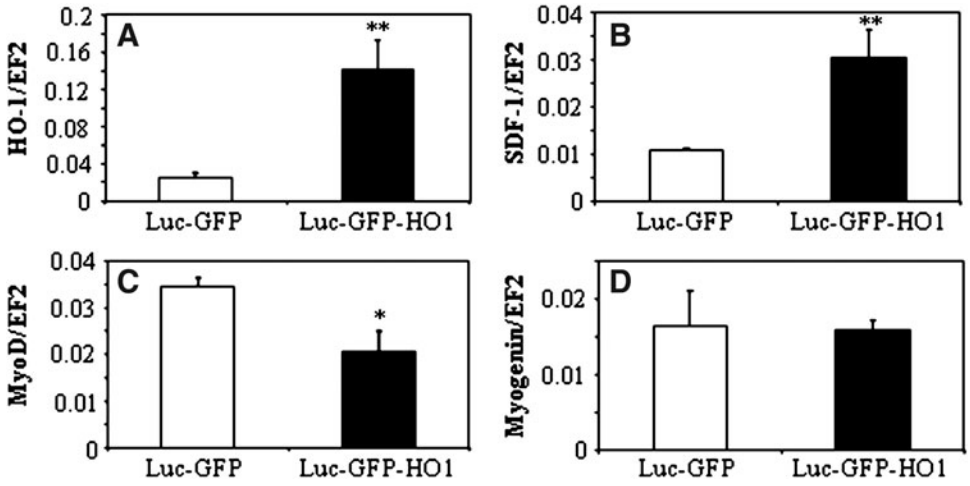
transiently transfected using siPORT. In short: 15 pmol of siRNA, pre-miRNA or scRNA were mixed with 23.5 μL of OptiMEM medium and added to 25 μL of OptiMEM with 1 μL of siPORT reagent, incubated earlier for 10 min in room temperature. Next day, the transfection medium was changed either for GrM or DfM, and cells were further cultured for 5 days.

Transfection with expression plasmids

Cells were transiently transfected using polyethylenimine (PEI). To achieve this, cells were seeded and grown to 70%–80% confluency for 24 h. For transfection, 2 μl of PEI and 2 μg of plasmid were used on 12-well plates. Efficacy of transgene transfection was assessed at mRNA (qRT-PCR) or protein (Western blot) level after 48 h.

Cell proliferation

Cells were seeded in 48-well plates (10,000 cells/well) in a serum-free medium for 24 h and then supplemented with 10% FCS. Proliferation was analyzed after next 48 h by detection of PCNA-positive cells. PCNA staining was performed on cells fixed in 4% paraformaldehyde (8 min) and 99% ethanol (2 min). After a triple washing with PBS, cells were permeabilized in 0.2% Triton X-100 in for 20 min. Next, nonspecific antigens were blocked (10% goat serum in PBS, 2 h), cells were washed (3 times, PBS), and primary antibody anti-PCNA (1:200 in 1% BSA in PBS) was applied overnight at 4°C. Negative controls were performed by

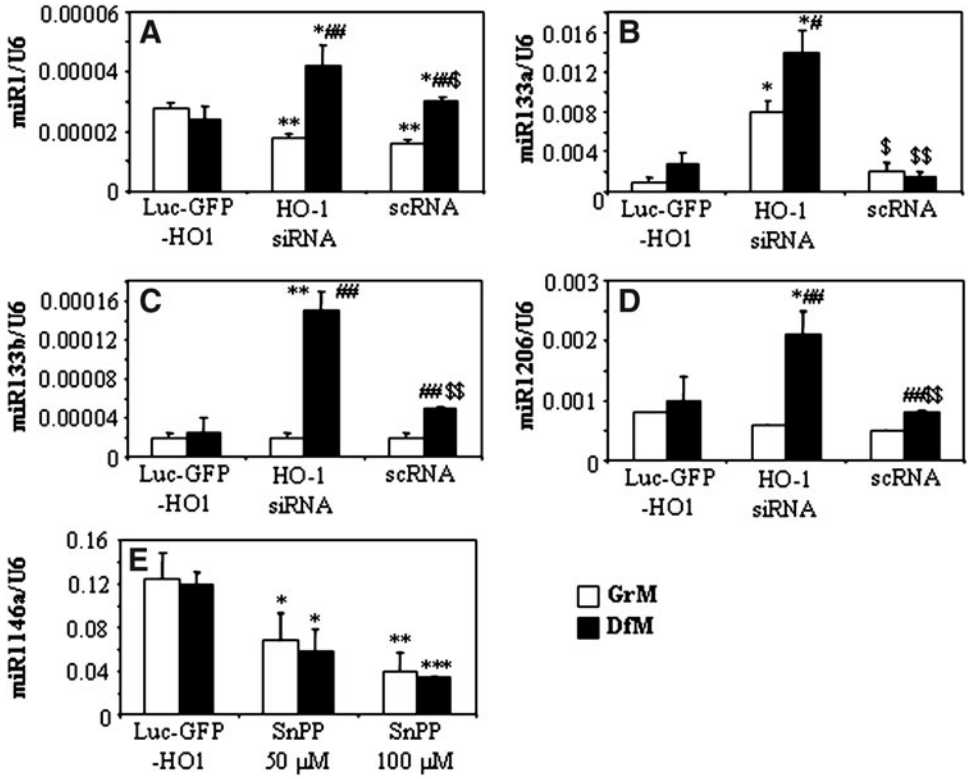


SUPPLEMENTARY FIG. S3. Gene expression profile in gastrocnemius muscles. Expression of HO-1 **(A)**, SDF-1 **(B)**, MyoD **(C)**, and myogenin **(D)** in the gastrocnemius muscles, 22 days after injection with C2C12-Luc-GFP or C2C12-Luc-GFP-HO1 cells. QRT-PCR. EF2 gene served as a constitutive control. *Each bar* represents mean ± SEM of 5 animals. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. C2C12-Luc-GFP injected muscles.

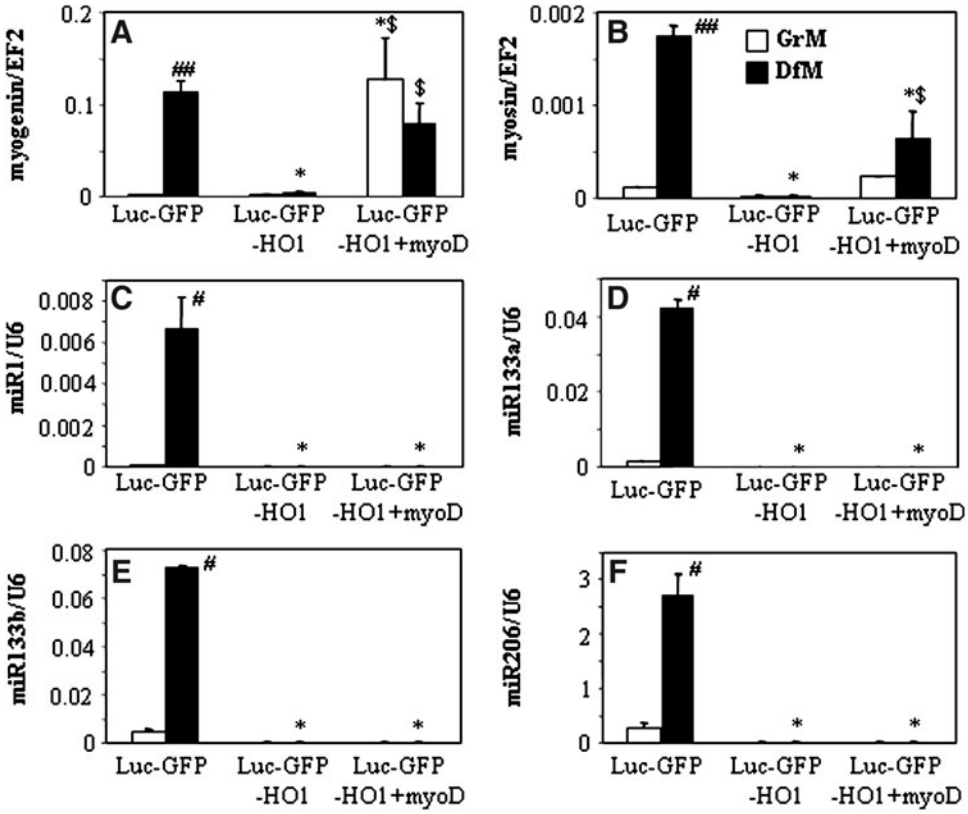
omitting primary antibody incubation. Next, cells were rinsed and incubated for 1 h with goat-anti mouse Alexa Fluor 546 secondary antibody (1:200 in PBS). Additionally, nuclei were visualized with Hoechst staining. Cells were rinsed and analyzed under fluorescence microscope Nikon Eclipse.

Cell viability

Cells were seeded in 96-well plates (10,000 cells/well) and cultured for 24 h. Activity of LDH in culture media was measured using CytoTox 96 Non-Radioactive Cytotoxicity Assay, according to vendor's instruction.



SUPPLEMENTARY FIG. S4. Effect of HO-1 inhibition on expression of myomirs. Expression of miR-1 **(A)**, miR-133a **(B)**, miR-133b **(C)**, and miR-206 **(D)** in C2C12-Luc-GFP-HO1 cells transfected with HO-1 or scrambled (sc) siRNAs and cultured for 5 days in GrM or DfM. **(E)** Expression of miR146a in C2C12-Luc-GFP-HO1 cells cultured for 5 days in GrM or DfM in the presence or absence of SnPP. QRT-PCR. U6 served as a constitutive control. *Each bar* represents mean ± SEM of 3 experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus C2C12-Luc-GFP-HO1 cells. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus cells cultured in GrM. $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus C2C12-Luc-GFP treated with siRNA.



SUPPLEMENTARY FIG. S5. Effect of myoD overexpression on C2C12-Luc-GFP cells cultured *in vitro*. Expression of myogenin **(A)**, myosin **(B)**, miR-1 **(C)**, miR-133a **(D)**, miR-133b **(E)**, and miR-206 **(F).** QRT-PCR. U6 served as a constitutive control. *Each bar* represents mean ± SEM of 3 experiments. $^{*}p < 0.05$ versus C2C12-Luc-GFP cells. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus cells cultured in GrM. $^{\$}p < 0.05$ versus C2C12-Luc-GFP-HO1 cells.

Protein isolation

Cells were washed twice with ice-cold PBS, covered with 750 μL of PBS, scratched from the plate, transferred to Eppendorf tubes and centrifuged (8000 *g*, 10 min, 4°C). Pellets were resuspended in 50 μL of Reporter Lysis Buffer, while fragments of gastrocnemius muscle, heart, lung, liver, spleen, and kidney were snap-frozen and homogenized in 200 μL of Reporter Lysis Buffer using Tissue Lyzer. Samples were incubated on ice for 30 min, and centrifuged (8000 *g*, 10 min, 4°C). Protein lysates were transferred to new Eppendorf tubes and stored at −20°C. Protein concentration was measured with bicinchoninic acid (BCA) assay, according to the vendor's protocol. The absorbance was read at wavelength of 562 nm using EL800 Universal Microplate Reader (BIO-TEK Instruments). Protein concentration was calculated according to the standard curve for bovine albumin.

Western blot analysis

Detection of HMOX1 (6) and DGCR8 (1) proteins were performed using Western blotting as described elsewhere. For densitometry analysis ImageJ® software was used.

Chromatin immunoprecipitation (ChIP) assay

ChIP analysis was performed as previously described (2), with minor changes. Briefly, cells were cultured without stimulants or with 10 μM of CORM or iCORM for 24 h, before they were fixed with 1% formaldehyde for 20 min. The immunoprecipitation was performed with antibodies against C/EBP delta (ab65081, Abcam), or IgG (cat. no. 309-005-003, Jackson ImmunoResearch) as a negative control. DNA was amplified by real time PCR using Power SYBR Green PCR Master Mix (Applied Biosystems). Primers used for PCR correspond to the putative C/EBPδ binding site within the MyoD promoter 5′- CAC GAC TGC TTT CTT CAC CA-3′ and 5′- CGG AAC CCC AAC AGT ACA AT-3′ (product length of 115 bp).

Luciferase activity measurement

Luciferase activity assay was performed according to vendor's instructions on protein isolated from cell culture, tissue samples, or sera collected from mice. It should be noted that activity of luciferase was slightly weaker in C2C12-Luc-GFP than in C2C12-Luc-GFP-HO1 cells (Fig. S1B). This discrepancy was negligible in the light of very strong and progressive differences between these two cell lines observed *in vivo* (Figs. 1B and 1C).

HMOX1 activity assays

HMOX1 activity was measured as described elsewhere (3).

CPK activity assay

Activity of creatine phosphokinase in cell lysates was measured using EnzyChrome™ Creatine Kinase Assay Kit according to vendor's instruction.

Supplementary Table S1. List of miRNAs Differently Expressed in C2C12-luc-GFP-HO1 Myoblasts When Compared to Control C2C12-luc-GFP myoblasts

MiR	*Fold of Induction (mean ± SD)*	*Statistical Significance*
miR-1	**0.000±0.000**	***p*<0.001**
miR-7a	0.285±0.121	*p*=0.014
miR-7b	**0.392±0.147**	***p*=0.028**
miR-9	**0.043±0.009**	***p*<0.001**
miR-10b	0.405±0.0.51	*p*=0.004
miR-15a	1.462±0.144	*p*=0.045
miR-16	**1.754±0.154**	***p*=0.020**
miR-18b	0.321±0.030	*p*=0.001
miR-27a	**0.551±0.136**	***p*=0.043**
miR-27b	**0.534±0.104**	***p*=0.024**
miR-29a	**0.569±0.094**	***p*=0.023**
miR-29b	0.389±0.102	*p*=0.014
miR-30a	1.530±0.077	*p*=0.010
miR-31	**0.730±0.083**	***p*=0.044**
miR-34a	0.683±0.091	*p*=0.039
miR-34c	**1.515±0.062**	***p*=0.007**
miR-96	**0.096±0.002**	***p*<0.001**
miR-99b	1.701±0.084	*p*=0.007
miR-125a	0.262±0.237	*p*=0.048
miR-133a	**0.020±0.007**	***p*<0.001**
miR-133b	**0.012±0.002**	***p*<0.001**
miR-135a	2.497±0.071	*p*=0.001
miR-136	**0.066±0.066**	***p*=0.009**
miR-139	0.826±0.003	*p*<0.001
miR-145	2.614±0.155	*p*=0.005
miR-146a	3.190±0.240	*p*=0.006
miR-146b	**1.556±0.095**	***p*=0.014**
miR-147	**0.391±0.096**	***p*=0.012**
miR-148a	5.469±1.098	*p*=0.029
miR-148b	3.081±0.524	*p*=0.030
miR-149	**0.447±0.177**	***p*=0.048**
miR-150	0.368±0.031	*p*=0.001
miR-152	2.349±0.401	*p*=0.019
miR-153	**0.523±0.094**	***p*=0.023**
miR-154	**0.098±0.123**	***p*=0.031**
miR-155	**0.534±0.102**	***p*=0.044**
miR-181b	0.727±0.084	*p*=0.008
miR-181d	0.786±0.027	*p*=0.001
miR-182	**0.164±0.038**	***p*=0.001**
miR-183	**0.100±0.039**	***p*=0.009**
miR-185	0.936±0.008	*p*=0.017
miR-187	0.512±0.090	*p*=0.019
miR-195	2.562±0.307	*p*=0.002
miR-196a	**3.743±0.179**	***p*=0.001**
miR-196b	**3.697±0.134**	***p*=0.002**
miR-204	0.396±0.035	*p*=0.001
miR-205	0.300±0.029	*p*<0.001
miR-206	**0.012±0.001**	***p*=0.001**
miR-210	1.491±0.016	*p*=0.002
miR-212	0.210±0.053	*p*=0.003
miR-217	0.198±0.060	*p*=0.039
miR-290	0.218±0.226	*p*=0.004
miR-293	**0.466±0.048**	***p*=0.008**
miR-295	0.391±0.076	*p*=0.028
miR-297c	0.820±0.044	*p*=0.001
miR-299	**0.201±0.034**	***p*=0.012**
miR-300	0.333±0.106	*p*=0.019
miR-302a	**0.207±0.155**	***p*=0.025**
miR-302b	0.212±0.179	*p*=0.003
miR-302c	**0.393±0.045**	***p*=0.007**
miR-323	0.673±0.039	*p*=0.034
miR-324	0.426±0.154	*p*=0.004
miR-325	**0.189±0.068**	**p=0.039**
miR-328	0.730±0.078	*p*=0.023
miR-330	0.331±0.148	*p*=0.021
miR-335	**0.322±0.140**	***p*<0.001**
miR-337	**0.359±0.009**	***p*=0.024**
miR-338	0.269±0.233	*p*=0.004
miR-340	0.278±0.069	*p*=0.006
miR-342	0.319±0.075	*p*=0.002
miR-345	0.390±0.041	*p*=0.005
miR-362	**0.265±0.072**	***p*=0.008**
miR-378	**0.599±0.121**	***p*=0.040**
miR-379	0.438±0.164	*p*=0.023
miR-380	0.303±0.151	*p*=0.021
miR-382	**0.116±0.003**	***p*<0.001**
miR-409	0.080±0.019	*p*<0.001
miR-410	0.394±0.002	*p*<0.001
miR-412	0.064±0.002	*p*=0.004
miR-423	0.655±0.029	*p*<0.001
miR-425	0.209±0.001	*p*=0.013
miR-431	0.312±0.110	*p*=0.016
miR-434	**0.193±0.144**	***p*=0.002**
miR-448	0.412±0.023	*p*=0.028
miR-449b	0.428±0.138	*p*=0.015
miR-449c	0.191±0.140	*p*=0.010
miR-451	**0.379±0.090**	***p*=0.002**
miR-463	0.053±0.052	*p*=0.001
miR-464	0.037±0.043	*p*=0.001
miR-465a	0.264±0.027	*p*=0.005
miR-465b	**0.103±0.088**	***p*=0.002**
miR-465c	0.181±0.045	*p*<0.001
miR-466b	8.202±0.050	*p*=0.026
miR-466c	7.020±1.409	*p*=0.004
miR-466d	2.682±0.154	*p*=0.016
miR-466h	**0.234±0.140**	***p*=0.042**
miR-467a	1.660±0.198	*p*=0.009
miR-467d	4.837±0.505	*p*=0.007
miR-467e	1.977±0.119	*p*=0.037
miR-485	**0.587±0.115**	***p*=0.006**
miR-486	**0.451±0.058**	***p*=0.046**
miR-488	0.551±0.141	*p*=0.018
miR-489	0.295±0.136	*p*=0.001
miR-500	**0.228±0.036**	***p*=0.009**
miR-501	**0.176±0.109**	***p*=0.014**
miR-504	0.563±0.073	*p*=0.027
miR-532	**0.388±0.145**	***p*=0.001**
miR-543	0.372±0.049	*p*=0.007
miR-544	0.334±0.077	*p*=0.001
miR-546	0.151±0.029	*p*=0.006
miR-551b	0.282±0.078	*p*=0.032
miR-574	0.433±0.148	*p*<0.001
miR-590	0.172±0.018	*p*=0.015
miR-615	0.233±0.133	*p*=0.047
miR-667	1.550±0.175	*p*=0.002
miR-668	0.390±0.034	*p*=0.031
miR-669f	2.590±0.405	*p*=0.003
miR-669g	0.334±0.053	*p*=0.038
miR-676	0.172±0.236	*p*=0.032
miR-679	0.160±0.216	*p*=0.036
miR-698	0.167±0.231	*p*=0.012
miR-707	0.105±0.141	*p*=0.025

(*continued*)

Supplementary Table S1. (Continued)

MiR	*Fold of Induction (mean ± SD)*	*Statistical Significance*
miR-743a	0.141 ± 0.195	$p = 0.012$
miR-743b	**0.107±0.141**	***p*=0.036**
miR-759	0.166 ± 0.231	$p = 0.015$
miR-877	0.136 ± 0.153	$p = 0.023$
miR-879	0.139 ± 0.189	$p = 0.008$
miR-880	0.087 ± 0.114	$p = 0.011$
miR-882	0.106 ± 0.133	$p = 0.019$
miR-1190	0.155 ± 0.166	$p = 0.018$
miR-1274a	2.877 ± 0.357	$p = 0.024$
miR-1952	0.149 ± 0.190	$p = 0.012$
miR-1960	0.104 ± 0.141	$p = 0.010$
miR-1963	0.099 ± 0.127	$p = 0.045$
miR-1983	0.215 ± 0.244	$p = 0.045$

The cells were cultured in GrM. **Bold**: miRNAs which differ significantly both under growth and differentiation conditions. *Gray backlight* indicates more than 10-fold difference.

ROS production assay

Cells were seeded into 12-well plates (100,000 cells/well). For ROS detection, the culture medium was changed and dichlorofluorescein (DCF, 10 μmol/L) was added for 1 h. To measure the fluorescence of DCF oxidation product, the cells were rinsed twice with cold PBS, overlaid with 100 μL of 1% Triton X-100 in PBS, and scratched from the plate. The obtained lysates were collected to pre-cooled Eppendorfs and spun down (12,000 *g*, 5 min, 4°C). Supernatants were transferred to the nontransparent plate and fluorescence was measured at 530 nm with excitation wavelength at 480 nm using EL800 Universal Microplate Reader. Results were normalized to total protein concentration.

Cytokine measurements

Cytokine concentrations in serum and gastrocnemius muscle lysates were assessed with the BD™ CBA Mouse Inflammation Kit, containing beads coated with capture antibodies specific for IL-6, IL-10, MCP-1, IFNγ, TNFα, and IL-12p70 proteins. Assay was performed according the vendor's instruction using BD FACSCalibur™ with BD CellQuest™ and BD™ CBA Software.

Histological analysis

Fragments of gastrocnemius muscles were fixed in 10% paraformaldehyde for 24 h and in 4% paraformaldehyde for next 24 h. Tissues were washed with water, dehydrated in alcohol solutions (50%, 70%, 95%, and 100%), and placed in xylene. Finally, the samples were embedded in paraffin. Sections (4 μm) were stained with hematoxylin (15 min), rinsed with tap water (10 min), counterstained with eosin (15 sec), and mounted in Canadian balsam.

Immunohistochemistry

To visualize subcellular localization of HMOX1, the cells were fixed in 4% paraformaldehyde (10 min) and in ice-cold 99% ethanol (10 min, −20°C). After a triple washing with PBS,

Supplementary Table S2. List of miRNAs Differently Expressed in C2C12-luc-GFP-HO1 Myoblasts When Compared to Control C2C12-luc-GFP Myoblasts

MiR	*Fold of Induction (mean ± SD)*	*Statistical Significance*
let-7b	0.287 ± 0.107	$p = 0.011$
let-7c	0.292 ± 0.013	$p < 0.001$
let-7e	0.390 ± 0.014	$p < 0.001$
miR-1	**0.000±0.000**	***p*<0.001**
miR-7b	**0.434±0.010**	***p*<0.001**
miR-9	**0.140±0.124**	***p*<0.010**
miR-16	**1.830±0.096**	***p*=0.006**
miR-19a	0.408 ± 0.181	$p = 0.044$
miR-19b	0.199 ± 0.131	$p = 0.013$
miR-20a	0.205 ± 0.022	$p < 0.001$
miR-20b	0.206 ± 0.067	$p = 0.004$
miR-21	0.325 ± 0.051	$p = 0.003$
miR-23a	0.300 ± 0.114	$p = 0.013$
miR-23b	0.235 ± 0.069	p 0.004
miR-27a	**0.261±0.045**	***p*=0.002**
miR-27b	**0.278±0.150**	***p*=0.021**
miR-29a	**0.328±0.052**	***p*=0.003**
miR-31	**0.399±0.078**	***p*=0.008**
miR-33	0.354 ± 0.005	$p < 0.001$
miR-34c	**1.520±0.129**	***p*=0.030**
miR-96	**0.185±0.167**	***p*=0.020**
miR-101b	0.375 ± 0.013	$p < 0.001$
miR-126	0.030 ± 0.027	$p < 0.001$
miR-128	0.242 ± 0.201	$p = 0.033$
miR-130b	0.356 ± 0.047	$p = 0.003$
miR-132	0.066 ± 0.025	$p < 0.001$
miR-133a	**0.003±0.002**	***p*<0.001**
miR-133b	**0.003±0.003**	***p*<0.001**
miR-136	**0.502±0.099**	***p*=0.019**
miR-137	0.199 ± 0.139	$p = 0.015$
miR-141	0.224 ± 0.217	$p = 0.037$
miR-146b	**0.333±0.041**	***p*=0.002**
miR-147	**0.226±0.059**	***p*=0.003**
miR-149	**0.142±0.082**	***p*=0.005**
miR-151	0.311 ± 0.125	$p = 0.016$
miR-153	**0.202±0.155**	***p*=0.018**
miR-154	**0.250±0.174**	***p*=0.026**
miR-155	**0.149±0.025**	***p*<0.001**
miR-181c	0.172 ± 0.114	$p = 0.009$
miR-182	**0.242±0.148**	***p*=0.019**
miR-183	**0.075±0.006**	***p*<0.001**
miR-190	0.025 ± 0.014	$p < 0.001$
miR-193	0.353 ± 0.167	$p = 0.032$
miR-194	0.260 ± 0.136	$p = 0.016$
miR-196a	**0.510±0.137**	***p*=0.037**
miR-196b	**0.457±0.178**	***p*=0.050**
miR-200c	0.180 ± 0.037	$p = 0.001$
miR-206	**0.001±0.000**	***p*<0.001**
miR-208b	0.031 ± 0.033	$p = 0.001$
miR-219	0.427 ± 0.036	$p = 0.002$
miR-221	0.387 ± 0.198	$p = 0.048$
miR-223	0.066 ± 0.017	$p < 0.001$
miR-291b	0.306 ± 0.149	$p = 0.022$
miR-292	0.257 ± 0.146	$p = 0.019$
miR-293	**0.152±0.207**	***p*=0.029**
miR-299	**0.185±0.028**	***p*=0.001**
miR-301a	0.390 ± 0.044	$p = 0.003$
miR-302a	**0.247±0.030**	***p*=0.001**
miR-302c	**0.131±0.141**	***p*=0.013**
miR-325	**0.383±0.048**	***p*=0.003**

(*continued*)

Supplementary Table S2. (Continued)

MiR	Fold of Induction (mean ± SD)	Statistical Significance
miR-331	0.471 ± 0.033	$p = 0.002$
miR-335	**0.180 ± 0.148**	***p* = 0.016**
miR-337	**0.172 ± 0.088**	***p* = 0.006**
miR-339	0.359 ± 0.062	$p = 0.005$
miR-344	0.353 ± 0.009	$p < 0.001$
miR-346	0.202 ± 0.146	$p = 0.016$
miR-350	0.338 ± 0.131	$p = 0.019$
miR-361	0.256 ± 0.006	$p < 0.001$
miR-362	**0.092 ± 0.044**	***p* = 0.001**
miR-369	0.059 ± 0.075	$p = 0.003$
miR-378	**0.190 ± 0.044**	***p* = 0.001**
miR-382	**0.054 ± 0.049**	***p* = 0.001**
miR-383	0.311 ± 0.203	$p = 0.041$
miR-421	0.150 ± 0.013	$p < 0.001$
miR-434	**1.992 ± 0.324**	***p* = 0.050**
miR-451	**0.285 ± 0.192**	***p* = 0.034**
miR-455	0.236 ± 0.168	$p = 0.023$
miR-465b	**0.179 ± 0.115**	***p* = 0.010**
miR-466e	0.538 ± 0.005	$p < 0.001$
miR-466f	0.210 ± 0.160	$p = 0.020$
miR-466h	**0.219 ± 0.177**	***p* = 0.025**
miR-466i	0.261 ± 0.135	$p = 0.016$
miR-467j	0.137 ± 0.098	$p = 0.006$
miR-466k	0.289 ± 0.147	$p = 0.021$
miR-485	**0.129 ± 0.165**	***p* = 0.017**
miR-486	**0.279 ± 0.214**	***p* = 0.041**
miR-490	0.338 ± 0.024	$p = 0.001$
miR-493	0.184 ± 0.048	$p = 0.002$
miR-495	0.279 ± 0.090	$p = 0.008$
miR-500	**0.384 ± 0.032**	***p* = 0.001**
miR-501	**0.293 ± 0.198**	***p* = 0.037**
miR-532	**0.124 ± 0.045**	***p* = 0.001**
miR-540	1.229 ± 0.065	$p = 0.038$
miR-547	0.212 ± 0.051	$p = 0.002$
miR-582	0.736 ± 0.007	$p < 0.001$
miR-669b	0.241 ± 0.180	$p = 0.027$
miR-673	0.576 ± 0.044	$p = 0.005$
miR-675	0.140 ± 0.069	$p = 0.003$
miR-680	0.673 ± 0.095	$p = 0.039$
miR-693	0.511 ± 0.043	$p = 0.004$
miR-695	0.436 ± 0.176	$p = 0.045$
miR-696	0.527 ± 0.122	$p = 0.032$
miR-697	0.434 ± 0.121	$p = 0.022$
miR-701	0.386 ± 0.001	$p < 0.001$
miR-702	0.405 ± 0.050	$p = 0.003$
miR-709	2.355 ± 0.371	$p = 0.035$
miR-710	0.544 ± 0.117	$p = 0.031$
miR-713	3.332 ± 0.006	$p < 0.001$
miR-714	0.584 ± 0.064	$p = 0.012$
miR-719	0.234 ± 0.041	$p = 0.001$
miR-741	0.163 ± 0.040	$p = 0.001$
miR-743b	**0.212 ± 0.072**	***p* = 0.004**
miR-764	0.209 ± 0.174	$p = 0.023$
miR-770	2.249 ± 0.350	$p = 0.037$
miR-873	4.814 ± 1.202	$p = 0.046$
miR-1197	0.420 ± 0.005	$p < 0.001$
miR-1936	1.336 ± 0.001	$p < 0.001$
miR-1938	0.395 ± 0.064	$p = 0.006$
miR-1940	1.526 ± 0.046	$p = 0.004$
miR-1943	1.871 ± 0.255	$p = 0.040$
miR-1944	0.627 ± 0.122	$p = 0.049$
miR-1946a	1.654 ± 0.128	$p = 0.019$

Supplementary Table S2. (Continued)

MiR	Fold of Induction (mean ± SD)	Statistical Significance
miR-1953	0.696 ± 0.036	$p = 0.007$
miR-1964	2.032 ± 0.180	$p = 0.015$

The cells were cultured in DfM. **Bold:** miRNAs which differ significantly both under growth and differentiation conditions. *Gray backlight* indicates more than 10-fold difference.

cells were permeabilized in 0.1% Triton X-100 for 20 min. Next, nonspecific antigens were blocked with 10% goat serum in PBS for 2 h. After a subsequent washing, rabbit anti-human HMOX1 primary antibody (1:200 in PBS) was applied overnight at 4°C. Negative controls were performed by omitting the primary antibody incubation. In next step, cells were rinsed three times and incubated for 2 h with goat anti-rabbit Alexa Fluor 562 secondary antibody (1:200 in PBS). Additionally, nuclei were visualized with DAPI staining. Cells were rinsed and analyzed under confocal microscope (Leica).

MHC staining was performed in cells fixed in 3% paraformaldehyde (10 min). After a triple washing with PBS, cells were permeabilized in 0.05% Triton X-100 for 3 min, and paraformaldehyde residues were removed by incubation with 0.25% glycine for 30 min. Then, nonspecific antigens were blocked with 3% BSA in PBS for 1 h and, after the next washing, primary anti-MHC antibody (1:100 in PBS) was applied overnight at 4°C. Next, cells were rinsed and incubated for 2 h with goat-anti mouse Alexa Fluor 562 secondary antibody (1:200 in PBS). Negative control was performed by omitting primary antibody incubation. Additionally, nuclei were visualized with Hoechst (1 μg/mL). Cells were analyzed under fluorescent microscope Nikon Eclipse.

Paraffin-embedded muscle specimens were analyzed for GFP expression. Samples were deparaffinized in xylene and rehydrated in alcohol solutions (100%, 90%, 70%). Antigens were retrieved by incubation in microwave oven for 12 min in citrate buffer (pH 6). After washing with TBS, specimens were blocked for 1 h in 10% goat serum in PBS and rabbit anti-GFP antibody (1:250 in 1% goat serum in PBS) was applied overnight at 4°C. Endogenous peroxidases were inhibited by a 20 min incubation with 0.003% H_2O_2 in methanol. Then, the secondary goat anti-rabbit IgG-HRP antibody (1:1000 in 1% goat serum in PBS) was applied for 1 h, followed by incubation with DAB Fast (5 min), and additional staining of nuclei with hematoxylin (15 min). The specimens were subsequently dehydrated in ascending alcohol solutions (70%, 90%, 100%), washed with xylene, and mounted with Canadian balsam.

IVIS analysis

Mice were analyzed using IVIS® Lumina II Imaging System (Caliper Life Science) at day 2, 9, and 22. Luciferin (150 μL, 5 mg/mL) was administered intraperitoneally and luciferase activity was measured for 5 min.

Supplementary Table S3. Primers Used for qRT-PCR Analysis of mRNAs

Primer		*Sequence*	*Annealing Temperature*	*Product Length*
EF-2	forward	5′-GACATCACCAAGGGTGTGCA-3′	60°C	218 bp
	reverse	5′-TCAGCACACTGGCATAGAGG-3′		
HMOX1	forward	5′-GTGGAGACGCTTTACGTAGTGC-3′	60°C	250 bp
	reverse	5′-CTTTCAGAAGGGTCAGGTGTCC-3′		
CyclinD1	forward	5′-ATGCGGATCAAACCTCACCAAGGC-3′	60°C	220 bp
	reverse	5′-TTAACTCAAGCTGCCTCGCCTTGC-3′		
SDF-1	forward	5′-CCTTCAGATTGTTGCACGGCTGA-3′	60°C	181 bp
	reverse	5′-CCCACCACTGCCCTTGCATC-3′		
p21	forward	5′-AAACCTCTGAGGCGTTTGGT-3′	60°C	149 bp
	reverse	5′-AGCAGGGTTCCTTGTTGGAG-3′		
Myf5	forward	5′-CCTGTCTGGTCCCGAAAGAAC-3′	55°C	131 bp
	reverse	5′-GACGTGATCCGATCCACAATG-3′		
Myf6	forward	5′-ATCAGCTACATTGAGCGTCTACA-3′	55°C	174 bp
	reverse	5′-CCTGGAATGATCCGAAACACTTG-3′		
MEF2	forward	5′-CAGGCGCTATGGGTCATCTG-3′	55°C	100 bp
	reverse	5′-GCTACTTGGATTGCTGAACTGC-3′		
MyoD	forward	5′-GCTGCCTTCTACGCACCTG-3′	55°C	120 bp
	reverse	5′-GCCGCTGTAATCCATCATGC-3′		
Myogenin	forward	5′-CAGTACATTGAGCGCCTACAG-3′	55°C	164 bp
	reverse	5′-GCACCGAACTCCAGTGCAT-3′		
Myosin	forward	5′-CCCAATGAGTAGGCTGGAGA-3′	60°C	125 bp
	reverse	5′-TCTGGACCCATTCCTTCTTG-3′		
Lin28	forward	5′-GTCCAATCCTGGTGATGTC-3′	60°C	407 bp
	reverse	5′-GTTTTCGGCCCTGAGATGT-3′		

Supplementary Table S4. Primers Used for qRT-PCR Analysis of miRNA

Primer	*Sequence*	*Annealing Temperature*
mU6	5′- CGC AAG GAT GAC ACG CAA ATT C-3′	60°C
microRNA 1	5′-GCTGGAATGTAAAGAAGTATGTAT-3′	60°C
microRNA 133a	5′-TTGGTCCCCTTCAACCAGCTGT-3′	60°C
microRNA 133b	5′-TTGGTCCCCTTCAACCAGCTA-3′	60°C
microRNA 206	5′-TGGAATGTAAGGAAGTGTGTGG-3′	60°C
microRNA 146a	5′-CGTGAGAACTGAATTCCATGGGTT-3′	60°C

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