Supplementary material

Nowaczyk M, Malcher A, Zimna A, et al. Effect of miR-195 inhibition on human skeletal muscle-derived stem/progenitor cells. Kardiol Pol. 2022.

Supplementary material No 1

Transfection of SkMDS/PCs with the miR-195 inhibitor

The miR-195 inhibitor (Genecopoeia, Carlsbad, CA, USA) was used for further experiments. An FAM dye–labelled synthetic oligonucleotide nonspecific miRNA inhibitor (Thermo Fisher, Waltham, MA, USA) was applied as a negative transfection control. The miR-195 inhibitor and FAM were diluted to 2.8 µM using Buffer Blue (Lipocalyx, Halle, Germany). The miRNAs were swiftly mixed with Viromer Blue and Buffer Blue solutions according to the manufacturer's instructions. Transfection complexes were added to human SkMDS/PC suspensions at a final concentration of 50 nM miRNA per well (6-well plate) in fresh medium. *In vitro* culture of wild-type human SkMDS/PCs was conducted in parallel, which was not treated with either transfection reagent or miRNA inhibitor. The transfected cells were cultured in Gibco Opti-Mem medium (Thermo Fisher, Waltham, MA, USA) with reduced foetal bovine serum and without antibiotics from the 7^{th} to 11^{th} cell passages.

Suplementary material No 2

miRNA expression analysis

To determine the expression, the taqMan Advanced MicroRNA Assay for hsa-miR-195-3p (478744_mir, Thermo Fisher Scientific, Waltham, MA USA) was used. Mean cycle threshold (Ct) values were estimated with BioRad CFX Manager 3.1) software. Relative expression levels were calculated using the 2−DCt formula. The 10 ng of total RNA isolated from SkMDS/PCs

Table S1. Primers sequences and expected length of the PCR product

	Sequence (5'->3')	Length of PCR product
CAT		
Forward primer	TATCCTGACACTCACCGCCA	277
Reverse primer	CGTTCACATAGAATGCCCGC	
FOXO1		
Forward primer	GAGGGTTAGTGAGCAGGTTAC	243
Reverse primer	TGGCACAGTCCTTATCTACAG	
SIRT1		
Forward primer	TGGTATTTATGCTCGCCTTGC	220
Reverse primer	CAGCGTGTCTATGTTCTGGGT	
SOD1		
Forward primer	TGGTTTGCGTCGTAGTCTCC	168
Reverse primer	GTCCATTACTTTCCTTCTGCTC	
SOD2		
Forward primer	ACCTGCCCTACGACTACGG	262
Reverse primer	AACTCCCCTTTGGGTTCTCC	
SOD3		
Forward primer	ATGCTGGCGCTACTGTGTTC	100
Reverse primer	ACTCCGCCGAGTCAGAGTT	
MyoD		
Forward primer	ACGGCATGATGGACTACAG	212
Reverse primer	CGACTCAGAAGGCACGTC	

MyoG		
Forward primer	GCTGTATGAGACATCCCCCTA	226
Reverse primer	CGACTTCCTCTTACACACCTT	
TERT1		
Forward primer	CCGATTGTGAACATGGACTACG	99
Reverse primer	CACGCTGAACAGTGCCTTC	

Supplementary material No 4

Characteristics of human muscle-derived stem/progenitor cell samples

The percentage of CD56-positive cells was defined in a human skeletal SkMDS/PC population by flow cytometry using an anti-CD56-specific antibody. The FACS results indicated that approx. 90% of the cells examined in each analysed SkMDS/PC sample were positive for CD56, in 90.8% in the wild-type population (WT), 90.4% in cells transfected with FAM™ Dye-Labelled Anti-miR™, and similarly in 90.4% in myogenic cells transfected with miR-195 inhibitor (Figure S1). The lipofection itself did not affect the main myoblast marker in the cell populations under study.

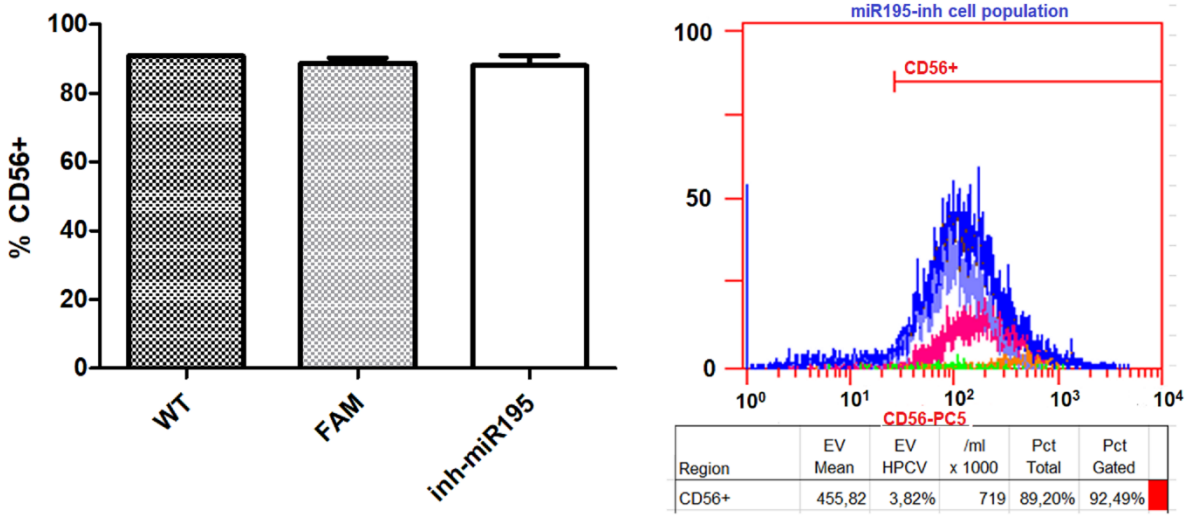


Figure S1. Percentage of CD56-positive cells in *in vitro* SkMDS/PC culture (at 4th cell passage). Values are given as the mean ± SD

Supplementary material No 5

Lipofection efficiency

The efficiency of miRNA inhibitor introduction procedure was evaluated by flow cytometry. An FAM dye-labelled synthetic oligonucleotide nonspecific miRNA inhibitor (excitation max (λmax) 494 nm; emission max (λmax) 520 nm) was used for this purpose. We determined a transfer rate of approximately 94 % efficacy (Figure S1)

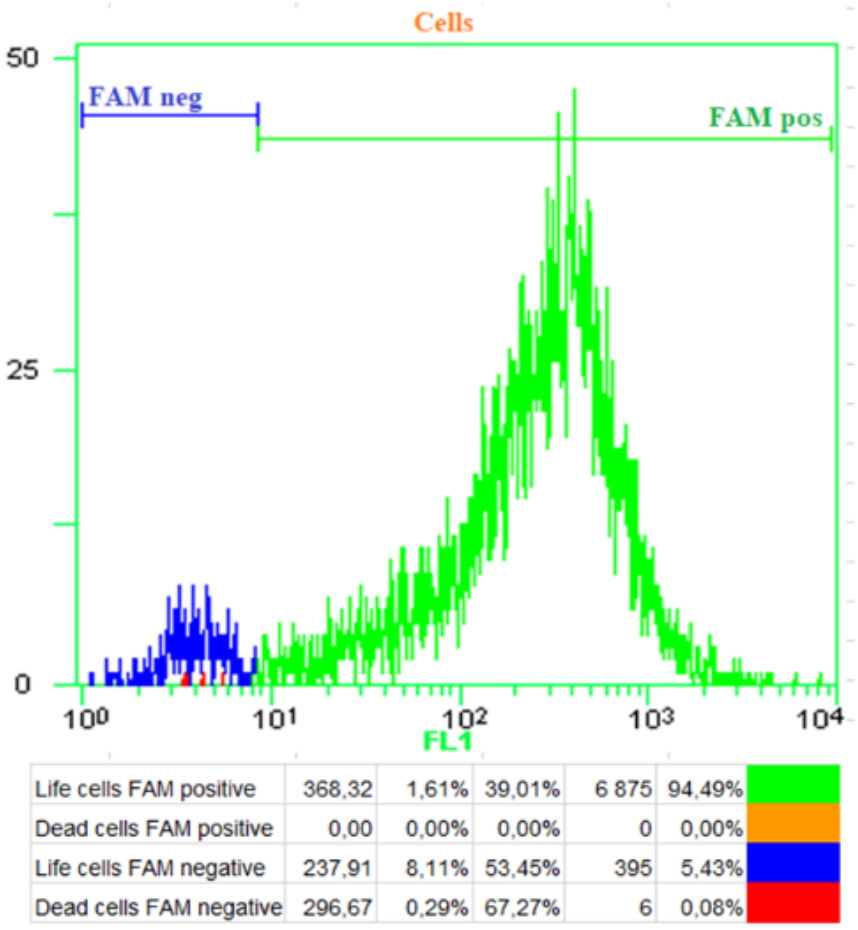


Figure S2. The efficiency of miRNA inhibitor introduction procedure. Percentage of transfected-FAM- positive cells, and non transfected- FAM- negative cells 72 hours after lipofection (transfection)- at 4th cell passage. FAM- dye-labelled synthetic oligonucleotide nonspecific miRNA inhibitor was a control efficiency of lipofection/transfection procedure.

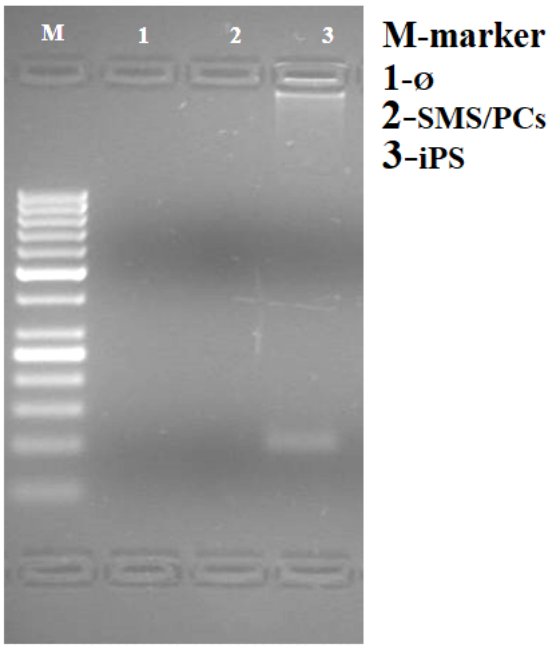
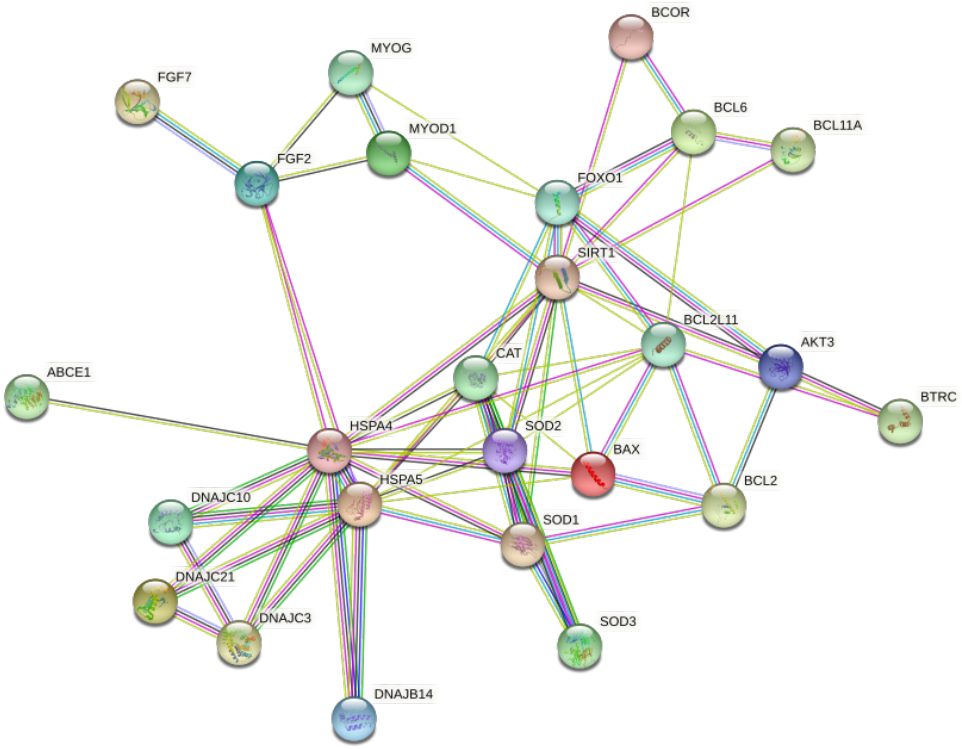


Figure S3. Electrophoresis of PCR product *TERT*. Product is present in ips patch; we did not observe this product in SkMDS/PCells. Isolation of DNA was performed after the 7th cell passage

Supplementary material No 6

Visualization of miR-195 regulatory role in skeletal muscle- derived stem/progenitor cells differentiation. Selected targets and pathway interactions.



Legend:

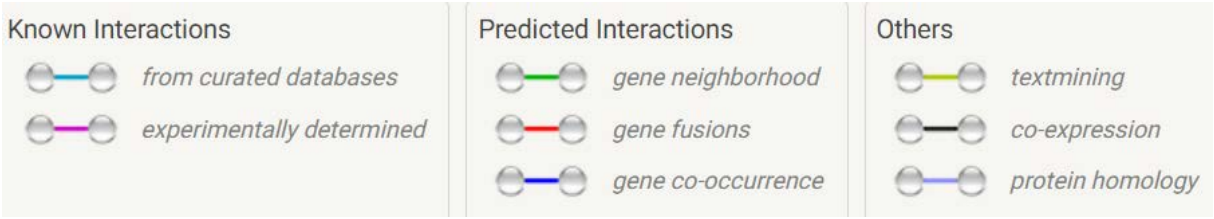


Figure S4. Visualization of interaction pathway of selected targets using STRING data base.

Abbreviation:

BCL2L11 Bcl-2-like protein 11; Induces apoptosis and anoikis. Isoform Bim-alpha2 and isoform Bim-alpha3 induce apoptosis, although less potent than isoform BimEL, isoform BimL and isoform BimS. Isoform Bim-gamma induces apoptosis.

BCL6 B-cell lymphoma 6 protein; Transcriptional repressor mainly required for germinal center (GC) formation and antibody affinity maturation which has different mechanisms of action specific to the lineage and biological functions. Forms complexes with different corepressors and histone deacetylases to repress the transcriptional expression of different subsets of target genes.

MYOD1 Myoblast determination protein 1; Acts as a transcriptional activator that promotes transcription of muscle-specific target genes and plays a role in muscle differentiation. Together with MYF5 and MYOG, co-occupies muscle-specific gene promoter core region during myogenesis. Induces fibroblasts to differentiate into myoblasts. Interacts with and is inhibited by the twist protein.

BTRC F-box/WD repeat-containing protein 1A; Substrate recognition component of a SCF (SKP1-CUL1-F- box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins. Recognizes and binds to phosphorylated target proteins.

SOD3 Extracellular superoxide dismutase [Cu-Zn]; Protect the extracellular space from toxic effect of reactive oxygen intermediates by converting superoxide radicals into hydrogen peroxide and oxygen.

DNAJB14 DnaJ homolog subfamily B member 14; Acts as a co-chaperone with HSPA8/Hsc70; required to promote protein folding and trafficking, prevent aggregation of client proteins, and promote unfolded proteins to endoplasmic reticulum-associated degradation (ERAD) pathway.

SOD2 Superoxide dismutase [Mn], mitochondrial; Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems.

BCL2 Apoptosis regulator Bcl-2; Suppresses apoptosis in a variety of cell systems including factor-dependent lymphohematopoietic and neural cells. Regulates cell death by controlling the mitochondrial membrane permeability.

BCL11A Baf chromatin remodeling complex subunit bcl11a; B-cell lymphoma/leukemia 11A; Transcription factor associated with the BAF SWI/SNF chromatin remodeling complex (By similarity). Repressor of fetal hemoglobin (HbF) level. Involved in brain development. Functions as a myeloid and B-cell proto-oncogene. May play important roles in leukemogenesis and hematopoiesis.

BCOR BCL-6 corepressor; Transcriptional corepressor. May specifically inhibit gene expression when recruited to promoter regions by sequence- specific DNA-binding proteins such as BCL6 and MLLT3. This repression may be mediated at least in part by histone deacetylase activities which can associate with this corepressor.

BAX Apoptosis regulator BAX; Accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 or its adenovirus homolog E1B 19k protein. Under stress conditions, undergoes a conformation change that causes translocation to the mitochondrion membrane, leading to the release of cytochrome c that then triggers apoptosis. Promotes activation of CASP3, and thereby apoptosis.

FOXO1 Forkhead box protein O1; Transcription factor that is the main target of insulin signaling and regulates metabolic homeostasis in response to oxidative stress. Binds to the insulin response element (IRE) with consensus sequence 5'-TT[G/A]TTTTG-3' and the related Daf-16

family binding element (DBE) with consensus sequence 5'- TT[G/A]TTTAC-3'. Activity suppressed by insulin. Main regulator of redox balance and osteoblast numbers and controls bone mass.

DNAJC3 DnaJ homolog subfamily C member 3; Involved in the unfolded protein response (UPR) during endoplasmic reticulum (ER) stress. Acts as a negative regulator of the EIF2AK4/GCN2 kinase activity by preventing the phosphorylation of eIF-2-alpha at 'Ser-52' and hence attenuating general protein synthesis under ER stress, hypothermic and amino acid starving stress conditions (By similarity).

HSPA4 Heat shock protein family A member 4; Belongs to the heat shock protein 70 family.

HSPA5 78 kDa glucose-regulated protein; Plays a role in facilitating the assembly of multimeric protein complexes inside the endoplasmic reticulum. Involved in the correct folding of proteins and degradation of misfolded proteins via its interaction with DNAJC10, probably to facilitate the release of DNAJC10 from its substrate (By similarity); Belongs to the heat shock protein 70 family.

ABCE1 ATP-binding cassette sub-family E member 1; Antagonizes the binding of 2-5A (5'-phosphorylated 2',5'-linked oligoadenylates) by RNase L through direct interaction with RNase L and therefore inhibits its endoribonuclease activity. May play a central role in the regulation of mRNA turnover.

DNAJC21 DnaJ homolog subfamily C member 21; May act as a co-chaperone for HSP70. May play a role in ribosomal RNA (rRNA) biogenesis, possibly in the maturation of the 60S subunit.

MYOG Myogenin; Acts as a transcriptional activator that promotes transcription of muscle-specific target genes and plays a role in muscle differentiation, cell cycle exit and muscle atrophy. Essential for the development of functional embryonic skeletal fiber muscle differentiation. However, is dispensable for postnatal skeletal muscle growth; phosphorylation by CAMK2G inhibits its transcriptional activity in respons to muscle activity.

FGF2 Fibroblast growth factor 2; Plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. Functions as potent mitogen in vitro. Can induce angiogenesis.

FGF7 Fibroblast growth factor 7; Plays an important role in the regulation of embryonic development, cell proliferation and cell differentiation. Required for normal branching morphogenesis. Growth factor active on keratinocytes. Possible major paracrine effector of normal epithelial cell proliferation.

CAT Catalase; Occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Promotes growth of cells including T-cells, B-cells, myeloid leukemia cells, melanoma cells, mastocytoma cells and normal and transformed fibroblast cells.

SIRT1 NAD-dependent protein deacetylase sirtuin-1; NAD-dependent protein deacetylase that links transcriptional regulation directly to intracellular energetics and participates in the coordination of several separated cellular functions such as cell cycle, response to DNA damage, metobolism, apoptosis and autophagy.

SOD1 Superoxide dismutase, cu-zn family; Superoxide dismutase [Cu-Zn]; Destroys radicals which are normally produced within the cells and which are toxic to biological systems.

DNAJC10 Dnaj heat shock protein family (hsp40) member c10; DnaJ homolog subfamily C member 10; Endoplasmic reticulum disulfide reductase involved both in the correct folding of proteins and degradation of misfolded proteins. Required for efficient folding of proteins in the endoplasmic reticulum by catalyzing the removal of non-native disulfide bonds formed during the folding of proteins, such as LDLR.