Α

Mean adipocyte size (µM)

С





-0.4

-0.2

0

0.2

0.4

0.6

0.8

-0.8

-1

-0.6

HDL.C -0.09-0.04 0 -0.27 -0.24 -0.06 -0.39 -0.29 -0.32 -0.35 -0.14 -0.05 -0.12 -0.12 J LDL.C -0.24 -0.25 0.15 0.67 0.31 0.16 0.36 0.07 0.36 0.57 -0.11 0.09 0.21 0.18 -0.23

ALAT 0.04 0.16 0.11 -0.06 0.01 -0.18 0.14 0.19 0.1 0.08 -0.05 0.06 0 -0.11 0.01 0.06 ASAT -0.03 0.03 0.1 -0.05 0.05 -0.2 0.02 0.17 -0.03 -0.07 -0.02 0.22 -0.01 -0.13 -0.11 0.03 95 y

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Maximal_Adipocyte_volume_visceral

Figure S1. Correlation of PPARG∆5 and cPPARG levels with clinical and biochemical parameters

(A) Scatterplot resulting from regression analysis and showing correlation between PPARG Δ 5 levels and mean diameter of subcutaneous adipocyte size (N=86). Pearson correlation coefficient (r) and p value (p) are shown.

(B) Boxplot showing cPPARG levels in two subgroups, defined according to the mean diameter of subcutaneous adipocytes as "Low Mean Diameter" (LMD; mean diameter <115μm, N=63) and "High Mean Diameter" (HMD; mean diameter >115μm, N=23) group. *p<0.05.

(C) Corrplot reporting the Pearson's correlation coefficients for cPPARG, PPARG Δ 5, PPARG Δ 5/cPPARG and several clinical and biochemical parameters. CT_ratio=VAT/SAT ratio by computing tomography, CREA=Creatinine, CRP=C-reactive protein, FPG=fasting plasma glucose, HbA1c=Hemoglobin A1c, HDL.C=High-Density Lipoprotein-Cholesterol, LDL.C=Low-Density Lipoprotein-Cholesterol, ALAT=Alanine aminotransferase, ASAT=Aspartate transaminase, gGT=gamma-glutamyl transpeptidase, SC=subcutaneous.



Figure S2. Adipocyte differentiation of hMSCs is affected by densities of cell plating

(A) Representative phase-contrast images of hMSCs plated at different densities (10³, 2x10³, $3x10^3$ and $4x10^3$ /cm²; scale bars, 100 µm).

(B) Lipid accumulation by oil Red O staining (optical density) of mature adipocytes differentiated from hMSCs with adipogenic mixes supplemented with increasing doses (i.e. 10, 20 and 50 ng/mL) of Bone Morphogenic Protein 4 (BMP4). Data are reported as mean ±SEM *vs* cells treated with vehicle (0 ng/mL).



Figure S3. Stemness markers expression in terminally differentiated hMSCs and in hypertrophic-like cells

Flow cytometry analysis of CD73 and CD90 markers in hMSCs at starting point (hMSCs), in mature (MA) and hypertrophic-like adipocytes (HA). PE and FITC fluorophores were conjugated to anti-CD73 and anti-CD90 antibodies, respectively. Left boxes show expression values of FITC- and PE-isotype controls.



5x magnification

10x magnification

20x magnification

Figure S4. Hypertrophic-like adipocytes are less prone to dedifferentiation in vitro

Representative phase-contrast images at different magnifications cultured of hypertrophic-like adipocytes in standard conditions for additional 30 days (scale bars, 100 µm).

Figure S5



Figure S5. Expression trends of PPAR γ and its target genes

(A) Gray-scale heatmap of normalized mRNA expression values (i.e. Δ Ct= Ct gene - Ct *PPIA*) of canonical (PPARG1 and PPARG2) and dominant negative (PPARG Δ 5) *PPARG* transcripts determined by qPCR in hMSCs (T=0 hours). *PPIA* was used as reference gene.

(B) Representative gel images of RT-PCR assays in hMSCs at different time points upon adipogenic induction (T=0 hours, T=6 and T=12 days) and in mature and hypertrophic-like adipocytes (MAs and HAs, respectively).

(C) Line charts displaying gene expression trends (normalized mRNA expression values; $\Delta\Delta Ct = \Delta Ct$ sample- ΔCt reference sample) measured by qPCR. *PPIA* was used as reference gene for all analyzed genes. For each gene, the first time point showing detectable mRNA levels was used as reference ($\Delta\Delta Ct=0$). Data are reported as mean from three independent experiments.

(D) Line charts showing gene expression variations between two subsequent time points ($\Delta\Delta$ Ct time point 2 - $\Delta\Delta$ Ct time point 1) along hMSCs differentiation determined by qPCR. *PPIA* was used as reference gene and data are reported as mean from three independent experiments.

(E) Line charts comparing gene expression trends - analyzed as in panel C - of cPPARG and *MRTFA* genes along hMSCs differentiation. Similar expression trends at specific time points are indicated by light green boxes (from T=0 hours to T=6 days, from MA to HA) and opposite trends in stages indicated by yellow box (from T=6 days to MA).



Figure S6. Morphometric characteristics of hypertrophic-like adipocytes

(A) Representative confocal microscopy images displaying a marked *nuclei* pressure in hypertrophic-like adipocytes stained with DAPI (*nuclei*, blue), Bodipy 495/503 (lipid droplets, green) and WGA 632/647(cell membranes, red; scale bar, 50 μ m).

(B-C) Density plots showing value distribution of (B) adipocyte area - analyzed on MAs (n=30) and HAs (n=30) - and (C) lipid droplets area measured on 2973 LDs (from 214 MAs) and on 1168 LDs (from 206 HAs).



Figure S7. The expression of canonical *PPARG* transcripts correlates with *SLC2A4* levels

cPPARG expression was previously measured in Aprile et al., 2018.

(A-C) Scatterplot reporting the correlation by linear regression analysis between *SLC2A4* and cPPARG expression levels (qPCR) in the SAT of a subset of individuals (N=56); stratified in subgroups according to BMI in normal weight (N=14) and overweight/obese (N=42), or to glucose-metabolizing capacity in NGT (N=27), IGT and T2D (N=29). *RPS23* was used as reference gene. Pearson's correlation coefficient (r) and p values (p) are shown.

Gene	Sense Primer (5'-3')	Antisense Primer (5'-3')	Melting Temperature (°C)	PCR product (bp)	Reference figure
cPPARG	GAGAAGGAGAAGCTGTTGGC	ATGGCCACCTCTTTGCTCT	60	272	1B-C-D; 3C; 4A-B; 6F-G; 7A-B-C; S5B-C-D-E; S7A-B-C
ADIPOQ	CTGGTGAGAAGGGTGAGAAA	GTTCTCCTTTCCTGCCTTGG	60	126	3C; 4A-B; 6G; S5B-C- D
FABP4	TGTGTGATGCTTTTGTAGGTAC	CTTCGTCAAATTCCTGGCCC	60	215	3C; 4A-B; 6G; S5B-C- D
LPL	CGCCGCCGACCAAAGAAGA	AGGTAGCCACGGACTCTGC	60	122	4A-B; 6G
PPARG∆5	CTTGCAGTGGGGATGTCTCA	CAGCAAACCTGGGCGGTTGA	60	242	1A-C-D; 6F- G; S5A
PPIA	TACGGGTCCTGGCATCTTGT	GGTGATCTTCTTGCTGGTCT	60	196	3C; 4A-B; 6F-G; S5A- B-C-D-E
MRTFA	TTGAAACTCCAGCAGCGCC	GCTCCTTCTCTGCTCATGA	60	99	4A-B; 6G; S5B-E
SLC2A4	CGTCGGGCTTCCAACAGAT	GAGCCAAGCACCGCAGAGA	60	100	3C; 4A-B; 6G; 7A-B-C; S5B-C-D; S7A-B-C
PLIN1	GCCTCACCTTGCTGGATGG	GTGGGCTTCCTTAGTGCTG	60	128	3C; 4A-B; 6G; S5B-C- D
PLIN2	GGGCTAGACAGGATTGAGGA	TCACTGCCCCTTTGGTCTTG	60	181	3C; 4A-B; 6G; S5B-C- D
IRS2	TACATCGCCATCGACGTGAG	TCAATGCTGGCGTAGGTGTT	60	215	3C; 4A-B; 6G
RPS23	TCGTGGACTTCGTACTGCT	GCTGTGATTTTCTTGCCATTC	60	237	1A-B-C-D; 7A-B-C; S7A-B-C
PPARG1	CGAGGACACCGGAGAGGG	TGTGTCAACCATGGTCATTTC	60	135	S5A
PPARG2	TCCATGCTGTTATGGGTGAA	TGTGTCAACCATGGTCATTTC	60	113	S5A

Table S1. Sequences of oligonucleotides used in RT-PCR and qPCR assays

Table S2. Reagents and resources information

Antibodies PPARy (C26H12) Rabbit mAb (N terminus) Cell Signaling Technology Catt 2435 CUTU-7 Aptyclonal Antibody Elabscience Catt#CAB-30268 ADIPOQ Polyclonal Antibody Elabscience Catt#CAB-30268 ADIPOQ Polyclonal Antibody Elabscience Catt#CAB-30268 FABP4 Polyclonal Antibody Elabscience Catt#CAB-00281 HS2 Polyclonal Antibody Citats CAB-00281 Elabscience Catt#CAB-00281 HS980A1 Mouse Monocional Antibody Origene Catt# TA500883 Gast Anti-Mouse lgG (H L)-HRP Conjugate Bio-Rad Catt# 170-6516 Gast Anti-Rabbit IgG (H L)-HRP Conjugate Bio-Rad Catt# 170-6516 Subcutaneous adipose tissue CDNA Subcutaneous adipose tissue CDNA End of the Catt# 100-6515 N/A Subcutaneous adipose tissue CDNA End of the Scientific Catt# 100-9114 N/A DMEMF-12, no glutamine Thermo Fisher Scientific Catt# 100-9141 Parter Tast# Scientific Catt# 100-9141 Ponicillin-Streptomycin (G,000 UmL) Thermo Fisher Scientific Catt# 100-9141 Parter Tast# Scientific Catt# 2433 BMP4 Recombinant Human Protein Thermo Fisher Scientific	REAGENT	SOURCE	IDENTIFIER					
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Software and Algorithms Tarazona et al. 2011 https://www.bioconductor.org/	R Bioconductor package	Tarazona et al 2011	https://www.bioconductor.org/					
FACSDiva software			https://www.bidconducion.org/					
GelOuant NET software	GelOuant NET software		https://www.biochamlahsolutions.com					
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