

Supplemental information

**PGC-1s shape epidermal physiology
by modulating keratinocyte proliferation
and terminal differentiation**

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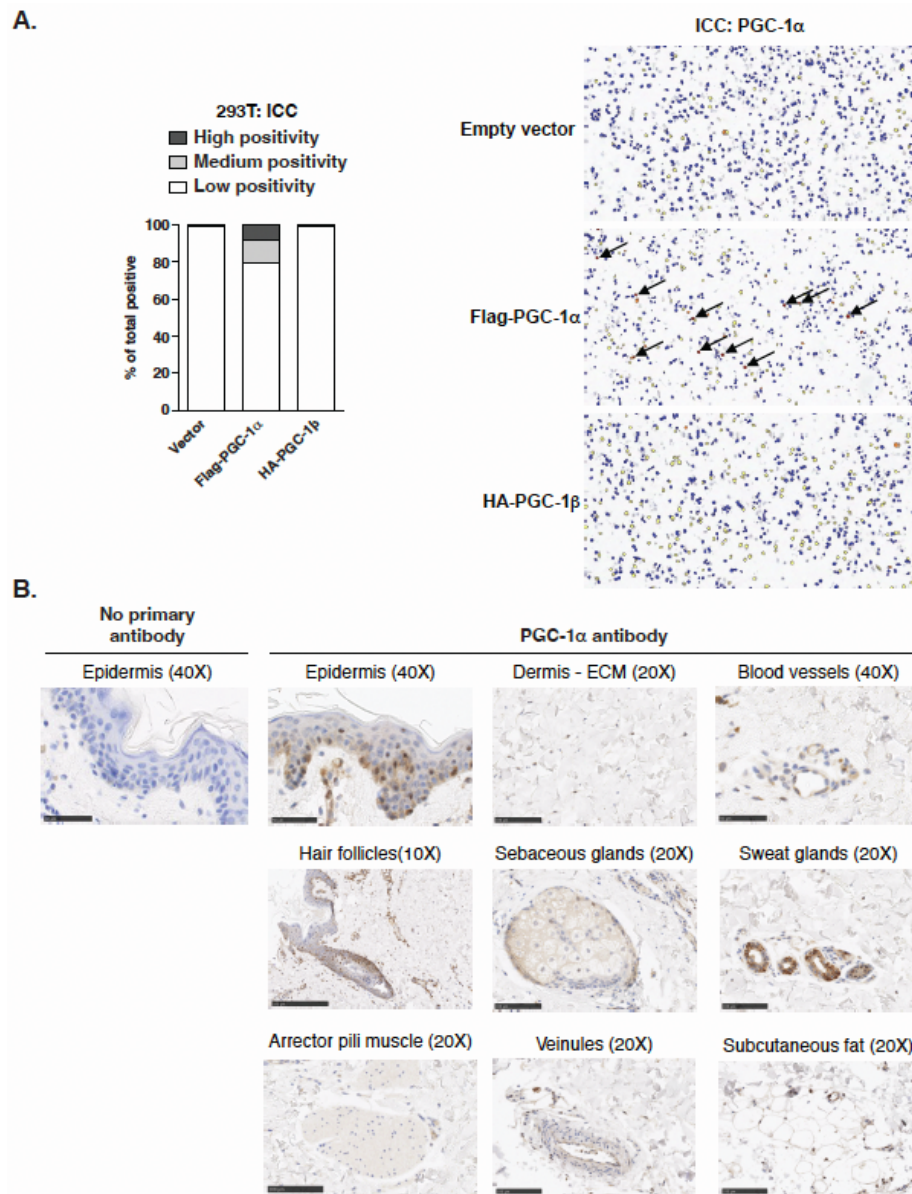
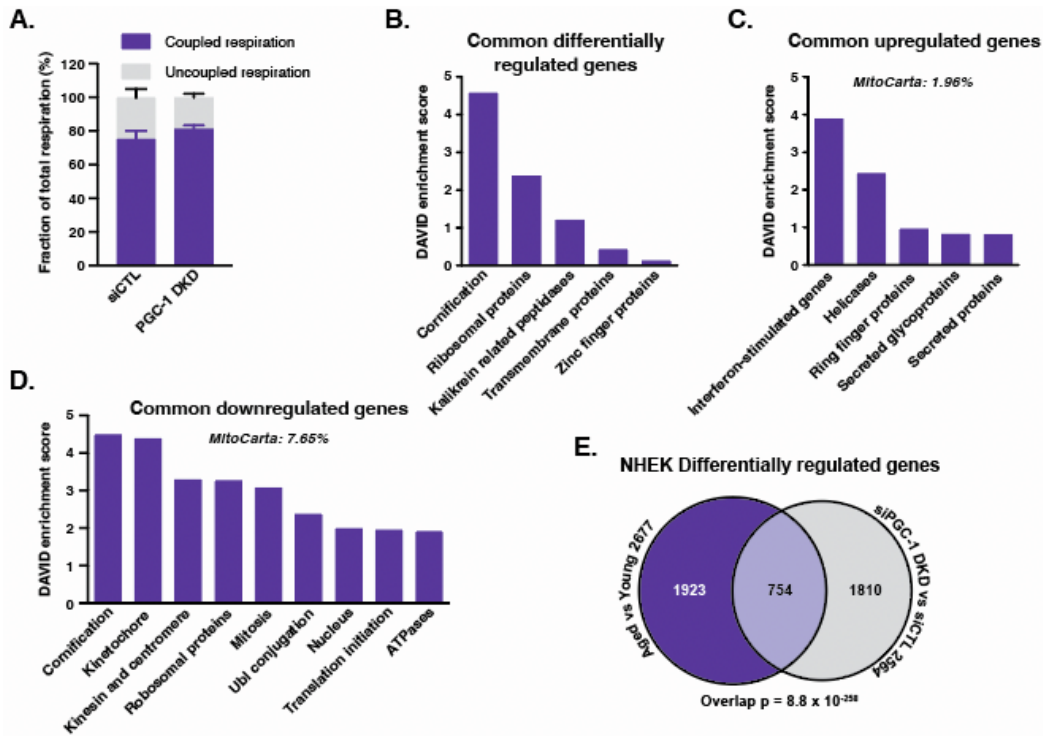


Figure S1. Validation of the anti-PGC-1 α antibody. Related to Figure 1.

(A) Immunocytochemistry for PGC-1 α in 293T cells transfected with Flag-PGC-1 α , HA-PGC-1 β or equivalent concentration of the corresponding empty plasmid. Only 293T cells transfected with Flag-PGC-1 α showed medium and high positivity staining. (B) Immunohistochemistry for PGC-1 α in various human skin features. Panel B is a complement to Figure 1D.



Analysis of upregulated genes in Aged vs Young		Analysis of downregulated genes in Aged vs Young	
Over-represented TFBS	P-value	Over-represented TFBS	P-value
MAFG:NFE2L1	0.67E-04	E2F4	1.64E-06
Stat4	1.30E-03	E2F1	2.01E-06
ATF4	0.00129942	TFDP1	2.78499E-06
JDP2	0.00167059	E2F6	2.27123E-05
TBK5	0.00226745	MYA	0.000110547
Stat3a:Stat5b	0.00251769	ZBTB7A	0.000296722
NFE2	0.00302704	MYB	0.000332058
JUN	0.00319455	SP2	0.000551221
ZBTB18	0.00329712	SP1	0.000647763
ESR2	0.00340408	KLF5	0.000687544
BATF:JUN	0.00371306	ETV5	0.0010241
NRL	0.00465977	SP3	0.0012252
TBK4	0.00545705	ELK4	0.00126166
SOM5	0.00550016	EGR4	0.00169567
JUN(wr.2)	0.00602046	Smad4	0.00169946
IRF3	0.00790523	ETV1	0.00185386
FOSL1	0.00798434	LxR	0.00185995
STAT3	0.00812194	SMAD3	0.00217648
IRF8	0.00874022	Gmab1	0.00221116
TBK1	0.00910568	ETV6	0.0024209
NR3C2	0.00923449	EGR2	0.00261472
STAT1	0.0098789	Gabpa	0.00264632
MEOX1	0.0100219	KLF16	0.00277719
TBK2	0.0104227	ERG	0.00287955
TBK20	0.010675	NR1	0.00327699

Analysis of shared upregulated genes		Analysis of shared downregulated genes	
Over-represented TFBS	P-value	Over-represented TFBS	P-value
ZBTB18	5.03E-04	ELK3	1.61E-05
MAFG:NFE2L1	6.38E-04	ELK1	2.40E-05
JUN	0.000940986	ETV5	3.25666E-05
IRF3	0.00100962	MYB	3.96496E-05
ESR2	0.0010319	ETV1	5.88389E-05
NR3C2	0.00195509	ETV3	6.04624E-05
Stat3a:Stat5b	0.00196193	ETV4	9.70354E-05
BATF:JUN	0.00242824	FEV	0.000114488
NRL	0.00249255	PAX4	0.000151861
IRF9	0.00398833	STAT1:STAT2	0.000174991
ATF4	0.00437921	GMEB2	0.000194828
NRL	0.00506708	TEAD4	0.000246914
NR3C1	0.00514178	FLI1	0.000252903
JDP2	0.00547555	ETV2	0.000260464
TWIST1	0.00592023	ELF1	0.000261062
FOXA1	0.00642341	ERF	0.000363852
TBK5	0.00705105	ERG	0.000364484
IRF8	0.00744711	Hes6	0.000401243
TP53	0.00764341	LMO9	0.000411718
Sox17	0.00775251	TEAD1	0.00043063
JUN(wr.2)	0.00793771	ETV6	0.000455334
NFE2	0.00837516	EMX1	0.000579516
JUN(wr.2)	0.00854396	ETS1	0.000609914
MEIS1	0.0092247	SHOX	0.000607548
TBK19	0.00992113	ATF4	0.000621479

Analysis of upregulated genes in siPGC-1 DKD vs siCTL		Analysis of downregulated genes in siPGC-1 DKD vs siCTL	
Over-represented TFBS	P-value	Over-represented TFBS	P-value
JDP2	8.44E-05	ELK3	3.01E-11
RELA	8.47E-05	ELK1	4.32E-11
JUND	0.000107842	ETV5	3.15283E-10
NRA2	0.00011049	ETV1	5.6653E-10
NFE2	0.00011241	ETV4	1.52029E-09
Stat3a:Stat5b	0.000213703	FEV	1.814E-09
FOSL2	0.000299243	GMEB2	4.71577E-09
JUN(wr.2)	0.000357456	FLI1	2.71597E-08
FOSL1	0.000401932	ERF	5.88127E-08
ZBTB18	0.000688942	ETV3	9.75915E-08
JUNB	0.00109502	ERG	1.36251E-07
FOS	0.00127252	ELK4	1.48174E-07
FOS:JUND	0.00144144	ETS1	1.82201E-07
RORA	0.00153528	Gabpa	1.93471E-07
REL	0.00184633	ELF1	3.495E-07
FOSL2:JUN	0.0021201	ELF4	8.21314E-07
FOXA1	0.00229877	Gmab1	3.3788E-06
BATF:JUN	0.0024058	ETV6	5.25294E-06
NR2F2	0.00241138	ZBTB7A	8.35212E-06
HSF2	0.002708	MYB	1.04897E-05
MYF6	0.00296827	AMD	1.51583E-05
FOXO1	0.00307664	SHF	1.66277E-05
PKNOK2	0.00372504	Crem	1.81663E-05
FOXO1	0.00378216	HOKD11	2.11119E-05
Foxo2	0.00385645	CEBPD	0.000054904

Figure S2. Characterization of metabolic and gene expression changes by PGC-1s knockdown and aging in keratinocytes. Related to figure 2. (A) Fraction of mitochondrial respiration that is coupled and uncoupled to ATP production in PGC-1 DKD or control HaCaT keratinocytes. Data are shown as mean from 3 independent experiments + SEM. (B) Gene functional classification analysis of differentially regulated genes shown in Figure 1D and shared between aging and PGC-1 DKD groups. (C) Analysis of upregulated genes shown in Figure 1D and shared between aging and PGC-1 DKD groups. (D) Analysis of downregulated genes shown in Figure 1D and shared between aging and PGC-1 DKD groups. For panels C and D, the % of identified genes that code for mitochondrial proteins is shown (human MitoCarta2.0 database). (E) Transcription factor binding site (TFBS) over-representation in NHEK from aged versus young donors, siPGC-1 DKD versus siCTL, and for the overlap between groups. Top: groups of differentially regulated genes and overlap. Bottom: the top 25 over-represented TFBS (p -value below 0.05) are presented for each group and overlap.

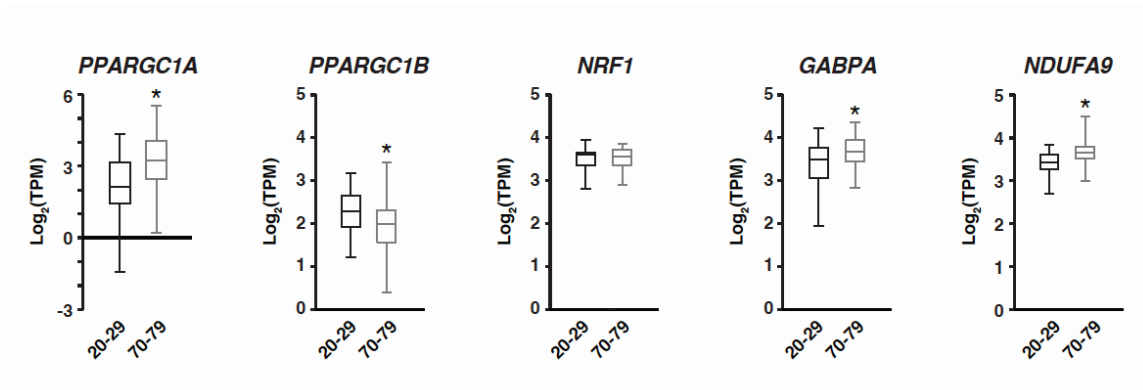


Figure S3. Gene expression analyses of skin samples from young (20-29 years) and aged (70-79 years) donors. Related to Figure 2. Analyses were performed using openly accessible RNA-seq data from the Genotype-Tissue Expression (GTEx) Project. Gene transcripts per million (TPMs) from suprapubic skin samples were sourced from GTEx Analysis V8 (dbGaP Accession phs000424.v8.p2). Data are shown as mean \pm STDEV using box plots. There are 35 young donors and 22 aged donors. * $p < 0.05$, unpaired Student's t -test.

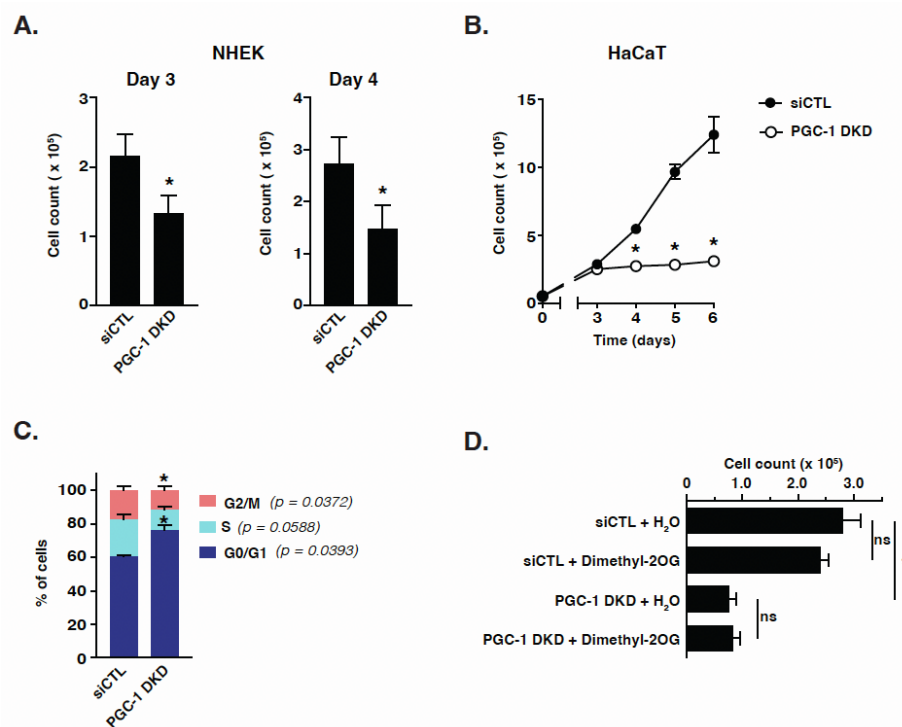


Figure S4. Impact of PGC-1s knockdown on the proliferation of keratinocytes. Related to Figure 4. (A) Proliferation curve of normal human epidermal keratinocytes (NHEK) transfected with control siRNA or PGC-1 DKD. Data are shown as mean + SEM from 3 independent experiments. * $p < 0.05$, paired Student's t -test. (B) Proliferation curve of HaCaT cells transfected with control siRNA or PGC-1 DKD. Data is shown as mean \pm SEM from 3 independent experiments. * $p < 0.05$, paired Student's t -test. (C) Cell cycle analysis of keratinocyte HaCaT cells transfected with control siRNA or PGC-1 DKD. Data are shown as mean + SEM from 3 independent experiments. * $p < 0.05$, paired Student's t -test. (D) Dimethyl-2OG cannot rescue the proliferation of PGC-1s depleted keratinocyte HaCaT cells. Data are shown as mean + SEM from 3 biological replicates. Data are representative of 2 independent experiments. * $p < 0.05$, Two-Way ANOVA, Tukey post-test.

Table S1. List of oligonucleotides used for qPCR. Related to STAR Methods.

Primer name	Sequence (5'-->3')	Source	Identifyer
<i>PPARGCIA</i> -FWD	CCTGTGATGCTTTTGCTGCTCTTG	This paper	N/A
<i>PPARGCIA</i> -REV	AAACTATCAAAATCCAGAGAGTCA	This paper	N/A
<i>PPARGCIB</i> -FWD	GTACATTCAAAATCTCTCCAGCGACATG	This paper	N/A
<i>PPARGCIB</i> -REV	GAGGGCTCGTTGCGCTTCTCAGGGCAG	This paper	N/A
<i>B2M</i> -FWD	GTGCTCGCGCTACTCTCTC	This paper	N/A
<i>B2M</i> -REV	GTCAACTTCAATGTCCGGAT	This paper	N/A
<i>POLR2A</i> -FWD	ATCTCTCCTGCCATGACACC	This paper	N/A
<i>POLR2A</i> -REV	AGACCAGGCAGGGGAGTAAC	This paper	N/A
<i>TGMI</i> -FWD	CCCCAAGAGACTAGCAGTG	This paper	N/A
<i>TGMI</i> -REV	AAAATGAAAGGCGTGTGTA	This paper	N/A
<i>IVL</i> -FWD	CCTCAGCCTTACTGTGAGT	This paper	N/A
<i>IVL</i> -REV	TGCTCCTGATGGGTATTGAC	This paper	N/A
<i>KRT1</i> -FWD	CGAGAAAGGGAGCAAATCAA	This paper	N/A
<i>KRT1</i> -REV	TCCCATTTTGTTCAGTACC	This paper	N/A
<i>KRT10</i> -FWD	GGGACCAAGATACTAACAAAACC	This paper	N/A
<i>KRT10</i> -REV	GAAAGAACTCTACCGTCGGG	This paper	N/A
<i>CS</i> -FWD	CAACTCAGGACGGGTTGTTCCAGG	This paper	N/A
<i>CS</i> -REV	GCATGAATAAAGCAGCCAAGTGAG	This paper	N/A
<i>FAS</i> -FWD	AGGTTTGATGCCTCCTTCTTCGGA	This paper	N/A
<i>FAS</i> -REV	TGGCTTCATAGGTGACTTCCAGCA	This paper	N/A
<i>GSS</i> -FWD	CCTGGCTGAGGGAGTATTG	This paper	N/A
<i>GSS</i> -REV	TTTGATGGTGCTGGAAAGAGT	This paper	N/A
<i>GSR</i> -FWD	TGATTCAATGATCAGCACCAAC	This paper	N/A
<i>GSR</i> -REV	CAGTAACCATGCTGACTTCCA	This paper	N/A
<i>VEGFA</i> -FWD	TACCTCCACCATGCAAAGTG	This paper	N/A
<i>VEGFA</i> -REV	ATGATTCTGCCCTCCTCCTTC	This paper	N/A
<i>C3</i> -FWD	AAGATAAGAACCGCTGGGAG	This paper	N/A
<i>C3</i> -REV	GGAGGCACAAAGTCAAAGTC	This paper	N/A
<i>SGK1</i> -FWD	GGTGATGACGGTGAAAAGTCTG	This paper	N/A
<i>SGK1</i> -REV	AAAGCGATGAGAATTGCCAC	This paper	N/A
<i>IFIT1</i> -FWD	TCCACAAGACAGAATAGCCA	This paper	N/A
<i>IFIT1</i> -REV	CCATTTGTACTCATGGTTGCT	This paper	N/A
<i>PPARGCIA</i> (TaqMan)	N/A	Thermo Scientific	Fisher Hs00173304 m1
<i>PPARGCIB</i> (TaqMan)	N/A	Thermo Scientific	Fisher Hs00993805 m1
<i>IVL</i> (TaqMan)	N/A	Thermo Scientific	Fisher Hs00846307_s1
<i>TGMI</i> (TaqMan)	N/A	Thermo Scientific	Fisher Hs00165929 m1
<i>TBP</i> (TaqMan)	N/A	Thermo Scientific	Fisher Hs00427620 m1

Table S2. List of shRNA sequences used for the preparation of lentiviruses. Related to STAR Methods.

shRNA	21mer sequence	Position on mRNA	Region	% of knockdown	Source	Identifyer
Non targeting (NTshRNA)	GGAATCTCATTTCGATGCATAC	N/A	N/A	N/A	This paper	N/A
shPGC1α	GCTATGGTTTCATTACCTACC	2181	CDS	68	This paper	N/A
shPGC1β	GCTGCTGAGAGACCATGAGAT	2233	CDS	72	This paper	N/A