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Supplemental Information

SNEV$^{hPrp19/hPso4}$ Regulates Adipogenesis of Human Adipose Stromal Cells

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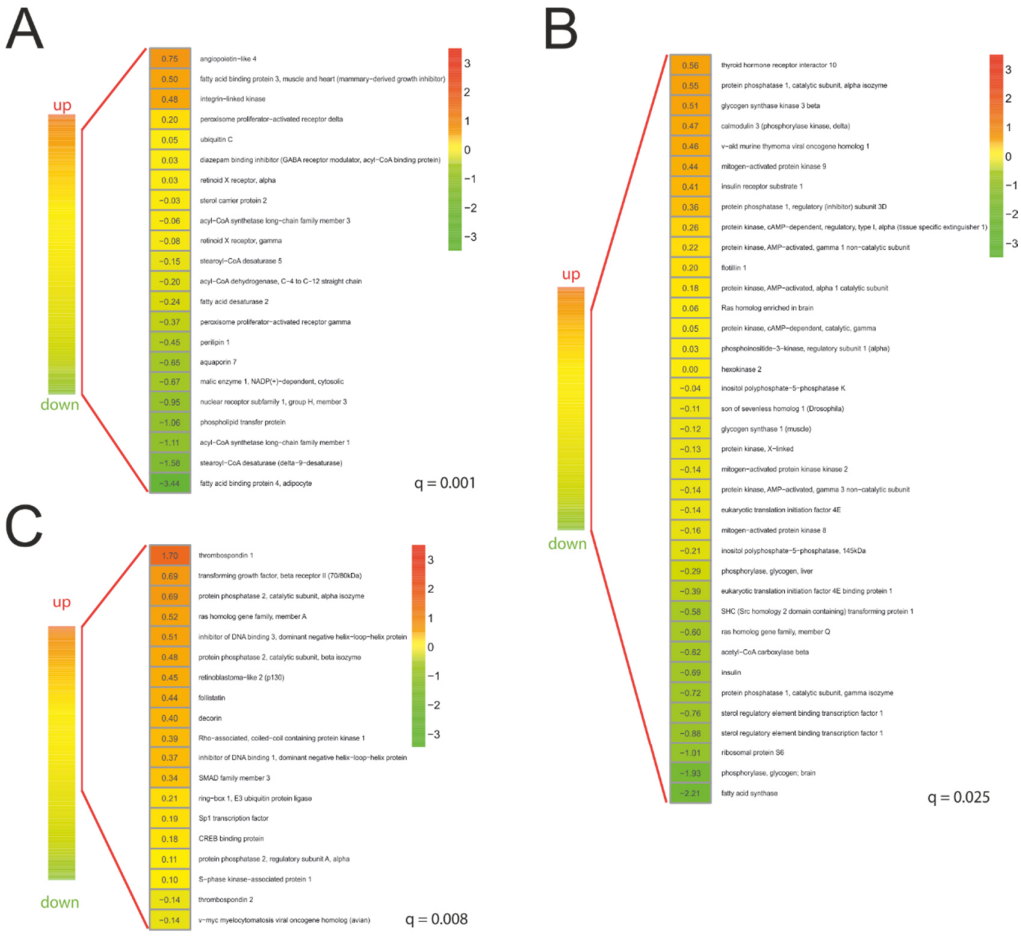
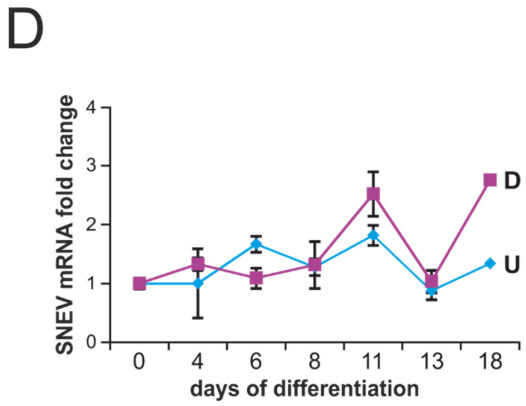
Supplemental Experimental Data Items:

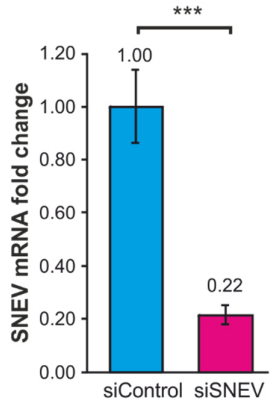
Fig. S1 (related to Fig. 2)

Knock-down of SNEV downregulates components of signalling pathways involved in adipogenic differentiation and does not interfere with osteogenic differentiation.

(A) Micro-array based analysis of transcript expression assorted to the KEGG PPARγ signalling pathway is shown as heat map after GSEA. **(B)** Expression of transcripts assorted to the KEGG insulin signaling pathway is shown as heat map. **(C)** Expression of transcripts assorted to the KEGG TGF-β signaling pathway is shown as heat map. Numbers within cells are log2-transformed expression ratios (siSNEV /siControl). The red vertical line denotes the range of this gene set relative to all 3,250 transcripts that could be detected (small heat map). FDR q-value obtained from GSEA is depicted bottom right. **(D)** *SNEV* mRNA fluctuates during osteogenic differentiation. Osteogenic differentiation of hASCs was induced and *SNEV* mRNA expression was determined by qPCR. **(E)** hASCs were transfected with siRNA against SNEV or control siRNA, leading to a knockdown of almost 80%. **(F)** SNEV knockdown does not interfere with osteogenic differentiation of hASCs. Alizarin Red staining for calcium deposition as end-point measurement for siControl (left panel) and siSNEV (right panel) transfected hASCs.


A
up
down
0.75 angiopoietin−like 4
0.50 fatty acid binding protein 3, muscle and heart (mammary−derived growth inhibitor)
0.48 integrin−linked kinase
0.20 peroxisome proliferator−activated receptor delta
0.05 ubiquitin C
0.03 diazepam binding inhibitor (GABA receptor modulator, acyl−CoA binding protein)
0.03 retinoid X receptor, alpha
−0.03 sterol carrier protein 2
−0.06 acyl−CoA synthetase long−chain family member 3
−0.08 retinoid X receptor, gamma
−0.15 stearoyl−CoA desaturase 5
−0.20 acyl−CoA dehydrogenase, C−4 to C−12 straight chain
−0.24 fatty acid desaturase 2
−0.37 peroxisome proliferator−activated receptor gamma
−0.45 perilipin 1
−0.65 aquaporin 7
−0.87 malic enzyme 1, NADP(+)−dependent, cytosolic
−0.95 nuclear receptor subfamily 1, group H, member 3
−1.06 phospholipid transfer protein
−1.11 acyl−CoA synthetase long−chain family member 1
−1.58 stearoyl−CoA desaturase (delta−9−desaturase)
−3.44 fatty acid binding protein 4, adipocyte
3 2 1 0 −1 −2 −3
q = 0.001
B
up
down
0.56 thyroid hormone receptor interactor 10
0.55 protein phosphatase 1, catalytic subunit, alpha isozyme
0.51 glycogen synthase kinase 3 beta
0.47 calmodulin 3 (phosphorylase kinase, delta)
0.46 v−akt murine thymoma viral oncogene homolog 1
0.44 mitogen−activated protein kinase 9
0.41 insulin receptor substrate 1
0.36 protein phosphatase 1, regulatory (inhibitor) subunit 3D
0.26 protein kinase, cAMP−dependent, regulatory, type I, alpha (tissue specific extinguisher 1)
0.22 protein kinase, AMP−activated, gamma 1 non−catalytic subunit
0.20 flotillin 1
0.18 protein kinase, AMP−activated, alpha 1 catalytic subunit
0.06 Ras homolog enriched in brain
0.05 protein kinase, cAMP−dependent, catalytic, gamma
0.03 phosphoinositide−3−kinase, regulatory subunit 1 (alpha)
0.00 hexokinase 2
−0.04 inositol polyphosphate−5−phosphatase K
−0.11 son of sevenless homolog 1 (Drosophila)
−0.12 glycogen synthase 1 (muscle)
−0.13 protein kinase, X−linked
−0.14 mitogen−activated protein kinase kinase 2
−0.14 protein kinase, AMP−activated, gamma 3 non−catalytic subunit
−0.14 eukaryotic translation initiation factor 4E
−0.16 mitogen−activated protein kinase 8
−0.21 inositol polyphosphate−5−phosphatase, 145kDa
−0.29 phosphorylase, glycogen, liver
−0.39 eukaryotic translation initiation factor 4E binding protein 1
−0.58 SHC (Src homology 2 domain containing) transforming protein 1
−0.60 ras homolog gene family, member Q
−0.62 acetyl−CoA carboxylase beta
−0.69 insulin
−0.72 protein phosphatase 1, catalytic subunit, gamma isozyme
−0.76 sterol regulatory element binding transcription factor 1
−0.88 sterol regulatory element binding transcription factor 1
−1.01 ribosomal protein S6
−1.93 phosphorylase, glycogen; brain
−2.21 fatty acid synthase
3 2 1 0 −1 −2 −3
q = 0.025
C
up
down
1.70 thrombospondin 1
0.69 transforming growth factor, beta receptor II (70/80kDa)
0.69 protein phosphatase 2, catalytic subunit, alpha isozyme
0.52 ras homolog gene family, member A
0.51 inhibitor of DNA binding 3, dominant negative helix−loop−helix protein
0.48 protein phosphatase 2, catalytic subunit, beta isozyme
0.45 retinoblastoma−like 2 (p130)
0.44 follistatin
0.40 decorin
0.39 Rho−associated, coiled−coil containing protein kinase 1
0.37 inhibitor of DNA binding 1, dominant negative helix−loop−helix protein
0.34 SMAD family member 3
0.21 ring−box 1, E3 ubiquitin protein ligase
0.19 Sp1 transcription factor
0.18 CREB binding protein
0.11 protein phosphatase 2, regulatory subunit A, alpha
0.10 S−phase kinase−associated protein 1
−0.14 thrombospondin 2
−0.14 v−myc myelocytomatosis viral oncogene homolog (avian)
3 2 1 0 −1 −2 −3
q = 0.008


D
SNEV mRNA fold change
0 1 2 3 4
0 4 6 8 11 13 18
days of differentiation
D
U


E
***
SNEV mRNA fold change
0.00 0.20 0.40 0.60 0.80 1.00 1.20
1.00
0.22
siControl siSNEV

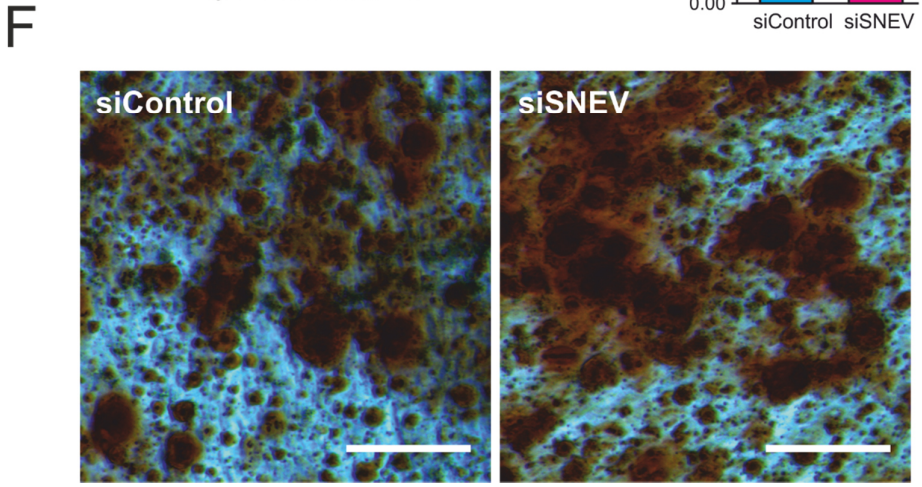

F
siControl
siSNEV

Fig. S2 (related to Fig. 2)

SNEV overexpression accelerates adipogenic differentiation of hASCs.

(A) Schematic representation of experimental design. hASCs were transduced with *SNEV* or empty vector and were sequentially differentiated for 10 days. **(B)** Total RNA isolated at specified time points was subjected to qPCR to analyze *SNEV* mRNA levels to confirm overexpression. The data was normalized to *GAPDH*. N = 4 (four technical replicates from donor 812 are shown. The experiment was repeated twice with cells from the other two other donors with a similar outcome). **(C)** Oil red O staining of intracellular lipids after 6 days of differentiation. Scale bar = 100 µm. Exemplary images of cells from donor 812 are shown. **(D)** Intracellular triglyceride levels in transduced hASCs after 6 days of differentiation. The triglyceride content was normalized to total protein content, the mean values of four technical replicates (cells were seeded into four wells of a plate and grown and analysed independently) are shown. Two independent differentiation experiments from two different donors (803 and 812) are shown side-by-side. **(E)** Quantitation of *PPARγ* and **(F)** *FASN* mRNA. N = 4 (four technical replicates from donor 812 are shown. The experiment was repeated twice with cells from the other two other donors with a similar outcome).

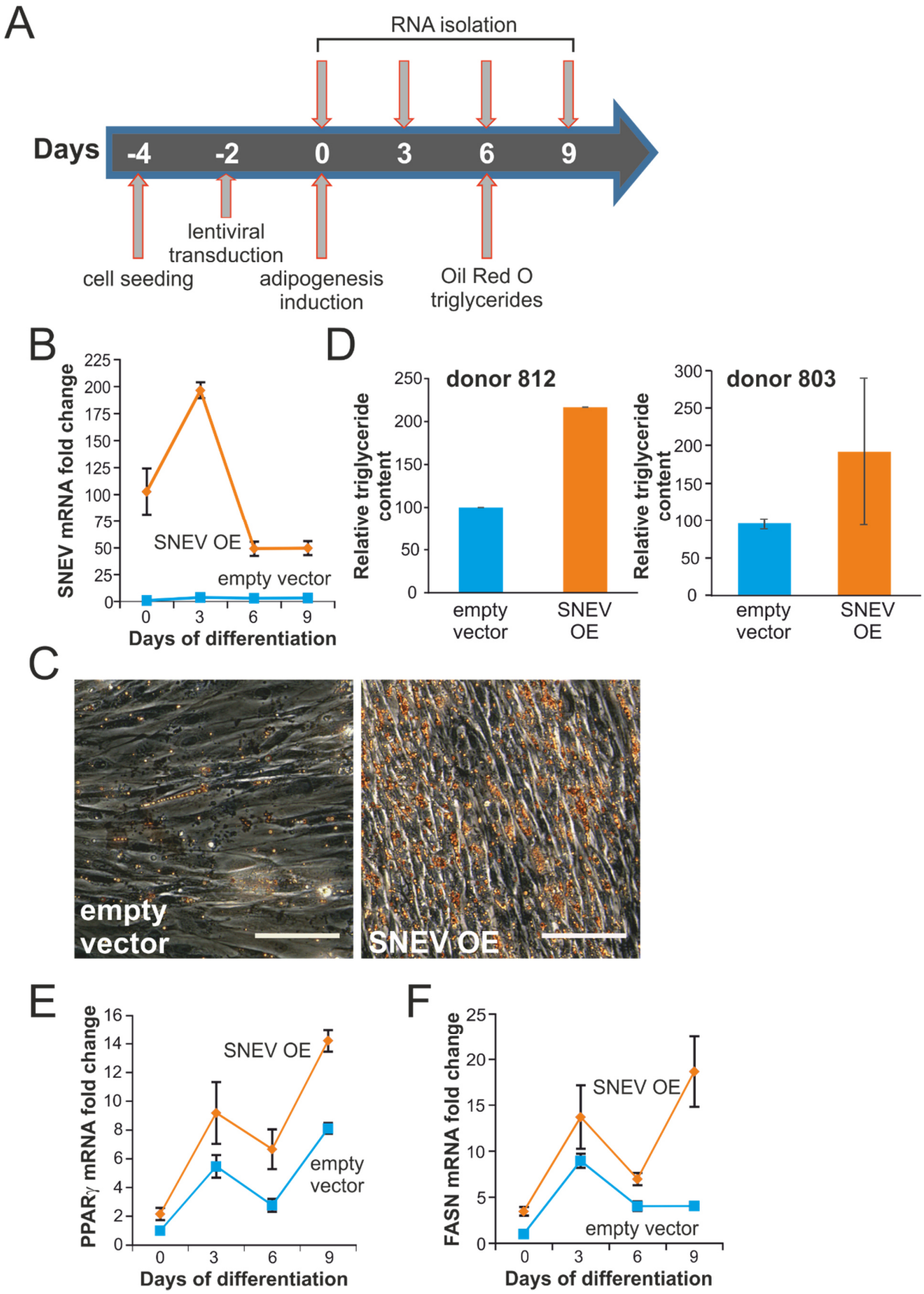
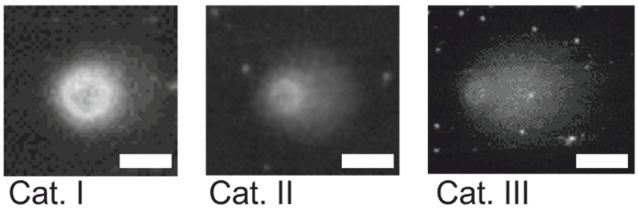

A
RNA isolation
Days
-4
-2
0
3
6
9
lentiviral transduction
cell seeding
adipogenesis induction
Oil Red O triglycerides
B
SNEV mRNA fold change
SNEV OE
empty vector
Days of differentiation
D
donor 812
donor 803
Relative triglyceride content
empty vector
SNEV OE
C
empty vector
SNEV OE
E
PPARγ mRNA fold change
SNEV OE
empty vector
Days of differentiation
F
FASN mRNA fold change
SNEV OE
empty vector
Days of differentiation

Fig. S3 (related to Fig. 3)

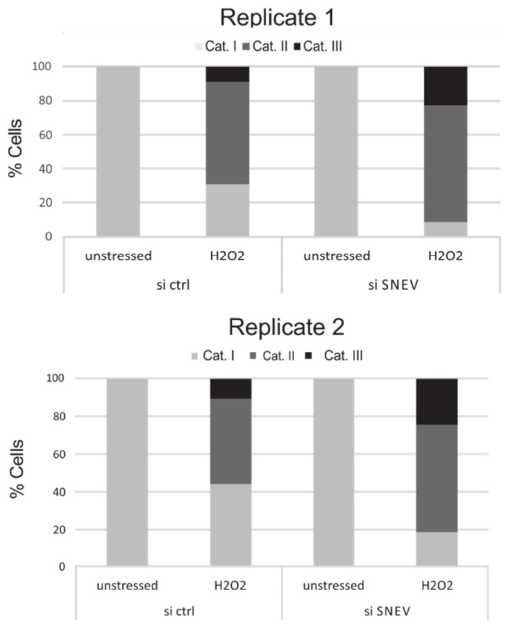
Results of comet assays analysed by two different methods are similar.

(A) hASCs derived from donor 812 were transfected with siRNA targeting *SNEV* mRNA, followed by oxidative stress treatment using 500 µM H_2O_2 for 90 min and 60 min recovery. DNA damage induced by that treatment was assessed by a comet assay. Cells were classified into three groups according to tail size, representing no, low to medium or high levels of DNA breaks. Representative pictures of cells in category 1 (no or very small tail), category 2 (small to medium size tail) and category 3 (large tail) are shown. Scale bar = 20 µm. **(B)** Separate results for two independent siRNA transfections (both with cells from donor 812) are shown. **(C)** An alternative data analysis based on calculation of tail size using ImageJ yielded similar results as in (B). Pooled data from two replicates are shown. **(D)** Separate results for the two replicates.

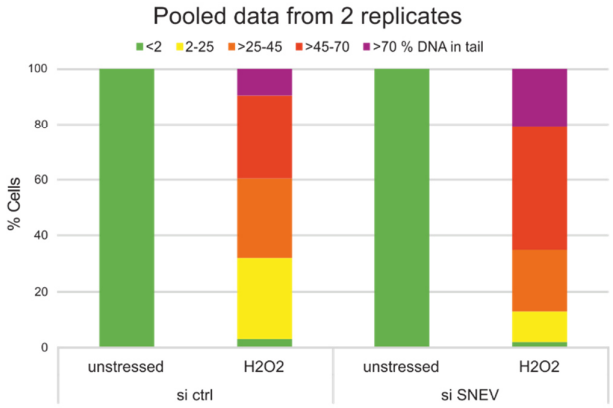
A


Cat. I
Cat. II
Cat. III

B


Replicate 1
Cat. I Cat. II Cat. III
% Cells
unstressed
H2O2
si ctrl
si SNEV
Replicate 2
Cat. I Cat. II Cat. III
% Cells
unstressed
H2O2
si ctrl
si SNEV

C


Pooled data from 2 replicates
<2 2-25 >25-45 >45-70 >70 % DNA in tail
% Cells
unstressed
H2O2
si ctrl
si SNEV

D

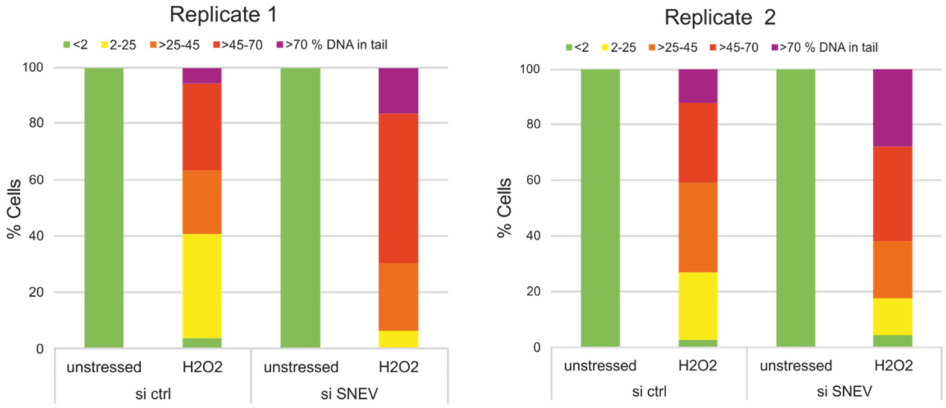

Replicate 1
<2 2-25 >25-45 >45-70 >70 % DNA in tail
% Cells
unstressed
H2O2
si ctrl
si SNEV
Replicate 2
<2 2-25 >25-45 >45-70 >70 % DNA in tail
% Cells
unstressed
H2O2
si ctrl
si SNEV

Fig. S4 (related to Fig. 3)

Knock-down of WRN reduces adipogenic differentiation in hASCs.

(A) Heat map of differentially transcribed genes during adipogenesis. Fold changes were calculated relative to reference day 2. RT-qPCR confirmed regulation of **(B)** *WRN*, **(C)** *CSA* and **(D)** *XPE* during adipogenic differentiation. N = 4 (four technical replicates, the experiment was once repeated independently with cells from a different donor with the same outcome). **(E)** Knock-down of *WRN*, *CSA*, *XPE* mRNAs was confirmed by qPCR and normalized to *GAPDH*. N = 4 (four technical replicates from one donor are shown. The experiment was repeated twice with cells from two other donors with a similar outcome). **(F)** Triglyceride content was quantified at day 10 of adipogenic differentiation and normalized to total protein content. N = 4 (cells were seeded into four wells of a plate and grown and analysed independently).

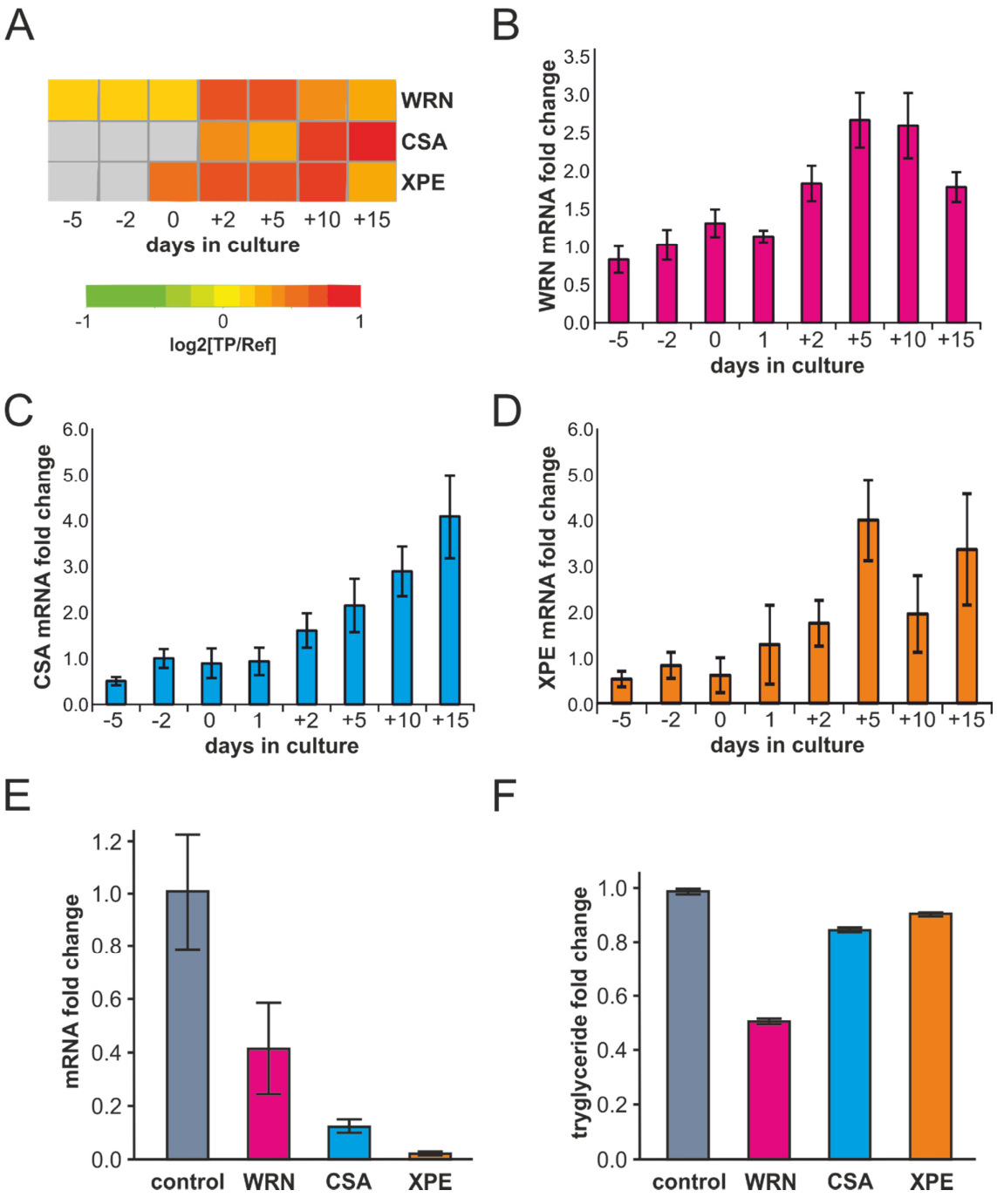

A
WRN
CSA
XPE
-5 -2 0 +2 +5 +10 +15
days in culture
-1 0 1
log2[TP/Ref]
B
WRN mRNA fold change
3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
-5 -2 0 1 +2 +5 +10 +15
days in culture
C
CSA mRNA fold change
6.0 5.0 4.0 3.0 2.0 1.0 0.0
-5 -2 0 1 +2 +5 +10 +15
days in culture
D
XPE mRNA fold change
6.0 5.0 4.0 3.0 2.0 1.0 0.0
-5 -2 0 1 +2 +5 +10 +15
days in culture
E
mRNA fold change
1.2 1.0 0.8 0.6 0.4 0.2 0.0
control WRN CSA XPE
F
tryglyceride fold change
1.0 0.8 0.6 0.4 0.2 0.0
control WRN CSA XPE

Table S1 (related to Fig. 2): Significantly enriched gene sets among upregulated and downregulated mRNAs upon SNEV knockdown.

gene sets database	significantly enriched gene sets amung UPREGULATED mRNAs	FDR q-val
KEGG pathways	KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	0.000
	KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	0.000
	KEGG_FOCAL_ADHESION	0.001
	KEGG_CELL_ADHESION_MOLECULES_CAMS	0.005
	KEGG_LYSOSOME	0.006
	KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	0.006
	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	0.007
	KEGG_HEMATOPOIETIC_CELL_LINEAGE	0.008
	KEGG_ECM_RECEPTOR_INTERACTION	0.008
	KEGG_TGF_BETA_SIGNALING_PATHWAY	**0.008**
	KEGG_TIGHT_JUNCTION	0.008
	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	0.045
	KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.058
	KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.062
	KEGG_COLORECTAL_CANCER	0.064
	KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	0.066
	KEGG_VIBRIO_CHOLERAE_INFECTION	0.069
	KEGG_DILATED_CARDIOMYOPATHY	0.073
	KEGG_ADHERENS_JUNCTION	0.091
	KEGG_SMALL_CELL_LUNG_CANCER	0.092
	KEGG_BLADDER_CANCER	0.092
BP: GO biological process	ACTIN_FILAMENT_BASED_PROCESS	0.000
	ACTIN_CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	0.000
	CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	0.020
CC: GO cellular component	ACTIN_CYTOSKELETON	0.000
	ER_GOLGI_INTERMEDIATE_COMPARTMENT	0.026
	CYTOSKELETON	0.027
	ENDOPLASMIC_RETICULUM	0.027
	MEMBRANE_FRACTION	0.048
	INTEGRAL_TO_MEMBRANE	0.050
	INTRINSIC_TO_MEMBRANE	0.053
	CELL_FRACTION	0.053
	ENDOPLASMIC_RETICULUM_PART	0.055
	EXTRACELLULAR_REGION_PART	0.057
	MEMBRANE	0.057
	PLASMA_MEMBRANE	0.058
	EXTRACELLULAR_REGION	0.059
	ENDOMEMBRANE_SYSTEM	0.063
	GOLGI_APPARATUS	0.069
	MEMBRANE_PART	0.070
	CYTOSKELETAL_PART	0.071
	PROTEINACEOUS_EXTRACELLULAR_MATRIX	0.072
	ENDOPLASMIC_RETICULUM_MEMBRANE	0.074
	EXTRACELLULAR_MATRIX	0.075
	CELL_PROJECTION	0.082
	CYTOPLASMIC_PART	0.087
	CELL_CORTEX	0.087
	PLASMA_MEMBRANE_PART	0.096
MF: GO molecular function	CYTOSKELETAL_PROTEIN_BINDING	0.004
	ACTIN_BINDING	0.010
	PEPTIDE_BINDING	0.024
	NUCLEOSIDE_TRIPHOSPHATASE_ACTIVITY	0.027
	HYDROLASE_ACTIVITY_ACTING_ON_ACID_ANHYDRIDES	0.034
	ATPASE_ACTIVITY_COUPLED	0.057
	ATPASE_ACTIVITY	0.091
	GTPASE_ACTIVITY	0.091
Reactome pathway database	REACTOME_HEMOSTASIS	0.003
	REACTOME_AXON_GUIDANCE	0.005
	REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	0.051
transcription factor targets	CCAWWNAAGG_V$SRF_Q4	0.004

gene sets database	significantly enriched gene sets amung DOWNREGULATED mRNAs	FDR q-val
KEGG pathways	KEGG_RIBOSOME	0.000
	KEGG_PPAR_SIGNALING_PATHWAY	0.001
	KEGG_INSULIN_SIGNALING_PATHWAY	0.025
BP: GO biological process	CELLULAR_BIOSYNTHETIC_PROCESS	0.000
	BIOSYNTHETIC_PROCESS	0.000
CC: GO cellular component	RIBONUCLEOPROTEIN_COMPLEX	0.080
MF: GO molecular function	OXIDOREDUCTASE_ACTIVITY_GO_0016616	0.085
Reactome pathway database	REACTOME_3_UTR_MEDIATED_TRANSLATIONAL_REGULATION	0.000
	REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	0.000
	REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	0.000
transcription factor targets	no significantly (q<0.1) enriched gene sets	
chemical and genetic perturbations	BHATI_G2M_ARREST_BY_2METHOXYESTRADIOL_DN	0.036
	ABE_INNER_EAR	0.081

Table S2 (related to Experimental Procedures): Donor information

hASCs were isolated by liposuction. Basic information on the donors is provided.

Donor Number	**803**	**812**	**851**
Gender	female	female	female
Age (years)	45	39	25
Site of liposuction	femoral	femoral/abdominal	femoral
BMI	30.6	31.5	26.9

Table S3 (related to Experimental Procedures): **Characterization of hASCs**

Typical markers of mesenchymal (CD73, CD90, CD105 and HLA-ABC) and hematopoietic (CD14, CD34, CD45 and HLA-DR) stem cells were analyzed by flow cytometry. The percentage of cells positive for each of the markers is shown.

Donor	**803**	**812**	**851**
CD73 [% positive]	99.23	95.23	99.88
CD90 [% positive]	99.27	80.55	99.91
CD105 [% positive]	91.12	99.68	98.84
HLA-ABC [% positive]	94.72	97.57	97.54
CD14 [% positive]	1.03	0.86	1.5
CD34 [% positive]	4.96	1.25	1.03
CD45 [% positive]	1.95	1.19	0.95
HLA-DR [% positive]	1.87	0.75	0.73

Table S4 (related to Experimental Procedures): Primers used in this study

Sequences of primers used in this study are provided.

Gene name	Forward primer	Reverse primer
SNEV	TCATTGCCCGTCTCACCAAG	GGCACAGTCTTCCCTCTCTTC
PPARγ	AGCCTGCGAAAGCCTTTTGGTGA	GCAGTAGCTGCACGTGTTCCGT
FASN	AACTTGCAGGAGTTCTGGGAC	TGAATCTGGGTTGATGCCTCCG
GAPDH	TGTGAGGAGGGGAGATTCAG	CGACCACTTTGTCAAGCTCA
WRN	GTGGCGCTCCACAGTCAT	TCTTCCGAACACATGCCTTTC
CSA	GAGGACACGATATGCTGGGG	CCAGTCCCAAAACTCTCCGT
XPE	AAGAAACGCCCAGAAACCCA	ACATCTTCTGCTAGGACCGGA
prp-19 (C. elegans)	TCGTGTGCGGAATCAGTGGTGA	TGGTGCTGATCCAGTGCCGC
wrn-1 (C. elegans)	AGGAAGACACTTTGGTGGACCT	ACCAAATGCAACTGTCGCAACGA
xpa-1 (C. elegans)	TGCAGGAACGTCGCGAGCAA	TGCGCAGATCCAGATCGCAA
M18.5 (C. elegans)	CTCGCTGAGTTCCAGCGCCT	CCATTGGAATCGAGCGAACGTGG
act-1 (C. elegans)	CTACGAACTTCCTGACGGACAAG	CCGGCGGACTCCATACC

Supplemental Experimental Procedures:

Adipogenic differentiation

14,000 hASCs were seeded into 1.9 cm^2 plates in growth medium two days prior to adipogenic induction. Adipogenesis was induced by adding DMEM high glucose (GE Healthcare) supplemented with 549 µM 3-Isobutyl-1-methylxanthine (Sigma), 1 µM Dexamethasone (Sigma), 549 nM hydrocortisone (Sigma), 66 µM Indomethacin (Sigma). Medium was exchanged twice per week. Undifferentiated control hASCs were grown in DMEM high glucose/Ham's F12 (GE Healthcare), supplemented with 10% FCS, 4 mM L-Glutamine and 0.1 ng/ml β-FGF (R&D systems). Total RNA and protein were isolated at specified time points during differentiation. Triglycerides were quantified by Infinity Triglyceride quantification kit (ThermoScientific) according to the manufacturer's recommended protocol and normalized to total protein concentration, as measured using the BCA kit (ThermoScientific).

Oil red O staining of hASCs was performed on day 10 of differentiation. Medium was removed, cells were washed twice with PBS, and fixed by incubating in 3.6% formaldehyde in PBS for 1 h at room temperature. Cells were then washed twice for 5 min with PBS for 5 min, incubated and fixed with 70% ethanol for 2 min followed by 10 min incubation in Oil red O working solution (1.8 mg/ml, Sigma). Cells were washed with PBS until all visible traces of dye were removed. Pictures were taken on a Leica DM IL LED Inverted Microscope with a 10x dry objective (Leica Microsystems).

Osteogenic differentiation

2,000 hASCs were seeded into 1.9 cm^2 plates 72 hours prior to induction of osteogenesis. 72 h post seeding, osteogenesis was induced by low glucose DMEM (GE Healthcare) supplemented with 10% FCS, 4 mM L-Glutamine, 10 mM L-Glycerophosphate (Sigma), 150 µM Ascorbate-2-phosphate (Sigma), 10 nM vitamin-D3 (Sigma), 10 nM Dexamethasone (Sigma) and 100 µg/ml Primocin. hASCs grown in DMEM low glucose/Ham`s F12, 1:1 (PAA) supplemented with 10% FCS, 4 mM L-Glutamine and 1x Primocin were used as control. The medium was replaced every third day until day 12. Osteogenic differentiation was assessed by Alizarin Red staining.

To perform Alizarin Red staining, medium was removed and cells were washed thrice with PBS, fixed with 70% ethanol for 1 h at -20 °C, washed again thrice with PBS, and incubated for 10 min with gentle shaking with Alizarin Red solution (200 mg/ml, Applichem). Cells were washed with PBS until all visible traces of the dye were removed. Pictures were taken on a Leica DM IL LED Inverted Microscope with a 10x dry objective.

Antibodies for Western Blot

Prp19/Pso4 rabbit polyclonal antibody was from Bethyl Laboratories (Montgomery, TX, USA) #A300-102A. As loading controls, ß-Actin mouse monoclonal antibody from Sigma-Aldrich (St.Louis, MO, USA; #A-5441), and GAPDH rabbit antibody FL-335 from Santa Cruz (Santa Cruz, CA, USA; #sc-25778) were used.

Immunofluorescence

Cells were washed twice with PBS and fixed in 4% formaldehyde in PBS for 20 min at room temperature. After washing with PBS, cells were permeabilized in 0.25% Triton in PBS for 20 min and blocked in 20% FCS in PBS for 1 h. Cells were washed thrice with PBS and stained with anti-Prp19 antibody (Bethyllab) diluted 1:500 in PBS containing 20% FCS for 1 h. Cells were again washed with PBS prior to application of Dyelight 649 anti-rabbit antibody (Jackson Immunoresearch) diluted 1:500 in PBS containing 20% FCS for 1 h, followed by three washes with PBS. Nuclei were counterstained with DAPI (200 ng/ml). Slides were mounted with Vectashield mounting medium (Vector Laboratories) and sealed with nail polish. Microscopy and image analysis were performed on a Leica SP5 II laser scanning confocal microscope.

Comet Assays

DNA was stained with 0.5 µg/ml DAPI for 10 min. Between 69 and 500 randomly selected cells per slide were examined for the presence or absence of comets using fluorescence microscopy. The cells were assigned to three different categories according to their tail size. Percentage of cells in each category was calculated for two biological replicates separately and for pooled data. To evaluate analysis by visual inspection, comets were analysed by measuring integrated density of DAPI signal in comet heads and entire comets using ImageJ and calculating % DNA in comet tails after subtracting the background fluorescence from a neighboring dark area of equivalent size. To assess DNA damage accumulation during adipogenic differentiation, undifferentiated hASCs were transfected with siSNEV and control siRNA and induced to differentiate 48 h post transfection as described. Comet assays were carried out using differentiated and undifferentiated cells, as described.

***C. elegans* strains**

Strains used in this work include N2 (provided by V. Jantsch) and CF1814 [*rrf-3*(pk1426) II, *daf-2*(e1370) III]. All strains are available through the Caenorhabditis Genetics Center (CGC).

RNA interference in *C. elegans*

The HT115 strain of *E. coli* carrying the RNAi-construct or the empty vector (L4440) as control, was cultured overnight in liquid LB medium with ampicillin and tetracyclin at 37 °C. The bacteria were harvested by centrifugation, re-suspended in LB to a concentration of 60 mg/ml and 200 µl of this suspension was seeded on NGM plates containing 1 mM IPTG and 25 µg/ml Carbenicillin. Plates were incubated at 37 °C overnight and used within one week.