

Supplemental Information

The Histone H2B Monoubiquitination Regulatory Pathway Is Required for Differentiation of Multipotent Stem Cells

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SUPPLEMENTAL INVENTORY

1) Supplemental figures:

- Figure S1 related to Figure 1. H2A and RNF40 levels during hMSC differentiation.
- Figure S2 related to Figure 2. RNF40 depletion decreases H2Bub1 levels and affects marker gene expression in differentiating hMSC.
- Figure S3 related to Figure 3. CDK9 and WAC depletion impair hMSC differentiation and decrease H2Bub1 levels.
- Figure S4 related to Figure 4. H3K4me3 after depletion of RNF40 or RNF20.

2) Supplemental Experimental Procedures:

- Cell culture, differentiation and knockdowns;
- Chromatin Immunoprecipitation (ChIP) and qRT-PCR;
- Immunofluorescence staining of *Drosophila* ovaries;
- Immunofluorescence staining of oligodendrocytes;
- Monolayer-embedding in Epon for electron microscopy;
- Pathway analysis;
- Oil Red O staining;
- Quantification of Oil Red O and alkaline phosphatase staining;
- List of siRNAs used in the study;
- List of antibodies used for ChIP, immunofluorescence and Western blot;
- List of qRT-PCR primers used in the study;
- List of ChIP primers used in the study.

3) Supplemental References

Figure S1 related to Figure 1

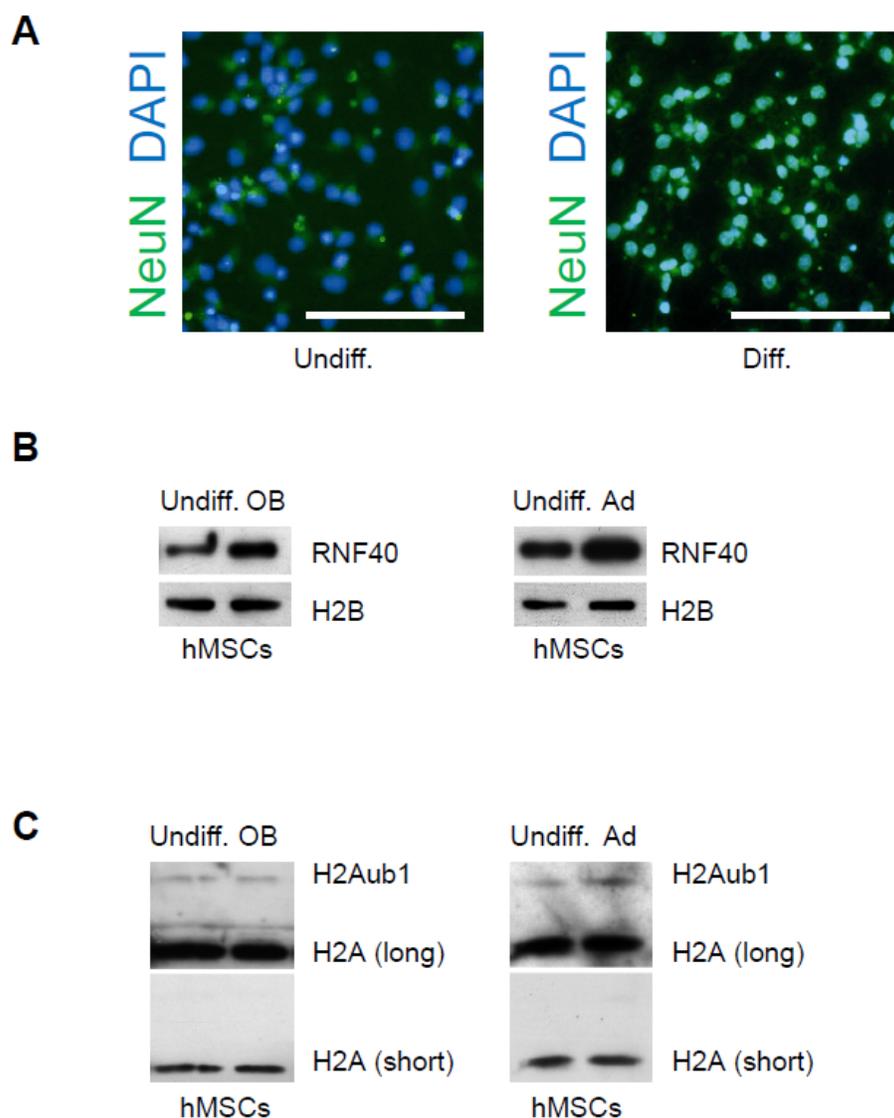


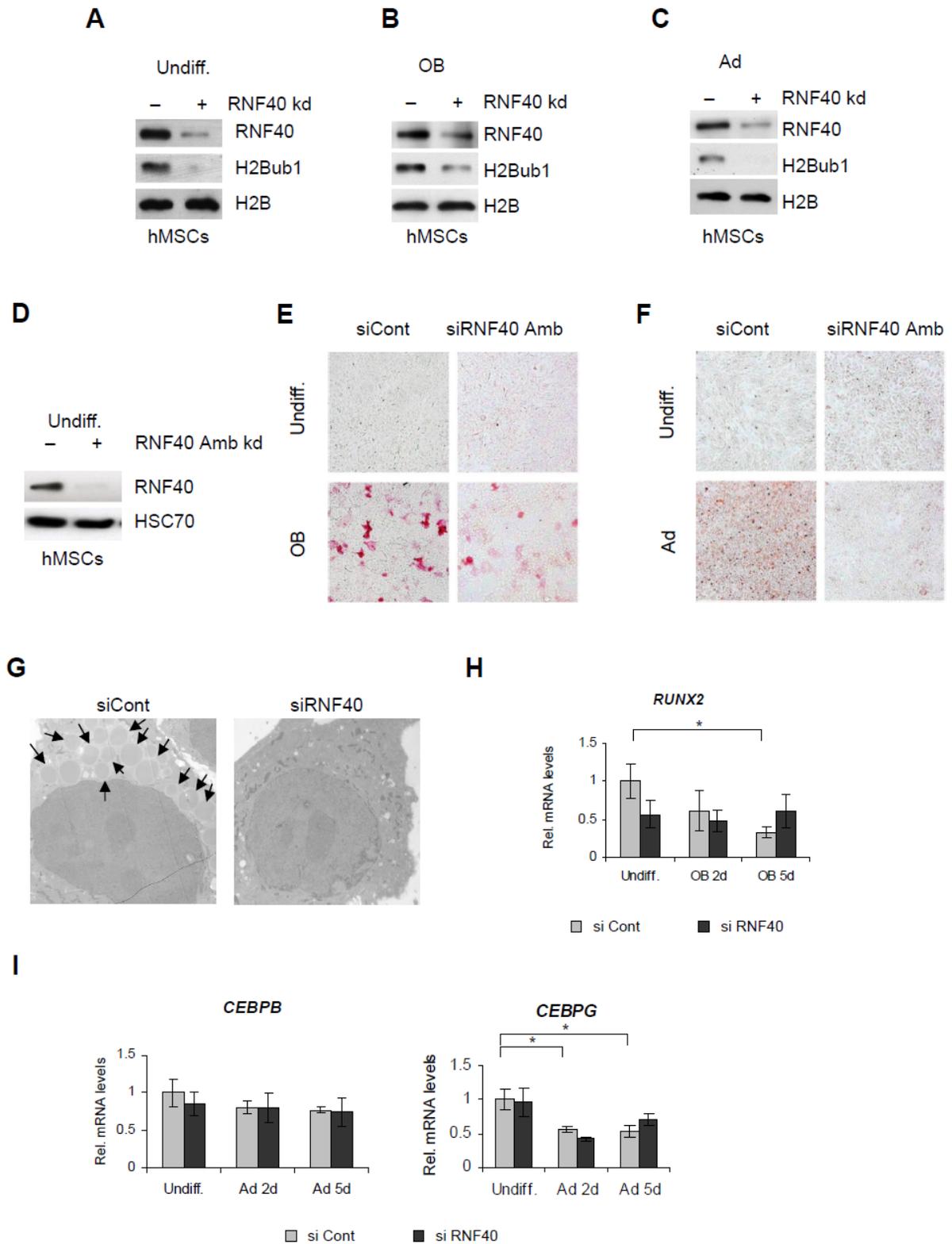
Figure S1

(A) Mouse neurospheres were differentiated as described in Supplemental Materials and Methods. Immunofluorescence staining against NeuN (green) was used to verify the differentiation. Nuclei were stained with DAPI.

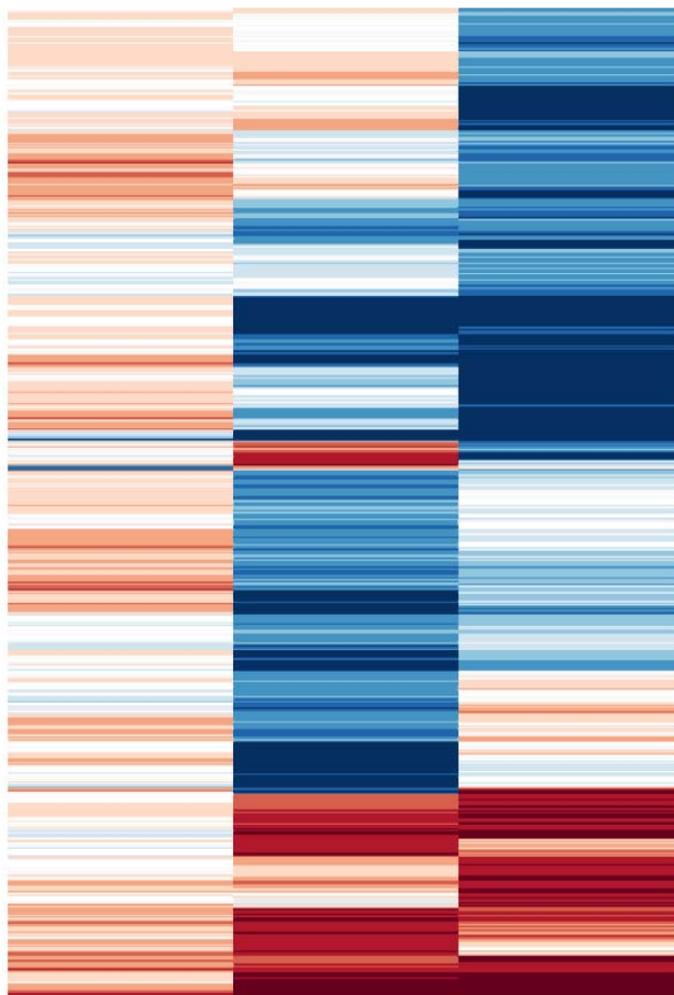
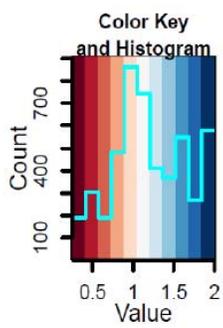
(B) hMSCs were differentiated to osteoblasts (OB) or adipocytes (Ad) for 2 d as described in Supplemental Materials and Methods. Protein extracts were analyzed by Western blot using antibodies against RNF40 and H2B (loading control).

(C) hMSCs were differentiated as in S1B. Western blot against H2A was performed on protein extracts. H2Aub1 was visualized as a higher migrating band. Low exposure of H2A is shown as a loading control.

Figure S2 related to Figure 2



J

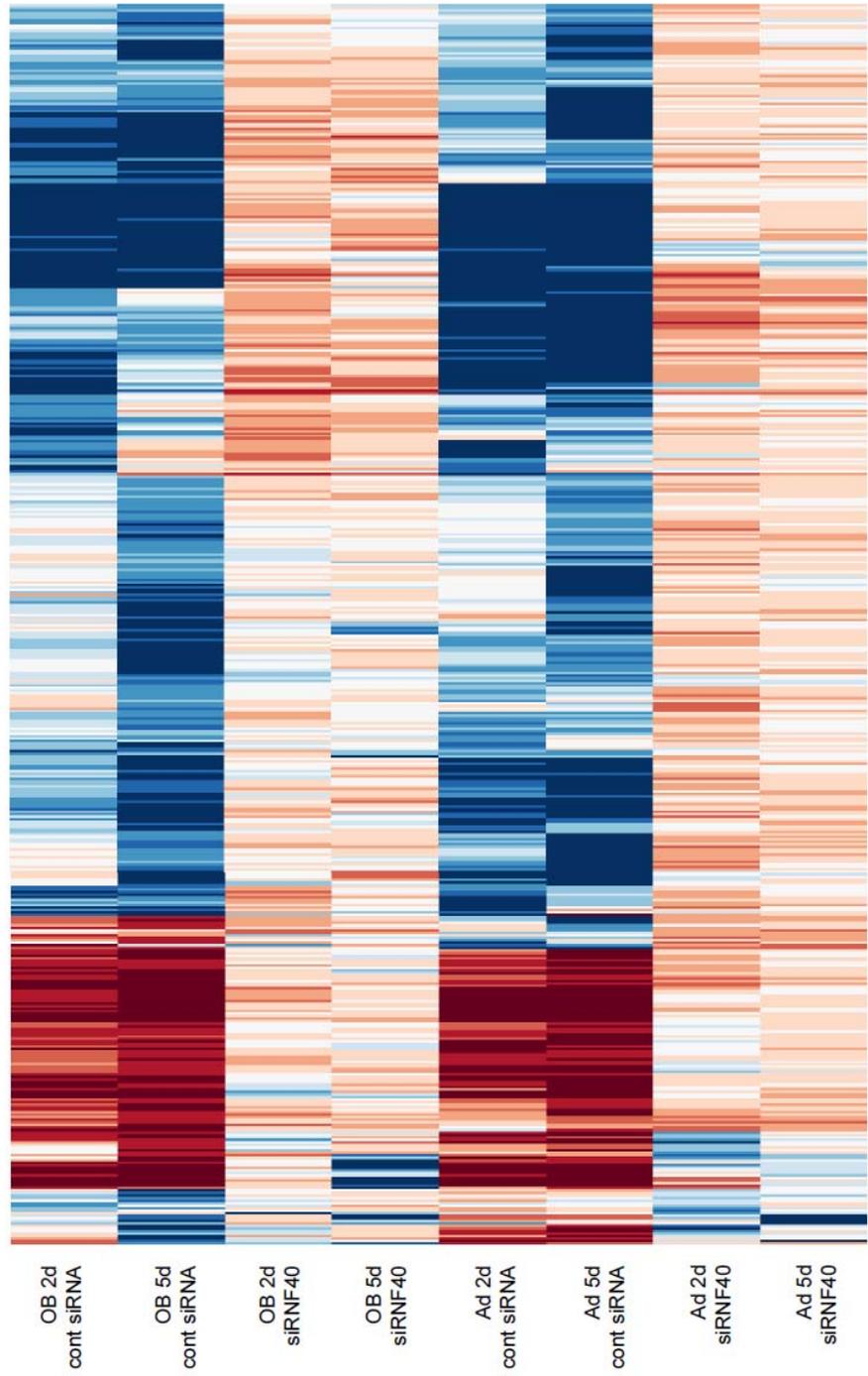
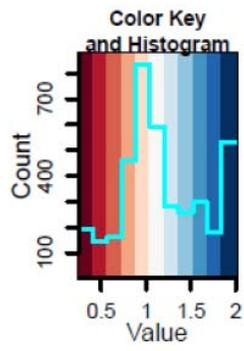


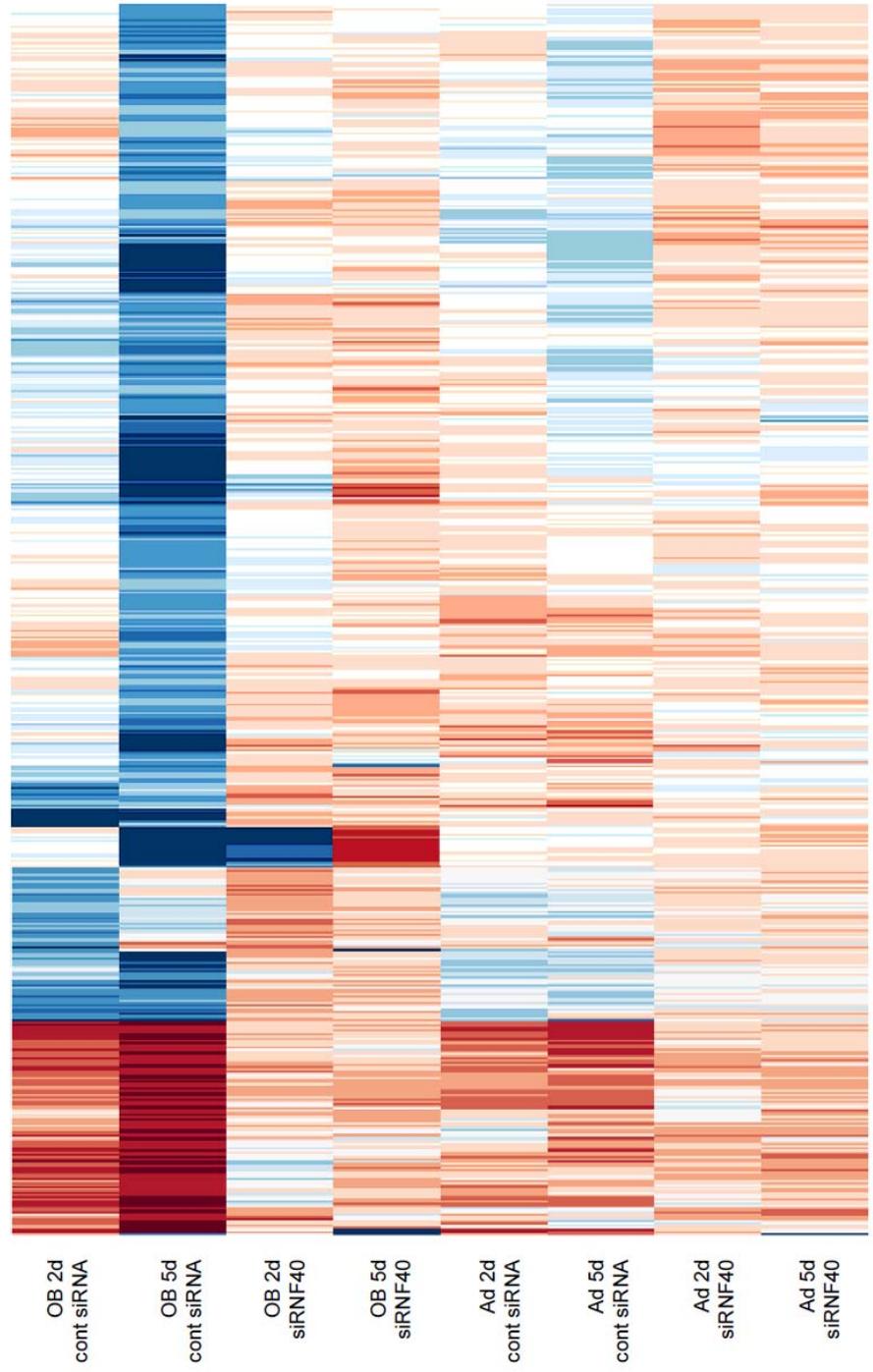
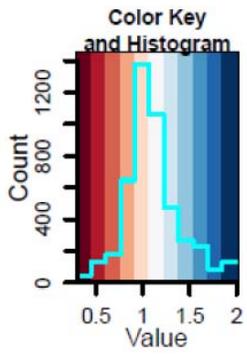
Undiff.
siRNF40

OB 2d
cont siRNA

Ad 2d
cont siRNA

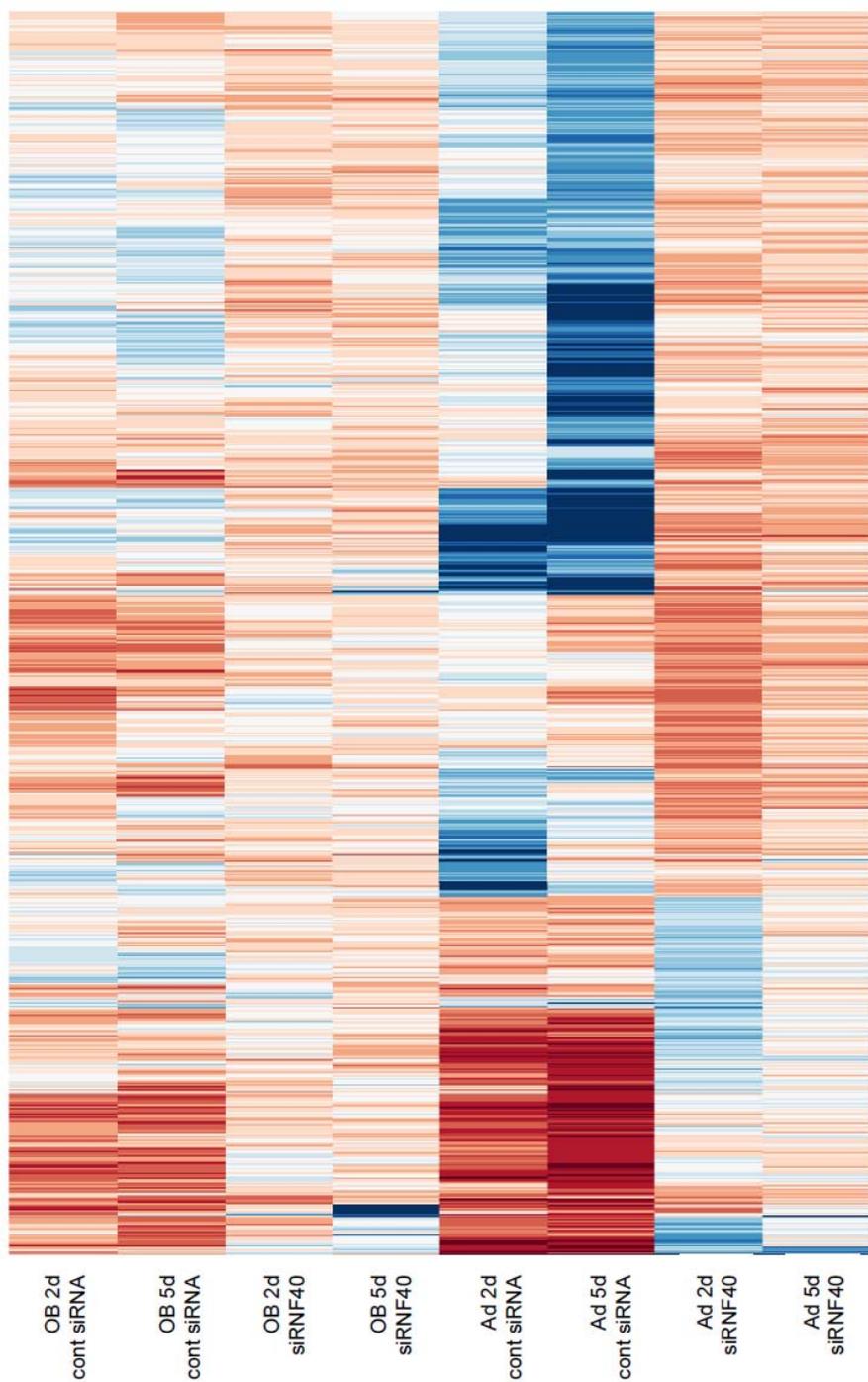
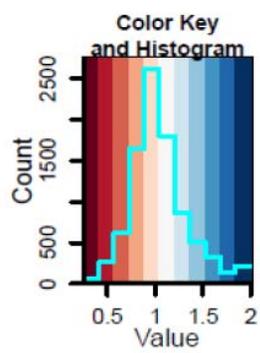
K





L

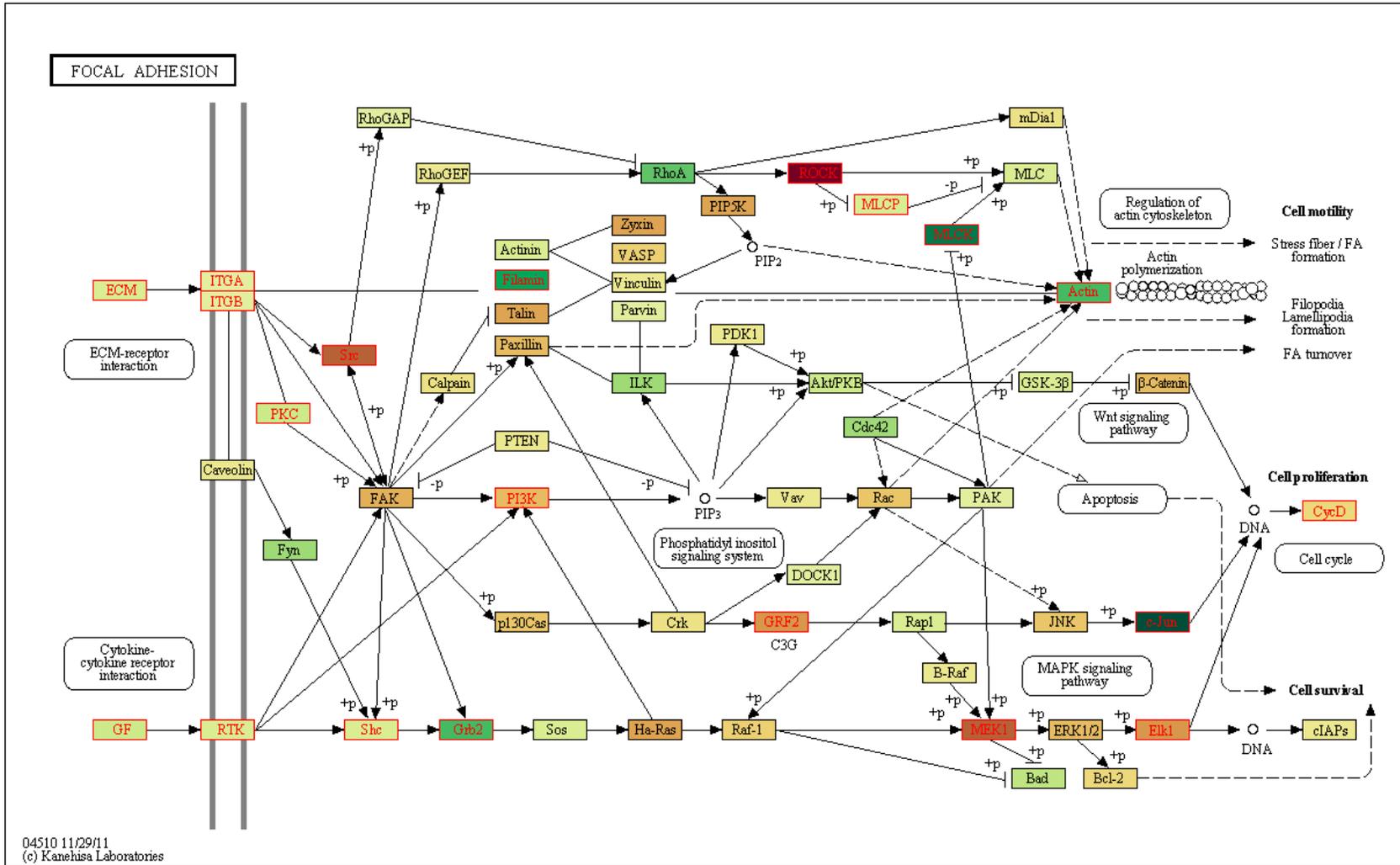
M



Undiff. vs. 5 day osteoblast differentiation

N

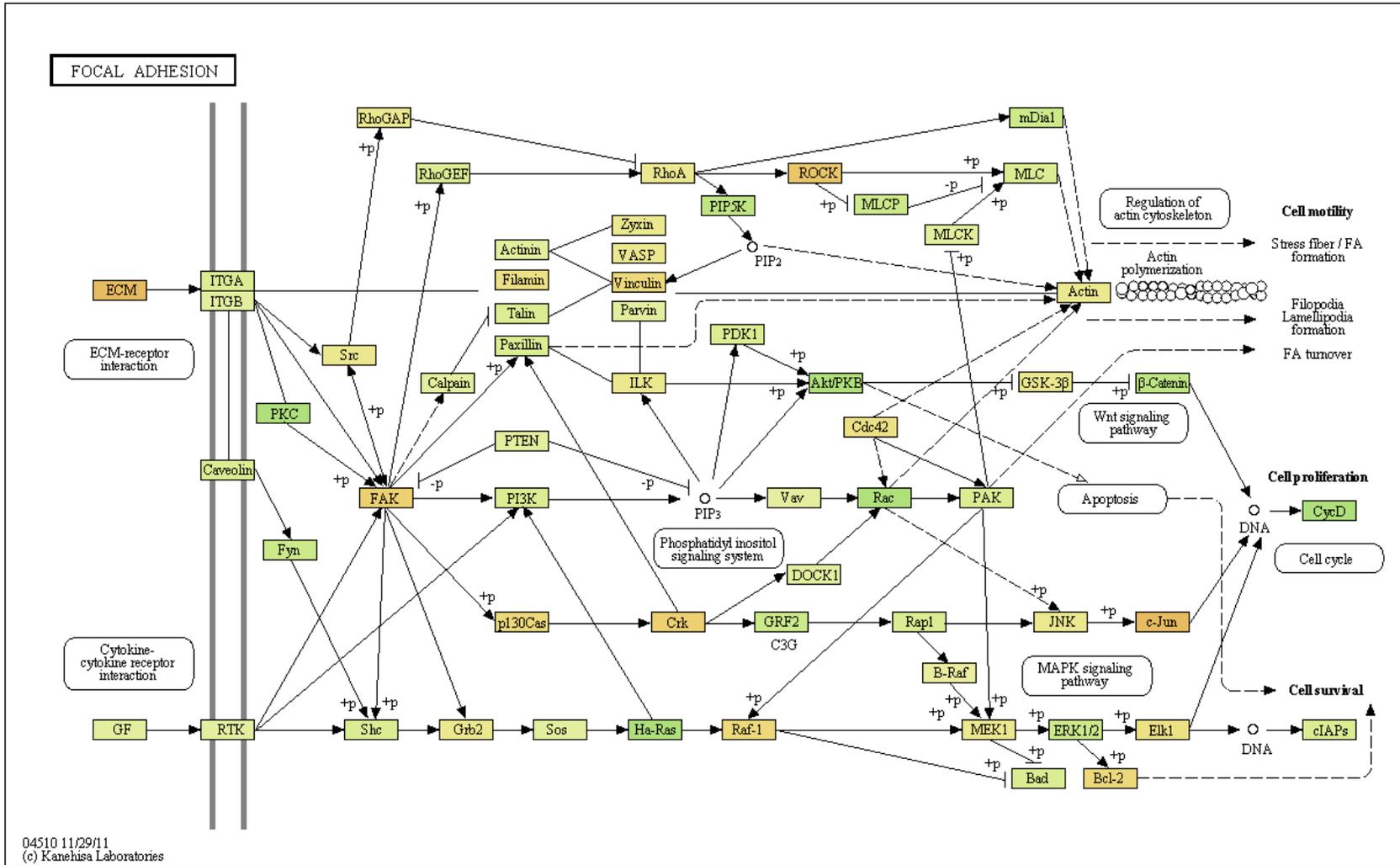
Focal adhesion (hsa04510); P = 1.49E-07



siCont vs. siRNF40 5 day osteoblast differentiation

0

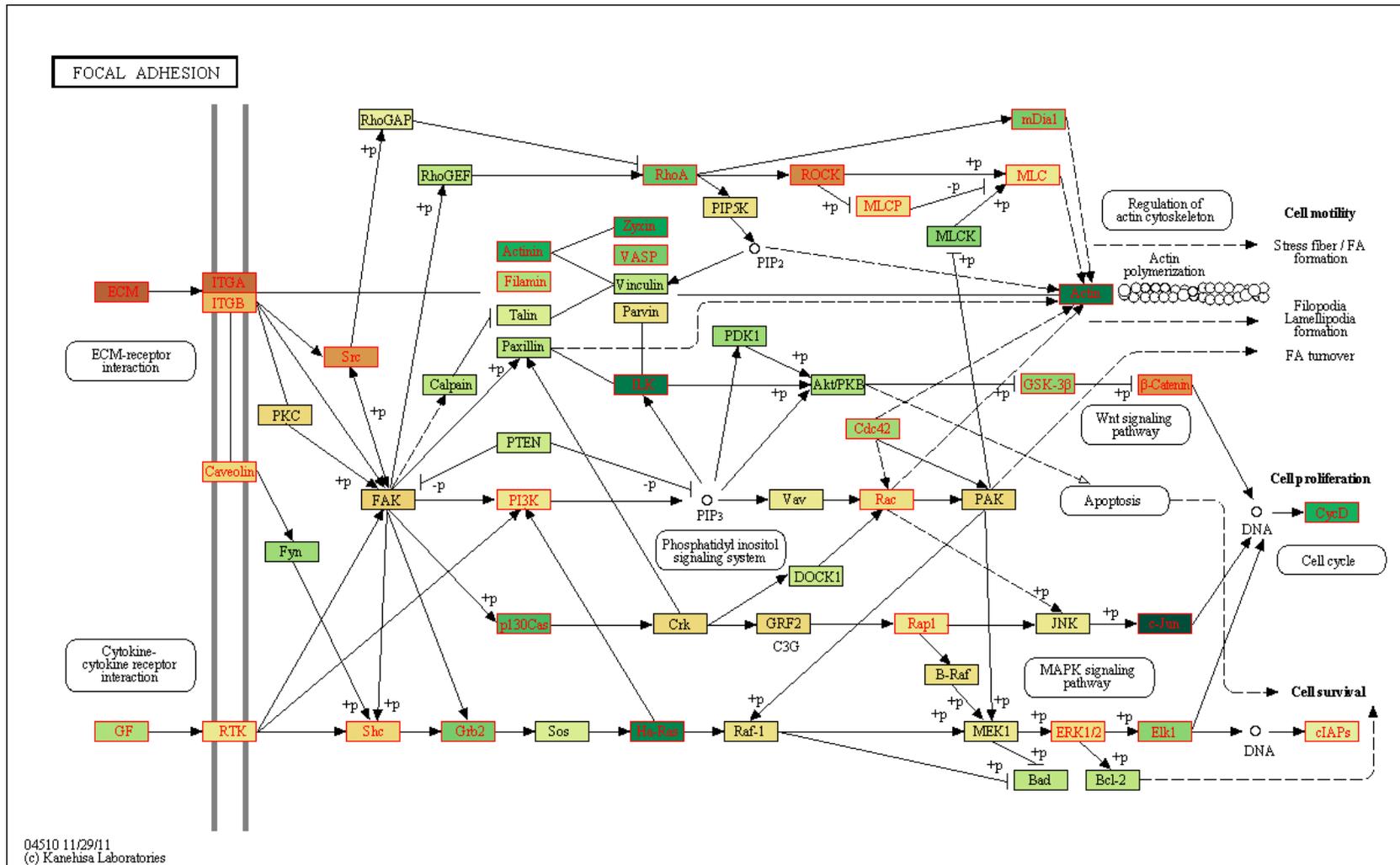
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Undiff. vs. 5 day adipocyte differentiation

P

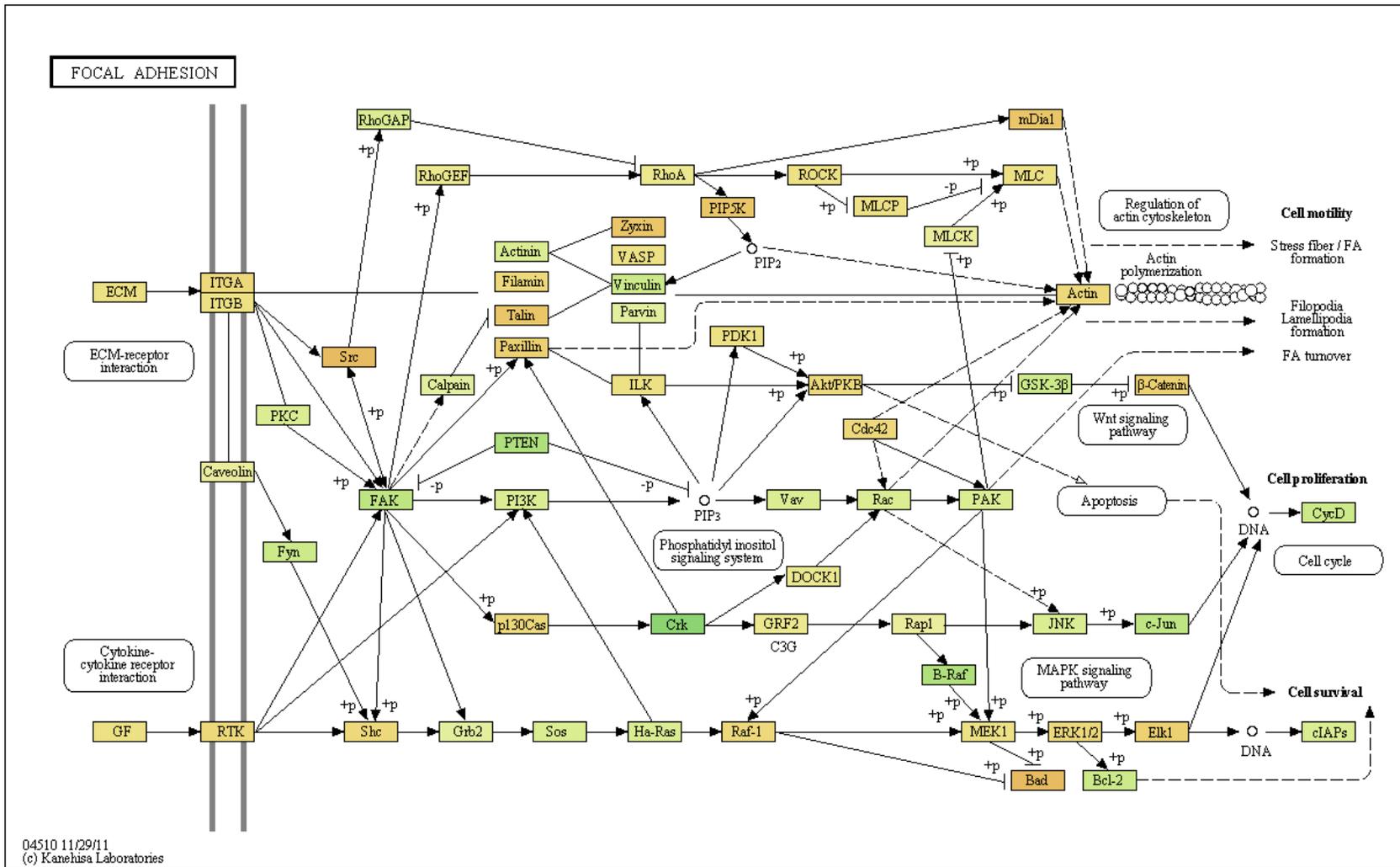
Focal adhesion (hsa04510); P = 2.70E-11



siCont vs. siRNF40 5 day adipocyte differentiation

Q

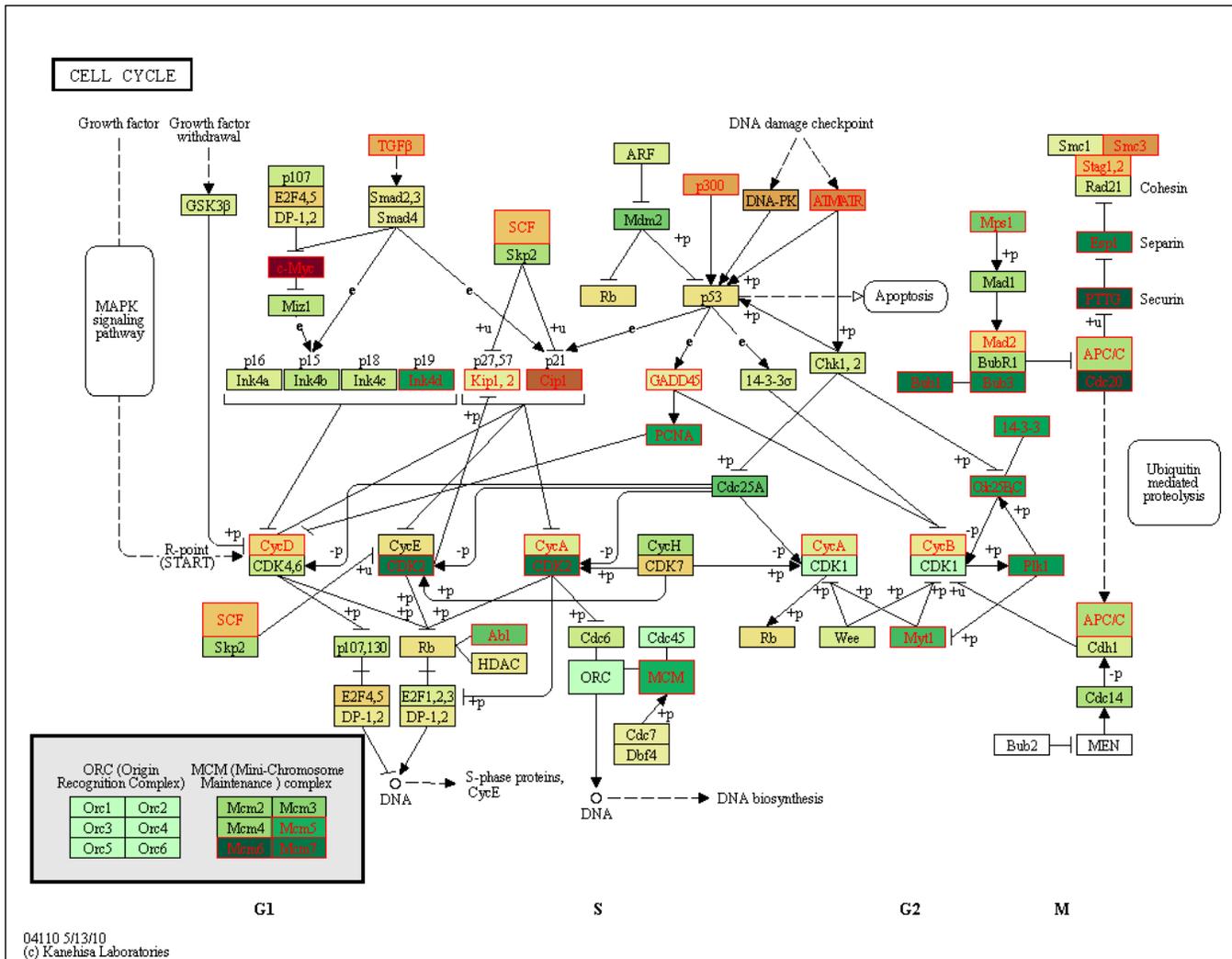
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Undiff. vs. 5 day osteoblast differentiation

R

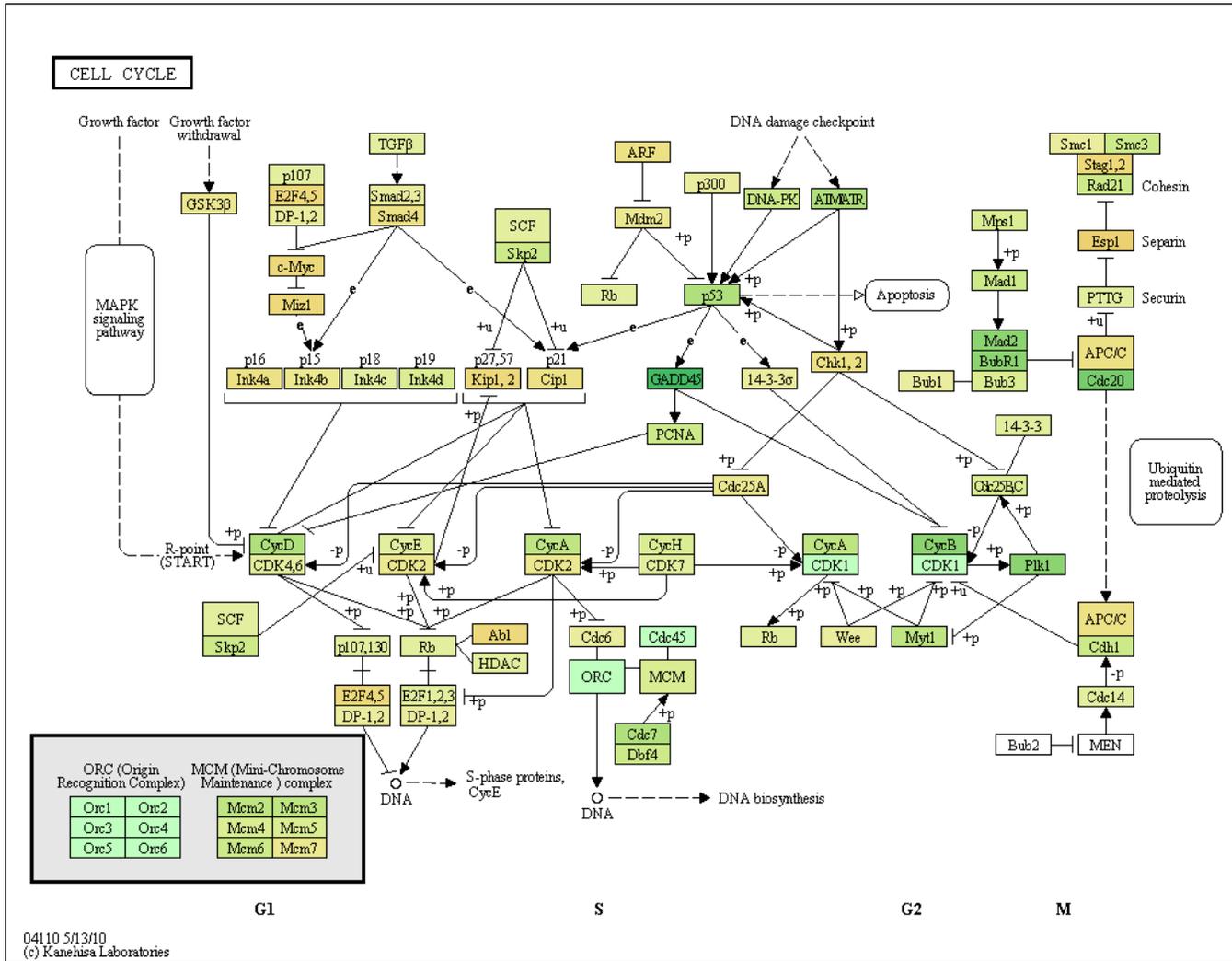
Cell cycle (hsa04110); P = 2.89E-14



Cont. vs. siRNF40 5 day osteoblast differentiation

S

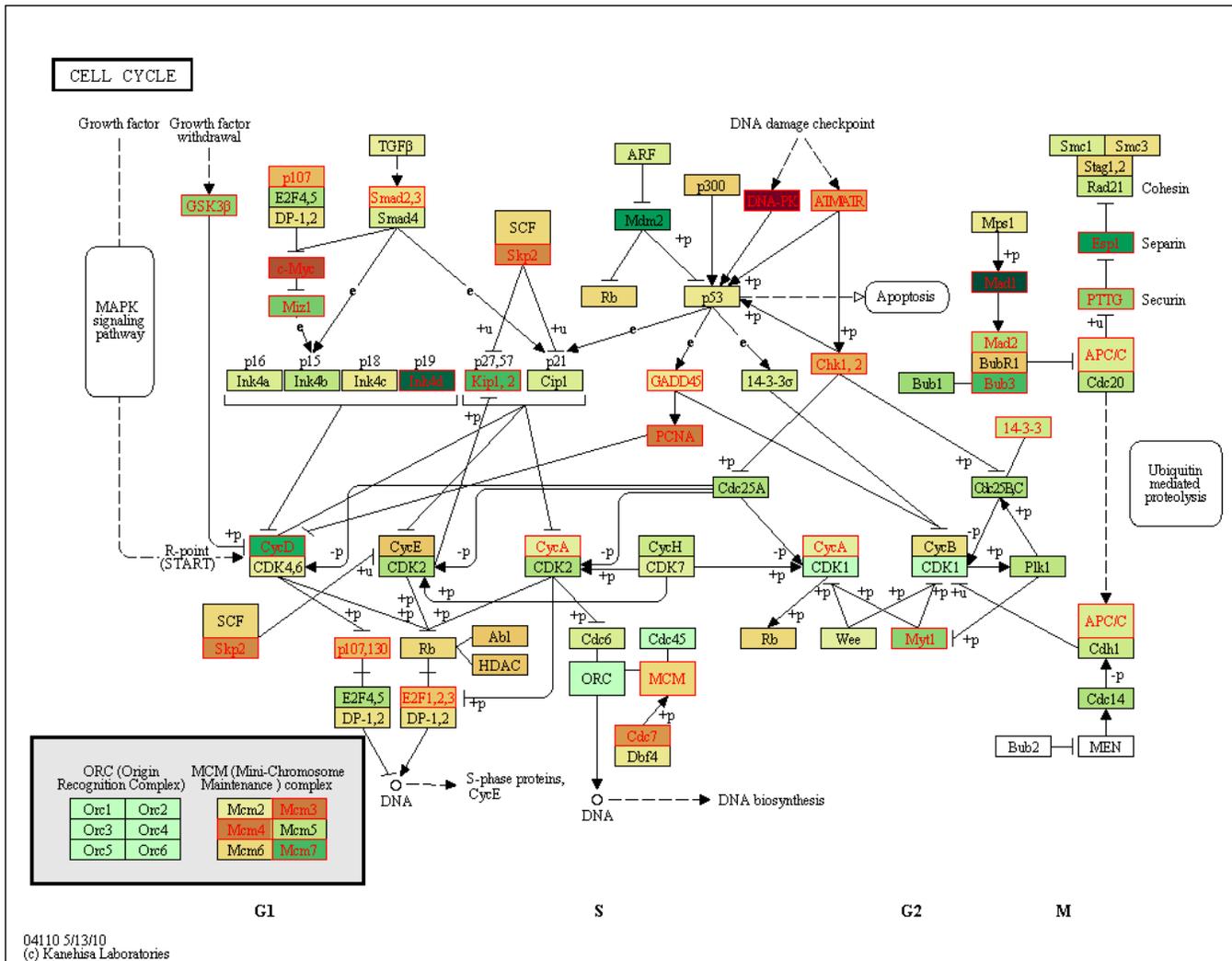
Cell cycle (hsa04110); P = 1.21E-03



Undiff. vs. 5 day adipocyte differentiation

T

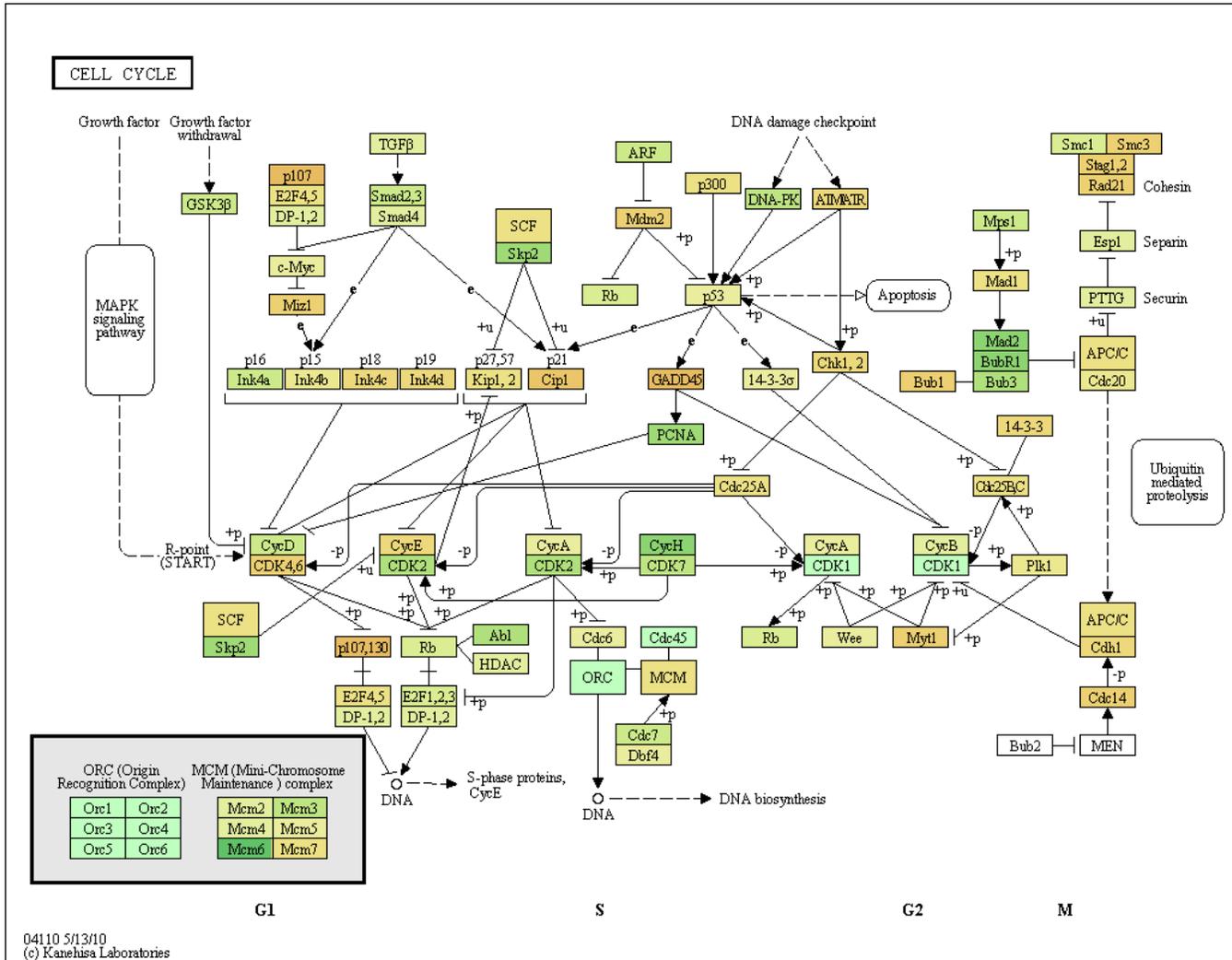
Cell cycle (hsa04110); P = 3.10E-10



Cont. vs. siRNF40 5 day adipocyte differentiation

U

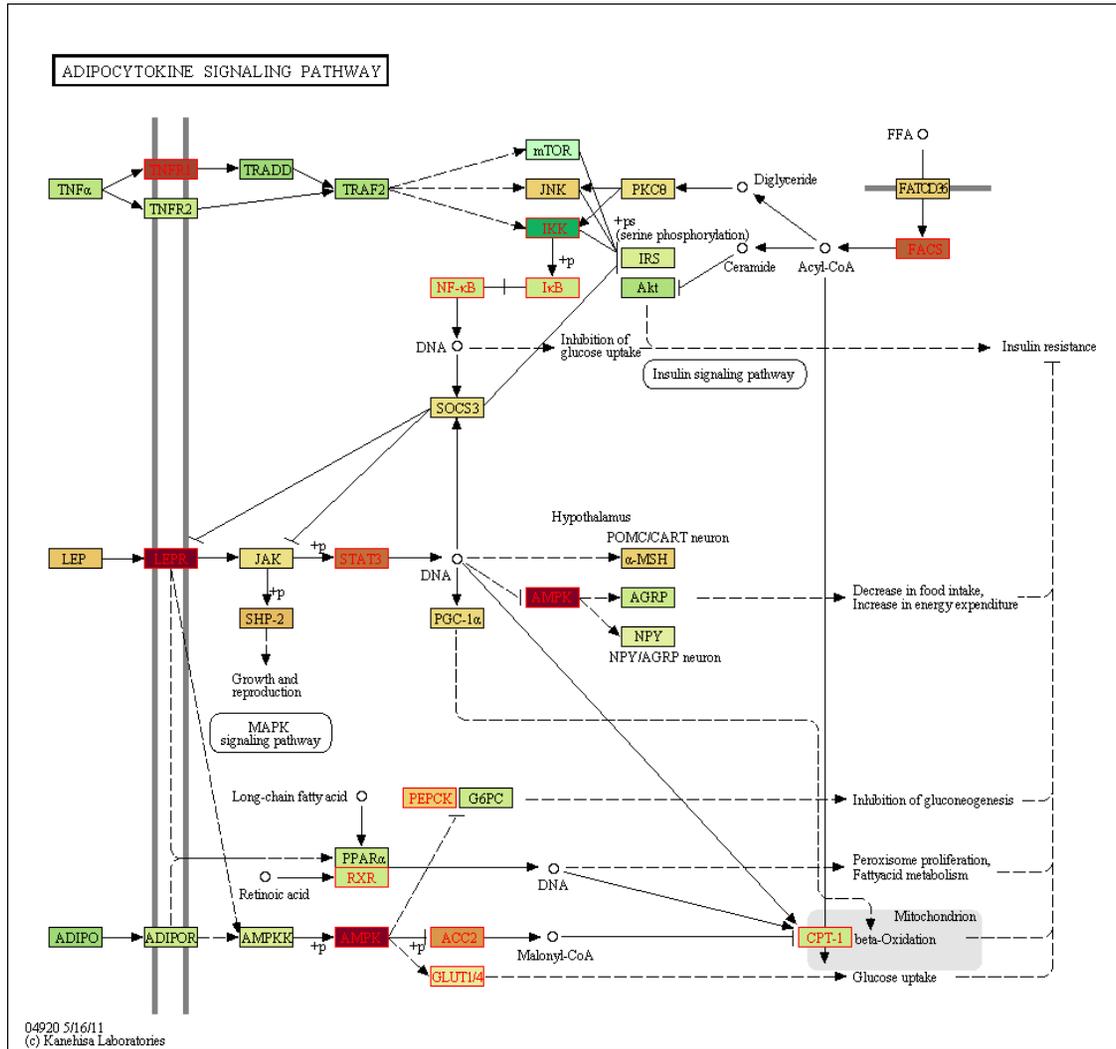
Cell cycle (hsa04110); P = 1.72E-04



Undiff. vs. 5 day adipocyte differentiation

v

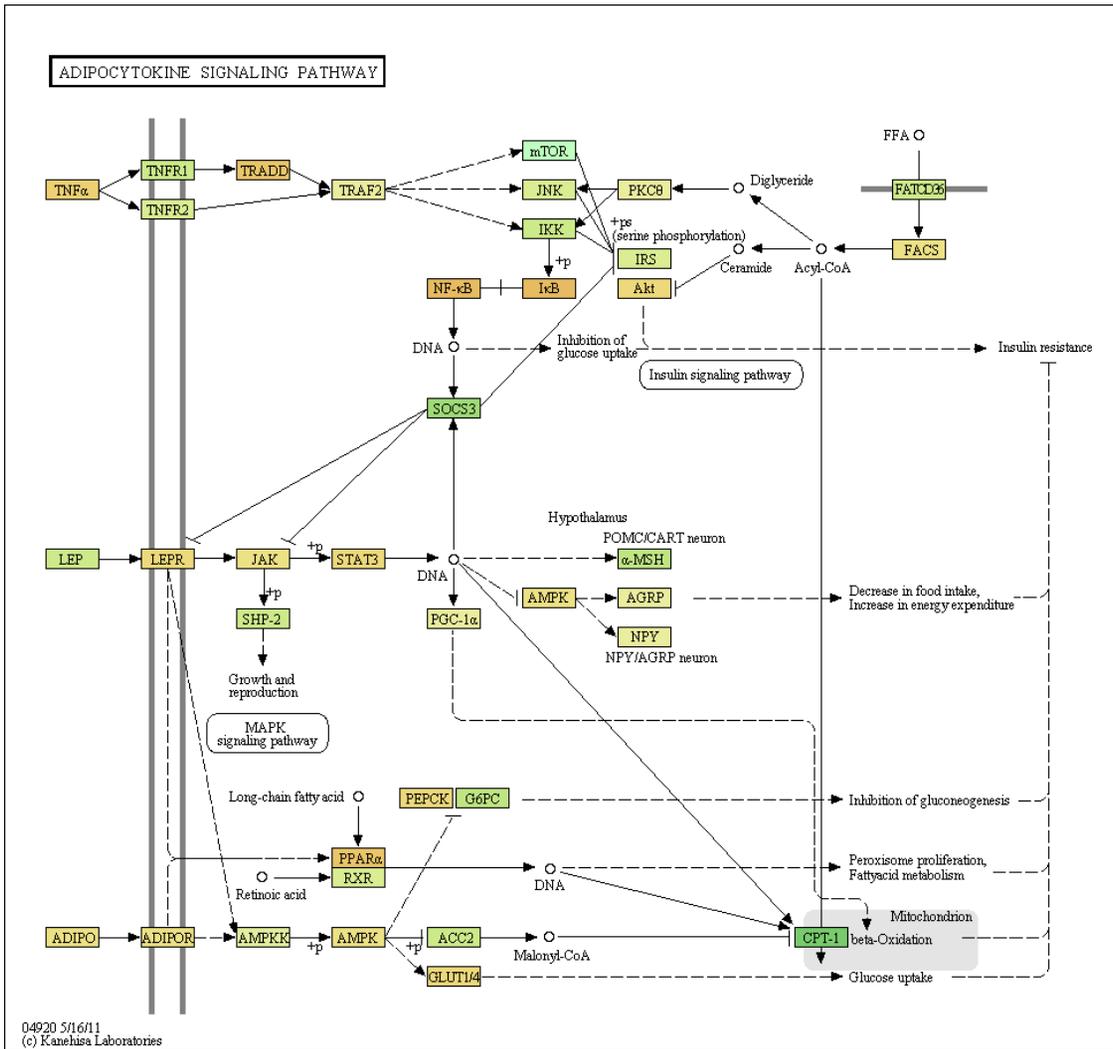
Adipocytokine signaling pathway (hsa04920); P = 4.97E-7



siCont. vs. siRNF40 5 day adipocyte differentiation

W

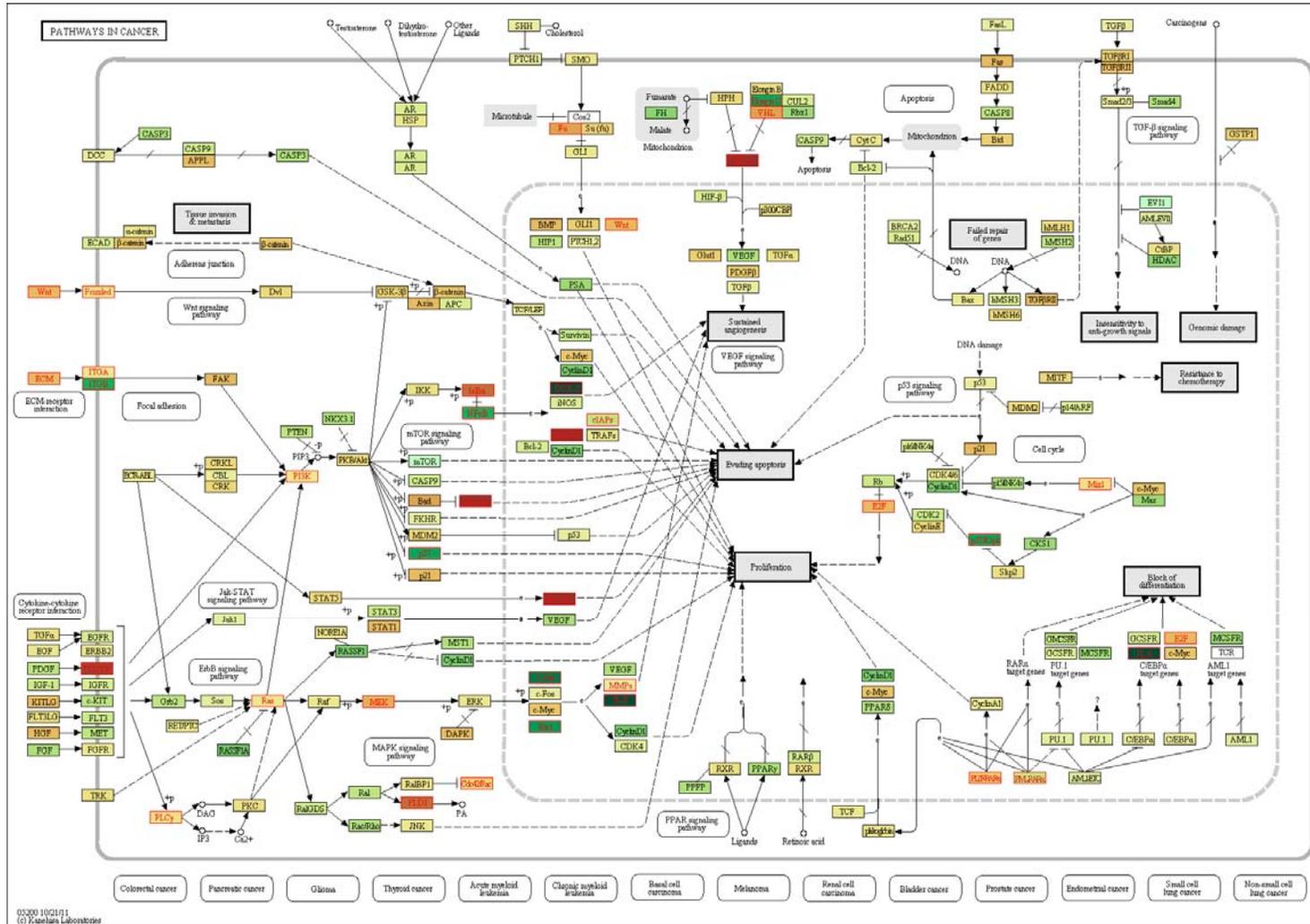
Adipocytokine signaling pathway (hsa04920); P = 2.84E-2



Undiff. vs. 2 day osteoblast differentiation

X

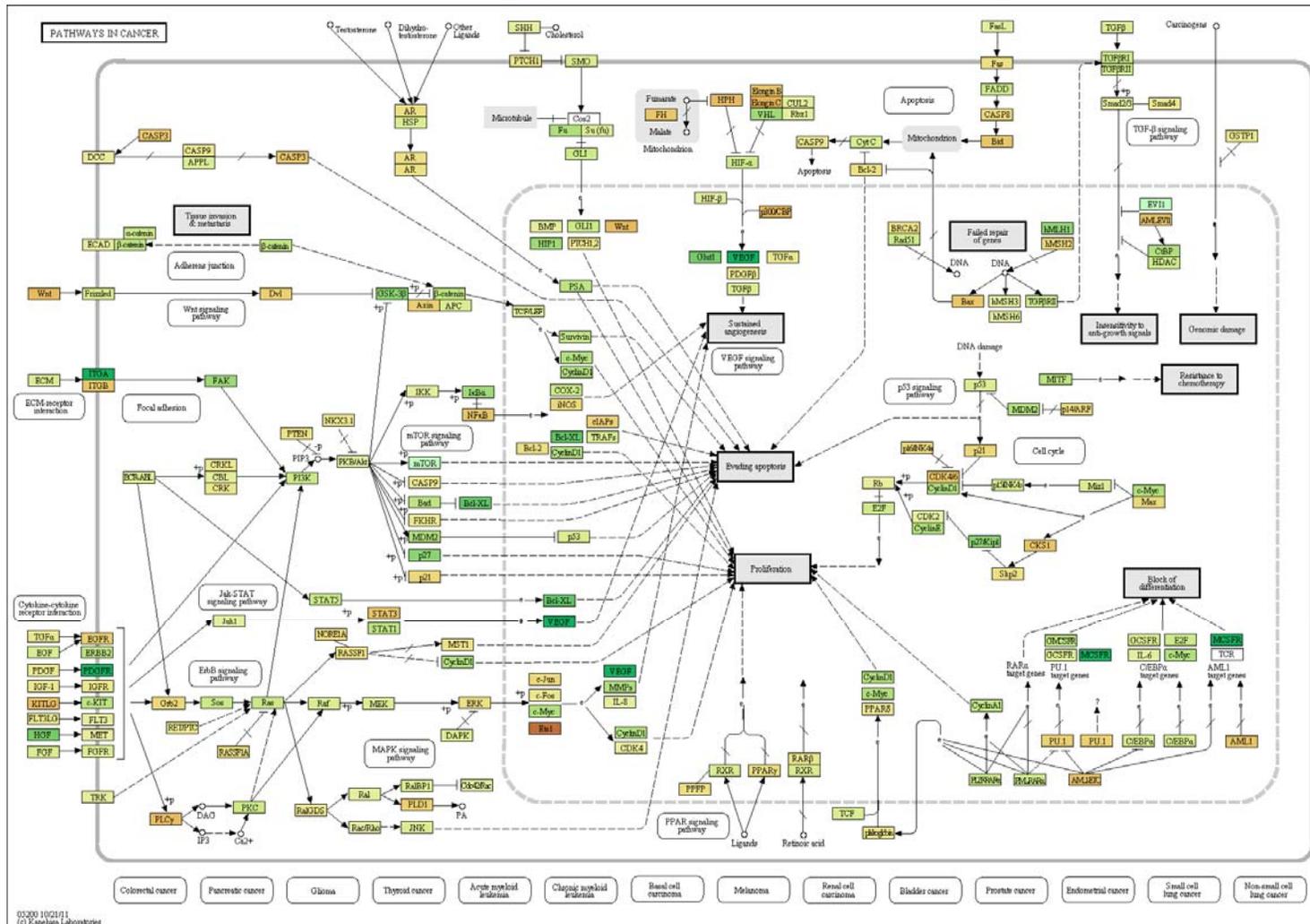
Pathways in cancer (hsa05200); P = 1.46E-10



Cont. vs. siRNF40 2 day osteoblast differentiation

Y

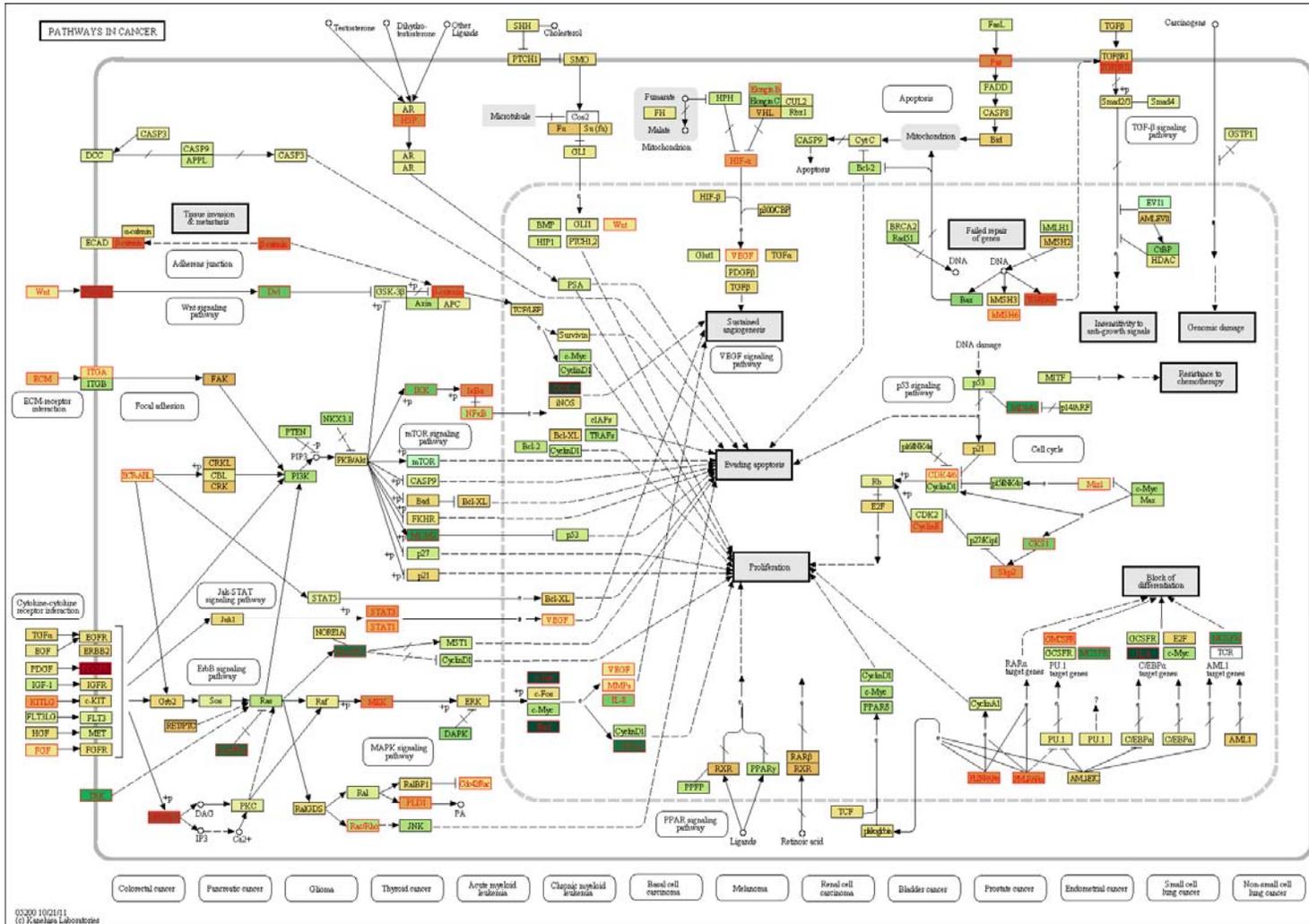
Pathways in cancer (hsa05200); P = 1.47E-05



Undiff. vs. 2 day adipocyte differentiation

Z

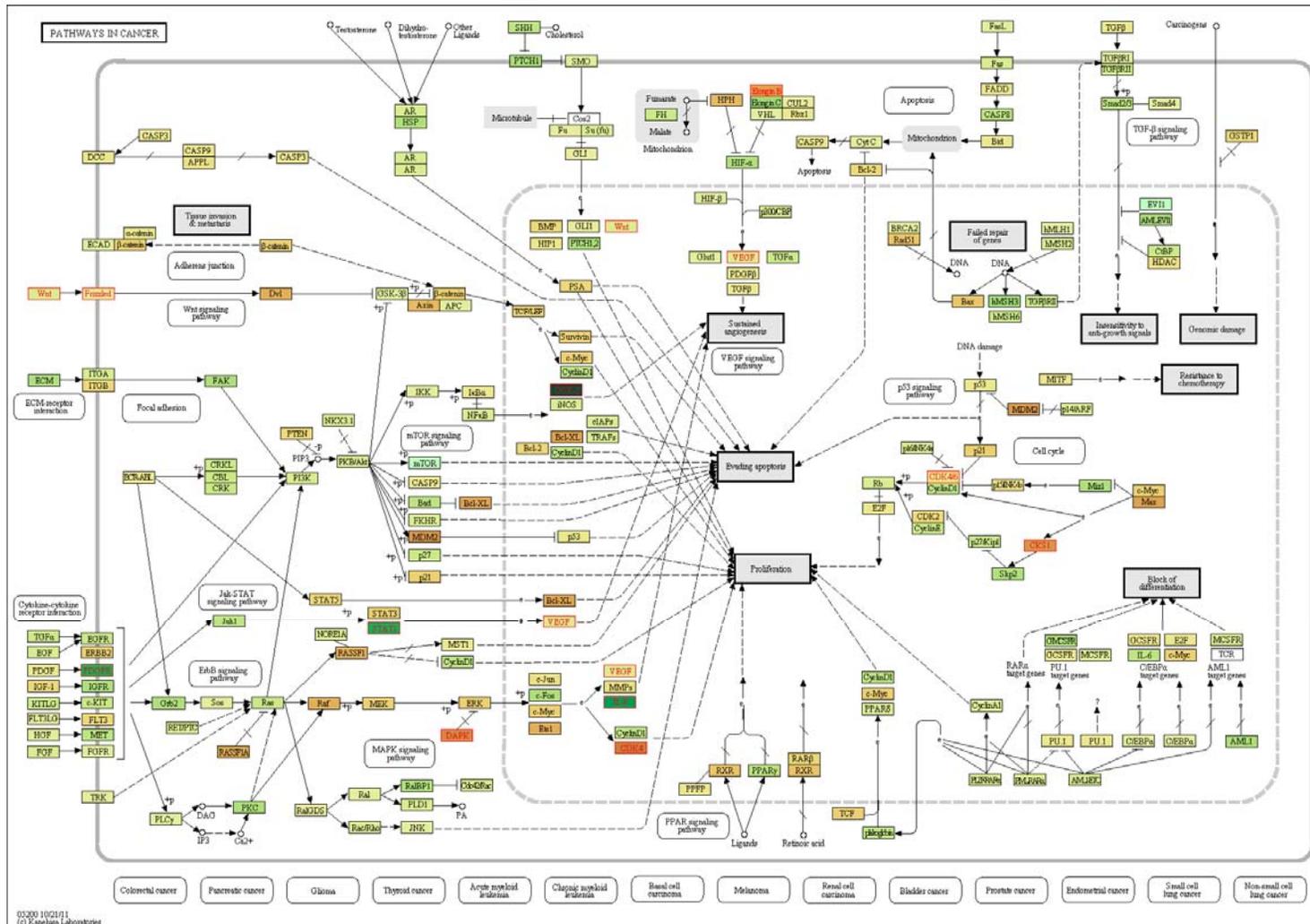
Pathways in cancer (hsa05200); P = 7.81E-11

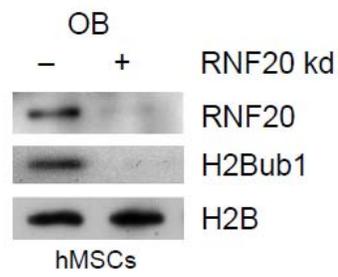
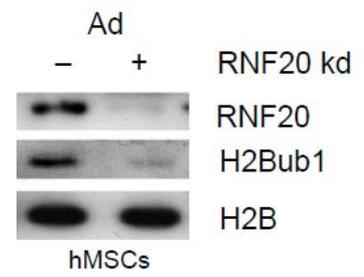
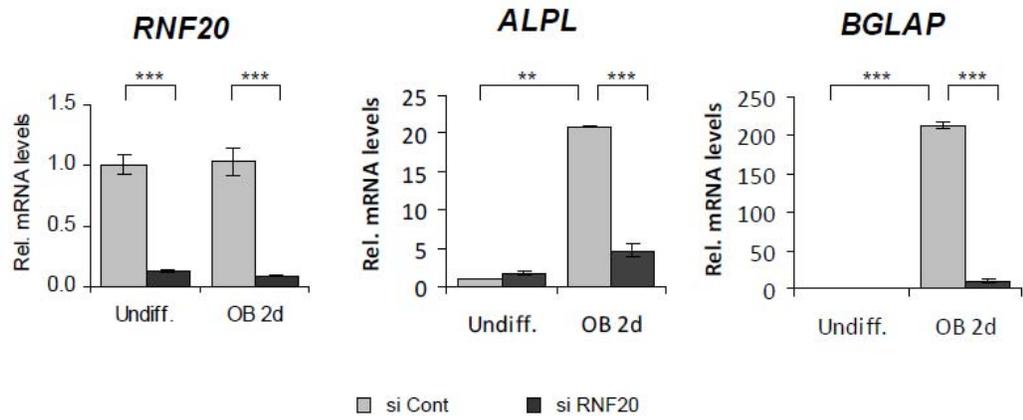
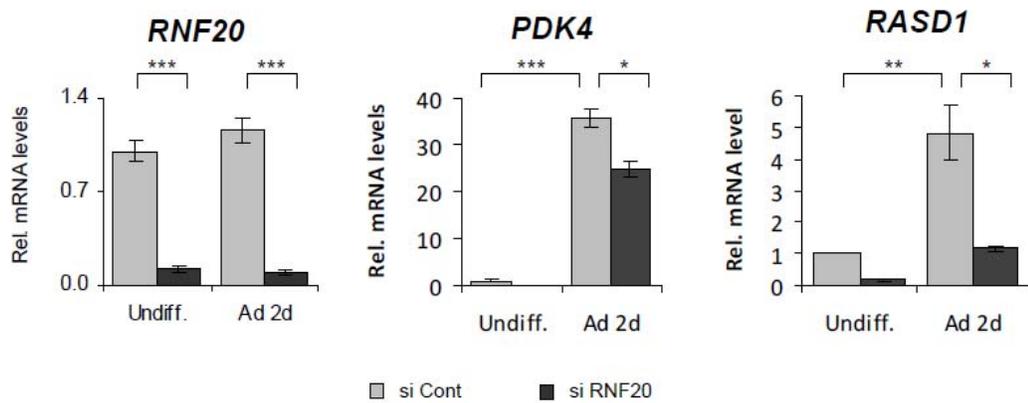


Cont. vs. siRNF40 2 day adipocyte differentiation

AA

Pathways in cancer (hsa05200); P = 5.46E-04



AB**AC****AD****AE**

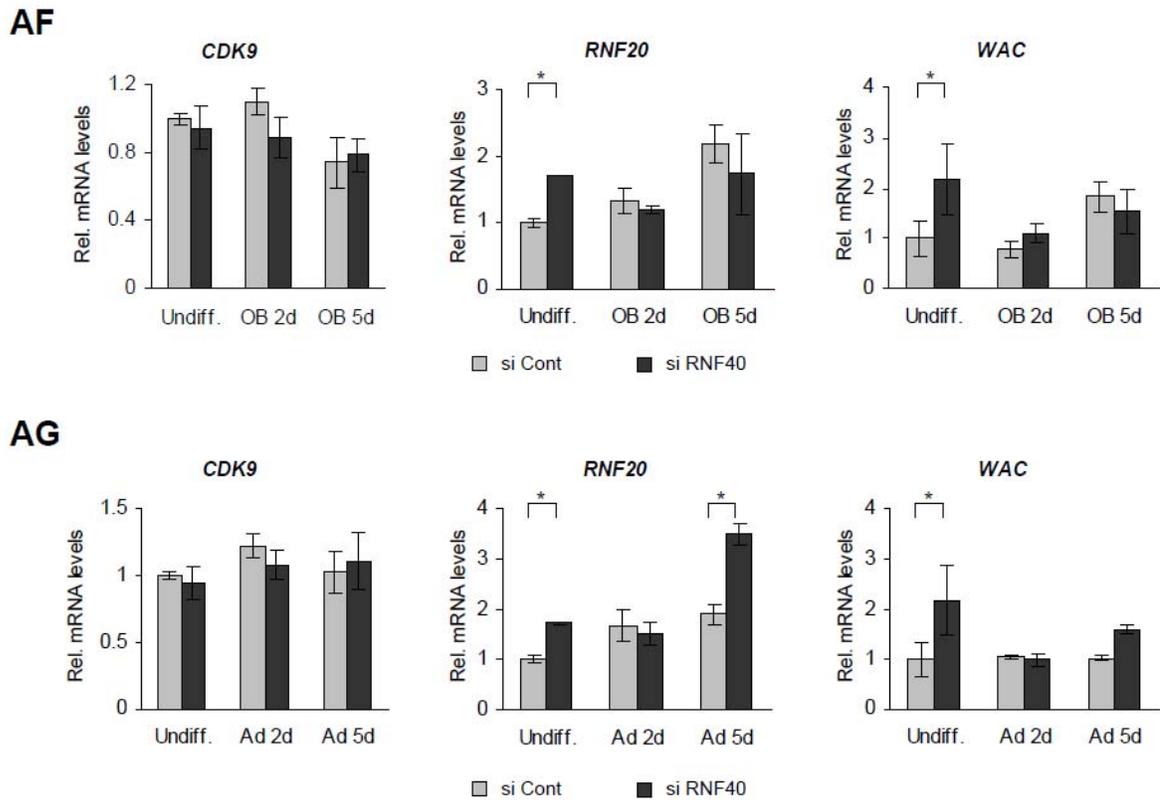


Figure S2

(A-C) hMSCs were transfected with control or RNF40 siRNAs (SmartPool) for 24 h and induced to differentiate into osteoblasts (B), adipocytes (C) or left undifferentiated (A) for 5 d. Protein extracts were analyzed by western blot using antibodies against RNF40, monoubiquitinated H2B (H2Bub1) and H2B as a loading control.

(D) RNF40 knockdown (siRNA from Ambion, see List of siRNAs) was performed in undifferentiated hMSCs for 2 d. Proteins were analyzed by Western blot for RNF40 and Hsc70 (loading control).

(E-F) hMSCs were transfected with control or RNF40 siRNAs (Ambion) for 24 h, induced to differentiate into osteoblasts (E) or adipocytes (F) for 5 d and then stained for alkaline phosphatase activity (E) or the presence of lipid drops (F) as described in Materials and Methods.

(G) Electron microscopy of differentiated adipocytes treated with control siRNA (left) and RNF40 siRNA (right). Arrows indicate lipid droplets.

(H-I) RNF40 knockdown was performed in hMSCs followed by osteoblast (H) or adipocyte (I) differentiation for 2 and 5 d. Expression of osteoblast (RUNX2; S2H) or adipocyte (CEBPB and CEBPG; S2I) marker genes was examined by qRT-PCR. Statistical analysis: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean \pm SD, $n = 3$.

(J) Transcriptome-wide gene expression microarray analysis of hMSC induced to differentiate into the osteoblast or adipocyte lineages for 2 d after RNF40 knockdown versus undifferentiated hMSC under the same siRNA treatment. Color code indicates downregulated genes in red and up-regulated genes in blue. Mean values, $n = 3$.

(K) hMSCs were transfected with control or RNF40 siRNAs (SmartPool) for 24 h and induced to differentiate into osteoblasts or adipocytes for 2 and 5 d. Heat map was composed of the genes that were similarly regulated in both osteoblast and adipocyte differentiations. Color code indicates downregulated genes in red and upregulated genes in blue. Mean values, $n = 3$.

(L-M) Microarray analysis was performed as in (K). Genes that were specifically regulated in osteoblasts (L) or adipocytes (M) were used for the heat-maps. Color code indicates downregulated genes in red and up-regulated genes in blue. Mean values, $n = 3$.

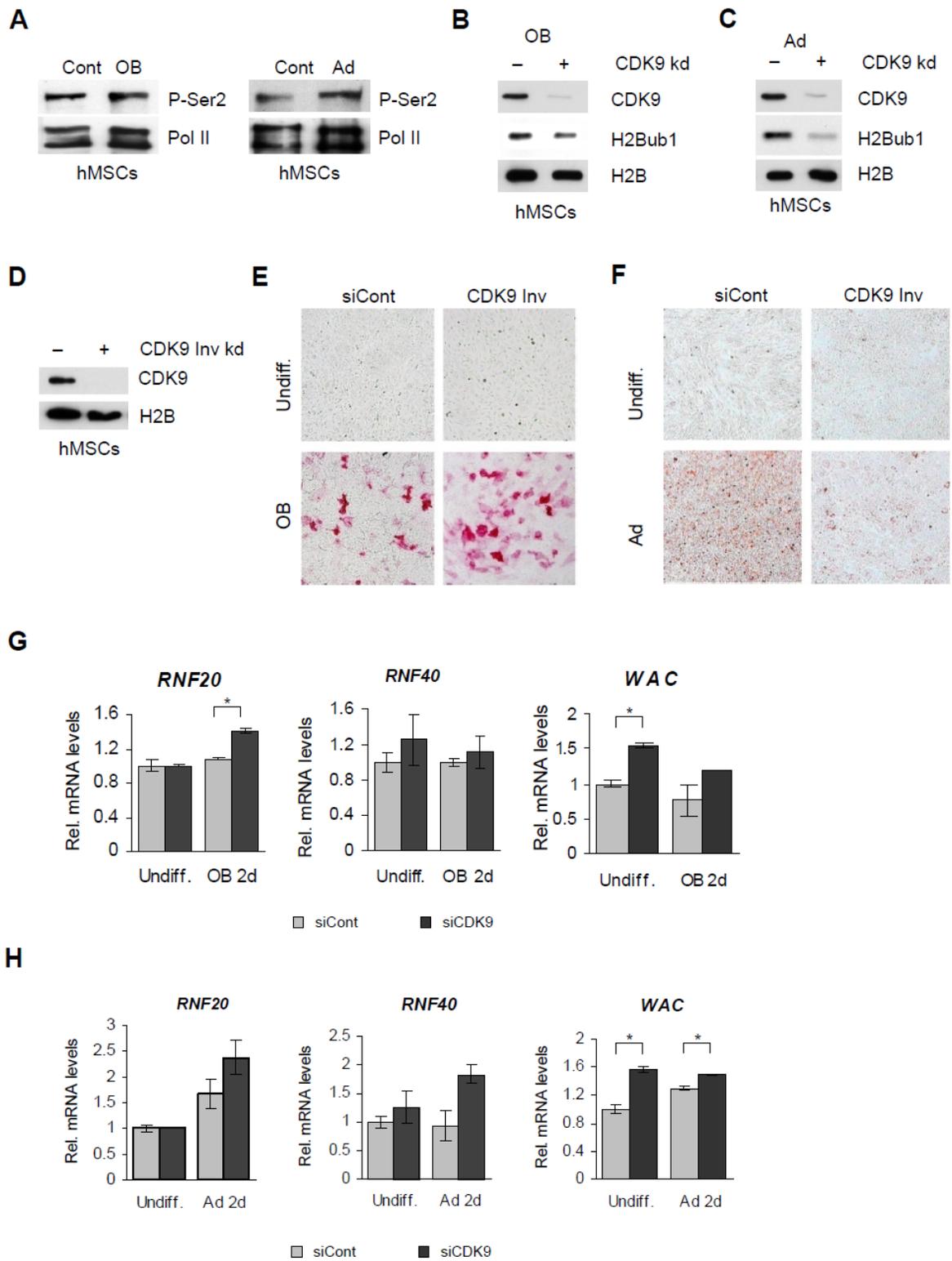
(N-AA) Pathway analysis was performed as indicated in Supplemental Materials and Methods using various comparisons from the hMSC differentiation microarray data with control or RNF40 siRNAs. Downregulated genes are shown in green squares, up-regulated in red squares. Red color for the text indicates significantly regulated genes. Mean values, $n = 3$.

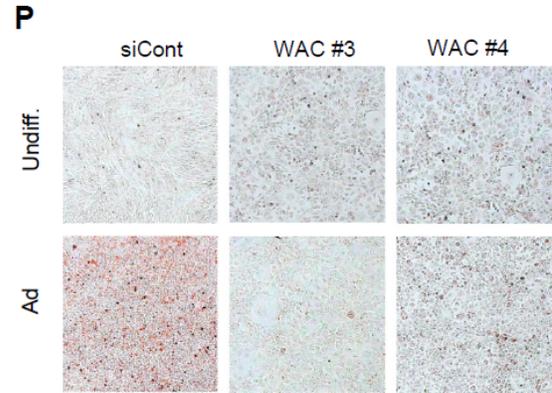
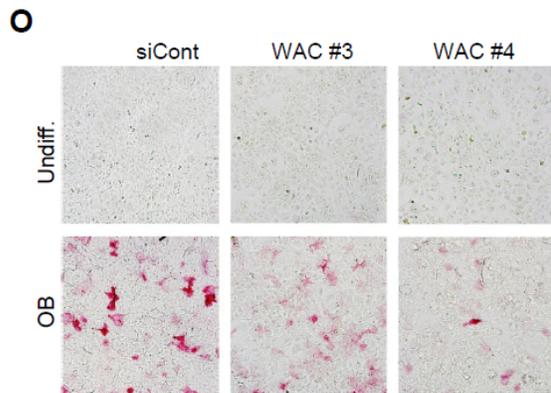
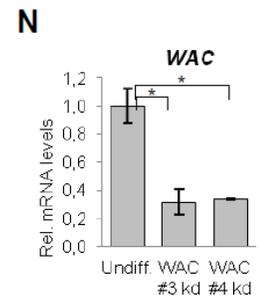
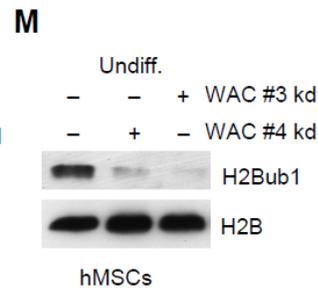
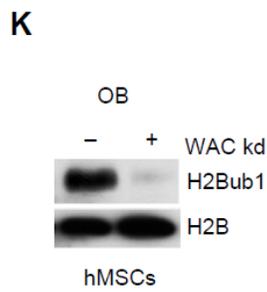
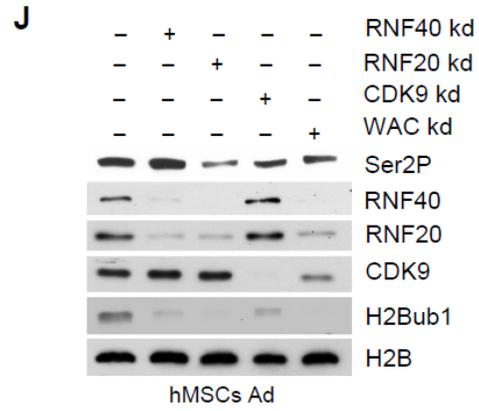
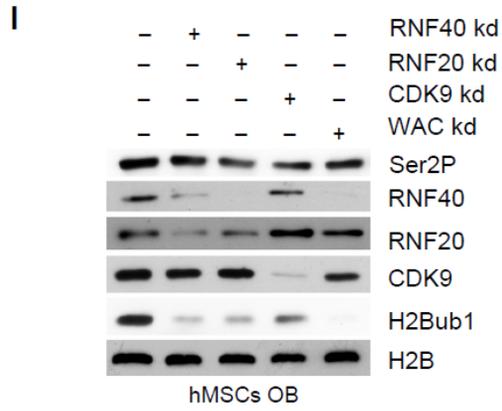
(AB-AC) hMSCs were transfected with control or RNF20 siRNAs (SmartPool) for 24 h and differentiated to either osteoblasts (OB) or adipocytes (Ad) for 2 d. Protein lysates were analyzed by Western blot for RNF20, monoubiquitinated H2B (H2Bub1) and H2B as a loading control.

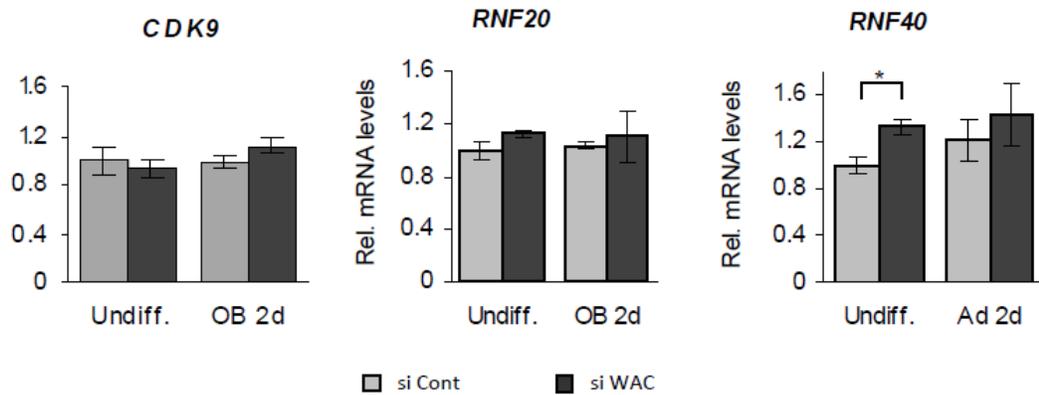
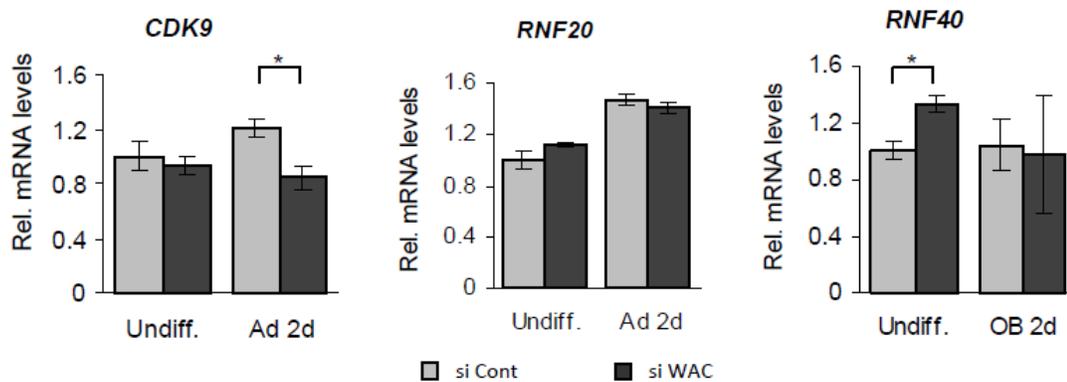
(AD-AE) Knockdowns and differentiation were performed as in (AB). The efficiency of knockdown and the changes in osteoblast (AD) and adipocyte (AE) gene expression were determined by qRT-PCR. Statistical analysis: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean \pm SD, $n = 3$.

(AF-AG) hMSCs were transfected with control or RNF40 siRNAs (SmartPool) for 24 h and induced to differentiate into osteoblasts (AF) or adipocytes (AG) for 2 or 5 d. qRT-PCR was used to monitor osteoblast (AF) and adipocyte (AG)-specific gene expression. Statistical analysis: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean \pm SD, $n = 3$.

Figure S3 related to Figure 3





Q**R****Figure S3**

(A) hMSCs were differentiated for 2 d to osteoblasts (OB) or adipocytes (Ad). Protein extracts were analyzed by Western blot for Ser2 phosphorylation of CTD (P-Ser2) and total polymerase II (loading control).

(B-C) hMSCs were transfected with control or CDK9 siRNAs (Ambion) for 24 h and differentiated to either osteoblasts (OB) or adipocytes (Ad) for 2 d. Protein lysates were analyzed by Western blot with antibodies to CDK9, monoubiquitinated H2B (H2Bub1) and H2B as a loading control.

(D) CDK9 knockdown was performed using an independent siRNA (Invitrogen, Inv) in undifferentiated hMSCs for 2 d. Proteins were analyzed by Western blot for CDK9 and H2B (loading control).

(E-F) hMSCs were transfected with control or CDK9 siRNAs (Invitrogen) for 24 h and induced to differentiate into osteoblasts (E) or adipocytes (F) for 5 d and stained for alkaline phosphatase activity (E) or presence of lipid drops (F) as described in Materials and Methods.

(G-H) Knockdowns with control or CDK9 siRNAs (Ambion) were performed in hMSCs for 24 h. After that cells were induced to differentiate into osteoblasts (G) or adipocytes (H) for 2 d. qRT-PCR was used to monitor osteoblast (G) and adipocyte (H)-specific gene expression. Statistical analysis: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean \pm SD, $n = 3$.

(I-J) hMSC were transfected with siRNAs to RNF40 (SmartPool), RNF20 (SmartPool), CDK9 (Ambion), WAC (SmartPool) and control siRNAs for 24 h and differentiate to osteoblasts (I) or adipocytes (J) for 2 d. Protein lysates were checked for the levels of Ser2 phosphorylation of CTD (P-Ser2), RNF40, RNF20, CDK9, monoubiquitinated H2B (H2Bub1) and H2B (loading control) by Western blot.

(K-L) WAC knockdown (SmartPool) was performed in hMSCs for 24 h. After that cells were differentiated in either osteoblasts (K) or adipocytes (L). Protein extracts were analysed by Western blot for monoubiquitinated H2B (H2Bub1) and H2B as a loading control.

(M) Undifferentiated hMSCs were transfected with control siRNA and siRNAs for WAC (#3 and #4, Dharmacon) for 48 h. Protein extracts were examined for levels of monoubiquitinated H2B (H2Bub1) and H2B (loading control) by Western blot.

(N) Knockdowns were performed as in (M). Knockdown efficiency was verified by WAC expression using qRT-PCR. Statistical analysis: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean \pm SD, $n = 3$.

(O-P) hMSCs were transfected with control siRNA and siRNAs for WAC (#3 and #4, Dharmacon) for 24 h and then differentiated to osteoblast (O) or adipocytes (P) for 5 d. Cells were stained for alkaline phosphatase activity (O) or presence of lipid drops (P).

(Q-R) WAC knockdown (SmartPool) was performed in hMSCs for 24 h, followed by differentiation to osteoblasts (Q) or adipocytes (R). qRT-PCR was used to monitor expression of the osteoblast (Q) or adipocyte (R)-specific genes. Statistical analysis: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean \pm SD, $n = 3$.

Figure S4 related to Figure 4

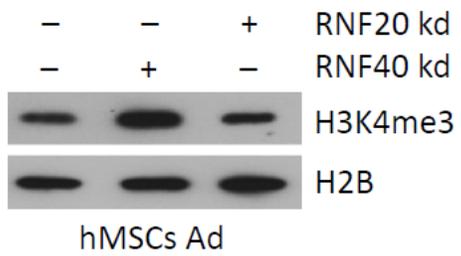


Figure S4

hMSC were transfected with control, RNF40 (SmartPool) or RNF20 (SmartPool) siRNAs for 24h and differentiated to adipocytes for 2d. Protein lysates were analyzed by Western blot using antibodies to H3K4me3 and H2B (loading control).

2) SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture, differentiation and knockdowns

hMSC-Tert cells (Simonsen et al., 2002) were cultured in low glucose, phenol red free MEM (Invitrogen, Carlsbad, CA), supplemented with 10% bovine growth serum (BGS; HyClone, USA) and 1X penicillin/streptomycin (Invitrogen). For osteoblast differentiation growth medium was supplemented with 10 mM β -glycerophosphate, 0.2 mM ascorbate, 10^{-8} M calcitriol, and 10^{-7} M dexamethasone. For adipocyte differentiation normal growth

medium containing 15% BGS was supplemented with 2×10^{-6} M insulin, 0.45 mM isobutylmethyl-xanthine, 10^{-5} M troglitazone, and 10^{-7} M dexamethasone. hFOB1.17 cells (Harris et al., 1995) were provided by Tom Spelsberg (Mayo Clinic, Rochester, Minnesota) and cultured at the permissive temperature (33°C) in high glucose, phenol red free DMEM/F12 (Invitrogen) supplemented with 10% BGS (Hyclone) and 1X penicillin/streptomycin (Invitrogen). Osteoblast differentiation was induced by shifting to the restrictive temperature (39°C) and growing for 7 d. Neurospheres were prepared from E17.5 mouse brains according to standard procedures. Spheres were maintained in proliferation medium (NB with B27 and N2 supplement, 20 ng/ml FGF2, 20 ng/ml EGF). Spheres were differentiated by culturing in NB with B27 and N2 supplement containing 2% horse serum for 5 d. Primary mouse oligodendrocyte cultures were prepared as described previously (Trajkovic et al., 2006). For differentiation the progenitors of oligodendrocytes were cultured for 5 d in MEM containing B27 supplement, 1% horse serum, L-thyroxine, tri-iodothyroxine, glucose, glutamine, penicillin, streptomycin, gentamycin, pyruvate, and bicarbonate (Sato-B27) on poly-L-lysine-coated dishes or glass-coverslips.

Knockdowns were performed using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. siRNAs utilized are listed in Table S1.

Cell culture for microarray studies. Total RNA for microarray experiments was isolated from control or RNF40 siRNA transfected cells prior to differentiation or after differentiation for 2 or 5 d into osteoblasts or adipocytes.

Chromatin immunoprecipitation (ChIP) and qPT-PCR

ChIP was performed using a modified protocol based on (Gomes et al., 2006; Nelson et al., 2006). Adherent cells were crosslinked for 10 min in PBS containing 1% formaldehyde. Crosslinking was quenched by adding glycine to a final concentration of 156 mM for 5 min.

Fixed cells were washed twice with PBS and scraped in a buffer containing 150 mM NaCl, 5 mM EDTA, 50 mM Tris (pH 8), 0.5% (v/v) NP-40, 1% (v/v) Triton X-100, 20 mM NaF and inhibitor cocktail (1 mM N-ethylmaleimide, 10 mM β -glycerophosphate, 1 ng/ μ l Aprotinin, 1 ng/ μ l Leupeptin, 1 mM Pefabloc, 10 μ M iodoacetamide and 1 mM nickel chloride). The nuclei were pelleted and washed with the same buffer before lysing in 300 μ l of Lysis Buffer (150 mM NaCl, 1% (v/v) NP-40, 0.5% w/v sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 8), 20 mM EDTA, 20 mM sodium fluoride and inhibitor cocktail). Pre-clearing was performed with 100 μ l of Sepharose 4B (GE Healthcare, Uppsala, Sweden) in Lysis Buffer (50% slurry) for 1 hour at 4°C. After that chromatin was diluted with Lysis Buffer, aliquoted in 50 μ l aliquots and stored at -80 C. After thawing, each 50 μ l aliquot was brought to the final volume of 1 ml with Lysis Buffer and 2 μ g of corresponding antibodies (Supplemental Table 4) were added. Samples were incubated with the antibodies overnight at 4°C before adding 30 μ l of Protein-G Sepharose slurry (GE Healthcare) and incubating for another 2 hours at 4°C. After centrifugation (2000g, 2 min, 4°C) beads were washed three times with Lysis Buffer, three times with Wash Buffer (100 mM Tris (pH 8,5), 500 mM LiCl, 1% (v/v) NP-40, 1% w/v sodium deoxycholate, 20 mM EDTA, 20 mM NaF and inhibitor cocktail), three additional times with Lysis Buffer and twice with TE-buffer. Reverse crosslinking was performed by adding 100 μ l of 10% (w/v) Chelex 100 slurry (Bio-Rad) and incubating at 95 °C for 10 min. After that 2 μ l of Proteinase K (20 μ g/ μ l, Invitrogen) were added to each sample, followed by the incubation at 55°C for 30 minutes and inactivation by heating to 95 °C for 10 min. Samples were centrifuged at 12,000 X g for 1 min at 4°C and the supernatant containing DNA was utilized for quantitative real-time PCR. The background binding was determined by performing a ChIP with a non-specific IgG antibody. To prepare inputs 5 μ l (10% relative to ChIPs) of chromatin extracts were precipitated by adding 100% EtOH and 1 μ l of Pink Precipitant (5 mg/ml, Bioline, Luckenwalde, Germany) and incubating overnight at -20°C. The pellets were washed twice with 70% EtOH and processed as described above for ChIP samples. ChIP samples were normalized to input DNA samples, and displayed as “% of input”.

RNA isolation, reverse transcription and quantitative RT-PCR was performed as described previously (Prenzel et al., 2011). All qRT-PCR samples were normalized to an internal reference gene (*HNRNPK*) and displayed relative to the control non-differentiated sample.

Statistical analysis was done with ANOVA test.

Primers utilized for gene expression and ChIP analyses are indicated in corresponding lists in Supplemental Materials and Methods.

Immunofluorescence staining of *Drosophila* ovaries

Drosophila melanogaster OregonR stocks were raised on standard cornmeal-yeast-agar-medium at 25°C. Ovaries were fixed in 5% formaldehyde (Polysciences, Inc.) for 10 min and the staining procedure was essentially performed as described (Konig et al., 2011; Shcherbata et al., 2004). Additionally, after fixation ovaries were permeabilized (3 washes in PBT (PBS with 0.2% Triton X-100), followed by 30 min incubation in 2% Triton X-100 in PBS) and incubated in HCl to allow for access of the anti-H2Bub1 antibody to the chromatin. Samples were washed 3 times in PBT, then incubated in 2N HCl at 37°C for 30 min). The following antibodies were used: mouse monoclonal H2Bub1 (1:500, Millipore); rabbit polyclonal pMad (1:1000, D. Vasiliauskas, S. Morton, T. Jessell and E. Laufer), Alexa 488, 568 goat anti-mouse, anti-rabbit (1:500, Molecular Probes).

Immunofluorescence staining of oligodendrocytes

Oligodendrocyte precursors or mature oligodendrocytes were fixed with 4% formaldehyde, 0.2% glutaraldehyde in PBS for 15 minutes. After that cells were washed 3 times with PBS, permeabilized for 1 min with 0.1% Triton X-100, again washed 3x with PBS followed by 30 min blocking with blocking solution (2% BSA, 2% Fetal Calf Serum Gold, 20 µl/ml fish gelatine in PBS). Then samples were incubated for 1h with MBP antibody (1:200, DakoCytomation, Carpinteria, CA, USA) in 10% blocking solution at room temperature, washed 3x with PBS and incubated with Alexa 488 goat anti-rabbit (1:200, Invitrogen) and Phalloidin rodamin (1:200, Invitrogen) in 10% blocking solution followed by 3x PBS wash and mounting in mounting media (0.4 g/ml Mowiol, Merck; 1 g/ml glycerol; 24 mg/ml Anti-fading reagent, DABCO in 0.1M Tris-HCl).

Monolayer-embedding in Epon for electron microscopy

For electron microscopy hMSCs were differentiated to adipocytes for 5 d. Cells were washed in 0.1 M phosphate buffer and fixed for 1h in 1% OsO₄ in 0.1 M PB at 4°C. After that cells were washed (10 min each time) 3 times with DI water, twice with 50% EtOH and once with 70% EtOH. Contrasting was done with 1.5 % uranylacetate and 1.5 % tungstophosphoric acid in 70 % EtOH for 90 min. Then cells were washed twice in 90% EtOH (for 10 min) and 5 times in 99.9% EtOH (for 5 min). After that EtOH was replaced with Epon in EtOH (3 washes, 30 min each): 1st wash – 1:2 Epon to EtOH, 2nd wash – 1:1 Epon to EtOH, 3rd wash – 2:1 Epon to EtOH. Finally cells were incubated in 100% Epon for 1h and then enclosed in

BEEM-capsules. After 24 h of Epon polymerization at 60°C BEEM-capsules were disassembled from the plates and prepared for ultrathin sectioning. Sections were prepared and imaged with a Leo 912AB electron microscope equipped with

a CCD camera 2048 x 2048 (Proscan, Scheuring, Germany) as described (Aggarwal et al., 2011).

Pathway analysis

Pathway analysis was performed to assess biological functions influenced by cell differentiations and knockdowns. All non-metabolic pathways of the KEGG database (www.genome.jp/kegg/) were tested for enrichment using a one-sided wilcoxon rank-sum test on the ranked p-values of differential gene expression analyses. Results were adjusted for multiple testing using Benjamini-Hochbergs method. All analyses were performed using the free statistical software R (version 2.14.1).

Oil Red O staining

Oil Red O staining was performed to visualize lipid drops accumulation. Oil Red O working solution was prepared by mixing 3 parts of Oil Red O stock solution (3mg/ml Oil Red O in 99% isopropanol) and 2 parts of DI water followed by 10 min incubation at RT and filtering. Cells were fixed with 10% formaldehyde in PBS for 30 min, washed 3 times in DI water and incubated with 60% isopropanol for 5 min. After that cells were incubated with Oil Red O working solution for 5 min and rinsed in DI water.

Quantification of Oil Red O and alkaline phosphatase staining

Pictures of the stained plates were taken under microscope using 10x magnification. Each picture showed approximately 500-1000 cells. The staining was quantified using the Threshold_Color plugin (<http://www.dentistry.bham.ac.uk/landinig/software/software.html>) of ImageJ software (<http://rsb.info.nih.gov/ij/>). For Oil Red O staining, the threshold was defined in RGB color space for each experiment (typically : R:150-255 ; G:0-140 ; B:0-140) and the positive area of each picture was measured. For alkaline phosphatase staining, the threshold was defined in CIE Lab color space for each experiment (typically : L*:5-255 ; a*:125-255 ; b*:0-255) and the positive area of each picture was measured.

List of siRNAs used in this study.

Target Gene	siRNA target sequence	Source	Cat.No.
siGENOME Non-targeting siRNA pool # 1	-	Dharmacon	D-001206-13
RNF20 si Genome (#1)	CCAAUGAAAUCAAGUCUAA	Dharmacon	D-007027-01
RNF20 si Genome (#2)	UAAGGAAACUCCAGAAUAU	Dharmacon	D-007027-02
RNF20 si Genome (#3)	GCAAUGUCCCAAGUGUAA	Dharmacon	D-007027-03
RNF20 si Genome (#4)	AGAAGAAGCUACAUGAUUU	Dharmacon	D-007027-04
RNF40	UGAGGACAUGCAGGAACAGAA	Ambion	s18960
RNF40 siGENOME (# 1)	GAGAUGC GCCACCUGAUUUAUU	Dharmacon	D-006913-01
RNF40 siGENOME (# 2)	GAUGCCAACUJUAAGCUAAJU	Dharmacon	D-006913-02
RNF40 siGENOME (# 3)	GAUCAAGGCCAACCCAGAUUUU	Dharmacon	D-006913-03
RNF40 siGENOME (# 4)	CAACGAGUCUCUGCAAGUGUU	Dharmacon	D-006913-04
CDK9	UGAGAUUUGUCGAACCAAAtt	Ambion	S2834
CDK9	GUCAACUUGAUUGAGAUUUGU CGAA	Invitrogen Val. Stealth	
WAC siGENOME (# 1)	CAACAUACGUCUCUGAUU	Dharmacon	D-013325-01
WAC siGENOME (# 2)	UAAGCACACCUCAAACUAA	Dharmacon	D-013325-02
WAC siGENOME (# 3)	GAGACAAACCCGUAUCACA	Dharmacon	D-013325-03
WAC siGENOME (# 4)	CGAUCCACGUGUUCAUUA	Dharmacon	D-013325-04

List of antibodies used for ChIP and Western blot analysis.

Target protein	ChIP	WB	IF	Clone	Cat. No.	Source
CDK9		1:1000		C-20	sc-484	Santa Cruz
H2B		1:3000			07-371	Millipore
H2Bub1		1:100		7B4		(Prenzel et al., 2011)
H2Bub1			1:500	56	05-1312	Millipore
H2Bub1	2 µl			D11	5546	Cell Signaling
H3K4me3	2 µg	1:1000			MAB-003-050	Diagenode
H3K27me3	2 µg				pAb-069-050	Diagenode
GFAP			1:400		MAB360	Chemicon International
IgG	2 µg				ab46540-1	Abcam
MBP			1:200		A0623	Dako
NeuN			1:500		NUN	Aves Labs
pMAD			1:1000			D. Vasiliauskas, et al.
P-Ser2 RNAPII		1:10		3E10		(Chapman et al., 2007)
RNF40		1:1000		KA7-27	R9029	Sigma
Goat anti-mouse IgG-HRP		1:5000			sc-2005	Santa Cruz
Goat anti-rabbit IgG-HRP		1:5000			sc-2004	Santa Cruz
Goat Anti-Rat IgG + IgM-HRP		1:10000			112-035-068	Jackson ImmunoResearch
Goat anti-mouse Alexa 488			1:500			Molecular Probes
Goat anti-rabbit Alexa 568			1:500			Molecular Probes
Fluorescein-Labeled goat anti-Chicken			1:500		F-1005	Aves Labs
Goat anti-Mouse 568			1:500			Invitrogen

Goat anti-rabbit 488			1:200		A-11070	Invitrogen
Phalloidin rodamin			1:200		R415	Invitrogen

List of qRT-PCR primers used in this study.

Name	Sequence	Source
ALPL F	TGGGCCAAGGACGCTGGGAA	This study.
ALPL R	AAGGCCTCAGGGGGCATCTCG	This study.
BGLAP F	GCCCTCACACTCCTCGCCCT	This study.
BGLAP R	CGGGTAGGGGACTGGGGCTC	This study.
CDK9 F	AGAGGGTTTCCATGGGGTAG	This study.
CDK9 R	TCAGCCCGAGAATAGGATTG	This study.
G6PD F	CGACGAAGCGCAGACAGCGTCA	This study.
G6PD R	CAGCCACATAGGAGTTGCGGGC	This study.
HNRNPK F	ATCCGCCCTGAACGCCCAT	This study.
HNRNPK R	ACATACCGCTCGGGGCCACT	This study.
LPL F	TCAGCCGGCTCATCAGTCGGT	This study.
LPL R	AGAGTCAGCACGAGCAGGGCT	This study.
PDK4 F	TTCACTCCGCGGCACCCTCA	This study.
PDK4 R	TCGGAGCAGAGCCTGGTTCCG	This study.
PPARG F	ACCTCCGGGCCCTGGCAAAA	This study.
PPARG R	TGCTCTGCTCCTGCAGGGGG	This study.
RASD1 F	CAAGACGGCCATCGTGTGCGG	This study.
RASD1 R	GCTGCACCTCCTCGAAGGAGTCG	This study.
RNF20 F	TGGCCAAGCAGGAAGAAG	This study.
RNF20 R	ACGCTCTGACATGAGCTTGA	This study.
RNF40 F	AGTACAAGGCGCGGTTGA	(Prenzel et al., 2011)
RNF40 R	GAAGCAGAAAACGTGGAAGC	(Prenzel et al., 2011)
RPLP F	GATTGGCTACCCAAGTGTG	(Fritah et al., 2005)
RPLP R	CAGGGGCAGCAGCCACAAA	(Fritah et al., 2005)
WAC F	AGTGGGTTTGCCAGTGGAATGGAAGA	This study.
WAC R	ACAGTGCTTGGGGTAGCAGTTGGA	This study.

List of ChIP primers used in this study

Name	Sequence	Source
HIST1H2AC F	AAAAGCGGCCATGTTTTACA	(Pirngruber et al., 2009)
HIST1H2AC R	AAAAATCACCAAACCAGCG	(Pirngruber et al., 2009)
GAPDH +1061F	CCGGGAGAAGCTGAGTCATG	(Shema et al., 2008)
GAPDH +1111R	TTTGCGGTGAAATGTCCTT	(Shema et al., 2008)
PDK4-BV+468F	GCGTCGAGGCTCCAGGGCT	This study.
PDK4-BV+570R	GCCCAAGCTGGGTCCTAGGGTT	This study.
PDK4 +3831F	CTCGGATGCTGATGAACCAGCACAGTAAG	This study.
PDK4 +3963R	AGTACTATCACTGAGAATGTGACCCGCTGAT	This study.
PPARG-BV+655F	AGCCGCTCCGGGGGAACTT	This study.
PPARG-BV+850R	ACAGGGCCTGGCCAGCTACAA	This study.
PPARG +91888F	GGCCACCAACTTTGGGATCAGC	This study.
PPARG +91922R	GAGTGGGAGTGGTCTTCCATTACGGAG	This study.
RASD1-BV +768R	GATCTGCTGCCTGAGCCGCTG	This study.
RASD1-BV +666F	CGGCCACCCTCACCTTCTCCT	This study.
TFF1 +6KB F	CAGGCTTCTCCCTTGATGAAT	(Pirngruber and Johnsen, 2010)
TFF1 +6KB R	ACACCCACCTTCCACAACAC	(Pirngruber and Johnsen, 2010)

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