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multimedia online data

SUPPLEMENTARY ONLINE DATA

Regulation of adipogenesis by cytoskeleton remodelling is facilitated by acetyltransferase MEC-17-dependent acetylation of α-tubulin

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Supplementary Figures S1–S6, and Supplementary Tables S1 and S2 are on the following pages.

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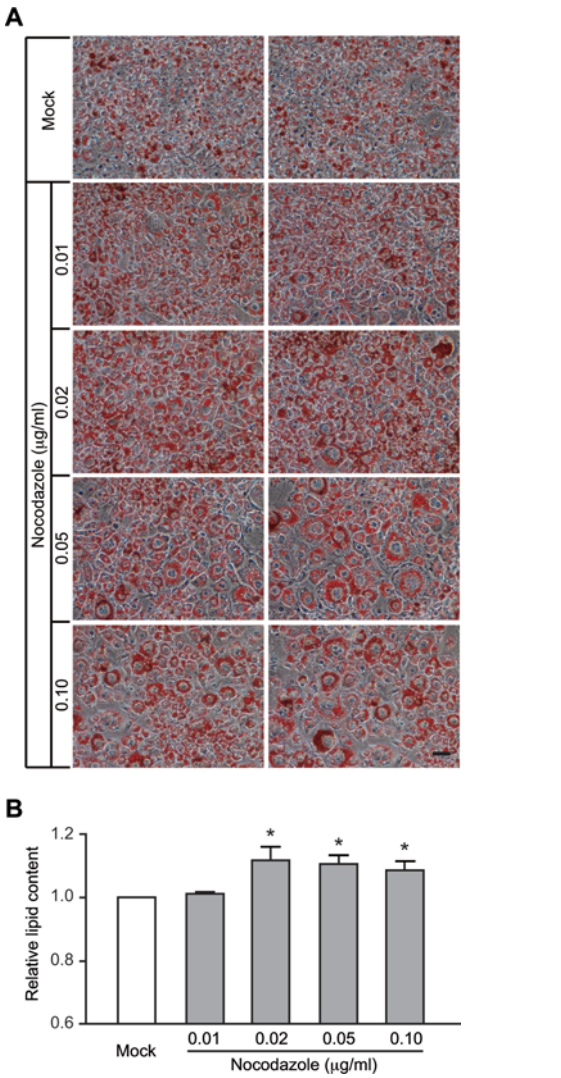

Figure S1 Disruption of MTs by nocodazole enhances lipid accumulation during adipogenesis

(**A**) Treatment with nocodazole increased triacylglycerol accumulation after adipogenic cocktail treatment. 3T3-L1 cells were treated with various concentrations of nocodazole or vehicle (Mock) for 2 h each on days 2, 4 and 6 after adipogenic cocktail treatment. Lipid accumulation was assessed by microscopy (20×). Two different fields are shown for each condition to illustrate reproducibility. (**B**) Propan-2-ol extracts of Oil-Red O from the nocodazole experiments were used to determine relative total lipid content. Values are means±S.E.M. ($n = 3$–4 independent experiments). *$P < 0.05$. Scale bar in (**C**) = 50 μm.

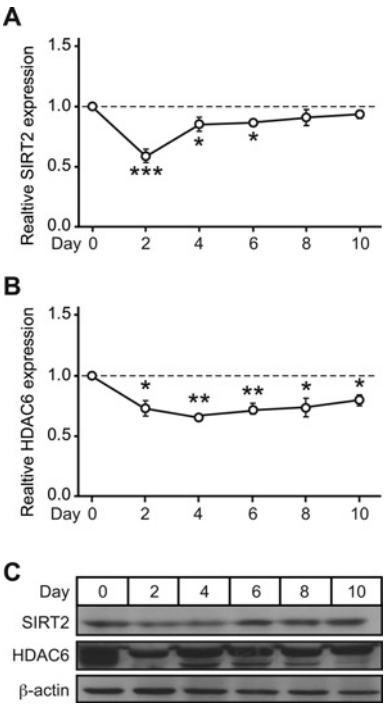

Figure S3 Expression profile of deacetylases, SIRT2 and HDAC6 during adipogenesis

Expression levels of SIRT2 (**A**) and HDAC6 (**B**) were evaluated in 3T3-L1 cells at the time points indicated during adipogenesis by qPCR. Values are means±S.E.M. ($n = 3$ independent experiments, each measured in triplicate). (**C**) Western blots showing protein levels of SIRT2 and HDAC6 in 3T3-L1 cells at the time points indicated after adipogenic cocktail treatment.

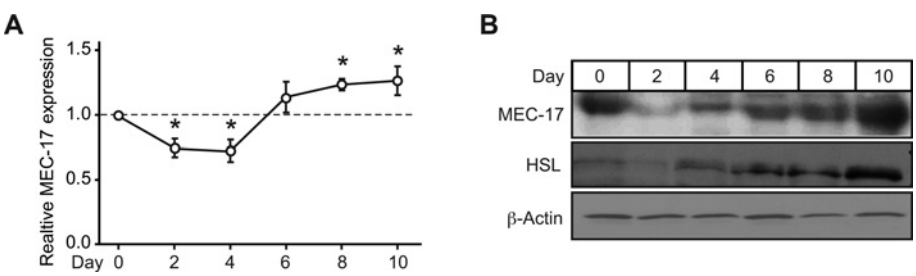

Figure S2 Expression profile of MEC-17 in 3T3-L1 cells during adipogenesis

(**A**) qPCR was used to assess mRNA levels of the acetyltransferase MEC-17 in 3T3-L1 cells at different time points after adipogenic cocktail treatment. Values are means±S.E.M. $n = 3$ independent experiments, each measured in triplicate. (**B**) Western blots showing protein levels of MEC-17 in 3T3-L1 cells at the time points indicated after adipogenic cocktail treatment.

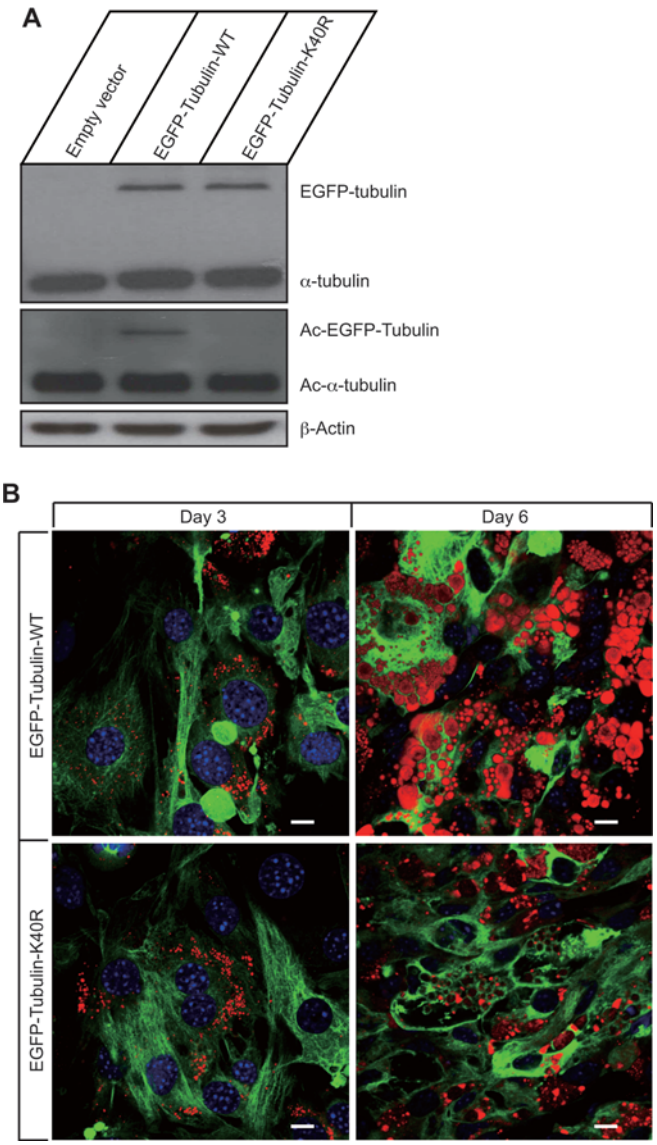

Figure S4 Impaired morphological transition in acetylation-resistant tubulin-expressing cells during adipogenesis

(**A**) Lysates from stable 3T3-L1 cells expressing EGFP–α-tubulin (EGFP-Tubulin-WT) or EGFP–α-tubulin-K40R mutant (EGFP-Tubulin-K40R) were examined by immunoblotting using anti-α-tubulin, anti-(acetylated α-tubulin) and anti-β-actin antibody. (**B**) Stable 3T3-L1 cells expressing EGFP–α-tubulin (EGFP-Tubulin-WT) or EGFP–α-tubulin-K40R (EGFP-Tubulin-K40R) were fixed and LDs were stained with LipidTOX reagent (red), and imaged on a confocal microscope. Note the diffuse staining pattern of EGFP–Tubulin-WT and increased LD staining at a late stage of differentiation. In contrast, the majority of EGFP–Tubulin-K40R expressing cells retained a fibroblast-like morphology and relatively intact MT network. Scale bars = 10 μm.

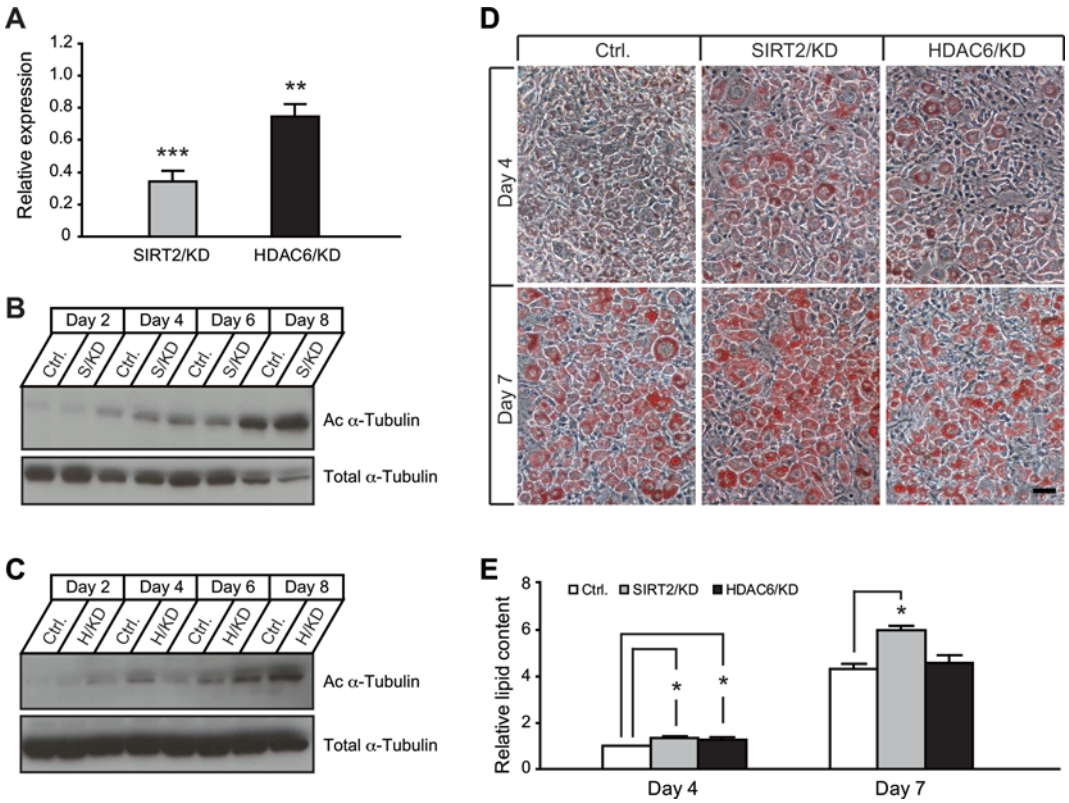

Figure S5 Enhanced adipogenesis in SIRT2- or HDAC6-KD cells is associated with increased acetylation of α-tubulin

(**A**) 3T3-L1 cells with efficient KD of SIRT2 or HDAC6. (**B** and **C**) The levels of acetylated α-tubulin were detected in SIRT2- (**B**) or HDAC6- (**C**) KD cells at different time points after adipogenic cocktail treatment. (**D**) Stable 3T3-L1 cells with KD of SIRT2 or HDAC6 were stained with Oil-Red O and imaged on a microscope at days 4 and 7 after adipogenic cocktail treatment. Note both cell lines showed increased Oil-Red O staining at day 4, but only SIRT2-KD cells showed more staining at day 7. (**E**) Propan-2-ol extracts of Oil-Red O cells from (**D**) were used to determine the relative lipid content. As was found in (**D**), lipid content was increased in both cell lines at day 4, but only SIRT2-KD cells showed increased lipid content at day 7. Values are means$\pm$S.E.M. ($n = 3$–4 independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Scale bar in (**D**) $= 50$ μm. Ctrl., control.

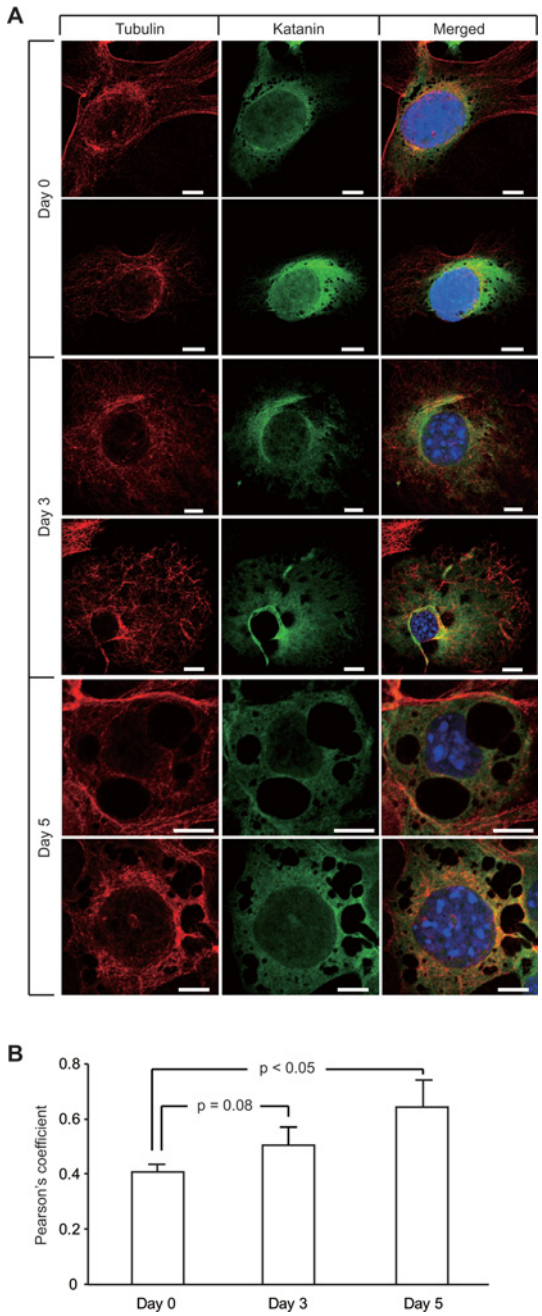

Figure S6 Increased co-localization of katanin with α-tubulin during adipogenesis

(**A**) 3T3-L1 cells expressing EGFP-tagged katanin were fixed, stained with the anti-tubulin antibody at days 0, 3 and 5 after adipogenic cocktail treatment, and imaged on a confocal microscope. Scale bars = 10 μm. (**B**) Pearson's coefficients at the time points indicated. Values are means±S.E.M. (n = 3–4 independent experiments).

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Table S1 Target sequences for KD of MEC-17, SIRT2, HDAC6 and katanin

Target	Oligonucleotide name	Sequence (5′→3′)
MEC-17	M/KD1	GCCATACTCTTCCAGTGAC
MEC-17	M/KD2	AGTTGGATACAAGAAGCTC
SIRT2	S/KD	GAAGGAGTGACACGCTACA
HDAC6	H/KD	GGGCTGGATCTGAACCCTG
Katanin	K/KD1	GCAGTGGTGTTACCAATGT
Katanin	K/KD2	GACAACGTTCTTCAATGTC

Table S2 Real-time qPCR primers

aP2, adipocyte P2; GLUT4, glucose transporter 4; LDLR, low-density lipoprotein receptor; TBP, TATA-box binding protein.

Target	Forward primer (5′→3′)	Reverse primer (5′→3′)
MEC-17	GAGCCATTATTGGTTTCCTCAAAG	AGCCTCCCGGTCATCCA
SIRT2	AGCCAACCATCTGCCACTAC	CCAGCCCATCGTGTATTCTT
HDAC6	GACAGCGAAAGAGTAGGCACAA	AGGTGGCGCTGGATTCC
Katanin	GCCAGCCGAAGGATGAAA	GCACCTCCAACACCATCCAT
PPARγ	CATGACCAGGGAGTTCCTCAA	AGCAAACTCAAACTTAGGCTCCAT
C/EBPα	CCAAGAAGTCGGTGGACAAGA	CGGTCATTGTCACTGGTCAACT
GLUT4	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
Perilipin A	GGGACCTGTGAGTGCTTCC	GTATTGAAGAGCCGGGATCTTTT
aP2	GCCAAGCCCAACATGATCA	TTCCACGCCCAGTTTGAAG
LDLR	CCACTTCCGCTGCAACTCA	CGTCGCAGGCCCAAAG
TBP	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG