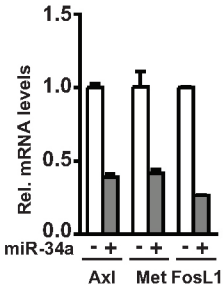
Supplementary Figure S6

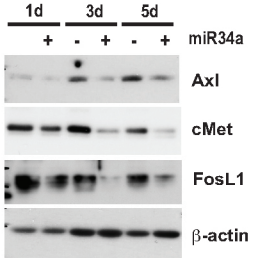
A

Symbol	Name	FC	P-value
ACSL4	acyl-CoA synthetase long-chain family member 4	-3.04	5.85E-14
RRAS	related RAS viral (r-ras) oncogene homolog	-2.18	4.47E-15
LGR4	leucine-rich repeat-containing G protein-coupled receptor 4	-2.63	3.24E-10
MET	met proto-oncogene (hepatocyte growth factor receptor)	-4.13	1.36E-21
AXL	AXL receptor tyrosine kinase	-2.62	0.00E+00
FGD6	FYVE, RhoGEF and PH domain containing 6	-2.22	7.33E-23
FOSL1	FOS-like antigen 1	-4.55	5.91E-26
SYT1	synaptotagmin I	-12.14	2.10E-10
NAV3	neuron navigator 3	-5.74	1.20E-04
SLC4A7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	-5.43	1.57E-06

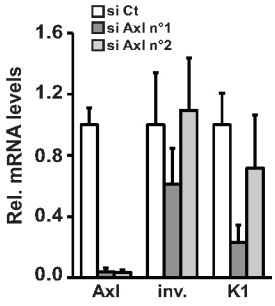
B

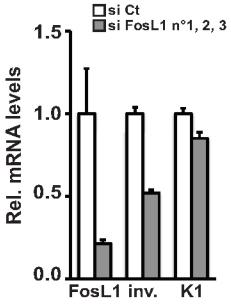


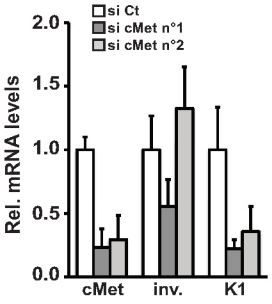
C



D

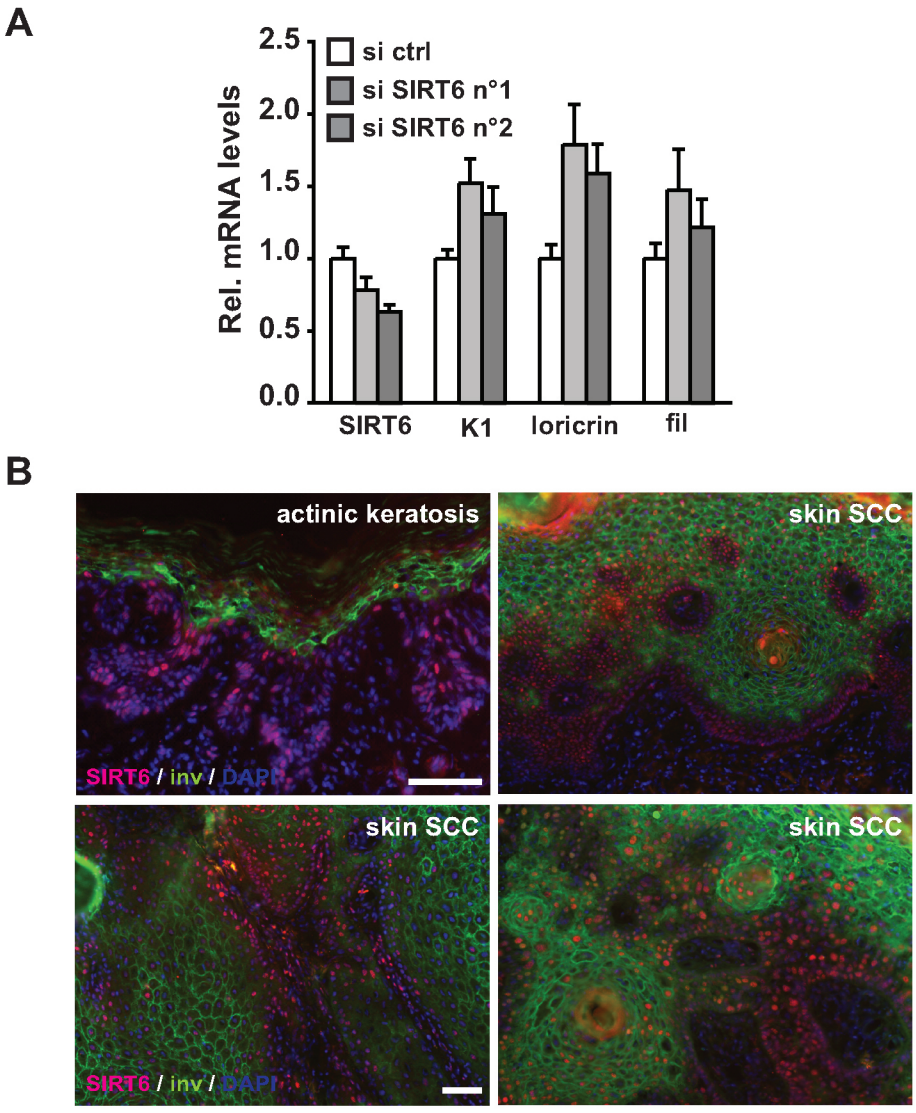






Supplementary Figure S6. Identification of putative miR-34a target genes with a possible role in differentiation. (A) Among the 168 transcripts commonly down-regulated by elevated miR-34a levels and in differentiating HKCs (as analyzed in Figure 3F, right panel), 11 genes were predicted targets of miR-34a as determined by the presence of miR-34a recognition site(s) in their 3' UTR regions. For each of these genes, are shown their fold change expression in miR34a-overexpressing HKCs versus control as well as the corresponding P value. (B) HKCs transfected with precursor miR-34a (+) or scrambled control oligonucleotides (-) for 3 days were analyzed for expression of the indicated genes by qRT-PCR. (C) HKCs transfected as in the previous panel were analyzed at various times (days) by immuno-blotting for the indicated proteins. Given the equivalent molecular weight of the two proteins c-Met and Axl, their expression was assessed by a parallel gel/blot, while FosL1 protein levels were assessed on a different blot. All the gels were probed with an antibody against β-actin giving the same pattern of expression as the one shown. (D) HKCs were transfected for 3 days with multiple sets of siRNAs against Axl (si Axl), FosL1 (si FosL1; 3 different siRNAs pooled together), cMet (si cMet) in parallel with scrambled control (si Ct), followed by qRT-PCR analysis of the indicated genes.

Supplementary Figure S7



Supplementary Figure S7. SIRT6 silencing promotes differentiation in SCC13 cells and HKCs while its expression is elevated in skin cancerous lesions. (A) HKCs transfected for 3 days with siRNAs against SIRT6 (si SIRT6 n°1 and n°2) or scrambled control (si ctrl) were analyzed for expression of the indicated genes by qRT-PCR using 36β4 for normalization. (B) Double immunofluorescence analysis of SIRT6 (red) and involucrin (green) expression in a human actinic keratosis sample (same as S4 used for LCM analysis in Figure 1F) and skin SCCs, with DAPI for counterstaining. Bar = 200 μm.

Supplementary Table 3: Primers used for real time RT-PCR reactions, methylation specific PCR and si-oligonucleotides

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
5s GS/LNA	CATGATCAGCTGGGCCAAGAAAGCC TACAGCACCCGGTATT	TGGAAGCTAAGCAGGGTCGG
MIR-34A-GS/LNA	CATGATCAGCTGGGCCAAGAAACAA CCAGCTA	TGGCAGTGTCTTAGCT
INTEGRIN a6	ATAAATTTTGCACCCGAGAAGGAA	GTTGGAAGGGCTGTTTGTCACTGT
C-MET	CACGAAGATCCCATTGAATGGCTTG	CCAGCCACATATGGTCAGCCTTGTC
INVOLUCRIN	AGGGAAGAGGGGATGCTAAA	GTGTGTGTTGCTGGGACATC
PRE-MIR34A	CCAGCTGTGAGTGTTTCTTTGG	GGGCAGTATACTTGCTGATTGC
PRI-MIR34A	CCTCCAAGCCAGCTCAGTTG	TGACTTTGGTCCAATTCCTGTTG
36B4	GCAATGTTGCCAGTGTCTGT	GCCTTGACCTTTTCAGCAAG
B-ACTIN	GCGTTGTTACAGGAAGTCCCTTGCC	TGCTATCACCTCCCCTGTGTGGA
DNP63a	ATTGCATCACTGTATCATTTTCT	TGCTCTGTGGGGACCTTTCA
KERATIN 1	GTTCCAGCGTGAGGTTTGTT	TAAGGCTGGGACAAATCGAC
NOTCH 1	GAACCAATACAACCCTCTGC	AGCTCATCATCTGGGACAGG
HERP1	GCATATGATTCCGAGAGTGC	CGCAAGTGCTGAGATGAGAC
P21WAF1/CIP1	AGCAGAGGAAGACCATGTGGACCT	GAAGATGTAGAGCGGGCCTTTGAGG
AXL	TGAACGAGAGAGCTTCCCAGCACC	TCCATGCCACTGGCGATGTCTG
FOSL1	TCCAGGCTACACAAAGCTAC	TTGTTCCGCTCGCGCCTTACTC
KERATIN 10	GAAAAGCATGGGCAACTCACA	TGTCGATCTGAAGCAGGATG
SPRR3	CCAGGCTACACAAAGCTAC	GCTTAATTCAGGGGCTTAC
LORICRIN	ATGATGCTACCCGAGGTTTG	ACTGGGGTTGGGAGGTAGTT
FILAGGRIN	TGGACACCCGGGGTCAAGCA	TGCCCACGGGAGGCATCAGA
P16INK4A	CCCAACGCACCGAATAGTTAC	ATTCCAATTCCCCTGCAAACT
P15INK4B	GCGGGGACTAGTGGAGAAGGTGC	GTCGGGTGAGAGTGGCAGGGTC
CDK4	TCCTCTGTTTGGCTTTGCCA	GACTTCCTAGGCCCTGTAAT
PAI1	TGAAGATCGAGGTGAACGAG	GAAAAGGACTGTTCCTGTGG
GRO1	ACTCAAGAATGGGCGGAAAGCTTG	AGCGATGCTCAAACACATTAGGCAC
DCR2	TTGCCTTCTTGCCTGCTATG	TACTGACCTTGACCACCTCT
KI67	CTGCTTGTTTGGAAGGGGTA	AGCCGTACAGGCTCATCAAT
SIRT6	TACGCGGACAAGGGCAAG	ACTTGGGGGCCAGACCTCGC
FGF21	AAGACATCCAGGTTCCTGTG	TATCCGTCCTCAAGAAGCAG
P53	AGGCCTTGGAACTCAAGGAT	CTGAGTCAGGCCCTTCTGTC
BAX	TAACATGGAGCTGCAGAGGA	CAGTTGAAGTTGCCGTCAG
BIM	TGCAGACATTTTGCTTGTTCAA	GAACCGCTGGCTGCATAATAAT
UNMET. MIR34A	IIGGTTTTGGGTAGGTGTGTTTT	AATCCTCATCCCCTTCACCACCA
MET. MIR34A	GGTTTGGGTAGGCGCGTTTC	TCCTCATCCCCTTCACCGCCG

Si RNA	targeted sequence
si Axl n°1	GGAACUGCAUGCUGAAUGA
si Axl n°2	CAGCGAGAUUUAUGACUAU
si FosL1 n°1	CGAAGGCCUUGUGAACAGA
si FosL1 n°2	GGAAGGAACUGACCGACUU
si FosL1 n°3	CCAUCUGCAAAAUCCCGGA
si cMet n°1	GCUACUUAUGUGAACGUAA
si cMet n°2	GCACUAGCAAAGUCCGAGA

si SIRT6 n°1	AAGCUGGAGCCCAAGGAGGAAUCUC
si SIRT6 n°2	AAGAAUGUGCCAAGUGUAAGACGUG