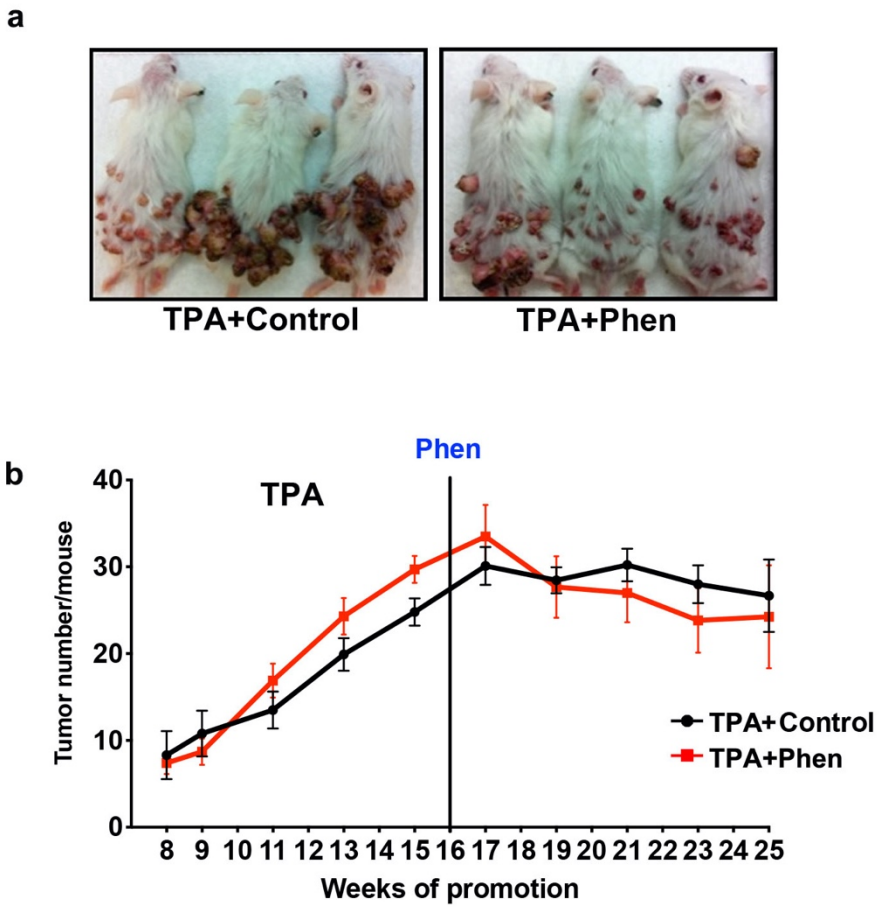
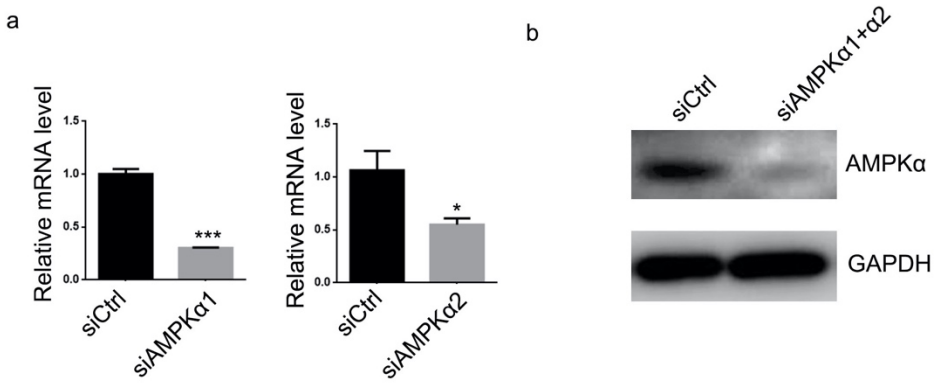
Supplementary Figures and Figure Legends



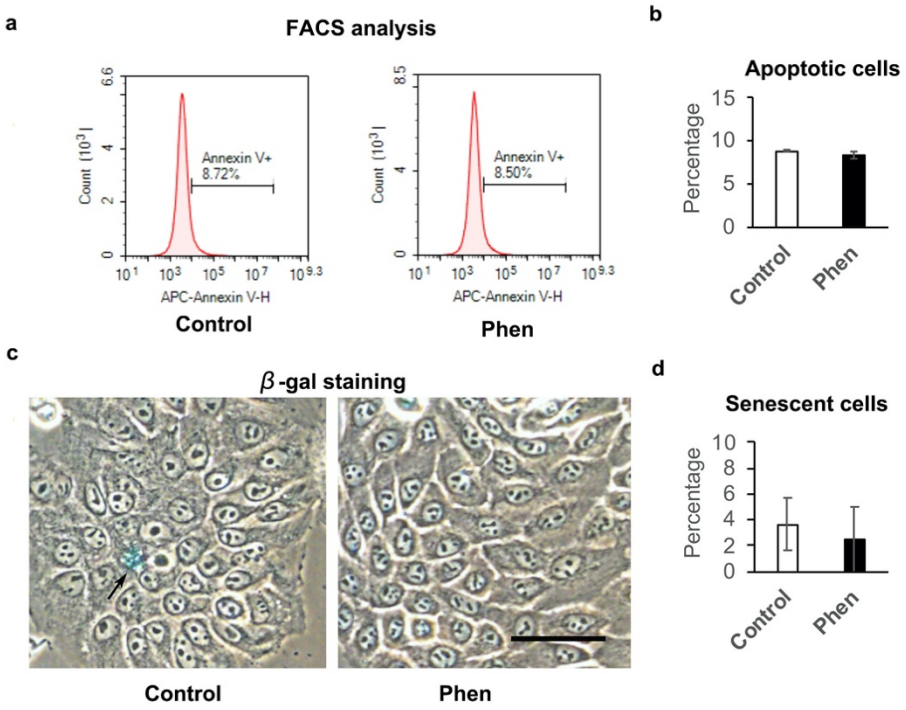
Supplementary Figure S1. Phenformin has an anti-tumor function in mouse keratinocytes *in vivo*.

a. Representative images of mice at 25 weeks from the DMBA-TPA model experiment described in **Figure 1a**. **b.** The number of tumors in mice was counted at 17 weeks, one week after starting the treatment with phenformin (see **Figure 1a**) and the average number of tumors per mouse at each time point is shown.



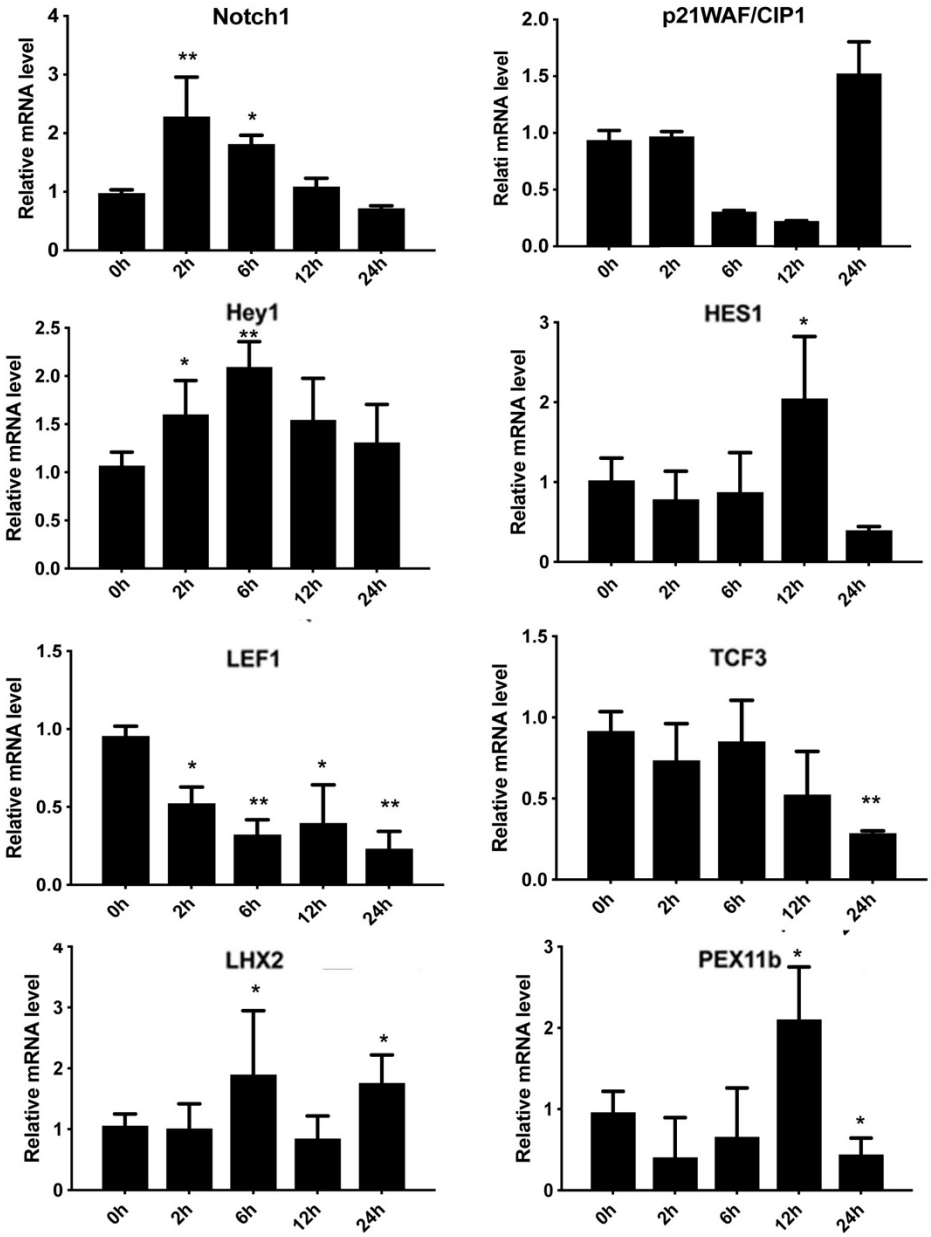
Supplementary Figure S2. AMPKα expression is efficiently knocked down by transfection of double siRNAs of AMPKα1 and AMPKα2.

a-b: Human keratinocytes were transfected with siRNAs targeting AMPKα1/AMPKα 2 or a scrambled control siRNA (siCtrl). 72 h after transfection, cells were lysed for RT-PCR analysis of AMPKα1 and AMPKα2 expression (**a**) and western blot analysis of AMPKα (**b**); GAPDH was used as a loading control. * $P<0.05$, *** $P<0.005$. Student's two tailed *t*-test.



Supplementary Figure S3. Phenformin does not significantly affect keratinocyte apopotosis or senescence.

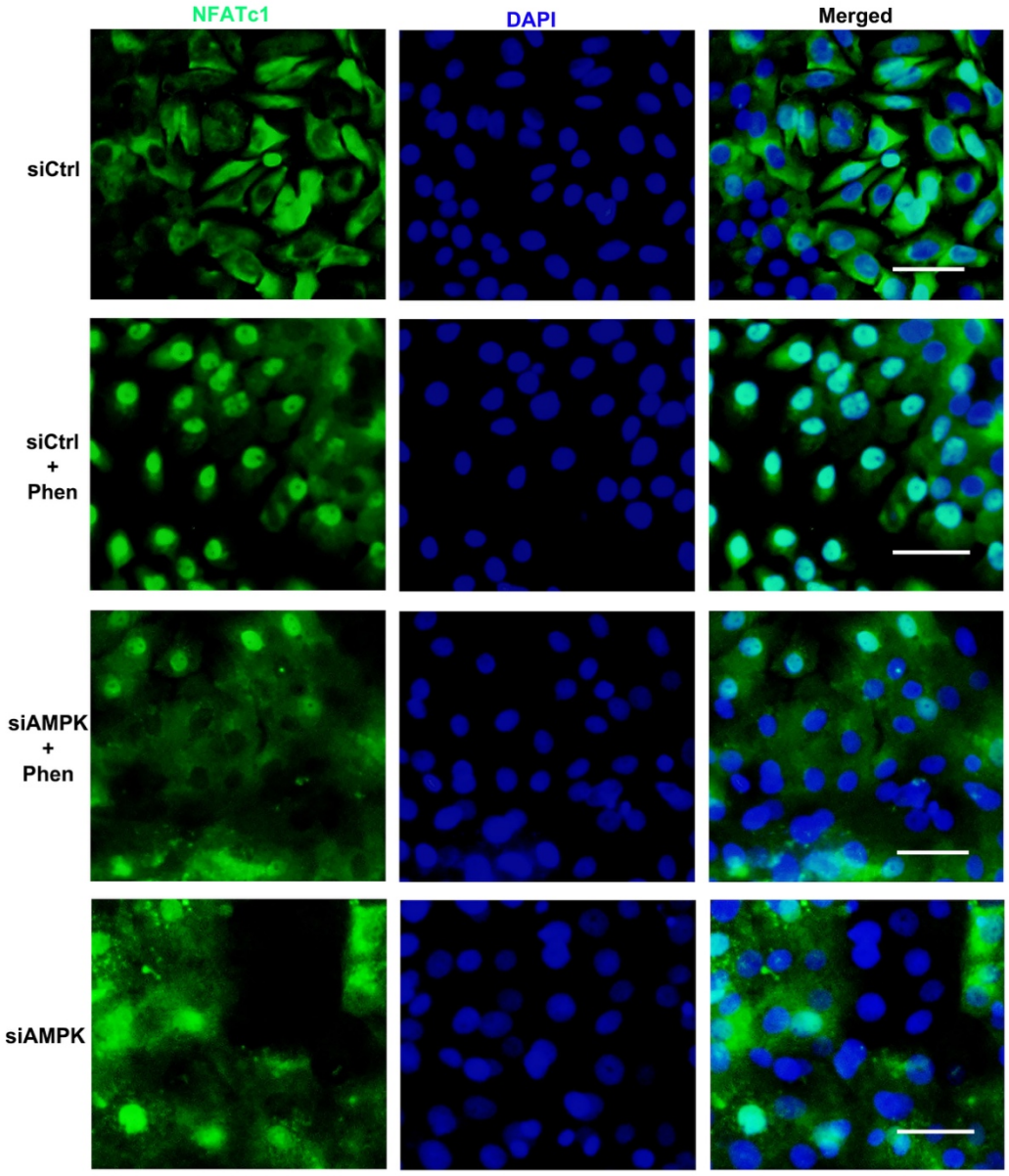
a. Human keratinocytes were treated with vehicle (control) or 1 mM phenformin (phen) for 24 h, followed by staining with annexin V-FITC and propidium iodide (PI) and FACS analysis for apoptotic cells. Represenative FACS data are shown. **b.** Quantification of apoptotic cells from three independent experiments as shown in **a**. **c.** Human keratinocytes were treated without (control) or with 1 mM phenformin (Phen) for 24 h, followed by β-gal staining (blue) for senescent cells. scale bar = 50 μm. **d**. Percentages of β-gal positive cells (black arrow) was quantified based on a total of 200 cells from **c**.



Supplementary Figure S4. RT-PCR analysis of the expression of candidate genes that regulate keratinocyte differentiation.

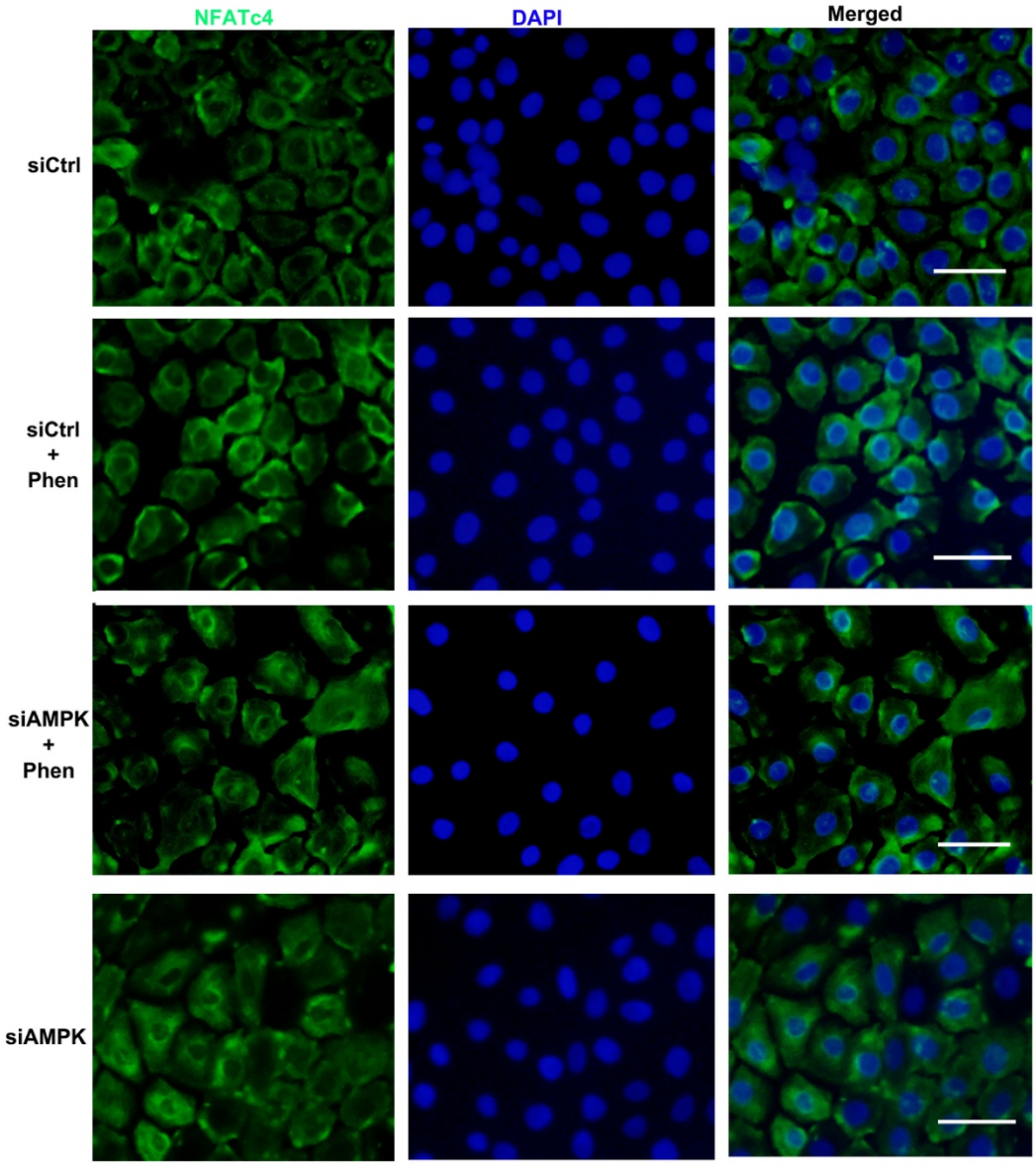
Human keratinocytes were seeded in non-adhesive 6-well plates in suspension to induce differentiation. Cells were collected at the indicated time points for qRT-PCR analysis

of various mRNAs as indicated. Expression levels of different markers were calculated relative to the control at 0 h in suspension. The experiment was repeated at least 3 times, and * $P<0.05$, ** $P<0.01$ by Student's two tailed *t*-test (compared to 0 h).



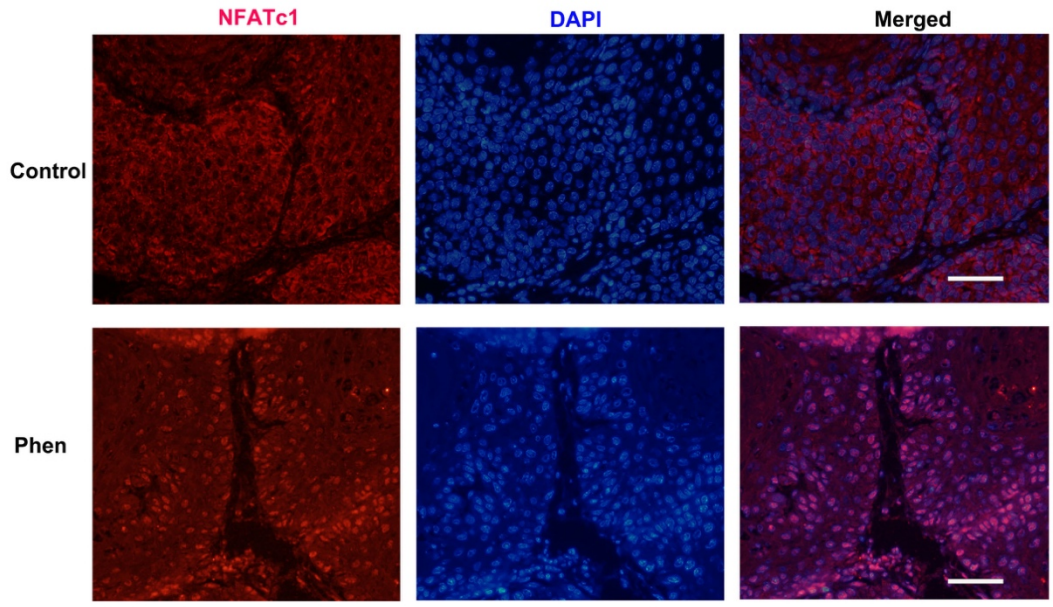
Supplementary Figure S5. Phenformin enhances the nuclear localization of NFATc1 *in vitro*.

Human keratinocytes transfected with siRNAs against AMPKα1/α2 (siAMPK) or with a scrambled control siRNA (siCtrl), were cultured in the presence or absence of 1 mM phenformin, followed by IF analyses with anti-NFATc1 (green, left panels) and DAPI staining for nuclei (blue, middle panels). Merged images of NFATc1 and DAPI staining are shown in the right panels, which is also shown in **Figure 3c**. Bars = 50 μm.



Supplementary Figure S6. Phenformin does not significantly affect the cellular localization of NFATc4 *in vitro*.

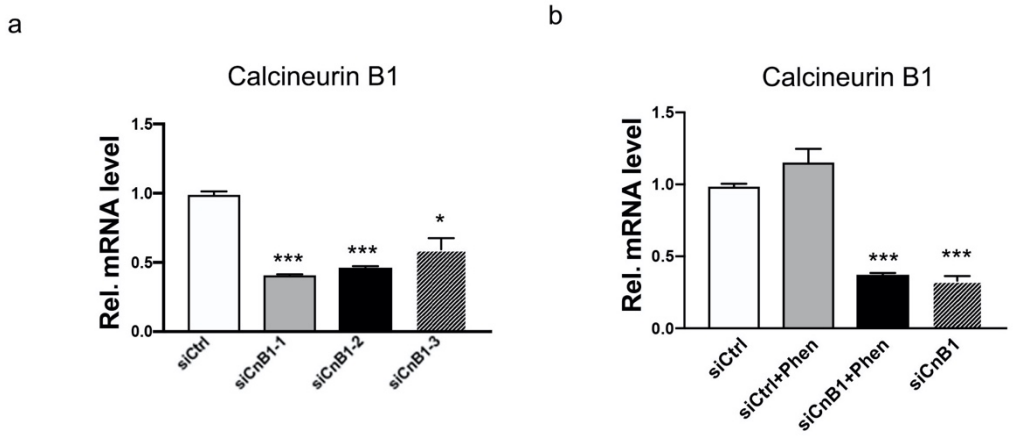
Human keratinocytes transfected with siRNAs against AMPKα1/α2 (siAMPK) or a scrambled control siRNA (siCtrl) were cultured in the presence or absence of 1 mM phenformin as described for **Supplementary Figure S5**, followed by IF analyses with anti-NFATc4 (green, left panels) and DAPI staining of nuclei (blue, middle panels). Merged images of NFATc4 and DAPI staining are shown in the right panels, which is also shown in **Figure 3e**. Bars = 50 μm.



Supplementary Figure S7. Phenformin enhances the nuclear localization of NFATc1 *in vivo*.

Tumor sections from **Figure 1** were used for immunofluorescence analyses of NFATc1 (red, left panels) and DAPI staining of nuclei (blue, middle panels). Merged images of

NFATc1 and DAPI staining are shown in the right panels, which is also shown in **Figure 3g**. Bars = 50 μm.



Supplementary Figure S8. Calcineurin B1 expression is efficiently knocked down by transfection of siRNAs targeting CnB1.

a: Human keratinocytes were transfected with three independent siRNAs targeting calcineurin B1 (CnB1) or a scrambled control siRNA (siCtrl). 72 h after transfection, cells were lysed for RT-PCR analyses of calcineurin B1 expression. **b.** Human keratinocytes transfected with siRNAs targeting calcineurin B1 (siCnB1) and a scrambled siRNA as a control (siCtrl) were cultured in suspension in the presence or absence of 1 mM phenformin (Phen) for 24 h. Cells were lysed for qRT-PCR analysis of Calcineurin B1 expression. **a-b.** All experiments were repeated three times, and * $P<0.05$, *** $P<0.005$ by Student's two tailed *t*-test (compared to the siRNA control group).

Supplementary Tables

Supplementary Table 1. siRNA sequences

PPP3R1(CnB1)	PPP3R1-homo-405	Sense	5'-GCA AGU UAU CCU UUG GAA ATT-3'
		Antisense	5'-UUU CCA AAG GAU AAC UUG CTT-3'
	PPP3R1-homo-594	Sense	5'-CCU UUA GUA CAG CGA GUA ATT-3'
		Antisense	5'-UUA CUC GCU GUA CUA AAG GTT-3'
	PPP3R1-homo-656	Sense	5'-GGA GCA GAA AUU GAG GUU UTT-3'
		Antisense	5'-AAA CCU CAA UUU CUG CUC CTT-3'
PRKAA1（AMPKα1/2）	PRKAA1-homo-1333	Sense	5'-GCG UGU ACG AAG GAA GAA UTT-3'
		Antisense	5'-AUU CUU CCU UCG UAC ACG CTT-3'
	PRKAA1-homo-330	Sense	5'-GAG GAG AGC UAU UUG AUU ATT-3'
		Antisense	5'-UAA UCA AAU AGC UCU CCU CTT-3'
	PRKAA1-homo-1485	Sense	5'-CGG GAU CAG UUA GCA ACU ATT-3'
		Antisense	5'-UAG UUG CUA ACU GAU CCC GTT-3'
	PRKAA2-homo-796	Sense	5'-CCA CUC UCC UGA UGC AUA UTT-3'
		Antisense	5'-AUA UGC AUC AGG AGA GUG GTT-3'
	PRKAA2-homo-1551	Sense	5'-GGC UUA CAC AGA CCA AGA UTT-3'
		Antisense	5'-AUC UUG GUC UGU GUA AGC CTT-3'
	PRKAA2-homo-1032	Sense	5'-GCA GUG GCU UAU CAU CUU ATT-3'
		Antisense	5'-UAA GAU GAU AAG CCA CUG CTT-3'

Supplementary Table 2： Oligos sequences for RT-PCR analysis

Gene	Human	Mouse
CK1	Forward： 5’-GTTCCAGCGTGAGGTTTGTT-3’ Reverse： 5’-TAAGGCTGGGACAAATCGAC-3’	Forward： 5’-GAGCAGATCAAGTCACTCAATGA-3’ Reverse: 5’-CCCATTTGGTTTGTAGCACCT-3’
CK10	Forward： 5’- GAAAAGCATGGGCAACTCACA-3’ Reverse： 5’-TGTCGATCTGAAGCAGGATG-3’	Forward 5’-GCCTCCTACATGGACAAAGTC-3’ Reverse： 5’-GCTTCTCGTACCACTCCTTGA-3’
CK5	Forward: 5’-AAATCAACAAGCGTACCACT-3’ Reverse: 5’-AATCTCATCCATCAGTGCAT-3’	
Loricrin	Forward: 5’-ATGATGCTACCCAGGTTTG-3’ Reverse: 5’-ACTGGGGTTGGGAGGTAGTT-3’	Forward： 5’-CTCCTGTGGGTTGTGGAAAGA-3’ Reverse： 5’-TGGAACCACCTCCATAGGAAC-3’
36B4	Forward： 5’-GCAATGTTGCCAGTGTCTGT-3’ Reverse： 5’-GCCTTGACCTTTTCAGCAAG-3’	Forward： 5’- TGAGATTCGGGATATGCTGTTGG-3’ Reverse： 5’- CGGGTCCTAGACCAGTGTTCT-3’

NOTCH 1	Forward： 5’-TTGGGAGGAGCAGATTTTTG-3’ Reverse： 5’-CACTGGCATGACACACAACA-3’	
p21WAF1/CIP1	Forward： 5’- GATTAGCAGCGGAACAAGGA-3’ Reverse： 5’- CAACTACTCCCAGCCCCATA-3’	
Hey1	Forward： 5’- TCATTTGGAGTGTTGGTGGA-3’ Reverse： 5’- CTCGCACACCATGATCACTT-3’	
HES1	Forward： 5’- GGTGCTGATAACAGCGGAAT-3’ Reverse： 5’- TGAGCAAGTGCTGAGGGTTT-3’	
LEF1	Forward： 5’- TCACACCCGTCACACATCCC-3’ Reverse： 5’- CAGCCAAGAGGTGGGGTGAT-3’	
TCF3	Forward： 5’- CCTGTGGGCACAGACAAGGA-3’ Reverse： 5’- GCCGGTCCTCAAGACCTGAA-3’	
LHX2	Forward： 5’- CTCACCAAGCGGGTCCTC-3’ Reverse： 5’- AGCTCCGAGGCCGGG-3’	
PEX11b	Forward： 5’- CCGTGTGGATCAGGAGAAGT-3’ Reverse： 5’- GCCGGCTACAAGCAGAAGA-3’	