SUPPLEMENTARY INFORMATION

This file (in PDF format) contains Supplementary Figure S1 - S6, Supplementary Table S1 and S2, and Legends for Supplementary Table S3 and S4. Supplementary Table S3 and S4 are listed as separate Microsoft Excel spreadsheet documents.

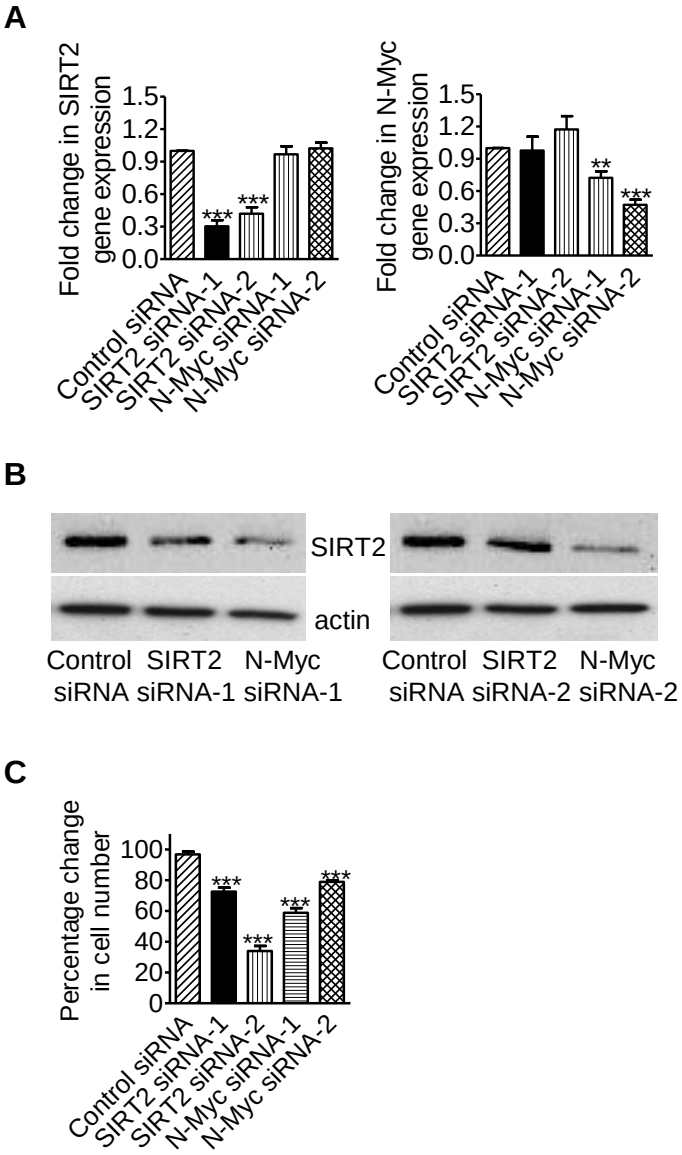

Figure S1. Up-regulation of SIRT2 expression promotes neuroblastoma cell proliferation. (A, B) CHP134 cells were transfected with scrambled control siRNA, N-Myc siRNA-1, N-Myc siRNA-2, SIRT2 siRNA-1 or SIRT2 siRNA-2 for 48 hours, followed by RNA and protein extraction, real-time RT-PCR (A) and/or immunoblot (B) analyses of N-Myc and SIRT2 mRNA and/or protein expression. (C) CHP134 cells were transfected with scrambled control siRNA, N-Myc siRNA-1, N-Myc siRNA-2, SIRT2 siRNA-1 or SIRT2 siRNA-2. Seventy-two hours later, relative cell numbers were examined by the Alamar blue assay, and expressed as percentage change in cell numbers. Error bars represented standard error. ** indicated $P < 0.01$ and *** $P < 0.001$.

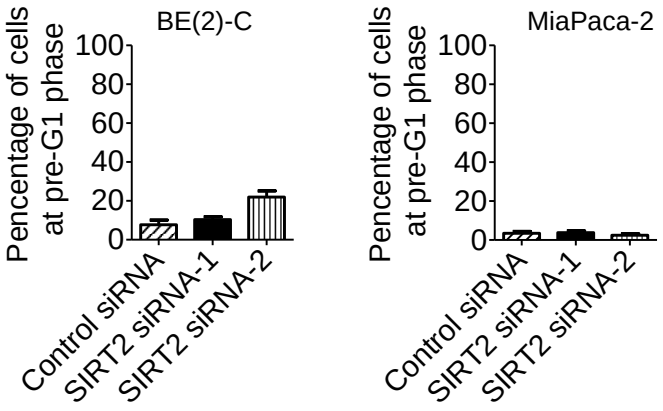

Figure S2. Repression of SIRT2 does not induce apoptosis. BE(2)-C and MiaPaca-2 cells were transfected with scrambled control siRNA, SIRT2 siRNA-1 or SIRT2 siRNA-2. Seventy-two hours after siRNA transfection, cells were stained with propodium iodide reagents, and subjected to flow cytometry analyses of cell cycle. The percentages of dead cells at pre-G1 phase were calculated with CellQuest cell cycle analysis program.

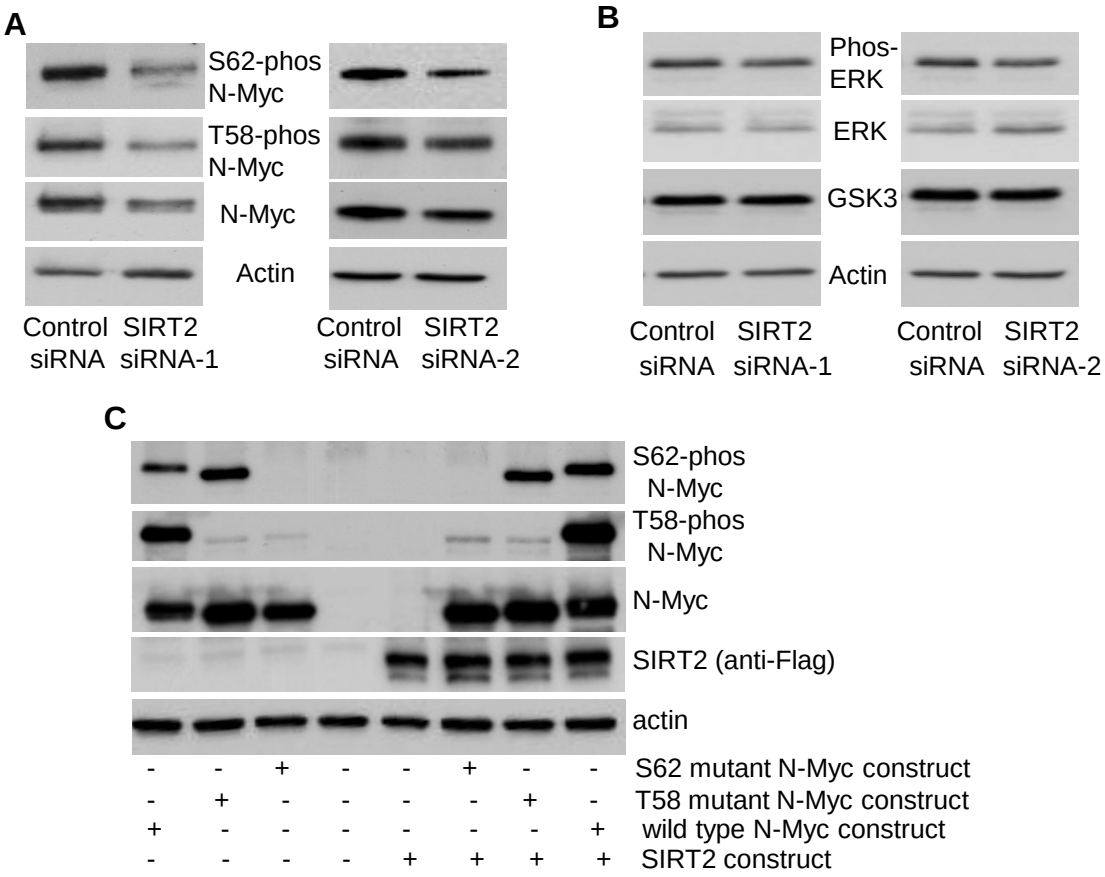

Figure S3. SIRT2 stabilizes N-Myc protein without modulating ERK protein phosphorylation and GSK3 protein expression. (A, B) CHP134 neuroblastoma cells were transfected with scrambled control siRNA, SIRT2 siRNA-1 or SIRT2 siRNA-2, followed by protein extraction. The expression of total N-Myc protein, N-Myc protein phosphorylated at S62 (S62-phos) and N-Myc protein phosphorylated at T58 (T58-phos) (A), GSK3 protein, total ERK protein and phosphorylated ERK protein (phos-ERK) (B) was analysed by immunoblot with specific antibodies. (C) Primary HEK293 human embryonic kidney cells were co-transfected with a construct expressing empty vector or Flag-tagged SIRT2, together with a construct expressing empty vector, S62-mutant (S62A) N-Myc or T58-mutant (T58A) N-Myc. The expression of total, S-62-phos and T58-phos N-Myc protein was analysed by immunoblot with specific antibodies, and the expression of SIRT2 was analysed with an anti-Flag antibody.

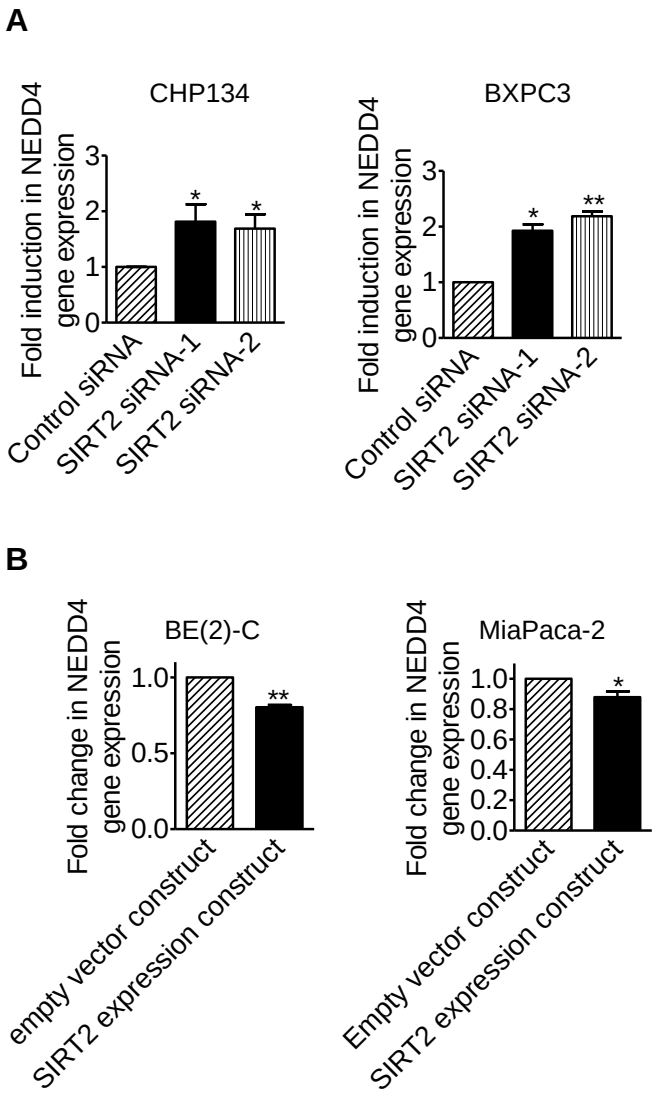

Figure S4. SIRT2 represses NEDD4 gene expression in neuroblastoma and pancreatic cancer cells. (A) CHP134 neuroblastoma and BXPC3 pancreatic cancer cells were transfected with scrambled control siRNA, N-Myc siRNA-1, N-Myc siRNA-2, SIRT2 siRNA-1 or SIRT2 siRNA-2 for 48 hours, followed by RNA extraction and real-time RT-PCR analyses of NEDD4 gene expression. (B) BE(2)-C neuroblastoma and MiaPaca-2 pancreatic cancer cells were transfected with a construct expressing empty vector or SIRT2 for 48 hours, followed by RNA extraction and real-time RT-PCR analyses of NEDD4 gene expression. Error bars represented standard error. * indicated $P < 0.05$ and ** $P < 0.01$.

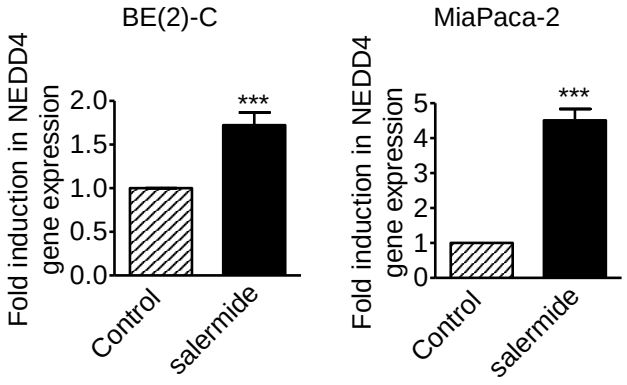

Figure S5. The SIRT1/SIRT2 inhibitor Salermide induces NEDD4 gene expression in neuroblastoma and pancreatic cancer cells. BE(2)-C and MiaPaca-2 cells were treated with vehicle control or Salermide at 50μM, at which Salermide inhibited SIRT2 but not SIRT1, for 48 hours, followed by RNA extraction and real-time RT-PCR analysis of NEDD4 gene expression. Error bars represented standard error. *** indicated $P < 0.001$.

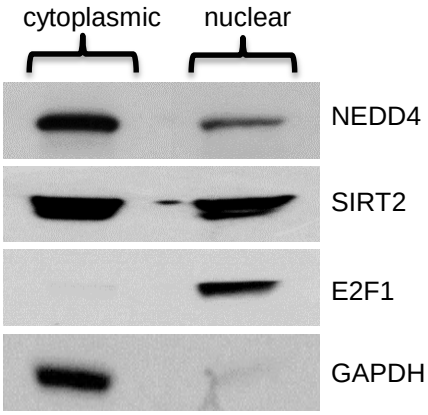

Figure S6. SIRT2 protein and NEDD4 protein are localized in both cytoplasm and nucleus. Cytoplasmic and nuclear protein was extracted from MiaPaca-2 cells, separated and analysed by immunoblot with anti-NEDD4, anti-SIRT2, anti-GAPDH (marker for cytoplasmic protein) and anti-E2F1 (marker for nuclear protein) antibodies.

Table S1. Cell cycle analysis of BE(2)-C neuroblastoma cells 72 hours after transfection with control siRNA, SIRT2 siRNA-1 or SIRT2 siRNA-2. Results of cell cycle distribution were expressed as % ± standard error.

Cell cycle distribution	Pre-G1	G0/G1	S	G2/M
Control siRNA	7.69 ± 2.40	59.79 ± 2.45	17.16 ± 0.98	15.35 ± 1.37
SIRT2 siRNA-1	10.35 ± 1.46	57.02 ± 0.72	12.31 ± 0.91	20.32 ± 0.89
SIRT2 siRNA-2	21.96 ± 3.19	54.99 ± 3.08	11.81 ± 0.74	11.24 ± 1.41

Table S2. Cell cycle analysis of MiaPaca-2 pancreatic cancer cells 72 hours after transfection with control siRNA, SIRT2 siRNA-1 or SIRT2 siRNA-2. Results of cell cycle distribution were expressed as % ± standard error.

Cell cycle distribution	Pre-G1	G0/G1	S	G2/M
Control siRNA	3.42 ± 1.05	38.61 ± 4.55	42.68 ± 6.13	15.27 ± 4.79
SIRT2 siRNA-1	3.76 ± 0.95	42.95 ± 2.41	29.92 ± 3.83	23.38 ± 1.36
SIRT2 siRNA-2	2.42 ± 0.78	53.53 ± 3.20	30.91 ± 3.52	13.16 ± 4.24

Legends for Supplementary Table S3 and S4

Table S3. Genes up-regulated by SIRT2 siRNA-1 by more than 2 fold, as identified by Affymetrix gene array analysis, in neuroblastoma BE(2)-C cells 30 hours after transfection with SIRT2 siRNA-1 or control siRNA.

Table S4. Genes down-regulated by SIRT2 siRNA-1 by more than 2 fold, as identified by Affymetrix gene array analysis, in neuroblastoma BE(2)-C cells 30 hours after transfection with SIRT2 siRNA-1 or control siRNA.