Supplementary information

Nuclear factor erythroid 2-related factor 2 enhances carcinogenesis by suppressing apoptosis and promoting autophagy in nickel-transformed cells

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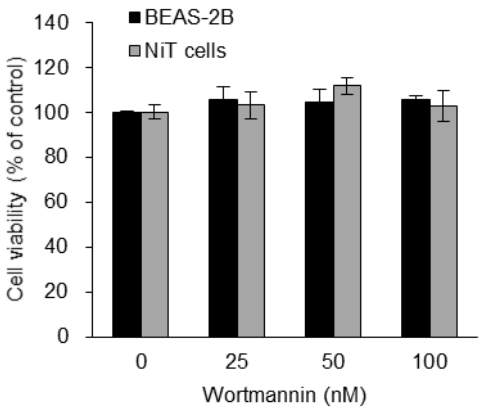
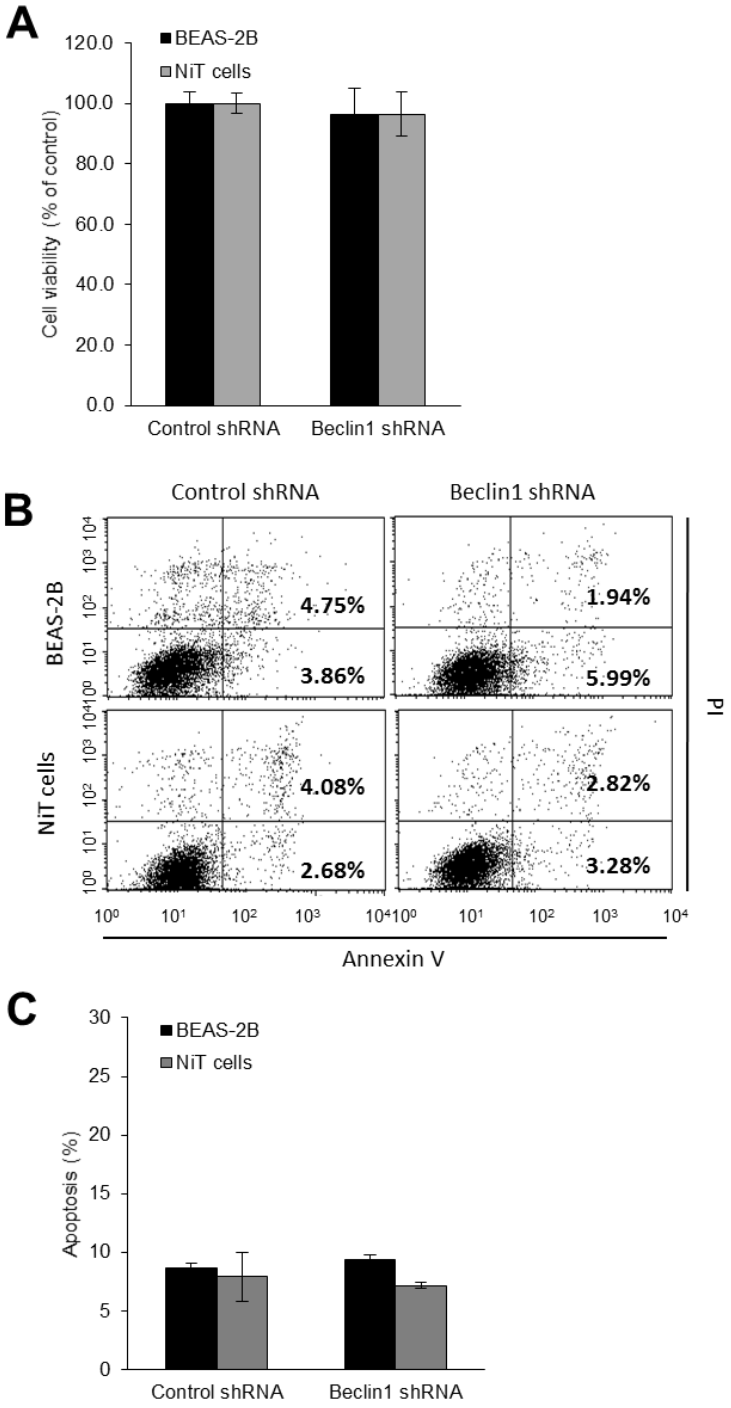
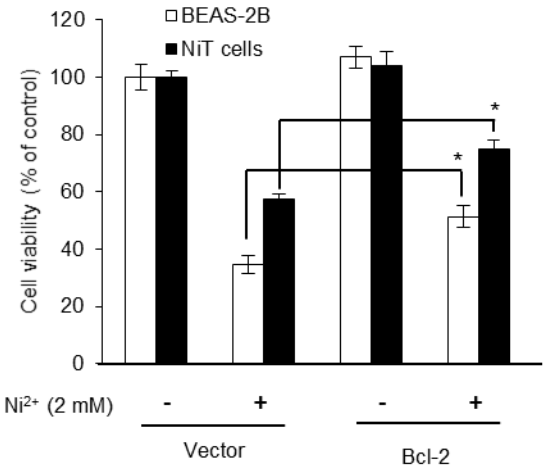
Supplementary Figures 1–4



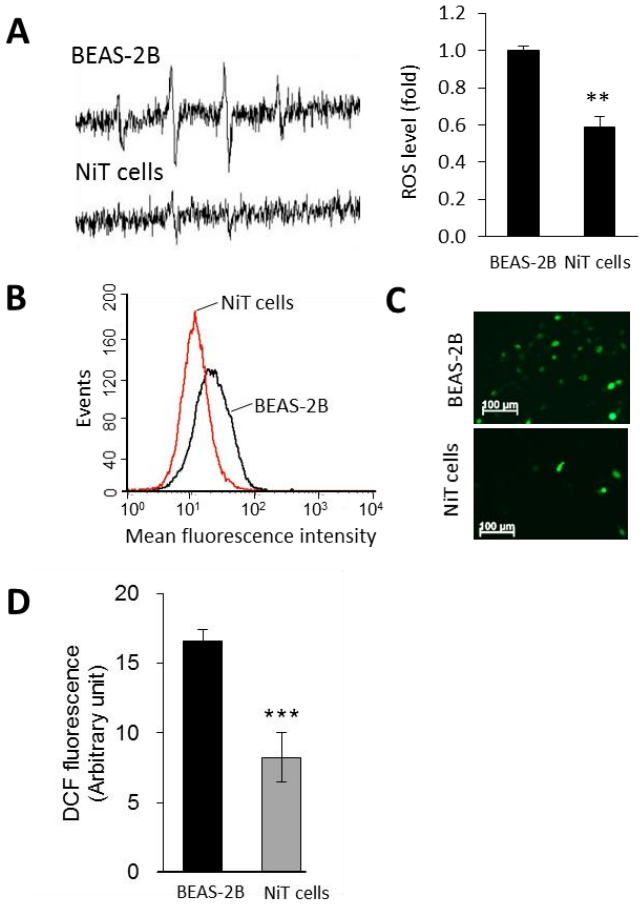
Supplementary Figure 1. The effect of wortmannin on survival in normal BEAS-2B and NiT cells. BEAS-2B and NiT cells (0.2×10^4) were seeded into a 96-well plate. After overnight incubation, these cells were incubated with Wortmannin (0 - 100 nM) for 24 h. Cell viability was assessed by MTT assay.



Supplementary Figure 2. The effect of shRNA Beclin1 on survival in normal BEAS-2B and NiT cells. BEAS-2B and NiT cells were transfected with control or Beclin-1 shRNA (500 nM) overnight, and cell viability (**A**) and apoptotic cells (**B**) were evaluated by MTT assay and annexin V/PI staining followed by flow cytometry, respectively. (**C**) Quantification of the apoptosis assay is presented.



Supplementary Figure 3. Bcl-2 overexpression protects Ni^{2+}-induced cell death in BEAS-2B and NiT cells. BEAS-2B and NiT cells were transfected with control or Bcl-2 vector overnight. The next day cells were exposed to Ni^{2+} (2 mM) for 24 h. Cell viability was evaluated by MTT assay.



Supplementary Figure 4. NiT cells have lower ROS levels than normal BEAS-2B cells. To measure basal ROS levels, cell suspensions were prepared from BEAS-2B and NiT cells, and ESR spectra were recorded. The generation of a 1:2:2:1 quartet ESR signal and the signal intensity of DMPO-OH was demonstrated (**A**). ROS levels in BEAS-2B and NiT cells were also examined by flow cytometry (**B**), fluorescence microscopy (**C**), and fluorescence spectroscopy (**D**) after staining with CM-H_2DCFDA (5 μM) for 30 min. The ESR spectrometer settings were as follows: frequency, 9.8 GHz; power, 39.91 mW; modulation frequency, 100 kHz; receiver gain, 5.02×10^5; time constant, 40.96 ms; modulation amplitude, 1.00 G; scan time, 60 s; and magnetic field, 3,451 ± 100 G. All spectra shown are an accumulation of ten scans. Photomicrographs are representative images of each experimental design. Presented results are the mean ± SE of three separate experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences determined by ANOVA and Scheffe's test.