Figure S1. The effect of NTZ on relative viability of normal cells. Relative cell viability of (A) HEMa and (B) NIH-3T3 cells respectively. Cells were treated with different concentrations of NTZ (μM) and DMSO control for 48 h, and relative cell viability was determined with an SRB assay. Results are presented as mean ± SD from three independent experiments. One-way ANOVA test followed by Dunnett's post-hoc test used to determine the statistical significance. ns, not significant; NTZ, nitazoxanide; HEMa, human epidermal melanocytes.

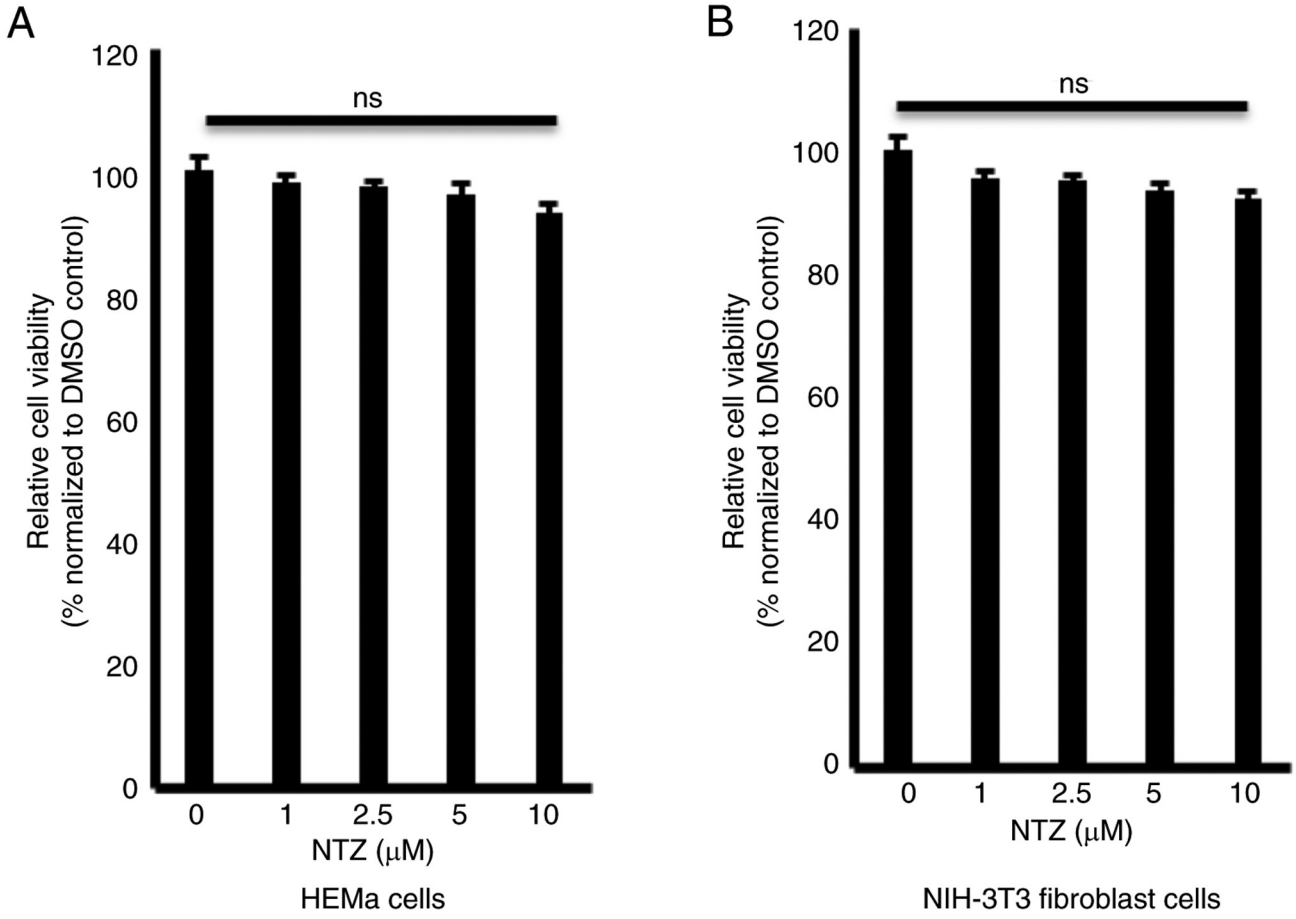


Figure S2. NTZ reduces the migration of human uveal melanoma cells. (A) Representative images showing the differences of wound closure between the DMSO vehicle group and NTZ-treated group at 0, 24 and 48 h timepoints. (B) Quantification of the wound closure percentage showing the differences between the DMSO control group and NTZ treated group at five different areas of the wound at different times points. Two-way ANOVA followed by Šídák's test was used to determine the statistical significance (*P<0.01, **P<0.01, ***P<0.001). Data are presented as mean ± SD. The data show a representative result from three independent experiments. NTZ, nitazoxanide.

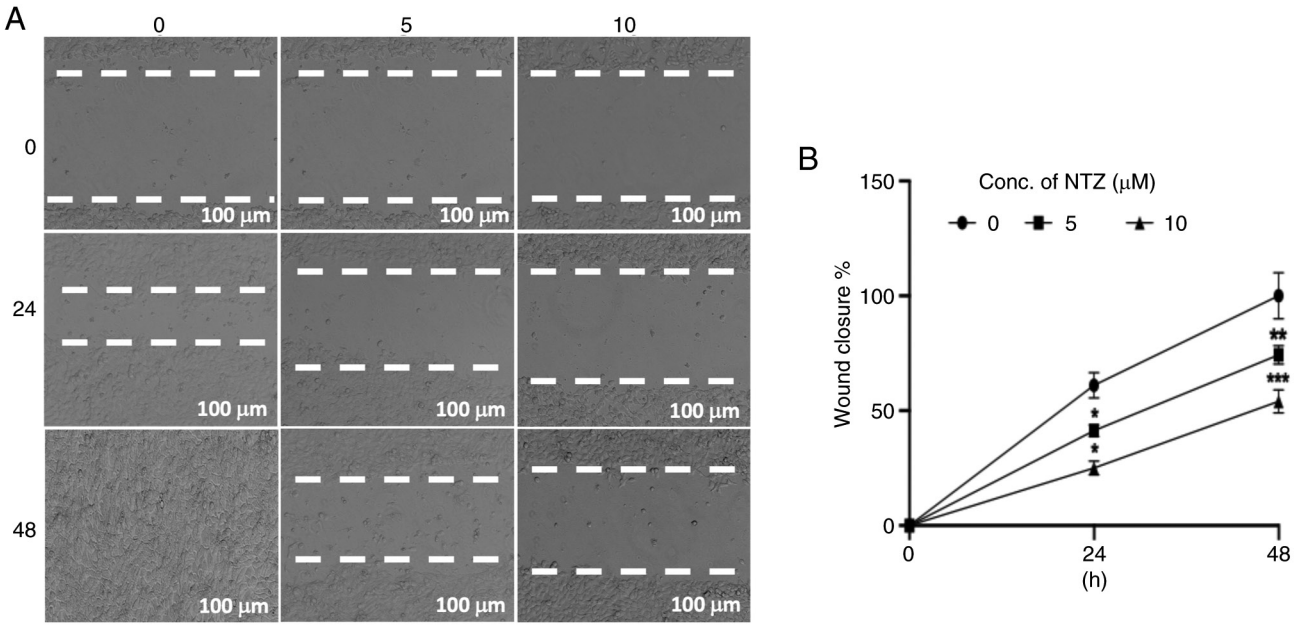


Figure S3. NTZ reduces hepatic metastasis of uveal melanoma cells. Quantification of (A) body weight and (B) liver weight in NOD mice with/without NTZ (200 mg/kg/body weight). Data are presented as mean ± SD from duplicate separate experiments. Student's t-test was used to detect the statistical significance. ns, not significant; NTZ, nitazoxanide.

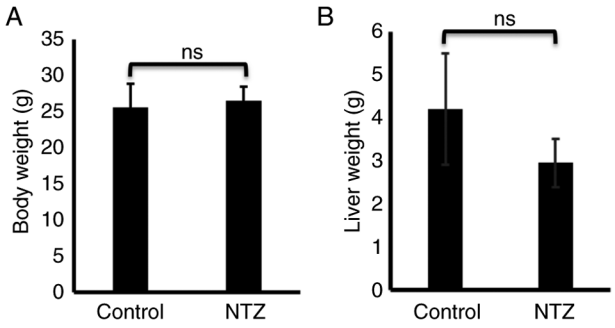


Figure S4. Toxicity study of NTZ. Quantification of plasma levels (U/l) of (A) ALT, (B) AST and (C) ALP in the control group and NTZ-treated group. (D) Quantification of body weight (g) in the control group and NTZ-treated group. Representative liver tissue sections of H&E stain showing the liver histological structure of (E) control and (F) NTZ-treated mice. Data are presented as means ± SD. Student's t-test was used to detect the statistical significance. Data obtained from two independent experiments. NTZ, nitazoxanide; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; ns, not significant.

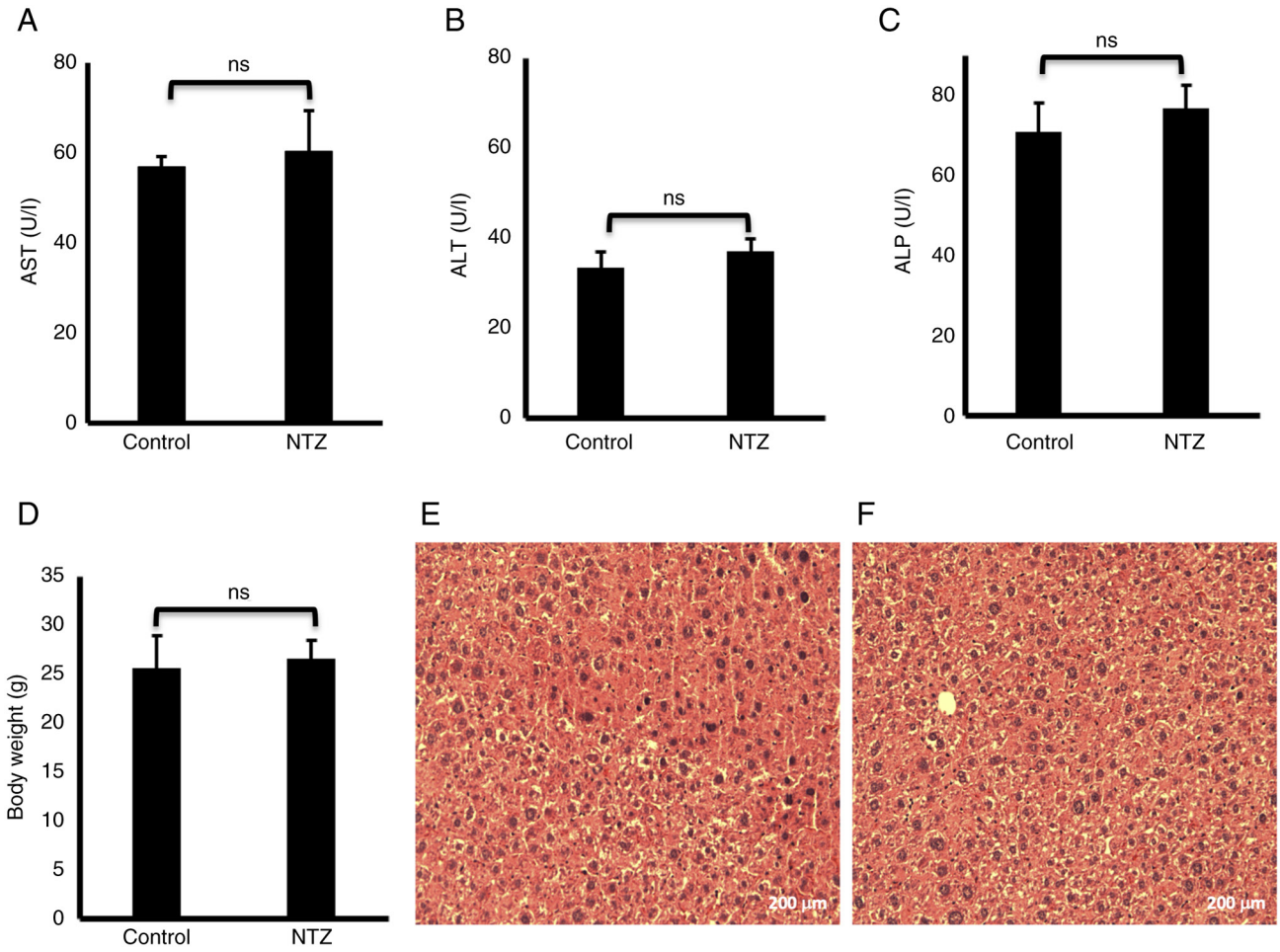


Figure S5. NTZ activates AMPK and reduces the activity of the mTOR and ACC pathways in uveal melanoma cells. Quantification of western blot analyses of (A) p-ACC/ACC ratio, (B) p-p70S6K/p70S6K ratio, (C) p-ERK/ERK ratio, (D) p-AMPK/AMPK, (E) p-ACC/Actin ratio and (F) p-AMPK/Actin in Mel270 cells. Quantification of western blot analyses of (G) p-AMPK/AMPK ratio, (H) p-ACC/ACC ratio, (I) p-AMPK/Actin and (J) p-p70S6K/p70S6K ratio in Mel290 cells. The cells were treated for 4 h with DMSO or different concentrations of NTZ. Data are presented as mean ± SD from two separate experiments. One-way ANOVA test followed by Dunnett's post-hoc test used to determine the statistical significance ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). NTZ, nitazoxanide; p, phosphorylated.

