Figure S1. Correlation of Akt status with phosphorylation of Cables1 at pT44 and T150 in an A549 tumor xenograft mouse model. Tumor tissue lysates (10 µg) from mice that received various treatment as indicated were applied to SDS-PAGE (12.5%) for separation. Separated proteins were transferred to PVDF membrane for Western blotting with indicated antibodies.

The working model, as proposed in Figure 7, suggests that Cables1 tumor suppressor activity is antagonized by oncogenic kinases, such as Akt, through phosphorylation of Cables1 at T44 and T150. To test this model, we examined whether Akt status was correlated with Cables1 phosphorylation at these two sites in vivo using a lung cancer A549 xenograft mouse model (2). A mTOR kinase inhibitor, INK128, was used to reduce Akt activity through its inhibition of
TORC2 activity (3). SB216763, a GSK3beta inhibitor, has previously shown to inhibit INK128 effects and was shown to modulate Akt activity (2). Treatment of A549 xenograft animals with INK128 (1 mg/kg, orally) effectively inhibited the growth of the xenograft, reducing both tumor size and tumor weight. The addition of SB216763 attenuated the tumor inhibition effect of INK128. To evaluate the molecular events in these experiments, tumor lysates were prepared and used to blot for the status of Akt and Cables1. As shown in Figure S1 (next page), lysates from tumors treated with vehicle exhibited relatively high Akt phosphorylation at T473, a TORC2 phosphorylation site, and phosphorylated Cables1 at T44 and T150 Conversely, tumors treated with INK128 showed reduced phosphorylation of Akt at T473, which correlated with decreased phosphorylation of Cables1 at T44 and T150. When tumors were treated with both INK128 and SB216763, both the Akt phosphorylation level and the Cables1 phosphorylation level were reversed. To quantify these results, the band intensities of phosphorylated samples were normalized to the band intensities of total protein (e.g. S = Cables1-pT44-unreated-1/Cables-pan-untreated-1). The normalized values were used to calculate the statistical significance of the correlation between the phosphorylation level of Akt and Cables1 in a collective manner. The calculations were performed in MatLab using the corrcoef command. The following values were obtained (R: correlation coefficient):

pAKT/pT44: R = 0.717, p = 0.009;
pAKT/pT150: R = 0.832, p = 0.001

These data strongly support the statistically significant correlation between phosphorylated Akt at T473 and Cables1 at T44 and pT150, supporting our proposed working model in Figure 7.