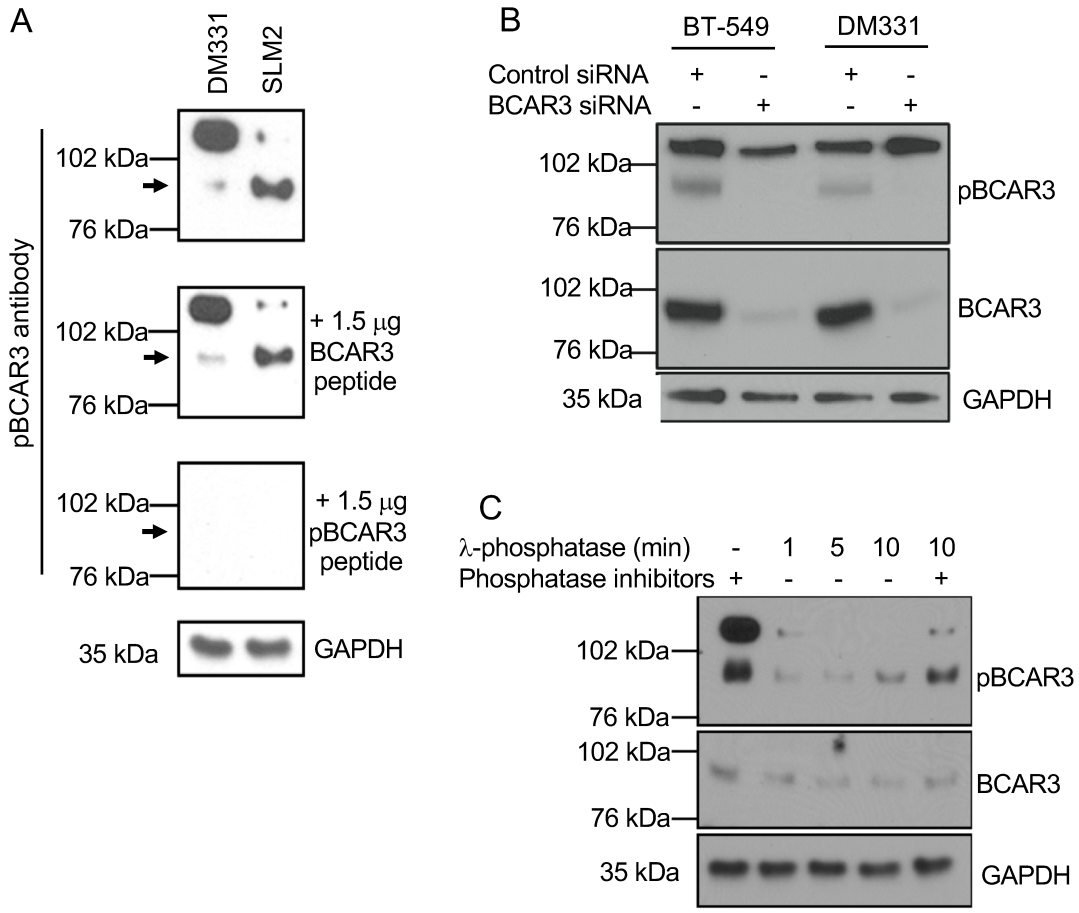

Supplemental Figure 1. Specificity of anti-phosphoT130-BCAR3 antibody. (**A**) Detection of whole protein by an affinity purified antibody raised against an epitope spanning amino acids 123 – 135 of BCAR3 and phosphorylated at T130 (phosphoT_{130}-BCAR3 antibody) was assessed by Western blot. Excess amounts of the unphosphorylated peptide or the immunizing phosphopeptide were added during overnight incubation to compete for antibody binding. (**B**) Protein lysates from cells in which BCAR3 had been knocked down by siRNA for 48hrs were probed with the phosphoT_{130}-BCAR3 antibody and a BCAR3 antibody. GAPDH expression served as a loading control. Results are representative of two independent experiments. (C) Verification that the phospho-BCAR3 antibody detects phosphorylated proteins was carried out using protein lysates from DM331 were incubated with purified λ-phosphatase over several minutes. GAPDH expression served as a loading control. Results are representative of 2 independent experiments.

Supplemental Methods

siRNA knockdown of BCAR3. 600pmol of small interfering RNA oligonucleotide against human BCAR3 (AAAUCAACCGGACAGUUCU, Dharmacon) or silencer negative control #1 siRNA (cat# AM4611, Ambion) was diluted in 2ml of Opti-MEM I media (cat# 31985-062, Invitrogen) in 100mm culture dishes. 35μl of Lipofectamine RNAiMAX (cat# 13778, Invitrogen) was added to the culture dish and mixed gently. The culture dish was incubated at room temperature for 20 min. Breast cancer cell line BT-549 or melanoma cell line DM331 cells (3 – 5 x 10^5) were resuspended in 10ml of growth media with antibiotics, added to the culture dish, and incubated at 37°C for 48 h. The cells were then harvested for Western analysis.