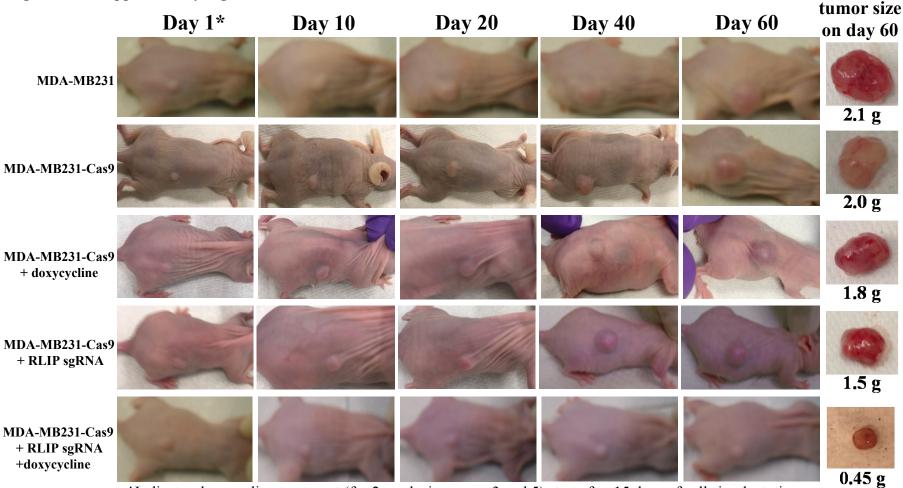
Singhal et al., Supplementary Figure



*Indicates doxycycline treatment (for 2 weeks in groups 3 and 5) start after 15 days of cells implantation.

Effect of CRISPR/Cas9 dependent sgRNA-mediated RLIP knockdown on the size of subcutaneously implanted human breast cancer cells (MDA-MB231) in nude mice

Hsd: Athymic female nude nu/nu mice were obtained from Charles River, Wilmington, MA. All animal experiments were carried out in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). Thirty 10-weeks-old mice were divided into five groups: 1) MDA-MB231, 2) MDA-MB231-Cas9 sgRNA, 3) MDA-MB231-Cas9 sgRNA + doxycycline, 4) MDA-MB231-Cas9 + RLIP sgRNA, and 5) MDA-MB231-Cas9 + RLIP sgRNA +doxycycline; (5 mice in groups # 1-4; and 10 mice in group # 5). Respective cells were suspended in PBS and mixed in a 1:1 ratio with Matrigel. All 30 animals were injected with respective 2 x 10⁶ cells subcutaneously into one flank of each nu/nu nude mouse in respective groups. Animals were examined daily for signs of tumor growth. When tumors reached a cross-sectional area of ~ 30 mm² (15 days later), doxycycline (2 mg/ml in drinking water containing 1% sucrose) treatment was started in groups 3 and 5 for two weeks as indicated in the figure. Tumors were measured in two dimensions using calipers. Photographs of animals were taken at day 1, day 10, day 20, day 40 and day 60 are shown for all groups. Photographs of tumor were also taken at day 60.