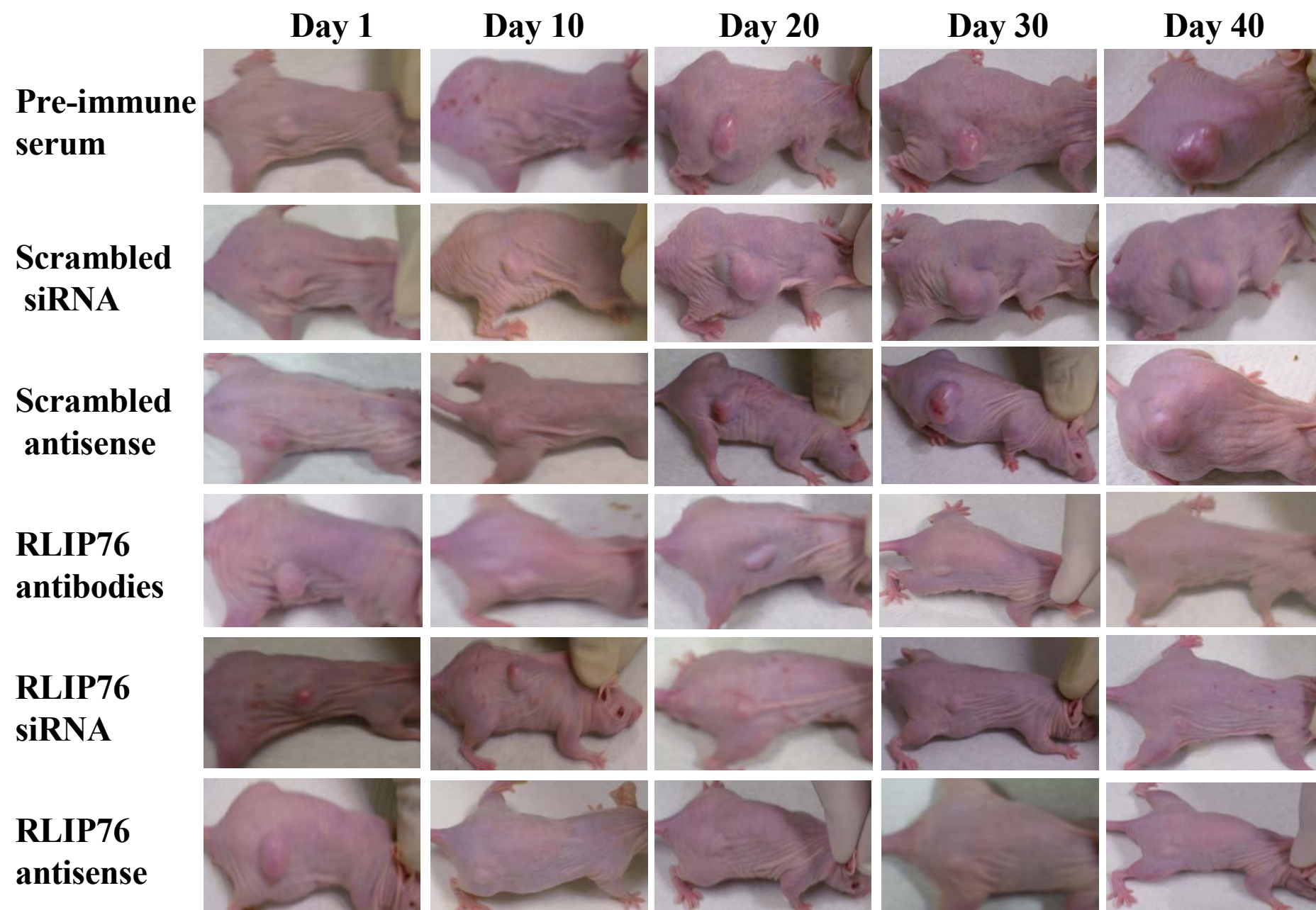


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Singhal et al., Supplementary figure legends

Figure Effect of anti-RLIP76 IgG, RLIP76 siRNA and RLIP76 antisense on the size of subcutaneously implanted human prostate cancer cells (PC-3) in nude mice

Human prostate cancer cells (PC-3) were purchased from American Type Culture Collection, Manassas, VA, and cultured at 37 °C in a humidified atmosphere of 5 % CO₂ in Ham's F12K medium supplemented with 10 % (v/v) heat-inactivated FBS, 1% (v/v) P/S solution, 2 mM L-glutamine, 10 mM HEPES, 1mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate.

Hsd: Athymic nude nu/nu mice were obtained from Harlan, Indianapolis, IN. All animal experiments were carried out in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). Twenty-four 12-weeks-old male mice were divided into six groups of 4 animals (treated with pre-immune serum, scrambled siRNA, scrambled anti-sense DNA, RLIP76 antibodies, RLIP76 siRNA and RLIP76 antisense). All 24 animals were injected with 2×10^6 human prostate cancer cells (PC-3) suspensions in 100 μ l of PBS, subcutaneously into one flank of each nu/nu nude mouse. Animals were examined daily for signs of tumor growth. When tumors reached a cross-sectional area of $\sim 42 \text{ mm}^2$ (27 days later), animals were randomized treatment groups as indicated in the figure. Treatment consisted of 200 μ g of RLIP76 antibodies, siRNA or antisense in 100 μ l PBS. Control groups were treated with 200 μ g/100 μ l pre-immune serum, scrambled siRNA or scrambled anti-sense DNA. Tumors were measured in two dimensions by calipers using Study Director Software V1.6, from Studylog System, San Francisco, CA. We have obtained the software that automates this analysis. Photographs of animals were taken at day 1, day 10, day 20, day 30, and day 40 after treatment are shown for all groups.