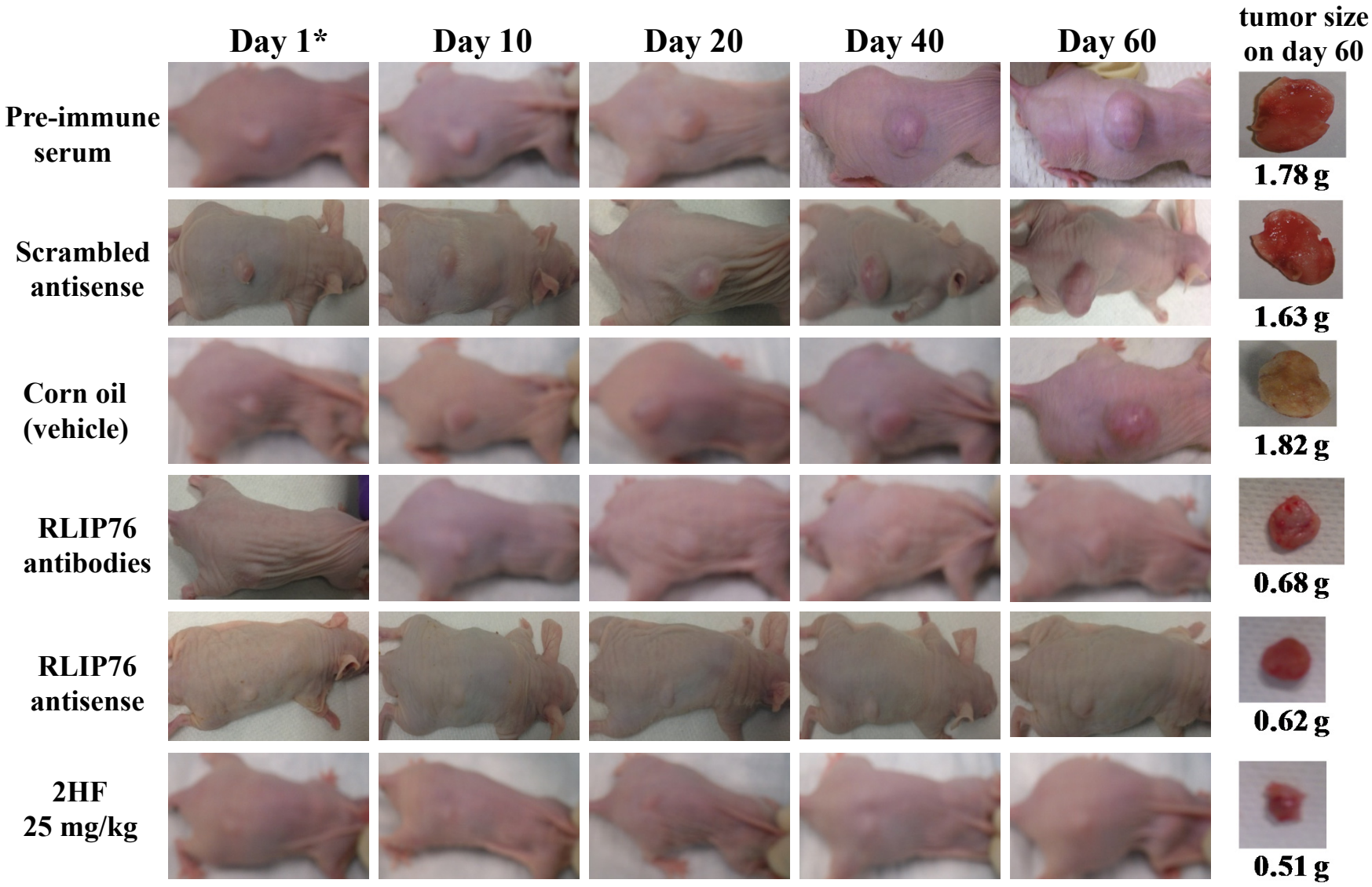
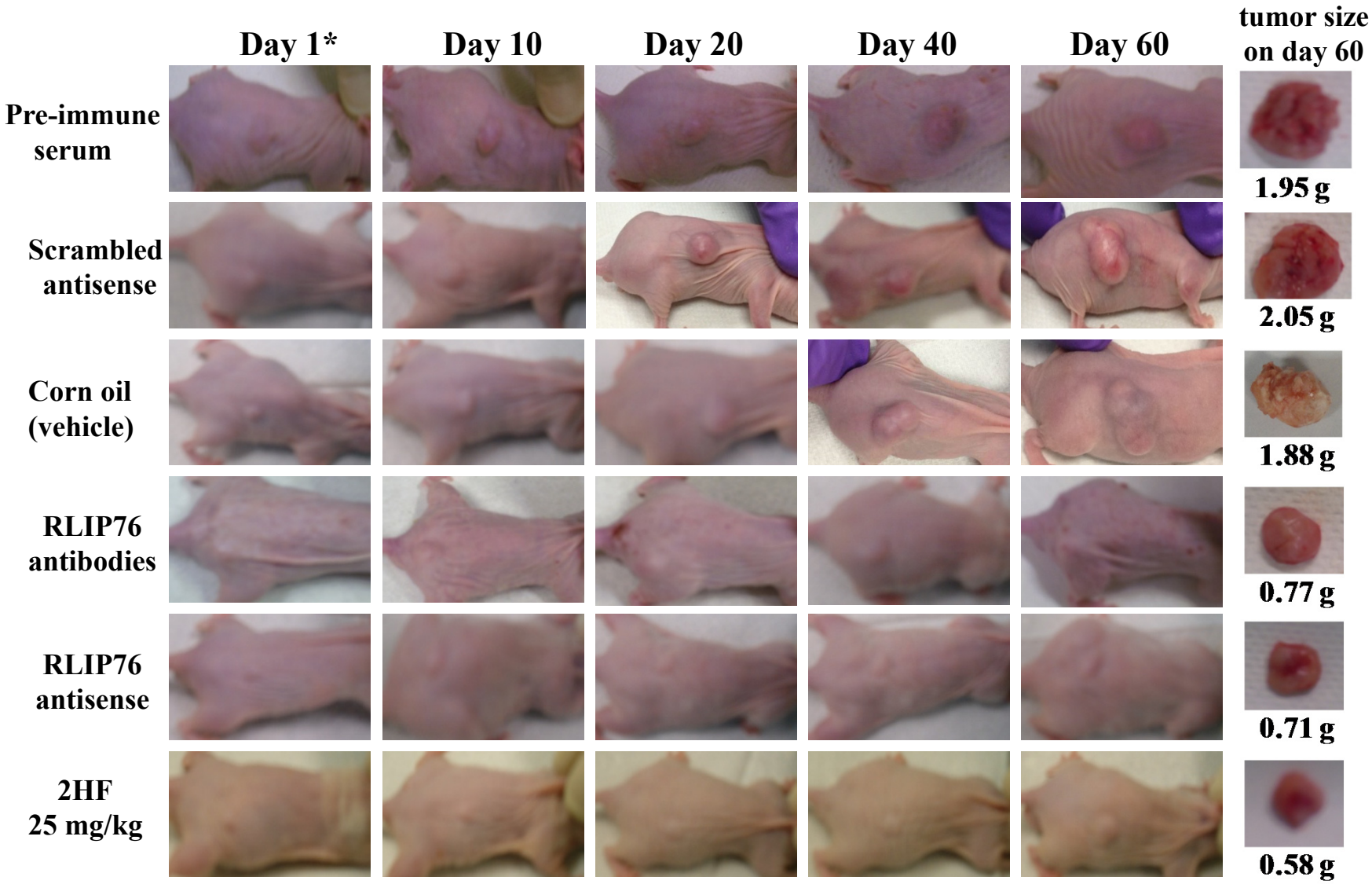
Singhal J *et al*., Supplementary Figure 1



*Indicates treatment start after 25 days of MCF7 cells implantation.

Supplementary Figure 1 Effect of anti-RLIP76 IgG, RLIP76 antisense, and 2'-hydroxyflavanone (2HF) on the size of subcutaneously implanted human breast cancer cells (MCF7) 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 six groups of 5 animals (treatment with pre-immune serum, scrambled antisense, corn oil, RLIP76 antibodies, RLIP76 antisense, and 2HF). In the MCF7 xenografts model, a pellet containing 0.72 mg of 17β-estradiol (90 days release, Innovative Research of America, Sarasota, FL) was implanted *s.c.* into the shoulder area of mice 5 days before tumor cell injection. After five days, all 30 animals were injected with 2 x 10^6 MCF7 cells suspensions in 100 µl 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 45 $\pm$ 4.3 mm^2 (25 days later), animals were randomized treatment groups as indicated in the figure. Treatment consisted of 5 mg/kg b.w. of either RLIP76 antibodies or antisense in 100 µl PBS once a week for 8 weeks intraperitoneally; and 25 mg/kg b.w. of 2HF in 100 µl corn oil by oral gavage alternate day for 8 weeks. Control groups were treated with100 µl of either pre-immune serum, scrambled antisense, or 100 µl corn oil. 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 after treatment are shown for all groups. Photographs of tumor were also taken at day 60 after treatment.

Singhal J *et al*., Supplementary Figure 2



*Indicates treatment start after 18 days of SKBR3 cells implantation.

Supplementary Figure 2 Effect of anti-RLIP76 IgG, RLIP76 antisense, and 2'-hydroxyflavanone (2HF) on the size of subcutaneously implanted human breast cancer cells (SKBR3) 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 six groups of 5 animals (treatment with pre-immune serum, scrambled antisense, corn oil, RLIP76 antibodies, RLIP76 antisense, and 2HF). SKBR3 cells were suspended in PBS and mixed in a 1:1 ratio with Matrigel. All 30 animals were injected with 2 x 10^6 SKBR3 cells 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 42 $\pm$ 5.6 mm^2 (18 days later), animals were randomized treatment groups as indicated in the figure. Treatment consisted of 5 mg/kg b.w. of either RLIP76 antibodies or antisense in 100 µl PBS once a week for 8 weeks intraperitoneally; and 25 mg/kg b.w. of 2HF in 100 µl corn oil by oral gavage alternate day for 8 weeks. Control groups were treated with100 µl of either pre-immune serum, scrambled antisense, or 100 µl corn oil. 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 after treatment are shown for all groups. Photographs of tumor were also taken at day 60 after treatment.

Supplementary Table 1 **Regulation of blood and serum chemistry parameters following 2HF and RLIP76 antisense treatment**

Blood/Serum Profile	Control-corn oil	Treatment		
		2HF	Scrambled antisense	RLIP76 antisense
BLOOD				
RBCs (10^6/μL)	8.6 ± 1.2*	8.9 ± 1.1	8.4 ± 1.3	7.7 ± 1.0
WBCs (10^3/μL)	9.1 ± 1.4	8.3 ± 1.2	8.6 ± 1.1	7.4 ± 1.1
Platelets (10^3/μL)	1014 ± 122	905 ± 113	728 ± 94	608 ± 91
Hemoglobin (g/dL)	13.2 ± 0.9	12.4 ± 1.1	11.8 ± 1.2	9.9 ± 0.8
Hematocrit (%)	41.2 ± 5.2	43.5 ± 3.6	44.4 ± 3.2	39.7 ± 4.8
SERUM				
Cholesterol (mg/dL)	96 ± 6	80 ± 4	89 ± 5	66 ± 6
Triglycerides (mg/dL)	91 ± 12	79 ± 7	109 ± 12	78 ± 8
Glucose (mg/dL)	197 ± 18	158 ± 12	165 ± 17	119 ± 14
Creatinine (mg/dL)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
SGPT (ALT) (U/L)	69 ± 5	61 ± 8	58 ± 3	71 ± 3
SGOT (AST) (U/L)	98 ± 6	91 ± 4	111 ± 5	139 ± 11
LDH (U/L)	1108 ± 142	921 ± 121	1104 ± 154	1288 ± 137
Alkaline phosphatase (U/L)	111 ± 12	104 ± 12	167 ± 15	135 ± 11
Albumin (g/dL)	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
Bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

*n = 3 mice/group