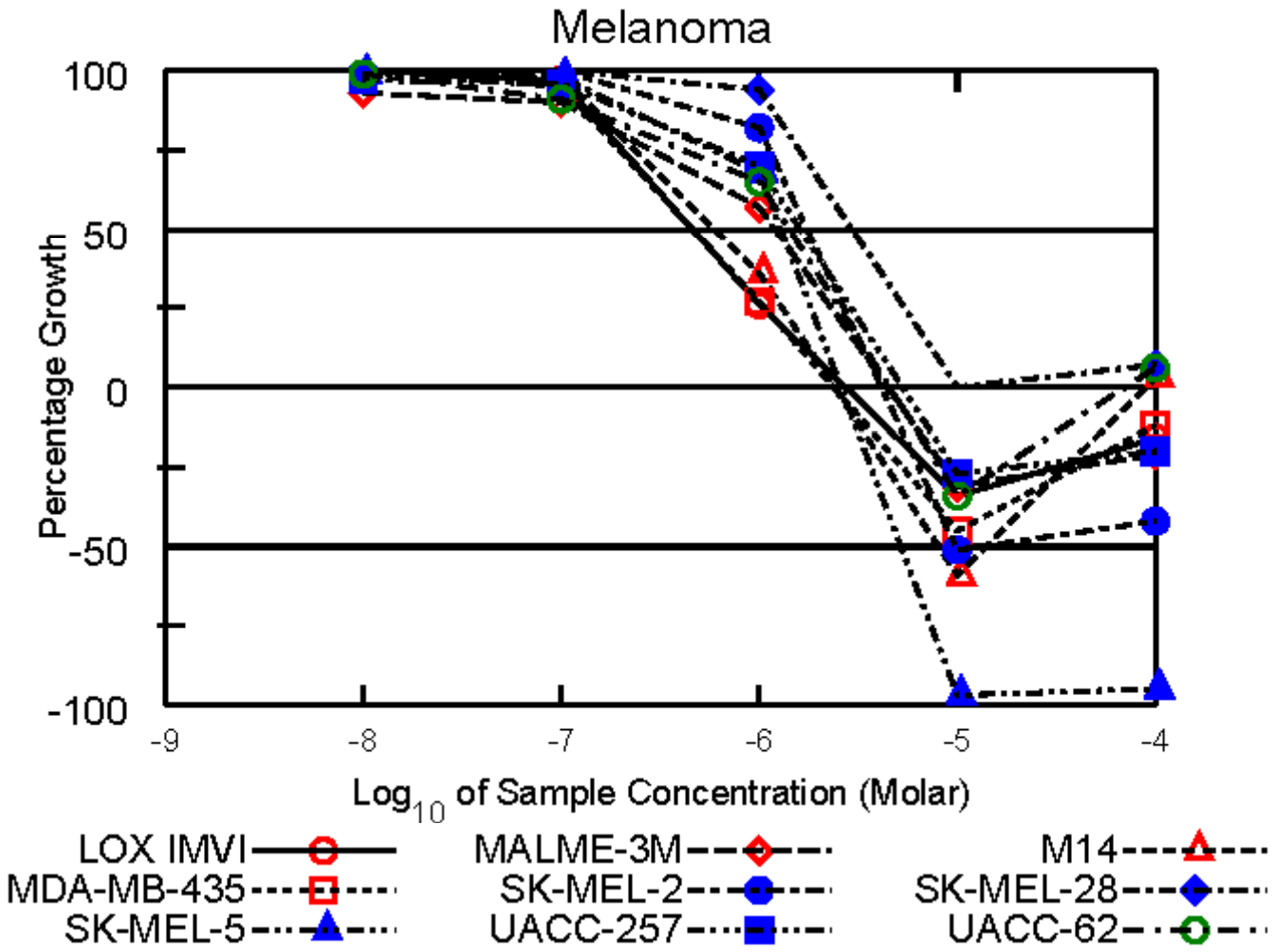
Singhal et al., Supplementary Figure 1

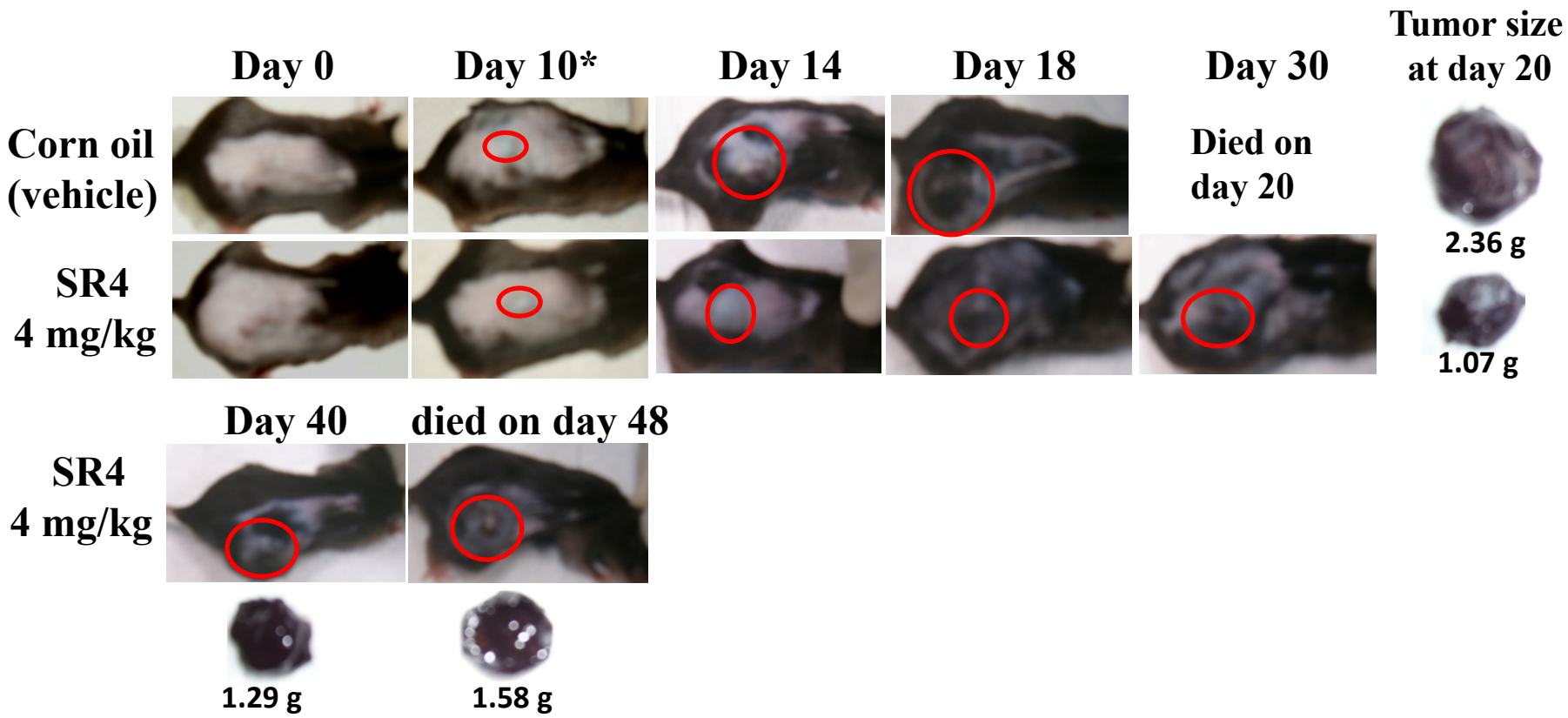


Singhal et al., Supplementary Figure 1 Legend

Dose-dependent growth inhibition of various melanoma cell lines by COH-SR4 (NIH/NCI DTP60 screening data).

Singhal et al., Supplementary Figure 2

B16-F0 syngeneic mouse melanoma model



Tumor size

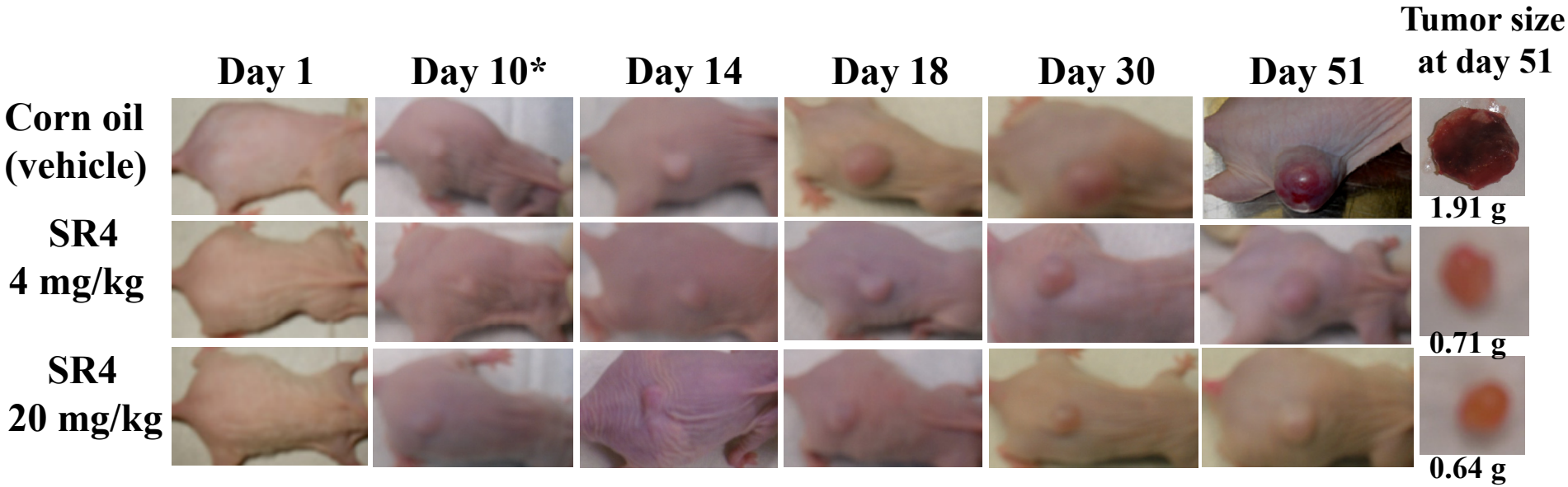
*Indicates SR4 treatment start alternate day by oral gavage after 10 days of B16-F0 cells implantation.

Singhal et al., Supplementary Figure 2 Legend

Effect of SR4 on B16-F0 melanoma mice model C57B mice (for syngeneic model) 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). Ten 10-weeks-old C57B mice were divided into two groups of 5 animals (treated with corn oil (vehicle), and SR4 compound 4 mg / kg b.w.). All 10 animals were injected with 2 x 10^6 B16-F0 melanoma cells suspensions in 100 µl of PBS, subcutaneously into one flank of each C57B mouse. At the same time, animals were randomized treatment groups as indicated in the figure. Treatment was started 10 days after the B16-F0 cells implantation to see palpable tumor growth. Treatment consisted of 0.1 mg of SR4/mice in 200 µl corn oil by oral gavage alternate day. Control groups were treated with 200 µl corn oil by oral gavage alternate day. Animals were examined daily for signs of tumor growth. Tumors were measured in two dimensions using calipers. Photographs of animals were taken at day 1, day 10, day 14, day 18, day 20, day 30, day 40 ,and day 48 after subcutaneous injection, are shown for all groups. Photographs of tumors were also taken at day 20.

Singhal et al., Supplementary Figure 3

A2058 human melanoma xenograft model



*Indicates SR4 treatment start alternate day by oral gavage after 10 days of A2058 cells implantation.

Singhal et al., Supplementary Figure 3 Legend

Effect of SR4 on A2058 human melanoma nude mice model Hsd: Athymic nude nu/nu mice (for xenografts model),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). Ten 10-weeks-old nude mice were divided into two groups of 5 animals (treated with corn oil (vehicle), and SR4 compound 4 mg / kg b.w.). All 10 animals were injected with 2 x 10^6 A2058 human melanoma cells suspensions in 100 µl of PBS, subcutaneously into one flank of each nu/nu nude mouse. At the same time, animals were randomized treatment groups as indicated in the figure. Treatment was started 10 days after the A2058 cells implantation to see palpable tumor growth. Treatment consisted of 0.1 mg of SR4/mice in 200 µl corn oil by oral gavage alternate day. Control groups were treated with 200 µl corn oil by oral gavage alternate day. Animals were examined daily for signs of tumor growth. Tumors were measured in two dimensions using calipers. Photographs of animals were taken at day 1, day 10, day 14, day 18, day 20, day 30, and day 51 after subcutaneous injection, are shown for all groups. Photographs of tumors were also taken at day 51. In parallel studies, we also used 20 mg / kg b.w. SR4 to see better regression and animal toxicity.

Supplementary Table 1 Effects of SR4 on some key metabolic parameters on C57B mice (mean ± S.E.

	Vehicle Control	COH-SR4 treated	*P*-value
CBC			
RBC (x 10^6/uL)	8.8 ± 0.1	8.7 ± 0.2	0.675
WBC (x 10^3/uL)	9.1 ± 0.3	7.4 ± 0.9	0.219
Platelets (x 10^3/uL)	1207 ± 71	1055 ± 124	0.350
Hemoglobin (g/dL)	14.0 ± 0.1	13.9 ± 0.4	0.818
Hematocrit (%)	41.2 ± 0.1	41.0 ± 0.7	0.737
Plasma/Serum			
Glucose (mg/dL)	336.3 ± 14.8	304.2 ± 21.1	0.249
Creatinine (mg/dL)	0.2 ± 0.0	0.2 ± 0.0	1.000
Albumin (g/dL)	2.4 ± 0.1	2.4 ± 0.1	1.000
ALT (units/L)	63.3 ± 2.4	73.3 ± 2.4	*0.042*
AST (units/L)	54.0 ± 1.2	48.0 ± 3.0	0.140
ALP (units/L)	81.3 ± 4.1	119.3 ± 2.4	*0.001*
LDH (units/L)	943.3 ± 138.0	822.7 ± 56.0	0.463
Triglycerides (mg/dL)	94.6 ± 1.3	78.7 ± 2.4	0.004
Cholesterol (mg/dL)	92.0 ± 3.5	82.0 ± 0.1	0.114

n = 6 mice in each group