

OMTO, Volume 8

Supplemental Information

A Retroviral Replicating Vector Encoding

Cytosine Deaminase and 5-FC Induces Immune

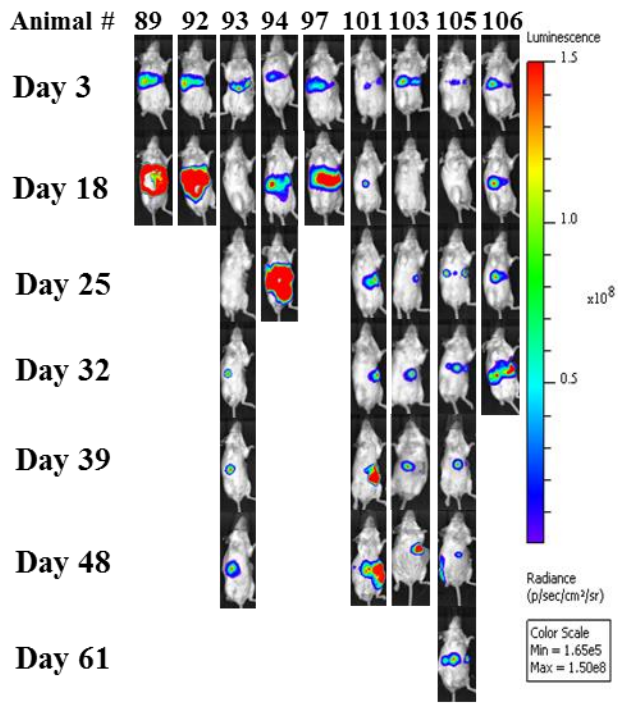
Memory in Metastatic Colorectal Cancer Models

Kader Yagiz, Maria E. Rodriguez-Aguirre, Fernando Lopez Espinoza, Tiffany T. Montellano, Daniel Mendoza, Leah A. Mitchell, Carlos E. Ibanez, Noriyuki Kasahara, Harry E. Gruber, Douglas J. Jolly, and Joan M. Robbins

SUPPLEMENTARY FIGURES AND LEGENDS:

Figure S1

A



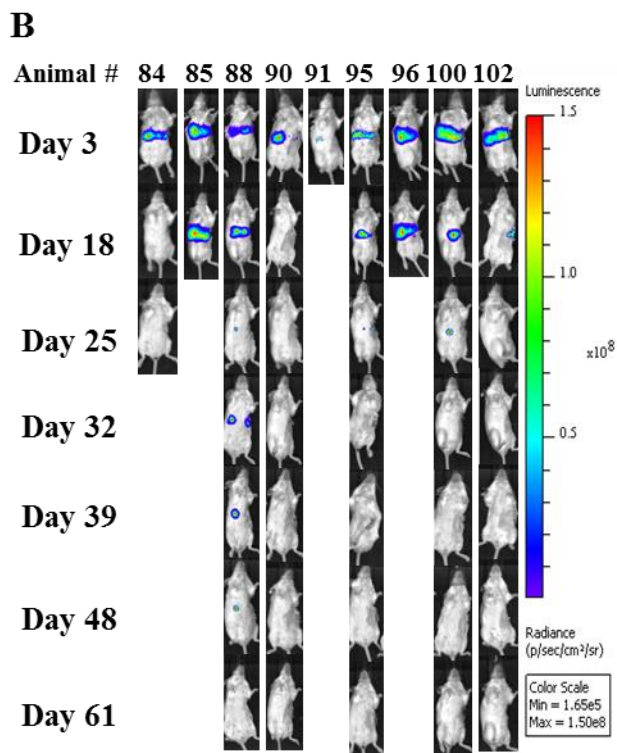


Figure S1: Tumor progression in the Toca 511 and 5-FC treatment group was blunted over time

Representative radiance intensities (photon/sec/mouse) from **Fig. 1**. (A) PBS and (B) 5-FC treatment group at various time points throughout the study. After liver metastases were established with Toca 511 pre-transduced CT26-Luc cells delivered intrasplenically, each mouse was imaged by IVIS at indicated time points. 5-FC treatment (500 mg/kg, IP, BID) was initiated on day 13 for 5 days on and 2 days off for total of six cycles. Control group received PBS.

Figure S2

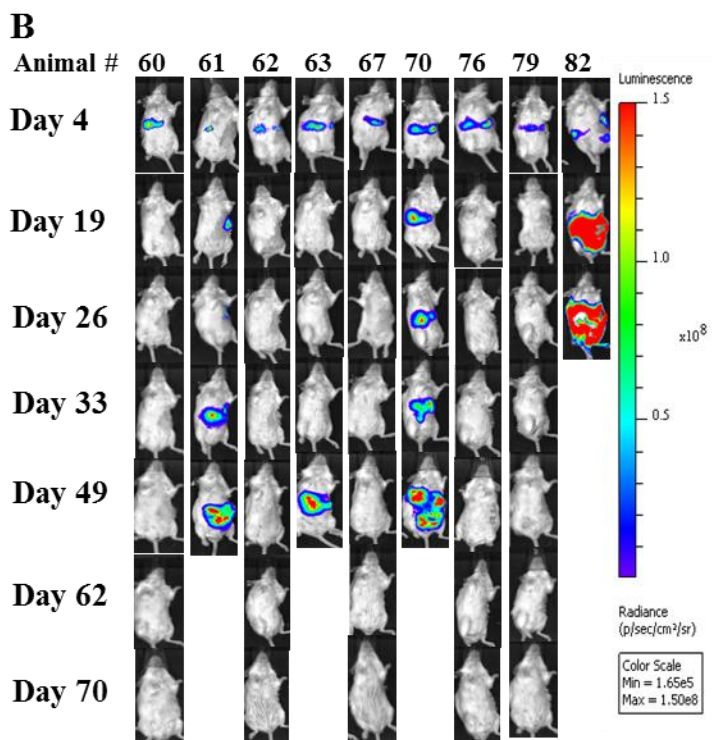
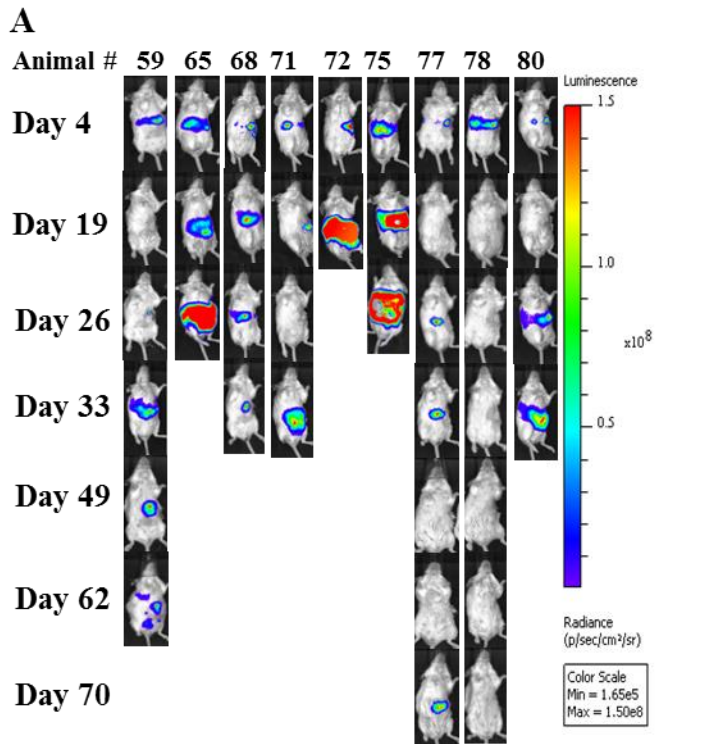


Figure S2: Tumor progression after IV Toca 511 administration and cycles of 5-FC treatment group

Representative radiance intensities (photon/sec/mouse) from Fig. 3. (A) PBS and (B) 5-FC treatment group at various time points throughout the study. Mice inoculated with CT26-Luc cells intrasplenically were then administered Toca 511 IV for 5 consecutive days starting on day 4 post cell inoculation. Each mouse was imaged by IVIS at indicated time points. 5-FC treatment (500 mg/kg, IP, BID) was initiated on day 13 for 5 days on and 2 days off for a total of six cycles. Control group received PBS.

Figure S3

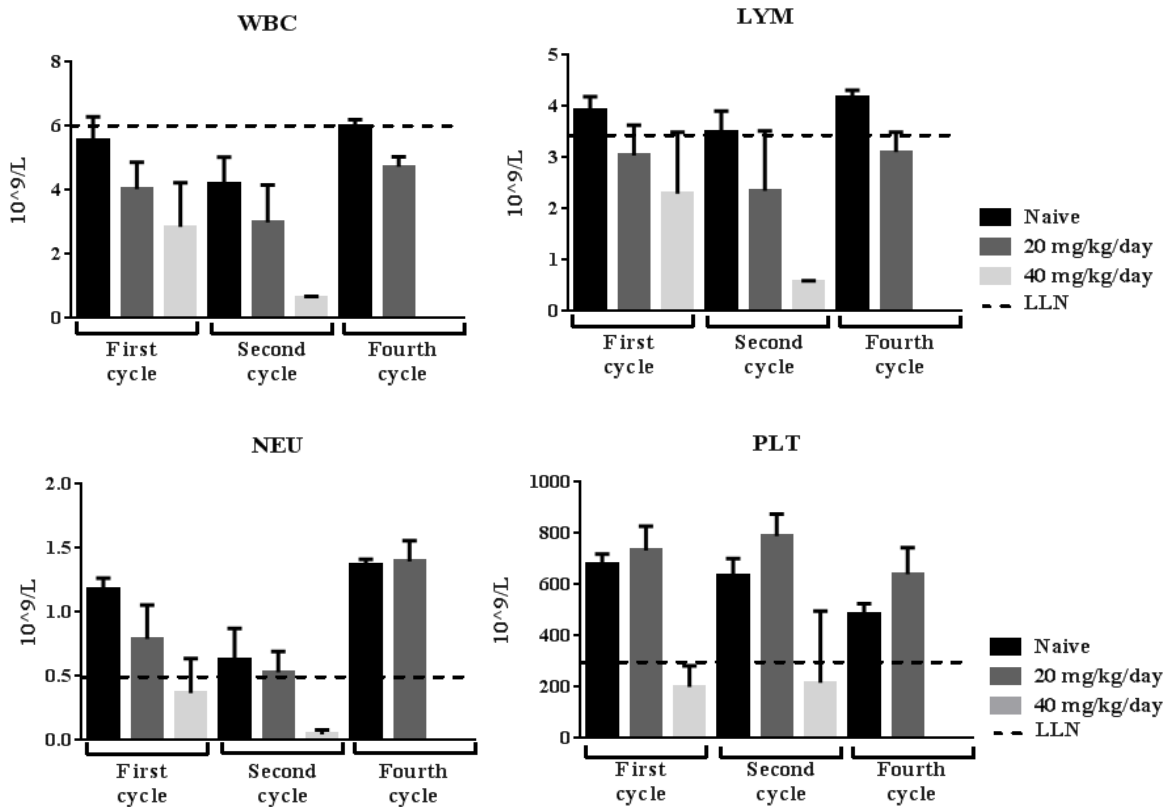


Figure S3: Hematological toxicity of systemic 5-FU treatment in non-tumor bearing animals A group of non-tumor bearing BALB/c mice received 20 mg/kg/day or 40 mg/kg/day 5-

FU injections. Cycles were 5 days on and 2 days off for a total of four cycles. WBC, LYM, NEU and PLT were determined at indicated time points. Dotted line represents LLN for age-matched mice. Age-matched mouse LLN = WBC: 6×10^9 cells/L; LYM: 3.4×10^9 cells/L; PLT: 200×10^9 cells/L; NEU: 0.5×10^9 cells/L.

Figure S4

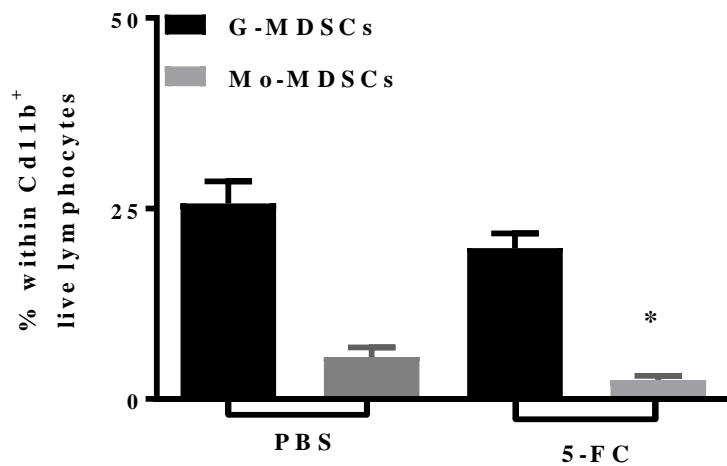


Figure S4: Distribution of monocytic and granulocytic myeloid derived suppressor cells in a brain metastasis model of mCRC BALB/c mice received Toca 511 pre-transduced CT26 cells intracranially and were treated with 5-FC (500 mg/kg, IP, BID) or PBS. Treatments started at day 10 post cell inoculation and were for 7 days on and 7 days off for one cycle. Tumor was collected for flow cytometric analysis 14 days post cell implantation. CD11b⁺ live lymphocytes were stained for Ly6G and Ly6C. Monocytic (CD11b⁺Ly6C⁺) and granulocytic (CD11b⁺Ly6G⁺) myeloid cells determined. Data are depicted as mean +/- SEM. *Statistical significance was defined as $P < .05$. (* $p < 0.0001$ vs. PBS).

Figure S5

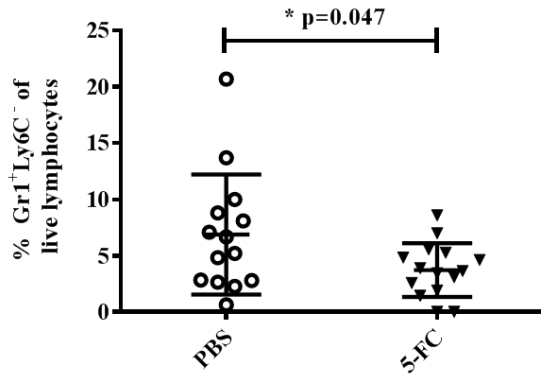


Figure S5: Neutrophils after one cycle of 5-FC treatment in a brain metastasis model of mCRC BALB/c mice received Toca 511 pre-transduced CT26 cells intracranially and were treated with 5-FC (500 mg/kg, IP, BID) or PBS. Treatments started at day 10 post cell inoculation and were for 7 days on and 7 days off for one cycle. Tumor was collected for flow cytometric analysis 14 days post cell implantation. Neutrophils (Gr1⁺Ly6C⁻) were analyzed as a percentage of total live lymphocytes in the tumor. *Statistical significance was defined as $P < .05$.

Figure S6

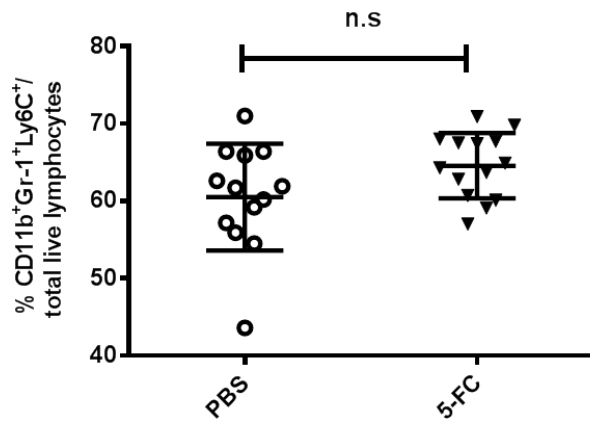


Figure S6: Myeloid cell populations in bone marrow of tumor-bearing animals after Toca 511 and 5-FC treatment BALB/c mice received Toca 511 pre-transduced CT26 cells intracranially and were treated with 5-FC (500 mg/kg, IP, BID) or PBS. Treatments started at day 10 post cell inoculation and were for 7 days on and 7 days off for one cycle. Bone marrow was collected for flow cytometric analysis 14 days post cell implantation. Myeloid cell populations (Cd11b⁺Gr-1⁺ Ly6C⁺) were detected from PBS and 5-FC treatment groups. Data are depicted as mean +/- SEM. *Statistical significance was defined as $P < .05$.

SUPPLEMENTARY TABLES:

Table S1. Body weights of Toca 511 pre-transduced CT26-Luc tumor-bearing B6C3F1 mice that received 5-FC cycles

Summary of the body weights from Fig. 1

Summary of Body Weights (Gr)				
Period	Group:	CT26-Toca511 +PBS	CT26-Toca511 + 5-FC	Naïve + 5-FC
Day 14	N	14	14	1
	Mean	19.24±1.04	19.01±1.63	17.3±0
Day 21	N	12	13	1
	Mean	19.58±1.22	18.5±2.53	17.98±0
Day 28	N	11	10	1
	Mean	20.46±1.7	18.02±2.42	18.77±0
Day 35	N	2	8	1
	Mean	19.8±0.3	17.73±2.74	18.03±0
Day 42	N	1	8	1
	Mean	19.66±0	17.78±3.28	18.17±0
Day 49	N	1	8	1
	Mean	22.47±0	17.58±1.83	19.24±0
Day 63	N	N/A	6	1
	Mean		18.91±1.24	19.61±0
Day 70	N	N/A	6	1
	Mean		20.14±1.37	19.59±0
Day 81	N	N/A	6	1
	Mean		19.77±1.1	20.44±0
Day 91	N	N/A	6	1
	Mean		20.39±1.08	19.8±0
Day 102	N	N/A	6	1
	Mean		20.84±1.5	19.82±0
Day 112	N	N/A	6	1
	Mean		21.8±1.6	21.1±0
Day 119	N	N/A	6	1
	Mean		22.86±1.81	22.13±0