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Supplementary Materials

Lead Validation of the Olive Phenolic *S*-(-)-Oleocanthal for Effective Control of *KRAS*^{G13D}-Mutant Colorectal Cancer Progression and Metastasis

Md Towhidul Islam Tarun [1], Hassan Y. Ebrahim [2], Khalid A. El Sayed [1]*

[1] School of Basic Pharmaceutical and Toxicological Sciences, College of Pharmacy, University of Louisiana at Monroe, 1800 Bienville Drive, Monroe, LA 71201, USA; tarunmt@warhawks.ulm.edu (M.T.T.).
[2] Department of Biomedical Sciences, Discipline of Pharmacology, Edward Via College of Osteopathic Medicine, Monroe, LA 71203, USA; hebrahim@ulm.vcom.edu (H.Y.E.).

* Correspondence: elsayed@ulm.edu (K.A.E.); Tel.: +1-318-342-1725

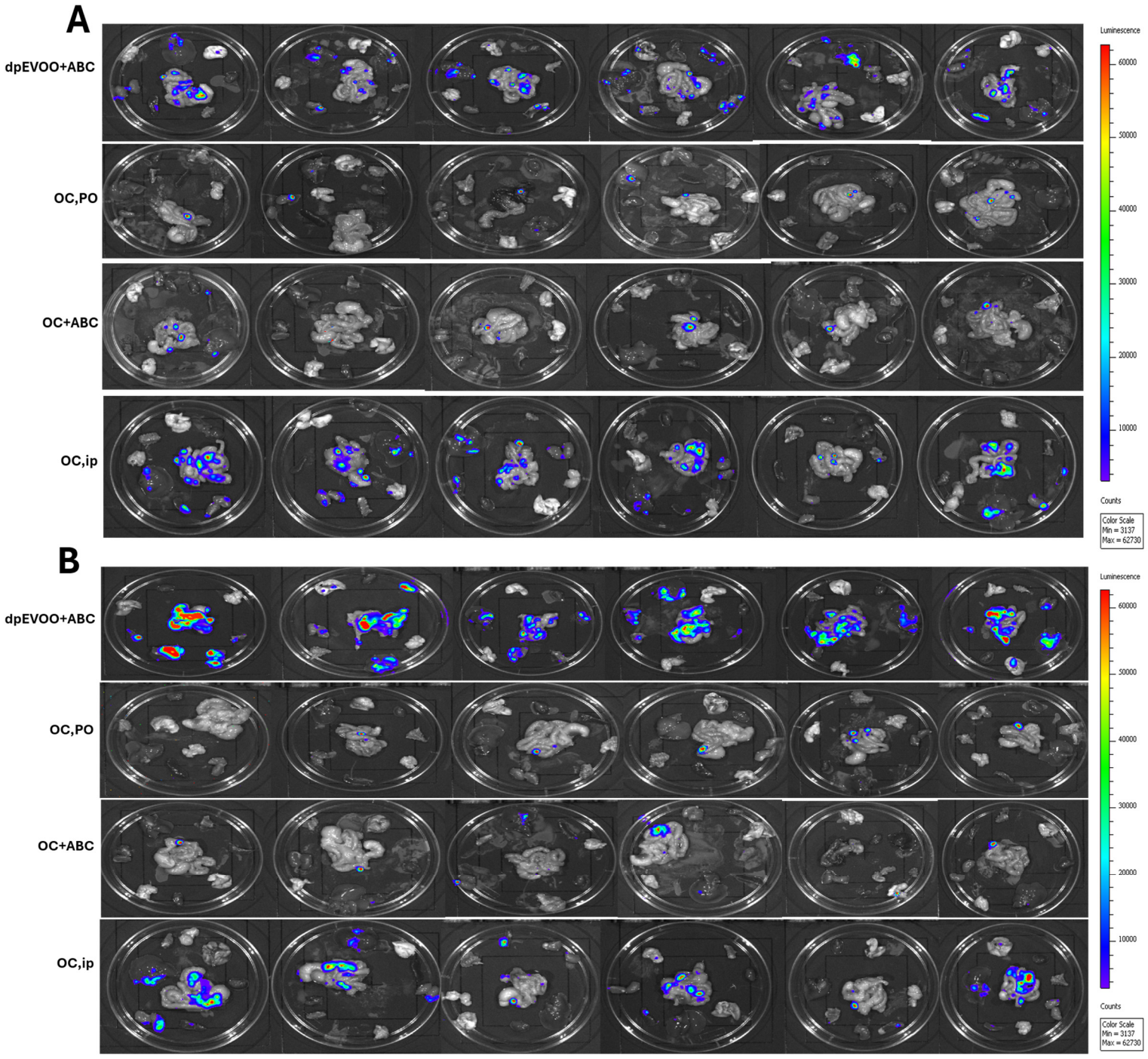

Figure S1. Representative bioluminescence images of metastatic dissemination in major organs from (A) Male and (B) Female orthotopic HCT-116-Luc CRC-bearing nude mice following ABC study groups.

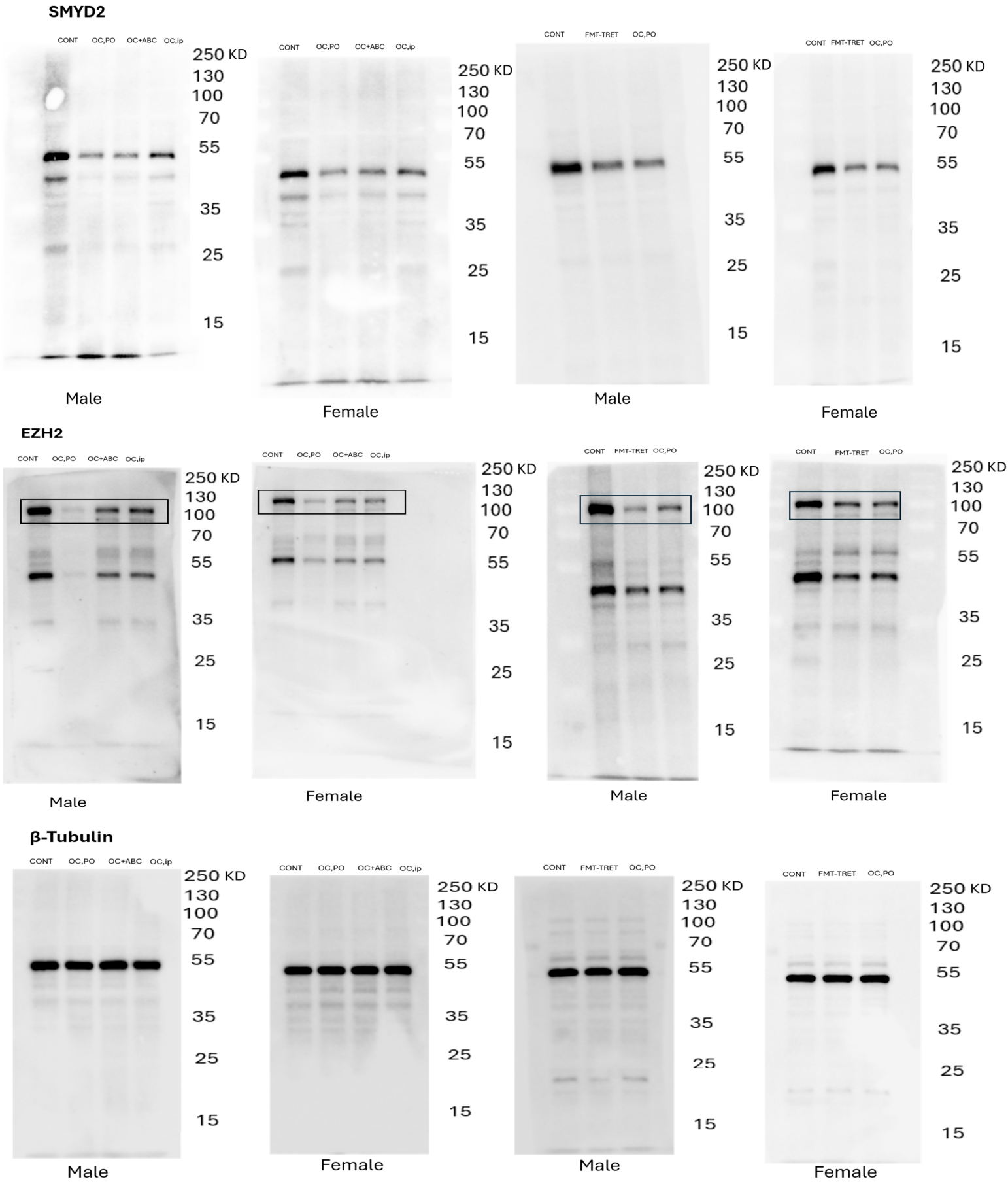

Figure S2. Raw Western blot images demonstrate the effect of OC and OC modulated GM on SMYD2, EZH2. β-Tubulin used as loading control.

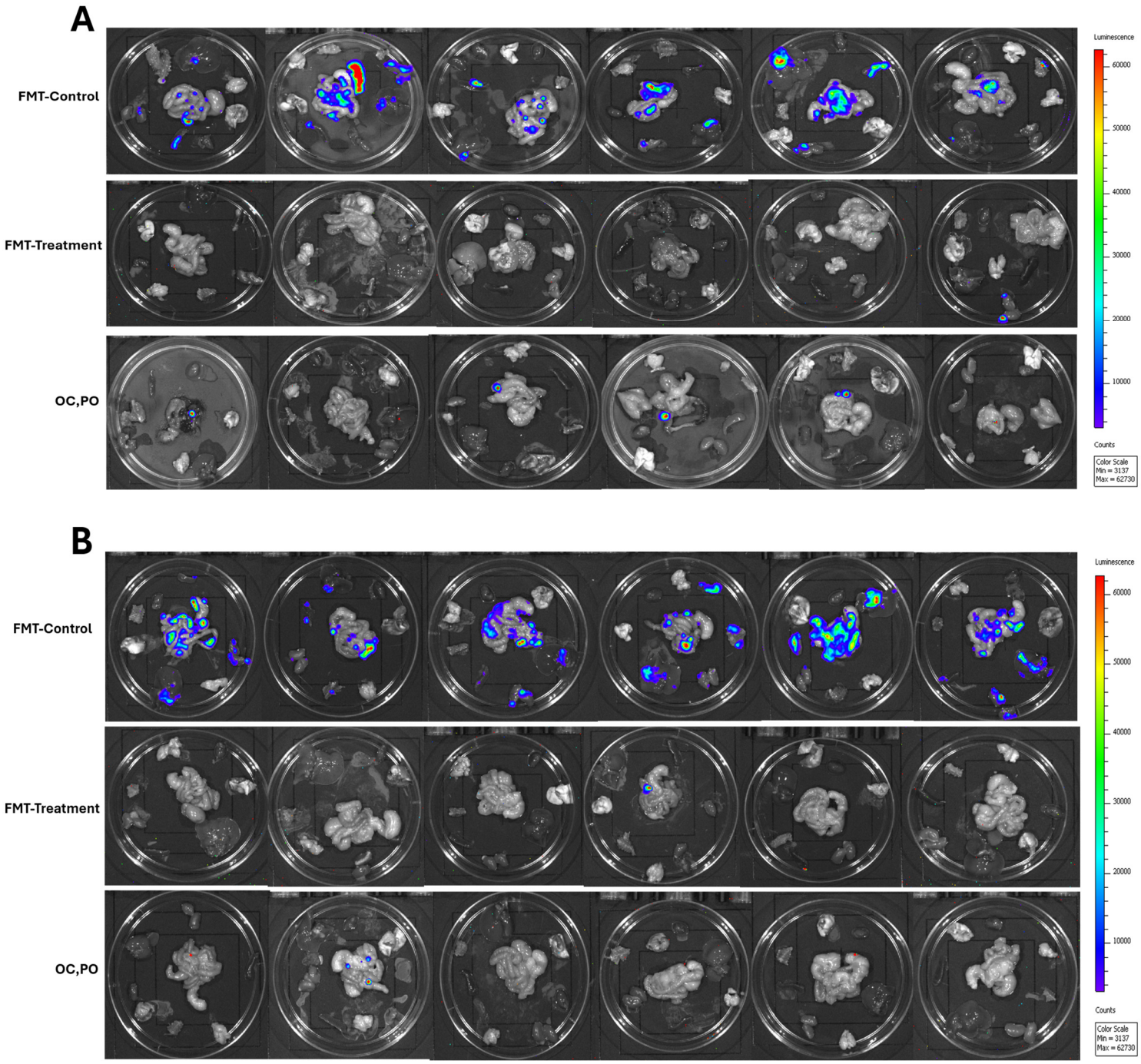

Figure S3. Representative bioluminescence images of metastatic dissemination in major organs from (A) Male and (B) Female orthotopic HCT-116-Luc CRC-bearing nude mice following FMT study groups.

Table S1. Individual primary tumor weights in mg across the treatment groups.

A. Male mice (ABC study)

Mouse	dpEVOO+ABC (Control)	OC, PO	OC+ABC	OC, ip
Mouse 1	1183.2	24.8	97.2	429
Mouse 2	1878.7	10.4	9.7	428.5
Mouse 3	955	69.9	30.5	358.7
Mouse 4	1083.3	43	17.5	521.6
Mouse 5	1205.3	9	47.5	437.7
Mouse 6	1099	3.1	10.4	442.2
Mouse 7	—	5.6	—	—
Mouse 8	—	12.6	—	—

B. Female mice (ABC study)

Mouse	dpEVOO+ABC (Control)	OC, PO	OC+ABC	OC, ip
Mouse 1	717.4	29.1	31.1	223.1
Mouse 2	1380.5	17.5	30	428
Mouse 3	850	3	111.9	399.4
Mouse 4	1547.9	5	58.9	256.3
Mouse 5	608.3	3	20.4	309
Mouse 6	1436	5	10.3	352.2

C. Male mice (FMT study)

Mouse	FMT Control (n=6)	FMT Treatment (n=6)	OC, PO (n=6)
Mouse 1	1745.3	20.1	22.2
Mouse 2	1801.5	11	55.2
Mouse 3	1445.2	4.2	50.4
Mouse 4	1523.1	5	48.3
Mouse 5	1498.4	5	20.1
Mouse 6	1238.7	3.2	15

D. Female mice (FMT study)

Mouse	FMT Control (n=6)	FMT Treatment (n=6)	OC, PO (n=6)
Mouse 1	1534.2	10	45.3
Mouse 2	1267	12.2	40.2
Mouse 3	1133.3	5.2	38.4
Mouse 4	988.8	3	37.3
Mouse 5	786.3	5	20.1
Mouse 6	885.2	3	5.5

Table S2. Individual body weight measurements of mice in gram at different time points across the treatment groups.

A. Male mice (ABC study)

Day	dpEVOO+ABC (Control)	OC, PO	OC+ABC	OC, ip
3	24.5, 25.6, 23.4, 23.8, 26.6, 27.7	23.6, 24.6, 26.4, 22.8, 22.9, 23.9	23.3, 23.5, 23.7, 23.2, 25.8, 26.5	26.7, 22.8, 23.8, 25.8, 22.5
7	24.0, 25.0, 23.1, 23.5, 26.5, 27.5	23.7, 24.6, 26.4, 22.8, 23.0, 23.9	23.4, 23.5, 23.7, 23.2, 26.0, 26.5	26.8, 22.8, 23.8, 25.8, 22.5
10	23.5, 24.4, 22.5, 23.0, 25.2, 26.6	23.7, 24.6, 26.5, 22.9, 23.0, 24.0	23.5, 23.5, 23.7, 23.2, 26.1, 26.5	26.0, 22.4, 23.8, 25.8, 22.0
14	23.0, 24.2, 22.0, 21.0, 24.8, 25.0	23.8, 24.7, 26.5, 23.0, 23.0, 25.2	23.9, 23.7, 24.1, 23.2, 26.2, 26.6	25.5, 22.4, 23.7, 25.8, 22.0
17	22.0, 22.0, 21.2, 20.2, 23.0, 22.8	23.7, 24.7, 26.8, 23.0, 23.2, 25.2	23.8, 23.8, 24.2, 23.2, 26.2, 26.7	24.0, 20.3, 23.7, 25.9, 22.0
21	20.0, 20.2, 19.0, 18.9, 22.6, 22.2	23.8, 24.7, 26.8, 23.2, 23.2, 25.2	23.9, 23.8, 24.5, 23.2, 26.2, 26.7	24.0, 20.3, 23.6, 25.8, 22.2

B. Female mice (Antibiotic study)

Day	dpEVOO+ABC (Control)	OC, PO	OC+ABC	OC, ip
3	23.4, 23.6, 22.5, 26.6, 27.7, 25.5	23.5, 23.6, 26.5, 24.7, 22.8, 25.9	23.3, 23.4, 23.2, 23.2, 25.6, 27.0	26.6, 22.7, 23.5, 25.6, 22.5, 23.9
7	23.0, 23.2, 22.4, 26.0, 26.5, 25.2	23.5, 23.6, 26.5, 24.8, 22.9, 25.9	23.4, 23.5, 23.7, 23.2, 26.0, 26.9	26.8, 22.8, 23.8, 25.7, 22.5, 23.8

Day	dpEVOO+ABC (Control)	OC, PO	OC+ABC	OC, ip
10	23.2, 22.5, 21.2, 25.0, 26.0, 25.2	23.5, 23.8, 26.8, 24.8, 22.9, 26.0	23.5, 23.5, 23.7, 23.0, 26.1, 27.1	26.0, 22.5, 23.6, 24.4, 22.0, 23.9
14	22.5, 22.1, 20.4, 24.2, 25.9, 24.1	23.4, 23.8, 26.8, 24.8, 22.8, 26.2	23.9, 23.7, 24.1, 23.0, 26.2, 27.0	25.5, 21.0, 22.7, 23.0, 22.0, 22.7
17	22.0, 19.5, 19.7, 23.5, 24.3, 23.8	23.6, 23.9, 26.9, 25.0, 22.9, 26.2	23.4, 23.7, 24.1, 23.5, 26.0, 26.8	25.0, 21.0, 22.7, 23.0, 21.9, 22.5
21	20.0, 19.0, 17.8, 22.0, 24.0, 22.2	23.6, 24.0, 27.2, 25.2, 22.9, 26.3	23.5, 23.7, 24.2, 23.5, 26.1, 27.0	25.0, 21.4, 22.5, 23.2, 21.8, 22.5

C. Male mice (FMT study)

Day	FMT Control (*n*=6)	FMT Treatment (*n*=6)	OC, PO (*n*=6)
3	23.0, 25.5, 25.7, 24.8, 22.5, 23.5	23.4, 23.2, 23.8, 25.0, 26.6, 24.4	24.7, 23.7, 24.3, 23.5, 23.8, 23.1
7	23.0, 24.8, 25.5, 23.6, 21.0, 22.0	23.4, 23.2, 23.8, 25.0, 26.6, 24.4	24.7, 23.7, 24.3, 23.5, 23.8, 23.1
10	21.0, 22.5, 24.7, 21.5, 20.0, 21.0	23.3, 23.3, 23.8, 25.1, 26.7, 24.4	24.9, 23.7, 24.3, 23.9, 23.7, 23.1
14	19.6, 22.0, 24.0, 21.1, 19.0, 20.0	23.6, 23.4, 23.8, 25.3, 26.9, 24.5	25.2, 23.7, 24.5, 23.9, 23.9, 23.1
17	18.0, 21.0, 23.8, 21.0, 18.0, 19.0	24.0, 23.8, 23.9, 25.6, 27.0, 24.6	25.3, 23.8, 24.5, 24.2, 24.4, 23.0
21	17.0, 20.0, 22.0, 19.5, 17.0, 18.0	24.1, 24.0, 24.3, 25.6, 27.0, 24.6	25.3, 24.0, 24.5, 24.4, 24.3, 23.5

D. Female mice (FMT study)

Day	FMT Control (*n*=6)	FMT Treatment (*n*=6)	OC, PO (*n*=6)
3	27.5, 22.5, 23.8, 23.7, 20.8, 26.8	24.3, 22.9, 22.8, 27.5, 25.2, 23.0	23.5, 23.1, 22.7, 27.5, 26.5, 24.2
7	27.0, 22.1, 23.8, 23.5, 20.1, 26.5	24.3, 23.0, 22.8, 27.5, 25.3, 23.0	23.5, 23.1, 22.7, 27.5, 26.5, 24.2
10	26.1, 21.7, 23.0, 23.1, 19.5, 26.1	24.4, 23.0, 22.8, 27.7, 25.5, 23.0	23.5, 23.5, 23.0, 27.7, 26.9, 24.2
14	25.5, 21.0, 21.0, 21.0, 18.2, 25.0	24.3, 23.0, 22.9, 27.9, 25.5, 23.5	23.6, 23.5, 23.0, 27.7, 26.9, 24.5
17	24.7, 19.0, 19.0, 20.5, 17.0, 24.0	24.4, 23.1, 22.9, 28.2, 25.5, 23.5	23.6, 23.4, 23.0, 27.8, 27.1, 24.5
21	24.2, 17.5, 18.7, 19.2, 17.0, 23.2	24.4, 23.2, 22.9, 28.2, 25.5, 23.8	23.6, 23.5, 23.2, 27.8, 27.1, 24.5

Table S3. Individual tumor bioluminescence average radiance (photons/s/cm²/sr) values for male and female mice across all treatment groups in ABC study.

Sex	dpEVOO+ABC	OC, PO	OC+ABC	OC, ip
Male	7.62×10^8	7.83×10^7	1.19×10^8	2.43×10^8
	4.73×10^8	5.24×10^7	2.65×10^7	9.84×10^7
	2.16×10^9	5.70×10^7	2.19×10^7	5.12×10^8
	9.93×10^8	1.04×10^7	2.67×10^7	7.83×10^7
	1.17×10^9	1.92×10^6	1.55×10^8	3.38×10^8
	1.16×10^9	1.04×10^7	7.40×10^7	2.21×10^8
Female	3.12×10^9	9.13×10^6	5.70×10^7	1.19×10^8
	2.86×10^9	1.29×10^7	2.23×10^7	2.43×10^8
	1.34×10^9	5.14×10^7	3.27×10^8	2.34×10^8
	1.12×10^9	9.16×10^7	1.29×10^7	4.85×10^8
	1.20×10^9	5.63×10^6	2.34×10^7	6.84×10^8
	1.00×10^9	1.32×10^7	1.27×10^7	7.62×10^8

Table S4. Individual Western Blot densitometry values for different treatment groups.

A. Male mice (ABC study)

Group	SMYD2-1	SMYD2-2	SMYD2-3	EZH2-1	EZH2-2	EZH2-3
dpEVOO+ABC	1.10	0.97	0.90	1.10	0.97	0.90
OC, PO	0.32	0.45	0.29	0.06	0.04	0.23
OC+ABC	0.36	0.48	0.32	0.29	0.32	0.32
OC, ip	0.54	0.52	0.31	0.42	0.26	0.57

B. Female mice (ABC study)

Group	SMYD2-1	SMYD2-2	SMYD2-3	EZH2-1	EZH2-2	EZH2-3
dpEVOO+ABC	1.00	1.10	1.09	1.00	1.10	1.09
OC, PO	0.27	0.30	0.43	0.13	0.19	0.15
OC+ABC	0.38	0.41	0.54	0.28	0.26	0.14

Group	SMYD2-1	SMYD2-2	SMYD2-3	EZH2-1	EZH2-2	EZH2-3
OC, ip	0.42	0.50	0.63	0.25	0.22	0.51

C. Male mice (FMT study)

Group	SMYD2-1	SMYD2-2	SMYD2-3	EZH2-1	EZH2-2	EZH2-3
Control	1.00	0.92	1.12	1.00	0.92	1.12
FMT Treatment	0.35	0.30	0.35	0.37	0.18	0.22
OC, PO	0.32	0.29	0.36	0.34	0.23	0.22

D. Female mice (FMT study)

Group	SMYD2-1	SMYD2-2	SMYD2-3	EZH2-1	EZH2-2	EZH2-3
Control	1.00	1.09	0.90	1.00	1.09	0.90
FMT Treatment	0.43	0.38	0.39	0.14	0.14	0.22
OC, PO	0.37	0.41	0.35	0.21	0.16	0.22

Table S5. Individual tumor bioluminescence average radiance (photons/s/cm²/sr) values for male and female mice across all treatment groups in FMT study.

Sex	FMT Cont	FMT Treatment	OC, PO
Male	2.18×10^9	8.90×10^6	1.18×10^8
	1.95×10^9	1.26×10^7	1.42×10^8
	1.67×10^9	1.74×10^7	1.67×10^8
	8.81×10^8	2.08×10^7	1.93×10^8
	2.19×10^9	2.53×10^7	2.21×10^8
	1.98×10^9	3.02×10^7	2.48×10^8
Female	7.94×10^8	7.80×10^6	1.09×10^8
	2.71×10^9	1.11×10^7	1.31×10^8
	2.48×10^9	1.59×10^7	1.56×10^8
	2.22×10^9	1.98×10^7	1.84×10^8
	2.06×10^9	2.37×10^7	2.12×10^8
	1.87×10^9	2.28×10^8	2.39×10^8