Bioengineered Adipose-Derived Stem Cells for Targeted Enzyme-Prodrug Therapy of Ovarian Cancer Intraperitoneal Metastasis

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Supporting Information

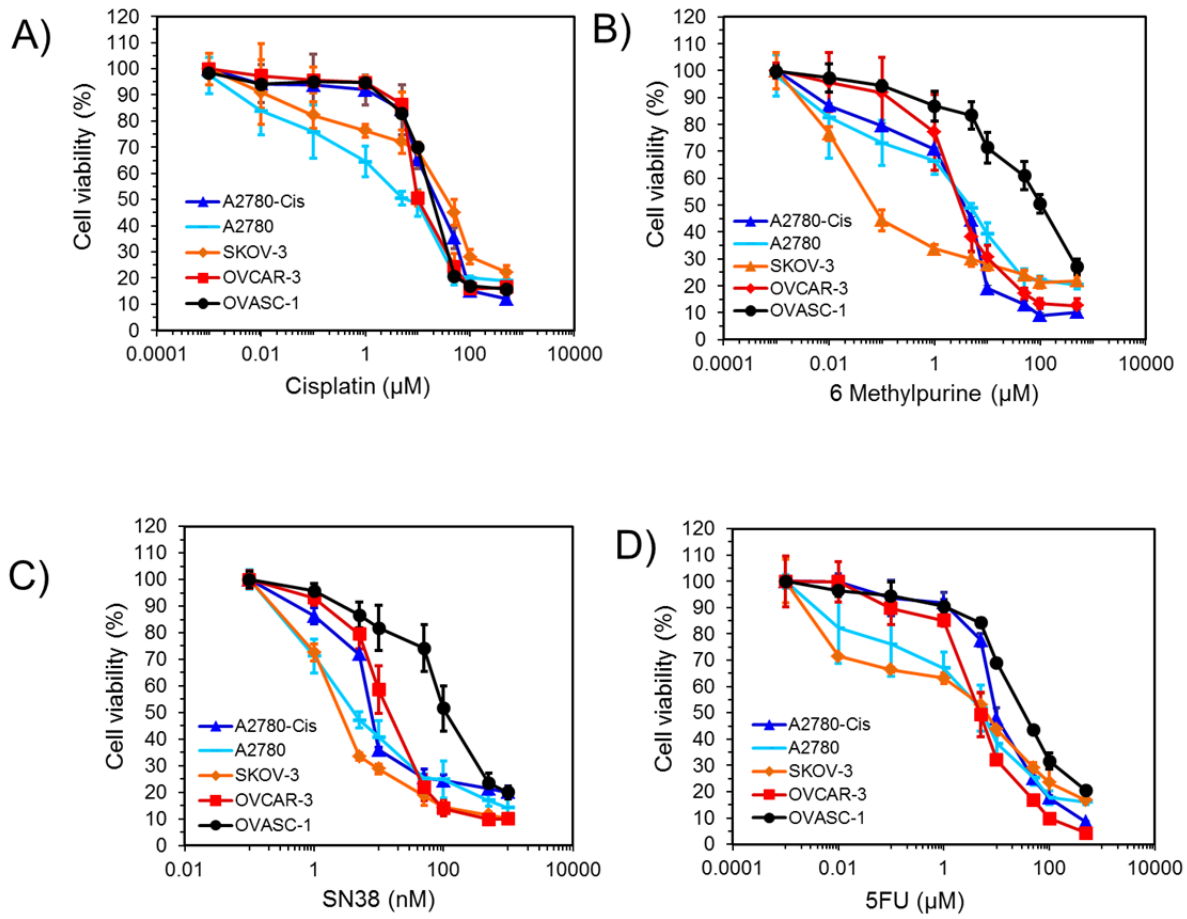


Figure S1: Evaluation of the cell viability by WST-1 cell toxicity assay. A-D) Ovarian cancer cells were exposed to different concentrations of the drugs and the viability of the cells was measured. The X-axis is plotted in Log scale. The data are reported as mean±SD (n=4).

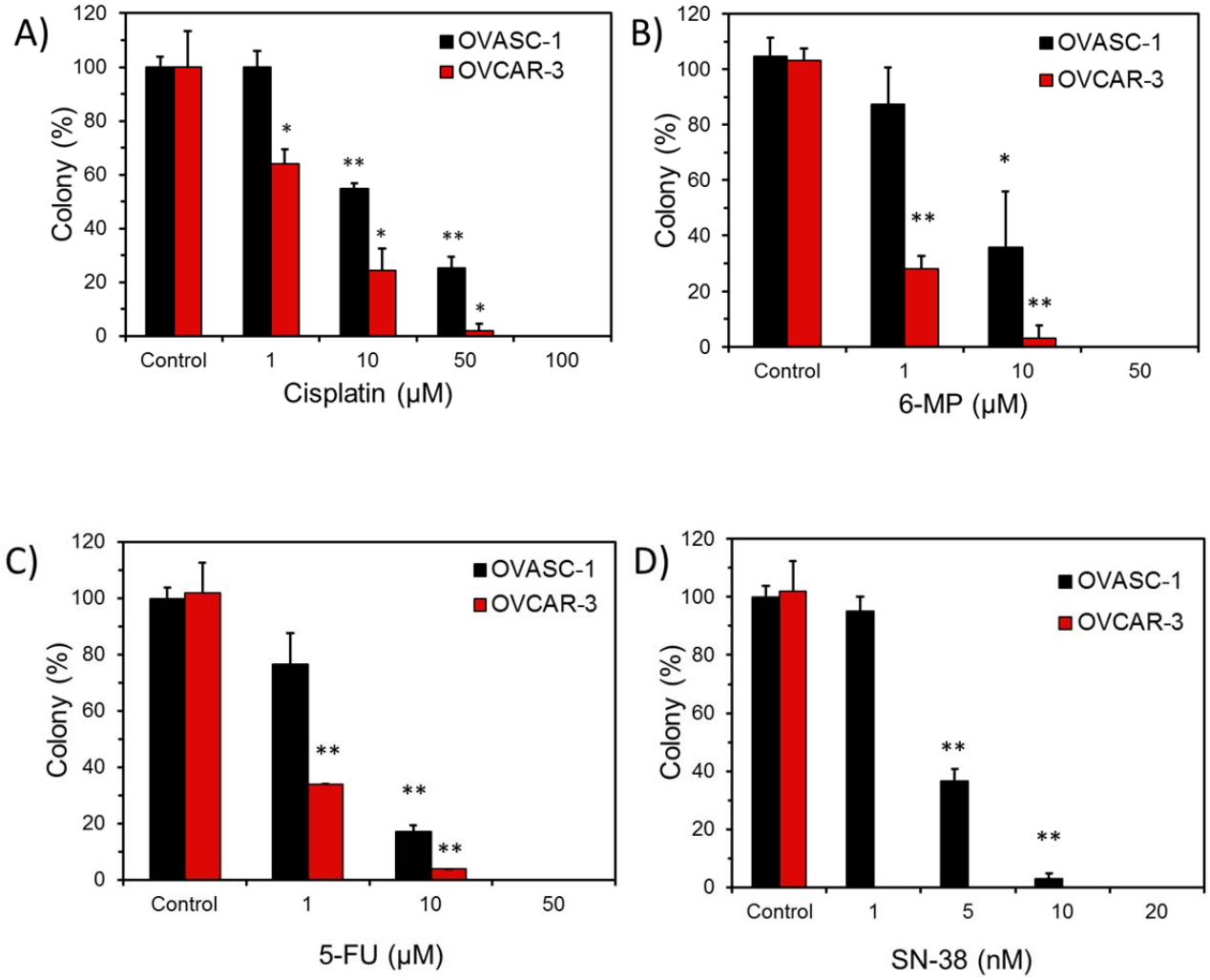
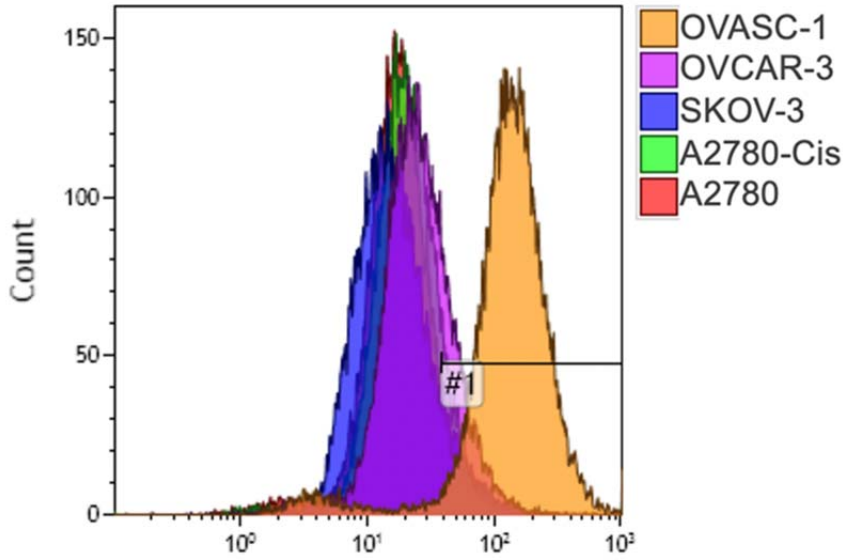


Figure S2: The measurement of drugs' chronic toxicities to OVASC-1 and OVCAR-3 cells by clonogenic assay. Ovarian cancer cells were exposed to different concentrations of the drugs and the number of colonies in each well was counted after 14 days. This figure shows that the OVASC-1 cells are the most drug-resistant cells and SN-38 is the most potent anticancer drug. The data are presented as mean±SD (n=3).*Statistical significance, t-test (p<0.05).

A)

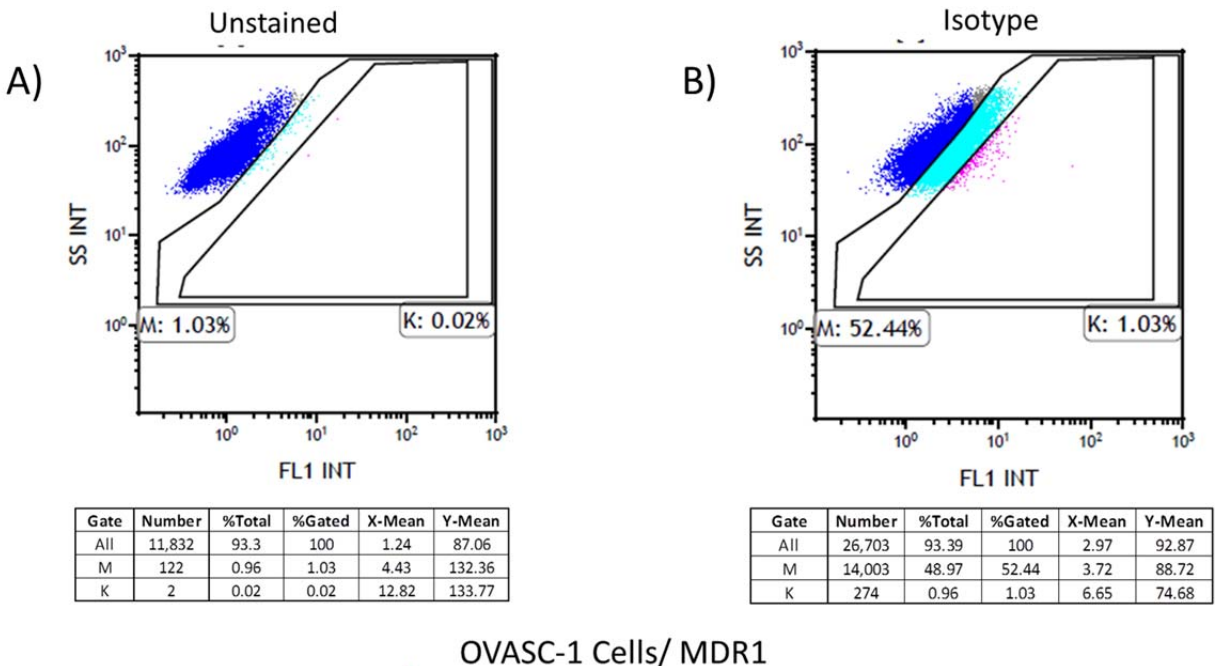


B)

0.95 Confidence interval
(1/colony forming cells frequency)

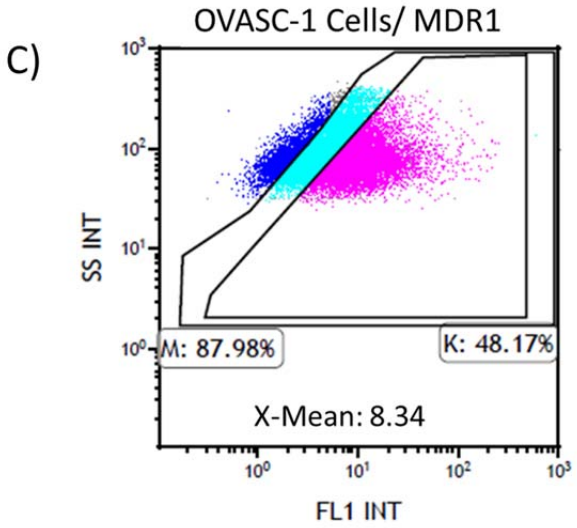
Cell line	Lower	Estimate	Upper
OVASC-1	31.7	20.3	13.1
OVCAR-3	49.4	31.7	20.3
A2780-CIS	73.4	47	30.2
SKOV-3	74.8	47.9	30.7
A2780	108	69.2	44.3

Figure S3: A) Flow cytometry histograms of the ALDH+ ovarian cancer cells. Cells were treated with ALDHFLUOR substrate and the intensity of the fluorescent signal was detected by flow cytometry. This figure shows that OVASC-1 cells have the highest population of ALDH+ cells. B) Estimation of the frequency of CICs in different ovarian cancer cell populations (0.95 Confidence Interval) by limiting dilution assay using ELDA online software (http://bioinf.wehi.edu.au/software/elda/index.html).



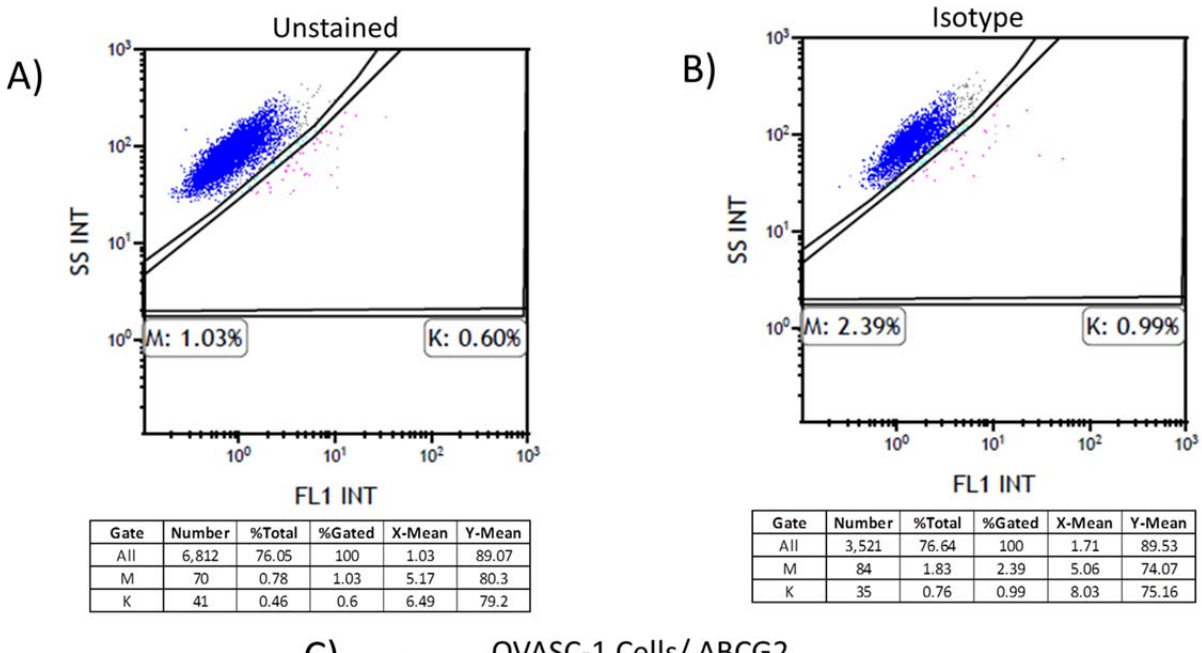
Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	11,832	93.3	100	1.24	87.06
M	122	0.96	1.03	4.43	132.36
K	2	0.02	0.02	12.82	133.77

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	26,703	93.39	100	2.97	92.87
M	14,003	48.97	52.44	3.72	88.72
K	274	0.96	1.03	6.65	74.68



Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	26,717	93.27	100	8.34	92.05
M	23,506	82.06	87.98	9.11	90.55
K	12,870	44.93	48.17	12.42	76.51

Figure S4: Evaluation of the expression of MDR1 transporters on the surface of OVASC-1 cells. A-B) Unstained OVASC-1 cells and cells labeled with fluorescent IgG1 isotype (controls). C) OVASC-1 cells were labeled with fluorescent anti-MDR1 primary antibody and analyzed by flow cytometry. X-mean (Mean Fluorescence Intensity) denotes the fold difference in between the expression of the transporter on the surface of OVASC-1 cells stained with the anti-MDR1 antibody and the IgG isotype control indicating the transporter density.

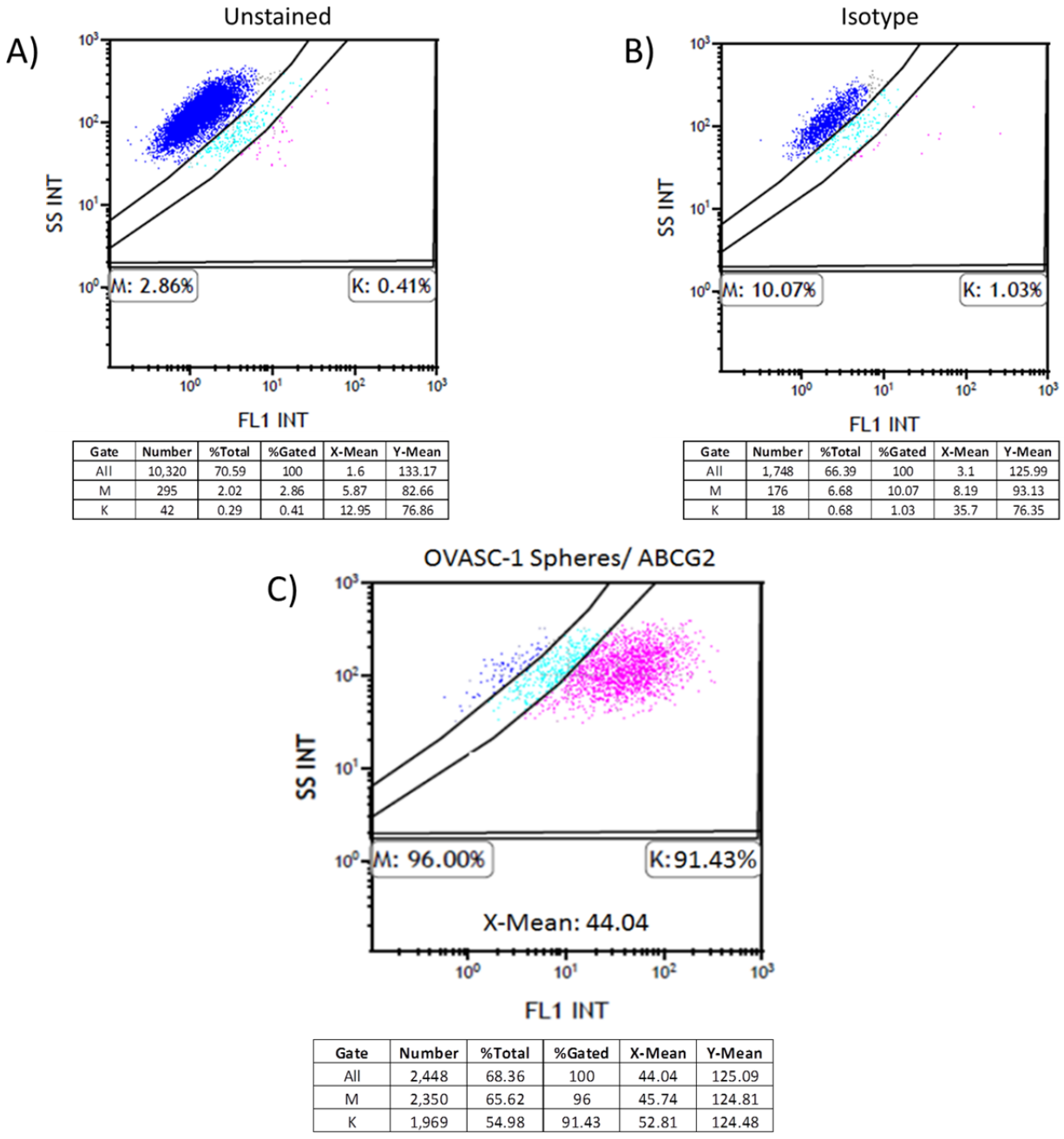


Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	6,812	76.05	100	1.03	89.07
M	70	0.78	1.03	5.17	80.3
K	41	0.46	0.6	6.49	79.2

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	3,521	76.64	100	1.71	89.53
M	84	1.83	2.39	5.06	74.07
K	35	0.76	0.99	8.03	75.16

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	3,302	73.69	100	21.7	89.14
M	3,186	71.1	96.49	22.38	88.74
K	3,093	69.02	93.67	22.93	88.43

Figure S5: Evaluation of the expression of ABCG2 transporters on the surface of OVASC-1 cells. A-B) Unstained OVASC-1 cells and cells labeled with fluorescent IgG1 isotype were used as controls. C) OVASC-1 cells were labeled with fluorescent anti-ABCG2 primary antibody and analyzed by flow cytometry. X-mean (Mean Fluorescence Intensity) denotes the fold difference in between the expression of the transporter on the surface of OVASC-1 cells stained with the anti-ABCG2 antibody and the IgG isotype control indicating the transporter density.

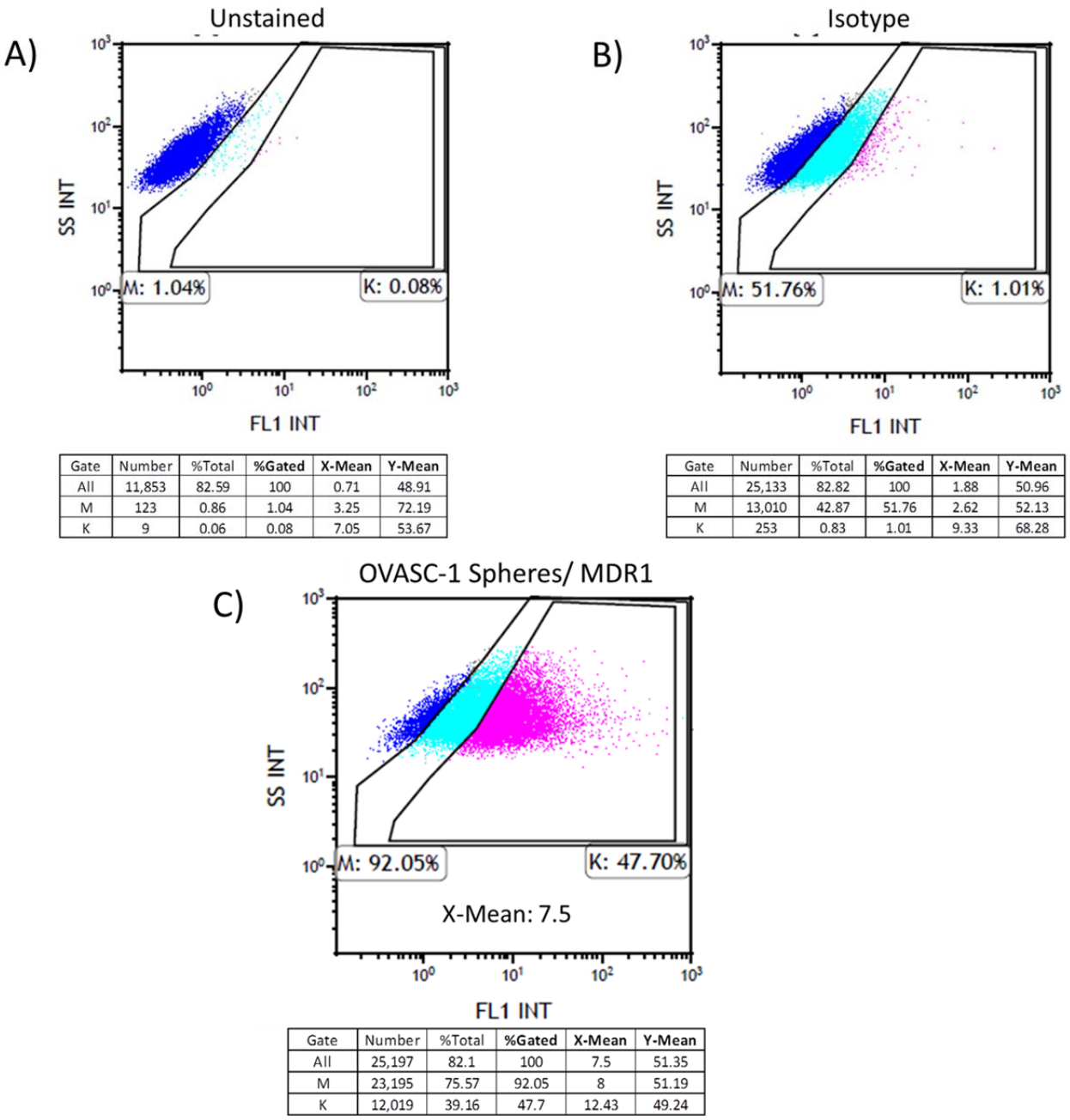


Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	10,320	70.59	100	1.6	133.17
M	295	2.02	2.86	5.87	82.66
K	42	0.29	0.41	12.95	76.86

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	1,748	66.39	100	3.1	125.99
M	176	6.68	10.07	8.19	93.13
K	18	0.68	1.03	35.7	76.35

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	2,448	68.36	100	44.04	125.09
M	2,350	65.62	96	45.74	124.81
K	1,969	54.98	91.43	52.81	124.48

Figure S6: Evaluation of the expression of ABCG2 transporters on the surface of OVASC-1 cells in tumorspheres. A-B) Unstained OVASC-1 cells and cells labeled with fluorescent IgG1 isotype were used as controls. C) OVASC-1 cells from tumorspheres were labeled with fluorescent anti-ABCG2 primary antibody and analyzed by flow cytometry. X-mean (Mean Fluorescence Intensity) denotes the fold difference in between the expression of the transporter on the surface of OVASC-1 cells stained with the anti-ABCG2 antibody and the IgG isotype control indicating the transporter density.



Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	11,853	82.59	100	0.71	48.91
M	123	0.86	1.04	3.25	72.19
K	9	0.06	0.08	7.05	53.67

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	25,133	82.82	100	1.88	50.96
M	13,010	42.87	51.76	2.62	52.13
K	253	0.83	1.01	9.33	68.28

Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	25,197	82.1	100	7.5	51.35
M	23,195	75.57	92.05	8	51.19
K	12,019	39.16	47.7	12.43	49.24

Figure S7: Evaluation of the expression of MDR1 transporters on the surface of OVASC-1 cells in tumorspheres. A-B) Unstained OVASC-1 cells and cells labeled with fluorescent IgG1 isotype were used as controls. C) OVASC-1 cells from tumorspheres were labeled with fluorescent anti-MDR1 primary antibody and analyzed by flow cytometry. X-mean (Mean Fluorescence Intensity) denotes the fold difference in expression of the transporter on the surface of OVASC-1 cells stained with the anti-MDR1 antibody in comparison to the IgG isotype control indicating the transporter density.

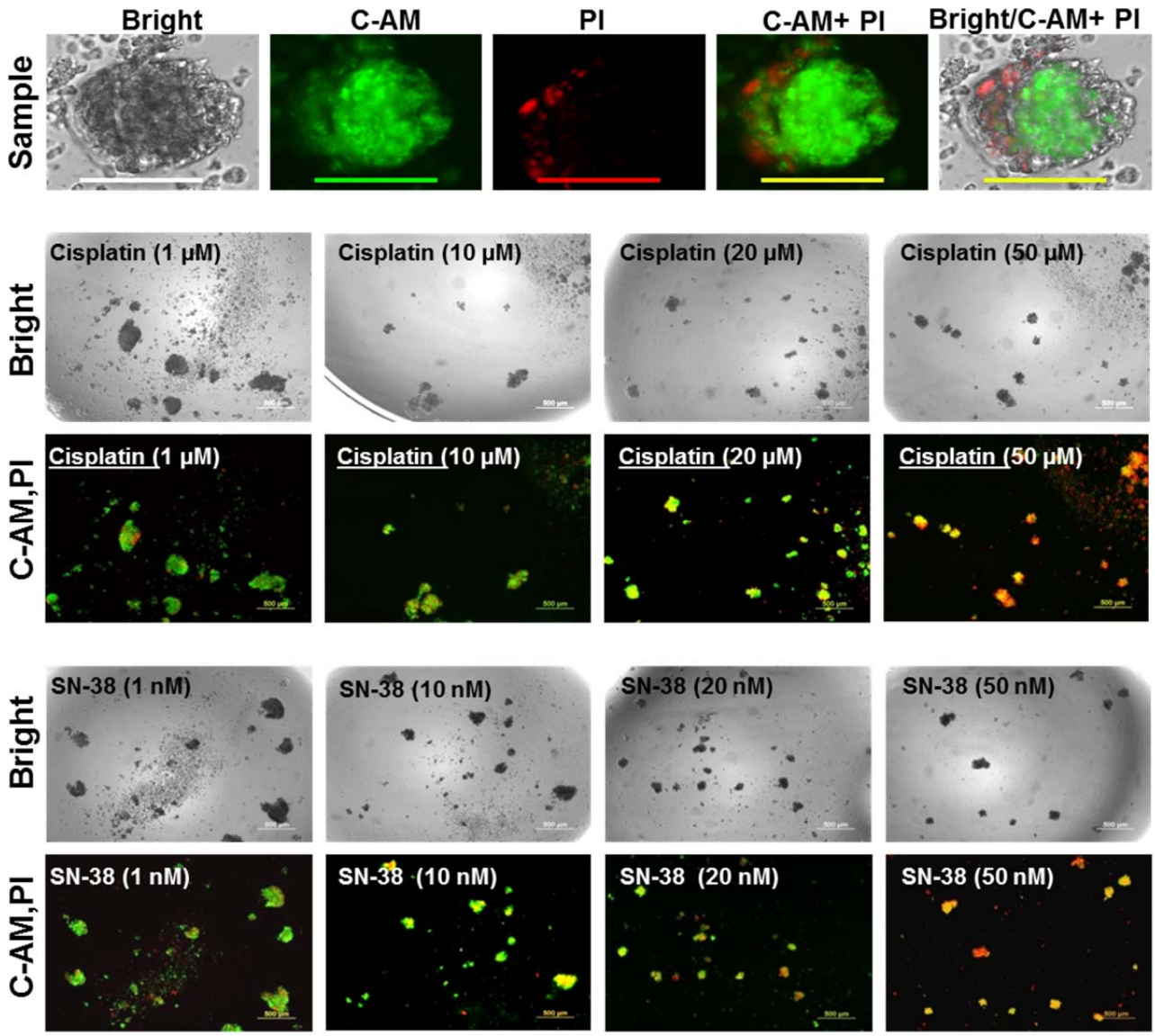


Figure S8: Evaluation of the OVASC-1 tumorspheres sensitivity to SN-38 and cisplatin. Tumorspheres were stained with the fluorophores calcein-AM (CAM) and propidium iodide (PI) to visualize live (green) and dead (red) cells, respectively. Scale bar= 500 µm. This figure shows that the tumorspheres consisted of mostly live cells when exposed to SN-38 at less than 50 nM and cisplatin at less than 50 µM.

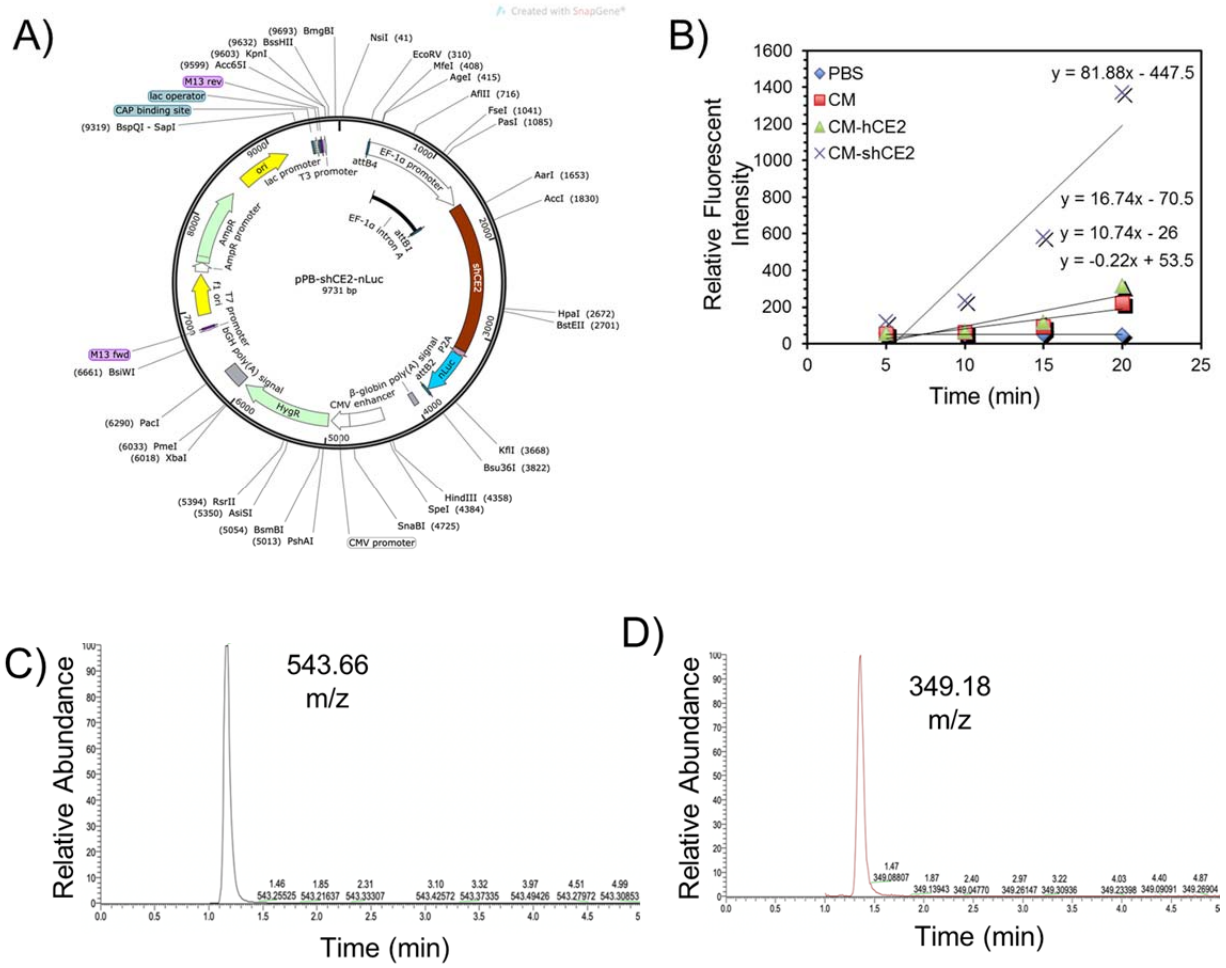


Figure S9: Genetic engineering and evaluation of the functionality of the genetically engineered ASC-shCE2:nLuc cells. A) The map of the plasmid DNA encoding shCE2 in fusion with nLuc. B) Measurement of the kinetics of FDA conversion over time. The ASC-shCE2:nLuc cells that secreted CE2 into the media could effectively convert FDA into its fluorescent byproduct (Y-axis). CM stands for culture media and PBS is phosphate buffer saline. CM-shCE2 stands for culture media from the ASC-shCE2:nLuc cells (secretory), whereas CM-hCE2 stands for culture media from the ASC-hCE2:nLuc cells (non-secretory). C) The mass spectroscopy chromatogram with major fragment ion of 543.66 m/z corresponding to irinotecan. D) The mass spectroscopy chromatogram with major fragment ion of 349.18 m/z corresponding to SN-38.

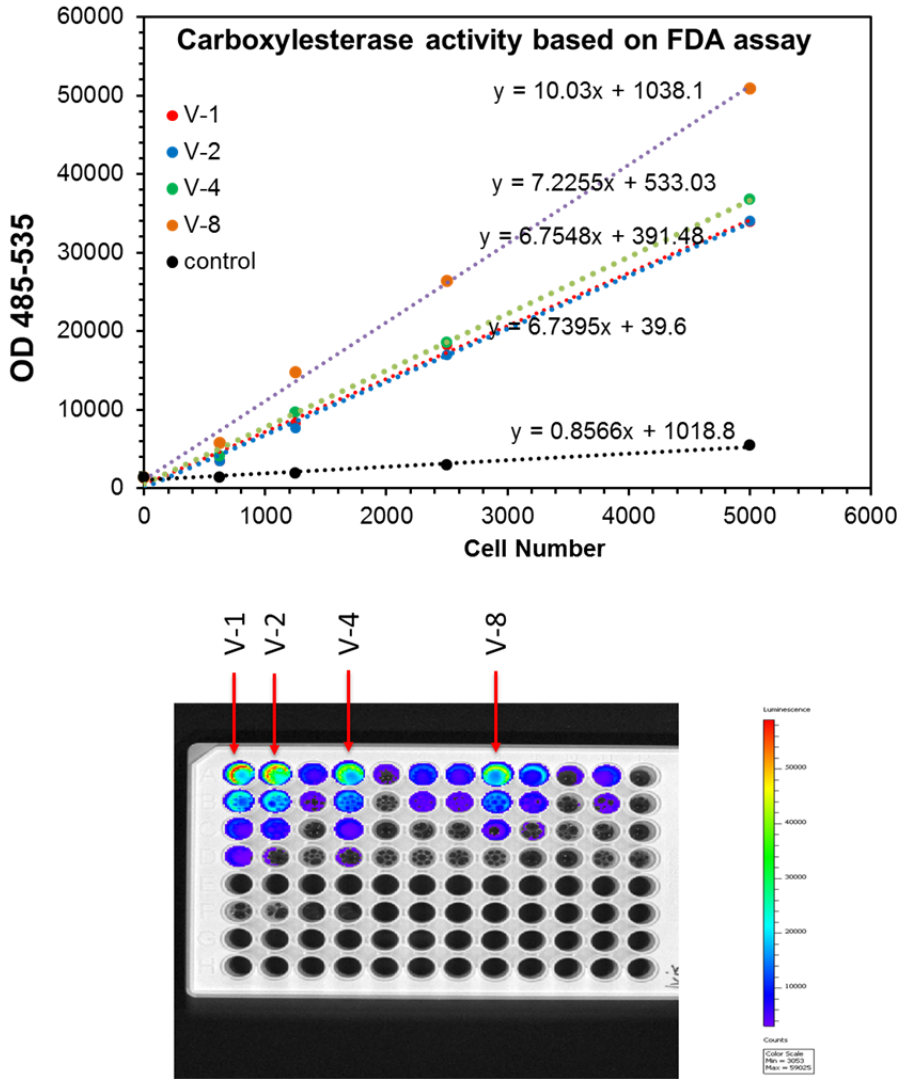


Figure S10: Selection of a clone of ASC-shCE2:nLuc cells that have high expression of both carboxylesterase and nanoluciferase genes. Top panel) FDA conversion assay that shows clone V8 had the highest carboxylesterase activity. Lower panel) nanoluciferase expression assay which shows clone V8 had significant luciferase expression levels (third highest). Considering the carboxylesterase expression as the priority, clone V8 was selected for the in vivo studies.

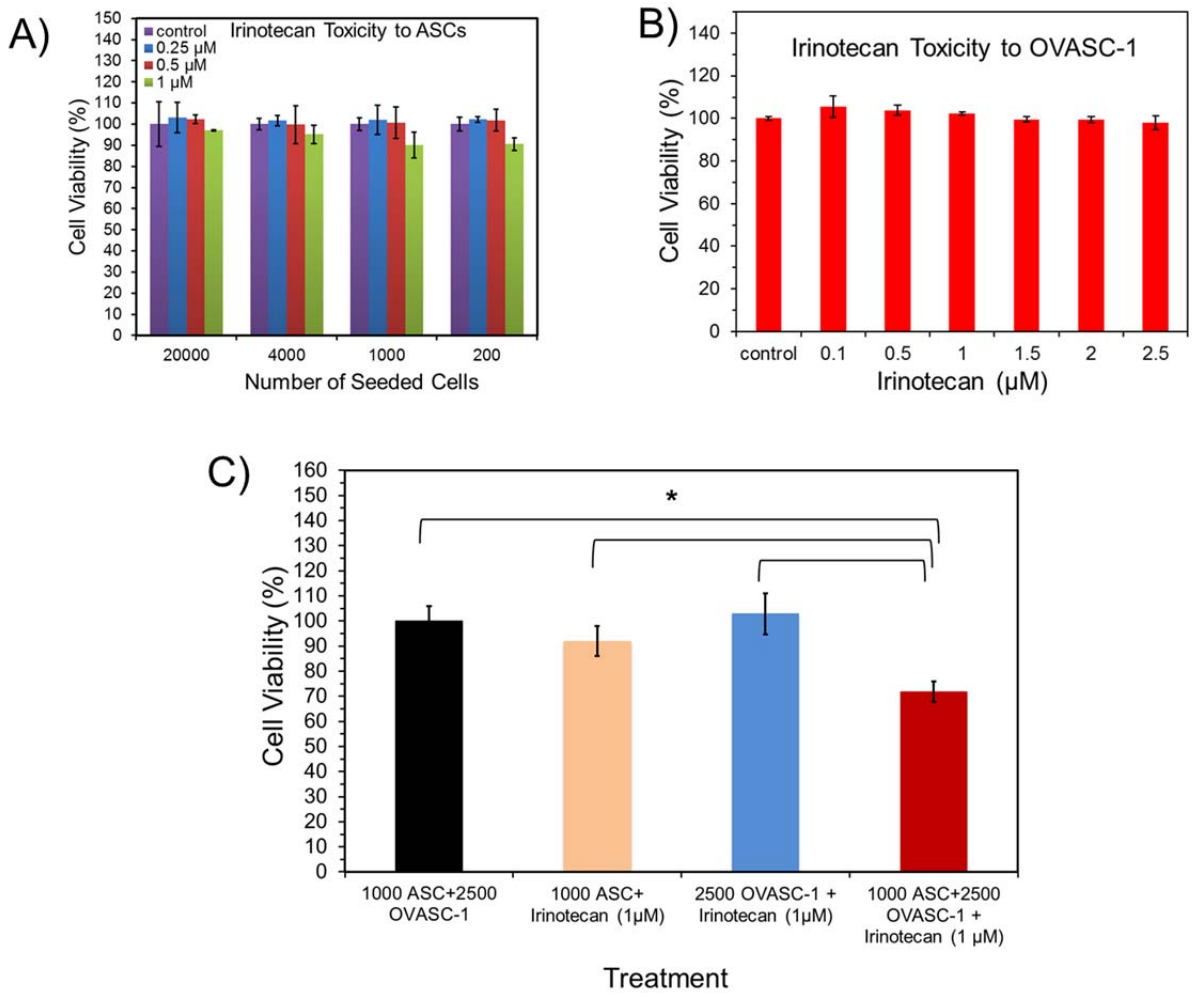


Figure S11: A) Evaluation of the viability of OVASC-1 cells treated with different concentrations of irinotecan. Irinotecan was not toxic to OVASC-1 cell up to 2.5 µM. B) Evaluation of the viability of ASCs seeded at different numbers and treated with different concentrations of irinotecan. Irinotecan at 1µM concentration was not toxic to ASCs when at least 1000 cells were seeded. C) Evaluation of the cytotoxicity of the ASC-shCE2:nLuc cells in combination with CPT-11 to OVASC-1 cells. The absorbance of the 1000 ASCs co-cultured with 2500 OVASC-1 cells (without irinotecan) was considered as 100% viable. (*ANOVA followed by a Post hoc Tukey test, $p<0.05$)

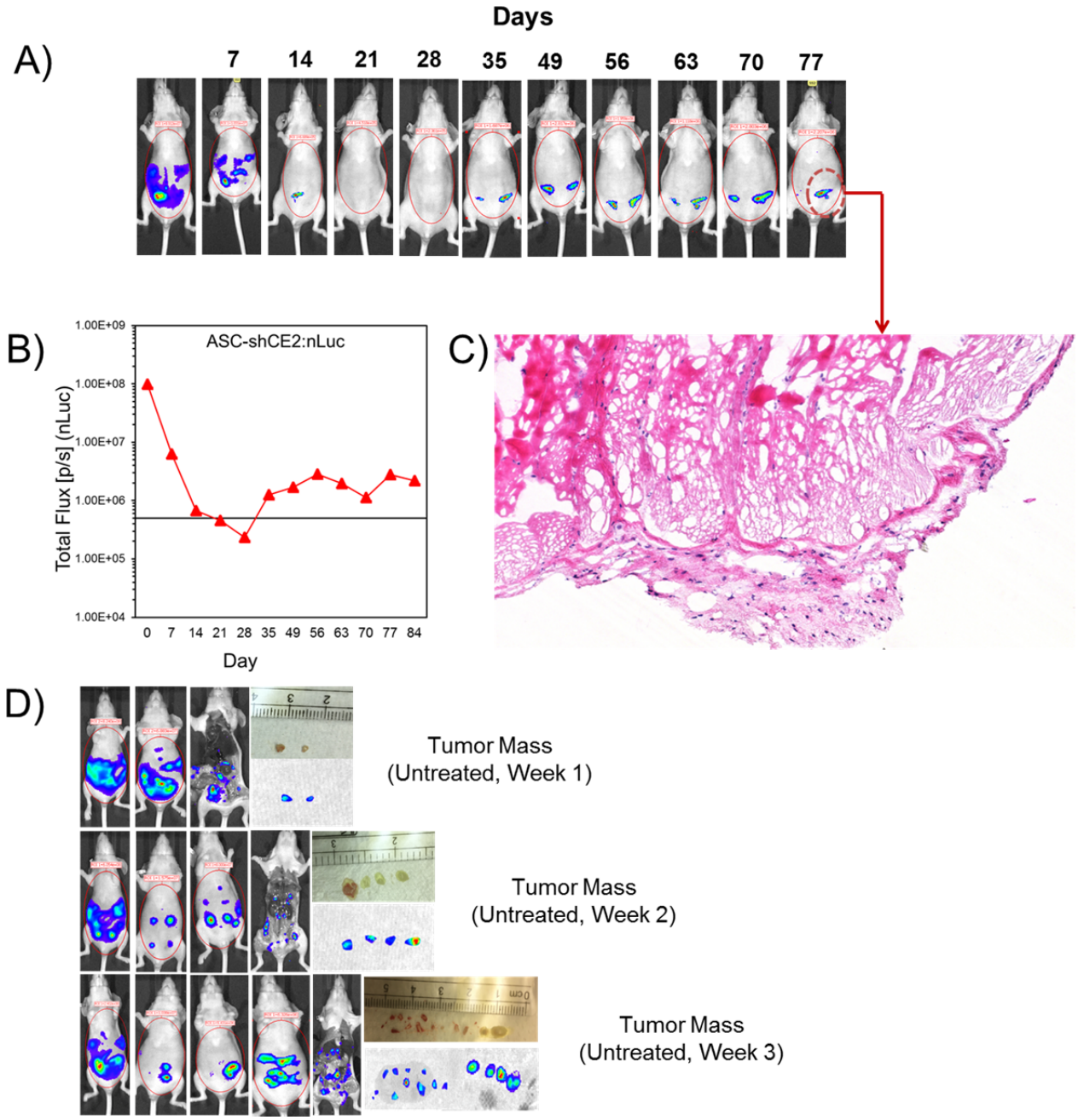


Figure S12: A) Bioluminescent imaging of the live mouse that was injected intraperitoneally with ASC-shCE2:nLuc cells. B) The bioluminescent signal intensity quantification over the 12 weeks period. C) Tissue section slide of the bioluminescent area showing the healthy mesentery and absence of malignancy. D) The growth of tumor mass inside mouse peritoneum over the course of three weeks. Mice were studied by BLI and then dissected to remove the tumors. The sizes of the tumors were measured and the numbers counted.

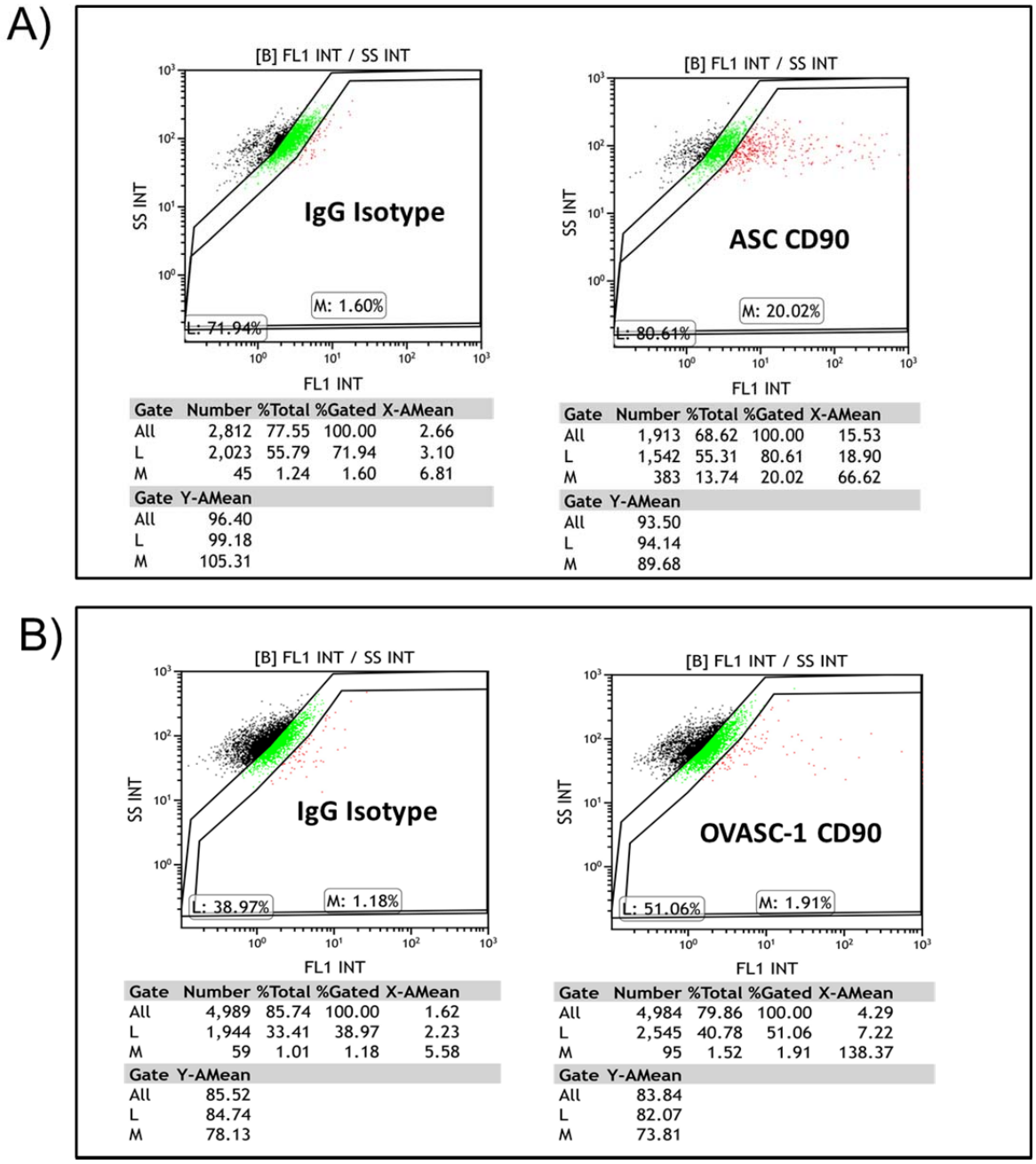


Figure S13: Evaluation of the expression of CD90 biomarker on the surfaces of ASC-shCE2:nLuc and OVASC-1 cells by flow cytometry. A) ASC-shCE2:nLuc cells labeled with fluorescent IgG1 isotype or anti-CD90 primary antibodies. B) OVASC-1 cells labeled with fluorescent IgG1 or anti-CD90 primary antibodies.

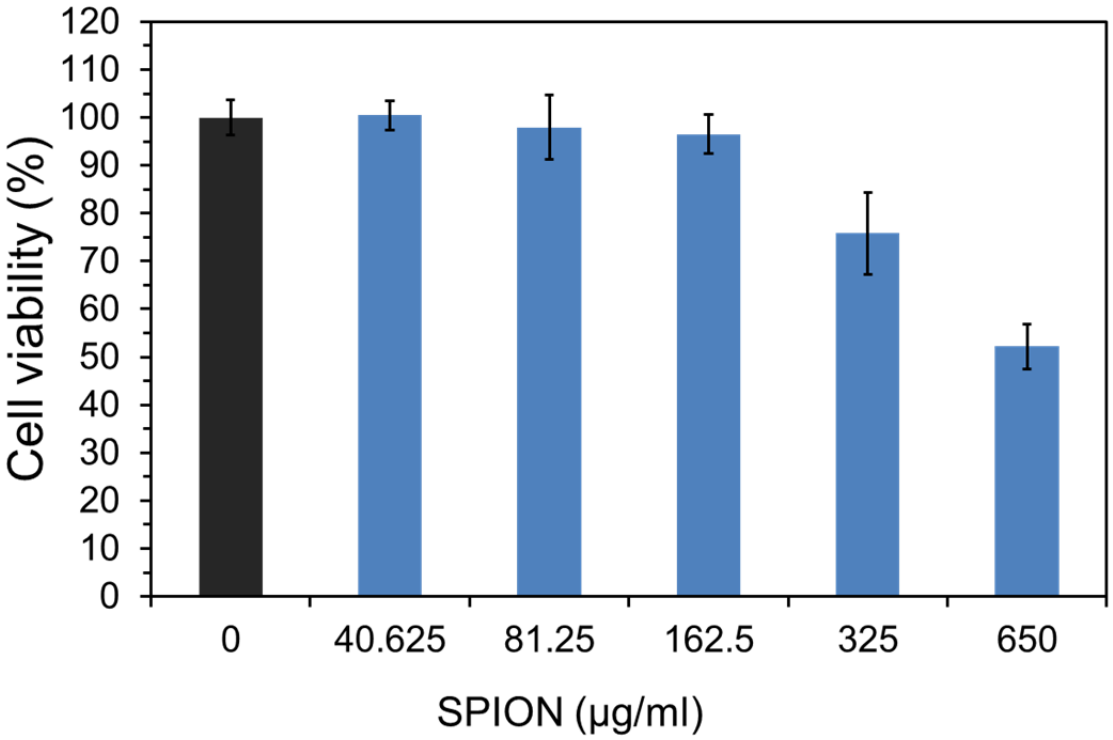


Figure S14: Evaluation of the toxicity of FluidMAG-D SPIONs to ASC-shCE2:nLuc cells. Cells were incubated with FluidMAG-D for 24 hours at different concentrations and the viability of the ASCs was measured by WST-1 cell toxicity assay. This figure shows that FluidMAG-D SPIONs are not toxic to ASCs at concentrations equal to or less than 162.5 µg/ml.

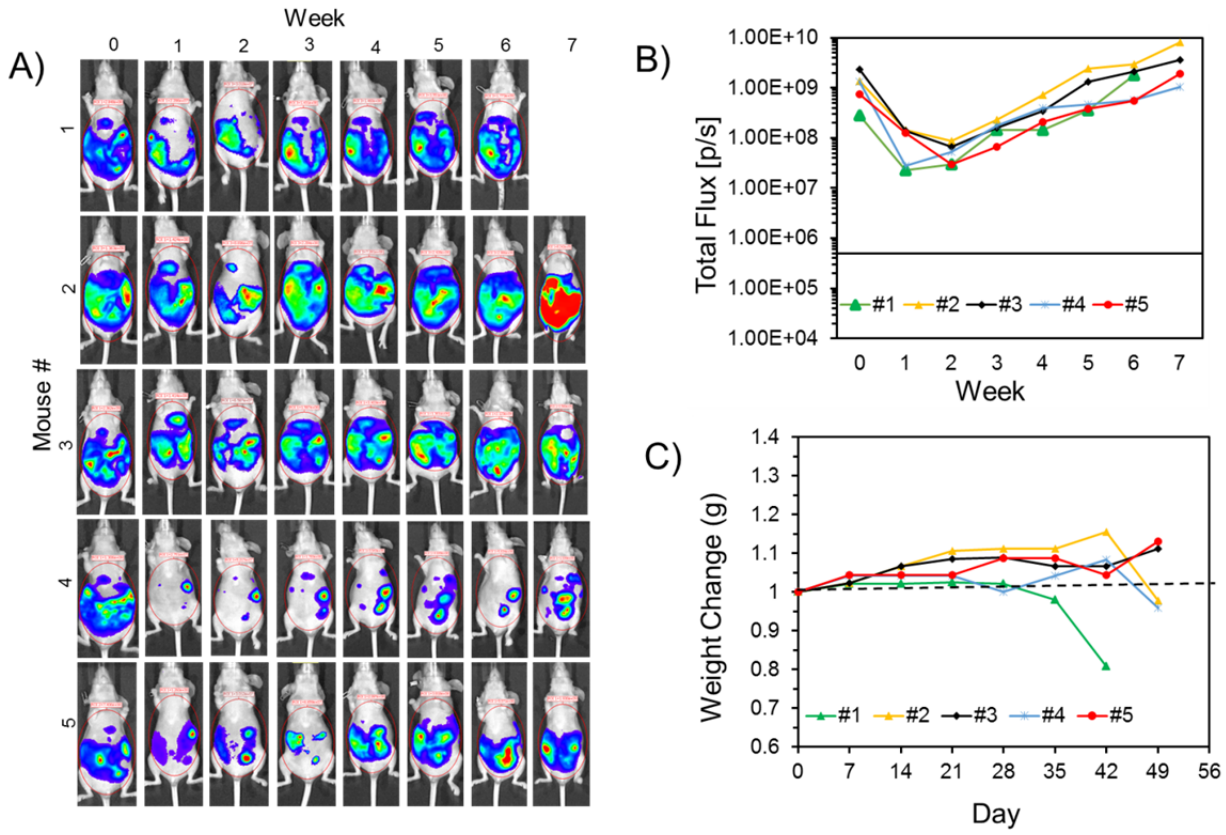


Figure S15: A) BLI of nude mice with intraperitoneal tumors (firefly luciferase imaging). B) The quantification of the bioluminescent signal over the period of seven weeks. The line at 5×10^5 (p/s) shows the background bioluminescence of mice body without any tumors. C) Change in mice weight over the period of seven weeks. The weights of the mice before the treatment are normalized and considered as 1.

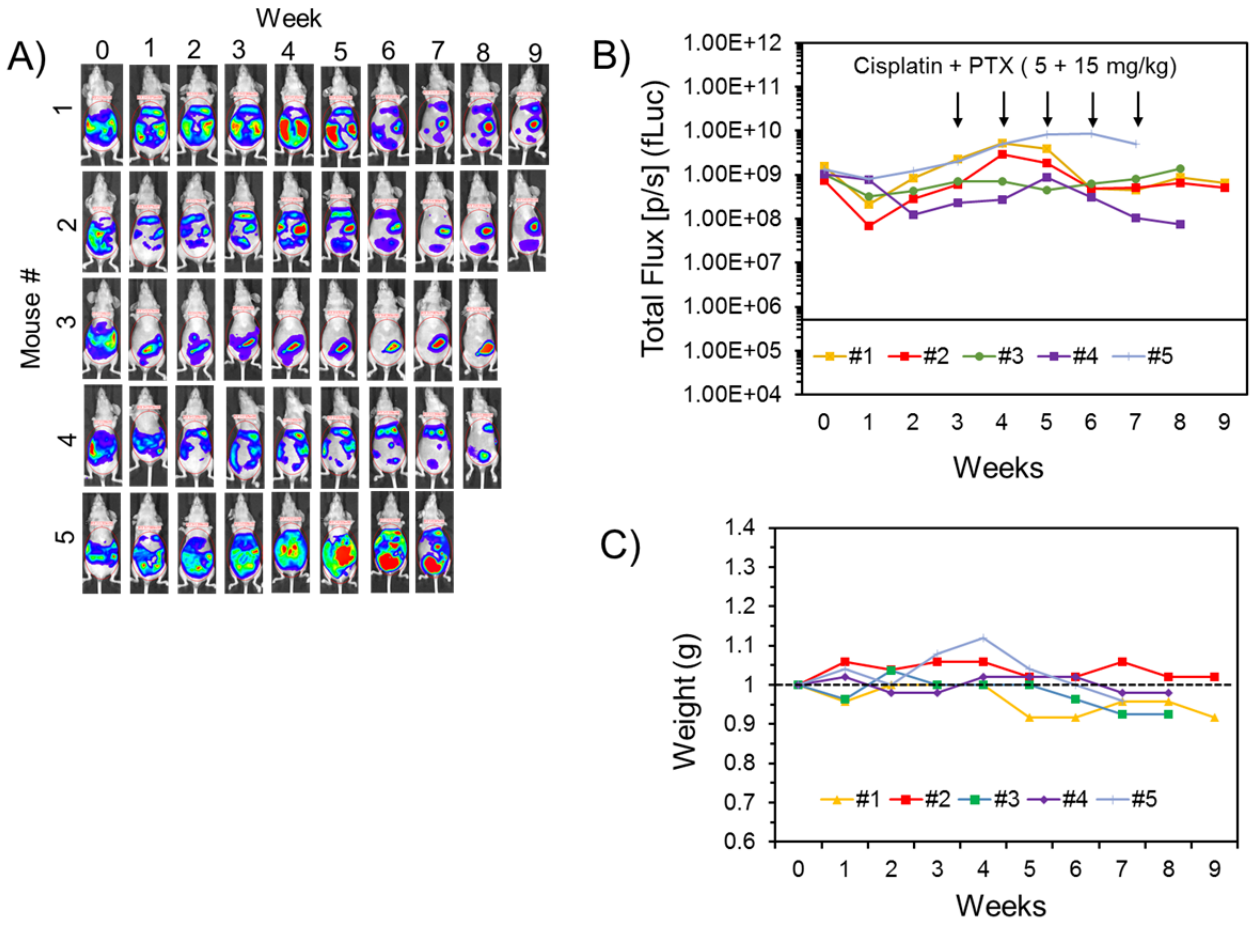


Figure S16: A) BLI of nude mice with intraperitoneal tumors (firefly luciferase imaging). B) The quantification of the bioluminescent signal over the treatment period with cisplatin and PTX. Arrows denote the points at which the mice received treatment. The line at 5×10^5 (p/s) shows the background bioluminescence of mice body without any tumors. C) Change in mice weight over the period of nine weeks. The weights of the mice before the treatment are normalized and considered as 1.

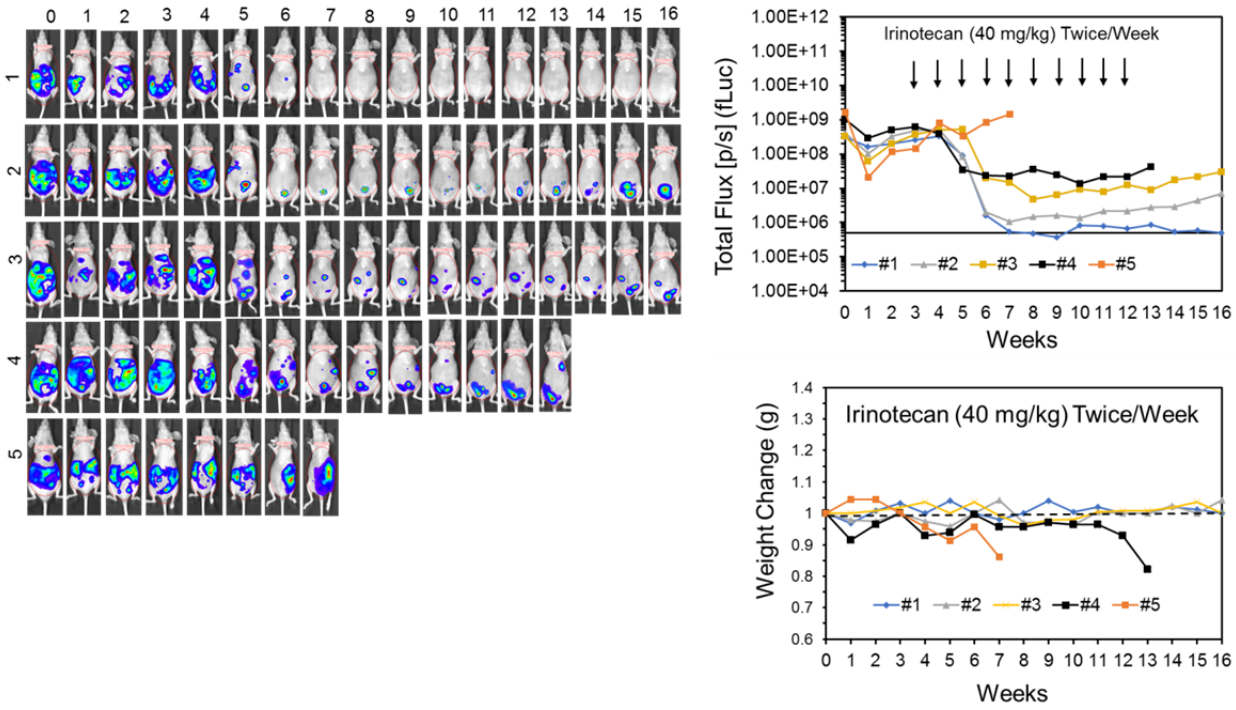
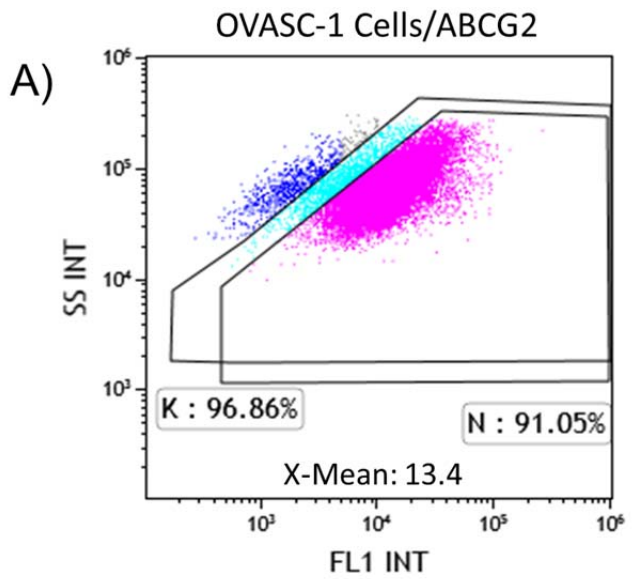
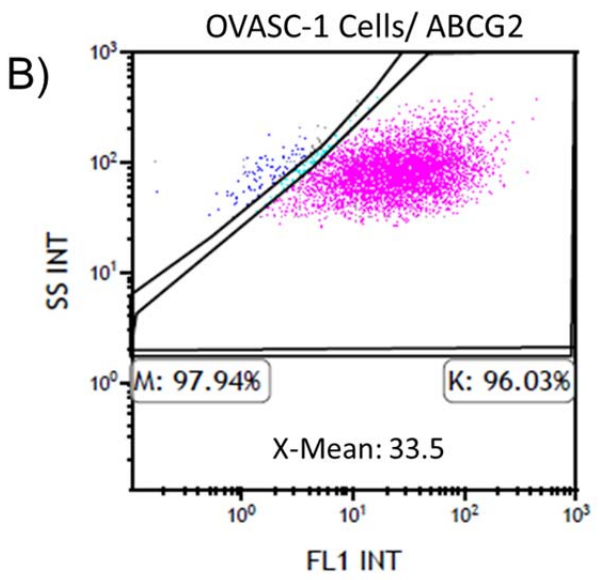


Figure S17: A) BLI of nude mice with intraperitoneal tumors (firefly luciferase imaging). B) The quantification of the bioluminescent signal over the treatment period with irinotecan (40 mg/kg). Arrows denote the points at which the mice received treatment. The line at 5×10^5 (p/s) shows the background bioluminescence of mice body without any tumors. C) Change in mice weight over the treatment period. The weights of the mice before the treatment are normalized and considered as 1.



Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	25000	85.44	100	13.4	69.3
K	24219	82.77	96.86	13.8	68.7
N	22737	77.71	91.05	14.3	67.2



Gate	Number	%Total	%Gated	X-Mean	Y-Mean
All	5,837	76.69	100	33.5	88.48
M	5,717	75.11	97.94	34.15	88.29
K	5,605	73.64	96.03	34.74	88

Figure S18: Evaluation of the expression of ABCG2 transporters on the surface of OVASC-1 cells. A) OVASC-1 cells grown in cell culture (in vitro) were labeled with fluorescent anti-ABCG2 primary antibody and analyzed by flow cytometry. X-mean (Mean Fluorescence Intensity) denotes the fold difference in expression of the transporter on the surface of OVASC-1 cells stained with the anti-AGCG2 antibody in comparison to the IgG isotype control indicating the transporter density. B) OVASC-1 cells were harvested from the tumor of the non-responsive mouse that was treated with irinotecan (40 mg/kg). Cells were propagated in vitro, labeled with fluorescent anti-ABCG2 primary antibody, and then analyzed by flow cytometry. X-mean denotes the fold difference in expression of the transporter on the surface of OVASC-1 cells stained with the anti-AGCG2 antibody in comparison to the IgG isotype control indicating the transporter density.

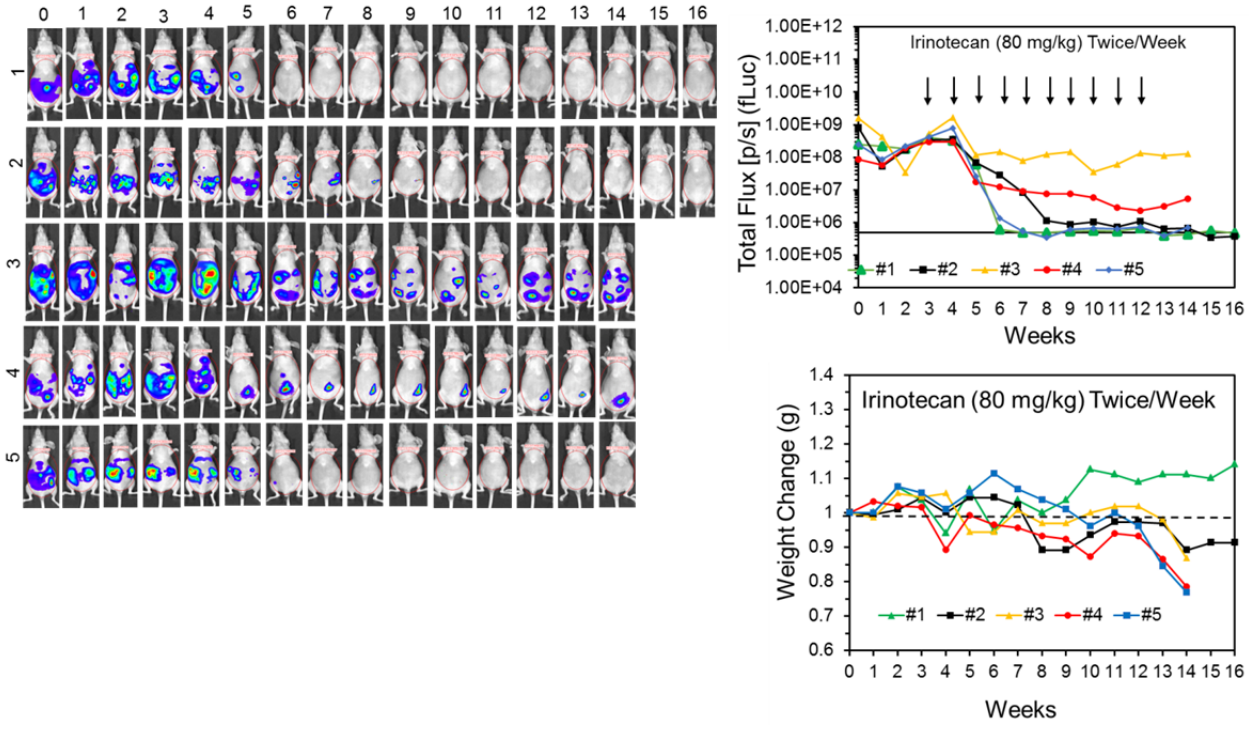


Figure S19: A) BLI of nude mice with intraperitoneal tumors (firefly luciferase imaging). B) The quantification of bioluminescent signal over the treatment period with irinotecan (80 mg/kg). Arrows denote the points at which the mice received treatment. The line at 5×10^5 (p/s) shows the background bioluminescence of mice body without any tumors. C) Change in mice weight over the treatment period. The weights of the mice before the treatment are normalized and considered as 1.

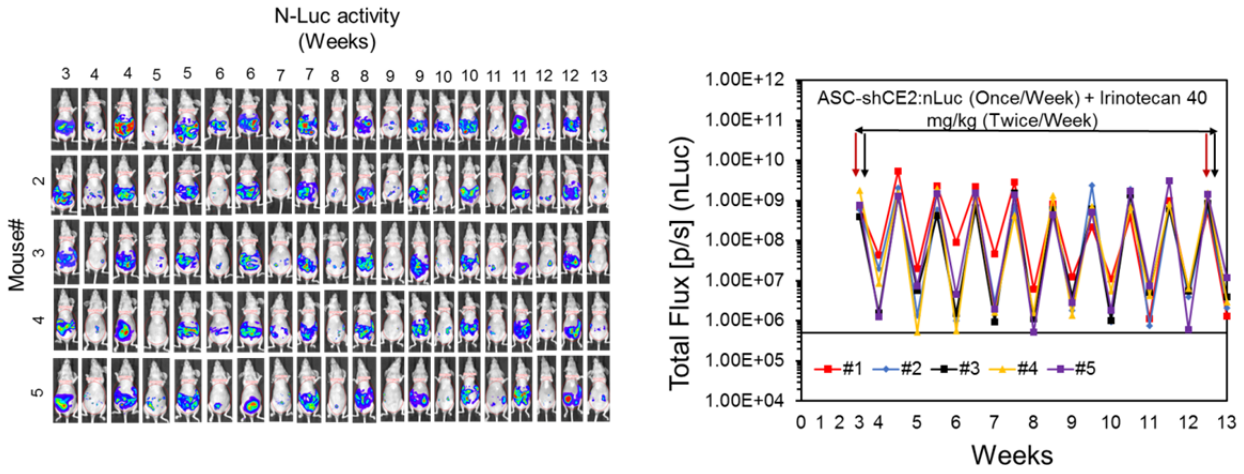


Figure S20: A) BLI of nude mice injected with ASC-shCE2:nLuc cells once a week for ten weeks and irinotecan (40 mg/kg) twice a week for ten weeks. B) The quantification of bioluminescent signal (nanoluciferase) from the ASC-shCE2:nLuc cells over the treatment period with irinotecan (40 mg/kg). Red arrow denotes the point at which the mice received cells and black arrow denotes the point at which the mice received irinotecan. The line at 5 $\times 10^5$ (p/s) shows the background bioluminescence of mice body without any tumors. The significant drop in bioluminescent signal intensity indicates the death of ASC-shCE2:nLuc cells after irinotecan injection.

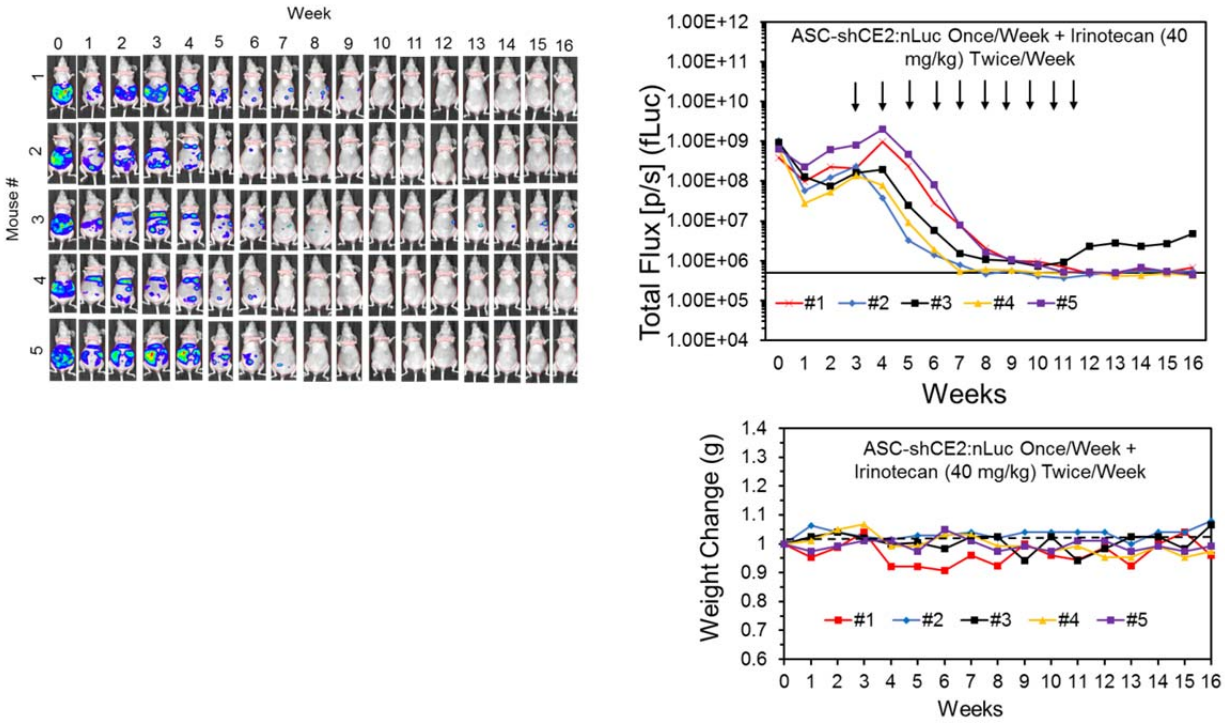


Figure S21: A) BLI of nude mice with intraperitoneal tumors (firefly luciferase imaging). B) The quantification of the bioluminescent signal over the treatment period with irinotecan (40 mg/kg). Arrows denote the points at which the mice received treatment. The line at 5×10^5 (p/s) shows the background bioluminescence of mice body without any tumors. C) Change in mice weight over the treatment period. The weights of the mice before the treatment are normalized and considered as 1.

Treatment	Mean (Weeks)				Survival
	Estimate	Std. Error	95% Confidence Interval		Percent
			Lower Bound	Upper Bound	
Control, untreated	6.800	.200	6.408	7.192	0%
Cisplatin + PTX (5 + 15 mg/kg)	8.200	.374	7.467	8.933	0%
Irinotecan (40 mg/kg)	14.000	1.720	10.628	17.372	20%
Irinotecan (80 mg/kg)	15.800	.820	14.193	17.407	40%
ASC + Irinotecan (40 mg/kg)	17.600	.358	16.899	18.301	80%

Figure S22: Kaplan-Meier survival estimator data highlighting the increase in survival time and percent survival rate among the groups.

Test	Vehicle control	CPT-11 40 mg/kg	CPT-11 80 mg/kg	ASC-shCE2 + CPT-11 (40 mg/kg)	Normal range	Units
WBC	2.37	5.82	3.2	5.29	0.80 - 10.60	10^3/µL
NEU	0.42	1.17	0.52	1.59	0.23 - 3.60	10^3/µL
LYM	1.80	3.51	2.03	2.47	0.60 - 8.90	10^3/µL
MONO	0.12	0.95	0.31	0.57	0.04 - 1.40	10^3/µL
EOS	0.03	0.13	0.29	**0.52**	0.00 - 0.51	10^3/µL
BAS	0.01	0.06	0.05	0.01	0.00 - 0.12	10^3/µL
RBC	7.73	8.22	7.79	7.81	6.50 - 11.50	10^6/µL
HGB	15.5	14.7	14.6	14.8	11.00 - 16.50	g/dL
HCT	42.8	43.8	43	43.7	35.00 - 55.00	%
MCV	**55.3**	53.3	55.2	**55.65**	41.00 - 55.00	fL
MCH	19.3	17.9	18.7	18.65	13.00 - 18.00	pg
MCHC	35.1	33.5	33.9	33.7	30.00 - 36.00	g/dL
RDW %	12.05	13.7	14.1	12.85	12.00 - 19.00	%
PLT	**356**	1347	778	533	400 - 1600	10^3/µL
MPV	6.15	5.4	7.3	5.9	4.00 - 6.2	fL

Figure S23: Analysis of blood factors in mice groups 1 to 5. No significant hematological toxicity was observed in mice of any group. Irinotecan (CPT-11).