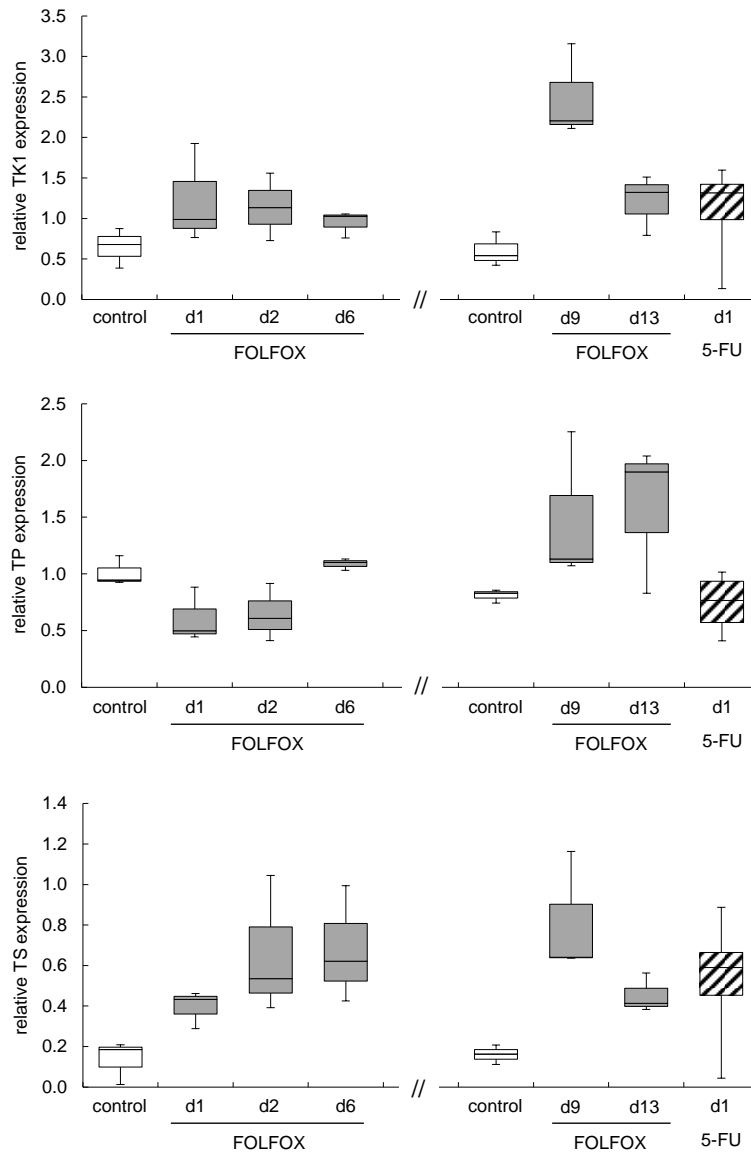
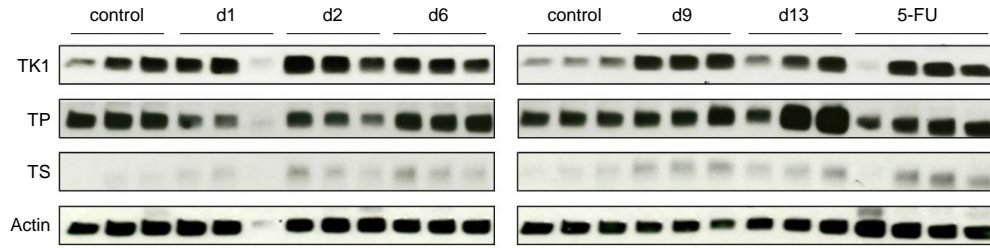
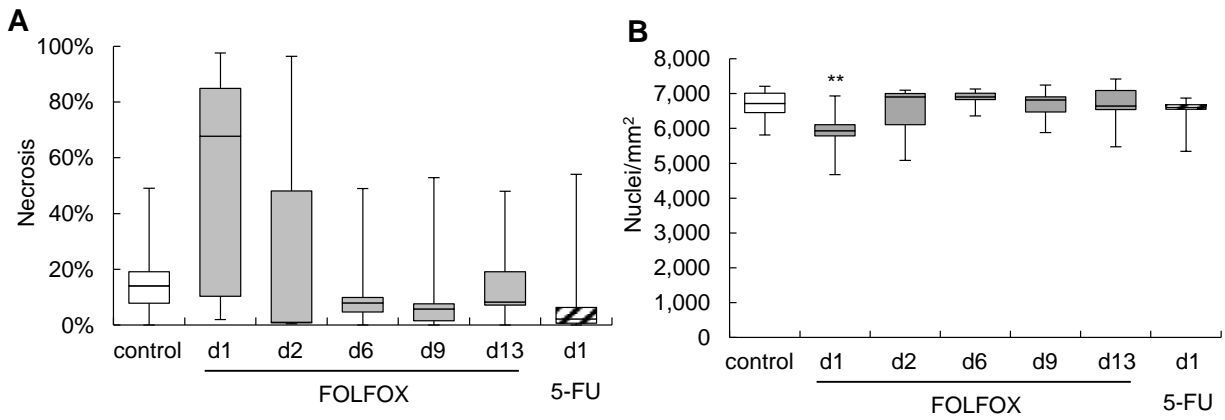


SUPPLEMENTAL FIGURE 1. MR images of a FOLFOX treated tumor reflect variability of ADC. Transverse slices of a tumor at different time points of FOLFOX therapy are depicted here. The upper panel shows T2 weighted images, which were used for the definition of ROIs. ADC maps in the lower panel reveal a variable and heterogeneous distribution of water diffusion and show a trend for reduced ADC_{mean} . Scale bar = 2 mm.

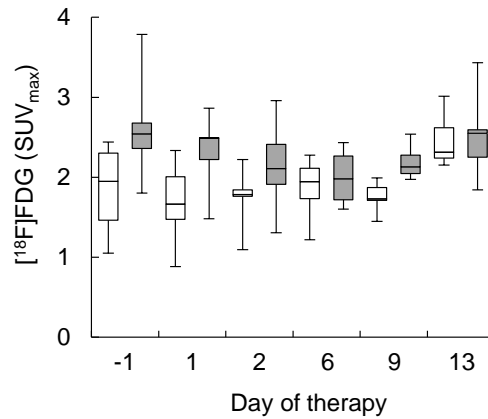
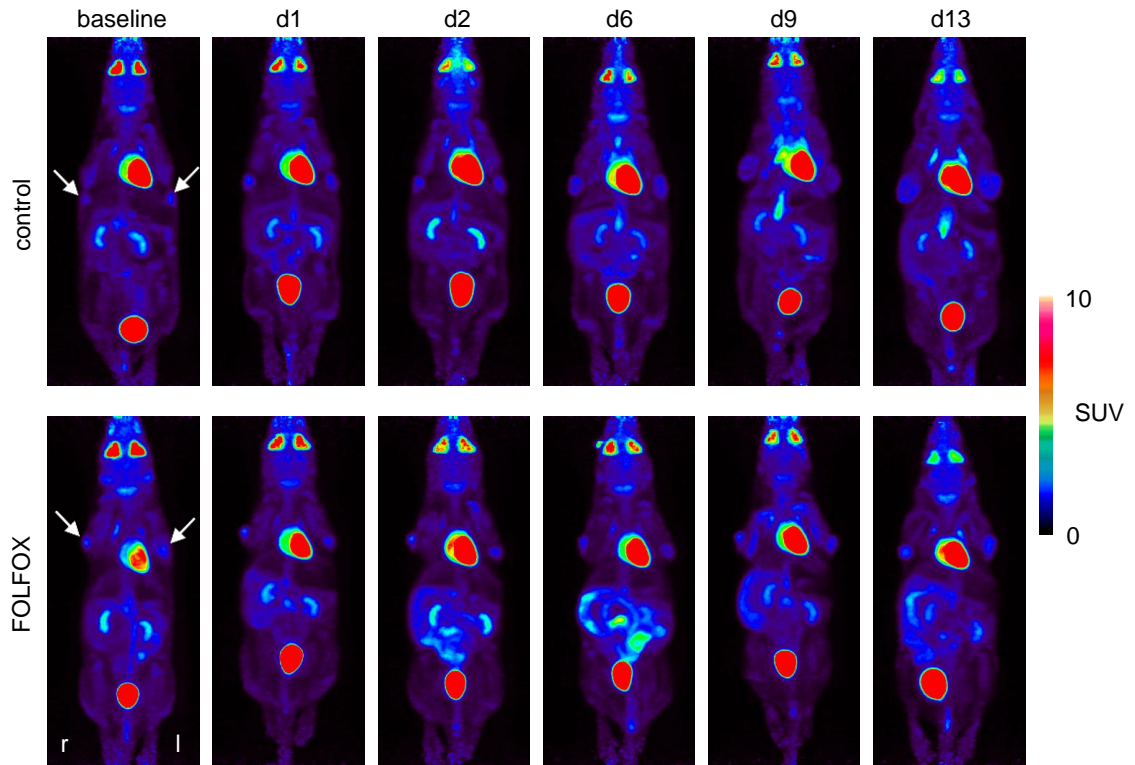


SUPPLEMENTAL FIGURE 2. Western blots of Colo205 xenografts revealed upregulation of different proteins involved in thymidine metabolism after FOLFOX therapy. However, non-parametric testing of such low sample sizes does not allow detection of statistically significant differences relative to controls

on the respective gel. Expression levels are expressed relative to actin loading control. White: control, grey: FOLFOX, striped: 5-FU.



SUPPLEMENTAL FIGURE 3. (A) The percent necrotic area, determined on H&E sections, was highly variable but not significantly altered upon FOLFOX or 5-FU therapy. (B) Cellular density, determined from Ki67 immunohistochemistry, which can also be interpreted as an indicator of cell death, was not altered in a biologically relevant manner. White: control, grey: FOLFOX, striped: 5-FU; **: $P < 0.01$ relative to control.



SUPPLEMENTAL FIGURE 4. Uptake of $[^{18}\text{F}]\text{FDG}$ was not affected by FOLFOX therapy in Colo205 xenografts. Tumors are indicated by white arrows in the maximum-intensity-projections of representative mice. White: control, grey: FOLFOX.

Supplemental Table S2 – S12 show means \pm standard deviation of results presented in this manuscript. Numbers of samples are presented in brackets, if not indicated otherwise.

SUPPLEMENTAL TABLE 1. Experimental schedule.

tumor	readout	therapy	# of mice and time points
Colo205	[¹⁸ F]FLT, ADC	control	4M repetitively (bl [‡] , d1, d2, d6, d9 [§] , d13 ^{†, ¶})
		FOLFOX	4M repetitively (bl [‡] , d1, d2, d6, d9 [§] , d13 ^{†, ¶})
		5-FU	2M d1*
	[¹⁸ F]FDG	control	3M repetitively (bl, d1, d2, d6, d9, d13*)
		FOLFOX	3M repetitively (bl, d1, d2, d6, d9, d13*)
		<i>ex vivo</i> analyses*	control
HCT116	[¹⁸ F]FLT	FOLFOX	4M d1, 4M d2, 2M d6, 2M d9, 2M d13
		5-FU	2M d1
		control	3M bl and d1
		FOLFOX	3M bl and d1

*: Mice used for *ex vivo* analyses: tumors were cut in three pieces and subjected to histology, western blot and thymidine analysis. Plasma thymidine was also analyzed.

†: 2M were subjected to *ex vivo* analyses after completion of the imaging schedule.

‡: due to problems in tracer synthesis, only 2M obtained a baseline PET scan

§: 2M only for FLT

¶: 2M only for MRI

bl: baseline; M: mice (with two tumors each)

SUPPLEMENTAL TABLE 2. Growth of FOLFOX and control treated Colo205 tumors, expressed as change in volume relative to treatment initiation (see Fig. 1A). *n* = 8 tumors were followed per treatment group.

	d2	d4	d7	d9	d11	d14	d16
control	1.16 ± 0.26	1.49 ± 0.56	1.92 ± 0.82	2.13 ± 0.91	2.41 ± 1.07	3.00 ± 1.05	3.36 ± 1.18
FOLFOX	0.90 ± 0.18*	0.87 ± 0.14**	0.90 ± 0.17**	0.96 ± 0.25**	1.00 ± 0.28**	1.29 ± 0.47**	1.35 ± 0.47***

*: *P* < 0.05, **: *P* < 0.01, ***: *P* < 0.001 relative to control

SUPPLEMENTAL TABLE 3. [¹⁸F]FLT PET results of Colo205 xenografts (see Fig. 1C).

		baseline	d1	d2	d6	d9	d13
<i>n</i> =	control	4	5	5	4	8	8
	FOLFOX	4	8	8	4	8	8
	5-FU		4				
SUV _{max}	control	1.39 ± 0.46	1.63 ± 0.41	1.61 ± 0.39	1.69 ± 0.23	1.68 ± 0.24	1.70 ± 0.32
	FOLFOX	2.24 ± 0.89	16.54 ± 1.97 †† ***	10.44 ± 1.42 †† ***	3.65 ± 0.57 † *	7.32 ± 1.59 †† ***	5.08 ± 1.51 †† ***
	5-FU		11.63 ± 2.48 ** ##				
SUV _{mean}	control	0.89 ± 0.20	0.94 ± 0.15	0.93 ± 0.20	1.06 ± 0.13	1.02 ± 0.09	1.02 ± 0.20
	FOLFOX	1.13 ± 0.37	4.24 ± 1.28 †† ***	3.39 ± 0.64 †† ***	1.54 ± 0.42	2.50 ± 0.68 †† ***	2.10 ± 0.57 †† ***
	5-FU		2.95 ± 0.97 **				
%ID _{max} /mL	control	6.37 ± 2.57	7.13 ± 1.80	7.15 ± 1.92	6.47 ± 1.11	6.99 ± 1.21	6.95 ± 1.58
	FOLFOX	9.69 ± 3.91	69.40 ± 8.94 †† ***	45.14 ± 6.53 †† ***	15.84 ± 4.47 *	31.90 ± 8.26 †† ***	21.68 ± 8.24 †† ***
	5-FU		50.41 ± 9.78 ** ##				
%ID _{mean} /mL	control	4.07 ± 1.26	4.11 ± 0.66	4.09 ± 0.86	4.07 ± 0.72	4.25 ± 0.63	4.19 ± 1.04
	FOLFOX	4.90 ± 1.62	17.88 ± 5.64 †† ***	14.70 ± 2.95 †† ***	6.70 ± 2.52	11.02 ± 3.85 †† ***	8.93 ± 3.09 †† ***
	5-FU		12.74 ± 4.20 **				
T-M	control	1.71 ± 0.63	2.02 ± 0.48	1.90 ± 0.44	2.01 ± 0.27	1.92 ± 0.28	2.08 ± 0.45
	FOLFOX	2.75 ± 1.23	20.52 ± 3.73 †† ***	13.40 ± 1.72 †† ***	4.90 ± 0.63 † *	10.19 ± 1.98 †† ***	7.22 ± 2.63 †† ***
	5-FU		17.15 ± 5.32 **				
T-L	control	1.40 ± 0.57	1.63 ± 0.32	1.65 ± 0.37	1.65 ± 0.20	1.62 ± 0.27	1.67 ± 0.35
	FOLFOX	2.14 ± 0.859	17.64 ± 3.39 †† ***	10.84 ± 2.46 †† ***	3.58 ± 0.32 † *	8.28 ± 2.03 †† ***	5.79 ± 2.03 †† ***
	5-FU		14.08 ± 4.07 **				

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ relative to NaCl; ††: $P < 0.01$, †††: $P < 0.001$ relative to baseline; ##: $P < 0.01$ relative to FOLFOX. T-M: tumor-to-muscle ratio, T-L: tumor-to-liver ratio.

SUPPLEMENTAL TABLE 4. ADC_{mean} of FOLFOX / 5-FU treated Colo205 xenografts, displayed in 10⁻³ mm²/s (see Fig. 1D).

	baseline	d1	d2	d6	d9	d13
control	1.004 ± 0.141 (5)	0.927 ± 0.091 (7)	0.899 ± 0.081 (7)	0.824 ± 0.081 (6)	0.780 ± 0.101 (10)	0.784 ± 0.083 (2)
FOLFOX	1.163 ± 0.200 (7)	1.035 ± 0.110 (8)	0.986 ± 0.074 (7) [†]	0.892 ± 0.087 (6) [†]	0.995 ± 0.079 (8) ^{†**}	0.799 ± 0.082 (4) [†]
5-FU	0.926 ± 0.107 (4)	0.975 ± 0.200 (4)				

***P* < 0.01 relative to control; [†]*P* < 0.05 relative to baseline

SUPPLEMENTAL TABLE 5. Immunohistochemical analysis of proliferation in FOLFOX, 5-FU and control treated tumors (see Fig. 2).

	control	d1 FOLFOX	d2 FOLFOX	d6 FOLFOX	d9 FOLFOX	d13 FOLFOX	d1 5-FU
% Ki67-positive nuclei	69.3 ± 7.5 (18)	68.4 ± 12.9 (8)	86.7 ± 3.2 (7) ^{***}	75.9 ± 5.3 (8) [*]	76.9 ± 6.0 (8) [*]	75.1 ± 5.3 (14) [*]	72.4 ± 14.9 (7)
% TK1-positive area	30.3 ± 1.5 (5)	53.5 ± 3.8 (3) [*]	55.2 ± 4.1 (4) [*]	35.5 ± 5.9 (5)	48.0 ± 2.8 (4) [*]	36.3 ± 1.8 (4) [*]	59.9 ± 6.8 (3) [*]

P* < 0.05, **P* < 0.001 relative to control

SUPPLEMENTAL TABLE 6. Western blot analysis of TK1, TP and TS expression in FOLFOX, 5-FU and control treated Colo205 tumors (see Supplemental Fig. S2), expressed as band intensity relative to actin. Protein levels were not statistically different from respective controls.

	control (3)	d1 FOLFOX (3)	d2 FOLFOX (3)	d6 FOLFOX (3)	control (3)	d9 FOLFOX (3)	d13 FOLFOX (3)	d1 5-FU (4)
TK1	0.65 ± 0.25	1.23 ± 0.62	1.14 ± 0.42	0.95 ± 0.16	0.60 ± 0.21	2.49 ± 0.58	1.21 ± 0.37	1.09 ± 0.65
TP	1.01 ± 0.13	0.61 ± 0.24	0.65 ± 0.25	1.09 ± 0.05	0.81 ± 0.06	1.49 ± 0.67	1.59 ± 0.66	0.74 ± 0.27
TS	0.14 ± 0.11	0.39 ± 0.09	0.66 ± 0.34	0.68 ± 0.29	0.16 ± 0.05	0.81 ± 0.30	0.45 ± 0.10	0.53 ± 0.35

SUPPLEMENTAL TABLE 7. Thymidine levels of plasma and Colo205 tumors from FOLFOX treated mice (see Fig. 3), expressed in μM.

	control	d1 FOLFOX	d2 FOLFOX	d6 FOLFOX	d9 FOLFOX	d13 FOLFOX	d1 5-FU
Plasma	1.03 ± 0.31 (9)	0.95 ± 0.19 (4)	0.71 ± 0.26 (4)	0.54 ± 0.20 (4) [*]	0.51 ± 0.08 (4) ^{**}	0.65 ± 0.20 (7) [*]	1.11 ± 0.25 (4)
Tumor	3.51 ± 1.87 (6)	4.71 ± 3.68 (4)	2.06 ± 4.40 (3)	1.85 ± 0.76 (3) [*]	1.54 ± 0.57 (4) [*]	1.69 ± 0.48 (4) [*]	2.03 ± 1.53 (4)

P* < 0.05, *P* < 0.01 relative to control

SUPPLEMENTAL TABLE 8. Immunohistochemical analysis of cell death in Colo205 tumors (see Fig. 4 and Supplemental Fig. 3)

	control	d1 FOLFOX	d2 FOLFOX	d6 FOLFOX	d9 FOLFOX	d13 FOLFOX	d1 5-FU
% Casp3-positive area	1.35 ± 0.25 (6)	1.24 ± 0.07 (4)	1.33 ± 0.29 (3)	1.20 ± 0.31 (4)	1.45 ± 0.41 (5)	3.24 ± 0.71 (7)**	1.30 ± 0.12 (5)
% TUNEL-positive area	0.57 ± 0.6 (4)	0.50 ± 0.04 (3)	0.51 ± 0.05 (3)	0.53 ± 0.18 (4)	1.08 ± 0.08 (4)*	1.15 ± 0.04 (5)*	0.51 ± 0.13 (5)
% Necrotic area	14.3 ± 11.9 (18)	52.9 ± 40.7 (8)***	27.9 ± 38.5 (7)	11.6 ± 15.5 (8)	10.3 ± 17.5 (8)	13.7 ± 12.3 (14)	10.0 ± 19.6 (7)
Nuclei/mm ²	6,682 ± 411 (18)	5,890 ± 648 (8)**	6,471 ± 833 (7)	6,876 ± 239 (8)	6,656 ± 478 (8)	6,745 ± 505 (14)	6,467 ± 507 (7)

*: $P < 0.05$, **: $P < 0.01$ relative to control

SUPPLEMENTAL TABLE 9. Immunohistochemical analysis of γ H2AX in Colo205 tumors (see Fig. 5)

	control	d1 FOLFOX	d2 FOLFOX	d6 FOLFOX	d9 FOLFOX	d13 FOLFOX	d1 5-FU
% γ H2AX-positive area	9.3 ± 0.7 (6)	34.7 ± 3.5 (4)	32.1 ± 3.2 (5)	19.6 ± 1.9 (5)	28.2 ± 5.3 (4)	28.2 ± 4.1 (4)	37.8 ± 3.1 (4)

*: $P < 0.05$, **: $P < 0.01$ relative to control

SUPPLEMENTAL TABLE 10. Growth of FOLFOX treated HCT116 xenografts, expressed as change in volume relative to treatment initiation (see Fig. 6A).

	d7	d14	d21	d28	d35
control	1.52 ± 0.87 (12)	2.25 ± 1.03 (12)	3.21 ± 1.27 (12)	5.33 ± 3.09 (12)	8.47 ± 5.36 (12)
FOLFOX	1.34 ± 0.57 (15)	2.26 ± 1.26 (15)	3.29 ± 2.10 (13)	3.89 ± 1.49 (10)	6.78 ± 3.33 (10)

SUPPLEMENTAL TABLE 11. [¹⁸F]FLT PET results of FOLFOX treated HCT116 xenografts (see Fig. 6B).

		baseline	d1
<i>n</i> =	control	3	3
	FOLFOX	4	4
SUV _{max}	control	2.14 ± 0.82	2.77 ± 0.32
	FOLFOX	2.66 ± 0.38	3.08 ± 0.32
SUV _{mean}	control	1.06 ± 0.35	1.36 ± 0.10
	FOLFOX	1.45 ± 0.10	1.42 ± 0.22
%ID _{max} /mL	control	7.68 ± 2.41	10.49 ± 1.21 †
	FOLFOX	10.62 ± 1.17	13.27 ± 1.22
%ID _{mean} /mL	control	3.82 ± 0.98	5.19 ± 0.86
	FOLFOX	5.82 ± 0.38	6.11 ± 0.90
T-M	control	2.89 ± 1.42	3.09 ± 0.43
	FOLFOX	3.44 ± 0.66	3.44 ± 0.27
T-L	control	2.20 ± 0.92	2.79 ± 0.81
	FOLFOX	2.50 ± 0.45	2.84 ± 0.23

†: *P* < 0.05 relative to baseline

SUPPLEMENTAL TABLE 12. [¹⁸F]FDG PET results of FOLFOX treated Colo205 tumors (see Supplemental Fig. 4).

		baseline	d1	d2	d6	d9	d13
<i>n</i> =	control	6	6	6	6	6	6
	FOLFOX	5	5	5	4	4	5
SUV _{max}	control	1.85 ± 0.56	1.68 ± 0.52	1.75 ± 0.37	1.87 ± 0.38	1.75 ± 0.19	2.45 ± 0.34
	FOLFOX	2.63 ± 0.72	2.31 ± 0.52	2.14 ± 0.61	2.00 ± 0.39	2.19 ± 0.25 *	2.53 ± 0.58
SUV _{mean}	control	0.91 ± 0.26	0.84 ± 0.16	0.81 ± 0.14	0.87 ± 0.22	0.86 ± 0.15	1.26 ± 0.16
	FOLFOX	1.28 ± 0.13	0.83 ± 0.10 ††	0.95 ± 0.13 ††	0.84 ± 0.12 †	1.07 ± 0.07 *	1.23 ± 0.24
%ID _{max} /mL	control	8.18 ± 2.49	7.47 ± 2.00	7.76 ± 1.48	8.13 ± 1.88	7.83 ± 1.42	10.57 ± 1.80
	FOLFOX	11.32 ± 4.13	9.79 ± 2.23	9.44 ± 2.51	8.75 ± 1.93	9.49 ± 1.12	10.78 ± 2.43
%ID _{mean} /mL	control	4.03 ± 1.21	3.75 ± 0.64 ††	3.59 ± 0.59 †	3.80 ± 1.00 †	3.81 ± 0.78	5.40 ± 0.79
	FOLFOX	5.43 ± 0.74	3.52 ± 0.55	4.22 ± 0.53	3.69 ± 0.65	4.62 ± 0.25	5.25 ± 0.99
T-M	control	7.39 ± 2.19	5.86 ± 2.35	8.23 ± 2.58	10.40 ± 3.69	7.16 ± 2.12	10.12 ± 1.14
	FOLFOX	9.92 ± 4.52	11.59 ± 3.37 *	12.25 ± 4.25	10.56 ± 1.54	13.73 ± 1.91 *	13.70 ± 4.25
T-L	control	5.95 ± 2.17	5.43 ± 2.68	4.90 ± 1.49	4.46 ± 2.38	4.11 ± 0.92	5.16 ± 1.03
	FOLFOX	6.38 ± 1.91	6.43 ± 1.48	5.73 ± 1.64	5.80 ± 1.57	5.70 ± 1.24	4.92 ± 1.49

*: *P* < 0.05 relative to NaCl; †: *P* < 0.05, ††: *P* < 0.01 relative to baseline; T-M: tumor-to-muscle ratio, T-L: tumor-to-liver ratio.