Supplemental Methods

^{123}I-IIMU synthesis

The precursor 6-(1-(2-iminoimidazolidinyl)-methyl)-uracil trifluoroacetate was synthesized as described previously *(24)*. Radiotracer synthesis was achieved by radioiodination of the precursor at the C-5 position. A solution of ^{123}I-NH_4I (587 MBq/mL) was added to 1 M NaOH (8 μL), and the solvent was removed at 110°C. An acetone solution (20 μL) containing acetate (0.2 M) and an acetone solution (150 μL) containing *N*-chlorosuccinimide (0.29 μg/μL) were mixed in a reaction vial and allowed to stand for 10 min at room temperature. Acetone was then removed under argon gas. A solution of the precursor (2.3 μg/μL) in aqueous acetonitrile (H_2O/CH_3CN = 1:3, 150 μL) was added to the residue, and the vial was heated for 35 min at 50°C. After removing the solvent, a hydrochloric acid solution (20 mM, 0.8 mL) was added to convert trifluoroacetate to hydrochloride. The mixture was allowed to stand for 5 min at room temperature. The crude product was purified using reversed-phase high performance liquid chromatography (Waters Corporation, Milford, MA) on a Develosil PRAQUEOUS column with a 10-mm diameter and 250-mm length (Nomura Chemical, Seto, Japan); the mobile phase for elution consisted of 0.01 M hydrochloric acid at a flow rate of 4.0 mL/min. The radioactive fraction corresponding to the authentic IIMU retention time (47.0 min) was separated from the precursor and by-products. The separated fraction was added to a 7% sodium bicarbonate solution (90 μL) (Otsuka Pharmaceutical Factory, Naruto, Japan). The radiochemical purity of the labeled compound was determined using Silica Gel 60 F254 thin-

layer chromatography with a mobile phase containing $CHCl_3$/MeOH/H_2O = 4:4:1. Radioactivity was detected using a radioactivity thin-layer chromatography detector (RITA*, Raytest, Straubenhardt, Germany). The radiochemical yield of ^{123}I-IIMU was 10.9%, and radiochemical purity was >99%. Figure 1 shows the ^{123}I-IIMU structure.

Electrophoresis and western blot

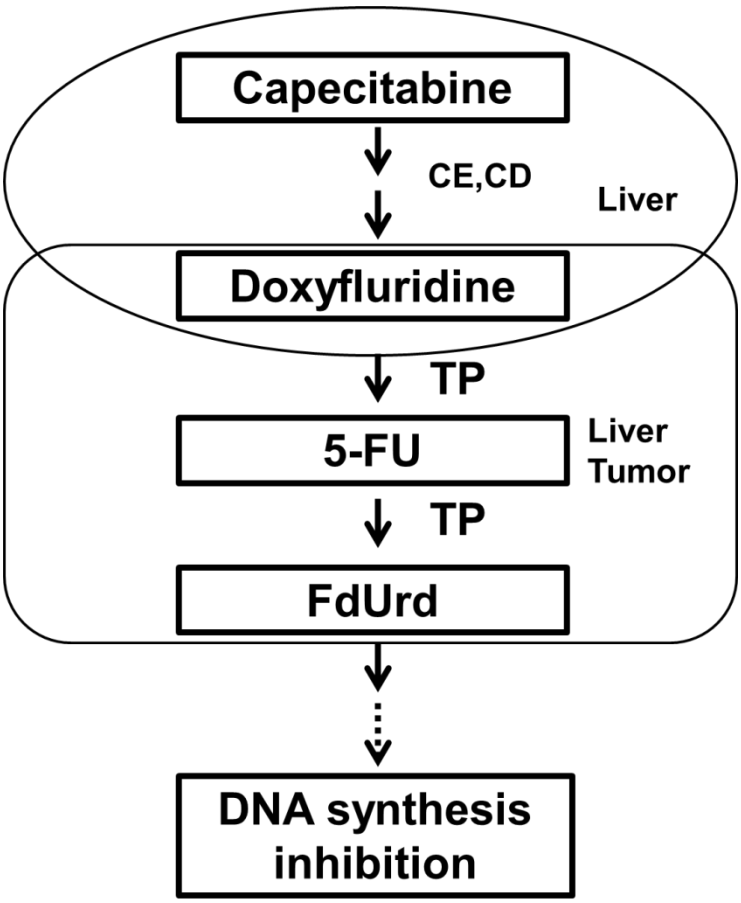
Cells were lysed in cell lysis buffer (Cell Signaling Technology, Beverly, MA). After sonication, cell lysates were centrifuged (15,000 × *g*, 15 min), and supernatants were collected. A three-fold volume of blue loading buffer (Cell Signaling Technology) was added to the supernatants, and samples were boiled for 5 min. The proteins in samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with ImmunoBlock (DS Pharma Biomedical, Suita, Japan) and then incubated with mouse monoclonal anti-human TP (THP11-M, Alpha Diagnostic International, San Antonio, TX) or mouse monoclonal anti-actin (A5441, Sigma-Aldrich, St Louis, MO) antibodies in Tris-buffered saline plus 0.1% Tween-20 and 10% ImmunoBlock (TBST, TBS from Bio-Rad Laboratories). After membrane incubation with anti-mouse IgG peroxidase (A0168, Sigma-Aldrich), immunoreactive bands were detected using ECL Prime Western Blotting Detection Reagent (GE Healthcare UK, Buckinghamshire, England). Recombinant

TP protein (R&D Systems, Minneapolis, MN) was used as a reference. Western blot analysis was repeated three times on different days.

Supplemental Table 1. **Biodistribution of radioactivity 30 min after administration of ^{123}I-IIMU to tumor-bearing mice.** Results are expressed as the mean ± SEM of three independent experiments. ^{123}I-IIMU uptake is indicated as %ID/g, except for in the urinary bladder.

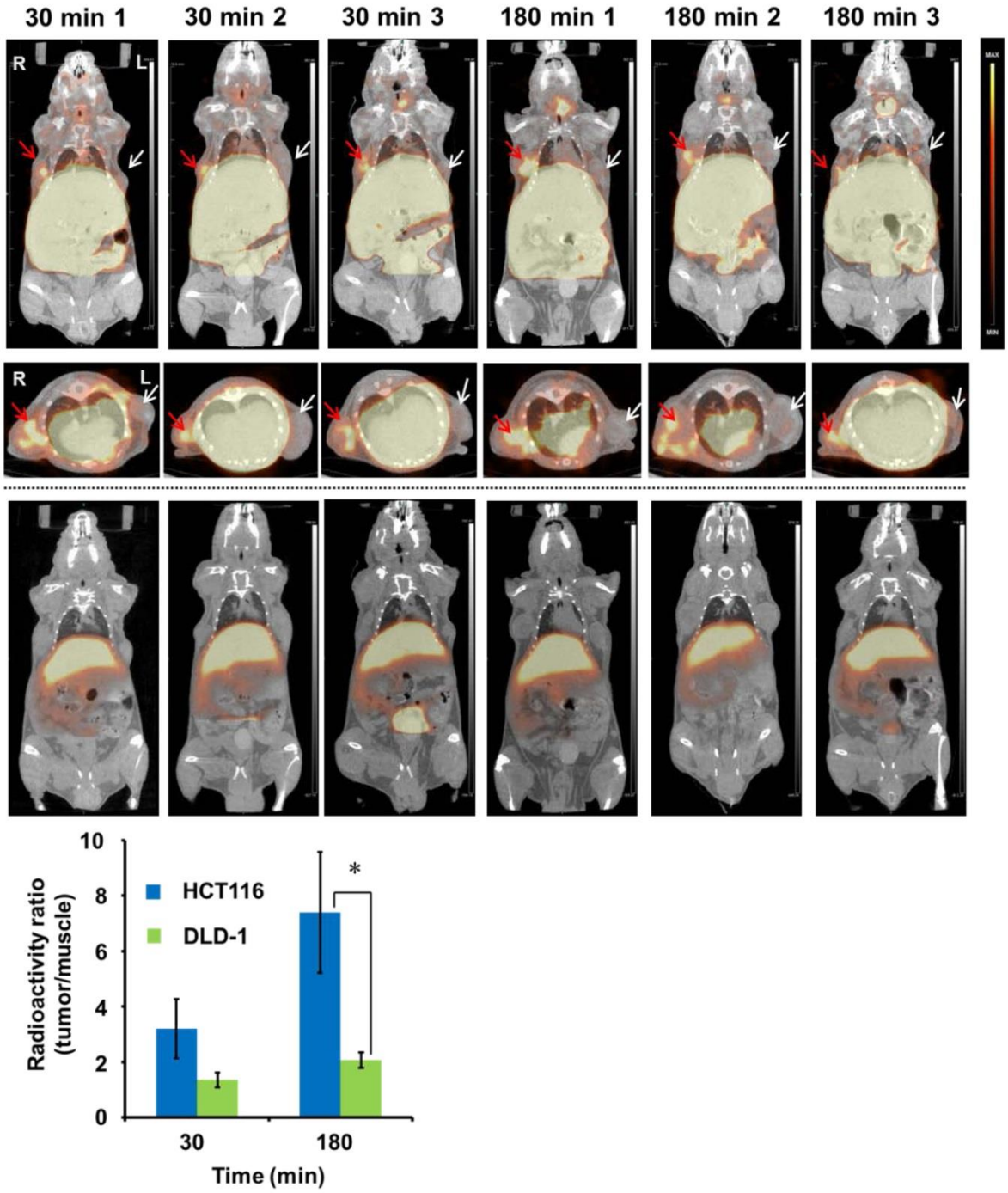
[*] ^{123}I-IIMU uptake is indicated as % ID. [†] vs. WiDr, $P < 0.05$. [‡] vs. DLD-1, $P < 0.01$. [§] vs. DLD-1, $P < 0.05$.

	Cell line		
Tissue	HCT116	WiDr	DLD-1
Tumor	0.99 ± 0.17 [†,‡]	0.38 ± 0.03	0.22 ± 0.01
Blood	0.66 ± 0.09	0.40 ± 0.01	0.61 ± 0.15
Muscle	0.19 ± 0.05	0.15 ± 0.00	0.16 ± 0.01
Heart	0.20 ± 0.03	0.18 ± 0.01	0.24 ± 0.03
Lung	0.61 ± 0.07	0.60 ± 0.11	0.66 ± 0.09
Thyroid	4.51 ± 0.61	4.28 ± 0.24	5.72 ± 0.47
Stomach	1.40 ± 0.28	1.37 ± 0.22	1.37 ± 0.16
Liver	39.6 ± 0.35	38.4 ± 3.51	32.9 ± 3.19
Spleen	0.43 ± 0.03	0.34 ± 0.03	0.43 ± 0.06
Small intestine	10.8 ± 1.08	7.79 ± 0.87	8.99 ± 1.17
Large intestine	0.66 ± 0.03	0.58 ± 0.10	0.63 ± 0.08
Kidney	0.57 ± 0.08	0.48 ± 0.06	0.81 ± 0.24
Urinary bladder [*]	40.4 ± 0.12	43.7 ± 4.00	48.8 ± 4.14
Tumor/Blood	1.53 ± 0.22 [‡]	0.96 ± 0.07	0.39 ± 0.07
Tumor/Muscle	5.91 ± 1.38 [§]	2.59 ± 0.26	1.34 ± 0.11



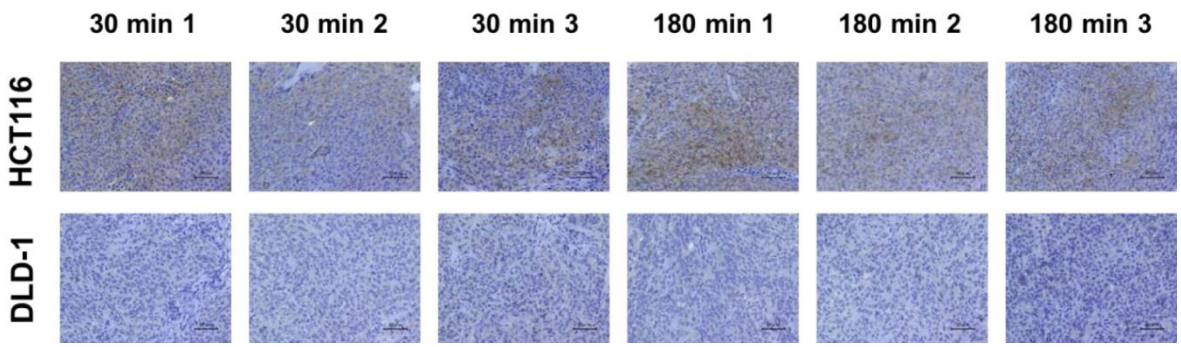
Supplemental Figure 1. The metabolic pathway of capecitabine. Capecitabine is metabolized by carboxylesterases in the liver before being metabolized to doxifluridine by cytidine deaminases in the liver. Doxifluridine is metabolized to active forms by TP in the liver and tumor tissues.

Abbreviations: CE, carboxylesterase; CD, cytidine deaminase; TP, thymidine phosphorylase; 5-FU, 5-fluorouracil; FdUrd, 5-fluorodeoxyuridine.



Supplemental Figure 2. ^{123}I-IIMU imaging of mice inoculated with tumor cells at 30 min or 180 min postinjection. ^{123}I-IIMU accumulation in tumor tissue depended on TP levels. Coronal (top row) and transverse (middle row) images of ^{123}I-IIMU SPECT/CT. Red arrows indicate HCT116 tumor. White

arrows indicate DLD-1 tumor. Coronal images of ^{123}I-IIMU SPECT/CT shown in different color scale (bottom row). Radioactivity ratios (tumor/muscle) of HCT116 and DLD-1are indicated as bar graph. Results are expressed as mean ± SEM (n = 3). Statistical analysis was performed using unpaired Student's t-test (*$P < 0.05$)



Supplemental Figure 3. Immunohistochemistry for thymidine phosphorylase in inoculated tumor at 30 min or 180 min postinjection. Immunohistochemistry for TP in HCT116 (upper rows) and DLD-1 (lower rows) tumor tissue sections from mice that underwent SPECT/CT. HCT116 cells expressed higher TP levels, and DLD-1 cells expressed lower TP levels. Scale bars = 50 μM.