Supplemental Materials

Kojima et al.
A simple biological imaging system for viable human circulating tumor cells

Supplemental Video 1
Time-lapse images of H1299 human lung cancer cells were recorded for 48 hours after OBP-401 infection at 10 MOI.

Supplemental Video 2
Time-lapse images of OST human sarcoma cell lines were recorded for 72 hours after OBP-401 infection at 10 MOI.

Supplemental Figure 1
Time-lapse images of human sarcoma cell lines (NDCS-1, OST, and NOS-10) were recorded for 72 hours after OBP-401 infection at 10 MOI.

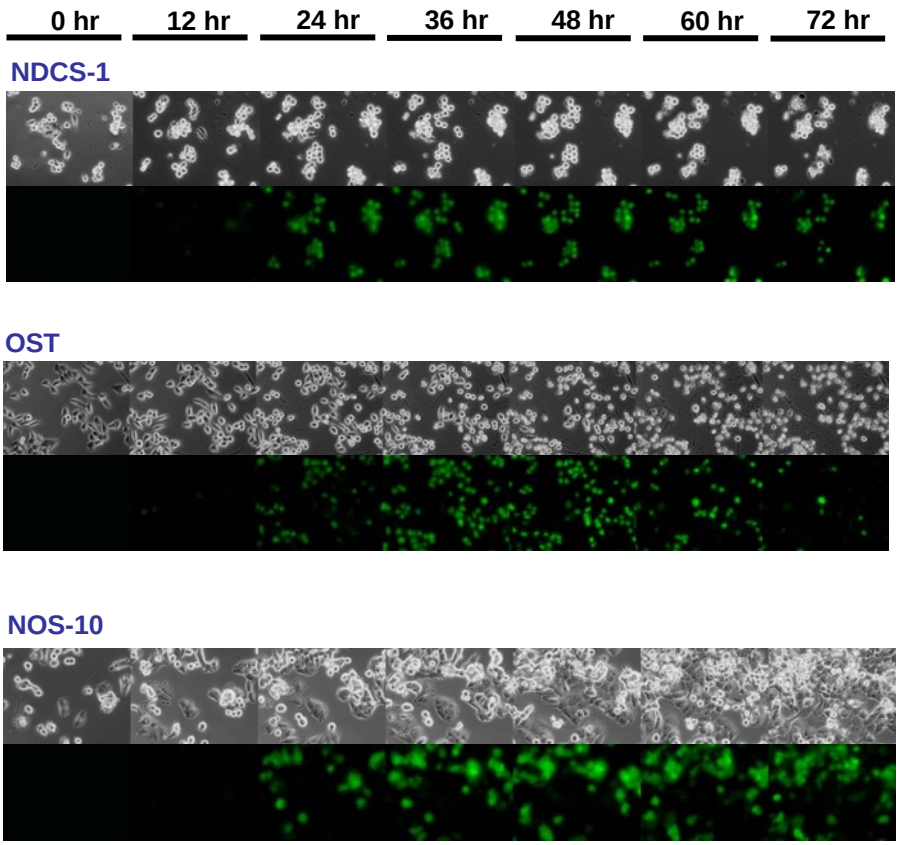
Supplemental Figure 2
Quantitative assessment of GFP labeling by OBP-401 in a variety of human cancer cell lines.

Supplemental Figure 3
Immunohistochemical staining for cytokeratin in H1299 cells stably expressing GFP.

Supplemental Figure 4
Flowcytometric detection of GFP-positive human tumor cells following *ex vivo* OBP-401 infection.

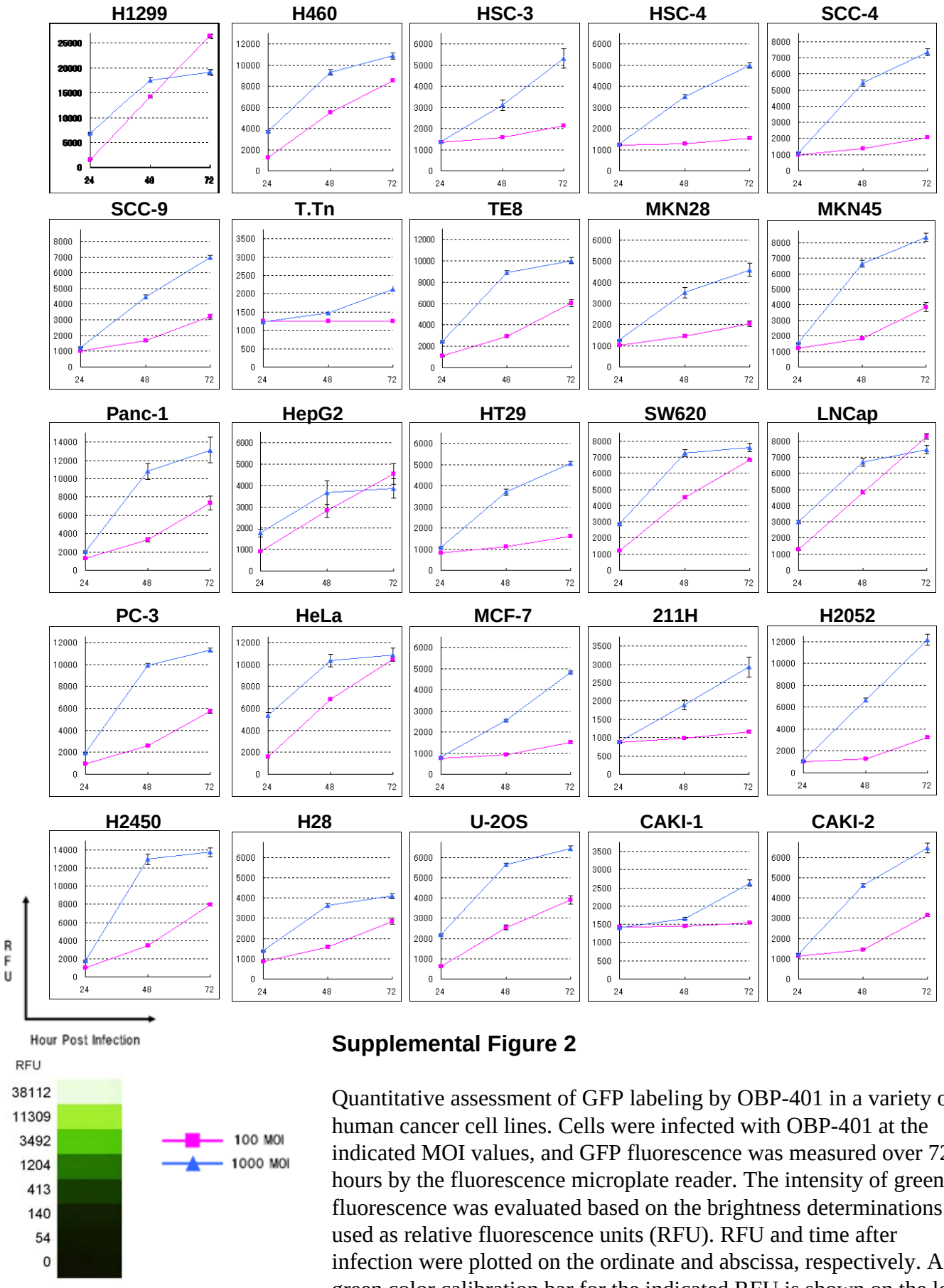
Supplemental Table 1
Characteristics of patients with gastric cancer and CTC numbers.

Supplemental Table 2
Characteristics of patients with other cancers and CTC numbers.



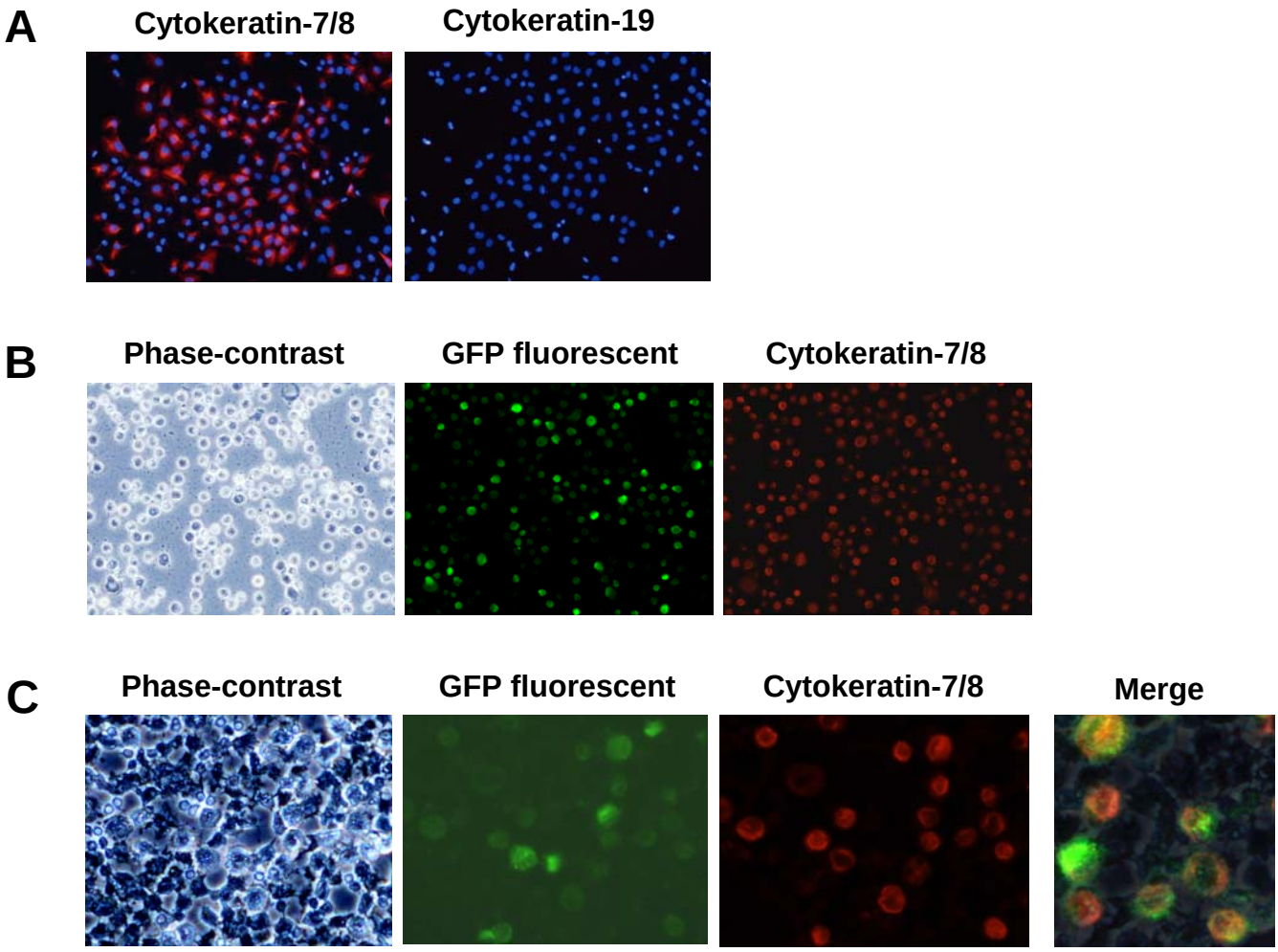
Supplemental Figure 1

Time-lapse images of human sarcoma cell lines (NDCS-1, OST, and NOS-10) were recorded for 72 hours after OBP-401 infection at 10 MOI. Selected images taken at the indicated time points showing cell morphology by phase-contrast microscopy (top panels) and GFP expression under fluorescence microscopy (bottom panels). Magnification × 200.



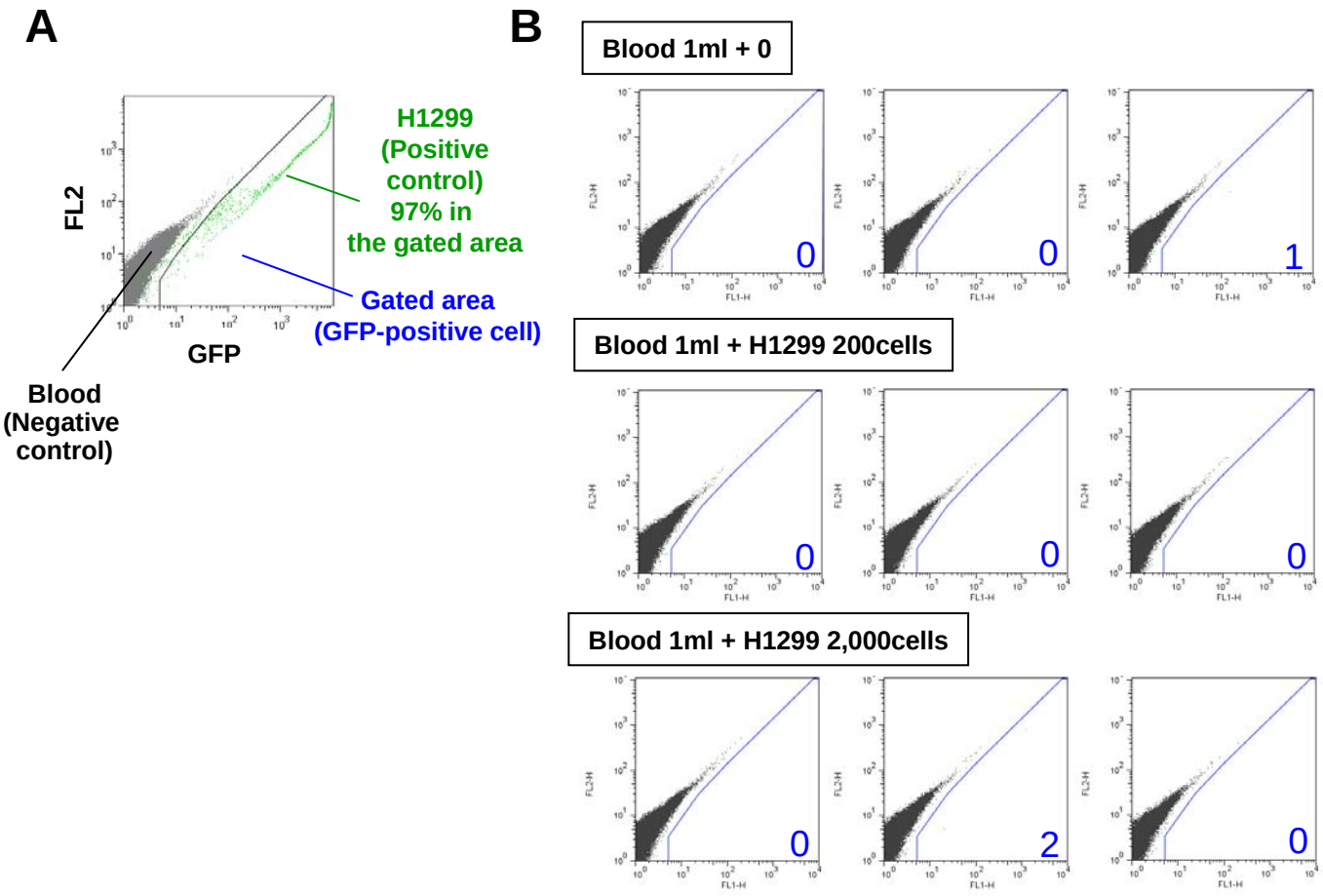
Supplemental Figure 2

Quantitative assessment of GFP labeling by OBP-401 in a variety of human cancer cell lines. Cells were infected with OBP-401 at the indicated MOI values, and GFP fluorescence was measured over 72 hours by the fluorescence microplate reader. The intensity of green fluorescence was evaluated based on the brightness determinations used as relative fluorescence units (RFU). RFU and time after infection were plotted on the ordinate and abscissa, respectively. A green color calibration bar for the indicated RFU is shown on the left.



Supplemental Figure 3

Immunohistochemical staining for CK-7/8 and CK-19 in H1299 cells stably expressing GFP. (**A**) H1299 human lung cancer cells were immunohistochemically stained with rhodamine-labeled anti-CK-7/8 or CK-19 antibody, and also counterstained with DAPI. H1299 cells express CK-7/8, but not CK-19. (**B**) H1299 cells were transfected with the CMV promoter-driven GFP expression construct and selected with G418 for 56 days. Cells expressing GFP were positive for CK-7/8 by immunohistochemical staining. (**C**) Blood samples (5 ml) obtained from healthy donors were mixed with 1×10^4 H1299 cells stably expressing GFP, exposed to erythrocyte lysis buffer, and then photographed by fluorescent microscopy. After fixation, the cells were immunohitochemically stained for CK-7/8. The merged image demonstrates that GFP-expressing cells were positive for CK-7/8.



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	Total cell	GFP-positive cells	Dilution (/ 0.7 x 20)	Average	S. D.
Blood 1 ml + 0	54450	0	0.0	9.5	16.5
	87675	0	0.0		
	92730	1	28.6		
Blood 1 ml + 200	98460	0	0.0	0.0	0.0
	82575	0	0.0		
	68040	0	0.0		
Blood 1 ml + 2,000	80625	0	0.0	19.0	33.0
	70185	2	57.1		
	56820	0	0.0		

Supplemental Figure 4

Flowcytometric detection of GFP-positive human tumor cells following *ex vivo* OBP-401 infection. (**A**) Gates were set for GFP-positive cells according to the blood (negative control) and GFP-expressing H1299 cells (positive control). (**B**) After the lysis of RBC, blood samples (1 ml) spiked with 0, 200, or 2,000 H1299 cells were infected with 10^4 PFU of OBP-401 for 24 hours, and then subjected to flowcytometric analysis. (**C**) The numbers of GFP-positive cells were identified in three samples in each group, and averaged with SD.

Supplemental Table 1

Characteristics of patients with gastric cancer and CTC numbers

Case No.	Sex	Age	Tumor status			Stage	CTC numbers
			T	N	M		
1	M	62	3	3	1	IV	6
2	M	69	3	3	1	IV	2
3	M	70	2	1	0	II	2
4	M	60	2	2	0	IIIA	0
5	F	52	2	1	0	II	23
6	F	58	1	0	0	IA	3
7	F	70	1	0	0	IA	0
8	F	70	1	0	0	IA	2
9	M	62	1	1	0	IA	47
10	M	77	2	1	1	IV	16
11	F	46	4	1	1	IV	0
12	M	64	3	1	1	IV	0
13	M	55	2	0	0	IB	0
14	F	66	1	0	0	IA	4
15	M	79	2	1	0	II	25
16	M	77	2	1	0	II	2
17	F	73	1	0	0	IA	0
18	F	61	3	1	0	IIIA	0
19	M	83	2	1	0	II	4
20	M	63	1	0	0	IA	0
21	F	57	1	0	0	IA	5
22	M	63	2	0	0	IB	5
23	M	59	3	0	0	II	2
24	F	78	3	1	1	IV	1
25	M	73	4	2	1	IV	6
26	M	58	2	2	1	IV	11
27	F	72	1	1	0	IB	29
28	M	83	1	0	0	IA	6
29	M	54	3	1	1	IV	0
30	M	67	3	1	0	IIIA	1
31	F	64	1	0	0	IA	8
32	M	80	3	1	1	IV	0
33	M	70	2	2	0	IIIA	0
34	M	60	3	1	0	IIIA	5
35	F	69	2	0	0	IB	7
36	M	70	3	1	1	IV	1
37	F	56	3	1	1	IV	2

Supplemental Table 2

Characteristics of patients with other cancers and CTC numbers

Case No.	Sex	Age	Cancer type	Stage	CTC numbers
1	F	69	Colon	IV	56
2	M	56	Colon	IV	1
3	M	77	Colon	IIIA	17
4	F	60	Colon	I	18
5	M	51	Colon	IIIB	6
6	M	70	HCC	II	0
7	F	59	HCC	IV	0
8	F	52	Breast	IV	19
9	M	63	Lung	IA	0