Figure S5

A

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B

![Bar chart](chart)

Mean fluorescence intensity (cps/cell)

- **12A8**
- **K45**
- **YB5.B8**
- **104D2**

GIST-T1 | GIST-882M

**Significance levels:**

- **p < 0.01**
Figure S6

The figure shows a bar chart comparing cell viability (%) between two cell lines, GIST-T1 and GIST-882M, treated with different compounds. The treatments include Vehicle, 12A8, K45, YB5.B8, and 104D2. Significant differences are indicated by **.
A  Temperature change in vitro (cells)

Measure  Measure  Measure  Measure  Measure  Measure

50 J/cm² of irradiation at 27.8 mW/cm²

0 min  7.5 min  15 min  22.5 min  30 min

**Graph:**
- IR700-12A8 + laser
- Control

B  Temperature change in vivo (mice)

Measure  Measure  Measure  Measure  Measure  Measure  Measure  Measure

100 J/cm² of irradiation at 55.5 mW/cm²

0 min  5 min  10 min  15 min  20 min  25 min  30 min

**Graph:**
- IR700-12A8 + laser
- Control
Supplementary Figure Legends

Figure S1. Characterization of IR700-labeled 12A8 or K45 antibody by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. (A) Non-reducing SDS-PAGE analysis of IR700-labeled 12A8 or K45 antibody. Coomassie brilliant blue (CBB) staining revealed a clear single band of unlabeled or labeled 12A8 and K45 antibodies at approximately 150 kDa. Moreover, NIR fluorescence imaging of this gel exhibited a clear single band of IR700-labeled 12A8 or IR700-labeled K45 at the corresponding location (approximately 150 kDa). (B) MALDI-TOF spectra of unlabeled 12A8, IR700-12A8, unlabeled K45, and IR700-K45. The unlabeled 12A8 showed a peak at approximately 150,000 (m/z), and the IR700-labeled 12A8 showed a peak at a slightly higher molecular weight compared with unlabeled 12A8 antibody. Likewise, the IR700-labeled K45 showed a peak at a slightly higher molecular weight compared with unlabeled K45 antibody. The results strongly suggest that the antibodies had been properly labeled and prepared.

Figure S2. Affinity of AF488-conjugated 12A8 (AF488-12A8) and IR700-conjugated AF488-12A8 (IR700-AF488-12A8) for c-KIT on GIST cells. (A) Immunofluorescence staining of GIST-T1 cells with AF488-conjugated 12A8 (AF488-12A8) or IR700-conjugated AF488-12A8 (IR700-AF488-12A8). Nuclei were stained with DAPI (scale bar, 100 \( \mu \)m). (B) Flow cytometric analysis for evaluating the fluorescence intensity of AF488 in GIST-T1 cells stained with
AF488-12A8 (green), AF488-IR700-12A8 (red), and non-staining control (gray). Mean fluorescence intensities in the cells with AF488-12A8 and IR700-AF488-12A8 were 659.0 ± 9.3 a.u. and 649.6 ± 1.9 a.u., respectively, indicating that IR700 conjugation did not essentially change affinity for c-KIT.

**Figure S3.** Laser devices used in this study. (A) NIR light with a diameter of 5 mm was emitted from a fiber-pigtailed laser diode (LP685-SF15, Thorlabs Inc., Newton, NJ). The red dashed arrow indicates NIR light emission from the laser diode to the irradiated object. (B) Power density was measured with an optical power meter (PM 100, Thorlabs). When we irradiated GIST tumors with NIR light, the power meter showed a power density of 55.5 mW/cm².

**Figure S4.** Treatment protocol for in vivo IR700-conjugated anti-c-KIT antibody (IR700-12A8) combined with NIR laser irradiation therapy for groups of mice receiving IR700-12A8 + laser, IR700-12A8 alone, NIR laser alone, and vehicle alone. Mice were injected with IR700-12A8 or vehicle on day 0 and day 7, and NIR laser irradiation was performed on days 1, 2, 3, 8, 9, and 10. Mice were injected with Alexa Fluor 647-conjugated anti-c-KIT antibody (AF647-antibody) at day 28 for observation of the tumor using the IVIS Spectrum at day 30. The mice were then sacrificed for histological analysis.

**Figure S5.** Immunofluorescence staining of GIST cells using 4 types of anti-c-KIT monoclonal antibodies. (A) Representative staining patterns of GIST-T1 and GIST-882M cells for c-KIT. The cells were incubated with each
anti-c-KIT antibody (12A8, K45, YB5.B8, or 104D2; 4 µg/mL) as the primary antibody, and then incubated with AF488-conjugated antibody (green) as the secondary antibody. Nuclei were stained with DAPI (blue) and the cells were observed under confocal laser microscopy (original magnification, ×100) (scale bar, 500 µm). (B) Mean fluorescence intensity of AF488 in individual cells incubated with each anti-c-KIT antibody. The mean fluorescence intensity of AF488 was quantified using Image J software. At least 100 cells were quantified for each cell line and each experiment was repeated 3 times. Data represent mean ± standard deviation (SD) (**p<0.01).

**Figure S6.** Growth inhibitory effect of anti-c-KIT antibodies on GIST cells. The cells were incubated with each anti-c-KIT antibody (12A8, K45, YB5.B8, or 104D2; 20 µg/mL) at 37°C for 6 days, and cell viability was assessed by WST-8 assay. Data represent mean ± SD (n=3, **p<0.01).

**Figure S7.** Competition assay of IR700-12A8 fluorescence imaging with unlabeled 12A8 in GIST-T1-xenografted mice. (A) Representative fluorescence images of mice injected with IR700-12A8 (30 µg) alone (left), or pre-injected with unlabeled 12A8 (300 µg) and injected with IR700-12A8 (30 µg) (right). The yellow arrows indicate GIST-T1 tumors. The tumor from a mouse pre-injected with unlabeled 12A8 showed a faint fluorescent signal, whereas the tumor from a mouse treated with vehicle alone showed a clear fluorescent signal 24 h after IR700-12A8 injection. (B) Chronological changes in the mean fluorescence intensity in GIST-T1 tumors. The mean fluorescence intensity of the tumor in the
pre-injected mouse was significantly lower than that in mice treated with vehicle alone at all timepoints. Data represent mean ± SD (n=3, **p<0.01).

**Figure S8.** Ex vivo fluorescent imaging of xenografted GIST-T1 and SW620 tumors and of various organs from a mouse injected with IR700-12A8. (A) Representative ex vivo images of tumors and organs resected at 120 h after injection. The GIST-T1 tumor showed a strong fluorescent signal, whereas the SW620 tumor, liver, and kidney showed weak fluorescent signals. (B) The mean fluorescence intensities of the tumors and organs. The respective fluorescence intensities in GIST-T1 tumors, SW620 tumors, liver, and kidney were 8083 ± 190, 2405 ± 484, 1985 ± 119 and 1885 ± 98 a.u.. Data represent mean ± SD (n=3, **p<0.01).

**Figure S9.** Preliminary study of in vivo IR700-12A8-mediated PIT for GIST-xenografted mice. (A) Treatment regimen for IR700-12A8 injection and NIR light irradiation. Mice were injected with IR700-12A8 (100 µg) at day 0 and the tumors were irradiated with 50 J/cm² of NIR light at day 1 and 100 J/cm² at day 2. The mice were finally sacrificed at day 28. (B) Chronological changes in relative tumor volume in the IR700-12A8 + laser group, IR700-12A8 alone group, laser alone group, and vehicle alone group (n=4 per group). Although the tumors did not regress, tumor growth was significantly suppressed in the IR700-12A8 + laser group as compared with the other groups. Data represent mean ± SD (*p<0.05, **p<0.01). (C) Tumors resected from each group of mice at day 28.
**Figure S10.** IR700 fluorescent signal in tumors before each NIR light irradiation at day 1, 2, and 3. (A) Representative fluorescence images of the tumors before the first (day 1), second (day 2), and third irradiation (day 3). The left tumor was treated with IR700-12A8 alone and the right tumor was treated with IR700-12A8-mediated NIR irradiation (IR700-12A8 + laser). (B) The mean fluorescence intensity in the tumors before each NIR light irradiation at day 1, 2, and 3. The intensity in the right tumors (IR700-12A8 + laser) was effectively decreased by PIT, whereas that in the left tumors (IR700-12A8 alone) was gradually decreased with fading of fluorescence. The intact fluorescence of IR700 photosensitizer was still present before the second and even the third irradiation. Data represent mean ± SD (n=7, *p<0.05, **p<0.01).

**Figure S11.** Chronological changes in absolute tumor volumes in the 4 groups of mice treated with IR700-12A8 + laser, IR700-12A8 alone, laser alone, or vehicle alone.

**Figure S12.** Temperature changes during irradiation with NIR light in vitro (A) and in vivo (B). (A) GIST-T1 cells in 96-well plates were treated with IR700-12A8 for 6 h and then irradiated with NIR light at 50 J/cm² (27.8 mW/cm²) for 30 min. The temperature of the medium for GIST-T1 cells was measured every 7.5 min during irradiation using a contactless thermometer (MT-7, AS ONE, Osaka, Japan). (B) Mice with GIST-T1 xenograft tumors were injected with IR700-12A8 and the tumors were then irradiated with NIR light at 100 J/cm² (55.5 mW/cm²) for 30 min. The temperature of the tumor was measured every 5 min during
irradiation. Data represent mean ± SD (n=3).

**Figure S13.** Apoptosis and autophagic activity in GIST-T1 cells after treatment with IR700-12A8 and NIR laser irradiation. (A) Immunohistochemistry for LC3 in sections of tumor treated with 100, 200, and 300 J/cm² of IR700-12A8-mediated NIR irradiation in vivo. A rabbit anti-human LC3B monoclonal antibody (D11, Cell Signaling Technology) was used as a primary antibody. Original magnification, × 200. High-magnification images are shown in the inset at the upper right (× 400). (B) Chronological changes in PARP and LC3 expression in GIST-T1 cells after treatment with IR700-12A8-mediated NIR laser irradiation in vitro. The cells were incubated with IR700-12A8 in the presence or absence of 25 µM of chloroquine (CQ) and irradiated by 20 J/cm² of NIR light. The expression of PARP and LC3 was evaluated by Western blotting using anti-human PARP polyclonal antibody (Cell Signaling Technology) and D11, respectively, at 6, 12, and 24 h after irradiation. β-actin was used as a loading control.