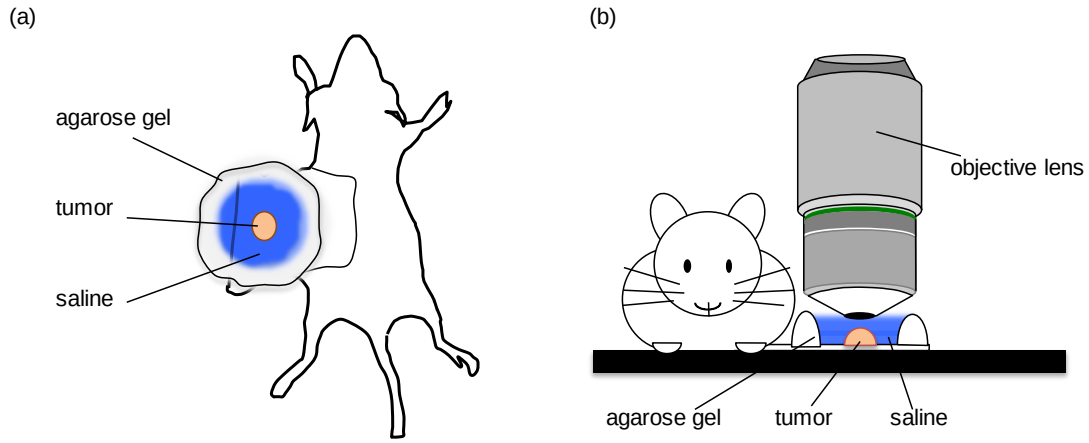
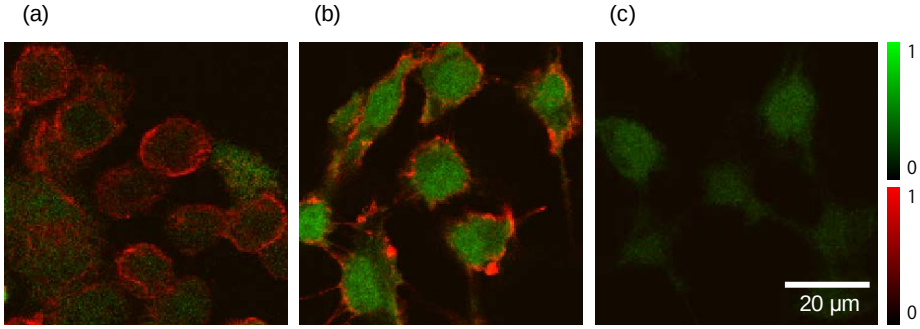
Supplementary Figure 1



Supplementary Figure 1. Schematic diagram of *in vivo* fluorescence micro imaging of tumor mass on skin flap of a mouse

(a, b) The tumor mass on the skin flap was observed by upright microscopic system. Overhead view (a), and side view (b) of *in vivo* observation of tumor mass on skin flap of a mouse on the microscope stage. Image acquisition was performed by a water-immersion objective lens. Tumors were surrounded by agarose gel and submerged in saline during the observation.

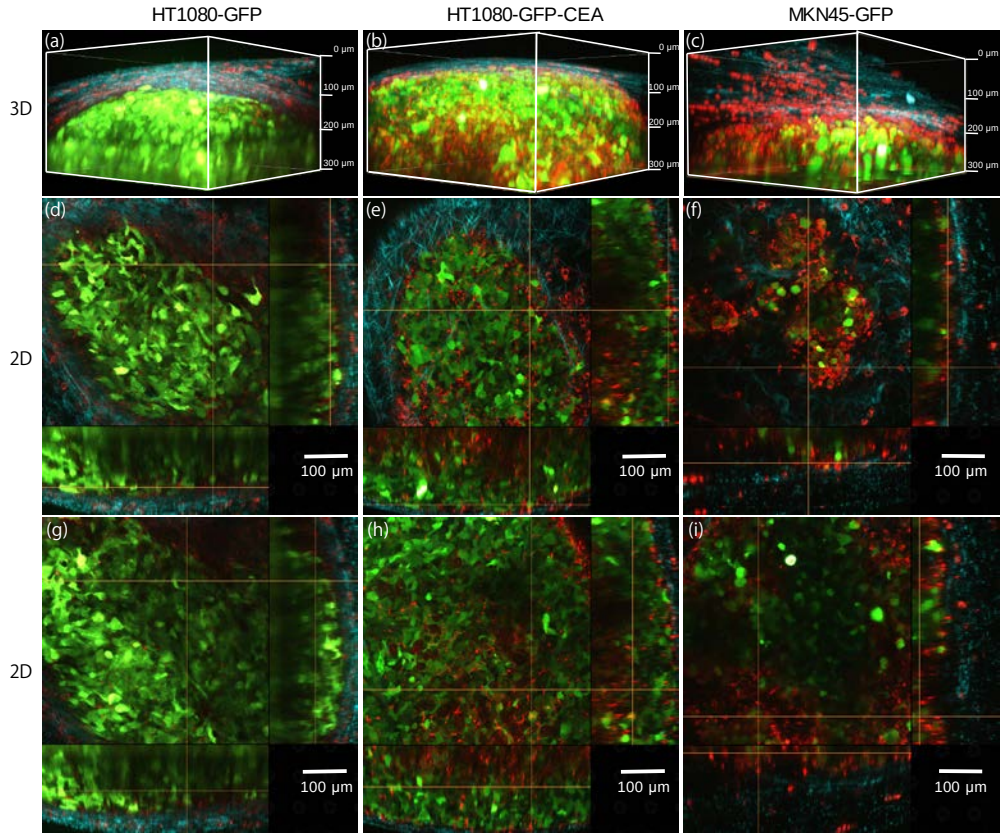
Supplementary Figure 2



Supplementary Figure 2. Immunostaining of GFP- and CEA-expressing human cancer cells *in vitro*

Magnified images of immunostaining in Figure 1(c–e). CEA could be detected on the cell surface in MKN45-GFP (a) and HT1080-GFP-CEA (sorted twice) (b) cells, but not in HT1080-GFP (c) cells. Red and green indicate fluorescent signals of Alexa594 and GFP, respectively.

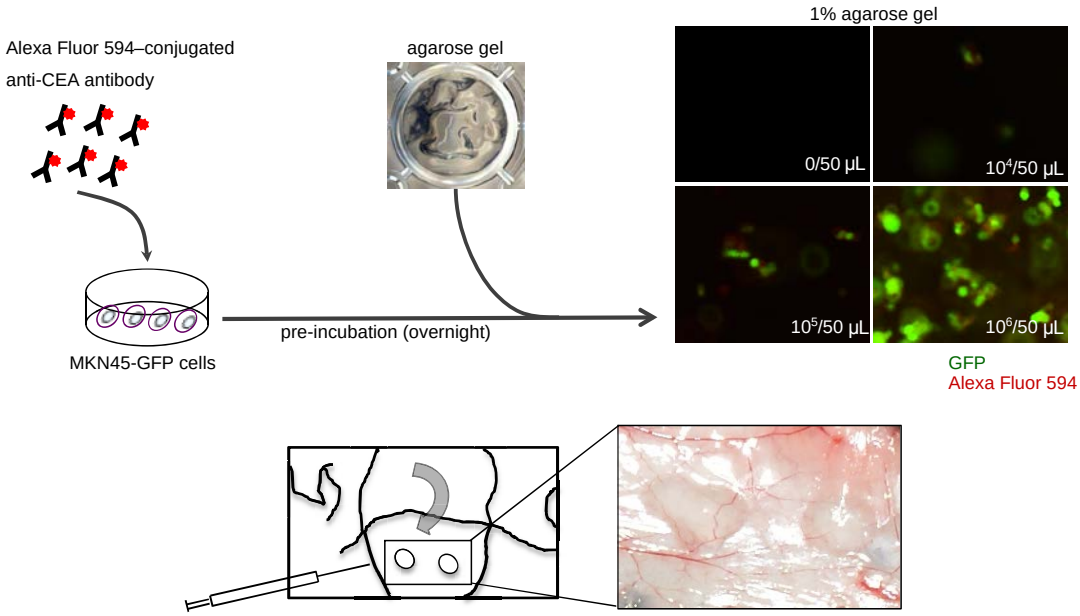
Supplementary Figure 3



Supplementary Figure 3. ***In vivo*** **fluorescence micro imaging by two-photon microscopy using a low-dose fluorescent probe**

In vivo micro imaging of tumor masses was performed using a two-photon excitation microscope 24 hours after injection of Alexa Fluor 594–conjugated anti-CEA antibody (10 μg/mouse). Three-dimensional and 2D images of HT1080-GFP (a, d, g), HT1080-GFP-CEA (b, e, h), and MKN45 cells (c, f, i) were obtained in the same manner as Figure 3. The middle (d, e, f) and lower panels (g, h, i) depicted, respectively, regions near the surface and in the deep regions of the tumors.

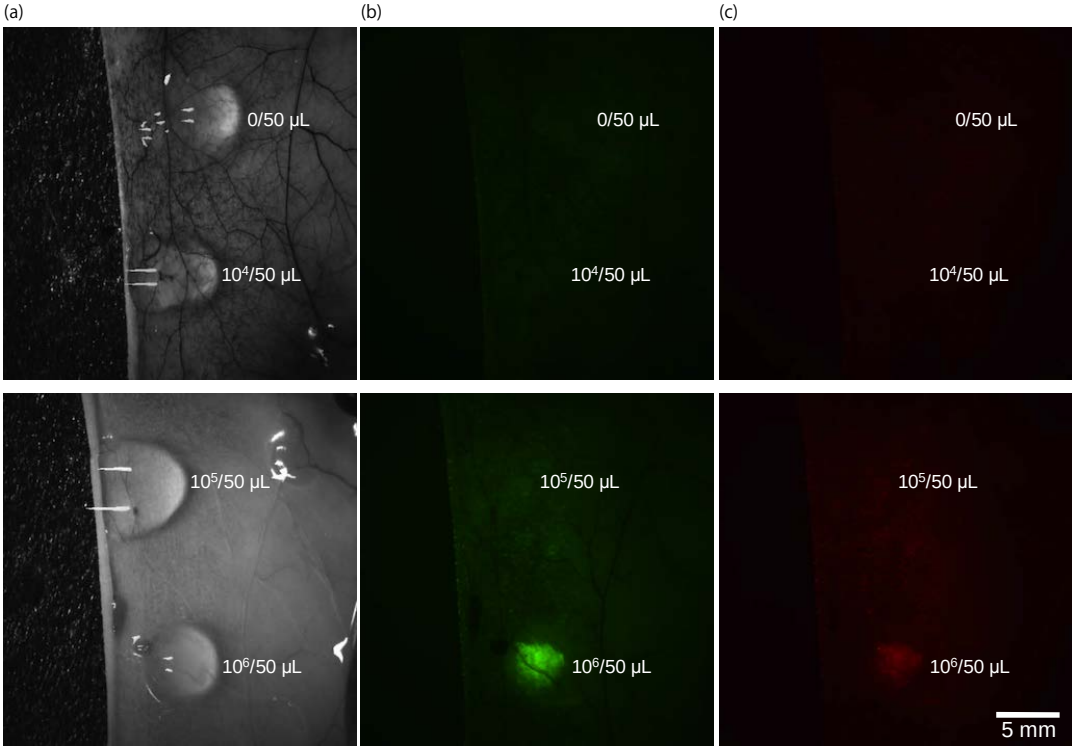
Supplementary Figure 4



Supplementary Figure 4. Experimental flow of pre-incubation assay

MKN45-GFP cells were pre-incubated overnight with Alexa Fluor 594–conjugated anti-CEA antibody. The immunostained cells were inoculated into the connective tissue of skin flaps at densities of 0, 10^4, 10^5, or 10^6 cells in 50 μL of 1% agarose.

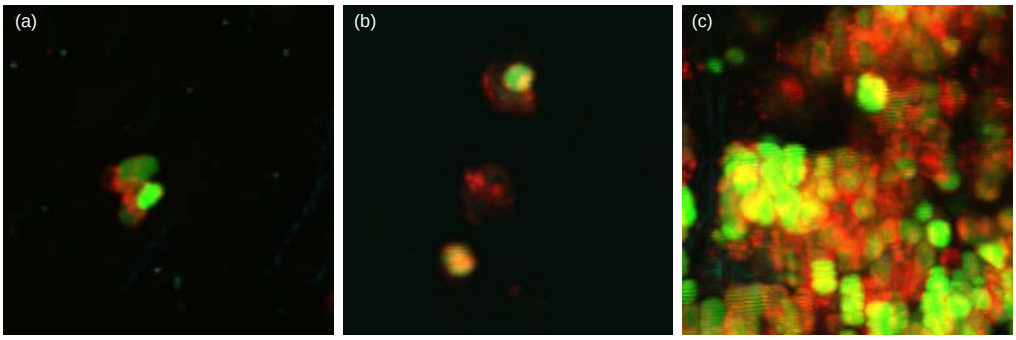
Supplementary Figure 5



Supplementary Figure 5. ***In vivo*** **macroscopic fluorescence imaging of small numbers of cancer cells by fluorescence zoom microscopy**

In vivo macroscopic imaging of the inoculation site was performed immediately after injection of immunostained MKN45-GFP cells pre-incubated with Alexa Fluor 594–conjugated anti-CEA antibody (see Supplementary Figure 4). Bright field (BF) (a), GFP (b), and Alexa Fluor 594 (c) images were obtained using a fluorescence zoom microscope. Exposure times for the GFP and Alexa Fluor 594 fluorescence images were 1000 ms and 3000 ms, respectively.

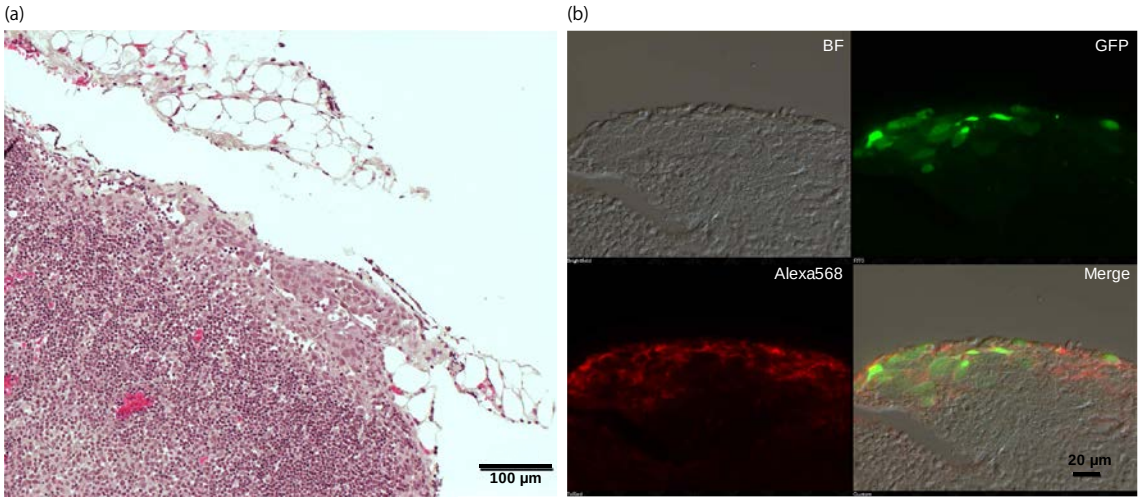
Supplementary Figure 6



Supplementary Figure 6. *In vivo* fluorescence micro imaging of small numbers of cancer cells by two-photon excitation microscopy

(a-c) Magnified images of immunostained MKN45-GFP cells pre-incubated with Alexa Fluor 594–conjugated anti-CEA antibody in Figure 4(f–h). Red, green, and blue indicate Alexa Fluor 594 fluorescence, GFP fluorescence, and second harmonic generation (SHG), respectively.

Supplementary Figure 7



Supplementary Figure 7. Immunofluorescence and HE staining of tissue sections

The footpad spontaneous metastasis model using HT1080-GFP-CEA was validated by hematoxylin and eosin (HE) staining (a) and immunofluorescence imaging (b) in resected lymph nodes.