SUPPLEMENTAL FIGURE AND VIDEO LEGENDS

FIGURE LEGENDS

FIGURE S1. **Preparation of tumor-bearing SCID mice.** *A*, PAR1-KPL cells were transplanted under the skin of SCID mice. Five to ten weeks after transplantation, mice were used for *in vivo* nano-imaging. *B*, When 1 x 10^6 PAR1-KPL cells were transplanted into mice, liver metastasis was seen within 5-10 weeks (blue circle). PAR1 expression induced metastasis of KPL cells in SCID mice, whereas KPL cells alone did not show metastasis.

FIGURE S2**. Effect of anti-PAR1 antibody on the endocytosis of PAR1.** *A*, Fluorescent imaging of PAR1-GFP and anti-PAR1-QDs (10nM) in PAR1-KPL cell. Orange line shows the perinuclear region of the cell. PAR1 on cell membrane is transported to the perinuclear region after endocytosis. Bar, 10 μm. *B*, Comparison of GFP-PAR1-fluorescence intensity of perinuclear region in the conditions with and without 10 nM anti-PAR1-QDs. In the both conditions, GFP-fluorescent intensity in the regions is similar, showing that 10nM anti-PAR1-QDs does not affect the endocytosis of PAR1. Error bars indicate SEM. 0 nM, n=10. 10 nM, n=9.

FIGURE S3. **Preparation of exposed tumor for *in vivo* nano-imaging.** We previously imaged surfaces of upper sections of tumors *in vivo* (20). After preparation of tumor-bearing mice by transplantation of PAR1-KPL cells, tumors were excised and three sections, upper, middle, and lower, were stained with anti-PAR1-QDs. The results showed that the tumor cells of an area near the large vessel in the lower section were stained most strongly with anti-PAR1-QDs among the three sections. Therefore, in the preparation of the dorsal skin fold chamber, the tumor was stripped (*A*, *B*), turned over (*C*), and fixed with thread (*D*). By way of this surgical procedure, the area near the large vessel in the lower section of the tumor was exposed. After that, a polyvinyl chloride plate containing a small window (10 mm X 10 mm) was mounted on the exposed tumor, and then the skin around the tumor was bonded to the plate using instant superglue (see Fig. 2*D*). An example of a tumor-bearing nude mouse is provided.

FIGURE S4**. Cell image superimposed for 0-2 s, 16-18 s, and 40-42 s in Fig. 4*A***. Yellow lines represent outlines of cancer cells. Red dotted lines show outlines of the vessel. Bar, 10 μm.

FIGURE S5**. Membrane dynamics in cancer cells *in vitro*.** *A*, Imaging of PAR1-KPL cells with anti-PAR1-QDs (2.5 nM QDs). Membrane fluidity in PAR1-KPL cells *in vitro* was investigated by imaging the movement of PAR1 labeled with anti-PAR1-QDs. Each orange and green square shows typical QDs bound to PAR1 on the immobile edge of a PAR1-KPL cell and on the mobile edge of a PAR1-KPL cell, respectively. Yellow lines represent the outline of the cells, delineated using phase contrast images. Excitation, 532 nm; emission, >580 nm; exposure time, 0.2 s. Bar, 10 μm. *B*, Traces of orange and green squares as shown with arrowheads in *A*. The center of anti-PAR1-QD fluorescence was calculated by fitting the intensity profiles to two-dimensional Gaussian curves. This center was observed to move randomly along the membrane. Numbers show the tracking order. *C*, MSD plots of QDs bound to PAR1 on the immobile

edge of PAR1-KPL cells (orange) and on the mobile edge of PAR1-KPL cells (green). D shows the diffusion constant. The diffusion constant of QDs on mobile lamellipodia of the PAR1-KPL cell (1.2×10^5 nm^2/s) was five-fold greater than that on immobile cell edges (2.2×10^4 nm^2/s). Error bars show SEM. Orange data, n=70 (10 trajectories/cell X 7 cells). Green data, n=70 (10 trajectories/cell X 7 cells). *D*, A model for membrane dynamics in metastatic cancer cells *in vitro*. Numerical values show the diffusion (nm^2/s) of PAR1 labeled with anti-PAR1-QDs.

VIDEO LEGENDS

Video 1. **Metastatic cancer cells far from vessels**. These cells are several hundred μm away from vessels. Excitation, 532 nm; emission, >580 nm; exposure time, 0.2 s. 1 s (movie) = 10 s (real time).

Video 2. **Metastatic cancer cell near a vessel**. Auto-fluorescence of red blood cells is seen in a vessel. Excitation, 532 nm; emission, >580 nm; exposure time, 0.2 s. 1 s (movie) = 2 s (real time).

Video 3. **Metastatic cancer cell in bloodstream**. The cancer cell passes through a vessel 2.5 μm wide and travels, changing velocity. Auto-fluorescence of two red blood cells is seen in the vessel. The two red blood cells follow the cancer cell. Excitation, 532 nm; emission, >580 nm; exposure time, 0.2 s. 1 s (movie) = 2 s (real time).

Video 4. **Metastatic cancer cell adhering to the inner vascular surface**. A cancer cell adhering to the surface without directional migration is shown. Excitation, 532nm; emission, >580nm; exposure time, 0.2 s. 1 s (movie) = 2 s (real time).

Video 5. **Directional cell migration on the inner vascular surface**. The cancer cell migrates in the direction of blood flow, pauses after migration, and then migrates in direction opposite to that of blood flow. Auto-fluorescence of many red blood cells is seen in the vessel. Excitation, 532nm; emission, >580 nm; exposure time, 0.2 s. 1 s (movie) = 10 s (real time).