Supplymentary Information

Real-time longitudinal evaluation of tumor blood vessels using a compact preclinical fluorescence imaging system

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Supplementary figures

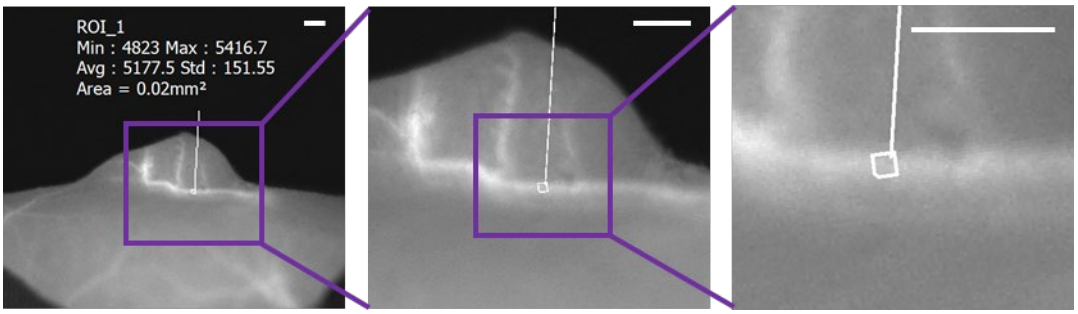

Figure S1. ROI drawn to include only the blood vessel area. Purple rectangles are enlarged on the right-hand side. Scale bar, 1 mm

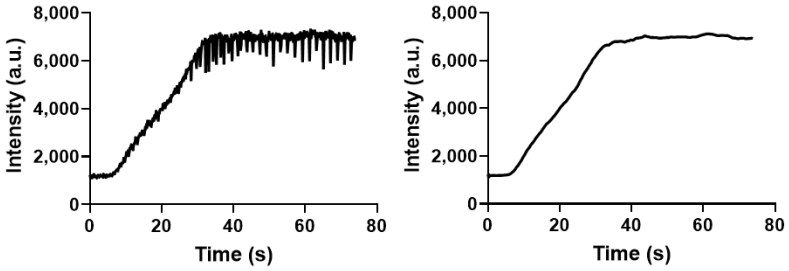

Figure S2. ICG dynamics of a pixel on tumor blood vessel when mouse movement due to breathing is reflected (left) and when artifacts are removed using Savitzky-Golay Filter (right).

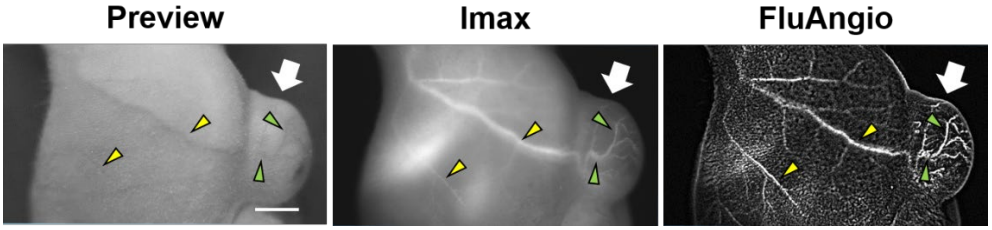

Figure S3. Preview (photograph), Imax, and FluAngio maps in an A431 tumor-bearing mouse. White arrows indicate the A431 tumor. Yellow or green arrowheads indicate blood vessels in the tumor periphery or within the tumor, respectively. Scale bar, 5 mm.

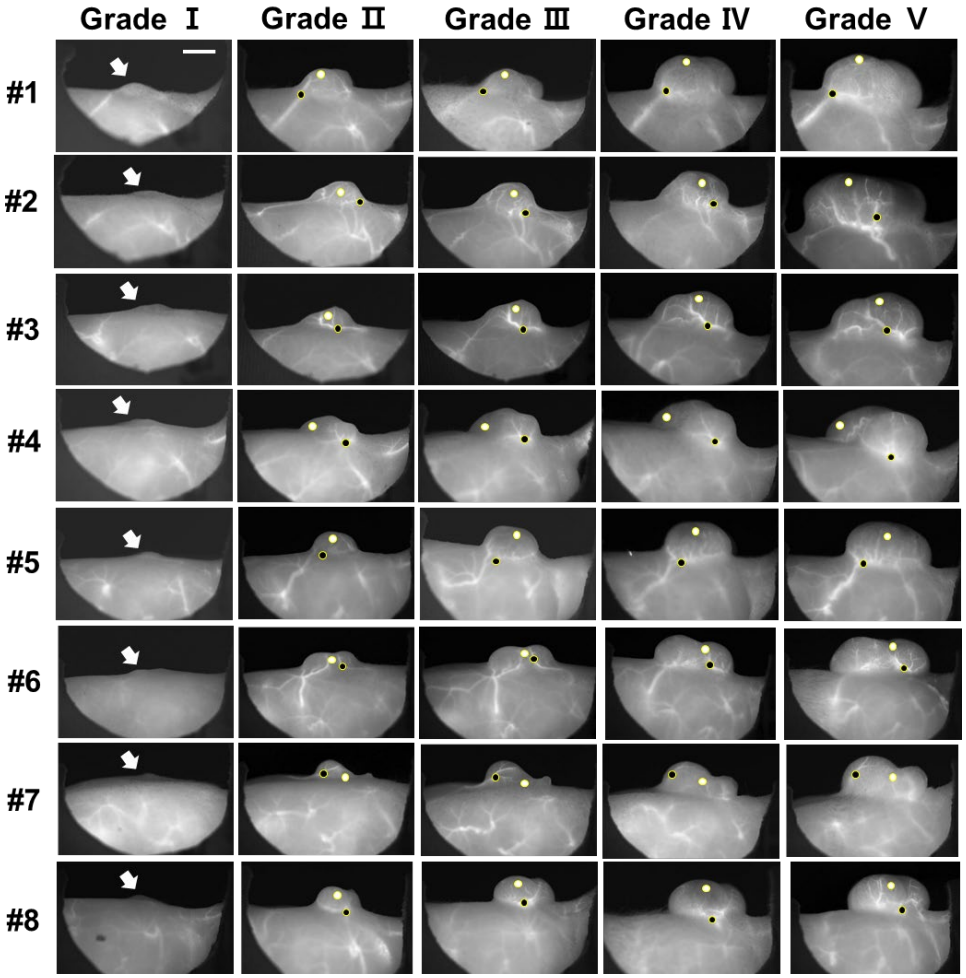

Figure S4. Fluorescence images of A431 tumors for each grade determined based on the tumor size. The white arrow on Grade I indicates the location of the small palpable tumor. In Grades II, III, IV, and V, black circles indicate blood vessels and white circles indicate outer areas. Tumor #3 is the one in Figure 4A. Scale bar, 5 mm.

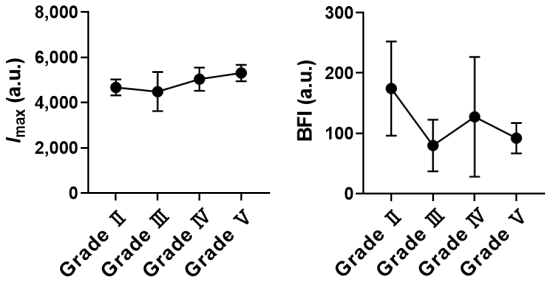

Figure S5. Changes in I_{max} and BFI when the entire tumor area is designated as an ROI by grade. Data are the mean ± SD for n = 8 mice per grade.

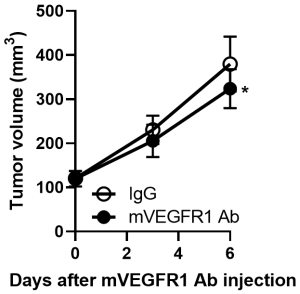

Figure S6. Growth of A431 tumors in mice injected with IgG or mVEGFR1 Ab twice every three days. Data are the mean ± SD for $n \geq 7$ mice per group. *, $P < 0.05$ determined via two-way ANOVA.

Supplementary tables

	Ultrasound Doppler Flowmetry	Laser Doppler Flowmetry	Laser Speckle Contrast Imager	VISQUE® InVivo Smart-LF
Temporal Resolution	A few seconds	A few minutes	a few seconds	Once at a time
Spatial Resolution	-	1 mm – 0.1 mm	tens of micrometer	27 µm (@3×)
Imaging Depth	A few mm depth	Less than 1 mm depth	Less than 1 mm depth	According to the FL dye
Imaging Target	RBC	RBC	RBC	Fluorescence dye in blood

Table S1. Comparison of VISQUE® InVivo Smart-LF equipment and other optical *in vivo* imaging systems for small animals with resolution, imaging depth and target.

Model	VSP-4MU
Resolution	2048(H) × 2048(V)
Sensor Type	Backside Illuminated Scientific CMOS Image Sensor

Pixel Size	6.5 μm × 6.5 μm
Optical Format	1.2”
Cooling Method	Thermoelectric Peltier Cooling
Cooling Performance	50°C below ambient temperature
Quantum Efficiency	Max. 94 % @ 550 nm
Full Well Capacity	54 ke^-
Max. Frame Rate	37 fps
Exposure Time (1μs step)	11 μs ~ 6 s
Binning	2 × 2, 4 × 4
Output Format	Mono 16
Lens Mount	C-mount
Interface	USB 3.0

Table S2. Specifications for the sCMOS camera, VSP-4MU.

Model	**VISQUE® InVivo Smart LF**
Weight and Dimension	About 20 kg (44 lbs), 40 cm × 40 cm × 57 cm
Sensor	1.2“ Backside Illuminated sCMOS
Cooling	-20°C, Thermoelectric Peltier Cooling
Resolution (H × V)	1824 × 1824
Pixel Size	6.5 μm × 6.5 μm
Exposure Time	25 ms – 30 mins
Maximum Frame Rate	37 fps
Digital Output	16 bit
Binning	1 × 1, 2 × 2, 4 × 4
Fluorescence Light Source	LED
Fluorescence Emission Filter	Up to 9, Optional
Lens Control	Motorized Iris / Zoom / Focus
Zoom (Field of View, H × V)	15 cm x 15 cm (1×) – 5 cm x 5 cm (3×)
Stage Type	Sliding stage, Up to 3 mice
Accessory	Heating stage, Anesthesia Ventilator Adaptor

Table S3. Specifications for the VISQUE® InVivo Smart-LF equipment.