Supporting Information

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SI Text

Preparation of Biotinylated CLuc. The Avi-Tag encoding a 16-residue peptide SGLNDIFEAQKIEWHE was introduced to the C terminus of the pcDNA-CLuc gene with a KOD-Plus-Mutagenesis kit. The DNA fragment flanked by *Kpn*I and *Xho*I restriction sites was used to construct the plasmid pM01-CLuc-Avi, which was a polyhedrin promoter-based baculovirus transfer vectors. The recombinant CLuc-Avi protein was produced by Katakura industry Corporation using the Kaiko-Express system. Fifteen ml of body fluid of infected silkworms containing CLuc was withdrawn and concentrated by ultra filtration using an Ultrafree-15 centrifugal device (Millipore). The retentate was washed with 10 mM Tris buffer (pH 8). Biotinylation of the lysine residue in Avi-Tag was performed according to the manufacturer's instruction with biotin-protein ligase (Avidity). To remove excess reagents, the mixture was loaded onto a size-exclusion column and biotinylated CLuc-Avi was eluted with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.15 M NaCl. The resultant solution was loaded onto a monomeric avidin column, washed with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.15 M NaCl, and eluted with 2 mM biotin in phosphate buffer. Fractions giving strong bioluminescence signals were pooled and concentrated. CLuc in 20 mM Tris-HCl buffer was applied to an HP Q column and eluted with the same buffer at a linear gradient of 0–1.0 M NaCl. The concentration of purified recombinant CLuc was calculated from the extinction coefficient at 280 nm and purity was determined by SDS/PAGE. The purity of biotinylated CLuc was estimated to be >90%. The yield of purified biotinylated CLuc was 33 μg/mL of the original body fluid.

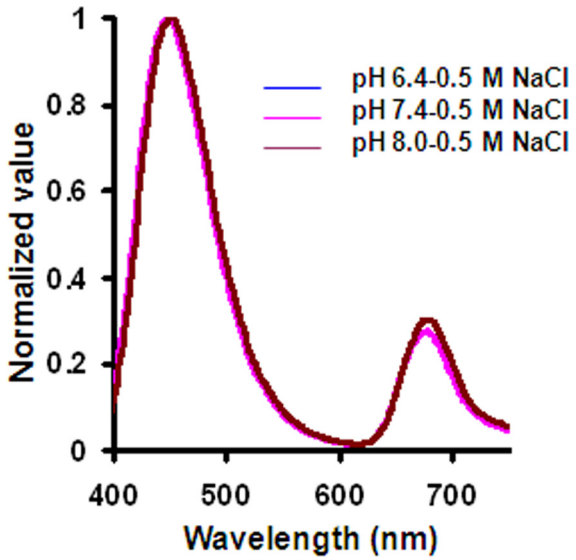

Fig. S1. Bioluminescence spectra of FBP in various pH buffer solutions containing 0.5 M NaCl.

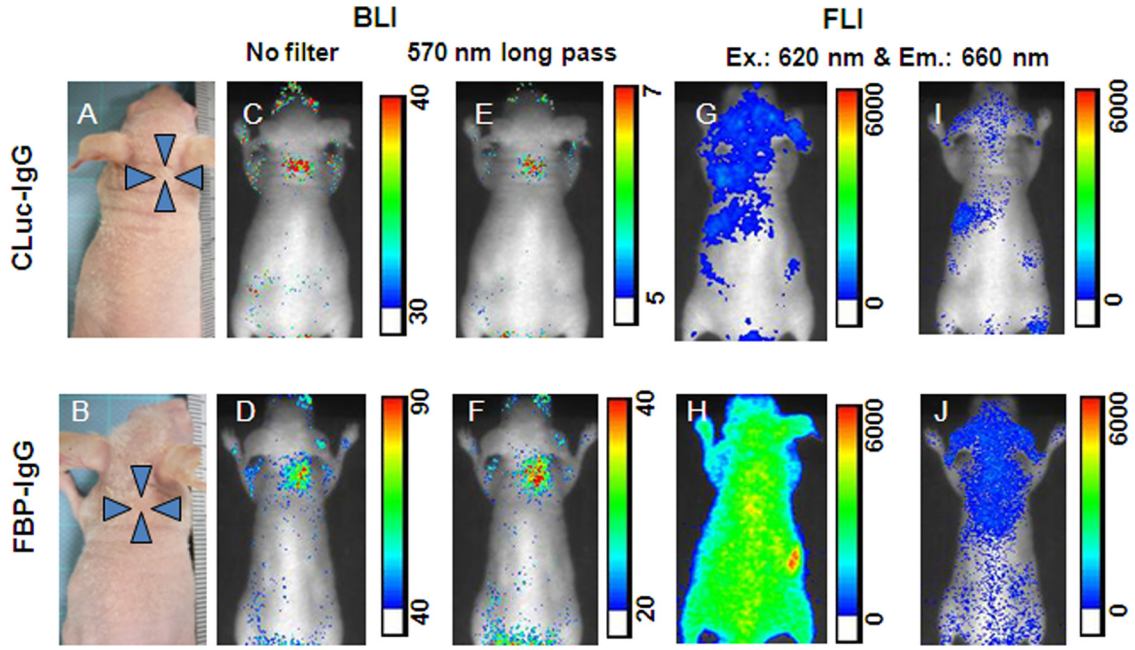

Fig. S2. Images of additional live animals. (*A* and *B*) Photographs of another two tumor-bearing mice. (*C* and *D*) Forty-eight hours after the administration of CLuc-IgG or FBP-IgG, luciferin was injected and bioluminescence images (BLIs) were obtained using a CCD photon imaging system. Color scale represents photons/s/steradian. (*E* and *F*) The bioluminescence images were obtained with 570 nm long-pass filter. (*G* and *H*) Fluorescence images (FLIs) of tumor-bearing mice shown in panels *A* and *B* immediately after the administration of CLuc-IgG or FBP-IgG were obtained using the same CCD photon imaging system and a set of Cy5 filters. (*I* and *J*) FLIs of the tumor-bearing mice shown in panels *A* and *B* 48 h after administration of CLuc-IgG or FBP-IgG but before luciferin injection were obtained under same conditions.

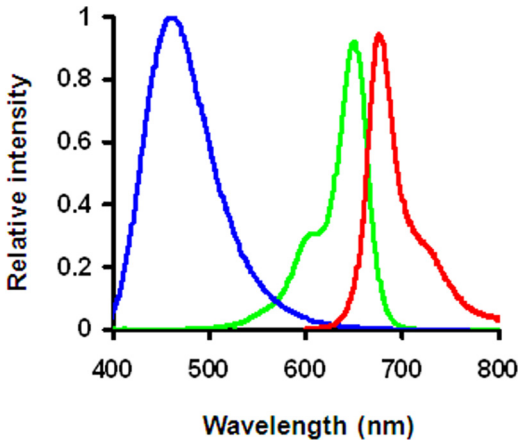

Fig. S3. Bioluminescence spectrum of CLuc (blue), excitation spectrum (green), and emission spectrum (red) of the organic dye.

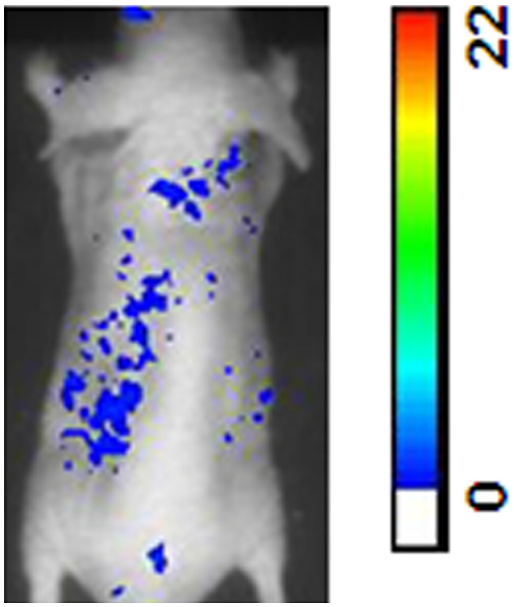

Fig. S4. Bioluminescence image of the tumor-bearing mouse using avidin-bound antibody and FBP at 24 h.