Supporting Information

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SI Materials and Methods

Plasmid Construction. The cDNA sequence of *Avena sativa* Phototropin1 was synthesized from oligonucleotides by PCR. An N-terminal fragment of firefly luciferase and McLuc1 cDNAs were amplified by PCR with sources of cDNA constructs (1). Human microtube-associated protein1 light chain 3 (LC3)-A cDNA was amplified from cDNA clone vector (Kazusa original ORF clone, ORH5916). PCR products were inserted into a mammalian expression vector, pcDNA/V5-His (B) (Invitrogen) and were sequenced using a genetic analyzer (AB310; Applied Biosystems). A replication-deficient adenoviral vector encoding the fusion protein photo-inactivatable luciferase (PI-Luc) was constructed with the cosmid vector pAxCAwtit2 (TaKaRa).

Selection of a Stable Cell Line. For establishing respective cell lines that stably expressed a series of PI-Luc, HEK293 cells were subcultured in DMEM (Wako) supplemented with 10% FBS (Gibco), 100 units/mL penicillin and 100 μg/mL streptomycin (Gibco) at 37 °C in an incubator with 5% CO_2. The plasmids were transfected into HEK293 cells with a Lipofectamine 2000 reagent (Invitrogen). Stable expressing cells were obtained after ∼7 d of selection in 1.0 mg/mL Zeocin (Invitrogen) containing the growth medium. A single clone expressing PI-Luc was cultured into the subpopulation for additional experiments.

Sample Preparation. All experimental buffers for living cell measurement except for pH calibration were based on HBSS (Gibco) supplemented with 10% FBS, 10 mM Hepes, and 2 mM D-luciferin (potassium salt; Wako). Staurosporine (Sigma) was provided directly into the buffer. The subcultured cell line was exchanged to the experimental buffer immediately before experiments. When the half-recovery time (RT) values were calibrated for pH, the stable cells were dipped into a phosphate-based pH-adjusting buffer supplemented with 10% (vol/vol) FBS, 2 mM D-luciferin, 1μM 5-(and-6)-carboxy seminaphthorhodafluor-1-acetoxymethylester (SNARF-1-AM), and 20 μM nigericin (sodium salt; Sigma) as a proton ionophore. The pH condition of the buffer was adjusted to 37 °C before experiments.

Immunostaining. Cell lines cultured on a cover glass with or without serum starvation were incubated for 2 h. Cells were washed with PBS and were fixed with 3.7% formaldehyde at 37 °C for 15 min. The cells were washed and permeabilized with 0.2% Triton X-100 in PBS for 5 min with subsequent washing three times with PBS. The cells were blocked by 0.2% fish skin gelatin (FSG) in PBS overnight at 4 °C. The buffer was exchanged to PBS (0.2% FSG) containing 1/2,000 dilution of rabbit anti-firefly luciferase antibody (Abcam) and mouse Anti-LAMP2 antibody (Abcam), and incubated for 1 h at room temperature, shaking gently. After washing with PBS, the cells were filled with PBS (0.2% FSG) containing 1/2,000 dilution of a goat anti-rabbit IgG labeled with Cy3 (Molecular Probes, Inc.) and a donkey anti-mouse IgG labeled with AlexaFluoro 488 (Molecular Probes, Inc.) and incubated for 1 h at room temperature. Cells were washed with PBS (0.2% FSG) and fixed on a cover glass with Mowiol. The samples were observed under a confocal fluorescence microscope (FV-1000; Olympus).

Preparation and Infection of Adenovirus. The cosmid vector pAxCAwtit2 including the cDNA of PI-Luc was transfected into HEK293 cells. After 7–14 d of culture, high-titer adenoviruses were collected and purified by ultracentrifugation with a cesium chloride density gradient. The titer was 3.6×10^9 pfu/mL, evaluated with Adeno-X Rapid Titer kit (Clontech). The adenoviruses were infected to a mouse footpad by s.c. injection 3 d before experiments (1×10^6 pfu/footpad).

1. Hida N, et al. (2009) High-sensitivity real-time imaging of dual protein-protein interactions in living subjects using multicolor luciferases. *PLoS ONE* 4(6):e5868.

LOV2 region (404-546 a.a.) in *Avena sativa* Phototropin1 (1-923 a.a.)

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     TTG GCT ACT ACA CTT GAA CGT ATT GAG AAG AAC TTT GTC ATT ACT GAC CCA AGG TTG CCA
404  L   A   T   T   L   E   R   I   E   K   N   F   V   I   T   D   P   R   L   P    423
                                                    ↓
                                                (416)ATC
                                                     I

     GAT AAT CCC ATT ATA TTC GCG TCC GAT AGT TTC TTG CAG TTG ACA GAA TAT AGC CGT GAA
424  D   N   P   I   I   F   A   S   D   S   F   L   Q   L   T   E   Y   S   R   E    443


     GAA ATT TTG GGA AGA AAC TGC AGG TTT CTA CAA GGT CCT GAA ACT GAT CGC GCG ACA GTG
444  E   I   L   G   R   N   C   R   F   L   Q   G   P   E   T   D   R   A   T   V    463


     AGA AAA ATT AGA GAT GCC ATA GAT AAC CAA ACA GAG GTC ACT GTT CAG CTG ATT AAT TAT
464  R   K   I   R   D   A   I   D   N   Q   T   E   V   T   V   Q   L   I   N   Y    483


     ACA AAG AGT GGT AAA AAG TTC TGG AAC CTC TTT CAC TTG CAG CCT ATG CGA GAT CAG AAG
484  T   K   S   G   K   K   F   W   N   L   F   H   L   Q   P   M   R   D   Q   K    503
                                                    ↓
                                                (496)ATT
                                                     I

     GGA GAT GTC CAG TAC TTT ATT GGG GTT CAG TTG GAT GGA ACT GAG CAT GTC CGA GAT GCT
504  G   D   V   Q   Y   F   I   G   V   Q   L   D   G   T   E   H   V   R   D   A    523
                                        ↓
                                    (513)CTG
                                         L

     GCC GAG AGA GAG GGA GTC ATG CTG ATT AAG AAA ACT GCA GAA AAT ATT GAT GAG GCG GCA
524  A   E   R   E   G   V   M   L   I   K   K   T   A   E   N   I   D   E   A   A    543
                    ↓                                       ↓
                (528)GCA                                (538)GAA
                     A                                       E

     AAA GAA CTT
544  K   E   L    546 (a.a.)
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Fig. S1. Mutated amino acids in a light, oxygen, and voltage domain from *Avena sativa* Phototropin1 (LOV2). DNA and amino acid sequences of LOV2 protein region in *A. sativa* Phototropin1 are shown. Substituted amino acid residues and their corresponding DNA sequences are indicated with arrows (Q513L, V416I&L496I, and G528E&N538E). Numbers are positions of the amino acid sequence in Phototropin1.

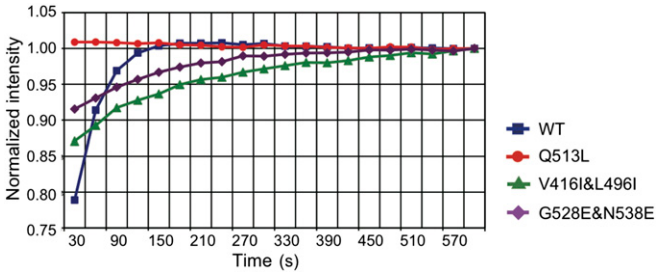
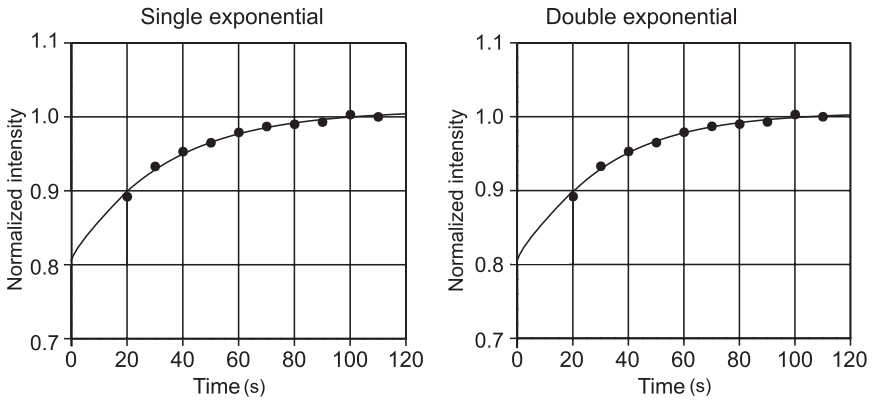


Fig. S2. Time-course measurements of bioluminescence for LOV2 domain mutants. Bioluminescence intensities of PI-Luc expressed in HEK293 cells were measured after blue light irradiation. The bioluminescence intensities were normalized against initial values of bioluminescence intensities. Data are shown for 10 min.



$$Intensity = -[Int]_0 e^{-k_1 T} + [Int]_{\max}$$

$$Recovery\ Time\,(RT) = \frac{\ln 2}{k_1}$$

$$Intensity = -[Int]_0\left(1 - \frac{k_2}{k_2 - k_1}e^{-k_1 T} + \frac{k_1}{k_2 - k_1}e^{-k_2 T}\right) + [Int]_{\max}$$

Fig. S3. Curve fitting for PI-Luc bioluminescence recovery. Black circles show bioluminescence intensities at each time point measured using PI-Luc–expressed HEK293 cells. Cells were irradiated at time 0. Single and double exponential curves were fitted from the time of 20 s to 110 s. Equations of curve fitting are shown under the graphs. $[Int]_{max}$, maximum value of normalized intensity; $[Int]_0$, value of *y*-intercept at time 0; k_1, rate constant of LOV–Jα interaction; k_2, rate constant of luciferase-fragment complementation; *T*, time (seconds).

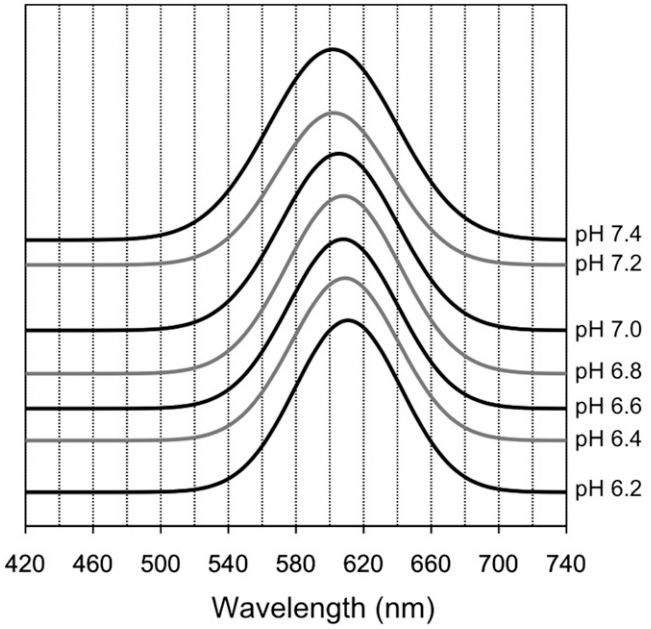


Fig. S4. Bioluminescence spectra of PI-Luc at different pHs. Bioluminescence spectra were obtained using lysates of HEK293 cells expressing PI-Luc. The spectra for respective pH conditions are displayed alternately in black and gray.

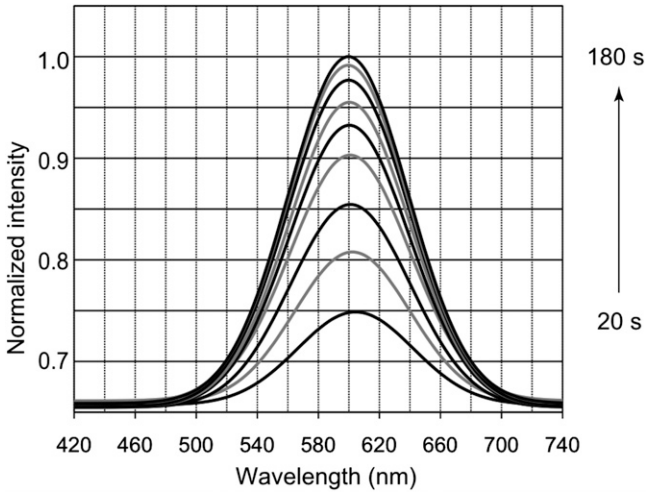


Fig. S5. Time-dependent bioluminescence spectra of PI-Luc after light stimulation. Bioluminescence spectra were obtained using cell lysates that include PI-Luc. Bioluminescence was measured every 20 s after blue light irradiation. The sequential spectra are displayed alternately in black and gray.

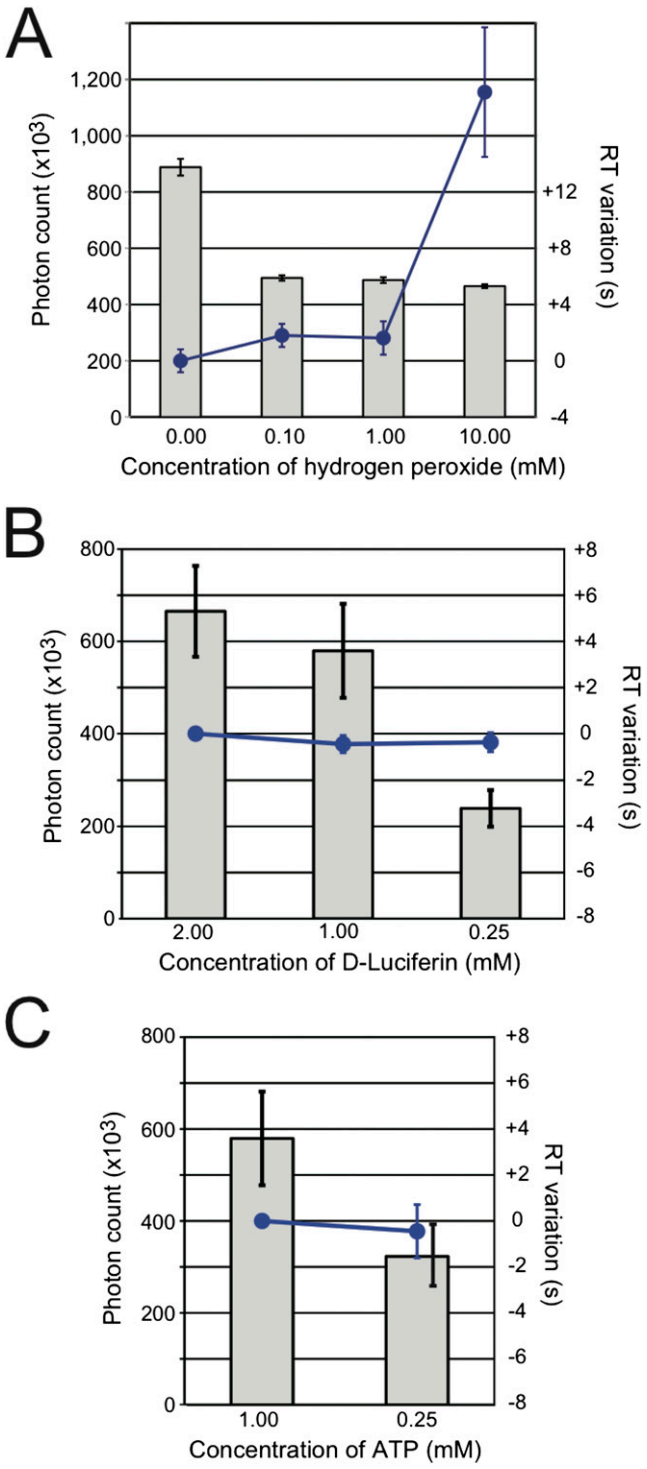


Fig. S6. Pl-Luc responses for different buffer conditions. Each figure shows effects of hydrogen peroxide (*A*), D-luciferin (*B*), and ATP (*C*) concentrations on absolute photon counts of bioluminescence (gray bars) and the RT values (blue circles). Variations of the RT values are indicated, assuming that the RT values in the absence of hydrogen peroxide and the presence of 2.0 mM D-luciferin and 1.0 mM ATP were zero, respectively ($n = 5$). Error bars, SD.

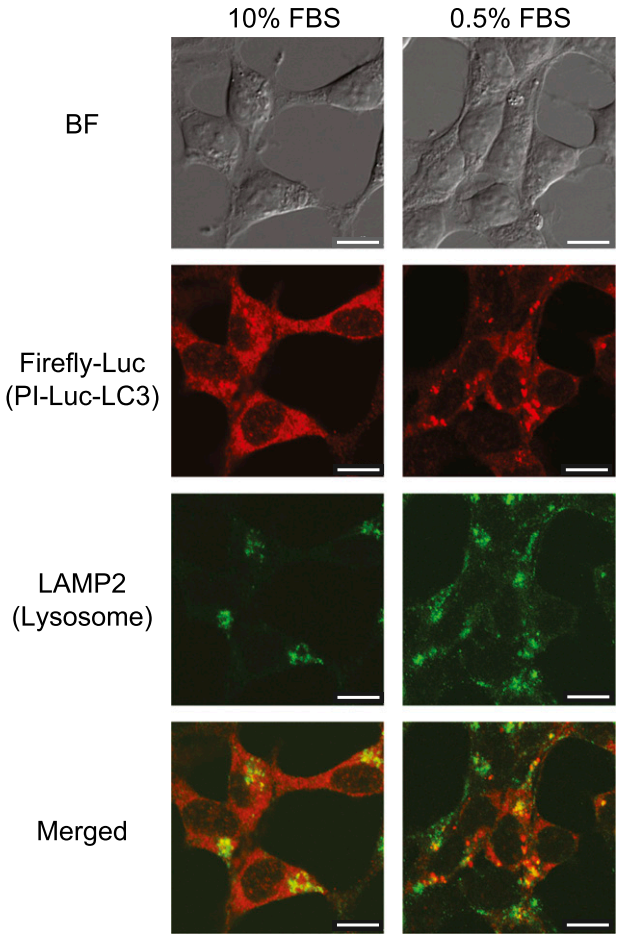


Fig. S7. Intracellular localization of PI-Luc–LC3 under serum starvation. HEK293 cells containing PI-Luc–LC3 were cultured under serum starvation (0.5%) for 2 h. Localization of PI-Luc–LC3 was detected by immunostaining with a rabbit anti-firefly luciferase antibody. Lysosomes were detected with mouse anti-LAMP2 antibody. (Scale bar, 10 μm.)

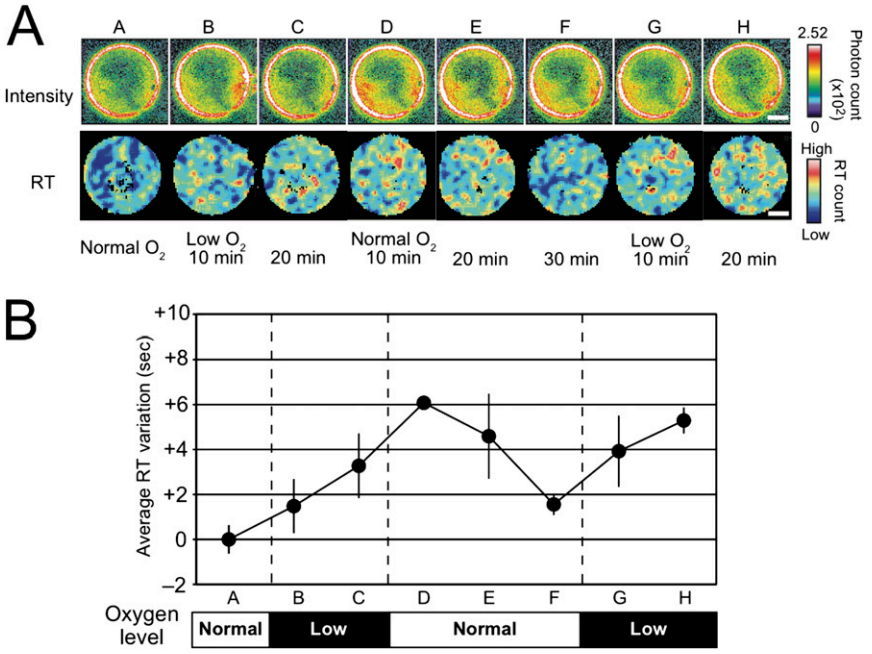


Fig. S8. Variation of pH under hypoxic conditions. (*A*) Hypoxic effects for bioluminescence intensity and the RT values. HEK293 cells harboring PI-Luc were cultured on a dish and were incubated under a low-oxygen condition. Sequential images of bioluminescence were taken every 20 s using a cooled CCD camera. The circlular strong bioluminescence originated from reflection off the edge of the culture dish. (Scale bar, 10 mm.) (*B*) Variation of average RT values with changing oxygen level. Average RT values were evaluated using data of the whole dish (*A*). Average RT variations are shown, assuming that the RT value for the starting time was zero (n = 3). Error bars, SD. Each oxygen status is indicated below the graph.

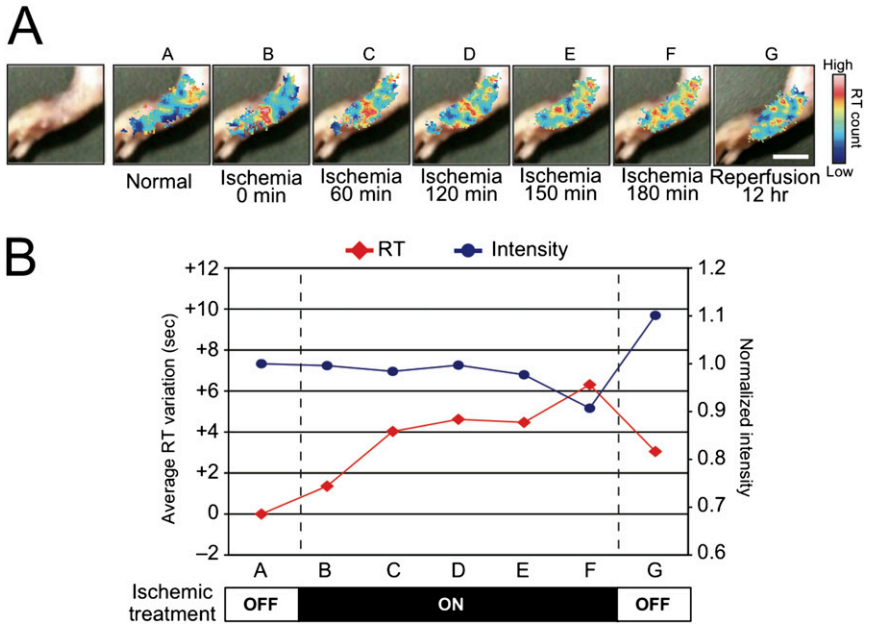
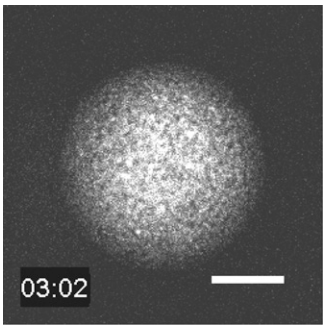
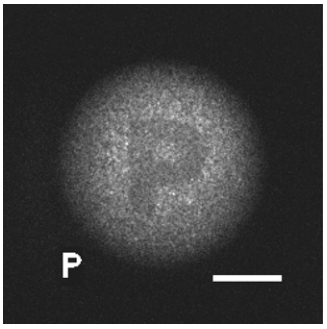


Fig. S9. RT imaging of a mouse footpad treated with ischemia for a long time. (*A*) Bright-field and RT images of mouse footpad with ischemia and reperfusion treatments. The mouse footpad was treated with vessel clipping and detected bioluminescence images. The RT images were overlaid with the bright-field image. (Scale bar, 10 mm.) (*B*) Variation of average RT and bioluminescence intensity. Average RT values were evaluated using data from *A*. The bioluminescence intensities were normalized against the initial value of bioluminescence intensities. Average RT variations are shown, assuming that the RT value for the starting time was zero. Each oxygen status is shown at the bottom of the graph.



Movie S1. Time-lapse bioluminescence recovery of cell population with light irradiation. A PI-Luc–expressed cell population in a cultured dish was set on a microscope and irradiated with blue light. Each bioluminescence image was taken in sequence using a bioluminescence microscope for 10-s exposure. The displayed count shows the elapsed time from the start of measurement. (Scale bar, 1 mm.)

Movie S1



Movie S2. Time-lapse bioluminescence recovery of the cell population with letter-shaped irradiation. A PI-Luc–expressed cell population in a cultured dish was irradiated with light having the following letter shapes: P, I, -, L, U, C. In each letter, the time-lapse recovery video was composed of five images, for 30 images in all. Each image was taken sequentially using a bioluminescence microscope for 10-s exposure. The interval time between individual letters was 20 s. (Scale bar, 1 mm.)

Movie S2