Supporting Information

An Activatable Photosensitizer for Targeted Ablation of *lac*Z-Positive Cells with Single-Cell Resolution

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Safety statement.

No unexpected or unusually high safety hazards were encountered.

Materials.

General chemicals were of the best grade available, supplied by Tokyo Chemical Industries, Wako Pure Chemical or Aldrich Chemical Company, and were used without further purification. Dimethyl sulfoxide (DMSO, fluorometric grade) for the spectrometric measurements was purchased from Dojindo.

Instruments.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 instrument (400 MHz for ^{1}H, 101 MHz for ^{13}C). High-resolution mass spectra were recorded on a Bruker micrOTOF II, using electron spray ionization (ESI). HPLC purification and analyses were performed on an HPLC system composed of reverse-phase columns of Inertsil ODS-3 10.0 mm × 250 mm or 4.5 mm × 250 mm for purification and Inertsil ODS-3 4.6 mm × 250 mm for analyses (GL Sciences, Tokyo, Japan), with a pump (PU-2080, JASCO) and a detector (MD-2015, JASCO). All experiments were carried out at 298 K, unless otherwise specified.

Spectral measurements.

Absorption spectra were obtained with a UV-2450 UV/Vis spectrometer (Shimadzu) or with a UV-1650PC UV/Vis spectrometer (Shimadzu), and fluorescence spectra were obtained with a FP-6600 fluorescence spectrometer (JASCO) or with a F7000 fluorescence spectrometer (Hitachi). Probes were dissolved in DMSO (fluorometric grade, Dojindo) to obtain stock solutions. Optical properties of probes (1 μM) were examined in 200 mM sodium phosphate buffer containing less than 1% (v/v) DMSO as a cosolvent. Absolute fluorescence quantum efficiency was determined with an absolute PL quantum yield spectrometer, Quantaurus-QY (Hamamatsu Photonics).

Determination of pK_{cycl} values of test compounds.

Absorption and fluorescence emission spectra of test compounds were measured in 200 mM sodium phosphate buffer at various pH values. For test compounds with *n* acid-base equilibrium structures (n = 1 or 2), pH profiles of absorbance (Abs) were fitted to the following formula to determine pK_{cycl}.

$$\text{Absorbance or FI} = \frac{c_0 + \sum_{k=1}^{n} c_k \cdot 10^{k\cdot pH - \sum_{l=1}^{k} pK_{al}}}{1 + \sum_{k=1}^{n} 10^{k\cdot pH - \sum_{l=1}^{k} pK_{al}}}$$

$$(pK_{a1} < pK_{a2} < \bullet\bullet\bullet < pK_{an})$$

$$(c_n = \text{constant}).$$

Detection of singlet oxygen by near-infrared spectroscopy

Singlet oxygen was detected by measuring $^1O_2$ luminescence around 1270 nm in response to light irradiation, using a near-infrared emission spectrometer (Fluorolog-3, HORIBA, Japan). Dyes were dissolved in PBS containing 1% DMSO as a cosolvent and irradiated at 532 nm. To calculate the quantum yield of $^1O_2$, the luminescence signal was integrated for 7 seconds at each wavelength. The quantum yield was calculated by using Rose bengal in PBS as a reference (0.75)[1]. For other spectra, the luminescence signal from 0.2 – 5 μs was accumulated.

Cell cultures

HEK293 and HEK/*lacZ* cells were purchased from JCRB (JCRB1414/Murakami, T.) and cultured in DMEM (GIBCO) containing 10% fetal bovine serum (GIBCO), 100 U/mL penicillin, 100 μg/ml streptomycin, at 37 ℃ in humidified air containing 5% CO_2.

Cell viability assay

HEK293 and HEK/*lacZ* cells were seeded in a plastic-bottomed 96-well plate and cultured overnight. The medium was changed to fresh medium containing SPiDER-killer-βGal at various concentrations, followed by incubation for 1 hour at 37 ℃. The cells were irradiated at 550 nm (550±5 nm, 8 mW/cm^2) for 3 min by using a Xe light source, MAX301 (Asahi Spectra Co., Ltd.), then the medium in each well was replaced with 100 μL of fresh medium, and the cells were cultured for 24 hours. The cells were further incubated in medium containing 10% Cell Counting Kit-8 (Dojindo), and the absorbance at 450 nm was measured using an Envision plate reader (Perkin Elmer) to determine the cell viability. Values from wells containing cells without photosensitizer and without photoirradiation were taken as representing 100% cell viability. Cell toxicity in the absence of irradiation (dark toxicity) was similarly calculated, without light irradiation.

Preparation of cell lysates and SDS-PAGE

HEK-*lac*Z(+) cells were incubated with 400 μM SPiDER-killer-βGal in culture medium for 1 hour at 37 ℃, and the cell lysate was prepared with CelLytic M (Sigma-Aldrich, #2978) according to the manufacturer's protocol. Protein concentrations of lysates were determined by BCA assay. SDS-PAGE analysis was carried out on 10 % polyacrylamide gel with a Mini-PROTEAN tetra Cell (Bio-Rad). 4-CH_2OH HMDESeR was used as the control. Fluorescence images of the gels were obtained with a LAS4000, at excitation and emission wavelengths of 520 nm and 575/20 nm, respectively.

Cell ablation of HEK-*lacZ*(+) cells in a co-culture of HEK293 and HEK-*lacZ*(+) cells

For Figure 2b, HEK/*lacZ*(+) cells were pre-stained with 5 μM CellTracker Blue CMAC (Thermo Fisher Scientific) and HEK293 cells were pre-stained with 1 μM CellTracker™ Green CMFDA (Thermo Fisher Scientific) for 1 hour, and then co-cultured for 1 day on an 8-chamber plate (Ibidi, μ-slide). For Figure S7, HEK293 cells were incubated with 10 μM CellTracker™ Green CMFDA for 1 hour, and then co-cultured with unstained HEK-*lacZ*(+) cells for 1 days on an 8-chamber plate. DMEM was replaced with SPiDER-killer-βGal (1 μM for Figure 2 and 0.7 μM for Figure S7) solution in DMEM (phenol red-free) and incubation was continued for 1 hour at 37 °C. For Figure S7, the medium was replaced with DMEM containing 2 μM EthD-1 and 1 μM Hoechst 33342. Then, light irradiation was carried out under a confocal fluorescence microscope (TCS SP8 STED, Leica) equipped with a white light laser (561 nm, 100%, 5 min) and objective lens (HCX PL APO CS 63x/1.40 Oil, Leica). For the comparison with our previously reported activatable photosensitizer, HMDESeR-βGal (Figure S8), the co-culture was incubated with 2 μM SPiDER-killer-βGal or HMDESeR-βGal for 1 hour at 37 °C. Then, the cells were irradiated at 550 nm (550±5 nm, 10 mW/cm^2) for 5 min using a Xe light source, MAX301 (Asahi Spectra Co., Ltd.) without washing, and the medium was replaced with DMEM containing 2 μM EthD-1 and 1 μM Hoechst 33342. Time-lapse imaging was performed under a confocal fluorescence microscope (TCS SP8 STED, Leica) equipped with a white light laser and objective lens (HCX PL APO CS 63x/1.40 Oil, Leica). Excitation/emission: 405 nm/430-480 nm for Hoechst 33342 or CellTracker Blue, 490 nm/500-550 nm for CellTracker™ Green CMFDA, 561 nm/600-650 nm for EthD-1.

***Drosophila* culture conditions and strains.**

The following fly strains were used in this study: *Hs-flp^{122}*(#23649), *AyGal4^{25} (Act>y$^+$, stop>Gal4) UAS-LacZ* (# 4410) from the Bloomington *Drosophila* Stock Center; *engrailed-lacZXho25 (en-lacZ)* (# 101554) from the Kyoto Stock Center (DGRC); *UAS-H2B::ECFP*, a gift from S. Kondo[2], *UAS-VC3Ai*, a gift from M. Suzanne[3]. Flies were maintained at 25 °C on a standard diet containing 4% cornmeal, 4% baker's yeast, and 10% glucose, and 0.8% agar with propionic acid and nipagin. Clones were induced by heat-shock (37 °C) for 7 min. Images were taken at 20 hr APF (after puparium formation), 24 hr after the induction. We thank S. Kondo, M. Suzanne, and the Bloomington Stock Center for providing the fly strains.

Cell ablation in *Drosophila* wing disc.

Ex vivo imaging of *en-lacZ* wing disc was carried out according to the previous report[4]. Third instar larvae were dissected in culture medium (Schneider's *Drosophila* Medium (GIBCO 21720-024) containing 10% fetal bovine serum and 1% penicillin/streptomycin) and wing discs and attached

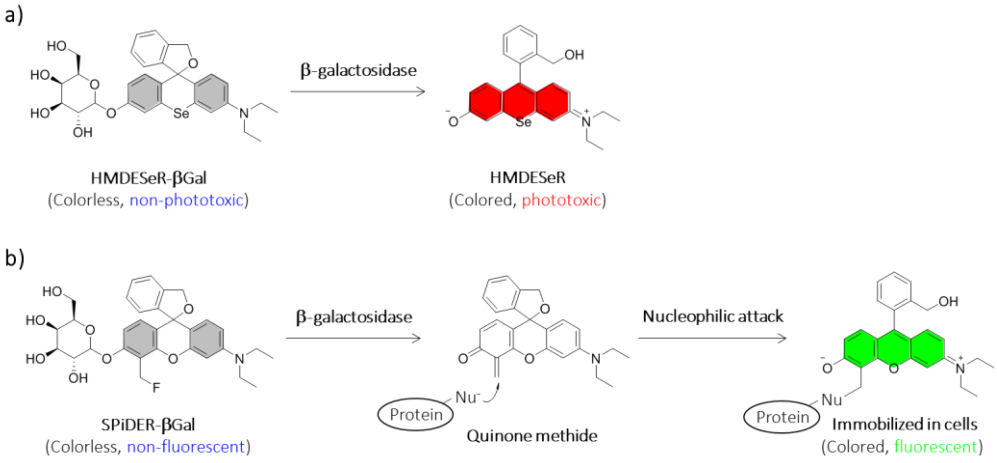
haltere discs were incubated for 2 hours with 3 μM SPiDER-killer-βGal in PBS containing 1% DMSO. The tissue was moved onto a 35 mm dish in culture medium and irradiated at 550 nm (10 mW/cm^2, 5 min) with a Xe lamp (MAX301, Asahi Spectra Co., Ltd.) without washing. The tissue was moved to fresh culture medium and incubated at r.t. for 4 hours, then moved to culture medium containing 2 μM Calcein-AM and further incubated for 1 hour. Microscopic imaging was carried out with a confocal fluorescence microscope SP8 at excitation and emission wavelengths of 490 nm and 500-550 nm, respectively.

Time-lapse fluorescence imaging of pupal notum of live *Drosophila*.

Pupae at 16-20 hr APF were fixed on slide glass with double-sided tape, and the pupal case was removed at the head and thorax region. Wet filter paper was placed around each pupa to keep it hydrated, and it was covered with a cover-glass bearing a small drop of water to prevent desiccation. Silicon (Shinetsu) was used to seal the chamber. A 0.2-0.5 μL aliquot of DMSO solution of Hoechst 33342 (5 mM) and SPiDER-killer-βGal (5 mM) was injected into the abdomen with a glass capillary, followed by incubation at r.t. for 1.5 hours. Then, the pupa was moved under a confocal fluorescence microscope equipped with a white-light laser, a 405 nm LED, and an objective lens (HCX PL APO CS 63x/1.40 Oil). After laser irradiation of the pupal notum at 561 nm (WLL 100%, 10 min), time-lapse fluorescence imaging was carried out for 10 hours. Excitation/emission: 405 nm/410-460 nm for Hoechst 33342, 458 nm/460-490 nm for ECFP, and 490 nm/540-590 nm for VC3Ai.

Immunohistochemical staining of *Drosophila* pupal notum

Immunohistochemical staining was carried out as described previously[2]. At 10 hours after photo-irradiation at r.t., each *Drosophila* pupa was dissected and fixed with 4% PFA for 20 min. After blocking, the tissue was stained with rabbit polyclonal anti-cleaved Dcp-1 antibody (diluted 1:100 with 0.1% Triton containing PBS (PBST); Cell Signaling Technology #9578S) at 4℃ overnight. The tissue was washed twice with 500 μL PBST, stained for 2 hours at r.t. with goat anti-rabbit Cy5 (diluted with PBST, Abcam (#ab97077), washed again with PBST 3 times, and mounted on slide glass. SlowFade® (Thermo Fisher Scientific) was dropped onto the sample and a cover glass was placed carefully over it. Fluorescence imaging of the tissue was carried out with a confocal fluorescence microscope SP8. Excitation/emission: 405 nm/410-460 nm for Hoechst 33342, 458 nm/460-490 nm for CFP, 640 nm/650-690 nm for Cy5.



c)

Compound	Absorption maximum / nm	Emission maximum / nm	Φ_{fl}	Φ_{Δ}	pK_{cycl}	pK_a
HMDESeR-βGal[a]	543, 580	n.d.	n.d.	-	5.8	-
HMDESeR[a]	558	590	0.006	0.99	5.2	10.3
SPiDER-βGal[b]	493, 525	560	<0.01	-	6.3	-
4-CH_2OH HMDER[b]	526	553	0.13[b]	-	11.4	4.9

Figure S1. Chemical structures of HMDESeR-βGal and SPiDER-βGal. (a) HMDESeR-βGal, (b) SPiDER-βGal. (c) Photochemical properties of HMDESeR-βGal, SPiDER-βGals, and the enzyme-catalyzed hydrolysis product. n.d.: not detectable. [a] Reference[4]. [b] Reference[5].

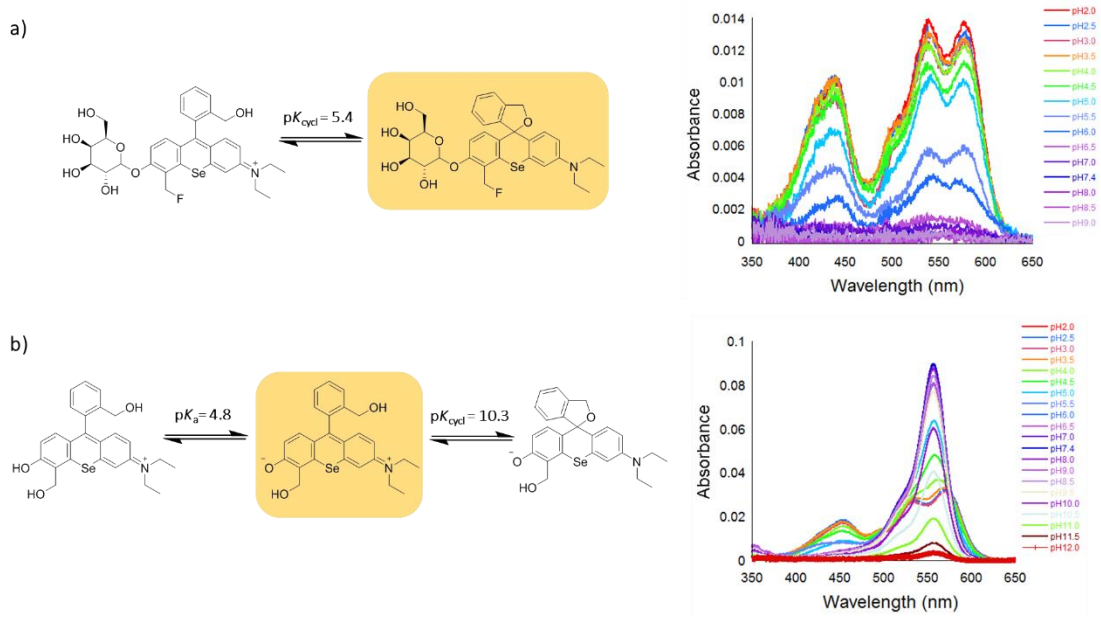


Figure S2. pH-dependence of the optical properties of SPiDER-killer-βGal and 4-CH_2OH HMDESeR. Acid-base equilibrium of SPiDER-killer-βGal (a, left) and 4-CH_2OH HMDESeR (b, left). The highlighted structures are the forms expected to be predominantly present at physiological pH. Absorption spectra of 1 μM SPiDER-killer-βGal (a, right) or 4-CH_2OH HMDESeR (b, right) in 200 mM sodium phosphate buffer at various pHs, containing 0.1% DMSO as a cosolvent.

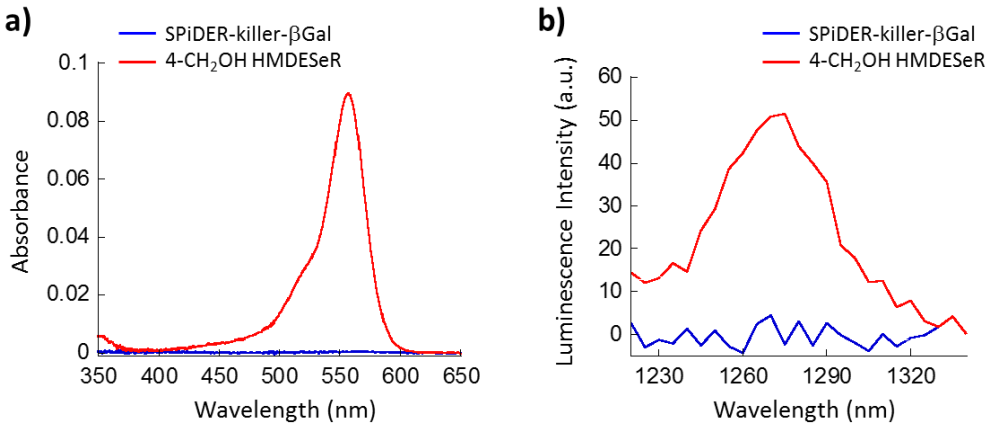


Figure S3. Comparison of absorption and luminescence spectra of SPiDER-killer-βGal and 4-CH_2OH HMDESeR. (a) Absorption spectra of 1 μM SPiDER-killer-βGal (blue) or 4-CH_2OH HMDESeR (red) in 200 mM sodium phosphate buffer at pH 7.4, containing 0.1% DMSO as a cosolvent. (b) Luminescence spectra of $^1O_2$ generated by exciting 0.6 μM SPiDER-killer-βGal (blue) and 4-CH_2OH HMDESeR (red) in PBS (-), pH 7.4, containing 0.1% DMSO as a cosolvent, at 532 nm.

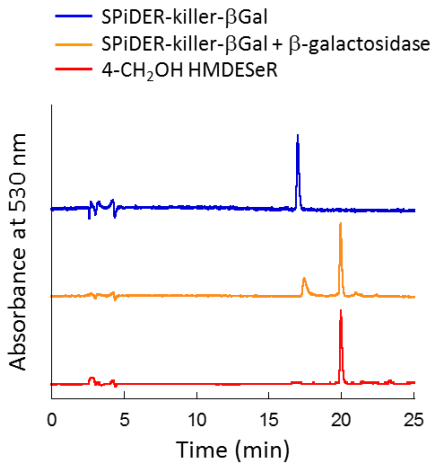


Figure S4. HPLC analysis of *in vitro* reaction products of SPiDER-killer-βGals with β-galactosidase. To a 25 μM solution of SPiDER-killer-βGal in PBS(-), pH 7.4, containing 1% DMSO as a co-solvent, 4 U of β-galactosidase was added, and the reaction mixture was incubated at 37 °C for 30 min. HPLC analysis of reaction solution before (blue) and after (orange) addition of β-galactosidase, and its putative hydrolysis product 4-CH_2OH HMDESeR, was performed under a gradient condition (A: H_2O containing 0.1% TFA; B: acetonitrile containing 1% H_2O; A/B = 95/5 to 5/95 in 30 minutes). Absorbance at 530 nm was monitored.

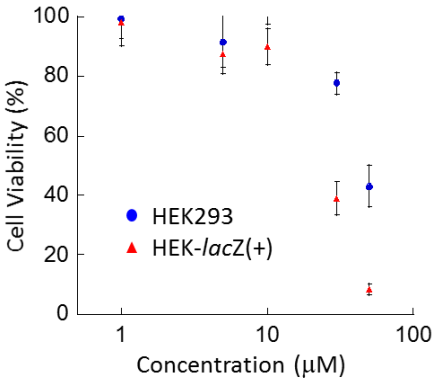
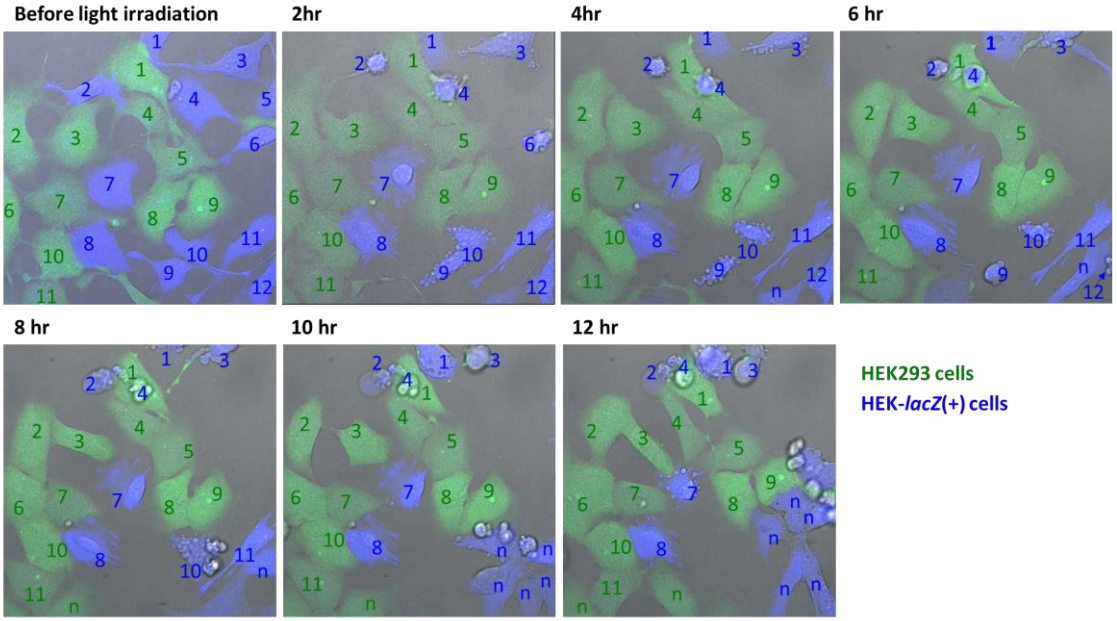


Figure S5. Cytotoxicity of SPiDER-killer-βGal to HEK-*lac*Z(+) cells and HEK293 cells. HEK-*lac*Z(+) cells and HEK293 cells were incubated with SPiDER-killer-βGal for 1 hour at 37 °C under 5% CO_2, then the medium was replaced with fresh culture medium, and culture was continued overnight. Cell viability was measured by means of CCK-8 assay. Data represent mean and s.d. from a single experiment in triplicate.

a)

ROI 1



HEK293 (green)	2 hr after irradiation	12 hr after irradiation
#1	intact	intact
#2	intact	intact
#3	intact	intact
#4	intact	intact
#5	intact	intact
#6	intact	intact
#7	intact	intact
#8	intact	intact
#9	intact	intact
#10	intact	intact
#11	intact	intact

HEK-*lacZ*(+) (blue)	2 hr after irradiation	12 hr after irradiation
#1	intact	dead
#2	dead	dead
#3	dead	dead
#4	dead	dead
#5	dead	dead
#6	dead	dead
#7	dead	dead
#8	intact	intact
#9	dead	dead
#10	dead	dead
#11	intact	untraceable after 10 hr
#12	intact	dead

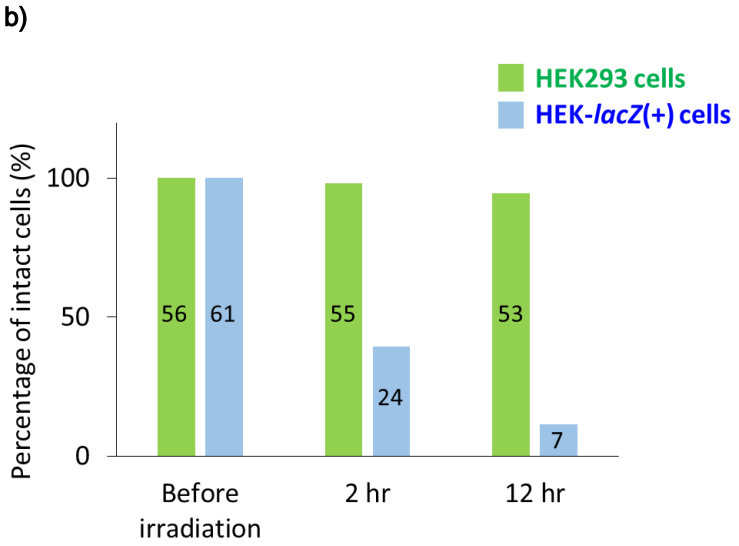
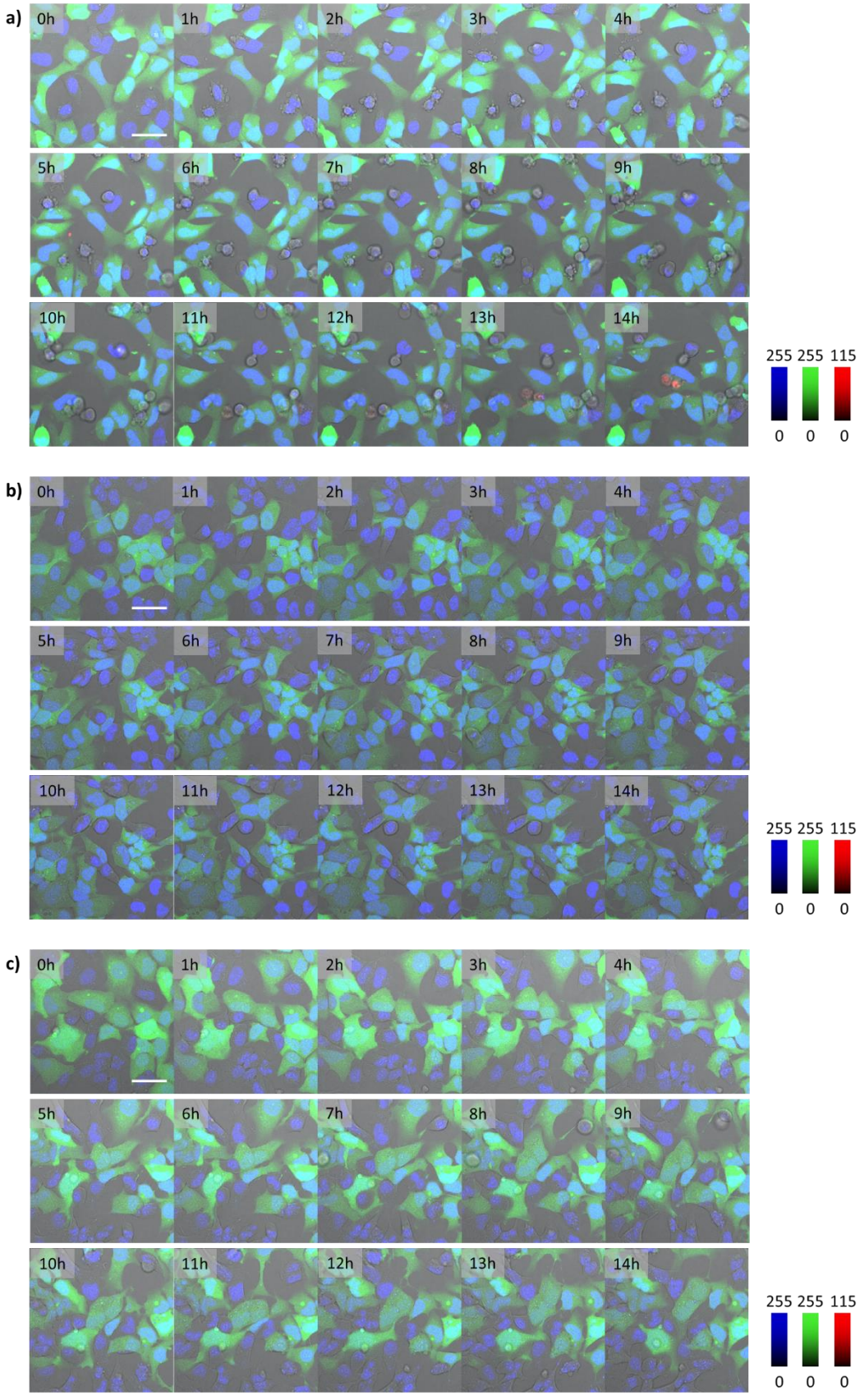


Figure S6. Quantification of the number of cells which remained intact or those which formed blebs or showed cell shrinkage after light irradiation. A co-culture of HEK293 (pre-stained with CellTracker Green) and HEK-*lacZ*(+) cells (pre-stained with CellTracker Blue) was incubated with 1 μM SPiDER-killer-βGal for 1 hour at 37 °C. The medium was replaced with culture medium, and the cells were irradiated at 561 nm (WLL100%, 5 min). Time-lapse fluorescence imaging was carried out every 15 min using a confocal microscope for 12 hrs, and the numbers of cells which remained intact or those which formed blebs or showed cell shrinkage (dead cells) during 12 hrs were counted at 5 different regions of interest (ROIs). a) Representative data at ROI #1. Untraceable cells during 12 hrs were omitted from counting. "n" represents newly appeared cells (cells moved from outside the field of view (non-irradiated area)) during time-lapse imaging. b) The percentages of the number of cells which remained intact in 5 different ROIs. The values were 95% for HEK293 cells (53 cells out of 56 cells), and 12 % for HEK-*lacZ*(+) cells (7 cells out of 61 cells) at 12 hrs after light irradiation, demonstrating that selective death of *lacZ*-positive cells was induced by SPiDER-killer-βGal and light irradiation.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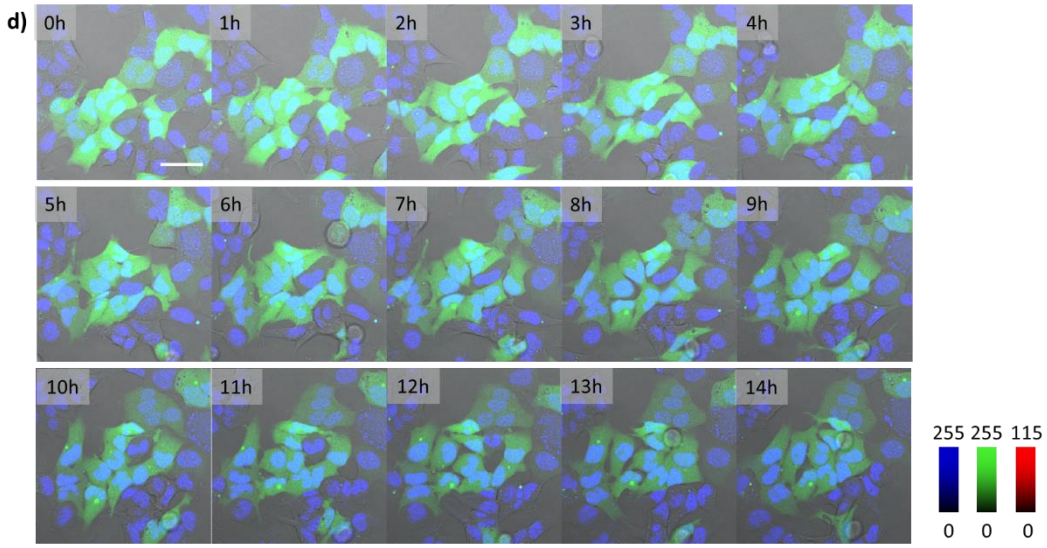


Figure S7. Time-lapse fluorescence imaging of co-cultured HEK293 and HEK-*lac*Z(+) cells. HEK293 cells were pre-incubated with Cell-Tracker Green and HEK-*lac*Z(+) cells were unstained. The co-cultured cells were incubated with 0.7 μM SPiDER-killer-βGal for 1 hour at 37 °C. The medium was replaced with culture medium containing 2 μM EthD-1 and 1 μM Hoechst 3334, and the cells were irradiated at 561 nm (WLL100%, 5 min). Immediately thereafter, time-lapse fluorescence imaging was carried out using a confocal microscope. (a) SPiDER-killer-βGal+, light:+, (b) SPiDER-killer-βGal:+, light:-, (c) SPiDER-killer-βGal:-, light:-, (d) SPiDER-killer-βGal:-, light:+. Excitation/emission: 405 nm/430-480 nm for Hoechst 33342 (blue), 490 nm/500-550 nm for CellTracker Green (green), and 561 nm/600-650 nm for EthD-1 (red). Scale bars, 40 mm.

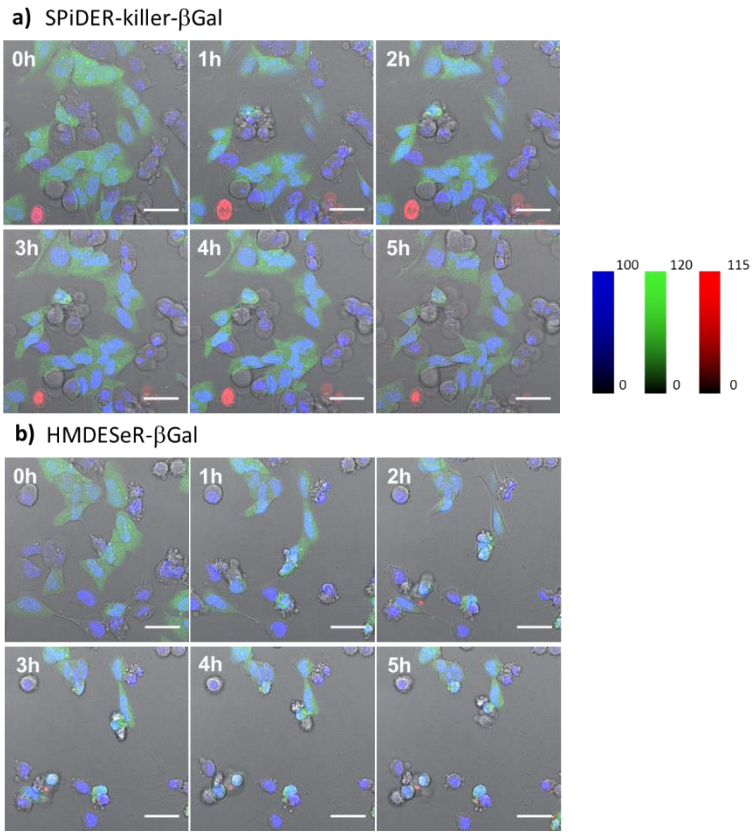


Figure S8. Comparison of SPiDER-killer-βGal and HMDESeR-βGal. A co-culture of HEK293 and HEK-*lac*Z(+) cells was incubated with 2 μM SPiDER-killer-βGal or 2 μM HMDESeR-βGal for 1 hour at 37 °C, then the cells were irradiated at 550 nm (550±5 nm, 10 mW/cm^2) for 5 min with a Xe light source, MAX301 (Asahi Spectra Co. Ltd.). Immediately thereafter, the medium was replaced with culture medium containing 2 μM EthD-1 and 1 μM Hoechst 3334, and time-lapse fluorescence imaging was carried out using a confocal microscope. (a) SPiDER-killer-βGal, (b) HMDESeR-βGal. Excitation/emission: 405 nm/430-480 nm for Hoechst 33342 (blue), 490 nm/500-550 nm for CellTracker Green (green), and 561 nm/600-650 nm for EthD-1 (red). Scale bars, 40 μm.

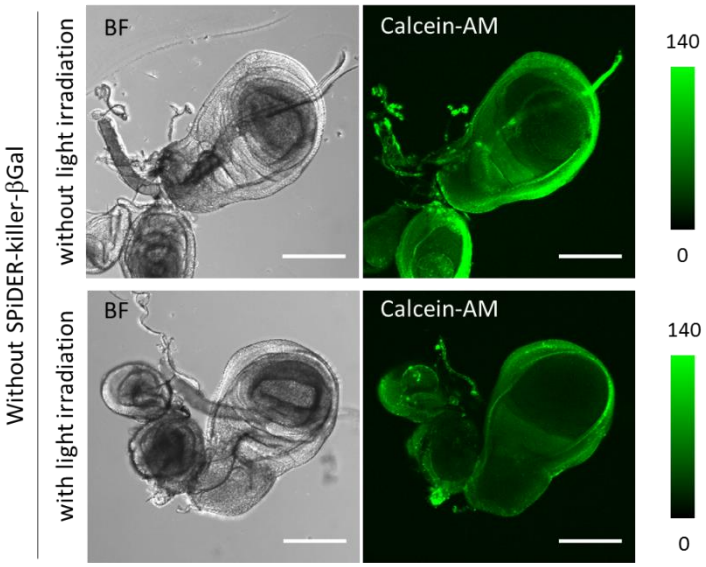


Figure S9. Fluorescence imaging of *en-lacZ Drosophila* third larval wing discs. *Drosophila* wing disc was incubated in the absence SPiDER-killer-βGal for 2 hrs at r.t., followed by irradiation with a Xe lamp (550 nm, 10 mW/cm^2, 5 min)(bottom) or incubation without illumination (top). After 4 hours incubation, wing discs were stained with 2 μM Calcein-AM and fluorescence images were acquired. Excitation/emission: 490 nm/450-500 nm. Scale bars, 150 μm.

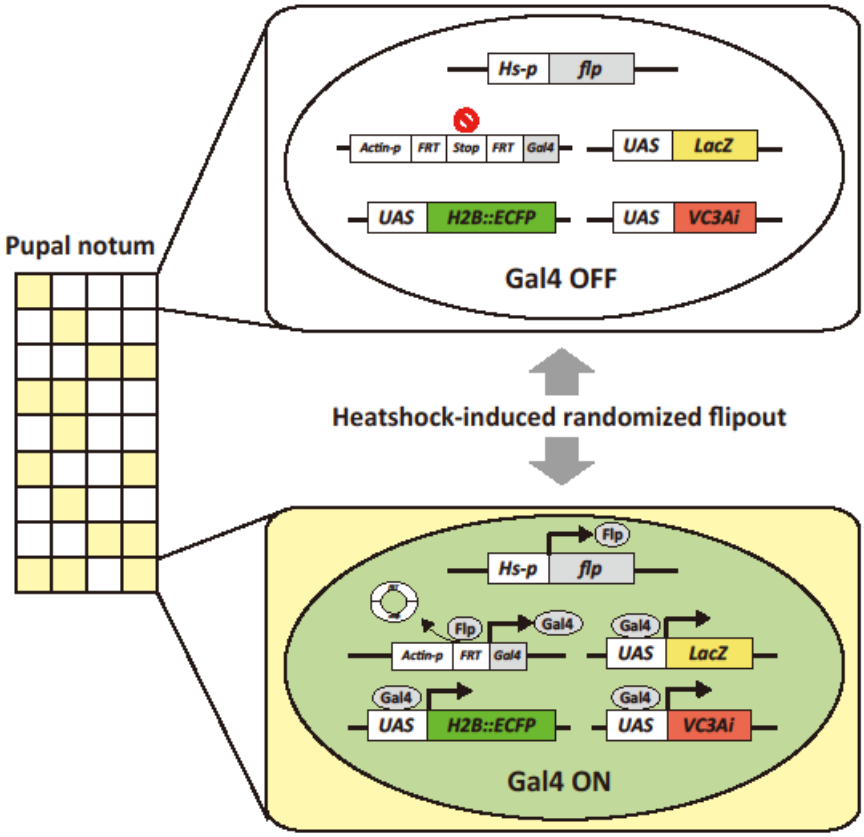


Figure S10. Flipout clone in pupal notum. Mild heatshock induces expression of flippase (flp) and randomized flipout of the FRT-Stop-FRT cassette. In the Flipout clone, Gal4 activates LacZ, H2B::ECFP and VC3Ai expression.

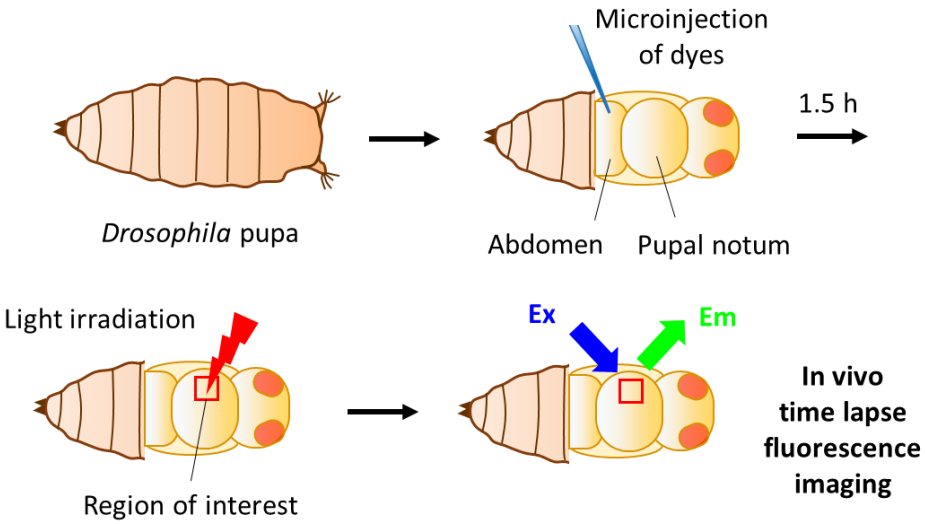
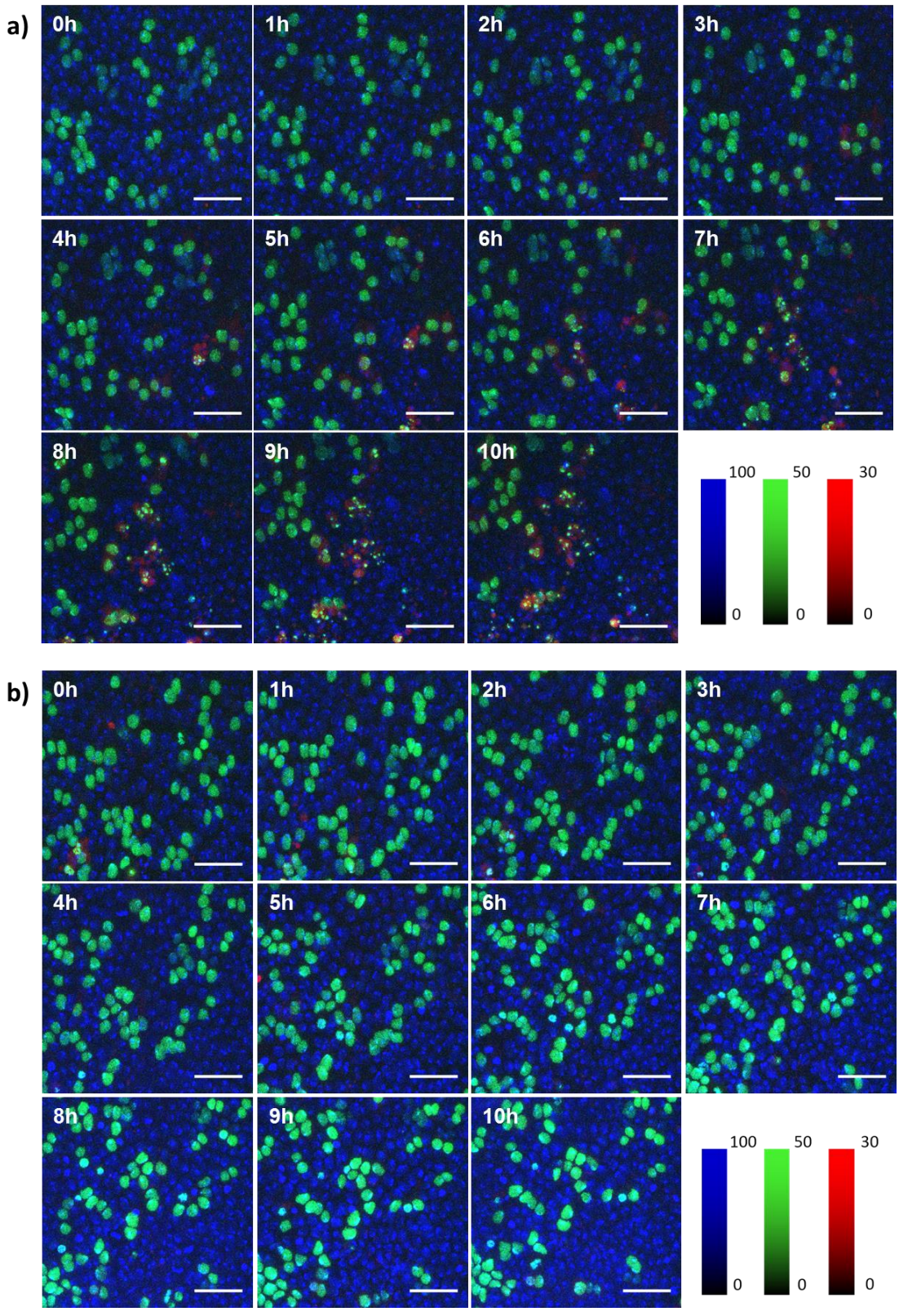


Figure S11. Experimental scheme for *in vivo* imaging of *Drosophila* pupal notum.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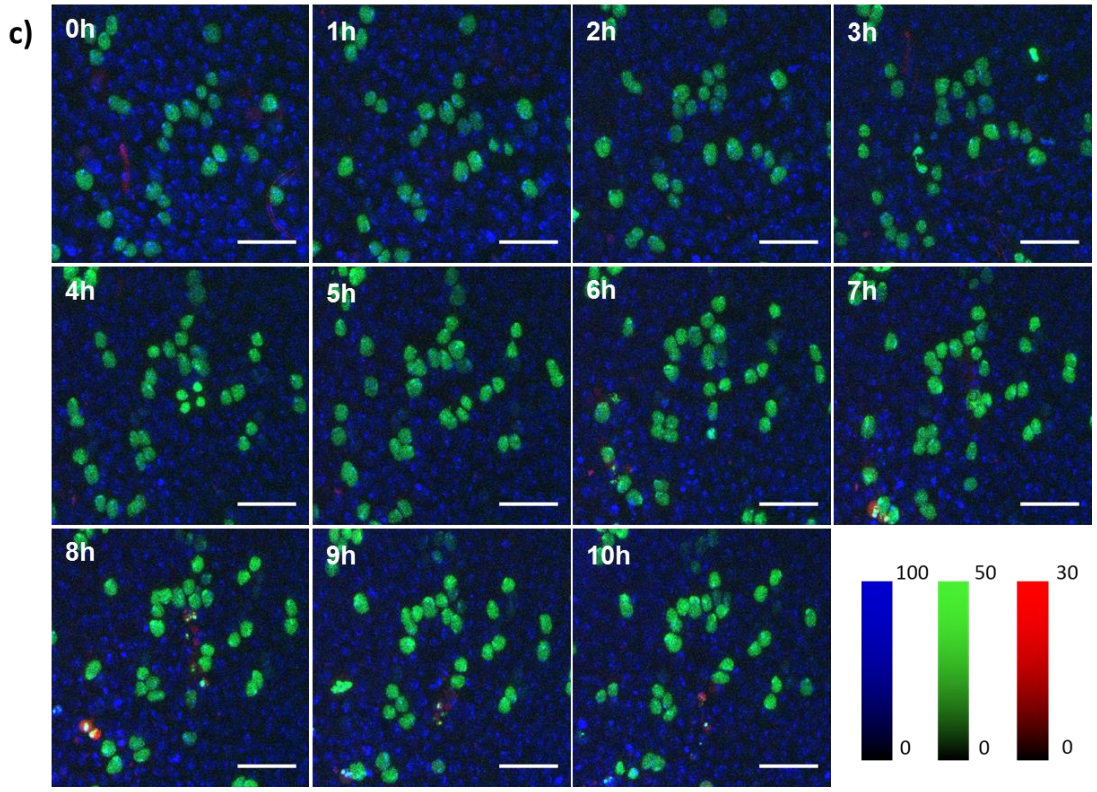
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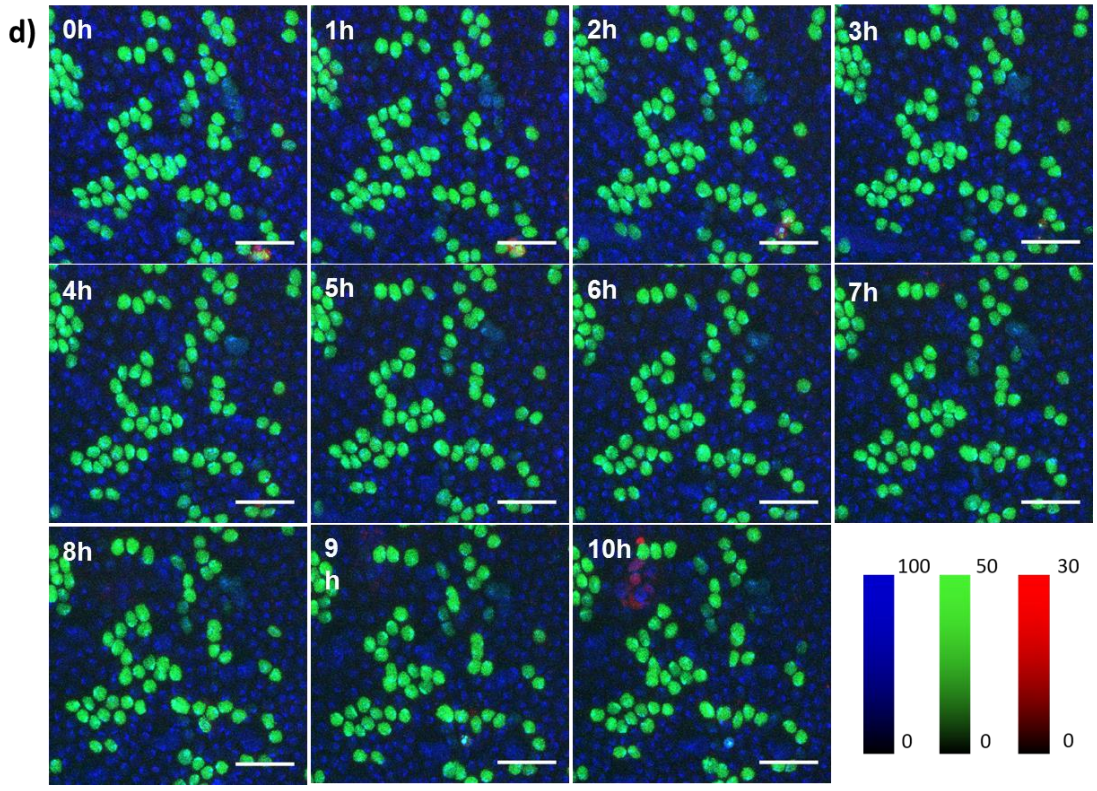
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Figure S12. Time-lapse fluorescence imaging of flip-out clones of live pupal notum. At 1.5 hours after injection of a mixture of SPiDER-killer-βGal and Hoechst33342 (5 μM) into pupae, the pupal notum was irradiated at 561 nm (WLL 100%, 10 min), and time-lapse fluorescence imaging was carried out. (a) SPiDER-killer-βGal+, light:+, (b) SPiDER-killer-βGal:+, light:-, (c) SPiDER-killer-βGal:-, light:-, (d) SPiDER-killer-βGal:-, light:+. Excitation/emission: 405 nm/410-460 nm for Hoechst33342 (blue), 458 nm/460-490 nm for ECFP (green), and 490 nm/540-590 nm for VC3Ai (red). Scale bars, 30 μm.

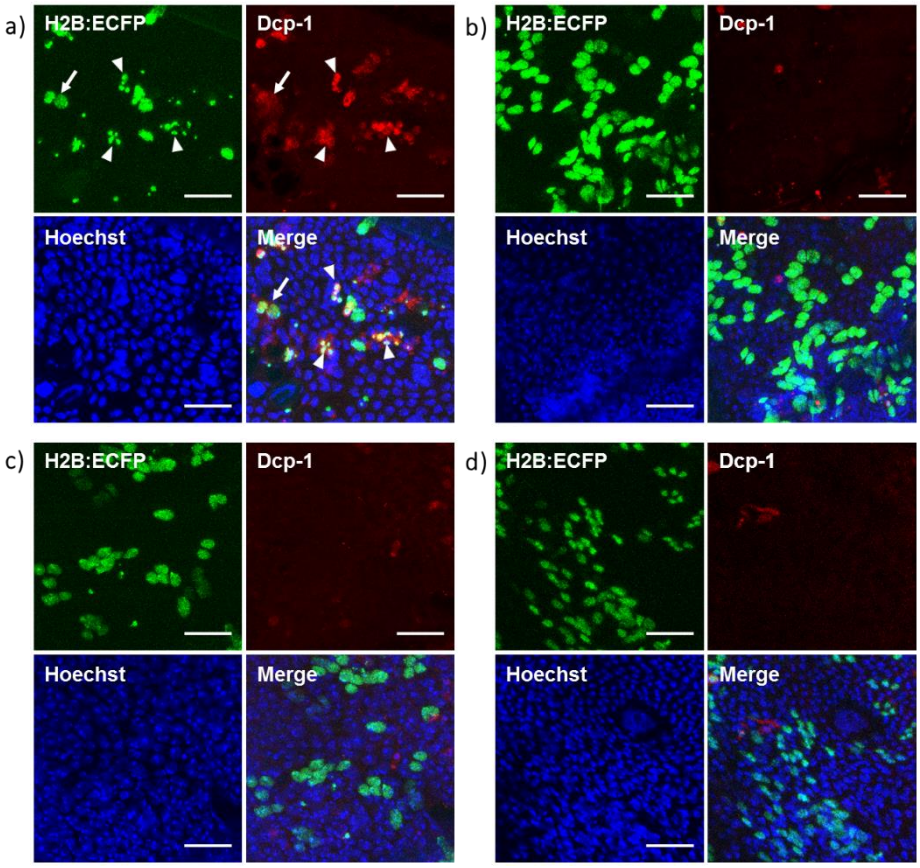


Figure S13. Immunohistochemical staining of dissected pupal notum. At 10 hrs after dye injection or photoirradiation, the pupal notum was dissected and fixed with 4% PFA. Immunohistochemical staining was performed with anti-cleaved Dcp-1 antibody. (a) SPiDER-killer-βGal+, light:+ (reproduced from Figure 4c in the main text for reference), (b) SPiDER-killer-βGal:+, light:-, (c) SPiDER-killer-βGal:-, light:-, (d) SPiDER-killer-βGal:-, light:+. Excitation/emission: 405 nm/410-460 nm for Hoechst33342 (blue), 458 nm/460-490 nm for ECFP (green), and 640 nm/650-690 nm for anti-cleaved Dcp-1 antibody (red). Scale bars, 30 μm.

Table S1. Kinetic characteristics of SPiDER-βGal and SPiDER-killer-βGal.

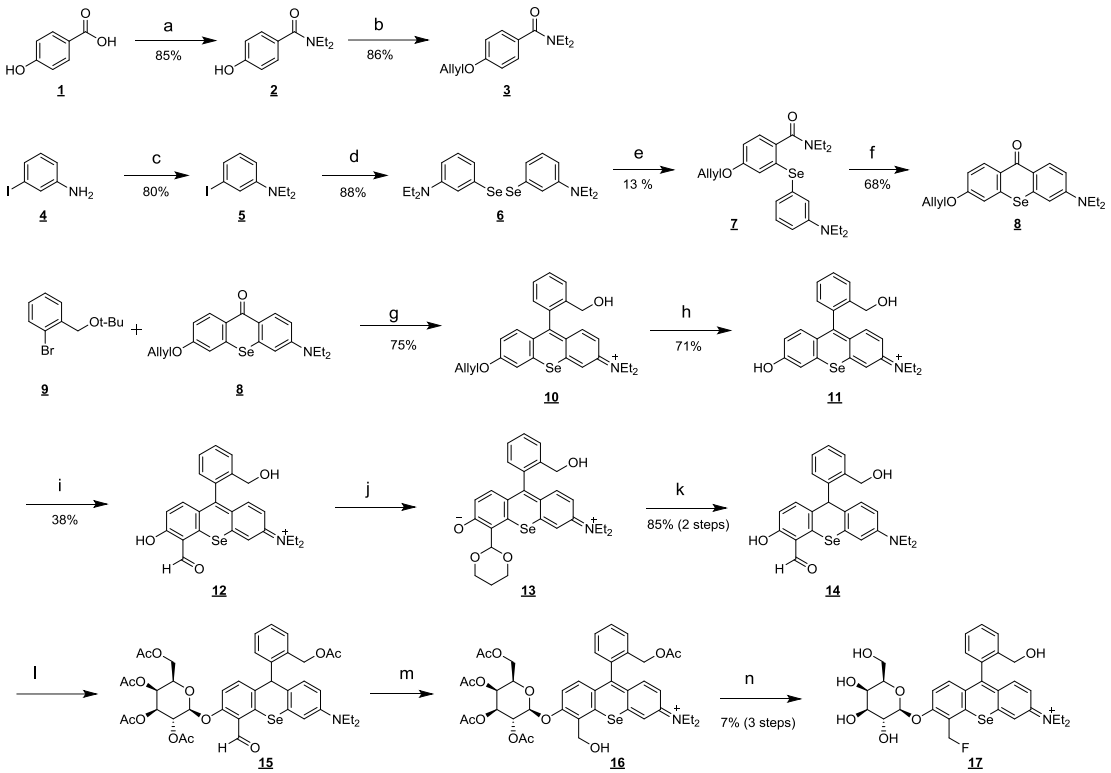
	SPiDER-βGal[a]	**SPiDER-killer-βGal**
K_m (μM)	16.9	10.1
V_{max} (nM s^{-1})	6.10	7.85
k_{cat} (s^{-1})	3.38	3.32
k_{cat}/K_m (μM^{-1} s^{-1})	0.200	0.33

β-Galactosidase (4 U) was added to solutions of various concentrations of SPiDER-killer-βGal in PBS(-), pH 7.4, and the mixtures were incubated at 37 °C. Initial velocities were calculated from the change in absorbance at 557 nm. [a] Reference[5].

Supplementary Movies

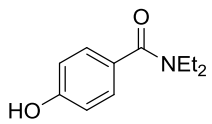
Supplementary Movie 1. Time-lapse fluorescence imaging of flip-out clones of live pupal notum which was injected with a mixture of SPiDER-killer-βGal and Hoechst33342 (5 mM), followed by 561 nm light irradiation. Ex/Em = 405 nm/410-460 nm for Hoechst33342 (blue), 458 nm/460-490 nm for ECFP (green), and 490 nm/540-590 nm for VC3Ai (red). Scale bars, 30 μm.

Synthesis and characterization



Scheme S1. Synthesis of SPiDER-killer-βGal. (a) i) $SOCl_2$, 85 °C, 1 h, ii) DEA, DCM, 85 °C, 18 h; (b) K_2CO_3, allyl bromide, CH_3CN, 85 °C, 18 h; (c) K_2CO_3, iodoethane, CH_3CN, 85 °C, 18 h; (d) Se, CuI, K_3PO_4, DMSO, 90 °C, 21 h; (e) *tert*-BuLi, TMEDA, THF, -78 °C to r.t.; (f) $POCl_3$, DIEA, CH_3CN, 90 °C, 12 h, then add NaOH, 0 °C; (g) i) *sec*-BuLi, TMEDA, THF, -78 °C to r.t., ii) 1 N HCl, r.t.; iii) TFA, DCM, 15 h; (h) K_2CO_3, $Pd(PPh_3)_4$, MeOH, r.t., 15 h; (i) hexamethylenetetramine, TFA, 95 °C, 20 h, then add H_2O, TFA, 95 °C, 15 h; (j) 1,3-propanediol, TsOH, Na_2SO_4, 60 °C; (k) i) $NaBH_4$, MeOH, r.t., 30 min; ii) TFA, H_2O, r.t., 1 h; (l) i) 2,3,4,6-tetra-O-acetyl-D-galactopyranosyl bromide, Cs_2CO_3, Na_2SO_4, DMF, r.t., 30 min; ii) Ac_2O, r.t., 30 min; (m) i) $LiAlH(OtBu)_3$, THF, 0 °C, 30 min, ii) p-chloranil, r.t., 20 min; (n) i) DAST, DCM, -20 °C, 20 min; ii) NaOMe, MeOH, r.t., 1 h.

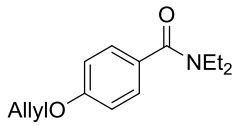
Compound 2.



Thionyl chloride (15 mL, 0.20 mol) was added to 4-hydroxybenzoic acid (1) (3.07 g, 22.2 mmol, 1 eq). The mixture was refluxed for 1 h at 85 °C, then cooled to ambient temperature, and remaining thionyl chloride was evaporated. Dichloromethane (50 mL) and diethylamine (20 mL, 0.19 mol, 8.5

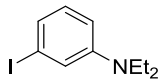
eq) were added. The mixture was refluxed at 85 °C for 18 h, then cooled to 0 °C, and neutralized by adding dropwise 1 N HClaq.. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (dichloromethane/methanol = 99/1) to obtain *N*,*N*-4-hydroxybenzamide (2) (3.67 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 – 1.16 (br t, 6H), 3.29 - 3.48 (br q, 4H), 6.70 (d, 2H, *J* = 8.6 Hz), 7.15 (d, 2H, *J* = 8.6 Hz), 9.53 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): 12.8, 14.0, 39.8, 43.7, 115.4, 126.7, 128.0, 158.6, 172.5.

Compound 3.



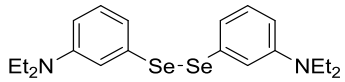
Compound (2) (4.20 g, 21.7 mmol, 1 eq) was dissolved in 40 mL of acetonitrile. Allyl bromide (8 mL, 92.4 mol, 4 eq) and K_2CO_3 (6.18 g, 44.7 mmol, 2 eq) were added to the flask. The mixture was refluxed at 85 °C for 20 h, then cooled to ambient temperature, and evaporated. Dichloromethane was added to the residue. The resulting solution was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (hexane/EtOAc = 90/10 – 70/30) to obtain 4-allyloxy-*N*,*N*-diethylbenzamide (3) （4.38 g, 86%）. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.17 (br t, 6H), 3.39 (br q, 4H), 4.54 (d, 2H, *J* = 5.2 Hz), 5.28 (d, 1H, *J* = 8.8 Hz), 5.41 (s, 1H, *J* = 17.2 Hz), 6.08 -5.99 (m, 1H), 6.91 (d, 2H, *J* = 8.8 Hz), 7.33 (d, 2H, *J* = 8.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): 12.8, 13.9, 39.2, 43.2, 129.5, 132.8, 159.1, 171.0.

Compound 5.



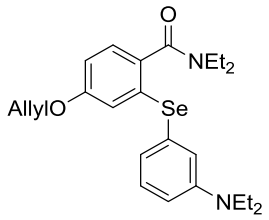
3-Iodaniline (4) (4.23 g, 19.3 mmol, 1 eq) was dissolved in acetonitrile (30 mL). Iodoethane (9.73 g, 62.4 mmol, 3 eq) and K_2CO_3 (5.32 g, 38.5 mmol, 2 eq) were added. The mixture was refluxed at 85 °C for 18 h, then cooled to ambient temperature, and filtered. The filtrate was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (hexane/dichloromethane = 90/10 – 80/20) to obtain *N*,*N*-diethyl-3-iodoaniline (5) （4.22 g, 80%）. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 1.10 (t, 6H, *J* = 7.0 Hz), 3.25 (q, 4H, *J* = 7.0 Hz), 6.56 (d, 1H, *J* = 8.3 Hz), 6.85 (t, 1H, *J* = 8.3 Hz), 6.91 (d, 1H, *J* = 8.3 Hz), 6.95 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): 12.7, 44.4, 96.1, 111.0, 120.4, 124.2, 130.8, 149.0.

Compound 6.



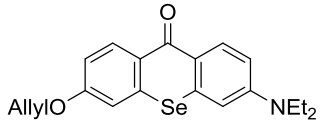
To a solution of compound (5) (7.27 g, 26.4 mmol, 1 eq) in DMSO (40 mL), Se powder (6.25 g, 79.2 mmol, 3 eq), K_3PO_4 (16.8 g, 79.2 mmol, 3 eq)、CuI (0.51 g, 2.64 mmol, 0.1 eq) were added. The mixture was refluxed at 90 °C for 21 h, then cooled to room temperature, and filtered. The filtrate was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel chromatography (hexane/dichloromethane = 90/10 – 70/30) to obtain compound (6)（5.27 g, 88%）. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (t, 6H, *J* = 7.0 Hz), 3.28 (q, 4H, *J* = 7.0 Hz), 6.51 (dd, 1H, *J* = 2.4, 8.4 Hz), 6.89 (d, 1H, *J* = 8.4 Hz), 6.91 (d, 1H, *J* = 2.4 Hz), 7.06 (t, 1H, *J* = 8.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): 12.6, 44.5, 111.1, 114.4, 117.9, 129.7, 132.2, 148.4.

Compound 7.



Compound (3) (1.46 g, 6.24 mmol, 1 eq) and tetramethylethylenediamine (0.81 g, 6.97 mmol, 1.1 eq) were dissolved in THF (7 mL). The solution was cooled to -78°C under an Ar atmosphere, then 1.9 M *tert*-butyllithium in cyclohexane (4.9 ml, 9.3 mmol, 1.5 eq.) was added dropwise, followed by the addition of a solution of compound (6) in THF. The reaction mixture was cooled to ambient temperature, and then saturated NaH_2PO_4 solution and EtOAc were added. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (hexane/EtOAc = 90/10 – 60/40) to obtain compound (7) （1.43 g, 13%）. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, 9H, *J* = 7.0 Hz), 1.26 (t, 3H, *J* = 7.0 Hz), 3.23 (br q, 2H), 3.31 (q, 4H, *J* = 7.0 Hz), 3.57 (br q, 2H), 4.38 (dt, 2H, *J* = 1.4, 5.4 Hz), 5.20 (dd, 1H, *J* = 1.4, 8.0 Hz), 5.25 (dd, 1H, *J* = 1.4, 16.0 Hz), 5.86 - 5.96 (m, 1H), 6.73 (dd, 1H, *J* = 2.5, 8.0 Hz), 6.80 - 6.81 (m, 2H), 6.89 (s, 1H), 7.11- 7.15 (m, 2H) ^{13}C NMR (101 MHz, $CDCl_3$): 12.5, 14.2, 39.2, 43.1, 44.3, 68.8, 111.6, 113.0, 114.3, 114.8, 117.8, 118.1, 118.2, 121.7, 127.3, 129.7, 130.1, 131.1, 132.2, 132.4, 132.8, 148.5, 158.9, 170.0.

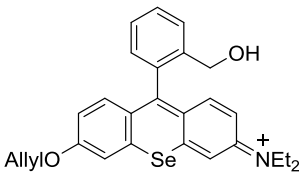
Compound 8.



Compound (7) (214.3 mg, 0.466 mmol, 1 eq) was dissolved in acetonitrile (10 mL). Phosphoryl chloride (434 μL, 9.32 mmol, 20 eq) and *N*,*N*-diisopropylethylamine (812 μL, 4.66 mmol, 10 eq)

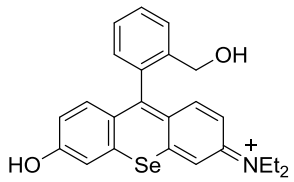
were added. The mixture was refluxed at 90°C for 12 h, then cooled to 0°C, and neutralized by adding dropwise 1 N NaOH, and evaporated. Dichloromethane was added to the residue, and the resulting solution was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (hexane/EtOAc = 90/10 – 60/40) to obtain compound (8) (123.1 mg, 68%）. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (br m, 6H), 3.42 (br q, 4H), 4.62 (dt, 2H, *J* = 1.5, 5.3 Hz), 5.33 (dd, 1H, *J* = 1.5, 10.4 Hz), 5.44 (dd, 1H, *J* = 1.5, 17.2 Hz), 6.62 (d, 1H, *J* = 2.6 Hz), 6.74 (dd, 1H, J = 2.6, 8.8 Hz), 6.97(dd, 1H, *J* = 2.6, 8.8 Hz), 7.00 (d, 1H, *J* = 2.6 Hz), 8.46 (d, 1H, *J* = 8.8 Hz), 8.55 (d, 1H, *J* = 8.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): 12.6, 44.5, 69.0, 107.0, 111.4, 111.8, 114.5, 118.3, 119.4, 125.1, 132.4, 136.3, 137.1, 149.8, 160.7, 179.8. HRMS-ESI (m/z): $[M+Na]^+$ calcd for 410.06305 ($C_{20}H_{21}NO_2Se$), found 410.06372 (- 0.7 mDa).

Compound 10.



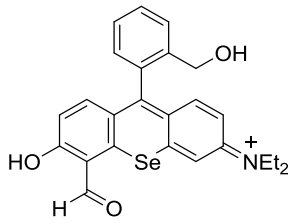
1-Bromo-2-(*tert*-butoxymethyl)benzene (9)[6] (0.24 g, 0.99 mmol, 3 eq) and tetramethylethylenediamine (0.11 g, 0.95 mmol, 3 eq) were dissolved in THF (7 mL) and the solution was cooled to -78°C under an Ar atmosphere. Next, a 1 M solution of *sec*-butyllithiumcyclohexane in n-hexane (1.0 mL, 1.00 mmol, 3 eq) was added dropwise, followed by the addition of a solution of compound (8) in THF (110.3 mg, 0.29 mmol, 1 eq). The mixture was stirred for 1 h at ambient temperature, and then 2 N HCl and dichloromethane were added. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. TFA (3 mL) and dichloromethane (3 mL) were added to the residue. The mixture was stirred for 15 h, and then evaporated. The residue was purified by means of HPLC (Solvent A: water, 0.1% TFA, 1% acetonitrile, Solvent B: acetonitrile, 1% water, gradient: A/B = 95/5-5/95 (40 min)) to obtain compound (10) (102.2 mg, 75%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.34 (br t, 6H), 1.38 (br t, 6H), 3.76 (br q, 4H), 4.24 (s, 2H), 4.80 (s, 1H), 4.81 (s, 1H), 5.35 (d, 1H, *J* = 10.6 Hz), 5.47 (d, 1H, *J* = 10.6 Hz), 6.05 - 6.15 (m, 1H), 7.12 (d, 1H, *J* = 9.3 Hz), 7.21 - 7.25 (m, 2H), 7.50 (d, 1H, *J* = 9.3 Hz), 7.54 - 7.59 (m, 1H), 7.69 (t, 1H, *J* = 7.6 Hz), 7.77 (d, 1H, *J* = 7.6 Hz), 7.86 (s, 2H); ^{13}C NMR (101 MHz, CD_3OD): 12.9, 24.2, 47.3, 62.8, 71.0, 111.0, 113.3, 119.0, 119.1, 119.4, 122.7, 124.8, 128.8, 129.3, 130.4, 131.0, 133.2, 136.5, 138.9, 140.7, 145.6, 151.5, 154.4, 162.7, 164.0. HRMS-ESI (m/z): $[M+H]^+$ calcd for 478.12812 ($C_{27}H_{28}NO_2Se$), found 478.12843 (+ 0.3 mDa).

Compound 11.



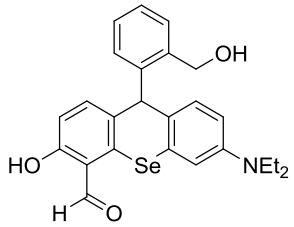
Compound 10 (102.2 mg, 0.21 mmol, 1 eq) was dissolved in deoxygenated methanol (10 mL) and the solution was added to a reaction flask containing K_2CO_3 (42.3 mg, 0.31 mmol) and tetrakis(triphenylphosphine)palladium(0) (24.7 mg, 0.02 mmol, 0.1 eq). The mixture was stirred at ambient temperature for 15 h, then evaporated, and the residue was purified by means of HPLC (Solvent A: water, 0.1% TFA, 1% acetonitrile, Solvent B: acetonitrile, 1% water, gradient: A/B = 95/5-5/95 (30 min)) to obtain compound (11) (66.3 mg, 71%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.23 (t, 6H, *J* = 7.2 Hz), 3.64 (q, 4H, J = 7.2 Hz), 4.13 (s, 2H), 6.87 (dd, 1H, *J* = 2.4, 9.2 Hz), 7.08 (dd, 1H, *J* = 2.4, 10.0 Hz), 7.13 (d, 1H, *J* = 7.6 Hz), 7.36 (d, 1H, *J* = 10.0 Hz), 7.43 - 7.49 (m, 3H), 7.58 (t, 1H, *J* = 7.6 Hz), 7.67 (d, 1H, *J* = 7.6 Hz), 7.69 (s, 1H); ^{13}C NMR (101 MHz, CD_3OD): 13.1, 47.3, 49.9, 62.7, 110.8, 111.0, 114.8, 118.6, 119.8, 122.2, 123.6, 128.8, 129.2, 130.4, 131.0, 136.5, 139.6, 140.6, 140.9, 146.0, 150.9, 154.2. HRMS-ESI (m/z): $[M+H]^+$ calcd for 478.12812 ($C_{27}H_{28}NO_2Se$), found 478.12843 (+ 0.3 mDa).

Compound 12.



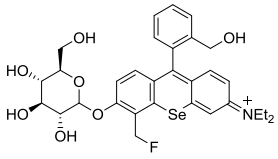
Compound 11 (79.7 mg, 0.18 mmol, 1 eq) and hexamethylenetetramine (25.5 mg, 0.18 mmol, 1 eq) were dissolved in TFA (10 mL). The solution was heated at 95 °C for 20 h, and 4 mL of water was added. The mixture was refluxed at 95 °C for 15 h, then evaporated, and dichloromethane and sat. K_2CO_3 were added to the residue. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by means of HPLC (Solvent A: water, 0.1% TFA, 1% acetonitrile, Solvent B: acetonitrile, 1% water, gradient: A/B = 95/5-5/95 (30 min)) to obtain compound (12) (32.3 mg, 38%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.21 (t, 6H, *J* = 7.0 Hz), 3.60 (q, 4H, *J* = 7.0 Hz), 4.14 (s, 2H), 6.73 (d, 1H, *J* = 9.8 Hz), 7.04 (d, 1H, *J* = 9.8 Hz), 7.13 (s, 1H, *J* = 7.7 Hz), 7.31 (d, 1H, *J* = 9.8 Hz), 7.44 (d, 1H, *J* = 7.7 Hz), 7.47 (d, 1H, J = 9.8 Hz), 7.56 (d, 1H, J = 7.7 Hz), 7.60 (d, 1H, J = 7.7 Hz), 7.66 (d, 1H, J = 7.7 Hz), 10.6 (s, 1H); ^{13}C NMR (101 MHz, CD_3OD): 11.5, 22.7, 29.2, 45.8, 61.2, 101.2, 108.2, 117.0, 117.5, 120.8, 121.6, 123.1, 127.3, 127.7, 129.0, 129.4, 135.6, 138.4, 138.4, 139.4, 143.3, 152.6, 161.3. HRMS-ESI (m/z): $[M+H]^+$ calcd for 466.09172 ($C_{25}H_{24}NO_3Se$), found 466.09268 (+ 1.0 mDa).

Compound 14.



A mixture of compound (12) (32.3 mg, 0.069 mmol, 1 eq), 1,3-propanediol (10 mL), p-toluenesulfonic acid (12.4 mg, 0.065 mmol, 1 eq), and sodium sulfate (28.2 mg, 0.199 mmol, 2eq) was heated at 60°C for 12 h, and then sat. $NaHCO_3$ solution was added. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated, affording crude compound (13) (27.8 mg). HRMS-ESI (m/z): $[M+H]^+$ calcd for 524.13363 ($C_{28}H_{30}NO_4Se$), found 524.13399 (+0.4 mDa). Crude compound (13) (94.7 mg, 0.18 mmol, 1 eq) was dissolved in methanol (10 mL). Sodium borohydride (100 mg, 2.64 mmol, 15 eq) was added to the solution under an Ar atmosphere. The mixture was stirred for 30 min at ambient temperature, and then evaporated. Saturated $NaHCO_3$ solution and dichloromethane were added to the residue. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. TFA (5 mL) and water (5 mL) were added to the residue and the mixture was stirred at ambient temperature for 1 h. TFA was evaporated, and the residue was purified by means of HPLC (Solvent A: water, 0.1% TFA, 1% acetonitrile, Solvent B: acetonitrile, 1% water, gradient: A/B = 90/10-10/90 (30 min)) to obtain compound (14) (72.0 mg, 85% in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (t, 6H, J = 7.1 Hz), 3.51 (q, 4H, J = 7.1 Hz), 4.68 (s, 2H), 5.26 (s, 1H), 6.80 (d, 1H, J = 8.8 Hz), 7.23 (d, 1H, J = 8.8 Hz), 7.30 (t, 1H, J = 8.8 Hz), 7.39 – 7.46 (m 4H), 7.55 - 7.57 (m, 1H), 7.73 (s, 1H), 10.5 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): 10.7, 50.6, 52.5, 63.9, 116.8, 118.4, 120.5, 122.0, 128.5, 128.8, 130.3, 130.4, 131.2, 131.4, 131.9, 136.4, 136.9, 137.4, 137.7, 139.5, 140.5, 162.4, 195.6. HRMS-ESI (m/z): $[M+H]^+$ calcd for 468.10737 ($C_{25}H_{26}NO_3Se$), found 468.10505 (- 2.3 mDa).

Compound 17.



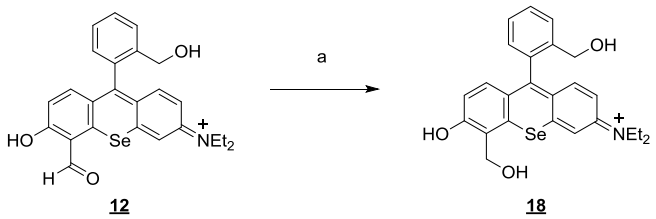
Compound (14) (44.7 mg, 0.096 mmol, 1 eq) was dissolved in dehydrated dimethylformamide (10 mL). 2,3,4,6-Tetra-*O*-acetyl-*D*-galactopyranosyl bromide (81.4 mg, 0.84 mmol, 2 eq), caesium carbonate (274.2 mg, 1.23 mmol, 8 eq), and sodium sulfate (89.5 mg, 0.63 mmol, 5 eq) were added. The mixture was stirred at ambient temperature for 30 min, then anhydrous acetic acid (240 μL, 2.54

mmol, 27 eq) was added, and stirring was continued at ambient temperature for 30 min. The mixture was evaporated, and sat. NH_4Cl solution and dichloromethane were added to the residue. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was roughly purified by silica gel chromatography (dichloromethane/methanol = 90/10), affording crude compound (15) (73.6 mg). HRMS-ESI (m/z): $[M+H]^+$ calcd for 840.21328 ($C_{41}H_{46}NO_{13}Se$), found 840.21223 (- 1.0 mDa).

Compound (15) was dissolved in dichloromethane. *p*-Chloranil (23.6 mg, 0.096 mmol, 1 eq) was added, and the mixture was stirred at ambient temperature for 20 min, and then evaporated. The residue was roughly purified by silica gel chromatography (dichloromethane/methanol = 90/10). The crude product was dissolved in dyhydrated THF (10 mL). This solution was cooled to 0°C, and lithium-tri-*tert*-butoxyaluminum hydride (53.2 mg, 0.21 mmol, 2 eq) was added to it. The mixture was stirred at 0°C for 30 min. Then sat. NH_4Cl and ethyl acetate were added at 0°C. After 1 h, the mixture was filtered, and the organic layer was evaporated. Dichloromethane and sat. Rochelle salt solution were added to the residue. The organic layer was dried over Na_2SO_4 and evaporated, affording crude compound (16). HRMS-ESI (m/z): $[M+H]^+$ calcd for 840.21328 ($C_{41}H_{46}NO_{13}Se$), found 840.21223 (- 1.0 mDa).

Compound (16) was dissolved in dichloromethane (10 mL) and the solution was cooled to - 20°C. DAST (diethylaminosulfur trifluoride) (130 μL, 0.98 mmol, 10 eq) was added to it. The mixture was stirred for 15 min, and then 6 mL of methanol was added. Stirring was continued for a while, then the mixture was evaporated. Saturated $NaHCO_3$ solution and dichloromethane were added to the residue. The organic layer was dried over Na_2SO_4 and evaporated. The residue was dissolved in methanol (10 mL), and sodium methoxide (39.5 mg, 0.73 mmol, 8 eq) was added. The mixture was stirred at ambient temperature for 1 h, and sat. NH_4Cl solution was added. The organic layer was evaporated, and dichloromethane was added to the residue. The resulting solution was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by means of HPLC (Solvent A: water, 100 mM TFAA, 1% acetonitrile, Solvent B: acetonitrile, 1% water, gradient: A/B = 95/5-5/95 (40 min)) to obtain compound (17) (4.3 mg, 7 % in 3 steps). Isomer 1 ^{1}H NMR (400 MHz, CD_3CN): δ 1.10 (t, 6H, J = 7.0 Hz), 3.34 (q, 4H, J = 7.0 Hz), 3.47 - 3.71 (m, 6H), 3.82 (s, 1H), 5.59 (dd, 1H, J = 10.6, 48.9 Hz), 5.93 (dd, 1H, J = 10.6, 48.9 Hz), 6.58 (d, 1H, J = 8.0 Hz), 6.88 (s, 1H), 7.07 (t, 1H, J = 8.0 Hz), 7.14 (t, 1H, J = 7.7 Hz), 7.26 – 7.35 (m, 3H), 7.55 - 7.59 (m, 1H), 7.79 (d, 1H, J = 7.7 Hz); ^{13}C NMR (101 MHz, CD_3CN): 12.7, 44.9, 62.2, 70.8 (d, J = 224.2 Hz), 78.7 (d, J = 14.1 Hz), 90.9, 102.4, 102.9, 111.0, 112.0, 114.9, 115.3, 122.6, 123.0, 124.1, 124.2, 127.5, 128.0, 128.1, 128.7, 128.8, 129.2, 130.0, 130.1, 130.2, 132.5, 137.3, 137.7, 137.8, 147.4, 147.5, 148.1, 156.2. Isomer 2 δ 1.10 (t, 6H, J = 7.0 Hz), 3.34 (q, 4H, J = 7.0 Hz), 3.47 - 3.71 (m, 6H), 3.82 (s, 1H), 5.61 (dd, 1H, J = 10.6, 48.9 Hz), 5.94 (dd, 1H, J = 10.6, 48.9 Hz), 6.58 (d, 1H, J = 8.0 Hz), 6.88 (s, 1H), 7.07 (t, 1H, J = 8.0 Hz), 7.14 (t, 1H, J = 7.7 Hz), 7.26 – 7.35 (m, 3H), 7.55 - 7.59 (m, 1H), 7.79

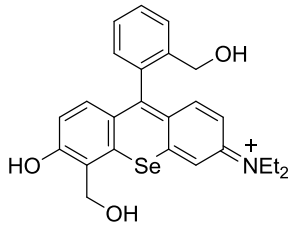
(d, 1H, J = 7.7 Hz); ^{13}C NMR (101 MHz, CD_3CN): 12.7, 44.9, 62.2, 75.3 (d, J = 224.2 Hz), 80.2 (d, J = 14.1 Hz), 90.9, 102.4, 102.9, 111.0, 112.0, 114.9, 115.3, 122.6, 123.0, 124.1, 124.2, 127.5, 128.0, 128.1, 128.7, 128.8, 129.2, 130.0, 130.1, 130.2, 132.5, 137.3, 137.7, 137.8, 147.4, 147.5, 148.1, 156.2; ^{19}F NMR (376 MHz, CD_3CN): δ 207.66 (t, J = 48.9 Hz). HRMS-ESI (m/z): $[M+H]^+$ calcd for 632.15595 ($C_{31}H_{35}FNO_7Se$), found 632.15517 (- 0.8 mDa).



12 **18**

Scheme S2. Synthesis of 4-CH_2OH HMDESeR. (a) $LiAlH(OtBu)_3$, THF, 0 °C, 30 min.

Compound 18.



Compound (12) (24.6 mg, 0.053 mmol, 1 eq) was dissolved in THF (5 mL) and the solution was cooled to 0°C. Lithium-tri-*tert*-butoxyaluminum hydride (29.7 mg, 0.12 mmol, 2 eq) was added, and the mixture was stirred for 30 min. Saturated NH_4Cl solution and ethyl acetate were added and stirring was continued for 1 h. The reaction mixture was filtered. Dichloromethane and sat. Rochelle salt solution were added to the residue. The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by HPLC (Solvent A: water, 0.1% TFA, 1% acetonitrile, Solvent B: acetonitrile, 1% water, gradient: A/B = 95/5-5/95 (40 min)) to obtain compound (18) (1.9 mg, 4 %). ^{1}H NMR (400 MHz, CD_3OD): δ 1.33 (t, 6H, J = 7.1 Hz), 3.74 (q, 4H, J = 7.1 Hz), 4.22 (s, 2H), 5.06 (s, 2H), 7.00 (d, 1H, J = 9.3 Hz), 7.18 (d, 1H, J = 10.0 Hz), 7.23 (d, 1H, J = 7.4 Hz), 7.45 (d, 1H, J = 9.3 Hz), 7.51 (d, 1H, J = 10.0 Hz), 7.55 (t, 1H, J = 7.1 Hz), 7.68 (t, 1H, J = 7.5 Hz), 7.77 (d, 1H, J = 7.5 Hz), 7.81 (s, 1H); ^{13}C NMR (101 MHz, CD_3OD): 1.46, 13.0, 57.9, 62.6, 110.43, 118.5, 118.8, 121.9, 124.1, 125.9, 128.7, 129.1, 130.5, 130.9, 136.8, 139.3, 140.1, 140.9, 147.0, 151.7, 154.0, 162.8, 163.0. HRMS-ESI (m/z): $[M+H]^+$ calcd for 468.10737 ($C_{25}H_{26}NO_3Se$), found 468.10734 (+ 0.0 mDa).

Supporting References

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