Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

A Yellowish-Green-Light-Controllable Nitric Oxide Donor Based on *N*-Nitrosoaminophenol Applicable for Photocontrolled Vasodilation

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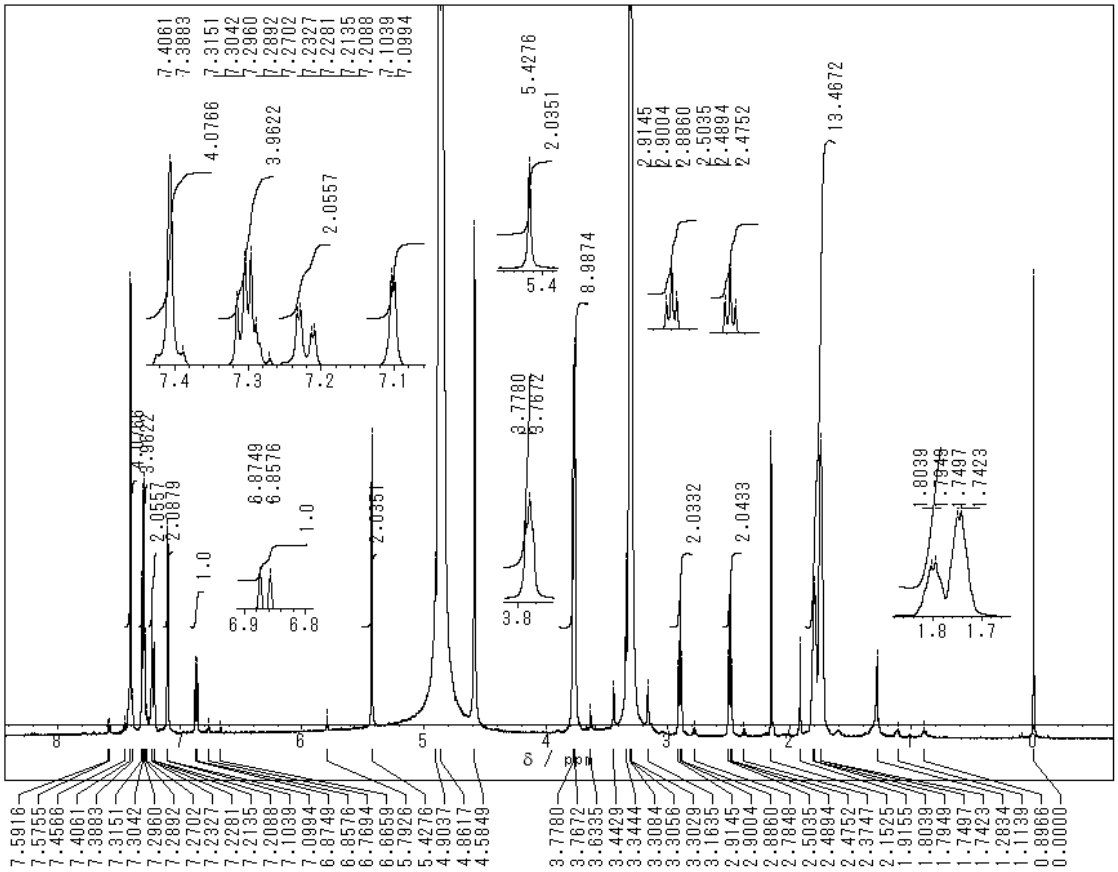
Contents

1. Experimental Section

A. ^{1}H NMR chart of NO-Rosa



B. Experimental procedure

NO_2^-/NO_3^- detection with a NO fluorescence probe DAN

Sample solutions of **NO-Rosa**, (10 μM, 1 mL) were photoirradiated at room temperature for 60 min. Photoirradiation was performed with an MAX-302 (Asahi spectra) equipped with a 530–590 nm band-pass filter (190 mW/cm^2 at 570 nm) for **NO-Rosa**. The calibration curve was prepared by using $NaNO_3$ standard solution and DAN. After reducing NO_2^- to NO_3^- by NO_3 reductase, concentration of NO_3^- in the photoirradiated **NO-Rosa** solution was determined by comparing the fluorescence intensity of naphthalenetriazole with the calibration curve. The fluorescence intensity was recorded on an ARVO-X5 (Perkinelmer) at λ_{ex} = 355 nm/λ_{fl} = 460 nm.

HPLC analysis of photodecomposition of NO-Rosa

NO-Rosa was dissolved in MilliQ water containing 1% DMSO as a cosolvent to prepare 100 μM **NO-Rosa** solution, and this solution was photoirradiated (530–590 nm, 60 mW/cm^2, 15 min).

Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H_2O (0.1% FA).

LC-MS analysis of photodecomposition products of NO-Rosa

NO-Rosa (100 μM in MilliQ water containing 10% DMSO, 1 mL) was irradiated with yellowish green light (530–590 nm, 140 mW/cm^2, 15 min), and the solution was analyzed by LC-MS. Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H_2O (0.1% FA).

Light-toxicity assay for HEK293 cells

Cell viability was verified by Cell Counting Kit-8 (Dojindo, Kumamoto). HEK293 cells were plated on 96-well plates (100 μL, 1.0×10^4 cells /well). After incubation under 5% CO_2 atmosphere at 37 °C for 24 hr, the cells were irradiated by yellowish green light (530–590 nm, 40 mW/cm^2, 15 min) or blue light (470–500 nm, 40 mW/cm^2, 15 min) with MAX-302 (Asahi Spectra). After irradiation, the cells were incubated another 48 hr under 5% CO_2 atmosphere at 37 °C. CCK solution (10 μL) was added to each well and the cells were incubated for 2 hr under 5% CO_2 atmosphere at 37 °C. The absorption intensity at 450 nm was recorded on an ARVO-X5 (Perkinelmer). Cell viability (%) was determined by dividing the absorption intensity of irradiated group by the absorption intensity of the group without photoirradiation.

2. Figures for supporting data

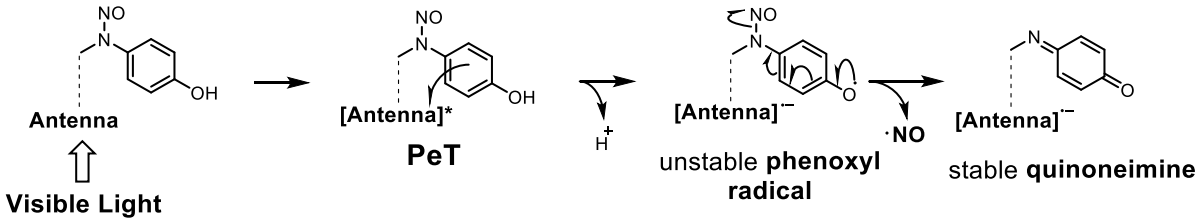
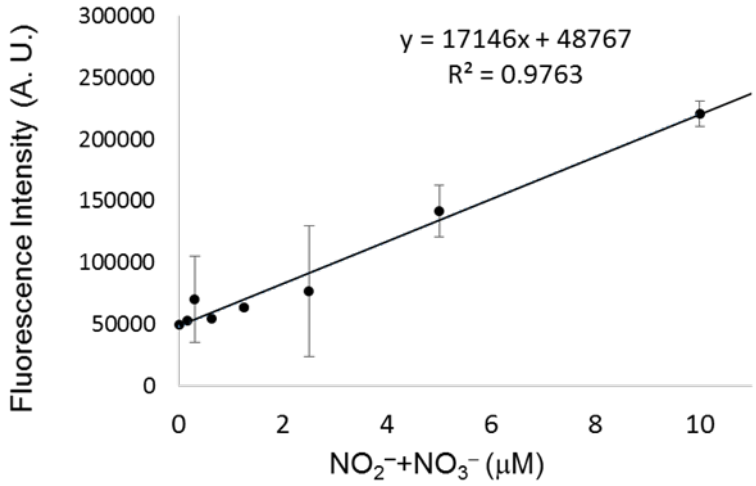


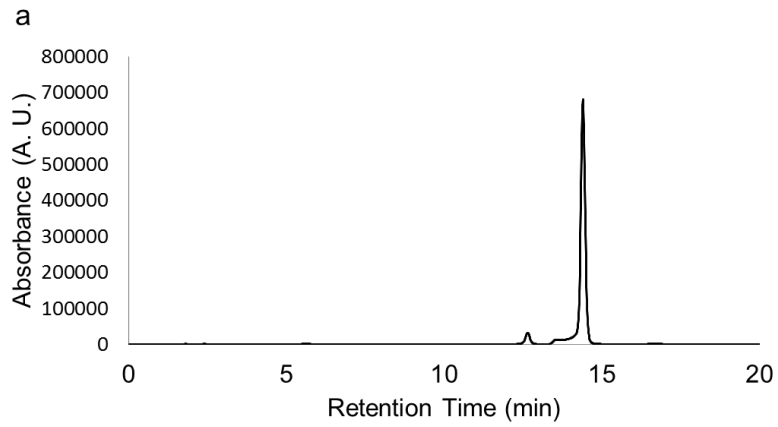
Figure S1 Proposed mechanism of NO release from **NOBL-1** (**1**) / **NO-Rosa** (**2**)

Figure S2 ESR spectrum of a solution of Fe-MGD and **NO-Rosa** without photoirradiation**.** **NO-Rosa** (100 μM), MGD (6 mM), and $FeSO_4$ (1.5 mM) were dissolved in MilliQ water containing 15% DMSO, and the ESR spectrum of the solution was measured without photoirradiation. ESR conditions: microwave power, 10 mW; frequency, 9.4 GHz; field, 330 mT; sweep width, 7.5 mT; sweep time, 4 min; modulation width, 0.125 mT; time constant, 0.10 s.



	Fluorescence Intensity (A. U.) Mean±SD	NO Concentration (μM)
NO-Rosa	216068±3522	9.8±0.2

Figure S3 Quantitative analysis of NO release from **NO-Rosa** by DAN. A solution of **NO-Rosa** (10 μM, 1 mL) was photoirradiated at room temperature for 60 min. Visible light photoirradiation was performed with an Asahi Spectra MAX-302 equipped with a 530–590 nm band-pass filter (190 mW/cm^2 at 570 nm). NO_2/NO_3 were detected by using a 2,3-diaminonaphthalene (DAN) fluorometric assay kit.



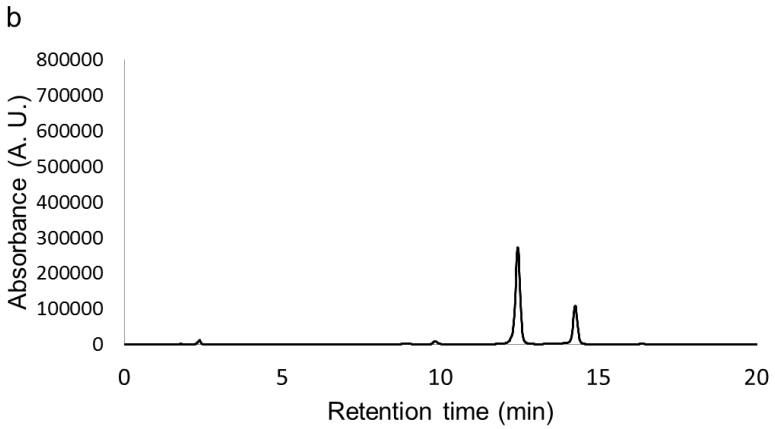
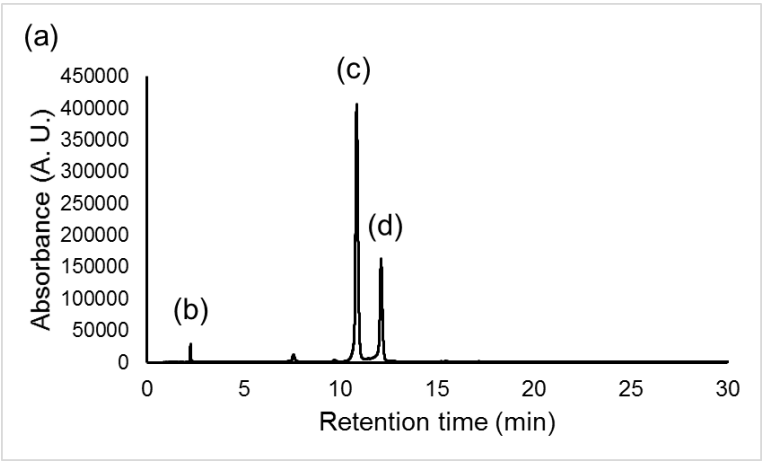


Figure S4 HPLC analysis of photodecomposition. Chromatograms of solution containing NO-Rosa (a) before photoirradiation, and (b) after photoirradiation. **NO-Rosa** was dissolved in MilliQ water containing 1% DMSO as a cosolvent to prepare 100 μM **NO-Rosa** solution, and this solution was photoirradiated (530–590 nm, 60 mW/cm^2, 15 min). Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H_2O (0.1% FA).



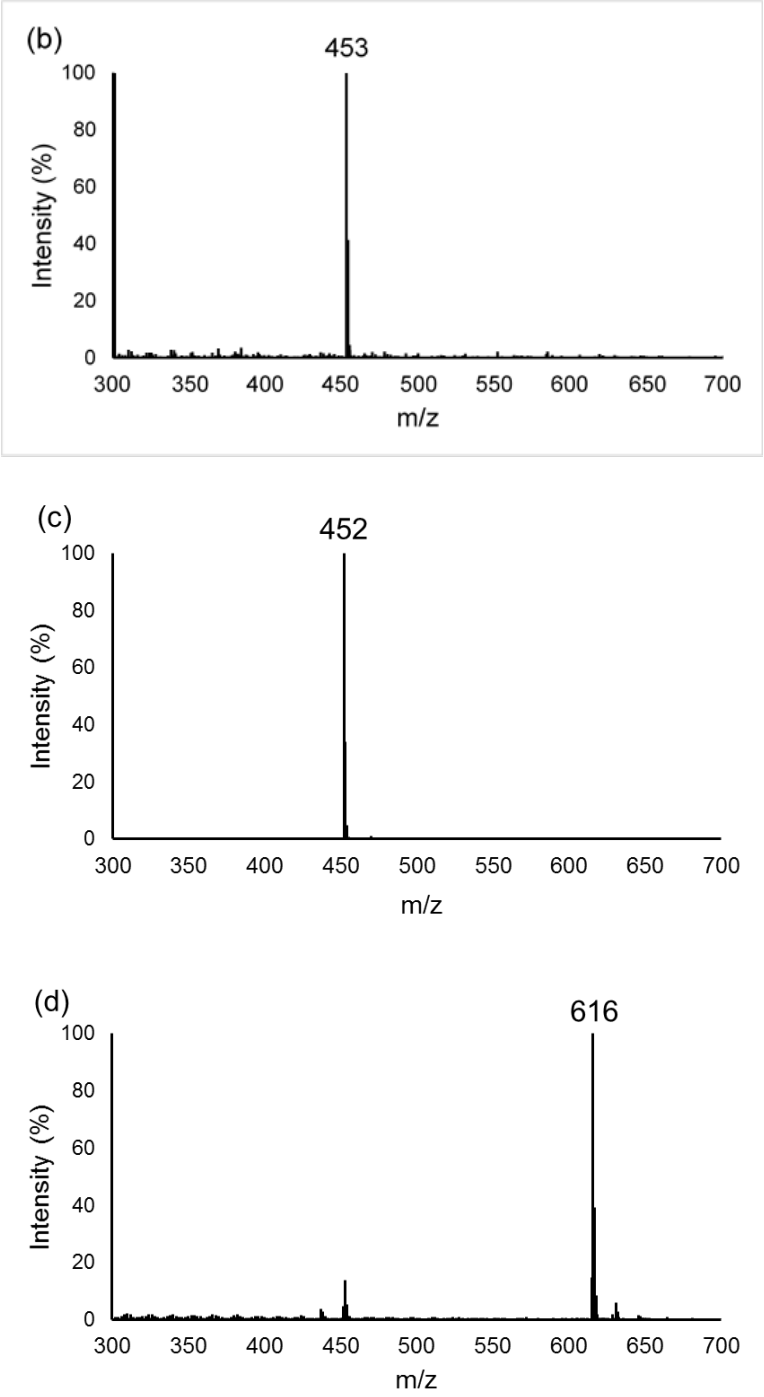


Figure S5 LC-MS analysis of photodecomposition products of **NO-Rosa**

NO-Rosa (100 μM in MilliQ water containing 10% DMSO, 1 mL) was irradiated with yellowish green light (530–590 nm, 140 mW/cm^2, 15 min), and the solution was analyzed by LC-MS. (a) Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H_2O (0.1% FA). Mass spectra of photodecomposition products at (b) 2 min (**S1**), (c) 11 min (intermediate **9**), (d) 12 min

(intermediate **11**) are shown.

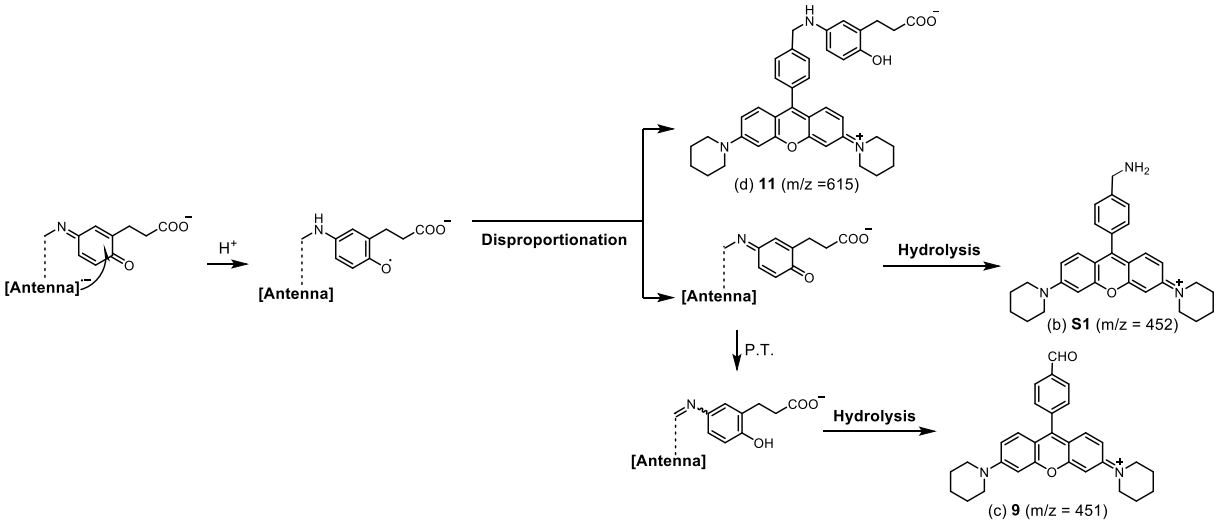


Figure S6 Proposed decomposition mechanism of **NO-Rosa**

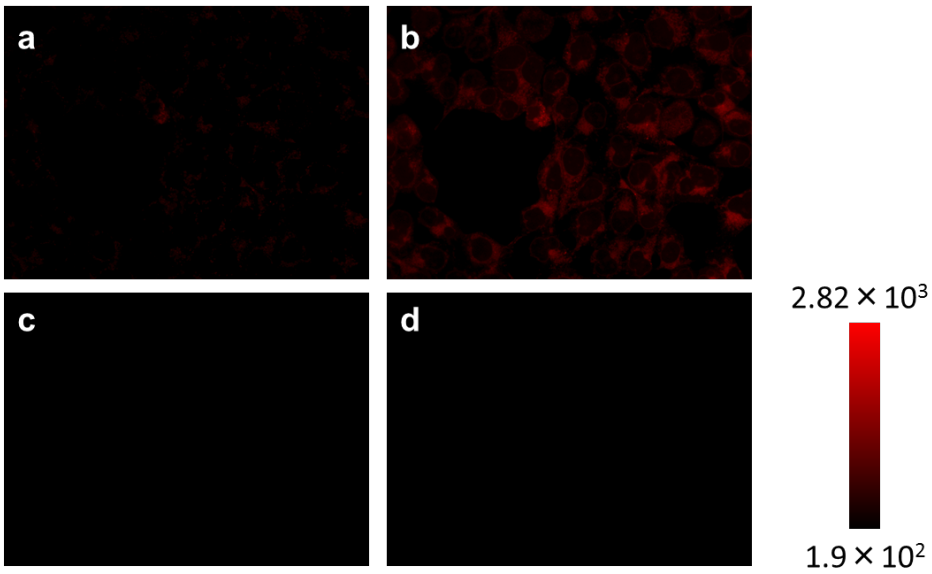


Figure S7 Red fluorescence images of each sample corresponding to (a)–(d) of Figure 4 (photocontrolled NO release in HEK293 cells).

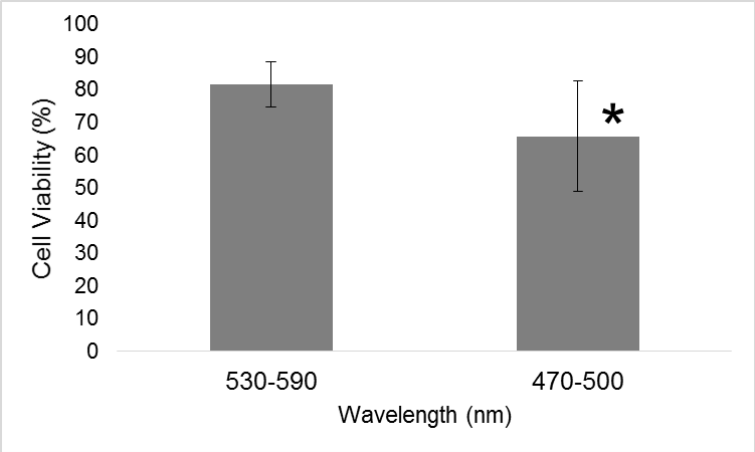


Figure S8 HEK293 cell viability determined by Cell Counting Kit-8 after irradiation by different wavelength light. HEK293 cells were irradiated by yellowish green light (530–590 nm, 40 mW/cm^2, 15 min) or by blue light (470–500 nm, 40 mW/cm^2, 15 min). Cell viability was determined by Cell Counting Kit-8. Cell viability after each wavelength range was; 530–590 nm: 82±7%, 470–500 nm: 66±17%, n = 9, **p* = 0.026 (Studest's t-test).

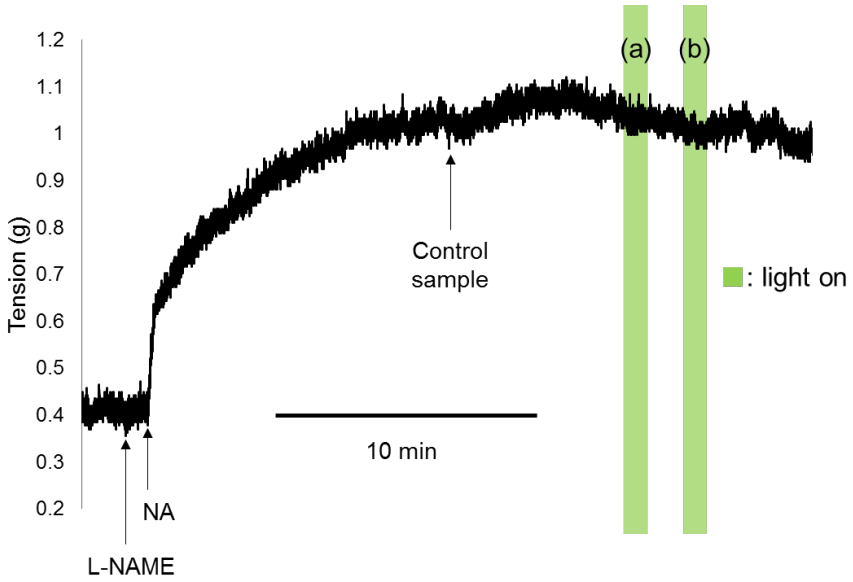


Figure S9 Vasodilation test with photodecomposed sample**. NO-Rosa** (1 mM in MilliQ water containing 10% DMSO) was decomposed by pre-irradiation with yellowish green light (530–590 nm, 170 mW/cm^2, 30 min). A rat aortic strip was placed in a Magnus tube filled with Krebs buffer at 37 °C. The strip was then pretreated with L-NAME (10 μM) and noradrenaline (10 μM). After equilibration, the photodecomposed sample (originally 10 μM **NO-Rosa**) was added to the tube. The strip was irradiated with a light source (MAX-303, Asahi Spectra) equipped with a 530–590 nm band-pass filter for 1 min periods as shown. The light intensity at the each irradiation of was (a) 65 mW/cm^2, (b) 130 mW/cm^2.