**Supplementary information** 

## Direct analysis of stress-fiber formation effect in photodynamic therapy

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Fig. S1 Model for the mechanism of stress fiber formation by PDT. (a) When a photosensitizer (TS) taken up into cells is irradiated with light, singlet oxygen is generated and converted to active oxygen (ROS)<sup>1–3</sup>. ROS is known to activate RhoA<sup>2,4–7</sup>. (b) RhoA activation is regulated by guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP)<sup>4,8,9</sup>. (c) RhoA\* activated by GEF acts on Mammalian diaphanous-related formin (mDia) and promotes actin polymerization<sup>10–13</sup>. (d), (e) On the other hand, activated RhoA\* binds to Rho-associated protein kinase (ROCK) and promotes phosphorylation of the myosin light chain<sup>4,6,7,9,12,14–16</sup>. (f) S-fiber is formed by the formed A-filament and myosin-light chain. S-fiber binds to desmosomes, and their tension determines the elastic modulus of the cell.



Fig. S2 Results of quantifying the intensity of the fluorescence images in the text. The CCD measurement intensity values of one pixel ( $6.45 \times 6.45 \ \mu m^2$ ) were averaged over each image.

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(a)	0 min	(b)	5 min	(c)	10 min	(d)	15 min	(e)	20 min	(f)	25 min	(g)	30 min
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Fig. S3 Phase difference observation images (upper) and fluorescence observation images

(lower) when only SPY555-actin fluorescent probe is inserted.



Fig. S4 Western blotting data used to make Fig. 3b.



Phase contrast images ((a)-(d)) and fluorescence images ((e)-(h)) immediately, 4, and 9 minutes after light irradiation with an intensity of 34 mW/cm<sup>2</sup> for 1 min.



Ultraviolet-visible (UV-Vis) range absorption spectrum of aqueous solution of taraporphyrin sodium.  $A_{(545)}$  and  $A_{(655)}$  indicate the areas for the irradiation with center wavelengths of 545 and 655 nm, respectively.

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(a) phase contrast	(b)	before irradiation	(c)	0 min	(d)	5 min	(e)	10 min	
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(f) phase contrast	fluo (g)	rescence —> before irradiation	(h)	0 min	(i)	5 min	(j)	10 min	

Results obtained by irradiating RGK1 with light with a center wavelength of 545 nm and an intensity of 235 mW/cm<sup>2</sup> for 5 minutes ((a) to (e)) and 10 minutes ((f)-(j)), (a), (f) Phase contrast images before light irradiation and fluorescence images (b), (g) before light irradiation, (c), (h) immediately after light irradiation, (d), (i) 5 minutes after light irradiation, and (e), (j) 10 minutes after light irradiation.



Phase contrast images (middle row) and their magnifications (top row), and fluorescence images (bottom row) of RGK1 when light with a central wavelength of 545 nm and an intensity of 235 mW/cm<sup>2</sup> was irradiated for 5 minutes. (a) Immediately, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min, (f) 25 min, and (g) 30 min after irradiation.

## Movie S1

Movie showing the effect of PDT on RGK1 obtained by irradiation with light with a center wavelength of 655 nm and an intensity of 87 mW/cm<sup>2</sup> for 1 minute.

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