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Supplementary Information

Applicability and Limitations of Fluorescence Intensity-Based Thermometry Using a Palette of Organelle Thermometers

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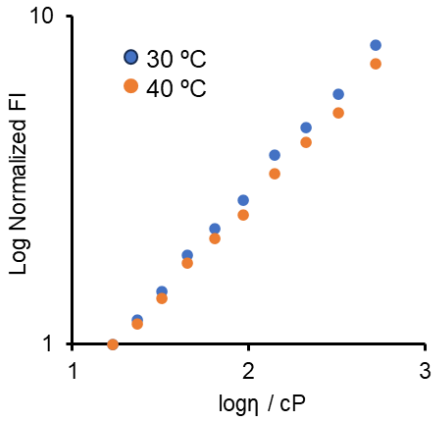

Figure S1. Relationship between fluorescence intensity (FI) of ER Thermo Green (ETG) and viscosity. The normalized fluorescence intensity (FI) at 512 nm was plotted against viscosity. Viscosity was varied from 17 to 529 cP by preparing mixtures of glycerol and ethylene glycol at different volume ratios.

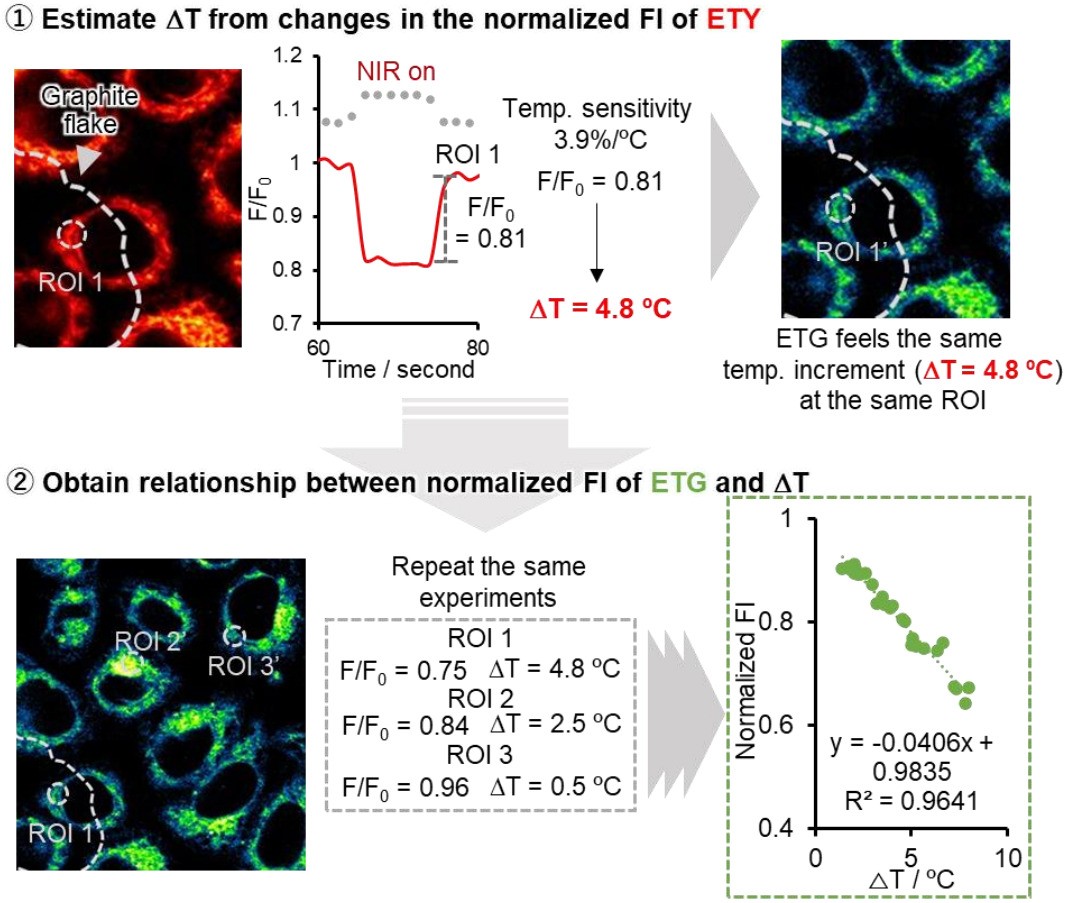

Figure S2. How to evaluate the temperature sensitivity of OTGs. 1) Temperature increment (ΔT) was estimated from the step depth in the normalized FI of ETY (F/F_0) when a graphite flake was illuminated with an NIR laser. 2) The experiment of 1 was repeated to investigate the relationship between normalized FI (F/F_0) of ETG and ΔT. F/F_0 was plotted against ΔT to obtain the calibration curve and the temperature sensitivity of ETG.

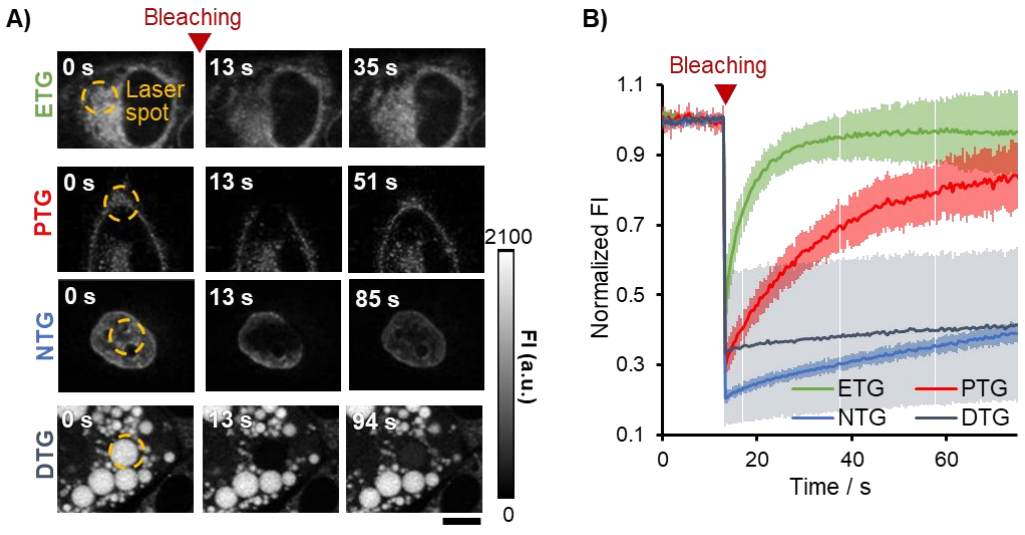

Figure S3. Lateral diffusion of Organelle Thermo Greens (OTGs) analyzed by fluorescence recovery after photobleaching (FRAP). A) Fluorescence images of ETG, PTG, NTG, and DTG in the FRAP experiments. Scale bar: 10 μm. B) Normalized

fluorescence intensities (FIs) of ETG, PTG, NTG, and DTG ware plotted against time, respectively, showing the recovery of fluorescence intensity after the bleaching.

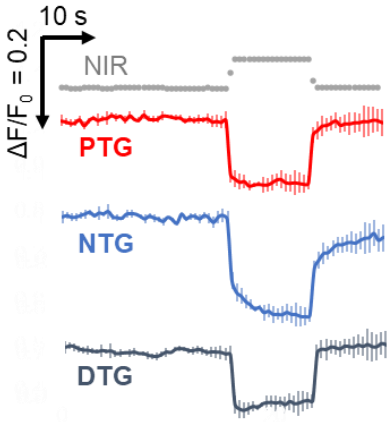

Figure S4. Step-like response of the fluorescence intensity of PTG, NTG, and DTG after photothermal heating using a nanoheater (**nanoHT**). The step-like responses of the normalized FIs of the PTG, NTG, and DTG were plotted. Images with negligible photobleaching were selected for analyzing the step like responses.

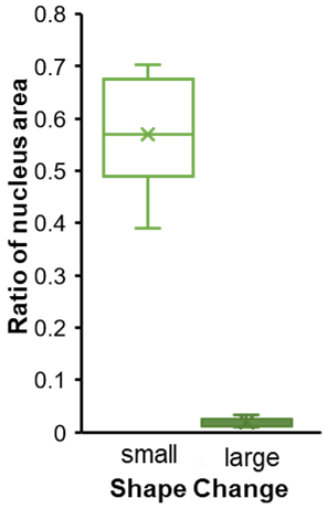

Figure S5. Definition of induced shape change in brown adipocytes after stimulation. The nuclear area in brown adipocytes was evaluated before and after treatment with isoproterenol, after which, the "large" shape change was defined when its ratio of the area was less than 0.1 and the other as "small" shape change. The nuclear area was measured using ImageJ software. The line and x in the boxes show the median and the average, respectively.

	Intensity-based thermometry	Lifetime-based thermometry
Availability of instrument	widespread	limited
Availability of materials	A varieties of sensors	Limited number
Concentration of fluorophore	dependent	independent
Morphology of samples	dependent	independent
Changes in focal plane	dependent	independent
Photobleaching	dependent	independent

Figure S6. Pros and cons in fluorescence intensity-based and fluorescence lifetime-based thermometry.