PNAS
www.pnas.org

Supplementary Information for

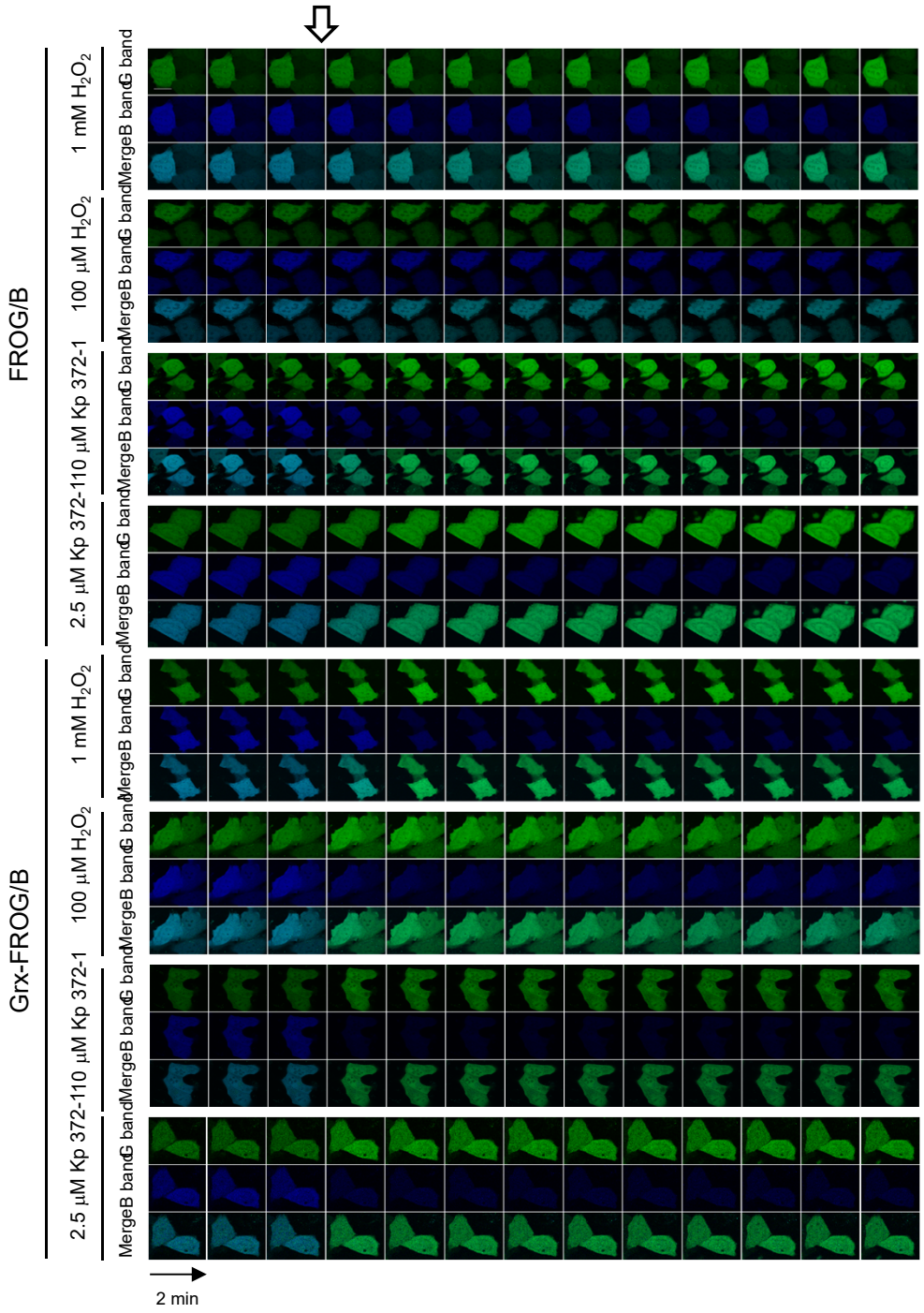
Real-time monitoring of the *in vivo* redox state transition using the ratiometric redox state sensor protein FROG/B

Kazunori Sugiura, Shoko Mihara, Nae Fu, and Toru Hisabori

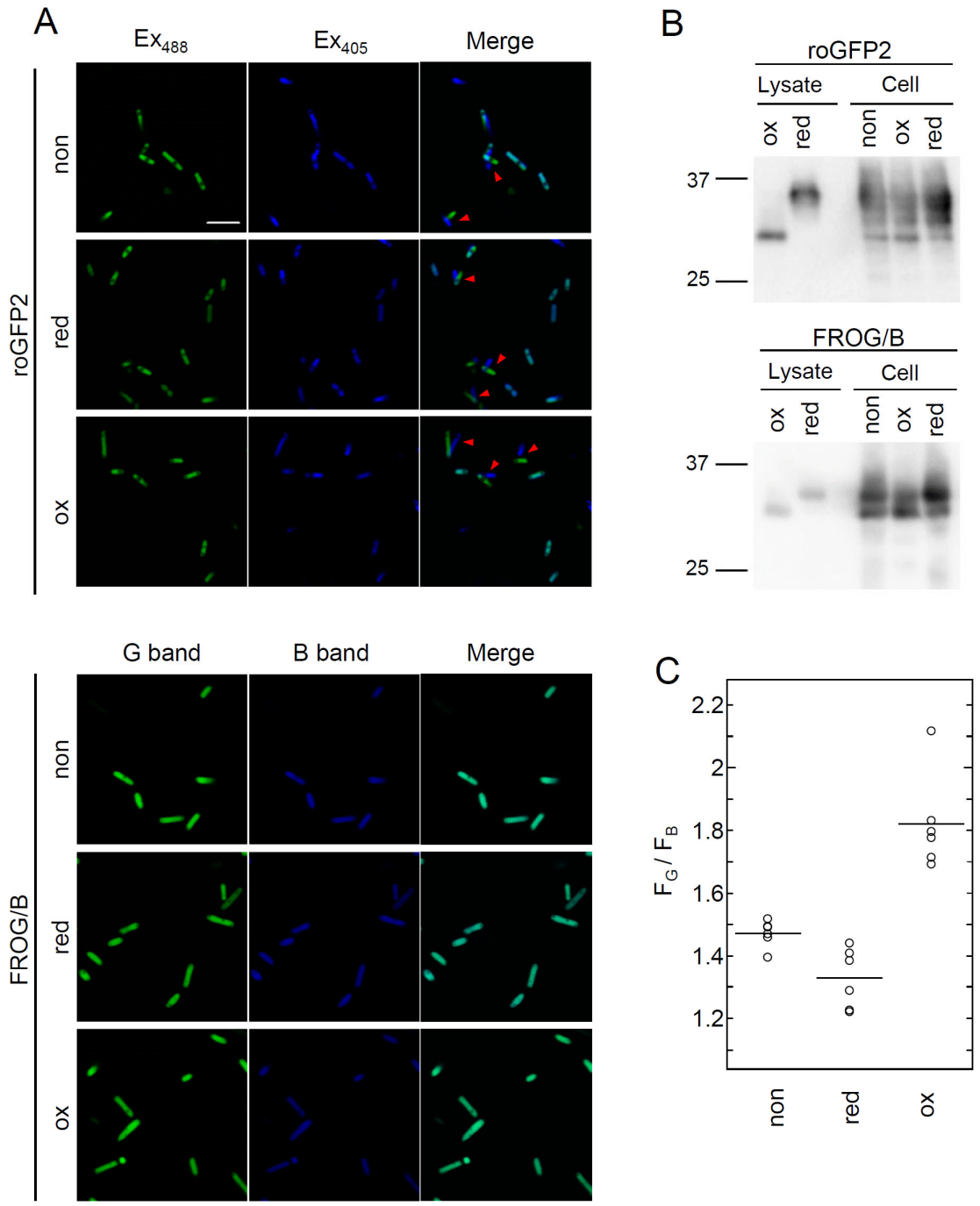
Toru Hisabori
Email: thisabor@res.titech.ac.jp

This PDF file includes:

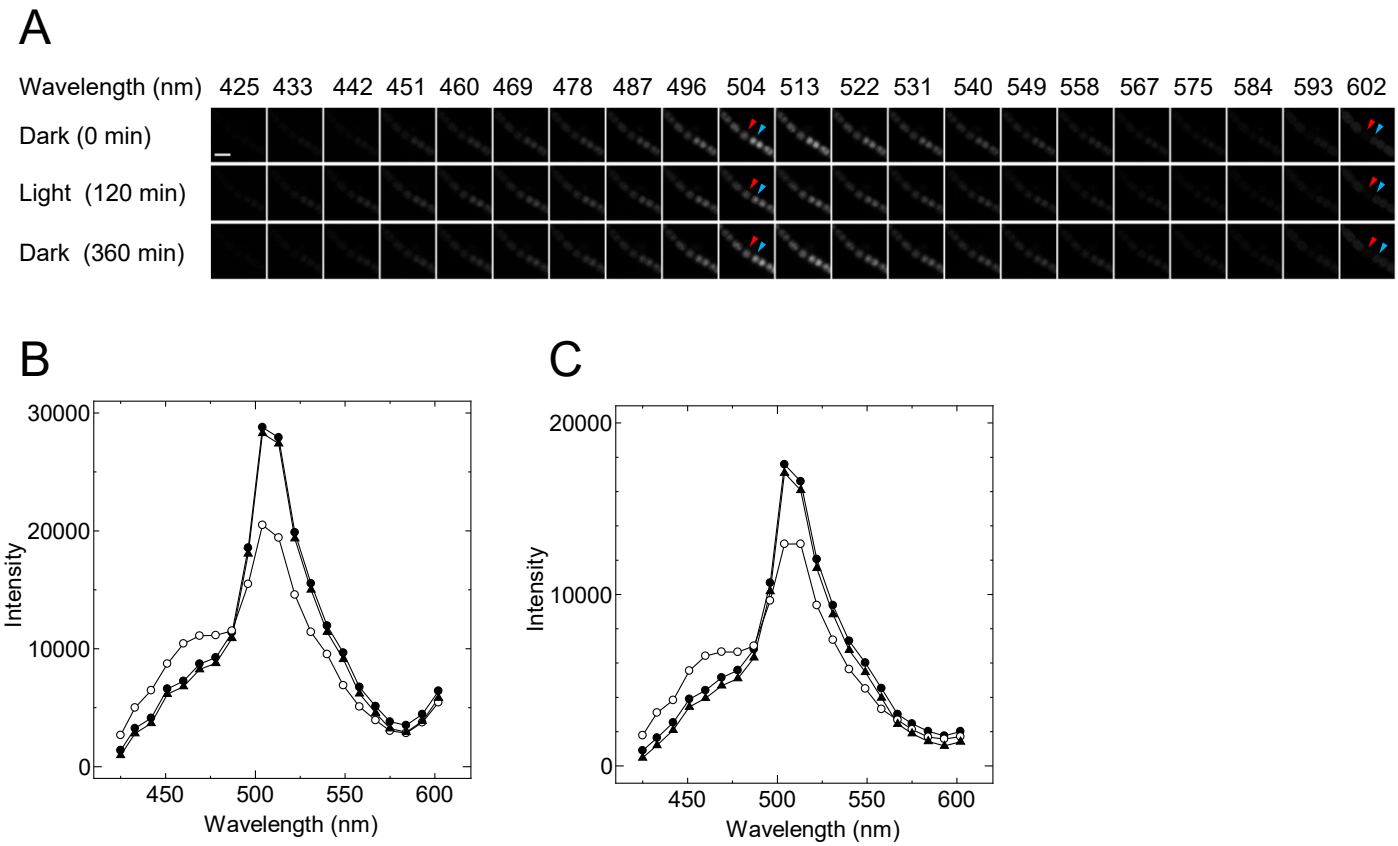
Figs. S1 to S3



Supplementary Figure 1. Redox imaging of FROG/B in HeLa cells. Imaging of the ROS induction in HeLa cells. Cells expressing FROG/B and Grx-FROG/B were observed under a confocal fluorescence microscope. Fluorescence images of the blue range (420–482 nm), the green range (500–562 nm), and merged images at the indicated time periods are shown at 2-min intervals. Scale bar, 20 μm. At 5 min (indicated as an open arrow), 1 mM H_2O_2, 0.1 mM H_2O_2, 10 μM Kp372-1, or 2.5 μM Kp372-1 was added.



Supplementary Figure 2. Redox imaging in *E. coli* cells. (A) Fluorescent imaging of redox changes in *E. coli* cells expressing roGFP or FROG/B. Cells expressing roGFP2 that migrate during measurements are indicated by red arrows. (B) Confirmation of the redox state of sensor proteins in *E. coli* cells. Cells used for the fluorescent imaging (A) were collected and used for the SDS-PAGE analysis of the redox states of sensor proteins using AMS. The protein bands were visualized using anti-GFP antibodies. Fully oxidized or reduced cell lysates were used as controls. (C) F_G/F_B signal ratios of FROG/B in *E. coli* cells. The short bars indicate the averaged values. For oxidation, cells were incubated with 1 mM diamide for 1h, and for reduction 10 mM DTT_{red} was used.



Supplementary Figure 3. Spectrum images of FROG/B in *Anabaena* 7120. Emission spectra of FROG/B in *Anabaena* 7120 cells measured using confocal microscopy. (A) Fluorescent images of *Anabaena* 7120 cells of each channels under mutual light (50 μmol photons m^{-2} s^{-1})/dark conditions. Scale bar, 5 μm. (B, C) Emission spectra of FROG/B in the typical vegetative cell (B) and heterocyst (C) indicated by cyan arrows and a red arrows in Fig. S2A respectively at 0 min (closed circle), 120 min (open circle) and 360 min (closed triangle). Time scale corresponds with that in Fig. 4D.