Supplementary Information with:

Imaging of photosystem energy distribution and grana macro-organization during state transitions

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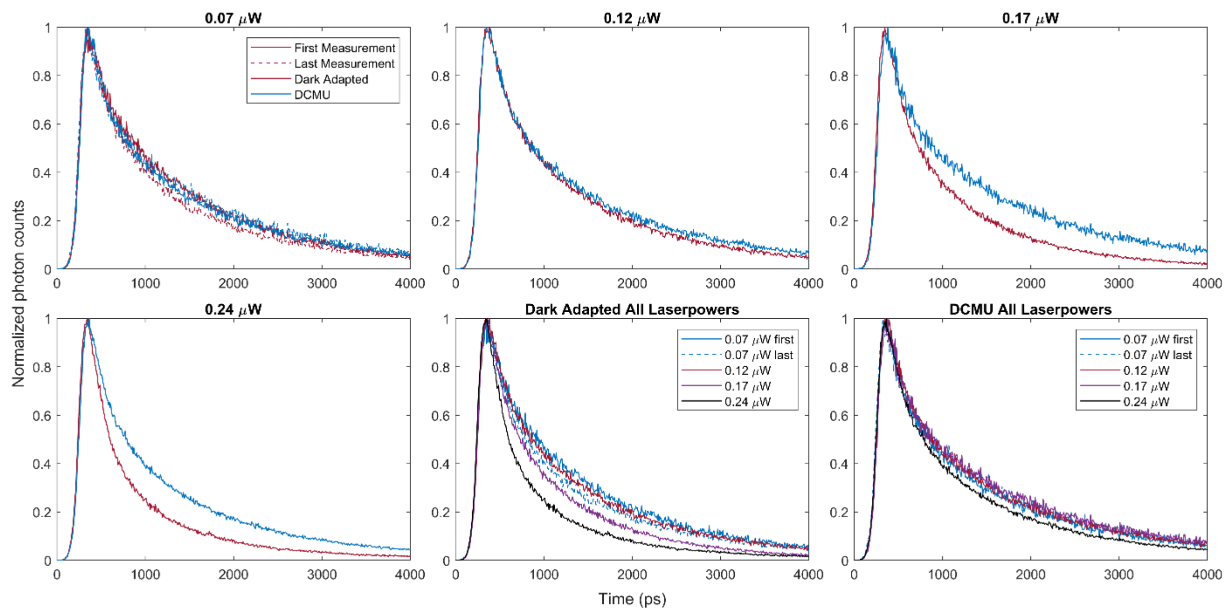
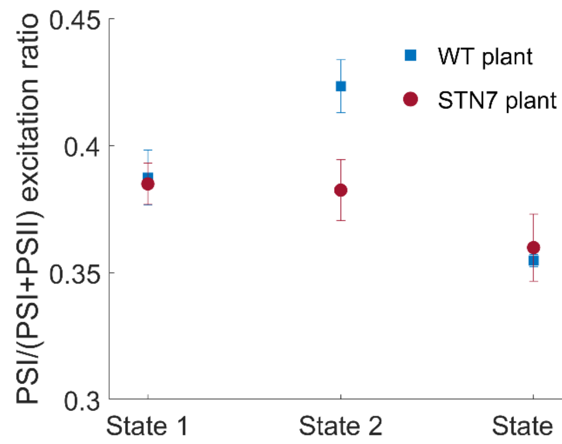
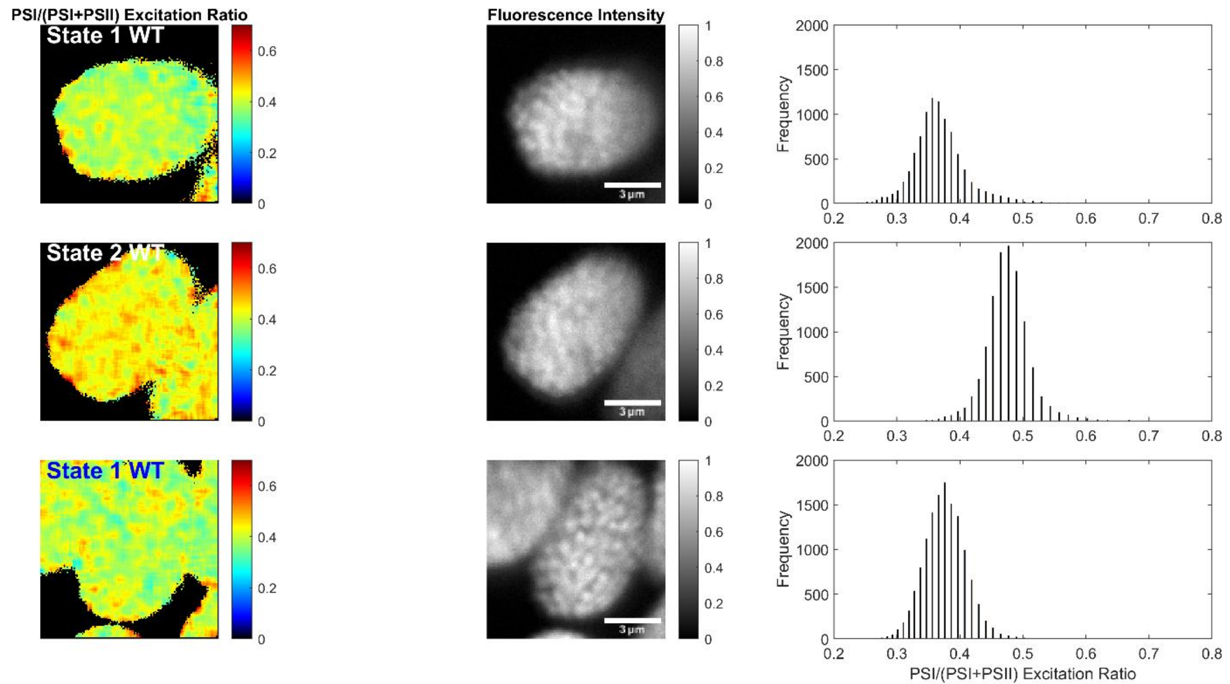


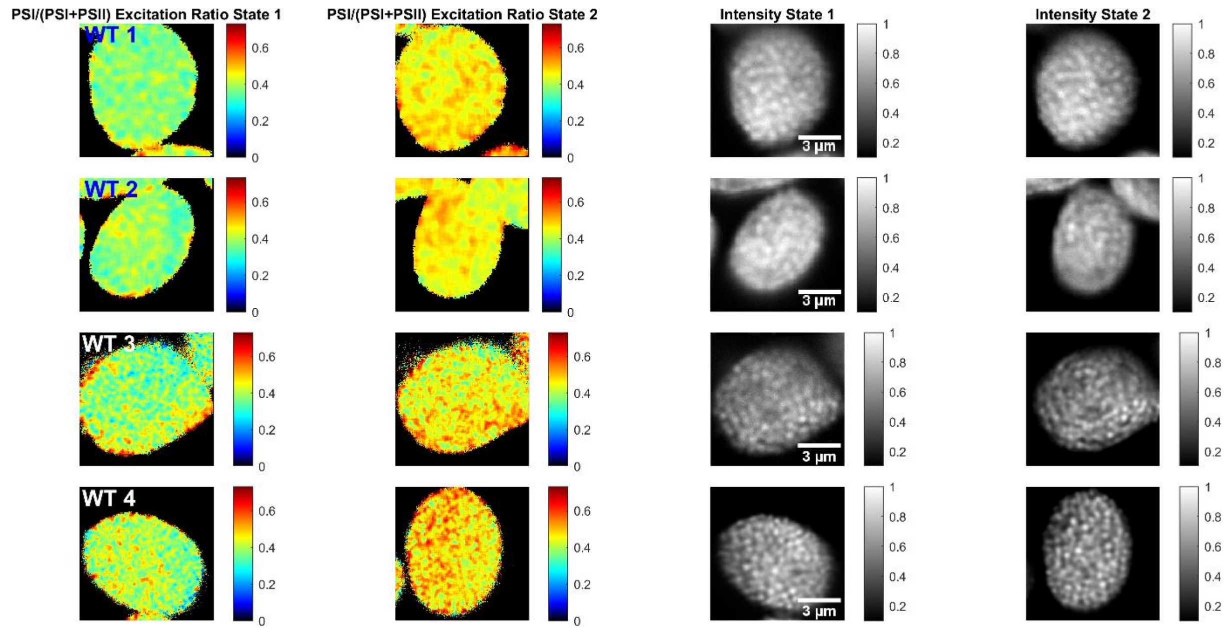
Supplemental Figure S1. Effect of laser intensity. Fluorescence decay of a dark-adapted (red) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) infiltrated dark-adapted (blue) wild type (WT) *A. thaliana* leaf. The decay traces have been recorded with different laser powers, the excitation wavelength was 488 nm. The lowest laser power of 0.07 µW was used as a first and last measurement, to indicate the potential permanent damage on the leaf caused by the higher laser powers. In the last two tiles all measurements in the dark-adapted and DCMU-infiltrated leaf are shown for comparison. Higher laser powers resulted in faster decay kinetics for the untreated dark adapted leaf. Instead, the decay kinetics was largely unchanged for the DCMU-treated leaf. At 0.07 and 0.12 µW the decay kinetics of the untreated leaf was very similar to that of the DCMU treated leaf, indicating that the photosystem II (PSII) reaction centres were closed by the laser light. Similar results were obtained for other excitation wavelengths.



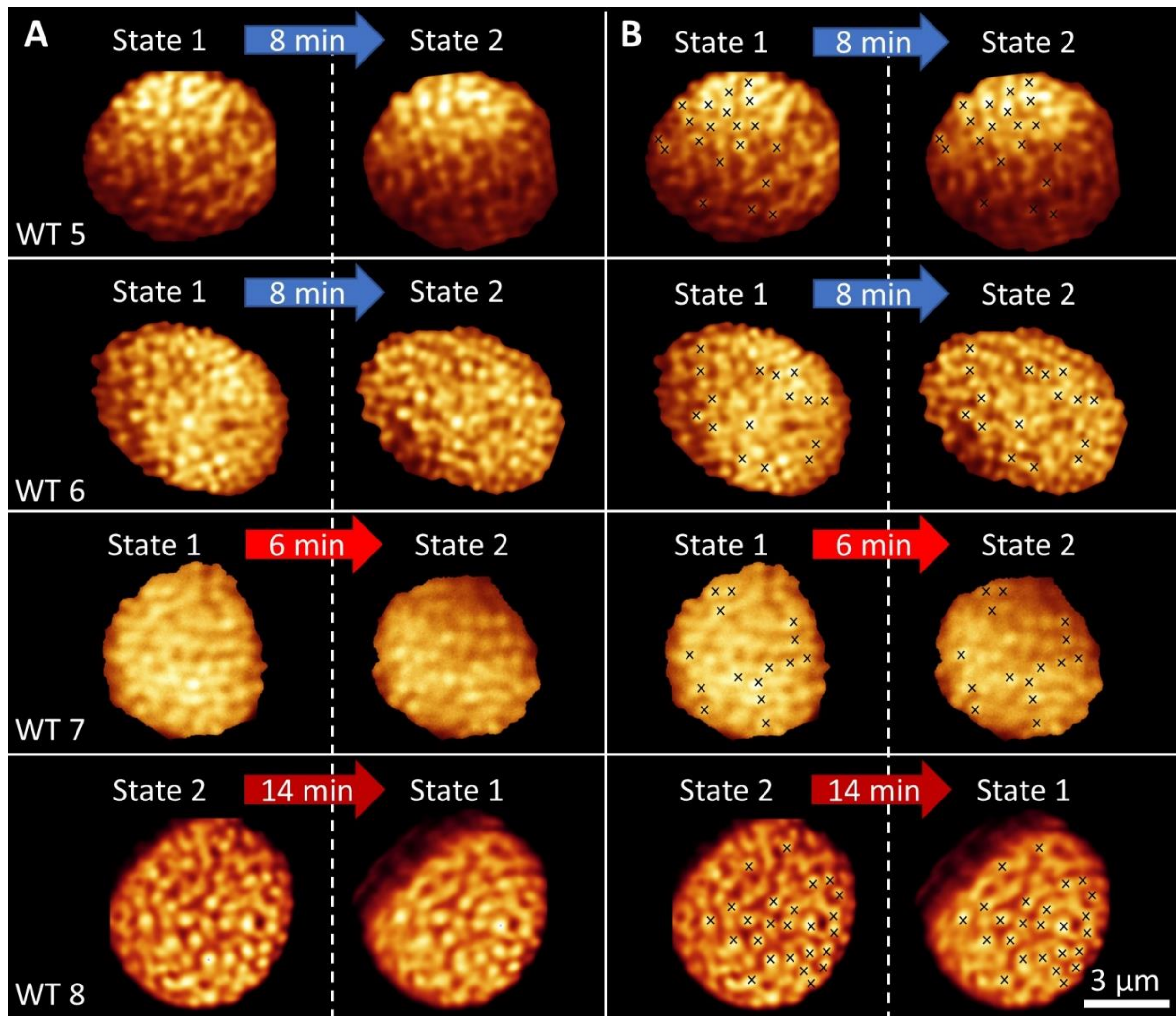
Supplemental Figure S2. Photosystem I/(Photosystem I + Photosystem II) (PSI/(PSI+PSII)) excitation ratio of wild type (WT) and STN7 samples brought to state 1, then to state 2 and again back to state 1. The measurements were done at excitation wavelength 488 nm. Standard deviations are indicated, number of chloroplasts imaged per light treatment is 3.



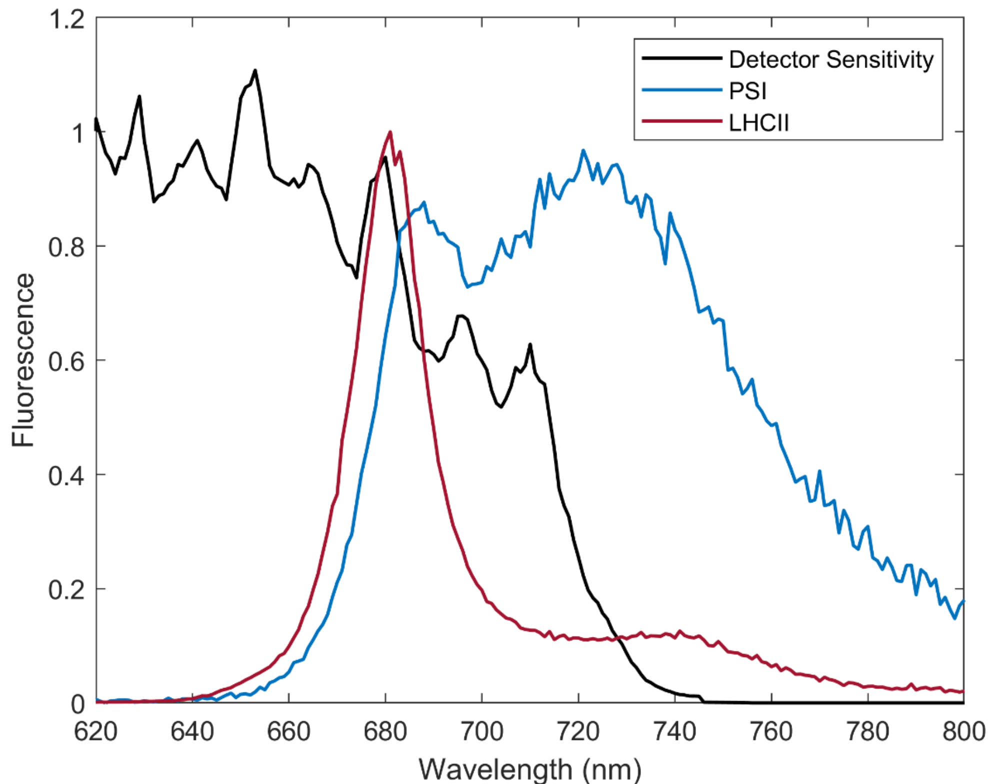
Supplemental Figure S3. Photosystem I/(Photosystem I + Photosystem II) (PSI/(PSI+PSII)) excitation ratio and fluorescence intensity images of the same chloroplast from WT *Arabidopsis* which is first brought to state 1, then to state 2 and again to state 1. To induce state 1 plants were illuminated for 30 minutes with 78 µmol photons m^{-2} s^{-1} of far-red light. State 2 was induced by illumination the sample on the microscope with 25 µmol photons m^{-2} s^{-1} of blue light for 8 minutes. Next, the sample was left for 30 minutes in the dark to bring it back to state 1. In the third column a histogram of the PSI/(PSI+PSII) excitation ratio as measured in the first column are shown. The excitation wavelength was 488 nm.



Supplemental Figure S4. Photosystem I/(Photosystem I + Photosystem II) (PSI/(PSI+PSII)) excitation ratio and fluorescence intensity images of WT chloroplasts which are first brought to state 1 and next to state 2. The excitation wavelength was 488 nm. The higher PSI/(PSI+PSII) excitation ratio in state 2 shows that state transitions were successfully induced. The scalebar of 3 µm is the same for all figures.



Supplemental Figure S5 fluorescence intensity images of WT chloroplasts in state 1 and state 2. The excitation wavelength was 488 nm. State transitions were induced with different light treatments. FLIM was used to confirm that state transitions were successfully induced. Chloroplasts WT5 and WT6 were imaged in state 1 and next brought to state 2 with blue light. Chloroplast WT7 was brought to state 2 by illumination with red (λ_{max} = 650 nm) light. Chloroplast WT8 was first brought to state 2 with blue light and next to state 1 by illumination with far-red light. Individual chloroplast images were digitally extracted for comparison. The scalebar is the same for all images. Leica HyVolution software was used to increase the image contrast and signal to noise ratio of chloroplasts WT5, WT6 and WT8.



Supplemental Figure S6. **Spectra used to calculate the PSI/(PSI+PSII) excitation ratio based on the ~100 ps amplitude of the thylakoid fluorescence decay kinetics.** Fluorescence emission of photosystem I (PSI), photosystem II (PSII) and sensitivity of the detector used for the FLIM measurements. All spectra are normalized to 1 at their maxima. PSI was isolated from Arabidopsis according to (Wientjes et al., 2009). For the PSII spectrum we recorded the emission of freshly prepared grana membranes, prepared according to (Barbato et al., 2000). To measure the detector sensitivity, spectra of Atto 594 (Atto-Tec GmbH) dissolved in dimethyl sulfoxide (Sigma-Aldrich) recorded on a Fluorolog 3.22 spectrofluorimeter (Jobin Yvon-Spex) and on the Leica TCS SP8 were compared.

References

Barbato R, Bergo E, Szabo I, Dalla Vecchia F, Giacometti GM (2000) Ultraviolet B exposure of whole leaves of barley affects structure and functional organization of photosystem II. Journal of Biological Chemistry **275:** 10976-10982

Wientjes E, Oostergetel GT, Jansson S, Boekema EJ, Croce R (2009) The Role of Lhca Complexes in the Supramolecular Organization of Higher Plant Photosystem I. Journal of Biological Chemistry **284:** 7803-7810