

Supplementary Material.

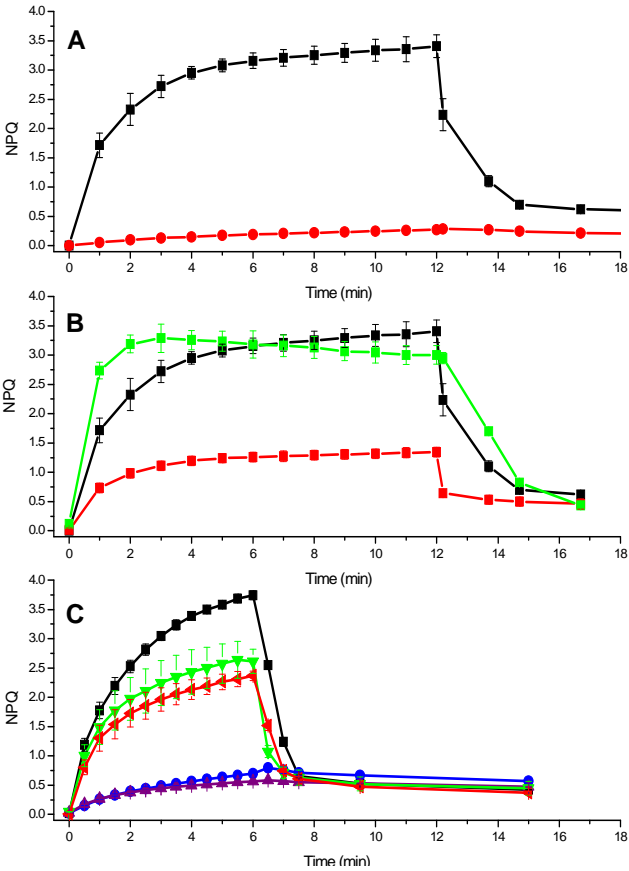
Supplementary Figure S1. NPQ kinetics dependence from nigericine and genotype. A) NPQ measured in leaves infiltrated with nigericine (red), an uncoupler dissipating ΔpH across the thylakoids membranes, as compared with control leaves infiltrated with buffer only (black). B) NPQ Dependence on carotenoid composition, analyzed by comparing plants depleted (*npq1*, red) or accumulating constitutively zeaxanthin (*npq2*, green). C) NPQ dependence on PsbS is analyzed by comparing PsbS-less plants (*npq4*, blue) with *npq4* plants complemented with PsbS in the WT form (*npq4*+PsbS, black) and PsbS where either one or both lumen exposed glutamate residues binding DCCD were mutated into glutamine (E122Q, E226Q and E122Q/E226Q, respectively green and red and purple).

Supplementary Figure S2. PSII recovery and zeaxanthin content in leaves after prolonged NPQ measurements. A) PSII yield (Fv/Fm) in dark adapted conditions was measured in leaves treated with 30 (black squares) and 90 minutes (red circles) of high light following light treatment leaves were incubated in the dark for 60 minutes before measurement. B) Zeaxanthin content in leaves was also analyzed during the prolonged NPQ measurements. Values for samples treated for 30 and 90 minutes of actinic light are shown.

Supplementary Figure S3. Analysis of PSII core distribution, considering only the closest neighbor. Distribution of PSII complexes in grana membranes was analyzed as shown in figure 4 of the main text, but considering only the distance of the closest PSII core rather than the average of the closest four. A) Distribution in dark adapted sample. B) Comparison of PSII distribution in samples dark adapted (black squares) or treated with 30 (red circles) and 90 minutes (green triangles) of light before grana isolation. C) Comparison of sample either light adapted (green triangles) or incubated in the dark for 90 minutes following light treatment (blue diamonds). D) effect of light treatment in PSII distribution in *npq4* grana. Dark adapted sample is shown in black triangles, sample light treated for 90 minutes in green diamonds.

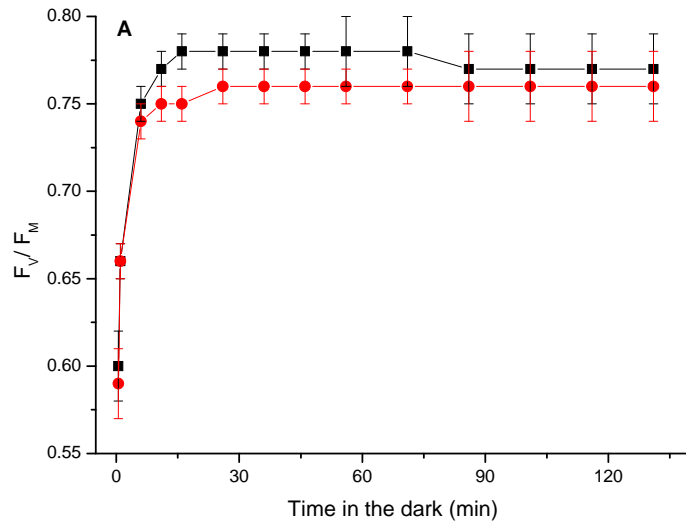
Supplemental Figure S4. EM images from KoCP24. EM pictures of negatively stained grana membranes purified from KOCP24 plants. Regions with high or low density of PSII core complexes are indicated with H and L respectively.

Figure S1.



Supplementary figure S2.

Supplementary Figure S2. PSII recovery and zeaxanthin content in leaves after prolonged NPQ measurements. A) PSII yield (F_v/F_m) in the dark was measured in leaves treated with 30 (black squares) and 90 minutes (red circles) of high light. B) Zeaxanthin content (moles / 100 Chl moles) in leaves was also analyzed during the prolonged NPQ measurements. Values for samples treated for 30 and 90 minutes of actinic light are shown.



	30 minutes light	90 minutes light
Dark	n.d	n.d.
End light treatment	2.29± 0.10	2.43±0.23
15' recovery	1.50±0.13	1.72±0.30
90' recovery	1.03±0.04	1.06±0.05

Supplementary Figure S3

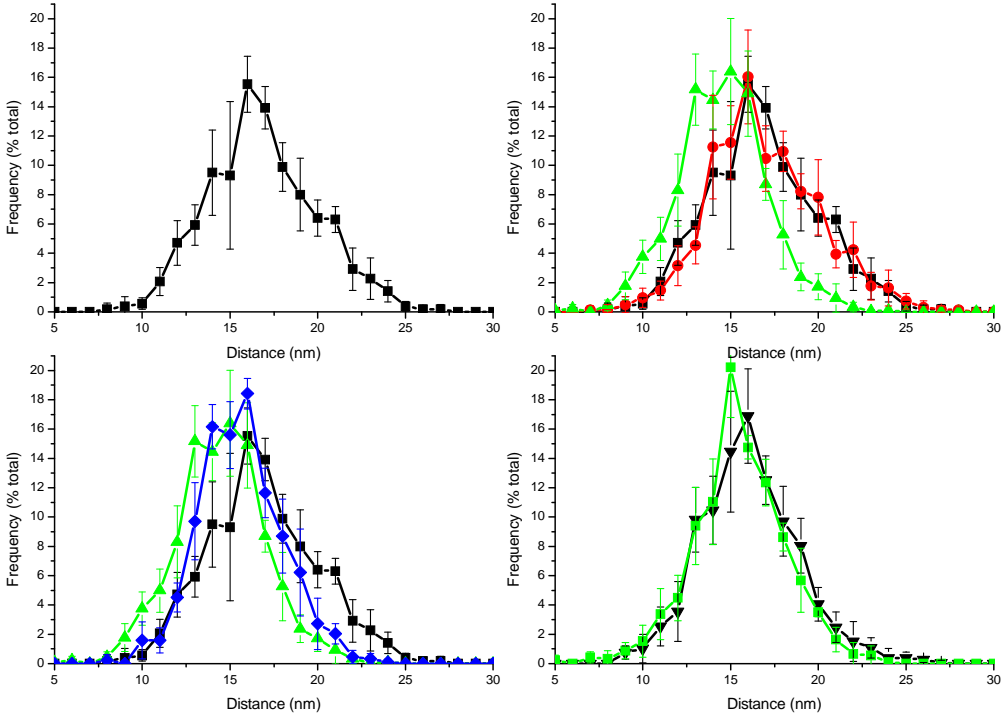


Figure S4.

