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| **Supplementary File 2a. Table of Mixed Effects Model investigating changes in FNR density between different chloroplast sub-compartments in WT Arabidopsis.** Analysis of data presented in Figure 1 Supplement 2. Fixed Effects taking either label density in the stroma as the intercept or label density in the margins/lamellae as the intercept. Linear mixed model fit by REML. Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1 | | | | | | | | | | | | | | | | | | | |
| **Deletion test** carried out using using Satterthwaite’s method with the R package lmerTest (Kuznetsova, Brockhoff & Christensen 2017). The model is a mixed effects model with random intercepts. The square root of response is the response variable, tissue is the fixed effect and individual the random effect. | | | | | | | | | | | | | | | | | | | |
| Fixed effect deleted | Sum Sq | | Mean Sq | | | Num DF | | | Den DF | | | | F value | | | Pr (>F) | | |  |
| sub-compartment | 284.46 | | 94.82 | | | 3 | | | 66.825 | | | | 118.88 | | | 2.20^-16 | | | \*\*\* |
|  | | | | | | | | | | | | | | |  | |  | |  |
| **Model summary:** | | | | |  | | |  | | | |  | | |  | |  | |  |
| Random effects: | |  | | |  | | |  | | | |  | | |  | |  | |  |
| Groups | | Name | | | Variance | | | | | Std. Dev. | | | |  | |  |  | |  |
| individual | | (Intercept) | | | 0.01648 | | | | | 0.1284 | | | |  | |  |  | |  |
| Residual | |  | | | 0.79758 | | | | | 0.8931 | | | |  | |  |  | |  |
| Number of obs: 91, groups: individual, 25 | | | | | | | | | | | | | | |  | |  | |
| Fixed effects when stroma is set as the intercept: | | | | | | | | | | | | | | | | | | | |
|  | | Estimate | | Std. Error | | | DF | | | | t value | | | | Pr (>|t|) | | |  |  |
| (Intercept) | | 2.3925 | | 0.1924 | | | 86.9198 | | | | 12.438 | | | | <2.00^-16 | | | \*\*\* |  |
| cytosol | | -2.3791 | | 0.2612 | | | 67.8887 | | | | -9.107 | | | | 2.19^-13 | | | \*\*\* |  |
| grana | | -0.2515 | | 0.2693 | | | 65.7707 | | | | -0.934 | | | | 0.354 | | |  |  |
| margin/lamellae | | 2.5477 | | 0.2693 | | | 65.7707 | | | | 9.462 | | | | 6.86^-14 | | | \*\*\* |  |
|  | |  | | |  | | |  | | | |  | | |  | |  | |  |
| Fixed effects when margin/lamellae is set as the intercept: | | | | | | | | | | | | | | | | | | | |
|  | | Estimate | | Std. Error | | | DF | | | | | t value | | | Pr (>|t|) | | | |  |
| (Intercept) | | 4.9402 | | 0.1924 | | | 86.9198 | | | | | 25.683 | | | <2.00^-16 | | | \*\*\* | |
| cytosol | | -4.9269 | | 0.2612 | | | 67.8887 | | | | | -18.86 | | | <2.00^-16 | | | \*\*\* | |
| grana | | -2.7993 | | 0.2693 | | | 65.7707 | | | | | -10.396 | | | 1.62^-15 | | | \*\*\* | |
| stroma | | -2.5477 | | 0.2693 | | | 65.7707 | | | | | -9.462 | | | 6.86^-14 | | | \*\*\* | |

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| **Supplementary File 2b. Mixed Effects Model investigating changes in cytochrome *f* density between different chloroplast sub-compartments in WT Arabidopsis.** Analysis of data presented in Figure 1 Supplement 2. Fixed Effects taking either label density in the stroma as the intercept or label density in the margins/lamellae as the intercept. Linear mixed model fit by REML. Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1 | | | | | | | | | | | | | | | | | | | |
| **Deletion test** carried out using using Satterthwaite’s method with the R package lmerTest (Kuznetsova, Brockhoff & Christensen 2017). The model is a mixed effects model with random intercepts. The square root of response is the response variable, tissue is the fixed effect and individual the random effect. | | | | | | | | | | | | | | | | | | | |
| Fixed effect deleted | Sum Sq | | Mean Sq | | | Num DF | | | Den DF | | | | F value | | | Pr (>F) | | |  |
| sub-compartment | 15.211 | | 5.0702 | | | 3 | | | 39 | | | | 23.586 | | | 7.135e-09 | | | \*\*\* |
|  | |  | | |  | | |  | | | |  | | |  | |  | |  |
| **Model summary**: | | | | | | | | | | | | | | | | | | | |
| Random effects: | |  | | |  | | |  | | | |  | | |  | |  | |  |
| Groups | | Name | | | Variance | | | | | Std. Dev. | | | |  | |  |  | |  |
| individual | | (Intercept) | | | 0.007045 | | | | | 0.08393 | | | |  | |  |  | |  |
| Residual | |  | | | 0.214969 | | | | | 0.46365 | | | |  | |  |  | |  |
| Number of obs: 56, groups: individual, 14 | | | | | | | | | | | | | | |  | |  | |
|  | |  | | |  | | |  | | | |  | | |  | |  | |  |
| Fixed effects when stroma is set as the intercept: | | | | | | | | | | | | | | | | | | | |
|  | | Estimate | | Std. Error | | | DF | | | | t value | | | | Pr (>|t|) | | |  |  |
| (Intercept) | | 0.283 | | 0.1259 | | | 51.8434 | | | | 2.247 | | | | 0.0289 | | | \* |  |
| relevel cytosol | | -0.2765 | | 0.1752 | | | 39 | | | | -1.578 | | | | 0.1227 | | |  |  |
| relevel grana | | 0.863 | | 0.1752 | | | 39 | | | | 4.925 | | | | 1.59^-05 | | | \*\*\* |  |
| relevel margin/lamellae | | 0.9072 | | 0.1752 | | | 39 | | | | 5.177 | | | | 7.15^-06 | | | \*\*\* |  |
|  | |  | | |  | | |  | | | |  | | |  | |  | |  |
| Fixed effects when margin/lamellae is set as the intercept: | | | | | | | | | | | | | | | | | | | |
|  | | Estimate | | Std. Error | | | DF | | | | | t value | | | Pr (>|t|) | | | |  |
| (Intercept) | | 1.19021 | | 0.12593 | | | 51.84340 | | | | | 9.451 | | | 7.24^-13 | | | \*\*\* | |
| relevel cytosol | | 1.18371 | | 0.17524 | | | 39.00000 | | | | | -6.755 | | | 4.66^-08 | | | \*\*\* | |
| relevel grana | | -0.04422 | | 0.17524 | | | 39.00000 | | | | | -0.252 | | | 0.802 | | |  | |
| relevel stroma | | -0.90724 | | 0.17524 | | | 39.00000 | | | | | -5.177 | | | 7.15^-06 | | | \*\*\* | |

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| **Supplementary File 2c. Fitting parameters and errors in comparison of light dependent NADP+ reduction by different genotypes.**  Analysis performed using the data in Figure 4. Fits were calculated from experiments on individual chloroplast preparations, and then the parameters, and the fitting errors averaged. | | | | | | | | | | | | |
|  | Fitting parameters  Parameters calculated individually for 3-6 biological replicates and then averaged ± s.d. | | | | | | Errors (absolute, 95% confidence)  Errors in the fit calculated individually for 3-6 biological replicates and then averaged ± s.d. | | | | | |
|  |  |  |  |  |  |  | |  |  |  |  |  |
| wt | 0.0134  ±0.0072 | 0.697  ±0.048 | 0.303  ±0.048 | 3.74  ±1.62 | 0.111  ±0.052 |  | | 0.000677  ±0.000465 | 0.0674  ±0.0455 | 0.0272  ±0.0144 | 1.51  ±0.45 | 0.0758  ±0.0576 |
| *fnr1* | 0.0155  ±0.0075 | 0.865  ±0.117 | 0.135  ±0.117 | 10.8  ±6.62 | 0.235  ±0.252 |  | | 0.000185  ±6.48E-05 | 0.0371  ±0.0338 | 0.00949  ±0.00889 | 4.88  ±3.40 | 0.100  ±0.110 |
| *fnr1*-ZmFNR1 | 0.0201  ±0.0082 | 0.701  ±0.060 | 0.298  ±0.06 | 10.2  ±5.62 | 0.300  ±0.122 |  | | 0.000243  ±0.000101 | 0.0597  ±0.0390 | 0.0239  ±0.0196 | 3.46  ±1.70 | 0.0842  ±0.0384 |
| *fnr1-*ZmFNR2 | 0.0158  ±0.0037 | 0.696  ±0.084 | 0.304  ±0.085 | 8.60  ±3.12 | 0.388  ±0.225 |  | | 0.00021  ±2.5E-05 | 0.0705  ±0.0230 | 0.0367  ±0.0187 | 5.44  ±3.26 | 0.106  ±0.076 |
| *fnr1-*ZmFNR3 | 0.0164  ±0.0067 | 0.833  ±0.109 | 0.167  ±0.120 | 9.04  ±5.18 | 0.209  ±0.119 |  | | 0.000213  ±1.31E-05 | 0.0552  ±0.0120 | 0.0159  ±0.0049 | 4.34  ±3.34 | 0.106  ±0.053 |

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| **Supplementary File 2d. Statistical analysis on the contribution of the fast phase to total amplitude of light dependent fluorescence change in the chloroplast assay of NADP+ reduction.** Analysis performed using the data in Figure 4 and Averaged in Supplementary File 2c.  One Way Analysis of Variance | | | | | | | | | | | | | |
| Normality Test (Shapiro-Wilk): Passed (P = 0.642) | | | | | | | | | | | | | |
| Equal Variance Test (Brown-Forsythe): Passed (P = 0.629) | | | | | | | | | | | | | |
|  | *n* | | *missing* | | | *mean* | | *Std Dev* | | | *SEM* | | |
| wt | 3 | | 0 | | | 0.697 | | 0.0479 | | | 0.0276 | | |
| *fnr1* | 3 | | 0 | | | 0.865 | | 0.117 | | | 0.0675 | | |
| *fnr1*-ZmFNR1 | 4 | | 0 | | | 0.702 | | 0.0600 | | | 0.0300 | | |
| *fnr1-*ZmFNR2 | 5 | | 0 | | | 0.696 | | 0.0848 | | | 0.0379 | | |
| *fnr1-*ZmFNR3 | 5 | | 0 | | | 0.833 | | 0.110 | | | 0.0490 | | |
|  | | | | | | | | | | | | | |
| Source of Variation | | *DF* | | | *SS* | | *MS* | | *F* | | | *P* | |
| Between Groups | | 5 | | | 0.114 | | 0.0228 | | 2.995 | | | 0.043 | |
| Residual | | 16 | | | 0.122 | | 0.00761 | |  | |  | | |
| Total | | 21 | | | 0.236 | |  | |  | |  | | |
|  | | | | | | | | | | | | | |
| The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.043). | | | | | | | | | | | | | |
| Power of performed test with alpha = 0.050: 0.514 | | | | | | | | | | | | | |
|  | | | | | | | | | | | | | |
| Multiple Comparisons versus Control Group (Holm-Sidak method): | | | | | | | | | | | | | |
|  | | | | | | | | | | | | | |
| Comparison | | | | Diff of Means | | | | | | t | | | P |
| wt vs. *fnr1* | | | | 0.169 | | | | | | 2.368 | | | 0.145 |
| wt vs. *fnr1-*ZmFNR3 | | | | 0.136 | | | | | | 2.136 | | | 0.180 |
| wt vs. *fnr1*-ZmFNR1 | | | | 0.00503 | | | | | | 0.0756 | | | 0.996 |
| wt vs. *fnr1*-ZmFNR2 | | | | 0.000679 | | | | | | 0.0107 | | | 0.992 |

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| **Supplementary File 2e. Fitting parameters and errors in comparison of dark NADPH oxidation by different genotypes.**  Analysis performed using the data in Figure 4. Fits were calculated from experiments on individual chloroplast preparations, and then the parameters, and the fitting errors averaged. | | | | | | | |
|  | Fitting parameters (Hill)  Parameters calculated individually for 3-6 biological replicates and then averaged ± s.d. | |  | Errors (absolute, 95% confidence)  Errors in the fit calculated individually for 3-6 biological replicates and then averaged ± s.d. | | | |
|  |  |  |  |  |  |  |  |
| wt | -0.00344 ±0.00112 | 0.415±0.0980 | 1 |  | 0.000619  ±7.2E-05 | 0.109  ±0.038 | - |
| *fnr1* | -0.00341±0.00414 | 0.455±0.410 | 1 |  | 0.000694  ±0.00013 | 0.141  ±0.1812 | - |
| *fnr1-* ZmFNR1 | -6.38E-04±0.00147 | 0.202±0.098 | 1 |  | 0.00103  ±0.00052 | 0.0406  ±0.0115 | - |
| *fnr1-* ZmFNR2 | -0.00361±0.00196 | 0.373±0.034 | 1 |  | 0.00070  ±0.00013 | 0.0966  ±0.0145 | - |
| *fnr1-* ZmFNR3 | -0.00287±0.0029 | 0.185±0.034 | 1 |  | 0.00089  ±0.00011 | 0.0312  ±0.0005 | - |

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| **Supplementary File 2f. Pm values and statistical analysis of plants used for PAM analysis of the high light response.** Analysis performed using the data in Figure 5, with example traces given in Figure 5 Supplement 1. Pm determination of dark-adapted leaves in order to calculate PSI parameters in response to high light treatment of Wt, *fnr1*, and *fnr1* plants expressing either ZmFNR1, ZmFNR2 or ZmFNR3 Arabidopsis plants (see Figure 5). n = 5-7 replicates. Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1 | | | | | | | |
|  | | | Mean ± sd | | | | |
| wt | | | 1.04 | ±0.07 | |  |  |
| *fnr1* | | | 0.91 | ±0.25 | |  |  |
| *fnr1-* ZmFNR1 | | | 1.11 | ±0.22 | |  |  |
| *fnr1-* ZmFNR2 | | | 1.05 | ±0.23 | |  |  |
| *fnr1-* ZmFNR3 | | | 1.11 | ±0.23 | |  |  |
|  | | |  |  | |  |  |
| ANOVA (One - way ) and Post hoc TSD Tukey test of the Pm determination for assessing the PSI responses to high light treatment on leaves from wild type, *fnr1*, and *fnr1* plants expressing either ZmFNR1, ZmFNR2 or ZmFNR3 (n>6 replicates). Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1 in .  Overall ANOVA | | | | | | | |
|  | Df | | Sum Sq | | Mean Sq | F value | Pr(>F) |
| genotype | 4 | | 0.3142 | | 0.07854 | 1.719 | 0.16 |
| Residuals | 52 | | 2.3757 | | 0.04569 |  |  |
| Post hoc TSD Tukey | | | | |  |  |  |
|  | | Estimate | | | Std. Error | t value | Pr(>|t|) |
| ZmFNR1-fnr1 | | 0.193861 | | | 0.094252 | 2.057 | 0.254 |
| ZmFNR2-*fnr1* | | 0.135583 | | | 0.08726 | 1.554 | 0.533 |
| ZmFNR3-*fnr1* | | 0.200417 | | | 0.085565 | 2.342 | 0.148 |
| wt-*fnr1* | | 0.096689 | | | 0.089221 | 1.084 | 0.814 |
| ZmFNR2-ZmFNR1 | | -0.058278 | | | 0.094252 | -0.618 | 0.971 |
| ZmFNR3-ZmFNR1 | | 0.006556 | | | 0.092685 | 0.071 | 1 |
| wt-ZmFNR1 | | -0.097172 | | | 0.09607 | -1.011 | 0.849 |
| ZmFNR3-ZmFNR2 | | 0.064833 | | | 0.085565 | 0.758 | 0.941 |
| wt-ZmFNR2 | | -0.038894 | | | 0.089221 | -0.436 | 0.992 |
| wt-ZmFNR3 | | -0.103727 | | | 0.087565 | -1.185 | 0.76 |