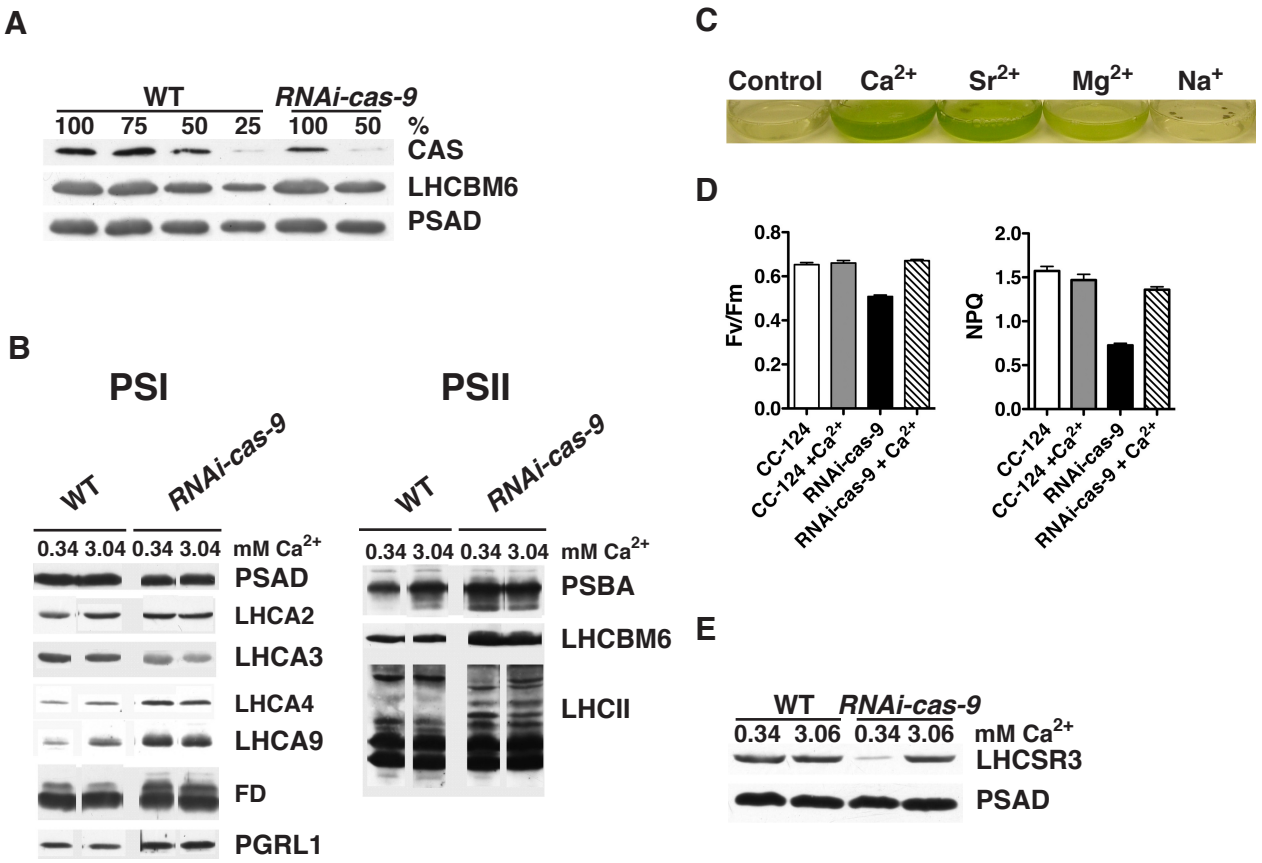
Supplemental Figure 1



Analysis of the HL response of the *cas-kd* line *RNAi-cas-9*

(**A**) Quantification of CAS amounts in thylakoids isolated from WT (CC-124) and *RNAi-cas-9*, grown in TAP under LL, upon SDS PAGE fractionation and immunoblot analysis (100% equals 5 µg of chlorophyll per lane; LHCBM6 and PSAD signals served as loading controls).

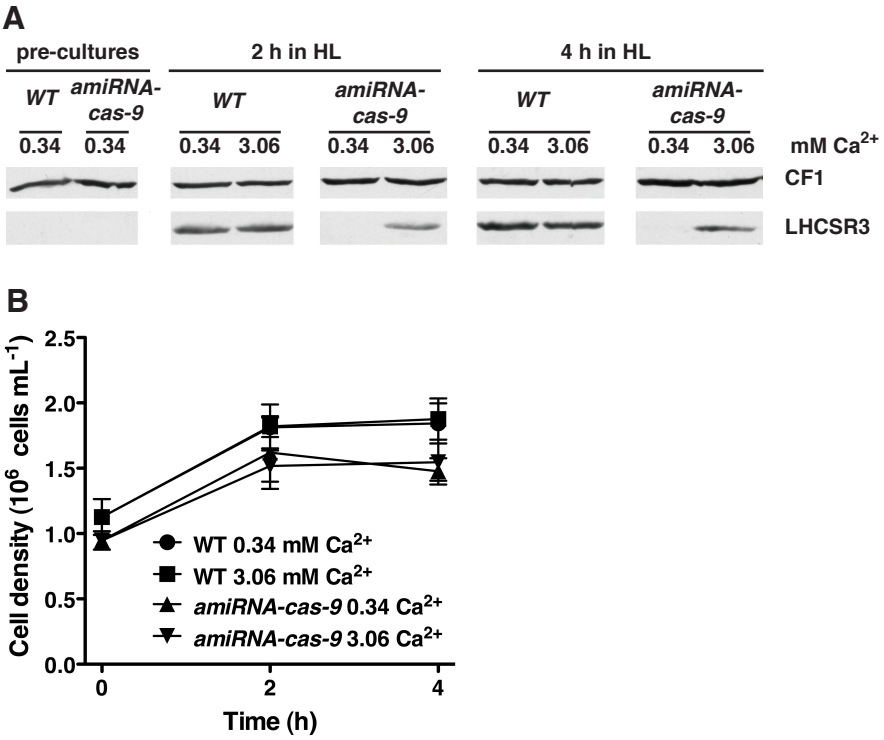
(**B**) Quantification of PSI and PSII subunits in WT (CC-124) and *RNAi-cas-9* grown under LL in TAP containing 0.34 or 3.06 mM $CaCl_2$. Five µg of chlorophyll of whole cell extracts were fractionated on a 13% SDS-PAGE and PSAD, LHCA2, LHCA3, LHCA4 LHCA9, FD, PGRL1 (for PSI), PSBA, LHCBM6 and P25K (for PSII) abundance were analyzed by immunoblot. CF1 signal served as a loading control whereas P25K is a polyclonal antibody against LHCII proteins.

(**C**) *RNAi-cas-9* strain after 24 h under HL in TAP. Cultures were supplemented with 3.06 mM of the indicated ion in the form of its chloride salt. Control contains standard concentration of 0.34 mM $CaCl_2$. Starting cell density of the cultures was $1x10^6$ cells/mL.

(**D**) Fv/Fm and maximal NPQ in WT (CC-124) and in *RNAi-cas-9*. Cells were grown in TAP under LL and then exposed for 16 h under HL in HSM containing 0.34 or 3.06 mM $CaCl_2$. Black bar = dark, white bar = 800 $\mu Em^{-2}s^{-1}$.Values plotted are the means of three samples ± standard deviation, PAR stands for photosynthetic active radiation.

(**E**) Quantification of LHCSR3 amounts in WT (CC-124) and *RNAi-cas-9*. Cells were grown in TAP under LL and then shifted for 24 h under HL (200 $\mu Em^{-2}s^{-1}$) in 80% HSM containing normal (0.34 mM) or high (3.06 mM) $CaCl_2$ concentration. Five µg of chlorophyll of whole cell extracts were fractionated on a 13% SDS-PAGE and LHCSR3 abundance was analyzed by immunoblot. PSAD signal served as a loading control.

Supplemental Figure 2

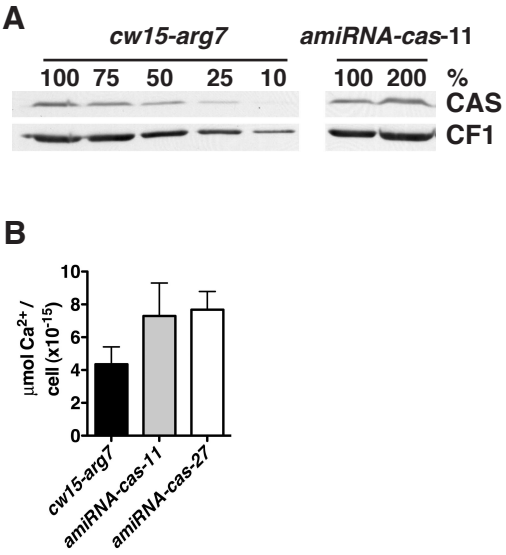


The differences in LHCSR3 expression between WT and *cas-kd* strains in 0.34 and 3.06 mM Ca^{2+} are not due to altered growth.

(A) WT and *amiRNA-cas-9* cells were shifted from LL TAP to HL 80% HSM containing 0.34 or 3.06 mM Ca^{2+}. Whole cell extracts of samples were taken at 0, 2 and 4h were fractionated on a 13% SDS-PAGE and LHCSR3 and CF1 abundances were analyzed by immunoblot. CF1 signal served as a loading control.

(B) Cell densities of WT and *amiRNA-cas-9* cultures from the experiment described above (Figure S2A). The values are the means ± S.D. of three cell counts.

Supplemental Figure 3

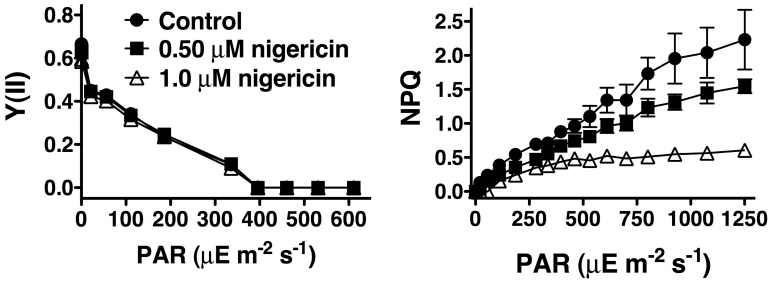


Down-regulation of CAS does not lead to a decrease in cellular calcium content in *amiRNA-cas-11* and *amiRNA-cas-27*.

(A) Quantification of CAS amounts in thylakoids isolated from WT (*cw15-arg7*) and *amiRNA-cas-11*, grown in TAP under LL, upon SDS-PAGE fractionation and immunoblot analysis (100% equals 4 µg of chlorophyll per lane; CF1 signal served as loading control).

(**B**) Calcium content of the *cas-kd* strains *amiRNA-cas-11* and *amiRNA-cas-27* compared with their WT background *cw15-arg7* as assessed by ion chromatography (IC). Data are presented on cell basis. The values are the means ± S.D. of three biological samples. The differences displayed in the graph are statistically not significant (paired t-test, 95% confidence level).

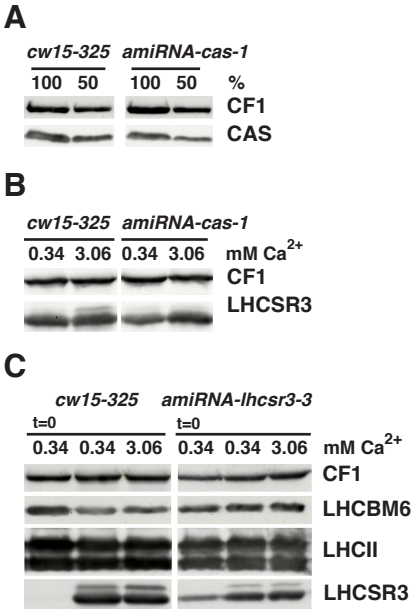
Supplemental Figure 4



Nigericin at 1 μM selectively impacts NPQ but not photosynthetic electron transfer in WT cells (CC-124)

Irradiance dependence of quantum yield of PSII and NPQ of WT (CC-124) cells shifted from TAP medium in LL to 80% HSM medium HL for two hours in the presence or absence of nigericin. Values plotted are the means of three samples ± standard deviation, PAR stands for photosynthetic active radiation.

Supplemental Figure 5



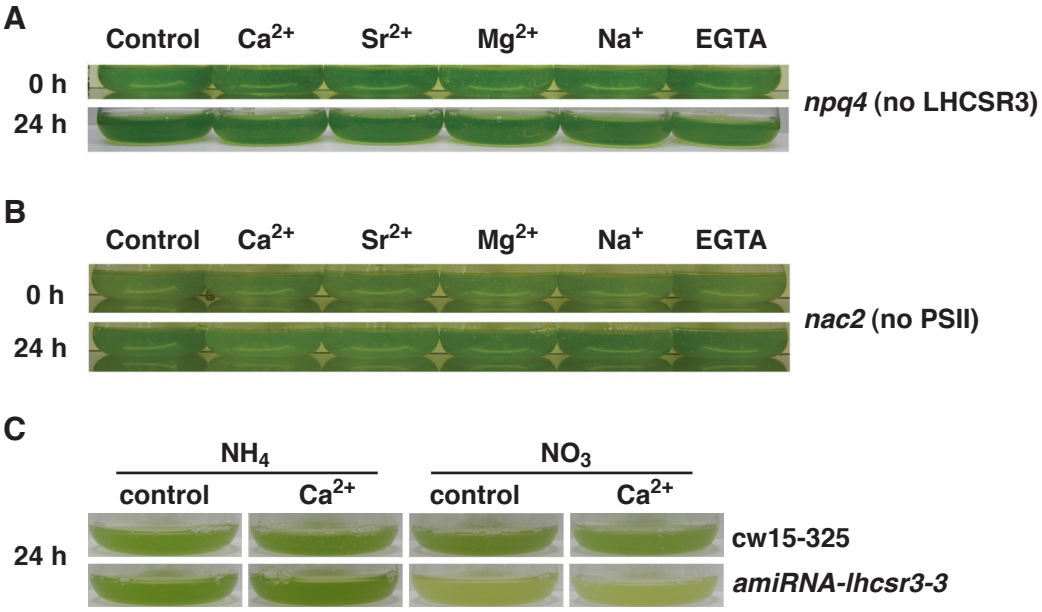
Characterization of *amiRNA-cas-1* and *amiRNA-lhcsr3-3* strains.

(A) Quantification of CAS amounts in thylakoids isolated from WT (*cw15-325*) and *amiRNA-cas-1* grown under LL in TAP-NO_3 (amiRNA expression activated) upon SDS-PAGE fractionation and immunoblot analysis (100% equals 70 µg of protein per lane; CF1 signal served as loading control). Densitometric analyses showed that CAS levels in *amiRNA-cas-1* are 40% down compared to the WT levels.

(B) Quantification of LHCSR3 amounts in whole cell extracts of WT (*cw15-325*) and *amiRNA-cas-1* upon SDS-PAGE fractionation and immunoblot analysis (100% equals 2.5 µg of chlorophyll per lane; CF1 signal served as loading control). WT and *amiRNA-cas-1* were initially shifted from LL TAP-NH_4 to LL TAP-NO_3 for 40 h to activate the amiRNA expression and subsequently shifted for 24 h to HL in either HSM-NH_4 or HSM-NO_3 containing 0.34 or 3.06 mM Ca^{2+}.

(C) Quantification of LHCSR3, LHCBM6 and LHCII amounts in whole cell extracts of WT (*cw15-325*) and *amiRNA-lhcsr3-3* upon SDS-PAGE fractionation and immunoblot analysis (100% equals 2.5 µg of chlorophyll per lane; CF1 signal served as loading control). WT and *amiRNA-lhcsr3-2* were initially shifted from LL TAP-NH_4 to LL TAP-NO_3 for 40 h to activate the amiRNA expression and subsequently shifted for 2 h to HL in either HSM-NH_4 or HSM-NO_3 containing 0.34 or 3.06 mM Ca^{2+}.

Supplemental Figure 6



Growth phenotypes of *npq4*, *nac2* and *amiRNA-lhcsr3-3* after 24h in HL.

(A) *npq4* (LHCSR3 knock-out strain; (Peers et al., 2009) and (B) *nac2* (PSII deficient mutant; (Kuchka et al, 1989)) 24 h after being shifted from LL TAP to HL 80% HSM with the addition of the indicated ions in their chloride salt form (final concentration 3.06 mM) or of EGTA (2 mM). Control contained 0.34mM Ca^{2+}, 0.41 mM Mg^{2+} and 0.27 mM Na^{+}. Starting cell density of the cultures was $1x10^{6}$ cells/mL.

(C) WT and *amiRNA-lhcsr3-3* were initially shifted from LL TAP-NH_4 to LL TAP-NO_3 for 40 h to activate the amiRNA expression and subsequently shifted for 24 h to HL in either HSM-NH_4 or HSM-NO_3 containing 0.34 or 3.06 mM Ca^{2+}. Before exposure to HL the cultures were set to 2.5 μg chl/mL.

Supplemental Table 1: Fm, Fo and Fv/Fm values of Figure 2D

	cw15-arg7 HL	*cw15-arg7* HL +Ca^{2+}	*amiRNA-cas-27* HL	*amiRNA-cas-27* HL +Ca^{2+}
Fm	0.190	0.191	0.065	0.075
	±0.012	±0.011	±0.003	±0.003
Fo	0.059	0.058	0.030	0.030
	±0.004	±0.003	±0.001	±0.001
Fv/Fm	0.688	0.696	0.534	0.608
	±0.003	±0.003	±0.005	±0.004

Supplemental Table 2: Fm, Fo and Fv/Fm values of Figure 3E

	CC-124	*amiRNA-cas-9*	*amiRNA-cas-9*+Ca^{2+}
Fm	0.222	0.107	0.179
	±0.09	±0.008	±0.008
Fo	0.059	0.054	0.047
	±0.002	±0.004	±0.003
Fv/Fm	0.735	0.495	0.735
	±0.002	±0.010	±0.005

Supplemental Table 3: Fm, Fo and Fv/Fm values of Figures 6B, C

	ctrl	W7 25 µM	W7 50 µM	W7 75 µM	W7 100 µM	W5 50 µM	W5 75 µM	W5 100 µM
Fm	0.091	0.079	0.073	0.042	0.035	0.097	0.092	0.088
	±0.011	±0.005	±0.006	±0.002	±0.003	±0.002	±0.001	±0.003
Fo	0.033	0.031	0.032	0.035	0.034	0.034	0.032	0.033
	±0.004	±0.002	±0.003	±0.001	±0.003	±0.000	±0.000	±0.001
Fv/Fm	0.639	0.605	0.563	0.157	0.055	0.652	0.646	0.631
	±0.002	±0.002	±0.008	±0.008	±0.004	±0.007	±0.001	±0.004

Supplemental Table 4: Fm, Fo and Fv/Fm values of Figures 6E, F

	Ctrl	W7 50 µM	W7 75 µM
Fm	0.075 ±0.006	0.070 ±0.004	0.052 ±0.002
Fo	0.029 ±0.002	0.029 ±0.002	0.030 ±0.000
Fv/Fm	0.614 ±0.002	0.586 ±0.009	0.441 ±0.028

Supplemental Table 5: Fm, Fo and Fv/Fm values of Figures 6H, I

	ctrl	1 µM mas	2 µM mas	2 µM maS17
Fm	0.126 ±0.005	0.076 ±0.003	0.035 ±0.002	0.158 ±0.007
Fo	0.044 ±0.002	0.038 ±0.001	0.029 ±0.002	0.052 ±0.003
Fv/Fm	0.650 ±0.003	0.501 ±0.007	0.164 ±0.027	0.671 ±0.003

Supplemental Table 6: Fm, Fo and Fv/Fm values of Figures 8B, C

	WT NH_4	WT NH_4 $+Ca^{2+}$	WT NO_3	WT NO_3 $+Ca^{2+}$	*amiRNA-cas-1* NH_4	*amiRNA-cas-1* NH_4 $+Ca^{2+}$	*amiRNA-cas-1* NO_3	*amiRNA-cas-1* NO_3 $+Ca^{2+}$	*amiRNA-cas-6* NH_4	*amiRNA-cas-6* NH_4 $+Ca^{2+}$	*amiRNA-cas-6* NO_3	*amiRNA-cas-6* NO_3 $+Ca^{2+}$
Fm	0.180 ±0.013	0.156 ±0.002	0.176 ±0.005	0.151 ±0.005	0.168 ±0.004	0.143 ±0.002	0.123 ±0.004	0.185 ±0.011	0.145 ±0.006	0.151 ±0.003	0.140 ±0.005	0.162 ±0.013
Fo	0.050 ±0.002	0.043 ±0.000	0.048 ±0.002	0.044 ±0.001	0.046 ±0.001	0.040 ±0.000	0.039 ±0.001	0.051 ±0.002	0.040 ±0.001	0.040 ±0.000	0.038 ±0.001	0.044 ±0.004
Fv/Fm	0.724 ±0.006	0.726 ±0.006	0.729 ±0.007	0.709 ±0.001	0.724 ±0.001	0.721 ±0.001	0.686 ±0.001	0.725 ±0.005	0.725 ±0.006	0.732 ±0.003	0.729 ±0.002	0.729 ±0.004

Supplemental Table 7: Fm, Fo and Fv/Fm values of Figures 9B, C

	WT NH_4	WT NH_4 $+Ca^{2+}$	WT NO_3	WT NO_3 $+Ca^{2+}$	*amiRNA-lhcsr3-2* NH_4	*amiRNA-lhcsr3-2* NH_4 $+Ca^{2+}$	*amiRNA-lhcsr3-2* NO_3	*amiRNA-lhcsr3-2* NO_3 $+Ca^{2+}$	*amiRNA-lhcsr3-3* NH_4	*amiRNA-lhcsr3-3* NH_4 $+Ca^{2+}$	*amiRNA-lhcsr3-3* NO_3	*amiRNA-lhcsr3-3* NO_3 $+Ca^{2+}$
Fm	0.123 ±0.012	0.129 ±0.007	0.149 ±0.001	0.157 ±0.001	0.145 ±0.001	0.150 ±0.008	0.131 ±0.016	0.148 ±0.000	0.187 ±0.004	0.189 ±0.000	0.155 ±0.008	0.160 ±0.003
Fo	0.042 ±0.003	0.041 ±0.001	0.049 ±0.001	0.050 ±0.000	0.047 ±0.001	0.048 ±0.003	0.066 ±0.007	0.076 ±0.000	0.057 ±0.001	0.058 ±0.000	0.063 ±0.004	0.064 ±0.002
Fv/Fm	0.657 ±0.010	0.682 ±0.006	0.673 ±0.009	0.684 ±0.002	0.679 ±0.008	0.683 ±0.001	0.494 ±0.006	0.488 ±0.001	0.693 ±0.002	0.694 ±0.000	0.597 ±0.005	0.601 ±0.004

Supplemental Table 8: Fm, Fo and Fv/Fm values of Supplemental Figure 4 online

	ctrl	0.5 µM nigericin	1.0 µM nigericin
Fm	0.086 ±0.005	0.091 ±0.003	0.084 ±0.002
Fo	0.029 ±0.001	0.033 ±0.001	0.034 ±0.001
Fv/Fm	0.665 ±0.004	0.639 ±0.005	0.597 ±0.001