



Supplemental Fig. S1

Supplemental Figure S1. Chloroplast mis-targeted Δ CpIscA-CA4-GFP does not complement CO_2 responsiveness of the *calca4* double mutant.

(A) Cartoon depicting the cloning scheme for mis-targeting CA4 to the chloroplast (Δ CpIscA-CA4-GFP). CpIscA is a chloroplast-localized protein with a chloroplast transit peptide in the N-terminal 55 amino acids (Abdel-Ghany et al., 2005). *pGC1* is a mature guard cell preferential promoter (Yang et al., 2008). (B) Confocal imaging of Δ CpIscA-CA4-GFP expression in guard cell chloroplasts. i, bright field; ii, GFP; iii, merged image of i and ii. (C-H) Time-resolved relative stomatal conductance responses in three independent chloroplast-expressing Δ CpIscA-CA4-GFP transgenic *calca4* lines, *calca4* and wild-type control plants. These data show that chloroplast mis-targeting of CA4 does not rescue the CO_2 -insensitive stomatal responses of *calca4* plants. Absolute stomatal conductance data are shown in D, F, H and the corresponding relative stomatal conductance data are shown in C, E and G. $n = 4$ plants for each genotype in panels C to H; data represent mean \pm s.e.m.

Author contributions: Experiments were conceived by H.H., W.J.R. & J.I.S. and designed by all authors. Experiments were performed and data analyzed by authors at UCSD (Figures 1, 2, 4, 5B to 5E, 6A; S1), HZAU (Figures 3, 5A, 7) and Case Western Reserve University (Figure 6B).