A

B

C

PHE345 (342)

16.7Å HIS294

GLU204

MET262 (259)

20.3Å

ASN123

2CABP Mg2+ LYS201

LYS334 ASP203

GLU60 LYS177

LYS175

24.3Å

ALA11 (8)

PHE345 (342)

16.7Å HIS294

GLU204

MET262 (259)

20.3Å

ASN123

2CABP Mg2+ LYS201

LYS334 ASP203

GLU60 LYS177

LYS175

24.3Å

ALA11 (8)

Supplementary Figure 1. The structure of L_8S_8 Rubisco. (A) Arrangement of the eight large (L, light and dark blue) and eight small (S, yellow and orange) subunits in the *Synechococcus* PCC6301 Rubisco hexadecamer viewed down the twofold and (B) fourfold axes (Protein Data Bank code 1RBL; [1]). (C) Stereo view of the positioning of the L subunit mutations Ala-8-Ser (pink), Met-259-Thr (red) and Phe-342-Ser (green) relative to the bound substrate intermediate analogue 2-carboxyarabinitol-P_2 (2CABP, orange) and key conserved residues in a CO_2-Mg^{2+} 'activated' (CO_2 carbamate moiety in grey, bound Mg^{2+} in yellow) active site that is formed at the interface between the C-terminal domain of one L (dark blue) and the N-terminal residues of its paired L (light blue). The distance from the closest carbon atom in each mutation to the Mg^{2+} is shown. The relative position of the active site to the structure in panel A is indicated by a

white circle. The mutated L residues are uniformly colored in each panel (Phe-342 is not a surface residue and is not visible in panel A or B). Residue numbering is relative to spinach Rubisco L.

1 Newman, J. and Gutteridge, S. (1993) The X-ray structure of *Synechococcus* ribulose-bisphosphate carboxylase/oxygenase-activated quaternary complex at 2.2- Á resolution. J. Biol. Chem. **268**, 25876-25886

```
                                            250              266
                                             |                |
                Synechococcus PCC6301-  RAEFAKELGMPIIMHDFLT
                Synechocystis PCC6803-           I T
          Gleobacter violaceus  PCC7421-          KT         Y
         Crocosphaera watsonii WH8501-           I T  V      I
    Trichodesmium erythraeum IMS101-              T
      Anabaena variabilis ATCC29423-       Y     NQ         Y
                Anabaena sp PCC7120-       Y     --Q         Y
          Nostoc punctiforma PCC73102-     Y     KQ         Y
              Synechococcus sp CC9902-           NQ
              Synechococcus sp CC9605-                       I
              Synechococcus sp PCC7942-
              Synechococcus sp WH8102-                       I
          Thermosynechococcus sp BP-1-          E
          (9x) Prochlorococcus marinus-         D           YI
```

Supplemental Figure 2. Cyanobacterial L subunit comparison. Amino acids residues 250 to 266 in the *Synechococcus* PCC6301 Rubisco large subunit (PCC6301, GenBank Acc No. (GB) AP008231.1) compared with cyanobacterial Rubisco sequences containing threonine (T) or glutamine (Q) at the equivalent Met-259 codon (in red). The cyanobacterial genome sequences were obtained from the NCBI (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi) and JGI (http://genome.jgi-psf.org/mic_home.html) genome databases. Dashes are included to optimize the alignment. The sequences were aligned by the Clustal V method and only residues differing from the PCC6301 sequence are shown. PCC6803, (GB: BA000022); PCC7421, (GB: BA000045); WH8501, (JGI: 400856410); IMS101, (JGI: 403238160); ATCC29423, (GB: CP000117): PCC7120, (GB: BA000019); PCC73102 (GB: NZ_AAAY02000040); CC9902, (GB: CP000097); CC9605, (GB: CP000435); PCC7942, (GB: CP000100); PCC8102, (GB: BX548020); BP-1 (GB: BA000039); *P. marinus* (9 strains, GB: CP000095 (str. NATL2A), CP000111 (str. MIT 9312), CP000551 (str. AS9601), CP000552 (str. MIT 9515), CP000553 (str. NATL1A), CP000554 (str. MIT 9303), AE017126 (str. CCMP1375), BX548174 (str. MED4) and BX548175 (str. MIT9313)).