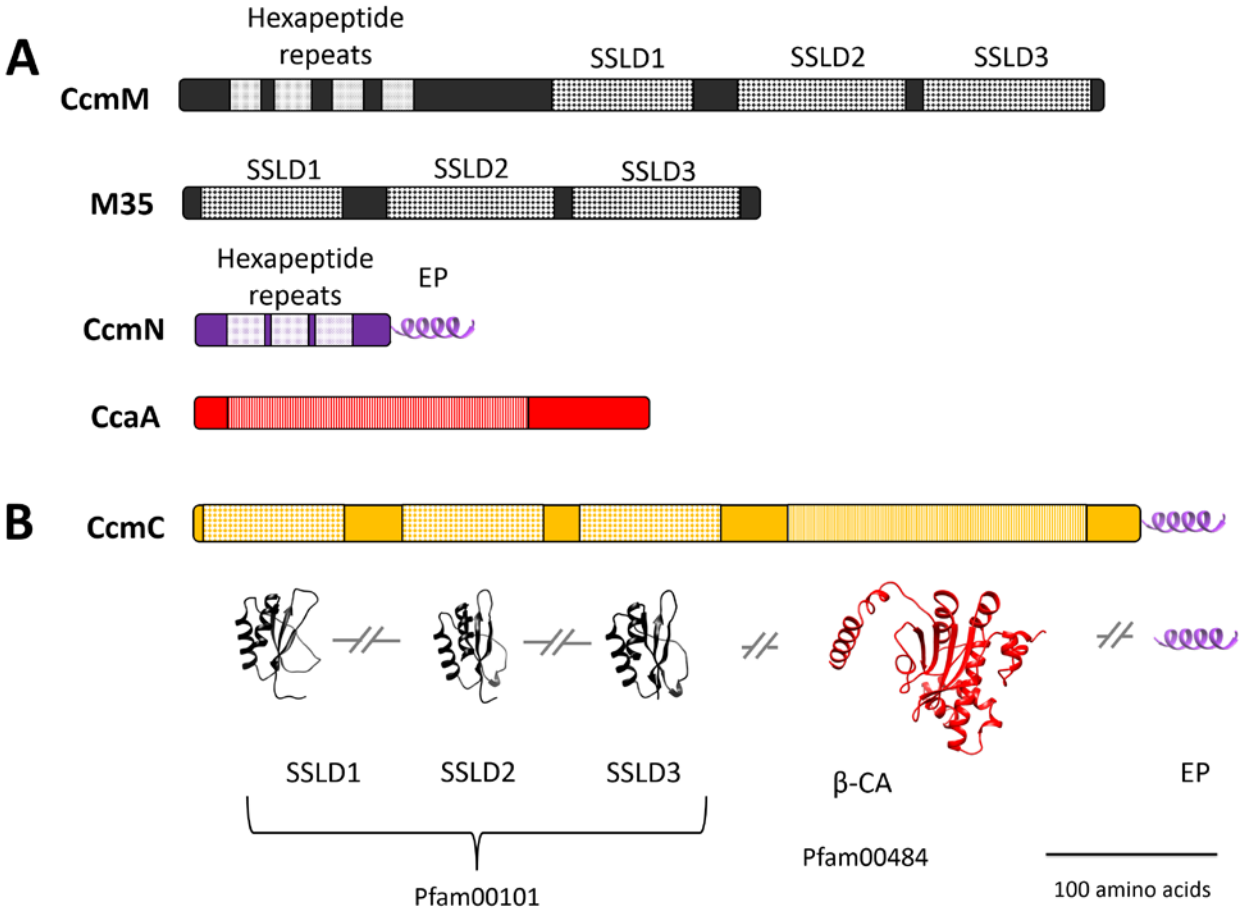
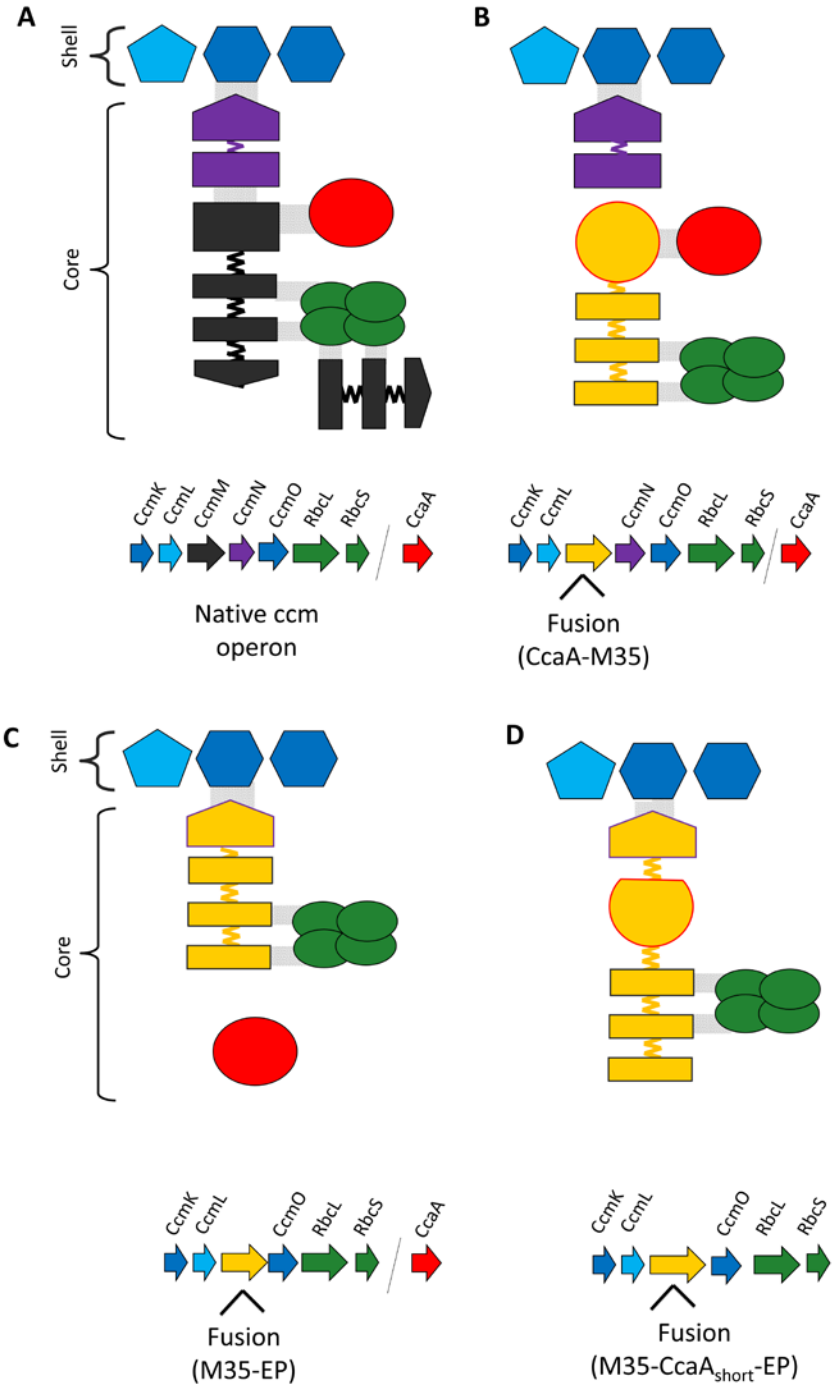
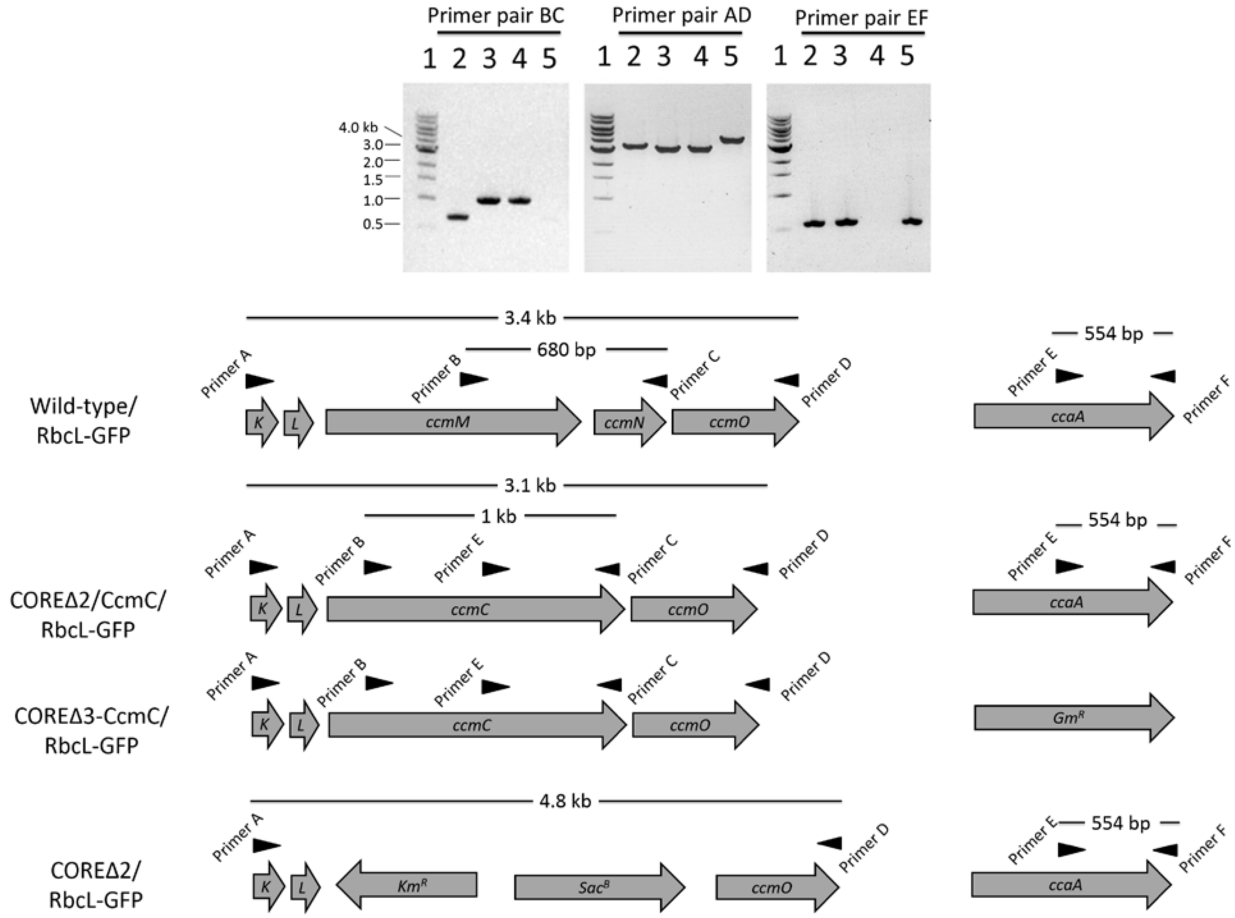


Supplemental Figure 1. Schematic of domain architectures in CcmM, CcmN, CcaA and CcmC. (A) Domain architectures of the four proteins that recruit RuBisCO and assemble the carboxysome core. (B) The architecture and (predicted) structure of the domains of the chimeric protein, CcmC. SSLD: Small subunit-like domain. EP: Encapsulation peptide. CA: Carbonic anhydrase.



Supplemental Figure 2. Schematics of native and designed chimeric carboxysome cores used in this study. (A): Native β-carboxysome core and (B): CcaA-M35 (a fusion of CcaA and 3xSSLDs). (C): M35-EP (a fusion of the EP of CcmN and 3xSSLDs). (D): CcmC (a fusion of the EP of CcmN, a shortened version of CcaA and 3xSSLDs). Colors correspond to the gene depicted on the bottom. Gray shading denotes known non-covalent domain interactions. SSLD: small subunit-like domain. EP: Encapsulation peptide.



Supplemental Figure 3. Confirmation of genomic integration. Top: DNA fragments amplified by PCR were run on 1.2% agarose gels. Bottom: Primer location and expected DNA fragment size based on the DNA template. 1: 1 kb DNA band; 2: Wild-type/RbcL-GFP; 3: COREΔ2/CcmC/RbcL-GFP; 4: COREΔ3/CcmC/RbcL-GFP; 5: COREΔ2/RbcL-GFP.

AGCCGCGGCAGTCAAGCGCGCCATGTGCGCGATTGTCAGGAACGACCGGTTGATGCAGCTGTCATTGCCA
TCATCGATACGGTCAACGTGGAAAACCGCTCCGTCTACGACAAACGCGAGCACAGCTAATGGGCAGGGAT
TGAATCCCTGCTGGTCATTGATCTGGATTGAGCCCAGGCTTGGGAGGTTAGCAT**ATGACCGTGAGCGCTT**
ATAACGGCCAAGGCCGACTCAGTTCCGAAGTCATCACCCAAGTCCGGAGTTTGCTGAACCAGGGCTATCG
GATTGGGACGGAACATGCGGACAAGCGCCGCTTCCGGACTAGCTCTTGGCAGCCCTGCGCGCCGATTCAA
AGCACGAACGAGCGCCAGGTCTTGAGCGAACTGGAAAATTGTCTGAGCGAACACGAAGGTGAATACGTTC
GCTTGCTCGGCATCGATACCAATACTCGCAGCCGTGTTTTTGAAGCCCTGATTCAACGGCCCGATGGTTC
GGTTCCTGAATCGCTGGGGAGCCAACCGGTGGCAGTCGCTTCCGGTGGTGGCCGTCAGAGCAGCTATGCC
AGCGTCAGCGGCAACCTCTCAGCAGAAGTGGTCAATAAAGTCCGCAACCTCTTAGCCCAAGGCTATCGGA
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TTCGAATGAGCGCCAGGTTCTGGCTGAACTGGAAAACTGTCTGAGCGAGCACGAAGGTGAGTACGTTCGC
CTGCTGGGCATCGACACTGCTAGCCGCAGTCGTGTTTTTGAAGCCCTGATCCAAGATCCCCAAGGACCGG
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TGGATCGAGTTCGAGCGATGTCGCTGGACAGGTTCGGGGTCTGCTAGCCCAAGGCTACCGGATCAGTGCG
GAAGTCGCCGATAAGCGTCGCTTCCAAACCAGCTCTTGGCAGAGTTTGCCGGCTCTGAGTGGCCAGAGCG
AAGCAACTGTCTTGCCTGCTTTGGAGTCAATTCTGCAAGAGCACAAGGGTAAGTATGTGCGCCTGATTGG
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GGGCATGGGTGAGCTGTTCGTCATTCGCAACGCTGGCAATCTGATCCCGCCCTTCGGTGCCGCCAACGGT
GGTGAAGGGGCATCGATCGAATACGCGATCGCAGCTTTGAACATTGAGCATGTTGTGGTCTGCGGTCACT
CGCACTGCGGTGCGATGAAAGGGCTGCTCAAGCTCAATCAGCTGCAAGAGGACATGCCGCTGGTCTATGA
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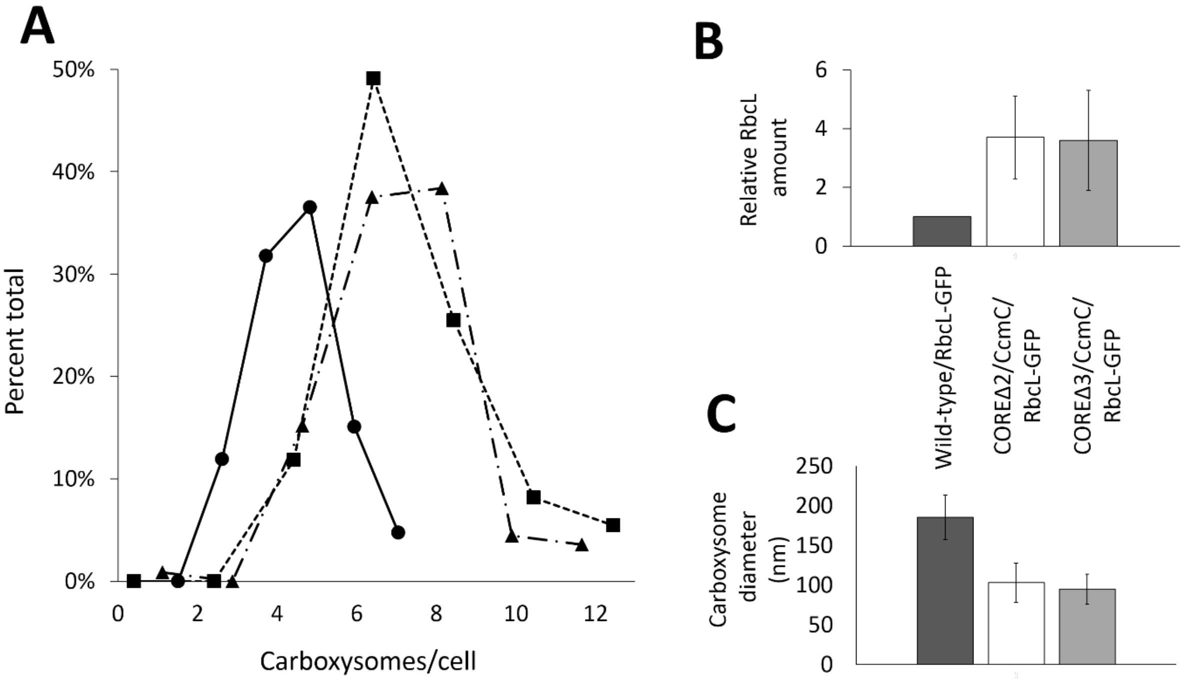
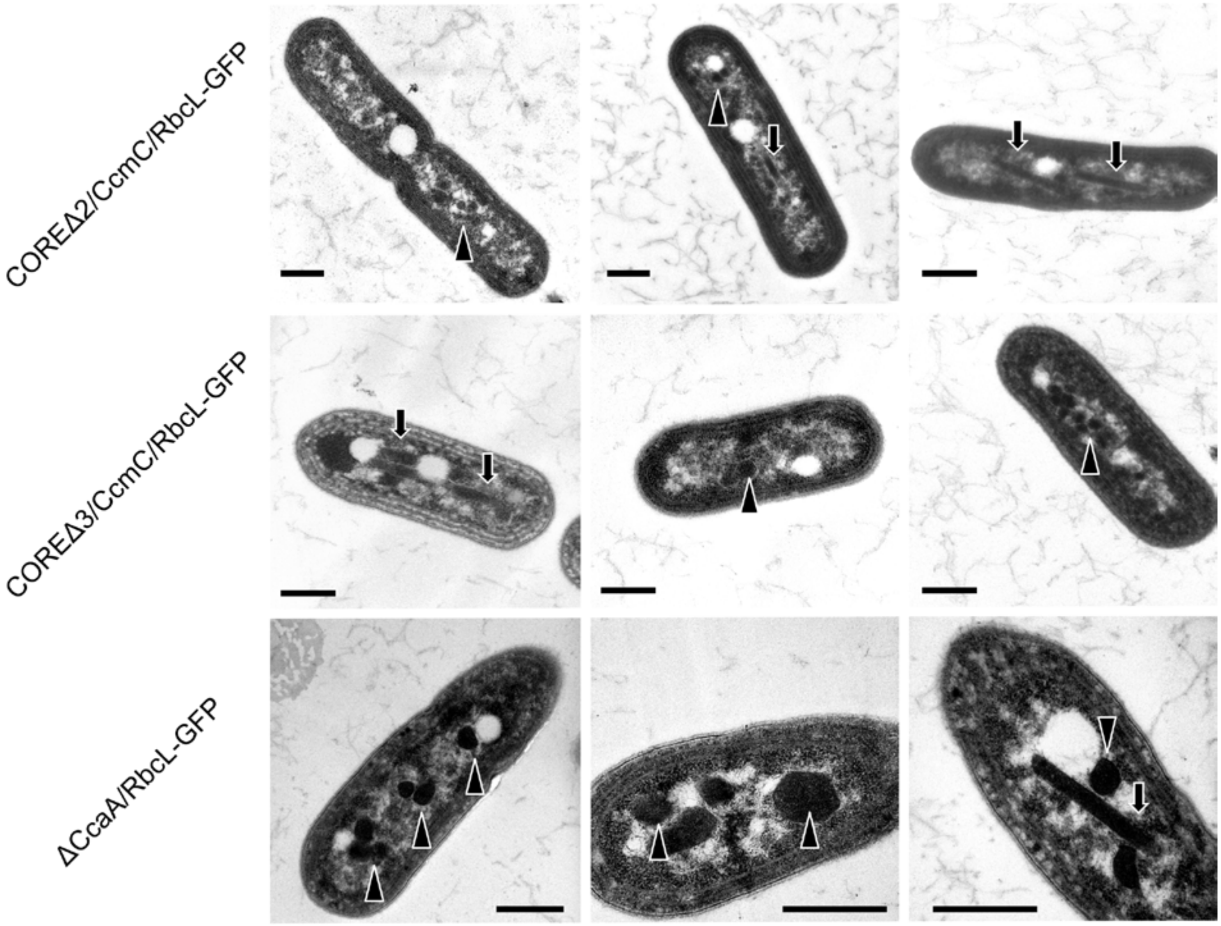
CAGTGTCTACGGCAAGGAACAGTTTTTGCGGATGCGCCAGAGCATGTTCCCCGATCGCTAAGATGTGCAC
AGCAGCTCTAGGAGCTGCAGGGTACT

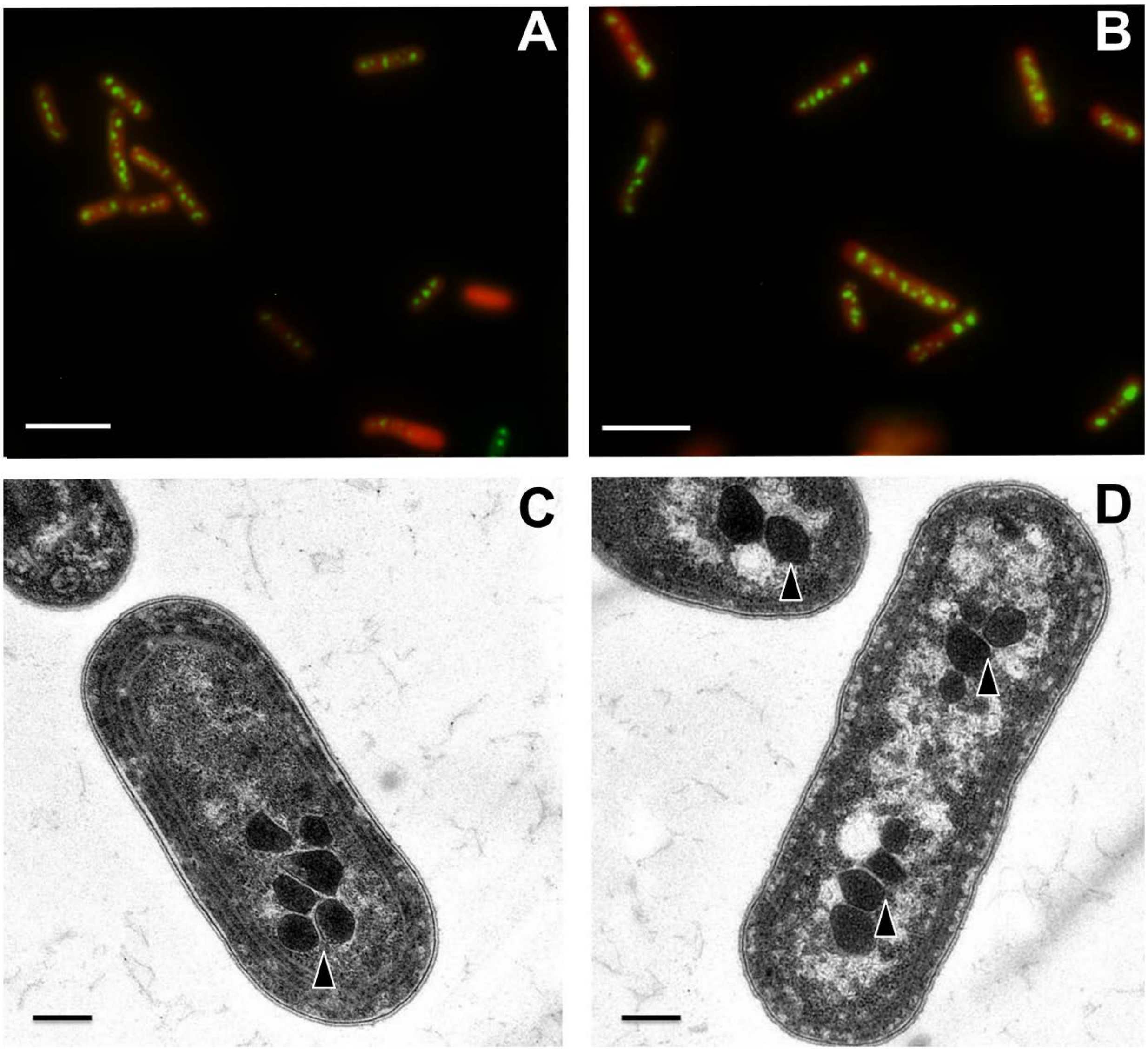
Supplemental Figure 4. DNA sequence of *ccmC* integrated into the CCM locus. Confirmation of the sequence of *ccmC* by Sanger sequencing. Blue: *ccmK2* gene; Black: Intergenic regions; Red: *ccmC* gene.



Supplemental Figure 5. Structural characterization of native and minimized carboxysomes. (A): Distribution of the number of carboxysomes per cell (n≤100). (B) Relative RbcL content in protein samples normalized to Chl *a* (n=3). (C): Carboxysome diameters measured from electron micrographs (n=50). Solid line and dark grey bar: Wild-type/RbcL-GFP; Dash-dotted line: COREΔ2/CcmC/RbcL-GFP and Dashed line and light grey bar: COREΔ3/CcmC/RbcL-GFP. Error bars= std. dev.



Supplemental Figure 6. Additional transmission electron micrographs of carboxysomal core mutants. Top panels: COREΔ2/CcmC/RbcL-GFP; Middle panels: COREΔ3/CcmC/RbcL-GFP; Bottom panels: ΔCcaA/RbcL-GFP. Cells incubated for at least 12 hours in air. Arrowhead: carboxysomes; Arrow: rod carboxysomes. Scale bar: 500 nm.



Supplemental Figure 7. Carboxysome aggregation in the ΔCcaA/RbcL-GFP mutant. Fluorescence (A and B) and transmission electron (C and D) micrographs of the hcr strain ΔCcaA/RbcL-GFP. Scale bars: 5 µm for A-B; 200 nm for C-D. Arrowhead: carboxysomes.

CcaA-M35

MRKLIEGLRHFRTSYYPSHRDLFEQFAKGQHPRVLFITCSDSRIDPNLITQSGMGELFVI
RNAGNLIPPFGAANGGEGASIEYAIAALNIEHVVVCGHSHCGAMKGLLKLNQLQEDMPLV
YDWLQHAQATRRLVLDNYSGYETDDLVEILVAENVLTQIENLKTYPIVRSRLFQGKLQIF
GWIYEVESGEVLQISRTSSDDTGIDECPVRLPGSQEKAILGRCVVPLTEEVAVAPPEPEP
VIAAVAAPPANYSSRGWLAPEQQQRIYRGNASGSVSAYNGQGRLSSEVITQVRSLLNQGY
RIGTEHADKRRFRTSSWQPCAPIQSTNERQVLSELENCLSEHEGEYVRLLGIDTNTRSRV
FEALIQRPDGSVPESLGSQPVAVASGGGRQSSYASVSGNLSAEVVNKVRNLLAQGYRIGT
EHADKRRFRTSSWQSCAPIQSSNERQVLAELENCLSEHEGEYVRLLGIDTASRSRVFEAL
IQDPQGPVGSAKAAAAPVSSATPSSHSYTSNGSSSSDVAGQVRGLLAQGYRISAEVADKR
RFQTSSWQSLPALSGQSEATVLPALESILQEHKGKYVRLIGIDPAARRRVAELLIQKP

M35-EP

MTVSAYNGQGRLSSEVITQVRSLLNQGYRIGTEHADKRRFRTSSWQPCAPIQSTNERQVL
SELENCLSEHEGEYVRLLGIDTNTRSRVFEALIQRPDGSVPESLGSQPVAVASGGGRQSS
YASVSGNLSAEVVNKVRNLLAQGYRIGTEHADKRRFRTSSWQSCAPIQSSNERQVLAELE
NCLSEHEGEYVRLLGIDTASRSRVFEALIQDPQGPVGSAKAAAAPVSSATPSSHSYTSNG
SSSSDVAGQVRGLLAQGYRISAEVADKRRFQTSSWQSLPALSGQSEATVLPALESILQEH
KGKYVRLIGIDPAARRRVAELLIQKPGSGGSVYGKEQFLRMRQSMFPDR

CcmC

MTVSAYNGQGRLSSEVITQVRSLLNQGYRIGTEHADKRRFRTSSWQPCAPIQSTNERQVL
SELENCLSEHEGEYVRLLGIDTNTRSRVFEALIQRPDGSVPESLGSQPVAVASGGGRQSS
YASVSGNLSAEVVNKVRNLLAQGYRIGTEHADKRRFRTSSWQSCAPIQSSNERQVLAELE
NCLSEHEGEYVRLLGIDTASRSRVFEALIQDPQGPVGSAKAAAAPVSSATPSSHSYTSNG
SSSSDVAGQVRGLLAQGYRISAEVADKRRFQTSSWQSLPALSGQSEATVLPALESILQEH
KGKYVRLIGIDPAARRRVAELLIQKPGSRKLIEGLRHFRTSYYPSHRDLFEQFAKGQHPR
VLFITCSDSRIDPNLITQSGMGELFVIRNAGNLIPPFGAANGGEGASIEYAIAALNIEHV
VVCGHSHCGAMKGLLKLNQLQEDMPLVYDWLQHAQATRRLVLDNYSGYETDDLVEILVAE
NVLTQIENLKTYPIVRSRLFQGKLQIFGWIYEVESGEVLQISRTSSDDTGIDECPVRLPG
SQEKAILGRCVVPLTEEVAVAPPEPEPVIAAVAAPPANYSSRGWLGSGGSVYGKEQFLRM
RQSMFPDR

Supplemental Figure 8. Amino acid sequences of the chimeric proteins. CcaA-M35 and M35-EP correspond to proteins that failed to rescue the high CO_2-requiring phenotype. CcmC forms a functional streamlined carboxysomes core. Color of the letters correspond to: black: SSLDs, grey: linkers, red: carbonic anhydrase, purple: encapsulation peptide.

Supplemental Table 1. Primers used in this study

Primer ID	Purpose	Sequence
pUC19 speI fwd	**CcmM-N deletion**	**GGTGCACTACTAGTACAATCTGC**
pUC19 speI rv	**CcmM-N deletion**	**GTGAAATACCGCACTAGTGCGTAAG**
FR left (ccmL-O) fwd	**CcmM-N deletion**	**CTTTCATCTTGAATTCCGACTCTTTAGG**
FR left (ccmL-O) rv	**CcmM-N deletion**	**GCTCGGCATATGCTAACCTC**
FR right (ccmL-O) fwd	**CcmM-N deletion**	**GGGAGGTTAGCATATGCTCTAGAAGCTGCAGG**
FR right (ccmL-O) rv	**CcmM-N deletion**	**CTACTGAGTCCGAAGCTTTCAGC**
Km^R/SacB fwd	**CcmM-N deletion**	**GAATTATAACCATATGCATCCTAGG**
Km^R/SacB rv	**CcmM-N deletion**	**TCCCGTCTAGACAGCGTAATG**
CcaA ndeI fwd	***ccmC, ccaA-M35***	**GAGTATCACTCATATGCGCAAGC**
CcaA bamHI rv	***ccaA-M35***	**CTTCGGGATCCGCTAGCATTG**
SSLDs-CcmN bglII fwd-	***ccaA-M35***	**TAGCGAGGCAAGATCTGTGAGC**
SSLDs-CcmN xhoI rv-	***ccaA-M35***	**CCTGCAGCTTCTAGAGCTGCTGTG**
CcaA$_{(short)}$ bamHI rv	***ccmC***	**GTTGTTGTTCGGATCCCAACCAAC**
EP bglII fwd	***ccmC, M35-EP***	**CCCAGATCTGGAGGCAGTGTCTACGGCAAGGAAC**
EP NcoI rv	***ccmC, M35-EP***	**CGTGGCCATGGCTTCTTGGGAGAGC**
ccaA$_{(short)}$ bglII fwd	***ccmC***	**GCCCTTGTCAGATCTCGCAAGCTCATCG**
SSLDs ndeI fwd	***ccmC, M35-EP***	**CTAGCGAGCATATGACCGTGAGCGC**
SSLDs bamHI rv	***ccmC, M35-EP***	**CAGGATCCTCCCGGCTTTTGTTAGAGC**
FR left (ccaA) notI fwd	**CcaA deletion**	**CAGCGGCCGCGCCTAGTGC**
FR left (ccaA) xhoI rv	**CcaA deletion**	**GCTTGCGCATCTCGAGTGATACTCGGGAC**
FR right (ccaA) xbaI fwd	**CcaA deletion**	**GCGGCAATTCTAGATAGGATCGAAGCATC**
FR right (ccaA) ncoI rv	**CcaA deletion**	**TACCCATGGACTCAAGCGCTCATTGCCAG**
Gm^R xhoI fwd	**CcaA deletion**	**GGTACCGAGCTCGAGTTGACATAAGC**
Gm^R xbaI rv	**CcaA deletion**	**TCCGCGGCTCTAGAGCCGATC**
Primer A	**Screening**	**TGCCTATTGCGGTTGGAATG**
Primer B	**Screening**	**AATCATGATGCACGCCCTTG**
Primer C	**Screening**	**AATCATGATGCACGCCCTTG**
Primer D	**Screening**	**TTAGCCGATTTGAGCATGGC**
Primer E	**Screening**	**CAGCTTTGAACATTGAGCATGTTGTG**
Primer F	**Screening**	**ATTGCCGCGATAAATCCGCTG**