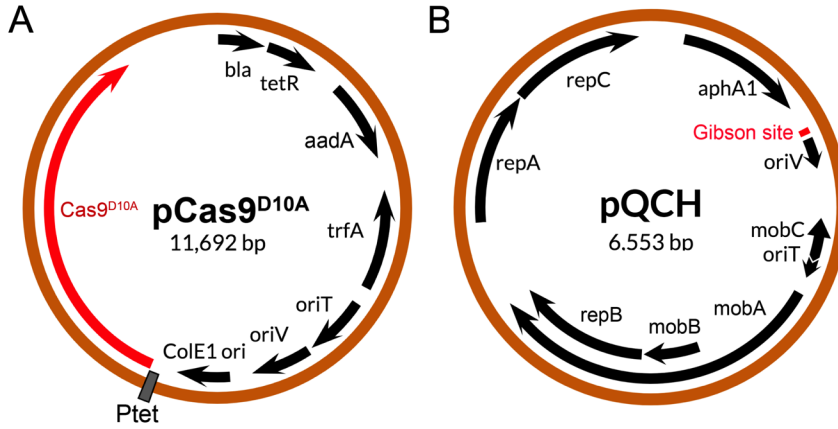


1 Supplemental Materials: Tapscott, *et al.*

2

3 Supplemental Figures



4

5 **Figure S1. Development of CRISPR/Cas9 genetic tools**

6 (A) Plasmid map of pCAH01::Cas9^{D10A} (pCas9^{D10A}). (B) Plasmid map of pQCH.

7

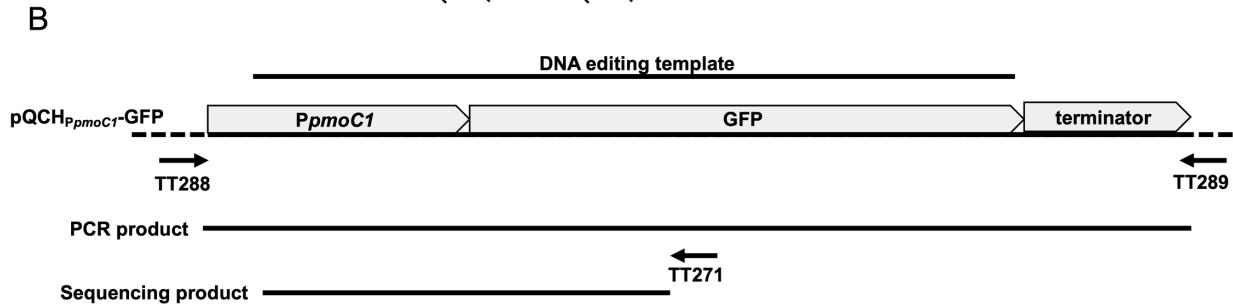
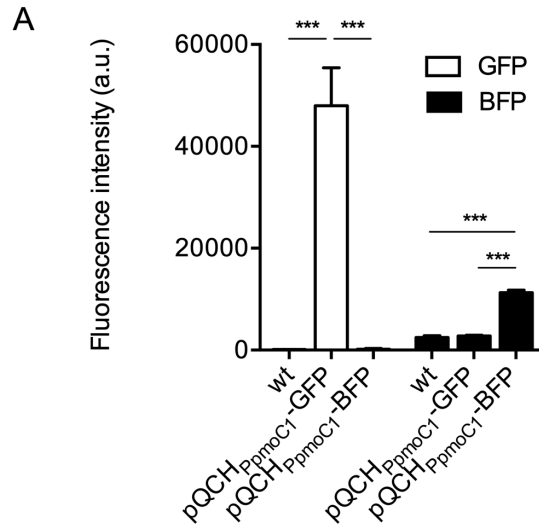
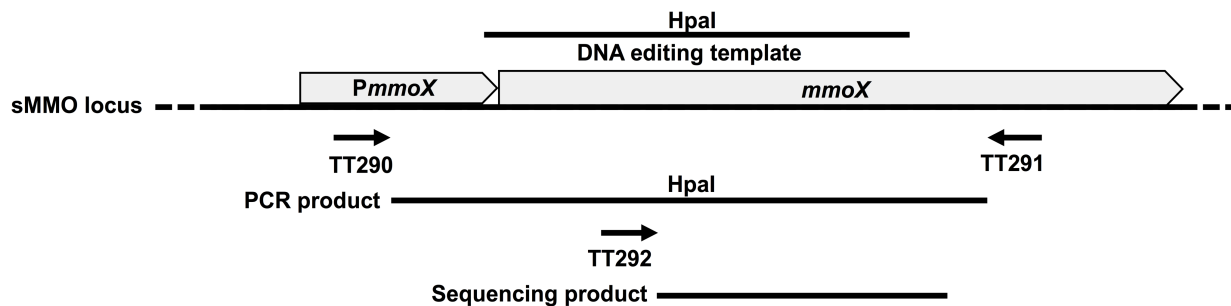


Figure S2. GFP to BFP editing

(A) Fluorescence intensity of *M. capsulatus* cells expressing pQCH::P_{pmoC1}-GFP and pQCH::P_{pmoC1}-BFP. Data depict mean arbitrary units (a.u.) ± S.D. from at least 3 unique CFUs (B) Schematic of pQCH::P_{pmoC1}-GFP locus that was targeted for gene editing. DNA editing template spanned 1 kb. For verification of genomic edits, primers TT288 and TT289 were used for genomic cPCR amplification and primer TT271 was used for Sanger sequencing. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. not significant.



18

19 **Figure S3. sMMO *mmoX* editing**

20 Schematic of soluble methane monooxygenase (sMMO) hydroxylase component *mmoX*
 21 that was targeted for gene editing. DNA editing template spanned 1 kb
 22 of *mmoX* containing *HpaI* endonuclease recognition site and nonsense codon
 23 substitution p.C151X. For verification of genomic edits, primers TT290 and TT291 were
 24 used for genomic PCR amplification and primer TT292 was used for Sanger sequencing.

25 **Supplemental Tables**

26 **Table S1. Media composition**

Component	NMS	Mating
KNO ₃	1 g/L	1 g/L
MgSO ₄ x 7H ₂ O	0.2 g/L	0.2 g/L
CaCl ₂ x 2H ₂ O	0.02 g/L	0.02 g/L
NaCl		2 g/L
Trace solution (1000x)	1 ml/L	1 ml/L
Nutrient Broth		1.2 g/L
Agar	15 g/L	15 g/L
Phosphate solution (pH 6.8)	50 m/L	50 m/L
Phosphate solution (pH 6.8)	g/L	
KH ₂ PO ₄	5.44	
Na ₂ HPO ₄	5.68	
Trace mineral solution (1000x)	g/L	
Na ₂ EDTA	5	
FeSO ₄ x 7 H ₂ O	2	
ZnSO ₄ x7 H ₂ O	0.3	
MnCl ₂ x 4 H ₂ O	0.03	
CoCl ₂ x 6 H ₂ O	0.2	
CuSO ₄ x 5 H ₂ O	1.2	
Na ₂ O ₄ W x 2H ₂ O	0.3	
NiCl ₂ x 6 H ₂ O	0.05	
Na ₂ MoO ₄ x 2 H ₂ O	0.05	
H ₃ BO ₃	0.03	

27

28

Table S2. Gene fragments

Name / Sequence	Source
29 gRNA-GFP	IDT gBlock
30 GAGGTT CAGGCGAAACCGCAGACTCAAGGGCGCTTGCTCCCGGAAAGATCGTATTAGTTTGCCTCGATCGGCGGTCCTT 31 GTGACAGGGAGATATTCCCGACGGATCCGGGGCATTTCGAGCGGAACCGCCCGCGTGGGAGTTTTTCCAGCGAGCATTCCG 32 AGAGTTTTTCAAGGCGGCTTCGAGGGGTATTCGGTAACGCCGCCGACATGATCTGTCCAGAATCTCCGCCGCTGTTCG 33 TAGAGCGCCGATGCAGGGTCGGCATCAATCATTCTTGGAGGAGACACGGAAAAGCATTGAACACCATGTTTTAGAGCTAG 34 AAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCCGGTGCTTTTTTTTGTCCGGGAAG 35 ATGCGTGATCTGATCC	
36 gRNA-GFP & GFP ^{BFP} editing template	PCR/Gibson
37 GAGGTT CAGGCGAAACCGCAGACTCAAGGGCGCTTGCTCCCGGAAAGATCGTATTAGTTTGCCTCGATCGGCGGTCCTT 38 GTGACAGGGAGATATTCCCGACGGATCCGGGGCATTTCGAGCGGAACCGCCCGCGTGGGAGTTTTTCCAGCGAGCATTCCG 39 AGAGTTTTTCAAGGCGGCTTCGAGGGGTATTCGGTAACGCCGCCGACATGATCTGTCCAGAATCTCCGCCGCTGTTCG 40 TAGAGCGCCGATGCAGGGTCGGCATCAATCATTCTTGGAGGAGACACGGAAAAGCATTGAACACCATGTTTTAGAGCTAG 41 AAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCCGGTGCTTTTTTTTGTCCGGGAAG 42 ATGCGTGATCTGATCCTTCGGACCGACGGATTGGACCGACGGATTTTATGCAAGCATGTCGCTTTGACCAAGCCGGGATT 43 CCATGGAAGGGATGTCATCGGGAGAGTTATTTATGTCGTTGATTATAAGAACTACCCCTGCGTCAAAATGTCGAGAT 44 TTTTCTTGACAGTTTGGGGGAGGGTGATAGACTCCCTCCACCGATGGACCGGTACCGCCTCTGTTCGGGGTCCATGAAA 45 TGCCCGTTAGAGGCAGAACCGATAGGGAATTAGAGAAGCGGGCGTCGGCGCGAATGCGGGCCCTGTCAACCATCACTT 46 TAGGAGGAACAAACAATGAGCAAAGGAGAAGAACCTTTCACCTGGAGTTGTCCCAATTCTTGTGAATTAGATGGTGTATGT 47 TAATGGGCACAAATTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACCTACCCCTTAAATTTATTTGCA 48 CTACTGGAACCTACCTGTTCCATGGCCAACACTTGTCACTACTCTGAGCCATGGGGTTCAATGCTTTTCCGTTATCCG 49 GATCACATGAAACGGCATGACTTTTCAAGAGTGCCATGCCGAAGGTTATGTACAGGAACGCACTATATCTTCAAAGA 50 TGACGGGACCTACAAGACGCGTCTGAAAGTCAAGTTTGAAGGTGATAACCTTGTTAATCGTATCGAGTTAAAAGGTATTG 51 ATTTTAAAGAAGATGGAACATCTCGGACACAACTCGAGTACAACCTTAACTCACACAATGTATACATCACGGCAGAC 52 AACAAAAGAATGGAATCAAAGCTAAGTTCAAATTCGCCACACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATT 53 TCAACAAAATACTCCAATTGGCGATGGCCCTGTCTTTTACCAGACAACCATTACCTGTGACACAATCTGTCTTTTCGA 54 AAGATCCCAACGAAAAGCGTGACCACATGGTCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCA 55	
56 gRNA- <i>mmoX</i>	IDT gBlock
57 GAGGTT CAGGCGAAACCGCAGACTCAAGGGCGCTTGCTCCCGGAAAGATCGTATTAGTTTGCCTCGATCGGCGGTCCTT 58 GTGACAGGGAGATATTCCCGACGGATCCGGGGCATTTCGAGCGGAACCGCCCGCGTGGGAGTTTTTCCAGCGAGCATTCCG 59 AGAGTTTTTCAAGGCGGCTTCGAGGGGTATTCGGTAACGCCGCCGACATGATCTGTCCAGAATCTCCGCCGCTGTTCG 60 TAGAGCGCCGATGCAGGGTCGGCATCAATCATTCTTGGAGGAGACACGATACGCCATCGGACATAAGTTTTAGAGCTAG 61 AAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCCGGTGCTTTTTTTTGTCCGGGAAG 62 ATGCGTGATCTGATCC 63	
64 gRNA- <i>mmoX</i> & <i>mmoX</i> ^{TAA} editing template	IDT gBlock
65 TCCAATTCGCCCTATAGTGAGAGGTT CAGGCGAAACCGCAGACTCAAGGGCGCTTGCTCCCGGAAAGATCGTATTAGTT 66 TGCTCGATCGGCGGTCCTTGTGACAGGGAGATATTCCCGACGGATCCGGGGCATTTCGAGCGGAACCGCCCGCGTGGGA 67 GTTTTTCCAGCGAGCATTTCGAGAGTTTTTCAAGGCGGCTTCGAGGGGTATTCGGTAACGCCGCCGACATGATCTGTCCC 68 AGAATCTCCCGCGCTGTTTCGTAGAGCGCCGATGCAGGGTCGGCATCAATCATTCTTGGAGGAGACACCGGCTATCTGGCC 69 CAGGTGTGTTTTAGAGCTAGAAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCCG 70 TGCTTTTTTTGTCCGGGAAGATGCGTGATCTGATCCGTCATCGGGCATTTCATCATTCATAGAAATGTGTTACGGAGGAAAC 71 AAGTAATGGCACTTAGCACCACCAAGGCCGACGACGCGCTGGCTGCCAATCGGGCACCACCAGCGTGAATGCA 72 CAGGAAGTGCACCGTTGGCTCCAGAGCTTCAACTGGGATTTCAAGAACAACCGGACCAAGTACGCCACCAAGTACAAGAT 73 GCGGAACGAGACCAAGGAACAGTTCAAGCTGATCGCAAGGAATATGCGCGCATGGAGGCAGTCAAGGACGAAAGGCAGT 74 TCGGTAGCCTGCAGGATGCGTGACCCGCTCAACGCGGTGTTCCGCTTCATCCGAAGTGGACGAGACCATGAAAGTG 75 GTTTCGAACTTCTGGAAGTGGCGAATAACAACGCCATCGCCGCTACCGGGATGCTGTGGGATTCGCCACGCGCGGA 76 ACAGAAGAACGGCTATCTGGCCAGGTGTTTAAATGAAATCCGCCACACCCACCGTTAACCCTACGTCAACTACTACTTCG 77 CGAAGAACGGCCAGGACCCGGCCGGTCAACGATGCTCGCCGACCCGTTACCATCGGTCCGCTGTGGAAGGGCATGAAG 78 CGCGTGTTCGACGGCTTCAATTCGGCGACGCGGTGAAATGCTCCCTCAACCTGCAGCTGGTGGGTGAGGCTGCTT 79 CACCAATCCGCTGATCGTCGAGTGACCGAATGGGTGCGCCACGCGCATGAAATCACCCGACGGTGTTCCTGTGCA 80 TCGAGACCGAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAGTCAAG 81 TATCTCAACACGGACCTGAACAACGCCTTCTGGACCCAGCAGAACTTACGCGCGGTGTGGGCATGCTGTTCGAGTA 82 TGGCTCCAAGTTCAAGTTCGAGCCGTGGGTCAAGACGTGGAACCGCTGGGTGTACGAGGACTGGGGCGGCATCTGGTAGC 83 CGCTTATGTCTATTGC	