Supplemental Figure S2

Sequence of the chimeric gene *ssg:yfp* as it is present in the vector pSSG-YFP

GAATTCACATGGCGAAGTATAAGCATTGGATGGGGCTGGCGGCCGAGCAAGCGGGCAGGAAGGAGGTG — ***Eco*RI**
CTCCCGCACATCACAATGTTTATCCCACGTTCGGCGAATTGCAAGCTTATGCAGTTTAGGCTTTGCTG
TTGGGCACTGGCGGCTAACAGAGCGCGTAAGGGACGGCTTTATATACCTAGGGAGGAGCGAGTATGCC
CCTGCTGCGGCACTGATAGCCACATCGAGGATGAGAAACACATTTTGCTGGACTGTCCGGTTTACGAC
GGAATCAGATCTGATATGGGCTTTCCACGCTTCGAACATATGAAGGACGCTATGAAGTGGGAACCTGG
ACGCCTGGCAAGAATGCTGGAACGTATATGGTCTCACCGCAACTCTATCGTTCCCTTTGGTCGTTAGT
ATACTTCATTACTTGTTTCGTTGGAAGTGCGAGGTAGGATGGTGCTTATAGGCGATTTGGCAGCCCTT
GAGCCCTCGGCTCGTACTAGGGTGGTTTGCAGGTACGGGGAACGCACCCACGACCATGTAACAACAAC
AACAATACATACGTGCCGTCTTCCCTGAAGCATATTTTCCCCCACGATGAATCGAATTATGACAAAGT
CAATATCAGGTGAACCATACGAACGGCGCCTCTTCGTGATGGGGCGACGCAGTGACTTGATCCGCCGA
GGGAAACGCCACCCCATAGAACGCCTGCAGAAGCTACAGCACGGCGGGCCGTAAAATGGAGTGTGGTT
TTGGTCCCCGACAACGATTACATTGACATGCAAGCAGCGGGTTGATTGCAAAGCTCAAGTTCATGCGA
CATCATCATTTGTATTTGACGCACATCGTGGCCGTTTTCCCCTCCTGAGGAATGAGAACGATCAGGAA
GCAAGCACGAAACTGACGCTGACTGTTGTTGCTTGCGGGTGCGAGTCAGCAACACATGCAACCCCAGT
TCAAGGGTCCAATTATGAATGCGGGGATGGGGTGGTAGGCGCGGCAGCGAGTATGCGCGTATCGGCGC
CCGGCTGGGCATCAGCCTGTTTGAGACGCCGTGCCATGCCGCTCCGCGCGGGTGCTTCGTTGGTCCAT
ACGTCTCGACTGGAATGTGTGTAGTCACAACTTCTGCCACATTCGATCTCCTTGGTCTTTGTAACACT
TTTGGGCGAGCAAATTTTAATCACTTTTTGTTGCCAAAGCGAAGGTATGGACTGGGCTTTCTAAGTCC
CGTTTCAAAAGCACATCTTTCGGAAGAAAAGGATACGAAAAGTTTTCATTTACGGCCGCATGTGCTAT
GTGGGTAGATTTTGCAGTGGCAGTTCCGCAATTCGACAACATGCCTTAGAAAATAAAATTAAGCTCCA
AGCCCAGGGCCGAAATAGAATACTGTCTATGTCTCGTGCAATCACCGGCAATTGAAGGAAGCAAGAAG
CTATTGAAATTGACCCTGAGATTCCTTTGAACGACTTATTCTCGATTGTTACGGCAGTCGCCCACGTG
AAACTGCCGCCGTTCATCAGCAGTCAGGGCAAGCTAGCGTGAAAAACAGTAACGCGCCGTATCAGTAG
TACATGTTTGAACGGCGTTTTGCTTGGATAAAATTATGAAGTTTTCTTTTGGCAAGCAGACCTTTGGT
GCATCACCCTTCTTTATAAGCAATTCATCGCTTGCAACGCACATACTAGCCAACTTCTTTTAGCGATA
TACTTATTGGTATTGTTCTAGTACTTTCTATGAACGTCGACTGTGTCCAATTGTGTTCAACTAGGATT
AAGCGTGCCACGTCAGCAGATAGACTGCTAGTACTGCTGTTCTCCCGTGTATGACTACGGTACTCTGA
AAAGGCGTTAACGCCTCATATTAACGTAAAGGTAACGTA**GATATC**AGTTGCTAAAATTACCTAGCATT — ***Eco*RV**
AGATTATGTTGCAAAACGGATCAATACGTATCGGATAGGGATGTGGCGAAATTGCACGACGCTCGGAA
TGAAAGGTAGAATGCGTAAATGTTCTTACCTTTCCCTAGACTGCGAAGAAACAAGCAAATGATGACTT
GATCTATTTATTTTATCCAAAGGCGCCAATATCCGACGTCACATCAACCGAGCACTCCATCCAAGTTA
GCCATTGAAGCAGGAGCAGCAGCACAACAACGTAATCACCTATGAGGATATAAGTCTCATCATATTTT
AAACTATATTATACTGCACTCAGGATGACTGCCGGAAGGGAAGTGAACCGCCGGCAATCTTTACGAAG
TAGCTGGCCAAATCCATAAGCCTCATGAAATTATTAAATCAAATTAACTCTGATTAAGTTTAACTTCA
TCGCGGATGTCACCGCGTACGTTGGACTTGTCGTTACCCATCAGCATTGCTTACTGTTAACTTGGCGG
AACGTTACAATCACATCCCAGGAGGCCACCATCCTCAATATACTCTATTTTATGGTGTTTTAAGCACA
TTAGTTGGCACGCTCGCCTTCTTTCCCGCGCTCGCGCCCACCGCTCTGTACACCGTCTGGACATGCCG
TACATTATTCGCCAGGGAGGGCGCGTTGCAAGCCGTCATAGAAGGATCGGGTTCTGCGATTGCATAAT
CGTAGCTGCACCCTTACAGGCTGAAGAGTTACGGCCTCGTGGGTTGCATAATTGGAATCCCTTTTTCA
CGAAAAGGCGTGGAGTGGTCCGTTATTCCATTTCTGTCACGA**CTCGAG**ACTTGGCTACGTAAGTCTGC — ***Xho*I**
ATGTGATGGATTCACGCGACATCGCTAGATTGTTTGATGTTGCATAATTGGCCAACATCTCAGCATTT
TAGCTGGAGAGGGTAGCTGGCAGACGCTTATCATGTCGCGGTTCCTTCCGTGCGCAGTGGTGCTCACC
GGGACCAATTTTATACGCAAAG**ATG**TCTAAGCTGCTGCTCGTGGCGCTTTTTGGCGCCATAGCAGTGG — **start**
TTGCTACGAGTGCTGGTGAGTGCGCTGCCTCCTTCGACCCTTACGATGCAACGTAAATTTTCGCTACC
TGAGTTAACCTGCTTCTTTCTGCCTATTCTTTTGCTTATAACAGAGGTACTCAACCTCAACGGAAGGT
AAGACGCGATTGCTTAAGATGTCGTTCCTCATCGTGAGTTACTACTGAAAGATGTGGTTTTGCAAGTG
CCATCGCATGCGCTAAGTTTTTGATGTTAGATGTCGGAGCCAAAATTTACATCGTTTTATTCTTTGTC
GGCAGGTCTCTTTTGAACAACGACGACCCGAACGCCTTCCCTTACTGCAAATGTACTTATCGTCAGCG
CCGGAGCCCATACCGCCTGAAGTATGTTGGCGCCGAGAACAACTACAAGGGCAATGACTGGCTGTGCT
ACAGCATTGTCCTTGACACCACGGGCACTGTTTGCCAGACAGTTCCGTTGACGGAGCCTTGCTGCAGC
GCTGACCTGTACAAGATTGAGTTTGACGTCAGTAAGTGGTGTGATCCAGGCACCCTTCTGTTCTTACC

CAGGCATGTCTGTAGGCACCTTGCCTGGAAAGGCAATTTCAATCTTGCGACACCCTGTCTGGCTCTTC
GTAGCTATGAGTTGGCATTCAATTCCTTCCATCGGCCAAGTAGCCCTTGCCTTATCTCTCTATCCAGT
CAACATCCTTGCGGCCTAAAGTAAGCTCATGGTCCCAGGCCTTGTGGCCCACTGTTACACAGCCTTAC
TAACTGACGCACGTTGTTCCCTTGCCATCCTTTTCCATGGCGCACGTTACAGAACCCTCCTGCAAGGG
CACTGTTACACGTGCTATGGTGTTCAAGGGCATTGACAGGACTGTTGGCGGCGTACGTGTGTTGGAGT
CCATCAGCACCGTCGGCATTGATGACGTCACCGGTGTTCCGGGCGCGGCCATCCTTCGCATCGTGAAG
GACCTTGCCCTCCCGTACAGCGTCGTGGCTTCCTTCTTGCCTAGTAAGTTGGGACTAAACTCAAATTT
TCGTTCTTGCCCAGCAGCTTGATGATTTTCTTCGTGGGGGAAGCGTCACGGTTTGCGATCGATGCCGA
TGTATAAAATGTTTTTTTTGTCTATCTCGCTTTTTAGCCTACGCTTTCTTAATCAGTTTTTTTGGGTC
TTCAGTGTTGTTTTGTGGTCCGTCCCGGCATGTGGACGTTCGTGCCGAAGTTATCTTACACCGAGGGA
TGTGAGGGATGTAAGAAAGGATGTTTGTACCAGACGAATCGTCCACTTCCCATCCAGAGCGCATACAG
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TCGCGTCGGACAAGTTAAGTCTTTTGCACCATAACGAACTTTCCAGCCGTTTAGATGTCAAATAGATA
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TACCTGCCTCCCTACCTGCCTCCCTACCTGCCTCCCAACCTCCCTCCCTACCTGCCTCCCTCCCTGCC
CATATCCTTGCAGATGGCTTGCCGGTCTGCATCAACAGGGTGCCCGGCTCGTGCACGTTCCCGGAACT
GTTCATGGACGTTAACGGCACTGCCTCGTACTCGGTCTTCAACTCTGATAAGGATTGCTGCCCAACCG
GCCTTTCCGGCCCCAATGTCAATCCAATTGGCCCGGCACCCAACAACAGCCCGTTGCCACCAAGCCCG
CAGCCAACAGCAAGCTCGCGGCCACCAAGCCCGCCGCCCAGCCC**GCGGCCGC**CAAGCCCGCCGCCTCC
CTCG**CCCAGCCCGCCGCCCCCACCGCCGCCGCCCCCTCCACCCCCACCGCCGCCCCCCCCAAGCCCCC**
CACCGCCGCCACCGCCTCCCCCACCTCCTCCTCCACCACCACCGCCACCCTCTCCCTCCCCGCCACGC
AAGCCACCGTCCCCATCCCCACCTGTTCCCCCACCGCCATCACCACCGAGCGTCTTGCCCGCTGCTAC
CGGCTTCCCCTTCTGCGAGTGAGTGGCAACGGCTTCCCTTCTCTCTCCACTTTTAAACGAACGTTCAA
ACTGTCAAAACGCTACACCAGTACGAAGGATACACAACTGCCGTATCATGGGCAAAGTATTTGTCTTC
AGGTCCATATGTGGTTTCGATACGTCGCTTAGTGCTTGACTATGCTATGCATGTTTAGCTGCGCAGTG
TCGTGCTGTGCTACATTCAGTCATCCAGCGCTGCACAGTGTTGTGCATGATTGACAAAAATACCCCCT
CGGCCATGCGCACCTTGCCCCGTCCAGCCCACGTCTACTTCCTTCAGATGTTCTGTGTACACCAACCC
CCTGCTCGATCACAGAAATATCACAGCACGAACTGGACTGTTCCTTGCTTTCAGCATGTGCGCGCGCG
CGCCTGTGTGTGCATATGTGTATATGTGTGTCGAAATGTGGCGGCAATATCGCTTTTTCTCTATTCCG
AGGGAACAGCATCTGCCAAGGAGCACCCCTGTGTCTCATTTTCGCAAGTGTCCTTGTAACCCGTCCCA
ACCCAAACCCACTCCGCGTCCCCTCATGGACCCCCCCCCACGCGCCATGGGCCCCTGGCCACATCCTG
ATACAACCAAAGCTTGCCCTCTTGAGGTGCTATATGACCTGCTTTTCACTGACCCCCTACAACCCCAA
AACTCACATCCTACGCGCCCTCCTCTTTTTCCCCCGCGCCCAGGTGCGTGTCCCGGTCCCCTTCCAGC
TACCCATGGCGCGTGACCGTCGCCAACGTTTCCGCGGTCACCATCTCTGGCGGCGCTGGCGAGCGCGT
GTGCCTGAAGATTTCGGTCGACAAC**GCGGCCGC**TGCCACCTGCAACAACGGCCTGGGCGGGTGCTGCT
CGGATGGACTGGAGAAGGTC**GAGCTC**TTTGCTAGTGAGTACCTCAGCGTTGCGGCGGCAATTTGTGTG
AATGTGTGTTTGTGTGTGTGTGGGGGGGGGGAATGGGGGGTGGGGGTGTAATGGCGGCGCGGGAGATT
GGGATCACGGGACTGACACTACCATCAGATGGCTTTGGGGGAGAGGATGGGATTTTCGGGTGAACTGG
AACAAGGATGTGTGTGTGTGTGTATGTGTGTATGTGTGTTGTTCATGCTGCTCTAGCAGCTGCGGAAA
CAGCTCCTGGGTGATGGGTGGCTTCCTAGGCACTACAGCTACGGGCTATGGCGAGCCCGTCATAGCAG
CCTGCTGGGAAGCCGATGTGGTGCGTAACGTTGGTCGCTTAGGAGGGGTCGTGGAGGGGTCGTGGAGG
GGTCGTGGAGGGGTCGTGGAAGTATCGATGTTGCGTTTGCGGTGACCCAGCGTGAAGAATAACAAATG
CCAACAGTGTCGGAGTGTTATCAGGTGCAAAGTCCTCCGCAGTGCTGATGCCTCCTCTTCAGCAGCAG
CCTGCCCTACGCGATTACACGGTGGTTGAAGTGGTTGCTGGTTGGCGCCGCCAGCATTATATTTTAAC
CACGTCAGGGCCTGATGCGCCGTGGGTTTAGGAAGCAGCTTCTCGCCTGAAGGGAGGGGGAGACGGGT
TCTGGGTTGGTTTGGTTCTGGTGCCCGCTAGGGTGAGAATTGCTGAAGGGCTGACGTGTACCAGGTCA
TATGTACCCTAGTGGCGCGAAGTAAACAGACGAGCTAGGGAACATAACAATAACAACAGCAAATACAA
TAAGCTTTAAAACGATCTATTGATATGCGCTGCATGTGCGTGCGTATGTATGTGTGTATACGGCGATG
CGAGTACGGCATCTTTCCATACTCTGGTCTTTAATCTCACCCCTGAAACACACCACCTTCGTTTGCGC
GTGCATCCATGCGTGTGTGGTGTGGGCTGGCGTGGCCTAGTAAGTGGAAATGATTTCAAAGCCAAAAC
AAGCTACCGGGATAGCATCAGTCTCTTCAATCATTCACCCGGTCTCCGCGTGTGGCGCTTGGGGGTAT
GTTCCCACTGCCGCCACTGTCACCTGCTGAGCTGTCGGCATGTCGTAATAACGCCTCTGCACGCCAGC

***Not*I**

previous sequence gap

***Not*I**

***Sac*I**

CCGTGGTATCGTTTTCCG**GGATCC**CGCTGTCGGGTCTAAGAATGGCTTCCGTTGCTGGCGCTGCTCGC ***Bam*HI**
TCGAACGCGAGACCAAGTTAACTTTTACTCCTGGAGCAGAAGGTTTAGTAAAACAAGCTATTGCTGAA
TATTTAGAA**GAATTC**AAATCAAGTGCTAAAGCTGCTTAATTAGTAATAATTTATATAATTTGGTGTCC ***Eco*RI**
ACAACATACATAGCTGCCGGTTGCAACCACATCTACACCCACATATGTCTGTGCGCGTGCGTGTTGCA
CCTTGGGCTGCTTATGTGCTACCCTACGAATGTATTAACCTAGAGCCCCATTTACATGTGCTCAACCA
TGCCTACCAACACAACACCAACACATTTCCCCTTCGCACCCGTTCTCTGTTCGAACCCCACAGACGGC
AAGTGCAAGGGCTCCATTTTGCCGTTCACTCTGTCCAACACCGCGGAGATTAGGTCGTCATTCTCCTG
GGACTCCACTCGCCCAGTCCTCAAATTCACCCGCCTGGGCCTCACCTATGCTCAAGGTGTCGCCGGCG
GCAGCCTCTGCTTCAACATCAAGGGTGCCGGCTGTACCAAGTTTGC**AGATCT**CTGTCCTGGCCGCGGC ***Bgl*II**
TGCACCGTCGCGGTATTCAACAACCCCGACAACACATGTTGCCCGCGGGTCGGCACCATCGCG**GGTAC** ***Kpn*I**
CGGCGGAGGCGGTGGCATGAGCAAGGGCGAGGAGCTGTTCACCGGCGTGGTGCCCATCCTGGTGGAGC gly5
TGGACGGCGACGTGAACGGCCACAAGTTCAGCGTGAGCGGCGAGGGCGAGGGCGACGCCACCTACGGC
AAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTGGTGACCAC
CCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGA
GCGCCATGCCCGAGGGCTACGTGCAGGAGCGCACCATCTTCTTCAAGGACGACGGTAACTACAAGACC *yfp*
CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA
GGAGGACGGCAACATCCTGGGCCACAAGCTGGAGTACAACTACAACAGCCACAACGTGTACATCACCG
CCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTG
CAGCTGGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCA
CTACCTGAGCTACCAGAGCAAGCTGAGCAAGGACCCCAACGAGAAGCGCGACCACATGGTGCTGCTGG
AGTTCGTGACCGCCGCCGGCATCACCCTGGGCATGGACGAGCTGTACAAG**GGTACC**TAAAGCGTTTCG ***Kpn*I**, **stop**
CATGACGATGCAAAAAAAAGGGTGGTGCAGCGCAACACCCCCCCGTGGGTGTGTGGGTGTGTGTGCCC
GCGCAAGAAGATTGTGCGTGAGTGGATGTGCGCGGATGTGGGGGGGAAAACCGCCAGTCCCTTTCTTA
TATGCTGCTACACATGACTGAGGTTGCTGCCGGGGAGGCGGAGGAGGCTTGTGGGTGTGGCGTCGGCG
TCGATGCCAAGTCACCGCTGTGCCACTGCTGCGGCGGGCTTGGATAGCAGCAGCAGCGGCGCAGCAGC
AGCAGCATCCGCAGCATCAGCAGCATAGGGACGCATGCATGCCTTTAGCCGGAATGGGAGGGCTGCGT
GCTGCTTTTGCAACCGCAGGGGTGAAAGTAGGTAGTCCTGCATAAATGATTAAGCACTGGTCAAATGT
CGCTGAGATGAACATAACACGATTATATATGATATGATGCGCGACATGATTGTTGGATGCGGCCAGTA
CTTTTTTCCGCTGCGCAGGTGTCAGTGTTGCACTTGGGTTTGATCCAGTTACGGGCCTCTCGGCGTCT
ACCAAACGCAAATTGTCGTTAATTGATATTCTCTTGCTTTTTGCGCATCTCGTCAAGTGAGGGCAGCG
TCCCCCTCCTGTGCTGCTGCACTTGACGTGGCTGTTGGAATGTTCCGTGCATGGCCGCTGGCCCGAAC
CATTTGTAGTTGAGTGACTACTGCGCTAACATTCAGTTATTTCGTAATAATCACCATCATAATTATCA
TCATCGCTACCTGCCTATGGCCGGGAAGTTGAGCAGGTTTTAACGTTTTACGTTCAATCCCGATGGCC
CACGTCACGTGTAGCAGGCCTTTGCGTTCCCGCTCTCTTGTAACTTATTTCTCATA**GATATC**AAATTT ***Eco*RV**
GGCTCATCTTTGCAGTGGAGTATTCCTACAAATAGAGGTGCATCTGCCAGATTTGGCAAATAGAGGTG
CATCTGCCAGATTTGGCCGGTGGGGGGACCTTATTAACCGTCGTTAACCA**GGATCC** ***Bam*HI**

The vector pSSG-YFP carries a 8.8-kb fragment of *V. carteri* genomic DNA containing the *ssg185* gene including its 6 introns, a short linker sequence, which codes for 5 glycines (Gly5) and the coding sequence of *yfp* (mVenus). The chimeric gene is controlled by the original *ssg185* 5' and 3' flanking sequences, including the promotor region. The corresponding gene entry in the current *Volvox carteri* genome version 2.1 (Prochnik et al., 2010) in Phytozome 12.1.6 (Goodstein et al., 2012) is Vocar.0002s0564. The *yfp* (mVenus) gene has been codon-adapted for *C. reinhardtii* (Lauersen et al., 2015) but also works well in *V. carteri* (Tian et al., 2018; von der Heyde and Hallmann, 2020). Artificial *Kpn*I and *Bam*HI sites have been introduced to facilitate cloning. The background colors indicate the following sequence features: gray, *ssg185* promoter region, green, 5' and 3' UTRs of *ssg185*; blue, coding sequences of *ssg185*; turquoise, sequence coding for 5 glycines (Gly5); yellow, coding sequence of *yfp* (mVenus). Start and stop codons are highlighted (violet font). A previous sequence gap, which we closed, is also indicated (blue font). Relevant restriction sites are marked (bold, underlined).

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