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pdsACFL1 (KX910691) MKHLVFKKDLSDLKNSPLSLF IGFTSLCETYSLKASEAESCGTDKLTFTLNTYLGKIIEDILKHGGDVLKFGDALLALWKAEYGC NLDDLTLINKAICCSIA
pdsACFL2 (KY853034) -----MPRVKHMVGVMCFADI SGFTSLCETYSLKASEAESCGTDKLTFTLNTYLGKIIEDILKHGGDVLKFGDALLALWKAEYGC NLDDLTLINKAICCSIA
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pdsACFL1 (KX910691) IQEEDCNMYMTDVGVLLRVKIALSVGKMQITHVGVTESKHFDSLGSASVDVNAAEKWAEPGSIILSLLAFSNCDQSLFLFETIDNGMYRVVAGARVEEESATVSTV
pdsACFL2 (KY853034) IQEEDCNMYMTDVGVLLRVKIALSVGKMQITHVGVTESKHFDSLGSASVDVNAAEKWAEPGSIILSLLAFSNCDQSLFLFETIDNGMYRVVAGARVEEESATVSTV
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pdsACFL1 (KX910691) HPTGVQLALMSGVLSTGAVLTSTPSVAKRIFAPHVGTLLSRYYPQMKLPRIRYRKKGQSEQKEQICKTTGNLLKSDSCHDPGHRTYIYSKLNDQDIEDLRAVVPKT
pdsACFL2 (KY853034) HPTGVQLALMSGVLSTGAVLTSTPSVAKRIFAPHVGTLLSRYYPQMKLPRIRYRKKGQSEQKEQICKTTGNLLKSDSCHDPGHRTYIYSKLNDQDIEDLRAVVPKT
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pdsACFL1 (KX910691) VLSRIDHGQDLEWLSEMRQSVLFINMELPIKGNSSH WALQRAFEIHECARRLRGNLNKVFSDKGCTFIVIFGLPGDKHDDDPTRALKAGHRIMDSLHQIMDIT
pdsACFL2 (KY853034) VLSRIDHGQDLEWLSEMRQSVLFINMELPIKGNSSH WALQRAFEIHECARRLRGNLNKVFSDKGCTFIVIFGLPGDKHDDDPTRALKAGHRIMDSLHQIMDIT
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pdsACFL1 (KX910691) TNESIGVTTGRAFCGVVGHDRDRHEYTVIGRKVNMAARLMVNYPGTLCDDDTYRSGKSKLKKEDFMVLPFIKLGKGVADPGTVREY NNHHDREEEEEYDY PILGR
pdsACFL2 (KY853034) TNESIGVTTGRAFCGVVGHDRDRHEYTVIGRKVNMAARLMVNYPGTLCDDDTYRSGKSKLKKEDFMVLPFIKLGKGVADPGTVREY NNHHDREEEEEYDY PILGR
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pdsACFL1 (KX910691) GKEIEEMKMLLHAIKCDDTRVSGSRRVVVIEGEGGVGKTRLLEAFMDIAEEDDFKVDYVEADMAHAHTPYHVVKTLIENLLELEACRTASEKENT LMEHITDQKL
pdsACFL2 (KY853034) GKEIEEMKMLLHAIKCDDTRVSGSRRVVVIEGEGGVGKTRLLEAFMDIAEEDDFKVDYVEADMAHAHTPYHVVKTLIENLLELEACRTASEKENT LMEHITDQKL
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pdsACFL1 (KX910691) REKMFLNLDLLGTHIPPAD FARMDLDKVTQFHLLFEIVHQFVVSQPCLFTVDNAQFIDQESWDFIEDLSADSHAILVLSLRPFSSSNPCEAAIRLTGHETTP
pdsACFL2 (KY853034) REKMFLNLDLLGTHIPPAD FARMDLDKVTQFHLLFEIVHQFVVSQPCLFTVDNAQFIDQESWDFIEDLSADSHAILVLSLRPFSSSNPCEAAIRLTGHETTP
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pdsACFL1 (KX910691) KKVKLGGLEASYMSDLACQFMDVMI PNELDGILREKSHGIASWCEQLIKDMLTSNVIQVNVESQAFLNKESLDLLSFGGSTTTTISQKHNNASSYLPRPSTLL
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*****

pdsACFL1 (KX910691) YGKATDGGWLGANSPEPRRSLTLGKGFASNTLSSGFQDTLDTLSLFLRDRRSTSAVESLQYNFANNPFEIKTKIAESDLKSNRSTRYFPEDDFIQEKIQYNLE
pdsACFL2 (KY853034) YGKATDGGWLGANSPEPRRSLTLGKGFASNTLSSGFQDTLDTLSLFLRDRRSTSAVESLQYNFANNPFEIKTKIAESDLKSNRSTRYFPEDDFIQEKIQYNLE
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pdsACFL1 (KX910691) RANFVSASENSMPLDTKKVCILSPGVDISKIVVPESVKDMVLARVDRMLPQEQTALKCASVLGCFYRDVLQAILPRSSQSCIDLVLVYLAQESILECASLALQH
pdsACFL2 (KY853034) RANFVSASENSMPLDTKKVCILSPGVDISKIVVPESVKDMVLARVDRMLPQEQTALKCASVLGCFYRDVLQAILPRSSQSCIDLVLVYLAQESILECASLALQH
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pdsACFL1 (KX910691) QNAHNHHGFYDFNDPVHAQHGHGHHHHHHH QNVASSI HASVYCGCYADEMTKVINLSRLMTPSGPRKHCLYMKFVNTYVQETTYSWLWLEDQKKELHERAAMFLES
pdsACFL2 (KY853034) QNAHNHHGFYDFNDPVHAQHGHGHHHHHHH QNVASSI HASVYCGCYADEMTKVINLSRLMTPSGPRKHCLYMKFVNTYVQETTYSWLWLEDQKKELHERAAMFLES
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46 pdsACFL1 (KX910691) QAHKCKSCGGGFVAKKADTLEEQGGHGKRTTEGRSQARVSSKMAKRRRTAEMREKVQSRGAFSSRTHERSSDISEAKHALKTKKLE SPVVNQKAGETEGIGSK

47 pdsACFL2 (KY853034) QAHKCKSCGGGFVAKKADTLEEQGGHGKRTTEGRSQARVSSKMAKRRRTAEMREKVQSRGAFSSRTHERSSDISEAKHALKTKKLE SPVVNQKAGETEGIGSK

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50 pdsACFL1 (KX910691) FANTAGFFNDAVVNRAVDHRTSWLQRLPCAKKFVSPGKEDGEQDEGVTRKKLRRFSSKKNSTMIIGGPETRRFSTPQIEEEEEEEEEVVDIDFE DSAESQQTVID

51 pdsACFL2 (KY853034) FANTAGFFNDAVVNRAVDHRTSWLQRLPCAKKFVSPGKEDGEQDEGVTRKKLRRFSSKKNSTMIIGGPETRRFSTPQIEEEEEEEEEVVDIDFE DSAESQQTVID

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54 pdsACFL1 (KX910691) LSNQCQAEVLASVFPQLVDHWRAAGNKLKTMRYLTESGAA ALATSSNMQALSYLYEVQTLIEETKDDEEPLAAEEETARVESLIGQALIR MNRPHEALRHLSAAL

55 pdsACFL2 (KY853034) LSNQCQAEVLASVFPQLVDHWRAAGNKLKTMRYLTESGAA ALATSSNMQALSYLYEVQTLIEETKDDEEPLAAEEETARVESLIGQALIR MNRPHEALRHLSAAL

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58 pdsACFL1 (KX910691) LILGKKQPKTDFGCVRLLEALRHYLHVFLPGYYIGGAGDKSARLIEQSRCLSHLSHVYHSLHLKKWSLMAALQEL NAEEEAENLHELINAYAGVVECSHLVG

59 pdsACFL2 (KY853034) LILGKKQPKTDFGCVRLLEALRHYLHVFLPGYYIGGAGDKSARLIEQSRCLSHLSHVYHSLHLKKWSLMAALQEL NAEEEAENLHELINAYAGVVECSHLVG

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62 pdsACFL1 (KX910691) WKGWGKTYEKIGKNRCEPDS LYSDPEDMMTVAHLYCVSLAFRLAIGEVSIANSGKHAF DLAQVHDQHMMTTC IPLLAQSLLEFTAKIPDCIDVLKELNAAALDS

63 pdsACFL2 (KY853034) WKGWGKTYEKIGKNRCEPDS LYSDPEDMMTVAHLYCVSLAFRLAIGEVSIANSGKHAF DLAQVHDQHMMTTC IPLLAQSLLEFTAKIPDCIDVLKELNAAALDS

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66 pdsACFL1 (KX910691) CDSHGRALYMCCCFDLILEAGWRLEDFDEIVQFTA KMSTDPAFSSDVAPKFYLNVSLALWYARRRDWKNADSLMSSASAVVPSKLEVFMSVHGYAKVVEFWLLKL

67 pdsACFL2 (KY853034) CDSHGRALYMCCCFDLILEAGWRLEDFDEIVQFTA KMSTDPAFSSDVAPKFYLNVSLALWYARRRDWKNADSLMSSASAVVPSKLEVFMSVHGYAKVVEFWLLKL

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70 pdsACFL1 (KX910691) QEDGKNRQNEDEVQKCLKKFKNALNHHPVFDGRRLLHLLAYHLLRGKKDKCKSKLRRFCFNKNKNLGLWLEVEWANKSFKEWFISWKESEPNYLGTSMFTLPKPKKD

71 pdsACFL2 (KY853034) QEDGKNRQNEDEVQKCLKKFKNALNHHPVFDGRRLLHLLAYHLLRGKKDKCKSKLRRFCFNKNKNLGLWLEVEWANKSFKEWFISWKESEPNYLGTSMFTLPKPKKD

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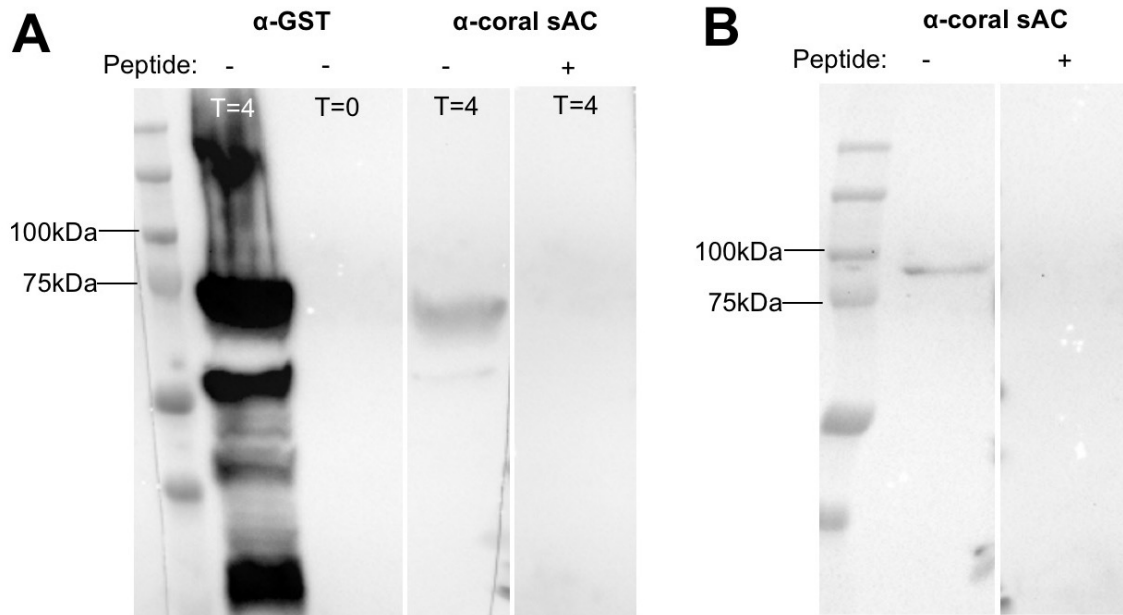
78 **Supplemental Figure 1.** Alignment of full length *Pocillopora damicornis* soluble adenylyl cyclases 1 and 2 (pdsACFL1 and pdsACFL1). The

79 two catalytic domains are highlighted in red, the two P-loop motifs in purple, the histidine rich region in light grey, the leucine zipper pattern in

80 dark grey, and the four tertraticopeptide (TPR) like helical domains in green. The 1061 amino acids that are missing in PDsAC94 (Figure 2 in the

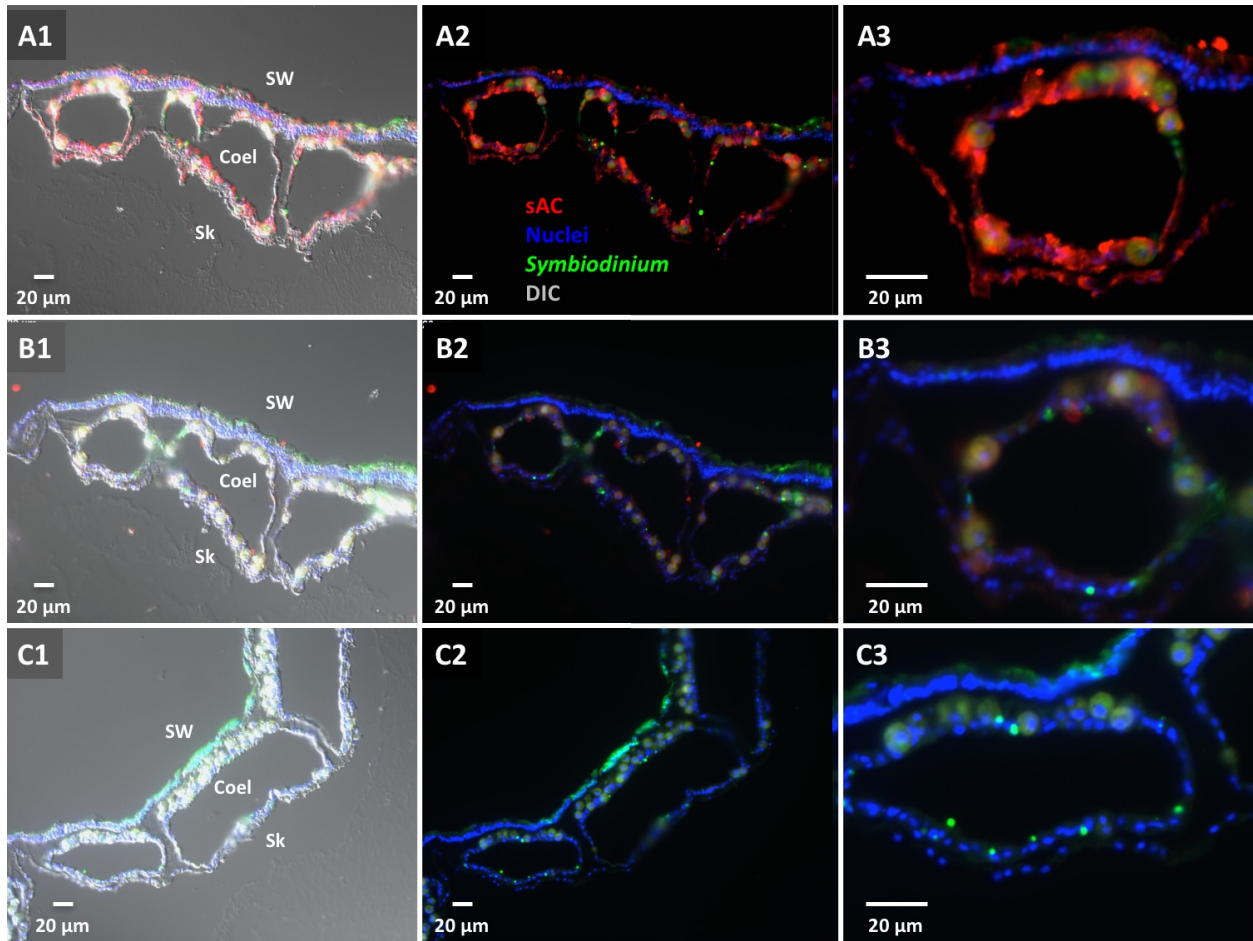
81 manuscript) are underlined. Made online using Clustal Omega Multiple Sequence Alignment (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

82 **Supplemental Information**



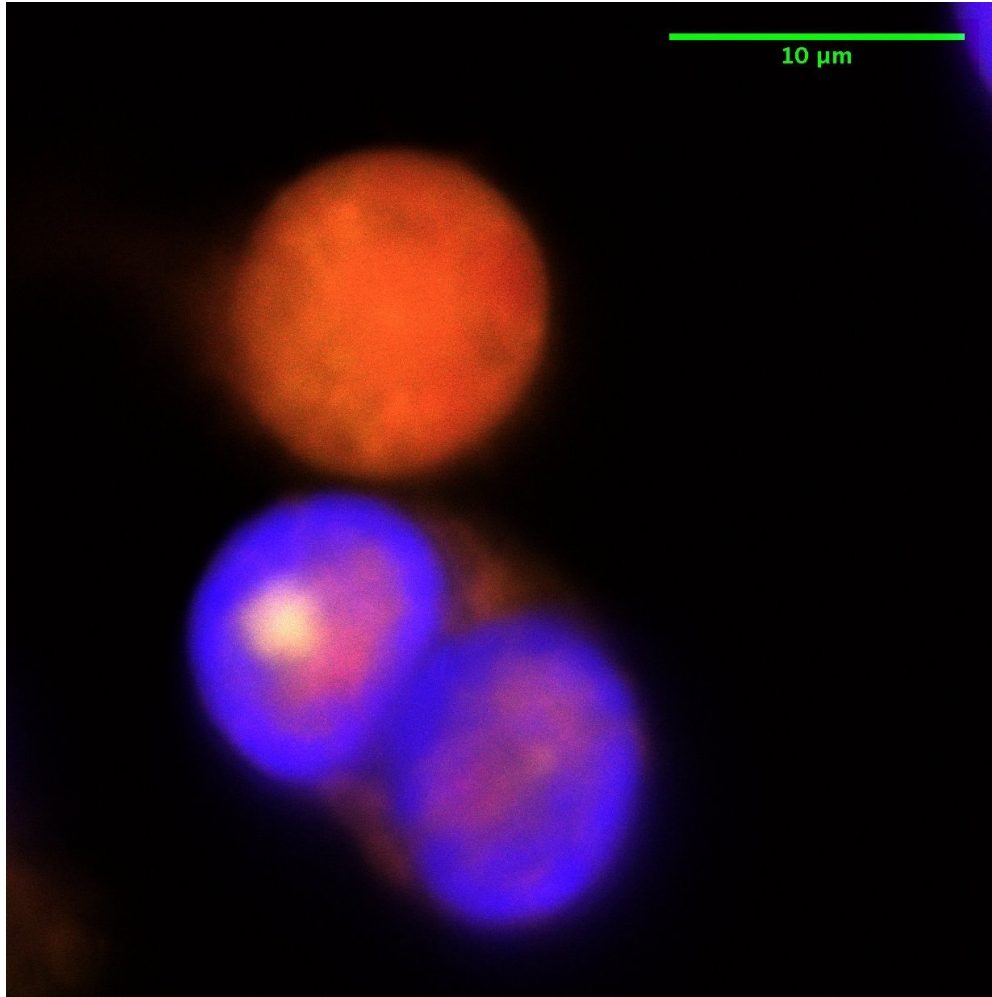
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Supplemental Figure 2. Specificity of anti-coral sAC antibodies. A) Western blots with either anti-GST or anti-coral sAC antibodies against recombinant pdsAC₉₄ before (T=0) and after (T=4) expression of the protein was induced in *E. coli*. B) Western blots using anti-coral sAC antibodies against native proteins prepared from whole tissue homogenates. Anti-coral sAC antibodies pre-adsorbed with antigen peptide are indicated by +. Molecular weight marker shown in the left-most well with sizes indicated.



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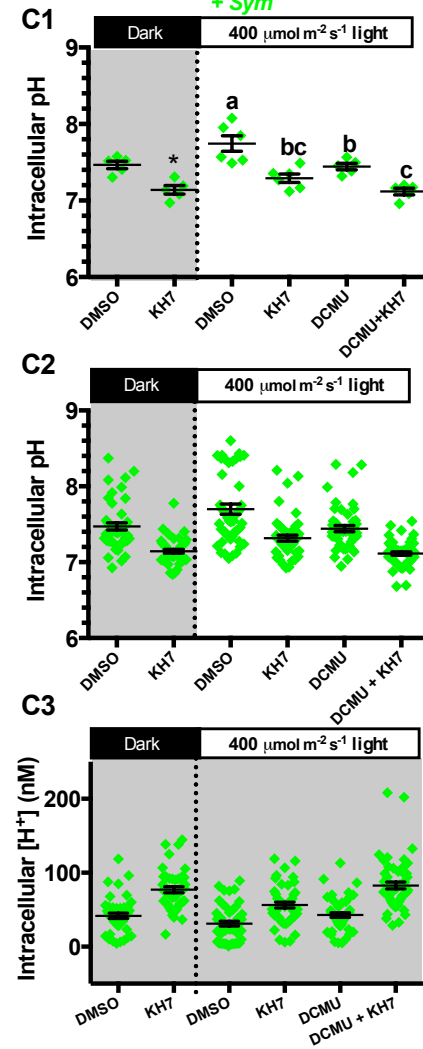
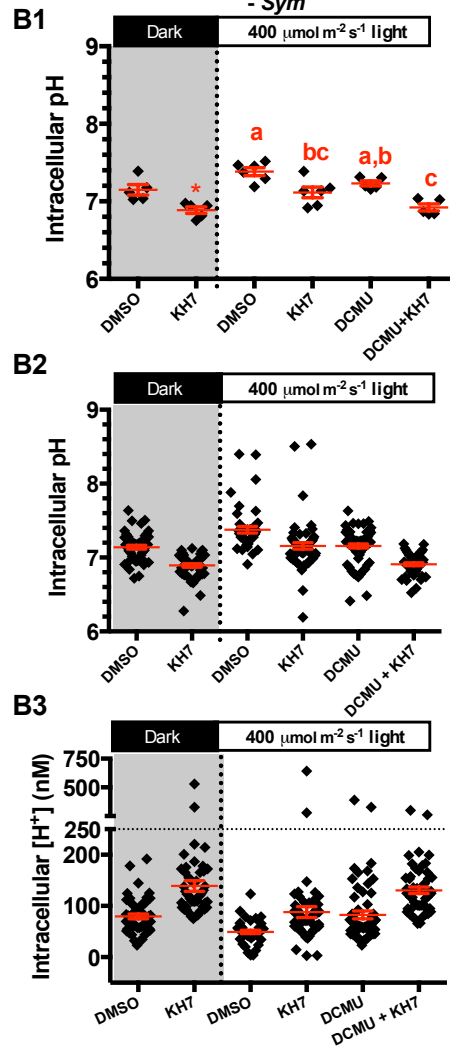
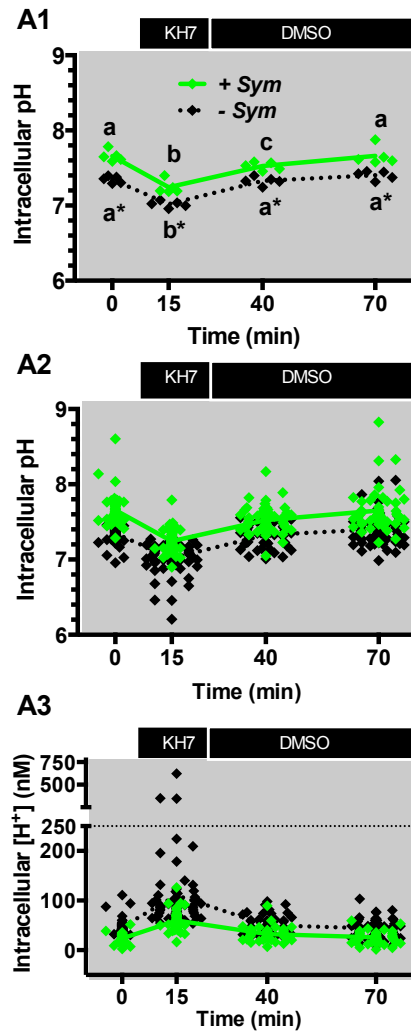
Supplemental Figure 3. Validation of anti-coral sAC antibodies in *P. damicornis*. A) anti-sAC antibodies, B) anti-sAC antibodies preadsorbed with antigen peptide, C) no primary antibody.



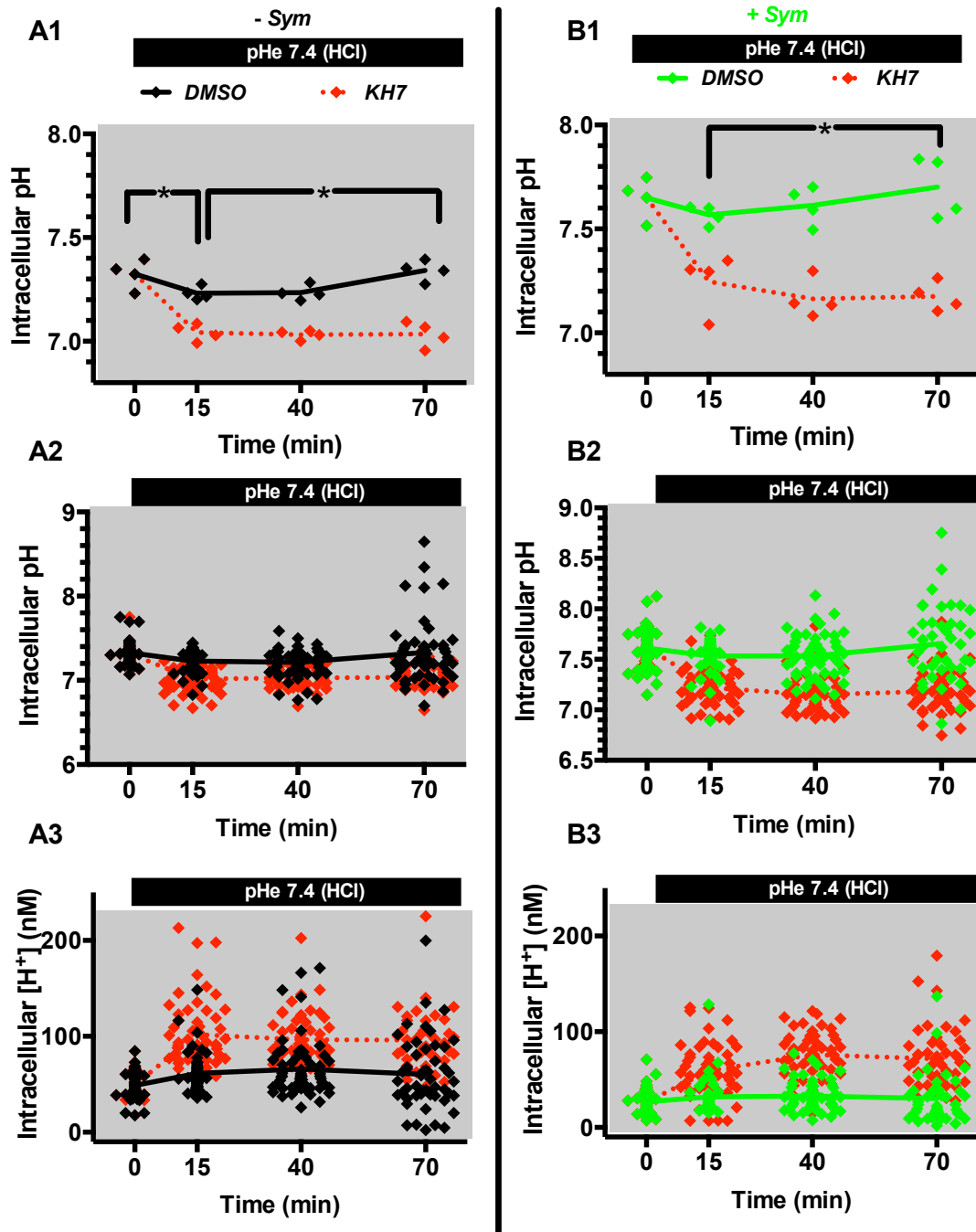
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Supplemental Figure 4. Representative image of coral cells with *Symbiodinium* (bottom left) and without *Symbiodinium* (top). Blue indicates *Symbiodinium* chlorophyll autofluorescence. SNARF1 fluorescence is shown in orange.

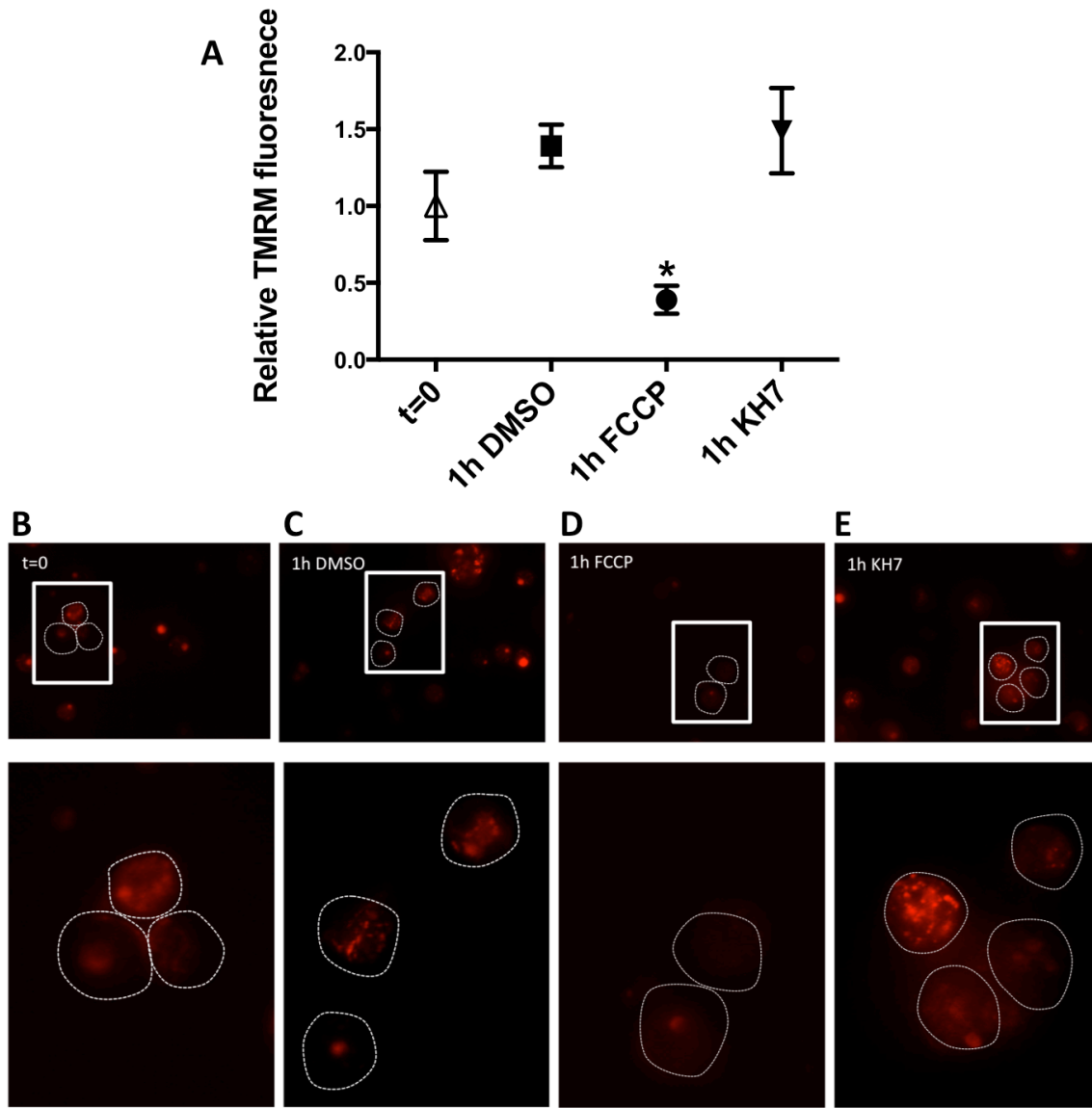
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Supplemental Figure 5. Intracellular pH (pHi) and intracellular [H⁺] in the experiments shown in Figure 4. A1, B1, C1: each point represents the average pHi value from 48-62 (A), 45-53 (B), and 47-67 (C) cells from each of five corals (N=5). A2, B2, C2: Each point represents the pHi value of a cell. A3, B3, C3: Each point is the intracellular [H⁺] of each cell shown in A2, B2, C2. The shaded boxes indicate dark conditions.

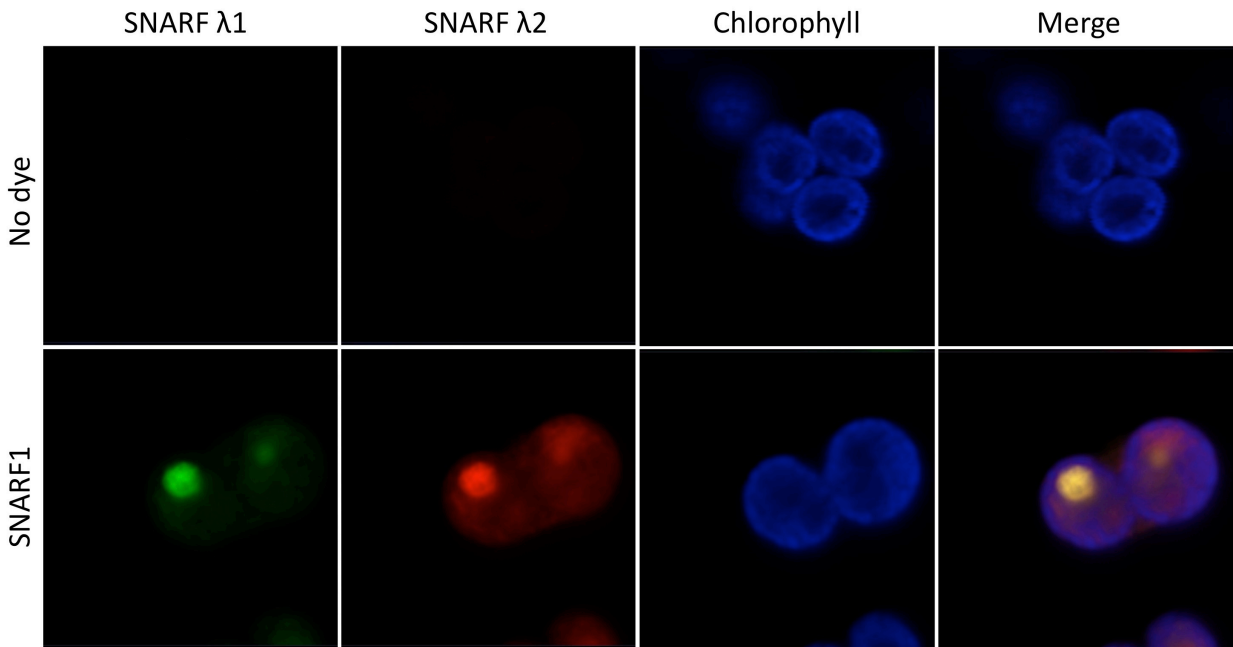


133 **Supplemental Figure 6.** Intracellular pH (pHi) and intracellular [H⁺] in the experiments shown
 134 in Figure 5. External pH (pHe) acidification was achieved by addition of HCl in the dark
 135 (indicated by the shaded boxes). A1, B1: each point represents the average pHi value from 50-59
 136 cells from four corals (N=4). A2, B2: Each point is the pHi value of each cell shown in A2, B2.
 137 A3, B3: Each point is the intracellular [H⁺] of each cell shown in A2, B2, C2.
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141 **Supplemental Figure 7.** KH7 does not induce mitochondrial uncoupling in isolated coral cells.
 142 Coral cells were isolated as described in Methods, incubated with 0.1 μM tetramethylrhodamine
 143 methyl ester (TMRE) for 30 min, pelleted down (5 min at 350 xg) and resuspended in filtered
 144 seawater to wash away excess dye, and 1 mL aliquots were incubated with either nothing (t=0h),
 145 DMSO, 5 μM FCCP (a classic mitochondrial uncoupler that served as positive control), or 50
 146 μM KH7. TMRE accumulation in mitochondria was quantified at t=0 and t=1h using a
 147 fluorescence microscope (Zeiss AxioObserver) (magnification=400x, excitation= 550 nm;
 148 emission 605 nm). All images were taken with the same exposure settings; for each field of view
 149 five z-images were stacked together and analyzed using Image J (National Institute of Health,
 150 USA). Per-cell TMRE fluorescence was calculated as the product of fluorescence area and
 151 average intensity. (A) Summary statistics (Kruskal-Wallis 1-way ANOVA followed by Dunn's
 152 multiple comparisons test. Mean \pm SEM of 30-34 cells per treatment, the asterisk indicates a
 153 significant difference compared to t=0, $p < 0.05$). (B-E) Representative images of TMRE signal in
 154 isolated coral cells from the different treatments, the white squares correspond to the zoomed in
 155 images shown in the bottom row.

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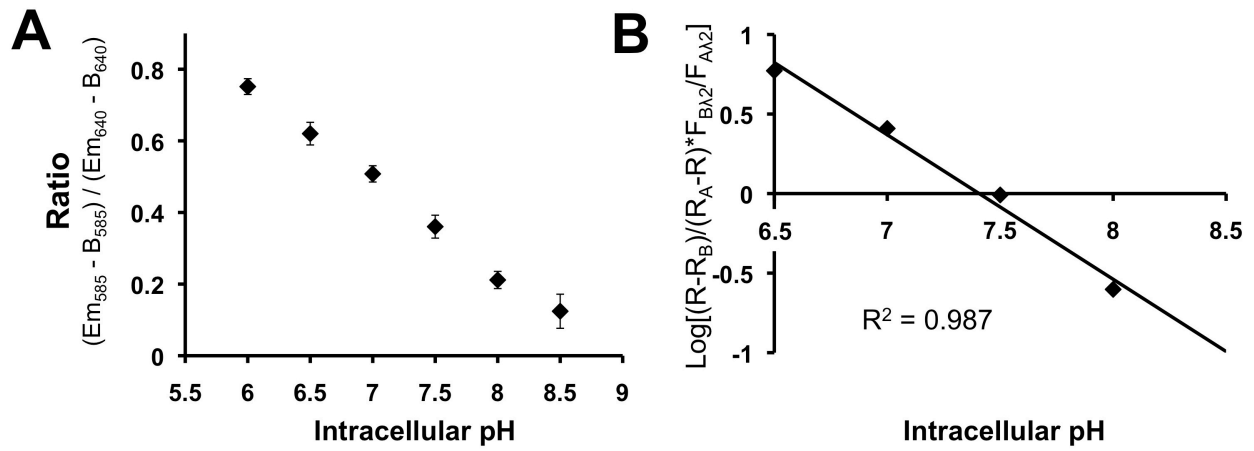


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158 **Supplemental Figure 8.** Comparison of endogenous fluorescence with SNARF1 fluorescence in
159 cells isolated from the coral *P. damicornis*. Cells were excited with a 561 nm laser (10% power)
160 and fluorescence emission was collected at 585 +/- 10 nm (SNARF λ1), 640 nm +/- 10 nm
161 (SNARF λ2), and 680 +/- 10 nm (chlorophyll).
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167 **Supplemental Figure 9.** Calibration of intracellular pH in isolated *P. damicornis* cells loaded
 168 with SNARF1-AM.

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