



## Supporting Information

for

### **The cement of the tube-dwelling polychaete *Sabellaria alveolata*: a complex composite adhesive material**

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## Detailed experimental results

**This PDF file includes:**

**Table S1, S2, S3 and S4**

**Figure S1, S2, S3, S4, S5 and S6**

**Other Supplementary Materials for this manuscript include the following:**

**Table S5:** Complete list of *Sabellaria alveolata* adhesive proteins and FAM20C kinases candidates identified through in silico analyses. Indicated are the NCBI accession number of the transcript if available, the transcript ID from the transcriptome of the anterior part of the worm, the amino acid length, proportion of transcripts in the transcriptome, completeness of the ORF, presence of a signal peptide, molecular weight, isoelectric point, the conserved domain, the top reciprocal BLAST hit, and the amino acid composition, with color coding indicating lower or higher amino acid concentrations.

**Table S1:** Elemental composition (in wt %) of the heterogeneous granules present in the parathoracic part of *S. alveolata* embedded in Spurr resin.

| <b>Elements</b> | <b>Hetero 1</b> | <b>Hetero 2</b> | <b>Hetero 3</b> | <b>Hetero 4</b> | <b>Mean</b> | <b>Standard deviation</b> |
|-----------------|-----------------|-----------------|-----------------|-----------------|-------------|---------------------------|
| <b>C</b>        | 53.7            | 43.9            | 45.9            | 58              | 50.4        | 6.6                       |
| <b>O</b>        | 30.7            | 40.2            | 36.8            | 30.5            | 34.6        | 4.8                       |
| <b>P</b>        | 7.4             | 7.2             | 8.7             | 5.7             | 7.3         | 1.2                       |
| <b>Na</b>       | 2.4             | 3.1             | 2.8             | 1.9             | 2.6         | 0.5                       |
| <b>Mg</b>       | 2.6             | 1.1             | 3.4             | 2.3             | 2.4         | 1.0                       |
| <b>Ca</b>       | 0.8             | 1.2             | 1.1             | 0.6             | 0.9         | 0.3                       |
| <b>Cl</b>       | 1.1             | 0.7             | 0.7             | 0.4             | 0.7         | 0.3                       |
| <b>S</b>        | 0.8             | 0.6             | 0.6             | 0.4             | 0.6         | 0.2                       |

**Table S2:** Elemental composition (in wt %) of the homogeneous granules present in the parathoracic part of *S. alveolata* embedded in Spurr resin.

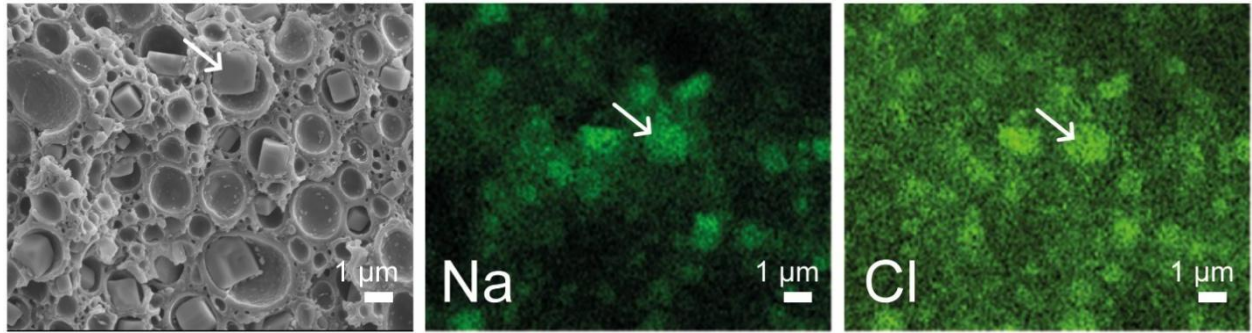
| Elements  | Homo 1 | Homo 2 | Homo 3 | Homo 4 | Mean | Standard deviation |
|-----------|--------|--------|--------|--------|------|--------------------|
| <b>C</b>  | 66.3   | 66.1   | 64.7   | 64     | 65.3 | 1.1                |
| <b>O</b>  | 28.4   | 28.7   | 29.1   | 29.5   | 28.9 | 0.5                |
| <b>P</b>  | 1.5    | 1.3    | 2      | 2      | 1.7  | 0.4                |
| <b>S</b>  | 1.2    | 1.3    | 1.3    | 1.4    | 1.3  | 0.1                |
| <b>Na</b> | 1.1    | 1.2    | 1      | 1.2    | 1.1  | 0.1                |
| <b>Cl</b> | 0.8    | 0.8    | 1.2    | 1.1    | 1.0  | 0.2                |
| <b>Mg</b> | 0.5    | 0.4    | 0.7    | 0.7    | 0.6  | 0.2                |

**Table S3:** Elemental composition (in wt %) of six different cement spots glueing mineral particles together in a natural tube embedded in epoxy resin.

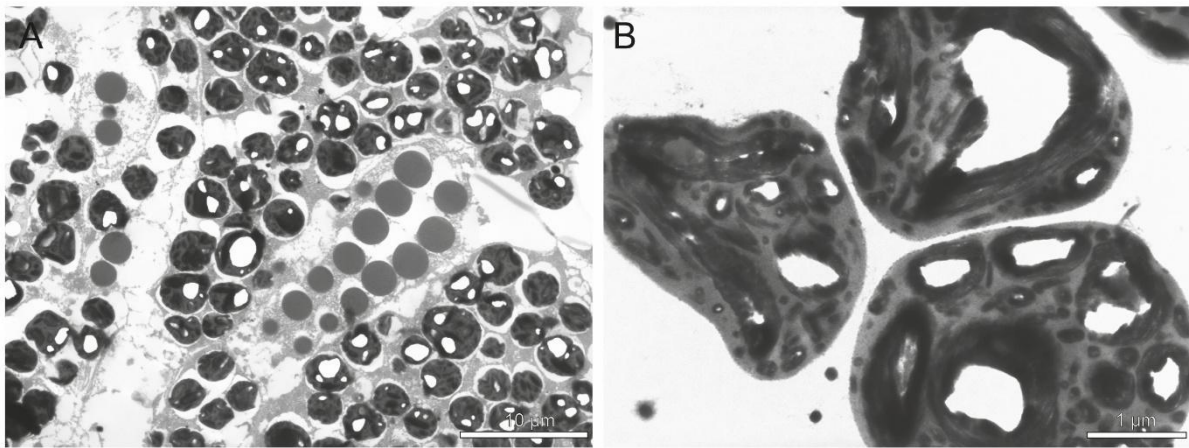
| Elements  | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Mean | Standard deviation |
|-----------|--------|--------|--------|--------|--------|--------|------|--------------------|
| <b>O</b>  | 39.6   | 33.0   | 43.2   | 44.2   | 33.1   | 43.6   | 39.4 | 5.2                |
| <b>C</b>  | 35.2   | 49.4   | 30.2   | 30.3   | 55.2   | 34.5   | 39.1 | 10.6               |
| <b>Ca</b> | 17.1   | 13.2   | 20.2   | 19.4   | 8.0    | 15.3   | 15.5 | 4.5                |
| <b>P</b>  | 5.6    | 2.8    | 3.8    | 3.8    | 2.5    | 3.9    | 3.7  | 1.1                |
| <b>S</b>  | 0.9    | 0.5    | 0.7    | 0.7    | 0.5    | 0.6    | 0.6  | 0.1                |
| <b>Mg</b> | 0.7    | 0.4    | 0.6    | 0.6    | 0.3    | 0.8    | 0.6  | 0.2                |
| <b>Na</b> | 0.3    | 0.2    | 0.2    | 0.2    | 0.2    | 0      | 0.2  | 0.1                |
| <b>Mn</b> | 0.3    | 0.1    | 0.2    | 0.3    | 0      | 0      | 0.2  | 0.1                |
| <b>Cl</b> | 0.2    | 0.1    | 0.2    | 0.1    | 0.1    | 0.1    | 0.1  | 0.03               |
| <b>Si</b> | 0.1    | 0      | 0      | 0.1    | 0      | 0      | 0.1  | 0.1                |
| <b>Al</b> | 0.1    | 0.03   | 0.03   | 0      | 0      | 0      | 0.02 | 0.02               |

**Table S4:** List of primers used to generate the in situ hybridization probes.

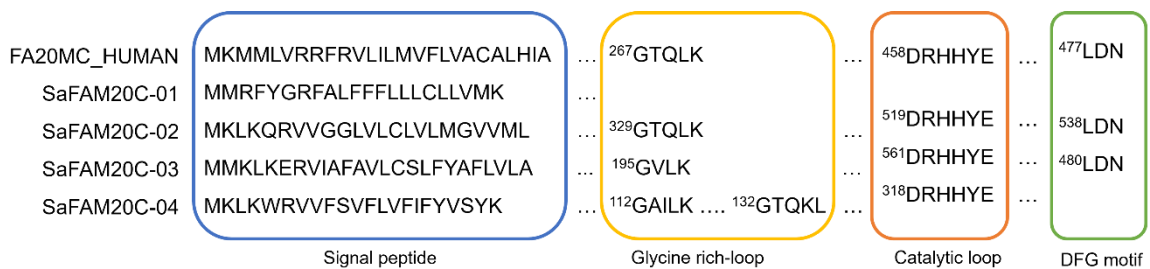
| Adhesive protein candidate | NCBI accessory number | In situ primer name | In situ FW primer sequence | In situ RV primer sequence | In situ RV primer sequence | Length in situ probe |
|----------------------------|-----------------------|---------------------|----------------------------|----------------------------|----------------------------|----------------------|
| Sa-1                       | HE599563              | Sa-1_LPA            | TCTCGCTTTGGTCTCTGCAG       | Sa-1_RPA                   | AAGCACCACCATATCCACCC       | 644                  |
| Sa-2                       | HE599584              | Sa-1_LPA            | TGGTCGCATTAGCTGCAA         | Sa-1_RPA                   | ACACCGTAA CCACCTGCAC       | 319                  |
| Sa-3A                      | HE599605              | Sa-3A_LPA           | TCTGCCTTGTACACTCTGAGG      | Sa-3A_RPA                  | TGAGCTTGAAGTTCTACCGC       | 605                  |
| Sa-3B                      | HE599626              | Sa-3B_LPA           | GAAGACTTTTGCCGTATTCG       | Sa-3B_RPA                  | AATGATGGCTGCTAGAACTAC      | 647                  |
| Sa-3C                      | NA                    | Sa-3C_LPA           | CCGCCTTACTATGGACGTGG       | Sa-3C_RPA                  | CTTTGCCGTATCCGCCTTTG       | 775                  |
| SaFAM20C-2                 | NA                    | SaFAM20C-2_LPA      | AGACCCCGCCATACAACAAG       | SaFAM20C-2_RPA             | CTATCCAGCGCCCTCAGATG       | 912                  |
| SaFAM20C-3                 | NA                    | SaFAM20C-3_LPA      | AGCCAGGACGAACAGAACTG       | SaFAM20C-3_RPA             | GGATGTCCGAAAGCTCTTCC       | 805                  |
| SaFAM20C-4                 | NA                    | SaFAM20C-4_LPA      | TCCGTACAATGCCTCTCGTG       | SaFAM20C-4_RPA             | AGTGGGGACTGGCAAACTC        | 857                  |



**Figure S1:** SEM secondary electron imaging and EDS spatial mapping of a fracture in an unfixed, air-dried cement spot from *Sabellaria alveolata* reveal NaCl crystals within the spheroids (one of the salt crystals is indicated by a white arrow).



**Figure S2:** TEM images of cement cells with heterogeneous granules containing expanded inclusions with an apparently empty cavity at their center.



**Figure S3:** The four candidate SaFAM20C protein sequences aligned with the human FAM20C reference sequence (NCBI accession number Q8IXL6.2). The FAM20C signatures include the N-terminal signal peptide (blue), the glycine-rich loop (yellow), the highly conserved aspartate residue in the DRHHYE catalytic loop (orange), and the DFG-binding motif (green).

>SaFAM20C-1

MMRFYGRFALFFFLLLCLVMK KQVITLFGTAREDTALRIVEGDTDLINKPTFIKNKENRNIESPKEGNGILQRENQV  
INKFMSTEFKLPQYGSSALPDKHMEVIDELQKHIQSLNISLLEIKELLSVNSTMTASSKHRMPTRVKHINTTNDNIITR  
APNDYVSSERSTVHTQATDEYFASDIVQYQKYRNIWNWANNSSISHESLFPGKSPEVDTVLNALATARILSIEWFTMG  
KKFYENGTSFKWIAMLEGGQKAVIKLAWEEFGGQKKGGRCDNGHELPAAEIAAFHLHRILGFYNTPTYVSGRWIDLI  
HEIYPVACEFVRKQITLLPNGDACVSSGILMYNDNQTLCSGGKIKASIGYWIPRPLKLYTWYPKYAPFSMARKEWL  
DIGFNKSYCDKIPKTIKPYDQVNYN NDL FDFGLLDMLMYHFDTKHYVIDDGSSANGLTIRLDHGRAFC CYERDSM  
NRVFAPIKQCCSLRKT TYERLKAFRHGNVLSLQLKSALKKDPLYPVLFEGWYSALERRLGVLFD TLEKCIQANGRD  
NVLLSS

> SaFAM20C-2

MKLKQRVVGGLVLCLVLMGVVML KGSLSFPSHLTDTDDTVLKSPHRVKPRHMDPFVRNSVRLSNDANNAGANH  
MPNAYANNMPNVDAPESQMKNGLNELMGHLNVMRQELDASNYTSNDRSPEGQRVQSRQSPSPDQLQKLILGD  
KYIMNRADDIIRLAHVIEETRAGGDVSKNDKPKEFIPNADVEFDKRSQFETGLNDDSDIPPGEHYIRIRKAKERELMA  
DQVEKYEREQKQIRMEVYILPLVMHELEDIATKMTKTRRPSHRRRGATFKIASGSIKNLTGWEEKFHNHITQFDMY  
DPKDPAIQQVLDLARQPLVELYQHDG GTQLK IVYWLENDGRAL FKP MRFRDHETLPNHFYFS DYE RHTAEIASF  
HLDKVLDFHRVPPTAGRAVNMTQDIKMLADSKLLKTFFISPA GNVCFHGQCDYYCDSGHAICGTPDMLLEGSLAAY  
LPSSRYVSRKTWRHPWRRSYHKRKA EWETDNTYCQRVRET PYPYDTGRRLDLMDMSVDFLQGNL DRHHYETF  
KDFGNYTFTMH LDN GRAFGKPRYDDL SILAPVYQCCLIRYSTFMKLV SFEQGPHLSEVIDKSM SHDMLYPILIDKHL  
RALDRRVRIILKTIHRCVKKNGYQAVIQDDGF

> SaFAM20C-3

MMKLKERVIAFAVLCSLFYAFLVLA KYTNILNNTPLRSHQFSADEIFKDSKSQDAHLSMLRRELSLQKEDSGSDSLK  
FDPKEALKVFILNDKVLQEADSIQIAEILKESRNP KKQKYPEYPNNTHYHFQTYGDYHIIRKKPNFNNESSNDHVAYR  
KQVESEIKEVRETQWQREQRHQKRISVNLPEHVIEELE GVLK KMKENDLTPVKKTAPNLDIWLKPGRTELRTWEV  
FHNHITPFDLYKPEDPIIDKILHDLTNLPVVELYQHAGGTQIKFVYWFENGRAL FKP MRFKRDMETLPDHYFSDY  
E RHNAEIASWHLDKVLGFHRVPPTAGRIMNITRDIQELADKDLHGTF FISPAGNLCFYGQCDYYCDSNHAVCGNPT  
MIEGSMAYLPSTNHFDRETWRHPWRRSYSRKKA EWETPDYCHRVKTPPYGSGRRLDIMDLAVFDLQDNL  
DRHHYETFQKFGNYTALLH LDN GRAFGHPRVDDMSILAPISHCCEIRYSTLQKLIGFERGPKLSEIFDKSM SKDPLYP  
LLLDKHLVAIDRRVKILLKTIYKCIAANGYKSVVKDDGF

> SaFAM20C-4

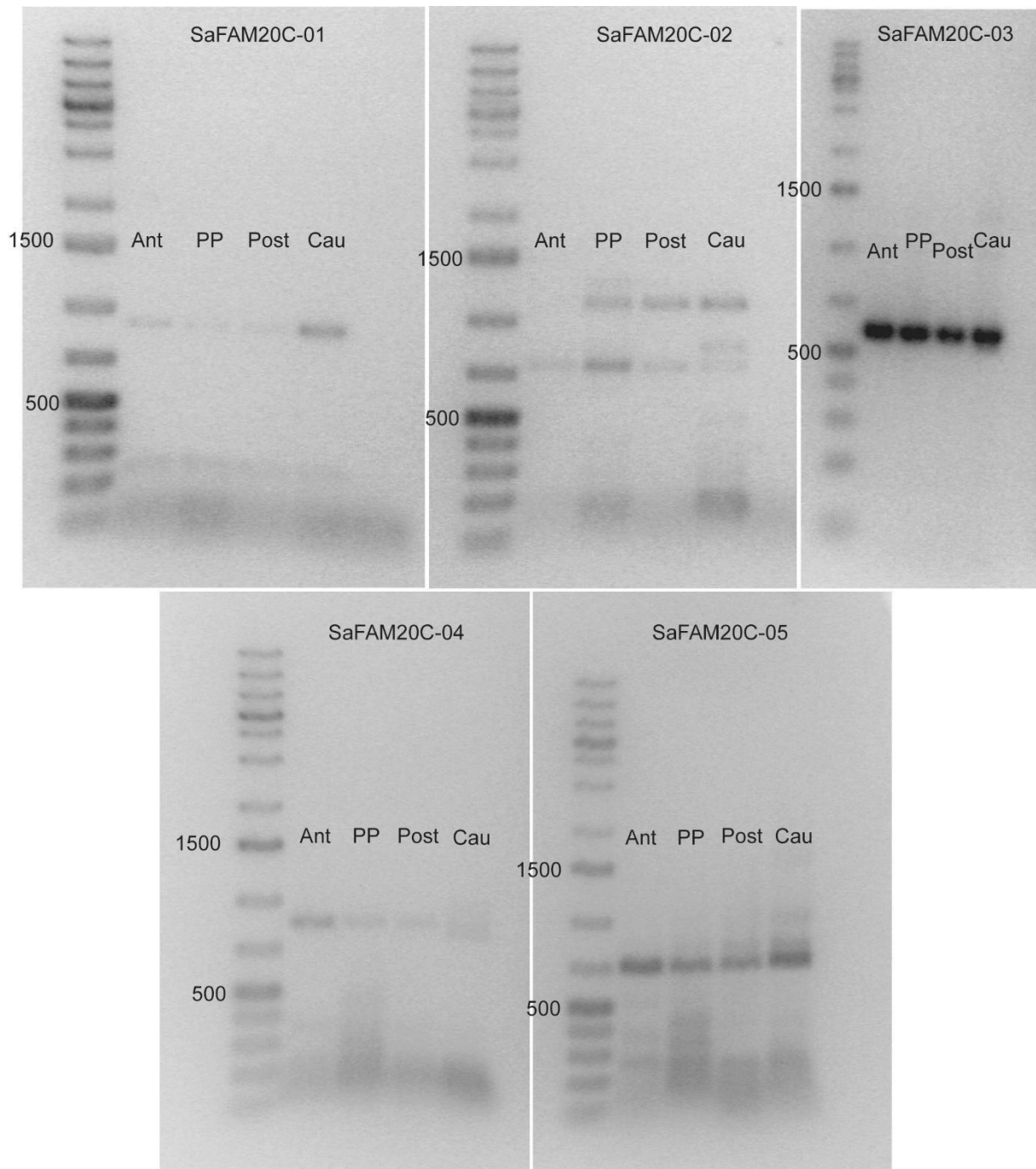
MKLKWRVVSFVFLVFIFYVSYK ILEPELHQGGRRDDGGIYDKADTGN SREFDSDIQEMPLPYNASRGVREKYLLQ  
MYDLQWDRPLKEDPWKVAESWVTHRQVHPDIPEL GAILK WMSVATIQLADVGYK GTQLK MLLQLQGNIPVAFK  
PKWFGRDEIIPGKAWNGAD RHFG EIA GFHLNRILGLNKVPLVVGRTLDLKEEIMPVAKKELLKTFYTKGANTCFYG  
KCLYCKNESTGVCGEFMEGAIVLWLPTKYKYQKWRHPYQRTYKEGKSARWEEDDSYCSLVVKQQLFQNGPR  
LGDILDAAVDYDYLILNAD RHHYE TMSEPWDSMLVMFDSGKSFASPHYDEESILAPLTQCCVIRKNLYERLLMLKDG  
VLSKVLKDILNNDPISVLSNLHLAAMDRLVRILEAVADCLEKWGP EIVFINDEYR

> SaFAM20C-5

MRFRIRGIRCQVAFFVVL MGLVSVYFILFESSTLPDPSSVYFSKDELEKLILNKNIFHNVTYKSPFEAVYKNIMERQAK  
NKS MWQLHPTESPVVREEKYRKVWQWANDSISQEG LFPGDAPQADLV LKALATARIVSVGG LDMSEYESGTSR  
VKWIAQLEGGQKAVIKLVWEKDEWSFSKAKNVIDGQLQSPCNAGHEMCFSEIAGWHLHRVLGFYNTPTYVSGRQLS  
LIEIYPVANEAVRKQISILPGGDACITAKCYLCKYPQRLCVARGMIDASIAYWIPRPLKLYTYPGYPYSTPRMDR  
WEHIGFNNTKYCKELRQTIEPYEKQRYYNDFDFAVLDTL MYHYDSKHYVDDNSKARGLTIRLDHGRAFCFFDE  
DNAEIFLAPITQCCSLRKT TY SRLQELRYGDR LTSRLRSAL EKDPLYPVLSEVWYPTLERRLKLIFAMLEKCIDANGL  
NNVMLKV FKP

Signal peptide / Glycine-rich loop / Beta-3 / Delta-C / the catalytic loop / DFG motif

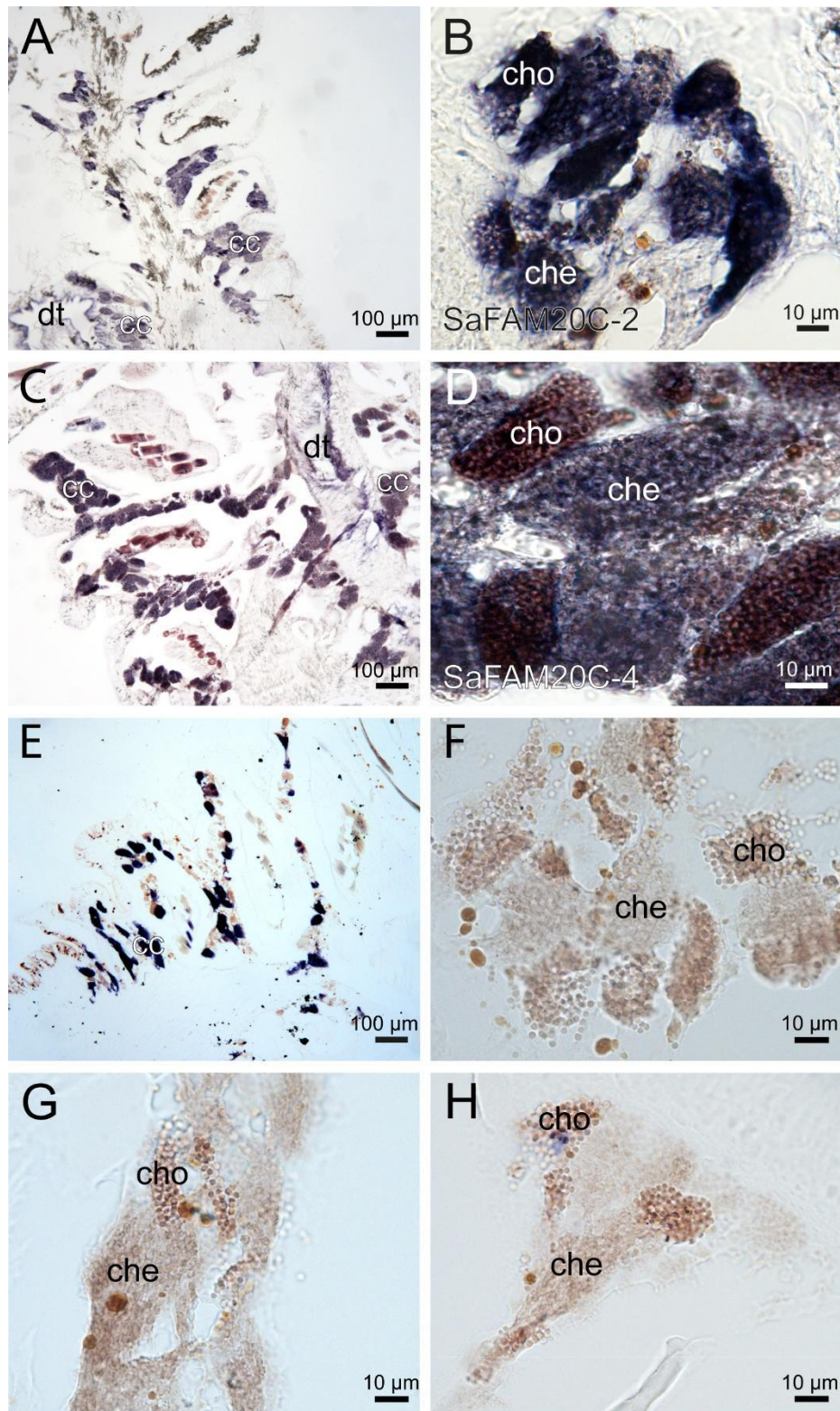
**Figure S4:** Amino acid sequences of the five SaFAM20C proteins retrieved from the transcriptome of *Sabellaria alveolata*. The signature motifs defining those proteins as FAM20C kinases are highlighted as follows: the signal peptide in turquoise, the glycine-rich loop in green, the Beta-3 in mallow, the catalytic loop in grey, and the DFG motif in dark teal.



**Figure S5:** Body region specificity of SaFAM20C candidate genes in *Sabellaria alveolata*. RT-PCR products obtained by using transcript specific primers are separated on an agarose gel. Bands are visible in each lane, indicating the expression of SaFAM20C genes in every part of the honeycomb worm.

Abbreviations: Ant - head region; PP - parathoracic region; Post - abdominal region ; Cau - caudal region.





**Figure S6:** In situ hybridization localization of adhesive and kinase candidates in the parathorax of *Sabellaria alveolata*. Localization of the mRNAs coding for the putative kinases FAM20C-2 (A, B) and FAM20C-4 (C, D) in the cement cells. Overview image of the localization of the mRNAs coding for the adhesive protein Sa-3C (E). Negative controls without probes (F), without antibodies (G), and with sense RNA probes (H).

Abbreviations: cc – cement cells; dt – digestive tract; che – cement cell with heterogeneous granules; cho – cement cell with homogeneous granules.