Supplementary material to "Production, characterization and application of an alginate lyase, AMOR_PL7A, from hot vents in the Arctic Mid-Ocean Ridge"

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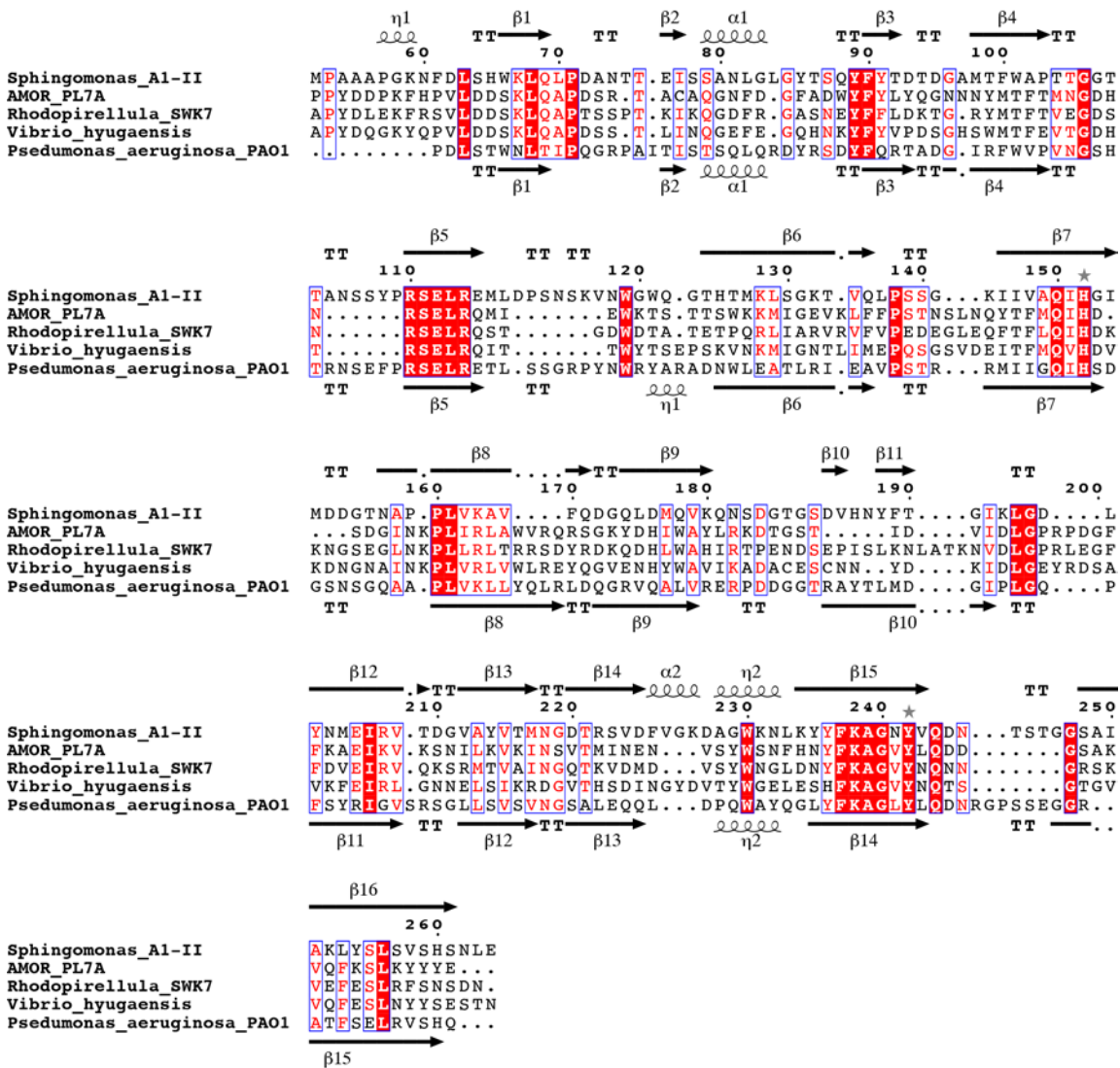
Block 1 (secondary structure: η1, β1, β2, α1, β3, β4)

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                η1        β1            β2    α1        β3        β4
               ℓℓℓ    TT →       TT      →   ℓℓℓℓℓ    TT →  TT    →   TT
                60          70          80          90         100
Sphingomonas_A1-II       MPAAAPGKNFDLSHWKLQLPDANTT.EISSANLGLGYTSQYFYTDTDGAMTFWAPTTGGT
AMOR_PL7A                PPYDDPKFHPVLDDSKLQAPDSR.T.ACAQGNFD.GFADWYFYLYQGNNNYMTFTMNGDH
Rhodopirellula_SWK7      APYDLEKFRSVLDDSKLQAPTSSPT.KIKQGDFR.GASNEYFFLDKTG.RYMTFTVEGDS
Vibrio_hyugaensis        APYDQGKYQPVLDDSKLQAPDSS.T.LINQGEFE.GQHNKYFYVPDSGHSWMTFEVTGDH
Pseudomonas_aeruginosa_PAO1 .........PDLSTWNLTIPQGRPAITISTSQLQRDYRSDYFQRTADG.IRFWVPVNGSH
                         TT →             →    ℓℓℓℓℓ   TT  →TT-. →    TT
                         β1             β2    α1        β3        β4
```

Block 2 (β5, β6, β7)

```
           TT       β5             TT TT             β6          TT        β7
          TT →      →            TT TT              →           TT         →      ★
              110          120          130         140         150
Sphingomonas_A1-II       TANSSYPRSELREMLDPSNSKVNWGWQ.GTHTMKLSGKT.VQLPSSG...KIIVAQIHGI
AMOR_PL7A                N......RSELRQMI.......EWKTS.TTSWKKMIGEVKLFFPSTNSLNQYTFMQIHD.
Rhodopirellula_SWK7      N......RSELRQST......GDWDTA.TETPQRLIARVRVFVPEDEGLEQFTFLQIHDK
Vibrio_hyugaensis        T......RSELRQIT.......TWYTSEPSKVNKMIGNTLIMEPQSGSVDEITFMQVHDV
Pseudomonas_aeruginosa_PAO1 TRNSEFPRSELRETL.SSGRPYNWRYARADNWLEATLRI.EAVPSTR...RMIIGQIHSD
                         TT →               TT          ℓℓℓ      →   . →       TT
                         β5                           η1         β6        β7
```

Block 3 (β8, β9, β10, β11)

```
           TT          β8          β9               β10  β11          TT
          TT →         →      .....→ TT →          →    →            TT
              160          170          180          190         200
Sphingomonas_A1-II       MDDGTNAP.PLVKAV....FQDGQLDMQVKQNSDGTGSDVHNYFT....GIKLGD....L
AMOR_PL7A                ...SDGINKPLIRLAWVRQRSGKYDHIWAYLRKDTGST.....ID....VIDLGPRPDGF
Rhodopirellula_SWK7      KNGSEGLNKPLLRLTRRSDYRDKQDHLWAHIRTPENDSEPISLKNLATKNVDLGPRLEGF
Vibrio_hyugaensis        KDNGNAINKPLVRLVWLREYQGVENHYWAVIKADACESCNN...YD....KIDLGEYRDSA
Pseudomonas_aeruginosa_PAO1 GSNSGQAA.PLVKLLYQLRLDQGRVQALVRERPDDGGTRAYTLMD....GIPLGQ....P
                         TT →                →   TT                →   ...→    TT
                         β8             β9               β10
```

Block 4 (β12, β13, β14, α2, η2, β15)

```
      β12           β13        β14     α2        η2          β15              TT
    →   .►TT →        →  TT →       →   ℓℓℓℓ    ℓℓℓℓℓ       →                 TT    →
        210          220          230          240         250
Sphingomonas_A1-II       YNMEIRV.TDGVAYVTMNGDTRSVDFVGKDAGWKNLKYYFKAGNYVQDN...TSTGCSAI
AMOR_PL7A                FKAEIKV.KSNILKVKINSVTMINEN...VSYWSNFHNYFKAGVYLQDD.......GSAK
Rhodopirellula_SWK7      FDVEIRV.QKSRMTVAINGQTKVDMD...VSYWNGLDNYFKAGVYNQNN.......GRSK
Vibrio_hyugaensis        VKFEIRL.GNNELSIKRDGVTHSDINGYDVTYWGELESHFKAGVYNQTS.......GTGV
Pseudomonas_aeruginosa_PAO1 FSYRIGVSRSGLLSVSVNGSALEQQL...DPQWAYQGLYFKAGLYLQDNRGPSSEGGR..
                         →       TT        →   TT      →       ℓℓℓℓℓ   →        TT  →  ..
                         β11            β12        β13           η2        β14
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Block 5 (β16)

```
           β16
          →
              260
Sphingomonas_A1-II       AKLYSLSVSHSNLE
AMOR_PL7A                VQFKSLKYYYE...
Rhodopirellula_SWK7      VEFESLRFSNSDN.
Vibrio_hyugaensis        VQFESLNYYSESTN
Pseudomonas_aeruginosa_PAO1 ATFSELRVSHQ...
                         →
                         β15
```

Figure S1. Domain structure and sequence of AMOR_PL7A. A) Domain structure of AMOR_PL7A, drawn to scale. The full-length enzyme contains a signal peptide (SP: residues 1-24) and a polysaccharide lyase family 7 domain (PL7: 66-257). B) Amino acid sequence alignment of the catalytic domain of AMOR_PL7A with the two most related PL7 alginate lyase domains with a known structure, from *Sphingomonas* sp A1-II (Taxon identifier: 90322; PDB id: 2CWS) and from *Pseudomonas aeruginosa* (strain ATCC 15692; PDB id: 1VAV) and the two PL7 alginate lyases with the highest sequence identity, from *Rhodopirellula* sp. SWK7 (EMI43849) and *Vibrio hyugaensis* (WP_052441931.1). The sequence numbering for AMOR_PL7A is shown above the alignment. Conserved residues appear in white on a red background, and conservatively substituted residues are printed in red. Putative catalytic residues[1] are indicated by black asterisks. Secondary structure assignments are based on the crystal structures of *Sphingomonas* sp. A1-

II (top; PDB id: 2CWS) and *Pseudomonas aeruginosa* (bottom; PDB id: 1VAV) and defined as follows: α1-3; α-helices, β1-16; β-sheets, η1-2; 3_{10}-helices and TT; strict β-turns.
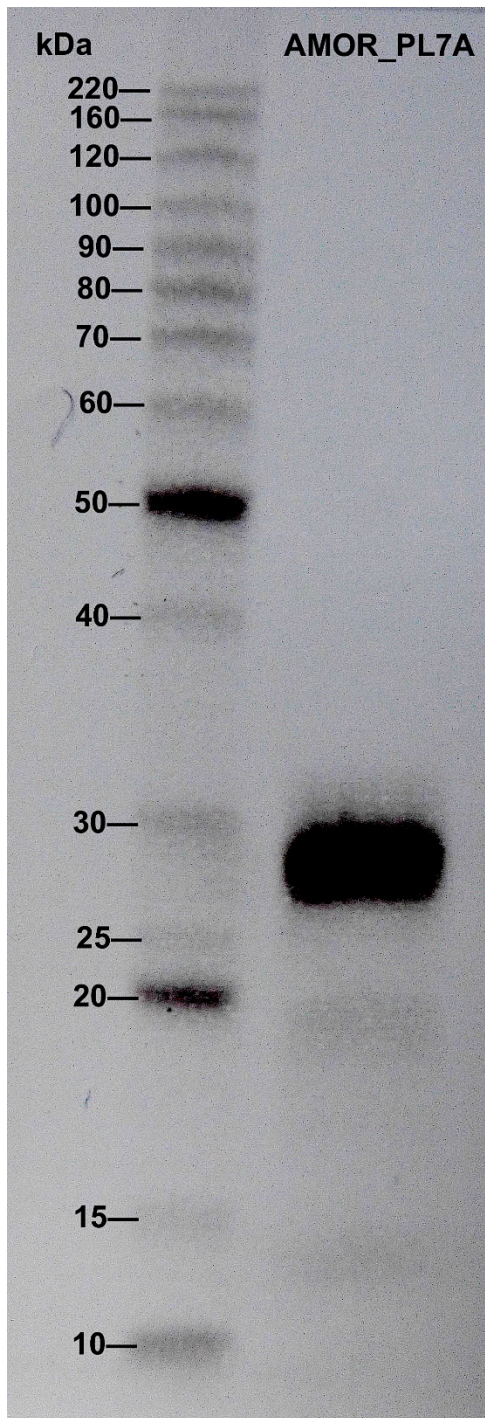
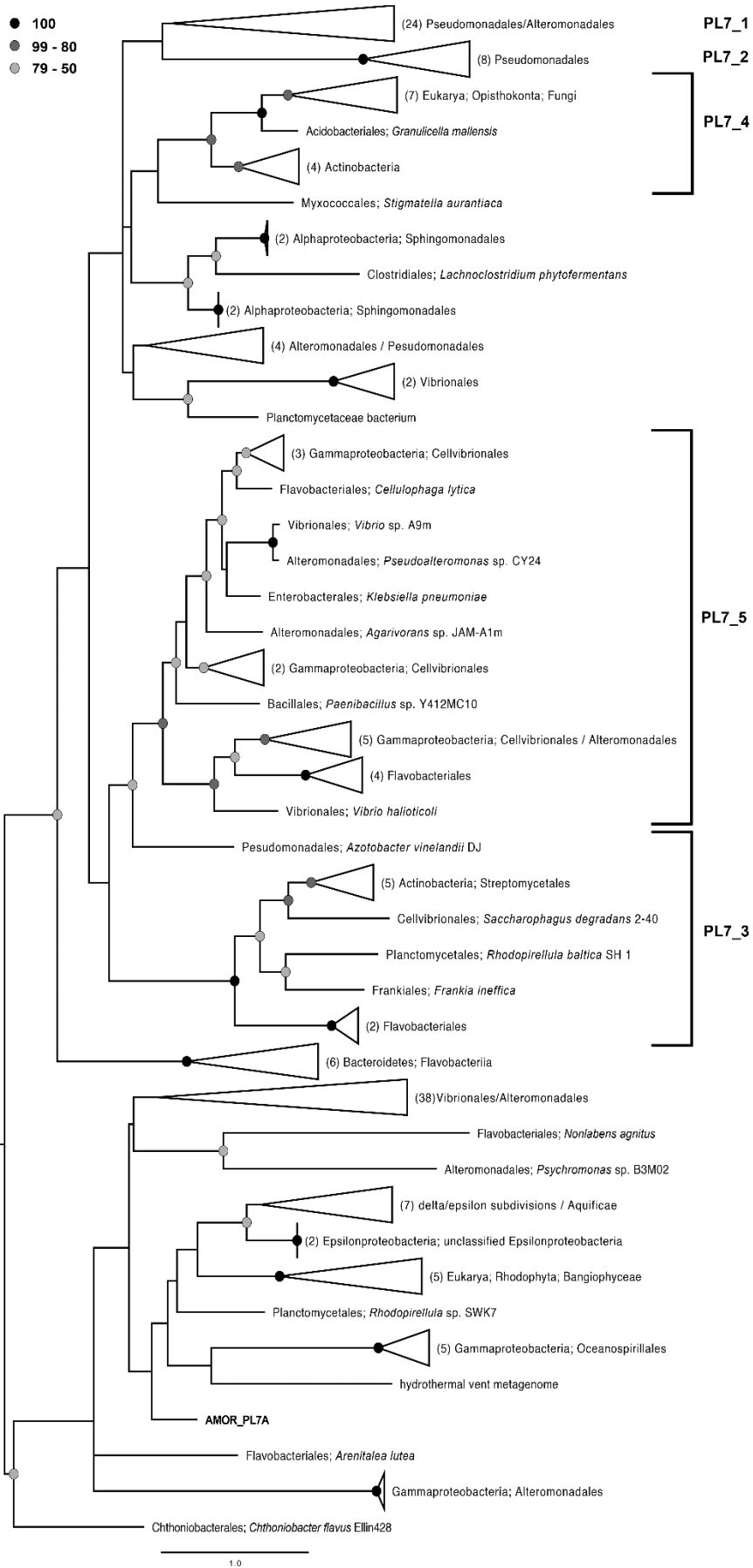**Figure S2. SDS-PAGE analysis of purified AMOR_PL7A including a C-terminal His-tag.**

- ● 100
- ● 99 - 80
- ● 79 - 50

(24) Pseudomonadales/Alteromonadales — PL7_1

(8) Pseudomonadales — PL7_2

(7) Eukarya; Opisthokonta; Fungi

Acidobacteriales; *Granulicella mallensis*

(4) Actinobacteria

Myxococcales; *Stigmatella aurantiaca*

PL7_4

(2) Alphaproteobacteria; Sphingomonadales

Clostridiales; *Lachnoclostridium phytofermentans*

(2) Alphaproteobacteria; Sphingomonadales

(4) Alteromonadales / Pesudomonadales

(2) Vibrionales

Planctomycetaceae bacterium

(3) Gammaproteobacteria; Cellvibrionales

Flavobacteriales; *Cellulophaga lytica*

Vibrionales; *Vibrio* sp. A9m

Alteromonadales; *Pseudoalteromonas* sp. CY24

Enterobacterales; *Klebsiella pneumoniae*

Alteromonadales; *Agarivorans* sp. JAM-A1m

(2) Gammaproteobacteria; Cellvibrionales

Bacillales; *Paenibacillus* sp. Y412MC10

(5) Gammaproteobacteria; Cellvibrionales / Alteromonadales

(4) Flavobacteriales

Vibrionales; *Vibrio halioticoli*

PL7_5

Pesudomonadales; *Azotobacter vinelandii* DJ

(5) Actinobacteria; Streptomycetales

Cellvibrionales; *Saccharophagus degradans* 2-40

Planctomycetales; *Rhodopirellula baltica* SH 1

Frankiales; *Frankia ineffica*

(2) Flavobacteriales

PL7_3

(6) Bacteroidetes; Flavobacteriia

(38) Vibrionales/Alteromonadales

Flavobacteriales; *Nonlabens agnitus*

Alteromonadales; *Psychromonas* sp. B3M02

(7) delta/epsilon subdivisions / Aquificae

(2) Epsilonproteobacteria; unclassified Epsilonproteobacteria

(5) Eukarya; Rhodophyta; Bangiophyceae

Planctomycetales; *Rhodopirellula* sp. SWK7

(5) Gammaproteobacteria; Oceanospirillales

hydrothermal vent metagenome

AMOR_PL7A

Flavobacteriales; *Arenitalea lutea*

Gammaproteobacteria; Alteromonadales

Chthoniobacterales; *Chthoniobacter flavus* Ellin428

1.0

4

Figure S3. Phylogenetic analysis of AMOR_PL7A. Sequences of PL7 were retrieved from dbCAN and domain architecture similarity searches using EMBL-EBI HMMER[2], aligned using MAFFT-LINSi[3,4], trimmed with trimal, and analyzed usingIQ-TREE[5]. The numbers at the nodes indicate bootstrap values based on 100 bootstrap replications. Branch support values above 50 are indicated by dots. Collapsed branches are indicated by the number of sequences in parenthesis. PL7 subfamilies are indicated based on the classification in CAZy[6]. The scale bar indicates the number of amino acid substitutions per site.
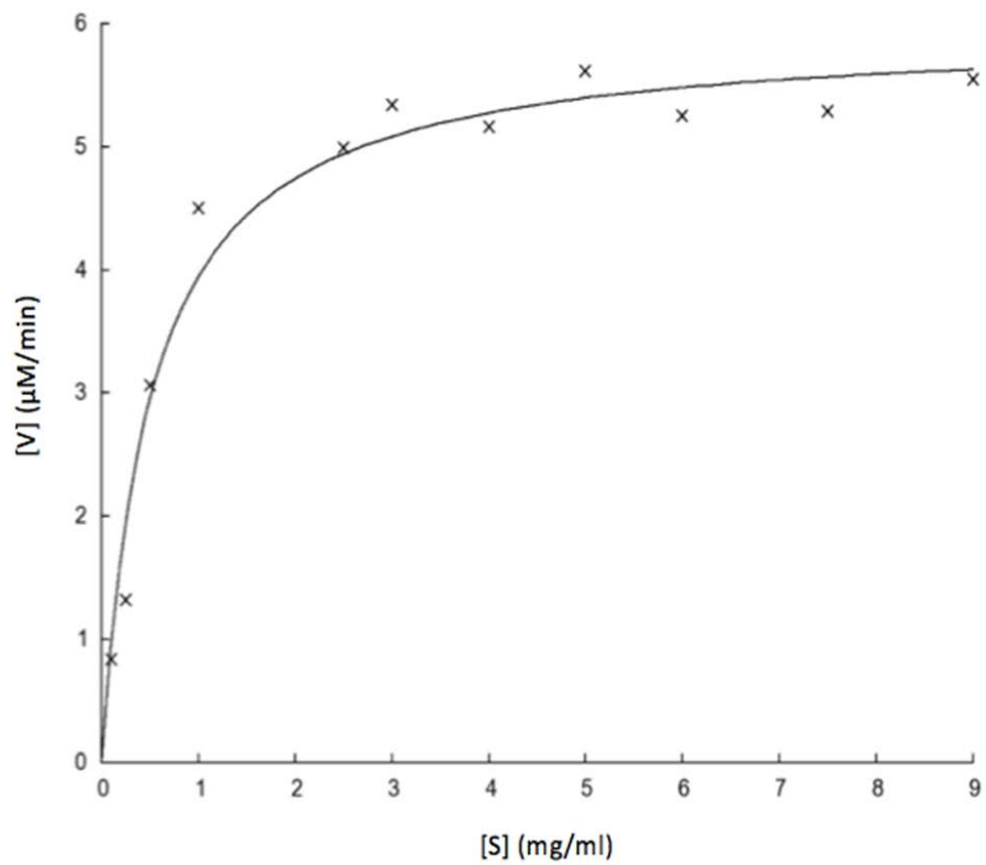
Figure S4. Steady state kinetics of AMOR_PL7A. The graph shows a non-linear fit for the hydrolysis of

sodium alginate by AMOR_PL7A.
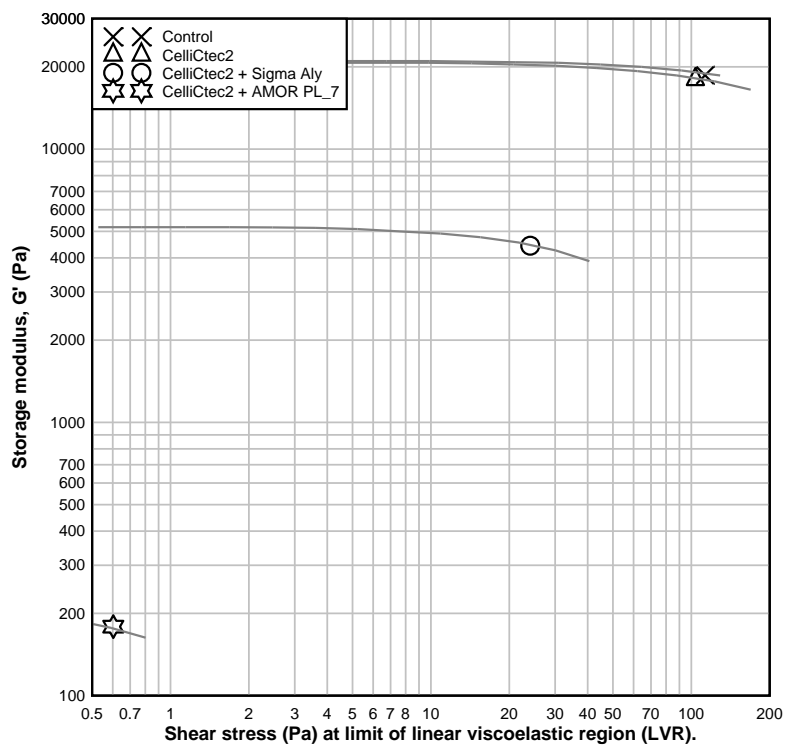
Figure S5. Oscillatory measurements. The picture shows the storage modulus, G', versus Shear stress (Pa) at the limit of the Linear Viscoelastic Region (LVR). The viscoelasticity of a material can be characterized by amplitude sweep measurements where the sample is exposed to an oscillatory deformation at a constant frequency. In oscillatory rheology, measurements are non-destructive when operating in the LVR where the applied strain or shear stress does not damage the structure. Amplitude sweeps at a constant frequency and increasing strain are used to characterize the sample. The limit of the LVR reflects the strength of the structure and shows how much strain it can be exposed to without damage. The limit of the linear LVR is here defined by a reduction of the storage modulus, G', by 3%. The storage modulus, G', is an elastic modulus representing how stiff the structure is, so a lower value of G' represents a softer sample.
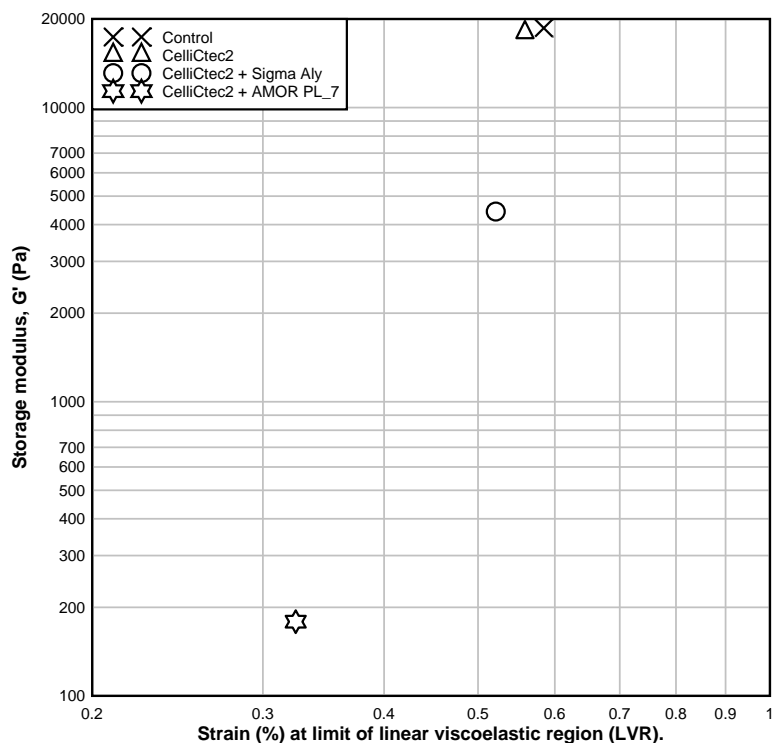
Figure S6. Oscillatory measurements. The picture shows the storage modulus, G', versus strain % at the limit

of the LVR.

References

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