Supplemental Figures for

Lithotrophic Fe-oxidizing bacteria form organic stalks to control iron mineral growth: implications for biosignature genesis

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8 Figures

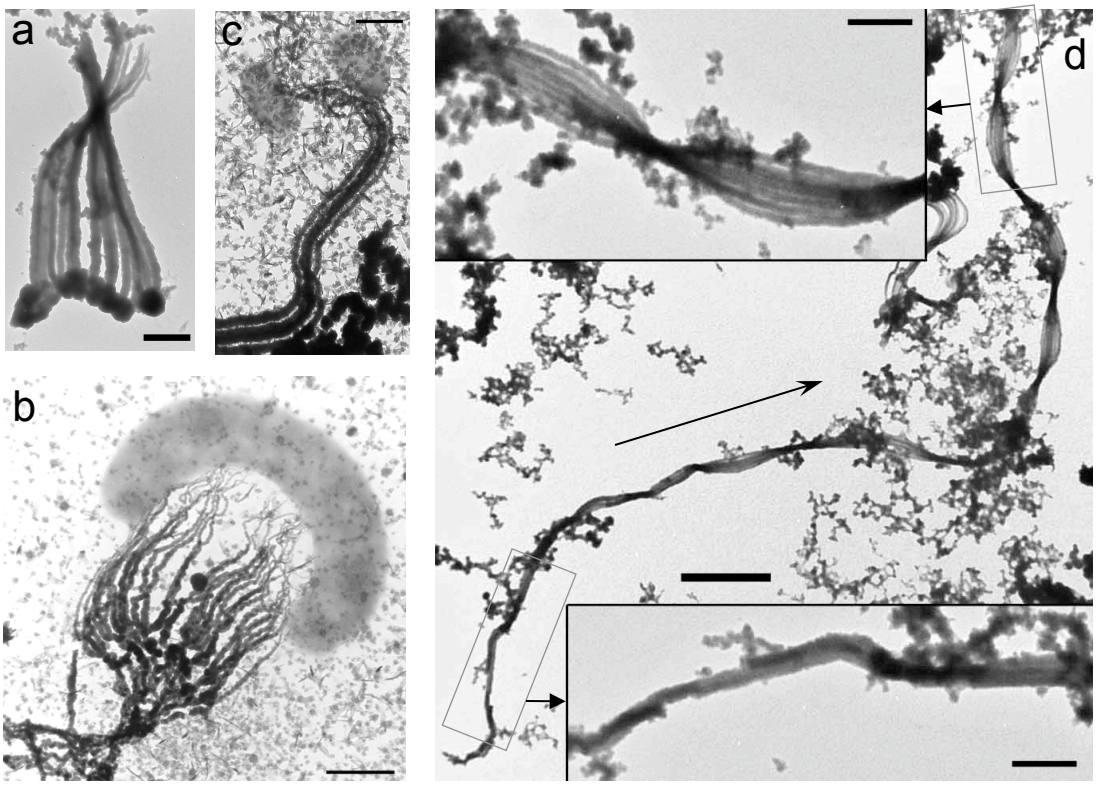

Figure S1. TEM images showing: a) short stalk exhibiting denser mineralization on holdfast end, scale bar = 0.5 μm, b) longer cell and stalk, scale bar = 0.5 μm, c) two cells on a stalk, possibly branched, scale bar = 0.5 μm, d) changes in stalk and filament width and number of filaments along the length of the stalk. Arrow indicates direction of stalk growth. Scale bars: main image = 2 μm, insets = 0.5 μm.

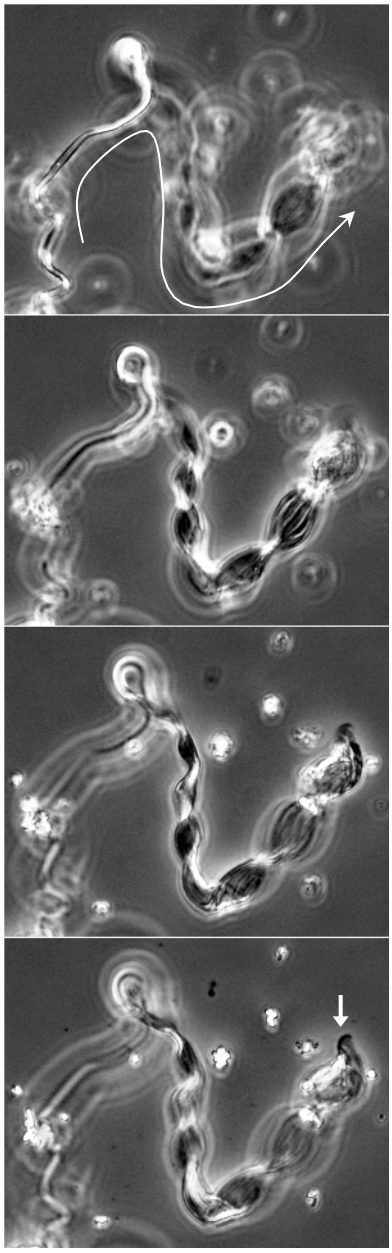

Figure S2. Focus series of phase contrast micrographs showing changes in stalk width and number of filaments along the length of the stalk. Arrow in top image shows direction of stalk growth. Arrow in bottom image points to curved rod-shaped cell (partially obscured).

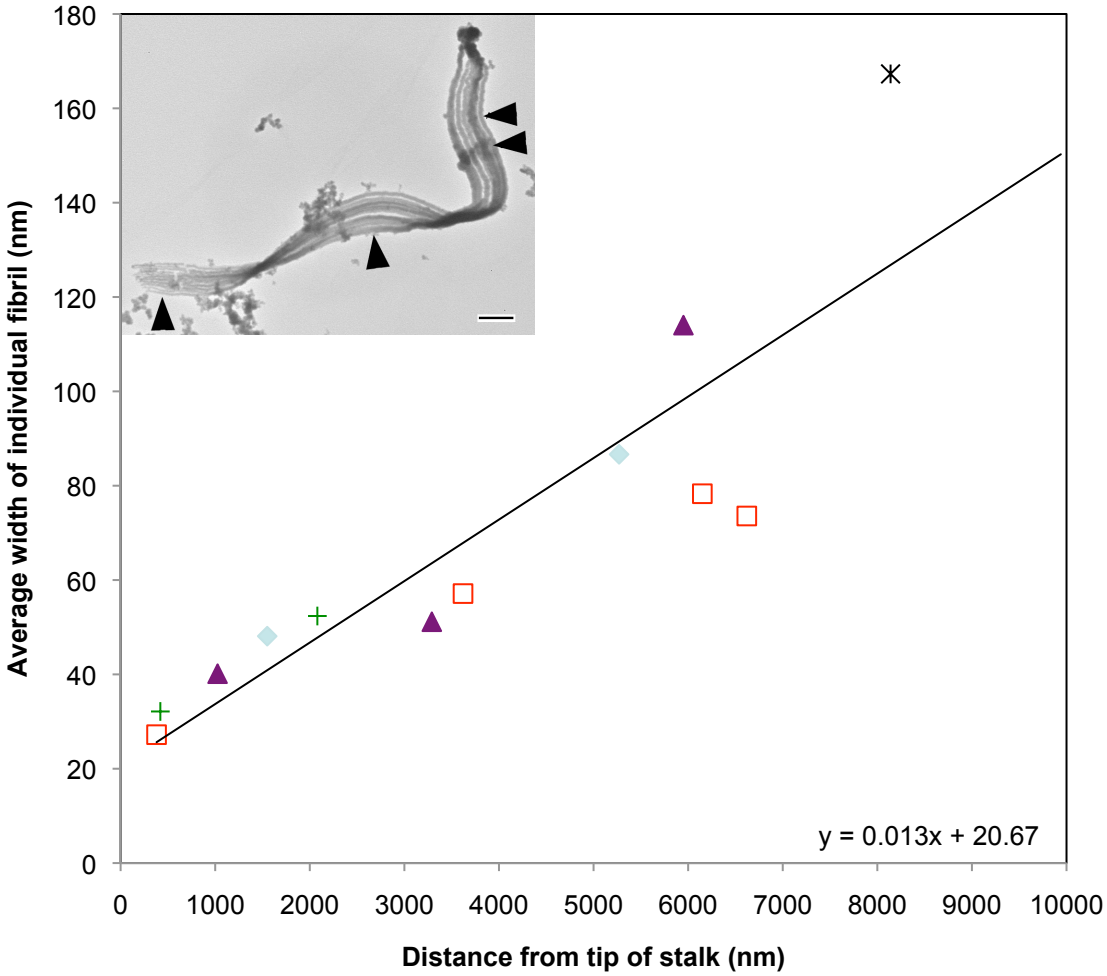

Figure S3. Average width of stalk fibril vs. distance from tip of stalk (i.e. end where cell is attached). Each point represents the average width of a fibril measured at a particular point on a TEM image. Each symbol type represents a different stalk measured; five stalks were measured from one sample. (Inset shows one example of stalk image, with arrows pointing to measurement locations. At each location, several fibril widths were measured and averaged. The stalk tip is on the left side of the image. This pictured stalk corresponds to the red open squares on the graph. Scale bar = 500 nm.) Measurements were only made where fibril width was clear (i.e. no overlap between fibrils); because this was rare, few stalks could be measured accurately.

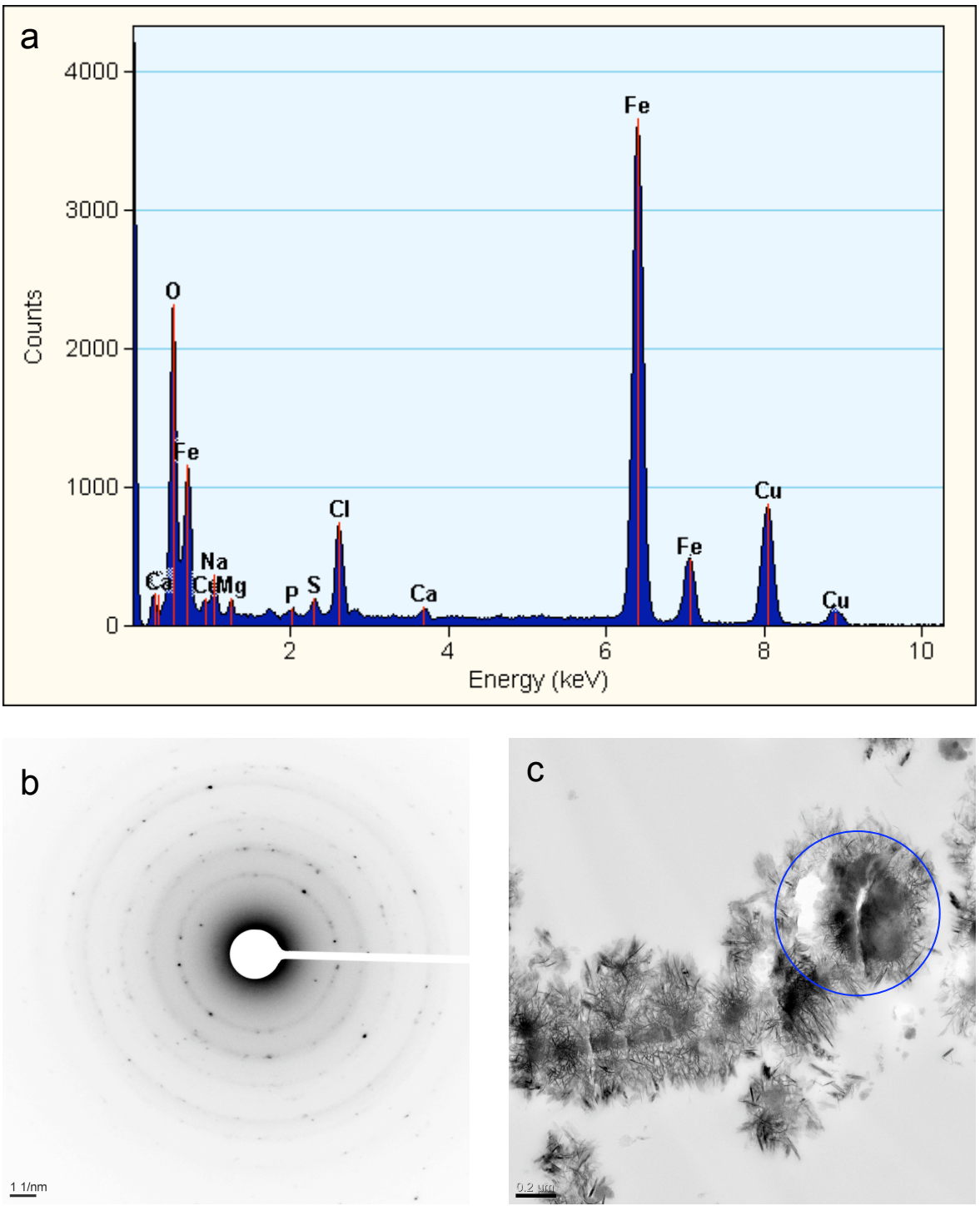

Figure S4. a) Energy dispersive X-ray spectrum showing elemental composition of lightly-mineralized stalk, showing that it is rich in Fe and O. Cu is part of the TEM grid; Cl and other elements are likely due to artificial seawater medium residue. b) Electron diffraction of highly mineralized stalk. c) TEM image of sectioned stalk showing diffracted area (circled). Scale bar = 0.2 µm.

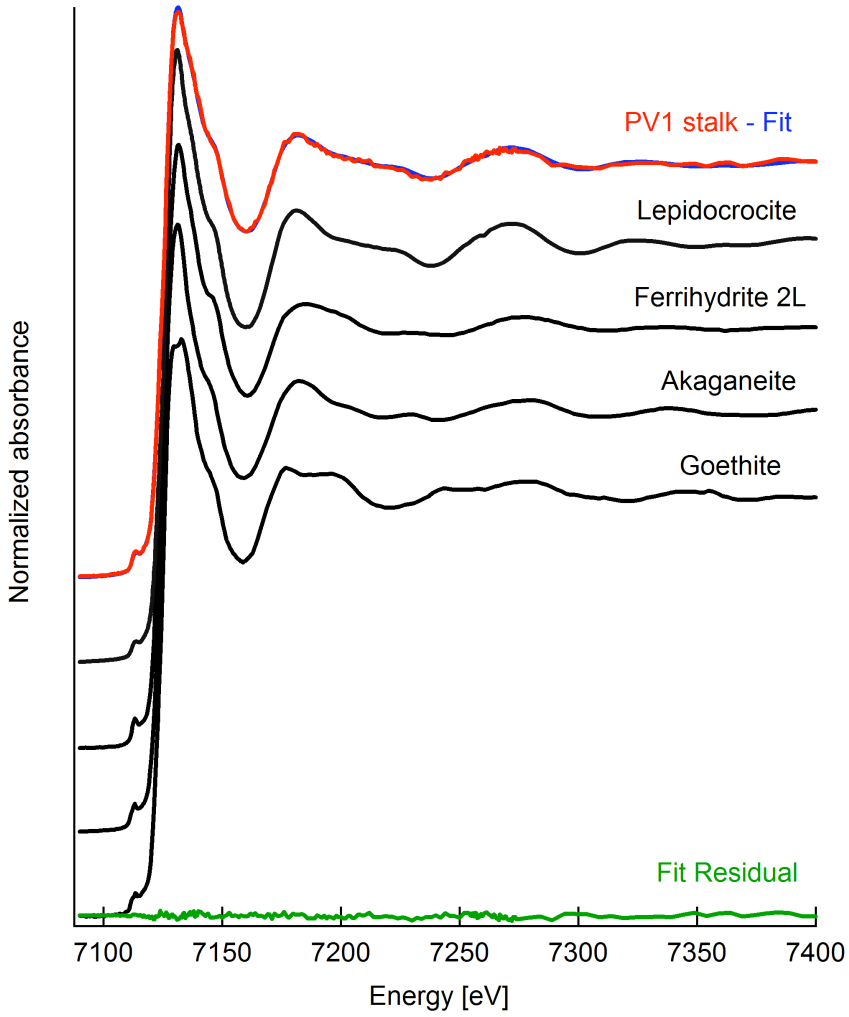

Figure S5. Fe K-edge micro-XANES spectrum of *M. ferrooxydans* stalk (in red), along with its best least squares fit (in blue) obtained with 76% lepidocrocite and 24% 2-line ferrihydrite (norm. sum square =2.48 x 10^{-5}). FeOOH standards (in black) are displayed for comparison.

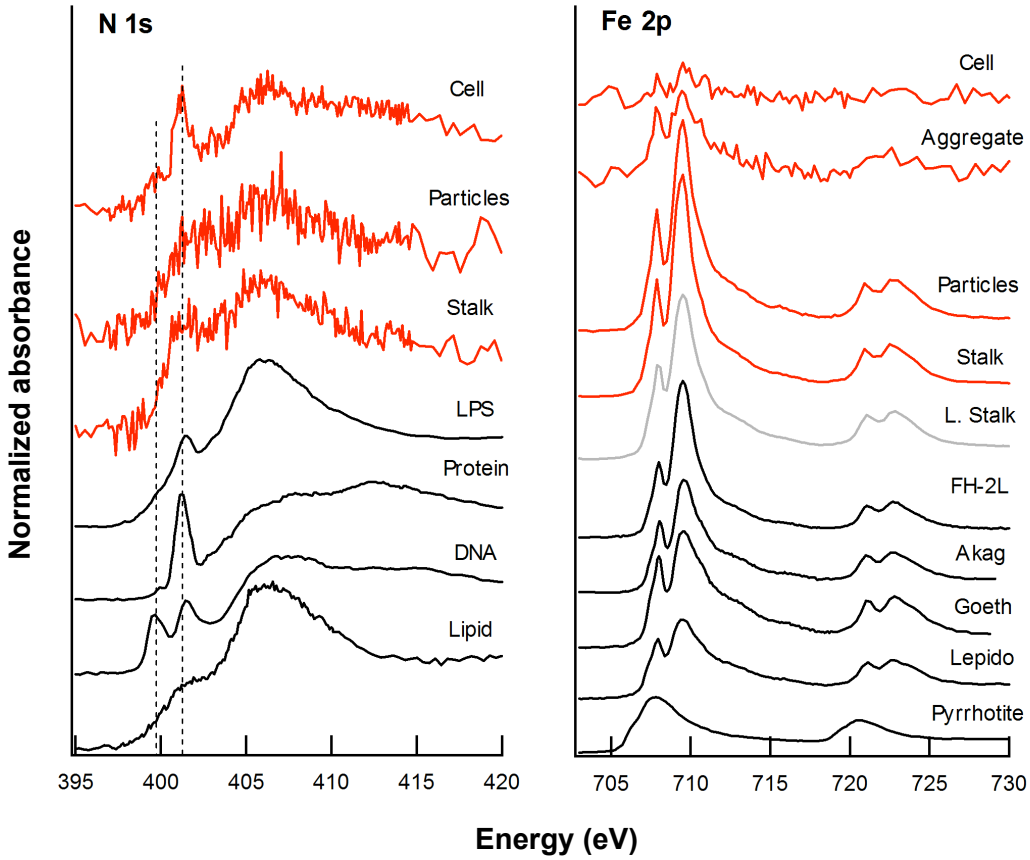

Figure S6. N 1s and Fe 2p NEXAFS spectra of culture (stalk, cells and surrounding mineral particles), Loihi microbial mat stalk, and standards. N standards include *E. coli* lipopolysaccharide (LPS), bovine serum albumin (protein), DNA, and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (phospholipid). Fe standards (confirmed by XRD) include the iron oxyhydroxides 2-line ferrihydrite, akaganeite, goethite, and lepidocrocite and the Fe(II) sulfide pyrrhotite. The presence of lepidocrocite in cultured stalks was confirmed by XRD (Fig. S4).

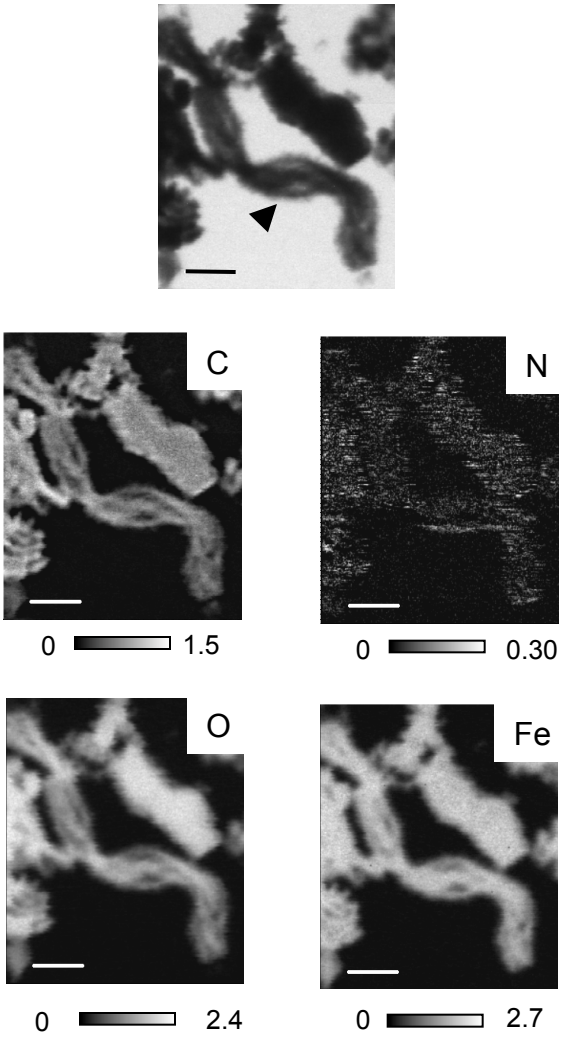

Figure S7. STXM image (C 1s edge, 300eV) and elemental maps of stalks in a Loihi microbial mat sample. Arrow points to twisted stalk. Intensity scales are in optical density units. Scale bars=1 μm.

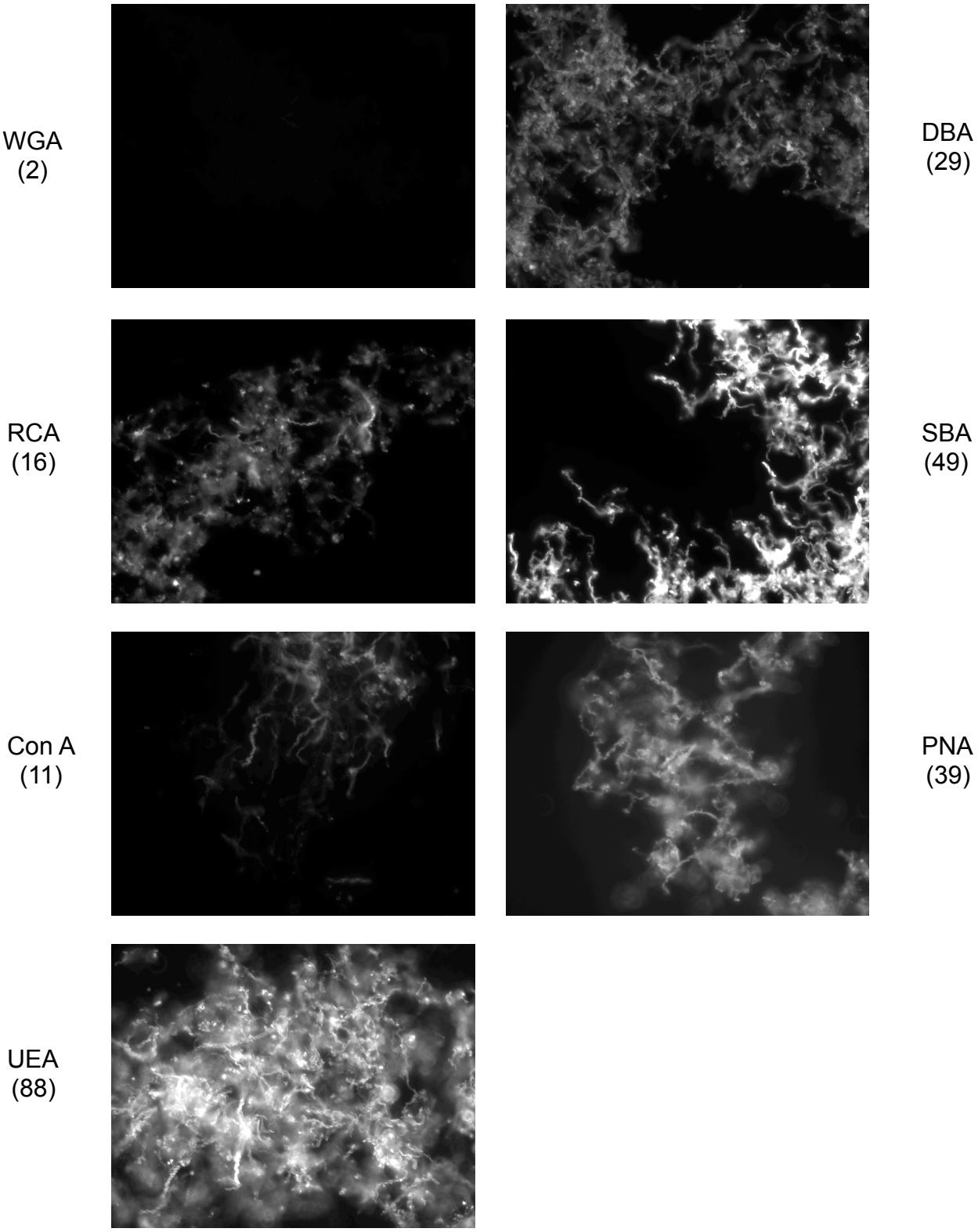

Figure S8. Epifluorescence images of rhodamine lectin-stained *Mariprofundus* stalks, showing that stalks contain polysaccharide. Text labels indicate lectin type; number in parentheses is the average intensity value. The WGA lectin bound poorly while the other six lectins bound to varying degrees. The poor binding of WGA indicates that rhodamine itself is not labeling the stalks. Exposure time for all images was 702 ms. Lectins used: WGA Wheat germ agglutinin, DBA *Dolichos biflorus* agglutinin, RCA *Ricinus communis* agglutinin I, SBA soybean agglutinin, Con A Concanavalin A, PNA Peanut agglutinin, UEA *Ulex europaeus* agglutinin I.