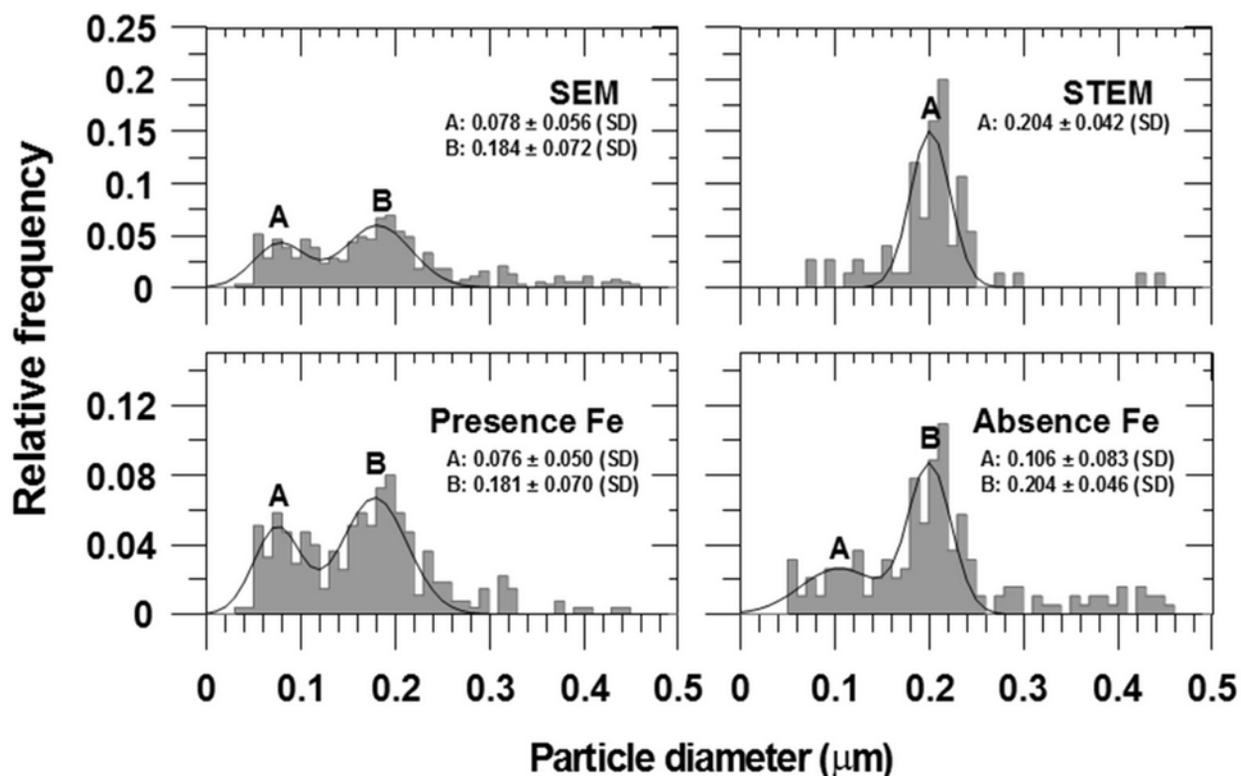
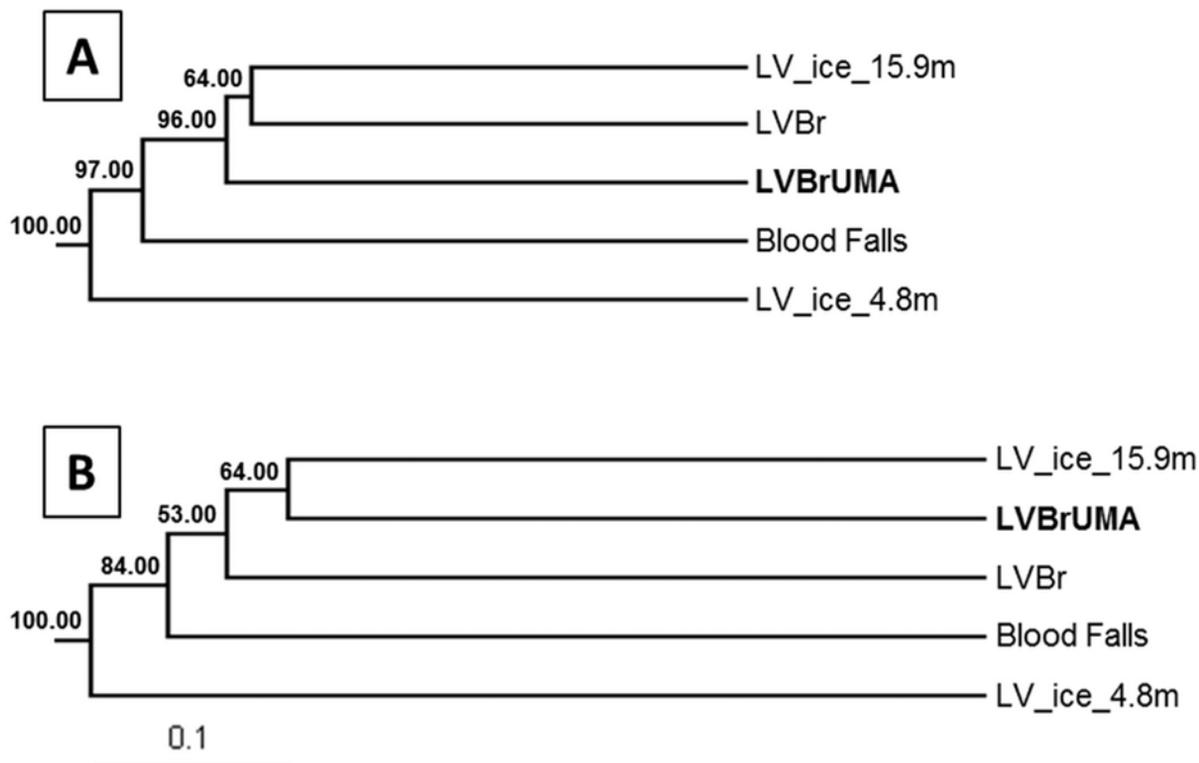


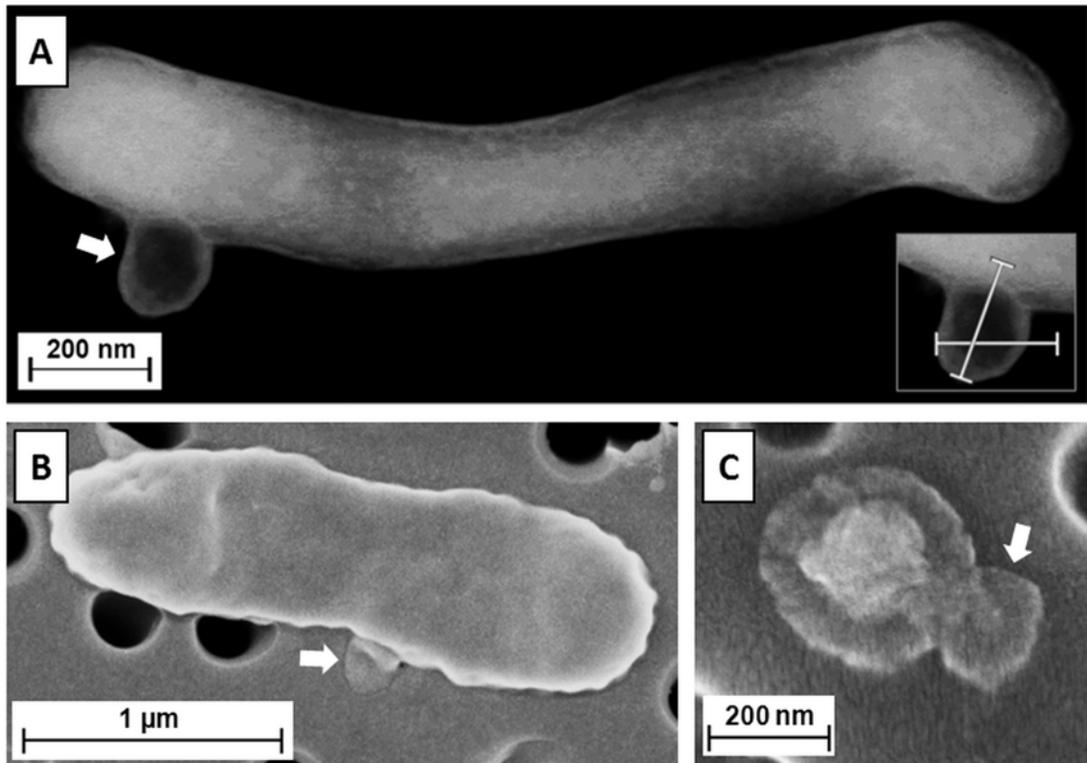
**Fig. S1.** Diagram of Lake Vida brine sampling system. A 3/8” ID PTFE tubing connected to a battery-powered submersible pump was used to bring the brine to the surface where it was directed to different sampling lines. One line was used for collecting the brine in bottles under nitrogen atmosphere (left), and the other line carried the brine into an anaerobic glove bag (right). The depth of the sampling hole was approximately 20.4 m; brine infiltrated the borehole until it reached the depth of approximately 11 m; the pump head was positioned at 18.40 m.



**Fig. S2** Size distribution of cells and unidentified nanoparticles in Lake Vida brine. Size distribution of 465 cells/particles measured from SEM and STEM observations and in the presence and the absence of amorphous Fe<sub>3</sub>O<sub>4</sub> are shown in five distinct graphs. Gaussian distribution fits and standard deviation are indicated in each graph.



**Fig. S3** Comparison of LVBrUMA with previously characterized microbial assemblages from Lake Vida and Blood Falls. (A) UniFrac cluster dendrogram considering the phylogenetic diversity and the normalized abundance weight (evenness) of the 16S rRNA gene sequences of the microbial assemblages compared. (B) UniFrac cluster dendrogram considering the phylogenetic diversity based on only the presence and absence of the 16S rRNA gene sequences of the microbial assemblages compared. The jack-knife statistic shown at the nodes represents the fraction of the random samples that the branching order was recovered in. LVBrUMA: Lake Vida brine ultra-small microbial assemblage; LVBr: Lake Vida brine microbial assemblage collected in 2005 (1); LV\_ice\_4.8 m and LV\_ice\_15.9 m: Lake Vida ice core microbial assemblages from the depth sections 4.8 m and 15.9 m (2); Blood Falls: microbial assemblage from Taylor Glacier’s subglacial outflow (3).



**Fig. S4** External cellular structures observed in Lake Vida brine cells that could be representative of membrane vesicles (indicated by arrows). (A) STEM dark field micrograph of a brine cell stained with UA pH4-5. Detail on the right shows the scale bar (200 nm) on the external cellular structure; (B) SEM micrograph of a brine cell presenting a similar structure as (A); (C) SEM micrograph of an ultra-small cell from the brine presenting a capsular structure surrounding the cell and an extracellular structure that could be also representative of a membrane vesicle. Scale bars are shown in each micrograph.

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