

Supplementary Fig. 1 Positive and negative control experiments for CARD-FISH hybridization and visualization of loki- and heimdallarchaeotal cells. **A-K** Non-sense probe NON338 was used in second hybridizations after the first hybridization with Loki- or Heimdallarchaeota specific probes to confirm that detected Loki- and Heimdallarchaeota morphotypes are not random probe-binding or auto-fluorescent particles. **L-O** As positive control for the hybridizations with Lokiarchaeota specific probes, general archaea probe ARC915 was used in second hybridizations. Note that ARC915 does not target Heimdallarchaeota. **P-R** Potential false-positive signals lacking double hybridization with the second probe, which resembles large ovoid and filamentous cells detected in Salcher et al. *mSphere*, 2020. Three-dimensional (3D) surface reconstructions from confocal laser scanning microscope (CLSM) imaging are depicted. All z-stack images for true-positive loki- and heimdallarchaeotal cells are included in Supplementary Fig. 2. Probe names and dyes for each panel are included. The scale bar is 1 μm .







