

## Supplemental text for

### **Subgroup characteristics of marine methane-oxidizing ANME-2 archaea and their syntrophic partners revealed by integrated multimodal analytical microscopy**

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### **Site location and sample description:**

Sediment sample 5133 was collected using a standard 30 cm push core within a white filamentous microbial mat from an active methane seep located at Hydrate Ridge North (station HR-7; 44° 40.02N, 125° 6.00 W; 600 m water depth). Processing and analysis of 5133 has been previously described in (Trembath-Reichert, Green-Saxena and Orphan, 2013; McGlynn *et al.*, 2015). The push core was processed shipboard following recovery by removing surface layers containing the bacterial mat and transferring the next ~9 cm into a sealed N<sub>2</sub> flushed mylar bag. This sample was stored at 4°C until transport back to the lab, where it was transferred into a sterile 1L pyrex bottle on ice in an anaerobic chamber (Coy), mixed with chilled N<sub>2</sub> sparged 0.22µm filtered bottom seawater from the Hydrate Ridge site at a ratio of 1:3 sediment

to liquid, and sealed with a large butyl stopper. The sediment incubation was then over-pressurized with a CH<sub>4</sub> headspace (2 bar) and incubated in the dark at 6°C. This large sediment microcosm maintained active sulfate-coupled methane oxidation in the laboratory and was sampled periodically to obtain fresh sediment for the investigations described here.

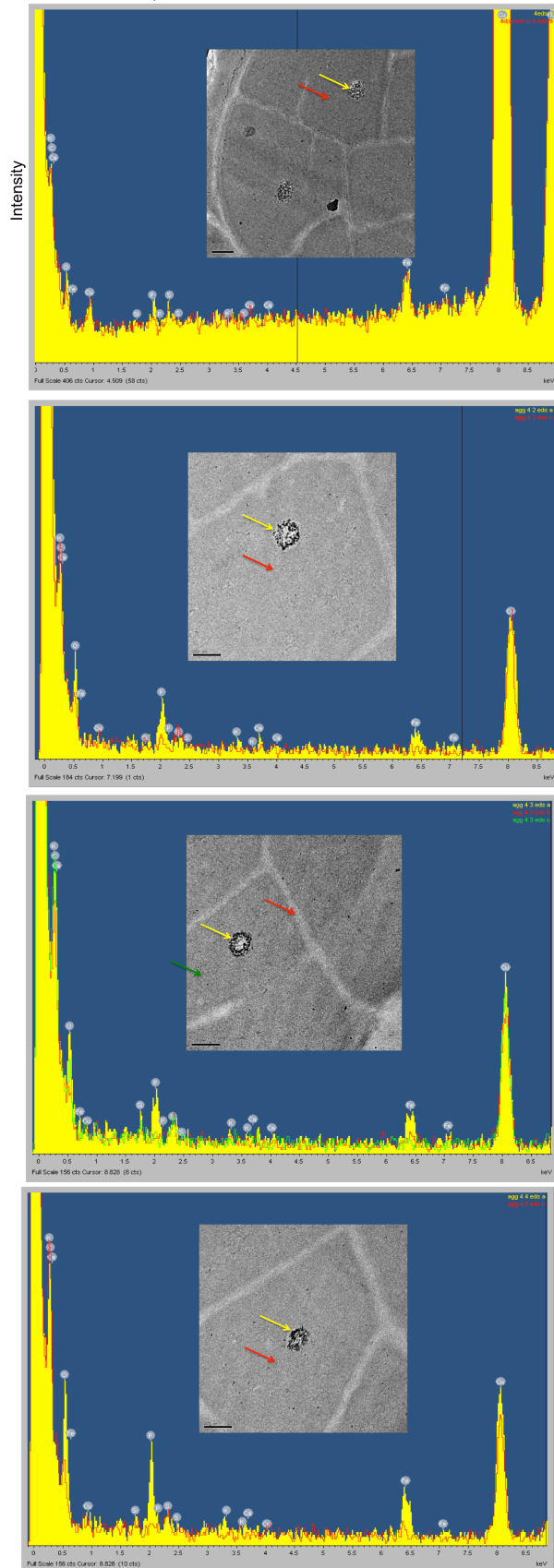
Sediment incubation “5546-5549 / 5556-5560” was also collected during the AT18-10 cruise and is comprised of pooled samples from two adjacent push cores (PC31; 5546-5549: at the edge of the clam bed), and PC26; 5556, 5558-5560 collected in an active chemosynthetic clam bed (*Calymptogena* sp); from Hydrate Ridge North (station HR-4, 44° 40.20N, 125° 5.88W; 596m depth). Here, each 4-digit serial number represents a discrete 3 cm thick depth section from each core, with #5546 and #5556 representing the 0-3 cm horizon from PC 31 and PC26, respectively. Sediments were transferred into 2 large pyrex bottles, flushed with nitrogen to remove methane and mixed with chilled 0.22µm filtered Hydrate Ridge bottom water in an anaerobic chamber as described above. The mud slurries from both bottles were then combined into a sterile 3L beaker in an anaerobic chamber (Coy) with a magnetic stir bar for continuous mixing. The settled mud volume in the slurry was 450mL. Filtered N<sub>2</sub> sparged bottom seawater collected from the field site was added to a final volume of 1.6 L. Individual aliquots of this mud slurry (40 ml) were subsequently added to 60 mL serum vials (for parallel experiments not included in this study), sealed with butyl rubber stoppers, over pressurized with methane (2 bar), and stored at 4°C in the dark.

Sample 3730 represented the top 0-6 cm of sediment collected by push core within a *Calypptogena* sp. clam bed exhibiting active methane bubbling (Hydrate Ridge South, 44° 34.09N; 125° 9.14; 775 m water depth). Sediments were immediately transferred into a 1 L Pyrex bottle onboard ship, flushed with N<sub>2</sub> gas, sealed with a large butyl rubber stopper and maintained at 4°C. A sediment slurry (500 mL) was subsequently created in an anaerobic chamber (Coy) by mixing one volume of the collected sediment with two volumes 0.22µm filtered and N<sub>2</sub> sparged bottom seawater from Hydrate Ridge. Samples were over-pressured with methane and incubated as described above. The 3730 incubation was comprised predominantly of ANME-2 and ANME-1 archaea representing different subgroups, with many forming large aggregations with bacteria (Hatzenpichler *et al.*, 2016). The relative abundance of archaea observed by 16S rRNA Illumina TAG sequencing included 58% ANME-1, 25% ANME-2a/2b, 16% ANME-2c, and less than 1% non-ANME archaea (Hatzenpichler *et al.*, 2016).

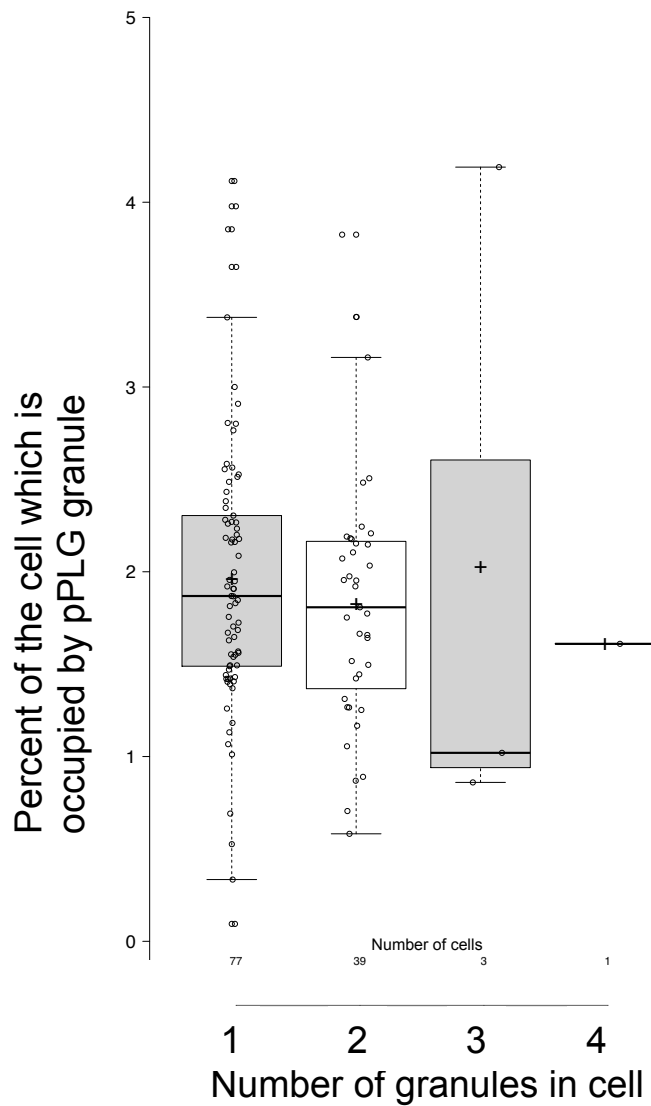
1. Trembath-Reichert E, Green-Saxena A, Orphan VJ. 2013. Chapter Two - Whole Cell Immunomagnetic Enrichment of Environmental Microbial Consortia Using rRNA-Targeted Magneto-FISH, p. 21–44. *In* DeLong, EF (ed.), *Methods in Enzymology*. Academic Press.
2. McGlynn SE, Chadwick GL, Kempes CP, Orphan VJ. 2015. Single cell activity reveals direct electron transfer in methanotrophic consortia. *Nature* 526:531–535.
3. Hatzenpichler R, Connon SA, Goudeau D, Malmstrom RR, Woyke T, Orphan VJ. 2016. Visualizing in situ translational activity for identifying

and sorting slow-growing archaeal–bacterial consortia. *Proc Natl Acad Sci*  
113:E4069–E4078.

Cells and EDS spectra of ANME2B-like cells from the 3730 incubation



**Supplemental Figure 1.** TEM-EDS spectra of pPLG containing cells from the 3730 incubation.



**Supplemental Figure 2.** Percent of cells volume occupied by pPLG for cells with different pPLG numbers. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; data points are plotted as open circles.