

**Supplementary Information for**  
**Comparative single-cell genomics of *Chloroflexi* from the Okinawa Trough**  
**deep subsurface biosphere**

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**Table S1.** Phylogeny and metabolism of select organisms in the phylum *Chloroflexi*.

| Organism                                    | Order                    | Metabolic Classification          | Accession Number                                |
|---|--------------------------|-----------------------------------|---|
| <i>Anaerolinea thermophila</i>              | <i>Anaerolineales</i>    | Anaerobic Heterotroph             | NC_014960                                       |
| <i>Caldilinea aerophila</i>                 | <i>Caldilineales</i>     | Facultative Heterotroph           | NC_017079                                       |
| <i>Chloroflexus aurantiacus</i>             | <i>Chloroflexales</i>    | Anoxygenic Photoautotroph         | NC_010175                                       |
| <i>Dehalogenimonas lykanthroporepellens</i> | <i>Dehalococcoidales</i> | Anaerobic Dehalorespirer          | GCA_000143165                                   |
| <i>Nitrolancetus hollandicus</i>            | <i>Sphaerobacterales</i> | Chemoautotrophic Nitrite Oxidizer | CAGS01000001                                    |
| <i>Thermomicrobium roseum</i>               | <i>Thermomicrobiales</i> | Aerobic Chemoheterotroph          | CP001275 (chromosome)<br>CP001276 (Megaplasmid) |

**Table S2.** One Carbon Pool Genes

| Carbon Utilization Pathway |   |                  | Dehalococcoidales | Thermoflexales | Anaerolineales |            |                           |            |                  |            |            |
|----------------------------|---|------------------|-------------------|----------------|----------------|------------|---------------------------|------------|------------------|------------|------------|
| Enzyme                     | EC number   |                  | AB-790-I04        | AB-790-L07     | AB-790-K11     | AC-715-B04 | AB-790-B16                | AC-711-E09 | AC-711-L07       | AC-711-J10 | AC-711-B22 |
| One Carbon pool by Folate  | Dihydrofolate reductase   | 1.5.1.3          |                   |                |                |            |                           | 0115       |                  |            |            |
| One Carbon pool by Folate  | Formyltetrahydrofolate dehydrogenase                              | 1.5.1.6          |                   |                |                |            |                           |            |                  |            |            |
| One Carbon pool by Folate  | Thymidylate synthase  | 2.1.1.45         |                   |                |                |            |                           | 0116       |                  |            |            |
| One Carbon pool by Folate  | Phosphoribosylglycinamide formyltransferase                       | 2.1.2.3/2.1.2.2  | 0159c             |                |                |            |                           |            | 0803             |            |            |
| One Carbon pool by Folate  | 5-methyltetrahydrofolate--homocysteine methyltransferase          | 2.1.1.13         |                   |                |                |            | 0599, 0651c, 0657c, 0998c |            | 0365             | 0400       | 0307       |
| One Carbon pool by Folate  | Formimidoyltetrahydrofolate cyclodeaminase                        | 4.3.1.4          |                   |                | 0064c          |            |                           |            |                  | 0682       |            |
| One Carbon pool by Folate  | methionyl-tRNA formyltransferase                                  | 2.1.2.9          | 0081c             |                |                | 0409c      |                           |            |                  | 0261       |            |
| Wood-Ljungdahl             | carbon-monoxide dehydrogenase catalytic subunit                   | 1.2.99.2/1.2.7.4 |                   |                | CooS=0059c     |            |                           |            | Large Chain=0723 |            |            |
| Wood-Ljungdahl             | 5,10-Methylenetetrahydrofolate reductase (NAD(P))                 | 1.5.1.20         |                   |                |                | 0343, 0347 | 0309                      |            |                  |            | 0375c      |
| Wood-Ljungdahl             | CO dehydrogenase/acetyl-CoA synthase, acetyl-CoA synthase subunit | 2.3.1.169        |                   |                | 0055c          |            |                           |            |                  |            |            |
| Wood-Ljungdahl             | Formate dehydrogenase, alpha and delta subunit                    | 1.2.1.2          |                   |                | 0279           |            | 0768c                     |            |                  | 0063       | 0854       |
| Wood-Ljungdahl             | Formyltetrahydrofolate synthetase                                 | 6.3.4.3          |                   |                | 0062c          |            |                           |            |                  |            |            |
| Wood-Ljungdahl             | 5,10-methylenetetrahydrofolate dehydrogenase                      | 1.5.1.5          |                   |                | 0063c          | 0351       |                           |            |                  | 0311       | 0370c      |
| Wood-Ljungdahl             | cyclohydrolase  | 3.5.4.9          |                   |                | 0063c          | 0351       |                           |            |                  | 0311       | 0370c      |

**Table S3.** Glycolysis Genes

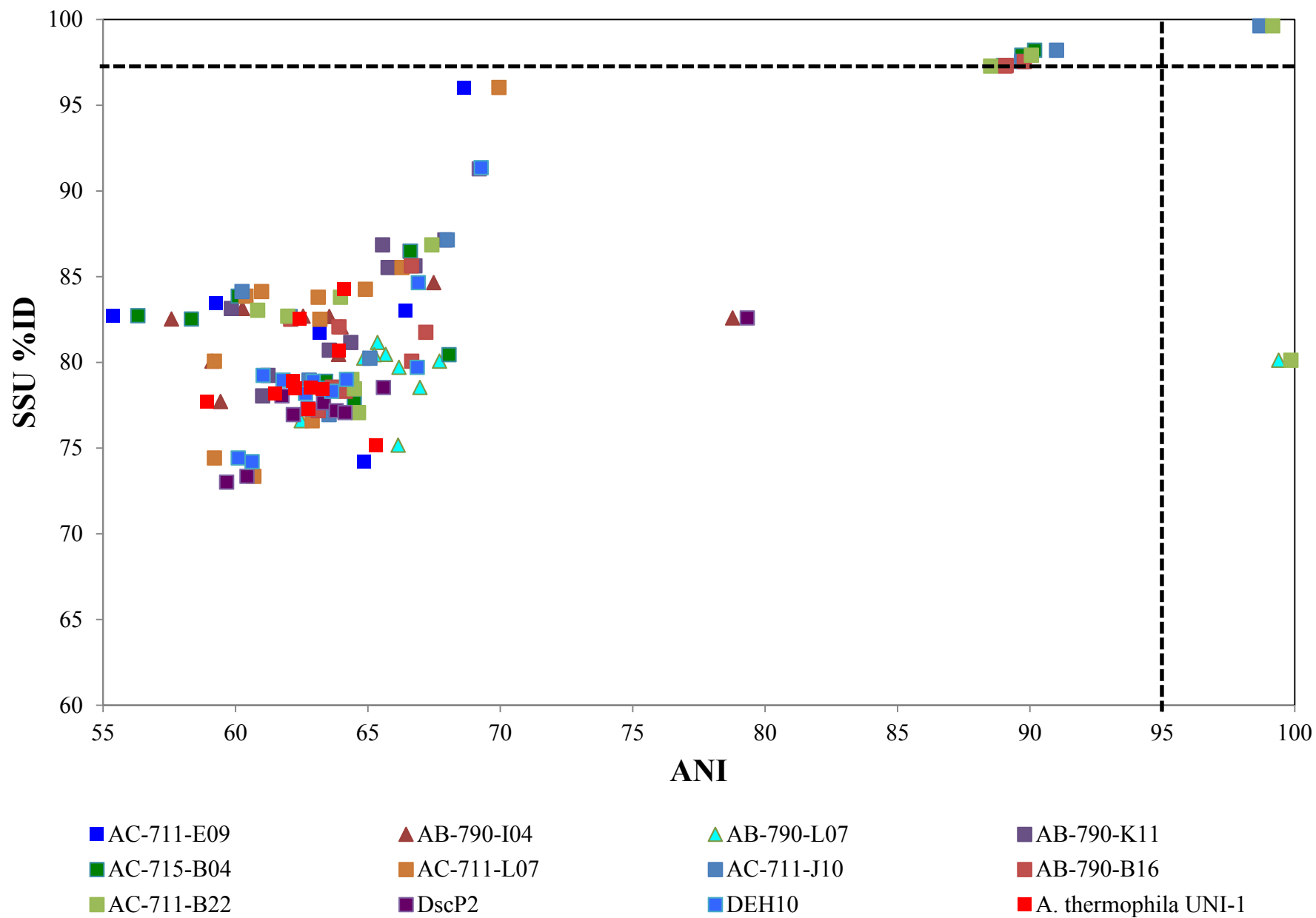
| Carbon Utilization |  |                 | Dehalococcoidales | Thermoflexales | Anaerolineales                  |   |               |                                   |                                   |   |            |
|--------------------|--|-----------------|-------------------|----------------|---------------------------------|---|---------------|-----------------------------------|-----------------------------------|---|------------|
| Pathway            | Enzyme   | EC number       | AB-790-I04        | AB-790-L07     | AB-790-K11                      | AC-715-B04  | AB-790-B16    | AC-711-E09                        | AC-711-L07                        | AC-711-J10  | AC-711-B22 |
| Glycolysis         | Phosphoglucomutase   | 5.4.2.2         |                   |                |                                 | 0194  | 0718c         |                                   |                                   |   | 0165c      |
| Glycolysis         | glucokinase  | 2.7.1.2         |                   |                | 0102                            |   |               |                                   | 0287c                             |   |            |
| Glycolysis         | isomerase/mannose-6-phosphate isomerase, archaeal                          | 5.3.1.9/5.3.1.8 |                   | 0333           |                                 |   |               |                                   |                                   | 0652c   | 1150c      |
| Glycolysis         | 6-phosphofructokinase  | 2.7.1.11        |                   |                | 0068c                           |   | 0943c         |                                   |                                   |   |            |
| Glycolysis         | fructose-bisphosphatase  | 3.1.3.11        |                   |                |                                 |   | 0505          |                                   |                                   |   |            |
| Glycolysis         | fructose-bisphosphate aldolase   | 4.1.2.13        |                   |                |                                 |   | 0817c         |                                   |                                   |   |            |
| Glycolysis         | Triosephosphate isomerase  | 5.3.1.1         |                   |                |                                 |   | 0095c         |                                   |                                   |   |            |
| Glycolysis         | glyceraldehyde-3-phosphate dehydrogenase                                   | 1.2.1.12        |                   |                |                                 |   | 0944c         |                                   |                                   |   |            |
| Glycolysis         | glyceraldehyde-3-phosphate dehydrogenase                                   | 1.2.1.59        |                   |                |                                 |   |               |                                   |                                   |   | 1092       |
| Glycolysis         | Phosphoglycerate kinase  | 2.7.2.3         |                   |                |                                 |   |               |                                   | 0775c                             |   |            |
| Glycolysis         | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, archaeal type | 5.4.2.1         |                   |                | 0018c                           |   | 0606c         |                                   | 0541                              | 0831c   | 1279       |
| Glycolysis         | Enolase  | 4.2.1.11        |                   |                |                                 | 0143c   | 0294          |                                   |                                   |   |            |
| Glycolysis         | Pyruvate Kinase  | 2.7.1.40        |                   |                |                                 |   | 0945c         |                                   |                                   |   |            |
| Glycolysis         | Pyruvate dehydrogenase E1 component  | 1.2.4.1         |                   |                |                                 |   |               | $\alpha=0146c$ ,<br>$\beta=0147c$ | $\alpha=0012c$ ,<br>$\beta=0013c$ |   |            |
| Glycolysis         | Pyruvate:ferredoxin oxidoreductase   | 1.2.7.1         |                   |                | $\alpha=0317$ ,<br>$\beta=0318$ | $\alpha=0532c$ ,<br>$\beta=0531c$ ,<br>$\gamma=0534c$ ,<br>$\delta=0533c$ | $\beta=1197c$ |                                   |                                   | $\alpha=0200c$ ,<br>$\beta=0199c$ ,<br>$\gamma=0202c$ ,<br>$\delta=0201c$ |            |
| Glycolysis         | Dihydrolipoamide dehydrogenase   | 1.8.1.4         |                   |                | 0338                            |   | 0174c         |                                   | 0297c                             |   |            |
| Glycolysis         | Acetate-CoA ligase   | 6.2.1.1         |                   |                |                                 | 0158  | 0222c         |                                   |                                   | 0250c   |            |

**Table S4.** TCA Cycle Genes

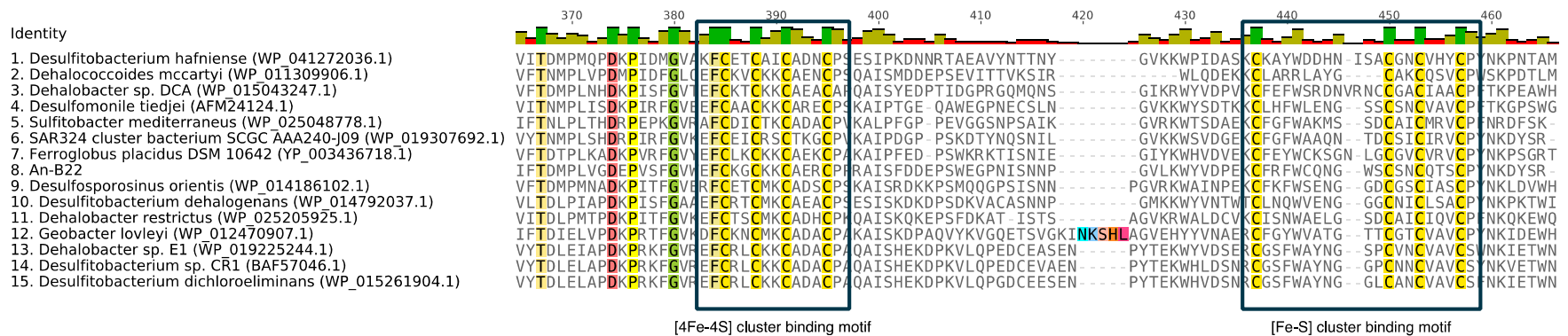
| Carbon Utilization |   |                   | Dehalococcoidales | Thermoflexales | Anaerolineales |   |  |            |                     |   |   |
|--------------------|---|-------------------|-------------------|----------------|----------------|---|--|------------|---------------------|---|---|
| Pathway            | Enzyme                                    | EC number         | AB-790-I04        | AB-790-L07     | AB-790-K11     | AC-715-B04  | AB-790-B16   | AC-711-E09 | AC-711-L07          | AC-711-J10  | AC-711-B22  |
| TCA                | Citrate Synthase                          | 2.3.3.1           |                   |                |                |   |  | 0089       |                     |   |   |
| TCA                | Aconitase                                 | 4.2.1.3           |                   |                |                |   |  |            |                     |   |   |
| TCA                | Isocitrate dehydrogenase                  | 1.1.1.42/1.1.1.41 |                   | 0084           |                |   |  |            |                     |   | 0381c   |
| TCA                | 2-Oxoacid:acceptor oxidoreductase         | 1.2.7.3           |                   |                |                | $\alpha=0512c$ ,<br>$\beta=0488c/0513$ ,<br>$\gamma=0514c$ ,<br>$\delta=0511$ | $\alpha=0609/0610$ ,<br>$\beta=0134c/0611$ ,<br>$\gamma=0612$ ,<br>$\delta=0608$ |            |                     | $\alpha=0075/0544c$ ,<br>$\beta=0076$ ,<br>$\gamma=0077$ ,<br>$\delta=0074$ | $\alpha=1089$ ,<br>$\beta=1090$ ,<br>$\gamma=0588$ ,<br>$\delta=0587$ |
| TCA                | Succinyl-CoA synthetase (ADP-forming)     | 6.2.1.5           |                   |                |                |   |  |            |                     |   | $\alpha=1198$ ,<br>$\beta=1196$                                       |
| TCA                | Fumarate Reductase                        | 1.3.5.4           |                   |                |                |   |  |            | C=0522c,<br>D=0521c |   |   |
| TCA                | Succinate Dehydrogenase                   | 1.3.5.1           |                   |                |                |   |  |            | 0523c,<br>0524c,    | 0756c   | 1176c   |
| TCA                | Fumarase, class I, aerobic                | 4.2.1.2           |                   |                |                | 0072, 0073  | 0778, 0780   |            |                     |   | 0317, 0319  |
| TCA                | Malate Dehydrogenase                      | 1.1.1.37          |                   | 0085           |                |   |  |            |                     |   | 0249c   |
| TCA                | Pyruvate/oxaloacetate carboxyltransferase | 6.4.1.1           |                   |                |                |   | 0801c  |            |                     |   |   |

**Table S5.** Estimated genome size based percent genome recovery as determined by tRNAs of nearest neighbor.

| SAG Name   | Nearest Sequenced Neighbor                          | SSU % ID to Nearest Neighbor | Estimated Genome Recovery (Based on Nearest Neighbor tRNA Count) | Estimated Size of Genome (Based on tRNAs) |
|------------|---|------------------------------|--|---|
| AC-711-E09 | <i>Anaerolinea thermophila</i> UNI-1                | 82.54%                       | 4%   | 3,831,175                                 |
| AB-790-I04 | <i>Dehalogenimonas lykanthroporepellens</i> BL-DC-9 | 87.41%                       | 11%  | 1,768,736                                 |
| AB-790-L07 | <i>Thermoflexus hugenholtzii</i> JAD2               | 85.62%                       | 16%  | 2,908,579                                 |
| AB-790-K11 | <i>Anaerolinea thermophila</i> UNI-1                | 80.72%                       | 36%  | 1,371,942                                 |
| AC-715-B04 | <i>Anaerolinea thermophila</i> UNI-1                | 78.50%                       | 8%   | 7,697,700                                 |
| AC-711-L07 | <i>Anaerolinea thermophila</i> UNI-1                | 84.27%                       | 22%  | 3,639,645                                 |
| AC-711-J10 | <i>Anaerolinea thermophila</i> UNI-1                | 78.95%                       | 40%  | 2,527,060                                 |
| AB-790-B16 | <i>Anaerolinea thermophila</i> UNI-1                | 78.56%                       | 66%  | 1,906,780                                 |
| AC-711-B22 | <i>Anaerolinea thermophila</i> UNI-1                | 78.47%                       | 52%  | 2,737,423                                 |



**Figure S1.** Pairwise comparisons of *Chloroflexi* genomes ANI vs. SSU percent identity. Dashed lines represent species level cutoff values.



**Figure S2.** N-terminal amino acid alignment of RdhA sequences, including the one found in SAG An-B22 with boxes highlighting the Fe-S cluster binding motif. This is a subset of the amino acid alignment that was used to create Figure 4.