Nickel limited methanogens shaped Precambrian climate

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Supplementary information

Environmental distribution and methane-metabolisms of Class II methanogens

SC-*Methanomicrobia* has 11 family-level members. Based on previous research, they are considered as hydrogenotrophic methanogens that utilizing hydrogen to reduce carbon dioxide into methane[83-86]. They are distributed in various environments including freshwater or marine sediments[86-89], wastewater treatment plant[90], oil well[91], gas fields[92], swamp[93], sludge[94] and are even animal-associated[95].

SC-*Methanosarcinia* is classified into four main clades (in total eight families) (Figure 1). (i)The clade *Methanosarcinia_A* comprises one family named *Methermicoccaceae*. This clade is considered to use methoxylated coal compounds ($R\text{-}O\text{-}CH_3$) to produce methane and it was discovered in extreme environments such as Shengli oilfield in China[96], as well as hydrothermal vent sediments from Guaymas Basin[97]. (ii)*Methanotrichales* clade comprises one family but 11 genus-level lineages. They are considered obligate acetotrophic methanogens that thrive in freshwater environments but can grow in a diverse range of habitats[95,98-100]. (iii)*Methanosarcinaceae* is a metabolically versatile group[101], containing four types of methanogenic pathways including carbon dioxide-reducing, methyl-reducing, methyl-dismutating and acetate-cleaving[101,102], and one type of methanotroph. Methanogens of *Methanosarcinaceae* normally dominates in marine water and sediment[103], and also live in multiple niches such as freshwater[103], terrestrial soil systems[104], wetland[105], host-related[106], and bioreactors[107]. Methanotroph of *Methanosarcinaceae*, ANME-3, lives in methane-rich mud volcanoes[108]. (iv)Intriguingly, there is also an entire major multi-order-level clade in SC-*Methanosarcinia* that function as anaerobic methane oxidizers[109]. These archaea use reversed methanogenic pathway to oxidize methane coupled with sulfate[110,111], nitrate/nitrite, and iron and manganese oxides[112,113].

Comparative genomics of SC-*Methanomicrobia* and SC-*Methanosarcinia*

SC-*Methanomicrobia* and SC-*Methanosarcinia* are phylogenetically closely related clades and share comparable average genomic sizes, measuring 2.09 and 2.16 Mb, respectively (Supplementary Figure 13). Nevertheless, they exhibit significant differences in the GC content ($p<0.0001$) and the frequency of amino acid (AA) utilization (Supplementary Figure 14). The GC content is considered to be related to various ecological factors, such as host association[114], oxidative adaption[115,116], nitrogen fixing[117], and thermal adaptability[118]. In this context, SC-*Methanomicrobia*

displays a higher average GC content than SC-*Methanosarcinia*, suggesting that the divergence of these major clades has undergone specific evolutionary selective pressures. In addition, substantial alterations in AA utilization frequencies, including glutamic acid, threonine, lysine, proline, asparagine, cystine and alanine, imply that these two superclasses may have adapted to distinct ecological niches, leading to changes in their preferences for these amino acids.

For comparative genomics, a total of 6,851 orthogroups were derived from 70 genomes through OrthoFinder[119] and annotated by eggNOG mapper[120] (Supplementary Figure 13). Among these orthogroups, 924 were identified as the core genome of SC-*Methanosarcinia*, while 1,041 orthogroups constituted the core genome of SC-*Methanomicrobia*. Whereas a total of 4,492 and 3,892 orthogroups compromised the accessory genomes of SC-*Methanosarcinia* and SC-*Methanomicrobia*, respectively. Additionally, these two superclasses shared 789 orthogroups categorized as "common core orthogroups". For each superclass-specific gene, using a relaxed definition that orthogroups are present in at least 70% of genomes from one superclass but fewer than 30% of genomes from the other superclass, we found 86 and 97 orthogroups form superclass-specific genomes of SC-*Methanosarcinia* and SC-*Methanomicrobia*, respectively (Supplementary Figure 13).

In general, the common core genes found in SC-*Methanosarcinia* and SC-*Methanomicrobia* predominantly participate in fundamental processes such as cell division, vitamin synthesis, transcription, translation, and carbon fixation (Supplementary Table 4). Notably, the core genome of SC-*Methanosarcinia* is smaller than that of SC-*Methanomicrobia* but exhibits a larger set of accessory genes. This discrepancy suggests that the SC-*Methanosarcinia* lineage possesses a more extensive and diversified metabolism potentials compared to SC-*Methanomicrobia*. Indeed, most accessory genes in both superclasses are classified as metabolic substrate transporters, indicating their distinct habitats. Within SC-*Methanosarcinia*, there are distinctive accessory genes related to cytochrome, including Ccm proteins for cytochrome *c* assembly, polyvinyl alcohol dehydrogenase (cytochrome), cytochrome *c*, and cytochrome *c* peroxidase (Supplementary Table 5). In contrast, SC-*Methanomicrobia* contains specific accessory genes for lactate permease, alanine dehydrogenase, and subunits of energy-converting hydrogenase A (Eha) (Supplementary Table 6).

Regarding the genes specific to each superclass, SC-*Methanosarcinia* exhibits significant enrichment in glycerophospholipid, cysteine, and methionine metabolism (p<0.05) (Supplementary Table 7). We observed that members of this superclass also

encode the exosome complex component CSL4/RRP41, which functions as a digester of exogenous RNA, and membrane-bound serine protease that cleaves extracellular substrates. These components play roles in maintaining small molecule homeostasis, self-protection, and the supplementation of growth materials. Moreover, SC-*Methanosarcinia* uniquely possesses membrane-bound proteins for methanogenesis, including the cytochrome subunit (HdrE) of heterodisulfide reductase (HdrDE) and subunits of Fd or F_{420}:methanophenazine oxidoreductase (Fpo). In SC-*Methanomicrobia*, genes specific to this superclass significantly enrich in bacterial motility proteins and seleno-compound metabolism (p<0.05) (Supplementary Table 8). Additionally, some archaeal flagellin and chemotaxis proteins are conserved, indicating a strong motility trait within this superclass. Similarly, proteins related to hydrogenase synthesis (Hyp complex) and certain hydrogenases like F_{420}-reducing hydrogenase complex (Frh) and methyl-viologen-reducing hydrogenase (Mvh) are conserved.

Contrast methane metabolic pathways between SC-*Methanomicrobia* and SC-*Methanosarcinia*

For SC-*Methanomicrobia*, nearly all genomes contain genes coding for enzymes responsible for catalyzing methane production from hydrogen and carbon dioxide. These genes code for the Wood-Ljungdahl methyl branch (WL-MB) pathway, tetrahydromethanopterin S-methyl-transferase (Mtr) complex, and Mcr, to compose carbon dioxide-reducing hydrogenotrophic methanogenesis pathway (Supplementary Figure 5). However, two exceptions are observed in the genera *JAFGOM01* and *Methanocella_A* which possesses the *mtaB* gene potentially coding for methyltransferase, although experimental verification is lacking.

In SC-*Methanosarcinia*, six families (*Methermicoccaceae*, *Methanotrichaceae*, *Methanocomedenaceae*, *Methanogasteraceae*, *Methanoperedenaceae*, *Methanosarciniaceae*) contain genes associated with the carbon dioxide-reducing hydrogenotrophic methanogenesis pathway (Supplementary Figure 5). Two families, *Methanosarcinaceae* and *Methermicoccaceae*, demonstrate the ability to dismutate methyl compounds for methane production. Almost all species within *Methanosarcinaceae* contain methyltransferases for methylamine (MtmB, MtbB, MttB) and methanol (MtaB), implying a coherent occurrence of methanogenesis from methylamine and methanol within this family. *Methermicoccaceae* species also have *mtmB*, *mtbB*, and *mttB* genes, indicating the potential for methane production from methylamine compounds.

Interestingly, most species from both superclasses contain either two potential pathways of the acetate-cleaving methanogenesis related genes. These pathways include one with genes coding for acetyl-CoA synthase (Acs) and carbon dioxide dehydrogenase complex (Cdh), as well as another with genes for acetate kinase (AckA), phosphoacetyl-transferase (Pta) and Cdh (Supplementary Figure 5). Within SC-*Methanomicrobia*, eight families (*Ca*. *Methanoflorenceae*, *Methanocellaceae*, *Methanofollaceae*, *JACTUA01*, *Methanosphaerulaceae*, *Methanoculleaceae*, *Methanospirillaceae*, and *Methanoregulaceae*) possess genes necessary for the first type acetate-cleaving methanogenesis pathway. They contain the *acs* gene obtained from the *Bacteria* domain and *cdhABCDE* obtained from *Thermoproteota* or *Hadarchaeota* (Supplementary Figure 15). In SC-*Methanosarcinia*, seven families (*Methermicoccaceae*, *Methanotrichaceae*, *EX4572-44*, *Methanocomedenaceae*, *Methanogasteraceae*, *Methanoperedenaceae*, and *Methanosarciniaceae*) encode the Acs acquired from the *Bacteria* domain and the Cdh complex acquired from *Archaeoglobi* (*cdhABC*) and *Methanoliparia* (*cdhDE*) (Supplementary Figure 15). However, as for now, only *Methanotrichaceae* has been confirmed capable of utilizing acetate as a methanogenic substrate in the first type of acetate-metabolizing pathway[98-100], whereas other members with these genes, such as *Methanolobus*[121] and *Methanococcoides*[122], have not been experimentally verified. In addition, two genera, *Methanosarcina* and *MTP4* from *Methanosarcinaceae*, are found to encode genes for the second type of acetate-cleaving methanogenesis with enzymes AckA, and this has been experimentally confirmed in *Methanosarcina*.

Contrast energy system between SC-*Methanomicrobia* and SC-*Methanosarcinia*

Methanogens derive energy from methanogenesis through chemiosmotic energy conservation, coupling the exergonic reactions in the pathway and cofactor system with the establishment of an ionic motive force for ATP production[123]. In methanogenesis, the energy system is composed of two parts:

(1) *energy conversion during carbon substrate conversion*. During the process of gradually catalyzing carbon substrate into methane, MTR complex catalyzes the transfer of methyl-groups from H_4MPT to CoM-SH, and couples this reaction with build-up of Na^+ motive force for ATP synthesis. MTR is shared by both superclasses (Supplementary Figure 5).

(2) *energy conversion during cofactors redox reactions*. The Class II methanogens primarily utilize four cofactors in methanogenesis, *i.e.* ferredoxin ($Fd_{red/ox}$), deazaflavin hydride carrier coenzyme (F_{420}), coenzyme B, and coenzyme M

(CoB/CoM). These two superclasses use distinct sets of cofactor enzymes. In SC-*Methanomicrobia*, hydrogenases such as Mvh and Ech are widely utilized for redox reactions of $Fd_{red/ox}$, while Frh is for F_{420}. The cytosolic heterodisulfide reductase HdrABC, coupled with Mvh, regenerates CoB-SH and CoM-SH[124]. Eha is involved in replenishing Fd_{red}[125], a process likely to have evolved independently in the class *Methanomicrobia*. In SC-*Methanosarcinia*, hydrogenases are either universally absent or incomplete. Instead, the $Fd_{red/ox}$:methanophenazine oxidoreductase complex (Rnf) and $Fd_{red/ox}$ or F_{420}:methanophenazine oxidoreductase complex (Fd_Fpo, F_{420}_Fpo) are involved in redox reactions between cofactors ($Fd_{red/ox}$ or F_{420}) and fat-soluble electron carriers such as methanophenazine, MP)[126,127]. HdrABC and the membrane-bound heterodisulfide reductase HdrDE may participate in redox reactions of CoB and CoM. Among the above enzymes, the hydrogenases Eha and Ech in SC-*Methanomicrobia* are involved in energy conversion; the membrane-bound enzymes Rnf, Fd_Fpo, F_{420}_Fpo, HdrDE in SC-*Methanosarcinia* are involved in energy conversion. Notably, theses membrane-bound enzymes in SC-*Methanosarcinia* are working in form of ETC. ETC is defined as a set of membrane bound protein complexes arranged in a specific order. It receives electrons from reduced cofactors generated in other metabolic pathways and transfers them from carriers with high redox potential to those with lower potential, using the change in reduction potential to pump protons or sodium ions across the membrane[128].

The energetc efficiency when combining distinct energy systems with three methanogeneic pathways.

The comparison of methanogenesis pathways above reveals that both SC-*Methanosarcinia* and SC-*Methanomicrobia* encompass three types of methanogenic pathways. The differentiation in metabolic types between these two superclasses likely stems from variances of energy systems during cofacter redox reactions. To evaluate the energetic efficient of hydrogenase-based energy system and ETC-based energy system, we calculate the net standard Gibbs free energy change ($\Delta G^{0\prime}$) for key steps in organic substrate utilization (methyl compounds or acetate) between SC-*Methanosarcinia* and SC-*Methanomicrobia* (Supplementary Figure 6).

In both methyl-dismutating and acetate-cleaving methanogenesis, organic electron donors follow the oxidizing direction of the WL (WL-MB or WL-CB) pathway, releasing electrons to form Fd_{red} or $F_{420}H_2$. These electron carriers require specific pathways to deliver electrons to the final acceptor CoM-S-S-CoB. In SC-*Methanosarcinia*, Rnf or Fpo facilitate the delivery of electrons from Fd_{red} and $F_{420}H_2$ to MP. Subsequently, these fat-soluble electron carriers deliver electrons to HdrDE,

reducing CoM-S-S-CoB. Consequently, members of SC-*Methanosarcinia* can effectively channel electrons from an organic source into energy through methane production. And the total standard Gibbs free energy change with ETC-based energy system shows the whole process are energy-producing.

Conversely, in SC-*Methanomicrobia*, when considering a methyl compound as the energy source (Supplementary Figure 6), the only energy-conserving step involves the reaction catalyzed by Ech in oxidizing Fd_{red} to Fd_{ox} ($\Delta G^{0\prime} \approx$ -15.44 kJ/mol)[129]. In contrast, the energy-consuming step occurs in the reaction catalyzed by the MTR complex during methyl transfer from CoM-SH to H_4MPT, coupled with the consumption of the sodium motive force ($\Delta G^{0\prime} \approx$ 30 kJ/mol)[130]. When acetate is the energy source, the sole energy-conserving reaction involving the transfer of a methyl-group from H_4MPT to CoM-SH via MTR shows only a slight energy gain ($\Delta G^{0\prime} \approx$ -30 kJ/mol). However, activating acetate into acetyl-CoA via Acs is energy-consuming, costing two ATP ($\Delta G^{0\prime} \approx$ 30.54 kJ/mol)[131]. It is evident that the conserved energy is insufficient to cover the consumed energy in both scenarios. Therefore, utilizing organic energy sources for methanogenesis appears impractical in SC-*Methanomicrobia*.

Tracing the energy system differentiation to the common ancestor of Class II methanogens

Within all ETCs utilized in methanogenesis by SC-*Methanosarcinia* (Figure 2), HdrDE represents an essential component, acting as the terminal complex. It receives electrons from MPH_2 to reduce the terminal acceptor CoB-S-S-CoM and is involved in generating a proton chemiosmotic gradient across the cell membrane[132,133]. The HdrDE complex contains an iron-sulfur protein, HdrD, with catalytic activity, and a cytochrome *b* subunit, HdrE, that mediates electron transmission and proton translocation[132]. We found that the gene coding for HdrDE is ubiquitously distributed across nearly all genomes of SC-*Methanosarcinia* (Supplementary Figure 5). However, HdrD sequences from SC-*Methanosarcinia* form two distinct clades on the phylogenetic tree (Supplementary Figure 7). One clade clusters with *Methanonatronarchaeales*, while the other clade clusters with *Syntropharchaeaceae*, implying that SC-*Methanosarcinia* acquired the HdrD-encoding gene via at least two HGT events. Remarkably, only the gene coding for HdrD homologs to *Methanonatronarchaeales* is co-located with the HdrE-encoding gene (Supplementary Figure 7), suggesting their likely functionality in the normal HdrDE complex. In contrast, HdrD from *Syntropharchaeaceae*, lacking HdrE, may have a different function or be nonfunctional.

Regarding HdrE, it belongs to the protein family PF02665, recognized as a *b*-type cytochrome and the gamma subunit of the nitrate reductase responsible for receiving electrons from the quinone pool in various bacteria[133]. By detecting and annotating PF02665 sequences across representative archaeal genomes from the GTDB database release 207, we identified roughly four major clades of PF02665 sequences (Supplementary Figure 16). In the Class II methanogens, sequences annotated as HdrE belong to classes *Methanosarcinia* and *Methanosarcinia_A*, as well as in the *Methanoculleaceae*, *Methanosphaerulaceae* and *Methanospirillaceae* families within the class *Methanomicrobia*. HdrE is also present in *Methanonatronarchaeales* and *JACAEJ01* (formerly called *Ca*. Methylarchaeales), anaerobic alkane-oxidizing archaea from families *Syntropharchaeaceae* and *JdFR-42* in *Archaeoglobi*, and isoprenoids producer belonging to the order *UBA10834* in *Thermoplasmata* (formerly called *Ca*. Gimiplasmatales[134]). The phylogenetic tree of the PF02665 family (Supplementary Figure 16) shows that almost all HdrE sequences cluster together, with the exception being the HdrE sequences from *Ca*. Methylarchaeales. They cluster with HmeC sequences from *Thermoproteota*, indicating nonhomology to other HdrE sequences (e-value > 1e-5). For the HdrE-like sequences of unknown function closely clustered with the known HdrE sequences, they also exhibit the domain for HdrD (PF13183: Fer4_8; PF02754: CCG) adjacent to the domain for HdrE (PF02665: Nitrate_red_gam). This suggests that HdrE-like sequencese may also have an ETC-related function, even though these microbes lack methane-related metabolisms.

To further investigate the origins of HdrE, we conducted a phylogenetic analysis of HdrE and HdrE-like sequences across the *Archaea* and *Bacteria* domains (Supplementary Figure 8). Most sequences of HdrE and HdrE-like exhibit separative evolutionary histories, forming distinct clusters. However, certain HdrE-like sequences from *Hermodarchaeia* occasionally cluster with HdrE (Supplementary Figure 8). Additionally, domain-crossing HGTs can be observed in both HdrE and HdrE-like clusters. Some bacterial HdrE sequences frequently cluster with archaeal HdrE sequences from *Syntropharchaeaceae*, *Archaeoglobi* and *Thermoplasmata*, suggesting a potential bacterial origin of HdrE-encoding gene. Examining the detailed evolutionary history of HdrE within SC-*Methanosarcinia* (Figure 2), it is estimated that the earliest node at which HdrE in SC-*Methanosarcinia* originated from a HGT event. Specifically, the ancestor of *Methanosarcinia_A* received HdrE transferred from *Methanonatronarchaeales* or another donor that has not yet been identified or is already extinct. Subsequently, this cytochrome spread throughout SC-*Methanosarcinia* primarily through speciation events. Interestingly, *Methanoculleus_A* from SC-*Methanomicrobia* also acquired the HdrE-encoding gene

from *Methanotrichaceae* via an HGT event, thus expanding their metabolic potentials (Figure 2).

The HdrDE complex was first discovered in *Methanosarcina barkeri*[135], where it played a role in assisting the MCR complex in reducing methyl-coenzyme M to produce CoB-S-S-CoM. Subsequently, genes coding for HdrDE have been found in various methanogens, including those from *Methanosarcinales*[136], *Methanotrichales*[137], *Methermicoccus*[96], and *Ca.* Methylarchaeales[138] in *Thermoproteota*. Additionally, HdrDE homologues have been found in methanotrophs from *Methansarcinales*[29,139,140], alkanotrophs such as *Ca*. Polytropus marinifundus in *Archaeoglobi*[141], sulfate reducers from *Archaeoglobus* involved in catalyzing disulfide/thiol conversions of DsrC (a heterodisulfide protein) during sulfate reduction[142-144], and assisting a specific fumarate reductase using CoM-SH and CoB-SH in *Hermodarchaeota* from Asgard archaea[145]. Despite being identified in various microorganisms, the HdrDE complex has only been purified from methanogens[135], and experimentally verified as functioning in the process of methanogenesis by coupling the heterodisulfide reducing reaction with energy conservation[146,147]. Owing to HdrDE, hydrogenotrophic SC-*Methanosarcina* species, such as *Methanosarcina barkeri* and *Methanosarcina mazei*, achieve higher energy efficiency by converting the released free energy into a proton motive force during the heterodisulfide reducing reaction. This efficiency, however, requires a higher hydrogen threshold concentration (>100 Pa) compared to SC-*Methanomicrobia* species (<10 Pa)[102,148]. Considering that diverse metabolic types of methanogenesis that utilize organic energy are characterized for SC-*Methanosarcinia*, in contrast to their sister lineage SC-*Methanomicrobia* and other methanogens (like Class I methanogens) which can only use hydrogen, we propose that the ancestor of Class II methanogens likely conducted hydrogenotrophic methanogenesis. The horizontal transfer of essential ETC genes (*hdrDE*) to the ancestor of SC-*Methanosarcinia* marks the onset of energy system differentiation between two superclasses, allowing SC-*Methanosarcinia* to develop its own ETC.

History of losing ability of hydrogenotrophic living

In SC-*Methanosarcinia*, although the complete gene cluster coding for Hyp is detected in several groups such as *JdFR-19* from *Methanosarcinia_A*, ANME group, and *Methanosarcinaceae*, it is mostly acquired through horizontal gene transfer (Supplementary Figure 3). The phylogeny of Hyp shows that *JdFR-19* obtained Hyp complex genes from *Thermoproteota*. For *Methanosarcinales* (ANME group and *Methanosarcinaceae*), Hyp subunits originated from diverse sources, including the

Class I methanogen (HypA and HypB), SC-*Methanomicrobia* (HypC, HypF), even the Class III methanogen (HypD and HypE). The varied origins of the Hyp complex suggest that SC-*Methanosarcinia* might have lost the *hpyABCDEF* at the period of common ancestor, or at the early stages of sublineages (like ancestor of *Methanosarcinia_A*, ancestor of *Methanotrichales*).

The diversification of ETCs is consistent with metabolic diversification in SC-Methanosarcinia

The shift of methanogenesis in SC-*Methanosarcinia* is closely related to ETCs' reforming (Figure 1). Therefore, through phylogeneitc analysis of all ETC-concerned enzymes, we conclude the history of methanogenesis diversification along with the phylogenetic diversification of SC-*Methanosarcinia*. The last common ancestor of SC-*Methanosarcinia* has constructed ETC "Fd_Fpo-HdrDE" by acquiring genes of Fpo from archaea and bacteria via HGTs (Supplemenary Figure 9). *Methanosarcinia_A* inherited this and utilizes it today to conserve energy by oxidizing Fd_{red} which is formed in the oxidizing direction of the WL-MB pathway and transmitting the electrons to reduce CoM-S-S-CoB in methoxyl-dismutating methanogenesis. Simultaneously, it supplements this process with the electron bifurcation mechanism conducted by HdrABC and FdhB, which transfers electrons from $F_{420}H_2$ to CoM-S-S-CoB (Figure 2). *Methanotrichales* also inherited the "Fd_Fpo-HdrDE" ETC, connecting it to the WL-CB pathway and MCR. This lineage conserves energy by oxidizing Fd_{red} produced by the WL-CB pathway and transmitting the electrons to reduce CoM-S-S-CoB in the acetate-cleaving methanogenesis. The ancestor of *Methanosarcinales* acquired genes coding for Rnf (Supplementary Figure 10) from bacteria and replaced Fd_Fpo with F_{420}_Fpo by acquiring the gene coding for FpoF subunit (Supplementary Figure 9). Within *Methanosarcinales*, the evolution of ANME group was estimated to be triggered by the acquisition of genes for cytochrome *b* and cytochrome *c*[126]. This lineage reversed the methanogenic pathway to oxidize methane into carbon dioxide, generating Fd_{red}, $F_{420}H_2$, CoB-SH, and CoM-SH during this process. To access electrons from these reduced carriers to their final electron acceptors, such as sulfate and nitrate, the ANME group reconfigured ETCs into extracellular electron transfer (EETs)[126] "F_{420}_Fpo-cytb-cytc", "Rnf-cytb-cytc", and "HdrDE-cytb-cytc" to transfer electrons from $F_{420}H_2$, Fd_{red}, CoB-SH, and CoM-SH to extracellular sulfate or nitrate (Supplementary Figure 17, Figure 2). In their sister group, *Methanosarcinaceae* effectivley inherited Rnf and F_{420}_Fpo to construct new ETCs, such as "Rnf-HdrDE" and "F_{420}_Fpo-HdrDE", or to conserve energy by oxidizing Fd_{red} and $F_{420}H_2$ for reducing CoM-S-S-CoB, respectively. Moreover, within *Methanosarcinaceae*, three

genera (*Methanimicrococcus*, *Methanosarcina,* and *MTP4*) reacquired the Hyp complex, thereby recovering their ability to synthesize [NiFe] hydrogenase. Based on this, these three genera innovated the new ETC "Vht-HdrDE", allowing hydric electrons to be reaccepted by final electron accepter CoM-S-S-CoB. *Methanimicrococcus* and *Methanosarcina* both use "Vht-HdrDE" in methyl-reducing methanogenesis, and some species of *Methanosarcina*, such as *Methanosarcina mazei*[151] and *Methanosarcina barkeri*[152] from fresh water, were reported to utilize ETC "Vht-HdrDE" in carbon dioxide-reducing methanogenesis or combine Ech with "Vht-HdrDE" in methyl-dismutating methanogenesis (Supplementary Figure 18).

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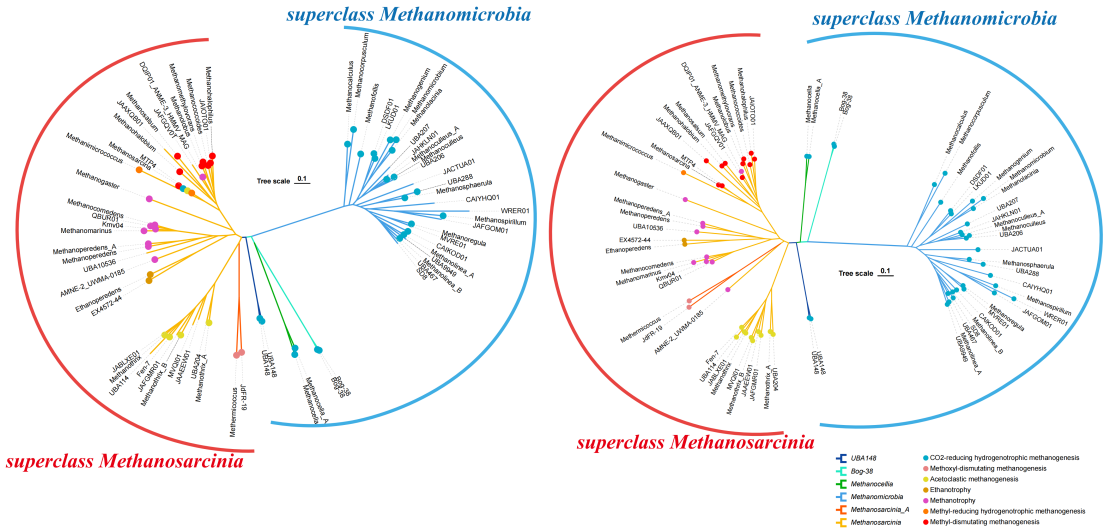
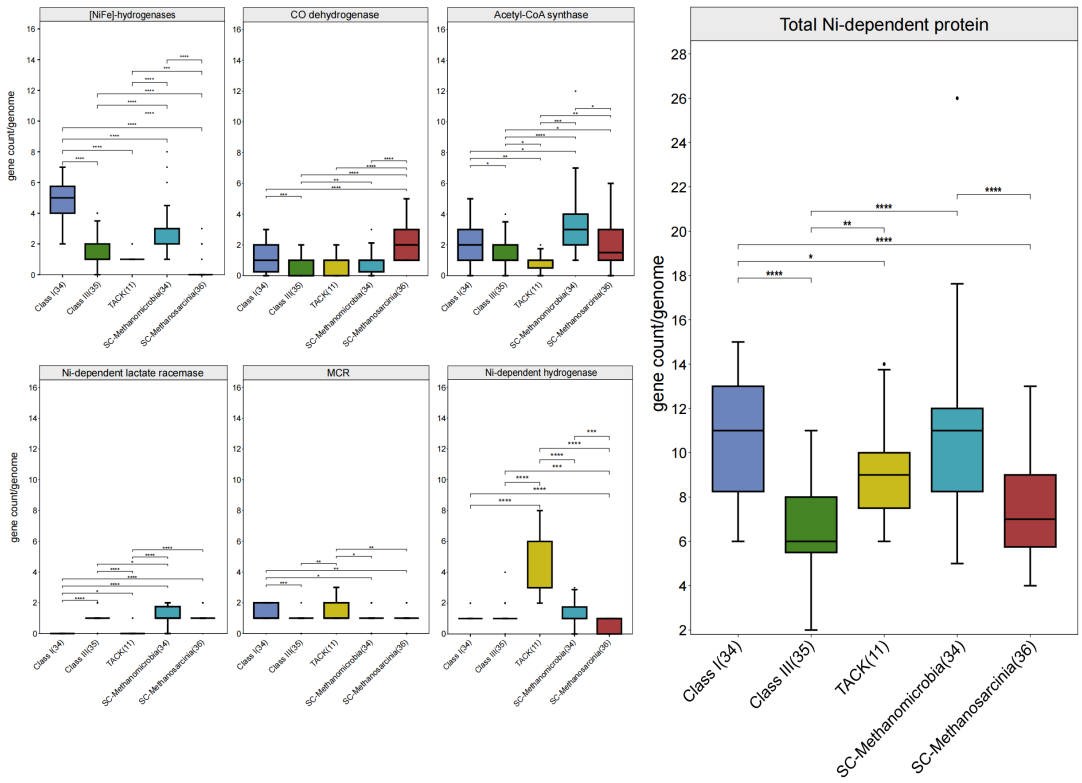
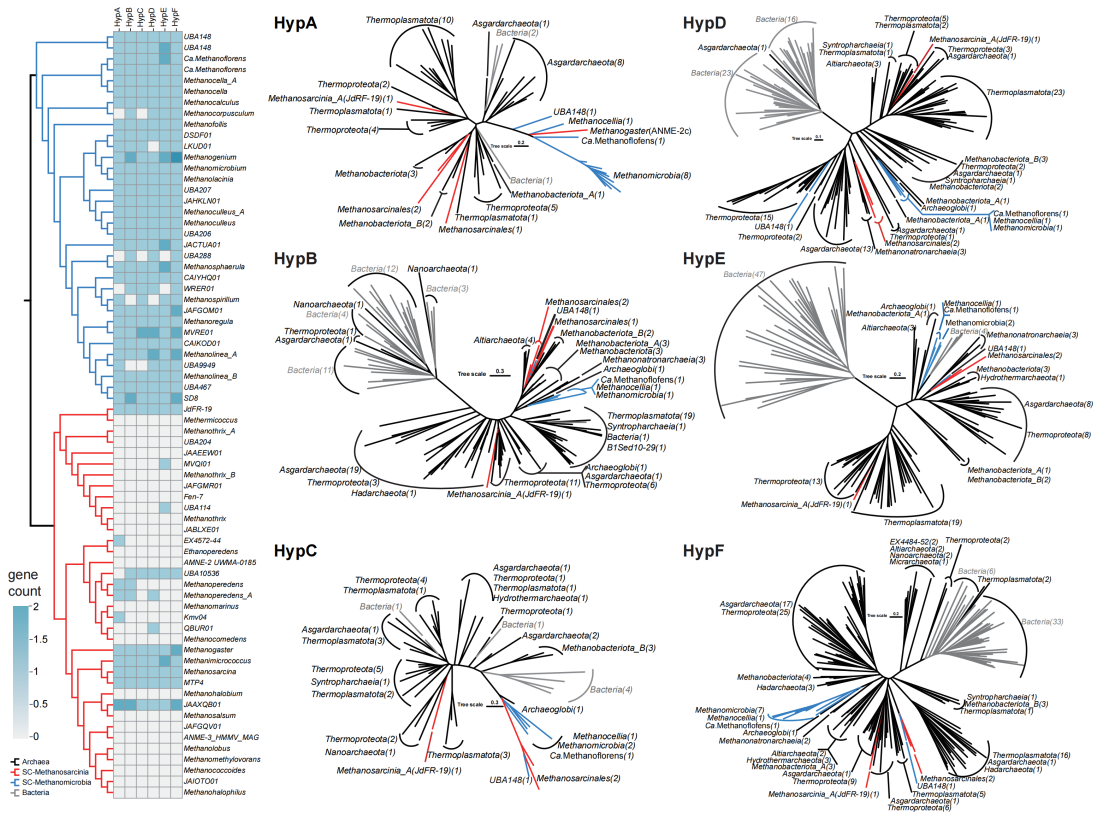


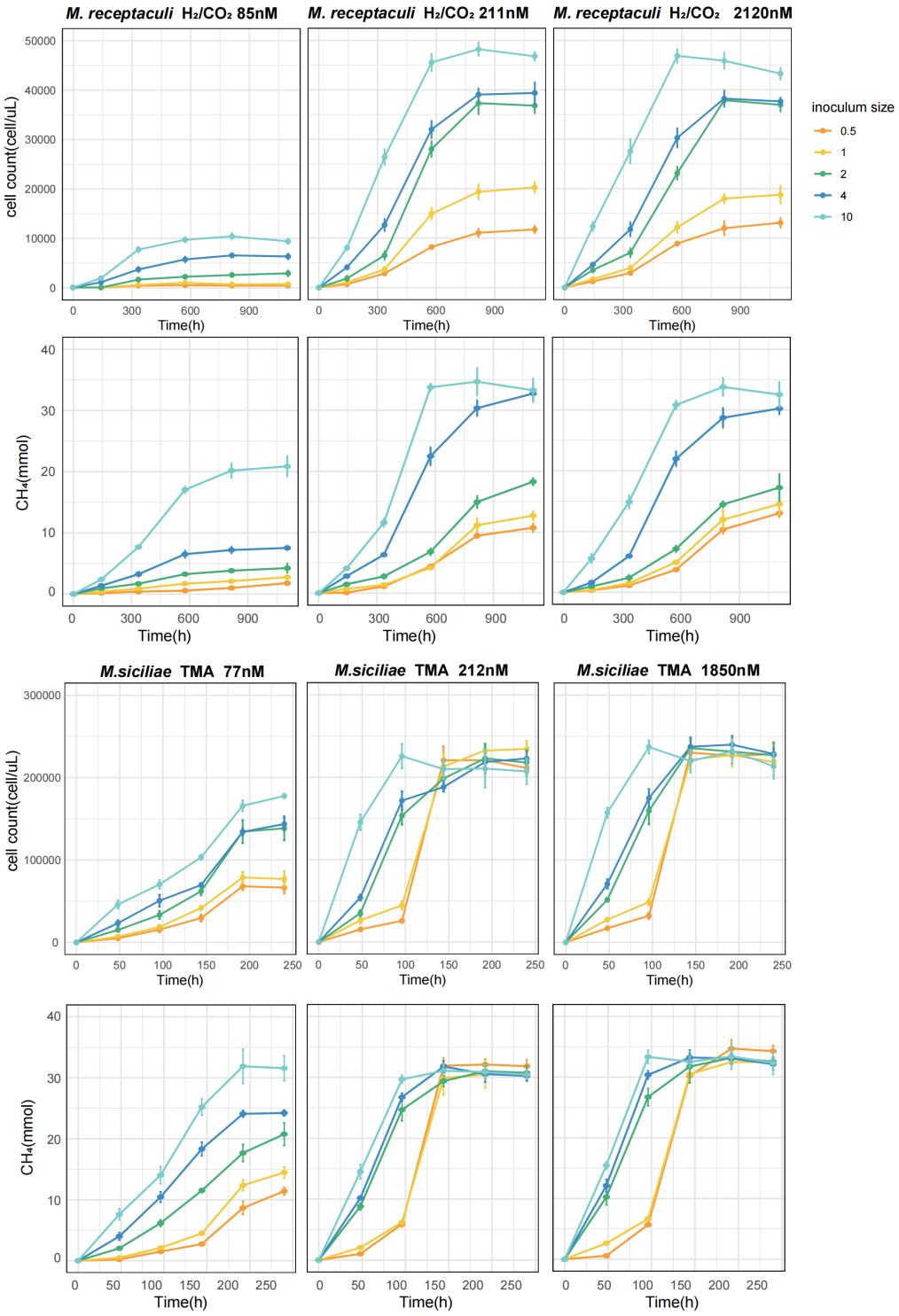
Supplementary Figure 1. Unrooted phylogenetic trees of Class II methanogen. On the basis of the concatenated alignment of a set of 37 (left) and 53 (right) conserved marker genes, the tree is constructed with model LG+R8+C60 and LG+F+R8+C60, respectively, by IQ-Tree.



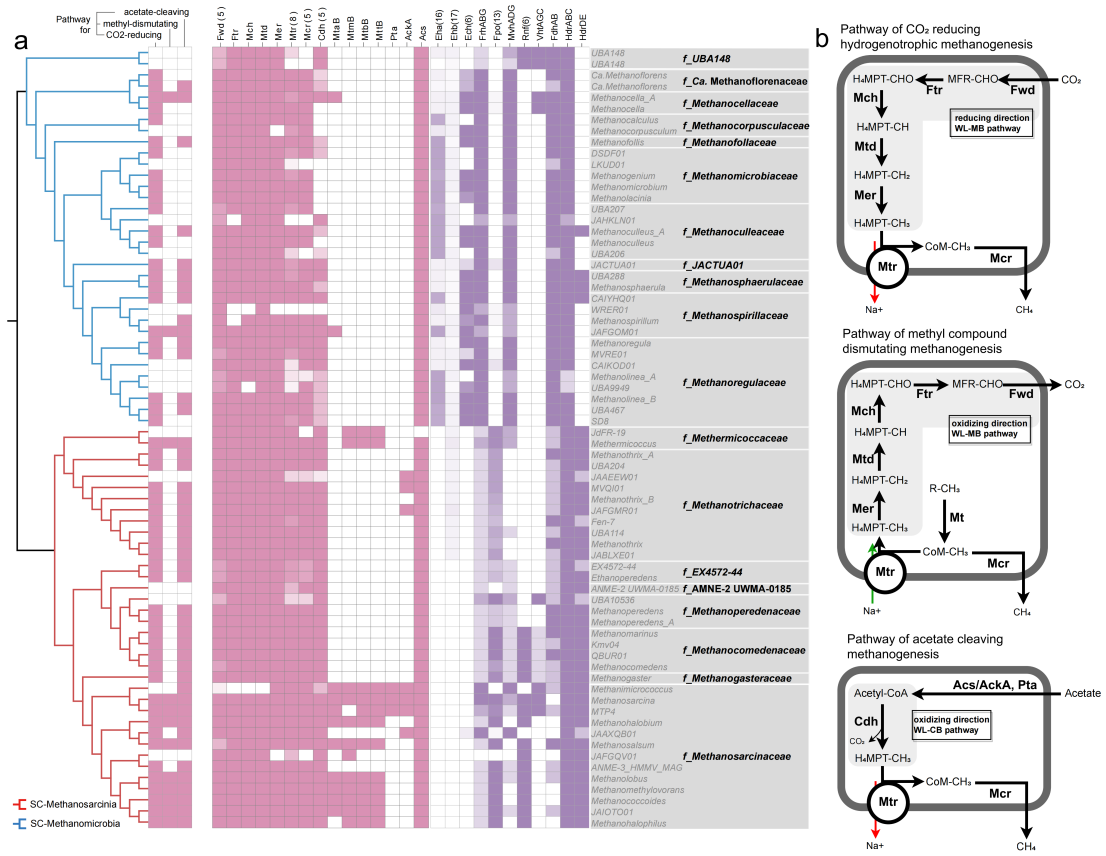
Supplementary Figure 2. Boxplots of gene count of nickel-dependent enzymes in different methanogen groups, including Class I, Class III, TACK, SC-*Methanosarcinia* and SC-*Methanomicrobia*. The nickel-containing proteins from [NiFe]-hydrogenases (EchE, EhaO, FrhA, MvhA,VhoA), CO dehydrogenase (CdhA, CooS), MCR (McrA), Acetyl-CoA synthase (Acs), Ni-dependent lactate racemase (LarA) and Ni-dependent hydrogenase (AraM, MbhL etc.) are calculated respectively, and the total gene count of above proteins are also calculated. Stars represent the significant differences between two superclasses, determined using a Wilcoxon test.



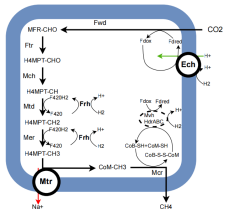
Supplementary Figure 3. Taxonomically distribution of genes coding for Hyp complex, and the phylogeny of HypA, HypB, HypC, HypD, HypE and HypF, being constructed with models LG+I+G4+C60, LG+R9+C60, LG+I+G4+C60, LG+R8+C60, LG+R8+C60 and LG+R10+C60 by IQ-Tree, respectively.



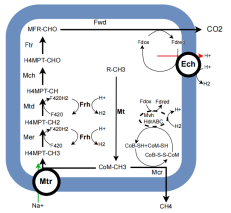
Supplementary Figure 4. Methane production and cell proliferation of *M.siciliae* and *M.receptaculi* growing under different nickel level. Both strains are cultured with 0.5%, 1%, 2%, 4%, 10% inoculum size.



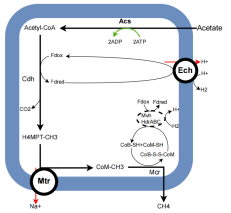
Supplementary Figure 5. Comparative analysis of three types of methanogenesis. (a) Heatmaps of methanogenesis-relate genes, showing the taxonomically distribution of carbon dioxide reducing hydrogenotrophic, methyl-dismutating and acetate-cleaving methanogenesis across the phylogeny of SC-*Methanomicrobia* (blue branches) and SC-*Methanosarcinia* (red branches). The left heatmap presents the existence of complete C1 pathway of each type of methanogenesis. We define that the "complete C1 pathway" as: all the enzymes involving carbon substrate conversion are contained, and absent subunits ≤2. The right heatmap shows the coding of cofactor enzymes involving in methanogenesis. The same heatmap presenting distribution of genes for methane-related metabolism with more detailed information is shown in Supplementary Figure 6. (b) schematic diagrams for three types of methanogenesis.



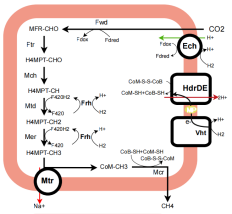
	reactions	enzyme	status	energy conservation	nΔG°'(kJ)
reactions of carbon substrate	$CO_2 + Fd_{red} + MFR \rightarrow CHO\text{-}MFR + Fd_{ox}$	Fwd	cytosolic	no	
	$CHO\text{-}MFR + H_4MPT \rightarrow CHO\text{-}H_4MPT + MFR$	Ftr	cytosolic	no	
	$CHO\text{-}H_4MPT \rightarrow CH\text{-}H_4MPT$	Mch	cytosolic	no	
	$CH\text{-}H_4MPT + F_{420}H_2 \rightarrow CH_2\text{-}H_4MPT + F_{420}$	Mtd	cytosolic	no	
	$CH_2\text{-}H_4MPT + F_{420}H_2 \rightarrow CH_3\text{-}H_4MPT + F_{420}$	Mer	cytosolic	no	
	$CH_3\text{-}H_4MPT + CoM\text{-}SH \rightarrow CH_3\text{-}CoM + H_4MPT$	Mtr	membrane bound	yes(Na^+)	-30
	$CH_3\text{-}CoM + CoB\text{-}SH \rightarrow CH_4 + CoM\text{-}S\text{-}S\text{-}CoB$	Mcr	cytosolic	no	
reactions of cofactors	$H_2 + Fd_{ox} \rightarrow 2H^+ + Fd_{red}$	Ech	membrane bound	yes(H^+)	15.44
	$2H_2 + 2F_{420} \rightarrow 2F_{420}H_2$	Frh	cytosolic	no	
	$CoM\text{-}S\text{-}S\text{-}CoB + H_2 + Fd_{red} \rightarrow CoM\text{-}SH + CoB\text{-}SH + Fd_{ox}$	HdrABC+MvhACG	cytosolic	no	
net $\Delta G^{0'}$ of energy conservation process of carbon dioxide reducing hydrogenotrophic methanogenesis in SC-*Methanomicrobia* (per mole CH4)					**-14.56**



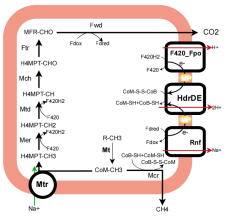
	reactions	enzyme	status	energy conservation	nΔG°'(kJ)
reactions of carbon substrate	$4CH_3\text{-}R + 4CoM\text{-}SH \rightarrow 4CH_3\text{-}CoM + 4RH$	Mt	cytosolic	no	
	$CH_3\text{-}CoM + H_4MPT \rightarrow CH_3\text{-}H_4MPT + CoM\text{-}SH$	Mtr	membrane bound	yes(Na^+)	30
	$CH_3\text{-}H_4MPT + F_{420} \rightarrow CH_2\text{-}H_4MPT + F_{420}H_2$	Mer	cytosolic	no	
	$CH_2\text{-}H_4MPT + F_{420} \rightarrow CH\text{-}H_4MPT + F_{420}H_2$	Mtd	cytosolic	no	
	$CH\text{-}H_4MPT \rightarrow CHO\text{-}H_4MPT$	Mch	cytosolic	no	
	$CHO\text{-}H_4MPT + MFR \rightarrow CHO\text{-}MFR + H_4MPT$	Ftr	cytosolic	no	
	$CHO\text{-}MFR + Fd_{ox} \rightarrow CO_2 + Fd_{red} + MFR$	Fwd	cytosolic	no	
	$3CH_3\text{-}CoM + 3CoB\text{-}SH \rightarrow 3CH_4 + 3CoM\text{-}S\text{-}S\text{-}CoB$	Mcr	cytosolic	no	
reactions of cofactors	$2H^+ + Fd_{red} \rightarrow H_2 + Fd_{ox}$	Ech	membrane bound	yes(H^+)	-15.44
	$2F_{420}H_2 \rightarrow 2H_2 + 2F_{420}$	Frh	cytosolic	no	
	$3CoM\text{-}S\text{-}S\text{-}CoB + 3H_2 + 3Fd_{red} \rightarrow 3CoM\text{-}SH + 3CoB\text{-}SH + 3Fd_{ox}$	HdrABC+MvhACG	cytosolic	no	
net $\Delta G^{0'}$ of energy conservation process of assumed methyl-compounds dismutating methanogenesis in SC-*Methanomicrobia* (per mole CH_4)					**4.85**



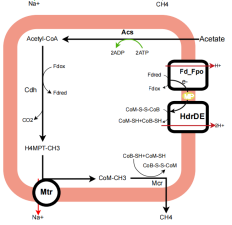
	reactions	enzyme	status	energy conservation	nΔG°'(kJ)
reactions of carbon substrate	$CH_3COO^- + CoA\text{-}SH + 2ATP \rightarrow CH_3CO\text{-}CoA + 2ADP + 2Pi$	Acs	cytosolic	yes(ATP)	61.08
	$CH_3CO\text{-}CoA + Fd_{ox} + H_4MPT \rightarrow CH_3\text{-}H_4MPT + CO_2 + Fd_{red}$	Cdh	cytosolic	no	
	$CH_3\text{-}H_4MPT + CoM\text{-}SH \rightarrow CH_3\text{-}CoM + H_4MPT$	Mtr	membrane bound	yes(Na^+)	-30
	$CH_3\text{-}CoM + CoB\text{-}SH \rightarrow CH_4 + CoM\text{-}S\text{-}S\text{-}CoB$	Mcr	cytosolic	no	
reactions of cofactors	$4H^+ + 2Fd_{red} \rightarrow 2H_2 + 2Fd_{ox}$	Ech	membrane bound	yes(H^+)	-30.88
	$CoM\text{-}S\text{-}S\text{-}CoB + H_2 + Fd_{ox} \rightarrow CoM\text{-}SH + CoB\text{-}SH + Fd_{red}$	HdrABC+MvhACG	cytosolic	no	
net $\Delta G^{0'}$ of energy conservation process of assumed aceate cleaving methanogenesis in SC-*Methanomicrobia* (per mole CH_4)					**0.2**



	reactions	enzyme	status	energy conservation	nΔG°'(kJ)
reactions of carbon substrate	$CO_2 + Fd_{red} + MFR \rightarrow CHO\text{-}MFR + Fd_{ox}$	Fwd	cytosolic	no	
	$CHO\text{-}MFR + H_4MPT \rightarrow CHO\text{-}H_4MPT + MFR$	Ftr	cytosolic	no	
	$CHO\text{-}H_4MPT \rightarrow CH\text{-}H_4MPT$	Mch	cytosolic	no	
	$CH\text{-}H_4MPT + F_{420}H_2 \rightarrow CH_2\text{-}H_4MPT + F_{420}$	Mtd	cytosolic	no	
	$CH_2\text{-}H_4MPT + F_{420}H_2 \rightarrow CH_3\text{-}H_4MPT + F_{420}$	Mer	cytosolic	no	
	$CH_3\text{-}H_4MPT + CoM\text{-}SH \rightarrow CH_3\text{-}CoM + H_4MPT$	Mtr	membrane bound	yes(Na^+)	-30
	$CH_3\text{-}CoM + CoB\text{-}SH \rightarrow CH_4 + CoM\text{-}S\text{-}S\text{-}CoB$	Mcr	cytosolic	no	
reactions of cofactors	$H_2 + Fd_{ox} \rightarrow 2H^+ + Fd_{red}$	Ech	membrane bound	yes(H^+)	15.44
	$2H_2 + 2F_{420} \rightarrow 2F_{420}H_2$	Frh	cytosolic	no	
	$CoM\text{-}S\text{-}S\text{-}CoB + MP + H_2 \rightarrow CoM\text{-}SH + CoB\text{-}SH + MP$	HdrDE-VhtACG	membrane bound	yes(H^+)	-53.07
net $\Delta G^{0'}$ of energy conservation process of carbon dioxide reducing hydrogenotrophic methanogenesis in SC-*Methanosarcinia* (per mole CH4)					**-67.63**

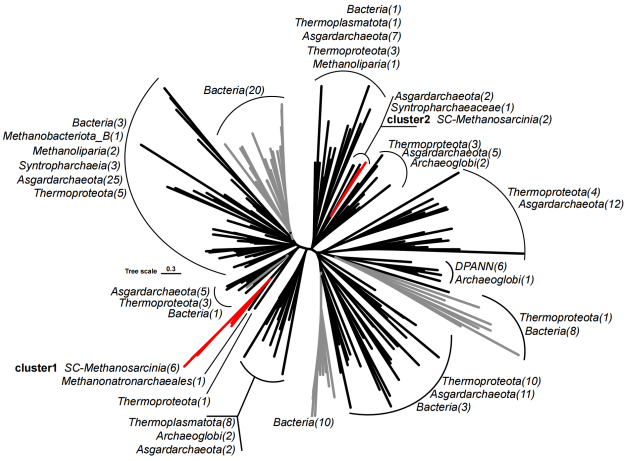


	reactions	enzyme	status	energy conservation	nΔG°'(kJ)
reactions of carbon substrate	$4CH_3\text{-}R + 4CoM\text{-}SH \rightarrow 4CH_3\text{-}CoM + 4RH$	Mt	cytosolic	no	
	$CH_3\text{-}CoM + H_4MPT \rightarrow CH_3\text{-}H_4MPT + CoM\text{-}SH$	Mtr	membrane bound	yes(Na^+)	30
	$CH_3\text{-}H_4MPT + F_{420} + 2H^+ \rightarrow CH_2\text{-}H_4MPT + F_{420}H_2$	Mer	cytosolic	no	
	$CH_2\text{-}H_4MPT + F_{420} + 2H^+ \rightarrow CH\text{-}H_4MPT + F_{420}H_2$	Mtd	cytosolic	no	
	$CH\text{-}H_4MPT \rightarrow CHO\text{-}H_4MPT$	Mch	cytosolic	no	
	$CHO\text{-}H_4MPT + MFR \rightarrow CHO\text{-}MFR + H_4MPT$	Ftr	cytosolic	no	
	$CHO\text{-}MFR + Fd_{ox} \rightarrow CO_2 + Fd_{red} + MFR$	Fwd	cytosolic	no	
	$3CH_3\text{-}CoM + 3CoB\text{-}SH \rightarrow 3CH_4 + 3CoM\text{-}S\text{-}S\text{-}CoB$	Mcr	cytosolic	no	
reactions of cofactors	$CoM\text{-}S\text{-}S\text{-}CoB + Fd_{red} + MP \rightarrow CoM\text{-}SH + CoB\text{-}SH + MP + Fd_{ox}$	HdrDE-Rnf	membrane bound	yes(Na^+,H^+)	-68.5
	$2CoM\text{-}S\text{-}S\text{-}CoB + 2F_{420}H_2 + 2MP \rightarrow 2CoM\text{-}SH + 2CoB\text{-}SH + 2MP + 2F_{420} + 4H^+$	HdrDE-F_{420}_Fpo	membrane bound	yes(H^+)	-82.97
net $\Delta G^{0'}$ of energy conservation process of methyl-compounds dismutating methanogenesis in SC-*Methanosarcinia* (per mole CH_4)					**-40.49**



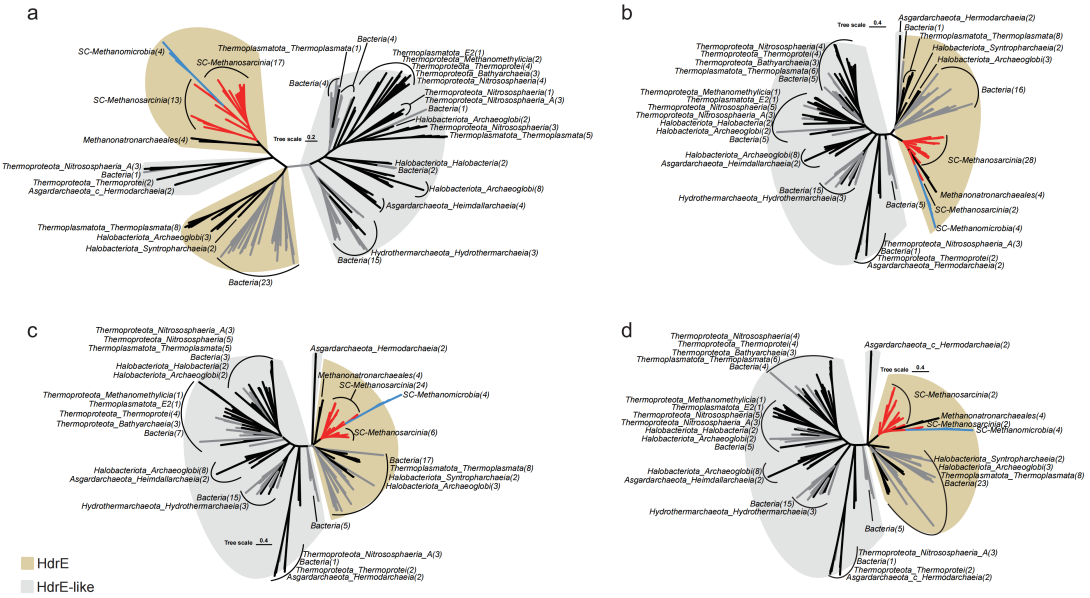
	reactions	enzyme	status	energy conservation	nΔG°'(kJ)
reactions of carbon substrate	$CH_3COO^- + CoA\text{-}SH + 2ATP \rightarrow CH_3CO\text{-}CoA + 2ADP + 2Pi$	Acs	cytosolic	yes(ATP)	61.08
	$CH_3CO\text{-}CoA + Fd_{ox} + H_4MPT \rightarrow CH_3\text{-}H_4MPT + CO_2 + Fd_{red}$	Cdh	cytosolic	no	
	$CH_3\text{-}H_4MPT + CoM\text{-}SH \rightarrow CH_3\text{-}CoM + H_4MPT$	Mtr	membrane bound	yes(Na^+)	-30
	$CH_3\text{-}CoM + CoB\text{-}SH \rightarrow CH_4 + CoM\text{-}S\text{-}S\text{-}CoB$	Mcr	cytosolic	no	
reactions of cofactors	$CoM\text{-}S\text{-}S\text{-}CoB + Fd_{red} + MP \rightarrow CoM\text{-}SH + CoB\text{-}SH + MP + Fd_{ox} + 2H^+$	HdrDE-Fd_Fpo	membrane bound	yes(H^+)	-68.5
net $\Delta G^{0'}$ of energy conservation process of aceate cleaving methanogenesis in SC-*Methanosarcinia* (per mole CH_4)					**-37.42**

Supplementary Figure 6. The standard Gibbs free energy change when combine the hydrogenotrophic carbon dioxide reducing methanogenesis, methyl-dismutating methanogenesis and acetoclastic methanogenesis with hydrogenases-based (upper) or ETCs-based (below) energy system.

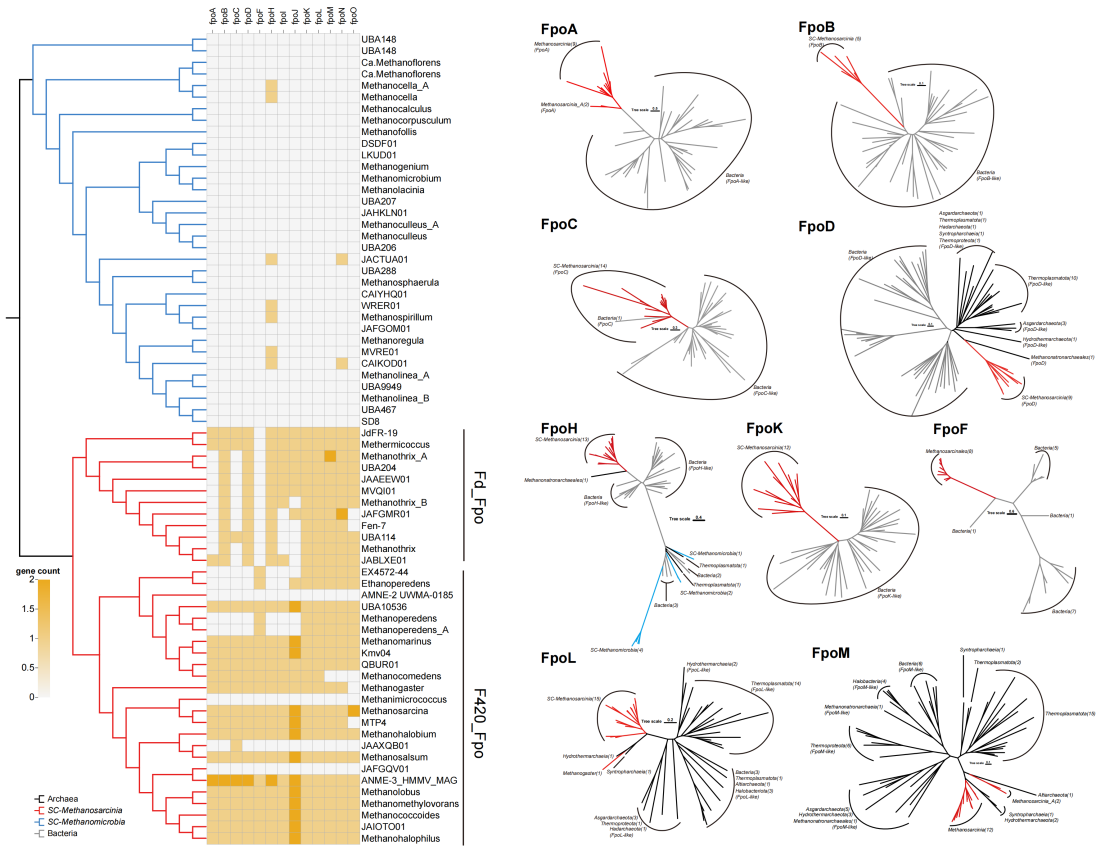


	genome	hdrD	co-located hdrE
cluster 1	DQIP01_ANME-3_HMMV_MAG	DQIP01000130.1_5	DQIP01000130.1_4
	DQIP01_ANME-3_HMMV_MAG	DQIP01000130.1_7	DQIP01000130.1_6
	DQIP01_ANME-3_HMMV_MAG	DQIP01000194.1_7	DQIP01000194.1_8
	GCA_000013725.1_Methanococcoides_burtonii	CP000300.1_2608	CP000300.1_2607
	GCA_000014945.1_Methanothrix_B_thermoacetophila	NC_008553.1_1025	NC_008553.1_1026
	GCA_000021965.1_Methanosphaerula_palustris	NC_011832.1_769	NC_011832.1_768
	GCA_000196655.1_Methanohalobium_evestigatum	CP002069.1_545	CP002069.1_544
	GCA_000306725.1_Methanolobus_psychrophilus	CP003083.1_882	CP003083.1_881
	GCA_000328665.1_Methanomethylovorans_hollandica	CP003362.1_1458	CP003362.1_1457
	GCA_000970085.1_Methanosarcina_siciliae	NZ_CP009506.1_653	NZ_CP009506.1_652
	GCA_002010075.1_bFR-19_sp002010075	MTMG01000004.1_58	MTMG01000004.1_59
	GCA_002067365.1_Methanothrix_sp002067365	MVRK01000083.1_9	MVRK01000083.1_8
	GCA_002067795.1_MVQI01_sp002067795	MVQI01000051.1_3	MVQI01000051.1_2
	GCA_002501765.1_UBA204_sp002501765	DAOG01000151.1_18	DAOG01000151.1_19
	GCA_002506335.1_UBA114_sp002506335	DAXI01000061.1_10	DAXI01000061.1_9
	GCA_002506335.1_UBA114_sp002506335	DAXI01000061.1_8	DAXI01000061.1_9
	GCA_002506535.1_Methanothrix_A_harundinacea_B	DAXT01000019.1_30	DAXT01000019.1_29
	GCA_002926195.1_Methanomarinus_sp002926195	MZXQ01000115.1_4	MZXQ01000115.1_3
	GCA_002926195.1_Methanomarinus_sp002926195	MZXQ01000203.1_5	MZXQ01000115.1_3
	GCA_003104905.1_Methanoperedens_sp003104905	PQAS01000047.1_67	PQAS01000047.1_68
	GCA_003555325.1_Methanosalsum_natronophilum	PWGQ01000102.1_54	PWGQ01000102.1_53
	GCA_004102725.1_Methanoculleus_A_taiwanensis	NZ_LHQS01000002.1_1131	NZ_LHQS01000002.1_1132
	GCA_004211975.1_Methanogaster_sp004211975	RXIK01000125.1_24	RXIK01000125.1_23
	GCA_004212095.1_Ethanoperedens_sp004212095	RXIO01000030.1_8	RXIO01000030.1_7
	GCA_004332335.1_UBA288_sp004332335	SISS01000014.1_4	SISS01000014.1_5
	GCA_004363215.1_Methanimicrococcus_blatticola	NZ_SNYS01000005.1_247	NZ_SNYS01000005.1_248
	GCA_009649835.1_Methanocomedens_sp009649835	PYCK01000061.1_6	PYCK01000061.1_5
	GCA_012962625.1_AMNE-2_UWMA-0185	DTXF01000607.1_4	DTXF01000607.1_3
	GCA_013178225.1_JABLXE01_sp013178225	JABLXE010000008.1_84	JABLXE010000008.1_83
	GCA_013329415.1_MTP4_sp9310u	DUKH01000082.1_10	DUKH01000082.1_11
	GCA_013330515.1_Methermicoccus_shengliensis	DUIH01000017.1_55	DUIH01000017.1_54
	GCA_014859725.1_Kmv04_sp014859725	JACUTQ010000075.1_9	JACUTQ010000075.1_8
	GCA_014859785.1_Methanoperedens_A_sp014859785	JACUTP010000124.1_4	JACUTP010000124.1_5
	GCA_016926455.1_JAFGQV01_sp016926455	JAFGOO010000154.1_3	JAFGOO010000154.1_2
	GCA_016927425.1_JAFGMR01_sp016927425	JAFGMR010000047.1_12	JAFGMR010000047.1_13
	GCA_017874375.1_Methanohalophilus_levihalophilus	NZ_JAGGLK010000001.1_72	NZ_JAGGLK010000001.1_71
	GCA_019429385.1_QBUR01	JAHYIS010000019.1_25	JAHYIS010000019.1_24
	GCA_021106765.1_JAIOTC01	JAIOTO010000125.1_8	JAIOTO010000125.1_9
	GCA_903926075.1_CAIYHQ01_sp903926075	CAIYHQ010000109.1_29	CAIYHQ010000109.1_30
cluster 2	GCA_000013725.1_Methanococcoides_burtonii	CP000300.1_497	none
	GCA_000196655.1_Methanohalobium_evestigatum	CP002069.1_1549	none
	GCA_000328665.1_Methanomethylovorans_hollandica	CP003362.1_1012	none
	GCA_002010075.1_bFR-19_sp002010075	MTMG01000017.1_114	none
	GCA_002501765.1_UBA204_sp002501765	DAOG01000075.1_10	none
	GCA_003555325.1_Methanosalsum_natronophilum	PWGQ01000084.1_2	none
	GCA_004211975.1_Methanogaster_sp004211975	RXIK01000041.1_5	none
	GCA_004212095.1_Ethanoperedens_sp004212095	RXIO01000046.1_18	none
	GCA_012962625.1_AMNE-2_UWMA-0185	DTXF01000585.1_2	none
	GCA_013330515.1_Methermicoccus_shengliensis	DUIH01000021.1_142	none
	GCA_014859785.1_Methanoperedens_A_sp014859785	JACUTP010000177.1_5	none
	GCA_905171695.1_EX4572-44_sp905171695	LR991653.1_1202	none

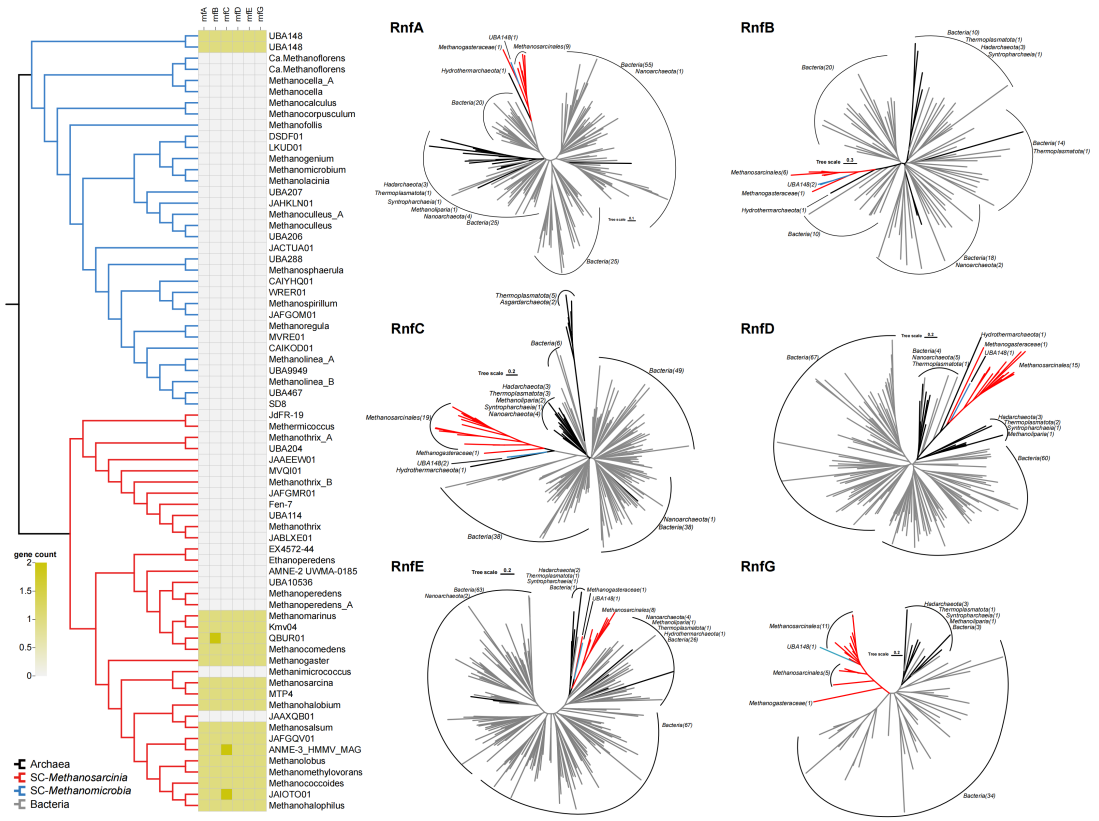
Supplementary Figure 7. The phylogenetic tree of HdrD, constructed with model LG+R8+C60 by IQ-Tree. The right table shows the coding position of HdrD as well as the co-located HdrE in the relevant genome.



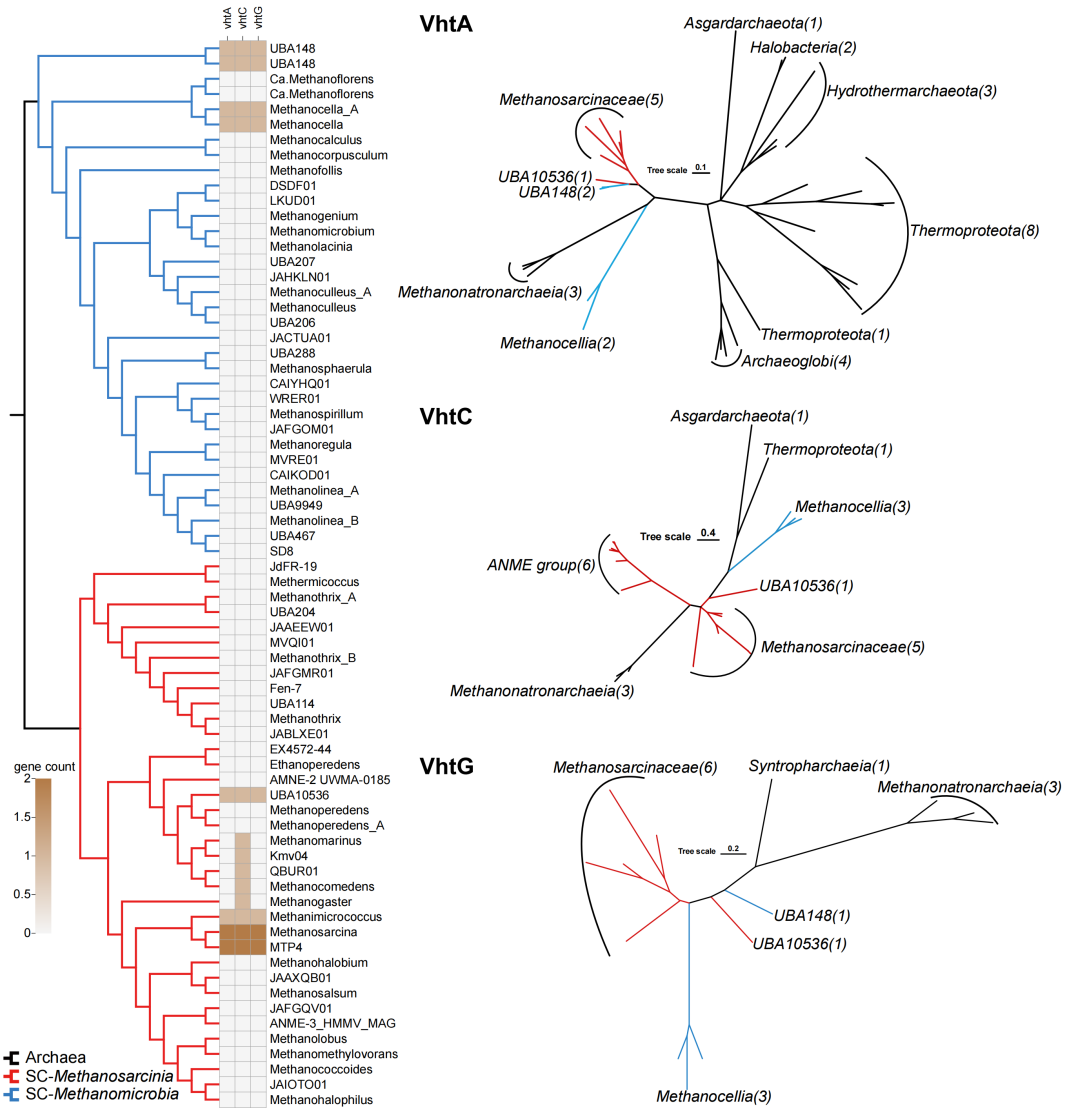
Supplementary Figure 8. The phylogenetic trees of HdrE and HdrE-lile proteins. (a) the tree constructed by FastTree. (b) the tree constructed by IQ-Tree with model mtlnv+F+R. (c) the tree constructed by IQ-Tree with model mtlnv+F+R+C30. (d) the tree constructed by IQ-Tree with model mtlnv+F+R+C60.



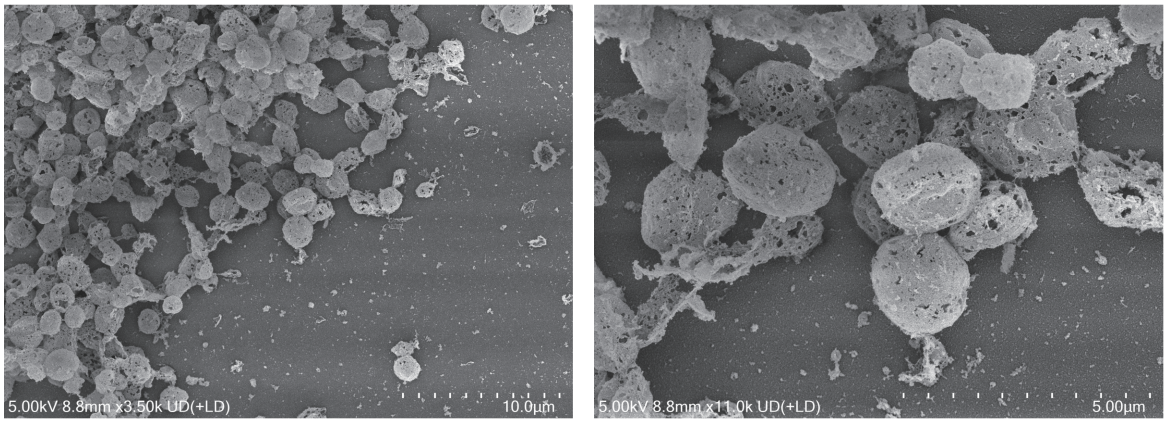
Supplementary Figure 9. Taxonomically distribution of genes coding for Fpo complex, and the phylogeny of FpoA, FpoB, FpoC, FpoD, FpoF, FpoH, FpoK, FpoL and FpoM, being constructed with the models LG+G4+C60, LG+I+G4+C60, LG+I+G4+C60, LG+R5+C60, LG+I+G4+C60, LG+F+R5+C60, LG+F+R5+C60, LG+F+I+G4+C60 and LG+F+R6+C60 by IQ-Tree, respectively. The subunits I, J, N, O are failed in acquiring homologs from GTDB archaeal and bacterial genome database, so they have no phylogenetic tree constructed here.



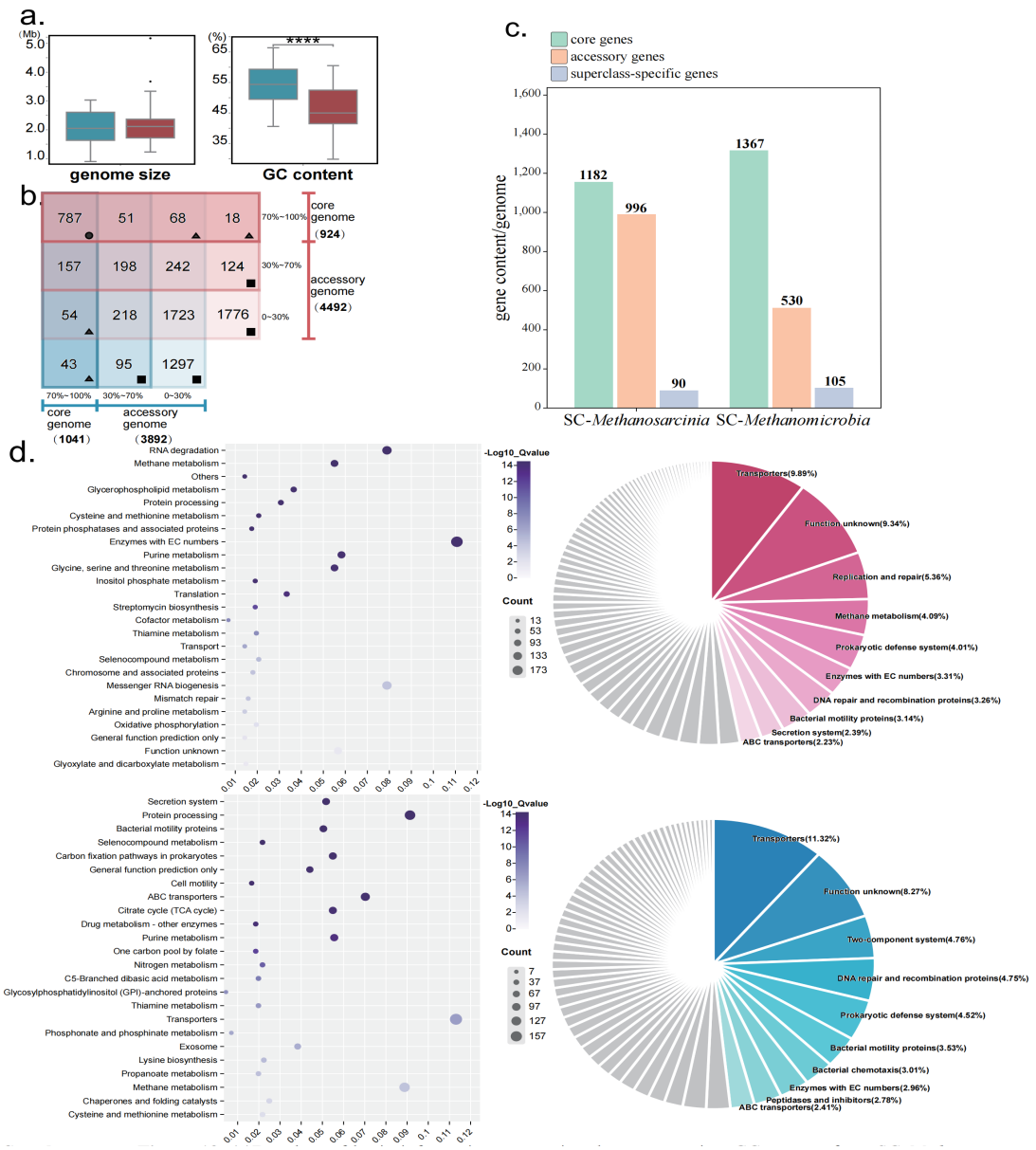
Supplementary Figure 10. Taxonomically distribution of genes coding for Rnf complex, and the phylogeny of RnfA, RnfB, RnfC, RnfD, RnfE and RnfF, being constructed with models LG+F+R7+C60, LG+I+G4+C60, LG+R8+C60, LG+F+R8+C60, LG+F+R7+C60 and LG+I+G4+C60 by IQ-Tree, respectively



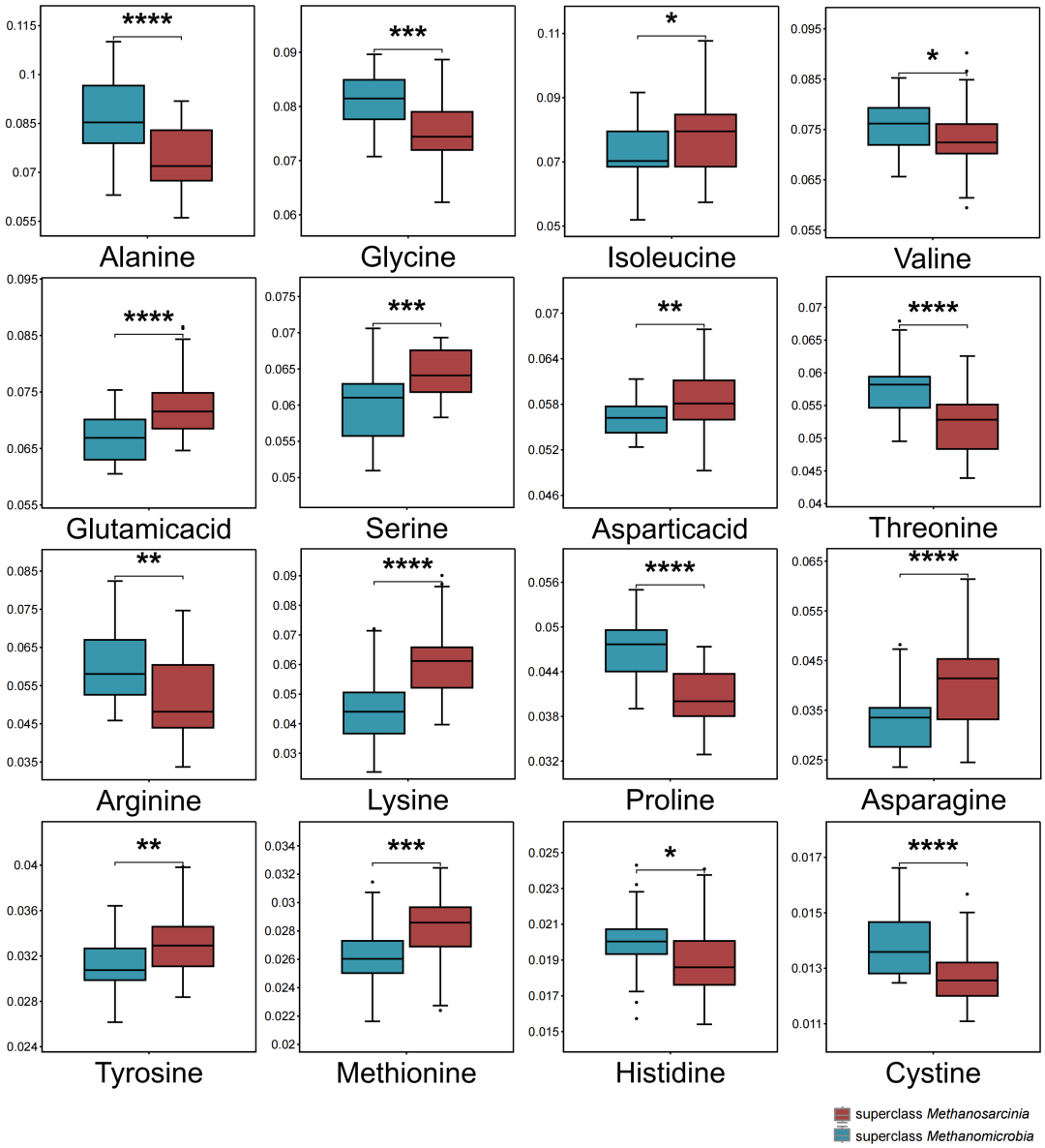
Supplementary Figure 11. Taxonomically distribution of genes coding for Vht complex, and the phylogeny of VhtA, VhtC and VhtG, being constructed with models LG+I+G4+C60, LG+I+G4+C60 and LG+I+G4+C60 by IQ-Tree, respectively.



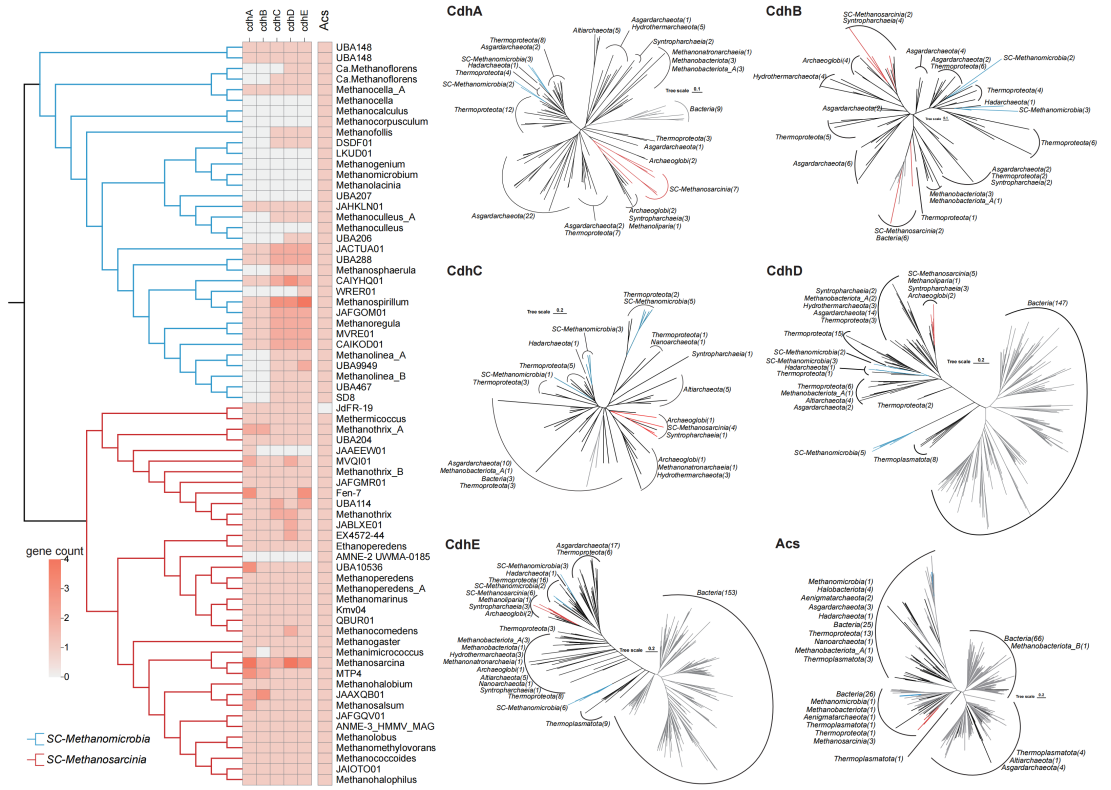
Supplementary Figure 12. Scanning electron microscopy (SEM) image of *Methanosarcina siciliae E3*.



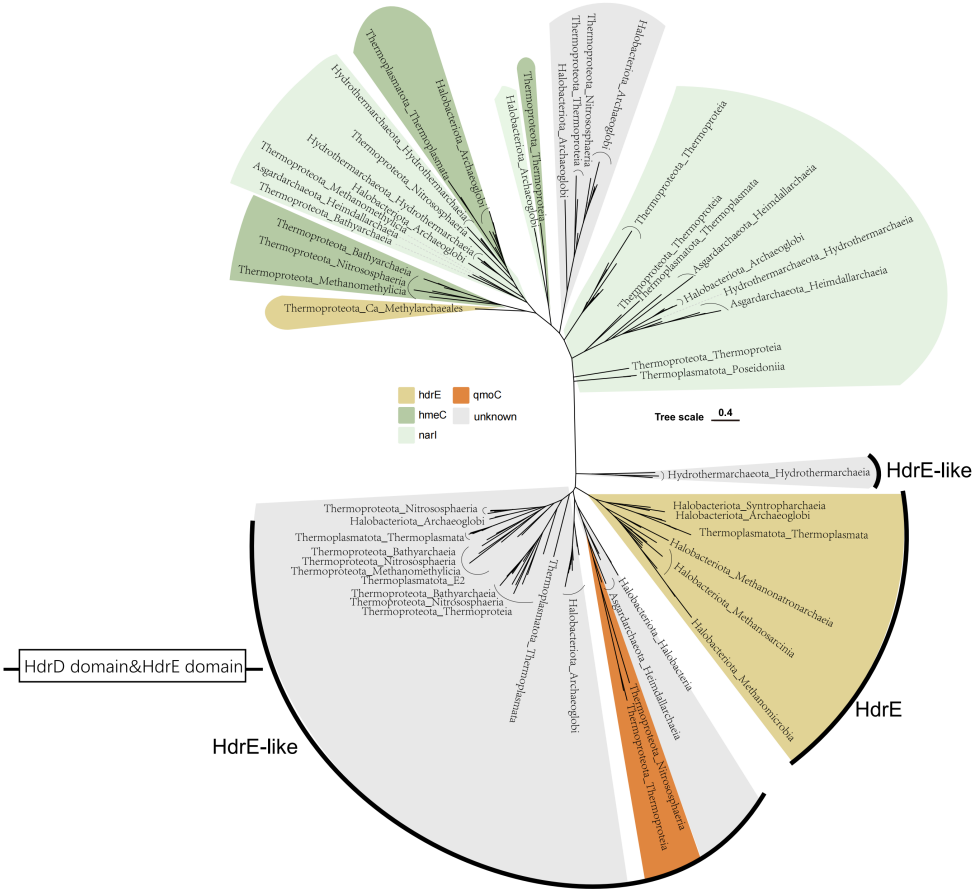
Supplementary Figure 13. (a) Boxplots of basic information, presenting the genome size, GC content from SC-*Methanosarcinia* (n=36) and SC-*Methanomicrobia* (n=34). Stars represent the significant differences between two superclasses, determined using a Wilcoxon test (****: $p < 1e{-}4$). (b)Venn figure of pangenome composition. The pangenome of each superclass is divided into three parts: the orthogroups contained by >70% members, by 30~70% members and by 0~30% members. The composition of pangenome of SC-*Methanosarcinia* is represented by three red rows, and those of SC-*Methanomicrobia* is represented by three blue columns. The number in each cell refer to counts of orthogroups. (c)The average contents of core genes, accessory genes and superclass-specific genes in SC-*Methanosarcinia* and SC-*Methanomicrobia*. (d)Bubble plots of KEGG enrichment for superclass-specific genomes of SC-*Methanosarcinia* (up) and SC-*Methanomicrobia* (below), at KEGG level three category. The pie plot shows the KEGG functional composition of accessary genomes from SC-*Methanosarcinia* (up) and SC-*Methanomicrobia* (below).



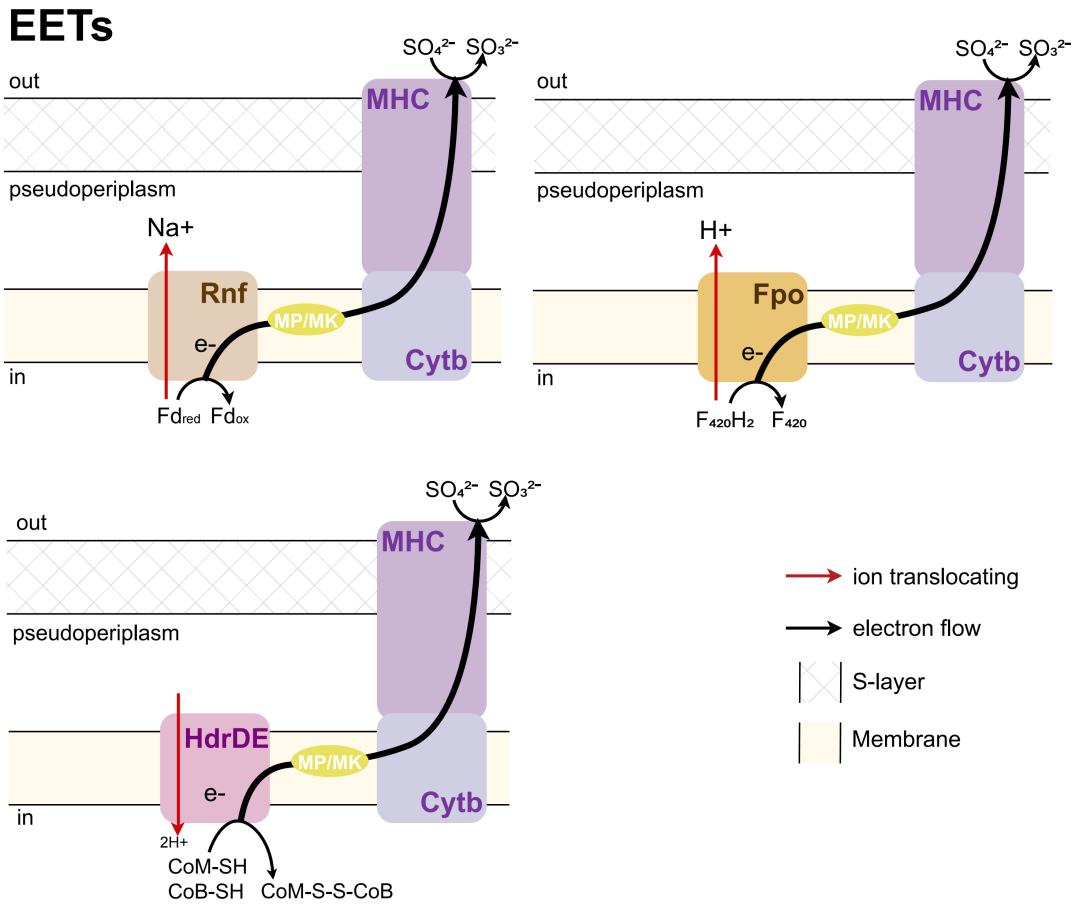
Supplementary Figure 14. Boxplots of amino acids utilization from SC-*Methanosarcinia* (n=36) (red) and SC-*Methanomicrobia* (n=34) (blue). Stars represent the significant differences between two superclasses, determined using a Wilcoxon test (****: p< 1e−4, ***: p< 1e-3, **: p< 1e-2, *: p< 0.05).



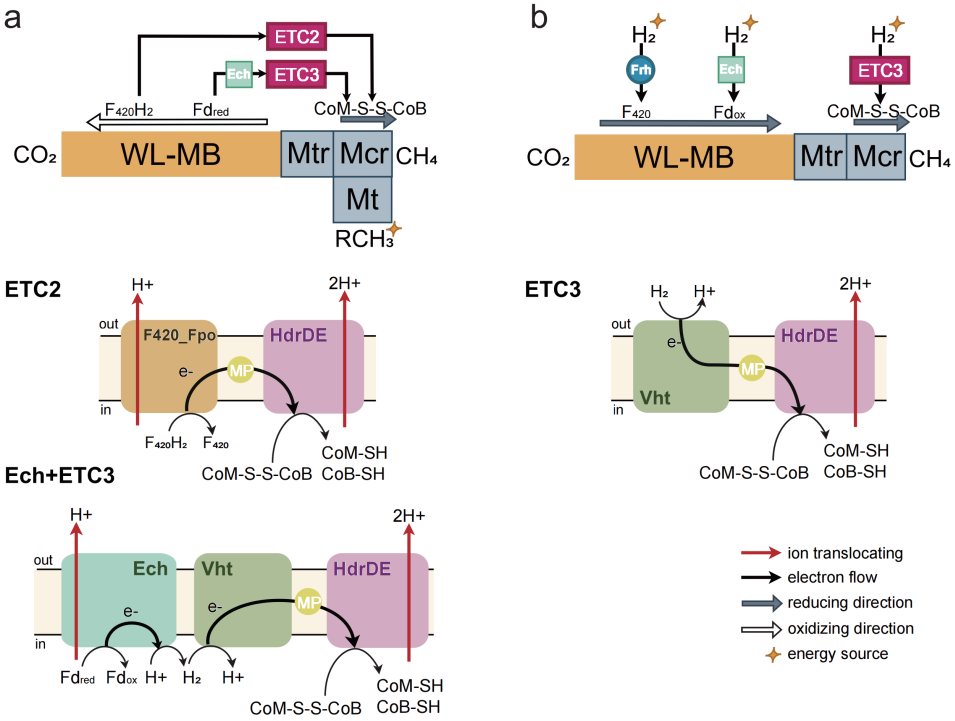
Supplementary Figure 15. Taxonomically distribution of genes coding for Cdh complex, and the phylogeny of CdhA, ChdB, CdhC, CdhD and CdhE, being constructed with the models LG+R5+C60, LG+I+G4+C60, LG+R7+C60, LG+R7+C60 and LG+F+R10+C60, respectively.



Supplementary Figure 16. The phylogeny of PF02665 family.



Supplementary Figure 17. The composition and electron transfer process of extracellular electron transfers (EETs) in ANME group from SC-*Methanosarcinia*.



Supplementary Figure 18. The electron transfer process of methyl dismutating methanogenesis (a) and carbon dioxide reducing hydrogenotrophic methanogenesis (b) in fresh water species of *Methanosarcina*.