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Expanding anaerobic alkane metabolism in the domain of Archaea

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Methanogenesis and anaerobic methane oxidation through methyl-coenzyme M reductase (MCR) as a key enzyme have been suggested to be basal pathways of archaea[1]. How widespread MCR-based alkane metabolism is among archaea, where it occurs and how it evolved remain elusive. Here, we performed a global survey of MCR-encoding genomes based on metagenomic data from various environments. Eleven high-quality *mcr*-containing metagenomic-assembled genomes were obtained belonging to the Archaeoglobi in the Euryarchaeota, Hadesarchaeota and different TACK superphylum archaea, including the Nezhaarchaeota, Korarchaeota and Verstraetearchaeota. Archaeoglobi WYZ-LMO1 and WYZ-LMO3 and Korarchaeota WYZ-LMO9 encode both the (reverse) methanogenesis and the dissimilatory sulfate reduction pathway, suggesting that they have the genomic potential to couple both pathways in individual organisms. The Hadesarchaeota WYZ-LMO4–6 and Archaeoglobi JdFR-42 encode highly divergent MCRs, enzymes that may enable them to thrive on non-methane alkanes. The occurrence of *mcr* genes in different archaeal phyla indicates that MCR-based alkane metabolism is common in the domain of Archaea.

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Table 1 | MAGs described in this study and their main potential metabolic features

MAG name	Sampling location	MCR group	Potential alkane metabolic pathways
Archaeoglobi WYZ-LMO1	Washburn Spring, WY, USA	II	MAO coupled to DSR in one cell or methanogenesis
Archaeoglobi WYZ-LMO2	Obsidian Pool, WY, USA	II	Methanogenesis from CO_2 and H_2 or MAO coupled to DSR
Archaeoglobi WYZ-LMO3	Obsidian Pool, WY, USA	II	MAO coupled to DSR in one cell or methanogenesis
Archaeoglobi JdFR-42[a]	Juan de Fuca Ridge, Pacific	III	MAO coupled to nitrate reduction or external e sinks
Hadesarchaeota WYZ-LMO4	Jinze Hot Spring, Yunnan, China	III	MAO with unknown or external e sink or alkane production
Hadesarchaeota WYZ-LMO5	Jinze Hot Spring, Yunnan, China	III	MAO with unknown or external e sink or alkane production
Hadesarchaeota WYZ-LMO6	Washburn Spring, WY, USA	III	MAO with unknown or external e sink or alkane production
Nezhaarchaeota WYZ-LMO7	Jinze Hot Spring, Yunnan, China	II	Methanogenesis from CO_2 and H_2
Nezhaarchaeota WYZ-LMO8	Jinze Hot Spring, Yunnan, China	II	Methanogenesis from CO_2 and H_2
Korarchaeota WYZ-LMO9	Washburn Spring, WY, USA	II	MAO coupled to sulfite reduction; methanogenesis from methyl groups and H_2 or reduced sulfur species as an e source
Verstraetearchaeota WYZ-LMO10	Washburn Spring, WY, USA	II	Methanogenesis from methyl groups and H_2
Verstraetearchaeota WYZ-LMO11	Washburn Spring, WY, USA	II	Methanogenesis from methyl groups and H_2

DSR, dissimilatory sulfate/sulfite reduction; MAO, MCR-based alkane oxidation. [a]Origin of this bin from ref. [53].

[illegible]

[illegible]

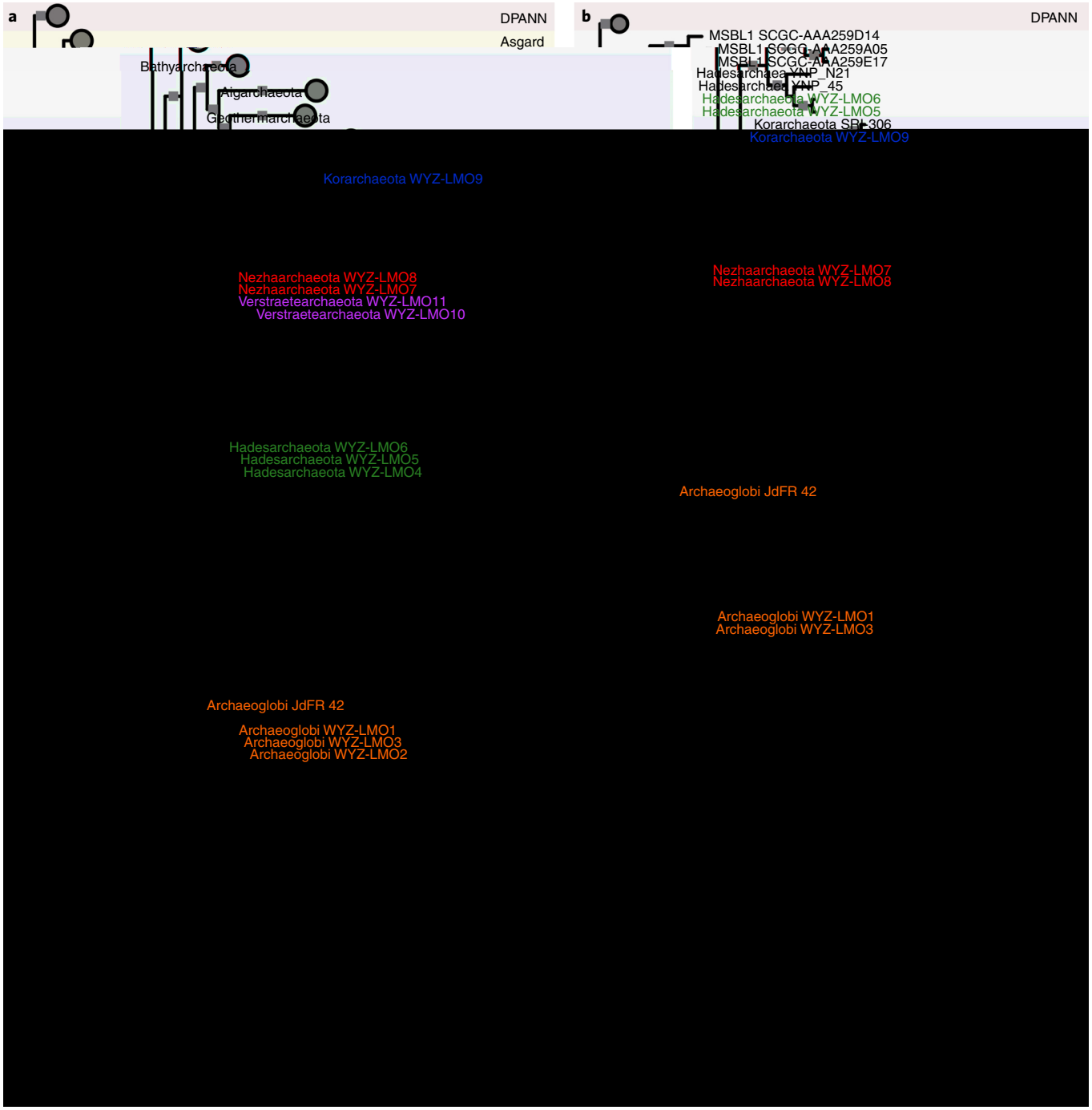

Fig. 1 | Classification of the 12 described MAGs. **a**, Phylogenomic affiliation of the MAGs based on 37 conserved protein sequences and using 233 representative archaeal genomes from 4 superphyla. **b**, Phylogenetic affiliation of the MAGs and representative archaea based on their 16S rRNA genes. Both alignments were based on MAFFT and then filtered with trimAl, and the trees were built by the IQ-Tree method with the model LG C60 F G or HKY F G with 1,000 bootstrap replicates. The phylogenetic trees were rooted at the DPANN superphylum and all four superphyla were assigned different background colours. Distinct *mcr* genes containing archaea are shown with coloured text (the Archaeoglobi WYZ-LMO1–3/JdFR-42, orange; Hadesarchaeota WYZ4–6, green; Nezhaarchaeota WYZ-LMO7 and WYZ-LMO8, red Korarchaeota WYZ-LMO9, blue; Verstraetearchaeota WYZ-LMO10 and WYZ-LMO11, purple).

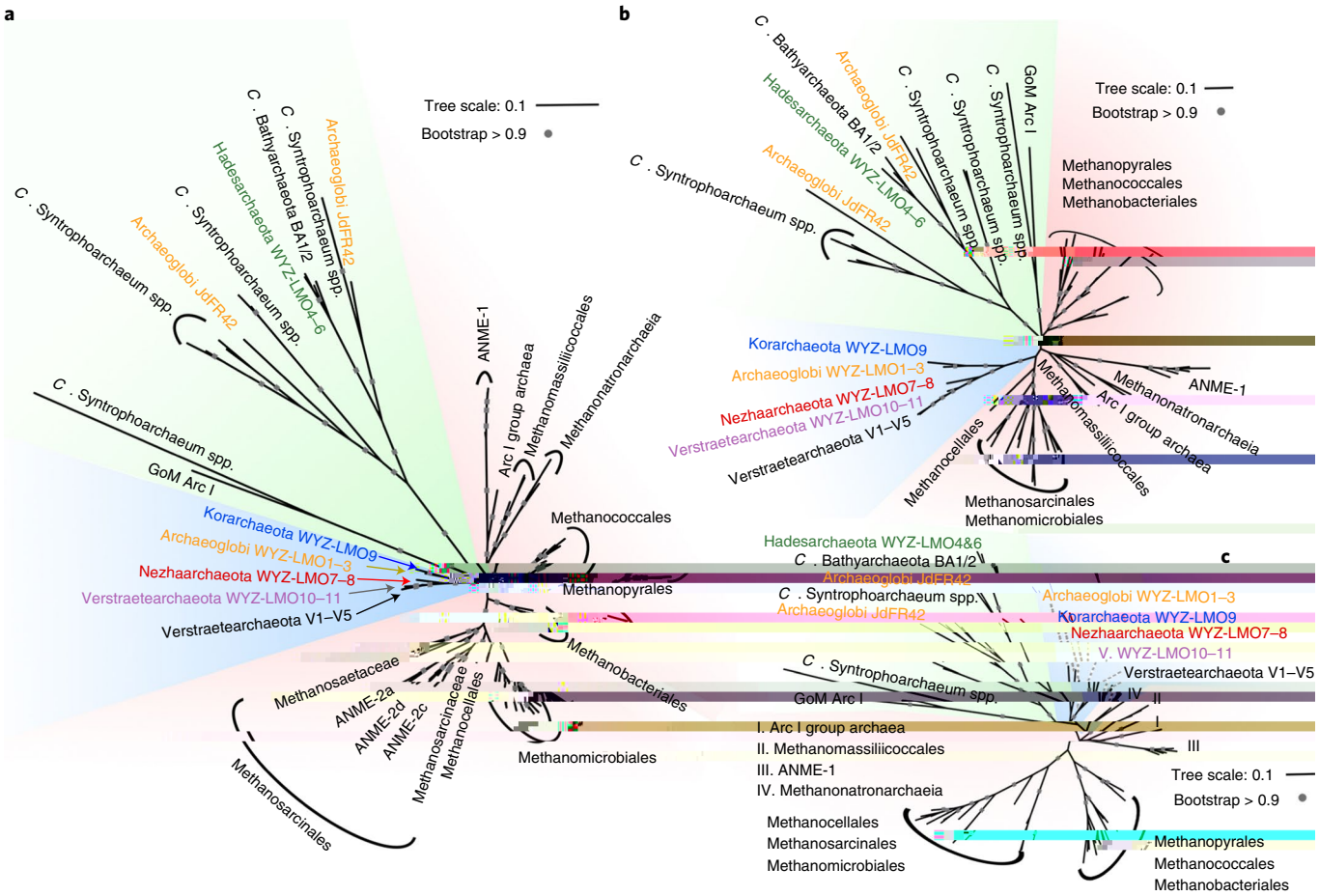

Fig. 2 | Phylogenetic affiliations of the McrA, McrB and McrG protein sequences of the 12 studied archaeal MAGs. **a**–**c**, The phylogenetic tree was constructed based on the alignments of McrA with 461 aligned positions (**a**), McrB with 351 aligned positions (**b**) and McrG with 221 aligned positions (**c**). Alignments were generated using MAFFT and then filtered with trimAl, and the trees were built by the IQ-Tree method with the model LG C60 F G with 1,000 bootstrap replicates. The tree branches were classified into three distinct MCR groups: canonical euryarchaeotal MCRs (group I, red shaded background), the MCRs of group II dominated by sequences of the TACK superphylum archaea but including the Archaeoglobi WYZ-LMO1–3 Mcr sequences (group II, blue shaded background) and the putative multi-carbon alkane oxidization MCRs (group III, green shaded background) that seem to be laterally transferred between different Archaeoglobi, Hadesarchaeota, Bathyarchaeota and *Ca*. Syntrophoarchaeum spp. Previously unidentified MCR protein sequences are also coloured (the Archaeoglobi WYZ-LMO1–3/JdFR-42, orange; Hadesarchaeota WYZ4–6, green; Nezhaarchaeota WYZ-LMO7 and WYZ-LMO8, red; Korarchaeota WYZ-LMO9, blue; Verstraetearchaeota WYZ-LMO10 and WYZ-LMO11, purple).

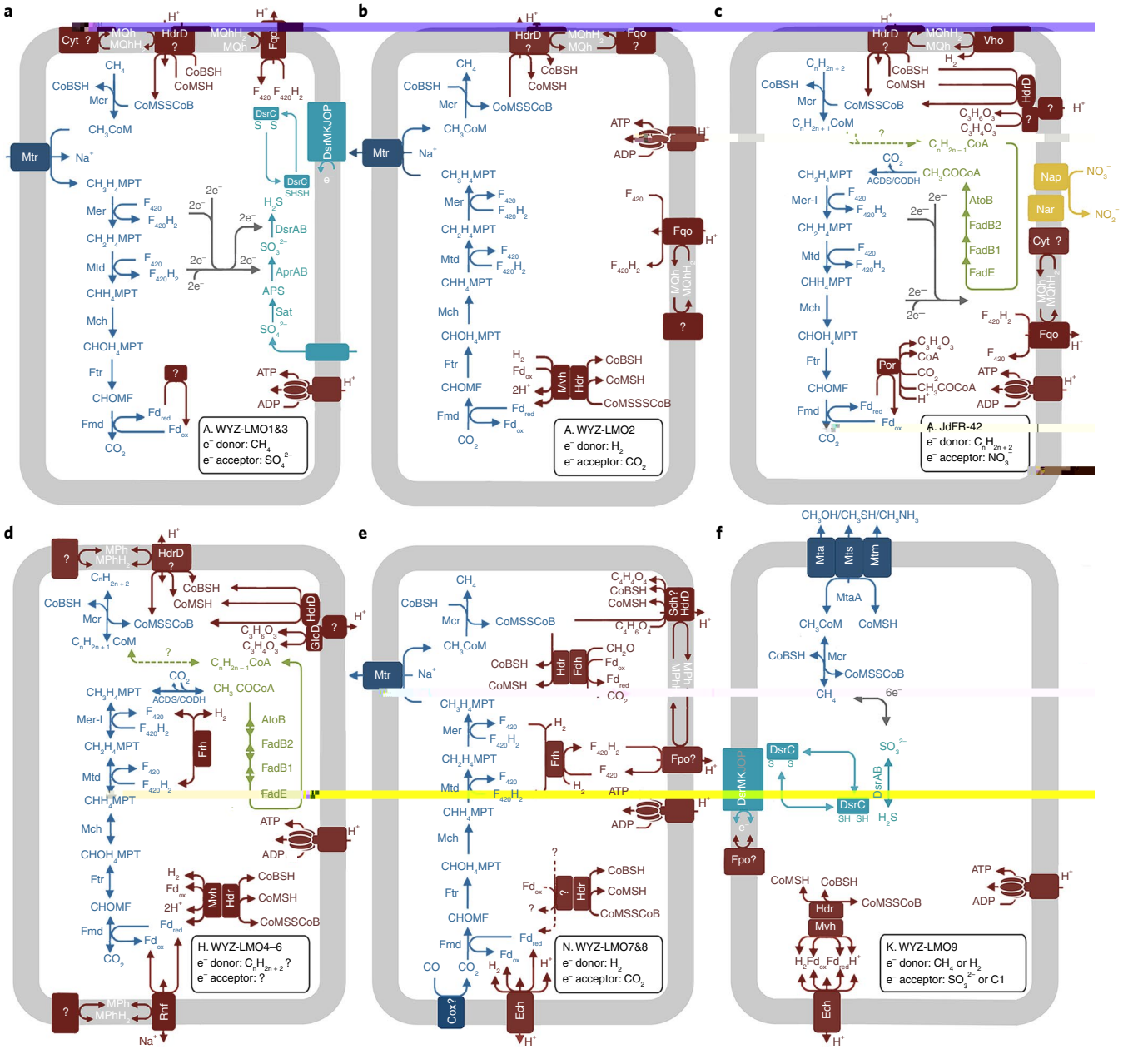

Fig. 3 | Alkane metabolic schemes of the studied MAGs with previously unidentified ***mcr*** genes. **a**–**c**, The MAGs demonstrate the large metabolic diversity of *mcr*-containing Archaeoglobi. WYZ-LMO1 (88.89% genome completeness) and WYZ-LMO3 (87.40% completeness) are probably methane oxidizers capable of sulfate reduction (**a**). WYZ-LMO2 (97.60% completeness), with a hydrogenase *mvh* gene cluster but no sulfate-reducing genes, is probably a methanogen or partner-bacterium-dependent methane oxidizer (**b**). JdFR-42 (99.84% completeness) contains the multi-carbon alkane oxidation group III *mcr* gene cluster, genes for fatty acid degradation and genes for nitrate/nitrite reduction; hence, it is probably a nitrate/nitrite- or partner-bacterium-dependent alkane oxidizer (**c**). **d**, The MAGs of Hadesarchaeota WYZ-LMO4–6 (88.89%, 83.26% and 92.37% completeness, respectively) encode sets of enzymes for multi-carbon alkane metabolism; the absence of reductive pathways suggests a need for partner bacteria. **e**, The Nezhaarchaeota WYZ-LMO7 (93.90% completeness) and WYZ-LMO8 (97.20% completeness) contain the genes for hydrogenotrophic methanogenesis. **f**, The Korarchaeota WYZ-LMO9 has both *mcr* and *dsr* genes but lacks the Wood–Ljungdahl pathway, it might also oxidize methane with sulfite reduction; alternatively, it is probably a methylotrophic methanogen that uses electrons from sulfide or hydrogen oxidation. Question marks indicate unknown enzymes and substrates; dashed arrows indicate unknown reactions. Dark blue reactions are those involved in hydrocarbon metabolism; light blue reactions are those involved in dissimilatory sulfate reduction; red reactions are those involved in energy conservation; green reactions are those involved in -oxidation; and yellow reactions are those involved in dissimilatory nitrate reduction. ACDS, acetyl-CoA decarbonylase/synthase; CODH, carbon-monoxide dehydrogenase; Cox, carbon monoxide dehydrogenase; Cytb, cytochrome b reductase; CHOMF, formylmethanofuran; Fmd, formylmethanofuran dehydrogenase; Fpo, $F_{420}H_2$ dehydrogenase; Fqo, $F_{420}H_2$:quinone oxidoreductase; Ftr, formylmethanofuran-tetrahydromethanopterin N-formyltransferase; GlcD, glycolate oxidase; Mch, methenyltetrahydromethanopterin cyclohydrolase; Mer, 5,10-methylenetetrahydromethanopterin reductase; Mer-l, 5,10-methylenetetrahydromethanopterin reductase-like enzyme; Mtd, methylenetetrahydromethanopterin dehydrogenase; NarDHI, nitrate reductase/nitrite oxidoreductase; Por, pyruvate ferredoxin oxidoreductase; Rnf, Na -translocating ferredoxin:NAD oxidoreductase; Sat, sulfate adenylyltransferase; Vho, methanophenazine-dependent hydrogenase; APS, adenosine phosphosulfate; H_4MPT, tetrahydromethanonopterin; CoMSSCoB, heterodisulfide coenzyme B coenzme M; Fd_{ox} oxidized ferrodoxin; Fd_{red}, reduced type ferrodoxin; MPh, methanophenazine; MQh, menaquinone; MPT, methanopterin.

Methods

Data selection and treatments of raw sequencing reads.

[illegible]

Phylogenetic analyses based on conserved proteins, 16S rRNA genes and McrABG/DsrA/AprA subunits. [illegible]

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Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
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☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging