In plaque-mass spectrometry imaging of a bloom-forming alga during viral infection reveals a metabolic shift towards odd-chain fatty acid lipids

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Tapping into the metabolic crosstalk between a host and its virus can reveal unique strategies employed during infection. Viral infection is a dynamic process that generates an evolving metabolic landscape. Gaining a continuous view into the infection process is highly challenging and is limited by current metabolomics approaches, which typically measure the average of the entire population at various stages of infection. Here, we took an innovative approach to study the metabolic basis of host-virus interactions between the bloom-forming alga *Emiliania huxleyi* and its specific virus. We combined a classical method in virology, the plaque assay, with advanced mass spectrometry imaging (MSI), an approach we termed ‘in plaque-MSI’. Taking advantage of the spatial characteristics of the plaque, we mapped the metabolic landscape induced during infection in a high spatiotemporal resolution, unfolding the infection process in a continuous manner. Further unsupervised spatially aware clustering, combined with known lipid biomarkers, revealed a systematic metabolic shift during infection towards lipids containing the odd-chain fatty acid pentadecanoic acid (C15:0). Applying ‘in plaque-MSI’ may facilitate the discovery of bioactive compounds that mediate the chemical arms race of host-virus interactions in diverse model systems.

Microbial interactions, such as those between a host and its pathogen, are shaped by a dynamic metabolic crosstalk and chemical communication1,2. Metabolomics tools allow simultaneous identification of thousands of molecules in a given sample1 and have been used to monitor the metabolic fluctuations in diverse host-symbiont and host-pathogen model systems3,4.

Mapping the metabolic landscape of dynamic processes, such as viral infection, requires high temporal resolution of the different stages of infection5-7. Most metabolic studies of viral infection are performed in bulk liquid cultures, measuring the average of the entire population at various time points during infection6,7. However, infection is not always a synchronous process, in which all cells are infected at the same time. Asynchronous infection can lead to a mixed population of cells at different stages of infection. Consequently, bulk analysis might overlook rare subpopulations (for example, resistant cells)3 and mask metabolic alterations that originate within them5.

Rewiring of host metabolic pathways by the virus during infection generates multiple metabolic states that may affect the infection outcome (that is, cell death or resistance)3. Mapping these metabolic states into infection states might uncover unique strategies employed by the virus, which are essential for an optimal infection process6. Furthermore, the occurrence of specialized metabolic capabilities encoded by viruses (that is, auxiliary metabolic genes), common in the marine environment, provides an opportunity to reveal metabolic innovation during viral infection6. Recent advances in mass spectrometry imaging (MSI) technologies allow the localization of specific metabolites of various dynamic processes and biotic interactions at the microscale level and, consequently, monitoring of metabolic changes in high spatiotemporal resolution2,11. MSI-based approaches were used to study the metabolic footprints of host-associated bacteria12, bacterial population dynamics3 and to detect human pathogens5,6.

An attractive host–virus model system is the cosmopolitan alga *Emiliania huxleyi* and its specific virus, *E. huxleyi* virus (EhV), which plays a key role in regulating the fate of carbon and sulfur in contemporary oceans17. *E. huxleyi* forms massive annual blooms that cover vast oceanic areas and are terminated following infection by EhV18. EhV infection causes profound remodelling of the transcriptome of *E. huxleyi*, which leads to massive changes in the metabolome and lipidome of infected cells. For example, changes in glycolysis, de novo fatty acid (FA) synthesis19, induction of triacylglycerols (TAGs) and rewiring of sphingolipid biosynthesis8,20,21. EhV is a large double-stranded DNA virus with a genome harbouring 472 predicted protein-coding sequences, and it encodes almost a full biosynthetic pathway for sphingolipids, a pathway that was not detected in any other viral genome to date22-24. Virus-derived glycosphingolipids (vGSLs), the products of this virus-encoded pathway, were found to be central components of the EhV membranes and to trigger host programmed cell death19,23,24. Hallmarks of programmed cell death include the production of reactive oxygen species25, induction of caspase activity, metacaspase expression and compromised membrane integrity26,27. vGSLs are produced exclusively during viral infection and are used as an effective metabolic biomarker to detect viral infection during natural blooms of *E. huxleyi* in the ocean17,18.

In this study, we sought to gain higher spatiotemporal resolution of metabolic biomarkers that are associated with viral infection and,
in addition, to discover the metabolites that are induced or reduced throughout this dynamic process. We combined a classical method in virology, the plaque assay, with advanced MSI technologies, a unique approach we termed ‘in plaque-MSI’. This approach increased the spatiotemporal resolution by which we could study the metabolic profile of viral infection, taking advantage of the characteristics of the plaque. MSI data were used to visualize the changes of known lipid biomarkers for viral infection across the plaque. In parallel, unsupervised spatially aware clustering and colocalization allowed for the identification of lipids that were previously undescribed in this host–virus model system, in addition to the discovery of lipids of an unknown class. This approach uncovered a profound metabolic shift towards pentadecanoic acid (C15:0)-based lipids during infection.

Results
To gain high spatiotemporal resolution of the metabolic alterations during host–virus interactions, we mapped the metabolic profile of viral plaques (Fig. 1). As opposed to infection in liquid medium, a plaque originates in a single infected cell. The plaque expands via concentric rings, and each ring corresponds to the plaque circumference at a different time point, keeping a metabolic record of the infection. Thus, a snapshot of a plaque unfolds the infection in a continuous manner based on a snapshot view of the plaque. Despite the differences in resolution and sample preparation, a unique approach we termed ‘in plaque-MSI’. This approach increased the spatiotemporal resolution by which we could study the metabolic profile of viral infection, taking advantage of the characteristics of the plaque. MSI data were used to visualize the changes of known lipid biomarkers for viral infection across the plaque. In parallel, unsupervised spatially aware clustering and colocalization allowed for the identification of lipids that were previously undescribed in this host–virus model system, in addition to the discovery of lipids of an unknown class. This approach uncovered a profound metabolic shift towards pentadecanoic acid (C15:0)-based lipids during infection.

**Targeted MSI analysis of known lipid biomarkers across a viral plaque.** We focused on known lipid biomarkers for viral infection, which were shown to be remodelled during infection in previous liquid chromatography–MS (LC–MS)-based lipidomics experiments performed in liquid cultures.

These lipid biomarkers are usually classified based on their chemical category, such as glycerolipids, glycerophospholipids and sphingolipids. As the viral genome also encodes the biosynthetic pathway for sphingolipids, the lipids can also be classified based on their biosynthetic origin (that is, produced by alga-encoded or virus-encoded enzymes). In addition, these lipid biomarkers can be classified based on their relative abundance during infection (that is, induction or reduction). Thus, we grouped the lipids into three biological categories: virus-derived (encoded by the virus and induced during infection), host-induced and host-reduced (encoded by the host induced or reduced during infection). VGLS are the only virus-derived lipids known to date, with the majority of enzymes in their biosynthetic pathway encoded by the viral genome. Conversely, remodelled host lipids include diverse classes: glycerolipids, such as betaine-like lipid (BLL) 36:6, TAG 48:1 and diacylglyceryl carboxyhydroxymethyllycholine (DGCC) 40:7; and glycerophospholipids, such as phosphatidyl-1,2-diacyl-3-methylpropanethiol (PDPT) 40:7 and phosphatidylethanolamine (PC) 32:1; these were all found to be induced during infection (that is, ‘host induced’), whereas others, such as BLL 38:6, were found to be reduced (that is, ‘host reduced’).

MS images of these lipid biomarkers across a plaque (Fig. 2) allowed us to follow their induction and reduction patterns during infection in a continuous manner based on a snapshot view of the plaque. Despite the differences in resolution and sample preparation, in most cases, both MALDI-MS (Fig. 2a) and Flow-probe-MS (Fig. 2b) presented comparable spatial patterns (correlation > 0.8 for most lipids; see Supplementary Table 1) and highlighted the differences in the intensity of various lipids across the plaque. For example, vGSL (17:0/h22:0) showed high relative intensity across the plaque area, whereas BLL 36:6 showed a different pattern, with lower intensity at the centre of the plaque. Both lipids were detected only inside the plaque, whereas others, such as DGCC 40:7, PDPT 40:7 and TAG 48:1, were also detected outside the plaque area, albeit at a lower intensity, indicating a basal production in uninfected cells. Cross-section profiles (Fig. 2) were used to present the relative abundance of each lipid along the plaque and revealed even finer differences, such as distance and degree of induction, between lipids with similar MS images. Interestingly, TAG 48:1 presented a unique ring-like pattern inside the plaque (visible only in the MALDI-MS, owing to its higher spatial resolution), which was not identified in other known lipid species.

Taken together, the targeted analysis indicates the existence of chemotypic heterogeneity during infection, as visible from the diverse intensity patterns of specific lipids across the plaque. This continuous metabolic view of the infection process would not have been possible in bulk LC–MS-based lipidomics.
Untargeted data analysis using spatially aware clustering. We applied an unsupervised spatially aware clustering approach to Flow-probe-MS data generated from a plaque at 6 days post-infection (d.p.i.; Fig. 3a) to discover metabolites that are altered during infection\(^{34,35}\). First, m/z images were generated for each m/z value recorded, depicting the intensity of the m/z values in each of the pixels analysed in the sample. The m/z images were clustered based on correlations between their spatial intensity profiles (Fig. 3b).
Fig. 3 | Unsupervised spatially aware clustering allows the identification of unknown lipids. a, Chlorophyll autofluorescence (red pseudo-colour) of the plaque sample at 6 d.p.i. b, Dendrogram clustering of all m/z values based on their spatial Flow-probe-MS distribution along the plaque (performed on a single experiment, as described in the Methods section). Nine clusters that presented distinct induction or reduction patterns across the plaque (as was visible by the representative average images of the clusters; see Supplementary Figs. 2 and 3) were classified and labelled. The category of each cluster was determined by the inclusion of known lipid biomarkers for viral infection. c, Four selected clusters are presented in detail, including their respective cluster-representative images and putatively annotated mass features. Mass features in blue indicate lipids that were previously described in the E. huxleyi–EhV model system; mass features in black indicate either lipids previously undescribed in this model system or lipids of an unknown class, which were not found in known repositories (termed sulfonioglycerolipids; see Table 1). *Mass features of vGSL t17:0/h23:1 and vGSL t17:0/h23:0 were also putatively annotated as glucosylceramide t18:0/h22:1 and glucosylceramide t18:0/h22:0, respectively. **Partial representation of CL5 (see Supplementary Fig. 3). A full m/z list of the four clusters can be found in Supplementary Table 2. Scale bars, 1 mm. (i), isotope; (f), fragment.
A representative image was then generated for each cluster in the dendrogram, averaging the m/z images of the cluster members. Based on these cluster-representative images, we selected clusters that presented distinct induction or reduction patterns across the plaque (Supplementary Figs. 2 and 3). This allowed us to focus on clusters with biological relevance to the infection dynamics. Next, we matched the m/z values in each cluster with the known annotations of lipid biomarkers and used them as markers for the cluster category (that is, virus derived, host induced or host reduced). For example, cluster two (CL2) included several vGSL species (Supplementary Fig. 4), as well as other host-induced lipids, and therefore was classified as a ‘virus-derived and host-induced’ cluster. We then focused on four clusters that presented more pronounced changes across the plaque (Fig. 3c): three clusters (CL2, CL4 and CL5) that were induced in the plaque and one (CL7) that was reduced. In total, the four clusters contained 129 mass features (see the Methods section and Supplementary Table 2). Nineteen mass features were putatively annotated as lipids previously undescribed in this host–virus model system (18 induced and 1 reduced; see Table 1 and Supplementary Fig. 5), one of which was putatively identified as vGSL-like t16:0/h22:0 (as previously suggested by long-chain base analysis24; see Supplementary Fig. 6 and Supplementary Table 3). Five features that shared the same head group were not found in known repositories and were termed sulfonioglycerolipids (all decreased; see Table 1, Supplementary Figs. 5 and 7 and Supplementary Table 4).

Interestingly, 16 of the lipids that increased across the plaque were putatively identified as odd-chain fatty acid (OC-FA) lipids based on the LIPID MAPS computationally generated database of lipid classes and structure database (LMSD)14. Fourteen were previously undescribed in the E. huxleyi–EhV model system, whereas two were previously detected; however, their unique OC-FA characteristic was not identified15. MS/MS analyses of the OC-FA lipids using ‘in plaque-Flow-probe’ revealed the existence of a C15:0 FA chain (pentadecanoic acid; see Fig. 4a and Supplementary Data 1). The presence of C15:0 FA was further suggested by targeted LC–MS/MS analysis of infected cultures growing in liquid medium (Supplementary Figs. 8 and 9, Supplementary Tables 5 and 6 and Supplementary Data 2). The occurrence of C15:0 FA rather than a branched C14:0 FA (methyltetradecanoic acid) was confirmed by fatty acid methyl ester (FAME) analysis using gas chromatography–MS (GC–MS; see Fig. 4b and Supplementary Fig. 10).

LC–MS-based analysis of these specific lipids during infection in liquid culture revealed a profound induction compared to control uninfected cells (Fig. 4c and Supplementary Table 7). These OC-FA lipids were also detected in LC–MS-based analysis of the plaques, as well as in purified virions and in extracellular vesicles that originated from infected cultures (Supplementary Table 7). To explore the global distribution of C15:0-based lipids, we compared the abundance of C15:0 FA fragments in the lipidome of infected and uninfected cultures using LC–MS–based lipidomics of liquid cultures (Fig. 4d). This comparison revealed massive induction of C15:0-based lipids during lytic infection in diverse lipid classes, suggesting a systemic-level metabolic switch towards a C15:0-based lipidome. Following a targeted search, we were able to putatively identify seven more lipids containing a C15:0 FA (Supplementary Data 3). One of these lipids was TAG 49:1, which presented a unique ring-like intensity pattern across the plaque (Fig. 2) and has been shown to be highly induced during viral infection2; however, its FA composition was never explored. Our LC–MS/MS analyses indicated the existence of two structural isomers differing in their FA composition. Whereas one isomer contained only even-chain FAs (C14:0, C16:0, C18:1) and was found in both uninfected and infected cells, the second isomer contained two OC-FAs (C15:0, C15:0, C18:1) and was found only in infected cells (Supplementary Fig. 11 and Supplementary Data 1).

### Table 1 | Putative identification of previously undescribed lipids in the E. huxleyi–EhV model system

| CL | Putative annotation (lipid composition) | Theoretical m/z | Chemical formula | Change |
|---|---|---|---|---|
| 2 | DGCC 32:1 (14:0/18:1) | 726.5884 | C_{26}H_{40}O_{8}N_{3} | Induced |
| 2 | DGCC 37:6 (15:0/22:6) | 786.5884 | C_{28}H_{60}O_{8}N_{3} | Induced |
| 2 | TAG 47:1 (14:0/15:0/18:1) | 791.7129 | C_{29}H_{56}O_{8}N_{3} | Induced |
| 2 | vGSL-like (16:0/22:0) | 790.6408 | C_{30}H_{60}O_{8}N_{3} | Induced |
| 4 | DGTS 33:1 (15:0/18:1) | 724.6091 | C_{29}H_{56}O_{8}N_{2} | Induced |
| 4 | PDPT 32:1 (14:0/18:1) | 749.5155 | C_{26}H_{40}O_{8}P_{2} | Induced |
| 4 | PDPT 33:1 (15:0/18:1) | 763.5132 | C_{27}H_{44}O_{8}P_{2} | Induced |
| 4 | PDPT 34:1 (16:0/18:1) | 777.5468 | C_{28}H_{48}O_{8}P_{2} | Induced |
| 4 | PDPT 37:6 (15:0/22:6) | 809.5155 | C_{29}H_{56}O_{8}P_{2} | Induced |
| 4 | TAG 49:1 (15:0/16:0/18:1) | 819.7442 | C_{31}H_{56}O_{8} | Induced |
| 4 | TAG 51:6 (15:0/18:1/18:5) | 837.6972 | C_{33}H_{58}O_{8} | Induced |
| 4 | TAG 51:2 (15:0/18:1/18:1) | 845.7598 | C_{33}H_{58}O_{8} | Induced |
| 4 | TAG 53:6 (15:0/16:0/22:0) | 865.7285 | C_{34}H_{60}O_{8} | Induced |
| 4 | TAG 55:1 (15:0/18:1/22:0) | 903.8381 | C_{36}H_{62}O_{8} | Induced |
| 4 | TAG 55:6 (15:0/18:0/22:6) | 893.7598 | C_{36}H_{62}O_{8} | Induced |
| 4 | TAG 55:7 (15:0/18:1/22:6) | 891.7442 | C_{36}H_{62}O_{8} | Induced |
| 4 | TAG 59:12 (15:0/22:6/22:6) | 937.7285 | C_{36}H_{62}O_{8} | Induced |
| 7 | PDPT 28:0 (14:0/14:0) | 695.4686 | C_{28}H_{46}O_{8}P_{2} | Reduced |
| 7 | Sulfonioglycerolipid 28:0 (14:0/14:0)* | 689.5026 | C_{29}H_{52}O_{8}S | Reduced |
| 7 | Sulfonioglycerolipid 30:0 (14:0/16:0)* | 717.5339 | C_{31}H_{54}O_{8}S | Reduced |
| 7 | Sulfonioglycerolipid 36:6 (14:0/22:6)* | 789.5339 | C_{38}H_{58}O_{8}S | Reduced |
| 7 | Sulfonioglycerolipid 38:6 (16:0/22:6)* | 817.5652 | C_{38}H_{58}O_{8}S | Reduced |
| 7 | Sulfonioglycerolipid 40:7 (18:1/22:6)* | 843.5797 | C_{39}H_{60}O_{8}S | Reduced |

Nineteen lipids were previously undescribed in the E. huxleyi–EhV model system and five belong to an unknown class of lipids (termed sulfonioglycerolipids). The lipids were putatively annotated based on MS/MS spectra (Metabolomics Standards Initiative level 2 annotation73), except for the sulfonioglycerolipids (marked with an asterisk), which belong to the same putatively characterized compound class based on MS/MS spectra (level 3 annotation). ‘CL’ cluster (Fig. 3c). ‘Change’, as visible in the Flow-probe-MS images (Supplementary Fig. 5). The [M + H]^+ adduct is presented for all lipids.

A parallel analysis of MALDI-MS data by a computational colocalization approach was applied to three known lipid biomarkers: vGSL (117:0/h22:0), BLL 36:6 and BLL 38:6 (Supplementary Tables 8–10). Most of the lipids that colocalized with vGSL and BLL 36:6 were also found in the two clusters that originated from the Flow-probe-MS data (CL2 and CL4; see Fig. 3), including many of the C15:0-based lipids (Table 1). BLL 38:6, which is reduced during infection, was colocalized with all sulfonioglycerolipids found in the host-reduced cluster in the Flow-probe-MS data set (CL7; see Fig. 3 and Supplementary Table 10). Colocalization of TAG 48:1, which presented a unique ring-like intensity pattern, revealed seven additional mass features harbouring a similar pattern, one of which was identified as TAG 46:1, and the remaining six features are currently unknown (Supplementary Fig. 12 and Supplementary Table 11).

**Discussion**

Tapping into the metabolic crosstalk between a host and its infecting virus can provide a unique way for the identification of metabolic...
Fig. 4 | Induction of C15:0-based lipids during viral infection. a, MS/MS-based putative identification of DGCC 37:6 with C15:0 FA (theoretical [M + H]+ = 786.5884 Da, observed = 786.5862 Da). The analysis was performed using both Flow-probe-MS/MS and LC-MS/MS based on two independent experiments in each technique, yielding similar results (Supplementary Data 1). The inset shows magnification of the mass range 450–570 Da. Double bonds in C22:6 FA were assigned based on the most common structure in the LMSD. b, GC–MS identification of C15:0 methyl ester in four different derivatized fractions collected from LC–MS runs of an infected culture at 48 h.p.i. Fraction 1, DGCC 37:6 and PDPT 37:6; fraction 2, PDPT 33:1; fraction 3, DGTS 33:1; fraction 4, several TAGs. Methyl-C15:0 FA, methyl pentadecanoate standard; methyl-12-methyl-C14:0 FA, methyl 12-methylmyristate standard. FAME analysis was performed on two independent experiments, yielding similar results. c, Heatmap representation of the alterations in C15:0-based lipids in uninfected and infected cells at different h.p.i. (left) and MALDI-MS images of three representative lipids (right). Differences between control and infected samples were significant for all lipid species (two-way ANOVA, false discovery rate-corrected \( P < 0.02, n = 3 \)). Scale bars, 2 mm. d, Systemic remodelling in C15:0-based lipids during viral infection presented by LC–MS extracted ion chromatogram of the C15:0 fragment ([M+H]+ = 255.52 Da, negative-ion mode, energy ramp of 10–40 eV) in uninfected and infected cells after 48 h. Values are presented as the mean ± s.d. (light red or blue colour), \( n = 3 \).

pathways that are essential for viral infection or host defence strategies. *E. huxleyi*-EhV is an important host–pathogen model system with great ecological importance in the marine environment\(^{17,19}\). Viral infection leads to substantial rewiring of the lipidome of infected cells\(^{8,20,21}\). We could identify differences between lipidomics experiments performed in liquid cultures and represent to be remodelled during viral infection in previous LC–MS-based biomarkers for viral infection. These lipid biomarkers were shown to be remodelled during viral infection in previous LC–MS-based lipidomics experiments performed in liquid cultures and represent diverse lipid classes\(^{19,30,33}\). We could identify differences between lipids that previously presented similar trends during infection. vGSL and BLL 36:6, which are used as lipid biomarkers for *E. huxleyi* infection in the natural environment\(^{23,28,39}\), were both induced in the plaque. However, BLL 36:6 was reduced at the centre of the plaque and was induced at a greater distance from the centre than vGSL (Fig. 2). Lower intensity of BLL 36:6 at the centre might suggest modification of this lipid or its consumption and recycling for the synthesis of other metabolites at later stages of infection. The different localization of induction might be explained by the production of BLL 36:6 in cells at earlier stages of infection than vGSL, or even in uninfected cells due to a chemical
signal diffusing from the infected cells. It is also possible that BLL diffuses at a faster rate than vGSL in the plaque. It is important to note that, as the center of the plaque consists of mainly lysed cells, the detected lipids in this area and their signal might originate not only from the lysed cells and virions but also from different biodegradation processes that can occur in the agarose.

Conversely, the chemical stability of lipids does not seem to play a significant role in the relative abundance measured across the plaque (Supplementary Fig. 13). The structure of the plaque and the identification of distinct induction and reduction patterns across the plaque allowed us to apply unsupervised spatially aware clustering on Flow-probe-MS.
data to discover metabolites that are induced or reduced during infection. The representative average image of each cluster was used to reduce the complexity of the untargeted data, allowing us to select clusters with biological relevance to the infection dynamics. A selected number of clusters, all of them with distinct patterns, enabled us to concentrate our identification efforts on a smaller number of infection-related mass features (Fig. 3). Thus, we were able to detect a group of functionally relevant metabolites from a plethora of unknown mass features based on their spatial intensity patterns. Such a dedicated identification might help to uncover metabolites that are masked in untargeted LC–MS-based metabolomics analyses. Applying ‘in plaque-MSI’ to other host–virus interactions might allow the discovery of bioactive molecules and potential antiviral compounds.

We putatively annotated 19 lipid species that were previously undescribed in this host–virus model system and 5 of an unknown class of lipids, termed sulfonioglycerolipids. The sulfonioglycerolipids share the same head group and were highly reduced along the plaque and in infected liquid cultures (Supplementary Table 7). More than half of the lipids previously undescribed in this model system were putatively identified as C15:0-based lipids and were highly increased across the plaque and in infected liquid cultures (Fig. 4c). They were also detected in purified virions and in extracellular vesicles that originated from infected cultures (Supplementary Table 7) and represent a fraction of a systematic induction of C15:0-based lipids during infection (Fig. 4d). Interestingly, C15:0 FAs were incorporated in lipids usually found in the cytoplasmic membrane (for example, sphingolipids, DGCCs, diacylglycerol trimethylhydroxymoserines (DGTSs) and PDPTs) or in lipid droplets (for example, TAGs), but not in lipids found in the chloroplast (for example, glycosylated glycolipids; see Fig. 5). Although the biological function of OC-FA lipids is still unknown, C15:0 FA has been previously shown to be less preferred for β-oxidation than even-numbered FAs. Thus, selective incorporation of OC-FA lipids in the viral membrane might increase the durability of the viral particles and, consequently, attenuate their decay rate due to UV stress in the marine environment. Induction of C15:0-based lipids during viral infection is also consistent with a previous report that indicated an increase in the levels of C15:0 FA during viral infection, although the specific lipids were not investigated. Interestingly, C15:0 FA is also involved in the production of vGSLs during viral infection of E. huxleyi by EhV. vGSLs are products of a virus-encoded biosynthetic pathway and were found to be central components of the EhV membrane and to trigger E. huxleyi programmed cell death. EhV causes a shift in the substrate specificity of the virus-encoded enzyme pyridoxal 5'-phosphate-dependent serine palmitoyl transferase (SPT; EC 2.3.1.50), which is the rate-limiting enzyme of the entire sphingolipid biosynthetic pathway. In infected cells, the virus-derived SPT enzyme was shown to use C15:0-FA-CoA, whereas in uninfected cells, the host-derived SPT typically utilizes the canonical C16:0-FA-CoA. The C15:0-FA-CoA used by virus-derived SPT serves as a substrate for the unusual hydroxylated C17 long-chain base found in vGSLs.

Recent years have witnessed a growing interest in lipids containing OC-FA and their role as markers for metabolic disorders in humans. OC-FA lipids, including C15:0-based lipids, have been reported in humans, animals, microorganisms and plants. Nevertheless, understanding of their biological function is still elusive. Several biosynthetic pathways have been proposed for OC-FAs, either de novo or by chain shortening of longer FA chains (Fig. 5). De novo synthesis involves the conversion of propionyl-CoA to propionyl-CoA, which, in turn, can replace acetyl-CoA in the initial step of fatty acid synthesis. Excess levels of propionyl-CoA were reported to increase the levels of C15 and C17 FAs in humans. In addition, phytosphingosine, a sphingoid base of GSLs, was previously reported as a source of C15:0 FAs in yeast. The odd-numbered FAs are later incorporated into glycerophospholipids. Future studies will enable elucidation of the biochemical origin and function of C15:0-based lipids during viral infection in the ocean.

Taken together, the ‘in plaque-MSI’ approach revealed a systemic metabolic shift towards C15:0-based lipids as a result of viral infection. Identification of this profound metabolic shift following an untargeted analysis might not have been possible in LC–MS-based analysis of bulk samples, as C15:0-based lipids are represented in diverse lipid classes. This shift might be part of the viral strategy to hijack host metabolism during infection, by inducing an orthogonal biosynthetic pathway for the synthesis of these lipids. Given their high and specific abundance during infection, C15:0-based lipids might serve as unique biomarkers for E. huxleyi–EhV interactions in the ocean.

Methods
Culture growth and viral infection dynamics. E. huxleyi strain CCMP2909 was used for this study. Cells were cultured in K/2 medium in artificial seawater (ASW) supplemented with ampicillin (100 µg ml⁻¹) and kanamycin (50 µg ml⁻¹) and incubated at 18 °C with a 16/8 h light/dark illumination cycle. A light intensity of 100 µmol photons m⁻² s⁻¹ was provided by cool white light-emitting diode lights. The virus used in this study was the lytic EhV201 (ref. ). Growing liquid cultures in the presence of antibiotics maintained a low basal abundance of bacteria throughout the experiments in both control and infected samples (Supplementary Fig. 14a). The lipid profile of the samples did not change significantly upon adding antibiotics, suggesting that the contribution of bacteria to the production of these specific lipids was insignificant (Supplementary Fig. 14b). The bacterial abundance was measured also in agarose samples, and no significant difference was detected between control and plaque samples (Supplementary Fig. 14c).

Plaque assay. Cells (150 ml) at 2–3×10⁶ cells per ml were concentrated (2,000 g at 5 min at 18 °C) to 2.7 ml. Virions (300 µl) at a concentration of 10⁴ virions per ml were added to the cells. After 2 h of incubation under normal growth conditions, the host–virus mixture was mixed with 9 ml K/2 medium in ASW that contained 300 µl of 1 µM SeaKem LE Agarose (LMP) and then poured onto a solidified plate (12×12 cm) containing K/2 medium in ASW solidified by 1.5% agarose, supplemented with ampicillin (100 µg ml⁻¹) and kanamycin (50 µg ml⁻¹). Plaques were visible to the naked eye usually at 2 d.p.i. Plaques were subjected to MSI analysis at 5–6 d.p.i. when they were at a size large enough for spatial analysis (diameter of 4–8 mm), yet were still active and growing. Control samples were prepared following the same procedure, adding 300 µl ASW instead of virions. Blank samples were prepared following the same procedure, using K/2 medium only (that is, without the host–virus mixture).

Enumeration of cells, viruses and bacteria. Cells were quantified using a MultiSizer 4 Coulter Counter (Beckman Coulter, version 4.01) and an Eclipse (iCyt) flow cytometer (Sony Biotechnology, using ec800 software, version 1.3.7), equipped with 405-nm and 488-nm solid-state air-cooled lasers (both 25 mW on the flow cell) and standard filter setup. Algae were identified by plotting chlorophyll autofluorescence in the red channel (663–737 nm) versus green autofluorescence (500–530 nm) or side scatter (Supplementary Fig. 15a). Extracellular viral particles were quantified as described previously (Supplementary Fig. 15b).

Quantification of E. huxleyi and bacterial cells from agarose samples was performed by suspending agarose samples in ASW. A sterile biopsy punch (8-mm diameter) was used for sampling. Each punched sample was added to 300 µl ASW, vortexed, pipetted and placed at 18 °C for 15 min to allow transfer of cells from the agarose to the ASW. Samples were then filtered through a 35-µm cell strainer. Plaques were smaller than 8 mm in diameter, and hence also contained uninfected areas. E. huxleyi cells were quantified as described above. Bacterial cells were quantified as described previously (Supplementary Fig. 15b).

Sample preparation for epifluorescence microscopy and MSI. Plaque samples were cut using a metal square punch and transferred to a glass slide. The upper layer of 0.2% agarose was blotted onto a 0.22-µm PVDF (polyvinylidene difluoride) filter (Merck Millipore). The filter containing the plaque was then dried for 10 min at 30°C. The filter was immobilized on a microscope slide with double-sided adhesive tape for further analyses using epifluorescence microscopy and MSI. Epifluorescence microscopy. Microscopy images used for both Flow-probe-MS and MALDI-MS samples where obtained using Olympus IX81 motorized Epifluorescence microscope. The representative average image of each cluster was used to reduce the complexity of the untargeted data, allowing us to select clusters with biological relevance to the infection dynamics.
Flow-probe-MSI. The analysis was performed at ambient conditions using a 'Flow-probe' in situ microextraction system ('Proisola', connected to a Thermo Scientific 'Q Exactive' mass spectrometer. The 'Flow-probe' was operated using Flowprobe nMotion software (version 1.0.0.58, Proisola). The instrument was operated in positive-ionization mode, with spray voltage of 3 kV and a capillary temperature of 250 °C. For data acquisition, 100 µl of the sample was applied to the sample plate, and for each scan, a 70,000 at 400 m/z mass spectrum was acquired. Mass peaks in the MS1 scan were fragmented in the absence of listed ions, or without an inclusion list and using precursor ions per MS1 scan at a resolution of 17,500 at 400 m/z. Simultaneous Flow-probe imaging and data-dependent acquisitions were performed either using an inclusion list of known lipids, allowing ions not included in the inclusion list to be fragmented in the absence of listed ions, or without an inclusion list and using a dynamic exclusion window of 3 s. The full list of the lipids annotated using Flow-probe-MS/MS and their fragments can be found in Supplementary Data 1.

Processing of Flow-probe-MSI data. Raw Flow-probe-MSI data generated from a plaque at 6 d.p.i. was converted from the vendor’s raw format to the open format mZML using the 'msconvert' software (part of ProteoWizard version 3.0)61 and 'msconvert' software (version 1.3)63. Next, mZML files corresponding with each experimental run were imported into the R open source programming environment (www.r-project.org) using the R package 'MALDIquant Foreign'64. Initially, 75 heading and tailing scans were removed from each horizontal acquisition line over to high levels of noise issues, and in addition, the first horizontal acquisition line (out of 11), which was detected in manual inspection of the raw data as an outlier, showing irregular visual pattern due to poor instrumental performance, was completely removed from analysis. This particular acquisition line was spatially located away from the centre of infection, and we assume that no meaningful data were removed and that any subsequent data extraction was not affected.

Next, matrix intensity values were log transformed and then scaled using a series of 25 specific mass features corresponding with different clusters were done using dedicated in-house R scripts based on the R function 'dendrapply'. For plotting the average spatial representations, a simple moving average smoothing was applied using a rectangular box of 3 horizontal lines by 11 vertical scans. The detection of meaningful clusters was supported by tagging each cluster by its height in the dendrogram tree and the number of its members, using a minimum height of four and minimum number member of five (Supplementary Fig. 3). Plotting the average spatial representations was performed using the 'kXkBu' in the R package 'RCenterBrewer'65. The uniqueness is each of the nine detected clusters was evaluated by generating a silhouette index as a score of ‘goodness’ of clustering66 (Supplementary Fig. 17).

Annotation of mass features to masses of known lipids was done using a dedicated in-house R script, which performed mass-to-mass comparisons of points in mass features with the theoretical accurate mass of the protonated ([M + H]+), sodium ([M + Na]+) and ammonium ([M + NH₄]+) ions of each known lipid, using a mass error tolerance of 6 parts per million (ppm). The annotation script further extracted the natural heavy isotope pattern of each putatively annotated parent ion and performed chemical formula decomposition using the 'chemmine' package on the R package 'Picolor'. Putative mass features to known lipids were then assigned using the combined criteria of: mass-to-mass, chemical formula decomposition and manual inspection of the corresponding mass peak in the raw data.

In total, the 4 clusters we focused on contained 129 mass features (Fig. 3c and Supplementary Table 2). Out of which, 94 mass features were putatively annotated as molecular ions, isotopes or fragments of lipids, corresponding with 41 mass feature groups (of which, 33 were induced and 8 reduced; see Supplementary Data 1). Seventeen m/z feature groups were putatively annotated as lipids known from previous LC-MS-based studies (15 induced and 2 reduced)44,45.

MS images of specific m/z values derived from the raw data (used in Fig. 2 and Supplementary Figs. 1, 4, 5, 6 and 16) were generated using the open-source software package MSiReader v1.00 (refs. 79, 80), built on the platform of MATLAB (Mathworks), allowing mass error of 5 ppm, using total ion current normalization and a 'Viridis' colourmap. Pixel smoothing was not applied to the images.

Matrix sublimation for MALDI-MSI. A 2.5-dihydroxybenzoic acid (DHB) matrix was deposited using a sublimation apparatus (height × inner diameter, 250 × 152 mm, Sigma-Aldrich). The sublimator was coupled to a rough pump and a digital thermocouple vacuum gauge controller and was placed on a sand bath heated by a hot plate. The temperature was monitored by a digital thermometer. DHB matrix (200 mg) was sublimated under a fixed pressure of 8 × 10⁻¹ Torr at 140 °C for 4 min.

MALDI-MSI and data processing. MALDI imaging experiments were performed using a 7T Solarix Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics). Data sets were recorded in positive-ionization mode using a 7 T Solarix FT-ICR mass spectrometer. Data were processed using FlexImaging software (version 4.0, Bruker Daltonics) and SCiLS Lab 2015b (SCiLS). Data sets were normalized to root mean square intensity, and MALDI images were plotted at the theoretical m/z ± 0.001% (FlexImaging) and m/z ± 5 ppm (SCiLS Lab), with pixel interpolation on. MS images were produced using SCiLS Lab 2015b using ‘Viridis’ colourmap. Coloization was carried out using SCiLS Lab based on Pearson’s correlation analysis (P = 0.05), using root mean square normalization, and an m/z interval of 0.5 ppm. Pixel smoothing was not applied to the images or to the colocalization analysis.

Cross-section graphs. Cross-section graphs were plotted by averaging the intensity of each lipid or chlorophyll signal across several lines from the outer part of the plaque to its centre. The centre was selected to be the minimum of the chlorophyll signal. Ten lines were used in epifluorescence microscopy images and MALDI-MS and two in Flow-Probe-MSI images. Intensity values for each line were extracted from the images using Fiji. Graphs were plotted using MATLAB R2016b.

Chemical stability of lipid standards in MALDI-MS. A lipid standard solution containing 12.5 µM of ceramide/sphingoid internal standard mixture (LM6002, Avanti Polar Lipids) was mixed with freshly prepared DHB matrix solution (10 mg/ml in 100% MeOH and 0.1% TFA) in a 1/1 ratio (v/v). Of the mix, 1 µl was spotted onto a MALDI AnchorChip target (Bruker Daltonics) with six technical repeats per day, and the spotted target was allowed to air dry before MALDI-MS analysis. Each MALDI spot was analysed with the same instrumental method as
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Chemicals and internal standards for LC-MS analyses. LC-grade solvents were purchased from Merck and Bio-Lab. Ammonium acetate was purchased from Sigma-Aldrich. Internal standards for lipidomics analysis were purchased from Avanti Polar Lipids. The lipid internal standards were added to the extraction solution employed in the initial extraction step.

Lipidomics analyses. Lipids were extracted from liquid internal cultures of E. huxleyi cells infected with EhV201 and from non-infected cells harvested at 4, 24, 48 and 72 hours post-infection (h.p.i.) in three biological replicates. Infection was performed on exponential-phase cultures (5×10^9 to 10^10 cells per ml), which were infected 2–3 h after the onset of the light period, with a 1/100 volumetric ratio of viral lysate to culture (multiplicity of infection of 1/1 viral particles per cell). The samples (30–150 ml of each culture, equivalent to 5×10^9 cells per sample) were collected on GF/C filters (pre-combusted at 400 °C for 4 h), plunged into liquid nitrogen and stored at −80 °C until analysis.

Lipid extraction and analysis were performed as previously described and some modifications: filters containing algae were placed in a 15-ml glass tube and extracted with 3 ml of a pre-cooled (−20 °C) homogenus methanol: methyl tert-butyl-ether (MTBE) 1/3 (v/v) mixture containing 0.1 µg/ml glucosylceramide (d18:1/12:0), which was used as an internal standard. The tubes were shaken for 30 min at 4 °C and then sonicated for 30 min. Ultra-performance LC (UPLC) grade water/methanol (3/1, v/v) solution (1.5 ml) was added to the tubes followed by centrifugation.

The organic phase (1.2 ml) was transferred to an Eppendorf tubes. The polar phase containing algae debris and filter pieces was re-extracted with 0.5 ml MTBE. Organic phases were combined and dried under N2 flow and then stored at −80 °C until analysis.

Extraction of lipids from plaque samples was performed by suspending the upper soft layer containing the plaque in lipid extraction solution, performing the same procedure described above (using one-third of the mentioned volumes). As a blank, a soft layer without E. huxleyi cells (that is, ASW + K2 containing 0.2% agarose only) was extracted as well. Samples were extracted in three replicates. Isolation, concentration and lipid extraction of vesicles and virions were performed as previously described.

All dried lipid extracts were resuspended in 300 µl mobile phase B and centrifuged again at 18,000g and 4 °C for 5 min for LC–MS and LC–MS/MS analyses.

UPLC–quadrupole-time-of-flight MS for lipidomics analysis. Post-extraction, the supernatant was transferred to an autosampler vial and an aliquot of 1 µl was subjected to UPLC–MS analysis. Lipid extracts were analysed using a Waters ACQUITY UPLC 1–Class system coupled to a SYNAPT G2 HDMS mass spectrometer (Waters). Chromatographic conditions were as described previously. Briefly, the chromatographic separation was performed on an ACQUITY UPLC BEH C8 column (2.1×100 mm, i.d., 1.7 µm) (Waters). The mobile phase consisted of water (UPLC grade) with 1% M NH4Ac, 0.1% acetic acid (mobile phase A) and acetonitrile/isopropanol (7/3) with 1% 1 M NH4Ac, 0.1% acetic acid (mobile phase B). The column was maintained at 40 °C and the flow rate of the mobile phase was 0.4 µl min⁻¹. The gradient separation was as follows: 1 min 45% A, 3-min linear gradient from 45% to 35% A, 8-min linear gradient from 35% A to 10% A. After washing the column with 0% A, the method was set back to the initial condition and the column was re-equilibrated for 4 min (22 min total run time). MS parameters were as follows: the source and desolvation temperatures were maintained at 120 °C and 450 °C, respectively. The capillary voltage and cone voltage were set to 1.0kV and 40 V, respectively. Nitrogen was used as the desolvation gas and cone gas at a flow rate of 800 l/h⁻¹ and 20 l/h⁻¹, respectively. The mass spectrometer was operated in full scan positive ionization mode over a mass range of 50–1,500 Da. For the high-energy scan function, a collision energy range of 15–35 eV was applied. For the low-energy scan function, −4 eV was applied. Leucine-enkephalin was used as lock-mass reference standard. The major ions and specific fragment ions of the lipids were analysed using positive-ionization mode. For the detection of C15:0 FA fragments, two sets of the lipids were injected also in negative-ionization mode. The MS⁵ energies applied for the negative ionization were 15–40 eV. For MS/MS (in positive-ionization mode), a collision energy range of 10–45 eV was applied.

LC–MS and LC–MS/MS data analysis, lipid identification and quantification. LC–MS data were analysed and processed with MassLynx and QuanLynx (version 4.1, Waters). The peaks and lipid identifications of the various lipid species identified were performed according to the lipid accurate mass and LC–MS/MS fragmentation pattern, as described previously. The putative identification of lipid species that were previously undescribed in this host–virus model system was based on the LMDSD and carried out following the Metabolomics Standards Initiative guidelines and LMDSD (see Fig. 1 and Supplementary Figs. 8, 9 and 11 for MS/MS spectra and the list of fragments of representative species of each class). Five unidentified mass features, which we termed sulfonioglycerolipids (listed in Table 1), did not exist in our in-house repository or in public repositories such as LMDSD and METLIN. Putative structures of these sulfonioglycerolipids, which belong to the same putatively characterized compound class, were based on accurate mass matching and manual annotation of LC–MS/MS fragments (corresponding with level 3 annotation), as elaborated in Supplementary Fig. 7 and Supplementary Table 4. A full list of all of the lipids annotated using LC–MS/MS and their detected fragments can be found in Supplementary Data 1–3. Relative levels of lipids extracted from liquid cultures were normalized to the internal standard and the number of algal cells used for analysis. The heatmap was generated using MATLAB R2016b. Mean intensity values (normalized peak intensity per cell) were log-transformed and standardized per lipid (row-wise). Zero values were replaced with 0.5 of the minimal value prior to log₁₀ transformation.

Fatty acid composition. Four fractions enriched with potential C15:0-based lipids were collected manually from LC–MS runs of lipidics extracts from infected cells at 48 h.p.i. Five unidentified mass features listed in Table 1 were dried. At 4 °C, the last fractions were dissolved in 500 µl 1.25 M methanolic hydrochloric (MeOH/HCI Sigma-Aldrich), incubated for 1 h at 80 °C while shaking and then dried under N2 flow. The resulting FAMEs were extracted twice with 250 µl hexane/chloroform (1/1) and once with 250 µl chloroform. Each extraction for 30 min at room temperature while shaking. The extracts were dried under N2 flow. The dried FAME extracts were resuspended in 100 µl hexane and centrifuged at 18,000g for 4 °C for 5 min for GC–MS analysis.

GC–MS for FAME analysis. Post-extraction, the supernatant was transferred to an autosampler vial and an aliquot of 1 µl was subjected to GC–MS analysis. The GC–MS system comprised an Agilent 7890A gas chromatograph equipped with split/splitless injector and a LECO Pegasus HT Time–of–Flight Mass Spectrometer (LECO). GC was performed on a 30 m × 0.25 mm × 0.25-µm Rxi–5Sil MS column (Restek). Samples were analysed in the splitless mode; the injector temperature was set at 280 °C. Analytes by 1 µl injected were separated using the following chromatographic conditions: helium was used as carrier gas at a flow rate of 1.0 ml min⁻¹. The thermal gradient started at 80 °C and was held at this temperature for 2 min, ramped to 330 °C at 15 °C per min and then held at 330 °C for 6 min. Eluents were fragmented in the electron impact mode with an ionization voltage of 70 eV. The MS range was set to 45–800 m/z with an acquisition rate of 20 spectra per s. The ion source chamber was set to 250 °C and the transfer line to 250 °C. LECO ChromaTOF (version 4.50.8.0, LECO) was used for acquisition control and data processing. FAMEs were identified by comparison of their mass spectra and retention times to the corresponding analytical standards, which was done in the same GC–MS conditions. Two standards were used: 12C16-palmitic acid (methyl-C15:0 FA; Sigma) and methyl-12-methylmyristate (methyl-12-methyl-C14:0 FA; Santa Cruz Biotechnology), at a concentration of 10 µg ml⁻¹ in hexane (Fig. 4b and Supplementary Fig. 10).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data supporting the findings of this study are available within the paper (and Supplementary Information files). Raw data generated or analysed in this study have been deposited in the EMBL-EBI MetaboLights repository with the identifiers MTBLS676 (including LC–MS of plaque samples, LC–MS/MS of specific lipids) and MTBLS677 (including LC–MS and LC–MS/MS). The data can be accessed with the following links: http://www.ebi.ac.uk/metabolights/MTBLS676 and http://www.ebi.ac.uk/metabolights/MTBLS677.

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Author contributions
G.S. and A.V. conceptualized the project and conceived and designed the experiments. G.S. and A.V. wrote the manuscript. G.S. performed all experiments. N.S. developed the computational analysis of MS data. C.Z. conducted lipid extractions and the LC–MS experiments. R.A.M. and E.J.N.H. conducted the Flow-probe-MS experiments. Y.D. conducted the MALDI-MS experiments. I.R. conducted the GC–MS experiments. D.S. isolated the vesicles and virions for lipidomics analysis. All authors provided useful feedback on the experimental design and comments on the manuscript.

Competing interests
The authors declare no competing interests.

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Software and code

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Data collection

- Multisizer 4 Coulter Counter (version 4.01, Beckman Coulter, for E. huxleyi cell counts); ec800 (version 1.3.7, Sony Biotechnology Inc, Sony Biotechnology, Champaign, IL, USA, for E. huxleyi cell counts and viral counts); MetaMorph (version 7.8.7, Molecular Devices, for epi-fluorescence microscopy); CellSens Dimension (version 1.11 Olympus, for epi-fluorescence microscopy, for MALDI-MS data); Flowprobe nMotion (version 1.0.0.58, Prosolia, Indianapolis, IN, USA, for Flow-probe-MS data); FTMS Control (version 2.1, Bruker Daltonics, Bremen, Germany); MassLynx (version 4.1, Waters Corp., Milford, MA, USA, for UPLC-q-TOF MS data); ECO ChromaTOF (LECO Corp., St Joseph, MI, USA, for GC-MS data);

Data analysis

- ec800 (version 1.3.7, Sony Biotechnology Inc, Sony Biotechnology, Champaign, IL, USA, for E. huxleyi cell counts and viral counts); msconvert (part of ProteoWizard version 3.0, for Flow-probe-MS data); imzMLConverter (version 1.3, for Flow-probe-MS data); R (version 3.3.1) and R packages: ‘MALDIquant’, ‘cluster’, ‘Rdisop’ and ‘RColorBrewer’ (for Flow-probe-MS data); MSiReader v1.00 (built on the platform of Matlab, for Flow-probe-MS data); FlexImaging (version 4.0, Bruker Daltonics, Bremen, Germany), SCILS Lab 2015b (SCILS GmbH, Bremen, Germany, for MALDI-MS data); MassLynx and QuanLynx (Version 4.1, Waters Corp., Milford, MA, USA, for UPLC-q-TOF MS data); LECO ChromaTOF (LECO Corp., St Joseph, MI, USA, for GC-MS data); Thermo Xcalibur 2.2 (Thermo Scientific, for GC-MS data); Matlab R2016b (Mathworks, Natick, MA, USA, for heatmaps); Fiji (Fiji Is Just ImageJ, version 1.51w, for epi-fluorescence microscopy data and cross sections), Microsoft Excel 2016

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data supporting the findings of this study are available within the paper (and supplementary information files). Raw data generated or analyzed in this study are available from the MetaboLights repository (http://www.ebi.ac.uk/metabolights/MTBLS767), including: MALDI-MSI and Flow-probe-MSI, LC-MS of plaque samples, LC-MS/MS of specific lipids and GC-MS.

Field-specific reporting
Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑️ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design
All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes were chosen based on previous experience that enabled repetitive significant results. No statistical method was used to predetermine sample size. |
| Data exclusions | Analysis of some MSI data required exclusion of low intensity and noisy scans, as specified in the Methods section, under "Processing of Flow-probe-MSI data". Exclusion criteria were established based on initial analysis of the data. |
| Replication | All experimental findings were reliably reproduced. Targeted analysis of lipids in MSI was performed in two biological replicates for each technique (Flow-probe-MSI and MALDI-MSI), yielding similar results. LC-MS analysis was performed on three biological replicates, yielding similar results. No statistical tests were performed to determine the required replicate number. |
| Randomization | Randomization was performed in experiments where uninfected in infected cultures were compared. The initial culture was divided randomly to two groups (further divided to different replicates), one of which was then infected with a virus. Plaques used for MSI analyses were randomly chosen. No statistical parameters were used for randomization. |
| Blinding | Blinding was not relevant in the study, since samples were prepared for specific analyses - Flow-probe-MSI, MALDI-MSI, LC-MS, GC-MS, etc. |

Reporting for specific materials, systems and methods

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
☒ ☒ ChIP-seq
☒ ☒ Flow cytometry
☒ ☒ MRI-based neuroimaging

Unique biological materials
Policy information about availability of materials
Obtaining unique materials Emiliania huxleyi CCMP2090 cultures were obtained from the National Center of Marine Algae and Microbiota (NCMA).
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  Emiliana huxleyi CCMP2090 cultures were obtained from the National Center of Marine Algae and Microbiota (NCMA).

Authentication  Have not been authenticated

Mycoplasma contamination  Cell lines were not tested for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)  Not relevant for marine single-cell alga

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☐ All plots are contour plots with outliers or pseudocolor plots.
☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation  Sample preparation is described in the Methods section, under "Enumeration of cells, viruses and bacteria".

Instrument  Eclipse (iCyt) flow cytometer (Sony Biotechnology, Champaign, IL, USA), as specified in the Methods section, under "Enumeration of cells and viruses"

Software  ec800 version 1.3.7 (Sony Biotechnology), as specified in the Methods section, under "Enumeration of cells and viruses"

Cell population abundance  Not relevant (not sorting was performed). No plots for sorting are presented

Gating strategy  Gating strategy is described in Supporting Figure 15.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.