

# Carbonate-sensitive phytotransferrin controls high-affinity iron uptake in diatoms

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In vast areas of the ocean, the scarcity of iron controls the growth and productivity of phytoplankton<sup>1,2</sup>. Although most dissolved iron in the marine environment is complexed with organic molecules<sup>3</sup>, picomolar amounts of labile inorganic iron species (labile iron) are maintained within the euphotic zone<sup>4</sup> and serve as an important source of iron for eukaryotic phytoplankton and particularly for diatoms<sup>5</sup>. Genome-enabled studies of labile iron utilization by diatoms have previously revealed novel iron-responsive transcripts<sup>6,7</sup>, including the ferric iron-concentrating protein ISIP2A<sup>8</sup>, but the mechanism behind the acquisition of picomolar labile iron remains unknown. Here we show that ISIP2A is a phytotransferrin that independently and convergently evolved carbonate ion-coordinated ferric iron binding. Deletion of *ISIP2A* disrupts high-affinity iron uptake in the diatom *Phaeodactylum tricornutum*, and uptake is restored by complementation with human transferrin. ISIP2A is internalized by endocytosis, and manipulation of the seawater carbonic acid system reveals a second-order dependence on the concentrations of labile iron and carbonate ions. In *P. tricornutum*, the synergistic interaction of labile iron and carbonate ions occurs at environmentally relevant concentrations, revealing that carbonate availability co-limits iron uptake. Phytotransferrin sequences have a broad taxonomic distribution<sup>8</sup> and are abundant in marine environmental genomic datasets<sup>9,10</sup>, suggesting that acidification-driven declines in the concentration of seawater carbonate ions will have a negative effect on this globally important eukaryotic iron acquisition mechanism.

The iron cargo protein transferrin is thought to have originated in metazoa as an adaptation to multicellularity<sup>11</sup>. In vertebrates, serum transferrin circulates in the blood, binding labile ferric iron (Fe<sup>3+</sup>) with high affinity ( $K_d^{\text{Fe}^{3+}} \approx 1\text{--}10\text{ pM}$ ) and distributing iron to cells via receptor-mediated endocytosis<sup>12</sup>. Transferrin binds iron tightly by coordinating ferric iron with an exogenous carbonate anion (CO<sub>3</sub><sup>2-</sup>). Following endocytosis, the carbonate anion is protonated and ferric iron is reduced, disrupting Fe coordination and initiating the release of iron<sup>13</sup>. Recent discoveries of ferric-iron-concentrating ISIP2A proteins in diatoms<sup>7,8</sup> and transferrin in the green alga *Dunaliella salina*<sup>14</sup> suggest that transferrin-like iron acquisition mechanisms are not limited to multicellular organisms. We used a marine microeukaryote transcriptional dataset to reconstruct the phylogenetic histories of ISIP2A and transferrin, revealing a common origin in phosphonate-binding periplasmic proteins (PBPs; Fig. 1). Transferrins and the ISIP2A clade of algal transferrin-like proteins (herein phytotransferrin, or pTF<sup>15</sup>) are functional analogues that have convergently added iron coordination sites onto an anion-binding precursor protein (Extended Data Fig. 1a). The evolution of phytotransferrin marks the third occasion in which anion-coordinated iron binding has arisen from the bacterial PBP family<sup>16</sup>, highlighting the fundamental role coordinating anions have within the transferrin superfamily<sup>13</sup>.

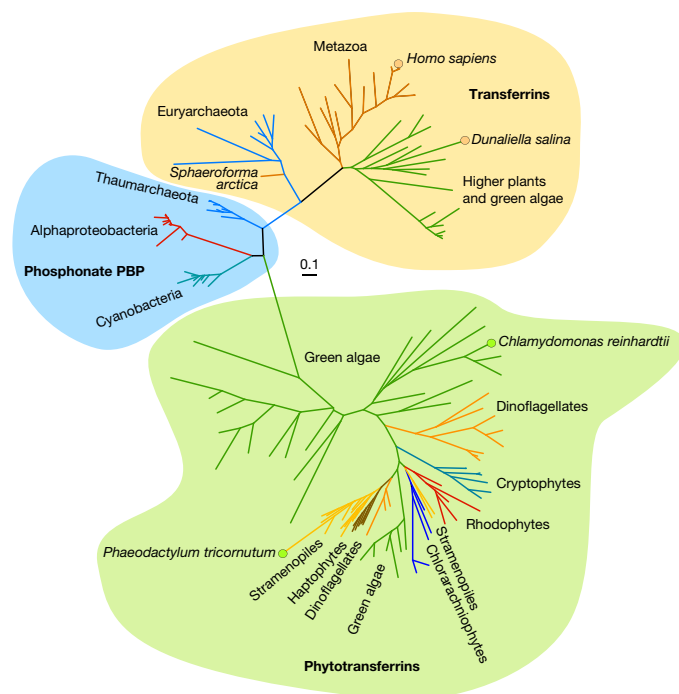
Molecular dating (by PhyloBayes) estimates the appearance of phytotransferrins to between 914 and 671 million years ago, which is consistent with the inferred changes to Neoproterozoic marine redox states<sup>17</sup> and the subsequent requirements to develop mechanisms to access dilute ferric iron (Extended Data Fig. 2). Transferrin and phytotransferrin are simultaneously present in the genomes of deeply branching chlorophyte algae (Extended Data Fig. 3), although our phylogenetic reconstruction suggests that these proteins were acquired through separate events. Whereas transferrin appears to have been vertically inherited from marine archaea, the chlorophyte acquisition of phytotransferrin is less clear, plausibly occurring through endosymbiotic or horizontal gene transfer. Unlike transferrins, phytotransferrins are often transmembrane anchored (Supplementary Table 2), a selective advantage for unicellular organisms that probably contributes to its wide phylogenetic distribution among marine plankton.

To examine the role of phytotransferrin in high-affinity iron uptake in diatoms, we used TALE nucleases to disrupt *ISIP2A* (hereafter  $\Delta$ ISIP2A) in *P. tricornutum* (Fig. 2a and Extended Data Fig. 4a). In artificial seawater in which the concentration of labile inorganic iron species ([Fe']) is controlled with excess EDTA,  $\Delta$ ISIP2A has significantly reduced growth rates at low [Fe'] (Extended Data Fig. 4b). Short-term <sup>59</sup>Fe uptake rates of  $\Delta$ ISIP2A are reduced by more than 90%, confirming the role of ISIP2A in high-affinity iron uptake (Fig. 2b). In *P. tricornutum*, ISIP2A is one of three transmembrane-anchored phytotransferrin homologues (Extended Data Fig. 1b); however, ISIP2A dominates transcriptional abundance, accounting for 95% of all phytotransferrin transcripts<sup>18</sup>. Uptake of <sup>59</sup>Fe-ferrioxamine B is not affected in  $\Delta$ ISIP2A, which suggests a separate mechanism for the uptake of complexed iron (Extended Data Fig. 4c).

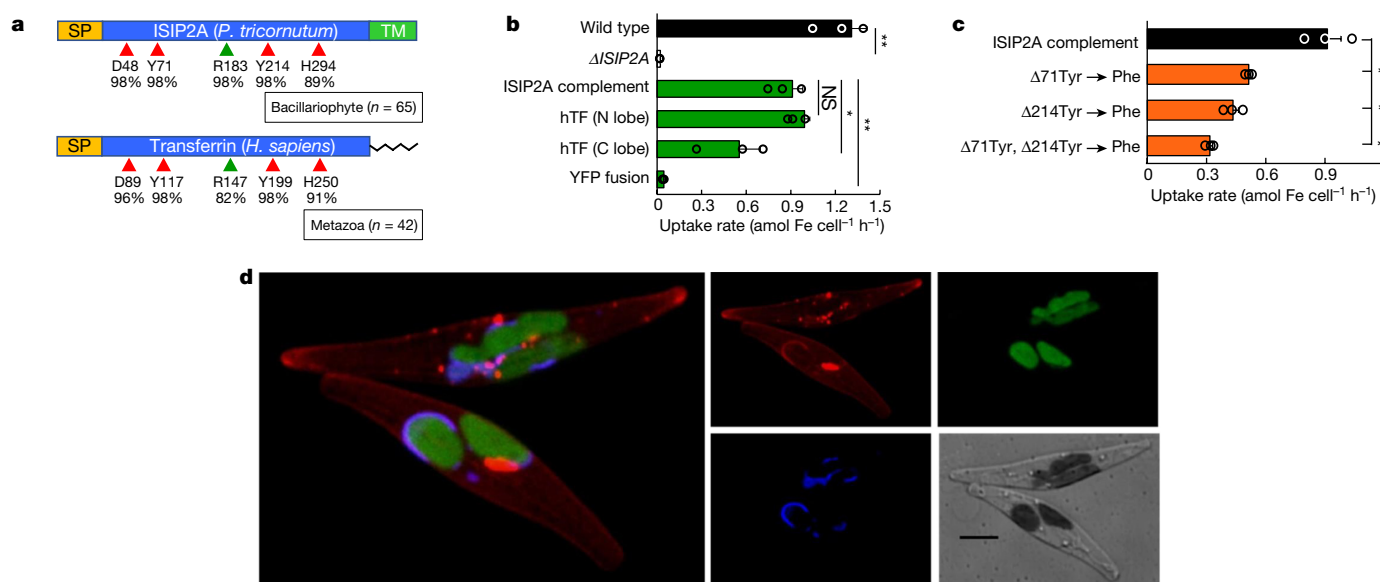
To determine whether ISIP2A is a functional analogue of transferrin, codon-optimized N- and C-terminal domains of human serum transferrin (hTF) were fused to the signal peptide and transmembrane anchor of ISIP2A and reintroduced into  $\Delta$ ISIP2A via conjugation<sup>19</sup>. The N-terminal domain of hTF fully restored high-affinity iron uptake equal to that of ISIP2A, whereas the C-domain partially restored uptake (Fig. 2b), confirming phytotransferrin to be a functional analogue of transferrin and suggesting that both proteins use similar iron-binding, -internalization and -release mechanisms. In hTF, mutation of the conserved tyrosine ligands disrupts iron coordination<sup>11</sup>. In ISIP2A, mutagenesis of the analogous tyrosines decreases short-term uptake rates by 50%, providing further evidence of a shared mechanism (Fig. 2c).

Internalization through endocytosis is required for the release of transferrin-bound iron<sup>20</sup>. In *P. tricornutum*, inclusion of an endocytosis inhibitor decreases short-term <sup>59</sup>Fe' uptake (Extended Data Fig. 4d). When ISIP2A is labelled with fluorescent protein, ISIP2A-RFP localizes to the outer membrane, small internal vesicles and endosome-like

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**Figure 1 | Transferrin and phytotransferrin are functional analogues with a common origin.** Maximum likelihood phylogenetic tree of the divergence of transferrin and phytotransferrin from ancestral PBPs as inferred from amino acid sequences. Branch colour denotes phylogenetic group, and circles at branch ends denote characterized iron-binding proteins: hTF (*H. sapiens*), triplicated transferrin (TTF1, *D. salina*), Fe-assimilation protein (FEA1, *Chlamydomonas reinhardtii*) and ISIP2A (*P. tricornutum*). Scale bar, 0.1 amino acid substitutions per position.



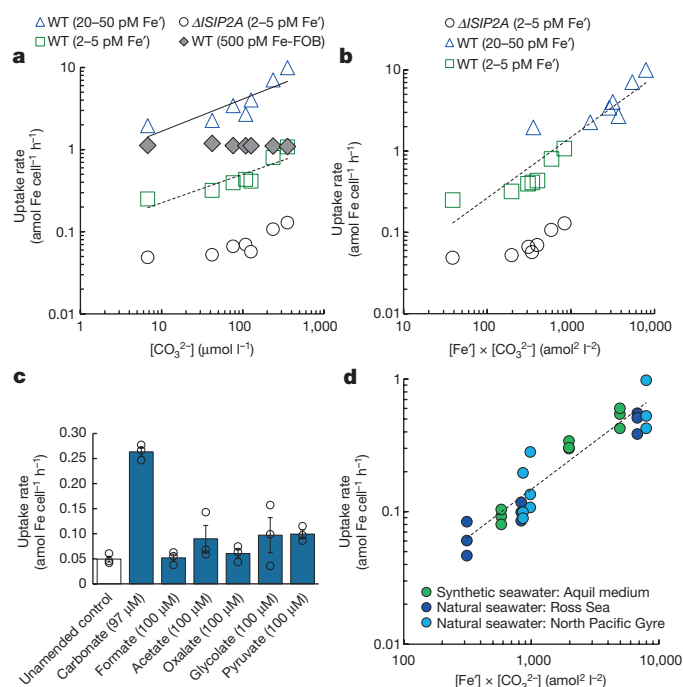
**Figure 2 | Characterization of phytotransferrin ISIP2A (*P. tricornutum*).** **a**, Schematic of phytotransferrin (ISIP2A) and hTF (N-terminal domain). Locations and per cent conservation of putative iron- and carbonate-coordinating amino acids (red and green triangles, respectively). SP, signal peptide; TM, transmembrane domain. **b**, High-affinity iron uptake (in attomole (amol) Fe cell<sup>-1</sup> h<sup>-1</sup>) is disrupted in  $\Delta$ ISIP2A and restored in hTF- and ISIP2A-complemented cultures. YFP, yellow fluorescent

structures, a pattern that is consistent with internalization through endocytosis<sup>20</sup> (Fig. 2d). The receptor-like iron-regulated protein ISIP1<sup>6,7</sup> was co-visualized, however cell-surface interactions with ISIP2A were not apparent (Fig. 2d). When iron-limited *P. tricornutum* is stained with a membrane-impermeable dye and pulsed with Fe<sup>3+</sup>, portions of the outer membrane are internalized (Extended Data Fig. 4e). Because endocytosis is restricted to eukaryotic organisms, phytotransferrin is a factor that probably contributes to the rapid response of diatoms and other eukaryotic plankton to iron fertilization events in high-nutrient low-chlorophyll regions<sup>2</sup>.

In both human<sup>12</sup> and algal<sup>21</sup> transferrin, CO<sub>3</sub><sup>2-</sup> is required for the efficient binding of iron. To investigate the role of CO<sub>3</sub><sup>2-</sup> in phytotransferrin-mediated Fe<sup>3+</sup> uptake, we resuspended cultures of *P. tricornutum* that were acclimatized to a low-iron environment into pH-controlled <sup>59</sup>Fe-uptake medium and manipulated carbonate ion availability [CO<sub>3</sub><sup>2-</sup>] by NaHCO<sub>3</sub> addition (Extended Data Table 1). At both low and high [Fe<sup>3+</sup>], short-term Fe<sup>3+</sup> uptake rates were positively correlated with [CO<sub>3</sub><sup>2-</sup>], consistent with a transferrin-like mechanism<sup>12</sup>, whereas the uptake of Fe-ferrioxamine B was unaffected (Fig. 3a and Extended Data Fig. 5a). In  $\Delta$ ISIP2A, <sup>59</sup>Fe<sup>3+</sup> uptake remained correlated with [CO<sub>3</sub><sup>2-</sup>]; this probably reflects the activity of ISIP2A homologues (Extended Data Fig. 1b).

Iron binds to transferrin through a synergistic interaction between Fe<sup>3+</sup> and CO<sub>3</sub><sup>2-</sup>, and neither substrate binds in the absence of the other<sup>13</sup>. This synergistic binding is a defining characteristic of transferrin<sup>12</sup>, and in the above experiments the interaction between Fe<sup>3+</sup> and CO<sub>3</sub><sup>2-</sup> is significant ( $P = 2 \times 10^{-6}$ ), demonstrating a second-order rate dependency (Fig. 3b). Using these as representative measurements, we derived approximations for the pseudo-first-order rate constants for the dependency of Fe uptake on Fe<sup>3+</sup> ( $0.78 \pm 0.05$  mol Fe per cell per h per M Fe<sup>3+</sup> (hereafter mol Fe cell<sup>-1</sup> h<sup>-1</sup> (M Fe<sup>3+</sup>)<sup>-1</sup>) and CO<sub>3</sub><sup>2-</sup> ( $0.0016 \pm 0.0009$  mol Fe per cell per h per M CO<sub>3</sub><sup>2-</sup> (hereafter mol Fe cell<sup>-1</sup> h<sup>-1</sup> (M CO<sub>3</sub><sup>2-</sup>)<sup>-1</sup>; Extended Data Fig. 6). Although organic

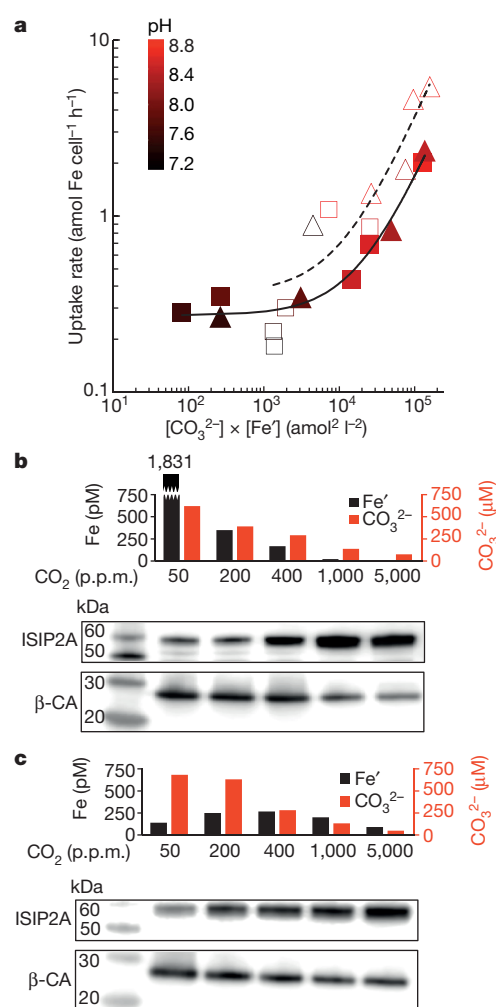
protein control. **c**, Site-directed mutagenesis of putative iron-coordinating residues. **d**, In *P. tricornutum* acclimatized to low-iron concentrations, RFP-labelled ISIP2A (red) localizes to the outer membrane and internal vesicles. Iron-regulated ISIP1 (blue) and plastid (green) are co-visualized. Scale bar, 5  $\mu$ m. **b**, **c**, Data are mean  $\pm$  s.e.m.;  $n = 3$  biological replicates; \* $P < 0.05$ ; \*\* $P < 0.01$ ; NS, not significant; paired *t*-test. **d**, Representative image of  $n = 5$  biological replicates.



**Figure 3 | The synergistic interaction of Fe' and CO<sub>3</sub><sup>2-</sup> determines Fe' uptake rates.** **a**, <sup>59</sup>Fe' uptake rates of *P. tricornutum* resuspended in pH-controlled synthetic seawater are correlated to [CO<sub>3</sub><sup>2-</sup>], whereas the uptake of <sup>59</sup>Fe-ferrioxamine B is not positively correlated.  $P = 8 \times 10^{-5}$  (solid line),  $P = 3 \times 10^{-5}$  (dashed) on  $n = 7$  individual experiments per regression. WT, wild type. **b**, Data from **a** replotted as a function of [Fe']  $\times$  [CO<sub>3</sub><sup>2-</sup>], revealing a synergistic interaction. Linear regression  $P = 2 \times 10^{-10}$ ,  $n = 14$  individual experiments. **c**, <sup>59</sup>Fe' uptake rates are not restored with organic acids, suggesting an *in vivo* specificity for carbonate. Data are mean  $\pm$  s.e.m.;  $n = 3$  biological replicates;  $**P < 0.01$ , all others  $P > 0.05$ ; paired *t*-test. **d**, The [Fe']  $\times$  [CO<sub>3</sub><sup>2-</sup>] synergistic interaction is also observed in CO<sub>2</sub>-manipulated natural seawater. Data are mean  $\pm$  s.e.m.;  $n = 3$  biological replicates;  $n = 27$  individual experiments; linear regression  $P = 6 \times 10^{-9}$ .

acids can replace CO<sub>3</sub><sup>2-</sup> in an anion–Fe–transferrin ternary complex<sup>22</sup>, evidence for *in vivo* iron assimilation is rare<sup>23</sup>, and a panel of surrogate anions failed to restore *P. tricornutum* uptake rates (Fig. 3c). When cells are resuspended in natural seawater, the synergistic interaction between Fe' and CO<sub>3</sub><sup>2-</sup> is also observed, further supporting carbonate ion as the most likely Fe-coordinating anion (Fig. 3d). The correlation between Fe' uptake rates and [CO<sub>3</sub><sup>2-</sup>] occurs at environmentally relevant concentrations of CO<sub>3</sub><sup>2-</sup>, indicating that [CO<sub>3</sub><sup>2-</sup>] co-limits Fe' uptake, and suggesting that the projected decline in seawater [CO<sub>3</sub><sup>2-</sup>] due to ocean acidification could negatively affect phytotransferrin-mediated iron acquisition<sup>24</sup>.

To investigate the effects of acidification on Fe' uptake rates, we aerated cultures of *P. tricornutum* with 50–5,000 p.p.m. CO<sub>2</sub>, generating cultures in which the pH ranged from 8.7 to 7.2 (Extended Data Table 3). In seawater, acidification increases [H<sup>+</sup>] and decreases [CO<sub>3</sub><sup>2-</sup>], two linked processes that separately influence each component of the synergistic interaction between Fe' and CO<sub>3</sub><sup>2-</sup>. In seawater in which [Fe'] is buffered with EDTA, the increase in [H<sup>+</sup>] decreases the favourability of Fe–EDTA dissociation<sup>25</sup>. Therefore, as pH decreases, [Fe'] decreases, and this change in iron chemistry has been documented to decrease *P. tricornutum* uptake rates<sup>26</sup>. However, here we show that the interactive effect between CO<sub>3</sub><sup>2-</sup> and Fe' in determining uptake rates is significant ( $P = 0.00375$ ), with the [Fe']  $\times$  [CO<sub>3</sub><sup>2-</sup>] product resulting in a better uptake model than [Fe'] alone (Fig. 4, solid line, and Extended Data Fig. 5b, c). Our results show that the effects of acidification are two-edged: whereas low pH decreases [Fe'], low [CO<sub>3</sub><sup>2-</sup>] decreases



**Figure 4 | pH and CO<sub>2</sub> manipulation of *P. tricornutum* induces expression of ISIP2A.** **a**, In *P. tricornutum* cultures, elevated CO<sub>2</sub> lowers pH, decreasing both [CO<sub>3</sub><sup>2-</sup>] and [Fe'] and resulting in decreased <sup>59</sup>Fe' uptake rates (filled shapes and solid line).  $n = 9$  individual experiments; linear regression  $P = 5 \times 10^{-8}$ . Constraining the change in [Fe'] relative to [CO<sub>3</sub><sup>2-</sup>] confirms the influence of [CO<sub>3</sub><sup>2-</sup>] on uptake rates (open shapes, dashed line).  $n = 10$  individual experiments, linear regression  $P = 2 \times 10^{-5}$ . **b**, Immunoblots of ISIP2A and  $\beta$ -carbonic anhydrase ( $\beta$ -CA) for conditions in which the change in [CO<sub>3</sub><sup>2-</sup>] and [Fe'] is unconstrained (solid triangles from **a**). **c**, Immunoblots of ISIP2A and  $\beta$ -carbonic anhydrase for conditions in which the change in [Fe'] is constrained in relation to the change in [CO<sub>3</sub><sup>2-</sup>] (open triangles from **a**). Graphs indicate [Fe'], [CO<sub>3</sub><sup>2-</sup>] and bubbled CO<sub>2</sub> per immunoblot,  $\beta$ -carbonic anhydrase was used as carbon chemistry indicator. Immunoblot experiments were replicated three times with independent cultures, each with equivalent results.

Fe' bioavailability, and both of these processes act individually and in tandem to reduce phytotransferrin-mediated iron uptake rates.

High expression levels of ISIP2A are an indication of iron-limited growth<sup>6</sup>, and in the high-CO<sub>2</sub> cultures, ISIP2A expression may be responding to [Fe'], [CO<sub>3</sub><sup>2-</sup>] or both (Fig. 4b). To demonstrate that changes in [CO<sub>3</sub><sup>2-</sup>] can induce the expression of ISIP2A, we constrained the change in [Fe'] by altering the Fe:EDTA ratio (Extended Data Table 4). We show that <sup>59</sup>Fe uptake rates are correlated with changes in [CO<sub>3</sub><sup>2-</sup>], but not with changes in [Fe'] (Extended Data Fig. 5d, e). As in the NaHCO<sub>3</sub>-manipulation experiments, the interaction between Fe' and CO<sub>3</sub><sup>2-</sup> remained a significant model for predicting Fe' uptake rates (Fig. 4a, dashed line), confirming that elevated CO<sub>2</sub> decreases the bioavailability of Fe' and induces the expression of ISIP2A (Fig. 4c). This link between CO<sub>2</sub> and iron acquisition resolves the puzzling



Fe–CO<sub>2</sub> co-regulation of FEA1 (also known as H43), an algal iron-binding protein<sup>27</sup> that was initially identified during high-CO<sub>2</sub> cultivation of the chlorophyte *Chlorococcum littorale*<sup>28</sup>. Our placement of FEA1 in the phytotransferrin family provides a biochemical basis for the observed influence of CO<sub>2</sub> on iron homeostasis while validating the CO<sub>2</sub> sensitivity of phytotransferrin in distantly related taxa (Fig. 1).

The requirement for synergistic binding reveals the existence of a previously undescribed form of iron–carbonate co-limitation<sup>29</sup> that may be relevant in environments in which primary productivity is limited by [Fe]. Like transferrin, phytotransferrin exploits carbonate chemistry to function as both coordination anion and internalization release trigger<sup>13</sup>, resulting in a mechanism that is exquisitely sensitive to acidification-induced changes in [CO<sub>3</sub><sup>2-</sup>]. Our results show that under constant [Fe'], the doubling of CO<sub>2</sub> to 800 p.p.m. CO<sub>2</sub> can reduce *P. tricornutum* Fe' uptake rates by 44%. While Fe' is an important component of phytoplankton nutrition<sup>5</sup>, we also show that *P. tricornutum* can use phytotransferrin-independent pathways to access organically complexed iron. In the marine environment, this pool of complexed iron is much larger<sup>3</sup>, although uptake rates can be orders of magnitude slower<sup>30</sup>, underscoring the trade-off inherent to the different iron acquisition strategies. As iron-limited regions exert an important influence on global biogeochemical cycles<sup>1</sup>, we view these results as a starting point for understanding the complex and interdependent influences of ocean acidification on phytoplankton iron uptake mechanisms and rates.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** J.B.M., A.B.K., M.O. and A.E.A. designed the study and interpreted the results. J.B.M., M.O. and A.H. generated and analysed phylogenetic and molecular clock data. J.B.M. and B.J.K. generated mutant cell lines, J.B.M. and A.B.K. with assistance from K.A.B. performed physiology experiments. J.B.M. performed microscopy, and H.Z. performed western blots. T.K. and A.J.A. analysed inorganic carbon species. J.B.M. and J.P.M. conducted statistical analyses. J.B.M. wrote the paper with input from A.E.A., A.B.K., M.O., J.P.M., A.J.A. and K.A.B. All authors discussed the results and commented on the manuscript.

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## METHODS

**Phylogenetic analysis.** To maximize the dimensions of our phylogenetic analysis, we built a series of hidden Markov models (HMM) from manually curated multiple peptide alignments. HMMs were assembled using transferrin and ISIP2A sequences obtained from NCBI via BLAST. Transferrin and ISIP2A HMMs were then used to iteratively obtain sequences from the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP)<sup>31</sup>. For inclusion into the transferrin and phytotransferrin clades, sequences were manually screened to ensure that they contained the conserved metal-binding residues and the carbonate-binding region (Extended Data Fig. 1a). Sequences were aligned using the genafpair exhaustive algorithm included in MAFFT<sup>32</sup>. The alignment was then manually inspected and ambiguous regions were removed in SEAVIEW v.4<sup>33</sup>. Phylogenetic analysis was conducted using maximum likelihood under the gamma-corrected LG matrix in RAXML v.8.2.8<sup>34</sup>. The best topology, as well as non-parametric branching support was inferred using fast algorithm (-fa option) from 1,000 replicates. Owing to the high divergence of the sequences, we have alternatively used Bayesian inference under the probabilistic CAT algorithm with numbers of site categories limited to 40 (C40 model), combined with empirical exchange rates as defined by the LG model in PhyloBayes v.4.1<sup>35</sup>. Two independent Markov chain Monte Carlo (MCMC) chains were run until they converged (that is, maximum observed discrepancy was below 0.2) and minimum effective size of model parameters exceeded 100.

**Divergence time estimation.** Fossil calibration points for multicellular taxa (metazoans and green plants) were compiled from <http://fossilcalibrations.org> (Extended Data Table 4). Calibration of diatoms and haptophytes (mineralizing protozoans) was adopted according previously published studies<sup>36,37</sup>. Respective values are summarized in Supplementary Table 4. The best-scoring topology from the Bayesian inference (see above) was then used for an estimation of divergence using UGAM and CIR relaxed clock models in PhyloBayes v.4.1. In addition, we used an uncorrelated log-normal clock model as implemented in BEAST v.2<sup>38</sup>. For this model, MCMC was run for 30 million generations. Convergence as well as effective sample size was evaluated using Tracer and the dating visualized on the tree using TreeAnnotator in the BEAST package.

**Predictions of signal peptides, transmembrane domains and glycosylphosphatidylinositol anchors.** All the sequences used for phylogenetic reconstructions were searched for the presence of signal peptide, transmembrane domains and glycosylphosphatidylinositol anchor (GPI). Endoplasmic reticulum signal peptides were predicted using SignalP<sup>39</sup> and TargetP<sup>40,41</sup>. Transmembrane domains were searched using TMHMM<sup>42</sup> and GPI anchors were predicted by PredGPI<sup>43</sup>. The results are shown in Supplementary Table 2.

**Cell lines and cultivation.** All cultivation and manipulation of *P. tricornutum* strain CCMP 632 was conducted using sterile trace-metal clean techniques and in Aquil medium<sup>44</sup>, unless otherwise noted. To remove contaminating iron, prepared medium was first passed through a Chelex 100 resin (Bio-Rad Laboratories) and microwave-sterilized in acid-cleaned polycarbonate bottles before addition of filter-sterilized, Chelex-passaged nutrient stocks and F/2 vitamin supplements.

**Cultures for short-term <sup>59</sup>Fe uptake rate experiments.** Cultures were grown in medium supplemented with macronutrients (880 μM NO<sub>3</sub><sup>-</sup>, 36 μM PO<sub>4</sub><sup>3-</sup>, 106 μM SiO<sub>4</sub><sup>2-</sup>) and trace metals (100 nM Zn, 48 nM Mn, 40 nM Co, 40 nM Cu, 10 nM Se and 100 nM Ni) with 100 μM EDTA. Vitamin and metal-EDTA solutions were added to artificial seawater and allowed to equilibrate overnight. Iron was pre-equilibrated overnight in a 1:1.25 ratio of iron:EDTA before addition. Cultures were maintained at 17 °C at 300 μmol quanta m<sup>-2</sup> s<sup>-1</sup> in a 10-h:14-h dark:light cycle, and all culture manipulations were done under a class-100 HEPA filter. Background contaminating iron was measured by flow injection analysis at multiple times and varied from 0.2–0.7 nM dissolved iron over the two-year course of experiments. Fe<sup>0</sup>, the sum of all labile inorganic iron species, was calculated as previously described<sup>44</sup>.

**Cultures grown for growth rate analysis.** Cultures of *P. tricornutum* (CCMP 632) were grown in a modified Aquil medium<sup>44</sup> containing EDTA, Co, Cu, Mo, Mn and Zn at final concentrations of  $3 \times 10^{-4}$  M,  $1.51 \times 10^{-7}$  M,  $1 \times 10^{-7}$  M,  $3.36 \times 10^{-7}$  M,  $1 \times 10^{-8}$  M and  $2.39 \times 10^{-7}$  M, with variable Fe concentrations. Cells were grown at 320 μmol photons m<sup>-2</sup> s<sup>-1</sup> under a 12-h:12-h light:dark cycle using cool-white fluorescent bulbs. Under these conditions, Fe<sup>0</sup> = 0.00068 of total Fe. A maximum of 700 pmol l<sup>-1</sup> Fe<sup>0</sup> was assumed, because of iron hydroxide precipitation, but for simplicity, we report the results as Fe<sup>0</sup> even at concentrations exceeding this maximum. Cell growth was monitored daily using a Turner AU-10 fluorometer for *in vivo* chlorophyll fluorescence, and steady-state growth rates were computed from linear regressions of ln(relative fluorescence) versus time.

**TALEN knockouts, transferrin complementation and site-directed mutagenesis.** Transcription activator-like effector nucleases (TALENs) were used to disrupt the *ISIP2A* gene. TALEN construction protocols followed the methods

as described for *P. tricornutum*<sup>49</sup>. In brief, a unique pair of 20-nucleotide length sequences was designed to span the first intron junction of ISIP2A (GenBank ID: XP\_002179762.1). TALEN constructs were assembled into the pTH vector<sup>49</sup> and the sequence was verified. A homologous recombination (HR) plasmid containing resistance to nourseothricin and driven by the promoter/terminator for the light harvesting promoter *FcpA*<sup>45</sup> was assembled into pUC-19 using Gibson cloning<sup>46</sup>. Both HR and pTH plasmids were introduced into *P. tricornutum* via particle bombardment<sup>47</sup>. Transformants were pre-screened by PCR for correct insertion of the nourseothricin-resistance cassette and  $\Delta$ ISIP2A gene knockouts were subsequently confirmed by western blot using custom-made Genomic Antibodies (OriGene) targeting the ISIP2A protein. Complementary sequences for the N- and C-terminal domains of hTF were codon-optimized for the diatom *Thalassiosira pseudonana* and designed using CLC software (Qiagen), and the signal peptide and C-terminal transmembrane anchor of ISIP2A were subsequently added before synthesis. The fusion products were then synthesized on a BioXp 3200 DNA synthesizer (SGI DNA). YFP was fused via Gibson cloning to the signal and transmembrane sequences of ISIP2A, while the ISIP2A complement was obtained by amplifying *P. tricornutum* cDNA (GeneScript). PCR-based site-directed mutagenesis strains were made with base-pair mismatch primers and amplified from the ISIP2A complement strain. All constructs were placed under control of the *P. tricornutum* nitrate reductase promoter and terminator<sup>19,48</sup>, sequence-verified, and maintained on pUC-19 vectors ( $n = 3$  for each construct). All complement sequences were Gibson-assembled into the cargo plasmid p0521s<sup>19</sup> (containing a selection marker for bleomycin) and introduced into the  $\Delta$ ISIP2A-knockout strain via conjugation with *Escherichia coli* Epi300 cells as previously described<sup>49</sup>. Positive transformants were selected on dual nourseothricin/zeocin antibiotic plates (200 and 100 μg ml<sup>-1</sup>, respectively) and verified by PCR and sequencing. Cell lines were maintained in 7.5 nM Fe-EDTA Aquil medium under positive antibiotic selection until testing.

**Short-term <sup>59</sup>Fe uptake assays.** Cell strains were grown without antibiotic selection and acclimatized in Aquil medium at 15 pM Fe<sup>0</sup> for a minimum of five transfers before assay. Growth in 15 pM Fe<sup>0</sup> was found to reduce the growth rate in wild-type *P. tricornutum* by approximately 15%, with an approximate  $F_d/F_m$  of 0.55, indicative of iron-limited (but not iron-starved) growth<sup>6</sup>. Cell counts were conducted on a Beckman Quanta cell cytometer and cross-calibrated by direct microscopic counts using a haemocytometer. To avoid changes to physiology and medium pH, cultures were grown and assayed at low cell densities ( $1\text{--}3 \times 10^5$  cells ml<sup>-1</sup>). <sup>59</sup>Fe was equilibrated with EDTA at a 1:2 ratio and pH 7 for 24 h before use. The specific activity of the <sup>59</sup>Fe was 42 Ci g<sup>-1</sup>, or about 0.08% pure. Desferrioxamine B (DFB) was complexed with <sup>59</sup>Fe by equilibrating at pH 3 overnight and adjusting to pH 8 with high-purity NaOH. The ratio of <sup>59</sup>Fe to uncomplexed DFB was 4:5. Precomplexed <sup>59</sup>Fe-EDTA and <sup>59</sup>Fe-DFB substrates were added to standard medium containing 100 μM EDTA and allowed to equilibrate overnight. Uptake assays were commenced by gravimetrically adding a 1:10 volume to the cell cultures. Uptake assay data were generated from uptake curves consisting of a minimum of five time points spaced 20–30 min apart. Assays were simultaneous and staggered, resulting in variations in the total length of assay; however, assays were no less than 2 h in length, and did not exceed 3 h in duration. ISIP2A protein expression has a strong diurnal pattern: for consistency and reproducibility, cells were collected in the light portion of the light:dark cycle. Room temperature fluctuations influence both carbon and iron speciation; temperature changes were accounted for in calculating Fe:EDTA dissociation constants<sup>25</sup> and CO<sub>2</sub> speciation<sup>54</sup>. During uptake assays, cells were shielded from actinic light and periodically filtered onto 2.0-μm PTFE filters, washed with Ti-citrate-EDTA solution<sup>50</sup> and preserved in Ecolite (MP Biomedicals) before liquid scintillation counting.

**Carbonic acid system manipulation and surrogate anion experiments.** Medium for the carbonic acid manipulation experiments was made by acidifying Chelex-treated bicarbonate-free Aquil medium to pH 3 with ultra-high purity HCl and bubbling with filtered N<sub>2</sub> for 60 min to strip out dissolved CO<sub>2</sub>. To buffer against large ( $\pm 0.1$  pH) changes in pH, Chelex-treated Tris-HCl was added at a final concentration of 2 mM, and the pH was adjusted back to 8.0 using ultra-high purity ammonium hydroxide. Ammonium hydroxide was used rather than sodium hydroxide to avoid contaminating the uptake medium with sodium bicarbonate, which is a common atmospherically derived contaminant in NaOH. Trace-metal clean F/2 nutrients were added, and all medium was stored in an N<sub>2</sub>/O<sub>2</sub>-purged, CO<sub>2</sub>-impermeable glove box before use. Carbonate ion availability was manipulated by adding NaHCO<sub>3</sub> to sub-aliquoted bottles of medium at concentrations of 0, 0.5, 1, 1.5, 2, 4 and 6 mM NaHCO<sub>3</sub> l<sup>-1</sup>, and allowed to equilibrate 24 h. <sup>59</sup>Fe-EDTA or <sup>59</sup>Fe-FOB were added 24 h before assay and allowed to equilibrate. *P. tricornutum* cells were grown in 2.7-l square polycarbonate bottles in Aquil medium containing 15 pM Fe<sup>0</sup> (7.5 nM Fe-EDTA), and were grown on a 10-h:14-h light:dark schedule. Cultures were grown to early exponential phase ( $3 \times 10^5$  cells ml<sup>-1</sup>) and collected



by centrifugation for 30 min at 1,000g at 17°C in acid-cleaned centrifuge bottles. Cells were washed and centrifuged twice in  $\text{NaHCO}_3$ -free Aquil synthetic seawater salts (without nutrients), and re-suspended in the carbonate chemistry-adjusted  $^{59}\text{Fe}$  medium. Uptake reaction volume was 150 ml, and to avoid unnecessary aeration, the medium was removed from the medium bottles using an acid-cleaned pipette. Volumes were determined gravimetrically. Uptake rates were assayed as outlined above (5-point curves, 2–3 h in duration), and samples for pH and total dissolved inorganic carbon (DIC) analysis removed from parallel (non-radioactive) cultures, poisoned with  $\text{HgCl}_2$ , sealed with Apiezon-L vacuum grease (M&I Materials) and stored in borosilicate-stoppered bottles for analysis<sup>51</sup>. Owing to the presence of Tris-HCl, total alkalinity was not analysed for this experiment and inorganic carbon speciation was calculated from pH and DIC (see below).

To test whether other anions could substitute for carbonate ions, bicarbonate-free medium was prepared as above. Sub-aliquots of this medium were used to create 100 mM stocks of the sodium salts of formate, acetate, oxalate, glycolate and pyruvate. These stocks were Chelex-treated for 48 h at 4°C before use. These alternate anion stocks were diluted 1:1,000 into 150-ml uptake medium reactions and the medium was allowed to equilibrate in an  $\text{N}_2/\text{O}_2$ -purged,  $\text{CO}_2$ -impermeable glove box as above. Cells were grown, collected and resuspended as above, and added to the 150-ml uptake reactions. Uptake rates were assayed as in the previous carbonic acid manipulation experiments: 5-point assay curves with a total uptake assay length of 2–3 h. Samples for pH and DIC analysis were removed from parallel (non-radioactive) cultures, poisoned with  $\text{HgCl}_2$ , sealed with Apiezon-L vacuum grease (M&I Materials) and stored in borosilicate-stoppered bottles for analysis<sup>51</sup>. Carbonate contamination in the unsupplemented control was 2.5  $\mu\text{M}$  (Extended Data Table 2).

**Demonstration of carbonate effect in natural seawater.** On 16 January 2013, seawater was collected from 3 m depth at the edge of the sea ice in McMurdo Sound Ross Sea, Antarctica (77° 36.999' S 165° 28.464' E). Water was pumped to the surface using a trace-metal clean diaphragm pump via acid-cleaned Teflon tubing and filtered on-site through an acid-cleaned 0.22- $\mu\text{m}$  filter. Filtered seawater was dispensed into trace-metal clean 50-l carboys and stored in the dark at 4°C. Phytoplankton grown in this batch of seawater are iron-limited<sup>10</sup>. For North Pacific Gyre water, seawater from station ALOHA (22° 45' N 158° 00' W) was collected in June 2016 using trace-metal clean techniques and similarly filtered and cold-stored before use. Samples of both types of natural seawater plus synthetic seawater (Aquil) were dispensed into 2.7-l acid-cleaned polycarbonate bottles. Seawater was amended with 100  $\mu\text{M}$  EDTA and Chelex-treated F/2 nutrients and allowed to equilibrate overnight. Two-litre samples of seawater were placed in 2.7-l acid-cleaned polycarbonate bottles and bubbled with filtered  $\text{N}_2/\text{O}_2$  mixtures containing 200 and 1,000 p.p.m.  $\text{CO}_2$ . Tubing and lines were acid-cleaned PTFE, with acid-soaked and -cleaned plastic aerators (Lee's Discard-a-Stone) used to maximize gas transfer. One hundred and fifty millilitres of each seawater sample ( $\text{CO}_2$ -modified samples and the unmodified controls) was transferred to small polycarbonate bottles as uptake medium, with 7.5 nM  $^{59}\text{Fe}$ -EDTA allowed to equilibrate overnight. Separately, *P. tricornutum* was grown in 7.5 nM Fe-EDTA Aquil to early exponential phase ( $3 \times 10^5$  cells  $\text{ml}^{-1}$ ) and harvested by centrifugation for 30 min at 1,000g at 17°C in acid-cleaned centrifuge bottles. This concentrate was resuspended in the uptake medium, and uptake rates were assayed as outlined above (5 point curves, 2–3 h in duration), and samples for total alkalinity and DIC analysis removed from parallel (non-radioactive) cultures, poisoned with  $\text{HgCl}_2$ , sealed with Apiezon-L vacuum grease (M&I Materials), and stored in borosilicate-stoppered bottles for analysis<sup>51</sup>. This experiment was repeated with three cultures on three separate days.

**Approximation of  $\text{Fe}'$  and carbonate rate constants ( $\text{NaHCO}_3$  addition experiments).** The short-term uptake rates determined from an iron-limited culture incubated across a range of  $[\text{CO}_3^{2-}]$  and  $\text{Fe}'$  levels (Extended Data Table 1) were used to determine a second-order rate constant for  $\text{Fe}'$  uptake and to solve for pseudo-first-order rate constants with respect to  $\text{Fe}'$  and  $[\text{CO}_3^{2-}]$ . These results are expressed on a per-cell basis, and pertain to cultures of *P. tricornutum* grown under this condition<sup>51</sup>, as the starting ISIP2A protein content per cell was identical (but unknown) in all short-term uptake experiments. Uptake rates determined from the two treatments to which no  $\text{NaHCO}_3$  was added were excluded. The slope of uptake versus the product of  $\text{Fe}'$  and  $\text{CO}_3^{2-}$  ( $1.22 \times 10^{-3}$  mol Fe cell $^{-1}$  h $^{-1}$  (M  $\text{Fe}'$ ) $^{-1}$  (M  $\text{CO}_3^{2-}$ ) $^{-1}$ ; Extended Data Fig. 6a), was used as the starting condition from which an initial  $\text{CO}_3^{2-}$  rate constant was determined. Assuming Fe uptake rate is first order with respect to  $\text{Fe}'$  at low  $^{59}\text{Fe}$  (and supported by the ratios of rates observed at varied  $\text{Fe}'$  at identical  $[\text{CO}_3^{2-}]$ ), the uptake rates measured at  $\text{Fe}'$  ranging from 2 to 45 pM (Extended Data Fig. 6b) and at variable  $[\text{CO}_3^{2-}]$  were collapsed by dividing these rates by the  $\text{Fe}'$  levels (Extended Data Fig. 6d). The slope of this quantity versus  $[\text{CO}_3^{2-}]$  revealed an initial constant for  $\text{Fe}'$ -normalized Fe uptake of  $1.27 \times 10^{-3}$  mol Fe cell $^{-1}$  h $^{-1}$  (M  $\text{CO}_3^{2-}$ ) $^{-1}$ . Then, a pseudo-first-order rate

constant of  $\text{Fe}'$  for Fe uptake,  $k = 0.962$  mol Fe cell $^{-1}$  h $^{-1}$  (M  $\text{Fe}'$ ) $^{-1}$ , was estimated from the second-order rate constant and the estimated  $\text{CO}_3^{2-}$  rate constant. The constituent rate constants for Fe uptake were solved by iteration using these initial conditions. Each round was performed with 16,000 pairs of trial rate constants, allowed to vary randomly from 0.33 to threefold, from the initial or previously iterated values. Residual sum of square values were calculated for each pair of trial rate constants. The median constants from the 20 pairs of trial constants with the lowest residual sum of square values (which had coefficients of variation of 0.01% or less) were used for the next trial. Trials were stopped when constants did not appreciably differ and no trend with trial number was apparent. For each constant, the mean of the last four trials are reported; the iterated constants were 0.782 mol ( $\pm 0.05$ ) mol Fe cell $^{-1}$  h $^{-1}$  (M  $\text{Fe}'$ ) $^{-1}$  and 0.00156 ( $\pm 0.00087$ ) mol Fe cell $^{-1}$  h $^{-1}$  (M  $\text{CO}_3^{2-}$ ) $^{-1}$  for  $\text{Fe}'$  and  $\text{CO}_3^{2-}$ , respectively (about 20% different from initial estimates). The fit of the data using the derived rate constants versus empirically measured rates is shown in Extended Data Fig. 6e.

**Carbonic acid system manipulation by  $\text{CO}_2$  bubbling, synthetic seawater.** Two litres of Chelex-treated Aquil medium in 2.7-l polycarbonate bottles was bubbled with filtered  $\text{N}_2/\text{O}_2$  mixtures containing 50, 200, 1,000 and 5000 p.p.m.  $\text{CO}_2$  plus an ambient air ( $\sim 400$  p.p.m.) control. Tubing and lines were acid-cleaned PTFE, with acid-soaked and -cleaned plastic aerators (Lee's Discard-a-Stone) used to maximize gas transfer. In the first experiment ( $\text{Fe}'$  unconstrained), both high (100 nM Fe-EDTA) and low (10 nM Fe-EDTA)  $\text{Fe}'$  media were pre-equilibrated with bubbled gas for 48 h before inoculation with a low-iron acclimatized culture of wild-type *P. tricornutum* cells. Cells were monitored twice daily for cell counts, chlorophyll and  $F_v/F_m$ . Cultures were grown to a low cell density ( $< 3 \times 10^5$  cells  $\text{ml}^{-1}$ ) before the uptake assay. The specific growth rates for each culture were determined from the last three cell counts.  $^{59}\text{Fe}$ -EDTA was pre-equilibrated overnight in filter-sterilized medium from each culture to assure that medium chemistry would be similar. Assays were a mix of 135 ml culture and 15 ml  $^{59}\text{Fe}$  uptake media, blended without aeration, periodically filtered onto 2.0- $\mu\text{m}$  PTFE filters, washed with Ti-citrate-EDTA solution<sup>50</sup> and preserved in Ecolite (MP Biomedicals) before liquid scintillation counting. Short-term uptake assays consisted of a 5-point curve, sampled 20 min apart, and not exceeding 3 h in duration. Culture aliquots of 500 ml were preserved for DIC and total alkalinity, and the rest of the culture was centrifuged and stored for protein expression analysis. Both ISIP2A and  $\beta$ -carbonic anhydrase were analysed on protein-per-cell normalized samples (Invitrogen).

For the  $\text{Fe}'$ -constrained experiments, the Fe:EDTA ratio was varied to constrain pH-induced changes in  $[\text{Fe}']$ . For the low-iron cultures, we attempted to constrain Fe to  $\sim 20$  pM  $\text{Fe}'$  across all five pH and  $\text{CO}_2$  manipulations, and in the high-iron cultures,  $\text{Fe}'$  was constrained to  $\sim 200$  pM  $\text{Fe}'$ . To keep the maximum concentration of EDTA to 1 mM or less, the total concentration of Fe-EDTA was decreased to 50 nM in the Fe-replete cultures, and 10 nM in the Fe-limited cultures. Cultures were analysed as above.

**Characterization of endocytosis.** To generate fluorescently labelled cell lines, full-length ISIP2A and ISIP1 genes (including the 600 base-pair native promoter and 300-bp native terminator regions) were amplified with Phusion high-fidelity polymerase (New England Biolabs). RFP and YFP protein tags were fused onto constructs using Gibson cloning and assembled into a pUC-19 vector. Full-length constructs were re-amplified, sequence-verified and assembled into p0521s using yeast assembly<sup>22</sup>. Clones were PCR-screened, with positive clones re-sequenced. For microscopy, cells were grown at a low density ( $2 \times 10^5$  cells  $\text{ml}^{-1}$ ) in 7.5 nM Fe-EDTA Aquil medium, centrifuged briefly and resuspended in Aquil medium containing 300 nM Fe-EDTA. Cells were immediately visualized on a Leica SP5 confocal microscope equipped with a 100 $\times$  oil immersion objective. A 514-nm laser was used to visualize YFP, RFP and plastid autofluorescence, with emission monitored at 520–550 nm, 610–635 nm and 700–740 nm, respectively. The membrane-impermeable dye FM 1-43 was used to stain the outer membrane; wild-type *P. tricornutum* was grown at 7.5 nM Fe-EDTA, centrifuged briefly and resuspended in Aquil medium containing 300 nM Fe-EDTA and 5  $\mu\text{g ml}^{-1}$  of FM 1-43 (Thermo Fisher). Cells were visualized within 10 min of dye addition (excitation 488 nm, emission 610 nm) by confocal microscopy. To evaluate the effect of endocytosis inhibitors, we added 5  $\mu\text{M}$  of the clathrin-mediated endocytosis inhibitor Pitstop2<sup>53</sup> (N-[5-(4-bromobenzylidene)-4-oxo-4,5-dihydro-1,3-thiazol-2-yl]-naphthalene-1-sulfonamide, Abcam) to short-term uptake medium containing 7.5 nM Fe-EDTA, mixed and assayed immediately, as outlined above. The toxic effects of Pitstop2 on *P. tricornutum* over the course of the 2-h uptake experiment were not evaluated.

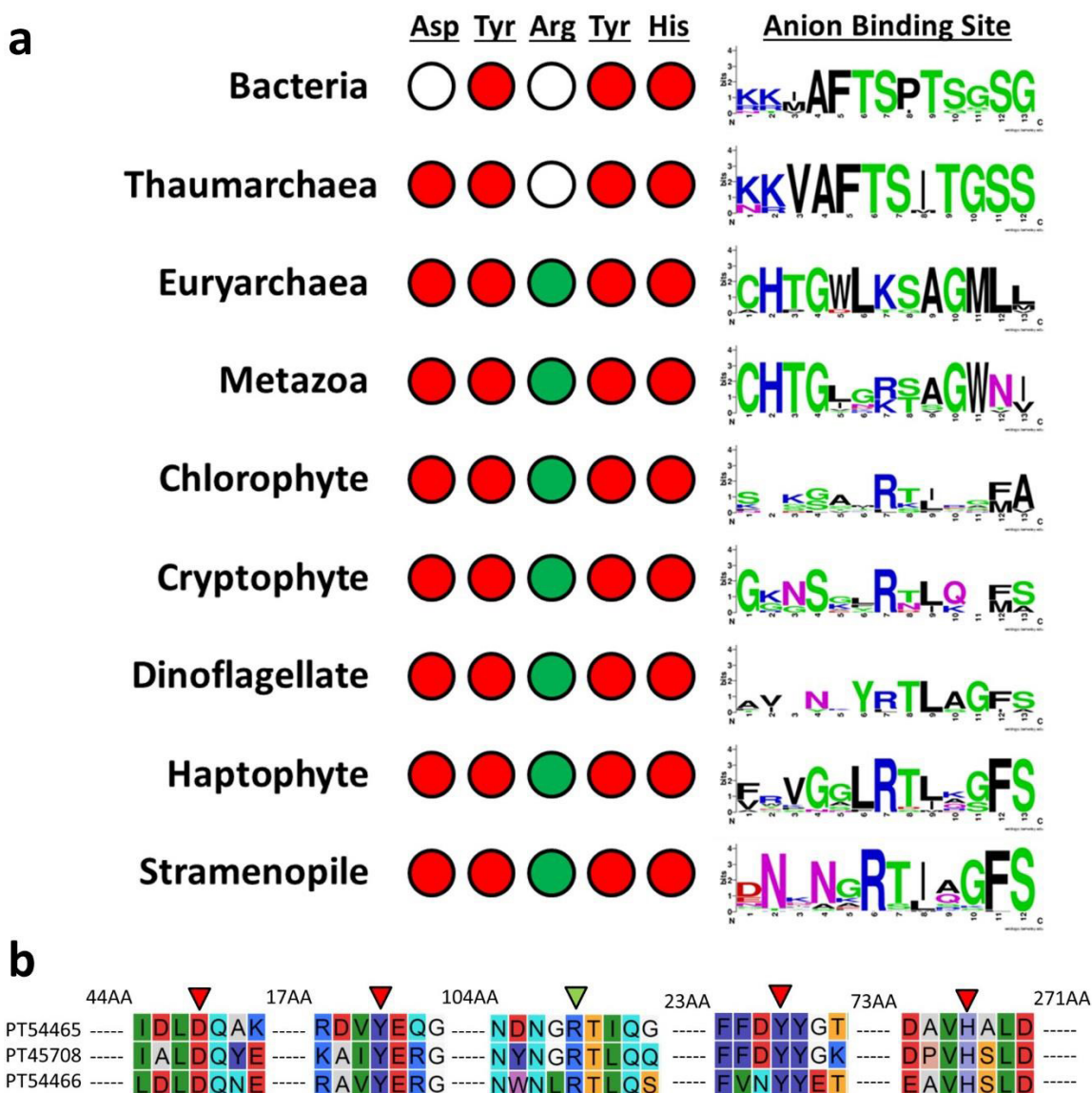
**Analysis of seawater carbonate chemistry.** Medium samples for pH, total alkalinity and DIC were collected following previously described protocols<sup>51</sup>. All samples were collected in 500-ml Pyrex borosilicate glass bottles and poisoned with 200  $\mu\text{l}$  of a saturated  $\text{HgCl}_2$  solution to arrest metabolic activity, and bottles were sealed using Apiezon-L sealant. For the  $\text{NaHCO}_3$ -manipulation experiments, pH and

DIC were measured: pH was measured on the total pH scale using meta-cresol purple<sup>54</sup>, and DIC analyses were carried out using an automated infrared inorganic carbon analyser (AIRICA, Marianda). For the CO<sub>2</sub> bubbling experiments, DIC and total alkalinity were measured. Total alkalinity was determined using an open-cell potentiometric acid titration system equipped with a Metrohm 876 Dosimat Plus automated titrator and a Metrohm Ecotrode Plus pH electrode, as developed by the Dickson Laboratory at Scripps Institution of Oceanography. Performance and accuracy of the analyses was evaluated through analysis of Certified Reference Material provided by the Dickson laboratory. Accuracy and precision  $\pm 2 \mu\text{mol kg}^{-1}$  was achieved for both DIC and total alkalinity analyses. The complete medium carbonic acid system including carbonate ion concentration was calculated based on pH and DIC (NaHCO<sub>3</sub> addition experiment) or DIC and total alkalinity (CO<sub>2</sub> bubbling experiment) data based on *in situ* temperature and salinity using CO2SYS<sup>55</sup>.

**Statistical analyses.** No statistical methods were used to predetermine sample size. All *n* numbers represent biological replicates, and the experiments were not randomized or blinded. All the data in bar graphs are expressed as mean  $\pm$  s.e.m., with paired Student's *t*-test (two-tailed). The ANOVA interaction effect between variables was used for evaluating synergism<sup>56</sup>. Statistical analysis was performed in R<sup>57</sup>, using linear regression (lm), two-way ANOVA (aov) and correlation tests (cor.test), where appropriate, to compare the relationship of Fe' uptake to [Fe'], [CO<sub>3</sub><sup>2-</sup>] and the [Fe']  $\times$  [CO<sub>3</sub><sup>2-</sup>] product. Test statistics and *P* values are summarized in Extended Data Table 3. *P* < 0.05 was considered significant.

**Data availability.** The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information.

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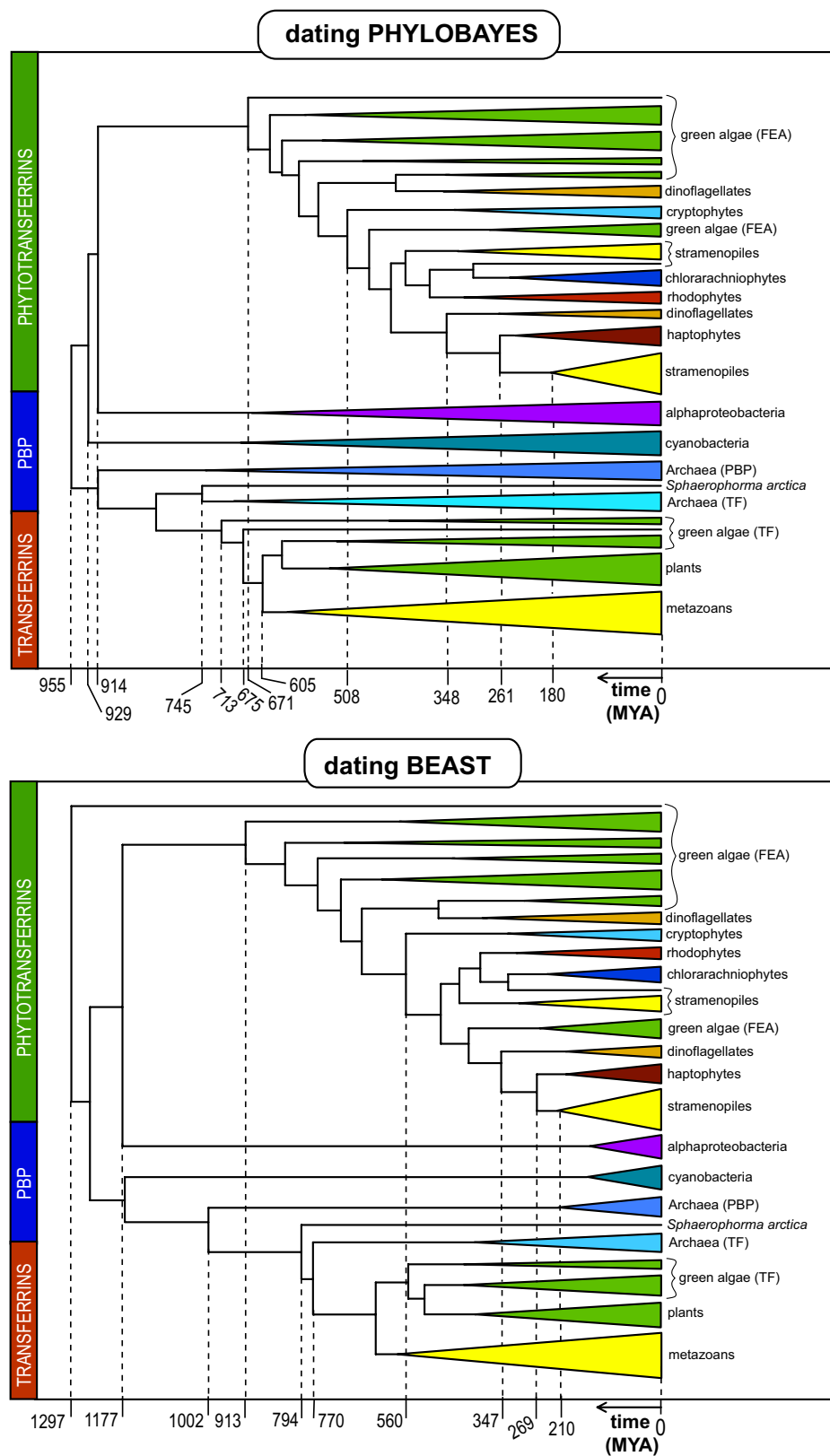


#### Extended Data Figure 1 | Conservation of active site amino acids.

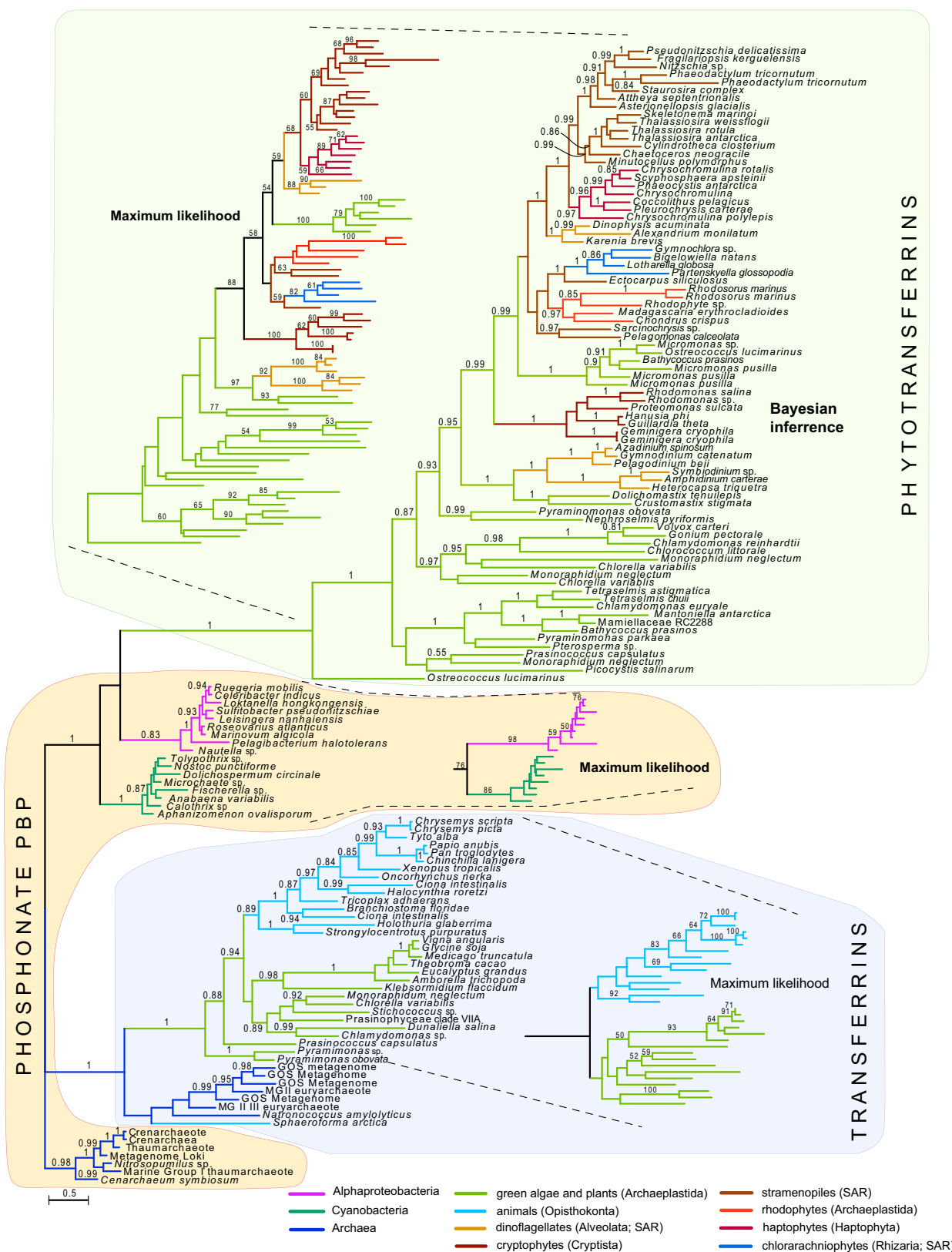
**a**, Left, conservation of putative iron-coordinating (red) and carbonate-coordinating (green) amino acids among phylogenetic groups, filled circles indicate >85% conservation, unfilled circles indicate <20% conservation; arginine and lysine were permitted at the carbonate-binding site<sup>11</sup>. Right, logo tag detailing alignment conservation at the anion-binding region. Phosphonate-binding proteins (Bacteria and Thaumarchaea) retain the S/T-rich phosphonate-binding area, whereas

transferrin (Euryarchaea and Metazoa) and phytotransferrin have the carbonate-coordinating K/R insertion. A downstream SAG region used to stabilize carbonate in transferrin<sup>24</sup> shows some conservation in phytotransferrin, but an upstream threonine (−4 amino acids from the conserved Arg) is not present. **b**, Active site homology among ISIP2A (PT54465) and two transmembrane-anchored *P. tricornutum* homologues, PT45708 and PT54466. Amino acid distances based on PT54465, red/green triangles are iron- and carbonate-coordinating amino acids.



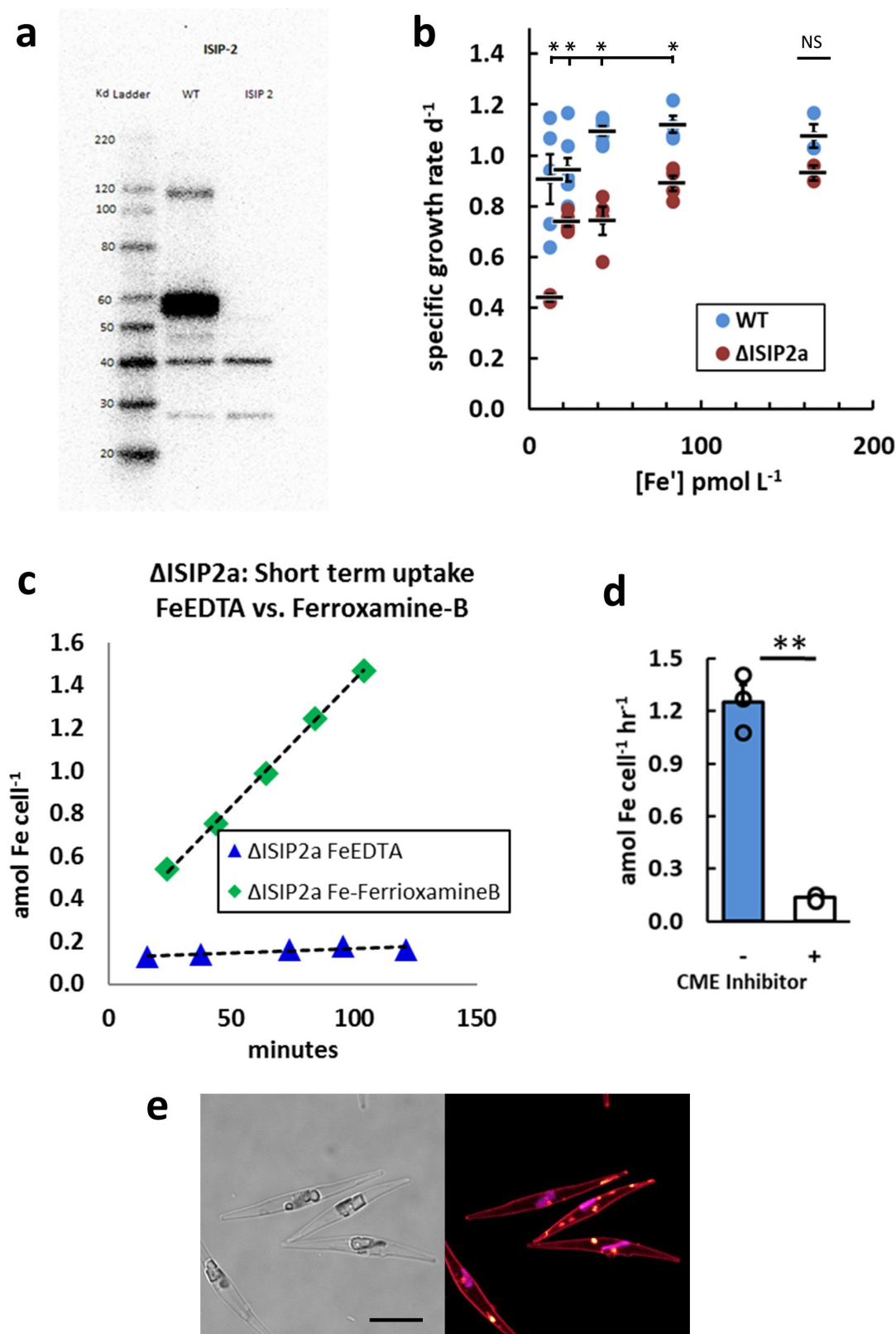


**Extended Data Figure 2 | Estimated divergence times between transferrin, phytotransferrin and PBPs.** Analyses were carried out using PhyloBayes (top) and BEAST (bottom). For the fossil calibration points used in generating the minimum and maximum constraints, see Supplementary Table 1. MYA, million years ago.



**Extended Data Figure 3 | Bayesian (PhyloBayes) phylogenetic tree contrasted with alternate topology derived using maximum likelihood. *Prasinococcus capsulatus*, *Pyramimonas obovata* and other chlorophyte**

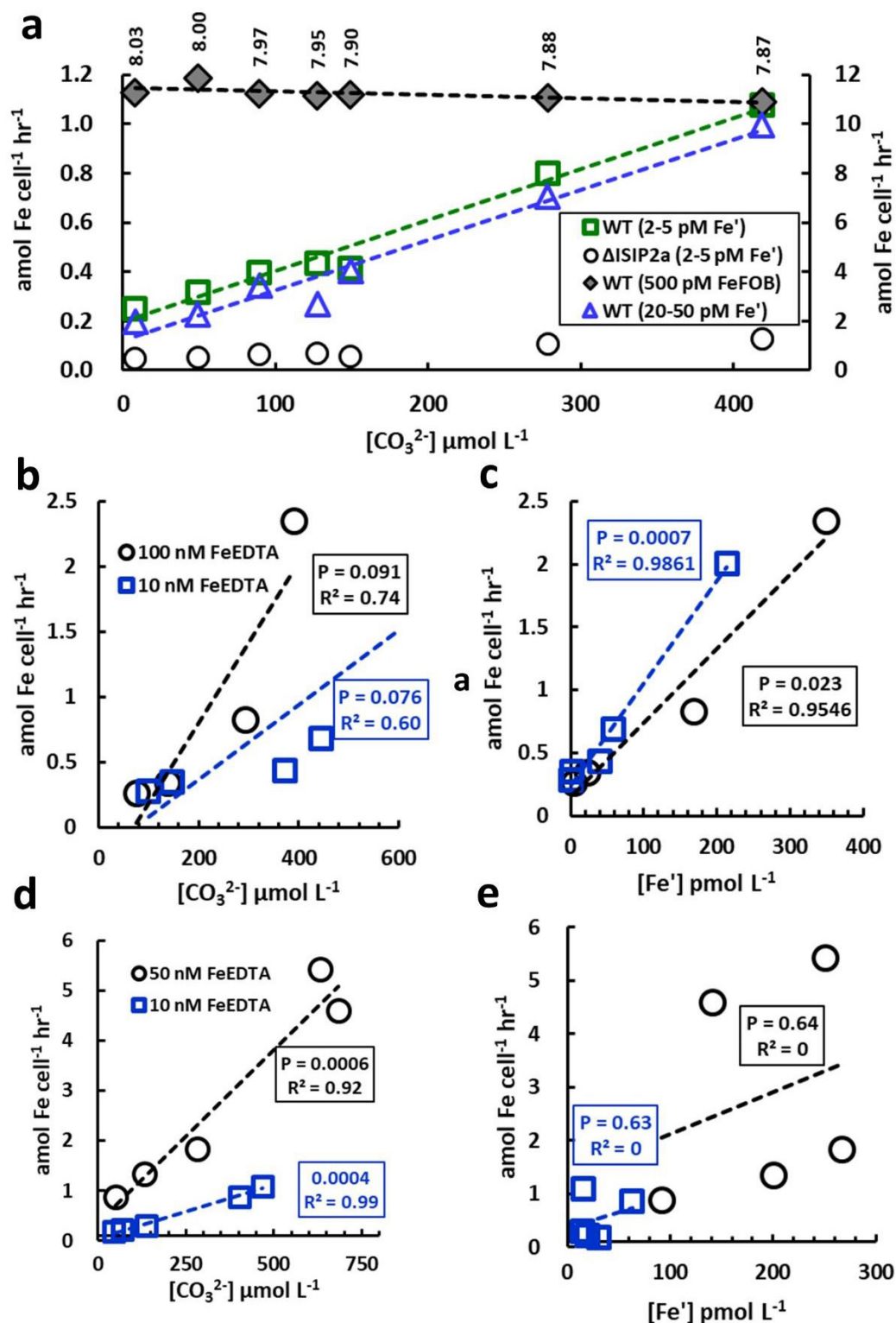
algae have copies of transferrin and phytotransferrin, whereas all other non-chlorophyte algae have only phytotransferrin. Branches are colour-coded by phylogenetic group. Scale bar, 0.5 substitutions per position.



**Extended Data Figure 4 | Characterization of ISIP2A.** **a**, Western blot of wild-type *P. tricornutum* compared to *ISIP2A*-knockout strains (*ΔISIP2A*). The estimated mass of ISIP2A protein is 57 kDa. **b**, Specific growth rates of wild type compared to *ΔISIP2A*. **c**, Uptake of  $^{59}Fe$ -ferrioxamine B is unaffected in *ΔISIP2A*, suggesting an alternate pathway for uptake of iron-siderophore complexes. **d**, Effect of a clathrin-mediated endocytosis (CME) inhibitor on short-term  $^{59}Fe'$  uptake rates, wild-type *P. tricornutum*. **e**, Upon addition of iron to iron-limited cells, the

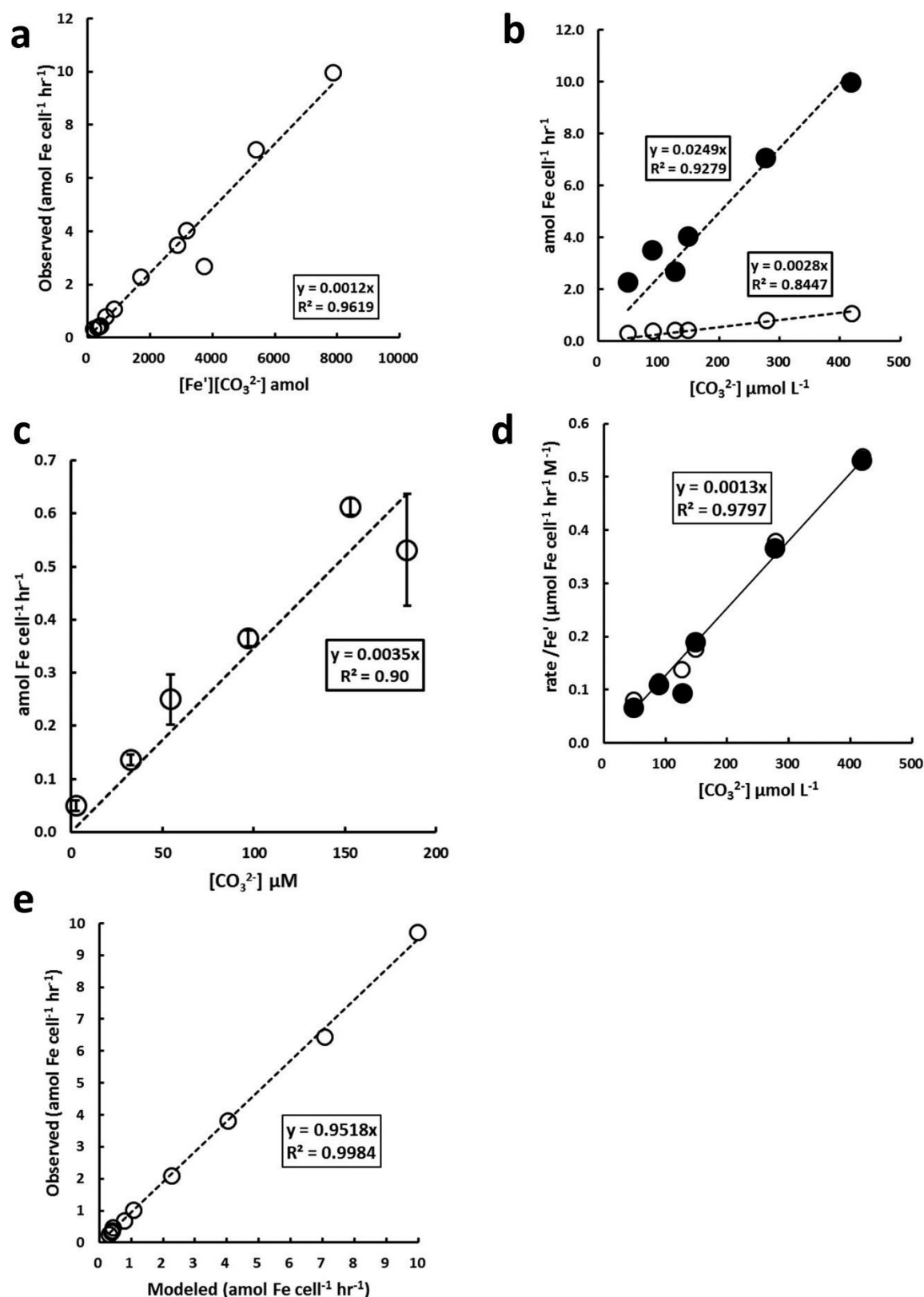
membrane-impermeable FM1-43 stain is internalized into vesicle-like inclusions. Scale bar, 5  $\mu m$ . Pink is plastid auto-fluorescence. **b**, Data are mean  $\pm$  s.e.m.; two-sided heteroscedastic *t*-test, *n* = biological replicates for wild-type and *ISIP2A* are indicated in brackets as (*WT*, *ISIP2A*) and *P* values are indicated. 12 pM  $Fe'$  (5, 3), *P* = 0.008; 22 pM  $Fe'$  (7, 5), *P* = 0.003; 43 pM  $Fe'$  (6, 4), *P* = 0.006; 83 pM  $Fe'$  (4, 4), *P* = 0.007; 165 pM  $Fe'$  (3, 3), *P* = 0.07. **d**, Data are mean  $\pm$  s.e.m.; *n* = 3 biological replicates; two-sided *t*-test, *P* = 0.009.





**Extended Data Figure 5 | Reconciliation of the carbonate effect with the influence of acidification on seawater iron chemistry.** **a**, Linearized representation of Fig. 3a, with high iron uptake rates (blue triangles) plotted on the secondary y axis, and pH values for each [CO<sub>3</sub><sup>2-</sup>] listed at top. Fe-FOB uptake rates decrease with pH, consistent with the findings of ref. 26, whereas Fe' uptake rates increase with decreasing pH, inconsistent with the hypothesized effects of acidification on iron-EDTA chemistry<sup>26</sup>. This inconsistency is resolved when uptake rates are plotted as a function

of the synergistic interaction between Fe' and CO<sub>3</sub><sup>2-</sup> (Fig. 3b). **b**, **c**, Under CO<sub>2</sub>-induced acidification, the strong influence of pH on [Fe'] results in a significant correlation of uptake to [Fe'], although the interaction Fe' and CO<sub>3</sub><sup>2-</sup> results in a better fit (Fig. 4a, solid line). **d**, **e**, When the change in [Fe'] is constrained relative to pH and [CO<sub>3</sub><sup>2-</sup>], uptake rates are positively correlated with [CO<sub>3</sub><sup>2-</sup>] and uncorrelated to [Fe'], revealing the influence of the carbonate ion on Fe' uptake rates. For statistical analyses of the linear regressions, see Extended Data Table 4.



**Extended Data Figure 6 | Derivation of second-order and constitutive rate constants from *P. tricornutum* resuspended in  $\text{NaHCO}_3$ -manipulated medium.** **a**, Regression of the interaction product of  $\text{Fe}'$  and  $\text{CO}_3^{2-}$ . Regression excludes the two observations in which the medium was not supplemented with  $\text{NaHCO}_3$ . **b**,  $^{59}\text{Fe}$  uptake rates for treatments incubated with low (2–5 pM  $\text{Fe}'$ , open symbols) and high (20–50 pM  $\text{Fe}'$ ,

closed symbols)  $^{59}\text{Fe}$  versus  $[\text{CO}_3^{2-}]$ . **c**, Demonstration of reproducibility at low  $[\text{Fe}']$ . Data are mean  $\pm$  s.e.m.;  $n = 3$  biological replicates. **d**, Uptake rates normalized to  $[\text{Fe}']$ , plotted against  $[\text{CO}_3^{2-}]$ . The slope has units of  $\text{mol Fe cell}^{-1} \text{ h}^{-1} (\text{M Fe}')^{-1} (\text{M CO}_3^{2-})^{-1}$ , equivalent to the pseudo-first-order uptake rate with respect to carbonate. **e**, Rates of uptake calculated as a function of  $[\text{Fe}']$  and  $[\text{CO}_3^{2-}]$  versus measured rates.

Extended Data Table 1 | Uptake rates compared to the measured (in bold) and derived concentrations of Fe' and carbonic acid species, in Aquil uptake medium

Treatment		Fe' uptake amol Fe' cell <sup>-1</sup> ·hr <sup>-1</sup>	[DIC] μmol kg <sup>-1</sup>	[TA] μmol kg <sup>-1</sup>	pH	[CO <sub>3</sub> <sup>2-</sup> ] μmol L <sup>-1</sup>	[Fe'] pmol L <sup>-1</sup>	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ] fmol <sup>2</sup> L <sup>-2</sup>	[EDTA] μmol L <sup>-1</sup>	[Fe <sub>TOTAL</sub> ] nM	log K' <sub>dark</sub> 20°C
10 nM FeEDTA <i>P. tricornutum</i>	6 mM NaHCO <sub>3</sub>	1.076	5557	-	7.87	419	2.0	0.84	100	10.8	-7.731
	4 mM NaHCO <sub>3</sub>	0.800	3647	-	7.88	278	2.1	0.59	100	10.9	-7.716
	2 mM NaHCO <sub>3</sub>	0.412	1884	-	7.90	149	2.3	0.35	100	11.0	-7.674
	1.5 mM NaHCO <sub>3</sub>	0.433	1437	-	7.95	127	3.1	0.40	100	11.0	-7.545
	1 mM NaHCO <sub>3</sub>	0.396	970	-	7.97	90	3.4	0.31	100	10.8	-7.499
	0.5 mM NaHCO <sub>3</sub>	0.318	505	-	8.00	49	4.0	0.20	100	10.9	-7.436
	unamended	0.250	75	-	8.03	8	4.9	0.04	100	10.9	-7.347
Treatment		Fe' uptake amol Fe' cell <sup>-1</sup> ·hr <sup>-1</sup>	[DIC] μmol kg <sup>-1</sup>	[TA] μmol kg <sup>-1</sup>	pH	[CO <sub>3</sub> <sup>2-</sup> ] μmol L <sup>-1</sup>	[Fe'] pmol L <sup>-1</sup>	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ] fmol <sup>2</sup> L <sup>-2</sup>	[EDTA] μmol L <sup>-1</sup>	[Fe <sub>TOTAL</sub> ] nM	log K' <sub>dark</sub> 20°C
100 nM FeEDTA <i>P. tricornutum</i>	6 mM NaHCO <sub>3</sub>	9.967	5557	-	7.87	419	18.8	7.89	100	101.4	-7.731
	4 mM NaHCO <sub>3</sub>	7.071	3647	-	7.88	278	19.4	5.42	100	101.1	-7.716
	2 mM NaHCO <sub>3</sub>	4.032	1884	-	7.90	149	21.3	3.17	100	100.5	-7.674
	1.5 mM NaHCO <sub>3</sub>	2.694	1437	-	7.95	127	29.1	3.71	100	102.0	-7.545
	1 mM NaHCO <sub>3</sub>	3.433	970	-	7.97	90	32.1	2.88	100	101.2	-7.499
	0.5 mM NaHCO <sub>3</sub>	2.264	505	-	8.00	49	34.7	1.71	100	94.7	-7.436
	unamended	1.960	75	-	8.03	8	45.2	0.36	100	100.4	-7.347
Treatment		Fe' uptake amol Fe' cell <sup>-1</sup> ·hr <sup>-1</sup>	[DIC] μmol kg <sup>-1</sup>	[TA] μmol kg <sup>-1</sup>	pH	[CO <sub>3</sub> <sup>2-</sup> ] μmol L <sup>-1</sup>	[Fe'] pmol L <sup>-1</sup>	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ] fmol <sup>2</sup> L <sup>-2</sup>	[EDTA] μmol L <sup>-1</sup>	[Fe <sub>TOTAL</sub> ] nM	log K' <sub>dark</sub> 20°C
10 nM FeEDTA <i>ΔISIP2a</i>	6 mM NaHCO <sub>3</sub>	0.129	5557	-	7.87	419	2.0	0.84	100	10.8	-7.731
	4 mM NaHCO <sub>3</sub>	0.107	3647	-	7.88	278	2.1	0.59	100	10.9	-7.716
	2 mM NaHCO <sub>3</sub>	0.057	1884	-	7.90	149	2.3	0.34	100	10.9	-7.674
	1.5 mM NaHCO <sub>3</sub>	0.070	1437	-	7.95	127	3.1	0.40	100	10.9	-7.545
	1 mM NaHCO <sub>3</sub>	0.066	970	-	7.97	90	3.5	0.31	100	11.0	-7.499
	0.5 mM NaHCO <sub>3</sub>	0.052	505	-	8.00	49	4.0	0.20	100	10.9	-7.436
	unamended	0.049	75	-	8.03	8	4.9	0.04	100	10.9	-7.347
Treatment		Fe' uptake amol Fe' cell <sup>-1</sup> ·hr <sup>-1</sup>	[DIC] μmol kg <sup>-1</sup>	[TA] μmol kg <sup>-1</sup>	pH	[CO <sub>3</sub> <sup>2-</sup> ] μmol L <sup>-1</sup>	[FeFOB] pmol L <sup>-1</sup>	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ] fmol <sup>2</sup> L <sup>-2</sup>			
500 pM FeFOB <i>P. tricornutum</i>	6 mM NaHCO <sub>3</sub>	1.090	5557	-	7.87	419	500	-			
	4 mM NaHCO <sub>3</sub>	1.106	3647	-	7.88	278	500	-			
	2 mM NaHCO <sub>3</sub>	1.117	1884	-	7.90	149	500	-			
	1.5 mM NaHCO <sub>3</sub>	1.116	1437	-	7.95	127	500	-			
	1 mM NaHCO <sub>3</sub>	1.123	970	-	7.97	90	500	-			
	0.5 mM NaHCO <sub>3</sub>	1.187	505	-	8.00	49	500	-			
	unamended	1.126	75	-	8.03	8	500	-			

These data were used to generate Fig. 3a, b. Note, total alkalinity (TA) was not measured in any of these samples because 2 mM Tris-HCl was used to maintain the pH at approximately 8.0.



Extended Data Table 2 | Uptake rates compared to measured (in bold) and derived concentrations for both Fe' and carbonic acid species

		CO <sub>2</sub> (ppm)	Fe' uptake amol cell <sup>-1</sup> hr <sup>-1</sup>	[DIC] μmol kg <sup>-1</sup>	[TA] μmol kg <sup>-1</sup>	pH	[CO <sub>3</sub> <sup>2-</sup> ] μmol L <sup>-1</sup>	[Fe'] pmol L <sup>-1</sup>	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ] amol <sup>2</sup> L <sup>-2</sup>	K' <sub>DARK</sub> 21.5°C	[EDTA] μmol L <sup>-1</sup>	[Fe:EDTA] nmol L <sup>-1</sup>
Nat. Seawater vs. Aquil (Fig. 3d)	Natural Seawater Ross Sea	200	<b>0.483</b>	<b>1837</b>	<b>2328</b>	8.36	320	21.1	6767	-6.550	100	7.5
		amb.	<b>0.064</b>	<b>2099</b>	<b>2324</b>	7.94	154	2.0	312	-7.570	100	7.5
		1000	<b>0.101</b>	<b>2045</b>	<b>2342</b>	8.07	199	4.2	832	-7.254	100	7.5
	Natural Seawater North Pac	200	<b>0.645</b>	<b>1874</b>	<b>2387</b>	8.38	337	23.6	7952	-6.502	100	7.5
		amb.	<b>0.175</b>	<b>2058</b>	<b>2371</b>	8.09	210	4.7	979	-7.206	100	7.5
		1000	<b>0.128</b>	<b>2091</b>	<b>2397</b>	8.07	206	4.2	860	-7.254	100	7.5
	Synthetic Seawater (Aquil)	200	<b>0.525</b>	<b>509</b>	<b>1012</b>	8.81	189	26.1	4943	-5.458	100	7.5
		amb.	<b>0.315</b>	<b>2070</b>	<b>2450</b>	8.18	255	7.7	1973	-6.987	100	7.5
		1000	<b>0.092</b>	<b>830</b>	<b>1047</b>	8.14	94	6.2	581	-7.084	100	7.5
Subs. Anions (Fig. 3c)	*Unamended		<b>0.049</b>	<b>27</b>	-	<b>8.059</b>	2.6	5.2	13.5	-7.282	100	7.5
	*Carbonate		<b>0.263</b>	<b>1149</b>	-	<b>8.003</b>	97.2	3.8	278.5	-7.418	100	7.5
	Formate		<b>0.052</b>	<b>27</b>	-	<b>8.063</b>	2.6	5.4	13.9	-7.271	100	7.5
	Acetate		<b>0.090</b>	<b>41</b>	-	<b>8.051</b>	3.9	5.0	19.5	-7.299	100	7.5
	Oxalate		<b>0.060</b>	<b>28</b>	-	<b>8.059</b>	2.8	5.2	14.8	-7.280	100	7.5
	Glycolate		<b>0.097</b>	<b>20</b>	-	<b>8.067</b>	2.2	5.5	11.7	-7.263	100	7.5
	Pyruvate		<b>0.099</b>	<b>36</b>	-	<b>8.019</b>	3.3	4.2	13.7	-7.377	100	7.5
NaHCO <sub>3</sub> (Fig. 6c)	*Unamended		<b>0.049</b>	<b>27</b>	-	<b>8.059</b>	2.6	5.2	10.1	-7.282	100	7.5
	0.5 mM NaHCO <sub>3</sub>		<b>0.096</b>	<b>358</b>	-	<b>8.036</b>	32.6	4.6	112.7	-7.337	100	7.5
	1.0 mM NaHCO <sub>3</sub>		<b>0.170</b>	<b>603</b>	-	<b>8.036</b>	54.5	4.6	250.9	-7.336	100	7.5
	*1.5 mM NaHCO <sub>3</sub>		<b>0.263</b>	<b>1149</b>	-	<b>8.003</b>	97.2	3.8	278.5	-7.418	100	7.5
	2.0 mM NaHCO <sub>3</sub>		<b>0.318</b>	<b>1806</b>	-	<b>8.004</b>	153.1	3.8	441.4	-7.415	100	7.5
	2.5 mM NaHCO <sub>3</sub>		<b>0.420</b>	<b>2423</b>	-	<b>7.953</b>	184.1	2.9	398.9	-7.539	100	7.5

\* duplicate data used in both experiments

Top, uptake rates of cells resuspended in two types of natural compared to synthetic seawater at ambient or modified with 200 and 1,000 p.p.m. CO<sub>2</sub>. Uptake rates are an mean of three biological replicates. These data were used to generate Fig. 3d. Bottom, uptake rates of cells resuspended in synthetic seawater (Aquil medium) supplemented with 100 μM of carbonate or alternate synergistic anions, and data show the reproducibility of the NaHCO<sub>3</sub> manipulation experiment. All uptake rates are an average of three biological replicates. These data were used to generate Fig. 3c and Extended Data Fig. 6c, respectively.

Extended Data Table 3 | Measured (in bold) and derived values for CO<sub>2</sub> and pH manipulation experiments

		CO <sub>2</sub>	Fe' uptake	[DIC]	[TA]		[CO <sub>3</sub> <sup>2-</sup> ]	[Fe']	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	[EDTA]	[Fe] <sub>TOTAL</sub>	log K' <sub>DARK</sub>		
		TREATMENT	amol cell <sup>-1</sup> hr <sup>-1</sup>	μmol kg <sup>-1</sup>	μmol kg <sup>-1</sup>	pH	μmol L <sup>-1</sup>	pmol L <sup>-1</sup>	amol <sup>2</sup> L <sup>-2</sup>	μmol L <sup>-1</sup>	nM	20°C	μ	Fv/Fm
pH/CO <sub>2</sub> manipulations Δ[Fe']unconstrained	10 nM FeEDTA	50 ppm	2.009	2052	2937	8.66	605	214	129.3	100	14.4	-5.829	1.21	0.606
		200 ppm	0.684	1903	2583	8.52	444	58	25.7	100	8.5	-6.164	1.02	0.613
		400 ppm	0.434	1802	2392	8.45	373	40	14.9	100	8.4	-6.325	1.13	0.577
		1000 ppm	0.349	2162	2391	7.90	146	2	0.3	100	8.5	-7.664	0.72	0.513
		5000 ppm	0.280	2488	2623	7.67	100	0.8	0.1	100	14.2	-8.237	0.48	0.431
	100 nM FeEDTA	50 ppm	7.358	1979	2891	8.70	620	1831§	1135.3	100	100.0	-5.737	1.14	0.587
		200 ppm	2.348	2000	2596	8.40	391	350	136.5	100	100.9	-6.460	1.22	0.605
		400 ppm	0.832	1989	2446	8.27	292	168	49.0	100	97.5	-6.764	1.29	0.611
		1000 ppm	0.345	2092	2311	7.89	138	22	3.1	100	109.4	-7.691	0.93	0.592
		5000 ppm	0.267	2288	2386	7.58	76	3	0.3	100	97.5	-8.446	0.70	0.504

§The calculated concentration of Fe' exceeds the 750 pmol Fe' L<sup>-1</sup> iron hydroxide precipitation limit, therefore this datapoint was not used in data models

		CO <sub>2</sub>	Fe' uptake	[DIC]	[TA]		[CO <sub>3</sub> <sup>2-</sup> ]	[Fe']	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	[EDTA]	[Fe] <sub>TOTAL</sub>	log K' <sub>DARK</sub>		
		TREATMENT	amol cell <sup>-1</sup> hr <sup>-1</sup>	μmol kg <sup>-1</sup>	μmol kg <sup>-1</sup>	pH	μmol L <sup>-1</sup>	pmol L <sup>-1</sup>	amol <sup>2</sup> L <sup>-2</sup>	μmol L <sup>-1</sup>	nM	20°C	μ	Fv/Fm
pH/CO <sub>2</sub> manipulations Δ[Fe']constrained	10 nM FeEDTA	50 ppm	1.089	1587	2335	8.63	468	16	8.4	1000	12.0	-5.888	1.05	0.584
		200 ppm	0.865	1803	2434	8.47	403	63	25.5	100	12.1	-6.281	1.11	0.598
		400 ppm	0.301	1874	2103	7.92	138	14	1.9	20	12.0	-7.630	1.02	0.578
		1000 ppm	0.221	2088	2183	7.56	69	19	1.3	2	12.2	-8.499	0.74	0.512
		5000 ppm	0.182	2900	2874	7.23	46	30	1.4	0.2	11.7	-9.286	0.77	0.517
	50 nM FeEDTA	50 ppm	4.605	1991	2987	8.75	683	141	96.0	1000	55.2	-5.594	1.12	0.603
		200 ppm	5.432	2315	3210	8.61	631	250	157.8	250	55.2	-5.946	1.23	0.617
		400 ppm	1.847	1632	2103	8.33	282	267	75.4	50	55.1	-6.616	1.06	0.581
		1000 ppm	1.348	1987	2201	7.87	132	200	26.4	5	55.4	-7.744	1.08	0.564
		5000 ppm	0.888	1918	1975	7.44	49	91	4.5	1	55.4	-8.783	0.92	0.541

Top, CO<sub>2</sub> manipulations where the Fe:EDTA ratio was held constant, and Fe' concentrations were allowed to change with changes in pH. Bottom, CO<sub>2</sub> manipulations in which the pH was allowed to change whereas the Fe:EDTA ratio was manipulated, constraining changes in [Fe'] to a narrow range (for full statistical analysis, see Extended Data Table 4).

Extended Data Table 4 | Statistical analyses of NaHCO<sub>3</sub> and pH and CO<sub>2</sub> manipulations**Linear regression analysis, Fe' uptake**

	FIGURE	Data set	term	DF	RSE	adj. R <sup>2</sup>	F-stat	P-value	comment
NaHCO <sub>3</sub> manipulation (ΔpH constrained)	3a	WT Low Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	5	4.97 × 10 <sup>-17</sup>	0.972	212	2.76 × 10 <sup>-5</sup>	
	3a	WT High Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	5	6.13 × 10 <sup>-16</sup>	0.957	135	8.29 × 10 <sup>-5</sup>	
	3a	ΔISIP2a Low Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	5	8.54 × 10 <sup>-18</sup>	0.921	71	3.85 × 10 <sup>-4</sup>	
		WT Low Fe'	[Fe']	5	1.86 × 10 <sup>-16</sup>	0.611	10	2.32 × 10 <sup>-2</sup>	
		WT High Fe'	[Fe']	5	1.95 × 10 <sup>-15</sup>	0.565	9	3.13 × 10 <sup>-2</sup>	
		ΔISIP2a Low Fe'	[Fe']	5	2.12 × 10 <sup>-17</sup>	0.514	7	4.23 × 10 <sup>-2</sup>	
	3b	WT (Combined Low/High)	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	12	5.96 × 10 <sup>-16</sup>	0.957	292	8.70 × 10 <sup>-10</sup>	
CO <sub>2</sub> /pH manipulation (ΔFe' unconstrained)	Ext. 5b	WT Low Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	3	4.54 × 10 <sup>-16</sup>	0.601	7	7.68 × 10 <sup>-2</sup>	Not Significant
	Ext. 5b	WT High Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	2	4.92 × 10 <sup>-16</sup>	0.741	10	9.06 × 10 <sup>-2</sup>	Not Significant
	Ext. 5c	WT Low Fe'	[Fe']	3	9.81 × 10 <sup>-17</sup>	0.981	212	7.02 × 10 <sup>-4</sup>	
	Ext. 5c	WT High Fe'	[Fe']	2	2.52 × 10 <sup>-16</sup>	0.932	42	2.30 × 10 <sup>-2</sup>	
	4a	WT (Combined Low/High)	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	7	9.13 × 10 <sup>-17</sup>	0.987	588	5.16 × 10 <sup>-8</sup>	Solid line
CO <sub>2</sub> /pH manipulation (ΔFe' constrained)	Ext. 5d	WT Low Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	3	4.85 × 10 <sup>-17</sup>	0.987	292	4.36 × 10 <sup>-4</sup>	
	Ext. 5d	WT High Fe'	[CO <sub>3</sub> <sup>2-</sup> ]	3	5.74 × 10 <sup>-16</sup>	0.922	48	6.15 × 10 <sup>-4</sup>	
	Ext. 5e	WT Low Fe'	[Fe']	3	4.60 × 10 <sup>-16</sup>	0	0	6.34 × 10 <sup>-1</sup>	Not Significant
	Ext. 5e	WT High Fe'	[Fe']	3	2.27 × 10 <sup>-15</sup>	0	0	6.47 × 10 <sup>-1</sup>	Not Significant
	4b	WT (Combined Low/High)	[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	8	5.90 × 10 <sup>-16</sup>	0.898	80	1.93 × 10 <sup>-5</sup>	Dashed line

**Significant interactions: 2-way Analysis of Variation (ANOVA), Fe' uptake**

FIGURE	Experiment type	term	DF	Mean Sq	F	p-value
3b	NaHCO <sub>3</sub> manipulation (pH constrained)	[Fe']	1	3.71 × 10 <sup>-29</sup>	131	4.55 × 10 <sup>-7</sup>
		[CO <sub>3</sub> <sup>2-</sup> ]	1	4.13 × 10 <sup>-29</sup>	146	2.73 × 10 <sup>-7</sup>
		[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	1	2.68 × 10 <sup>-29</sup>	95	2.02 × 10 <sup>-6</sup>
4a (Solid Line)	pH/CO <sub>2</sub> manipulation (Fe' unconstrained)	[Fe']	1	4.53 × 10 <sup>-30</sup>	450	4.33 × 10 <sup>-6</sup>
		[CO <sub>3</sub> <sup>2-</sup> ]	1	1.10 × 10 <sup>-31</sup>	11	2.14 × 10 <sup>-2</sup>
		[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	1	2.63 × 10 <sup>-31</sup>	26	3.75 × 10 <sup>-3</sup>
4a (Dash Line)	pH/CO <sub>2</sub> manipulation (Fe' constrained)	[Fe']	1	1.38 × 10 <sup>-29</sup>	43	5.89 × 10 <sup>-4</sup>
		[CO <sub>3</sub> <sup>2-</sup> ]	1	1.22 × 10 <sup>-29</sup>	38	8.10 × 10 <sup>-4</sup>
		[Fe']x[CO <sub>3</sub> <sup>2-</sup> ]	1	2.76 × 10 <sup>-30</sup>	9	2.58 × 10 <sup>-2</sup>



## Life Sciences Reporting Summary

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### ► Experimental design

#### 1. Sample size

Describe how sample size was determined.

No statistical methods were used to predetermine sample size. The sample sizes were determined, in part, based on availability of resources including time, cost, and personnel. All experiments for biochemical characterization of wild type and knockout cell lines and examination of the synergistic interaction of Fe' and carbonate on Fe' uptake rates were performed in biological triplicate. This level of replication was determined sufficient to reliably discern statistical reproducibility and correlations with p-values below a 0.05 threshold.

#### 2. Data exclusions

Describe any data exclusions.

One of the data points for the pH/pCO<sub>2</sub> Fe' uptake curve exceeded Fe' solubility. As the Fe' concentration was unknown, this data point was not used in generating the curve. This omission is noted in the methods and in the supplementary data tables.

In determining the first order rate constants, samples unamended with bicarbonate were omitted from the analysis. This omission is noted in the figure legend.

#### 3. Replication

Describe whether the experimental findings were reliably reproduced.

All experimental findings were reliably reproduced with sufficient biological replication.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

All n numbers represent biological replicates, and the experiments were not randomized or blinded. There was no biased use of particular tubes or reagents that were correlated with experimental conditions. Care was taken to repeat experimental measurements in an unbiased way so as to mitigate the effect of cofactors such as sampling tubes, assay instrumentation, and reagents.

#### 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Not applicable. Group allocation was not a feature for experiments and associated data.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

## 6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- ☐ ☒ The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- ☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ A statement indicating how many times each experiment was replicated
- ☐ ☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- ☐ ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☐ ☒ The test results (e.g.  $P$  values) given as exact values whenever possible and with confidence intervals noted
- ☐ ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- ☐ ☒ Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.

## ► Software

Policy information about [availability of computer code](#)

### 7. Software

Describe the software used to analyze the data in this study.

CO2SYS was used for calculation of carbonate ion, dissolved inorganic carbon (DIC) concentration, and total alkalinity (TA) based on in situ temperature and salinity.

MAFFT was used for multiple sequence alignments.

PhyloBayes and RAXML 8.2.8, BEAST 2, and SEAVIEW 4 were used for multiple sequence alignment and phylogenetic analyses.

CLC software was used for molecular construct design.

Statistical analysis was performed in R.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

## ► Materials and reagents

Policy information about [availability of materials](#)

### 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

### 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Custom-made genomic antibodies targeting *P. tricornutum* ISIP2a, ISIP1, and  $\beta$ -carbonic anhydrase proteins were developed by the commercial vendor OriGene. Antibodies were validated using Western Blotting experiments examining *P. tricornutum* wild-type and knock-out cultures. All cell lines were genotyped to confirm KO alleles.

## 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

*P. tricornutum* strain CCMP 632 was obtained from the National Center for Marine Algae and Microbiota. Transcription activator-like effector nucleases (TALENs) were used to disrupt the ISIP2a gene. Methods are described in the manuscript.

b. Describe the method of cell line authentication used.

Authentication for wild-type cells was confirmed by microscopy and by sequence verification of various alleles. (ISIP1, ISIP2a, ISIP3). Knockouts were confirmed by western blot using custom-made Genomic Antibodies (OriGene) targeting the ISIP2a protein.

c. Report whether the cell lines were tested for mycoplasma contamination.

The cell lines were confirmed to be free of heterotrophic bacterial contaminants, including *Mycoplasma*.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

## ► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

## 11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used in the study.

Policy information about [studies involving human research participants](#)

## 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.