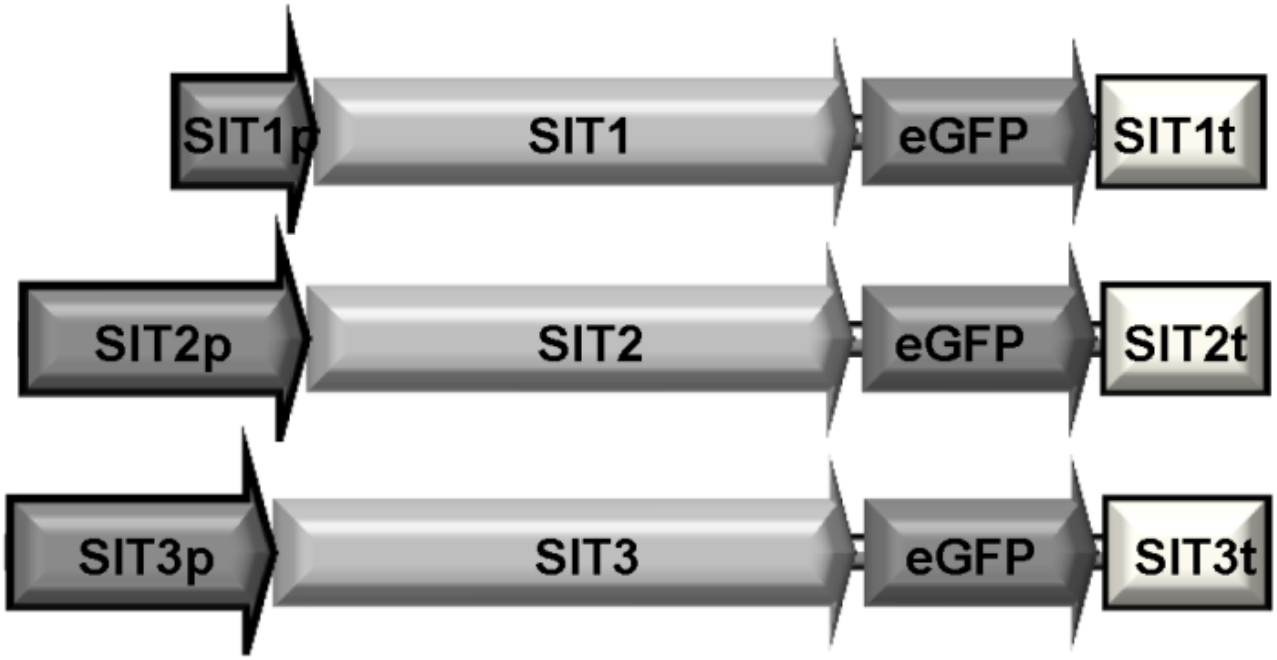
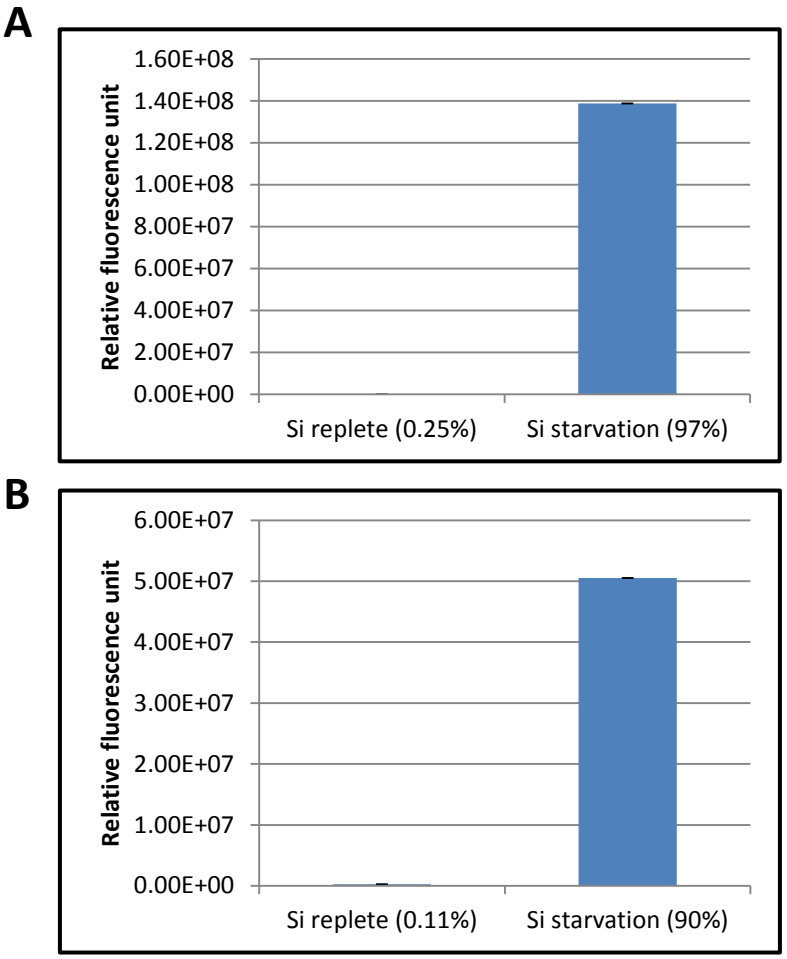
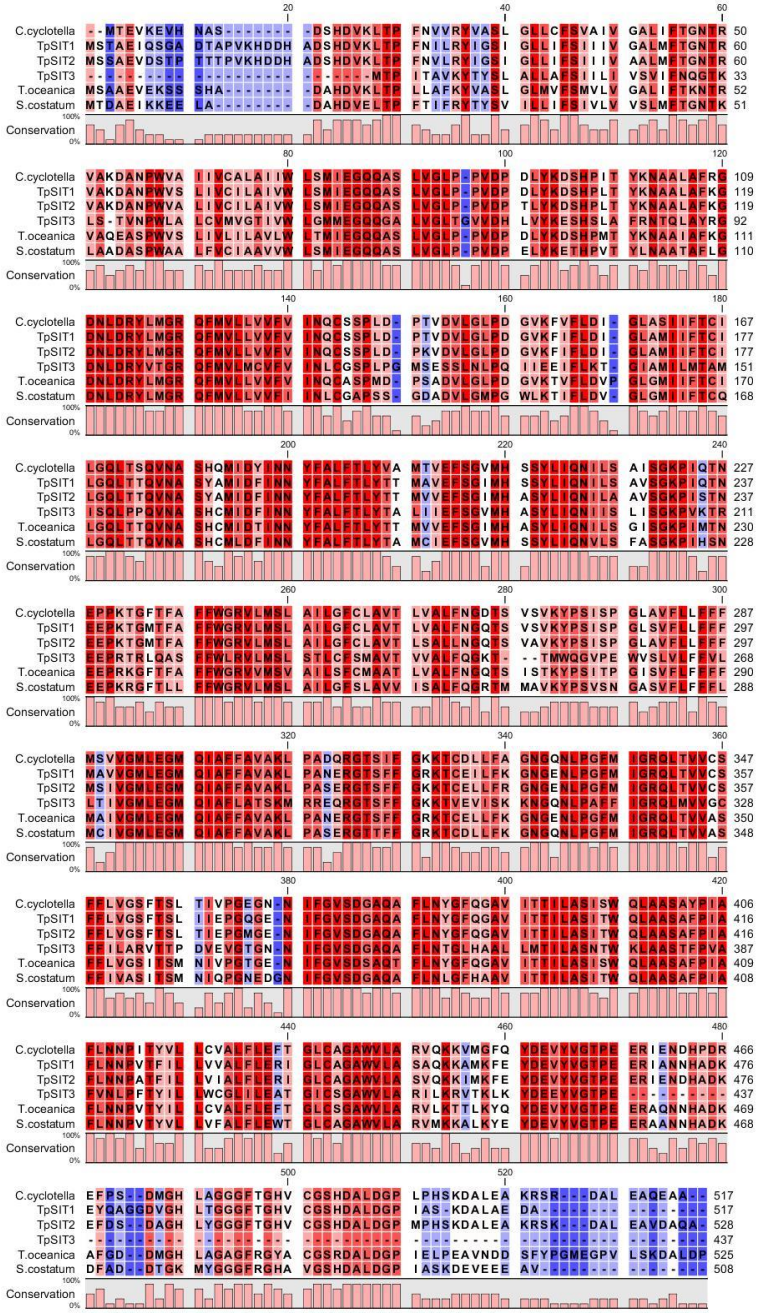
Supplementary Figures and Tables

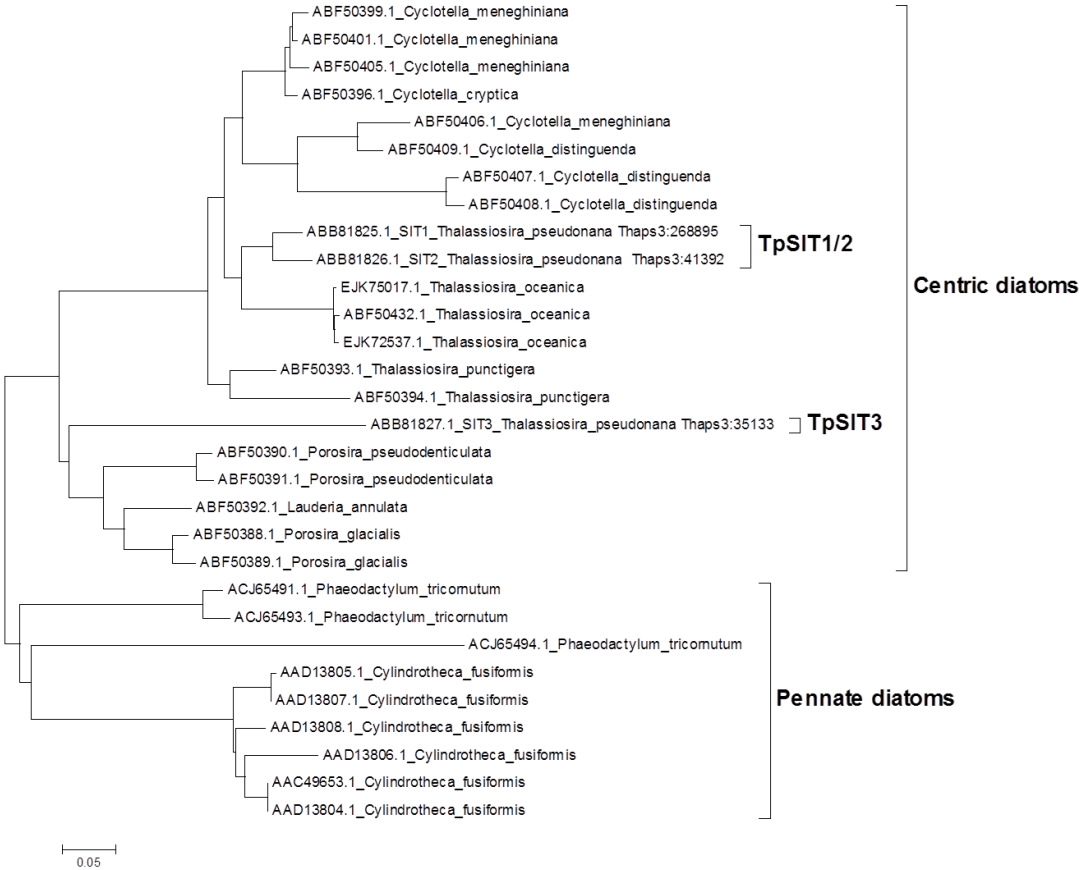


SI Fig. 1. Schematic diagrams of the Gateway-system based *T. pseudonana* transformation vectors. A-C, for expression and in vivo localization of eGFP-tagged *SIT* genes under the transcriptional control of respective native promoters. Upper, pMHL_243; middle, pMHL_290; lower, pMHL_293. These vectors were cotransformed with a plasmid vector expressing *Nat1* conferring resistance to the antibiotic nourseothricin.

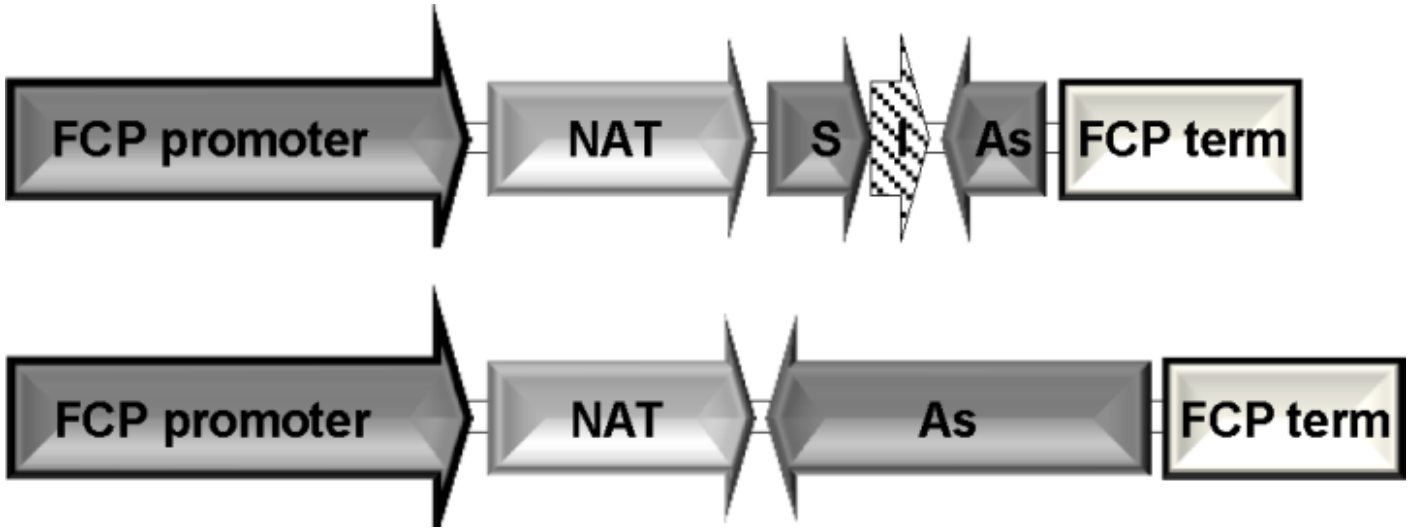


SI Fig. 2. Flow cytometry analysis of net eGFP expression . The relative whole cell fluorescence was measured, using a imaging flow cytometer, after incubating *Thalassiosira pseudonana* cells in silicon-replete and silicon-deplete media for 24 hr. *SIT1-eGFP* (A) and *SIT2-eGFP* (B) were transcribed under the transcriptional control of the respective native promoters to analyze expression level in response to Si starvation.
488nM laser with 100 mW power output was used to excite eGFP. Number in parentheses indicate the percentage of fluorescent cells in the gated population .





SI Fig. 3. Multiple sequence alignments and neighbor joining tree of SITs from centric and pennate diatoms. *T. pseudonana* SIT1 and SIT2 share 92% amino identities, and only 56% to SIT3.



SI Fig. 4. Schematic diagrams of the intron-spliced hairpin RNA (RNAi) and antisense RNA expression used for downregulation of silicon transporters in the diatom *T. pseudonana*. For efficient selection of transgenic clones, nourseothricin acetyl transferase gene (NAT) conferring resistance against herbicide nourseothricin was placed at the 3' end of the constitutive promoter FCP (fucoxanthin chlorophyll a/c binding protein).

SI TABLE 1 Primers used in vector constructions and RT-PCR

Gateway Vector Construction Primers

Primers	Sequence	Description
GW-13	GGGGACAACTTTGTATACAAAAGTTGTACATTCAACGGAATCTGATTATCAAC	SIT1 as-attB5
GW-14	GGGGACCACTTTGTACAAGAAAGCTGGGTAACCCTTCCATCTCTCCAGGAC	SIT1 as-attB2
GW-19	GGGGACAACTTTTCTATACAAAGTTGTAATGTCTACCGCTGAAATCCAATC	SIT1 +ve RNAi-attB4r
GW-20	GGGGACAACTTTATTATACAAAGTTGTCTGTAAGGTCAAAGAGAGCAACG	SIT1 +ve RNAi-attB3r
GW-21	GGGGACAACTTTGTATAATAAAGTTGTAACAGACGATAAGAGAGACCCATG	SIT1 -ve RNAi-attB3
GW-22	GGGGACCACTTTGTACAAGAAAGCTGGGTAATGTCTACCGCTGAAATCCAATC	SIT1 -ve RNAi-attB2
GW-25	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACCACTCTTGACGACACGGC	NAT-attB1
GW-26	GGGGACAACTTTTGTATACAAAGTTGTTCAGGGGCAGGGCATGCTCATG	NAT-attB5r
GW-35	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACCACTCTTGACGACACGGC	NAT-attB1
GW-36	GGGGACAACTTTGTATAGAAAAGTTGGGTGTCAGGGGCAGGGCATGCTCATG	NAT-attB4
GW-56	GGGGACAACTTTTCTATACAAAGTTGTAATGGTGAGCAAGGGCGAGGAG	eGFP-F
GW-57	GGGGACAACTTTATTATACAAAGTTGTTTACTTGTACAGCTCGTCCATGC	eGFP-R
GW-104	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATTACAATTACAGTGTACAGATAATG	SIT1 prom-F
GW-157	GGGGACAACTTTGTATAGAAAAGTTGGGTGGGCATCCTCGGCAAGAGCATC	SIT1 gene-R
GW-106	GGGGACAACTTTGTATAATAAAGTTGTAACTTCTGGCAATCTGTTGTAAATAG	SIT1-term-F
GW-107	GGGGACCACTTTGTACAAGAAAGCTGGGTAAGCAGAAACGAAATCATGCGGAG	SIT1-term-R
GW-182	GGGGACAAGTTTGTACAAAAAAGCAGGCTTATCACCAGTTTCATAACAACACAACC	SIT2 prom-F
GW-183	GGGGACAACTTTGTATAGAAAAGTTGGGTGAGCCTGCGCGTCAACAGCCTC	SIT2 gene-R
GW-184	GGGGACAACTTTGTATAATAAAGTTGTAGCAGTGTGTTTGTGTCATCGTG	SIT2 term F
GW-185	GGGGACCACTTTGTACAAGAAAGCTGGGTAATGATCTCGGGTGGAGGAGTC	SIT2 term R
GW-186	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAACTTCATCTGACAAACGCCGC	SIT3 prom-F
GW-187	GGGGACAACTTTGTATAGAAAAGTTGGGTGCCACTTCGCCAATCCACTTTCA	SIT3 gene-R
GW-188	GGGGACAACTTTGTATAATAAAGTTGTAGAAGCGTGATGATAATGCTCTGTC	SIT3 term-F
GW-189	GGGGACCACTTTGTACAAGAAAGCTGGGTAGTGGCTGTGTCAAAAAGCCCGC	SIT3 term-R

SI TABLE 1 Primers used in vector constructions and RT-PCR (cont'd)

RT-PCR Primers

Primers	Sequence	Description
RT-19	CTCTACATGAGCATGCCCTG	**RNAi**
RT-20	GAAGGATGTTGAAGGGAGTGAG	
RT-25	AGGTCACCAACGTCAACG	**Antisense RNA**
RT-26	TCGTCAGTTGCTCTCTATGTTG	
RT-30	CATGGGTCTCTCTTATCGTCTG	**RT qPCR**
RT-31	TCTTGTAAGTCAATGGGTGGC	
RT-32	TGTAAAGATCAGGGTCAACGG	

TABLE S2 Gateway vectors used to construct eGFP-tagged SITs

Fragments	**PCR primers**	**Donor vectors used**	**Entry vector created by BP reaction**	**pDestination vector used**	**pExpresssion vector created by LR reaction**
SIT1 promoter and coding region	GW-104/GW-157	pDONR-attP1-attP4	pMHL_241		
eGFP	GW-56/GW-57	pDONR-attP4r-attP3r	pMHL_139		
SIT1 terminator	GW-106/GW-107	pDONR-attP3-attP2	pMHL_207		
				pMHL_71	**pMHL_243**
SIT2 promoter and coding region	GW-182/GW-183	pDONR-attP1-attP4	pMHL_288		
eGFP	GW-56/GW-57	pDONR-attP4r-attP3r	pMHL_139		
SIT2 terminator	GW-184/GW-185	pDONR-attP3-attP2	pMHL_289		
				pMHL_71	**pMHL_290**
SIT3 promoter and coding region	GW-186/GW-187	pDONR-attP1-attP4	pMHL_291		
eGFP	GW-56/GW-57	pDONR-attP4r-attP3r	pMHL_139		
SIT3 terminator	GW-188/GW-189	pDONR-attP3-attP2	pMHL_292		
				pMHL_71	**pMHL_293**

SI TABLE 3 Signal peptide prediction for SIT1-3

	Hectar[1]	**Predotar[2]**	**pTARGET[3]**	**WoLF PSORT[4]**
SIT1	Signal peptide (0.5995)	-	Plasma membrane (87.6 %)	plas: 10
SIT2	Signal peptide (0.5999)	-	Plasma membrane (81.4 %)	plas: 10
SIT3	Type II signal anchor (0.4954)	Endoplasmic reticulum (0.95)	Peroxisomes (81.4 %)	plas: 7

1 http://www.sb-roscoff.fr/hectar/
2 http://urgi.versailles.inra.fr/predotar/predotar.html
3 http://golgi.unmc.edu/ptarget/
4 http://www.genscript.com/psort/wolf_psort.html