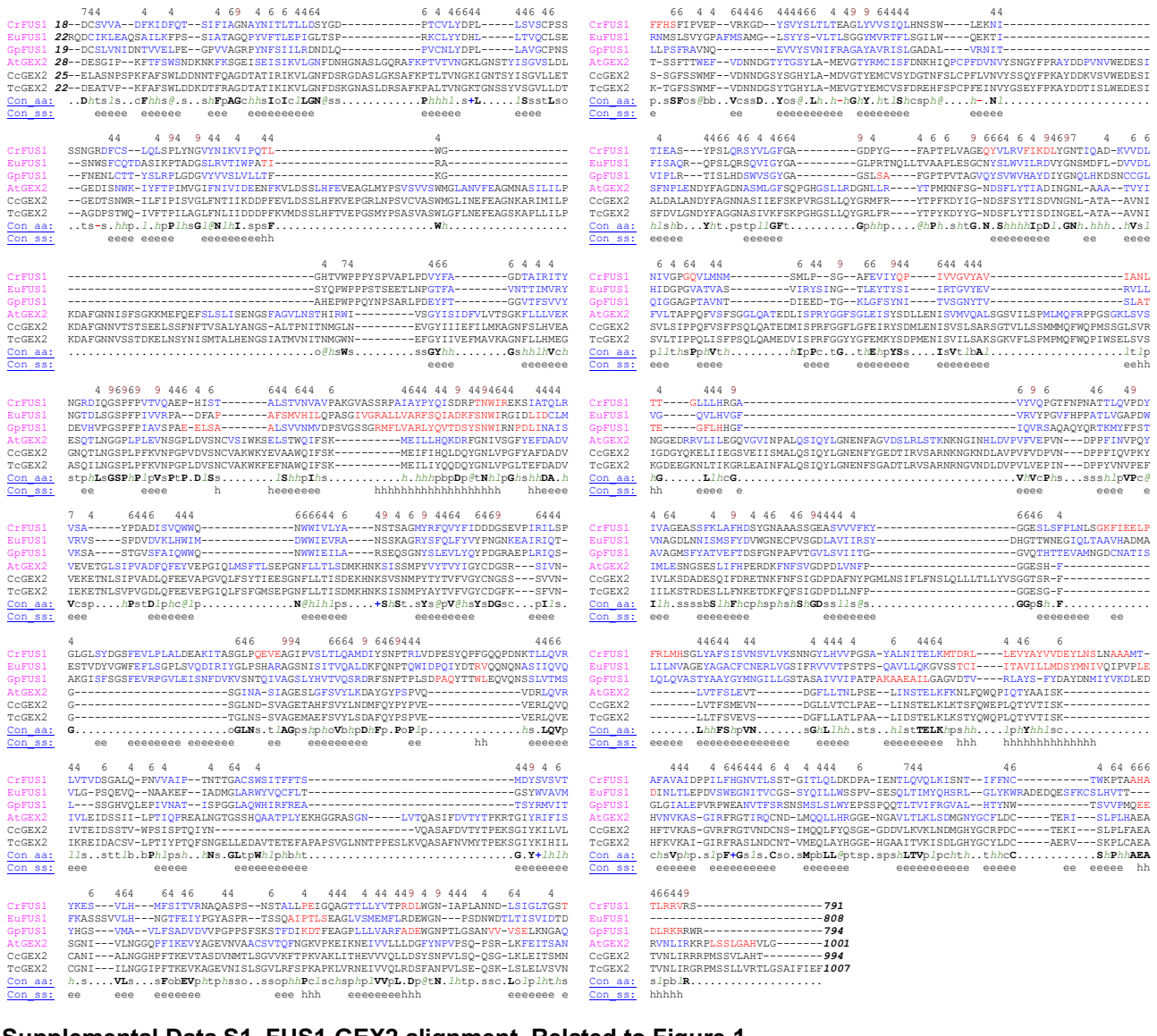
Table S1. Representative FUS1 Delta Blast Results. Related to Figure 1.

Description	Species	Lineage description / Common Name	Max Score	Query Cover	E value	Percent Identity	Accession
fus1 protein	*Chlamydomonas reinhardtii*	green alga	988	100%	0	99.6	AAC49416.1
gamete plasma membrane protein	*Gonium pectorale*	green alga	809	99%	0	31.5	BAU61607.1
plus gametic plasma membrane protein homolog	*Eudorina sp. NIES-3984*	green alga	814	97%	0	30.8	BBC28482.1
plus gametic plasma membrane protein homolog	*Yamagishiella unicocca*	green alga	734	98%	0	29.9	BBC28430.1
plus gametic plasma membrane protein	*Volvox reticuliferus*	green alga	809	99%	0	27.1	BCL66190.1
filamin	*Klebsormidium nitens*	green alga	311	84%	2.00E-87	23.6	GAQ86428.1
hypothetical protein DUNSADRAFT_11324	*Dunaliella salina*	green alga	563	96%	9.00E-180	22.0	KAF5841765.1
hypothetical protein HaLaN_10046	*Haematococcus lacustris*	green alga	91.1	68%	5.00E-17	21.0	GFH14067.1
hypothetical protein CBR_g31375	*Chara braunii*	green alga / stonewort	200	87%	2.00E-50	20.1	GBG80819.1
hypothetical protein MARPO_0070s0033	*Marchantia polymorpha*	liverwort	78.8	83%	5.00E-12	19.2	PTQ35564.1
hypothetical protein PPROV_000669800	*Pycnococcus provasolii*	green alga	55.3	17%	6.00E-05	19.2	GHP07956.1
hypothetical protein APUTEX25_001023	*Auxenochlorella protothecoides*	green alga	63	25%	3.00E-07	19.1	RMZ52904.1
PREDICTED: protein GAMETE EXPRESSED 2	*Raphanus sativus*	eudicots / radish	73.4	63%	2.00E-10	18.9	XP_018438118.1
protein GAMETE EXPRESSED 2-like isoform X2	*Panicum virgatum*	monocot / switchgrass	71.1	47%	8.00E-10	18.5	XP_039795268.1
protein gamete expressed 2	*Phtheirospermum japonicum*	eudicot / ko-shiogama	63.4	61%	2.00E-07	18.5	GFQ03406.1
protein GAMETE EXPRESSED 2	*Oryza sativa Japonica Group*	monocot / Japanese rice	54.9	31%	9.00E-05	18.2	XP_015651487.1
protein GAMETE EXPRESSED 2	*Panicum hallii*	monocot / Hall's panic grass	64.1	56%	1.00E-07	17.8	XP_025801577.1
predicted protein	*Micromonas commoda*	green alga	102	97%	2.00E-19	17.8	XP_002503840.1
hypothetical protein COCSUDRAFT_83706	*Coccomyxa subellipsoidea C-169*	green alga	94.9	84%	5.00E-17	17.8	XP_005647478.1
PREDICTED: protein GAMETE EXPRESSED 2	*Nicotiana sylvestris*	eudicot / wood tobacco	62.2	45%	4.00E-07	17.7	XP_009757468.1
PREDICTED: protein GAMETE EXPRESSED 2-like	*Nicotiana tabacum*	eudicot / common tobacco	62.2	45%	4.00E-07	17.7	XP_016487073.1
hypothetical protein M758_3G037800	*Ceratodon purpureus*	purple moss	78.8	39%	4.00E-12	17.7	KAG0621656.1
protein GAMETE EXPRESSED 2-like isoform X1	*Phalaenopsis equestris*	monocot / orchid	55.7	85%	4.00E-05	17.4	XP_020592212.1
protein GAMETE EXPRESSED 2 isoform X2	*Nicotiana tomentosiformis*	eudicot / wild tobacco	58	45%	9.00E-06	17.3	XP_033516539.1
protein GAMETE EXPRESSED 2	*Syzygium oleosum*	eudicot / blue cherry	56	72%	4.00E-05	17.3	XP_030463089.1
Protein GAMETE EXPRESSED 2	*Dichanthelium oligosanthes*	monocot / rosette grass	58	96%	9.00E-06	17.1	OEL30159.1
uncharacterized protein LOC111895799	*Lactuca sativa*	eudicots / lettuce	56.4	36%	3.00E-05	17.1	XP_023747630.1
conserved hypothetical protein	*Ricinus communis*	eudicot / castor bean	58	58%	1.00E-05	16.8	EEF32627.1

protein GAMETE EXPRESSED 2-like	*Papaver somniferum*	eudicots / opium poppy	71.8	66%	5.00E-10	16.6	XP_026444840.1
protein GAMETE EXPRESSED 2	*Carex littledalei*	monocot / sedges	61.4	61%	8.00E-07	16.4	KAF3339187.1
protein GAMETE EXPRESSED 2	*Citrus clementina*	eudicot / clementine	70.3	55%	2.00E-09	16.2	XP_024047282.1
protein GAMETE EXPRESSED 2	*Citrus sinensis*	eudicot / sweet orange	67.6	55%	9.00E-09	16.2	XP_024958580.1
protein GAMETE EXPRESSED 2	*Eucalyptus grandis*	eudicot / flooded gum	61.8	72%	6.00E-07	16.1	XP_010027085.2
Immunoglobulin-like fold	*Ostreococcus tauri*	green alga	100	83%	9.00E-19	16.1	XP_022839808.1
hypothetical protein ERO13_A02G050600v2	*Gossypium hirsutum*	eudicot / cotton	69.5	62%	3.00E-09	15.7	KAG4210466.1
Protein GAMETE EXPRESSED 2	*Capsicum annuum*	eudicot / pepper	65.3	55%	6.00E-08	15.6	PHT94469.1
Protein GAMETE EXPRESSED 2	*Capsicum baccatum*	eudicot / chili pepper	62.2	55%	5.00E-07	15.6	PHT59626.1
predicted protein	*Bathycoccus prasinos*	green alga	101	97%	5.00E-19	15.6	XP_007510093.1
protein GAMETE EXPRESSED 2 isoform X1	*Setaria viridis*	monocot / green bristlegrass	51.4	32%	8.00E-04	15.5	XP_034581122.1
protein GAMETE EXPRESSED 2	*Hordeum vulgare*	monocot / barley	58	86%	1.00E-05	15.4	KAE8807088.1
protein GAMETE EXPRESSED 2-like	*Triticum dicoccoides*	monocot / wheat	66.8	81%	2.00E-08	15.3	XP_037434559.1
PREDICTED: protein GAMETE EXPRESSED 2-like	*Camelina sativa*	eudicot / false flax	62.2	55%	5.00E-07	15.3	XP_019095519.1
hypothetical protein AXG93_4605s1010	*Marchantia polymorpha subsp. ruderalis*	liverwort	81.5	84%	8.00E-13	15.2	OAE34240.1
filamin like protein	*Micromonas pusilla CCMP1545*	green alga	71.4	86%	8.00E-10	15.2	XP_003056733.1
protein GAMETE EXPRESSED 2	*Aegilops tauschii subsp. strangulata*	monocot / goat grass	56.8	81%	2.00E-05	14.8	XP_020165207.1
hypothetical protein	*Adiantum capillus-veneris*	fern / maidenhair	106	87%	1.00E-20	14.7	MBC9837456.1
uncharacterized protein LOC9652715	*Selaginella moellendorffii*	club moss / spike moss	92.2	92%	3.00E-16	14.7	XP_024527230.1
protein GAMETE EXPRESSED 2	*Nymphaea colorata*	African water lily	55.3	56%	6.00E-05	14.2	XP_031479988.1
hypothetical protein A3770_09p56930	*Chloropicon primus*	green alga	96.5	97%	2.00E-17	13.9	QDZ23175.1
protein GAMETE EXPRESSED 2	*Senna tora*	eudicot / sensitive plant	58	47%	1.00E-05	13.2	KAF7837800.1

Representative hits from Delta-BLAST (iteration 2) search of *Viridiplantae* (taxid:33090) using the FUS1 ectodomain (residues 18-791) as a query protein sequence. Results are sorted from highest to lowest amino acid sequence identity between FUS1 and target sequence (Percent Identity). E-value; Query Coverage; Maximum Score; and descriptive information about the protein names and organisms are shown.

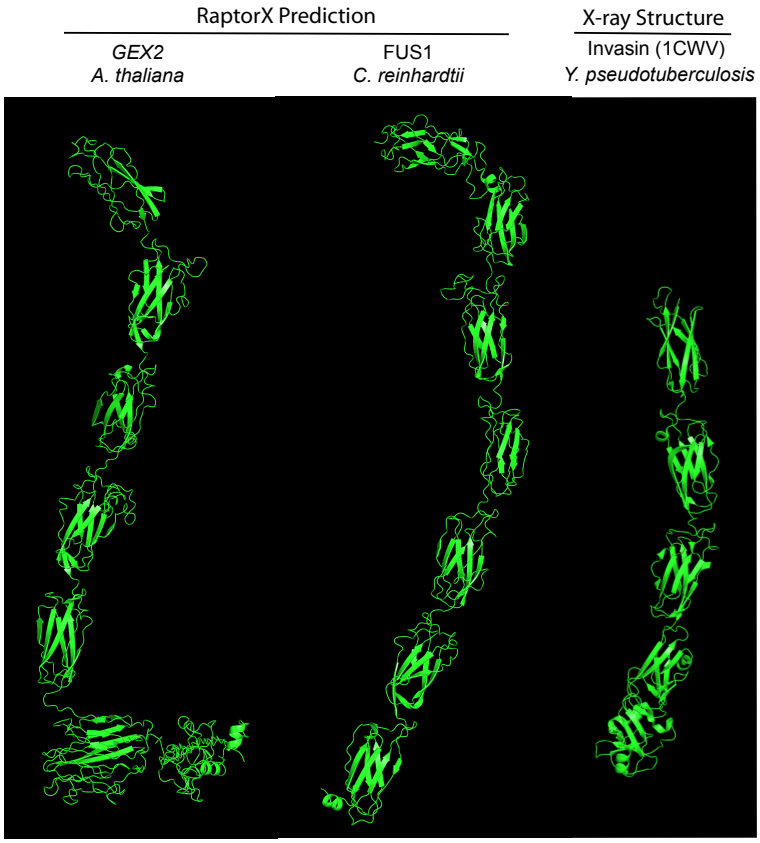
Supplemental Data S1



Supplemental Data S1. FUS1-GEX2 alignment. Related to Figure 1.

A PROMALS3D (Pei and Grishin, 2007) alignment of the ectodomain amino acid sequences from *Chlamydomonas reinhardtii* FUS1 (CrFUS1, GenBank#: AAC49416.1), *Eudorina sp. NIES-3984* FUS1 (EuFUS1, GenBank#: BBC28482.1), *Gonium pectorale* FUS1 (GpFUS1, GenBank#: BAU61607.1), *Arabidopsis thaliana* GEX2 (AtGEX2, DDBJ#: AB743888), *Citrus clementina* GEX2 (CcGEX2, GenBank#: ESR32766.1) and *Theobroma cacao* GEX2 (TcGEX2, GenBank# XP_017975311.1). Conservation score (4-9; 9 signifying the highest conservation) are shown above the alignment and the consensus amino acid sequence (Con_aa) and consensus predicted secondary structure (Con_ss) are shown below (Jones, 1999). Residue numbers in bold italic at the beginning and end indicate the amino acid residue spans aligned for each sequence. The CrFUS1 ectodomain sequence was 15.9% identical to the AtGEX2 sequence, and about ¾ as long (72.3% coverage). Predicted secondary structure is color coded onto the residues of each sequence and the consensus is shown below the alignment as: beta strand (e) and alpha helix (h). For consensus_aa residue code: conserved (bold & uppercase); aliphatic (*l*); aromatic (@); hydrophobic (*h*); alcohol (o); polar (p); tiny (t); small (s); bulky (b); positively charged (+); negatively charged (-); charged (c).

Supplemental Figure S1



Supplemental Figure S1. Structural homology of FUS1, GEX2, and invasin proteins. Related to Figure 1. Images of RaptorX contact-based prediction (Xu, 2019; Xu et al., 2021) for the ectodomains of *A. thaliana* GEX2 (residues 28 – 1007) and *C. reinhardtii* FUS1 (residues 17 – 791) along with the X-ray structure of the surface adhesion protein invasin of *Yersinia pseudotuberculosis* (PDB: 1CWV) illustrate their predicted structural similarities. New analyses found that the FUS1 extracellular region contains seven Ig-like domains predicted by structural homology models, including InterProScan identified Immunoglobulin-like fold domain (IPR013783, CATH 2.60.40.10) and Filamin/ABP280 repeat-like domain (IPR017868). Partial structural homology of the *Chlamydomonas* FUS1 ectodomain with bacterial proteins was found with HHPRED, including the S-layer protein SbsC from *Geobacillus stearothermophilus* (PDB: 5FTX, prob: 99.83%, E-value 4e-15, score: 169.78, ident: 14%), invasin-like protein FdeC from *E. coli* (PDB: 4E9L, prob: 99.7%, E-value: 2.2e-13, score: 147.25, ident: 17%), and invasin from *Yersinia pseudotuberculosis* (PDB: 1CWV, prob: 99.14, E-value: 1.3e-6, score: 96.34, ident: 11%). The *Y. pseudotuberculosis* protein, whose structure is shown above, binds to integrins on vertebrate cells (Hamburger et al., 1999). PHYRE2 ranked the gelation factor rod domain from *Dictyostelium* as the top hit (PDB 1WLH, confidence: 98.7%, ident: 17%) closely followed by the invasin-like protein FdeC (PDB 4E9L, residues 381-620, confidence: 97.3, ident: 16%); and Swiss-Model also found bacterial templates from invasin-like and S-layer protein structures as top hits (PDB: 4E9L, coverage: 45%, ident: 19.4%; PDB: 5FTX, coverage: 49%, ident: 12.4%). The *Arabidopsis* GEX2 ectodomain also exhibited partial structural homology with the same bacterial proteins and *Dictyostelium* protein as FUS1. HHPRED showed GEX2 homology with the *Dictyostelium* gelation factor (PDB 1WLH, prob: 99.7%, E-value 9.5e-14, score: 149.33, ident: 14%), and bacterial proteins including the invasin-like protein FdeC from *E. coli* (PDB: 4E9L, prob: 99.6%, E-value: 4.6e-11, score: 134.9, ident: 13%); the S-Layer protein SbsC of *Geobacillus stearothermophilus* (PDB: 5FTX, prob: 99.3%, E-value: 9.3e-8, score: 114.9, ident: 11%); and invasin from *Yersinia pseudotuberculosis* (PDB: 1CWV, prob: 98.97%, E-value: 8.9e-6, score: 95.78, ident: 11%). PHYRE2 ranked the *Dictyostelium* gelation factor rod domain as the top hit for GEX2 (PDB 1WLH, confidence: 99.8%, ident: 21%), followed by hits to filamin and cadherin-1 structures before the invasin-like protein FdeC (PDB 4E9L, confidence: 99.2, ident: 14%). Swiss-Model also found the *Dictyostelium* gelation factor as the top hit for GEX2 (PDB: 1WLH, coverage: 33%, ident: 17.3%) followed by hits to filamin structures before the invasin-like and S-Layer protein structures (PDB: 3TKV -superseded by 4E9L, coverage: 44%, ident: 16.5%; PDB: 5FTX, coverage: 24%, ident: 15.5%).

A

>MAR1

MARMLVTMLAGELLLGCLFAQLAAAQVTLCNKFDSVAGQAQFDQACW
NKAPCGGANVTIPTAWNYSSPCTSNTSNTTSSCVPLPPGYASCADWQ
AVTNASPAPCRTLTCCASPYGVSYSRYNCAPANTTCLDTGPNSVACM
PTQACPPGYIPDASGSCAGAPPTQPPRPPWPPRPPPAPPPSPRPPRP
PSPLPPRPRPPPPTPPSPPDPPPAPQIPQAPRWPYPQLPSWPPASPP
APVSTTPRSPLPPWNPRPPFLPPTPNGPTIFVGSDVPPSPLRPPPPP
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SAASEGGGGSSKTVAVVVTVVVSVAVVLVLLAVGWWACRKPPPPLPA
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EGGDDGYGEYEHKPAAKGPYDCDPYKDEYSPYDKYDKYGAQDDDGAT
TATGATASRMSHDSWGPAAAAAAAAPPPGPWPPPPPPPSVYPHQQPP
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RSNVSRTGRGAGHDPFRAGAPPPPPPWTPPPPPMARGMAAAAAAGGM
ASPGRSPRGTGRLEHGAAERQHDHQQQQHRDHQHHQQCHQQHQQPPW
ERQESHSRRHPPPLPPPQPHSHFRPPAAHGENSGRDGGGSSVWRRAH
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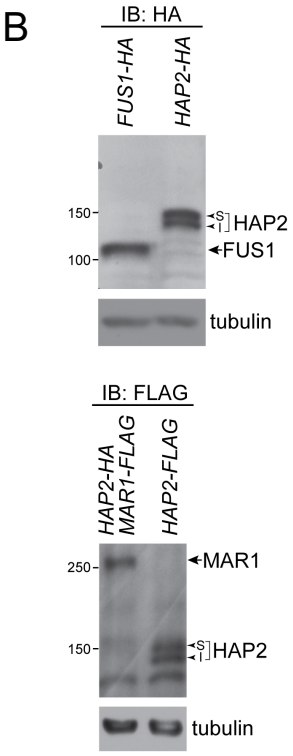
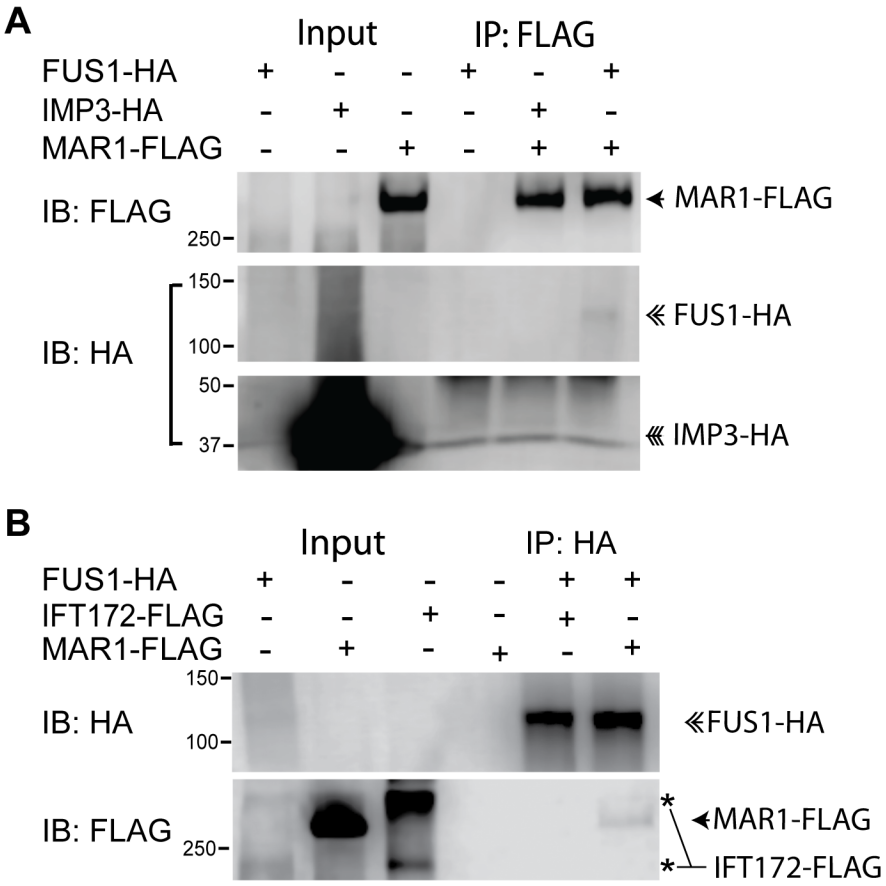
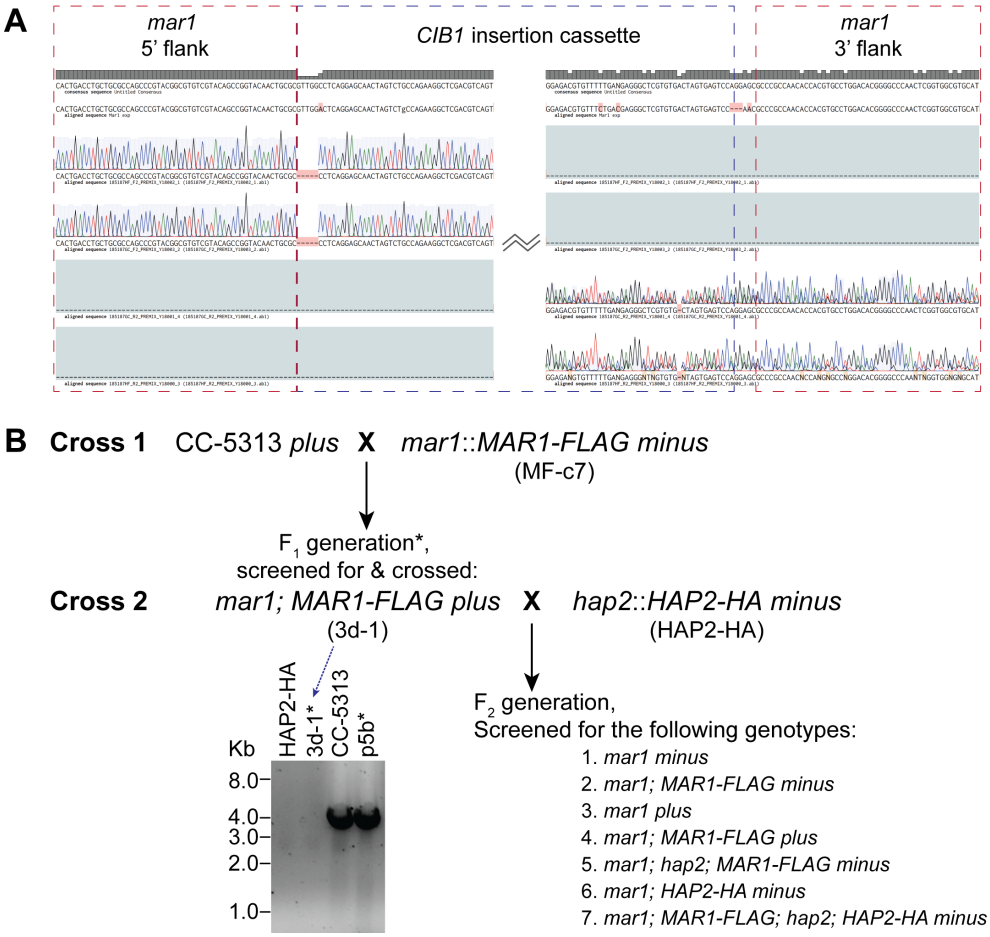


Supplemental Data S2. MAR1 identification and protein expression. Related to Figure 1.
(A) The streptavidin-staining protein of ~250 kDa indicated by the arrow in Fig. 1C is *Cre03.g176961*. The region corresponding to the streptavidin-stained band on the immunoblot shown in Fig. 1C was excised from an SDS-PAGE gel identical to the one used for the immunoblot (except that elution was done by boiling instead of by HA-peptide) and sent for mass spectrometry analysis by the Proteomics Core at UT Southwestern Medical Center (Dallas, TX). The *Cre03.g176961* peptides identified by mass spectrometry represented 21.8% of the MAR1 sequence and are underlined in the sequence above. The MAR1 sequence is also annotated to show other important features including the signal peptide called by SignalP (Nielsen, 2017), extracellular region, Growth factor receptor cysteine-rich domain superfamily (IPR009030) called by InterProScan, Putative tyrosine kinase phosphorylation sites (**Y**) called by both NetPhos3.1 (Blom et al., 2004) and MotifScan (Pagni et al., 2007), and *Disordered regions* called by MobiDB-lite (Necci et al., 2017). The following amino acids are enriched in the indicated sequence spans (MotifScan): Ala (A) 423-493; 845-921(15.1%) / Gln (Q) 773-802(3.6%) / Gly (G) 334-385; 625-979(15.6%) / His (H) 767-828(3.0%) / Pro (P) 142-365; 590-621; 726-738(19.1%). In the proline-rich (33% prolines) ectodomain, there are 5 repeats of a **PPSPX** motif found in other *Chlamydomonas* Hydroxy-Proline Rich Glycoproteins (HRGP). The Transmembrane domain was called by Phobius (Käll et al., 2007). Structural homology modeling of MAR1 offered only limited insights into the MAR1 intracellular region. RaptorX template-based models of residues 512-620 were based on an ABA receptor kinase protein structure from *Arabidopsis* (PDB: 5XD6, score:78, p-value: 5.77e-07) and included a predicted staurosporine binding site (pocket multiplicity of 52). PHYRE2 models of residues 619-1018 were based on a collagen template structure (PDB: 1YGV, confidence: 99.7%, identity: 15%). Sequence-based searches using BLAST for MAR1-related proteins found a limited number of putative homologs: *Vocar.0015s0371* and *Vocar.0015s0370* in *Volvox carteri, Cre03.g175926* in *C. reinhardtii*, and Genbank# KXZ56873.1 in *Gonium pectorale*. **(B)** In spite of differences in transcript RPKM levels (Fig. 1D), immunoblotting of *plus* and *minus* gametes indicated that the protein levels of HAP2, MAR1 and FUS1 are similar. Lysates from equal numbers of naive *fus1*::*FUS1-HA(+)*, *hap2*::*HAP2-HA(-)*, *hap2*::*MAR1-FLAG;HAP2-HA(-),* and *hap2*::*HAP2-FLAG(-)* gametes were analyzed by immunoblotting with anti-HA or anti-FLAG antibodies. The tubulin blots document equivalent loading. Proteins of interest, including surface (S) and internal (I) bands of HAP2-HA, are labeled.



Supplemental Figure S2. Immunoprecipitation controls. Related to Figure 2.
As additional controls to examine the specificity of the MAR1-FLAG and FUS1-HA interaction, we used lysates from cells expressing unrelated HA- and FLAG-tagged proteins. **(A)** Lysates from cells expressing MAR-FLAG were mixed with a lysate from cells expressing IMP3-HA (Lin et al., 2013), and separately mixed with a lysate from cells expressing FUS1-HA and subjected to FLAG immunoprecipitation**.** In the FLAG IP samples, FUS1-HA co-immunoprecipitated with MAR1-FLAG similarly to the results in Fig. 2 whereas IMP3-HA failed to co-immunoprecipitate with MAR1-FLAG. **(B)** Lysates from cells expressing FUS1-HA were mixed with a lysate from cells expressing IFT172-FLAG (Lin et al., 2018), and separately mixed with a lysate of cells expressing MAR1-FLAG and subjected to HA immunoprecipitation. In the HA IP samples, MAR1-FLAG co-immunoprecipitated with FUS1-HA, again as found in Fig. 2, whereas IFT172-FLAG failed to co-immunoprecipitate with FUS1-HA.

Supplemental Figure S3



Supplemental Figure S3. *mar1* insertion site and genetic crosses. Related to Figure 3 and STAR Methods. **(A)** Aligned sequence traces from across the *CIB1* insertion site of the *mar1(-)* deletion strain (CLiP mutant *LMJ.RY0402.185187*). PCR products amplified using *mar1(-)* genomic DNA as template were purified and single tube sequenced by Eurofins with the same primers (p1 and p2). A Benchling (San Francisco, CA) alignment of sequencing results shows the *mar1(-)* sequence directly flanking the CIB1 insertion site. The *CIB1* cassette inserted in the antisense direction at the location expected by its CLiP library annotation (Li et al., 2019). At the site of insertion, the junction of the *CIB1* cassette and *mar1* 5' flank lacked an expected "*GTTGGA*"*;* and the junction of the *CIB1* cassette with the *mar1* 3' flank contained a *"GG"* insertion. **(B)** Schematic depicting the *Chlamydomonas* genetic crosses performed. Resulting progeny were analyzed 1) for co-segregation of the *mar1* mutant genotype with the fusion-defective phenotype in *minus* gametes, and 2) to obtain specific genotypes in the F_2 generation (bottom right). The *mar1(-)* strain used in Cross 1 was transformed with *MAR1-FLAG* in order to obtain F_1 progeny. The *CC-5313(+)* gamete strain used in Cross 1 had a lesion in the gene *Cre12g541400* that was intentionally not selected for (to avoid possible confounding effects) in the *3d-1(+)* gamete F_1 strain used for Cross 2. Screening for the transmission of the *CC-5313* lesion by DNA gel electrophoresis of PCR products using primers RB1 and C12g541400.R2 is shown on the bottom left, with the 4013bp product indicating the presence of the insertional lesion. In the F_2 generation, single progeny colonies were picked and screened for the presence or absence of genetic and phenotypic markers (Supplemental Data S4) to isolate the indicated genotypes (bottom right).

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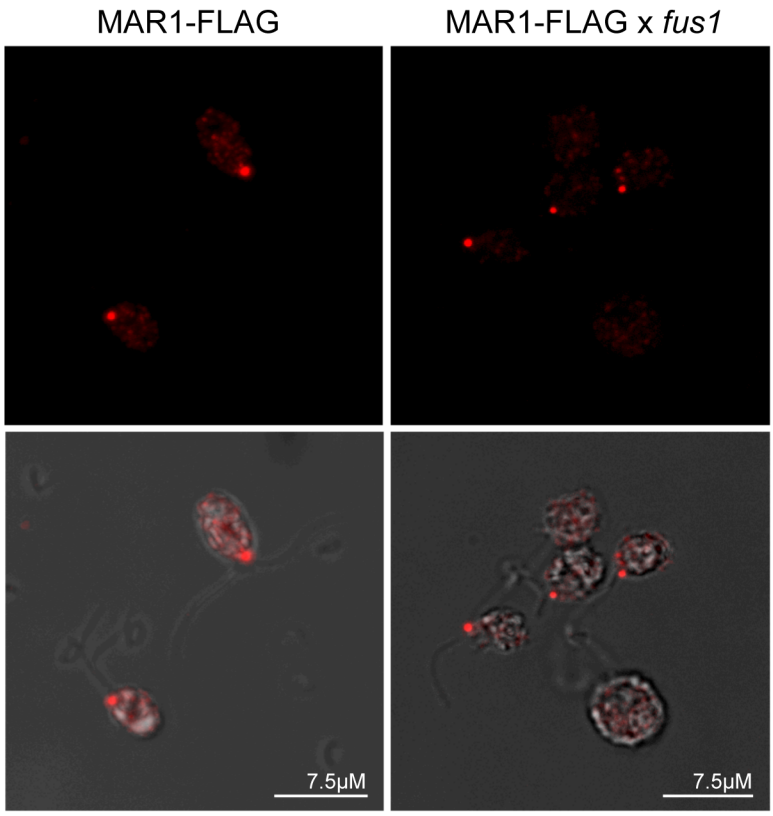
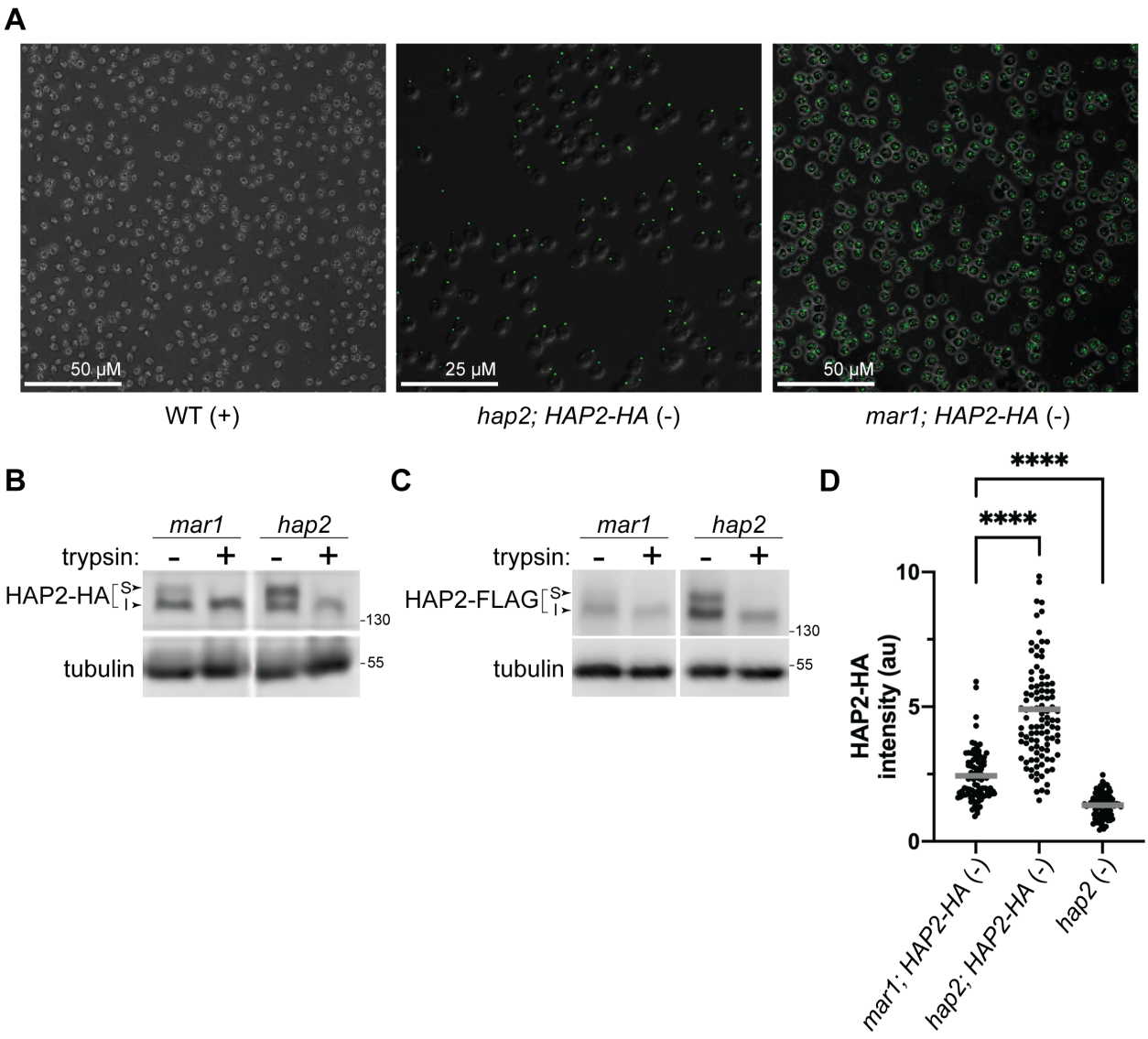
Supplemental Figure S4

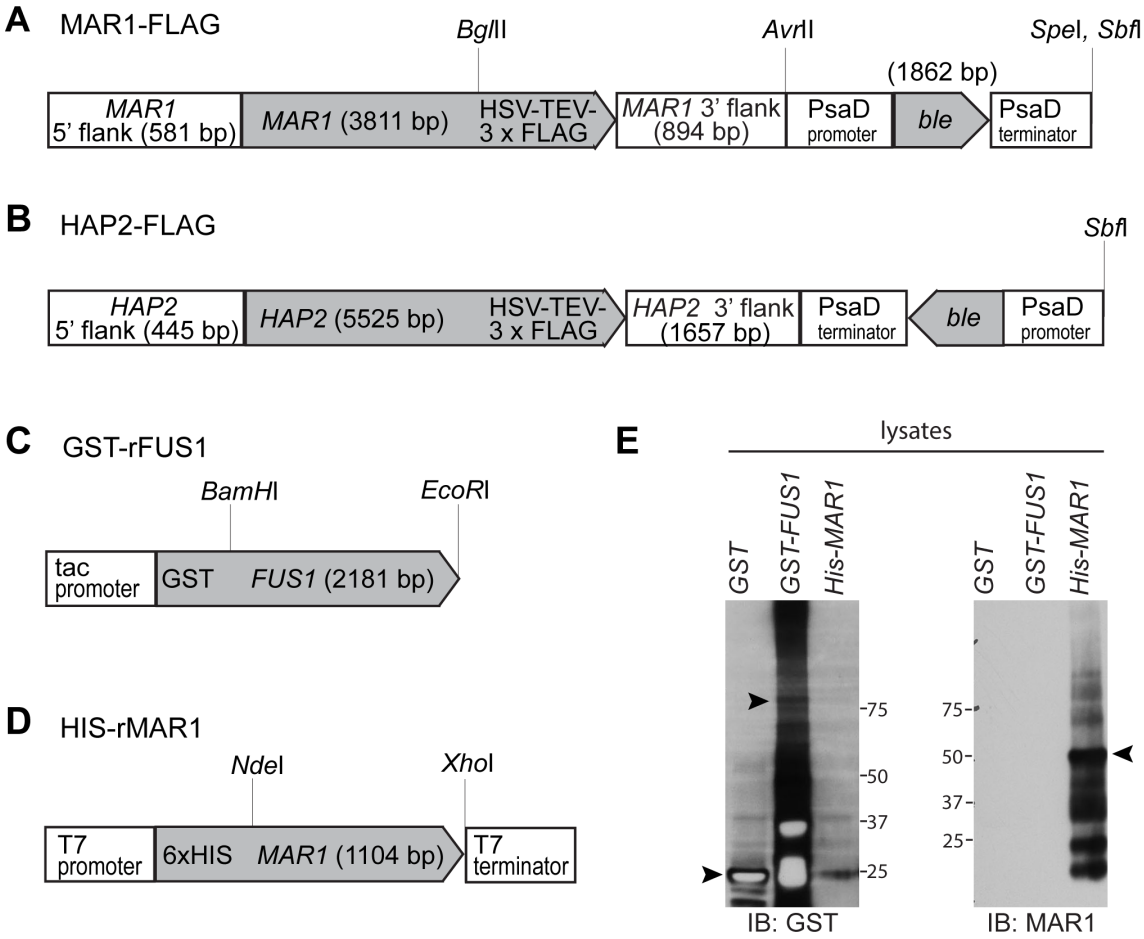


Supplemental Figure S4. MAR1-FLAG localization in unmixed and mixed (activated) *minus* gametes. Related to Figure 4. Confocal z-stack composite images of anti-FLAG immunostained *mar1; hap2; MAR1-FLAG; HAP2-HA*(-) gametes before (left panels), and after mix with *fus1(+)* gametes for 20 min to induce gamete activation (right panels). Top images show the fluorescence channel only and bottom images show fluorescence merged with DIC. No differences in the localization of MAR1-FLAG were detected between samples of unmixed (naive) and *fus1(+)*-mixed (activated) *minus* gametes.

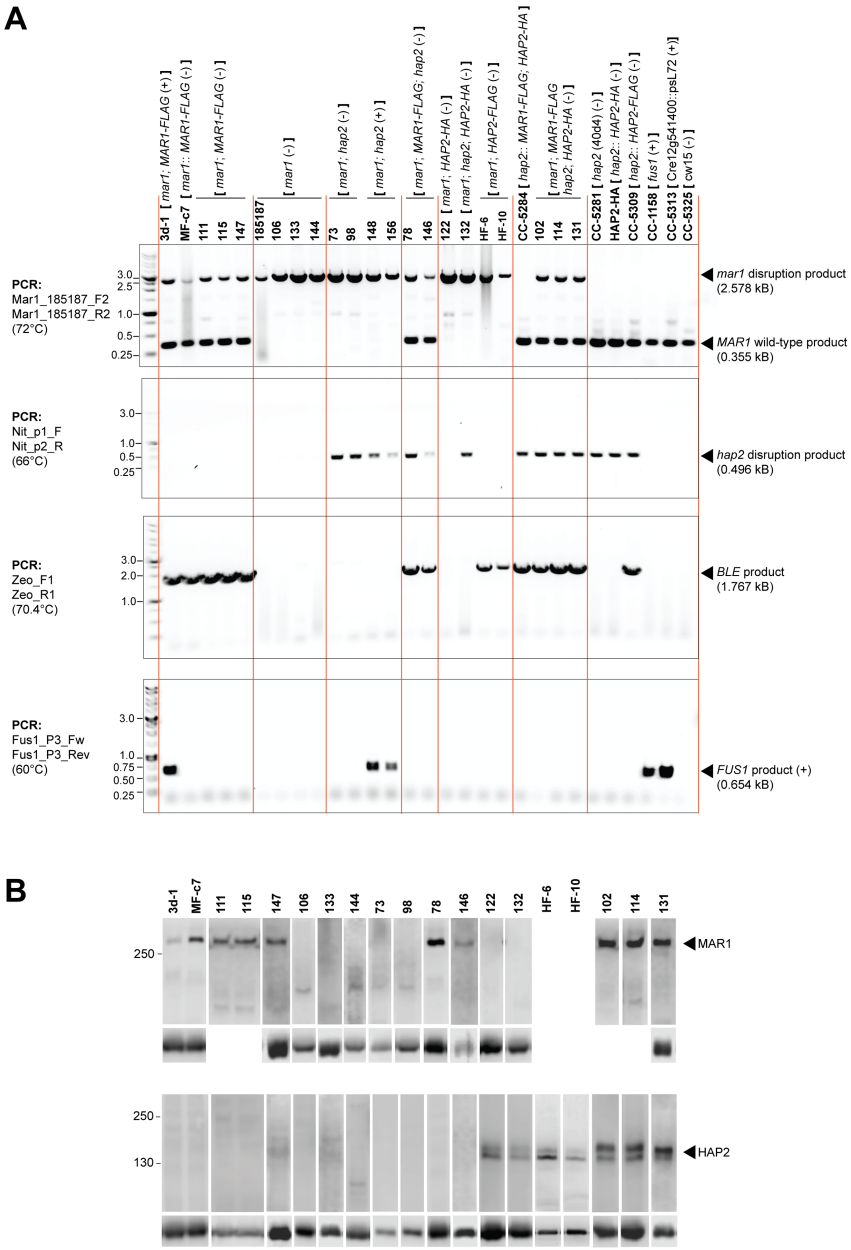
Supplemental Figure S5



Supplemental Figure S5. HAP2-HA expression in *mar1(-)* gametes. Related to Figure 5.
(A) Confirming results in Fig. 5F, wide field views of many gametes in merged bright field immunofluorescent confocal z-stack images show the broad mis-localization of HAP2-HA in *mar1(-)* gametes compared to the mating structure localization of HAP2-HA in positive control *hap2::HAP2-HA(-)* gametes (images were brightened to show differences in localization). Negative control WT*(+)* gametes (*21gr*) are shown on the left. **(B)** Trypsin treatment of live *mar1;HAP2-HA(-)* gametes and **(C)** of live *mar1::HAP2-FLAG(-)* gametes confirmed that the minimal amounts of the surface-expressed HAP2 isoform (upper band) in gametes lacking *MAR1* was present at the cell surface: *mar1(-)* gamete samples are shown in the left panels of (B) and (C). Immunoblots of trypsin-treated control samples of *hap2::HAP2-HA(-)* and *hap2::HAP2-FLAG(-)* gametes containing the wild-type *MAR1* gene are shown for comparison: *hap2(-)* gamete control samples are shown in the right panels of (B) and (C). **(D)** Quantification of the mean HAP2-HA immunofluorescent signal expressed in individual gamete cells with (n=104) and without (n=90) the wild-type *MAR1* gene. The mean signal intensity of *hap2(-)* gametes (n=76), which do not express HAP2-HA, served as a background signal. After subtracting the mean background signal from the means of both of the HAP2-HA expressing groups, the signal intensity of HAP2-HA in the *mar1* mutant gametes was found to be ~30% that of HAP2-HA in the wild-type *MAR1* gametes, which was a slightly greater reduction than that found with the immunoblot signal quantification shown in Fig. 5D. Results are displayed as a scatter plot of the mean HAP2-HA immunofluorescent signal from individual gamete cells and analyzed using one-way non-parametric ANOVA with Dunn's post-test; ****P<0.0001; grey bars are means.



Supplemental Data S3. Construction of plasmids and MAR1 antibody specificity tests. Related to STAR Methods. (A) The *MAR1-FLAG* plasmid was made by amplifying the *MAR1* gene (Cre03.g176961, 5981 bp) including its 581 bp 5' flanking promoter region and 1589 bp 3' flanking region using primers L8 and R5B, genomic DNA from *minus* strain *B215* as template, and the GC-rich PCR system (Roche). The PCR product was restricted at an *Avr*II site to truncate the 3' flanking termination region to 894 bp, and cloned into a *pGEM-T-easy* vector (Promega). An oligonucleotide encoding an HSV-TEV- 3 x FLAG epitope tag peptide (IDT) was added to the C-terminus of the *MAR1* gene using standard PCR weaving methods. The zeocin-resistance-encoding *ble* gene was inserted into the *MAR1-FLAG* plasmid at *Avr*II/*Spe*I sites using an In-Fusion Dry-Down PCR Cloning Kit (Takara). Primers L20 and G176961.R20 were used for amplification of the *ble* CDS and *PsaD* promoter and termination regions (1862 bp) using *pGenD-Ble* plasmid DNA as template; *Chlamydomonas* Resource Center (Fischer and Rochaix, 2001). **(B)** The *HAP2- FLAG* plasmid was made by inserting an oligonucleotide encoding tandemly arranged HSV-TEV- 3 x FLAG epitope tag into the C-terminus of the existing *HAP2* plasmid pYJ36 (Liu et al., 2008) using standard PCR weaving methods. The *ble* gene was inserted into the *HAP2-FLAG* plasmid at a *Spe*I & *Xba*I site using an Infusion Dry-down PCR Cloning Kit (Takara). Primers PGenD.s and PGenD.as were used for amplification of the *ble* CDS and *PsaD* promoter and termination regions using *pGenD-Ble* plasmid DNA as template. **(C)** The *GST-rFUS1* plasmid used to express the recombinant GST-tagged FUS1 ectodomain in B21 bacteria (NEB) was a pre-existing expression vector (Misamore et al., 2003) containing an oligonucleotide encoding residues 17 – 747 of the FUS1 ectodomain in a *pGEX-2T* vector (Amp^+) backbone. **(D)** The *HIS-rMAR1* plasmid used to express the recombinant His-tagged MAR1 ectodomain protein in BL21 bacteria was made by first amplifying an oligonucleotide encoding MAR1 residues 1 - 417 from *MAR1* cDNA using primers g176961-L3 and g176961-R10. This PCR product was cloned into a T-easy vector (Promega), and then a segment encoding residues 26 to 389 of the MAR1 ectodomain was further cloned by GenScript into the PET28a(+) vector (Kan^+) using *Nde*I & *Xho*I sites. **(E)** anti-MAR1 antibody (Yenzyme) specificity tests in lysates of induced *BL21/pGST, BL21/pHis-rMAR1* and *BL21/pGST-rFUS1* bacterial cells. Immunoblots were probed with MAR1 and GST antibody. Arrowheads show the recombinant MAR1, FUS1 and GST proteins.



Supplemental Data S4. Genotypic and phenotypic screening of strains. Related to STAR Methods.
(A) Images of DNA gel electrophoresis results from PCR screening of selected *Chlamydomonas* progeny obtained in crosses (Supplemental Figure S3). Genotypes are shown in the brackets above each progeny strain number (top). Strains previously deposited in the *Chlamydomonas* Resource Center are identified by their CC numbers. Ladder (kB), primers used in PCR, and annealing temperatures are shown on the left. Expected PCR product sizes (kB) and their identities are shown on the right. All primers used for this project (IDT) were stored as 100μM stock solutions, and diluted to 10μM working solutions prior to use. **(B)** Immunoblots showing the presence or absence of MAR1-FLAG, HAP2-HA and HAP2-FLAG proteins in naive gamete lysates from the strain number indicated (top) and tubulin loading control (below). For HAP2 blots, HAP2-FLAG was detected with anti-FLAG antibodies for samples from strains HF-6 and HF-10; HAP2-HA was detected with anti-HA antibodies for all other sample blots. For MAR1 blots, MAR1-FLAG was detected with anti-FLAG antibodies. Blots for progeny 111, 115, 102, and 114 were stripped and re-probed and the corresponding tubulin signal is shown below the HAP2 blot. Samples from *mar1*::*HAP2-FLAG(-)* strains were not screened for MAR1-FLAG expression.