

## Supplementary Materials for

Sex-linked deubiquitinase establishes uniparental transmission of chloroplast DNA

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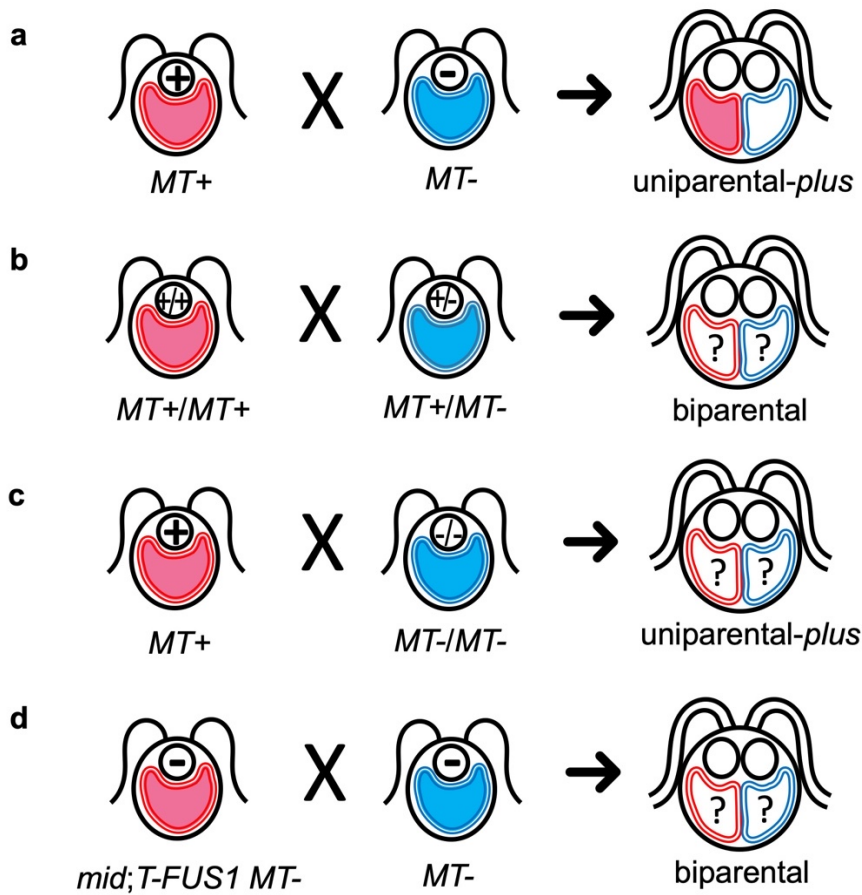
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Supplementary Figs. 1 to 13

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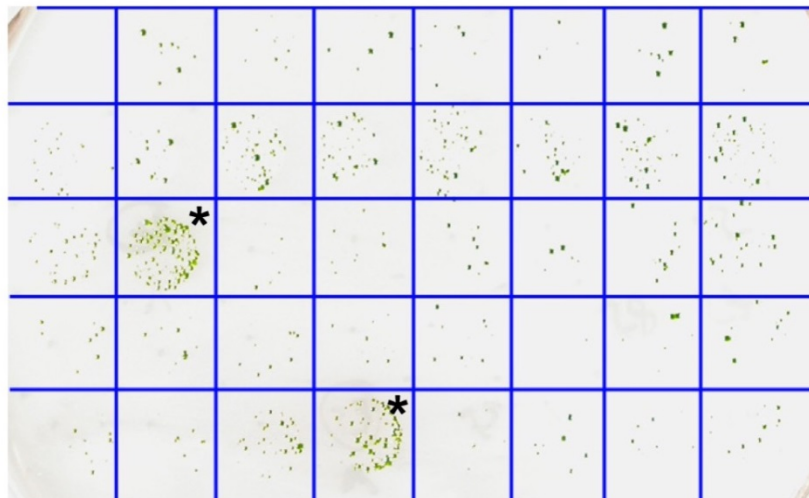
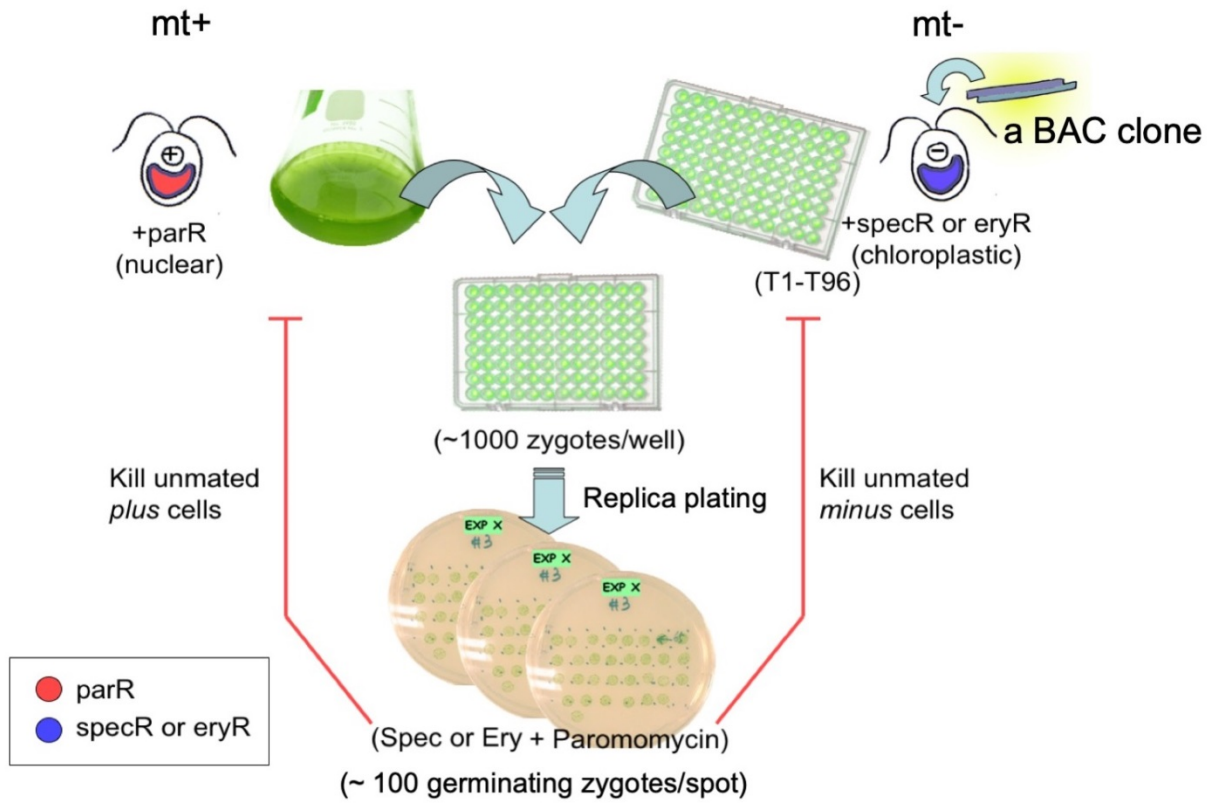
Supplementary References 1 to 9



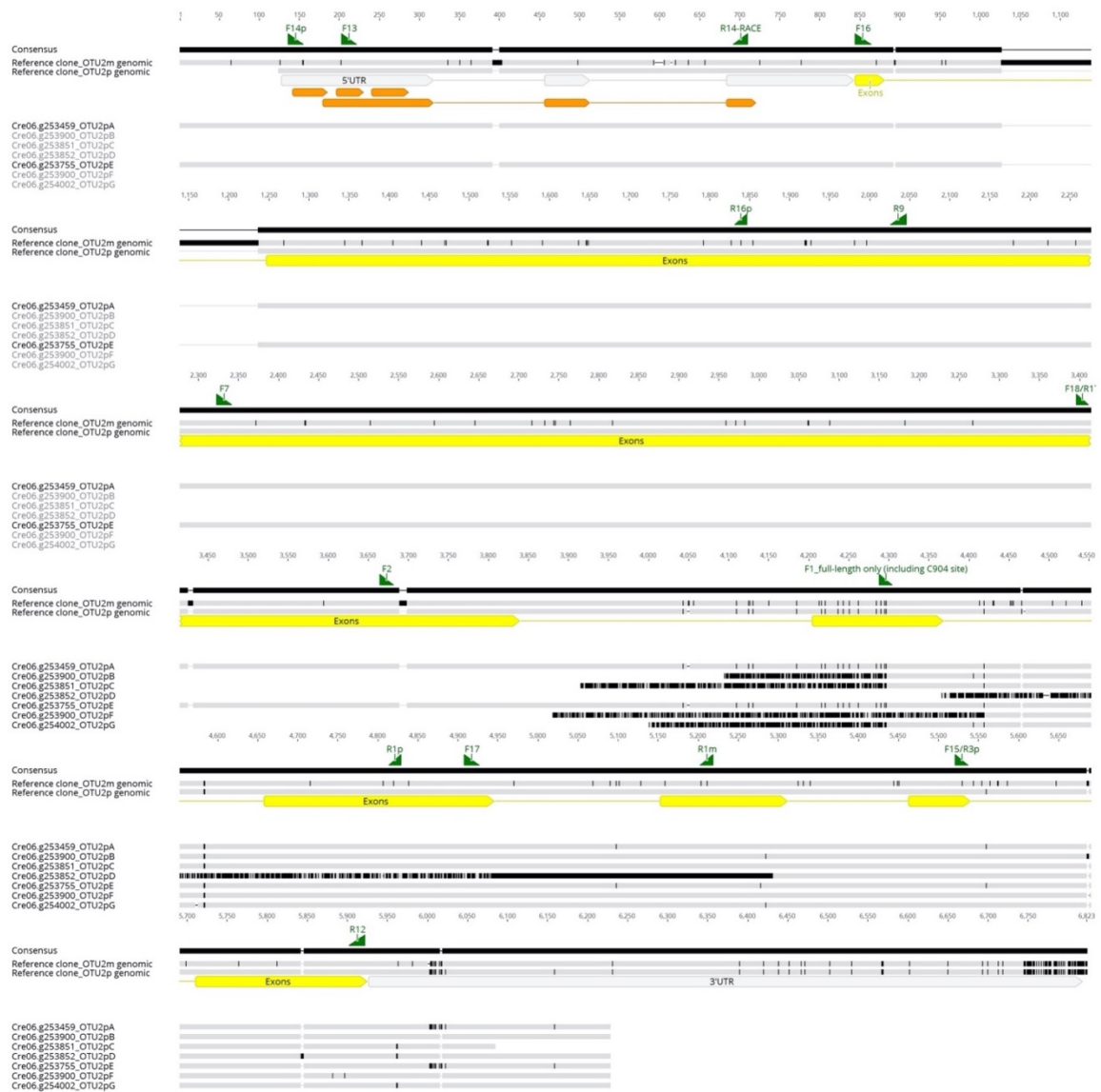
**Supplementary Figure 1.** The dominant control of  $MT^+$  locus for the uniparental inheritance of cpDNA in *C. reinhardtii*. The following genetic experiments postulate two functions genetically linked to  $MT^+$  for the uniparental inheritance of cpDNA: a “protector” function that protects cpDNA from degradation in zygotes and a “destroyer” function (elusive) that controls the onset of cpDNA degradation in early zygotes. **(a)** WT mating between *plus* and *minus* gametes produces uniparental progeny predominantly inheriting *plus*-derived cpDNA. *Minus*-derived cpDNA is completely degraded in zygotes (indicated by empty chloroplasts) (1). **(b)** Mating between  $MT^+/MT^+$  and  $MT^+/MT^-$  diploid gametes produces biparental progeny receiving a mixture of both cpDNAs (2), suggesting that the protection function is linked to  $MT^+$ . However, whether the *minus* cpDNA from  $MT^+/MT^-$  gametes escapes the zygotic degradation remains unknown (indicated by question marks in the zygotic chloroplasts). **(c)** Mating between haploid *plus* ( $MT^+$ ) and diploid *minus* homozygous at mating-type loci ( $MT^-/MT^-$ ) produces progeny predominantly receiving *plus*-contributed cpDNA, suggesting that an imbalance in the amount cpDNA cannot override the  $MT^+$ -dependent “protector” function (2). **(d)** Mating between WT *minus* gametes and *mid*-null  $MT^-$  mutants (thereby behaving as *plus*) harboring *T-FUS1* (allowing the fusion with WT *minus* gametes) produces biparental progeny (3). This phenotype has been explained by the  $MT^+$ -encoded “destroyer” function that is absent in  $MT^-$ .



**Supplementary Figure 2.** The gene content of the *OTU2p/EZY2* cluster in the R-domain of *MT+*. The *MT+* R-domain corresponds to 600–792.5 kb in chromosome 6 of v.236 genome at Phytozome (<https://phytozome-next.jgi.doe.gov>). At least seven copies of *OTU2p/EZY2*-containing 16-kb fragments were found, as reported earlier (3). Orange bars indicate the predicted locations of the three BAC clones used in this study. Gene annotations: green, *OTU2p*-A-F copies; magenta, *EZY2* copies; black, sequencing gaps; light blue, the repeating elements located between *OTU2p* and *EZY2* copies; brown, retrotransposon-like repeating elements; gray, genes outside the *OTU2p/EZY2* repeats.



**Supplementary Figure 3.** An experimental scheme for testing the “protector” function. Experimental details are found in Methods. Below the diagram is an example of the screening result, where two of 40 spots show an increase in the number of surviving zygotes (asterisks).



**Supplementary Figure 4.** *OTU2* gene structure and polymorphisms among the *OTU2* alleles. Reference *OTU2p* and *OTU2m* sequences were obtained from the BAC clone 33d13 and the lambda phage clone BJ2 (3), respectively. *OTU2p* and *OTU2m* exon-intron structures were confirmed by cDNA cloning. Nine exons were found with two introns in the 5'-UTR. The *OTU2p* 5'-UTR also contained four open reading frames (orange) that terminate before the ATG start codon of the *OTU2* main open reading frame (yellow). *OTU2p*-A/E copies contain a full-length gene structure, whereas *OTU2p*-B/C/D/F and -G copies are truncated by transposon-like repetitive sequences (indicated by dense black vertical lines between 3900 and 5300 bp), including only the upstream C-terminal segment. None of the truncated *OTU2p* copies include the Cys904 codon, essential for otubain catalysis. Sequences are shaded in gray, where black vertical lines indicate differences among *OTU2* copies. Primers used in this study are indicated by green wedges.

**a**

**Nt** (520)

```

MARKVRETHAALASAI EAAA RASAAA SAALLVPPEAHQLES LIRELQAA
RV GRAGGGNDGAWA SEGLRSGSNTY SRTATAATA STAAGDSYGYQE
VSTSTTDRNNAAC QYMYSSRSLEHAPAQPASPEHMYGLTSPSLAA
ASQRHASSRPGPAAPASSSSGLDYWEGAGPDPSASASASAASAGTAAVDEA
VHALRM REMQRLO QEAAAAARTPGAGGGGTVLGV GGFAREVWGHR
SQQQLAPAPYGG PLASSTSTLTYPWQREYVELYGGSCATAGGRTSNGSR
QQPAESVAIPQTVPASPEKTRPAAQYQASPSLNSAAYD GRSAGLYAAG
DPPATAYAAAPVAAATGQHLGGYSTLAGSSSGDGAGTVNGDYAAVRARLV
AHS SSSV YGSGIGSGYAG GSSSTAPAVAPAAV TVTSGGRSGSTVRL
RMQDAPPSTTAA TSLLSGAAGGGTDLQPQRSRRNHYSDILETAGRTSSR
ARTNGPTG YSA APQPART
  
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**Mid** (369)

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RDLSSSVSWRELEAEA EPAAPEAAAHAAA
TWPRGATSVSAVHATSTSRVRTVTQSAQEAPSP AYI PSL QQKPASRT
YDSRS AVPTAP ADSAARVQARFAGPAATNRDDNVSPAPPHASRAYPRG
RGGGDTGT SNGAEPAGAAAPAASPSLSPSPSPHRE HVSSQQLVADTDR
ALELYDEAQLQRAIQLSKQEARAALAAAGMTGLTDSSS RHRDGDKDG
GGDSLGPSSHPRPSSRLRSSP LLEHI RIGGATTPRRVAVAAAM SKAA
LQDDEVGRPGSVATPSRRRLGNQEAGA AASVSPRSTEVQAPPASQAA
AT EPGSSNSSGMLV SALAPLTALHQRYA LLPLLCA
  
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**Ct** (290)

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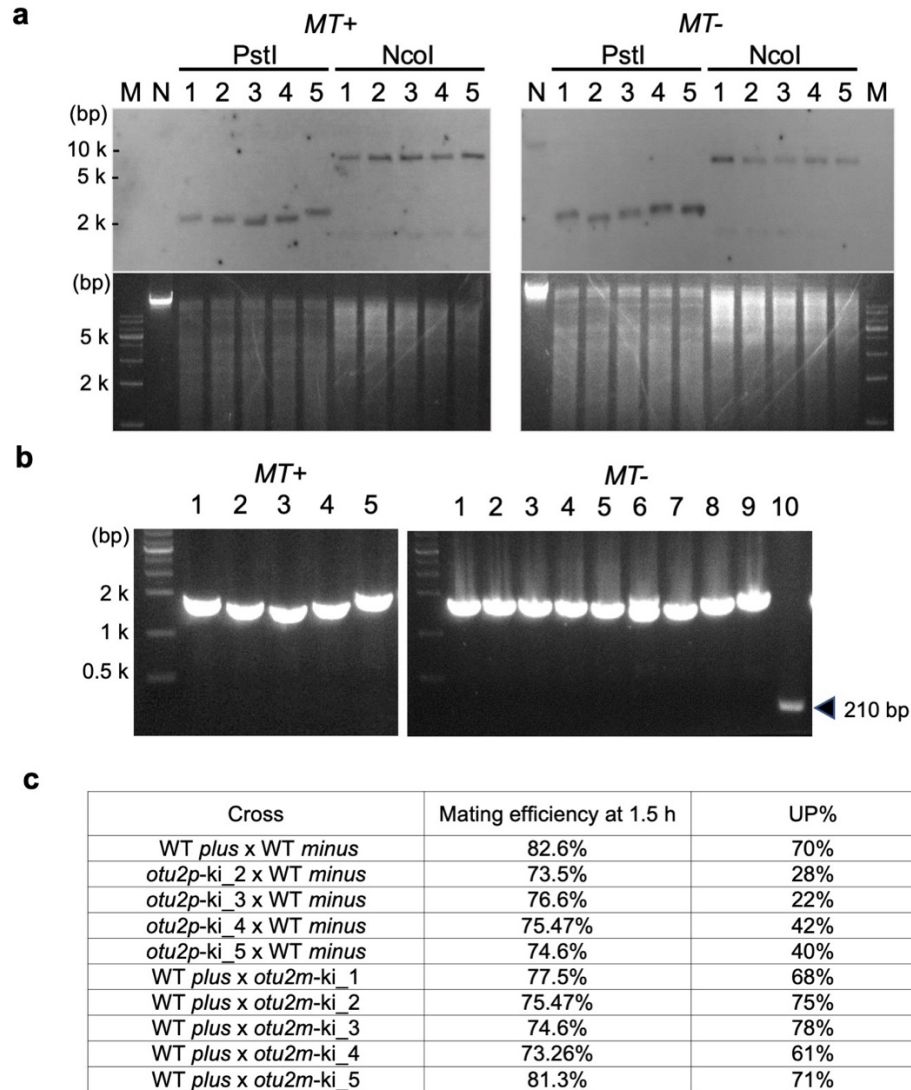
LVSL EDDFPIT
RRVAGDGN CFYRPF LFG LLEHVLAAPDPQLAERLDLVVSRHLARLDEPQF
QQQPPPTSPAANDGRGAGMSPLRLTPARGDPA APN ASPGQPAAAAAAA
GAQVPPRDDPAAYRGGQRLRLRLRTAWFKCPDAPRPSDV S SLERL FNS TE
QSGEVIRFARSLTARELMAAEEFYTFPIPGCGGDYTG LTLRQICERHVLP
MGVEVEQLQIIAACTALGVTLAVLDVAGSAVGAIKHGPAQQHGGPPVAWV
AHLPG HYDVITYR ARPLDVAPGGQLVAII*
  
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Charge changes ■  
 Radical changes ■  
 Similar changes ■ Otubain domain ■  
 Insertion/Deletion ■ Ub-interacting motif —

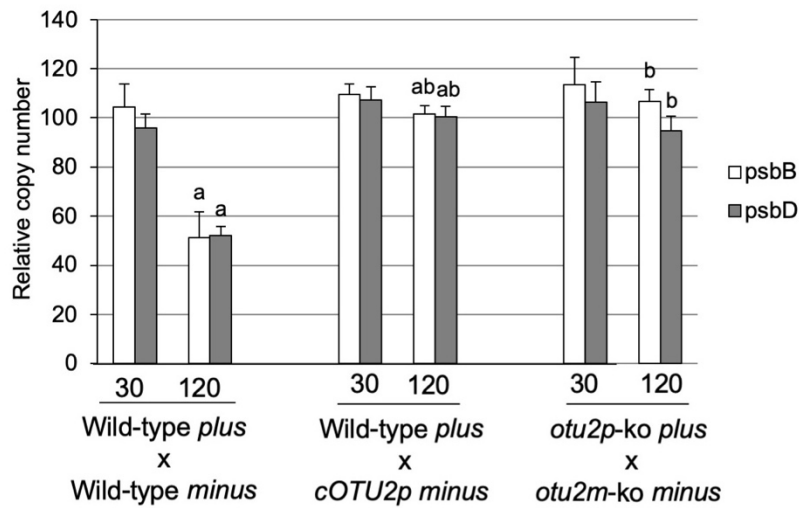
**b**

Changes	Charged	Radical	Similar
Nt	1.3%	1.7%	0.6%
Mid	<b>2.4%</b>	<b>1.9%</b>	0.0%
Ct	0.0%	1.0%	0.3%

**Supplementary Figure 5.** Protein sequence comparison between Otu2p and Otu2m. **(a)** A total of 39 changes were found in 1137 aa. Highlighted sequences indicate aa changes between Otu2p and Otu2m in charged, radical, similar, and insertion/deletion categories by navy, red, light blue, and yellow colors, respectively. Cys904, a critical cysteine for DUB activity, is marked in bold blue in the conserved OTU domain located at the beginning and end of Ct (light red shades). In total, 19, 16, and 4 aa positions differed between Otu2p and Otu2m in the N-terminal, middle, and C-terminal segments, respectively. **(b)** Summary of aa substitution rate per aa category in the three segments.



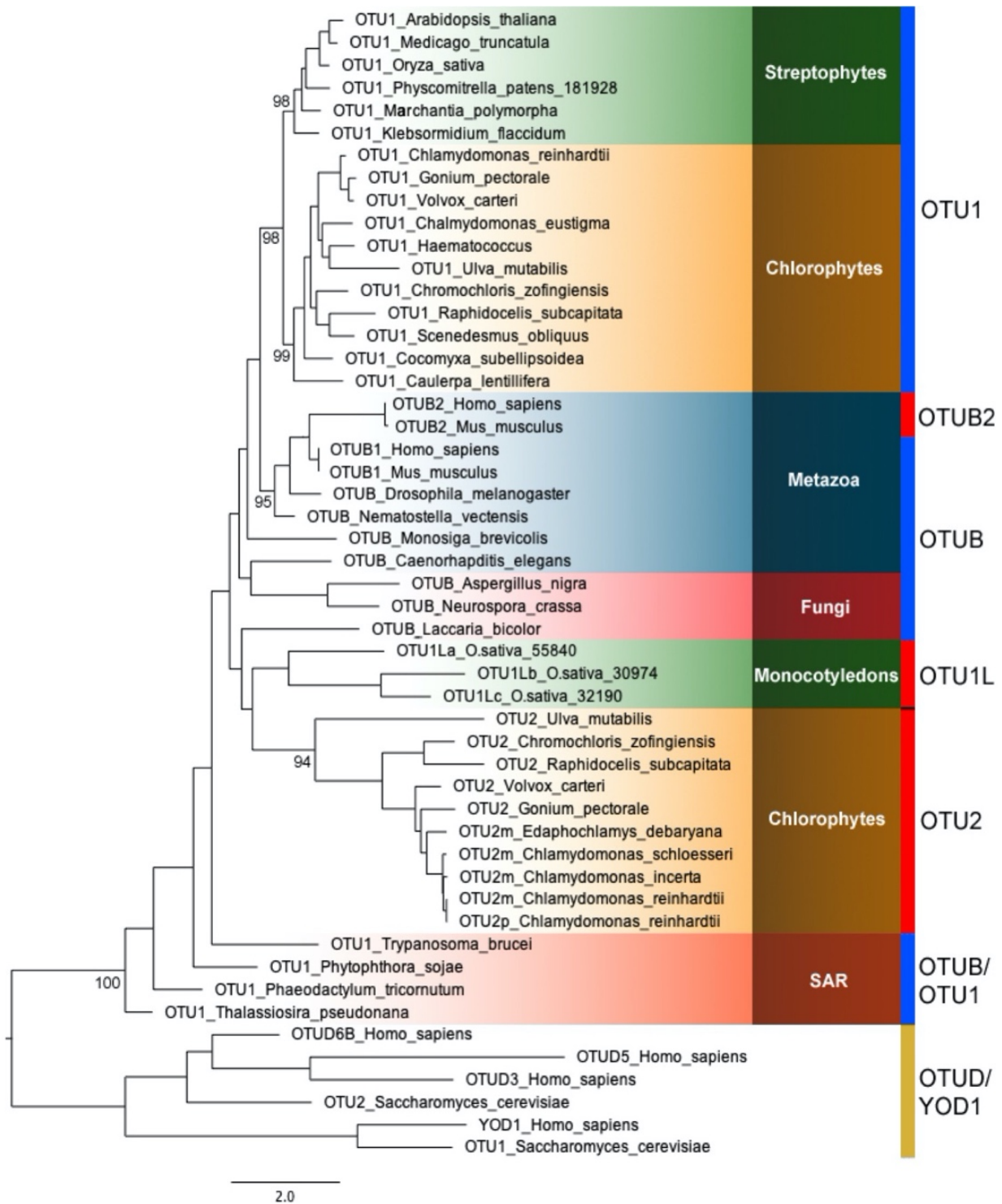
**Supplementary Figure 6.** CRISPR-Cas9-mediated generation of targeted knock-out mutants of *OTU2p* and *OTU2m*. **(a)** Southern blotting confirmed the single-locus insertion of *aphVII*, conferring hygromycin resistance. Wild-type gDNA did not hybridize with the *aphVII* probe. **(b)** gDNA amplification by PCR using the primer pair OTU2-ko-F1 and OTU2-ko-R1p/OTU2-ko-R1m (Supplementary Table 6), and sequencing confirmed insertion of a single copy of *aphVII* (1361 bp) at the 1-CCC/GAG-6 base in the targeted sequence in *otu2p* knock-out mutants #2–5 and all five *otu2m* knock-out mutants. Uninterrupted *OTU2p* or *OTU2m* copies should produce 210-bp products (#10 in *MT-* samples). The lack of 210-bp products indicates the absence of a full-length *OTU2* allele. *OTU2p* knock-out mutant #1 has an insertion at the last site of the targeted sequence (~ATC-23/GCT). The different band sizes are due to 5–101-bp deletions at the insertion sites. **(c)** The mating activities of *otu2p*-ko #2–#5 and *otu2m*-ko #1–#5 and selective cpDNA degradation in zygotes following mating with WT gametes were examined. All *otu2p*-ko mutants showed a significant reduction in the UP rate at 1.5 h after mating. For each sample, a total of 100 zygotes were examined.



		Wild-type 120 min	cOTU2p 120 min	otu2-ko 120 min
(a)	30 min zygotes	0.0005	0.0118	0.4338
(b)	wild-type 120 min	n.a.	0.0036	0.0018

**Supplementary Figure 7.** The lack of chloroplast DNA degradation in biparental zygotes. Changes in relative quantity between nuclear and chloroplastic DNA show that cpDNA mostly survive in biparental zygotes of *otu2p-ko* × *otu2m-ko* and wild-type *plus* × *cOTU2p minus*. gDNA was prepared from the zygotes at the indicated times. Quantitative PCR amplification of two chloroplastic loci (*psbB* and *psbD*) and one nuclear locus (*RCK2*). Relative copy numbers of *psbB* and *psbD* were calculated relative to *RCK2*. Experimental data represent the average ± s.d. from three biological replicates. Letters above the error bars indicate significant differences relative to samples at 30 min (a) or wild-type samples at the same time after mating (b) using student *t*-test (two-tailed,  $p < 0.05$ ). All *p*-values are provided in the table.

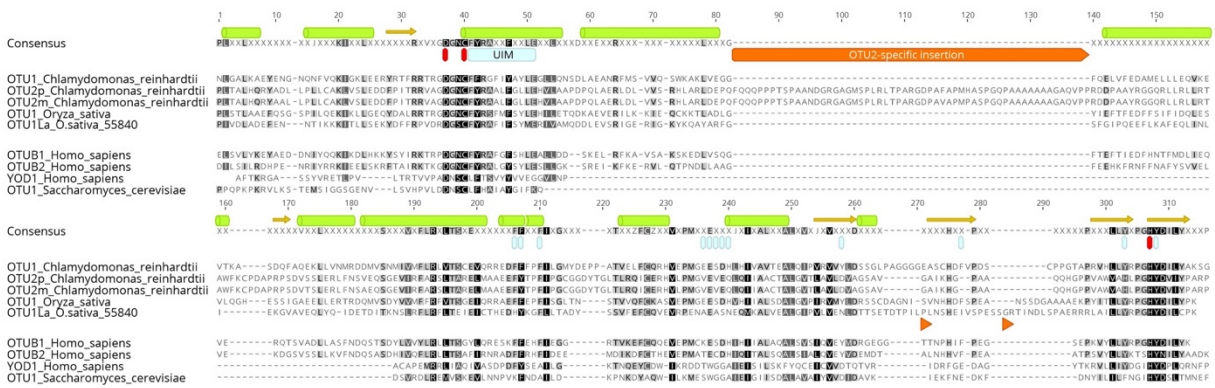




**Supplementary Figure 8.** Maximum likelihood phylogeny of OTU family DUBs. The HMMsearch identified a single member in the majority of genomes with three exceptions (red bars): mammals (OTUB1 and OTUB2), monocotyledonous plants (OTU1 and OTU1L), and Chlorophyceae, including *C. reinhardtii* (OTU1 and OTU2). Most single members formed a well-supported OTU1 clade for Viridiplantae and an OTUB clade for animals (blue bars). No

OTUB members were found in Rhodophyta and Glaucophyta. Single members in the SAR clade (Stramenopiles, Alveolates, and Rhizaria) and Euglenozoa were divergent. Outside the core-Reinhardtina lineage, *Chromochloris zofingiensis* and *Raphidocelis subcapitata* in the Sphaeropleales clade and *Ulva mutabilis* in the Ulvophyceae clade contained an OTU2 member, suggesting a deeper origin of OTU1/OTU2 diversification in Chlorophytes. The tree was reconstructed using IQ-TREE, based on the LG+I+G4 substitution model, and the bootstrap scores at the nodes were calculated by the Ultrafast method. Selected OTUD family members were included as outgroup sequences (yellow bar).

**a**



**Supplementary Figure 9.** Alignment of OTU domain aa sequences of the selected OTUB proteins. (a) Alignment of aa sequences for those depicted in Fig. 2a. OTU domains of OTU1 (aa 49–297Δ6), OTU2 (aa 866–1166), OsOTU1 (aa 33–274), OsOTU1La (aa 239–548Δ65), OTUB1 (human, 53–170 aa), OTUB2 (aa 14–234), YOD1 (human, aa 88–238, outgroup), and ScOTU1 (aa 41–232). Predicted alpha-helices and beta-sheets are indicated above as green rods and yellow arrows, respectively. The catalytic triad residues (D, C, and H) for cys-protease and ubiquitin-interacting residues are highlighted by red and light blue, respectively, below the consensus sequence. (b) Alignment of aa sequences of OTU domains used for the phylogeny in Supplementary Fig. 8 (pages 12–19).

b (1/8)

	1	10	20	30	40	50	60
OTU2p_Chlamydomonas_reinhardtii							
OTU2m_Chlamydomonas_reinhardtii	PLTALHQRYADL---	LPLLLCAKLV	SLEDDFPITRR	VAGDGNCFYRA	ALFGLLEHVLA	APD	
OTU2m_Chlamydomonas_incerta	PLTTLHQRYADL---	LPLLLCAKLV	SLEDDFPATRR	VAGDGNCFYRA	ALFGLLEHVLA	APD	
OTU2m_Chlamydomonas_schloesseri	PLNALHQRYADL---	LPLLLCAKLV	SLEDDFPITRR	VAGDGNCFYRA	ALFGLLEHVLA	APD	
OTU2m_Edaphochlamys_debaryana	PLQALHQRYAEP---	DPLLLCAKLV	SLEDDFPSVRR	VAGDGNCFYRA	CLFALLEHVLA	EPD	
OTU2_Volvox_carteri	PLTVLHQRYAQL---	DPLLSAKLV	SLEDDFPITRR	VAGDGNCFYRA	ALFAVLEHVLT	VPD	
OTU2_Gonium_pectorale	-----	-----	-----	-----	-----	-----	-----
OTU2_Chromochloris_zofingiensis	PLGQLIARYEDS---	SPLLAAKLLS	LEAEFPECRR	ICDGNCFYR	GFLFGILLEAL	LLEPN	
OTU2_Raphidocelis_subcapitata	PMSRLVERYRLS---	SPALAAARLA	ALAADFPTR	CRAVQDGNCF	YRGFVFALLE	ALLEQPH	
OTU2_Ulva_mutabilis	PIAHLISTCQH---	KPALKAKLE	MLLRHFSQYR	PILGDGNCFY	RSYAFGMIES	VLAWGQ	
OTUB1_Homo_sapiens	ELSVLYKEYAED---	DNIYQKIKD	LHKKYSYIRK	TRPDGNCFYR	AFGFSHLEAL	LDD--	
OTUB1_Mus_musculus	ELSVLYKEYAED---	DNIYQKIKD	LHKKYSYIRK	TRPDGNCFYR	AFGFSHLEAL	LDD--	
OTUB_Drosophila_melanogaster	PLTCLYAEYSG---	DEIFTAKIQ	DLSKKYKFR	RRTRPDGNCF	FRFAFYSYLE	YLISN--	
OTUB_Caenorhapditiis_elegans	PFSILCAEYDNET---	SAAFLSKATE	LSEVYGEIRY	IRGDGNCFYR	AILVGLIEIM	LKD--	
OTUB_Nematostella_vectensis	-----	-----	-----	-----	-----	-----	-----
OTUB2_Homo_sapiens	DILSILRDHPE---	NRIYRRKIE	ELSKRFTAIR	KTKDGNCFYR	ALGYSYLES	LGLK--	
OTUB2_Mus_musculus	DILSILRDHPE---	NRIYRQKIQ	ELSKRFTSIR	KTKDGNCFYR	ALGYSYLES	LGLK--	
OTUB_Monosiga_brevicollis	GIDVLLAEYP---	DPLFHTKLE	TLQRRQASFR	RRTRDGNCFY	RSYAFGILEH	LQLH--	
OTU1_Arabidopsis_thaliana	PLSSLAAYQSG---	SPILEKIKI	LDSQYIGIR	RRTRDGNCF	FRSFMFSYLE	HILESQD	
OTU1_Medicago_tranculata	PLSLLAAEYQSG---	SPILEKIKI	KVLDGQYAA	IRRRTRDGNCF	FRSFMFSYLE	HILEAQD	
OTU1_Oryza_sativa	PLSTLAAEFQSG---	SPILEKIKI	LGEQYDALR	RRTRDGNCFY	RSFMFSYLE	HILETQD	
OTU1La_O.sativa_55840	PIVDLADEFEN---	NTIKKKITL	LSEKYDFFR	PVDRDGS	CFYRAFI	FYSYMERIV	AMQD
OTU1Lb_O.sativa_30974	ANHYFRNIDNSFQ---	QPEVGLRL	KYLSHHYSE	FRPVHRDEE	CFYRSFI	FYSYLEQV	VDSIG
OTU1Lc_O.sativa_32190	PLASAPEHYGLR	SGLRRIMSAN	LILLNEYSDF	FRP-----	-----	-----	EQVLD
OTU1_Marchantia_polymorpha	PLSVLAAEYQSG---	NPVFRAKV	EKLGQTYVA	IRRRARGDGN	CFRFSFMFSY	LEHILETRD	
OTU1_Physcomitrella_patens_181928	PLSVLEEEYKAG---	SRVFLAKL	QLLQKQYGA	IRRRARGDGN	CFRFSFMFSY	LEHLLVTQD	
OTU1_Klebsormidium_flaccidum	PLSALAAEYERG---	NEVFKAKI	QKLGETYGS	IRRRTRDGN	CFRFGFMFAY	LERLLHSQD	
OTU1_Haematococcus_pluvialis	DLSVLKAEYEAG---	SDVWVRKI	EWLEHKF	SHFRTRDGN	CFYRAFI	FYSYLEG	LLQNSD
OTU1_Chlamydomonas_reinhardtii	NLGALKAEYENG---	NQNFVQKI	GKLEERYR	FRTRDGNCF	FRGFYAYLE	GLLQNSD	
OTU1_Gonium_pectorale	PMSALKEEYING---	NQNFVQKI	AKLEASYST	FRTRDGNCF	FRGFYYSY	LESLLARD	
OTU1_Volvox_carteri	PMSALKEEYING---	NQNFVQKI	GKLEASYGT	FRTRDGNCF	FRGFYAYLE	NLLTHD	
OTU1_Chlamydomonas_eustigma	DMSCLKTEYQNG---	NFVFLKKI	DLSLQATYSS	YRTRDGNCFY	RAFLRYLES	LLEDDEN	
OTU1_Chromochloris_zofingiensis	PLASLKAEYSAG---	SDVFIKIE	LLERDFTC	MRTRDGNCFY	RSFIYAYLE	WLLATDN	
OTU1_Raphidocelis_subcapitata	TLESMLQDYRA---	YPVFAKIR	SLASRYGG	IRRVSDGNCF	FRATAFGLLE	WLLVNEA	
OTU1_Scenedesmus_obliquus	SLDALKAEYANG---	SQVFVAKI	SNLQTYSS	MRTRDGNCF	FRSFIYGY	LEWLLHQ	
OTU1_Ulva_mutabilis	QVLSLREEYQDS---	SSTFVAKV	EYLCSDYTF	IRTRDGNCFY	RAFLYGLLE	ALLVTRA	
OTU1_Caulerpa_lentillifera	-----	-----	-----	-----	-----	-----	-----
OTU1_Cocomyxa_subellipsoidea	PLSALEEEYRQ---	SAVFRDKI	RSLETQYGS	MRTRDGNCF	FRSFMFAFME	QLVQNN	
OTU1_Thalassiosira_pseudonana	PIDALVAQYSSSN---	DGGFLQSA	NLLSQYTH	LRIRGDGNCFY	RAFLYLS	SEDNSKD--	
OTU1_Phaeodactylum_tricornutum	PILDCLKLEYNESN---	ESRFLDGV	DALSKEYQS	FRKVRDGNCFY	RAFLYRLVEE	IRQN--	
OTU1_Phytophthora_sojae	SPLCLKAEYVGNV---	NANFMHGIE	SLNARYEAL	RRVRDGNCF	FRGFIFALCER	LLPDS	
OTU1_Trypanosoma_brucei	DGCNLMTEFSD---	NPPMLVKV	VVSLKTYK	IGIRYARRDGN	CFYRSVVF	GMHESLLN	NKE
OTU1_Laccaria_bicolor	PMSNLREEYENG---	SAAFVNQI	DLSLTARY	DSVRRTRDGN	CFYRS	LAFAYVERI	LQSPE
OTU1_Aspergillus_nigra	SSSVIAMEYANA---	DPAFATKS	NALALTHP	WTRTMKGDGN	CGWRAVAF	GFENLL-L	QNL
OTU1_Neurospora_crassa	PSTAITEEYAKA---	DPVYVQKT	QSLP	PVYSHYR	PMLDGNCG	WRAIGFGY	FETLIK
YOD1_Homo_sapiens	---AFTKRGA---	SSYVRET	LPV---	LTRTVVPAD	NSCLFTSV	YVVVEGG	VLPN--
OTU1_Saccharomyces_cerevisiae	PPQPKPKRVLS---	TEMSIGGS	GENV---	LSVHPVLD	DNCLFHA	IAYGIF	FKQ---
OTUD6B_Homo_sapiens	EIENLTGARHME---	SEKLAQI	LAAARQ---	LEIKQIP	SDGHCMYKA	IEDQLKE	KDCA---
OTU2_Saccharomyces_cerevisiae	ALEASKQPD	LKMM---	EQESIDQ	LCELKK---	LKQFDI	QPDGH	CLFASILD
OTUD5_Homo_sapiens	IEAMD	PATVE	QQ---	EHWF	EKALR	DKK---	FIKQ
OTUD3_Homo_sapiens	PESGGGGG	CEEE---	FVSFAN	QLQALG---	LKLE	REVP	GDGNCL

**b (2/8)**

OTU2p\_Chlamydomonas\_reinhardtii ---PQLAERLDL-VVS-RHLARLDEPQFQQQP---PPTSP-AANDGRGAGMSPLRLTPAR  
OTU2m\_Chlamydomonas\_reinhardtii ---PQLAERLDL-VVS-RHLARLDEPQFQQQP---PPTSP-AANDGRGAGMSPLRLTPAR  
OTU2m\_Chlamydomonas\_incerta ---PKLAERLDL-VVS-RHLARLDEPQFQQQQQQQHPSPGPASDGRGAGVSPLR--SAR  
OTU2m\_Chlamydomonas\_schloesseri ---PQLAERLDL-VVS-RHLAQLDEPQFQQQQQQQPGSSP-AAGDARGAGLSPLR--SAR  
OTU2m\_Edaphochlamys\_debaryana ---PPLGARLEA-AFA-AHLRTLDEPPAASAT---TAAA--APAAGSAGGATSPR--TPR  
OTU2\_Volvox\_carteri AV-LPNRSRLHELRLLE-HLVGRYVRKLEALQDVGGVPQG--GGDEGAGGGRSRSR--AAT  
OTU2\_Gonium\_pectorale ---PSAPYSLPGDSLPG-SGARREAVRPE-----PAP---GAASAASGSRSAAR-----  
OTU2\_Chromochloris\_zofingiensis ---LTLYARLRA-SIE-HTWLEMRHAM-----  
OTU2\_Raphidocelis\_subcapitata ---VELHARLLQRVQEAWLARTRLP-----  
OTU2\_Ulva\_mutabilis RF-KLKSCTVAA-QTW-VWKAQALDIP-----  
OTUB1\_Homo\_sapiens ---SKEL-RFKA-VSA-KSKEDLVSQG-----  
OTUB1\_Mus\_musculus ---SKELQRFKA-VSA-KSKEDLVSQG-----  
OTUB\_Drosophila\_melanogaster ---TSAY-EFKK-LAE-ESKEKLVQLG-----  
OTUB\_Caenorhaptiditis\_elegans ---RARLEKFIA-SSR-DWTRTLVELG-----  
OTUB\_Nematostella\_vectensis ---EKEYQRFKD-LAA-RSKDELVELN-----  
OTUB2\_Homo\_sapiens ---SREI-KFKE-RVL-QTPNDLLAAG-----  
OTUB2\_Mus\_musculus ---SREI-KFKE-RVL-QTPNDLLAAG-----  
OTUB\_Monosiga\_brevicolis ---KDAIDGFL-RLR-ETYEMALAAG-----  
OTU1\_Arabidopsis\_thaliana ---RAEVDRIKV-NVE-KCRKTLQNLG-----  
OTU1\_Medicago\_trunculata ---SAEIDRIKA-NVE-RSRKALQTLG-----  
OTU1\_Oryza\_sativa ---KAEVERILK-KIE-QCKKTLADLG-----  
OTU1La\_O.sativa\_55840 ---DLEVSRIGE-RIG-KYKQAYARFG-----  
OTU1Lb\_O.sativa\_30974 ---TREDRLLA-AVR-ALATKAENLQ-----  
OTU1Lc\_O.sativa\_32190 ---TDEENRLLA-AVG-AIDHRQ-----  
OTU1\_Marchantia\_polymorpha ---ETEVKRIQK-NIE-QSKKALIELG-----  
OTU1\_Physcomitrella\_patens\_181928 ---VAEVARMEK-AIE-ICKKTLIDHG-----  
OTU1\_Klebsormidium\_flaccidum ---KTELPRMRD-RIE-RCKQELIAIG-----  
OTU1\_Haematococcus\_pluvialis ---TQETKRLAE-VLQ-GWRSKLVEAG-----  
OTU1\_Chlamydomonas\_reinhardtii ---LAEANRFMS-VVQ-SWKAKLVEGG-----  
OTU1\_Gonium\_pectorale ---LTECDRIKS-VIQ-EWKGKLVDDG-----  
OTU1\_Volvox\_carteri ---LPECSRFSMS-VVQ-AWKTRLVEGG-----  
OTU1\_Chlamydomonas\_eustigma ---IPECRRFIE-CIN-GWRQKLVEAH-----  
OTU1\_Chromochloris\_zofingiensis ---EQQCQRFSN-CVG-GWRQKLIDAG-----  
OTU1\_Raphidocelis\_subcapitata ---DSECQNFLR-TLE-ACKDRMVGAG-----  
OTU1\_Scenedesmus\_obliquus ---EQECQRFTK-CLQ-GWKQKIQDIG-----  
OTU1\_Ulva\_mutabilis ---VEATQAML-MLM-PLRQMLLDAG-----  
OTU1\_Caulerpa\_lentillifera ---QQNQLRMKD-HME-TCKKKLLDAG-----  
OTU1\_Cocomyxa\_subellipsoidea ---LSERNRVVT-CIR-QWKAKLVGVG-----  
OTU1\_Thalassiosira\_pseudonana ---DGEFERIKQ-LVS-DSLLWVCKFG-----  
OTU1\_Phaeodactylum\_tricornutum ---EKDSEKIID-WLKTWSWESVLAAG-----  
OTU1\_Phytophthora\_sojae GG-AALRGRIQH-KIQ-QSKSELVAIG-----  
OTU1\_Trypanosoma\_brucei RA-GAHLERITD----LSRQVVAD-----  
OTU1\_Laccaria\_bicolor P--DFAVASSVS-ALA-ATRDSLKAG-----  
OTU1\_Aspergillus\_nigra ---LMQVRHELA-RIK-SMNALLDQGT-----  
OTU1\_Neurospora\_crassa ---KAQVDGERE-RLE-SLNEYIQHHG-----  
YOD1\_Homo\_sapiens -----  
OTU1\_Saccharomyces\_cerevisiae -----  
OTUD6B\_Homo\_sapiens -----  
OTU2\_Saccharomyces\_cerevisiae -----  
OTUD5\_Homo\_sapiens -----  
OTUD3\_Homo\_sapiens -----

**b (3/8)**

OTU2p_Chlamydomonas_reinhardtii	GDPAFAPMHASPGQPAAAAAAGAQQVPPRDDPAAYRGGQRLRLRLRTAWFKCPDA-----
OTU2m_Chlamydomonas_reinhardtii	GDPAVAPMPASPGQPAAAAAAGAQQVPPRDDPAAYRGGQRLRLRLRTAWFKCPDA-----
OTU2m_Chlamydomonas_incerta	GDPAGTLAPASPGQPGEGAAA--AHIPPRDDPAAYRGGQRLRLRLRTAWFKCPDA-----
OTU2m_Chlamydomonas_schloesseri	GDTVGS-TPASPSRRESTAA--AQVPPRDDPAAYRGGQRLRLRLRTAWFKCPDA-----
OTU2m_Edaphochlamys_debaryana	T-----SPRAAGNGGTASAD--GPLPPSQDAAAYNGRLLRLRLRRRAWFKAPDA-----
OTU2_Volvox_carteri	PE-----RGGRSDSGGGGGGG--GGGGRSVDQVALRGGKRLRLRLRTAWFKAPDA-----
OTU2_Gonium_pectorale	-----PSSSSSATRLRQRSEPDAGARAAVPRGPD-----
OTU2_Chromochloris_zofingiensis	-----QVDADTERGFNVLANFLSRTWYVPAAQHADSR-----
OTU2_Raphidocelis_subcapitata	-----NPEVERGALLTRLLARVWYAPGAAGSVEAAAA-----
OTU2_Ulva_mutabilis	-----GHASFKARSDKLLKGYVKFRELKQDQVERA-----
OTUB1_Homo_sapiens	-----FTEFTIEDFHNTFMDLIEQVE-----
OTUB1_Mus_musculus	-----FTEFTIEDFHNTFMDLIEQVE-----
OTUB_Drosophila_melanogaster	-----FPSFTLEDFHETFMVILQVRVSPDNAG-----
OTUB_Caenorhaptiditis_elegans	-----FPDWTCTDFCFFIEFLEKIH-----
OTUB_Nematostella_vectensis	-----FPAFTIEDFHEVFMEVLDMMG-----
OTUB2_Homo_sapiens	-----FEEHKFRNFFNAFYSVVELVE-----
OTUB2_Mus_musculus	-----FEEHKFRNFFNAFYSVVELVE-----
OTUB_Monosiga_brevicollis	-----CPEFTTEDFYETVADMVTDLK-----
OTU1_Arabidopsis_thaliana	-----YTDFTFEDFFALFLEQLDDIILQGT-----
OTU1_Medicago_tranculata	-----YALTFEDFFTLFLEQLLEDVIGK-----
OTU1_Oryza_sativa	-----YIEFTFEDFFSIFIDQLESVLQGH-----
OTU1La_O.sativa_55840	-----SFGIPQEEFLKAFEQLINLI-----
OTU1Lb_O.sativa_30974	-----WASE-FSQKHKAFAERLEKIKGWKRMQEH---
OTU1Lc_O.sativa_32190	-----WASG-FSQSHKVFELIQNVMRWKRKQKVA-
OTU1_Marchantia_polymorpha	-----YQEFTFEDFLSIFVEQLDSVLPGR-----
OTU1_Physcomitrella_patens_181928	-----FAEFTFEDFLAIFVEQLQSVIQDK-----
OTU1_Klebsormidium_flacidum	-----YTPTFEDFLEIFMDQLESVLPPE-----
OTU1_Haematococcus_pluvialis	-----FQDLVFSDALDILLEQLNSIS-----
OTU1_Chlamydomonas_reinhardtii	-----FQELVFEDAMELLELQVKEVTKA-----
OTU1_Gonium_pectorale	-----FQALVFEDAMDVLLEQVAEISKP-----
OTU1_Volvox_carteri	-----FQELVFEDAMDVLLDQVKEITKP-----
OTU1_Chlamydomonas_eustigma	-----FQELVFEDAMELLELQVSTIGNN-----
OTU1_Chromochloris_zofingiensis	-----FQELVFEDAMDILQNMVNSISLGPQV-----
OTU1_Raphidocelis_subcapitata	-----FDPLVFEDALDMLAGMVNAICVGASVP-----
OTU1_Scenedesmus_obliquus	-----YDSIVFEDPLDMLLQQVQAILIGPGV-----
OTU1_Ulva_mutabilis	-----FMELVFHAMEVLEDDLKAVANT-----
OTU1_Caulerpa_lentillifera	-----FQELVFEDALAILQQRVEQIGT-----
OTU1_Cocomyxa_subellipsoidea	-----FQEMVFEDAMEIVIDQLNSLGT-----
OTU1_Thalassiosira_pseudonana	-----YDEITVDMFYELVDLLEFIESH-----
OTU1_Phaeodactylum_tricornutum	-----YDEVMLEAFHDTIVELLERILA-----
OTU1_Phytophthora_sojae	-----YSDVAIDAFWETFVDYLAAME-----
OTU1_Trypanosoma_brucei	-----YGNF-AEDFCHPAVEMAKKIES-----
OTU1_Laccaria_bicolor	-----IDKIVYEDIYDEFVGLISTIVQPNSA-----
OTU1_Aspergillus_nigra	-----QQEHLYEIFVDATEDVFIQIATAIQ-----
OTU1_Neurospora_crassa	-----GFDPMVFVDVFEETDELLKRISDKLN-----
YOD1_Homo_sapiens	-----
OTU1_Saccharomyces_cerevisiae	-----
OTUD6B_Homo_sapiens	-----
OTU2_Saccharomyces_cerevisiae	-----
OTUD5_Homo_sapiens	-----
OTUD3_Homo_sapiens	-----

b (4/8)

OTU2p\_Chlamydomonas\_reinhardtii -----PRPSDVSSLERLFNSVEQSGEVIRFARSLTARELMAAEFFYTPFIPGCG  
OTU2m\_Chlamydomonas\_reinhardtii -----PRPSDVTSLERLFNSAEQSGEVIRFARSLTARELMAAEFFYTPFIPGCG  
OTU2m\_Chlamydomonas\_incerta -----PRPSDVSSLERLFNSAEQSGEVIRFARSLTARELMAAEFFYTPFIPGCG  
OTU2m\_Chlamydomonas\_schloesseri -----PRPSDVSSLERLLNSAEQSGEVIRFARSLTARELMAAEFFYTPFIPGCG  
OTU2m\_Edaphochlamys\_debaryana -----PAPTDLASLEALLNAPGDSGDVIRFARSLTVRELLRAEDFYAPFIPGCG  
OTU2\_Volvox\_carteri -----PEPSDVP SRKRLFNSSAASSEVIRFARSLTVYELVSDAEFYAPFIPGCG  
OTU2\_Gonium\_pectorale -----DPSDDLSSLERLLNSGSASAEVIRFARSLTVAELMAGEEFYAPYIPGCG  
OTU2\_Chromochloris\_zofingiensis LPAAAQQEEG-NGPLYLPELEAALSSRDIFDPLVKFARSATSWEKLSNEQMYAPFIAGCC  
OTU2\_Raphidocelis\_subcapitata ARAGAGASTG-AGPLFLPELEAALACRDAFDPLVAFARAATSLELRANEAYAPFLAGCG  
OTU2\_Ulva\_mutabilis -----GENVDPMPLVLEFFQGVMGNSVMHLIRQLAAYEMLAHEDQYATFLPAIE  
OTUB1\_Homo\_sapiens -----KQTSVADLLASFNDQSTSDYLVVYLRLLTSGYLQRESKFFEHFIEGG-  
OTUB1\_Mus\_musculus -----KQTSVADLLASFNDQSTSDYLVVYLRLLTSGYLQRESKFFEHFIEGG-  
OTUB\_Drosophila\_melanogaster -----GHSTVQDELHKIFNEQGYSDYVVYLRLLTSGKLEQEEADFYQNFIEGD-  
OTUB\_Caenorhaptiditis\_elegans -----SGVHTEAVYTI LNDDGSANYILMFRLITSAFLKQNSEEYAPFIDEG-  
OTUB\_Nematostella\_vectensis -----NNATEDQLIQKFQDEGISNYLVVYLRLLTSAQLQRNAVFFENFIEGE-  
OTUB2\_Homo\_sapiens -----KDGSVSLLKVFNDQSASDHIVQFLRLLTSAFIRNRADFFRHFIDE-  
OTUB2\_Mus\_musculus -----KDSSVSSLLKVFNDQSSSDRIVQFLRLLTSAFIRNRADFFRHFIDE-  
OTUB\_Monosiga\_brevicollis -----ADEASLTTLAAVNDEGLSNYLVAVMRIITSAYMQLNADYFQPFIEGG-  
OTU1\_Arabidopsis\_thaliana -----EESISYDELVNRSRDQSVSDYIVMFFRFVTSAGDIRTRADFFPFITGLS  
OTU1\_Medicago\_trunculata -----ETSISHEELVLRSDQSVSDYVMMFFRFVTSAEIQKRSEFFPFIMGLT  
OTU1\_Oryza\_sativa -----ESSIGAEELLETRDQMVSDYVMMFFRFVTSGEIQRAEFPFISGLT  
OTU1La\_O.sativa\_55840 -----EKGVAVEQLYQ-IDETDITKNSLRFRLFLTEIEICTHEDHYKGFLLTAD  
OTU1Lb\_O.sativa\_30974 -----PISIIIRKILEFFSSYDITDDIFAFRLVAATWCTRIWNYEWCATNCG  
OTU1Lc\_O.sativa\_32190 -----SADSRKQLEFFSSYSKSDGILAFKLYAAANWICSHREEYEPNIAGLG  
OTU1\_Marchantia\_polymorpha -----ESSVDIDQLVEKCRDRYISDYVMMFFRFVTSAEIRKRAEFPFISGLS  
OTU1\_Physcomitrella\_patens\_181928 -----EVSVSLETLVERCRDQYISNSVMMFFRFVTSGEIGRRTEFFPFIQGMS  
OTU1\_Klebsormidium\_flaccidum -----GQPI SLSELEVRCDPNSVNWAVMFFRFVTSAEVQRRAEFPFIFILGLT  
OTU1\_Haematococcus\_pluvialis -----QGFLSSEALQEAYQDDTASNLVIMLLRFIVSAEIKRRDFFLFPILGMY  
OTU1\_Chlamydomonas\_reinhardtii -----SDQFAQEKLNVNMRDDMVSNMIVMFLRLVTSCEVQRREDFPFILGMY  
OTU1\_Gonium\_pectorale -----YTNFAHDKLLVDMRDTISNLIVMLLRLLTSCVQRREDFPFILGMY  
OTU1\_Volvox\_carteri -----YTNMHERLLVNMRRDTSVSNLVMLLRLLTSCVQRREDFPFIFIMGY  
OTU1\_Chlamydomonas\_eustigma -----DNPMTKETLTINMRDDMVSNMVMFLRMVTSAEIQRRSDFPFIFIMGY  
OTU1\_Chromochloris\_zofingiensis -----PDHMTQEQLENNFRDMLSNVVMFLRMVTSAEIQRRQDFLFPFIWGMT  
OTU1\_Raphidocelis\_subcapitata -----GQGADASTLLANFRDDTVSNMVMMLLRFITSAEIQARQDHFAPFVLGVA  
OTU1\_Scenedesmus\_obliquus -----EGGIDEQQLANFRDDTWSNTCVMLLRFITSAEIKRRVDHFEFPVVGQY  
OTU1\_Ulva\_mutabilis -----EDRMEPGELLVRLNDEVCNMLVMLVRFVSAHVSRAEHFLPFILGMV  
OTU1\_Caulerpa\_lentillifera -----ELTLEEWELVMN--DDNMSNYIVMFLRLLTSAEIQLRREFFPFIFIGIS  
OTU1\_Coccomyxa\_subellipsoidea -----AEPLSVQLEDNFCDAMLSPMIIMLLRMLTSAEIQRRSDFPFIFIMGMC  
OTU1\_Thalassiosira\_pseudonana -----ASSQLHSKLNE---ENATSDYCTWFMVRMTAAQMKSNPDRYLPFLFGGM  
OTU1\_Phaeodactylum\_tricornutum -----GTLDEAAFHEEMNQETATS DYCTWYLRVVTATHLQDPGRFLPFIAEPG  
OTU1\_Phytophthora\_sojae -----TRTHAELVQDFQTEGGESEYLVWYMRLLTAGYMKQHAETFQPFIDGLY  
OTU1\_Trypanosoma\_brucei -----GECSTVEQLYELS-TNGEAEYALYFYRYAVSHHIRTTHEDDFLFPVVGMG  
OTU1\_Laccaria\_bicolor -----GKKLDSAGLLAFQSPMSNAVVMRFLTSAEIRVHRENYEAFLIHPE  
OTU1\_Aspergillus\_nigra -----SGIRDETFLVDAFNEEYNSNAIITHFRLLTSAWMKLHPAQYEAFLSM--  
OTU1\_Neurospora\_crassa -----VPEEAMSEVMHAFTDAEKSNAI IYHLRLLASSWLKENRGSHEAFITTD-  
YOD1\_Homo\_sapiens -----ACAPEMRRLIAQIVASDPDFYSEAILG--  
OTU1\_Saccharomyces\_cerevisiae -----DSVRDLREMVSKVLLNPNVVFNDAILD--  
OTUD6B\_Homo\_sapiens -----LTVVALRSQTAEYMQSHVEDFLPFLTNP  
OTU2\_Saccharomyces\_cerevisiae -----KKLDQDMVMKLRWLSKNYVQEHRRDFIPLYFDEE  
OTUD5\_Homo\_sapiens -----DMHEVVRKHCDYLMKNADYFSNYVTE--  
OTUD3\_Homo\_sapiens -----RNHLKHRQETVDYMIKQREDFPFVDED-

**b (5/8)**

OTU2p\_Chlamydomonas\_reinhardtii GDYTGTLTLRQICERHVLPMGVEVEQLQIIAACTALGVTTLAVLDVAGSAV-----  
OTU2m\_Chlamydomonas\_reinhardtii GDYTGTLTLRQICERHVLPMGVEVEQLQIIAACTALGVTTLAVLDVAGSAV-----  
OTU2m\_Chlamydomonas\_incerta GDYAGLSLRQICERHVLPMGVEVEQLQIIAACTALGVTTLAVLDVAGSAV-----  
OTU2m\_Chlamydomonas\_schloesseri GDYTGLSLRQICERHVLPMGVEVEQLQIIAACTALGVTTLAVLDVAGSAV-----  
OTU2m\_Edaphochlamys\_debaryana RDYTGLSLRQICAHVLPPLGVEVEQLQMIALCTALGAVVAVLDVAGSQV-----  
OTU2\_Volvox\_carteri MDYSGMSLQWICSHHVLPMGVEVEQLQIIALCRALGITLGLVDVAGSQV-----  
OTU2\_Gonium\_pectorale GDYSGLSLRQVCVRHVLPMGVEVEQLQINALCSALGAPVAVLDVAGSQV-----  
OTU2\_Chromochloris\_zofingiensis QEY TALNIQQICERYVERMQEVEQLQMVVALAHAMKVAVGILDVAGSEI-----  
OTU2\_Raphidocelis\_subcapitata PGYGAASMAELCSRVERMGEEVEQVQMAALASAVAVSVGLDVAGSEV-----  
OTU2\_Ulva\_mutabilis GPYEQLTVPLVVGRLVAVDGVAAEHVMIQAIATLLGLCLAVVYADGQA-----  
OTUB1\_Homo\_sapiens -----RTVKEFCQQEVEPMCKESDHIHI IALAQALS SVSIQVEYMDRGE--  
OTUB1\_Mus\_musculus -----RTVKEFCQQEVEPMCKESDHIHI IALAQALS SVSIQVEYMDRGE--  
OTUB\_Drosophila\_melanogaster -----LTIEAFRHLVEPEMCKESDHIHI IALCTALGAGVRVEYLDREGG-----  
OTUB\_Caenorhaptiditis\_elegans -----MTVQYCEQEIEPMWKDADHLAINSLIKAAGTRVRIEYMDRTAAP-----  
OTUB\_Nematostella\_vectensis -----RTVKEFCNQEVEPMCKESDHIHI IGLTEALGVCIRVEYVDRGTG-----  
OTUB2\_Homo\_sapiens -----MDIKDFCTHEVEPMATECDHIQITALSQALSIALQVEYVDEM-----  
OTUB2\_Mus\_musculus -----MDIKDFCTHEVEPMAMECDHVQITALSQALNIALQVEYVDEM-----  
OTUB\_Monosiga\_brevicollis -----LTVKEYCSISVDPFGVESEHLQAQALISALRLGCRITYIDRSPGA-----  
OTU1\_Arabidopsis\_thaliana N----ATVDQFCKSSVEPMGEEEDHIHITALS DALGVAIRVVYLD RSSCDSGGV-----  
OTU1\_Medicago\_truncatula N----TTVEQFCKASVEPMGEEEDHVHITALS DALGPIRIRVVYLD RSSC DTGGV-----  
OTU1\_Oryza\_sativa N----STVVQFCKASVEPMGEEEDHVHI IALS DALGVP IRVMYLD RSSC DAGNI-----  
OTU1La\_O.sativa\_55840 Y----SSVFEFCQVEVRPENAEASNEQMKALVEALGIPVLVENLDTTSETDTP-----  
OTU1Lb\_O.sativa\_30974 EN----QNLEDWCSKHVIAPRVYATSAAVKACAEALRVTVQVENVDHGT-----  
OTU1Lc\_O.sativa\_32190 GG---YTLEAWCEIYLLQPREQTDHIQMTAVAAAALGVPLRVENLHNGP-----  
OTU1\_Marchantia\_polymorpha N----MNVQFCKTSVEPMGEEEDHVHITALS DALGVPVVRVVYLD RSVGADGKNT-----  
OTU1\_Physcomitrella\_patens\_181928 N----MSVQFRSSVEPMGEEEDHVHITALS DALGVPVVRVVYLD QSGDVNDKPV-----  
OTU1\_Klebsormidium\_flaccidum N----LDVKKFCQTSVEPMGEEEDHVHITAL TDALGPIRIRVVYLD RSGGASDDAL-----  
OTU1\_Haematococcus\_pluvialis DDPP--ACVDTFCQRVPEMAEESDNIHIVAL TDALQVPVTVVYLD RSGVESRASHLATRT  
OTU1\_Chlamydomonas\_reinhardtii DEPP--ATVELFCQRHVEPMGEEEDHLHIVAVTEALQIPVVRVVYLDSSGLPAGGGGGGAGA  
OTU1\_Gonium\_pectorale DEPP--ATVELFCQRHVEPMGEEEDHLHIVAITDALQIPVVRVVYLDSSGLPTGGGSGL--  
OTU1\_Volvox\_carteri DEPP--ATVELFCQRHVEPMGEEEDHLHIVAITDALQIPVVRVVYLDSSGMPTGGNGSGL--  
OTU1\_Chlamydomonas\_eustigma DDV--SCVEVFCQRSVEPMGEEEDHVHIVALSDALQVPIAVVYLD RSNVNRVNEGSSEV--  
OTU1\_Chromochloris\_zofingiensis EDES--MTADVFCRKFVEPMGEESEHIIHAQVLTDAFQVAVRVMYLD SRDHGTDN-----  
OTU1\_Raphidocelis\_subcapitata D----TDVQTFCCRFRVPEMAEESDHVHAQALCDALRVPI TVVYLD SHERSGVGGGAGGST  
OTU1\_Scenedesmus\_obliquus D----MDVATFCERSVDVPMGEEEDHIHAQALTD AVQVPVRI IQIDSSGGQ-----  
OTU1\_Ulva\_mutabilis DGC--DGVDDFRRHVEAMGEEEDHIQVVAVTEAFQVPLVVYLDNSSVTADGNGNGNGN  
OTU1\_Caulerpa\_lentillifera DV---LDVELFCQRMVEMDEESDEIHIRALTDAL EIPTRVYSLDSQPPI-----  
OTU1\_Cocomyxa\_subellipsoidea DDE--VSVEQFCRRYVEPMGEEEDHVHIVALTNALLVPIRIVVYLD RSMGAAMAGVGYDSA  
OTU1\_Thalassiosira\_pseudonana -----IDIPTFCTREVEPMNKECGMVQAALAEFCFVVRVAIEYMDGRR-----  
OTU1\_Phaeodactylum\_tricornutum -----LGINDFCQREVEPMGKECEQVQLALAEAFGVQVTIAYLDGHELLYG-----  
OTU1\_Phytophthora\_sojae PG---QTVAQFCAAEVEPMGKECDQPQIAALTEALQVGVKIEYLDGSAGPGQ-----  
OTU1\_Trypanosoma\_brucei H----ETVSEFCSEVD AVASEADNVQVFAQCFDVRVIVEYVDGREGD-----  
OTU1\_Laccaria\_bicolor THEP--MGVDAFCANIVEPLGKEADHVEIQALCAALQLNVDVAYLNGGSEE-----  
OTU1\_AspERGILLUS\_nigra -----PVEQYCATRIDVVRTEIDEIQLQALVDG VGF AVEIMYLD RSEGE-----  
OTU1\_Neurospora\_crassa -----ESVEKFCQRVIEVFNIEIDQLGLQALVSI LGFVLRVAYLDRSPGS-----  
YOD1\_Homo\_sapiens -----KTNQEYCDW- IKRDDTWGGAIEI S ILSKFYQCEICVVD TQTVR-----  
OTU1\_Saccharomyces\_cerevisiae -----KPNKDYAQW- I LKME SWGGAIEI G IISDALAVAIYVVDIDAVK-----  
OTUD6B\_Homo\_sapiens TGYTPPEEFQKYCED- IVNTAAWGGQLELRALSHILQTPIEI IQADSP-----  
OTU2\_Saccharomyces\_cerevisiae TMKM--KDI DEYTK E-MEHTAQWGG EIEI LALSHVFD C PISILMSGR-----  
OTUD5\_Homo\_sapiens -----DFTTYINR- KRKN NCHGNHIE MQAMAEMYNRPVEVYQYSTEP-----  
OTUD3\_Homo\_sapiens -----IPFEKHVAS- LAKPGTFAGND AIVAFARNHQLN VVIHQLNAPLW-----



**b (6/8)**

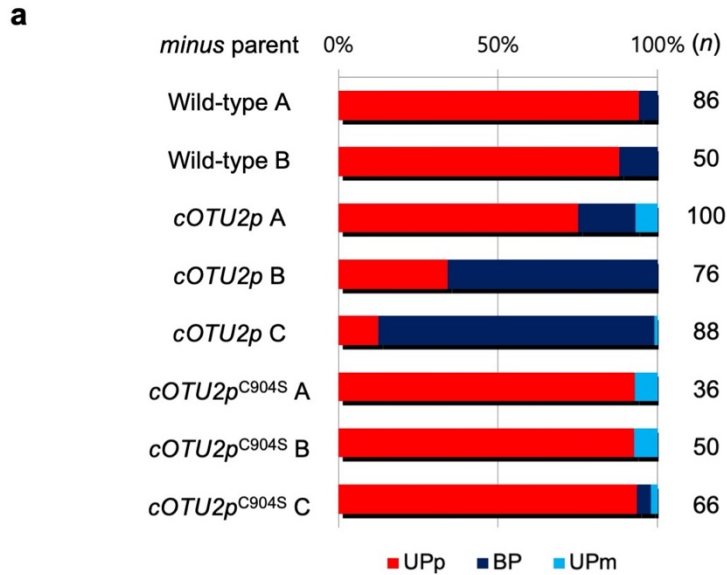
OTU2p_Chlamydomonas_reinhardtii	GAIKHG--PAA-----
OTU2m_Chlamydomonas_reinhardtii	GAIKHG--PAA-----
OTU2m_Chlamydomonas_incerta	GAIKHG--PAA-----
OTU2m_Chlamydomonas_schloesseri	GAIKHG--PAA-----
OTU2m_Edaphochlamys_debaryana	GAIKHG--PPG-----
OTU2_Volvox_carteri	GAIKHG--PTG-----
OTU2_Gonium_pectorale	GAIKHG--PPG-----
OTU2_Chromochloris_zofingiensis	GYIQH---PAG-----
OTU2_Raphidocelis_subcapitata	GMVRH---PAG-----
OTU2_Ulva_mutabilis	DPKVHISSQR-----
OTUB1_Homo_sapiens	TTNPHIF-PEG-----
OTUB1_Mus_musculus	TTNPHVF-PEG-----
OTUB_Drosophila_melanogaster	TVKAHDF-PEG-----
OTUB_Caenorhabditis_elegans	NGGWHYDIPSD-----
OTUB_Nematostella_vectensis	DVNHHDF-PED-----
OTUB2_Homo_sapiens	ALNHHVF-PEA-----
OTUB2_Mus_musculus	ALNHHVF-PEA-----
OTUB_Monosiga_brevicolis	EPPCHVLEGPD-----
OTU1_Arabidopsis_thaliana	TVNHHDFVPVG-----
OTU1_Medicago_trunculata	SVNHHDFTPVA-----
OTU1_Oryza_sativa	SVNHHDFSPEA-----
OTU1La_O.sativa_55840	ILNQHFYPRPESEEGTMLGPLNSHEIVSPESGGYHAARGELQNPSTSGSSTNSSTEAL
OTU1Lb_O.sativa_30974	CESTHYIVRGA-----
OTU1Lc_O.sativa_32190	AQDIYTADGVN-----
OTU1_Marchantia_polymorpha	DVNHHDFLPPD-----
OTU1_Physcomitrella_patens_181928	IVNNHDFIPEG-----
OTU1_Klebsormidium_flaccidum	EINHDFVPES-----
OTU1_Haematococcus_pluvialis	EVNEHTIVPDA-----
OTU1_Chlamydomonas_reinhardtii	EASCHDFVPDS-----
OTU1_Gonium_pectorale	DATVHDFVPDS-----
OTU1_Volvox_carteri	DATMHDFVPDS-----
OTU1_Chlamydomonas_eustigma	EVETYHFNPEP-----
OTU1_Chromochloris_zofingiensis	SVSKVDFIPEG-----
OTU1_Raphidocelis_subcapitata	ECEVHRFEPDS-----
OTU1_Scenedesmus_obliquus	EASVDMKPEG-----
OTU1_Ulva_mutabilis	QCNTHVFEPRE-----
OTU1_Caulerpa_lentillifera	EIQPTDYVPEE-----
OTU1_Cocomyxa_subellipsoidea	AVNHHDFVPDS-----
OTU1_Thalassiosira_pseudonana	-VATHVFGese-----
OTU1_Phaeodactylum_tricornutum	RLAQHTFGPDS-----
OTU1_Phytophthora_sojae	DLQSYVCSPTV-----
OTU1_Trypanosoma_brucei	CTTRHTFQQKD-----
OTU1_Laccaria_bicolor	GVVDFIPFRIA-----
OTU1_Aspergillus_nigra	AVTPHLL-TPP-----
OTU1_Neurospora_crassa	EVNTYSF-PEE-----
YOD1_Homo_sapiens	-IDRFGE-DAG-----
OTU1_Saccharomyces_cerevisiae	-IEKFNE-DKF-----
OTUD6B_Homo_sapiens	-IIVGEE-YSK-----
OTU2_Saccharomyces_cerevisiae	-IQVYNE-CGK-----
OTUD5_Homo_sapiens	-INTFHG-IHN-----
OTUD3_Homo_sapiens	-QIRGTE-KSS-----

**b (7/8)**

OTU2p\_Chlamydomonas\_reinhardtii -----QQHGPPVAWVAHL-----PGHYDVIYPARPLDV  
OTU2m\_Chlamydomonas\_reinhardtii -----QQHGPPVAWVAHL-----PGHYDVIYPARPLDV  
OTU2m\_Chlamydomonas\_incerta -----QQHGPPVAWVAHL-----PGHYDVIYPARALDV  
OTU2m\_Chlamydomonas\_schloesseri -----QQHSPPVAWVAHL-----PGHYDVIYPARPLDV  
OTU2m\_Edaphochlamys\_debaryana -----PAAAQPPAAWVAHL-----PGHYDVIYPARRMDV  
OTU2\_Volvox\_carteri -----HLGPPVAWVAHL-----PGHYDVVYPAKQLDV  
OTU2\_Gonium\_pectorale -----ARGPPAAWVAHL-----PGHYDVIYPARPLDV  
OTU2\_Chromochloris\_zofingiensis -----DATLAFWMIHL-----PGHYEIVYARPLDV  
OTU2\_Raphidocelis\_subcapitata -----GGEPLFYLVHL-----PGHYEICYS-----  
OTU2\_Ulva\_mutabilis -----EHVEEGEPSMFLFR-----DGHYDLIYPINNSWR  
OTUB1\_Homo\_sapiens -----SEPKVYLLYR-----PGHYDILYK-----  
OTUB1\_Mus\_musculus -----SEPKVYLLYR-----PGHYDILYK-----  
OTUB\_Drosophila\_melanogaster -----SEPRIYLIYR-----PGHYDILYPN-----  
OTUB\_Caenorhapditiis\_elegans -----DQQIAPETLLYR-----PGHYDVIYKKDSTEA  
OTUB\_Nematostella\_vectensis -----GSTPIVHLLYR-----PGHYDILYLANQ---  
OTUB2\_Homo\_sapiens -----ATPSVYLLYK-----TSHYNILYAADKH--  
OTUB2\_Mus\_musculus -----AIPSVYLLYK-----TSHYNILYAAEKH--  
OTUB\_Monosiga\_brevicollis -----GSGQPVCVHLMFR-----PGHYDVIYLPATAA  
OTU1\_Arabidopsis\_thaliana -----ITNEKDEEASAPFITLLYR-----PGHYDILYKPKPCKV  
OTU1\_Medicago\_trunculata -----GDLPSASGSSEKKNPFITLLYR-----PGHYDILYKPK-----  
OTU1\_Oryza\_sativa -----NSSDGA AAAEKPYITLLYR-----PGHYDILYKPK-----  
OTU1La\_O.sativa\_55840 GLQSIGTSSTPNERDGKDRITINDLSPAERRRLAILLYR-----PGHYDILCPK-----  
OTU1Lb\_O.sativa\_30974 -----PCVTLLRI-----ESHYDIYPLPSSSI  
OTU1Lc\_O.sativa\_32190 -----IPRVTLTY-----GVHYDILYPRHPSSG  
OTU1\_Marchantia\_polymorpha -----CPTVEMPPVLLYR-----PGHYDILYTRVQNL  
OTU1\_Physcomitrella\_patens\_181928 -----MDAAVEPNVLLYR-----PGHYDILYRKENSED  
OTU1\_Klebsormidium\_flaccidum -----APESFKPDTSGLLTLLYR-----PGHYDILYSL-----  
OTU1\_Haematococcus\_pluvialis -----CAAAGAPIRVHVLRY-----PGHYDILYKRPE---  
OTU1\_Chlamydomonas\_reinhardtii -----CPPGTAPRVHLLYR-----PGHYDILYAKSG--  
OTU1\_Gonium\_pectorale -----CVPGTHPRVHLLYR-----PGHYDILYAKGG--  
OTU1\_Volvox\_carteri -----CVPGTQPRVHLLYR-----PGHYDILYKPKGG--  
OTU1\_Chlamydomonas\_eustigma -----GASVLRSCVSLLYR-----PGHYDILYQQ-----  
OTU1\_Chromochloris\_zofingiensis -----CTGADAVPHVHVLRY-----PGHYDILYPL-----  
OTU1\_Raphidocelis\_subcapitata -----EGAGSIAAAAAGDGGGGAGTGRGRPSVHLLYR-----PGHYDISYPKLIA--  
OTU1\_Scenedesmus\_obliquus -----LSAEQQAALGPPKVHMLYR-----PGHYDILYPC-----  
OTU1\_Ulva\_mutabilis -----PVAGATIPQVAVLYR-----PGHYDILYK-----  
OTU1\_Caulerpa\_lentillifera -----ASIKSEIIVHLLYR-----PGHYDIYPKTTSTL  
OTU1\_Cocomyxa\_subellipsoidea -----LAESAAPVNRVHVLRY-----PGHYDILYK-----  
OTU1\_Thalassiosira\_pseudonana -----EEGSDEASTLTLTYR-----PGHYDILYQSECRYI  
OTU1\_Phaeodactylum\_tricornutum -----ASIEISLLYR-----PGHYDILYRCR----  
OTU1\_Phytophthora\_sojae -----PATEQQEVPVITLLYR-----PGHYDILYPREAGAE  
OTU1\_Trypanosoma\_brucei -----SHDANEYTTIEVTLLYR-----PGHYDILYK-----  
OTU1\_Laccaria\_bicolor -----SGSESLPLVLLYR-----PGHYDILYKKGQGEQ  
OTU1\_Aspergillus\_nigra -----RPTGGTICLLYR-----PGHYDILYKKGQGEQ  
OTU1\_Neurospora\_crassa -----AISQPTSPLDPVICLLYR-----PDHYDILYKKGQGEQ  
YOD1\_Homo\_sapiens -----YTKRVLLIYD-----GIHYDILYKKGQGEQ  
OTU1\_Saccharomyces\_cerevisiae -----DNYILILFN-----GIHYDSLTMNEFK--  
OTUD6B\_Homo\_sapiens -----KPLILVYMRHAYGLGEHYNSVTRLVNIVT  
OTU2\_Saccharomyces\_cerevisiae -----NPELKLVIYKHSYALGEHYNSLHDS-----  
OTUD5\_Homo\_sapiens -----EDEPIRVSYHR-----NIHYNSVVPNKATI  
OTUD3\_Homo\_sapiens -----VRELHIAYRY-----GEHYDSVRRINDNSE

**b (8/8)**

```
OTU2p_Chlamydomonas_reinhardtii APGGQLVAIII-----
OTU2m_Chlamydomonas_reinhardtii APGGQLVAIII-----
  OTU2m_Chlamydomonas_incerta APGGQLVAIVDM-----
OTU2m_Chlamydomonas_schloesseri APGGQLVPAAKKGIVAT
  OTU2m_Edaphochlamys_debaryana APGGMLVPA-----
    OTU2_Volvox_carteri APGGALVPVLLPEGF--
      OTU2_Gonium_pectorale APGGALIPV-----
OTU2_Chromochloris_zofingiensis STLTTLVKKAG-----
  OTU2_Raphidocelis_subcapitata -----
    OTU2_Ulva_mutabilis CLVAQTGTVSDY-----
      OTUB1_Homo_sapiens -----
        OTUB1_Mus_musculus -----
OTUB_Drosophila_melanogaster -----
  OTUB_Caenorhapditis_elegans SEIEN-----
  OTUB_Nematostella_vectensis -----
    OTUB2_Homo_sapiens -----
      OTUB2_Mus_musculus -----
OTUB_Monosiga_brevicolis -----
  OTU1_Arabidopsis_thaliana SDNVGK-----
  OTU1_Medicago_tranculata -----
    OTU1_Oryza_sativa -----
      OTU1La_O.sativa_55840 -----
      OTU1Lb_O.sativa_30974 NSSNPHEEKLLPIPSSI
      OTU1Lc_O.sativa_32190 SGSKSSTQR-----
    OTU1_Marchantia_polymorpha APVPRVEEPQDL-----
  OTU1_Physcomitrella_patens_181928 ALLSNLEQTG-----
  OTU1_Klebsormidium_flaccidum -----
  OTU1_Haematococcus_pluvialis -----
  OTU1_Chlamydomonas_reinhardtii -----
    OTU1_Gonium_pectorale -----
      OTU1_Volvox_carteri -----
        OTU1_Chlamydomonas_eustigma -----
  OTU1_Chromochloris_zofingiensis -----
  OTU1_Raphidocelis_subcapitata -----
    OTU1_Scenedesmus_obliquus -----
      OTU1_Ulva_mutabilis -----
        OTU1_Caulerpa_lentillifera -----
        OTU1_Cocomyxa_subellipsoidea -----
        OTU1_Thalassiosira_pseudonana R-----
  OTU1_Phaeodactylum_tricornutum -----
    OTU1_Phytophthora_sojae QEKAS-----
    OTU1_Trypanosoma_brucei -----
      OTU1_Laccaria_bicolor ELRH-----
      OTU1_Aspergillus_nigra MEPVVNYQYAMTSDYS-
      OTU1_Neurospora_crassa PAPMNIQVFRATIPQY-
        YOD1_Homo_sapiens TPPLTIFSSND-----
  OTU1_Saccharomyces_cerevisiae ----TVFNKNQP-----
    OTUD6B_Homo_sapiens ENCS-----
  OTU2_Saccharomyces_cerevisiae -----
    OTUD5_Homo_sapiens GVGLGLPSFKPG-----
    OTUD3_Homo_sapiens APAHLQTDFOML-----
```

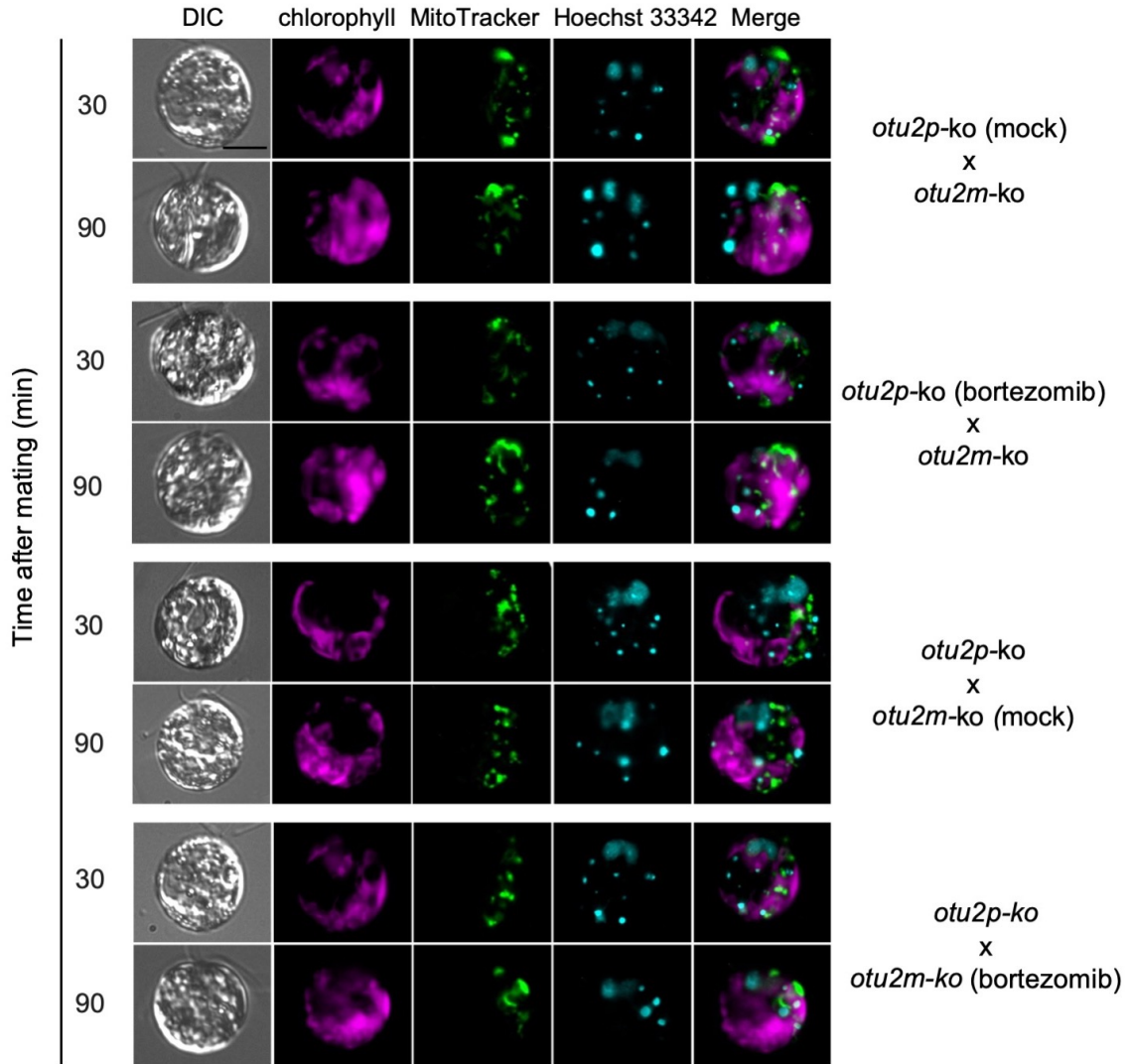


**b**

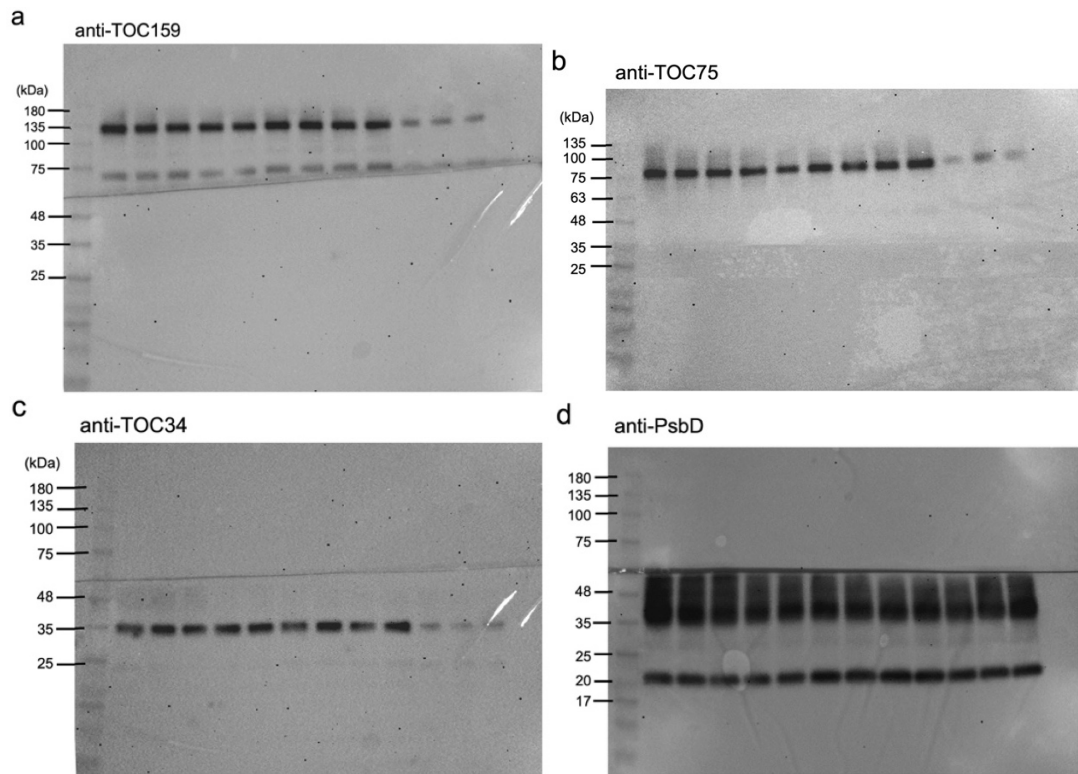
plus parent (eryR)	minus parent (specR)	Total (zygotes)	eryR	Both	specR	Exceptional	P-value
CC-333	Control-A	86	81	5	0	6%	n.a.
	Control-B	50	44	6	0	12%	n.a.
	cOTU2p-A	100	75	18	7	25%	7.77E-10
	cOTU2p-B*	76	26	50	0	66%	4.79E-49
	cOTU2p-C*	88	11	76	1	88%	9.66E-111
	cOTU2m-A	106	90	10	6	15%	5.12E-04
	cOTU2m-B	88	74	13	1	16%	3.31E-06
	cOTU2m-C	130	108	18	4	17%	2.64E-04
	cOTU2p <sup>C904S</sup> -A	36	35	0	1	3%	8.26E-18
	cOTU2p <sup>C904S</sup> -B	50	48	0	4	8%	5.88E-13
	cOTU2p <sup>C904S</sup> -C	66	63	2	1	5%	1.47E-09

**Supplementary Figure 10.** DUB activity of Otu2p is required for promoting chloroplast DNA inheritance. Individual zygote progeny from the mating of the erythromycin-resistant (eryR) WT *plus* strain with three independent transgenic *minus* strains that harbor *cOTU2p* or *cOTU2p*<sup>C904S</sup> (the non-catalytic Cys904Ser mutation) in a spectinomycin-resistant parental strain (specR) were examined for antibiotic resistance. (a) The C904S mutation abolished the increase in biparental inheritance caused by *cOTU2p*. (*n*) indicates the number of germinating zygotes examined. (b) Antibiotic resistance data depicted in Supplementary Fig. 10a. Asterisks indicate significant differences from the control mating in the exceptional zygote rate, as estimated with the chi-square test ( $p$ -value < 1.0E-25).





**Supplementary Figure 12.** Bortezomib treatment of *otu2-ko* mutant gametes phenocopied Otu2p expression for cpDNA degradation in zygotes. Differential interference contrast and overlaid fluorescent images of Hoechst 33342 (cyan)-stained zygotes with chlorophyll autofluorescence (magenta) at the indicated times after mating. Experiments were repeated three times. Quantitative results of three biological experiments are provided in Section “Figure 5d” of Supplementary Table 3. Only the *minus* gametes were stained with MitoTracker Green (green), staining *minus* chloroplasts in the zygotes. Bar = 5  $\mu$ m.



**Supplementary Figure 13.** The complete blots of Fig. 4a. **(a)** anti-TOC159. **(b)** anti-TOC75. **(c)** anti-TOC34. **(d)** anti-PsbD.

**Supplementary Table 1.** Genetic analysis of cpDNA inheritance for testing the “protector” function of *OTU2/EZY2* cluster in *MT+*

plus parent (specR)	minus parent (eryR)	Total (zygotes)	specR	Both	eryR	Exceptional	P-value
CJU10-J1	CC-2663	93	89	2	2	4%	n.a.
same as above	D13-03*	43	36	4	3	9%	2.06E-07
	D13-06	53	52	2	0	4%	1.22E-04
	D13-08*	73	43	30	0	28%	4.71E-39
	D13-10	84	84	0	0	0%	3.85E-02
	D13-13*	54	45	7	2	17%	1.21E-07
	D13-14*	66	49	11	6	26%	8.45E-15
	D13-19	94	87	4	3	7%	1.30E-01
	D13-20*	123	96	27	0	22%	1.00E-30
	D13-26	99	95	3	1	4%	5.25E-01
	D13-53	73	71	1	0	1%	1.90E-02
	D13-56	64	64	0	0	0%	9.00E-04
	D13-64	60	56	3	1	7%	4.69E-04
	D13-81	76	76	0	0	0%	1.52E-02
	P21-49	68	65	3	0	4%	9.52E-03
	O16-52	74	68	6	0	8%	1.47E-02

\*Asterisks next to the strain name indicate significant difference from wild-type zygotes (CJU10-J1 x CC-2663) based on *chi*-square test ( $p$ -value < 1.0E-6).



**Supplementary Table 2.** Genetic analysis of cpDNA inheritance for testing the “protector” function of the *OTU2p* and *OTU2m* coding segments

Strain ID	# zygote	UPp (eryR)	BP	UPm (specR)	(UPm + BP)	<i>P</i> -value
Control #1	40	32	2	6	20%	n.a.
AR::cOTU2p_#1	24	9	3	12	63%	1.94E-06
AR::cOTU2p_#7*	34	1	3	30	97%	2.48E-25
AR::cOTU2p_#8*	27	6	3	18	78%	8.03E-11
AR::cOTU2p_#9*	24	4	4	16	83%	7.07E-11
AR::cOTU2p_#10*	39	3	3	33	92%	7.31E-29
AR::cOTU2p_#11*	26	3	2	21	88%	1.63E-13
AR::cOTU2p_#15	98	79	7	12	19%	4.57E-20
AR::cOTU2p_#16	78	76	0	2	3%	7.49E-16
AR::cOTU2p_#17	31	22	1	8	29%	7.14E-02
AR::cOTU2p_#19	49	48	0	1	2%	1.71E-04
AR::cOTU2p_#20	61	51	3	7	16%	5.98E-04
AR::cOTU2p_#21	27	13	6	8	52%	7.11E-05
AR::cOTU2p_#22	29	13	11	5	55%	1.13E-05
AR::cOTU2p_#23*	39	13	13	13	67%	6.20E-13
AR::cOTU2p_#24	22	18	4	0	18%	4.37E-03

Strain ID	# zygote	UPp (eryR)	BP	UPm (specR)	(UPm + BP)	<i>P</i> -value
Control #1	83	72	8	3	13%	n.a.
Control #2	93	86	7	0	8%	n.a.
AR::cOTU2m_#2	96	92	2	2	4%	5.59E-10
AR::cOTU2m_#3	76	72	3	1	5%	3.05E-14
AR::cOTU2m_#4	98	85	3	10	13%	3.11E-09
AR::cOTU2m_#5	89	78	6	5	12%	4.87E-11
AR::cOTU2m_#6	96	89	1	6	7%	1.27E-09
AR::cOTU2m_#7	34	30	0	4	12%	9.70E-27
AR::cOTU2m_#8	92	86	5	1	7%	1.68E-10
AR::cOTU2m_#9	96	88	6	2	8%	1.47E-09
AR::cOTU2m_#10	41	39	2	0	5%	2.18E-24
AR::cOTU2m_#11	54	50	4	0	7%	3.45E-20
AR::cOTU2m_#12	81	72	4	5	11%	7.89E-13
AR::cOTU2m_#13	98	95	3	0	3%	8.59E-10
AR::cOTU2m_#14	78	70	6	2	10%	1.50E-13
AR::cOTU2m_#15	86	80	6	0	7%	9.13E-12
AR::cOTU2m_#16	104	90	12	2	13%	3.99E-08

(a) Asterisks indicate significant increase in exceptional zygote (UPm + BP) rates by chi-square test ( $p$ -value  $< 1.0E-6$ ). AR::cOTU2p #8, 10, and 23 and AR::cOTU2m #2, 6, and 12, showing comparable transgene expression with endogenous *OTU2*, were designated as A, B, and C, used in other experiments.

**Supplementary Table 3.** Summary of the detailed mating data described in this study

Cross		Mating efficiency	% <sup>a</sup>				Total QFC counted
<i>plus</i>	<i>minus</i>	% <sup>a</sup>	UP+ %	UP- %	BP %	No%	
<b>Figure 1</b>							
Haploid MT+	Haploid MT-	91.2 ± 1.3	85.0 ± 1.3	0.00	15.0 ± 1.3	0.0	608
Haploid MT+	MT+/MT-	88.5 ± 0.8	10.4 ± 4.1	1.3 ± 0.8	88.3 ± 4.8	0.0	1015
Haploid MT+	MT-/MT-	89.0 ± 1.6	78.5 ± 1.3	2.5 ± 0.5	19.0 ± 0.9	0.0	438
<b>Figure 2b</b>							
WT	cOTU2p	90.9 ± 1.1	12.2 ± 1.4	1.5 ± 0.8	86.3 ± 1.2	0.0	666
WT	cOTU2m	89.3 ± 0.8	81.1 ± 3.2	0.0	18.9 ± 3.2	0.0	416
<b>Figure 2c</b>							
WT	WT	92.5 ± 0.2	81.9 ± 1.8	0.2 ± 0.4	17.9 ± 2.2	0.0	543
otu2p	WT	89.2 ± 1.6	13.5 ± 2.8	1.1 ± 0.9	85.3 ± 2.1	0.0	770
otu2p	otu2m	87.2 ± 1.3	10.2 ± 1.2	3.6 ± 0.9	84.6 ± 0.6	1.6 ± 0.6	866
<b>Figure 2d</b>							
otu2p; cOTU2p	otu2m	89.4 ± 1.2	88.0 ± 0.2	1.2 ± 0.4	10.8 ± 0.4	0.0	866
otu2p	otu2m; cOTU2p	87.3 ± 0.4	2.6 ± 0.8	82.9 ± 3.3	14.6 ± 3.3	0.0	738
otu2p; cOTU2m	otu2m	87.6 ± 0.6	11.2 ± 0.3	0.8 ± 0.1	87.1 ± 0.4	0.9 ± 0.3	879
otu2p	otu2m; cOTU2m	88.5 ± 0.4	13.4 ± 0.7	1.3 ± 0.9	83.9 ± 1.6	1.3 ± 0.5	743
<b>Figure 5c</b>							
WT (mock)	WT (mock)	89.6 ± 2.2	82.4 ± 1.0	0.0	17.6 ± 1.0	0.0	1093
WT (MG132)	WT (mock)	88.8 ± 0.9	83.5 ± 1.3	0.0	16.5 ± 1.3	0.0	423
WT (bortezomib)	WT (mock)	88.1 ± 1.8	85.9 ± 1.8	0.0	83.9 ± 1.8	0.0	706
WT (mock)	WT (MG132)	88.6 ± 0.9	19.1 ± 1.7	0.0	80.9 ± 1.7	0.0	371
WT (mock)	WT (bortezomib)	87.2 ± 1.2	13.0 ± 0.7	0.0	87.0 ± 0.7	0.0	766
<b>Figure 5d</b>							
otu2p (mock)	otu2m	89.6 ± 0.9	15.5 ± 4.6	0.8 ± 0.4	83.7 ± 4.4	0.0	617
otu2p (MG132)	otu2m	88.2 ± 1.0	81.2 ± 0.4	1.8 ± 0.2	17.0 ± 0.4	0.0	441
otu2p	otu2m (mock)	89.6 ± 0.9	17.3 ± 1.7	4.1 ± 0.8	78.7 ± 1.7	0.0	435
otu2p	otu2m (MG132)	88.0 ± 0.5	1.0 ± 0.6	82.0 ± 1.8	17.1 ± 1.3	0.0	424
otu2p (bortezomib)	otu2m	86.1 ± 0.7	85.2 ± 0.8	1.2 ± 0.5	13.7 ± 0.5	0.00	689
otu2p	otu2m (bortezomib)	85.6 ± 1.5	0.8 ± 0.3	84.7 ± 1.0	14.6 ± 1.1	0.00	653

(a) % values show the average ± s.d. from three biological replicates.

**Supplementary Table 4.** Zygote-specific genes encoding chloroplast-localized proteins

Gene ID	FPKM (V-G-Z) <sup>a</sup>	Gene Name	Predicted locale <sup>b</sup>	(c)	(d)	Annotation
<b>a. Putative DNA-binding proteins</b>						
Cre06.g255750	0-0-1334	EZY1c	C	C	cTP	Localized to nucleoids
Cre06.g255650	0-1-411	EZY1b	C	C	cTP	Localized to nucleoids
Cre06.g255600	0-1-254	EZY1a	C	C	cTP	Localized to nucleoids
Cre02.g091550	46-33-868	EZY18/Whirly	CM	M	cTP	Localized to nucleoids
Cre07.g314650	41-36-408	EZY19/RECA	C	C	cTP	Localized to nucleoids
Cre12.g483720	0-0-127	OSB2/PTAC9	CM	C	mTP	Divergent
<b>b. Topoisomerases</b>						
Cre10.g442850	2-5-17	TOP-HMGbox, topo IA1	CM	C	mTP	
Cre04.g221650	1-1-13	Topo IV (GYRA)	C	C	cTP	
Cre10.g440750	4-1-45	Topo IV (GYRB)	O	O	mTP	
<b>c. Candidate helicases</b>						
Cre06.g296150	3-3-23	PIF1-type helicase	CM	C	mTP	PIF1 domain only
Cre06.g256700	1-1-38	AAA_helicase	C	C	cTP	Divergent
Cre06.g256650	0-0-13	AAA_helicase	M	M	mTP	Divergent
<b>d. DNA replication enzymes</b>						
Cre17.g736150	1-0-14	DNA polymerase	M	M	mTP	Divergent

(a) Annotation and RNA-seq-based expression measures (FPKM) are taken from Joo et al. (4).

(b) Predicted organellar localization is indicated as C (Chloroplast), M (Mitochondria), or O (others).

(c) Prediction by TargetP (<https://services.healthtech.dtu.dk/service.php?TargetP-2.0>).

(d) Prediction by PredAlgo (<http://lobosphaera.ibpc.fr/cgi-bin/predalgotdb2.perl?page=main>).

**Supplementary Table 5.** Genetic linkage of OTU2 homologs to the mating-type loci in Volvocine algae

Genome	OTU2	MT-linkage	mating-type (location)	MTD1	SAD1
<i>C. reinhardtii</i> <sup>a</sup>	MT+/MT-	Yes	MT+ (Ch6)	MT-	MT+/MT-
<i>C. incerta</i> <sup>b</sup>	C0033	Yes	MT- (C0033)	C0033	C0033
<i>C. schloesseri</i> <sup>b</sup>	C0045	Yes	MT- (C0045)	C0045	C0045
<i>Edaphochlamys debaryana</i> <sup>b</sup>	C0043	Yes	MT- (C0043)	C0043	C0043
<i>Gonium pectorale</i> <sup>c</sup>	C000113	<u>No</u>	MT- (C000001)	C000001	C000143
<i>Volvox carteri</i> <sup>d</sup>	Sc35	<u>No</u>	MT+ (Sc2, Sc38, Sc80)	Pseudogene	Sc27
<i>Dunaliella salina</i> <sup>e</sup>	C0044	Unknown	MT- (n.d.)	C0036	C0945

a) De Hoff et al. (2013) (5); b) Craig et al. (2021) (6); c) Hamaji et al. (2016) (7); d) Ferris et al. (2010) (8); e) Polle et al. (2017) (9).

**Supplementary Table 6. List of primers used in this study**

Name	Sequence	Restriction enzyme tag
psbB_qF1	AGCTGTTTTACCTGTACGTGG	
psbB_qR1	TCTGGAGTTATGAAGGTGTTGC	
psbD_qF1	GCTAACAGTATGGCTCACTCTC	
psbD_qR1	AAGCAACGAATGCCCATAAAC	
Cb1p_qF1	ACATTTCTGACCTCCACATC	
Cb1p_qR1	TGCTGGTGATGTTGAACTCG	
RCK1_qF1	GCCACACCGAGTGGGTGTCGTGCG	
RCK1_qR1	CCTTGCCGCCCCGAGGCGCACAGCG	
OTU2_F1	ACGGCAACTGCTTCTACC	
OTU2_F2	CTTGGCAATCAGGAGGCAG	
OTU2_F3	CATGACCGGGTTGACGGAC	
OTU2_F4	GCACTCGAGATCTGAGCAG	
OTU2_F5	GGTAGTACGGTTCGGCTAC	
OTU2_F6	CATGCATGCTTCGCCGG	
OTU2_F7	CTACAGTACCCTTGCTGGCAG	
OTU2_F9	TACCCGTGGCAACGCGAGTAC	
OTU2_F11	GAGGCGCATCAACTGGAATCG	
OTU2_F12	CTGGACTGTGACAAGCTCATC	
OTU2_F13	CTAGGACGGACCGACTTGGTC	
OTU2_F14	GCAGTCATGGTAGTCACCTTG	
OTU2_F16	ATGGCGCGCAAGGTCCGCGAGA	
OTU2_F17	CAGCGGCTGCTACGCCTTTTG	
OTU2_F18	CATATGACCGGGTTGACGGAC	NdeI
OTU2_F21	AGCAACGGCGCCGAGCCG	
OTU2p_R1	CCGGCGAAGCATGCATG	
OTU2m_R1	CTCTGCTCGGCGCTGTTG	
OTU2_R2	GGTAGAAGCAGTTGCCGTC	
OTU2_R3	CAGCTGCTCAACCTCCAC	
OTU2_R4	GCTGCTCAGATCTCGAGTG	
OTU2_R5	CGTAGCCGAACCGTACTAC	
OTU2_R12stop	gaattcTTATATAATTGCCGCCACTAG	EcoRI
OTU2_R12nostop	gaattcTATAATTGCCGCCACTAGTTGC	EcoRI
OTU2_R14	CAGCACACTGGCTTGCACAG	
OTU2_R16	AGGATCCCTGCAGGCGCTGCATCTC	BamHI
OTU2_R20	TGATGATGCCGGGCTGC	
OTU2_R21	GTGCGAAGCGGATCACCTC	
OTU2_C904S_F	GGGGCGACGGCAACAGCTTCTACCGCGCG	
OTU2_C904S_R	CGCGCGGTAGAAGCTGTTGCCGTCGCCCC	
rbcS_T_R1	AGCGCCTCCATTTACAC	
AtOTU1_F1	catATACGAACGCGTGCCGATTT	NdeI
AtOTU1_R1	gaattcTCATTTCCCCACATTGTCTG	EcoRI
OTU2_ko_F1	CGCGTCGGCCCGCCCTCTG	
OTU2_ko_R1p	GCAGCCGTACTTGGCGCAGTG	
OTU2_ko_R1m	GCAGCCGTACTCGACGCAGTG	

## Supplementary References 1–9

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