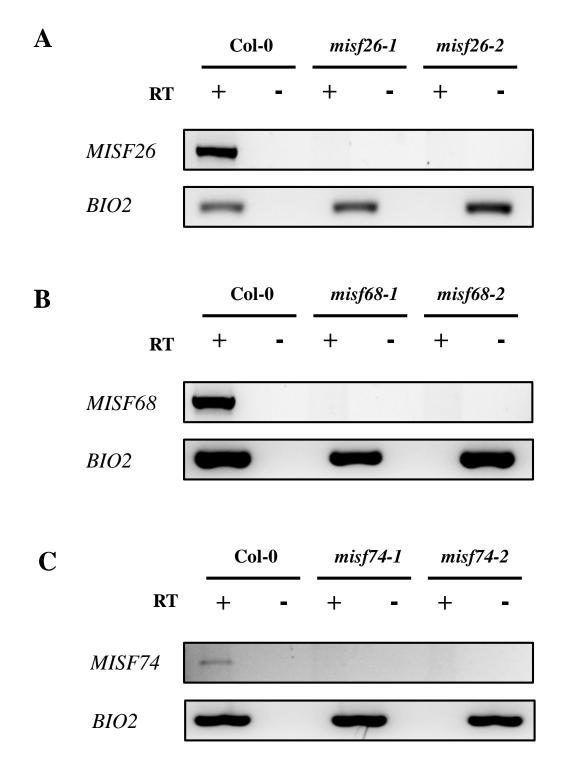
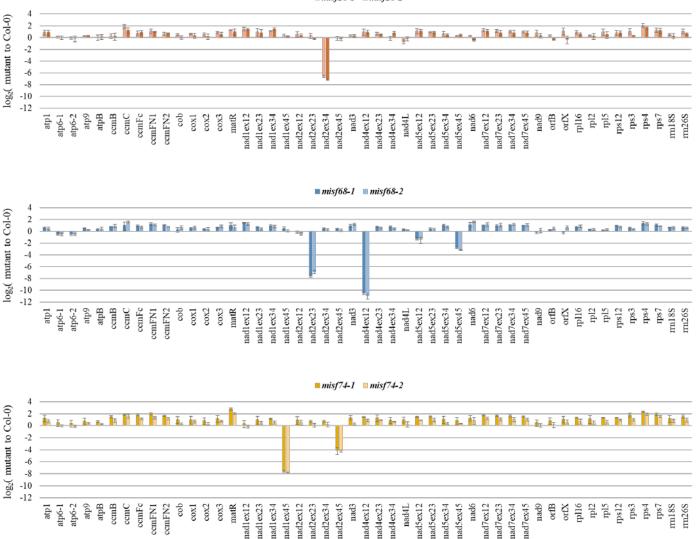
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Supplementary Table S1: Oligonucleotides used in this study.



Supplementary Figure S1: RT-PCR analysis of each *MISF* transcript in wild-type and *misf* mutants. cDNA prepared from both wild-type and *misf* plants were PCR amplified using primers located on both sides of the T-DNA insertion sites corresponding to each *misf* mutation, and the resulting amplification products were size fractionated on agarose gel. The amplification of *BIO2* cDNA was done separately to control the efficiency of the RT reaction. Reverse transcriptase was either added (RT+) or omitted (RT-) from the initial RT reaction.





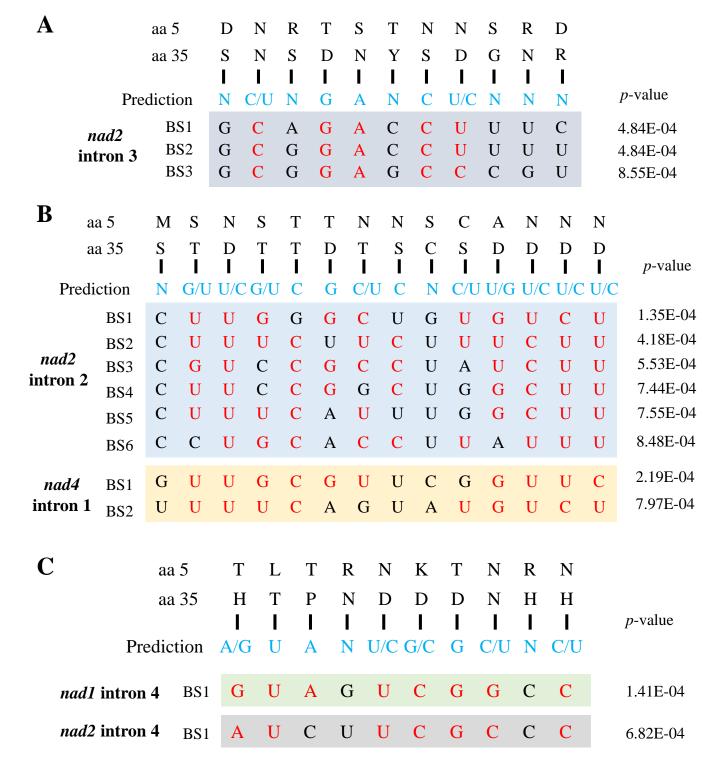
Supplementary Figure S2: Various mature mitochondrial transcripts underaccumulate in *misf* mutants. The steady state levels of mature mitochondrial mRNAs measured by quantitative RT-PCR in Col-0 and *misf* mutant plants. The histograms show the log₂ ratio of mature transcripts in mutants to the wild-type (Col-0). A single PCR was considered for mRNAs carrying no introns, whereas the accumulation of individual exons was analyzed for intron-containing transcripts. Three technical replicates of two independent biological repeats were used for each genotype; standard errors are indicated. The data were normalized to the nuclear 18S rRNA gene.

Supplementary Figure S3: Quantitative RT-PCR measuring the steady-state levels of mRNAs encoding alternative oxidases (AOX) and NADH-dehydrogenases (NDA, NDB and NDC). Three technical replicates of two independent biological repeats were used for each genotype; standard errors are indicated.

	Family	PPR										Maturase			Helicase		CRM	PORR	RCC1-like	mTERF	RAD52-like
	splicing factors	ABO5	ABO8	BIR6	MISF26	MISF68	MISF74	ОТР43	OTP439	SLO3	TANG2	nMAT1	nMAT2	nMAT4	РМН2	ABO6	mCSF1	WF9	RUG3	mTERF15	ODB1
	nad1 intron 1																				
	nad1 intron 2																				
	nad1 intron 3																				
	nad1 intron 4																				
	nad2 intron 1																				
	nad2 intron 2																				
	nad2 intron 3																				
	nad2 intron 4																				
	nad4 intron 1																				
	nad4 intron 2																				
group II introns	nad4 intron 3																				
	nad5 intron 1																				
	nad5 intron 2																				
	nad5 intron 3																				
	nad5 intron 4																				
	nad7 intron 1																				
	nad7 intron 2																				
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	nad7 intron 4																				
	ccmFC intron																				
	cox2 intron																				
	rpl2 intron																				
	rps3 intron																				

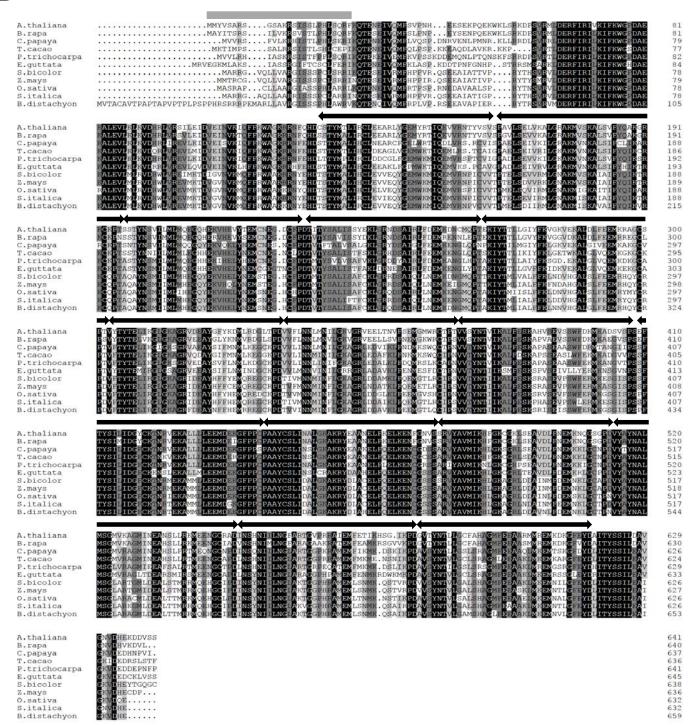
Supplementary Figure S4: Listing of known nucleus-encoded proteinaceous factors involved in the splicing of mitochondrial group Π introns in Arabidopsis.

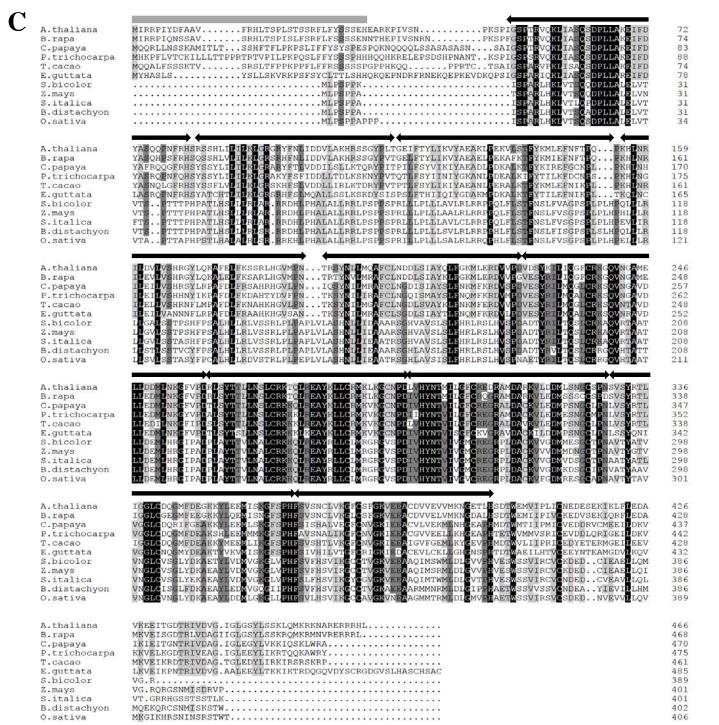
Different nucleus-encoded protein factors assisting mitochondrial intron splicing has been identified *via* genetic screens in Arabidopsis so far. Bold black indicate transspliced introns. Introns whose splicing is affected by the indicated transfactors are indicated by orange boxes. Corresponding references are cited in the introduction.



Supplementary Figure S5: Predictions of potential RNA target sites for each MISF protein according to the PPR code. The amino acids at positions 5 and 35 were extracted from each MISF PPR repeat and are listed from N- to C-terminus. These combinations were used to identify potential RNA recognition sites of each MISF protein within their intron targets according to the PPR code. The RNA targets within introns were shown in different background colors, and nucleotides that match the predicted RNA recognition code are marked in red. The *P-values* were determined using the FIMO program as previously described (Wang et al., 2017a). (A), (B) and (C) show the results for MISF26, MISF68 and MISF74, respectively.

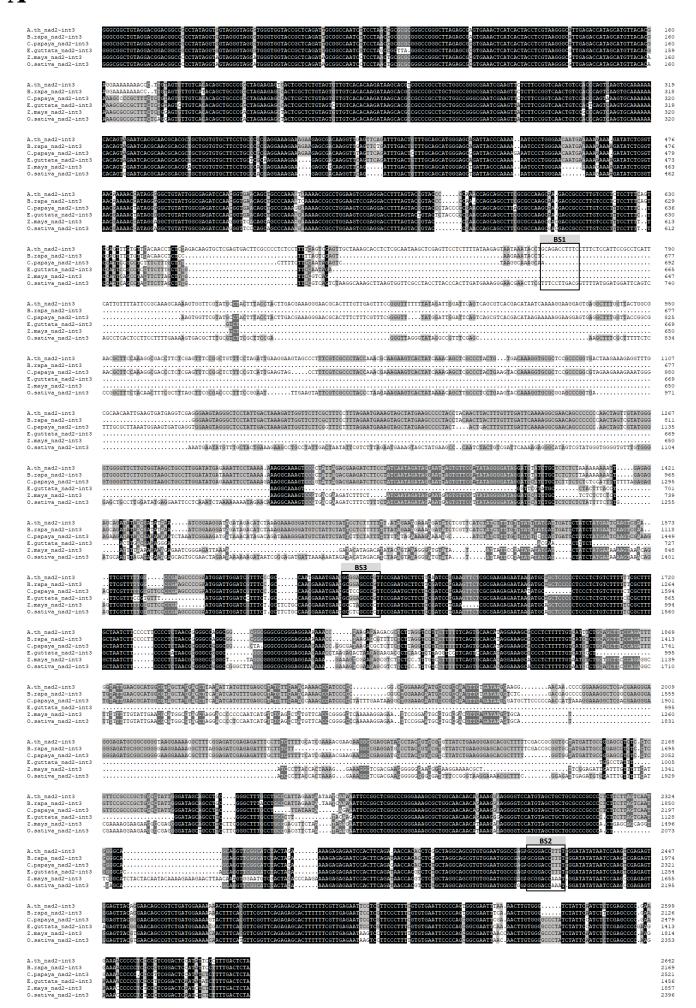
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	MASALRRLVEQQQWRYLVSQSTRSPKLIHGFFSFSSKTNPNPNKQQQILIDYISKSLQSNDTWETLSTKSS IDLSDSLIETILIFFK MRHLGEKQ.CRFFTLISKRSPRRTLGFFSSHSKD. QQQLLVDAISESLQNRDNFDTLSTKSS ANLSNSLVDQILIRFK MNSIRLPRPISSLSITRLLKTHTQIQPNTAT. TSLSTAICDSLRAASNWETLSAKSS IHLTDSLINTILIQIK MALINKRLGSLLVS. TSIHHNPFFALPKSTIIHSHS LSQLNNNDSIVNSISDSFKKTHNWGTITKTSSS VQLTHSLVQQVILQLK MALLRRTFPSLISTPLKYSIHPRVCSSWFEVARFLHD. GTKTESDTVVSSICDSLRRGYNWDTINRKSES LQLNNLLVKNVILELK MATTVGIFSREKETFIPFLPHIHTLTKFEINGNT. SSSSSITSLIDSLHERRSWKTISEKSS LKFTNQIVQNILIQLK MAVNA. VARRGGG. LHGRTPASALFTAATHAT.PQHISHYLAHQPRATWEAISAAPPAG. AVSHGHVDAVILSLA MAVNAG. GEVVARRGGG LLGRRPASALSTATTPAT.PQHISHYLAHQPRATWEAISAAPPAG. EMSHGHVDAVILSLA MAVNAG. GGVLAQRGGGGGNLFGLAAASALSASTSTAAATTPQRISHYLAHQPRATWEAISAAPPAG. SGAVPHGQVDAVILSLA MAVNAGAGGGGGGGGGGGLFGLAAASALSASTSTAAATTPQRISHYLAHPPRATWEAISAAPPAG. GGAVPHGQVDAVILSLA MTVRSWWGLSMALHAANCGGRVVARRG. LGRALASALL.CTTATT.PQRISHYLTHQPRATWEAISATEPAAG. GGAVPHGQVDAVILSLA MAAKSG. GRLVARRGG. AAVCAAAATALPTSTTAS.PQHIAHYLAHNPRVTWEAISATEPTAAVAAAAEAPDRQVVDAVILSLA	88 77 73 84 85 78 71 75 80 87 80
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	NPET. AKÇALSEF WSSHTRNLRHG. IKSYALTIK ILVKERLLIARADIESSLIN. SPPDSDLVDSLLDTEIS HPET. AKRALTEF WSSHARNLRHG. VSSVALAIR ILVKERLLIARADIESSLIN. SSPDSDLLDSLDTEVS EPTD. AKRALTEF WSSHARNLRHG. IRSYCIAIR ILVKERLLIARADIESSLIN. SSPDSDLLDSLDTY QVT. ARSALNEF WSSAKTKNHPPHG. IRSYCIAIR ILVKERLLIARADIESLKNTVIVH. GSREKKFSVVDSLLDTY QVT. ARSALNEF WSSAKSQNFKHQ. IYSYCIAIR ILVKERLILHSALKTSAP. DSTRSCILESLLGSVNVV. EPTD. AKRALGEF WSSAR, RNFYHG. VCSYCIMITI ILVKERLIKLAKADIESLIKKSVG. DPTKFLVLDSLLSSVKII. EPIN. SRKALNFF WSSAKANSSSS HSIRSYCILVE ILSKALTERNSVILESALSKSSS. APASCFILTFFAANEDSG RHPHASPE. PVAKNALTEF WSAAAASSSSPPPPSSHSLESYCILVE ILSKALTERNSVILESALSKROSSL. FSASCFILDFFAANEDSG KHLHSSSSSYSPELVARNALTEF WSAAAASSSSST. PHTLRAYCILVE ILSKALTRIASVILESALSKROSSL. FSASCFILDFFAANEDSG RHPHASPE. PVAKNALTEF WSAAAASSSSST. PHTLRAYCILVE ILSKALTRIASVILESALSKROSSL. FSASCFILDFFAANEDSG RHPHASPE. PVAKNALTEF WSAAAASSSSSST. PHTLRAYCILVE ILSKALTRIASVILESALAKHSSSS. PASSFILDAFFAANEDSG RHPHASPE. PVAKNALTEF WSAAAAVASLPS. SSHSLESYCILVE ILSKALTRIASVILESALAKHSSSS. PASSFILDAFFAANEDSG RHPHASPE. PVAKNALTEF WSAAAAASSPS. PHSLRSYCILVE ILSKALTRIASVILESALAKHSSSS. PASSFILDAFFAANEDSG KNSSPSSS. ETIAKNAHSFER SAAAAASSPS. PHSLRSYCILVE ILSKALTRIASVILESALARHSSST. PASSFILDAFFAANEDSG	160 149 151 159 159 155 155 167 169 176
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	.SSTPLVFLILVQCYRKIRYLELGFDVFKRICDCGFTLSVITINTLIHYSKSKIDDLVMRIYECAIDKRIYFNEITIRIMIQVICNEGRIKEVVDLLDR .CSTPLAFDILVQGYRKIRLLESGFDVFKNITDRGFSLSVITINTLIHYBSKSNRIDLVMRIYFDAAVDKRYVFNEITIRIMISAICKEGKIKEIVCLLDK .NSIPLVLLILVQGYRKLRMIEIGFDVCRIDEHGFSLTLLSFNALLHGILKSGENVMWWYYFNEITIRIMISAICKEGKIKIFIDMSDR .GSSTLVFLILVQGYRKLRMLEDGFEVCYJENHGFSLTLLSFNALLHGILKSGENVMWWYYFHMIEKRYFNEITIRIMISAICKEGKIQTVUDLLDK .ISSPLVFLLVQGYRKLRMEIGFDVCCTREEHRFTISLISFNTLIHVVGKSCKSPLAKKIYDHMLHRGTYFNEATIBESMISAICKEGKIQTIVMMLDK .ESVPFVFLIFIQTGAKLRMVDDILDACKLISRHDFPLSVISFNTILHVMIKSEKSRLVMSVYFHMISERMCFNEMTTRIMVSAICKEGKIGTIVMMLDK .ESVPFVFLIFIQTGAKLRMVDDILDACKLISRHDFPLSVISFNTILHVMIKSEKSRLVMSVYFHMISERMCFNEMTTRIMVSAICKEGKIERFLRIVDR TAATTRGLHILVHAYFRIRLPAEALEACRYIARRGVLFSLSAFNAALHAAQRAGSFGVAREVFILMTQKSMYANQSTUELVIGVISREGKIARTAALVER TAATTRGLHILVHAYFRIRLPGEALEACRYIARRGVLFSLSAFNAALHAAQRAGSFGVAREVFILMTQKSMYANQSTUELVIGVISREGKIARTAALVER TAATTRGLHILVHAYRRILPEEALEACRYIAQRGVVFSLPAFNAVLHAAQRTGRFGVAREVFILMTLKRYYANQSTUELVIGVISREGAIARMAALVER TGATTRGLHILVHAYRRILPEEALEACRYIAQRGVFSLPAFNAVLHAAQRTGRFGVAREVFILMTLKRYYANQSTUELVIGVISREGKIARTAALVER AAATTRGLHILVHSYROLFFEALEACRYIARRGVLFSLSAFNAALQSAQRAGAFGVAREVFILMTLKRYYANQSTUELVIGVISREGKIARTAALVER AAATTRGLHILVHSYROLFFEALEACRYIARRGVLFSLSAFNAALQSAQRAGAFGVAREVFILMTLKRYYANQSTUELVIGVISREGKIARTAALVER	259 248 250 258 258 254 255 267 269 276 264
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	ICERROL ESVIVNTS VERVLEEMRIEESMS LKRLUMKNMVVETTGYS IVWYAKAKEGDLVSARKVEDENLORGESANSE VYTVEVRVCEKGDVKEA HEGEROS. EPVIVNTS JALKVLDENRIDEGMS LKRLUMKNMVVETTGYS IVVYARIKEGDLVSARKVEDENLORGEDAAG WYTAFVKVYCHMIGDIDEA HEGROS. EPVIVNTS JETTAIEEGRIEEAMVETKRULOKNMILETIGES IFWYAKTRIGDESSAWOVEEM KREENRASELTSETGAYEEGRIEEA ILGAROS. EIVIVNTH VEKVIEEGRIEEGAMEE LKRMURKNILETVANYELIWYAKVRIGNINSAWOVEEM LKRGEIAASELFSSFIRAYEESGRIHEA HEGROS. EVVIVNTO VYRILEEGRVEEGALLKMMURKNMILETVANYELIWYAKVRIGNINSAWOVEEM LKRGEIAANSE VYTSETGAYKEERIEEDA MEGROS. ERIVINTO VYRMIEEGRVEEGALLKMMURKNMILETVANYELIWYAKVRIGNINSAWOVEEM LKRGEFEDA VEVSLEIGGAYKEERIEDA MEGROS. ERIVINTO VYRMIEER VEGILERA DORMALITISMS LVIFAKVRORIN DAAKEI YEEM LKRGEFEDA VEVSLEIGGAY EEGRIDDA HEGROS. EGIVAHVAUTIRMIEERE VEOQUILLIKRA JORNAILI DIAHSINVAH YORIGOLMSALEOODINVRRORRINAG VYTOLIRAHCOEGSMOKA HEGROOF. EGIVAHVAUTIRMIENSA VOOLIILIKRATORNILLI DIAHSI VHAHOOA DELKSACEORDINVRRORRINAG VYTOLIRAHCOEGSMOKA HEGROOF. EGIVAHVAUTIKHTEER KOOLIILIKRATORNILLI DIAHSI VHAHOOA DELKSACEORDINVRRORRINAG VYTOLIRAHCOEGSMOKA HEGROOF. ESIVAHVAUTIKHTEER KVEOVILLIKRATORNILLI DIAHSI VAA SLIVHAHOOA DELKSACEORDINVRROCRINAG VYTOLIRAHCORSVOKA HEGROOF. EGIVAHVAUTIKHTEER KVEOVILLIKRATORNILLI DIAHSI VAA SLIVHAYORDILKSA REQWIDIVRROCRINAG VYTOLIRAHCORSVOKA HEGROOF. EGIVAHVAUTIKHTEER KVEOVILLIKRATORNILLI DIAHSI LIVAY CRIGOLKAA FEORDINVRROCRINAG VYTOLIRAHCORSVOKA HEGROOF. EGIVAHVAUTIKHTEER KVEOVILLIKRATORNILLI DIAHSI LIVAY CRIGOLKAA FEORDINVRROCRINAG VYTOLIRAHCORSVOKA HEGROOF. EGIVAHVAUTIKHTEER KVEOVILLIKRATORNILLI DIAHSI LIVAY CRIGOLKAA FEORDINVRROCRINAG VYTORIGOLKAA TERMIOR TORDINAG KOOLIRAH T	358 347 349 357 357 354 354 366 368 375 363
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	ERILSENEESGVE YDEAFNOLIGG FAR. FGWEEKGLEYCEVMVTRGIMFSCSAFNEMVKSVSKIENVNRANEILAKSIDKG FVPDEHRYSHLIRG FIEG EKLIAEVESSGVIPYEETFNSTIVCAK, FGREGESLKYCEMVARGLIESCWAFNEMVERISKIENVNRANEILTKSIEKG FVPDEHRYSHLIEGFVRG NRLIEEVEIMGIKPYAETFNHLIHGCAR. AGRVEESLNLCEKMVKQGTLPSCSAFNEMVERGEEIGDSENANALIILLEKG FIPDEITYSHLIIGHVRG ENVIREVENMGIKPYODAFNYIIGEGAK. AGEWEKTSEYCKMMENGLVESCSFFNEMVRANGLGEIGDSENANALIILVIDKG FIPNETTYSHLIIGYGKE NQLIQEVENMGIKPYOTNFNILIEGGAK. AGRVEETSEYCKMMENGHVESLSAFNEMVGKLCRIEDVTRANEM NINLIEGFIADEITYSNLISYYAKN VGIFEEVESLGIKFFDEMFNHLIKGSSSYGRFEDGVVFCKKMMSMGLVPSCSSVNEMFGKLCENAKTKEADEILAILLEGFIADEITYSNLISYYAKN VGIFEEVESLGIKFFDEMFNHLIKGSSSYGRFEDGVVFCKKMMSMGLVPSCSSVNEMFGKLCENAKTKEADEILAILLEGFIADEITYSNLISYYAKN VGIFEEVESLGIKFFDEMFNHLIAGGFR. QGMIKEGLAYFDNMHEEGFVLDIGSCNEMLEGLONAGEVHRANNVITAMMOKGLIPDQUTYLSLINGYGKV ARCIQEVISMGIKFYDEMYNHLIAGGFR. QGMIKEGLAYFDNMHEEGFVLDIGSCNEMLEGLCSSGEVRKANNVITAMMOKGLIPDQUTYLSLINGYGKV MQIFEEVISIVIKMYDAMYSHITAGGFR. QGMIKEGLAYFDNMHEEGFVLDIGSCNEMLEALCDSGHVSKANEILTALMKGEVPDQNTYLEMTNAYGKV VQLICEVISMGIKPYDAMYSHITAGGFR. QGMKEGGSEYMDKNIHGGSVBDIGTCNDMLEALCDSGHVSKANEILTALMDKGEVPDQNTYLEMTNAYGKV VQLICEVISMGIKPYDAMYSHITAGGFR. QGMKEGGSEYMDKNIHGGSVBDISNCNDMIGGLSNAGEVRKVNDILTALMDKGIVPTRITYQKITLEYGKV	457 446 448 456 456 454 453 465 467 474 462
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	NDIDQALKLEYEMEYRKMSPGFEVERSITVGLCEKVEAGEKYLKIMKKRLIEENADIYDADIKAFQKIGDKTRADRVYNEKISVR. NDIEQALKMFYEMEYRNISPGFEVERSITVGLCEGGKVEAGEKYFRIMKRRLIGENAEIYEADIKAFRKTONKTRADRVYNEKISVR. GEVEEAFKLYYEMEYRALGIPLOVESELIGGLGRCEKPEGERYLRIMKDRFMVESVDVYHAIVGSYCRKGDKKRAVGVYREUMSEGLNPSGLYDLEDSE GNIQOVFRLYYEMEYRSLSPALFETSITRCLEHOCKIEEABRYLRIMKDRSVVLSEDIYEADITCHFEKGDKTCAGIIYNEVVARGMKPHKWGNFTKEV DVEGLTKLLFEMEYRSISPDALGESSVIVSFENHERLKEABRYLRINIGRSINFERDVYEADIKYFKEKOKRRALNIYNEVVSKGLKLCCSHYLGAST DDVEGLTKLLFEMEYRSISPDALGESSVIVSFENHERLKDABKYLGLMKSRSFIESPNVERDISGHLONGNETRARQIYGEVVGIG. GNNQGIVNLYHEMEHRCLDFGVEVETTIEKGLCCCGNLKEABKFLENKKTVALTSDLYDMFISGYCEKRNARRALWIYDMVVAENERLVFSAETFMML GDAQCIVNLYHEMEHRRLDPGVEVETTLEKGLCCGNLKEABKFLANKKKTLASTSDLYDMFISGYCEKRNARRALWIYDMVVAENERLVFSPETFMML GDAQGIIRIYHEMEHRCLNIGVDGSSIIRALCKGOLKEABKFLAILERKLLABTSSLYDLJISGYCEKRNAKRALWIYDMVMTENEKLVFSPETFMML GDAGGIIRIYHEMEHRCLNIGVDGSSIIRALCKGOLKEABKFLAILERKLLABTSSLYDLJISGNCEKGNTKKALWFYDMCMTENEKLVFSADFMML GDAGGIIRIYHEMBHRCLHFGVDGTTIIRGICCGGNLKEABKFLVWKKKAVAFTSDLYDLISSYSEKGNTKRALWFYDMCMTGDKLVFSADFFMML GDAGGIIRIYHEMBHRRLTHGVCGTSVIRGICCGGNLKEABKFLVWKKKAVAFTSDLYDLISSYSEKGNTKRALWFYDMCMTGDKLVFSADFFMML GDAGGIIRIYHEMBHRRLTHGVCGTSVIRGICCGGNLKEABKFLVWKKKAVAFTSDLYDLISSYSEKGNTKRALWFYDMCTAENEKLVFSADFFMML	544 533 548 556 556 541 553 565 567 574
A.thaliana B.rapa C.papaya T.cacao P.trichocarpa E.guttata S.bicolor Z.mays O.sativa S.italica B.distachyon	TVNMKALSAQKKIEYR TSMKDIAKTIPER VRRVIKVKNVCSPDS. VRRVIKVKNYYSPDS. VRRVIKVKNTYSPDS. VRRVIKVKNYYPPNN. VRRVIKVKNYYPPNN.	544 533 564 569 556 541 568 580 582 589 577

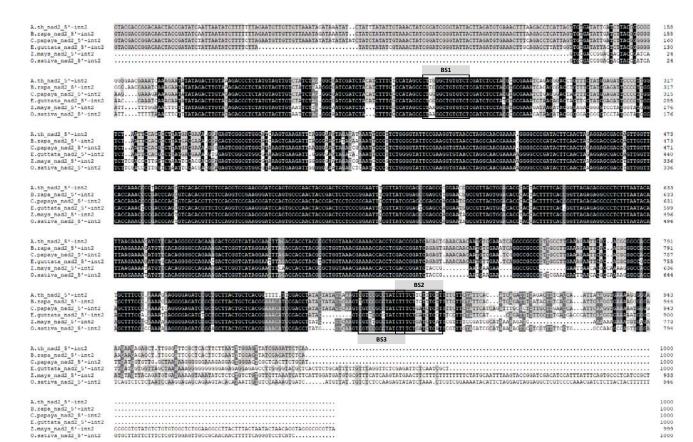


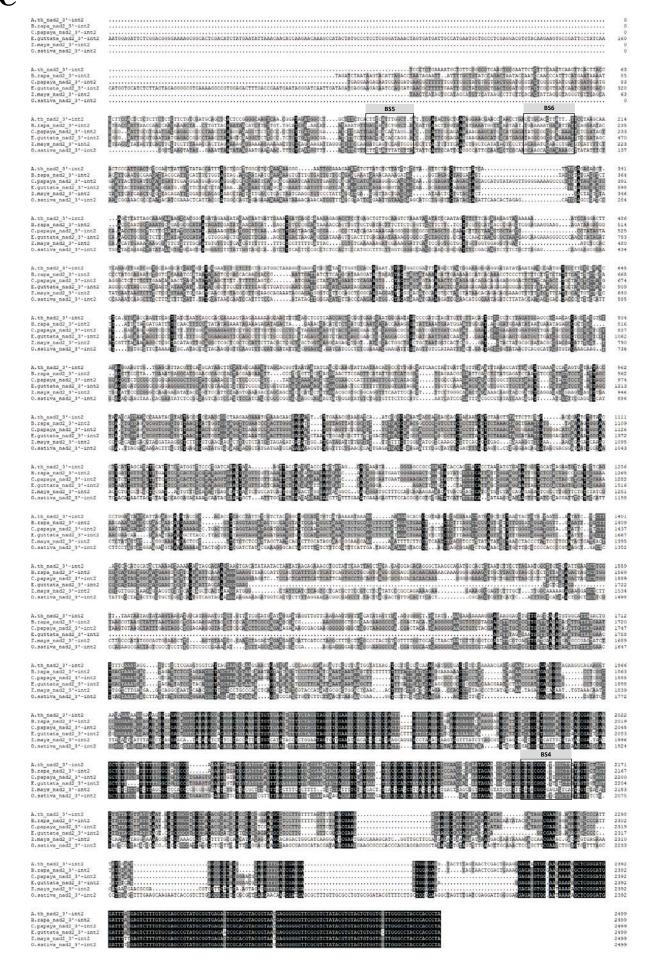


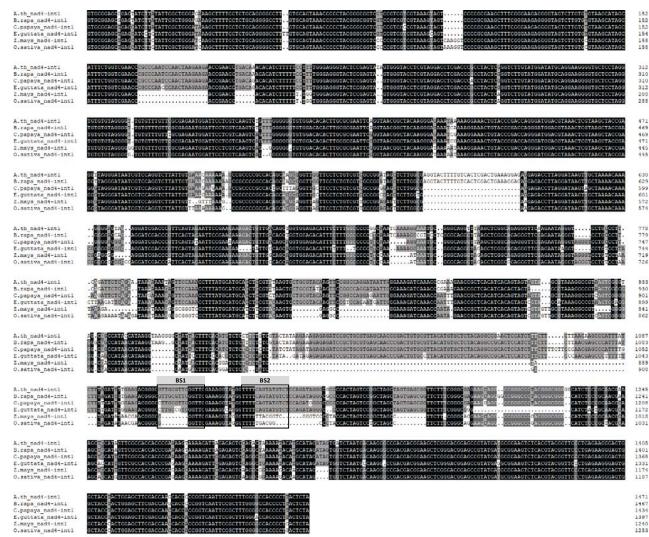
Supplementary Figure S6: Multiple sequence alignments of MISF protein homologs from a selection of dicot and monocot plant species. Amino acid sequence alignments of three MISF proteins from a representative selection of dicot (*Arabidopsis thaliana*, *Brassica rapa*, *Carica papaya*, *Theobroma cacao*, *Populus trichocarpa*, *Erythranthe guttata*) and monocot (*Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Setaria italic*, *Brachypodium distachyon*) plant species. Identical and conserved amino acids were shaded in black and grey respectively. PPR motifs are indicated by double arrow lines. Grey bars represent protein regions corresponding to mitochondrial targeting sequences, according to TargetP predictions. (A), (B) and (C) show the corresponding results from MISF26, MISF68 and MISF74 alignments.

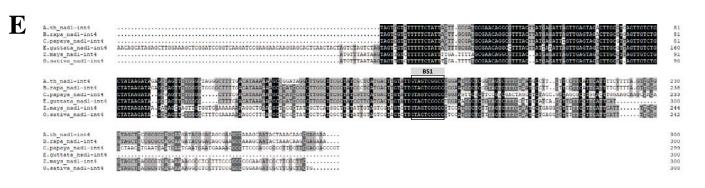
A



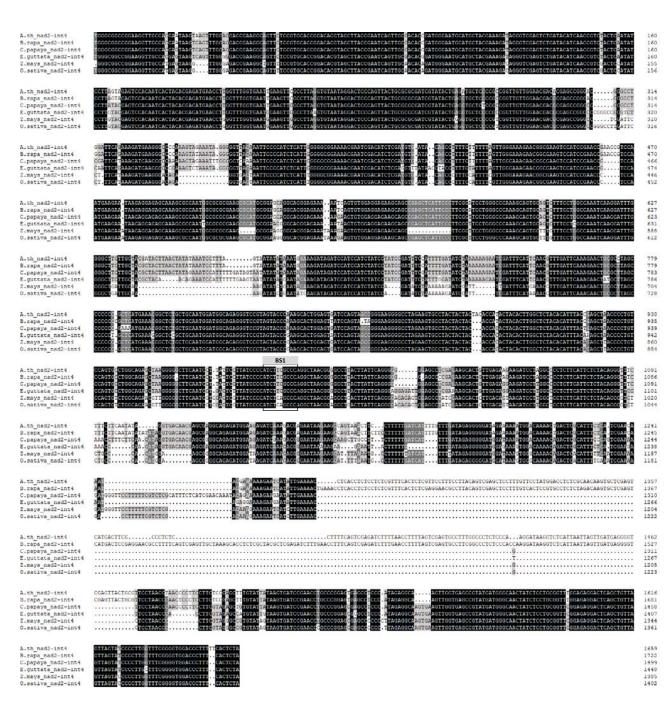








F



Supplementary Figure S7: Multiple sequence alignments analyzing conservation of the different predicted RNA targets of each MISF protein. Multiple sequence alignments of the different MISF protein intron targets from a representative selection of dicot (Arabidopsis thaliana, Brassica rapa and Carina papaya) and monocot (Oryza sativa, Zea mays) species. The sequences corresponding to the MISF predicted binding sites are shown in black boxes. (A): Alignment of *nad2* intron 3 sequences. BS1 to BS3 represent the three putative RNA binding sites of MISF26 in nad2 intron 3. (B) and (C): Sequence alignment of 5'- and 3'-portions of nad2 intron 2 (trans-intron). The six potential RNA binding sites of MISF68 are shown as BS1 to BS6. (D): Sequence alignment of nad4 intron 1. The two potential RNA binding sites of MISF68 are shown as BS1 to BS2. (E): Alignment of *nad1* intron 4 sequences. Since *nad1* intron 4 is fragmented in certain plant species, the sequences used for the alignment correspond only to the 300 nucleotides downstream of the matR open reading frame. The only putative RNA binding site of MISF74 is shown as BS1. (F): Alignment of nad2 intron 4 sequences. BS1 represents the single putative RNA binding site found for MISF74 in *nad2* intron 4.