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SUPPLEMENTARY ONLINE DATA

Different molecular bases underlie the mitochondrial respiratory activity in the homoeothermic spadices of *Symplocarpus renifolius* and the transiently thermogenic appendices of *Arum maculatum*

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SrNDA1     1 MGFSRIARSALRQSPGTNPASRIPSSNYMNQTASHCEGLPQSG--SYFFSVRYITTNSTLKHLP-SVGTMDRLSFESRGI  77
SrNDA2     1 MGFSRIARSALRQSPGTNPASRIPSSNYMNQTASHCKGLPQSG--SYFFSVRYITTNSTLKHLP-SVGTMDRLSFESRGI  77
AmNDA1     1 MGLSKIARSAWKTSLSADSVNRMLGSGHVIQTSVLLGDLLPSSARHHLLLARFVTTTGSTQRHPPGFESRDCEDRGSRGI  80
             ** * *****   *       *   *    **      *  *         *  **       *      *     ****

SrNDA1    78 SVTPQRQFPLAERVEEDPEVEVESRRLPGLGATKPGEKPRVVVLGTGWAGCRFLKGLDTKMYDVVCISPRNHMVFTPLLA 157
SrNDA2    78 SVTPQRQFPLAERVEEDSEVEVESRSLRGLGATKPGEKPRVVVLGTGWAGCRFLKGLDTKRYDVVCISPRNHMVFTPLLA 157
AmNDA1    81 SVTPHRQSPLAQPVEEEPDVENDRRQA-GLEATRPGEKPRVVVLGTGWAGCRFMKTLDTGVYDLVCISPRNHMVFTPLLA 159
             **** ** ***  ***   **   *   ** ** ******************* * ***  ** ****************
                                                    ++ + + +  +   +  +      + - -

SrNDA1   158 STCVGTLEFRSVAEPVSQIQAALGGAPNSYFYLASCMGIDTDKHEVYCEAVPG-AGLPNEPYGFTVAYDKLVIAAGAEPL 236
SrNDA2   158 STCVGTLEFRSVAEPVSQIQAALGGAPNSYFYLASCMGIDTDKHEVYCEAVPG-AGLPNEPYRFSVAYDKLVIAAGAEPL 236
AmNDA1   160 STCVGTLEFRSVAEPVSRIQTALATAPNSYFYLASCNGIDVDRHEVYCEAVPSSVGLSTEPYKFKVAYDKLVIAAGADPL 239
             ***************** ** **  *********** *** * *********   **  *** * ************ **

SrNDA1   237 TFNIKGVKEHAFFLREVNHAQEIRKKLLLNLMLSENPGISEEEKNRLLHCVVIGGGPTGVEFSGELSDFIMRDVRQRYSH 316
SrNDA2   237 TFNIKGVKEHAFFLREVNHAQEIRKKLLLNLMLSENPGISEEEKKRLLHCVVIGGGPTGVEFSGELSDFIMRDVRQRYSH 316
AmNDA1   240 TFNIKGVKEHAFFLREVNHAQEIRKKLLLNLMLSDNPGVSEEEKSRLLHCVVIGGGPTGVEFSGELSDFITRDVRQRYSH 319
             ********************************** *** ***** ************************* *********
                                                             +- + + +  +   -  +

SrNDA1   317 VKDYVRVTLIEANEILSSFDVSLRQYATNHLQKSGVRLVRGVVKEVLPKKIILNDGTDVPYGLLVWSTGVGASGFIKSLN 396
SrNDA2   317 VKDYVRVTLIEANEILSSFDVGLRQYATNHLQKSGVRLVRGVVKEVLPRKIILNDGTEVPYGLLVWSTGVGASGFIKSLN 396
AmNDA1   320 VKDYVRVTLIEANEILSSFDVSLRQYATNHLTKSGVRLVRGVVKEVMPKKILLSDGTEVPYGLLVWSTGVGPSGFTKSID 399
             ********************* ********* ************** * ** * *** ************* *** **
                   + + +

SrNDA1   397 LPKSPGGRIGIDEWMRVPSVEDVFALGDCAGFLEQTGRPVLPALAQVAEREGKYLAELFVKIGKQDGGRAFSAKDASLGD 476
SrNDA2   397 LPKSPGGRIGIDEWLRVPSVEDVFALGDCAGFLEQTGRPVLPALAQVAEREGKYLAELFVKIGKQDGGRAFSAKDASLGD 476
AmNDA1   400 LPKSPGGRIGIDEWLRVPSVDDVFALGDCAGFLQETGKPVLPALAQVAERQGKYLADLLNRIGKQNGGKAFASRGIDLRD 479
             ************** ***** ************  ** ************ ***** *   **** ** **      * *

SrNDA1   477 PFVYKHFGSMASVGGYKALVDLRQSKDAKGLTMAGFVSWFVWRSAYLTRVVSWRNRFYVATNWATTLVFGRDNSRIG    553
SrNDA1   477 PFVYKHFGSMASVGGYKALVDLRQSTDAKGPTLAGFGSWFIWRSAYLTRVVSWRNRFYVAANWATTLVFGRDNSRIG    553
AmNDA1   480 PFVYRHLGSMASVGRYKALVDLRQSKDAKGLAMAGFVSWFIWRSAYLTRVVSWRNRFYVAVNWATTLVFGRDNSRIG    556
             **** * ******* ********** ****   *** *** ******************* ****************
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Figure S1 Deduced amino acid sequences of SrNDA1, SrNDA2 and AmNDA1

Sequence alignments of SrNDA1, SrNDA2 and AmNDA1 were generated by GENETYX version 6.1. Underlined regions are mitochondrial targeting sequences predicted by Mitoprot. Asterisks indicate conserved amino acid residues among the three NDA sequences. Both the + and − signs under the alignments represent putative positions for two dinucleotide-fold fingerprints. Consistency of the respective positions in terms of these motifs is denoted by the different symbols: +, consistent; −, inconsistent.

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SrNDB1     1 MRFTGASSTIY----QAVRRNSAFSKLLVVFAVGSGGLVVYADANPNHGLITEQSEVSAKKKVVVLGTGWAGTSFLKNLD  76
SrNDB2     1 MRFTGASSTIY----QAVRRNSAFSKLLVVFAVGSGGLVVYADAKPNHSLITEQSEVPAKKKVVVLGTGWAGTSFLKNMD  76
AmNDB1     1 MPPTS---GFFDLASGLLRRNPGFSRLLVVFAVGGGGLVVYADSNADLGVPSEQNKMPTKKKVVILGTGWAGTSFLKNLD  77
             *  *              ***  ** ******** ********         **     ***** ************* *
                                                                          ++ + + +  +   +  +

SrNDB1    77 SSLYDVQVVSPRNYFAFTPLLPSVTCGTVDARSVVEPIRKIIKKKGGEIKFWEAECYNIDPGNKKVHCRSNIGTNLEGNG 156
SrNDB2    77 SSLYDVQVVSPRNYFAFTPLLPSVTCGTVDARSVVEPIRKIIKKKGGEIRFWEAECYNVDPVNKKVHCRSNIGTNLEGNG 156
AmNDB1    78 SSLYDVHVVSPRNYFAFTPLLPSVTCGTVDARSIVEPVRNIIRKKGGGVKFWEADCCKIDPTTKKVYCRSNVGTNLEGNG 157
             ****** ************************** *** * ** ****   **** *   **  *** **** ********
                  + + -

SrNDB1   157 EFVVEYDYLVVTLGAKANTFNTPGVVEHCHFLKEVEDALRIRRSVMDCFERASLPNLTDEERKTNLHFIIVGGGPTGVEF 236
SrNDB2   157 EFVVEYDYLVVALGAKANTFNTPGVVEHCHFLKEVEDALRIRRSVMDCFERASLPNLTDEERKTNLHFIIVGGGPTGVEF 236
AmNDB1   158 EFVVDYDYLVVAIGARANTFNTPGVTENCHFLKEVEDAQRIRRSVIDCFERASLPTLSDEERKKNLHFVIVGGGPTGVEF 237
             **** ******  ** ********* * ********** ****** ********* * ***** **** ***********
                                                                               ++ + + +  +

SrNDB1   237 AAELHDFVSEDIAKLYPRVRDLVKISVIEAGEHILTMFDKRITAFAEEKFKRDGIDVKTGYRVVKVSDKEIIMTGKATGE 316
SrNDB2   237 AAELHDFVSEDIAKLYPRVRDLVKISVIEAGEHILTMFDKRITAFAEEKFKREGIDVKTGYRVVKVSDKDIIMTGKATGE 316
AmNDB1   238 AAELHDFVNEDLSKLYPKVQELVKISVIEAGEHILTMFDKRITEFAEGKFQREGIDVKTGYKVVKVSDKSISMVSKEAGE 317
             ******** **  **** *  ********************** *** ** * ******** ******* * *  *  **
             +  +                    + + +

SrNDB1   317 TAVPYGMAVWSTGIGTRPVILDFMKQIGQVDRRVLATDEWLRVRGCDGVYALGDCATISQRKVMDDISAIFKVADKDNSG 396
SrNDB2   317 IAIPYGMAVWSTGIGTRPVILDFMKQIGQADRRVLATDEWLRVRGCDGVYALGDCATISQRKVMDDISAIFKVADKDNSG 396
AmNDB1   318 IDVPYGMAVWSTGIGTRPVILDFMKQIGQGDRRVLATDEWLRVRGCDGVYALGDCATITQRRVMDDISSIFRVADKDNSG 397
                ************************** **************************** ** ****** ** ********
                                                                                   # # ##

SrNDB1   397 TLTVKEIQDVLGDICERYPQVELYLKSKQMEDFVDLLEDSKGNAKKESIELDIEQFKKALAHVDFQVKNLPATAQVAAQQ 476
SrNDB2   397 TLTVKEIQDVLGDICERYPQVELYLKSKQMEDFVDLLEDSKGNAKKESIELDIEQFKKALAHVDFQVKNLPATAQVAAQQ 476
AmNDB1   398 TLTVKEINDVLGDICERYPQVELYLKSKQMKSLVDLLKESEGNAKKETMELNIEQFKKALENVDSQVKNLPATAQVASQQ 477
             ******* **********************   ****  * ******  ** ********  ** ************ **
              ##  ##

SrNDB1   477 GYYLARCFNVMKNVEENPEGPLRMRESGRHRFRPFRYKHLGQFAPLGGEQTAAQLPGDWISIGYGSQWLWYSVYASKQVS 556
SrNDB2   477 GYYLARCFNVMKNVEENPEGPLRMRESGRHRFRPFRYKHLGQFAPLGGEQTAAQLPGDWISIGYGSQWLWYSVYASKQVS 556
AmNDB1   478 GAYLARCFNLLQNIDVNPEGPIRIRESGRHRFRPFRYRHLGQFAPLGGEQTAAQLPGDWISIGYGSQWLWYSVYASKQVS 557
             * *******   *   ***** * ************* ******************************************

SrNDB1   557 WRTRLSVVSDWTRRFIFGRDSSCI                                                         580
SrNDB2   557 WRTRLSVVSDWTRRFIFGRDSSCI                                                         580
AmNDB1   558 WRTRVAVVSNWTRRFIFGRDSSSL                                                         581
             ****  *** ************
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Figure S2 Deduced amino acid sequences of SrNDB1, SrNDB2 and AmNDB1

Sequence alignments of SrNDB1, SrNDB2 and AmNDB1 shown in the same manner as described in Figure S1. In addition, # under the alignments indicates the putative positions for a Ca^{2+}-binding EF-hand motif.

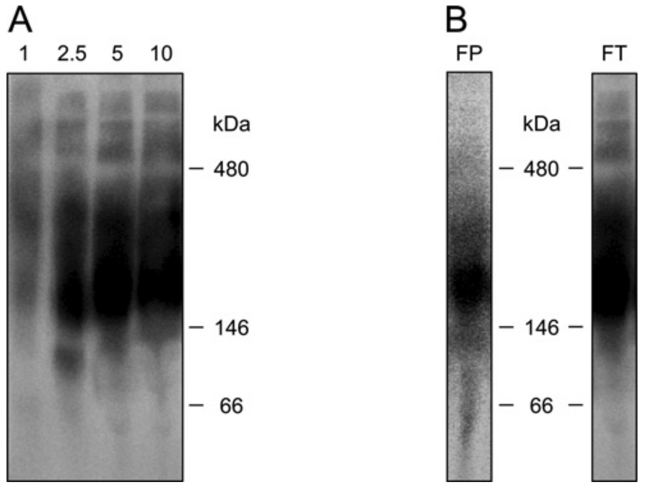

Figure S3 Immunological identification of AOX in *S. renifolius* mitochondria following 1D BN-PAGE under a range of experimental conditions

(**A**) *S. renifolius* mitochondria, which had experienced two freeze–thaw cycles, were solubilized using four different concentrations of digitonin, namely 1, 2.5, 5 and 10 g/g of protein, and were then resolved on to a 1D BN-PAGE gel. AOX was immunologically detected as shown in Figure 4(A) of the main text. The numbers above each lane indicate the digitonin concentrations. The molecular masses of standard proteins are indicated to the right-hand side of the image in kDa. The third lane from the left end is identical to the SR lane in Figure 4(A) of the main text. (**B**) Freshly-prepared *S. renifolius* mitochondria which had undergone no freeze–thaw cycles were solubilized using 5 g digitonin/g of protein, and were then run on a 1D BN-PAGE gel. AOX was immunologically detected as shown in Figure 4(A) of the main text. In the right image as a comparison, AOX was detected in mitochondria that had undergone two freeze–thaw cycles, which is identical to the SR lane in Figure 4(A) of the main text. The molecular masses of the standard proteins are shown between the two images in kDa. FP, freshly-prepared mitochondria; FT, mitochondria that had undergone two freeze–thaw cycles.

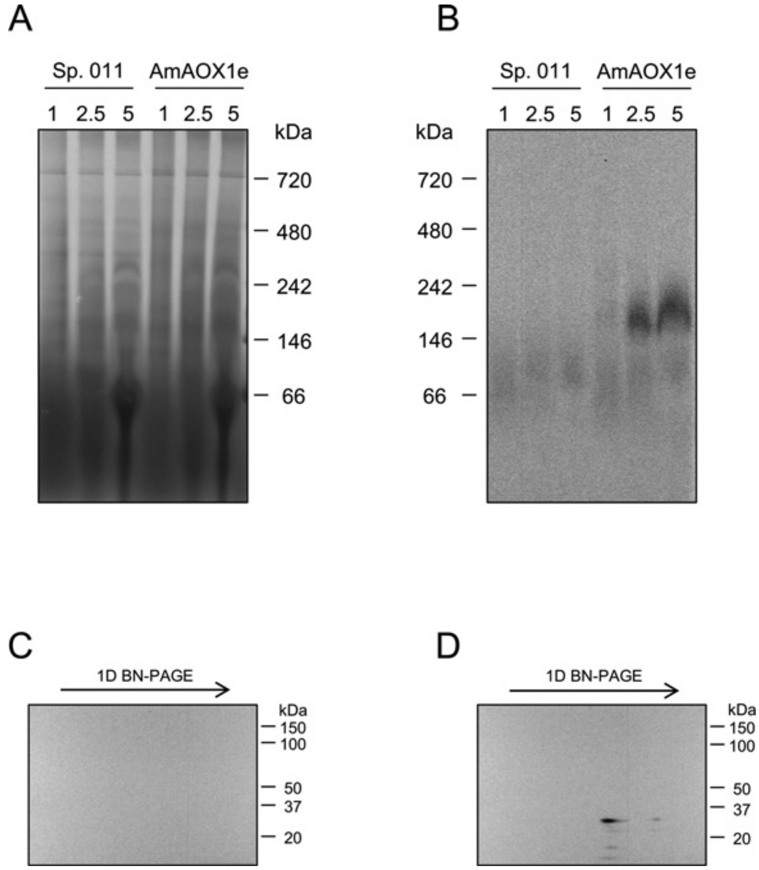

Figure S4 Immunological identification of AOX in mitochondria from non-transformed *S. pombe* and its transformant expressing AmAOX1e

Mitochondria that had undergone two freeze–thaw cycles were solubilized by digitonin and were subjected to Western blotting after either 1D BN-PAGE or 2D BN/SDS/PAGE. (**A**) The proteins were stained by Coomassie Brilliant Blue after 1D BN-PAGE to show their appropriate loading and separation. (**B**) Immunological detection of AOX after 1D BN-PAGE. Numbers above each lane represent digitonin concentrations in g/g of protein. (**C**) Immunological detection of AOX in mitochondria from the non-transformed yeast after 2D BN/SDS-PAGE. The mitochondria were solubilized using 5 g of digitonin/g of protein. The result is presented as in Figure 4(B) of the main text. (**D**) Immunological detection of AOX in mitochondria from the transformant after 2D BN/SDS/PAGE. The results were shown in the same manner as in (**C**).

Table S1 DNA sequences of the primers used for RT–PCR and real-time PCR

Name	Sequence (5′→3′)
NDAF1	AACCACATGGTNTTCAC
NDAF2	CAYGCTCAAGAAATAAGGAA
NDAF3	ATCTTCCTCCGCGAGGT
NDAR1	ACAGTWGCCATGCTTCC
NDAR2	CTTTCTGCAACCTGAGC
NDAR3	ACCTGTTCCTCCAGCTCAC
NDBF1	AAGGAAGTAGAGGATGCTCA
NDBF2	ACTGGWGTGGARTTTGC
NDBF3	CTGCWGAGCTTCATGAYT
NDBF4	CATGTTTGACAARAGAATYAC
NDBR1	GCRACCTGAGCTGTTGC
NDBR2	ACTTGYTTGCTKGCATA
NDBR3	CCTCCCARTGGAGCAAA
NDBR4	ATRTCTTCCATGACTTTGCG
EF1aF1	ACATTGTGGTCATTGGCCA
EF1aR1	ACCAGTTGGGTCCTTCTT
SrNDAF1	GTGAAGGATTACGTACGAGTTACCC
SrNDAF2	GATTACACAAGTGTTCGGCGTC
SrNDAR1	GGGTAACTCGTACGTAATCCTTCAC
SrNDAR2	CGTACAACAACTCATCAAGACACC
SrNDBF1	GGTATGGCTGTCTGGTCTACTGG
SrNDBF2	GTGATGGTGTGTATGCACTTGG
SrNDBF3	ATCTCCATTTCTGCTGTGGTTC
SrNDBR1	CCAAGTGCATACACACCATCAC
SrNDBR2	CCAGTAGACCAGACAGCCATACC
SrNDBR3	CAAGCATTCTGCAATTAGCAGC
AmNDAF1	GTAAAGGATTACGTTCGCGTGAC
AmNDAF2	GAACAAAGCGAGAAATTGACCTC
AmNDAF3	TGCGTTCTAGTGCCCTCCTC
AmNDAR1	GTCACGCGAACGTAATCCTTTAC
AmNDAR2	TTCCACACATCTGCAGTCTCC
AmNDAR3	TGTAACTAGTATCTGGTAGGTTTCTGC
AmNDBF1	GAATGGCTGTCTGGTCTACTGG
AmNDBF2	AGAATCTCTAGAGGGAACGCTTGG
AmNDBR1	CCAAGTGCATAAACCCCATCAC
AmNDBR2	ACCTGGGATTTTCGGTGATG
AmSrEF1aF1	CGTCAAGTTTGCCGAGATCC
AmSrEF1aR1	GCTCAGCCTTCAGCTTGTCAAG
AmSrEF1aR2	CTCTTGTTCATCTCAGCAGCTTC
SrEF1aF1	ACATGGGGCTCTTCAGCA
SrEF1aR1	CCACTCATTATAACTAGTCAACATGCG
AmEF1aF1	ACGGCCAATCTCTGGATC
AmEF1aR1	GGAACGGTGGACAAGGAG
rtNDAF1	GACTGTGCAGGTTTTCT
rtNDAR1	TAGAACCTGTTCCTCCAGC
rtNDBF1	CATATTTTGACCATGTTTGA
rtNDBR1	CAGTAGACCAGACAGCCAT
rtEF1aF1	AGCATTGTGGTCATTGG
rtEF1aR1	CTCTTGTTCATCTCAGCAG

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