MS ID#: JBC/2010/128611 Takenaka et al.

Legends to the supplemental figures

Figure S1. Growth of mutant plants in comparison to wild type plants. Homozygous mutant plants with the T-DNA insertions in the genes *MEF18*–*MEF22* grow in the greenhouse with phenotypes similar to the Col wild type plants. Pictures of several plants per pot are shown after three weeks of growth.

Figure S2. **Comparison of leaf numbers between mutant and wild type plants.** Numbers of leaves are counted in Col wt and in the mutant plants two and three weeks after seeding from five plants grown in two different pots. Overall numbers of leaves are similar between the mutants and the Col wt plants.

Figure S3. **Mutant plants produce viable pollen similar to wild type plants.** Individual pollen kernels were released from excised anthers from Col wt and the mutant plants and were stained for viability. Pollen viability was analysed by the staining procedure developed by Alexander (Alexander, M.P. (1969) *Stain Technol.* **44**, 117-122). The bar equals 20 µm.

Figure S4. Comparison of nucleotide and amino acid conservation around the editing sites affected in the *mef18* to *mef22* mutants. The left alignment compares the nucleotide conservation around the targeted editing sites between the respective mutant and edited sequences in *Arabidopsis* and other plants including dicots, monocots, a moss and a liverwort. The nucleotide whose editing is affected in the mutants is indicated by its position from the AUG. Shared nucleotide identities are highlighted in grey. Editing events are underlined and only the edited U-nucleotide is given. The amino acid alignments on right show the extensive conservation of shared amino acids between the different plants at and around the residue derived from the editing site altered in the mutants. The positions of these amino acids in the respective proteins are indicated by the numbers above. All sequences are shown 5' to 3' or N- to C-terminus from left to right.

Figure S5. Analysis of the T-DNA insertion sites in the mef18-mef22 mutant plants. The insertion sites of the T-DNA were investigated by PCR assays with right-border (RB) and left border (LB) primers and with primers in the predicted adjacent gene sequence. RB-gene primer combinations gave a product only for *mef22* with the downstream sequence. LB-gene primer combinations gave products with upstream and downstream sequences for *mef18-mef21* and only with the upstream sequence for mef22. All PCR products were sequenced which yielded the following information: In mef18 a left border - left border T-DNA fragment is inserted downstream of nucleotide 1148 from the A in the ATG start codon. Nucleotides 1149-1248 are deleted and instead 41 nucleotides of unknown origin are inserted. The lost nucleotides code for one of the central repeats (PPR). In mef19 a left border - left border T-DNA fragment is inserted in the E-domain downstream of nucleotide 1549 from the A in the ATG start codon. Nucleotides 1550-1599 are deleted. In mef20 a left border - left border T-DNA fragment is inserted just upstream of the first predicted L-type PPR downstream of nucleotide 228 from the A in the ATG start codon. Nucleotides 229-313 are deleted, while 773 nucleotides of plasmid vector origin are inserted just upstream of the T-DNA. In mef21 a left border - left border T-DNA fragment is inserted downstream of nucleotide 964 from the A in the ATG start codon. Nucleotides 965-979 are missing. In mef22 a left border - right border T-DNA fragment is inserted downstream of nucleotide 1840 from the A in the ATG start codon. Nucleotides 1841-2087 are deleted, which cover much of the E-domain and the N-terminus of the DYW-region. Downstream of the T-DNA 36 nucleotides of unknown origin are inserted.



Figure S1. Growth of mutant plants in comparison to wild type plants. Homozygous mutant plants with the T-DNA insertions in the genes *MEF18* –*MEF22* grow in the greenhouse with phenotypes similar to the Col wild type plants. Pictures of several plants per pot are shown after three weeks of growth.



Figure S2. Comparison of leaf numbers between mutant and wild type plants. Numbers of leaves are counted in Col wt and in the mutant plants two and three weeks after seeding from five plants grown in two different pots. Overall numbers of leaves are similar between the mutants and the Col wt plants.



Figure S3. **Mutant plants produce viable pollen similar to wild type plants.** Individual pollen kernels were released from excised anthers from Col wt and the mutant plants and were stained for viability. Pollen viability was analysed by the staining procedure developed by Alexander (Alexander, M.P. (1969) *Stain Technol.* **44,** 117-122). The bar equals 20 µm.

Figure S3. Takenaka et al.

mRNA

nad4

ccb206

rps4

сох3

nad3

amino acids

nad4- 1355	1355	445	452	459
mef18	A A C C U G A U U U C C U U C C A U A A A U U C U C	FLHK	FSD S NGR	ΕΥ <u></u> ΕΙ
Arabidopsis thaliana	A A C C U G A U U U C <mark>U U C C A U A A A U U C U C C G A U U <u>U</u> A A A U G G C A G A</mark>	FLHK	F	ΕV <u>F</u> Ι
Brassica oleracea	A A C C <mark>C</mark> G A U U U C <mark>C U C C A U A A A U U C U C C G A U U <u>U</u> A A A U G G C A G A</mark>	FLHK	F S D L N G R	e v <u>f</u> i
Vitis vinifera	A A C C C G A U U U C C U C C A U A A A U U C U C C G A U C <u>U</u> A A A U G G C A G A	FLHK	F S D L N G R	ΕVΕΙ
Triticum aestivum	A A C C <mark>C</mark> G A U U U C U U C C A U A A A U U C U C C G A U C U A A A U G G C A G A	FLYK	FSDLNGR	e v <u>f</u> i
Oryza sativa	A A C C C G A U U U C C U C C A U A A A U U C U C C G A U C U A A A U G G C A G A	FLHK	FSDLNGR	e v <u>f</u> i
Physcomitrella patens	A A C C C A A U U A C U U C C A A A A A	FILK	FSDLNGR	EVLI
Marchantia polymorpha	A A C C C A A U U U C U U U A U C A A A U U C U C	FILK	FSDLNRR	EVLI
<i>ccb206-</i> 566	566	182	189	196
mef19	G G U U U C A U G U U C U U U U U U G A U U G G U U A U U C U U U C U G U U U	VLLL	IGYSFLF	VSLF
Arabidopsis thaliana	<u> </u>	V T. T. T.	TGYFFLF	VSLF
Brassica oleracea	G G U U U C A U G U U U U U U U U G A U U G G U U A U U U C U U U C U G U U U	VLLL	IGYFFLF	VSLF
Vitis vinifera	GGUUUCAUGCUCCUUCAUCGAUUGGUUAUUUCUCUUUGUUC	APSS	IGYFSLF	VSFF
Triticum aestivum	G G U U U C A U G U U C U U U U U U G A U U G G G U A U U U U U	VLLL	IGYFFLF	VSLF
Oryza sativa	G G U U U C A U G U U C U U U U U G A U U G G G U A U U U U U U	VLLL	IGYFFLF	VSLF
Physcomitrella patens	G G U U U C A U G U U U G U U U U A A U G G G A U A U	VVLL	MGYLLLF	LFLY
Marchantia polymorpha	G G U U U C A U G U U C U U U U U A U U A A U G G G A U A U U U A C U U U U G U U U	VILL	MGYLLLF	LFFY
rpc4_ 226	000		70	
1034-220	226	69	/6	83
Mei2U	U A U A U A C A A U U A C A A A C U A C A C	LQTT	RKLPFFY	GDLP
Arabioopsis inaliana	U A U A C A A U U A C A A C U A C A C G A A G U U G U C C C U U U U U A	LQTT	RKLSFFY	GDLP
Brassica oleracea	U A C A U A C A A U U A C A A A C U A C A C	LQTT	RKLSFFY	GDLP
		LQTT	RKLSFFY	GDLP
Oryza sativa	A C A U A C C A U U A A A A A C U A C A C	LQTT	RKLSFFY	GDLP
Physcomitrella patens		LQTI	QKLSLFY	GKLP
магспапиа рокутогрпа	0 C C A U A C A A U U A C A A A C U A U A	ГÕЛІ	KKLSLFY	GNLP
<i>cox3</i> - 257	257	79	86	93
mef21	A A G U C G U A C A A U U A G G A C <u>U</u> U C G A U A U G G U U <mark>C</mark> U A U U C U G U U C	QLGL	RYGSILF	IVSE
Arabidopsis thaliana	A A G U C G U A C A A U U A G G A C <u>U</u> U C G A U A U G G U U <u>U</u> U A U U C U G U U C	QLGL	RYG <u>F</u> ILF	IVSE
Brassica oleracea	A A G U C G U A C A A U U A G G A C <u>U</u> U C G A U A U G G U U <u>U</u> U A U U C U G U U C	QLGL	RYG <u>F</u> ILF	IVSE
Vitis vinifera	A A G C U G U A C A A U U A G G A C <u>U</u> U C G A U A U G G U U <u>U</u> U A U U C U C U U	QLGL	RYG <u>F</u> ILF	IVSE
Triticum aestivum	A A G C U G U A C A A U U A G G A C <u>U</u> U C G A U A U G G U U <u>U</u> U A U U C U C U U	QLGL	RYG <u>F</u> ILF	IVSE
Oryza sativa	A A G C U G U A C A A U U A G G A C C U C G A U A U G G U U C U A U U C U C U U	QLGP	RYGSILF	IVSE
Physcomitrella patens	U U G U G G U C C A A U U A G G A C U U C G C U A U G G U A U G A U U U U G U U C	QLGL	RYGMILF	IVSE
Marchantia polymorpha	U U G U G G U C C A A U U A G G A C C U C G C U A U G G U A U A A U U C U U U U C	QLGL	RYGIILF	IVSE
nad3- 149	149	43	50	57
mef22	U G U C G G C C U A C G A A U G U G G U U U C G A U C C U U C G G U G A U G C C	YECG	FDPSGDA	RSRF
Arabidopsis thaliana	U G U C G G C C U A C G A A U G U G G U U U C G A U C C U U U C G G U G A U G C C	YECG	FDPFGDA	RSRF
Brassica oleracea	U G U C G G C C U A C G A U G U G G U U U C G A U C C U U U C G G U G A U G C C	YECG	FDPFGDA	RSRF
Vitis vinifera	UGUCGGCCUACGAAUGUGGUUUCGAUCCUUUCGGUGAUGCC	YECG	FDPFGDA	RSRF
Triticum aestivum	U G U C G G C C U A C G A U G U G G U U U C G A U C C C U U C G G U G A U G C C	YECG	FDPFGDA	RSRF
Orvza sativa	UGUCGGCCUACGAUGUGGUUUCGAUCCCUUCGUGAUGCC	YECG	FDPFGDA	RSRF
Physcomitrella patens	U G U C A G C U U A C G A A U G C G G G U U U G A U C C U U U G A U G A U G C C	YECG	FDPFDDA	RSRF
Marchantia polymorpha	U G U C A G C U U A C G A A U G U G G U U U G A U C C U U U G A U G A U G C U	YECG	FDPFDDA	RSRF

Figure S4. Comparison of nucleotide and amino acid conservation around the editing sites affected in the *mef18* to *mef22* mutants. The left alignment compares the nucleotide conservation around the targeted editing sites between the respective mutant and edited sequences in *Arabidopsis* and other plants including dicots, monocots, a moss and a liverwort. The nucleotide whose editing is affected in the mutants is indicated by its position from the AUG. Shared nucleotide identities are highlighted in grey. Editing events are underlined and only the edited U-nucleotide is given. The amino acid alignments on right show the extensive conservation of shared amino acids between the different plants at and around the residue derived from the editing site altered in the mutants. The positions of these amino acids in the respective proteins are indicated by the numbers above. All sequences are shown 5' to 3' or N- to C-terminus from left to right.

5

Figure S4. Takenaka et al.



Figure S5. Analysis of the T-DNA insertion sites in the *mef18-mef22* mutant plants. The insertion sites of the T-DNA were investigated by PCR assays with right-border (RB) and left border (LB) primers and with primers in the predicted adjacent gene sequence. RB-gene primer combinations gave a product only for *mef22* with the downstream sequence. LB-gene primer combinations gave products with upstream and downstream sequences for *mef18-mef21* and only with the upstream sequence for *mef22*. All PCR products were sequenced which yielded the following information: In *mef18* a left border - left border T-DNA fragment is inserted downstream of nucleotide 1148 from the A in the ATG start codon. Nucleotides 1149-1248 are deleted and instead 41 nucleotides of unknown origin are inserted. The lost nucleotides code for one of the central repeats (PPR). In *mef19* a left border - left border T-DNA fragment is inserted in the ATG start codon. Nucleotides 229 a left border - left border T-DNA fragment is inserted just upstream of nucleotide 228 from the A in the ATG start codon. Nucleotides 229-313 are deleted, while 773 nucleotides of plasmid vector origin are inserted just upstream of the T-DNA. In *mef21* a left border T-DNA fragment is inserted downstream of nucleotide 965-979 are missing. In *mef22* a left border T-DNA fragment is inserted downstream of nucleotide 1840 from the A in the ATG start codon. Nucleotides 1841-2087 are deleted, which cover much of the E-domain and the N-terminus of the DYW-region. Downstream of the T-DNA 36 nucleotides of unknown origin are inserted.

Figure S5. Takenaka et al.