

A

Sc	-----	0
At	-----	0
Pp	-----	0
Sv	-----	0
Ah	-----	0
Ls	-----	0
Cs	MIV-----YLTLFVELTLYFLVKSL---QEG-----TTIM---	27
Vv	MPTWLAEICSFELTLFE-LLRYAFRKNRETVRESKEIEAAVEEEEEEDSEDSVFAVMDHK	59
Ca	-----	0
Nb	-----	0
St	-----	0
Sc	-----	0
At	MDTGQSS-----IEPYQAKSFSRE--SSVASSLS-----LSRS--LPHLIDNDV---	40
Pp	---MAELVV-G-----GTGDESP-----	14
Sv	MDSSSARSNASAEENCL-GYTRSASMDSAAPPLAVRSGSMLSRSS-----RQG---	47
Ah	MDTGQSS-----IEPYRTKSFSRE---SSVASSLS-----LSRS--LPHLIDNDV---	40
Ls	MDYGHND-----CEPRRSGSFSRI---SSTNSSFRRQSFNFNQ-----SGANDDS--	42
Cs	DLE-----L-GVSGRRSFNRA-----GSAGSSLRRRSFGFTNA-ESTQK--DDDL--	68
Vv	TND-----L-EETRSGSFGRS-----SSAGSSLRRRSCGLPGP-VPPQM--DDDI--	100
Ca	MRKDR--EEMTLGGPRRSKSLNRA-----SSVKSSLRRHSFGS-FHV-SHNQG-DDDT--	48
Nb	MDCENKDS---DLRKIGS-----SRT---SSATSSLRRRSLSLSFSLPKRLNDDDDDE	46
St	MDSSENKDS---ELRKFGS-----SRT---SSASSLRRRSLSLSLSLPKQLNDDDD--	45
Sc	-----	0
At	DSESVSEAGDIGDRSLRRRHS-A-GR---SSR-L-----	68
Pp	DRLTLTQCMSLPDVAQ--RRHSWHADMSQNQATIPNF-----DILRAQKIEVTEV----	62
Sv	SRGSISLSREMGDSILNSMRHSLQS---ADQ-LLGDVDSVLAQLIESGRILAPEVDANE	103
Ah	DSESVSEAGDIGDRSLRRRHS-A-GR---SSR-L-----	68
Ls	DHLSVSEAGDIGDRALHSKRHS-GNE---SGR-P-----	71
Cs	ESETVSEAGDTGDRALRSNSYSESS---RQQ-L-----	97
Vv	ESETVSEQAGDIGDRALHSNRISSESS---SFR-L-----	129
Ca	ESEAVSEAGDIGDRALHSRRSSESG---RGR-I-----	77
Nb	ESESVSEAGDIGDRELQSNRYSGSG---RLR-L-----	75
St	ENESVSEAGDIGDRELQSNRYSGSG---RLR-L-----	74
Sc	-----	6
At	SADDFIEQGTHDTSRQEQ-----DILHDLRAFNTASVNKTLPEEDITASPLPTKSLLSPE	122
Pp	DIASGIELYQFRSGPEDASDTAASASAAEKRESQDDIICFDLPCTQNTFPLERREL----	116
Sv	DIANALEHHKVGPLPDDAMMPNNGKIAAAP-----VSSV-----ETKDIISNAP	147
Ah	SADDLMEQGTHDTSRQEQ-----DVLHDLRAFNTASVNKPLPEEDITASPLPTKSLLSPE	122
Ls	VFP--FEENLVLPIQEHFSKE-----SHL-PTPSPSPDAIL--H	106
Cs	PVDNVTETGSVVP I PEDALFRPHGFWSHDPTALAVASPVFPSTM-ETISPLSTEAVI--H	154
Vv	SFDNLLDNGVVVPI PENSLLKPHEIWFRRDATANT-VSPASPLPL-EIISPLSSDAMV--Y	185
Ca	SIDNVVESGVIVPILEDASLQPTGFSPHKTTTTFNTEALPSSTL-DIALGSSADGTA--Q	134
Nb	IGENAPEHGVVVPISVEWSDPNAMSSVA-----PV-----SSSKLIL--Q	114
St	IGENAPEHGVVVPISED-----TSSVT-----PV-----FPIKLIS--Q	106
Sc	ISN-----HKLSHYIHVFCTFTTFCILGTETRQAITALSTYTPAFVVTAP--TV---	52
At	INNSGKEEERVLPKSLEYISCLIH LAVFGIFGAITRYLLQK LFGPTGARVTS DGSILYLD	182
Pp	QSTNVEEESERLSPTLEYVSALAHLTTFGILGVCIRHGLEL LFS-GIANVTSENSPLFID	175
Sv	ADSSTKVEPYKLSLR LDYAAYMIHLAVFGFFGVFTRYGLQK LFGPECLALTS DQSPLYPD	207
Ah	INNPEKEEERVLPKSLDYISCLIH LAVFGIFGAITRYLLQK LFGPTGARVTS DGSILYLD	182
Ls	DKGQNDCKKELPWFMTYISSRVHLAVLGILGVLTRYLLEK LFGPQVVGATSDNSYMYVD	166
Cs	SDDKQENRKELPWL LDYTSCLLHLAVFGILGVLTRYLLQK LFGPGVIGATSDQSILYLD	214
Vv	SADKDEDNKKELPWLVEYISCLIH LAVLGILGALT RYLLQK LFGPGVASLTS NQSILYLD	245
Ca	LEDKKNENEKAIPWILEYISCLSFLALFGILGVITRFYLLQK LFGPRVIGATSDQSYMPD	194
Nb	TEHKKNEEKTEVPWVLEYASCLLFLAVFGILGVLLRYGLQILFGPRIVGATSDHSYMYLD	174
St	PDHKKNDEKTEVPWVLEYTSCLLFLAVFGILGVLLRYGLQK LFGPGIVGATSDHSYMYLD	166
	* * GxxxR * * *	
Sc	LWSNCSSCMLMGIMQSLNAYTWMKDHQVFLGVTTGYCGALSSFSSMLLEMFEHSTNLTN	112

At LPSNMVGSFSLMGWFGVVFKAADIARVSEFVAIGLSTGYLGSLLTTFSGWNQKMLDLSADGQ- 241
 Pp LPANMLGCFMGWVGVSLKKEIANFSELLAIGLSTGLMGSIITTYASWNQAMIFLVTKGF- 234
 Sv LPSNMLGSFSLMGWFGIIFKADIRHISDHLIVGITTYMGSLTTFSGWNQKMGVLSSTGH- 266
 Ah LPSNMVGSFSLMGWFGIVFKADIARVSEFVAIGLSTGYLGSLLTTFSGWNQKMLDLSADGQ- 241
 Ls LPPNMIGSFSLMGWFGVVFKGDISKFSPELAVGLTTGYLGSLLTTFSGWNQKMLELSVNGQ- 225
 Cs LPSNMVGSFSLMGWFGVVFKGDISRVDHLAIGLTTGYLGSLLTTFSGWNQKMLDLSVQVK- 273
 Vv LPSNMAGSFSLMGWVGWVVFKGDISSVSDQLAIGLTTGYLGSLLTTFSGWNQKMLDLSVDGQ- 304
 Ca LPSNMVGSFSLMGWFGVVFKGDISEVSDNLAIGLTTGFGLSLLTTFSGWNQKMLDLSVRGH- 253
 Nb LPSNMVGSFSLMGWFGAVFKEDISRFSSTLAIIGLSTGFGLSLLTTFSGWNQKMLDLSVEDQ- 233
 St LPSNMVGSFSLMGWFGVVFKEDISRISSQLAIGLSTGFGLSLLTTFSGWNQKMLELSVEGQ- 225

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Sc GNIANHTKLPNRAYGIMEFLSVLLVHLMVSMGSLIFGRQLGKEVIVAYGSSSFKPYTPP 172
 At -----WVYAVLGFLGLFLTSYSIILGVETAKGFKWLLHRRAS-SED--- 282
 Pp -----WVRSIVSLIIGMEMSQMSELLVIGIDSATFLKSGLMHPKRVRI-- 277
 Sv -----WVYAIAGIVLGMFIVNESITITIGAETGERLRSLLILKYIRERSSI-- 309
 Ah -----WVYAVLGFLGLFLTSYSIILGVETAKGFKWLLHRRAS-SEE--- 282
 Ls -----WVFSFLGFLGLFLVAYSFIFGVETAKGVKWFVFNKTNL-NSKC-- 267
 Cs -----WGFVVLGFLVGMILADYSIKFIRTAQGLKWILEMSNL-FPVS-- 315
 Vv -----WVFSVLGFLIGLFLAAYSIIIFGVETAKGFRWLLKRLNS-GGKR-- 346
 Ca -----WVFAILGILIGLFLAAYSIIIFGIETAKGFKWLLKRFIR-EMK--- 294
 Nb -----WVFSVLGFLIGLFLVAYSFIFGVETAKGVRWLYRRATM-NSF--- 274
 St -----WVFSVLGFLGLFLVAYSIIIFGIETAKGVNWLYKRTNM-NSS--- 266

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Sc SDTVKENAGDVDTQEMEKNILEFKFKTPAPFFKFFFDIVDKLAYALAFPLIILFVVLCA- 231
 At ----KFHCLKVNTFQSH-----IVSLTLMMLLLVALLTAS 313
 Pp GWQKHWN-SPETLHRR-----KAGMLVFLAISIIILWAAC 311
 Sv GHKYDWEQWRVDTRTKQ-----SVLLSVMMLMSFLWILS 344
 Ah ----KNSCIKVNTFRSH-----IVSMTLMMLLLVALLTAS 313
 Ls GFELKNN-----IISE-----SILIVLMITLLGLLWVVS 296
 Cs GLPNSKSNWKADYCKRQ-----VAVLVVLLLMLGLLWVVS 350
 Vv GILRSNGNWRVDSCKRH-----LAVMVVGMILIGLLYSLC 381
 Ca -FFSSIIYSWDLQYNTY-----LTGTVVLLLILGVVWVVC 328
 Nb -NSCTDYHWRVDSYKRH-----VLVILFLLFILFLLWVVS 308
 St -NSGTDCHWRVDSCKHH-----LIVILFLLLILASLVVVS 300

Sc -----YYENYSRGWTLPLCLFGIFAGFLRYWLAEMFNKTN-----KKFPLGTFLAN 277
 At SILLVKEFDKGTSEAQLWFGCLVAAPGVWLRWFLARLNGRGLGKDSQNLRWVPGFTLIAN 373
 Pp LVLTVTDVNSYV-RRRLWLA CMVGPVWLRWFLARLNGQIGRN-HHLKWLPIGTLITN 369
 Sv IALAVVKVRSADGAVLWVMGCSVPPGVWLRWFLARLNGQIGKQ-GSFKWLPIGTLAAN 403
 Ah SILFVKEFDKGTSEAQLWLGCLVAAPGVWLRWFLARLNGRGLGKDRQNLKWPFGFTLIAN 373
 Ls IALLKRDFESDKSTSQLWLG CIVGPIGVWIRWFYLAKFNGKGLGRQ-HIMKWPFGFTLIAN 355
 Cs AALEKRDFHSGSSEAQLWLA CIVGPFVWIRWFLARLNGRGLGKA-GLWKWLPIGTLIAN 409
 Vv GILEKREFQSGSSEAQLWLG CMVAPLVWIRWFLARFNGRGLGKA-GLFKWVPGFTLIAN 440
 Ca IAFTKKQFYSDHSHAELLLG CIVGPFVWIRWFLARLNGRGLGKA-PVLRWVPGFTLIAN 387
 Nb IGLEVTEFSSGSTKAQLWLA CIVGPFVWIRWFLARLNGRGLGKS-GLLKWLPIGTLIAN 366
 St IGLEVNEFSSGSPKAQLWLA CIVGPFVWIRWFLARLNGRGLGKS-GLLKWPFGFTLIAN 359

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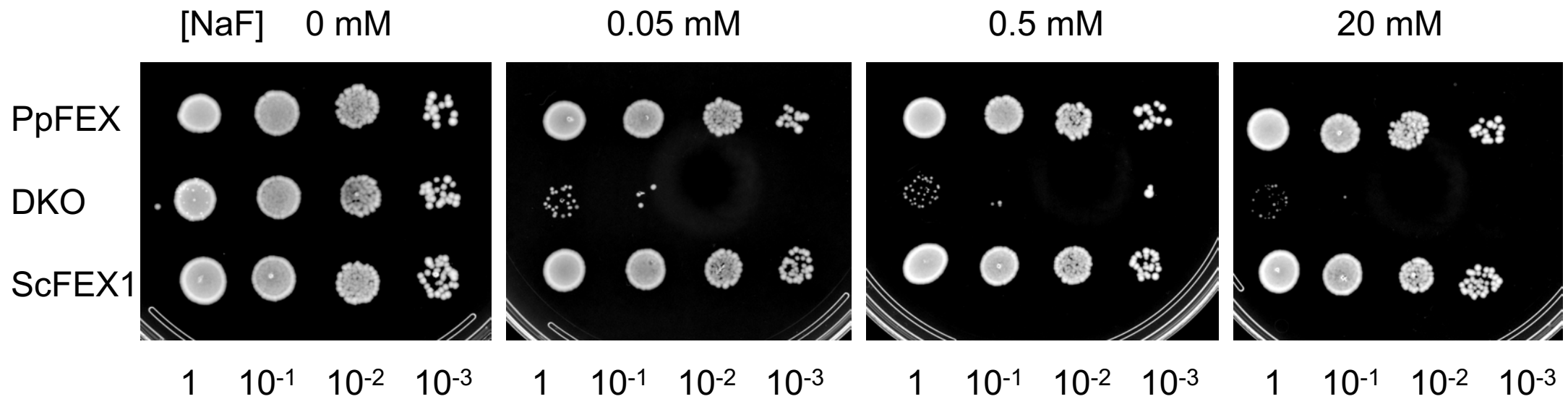
Sc VFATLLIGIFTMVQRGKKHFSTDVPIVNSLNSCHIVSALISGFCGTLSTISTFINEGYKL 337
 At VVAACVMAALATLKK-----SVNTRTCNTVASSIQFLLGCLSTVSTFMAEFNAM 423
 Pp LIASTLEAVLAVVIL-----V-SPDSSLARGLQVGLLGCMSTVSTFTTEVHSL 417
 Sv VLAAGVMAALAVTSK-----VVHTKQAMTILSGIQLGFLGCLSTVSTFAAEVYTM 453
 Ah VVAACVMAALATVKK-----SVNSRTCHTVASSIQFLLGCLSTVSTFMAEFNAM 423
 Ls VASACIMATFATLKK-----AVKDEHFDIVATGIQFGLGCLSTVSTFIAEFGAM 405
 Cs VSAACVMAALAIKK-----AVNTTTCHTVATGIQFGLMGCLSTVSTFMAEFHAM 459
 Vv VSAASVMAGLSTLKK-----AVDSKNCDTIVTGIQFGLGCLSTVSTFIAEFNAM 490
 Ca ILAADVMAALATLKK-----AVNTDKCNTVATGIQLGFLGCLSTVSTFIAEFNAM 437

| | | | | | | | |
|----|-----|---------------|---------------------------------|-------------------|----------------|-------------|-----|
| Nt | VL | AACVMAVLATLKK | ----- | AVKTQTCETVASGIQF | GLLGCLSTVSTFIA | EFHAM | 416 |
| St | VS | AACIMAALATLKK | ----- | AVNNTETCDTVASGIQF | GLLGCLSTVSTFIA | EFHAM | 409 |
| | | * | | | | *LSTxSTFxxE | |
| Sc | --- | SFINMLI | YYTVSIAISYCLLVITLGSYAWTRGLTNPIC | ----- | | | 375 |
| At | RES | DYPWRAYAY | ASFTIVVSFAIGTIIYSVPVWVIGFS | ----- | | | 461 |
| Pp | RRG | SQWRAYAY | FCIMVFSTYCMGVLVYTIAMTRHYSV | ----- | | | 456 |
| Sv | RRS | GQIARAFV | YAASTFLLSFVLGTLIYSVPVWEKHYG | ----- | | | 491 |
| Ah | RES | DYPWRAYAY | ASFTIAVSFAIGTVIYSVPVWVIGFS | ----- | | | 461 |
| Ls | RES | VDPWKAYV | YAFTTMIVSFVFGTFIYSVPVWAKSWS | ----- | | | 443 |
| Cs | SQS | NHPWRAEV | YAVITIALSFSLGTLIYSVPDWVMGYD | ----- | | | 497 |
| Vv | RES | HHPWRAYAY | AMLTIFLSFGLGTLIYSVPVWTKGYD | ----- | | | 528 |
| Ca | RQS | SHTWRAYAY | ATSTIVISFALGTLIYSVPVWVKGYD | ----- | | | 475 |
| Nb | RES | KYPWRAYV | YALSTMLMSFILGTLIYSVFWTENS | ----- | | | 455 |
| St | RG | SKYPWRAYV | YALCTTLISFILGTLIYSLPVWVENFN | ----- | | | 447 |
| | | * | | * | * | | |

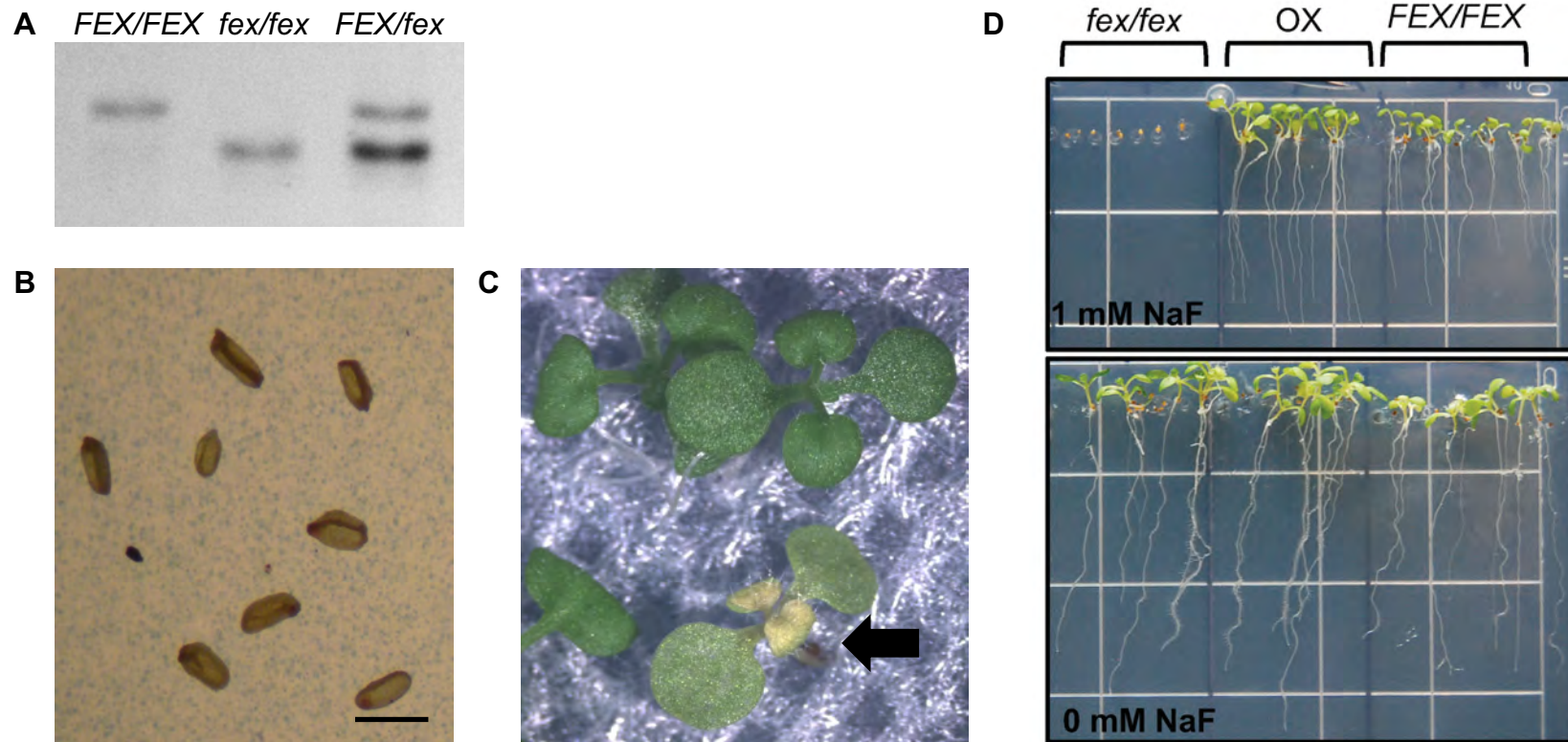
B

| | | |
|-------------|--|-----|
| AtFEX | MDTGQSSIEPYQAKSFSRESSVASSLSLSRSLPHLIDNDVDSESVSEAGDIGDRSLRRRH | 60 |
| Atfex | MDTGQSSIEPYQAKSFSRESSVASSLSLSRSLPHLIDNDVDSESVFRGRGYWGPLTSEKA | 60 |
| PlusIntron2 | ----- | 0 |
| Del17 | -----MDTSVASSLSLSRSLPHLIDNDVDSESVSEAGDIGDRSLRRRH | 43 |
| Del129 | ----- | 0 |
| | | |
| AtFEX | SAGRSSRLSADDFIEQGTHDTSRQEQDILHDLRAFNTASVNKTLPEEDITASPLPTKSLLS | 120 |
| Atfex | ----- | 60 |
| PlusIntron2 | ----- | 0 |
| Del17 | SAGRSSRLSADDFIEQGTHDTSRQEQDILHDLRAFNTASVNKTLPEEDITASPLPTKSLLS | 103 |
| Del129 | ----- | 0 |
| | | |
| AtFEX | PEINNSGKEEERVLPKSLEYISCLIH LAVFGIFGAI TRYLLQK LFGPTGARVTS DGSILY | 180 |
| Atfex | ----- | 60 |
| PlusIntron2 | -----MPNSF---GCF-----WDFW-GHYEIFAAKIVGPTGARVTS DGSILY | 38 |
| Del17 | PEINNSGKEEERVLPKSLEYISCLIH LAVFGIFGAI TRYLLQK LFGPTGARVTS DGSILY | 163 |
| Del129 | -----MDTEERVLPKSLEYISCLIH LAVFGIFGAI TRYLLQK LFGPTGATVTS DGSILY | 54 |

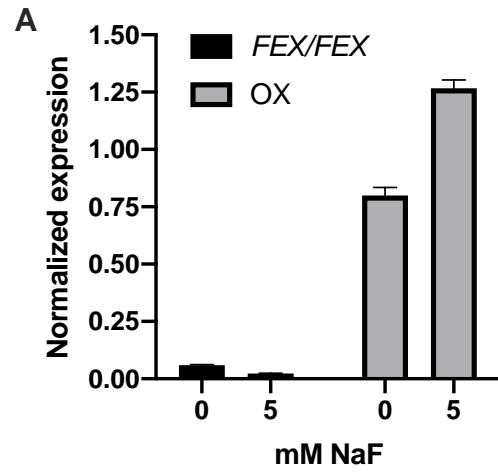
Supplemental Figure S1: Predicted protein alignment of putative plant and yeast FEX1 sequences used in this study. (A) Alignment of predicted FEX sequences. Grey boxes indicate proposed transmembrane domains as predicted by TCoffee PSI/TM (Notredame et al., 2000) and TMHMM2.0 (Möller et al., 2001). Red lettering denotes conserved amino acids in all sequences. Stars below an amino acid indicate conservation in all plant sequences, but not in yeast FEX1. Important conserved sequences for FEX proteins are indicated underneath the alignment. Conserved Asparagines (N56 and N277) in yeast (Sc) are blue. Corresponding blue N186 and N373 in AtFEX were changed to A to test functionality of Pores I and II respectively. In order of appearance: *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Physcomitrium patens*, *Setaria viridis*, *Arabidopsis halleri*, *Lactuca sativa*, *Camellia sinensis*, *Vitis vinifera*, *Coffea arabica*, *Nicotiana benthamiana*, and *Solanum tuberosum*. (B) Alignment of the predicted N-terminal regions encoded by the predicted sequence of the CRISPR/Cas9 mutation (*Atfex*) and the altered *AtFEX* constructs tested in yeast DKO. The CRISPR/Cas9 knock out causes a frameshift mutation which results in premature termination at amino acid 61. Del7 and Del129 are deletions of 7 and 129 AA respectively, from the AtFEX N-terminus, used to test rescue in yeast. PlusIntron2 represents a predicted protein that could be produced when the second intron is retained.



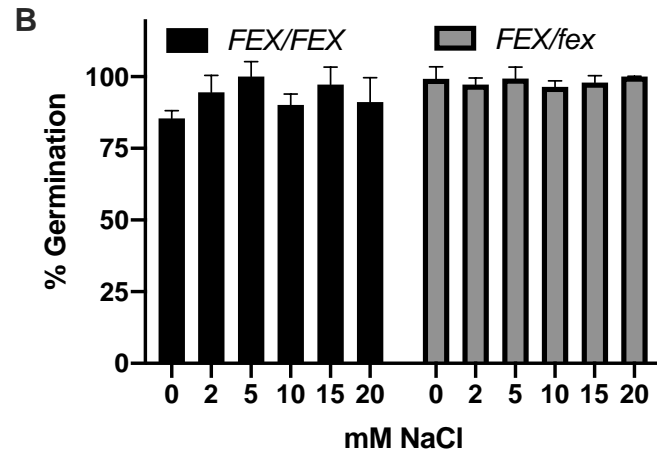
Supplemental Figure S2. An example of the serial dilution spot growth assay with plates containing different concentrations of NaF for DKO yeast rescued with either *P. patens* FEX or *S. cerevisiae* FEX1. The same data are presented in Figure 1 for *P. patens*.



Supplemental Figure S3. Examples of genotypic and phenotypic data. (A) dCAPS genotype analysis where *FEX/FEX* plants contained a *HinfI* site and *fex/fex* plants did not. (B) Seeds from *fex/fex* plants appear empty and do not produce seedlings. Bar is 500 μ m (C) Yellowing leaves on *fex/fex* seedling that has been transferred to plates containing 0.25 mM NaF for 10 d. Green seedlings above were *FEX/fex* and *FEX/FEX*. Arrow points to a chlorotic *fex/fex* plant. (D) Roots grown on 0 and 1 mM NaF. All grow the same on 0 mM NaF, but in 1 mM NaF the *fex/fex* root doesn't elongate after emergence from the seed coat. The primary roots of the OX are longer than *FEX/FEX* in NaF.



Supplemental Figure S4. Overexpression level of *AtFEX* and germination on NaCl. (A) OX seedlings had about 10 times the amount of *AtFEX* message as WT. The data represent three technical replicates of one trial of more than 3 trials which all had the same results. (B) NaCl had no effect on germination unlike similar concentrations of NaF. Each bar represents 2 trials of 40-70 seeds each. Error bars are SD.



Supplemental Table S1. Primer sequences.

| Primer | Sequence 5'-3' | Purpose |
|-----------|---|--|
| FLCDSYF1 | GATTCTAGAAGCTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAATGGATACGGGTCAAAGCAGCATAGAAC | Cloning in yeast vector pRS416GPD |
| FLCDSYR1 | GCGTGACATAACTAATTACATGACTCGAGGTGACGGTATCGATACTAACTGAATCCTATTACCCAGACAGG | <i>Arabidopsis thaliana</i> and <i>halleri</i> |
| SIATGF | GATTCTAGAAGCTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAatggatagctcctcagctaggagcaatg | <i>Setaria</i> |
| SITAGR | GCGTGACATAACTAATTACATGACTCGAGGTGACGGTATCGATAGAAAGTTGCCAAAGGTCATCCATAATGC | |
| TEAATGF | GATTCTAGAAGCTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAATGATTGTTTTATCTTACCTTGTTCGTTGA | CsFEX |
| TEAREV2 | GCGTGACATAACTAATTACATGACTCGAGGTGACGGTATCGATAgacacactaatcatagcccatgacc | |
| TOBATGF | GATTCTAGAAGCTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAATGGATTGTGAGAATAAGGATTCTGATCTC | NbFEX |
| TOBTAGR | GCGTGACATAACTAATTACATGACTCGAGGTGACGGTATCGATAGATCTTGGCTCTAATTGGAATTTTCAGTC | |
| ATATGF | CACCATGGATACGGGTCAAAGCAGCATAG | Cloning in pENTR/D |
| ATTAGR | CTAACTGAATCCTATTACCCAGAC | AtFEX |
| GLYNOATG | CACCGGTGGAGGTGGAGGTCAAAGCAGCATAGAACC | |
| AtpEGHF | GACCATCCTCCAAAATCGGATCTGGTCCGCGTGGATCCCCGGGAATGGATACGGGTCAAAGCAGCATAGAAC | Cloning in pEGH |
| AtpEGHR | GAAACGCGCGAGGCAGATCGTCAGTCAGTCACGATGAATTAAGCTTCTAACTGAATCCTATTACCCAGACAGG | AtFEX |
| CspEGHF | GACCATCCTCCAAAATCGGATCTGGTCCGCGTGGATCCCCGGGAATGATTGTTTTATCTTACCTTGTTCGTTGA | CsFEX |
| CspEGHR | GAAACGCGCGAGGCAGATCGTCAGTCAGTCACGATGAATTAAGCTTGACACACTAATCATAGCCCATGACCC | |
| L8015 | TGGCTGTGGCTTAAAATATGTG | CRISPR/Cas9 |
| L8126 | CTTTTAGTCCGGAGAGCAGTGT | |
| R8256 | GCTTCTACCAGCACTATGCCTT | |
| R8347 | TGAAAGCTCGAAGGTCATGTAA | |
| FEX2B | ATTGCGTCGATAGTGAGAGTGTCTCAG | |
| REX2B | AAACCTGAGACACTCTCACTATCGACG | |
| pYAOF | CCATGAGCTCATCTTCTCTTC | |
| pYAOR | GAAGAGGAAGATGAGCTCATGG | |
| HinfIF | AATTGACAATGACGTCGATAGTGAGAGTGACT | dCAPS |
| HinfIR | CGAAGGTCATGTAATATATCTTGTCTTGTGACGAG | |
| InHinfR | GTGACTATGCAAAGTTAGAGGTAGTCTC | |
| KCN186AF | CCTTGATCTTCCCTCCGCCATGGTAGGATCATTCTT | N to A conversion in AtFEX |
| KCN186AR | AAGAATGATCCTACCATGGCGGAGGGAAGATCAAGG | |
| KCN373AF | GGCACCTCATTGCAGCTGTAGTTGCAGCTTGCG | |
| KCN373AR | CGCAAGCTGCAACTACAGCTGCAATGAGGGTGCC | |
| CTPDEL | CACCATGGATACGAGTGTAGCCTCTTCTT | N terminal deletions of AtFEX |
| YCTPDEL | TCTAGAAGCTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAcaccATGGATACgagtgagcctcttctt | |
| YNTERMDEL | TCTAGAAGCTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAATGGATACGgaagagcgagtggttac | |
| QAT3338F | TTGAAAATTGGAGTACCGTACCAA | qPCR |
| QAT3338R | TCCCTCGTATACATCTGGCCA | At4g33380 |
| QTIPATR | TCAACTGGATACCCCTTTCGCA | |
| QTIPATF | GTGAAAAGCTGTTGGAGAGAAGCAA | At4g34270 (TIP41-like) |
| QFEXF1 | TGTAACACGGTTGCTTCGAG | |
| QFEXR1 | CAATCGAAAAGAAACCACA | 3' end of AtFEX |