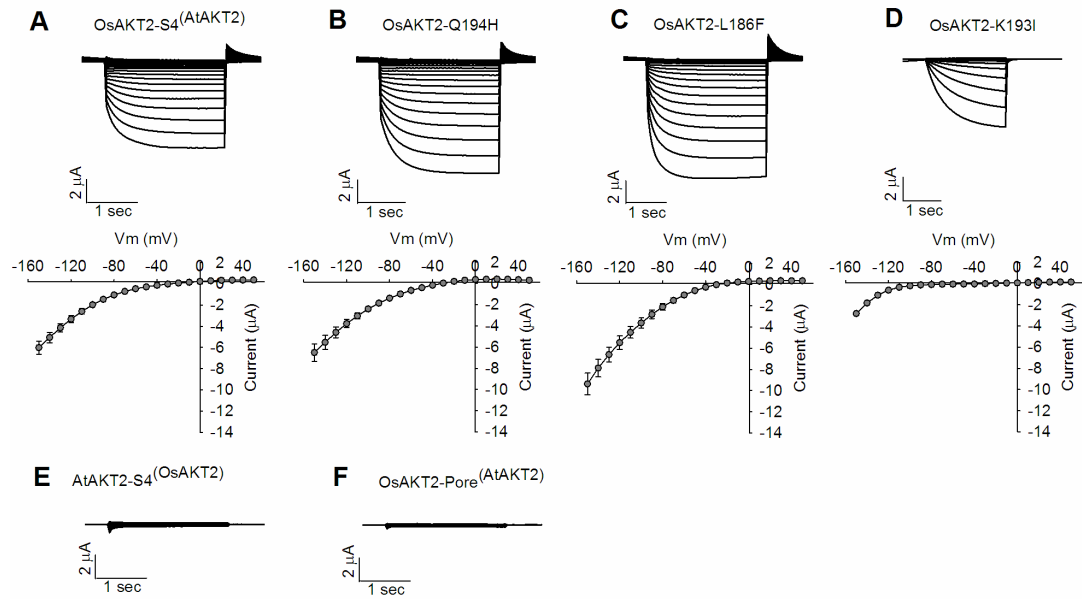


1 **Supplemental Data**

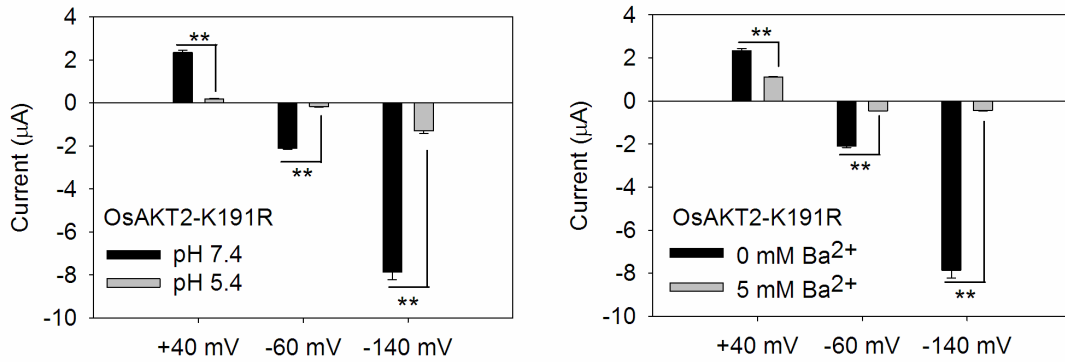


2

3 **Supplemental Figure S1. Effects of substitutions or point mutations that do not**
 4 **affect the rectification of OsAKT2 and AtAKT2.**

5 Representative recordings (upper panels) and currents-voltage relationships (lower
 6 panels) of the AKT2 mutants measured in 50 mM K⁺, pH 7.4: A, OsAKT2-S4^(AtAKT2),
 7 B, OsAKT2-Q194H, C, OsAKT2-L186F, D, AtAKT2-K193I. E, The intact
 8 substitution of S4 AtAKT2-S4^(OsAKT2) or F, pore motif OsAKT2-Pore^(AtAKT2) caused
 9 no detectable current with AtKAT2. Data were means \pm SE (n = 4 - 8).

10



11

12

13 **Supplemental Figure S2. Effect of external pH and Ba²⁺ on OsAKT2-K191R.**

14 Currents measured at different membrane potentials (+40, -60 and -140 mV) under 50
15 mM K⁺ for OsAKT2-K191R in pH 7.4 (black) or pH 5.4 (grey) baths (left).

16 Sensitivity to Ba²⁺ (5 mM, right). Currents were recorded in 50 mM K⁺ baths (pH 7.4).

17 Data were means ± SE (n = 4). *p < 0.05, **p < 0.01 (Student's *t*-test).

		Ankyrin domain			
Repeat1	OsAKT2	536	NIPCNLTVAATGNSSELEDLLKVGMDPDV	565	
	AtAKT2	540	NIASNTIAVVTTGNAALIDELLKAKLSPDI	569	
	OsAKT1	565	DLPITICFAVTRGDDEFHQLLKRGMDPNE	594	
	AtAKT1	517	DLPLNICFAAIREDDLHQLLKRGLDPNE	546	
Repeat2	OsAKT2	569	KGRITALHIAASKGYEDCVLVLLKQACNVNI	598	
	AtAKT2	573	KKTPPLHVAASRGYEDCVLVLLKHGCNIHI	602	
	OsAKT1	598	DGHTALHIAASKGNEQCVRLLEYGADPNA	627	
	AtAKT1	550	NGRTPPLHIAASKGTLNCVLLLEYHADPNC	579	
Repeat3	OsAKT2	602	QNTALWNAIAARHKKIFNIYHFARVSSP	631	
	AtAKT2	606	NGNSALWEAIIISKHYEIFRIYHFAAISDP	635	
	OsAKT1	631	EGKVPWLWEALCEKHAADVQLVEGGADLSS	660	
	AtAKT1	583	EGSVPLWEAMVEGHEKVVKVLEHGSTIDA	612	
Repeat4	OsAKT2	634	AAGDLICIAARRGDLDTLRELLKHGLAVDS	663	
	AtAKT2	637	IAGDLICEAAKQNNVEVMKALLKQCLNVDT	666	
	OsAKT1	662	DTGLYACIAVEESDTELLNDIIHYGDEVNR	691	
	AtAKT1	614	LVGHFACIAAEQGNLKLKEIVLHGDVTR	643	
Repeat5	OsAKT2	667	DCATAPVALAEGHADVARLIVINCASVER	696	
	AtAKT2	670	HCVTALCVMAEDQMDMVNLITATNCALVVC	699	
	OsAKT1	695	DGTTALHRAVCDGNVQMAELILEHGCALIDK	724	
	AtAKT1	647	TGTSALHTAVCEENIEMVKYLLEQCALVNK	676	
Repeat6	OsAKT2	699	HNEQQAAAA..VSVDELREIMKTR.....E	722	
	AtAKT2	702	HN.....EFTPLEK.....	711	
	OsAKT1	727	NGWTPRALAECCGHDDIQLIFRSRKAATAS	757	
	AtAKT1	679	HGWTPRDIAECCGHEDIKALIFREK.....L	704	

18

19 **Supplemental Figure S3. Amino acid sequence alignment of the ANK domains**
 20 **among AKT2 and AKT1 channels.**

21 The sequences were aligned using DNAMAN software. The access numbers of
 22 aligned sequences were: AtAKT1 (NP_180233), OsAKT1 (AK120308), OsAKT2
 23 (JN989970) and AtAKT2 (AAA97865). Conserved amino acids were highlighted in
 24 black.

S4

OsAKT2	179	L	LG	IL	RL	WR	LR	RV	KQ	EF	FTR	LEK	DIR	204
HvAKT2	171	L	LG	IL	RL	WR	LR	RV	KQ	EF	FTR	LEK	DIR	196
AtAKT2	183	L	LG	LL	RF	WR	LR	RV	KHL		FTR	LEK	DIR	208
ZMK2	177	M	LG	V	LR	WR	LR	RV	KQ	EF	FTR	LEK	DIR	202
NKT2	176	V	LG	M	LR	WR	LR	RV	KQ	EF	FTR	LEK	DMR	201
VFK1	163	L	LG	M	LR	WR	LR	RV	KQ	EF	FTR	LEK	DIR	188
PTK2	170	L	LG	LL	RF	WR	LR	RV	KQL		FTR	LEK	DIR	195
VvK3.1	188	L	LG	LL	RF	WR	LR	RV	KQL		FTR	LEK	DIR	213
GhAKT2	198	L	LG	LL	RF	WR	LR	RV	KQL		FTR	LEK	DIR	223
MhAKT2	210	F	LG	LL	RF	WR	LR	RA	KQL		FTR	LEK	DIR	235
SbAKT2	170	M	LG	V	LR	WR	LR	RV	KQ	EF	FTR	LEK	DIR	195
SPICK1	188	L	LG	M	LR	WR	LR	RM	KQ	EF	FTR	LEK	DIR	213
SPICK2	185	L	LG	M	LR	WR	LR	RV	KQ	Y	FTR	LEK	DIR	210

25

26

27 **Supplemental Figure S4. S4 amino acid sequence comparison among known**
 28 **AKT2 channels.**

29 Plant species and corresponding AKT2 channels analyzed were: *Oryza sativa*,
 30 OsAKT2 (JN989970); *Hordeum vulgare*, HvAKT2 (DQ465923); *Arabidopsis*
 31 *thaliana*, AtAKT2 (AAA97865); *Zea mays*, ZmK2.1 (AJ132686); *Nicotiana tabacum*,
 32 NKT2 (AB196789); *Vicia faba*, VFK1(Y10579); *Populus tremula*, PTK2
 33 (AJ271447.1); *Vitis vinifera*, VvK3.1 (XM_002268888.3); *Gossypium hirsutum*,
 34 GhAKT2 (NM_001327179); *Malus hupehensis*, MhAKT2 (JN632525); *Sorghum*
 35 *bicolor*, SbAKT2 (XM_002441096); *Samanea saman pulvinus*, SPICK1 (AF099095)
 36 and SPICK2 (AF145272). The red frame highlighted the distinct residue we focused
 37 on in this study.

38

39 **Supplemental Table S1. Primer sequences used in this study.**

40	Primer name	Primer sequence (5' - 3')
41	Primers used for OsAKT2 clone	
42	OsAKT2-F	GGCCCGGGATGAAGACCTCGAGCTTC
43	OsAKT2-R	TCCGCGGCCGCCTATGATCCAGACACCGAGTCCA
44	Primers used for site-directed mutagenesis construction	
45	OsAKT2-S4 ^(AtAKT2) -F1	cgtctcaggagagtcagcatctcTTCACAAGGCTGGAGAAGGA
46	CA	
47	OsAKT2-S4 ^(AtAKT2) -R1	ttgactctctgagacgccagaaccggagtaaGCCGAGGAGGCTGAA
48	CGC	
49	OsAKT2-S4 ^(AtAKT2) -F2	CAGGtactctTACTTCTGGATCCGCTGCGCCCG
50	OsAKT2-S4 ^(AtAKT2) -R2	CAGAAGTAagagtaCCTGATGTCCTTCTCCAGCCT
51	OsAKT2-Pore ^(AtAKT2) -F1	CACCgcagcaatcTACTGGTCCATCACCACCATGA
52	OsAKT2-Pore ^(AtAKT2) -R1	AGTAGattgtgcGGTGTAGCGGATCCACAGGC
53	OsAKT2-Pore ^(AtAKT2) -F2	cacgcctcaaacaccatcGAGATGATCTTCAACATCTTCTAC
54	ATGC	
55	OsAKT2-Pore ^(AtAKT2) -R2	atggtgtttgaggcgtgcaaGTCGCCGTAGCCGACGGT
56	OsAKT2-K193I-F1	cgagaattcacgcgtgttaccATGAAGACCTCGAGCTTCGAGA
57	OsAKT2-K193I-R1	tccagccttgTGAAGAACTGTATGACCTTCCTGAGA
58	OsAKT2-K193I-F1	cagttcttcaCAAGGCTGGAGAAGGACATCA
59	OsAKT2-K193I-R2	tcatgtctgctcgaagcggcgcCTATGATCCAGACACCGAGTCCA
60	OsAKT2-R190K-F	GCGTCTCaagAAGGTCAAGCAGTTCTTCACAAGG
61	OsAKT2-R190K-R	TGACCTTcttGAGACGCCATAGCCGGAGGATG
62	OsAKT2-K191R-F	AGGcgaGTCAAGCAGTTCTTCACAAGGCTGGA
63	OsAKT2-K191R-R	AACTGCTTGACtcgCCTGAGACGCCATAGCCGG
64	OsAKT2-Q194H-F	GGAAGGTCAAGcatTTCTTCACAAGGCTGGAGAAGG
65	OsAKT2-Q194H-R	GAAatgCTTGACCTTCCTGAGACGCCATAGCC
66	OsAKT2-L186F-F	ttcTGGCGTCTCAGGAAGGTCAAGCAGTTCTT
67	OsAKT2-L186F-R	TTCCTGAGACGCCAgaacCCGGAGGATGCCGAGGAG

68 OsAKT2-R190K-K191R-F TCTCaagcgaGTCAAGCAGTTCTTCACAAGGCT
69 OsAKT2-R190K-K191R-R TGCTTGACtcgcttGAGACGCCATAGCCGGAGG
70 OsAKT2-ANK^(OsAKT1)-F1 cgagaattcacgcgtggtaccATGAAGACCTCGAGCTTCGA
71 A
72 OsAKT2-ANK^(OsAKT1)-R1 aattggcaaatcGCCGTGGTCTGACTCGCC
73 OsAKT2-ANK^(OsAKT1)-F2 accacggcGATTTGCCAATTACACTCTGTTTTG
74 OsAKT2-ANK^(OsAKT1)-R2 agtcgacgacgatggtACTCGCCGTCGCCGCCTT
75 OsAKT2-ANK^(OsAKT1)-F3 gagtACCATCGTCGTCGACTCGC
76 OsAKT2-ANK^(OsAKT1)-R3 tcatgtctgctcgaagcggccgcCTATGATCCAGACACCGAGT
77 CCA
78 OsAKT1-ANK^(OsAKT2)-F1 cgagaattcacgcgtggtaccATGGCGAGGTGGGGCGCC
79 OsAKT1-ANK^(OsAKT2)-R1 cggtatggtCAGATTTCCCCGAGCTAGCA
80 OsAKT1-ANK^(OsAKT2)-F2 ggggaaatctgAACATAACCGTGCAATCTGCTGA
81 OsAKT1-ANK^(OsAKT2)-R2 tgggacgtgatggtggccGACCGGGTGCGCGAGCTC
82 OsAKT1-ANK^(OsAKT2)-F3 tcGGCCACCATCACGTCCCA
83 OsAKT1-ANK^(OsAKT2)-R3 tcatgtctgctcgaagcggccgcGCTCTTGCTTTTCATCTTCTC
84 TG
85 AtAKT2-F cgagaattcacgcgtggtaccATGGACCTCAAGTATTCAGCATCTC
86 AtAKT2-R tcatgtctgctcgaagcggccgcCTAAATTATCTTGTTTACGACAAAGTAGA
87 GTT
88 AtAKT2-S4^(OsAKT2)-F1 atggcgacttcgaaaggttaaacagttcTTCCTAGGCTCGAGAAGG
89 ACAT
90 AtAKT2-S4^(OsAKT2)-R1 cctttcgaagtcgcatagtctaaggatTCCCAAGAGATTACAAGTG
91 ATATTTAACG
92 AtAKT2-S4^(OsAKT2)-F2 AGAttcaacTATTTCTGGATTTCGCTGCTTTTCGA
93 AtAKT2-S4^(OsAKT2)-R2 CCAGAAATAgttgaaTCTTATGTCCTTCTCGAGCCTAGTG
94 AtAKT2-R194K-F GGCGACTTaagAGAGTTAAACACCTCTTCACTAGGCTC
95 AtAKT2-R194K-R AACTCTcttAAGTCGCCAAAATCTAAGTAATCCC
96 AtAKT2-R195K-F GCGACTTCGAaagGTTAAACACCTCTTCACTAGGCTCG
97 AtAKT2-R195K-R TAACcttTCGAAGTCGCCAAAATCTAAGTAAT

98 AtAKT2-R194K-R195K-F GGCGACTTaagaagGTAAACACCTCTTCACTAGGC
99 TCG

100 AtAKT2-R194K-R195K-R TAACcttcttAAGTCGCCAAAATCTAAGTAATCCC

101 **Primers used for qPCR**

102 OsAKT2-qF AGCCGTCATCCGGGAGGTCGGC

103 OsAKT2-qR TCAATCTCCGCTCCTTCGTCGTT

104 OsActin-qF TGGTCGTACCACAGGTATTGTGTT

105 OsActin-qR AAGGTCGAGACGAAGGATAGCAT

106 OsCBL1-qF CGATGGGCTCATCAATAAGG

107 OsCBL1-qR CCCCCTTTTCTTCACATCAA

108 OsCIPK23-qF GCCTCCAAGGTTTGAGACAG

109 OsCIPK23-qR GCCAAGATTGAGACCCTGAG

110 **Primers used for promoter clone**

111 OsAKT2-promoter-F gagctcggtagccggggatccCAGCTTGCCCAGGTCGAGG

112 OsAKT2-promoter-R ttaccctcagatctaccatggTGCTACCAGAAGACGTTGCACA

113 **Primers used for eGFP fluorescence observation**

114 OsAKT2-eGFP-F1 cgagaattcacgcgtggtaccATGAAGACCTCGAGCTTCGAGA

115 OsAKT2-eGFP-R1 tgctcaccatTGATCCAGACACCGAGTCCATC

116 OsAKT2-eGFP-F2 gtctggatcaATGGTGAGCAAGGGCGAGG

117 OsAKT2-eGFP-R2 tcatgtctgctgaagcggccgcTCACTTGTACAGCTCGTCCATGC
