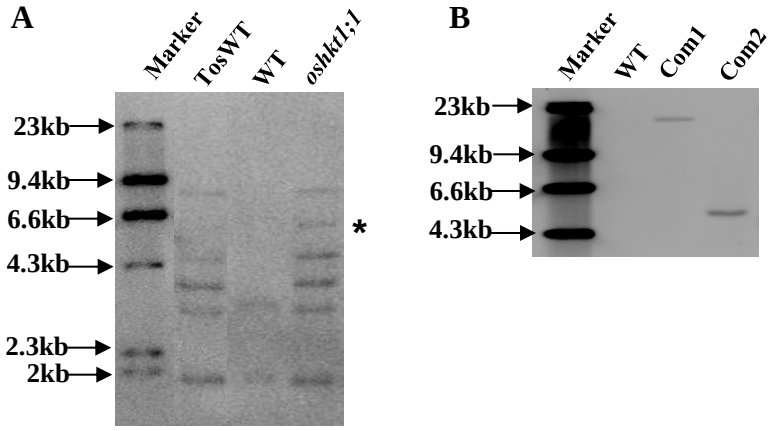
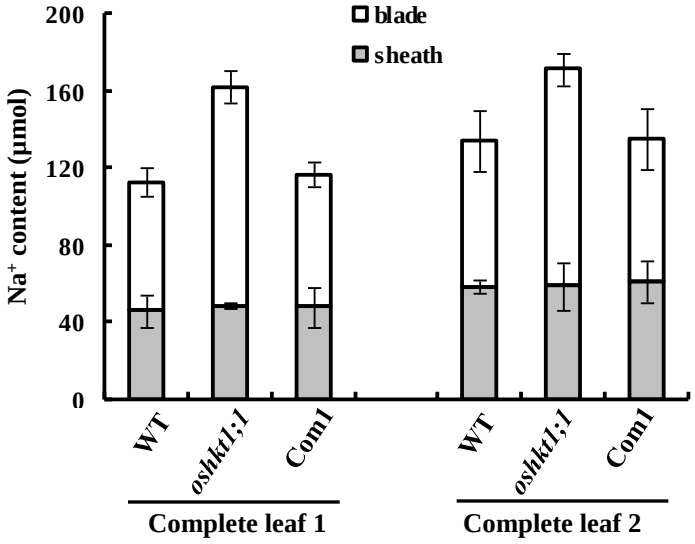


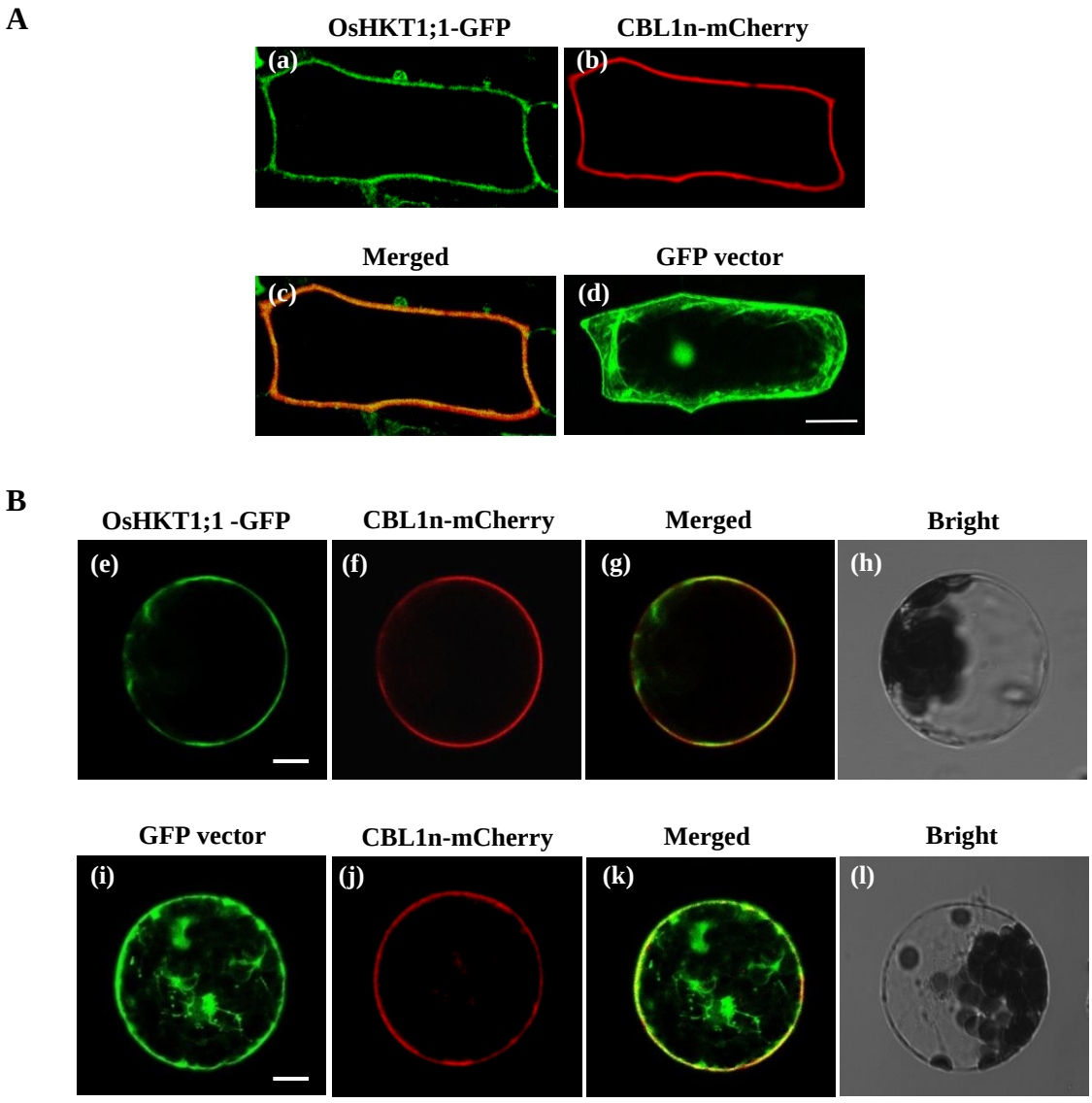
Supplemental Figure S1. Southern-blot analysis for the plant materials used in this study.
A, Genomic DNA was digested with restriction enzyme *BamHI*, a fragment of *Tos17* gene was used as probe. The original variety Nipponbare (WT), had two copies of *Tos17*. The *oshkt1;1* mutant had an additional copy with approximately 6-kb band (asterisk) as compared with TosWT.
B, Southern blot analysis of the copy number in transgenic plants with *OsHKT1;1-COM* (Com1 and Com2) and WT plants. Genomic DNA was digested with restriction enzyme *HindIII*, the DNA fragment of *Hygromycin* gene was used as probe.



Supplemental Figure S2. The Na^+ content in the leaf sheaths and leaf blades.
The Na^+ content in the sheath and the blade of complete leaves 1 and 2. The hydroponically grown seedlings were treated with 100 mM NaCl for 7 d, and the leaves were harvested for Na^+ content assay. Error bars represent SE (n=4).

Supplemental Table S1. The raw data of Na^+ content in the leaf sheaths and leaf blades

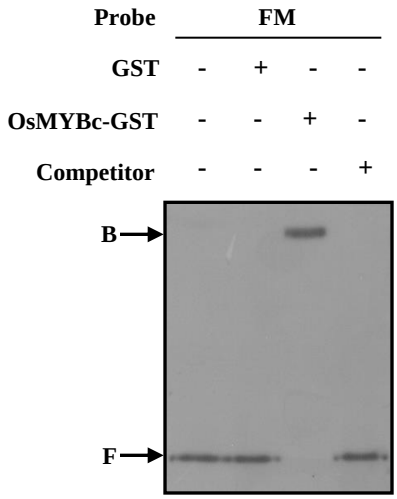
		sheath (μmol)	blade (μmol)	sheath/blade
Complete leaf 1	WT	45.96±8.54	66.82±6.99	0.68±0.031
	oshkt1;1	48.64±1.62	113.4±8.35	0.42±0.022
	Com	47.91±10.01	68.64±6.26	0.69±0.024
Complete leaf 2	WT	58.56±3.59	75.19±15.50	0.77±0.046
	oshkt1;1	58.76±12.13	112.3±8.25	0.52±0.057
	Com	61.08±11.18	73.79±15.57	0.82±0.063



Supplemental Figure S3. OsHKT1;1 localization in the cell.

A, Onion epidermal cells were transformed with the plasmid combinations indicated. Individual panels show (a) an epidermal cell expressed OsHKT1;1-GFP(green); (b) the same cell expressed PM-maker CBL1n-mcherry (red); (c) merged images of (a) and (b); (d) GFP vector -expressing cell. Bar=25 μm.

B, Subcellular localization of OsHKT1;1-GFP in *Arabidopsis* mesophyll protoplasts. (e) to (h), OsHKT1;1-GFP protein was transiently co-expressed with CBL1n-mcherry in a protoplast. (i) to (l), GFP vector was co-expressed with CBL1n-mcherry. Bars=10 μm.



Supplemental Figure S4. OsMYBc binds to the promoter of *OsHKT2;1*
EMSA shows the GST-OsMYBc protein binding with the fragment FM (-457 to -285) of *OsHKT2;1* promoter *in vitro*.

Supplemental Table S2. The raw data of Na^+ content in phloem sap.

	Na^+ (mM)	glutamine (mM)	Na^+:glutamine	Dry weight (g)
WT	1.27±0.13	0.151±0.019	8.49±0.38	0.41±0.02
oshkt1;1	0.79±0.08	0.130±0.011	6.44±0.48	0.44±0.033
Com1	1.34±0.15	0.149±0.013	9.01±0.53	0.44±0.027

Supplemental Table S3. Positive interactions from yeast One Hybrid screen

Locus identification no.	Description	The number of clones identified
LOC_Os06g50600	Putative protein	1
LOC_Os05g21180	Putative protein	1
LOC_Os02g07790	Protein kinase	1
LOC_Os05g35740	Subtilisin N-terminal Region family protein	2
LOC_Os11g01510	Ubiquitin-activating enzyme	2
LOC_Os02g35840	Expressed protein	1
LOC_Os01g49690	Putative protein	2
LOC_Os09g12770	Myb-like DNA-binding domain containing protein	7
LOC_Os04g50864	Expressed protein	1
LOC_Os01g21180	Translation machinery-associated protein 20	1
LOC_Os06g35814	Putative protein	1
LOC_Os12g42550	Methyl-CpG binding domain containing protein	1
LOC_Os11g05540	RhoGAP domain containing protein	2
LOC_Os08g03640	60S acidic ribosomal protein	1
LOC_Os02g10470	EF hand family protein	1
LOC_Os05g06310	60S ribosomal protein	1

Supplemental Table S4. *HKT* genes containing MYB-CC cis-element biding region in their promoters.

Nucleotide Accession No.	Description	No. of MYB cis-element	Position of MYB cis-element
AJ491816	*OsHKT1;1*	3	-76, -276, -1477
AJ491853	*OsHKT1;4*	1	-900
AK108663	*OsHKT1;5*	2	-2183, -2227
QB061311	*OsHKT2;1*	1	-395
U16709	*TaHKT2;1*	1	-1018
HQ845286	*ZmHKT-like*	1	-575

Os, *Oryza sativa*, Ta, *Triticum aestivum*, Zm, *Zea mays*.

Supplemental Table S5. The primers used in this study.

Primer name	Primer sequence（5’-3’）
OsHKT1;1-F	GATCCCGCAGATTCTAGCAG
OsHKT1;1-R	GGCAATTCGGATTTTCAGTG
tail 6	AGGTTGCAAGTTAGTTAAGA
OsHKT1;1-RT-F	TTCACCACTCTTGCGGCTATG
OsHKT1;1-RT-R	TGTTTGTAGCCAGTCTCCCCAG
OsHKT1;5-RT-F	CCACCTTTTCCTTTTCCATGC
OsHKT1;5-RT-R	GGTCTTCATCGGCAGAGCTTT
OsHKT2;1-RT-F	CACAGTCTCCTCGTTTGCGAA
OsHKT2;1-RT-R	GCAAGAATCTGGCCGATGAA
OsUBQ5-F	ACCACTTCGACCGCCACTACT
OsUBQ5-R	ACGCCTAAGCCTGCTGGTT
18S rRNA-F	CTACGTCCCTGCCCTTTATACA
18S rRNA-R	ACACTTCACCGGACCATTCAA
Com-OsHKT1;1-F	CGGGATCCTCCATTCTCTCTACCAACCTCAGAT
Com-OsHKT1;1-R	GCTCTAGATCATTTCAGGATGAACTCCTTGAGCC
GFP-OsHKT1;1-F	GCGGATCCATGCATCCACCAAGTTTAGTGCT
GFP-OsHKT1;1-R	GCGGATCCTTTCAGGATGAACTCCTTGA
Pro-OsHKT1;1-2120-F	CGGGATCCTCCTGGCAAAATGCTGGTTGA
Pro-OsHKT1;1-1623-F	CGGGATCCCATGTCATCATTCATGCAAAATC
Pro-OsHKT1;1-1126-F	CGGGATCCACGTGCTAACAAGTCGAGATAAAATC
Pro-OsHKT1;1-629-F	CGGGATCCTGAACTGGGGTCTGAACTGAACTG
Pro-OsHKT1;1-R	CATGCCATGGAAACTTGGTGGATGCATTCTTC
Pro-RD29A-F	CGGGATCCCCCGACCGACTACTAATAATAGTA
Pro-RD29A-R	CATGCCATGGGGTCCAAAGATTTTTTTCTTTCC
Bait-F	ACGCGTCGACTGAACTGGGGTCTGAACTGAACTG
Bait-R	CCGCTCGAGTTGGGCTTATAGAAGGTGGTA
Tos probe-F	GCTACCCGTTCTTGGACTAT
Tos probe-R	CTGAAATCGGAGCACTGACA
Hyg probe-F	TTCCACTATCGGCGAGTACT
Hyg probe-R	GGTGTCACGTTGCAAGACCT
CBL1n-F	GGACTAGTATGGGCTGCTTCCACTCAAA
CBL1n-R	CCGCTCGAGTTACTTGTACAGCTCGTCCA

Supplemental Table S5. The primers used in this study (continued) .

Primer name	Primer sequence（5'-3'）
GFP-OsMYBc-F	CGCCATGGATGGAATTAGGTGGCAACAATATGG
GFP-OsMYBc-R	CGCCATGGCTAATGACCTGAGTAGGACATGGAAC
GST-OsMYBc-F	CCCAAGCTTATGGAATTAGGTGGCAACAATATGG
GST-OsMYBc-R	GGGGTACCCTAATGACCTGAGTAGGACATGGAAC
F1-L	TGAACTGGGGTCTGAAC
F1-R	CTTCTTCCTGAGCAGTC
F2-L	GGAATCACATAGACCAATC
F2-R	AGAGTCCCAAGTGTTTGG
F3-L	AAAAAGTACACACCACC
F3-R	AAACTTGGTGGATGCA
FM-L	CGCTGAGCAGGTAATCATTAG
FM-R	CGGATTCTCAGAAGTGTAAAC
ChIP-I-F	GTATGGTTTGAAATGATATCT
ChIP-I-R	CATGTGGGCTAATATAGTCCT
ChIP-II-F	TGCTGGTTTCCTTGCTCTGAAG
ChIP-II-R	CACAGCGCAAGGATTATATGTTA
2715BP	GTTACGTCCTGTAGAA ACCCCAA
OsMYBc-F	AATTCCAACTTGGCTGCAAG
OsMYBc-R	TTGAAAGCCAAAAATGGTGG
OsMYBc-RT-F	CAAATGAGCTGCACGAACGA
OsMYBc-RT-R	GAGGGGTCAGTTCTTTCGGA
Point1-F	TTCCACAGCCACAAAAGCGGCTAACTAAGGTACCA
Point1-R	TGGTACCTTAGTTAGCCGCTTTTGTGGCTGTGGAA
Point2-F	TTAGAAACCTACCAAAGCGGCCATTGAAACACACT
Point2-R	AGTGTGTTTCAATGGCCGCTTTGGTAGGTTTCTAA
pGADT7-OsMYBc-F	GAATTCATGGAATTAGGTGGCAACAATATGG
pGADT7-OsMYBc-R	CTCGAGCTAATGACCTGAGTAGGACATGGAAC
Flag-OsMYBc-F	CGGAATTCATGGAATTAGGTGGCAACAATATGG
Flag-OsMYBc-R	TGCTCTAGACTAATGACCTGAGTAGGACATGGAAC

Supplemental Methods

Localization of OsHKT1;1

The localization assay in onion epidermal cells was performed as described by Campo et al. (2014). To construct the OsHKT1;1-GFP fusion expression vector, the full-length coding sequence of *OsHKT1;1* was cloned from rice cDNA by using the primers *OsHKT1;1-GFP-F/OsHKT1;1-GFP-R*. The PCR product was cloned into the HBT-sGFP plasmid (Sheen's Lab). The *CBL1n* (Held et al., 2011) sequence was cloned by using the primers *CBL1n-F/CBL1n-R*, and was constructed into the pUC-18-mCherry plasmid. Two constructions were co-transformed into the *Arabidopsis* mesophyll protoplasts.

Arabidopsis mesophyll protoplasts were isolated and transformed following a previous procedure (Zhang et al., 2004). Protoplasts were incubated at 23˚C in the dark for at least 16 h before confocal observation.

Reference

Held K, Pascaud F, Eckert C, Gajdanowicz P, Hashimoto K, Corratgé-Faillie C, Offenborn JN, Lacombe B, Dreyer I, Thibaud JB, **Kudla J** (2011) Calcium-dependent modulation and plasma membrane targeting of the AKT2 potassium channel by the CBL4/CIPK6 calcium sensor/protein kinase complex. Cell Research **21:** 1116-1130

Zhang W, Qin C, Zhao J, Wang X (2004) Phospholipase Dα1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc. Natl. Acad. Sci. USA **101:** 9508-9513