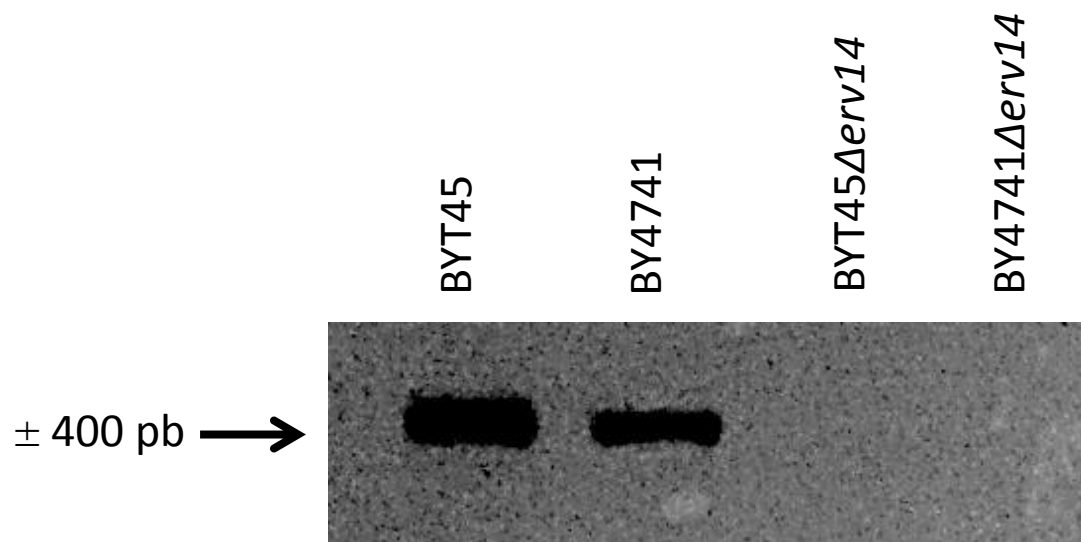


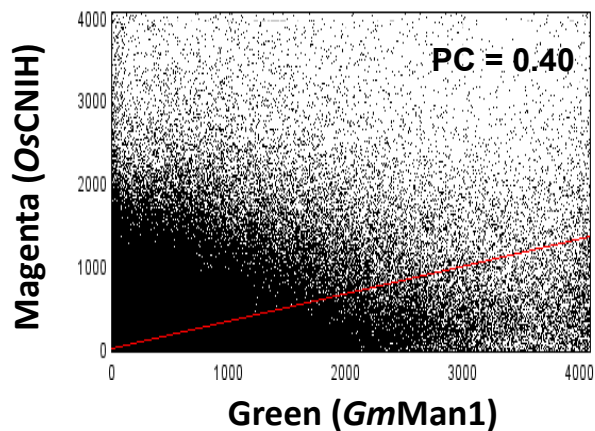
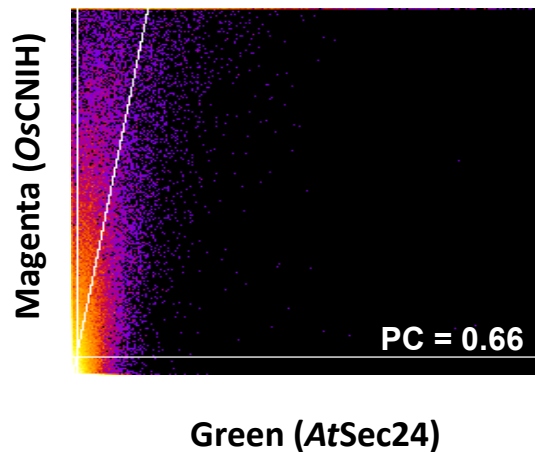
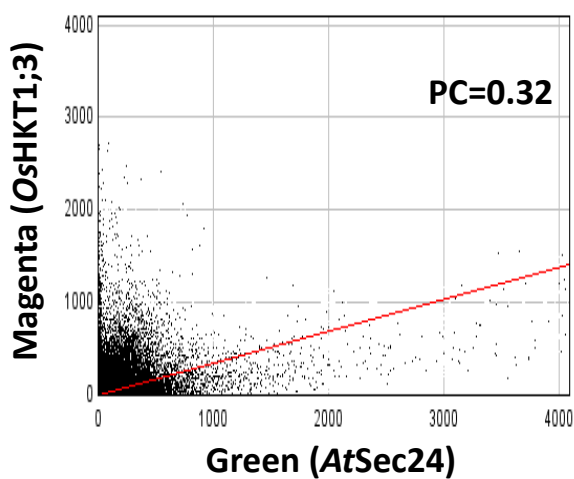
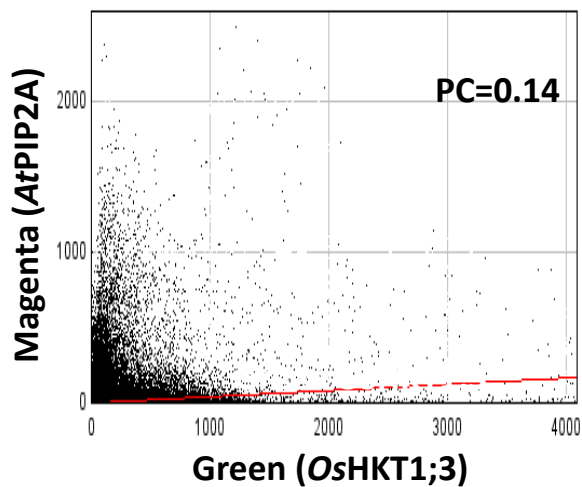
**Title:** Identification of Rice Cornichon as a possible cargo receptor for the sodium transporter OsHKT1;3

**Authors:** Paul Rosas-Santiago<sup>a,b</sup>, Daniel Lagunas-Gómez<sup>a</sup>, Bronwyn, J. Barkla<sup>a\*</sup>, Rosario Vera-Estrella<sup>a</sup>, Sylvie Lalonde<sup>b</sup>, Alexander Jones<sup>b</sup>, Wolf B. Frommer<sup>b</sup>, Hana Sychrová<sup>c</sup>, Olga Zimmermannova<sup>c</sup>, Omar Pantoja<sup>a,1</sup>

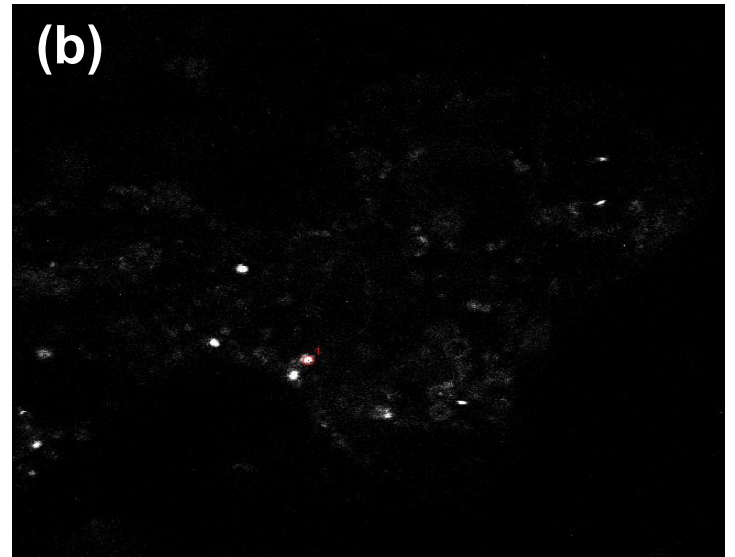
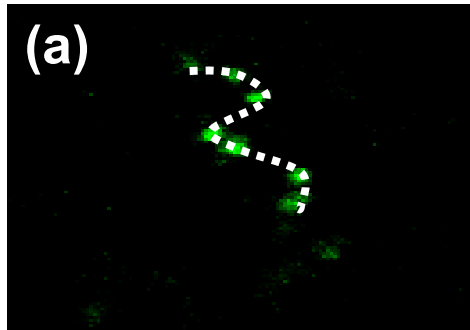
## SUPPLEMENTARY MATERIAL



**Figure S1. Deletion of *ERV14* in *S. cerevisiae*.** PCR amplification of the *ERV14* gene from the different yeast cells showing its absence in the BYT45 $\Delta$ erv14 mutants.

**A****B****C****D**

**Figure S2. Quantification of co-localization between *OsCNIH1* or *OsHKT1;3* with membrane markers.** Scatter plots of pixel distribution of the magenta (Y-axis) and green (X-axis) channels employing the Costes algorithm including in the plug-in JACoP (ImageJ Software) for *OsCNIH1*-mCherry and *GmMan1*-Citrine **A**; *OsCNIH1*-mCherry and *AtSec24*-YFP **B**; *OsHKT1;3*-mCherry and *AtSec24*-YFP **C**; and *AtPIP2A*-mCherry and *OsHKT1;3*-EYFP **D**.



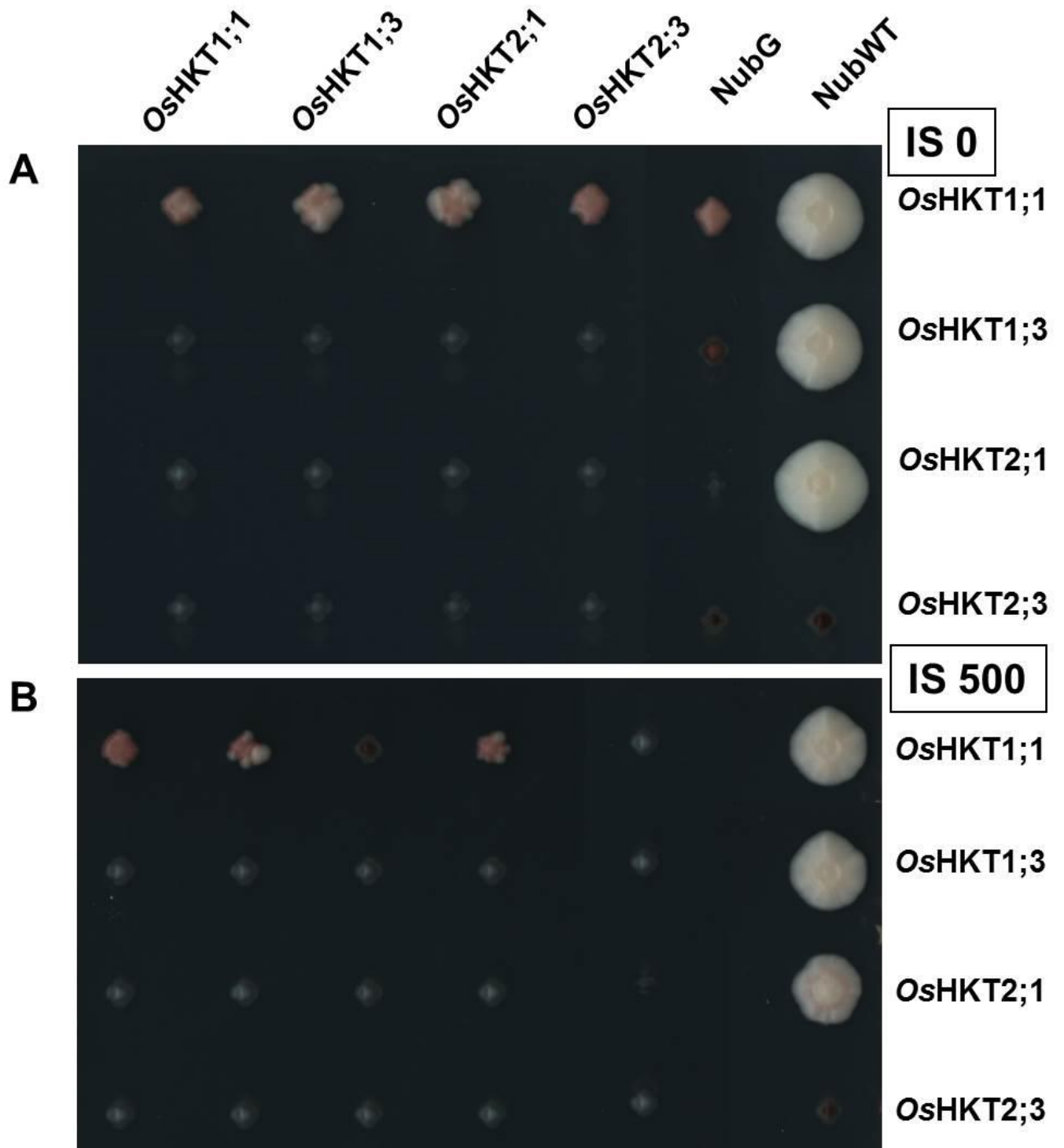
(c)

OsHKT1;3 Body Track Velocity

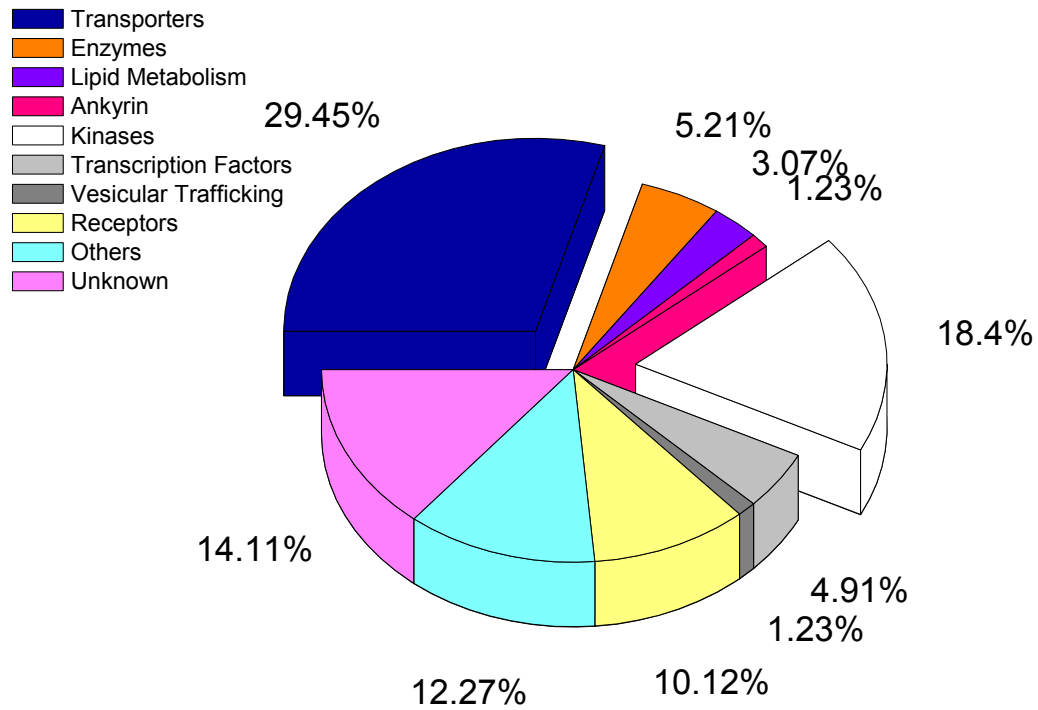
Velocity ( $\mu\text{m sec}^{-1}$ )

$0.14 \pm 0.02$

**Figure S3. Dynamics of OsHKT1;3-EYFP in tobacco epidermal cells.** (a) Trajectory of a single OsHKT1;3-EYFP body. (b) Dynamics of OsHKT1;3-EYFP bodies in an epidermal cell. (c) Mean velocity of OsHKT1;3-EYFP bodies derived from similar data as shown in (b). Values correspond to the mean $\pm$ SD from 9 measurements.



**Figure S4. Rice HKT transporters do not interact between them, indicating the absence of oligomerization.** OsHKT transporters do not seem to interact between them as indicated by the absence of yeast cell growth in selective medium in the absence (A) or in the presence of 0.5 mM methionine (B).



**Figure S5. Classification of the proteins interacting with AtCNIH1.** Thirty percent of the proteins included in the Arabidopsis interactome correspond to transporters. Data were obtained from the membrane-base associomics database: <https://associomics.dpb.carnegiescience.edu/Associomics/Home.html> (See also Table S3).

**Table S1. Primers Used for gene cloning into the Gateway (TOPO), pYeP352 and pOO2 plasmids.**

Gene	Primer Sequence (5'→3')	
	Forward	Reverse
<i>OsHKT1;3</i>	CACCATGAATCATTGTCTTGTAGTA TCCCACAAAAAECTCC	AGAACCACCACCAGAACCCACCACCTC TAAGCTTCCAGGCTCTTC
<i>OsCNIH1</i>	CACCATGGTTTTTCGTGTGGCT	CTCATCCTCCTCCAATAATACAG
pYeP352- <i>OsHKT1;3</i>	GTACATTATAAAAAAAATCCTGAA CTTAGCTAGATATTATGAATCATTG TCTTGTAG	CACGACGTTGTAACGACGGCCAGT GCCAAGCTTGCATGTTATCTAAGCTTC CAGGCTC
pYeP352- <i>OsHKT1;3-1</i>	TCC GTA TCT GCA TCA ACT G	
pYeP352- <i>OsHKT1;3-2</i>	CTT CAG CAA TCC TAG TCC T	
pOO2- <i>OsHKT1;3-EGFP</i>	CGGGATCCATGAATCATTGTCTTGT AGTATCCACA	CGGAATTCAGAACCCACCCTCTAAG CTTCCAGGCTCTTC
pOO2-EGFP	CGGAATTCGGTGGTGGTTCTATGG TGAGCAAGGGCGAGGA	CCAATGCATTGGTTCTGCAGTTACTTG TACAGCTCGTCCA

**Table S2. Primers Used for the deletion of *ERV14* in Yeast.**

<b>Gene</b>	<b>Primer Squence (5'→3')</b>	
	<i>Forward</i>	<i>Reverse</i>
<i>ScERV14-kanMX</i>	GTAAAGTAAAAAATTAAGAATAAA AAGAAAAATGGGTGCTTGGTTCGT ACGCTGCAGGTCGAC	CTTTGGATTTCAATGTCTTGTGGATTAGAA GTCATCACACAGCATAGGCCACTAGTGGA TCTG
<i>ScERV14</i>	CCCATTCTCACATTATC	TTTCACAGTCATGCTCACCC