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Supplemental Information

The Venus Flytrap *Dionaea muscipula* Counts Prey-Induced Action Potentials to Induce Sodium Uptake

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Figure S1

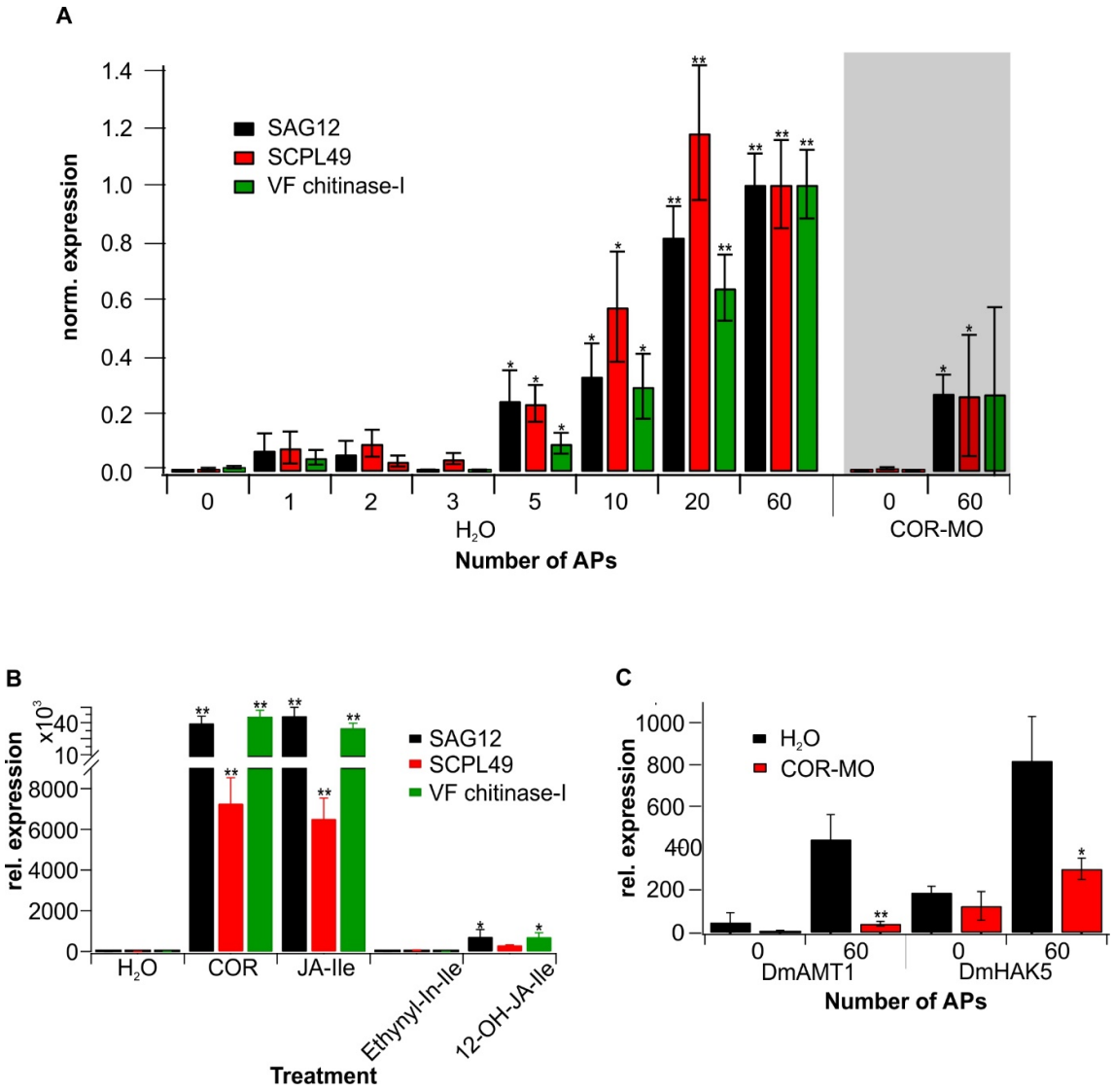

Fig. S1. Related to Figure 1

Transcript numbers were normalised to 10,000 molecules of DmACT1. (A): Transcript levels of the *Dionaea* hydrolases SAG12, SCPL49 and VF chitinase-I in response to different number of APs were quantified by qRT-PCR. Traps were pre-treated 4 h before initiating APs with H_2O (shaded in white) or the JA pathway inhibitor COR-MO (shaded in grey). Asterisks indicate a statistically significant difference to zero applied APs (* = $P < 0.05$; ** = $P < 0.01$ by one-way ANOVA) ($n \geq 3$, mean ± SE). (B): Expression level of hydrolases following chemical induction by COR, JA-Ile, Ethynyl-In-Ile and 12-OH-JA-Ile. Control plants were sprayed with water. Asterisks indicate a statistically significant difference to control plants (* = $P < 0.05$; ** = $P < 0.01$ by one-way ANOVA) ($n \geq 3$, mean ± SE). (C): Expression level of DmAMT1 and DmHAK5

after application of 0 and 60 APs. Mechanically-induced gene regulation was inhibited by application of 100 µM COR-MO (red). Control plants (black) were sprayed with water. Asterisks indicate a statistically significant difference to control plants (* = $P < 0.05$; ** = $P < 0.01$ by one-way ANOVA) ($n \geq 4$, mean ± SE).

Figure S2

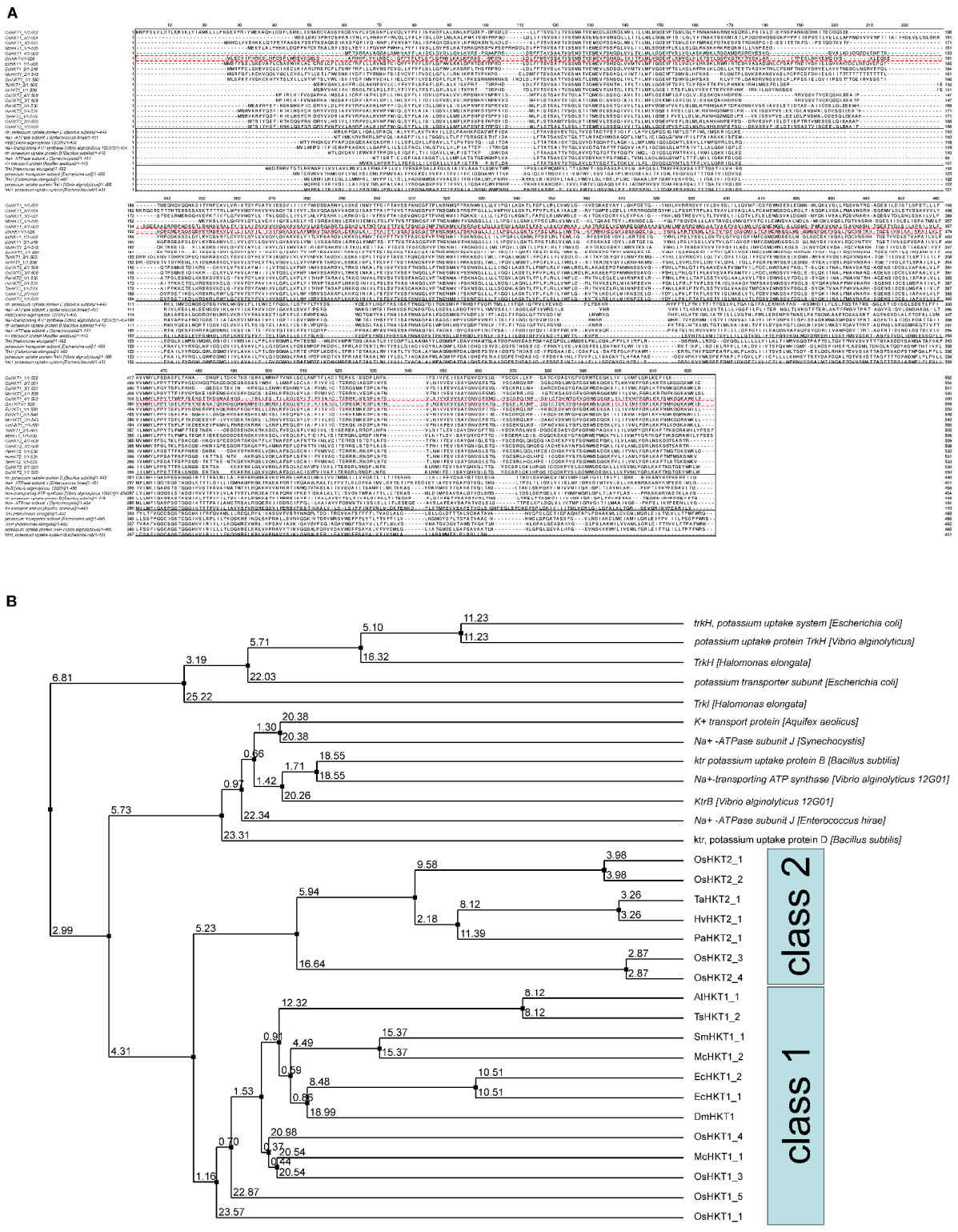

Fig. S2. Related to Figure 3

(A): Sequences of Trk/Ktr/HKT orthologues from various plant, bacterial and fungal species were aligned by ClustralW2. HKT1 from *Dionaea muscipula* is boxed in red (jalview 2.7). (B): Phylogenetic relation between Trk/Ktr/HKT transporters. The phylogenetic tree is based on an

alignment with selected protein sequences of plant, bacterial and fungal Trk/Ktr/HKT transporters (as indicated). The tree was constructed with Geneious using Neighbor-Joining [S1].

Figure S3

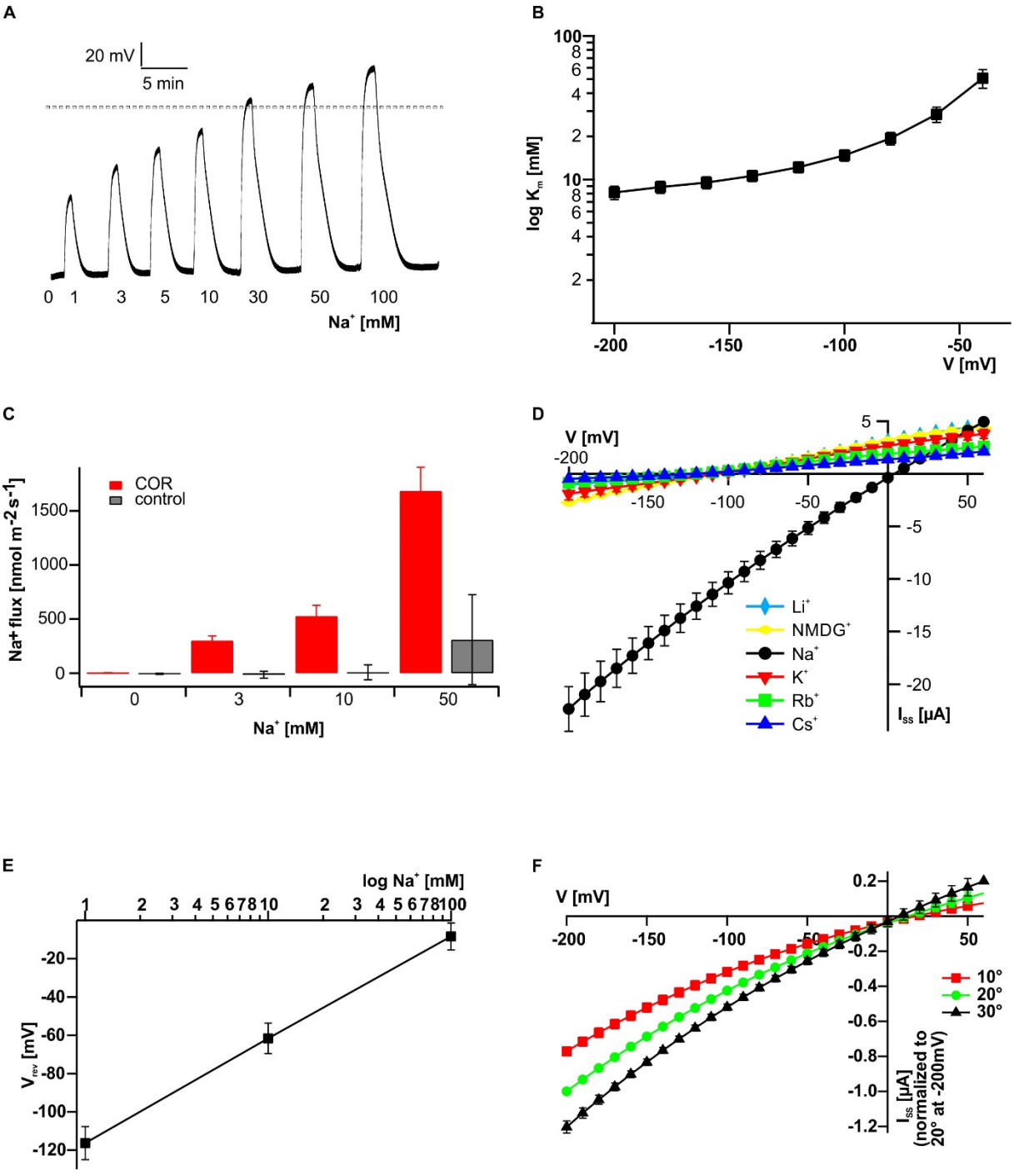

Fig. S3. Related to Figure 4

(A): Membrane potential measurements recorded in a representative DmHKT1-expressing oocyte in response to stepwise increases in sodium concentrations as indicated. Between applications of the different sodium concentrations, the oocyte was perfused with 100 mM LiCl. The dotted line indicates 0 mV. (B): K_m values derived from saturation curves recorded at different membrane potentials were fitted with a Michaelis-Menten equation. K_m values were

plotted as a function of the applied voltage, which indicated a pronounced voltage-dependent affinity of DmHKT1 towards sodium ions (n = 3, mean ± SD). (C): Net Na^+ fluxes derived from COR stimulated and unstimulated Venus flytraps using the MIFE-technique. COR-stimulated traps mediated Na^+ influx at all the tested external sodium concentrations. With 50 mM external NaCl, the sodium influx increased to 1683 ± 218 nmol m^{-2} s^{-1}. In contrast, non-stimulated traps showed a Na^+ uptake of only 310 ±415 nmol m^{-2} s^{-1} with 50 mM external NaCl (n ≥ 7, mean ± SD). (D): Current-voltage relation in the presence of various monovalent cations (100 mM each) illustrates the sodium-specific conductance of DmHKT1 (n = 3, mean ± SE). (E): The reversal potential (V_{rev}) recorded with DmHKT1-expressing oocytes were plotted against the applied sodium concentrations (logarithmic scale) (n = 3, mean ± SD). The reversal potential shifted in a sodium dependent manner with a slope of 54.7 ± 0.94 mV (1 – 10 mM Na^+) and 55.3 ± 0.94 (10 – 100 mM Na^+), respectively (n = 5, mean ± SD). (F): DmHKT1-mediated sodium currents were recorded at voltages ranging from +60 mV to -200 mV at bath temperatures of 10, 20 and 30 °C in 100 mM NaCl solutions. Data points were normalised to -200 mV at 20 °C and 100 mM Na^+. Q_{10} values were calculated at membrane potentials from -50 mV to -200 mV, and as the mean factor between currents recorded at the given temperatures. The mean of the resulting Q_{10} values calculated between 10 °C - 20 °C, 20 °C - 30 °C and √ 10 °C -30 °C was 1.27 ± 0.01 (n = 5, mean ± SD).

Table S1

Ions in moth powder:

µmol/g DW	**Mean±SD**
Ca_2^+	6.11±0.96
K^+	305.07±66.55
Mg^+	23.63±5.02
Na^+	42.64±8.58
PO_4^{3-}	110.09±32.28
SO_4^{2-}	6.35±2.1
NO_3^-	156.07±41.2

Table S1. Related to Figure 2

The ion content of the standardized animal powder used for feeding experiments shown in Fig 2A. DW: dry weight (n = 5 replicates, mean ± SD).

Supplemental References

S1. Ko JH, Yang SH, Han KH (2006) Upregulation of an *Arabidopsis* RING-H2 gene, *XERICO*, confers drought tolerance through increased abscisic acid biosynthesis. Plant J 47: 343-355.