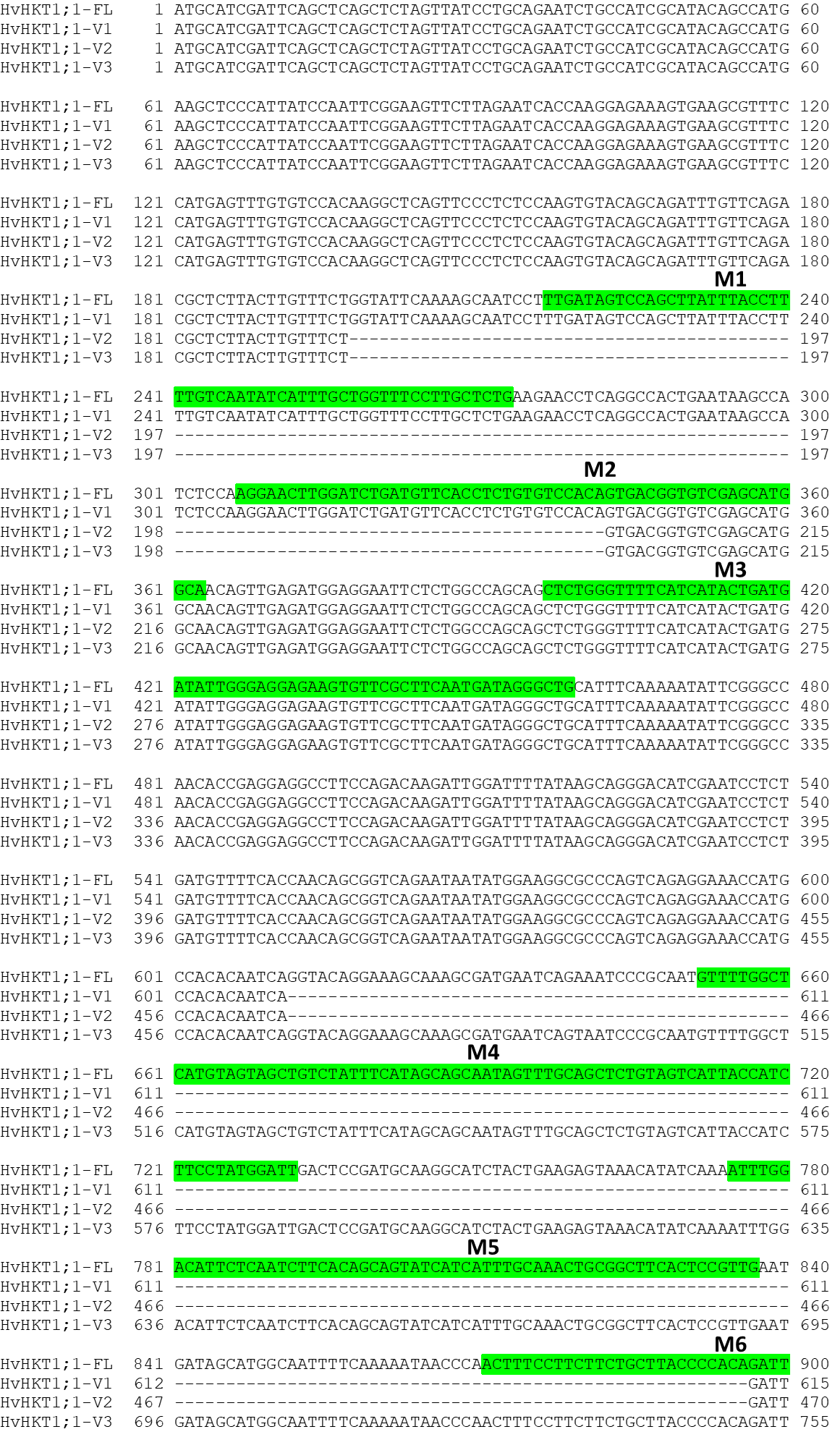
**Survey of Barley Sodium Transporter *HvHKT1;1* Variants and Their Functional Analysis**

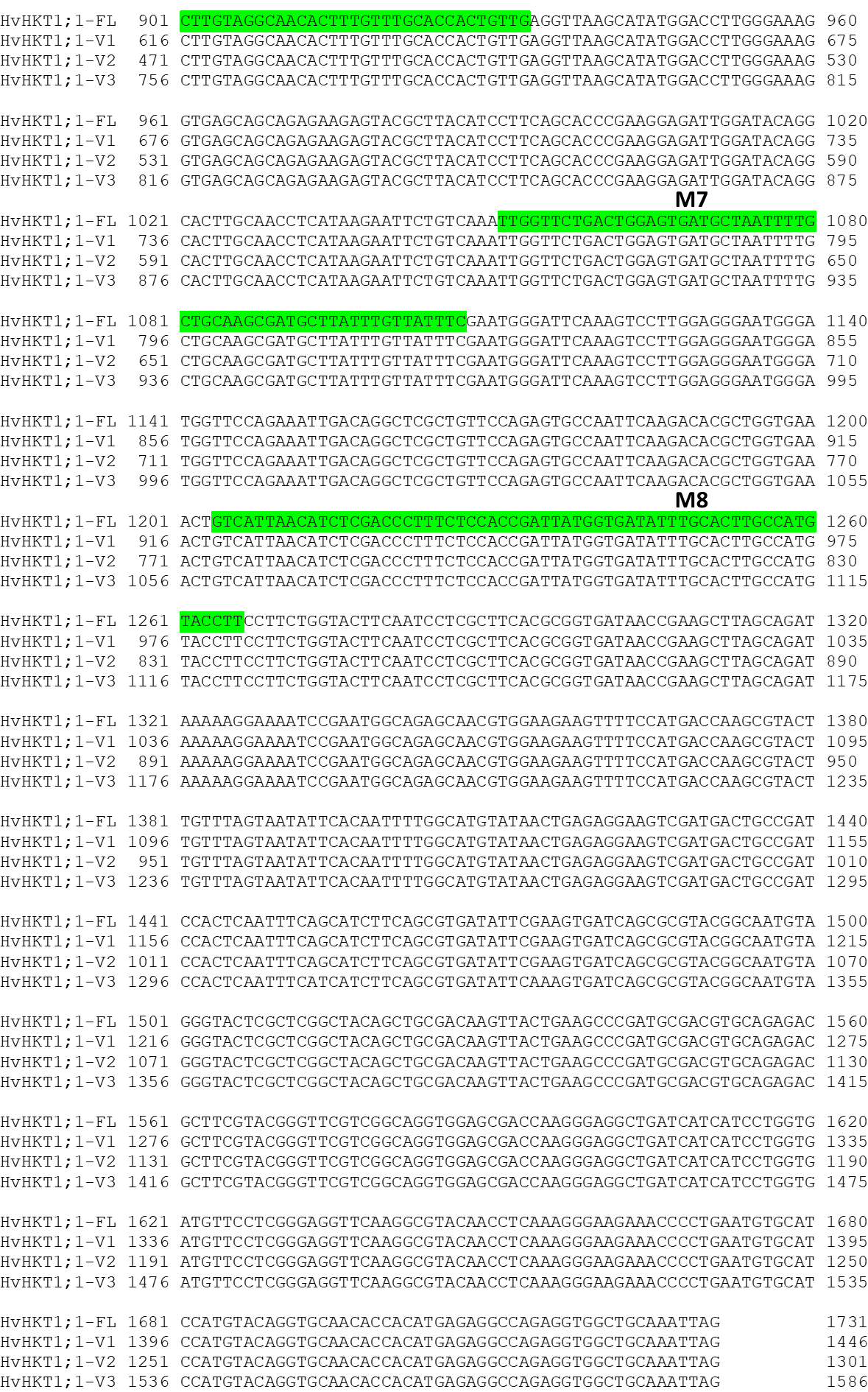
Shahin Imran and Maki Katsuhara

**Supplementary Materials:** **Table S1**. Gene-specific primer pairs used for the cloning and in the real-time PCR experiments; **Figure S1**. Nucleotide sequence alignment of *HvHKT1;1-FL* and its variants; **Figure S2**. Protein sequence alignment of *HvHKT1;1-FL* and its variants; **Figure S3**. Expression analysis of *HvHKT1;1-FL* and its variants; **Figure S4**. Na+ transport activity of HvHKT1;1-FL and its variants under different Na+ concentrations; **Figure S5**. Na+ selectivity of HvHKT1;1-FL and its variants.

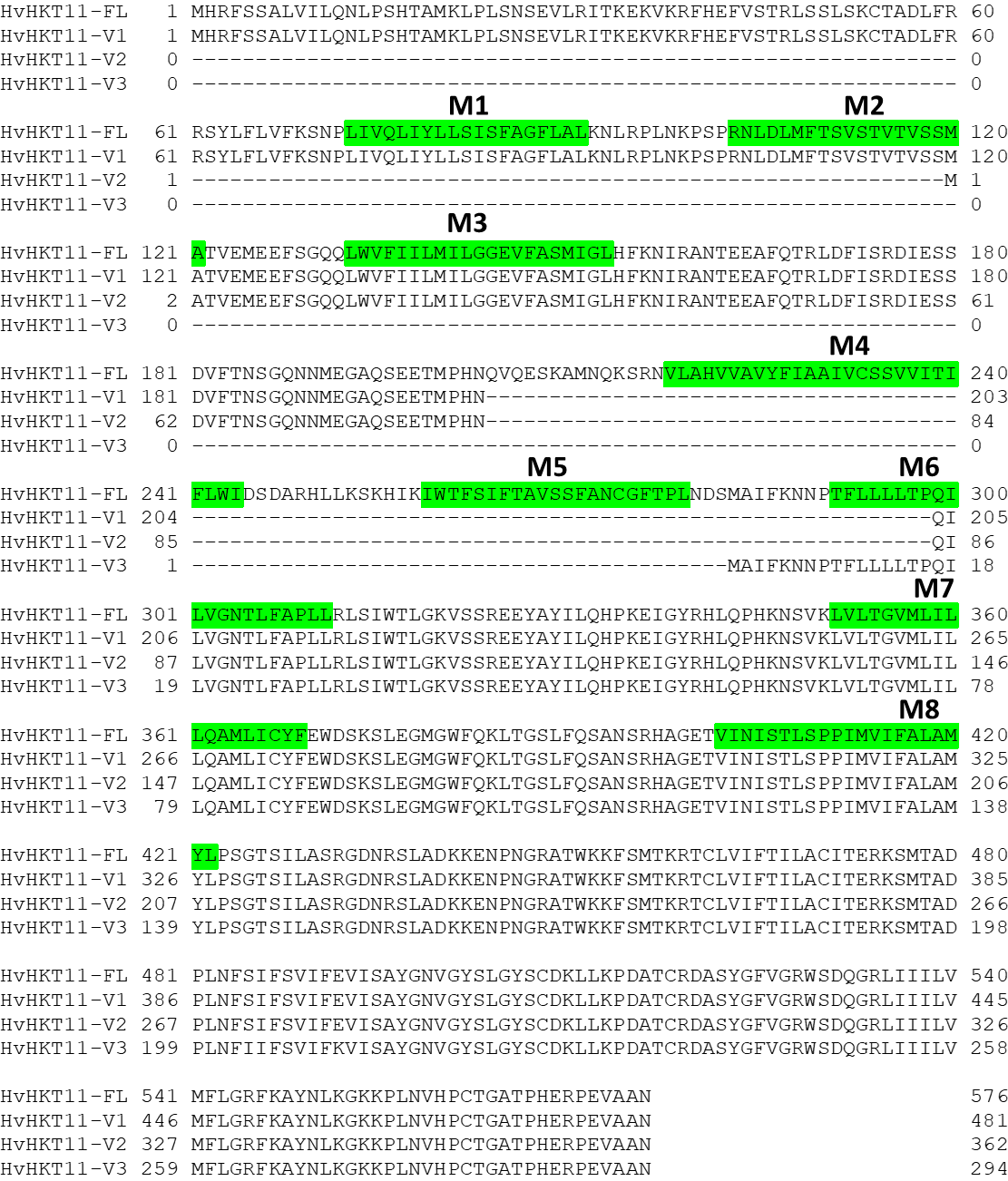
**Supplementary Table S1.** Gene-specific primer pairs were used for the cloning and in the real-time PCR experiments.

|  |  |  |
| --- | --- | --- |
| Primer Name | Sequence for full-length cloning | Sequence for real-time PCR |
| HvHKT1;1-F | ATGCATCGATTCAGCTCAGC |  |
| HvHKT1;1-R | CTAATTTGCAGCCACCTCTGG |  |
| HvHKT1;1-FL-F |  | ATGTTCACCTCTGTGTCCACAGT |
| HvHKT1;1-FL-R |  | CTGATTCATCGCTTTGCTTTCC |
| HvHKT1;1-V1-F |  | GGAACTTGGATCTGATGTTCACC |
| HvHKT1;1-V1-R |  | CTACAAGAATCTGATTGTGTGGC |
| HvHKT1;1-V2-F |  | CTTGTTTCTGTGACGGTGTCGA |
| HvHKT1;1-V2-R |  | CCTACAAGAATCTGATTGTGTGGC |
| HvHKT1;1-V3-F |  | CTTGTTTCTGTGACGGTGTCGA |
| HvHKT1;1-V3-R |  | GGATTACTGATTCATCGCTTTGC |

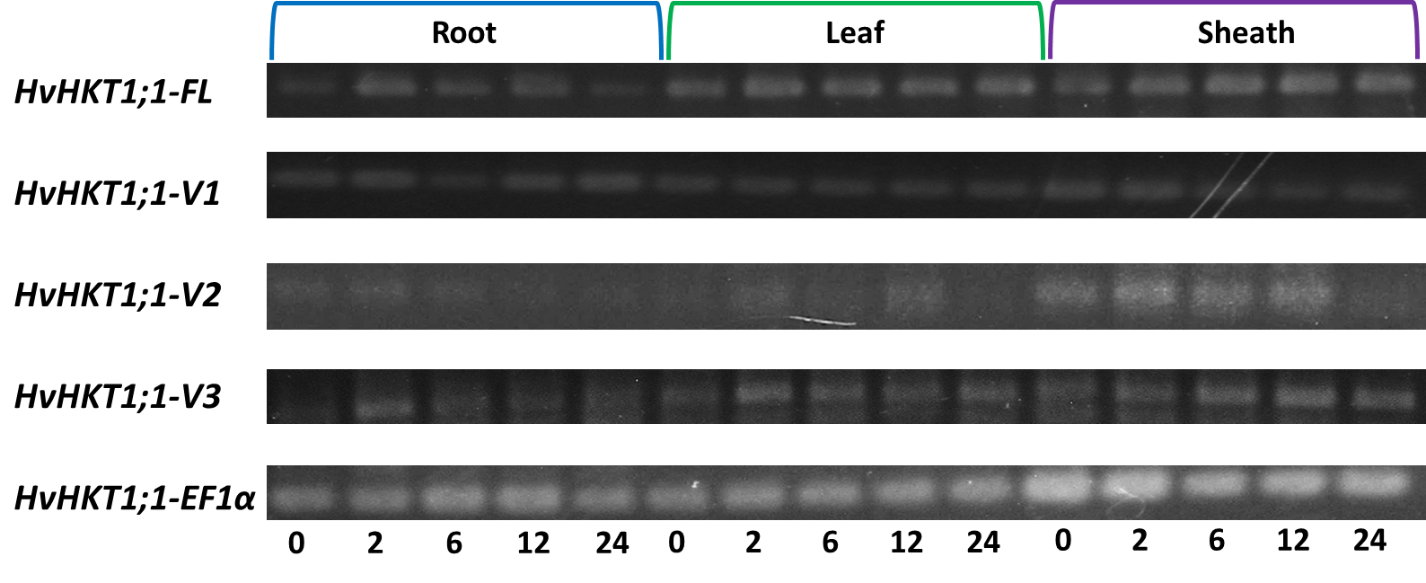




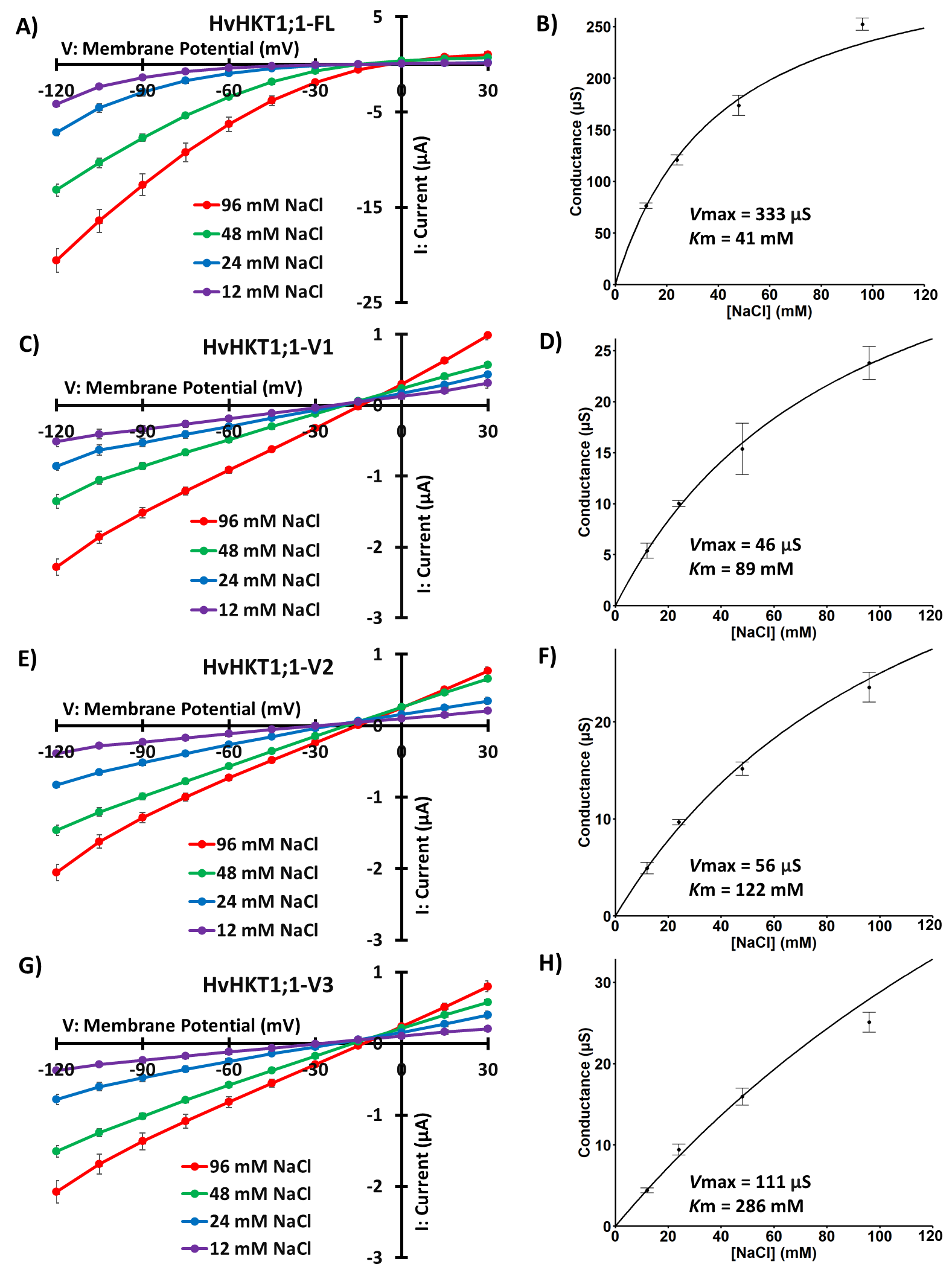
**Supplementary Figure S1.** Nucleotide sequence alignment of HvHKT1;1 and its variants using GENETYX ver. 16. M1–M8 indicate transmembrane domains predicted using previously registered data from the GeneBank database.



**Supplementary Figure S2.** Protein sequence alignment of HvHKT1;1 and its variants using GENETYX ver. 16. M1–M8 indicate transmembrane domains predicted using previously registered data from the GeneBank database.



**Supplementary Figure S3.** Expression analysis of *HvHKT1;1-FL* and its variants in root, leaf, and sheath of barley plants grown under stress conditions by reverse transcription polymerase chain reaction (RT-PCR). *HvHKT1;1-EF1α* was used as an internal control.



**Supplementary Figure S4.** Na+ transport activity of HvHKT1;1-FL and its variants under different Na+ concentrations. Current–voltage relationships (I–V curves) obtained from oocytes injected with either 50 ng of HvHKT1;1-FL cRNA (A), HvHKT1;1-V1 cRNA (C), HvHKT1;1-V2 cRNA (E), HvHKT1;1-V3 cRNA (G). Na+ concentration-dependent ionic conductance analysis for HvHKT1;1-FL (B), HvHKT1;1-V1 (D), HvHKT1;1-V2 (F), HvHKT1;1-V3 (H). External bath solutions contained 12, 24, 48, and 96 mM NaCl. 96 mM NaCl solution contains background elements as 1.8 mM CaCl2, 1.8 mM MgCl2, 1.8 mM mannitol, and 10 mM HEPES (pH 7.5 with Tris). 48 mM to 12 mM NaCl solutions contain background elements as 1.8 mM CaCl2, 1.8 mM MgCl2, 96-, 144-, or 168-mM mannitol, and 10 mM HEPES (pH 7.5 with Tris). Voltage steps ranged from −120 to +30 mV with 15 mV increments. Data are shown as means ± SE (*n* = 6-10).



**Supplementary Figure** **S5.** Na+ selectivity of HvHKT1;1 and its variants. The TEVC experiment using *X. laevis* oocytes was conducted. Current–voltage relationships (I–V curves) obtained from oocytes injected with either 50 ng of HvHKT1;1-FL cRNA (A), HvHKT1;1-V1 cRNA (C), HvHKT1;1-V2 cRNA (E), HvHKT1;1-V3 cRNA (G). Reversal potential shift analysis conducted by changing the external Na+ concentration from 96 mM to 0 mM for HvHKT1;1-FL (B), HvHKT1;1-V1 (D), HvHKT1;1-V2 (F), HvHKT1;1-V3 (H). External bath solutions contained 96 mM Na gluconate and 96 mM Choline-Cl. Basic background elements in the bath solution are 1.8 mM CaCl2, 1.8 mM MgCl2, 1.8 mM mannitol, and 10 mM HEPES (pH 7.5 with Tris). Voltage steps ranged from −120 to +30 mV with 15 mV increments. Data are shown as means ± SE (*n* = 10). Asterisks denote values significantly different from 9.6 mM NaCl + 86.4 mM Na gluconate as determined by one-way ANOVA (\*\*\**P* < 0.001). Post hoc tests were not applicable because only two groups were compared.