

Supplementary materials for

Article

Identification and characterization of rice *OsHKT1;3* variants

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

		For full-length	For RT-PCR and real-time RT-PCR
OsHKT1;3	Forward	TGCAAATAAGGAAGCACCCA	
	Reverse	AGGTGGAAGATGCATGGCTT	
OsHKT1;3_FL	Forward		AGTCAGCACAAGGGATCACTCAC
	Reverse		AGAAGATATCCCATCACTAC
OsHKT1;3_V1	Forward		GCGCTCAACTCTGGGTTTTGACTATTT
	Reverse		GATAGTTGCTCATTCCATCCA
OsHKT1;3_V2	Forward		TCCTCTCTTTGGAGTGGAGCT
	Reverse		GTCCCGTTCATCATGTCTGGGT
OsHKT1;3_V3	Forward		TGCTGATTGGTGGTATCTCCCTGGTTG
	Reverse		AACCGAGAACCCCACATTTCC
OsHKT1;3_V4	Forward		CTTCGTTTTTTACCCAGACATGATG
	Reverse		TGCTGATTGGTGGTATCTCCCTGGTTG
OsHKT1;3_V5	Forward		ATTGGTGGTGAGGTATCTCCCTGGTTA
	Reverse		TGCTGATTGGTGGTATCTCCCTGGTTG

Supplementary Table S2: R² values of all TEVC data

Supplementary Table S2.1. R² values of Oocytes injected with OsHKT1;3_FL and its variants (V1-V5) or water measured in 96 mM Na⁺ solution

Oocytes injected cRNAs or water measured in NaCl	R ² values for Figure 4A, B
OsHKT1;3_FL	0.9987
OsHKT1;3_V1	0.9981
OsHKT1;3_V2	0.9975
OsHKT1;3_V3	1.0000
OsHKT1;3_V4	0.9998
OsHKT1;3_V5	0.9998
Water	0.9971

Supplementary Table S2.2. R² values of Oocytes injected with OsHKT1;3_FL measured in 86.4 mM Na-Glu +9.6 mM NaCl and 86.4 mM choline-Cl +9.6 mM NaCl

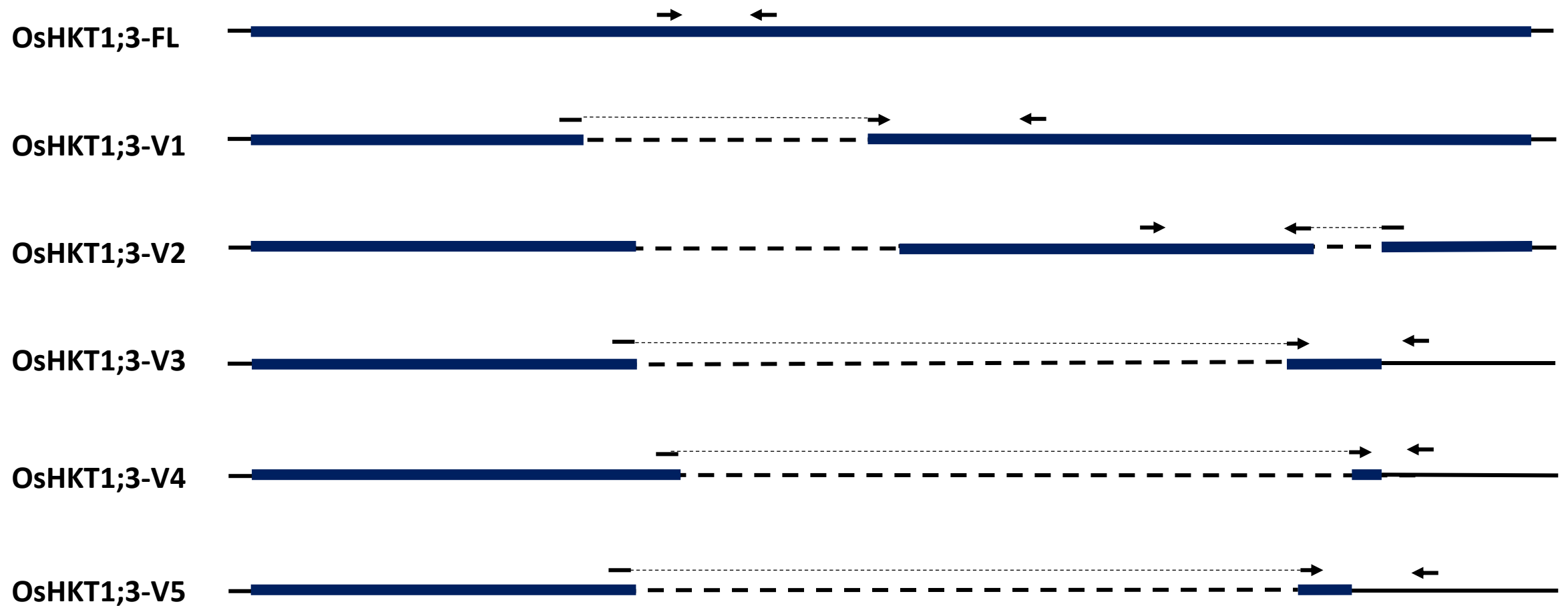
Oocytes injected with OsHKT1;3_FL measured in	R ² values for Figure 4C
86.4 mM Na-Glu +9.6 mM NaCl	0.9940
86.4 mM choline-Cl +9.6 mM NaCl	0.9956

Supplementary Table S2.3. R² values of Oocytes injected with OsHKT1;3_FL and its variants (V1-V5) or water measured in 96 mM K⁺ solution

Oocytes injected cRNAs or water measured in KCl	R ² values for Supplementary Figure S5
OsHKT1;3_FL	0.9926
OsHKT1;3_V1	0.9927
OsHKT1;3_V2	0.9934
OsHKT1;3_V3	0.9931
OsHKT1;3_V4	0.9933
OsHKT1;3_V5	0.9901
Water	0.9940

Supplementary Table S2.4. R² values of Oocytes injected with water measured in 86.4 mM Na-Glu +9.6 mM NaCl and 86.4 mM choline-Cl +9.6 mM NaCl

Oocytes injected with water measured in	R ² values for Supplementary Figure S6
86.4 mM Na-Glu +9.6 mM NaCl	0.9948
86.4 mM choline-Cl +9.6 mM NaCl	0.9932



Supplementary Figure S1.

Specific primer position of *OsHKT1;3*_FL and its five variant for qPCR assays. Right arrows indicate forward primers, and left arrows indicate reverse primers.

OsHKT1;3 Genomic DNA	1	CGGGGCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTGCAGAAGCTC	60
OsHKT1;3_FL	1	--GGGCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTGCAGAAGCTC	58
OsHKT1;3 Genomic DNA	61	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTTGTCTTGTAGTATCCACAAAAA	120
OsHKT1;3_FL	59	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTTGTCTTGTAGTATCCACAAAAA	118
OsHKT1;3 Genomic DNA	121	ACTCCAAACTTTCCGCACATTGCGAGTAGCAAGTTCTCTTCTTTTACCAAAATCTGCACA	180
OsHKT1;3_FL	119	ACTCCAAACTTTCCGCACATTGCGAGTAGCAAGTTCTCTTCTTTTACCAAAATCTGCACA	178
OsHKT1;3 Genomic DNA	181	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAAACAAATCCACTCTTTGTCCATGT	240
OsHKT1;3_FL	179	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAAACAAATCCACTCTTTGTCCATGT	238
OsHKT1;3 Genomic DNA	241	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGTCCTCAAGCCACG	300
OsHKT1;3_FL	239	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGTCCTCAAGCCACG	298
OsHKT1;3 Genomic DNA	301	AGACAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	360
OsHKT1;3_FL	299	AGACAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	358
OsHKT1;3 Genomic DNA	361	TGTTTCAAGCATGGCTACTGTGTGAAATGGAGGATTTCTCAAGCGCTCAACTCTGGGTTTT	420
OsHKT1;3_FL	359	TGTTTCAAGCATGGCTACTGTGTGAAATGGAGGATTTCTCAAGCGCTCAACTCTGGGTTTT	418
OsHKT1;3 Genomic DNA	421	GACTATTTTAAATGCTGATTGGTGGTGAGGTATTCACCTCAATGCTTGGCATTCACTTTAT	480
OsHKT1;3_FL	419	GACTATTTTAAATGCTGATTGGTGGTGAGGTATTCACCTCAATGCTTGGCATTCACTTTAT	478
OsHKT1;3 Genomic DNA	481	GAGAGCCGAATTTGGTACAAAAGAGTCAGTCAGCACAAAGGGATCACTCACCTTGCATTGA	540
OsHKT1;3_FL	479	GAGAGCCGAATTTGGTACAAAAGAGTCAGTCAGCACAAAGGGATCACTCACCTTGCATTGA	538
OsHKT1;3 Genomic DNA	541	TATTGAGTCTATTACTTCCACAAAATTTGGTCCCAGCACCCAGGGCACAAAAGTTACAGT	600
OsHKT1;3_FL	539	TATTGAGTCTATTACTTCCACAAAATTTGGTCCCAGCACCCAGGGCACAAAAGTTACAGT	598
OsHKT1;3 Genomic DNA	601	TTCATTTTCTGAACTCCGCATGGAAAATGGAGGACATGTAGAGCCCAAGACGATTAAATT	660
OsHKT1;3_FL	599	TTCATTTTCTGAACTCCGCATGGAAAATGGAGGACATGTAGAGCCCAAGACGATTAAATT	658
OsHKT1;3 Genomic DNA	661	TTTAGGTTTTGTAGTGTATGGGATATCTTCTAATAACAAACTTAGGCGGCTCCCTACTTAT	720
OsHKT1;3_FL	659	TTTAGGTTTTGTAGTGTATGGGATATCTTCTAATAACAAACTTAGGCGGCTCCCTACTTAT	718
OsHKT1;3 Genomic DNA	721	TTACCTCTACCTTAACCTGGTACCAAGTGCACATAAAATTCTAAAGAGAAAAGGCATTGG	780
OsHKT1;3_FL	719	TTACCTCTACCTTAACCTGGTACCAAGTGCACATAAAATTCTAAAGAGAAAAGGCATTGG	778
OsHKT1;3 Genomic DNA	781	GATCATCGTATTCTCAGTATTTACAGCCATCTCCTCAGTTGGAAATTGTGGCTTCACCTCC	840
OsHKT1;3_FL	779	GATCATCGTATTCTCAGTATTTACAGCCATCTCCTCAGTTGGAAATTGTGGCTTCACCTCC	838
OsHKT1;3 Genomic DNA	841	AGTAAATGAGAATATGATTATCTTTCAGAAGAACTCCATTCTTCTATTGCTAATTCCTCC	900
OsHKT1;3_FL	839	AGTAAATGAGAATATGATTATCTTTCAGAAGAACTCCATTCTTCTATTGCTAATTCCTCC	898
OsHKT1;3 Genomic DNA	901	TCAGATACTAGCAGGAAATACATTATTTGCACCATGCTTGAGATTAATGGTGTGGTCACT	960
OsHKT1;3_FL	899	TCAGATACTAGCAGGAAATACATTATTTGCACCATGCTTGAGATTAATGGTGTGGTCACT	958
OsHKT1;3 Genomic DNA	961	TGAGAAGATTACCGGAAAAAAGGATTGTCTGTACATTCTTGAATATCCAAAGGCCATTGG	1020
OsHKT1;3_FL	959	TGAGAAGATTACCGGAAAAAAGGATTGTCTGTACATTCTTGAATATCCAAAGGCCATTGG	1018
OsHKT1;3 Genomic DNA	1021	ATATAAACATCTTATGAGTACCAAGGGAAGTGTTTATTTGACTTTAACAGTTGTGAGCTT	1080
OsHKT1;3_FL	1019	ATATAAACATCTTATGAGTACCAAGGGAAGTGTTTATTTGACTTTAACAGTTGTGAGCTT	1078
OsHKT1;3 Genomic DNA	1081	GATCATTCTGC AAAACCGTATTGTTCCCTCTCTTTGGAGTGGAGCTCGGTAGCTTTGGATGG	1140
OsHKT1;3_FL	1079	GATCATTCTGC AAAACCGTATTGTTCCCTCTCTTTGGAGTGGAGCTCGGTAGCTTTGGATGG	1138
OsHKT1;3 Genomic DNA	1141	AATGAGCAACTATCAAAAGATAGTATCCGCTCTATTTCAGTCGGTCAATGCTAGGCATGC	1200
OsHKT1;3_FL	1139	AATGAGCAACTATCAAAAGATAGTATCCGCTCTATTTCAGTCGGTCAATGCTAGGCATGC	1198
OsHKT1;3 Genomic DNA	1201	AGGTGAATCTGTTACAGATCTGTCAAACCTCTCTTCAGCAATCCTAGTCCATACACCAT	1260
OsHKT1;3_FL	1199	AGGTGAATCTGTTACAGATCTGTCAAACCTCTCTTCAGCAATCCTAGTCCATACACCAT	1258
OsHKT1;3 Genomic DNA	1261	CATGATGTAAGTCCCTTCTCAAACCTTCTCCCCAAAATAAGAATGATCATAAGTAAGAAA	1320
OsHKT1;3_FL	1259	CATGATGTATCTCCCTGGTTACAC-TTC-----	1285
OsHKT1;3 Genomic DNA	1321	ACTTCCTGTATTTATTGCTCTAGTCAGTAACTATATAAATTTAACTACCTTATCATGGAC	1380
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1381	ATCACATTTGCCTTCCTTCACTTATAATAGCCCTATAGTGGTTCTCTCGAATATGTGCAG	1440
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1441	CAACGTTCAAAATATATTGTACTTCACCTAATAGGTAGGTTGCATATAGTAGAATGGAACAT	1500
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1501	AAGGACATCAGATAATCTTTAAGAAATACACAGGAAAGCTTCCTACCATAGTACTCCTACC	1560
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1561	TATTAAGAAAAGGAATTTTTTAATCACACAATCCCACAAGCCAGACTCAGTGCTAGATTTG	1620
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1621	GTTGAGGACTATAGTAGTTCAAGAACCACCTTACAGGCTCAACAGTATACTACATACAAAG	1680
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1681	CAGTTAGTCCAGTATATGTTATCATTGAGAGGAATTGTTCTTAATATGTAATCTTAACAC	1740
OsHKT1;3_FL	1285	-----	1285
OsHKT1;3 Genomic DNA	1741	ATACTTTGACTAATTTTTATGTAGGTATCTCCCTGGTTACACTTCGTTTTTACCCAGACA	1800
OsHKT1;3_FL	1286	-----GTTTTTACCCAGACA	1300
OsHKT1;3 Genomic DNA	1801	TGATGGTGAGGATTCTAAGACCGAGAAGATAAACAAAAGAAAAGGGCTATTGGAGAACTG	1860
OsHKT1;3_FL	1301	TGATGGTGAGGATTCTAAGACCGAGAAGATAAACAAAAGAAAAGGGCTATTGGAGAACTG	1360
OsHKT1;3 Genomic DNA	1861	GATCTTCTCACATATGTCCTTATTTGGCTATCTTTGTAATGCTAATTTGCATCACAGAACG	1920
OsHKT1;3_FL	1361	GATCTTCTCACATATGTCCTTATTTGGCTATCTTTGTAATGCTAATTTGCATCACAGAACG	1420
OsHKT1;3 Genomic DNA	1921	GGACTCGATGGCTACAGATCCACTTAATTTCAATGTTTTTCAGCATATTGTTTGAAGTCGT	1980
OsHKT1;3_FL	1421	GGACTCGATGGCTACAGATCCACTTAATTTCAATGTTTTTCAGCATATTGTTTGAAGTCGT	1480
OsHKT1;3 Genomic DNA	1981	CAGGCAAGCCACTGAAATTCACTGCATTCTGCAGAAAGCTCATGATTATTTTTTAACATG	2040
OsHKT1;3_FL	1480	-----	1480
OsHKT1;3 Genomic DNA	2041	AGTATCATCACTGAACATCTGGCTCTGATTGTTGCAGTGCATATGGAAATGTGGGGTTCT	2100
OsHKT1;3_FL	1481	-----CAGTGCATATGGAAATGTGGGGTTCT	1506
OsHKT1;3 Genomic DNA	2101	CGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGATGCCTCGT	2160
OsHKT1;3_FL	1507	CGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGATGCCTCGT	1566
OsHKT1;3 Genomic DNA	2161	ACGGGTTTGC GGGGAAATGGAGCGACAAATGGGAAAGCGATCCTGATCATCGTCATGCTTT	2220
OsHKT1;3_FL	1567	ACGGGTTTGC GGGGAAATGGAGCGACAAATGGGAAAGCGATCCTGATCATCGTCATGCTTT	1626
OsHKT1;3 Genomic DNA	2221	TCGGGAGGCTTAAAAACGTTTAAACATGAAGGGTGGAAAGACCTGGAAAGCTTAGATAAAGCG	2280
OsHKT1;3_FL	1627	TCGGGGGGCTTAAAAACGTTTAAACATGAAGGGTGGAAAGACCTGGAAAGCTTAGATAAAGCG	1686
OsHKT1;3 Genomic DNA	2281	GCCGCGACGGTACCACATAAACCGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACAT	2340
OsHKT1;3_FL	1687	GCCGCGACGGTACCACATAAACCGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACAT	1746
OsHKT1;3 Genomic DNA	2341	AATACCAACTTACACTTACAAA	2362
OsHKT1;3_FL	1747	AATACCAACTTACACTTACAAA	1768

Supplementary Figure S2.
Nucleotide sequence alignment of Nipponbare OsHKT1;3 genomic DNA and full-length cDNA. GENETYX ver. 13 was used for exon-intron analysis.

OsHKT1;3_FL	1	--GGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTTGCAGAAGCTC	58
OsHKT1;3_V1	1	--GGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTTGCAGAAGCTC	58
OsHKT1;3_V2	1	CGGGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTTGCAGAAGCTC	60
OsHKT1;3_V3	1	CGGGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTTGCAGAAGCTC	60
OsHKT1;3_V4	1	--GGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTTGCAGAAGCTC	58
OsHKT1;3_V5	1	--GGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTTGTTCTTTTTGCAGAAGCTC	58
Start codon			
OsHKT1;3_FL	59	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTGTCTTGTA GTATCCACAAAAA	118
OsHKT1;3_V1	59	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTGTCTTGTA GTATCCACAAAAA	118
OsHKT1;3_V2	61	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTGTCTTGTA GTATCCACAAAAA	120
OsHKT1;3_V3	61	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTGTCTTGTA GTATCCACAAAAA	120
OsHKT1;3_V4	59	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTGTCTTGTA GTATCCACAAAAA	118
OsHKT1;3_V5	59	AGAATAAACGCTCAACTTTGGCAGAACCATGAATCATTGTCTTGTA GTATCCACAAAAA	118
M1			
OsHKT1;3_FL	119	ACTCCAAACTTTCCGCACATTTGCAGCTAGCAA GTTCTCTTCTTTTACCAAATCTGCACA	178
OsHKT1;3_V1	119	ACTCCAAACTTTCCGCACATTTGCAGCTAGCAA GTTCTCTTCTTTTACCAAATCTGCACA	178
OsHKT1;3_V2	121	ACTCCAAACTTTCCGCACATTTGCAGCTAGCAA GTTCTCTTCTTTTACCAAATCTGCACA	180
OsHKT1;3_V3	121	ACTCCAAACTTTCCGCACATTTGCAGCTAGCAA GTTCTCTTCTTTTACCAAATCTGCACA	180
OsHKT1;3_V4	119	ACTCCAAACTTTCCGCACATTTGCAGCTAGCAA GTTCTCTTCTTTTACCAAATCTGCACA	178
OsHKT1;3_V5	119	ACTCCAAACTTTCCGCACATTTGCAGCTAGCAA GTTCTCTTCTTTTACCAAATCTGCACA	178
OsHKT1;3_FL	179	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAACAATCCA CTCTTTGTCCATGT	238
OsHKT1;3_V1	179	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAACAATCCA CTCTTTGTCCATGT	238
OsHKT1;3_V2	181	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAACAATCCA CTCTTTGTCCATGT	240
OsHKT1;3_V3	181	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAACAATCCA CTCTTTGTCCATGT	240
OsHKT1;3_V4	179	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAACAATCCA CTCTTTGTCCATGT	238
OsHKT1;3_V5	179	GAAGTCTATAAAATACTCCTTCCAGTTCATCTACCAAACAATCCA CTCTTTGTCCATGT	238
OsHKT1;3_FL	239	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGT CCTCAAGCCACG	298
OsHKT1;3_V1	239	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGT CCTCAAGCCACG	298
OsHKT1;3_V2	241	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGT CCTCAAGCCACG	300
OsHKT1;3_V3	241	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGT CCTCAAGCCACG	300
OsHKT1;3_V4	239	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGT CCTCAAGCCACG	298
OsHKT1;3_V5	239	AGCTTACTTTGCCCTGATCTCCTTTGCTGGATATGGATCTCTAAAGGT CCTCAAGCCACG	298
OsHKT1;3_FL	299	AGACAAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	358
OsHKT1;3_V1	299	AGACAAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	358
OsHKT1;3_V2	301	AGACAAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	360
OsHKT1;3_V3	301	AGACAAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	360
OsHKT1;3_V4	299	AGACAAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	358
OsHKT1;3_V5	299	AGACAAAGTCAAATACTCTGAAAGACTTGGACGTGCTATTTACTTCCGTATCTGCATCAAC	358
OsHKT1;3_FL	359	TGTTTCAAGCATGGCTACTGTTGAAATGGAGGATTTCTCAAGCGCTCAA CTCTGGGTTTT	418
OsHKT1;3_V1	359	TGTTTCAAGCATGGCTACTGTTGAAATGGAGGATTTCTCAAGCGCTCAA CTCTGGGTTTT	418
OsHKT1;3_V2	361	TGTTTCAAGCATGGCTACTGTTGAAATGGAGGATTTCTCAAGCGCTCAA CTCTGGGTTTT	420
OsHKT1;3_V3	361	TGTTTCAAGCATGGCTACTGTTGAAATGGAGGATTTCTCAAGCGCTCAA CTCTGGGTTTT	420
OsHKT1;3_V4	359	TGTTTCAAGCATGGCTACTGTTGAAATGGAGGATTTCTCAAGCGCTCAA CTCTGGGTTTT	418
OsHKT1;3_V5	359	TGTTTCAAGCATGGCTACTGTTGAAATGGAGGATTTCTCAAGCGCTCAA CTCTGGGTTTT	418
M2			
OsHKT1;3_FL	419	GACTATTTTAAATGCTGATTGGTG GTGAGGTATTCAC TTCAATGCTTGGCATTCACTTTAT	478
OsHKT1;3_V1	419	G-----	419
OsHKT1;3_V2	421	GACTATTTTAAATGCTGATTGGTG GTGAGATACT-----	453
OsHKT1;3_V3	421	GACTATTTTAAATGCTGATTGGTG GT-----	445
OsHKT1;3_V4	419	GACTATTTTAAATGCTGATTGGTG GTAGGTA---TCTCCCTGGTTACACTTCGTTTT--	472
OsHKT1;3_V5	419	GACTATTTTAAATGCTGATTGGTG GTAGGTT-----	448
OsHKT1;3_FL	479	GAGAGCCGAATTTGGTACAAAAGAGTCAGTCAGCACAAAGGGATCACTCACCTTGCA TTGA	538
OsHKT1;3_V1	419	-----	419
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	473	--TACCCAGA-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	539	TATTGAGTCTATTACTTCCACAAAATTTGGTCCCAGCACCCAGGGCACAAAAGTTACAGT	598
OsHKT1;3_V1	419	-----	419
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	599	TTCA TTTTCTGAACTCCGCATGGAAAA TGGAGGACATGTAGAGCCCAAGACGATTAAATT	658
OsHKT1;3_V1	419	-----	419
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448

M3

OsHKT1;3_FL	659	TTTAGGTTTTGTAGTGATGGGATATCTTCTAATAACAAACTTAGGCGGCTCCCTACTTAT	718
OsHKT1;3_V1	419	-----	419
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	719	TTACCTCTACCTTAACCTGGTACCAAGTGCACATAAAATTCTAAAGAGAAAAGGCATTGG	778
OsHKT1;3_V1	419	-----	419
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	779	GATCATCGTATTCTCAGTATTTACAGCCATCTCCTCAGTTGGAAATTGTGGCTTCACTCC	838
OsHKT1;3_V1	419	-----	419
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448

M4

OsHKT1;3_FL	839	AGTAAATGAGAAATATGATTATCTTTCAGAAGAACTCCATTCTTCTATTGCTAATTCTTCC	898
OsHKT1;3_V1	420	-----ACTATTTTAAATGCTGATTGGTGG	442
OsHKT1;3_V2	453	-----	453
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	899	TCAGATACTAGCAGGAAATACATTATTTGCACCATGCTTGAGATTAATGGTGTGGTCACT	958
OsHKT1;3_V1	443	TGAGATACTAGCAGGAAATACATTATTTGCACCATGCTTGAGATTAATGGTGTGGTCACT	502
OsHKT1;3_V2	454	-----AGCAGGAAATACATTATTTGCACCATGCTTGAGATTAATGGTGTGGTCACT	504
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	959	TGAGAAGATTACCGGAAAAAAGGATTGTCTGTTACATTCTTGAATATCCAAAGGCCATTGG	1018
OsHKT1;3_V1	503	TGAGAAGGTTACCGGAAAAAAGGATTGTCTGTTACATTCTTGAATATCCAAAGGCCATTGG	562
OsHKT1;3_V2	505	TGAGAAGATTACCGGAAAAAAGGATTGTCTGTTACATTCTTGAATATCCAAAGGCCATTGG	564
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448

M5

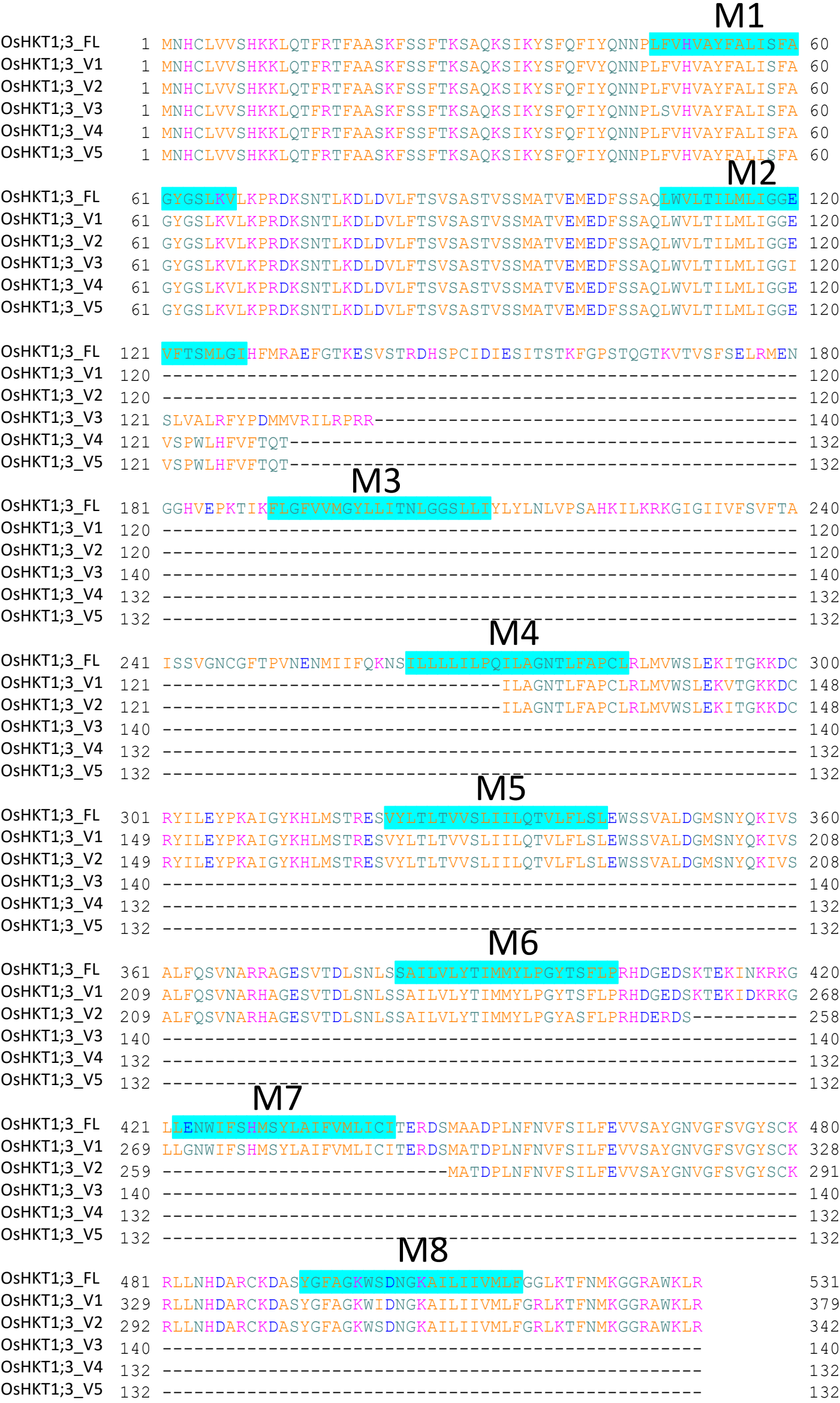
OsHKT1;3_FL	1019	ATATAAACATCTTATGAGTACCAGGGAAAAGTGTTTATTTGACTTTAACAGTTGTGAGCTT	1078
OsHKT1;3_V1	563	ATATAAACATCTTATGAGTACCAGGGAAAAGTGTTTATTTGACTTTAACAGTTGTGAGCTT	622
OsHKT1;3_V2	565	ATATAAACATCTTATGAGTACCAGGGAAAAGTGTTTATTTGACTTTAACAGTTGTGAGCTT	624
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	1079	GATCATTCTGCAAACCGTATTGTTCCCTCTCTTTGGAGTGGAGCTCGGTAGCTTTGGATGG	1138
OsHKT1;3_V1	623	GATCATTCTGCAAACCGTATTGTTCCCTCTCTTTGGAGTGGAGCTCGGTAGCTTTGGATGG	682
OsHKT1;3_V2	625	GATCATTCTGCAAACCGTATTGTTCCCTCTCTTTGGAGTGGAGCTCGGTAGCTTTGGATGG	684
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
OsHKT1;3_FL	1139	AATGAGCAACTATCAAAAGATAGTATCCGCTCTATTTCAGTCGGTCAATGCTAGGCGTGC	1198
OsHKT1;3_V1	683	AATGAGCAACTATCAAAAGATAGTATCCGCTCTATTTCAGTCGGTCAATGCTAGGCATGC	742
OsHKT1;3_V2	685	AATGAGCAACTATCAAAAGATAGTATCCGCTCTATTTCAGTCGGTCAATGCTAGGCATGC	744
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448

M6

OsHKT1;3_FL	1199	AGGTGAATCTGTTACAGATCTGTCAAACCTCTCTTCAGCAATCCTAGTCCTATACACCAT	1258
OsHKT1;3_V1	743	AGGTGAATCTGTTACAGATCTGTCAAACCTCTCTTCAGCAATCCTAGTCCTATACACCAT	802
OsHKT1;3_V2	745	AGGTGAATCTGTTACAGATCTGTCAAACCTCTCTTCAGCAATCCTAGTCCTATACACCAT	804
OsHKT1;3_V3	445	-----	445
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	448	-----	448
Stop codon			
OsHKT1;3_FL	1259	CATGATGTATCTCCCTGGTTACACTTCGTTTTTACCCAGACATGATGGTGAGGATTCTAA	1318
OsHKT1;3_V1	803	CATGATGTATCTCCCTGGTTACACTTCGTTTTTACCCAGACATGATGGTGAGGATTCTAA	862
OsHKT1;3_V2	805	CATGATGTATCTCCCTGGTTACGCTTCGTTTTTACCCAGACATGATGAACGGGACTCGAT	864
OsHKT1;3_V3	446	-----ATCTCCCTGGTTGCACTTCGTTTTTACCCAGACATGATGGTGAGGATTCTAA	497
OsHKT1;3_V4	480	-----	480
OsHKT1;3_V5	449	-----ATCTCCCTGGTTACACTTCGTTTTTACCCAGACATGATGGTGAGGATTCTAA	500

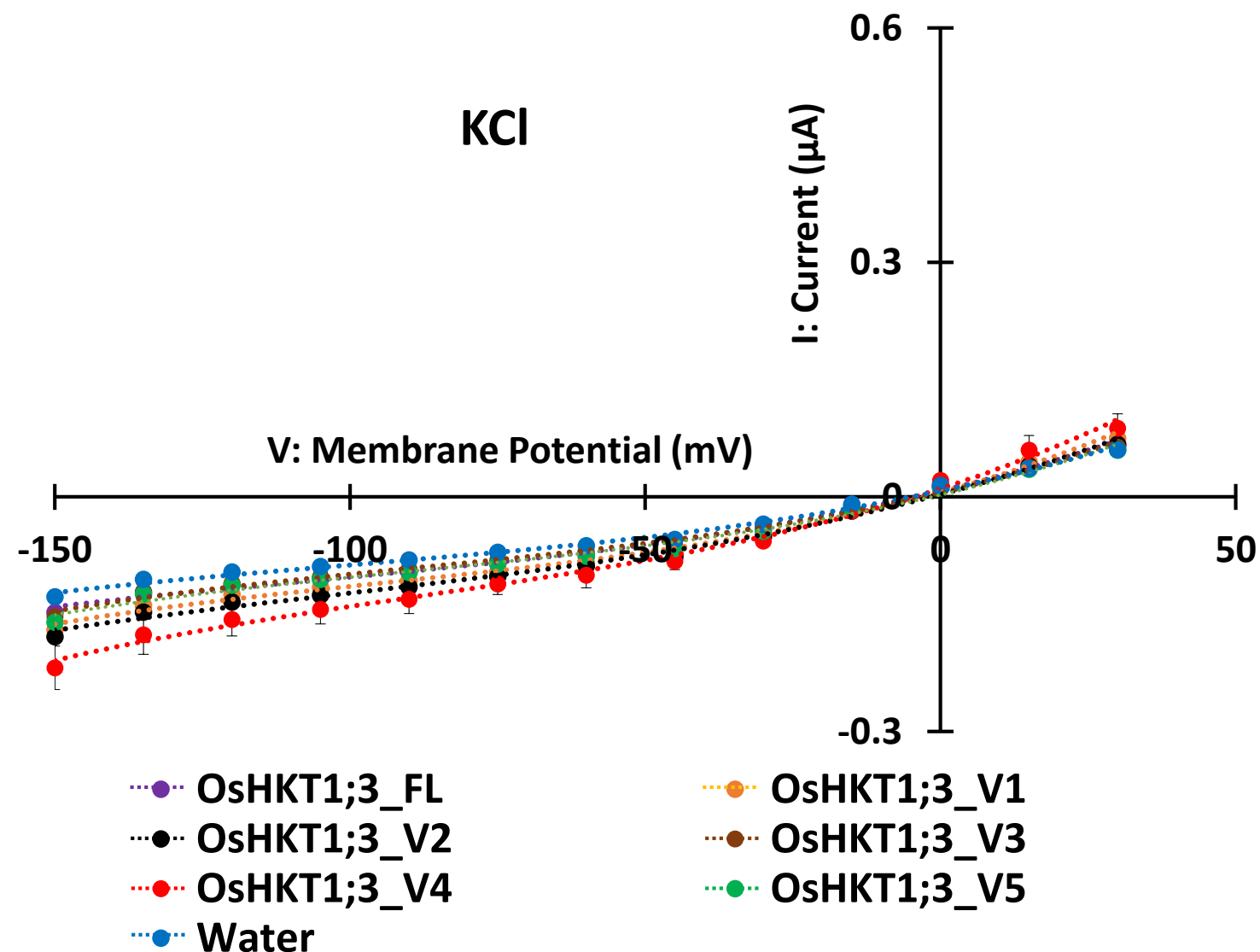
		Stop codon		M7
OsHKT1;3_FL	1319	GACCGAGAAGATAAAACAAAAGAAAAGGGCTATTGGAGAACTGGATCTTCTCACATATGTC	1378	
OsHKT1;3_V1	863	GACCGAGAAGATAGACAAAAGAAAAGGGCTATTGGGGAAC TGGATCTTCTCACATATGTC	922	
OsHKT1;3_V2	865	GGC-----	867	
OsHKT1;3_V3	498	GACCGAGAAGA TAAACAAAAGGAAAAGGGCTATTGGAGAACTGGATCTTCCCACATATGTC	557	
OsHKT1;3_V4	480	-----	480	
OsHKT1;3_V5	501	GACCGAGAAGATAAAACAAAAGAAAAGGGCTATTGGAGAACTGGATCTTCTCACATATGTC	560	
		Stop codon		
OsHKT1;3_FL	1379	TTATTTGGCTATCTTTGTAATGCTAATTTGCATCACAGAACGGGACTCGATGGCTGCAGA	1438	
OsHKT1;3_V1	923	TTATTTGGCTATCTTTGTAATGCTAATTTGCATTACAGAACGGGACTCGATGGCTACCGA	982	
OsHKT1;3_V2	868	-----TACAGA	873	
OsHKT1;3_V3	558	TTATTTGGCTATCTTTGTAATGCTAATTTGCATCACAGAACGGGACTCGATGGCTACAGA	617	
OsHKT1;3_V4	481	-----CATGATGAACGGGACTCGATGGCTACAGA	509	
OsHKT1;3_V5	561	TTATTTGGCTATCTTTGTAATGCTAATTTGCATCACAGAACGGGACTCGATGGCTACAGA	620	
OsHKT1;3_FL	1439	TCCACTTAATTTCAATGTTTTCAGCATATTGTTTGAAGTCGTCAGTGCATATGGAAATGT	1498	
OsHKT1;3_V1	983	TCCACTTAATTTCAATGTTTTCAGCATATTGTTTGAAGTTGTCAGTGCATATGGAAATGT	1042	
OsHKT1;3_V2	874	TCCACTTAATTTCAATGTTTTCAGCATATTGTTTGAAGTCGTCAGTGCATATGGAAATGT	933	
OsHKT1;3_V3	618	TCCACTTAATTTCAATGTTTTCAGCATATTGTTTGAAGTCGTCAGTGCATATGGAAATGT	677	
OsHKT1;3_V4	510	TCCACTTAATTTCAATGTTTTCAGCATATTGTTTGAAGTCGTCAGTGCATATGGAAATGT	569	
OsHKT1;3_V5	621	TCCACTTAAC TCAATGTTTTCAGCATATTGTTTGAAGTCGTCAGTGCATATGGAAATGT	680	
OsHKT1;3_FL	1499	GGGGTTCTCGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGA	1558	
OsHKT1;3_V1	1043	GGGGTTCTCGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGA	1102	
OsHKT1;3_V2	934	GGGGTTCTCGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGA	993	
OsHKT1;3_V3	678	GGGGTTCTCGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGA	737	
OsHKT1;3_V4	570	GGGGTTCTCGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGA	629	
OsHKT1;3_V5	681	GGGGTTCTCGGTTGGCTACAGCTGCAAGAGGCTACTGAACCATGATGCACGCTGCAAGGA	740	
		M8		
OsHKT1;3_FL	1559	TGCCTCGTACGGGTTTGCGGGGAAAATGGAGCGACAATGGGAAAGCGATCCTGATCATCGT	1618	
OsHKT1;3_V1	1103	TGCCTCGTACGGGTTTGCGGGGAAAATGGATCGACAATGGGAAAGCGATCCTGATCATCGT	1162	
OsHKT1;3_V2	994	TGCCTCGTACGGGTTTGCGGGGAAAATGGAGCGACAATGGGAAAGCGATCCTGATCATCGT	1053	
OsHKT1;3_V3	738	TGCCTCGTACGGGTTTGCGGGGAAAATGGAGCGACAATGGGAAAGCGATCCTGATCATCGT	797	
OsHKT1;3_V4	630	TGCCTCGTACGGGTTTGCGGGGAAAATGGAGCGACAATGGGAAAGCGATCCTGATCATCGT	689	
OsHKT1;3_V5	741	TGCCTCGTACGGGTTTGCGGGGAAAATGGAGCGACAATGGGAAAGCGATCCTGATCATCGT	800	
OsHKT1;3_FL	1619	CATGCTTTTCGGGGGGCTTAAACCGTTTAACATGAAGGGTGGAAGAGCCTGGAAGCCTTAG	1678	
OsHKT1;3_V1	1163	CATGCTTTTCGGGAGGCTTAAACCGTTTAACATGAAGGGTGGAAGAGCCTGGAAGCCTTAG	1222	
OsHKT1;3_V2	1054	CATGCTTTTCGGGAGGCTTAAACCGTTTAACATGAAGGGTGGAAGAGCCTGGAAGCCTTAG	1113	
OsHKT1;3_V3	798	CATGCTTTTCGGGAGGCTTAAACCGTTTAACATGAAGGGTGGAAGAGCCTGGAAGCCTTAG	857	
OsHKT1;3_V4	690	CATGCTTTTCGGGAGGCTTAAACCGTTTAACATGAAGGGTGGAAGAGCCTGGAAGCCTTAG	749	
OsHKT1;3_V5	801	CATGCTTTTCGGGAGGCTTAAACCGTTTAACATGAAGGGTGGAAGAGCCTGGAAGCCTTAG	860	
		Stop codon		
OsHKT1;3_FL	1679	ATAAAGCGGCCGCGACGGTACCAC TAAACAGCCTCAAGAACACCCGAATGGAGTCTCTA	1738	
OsHKT1;3_V1	1223	ATAAAGCGGCCGCGACGGTACCAC TAAACAGCCTCAAGAACACCCGAATGGAGTCTCTA	1282	
OsHKT1;3_V2	1114	ATAAAGCGGCCGCGACGGTACCAC TAAACAGCCTCAAGAACACCCGAATGGAGTCTCTA	1173	
OsHKT1;3_V3	858	ATAAAGCGGCCGCGACGGTACCAC TAAACAGCCTCAAGAACACCCGAATGGAGTCTCTA	917	
OsHKT1;3_V4	750	ATAAAGCGGCCGCGACGGTACCAC TAAACAGCCTCAAGAACACCCGAATGGAGTCTCTA	809	
OsHKT1;3_V5	861	ATAAAGCGGCCGCGACGGTACCAC TAAACAGCCTCAAGAACACCCGAATGGAGTCTCTA	920	
OsHKT1;3_FL	1739	AGCTACATAATACCAACTTACACTT-ACAAA-----	1768	
OsHKT1;3_V1	1283	AGCTACATAATACCAACTTACACTT-ACAAA-----	1312	
OsHKT1;3_V2	1174	AGCTACATAATACCAACTTACACTT-ACAAAATG-----	1206	
OsHKT1;3_V3	918	AGCTACATAATACCAACTTACACTT-TACAAATGTTGTCCCC-----	958	
OsHKT1;3_V4	810	AGCTACATAATACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTTCGT	869	
OsHKT1;3_V5	921	AGCTACATAATACCAACTTACACTT-TACAAATGTTGTCCCC--AAATGTAGCCATTTCGT	977	
OsHKT1;3_FL	1768	-----	1768	
OsHKT1;3_V1	1312	-----	1312	
OsHKT1;3_V2	1206	-----	1206	
OsHKT1;3_V3	958	-----	958	
OsHKT1;3_V4	870	ATCTGCTCCTAATAAAAAGAAAGTTTCTTC-----	899	
OsHKT1;3_V5	978	ATCTGCTC-TAATAAAA-GAAAGTT-CTTCCATTCT	1010	

Supplementary Figure S3.
Nucleotide sequence alignment of OsHKT1;3_FL and its five-variant using GENETYX ver. 13. Presumed membrane-spanning regions of OsHKT1;1_FL, and positions of start and stop codon for the full-length and variants are indicated.



Supplementary Figure S4.

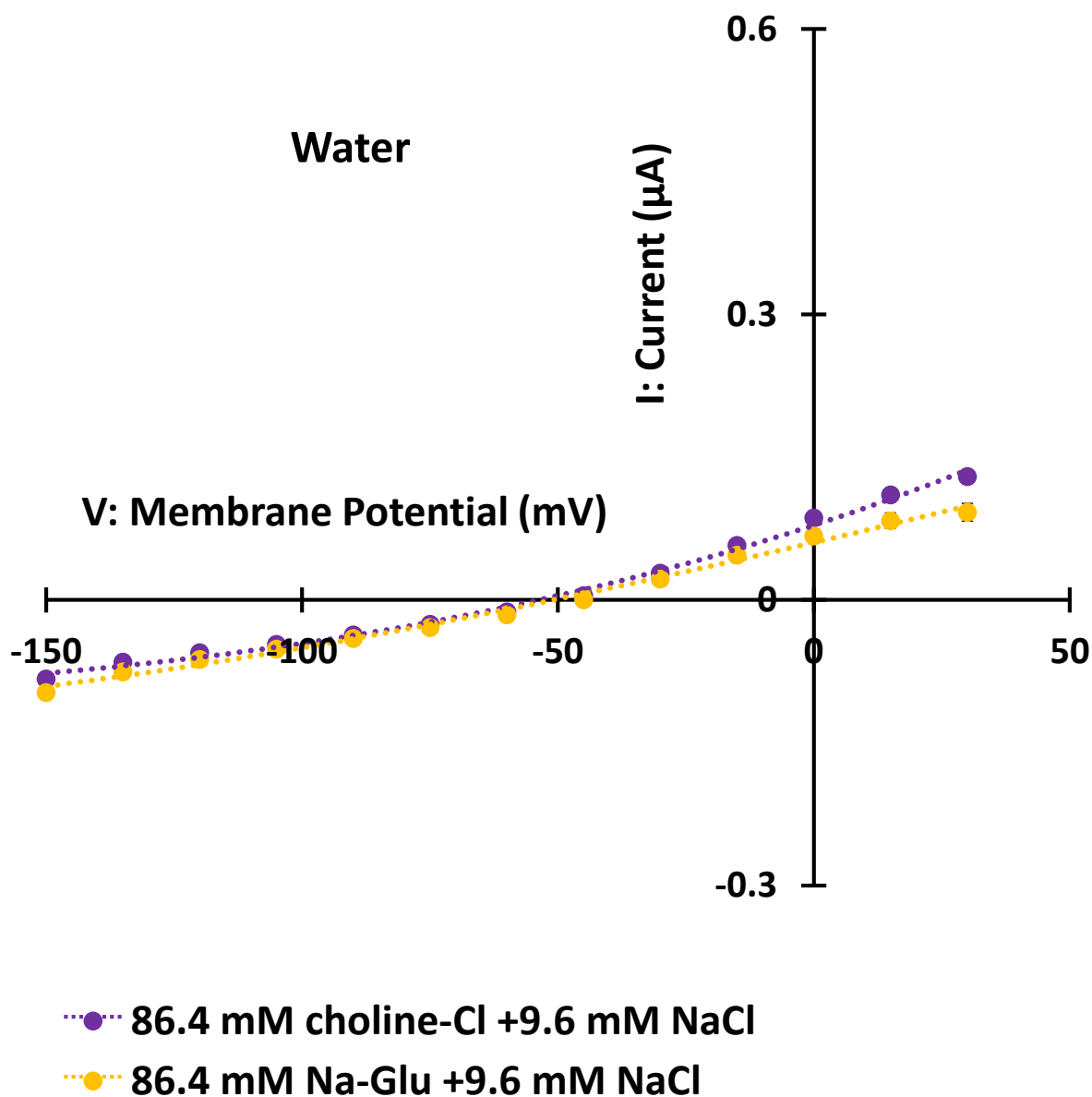
Protein sequence alignment of OsHKT1;3_FL and its five variant using GENETYXver.13. Presumed membrane-spanning regions of OsHKT1;3_FL and its five variants are indicated.



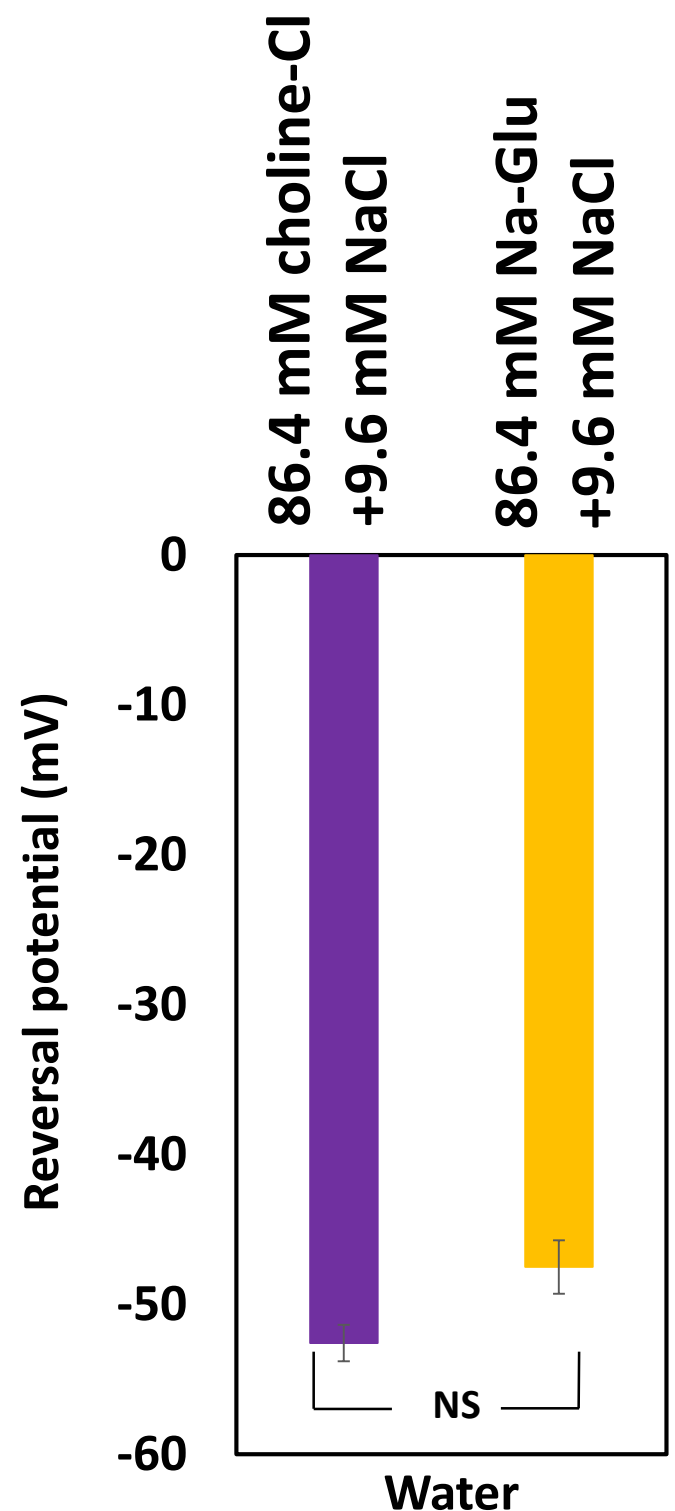
Supplementary Figure S5.

Current–voltage relationships from oocytes expressing OsHKT1;3_FL, _V1, _V2, _V3, _V4, _V5, and water-injection control in 96 mM KCl external solution. External solutions contain, as background elements, 1.8 mM CaCl_2 , 1.8 mM MgCl_2 , 1.8 mM mannitol, and 10 mM HEPES (pH 7.5 with Tris). Water was injected as a negative control. Data are means \pm SE, $n = 8$ –10. Regression analysis also performed with polynomial approximation (degree 3).

A)



B)



Supplementary Figure S6.

Current–voltage relationships from oocytes expressing water-injected negative control in 86.4 mM choline Cl + 8.6 mM NaCl external solution and 86.4 mM Na-gluconate + 8.6 mM NaCl external solution (A); Reversal potential shift analysis by changing external Na concentration from 96 mM to 9.6 mM (B). External solutions contain, as background elements, 1.8 mM CaCl_2 , 1.8 mM MgCl_2 , 1.8 mM mannitol, and 10 mM HEPES (pH 7.5 with Tris). Water was injected as a negative control. Data are means \pm SE, $n = 5$, Two independent experiments were performed, and similar results were obtained. Regression analysis also performed with polynomial approximation (degree 3). NS indicates data showing no significant difference ($p < 0.05$) detected.