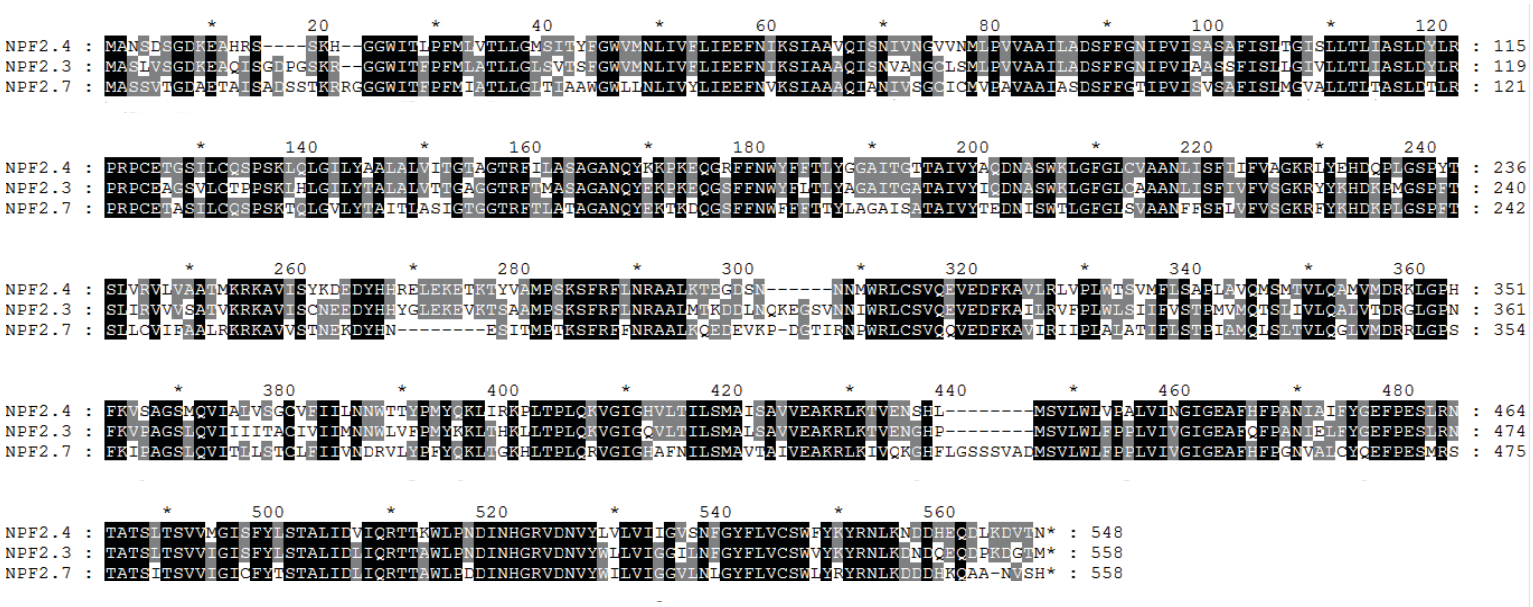
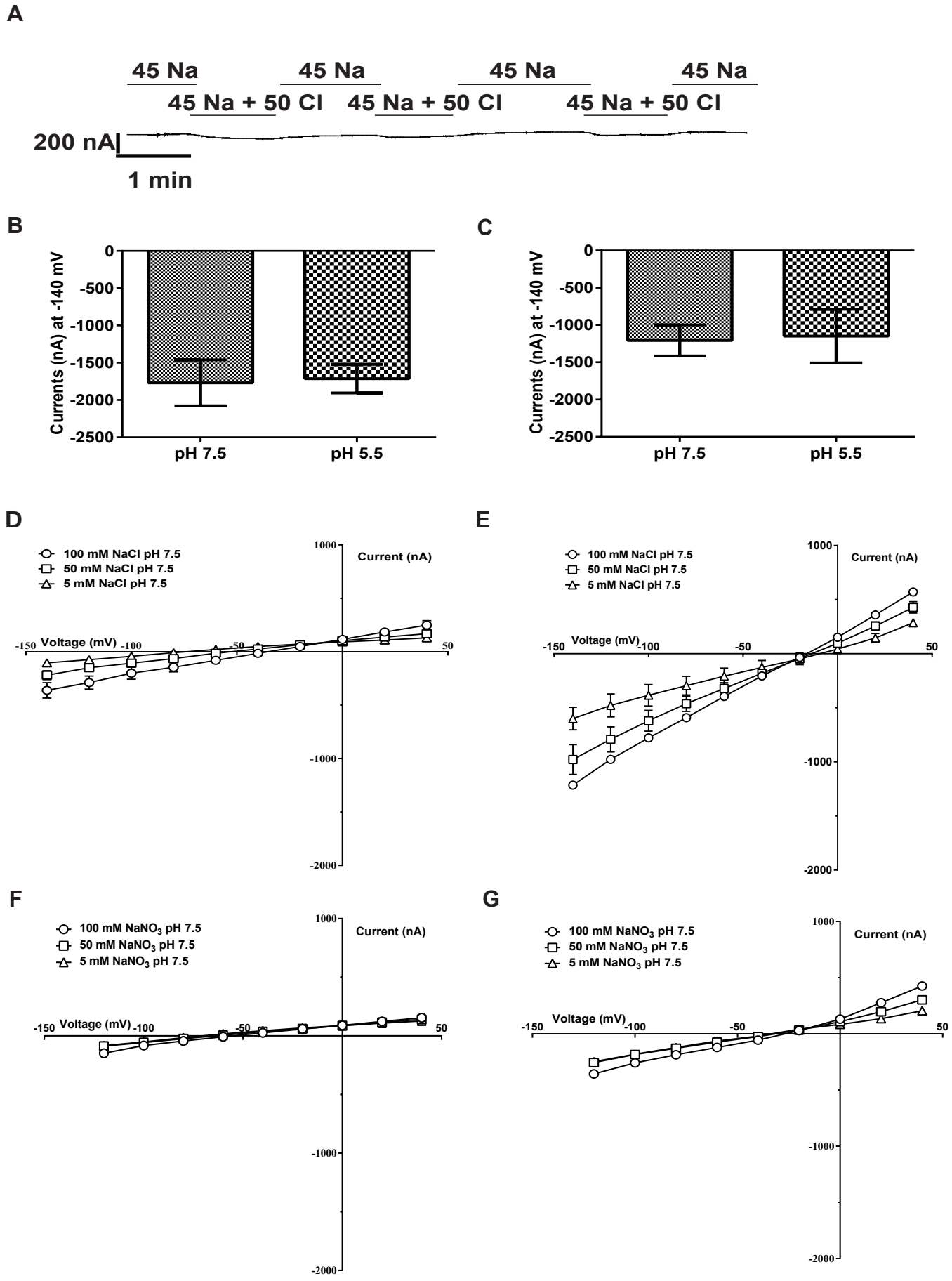


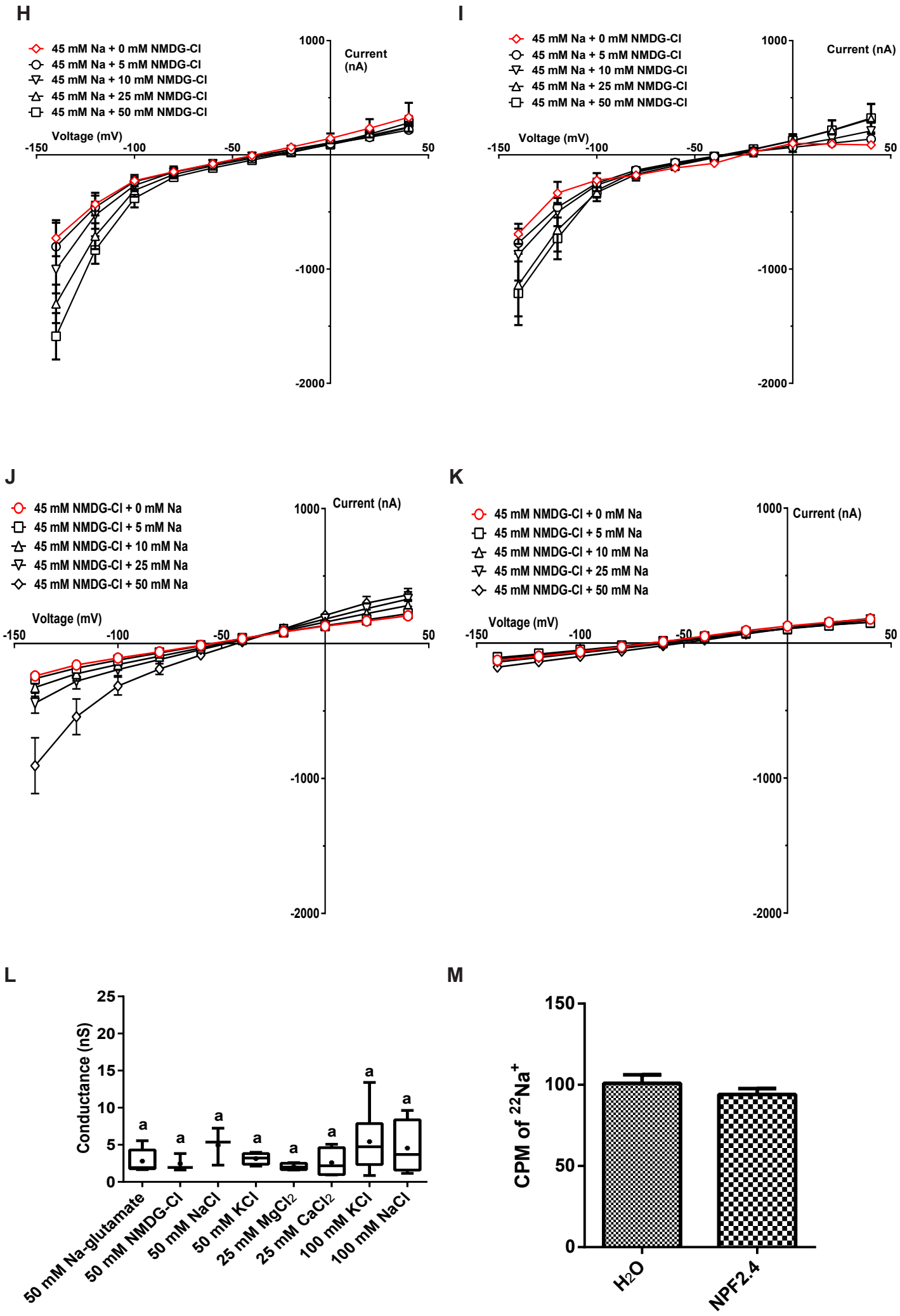
Supplemental Figure 1 Multiple sequence alignment of NPF2.4, NPF2.3 and NPF2.1/ NAXT1.

Protein sequence alignment was performed using ClustalW2 program with a gap open of 10 and a gap extension of 0.1. The black and shaded regions represent identical residues and conservative substitutions, respectively.

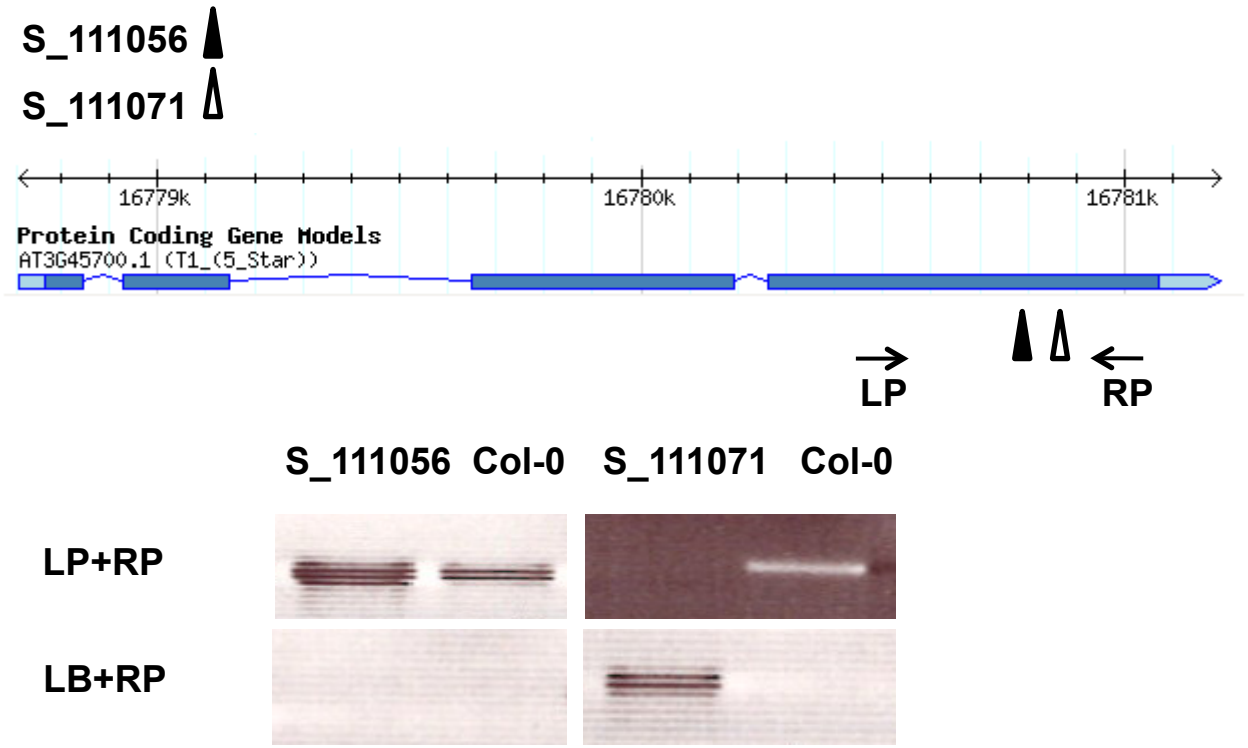

A
45 Na
45 Na
45 Na
45 Na
45 Na + 50 Cl
45 Na + 50 Cl
45 Na + 50 Cl
200 nA
1 min
B
Currents (nA) at -140 mV
0
-500
-1000
-1500
-2000
-2500
pH 7.5
pH 5.5
C
Currents (nA) at -140 mV
0
-500
-1000
-1500
-2000
-2500
pH 7.5
pH 5.5
D
100 mM NaCl pH 7.5
50 mM NaCl pH 7.5
5 mM NaCl pH 7.5
Current (nA)
Voltage (mV)
-150
-100
-50
50
1000
-1000
-2000
E
100 mM NaCl pH 7.5
50 mM NaCl pH 7.5
5 mM NaCl pH 7.5
Current (nA)
Voltage (mV)
-150
-100
-50
50
1000
-1000
-2000
F
100 mM NaNO3 pH 7.5
50 mM NaNO3 pH 7.5
5 mM NaNO3 pH 7.5
Current (nA)
Voltage (mV)
-150
-100
-50
50
1000
-1000
-2000
G
100 mM NaNO3 pH 7.5
50 mM NaNO3 pH 7.5
5 mM NaNO3 pH 7.5
Current (nA)
Voltage (mV)
-150
-100
-50
50
1000
-1000
-2000

Supplemental Figure 2 Raw and control data for expression of NPF2.4 in *Xenopus laevis* oocytes.

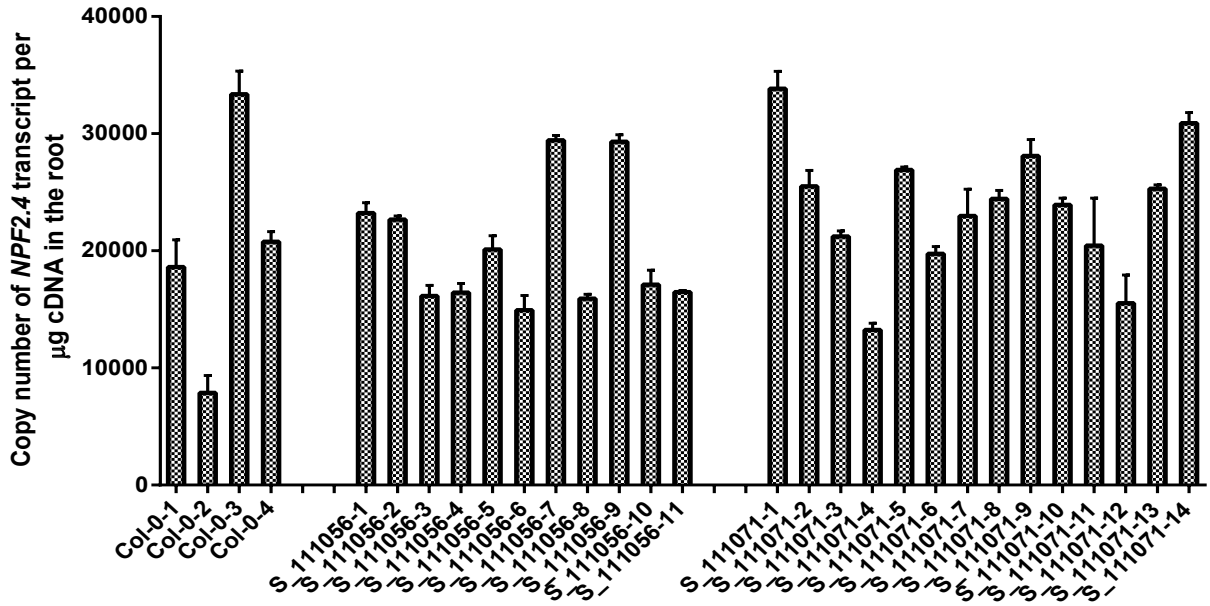
(A) Currents over time for water injected control oocytes, 2 d after injection, clamped at –60 mV, in the presence of 45 mM Na-glutamate (45 Na) and 45 mM Na-glutamate plus 50 mM NMDG-Cl (45 Na + 50 Cl). (B) Total currents (i.e. without leak subtraction) in oocytes expressing *NPF2.4*-cRNA, held at -140 mV in 50 mM NaCl, when exposed to pH 7.5 or 5.5 (n = 4). (C) Currents for water injected control oocytes, held at -140 mV in 50 mM NaCl, and exposed to pH 7.5 or 5.5 (n = 4). (D) Non-control subtracted currents elicited by *NPF2.4*-cRNA when oocytes were exposed to different concentrations of NaCl (n = 3). (E) Currents for water injected oocytes exposed to different concentrations of NaCl (n = 3). (F) Total currents (i.e. without leak subtraction) in oocytes expressing *NPF2.4*-cRNA when oocytes were exposed to different concentrations of $NaNO_3$ (n = 3). (G) Currents for water injected oocytes exposed to different concentrations of $NaNO_3$ (n = 3). (H) Total currents (i.e. without leak subtraction) in oocytes expressing *NPF2.4*-cRNA when oocytes were exposed to 45 mM Na-glutamate and varying concentrations of NMDG-Cl (n = 3–6). (I) Currents for water injected oocytes exposed to 45 mM Na-glutamate and varying concentrations of NMDG-Cl (n = 3–4). (J) Total currents (i.e. without leak subtraction) in oocytes expressing *NPF2.4*-cRNA when oocytes were exposed to 45 mM NMDG-Cl and varying concentrations of Na-glutamate (n = 5). (K) Currents for water injected oocytes exposed to 45 mM NMDG-Cl and varying concentrations of Na-glutamate (n = 5). (L) Conductance for water injected oocytes exposed to solutions containing various different ions; conductance was calculated for –80 mV to –100 mV, dots are means, maximum and minimum values ± SEM are represented by a vertical bar (n = 3) or box (n = 4–6); horizontal bars indicate the median; columns with different letters indicate statistically significant differences ($P \leq 0.05$). (M) $^{22}Na^+$ uptake measured in oocytes injected with either *NPF2.4*-cRNA or water, in a background of 100 mM $Na^+$ for 60 min; results are scintillation counts per minute, presented as mean ± SEM (n = 22 to 30).


H
45 mM Na + 0 mM NMDG-Cl
45 mM Na + 5 mM NMDG-Cl
45 mM Na + 10 mM NMDG-Cl
45 mM Na + 25 mM NMDG-Cl
45 mM Na + 50 mM NMDG-Cl
Current (nA)
Voltage (mV)
1000
-1000
-2000
-150
-100
-50
50
I
45 mM Na + 0 mM NMDG-Cl
45 mM Na + 5 mM NMDG-Cl
45 mM Na + 10 mM NMDG-Cl
45 mM Na + 25 mM NMDG-Cl
45 mM Na + 50 mM NMDG-Cl
Current (nA)
Voltage (mV)
J
45 mM NMDG-Cl + 0 mM Na
45 mM NMDG-Cl + 5 mM Na
45 mM NMDG-Cl + 10 mM Na
45 mM NMDG-Cl + 25 mM Na
45 mM NMDG-Cl + 50 mM Na
Current (nA)
Voltage (mV)
K
45 mM NMDG-Cl + 0 mM Na
45 mM NMDG-Cl + 5 mM Na
45 mM NMDG-Cl + 10 mM Na
45 mM NMDG-Cl + 25 mM Na
45 mM NMDG-Cl + 50 mM Na
Current (nA)
Voltage (mV)
L
Conductance (nS)
25
20
15
10
5
0
a
50 mM Na-glutamate
50 mM NMDG-Cl
50 mM NaCl
50 mM KCl
25 mM MgCl2
25 mM CaCl2
100 mM KCl
100 mM NaCl
M
CPM of 22Na+
150
100
50
0
H2O
NPF2.4

A

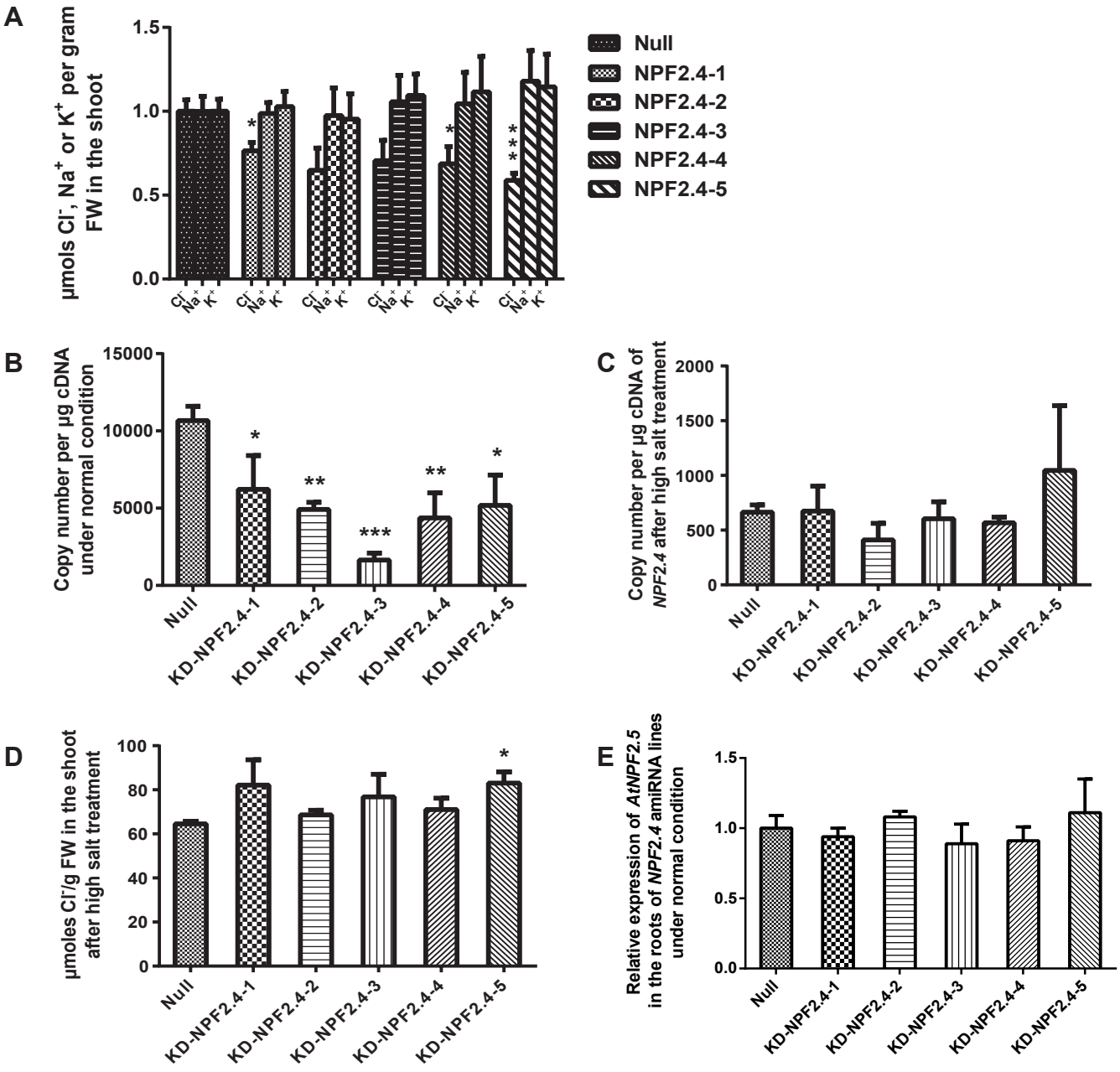



B



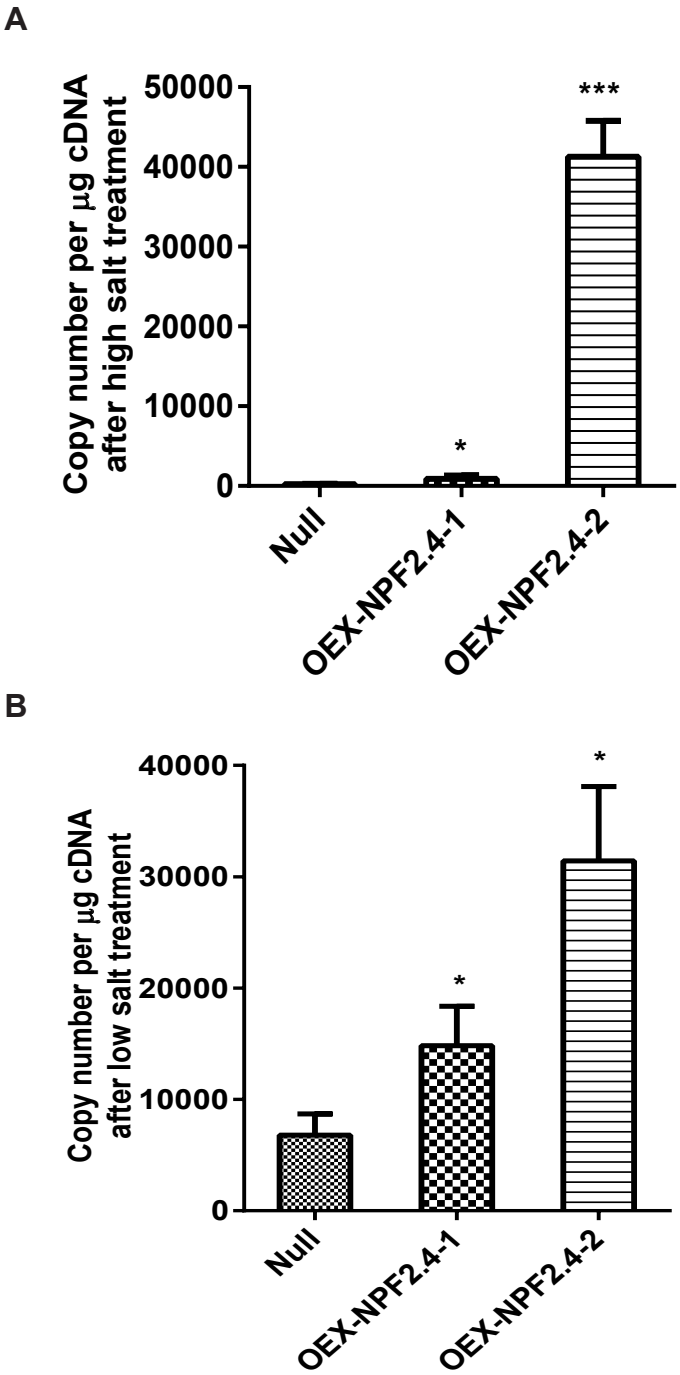

Supplemental Figure 3 *NPF2.4* transcript levels of lines SALK_111056 and SALK_111071 annotated as T-DNA knockouts for *NPF2.4* showed the failed disruption of *NPF2.4* expression in both lines.

(A) T-DNA insertions of both obtained SALK lines and results of genotyping PCRs showed that S_111056 line lost its T-DNA insertion within the ORF of *NPF2.4*. (B) Quantitative RT-PCR was performed on 11 and 14 progenies of $T_3$ SALK_111056 and SALK_111071, respectively and 4 Col-0 WT Arabidopsis plants. Plants were grown hydroponically for 4 weeks. Results are presented as mean ± SEM.



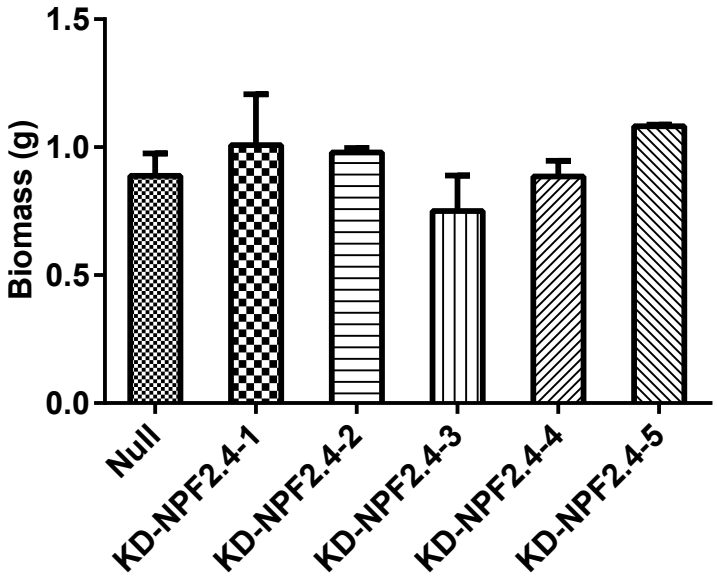

Supplemental Figure 4 Under low salt condition, shoot concentration of both $Na^+$ and $K^+$ were similar between *NPF2.4* knockdowns and null segregants. Expression of *NPF2.5* was not affected in the roots of *NPF2.4* knockdowns. Under high salt condition, shoot $Cl^-$ concentration of knockdowns remained similar to that of null segregants when *NPF2.4* expression in the knockdowns was down-regulated to similar low levels as null segregants.

Four-week old hydroponically grown plants were treated with salt for 5 d. (A) concentrations of $Cl^-$, $Na^+$ and $K^+$ in the shoot of *NPF2.4* knockdowns and null segregants after low salt treatment, data was normalized relative to nulls. (B) Expression levels of *NPF2.4* in knockdowns and null segregants after low salt treatment. (C) *NPF2.4* expression in the roots of *NPF2.4* knockdown lines after high salt treatment. (D) $Cl^-$ concentrations in the shoot of *NPF2.4* knockdowns and null segregants after high salt treatment. (E) Expression levels of *NPF2.5* in the roots of *NPF2.4* knockdowns after low salt treatment. Results are presented as mean ± SEM, n = 3 or 4. Significance is indicated by the asterisks (Single factor ANOVA and Tukey test, * $P \leq 0.05$).




Supplemental Figure 5 Expression validation of *NPF2.4* in the root of *NPF2.4* over-expression lines under both low salt and high salt conditions.

(A) Quantitative RT-PCR on the cDNA samples prepared from roots of *NPF2.4* over expression lines after high salt treatment (75 mM NaCl). (B) Quantitative RT-PCR on the cDNA samples prepared from roots of *NPF2.4* over expression lines after low salt treatment (2 mM NaCl). Results are presented as mean ± SEM, n = 4. Significance is indicated by the asterisks (Single factor ANOVA and Tukey test, * $P \leq 0.05$; *** $P \leq 0.001$).




Supplemental Figure 6 Biomass of *NPF2.4* knockdowns was unaffected by low salt condition compared to null segregants.

Four-week old hydroponically grown plants were treated with salt for 5 days. Result is presented as mean ± SEM, n = 3 or 4.

**Supplemental Table 1** The ABA signaling pathway associated *cis*-acting elements in the putative promoter region of *NPF2.4*. The 1.5 kb putative promoter region of *NPF2.4* was compared with the entries in a database of known plant *cis*-acting regulatory DNA elements (PLACE) at http://www.dna.affrc.go.jp/PLACE/. Date accessed: 7/4/2015.

| Cis-element | Sequence | Copies | Description | Reference |
|---|---|---|---|---|
| ABRELATERD1 | ACGTG | 3 | Early dehydration response in Arabidopsis | (Nakashima et al., 2006) |
| ABREOSRAB21 | ACGTSSSC | 1 | ABA-responsive element in wheat and rice | (Marcotte et al., 1989) |
| ABRERATCAL | MACGYGB | 3 | $Ca^{2+}$ responsive element in Arabidopsis | (Kaplan et al., 2006) |
| ACGTABREMOTIFA2OSEM | ACGTGKC | 1 | ABA responsive element in Arabidopsis and rice | (Narusaka et al., 2003) |
| ACGTATERD1 | ACGT | 8 | Dehydration responsive element in Arabidopsis | (Simpson et al., 2003) |
| MYB1AT | WAACA | 8 | MYB site of ABA responsive elements in Arabidopsis | (Abe et al., 2003) |
| MYB2AT | TAACTG | 2 | Dehydration responsive element in Arabidopsis | (Urao et al., 1993) |
| MYB2CONSENSUSAT | YAACKG | 3 | ABA responsive element in Arabidopsis | (Abe et al., 1997) |
| MYBCORE | CNGTTR | 7 | Dehydration responsive element in Arabidopsis | (Urao et al., 1993) |
| MYCCONSENSUSAT | CANNTG | 10 | ABA responsive element in Arabidopsis | (Abe et al., 2003) |

**S**: G/C, **M**: A/C, **Y**: T/C, **N**: A/T/C/G, **K**: A/C/G, **W**: A/T.

**Supplemental Table 2** Primers used for the generation of *NPF2.4*-amiRNA constructs.

| Target Sequence | Primer name | Primer Sequence |
|---|---|---|
| TATAAGTTATCGACATGCCTG | I miR-s | gaTATAAGTTATCGACATGCCTGtctct ctttgtattcc |
| | II miR-a | gaCAGGCATGTCGATAACTTATAtcaaa gagaatcaatga |
| | III miR-s | gaCAAGCATGTCGATTACTTATTtcaca ggtcgtgatatg |
| | IV miR-a | gaAATAAGTAATCGACATGCTTGtctac atatatattcct |
| TTAAGTATGATAAAGACGCAC | I miR-s | gaTTAAGTATGATAAAGACGCACtctct ctttgtattcc |
| | II miR-a | gaGTGCGTCTTTATCATACTTAAtcaaa gagaatcaatga |
| | III miR-s | gaGTACGTCTTTATCTTACTTATtcaca ggtcgtgatatg |
| | IV miR-a | gaATAAGTAAGATAAAGACGTACtctac atatatattcct |