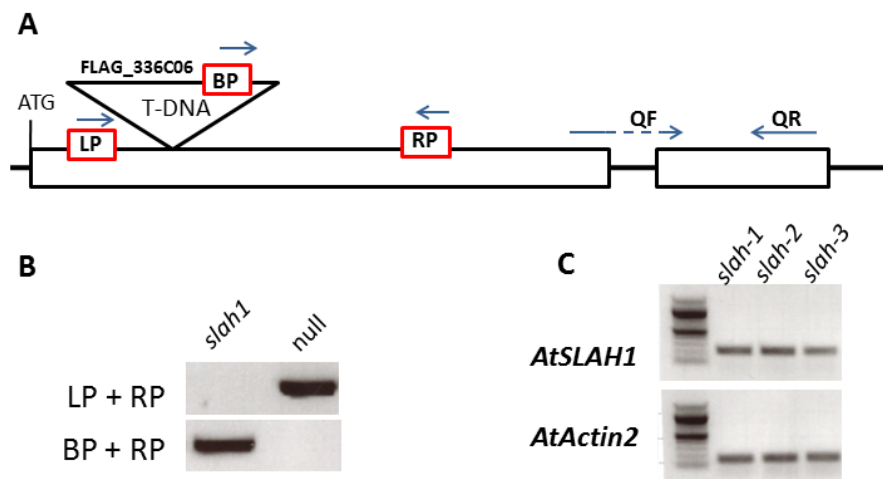
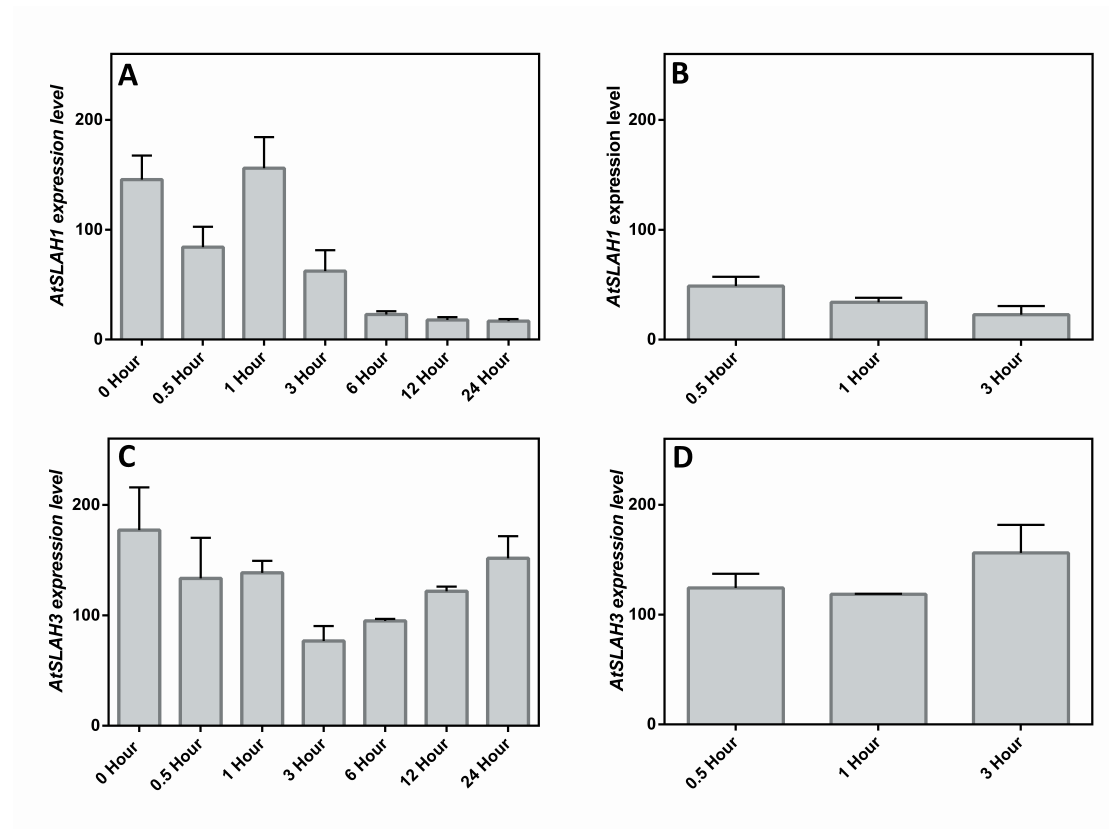


Figure S1



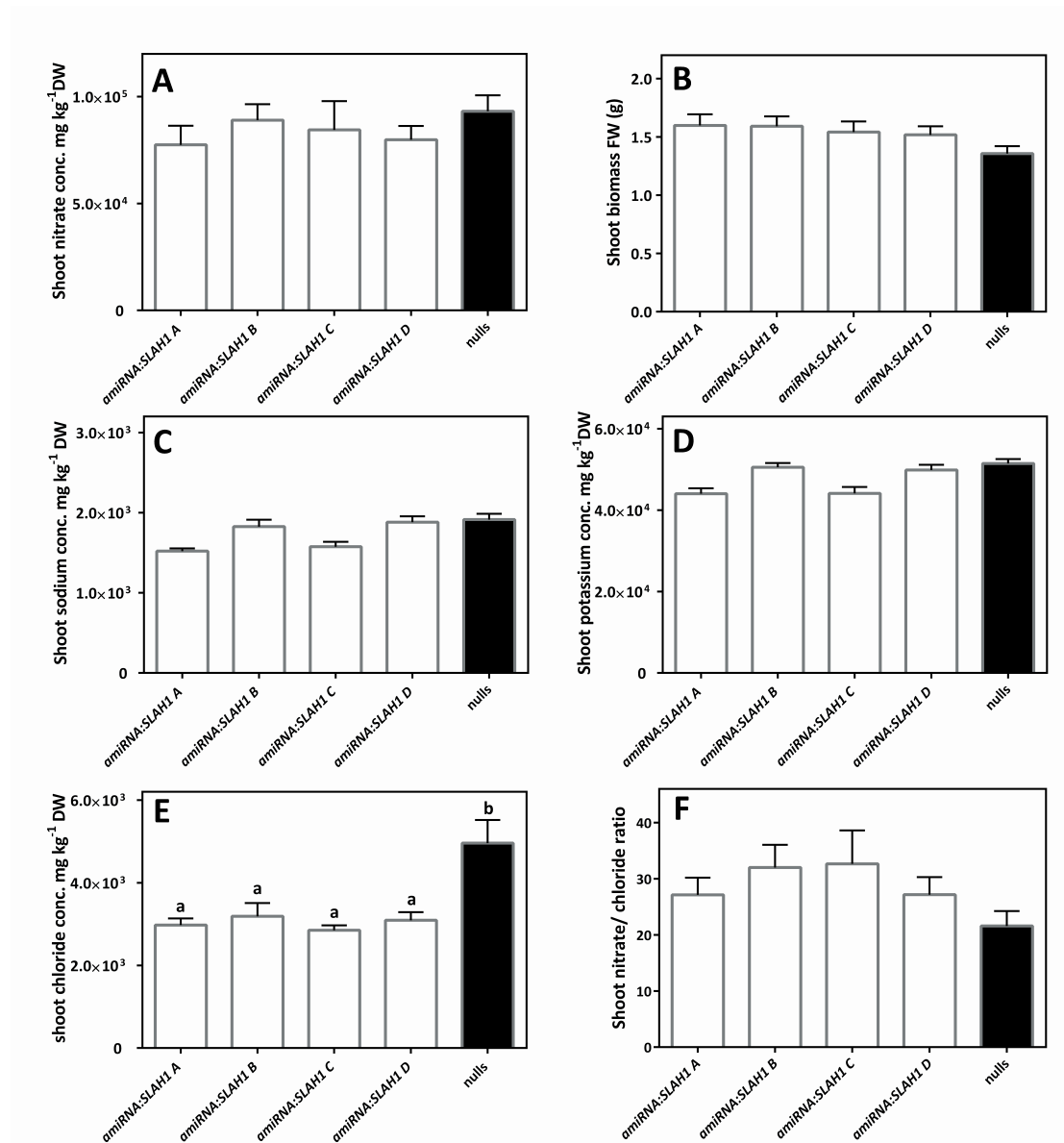
**Figure S1** *AtSLAH1* is still expressed in the *slah1* homozygous T-DNA insertion line FLAG\_336C06. (A) Map of the *slah1*T-DNA insertions in *AtSLAH1* (At1g62280). LP, T-DNA left border primer; RP, T-DNA right border primer; BP, T-DNA border primer; QF, *AtSLAH1* q-RT-PCR forward primer; QR, q-RT-PCR reverse primer; (B) RT-PCR detection of T-DNA insertion in *slah1* mutant gDNA. Primers used were listed in Supplementary Table S1; (C) RT-PCR suggested that *AtSLAH1*'s expression is still detectable in mutant's cDNA, bands in upper panel were sequenced and showed that the amplified PCR products are *AtSLAH1* rather than non-specific bands. Primers used for detecting *AtSLAH1* expression were listed in Supplementary Table S1.

**Figure S2**



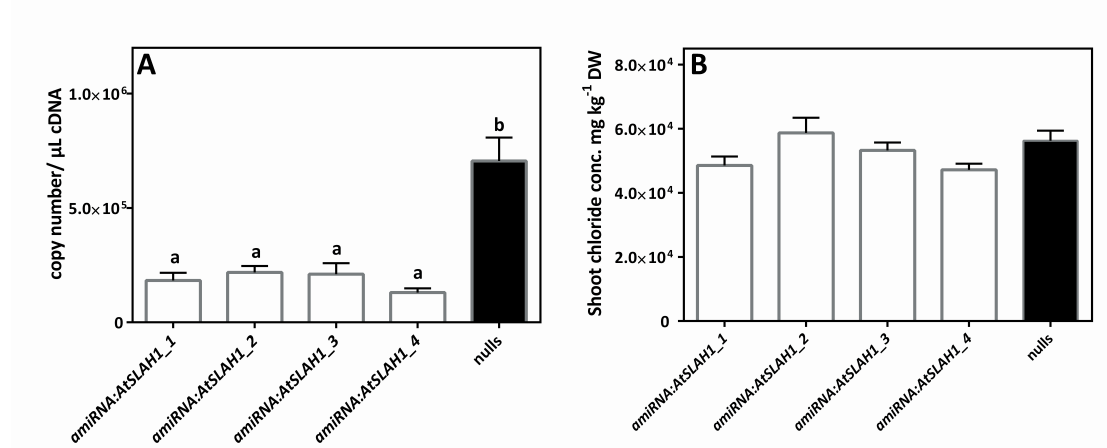
**Figure S2** The transcript level changes of *AtSLAH1* and *AtSLAH3* upon NaCl or ABA treatments. For NaCl treatment, 150 mM NaCl was applied for 0-24 hours and for ABA treatment, 20  $\mu$ M ABA was applied for 0.5-3 hours. (A) and (B) *AtSLAH1*, (C) and (D) *AtSLAH3*. Data was extracted from Arabidopsis eFP Browser database. Results were presented as mean + SD (n = 3). Y axis: GCOS signal (adapted from Kilian *et al.*, 2007).

Figure S3



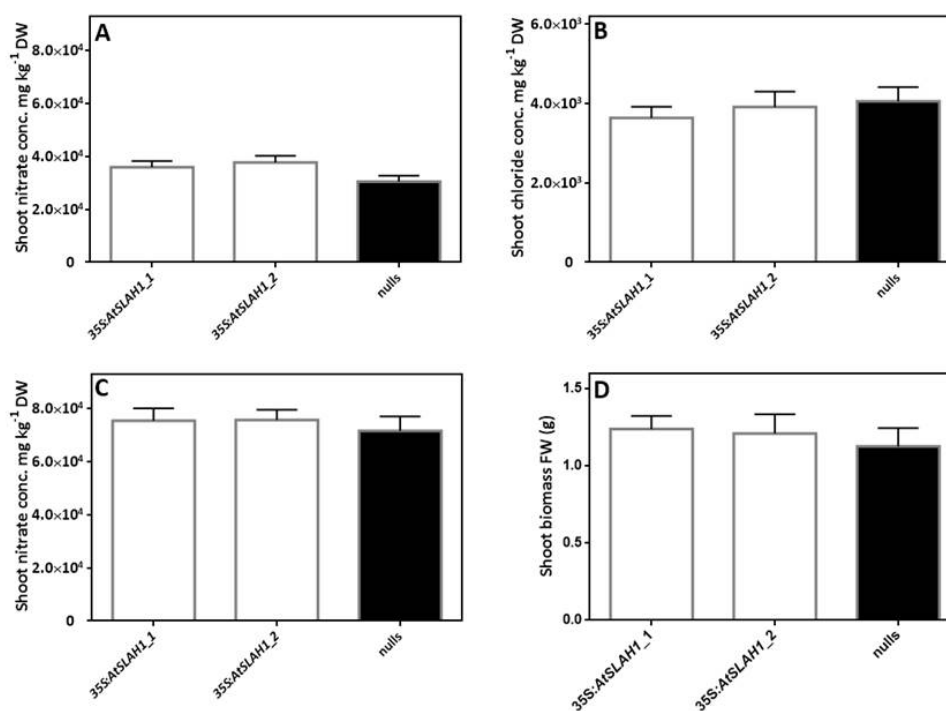
**Figure S3** Under low  $\text{Cl}^-$  conditions, the shoot  $\text{NO}_3^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$  concentrations and biomass were detected in all *amiRNA:AtSLAH1* mutants and null segregants (nulls). Hydroponically grown plants (6 weeks old) supplied with BNS containing 2 mM NaCl (low  $\text{Cl}^-$  conditions) were harvested at the same time point. (A) Shoot  $\text{NO}_3^-$  accumulation of *amiRNA:AtSLAH1* mutants and nulls under low  $\text{Cl}^-$  conditions. (B) Shoot biomass of *amiRNA:AtSLAH1* mutants and nulls under low  $\text{Cl}^-$  conditions. (C) Shoot  $\text{Na}^+$  accumulation of *amiRNA:AtSLAH1* mutants and nulls under low  $\text{Cl}^-$  conditions. (D) Shoot  $\text{K}^+$  accumulation of *amiRNA:AtSLAH1* mutants and nulls under low  $\text{Cl}^-$  conditions. (E) Shoot  $\text{Cl}^-$  accumulation of *amiRNA:AtSLAH1* mutants and nulls under low  $\text{Cl}^-$  conditions in a replicate experiment. (F) Shoot  $\text{NO}_3^-/\text{Cl}^-$  ratio of *amiRNA:AtSLAH1* mutants and nulls under low  $\text{Cl}^-$  conditions in a replicate experiment. Results are presented as mean + SEM (n > 8). Statistically significant differences were determined by one-way ANOVA (P < 0.005). Letters a and b denote data groups that are statistically significantly different from each other.

**Figure S4**



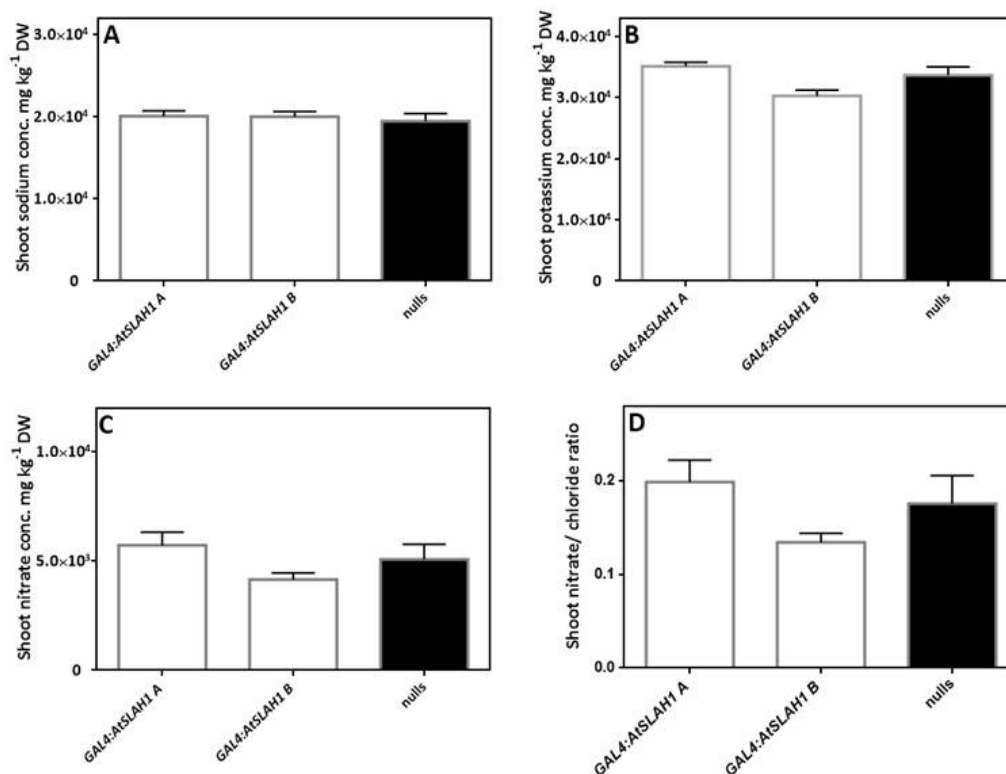
**Figure S4** Transcript abundance of *AtSLAH1* amiRNA containing lines ( $T_2$ ) and shoot  $\text{Cl}^-$  concentration under high  $\text{Cl}^-$  stress. Hydroponically grown plants (5 weeks old) were treated with BNS containing 75 mM NaCl (high  $\text{Cl}^-$  stress) for 7 days before harvest. (A) *AtSLAH1* transcript levels were determined in the root of all amiRNA-*AtSLAH1* mutants (amiRNA-*AtSLAH1*\_1, 2, 3 and 4) and null segregants. (B) Shoot  $\text{Cl}^-$  accumulation of amiRNA-*AtSLAH1* mutants and null segregants under high  $\text{Cl}^-$  conditions. Results are presented as mean + SEM ( $n > 10$ ). Statistically significant differences were determined by one-way ANOVA ( $P \leq 0.005$ ). Letters a and b denote data groups that are statistically significantly different from each other.

**Figure S5**



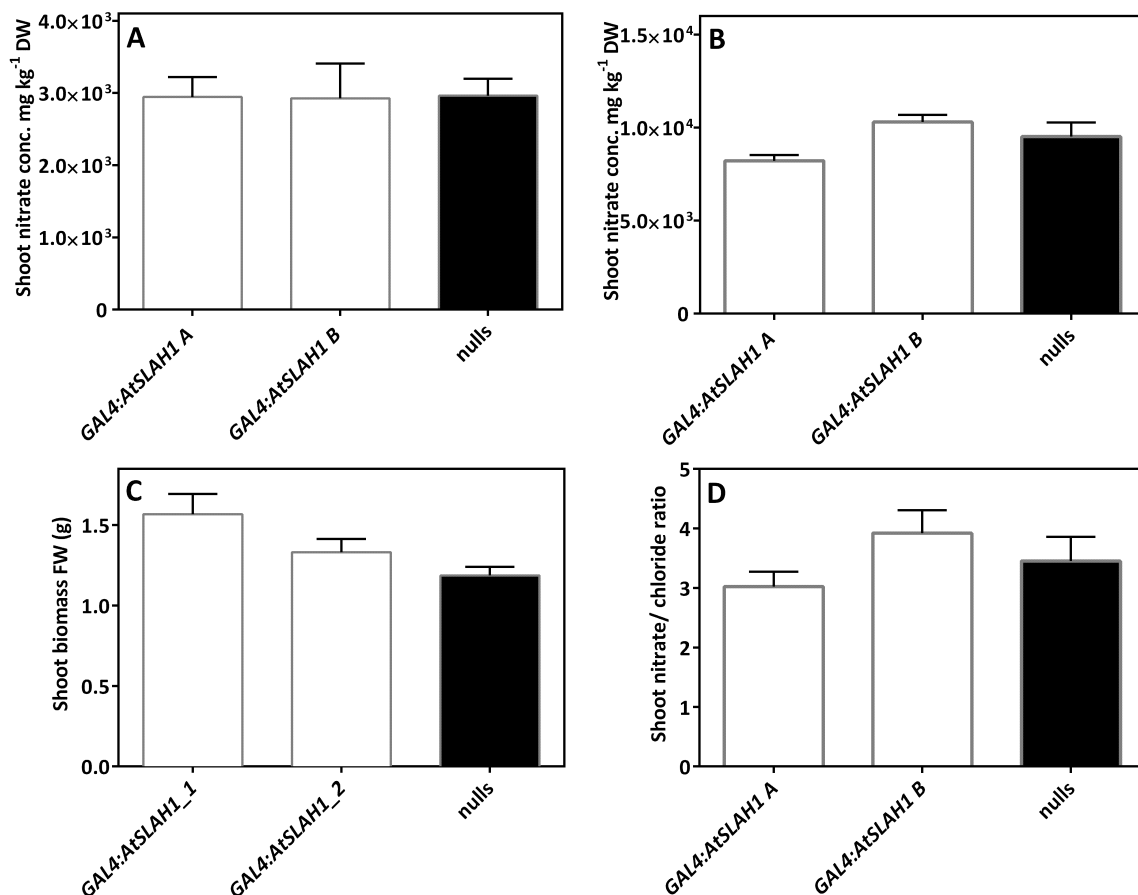
**Figure S5** The shoot NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup> concentrations and shoot biomass were detected under high and low Cl<sup>-</sup> supply in both *35S:AtSLAH1-1* and *35S:AtSLAH1\_2*, and null segregants (nulls). Hydroponically grown plants (6 weeks old) supplied with BNS containing 2 mM or 75 mM NaCl (low or high Cl<sup>-</sup> conditions) for 7 days were harvested at the same time point. (A) Shoot NO<sub>3</sub><sup>-</sup> accumulation of *35S:AtSLAH1* overexpression and null segregant lines under high Cl<sup>-</sup> conditions (75 mM NaCl). (B) Shoot Cl<sup>-</sup> accumulation of *35S:AtSLAH1* overexpression and null segregant lines under low Cl<sup>-</sup> conditions (2 mM NaCl). (C) Shoot NO<sub>3</sub><sup>-</sup> accumulation of *35S:AtSLAH1* overexpression and null segregant lines under low Cl<sup>-</sup> conditions (2 mM NaCl). (D) Whole shoot biomass of *35S:AtSLAH1* overexpression and null segregant lines under low Cl<sup>-</sup> conditions (2 mM NaCl). Results are presented as mean + SEM (n > 9). No significant differences were found using one-way ANOVA analysis or unpaired students t-tests.

Figure S6



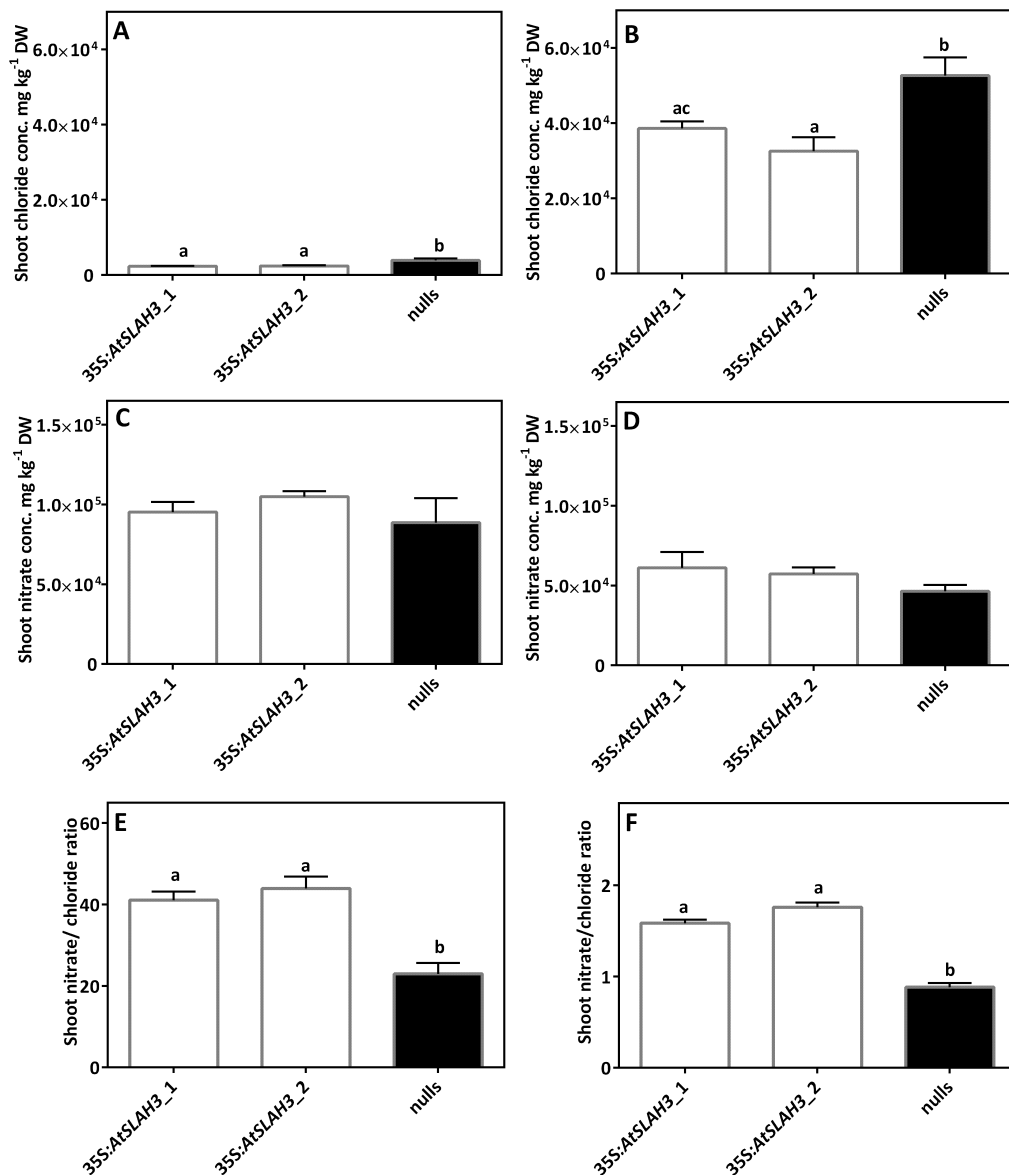
**Figure S6** Under high Cl<sup>-</sup> conditions, the shoot Na<sup>+</sup>, K<sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were detected in all GAL4:AtSLAH1 overexpression and null segregant (nulls) lines. Hydroponically grown plants (6 weeks old) supplied with BNS containing 75 mM NaCl (high Cl<sup>-</sup> conditions) were harvested at the same time point. (A) Shoot Na<sup>+</sup> accumulation of GAL4:AtSLAH1 mutants and nulls under high Cl<sup>-</sup> conditions. (B) Shoot K<sup>+</sup> of GAL4:AtSLAH1 overexpression and null lines under high Cl<sup>-</sup> conditions. (C) Shoot NO<sub>3</sub><sup>-</sup> accumulation of GAL4:AtSLAH1 overexpression and null lines under high Cl<sup>-</sup> conditions. (D) Shoot NO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> ratio of GAL4:AtSLAH1 overexpression and null lines under high Cl<sup>-</sup> conditions. Results are presented as mean + SEM (n > 9). No significant differences were found using one-way ANOVA analysis or unpaired students t-tests.

Figure S7



**Figure S7** Under low Cl<sup>-</sup> conditions, shoot Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> concentrations and whole shoot biomass were detected in all GAL4:AtSLAH1 overexpression and null segregant (null) lines. Hydroponically grown plants (7 weeks old) supplied with BNS containing 2 mM NaCl (low Cl<sup>-</sup> conditions) were harvested at the same time point. (A) Shoot Cl<sup>-</sup> accumulation of GAL4:AtSLAH1 overexpression and null lines under low Cl<sup>-</sup> conditions. (B) Shoot NO<sub>3</sub><sup>-</sup> accumulation of GAL4:AtSLAH1 overexpression and null lines under low Cl<sup>-</sup> conditions. (C) Whole shoot biomass of GAL4:AtSLAH1 overexpression and null lines under low Cl<sup>-</sup> conditions. (D) Shoot NO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> ratio of GAL4:AtSLAH1 overexpression and null lines under low Cl<sup>-</sup> conditions. Results are mean + SEM (n > 6). No significant differences were found using one-way ANOVA analysis or unpaired students t-tests.

Figure S8

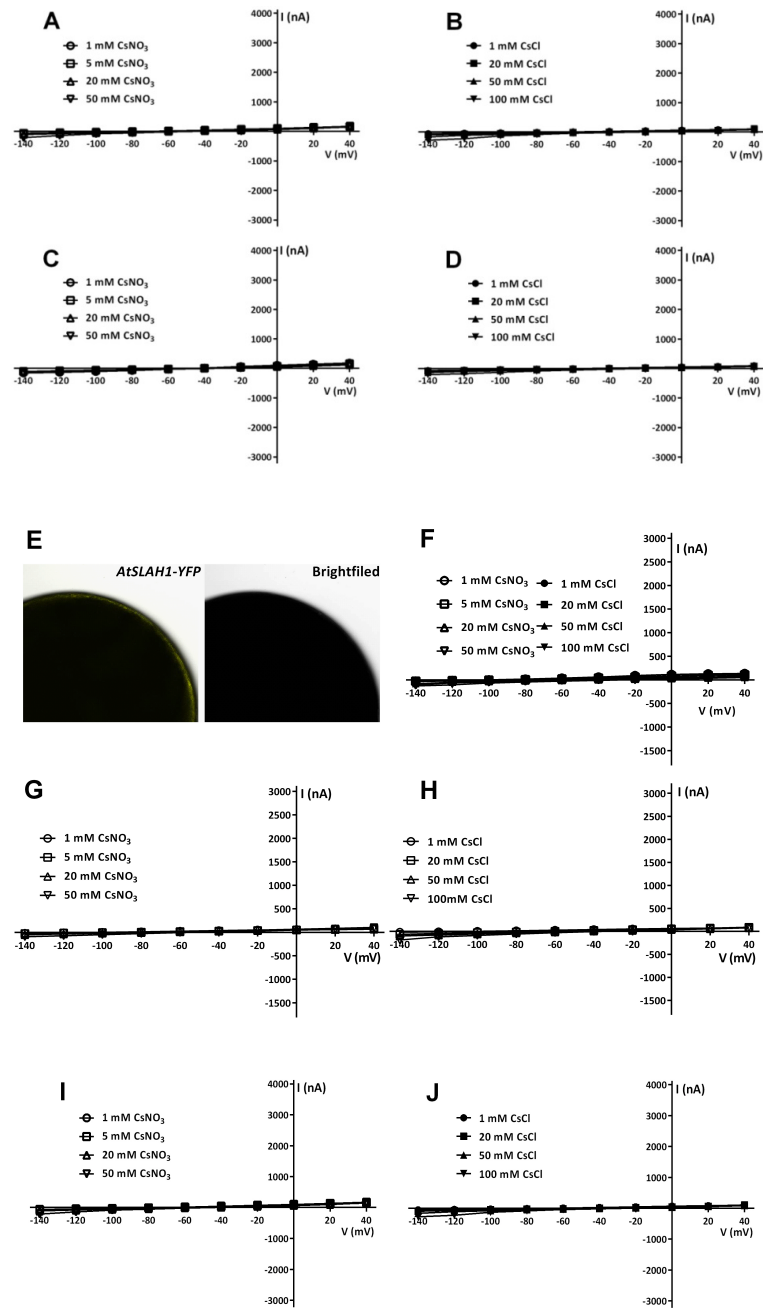


**Figure S8** The shoot  $\text{NO}_3^-$  and  $\text{Cl}^-$  concentrations were detected under low and high  $\text{Cl}^-$  supply in both *35S:AtSLAH3-1* and *35S:AtSLAH3\_2*, and null segregants (nulls). Hydroponically grown plants (6 weeks old) supplied with BNS containing 2 mM or 75 mM NaCl (low or high  $\text{Cl}^-$  conditions) for 7 days were harvested at the same time point. (A) Shoot  $\text{Cl}^-$  accumulation of *35S:AtSLAH3* overexpression and null lines under low  $\text{Cl}^-$  (2 mM) conditions. (B) Shoot  $\text{Cl}^-$  accumulation of *35S:AtSLAH3* overexpression and null lines under high  $\text{Cl}^-$  (75 mM) conditions. (C) Shoot  $\text{NO}_3^-$  accumulation of *35S:AtSLAH3* overexpression and null lines under low  $\text{Cl}^-$  conditions. (D) Shoot  $\text{NO}_3^-$  accumulation of *35S:AtSLAH3* overexpression and null lines under high  $\text{Cl}^-$  conditions. (E) The shoot  $\text{NO}_3^-/\text{Cl}^-$  ratio in all *35S:AtSLAH3* overexpression and null lines grown under low  $\text{Cl}^-$  conditions. (F) The shoot  $\text{NO}_3^-/\text{Cl}^-$  ratio in all *35S:AtSLAH3* overexpression and null lines grown under high  $\text{Cl}^-$  conditions. Results are mean + SEM ( $n > 6$ ). Statistical significance was determined by



one-way analysis of variance (ANOVA and Tukey test) ( $P \leq 0.05$ ), a, b and c represent data groups that are statistically different from each other.

Figure S9



**Figure S9** Electrophysiological characterisation of *AtSLAH1* in *X. laevis* oocytes. Whole cell currents (steady state) in response to 3 second voltage pluses from +40 mV to -140 mV for *AtSLAH1* /*AtSLAH1*+*SnRK2.3* cRNA and nuclease-free water injected oocytes were recorded. (A) water injected oocytes perfused with 1, 5, 20 and 50 mM CsNO<sub>3</sub> at pH 7.5 (mean ± SEM, n = 4); (B) water injected oocytes perfused with 1, 20, 50 and 100 mM CsCl at pH 7.5 (mean ± SEM, n = 4); (C) *SLAH1* injected oocytes perfused with 1, 5, 20 and 50 mM CsNO<sub>3</sub> at pH 7.5 (mean ± SEM, n = 3); (D) *SLAH1* injected oocytes perfused with 1, 20, 50 and 100 mM CsCl at pH 7.5 (mean ± SEM, n = 4); (E) Transient expression of *AtSLAH1*-YFP in *X. Laevis* oocytes. (F) RNA free water injected oocytes perfused with 1, 5, 20 and 50 mM CsNO<sub>3</sub> or with 1, 20, 50 and 100 mM CsCl at pH 7.5 (mean ± SEM, n = 3); (G) *SLAH1*-*SnRK2.2* injected oocytes perfused with 1, 20, 50 and 100 mM CsNO<sub>3</sub> at pH 7.5 (mean ± SEM, n = 5); (H) *SLAH1*-*SnRK2.2* complex injected oocytes perfused with 1, 5, 20 and 50 mM CsCl at pH 7.5 (mean ± SEM, n = 5); (I) *SLAH1*-*SnRK2.3* injected oocytes perfused with 1, 20, 50 and 100 mM CsNO<sub>3</sub> at pH 7.5 (mean ± SEM, n = 5); (J) *SLAH1*-*SnRK2.3* complex injected oocytes perfused with 1, 5, 20 and 50 mM CsCl at pH 7.5 (mean ± SEM, n = 5); Data are presented without water subtraction.

**Supplementary Table S1**

Primers used for generating *amiRNA:AtSLAH1* constructs, for screening homozygous *Atslah1* T-DNA mutant lines and for cloning *AtSLAH1/AtSLAH3/AtSnRk2.2/AtSnRk2.3* from Arabidopsis.

<b>Primers used for generating <i>amiRNA:AtSLAH1</i> mutant lines</b>	
Oligos for <i>amiRNA</i> "TAAAACGCTATTTGGTTCCGT"	
I miR-s	5' gaTAAAACGCTATTTGGTTCCGTtctctctttgtattcc 3'
II miR-a	5' gaACGGAACCAAATAGCGTTTTAtcaaagagaatcaatga 3'
III miR*s	5' gaACAGAACCAAATACCGTTTTTcacaggtcgtgatatg 3'
IV miR*a	5' gaAAAAACGGTATTTGGTTCTGTtctacatatattcct 3'
Oligos for <i>amiRNA</i> "TTATGTCTAGTGTGCGAGACTG"	
I miR-s	5' gaTTATGTCTAGTGTGCGAGACTGtctctctttgtattcc 3'
II miR-a	5' gaCAGTCTCGACACTAGACATAAtcaaagagaatcaatga 3'
III miR*s	5' gaCAATCTCGACACTTGACATATcacaggtcgtgatatg 3'
IV miR*a	5' gaATATGTCAAGTGTGCGAGATTGtctacatatattcct 3'
<b>Primers used for screening <i>Atslah1</i> knockout lines (FLAG_329G06)</b>	
Forward (FP)	5' TGGCCTACAGACCTGAAAATG 3'
Reverse (RP)	5' TTGGAATGACTTTGTGTGTGTG 3'
Border (BP)	5' CTACAAATTGCCTTTTCTTATCGAC 3'
<b>Primers used for detecting <i>AtSLAH1</i> expression in <i>Atslah1</i> knockout lines (FLAG_329G06)</b>	
Forward (QF)	5' TCTTCATGTCCCTGGTCTG 3'
Reverse (RF)	5' ATTGCTGTTTGCTGCTGTC 3'
<b>Primers used for cloning <i>AtSLAH1</i>, <i>AtSLAH3</i>, <i>AtSnRK2.2</i> and <i>AtSnRK2.3</i> from Arabidopsis</b>	
<i>AtSLAH1</i> (F)	5' ATGGAAATTCCGAGGCAA 3'
<i>AtSLAH1</i> (R)	5' CTAGTTTTGGTTAGTCGCATTG 3'
<i>AtSLAH3</i> (F)	5' ATGGAGGAGAAACCAAATAT 3'
<i>AtSLAH3</i> (R)	5' TTATGATGAATCACTCTCTTGAGT 3'
<i>AtSnRK2.2</i> (F)	5' ATGGATCCGGCGACTAAT3'
<i>AtSnRK2.2</i> (R)	5' TCAGAGAGCATAAACTATCTCTCC3'
<i>AtSnRK2.3</i> (F)	5' ATGGATCCGGCGACTAAT3'
<i>AtSnRK2.3</i> (R)	5' GAGAGCATAAACTATCTCTCCACT3'