FIGURE 1. *DSP3* mutation confers salt tolerance in *Arabidopsis*. (a) Upper: Schematic diagram of *dsp3* T-DNA insertion site in the mutant. Exons and introns are depicted to scale by boxes and lines, respectively. T-DNA insertion site is marked by inverted triangle, with arrow indicating the left-border. Bottom: Level of *DSP3* transcript in 7-day-old wild type and *dsp3* mutant seedlings analyzed by semi-quantitative RT-PCR. *UBQ5* was used as internal control. (b) Transcript level of *DSP3* in *dsp3* mutant analyzed by qRT-PCR. *PP2A* was used as internal control. (c) Germination analysis of wild type and *dsp3* mutant after 3 days of growth on ½ MS medium with or without 175 mM NaCl. (d-f) Salt sensitivity analysis of 3-week-old soil-grown Col and *dsp3* seedlings in terms of survival rate (e) and chlorophyII content (d, f). Seedlings were treated with 200 mM NaCl for 7 days for chlorophyll content analysis and 10 days for survival rate analysis. Error bars in (b), (c), (e) and (f) indicate standard division (SD) from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from one-way (b, e) or two-way (c, f) ANOVA.

FIGURE 2. The salt-insensitive phenotype of *dsp3* can be complemented by *pDSP3::DSP3-GUS* transgene. (a, b) Seed germination phenotype (a) and quantification (b) of *dsp3* mutant and complementation lines (*Com8-2* and *Com 4-15*) after 4 days of growth on ½ MS medium with or without 175 mM NaCl. (c-f) Salt sensitivity analysis of *dsp3* and complementation lines in 3-week-old soil-grown seedlings after 200 mM NaCl treatment. (c, d) Chlorophyll content after salt treatment for 8 days. (e) Survival rate after salt treatment for 12 days. (f) Ion leakage after salt treatment for 12 days. Error bars in (b), (d), (e) and (f) indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from two-way ANOVA.

FIGURE 3. Altering the expression of *DSP3* will result in the expression change of stress responsive genes under salt stress. The expression level of *RAB18*, *RD29B*, *KIN1*, *P5CS1*,

RD22 and *RD29A* were analyzed by qRT-PCR in 3-week-old soil-grown *dsp3* and *DSP3-GUS* complementation lines after 200 mM NaCl treatment for 3 days. Error bars indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from two-way ANOVA.

FIGURE4. Altering the expression of *DSP3* will result in the change of cellular redox homeostasis in *Arabidopsis*. Three-week-old soil-grown *dsp3* and *DSP3-GUS* complementation line seedlings were treated with 200 mM NaCl first before the cellular H_2O_2 content and gene expression level were measured. The cellular H_2O_2 level was measured by 3,3-diaminobenzidine (DAB) staining (a) and the expression of *AtrhboD*, *AtrhboF* and *CAT1* were analyzed by qRT-PCR. Error bars in (b-d) indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from two-way ANOVA.

FIGURE 5. DSP3 protein accumulation is negatively regulated by NaCl. (a) YFP fluorescence of 7-day-old root tip of *DSP-YFP* transgenic line (*Com6-2*) after 4 hours salt treatment. (b) The relative fluorescence intensity of YFP signal in (a). Error bars indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from two-way ANOVA. (c) DSP3 protein level was reduced after NaCl treatment (upper) and the reduction of DSP3 was suppressed by MG132 treatment (bottom). α-Tubulin was used as a loading control.

FIGURE 6. The tyrosine phosphatase activity of DSP3 is required for salt response. (a) DSP3 shows tyrosine phosphatase activity toward substrate P2 (Tyr Phosphopeptide-2). (b) The cysteine 112 to serine mutation totally disrupt the tyrosine phosphatase activity of DSP3. (c) Phenotypes of T1 plants overexpressing wild-type *DSP3* (*DSP3OX*) or *mDSP3* (*mDSP3OX*).

Results are displayed as percentage of phenotypes according to the severity of dwarfism and early senescence phenotype. (d) The *DSP3/mDSP3* expression level in *DSP3OX* or *mDSP3OX* plants analyzed by semi-quantitative RT-PCR. (e) Germination rate of *DSP3OX* or *mDSP3OX* seeds after 4 days of growth on ½ MS medium with or without 175 mM NaCl. (f) Seedling survival rate of 3-week-old soil-grown *DSP3OX* or *mDSP3OX* plants after NaCl treatment for 8 days. Error bars in (a), (b), (e) and (f) indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from one-way (a, b, f) or two-way (e) ANOVA.

FIGURE 7. DSP3 interacts with and de-phosphorylates MPK3/MPK6. (a) The direct interaction of DSP3 with MPK3/6 as detected by firefly luciferase complementation assay. (b) The interaction analysis of DSP3 with MPK3/6 using co-immunoprecipitation in transiently transformed *N. benthamiana* leaves. (c) The C112S mutation did not interfere the interaction of DSP3 with MPK3/6 in firefly luciferase complementation assay. (d) The MPK3 and MPK6 were over-phosphorylated in *dsp3* mutant as detected by immunoblotting using anti-phospho-p44/42 MAP kinase antibodies (α-p-MPK3/MPK6). α-MPK3 and α-MPK6 were used to probe for MPK3 and MPK6. α-Tubulin was used as the loading control.

FIGURE S1. The phenotype of *DSP3* over-expression lines. Three independent transgenic lines all display small stature together with an early senescence phenotype (a) and the severeness of the phenotype correlated with the expression level of *DSP3* gene analyzed by qRT-PCR in (b).

FIGURE S2. *pDSP3::DSP3-YFP* complements the salt tolerance phenotype of *dsp3*. (a) The transcript level of *DSP3* in *pDSP3::DSP3-YFP* transgenic lines (*Com6-2* and *Com10-4*) and *dsp3* mutant analyzed by qRT-PCR. (b, c) Salt sensitivity analysis of 3-week-old soil-grown

dsp3 mutant and *pDSP3::DSP3-YFP* complementation line seedlings in terms of chlorophyll content (b) and survival rate (c). Error bars in (a) and (c) indicated SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from one-way ANOVA.

FIGURE S3. The expression level of *DSP3* in *dsp3* mutant and *pDSP3::DSP3-GUS* complementation lines (*Com8-2* and *Com4-15*) analyzed by qRT-PCR. Error bars indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from one-way ANOVA.

FIGURE S4. The *dsp3* mutant is insensitive to KCl and mannitol. Col and *dsp3* mutant seeds were germinated on ½ MS medium containing different concentrations of KCl (a, b) or mannitol (c, d) for 4 days. The germination rates (b, d) were calculated from three biological repeats and error bars indicate SD. The different lowercase letters over each bar represent statistically significant difference ($P<0.05$) from two-way ANOVA.

FIGURE S5. Over expressing *DSP3* will result in an accumulation of ROS. The cellular H_2O_2 content and gene expression level were measured in three-week-old soil-grown Col and *DSP3OX* seedlings. The cellular H_2O_2 content was measured by 3,3-diaminobenzidine (DAB) staining (a) and the expression of *AtrhboD* and *AtrhboF* were analyzed by qRT-PCR. Error bars in (b, c) indicate SD from three biological repeats.

FIGURE S6. Sub-cellular and tissue specific localization of *DSP3*. (a) Sub-cellular localization of *DSP3-YFP* in root tip of 5-day-old *pDSP3::DSP3-YFP* transgenic line. The YFP signal (yellow) was visualized using confocal microscopy. The propidium iodide (PI) was used to stain cell outline (red). Scale bar=5 μm. (b-g) Expression pattern of *pDSP3::DSP3-GUS*

reporter in 1 day (b), 3 days (c), 7 days (d, f) and 3 weeks old seedlings (e), as well as the inflorescence from 4-week-old plant (g). Scale bar=0.5 mm.