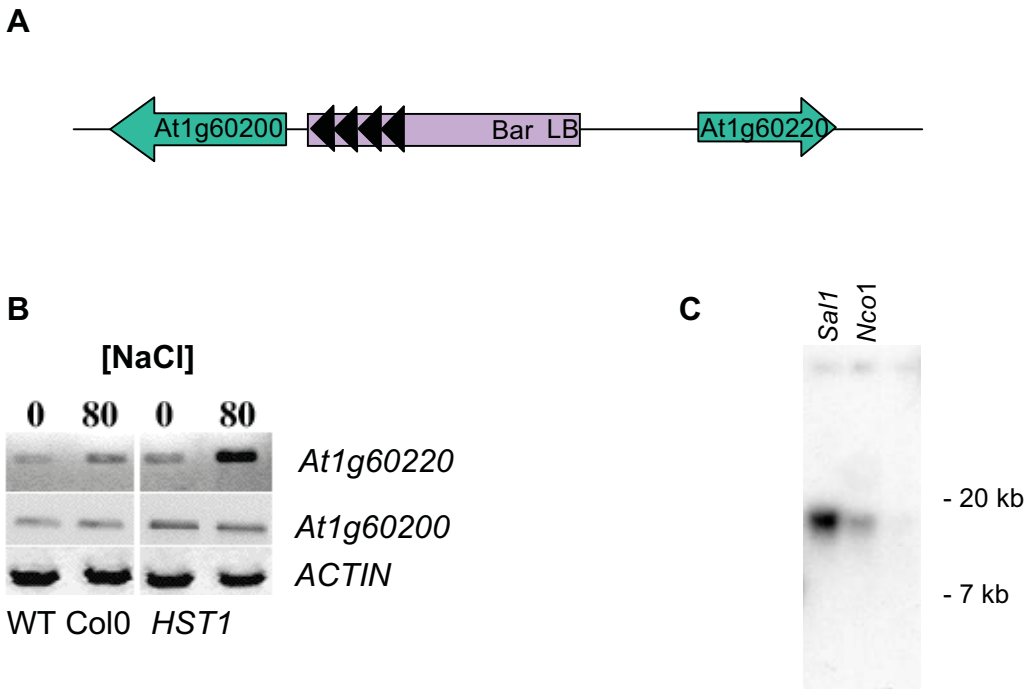
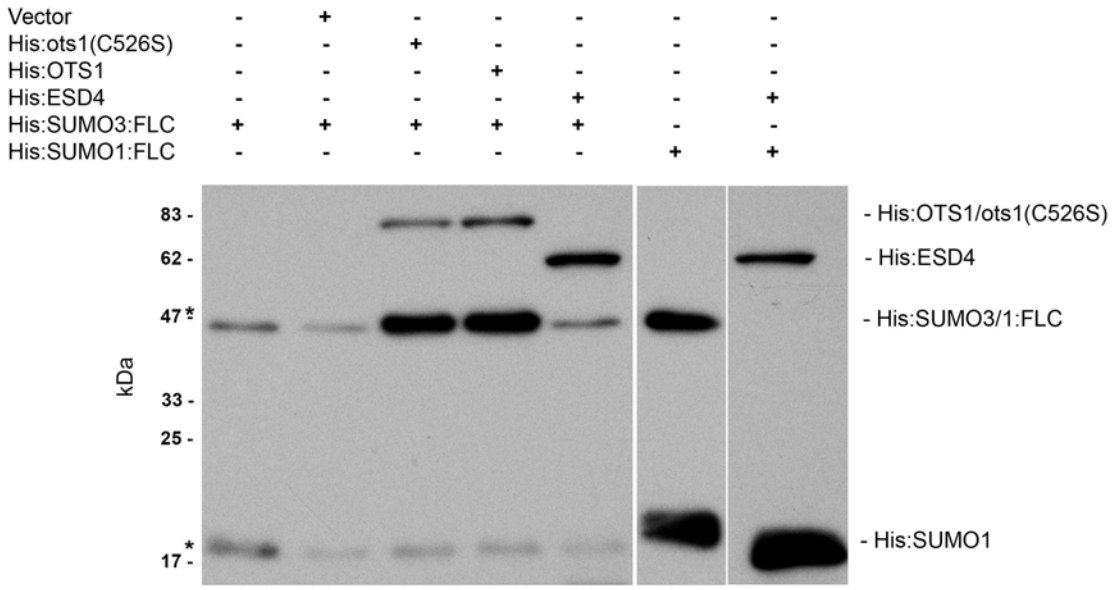


Supplemental Figure 1. Activation tagging of *Overly Tolerant to Salt 1* (*OTS1*).

(**A**) Cartoon representing the insertion site of the activation tagging vector pSKI015, in the *OTS1* activation tagging line (Weigel set N23153 pool N23846). The T-DNA insertion is shown in purple colour with arrowheads indicating the four copies of the *35S* promoter enhancers. The position of the Bar gene conferring Basta resistance and the left border (LB) on the T-DNA is shown. Green arrows represent annotated genes, At1g60220 (Ub-like protease) and At1g60200 (splicing factor PWI domain-containing protein/RNA recognition motif).

(**B**) RT-PCR analysis indicates that OTS1 is upregulated in the activation tagging line High Salt Tolerant 1 (*HST1*) but not in wild-type Columbia. The RT-PCR was repeated three times with similar results.

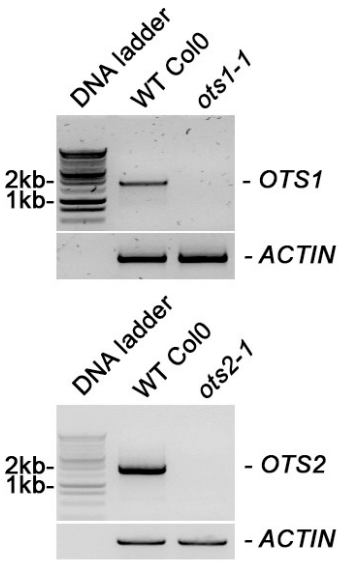
(**C**) Southern blot analysis of the *OTS1* activation tagging line. Genomic DNA was digested with the indicated restriction enzymes and probed with a fragment designed to the Bar gene of the pSKI015 vector. *Sal*I ad *Nco*I do not cut within the pSKI015 vector and produce a single band, indicating the presence of a single insertion in the genome.



Supplemental Figure 2. OTS1 does not cleave His:SUMO3:FLC.

In vitro SUMO protease activity assay. Equal amounts (20 μg) of total soluble proteins from *E. coli* cells transformed with expression vector control or fusion constructs His:ots1(C526S), His:OTS1 and His:ESD4 were mixed with 200 ng of purified SUMO substrates SUMO3 (His:SUMO3:FLC). To show overall SUMO protease activity of recombinant proteases, SUMO1 and ESD4 were loaded in parallel as a control.

The individual bands are shown on the right. Asterisks refer to cross-reacting bands likely derived from non-specific degradation of His:SUMO1/3:FLC or His:OTS1/His:ots1(C526S).

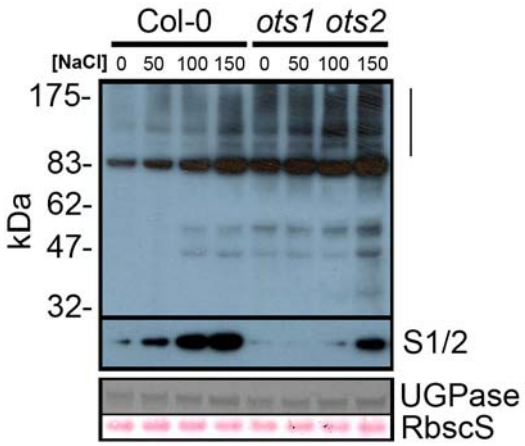


Supplemental Figure 3. *ots1-1* and *ots2-1* are knockout alleles.

RT-PCR analysis of *OTS1* and *OTS2* transcript levels from total RNA derived from young seedlings. cDNA of the indicated genotypes was subjected to 35 cycles of PCR with primers that amplify the full length coding sequence of OTS1 (primers lc1/OS12) and OTS2 (primers lc12/lc15). *ACTIN* was used as a loading control. PCR products were resolved on a 1% agarose gel in parallel with a DNA ladder. The experiment was repeated at least three times yielding similar results.

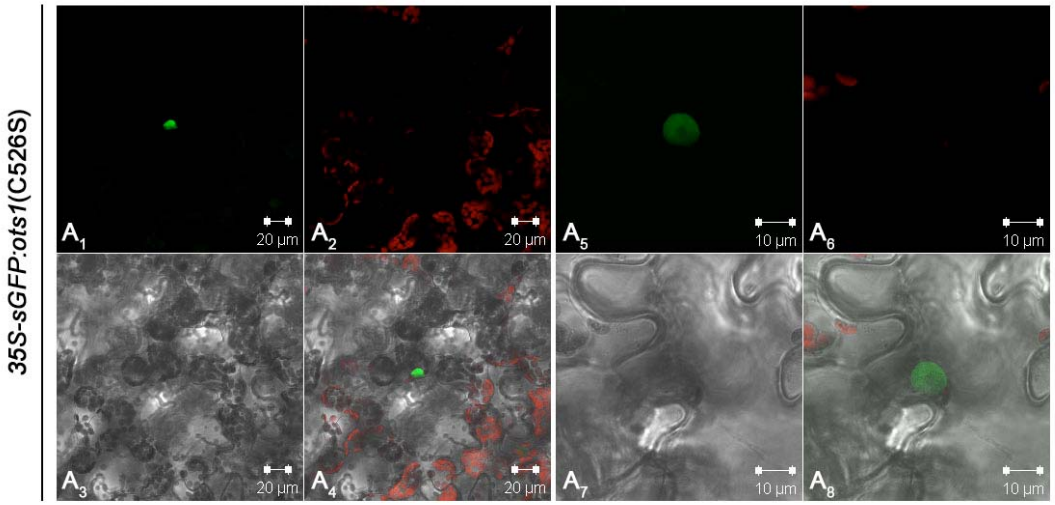
Supplemental Figure 4. Shoot growth is reduced in *ots1 ots2* double mutants in the presence of salt.

One week-old seedlings of the indicated genotype grown on normal medium were transferred to either normal or NaCl-supplemented medium plates and grown vertically in long day conditions. Picture shows a representative plate containing *ots1 ots2* double mutants and wild type Col-0 seedlings 15 days after transferring. Scale bar =1 cm.



Supplemental Figure 5. *ots1 ots2* double mutants over-accumulate SUMOylated proteins upon growth on high concentration of NaCl.

Upper panel, immunoblot with SUMO1/2 antibodies of 10 µg of total proteins derived from the indicated genotypes grown for 12 days in the presence of the indicated concentration of NaCl. The bar indicates the accumulation of SUMOylated proteins. The levels of free SUMO1/2 (S1/2) are shown below at lower exposure. UGPase immunostaining and Ponceau staining of Rubisco small sub-unit (RbscS) were used as loading controls.



Supplemental Figure 6. ots1(C526S) localises to the nucleus. Tobacco leaves were transiently transformed with vectors expressing ots1(C526S) and different fluorescent emissions were collected. ots1(C526S) protein localisation at low resolution (**A_1 to A_4**) and high resolution (**A_5 to A_8**). **A_1** and **A_5**, sGFP emission (green colour), **A_2** and **A_6**, chloroplast autofluorescence (red colour), **A_3** and **A_7**, bright field, **A_4** and **A_8**, merged image of **A_1-A_3** and **A_5-A_7**, respectively.