Supplemental Figures:

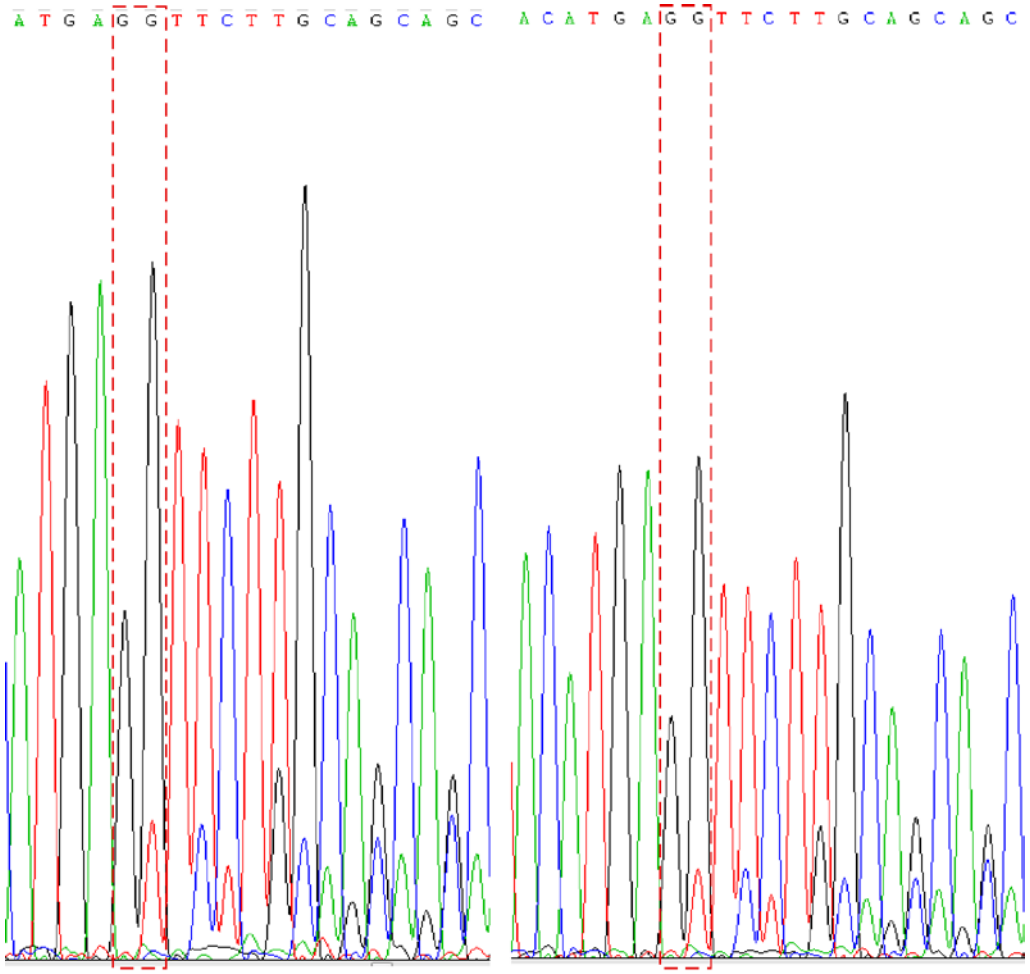

Figure S1. The sequencing chromatogram of *OTS1* cDNA of two independent complemented lines of mutant 438-1, com1 and com2.

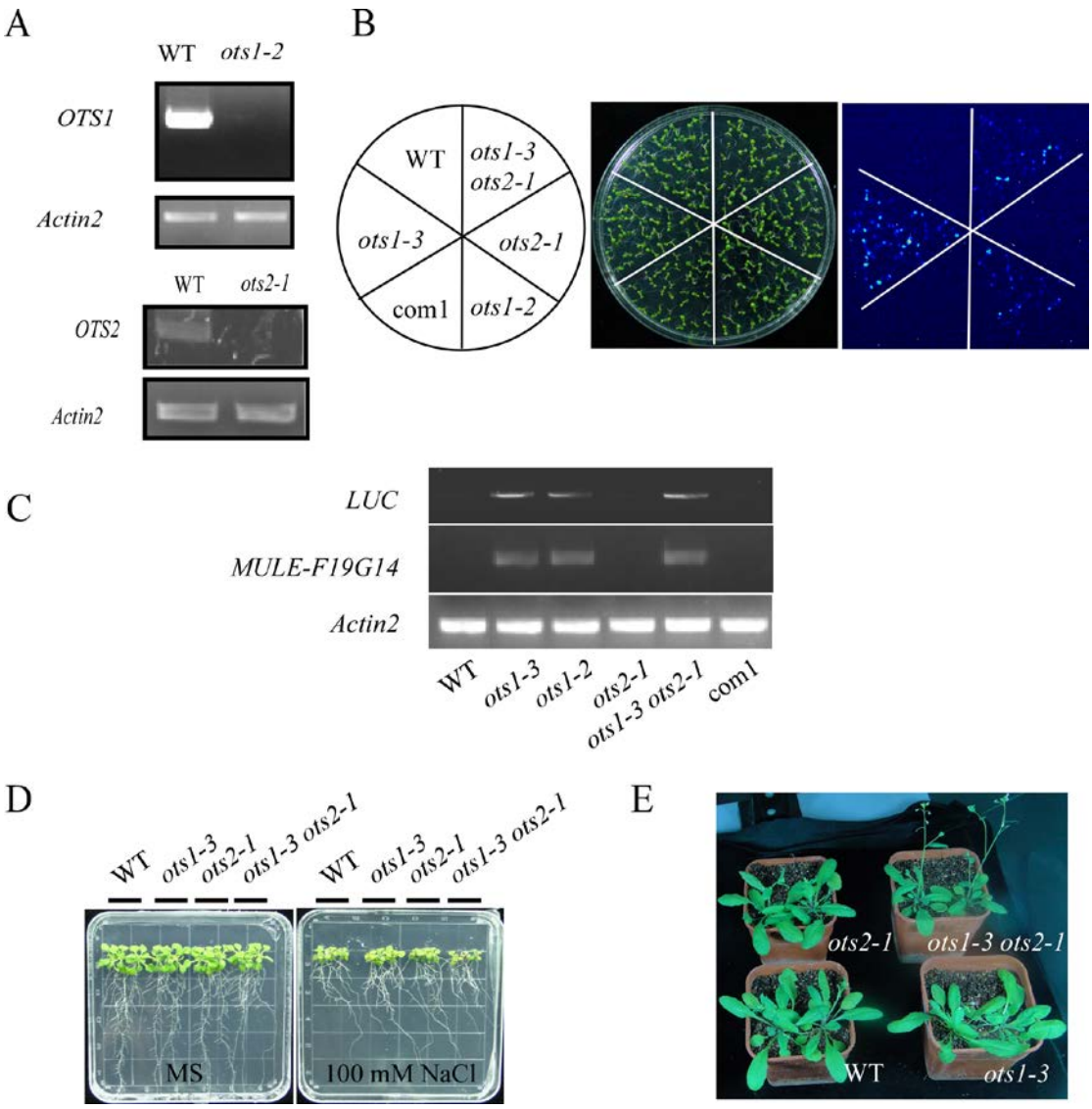

Figure S2. OTS2 has no functional redundancy with OTS1 for regulation of gene silence.

(A) RT-PCR analysis of expression of *35S-LUC* and *MULE-F19G14* in WT, *ots1-3*, *ots1-2*, *ots2-1*, *ots1-3 ots2-1*, and com1 plants. *Actin2* was used as an internal control.

(B) Luminescence images showing *LUC* expression in WT, *ots1-3*, *ots1-2*, *ots2-1*, *ots1-3 ots2-1*, and com1 plants, which all carry the transgene *35S-LUC*.

(C) RT-PCR analysis of expression of *35S-LUC* and *MULE-F19G14* in WT, *ots1-3*, *ots1-2*, *ots2-1*, *ots1-3 ots2-1*, and com1 plants. *Actin2* was used as an internal control.

(D) Analysis of t salt sensitivity of WT, *ots1-3*, *ots2-1*, and *ots1-3 ots2-1* seedlings. Left, Seedlings were grown in the regular MS medium. Right, seedlings were grown in the presence of 100 mM NaCl. The pictures were taken at 15 days.

(E) Analysis of flowering time of WT, *ots1-3*, *ots2-1*, and *ots1-3 ots2-1* plants grown

in long-day conditions (16-h light/8-h dark). Pictures were taken at 25 days.

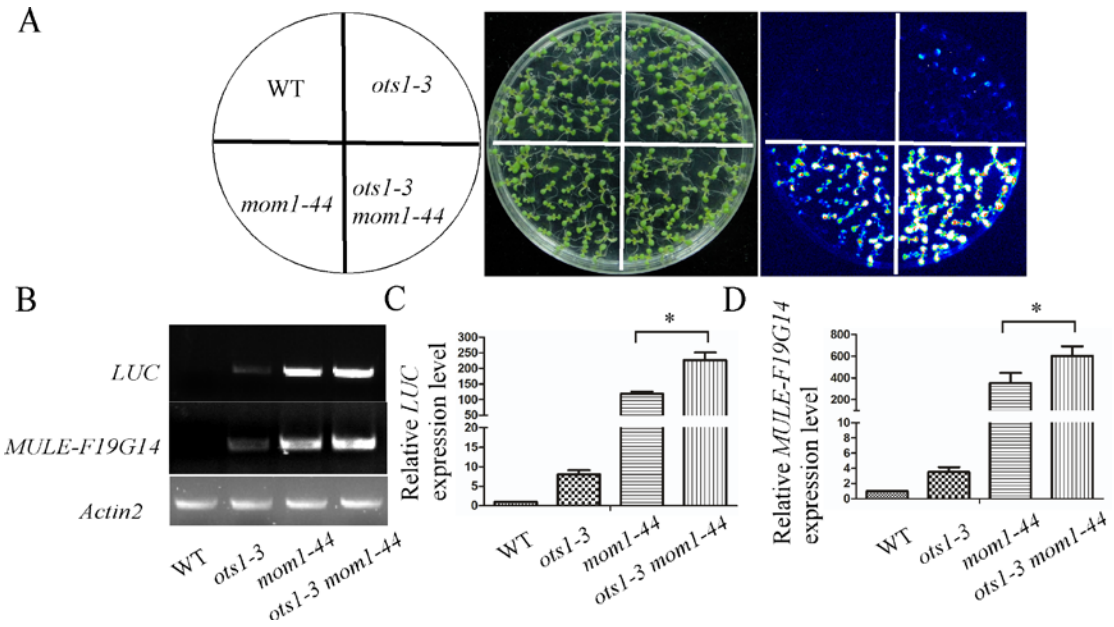

Figure S3. OTS1-mediated gene silence is independent of MOM1.

(A) Luminescence images showing *LUC* expression in WT, *ots1-3*, *mom1-44*, and *ots1-3 mom1-44* plants.

(B) RT-PCR analysis of expression of *LUC* and *MULE-F19G14* in WT, *ots1-3*, *mom1-44*, and *ots1-3 mom1-44* plants. *ACTIN2* was used as an internal control.

(C-D) Analysis of the polyadenylated *LUC* (C) and *MULE-F19G14* (D) expression level in WT, *ots1-3*, *mom1-44*, and *ots1-3 mom1-44* plants by real-time PCR using total RNA reverse transcribed by oligo (dT). *Actin2* was used as an internal control. Error bars represent SD (n=3) for at least three replicate experiments. According to Student's t-test, significant differences (p value ≤ 0.05) were indicated by different lowercase letters.

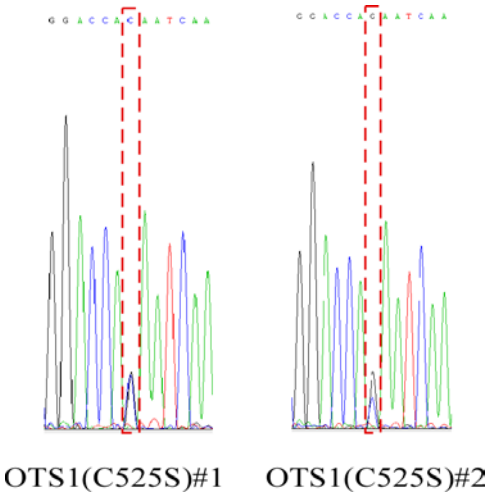

Figure S4. The sequencing chromatogram of *OTS1* cDNA in two independent transgenic lines, *OTS1*(C525S)#1 and #2. The cDNAs were reversely transcribed from the RNA extracted from *OTS1*(C525S)#1 and #2 plants. The OTS1 was amplified and sequenced. The mutation causing the C525S substitution was shown in red box.

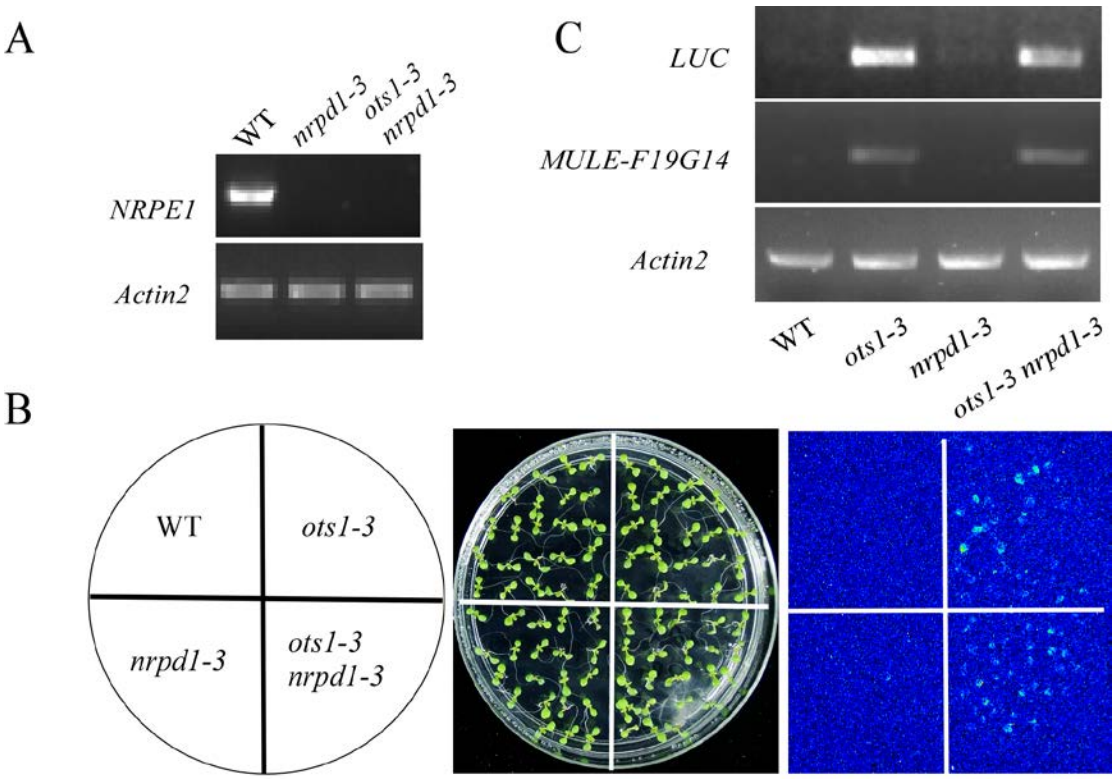

Figure S5. Mutation of Pol IV does not affect the gene silence in the *ots1-3* mutant.

(A) Expression of *NRPD1* in WT, *nrpd1-3* and *ots1-3 nrpd1-3* plants was analyzed by RT-PCR. *Actin2* was used as an internal control.

(B) LUC luminescence showing *LUC* expression in WT, *ots1-3*, *nrpd1-3*, and *ots1-3 nrpd1-3* plants.

(C) RT-PCR analysis of expression of *LUC* and *MULE-F19G14*. Total RNA were extracted from WT, *ots1-3*, *nrpd1-3*, and *ots1-3 nrpd1-3* plants. *Actin2* was used as an internal control.

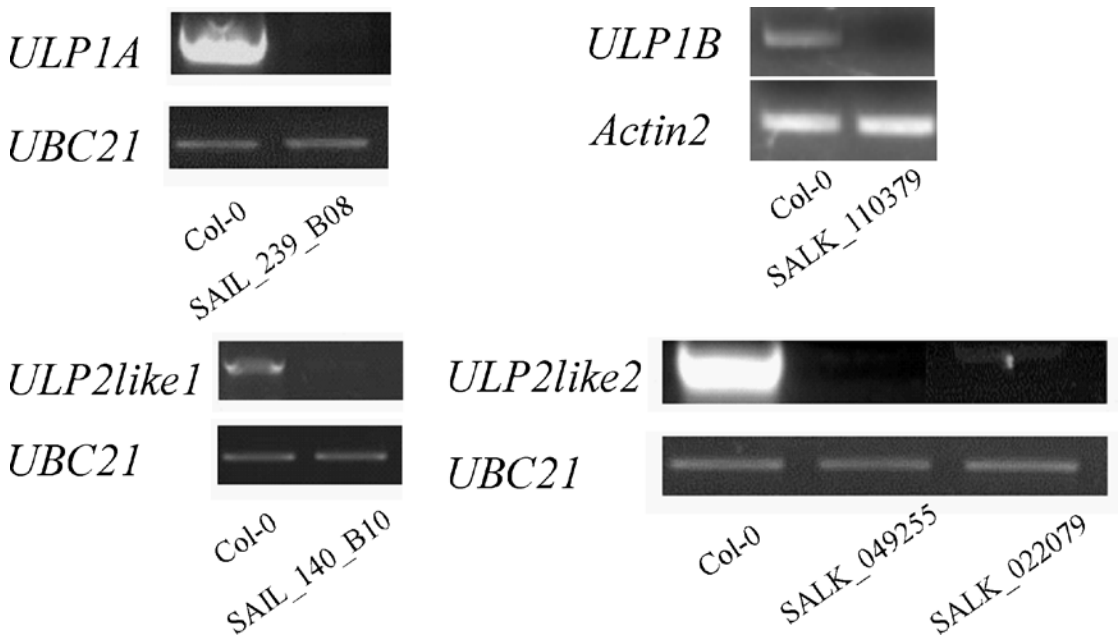

Figure S6. RT-PCR analysis of transcript levels of SUMO proteases, *ULP1A*, *ULP1B*, *ULP2like1* and *ULP2like2* in their corresponding T-DNA lines. *Actin2* or *UBIQUITIN CONJUGATING ENZYME 21* (*UBC21*) was used as an internal control.