



"The non-JAZ TIFY protein TIFY8 of *Arabidopsis thaliana* interacts with the HD-ZIP III transcription factor REVOLUTA and regulates leaf senescence."

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Supplemental Figures S1-S12

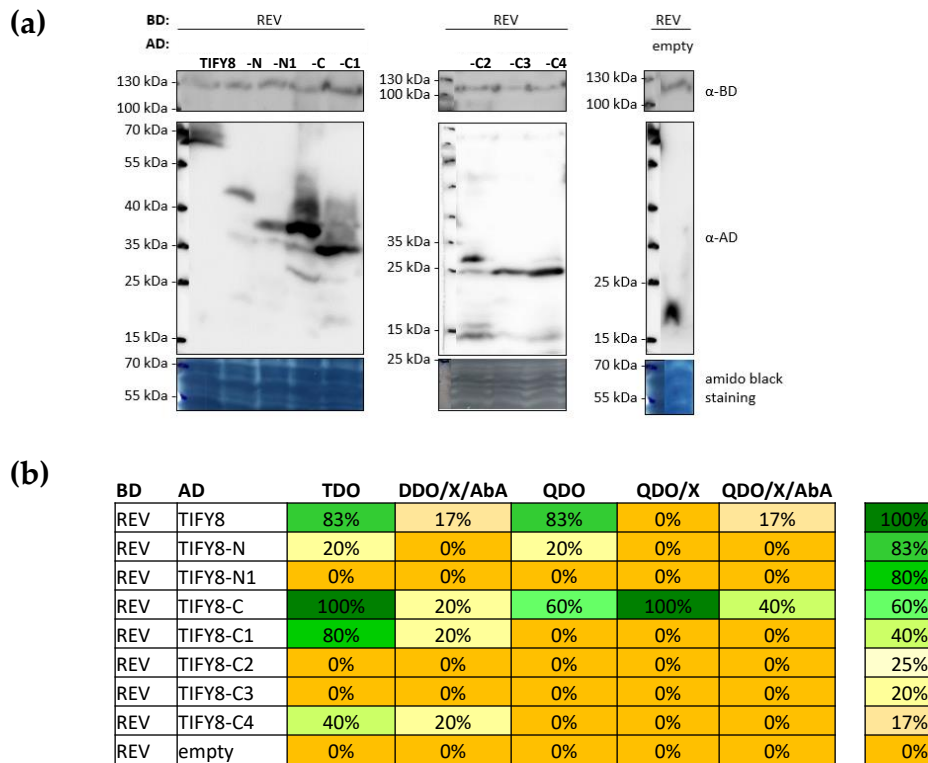
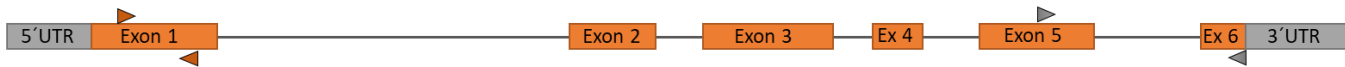


Figure S1: Yeast two-hybrid protein expression in yeast cells and heat map of positive results in independent mating experiments.

(a) Western blot analyzed with anti-BD and anti-AD antibodies (α) show expression of BD-REV and AD-TIFY8 fusion proteins in diploid yeast cells. Protein loading is indicated by a section of the Amido Black Staining (A.B.S) BD-REV: 114 kDa, AD TIFY8:62 kDa, AD-TIFY8-N: 48 kDa, AD-TIFY8-N1: 43 kDa, AD-TIFY8-C: 44 kDa, AD-TIFY8-C1: 38 kDa, AD-TIFY8-C2: 33 kDa, AD-TIFY8-C3: 30 kDa, AD-TIFY8-C4: 30 kDa, AD-empty: 22 kDa. **(b)** Heatmap of positive interaction results of 3 to 7 independent mating experiments in % positive results.

TIFY8 splice variant 1 (SV1)



TIFY8 splice variant 2 (SV2)



Figure S2: *Splicing variants of TIFY8.*

Two splicing variants of *TIFY8* differ in the 3' region in exon 5 and 6. Primer position used for qRT-PCR (orange) and splicing variant determination (grey) are indicated by the arrow heads).

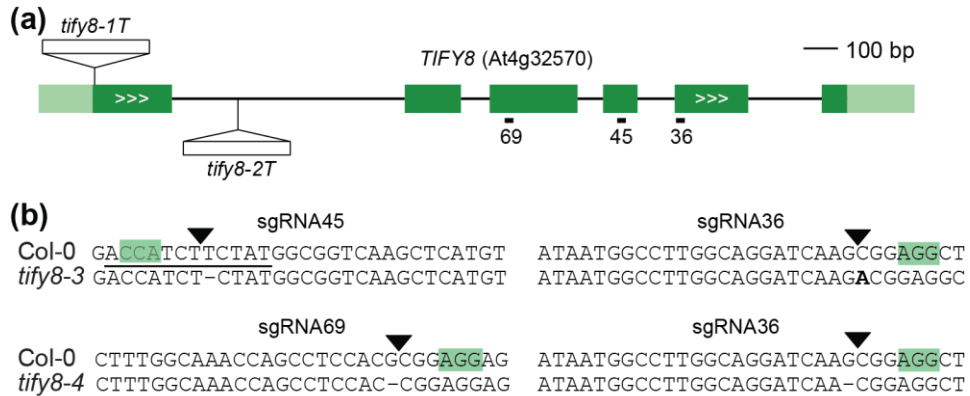
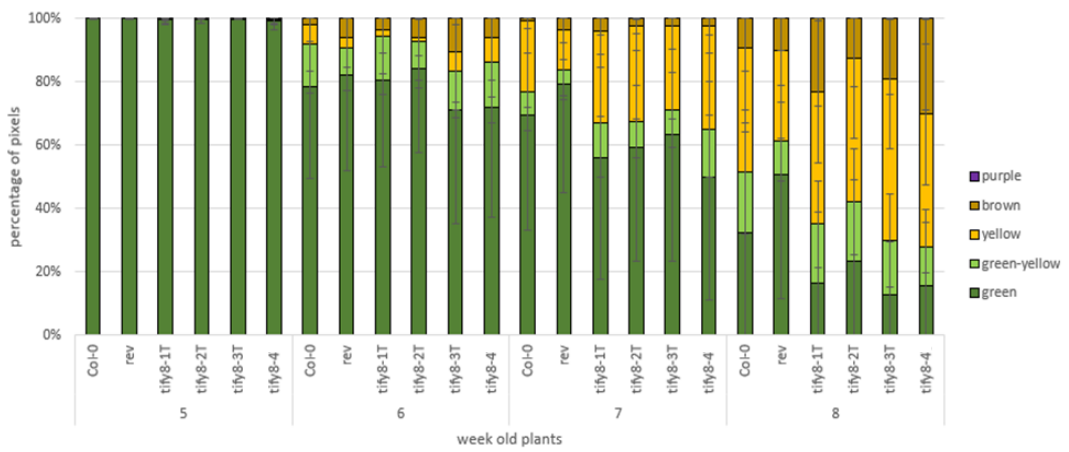


Figure S3: Generation of *tify8* CRISPR/Cas9 lines.

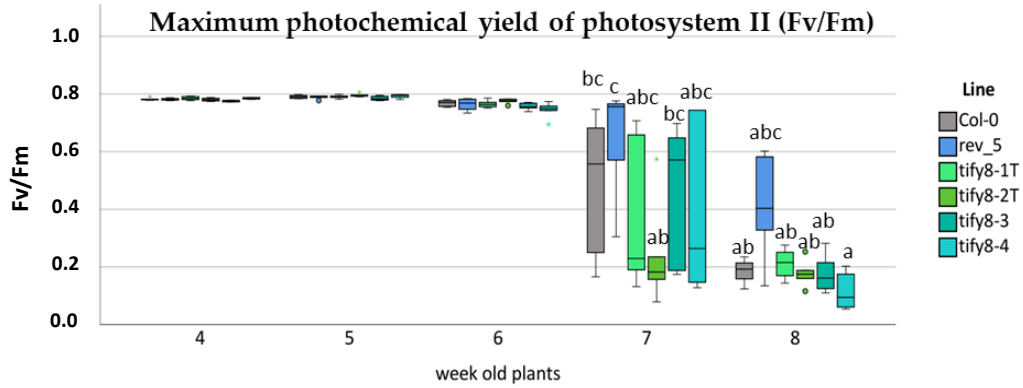
(a) Genomic structure of Arabidopsis *TIFY8* and location of the T-DNA inserts and sgRNAs. Dark green boxes designate exons; light green boxes, UTRs; solid lines, introns; white arrows gene orientation. sgRNA numbers are arbitrary identifiers. Locations of the *tify8-1T* and *tify8-2T* T-DNA are indicated. (b) Genotypes of homozygous *tify8-3* and *tify8-4* mutants, generated by combining sgRNA45 and 3, or sgRNA69 and sgRNA36, respectively. The PAM is highlighted in green, the triangle points to the Cas9 cut site; -, deleted base, bold, inserted base. The sequence encoding the TIFY motif is underlined.

(a)

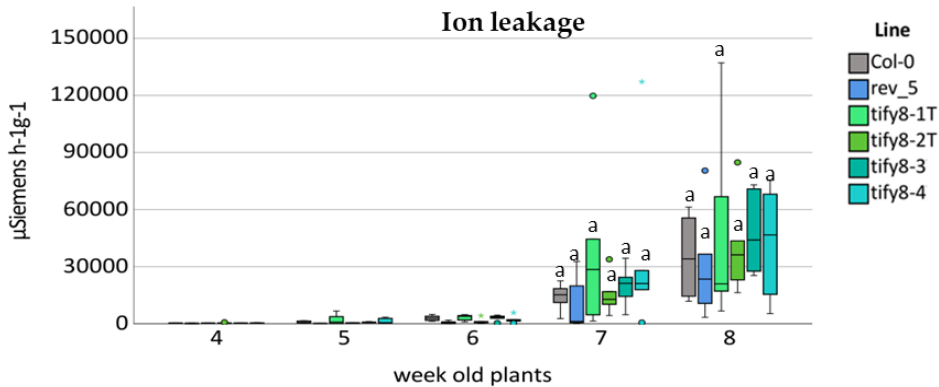
ACA



(b)



(c)



(d)

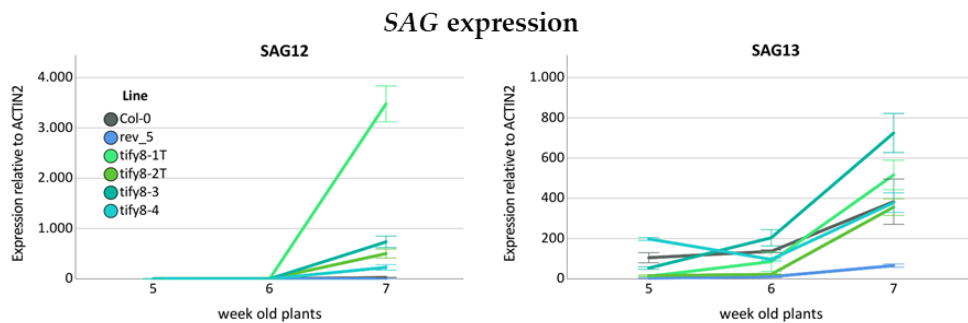


Figure S4: Senescence parameters of all mutant lines.

These parameters were analyzed for the senescence phenotyping of *tify8-1T*, *tify8-2T*, *tify8-3*, *tify8-4*, and *rev5* mutants compared to wildtype Col-0 plants. **(a)** Automated Colorimetric Assay (ACA) to categorize the color of individual leaves of at least six plants pixelwise into five groups: green, green-yellow, yellow, brown, and purple. The percentage of each group with respect to total pixel number of all leaves is presented ($n=6$). **(b)** Boxplot of Fv/Fm values measured with PAM for leaves No. 5 of 4- to 7-week-old plants (mean values \pm SE, $n=6-8$). **(c)** Boxplot of the decrease in solute retention determined through ion leakage in Leaves No. 4 of 4- to 7-week-old plants ($n=6-8$). One-way ANOVA test was performed, ($p \leq 0.05$). **(d)** Gene Expression of the senescence-associated marker genes *SAG12* and *SAG13* were analyzed by qRT-PCR and normalized to the expression of the *ACTIN2* gene. The expression of both senescence-associated marker genes was analyzed in 5- to 7-week-old plants (mean values \pm SD, $n=3$).

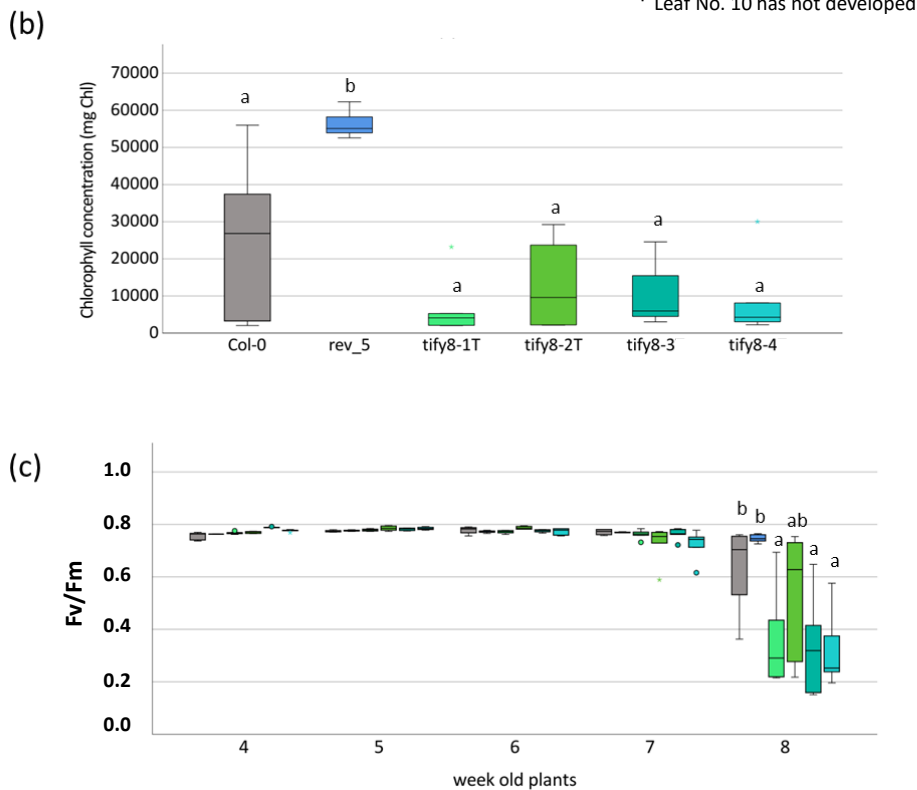
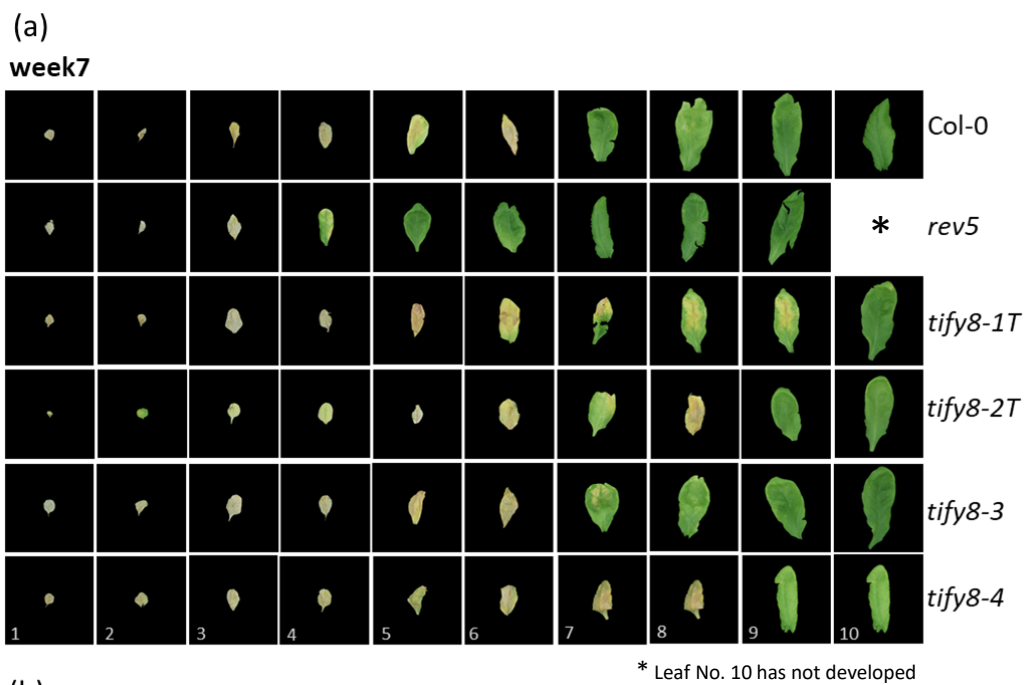
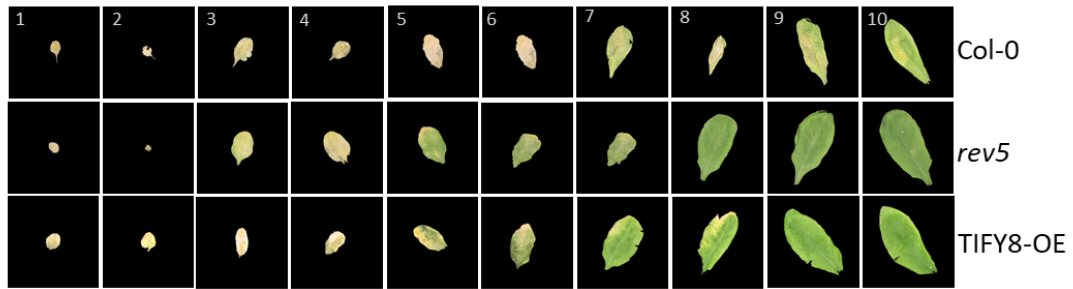


Figure S5: Phenotypic and photosynthetic parameters.

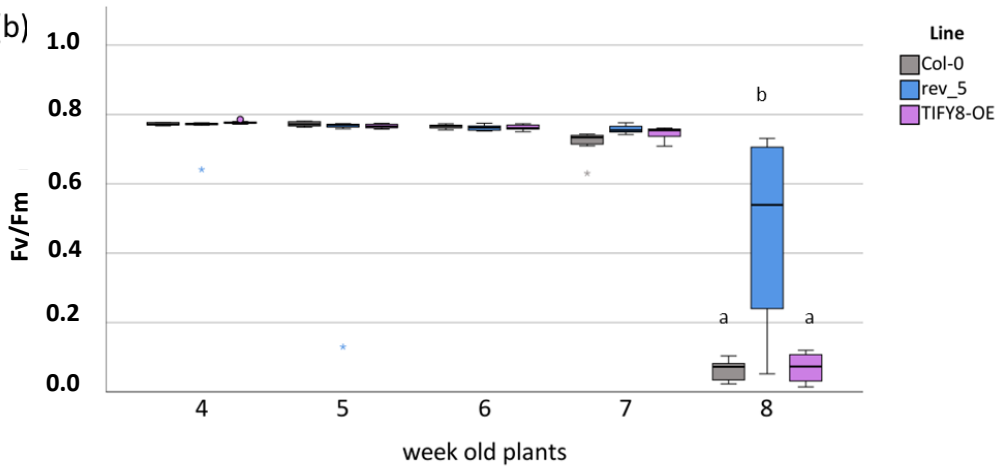
These parameters were analyzed for the senescence phenotyping of *tify8-1T*, *tify8-2T*, *tify8-3*, *tify8-4*, and *rev5* mutants compared to wildtype Col-0 plants. (a) Phenotypical appearance of leaves at positions 1 to 10 of 7-week-old plants. Boxplots of (b) the chlorophyll content per leaf determined for leaves No. 10 of 7-week-old plants ($n=6$) and (c) Fv/Fm values measured by PAM for the identical leaves (No. 10) of 4- to 7-week-old plants ($n=6$). One-way ANOVA test was performed, ($p \leq 0.05$).

(a)

week7



(b)



(c)

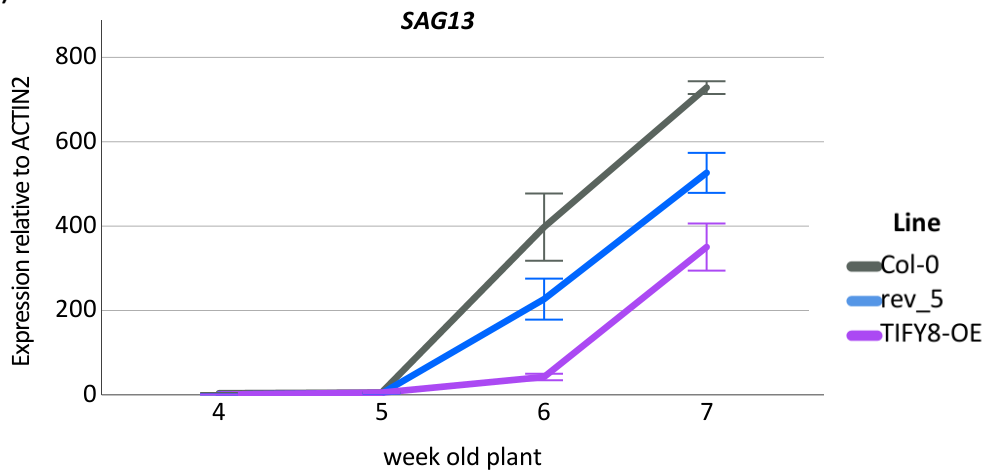


Figure S6: Senescence parameters.

These parameters were analyzed for the senescence phenotyping of *TIFY8*-OE and *rev5* mutants compared to wildtype Col-0 plants. **(a)** phenotypal appearance of leaves at positions 1 to 10 of 7-week-old plants, **(b)** Boxplot of F_v/F_m values measured with PAM for the leaves No. 10 of 4 to 7-week-old plants ($n=8$). One-way ANOVA test was performed, ($p \leq 0.05$). **(c)** Gene expression of the senescence-associated marker gene *SAG13* was analyzed by qRT-PCR and normalized to the expression of the *ACTIN2* gene. The expression was analyzed in 4- to 7-week-old plants in 2 pools of 4 leaves of 4 different plants (mean values \pm SD).

Lipid peroxidation

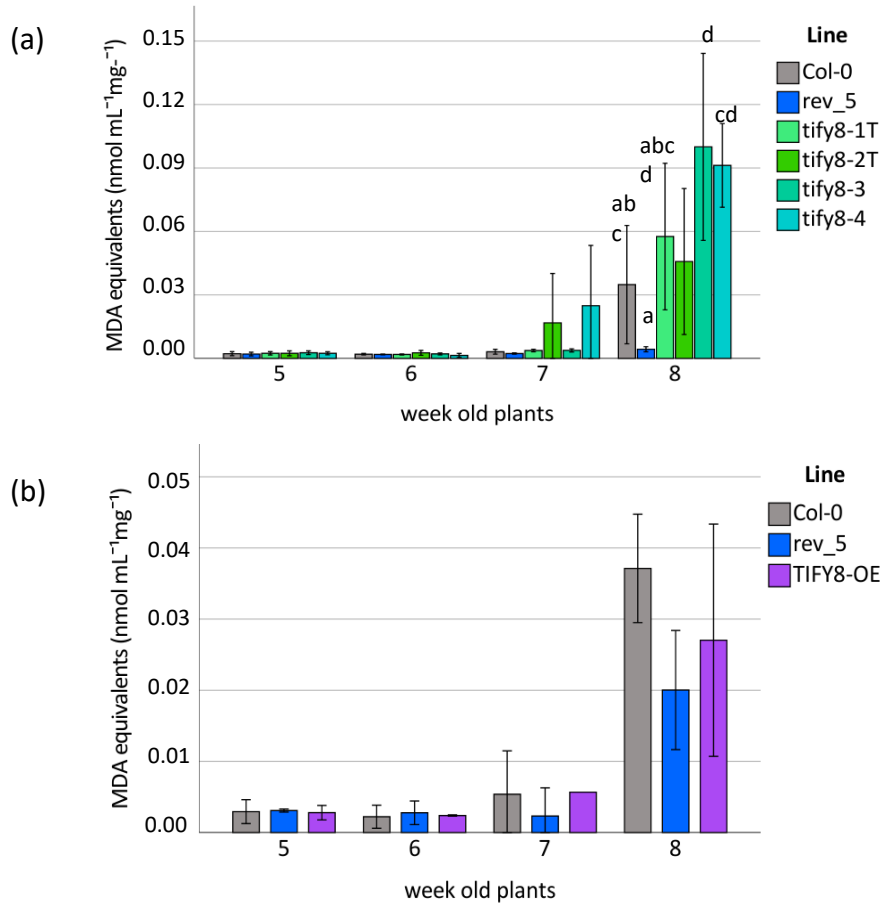


Figure S7: *Lipid peroxidation.*

Lipid peroxidation were determined by the quantification of the MDA concentration in leaves No. 9 of 5- to 7-week-old plants (a) *tify8-1T*, *tify8-2T*, *tify8-3*, *tify8-4*, and *rev5* mutants compared to wildtype Col-0 plants, mean \pm SE, $n=6$, One-way ANOVA test was performed, ($p \leq 0.05$) (b) *TIFY8-OE* and *rev5* mutants compared to wildtype Col-0 plants, mean \pm SE, (2 pools of 3 leaves No. 9 of 3 different plants each)

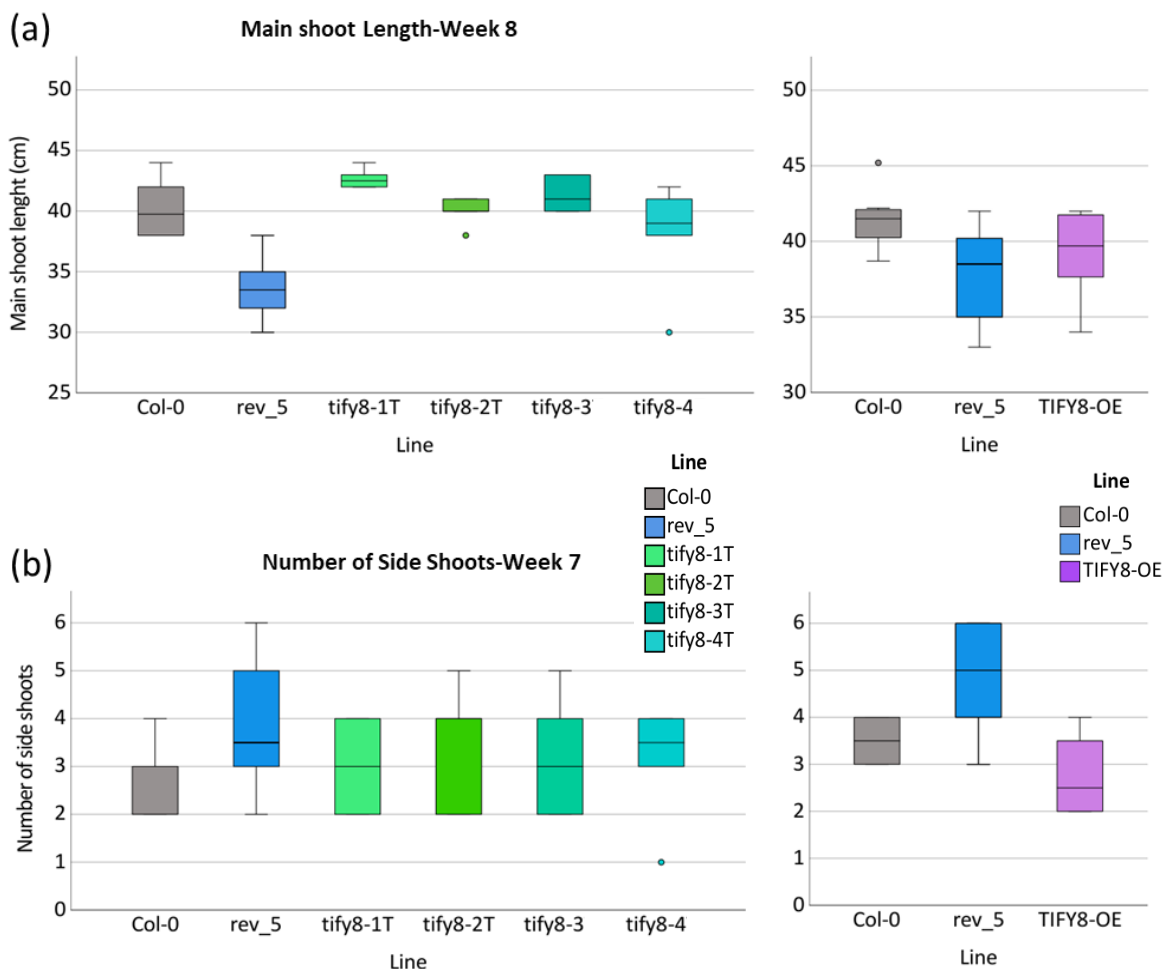


Figure S8: Phenotypic appearance.

Boxplots of **(a)** the length of the main shoot measured in 8-week-old plants in all *tify8* mutants as well as in the *TIFY8*-OE and the respective control Col-0 ($n=6-8$). **(b)** the number of side shoots determine in 7-week-old-plants in all the different lines previously mentioned ($n=6-8$)

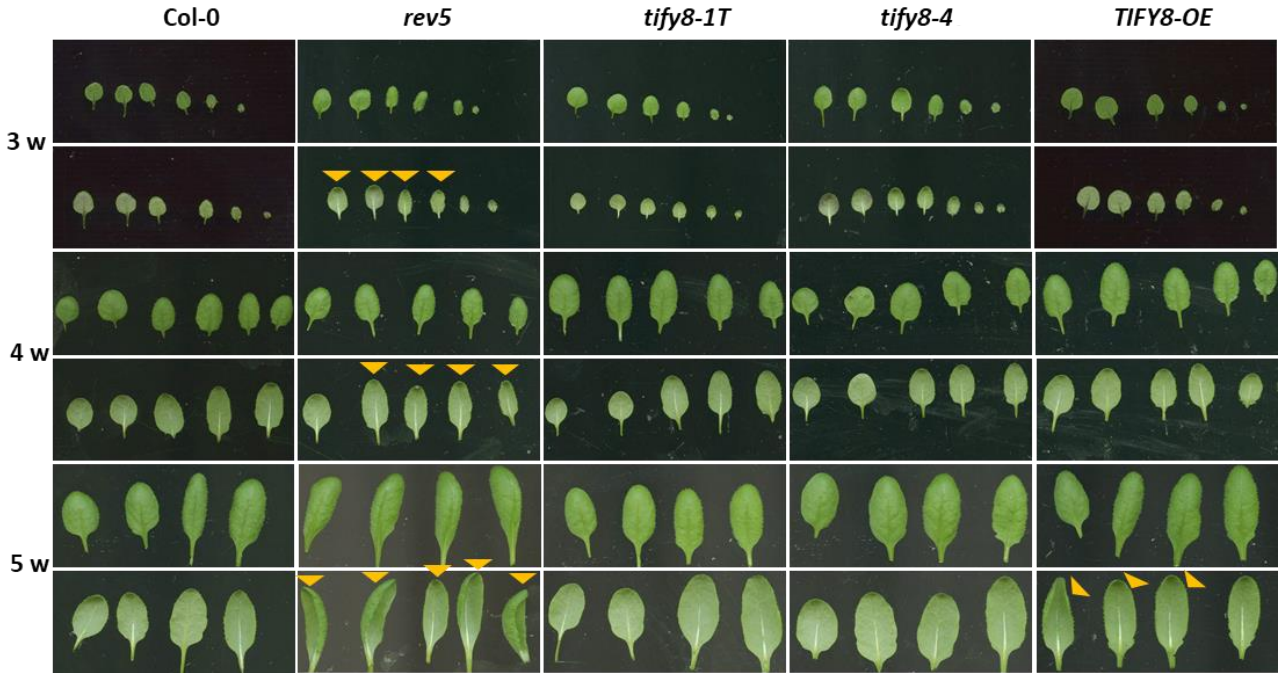


Figure S9: *Leaf phenotype in early stages.*

Leaf development of rosette leaves was documented in early stages of leaf and rosette development. Orange arrow heads point to the downward curled leaves which were typically observed in *rev5* mutant plants.

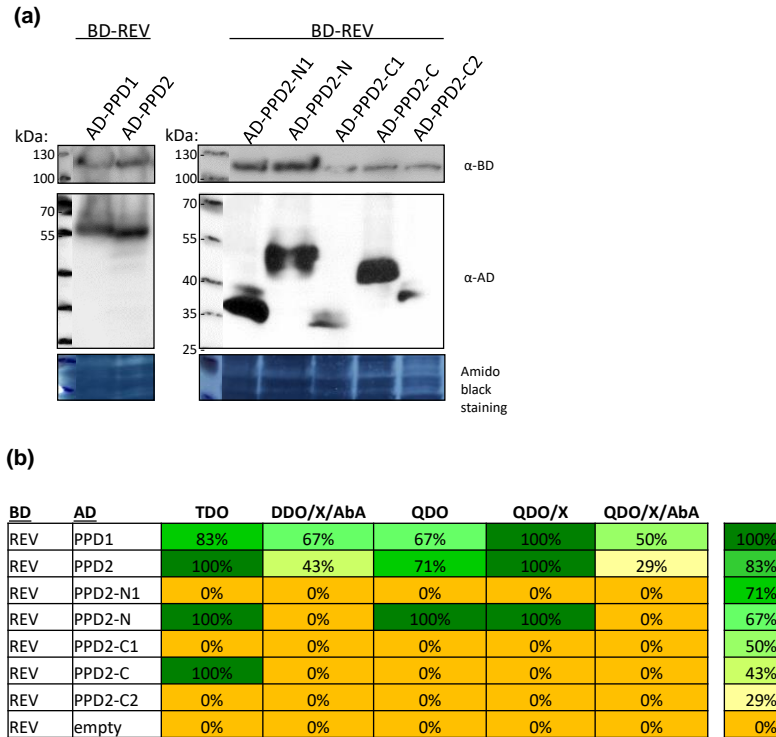


Figure S10: Yeast two-hybrid protein expression in yeast cells and heat map of positive results in independent mating experiments.

(a) Western blot analyzed with anti-BD and anti-AD antibodies (α) show expression of BD-REV and AD-PPD fusion proteins in diploid yeast cells. The amount of protein loaded onto the gel is indicated by a section of the Amido Black Staining. BD-REV: 114 kDa, AD-PPD1: 58 kDa, AD-PPD2: 58 kDa, AD-PPD2-N1: 37 kDa, AD-PPD2-N: 46 kDa, AD-PPD2-C1: 33 kDa, AD-PPD2-C: 46 kDa, AD-PPD2-C2: 36 kDa. **(b)** Heatmap of positive interaction results of 3 to 7 independent mating experiments in % positive results.

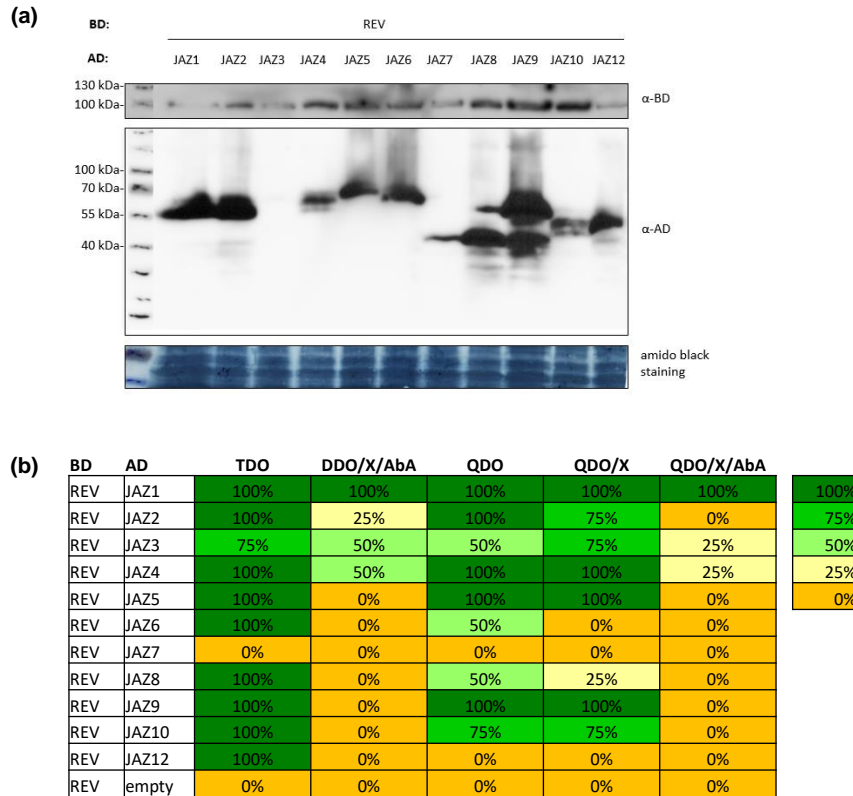


Figure S11: Yeast two-hybrid protein expression in yeast cells and heat map of positive results in independent mating experiments.

(a) Western blot analyzed with anti-BD and anti-AD antibodies (α) show expression of BD-REV and AD-JAZ fusion proteins in diploid yeast cells. The amount of protein loaded onto the gel is indicated by a section of the Amido Black Staining. BD-REV: 114 kDa, AD-JAZ1: 51 kDa, AD-JAZ2: 51 kDa, AD-JAZ3: 62 kDa, AD-JAZ4: 58 kDa, AD-JAZ5: 54 kDa, AD-JAZ6: 54 kDa, AD-JAZ7: 41 kDa, AD-JAZ8: 39 kDa, AD-JAZ9: 53 kDa, AD-JAZ10: 46 kDa, AD-JAZ12: 44 kDa. **(b)** Heatmap of positive interaction results of 3 to 7 independent mating experiments in % positive results.

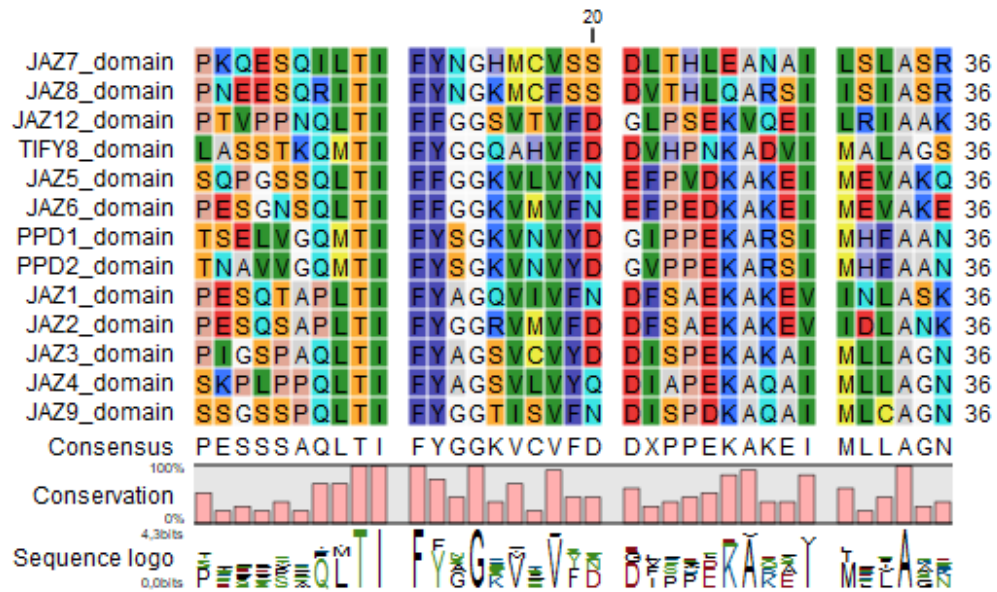


Figure S12: Alignment of all TIFY/JAZ domains of the class two TIFY proteins.