



"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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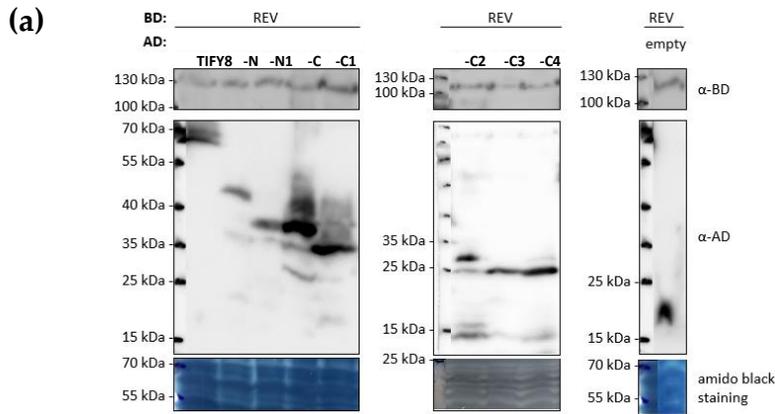
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Supplemental Figures S1-S12



(b)

BD	AD	TDO	DDO/X/AbA	QDO	QDO/X	QDO/X/AbA	
REV	TIFY8	83%	17%	83%	0%	17%	100%
REV	TIFY8-N	20%	0%	20%	0%	0%	83%
REV	TIFY8-N1	0%	0%	0%	0%	0%	80%
REV	TIFY8-C	100%	20%	60%	100%	40%	60%
REV	TIFY8-C1	80%	20%	0%	0%	0%	40%
REV	TIFY8-C2	0%	0%	0%	0%	0%	25%
REV	TIFY8-C3	0%	0%	0%	0%	0%	20%
REV	TIFY8-C4	40%	20%	0%	0%	0%	17%
REV	empty	0%	0%	0%	0%	0%	0%

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.

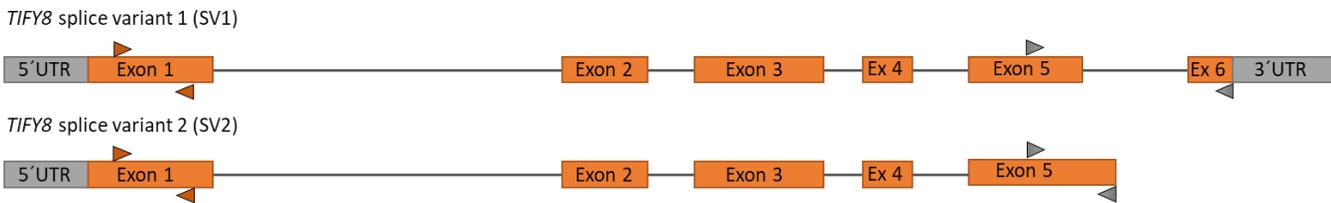


Figure S2: *Splicing variants of TIFY8.*

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).

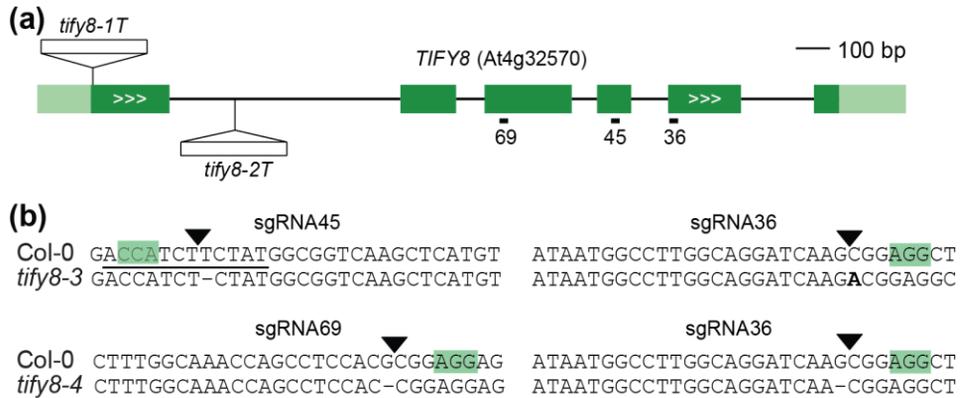
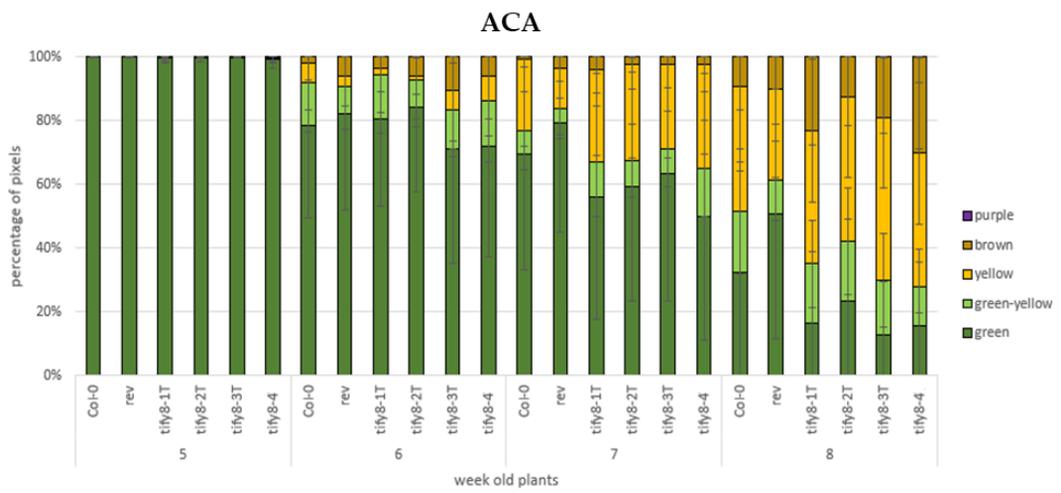


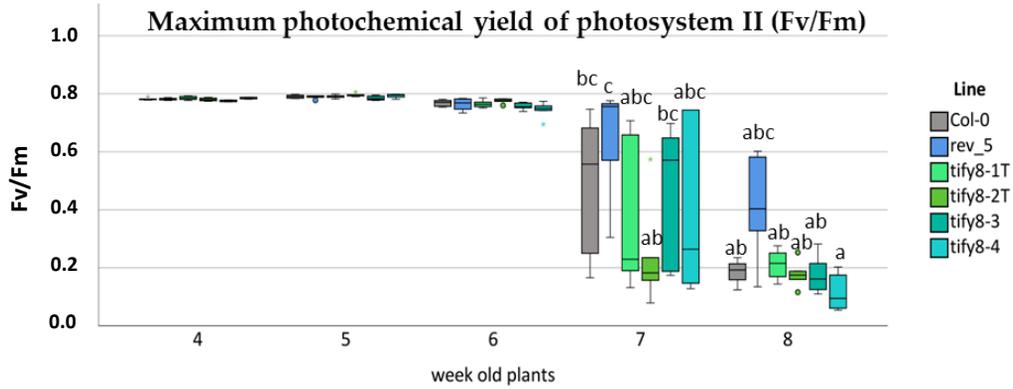
Figure S3: Generation of *tify8* CRISPR/Cas9 lines.

(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.

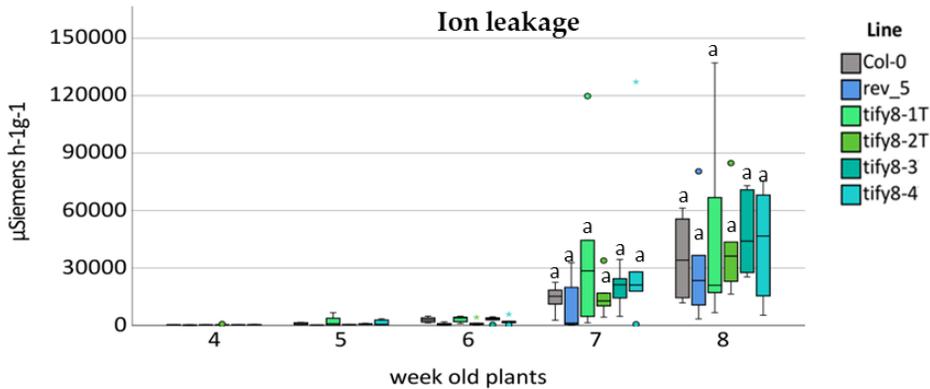
(a)



(b)



(c)



(d)

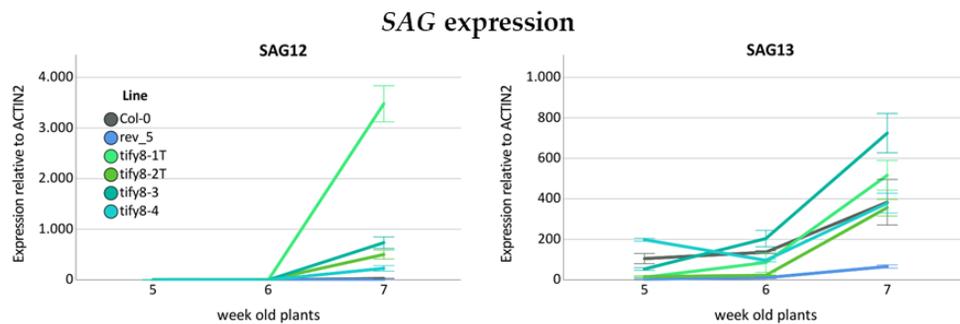
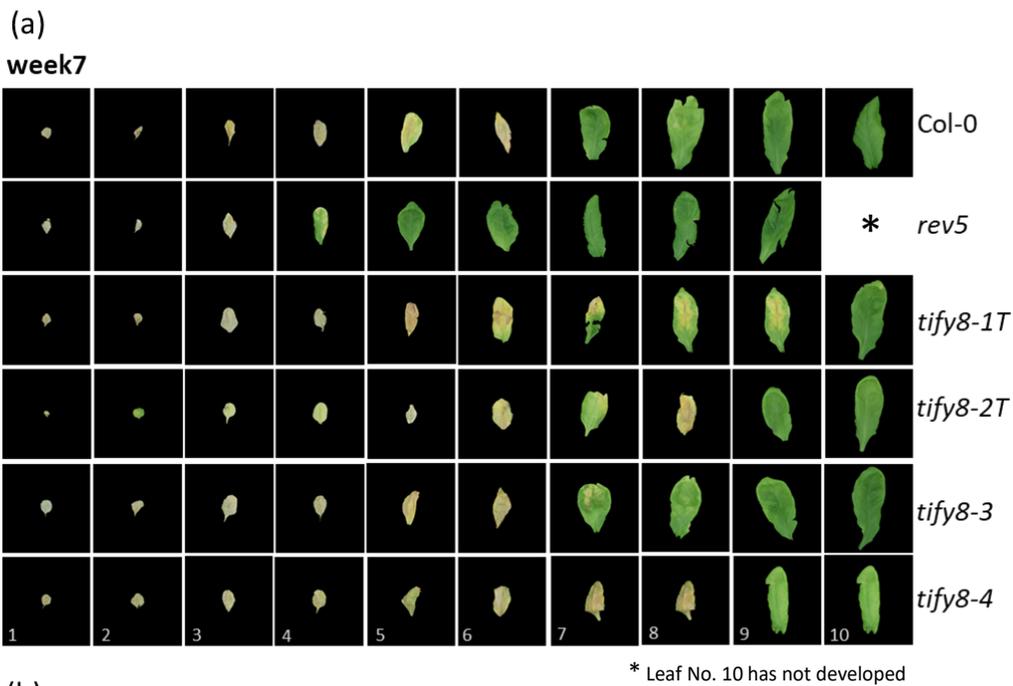


Figure S4: Senescence parameters of all mutant lines.

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, $n=3$).



* Leaf No. 10 has not developed

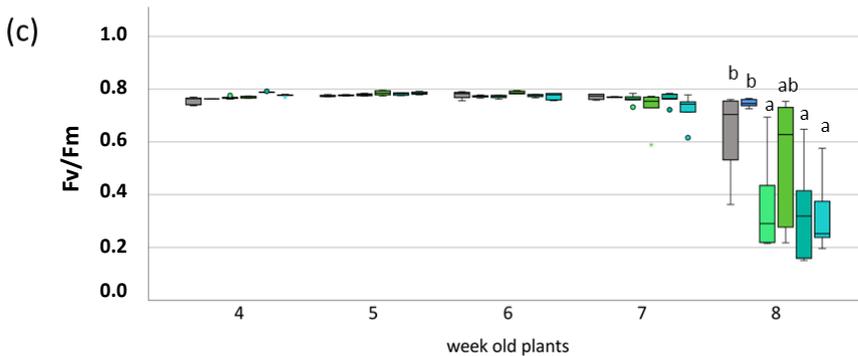
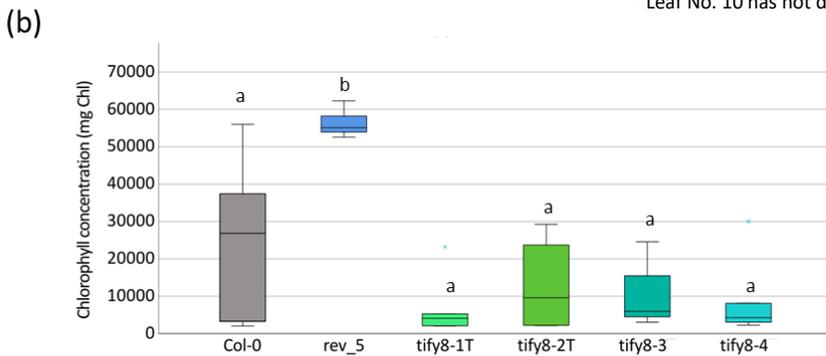


Figure S5: Phenotypic and photosynthetic parameters.

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, ($p \leq 0.05$).

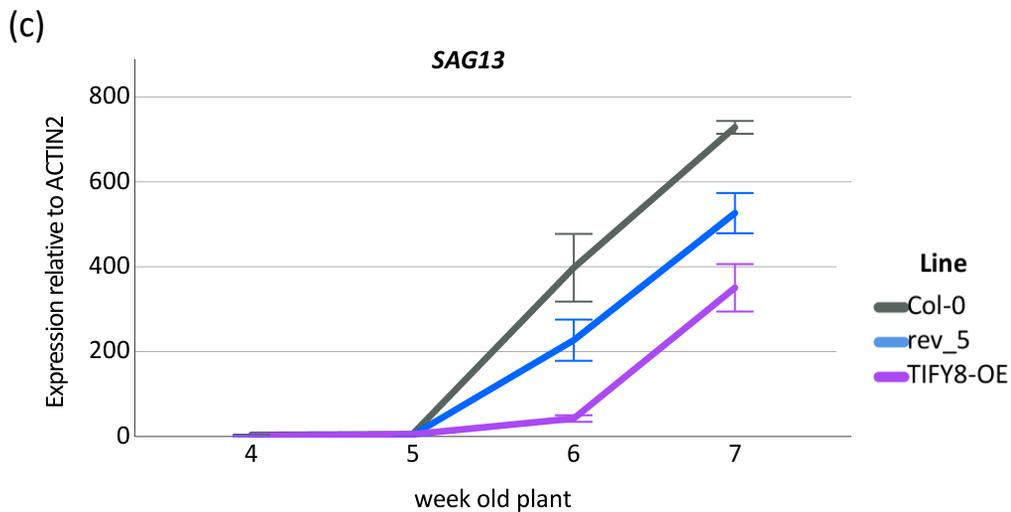
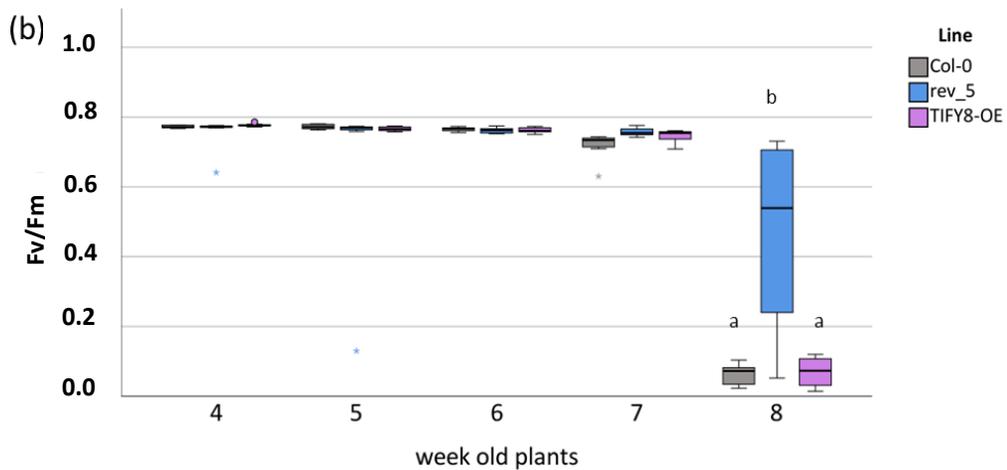
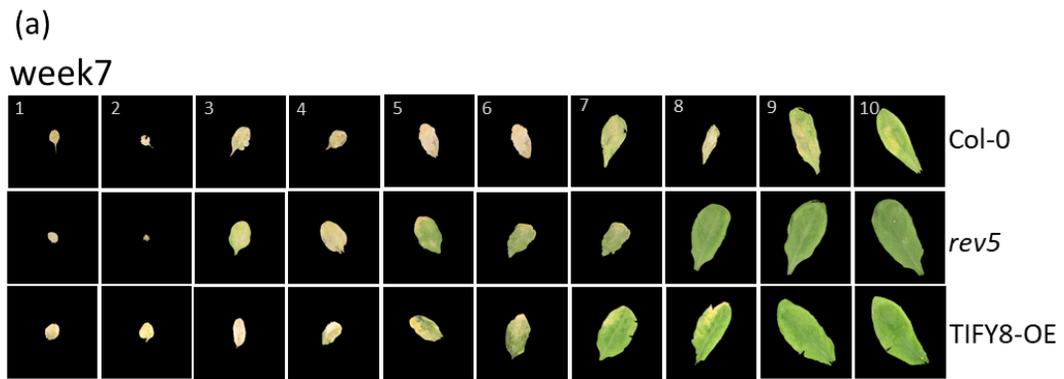


Figure S6: Senescence parameters.

These parameters were analyzed for the senescence phenotyping of *TIFY8*-OE and *rev5* mutants compared to wildtype Col-0 plants. (a) phenotypical appearance of leaves at positions 1 to 10 of 7-week-old plants, (b) Boxplot of Fv/Fm 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

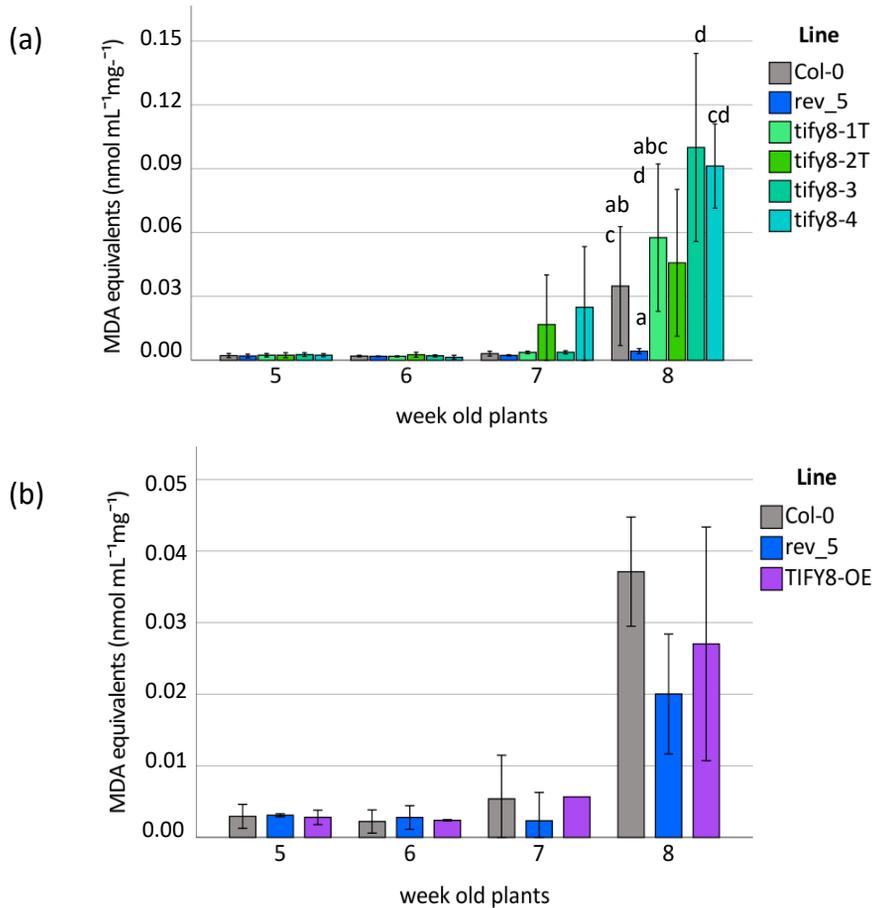


Figure S7: *Lipid peroxidation.*

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)

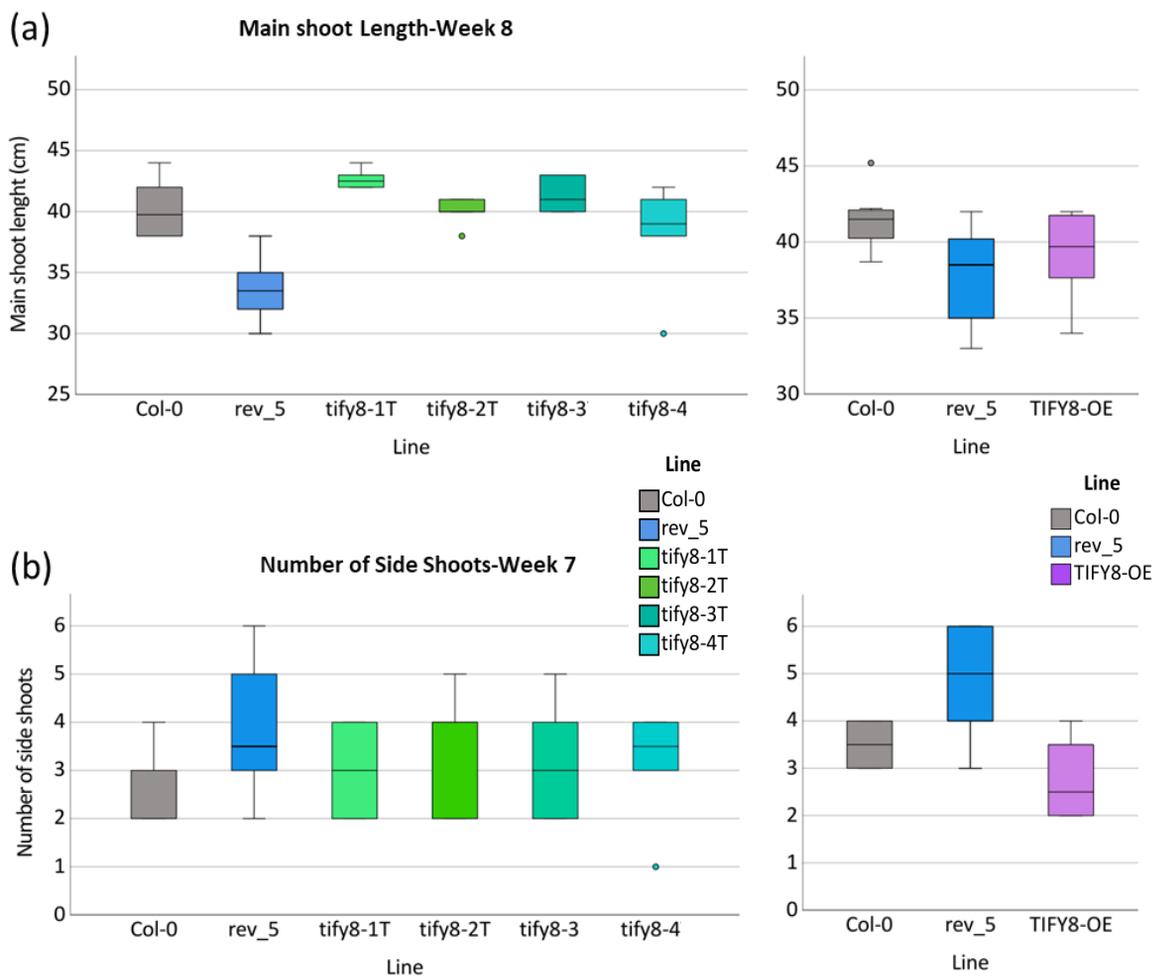


Figure S8: Phenotypic appearance.

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 ($n=6-8$)

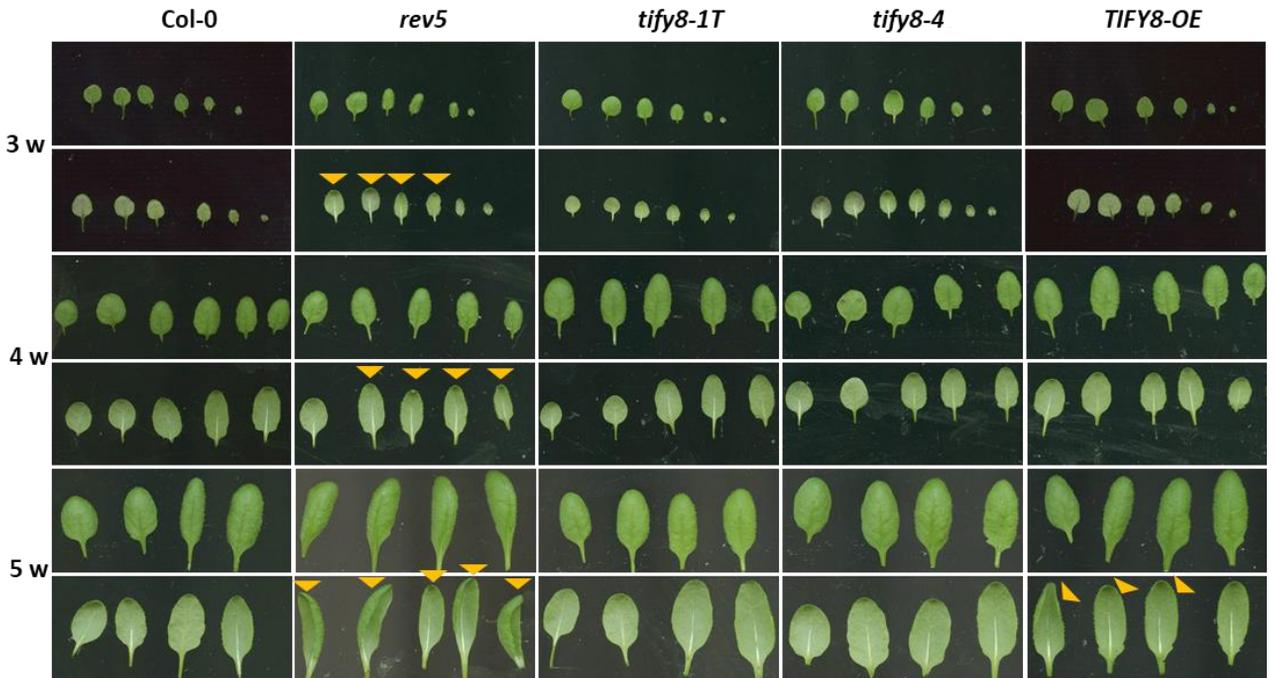


Figure S9: Leaf phenotype in early stages.

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.

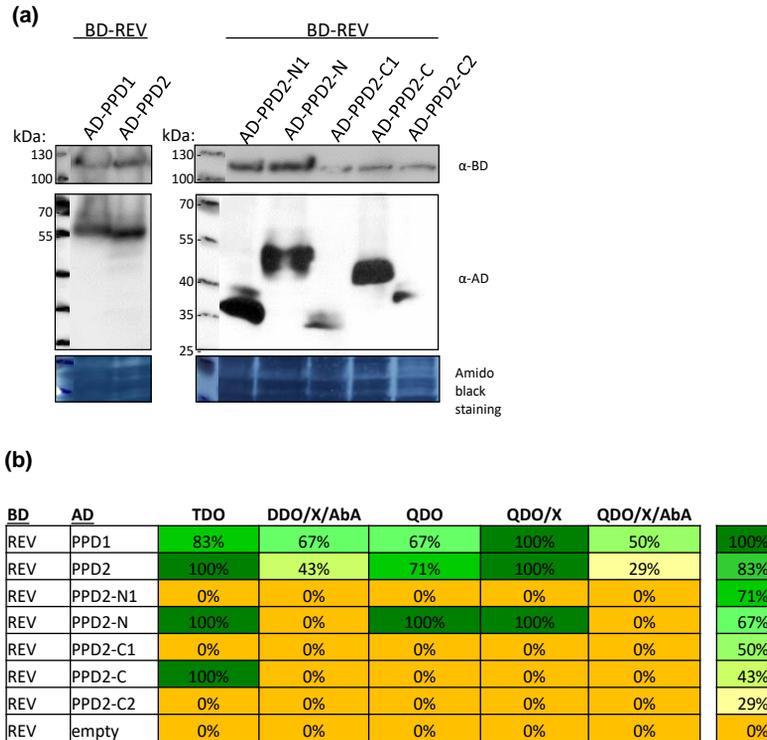


Figure S10: Yeast two-hybrid protein expression in yeast cells and heat map of positive results in independent mating experiments.

(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.

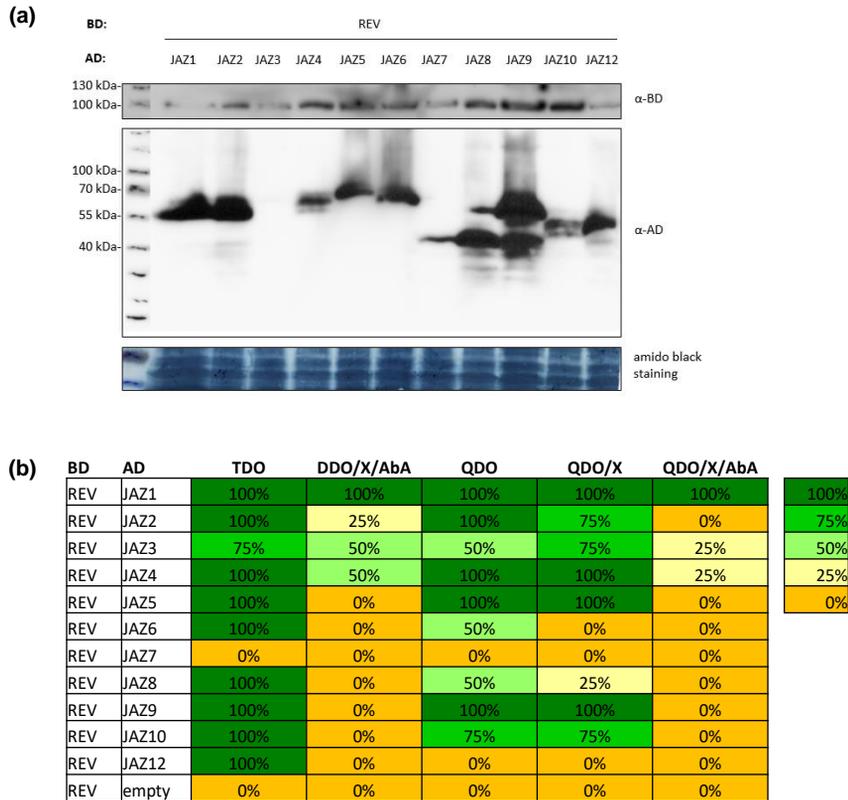


Figure S11: Yeast two-hybrid protein expression in yeast cells and heat map of positive results in independent mating experiments.

(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.

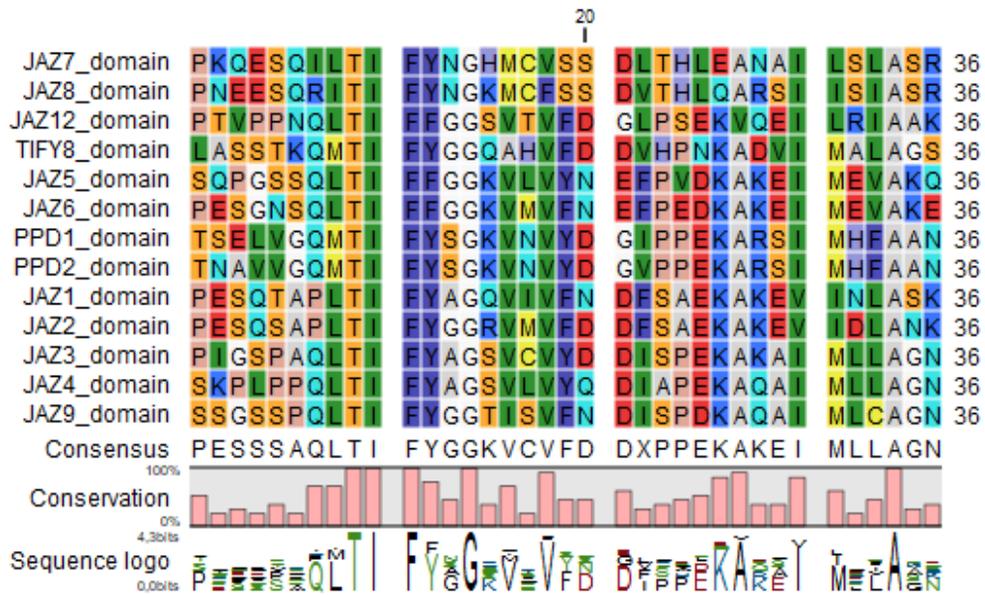


Figure S12: Alignment of all TIFY/JAZ domains of the class two TIFY proteins.