



Figures and figure supplements

Direct ETTIN-auxin interaction controls chromatin states in gynoecium development

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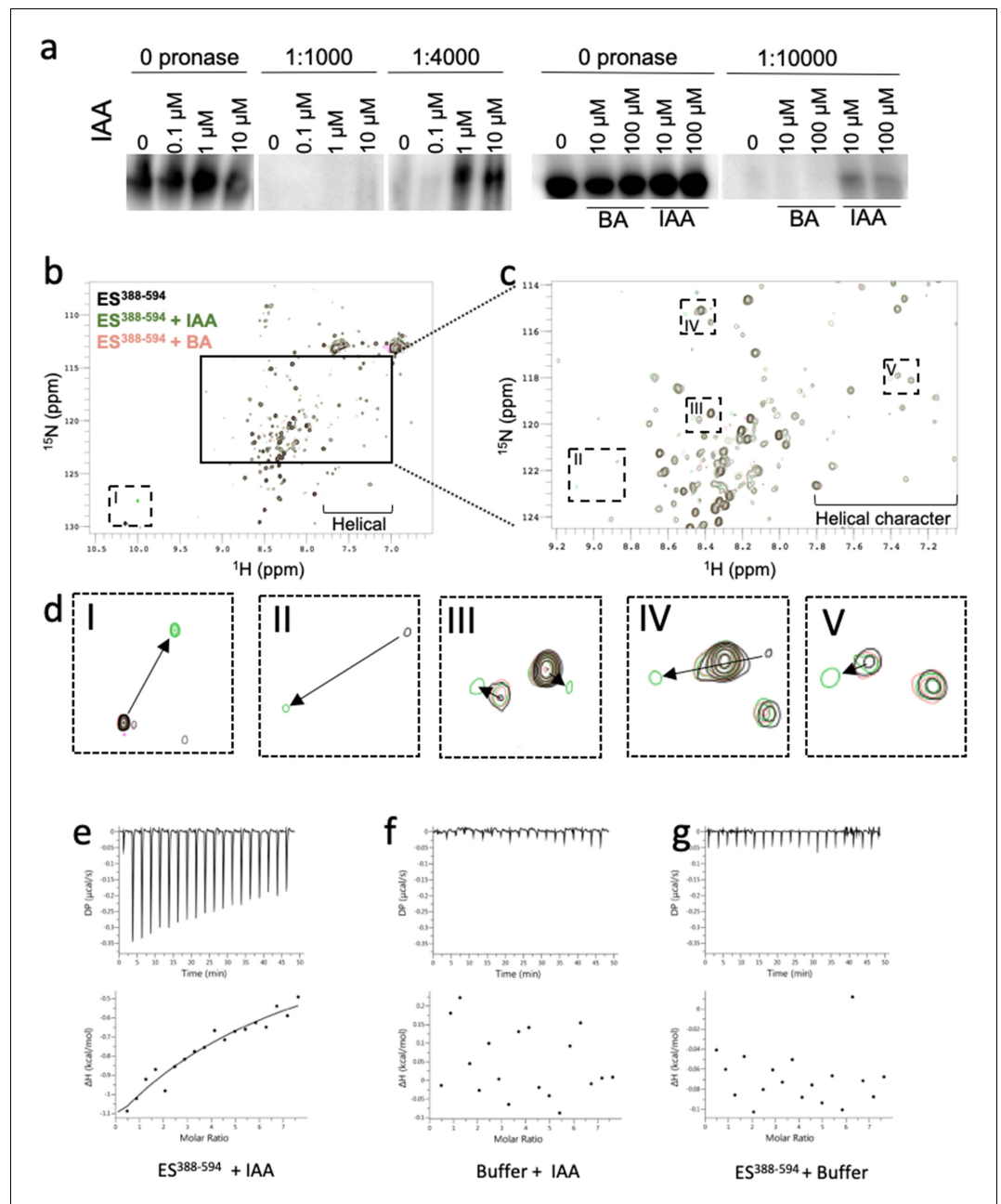


Figure 1. ETT directly binds auxin (IAA). (a) DARTS assay suggests that ETT binds IAA. Seedlings from an inducible two-component ETT-FLAG line were used for the protein isolation. Samples were treated with DMSO (mock), IAA and BA and digested by different concentrations of Pronase. Samples were further analysed by western blot with an anti-FLAG antibody. (b) HSQC-NMR performed with ES³⁸⁸⁻⁵⁹⁴ protein either alone (black), with indole-3-acetic acid (IAA, green) or benzoic acid (BA, pink). ES³⁸⁸⁻⁵⁹⁴: ligand, 1:10, 50 μ M:500 μ M. (c) Zoom-in of the indicated rectangular region in a. (d) Zoom-in of the specific shifts (labelled I-V) in the indicated dotted rectangles in b and c. Changes in chemical shifts are indicated by arrows from control to IAA treatment. (e–g) ITC spectra showing heat exchange between ES³⁸⁸⁻⁵⁹⁴ protein and IAA (e), but not in controls (f, g). See **Figure 1—figure supplement 1** for parameters used in the HSQC-NMR experiment.

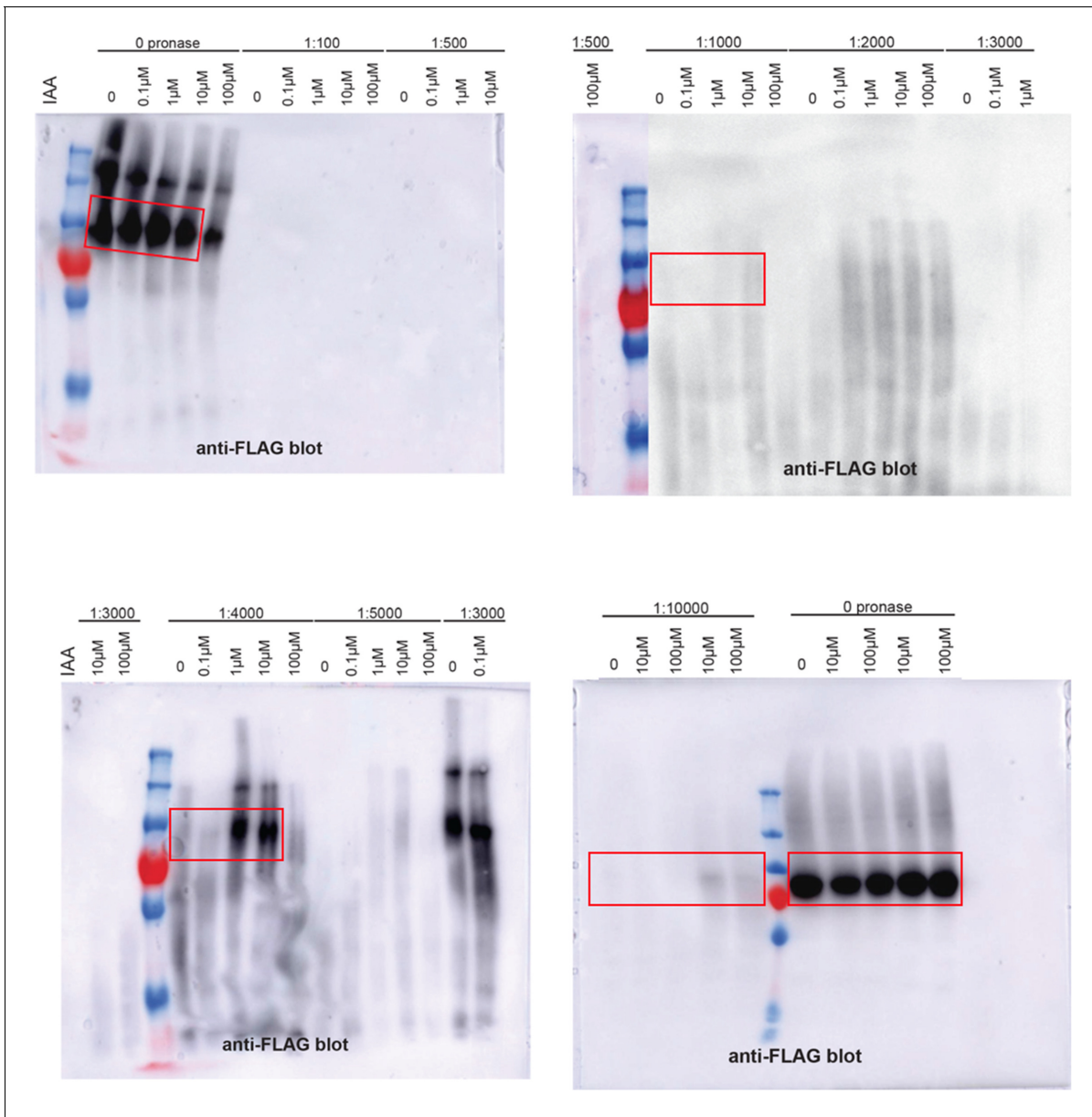


Figure 1—figure supplement 1. Original western blot images for DARTS assays. The red boxes indicate the regions used in *Figure 1a*.

Experiment	Recycling Delays (S)	Scans	Nuclei		Spectral width (Hz)		Number of complex points	
			t1	t2	t1	t2	t1	t2
HSQC	1		56^{15}N	^1H	2888.6	15243.9	256	2048

Figure 1—figure supplement 2. Parameters for HSQC NMR experiment.

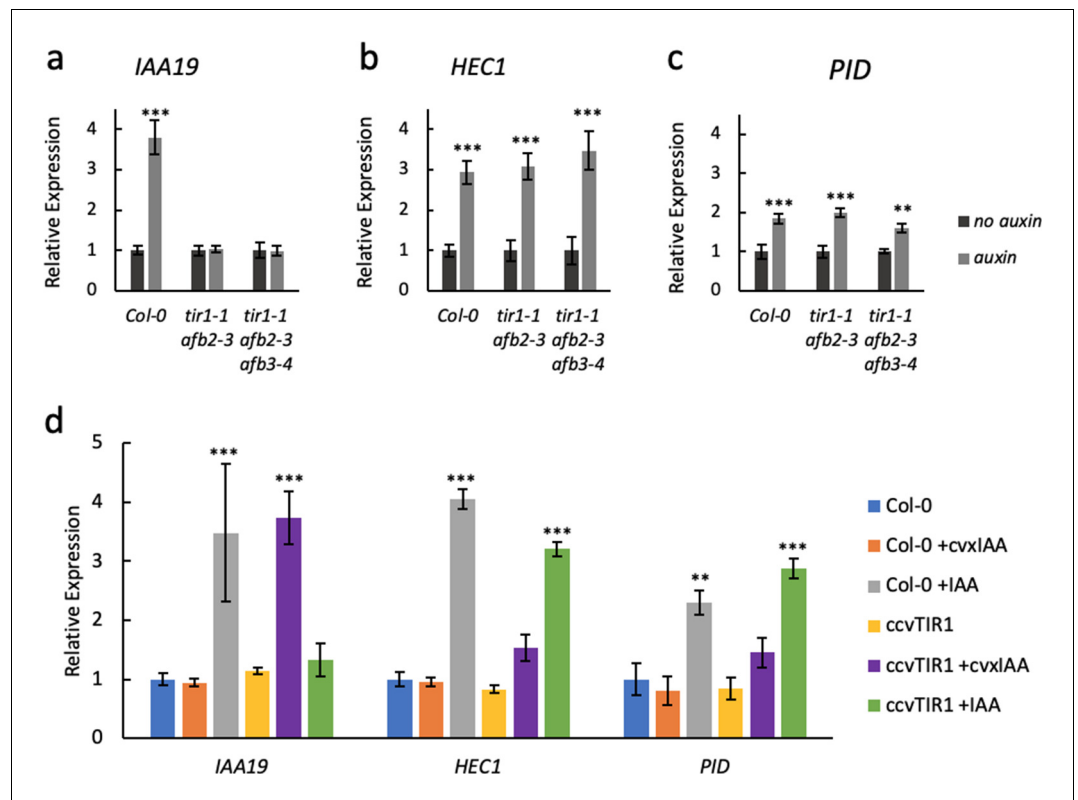


Figure 2. ETT regulates target gene expression independently of TIR1/AFB auxin receptors. Expression of the canonical auxin responsive *IAA19* gene (a) and the ETT-target genes *HEC1* (b) and *PID* (c) in control-treated (dark grey) or 100 μ M IAA-treated (light grey) gynoecia assayed using qRT-PCR. (a) *IAA19* expression is up-regulated in response to auxin in wild-type gynoecia (*Col-0*) but not in *tir1/afb* double and triple mutants. The ETT-target genes *HEC1* and *PID* are up-regulated in response to auxin in both wild-type and auxin receptor mutants (b c). This suggests a TIR1/AFB independent regulation of these genes. (d) Expression of *IAA19*, *HEC1* and *PID* in response to treatment with 100 μ M IAA and 100 μ M cvxIAA in wild-type (*Col-0*) and *pTIR1:ccvTIR1* gynoecia in the *tir1 afb2* double mutant (*ccvTIR1*). The data confirm TIR1/AFB independent regulation of *HEC1* and *PID* in the gynoecium. *** $p < 0.0001$; Shown are mean \pm standard deviation of three biological replicates. See **Figure 2—source data 2** for statistical analyses.

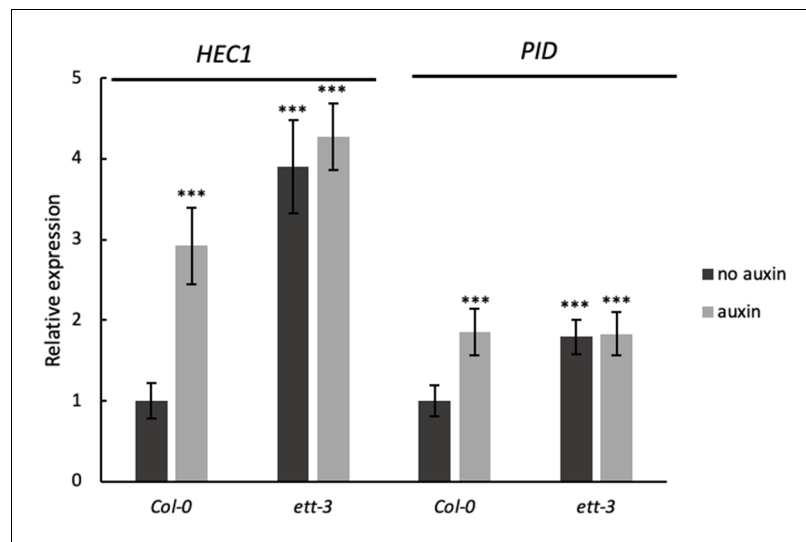


Figure 2—figure supplement 1. Expression of *HEC1* and *PID* in *Col-0* and *ett-3*. In wild-type gynoecia *HEC1* and *PID* are up-regulated upon auxin treatment while both genes are constitutively up-regulated in *ett-3*. Treatment with 100 μ M IAA does not affect *HEC1* and *PID* expression in the *ett-3* mutant suggesting that ETT acts as a transcriptional repressor. Asterisks indicate significant change upon auxin treatment compared to untreated *Col-0* (***) indicating $p < 0.0001$). Shown are mean \pm standard deviation of three biological replicates. See **Figure 2—source data 2** for statistical analyses.

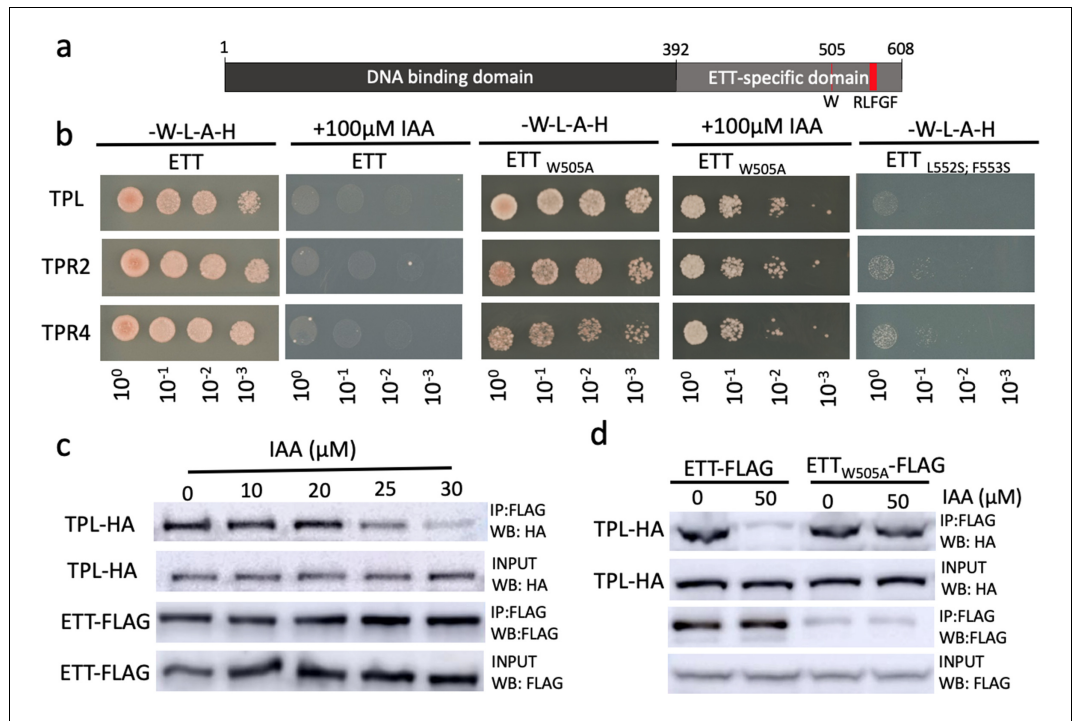


Figure 3. ETT interacts with members of the TPL/TPR co-repressor family in an auxin-sensitive manner. (a) Schematic representation of full-length ETT protein highlighting an EAR-like motif and tryptophan in position 505 (W) in the C-terminal ETT-specific domain. DBD, DNA-binding domain. (b) Y2H showing that ETT interacts with TPL, TPR2 and TPR4. These interactions depend on the identified C-terminal RLFGE motif and are auxin-sensitive. DBD, DNA-binding domain. (c) Co-IP revealing that ETT interacts with TPL in an auxin-sensitive manner with increasing IAA concentrations weakening the interaction. (d) Co-IP showing that mutating the tryptophan in position 505 of ETT (ETT_{W505A}) leads to loss of auxin sensitivity in the interaction between ETT and TPL.

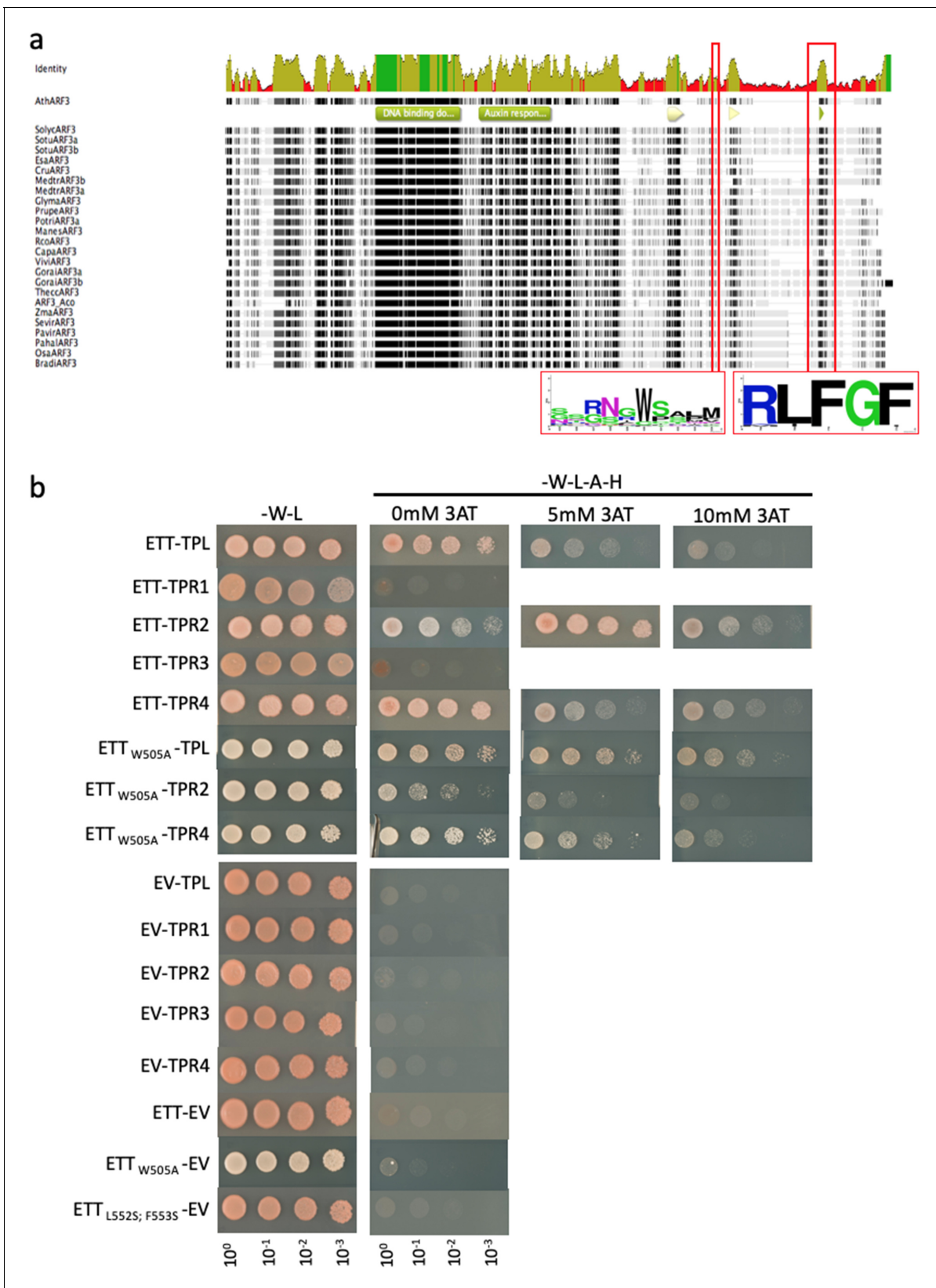


Figure 3—figure supplement 1. ETT can interact with several members of the TPL/TPR co-repressor family through a conserved EAR-like motif. (a) Alignment of ETT protein sequences of 22 species identified a conserved repressive motif (RLFGF) at its C-terminal domain. A logo of the sequence

Figure 3—figure supplement 1 continued on next page

Figure 3—figure supplement 1 continued

surrounding the conserved W505 is also shown. (b) ETT interacts with several members of the TPL/TPR co-repressor family in Y2H. Additionally, controls for **Figure 2** are shown.

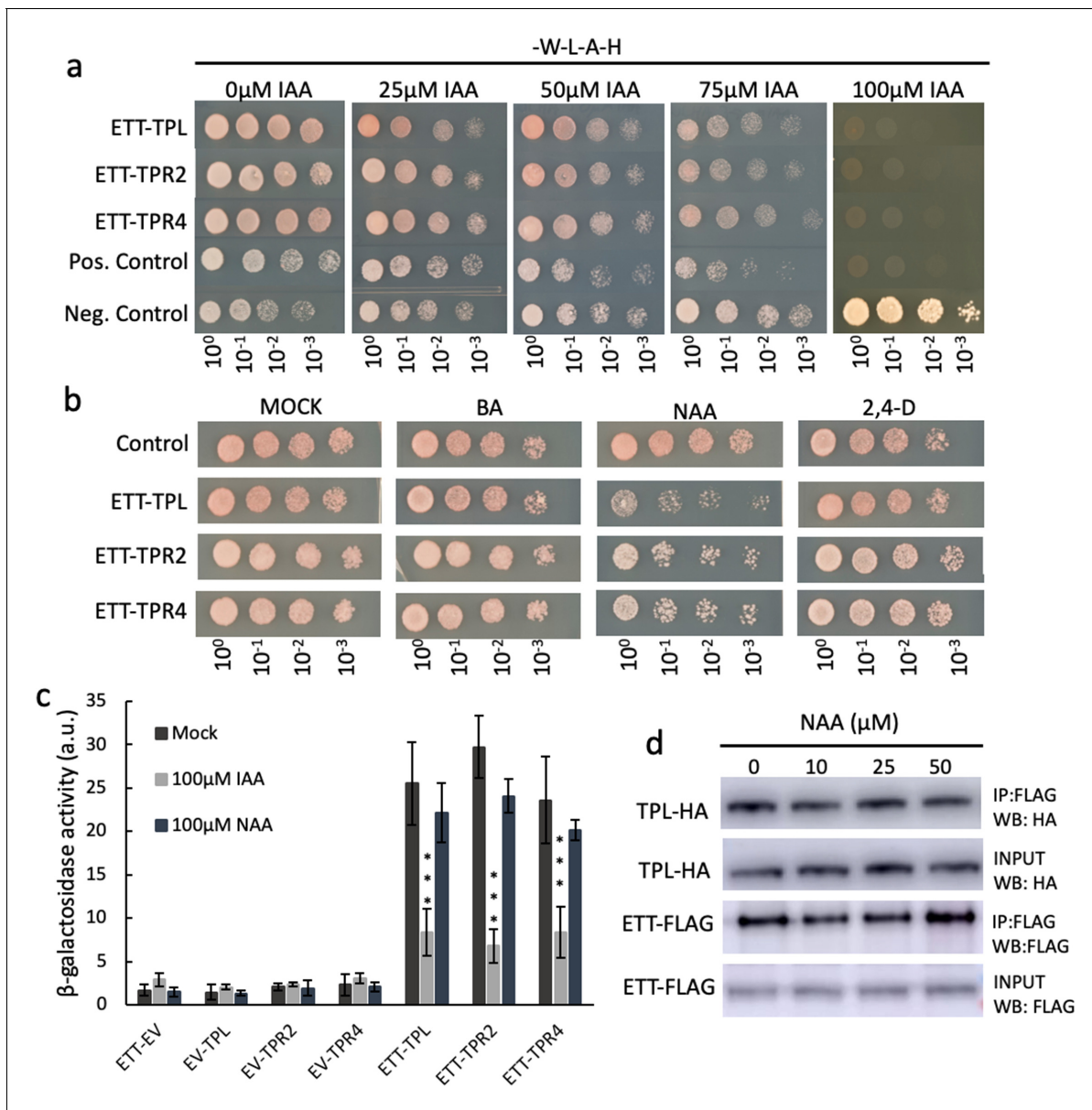


Figure 3—figure supplement 2. Interaction between ETT and TPL, TPR2 and TPR4 is auxin-sensitive and specific to IAA. (a) In Y2H increasing concentrations of IAA lead to reduction of yeast growth abolishing the interaction between ETT and its partners. The interactions are, therefore, auxin-sensitive. (b) Y2H to test specificity of auxin-sensitivity using benzoic acid (BA), NAA, and 2,4D in a yeast growth assay. The data suggest that the auxin-sensitivity observed in (a) is IAA-specific. (c) Y2H-based ONPG assay quantifying β -galactosidase activity as a measure of interaction strength. (d) Co-IP experiments show that the interaction between ETT and TPL cannot be disrupted by the synthetic auxin, NAA. The data support that the ETT TPL/TPR interactions are sensitive to IAA but not to NAA. *** $p < 0.0001$; Shown are mean \pm standard deviation of three biological replicates. See **Figure 3—source data 1** for statistical analyses.

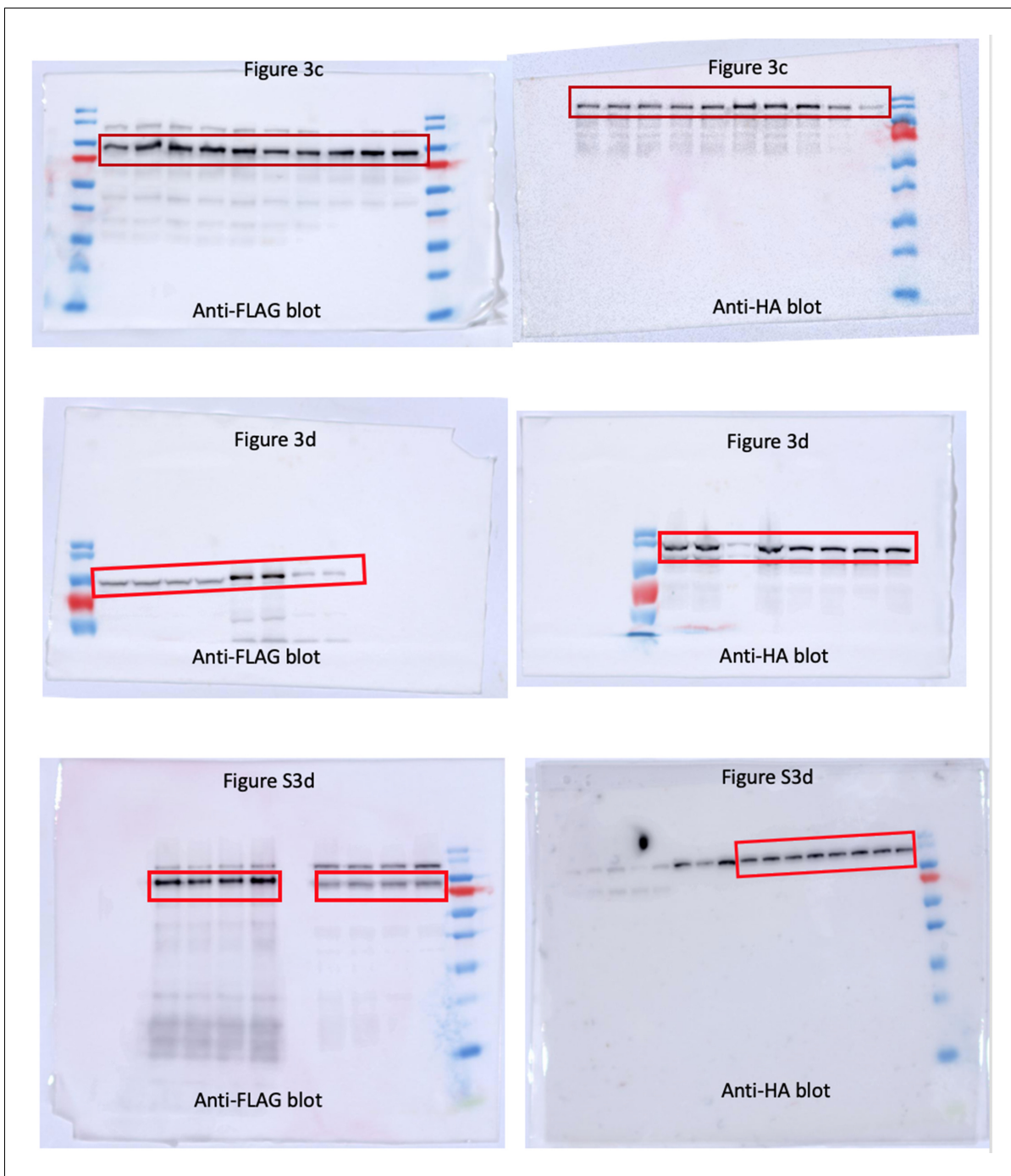


Figure 3—figure supplement 3. Original western blot images for Co-immunoprecipitation assays. The red boxes indicate the regions used in *Figure 3c,d* and *Figure 3—figure supplement 2d*.

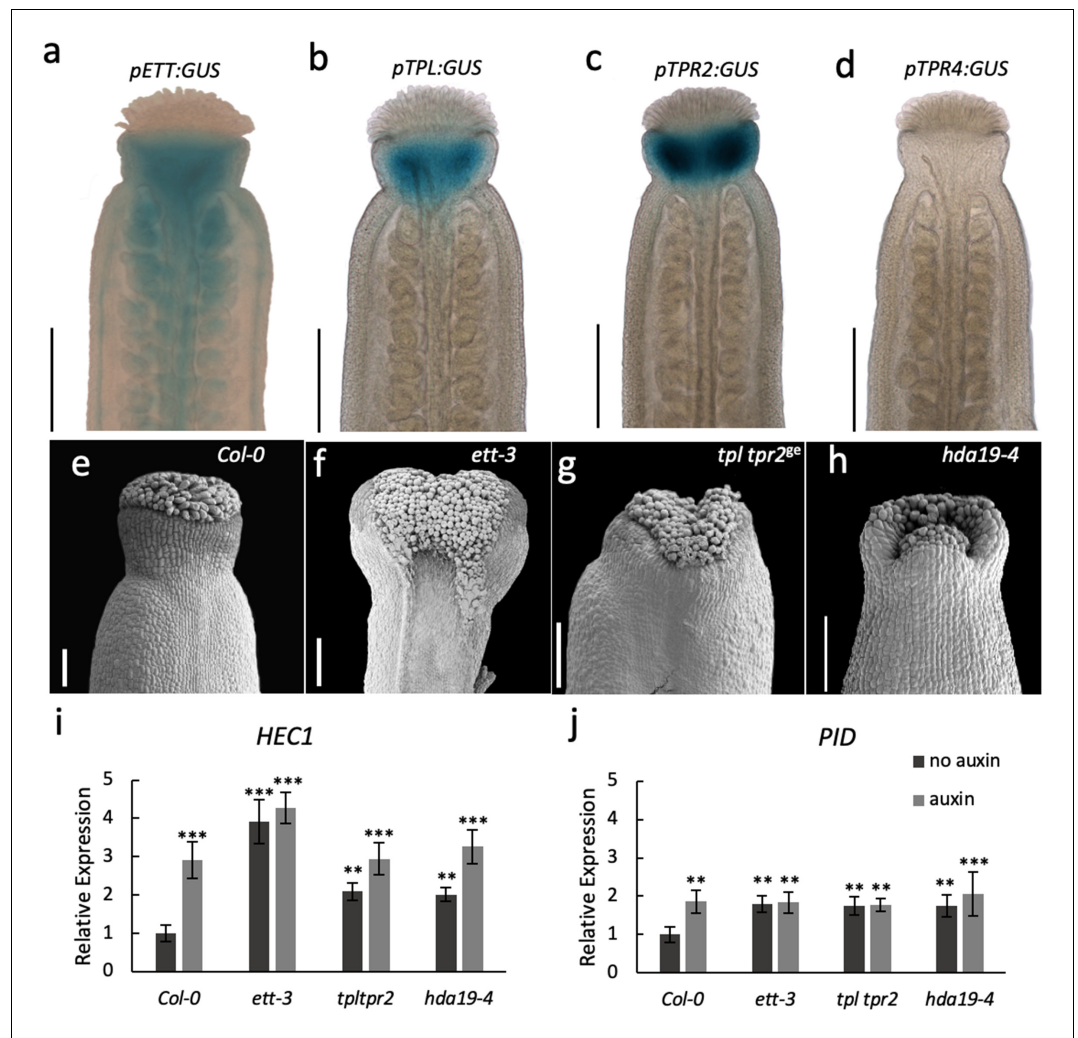


Figure 4. ETT, TPL/TPR2 and HDA19 co-operatively regulate gene expression to facilitate gynoecium development. (a–d) Promoter GUS expression analysis of *pETT:GUS* (a), *pTPL:GUS* (b), *pTPR2:GUS* (c) and *pTPR4:GUS* (d) revealed that *ETT*, *TPL* and *TPR2* but not *TPR4* are co-expressed in the Arabidopsis style. Scale bar = 300 μ m. (e–h) Gynoecium phenotypes of wild-type (e), *ett-3* (f) *tpl tpr2^{ge}* (g) and *hda19-4* (h). Scale bar = 100 μ m. (i j) *HEC1* (i) and *PID* (j) are constitutively mis-regulated in *ett-3*, *tpl tpr2^{ge}* and *hda19-4* gynoecia. This misregulation is unaffected by treatment with 100 μ M IAA. ***p-values<0.0001; Shown are mean \pm standard deviation of three biological replicates. Differences between untreated and IAA-treated mutants are not significant. See **Figure 4—source data 1** for statistical analyses.

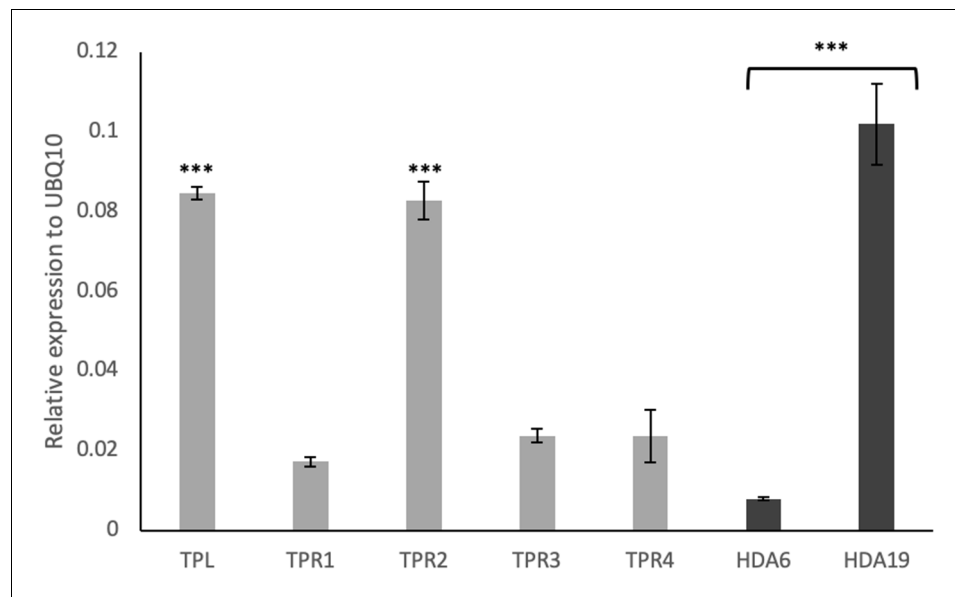


Figure 4—figure supplement 1. Expression of *TPL*, *TPRs* and *HDAs* genes in the gynoecium. Expression analysis using qRT-PCR in wild-type gynoecia showed that *TPL* and *TPR2* are more strongly expressed than *TPR1,3* and *4*. Likewise, *HDA19* exhibits higher expression compared to *HDA6*. ***p-values<0.0001; Shown are mean \pm standard deviation of three biological replicates. See **Figure 4—source data 1** for statistical analyses.

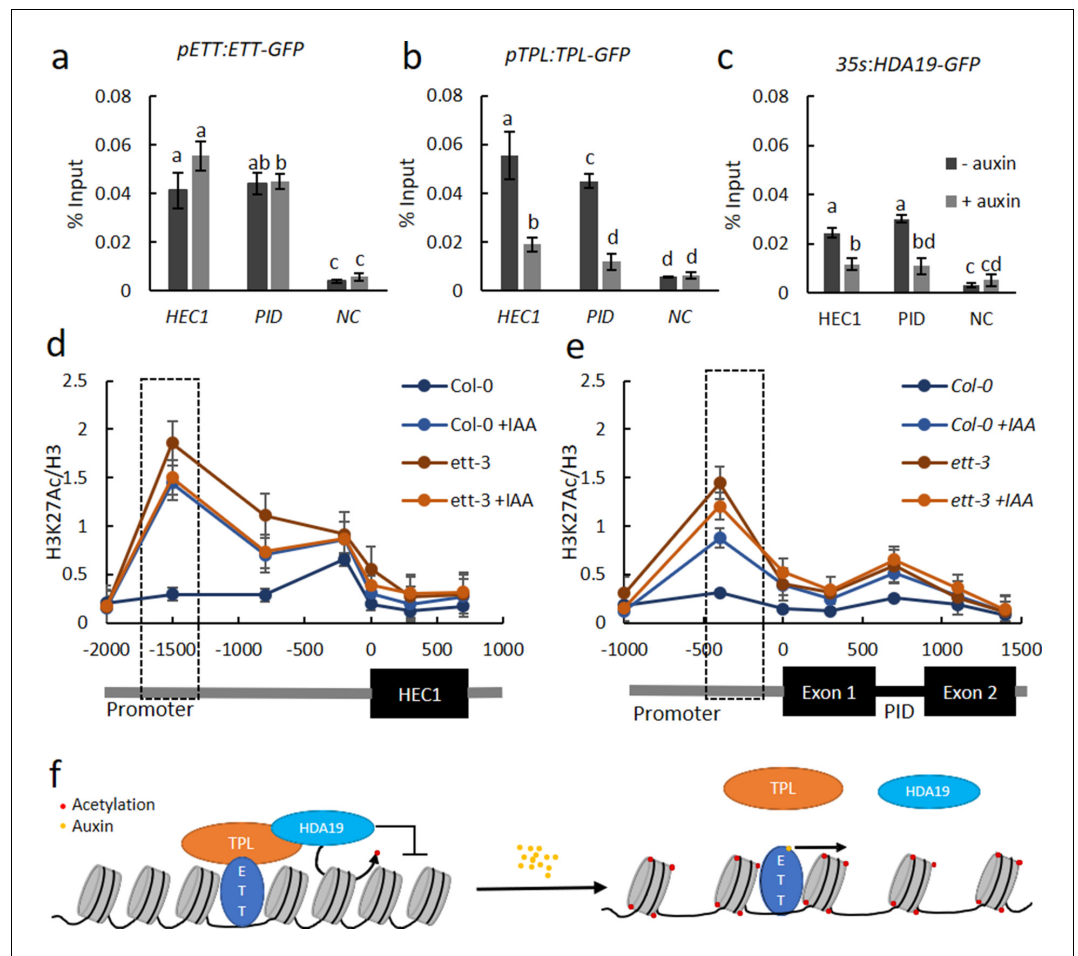


Figure 5. ETT, TPL and HDA19 co-operatively regulate *HEC1* and *PID* by modulating chromatin acetylation. (a–c) Chromatin immunoprecipitation (ChIP) shows ETT (a), TPL (b) and HDA19 (c) binding to conserved regions of *HEC1* and *PID* loci in the absence of IAA (dark grey columns). In the presence of IAA (light grey columns), ETT association is unchanged, while both TPL and HDA19 interactions are reduced. A *WUS* gene fragment served as negative control (NC). (d e) H3K27ac accumulation (from ChIP analysis) along the *HEC1* (d) and *PID* (e) loci in wild-type (*Col-0*) and *ett-3* plants ± treatment with 100 μM IAA. Numbers on the x axes are distances to the Transcription Start Site (TSS). The schematic of the loci is shown below each panel. Dashed boxes represent ETT binding regions. (f) Qualitative schematic illustration of alternative TIR1/AFB independent auxin signaling. Under low auxin conditions an ETT-TPL-HDA19 complex binds to ETT-target genes keeping their chromatin environments repressed, through de-acetylation. High nuclear auxin concentrations abolish the ETT-TPL-HDA19 complex through direct ETT-auxin interaction. This leads to an accumulation of histone acetylation and up-regulation of ETT-target genes. Values in a–e) are means ± standard deviation of three biological replicates. See **Figure 5—source data 1** for statistical analyses.