Title

HAIRY MERISTEM proteins regulate the WUSCHEL protein levels in mediating CLAVATA3 expression

Running Title: HAMs regulate the WUS protein

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ABSTRACT: The precise regulation of stem cells in the shoot apical meristems (SAMs) involves the function of the homeodomain transcription factor (TF)-WUSCHEL (WUS). WUS has been shown to move from the site of production-the rib-meristem (RM), into overlaying cells of the central zone (CZ), where it specifies stem cells and also regulates the transcription of *CLAVATA3 (CLV3)*. The secreted signaling peptide CLV3 activates a receptor kinase signaling that restricts *WUS* transcription and also regulates the nuclear gradient of WUS by offsetting nuclear export. WUS has been shown to regulate both *CLV3* levels and spatial activation, restricting its expression to a few cells in the CZ. The HAIRY MERISTEM (HAM), a GRASS-domain class of TFs, expressed in the RM, has been shown to physically interact with WUS and regulate *CLV3* expression. However, the mechanisms by which this interaction regulates *CLV3* expression non-cell autonomously remain unclear. Here we show that HAM function is required for regulating the WUS protein stability, and the *CLV3* expression responds to altered WUS protein levels in *ham* mutants. Thus, HAM proteins non-cell autonomously regulate *CLV3* expression.

INTRODUCTION: A spatiotemporal regulation of gene expression and growth patterns is critical for proper development across all multicellular organisms. In flowering plants, the shoot apical meristems (SAMs), located at the growing tip, harbor a set of stem cells that remain proliferative to provide cells for the development of all above-ground organs (Barton 2010). The SAM is a multilayered structure; the epidermal L1 and sub-epidermal L2 form monolayers. The underlying L3 forms a multilayered structure, which is also referred to as the corpus. Precise spatiotemporal control of gene expression sub-divides the multilayered SAM into functional zones. The stem cells are maintained in the central zone (CZ) located at the tip. The stem cell progeny displaced into the adjacent peripheral zone (PZ) differentiate as leaves or flowers in a specific spatiotemporal sequence. The rib meristem (RM) located beneath the CZ provides cues for stem cell maintenance, and the cells of the RM that are displaced basally differentiate as stem tissue.

In *Arabidopsis* SAMs, WUSCHEL (WUS), a homeodomain transcription factor (TF), is expressed in the RM and has been shown to specify stem cells in the overlying CZ (Mayer et al., 1998, Laux et al., 1996, Yadav et al., 2013). The non-autonomous regulation of stem cell specification requires WUS protein movement from the RM into the overlying cells of the CZ (Yadav et al., 2011). WUS protein accumulates at a higher level at the site of synthesis, the RM, then diffuses into neighboring cells, thus forming a concentration gradient (Mayer et al., 1998, Yadav et al., 2011, Rodriguez et al., 2016) (Fig. 1). WUS represses differentiation-promoting factors in the CZ stem cells (Yadav et al., 2013, Busch et al., 2010). As the stem cell descendants are displaced away from the influence of the WUS gradient, they differentiate into leaves/flowers in the PZ, and cells located beneath the RM differentiate into the stem (Barton 2010). The precise regulation of the WUS gradient is critical for stem cell homeostasis.

WUS protein gradient is controlled by CLAVATA3 (CLV3), a secreted peptide that activates receptor kinase signaling to repress *WUS* transcription and also offset nuclear export to regulate nuclear levels of WUS (Plong et al., 2021, Brand et al., 2000, Clark et al., 1997, Fletcher et al., 1999). WUS regulates *CLV3* transcription by directly binding a set of cis-elements located at the 3' end of the gene. An earlier study found that WUS binds five closely spaced cis-elements (CRM) [high (970), intermediate (997 and 1007), and low affinity (950 and 1060)] in the 3'

CRM of *CLV3* (Perales et al., 2016). Progressively mutating the cis-elements within the CRM leads to a gradual downregulation of *CLV3* in the CZ (lower WUS) and upregulation in the RM (higher WUS), showing that the same cis-elements activate and repress *CLV3* (Perlases et al., 2016). *In vitro* biochemical experiments showed that WUS binds cis-elements as monomers at low concentrations and switches with increasing concentration to a dimeric state. It suggests a concentration-based model where WUS could bind cis-elements as dimers at high WUS levels and monomers at low WUS levels to repress and activate *CLV3* expression, respectively (Rodriguez et al., 2022).

Moreover, increasing the affinity of a cis-element in the CRM represses *CLV3* even at lower WUS concentrations in the CZ (Perales et al., 2016, Rodriguez et al., 2022). These results show that WUS binding to the CRM regulates *CLV3* expression through a WUS concentration-dependent switch. Consistent with the model, a partial depletion of WUS results in *CLV3* upregulation, while a severe depletion and very high WUS levels lead to *CLV3* downregulation (Plong et al., 2021, Perales et al., 2016). Therefore, the precise regulation of the WUS protein gradient is critical to maintaining both the levels and the spatial activation of *CLV3*, which in turn regulates the WUS protein gradient.

The regulation of the WUS protein gradient is controlled by both the intrinsic protein signatures and the extrinsic layer-specific signals. The ethylene-responsive element binding factor-associated amphiphilic repression (EAR-like) domain resembles nuclear export signals (NES), and it has been shown to bind EXPORTIN proteins (Rodriguez et al., 2016, Plong et al., 2021). Mutating the NES led to a higher nuclear accumulation of WUS. In addition, treatment with nuclear export blocker-Leptomycin-B (LEP-B) led to a nuclear enrichment of WUS both in the wild-type and in *clv3* null mutants (Plong et al., 2021). A similar nuclear enrichment of the WUS protein was observed upon treatment with bioactive CLV3 peptide (Plong et al., 2021). These results suggested that CLV3 signaling could offset nuclear export in maintaining the WUS nuclear gradient.

Moreover, the conserved WUS-box domain has been shown to function as a nuclear retention signal (Rodriguez et al., 2016). The same WUS-box is also required for sensing the plant

hormone cytokinin (CK), which is active in the RM, suggesting that the CK acts as a positional signal to maintain higher nuclear levels (Snipes et al., 2018). Thus, the two opposing processes of nuclear export and the nuclear retention regulated by the two extrinsic signals maintain the nucleo-cytoplasmic (N-C) ratio of WUS. The N-C partitioning, in turn, regulates WUS protein diffusion into adjacent cells, thus the spatial gradient of the WUS protein (Plong et al., 2021).

Another class of RM-localized factors, HAIRY MERISTEM (HAM) (Engstrom et al., 2011) /LOST MERISTEM (LOM) (Schulze et al., 2010) class of putative DNA binding factors, has been shown to regulate *CLV3* expression. The double mutants of *lom1;lom2* have been shown to alter SAM structure subtly. The *lom1;lom2;lom3* triple mutants produced an extremely disorganized SAM with multiple tunica layers instead of the two observed in the wild type (Engstrom et al., 2011, Schulze et al., 2010). These defects were also accompanied by the misexpression of the *CLV3* in deeper cell layers, which overlapped with the *WUS* expression domain. Later studies showed that WUS physically interacted with the LOM proteins (Zhou et al., 2015). Among the three family members, LOM1/HAM1 and LOM2/HAM2 are expressed in the RM, and LOM3/HAM3 is expressed in the PZ (Schulze et al., 2010, Zhou et al., 2018). Moreover, the LOM/HAM proteins have been shown to accumulate in the same cells where these genes are expressed (Han et al., 2020). Therefore, how the physical interaction between WUS and HAM protein that occurs outside the CZ non-cell autonomously influences *CLV3* expression and growth patterns remains unclear.

By employing the reporter analysis, hormone-inducible fluorescently labeled WUS protein, and the structure-function analysis, we show that HAM proteins are required for maintaining the WUS protein stability. The low-level WUS accumulation in *ham* mutants leads to higher *CLV*3 activation. The higher affinity WUS-binding *CLV3* cis-element mutant repressed in the wild-type background is reactivated in the *ham* mutant background, showing the switch from repression to activation at lower WUS levels. Deleting the HAM binding domain destabilizes the WUS protein while increasing its diffusivity. However, the lower WUS is sufficient to rescue the SAM growth defect in the *wus* mutant phenotype and activate *CLV3,* showing that WUS could function at undetectable levels. Taken together, our results showing the requirement of HAM proteins in

regulating the WUS protein gradient provide an explanation for their non-cell autonomous regulation of *CLV3* expression.

MATERIAL AND METHODS:

Genotypes, plant growth, and Plant preparation for microscopy: The *Arabidopsis* plants were grown under continuous light in a plant growth room maintained at 22º Celsius. *ham1-1* and *ham2-1* have been described in earlier studies (Engstrom et al., 2011, Schulze et al., 2010, Zhou et al., 2015). *wus-1* mutant allele has been described in an earlier study (Laux et al., 1996). The *pCLV3::H2b-mYFP and pCLV3 (970M1)::H2b-mYFP* reporters were generated in Landsberg *erecta* (L*er*) background (Perales et al., 2016), and they were introduced into *ham1-1;ham2-1* double mutants through repeated back crossing, and genotyped to select for *ham1-1;ham2-1* double mutants carrying the reporters. *35S::eGFP-WUS-GR* was previously described (Rodriguez et al., 2016) and it was introduced into *ham1-1;ham2-1* mutants. The progeny from stable homozygous lines were treated with either mock or 10uM Dexamethasone (Dex) for 3 hours before imaging. Inflorescence meristems were dissected and planted in a clear plastic box containing solidified agarose. The meristems were stabilized by pouring molten agarose at the base of the stem. The older flower buds were removed, and the meristems were stained with a plasma membrane dye-FM 4-64.

DNA Constructs:

The previously described N'-terminal GFP-WUS translational fusion (Rodriguez et al., 2016) was used to introduce deletions of the HAM-binding domain (203 to 236) by employing appropriately-designed primers (Table S1) and expressed from the *WUS* promoter to create *pWUS::eGFP-WUS(ΔHAM)*. This construct and the wild type *pWUS::eGFP-WUS* were introduced into *wus1-1* mutants. *wus1-1* allele was followed by PCR genotyping as described in earlier studies (Gross-Hardt et al., 2002). The complemented *wus1-1* mutant lines were transformed with *pCLV3::H2b-mYFP* construct to follow the *CLV3* expression pattern.

RT-PCR analysis

For the semi-quantitative RT-PCR analysis, inflorescences of the lines *35S::eGFP-WUS-GR WT*; *35S::eGFP-WUS-GR*;*ham1-1;ham2-1*; *pWUS::eGFP-WUS(WT)* and *pWUS::eGFP-*

WUS(ΔHAM) were collected. RNA was extracted using TRIzol (Invitrogen) and Direct-zol RNA Microprep Kit (Cat. No. R2060), by including the DNAseI treatment, according to the manufacturer's instructions. cDNA was prepared using ProtoScript® II Reverse Transcriptase according to the manufacturer's instructions. The amplification of eGFP gene was done using specific designed oligos 5'- AGAACGGCATCAAGGTGAAC -3' and 5'- CTCAGGTAGTGGTTGTCGGG – 3' in triplicates. PCR product was run in an agarose gel (1.2%) and stained with ethidium bromide. Gel images were taken using the UV GelSolo Gel Documentation System (Analytik Jena). *ACTIN2* gene amplicons were used as a control of the expression levels. Each PCR was done at least two times.

Fluorescent microscopy and image analysis:

Imaging was performed on the Zeiss 880 upright (AIRYSCAN) microscope through a 40X objective lens as previously described (Rodriguez et al., 2022). eGFP-WUS was excited with the 488 nm laser, and the fluorescence emission was collected between 495-550 nm. H2B-mYFP was excited with the 514 nm laser, filtered through a main beam splitter (MBS) 458/514/561/633, and the emission was collected between 495-550 nm. FM4-64 was excited with the 561 nm laser and the emission was collected between 570-620 nm. Images were quantified using ZEN software. The nuclear fluorescence accumulation was quantified for multiple nuclei that were manually selected using a circle tool. The mean nuclear fluorescence was determined from the ten brightest cells in each cell layer. Two-tailed t-tests were applied across genotypes for each cell layer to determine the statistical significance. The source data is presented in the additional data files. This includes the means, N (number of samples), and P values.

RESULTS AND DISCUSSION: To test the function of the RM-localized HAM proteins, we analyzed the *CLV3* expression in *ham1-1;ham2-1* double mutants. The *pCLV3::H2b-mYFP* reporter expression revealed an elevated expression of *CLV3* in the CZ, and its expression domain also extended into the deeper cell layers of the RM (**Fig. 1A-D**). In addition, the double mutant SAMs were slightly flatter and wider than the wild-type SAMs. These results reveal a non-cell autonomous effect of the RM-localized HAM proteins on *CLV3* expression.

WUS has been shown to regulate *CLV3* expression in a non-cell autonomous fashion. Therefore, we analyzed the WUS protein levels in *ham1-1;ham2-1* double mutants. The pWUS::eGFP-WUS reporter failed to accumulate at detectable levels in the SAMs, while the floral meristems (FMs) revealed WUS protein accumulation (**Fig 2A and B**). These results suggest that HAM proteins play a role in regulating WUS protein levels either by regulating *WUS* transcription or by regulating the WUS protein stability. In dissecting these two possibilities, we analyzed the expression of the WUS transcriptional reporter-*pWUS::H2b-mYFP* in *ham1-1;ham2-1* double mutants (**Fig. 2C and D**). The WUS promoter was expressed at extremely low levels in the SAMs, which is attributable to elevated levels and misexpression of *CLV3* in the double mutants (**Fig 1C**).

However, the CLV3 negative regulation of the *WUS* transcription does not rule out the possibility of the role of HAM proteins in regulating WUS protein stability post-translationally. To analyze the effect of HAM proteins on the WUS protein, we analyzed the behavior of the WUS protein expressed from the heterologous promoter by using the *35S::eGFP-WUS-GR*, a Dexamethasone (Dex)-inducible form of eGFP-WUS expressed from the ubiquitous promoter, which allows analysis of WUS protein behavior both in the cytoplasm and the nucleus upon Dex-induced nuclear translocation (Rodriguez et al., 2016). The eGFP-WUS-GR accumulated at much lower levels in mock-treated *ham1-1;ham2-1* double mutant SAMs than in the wild-type background (**Fig. 3A and C and Fig. S1**). The 3-hour Dex-treatment led to a strikingly detectable accumulation of eGFP-WUS-GR in the nuclei of wild type SAMs (**Fig. 3B**). However, *ham1-1;ham2-1* double mutants failed to accumulate detectable levels of the eGFP-WUS-GR protein in the nuclei of most cells in the SAM expect for a scattered accumulation in few cells located in the periphery of the SAMs and in the deeper cell layers of the RM (**Fig. 3D**). To test the possible silencing of the *35S::eGFP-WUS-GR* transgene in *ham* double mutants, we performed RT-PCR analysis by using the eGFP specific primers. The results revealed comparable levels of transgene expression both in wild type and *ham1-1;ham2-1* mutant backgrounds (**Fig. S2**). Taken together, these results suggested that HAM proteins regulate both the cytoplasmic and the nuclear levels of the WUS protein.

HAM proteins have been shown to interact with the C-terminal region of the WUS protein, spanning amino acids 203-236 (Zhou et al., 2015). To test the importance of this region in regulating WUS protein levels, we introduced deletions of this region to create *pWUS::eGFP-WUS(ΔHAM)*. The *pWUS::eGFP-WUS(ΔHAM)* failed to accumulate at detectable levels in the outer cell layers of the SAM. At the same time, the deeper cell layers revealed an extremely low level of protein accumulation over an expanded domain (**Fig. 4B** and **D-G**). The RT-PCR analysis by using the eGFP specific primers revealed that the WUS protein lacking the HAM binding domain was expressed at similar levels to that of the wild type WUS protein ruling out the possibility of transgene silencing (Fig. S2). Moreover, *pWUS::eGFP-WUS(ΔHAM)* protein also accumulated at lower levels in *clv3-2* null mutant background (**Fig. 4 I-K** and **I'-K'**) compared to the wild type-*pWUS::eGFP-WUS* (**Fig 4H** and **H'**), ruling out the possibility of the repression of the *WUS* promoter due to the CLV3-mediated negative feedback loop. These results show the importance of the HAM-binding region in stabilizing the WUS protein and possibly restricting its diffusion.

To test the importance of the HAM binding region in the WUS function, we introduced *pWUS::eGFP-WUS(ΔHAM)* and *pWUS::eGFP-WUS* into the *wus-1* null mutant background. Five independent lines were generated for each construct and genotyped for *wus-1* homozygosity. Four lines carrying the *pWUS::eGFP-WUS(ΔHAM)* showed complete rescue and produced leaves and flowers resembling the wild type plants, and one line segregated *wus-1* mutant phenotypes in 4 of the 25 progeny screened. Our analysis showed that the WUS protein lacking the HAM binding domain was able to largely rescue the *wus-1* mutant phenotype. The SAMs of rescued *wus-1* null mutants resembled wild type SAMs (**Fig. 4C**). To test the importance of the HAM binding region in regulating the *CLV3* expression, we transformed rescued homozygous *pWUS::eGFP-WUS(ΔHAM);wus-1* and *pWUS::eGFP-WUS;wus-1*, with the *pCLV3::H2b-mYFP* reporter construct. In *wus-1* SAMs rescued with the *pWUS::eGFP-WUS,* the *CLV3* expression was detected in the CZ (Fig. 4L). Similarly, in the *wus-1* mutants rescued with the *pWUS::eGFP-WUS(ΔHAM)*, the *CLV3* expression was detected in the CZ; however, expression levels and the domain of expression varied widely, which could be grouped into two categories: one characterized by higher levels and a broader domain (**Fig. 4M**), and the other by lower levels (**Fig. 4N**). Notably, in none of these cases was the *CLV3* expression uncoupled from

the CZ similar to the *ham1-1;ham2-1* double mutants (**Fig. 1A-D**) and upon transient depletion of WUS levels as shown in an earlier study (Perales et al., 2016). The variable *CLV3* expression in *pWUS::eGFP-WUS(ΔHAM);wus-1* suggests that *CLV3* expression could be maintained over a broad range of WUS levels and consistent with the earlier work, which showed a higher *CLV3* expression upon partial depletion of WUS (Perales et al., 2016). In contrast, the severe depletion of WUS decreased *CLV3* expression (Perales et al., 2016). These results reiterate the requirement of HAM binding of the WUS protein in non-cell autonomous regulation of the *CLV3* expression in the CZ.

The elevated *CLV3* expression and the expansion of the *CLV3* expression domain observed in *ham1-1;ham2-1* double mutants reveal the non-cell autonomous effect of the HAM proteins on *CLV3* expression, which could be attributed to extremely low levels of the WUS protein accumulation in the double mutants. Such an increase in *CLV3* expression has been observed upon dilution of WUS through RNAi-mediated partial downregulation of WUS (Perales et al., 2016), revealing the concentration-dependent regulation of *CLV3* expression. To further test the WUS-concentration-dependent effect on *CLV3* expression, we analyzed the behavior of higher affinity cis-element mutants of *CLV3* reporters in *ham1-1;ham2-1* double mutants. Earlier work has shown that increasing the affinity of WUS binding to the cis-elements through point mutations-M1 and M4 induced WUS dimerization at much lower WUS concentrations (Perales et al., 2016). Moreover, the introduction of these mutants into the *CLV3* reporter revealed repression of *CLV3* expression, showing WUS concentration-dependent regulation of *CLV3* (**Fig. 1E**). Since WUS accumulated at a much lower level in *ham1;ham2* double mutants, we hypothesized that the higher affinity mutant reporter-*pCLV3 (970M1)::H2b-mYFP* may be reactivated. Consistent with this hypothesis, the *ham1-1;ham2-1* double mutants revealed reactivation of the mutant promoter, including in the outer cell layers of the CZ (**Fig. 1F**). The earlier studies have shown that HAM proteins are not detected in the outermost cell layers of the CZ (Han et al., 2020). Therefore, these results show the non-autonomous function of HAM proteins in regulating *CLV3* expression, likely by controlling the WUS protein stability.

WUS has been shown to activate and repress *CLV3* expression at lower and higher levels, respectively, leading to the maintenance of expression over a window bound by the activation

and repression thresholds (Perales et al., 2016). In this context, the current work reveals that the RM-localized HAM proteins that physically interact with WUS are required to maintain the WUS protein gradient by stabilizing the protein and restricting diffusion. The overall lower WUS protein levels found in *ham1-1;ham2-1* mutants can explain higher levels and expansion of the *CLV3* expression domain. Therefore, providing a mechanism for the non-cell autonomous effect of the HAM protein function in regulating *CLV3* expression in all cell layers, including the outer cell layers where HAM proteins do not accumulate. Moreover, Perales et al., 2016, showed that the *CLV3* promoter can be repressed in the outer cell layers by increasing the WUS binding affinity, which promotes WUS dimerization at lower WUS protein levels, suggesting that HAM proteins may not directly participate in the transcriptional repression of *CLV*3 in the CZ. Our work also shows that the *ham1-1;ham2-1* double mutants maintain SAM organization and continue producing leaves and flowers at extremely low WUS protein levels. An earlier study revealed that *ham1-1;ham2-1* double mutants maintain SAM structure compared to a severe reorganization of the SAM structure observed in the *ham1;ham2;ham3* triple mutants (Schulze et al., 2010). It has been shown that *HAM3* is expressed in the PZ (Schulze et. al., 2010), and the HAM3 protein was found to be localized to the PZ cells (Han et al., 2010). Taken together, these observations also argue for the non-cell autonomous roles of HAM proteins in maintaining the SAM structure and gene expression. Furthermore, the WUS protein, which lacks the HAM binding domain, accumulates at lower levels, can rescue the WUS null mutants and produce a functional SAM. These results show that SAM growth can be maintained over a wide range of WUS concentrations similar to the *CLV3* expression. Future studies to understand the underlying WUS concentration-mediated growth regulation may provide insights into the robustness associated with stem cell maintenance.

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DATA AND MATERIAL AVAILABILITY: Data sharing is not applicable to this article as all new created data is already contained within this article.

SUPPORTING INFORMATION: A separate file has been uploaded.

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Figures and legends:

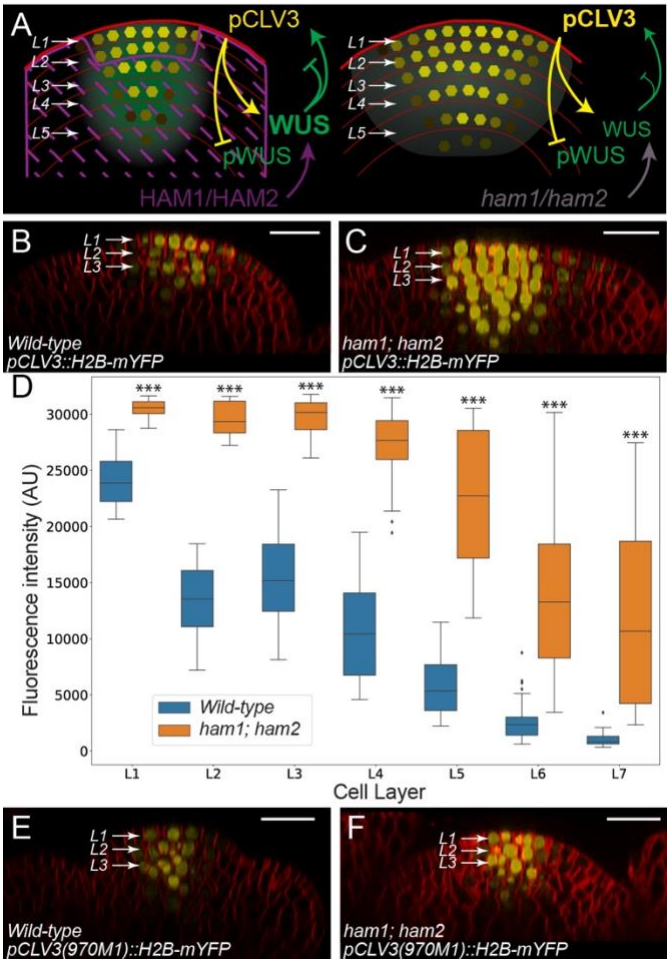

Fig. 1. HAM1 and HAM2 are critical to maintaining levels and spatial *CLV3* expression in the central zone. (**A**) A schematic showing the *CLV3* expression pattern in the wild-type (left) and *ham1-1;ham2-1* double mutants (right). In the wild-type background, the HAM1 and HAM2 proteins (purple) help maintain the WUS protein gradient (green). WUS at lower levels activates *CLV3* (*pCLV3::H2b-mYFP*) [yellow] in the outer layers and, at higher levels, represses *CLV3* in the inner layers. In the *ham1-1; ham2-1* homozygous background (grey), the WUS protein accumulates at much lower levels and possibly over a larger spatial domain, leading to higher activation of *CLV3* across all cell layers. Side views of the SAMs showing *pCLV3::H2b-mYFP* expression [yellow] in the *wild-type* (**B**) and *ham1-1; ham2-1* homozygous mutants (**C**). (**D**) Quantification of the *pCLV3::H2b-mYFP* fluorescence from 10 cells per layer from 4 independent SAMs of wild-type and *ham1-1; ham2-1* homozygous mutants. The central

box depicts the range of the central 50% of the data, the central line represents the median, and the extended lines represent the rest of the data, excluding outliers shown a black dots. $P < 0.001$ ***. *pCLV3::H2b-mYFP* expression in *CLAVATA3 promoter* containing a higher affinity WUS binding cis-element (970M1) in wild-type (**E**) and in *ham1-1; ham2-1* homozygous mutants (**F**). In all images, FM-4-64 stained plasma-membrane [red]. In all images, the scale bar = 20 μm.

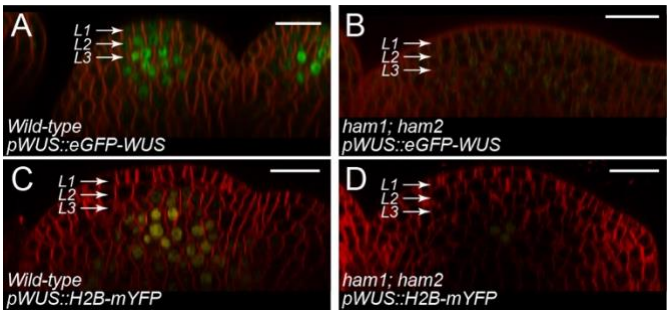

Fig. 2. HAM1 and HAM2 are critical to maintaining proper WUS levels. Side views of the *wild-type* (**A** and **C**) and *ham1-1; ham2-1* homozygous mutants (**B** and **D**). (**A**, **B**) GFP N'terminus- fused to the WUSCHEL protein expressed from the *WUSCHEL promoter* (*pWUS::eGFP-WUS*)[green]. (**C**, **D**) *WUSCHEL promoter* expressing a transcriptional reporter *Histone2b-modifiedYFP* (*pWUS::H2b-mYFP*)[yellow]. In all images, FM-4-64 stained plasma-membrane [red] and scale bar = 20 μm.

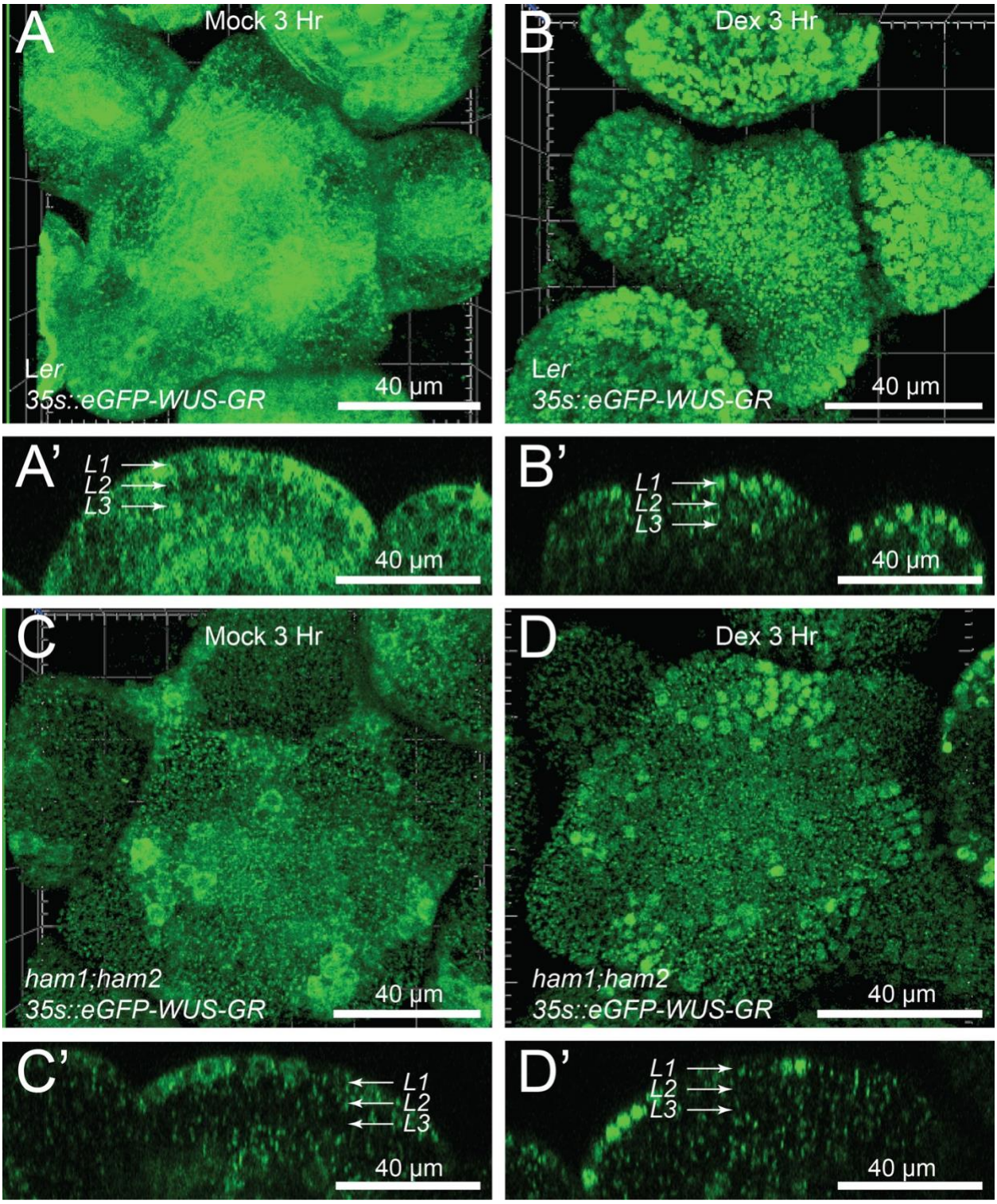

Fig. 3. HAM1 and HAM2 are critical for stable WUS protein accumulation. Top-view 3D reconstruction of SAM containing a translational fusion of GFP, WUS, and Glucocorticoid Receptor expressed from the *35s promoter* (p*35S::eGFP-WUS-GR)* in

wild-type (**A** and **B**) and *ham1-1; ham2-1* homozygous lines (**C** and **D**). Plants were mock-treated (**A** and **C**) or Dex-treated for 3 hours (**B** and **D**). Corresponding side views of the SAM are shown below each 3D reconstruction and labeled as **A'** - **D'**. In all images, the scale bar = 40 μm.

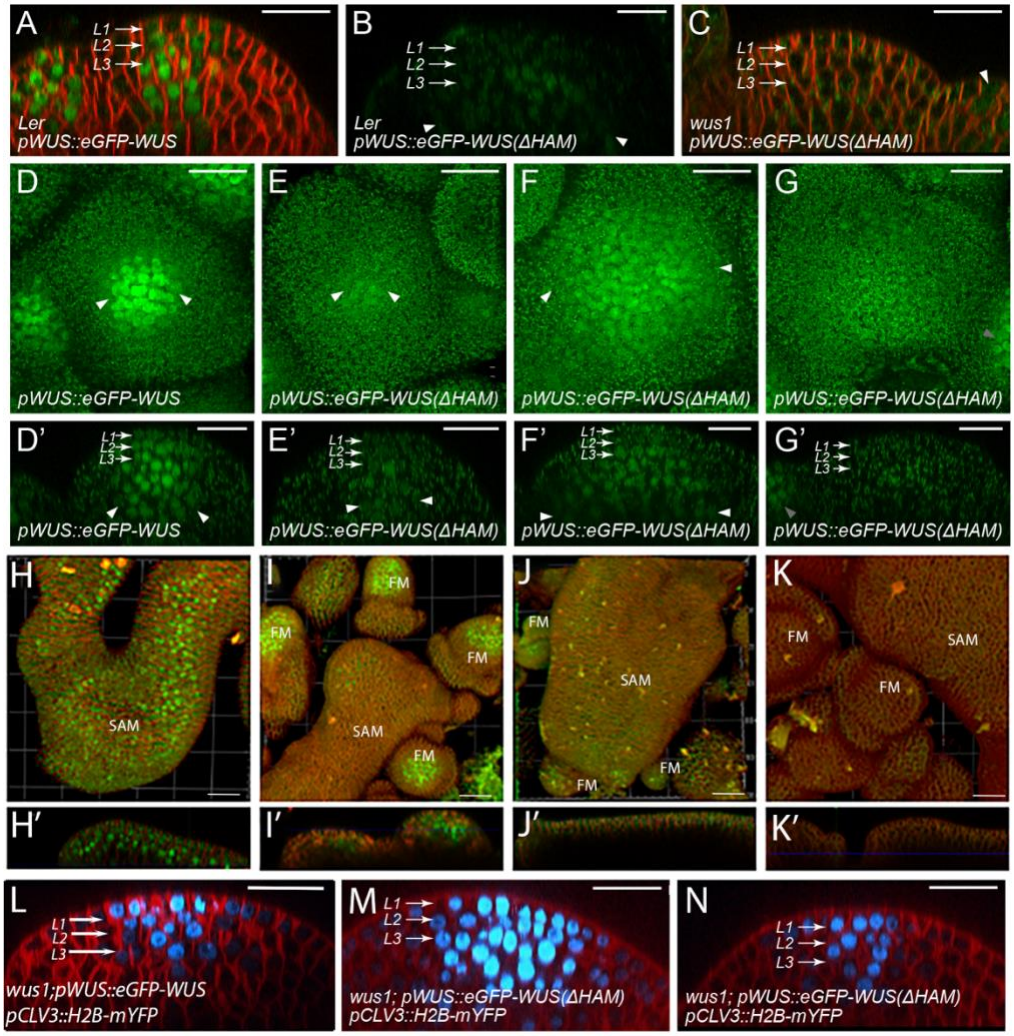

Fig. 4. The HAM binding domain is required to maintain WUS protein stability and *CLV3* expression. (**A**) Side view of the SAM in wild-type background with *WUSCHEL promoter* expressing a GFP N'terminus- fused to the WUSCHEL protein *pWUS::eGFP-WUS* [green]. Side view of the SAM in wild-type (**B**) and *wus1-1* (**C**) mutant background complemented with *WUSCHEL promoter* expressing a GFP N'terminus- fused to the WUSCHEL protein containing a deletion of amino acids (203 aa to 236 aa) [HAM-binding domain] *pWUS::eGFP-WUS(ΔHAM)* [green] (**B** and **C**). (**B**) In the wild-type background, WUS(ΔHAM) accumulates in a broader domain in the deeper layers of the SAM [arrowheads]. (**C**) WUS protein fails to accumulate visible levels in SAMs. However, WUS protein is visible in the floral meristem (FM) [arrowhead]. (**D** - **G**) 3D reconstructions of wild type SAMs showing the *WUSCHEL promoter* driving a eGFP-

WUS protein *pWUS::eGFP-WUS* [green] (**D**) and eGFP-WUS carrying deletions in the HAM binding domain *pWUS::eGFP-WUS(ΔHAM)* (**E-G**). (**E-G**) Shows images from three independent transgenic lines. In all images, white arrowheads denote the outer limits of the WUS protein accumulation in space, and gray arrowhead denotes the WUS protein in the floral meristem. (**D- G**) Images were acquired at a higher digital gain to highlight low WUS protein accumulation. (**D'-G'**) 3D reconstructed side views of 15 neighboring slices from images presented in (**D-G**). (H-K) 3D reconstructed top views of *clv3-2* shoot apex showing *pWUS::eGFP-WUS* [green] [n=5] (**H**) and three different shoot apex showing *pWUS::eGFP-WUS(ΔHAM)* [green] [n=5] (**I-K**). (**H'-K'**) Corresponding side views of images shown in (**H-K**). In all images, FM-4-64 stained plasma-membrane [red]. Side views of the SAMs showing *pCLV3::H2b-mYFP* expression (pseudo colored in Cyan) in the in *wus1-1* mutants complemented with the wild type WUS (**L**), WUS lacking the HAM-binding domain (**M** and **N**). *CLV3* expression was either higher [Bright, n=12] (**M**) or lower [Dim, n=7] (**N**) than the *wus 1-1* mutants complemented with the wild type WUS [n=5] (**L**). Scale bar = 20 μm for (**A-G**) and (**L-N**). 25 μm for (**H-K**). SAM=Shoot apical meristem. FM=Floral meristem. Arrows point to different cell layers-L1, L2 and L3.

SUPPLEMENTARY MATERIALS:

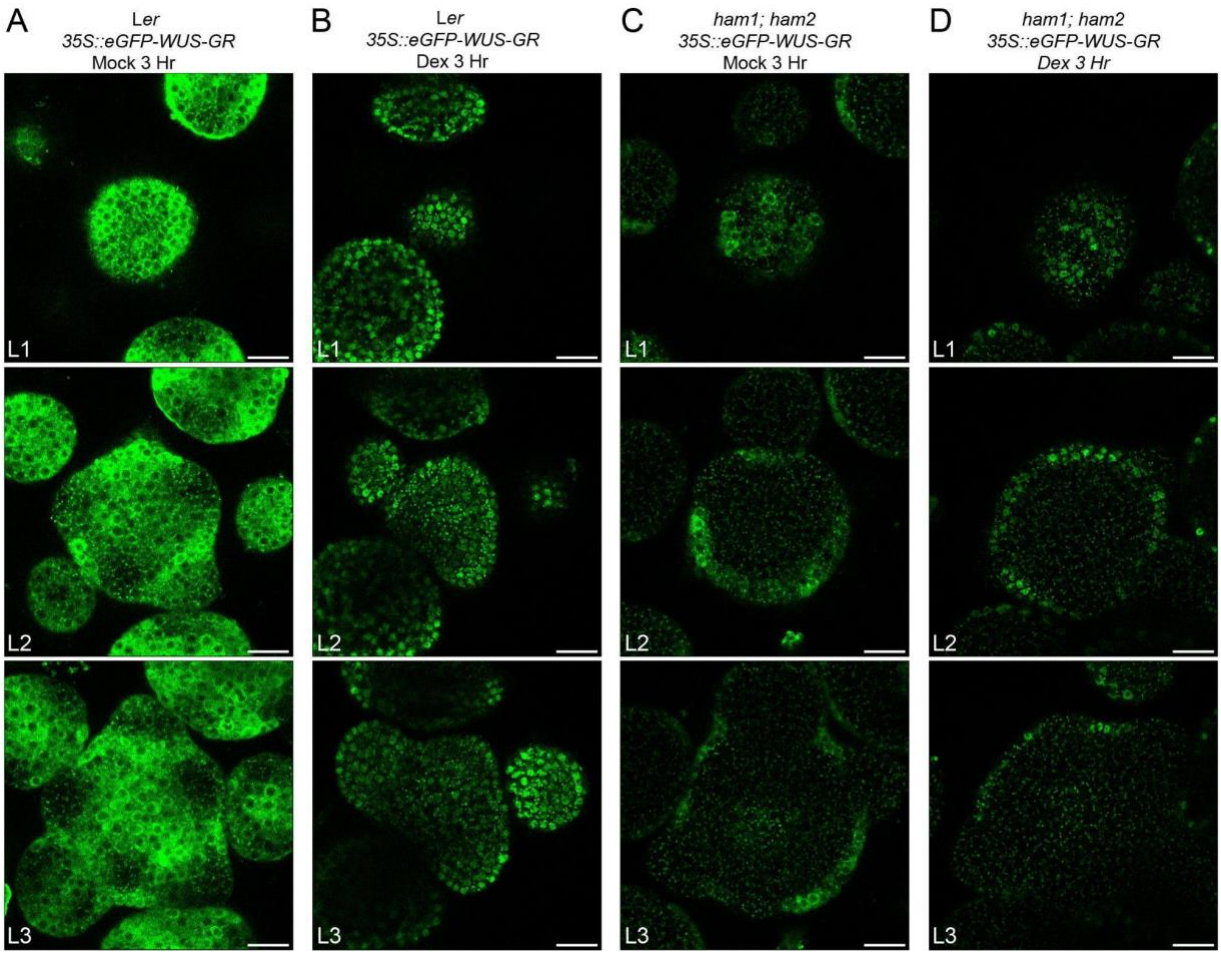

Fig. S1. HAM1 and HAM2 are critical for stable WUS protein accumulation. (**A** - **D**) Transverse sections of the SAMs showing translational fusion of eGFP-WUS with the hormone binding domain of the Glucocorticoid Receptor expressed from the *35s promoter* (p*35S::eGFP-WUS-GR)* [green] in wild-type (**A** and **B**) and *ham1; ham2* homozygous lines (**C** and **D**). Plants were mock-treated (**A** and **C**) or Dex-treated (**B** and **D**) for 3 hours before imaging. Sections corresponding to different cell layers-L1, L2, and L3 are shown. In all images, the scale bar = 20 μm.

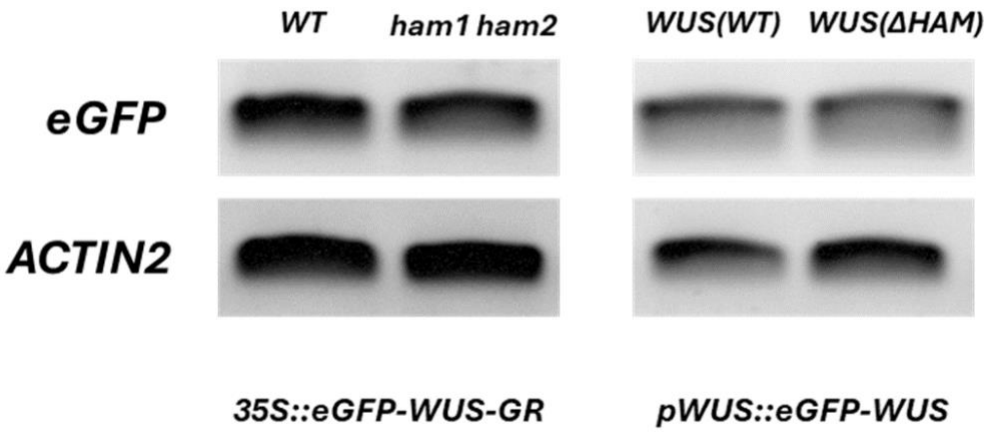

Fig. S2. Amplification of *eGFP* gene by semiquantitative RT-PCR. Samples represent the RNA extracted from inflorescences of the lines *35S::eGFP-WUS-GR* in wild type (*WT)*; *35S::eGFP-WUS-GR* in *ham1-1; ham2-1* [left panel]; *pWUS::eGFP-WUS(WT); pWUS::eGFP-WUS(ΔHAM)* [right panel]. The *ACTIN2* gene amplicon was used as a control.

Table S1. Primers used in this study.

Genotype mutants	Modification	Sequence
HAM1 Fwd		**5'-CAGATTCTCAGAAAATCTGGTCC-3'**
HAM1 Rev		**5'-CGGTGTGGTCGCCGTTGTTGTTTC-3'**
HAM2 Fwd		**5'-GGAGGTCAATGGGCGTCTCTG-3'**
HAM2 Rev		**5'-GGCGCGTCGTTGTTACGGTCG-3'**
***wus1-1* F**		**5' TTGAATTAATGAATTATAGTTTGATACG**
***wus1-1* R**		**5' TTGAAGTTATGGATCTTGATTGG**
dHAM (WUS 237 F)	**5' Phospho**	**5'CATCAAGACGAAGAAGAATGTGGTG**
dHAM (WUS 202 R)	**5' Phospho**	**5' ATTCATAGAACAGTCTTGTTCCATAGATCC**