Figures and figure supplements

The CLAVATA receptor FASCIATED EAR2 responds to distinct CLE peptides by signaling through two downstream effectors

Byoung Il Je *et al*

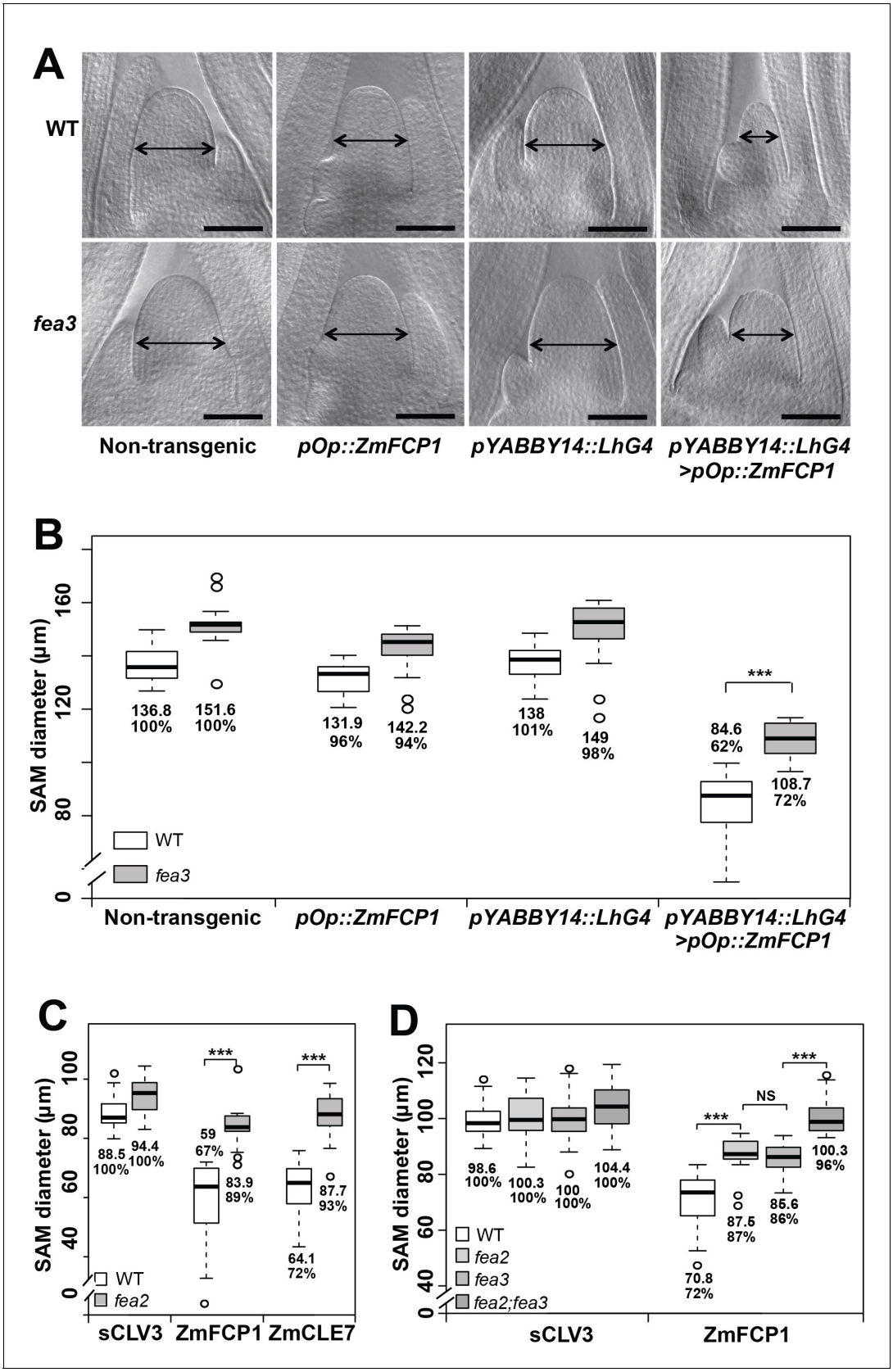

Figure 1. Both *fea3* and *fea2* mutants are resistant to ZmFCP1 peptide. (**A**) Transactivation of ZmFCP1 in primordia using a pYABBY14:LhG4 driver led to a strong reduction in vegetative SAM size as compared to a non-transgenic control, but this effect was only partially rescued in a *fea3* mutant

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background; SAM diameter was quantified (**B**). In CLE peptide treatments, *fea2* mutants were resistant to ZmFCP1, as well as to ZmCLE7 (**C**), and *fea3; fea2* double mutants showed additive resistance to ZmFCP1, restoring SAM size to normal (**D**). Scale bars; 100 μm in A. n = 20 (**B, C**) and 30 (**D**) plants for each genotype. Data in B, C and D are shown by box plots. The mean values as well as the relative % to each untreated control are listed for each genotype. The untreated controls are set to 100%: '***': P value < 0.0001, two-tailed, two-sample t test. 'NS': not significant.

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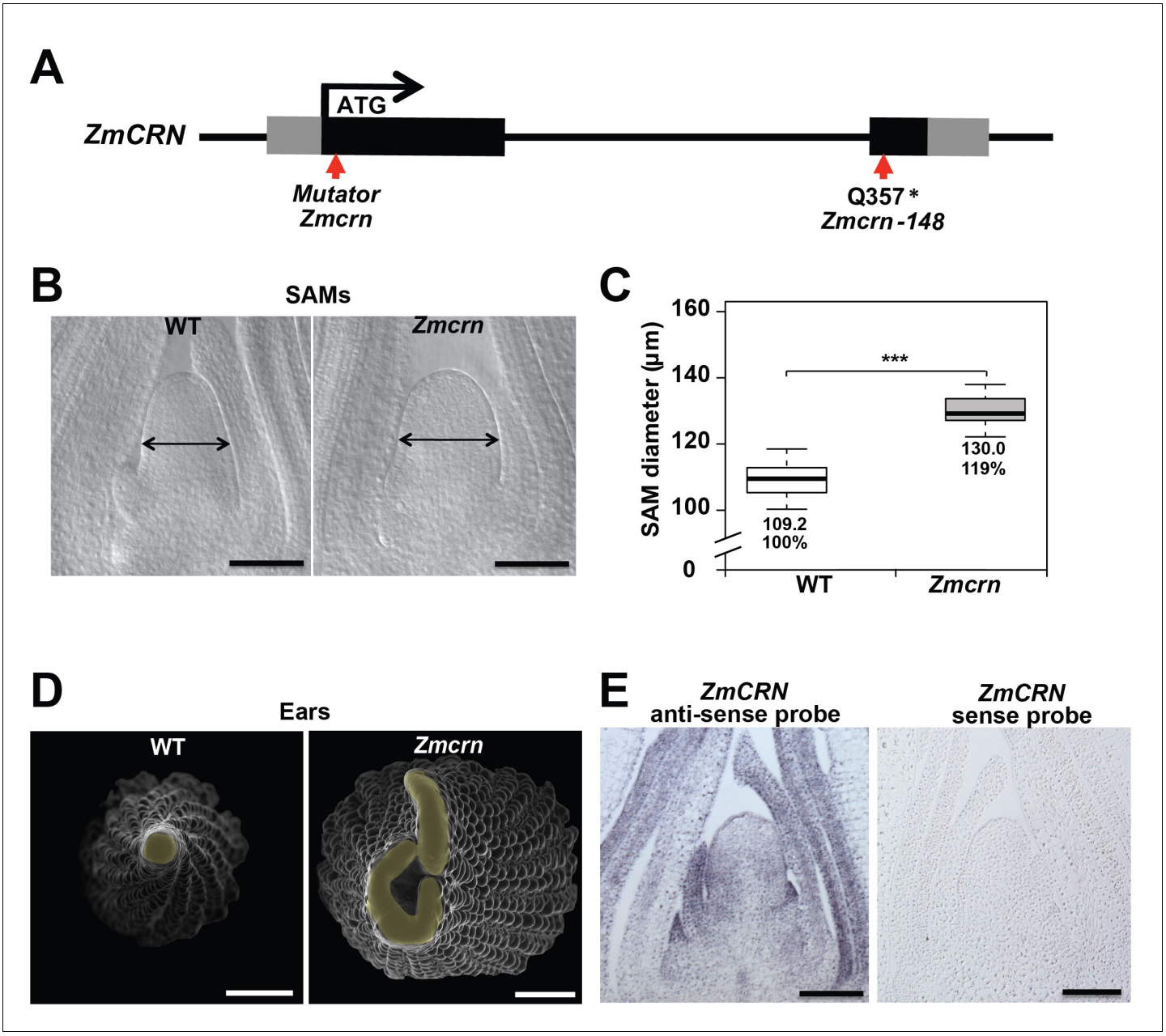

Figure 2. *Zmcrn* mutants develop fasciated ears. (**A**) Schematic of the *Zmcrn* mutant alleles. The arrows indicate the position of the Mutator transposon insertion and premature stop codon mutation. (**B**) Cleared SAMs from wild type (WT) and *Zmcrn* plants. The *Zmcrn* SAM has a larger diameter (double-headed arrows), SAM diameter was quantified (**C**). (**D**) Scanning electron microscopy images of WT and *Zmcrn* ear primordia (inflorescence meristems in yellow). The *Zmcrn* ear shows an enlarged and fasciated inflorescence meristem. (**E**) In-situ hybridization showing *ZmCRN* expression throughout the SAM, with higher expression in peripheral zone and leaf primordia. Scale bar: 100 µm in B and E, 500 µm in D. n = 30 (**C**) plants for each genotype. Data in C are shown by box plots. The mean values as well as the relative % to the WT control are listed. '***': P value < 0.0001, two-tailed, two-sample t test.

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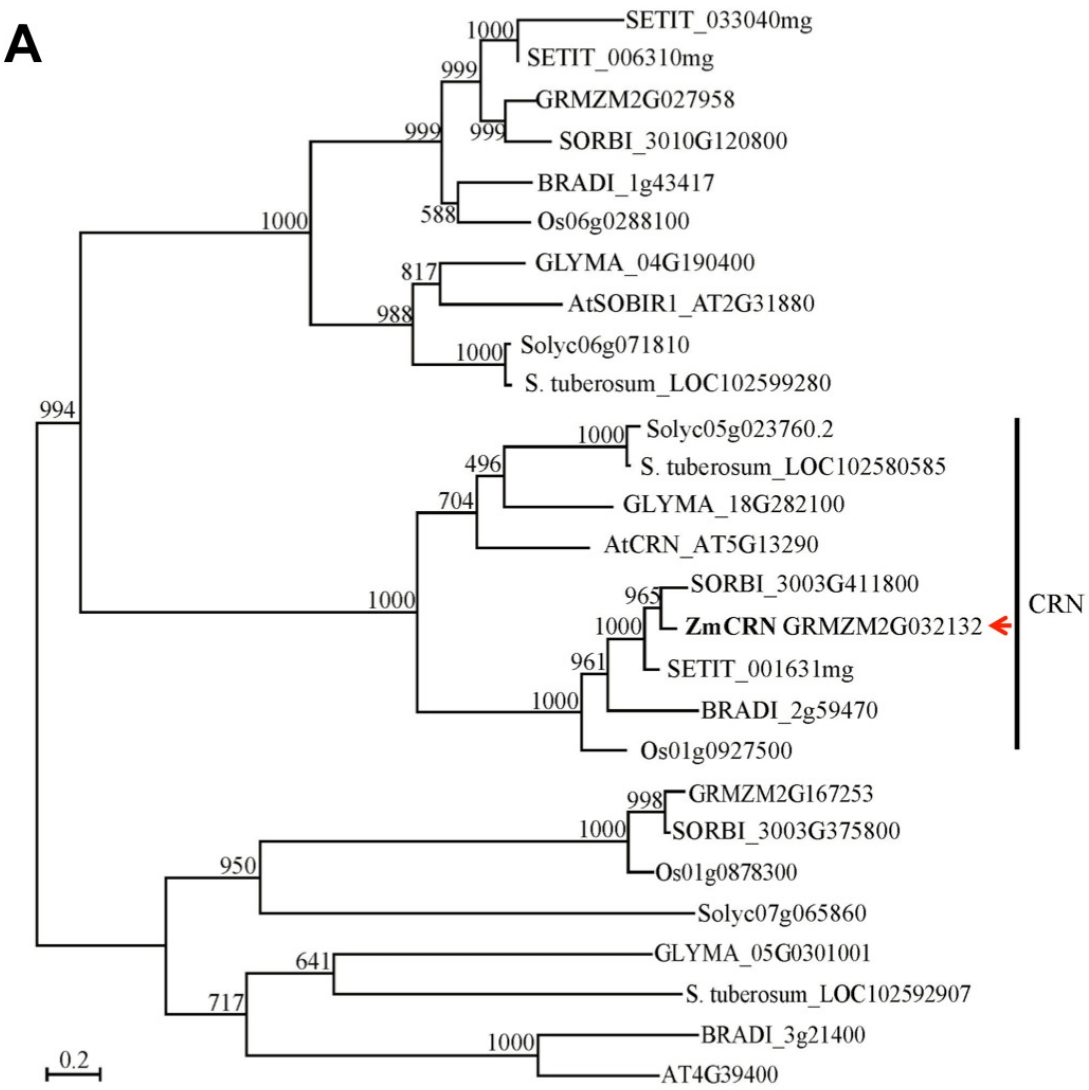

	Consensus of normal kinases	Pseudokinase of AtCRN	Pseudokinase of ZmCRN
G-Loop	GXGXXG	GSDLNG	AHGPHG
Catalytic Asp	HRD	HYN	HCN
DFG motif	DFG	DCG	DCG

Figure 2—figure supplement 1. Phylogeny of CRN related proteins, and ZmCRN features of a pseudokinase. (**A**) The phylogeny shows that the maize genome has one CRN ortholog, which we named ZmCRN (GRMZM2G032132). The top three hits chosen by BLASTP search of AtCRN against the NCBI

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database (www.ncbi.nlm.nih.gov) from nine representative species were aligned by Muscle and the phylogeny was made from PhyML 3.1 with 1000 bootstraps. (**B**) ZmCRN lacks consensus residues necessary for kinase activity (marked green, pink and yellow, top), the table shows the comparison with consensus of active kinases and Arabidopsis CRN (***Boudeau et al., 2006***; ***Nimchuk et al., 2011a***).

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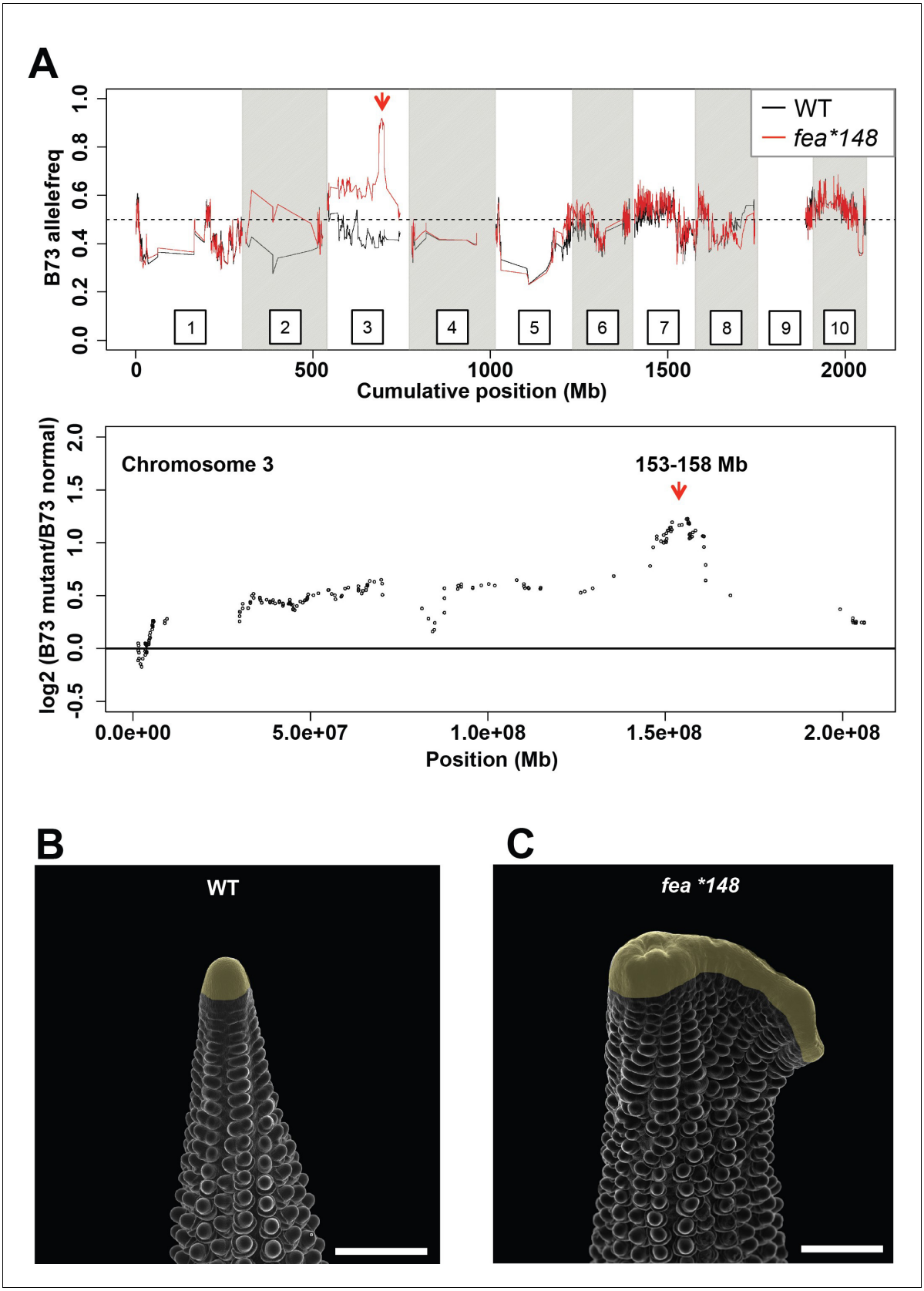
A
B73 allelefreq
0.0
0.2
0.4
0.6
0.8
1.0
WT
fea*148
1
2
3
4
5
6
7
8
9
10
0
500
1000
1500
2000
Cumulative position (Mb)
log2 (B73 mutant/B73 normal)
-0.5
0.0
0.5
1.0
1.5
2.0
Chromosome 3
153-158 Mb
0.0e+00
5.0e+07
1.0e+08
1.5e+08
2.0e+08
Position (Mb)
B
WT
C
fea *148

Figure 2—figure supplement 2. Mapping of the *fea*148* fasciated ear mutant. (**A**) Bulked segregant analysis of *fea*148* using Maize SNP50 chip data delineated a 5 Mb mapping interval surrounding *ZmCRN* on chromosome 3. Scanning electron microscopy images of wild type (**B**) and *Zmcrn-148* (**C**) ear primordia (inflorescence meristems in yellow). The *fea*148* ear shows an enlarged inflorescence meristem. Scale bars: 500 μm.
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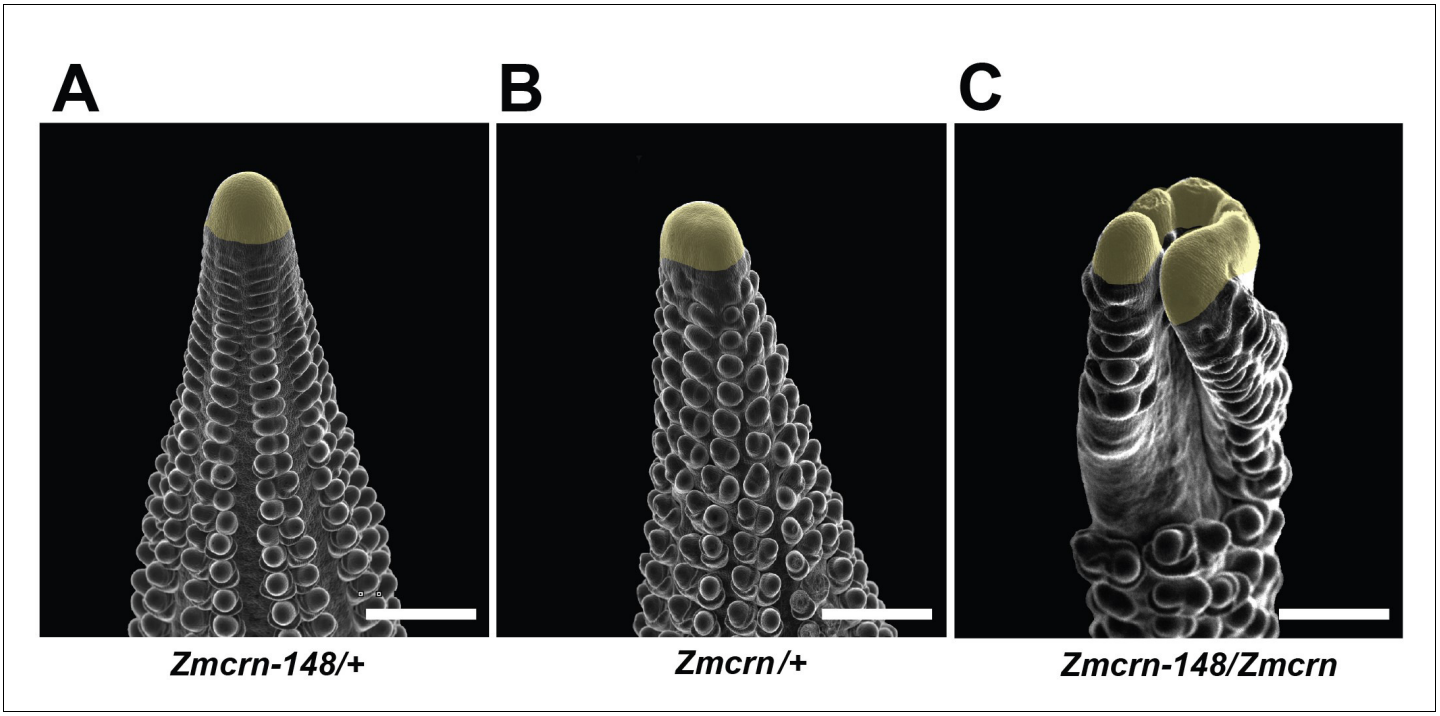

Figure 2—figure supplement 3. *Zmcrn/Zmcrn-148* F1 plants develop fasciated ears. Scanning electron microscopy image of a *Zmcrn/Zmcrn-148* F1 ear shows an enlarged ear inflorescence meristem (**C**), compared with normal ears of *Zmcrn-148/+* (**A**) or *Zmcrn/+* (**B**), suggesting that these mutations are allelic. Scale bars: 500 μm.

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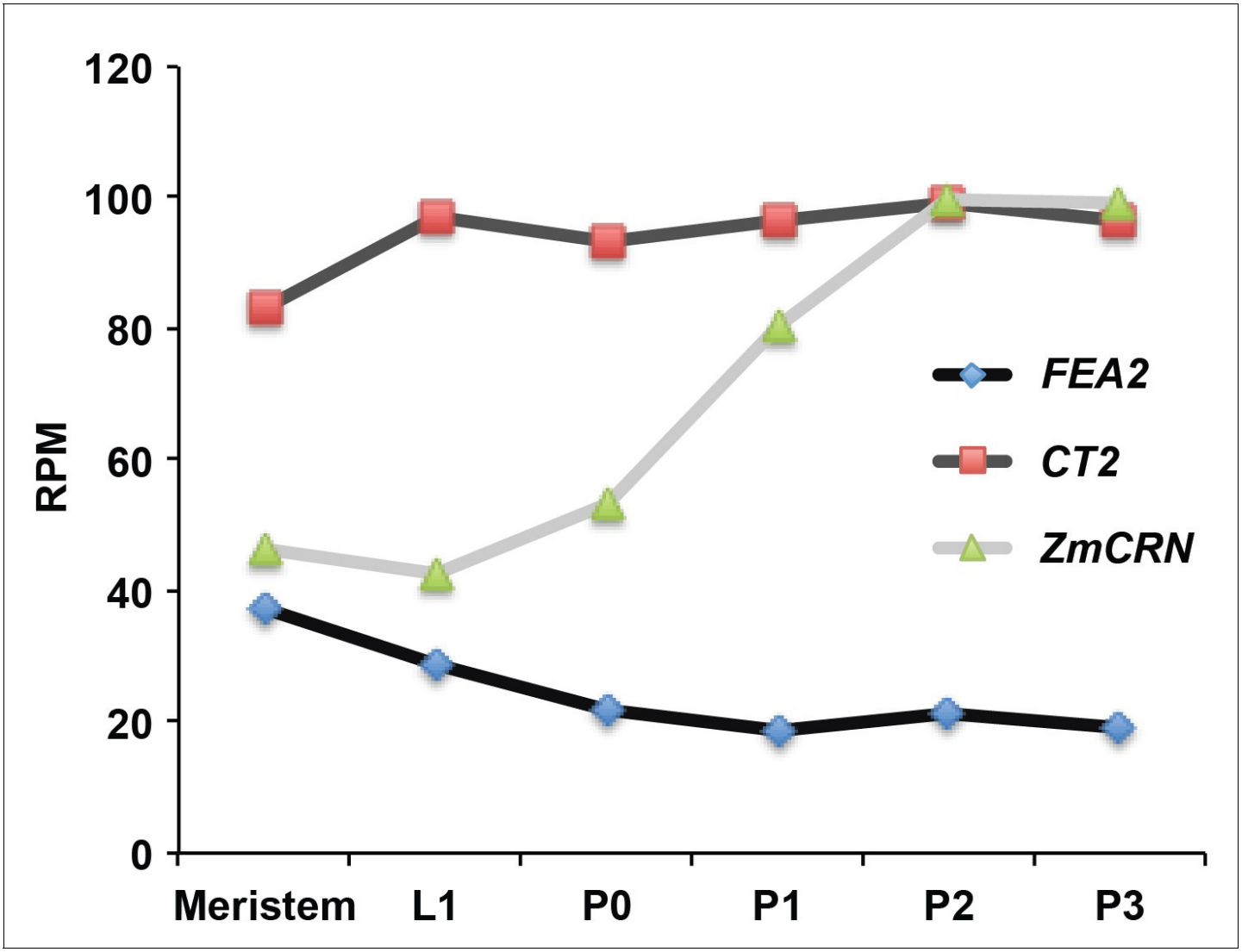

Figure 2—figure supplement 4. Expression of *FEA2, CT2* and *ZmCRN* in different domains of the SAM. Expression values were extracted from the maize shoot apex RNAseq Atlas from the Timmermans Lab (available at https://maizegdb.org/,). The samples are from B73 and different domains are captured by laser microdissection (LCM). L1 = epidermal (L1) layer of SAM, p=primordium number), RPM = reads per million.
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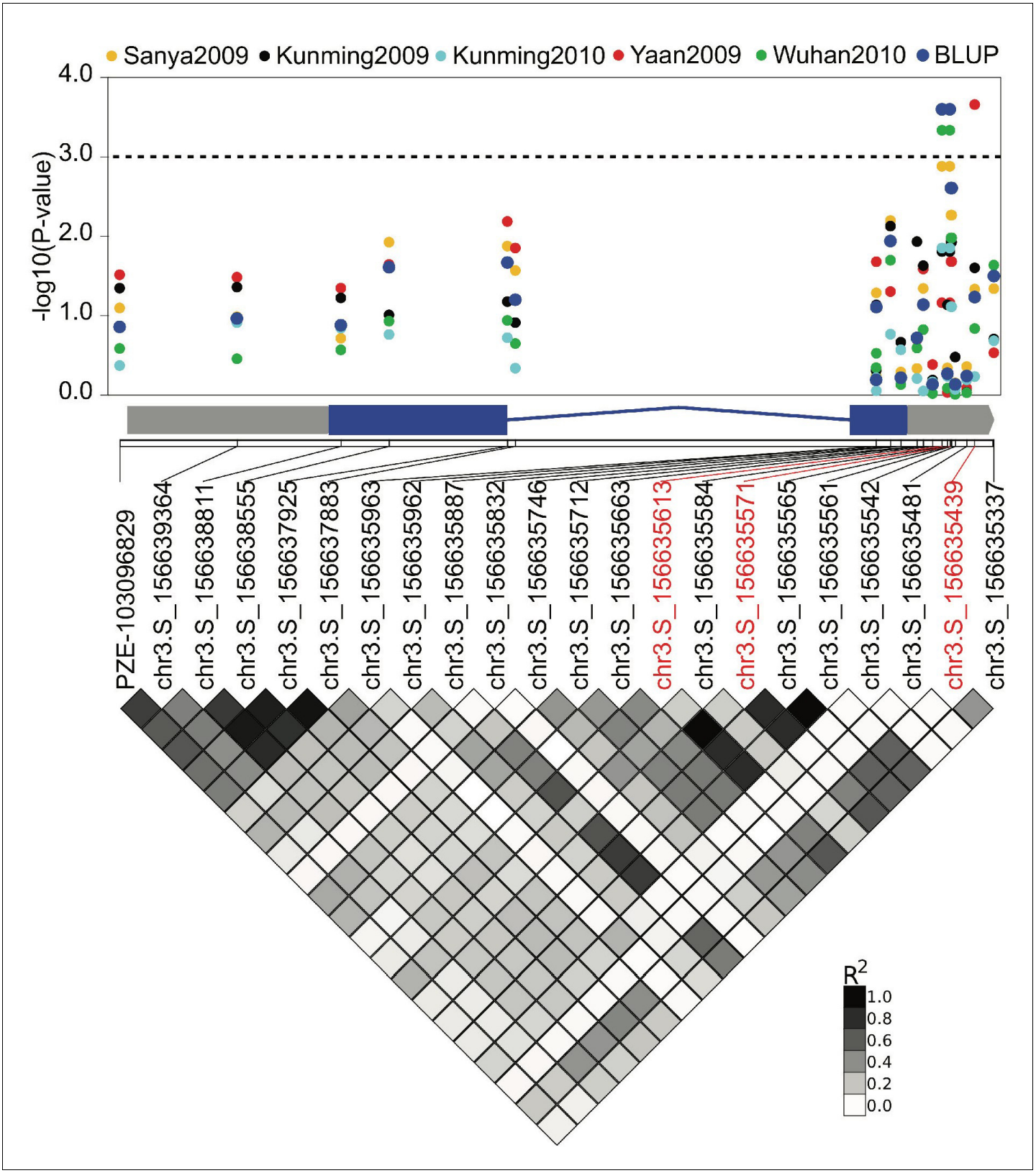

Figure 2—figure supplement 5. The association of *ZmCRN* locus with kernel row number (KRN). The dots show the association of SNPs in the *ZmCRN* locus with KRN evaluated in five environments, and their BLUP data. The 3'UTR region of *ZmCRN* locus is significantly associated KRN. BLUP = best linear unbiased prediction; the dotted line represents the threshold of significant association at p-value≤0.001; the x-axis represents the genomic

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distribution of these SNPs on *ZmCRN*, and the gray and blue boxes of the gene diagram below the x-axis represent the UTR and exon regions of *ZmCRN*, respectively; shaded diamonds below gene model show the linkage disequilibrium of those SNPs by their pairwise R^2.

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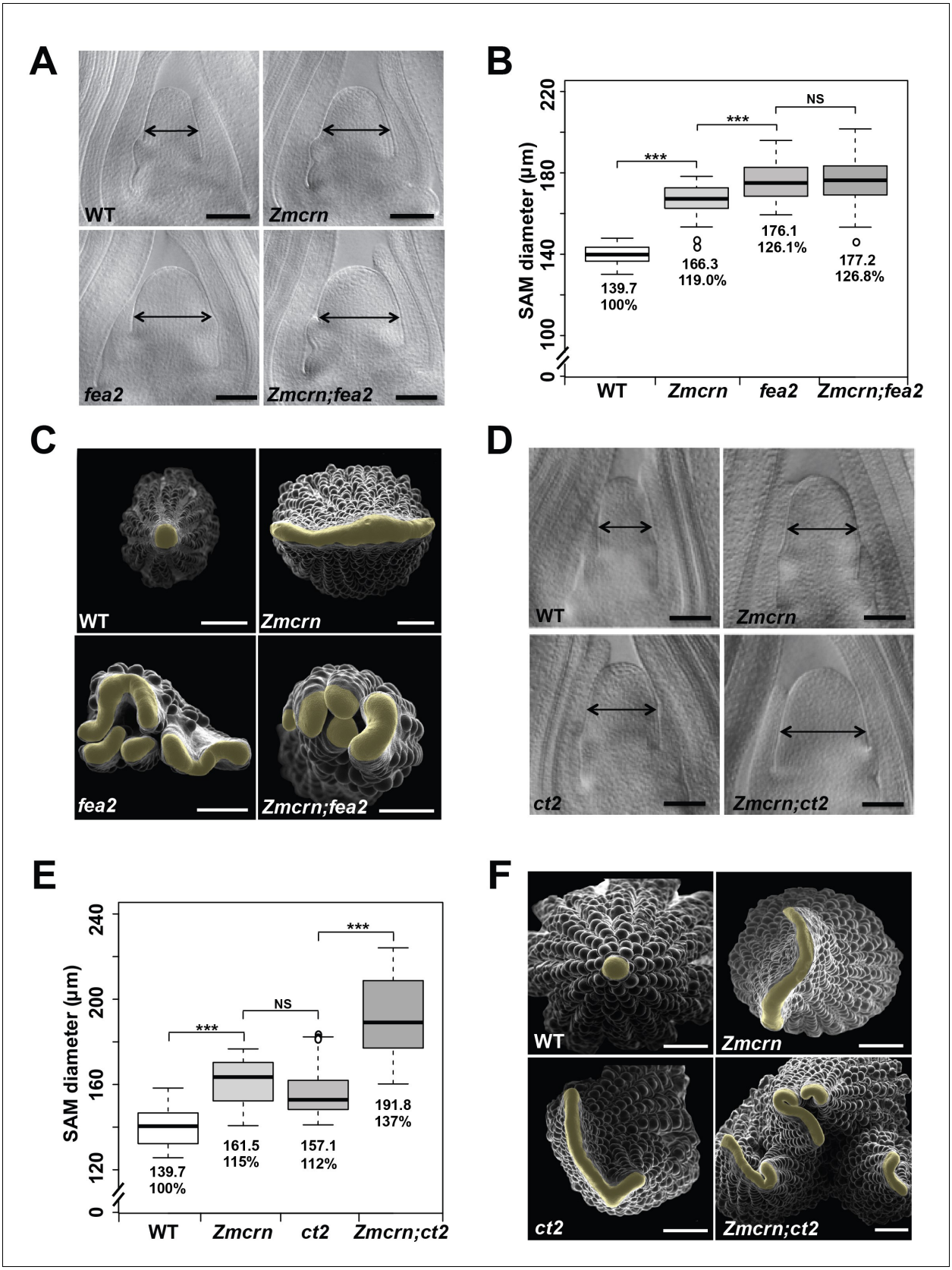

Figure 3. ZmCRN acts in a common pathway with FEA2, but not with CT2. (**A**) Cleared SAMs from wild type (WT), *Zmcrn*, *fea2*, and *Zmcrn;fea2* double-mutant plants. SAMs from *Zmcrn* and *fea2* plants were significantly wider than in wild type (double-headed arrows), but SAM size was not significantly

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different between *fea2* and *Zmcrn;fea2* double mutants, SAM diameter was quantified (**B**). (**C**) Ear meristems of *fea2;Zmcrn* double mutants resemble *fea2* single mutants. (**D**) Cleared SAMs from wild type, *Zmcrn*, *ct2*, and *Zmcrn;ct2* double-mutant plants. SAMs from *Zmcrn* and *ct2* plants were significantly wider than in wild type, and were additively increased in *Zmcrn;ct2* double mutants; SAM diameter was quantified (**E**). (**F**) *Zmcrn;ct2* double mutants had enhanced fasciation of ear primordia. Scale bars: 100 μm in A and D, 500 μm in C and F. n = 30 (**B, E**) plants for each genotype. Data in B and E are shown by box plots. The mean values as well as the relative % to the WT control are listed. '***': P value < 0.0001, two-tailed, two-sample t test, 'NS': not significant.

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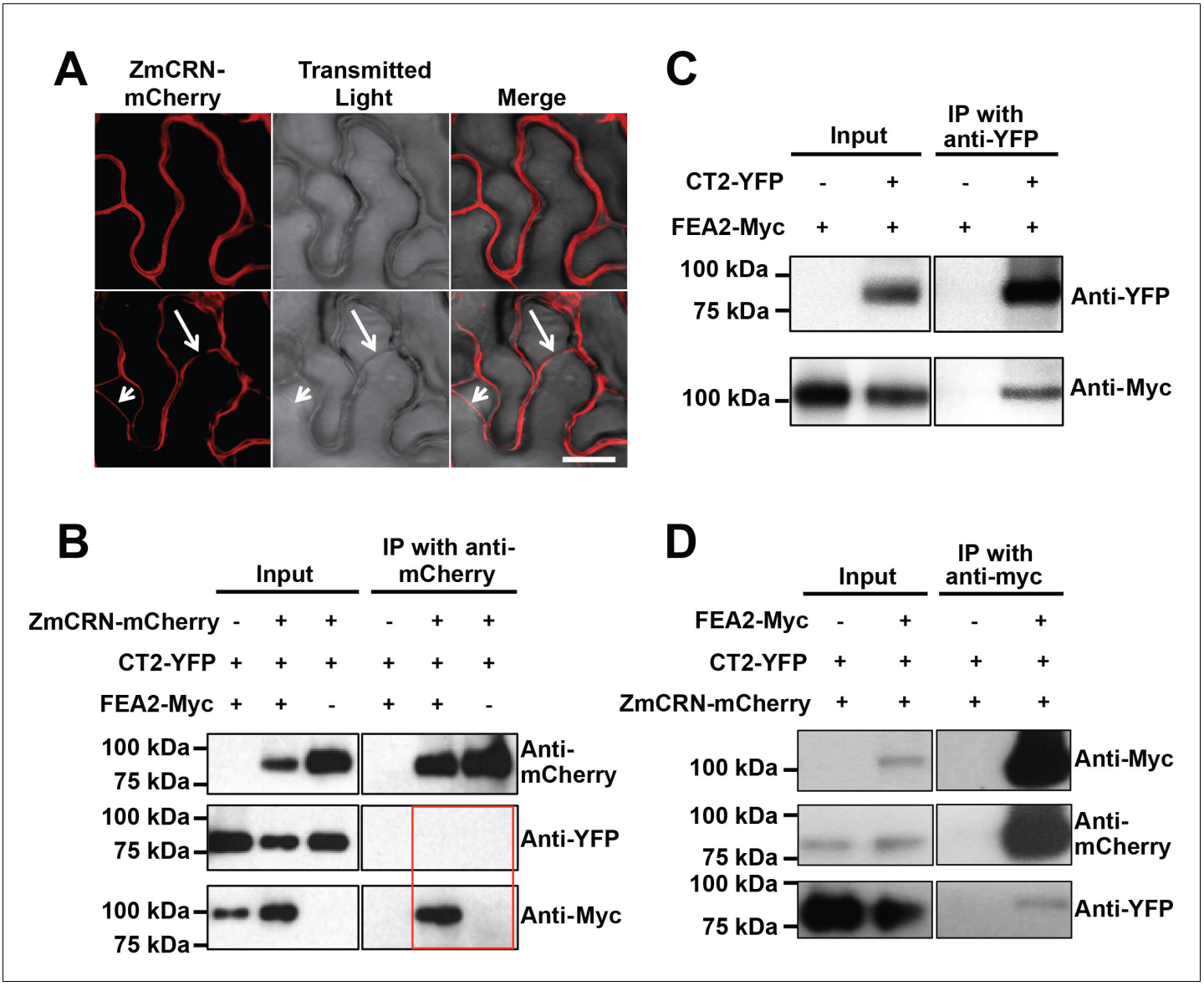

Figure 4. FEA2 is present in two different complexes. (**A**) ZmCRN-mCherry was localized at the plasma membrane following tobacco transient expression (top), and in subsequent plasmolysis (bottom). In transient expression followed by immunoprecipitation (IP) assay, ZmCRN-mCherry could IP FEA2-Myc, but not CT2-YFP (**B**), however CT2-YFP was able to IP FEA2-Myc, as expected (**C**). FEA2-Myc could also IP ZmCRN-mCherry and CT2-YFP, respectively (**D**). Scale bar: 20 μm in A.

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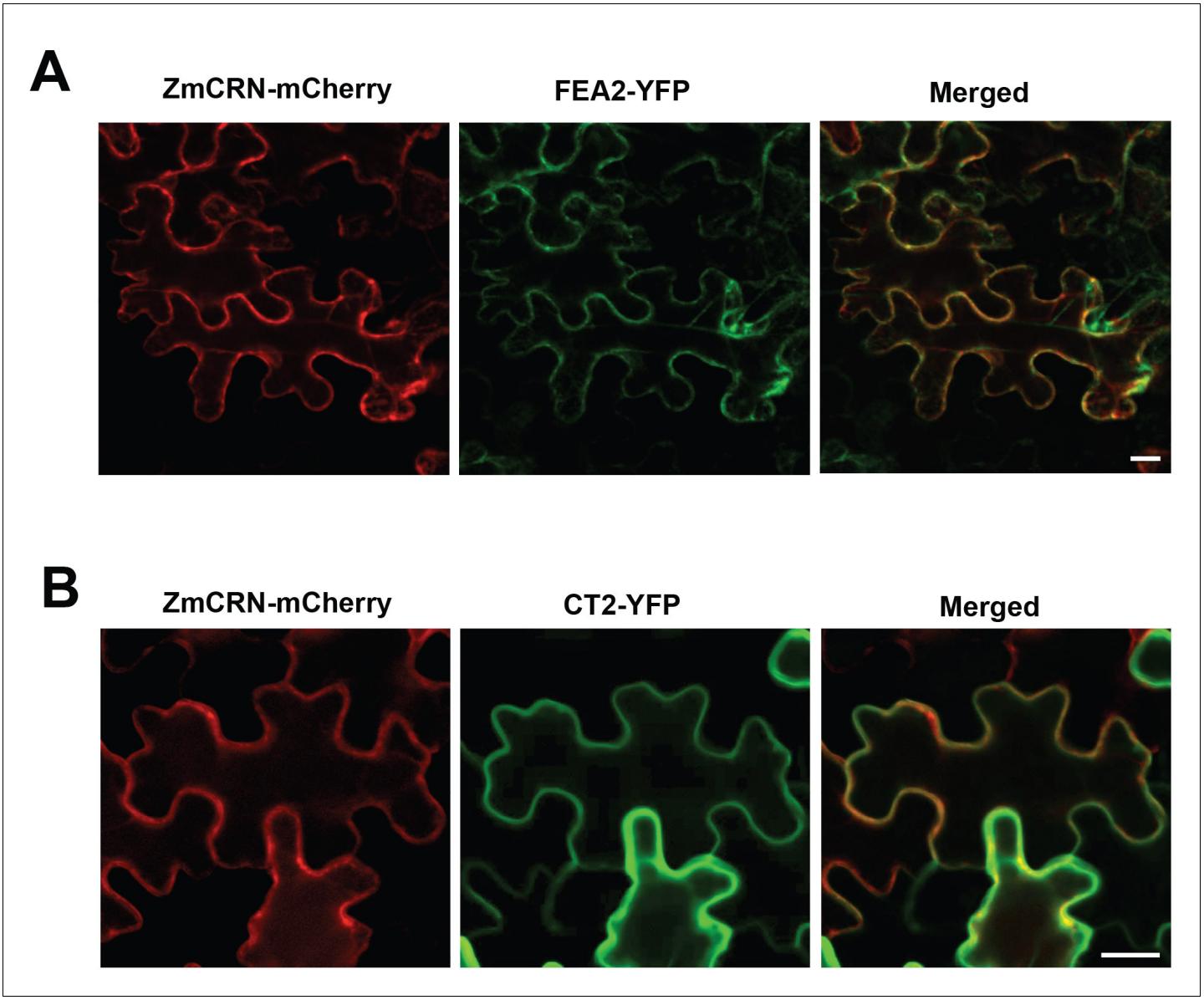

Figure 4—figure supplement 1. ZmCRN-mCherry co-localized with FEA2-YFP and CT2-YFP on the plasma membrane. ZmCRN-mCherry co-localized with FEA2-YFP (**A**) and with CT2-YFP (**B**) on the plasma membrane following transient expression in *N. benthamiana*. Scale bars: 20 μm.
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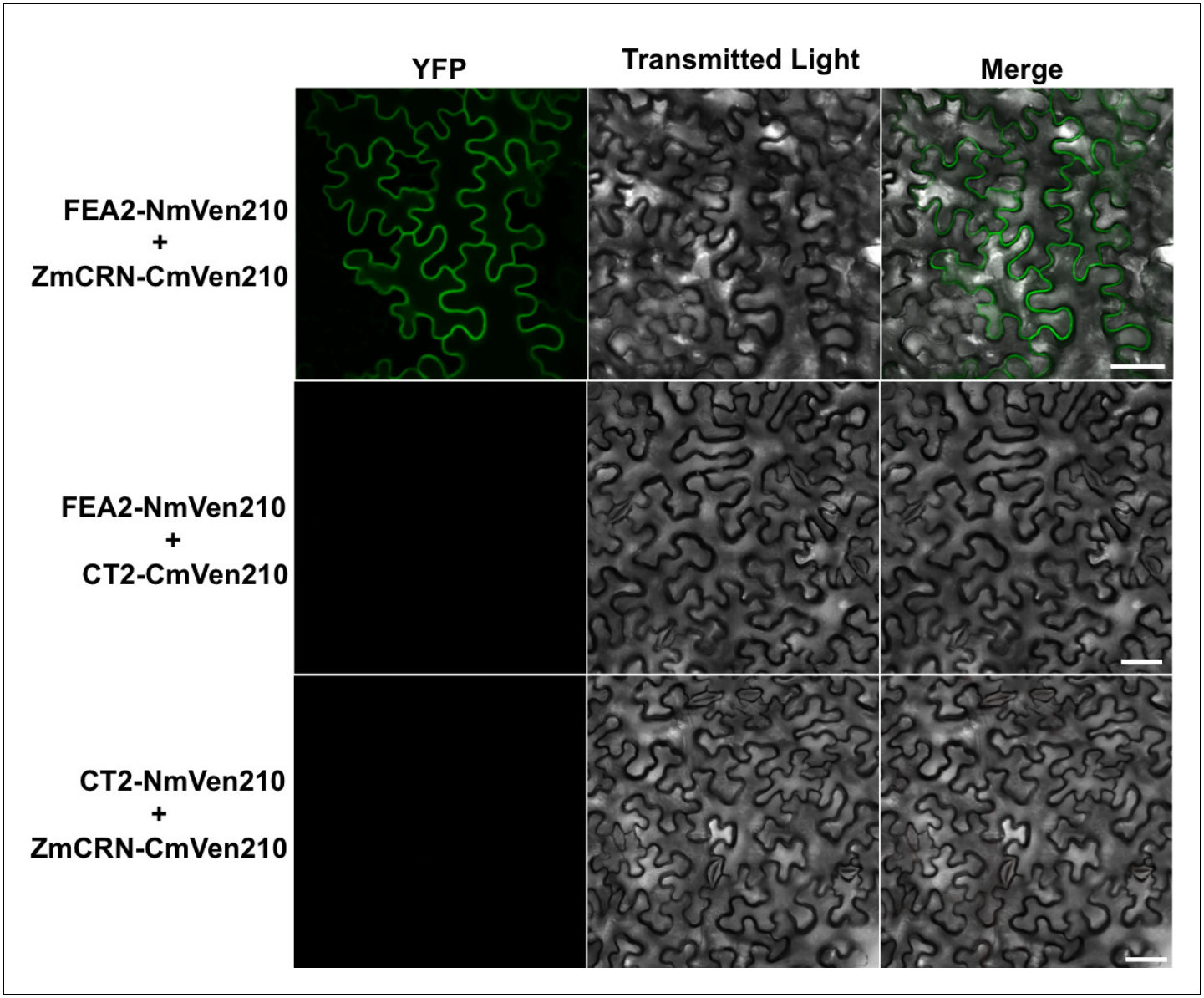

Figure 4—figure supplement 2. FEA2-NmVen210 interacts with ZmCRN-CmVen210 by BiFC. YFP signal was detected when FEA2-NmVen210 was co-expressed with ZmCRN-CmVen210. No YFP signal was detected when FEA2-NmVen210 was co-expressed with CT2-CmVen210, suggesting the interaction is indirect, and no YFP signal was detected when CT2-NmVen210 was co-expressed with ZmCRN-CmVen210, as expected from the Co-IP results. Scale bars: 50 μm.

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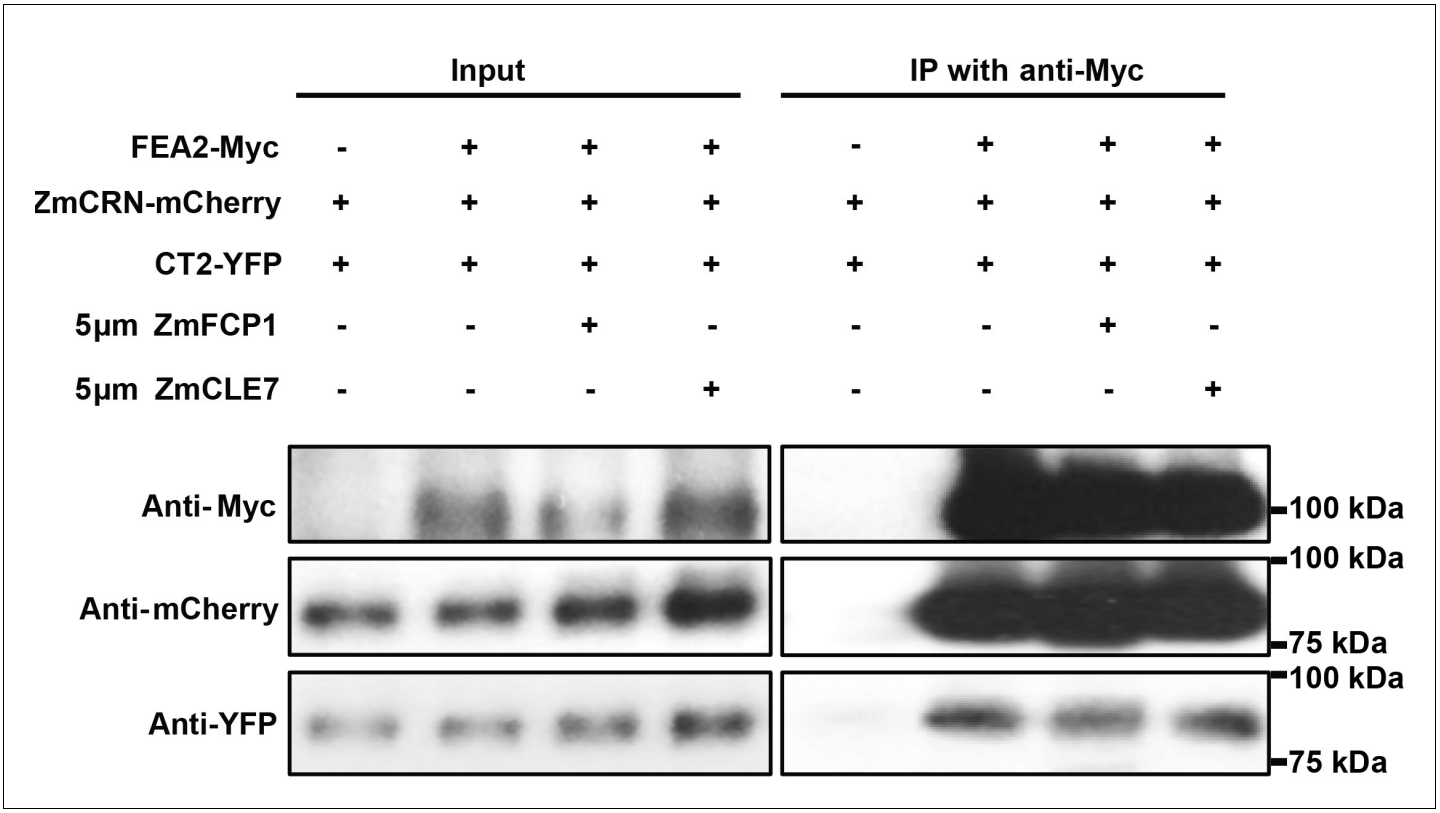

Figure 4—figure supplement 3. Treatment with ZmFCP1 or ZmCLE7 peptide didn't affect the protein complex formation. 5 μm ZmFCP1 or ZmCLE7 were infiltrated into the transformed *N. benthamiana* leaves expressing the indicated proteins following the protocol in ***Somssich et al. (2015)***. The presence of ZmFCP1 or ZmCLE7 didn't affect the interaction between FEA2-Myc and CT2-YFP or the interaction between FEA2-Myc and ZmCRN-mCherry.

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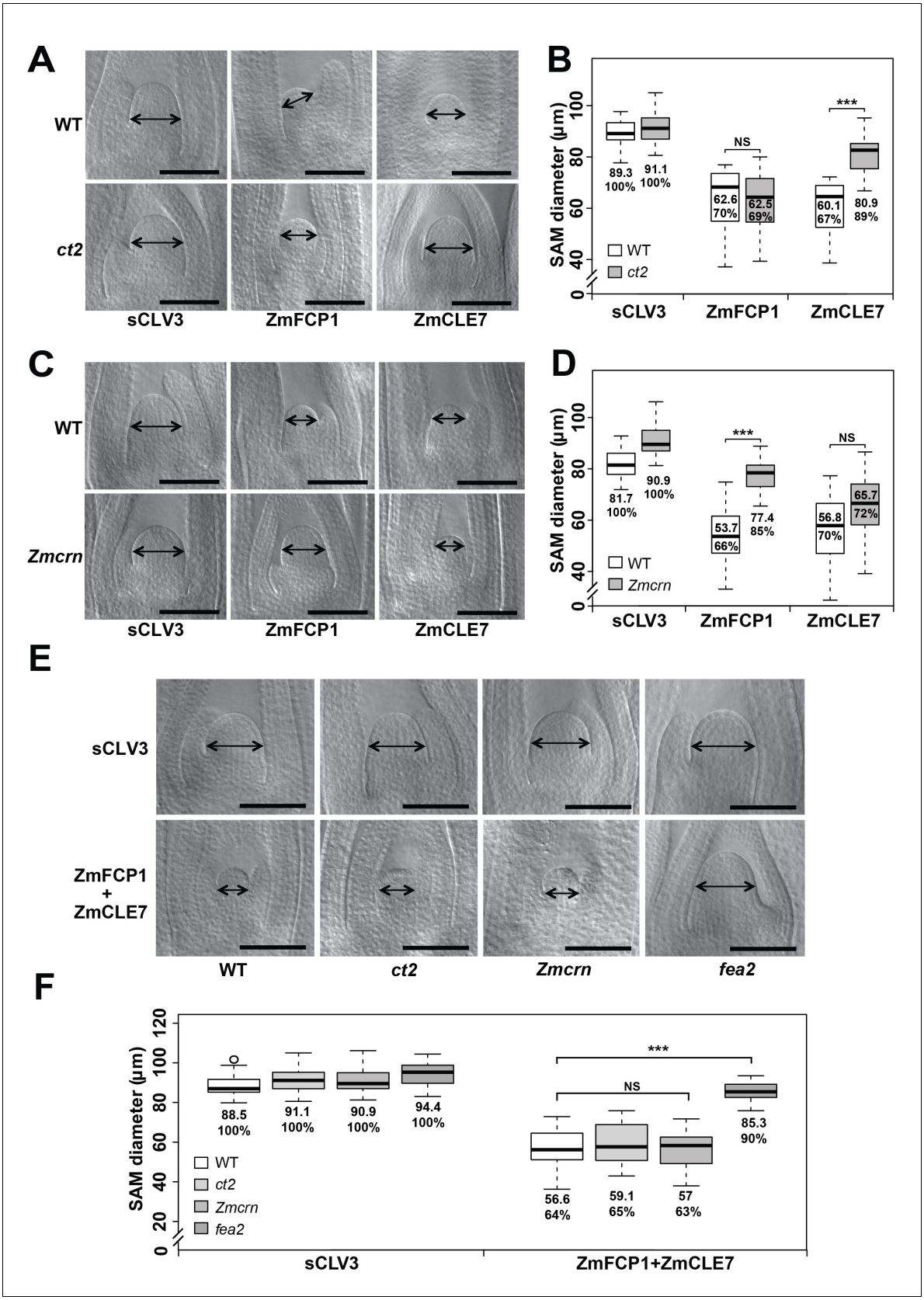

Figure 5. *ct2* and *Zmcrn* show different sensitivity to ZmCLE7 and ZmFCP1 peptides. Embryos of each genotype were cultured with control, scrambled peptide (sCLV3) or with ZmFCP1 or ZmCLE7. Wild type SAM growth (double-headed arrows) was strongly inhibited by all peptides except sCLV3, and

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ct2 growth was insensitive only to ZmCLE7 peptide (**A**), whereas *Zmcrn* was partially resistant only to ZmFCP1 peptide (**C**); SAM diameter was quantified (**B, D**). In treatments with both ZmFCP1 and ZmCLE7, only *fea2* showed resistance, but *Zmcrn* or *ct2* did not (**E, F**). Scale bars: 100 μm in A, C and E. N = 25 (**C**) plants for each genotype. Data in B, D and F are shown by box plots. The mean values as well as the relative % to each negative control are listed. '***': P value < 0.0001, two-tailed, two-sample t test, 'NS': not significant.

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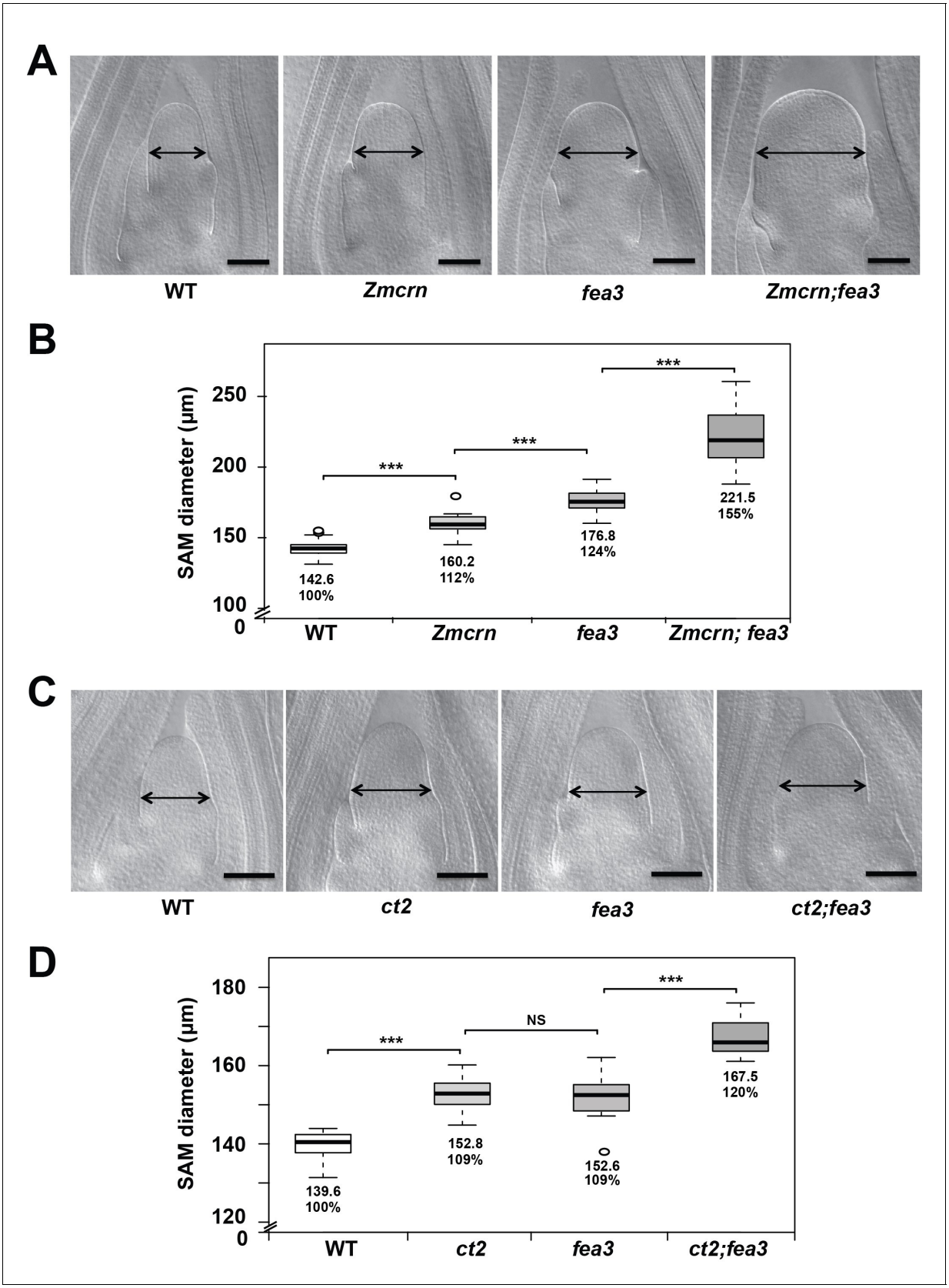

Figure 5—figure supplement 1. *ZmCRN* and *CT2* function in a different pathway to *FEA3*. (**A**) Cleared SAMs from wild-type (WT), *Zmcrn*, *fea3*, and double-mutant plants. SAMs from *Zmcrn* and *fea3* plants were wider than those in wild type (double-headed arrows), and *Zmcrn;fea3* double mutants

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were wider compared to the single mutants. SAM diameter was quantified in (**B**). (**C**) Cleared SAMs from wild-type (WT), *ct2*, *fea3*, and double-mutant plants. SAMs from *ct2* and *fea3* plants were wider than those in wild type (double-headed arrows), and the double mutants were wider compared to the single mutants. SAM diameter was quantified in (**D**). Scale bars: 100 μm in A. n = 28 plants for each genotype, except of *Zmcrn;fea3* double (n = 15). Data in B and D are shown by box plots. The mean values as well as the relative % to the WT control are listed. '***': P value < 0.0001, two-tailed, two-sample t test.

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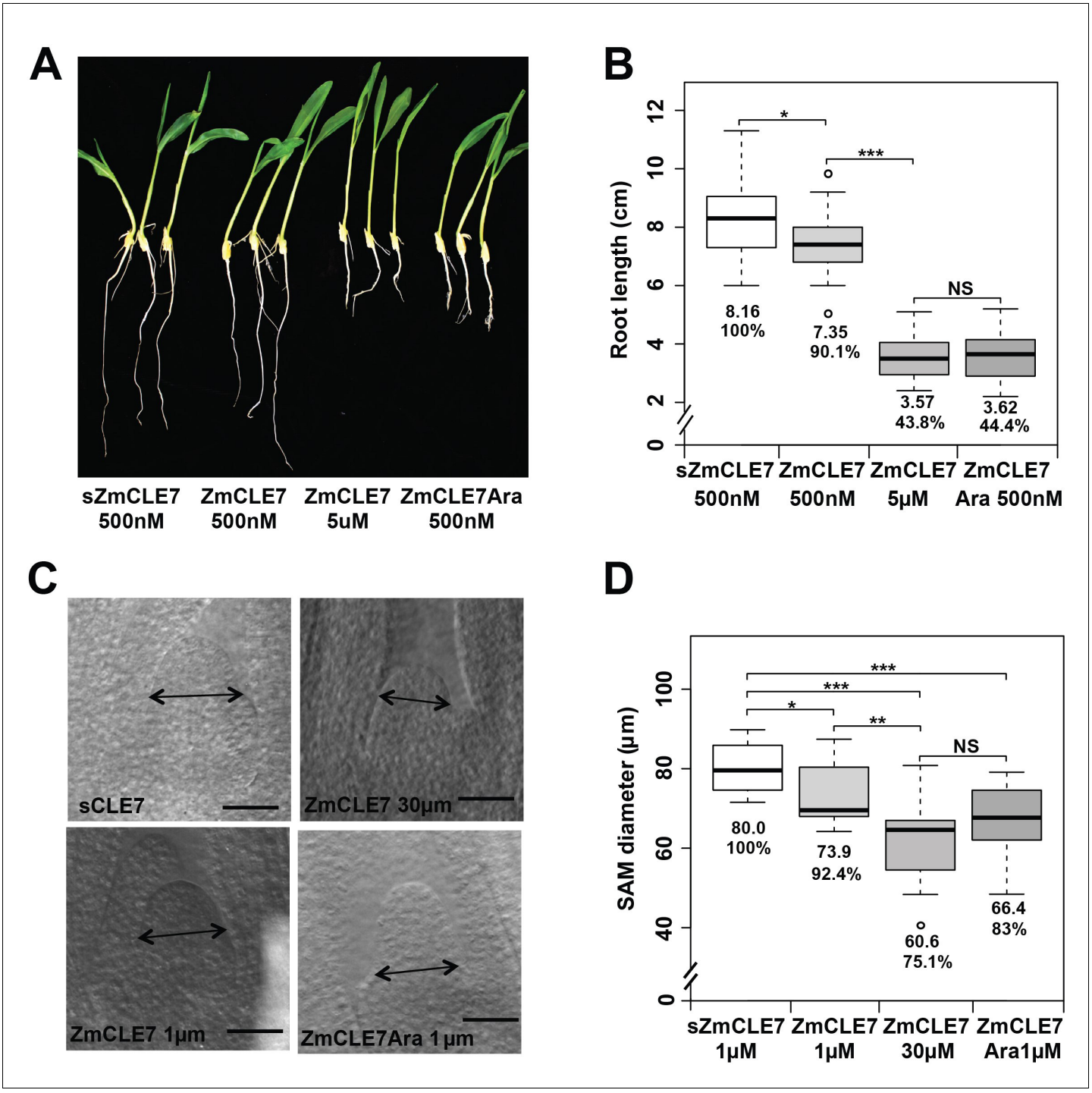

Figure 5—figure supplement 2. Triarabinosylated ZmCLE7 peptide is more potent. Germinating maize seedling roots or embryos were treated with non-modified or triarabinose modified ZmCLE7 peptide at the concentrations indicated. In each case, the modified peptide was significantly more potent than the non-modified form. Data in B and D are shown by box plots. The mean values as well as the relative % to the control scrambled peptide (sZmCLE7) treatment are listed. '*': P value < 0.05, '**': P value < 0.01, '***': P value < 0.001, 'NS': not significant, two-tailed, two-sample t test.
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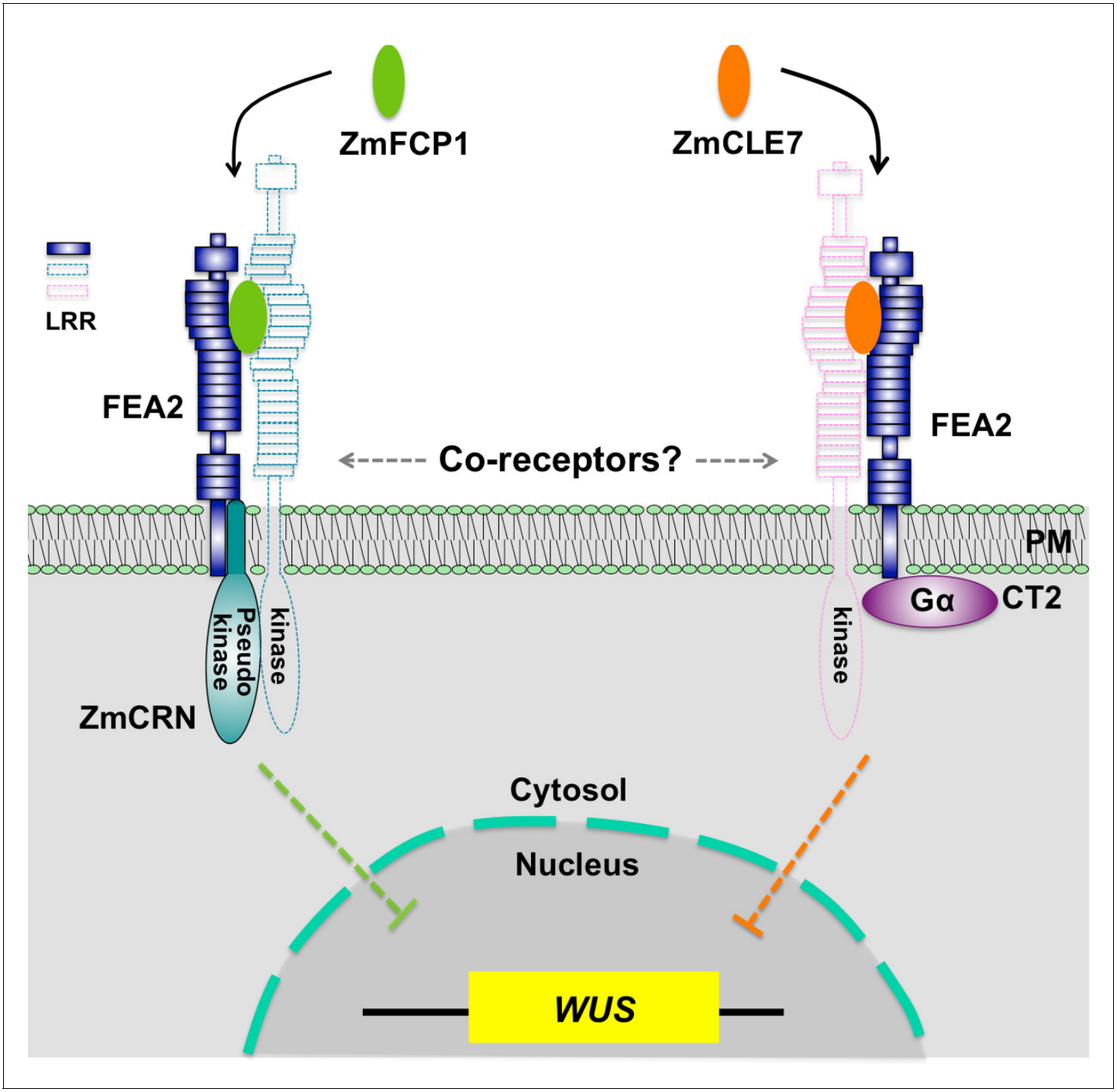

Figure 6. Hypothetical model for FEA2 signaling through two different pathways. Two different peptides, ZmFCP1 and ZmCLE7, are proposed to bind to two separate FEA2 receptor complexes, and the two signals are differentially transmitted to downstream components; with the ZmCLE7 signal passing through CT2, and the ZmFCP1 signal passing through ZmCRN.

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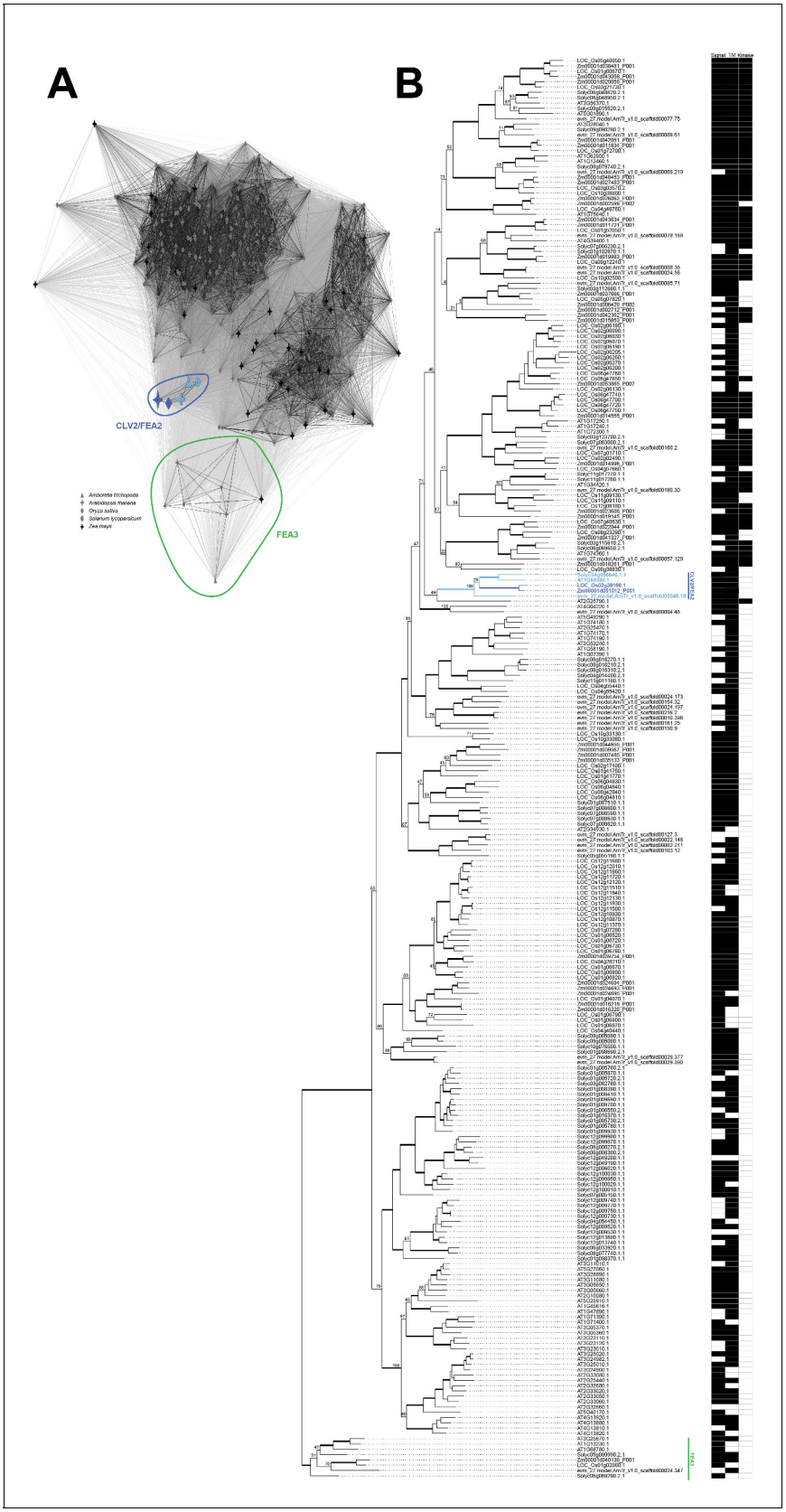

Figure 6—figure supplement 1. Clustering, phylogenetic tree, and functional annotations of CLV2/FEA2 genes and their close homologs from five species. CLANS clustering of CLV2/FEA2 and closely related homologs representing pairwise similarity (**A**). The clusters containing CLV2/FEA2 and

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FEA3 are circled in blue and green, respectively. The CLV2/FEA2 genes cluster separately from the remainder of the genes in the network. Maximum-likelihood phylogenetic tree of CLV2/FEA2 and the same closely related homologs as represented in the clustering, with functional annotations (**B**). Node values are bootstrap supports. The diagram shows which genes showed presence of a signal peptide domain ('signal') and a transmembrane domain ('TM'), as predicted by Phobius, and which genes showed the presence of a kinase domain ('Kinase'), as predicted by HMMER3; CLV2/FEA2 and FEA3 are colored the same as in (**A**). Thickened lines represent 90% or higher bootstrap support values.

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