Maize (*Zea mays* L.) nucleoskeletal proteins regulate nuclear envelope

remodeling and function in stomatal complex development and pollen viability.

McKenna JF*†, Gumber HK*, Turpin ZM, Jalovec AM, Kartick AC, Graumann K#, and

Bass HW#

*co-first authors, equal contributions

co-corresponding authors

7
8 email addresses:
9
10 Joseph F. McKenna, josephmckenna@brookes.ac.uk, Department of Biological and

11 Medical Sciences, Oxford Brookes University, Oxford, UK

12 † Current address: Joe.McKenna@Warwick.ac.uk, School of Life Sciences, University

13 of Warwick, Coventry, CV4 7AL, UK, ORCID 0000-0003-4838-6048

14
15 Hardeep K. Gumber, hkg12@my.fsu.edu, Department of Biological Science, Florida

16 State University, Tallahassee, FL, USA, ORCID 0000-0001-5250-7207

17
18 Zachary M. Turpin, zmt11@my.fsu.edu, Department of Biological Science, Florida State

19 University, Tallahassee, FL, USA

20
21 Alexis M. Jalovec, amj13e@my.fsu.edu, Department of Biological Science, Florida State

22 University, Tallahassee, FL, USA

23
Andre C. Kartick, ack17@my.fsu.edu, Department of Biological Science, Florida State

University, Tallahassee, FL, USA

Katja Graumann, kgraumann@brookes.ac.uk, Department of Biological and Medical

Sciences, Oxford Brookes University, Oxford, UK

Hank W. Bass, bass@bio.fsu.edu, Department of Biological Science, Florida State

University, Tallahassee, FL, USA, ORCID 0000-0003-0522-0881

Author Approval: All authors approve of this manuscript for preprint server biorxiv.

Competing Interests: All the authors declare no competing interest.

ABSTRACT

In eukaryotes, the nuclear envelope (NE) encloses chromatin and separates it from the rest of the cell. The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex physically bridges across the NE, linking nuclear and cytoplasmic components. In plants, these LINC complexes are beginning to be ascribed roles in cellular and nuclear functions, including chromatin organization, regulation of nuclei shape and movement, and cell division. Homologs of core LINC components, KASH and SUN proteins, have previously been identified in maize. Here, we characterized the presumed LINC-associated maize nucleoskeletal proteins NCH1 and NCH2, homologs of members of the plant NMCP/CRWN family, and MKAKU41, homologous to AtKAKU4. All three proteins localized to the nuclear periphery when transiently and heterologously expressed as fluorescent protein fusions in *Nicotiana benthamiana*. Overexpression of MKAKU41 caused dramatic changes in the organization of the nuclear periphery, including nuclear invaginations that stained positive for non-nucleoplasmic markers of the inner and outer NE, and the ER. The severity of these invaginations was altered by changes in LINC connections and the actin cytoskeleton. In maize, MKAKU41 appeared to share genetic functions with other LINC components, including control of nuclei shape, stomatal complex development, and pollen viability. Overall, our data show that NCH1, NCH2, and MKAKU41 have characteristic properties of LINC-associated plant nucleoskeletal proteins, including interactions with NE components suggestive of functions at the nuclear periphery that impact the overall nuclear architecture.

Keywords (4-6): Nucleus, Nuclear Envelope, Maize, Lamin, Nucleoskeleton, kaku4, Peripheral Nucleoplasm

INTRODUCTION

In plant cells, as with all eukaryotes, the nucleus is a conspicuous and characteristic organelle, housing the DNA (reviewed by (Meier et al. 2017)). The nucleus itself is a dynamic structure, organized largely by its enclosing membrane system - the nuclear envelope (NE). The NE is a double-membraned structure composed of an outer nuclear membrane (ONM) and an inner nuclear membrane (INM) connected at nuclear pores (reviewed by (Hetzer 2010; Graumann and Evans 2017). The functional properties of the NE are mediated by protein complexes linked to myriad cellular and nuclear processes, including cell division and gene expression (Kim et al. 2015; Meier et al. 2017; De Magistris and Antonin 2018; Pradillo et al. 2019). A major conserved NE complex is the linker of nucleoskeleton and cytoskeleton (LINC) complex (Crisp et al. 2006), known for it involvement in processes such as the maintenance of nuclear architecture and mechanical structures, signalling, nuclear motility, and nuclear positioning (reviewed by (Rothballer and Kutay 2013), (Chang et al. 2015; Tamura et al. 2015; Pradillo et al. 2019; Starr 2019). Well beyond the historically recognized role of compartmentalization, NE investigations increasingly address how the NE contributes to both general and specialized functions in plant growth and development. The underlying molecular mechanisms coordinating and regulating these fundamental NE processes remain, however, largely unknown.

The hallmark of LINC complexes is their ability to form direct connections bridging the cytoplasm and the nucleoplasm. This is accomplished by a core complex of two groups of proteins, residing on the two separate membranes of the NE. The INM

houses the Sad1/UNC84 homology (SUN) domain proteins and the ONM houses the

Klarsicht/ANC-1/Syne homology (KASH) proteins (Hagan and Yanagida 1995; Malone

et al. 1999; Starr 2002; Murphy et al. 2010). As a group, the KASH proteins are

numerous and diverse, reflecting their various cytoplasmic binding partners, such as

cytoskeletal and motor proteins (Starr and Fridolfsson 2010; Luxton and Starr 2014; Kim

et al. 2015; Evans and Graumann 2018; Starr 2019). In contrast, the SUN-domain

proteins are less diverse but still exhibit interactions with multiple components of the

nucleoplasm, such as nucleoskeletal components and chromatin proteins (Haque et al.

2006; Janin et al. 2017). While cytoskeletal components of LINC complexes and the

SUN proteins appear conserved in eukaryotes, plants have evolved unique KASH

proteins and nucleoskeletal components. Plant nucleoskeletal components, while

lacking sequence homology, share many of the animal lamin features, including

interactions with the NE and their ability to impact chromatin structure and gene

expression (Masuda et al. 1997; Dittmer et al. 2007; Wang et al. 2013; Ciska and

Moreno Dí-az de la Espina 2014; Goto et al. 2014; Zhao et al. 2016; Guo et al. 2017;

Choi et al. 2019; Ciska et al. 2019; Hu et al. 2019; Sakamoto 2020).

Plant nucleoskeletal proteins that interact directly or indirectly with the NE fall into

two families of proteins. One family, the Nuclear Matrix Constituent Proteins/Crowded

Nuclei (NMCP/CRWN) proteins, is found in plants with members encoded by the NMCP

genes in carrot (Masuda), CRWN genes in Arabidopsis (Dittmer et al. 2007), and NCH

genes in maize (Gumber et al. 2019a). The other family is also found in plants and

encoded by the AtKAKU4 gene in Arabidopsis (Goto et al. 2014) and the MKAKU41 and

MKAKU42 genes in maize (Gumber et al. 2019a). Here we refer to these two gene or

protein families generally as CRWN and KAKU4.

In Arabidopsis, CRWN1 is located primarily at the nuclear periphery and interacts

with SUN-domain proteins (Dittmer et al. 2007; Graumann 2014). CRWN proteins have

been implicated in the regulation of nuclear shape, nuclear size, chromatin organization,

regulation of gene expression, and nuclear body formation in plants (reviewed in

(Sakamoto 2020). KAKU4 was shown to interact with CRWN1 by yeast two-hybrid

analysis, supporting their cooperation in the form and function of a plant nucleoskeletal

system (Goto et al. 2014). Fluorescent protein fusions driven by native promoter

expression and electron microscopy showed that KAKU4 is localized to the inner

nuclear membrane. Interestingly, when high-expression stable lines were selected or

high-expression transient transformation was performed, KAKU4 appeared to induce

nuclear invaginations, which increased significantly when co-expressed with CRWN1

(Goto et al. 2014).

Together, the CRWN and KAKU proteins are known to affect phenotypes in

eudicots, yet their roles in crop species are less well understood. Two maize CRWN

homologs are known; NCH1 which is most closely related to AtCRWN1-3, and NCH2

which is most closely AtCRWN4. Maize MKAKU41 homologs are encoded by

MKAKU41 and *MKAKU42* (Gumber et al. 2019a). To investigate the functional

conservation of these presumed nucleoskeletal proteins in maize, we characterized

NCH1 and *NCH2* and *MKAKU4* using cytological or genetic approaches.

RESULTS

Maize NCH1, NCH2, and MKAKU41 localized to the nucleus, primarily at the nuclear periphery

In order to determine the cellular localization of the maize CRWN homologs NCH1, NCH2, and the KAKU4 homolog MKAKU41, we produced gene constructs with the protein coding region fused to either GFP or mCherry at the N-terminus. We then expressed these constructs transiently in *N. benthamiana* leaf tissue. All three constructs localized to the nuclear periphery with MKAKU41 also exhibiting internal structures as shown in Figure 1 for nuclei counterstained with DAPI. In order to confirm that NCH1 and NCH2 were localized at the nuclear periphery, we performed co-expression of NCH1 or NCH2 with AtCRWN1 (Fig. S1). The co-localization of NCH with CRWN confirmed that NCH1 and NCH2 did localize to the nuclear periphery when transiently expressed in *N. benthamiana*. Interestingly, MKAKU41 showed nuclear envelope labelling and inner nucleus labelling in most cells, including membrane invaginations (Fig. 1B, white arrowheads), as has been described for Arabidopsis KAKU4 when expressed under the control of the 35S promoter (Goto et al. 2014). These included internal structures and circular ring-like invaginations within the nucleus. The structures labelled with MKAKU41 colocalized with brighter DAPI staining regions, implying that MKAKU41 may associate with heterochromatin. The ring-like invaginations lacked internal DAPI staining and therefore appeared devoid of typical nucleoplasmic chromatin. Interestingly, the CRWN1 homolog NCH1 alone also caused these aberrant-looking intranuclear structures (Fig. 1B, white arrow), although to a lesser extent than

MKAKU41. However, NCH2, a CRWN4 homolog (Fig. 1A), did not induce ring-like

invaginations. The NCH1 and NCH2 also displayed different mobility proportions at the

nuclear periphery (Fig. 1C, S1B and S1C) as determined by Fluorescence Recovery

After Photobleaching (FRAP). NCH2 was significantly less mobile (16% mobile fraction)

than NCH1 (49% mobility) indicating that they might interact with different protein

complexes or structures. The lower mobility of NCH2 compared to NCH1 is consistent

with its reduced capacity to remodel the nuclear envelope membrane and cause

invaginations (Fig. 1A,C). The large immobile fraction of NCH2, but not NCH1, was

comparable to that of AtCRWN1 (Graumann 2014).

It has been demonstrated that the degree of nuclear invagination and

deformation is dependent on the expression level of KAKU4 in *A. thaliana* (Goto et al.

2014). We therefore asked if this causal relationship was conserved in the maize genes,

MKAKU41, NCH1, and NCH2, using the dose-response transient expression assays

summarized in Figure 2. We infiltrated *N. benthamiana* with three different

concentrations of Agrobacterium, OD 0.01, 0.05, or 0.1, and then classified live-imaged

nuclei (N≥30 per condition) into one of three previously described nuclear periphery

cytological image patterns (Goto et al. 2014): type I for normal nuclear periphery

localization, type II for minor invagintions or inclusions in the nucleus, and type III for

major invaginations and deformation of the nucleus. This classification assisted with a

comparative analysis of the severity of changes in nuclear morphology across the

various experiments used throughout this study. Increasing the infiltration concentration

of NCH1 resulted in progressively increased nuclear deformation illustrated by type II

pattern increases and the appearance of type III at the highest transfection dose (Fig.

2A). NCH2 also showed a transfection dose-dependent increase in nuclear aberration

coupled to type II increases, but less so and without any type III nuclei even at the

highest dose (Fig. 2B). For MKAKU41, increasing the transfecting plasmid

concentration led from most nuclei just showing peripheral localization initially to slightly

under half showing type III patterns with severe invaginations seen at the highest

concentration (Fig. 2C). We interpret these results as establishing that because all three

of the proteins tested showed they could cause dose-dependent increases in severity of

nuclear deformation patterns, they can all be considered to be part of the same process,

one needed to properly organize a nuclear periphery compartment within interphase

nuclei.

Co-expression of NCH1 or NCH2 with MKAKU41 showed a synergistic effect on

invagination and nuclear deformation phenotypes

The Arabidopsis CRWN1 and KAKU4 have previously been shown to result in

increased nuclear deformation and invaginations when co-expressed (Goto et al. 2014).

In order to determine whether the maize homologs similarly affect the nuclear

organization pathway, we co-expressed MKAKU41 with either NCH1 or NCH2 as

presented in Figure 3. Upon co-expression, the nuclear invaginations and deformations

were substantially enhanced compared to single-expression (Fig. 3A). Quantifying the

most severe phenotype (type III), we observed that NCH1 alone exhibited 7% and

MKAKU41 alone exhibited 42%. However, the combination of NCH1 and MKAKU41

exhibited 88% type III, well beyond the combined value of 49%, and thus synergistic for

this measurement. Similarly, the NCH2 and MKAKU41 co-expression reached a level of

66% type III nuclei, considerably more than 42% sum of the single-expressed levels.

Therefore, NCH proteins appeared to act cooperatively with MKAKU41 to affect nuclear

periphery organization as judged by changes in their localization patterns. This

cumulative effect on nuclear structure suggests that NCH1/NCH2 and MKAKU41 may

act in the same protein complex. We tested this idea by checking for evidence of

interaction between NCH and MKAKU41 *in planta* using acceptor photobleaching

fluorescence resonance energy transfer (apFRET). A significant rise in FRET

efficiencies would indicate interactions and such was measured when NCH1 and NCH2

were co-expressed with MKAKU41, compared to a non-interacting control, calnexin

(Fig. 3C). Internal control FRET efficiency % values were very low, as expected for non

bleached controls (Table S1). This demonstrated that NCH1 and NCH2 can interact

with MKAKU41, and is consistent with their synergistic effect on levels of nuclear

deformations. This observation is similar to that observed for Arabidopsis homologs

using yeast two-hybrid system and plant expression (Goto et al. 2014). Importantly, our

findings provide evidence from live imaging for *in planta* interactions between these

proteins at the nuclear periphery.

MKAKU41 overexpression remodeled other inner and outer nuclear membrane

proteins

Both AtKAKU4 and MKAKU41 cause deformation of the NE and disruption of nuclei

structure when co-expressed with AtCRWN1 (Goto et al. 2014), NCH1, or NCH2 (Fig.

3). To further investigate whether this involves the entire NE, we asked whether

overexpression of MKAKU41 can result in the invagination of other proteins not

previously tested but known to localize to either the INM, the ONM, or the ER. For this

we used AtSUN2-YFP to mark the INM, ZmMLKP1-GFP to mark the ONM, and

calnexin-GFP to mark the ER membrane. Figure 4 shows that each of these markers

showed normal nuclear periphery localization when expressed individually. However,

upon co-expression with MKAKU41, all of these proteins appeared in aberrant

intranuclear structures. Therefore, the expression of MKAKU41 appears to have caused

the internalisation of the entire NE, including the calnexin-GFP ER membrane marker,

demonstrating that MKAKU41-induced nuclei deformations and invaginations are not

limited to nucleoplasmic and INM LINC proteins.

Previously, it has been shown that AtSUN1 and AtSUN2 interact with AtCRWN1,

mediated by the SUN N-terminus (Graumann 2014) and that the deletion construct

SUN2ΔNterm could disrupt SUN-CRWN interactions (Graumann et al. 2010; Graumann

2014). To investigate more specifically the role of the LINC complex in producing these

aberrant nuclear structures, we overexpressed SUN2ΔNterm as a way to abrogate

CRWN-SUN binding and thereby possibly reduce the interaction of MKAKU41 with the

LINC complex. Notably, SUN2-MKAKU41 co-expression resulted in mostly type III

nuclei, whereas the SUN2ΔNterm-MKAKU41 co-expression led to less deformation,

with nuclei showing mostly type II phenotype (Fig. 4A). Interestingly, nuclear envelope

fluorescent labelling of full-length SUN2 appeared lower than that in SUN2ΔNterm when

co-expressed with MKAKU41 (Fig. 4A & 4B). Signal intensity line profiles drawn over

the nuclear invagination (Fig. 4A, dashed line) or nuclear envelope (Fig. 4A, solid line)

regions showed that the SUN2 full length signal was much stronger within the

invaginations but lower at the nuclear envelope when compared to those from

SUN2ΔNterm fluorescence (Fig. 4B). We quantified the effect of co-expressed

MKAKU41 on the SUN2's tendency for peripheral staining by determining the

percentage of the signal in the interior versus the entire nucleus (sub-periphery / whole

nucleus/, Fig. S2). For instance, if the signal was entirely peripheral, the percentage

would be at or near zero. Figure 4C shows an increase in internal fluorescence for

SUN2 in the SUN2 - MKAKU41 co-expression compared to SUN2 alone (p<0.0001).

Therefore, full length SUN2 is relocated away from the nuclear periphery when

coexpressed with MKAKU41. The relative internal nuclear fluorescence also increased

upon SUN2ΔNterm coexpression with MKAKU41 (p<0.001), but to a lesser extent than

for full length SUN2 (p<0.001). These observations implicate the LINC complex and

more specifically the nucleoplasmic N-terminal domain of the NE protein, SUN2, in the

formation of aberrant MKAKU41-dependent intranuclear structures.

Impairing Nuclear Actin anchoring enhances nuclear invaginations

In addition to the SUN2 NE protein which spans the INM and has direct contact with the

nucleoplasm, we also examined an ONM maker, MLKS2, and its ARM domain deletion

derivative previously shown to be impaired for actin binding (Gumber et al. 2019b).

Upon co-expression with MKAKU41, the ONM marker MLKS2 also appeared in

intranuclear invagination-like structures as shown in Figure 5 (panel A). Upon co-

expression of MKAKU4 and MLKS2ΔARM, nuclear deformations and invaginations

were observed (Fig. 5A) and found to much more severe and abundant (Fig. 5B)

compared to those from co-expression of MKAKU4 and the full length MLKS2.

Therefore, the ONM KASH protein MLKS2 is brought moved to intranuclear structures

by MKAKU41 co-expression, and loss of the actin-interacting ARM domain exacerbates

the situation and implicates cytoplasmic F-actin in NE and nuclear periphery

remodeling.

In order to further probe the interaction between peri-nuclear actin and

MKAKU41-induced nuclear deformations, we used Latrunculin-B (LatB) to depolymerise

the actin cytoskeleton as described previously (McKenna et al. 2019). Figure 5C shows

that upon expression of MKAKU41 at a moderate level (OD0.05), LatB depolymerisation

of the actin cytoskeleton resulted in a statistically significant increase in the number of

invaginations (P≤0.001). While this trend existed at lower (OD0.01) and higher

transfection concentrations (OD0.1), it was not statistically significant. To further explore

the connection between the actin cytoskeleton and MKAKU41 induced nuclear

deformations, we examined the interior versus peripheral signal ratios (Fig. S2) and

found that actin depolymerization quantitatively shifted the INM NE marker, LBR,

towards increased interior signal (Fig. 5D). This same effect was seen and found to be

statistically significant for two concentrations of MKAKU41 infiltration, OD0.01 (P≤0.01)

and 0.05 (P≤0.05). This demonstrates that at the moderate transfection concentration of

0.05OD, actin depolymerization with LatB increases the internalization of peripheral

markers. These findings corroborate those from the MLKS2ΔARM experiments in that

they implicate F-actin as a possible factor that can provide an opposing force or

counterbalance to nucleoskeletal proteins which can invaginate the NE and create

inclusion bodies in a concentration-dependent manner.

Transposon disruption of the MKAKU41 gene co-segregates with phenotypic

effects on nuclear shape and development.

Having seen that overexpression of maize MKAKU41 in a eudicot species resulted in

nuclear architecture disruption and severe nuclear envelope misplacement, we wanted

to examine the role of this gene in its native genetic background, maize. From the

UniformMu transposon mutagenesis project, we found and characterized a Mutator-

tagged allele of *MKAKU41*, allowing for a genetic examination of the biological

consequences of gene disruption in maize.

The transposon-tagged allele, here designated *mkaku41*, and its wild-type

counterpart, *MKAKU41*, are shown in Figure 6. The wild-type *MKAKU41* gene

(Zm00004b040444) from the color-converted W22 inbred is annotated as being

associated with three transcript models. The gene structure for transcript model T01

(Fig. 6A) spans 7.9 kb, with 11 exons producing an mRNA with a single large ORF

predicted to encode a protein of 579 AA. The transposon insertion site (mu1005806) is

located in the 5' UTR (Fig. 6B). The transposon insertion allele was characterized by

genomic PCR analysis (Fig. 6C) using various combinations of primers that were

flanking the insertion site, and were gene-specific and *Mutator*-specific (Fig. 6A, primers

F, T, R). These PCR products were visualized (Fig. 6C) and sequenced (Fig. 6D) from

the W22 wild type progenitor (W22+) as well as F2 individuals from families segregating

for *mkaku41* (+/+, +/-, or -/-), where the "-" symbol denotes the transposon-insertion

allele. These PCR primers and PCR gel products were used for plant genotyping in

subsequent analyses.

By inspection of the junction sequences between the wild-type reference W22

genome and the transposon (Fig. 6D, lower case letters), we identified the 9-bp target

site duplication as CTCCTCTC (color coded green in Fig. 6). These results confirmed

that the transposon was inserted in the 5' UTR at a position 23 bp upstream of the start

codon, disrupting the majority of the wild-type 95 bp 5' UTR. The location of this

insertion, while not in the protein coding region of the gene, is within the first exon and

its location is expected, therefore, to disrupt the expression or transcript structure of the

gene. Surprisingly, from transcriptome analysis we found that the gene expression

levels for MKAKU41, measured as total normalized reads across the gene model, were

similar in libraries made from wildtype and mutant leaf and tassel. We mined the

transcript data to investigate the effect of the transposon insertion on the 5' UTR region

by searching for the presence of a unique 25 bp 5' UTR sequence located just upstream

of the mapped insertion site (Fig. 6D,E, "Query sequence" highlighted in yellow). We

found a total of 70 matches to our 25 bp query sequence in our transcriptome, which

was sequenced at a depth of over 100M reads per tissue-genotype combination (Fig.

6E). All 70 occurrences of the query sequence were from the wildtype libraries except

for one, which was on the reverse strand relative to the gene (Fig. 6E). These results

indicate that the 5' UTR was indeed disrupted in the mutant plants. In addition to this

detailed analysis of MKAKU41, some differentially expressed genes (Table S2) were

observed in mutant versus wild-type leaf and meiotic-enriched whole tassel, but gene

ontology analysis did not reveal any clear and reproducible enrichments that differed

from those of randomized controls.

We next explored the phenotypic consequences of the *mkaku41* transposon

insertion on root hair nuclei, stomatal complex, and pollen viability, as summarized in

Figure 7. The root hair nuclei in W22+ (normal) and *mkaku41* mutant seedlings 5 days

after imbibition were imaged and their shapes were analyzed (Fig. 7A-F). The mutant

nuclei were visually and quantitatively more rounded than their wildtype counterparts.

The mutant nuclei had an average maximum length of 22 μm whereas their wild-type

counterparts averaged 34 μm (Fig. 7G). The mutant nuclei also exhibited a higher

circularity index than the wildtype nuclei (Fig. 7H). Both measures (n=50) were

statistically significant as determined using T-test, two-tailed with p<0.0001.

Next, we analyzed two above-ground phenotypes, the appearances of stomatal

complexes and pollen. In W22+ plants, a normal stomatal complex is composed of two

guard cells flanked by two subsidiary cells as shown for W22+ (Fig. 7I). In contrast,

mutant plants showed irregular stomatal complexes composed of two normal-looking

guard cells flanked by one or two extra and irregularly positioned subsidiary cells

(arrows, Fig.7 J-N). For the pollen phenotypes, we assayed viability and shape (Fig. 7O-

R). Using the modified Alexander's differential staining method, we found that the

percent of viable pollen was dramatically reduced in the mutant, from 84% to 46%. The

shape of the pollen was also affected in the mutant, where the average degree of

roundness decreased from 0.93 to 0.7. Both measures (n>1,000 for staining, n=100 for

roundness) were statistically significant as determined using a two-tailed T-test with

p<0.0001.

Taken together, these findings show that the *mkaku41* mutation was associated

with multiple phenotypes including root hair nuclear shape, stomatal complex

development, and pollen viability. Therefore, MKAKU41 appears to act in some of the

same genetic pathways as the NE-associated LINC complex proteins such as SUN and

KASH. This genetic data in maize is interesting when considered with our findings that

heterologous overexpression phenotypes and actin perturbations (Figs. 1-5) disrupt

nuclear architecture and nuclear envelope organization. Taken together, all of these

experiments establish biological roles for MKAKU41, NCH1, and NCH2 as

nucleoskeletal proteins that regulate fundamental nuclear processes in cellular structure

and function.

DISCUSSION

Regulation of nucleus size and shape is important for many fundamental cellular

processes in all eukaryotes. Nuclear architecture is controlled by multiple interactions

involving the NE, NE-associated complexes, and the nucleoskeleton. Here, we

characterized multiple maize nucleoskeletal proteins, which, like their animal

counterparts, controlled nuclear dynamics. An overarching goal motivating this study is

to establish the general rules that apply across the plant domain, an evolutionarily vast

space. Towards this goal, we have utilized the tobacco transient heterologous

expression assay as a powerful and versatile experimental platform for plant nuclear

envelope research. In this study and previously, we have established cellular

localization, protein-protein interactions, dose-response phenotypes, and live cell

imaging that allows for kymographic analysis, mobility via FRAP, and interactions via

AP-FRET, all of which have enabled and accelerated our understanding of grass and

model crop NE biology (Gumber et al. 2019b, a).

The maize nucleoskeletal proteins examined in this study are NCH1, NCH2 and

MKAKU41, each of which has one or more homologs (Fig. 1A) in eudicot species

(Gumber et al. 2019a) and all of which exhibit nuclear localization and NE enrichment in

heterologous expression systems. Interaction data from this (Fig. 3C) and prior studies

further indicate that these proteins are coupled to the NE via the LINC complex. These

findings, together with those from other plant species, point to the broad conservation of

plant nucleoskeletal proteins across angiosperms (Goto et al. 2014; Meier et al. 2017;

Ciska et al. 2019; Sakamoto 2020). The functional conservation of these components is

evidenced by previously reported cross-species functional rescue (Gumber et al. 2019b)

and by the current study where we show that the maize MKAKU41 (Fig. 4) interacts with

Arabidopsis AtSUN2, causing altered nuclear localization of the Arabidopsis AtSUN2.

Multiple lines of evidence for conservation of plant LINC complexes are also

seen at the organismal and phenotypic level. For instance, we (Fig. 7) and others found

that mutant phenotypes commonly include the rounding up of root hair nuclei, disruption

of stomatal complex development, and effects on pollen shape and viability (Dittmer et

al. 2007; Goto et al. 2014, 2020; Zhou et al. 2014; Gumber et al. 2019b; Newman-Griffis

et al. 2019). The nuclear shape defects in root hairs have become a hallmark of LINC

defects in plants, and as such were predicted. However, the stomatal complex and

pollen shape phenotypes have not been previously observed for plant mutants of

MKAKU4 genes, but they resemble to some extent those of the plant KASH mutant,

mlks2 (Gumber et al. 2019b). To gain genetic insight into these nucleoskeletal proteins

in the crop species maize, we searched for transposon-disrupted alleles of MKAKU41,

NCH1, and NCH2. Of these, we found that the *MKAKU41* gene was reported to have a

Mutator insertion (McCarty and Meeley 2009), described here as the first known mutant

allele, *mkaku41*. The insertion site was in the 5'-UTR (Fig. 6), a common hot-spot for Mu

insertion (Zhang et al. 2020). The transcript abundance was not significantly reduced in

the mutants, but the mutant allele produced an extremely truncated 5' UTR of 23 bp or

less. Given that the median 5' UTR length in maize was recently determined to be 132

bp (Leppek et al. 2018), such an extremely short 5' UTR in the *mkaku41* mutants may

abolish or greatly decrease the ability of the cell to utilize the native start codon for

translation of the full-length protein. If the mutant 5' UTR is too short for efficient

ribosome assembly and scanning, the next in-frame start codon is considerably farther

downstream, which would result in a loss of the first 125 AA. Additionally, the mutant

5'UTR may lack regulatory mRNA sequences in the first ~70 bases of the full-length

transcript. Further genetic and experimental analyses with new alleles, gene editing, or

application of specific biotic or abiotic stresses will be needed to gain a better

understanding of how these plant nucleoskeletal proteins functionally interact with the

genomes they help to organize.

The regulation of nuclear morphology and intra-nuclear organization was further

explored to gain mechanistic insight, using the tobacco transient expression assay with

fluorescently tagged proteins and quantitative microscopic analyses. We used this

approach to explore multiple aspects of the remarkable nuclear architecture disruption

caused by overexpression of each of the three maize nucleoskeletal proteins examined.

The severity of the nuclear disruption and of the NE invaginations was increased by co-

overexpression of two components (e.g. MKAKU41 with NCH1 or NCH2), or by

increasing the transfecting plasmid concentration, expected to increase their expression

levels. These findings (Fig. 2) and previous studies from plants and animals reveal that

proper nucleoskeleton protein concentration may be a primary determinant for overall

nuclear architecture (Legartová et al. 2014; Goto et al. 2014; Jorgens et al. 2017).

In addition to protein abundance, components of the nuclear invaginations were

tested for the presence of LINC and ER proteins. Knowing that SUN proteins interact

with ONM KASH proteins as part of the core LINC complex, we tested whether the

intranuclear foci of MKAKU41-FP reflected protein aggregates of entire NE, checking for

colocalization with two types of markers, those in the NE but not the LINC complex or

those in the ER membrane. All of these, including multiple ONM markers, colocalized

with the aberrant intranuclear structures (Figs. 4 and 5), demonstrating that these

intranuclear structures contain components from both the INM and ONM of the nuclear

envelope. These plant nuclei invaginations may contain, therefore, the entire NE

proteome as well as NE-associated chromatin that would normally be limited to the

nuclear periphery. Such invaginations are known to occur in plants and animals, which

can show grooves, deformations, actin, or ER in stable structures seen as deep

invaginations (Collings et al. 2000; Schermelleh et al. 2008). Interestingly, the

membrane invaginations and deformations caused by MKAKU41 also resemble to

some extent animal nuclear deformations associated with Lamin-A mis-expression

(Lammerding et al. 2004; Schreiber and Kennedy 2013; Swift et al. 2013; Legartová et

al. 2014).

In our experimental set up, we disrupted the LINC complex at two different

connections to investigate the effect of these disruptions on the NE structure. The first,

a SUN2ΔN, severed the LINC-to-nucleoskeleton connection; the second MLKS2ΔARM,

severed the LINC-to-cytoskeleton connection. It is quite interesting that these two

disruptions exhibited contrasting effects on the severity of invaginations. Our domain-

deletion analyses showed that perturbation of the LINC-to-nucleoskeleton connection

reduced the severity (Fig. 4A), whereas preventing the LINC-to-cytoskeleton connection

increased the severity of invaginations (Fig. 5). This has important mechanobiological

implications for the idea that plant nuclear shape involves a balance of forces between

actin-nucleus interactions and nucleoskeletal components. Interestingly, AtKAKU4,

arabidopsis KASH proteins WIPs, and NE-associated myosin are all involved in nuclear

migration in various cellular processes, such as in pollen-tube growth (Goto et al., 2020,

Meier et al., 2017), which also involves changes in actin dynamics.

Moving the nucleus exerts physical stress on the NE and the opposing forces of

the LINC components (nucleoskeletal and cytoskeletal) are expected to be, therefore,

important for maintaining NE integrity and stability (Enyedi and Niethammer 2017). The

contrasting effects on NE integrity that we observe in this study are an indicator that

maize nucleoskeletal components may be functionally associated with just such a tug-

of-war process that manifests as regulation of nuclear shape. Multiple lines of evidence

are consistent with this idea, including the change to spherical nuclei caused by genetic

knockouts of a nuclear-envelope-localized myosin (Tamura et al. 2013) and the

rounding up of root hair nuclei caused by the maize *mlks2* mutation (Gumber et al.

2019b). Along these lines, our study adds to the growing body of evidence that plants

deploy a general mechanism for nuclear shape in which a balance of forces is achieved

through LINC-interacting components on both sides of the NE, ensuring its structure

and function as a flexible cellular partition. In the current study, we note multiple

indications (Fig. 5) that support this tug-of-war type arrangement. These ideas align

with results from mammalian studies that identify roles for the LINC complex in

mediating mechanical crosstalk between the cytoplasm and nucleus (Alam et al. 2016;

Jorgens et al. 2017; Hieda 2019; Agrawal and Lele 2019; Bouzid et al. 2019).

Previous investigations of CRWN and KAKU4 have focused on chromatin

structure and nuclear architecture (Dittmer et al. 2007; Grob et al. 2014; Hu et al. 2019),

but our studies indicate that nucleoplasmic disruptions can also affect normal

developmental processes, an important finding for crop species. In animals, the

interplay between cellular-level structural integrity and genomic responses to

environmental and developmental processes is increasingly recognized as a complex

process involving lamins as central players (Gerbino et al. 2018). This study advances

our knowledge of the plant nucleoskeleton by identifying the components and their roles

in regulating fundamental dynamic processes of the plant nuclear envelope.

METHODS

Cloning

Maize gene constructs and sequence information for the clones used in this study are

listed in Table S3. NCH1 ORF was custom-synthesized with *Bam*HI and *Sbf*I at the 5'

and 3' ends, respectively (Genscript Biotech Corporation, NJ). The *Bam*HI-NCH1-*Sbf*I

construct was sub-cloned by restriction cloning into an ECGFP donor vector containing

eGFP-FLAG-HA (Gumber et al. 2019, JCB), to create the eGFP-FLAG-HA-NCH1 entry

vector, named NCH1ec. Similarly, the *Bam*HI-NCH2-*Pst*I construct was custom

synthesized and sub-cloned into an ECGFP donor vector to create the eGFP-FLAG-HA-

NCH2 entry vector, named NCH2ec. For construction of the mKAKU41vector, *Bam*HI-

mCherry-FLAG-HA-MKAKU41-*Bam*H1 was synthesized by Genscript and cloned in

pUC18 at the *Bam*HI restriction site. From this cloning vector, the mCherry-FLAG-HA-

MKAKU41 gene construct was amplified using KAKUattF (5'-

GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTAGCAAGGGAGAAGAGG-3')

and KAKUattR (5'-

GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACGTAGCCCGTCCCCGT-3')

primers and inserted into pDONR221 vector by BP cloning (Invitrogen), to generate the

MKAKU41 entry clone, named MKAKU41ec. For the generation of plant expression

vectors, the fluorescent fusion protein constructs from these three entry clones were

then transferred individually to the destination vector pH7WG2 (Karimi et al. 2002) by

Gate LR recombination (Invitrogen).

For production of the p35S::SP-mCherry-GFP-HDEL positive control for apFRET,

mCherry was PCR amplified with Q5 polymerase (NEB) using primers JM403 (5'-

GGGGACAAGTTTGTACAAAAAAGCAGGCTACAATGAAAGCCTTCACACTCGCTCTC

TTCTTAGCTCTTTCCCTCTATCTCCTGCCCAATCCAGCCATGGTGAGCAAGGGCGA

GGAGG-3') & JM404 (5'-acctccactgccaccCTTGTACAGCTCGTCCATGCCG-3'). The

primers included both the Gateway cloning attB1 site and secretion signal at the 5' end,

and a 15nt overhang for Gibson assembly at the 3' end, which contained a GGSGG

amino acid linker between mCherry and GFP upon fusion. GFP was amplified using

primers JM367

(5'GGGGACCACTTTGTACAAGAAAGCTGGGTGtcataattcatcatGCTTGTACAGCTCGT

CCATGCCGAGAG-3') & JM405

(5'GTACAAGggtggcagtggaggtATGGTGAGCAAGGGCGAGGAGC-3') which contained

the GGSGG linker at the 5' end, and the HDEL ER retention motif, an attB2 site, and a

stop codon at the 3' end. These two products were fused together using the NEB HIFI

Gibson assembly enzyme mix and incubated at 50°C for one hour. A Gateway BP

reaction into pDONR221 and subsequent LR reaction into pB7FWG2 was then

performed to produce the final vector. All steps confirmed by colony PCR and

sequencing.

Agrobacterium transformation

Constructs were transformed into A. tumefaciens GV3101. Transformation was

performed by incubating plasmid DNA and chemically competent agrobacterium on ice

for 30 minutes, followed by 5 minutes cold shock in liquid nitrogen, then 5 minutes heat

shock at 37 in a rotating incubator. After heat shock, 200μL LB media was added and

cells incubated at 28 for two hours. Cells were then plated in LB plates containing

Spectinomycin (50μg/mL), Gentamycin (10 μg/mL) and Rifampicin (25 μg/mL) and

incubated for two days at 28°C. Individual colonies were then picked, grown O/N and

transformed into *N. benthamiana*.

Plant growth conditions

533   *N. benthamiana* plants were grown in 16:8h light:dark cycle in a greenhouse maintained

535   at 21 °C. Infiltrated plants were 5-6 weeks old.

Live cell imaging

Fluorescently tagged proteins of interest were transiently transformed into *N.*

benthamiana as described previously (Sparkes et al. 2006). Protein expression

constructs first reported here are p35S::NCH1-GFP, p35S::NCH2-GFP and

p35S::MKAKU41-mCherry. All other markers have been previously published:

p35S::GFP-CNX (Irons et al. 2003), p35S::LBR-GFP (Irons et al., 2003), p35S::MLKP1-

GFP (Gumber et al. 2019a), p35S::YFP-AtSUN2 (Graumann et al. 2010), p35S::YFP-

AtSUN2ΔNterm (Graumann et al. 2010), p35S::MLKS2-GFP and p35S::MLKS2ΔARM-

GFP (Gumber et al. 2019b). An agrobacterium culture of OD 0.1 was used in all

conditions unless otherwise stated and cells were imaged three days after

transformation. The GFP / mCherry combinations were imaged using a Zeiss LSM 800

confocal microscope with line switching, 488nm and 561 nm excitation, and 500-550nm

and 565-620nm emissions, collected for GFP and mCherry respectively. For GFP / YFP

and YFP / mCherry imaging, a Zeiss LSM 880 was used with frame switching. For GFP

/ YFP imaging, 488nm and 514nm excitation was used with emission collected between

500-550 and 525-560 for GFP and YFP respectively. For YFP / mCherry imaging,

514nm and 561nm excitation was used, and emission collected between 517-560nm

and 561-624 nm respectively. An image size of 512x512 pixels with a scan zoom of 4

and a 63x 1.4NA lens was used for all imaging described above. All combinations were

performed with three independent experimental repeats; representative images are

shown. For Fluorescence recovery after photobleaching (FRAP) a 100x 1.4NA lens was

used with a 4μm ROI in the center of the image, encompassing the nucleus. Five scans

were taken pre-bleach and then the 488nm laser bleached the ROI by using 100%

transmission for 20 iterations. Recovery Images were then collected for one minute to

monitor recovery. Data was normalised and FRAP curves produced as described

previously (Martiniere et al. 2012).

For acceptor photobleaching förster resonance energy transfer (apFRET) a 100x

1.4NA lens was used with a 4μm ROI in the center of the image, encompassing the

nucleus. Five scans were performed with both GFP and mCherry emission / excitation,

and then the mCherry construct was bleached in the ROI by the 561nm laser at 100%

transmission for 20 iterations. Following this, five post-bleach scans were taken. Data

was normalized and apFRET efficiency (%) calculated as previously (Graumann et al.

2010; Graumann 2014; Pawar et al. 2016). A minimum of 30 nuclei per condition were

used for apFRET across three experimental repeats. A one-way ANOVA was performed

to determine statistically significant differences between samples. For Latrunculin-B

(LatB) treatment for depolymerisation of the actin cytoskeleton, samples were incubated

with 25 μM LatB for one hour, as this has previously been shown to depolymerise the

actin cytoskeleton sufficiently (McKenna et al. 2019). Graphs were generated with

graphpad and as described in the figure legends.

Maize plant material and genotyping

The wild-type W22 used in this study is a color-converted W22 line obtained from Hugo

Dooner (Waksman Inst., Rutgers, New Jersey, USA) derived by (Brink 1956). The UF-

Mu-00395 seed stock was obtained from the Maize Genetics Cooperation Stock Center

(http://maizecoop. cropsci.uiuc.edu/). The plants were grown at the Florida State

University Mission Road Research Facility (Tallahassee, FL, USA) during summer 2017

and 2018, and propagated by out- crossing to W22. In the fall of 2018, the progeny

seeds were grown in the greenhouse in the King Life Sciences Building (Biological

Science Dept, Florida State University, Tallahassee, FL, USA). The segregating plants

were self-crossed to obtain mutant plants from among the progeny.

DNA was isolated from 4-week old seedlings as described previously in (Gumber

et al. 2019b). PCR genotyping was carried out using a combination of gene-specific

forward (F, 5'-CCCGTGAAGCCGAAGGCAGA-3') and reverse (R, 5'-

CGCCTCACGCTCACGCTCAC-3') primers, or transposon-specific Tir6 primer (5'-

AGAGAAGCCAACGCCAWCGCCTCYATTTCGTC-3') in combination with F or R

primer. The PCR products were resolved by agarose gel electrophoresis and cloned in

pCRTM4Blunt-TOPO® Vector (Invitrogen cat # K2875-20) by TA cloning. The clones

were sequenced and the insert sequences were verified using M13F and M13R vector

primers at the Molecular Cloning Facility, Department of Biological Sciences, Florida

State University. The sequences were aligned with the W22v2 reference genome to

validate the transposon insertion site.

Microscopy in maize

Maize root hair imaging was carried out as described in (Gumber et al. 2019b). Briefly,

roots were harvested from 5-day old seedlings and fixed for 1 hour in Buffer A (Howe et

al. 2013) supplemented with 4% paraformaldehyde. Small sections of root tissue

containing root hair were stained with 3 µg/mL DAPI for 20 min at room temperature,

mounted with VECTASHIELD, and imaged on an EVOS fluorescence microscope

(Thermo Fisher Scientific). The images were processed using the Analyze Particle

function of ImageJ to measure the longest diameter and circularity of the nuclei.

For stomatal complex imaging, plants were grown in the greenhouse and the 4th

leaf was harvested at its first appearance. The harvested leaf was fixed in Buffer A with

4% paraformaldehyde for an hour at room temperature with rotation. The tissue was

rinsed thrice with and stored in Buffer A at 4C, until further use. The leaf tissue was

placed on a glass slide, chopped into small pieces and stained with 3 µg/mL DAPI for

20 min at room temperature, mounted with VECTASHIELD, and imaged on an EVOS

fluorescence microscope (Thermo Fisher Scientific).

Pollen grain staining was carried out as previously described in (Gumber et al.

2019b). Briefly, male flowers were harvested before dehiscence and fixed in Carnoy's

fixative (6 alcohol:3 chloroform:1 acetic acid) for a minimum of 2 hours at room

temperature. Anthers were extruded from flowers with the help of a micro scalpel and

forceps on a glass slide. Staining was carried out with modified Alexander's stain

containing Malachite green (0.01%), Acid Fuchsin (0.05%) and Orange G (0.005%) as

described to differentiate viable (magenta) pollen grains from aborted (green) pollen

grains. Bright field images of the pollen grains were collected on Revolve microscope

(Echo Labs). At least 300 pollen grains each from 3 plants of every genotype were

counted to calculate pollen viability. Pollen roundness was carried out using Fiji.

RNA isolation and library preparation

Segregating wildtype and mutant mkaku41 plants were grown in the greenhouse. From

two week-old plants, fourth leaves were harvested and from 6-8 week old plants, mid-

prophase meiotic-staged male flowers were harvested. The tissues were immediately

stored in liquid nitrogen. RNA was isolated from three biological replicates for each

genotype using Qiagen RNeasy Plant mini kit per manufacturer's instructions. Integrity

of the RNA was tested using the Bioanalyzer (Agilent) system. For library preparation,

sample input was 400 ng total RNA (determined by Qubit RNA HS reagents, Thermo)

with RIN >7 (Bioanalyzer RNA Nano, Agilent). Libraries were prepared with the Biomek

400 Automated Workstation (Beckman Coulter), using the NEBNEXT Ultra II RNA

Library Prep kit for Illumina (New England Biolabs) according to manufacturer's

instructions, with an RNA fragmentation time of 15 minutes, a $1/10^{th}$ dilution of NEB

adaptor and 11 cycles of PCR amplification with dual-indexing primers. Amplified

libraries were initially quantified by Qubit DNA HS reagents, checked for size and

artifacts using Bioanalyzer DNA HS reagents, and KAPA qPCR (KAPA Biosystems)

was used to determine molar quantities of each library. Individual libraries were diluted

and pooled equimolar, and the pool was again checked by Bioanalyzer and KAPA

qPCR before submission for sequencing.

RNA sequencing and data analysis

RNA-seq libraries were sequenced on a Novaseq 6000 at the Translational Science

Lab, College of Medicine, Florida State University. Approximately 40 million single-end

100 base reads were obtained for each biological replicate in this experiment and are

available from NCBI sequence read archive project, accession number PRJNA675860.

Contaminating 3' adapter sequences were trimmed from the demultiplexed raw reads

using cutadapt version 1.16. Raw and trimmed reads were subjected to quality control

testing with fastqc. Trimmed reads were aligned to the W22 genome assembly "Zm-

W22-REFERENCE-NRGENE-2.0" using the splice-aware aligner hisat2. Briefly, Hisat2

indices were constructed from known exons, and splice sites extracted from the W22

genome annotation (Zm00004b) and the reference genome assembly (Zm-W22-

REFERENCE-NRGENE-2.0.fasta). Trimmed reads were then aligned to the resulting

splice-aware hisat2 index using the following optional arguments: --rna-strandness R, --

dta-cufflinks, --summary-file. Predicted novel transcripts were assembled and merged

across replicates and samples using stringtie2 in "conservative" mode. Per-transcript

coverage tables were prepared by stringtie2 in "ballgown" format. Resulting coverage

tables were converted into count tables suitable for differential expression analysis by

DEseq2 in R using the tximport package. Differential expression analysis was

performed separately for each tissue group i.e (leaf mutant vs. WT and tassel mutant

vs. WT). Briefly, genes with fewer than 10 counts across all replicates were discarded

and DEseq2 results were generated for both tissue groups such that log2(fold-change)

estimates were reported for (mutant/WT) ratios. Statistically significant differentially

expressed (adjusted p-value <0.05) genes were subsequently extracted from each of

the resulting DEseq2 tables for further analysis (Table S2).

Acknowledgments

We would like to acknowledge the Bioimaging unit at Oxford Brookes for access to the

confocal microscopes. This work was performed as part of the Cost action # CA16212

'INDEPTH' whose members are appreciated for their fruitful discussions. This work was

supported in part by a grant to HWB from the National Science Foundation (NSF IOS

1444532).

FIGURE LEGENDS

Figure 1: The maize proteins NCH1, NCH2 and MKAKU41 localize to the nuclear

envelope. (A) Maximum likelihood phylogenetic tree showing maize NCH1 and NCH2

along with homologous proteins CRWN1-4 from Arabidopsis. (B) Confocal imaging of *N.*

benthamiana transiently expressing NCH1, NCH2 and MKAKU41 as fluorescent protein

fusions (green / magenta) and nuclei labelled with DAPI (blue). Both MKAKU41 and

NCH1 show nuclear invaginations (white arrowheads) and labelling at the nuclear

envelope (white boxes). NCH2 shows predominantly nuclear peripheral localization.

Scale bar denotes 5µm. (C) FRAP analysis demonstrates that NCH1 has a larger

mobile fraction than NCH2 at the nuclear periphery. N ≥ 30 nuclei imaged across three

experimental replicates.

Figure 2: The concentration of NCH1, NCH2 and MKAKU41 affects the level of

nuclei invagination and disruption. Confocal live cell imaging of NCH1, NCH2 and

MKAKU41 shows that increasing the concentration of NCH1 and MKAKU41 increases

nuclei disruption. Nuclei were classified as per (Goto et al. 2014) into Type I, Normal

nuclear envelope localization; Type II, Minor invaginations and predominantly nuclear

envelope localization; or Type III, major disruption of nuclei structure . (A) NCH1, (B)

NCH2 and (C) MKAKU41. Scale bar denotes 5µm. N≥ 30 nuclei imaged across three

experimental replicates.

Figure 3: Coexpression of NCH1 or NCH2 with MKAKU41 enhances nuclear

invaginations and NCH1 and NCH2 interact with MKAKU41. (A) Confocal images of

N. benthamiana coexpressing NCH1 or NCH2 with MKAKU41. (B) Quantification of

nuclear morphologies for nuclei expressing single MKAKU41, NCH1, NCH2; or co-

expression of NCH1 or NCH2 with MKAKU41; classified into types I, II, or III as

described in Figure 2. Scale bar denotes 5µm. (C) The apFRET efficiency (%)

demonstrates *in planta* interactions of NCH1 and NCH2 with MKAKU41 in comparison

with control (CNX). Solid lines underneath plots denote negative controls, hashed

positive control. Key statistical comparisons shown and more fully tabulated in Table

S1. Red line denotes mean, blue standard deviation. One-way ANOVA statistical test

performed. **** = P ≤ 0.0001. N≥ 30 nuclei imaged across three experimental replicates

for all experiments described.

Figure 4: MKAKU41-induced nuclear invaginations incorporate other nuclear

envelope- and ER-localized proteins. (A) Confocal imaging showing that the outer

nuclear Maize membrane markers MLKP1, Arabidopsis nuclear envelope proteins

SUN2 and SUN2ΔNterm, and the ER membrane marker CNX are incorporated into

invaginations when co-expressed with MKAKU41. Next to confocal images are

percentages of nuclei which show deformations as classified previously. (B) SUN2 and

SUN2ΔNterm show different incorporation into MKAKU41 induced nuclear

invaginations. Graphs show 4µm normalised line profiles over SUN2 or SUN2ΔNterm

Invaginations (solid white lines) and nuclear membrane (dotted white lines). Locations

of line profiles can be seen in confocal micrographs. (C) Ratio of Sub-periphery/whole

nuclei fluorescence of SUN2 and SUN2ΔNterm when expressed on their own or with

MKAKU41. **** = P ≤ 0.0001. N ≥ 30 for each condition.

Figure 5: Nuclear actin anchoring is important for regulating nuclei deformations

upon MKAKU41 overexpression. (A) Confocal live cell imaging of MLKS2 and

MLKS2ΔARM single and co-expresssion with MKAKU41. (B) All nuclei imaged were

categorised on the level of disruption as described previously. (C) Number of nuclei

invaginations at different MKAKU41 expression levels with actin depolymerised (LatB).

(D) Sub-periphery / whole nuclei fluorescence ratio from nuclei expressing different

levels of MKAKU41 (MK, from 0 to 0.1) with and without actin depolymerisation (LatB).

Nuclei were imaged with the NE marker LBR-GFP. Scale bar denotes 5μm. N≥ 30

nuclei imaged across three experimental replicates for panel A & B). For Panel C & D ≥

60 across three biological replicates.

Figure 6. Gene structure of wild-type and transposon-tagged alleles of *MKAKU41*.

(A) Gene model of MKAKU41 (transcript model "T01") showing the positions of exons

(black boxes), introns (grey), the 5' and 3' UTRs, the transposon-insertion (Mu),

transposon-specific Tir6 PCR primers (T arrows), and the Mu-flanking gene-specific

PCR primers (F, R arrows). (B) Diagram of the MuDR insertion site within the 95 bp 5'

UTR, showing the locations of the 9 bp target site repeat (green, bp 64-72 in the

mkaku41-T01 transcript model) and a 25 bp query sequence (yellow) used for transcript

analysis. (C) PCR Genotyping for presence or absence of the Mu-tagged allele. The

PCR products using various pairings of gene-specific (F, R) or transposon-specific

primer pairs (T). Ethidium bromide-stained agarose gel 100 bp size marker (lane M) and

single-plant PCR products (other lanes) from W22+ or select *mkaku41* F2 siblings to

illustrate wildtype (+/+), heterozygous (+/-), or mutant (-/-) PCR genotype patterns from

the PCR (arrows at right of gel). (D) Sequence of cloned and sequenced amplicons are

aligned and show the progenitor W22 (W22 reference) and F2 segregants. The +/+

individuals from the transposon mutagenesis stocks were found to be heterozygous for

a 2 bp indel (--). The sequences corresponding to a 25 bp query sequence (yellow

highlight), the 9 bp insertion site (green highlight), the transposon (lowercase, garnet

text), and the start codon (underlined ATG) are indicated. The Mu insertion occured in

the allele with the 2bp deletion and produced a flanking 9bp target site duplication. (E)

Strand-specific transcriptome analysis is summarized for perfect match occurrences of

the query sequence in libraries made from wildtype (top) or mutant (bottom) plants. All

of the sense transcripts from the mutant allele had an extremely short (~21 bp or less)

5'UTR.

Figure 7. Somatic phenotypes of *mkaku41*. DAPI-stained root hair nuclei in W22+ (A-

C) and mkaku41 (D-F) seedlings. (G) Longest diameter of W22+ and mkaku41 root hair

nuclei (n=50). (H) Nuclear circularity index measurements using $4\pi(area/perimeter^2)$,

where 1= perfect circle, of W22+ and mkaku41 root hair nuclei calculated using Fiji. (I)

Mature stomatal complex in W22+ DAPI-stained leaf where two central dumbbell-

shaped guard cells (GC) are surrounded by two subsidiary cells (SC). (J-N)

Representative images of stomatal complexes in *mkaku41* DAPI-stained leaves. Arrows

point to extra or irregularly-placed subsidiary cells. Scale bars are 10 μm. *****Student's

t-test two-tailed, p < 0.0001. (O-Q) Differential staining of anthers for testing pollen

viability from W22+ (O) and mkaku41 -/- (P,Q) tassels stained with modified Alexander's

stain where viable pollen grains appear magenta and aborted pollen grains appear

green. (R) Quantification of pollen viability, n>1000 per genotype. (S) Degree of pollen

roundness 4*area/(π*major_axis^2) calculated for W22+ and mkaku41-/- anthers using

Fiji. Scale bars are 50 μm. *****Student's t-test two-tailed, p < 0.0001.

Legends for Supplemental Figures

Figure S1: The Maize NCH1 and NCH2 proteins co-localize with their Arabidopsis homolog CRWN1. Confocal live cell imaging of cells co-expressing either NCH1 or NCH2 with Arabidopsis CRWN1 shows colocalization. B) Scatter dot plot of plateau recovery values from NCH1 and NCH2 FRAP time course experiment. C) Scatter dot plot of halftime recovery values from NCH1 and NCH2 FRAP time course experiment. Scatter dot plot of NCH2 Scale bar denotes 5µm. N≥ 30 nuclei imaged across three experimental replicates.

Figure S2: Description of Sub-periphery / whole nuclei fluorescence ratio measurement. Diagram describing how the sub-periphery over whole nuclei measurement was determined using image J. Two regions of interest (ROIs) were generated, one encompassing the whole nuclei and one only sub-periphery nuclear fluorescence (yellow ROIs with hashed boundaries). The sub-periphery fluorescence value was then divided by the whole nuclei fluorescence value in order to obtain the ratio. If the majority of fluorescence is located at the periphery / nuclear envelope, this would result in a low ratio, conversely if most fluorescence was internal, this would result in a higher ratio.

Figure S3: Multiple Seq Alignment of Transcripts. A) The 5' UTR region and a small portion of the CDS are diagrammed as shown in Figure 5, and reversed (bottom

configuration) as aligned in the multiple sequence alignment. B) The multiple sequence

alignment displays all of the RNA-seq reads with a perfect match to the 25 bp query

sequence (yellow) using grep of the fastq files. All matches were converted to FASTA

sequences for multiple sequence alignment. The reference genome sequence is shown

at top for comparison. The sequence identifiers start with single characters for tissue

("L" for leaf; "T" for tassel), genotype ("1" for wildtype, "2" for mkaku41 homozygous

mutant), or bioreplicate ("A", "B", or "C" for bioreplicate 1, 2, or 3, respectively), followed

by unique identifier from Illumina sequence read name. The strandedness is indicated

relative to the gene model, with all antisense RNAs indicated (ANTISENSE, red text).

Supplementary Tables:

Table S1: Values for apFRET efficiency with Internal controls and multiple

comparisons of all treatments.

A) All apFRET efficiency % values for data presented in figure 3C including internal

control values. B) Tukey multiple comparison one way Anova dataset from all apFRET

data presented, including that presented in figure 3C.

Table S2: Differentially expressed genes between *mkaku41* mutant versus

wildtype plants for maize leaf and tassel.

Table S3: Plasmid information and Addgene IDs.

References

Agrawal A, Lele TP (2019) Mechanics of nuclear membranes. J Cell Sci 132:jcs229245.
https://doi.org/10.1242/jcs.229245

Alam SG, Zhang Q, Prasad N, et al (2016) The mammalian LINC complex regulates genome
transcriptional responses to substrate rigidity. Sci Rep 6:38063.
https://doi.org/10.1038/srep38063

Bouzid T, Kim E, Riehl BD, et al (2019) The LINC complex, mechanotransduction, and
mesenchymal stem cell function and fate. J Biol Eng 13:68.
https://doi.org/10.1186/s13036-019-0197-9

Brink RA (1956) A Genetic Change Associated with the R Locus in Maize Which Is Directed and
Potentially Reversible. Genetics 41:872–889

Chang W, Worman HJ, Gundersen GG (2015) Accessorizing and anchoring the LINC complex
for multifunctionality. J Cell Biol 208:11–22. https://doi.org/10.1083/jcb.201409047

Choi J, Strickler SR, Richards EJ (2019) Loss of CRWN Nuclear Proteins Induces Cell Death
and Salicylic Acid Defense Signaling. Plant Physiol 179:1315–1329.
https://doi.org/10.1104/pp.18.01020

Ciska M, Hikida R, Masuda K, Moreno Díaz de la Espina S (2019) Evolutionary history and
structure of nuclear matrix constituent proteins, the plant analogues of lamins. J Exp Bot
70:2651–2664. https://doi.org/10.1093/jxb/erz102

Ciska M, Moreno Dí-az de la Espina S (2014) The intriguing plant nuclear lamina. Front Plant
Sci 5:. https://doi.org/10.3389/fpls.2014.00166

Collings DA, Carter CN, Rink JC, et al (2000) Plant Nuclei Can Contain Extensive Grooves and
Invaginations. 16. https://doi.org/10.1105/tpc.12.12.2425

Crisp M, Liu Q, Roux K, et al (2006) Coupling of the nucleus and cytoplasm: Role of the LINC
complex. J Cell Biol 172:41–53. https://doi.org/10.1083/jcb.200509124

De Magistris P, Antonin W (2018) The Dynamic Nature of the Nuclear Envelope. Curr Biol
28:R487–R497. https://doi.org/10.1016/j.cub.2018.01.073

Dittmer TA, Stacey NJ, Sugimoto-Shirasu K, Richards EJ (2007) *LITTLE NUCLEI* Genes
Affecting Nuclear Morphology in *Arabidopsis thaliana*. Plant Cell 19:2793–2803.
https://doi.org/10.1105/tpc.107.053231

Enyedi B, Niethammer P (2017) Nuclear membrane stretch and its role in mechanotransduction.

Nucleus 8:156–161. https://doi.org/10.1080/19491034.2016.1263411

Evans DE, Graumann K (2018) THE LINKER OF NUCLEOSKELETON AND CYTOSKELETON

COMPLEX IN HIGHER PLANTS. https://doi.org/10.1002/9781119312994.apr0617

Gerbino A, Procino G, Svelto M, Carmosino M (2018) Role of Lamin A/C Gene Mutations in the

Signaling Defects Leading to Cardiomyopathies. Front Physiol 9:1356.

https://doi.org/10.3389/fphys.2018.01356

Goto C, Tamura K, Fukao Y, et al (2014) The Novel Nuclear Envelope Protein KAKU4

Modulates Nuclear Morphology in *Arabidopsis*. Plant Cell 26:2143–2155.

https://doi.org/10.1105/tpc.113.122168

Goto C, Tamura K, Nishimaki S, et al (2020) The nuclear envelope protein KAKU4 determines

the migration order of the vegetative nucleus and sperm cells in pollen tubes. J Exp Bot

71:6273–6281. https://doi.org/10.1093/jxb/eraa367

Graumann K (2014) Evidence for LINC1-SUN Associations at the Plant Nuclear Periphery.

PLoS ONE 9:e93406. https://doi.org/10.1371/journal.pone.0093406

Graumann K, Evans DE (2017) The Nuclear Envelope - Structure and Protein Interactions. In:

Roberts JA (ed) Annual Plant Reviews online. John Wiley & Sons, Ltd, Chichester, UK,

pp 19–56

Graumann K, Runions J, Evans DE (2010) Characterization of SUN-domain proteins at the

higher plant nuclear envelope. Plant J 61:134–144. https://doi.org/10.1111/j.1365-

313X.2009.04038.x

Grob S, Schmid MW, Grossniklaus U (2014) Hi-C Analysis in Arabidopsis Identifies the KNOT,

a Structure with Similarities to the flamenco Locus of Drosophila. Mol Cell 55:678–693.

https://doi.org/10.1016/j.molcel.2014.07.009

Gumber HK, McKenna JF, Estrada AL, et al (2019a) Identification and characterization of genes

encoding the nuclear envelope LINC complex in the monocot species *Zea mays*. J Cell

Sci 132:jcs221390. https://doi.org/10.1242/jcs.221390

Gumber HK, McKenna JF, Tolmie AF, et al (2019b) MLKS2 is an ARM domain and F-actin-

associated KASH protein that functions in stomatal complex development and meiotic

chromosome segregation. Nucleus 10:144–166.

https://doi.org/10.1080/19491034.2019.1629795

Guo T, Mao X, Zhang H, et al (2017) Lamin-like Proteins Negatively Regulate Plant Immunity

through NAC WITH TRANSMEMBRANE MOTIF1-LIKE9 and NONEXPRESSOR OF PR

GENES1 in Arabidopsis thaliana. Mol Plant 10:1334–1348.

https://doi.org/10.1016/j.molp.2017.09.008

Hagan I, Yanagida M (1995) The Product of the Spindle Formation Gene sad1+Associates with

887   the Fission Yeast Spindle Pole Body and Is Essential for Viability. 15

888   Haque F, Lloyd DJ, Smallwood DT, et al (2006) SUN1 Interacts with Nuclear Lamin A and

889   Cytoplasmic Nesprins To Provide a Physical Connection between the Nuclear Lamina

890   and the Cytoskeleton. Mol Cell Biol 26:3738–3751.

891   https://doi.org/10.1128/MCB.26.10.3738-3751.2006

892   Hetzer MW (2010) The Nuclear Envelope. Cold Spring Harb Perspect Biol 2:a000539–a000539.

893   https://doi.org/10.1101/cshperspect.a000539

894   Hieda M (2019) Signal Transduction across the Nuclear Envelope: Role of the LINC Complex in

895   Bidirectional Signaling. 12

896   Howe ES, Murphy SP, Bass HW (2013) Three-Dimensional Acrylamide Fluorescence In Situ

897   Hybridization for Plant Cells. In: Pawlowski WP, Grelon M, Armstrong S (eds) Plant

898   Meiosis: Methods and Protocols. Humana Press, Totowa, NJ, pp 53–66

899   Hu B, Wang N, Bi X, et al (2019) Plant lamin-like proteins mediate chromatin tethering at the

900   nuclear periphery. Genome Biol 20:87. https://doi.org/10.1186/s13059-019-1694-3

901   Irons SL, Evans DE, Brandizzi F (2003) The first 238 amino acids of the human lamin B

902   receptor are targeted to the nuclear envelope in plants. J Exp Bot 54:943–950.

903   https://doi.org/10.1093/jxb/erg102

904   Janin A, Bauer D, Ratti F, et al (2017) Nuclear envelopathies: a complex LINC between nuclear

905   envelope and pathology. Orphanet J Rare Dis 12:147. https://doi.org/10.1186/s13023-

906   017-0698-x

907   Jorgens DM, Inman JL, Wojcik M, et al (2017) Deep nuclear invaginations are linked to

908   cytoskeletal filaments – integrated bioimaging of epithelial cells in 3D culture. J Cell Sci

909   130:177–189. https://doi.org/10.1242/jcs.190967

910   Karimi M, Inzé D, Depicker A (2002) GATEWAY™ vectors for Agrobacterium-mediated plant

911   transformation. Trends Plant Sci 7:193–195. https://doi.org/10.1016/S1360-

912   1385(02)02251-3

913   Kim DI, Kc B, Roux KJ (2015) Making the LINC: SUN and KASH protein interactions. Biol Chem

914   396:295–310. https://doi.org/10.1515/hsz-2014-0267

915   Lammerding J, Schulze PC, Takahashi T, et al (2004) Lamin A/C deficiency causes defective

916   nuclear mechanics and mechanotransduction. J Clin Invest 113:370–378.

917   https://doi.org/10.1172/JCI200419670

918   Legartová S, Stixová L, Laur O, et al (2014) Nuclear Structures Surrounding Internal Lamin

919   Invaginations: MORPHOLOGY OF INTERNAL LAMINS. J Cell Biochem 115:476–487.

https://doi.org/10.1002/jcb.24681

Leppek K, Das R, Barna M (2018) Functional 5′ UTR mRNA structures in eukaryotic translation

regulation and how to find them. Nat Rev Mol Cell Biol 19:158–174.

https://doi.org/10.1038/nrm.2017.103

Luxton GG, Starr DA (2014) KASHing up with the nucleus: novel functional roles of KASH

proteins at the cytoplasmic surface of the nucleus. Curr Opin Cell Biol 28:69–75.

https://doi.org/10.1016/j.ceb.2014.03.002

Malone CJ, Fixsen WD, Horvitz HR, Han M (1999) UNC-84 localizes to the nuclear envelope

and is required for nuclear migration and anchoring during C. elegans development. Dev

Camb Engl 126:3171–3181

Martiniere A, Lavagi I, Nageswaran G, et al (2012) Cell wall constrains lateral diffusion of plant

plasma-membrane proteins. Proc Natl Acad Sci 109:12805–12810.

https://doi.org/10.1073/pnas.1202040109

Masuda K Peripheral Framework of Carrot Cell Nucleus Contains a Novel Protein Predicted to

Exhibit a Long a-Helical Domain. 9. https://doi.org/10.1006/excr.1997.3531

Masuda K, Xu ZJ, Takahashi S, et al (1997) Peripheral framework of carrot cell nucleus

contains a novel protein predicted to exhibit a long alpha-helical domain. Exp Cell Res

232:173–181. https://doi.org/10.1006/excr.1997.3531

McCarty DR, Meeley RB (2009) Transposon Resources for Forward and Reverse Genetics in

Maize. In: Bennetzen JL, Hake S (eds) Handbook of Maize. Springer New York, New

York, NY, pp 561–584

McKenna JF, Rolfe DJ, Webb SED, et al (2019) The cell wall regulates dynamics and size of

plasma-membrane nanodomains in *Arabidopsis*. Proc Natl Acad Sci 116:12857–12862.

https://doi.org/10.1073/pnas.1819077116

Meier I, Richards EJ, Evans DE (2017) Cell Biology of the Plant Nucleus. Annu Rev Plant Biol

68:139–172. https://doi.org/10.1146/annurev-arplant-042916-041115

Murphy SP, Simmons CR, Bass HW (2010) Structure and expression of the maize (Zea mays

L.) SUN-domain protein gene family: evidence for the existence of two divergent classes

of SUN proteins in plants. BMC Plant Biol 10:269. https://doi.org/10.1186/1471-2229-10-

269

Newman-Griffis AH, del Cerro P, Charpentier M, Meier I (2019) Medicago LINC Complexes

Function in Nuclear Morphology, Nuclear Movement, and Root Nodule Symbiosis. Plant

Physiol 179:491–506. https://doi.org/10.1104/pp.18.01111

Pawar V, Poulet A, Détourné G, et al (2016) A novel family of plant nuclear envelope-associated

proteins. J Exp Bot 67:5699–5710. https://doi.org/10.1093/jxb/erw332

Pradillo M, Evans D, Graumann K (2019) The nuclear envelope in higher plant mitosis and

meiosis. Nucleus 10:55–66. https://doi.org/10.1080/19491034.2019.1587277

Rothballer A, Kutay U (2013) The diverse functional LINCs of the nuclear envelope to the

cytoskeleton and chromatin. Chromosoma 122:415–429. https://doi.org/10.1007/s00412-

013-0417-x

Sakamoto Y (2020) Nuclear lamina CRWN proteins regulate chromatin organization, gene

expression, and nuclear body formation in plants. J Plant Res 133:457–462.

https://doi.org/10.1007/s10265-020-01184-1

Schermelleh L, Carlton PM, Haase S, et al (2008) Subdiffraction Multicolor Imaging of the

Nuclear Periphery with 3D Structured Illumination Microscopy. Science 320:1332–1336.

https://doi.org/10.1126/science.1156947

Schreiber KH, Kennedy BK (2013) When Lamins Go Bad: Nuclear Structure and Disease. Cell

152:1365–1375. https://doi.org/10.1016/j.cell.2013.02.015

Sparkes IA, Runions J, Kearns A, Hawes C (2006) Rapid, transient expression of fluorescent

fusion proteins in tobacco plants and generation of stably transformed plants. Nat Protoc

1:2019–2025. https://doi.org/10.1038/nprot.2006.286

Starr DA (2002) Role of ANC-1 in Tethering Nuclei to the Actin Cytoskeleton. Science 298:406–

409. https://doi.org/10.1126/science.1075119

Starr DA (2019) A network of nuclear envelope proteins and cytoskeletal force generators

mediates movements of and within nuclei throughout *Caenorhabditis elegans*

development. Exp Biol Med 244:1323–1332. https://doi.org/10.1177/1535370219871965

Starr DA, Fridolfsson HN (2010) Interactions Between Nuclei and the Cytoskeleton Are

Mediated by SUN-KASH Nuclear-Envelope Bridges. Annu Rev Cell Dev Biol 26:421–

444. https://doi.org/10.1146/annurev-cellbio-100109-104037

Swift J, Ivanovska IL, Buxboim A, et al (2013) Nuclear Lamin-A Scales with Tissue Stiffness and

Enhances Matrix-Directed Differentiation. Science 341:1240104–1240104.

https://doi.org/10.1126/science.1240104

Tamura K, Goto C, Hara-Nishimura I (2015) Recent advances in understanding plant nuclear

envelope proteins involved in nuclear morphology. J Exp Bot 66:1641–1647.

https://doi.org/10.1093/jxb/erv036

Tamura K, Iwabuchi K, Fukao Y, et al (2013) Myosin XI-i Links the Nuclear Membrane to the

Cytoskeleton to Control Nuclear Movement and Shape in Arabidopsis. Curr Biol

23:1776–1781. https://doi.org/10.1016/j.cub.2013.07.035

Wang H, Dittmer TA, Richards EJ (2013) Arabidopsis CROWDED NUCLEI (CRWN) proteins
are required for nuclear size control and heterochromatin organization. BMC Plant Biol
13:200. https://doi.org/10.1186/1471-2229-13-200

Zhang X, Zhao M, McCarty DR, Lisch D (2020) Transposable elements employ distinct
integration strategies with respect to transcriptional landscapes in eukaryotic genomes.
Nucleic Acids Res 48:6685–6698. https://doi.org/10.1093/nar/gkaa370

Zhao W, Guan C, Feng J, et al (2016) The *Arabidopsis CROWDED NUCLEI* genes regulate
seed germination by modulating degradation of ABI5 protein: CRWNs regulate seed
germination. J Integr Plant Biol 58:669–678. https://doi.org/10.1111/jipb.12448

Zhou X, Graumann K, Wirthmueller L, et al (2014) Identification of unique SUN-interacting
nuclear envelope proteins with diverse functions in plants. J Cell Biol 205:677–692.
https://doi.org/10.1083/jcb.201401138

1000

Figure 1 (McKenna, Gumber, et al., submitted Dec. 2020)

Figure 2
(McKenna, Gumber, et al., submitted Dec. 2020)

Figure 3 (McKenna, Gumber, et al., submitted Dec. 2020)

Figure 4
(McKenna, Gumber, et al., submitted Dec. 2020)

Figure 5
(McKenna, Gumber, et al., submitted Dec. 2020)

Figure 6 (McKenna, Gumber, et al., submitted Dec. 2020)

Figure 7
(McKenna, Gumber, et al., submitted Dec. 2020)

A)

B) Plateau value   **C)** Half-time

Figure S1
(McKenna, Gumber, et al., submitted Dec. 2020)

Figure S2
(McKenna, Gumber, et al., submitted Dec. 2020)

A. Gene orientation.

B. Multiple sequence alignment of all transcripts containing the 25 bp query sequence.

```
                                              (<start codon)                    (TE site)  (....25 bp query........)                                              (<TSS)
W22 REFERENCE SEQUENCE, T01    CGGCGATCGCCGGCGGCGAGCCCCGAAGAGGGACGCCATGGGAGGGGCGGAGGGATGGGGGGAGGAGGAGCAAGCAGCGTGAGCGTGAGCGTGAGGCGACGAAGGATTCGAAACGGAAGCGTGAGGCGATTTTGGGAGGGGCGCCAAGAAC

>T1C_SENSE_2232_9471_24972
>T1C_SENSE_2275_13792_35618
>T1B_SENSE_2234_7500_25066
>T1B_SENSE_2156_29125_22028
>T1B_SENSE_2240_31114_30013
>T1B_SENSE_2158_28447_2628
>T1B_SENSE_2210_1344_24549
>L1B_SENSE_2106_31060_8406
>L1B_SENSE_2244_6985_20635
>L1C_SENSE_2133_25201_14481
>L1C_SENSE_2133_24207_15107
>T1B_SENSE_2117_24813_11553
>T1C_SENSE_2144_1136_27383
>T1C_SENSE_2144_1118_27383
>T1C_SENSE_2144_1316_27289
>T1B_ANTISENSE_2267_13304_8563
>T1C_ANTISENSE_2267_14724_3818
>T1B_SENSE_2204_25843_30185
>T1B_SENSE_2127_9697_22232
>T1C_SENSE_2106_18873_11350
>L1B_ANTISENSE_2152_5493_30232
>T1B_SENSE_2262_27308_36198
>T1C_SENSE_2126_8847_1783
>T1C_SENSE_2126_8811_1752
>T2B_ANTISENSE_2243_5177_3724
>T1B_SENSE_2122_30083_34648
>T1B_SENSE_2158_5927_31829
>T1B_SENSE_2233_27579_4257
>T1B_SENSE_2245_16631_33176
>T1B_SENSE_2144_2094_4460
>T1C_SENSE_2136_3676_26866
>T1C_SENSE_2217_7581_33379
>L1C_SENSE_2260_24623_7247
>L1A_SENSE_2149_16514_30968
>T1C_SENSE_2175_28103_36542
>L1C_SENSE_2260_23466_6840
>T1B_SENSE_2170_26883_35149
>T1C_SENSE_2105_16315_16188
>T1C_SENSE_2132_29441_31062
>T1C_SENSE_2171_7771_28322
>T1C_SENSE_2101_30463_36119
>L1C_SENSE_2207_15185_32017
>T1B_SENSE_2144_31756_27336
>T1B_SENSE_2227_16740_10974
>T1B_SENSE_2277_16595_21527
>T1B_SENSE_2159_8350_14857
>L1B_SENSE_2218_25238_13823
>L1C_SENSE_2133_31087_28432
>L1C_SENSE_2133_31584_29230
>T1C_SENSE_2257_21594_5353
>T1B_SENSE_2250_10502_14638
>T1C_SENSE_2106_7346_19883
>T1B_SENSE_2123_6849_20431
>T1C_SENSE_2273_2257_12038
>T1C_SENSE_2273_2953_12305
>L1A_SENSE_2104_15148_9220
>T1C_SENSE_2228_15790_25614
>T1B_SENSE_2236_23339_24972
>L1B_SENSE_2244_15103_10238
>T1B_SENSE_2152_18548_18176
>T1B_SENSE_2151_22390_1908
>T1B_ANTISENSE_2224_8196_4852
>T1B_SENSE_2170_11089_32002
>L1A_SENSE_2224_6931_21324
>T1B_SENSE_2104_29261_22294
>L1C_ANTISENSE_2120_27489_3912
>T1B_SENSE_2222_23439_21731
>T1B_ANTISENSE_2212_26811_6872
>T1B_ANTISENSE_2242_28447_21449
>L1B_SENSE_2112_31430_337
```

Figure S3 (McKenna, Gumber, et al., submitted Dec. 2020)

Table S1: Values for apFRET efficiency with Internal controls and multiple comparisons of all treatments.

A)

	NCH1 & CNX	NCH1 & CNX IC	NCH1 & KAKU41	NCH1 & KAKU41 IC	NCH2 & CNX	NCH2 & CNX IC	NCH2 & KAKU41	NCH2 & KAKU41 IC	CNX & KAKU41	CNX & KAKU41 IC	mCherry-GFP-HDEL	mCherry-GFP-HDEL IC
Number of values	30	30	30	30	30	30	33	33	30	30	30	30
Mean FRET Efficiency (%)	1.4	-0.67	10	-0.94	3.3	-0.86	11	-0.76	6.6	-2.1	19	-0.9
Std. Deviation	3.7	0.53	2.4	0.3	2.7	0.4	3	0.35	4.6	0.63	6.8	1.3
Std. Error of Mean	0.67	0.097	0.43	0.055	0.5	0.074	0.53	0.061	0.84	0.11	1.2	0.23

B)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below thr	Summarized	P Value
NCH1 & CNX vs. NCH1 & CNX IC	2.073	-0.4343 to 4.580	No	ns	0.2208
NCH1 & CNX vs. NCH1 & KAKU41	-8.939	-11.45 to -6.432	Yes	****	<0.0001
NCH1 & CNX vs. NCH1 & KAKU41 IC	2.343	-0.1640 to 4.850	No	ns	0.0925
NCH1 & CNX vs. NCH2 & CNX	-1.874	-4.381 to 0.6331	No	ns	0.3694
NCH1 & CNX vs. NCH2 & CNX IC	2.263	-0.2439 to 4.770	No	ns	0.122
NCH1 & CNX vs. NCH2 & KAKU41	-9.607	-12.06 to -7.158	Yes	****	<0.0001
NCH1 & CNX vs. NCH2 & KAKU41 IC	2.163	-0.2862 to 4.612	No	ns	0.1434
NCH1 & CNX vs. CNX & KAKU41	-5.199	-7.706 to -2.692	Yes	****	<0.0001
NCH1 & CNX vs. CNX & KAKU41 IC	3.473	0.9662 to 5.980	Yes	***	0.0004
NCH1 & CNX vs. mCherry -GFP -HDEL	-17.14	-19.65 to -14.64	Yes	****	<0.0001
NCH1 & CNX vs. mCherry -GFP -HDEL IC	2.303	-0.2041 to 4.810	No	ns	0.1065
NCH1 & CNX IC vs. NCH1 & KAKU41	-11.01	-13.52 to -8.505	Yes	****	<0.0001
NCH1 & CNX IC vs. NCH1 & KAKU41 IC	0.2703	-2.237 to 2.777	No	ns	>0.9999
NCH1 & CNX IC vs. NCH2 & CNX	-3.946	-6.453 to -1.440	Yes	****	<0.0001
NCH1 & CNX IC vs. NCH2 & CNX IC	0.1905	-2.316 to 2.697	No	ns	>0.9999
NCH1 & CNX IC vs. NCH2 & KAKU41	-11.68	-14.13 to -9.230	Yes	****	<0.0001
NCH1 & CNX IC vs. NCH2 & KAKU41 IC	0.09053	-2.359 to 2.540	No	ns	>0.9999
NCH1 & CNX IC vs. CNX & KAKU41	-7.272	-9.779 to -4.765	Yes	****	<0.0001
NCH1 & CNX IC vs. CNX & KAKU41 IC	1.401	-1.106 to 3.907	No	ns	0.7958
NCH1 & CNX IC vs. mCherry -GFP -HDEL	-19.22	-21.72 to -16.71	Yes	****	<0.0001
NCH1 & CNX IC vs. mCherry -GFP -HDEL IC	0.2303	-2.277 to 2.737	No	ns	>0.9999
NCH1 & KAKU41 vs. NCH1 & KAKU41 IC	11.28	8.775 to 13.79	Yes	****	<0.0001
NCH1 & KAKU41 vs. NCH2 & CNX	7.065	4.558 to 9.572	Yes	****	<0.0001
NCH1 & KAKU41 vs. NCH2 & CNX IC	11.2	8.695 to 13.71	Yes	****	<0.0001
NCH1 & KAKU41 vs. NCH2 & KAKU41	-0.6675	-3.117 to 1.782	No	ns	0.9991
NCH1 & KAKU41 vs. NCH2 & KAKU41 IC	11.1	8.653 to 13.55	Yes	****	<0.0001
NCH1 & KAKU41 vs. CNX & KAKU41	3.74	1.233 to 6.247	Yes	****	<0.0001
NCH1 & KAKU41 vs. CNX & KAKU41 IC	12.41	9.905 to 14.92	Yes	****	<0.0001
NCH1 & KAKU41 vs. mCherry -GFP -HDEL	-8.204	-10.71 to -5.698	Yes	****	<0.0001
NCH1 & KAKU41 vs. mCherry -GFP -HDEL IC	11.24	8.735 to 13.75	Yes	****	<0.0001
NCH1 & KAKU41 IC vs. NCH2 & CNX	-4.217	-6.724 to -1.710	Yes	****	<0.0001
NCH1 & KAKU41 IC vs. NCH2 & CNX IC	-0.07984	-2.587 to 2.427	No	ns	>0.9999
NCH1 & KAKU41 IC vs. NCH2 & KAKU41	-11.95	-14.40 to -9.500	Yes	****	<0.0001
NCH1 & KAKU41 IC vs. NCH2 & KAKU41 IC	-0.1798	-2.629 to 2.270	No	ns	>0.9999
NCH1 & KAKU41 IC vs. CNX & KAKU41	-7.542	-10.05 to -5.035	Yes	****	<0.0001
NCH1 & KAKU41 IC vs. CNX & KAKU41 IC	1.13	-1.377 to 3.637	No	ns	0.9444
NCH1 & KAKU41 IC vs. mCherry -GFP -HDEL	-19.49	-21.99 to -16.98	Yes	****	<0.0001
NCH1 & KAKU41 IC vs. mCherry -GFP -HDEL IC	-0.04004	-2.547 to 2.467	No	ns	>0.9999
NCH2 & CNX vs. NCH2 & CNX IC	4.137	1.630 to 6.644	Yes	****	<0.0001
NCH2 & CNX vs. NCH2 & KAKU41	-7.733	-10.18 to -5.284	Yes	****	<0.0001
NCH2 & CNX vs. NCH2 & KAKU41 IC	4.037	1.588 to 6.486	Yes	****	<0.0001
NCH2 & CNX vs. CNX & KAKU41	-3.325	-5.832 to -0.8183	Yes	**	0.001
NCH2 & CNX vs. CNX & KAKU41 IC	5.347	2.840 to 7.854	Yes	****	<0.0001
NCH2 & CNX vs. mCherry -GFP -HDEL	-15.27	-17.78 to -12.76	Yes	****	<0.0001
NCH2 & CNX vs. mCherry -GFP -HDEL IC	4.177	1.670 to 6.684	Yes	****	<0.0001
NCH2 & CNX IC vs. NCH2 & KAKU41	-11.87	-14.32 to -9.421	Yes	****	<0.0001
NCH2 & CNX IC vs. NCH2 & KAKU41 IC	-0.09992	-2.549 to 2.349	No	ns	>0.9999
NCH2 & CNX IC vs. CNX & KAKU41	-7.462	-9.969 to -4.955	Yes	****	<0.0001
NCH2 & CNX IC vs. CNX & KAKU41 IC	1.21	-1.297 to 3.717	No	ns	0.9125
NCH2 & CNX IC vs. mCherry -GFP -HDEL	-19.41	-21.91 to -16.90	Yes	****	<0.0001
NCH2 & CNX IC vs. mCherry -GFP -HDEL IC	0.0398	-2.467 to 2.547	No	ns	>0.9999
NCH2 & KAKU41 vs. NCH2 & KAKU41 IC	11.77	9.380 to 14.16	Yes	****	<0.0001
NCH2 & KAKU41 vs. CNX & KAKU41	4.408	1.958 to 6.857	Yes	****	<0.0001
NCH2 & KAKU41 vs. CNX & KAKU41 IC	13.08	10.63 to 15.53	Yes	****	<0.0001
NCH2 & KAKU41 vs. mCherry -GFP -HDEL	-7.537	-9.986 to -5.088	Yes	****	<0.0001
NCH2 & KAKU41 vs. mCherry -GFP -HDEL IC	11.91	9.460 to 14.36	Yes	****	<0.0001
NCH2 & KAKU41 IC vs. CNX & KAKU41	-7.362	-9.812 to -4.913	Yes	****	<0.0001
NCH2 & KAKU41 IC vs. CNX & KAKU41 IC	1.31	-1.139 to 3.759	No	ns	0.8385
NCH2 & KAKU41 IC vs. mCherry -GFP -HDEL	-19.31	-21.76 to -16.86	Yes	****	<0.0001
NCH2 & KAKU41 IC vs. mCherry -GFP -HDEL IC	0.1397	-2.310 to 2.589	No	ns	>0.9999
CNX & KAKU41 vs. CNX & KAKU41 IC	8.672	6.165 to 11.18	Yes	****	<0.0001
CNX & KAKU41 vs. mCherry -GFP -HDEL	-11.94	-14.45 to -9.438	Yes	****	<0.0001
CNX & KAKU41 vs. mCherry -GFP -HDEL IC	7.502	4.995 to 10.01	Yes	****	<0.0001
CNX & KAKU41 IC vs. mCherry -GFP -HDEL	-20.62	-23.12 to -18.11	Yes	****	<0.0001
CNX & KAKU41 IC vs. mCherry -GFP -HDEL IC	-1.17	-3.677 to 1.337	No	ns	0.9296
mCherry -GFP -HDEL vs. mCherry -GFP -HDEL IC	19.45	16.94 to 21.95	Yes	****	<0.0001

* Note IC = Internal FRET efficiency control

Supplementary Table S1 is from Mckenna, Gumber, et al., "Maize (Zea mays L.) nucleoskeletal proteins regulate nuclear envelope remodeling…", submitted 12/2020

Table S1 (McKenna, Gumber, et al., submitted Dec. 2020)

Table S2 (Tab 1 of 2) LEAF DEGs. All **225** Differentially Expressed Genes from Leaf.

W22v2 GeneID	B73v3 GeneID	B73v4 GeneID	Phytomine description / Uniprot id	Uniprot descriptor	baseMean-LEAF	log2FoldChange (mutant/WT)	lfcSE	stat	pvalue	padj
Zm00004a022952	GRMZM2G079381	Zm00001d052165	(1 of 2) K00366 - ferr #N/A	#N/A	7467.22838	29.4246738	3.90677062	7.53171267	5.01E-14	4.72E-11
Zm00004a029478	GRMZM2G442804	Zm00001d036738	(1 of 2) 2.1.1.163 - D B4FF45	S-adenosyl-L-methio	828.430667	13.1270875	3.61827211	3.62799898	0.000286	0.02374659
Zm00004a031378	GRMZM2G163861	Zm00001d039141	(1 of 4) PTHR24055: A0A1D6MDU6	Mitogen-activated pr	801.772225	13.0798895	3.63809059	3.59526219	0.000324	0.02627982
Zm00004a032719	GRMZM2G039586	Zm00001d046354	(1 of 2) PTHR10071/ B4F991	GATA transcription fa	675.803091	12.8333192	3.58881725	3.57591882	0.000349	0.02800135
Zm00004a002158	GRMZM2G050961	Zm00001d029997	(1 of 3) PTHR10772: B4FBN1	Chloroplast chaperor	666.3195	12.8129119	3.61916377	3.54029624	4.00E-04	3.14E-02
Zm00004a031472	GRMZM2G154664	Zm00001d044745	(1 of 1) PTHR11777: A0A1D6NR62	Alanine--tRNA ligase	442.856807	12.2235394	3.60460055	3.39109402	6.96E-04	4.88E-02
Zm00004a038561	GRMZM2G052036	Zm00001d024241	A0A1D6IYD1	Uncharacterized prot	412.427315	12.1208047	1.25435573	9.6629723	4.33E-22	1.07E-18
Zm00004a024341	GRMZM2G119932	Zm00001d053848	(1 of 5) PF00646//PF K7U803	Protein SRG1	221.633368	11.2250547	1.22916079	9.13229154	6.71E-20	1.21E-16
Zm00004a040326	GRMZM2G148495	Zm00001d026354	K7TSM1	Uncharacterized prot	155.731123	10.7159853	2.56855691	4.17198672	0.0000302	0.00390509
Zm00004a030713	GRMZM2G150471	Zm00001d038361	(1 of 2) PTHR12822: A0A1D6M5Q9	Protein YIPF	144.117489	10.6047446	1.29299803	8.20167116	2.37E-16	3.35E-13
Zm00004a036977	GRMZM2G000361	Zm00001d021708	(1 of 146) PF13812 - A0A1D6IE64	Pentatricopeptide rep	91.6284647	9.95055033	2.55206086	3.89902548	0.0000966	0.01021058
Zm00004a002460	GRMZM2G018929	Zm00001d030398	(1 of 2) PTHR24015: A0A1D6KCE5	Pentatricopeptide rep	73.3024849	9.62871858	2.01775461	4.77199682	0.00000182	0.000344
Zm00004a037691	GRMZM2G020401	Zm00001d022604	(1 of 1) KOG3444 - LC4J9F3	SNARE-like superfan	65.4345211	9.46628094	1.2787588	7.40271029	1.33E-13	9.78E-11
Zm00004a028573	GRMZM2G133629	Zm00001d035319	A0A1D6LFF8	Uncharacterized prot	61.0798391	9.3646218	1.24462084	7.52407602	5.31E-14	4.78E-11
Zm00004a012339	GRMZM2G048904	Zm00001d014596	(1 of 3) 2.3.1.98 - Ch A0A1D6GUF2	Alpha-L-fucosidase 2	49.0004256	9.04879444	1.54416675	5.8599853	4.63E-09	0.00000144
Zm00004a012010	GRMZM2G042136	Zm00001d014157	(1 of 6) K03809 - NA B6SPB2	Flavoprotein wrbA	45.2989985	8.93336195	1.23319652	7.24407002	4.35E-13	2.87E-10
Zm00004a029741	GRMZM2G142443	Zm00001d037085	C4J5G0	Uncharacterized prot	43.6925677	8.88172812	1.25055644	7.10222093	1.23E-12	7.36E-10
Zm00004a030995	GRMZM2G044368	Zm00001d038709	(1 of 5) K07897 - Ras A0A1D6M9Y7	Ras-related protein F	854.281322	8.80825777	2.49236587	3.534095	0.000409	0.03185181
Zm00004a006605	GRMZM2G129261	Zm00001d002654	(1 of 3) PTHR10593: A0A1D6E325	Naked endosperm1	793.787161	8.69986561	0.80718841	10.7779863	4.37E-27	2.16E-23
Zm00004a015807	GRMZM2G380319	Zm00001d039492	A0A1D6MHP7	Myb-like transcription	892.089428	8.29050629	1.78182041	4.65282935	3.27E-06	0.000573
Zm00004a015016	GRMZM2G090419	Zm00001d017951	(1 of 4) 3.6.1.13//3.6. B4FG54	Nudix hydrolase 17 n	26.4253106	8.15810576	1.40186465	5.8194675	5.90E-09	1.74E-06
Zm00004a031301	GRMZM2G141252	Zm00001d039054	(1 of 5) PTHR10093 #N/A	#N/A	90.5160879	8.08452683	1.15322615	7.01035681	2.38E-12	1.38E-09
Zm00004a000717	GRMZM2G471253	Zm00001d028138	(1 of 5) PF04481 - Pr A0A1D6JSB5	Thiamine monophos	70.5305127	7.71803729	1.08519142	7.11214369	1.14E-12	7.06E-10
Zm00004a036815	GRMZM2G069928	Zm00001d021532	(1 of 1) K01931 - pro A0A1D6IBW0	RING/U-box superfai	67.482069	7.66883779	1.29122334	5.9392032	2.86E-09	9.89E-07
Zm00004a013399	GRMZM2G177404		(1 of 4) 1.1.1.35//4.2. #N/A	#N/A	16.2942554	7.46088293	1.8357165	4.0642893	0.0000482	0.00584858
Zm00004a002499	GRMZM5G876022	Zm00001d030507	A0A1D6KCL1	WAT1-related protein	16.1201044	7.44434477	1.29028755	5.76952384	7.95E-09	0.0000323
Zm00004a004054	GRMZM2G413853	Zm00001d032655	(1 of 1) PTHR14950/ A0A1D6KSK7	Dicer-like 102	8.91036307	6.59202137	1.39215016	4.73513676	0.00000219	0.000409
Zm00004a034409	GRMZM2G420926	Zm00001d048336	(1 of 2) PTHR11668/ A0A1D6PJC8	Serine/threonine-prot	268.748915	6.50183669	1.65029566	3.93980112	0.0000815	0.00886601
Zm00004a037350	GRMZM2G312954	Zm00001d022184	(1 of 4) K17964 - leu A0A1D6IK37	Putative pentatricope	7.96421792	6.43041896	1.59590284	4.02932986	0.0000559	0.0065516
Zm00004a036573	GRMZM2G052474	Zm00001d021248	(1 of 2) PTHR13943: B7ZZ58	NC domain-containin	7.83785058	6.40412598	1.6734301	3.82694562	0.00013	0.01277228
Zm00004a025146	GRMZM2G112686	Zm00001d008929	(1 of 1) PTHR22835: A0A1D6FGP9	GDSL esterase/lipas	6.32065121	6.09817703	1.64375468	3.70990701	0.000207	0.01831493
Zm00004a004163	GRMZM2G379656	Zm00001d032784	(1 of 1) PTHR26402: A0A1D6KTY7	Two-component resp	6.27782711	6.08247491	1.39072809	4.37359033	0.0000122	0.00175244
Zm00004a036551	GRMZM2G017013	Zm00001d021221	(1 of 6) PTHR12329/ B6TJD1	Protein binding prote	72.3287031	6.00954777	1.72986443	3.47399926	0.000513	0.03814297
Zm00004a018053	GRMZM2G432390	Zm00001d042397	(1 of 15) 3.6.3.43 - P A0A1D6N3I1	ABC transporter B fa	612.986352	5.99792177	1.29237744	4.64099851	0.00000347	0.000586
Zm00004a000836	GRMZM2G344416	Zm00001d028274	(1 of 3) PTHR10177: A0A1D6JU49	Cyclin-SDS	5.65464364	5.93097241	1.51373161	3.9181136	0.0000892	0.00954532
Zm00004a024850	GRMZM2G112968	Zm00001d008529	(1 of 7) 3.1.30.1 - As A0A1D6FDT0	Endonuclease 2	178.036732	5.89782245	1.22968922	4.79618944	0.00000162	0.000308
Zm00004a006533	GRMZM2G038851	Zm00001d002570	(1 of 7) K10577 - ubi A0A1D6E1Y4	SUMO-conjugating e	5.44743536	5.87866026	1.61415843	3.64193511	2.71E-04	2.29E-02
Zm00004a039730	GRMZM2G038934	Zm00001d025670	(1 of 2) PTHR23344/ A0A1D6J8H8	Glycerophosphodies	5.34966121	5.85600702	1.47791992	3.96233039	7.42E-05	8.23E-03
Zm00004a027306	GRMZM2G091973	Zm00001d011631	(1 of 5) PTHR10994: B7ZYI3	Reticulon-like protein	5.3177009	5.83749437	1.59890571	3.65093095	0.000261	0.02228524
Zm00004a007542	GRMZM5G858609	Zm00001d003783	#N/A	#N/A	4.99512044	5.75176401	1.53940107	3.73636484	1.87E-04	1.69E-02
Zm00004a009893	GRMZM2G096106	Zm00001d006845	(1 of 2) K14288 - exp A0A1D6F180	Exportin-T (Exportin(	4.74289835	5.67384186	1.57729911	3.59718828	3.22E-04	0.031019
Zm00004a021274	GRMZM2G085573	Zm00001d050051	(1 of 3) PTHR23056: A0A1D6PZJ6	Calcineurin subunit B	4.65262088	5.64877774	1.44916835	3.89794445	0.000097	0.01021058
Zm00004a016770	GRMZM5G895400		(1 of 2) PF07516 - S #N/A	#N/A	463.230769	5.52897694	1.52920236	3.61559535	0.0003	0.02460305
Zm00004a024230	GRMZM2G000976	Zm00001d053719	(1 of 2) PTHR11062: A0A1D6QRU0	Exostosin-like	3.6831895	5.30976093	1.53300346	3.46363272	0.000533	0.03920133
Zm00004a037809	GRMZM2G036918	Zm00001d023301	(1 of 20) K13648 - al A0A1D6IRJ1	Hexosyltransferase (	12.9621521	5.24927925	1.29524929	4.05271732	0.0000506	0.00599844
Zm00004a020694	GRMZM2G036829	Zm00001d049343	(1 of 2) PTHR13343: A0A1D6PTV7	Glutamyl-tRNA reduc	428.130074	5.2109914	0.56562155	9.21285867	3.18E-20	6.28E-17
Zm00004a017828	GRMZM2G170689	Zm00001d042150	(1 of 4) 3.1.2.12 - S-f A0A1D6N1Q6	S-formylglutathione h	28.6707786	5.05967754	0.88129422	5.74119001	9.40E-09	0.00000266
Zm00004a000729	GRMZM2G047513	Zm00001d028153	(1 of 1) PTHR11700: A0A1D6JSF1	30S ribosomal protei	214.661897	4.43378056	1.1150952	3.97614531	0.00007	0.00796499
Zm00004a013031	GRMZM2G058252	Zm00001d015460	(1 of 2) K11098 - sm A0A1D6H288	Putative small nucleu	89.9045646	4.40178796	1.16074364	3.79221374	0.00149	0.01420386
Zm00004a018106	GRMZM2G013016	Zm00001d042472	(1 of 99) PF03514 - C A0A1D6N495	Protein SCARECRO\	7.5468871	4.38419021	1.26130462	3.47591704	0.000509	0.03801417
Zm00004a021544	GRMZM2G068455	Zm00001d050409	(1 of 2) PTHR11540: B4FFV3	Malate dehydrogenal	154.545715	4.27985353	1.17886939	3.63047303	0.000283	0.02361869
Zm00004a010999	GRMZM5G854045	Zm00001d013012	(1 of 2) PTHR21561: A0A1D6GEW1	HIT zinc finger	46.8177556	3.90744209	1.03999381	3.75717822	0.000172	0.01574167
Zm00004a036912	GRMZM2G180082	Zm00001d021639	(1 of 1) 1.14.13.184 - A0A1D6ID92	Taxane 13-alpha-hyd	32.4905967	3.89379865	0.64241747	6.06116561	1.35E-09	4.95E-07
Zm00004a016491	GRMZM2G121404	Zm00001d040311	(1 of 5) K12619 - 5'-3 A0A1D6MQ12	5'-3' exoribonuclease	17.6527176	3.5797071	0.92159871	3.88423621	0.000103	0.01062619
Zm00004a027586	GRMZM2G460566	Zm00001d011938	K7VM12	Uncharacterized prot	474.944398	3.48226135	0.30138449	11.5542157	7.03E-31	6.95E-27
Zm00004a005210	GRMZM2G348578	Zm00001d034124	(1 of 1) PTHR10869: A0A1D6L5N8	Putative prolyl 4-hydr	13.4450367	3.42875627	0.8513486	4.02744102	5.64E-05	6.56E-03
Zm00004a040037	GRMZM2G322634	Zm00001d026025	A0A1D6JBS5	Uncharacterized prot	40.7950382	3.38071759	0.81575384	4.14428647	3.41E-05	4.32E-03
Zm00004a024374	GRMZM2G155370	Zm00001d035880	(1 of 52) PF06203 - C A0A1D6QT42	Protein CHLOROPLA	61.9716189	3.36826757	0.88969286	3.78587684	0.000153	0.01443203
Zm00004a038737	GRMZM2G133512	Zm00001d024470	(1 of 2) PTHR20856/ A0A1D6IZI0	DNA-directed RNA p	60.9752615	3.18589191	0.43383499	7.34355683	2.08E-13	1.42E-10
Zm00004a013023	GRMZM2G109818	Zm00001d015450	(1 of 2) K19347 - SU A0A1D6H268	SUN domain protein	163.827706	3.0935191	0.8914755	3.47011118	0.00052	0.03841063
Zm00004a032080	GRMZM2G350319	Zm00001d045492	(1 of 164) PF01535// A0A1D6NWB0	Pentatricopeptide rep	50.0640181	3.04795717	0.72194349	4.22187779	0.0000242	0.00315389
Zm00004a032780	GRMZM2G022275	Zm00001d046445	(1 of 2) K13137 - ser A0A1D6P2Q2	Transducin/WD40 rep	88.3284389	2.96716841	0.65562993	4.52567567	6.02E-06	0.000976
Zm00004a035113	GRMZM2G410033	Zm00001d019206	(1 of 1) PTHR32195: B4FDE9	Tryptophan/tyrosine p	18.7119239	2.90922297	0.74995711	3.87918579	0.000105	0.01068974
Zm00004a036531	GRMZM2G043600	Zm00001d021191	(1 of 113) PF00170 - A0A1D6I8V4	Basic-leucine zipper	45.4940578	2.76864759	0.69911159	3.96023703	0.0000749	0.00823089
Zm00004a028733	GRMZM2G137930	Zm00001d035616	(1 of 3) PTHR10286: B4FJX6	Soluble inorganic pyr	1152.17637	2.42705929	0.48414472	5.01308634	0.000000536	0.000116
Zm00004a026814	GRMZM2G005350	Zm00001d011037	(1 of 2) PTHR10159/ A0A1D6FVL3	MAP kinase phospha	139.669477	2.35883218	0.67501116	3.49450842	0.000475	0.03614443
Zm00004a040568	GRMZM2G138870	Zm00001d026625	(1 of 17) PF06749 - F A0A1D6GJ63	Fiber protein Fb34	38.8115476	2.24965091	0.51411253	4.37579478	1.21E-05	1.75E-03
Zm00004a033483	GRMZM2G138987	Zm00001d047289	(1 of 1) PTHR10693/ C0P7S7	Nuclear transport fac	309.501595	2.23643667	0.5744732	3.89302176	0.000099	0.01036496
Zm00004a000179	GRMZM2G089860	Zm00001d027445	(1 of 3) PTHR12358: B4FIE8	Sphingoid long-chain	313.055498	2.1844164	0.41009395	5.32662433	0.0000001	0.0000244
Zm00004a030867	GRMZM2G117410	Zm00001d038540	(1 of 1) KOG2896 - LC0HEV5	Uncharacterized prot	30.93028	2.18269367	0.58520405	3.72979935	1.92E-04	1.72E-02
Zm00004a020935	GRMZM2G046804	Zm00001d049641	(1 of 2) PTHR10836/ K7UBU0	Glyceraldehyde-3-ph	290.988979	1.89219468	0.29262898	6.46619032	1.01E-10	4.61E-08
Zm00004a009015	GRMZM2G175867	Zm00001d036052	(1 of 2) PTHR24031: #N/A	#N/A	920.642936	1.28068292	0.17925872	7.14432695	9.04E-13	5.77E-10
Zm00004a012112	GRMZM2G377341	Zm00001d014294	(1 of 3) K11262 - ace A0A1D6GRS8	Acetyl-CoA carboxyla	5379.52762	1.22873173	0.33739947	3.6417714	0.000271	0.02289614
Zm00004a039163	GRMZM2G330635	Zm00001d024963	(1 of 2) PTHR11260/ B6TL20	Glutathione S-transfe	821.559951	1.12368212	0.25566698	4.39510063	0.0000111	0.00162285
Zm00004a040552	GRMZM2G343149	Zm00001d026606	(1 of 2) KOG0716 - N C4J426	DNAJ heat shock N-t	175.191177	1.11399665	0.19553823	5.69707843	1.22E-08	0.0000335
Zm00004a006763	GRMZM2G394450	Zm00001d002830	(1 of 28) PF11837 - D A0A1D6E4N9	Beta-fructofuranosida	510.860825	1.04284275	0.22253867	4.68611918	0.00000278	0.000492
Zm00004a032888	GRMZM2G069630	Zm00001d046571	(1 of 4) PTHR23042/ A0A1D6P3H5	Putative HLH DNA-bi	435.078325	1.01298064	0.15126213	6.69685548	2.13E-11	1.11E-08
Zm00004a028605	GRMZM2G410757	Zm00001d035456	A0A1D6LGG0	Uncharacterized prot	1102.34203	0.94624438	0.24964589	3.79034631	0.00015	0.01424261
Zm00004a023489	GRMZM2G002220	Zm00001d052809	(1 of 4) K09487 - hez K7V364	Heat shock protein 9(	1163.42188	0.94580231	0.15748147	6.00580049	1.90E-09	6.73E-07
Zm00004a030504	GRMZM2G305851	Zm00001d038085	(1 of 10) PF11891 - E A0A1D6M325	Protein RETICULATA	1188.38199	0.9352027	0.22602528	4.13760226	0.0000351	0.00442314
Zm00004a005417	GRMZM2G074404	Zm00001d034432	(1 of 2) PTHR23291/ B6TIC8	Bax inhibitor-1 family	424.535285	0.86562979	0.19919175	4.34571109	1.39E-05	1.93E-03
Zm00004a028575	GRMZM2G143788	Zm00001d035321	(1 of 4) PTHR13844: B6T2N1	SWIB/MDM2 domain	489.708705	0.85679437	0.23070118	3.71387084	0.000204	0.01811114
Zm00004a018769	GRMZM2G173654	Zm00001d034234	(1 of 1) PTHR12612: A0A1D6N9M7	NTF2-like	282.736689	0.82559477	0.24273462	3.40122386	0.000671	0.04740744
Zm00004a016582	GRMZM2G111014	Zm00001d040416	(1 of 1) K02470 - DN A0A1D6MQM1	DNA topoisomerase :	573.22022	0.79663319	0.14929919	5.33581713	9.51E-08	2.35E-05
Zm00004a003782	GRMZM2G127396	Zm00001d032334	(1 of 1) PTHR24092: A0A1D6KQ39	Phospholipid-transpo	307.752055	0.79617809	0.17445876	4.56370378	5.03E-06	8.29E-04
Zm00004a027549	GRMZM2G046558	Zm00001d011891	(1 of 1) K01070 - S-f K7V5R6	S-formylglutathione h	527.285315	0.76070436	0.19834295	3.8352982	0.000125	0.01234172
Zm00004a021214	GRMZM2G090241	Zm00001d049956	(1 of 2) K00215 - 4-h A0A1D6PYZ9	4-hydroxy-tetrahydro	112.382554	0.73587653	0.19995354	3.68023762	0.000233	0.02013407
Zm00004a040222	GRMZM2G129071	Zm00001d026244	(1 of 2) PTHR30222 B7ZYY2	Putrescine-binding pe	282.623632	0.72752977	0.21154612	3.43910705	0.000584	0.04230108
Zm00004a026760	GRMZM2G086766	Zm00001d010969	(1 of 2) KOG3882 - TC0PGJ5	Tetraspanin-6	207.76703	0.69786407	0.18917115	3.68906184	2.25E-04	1.97E-02
Zm00004a025751	GRMZM2G158518	Zm00001d009719	(1 of 2) PTHR13832: A0A1D6FLA0	PPM-type phosphata	532.830662	0.63346597	0.17343334	3.65250396	0.00026	0.02224501
Zm00004a038221	GRMZM2G036427	Zm00001d023830	(1 of 1) PTHR31964: B6TLW3	Fiber protein Fb19	1562.27079	-0.4159396	0.11035905	-3.7689666	1.64E-04	1.52E-02
Zm00004a011092	GRMZM2G007260	Zm00001d013092	(1 of 5) K10573 - ubi B4FH08	Ubiquitin-conjugating	923.813124	-0.4178773	0.12114084	-3.4495159	0.000562	0.04100456
Zm00004a012273	AC207656.3_FG002	Zm00001d014507	(1 of 17) PF02362//P A0A1D6GTV0	Auxin response facto	516.855693	-0.4187385	0.12013197	-3.485654	0.000491	0.0369359
Zm00004a036722	AC207722.2_FG009	Zm00001d021435	(1 of 5) K08912 - ligh B4FNR1	Chlorophyll a-b bindi	170912.219	-0.4331024	0.11385239	-3.8040698	0.000142	0.01373825
Zm00004a023254	GRMZM2G119175	Zm00001d054294	(1 of 2) PTHR11817/ A0A1D6QHK1	Pyruvate kinase (EC	839.32923	-0.475611	0.12467717	-3.8147405	0.000136	0.01322297
Zm00004a012904	GRMZM2G425774	Zm00001d015293	(1 of 2) PTHR21736: A0A1D6H134	VIN3-like protein 2	197.522432	-0.6919321	0.19597291	-3.5307537	0.000414	0.03202847
Zm00004a019820	GRMZM2G339540	Zm00001d044434	(1 of 16) PF00069//P A0A1D6NLS2	Putative inactive rec	227.937947	-0.7314427	0.14663392	-4.9882229	0.000000609	0.000131
Zm00004a038201	GRMZM2G001500	Zm00001d023802	(1 of 3) PTHR19375/ A0A1D6IW15	Heat shock 70 kDa p	390.383872	-0.7427689	0.16663045	-4.4575823	8.29E-06	1.18E-03
Zm00004a040507	GRMZM2G124466	Zm00001d026543	(1 of 14) PF04755 - F A0A1D6JH82	Putative plastid-lipid-	880.51809	-0.8379358	0.16072008	-5.2136345	0.00000185	0.0000436
Zm00004a024839	GRMZM2G030384	Zm00001d008517	(1 of 5) PTHR19856 A0A1D6FDQ9	Actin-interacting prot	325.234613	-0.8419972	0.14369917	-5.8594439	4.64E-09	0.00000144
Zm00004a029016	GRMZM2G110983	Zm00001d036054	(1 of 5) K10573 - ubi A0A1D6LKH9	Ubiquitin-conjugating	428.180372	-0.9107724	0.14663329	-6.2112252	5.26E-10	2.21E-07
Zm00004a031450	GRMZM2G310569	Zm00001d044717	(1 of 2) PTHR10217: K7VQ87	Potassium outward re	776.082506	-0.9308469	0.17592059	-5.2912904	1.21E-07	2.90E-05
Zm00004a039612	GRMZM2G132875	Zm00001d025533	(1 of 3) PTHR11732: K7TPL8	NAD(P)-linked oxidor	470.476633	-0.9542234	0.25513441	-3.7400811	0.000184	0.01677435
Zm00004a019130	GRMZM2G158013	Zm00001d043614	(1 of 1) PTHR11814: A0A1D6NDM6	Putative sulfate trans	220.659598	-0.9996597	0.28788078	-3.4724782	5.16E-04	3.82E-02
Zm00004a021942	GRMZM2G165231	Zm00001d050947	(1 of 1) PTHR23257: C0P3M4	Protein kinase superf	268.98269	-1.1283649	0.30233028	-3.7322261	1.90E-04	1.71E-02
Zm00004a023416	GRMZM2G097043	Zm00001d052723	(1 of 1663) 2.7.11.1 - A0A1D6QJ79	Uncharacterized prot	483.959303	-1.1750113	0.3058207	-3.8421574	0.000122	0.01218770
Zm00004a031824	GRMZM2G301860	Zm00001d045204	(1 of 2) PTHR31729: K7W642	Putative AP2/EREBP	91.8123203	-1.1767874	0.26793309	-4.3920943	1.12E-05	1.63E-03

Table S2a1 (McKenna, Gumber, et al., submitted Dec. 2020)

Zm00004b020891	GRMZM2G145972	Zm00001d049597	(1 of 6) K17871 - NA|A0A1D6PWC3	External alternative N	425.719275	-1.2024434	0.34440027	-3.4914125	0.00048	0.03636419
Zm00004b014169	GRMZM2G132706	Zm00001d016942	(1 of 1) PTHR11926/:A0A1D6HBD6	Glycosyltransferase (	88.72755892	-1.2785948	0.33703593	-3.7936455	1.48E-04	0.01419039
Zm00004b012181	GRMZM2G035405	Zm00001d014377	(1 of 19) PF02309//P A0A1D6GSR8	Auxin response facto	150.839573	-1.3074657	0.2772201	-4.7163454	0.0000024	0.00044
Zm00004b028942	GRMZM2G145396	Zm00001d035948	(1 of 1) PF06592 - Pr A0A1D6LJT8	ATOZI1	154.479925	-1.3600375	0.30840566	-4.409898	0.0000103	0.00155027
Zm00004b025175	GRMZM2G464891	Zm00001d008963	(1 of 2) PTHR12608: A0A1D6FGW5	GDT1 family protein	130.802281	-1.3936956	0.2822666	-4.9374087	0.000000792	0.000163
Zm00004b040292	GRMZM2G145460	Zm00001d026311	(1 of 3) K12448 - UD A0A1D6JEl6	NAD(P)-binding Ross	64.5436053	-1.3980243	0.32470084	-4.3055765	0.0000167	0.00225723
Zm00004b000155	GRMZM2G105436	Zm00001d027416	(1 of 1) 1.3.99.22 - C A0A1D6JM25	Oxygen-independent	71.9333728	-1.4589143	0.32542419	-4.4831158	0.00000736	0.00117383
Zm00004b024901	GRMZM2G477503	Zm00001d008600	(1 of 39) PF00534 - C A0A1D6FE41	Sulfoquinovosyl trans	135.498723	-1.4788108	0.38764344	-3.8148739	0.00136	0.01322297
Zm00004b001193	GRMZM2G443265	Zm00001d028713	(1 of 2) PTHR11132/:B4F896	Putative sugar phosp	146.819959	-1.7099847	0.36230074	-4.7197936	0.00000236	0.000437
Zm00004b031260	GRMZM2G318180	Zm00001d039011	(1 of 2) PTHR10168/:A0A1D6MCV5	Grx_I1-glutaredoxin s	55.4235679	-1.728671	0.4362094	-3.9629385	0.000074	0.00823089
Zm00004b031606	GRMZM2G370915	Zm00001d050484	(1 of 4) PTHR10766/:A0A1D6Q1U4	Transmembrane 9 su	24.9518867	-1.7731962	0.51670068	-3.4317667	0.0006	0.04330487
Zm00004b031166	GRMZM2G010349	Zm00001d038894	(1 of 1) PTHR11584::A0A1D6MBR1	Serine/threonine-prot	7526.21654	-1.8467058	0.44452862	-4.1543013	0.0000326	0.00416527
Zm00004b030125	GRMZM2G009845	Zm00001d037606	(1 of 4) PF00514//PFC0P5C0	Importin subunit alph	672.163686	-1.8509677	0.41775015	-4.430801	0.00000939	0.00144006
Zm00004b031291	GRMZM2G065757	Zm00001d039043	(1 of 4) PTHR13683: A0A1D6MD15	Aspartic proteinase A	895.396897	-1.8789325	0.39154149	-4.7988081	0.0000016	0.000307
Zm00004b034200	GRMZM2G113295	Zm00001d048112	(1 of 4) PTHR13139 C4JAV5	RING/U-box superfar	14.9266987	-1.9881288	0.58232065	-3.4141479	0.00064	0.04570428
Zm00004b031703	GRMZM2G177098	Zm00001d045054	(1 of 2) 4.2.3.123 - B A0A1D6NT87	Sesquiterpene cyclas	563.977223	-2.083258	0.44239875	-4.7090051	0.00000249	0.000452
Zm00004b023346	GRMZM2G091258	Zm00001d052620	(1 of 3) PF15346 - Ar B4FUY0	Uncharacterized prot	84.8148844	-2.0969343	0.51434005	-4.0769415	0.0000456	0.0056082
Zm00004b009147	GRMZM2G066516	Zm00001d005917	(1 of 18) PF04784 - F A0A1D6ERJ3	Ternary complex fact	52.1992731	-2.1476846	0.60276371	-3.5630622	3.67E-04	2.91E-02
Zm00004b038557	GRMZM2G134613	Zm00001d024239	(1 of 4) K08341 - GA A0A1D6IYD0	Autophagy-related pr	220.308776	-2.1764262	0.29327857	-7.42102	1.16E-13	8.84E-11
Zm00004b010118	GRMZM2G473709	Zm00001d007174	A0A1D6F4F7	Uncharacterized prot	72.7028661	-2.2070216	0.60999614	-3.6180911	2.97E-04	2.45E-02
Zm00004b016247	GRMZM2G360023	Zm00001d039993	(1 of 5) 1.11.1.6 - Cal B6SUS4	Uncharacterized prot	54.6089476	-2.4961955	0.66210963	-3.7700637	0.000163	0.01522807
Zm00004b014100	GRMZM2G113453	Zm00001d016856	(1 of 15) PF07887 - A0A1D6HAV0	Calmodulin binding p	896.773245	-2.5156699	0.56352855	-4.4641393	0.00000804	0.00126316
Zm00004b039718	GRMZM2G173878	Zm00001d025660	(1 of 4) K07877 - Rar#N/A	#N/A	97.940301	-2.5271222	0.5819074	-4.3428253	0.0000141	0.00194146
Zm00004b009434	GRMZM2G158629	Zm00001d006270	(1 of 2) KOG1206 - FC4JA75	Enoyl-CoA hydratase	85.256169	-2.5476031	0.72103652	-3.5332512	0.000441	0.03185181
Zm00004b001295	GRMZM2G076537	Zm00001d028827	(1 of 1) PTHR13058 A0A1D6K021	Exonuclease DPD1 c	11.6682028	-2.589785	0.67760974	-3.8219417	1.32E-04	1.30E-02
Zm00004b011960	GRMZM2G146446	Zm00001d014109	(1 of 1) PTHR14319 A0A1D6GPY5	Transmembrane prot	47.2813877	-2.634432	0.57927246	-4.5478289	5.42E-06	8.86E-04
Zm00004b000177	GRMZM2G090051	Zm00001d027443	(1 of 9) 1.14.13.129 - A0A1D6JM56	Hydroxylase8	165.877537	-2.6361802	0.48594064	-5.4249018	0.000000058	0.0000149
Zm00004b018443	GRMZM5G856598	Zm00001d042841	(1 of 2) K08193 - MF B6SWK6	Putative anion transp	69.729378	-2.6900638	0.43660711	-6.1612919	7.22E-10	2.69E-07
Zm00004b039626	GRMZM2G104603	Zm00001d025549	B4FV84	Putative carboxyleste	16.7476499	-2.7905884	0.70460708	-3.9604887	0.0000748	0.00823089
Zm00004b038619	GRMZM2G315767	Zm00001d024317	(1 of 2) PTHR31642:A0A1D6IYR6	Transferase	26.921785	-3.006855	0.4854769	-6.1936108	5.88E-10	2.33E-07
Zm00004b000094	GRMZM2G077769	Zm00001d027346	(1 of 61) PF02298 - F A0A1D6JL89	Early nodulin-like pro	16.7445831	-3.0378518	0.61567127	-4.9342107	8.05E-07	1.64E-04
Zm00004b019474	GRMZM2G004183	Zm00001d042033	(1 of 56) PF13839//P A0A1D6NHE8	TRICHOME BIREFR	32.4917945	-3.0584748	0.85118053	-3.5932152	3.27E-04	2.64E-02
Zm00004b025514	GRMZM2G387603	Zm00001d009416	(1 of 4) PTHR23244/:A0A1D6FJA2	Jacalin-related lectin	17.2901888	-3.0705361	0.63395466	-4.8434632	1.28E-06	2.50E-04
Zm00004b034863	GRMZM2G074323	Zm00001d018887	(1 of 5) PTHR24441::A0A1D6HT38	Speckle-type POZ pr	86.5699368	-3.2373495	0.78449684	-4.1266572	0.0000368	0.00460955
Zm00004b018449	GRMZM2G400390	Zm00001d042848	(1 of 1) PTHR11709/:A0A1D6N727	Laccase-7	209.16183	-3.2745927	0.88640694	-3.6942317	0.000221	0.01939589
Zm00004b012332	GRMZM2G168890	Zm00001d014587	(1 of 14) PF07800 - FB4FED2	C2H2-type domain-co	44.3866773	-3.3437481	1.01336395	-3.3983329	0.000678	0.04774079
Zm00004b001084	GRMZM2G160428	Zm00001d028570	(1 of 2) K08269 - seri A0A1D6JXN6	Serine/threonine-prot	145.887331	-3.5479438	0.83560926	-4.2459364	0.0000218	0.00287152
Zm00004b005839	GRMZM2G077596	Zm00001d034931	(1 of 3) PTHR31008: A0A1D6LCN7	COP1-interacting pro	22.8653413	-3.7412166	0.97284789	-3.8456336	0.00012	0.01207723
Zm00004b037811	GRMZM2G004412	Zm00001d023304	(1 of 16) PTHR23155:A0A1D6IRP3	Uncharacterized prot	94.358603	-3.9761305	0.62966583	-6.3146677	2.71E-10	1.16E-07
Zm00004b017793	GRMZM2G034502	Zm00001d024114	(1 of 9) PF12734 - C; B6SGH6	CYSTM domain-cont	80.0975862	-4.0257871	0.6645981	-6.0574761	1.38E-09	4.97E-07
Zm00004b001875	GRMZM2G077316	Zm00001d029594	(1 of 2) 5.3.99.6 - AllcQ6RW09	Allene oxide cyclase	59.2744867	-4.202648	1.2210899	-3.4417188	5.78E-04	4.20E-02
Zm00004b037351	GRMZM2G017368	Zm00001d022185	(1 of 1) PF00612//PF A0A1D6IK54	Calmodulin-binding tr	134.853653	-4.2552721	1.0466383	-4.0656568	0.0000479	0.00584858
Zm00004b002146	GRMZM2G098208	Zm00001d029978	(1 of 2) PTHR11740/:A0A1D6K8R9	Casein kinase II sub	100.91157	-4.2736018	1.05206984	-4.0620894	0.0000486	0.00586798
Zm00004b018757	GRMZM5G867518	Zm00001d043223	(1 of 4) K02975 - smiC0HDX5	40S ribosomal protei	43.0334973	-4.4208247	1.00152775	-4.4140811	0.0000101	0.00153321
Zm00004b011289	GRMZM2G312738	Zm00001d013318	(1 of 2) K16278 - E3 A0A1D6GI43	E3 ubiquitin-protein li	65.6800622	-4.5080077	1.14236326	-3.9462121	7.94E-05	8.68E-03
Zm00004b031913	GRMZM2G052562	Zm00001d045302	(1 of 2) PTHR22835/:A0A1D6NV48	Zea nodulation hom	245.889628	-4.5271323	0.99130819	-4.5668263	0.0000495	0.000823
Zm00004b030754	GRMZM2G318992	Zm00001d038407	(1 of 18) PF07765 - A0A1D6M671	Protein NETWORKE	45.0447659	-4.6916618	1.37776484	-3.4052704	6.61E-04	4.69E-02
Zm00004b006266	GRMZM2G012628	Zm00001d022244	(1 of 6) PHR24012::A0A1D6DYK9	Polyadenylate-bindin	207.03206	-5.2517456	1.36955945	-3.834624	1.26E-04	0.01244172
Zm00004b025060	GRMZM2G112681	Zm00001d008817	(1 of 3) PTHR31079::A0A1D6FFQ8	NAC domain containi	44.5506993	-5.5508241	1.27422831	-4.3562241	0.000132	0.00185697
Zm00004b024033	GRMZM2G081644	Zm00001d053447	A0A1D6QPH7	Uncharacterized prot	4.30518018	-5.560515	1.57323927	-3.5344369	4.09E-04	0.03185181
Zm00004b011936	GRMZM2G027135	Zm00001d014080	(1 of 1) PTHR12181/:B4FXM3	Uncharacterized prot	4.38609406	-5.5827533	1.47039337	-3.7967754	0.000147	0.01408052
Zm00004b011171	GRMZM2G033198	Zm00001d013184	(1 of 1) PTHR10438: A0A096QB26	Thiol disulfide interch	4.48040404	-5.6103985	1.60717577	-3.4908431	0.000481	0.03636419
Zm00004b039392	GRMZM2G151041	Zm00001d025240	(1 of 3) K00164 - 2-o A0A1D6J5Q7	2-oxoglutarate dehyd	1138.01393	-5.7409501	1.6081364	-3.5699398	0.000357	0.02848875
Zm00004b025158	GRMZM2G161988	Zm00001d008944	(1 of 4) KOG2887 - N A0A1D6FGT7	Uncharacterized prot	4.97261301	-5.7679222	1.5915362	-3.6241226	2.90E-04	0.0240047
Zm00004b018231	GRMZM2G025703	Zm00001d042613	(1 of 1) PF00615//PF A0A1D6N5E7	RING/FYVE/PHD-typ	5.558418	-5.931268	1.52727424	-3.8835645	0.000103	0.01062619
Zm00004b015220	GRMZM2G021514	Zm00001d018189	(1 of 1) PF00400//PF A0A1D6HLB9	Major facilitator supe	5.65292164	-5.9470806	1.54429679	-3.8509959	0.000118	0.01187608
Zm00004b004118	GRMZM2G167438	Zm00001d032728	(1 of 30) K15397 - 3- B4FQN3	3-ketoacyl-CoA synth	5.68148542	-5.9587649	1.39894683	-4.2594649	0.0000205	0.00273966
Zm00004b009098	GRMZM2G134351	Zm00001d005859	(1 of 1) PTHR13683/:A0A1D6ERB3	Eukaryotic aspartyl p	5.80723206	-5.9856396	1.47557762	-4.0564722	4.98E-05	5.94E-03
Zm00004b030863	GRMZM2G044457	Zm00001d038536	(1 of 3) PTHR10984/:A0A1D6M733	Endoplasmic reticulu	21.3259172	-6.0051667	1.52724447	-3.9320271	0.000842	0.0091077
Zm00004b038273	GRMZM2G316593	Zm00001d023907	K7TJX6	Uncharacterized prot	6.29336864	-6.1070367	1.73815496	-3.5135168	0.000442	0.03378431
Zm00004b040445	GRMZM2G007477	Zm00001d026489	(1 of 1663) 2.7.11.1 - K7TP92	OSJNBb0022F16.11-	39.149517	-6.3328095	1.31171634	-4.8278803	0.00000138	0.000268
Zm00004b006220	GRMZM2G114861	Zm00001d002186	(1 of 1) PTHR24115::A0A1D6DXK1	Kinesin-like protein	7.4353572	-6.3452997	1.54048688	-4.1190222	0.000038	0.00473499
Zm00004b039285	GRMZM2G081585	Zm00001d025106	(1 of 9) K04564 - suK7U2E7	Superoxide dismutas	7.93221065	-6.4429844	1.37152492	-4.6976794	0.00000263	0.000473
Zm00004b000181	GRMZM2G176585	Zm00001d027447	(1 of 3) PTHR31879:A0A1D6JM71	Det1 complexing ubi	67.4247474	-6.7039036	1.08801441	-6.1615945	7.20E-10	2.69E-07
Zm00004b034890	GRMZM2G107408	Zm00001d018906	B4FF39	Uncharacterized prot	200.726775	-6.8974602	1.62732124	-4.2385363	0.0000225	0.00294816
Zm00004b038011	GRMZM2G381429	Zm00001d023553	(1 of 2) PTHR23155/:A0A1D6IU36	Disease resistance p	14.1893652	-7.2758491	1.37114923	-5.3063875	0.000000112	0.000027
Zm00004b026342	GRMZM2G157705	Zm00001d010455	A0A1D6FR41	Reticulon-like protein	14.4386388	-7.3062458	1.43186922	-5.102593	0.000000335	0.0000737
Zm00004b038742	GRMZM2G148316	Zm00001d024476	A0A1D6IZl6	UDP-glycosyltransfer	15.6056043	-7.4185534	1.49455082	-4.9637344	0.000000692	0.000146
Zm00004b026136	GRMZM2G149808	Zm00001d010206	(1 of 3) PTHR13286 B4FT44	SAP30_Sin3_bdg do	16.0010348	-7.4530717	1.89630859	-3.9303053	8.48E-05	9.12E-03
Zm00004b013766	GRMZM2G137495	Zm00001d016440	(1 of 2) PTHR24078/:A0A1D6H7L4	DNAJ heat shock fam	35.5552311	-7.6372363	1.23540564	-6.1819665	6.33E-10	2.46E-07
Zm00004b037949	GRMZM5G839644	Zm00001d023470	(1 of 21) PF00560//P A0A1D6ITF5	Putative leucine-rich	19.8102521	-7.7609484	1.69935714	-4.5669908	0.00000495	0.000823
Zm00004b001530	GRMZM2G134671	Zm00001d029149	(1 of 52) PF06203 - CC0PI33	C2C2-CO-like transc	19.836807	-7.763756	1.7767038	-4.3697526	1.24E-05	1.76E-03
Zm00004b039025	GRMZM2G016312	Zm00001d024798	K7TMI5	Uncharacterized prot	19.9227091	-7.7693616	2.19202511	-3.5443762	0.000394	0.03102407
Zm00004b017717	GRMZM5G813403	Zm00001d042016	(1 of 309) 6.3.2.19 - `B6TIZ5	Uncharacterized prot	20.0343	-7.7752321	1.58789062	-4.8965791	0.000000975	0.000193
Zm00004b005561	GRMZM2G347056	Zm00001d034602	(1 of 1) K00297 - metA0A1D6L979	Brown midrib2	21.7107574	-7.8927693	1.98910339	-3.9680035	7.25E-05	0.0081949
Zm00004b031719	GRMZM2G455243	Zm00001d045080	(1 of 2) K02603 - origA0A1D6NTH3	Origin recognition co	24.1413339	-8.0473034	1.29821421	-6.1987485	5.69E-10	0.00000023
Zm00004b037473	GRMZM2G018771	Zm00001d022337	(1 of 2) 3.5.1.88 - PerA0A1D6IL69	Peptide deformylase	25.1511161	-8.1065104	1.405358	-5.7682885	8.01E-09	2.30E-06
Zm00004b022114	GRMZM2G148604	Zm00001d051183	(1 of 1) K14327 - reg A0A1D6Q5H4	Regulator of nonsens	25.6683286	-8.1347867	1.87357723	-4.3418476	0.0001141	0.00194146
Zm00004b018825	GRMZM2G093101	Zm00001d043291	(1 of 1) PTHR22953: A0A1D6NA73	Purple acid phosphat	29.2236183	-8.3224876	1.26522035	-6.5778958	4.77E-11	2.25E-08
Zm00004b007010	GRMZM2G086486	Zm00001d003117	(1 of 3) PTHR10744/:A0A1D6E6T4	40S ribosomal protei	29.5062216	-8.3337836	1.31279641	-6.3481157	2.18E-10	9.58E-08
Zm00004b005777	GRMZM2G164547	Zm00001d034859	B7ZZ71	Cobalt ion binding	30.292121	-8.3727734	2.14441788	-3.9044504	0.0000944	0.01004666
Zm00004b017733	GRMZM2G059083	Zm00001d042049	(1 of 3) 1.6.99.1 - NA A0A1D6N0R1	Ferredoxin--NADP re	10464.5512	-8.4011245	1.56595333	-5.3648626	0.000000081	0.0000203
Zm00004b005843	GRMZM2G077222	Zm00001d034935	(1 of 2) KOG3251 - C A0A1D6LCQ1	Uncharacterized prot	37.6670904	-8.6870873	1.69872918	-5.1138742	0.000000316	0.000071
Zm00004b019700	GRMZM2G070961	Zm00001d044293	(1 of 4) PF00069//PF A0A1D6NKI4	Protein kinase protei	38.4859778	-8.716857	1.32060373	-6.6006606	4.09E-11	1.98E-08
Zm00004b031672	GRMZM2G447806	Zm00001d045009	A0A1D6NST3	Uncharacterized prot	42.7021894	-8.8681762	1.51122296	-5.8682116	4.41E-09	0.00000141
Zm00004b000867	GRMZM2G043584	Zm00001d042317	(1 of 34) PF00069//P A0A1D6JUB4	Leucine-rich repeat r	50.3398948	-9.1050678	1.62156852	-5.6149757	1.97E-08	5.26E-06
Zm00004b020252	GRMZM2G468260	Zm00001d048796	(1 of 3) PTHR22763/:A0A1D6PQ59	RING/U-box superfar	53.9469078	-9.2054014	2.53250159	-3.6349045	2.78E-04	2.34E-02
Zm00004b015124	GRMZM2G036418	Zm00001d018057	(1 of 1) PTHR13520/:A0A1D6HKI4	RINT1-like protein M	55.1481017	-9.2386971	1.2420628	-7.4381884	1.02E-13	8.08E-11
Zm00004b003465	AC234528.1_FG004	Zm00001d031928	(1 of 1) PF11595 - PrB6TZS4	Uncharacterized prot	60.0901134	-9.3602792	1.25718448	-7.4454301	9.66E-14	7.97E-11
Zm00004b030599	GRMZM5G881803	Zm00001d038205	(1 of 1) PTHR31942:A0A1D6M4I8	MLO-like protein	77.0738763	-9.7205791	1.63722392	-5.9372325	2.90E-09	9.89E-07
Zm00004b006558	GRMZM2G114220	Zm00001d002598	(1 of 1) PTHR12555/:B6T4L6	Ubiquitin fusion degr	82.6579962	-9.8207032	1.42068652	-6.9126462	4.76E-12	2.69E-09
Zm00004b012058	GRMZM2G366638	Zm00001d014207	A0A1D6GR10	Uncharacterized prot	85.6508137	-9.8719883	1.23584383	-7.9880549	1.37E-15	1.36E-12
Zm00004b024150	GRMZM2G171452	Zm00001d053625	(1 of 21) PF01918 - FB6T175	Alba DNA/RNA-bindi	93.8008006	-10.004327	1.24530628	-8.0336274	9.46E-16	1.01E-12
Zm00004b031781	GRMZM2G223636	Zm00001d045147	(1 of 2) PTHR18934:A0A1D6NU12	Putative pre-mRNA-s	99.6267326	-10.090917	2.06077138	-4.8966697	0.000000975	0.000193
Zm00004b031625	GRMZM2G088053	Zm00001d044321	(1 of 54) PF00892 - EB4FTK8	WAT1-related protein	124.401544	-10.411613	1.27855043	-8.1432945	3.85E-16	5.07E-13
Zm00004b024037	GRMZM2G029058	Zm00001d053452	(1 of 7) PF00076//PF A0A1D6QPS5	Polyadenylate-bindin	203.284528	-11.119527	1.67867824	-6.6239776	3.50E-11	1.77E-08
Zm00004b003203	GRMZM2G386209	Zm00001d031555	(1 of 8) KOG4293 - F A0A1D6KJM6	Cytochrome b561 an	221.282815	-11.242145	1.39757195	-8.0440548	8.69E-16	1.01E-12
Zm00004b007229	GRMZM2G029001	Zm00001d003394	(1 of 7) PTHR12603 A0A1D6E920	RING/U-box superfar	221.630087	-11.244202	1.92064065	-5.8544018	4.79E-09	0.00000146
Zm00004b030622	GRMZM2G175676	Zm00001d038248	(1 of 4) K12831 - spli A0A1D6M4R6	RNA-binding (RRM/F	322.560854	-11.785783	1.82352341	-6.4631924	1.03E-10	4.61E-08
Zm00004b018149	GRMZM2G064914	Zm00001d042512	(1 of 2) PTHR11240/:A0A1D6N4Q6	Ribonuclease 2	421.632858	-12.171961	3.56098798	-3.4181417	0.000631	0.04536639
Zm00004b001184	GRMZM2G114557	Zm00001d013195	(1 of 3) PTHR12383/:A0A1D6GGF2	Thylakoid processing	443.480025	-12.24485	3.58774162	-3.4129687	0.000643	0.04573738
Zm00004b019678	GRMZM5G848696	Zm00001d044267	(1 of 2) K18121 - glycA0A1D6NK13	Glyoxylate/succinic s	469.510211	-12.327109	1.22910896	-10.029306	1.13E-23	3.20E-20
Zm00004b011184	GRMZM2G167932	Zm00001d021999	(1 of 2) PF02136//PF A0A1D6IIC4	Nuclear transport fac	497.082753	-12.409565	1.38506885	-8.9595291	3.26E-19	4.96E-16
Zm00004b019828	GRMZM2G143128	Zm00001d044445	(1 of 2) K03661 - V-ty#N/A	#N/A	507.068603	-12.438167	3.57743815	-3.4768365	0.000507	0.03801417
Zm00004b003707	GRMZM2G092595	Zm00001d032238	(1 of 1) PTHR11353/:A0A1D6KPG0	Putative 1-phosphati	598.995823	-12.678531	3.6072989	-3.5146883	0.000448	0.03378431
Zm00004b037850	GRMZM2G129804	Zm00001d023340	(1 of 1) K02356 - elorA0A1D6ISX4	Elongation factor P	830.618221	-13.150242	1.20932357	-10.874048	1.53E-27	1.01E-23
Zm00004b031504	GRMZM2G026833	Zm00001d044785	(1 of 334) PF00249 - K7W4V3	G2-like1	1385.73578	-13.888696	1.34835579	-10.300468	7.01E-25	2.77E-21
Zm00004b026230	GRMZM2G086464	Zm00001d010314	A0A1D6FQC3	Uncharacterized prot	1530.26023	-14.031661	3.71963824	-3.7723187	1.62E-04	0.0151673
Zm00004b000190	GRMZM2G177508	Zm00001d027456	(1 of 3) PTHR33222:B4FTJ9	PBA1 homolog1 (Thr	9043.16785	-16.594887	1.31130707	-12.655226	1.05E-36	2.07E-32

TABLE NOTES:

GENEIDs B73v3 GeneIDs were obtained from the W22 GeneIDs using a MaizeGDB syntelogs lookup table made with SynMap

TRANSCRIPTOME: Trimmed reads were aligned using the splice-aware aligner Hisat2, indices were constructed from known exons and splice sites (W22 annotation Zm00004b & assembly Zm-W22-REFERENCE-NRGENE-2.0 as described in the Methods.

DEG & TABULATION: Differential expression analysis was performed separately per tissue (leaf mutant vs. WT or tassel mutant vs. WT) as described in the Methods

TABLULATION: This entire table, sorted on col-H, log2(fold-change), includes statistically significant differentially expressed (adjusted p-value <0.05) genes and excludes any genes with fewer than 10 counts across sum of all replicates.

COLUMNS G-L: Columns G-L tabulate output values for the baseMean, the log2(fold-change), along with the associated values for standard error (lfcSE), Wald statistic (stat), pvalue, and adjusted pvalue (padj).

PROVENANCE: Supplementary Table S2 from Mckenna, Gumber, et al., "Maize (Zea mays L.) nucleoskeletal proteins regulate nuclear envelope remodeling…", submitted 12/2020

Table S2a2 (McKenna, Gumber, et al., submitted Dec. 2020)

Table S2b1 (McKenna, Gumber, et al., submitted Dec. 2020)

Zm00004b000914	GRMZM2G157177	Zm00001d028372	(1 of 4) PTHR22952/ A0A1D6JVI5	BZIP transcription fac	71.0752415	-2.0593793	0.30429553	-6.7676948	1.31E-11	7.81E-09
Zm00004b030613	GRMZM2G153075	Zm00001d038224	(1 of 2) K03875 - F-b B4F7T2	F-box protein FBL2 (	218.253808	-2.0984351	0.57522464	-3.648027	0.000264	0.03496643
Zm00004b034784	GRMZM2G012031	Zm00001d018795	(1 of 1) PTHR24365/ A0A1D6HSC5	187-kDa microtubule	1281.82697	-2.5686201	0.38603854	-6.6537919	2.86E-11	1.62E-08
Zm00004b029454	GRMZM5G829955	Zm00001d036709	(1 of 1) K12857 - Prp A0A1D6LQJ4	Transducin/WD40 rep	72.5753958	-2.5762318	0.34823181	-7.398037	1.38E-13	9.85E-11
Zm00004b037334	GRMZM2G371721	Zm00001d022168	(1 of 5) PTHR24078/ Q5GAN9	AT hook-containing N	66.8457668	-2.7007364	0.36102147	-7.4808194	7.39E-14	5.63E-11
Zm00004b011381	GRMZM2G090172	Zm00001d013431	(1 of 2) PTHR24067: A0A1D6GJ80	Ubiquitin-conjugating	178.729135	-2.7762387	0.68518628	-4.0518014	0.0000508	0.0089133
Zm00004b016600	GRMZM2G305264	Zm00001d040445	(1 of 2) PTHR22763/ C0PF84	RING/U-box superfar	134.364196	-3.1863228	0.66813468	-4.7689828	0.00000185	0.000426
Zm00004b005951	GRMZM2G040115	Zm00001d001858	(1 of 45) PF03000 - M B8A094	BTB/POZ domain-co	95.666743	-3.2570704	0.84535642	-3.852896	0.000117	0.01803748
Zm00004b006866	GRMZM2G176677	Zm00001d002945	(1 of 151) PF02365 - A0A1D6E5L0	Ras-related protein1	359.948291	-3.3304859	0.61989729	-5.3726415	7.76E-08	0.0000227
Zm00004b007683	AC235541.1_FG002	Zm00001d003948	(1 of 3) PTHR24078/ C4J193	DNAJ heat shock N-t	109.280361	-3.4489189	0.97099931	-3.5519273	0.000382	0.04654892
Zm00004b032487	GRMZM2G057743	Zm00001d046033	(1 of 2) PTHR10288/ K7W8X7	RNA-binding KH dom	35.6783979	-3.4498949	0.56344471	-6.1228631	9.19E-10	3.98E-07
Zm00004b030589	GRMZM2G133021	Zm00001d038194	(1 of 127) PF00646 - C0P6L8	F-box/FBD/LRR-repe	212.536727	-3.4882886	0.6674719	-5.2261206	1.73E-07	0.0000461
Zm00004b019813	GRMZM5G812270	Zm00001d044429	(1 of 44) 5.3.4.1 - PrcB6TH36	Thioredoxin superfan	158.728386	-3.5236272	0.97410544	-3.6172955	0.000298	0.03846912
Zm00004b001427	GRMZM2G312661	Zm00001d029028	(1 of 2) PTHR10891/ C0P445	Calcium-binding prot	49.7443658	-3.5317195	0.67313631	-5.2466631	1.55E-07	0.0000417
Zm00004b001119		Zm00001d028612	A0A1D6JY27	Uncharacterized prot	255.883023	-3.5347045	0.67840253	-5.210335	1.88E-07	0.000049
Zm00004b034015	GRMZM2G013814	Zm00001d047894	(1 of 7) PF11833 - PrB6TKC3	Protein CHAPERON!	119.776564	-3.546751	0.54232152	-6.5399414	6.15E-11	3.32E-08
Zm00004b037413	GRMZM2G180568	Zm00001d022256	(1 of 46) PF03634 - 1A0A060CZ25	TCP transcription fac	126.838844	-4.1765009	0.41628918	-10.032691	1.09E-23	2.69E-20
Zm00004b014162	GRMZM2G075637	Zm00001d016935	(1 of 2) K11290 - temQ94F78	Nucleosome/chromat	774.018655	-4.3893148	0.65387115	-6.712813	1.91E-11	1.11E-08
Zm00004b031562	GRMZM2G058560	Zm00001d044866	(1 of 1) K17605 - seriA0A1D6NRY3	Serine/threonine-prot	158.722343	-4.6022265	0.85675423	-5.3716998	7.80E-08	0.0000227
Zm00004b011362	GRMZM2G152328	Zm00001d013410	(1 of 2) PTHR11937/‌#N/A	#N/A	1408.63065	-4.8578728	0.62713761	-7.7461035	9.48E-15	9.10E-12
Zm00004b005296	GRMZM2G170727	Zm00001d034254	(1 of 3) KOG3381 - LA0A1D6L6F1	Protein AE7-like 1	88.6494724	-5.7478947	1.45627857	-3.9469747	0.0000791	0.01305123
Zm00004b000303	GRMZM2G009014	Zm00001d027588	A0A1D6JN74	Uncharacterized prot	47.4651127	-6.0922781	1.65823335	-3.6739571	0.000239	0.03217847
Zm00004b036142	GRMZM5G832772	Zm00001d020666	(1 of 58) 3.6.3.44 - X/ A0A1D6I5K5	Multidrug resistance	85.8998549	-6.1525908	1.13848309	-5.4042004	6.51E-08	0.00002
Zm00004b040245	GRMZM2G018462	Zm00001d026259	(1 of 2) PTHR33972/ A0A1D6JDS9	Uncharacterized prot	69.2136647	-6.6238048	1.70248072	-3.8906783	0.0001	0.01577795
Zm00004b039476	GRMZM2G095826	Zm00001d025352	(1 of 2) PTHR19370: B4FYW4	Ferredoxin	163.683221	-6.6902767	0.64379504	-10.391936	2.70E-25	8.52E-22
Zm00004b038507	GRMZM2G094452	Zm00001d024212	(1 of 7) K00901 - diacA8QM11	diacylglycerol kinase	14.1787349	-7.2001486	1.36678595	-5.2679417	1.38E-07	0.0000376
Zm00004b031692	GRMZM2G029559	Zm00001d016358	(1 of 1) PF00043//PF A0A1D6H6X9	Putative elongation f	528.862948	-7.3168313	1.9781414	-3.6988414	0.000217	0.02936134
Zm00004b003529	GRMZM2G084583	Zm00001d032024	(1 of 2) PTHR10641/ A0A1D6KN59	Myb transcription fac	15.7016476	-7.351001	1.59883276	-4.5977298	0.00000427	0.000925
Zm00004b032897	GRMZM5G805627	Zm00001d046583	(1 of 5) K02955 - sm/ A0A1D6P3R8	40S ribosomal protei	2049.55018	-7.3664354	1.37568503	-5.3547398	8.57E-08	0.0000243
Zm00004b004185	GRMZM2G144782	Zm00001d032810	(1 of 1) PTHR21319/ A0A1D6KU49	CHY-type/CTCHY-typ	16.6392848	-7.4346907	1.64434719	-4.5213631	0.00000614	0.00130548
Zm00004b017739	GRMZM2G162250	Zm00001d042055	(1 of 3) PTHR31326: A0A1D6N0T2	ARGOS6	18.9738992	-7.6208123	1.68744911	-4.5161731	0.0000063	0.00132513
Zm00004b011561	GRMZM2G038801	Zm00001d013639	(1 of 2) PTHR15852: A0A1D6LJ71	DnaJ/Hsp40 cysteine	19.1338892	-7.6335474	2.14431334	-3.5599029	0.000371	0.04619789
Zm00004b013627	GRMZM2G121309	Zm00001d016277	(1 of 32) K14484 - auA0A1D6H6I5	Auxin-responsive pro	20.9605007	-7.7652893	2.16172134	-3.5921787	0.000328	0.04180513
Zm00004b039120	GRMZM2G174671	Zm00001d024908	(1 of 1) K01476 - arg A0A1D6J2P7	Arginase 1 mitochond	124.634851	-7.8105926	2.01527474	-3.8756962	0.000106	0.01666212
Zm00004b038454	GRMZM2G062585	Zm00001d024155	(1 of 2) 3.1.3.21 - Gl)B4FM45	(DL)-glycerol-3-phosr	22.7030084	-7.8802551	1.29576777	-6.0815335	1.19E-09	5.06E-07
Zm00004b000737	GRMZM2G027272	Zm00001d004022	(1 of 3) K13456 - RP/ A0A1D6ED15	RPM1-interacting prot	85.7103695	-7.9584404	2.12649259	-3.7425197	0.000182	0.02564156
Zm00004b033732	GRMZM5G872256	Zm00001d047582	(1 of 3) 2.4.1.123 - InQ7XYY1	Hexosyltransferase (	31.5946066	-8.3574989	1.55936046	-5.3595683	8.34E-08	0.0000239
Zm00004b015097	GRMZM2G028325	Zm00001d018037	(1 of 5) PTHR19139/ A0A1Q1ADS4	NOD26-like membrar	37.5124823	-8.6059927	1.76417839	-4.8781873	0.00000107	0.000257
Zm00004b029343	GRMZM2G126920	Zm00001d000360	#N/A	#N/A	48.7785425	-8.9851403	1.817047	-4.9449135	7.62E-07	0.000185
Zm00004b018250	GRMZM2G135743	Zm00001d042627	(1 of 8) 2.4.1.17 - GI\ A0A1D6N5J7	Hexosyltransferase (	982.742606	-9.016643	0.77835005	-11.584303	4.95E-31	5.47E-27
Zm00004b036596	GRMZM2G048165	Zm00001d021280	(1 of 1) PTHR22298: A0A1D6I9L5	Endoglucanase (EC :	50.8729068	-9.0456557	1.81462784	-4.9848545	0.00000062	0.000152
Zm00004b011870	GRMZM2G001887	Zm00001d013999	(1 of 5) K01527 - nasA0A1D6GPC3	Basic transcription fa	53.8848989	-9.1296606	1.47437162	-6.1922384	5.93E-10	2.67E-07
Zm00004b039616	GRMZM2G097499	Zm00001d025538	A0A1D6HZ9	Uncharacterized prot	61.4228893	-9.3179732	2.14512664	-4.343787	0.000014	0.00276306
Zm00004b003293	GRMZM2G452523	Zm00001d031691	(1 of 2) PF00055//PF A0A1D6KKQ0	Biotin synthase	122.070016	-9.3438471	1.50000812	-6.2291977	4.69E-10	2.16E-07
Zm00004b040319	GRMZM2G150950	Zm00001d026348	(M=2) PF02178 - AT K7TSL3	AT hook motif family	66.1902783	-9.4249272	2.11675509	-4.4525355	0.00000849	0.00175252
Zm00004b000181	GRMZM2G176585	Zm00001d027447	(1 of 3) PTHR31879: A0A1D6JM71	Det1 complexing ubic	66.744177	-9.4374569	2.07279808	-4.5530035	0.00000529	0.00113457
Zm00004b009722	GRMZM2G099239	Zm00001d006626	(1 of 32) PF03763 - F A0A1D6EZ75	Remorin family protei	134.038381	-9.4798187	2.21758152	-4.2748456	0.0000191	0.00367521
Zm00004b031639	GRMZM2G142072	Zm00001d044971	(1 of 3) K15172 - trar A0A1D6NSG9	Transcription elongat	85.8177719	-9.7999257	1.58831871	-6.1699996	6.83E-10	3.02E-07
Zm00004b036768	GRMZM2G131275	Zm00001d021489	(1 of 23) PF03759 - F A0A1D6IBJ1	Rop guanine nucleoti	92.4669284	-9.9071476	1.42087283	-6.9725786	3.11E-12	1.91E-09
Zm00004b029791	GRMZM2G048194	Zm00001d037151	(1 of 3) PTHR23354: A0A1D6LUX5	Erwinia induced prote	503.899342	-9.9154886	0.93584643	-10.595209	3.14E-26	2.31E-22
Zm00004b015296	GRMZM2G095778	Zm00001d018278	(1 of 2) PTHR28039: B4F9M5	Fatty-acid-binding pro	96.9447582	-9.9763143	1.54298423	-6.4655971	1.01E-10	5.19E-08
Zm00004b011028	AC210013.4_FG014	Zm00001d013030	(1 of 3) PTHR24349/ B4FF99	Calcium-dependent p	100.245209	-10.023864	1.99183346	-5.0324812	4.84E-07	0.00012
Zm00004b026211	GRMZM2G007384	Zm00001d010294	(M=25) PF00627 - LIA0A1D6FQ91	Ubiquitin-associated/	109.832559	-10.155952	2.31126258	-4.3941144	0.0000111	0.00223943
Zm00004b035448	GRMZM2G515595	Zm00001d019671	(1 of 1) PTHR13527: A0A1D6HZP9	Ubiquitin family prote	128.430769	-10.382079	1.33796142	-7.7596254	8.52E-15	8.96E-12
Zm00004b031625	GRMZM2G088053	Zm00001d044951	(1 of 54) PF00892 - E B4FTK8	WAT1-related protein	132.976976	-10.431711	1.93391696	-5.3940843	6.89E-08	0.0000208
Zm00004b012800	GRMZM2G017229	Zm00001d015138	A0A1D6GZI4	Uncharacterized prot	263.006615	-11.415653	2.71423684	-4.2058427	0.000026	0.00487091
Zm00004b004985	GRMZM2G143238	Zm00001d033839	(1 of 10) PF07714//P C4J1X5	Putative LRR recepto	443.323259	-12.16883	3.42896934	-3.5488303	0.000387	0.04672324
Zm00004b033257	GRMZM2G144730	Zm00001d047054	(1 of 2) PTHR11817/ K7VGL4	Pyruvate kinase (EC	580.352579	-12.557533	1.30571317	-9.6173746	6.75E-22	1.49E-18
Zm00004b037132	GRMZM2G374969	Zm00001d021902	(1 of 1) PTHR16220 B6T966	Transducin/WD40 rep	723.238902	-12.875086	1.22507056	-10.509669	7.80E-26	3.45E-22
Zm00004b039391	GRMZM2G159675	Zm00001d025239	(1 of 1) K12625 - U6 B4FCE3	Sm-like protein LSM€	856.966885	-13.119928	1.24673117	-10.523462	6.74E-26	3.45E-22

TABLE NOTES:

GENEIDs B73v3 GeneIDs were obtained from the W22 GeneIDs using a MaizeGDB syntelogs lookup table made with SynMap

TRANSCRIPTOME: Trimmed reads were aligned using the splice-aware aligner Hisat2, indices were constructed from known exons and splice sites (W22 annotation Zm00004b & assembly Zm-W22-REFERENCE-NRGENE-2.0 as described in the Methods.

DEG & TABULATION: Differential expression analysis was performed separately per tissue (leaf mutant vs. WT or tassel mutant vs. WT) as described in the Methods

TABULATION: This entire table, sorted on col-H, log2(fold-change), includes statistically significant differentially expressed (adjusted p-value <0.05) genes and excludes any genes with fewer than 10 counts across sum of all replicates.

COLUMNS G-L: Columns G-L tabulate output values for the baseMean, the log2(fold-change), along with the associated values for standard error (lfcSE), Wald statistic (stat), pvalue, and adjusted pvalue (padj).

PROVENANCE: Supplementary Table S2 from Mckenna, Gumber, et al., "Maize (Zea mays L.) nucleoskeletal proteins regulate nuclear envelope remodeling…", submitted 12/2020

Table S2b2 (McKenna, Gumber, et al., submitted Dec. 2020)

Figure S3: Multiple Seq Alignment of Transcripts.

Addgene gene ID	Plasmid name	Plasmid type	N-terminal Tag	Backbone Name	Bacterial Resistance
131014	MKAKU41ec	entry vector	mCherry-FLAG-HA	pDONR221	Kanamycin
131015	MKAKU41exp	expression vector	mCherry-FLAG-HA	pH7WG2	Spectinomycin
131016	NCH1ec	entry vector	eGFP-FLAG-HA	pDONR221	Kanamycin
131017	NCH1exp	expression vector	eGFP-FLAG-HA	pH7WG2	Streptomycin
131018	NCH2ec	entry vector	eGFP-FLAG-HA	pDONR221	Kanamycin
131019	NCH2exp	expression vector	eGFP-FLAG-HA	pH7WG2	Spectinomycin
159097	p35S::mCherry-GFP-H	expression vector	mCherry-GFP	pB7FWG2	Spectinomycin

Table S3 is from Mckenna, Gumber, et al., "Maize (Zea mays L.) nucleoskeletal proteins…", submitted 12/2020

Table S3 (McKenna, Gumber, et al., submitted Dec. 2020)