# Autophagy functions as an antiviral mechanism against geminiviruses in plants

Yakupjan Haxim#, **Asigul Ismayil#**, Qi Jia1, Yan Wang, Xiyin Zheng, Tianyuan Chen, Lichao Qian, Na Liu, Yunjing Wang, Jiaxuan Cheng, Yiguo Hong, Yule Liu*

**School of Life Sciences, Tsinghua University, Beijing 100084, China. #Cof-rist author, *Corresponding author**

## Abstract

**Autophagy is an evolutionarily conserved process that recycles damaged or unwanted cellular components, and has been linked to plant immunity. However, how autophagy contributes to plant immunity is unknown. Here we reported that the plant autophagic machinery targets the virulence factor βC1 of Cotton leaf curl Multan virus (CLCuMuV) for degradation through its interaction with the key autophagy protein ATG8. A V32A mutation in βC1 abolished its interactionwith NbATG8f, and virus carrying βC1$^{V32A}$ showed increased symptoms and viral DNAaccumulation in plants. Furthermore, silencing of autophagy -related genes ATG5 and ATG7 reduced plant resistance to the DNA viruses CLCuMuV, Tomato yellow leaf curl virus, and Tomato yellow leafcurl China virus, whereas activating autophagy by silencing GAPC genes enhanced plant resistance to viral infection. Thus, autophagy represents a novel anti-pathogeicmechanism that plays an important role in antiviral immunity in plants.**

**Contacts**

**Asigul Ismayil**

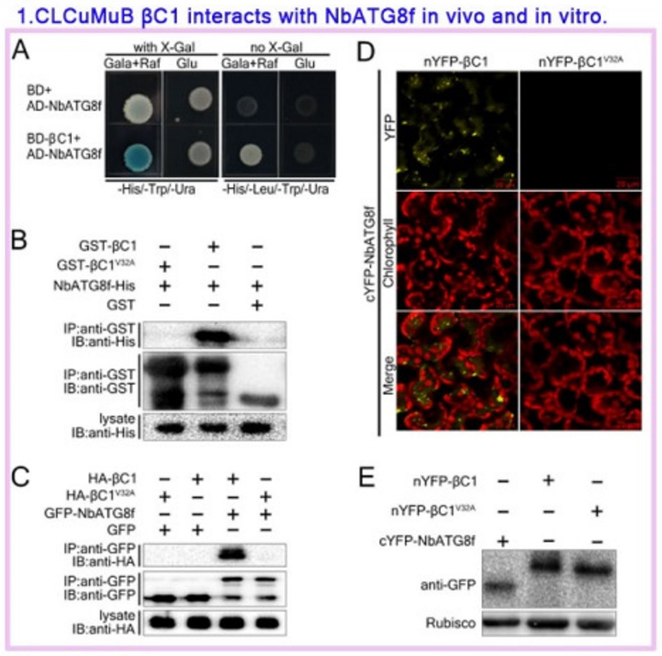
School of Life Sciences,
Tsinghua University
Email: asgli12@163.com

## Introduction

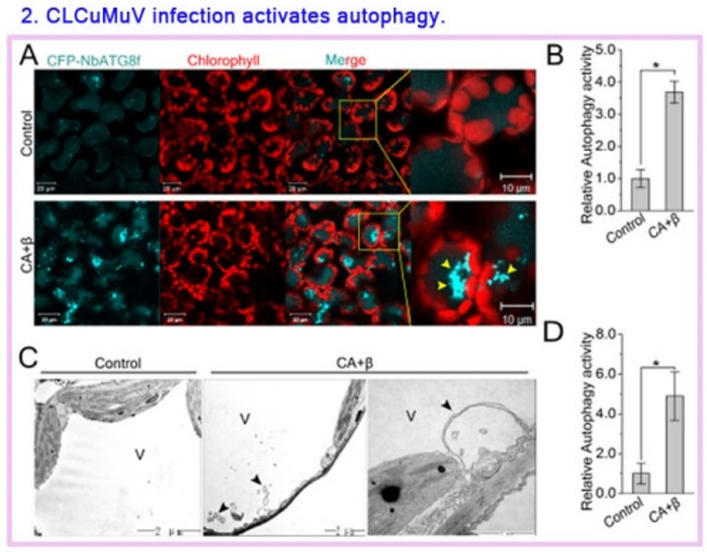
Plants have evolved various defense mechanisms to combat plant pathogens, including viruses. Autophagy is an evolutionarily conserved mechanism that recycles damaged or unwanted cellular materials under stress conditions or during specific developmental processes (Liu and Bassham,2012), and plays a critical role in multiple physiological processes, including plant biotic stress responses (Han et al., 2011). Autophagy may link plant immunity in different ways, with autophagy playing a role in degradingpathogen effectors or defense-related plant proteins, or pathogen effectors interfering with autophagy. Indeed, viral proteins are reported to promote autophagic degradation of plant host RNAi-related components (Derrien et al., 2012; Cheng and Wang, 2016). In addition, 2b protein from Cucumber mosaic virus is thought to be targeted for degradation by autophagy through the cal-modulin-like protein rgsCaM (Nakahara et al., 2012). Recently, an oomycete effector is reported tointerfere with autophagy by depleting the putative selective autophagy cargo receptor Joka2 out of ATG8 complexes (Dagdas et al., 2016). However, the role of autophagy in degrading pathogen effectors or plant defense-related proteins and the effect of viral effectors on autophagy remain uncertain in plants.

## Results

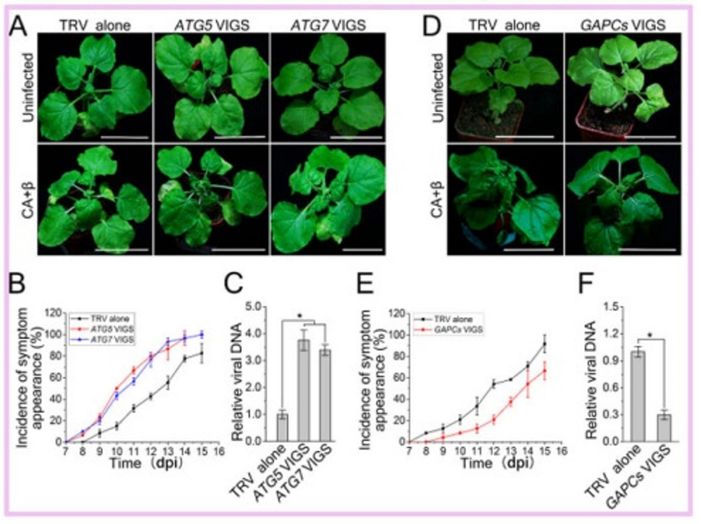
### 1.CLCuMuB βC1 interacts with NbATG8f in vivo and in vitro.



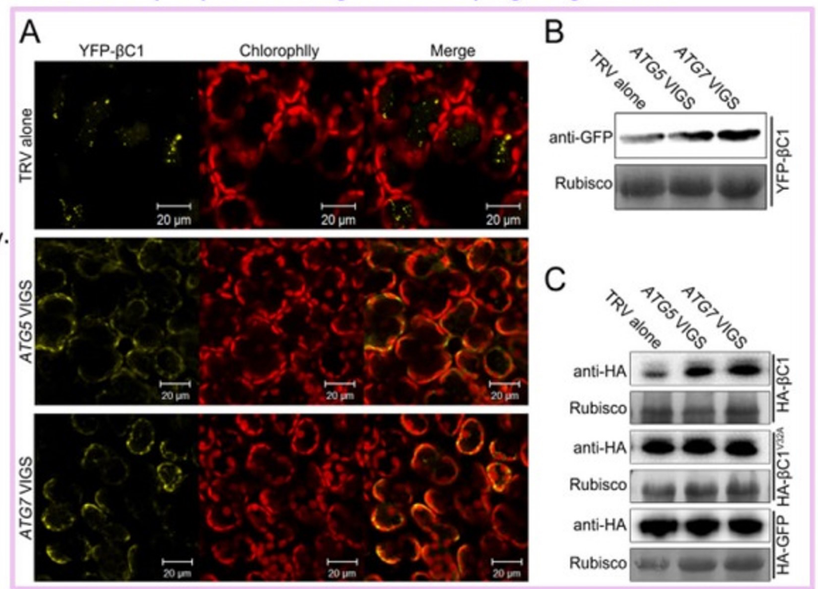

### 2. CLCuMuV infection activates autophagy.



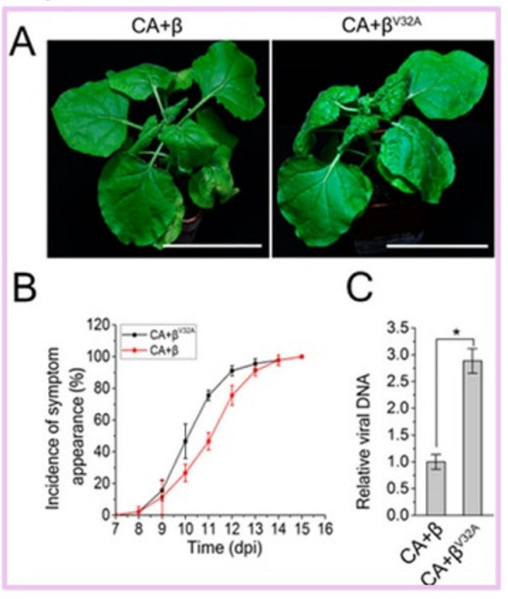

### 3. CLCuMuV DNA accumulation is affected by host cell autophagy.




### 4. CLCuMuB βC1 proteins is targeted for autophagic degradation.




### 5. βC1$^{V32A}$ enhanced CLCuMuV infection.




## Conclusions

Autophagy is known to play an important role in disease resistance or susceptibility to various pathogens in plants (Han et al., 2011). However, how autophagy is linked to plant immunity remains unknown. In this study, we show that geminivirus CLCuMuV infection activates autophagy and that autophagy targets the virulence protein βC1 for degradation. Further, we demonstrated for the first time that autophagy plays an active role as an antiviral mechanism in compatible plant-virus interactions. Interfering with autophagy made the *N.benthamiana* plants less resistant to the cotton leaf curl disease virus, and to two other geminiviruses that often infect tomatoes. Activating autophagy had the opposite effect, and made the plants more resistant to all three viruses.

In summary, we provide direct evidence that autophagy functions as a novel antiviral mechanism in plants.

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### CLCuMuV βC1 induces autophagy for its degradation




# CLCuMuB βC1 induces autophagy by disrupting the interaction of ATG3 with GAPCs

**Asigul Ismayil**, Meng Yang, Yunjing Wang, Jinlin Li, Lu Han, Yan Wang, Xiyin Zheng, Xiang Wei, Ugrappa Nagalakshmi, Yiguo Hong, Linda Hanley-Bowdoin, Yule Liu*

## Abstract

**Autophagy is known to play an important role in plant-pathogen interactions. Several pathogens including viruses are reported to induce autophagy in plants, but the underpinning mechanism remains largely unclear. Further, in virus-plant interplay it is yet to identify any viral factor(s) responsible for induction of autophagy. Here, we report that βC1 protein of Cotton leaf curl Multan betasatellite (CLCuMuB) interacts with cytosolic glyceraldehyde-3-phosphate dehydrogenases (GAPCs), a negative autophagic regulator, to induce autophagy in Nicotiana benthamiana. CLCuMuB βC1 bound to GAPCs and disrupted the interaction between GAPCs and ATG3. A mutant βC1 protein (βC1$^{3A}$) in which I45, Y48, and I53 were all substituted with alanine (A), dramatically reduced its binding capacity with NbGAPCs, failed to disrupt the NbGAPCs-NbATG3 interactions and failed to induce autophagy. Furthermore, mutant virus carrying βC1$^{3A}$ showed increased symptoms and viral DNA accumulation associated with decreased autophagy. These results suggest that CLCuMuB βC1 activates autophagy by disrupting GAPCs-ATG3 interactions.**

**Contacts**

**Asigul Ismayil**

Email: asgli12@163.com

## Introduction

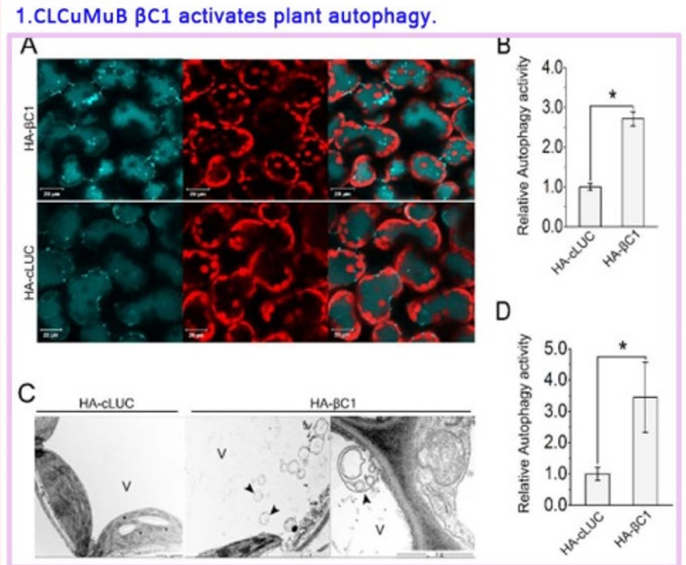
In plants, autophagy plays an important role in disease resistance or susceptibility to various pathogens (1,2). It contributes to the hypersensitive response cell death but restricts the spread of programmed cell death beyond the initial infection site (3-6). Autophagy has also recently been shown to represent an antiviral mechanism in plants (7-11). In addition, autophagy may also directly or indirectly promote virus infection (9,12).

Given that autophagy has an important role in host-virus interaction, it is not surprising that some viruses have evolved strategies to modulate host autophagy for their own benefit. For instance, Turnip mosaic virus (TuMV) antagonizes NBR1 -dependent autophagy during infection, thereby limiting its antiviral capacity.NBR1 -independent bulk autophagy prevents premature plant death, extending the lifespan of virus reservoirs and particle production (11). Cauliflower mosaic virus (CaMV) P6 protein disrupts the interaction between viral P4 and host NBR1 to protect viral replication factory inclusions from autophagic degradation (9). Barley stripe mosaic virus (BSMV) γb interferes with the interaction of ATG7 with ATG8 in a competitive manner to suppress autophagy, thereby promoting viral infection (13). Viral proteins can also promote autophagic degradation of components in RNA silencing pathway (14,15).

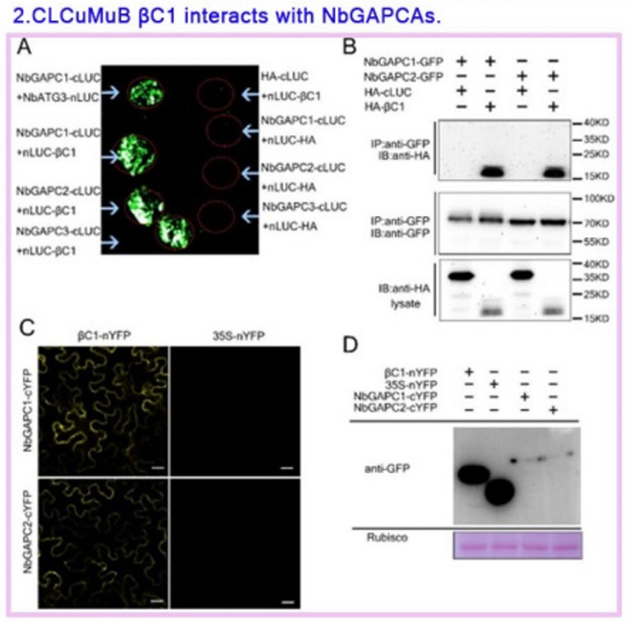
On the other hand, it is well-established that various plant pathogens can trigger autophagy (3,4, 8–10). However, in plants only one bacterial and one fungal protein have been shown to be responsible for autophagic activation despite no mechanism is characterized (16,17). In addition, no viral protein has been reported to activate autophagy in plants. In this study, we demonstrate that CLCuMuB βC1 activates autophagy by interfering with the interaction between NbGAPCs and NbATG3.

## Results

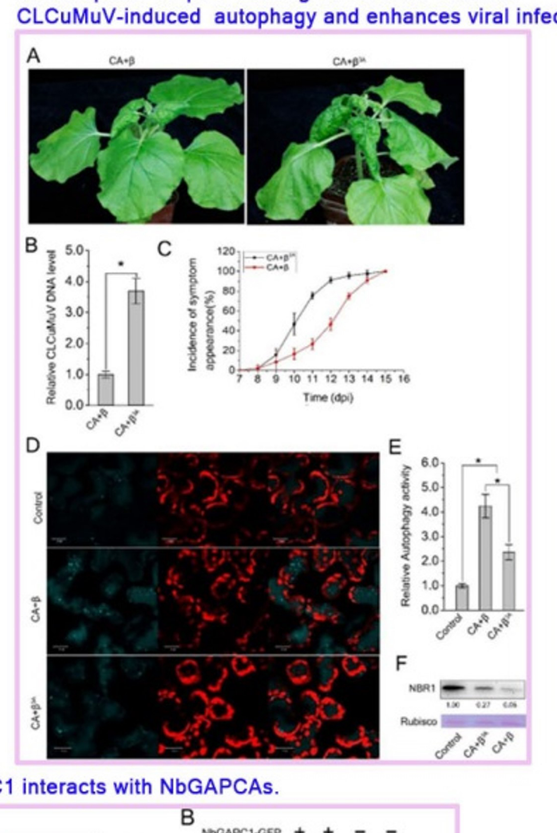
### 1.CLCuMuB βC1 activates plant autophagy.



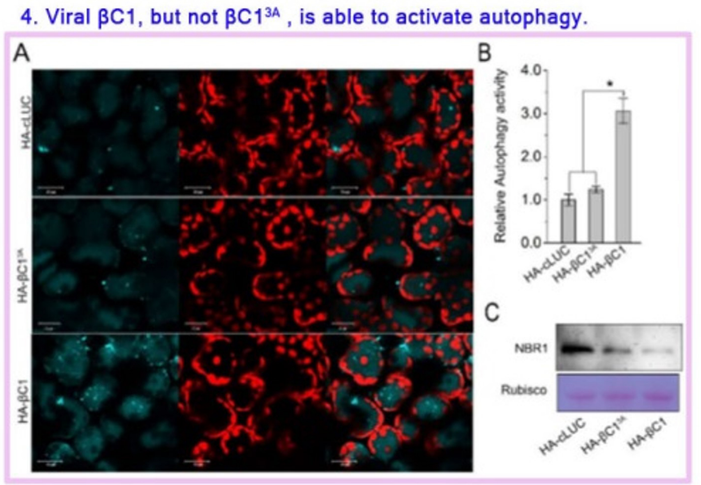

### 2.CLCuMuB βC1 interacts with NbGAPCAs.



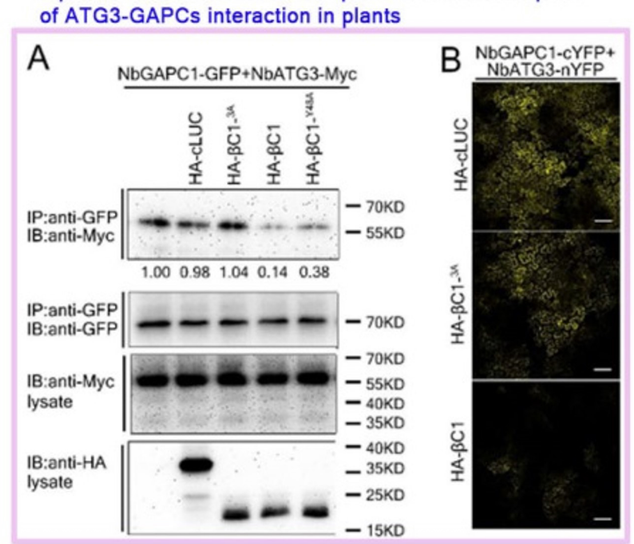

### 5. Disruption of βC1 binding to GAPCs reduces CLCuMuV-induced autophagy and enhances viral infection.




### 4. Viral βC1, but not βC1$^{3A}$ , is able to activate autophagy.




### 3. βC1-GAPCs interaction is important for the disruption of ATG3-GAPCs interaction in plants




## Conclusions and Discussions

In this study, we found that CLCuMuB βC1 induces autophagy by interacting with the negative autophagy regulator GAPCs to disrupt the interaction between NbGAPCs and NbATG3. To our knowledge, this is the first report of a viral effector that activates autophagy and the underlying mechanistic basis of pathogen-mediated autophagy activation in plants.

Since CLCuMuB βC1 is a viral virulence factor and is targeted by autophagy for the degradation, CLCuMuB βC1-mediated autophagy may reduce its viral virulence and enable successful infection during the plant-virus coevolution. In addition, it is also possible that CLCuMuB βC1-mediated autophagy helps host cell survival for better viral propagation and transmission. Indeed, autophagy is reported to extend plant lifespan and to play a proviral role during some virus infections

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