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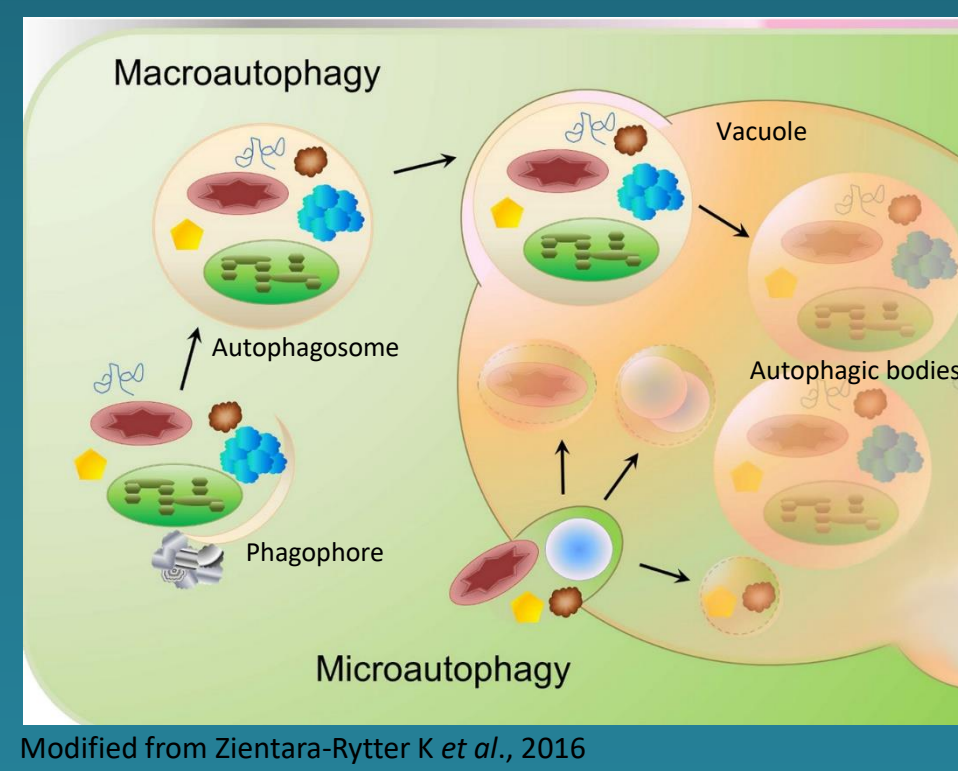
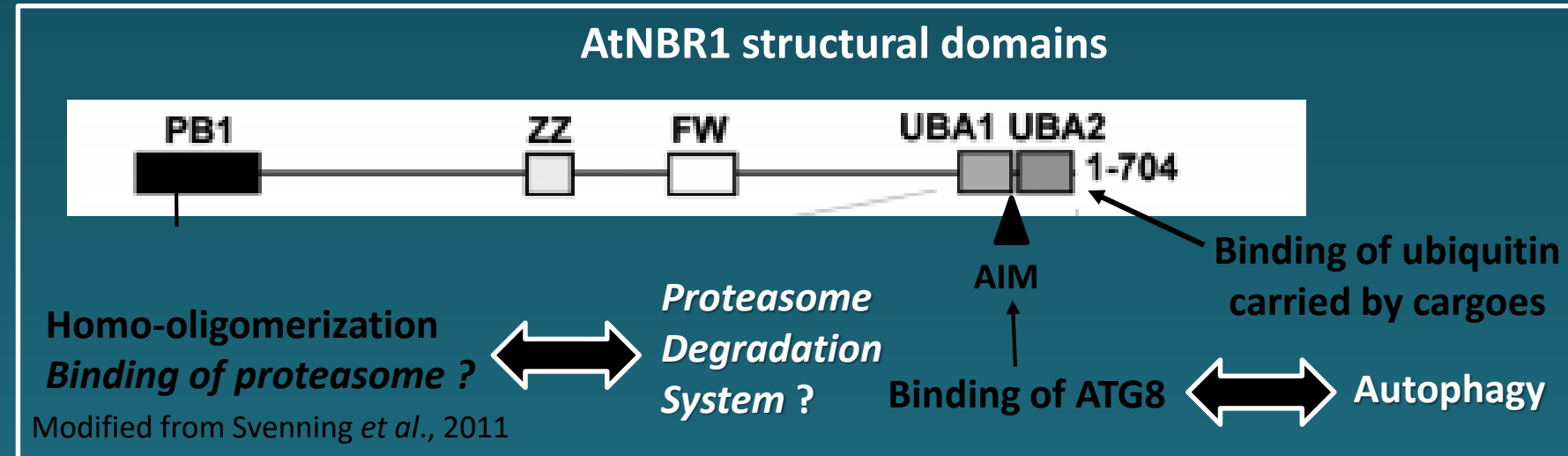
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NBR1, a plant selective autophagy receptor, associates with lipid droplets in germinating seeds and contributes to seed longevity

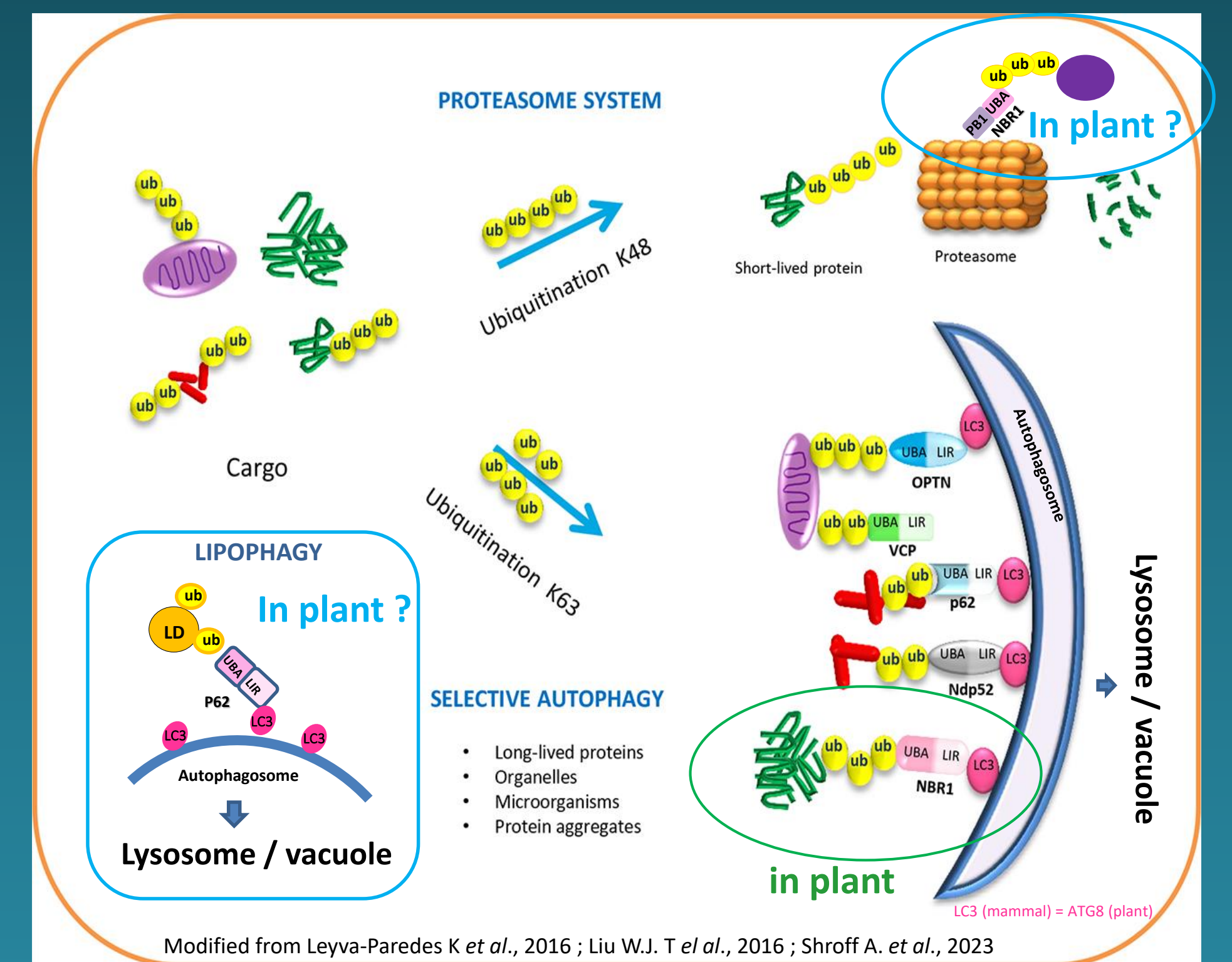
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BACKGROUND

Plant Neighbor of BRCA1 gene 1 (NBR1) is a functional hybrid of the mammalian selective autophagy receptors (SAR) NBR1 and p62/SQSTM1 (Svenning *et al.*, 2011), which share similar domains. It is the main SAR in plants, that do not contain p62/SQSTM1. It binds to ubiquitin via its UBA2 domain and to LC3/ATG8 via its LIR/AIM domain and targets its cargo towards the vacuole/lysosome for degradation. In plantlets, NBR1 is especially involved in aggrephagy upon heat-, oxidative- and drought stress. In contrast to the numerous data published on plantlets, the role of NBR1 has been poorly investigated in seedlings. Moreover, its involvement in lipophagy or in autophagic/proteasomal degradation of LD (lipid droplet) proteins has never been studied. Besides, it has been shown that mammal p62 could act as a receptor for the proteasomal degradation of ubiquitinated proteins via its PB1 domain (Liu W.J. *et al.*, 2016). This has never been described in plants.



Two distinct types of autophagy in plants : micro- and macroautophagy. The first one is characterized by direct invagination of the tonoplast to trap cytoplasmic material and the second one by the formation of an autophagosome that sequesters cytoplasmic components before fusing with the tonoplast to release its content. Macroautophagy can be selective (via the SARs) or bulk.



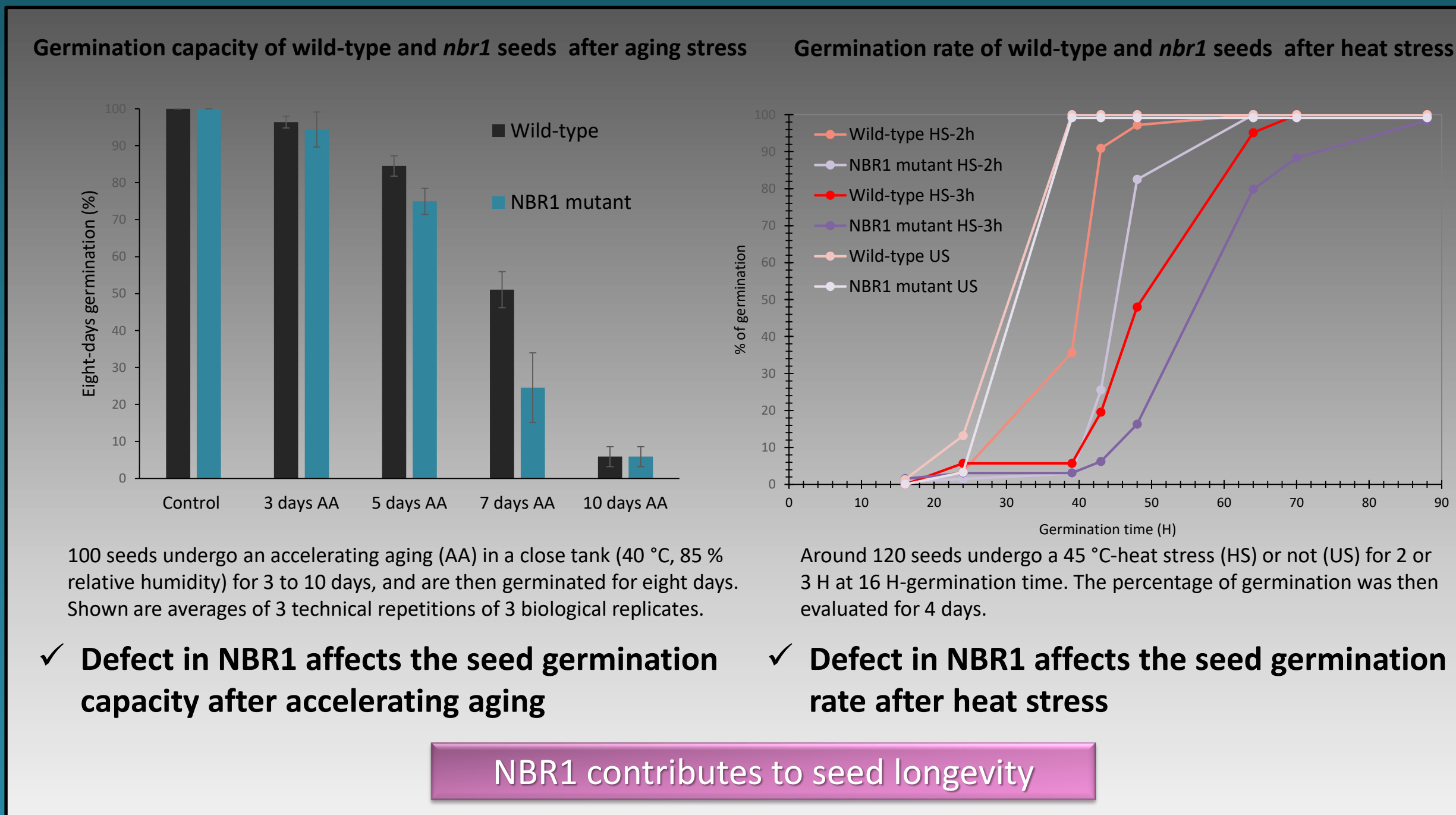
Two degradation pathways involve p62 and NBR1 in mammals. The circles emphasize a known similar role of NBR1 in plant (green) or an hypothetical role of NBR1 in plant (blue).

OBJECTIVE : We aimed to characterize the effect of stress on the germination of *nbr1* mutant seeds in *Arabidopsis thaliana*. Likewise, we investigated the role, expression and sub-cellular localization of NBR1 during seed germination.

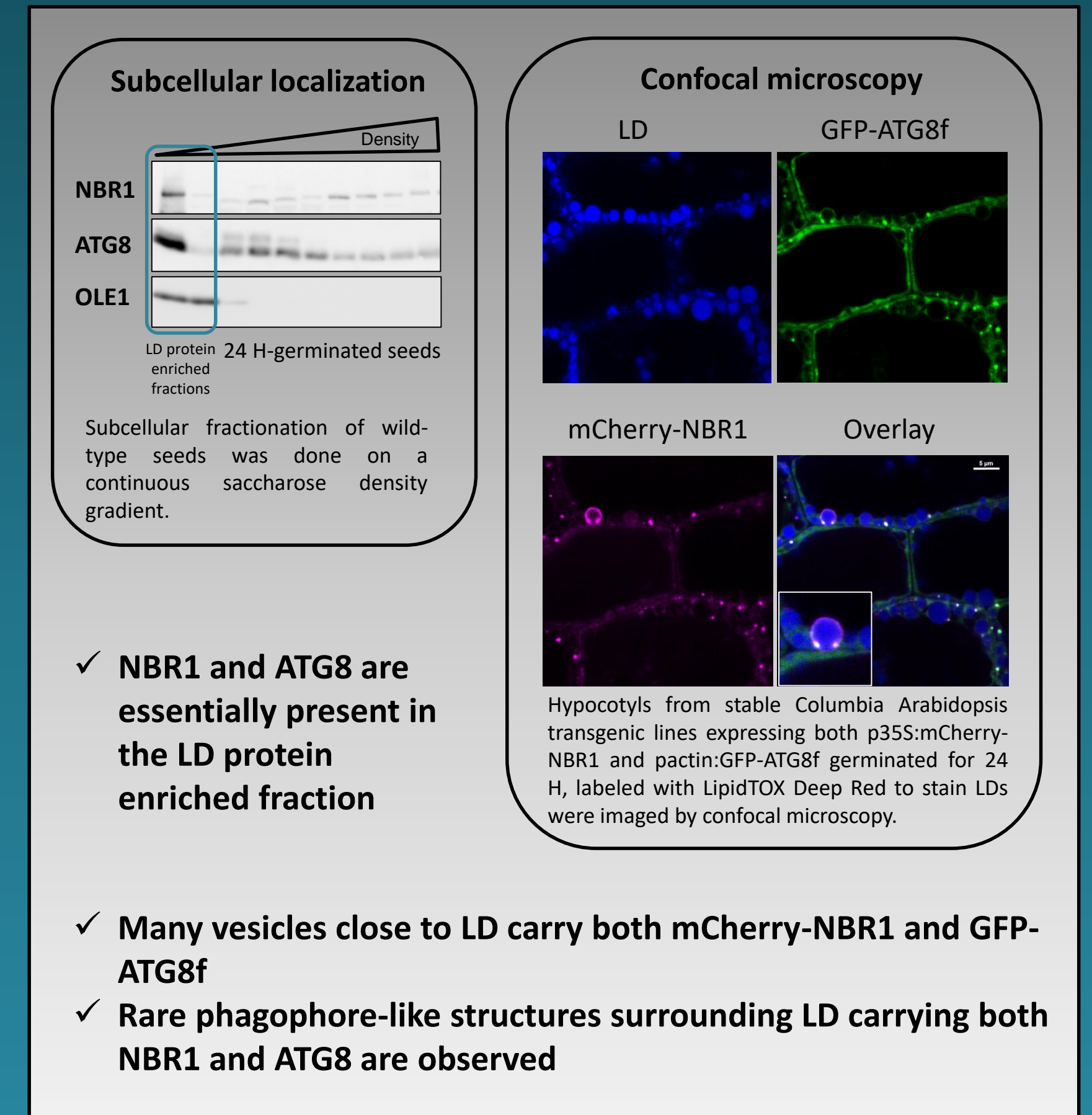
A In seeds, NBR1 may be involved in the clearance of...

...damaged proteins induced by stress

...proteins not required for the germination program

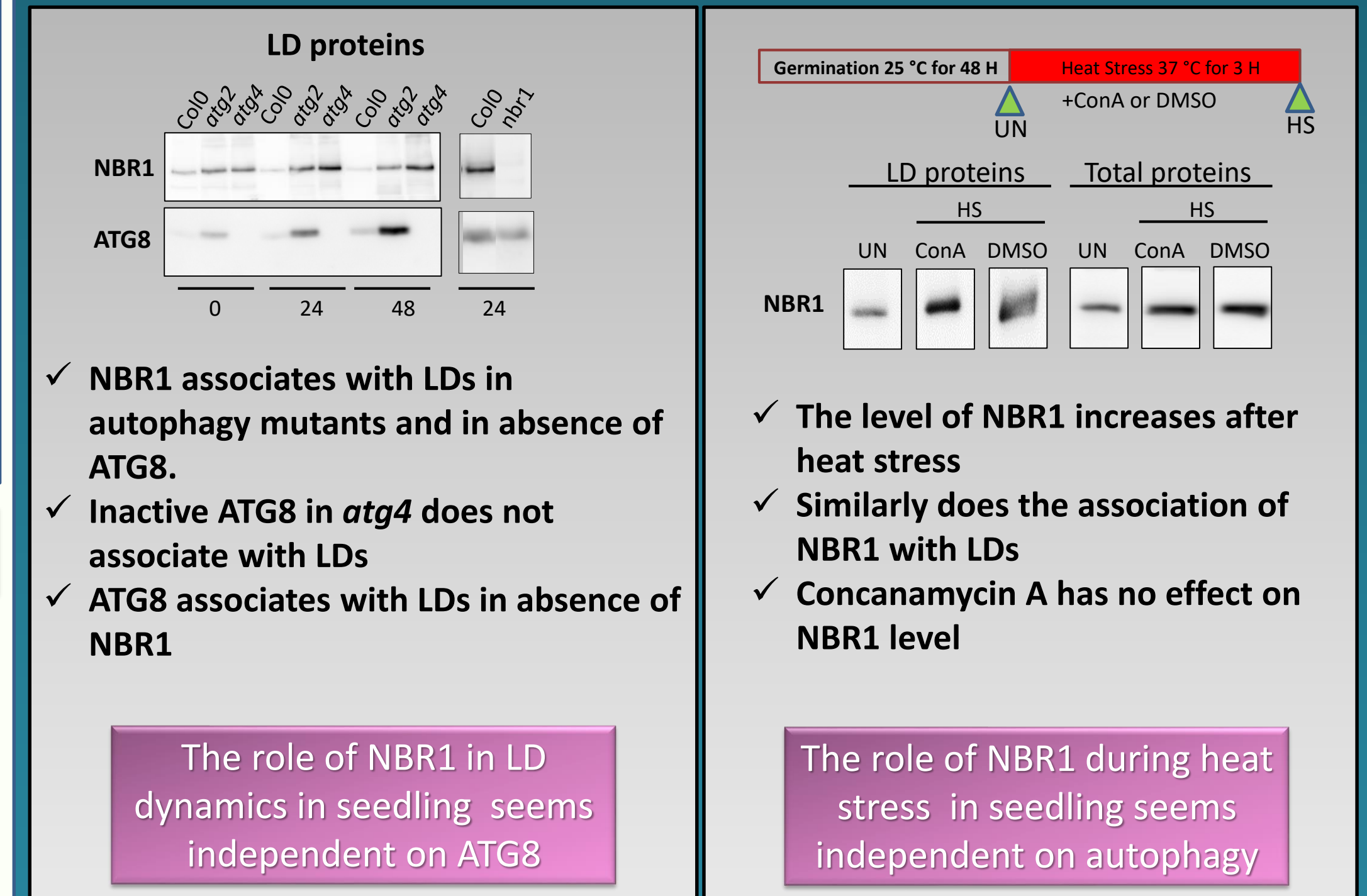
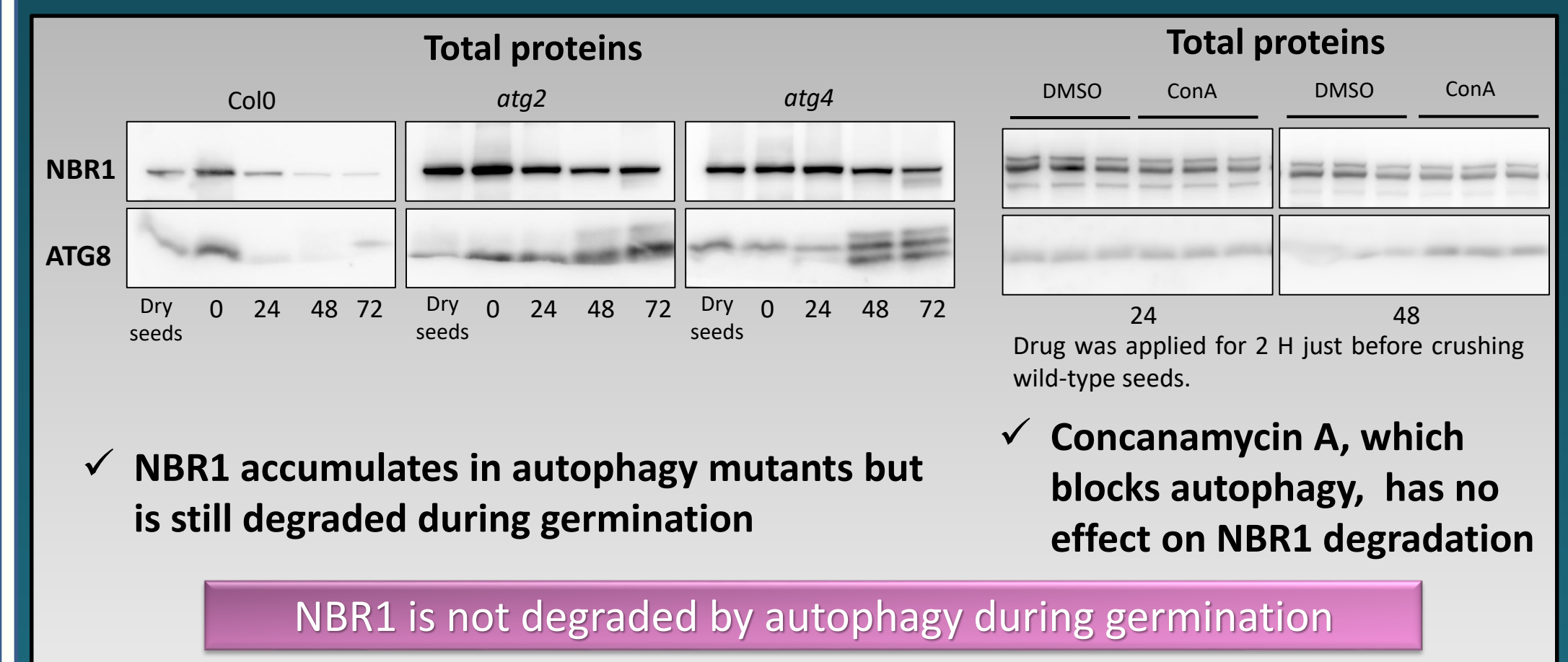
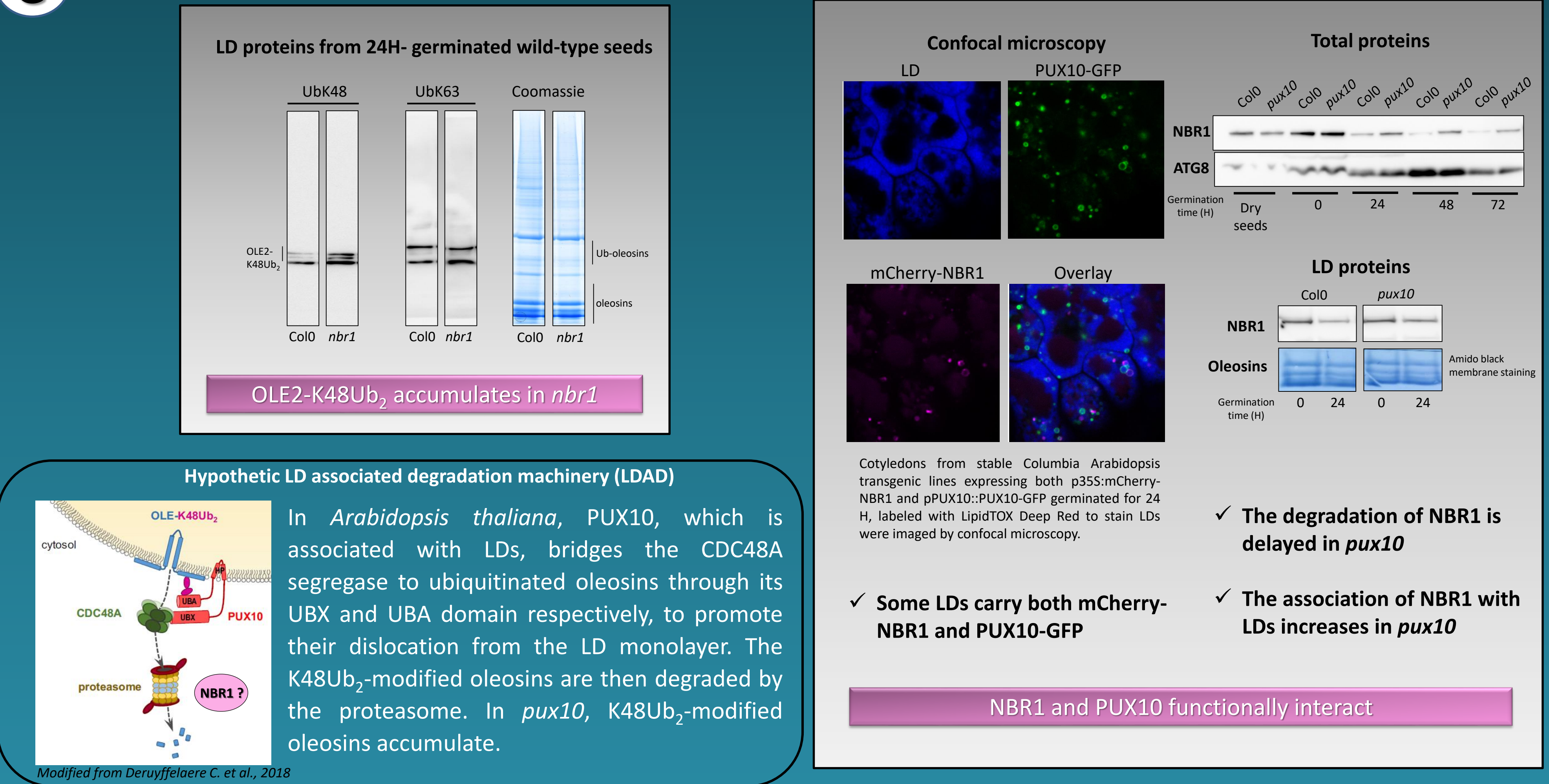


B Both NBR1 and ATG8 associate with LDs



Is NBR1 involved in autophagy in seedlings? ↓

C NBR1 may be involved in the proteasomal LD protein degradation in seedling



CONCLUSION

NBR1 is the first SAR shown to be associated with LDs in plants, which is consistent with recent findings in mammals where NBR1 and p62/SQSTM1 have been shown to be associated with LDs and involved in lipophagy (Robichaud *et al.*, 2021 ; Shroff *et al.*, 2023). Our microscopic observations clearly showed the presence of numerous vesicles in the vicinity of LDs and phagophore-like structures surrounding LDs carrying both NBR1 and ATG8. The involvement of NBR1 in lipophagy can therefore be clearly envisaged. Alternatively, it could also be involved in the autophagy degradation of LD proteins. As a SAR, NBR1 is degraded with its cargo in the vacuole. However, we did not demonstrate any major effect of autophagy inhibition on NBR1 degradation in seedlings. So, while we can not exclude the involvement of a selective autophagy mediated by NBR1 in LD dynamics, it is probably minor and not exclusive at these stages. Thus, we have explored another degradation pathway, the proteasome one. First of all, we showed that some oleosins linked to K48Ub₂, a classical mark for proteasome degradation, accumulate in *nbr1*. This mimics *pux10* phenotype. In *pux10*, the degradation of oleosins is delayed but not totally stopped (Deruyffelaere *et al.*, 2018). That leads to the hypothesis that other actors would play a role in this machinery. Consistently, our observations suggest that NBR1 could act, together with PUX10, in the LDAD machinery. Overall, our results showed that NBR1 might play a role in the LD protein turnover.

Acknowledgement : We greatly thank Agnieszka Sirko for the gift of the KO NBR1 mutant.

Autophagy may not be the major pathway for NBR1 degradation during seed germination

References : Deruyffelaere *et al.*, *The Plant Cell*, 2018 ; Leyva-Parades *et al.*, *Intech*, 2016; Liu W.J. *et al.*, *Cell. Mol. Biol. Lett.*, 2016; Robichaud *et al.*, *Autophagy*, 2021; Shroff *et al.*, *Autophagy*, 2023; Svenning *et al.*, *Autophagy*, 2011 ; Zientara-Rytter *et al.*, *The FEBS J.*, 2016.

