## **Supplementary Material**

## The levels of peroxisomal catalase protein and activity modulate the onset of cell death in tobacco BY-2 cells via reactive oxygen species levels and autophagy

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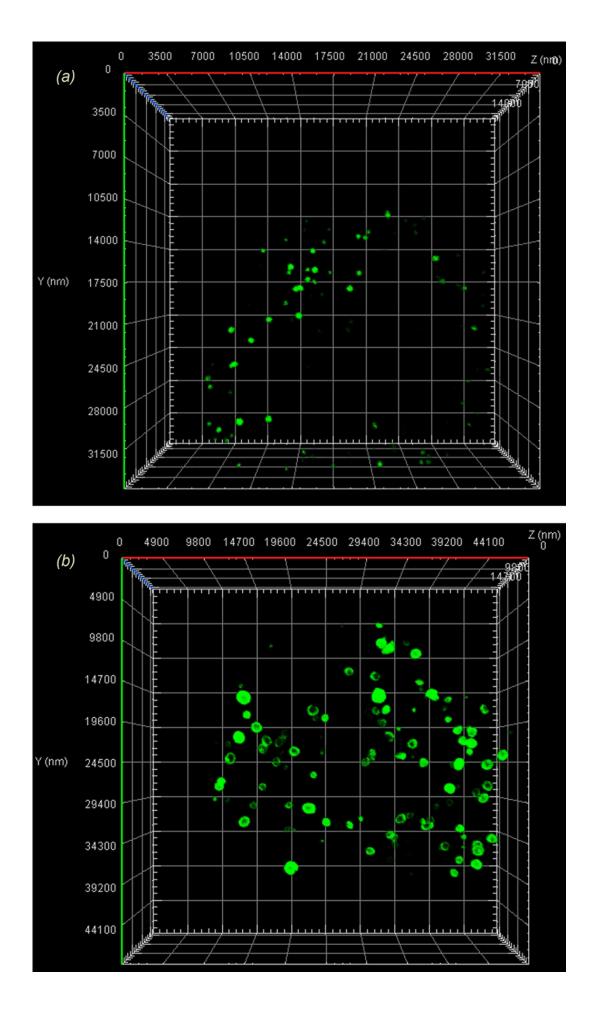
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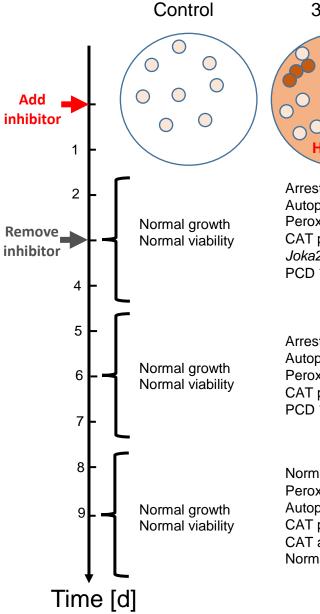
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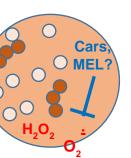
Genes	Forward primer	Reverse primer
NtUBC2	5' CTGGACAGCAGACTGACATC 3'	5' CAGGATAATTTGCTGTAACAGATTA 3'
NtAOX1	5' CTGTGTTGGGTCACATGGGT 3'	5' AGCCATAGAACTAGCGCTGC 3'
NtAPXc	5' CTGATGTTCCCTTTCACCCTG 3'	5' CACTCCCAACTCTTCCTCCT 3'
NtAPXp	5' CAGACGCGGCTCTTTTTGAA 3'	5' GGCAAATTAAAACAAACGGCA 3'
NtJOKA2	5' CTGGAGAGCAGTAGGGGAGA 3'	5' TTTGGGATACGACTACTTACCATAG 3'
NtPDS	5' GCTTTTGTGTTTGCCACTCCA 3'	5' ACGCAGAAACTGCTCTGGAA 3'
NtSOD	5' ATGTCACGGGACCACATTAC 3'	5' ATGCAGTACCATCTTCCCCAAC 3'

 Table S1.
 Primers used to amplify genes by RT-qPCR



**Fig. S1.** 3D-projections of Z-stacks obtained with single tobacco BY-2 cells showing cellular peroxisomes (green color due to eYFP fluorescence). (*a*) A control cell grown in MS-medium supplemented with 3% sucrose as described in Materials and methods. (*b*) A cell exposed to 0.2 mM aminotriazole for three days, after three days since the removal of inhibitor (i.e., at day 6 of the experiment). Both Z-stacks were obtained using a  $63 \times$  objective. The axes show the scale in nm.





3MA

Arrested growth Autophagy  $\downarrow\downarrow$ Perox. aggregation CAT protein ↑↑ Joka2 ↓↓ PCD ↑

Arrested growth Autophagy 11 Perox. biogenesis ↑ CAT protein ↑↑ PCD ↑

Normal growth Perox. biogenesis ↑ Autophagy ↑↑ CAT protein ↑ CAT activity  $\uparrow$ Normal viability

Arrested growth Autophagy 11 CAT activity  $\downarrow \downarrow$ CAT protein  $\uparrow\uparrow$ PCD ↑↑

Arrested growth

CAT protein  $\downarrow\downarrow$ 

CAT activity  $\downarrow \downarrow$ 

Normal viability

Joka2 ↓↓

AT

0,

Arrested growth Perox. biogenesis  $\downarrow$ Autophagy ↓↓ Perox. aggregation CAT protein ↑↑ CAT activity  $\downarrow \downarrow$ PCD ↑

Arrested growth Perox. biogenesis  $\downarrow$ Autophagy 4 Perox. aggregation CAT protein ↑ CAT activity  $\downarrow \downarrow$ 

Normal viability

Arrested growth Autophagy  $\downarrow\downarrow$ CAT protein  $\downarrow$ CAT activity  $\downarrow \downarrow$ Joka2↓ Normal viability

3MA+AT

APX Cars.

MEL

Arrested growth Perox. biogenesis  $\downarrow$ Autophagy  $\downarrow \downarrow$ Perox. aggregation CAT protein ↑↑ CAT activity  $\downarrow \downarrow$ Normal viability

**Fig. S2.** Scheme illustrating the time course of the experiment and changes in peroxisome numbers, ROS levels, autophagy levels, cell viability and growth. Schemes of tobacco cells are depicted for the end of exposure to inhibitors (day 3). Higher and lower ROS levels in the cytosol and peroxisomes are indicated by dark and weak red coloration, respectively. Abbreviations: AT, aminotriazole; 3-MA,3-methyladenine; Cars, carotenoids; Mel, melanin; APX, ascorbate peroxidase; CAT, catalase.