

Supplemental Material to:

**David Munch, Eleazar Rodriguez, Simon Bressendorff,
Ohkmae K Park, Daniel Hofius, and Morten Petersen**

**Autophagy deficiency leads to accumulation
of ubiquitinated proteins, ER stress, and cell death
in *Arabidopsis***

Autophagy 2014; 10(9)

<http://dx.doi.org/10.4161/auto.29406>

www.landesbioscience.com/journals/autophagy/article/29406

Figure S1

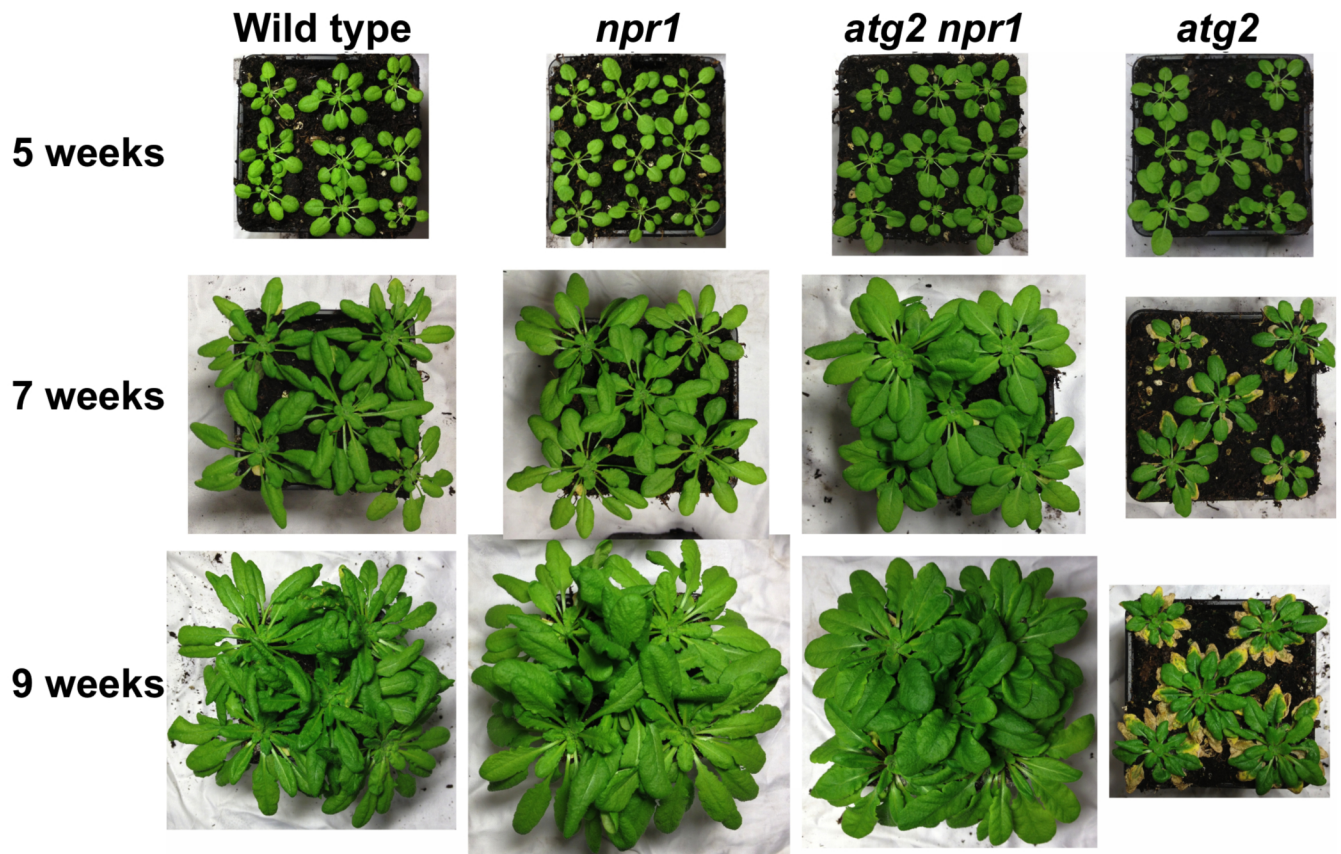


Figure S2

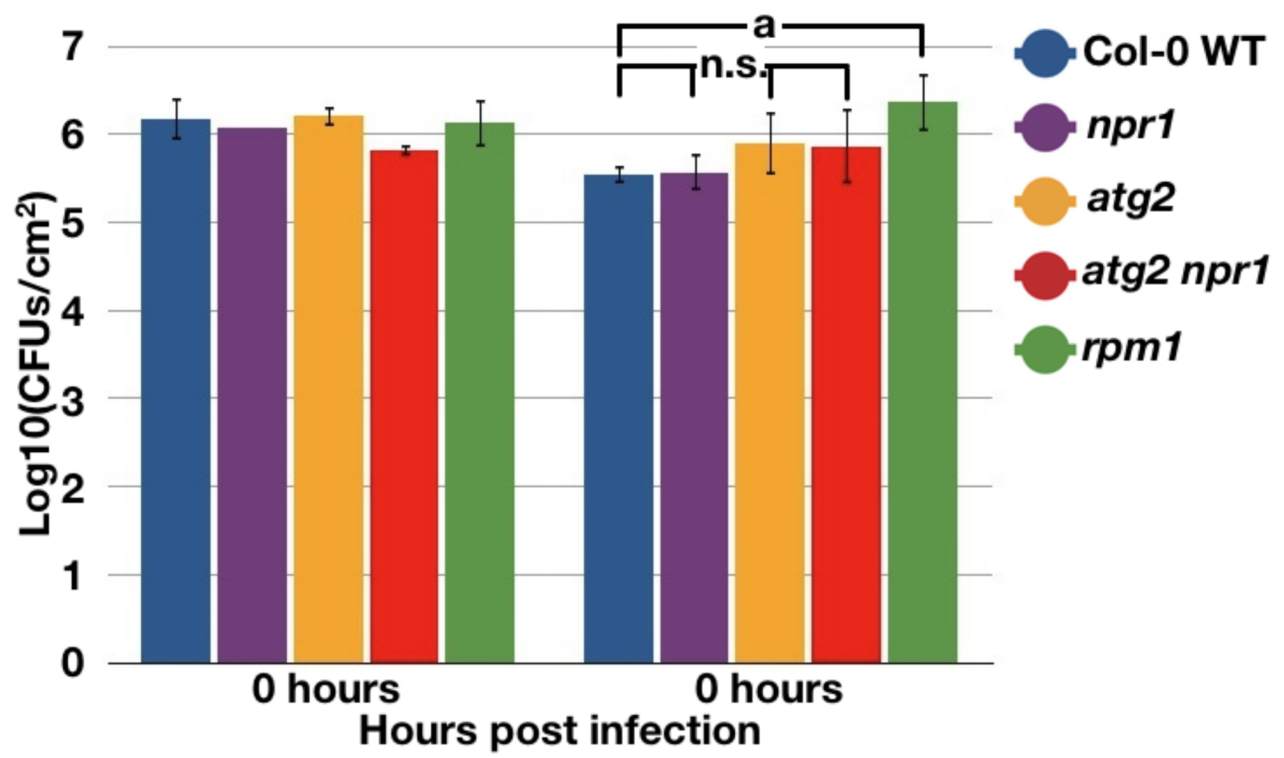


Figure S3

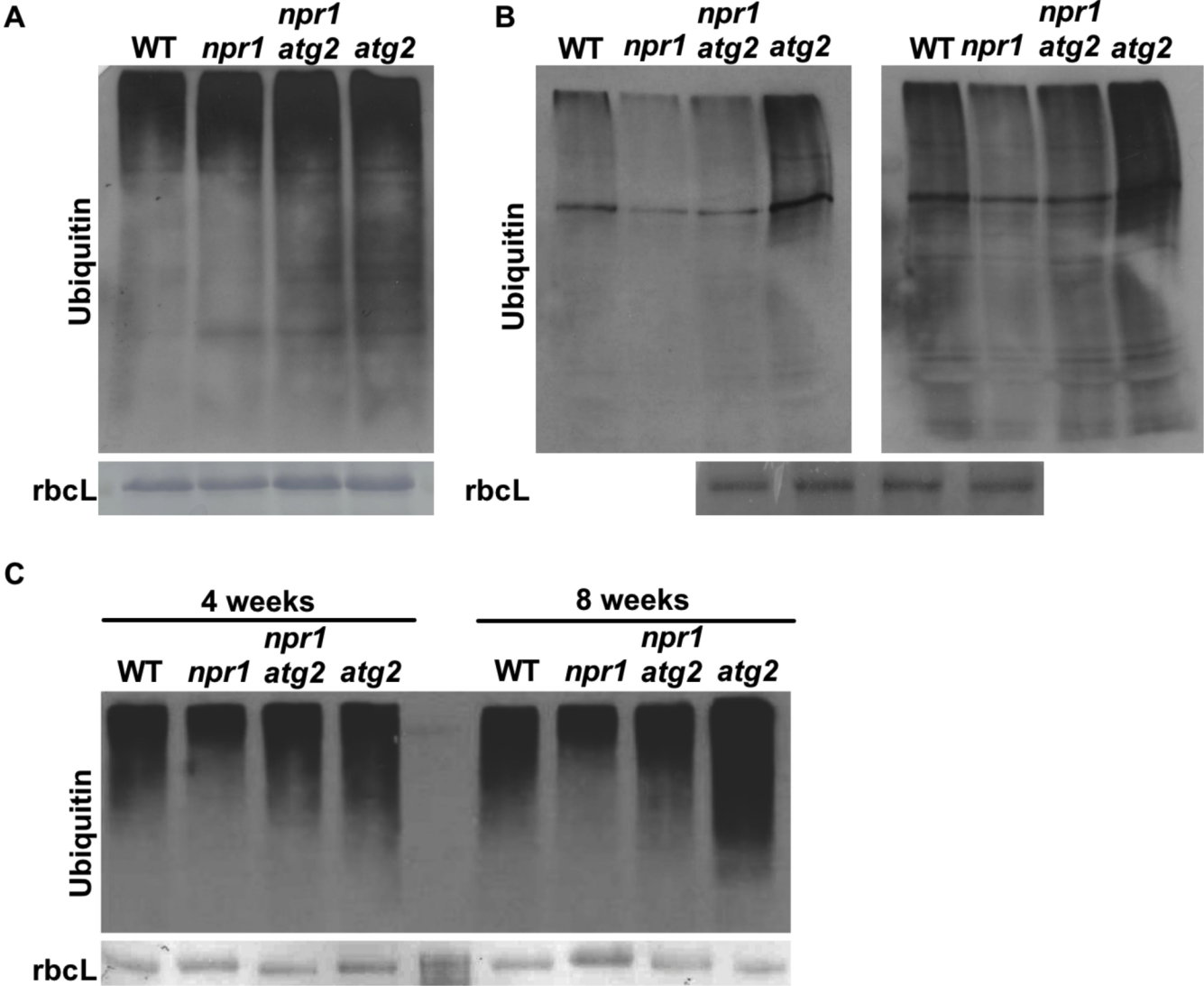
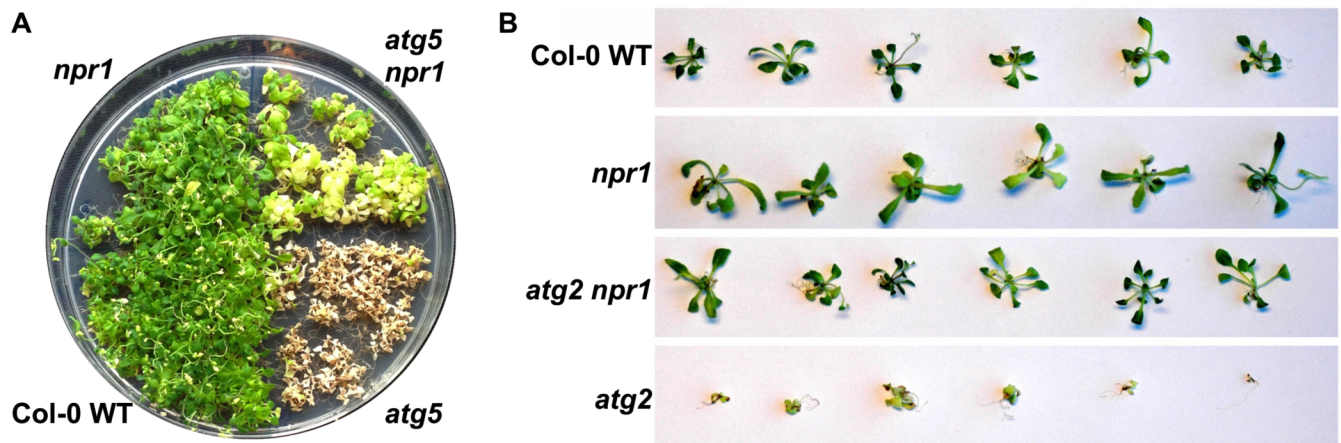


Figure S4



1 **Figure S1.** Phenotypes of 5-, 7- and 9-wk-old Col-0 WT, *npr1*, *atg2 npr1*, and *atg2* plants. Significant
2 morphological differences were not detected among the genotypes at 3 wk. At 7 wk, early senescence is
3 established in *atg2* mutants, which can be alleviated by a loss of NPR1 function.

4 **Figure S2.** Growth of avirulent *Pst* DC3000 (*AvrRpm1*) in 5-wk-old WT, *npr1*, *atg2*, *atg2 npr1*, and
5 *rpm1* plants. Plants were inoculated with 1×10^8 colony forming units (CFU) mL⁻¹ and log₁₀ bacterial
6 counts per area of leaf plotted against 0 and 6 h post-infection. Recognition of RPM1 triggers HR to fight
7 bacterial growth, but *rpm1* mutants do not recognize the effector and allow significant growth of the
8 pathogen. Error bars indicate standard deviation calculated from the mean of 3 samples per genotype.
9 Pairwise comparisons for all means at the 6-h time point post-infection were performed with a one-way
10 ANOVA test followed by the Tukey post-hoc test. n.s., not significant. a, $P \leq 0.05$.

11 **Figure S3.** Age-dependent accumulation of ubiquitinated proteins in *atg* mutants is alleviated by
12 mutations in *npr1* (A) Increased exposure of the HRP-developed immunoblot of ubiquitin from 3 wk-old
13 tissue from WT, *npr1*, *atg2 npr1*, and *atg2* plants, shown in Figure 3A. Amido black staining of the large
14 subunit of RuBisCO serves as loading control. Anti-ubiquitin antibody was from Dako. (B) Confirmation
15 of the results presented in Fig. 3 by probing the blots using an alternative anti-ubiquitin antibody (from
16 Agrisera). Two different exposures of the same HRP-developed immunoblot of ubiquitin from 6-wk-old
17 WT, *npr1*, *atg2 npr1*, and *atg2* plants. (C) Immunoblot detection of ubiquitin (antibody from Dako) in 4-
18 and 8-wk-old WT, *npr1*, *atg2 npr1*, and *atg2* plants respectively. Amido black staining of the large
19 subunit of RuBisCO serves as loading control.

20 **Figure S4.** Combinatorial treatments with tunicamycin and BTH mimic senescence in young autophagy-
21 deficient mutants (A) Early senescence in *atg5* mutants induced by combinatory TM/BTH stress is
22 alleviated by loss of NPR1 function. Five-wk-old Col-0 WT, *npr1*, *atg5*, and *atg5 npr1* plants grown on
23 MS plates supplemented with 5 ng/mL TM and 50 μ M BTH. (B) Single seedlings of Col-0 WT, *npr1*,

24 *atg2 npr1*, and *atg2* grown 12 d on MS plates, followed by the transfer of single seedlings to MS plates
25 supplemented with 5 ng mL⁻¹ TM and 50 μM BTH, and subsequently grown for an additional 16 d before
26 pictures were taken.