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Supplemental Material to:

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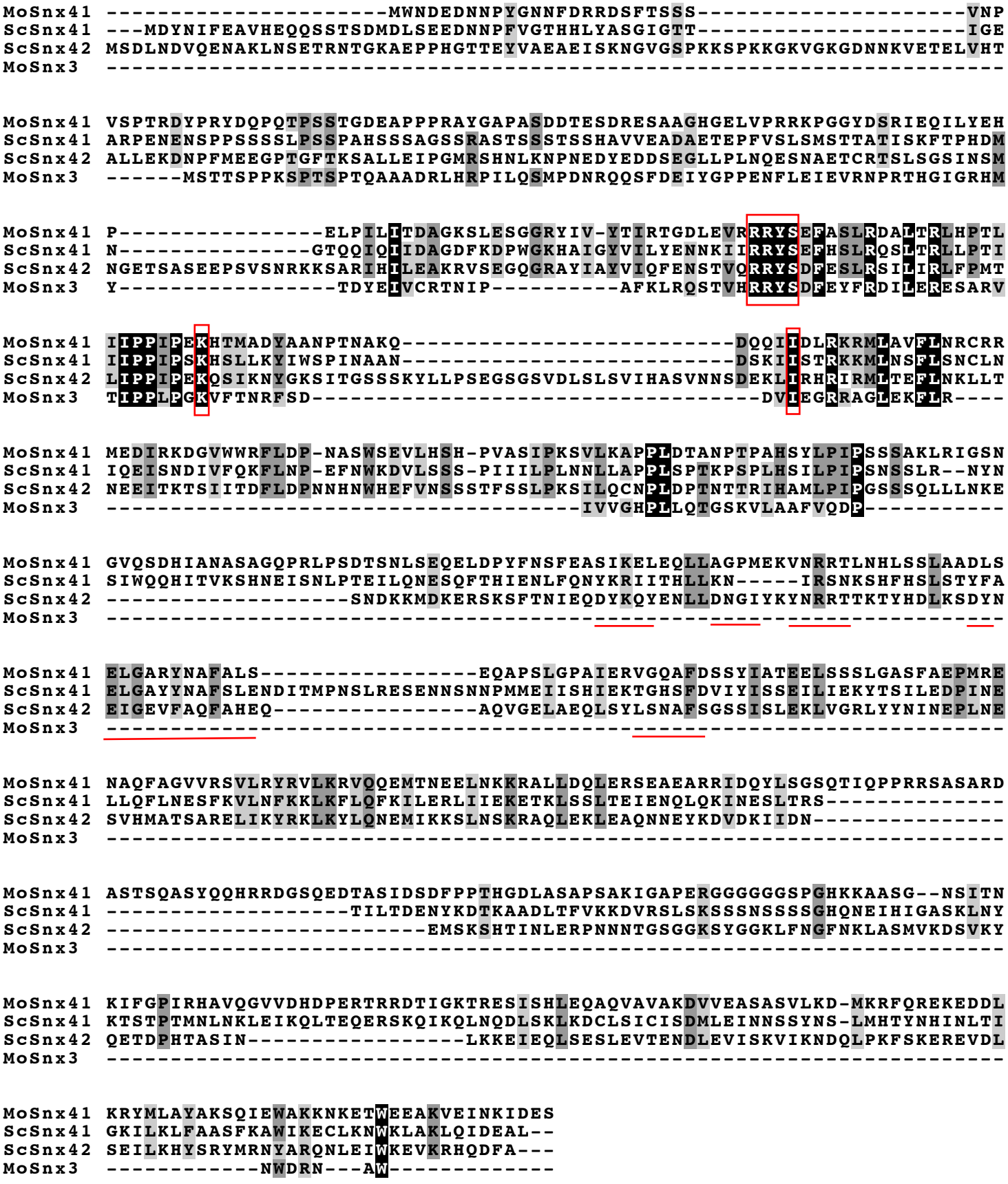
Sorting nexin Snx41 is essential for conidiation and mediates glutathione-based antioxidant defense during invasive growth in *Magnaporthe oryzae*

Autophagy 2012; 8(7)
http://dx.doi.org/10.4161/auto.20217

www.landesbioscience.com/journals/autophagy/article/20217

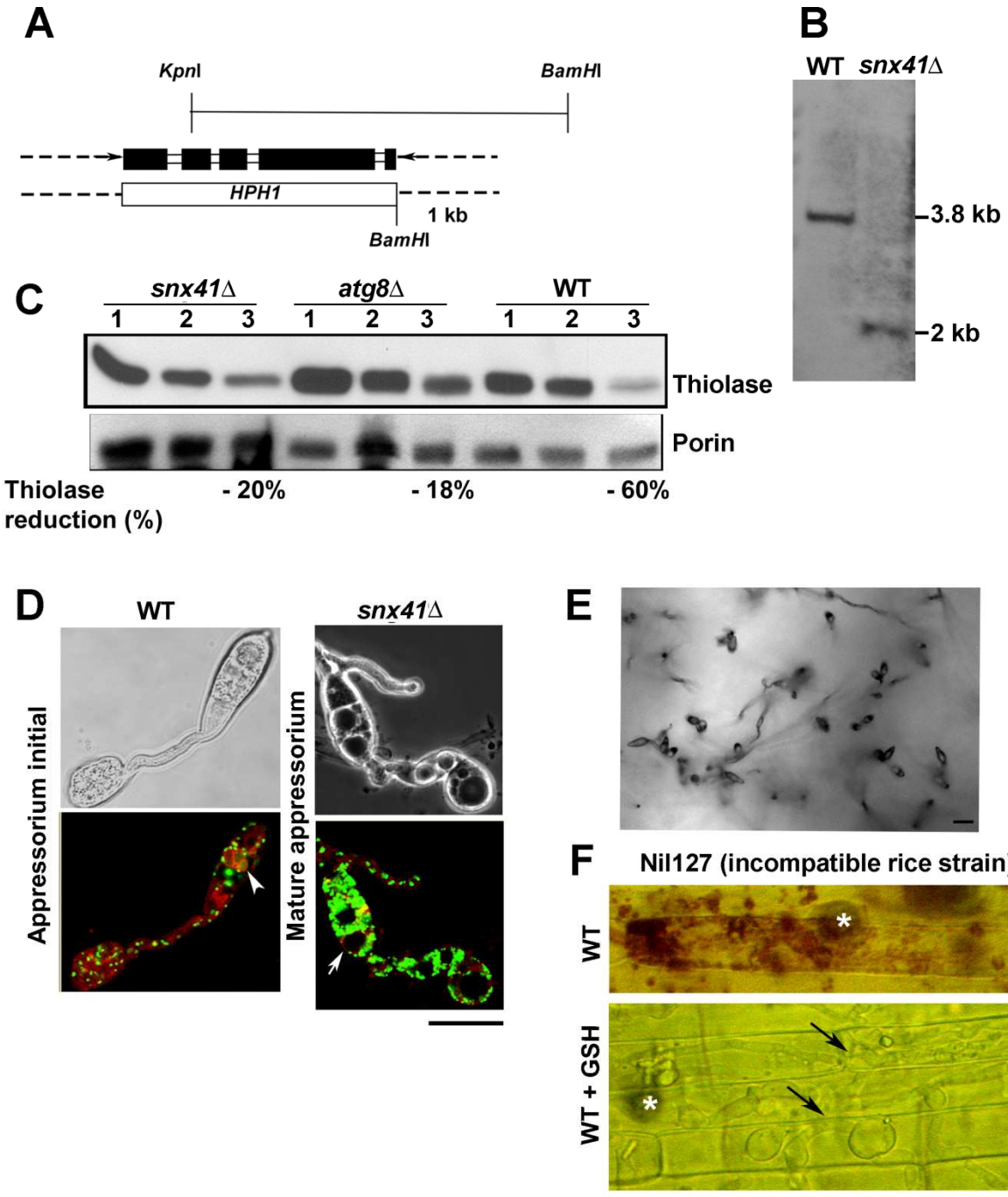
Deng et al., Figure S1



Deng et al, Figure S1:

Sequence comparison between MoSnx41 and ScSnx41, ScSnx42 and MoSnx3. ClustalW (Thompson et al., 1997) assisted multiple sequence alignment of relevant sorting nexins from *Magnaporthe oryzae* (MoSnx41, NCBI EHA50783.1; and MoSnx3, XP_001522621.1) and *Saccharomyces cerevisiae* Snx41 (NP_010713.1) and Snx42 (NP_010170.1). Boxshade was used for graphical rendering and presentation. Residues that are similar in at least three out of the four sequences are boxed in black. Solid rectangles highlight the residues for phosphoinositide binding from the PX domain, while residues in the dashed rectangles are for dimerization of BAR domain.

Deng et al., Figure S2

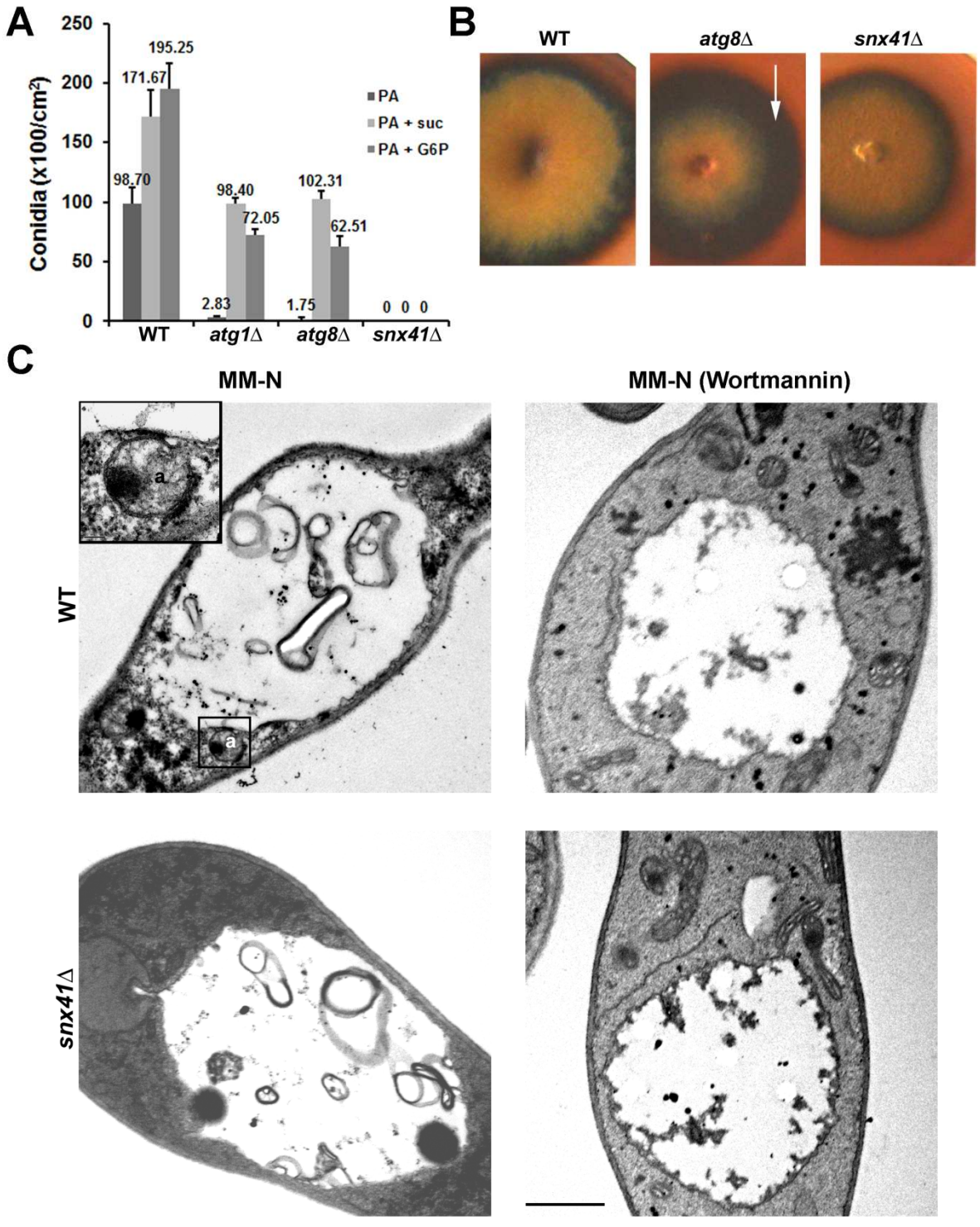


Deng et al, Figure S2:

Generation of *snx41*Δ mutant and investigation of pexophagy.

(A) Schematic representation of the Magnaporthe *SNX41* locus drawn to scale, wherein, solid bars represent coding regions and short open boxes represent introns. Opposing arrows denote the genomic region deleted in the *snx41*Δ strain using flanking homology (dashed lines) based gene replacement with the hygromycin-resistance cassette (*HPH1*). (B) Southern blot analysis to confirm *SNX41* deletion. Genomic DNA from B157 (WT) or *snx41*Δ was digested with *Kpn*I and *BamH*I and probed with the denoted 1 kb flank in (A). The wild-type 3.8 kb *SNX41* locus was lost while a 2 kb band detected in the *snx41*Δ strain, which is diagnostic of correct gene replacement event. (C) The *snx41*Δ mutant is defective in pexophagy. Total protein lysates from the WT, *atg8*Δ or *snx41*Δ straingrown in peroxisome induction or pexophagy induction conditions were analyzed by immunoblotting with antithiolase. The immunoblot was reprobed with anti-Porin antisera. 1. Basal medium (BM) + olive oil (OL) culture; 2. CM with PMSF; 3. CM without PMSF. (D) Snx41 is essential for pexophagy in Magnaporthe. Conidia of the wild type or the *snx41*Δ (expressing GFP-SRL as a peroxisomal marker) were analyzed by confocal microscopy at early (4-7 hpi) or late (20-22 hpi) stage of appressorium development. Co-staining with Lysotracker Red DND99 to label the vacuolar compartments was performed 5 min before microscopic observation. Arrowheads denote vacuolar GFP signal, an indication of pexophagy. Arrows denote vacuolar compartments without GFP, indicative of a block in pexophagy. Bar = 10 µm. (E) *In planta* conidiation of the *snx41*Δ. Microscopic examination was carried out at 5 dpi. Bar = 10 µm. (F) Microscopic observation of WT invasive hyphae developing in rice leaf sheath of an incompatible rice line NIL127, at 48 hpi. "*" denotes an appressorium. Arrows denote the invasive hyphae capable of crossing host cell wall. Bar = 10 µm.

Deng et al., Figure S3

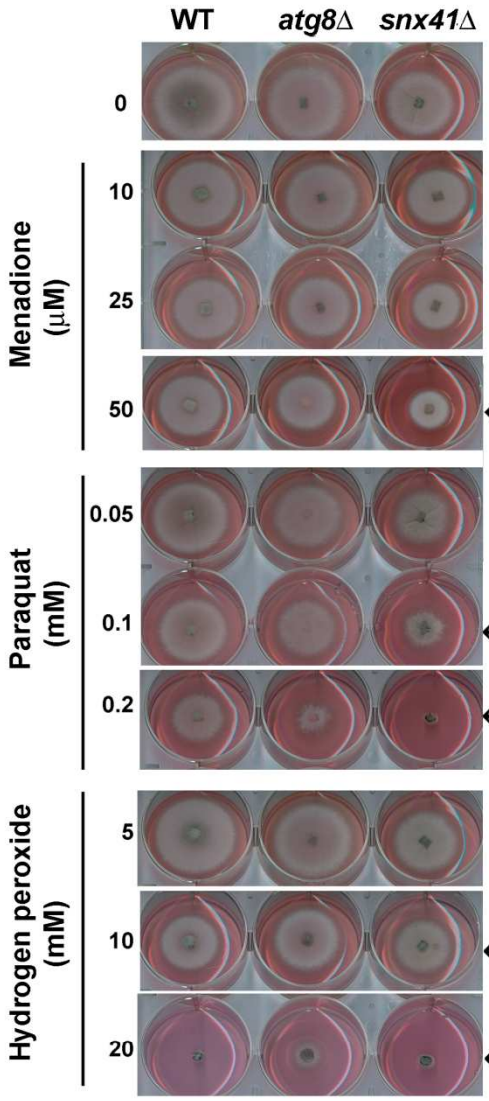


Deng et al, Figure S3:

Glycogen catabolism and autophagy is not defective in *snx41*Δ mutant.

(A) Exougenous addition of sucrose or G6P could restore conidiation defects in the *atg8*Δ or *atg1*Δ mutant, but not in the *snx41*Δ mutant. Results (mean ± SE) represent three independent experiments involving a total of 12 colonies per strain. (B) Iodine staining for analysis of glycogen accumulation. The indicated strains grown in dark for 2 days and subject to constant illumination for 2 days were exposed to iodine vapor for 20 min and photographed immediately. Arrow indicates that the outer margin of the *atg8*Δ colony was heavily stained, corresponding to high level of total glycogen accumulation. (C) TEM analysis of vegetative mycelia. Wild type and *snx41*Δ mycelia were grown in Complete medium for 2 days and subject to nitrogen starvation for 6 hours, and processed for thin-section transmission electron microscopy. Wortmannin pre-treatment was carried out for 1 hour, before nitrogen starvation. Inset depicts an autophagosome docking to the vacuolar membrane. a, autophagosome. Bar = 1 mm. Bar for inset: 100 nm.

Deng et al., Figure S4



Reduction in colony growth (%)

	WT	*atg8*Δ	*snx41*Δ
Menadione 50 μM	27.78%	27.78%	46.67%
Paraquat 0.1 mM	11.11%	16.67%	26.67%
Paraquat 0.2 mM	16.67%	38.89%	no growth
Hydrogen peroxide 10 mM	23.3%	16.67%	13.33%
Hydrogen peroxide 20 mM	no growth	80.89%	no growth

Deng et al, Figure S4:

***snx41*Δ is sensitive to GSH-repressible oxidative species.**

Mycelial plugs from the wild type (WT), *atg8*Δ and *snx41*Δ were subcultured on CM with indicated amounts of menadione, paraquat or hydrogen peroxide. Phloxine B staining was used as a cell death indicator. Arrows denote the concentration of the indicated oxidant, at which the sensitivity of the *snx41*Δ mutant was compared to the wild type or the *atg8*Δ mutant by quantification of the percentage reduction in colony radius: (R0 - Rconc) / R0, where R0 represents the radius of the colony grown on CM containing no oxidant; Rconc represents the radius of the colony grown on CM with one particular concentration of an oxidant.

Supplementary Movie 1: Live imaging of Snx41-GFP and RFP-Atg8 in Magnaporthe.