

## SUPPLEMENTAL FIGURES AND TABLE

### A Role of the *FUZZY ONIONS LIKE* Gene in Regulating Cell Death and Defense in *Arabidopsis*

Arianne Tremblay<sup>1\*+</sup>, Savanna Seabolt<sup>1\*</sup>, Hongyun Zeng<sup>2</sup>, Chong Zhang<sup>1</sup>, Stefan Böckler<sup>3</sup>, Dominique N. Tate<sup>1&</sup>, Vy Thuy Duong<sup>1</sup>, Nan Yao<sup>2</sup>, and Hua Lu<sup>1#</sup>

<sup>1</sup> Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, USA.

<sup>2</sup> State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, P.R. China

<sup>3</sup> Institut für Zellbiologie, Universität Bayreuth, Bayreuth 95440, Germany

\* These authors contributed equally to this work.

<sup>+</sup> Current address: AgBio Division, Intrexon Corporation, 1920 5th street, Davis, CA 95616

<sup>&</sup> Current address: Department of Plant Pathology, 201 Kottman Hall, 2021 Coffey Road, Columbus, OH 43210

<sup>#</sup> **Correspondence author:** Hua Lu, Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250. Tel: 410-455-5972 (O), 410-455-2263 (L), Fax: 410-455-3875. Email: [hualu@umbc.edu](mailto:hualu@umbc.edu).

**Running title:** FZL regulates cell death and defense

**Key words:** programmed cell death, disease resistance, reactive oxygen species, autophagy, mitochondrial fusion, salicylic acid

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1.** Complementation of *fzl-Ler*-conferred cell death by *FZL-YFP*. (A) Pictures of Ler and *fzl-Ler* leaves at different developmental stages. (B) Pictures of 21-day old plants. (C) Pictures of leaves from 21-day old plants. (D) Pictures of cell death in the leaves. Leaves were stained with trypan blue to visualize cell death and photographed.

**Figure S2.** *fzl-Ler*-conferred cell death phenotype is largely *EDS1*-dependent. The *fzl-Ler* mutant was crossed to *eds1-2* (a Ler mutant) to obtain the double mutant. (A) Pictures of 21-day old plants. (B) Cell death stained with the trypan blue dye. Arrows indicate dead cells in *fzl-Ler*. (C) SA quantification by HPLC analysis. These experiments were repeated two times with similar results.

**Figure S3.** SA treatment enhanced cell death in *fzl* mutants in Col-0 background. Protoplasts were prepared from 21-day old plants and treated with or without 100  $\mu$ M BTH. The number of living cells was determined by fluorescein diacetate staining and cell survival rate was calculated as the follow: number of living cells/total number of protoplast cells \*100. Different letters indicate significant difference among the samples at the same time point ( $P < 0.01$ ; Mann-Whitney test).

**Figure S4.** Pictures of the chloroplast ultrastructure from Ler (A) and *fzl-Ler* cells (B). Ch: chloroplast; CW: cell wall. Bar=1 $\mu$ m.

**Figure S5.** FZL-YFP localizes to the outer membrane of the chloroplast. Protoplasts were isolated from plants expressing *FZL-YFP* and stained with MitoTracker Red CMXRos for a co-localization study. The cells were imaged by confocal microscopy. Bars = 5  $\mu$ m. (A) YFP fluorescence. (B) YFP 3D reconstruction. The Imaris software (version 7.0.0) was used for image analysis. (C) YFP and MitoTracker Red fluorescence.

**Figure S6.** FZL-YFP does not co-localize with the mitochondrial marker mCherry. Protoplasts were isolated from plants expressing FZL-YFP and were further transfected with the mitochondrial marker gene tagged with the mCherry reporter for a co-localization study. (A) YFP fluorescence. (B) mCherry fluorescence. (C) Chloroplast

autofluorescence. (D) Cell morphology viewed with transmitted light. (E) Overlay of YFP and mCherry fluorescence.

**Table S1.** Primers used in this paper

<b>Purpose</b>	<b>Primer Name</b>	<b>Primer Sequence (5' &gt; 3')</b>
<b>Complementation</b>		
Arabidopsis <i>fzl-Ler</i>	FZL1forpGR117_F	ATGAGAACTCTAATCTCTCACCG
	FZL1forpGR117_R	GAAGTCTCATCTCGTCTCGTG
Yeast <i>fzo1</i>	FZL1forpYX_F	ATGAGAACTCTAATCTCTCACCG
	FZL1forpYX_R	CTAAAGTCTCATCTCGTCTCGT
	FZL1CDS-CTP_F	ATGCAGACTTCTTCTTCTAGGCCG
<b>Gene Expression</b>		
ATG5	ATG5_qPCR_for	GTGGCTGGACAAGTTAAGACAGC
	ATG5_qPCR_rev	ACTCAACAGGGCGATTAAGGTACG
ATG6	ATG6_qPCR_for	TTGCTAGAACTCAGACACAGGTTG
	ATG6_qPCR_rev	CGCATGCTTCGTATGCTTCCAC
ATG7	ATG7_qPCR_for	ACGTGGTTGCACCTCAGGATTC
	ATG7_qPCR_rev	ACTAAGAGTTCAACGGCGAGAGC
ATG8c	ATG8C_qPCR_for	TCGCCATGGCTAATAGCTCTTTC
	ATG8C_qPCR_rev	TCAGCTCTCTCTACGATCACTGG
ATG8i	ATG8I_qPCR_for	GGGCGGTCGAAGAAGATGAAATCG
	ATG8I_qPCR_rev	TTTCTCGATGGCAGGCAGATCC
ATG8f	ATG8F_qPCR_for	AACGATAGCGTTGTGCTCTGG
	ATG8F_qPCR_rev	GCCTCTGCCCTTCTCTTCTCTAAG
ATG9	ATG9_qPCR_for	TGATGTTTGTCTGTGCCAAAGCG
	ATG9_qPCR_rev	TGCAAACATGGCCTACACCTTC
ATG10	ATG10_qPCR_for	ACCAATCGTTCCCTCCATGGTC
	ATG10_qPCR_rev	AGCGACAAGTAACCTTCCTCCTTC
FZL	FZL-qPCR-forward	TGTTGACACACCTGGGACCAATG
	FZL-qPCR-reverse	TGTACCGGAGAAACGCAACCTC
PR1	PR1_qPCR_for	ACACGTGCAATGGAGTTTGTGG
	PR1_qPCR_rev	TTGGCACATCCGAGTCTCACTG













