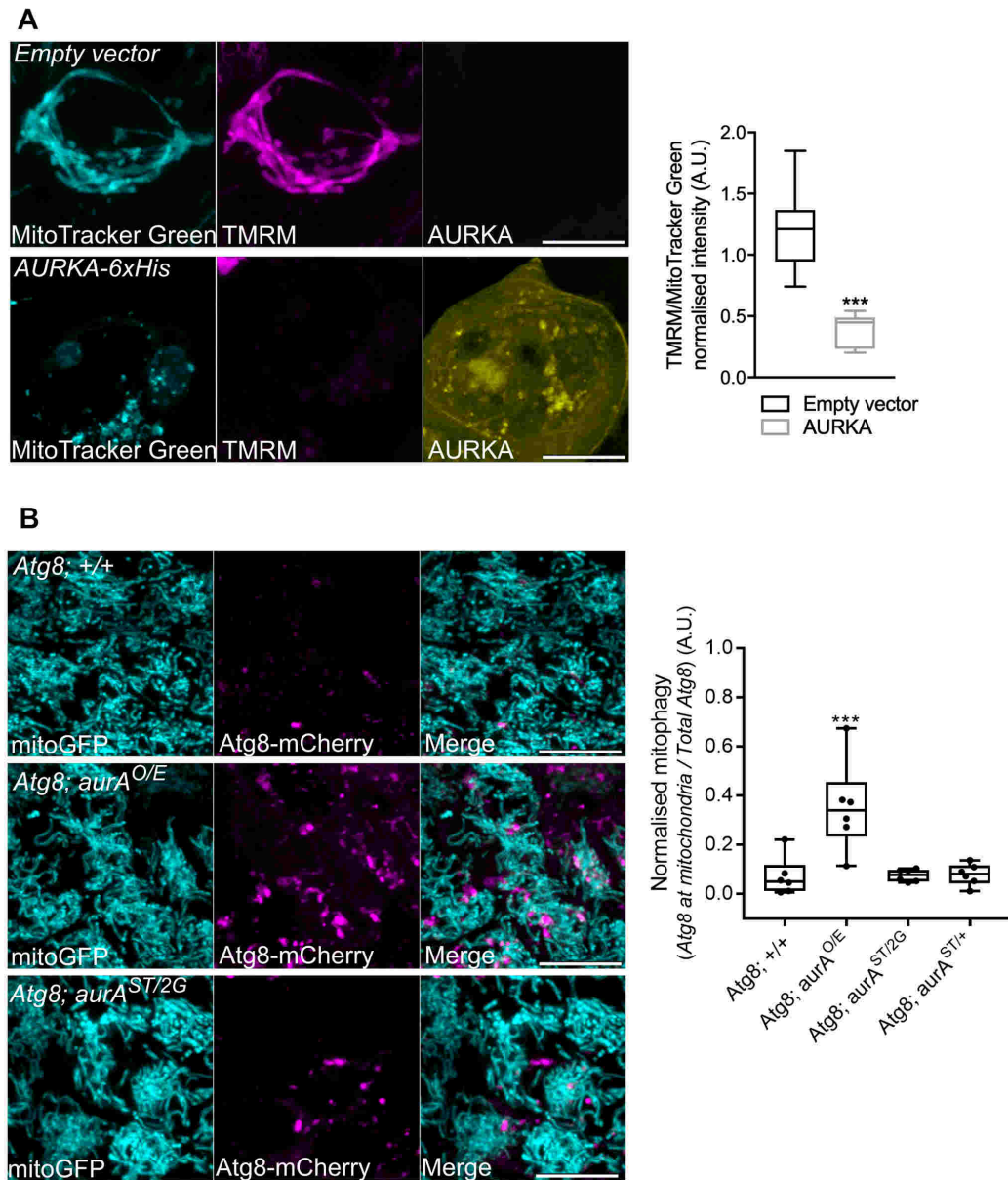


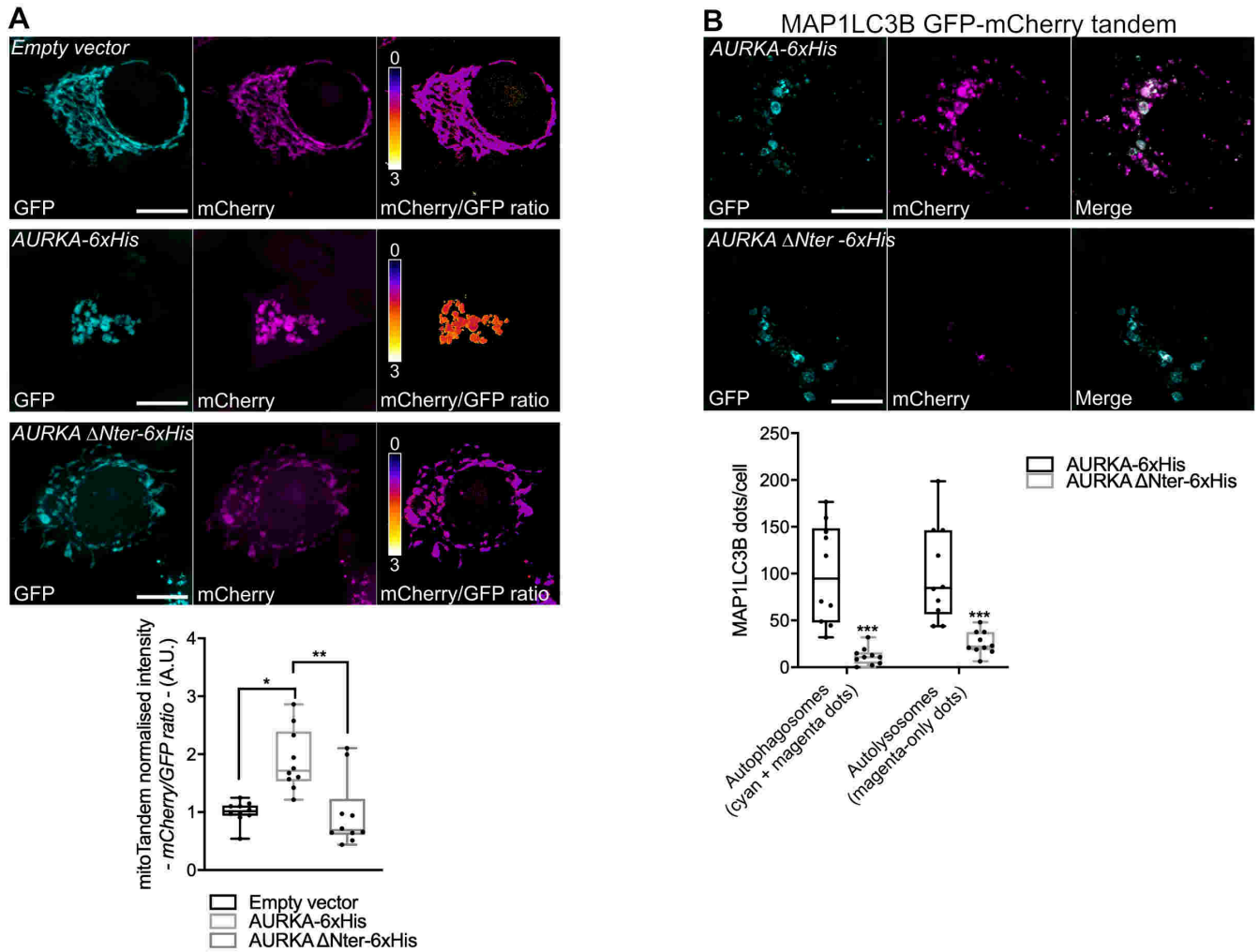
# **Mitochondrial Aurora kinase A induces mitophagy by interacting with MAP1LC3 and Prohibitin 2.**

**Giulia Bertolin et al.**

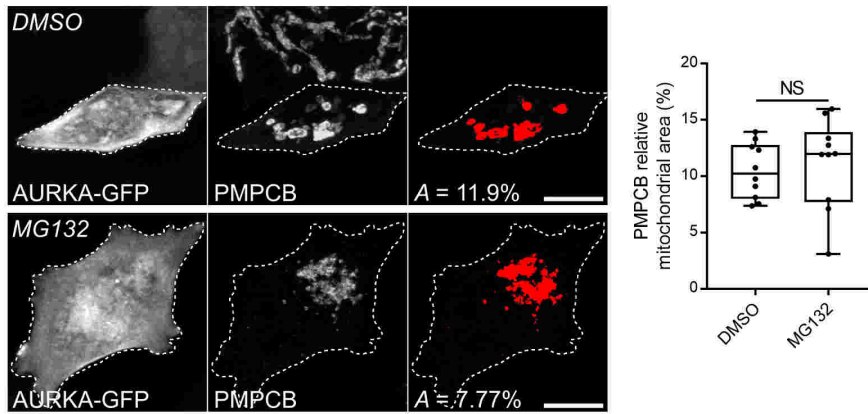
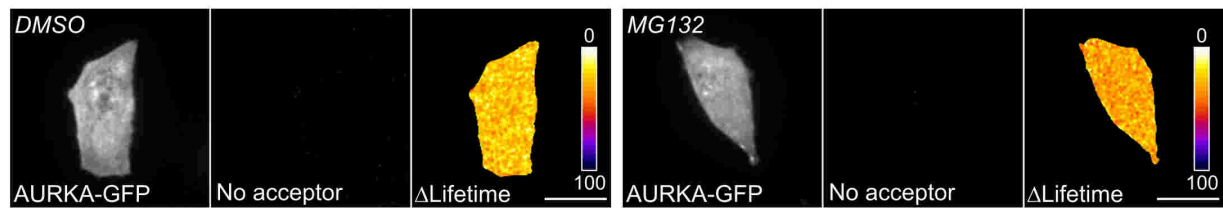
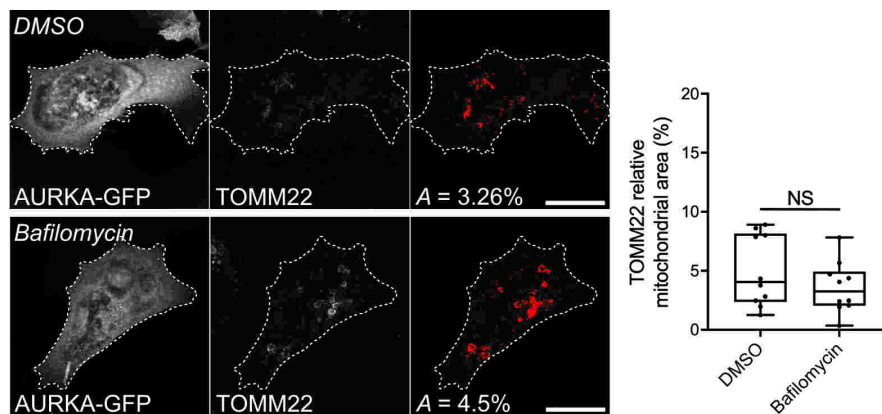
## **Supplementary information**



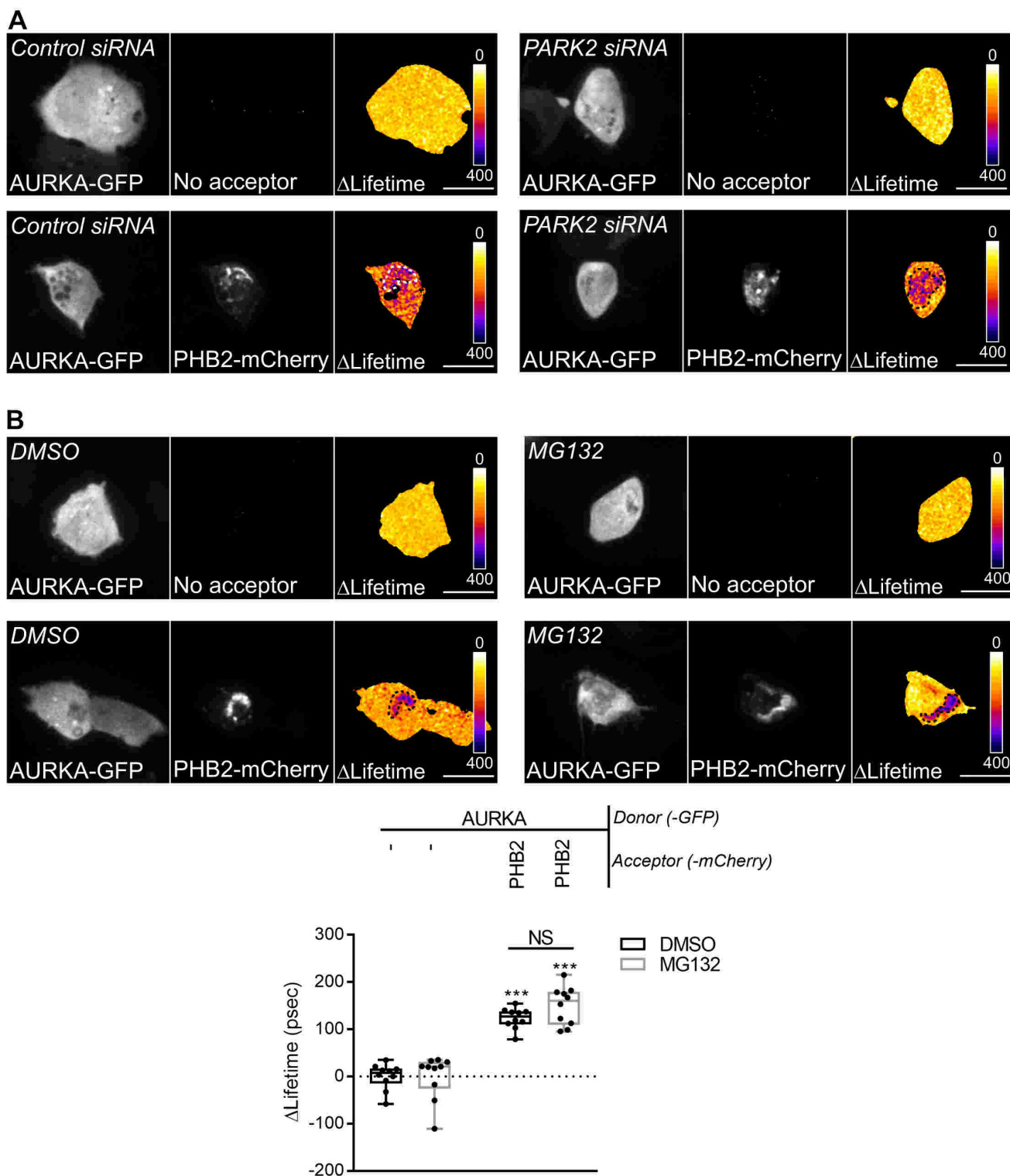
**Supplementary Fig. 1 The over-expression of AURKA induces  $\Delta\Psi$  loss and increases the mitochondrial localisation of Atg8 in *Drosophila melanogaster*.** (A) (Left panels) Representative images of MitoTracker Green, TMRM and AURKA-iRFP670 and (right panels) quantification of the TMRM/MitoTracker Green ratio in cells transfected with an empty vector or with AURKA-iRFP670.  $n=10$  images per condition on one representative experiment (of three). mitoGFP and MitoTracker Green were pseudocoloured cyan, AURKA-iRFP670 was pseudocoloured yellow, and Atg8-mCherry and TMRM were pseudocoloured magenta. (B) (Left panels) Representative images of a mitochondrially-targeted GFP (mitoGFP) and Atg8-mCherry, and (right panels) corresponding quantification of the number of Atg8-mCherry spots co-localising with mitoGFP and normalised to the total number of Atg8-mCherry spots in a wild-type background (Atg8; +/+), gain-of-function (Atg8; *aurA*<sup>O/E</sup>), AURKA null (*aurA*<sup>ST/2G</sup>) and heterozygous (*aurA*<sup>ST/+</sup>) *Drosophila* mutants.  $n=60$  images per condition from six independent pupae obtained from three independent crossings. Scale bar: 10 $\mu$ m. Data extend from the min to max. \*\* $P<0.01$ , \*\*\* $P<0.001$  compared to the 'Atg8; +/+' (A) or the 'Empty vector' (B) conditions. A.U.: arbitrary units.



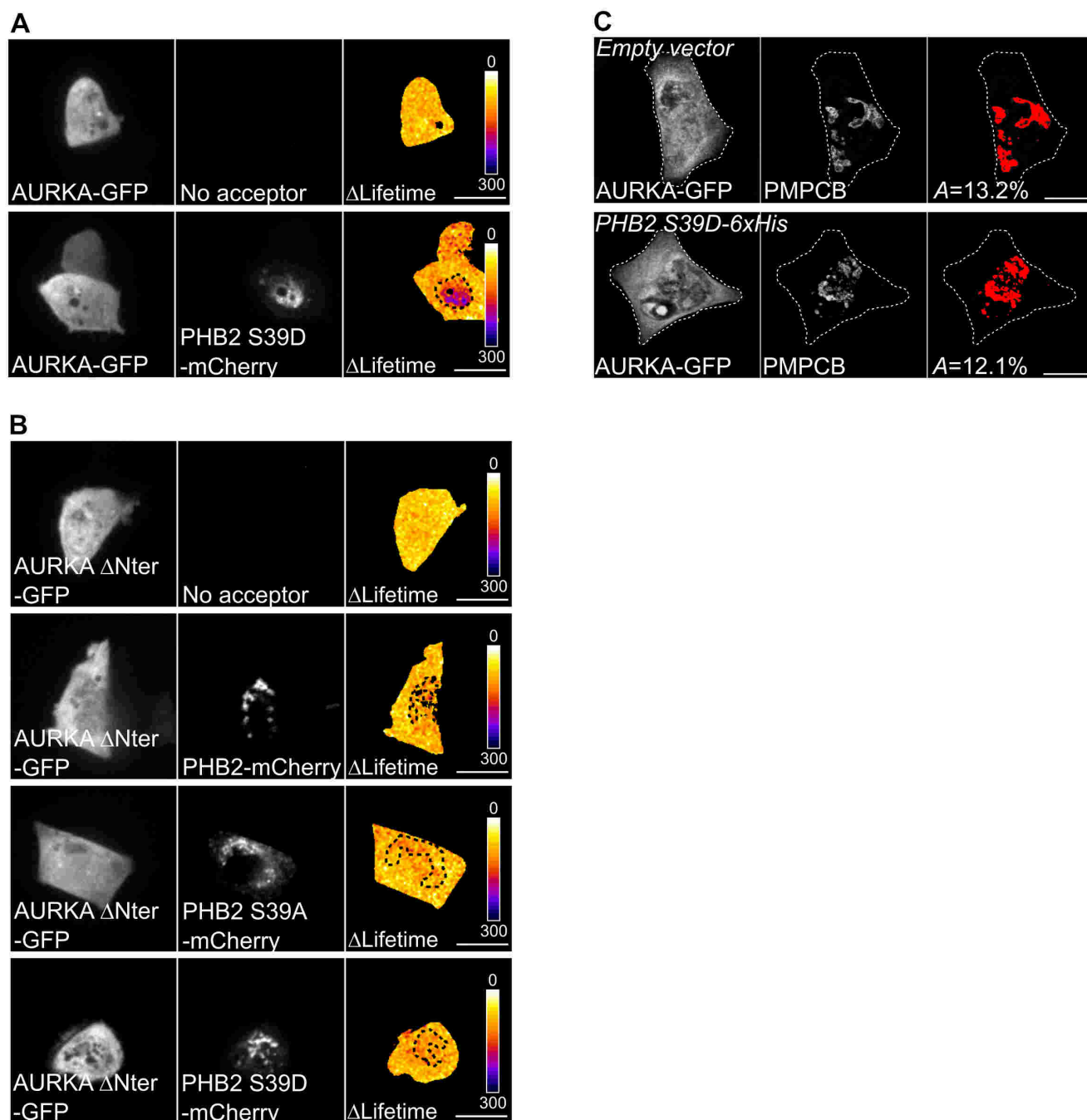
**Supplementary Fig. 2 The over-expression of AURKA induces an autophagy-related drop of mitochondrial pH.** (A) (Upper panels) Representative images of a mitochondrially-targeted GFP/mCherry fluorescent tandem co-transfected with an empty vector, AURKA-6xHis or AURKA  $\Delta$ Nter-6xHis plasmids. Pseudocolour scale: mCherry/GFP ratio. (Lower panel) Quantification of the mCherry/GFP fluorescence intensity ratio.  $n=10$  images per condition on one representative experiment (of three). (B) (Upper panels) Representative images of a GFP/mCherry-MAP1LC3 fluorescent tandem co-transfected with AURKA-6xHis or AURKA  $\Delta$ Nter-6xHis plasmids. (Lower panel) Quantification of the number of MAP1LC3 fluorescent in both the GFP and the mCherry channels or in the mCherry channel only. The dots that were fluorescent in both channels were classified as autophagosomes, the mCherry-only dots were considered as autolysosomes.  $n=10$  images per condition on one representative experiment (of three). GFP was pseudocoloured cyan and mCherry was pseudocoloured magenta. Scale bar:  $10\mu\text{m}$ . Data extend from the min to max. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  compared to the 'Empty vector' (A), or each corresponding 'AURKA' (B) conditions. A.U.: arbitrary units.

**A****B****C**

**Supplementary Fig. 3. Proteasomal inhibition does not prevent the loss of matrix markers and the interaction of AURKA with MAP1LC3.** (A) Loss of PMPCB staining (threshold mask and corresponding quantification) in MCF7 cells transfected with AURKA-GFP and treated with MG132. (B) Representative FRET by FLIM images of control MCF7 cells expressing AURKA-GFP and treated with DMSO or with MG132. Pseudocolour scale: pixel-by-pixel  $\Delta$ Lifetime. (C) Loss of TOMM22 staining (threshold mask and corresponding quantification) in MCF7 cells transfected with AURKA-GFP and treated with DMSO or Bafilomycin. A=mitochondrial area normalised against total cell area (%).  $n=10$  cells per condition from one representative experiment (of three). Data extend from the min to the max. Scale bar: 10 $\mu$ m. NS: not significant.



**Supplementary Fig. 4. The AURKA-PHB2 interaction is maintained upon *PARK2* silencing and proteasomal inhibition.** (A) Representative FRET by FLIM images of MCF7 cells expressing AURKA-GFP alone or together with PHB2-mCherry, and co-transfected with a control- or a *PARK2*-specific siRNA. (B) FRET by FLIM analyses of MCF7 cells expressing AURKA-GFP alone or together with PHB2-mCherry, and treated with DMSO or with MG132.  $n=10$  cells per condition from one representative experiment (of three). Data extend from the min to the max. Pseudocolour scale: pixel-by-pixel  $\Delta$ Lifetime. Scale bar:  $10\mu\text{m}$ . \*\*\* $P<0.001$  compared to each corresponding 'No acceptor' condition.



**Supplementary Fig. 5. The AURKA-PHB2 interaction is maintained with PHB2 S39D, but not with AURKA  $\Delta$ Nter.** (A-B) Representative FRET by FLIM images of MCF7 cells expressing AURKA-GFP alone or together with PHB2 S39D-mCherry (A), or expressing AURKA  $\Delta$ Nter alone or with PHB2-mCherry, PHB2 S39A-mCherry or PHB2 S39D-mCherry (B). Pseudocolour scale: pixel-by-pixel  $\Delta$ Lifetime. (C) Loss of PMPCB staining (threshold mask) in MCF7 cells co-transfected with AURKA-GFP and an empty vector or PHB2 S39D-6xHis, as indicated. A=mitochondrial area normalised against total cell area (%). Scale bar: 10 $\mu$ m.

Plasmid	Cloning sites	Remarks	Primers for mutagenesis (5' to 3'): sense	Primers for mutagenesis (5' to 3'): anti-sense
pcDNA 3.1 AURKA 6xHis		<i>From Bertolin et al, 2018.</i>		
pcDNA 3.1 AURKA ΔNter 6xHis		<i>the first 30 amino acids of AURKA are lacking; from Bertolin et al, 2018.</i>		
pcDNA3 AURKA-GFP		<i>From Bertolin et al, 2018.</i>		
pEGFP N1 AURKA ΔNter		<i>the first 30 amino acids of AURKA are lacking; from Bertolin et al, 2018.</i>		
pLKO Puro shControl		<i>purchased from Sigma-Aldrich (see materials and methods)</i>		
pLKO Puro shAURKA		<i>purchased from Sigma-Aldrich (see materials and methods)</i>		
pEGFP N1 mitoGFP		<i>From Bertolin et al, 2018.</i>		
pCMV7 mitomCherry		<i>Kind gift of O. Corti, Brain and Spine institute, France.</i>		
pcDNA 3.1 mCherry-hLC3B		<i>Addgene plasmid: #40827</i>		
pDEST GFP-mCherry MAP1LC3B		<i>Kind gift of M. Marinello, Genethon, France</i>		
pmCherry LAMP1		<i>Addgene plasmid: #45147</i>		
pEGFP-parkin WT		<i>Addgene plasmid #45875</i>		
pmCherry N1 PHB	XhoI/HindIII			
pmCherry N1 PHB2	XhoI/HindIII			
pmCherry N1 PHB2 S39A		<i>obtained from pmCherry N1 PHB2</i>	<i>cacggtgaacacagcttcgcgcacaccgta</i>	<i>tacggtgtgcggaagctgtgtcaccgtg</i>
pmCherry N1 PHB2 S39D		<i>obtained from pmCherry N1 PHB2</i>	<i>tccacggtgaacacatcttcgcgcacaccgtagg</i>	<i>cctacggtgtgcggaagatgtgtcaccgtgga</i>
pcDNA 3.1 (-) PHB2	NheI/EcoRI	<i>PHB2 template obtained from pmCherry N1 PHB2</i>		
pcDNA 3.1 (-) PHB2 S39A	NheI/EcoRI	<i>PHB2 S39A template obtained from pmCherry N1 PHB2 S39A</i>		
pcDNA 3.1 (-) PHB2 S39D	NheI/EcoRI	<i>PHB2 S39D template obtained from pmCherry N1 PHB2 S39D</i>		
pEGFP N1 PHB2	AgeI/NotI	<i>PHB2 template obtained from pmCherry N1 PHB2</i>		
pEGFP N1 PHB2 S39A	AgeI/NotI	<i>PHB2 S39A template obtained from pmCherry N1 PHB2 S39A</i>		
pEGFP N1 PHB2 S39D	AgeI/NotI	<i>PHB2 S39D template obtained from pmCherry N1 PHB2 S39D</i>		
pmCherry N1 mitoGFP	XhoI/HindIII	<i>mitoTandem (mitoGFP fused to mCherry)</i>		

**Supplementary Table 1. List of plasmid vectors used in this study.** Table illustrating the source of each plasmid, cloning sites (when applicable) and primers used for site-directed mutagenesis.

Antibody	Brand	Catalogue n°	Dilution
Actin	Sigma-Aldrich	A5060	1/1000
AURKA clone 5C3	home made	N.A. as in Bertolin et al, 2018	1/20
GFP	Roche/ Sigma Aldrich	11814460001	1/1000
LC3	Novus Biologicals	NB100-2331	1/1000
MitoBiogenesis Western Blot Cocktail	abcam	ab123545	1/250
MT-CO2	home made	N.A. (From Agier et al, 2012)	1/2000
PARKIN clone PARK8	Millipore	MAB5512	1/1000
PGC1 $\alpha$	abcam	ab188102	1/1000
PHB	Thermo Scientific	PA5-19556	1/500
PHB2/REA	Thermo Scientific	PA5-79817	1/1000
Tim50	abcam	ab109436	1/2000
Tom22	abcam	ab10436	1/500
Tubulin alpha clone YL1/2	Millipore	MAB1864	1/5000

**Supplementary Table 2. List of primary antibodies used for western blotting procedures.** The table contains the name of the antibody used, the supplier, the catalogue number and the final dilution used



Genotypes	Name used in publication
Sca-GAL4, UAS-mito-GFP / UAS-mCherry Atg8a ; +	(Atg8; +)
Sca-GAL4, UAS-mito-GFP / UAS-mCherry Atg8a ; UAS- <i>aurA</i> .Exel / +	(Atg8; <i>aurA</i> <sup>O/E</sup> )
Sca-GAL4, UAS-mito-GFP / UAS-mCherry Atg8a ; <i>aurA</i> <sup>ST/+</sup>	(Atg8; <i>aurA</i> <sup>ST/+</sup> )
Sca-GAL4, UAS-mito-GFP / UAS-mCherry Atg8a ; <i>aurA</i> <sup>ST/2G</sup>	(Atg8; <i>aurA</i> <sup>ST/2G</sup> )

**Supplementary Table 3. List of *Drosophila* crossings used in the study.** List of the genotype of the *Drosophila* crossings used in this study, along with the corresponding name used in the publication.