Deletion of Autophagy gene *ATG1* and F-box motif encoding gene *YDR131C* together leads to Synthetic Growth Defects and Flocculation behaviour in *Saccharomyces cerevisiae*

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Running title: Genetic Interactions between *YDR131C* and *ATG1* shows ubiquitination and autophagy pathway cross talk governing growth fitness

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Abstract:

F-box motif encoding *YDR131C* is functionally uncharacterized gene which forms the complex with the SCF-E3 ligase. The F-box motif containing proteins are involved in substrate recruitment for the ubiquitination and subsequent degradation through 26S proteasome. Autophagy gene, *ATG1* (*ULK1* in human) is a well conserved serine-threonine kinase, required for vesicle formation and cytoplasm to vacuole targeting pathway. Atg1p forms the complex with Atg13p and Atg17p during autophagy. The understanding of crosstalk between ubiquitin and autophagy pathways is crucial for synthetic lethality screen and drug targeting. Here we have conducted the study for genetic interaction between uncharacterized *YDR131C* and *ATG1* gene representing both specific and non-specific protein degradation pathways. The single and double gene knockout strains of *YDR131C* and *ATG1* genes were constructed in the BY4741 genetic background and analysed for growth fitness. The strains were also evaluated for cellular growth response in presence of hydroxyurea (HU), methyl methane sulfonate (MMS), and hydrogen peroxide (H₂O₂) stress causing agents by spot assay. The *ydr131cΔatg1Δ* showed the synthetic growth defect phenotype with floc formation in rich medium which showed floc disruption in presence of EDTA. The *ydr131cΔatg1Δ* cells showed the sensitivity to stress agents HU, MMS, and H₂O₂ when compared with *ydr131cΔ*, *atg1Δ*, and WT cells.. Based on the observations, we report that *YDR131C* and *ATG1* functions in parallel pathways for growth fitness and cellular growth response to stress agents. Interestingly this study also revealed the crosstalk between ubiquitination and autophagy pathways. The defects in both the pathways could lead to synthetic growth defects which may have implication for the precision medicine initiatives.

Introduction

Ubiquitin proteasome pathway is very specific for degradation of smaller and specific poly-ubiquitinated proteins whereas non-specific and larger misfolded proteins requires autophagy pathway. Autophagy is highly conserved pathway for degradation of large protein complexes which include organelle material through lysosomal hydrolytic enzymes (BUDOVSKAYA *et al.* 2004). The autophagy pathway is well characterised which regulates various physiological processes and maintains the cellular homeostasis. The starvation and endoplasmic reticulum stress leads to induction of autophagy which involves formation of double membrane structure, known as auto-phagosome. Auto-phagosome encapsulates cytosolic components (organelles or proteins) to be fused with lysosome for degradation through hydrolases (TAKESHIGE *et al.* 1992). In *Saccharomyces cerevisiae*, approximately 32 autophagy related genes (*ATG*) have been identified and one of these genes, *ATG1*, codes for cytosolic serine-threonine protein kinase, conserved from yeast to humans (SUZUKI and OHSUMI 2007). The Atg1p is crucial for vesical formation and cytoplasm to vacuole targeting of the substrate (MATSUURA *et al.* 1997; STRAUB *et al.* 1997). The Atg1p forms the complex with Atg13p and Atg17p for kinase activity during autophagy and is crucial for initiation of autophagy (KABEYA *et al.* 2005). It is also required for the localisation of the autophagy proteins such as Atg23p (REGGIORI *et al.* 2004). Besides its role in autophagy as kinase factor, it is also function as structural protein which is involved in the PAS organization and assembly (ABELIOVICH *et al.* 2003). *S. cerevisiae* with null mutant of *ATG1* gene showed viability in nutrient rich medium (GIAEVER *et al.* 2002) and defects in autophagy (MATSUURA *et al.* 1997), mitophagy (ZHANG *et al.* 2007), pexophagy (HUTCHINS *et al.* 1999), cell death (LISA-SANTAMARIA *et al.* 2012), cytosol to vacuole transport (SUZUKI *et al.* 2010), and sporulation. The *ATG1* is well conserved and its homologs have been reported in

65 *C.elegans* (UNC-51) (MATSUURA *et al.* 1997) and human (*ULK1*) (KUROYANAGI *et al.* 1998).
66 The high throughput studies have reported many genes showing genetic interactions with *ATG1*
67 (<https://www.yeastgenome.org/locus/S000003148/interaction>) involved in various biological
68 processes. The genetic interaction of *ATG1* with mitochondrial protein encoding gene *AIM25*
69 (AGUILAR-LOPEZ *et al.* 2016) involved in maintaining the integrity of mitochondrial network and
70 histone deacetylase, *HDA1* (ROBERT *et al.* 2011) and *RPD3* showed synthetic growth defects.
71 The Rpd3 histone deacetylase regulates the transcriptional silencing and autophagy by regulating
72 the chromatin remodelling activity (ROBERT *et al.* 2011).
73 F-box proteins are component of the SCF E3 ubiquitin ligase complex which recognizes the
74 phosphorylated target proteins for ubiquitination and subsequent degradation by 26S proteasome.
75 *YDR131C* of *S.cerevisiae* is non-essential gene, encodes for F-box motif protein (GIAEVER *et al.*
76 2002). The over expression of it leads to resistance against methyl mercury chloride in the W303
77 genetic background (HWANG *et al.* 2006). The null mutant of *ydr131cΔ* showed sensitivity to
78 antimicrobial peptide, lacto-ferricin amino acids 1-11 (LIS *et al.* 2013) and methyl methane
79 sulphonate (MMS) (Unpublished data from our lab). Genetic interactions between *YDR131C*
80 with *ATG* genes have not been reported so far. Here, we report the genetic interaction between
81 *YDR131C* and *ATG1* genes which regulates growth fitness, flocculation behaviour, and cellular
82 response to genotoxic stress agents.

83 **Materials and Methods**

84 **Yeast strain, plasmids, and growth conditions**

85 *Saccharomyces cerevisiae* strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was used for
86 construction of single and double gene deletions (**Table 1**). Plasmids, pFA6a-KanMX6, and

pFA6a- His3MX6 (**Table 2**) was used for amplification of the deletion cassettes for knockout of the *YDR131C* and *ATG1* ORFs. For ORF deletion PCR primers (**Table 3**) and method mentioned in (LONGTINE *et al.* 1998) was adopted.

Growth Assay and Microscopy

Comparative growth assessment of WT, *ydr131cΔ*, *atg1Δ* and *ydr131cΔatg1Δ* was carried out both on solid and liquid media as mentioned in the (SHARMA *et al.* 2019) and (SALARI and SALARI 2017). For comparative growth assessment on solid media each strains was streaked on YPD + agar plates and incubated for 36-48 hrs at 30° C. For comparative growth kinetics of strains in the liquid media, optical density measurement at 600nm for 14 hrs was carried out. For Comparative morphological assessment, an aliquot of each strain from liquid media was observed under Leica DM3000 microscope at 100X magnification.

Growth in YPD medium and Flocculation behaviour

The WT, *ydr131cΔ*, *atg1Δ* and *ydr131cΔatg1Δ* cells grown in YPD medium overnight and observed for floc formation visually and using microscope. The dependence of the floc formation on EDTA was tested by adding the 30mM of EDTA (chelating agent) to growth medium (STEWART 2018).

Spot Assay

To compare the cellular growth response of WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* cells in presence of stress causing agents spot assay was performed as mentioned in (SHARMA *et al.* 2019). Briefly Wild type (BY4741) and deletion derivatives (WT, *ydr131cΔ*, *atg1Δ* and *ydr131cΔatg1Δ*) were grown to log phase (OD⁶⁰⁰ 0.8-1.0) and equal number of cell were serially

diluted. From each dilution a 3µl aliquot was spotted onto agar plates containing YPD, YPD + stress causing agents such as a hydroxyurea (200mM), MMS (0.035%) and hydrogen peroxide (4mM). The plates were incubated at 30°C for 2-3 days and imaged.

Fluorescence Microscopy for Cell Wall and Nuclear status

To compare the cell wall status of WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* cells, Calcofluor white staining assay described in (PRINGLE 1991; PREECHASUTH *et al.* 2015; SHARMA *et al.* 2019) was adopted. Briefly, WT and mutant strains were grown overnight at 30°C and next day transferred to fresh culture in 1:10 ratio. Cells were grown to log phase and collected by centrifugation. Further, cells were suspended in 100µl of solution containing Calcofluor white (50 µg/ml solution) fluorescent dye. For nuclear status by DAPI staining log phase grown cells were harvested by centrifugation, washed with distilled water, and suspended in 1X Phosphate Buffer Saline (PBS). Further, fixation was done by adding 70% ethanol for 10 minutes and centrifuged for 2.5 minutes at 2500 rpm. DAPI stain (1mg/ml stock) to final concentration 2.5µg/ml was added and incubated for 5 minutes at room temperature and visualized under UV light of fluorescent microscope with 100X magnification using Leica DM3000 fluorescence microscope.

Results

Loss of *YDR131C* and *ATG1* together leads to synthetic growth defect and Flocculation behaviour

The null mutant of both the gene *YDR131C* and *ATG1* have been reported to be viable. The comparative growth assessment of both *ydr131cΔ* and *atg1Δ* strains was similar to the growth of WT cells on solid and liquid media (**Figure 1A, B**). However *ydr131cΔ atg1Δ* cells showed slow

growth phenotype when compared with WT, *ydr131cΔ*, and *atg1Δ* mutants indicating synthetic growth defect phenotype. The microscopy analysis of the WT, *ydr131cΔ*, *atg1Δ* and *ydr131cΔ atg1Δ* cells when grown in rich media (YPD), the *ydr131cΔ atg1Δ* cells showed the floc formation during growth kinetics (**Figure 1C, 2A,B**). Flocculation is cell surface characteristics and a process in which the cells have the ability to form clumps. The flocculation is regulated by genetic, physiological, and environmental factors. Further when cells *ydr131cΔ atg1Δ* were grown in YPD media supplemented with 30mM EDTA, the floc formation behaviour was reduced (**Figure 2C**). This indicated, the floc formation of the *ydr131cΔ atg1Δ* was EDTA dependent.

Loss of *YDR131C* and *ATG1* together does not affect chitin distribution

Calcofluor white is a flouorochrome stain, which is specific for the cell wall component chitin and has been used for detection of cell wall defect (DE GROOT et al. 2001). The Calcofluor white staining of WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔ atg1Δ* showed clear septa formation however *ydr131cΔ atg1Δ* showed aggregated cells and altered bud morphology (**Figure 3**).

Loss of *YDR131C* and *ATG1* together leads to sensitivity to genotoxic and oxidative stress.

Genotoxic stress agents, hydroxyurea (HU) and methyl methane sulphonate (MMS) act as DNA damaging agent whereas hydrogen peroxide leads to oxidative stress, respectively. The cellular growth response of WT, *ydr131cΔ*, *atg1Δ* and *ydr131cΔ atg1Δ* by spot assay in presence of 200mMHU, 0.035%MMS and 4mM H₂O₂ indicated, extremely slow growth of *ydr131cΔ atg1Δ* cells when compared with the WT and *ydr131cΔ*, *atg1Δ* (**Figure 4 A,B,C**).

Loss of *YDR131C* and *ATG1* together leads to altered nuclear morphology

DAPI stain is specific to the nuclear DNA which allows the visualization of the DNA in the form of single or defective nuclei depending upon the cells. The comparative analysis of nuclei in WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* of the log phase culture stained with DAPI showed compact nuclei in WT, *ydr131cΔ*, *atg1Δ* whereas *ydr131cΔatg1Δ* cells showed alerted nuclear morphology (**Figure 5**)

Discussion

The novel binary interactions between gene pairs and their functional relevance are crucial for better understanding of wiring of the pathways in the cell system. The Skp1-Cul1-F-box (SCF) E3 ligase complex recruits the target proteins for ubiquitination by its one of subunit protein which contains F-box motif, for degradation by the 26S proteasome (BAI *et al.* 1996; HERSHKO and CIECHANOVER 1998). The Ydr131c is F-box protein whose target has not been reported so far (KIPREOS and PAGANO 2000) however overexpression of it lead to resistance to methylmercury (HWANG *et al.* 2006). Here we observed the null mutants of *YDR131C* showed sensitivity to genotoxic agents such as hydroxyurea and MMS. Autophagy gene *ATG1* is crucial for autophagy response, the lack of it leads to absence of apoptosis, autophagy, and pexophagy in the cells. The combined mutation in autophagy gene *ATG1* and *YDR131C* showed synthetic growth defects and altered nuclear morphology suggesting that both the genes are required for growth fitness and cellular response to the hydroxyurea, MMS, and oxidative stress. In addition the flocculation behaviour observed with *ydr131cΔatg1Δ* cells is an interesting finding which needs to be investigated in the future. The investigated genetic interaction may have implication for synthetic lethal pair discovery for cancer, as there is high degree of functional homology of

ATG1 with mammalian *ULK1*. The mechanism of crosstalk between the specific protein degradation pathway mediated by *YDR131C* and non-specific proteins degradation pathway mediated by *ATG1* reported here would be investigated in future.

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References

ABELIOVICH, H., C. ZHANG, W. A. DUNN, JR., K. M. SHOKAT and D. J. KLIONSKY, 2003
Chemical genetic analysis of Apg1 reveals a non-kinase role in the induction of
autophagy. *Mol Biol Cell* **14**: 477-490.

197 AGUILAR-LOPEZ, J. L., R. LABOY, F. JAIMES-MIRANDA, E. GARAY, A. DELUNA *et al.*, 2016
 198 Slm35 links mitochondrial stress response and longevity through TOR signaling
 199 pathway. *Aging (Albany NY)* **8**: 3255-3271.

200 BAI, C., P. SEN, K. HOFMANN, L. MA, M. GOEBL *et al.*, 1996 SKP1 connects cell cycle
 201 regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell*
 202 **86**: 263-274.

203 BUDOVSKAYA, Y. V., J. S. STEPHAN, F. REGGIORI, D. J. KLIONSKY and P. K. HERMAN, 2004 The
 204 Ras/cAMP-dependent protein kinase signaling pathway regulates an early step of the
 205 autophagy process in *Saccharomyces cerevisiae*. *J Biol Chem* **279**: 20663-20671.

206 GIAEVER, G., A. M. CHU, L. NI, C. CONNELLY, L. RILES *et al.*, 2002 Functional profiling of the
 207 *Saccharomyces cerevisiae* genome. *Nature* **418**: 387-391.

208 HERSHKO, A., and A. CIECHANOVER, 1998 The ubiquitin system. *Annu Rev Biochem* **67**: 425-
 209 479.

210 HUTCHINS, M. U., M. VEENHUIS and D. J. KLIONSKY, 1999 Peroxisome degradation in
 211 *Saccharomyces cerevisiae* is dependent on machinery of macroautophagy and the Cvt
 212 pathway. *J Cell Sci* **112 (Pt 22)**: 4079-4087.

213 HWANG, G. W., Y. ISHIDA and A. NAGANUMA, 2006 Identification of F-box proteins that are
 214 involved in resistance to methylmercury in *Saccharomyces cerevisiae*. *FEBS Lett* **580**:
 215 6813-6818.

216 KABEYA, Y., Y. KAMADA, M. BABA, H. TAKIKAWA, M. SASAKI *et al.*, 2005 Atg17 functions in
 217 cooperation with Atg1 and Atg13 in yeast autophagy. *Mol Biol Cell* **16**: 2544-2553.

218 KIPREOS, E. T., and M. PAGANO, 2000 The F-box protein family. *Genome Biol* **1**:
 219 REVIEWS3002.

- 220 KUROYANAGI, H., J. YAN, N. SEKI, Y. YAMANOUCHI, Y. SUZUKI *et al.*, 1998 Human ULK1, a
221 novel serine/threonine kinase related to UNC-51 kinase of *Caenorhabditis elegans*: cDNA
222 cloning, expression, and chromosomal assignment. *Genomics* **51**: 76-85.
- 223 LIS, M., S. BHATT, N. E. SCHOENLY, A. Y. LEE, C. NISLOW *et al.*, 2013 Chemical genomic
224 screening of a *Saccharomyces cerevisiae* genomewide mutant collection reveals genes
225 required for defense against four antimicrobial peptides derived from proteins found in
226 human saliva. *Antimicrob Agents Chemother* **57**: 840-847.
- 227 LISA-SANTAMARIA, P., A. JIMENEZ and J. L. REVUELTA, 2012 The protein factor-arrest 11
228 (Far11) is essential for the toxicity of human caspase-10 in yeast and participates in the
229 regulation of autophagy and the DNA damage signaling. *J Biol Chem* **287**: 29636-29647.
- 230 LONGTINE, M. S., A. MCKENZIE, 3RD, D. J. DEMARINI, N. G. SHAH, A. WACH *et al.*, 1998
231 Additional modules for versatile and economical PCR-based gene deletion and
232 modification in *Saccharomyces cerevisiae*. *Yeast* **14**: 953-961.
- 233 MATSUURA, A., M. TSUKADA, Y. WADA and Y. OHSUMI, 1997 Apg1p, a novel protein kinase
234 required for the autophagic process in *Saccharomyces cerevisiae*. *Gene* **192**: 245-250.
- 235 PREECHASUTH, K., J. C. ANDERSON, S. C. PECK, A. J. BROWN, N. A. GOW *et al.*, 2015 Cell wall
236 protection by the *Candida albicans* class I chitin synthases. *Fungal Genetics and Biology*
237 **82**: 264-276.
- 238 PRINGLE, J. R., 1991 [52] Staining of bud scars and other cell wall chitin with Calcofluor, pp.
239 732-735 in *Methods in enzymology*. Elsevier.
- 240 REGGIORI, F., K. A. TUCKER, P. E. STROMHAUG and D. J. KLIONSKY, 2004 The Atg1-Atg13
241 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal
242 structure. *Dev Cell* **6**: 79-90.

243 ROBERT, T., F. VANOLI, I. CHIOLO, G. SHUBASSI, K. A. BERNSTEIN *et al.*, 2011 HDACs link the
244 DNA damage response, processing of double-strand breaks and autophagy. *Nature* **471**:
245 74-79.

246 SALARI, R., and R. SALARI, 2017 Investigation of the Best *Saccharomyces cerevisiae* Growth
247 Condition. *Electronic physician* **9**: 3592-3597.

248 SHARMA, M., S. SINGH, V. VERMA and N. K. BAIRWA, 2019 Absence of Replication fork
249 associated factor CTF4 and F-box motif Encoding Gene SAF1 leads to reduction in Cell
250 Size and Stress Tolerance Phenotype in *S. cerevisiae*. *bioRxiv*:
251 664185.

252 STEWART, G. G., 2018 Yeast flocculation—sedimentation and flotation. *Fermentation* **4**: 28.

253 STRAUB, M., M. BREDSCHEIDER and M. THUMM, 1997 AUT3, a serine/threonine kinase gene, is
254 essential for autophagocytosis in *Saccharomyces cerevisiae*. *J Bacteriol* **179**: 3875-3883.

255 SUZUKI, K., C. KONDO, M. MORIMOTO and Y. OHSUMI, 2010 Selective transport of alpha-
256 mannosidase by autophagic pathways: identification of a novel receptor, Atg34p. *J Biol*
257 *Chem* **285**: 30019-30025.

258 SUZUKI, K., and Y. OHSUMI, 2007 Molecular machinery of autophagosome formation in yeast,
259 *Saccharomyces cerevisiae*. *FEBS Lett* **581**: 2156-2161.

260 TAKESHIGE, K., M. BABA, S. TSUBOI, T. NODA and Y. OHSUMI, 1992 Autophagy in yeast
261 demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell*
262 *Biol* **119**: 301-311.

263 ZHANG, Y., H. QI, R. TAYLOR, W. XU, L. F. LIU *et al.*, 2007 The role of autophagy in
264 mitochondria maintenance: characterization of mitochondrial functions in autophagy-
265 deficient *S. cerevisiae* strains. *Autophagy* **3**: 337-346.

266 **Tables:**

267 **Table 1: List of Yeast strains and their genotype used in this study**

S.no.	Strain	Genotype	Source
1	BY4741 (WT)	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Dr. Deepak Sharma, IMTECH, India
2	HS1	<i>ydr131cΔ::KanMX</i>	This study
3	HNK1	<i>atg1Δ::KanMX</i>	Dr. Ravi Manjithya, JNCASR, India
4	HNK2	<i>ydr131cΔ::His3MX, atg1Δ::KanMX</i>	This study

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269 **Table 2: List of plasmids used in this study**

S.no.	Plasmid Name	Deletion cassette	PCR product size	Selection Media
1	pFA6a- KanMX6	KanMX6	1559 bp	YPD + G418
2	pFA6a- His3MX6	His3M X6	1403 bp	SC His -

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274 **Table 3: List of primers used in this study**

S.no	Primer name	Primer Sequence (5'-3')
1	<i>YDR131C</i>	<p>F-</p> <p>ATAAAAATAACCCTACTGCCAATTTGAAAGGGCCCGAAAACGGATCCCCGGGTT</p> <p>AATTAA</p> <p>R-</p> <p>TATCATTATGTACGTGTTATAACCGCCATGTCTCACAGTAGAATTCGAGCTCGTTT</p> <p>AAAC</p>

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284 **Figure Legends;**

285 **Figure 1. Comparative assessment of growth and morphological characteristics of WT,**
 286 ***ydr131cΔ*, *atg1Δ* and *ydr131cΔatg1Δ* cells** **A.** Comparative growth on YPD solid medium **B.**
 287 Growth kinetics of WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* strains YPD liquid medium. The
 288 error bars represent the standard deviation of three independent experiments **C.** Phase contrast
 289 images of WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* strains taken during analysis of growth
 290 kinetics at indicated time point. Images were acquired at 100X magnification using Leica
 291 DM3000 microscope. The *ydr131cΔatg1Δ* cells showed floc formation behaviour.

292 **Figure 2. Comparative assessment of the floc formation in WT, *ydr131cΔ*, *atg1Δ*,**
 293 ***ydr131cΔatg1Δ* strains** **A.** Overnight grown WT, *ydr131cΔ*, *atg1Δ*, *ydr131cΔatg1Δ* strains were
 294 poured in individual tubes and allowed to settle for 30 mins and then imaged. The
 295 *ydr131cΔatg1Δ* cells showed the diffused settlement **B.** Overnight grown cultures was gently
 296 poured in the centre of petri dish and allowed to settle, *ydr131cΔatg1Δ* cells formed the floc. **C.**
 297 WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* cells grown in presence of 30mM EDTA overnight
 298 showed disappearance of floc formation.

299 **Figure 3. Comparative assessment of chitin distribution in WT, *ydr131cΔ*, *atg1Δ*, and**
 300 ***ydr131cΔatg1Δ* by Calcofluor white staining.** The *ydr131cΔatg1Δ* showed no altered chitin
 301 distribution in comparison to WT, *ydr131cΔ*, and *atg1Δ* cells.

302 **Figure 4. Comparative assessment of cellular growth response of WT, *ydr131cΔ*, *atg1Δ* and**
 303 ***ydr131cΔatg1Δ* cells to genotoxic and oxidative stress agents.** Equal number of Log phase
 304 grown cells of each strain serially diluted and spotted on YPD agar plate and plates containing
 305 0.035% methyl methane sulfonate, 200mM hydroxyurea and 4mM hydrogen peroxide. The

ydr131cΔatg1Δ cells showed sensitivity to the genotoxic and oxidative stress agents in comparison to WT, *ydr131cΔ*, and *atg1Δ* cells.

Figure 5. Comparative assessment of nuclear status in WT, *ydr131cΔ*, *atg1Δ*, and *ydr131cΔatg1Δ* using DAPI (4', 6-diamidino-2-phenylindole) staining and fluorescence microscopy. The *ydr131cΔatg1Δ* cells exhibited diffused nucleus as compared to WT, *ydr131cΔ* and *atg1Δ*.

324 **Figure 1.**

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329 **Figure 4**

331 **Figure 5**