The absence of F-box motif Encoding Gene SAF1 and Chromatin Associated factor CTF8 together contributes to MMS Resistant and HU Sensitive phenotype in *S. cerevisiae*

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Running title: Double mutant of SAF1 and CTF8 confer MMS resistant phenotype

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Abstract:

The Replication factor-C complex which related to cohesion, constitutes, three subunits called Ctf18, Ctf8 and Dcc1. These three subunit complex assist the loading of PCNA onto the chromosome. None of the replication factor C components are essential for cell viability. The null mutant of the CTF8 in *S.cerevisiae* shows the chromosome instability and high frequency of chromosome loss. The SAF1 gene product of *S. cerevisiae* involved in the degradation of adenine deaminase factor Aah1p by SCF-E3 ligase, which itself is the part of E3 ligase. The ubiquitin marked degradation of Aah1p occurs during nutrient stress which lead to cell enter into the quiescent state. The N-terminus of Saf1p interacts with the Skp1 of SCF-E3 ligase and at C-terminus recruits with Aah1p. Here we have investigated about the binary genetic interaction

between the SAF1 and CTF8 genes. The strains containing single and double gene deletions of SAF1 and CTF8 were constructed in the BY4741 genetic background. Further the mutant strains were evaluated for growth fitness, genome stability and response to genotoxic stress caused by hydroxyurea (HU) and methyl methane sulfonate (MMS). The *saf1Δctf8Δ* strain showed the increased growth phenotype in comparison to *saf1Δ*, *ctf8Δ*, and WT strain on YPD medium. However *saf1Δctf8Δ* strain when grown in the presence MMS showed resistance and HU sensitive phenotype when compared with *saf1Δ*, *ctf8Δ*. The frequency of Ty1 retro-transposition was also elevated in *saf1Δctf8Δ* in comparison to either *saf1Δ* or *ctf8Δ*. The number of cells showing the two or multi-nuclei phenotype was also increased in *saf1Δctf8Δ* cells when compared with the either *saf1Δ* or *ctf8Δ*. Based on these observations, we report that the absence of both the gene SAF1 and CTF8 together leads to MMS resistance, HU sensitivity, and genome instability. This report warrants the investigation of mechanisms of differential growth phenotype due to loss of SAF1 and CTF8 together in presence of genotoxic stress in future.

Introduction:

The ubiquitin proteasome system regulates the phase transition due to nutrient availability in *Saccharomyces cerevisiae* (FINLEY *et al.* 1987). The nutrient deprivation condition induces stress, which leads to cell enter into the quiescent phase (FINLEY *et al.* 1987). The Saf1, of *S.cerevisiae* which constitutes the SCF E3-ligase component, recruits the Aah1p, for proteasomal mediated degradation during nutrient deprivation conditions (ESCUSA *et al.* 2006; ESCUSA *et al.* 2007). The Aah1, adenine deaminase of *S.cerevisiae*, converts adenine to hypoxanthine, implicating the role of Saf1 in nucleotide metabolism. The genetic interaction studies reported negative interaction of SAF1 with CDC10, CDC11, CDC12, and HYP2 (COSTANZO *et al.* 2016). The null mutant of SAF1 showed the synthetic growth defects with HSP82 (ZHAO *et al.* 2005), POL2 (DUBARRY *et*

al. 2015) and RTT109 (FILLINGHAM *et al.* 2008) RRM3 (Sharma, *et al.* 2019, bioRxiv archived data).

SAF1 also showed the negative genetic interaction with RFC- Ctf18 complex member DCC1 (COSTANZO *et al.* 2016). RFC helps in loading of PCNA (a processivity factor), known as sliding clamp, onto the DNA. The PCNA clamps the DNA polymerase onto its template (PETRONCZKI *et al.* 2004). The replicative RF-C helps in switching between DNA polymerases (delta and epsilon) during lagging strand DNA synthesis (BYLUND and BURGERS 2005). There are four types of RF-Cs (SHIOMI *et al.* 2007). The RF-C which related to cohesion, constitutes, three subunits called Ctf8, Ctf18, and Dcc1 proteins (BERMUDEZ *et al.* 2003). None of these proteins are essential for viability. The absence of RF-C complex showed the cohesion defects. Ctf8 forms the part of a complex (Ctf18p, Ctf8p, Dcc1p) called RFC which help in sister chromatid cohesion and chromosome transmission (MAYER *et al.* 2004). The deletion of any member of the complex individually or in combination causes cell to be sensitive to microtubule depolymerizing drugs and showed sister chromatid cohesion defects (MAYER *et al.* 2004). The complex help in loading of proliferating cell nuclear antigen (PCNA) onto primed or gapped DNA in ATP dependent manner. The loading of PCNA is mediated by the single-stranded DNA binding protein RPA (BYLUND and BURGERS 2005). The complex fails to load PCNA onto nicked or single-stranded circular DNA. There is evolutionary functional homologue complex reported in the nucleus of human cells carrying out the similar function (MERKLE *et al.* 2003). One of the Ctf18-RFC complex components, Ctf8 was first discovered as a mutant who showed the decreased chromosome transmission fidelity in mitosis in a haploid yeast strains with normal sensitivity to ultraviolet and gamma-irradiation (SPENCER *et al.* 1990). The null mutant of CTF8 in yeast showed the severe chromosome instability and high frequency of chromosome loss. The

mutant showed lower competitive fitness, slow growth, abnormal bud morphology (WATANABE *et al.* 2009) and increased sensitivity to multiple chemicals, including MMS, hydroxyurea. Synthetic genetic arrays (SGA) study with the CTF8 deletion reported the role of other genes such as CHL1, CSM3, BIM1, KAR3, TOF1, CTF4, and VIK1 in sister chromatid cohesion (MAYER *et al.* 2004). The RFC, Ctf18 has been proposed to be candidate cancer drug target in a synthetic lethal genetic interaction study carried out in *Caenorhabditis elegans* (MCLELLAN *et al.* 2009). The complex also involved in preserving genome stability through the suppression of the triplet repeat instability which is a hallmark of the hereditary neurological disorders (GELLON *et al.* 2011; OKIMOTO *et al.* 2016). The mutants of the Ctf18-RFC complex showed the triplet repeat instability which included expansions, contractions, and fragility.

Ty1 are the retrotransposons element which constitutes the part of yeast genome. They are flanked by long terminal repeats and contains genomic feature which encodes structural components of the virus like particles and the reverse transcriptase (BOEKE *et al.* 1985; GARFINKEL *et al.* 1985). The Ty1 element remains dormant in wild type cells due to host encoded factors and transpose infrequently due to inactivity of the repressive factors (SCHOLLES *et al.* 2001). The transposition events are considered to be mutagenic hence indicator of the defect in the genome maintenance.

The duplicated nuclei need to be segregated accurately in the all eukaryotic cells for precise distribution of genetic material. In *Saccharomyces cerevisiae*, nuclear migration from mother cell to daughter cell after duplication involves moving of nucleus closer to budneck then insertion of elongating nucleus into the daughter cell during anaphase (COTTINGHAM and HOYT 1997). The migration of nucleus from mother to daughter cells depends on the action of cytoplasmic microtubules (CARMINATI and STEARNS 1997). Mutations in tubulin affects the

cytoplasmic microtubules which affects the nuclear migration and causes abnormal nuclear division within the mother cells (HUFFAKER *et al.* 1988). The abnormal nuclear division leads to accumulation of two or more nuclei within the mother cells which could lead to genome instability.

The genetic interaction between SAF1 gene and RFC-complex member CTF8 has not been investigated so far. Here, we report the binary genetic interaction between the two genes SAF1 and CTF8. The simultaneous deletion of both genes (SAF1 and CTF8) leads to increased growth phenotype and elevated frequency of Ty1 retro-transposition. The nuclei in the double mutant showed the nuclear migration defects. The double mutant also exhibited the resistance to MMS and sensitivity to HU in comparison to single gene mutant or WT.

EXPERIMENTAL PROCEDURES

Yeast Strains and plasmid. The yeast strains with their genotypes and plasmids used in this study are mentioned in the **Table 1** and **Table 2**.

Growth Assay – For growth assessment of the WT and mutants in YPD broth and on solid media the method mentioned in (Sharma *et al.* 2019, biorxiv achived data; doi 1101/636902, www.biorxiv.org) was adopted. Briefly, The growth of strains in broth was measured at 2 hrs of interval for 14 hrs. The OD of three independent replicates of each culture at every time point was measured and average of that was plotted against the time. Growth of the strains was also compared by streaking on the YPD plates followed by incubation at 30°C for 2-3 days.

Phase Contrast Microscopy - To compare the morphology of WT and deletion strain, phase contrast microscopy was conducted using Leica DM3000 microscope at 100X magnification. Each strain was grown up to log phase in YPD medium at 30°C and imaged.

Spot Assay- To compare the growth fitness of WT and mutants in presence of genotoxic agents such as hydroxy urea (HU) and methyl methane sulfonate (MMS) the method was followed as mentioned in (Sharma *et al.* 2019, biorxiv achived data; doi 1101/636902, www.biorxiv.org). Briefly, wild type (BY4741) and its deletion derivatives strains (*saf1Δ*, *ctf8Δ*, and *saf1Δctf8Δ*) were grown in the 25 ml YPD (Yeast Extract 1% w/v, Peptone 2% w/v, dextrose 2% w/v) medium overnight at 30°C. The next day the cultures were diluted and grown in fresh YPD medium for 3-4 hrs so as to reach log phase (OD600 0.8-1.0). The cultures were equalized by OD at 600nm. A ten-fold serially diluted sample (3μl) was spotted onto agar plates containing (YPD and YPD + HU and MMS). The plates were incubated at 30°C for 2-3 days and imaged.

Assay for Ty1 retro-mobility - To measure the retro-mobility of Ty1 element methos mentioned in (SCHOLE *et al.* 2001; BAIRWA *et al.* 2011) and (Sharma *et al.* 2019, biorxiv achived data; doi 1101/636902, www.biorxiv.org) was followed. Briefly, the assay was carried out using JC2326 as WT and deletion derivatives (*saf1Δ*, *ctf8Δ*, and *saf1Δctf8Δ*). Each strain carries a single Ty1 element which is marked by an indicator gene HIS3AI. The reporter gene HIS3 is split by artificial intron (AI) in an orientation opposite to the HIS3. When Ty1 which is marked with this arrangement undergoes transcription, splicing followed reverse transcription to generated cDNA copy and finally integration into genomic locations. The integration of the cDNA would generate HIS3 positive phototrophs. The quantitative measurement of the HIS3 positive colonies is considered as frequency of retro-mobility. To measure the Ty1 retro-transposition frequency in the WT (JC2326; reporter strain) and the deletion derivatives (*saf1Δ* and *ctf8Δ* and *saf1Δctf8Δ*), a single colony of each strains was inoculated into 10 ml YPD broth and grown overnight at 30°C. The overnight grown cultures were again inoculated in 5 ml YPD at 1:1000 dilutions. The cultures were allowed to grow up to saturation point (144hrs) at

20°C. The saturated culture was serially diluted and plated on minimal media (SD/His⁻ plates) followed by incubation at 30°C for 3-7 days. The frequency of appearance of HIS⁺ colonies was measured for Ty1 retro-mobility.

Assay for Nuclear Migration defects using Fluorescence microscopy - For detection of nuclear migration defects, assay described by (PALMER *et al.* 1992; BRACHAT *et al.* 1998) and (Sharma *et al.* 2019, biorxiv achived data; doi 1101/636902, www.biorxiv.org) was adopted. Briefly, strains were grown to early log phase (OD₆₀₀ ~ 0.8) at 30°C. Yeast cells were washed with distilled water and suspended in 1X PBS (Phosphate Buffer Saline). Further, fixation was done by addition of 70% ethanol before DAPI staining. Cells were washed with 1X PBS then again centrifuged for 1 minute at 2500 rpm. DAPI stain (1mg/ml stock) to final concentration of 2.5µg/ml was added and incubated for 5 minutes at room temperature and visualized under UV light of fluorescent microscope with 100X magnification. A total of 200 cells were counted and grouped according 0, 1, 2, and multi nuclei per cell, more than two nuclei per cell indicated the nuclear migration defect.

Statistical methods- Statistical significance of observations was determined using paired student t-test. P-value less than 0.05 indicated significant.

RESULTS

Deletion of both the genes SAF1 and CTF8 together leads to growth advantage

The deletion of an ORF may compromise the growth fitness of the mutant depending on whether the gene is essential or non-essential. The essential gene cannot be deleted however the deletion of non-essential gene does not affect the growth fitness on the rich medium to a large extent. Both the gene SAF1 and CTF8, the non-essential therefore the null mutant of the SAF1 and CTF8 was reported to be viable (GIAEVER *et al.* 2002). We wished to determine the impact of deletion of both the gene together on growth fitness in rich medium. We constructed the single (*saf1Δ*, *ctf8Δ*) and double gene deletion (*saf1Δctf8Δ*) in the BY4741 genetic background and assessed the growth in the YPD broth and solid medium. We observed that single gene mutant (*saf1Δ*, *ctf8Δ*) were viable. The growth of the single gene mutants (*saf1Δ*, *ctf8Δ*) was slightly reduced in comparison to WT. In contrast, the double mutant (*saf1Δctf8Δ*) growth was observed better than the WT (**Figure 1 A, C**). The phase contrast imaging of the single gene mutant (*saf1Δ*, *ctf8Δ*) appeared normal. However, the (*saf1Δ*, *ctf8Δ*) mutant showed the slight enlargement of the cell size (**Figure1B**). The *saf1Δctf8Δ* showed the synthetic growth advantage as indicated by streaking and growth kinetics.

Absence of both the gene SAF1 and CTF8 together leads to MMS resistance and HU sensitivity

The genotoxic stress causing agents such as hydroxyurea and MMS have been utilized for mutant screen and clinical use. The hydroxyurea (HU) act as replication checkpoint inhibitor reduces the dNTP pool in the cell. The mechanism involves the inhibition of the ribonucleotide reductase (RNR) activity. We wished to determine the growth response of WT and mutants

(*saf1Δ*, *ctf8Δ*, and *saf1Δctf8Δ*) in the presence of 200mM HU by semi-quantitative spot analysis. Previously it has been reported that *ctf8Δ* cells showed sensitive phenotype in presence of hydroxyurea. In spot assay, WT, *saf1Δ*, *ctf8Δ* appeared slightly sensitive to HU however *saf1Δctf8Δ* cells showed the increased sensitivity to HU when compared with the growth on YPD agar medium without the HU (**Figure 2A**). However the *saf1Δctf8Δ* cells showed the resistance phenotype in comparison to WT or single gene mutant when grown on YPD agar plates containing 0.035% MMS (**Figure 2B**). Further, mechanism of DNA damage repair need to be investigated in *saf1Δctf8Δ* cells treated with MMS.

SAF1 and CTF8 together contributes to nuclear migration defects

The transfer of the newly replicated nuclear DNA into the daughter cells is very delicate process. The DAPI stain which is very specific to the nuclear DNA allows the visualization of the DNA in the form single nuclei in case of WT cells having intact DNA or the multi-nuclei which may indicate the defects in the nuclear migration from mother to daughter cells. We wished to investigate the status of nuclear DNA migration in *saf1Δ*, *ctf8Δ*, *saf1Δctf8Δ* by staining of the log phase culture with DAPI staining. The stained cells were visualized under the fluorescence microscope using appropriate filter. We observed that WT cells showed the compact nuclei (**Figure 3 A**) and majority of cells (80%) showed single nuclei representing budded cells, in case of *saf1Δ* percentage of cell showing the multinuclei phenotype was increased (**Figure 3 B**). However *ctf8Δ* showed increased percentage (20%) of cells showing multi-nuclei phenotype. However *saf1Δctf8Δ* cells showed nuclear DNA at the bud neck junction (**Figure 3A**) and multi nuclei phenotype in nearly 29% of the cells (**Figure 3B**). The multi-nuclei phenotype indicated the defect in the nuclear migration. The data indicates both the gene SAF1 and CTF8 together

contributes to accurate nuclear distribution between mother and daughter cell however mechanism need to investigated.

Deletion of both SAF1 and CTF8 induced high frequency of Ty1 retro-transposition

Replication stress and DNA damage known to induce the Ty retro-mobility in the *S. cerevisiae*. Ctf8 constitutes the part of RFC-Ctf18 complex which helps in the loading of PCNA. The PCNA clamps the DNA polymerases epsilon onto replicating leading strand. We wished to find out whether the absence of both genes could impact the genome stability as their absence showed the faster growth phenotype in comparison to WT. The increase in the frequency of Ty1 retro-transposition is an indicator of the genome instability phenotype. Previously the highest frequency of Ty retro-transposition reported in the *rrm3Δtof1Δ* background was 550 fold above the WT level(BAIRWA *et al.* 2011). We measured the Ty1 retro-mobility in the WT, *saf1Δ*, *ctf8Δ* and *saf1Δctf8Δ* cells using the previously described split HIS3 reporter assay, which allowed the counting of HIS⁺ phototrophs formation on the SD/his⁻ plates. Using this assay we observed that WT and *ctf8Δ* cells did not show the detectable level of His⁺ phototrophs formation however the *saf1Δ* cell showed the nearly 8-10 fold increase in the Ty1 retro-transposition in comparison to WT (**Figure 4 A, B**), however when both the mutant combined (*saf1Δctf8Δ*) showed nearly 170 fold increase in the Ty1 retro-transposition (**Figure 4A, B**). However the mechanism of increased Ty1 retro transposition needs further investigation in future.

Discussion

In this paper we have investigated the genetic interaction between the SAF1 and chromosome associated genome stability regulator CTF8. The search for the genetic interactions and their impact on phenotype and biological processes is important for the precision medicine

development. The investigations on the binary interactions are crucial for better understanding of the system biology and gene networks. This work reports that SAF1 and CTF8 genes together regulate the growth fitness and loss of both leads to the additive growth in rich medium and resistance to genotoxic agent MMS and sensitivity to HU. The Dcc1 component of the Rfc18 complex (Ctf18, CTF8, and Dcc1) reported to have negative genetic interaction in a high-throughput study (COSTANZO *et al.* 2016). Previously the *ctf8Δ* cell showed the decreased growth in the presence of MMS (CHANG *et al.* 2002) and HU (HARTMAN and TIPPERY 2004; PARSONS *et al.* 2004). The *saf1Δ* cells showed the resistance to histone deacetylase inhibitor CG-1521 drug (GAUPEL *et al.* 2014). However the loss of both the genes SAF1 and CTF8 together showed the resistance to MMS and sensitivity to HU suggesting their functioning in different pathways. This is the first report on the genetic interactions between the SAF1 and CTF8 gene which showed positive interactions during normal growth conditions. It would interest to investigate the trigenic interaction among the SAF1, CTF8 and DCC1 and impact on the growth fitness in the presence of HU and MMS. How the loss of genes affects the biological process is the next step in understanding of the overall system biology. Further the data presented in this study on Ty1 retro transposition suggest that loss of either CTF8 or SAF1, elicit the weak retro transposition event. However deletion of both the gene together leads to increased Ty1 retro-transpositions, indicating genome instability phenotype. The absence of both the gene together also showed the increased percentage of cells showing the nuclear migration defects, thereby suggesting the role of SAF1 and CTF8 interaction in faithful segregation of the duplicated genome from mother to daughter cells. We propose that the increased growth phenotype in the absence of both the gene leads to increase in the genome instability and drug resistance which coincide with the cancer cell phenotype. The double mutant (*saf1Δctf8Δ*) can be

used for screening of the chemical compound. It also be interesting to investigate the RNAi mediated knockdown of the hCTF8 and HERC2 in the human cell for fast growth phenotype and resistance to genotoxic agents.

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Author's contributions: NKB conceived and directed the study and wrote the paper with MS and VV. MS performed the experiments and analysed with NKB. VV provided the bioinformatics facility and analysed the data. All the authors reviewed the results and approved the final version of manuscript.

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Tables:

Table 1: List of primers used for construction of deletion strains

S.n o.	Primer code	Sequence
1	SAF1	F-5'CCAAAGGATATACTCTCAATTATAAATGGAAAAGCACATC CGGATCCCCGGGTTAATTAA-3'
		R-5'ACGGAATCCAAAATGCAAAATCGAAATGACACCTAAAA TGAATTCGAGCTCGTTTAAAC-3'
2	CTF8	F-5'AATTATCACTATCATTCAGCCCAATAAACAGCTGAAAAGA CGGATCCCCGGGTTAATTAA-3'
		R-5'AAATAACATAAAACAAACAACTCCGAACAATAACTAAGTA CGAATTCGAGCTCGTTTAAAC-3'
	CTF8 Leu	F-5'AATTATCACTATCATTCAGCCCAATAAACAGCTGAAAAGA CCAACTGTGGGAATACTCAG-3'
		R-5'AAATAACATAAAACAAACAACTCCGAACAATAACTAAGTAC TTGGCCCGAAATTCCCCTAC-3'

Table 2: List of plasmids used for generating deletion cassette

S.no.	Plasmid Name	Deletion casttee	PCR product size	Selection Media
1	pFA6a- KanMX6	<i>KanMX6</i>	1559 bp	YPD + G418
2	pFA6a- His3MX6	<i>HIS3MX6</i>	1403 bp	SD / His -

Table 3: Yeast strains and their genotype used in the study

S.no.	Strain	Genotype	source
1	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Dr. Deepak Sharma IMTECH
2	<i>saf1Δ</i>	<i>saf1Δ::HIS3</i>	This study
3	<i>ctf8Δ</i>	<i>ctf8Δ::KanMX</i>	This study
4	<i>saf1Δctf8Δ</i>	<i>saf1Δ::HIS3, ctf8Δ::KanMX</i>	This study
5	JC2326	<i>MAT-ura3, cir0, ura3-167, leu::hisG, his32</i> <i>Ty1his3AI-270, Ty1NEO-588, Ty1 (tyb::lacZ)-146</i>	Prof. M. Joan Curcio USA
6	<i>saf1Δ</i>	<i>saf1Δ::KanMX</i>	This study
7	<i>ctf8Δ</i>	<i>ctf8Δ::KanMX</i>	This study
8	<i>saf1Δctf8Δ</i>	<i>saf1Δ::KanMX, ctf8::LEU2</i>	This study

Legends and Figures:

Figure 1. Comparative analysis of growth and morphology of WT, *saf1Δ*, *ctf8Δ*, *saf1Δctf8Δ* strains. **A.** Growth of streaked strain on YPD plates which were incubated for 2 days at 30°C and then photographed. **B.** Phase contrast images of log phase cultures at 100X magnification using Leica DM3000. **C.** Growth Kinetics of strains (WT, *saf1Δ*, *ctf8Δ*, *saf1Δctf8Δ*). Cells were collected every 2 hour period and cellular growth was measured by optical density (OD) at 600 nm using TOSHVIN UV- 1800 SHIMADZU. The data shown represent the average of three independent experiments. The error bars seen represent the standard deviation for each set of data.

Figure 2. Comparative assessment of growth phenotype of WT *saf1Δ*, *ctf8Δ*, *saf1Δctf8Δ* strains in presence of methyl methane sulfonate and hydroxyurea by spot analysis. Each strains was grown to log phase was equalized by O.D 600nm and ten fold serially dilution was made. From each dilution 3μl spotted on YPD, YPD + HU/ MMS containing agar plates. The *saf1Δctf8Δ* showed the resistance to MMS and sensitivity to HU

Figure 3. Assessment of nuclear migration phenotype using DAPI (4',6-diamidino-2-phenylindole) staining of WT, *saf1Δ*, *rrm3Δ*, *saf1Δctf8Δ*. **A** Representative fluorescent images of WT, *saf1Δ*, *ctf8Δ*, *saf1Δctf8Δ* cells showing the status of Nuclear DNA migration. The images were acquired at the 100X magnification using Leica DM3000 fluorescent microscope. **B** Table showing the percentage from the count of 200 cell as, 0, 1, 2 and multi- nucleus in each strains, more than two nuclei indicate the nuclear migration defect..

Figure 4. Assay for genome instability by measuring of HIS3AI marked Ty1 transposition frequency in WT, *saf1Δ*, *ctf8Δ*, *saf1Δctf8Δ*. A Images of plates showing the Ty1 transposition induced colonies on SD plate lacking His media. B Bar diagram showing the frequency of Ty1his3AI transposition in each strains. The data shown represent the average of three independent experiments. The significance of transposition was determined by using two tailed t-test. P - value (p) less than 0.05 indicate significant difference and the symbol * represent to p<0.05.

A **B**

A**WT*****saf1Δ*** ***ctf8Δ******saf1Δ ctf8Δ*****B Nuclear Migration Phenotype**

Yeast Strain	0 Nucleus per cell	1 Nucleus per cell	2 Nucleus per cell	Multi Nucleus per cell
BY4741 WT	0%	80%	20%	0%
<i>saf1Δ</i>	5%	45%	30%	20%
<i>ctf8Δ</i>	3.5%	52.5%	23%	21%
<i>saf1Δ ctf8Δ</i>	3%	47%	21%	29%

A **B**