SIR telomere silencing depends on nuclear envelope lipids and modulates sensitivity to a lysolipid drug

Condensed title: LysoPC alters NE shape and telomere silencing

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Highlights:

Keywords: Nuclear morphology, lysophosphatidylcholine, edelfosine, SIR complex, Rap1, transcription, nuclear envelope, Spt23/Mga2, Opi1, lipid metabolism, lipid droplets

Author contributions

M.L.S.P., J.A.C. and V.Z. designed the research.

M.L.S.P. and S.M.F. performed experiments.

M.L.S.P., S.M.F., J.A.C. and V.Z. analyzed the data.

M.L.S.P., J.A.C. and V.Z. wrote the manuscript.

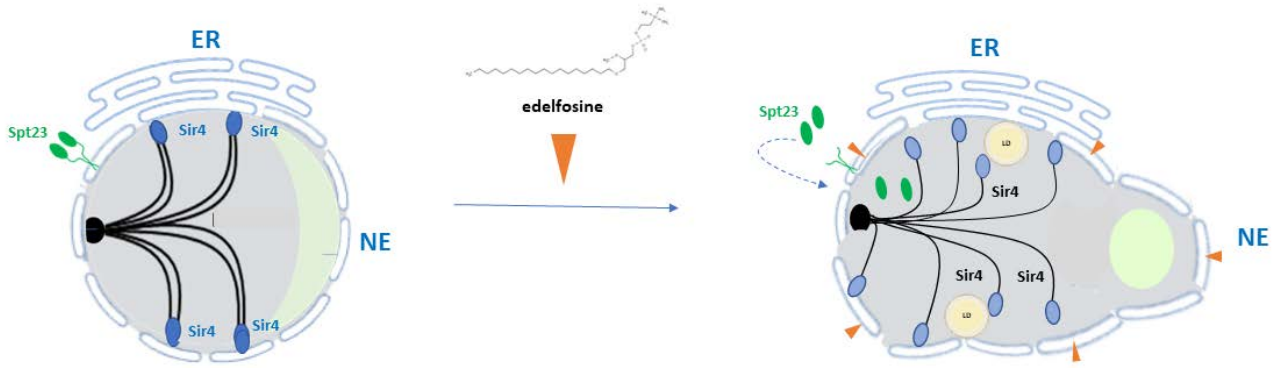
Summary

The nuclear envelope (NE) is important for nuclear organization. This study shows that changes in NE lipid composition from lysolipid treatment disrupts SIR-mediated telomere silencing and triggers changes in transcriptional circuits regulated by membrane sensing factors.

Abstract

The nuclear envelope (NE) is important in maintaining genome organization. The role of lipids in the communication between the NE and telomere silencing was investigated, including how changes in lipid composition impact gene expression and overall nuclear architecture. For this purpose, yeast cells were treated with the non-metabolizable lysophosphatidylcholine analog edelfosine, known to accumulate at the perinuclear endoplasmic reticulum. Edelfosine treatment induced NE deformation and disrupted telomere clustering but not anchoring. In addition, the association of Sir4 at telomeres measured by ChIP decreased. RNA-seq analysis showed that the expression of sub-telomeric regions increased, which was consistent with Sir4 dispersion. Transcriptomic analysis revealed that two lipid metabolic circuits were activated in response to edelfosine, one mediated by the membrane sensing transcription factors, Spt23/Mga2, and the other by a transcriptional repressor, Opi1. Activation of these combined transcriptional programs resulted in higher levels of oleate and the formation of nuclear lipid droplets. Interestingly, cells lacking Sir proteins displayed resistance to oleate and edelfosine and this phenotype was dependent on Rap1 and histone $H4^{K16}$ acetylation.

GRAPHICAL ABSTRACT



Introduction

The nuclear envelope (NE) is a double membrane layer contiguous with the endoplasmic reticulum (ER) that protects genomic material from the rest of the cell. The envelope is strategically positioned for sensing stress and transducing signals about lipid homeostasis into the nucleus, and is structurally critical for organizing the genome (Bahmanyar and Schlieker, 2020).

As with all eukaryotes, the nucleus of budding yeast is highly organized. The yeast nucleus is arranged in a Rabl configuration, where centromere clustering at the NE causes chromosomes to occupy spatially constrained territories, promoting clustering between telomeres anchored at the NE (Bystricky et al., 2005; Hozé et al., 2013; Jin et al., 1998). The nucleolus, which is the site of rDNA transcription and ribosome biogenesis, is also tethered to the NE and forms a crescent-shaped subnuclear compartment (Oakes et al., 1993; Taddei and Gasser, 2012; Yang et al., 1989). Therefore, the NE is a key player in the organization of the nucleus.

Interaction with the NE promotes the local accumulation of transcription factors at telomeres and rDNA, facilitating silencing (Taddei and Gasser, 2012). The most thoroughly characterized example of proteins that interact with NE-bound anchors involves the formation of heterochromatin by the Silent Information Regulator (SIR) protein complex, which includes Sir2, Sir3, and Sir4 for deacetylation of lysine 16 of histone H4 (Grunstein and Gasser, 2013). In budding yeast, heterochromatin forms at telomeres and the mating type loci *HML* and *HMR* on chromosome 3. Another silencing factor, Sir1, contributes to transcriptional repression at the silent *HM* mating-type loci, but not at telomeres (Aparicio et al., 1991; Pillus and Rine, 1989). Telomere positioning at the NE occurs through two redundant pathways, where telomere-associated Sir4 binds with the inner nuclear membrane (INM) proteins Esc1 and Mps3, or alternatively where yKu70/80 binds with Mps3 (Hediger et al., 2002). Sir4 and Sir3 bind telomeres through

interactions with Rap1 (Luo et al., 2002; Moretti et al., 1994), which itself is bound to the double-stranded TG_{1-3} moiety (Buchman et al., 1988a), and together with Sir2, a highly conserved NAD-dependent histone deacetylase (Imai et al., 2000), they form the SIR repressive complex (Grunstein and Gasser, 2013). Telomere anchoring at the periphery increases the relative concentration of SIR proteins, which can also interact in *trans* with SIR proteins bound to other telomeres to form clusters (Gotta et al., 1996; Taddei et al., 2009). The 32 telomeres of a yeast haploid cell typically cluster in ~3-5 foci during log phase (Laroche et al., 1998; Moradi-Fard et al., 2016). Although anchoring facilitates the formation of repressive compartments at the periphery, which silences sub-telomeric genes, anchoring can occur in the absence of SIR proteins and association with the nuclear periphery is not a prerequisite for transcriptional silencing (Taddei and Gasser, 2012; Taddei et al., 2009).

As described above, the pathways that regulate NE anchoring and clustering of telomeres depend on reversible interactions between proteins bound to chromosomes and NE membrane proteins. The impact of NE lipids on these pathways and gene expression is currently unknown. However, some links between lipid metabolism and chromatin organization have been previously identified. For example, decreased fatty acid synthesis due to mutations in acetyl-CoA carboxylase coincided with elevated histone acetylation and contributed to the increased expression of factors regulated by histone deacetylases, similar to cells where *SIR2* is deleted (Galdieri and Vancura, 2012; Papsdorf and Brunet, 2019). Acetyl-CoA derived from the breakdown of fatty acids accounts for a significant portion of the carbon source used in histone acetylation, indicating that transcriptional regulation is impacted by changes in the lipid landscape (McDonnell et al., 2016). Changes in lipid metabolic pathways have also been shown to affect NE size and shape, most notably with the accumulation of phosphatidic acid (PA), a key metabolic intermediate in

membrane lipid synthesis, causing a large expansion of the NE, particularly at the nucleolus (Campbell et al., 2006; Witkin et al., 2012; Carman and Han, 2019; Wolinski et al., 2015). The nucleolus-associated NE also defines a membrane subdomain that becomes actively involved in triacylglycerol (TAG) metabolism in response to cell cycle and nutrient signals (Barbosa et al., 2019).

Not much is known about how other nuclear territories, besides the nucleolus, respond to lipid changes at the nuclear envelope. Previous work has implicated sterols and sphingolipids in the regulation of telomere clustering. Cells lacking Arv1, a factor required for normal intracellular sterol distribution in yeast and mammalian cells (Tinkelenberg et al., 2000), displayed abnormal nuclear morphology and compromised telomere clustering (Ikeda et al., 2015; Papagiannidis et al., 2021). Similar effects were observed when sphingolipid levels decreased in response to pharmacological tools or mutations that inhibited enzymes of the ceramide biosynthetic pathway (Kajiwara et al., 2012; Ikeda et al., 2015). Sterols and sphingolipids are known to form domains in biological membranes based on their preferential interaction, and yeast cells respond to changes in sterol composition of their membranes by adjusting sphingolipids levels (Guan et al., 2009). Lysophosphatidylcholine (lysoPC) lipid analogues disrupt sterol-sphingolipid domains in yeast (Zaremberg et al., 2005). Cells with mutations in ergosterol (*erg3*Δ mutant) or sphingolipid biosynthesis (*lcb1-100* mutant) were hypersensitive to lysoPC related drugs (Zaremberg et al., 2005), while cells lacking chromatin modifiers like Sir4 displayed resistance to these lysoPC analogues (Cuesta-Marbán et al., 2013), pointing to a possible link between membrane lipid alterations and nuclear organization and gene silencing.

In this work we investigated how alterations in membrane lipid composition imposed by a lysoPC burden impact nuclear architecture and gene expression. With this intention, yeast cells

were treated with edelfosine, a non-metabolizable lysoPC analogue that accumulates at the NE. Edelfosine belongs to a group of antineoplastics and antiprotozoals known to target membranes. We previously validated budding yeast as a model organism to study this family of non-metabolizable lysoPC lipid analogues using edelfosine as the prototype (Zaremberg et al., 2005). This antitumor therapeutic was shown to function through a novel mechanism involving selective disruption of lipid rafts at the plasma membrane, which in yeast was characterized by sterol internalization and degradation of the lipid raft-associated proton pump Pma1 (Zaremberg et al., 2005; Czyz et al., 2013). Once internalized, edelfosine accumulated at the perinuclear ER (pER) in both yeast (Cuesta-Marbán et al., 2013) and mammalian cells (Bonilla et al., 2015; Gajate et al., 2012). We were intrigued by the resistance to edelfosine displayed by cells lacking components of the SIR complex like Sir4 (Cuesta-Marbán et al., 2013), opening the possibility lipid composition at the ER/NE could impact nuclear architecture.

We report that edelfosine led to severe changes in the structure of the NE and nuclear associated compartments. We quantified significant changes in nucleolar morphology and telomere clustering, but not telomere anchoring. Using an unbiased RNA-sequencing approach we showed that edelfosine treatment led to a loss of silencing in sub-telomeric regions and increased transcription of genes controlled by Spt23 and Mga2 membrane-sensing transcription factors. The inactive membrane-bound forms of Spt23/Mga2 were cleaved in response to edelfosine, resulting in their translocation from the ER membrane into the nucleus. In addition, a dispersion of the SIR complex from telomeres accompanied the transcriptional changes initiated at the NE in the presence of edelfosine. Our work supports a model where disruption of the NE shape by a lysoPC drug analogue is sufficient to trigger changes in membrane-associated transcription factors and

chromatin regulators, connecting nuclear membrane lipid composition to genome organization and transcriptional regulation.

Results

Nuclear envelope deformation results in disruption of nuclear architecture

Due to its large phosphocholine head group and a single long saturated acyl tail (18:0), edelfosine has an "inverted cone shape" geometry, inducing membrane bending and disrupting lipid packing (Bierbaum et al., 1979). In addition, the presence of ether linkages to the *sn-1* and *sn-2* positions of the glycerol backbone render this lysoPC analogue metabolically stable, as it cannot be remodelled (Kny, 1969). Given that edelfosine accumulates at the NE (Cuesta-Marbán et al., 2013), we treated yeast cells with edelfosine to alter NE lipid composition in a controlled manner, so that we could investigate its impact on nuclear architecture and transcriptional activity.

Using live imaging in cells expressing the ER marker Sec63^{GFP}, we observed a 3-fold increase in non-spherical nuclei after treatment with 20μM edelfosine for 90 minutes (Figure 1A). These deformations in the NE resulted in diverse nuclear shapes deviating from sphericity, with some resembling those seen in yeast with altered PA homeostasis (Campbell et al., 2006; Witkin et al., 2012; Carman and Han, 2019; Wolinski et al., 2015). Asymmetric NE expansion from high PA levels is known to occur in a region of the NE adjacent to the nucleolus, dubbed a nuclear flare (Witkin et al., 2012). Thus, we next examined if deformation of the NE by edelfosine affected nucleolar morphology. Using the nucleolar marker Nop1^{CFP} in combination with the NE marker Nup49^{GFP}, we observed condensation and a significant shrinkage of the nucleoli in edelfosine treated cells (Figure 1B). Interestingly, condensed nucleoli were positioned within a flare-like region of the NE induced by edelfosine.

Unlike other chemotherapeutic drugs, edelfosine does not target DNA, despite accumulating at the NE. However, edelfosine has been shown to induce DNA fragmentation by production of reactive oxygen species after extended treatment (Renis et al., 2000; Zhang et al., 2007). To determine whether DNA damage was being induced in the timescale of our experiments, the phosphorylation of Rad53 and histone $H2A^{Ser129}$ were analyzed by western blot (Figure S1). We observed no checkpoint signaling response to DNA damage with either of these markers.

To gather further insight about the overall impact of NE deformation, we next investigated other territories and compartments with links to the periphery. Since telomeres show perinuclear anchoring and clustering (Figure 2A), we next determined whether these properties were altered by edelfosine. First, anchoring was assessed by visualizing individual telomeres using the system where lacI-GFP conjugates to lacO arrays integrated in either Tel06R or Tel08L (Figure 2B) (Meister et al., 2010). No significant change in telomere association with the periphery was detected after edelfosine treatment, indicating anchoring continued regardless of the extensive alterations in NE shape, delineated by $Nup49^{GFP}$ (Figure 2C and Figure S2A). We next determined telomere clustering by performing live cell imaging of $Rap1^{GFP}$ foci. Consistent with previous reports, 32 telomeres in haploid cells clustered in ~ 3-5 foci (Figure 2D) (Laroche et al., 1998; Moradi-Fard et al., 2016). In edelfosine-treated cells, this increased to ~6-7 foci, suggestive of a defect in clustering, where Rap1-bound telomeres are not spatially bundled as close to one another (Figure 2D). Consistent with this interpretation, Rap1 recovery by ChIP at three different telomeres, Tel01L, Tel06R, and Tel15L, was statistically similar in the presence and absence of edelfosine, indicating that the increased number of foci did not result from massive changes in the association of Rap1 with telomeres (Figure 2E). Moreover, Rap1 protein levels (Figure 2F) and

the fraction of Rap1 associated with the membrane fraction (Figure S2B) did not change, indicating that Rap1 bound telomeres anchored at the NE distorted by edelfosine treatment.

Altogether, these findings support the notion that NE deformations induced by edelfosine resulted in the loss of NE sphericity affecting all regions of the nuclear membrane, from flares associated with the nucleolus to regions interacting with the bulk of the DNA. These NE deformations, in turn, impacted nuclear architecture, inducing nucleolar compaction and altering telomere clustering, but not anchoring.

The SIR complex is susceptible to lipid alterations at the NE

Defects in clustering can be indicative of disruptions in the association of the SIR complex with telomeres, independently of anchoring (Ruault et al., 2011), therefore we next investigated the SIR complex in edelfosine. As mentioned above, a chemogenomic screen previously identified *sir4*Δ mutant cells as edelfosine resistant. (Czyz et al., 2013; Cuesta-Marbán et al., 2013). Since the screen was performed in the BY4741 background, we first manually verified these results in W303, the background used in the current study. As with *sir4*Δ, deletion of *SIR2* or *SIR3* led to edelfosine resistance compared to wild type (Figure 3A). The canonical deacetylation target of the SIR complex is lysine 16 of histone H4 (Imai et al., 2000). Therefore, we assessed edelfosine sensitivity of cells expressing histone $H4^{K16A}$, a mutant preventing acetylation. The K16A mutant was hypersensitive to edelfosine (Figure 3B), which aligns edelfosine resistance in *sir* deletion mutants with histone $H4^{K16}$ hyper-acetylation (Braunstein et al., 1996). Acetylated K56 and K79 in histone H3 are also key epigenetic marks in yeast (Grunstein and Gasser, 2013). However, unlike $H4^{K16A}$, mutagenesis at these sites did not result in edelfosine hyper-sensitivity (Figure S3). Moreover, edelfosine resistance from *SIR4* deletion was supressed in combination with $H4^{K16A}$,

indicating the impact of the SIR complex on edelfosine resistance was mediated by chromatin rather than off-target effects (Figure 3C).

To explore how edelfosine impacted SIR interactions with telomeres, we next performed ChIP with Sir4^{Myc}. Although overall Sir4 levels remained constant (Figure 3D), there was a marked decrease in its recovery at the three telomeres we monitored (Figure 3E). To complement ChIP, we also performed live cell imaging with Sir4^{GFP}, which forms punctate foci when clustering is intact (Moradi-Fard et al., 2016; Palladino et al., 1993). In edelfosine-treated cells, Sir4^{GFP} was dispersed and in some cells appeared outside the nucleus (Figure 3F).

Altogether, these results suggest deformation of the NE by edelfosine destabilizes the protein-protein interactions of SIR complex components at telomeres resulting in Sir4 dispersion.

Transcriptional changes induced by NE deformation

So far, we have shown that edelfosine induced NE membrane deformations with consequential changes in nuclear architecture, impacting Sir4 association with telomeres and their clustering, as well as nucleolar compaction. These results suggest a loss of silencing at telomeres and decreased transcriptional activity in the nucleolus. Yeast cells exposed to various stressors have shown rDNA compaction (reviewed in Matos-Perdomo & Machín, 2019). This phenotype is linked with epigenetic changes as a consequence of altered protein interactions with the NE, as exemplified by rDNA tethering to the NE through the chromosome linkage INM proteins (CLIP) complex (Golam Mostofa et al., 2018; Mekhail et al., 2008; Chan et al., 2011) in combination with the Sir2 histone deacetylase (Gottlieb and Esposito, 1989; Bryk et al., 1997; Fritze et al., 1997; Smith and Boeke, 1997). Similarly, telomere clustering at the NE serves as a platform where protein-protein interactions are strengthened, reinforcing transcriptional silencing (Taddei et al., 2009). Many different NE scaffolding proteins and DNA interacting factors could collaborate to

modulate the NE changes imposed by edelfosine at telomeres and the nucleoli. In order to assess the overall transcriptional response, we next performed RNA-sequencing (RNA-seq) in the presence of edelfosine. We reasoned that information acquired by this unbiased approach would not only be a resource for understanding the full physiological impact of edelfosine at the molecular level, but also had the potential to reveal unique edelfosine-induced transcriptional changes associated with abnormal NE morphology distinct from the 'general response' accompanying most stressors.

A total of 224 genes were differentially expressed in edelfosine, using the stringent cut-off of > (ln)2-fold change and a false discovery rate of $p < 0.01$. Of these, 119 genes were upregulated and 105 were downregulated (complete list in Table S1). We hypothesized that loss of SIR from telomeres would induce transcription from sub-telomeric genes. Indeed, 12.6% of genes upregulated > 2-fold were sub-telomeric (p-value 0.0001 by Fisher's exact test; complete list in Table S2) while only 4.2% of encoded genes reside in sub-telomeric regions located < 20 kb from the end of the chromosome (Taddei et al., 2009). Furthermore, as expected for Sir4 dispersion, known internal (non-sub-telomeric) Sir4 targets represented 22.9% of the downregulated genes in edelfosine (p-value 2.56E-8 by Fisher's exact test; complete list in Table S3) (Taddei et al., 2009). In addition, ribosome protein genes emerged as the most significantly enriched category of downregulated genes (Figure S4), consistent with the accompanied rDNA compaction and an expected overall decrease of ribosomal biogenesis.

Next, we looked for over-represented transcription factors (TFs) that could bind to the promoter regions of the two sets of up-and down-regulated genes after edelfosine treatment. Gene promoter analysis with TFs and transcriptional regulators based on DNA binding was performed using Yeastract (Monteiro et al., 2020). This analysis identified a discrete set of 6 and 7 TFs/

transcription regulators for the up- and down- regulated gene clusters respectively (p-value <1E-05; Tables 1 and 2). Binding sites for transcriptional regulators involved in the response to oxidative stress and metals were among those identified in the cluster of upregulated genes. For the cluster of down regulated genes, we identified Rap1 and its co-regulators of ribosomal protein genes Ifh1 and Fhl1 (Cherel and Thuriaux, 1995; Wade et al., 2004; Rudra et al., 2007), as well as transcription factors Fkh1 and Fkh2. Indeed, 36.2% of down-regulated genes overlapped with known Rap1 binding sites (Figure 4A) (Lieb et al., 2001). Of these targets, 89.5% were ribosomal protein genes (Figure 4B and Table S4). Representative Rap1 target genes were selected for validation by qPCR and values were found to be consistent with the RNA-seq data (Figure 4C). Taken together with the nucleolar condensation we observed by microscopy (Figure 1B), our data point to a decrease in ribosome biogenesis after edelfosine treatment.

RAP1 is an essential gene, however a truncated allele missing 165 amino acids at the C-terminus, *rap1-17*, attenuates the repression of ribosomal protein genes in response to secretory pathway defects and disrupts silencing of sub-telomeric regions (Mizuta et al., 1998; Kyrion et al., 1992; Buck and Shore, 1995). Another characterized allele carrying two missense mutations at amino acids 726 and 727, *rap1-12*, results in loss of silencing at the homothallic mating (*HM*) loci, but in contrast to *rap1-17*, does not alter transcription from ribosomal protein genes (Mizuta et al., 1998; Sussel and Shore, 1991; Buck and Shore, 1995). To gain insight into the contribution of Rap1 in the transcriptional response to edelfosine, we tested these two loss-of-function *rap1* alleles in edelfosine sensitivity assays. Cell growth on plates containing edelfosine showed that *rap1-17*, but not *rap1-12*, increased edelfosine resistance (Figure 4D). Furthermore, the *rap1-17* C-terminal truncation mutant described above also lacks the domain responsible for interacting with and recruiting Sir4 to telomeres (Cockell et al., 1995; Moretti and Shore, 2001; Luo et al., 2002). In

sum, these results support and expand our findings with the SIR complex, and suggest the C-terminus of Rap1 facilitates interactions with Sir4 that mediate edelfosine sensitivity.

Spt23 and Mga2 are activated in response to edelfosine

To understand the mechanism by which changes in the lipid composition at the NE are sensed and communicated to the nucleus, we directed our RNA-seq analysis to differentially expressed genes related to lipid metabolism and transport in response to edelfosine. For these combined categories, an estimated ~ 9% (109 genes) and ~ 4.5% (60 genes) of the total transcripts differentially expressed [> 0.5 (ln)-fold change cutoff and $p < 0.01$] were upregulated and downregulated, respectively in edelfosine. Distinct metabolic branches affecting all lipid classes, including glycerolipids, sphingolipids and sterols, were mapped for each of the up and down datasets (Figure 5A and Table S5). Two critical transcriptional circuits that regulate lipid metabolic pathways clearly emerged from this analysis: Opi1/Ino2/4-mediated repression and Spt23/Mga2 activation. Furthermore, Spt23 was identified as a transcription factor enriched from the RNA-seq analysis (Table 1) and targets of Spt23 were among the group of transcripts displaying the most dramatic changes in response to edelfosine with >2(ln)-fold change (Figure 5B and Table S5). Spt23, together with its paralog Mga2, maintains NE integrity by way of an ER to nucleus signaling pathway (Zhang et al., 1999). Spt23/Mga2 regulates lipid remodelling by activating expression of *OLE1*, the gene encoding the only fatty acid desaturase present in budding yeast (Zhang et al., 1999; Chellappa et al., 2001; Auld et al., 2006; Martin et al., 2007; Fang et al., 2017; Romero et al., 2018). To confirm activation of the Spt23/Mga2 circuit, we measured transcription of *OLE1* and other targets of Mga2 and Spt23 by qPCR upon edelfosine treatment. Consistent with the RNA-seq results, *OLE1, ICT1, IZH1, IZH2, IZH4* and *MGA2* itself were upregulated while no changes were observed for *IZH3* and *SPT23* (Figure 5B, inset). In addition, we also confirmed by

qPCR repression of *INO1*, a representative target of the transcriptional repressor Opi1, which was also downregulated (Figure 5B, inset).

It is worth noting that Spt23 and Mga2 have been previously proposed to be antagonists of silencing (Dula and Holmes, 2000). In fact, 40% of the sub-telomeric genes differentially expressed by treatment with edelfosine can be mapped to the Spt23 regulated network (Table S5). Therefore, Spt23/Mga2 emerged as strong candidates to sense the lipid changes imposed by edelfosine at the NE.

Spt23 and Mga2 form homodimers that insert into the ER membrane by a single transmembrane helix, which is sensitive to the lipid environment (Ballweg et al., 2020). High lipid saturation triggers ubiquitylation, which then signals for the subsequent proteolytic release of a transcriptionally active N-terminal fragment that translocates into the nucleus, inducing *OLE1* expression (Figure 6A) (Hoppe et al., 2000). Based on the enrichment of Spt23 targets in the transcriptomic analysis, we next tested whether edelfosine treatment triggered the processing and subsequent translocation of Spt23 and Mga2 into the nucleus. To this end, we performed live cell imaging of cells expressing either ^{GFP}Spt23 or ^{GFP}Mga2 from a constitutive promoter wherein proteins were GFP tagged at their N-terminus. Cells harboring either construct showed ER localization (Figure S5), but after a short 30-minute treatment with edelfosine, 98% and 63% of the GFP signal from cells expressing ^{GFP}Spt23 and ^{GFP}Mga2 respectively were found in the nucleus, indicative of protein processing and translocation (Figure 6B, C). Importantly, the nuclear localization of ^{GFP}Spt23 or ^{GFP}Mga2 was not a general stress response, as no changes were observed when cells were treated with high levels of methyl methane sulfonate (MMS), an alkylating agent that causes DNA damage. ^{GFP}Spt23 and ^{GFP}Mga2 processing was further corroborated by western blot, as the N-terminus of Spt23 fused to GFP was cleaved, with ^{GFP}Spt23

being almost undetectable after a 60-minute treatment with edelfosine (Figure 6D). ^{GFP}Mga2 processing appeared slower (Figure 6E), as both the membrane bound precursor and the soluble active forms were detected after 60 minutes of edelfosine treatment. These results support a role for Spt23 and Mga2 as sensors for changes in the lipid environment imposed by edelfosine accumulation in the NE, and as mediators of the transcriptional response aimed at triggering lipid remodeling in a landscape where the *de novo* synthesis of glycerolipids was repressed.

Membrane alterations by edelfosine precede changes in nuclear architecture

As mentioned above, Spt23 and Mga2 are believed to function as silencing antagonists (Dula and Holmes, 2000). Edelfosine triggers Spt23/Mga2 processing and subsequent translocation into the nucleus and, as demonstrated above, it also triggers SIR complex delocalization within the nucleus (Figure 3). The lipid changes associated with NE deformation and Spt23/Mga2 activation could subsequently induce alterations in nuclear organization, including telomere clustering and Sir4 dispersion. Alternatively, a loss of the SIR complex from telomeres in response to edelfosine treatment could result in altered transcription, sensitizing the membrane, resulting in NE conformational changes. To bring insight to these possibilities, we wanted to determine which occurs first: membrane deformation or SIR dispersion. Spt23 and Mga2 are functionally redundant and loss of both is lethal (Zhang et al., 1997, 1999), precluding us from determining Sir4 recovery and localization in cells where both are deleted. However, we reasoned that if morphological alterations of the NE and lipid sensing by Spt23 and Mga2 occurred upstream, then changes in nuclear shape and Spt23/Mga2 processing would still occur in the absence of Sir4. Indeed, edelfosine induced NE deformations in *sir4*Δ mutants similarly to *SIR4*$^{+}$cells (Figure 7A). Additionally, ^{GFP}Spt23 and ^{GFP}Mga2 translocation was similar in *sir4*Δ and wild-type cells upon edelfosine treatment (Figures 7B, C). Interestingly, the level of

upregulation in some of Spt23/Mga2 target genes reproducibly decreased in *sir4*Δ mutant cells after edelfosine treatment including the transcription of *OLE1* (Figure 7D).

We next sought to assess if fatty acid unsaturation was augmented in response to edelfosine and if this increase was dependent on Sir4. In line with the upregulation of *OLE1* transcription, neutral lipid analysis of cells treated with edelfosine exhibited higher levels of oleic acid (OA) and this change was also observed in *sir4*Δ mutants (Figure 8A). Hence, these results support a model whereby edelfosine-induced alterations in lipid composition at the NE are sensed by Spt23/Mga2, resulting in the activation of *OLE1* and a concomitant accumulation of free oleate, independently of Sir4.

A build-up of edelfosine together with free oleate could result in a synergistic lipotoxic effect, suggesting that the presence of Sir4, and the associated deacetylase activity of the SIR complex, decreases the lipid detoxifying capacity of yeast. To challenge this idea, we tested the growth of *sir* and $H4^{K16A}$ mutants on oleate. Fitness of *sir3*Δ on oleate was better than wild type, followed by *sir4*Δ, while growth of the *sir2*Δ strain was comparable to the wild type (Figure 8B). As with edelfosine (Figure 3C), introducing the $H4^{K16A}$ mutation in a *sir4*Δ background dramatically impaired growth on oleate. Overall, these results strongly suggest that the SIR complex and concomitant deacetylation of $H4^{K16}$ compromise the lipid detoxifying capacity of yeast.

One cellular strategy behind lipid detoxification at the NE is to direct excess fatty acids towards TAG synthesis and lipid droplet (LD) biogenesis (Barbosa et al., 2019; Romanauska and Köhler, 2021). Depending on the status of lipid-related transcriptional circuits, these LDs could accumulate inside the nucleus (nLDs) or face the cytosol (cLDs). When the Opi1/Ino2-4 circuit was repressed by deletion of *INO4* ~30% of cells displayed nLDs in addition to cLDs. By contrast,

when *OLE1* was overexpressed, either directly or through constitutive activation of Mga2, only cLDs were produced (Romanauska and Köhler, 2021). Furthermore, when Mga2 was constitutively activated in *ino4*Δ cells, nLDs were still produced. The latter scenario resembles the transcriptional status of cells treated with edelfosine. Since our transcriptomic analysis showed simultaneous Spt23/Mga2 activation and Opi1/Ino2/4 repression, we reasoned the inner nuclear membrane would induce production of nLDs in response to a lysoPC burden. To this end, the number of LDs and their localization were assessed by staining cells expressing the ER marker ^{DsRed}HDEL with BODIPY 493/503 (Szymanski et al., 2007) (Figure 8C). In wild type cells, the number of LDs increased from 10.04 ± 3.33 LDs/cell to 12.29 ± 3.61 LDs/cell after edelfosine treatment (Figure 8D). A strong contributor to the increase in LDs was the formation of nLDs inside the nucleus, as ~25% of wild type cells treated with edelfosine had at least one nLD (Figure 8E). Although cells lacking Sir4 displayed fewer LDs in control (7.92 ± 3.06 LDs/cell), there was a similar overall increase to 11.07 ± 4.54 LDs/cell upon edelfosine treatment (Figure 8D).

In all, LD bulging from the nuclear membrane towards the nucleoplasm could be a significant contributor to NE deformation, disrupting telomere clustering and overall nuclear architecture independently of transcriptional silencing. These results indicate nLD formation is part of the cellular response to edelfosine, and that it occurs independently of Sir4. While deacetylation by the SIR complex appears to decrease the lipid detoxifying capacity of yeast as edelfosine and oleate tolerance increases in sir mutant cells, deletion of *SIR4* did not reverse LD formation after edelfosine.

Discussion

The nucleus, the defining feature of eukaryotic cells, is delimited by a double membrane structure that contains associated integral and peripheral proteins and is vital for the

compartmentalization of nuclear processes. Inactive chromatin regions are silenced by association with the nuclear membrane. Whereas other regions of active transcription, such as in the nucleolus, are closely associated to the nuclear envelope, facilitating product export. The architecture of the nucleus is highly regulated, and disruption of its structure has been linked to functional defects such as aberrant gene silencing, loss of chromatin anchoring, incomplete chromosome segregation and faulty cellular differentiation (Golden et al., 2009; Schreiner et al., 2015; Smith et al., 2017; Teixeira et al., 2002). Important, but seemingly often overlooked players in nuclear architecture and regulation, are the lipids that comprise the NE, although perturbations directed at glycerolipid metabolism have been shown to affect NE shape, impacting mainly the NE membrane closely associated with the nucleolus (Santos-Rosa et al., 2005; Siniossoglou et al., 1998; Campbell et al., 2006; Barbosa et al., 2019).

In this study, our work revealed that changing the lipid composition of the NE through a lysolipid burden impacted nuclear architecture and gene expression. Lysolipids are known to introduce membrane deformations and are usually present in small amounts in biological membranes, as they are rapidly re-acylated or metabolized (Fuller and Rand, 2001). Therefore, we manipulated the NE lipid composition by treating cells with the non-metabolizable lysoPC analogue edelfosine, which cannot be altered or metabolized. Our data supports a model whereby contacts between chromatin-associated factors and the nuclear membrane are functionally sensitive to variations in the lipid environment, triggering distinct transcriptional responses driven by changes arising within the membrane. Our work unveiled a mechanism by which intracellular accumulation of edelfosine induced deformation of the NE, which in turn led to changes in genome organization, supporting transcriptional programs aimed at correcting membrane defects and lipid detoxification. Specifically, two subnuclear compartments, the nucleolus and telomeres, switched

their transcriptional status in response to edelfosine. While telomere clustering decreased, resulting in transcriptional activation of sub-telomeric regions, nucleoli experienced compaction, accompanied by a general repression of ribosome biogenesis.

The SIR complex is susceptible to a lysolipid burden

NE deformations induced by edelfosine affected the NE associated with several nuclear areas, including both the nucleolus and bulk DNA. Components of the SIR complex are known to shuttle between these two nuclear territories (Gotta et al., 1997; Kennedy et al., 1997; Radman-Livaja et al., 2011). For example, in addition to promoting telomere clustering, Sir3 directly promotes long-range contacts between distant chromatin regions, acting as a molecular bridge between the rDNA and telomeres (Ruault et al., 2021). Furthermore, association of Sir3 with these compartments requires Sir2 deacetylase activity (Hoppe et al., 2002; Radman-Livaja et al., 2011). Our results indicate that unlike Rap1, which binds to telomeric DNA directly, SIR association with chromatin is highly reactive to an increased burden of lysolipids, like the one imposed by edelfosine. Precisely, alterations in NE shape did not affect anchoring of telomeres to the membrane but led to loss of telomere clustering as shown by increased Rap1 foci at the nuclear periphery, with a concomitant release of Sir4 from telomeres and lessening of silencing of sub-telomeric regions (Figures 2D & 3E).

Our view is that the fluid nature of the membrane where telomeres are anchored, allows clusters to be dynamic, enabling cluster-fusion or separation (Schober et al., 2008; Hozé et al., 2013). That is to say, while telomere anchoring to the membrane relies on the interaction of chromatin factors with peripheral and integral membrane proteins like Esc1 and Mps3, respectively, clustering relies more on membrane fluidity, allowing the lateral movement of these

proteins to bundle, perhaps propelled by lipid subdomains at the NE and further stabilized by Sir-mediated *trans* interactions.

Edelfosine is known to disturb lipid rafts at the PM, altering membrane domain organization (Ausili et al., 2008; Zaremberg et al., 2005; Cuesta-Marbán et al., 2013; Czyz et al., 2013). It is therefore reasonable to propose that edelfosine is acting similarly once reaching the ER/NE. Its incorporation in the ER/NE changes the lipid microenvironment, decreasing telomere clustering. An additional contribution to NE deformation and obstruction for telomere clustering may come from the bulging of nuclear LDs in an attempt of cells to recalibrate the fatty acyl composition of the membrane to ease the lysoPC burden.

In addition to loss of telomere clustering and nucleolar compaction, our study determined that edelfosine induced repression of ribosomal protein genes, known to be regulated by Rap1. While a reduction in ribosome biogenesis is part of a general stress response, the signaling pathways leading to a coordinated Rap1-dependent repression of ribosomal protein expression differs depending on the stress. For example, it was previously demonstrated that the *rap1-17* allele prevents ribosomal repression during the secretory stress response, but not the heat shock (Mizuta et al., 1998) or nitrogen starvation responses (Miyoshi et al., 2001). As with the secretory pathway, edelfosine resistance of *rap1-17*, but not *rap1-12,* suggests that repression of ribosomal proteins induced by the lipid drug requires Rap1 C-terminus interaction with Sir4. Altogether, the observed resistance of cells lacking SIR complex components or the C-terminal end of Rap1 suggests that the Rap1-Sir4 binding contributes to edelfosine toxicity (Figures 3A & 4D).

Sensing lipid changes by Spt23/Mga2 and Opi1, what is being sensed?

Our unbiased RNA-seq approach identified Spt23 and Mga2 transcription factors as membrane sensors that are sensitive to the lipid changes induced by edelfosine. The inactive forms of Spt23/Mga2 reside in the ER/NE membranes and have been previously shown to sense membrane fluidity (Covino et al., 2016), although this concept has been recently challenged by uncovering a particular sensitivity of Mga2 to the degree of lipid saturation instead of viscosity (Ballweg et al., 2020).

What could Spt23 and Mga2 be sensing in edelfosine treated cells? Activation of these transcription factors was an early event noticed in live cells as early as 30 minutes after edelfosine treatment. One possible explanation for their activation is that they are capable of sensing the saturated C18:0 tail of this lysoPC analogue, therefore responding by increasing desaturation through *OLE1*. Edelfosine is also a lipid with non-cylindrical shape, expected to introduce curvature in a bilayer. Therefore, in addition to their known ability to sense lipid packing, Spt23/Mga2 may be able to detect changes in membrane curvature directly introduced by edelfosine. Indeed, it is the combination of membrane curvature and composition that modulates lipid packing and protein recruitment (Vanni et al., 2014), opening the possibility that Spt23/Mga2 are sensitive to both membrane properties.

Another, non-exclusive, explanation relates to the fact that edelfosine induces a rapid (within 15 minutes) internalization of sterols from the PM in yeast (Zaremberg et al., 2005). It is well known that lysoPC interacts with sterols forming lamellar structures due to their complementary geometry (Rand et al., 1975; Lange and Slayton, 1982). Although it is not clear where ergosterol localizes once it is internalized, it is possible that it complexes with edelfosine in internal membranes, and this could be sensed by Spt23/Mga2. Furthermore, ergosterol transport and biosynthetic genes are targets of these transcription factors in addition to *OLE1,* all of which

are upregulated in response to edelfosine (Zhang et al., 1999). Thus, the contribution of sterols to the lipid changes sensed at the ER/NE by Spt23/Mga2 will be an area of future exploration beyond this study.

Importantly, it is known that edelfosine reduces the stored membrane curvature elastic stress, which is a membrane property that plays a role in the recruitment of several proteins to membranes (Dymond et al., 2008). One such protein is the CTP:phosphocholine cytidylylphosphotransferase (CCT) which localizes to the nucleus and senses curvature through an amphipathic helix (Haider et al., 2018; Taneva et al., 2012; Attard et al., 2000). CCT represents the rate limiting and committed step for the synthesis of PC through the Kennedy pathway, and edelfosine alters its recruitment to membranes while inhibiting the pathway in both yeast and mammalian cells (Boggs et al., 1995; Zaremberg and McMaster, 2002). Of note, it has been shown that in hepatocytes nLDs recruit CCTα (Fujimoto, 2022; Sołtysik et al., 2019). Since we show herein that edelfosine induces nLD biogenesis (Fig 8E), this mechanism could also contribute to the effects previously associated with edelfosine treatment and its impact on CCT activity and the Kennedy pathway.

The transcriptional repressor Opi1 is another lipid sensor that reacts to edelfosine. Downregulation of Opi1 targets identified by our RNA-seq analysis implicates the release of Opi1 from the ER and translocation to the nucleus, which occurs in conditions of low PA or in conditions where cytosolic acidification leads to protonation of the phosphate group in PA (Loewen et al., 2004; Young et al., 2010). Although no changes in PA levels were previously detected (Tambellini et al., 2017), cytosolic acidification was indeed a rapid effect induced by edelfosine (Czyz et al., 2013; Zaremberg et al., 2005). Opi1 has also been shown to be sensitive to acyl-chain length, opening the possibility that it could have a role in the cell's response to edelfosine (Hofbauer et

al., 2014). The combined activation of Spt23/Mga2 and Opi1 circuits by edelfosine resulted in the upregulation of FA and glycerolipid remodelling pathways and a shutdown of *de novo* glycerolipid synthesis.

Lipid changes and nuclear territories: insights from edelfosine transcriptomics

NE deformations induced by edelfosine affected both the nucleolus and the bulk DNA. In the case of the nucleolus, compaction was accompanied by expansion of the associated nuclear membrane leading to the formation of a flare-like structure (Figure 1). The nuclear membrane associated with the nucleolus is known to be particularly susceptible to expansion in conditions of excess PA and phospholipid synthesis (Campbell et al., 2006; Karanasios et al., 2010; Witkin et al., 2012). It is interesting to note however, that the nuclear flare associated with the nucleolus in edelfosine is unlikely to be the result of PA accumulation or increased *de novo* synthesis of phospholipids, as all biosynthetic pathways were downregulated.

The combined analysis of previous lipidomic (Tambellini et al., 2017) and current transcriptomic and neutral lipid profile analysis point to major changes in neutral lipid metabolism leading to FA and DAG accumulation (Figure 8A). Importantly, a membrane subdomain linked to nuclear shape and active in fat storage and lipid droplet biogenesis has been defined at the nuclear envelope tightly connected to the nucleolus (Romanauska and Köhler, 2018). Interestingly, the transacylase Lro1 was recently shown to be enriched in this subdomain in response to cell cycle and nutrient signals (Barbosa et al., 2019). Lro1 consumes DAG while generating TAG and LysoPC, which is re-acylated by a PC remodelling pathway (Oelkers et al., 2000). The accumulation of a non-metabolizable LysoPC analogue like edelfosine at the NE would frustrate cellular attempts for re-acylation, resulting in membrane deformation and possible inhibition of TAG synthesis, inducing local DAG accumulation. The lipid metabolic landscape emerging from

edelfosine transcriptomic results certainly supports accumulation of DAG through the repression of biosynthetic pathways that consume DAG and the upregulation of several catabolic branches directly producing DAG through TAG lipolysis, phospholipase C, and PA phosphatase Pah1 activities (Figure 5A). In addition, upregulation of several remodelling pathways including FA desaturation, activation and peroxisomal shortening as well as phospholipases and acyltransferases all point to a major cellular effort to change the acyl tails of phospholipids in response to edelfosine.

Once the transcriptional programs are triggered by the membrane sensors Spt23/Mga2 and Opi1, hindered attempts to re-acylate edelfosine induce accumulation of DAG and FAs (Fig 8A), potentiating the effect of the drug on the different nuclear territories. Falling of the SIR complex from silencing at telomeres opens their targeting to new cellular areas, even outside the nucleus, as seen for Sir4 in edelfosine. Interestingly, the human ortholog of Sir2 in yeast, the sirtuin SIRT1, regulates the mammalian sterol response element-binding protein (SREBP) through deacetylation (Ponugoti et al., 2010). SREBP is a transcription factor trapped in the ER which senses membrane sterol depletion in the ER, triggering its transport to the Golgi for proteolysis and translocation to the nucleus in an analogous mechanism to that reported for Spt23/Mga2 activation. Furthermore, it has been shown that lipid consumption modulates sirtuins in humans, with the best-studied mechanism highlighting oleic acid as a natural activator of SIRT1 (Caldas et al., 2021). Similar regulation could operate in yeast, where an increase in oleate could result in Sir2 activation, contributing to edelfosine toxicity.

Targeting the NE lipid composition could therefore be a novel strategy to regulate the activity of sirtuins, relevant in the development of chronic diseases including cancer, diabetes, cardiovascular disease, insulin resistance, and metabolic syndrome (Wang et al., 2012; Bindu et

al., 2016; Winnik et al., 2015; Kurylowicz, 2016). Importantly, edelfosine has been studied as a chemotherapeutic, particularly in leukemic bone marrow purging (Vogler and Berdel, 2009; Mollinedo et al., 2004). Our data suggest edelfosine should not be used in combination with the popular histone deacetylase inhibitor class of cancer therapeutics (Shirbhate et al., 2022; Palamaris et al., 2022; Conery et al., 2022), as this may desensitize cells to the toxicity of edelfosine.

In summary, our work suggests that disruption of the nuclear membrane is sufficient to trigger changes in membrane-associated transcription factors and chromatin remodellers. Our data indicate that nuclear membrane integrity is linked to transcriptional regulation. The NE could represent a novel target for chemotherapeutics, since lipid drugs like edelfosine are not mutagenic.

Materials and Methods

Reagents. Unless otherwise indicated, all reagents were purchased from Fisher or Sigma. Yeast extract, peptone, and yeast nitrogen base were from MP Biomedicals (Santa Ana, CA). BODIPY™ 493/503 was ordered from Fisher Invitrogen (D3922). All lipids were ordered from Sigma: 1-2-dioleyl-*sn*-glycerol (800811C), oleic acid (O1008-1G), 1,2,3,-trioleylglycerol (870110O) and cholesteryl oleate (C9253), except for ergosterol (Fluka Analytical, 45480). Edelfosine was the kind gift of Medmark Pharma GmbH.

Yeast strains, plasmids and primers. Detailed information on yeast strains, plasmids and primers used in this study is provided in Tables 1, 2 and 3 respectively.

Strains expressing DsRed-HDEL were transformed with the integrating plasmid pAM41 using the standard transformation protocol (Guthrie and Fink, 1991). Strains VZY452, JC4509 and JC4881 were created by mating and tetrad dissection, as described previously (Morin et al., 2009). Briefly, parental strains of opposite mating types were streaked on YPD with one intersecting area to allow mating and produce diploids. Diploids were selected and sporulation was then induced by streaking on 2% potassium acetate plates Tetrads were identified by light microscopy and their cell walls were digested with 5 ug/mL Zymolyase (amsbio, 120491-1) in 1M sorbitol for 5 minutes at 30°C. Tetrads were then dissected using a Zeiss Axioskop 40 dissecting microscope and progeny expressing only the markers of interest were saved as the indicated strains. Genotypes were confirmed by colony PCR and sequencing.

SIR4, *MGA2* and *SPT23* coding sequences were PCR amplified from yeast genomic DNA (lab strain W303) using gateway compatible primers (Table 3). The amplified product was then cloned into Gateway™ pDONR™221 donor vector (ThermoFisher) to generate the corresponding entry clones. Entry clones for each indicated gene were confirmed by standard DNA sequencing (DNA sequencing facility, University of Calgary). *SIR4* was then subcloned into the *S. cerevisiae* Advanced Gateway™ Destination Vector pAG415GPD-ccdB, while *MGA2* and *SPT23* were subcloned into *S. cerevisiae* Advanced Gateway™ Destination Vector pAG415GPD-EGFP-ccdB (Alberti et al., 2007). The *S. cerevisiae* Advanced Gateway™ Destination Vectors were a gift from Susan Lindquist (Addgene kit #1000000011).

Table 1- Yeast strains used in this study

Strain	**Genotype**	**Source**
JC0048	*MAT a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::KanMX3 [YCp22 HHT1 HHF1-TRP1]* Kind gift of Dr. Alain Verreault	Masumoto et al., 2005 (W303 with *RAD5*$^{-}$)
JC0049	*MAT a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::KanMX3 [YCp22 hht1*^{K56A} *HHF1-TRP1]* Kind gift of Dr. Alain Verreault	Masumoto et al., 2005 (W303 with *RAD5*$^{-}$)

JC0050	*MAT a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::KanMX3 [YCp22 hht1^{K56Q} HHF1-TRP1]* Kind gift of Dr. Alain Verreault	Masumoto et al., 2005 (W303 with *RAD5*$^{-}$)
JC0051	*MAT a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::KanMX3 [YCp22 hht1^{K56R} HHF1-TRP1]* Kind gift of Dr. Alain Verreault	Masumoto et al., 2005 (W303 with *RAD5*$^{-}$)
JC470	*Mat a ura3-1 his3-11,15 leu2-3,112 trp1-1 ade2-1 can1-100 RAD5*$^{+}$	R. Rothstein (W303 with *RAD5*$^{+}$)
JC471	*Mat α ura3-1 his3-11,15 leu2-3,112 trp1-1 ade2-1 can1-100 RAD5*$^{+}$	R. Rothstein (W303 with *RAD5*$^{+}$)
JC1822	JC471 with *RAP1-GFP::LEU2*	Moradi-Fard et al., 2016
JC2494	JC471 with *8HIS-Smt3::TRP1 RAP1-13MYC:HIS*	This study
JC3737	JC470 with *sir4Δ::HIS3*	Moradi-Fard et al., 2016
JC3786	JC470 with *sir2Δ::TRP1*	This study
JC4022	JC470 with *HEH1-13MYC::KanMX6*	Moradi-Fard et al., 2021
JC4311	JC470 with *sir3Δ::HIS3*	This study
JC4509	*MAT a can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::HHT2-hhf2^{K16A}-URA3* Triple backcross of JC4338 x JC471	This study
JC4263	JC471 with *nup49::NUP49-GFP*	Moradi-Fard et al., 2021
JC4881	JC470 with *RAP1-13MYC::HIS3* Cross of JC2494 x JC470	This study
JC5047	JC470 with *SIR4-GFP::KanMX6 HDEL-DsRed::LEU2*	This study
GA1275	JC470 with *SIR4-13MYC::KanMX6*	Perrod et al., 2001
GA1459	*MATa ade2-1 can1-100 his3-11,15::GFP-LacI-HIS3 trp1-1 ura3-1 leu2-3,112 nup49::NUP49-GFP-URA3 TELVI-R::lacO-lexA-TRP1* Kind gift of Dr. Susan Gasser	Hediger et al., 2002
GA1986	*MATa ade2-1 can1-100 his3-11,15::GFP-LacI-HIS3 trp1-1 ura3-1 leu2-3,112 nup49::NUP49-GFP-URA3 TELVIII-L::lacO-lexA-TRP1* Kind gift of Dr. Susan Gasser	Hediger et al., 2002
VZY452	*MAT a sir4Δ::HIS3 hht1-hhf1::NatMX4 hht2-hhf2::HHT2-hhf2^{K16A}-URA3* Cross of JC4509 x JC3737	This study
VZY452	JC470 with *DsRed-HDEL::LEU2*	This study

KM014	*MATa* with *rap1::LEU2 [pRS313-RAP1]*	Yeast Genome Resource Centre* (W303 with *RAD5*$^{-}$)
KM015	*MATa* with *rap1::LEU2 [pRS313-rap1-12]*	Yeast Genome Resource Centre* (W303 with *RAD5*$^{-}$)
KM016	*MATa* with *rap1::LEU2 [pRS313-rap1-17]*	Yeast Genome Resource Centre* (W303 with *RAD5*$^{-}$)
M1360	*Mat a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3* [*pDP378-HHT2,HHF2 CEN6 ARSH4 URA3]* Kind gift of Dr. Michael Weinreic	Hoggard et al., 2018 (W303 with *RAD5*$^{-}$)
M4654	*Mat a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 [pRS414-HHF2-hht2*^{K79A}*-TRP1]* Kind gift of Dr. Michael Weinreic	Hoggard et al., 2018 (W303 with *RAD5*$^{-}$)
M4655	*Mat a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 [pRS414-HHF2-hht2*^{K79E}*-TRP1]* Kind gift of Dr. Michael Weinreic	Hoggard et al., 2018 (W303 with *RAD5*$^{-}$)
M4671	*Mat a hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 [pRS414-HHF2-hht2*^{K79R}*-TRP1]* Kind gift of Dr. Michael Weinreic	Hoggard et al., 2018 (W303 with *RAD5*$^{-}$)
JC4338	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::HHT2-hhf2*^{K16A}*-URA3*	Dharmacon Dai et al., 2008
BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ*	Euroscarf
mga2Δ	BY4741 with *mga2Δ::KanMX4*	Euroscarf
spt23Δ	BY4741 with *spt23Δ::KanMX4*	Euroscarf
Spt23-TAP	BY4741 with *SPT23-TAP::KanMX4*	Euroscarf
VZY397	BY4741 with *DsRed-HDEL::LEU2*	This study
VZY453	BY4741 with *sir4Δ::KanMX4 DsRed-HDEL::LEU2*	This study

*Strain provided by the NBRP, Japan.

Table 2 – Plasmids used in this study

Plasmid	**Description**	**Source**
Ycplac111-Sec63-GFP	GPD promoter, Sec63-GFP (CEN, *LEU2*)	Santos-Rosa et al., 2005
pAG415-ccdB	GPD promoter (CEN, *LEU2*)	Alberti et al., 2007

pAG415-Sir4	GPD promoter, Sir4 (CEN, *LEU2*)	This study
pAG415-EGFP-ccdB	GPD promoter, EGFP (CEN, *LEU2*)	Alberti et al., 2007
pAG415-EGFP-Mga2	GPD promoter, EGFP-Mga2 (CEN, *LEU2*)	This study
pAG415-EGFP-Spt23	GPD promoter, EGFP-Spt23 (CEN, *LEU2*)	This study
pAM41	pRS305-DsRED-HDEL Kind gift of Dr. Scott D. Emr	Manford et al., 2012
pRS426-Nop1-CFP	GPD promoter, Nop1-CFP (2μ, *URA3*)	Moradi-Fard et al., 2021

Table 3 – Primers used in this study

Primer	**Sequence**
SIR4-Gateway-F	GGGG ACA AGT TTG TAC AAA AAA GCA GGC TCT ATG CCA AAT GAC AAT AAG
SIR4-Gateway-R	GGGG AC CAC TTT GTA CAA GAA AGC TGG GTC ATA CGG TTT TAT CTC CTT
MGA2-Gateway-F	GGGG ACA AGT TTG TAC AAA AAA GCA GGC TCT ATG CAG CAG AAC AGT GAG TTC
MGA2-Gateway-R	GGGG AC CAC TTT GTA CAA GAA AGC TGG GTC CTA ACT GAC AAT TAA ATC GTT
SPT23-Gateway-F	GGGG ACA AGT TTG TAC AAA AAA GCA GGC TCT ATG ATG AGT GGC ACA GGA AAC
SPT23-Gateway-R	GGGG AC CAC TTT GTA CAA GAA AGC TGG GTC TTA ATT GAC TCG CAT GTC GTT
RPL9A-qPCR-F	GTCACCAAGGGTTACAAGTACA
RPL9A-qPCR-R	CAATTTCGTCCTTAACGTTGGTG
RPS26B-qPCR-F	TACCCAGAATACGCCTTGCC
RPS26B-qPCR-R	CAAAGCTTTCTTTGCGGCAG
SSB1-qPCR-F	TTCAAGGTGCTATCTTGACC
SSB1-qPCR-R	GGTTGTCAGCACATGTAGTA
RPP1B-qPCR-F	GTCTGGGCTGATGTTTACGC
RPP1B-qPCR-R	GAAACCCATGTCGTCGTCAG
RPS12-qPCR-F	AGTTGGTTGAAGGTTTGGCT
RPS12-qPCR-R	AGTTTCAGCACCCCAGTTCT
RPL8B-qPCR-F	AGCAAGACGCTTCTCCAAAG
RPL8B-qPCR-R	TCTTACACAAAGCTGGTAGGAA
RPS22A-qPCR-F	AGCACGGTTACATTGGTGAA
RPS22A-qPCR-R	CAGAGGTGGTCAAGATGACG

OPI1-qPCR-F	CCAGCGAGCAGTCTATTGTG
OPI1-qPCR-R	CAGTGATGTACTGTGGACGC
INO1-qPCR-F	GATGTAACGCCCACTGTTCA
INO1-qPCR-R	AGTCATGGAGCCGAAGTAGT
IZH1-qPCR-F	TCGGTATCCAAGGGGTTCTT
IZH1-qPCR-R	TGGCTTTCAAGTGGCATACA
IZH2-qPCR-F	CCGGAATGGCAAAGAGACAA
IZH2-qPCR-R	AGTGAAGAACCCGAGAGCAG
IZH3-qPCR-F	GCTGGGTGTATTTGGTGTGT
IZH3-qPCR-R	GATTTGTATGTGACGGGGCT
IZH4-qPCR-F	TCGAACGAGAATGCAGAGAC
IZH4-qPCR-R	GTGGTGGGGAACAAAGGTATC
OLE1-qPCR-F	ACCGCTTTCGTCATTCCAAC
OLE1-qPCR-R	TTGTCACGAGGGGTTCTTCT
SPT23-qPCR-F	CTAGCATAGAGCCTGACGGA
SPT23-qPCR-R	ATCATCCACGGTCATTTGGG
ICT1-qPCR-F	ATGGCGGAAGGATAACAAGC
ICT1-qPCR-R	AGTGGATAGGTGGTGTTGGG
MGA2-qPCR-F	CGAGAACAAAGAAGAGCCGC
MGA2-qPCR-R	TGCCTTATGGTGCCTGCAAT

Growth conditions. Yeast strains were grown in synthetic defined minimal medium (SD) containing 0.67% yeast nitrogen base without amino acids, 2% filtered sterilized glucose and 0.1% tyrosine, 0.1% methionine, 0.1% leucine, 0.1% lysine, 0.1% arginine, 0.1% histidine, 0.02% adenine, and 0.02% uracil to fulfill strain auxotrophies. Additional adenine (0.004%, SD+Ade) was added to prevent vacuolar fluorescence in *ade2* strains used for live microscopy. Yeast cultures were routinely grown at 30 °C with shaking (200 rpm). Standard transformation protocol was used and cells carrying plasmids of interest were selected on selective media (Guthrie and Fink, 1991).

All experiments were conducted in exponential growth phase. Cells were routinely grown overnight in SD+Ade unless otherwise indicated. The absorbance (600-nm filter) of the cultures was monitored with a ThermoScientific™ GENESYS™ 30 Visible Spectrophotometer. Cultures were diluted the following day in fresh media and cells were grown into log phase for ~4 hours. For experiments with edelfosine, the lyophilized drug was dissolved in ethanol as a 20 mM fresh

stock. Log phase cells were standardized to A_{600} ~0.4, then incubated with the vehicle (0.095% ethanol) or 20 μM edelfosine at 30 °C with shaking for the indicated time in each experiment.

For experiments determining sensitivity to oleate, semi-complete media (0.67% yeast nitrogen base without amino acids, 2% filtered sterilized glucose 0.1% tyrosine, 0.1% methionine, 0.1% leucine, 0.1% lysine, 0.1% arginine, 0.1% histidine, 0.02% adenine, 0.02% uracil) was prepared, with glucose replaced by oleic acid in plates containing oleate. For every experiment, fresh oleic acid from a -20°C stock was dissolved in 50% ethanol and 20% Tergitol™ (Sigma, NP40S) then diluted 1:10 into autoclaved warm medium to reach the indicated oleate final concentration.

Fluorescence Microscopy. Cells expressing the indicated fluorescent protein conjugates were grown into log phase as described above, then diluted to A_{600} ~0.4 and treated with 20 μM edelfosine or ethanol for the indicated time periods. Cells were pelleted at the indicated timepoints, resuspended in ~10 μL of cleared media and mounted on agarose pads prepared from the same media in which the cells were cultured. Images were acquired with a Zeiss AxioImager Z2 upright epifluorescence microscope. ZEISS Zen blue imaging software and Zeiss plan Apochromat 100x/1.4 oil immersion objective lens were used for image acquisition. Z-stacks were taken in seventeen 0.24 μm steps and deconvolved using the constrained iterative algorithm available in the Zen 2.3 Pro software. Colibri 7 LED light and 90 High Efficiency filter sets were used for excitation of CFP, GFP, and DsRed. For CFP signal, samples were excited at 423/44 nm and emission was captured at 475 nm, while for GFP signal, samples were excited at 469/38 nm and emission was captured at 509 nm, and for DsRed signal, samples were excited at 555/30 nm and emission was captured at 610 nm. Image analysis was performed manually using FIJI (Schindelin et al., 2012). For microscopy using BODIPY™ 493/503, 1mL aliquots of cells that had been treated with edelfosine or the vehicle for 90 minutes were incubated with 1μL of 5 mM BODIPY in DMSO for 5 minutes at 30 °C. Cells were then washed with 500 μL PBS and BODIPY signal was captured in the GFP channel. All quantitation of phenotypes was done by manual analysis of Z-stacks in FIJI, with the exception of nucleolar volume. For nucleolar volume quantification, three dimensional (X, Y, Z) stacks of yeast cells carrying $Nop1^{CFP}$ were acquired using the CFP channel. After image deconvolution, the 3D Object Counter Volume plug-in of FIJI (Bolte and Cordelières, 2006) was used to measure the volume of the nucleolus (CFP signal) in μm^3.

Chromatin Immunoprecipitation. Cells expressing $Sir4^{MYC}$ or $Rap1^{MYC}$ were grown into log phase as described above, then diluted to A_{600} ~ 0.4 in 50 mL liquid media and treated with 20 μM edelfosine or ethanol for 60 minutes before crosslinking with 1.1% Formaldehyde Solution (Sigma) for 15 minutes followed by quenching with 0.125 M glycine for 5 minutes at room temperature. ChIP of yeast cells was performed as described in (Lee and Keung, 2018). Briefly, cells were digested with 0.5 mg/mL zymolyase at 30 °C until spheroblasts were detected using a light microscope. Spheroblasts were lysed in NP-S buffer [0.5 mM spermidine, 0.075 % NP-40, 10 mM Tris-HCl pH 7.4, 50 mM NaCl, 5 mM MgCl2, 1 mM CaCl2, 1 mM 2-mercaptoethanol, supplemented with protease inhibitor cocktail (Roche), 0.5% (w/v) Sodium Deoxycholate and 1mM PMSF] for 10 minutes on ice. DNA was sheared using a Bioruptor sonicator for four sets of 15 × 30s at amplitude of 50% with 30s shut off intervals and immunoprecipitated using sheep anti-mouse conjugated Dynabeads (Invitrogen) coupled with 1:10 mouse anti MYC antibody (Abcam, 9E10 ab32) for 2 hrs at 4°C. Immunoprecipitates were washed once with NP-S buffer and twice

with wash buffer [100 mM Tris pH 8, 0.5% NP-40, 1 mM EDTA, 500 mM NaCl, 500 mM LiCl, supplemented with 0.5% (w/v) Sodium Deoxycholate, 1mM PMSF and protease inhibitor pellet (Roche)] at 4°C, each for 5 minutes with shaking at 2,200 g. Real-time qPCR reactions were carried out in technical triplicates using SYBR Green with a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Life Technologies Inc.). Ct (cycle threshold) values of Ab-coupled beads and uncoupled beads were used to calculate the fold enrichment of protein at three native sub-telomeres (Tel01L, Tel06R and Tel15L) compared to a control late replicating region on Chromosome V (469104–469177).

RNA-sequencing. Wild-type cells (two W303 replicates, one BY4741 replicate) were grown into log phase as described above, then diluted to A_{600} ~0.4 in 20 mL liquid media and treated with 20 µM edelfosine or ethanol for 60 minutes before flash-freezing**.** RNA was then extracted using the hot acid phenol protocol as previously described (Green and Sambrook, 2021). Briefly, yeast pellets were resuspended in 0.5 mL cold AE buffer (50mM NaOAc pH 5.2, 10mM EDTA) and lysed at 65 ^{o}C by vortexing in 1.5% SDS and acid phenol. RNA and proteins were then separated with chloroform and RNA was precipitated from the supernatant using glycogen and ethanol.

RNA-sequencing and subsequent statistical analysis was performed by the Cumming School of Medicine's Centre for Health Genomics and Informatics, University of Calgary. All samples were found to have a RIN >8 as assessed via Agilent TapeStation assay. Fluorometric assays were used to obtain the concentration in ng/µl. One µg of total RNA input was used for all libraries. NEBNext® Poly(A) mRNA Magnetic Isolation Module (E7490) and NEBNext® Ultra™ II RNA Library Prep Kit for Illumina® (E7770, E7775) were used to prepare the libraries according to the manufacturer's instructions. Final libraries were again assessed via Agilent TapeStation assay, Qubit to obtain the concentration and Kapa qPCR to obtain the exact molarity of Illumina adapter ligated fragments according to the manufacturer's instructions. Sequencing was then performed on an Illumina NextSeq500 system using 150 cycle mid-output 2x75 bp paired end sequencing.

Gene Ontology analysis was performed using the ClueGo plug-in for Cytoscape (Shannon et al., 2003; Bindea et al., 2009) selecting for significantly overrepresented pathways with medium network specificity and GO term fusion enabled. Transcription factor enrichment was analyzed using Yeastract (Monteiro et al., 2020), selecting for transcription factors whose targets were significantly enriched by DNA binding evidence only. Additional analysis of the RNA-sequencing data was performed with the R/Rstudio computational platform (R Core Team, 2021). Volcano plots were made with the ggplot2 package (Wickham, 2016) as a scatter plot of ln(FC) vs -$\log_{10}$(FDR). Venn diagrams were made with the venndir package (jmw86069, 2020). Telomere enrichment was calculated using the hypergeometric distribution function in base R. Principal component analysis, which concluded that genetic background minimally contributed to differences in gene expression and it was therefore sound to average W303 and BY4741 (Figure S4A), was performed using base R functions and scores were plotted with the ggplot2 package.

qPCR. Wild-type or *sir4*Δ cells (W303 background) were grown into log phase as described above, then diluted to A_{600} ~ 0.4 in 50 mL liquid media and treated with 20 µM edelfosine or ethanol for 60 minutes before flash-freezing. RNA was then extracted using the RNeasy Mini Kit (Qiagen) as per manufacturer directions from three biological replicates. Complementary DNA (cDNA) was

prepared using QuantiTect Reverse Transcription Kit (Qiagen) as indicated by the manufacturer. RNA abundance was quantified using real-time qPCR reactions, which were carried out using SYBR Green (Applied Biosystems) with a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Life Technologies Inc.). Ct (cycle threshold) values of target genes were used to calculate the fold enrichment of mRNA abundance in treated cells compared to untreated cells. Primers used are listed in Table 3.

Spot-plating. For growth assays on solid media, cells were grown into log phase as described above and serially diluted 1:10 beginning with A_{600} ~0.5 unless otherwise noted in the figure caption. For experiments determining sensitivity to edelfosine, cells were spotted on SD+Ade plates with 2% glucose and either 0.095% ethanol or 20 µM edelfosine using a bolt replicator and incubated at 30°C for the indicated number of days. For experiments determining sensitivity to oleate, cells were spotted on SD+Tergitol plates with 2% glucose or SD+Tergitol containing 12 mM oleic acid using a bolt replicator and incubated at 30 °C for the indicated number of days. Images shown are representative plates from a minimum of three independent experiments with two technical replicates per experiment.

Subcellular Fractionation. Membrane fractions were prepared as described previously (Marr et al., 2012). Briefly, cells were lysed using glass beads in 0.7 mL of GTE buffer [20% glycerol, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, Complete EDTA-free protease inhibitor mixture (Roche), 1 mM PMSF, 3 µg/mL pepstatin, and 1 mM phosphatase inhibitor mix (Sigma)]. Lysis was done by vortexing samples with glass beads five times for 30 s with 30 s intervals on ice in-between. Beads were washed with 0.5 mL of GTE buffer and the combined lysate was centrifuged at 16,000 × g for 15 min at 4 °C. The supernatant was then centrifuged at 450,000 × g for 15 min at 4 °C in a Beckman fixed angle rotor ultracentrifuge. Membranes were homogenized in GTE buffer using a Dounce homogenizer. Protein concentration was determined using the BCA assay (Thermo Scientific) with bovine serum albumin as a standard. Lysates and fractions (40 µg) were then analyzed by Western blot.

Western Blot. Ten A_{600} units of cells were grown under the indicated conditions, harvested, washed once with water, and flash-frozen before being resuspended in 1 mL cold 20% trichloroacetic acid (TCA) solution. Acid-washed glass beads were added to each sample and cells were disrupted using a Mini BeadBeater (BioSpec Products) two times, 1 min each at 4 °C. The liquid was collected and combined with cold 400 uL of 5% TCA to finalize protein precipitation. Samples were then centrifuged at 3,000 rpm in a pre-cooled microcentrifuge (Eppendorf) for 10 min at 4 °C. Pellets were resuspended in 150 µL of 1x Laemmli buffer [0.2 M Tris–HCl (pH 6.8), 8% SDS, 0.4% bromophenol blue, and 40% glycerol], neutralized with 50 µL of 2M TRIS and then boiled for 5 minutes. Samples were centrifuged again for 10 min at 3,000 rpm, 40 µL of sample was loaded onto each lane and proteins were separated by 8% or 12% resolving gel as indicated, containing trichloroethanol (TCE, Sigma) to visualize proteins (Ladner et al., 2004). Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore) using a Bio-Rad transfer system at 100 V for 1 h or 25 V for 16 hrs, and then stained with Red Ponceau (Sigma) to confirm transfer. For western blot analysis, the following primary antibodies were used: 1:5,000 mouse αMYC (Abcam, 9E10 ab32), 1:1,000 mouse αRap1 (Santa Cruz, sc374297), 1:1,000 mouse αGFP

(Roche, 11814460001), 1:500 mouse αPgk1 (Molecular Probes), 1:1,000 rabbit αH2A (Active Motif, 39235), 1:1,000 rabbit αH2AS129ph (Active Motif, 39271) and 1:2,000 mouse αRad53 (Abcam, ab104232). Horseradish peroxidase conjugated secondary antibodies (Invitrogen, Thermo Fisher) and enhanced chemiluminescence (Amersham, GE Healthcare) were used for detection using an Invitrogen iBright FL1500 Imaging System.

Lipid extraction and neutral lipids analysis. Lipid extracts were prepared as described previously (Zaremberg and McMaster, 2002). Briefly, cells were concentrated by centrifugation, washed twice with water, and resuspended in 1 mL of $CHCl_3/CH_3OH$ (1/1, v/v), then stored overnight at -20 °C. Cells were disrupted for 1 minute at 4 °C in a Mini BeadBeater (BioSpec Products) with acid-washed glass beads. Beads were washed with 1.5 mL of $CHCl_3/CH_3OH$ (2/1, v/v), then 0.5 mL of $CHCl_3$ were added to the combined supernatant followed by 1.5 mL of water to facilitate phase separation. Lipids were dried under nitrogen, resuspended in 30 µL chloroform and 15 µL of this resuspension was loaded for separation on silica gel on TLC aluminum foils (Sigma-Aldrich, 60805-25EA) using a solvent system containing 80:20:1 petroleum ether/diethyl ether/acetic acid for separation of neutral lipids. Standard mix (10 µg/ each lipid) was loaded onto the plate. Standard mix consisted of a mix of 2 mg/mL solution of DAG, ergosterol, oleic acid, TAG, and cholesteryl oleate. Plates were developed with iodine vapors and imaged using a BioRad GelDoc. Images shown are representative of two independent experiments with two technical replicates per experiment.

Statistical analysis. All statistical analysis was performed in GraphPad Prism 9.3.1. The following analyses were conducted depending on the experiment as indicated in each figure legend. A minimum of three independent experiments were analyzed in each case.

For categorical quantification analyzing the difference between only two variables, two-tailed unpaired t-tests were performed. For analyzing the difference between more than two variables, standard one-way ANOVA with Tukey's multiple comparisons post-test was used. For multiple comparisons, multiple unpaired t-tests were performed and corrected for multiple comparisons using the Holm-Šídák post-test (Holm, 1979). Additionally the Welch’s correction for unequal variance (Welch, 1947) was used when appropriate. For analysis of differences between wildtype and mutants in treated and untreated conditions, 2-way ANOVA with Šídák's multiple comparisons test was applied (Sidak, 1967).

For numerical quantification analyzing the difference between only two variables, two-tailed nested t-tests were performed. For analysis of differences between wildtype and mutants in treated and untreated conditions, nested one-way ANOVA with Šídák's multiple comparisons post-test were performed.

Table 1- Transcription factors with DNA binding sites over-represented in upregulated genes Analysis of genes with >2-fold increase in response to edelfosine (119 genes) as determined by Yeastract (Monteiro et al., 2020) using DNA binding evidence only. P-values calculated using the hypergeometric test.

TF	Description	# of genes & (p-value)	References
Sko1	Basic leucine zipper transcription factor of the ATF/CREB family; forms a complex with Tup1p and Cyc8p to both activate and repress transcription; cytosolic and nuclear protein involved in osmotic and oxidative stress responses	44 (4.7E-12)	(Reimand et al., 2010; Niu et al., 2008; Ni et al., 2009)
Hot1	Transcription factor for glycerol biosynthetic genes; required for the transient induction of glycerol biosynthetic genes GPD1 and GPP2 in response to high osmolarity; targets Hog1p to osmostress responsive promoters; has similarity to Msn1p and Gcr1p	13 (1.2E-09)	
Spt23	ER membrane protein involved in regulation of OLE1 transcription; inactive ER form dimerizes and one subunit is then activated by ubiquitin/proteasome-dependent processing followed by nuclear targeting; SPT23 has a paralog, MGA2, that arose from the whole genome duplication	58 (1.3E-08)	(Auld et al., 2006; Reimand et al., 2010)
Yap6	Basic leucine zipper (bZIP) transcription factor; physically interacts with the Tup1-Cyc8 complex and recruits Tup1p to its targets; overexpression increases sodium and lithium tolerance; computational analysis suggests a role in regulation of expression of genes involved in carbohydrate metabolism; YAP6 has a paralog, CIN5, that arose from the whole genome duplication	38 (6.22E-08)	(Lee et al., 2002; Harbison et al., 2004; Ni et al., 2009; Reimand et al., 2010)
Cad1	AP-1-like basic leucine zipper (bZIP) transcriptional activator; involved in stress responses, iron metabolism, and pleiotropic drug resistance; controls a set of genes involved in stabilizing proteins; binds consensus sequence TTACTAA; CAD1 has a	27 (1.01E-07)	(Mazzola et al., 2015; Reimand et al., 2010; Haugen et al., 2004; Lee et al., 2002; Azevedo et al., 2007)

	paralog, YAP1, that arose from the whole genome duplication		
Adr1	Carbon source-responsive zinc-finger transcription factor; required for transcription of the glucose-repressed gene ADH2, of peroxisomal protein genes, and of genes required for ethanol, glycerol, and fatty acid utilization	31 (8.55E-07)	(Chua et al., 2006; Reimand et al., 2010; Karpichev et al., 2008)

Table 2- Transcription factors with DNA binding sites over-represented in downregulated genes. Analysis of genes with >2-fold decrease in response to edelfosine (115 genes) as determined by Yeastract (Monteiro et al., 2020) using DNA binding evidence only. P-values calculated using the hypergeometric test.

TF	Description	# of genes & (p-value)	Reference
Fkh1	Forkhead family transcription factor with a minor role in the expression of G2/M phase genes; negatively regulates transcriptional elongation; positive role in chromatin silencing at HML and HMR; regulates donor preference during switching	80 (0)	(Pondugula et al., 2009; Knott et al., 2012; Ostrow et al., 2014; Reimand et al., 2010; Workman et al., 2006; Harbison et al., 2004; Lee et al., 2002)
Ifh1	Coactivator that regulates transcription of ribosomal protein (RP) genes; recruited to RP gene promoters during optimal growth conditions via Fhl1p; subunit of CURI, a complex that coordinates RP production and pre-rRNA processing	42 (0)	(Rudra et al., 2005; Schawalder et al., 2004; Lavoie et al., 2010; Cai et al., 2013; Venters and Pugh, 2009; Wade et al., 2004; Reja et al., 2015)
Spt23	ER membrane protein involved in regulation of OLE1 transcription; inactive ER form dimerizes and one subunit is then activated by ubiquitin/proteasome-dependent processing followed by nuclear targeting; SPT23 has a paralog, MGA2, that arose from the whole genome duplication	62 (6.6E-14)	(Auld et al., 2006; Reimand et al., 2010)
Fhl1	Regulator of ribosomal protein transcription; has forkhead associated domain that binds phosphorylated proteins; also has forkhead DNA-binding domain but does not bind DNA in vitro; suppresses RNA pol III and splicing factor prp4 mutants	53 (1.11E-07)	(Rudra et al., 2005; Lee et al., 2002; Tai et al., 2017; Harbison et al., 2004; Kasahara et al., 2007; Lavoie et al., 2010)

Yap6	Basic leucine zipper (bZIP) transcription factor; physically interacts with the Tup1-Cyc8 complex and recruits Tup1p to its targets; overexpression increases sodium and lithium tolerance; computational analysis suggests a role in regulation of expression of genes involved in carbohydrate metabolism; YAP6 has a paralog, CIN5, that arose from the whole genome duplication	33 (6.0E-07)	(Lee et al., 2002; Harbison et al., 2004; Ni et al., 2009; Reimand et al., 2010)
Fkh2	Forkhead family transcription factor with a major role in the expression of G2/M phase genes; positively regulates transcriptional elongation; negative role in chromatin silencing at HML and HMR; substrate of the Cdc28p/Clb5p kinase	53 (7.82E-07)	(Pondugula et al., 2009; Harbison et al., 2004; Ostrow et al., 2014; Reimand et al., 2010; Linke et al., 2017; Ghavidel et al., 2018; Gefeng et al., 2000; Simon et al., 2001; Shapira et al., 2004; Workman et al., 2006)
Rap1	DNA-binding protein involved in either activation or repression of transcription, depending on binding site context; also binds telomere sequences and plays a role in telomeric position effect (silencing) and telomere structure	59 (9.6E-05)	(Chua et al., 2006; Reimand et al., 2010; Lavoie et al., 2010; Workman et al., 2006; Kasahara et al., 2007; Venters and Pugh, 2009; Lieb et al., 2001; De Sanctis et al., 2002; Buchman et al., 1988b; Yarragudi et al., 2007; Lee et al., 2002; Harbison et al., 2004; Hall et al., 2006; Reja et al., 2015)
Ndt80	Meiosis-specific transcription factor required for exit from pachytene and for full meiotic recombination; activates middle sporulation genes; competes with Sum1p for binding to promoters containing middle sporulation elements (MSE)	49 (1.4E-04)	(Nocedal et al., 2017; Reimand et al., 2010; Chu and Herskowitz, 1998)

Figure legends:

Figure 1 – The metabolically stable lysophosphatidylcholine analogue edelfosine alters nuclear envelope morphology and nuclear architecture. (**A**) Representative images of wild-type cells expressing the ER marker Sec63^{GFP} from a centromeric plasmid under the constitutive GPD promoter. Cells were imaged using live fluorescence microscopy after growth in SD-Leu+Ade and 90 minutes in the presence of edelfosine (EDLF) or the vehicle (Ctrl). Scale bar represents 2 µm. Quantification of cells displaying abnormal nuclear membrane morphology is shown beside the microscopy images. Circles represent the percentage of cells displaying abnormal nuclear morphology in each experiment (two-sided Fisher's exact test gave a p-value < 0.0001 for each experiment (n=100 cells per treatment)). The bar represents the mean of the three experiments ± SD. ** indicates a p-value < 0.01 as determined by two-tailed unpaired t-test (N=3). (**B**) Representative images of wild-type cells expressing the Nop1^{CFP} nucleolar marker expressed from a 2µ plasmid under the constitutive GPD promoter in cells with Nup49^{GFP} (nuclear envelope marker) endogenously tagged. Cells were imaged using live fluorescence microscopy after growth in SD-Ura+Ade and 90 minutes in the presence of edelfosine (EDLF) or the vehicle (Ctrl). Scale bar represents 2 µm. The distribution of values from the quantification of nucleolar volume of cells (described in Materials and Methods) is shown beside the microscopy images. Values cumulative from three independent experiments (n = 163 Ctrl, 190 EDLF) are shown as small circles, with the means of each experiment shown as large circles. Bars represent the mean with 95% confidence interval from the pooled values. **** indicates a p-value < 0.0001 as determined by two-tailed nested t test (N=3).

Figure 2 – Altered nuclear envelope shape disrupts telomere clustering but not tethering. (**A**) Schematic showing the Rabl conformation of yeast nuclei, where centromeres, telomeres and the nucleolus are tethered to the NE, and telomeres are clustered into 3-5 foci per cell that can be visualized by Rap1. (**B**) Schematic created with BioRender.com explaining the GFP constructs allowing for analysis of telomere tethering. LacO repeats (6-10 kb) are incorporated into either Tel06R or Tel08L which are then bound by lacI^{GFP} conjugates. The large amount of lacO sites produces a bright focus at the location of the tagged telomere, which stands out against the nuclear membrane marked by Nup49^{GFP}. (**C**) Representative images of cells expressing the Tel08L constructs described in (B) that were imaged using live fluorescence microscopy after 90 minutes in edelfosine (EDLF) or the vehicle (Ctrl). Scale bar represents 2 µm. Quantification of cells showing the telomere at the periphery for both Tel06R and Tel08L is shown beside the microscopy images. Circles represent the percentage of cells displaying telomere localization at the NE in each experiment [two-sided Fisher's exact test gave the p-values 0.2545, 0.8884 and 0.5461 for each Tel06R experiment ($n \geq 80$ cells per treatment), and the p-values 0.0710, 0.1873, 0.3210, 0.6050, and 0.7854 for each Tel08L experiment ($n \geq 45$ cells per treatment)]. The bar represents the mean of the experiments ± SD. Differences between control and edelfosine treatments were found to be non-significant by unpaired t tests with Holm-Šídák correction for multiple comparisons (N=3 for Tel06R, N=5 for Tel08L). (**D**) Representative images of wild-type cells expressing Rap1^{GFP} that were imaged by live fluorescence microscopy after 90 minutes in edelfosine or the vehicle. Scale bar represents 2 µm. The distribution of values from the quantification of the number of Rap1 foci per cell is shown beside the microscopy images. Values cumulative from three independent experiments (n=180 per treatment) are shown as small circles, with the means of each experiment shown as large circles. Bars represent the mean with 95% confidence interval from the pooled values. * indicates a p-value < 0.05 as determined by two-tailed nested t test (N=3). (**E**) ChIP-

qPCR of Rap1^{MYC} after 60 minutes in edelfosine or control. The fold enrichment at three native sub-telomeres (Tel01L, Tel06R and Tel15L) is shown, normalized to a late replicating region on Chromosome V (469104–469177). Bars represent mean ± SD for four independent experiments while circles represent individual experiments. Differences between control and edelfosine treatments were found to be non-significant by unpaired t tests with Holm-Šídák correction for multiple comparisons and Welch's correction for unequal variance (N=4). (**F**) Western blot of endogenous Rap1 after 60 minutes with edelfosine or control. Bottom panel shows protein loading by red ponceau.

Figure 3 – The SIR complex is susceptible to lipid alterations at the NE. Growth on synthetic solid media containing 20 µM edelfosine (EDLF) or vehicle (Ctrl) at 30°C for (**A**) SIR complex mutants (two days of growth), (**B**) histone acetylation mutants (three days of growth), and (**C**) the H4^{K16A} *sir4*Δ double mutant (two days of growth) (**D**) Western blot of Sir4^{MYC} after 60 minutes with edelfosine or control. Bottom panel shows protein loading by TCE. (**E**) ChIP-qPCR of Sir4^{MYC} after 60 minutes in edelfosine or control. The fold enrichment at three native subtelomeres (Tel01L, Tel06R and Tel15L) is shown, normalized to a late replicating region on Chromosome V (469104–469177). Bars represent mean ± SD for four independent experiments while circles represent individual experiments. * indicates p-value <0.05; ** indicates p-value <0.01 as determined by unpaired t tests with Holm-Šídák correction for multiple comparisons. (**F**) Representative images of cells expressing Sir4^{GFP} and the ER marker ^{DsRed}HDEL that were visualized by live fluorescence microscopy after 90 minutes with edelfosine (EDLF) or the vehicle (Ctrl). Scale bar represents 2 µm. The distribution of values from the quantification of the number of Sir4 foci per cell is shown beside the microscopy images. Values cumulative from three independent experiments (n=180 cells per treatment) are shown as small circles, with the means of each experiment shown as large circles. Bars represent the mean with 95% confidence interval from the pooled values. * indicates a p-value < 0.05 as determined by two-tailed nested t test (N=3).

Figure 4 – Ribosomal protein targets of Rap1 are repressed in edelfosine. (**A**) Volcano plot of genes downregulated in response to edelfosine as identified by the RNA-Seq transcriptome analysis. Targets of Rap1 are coloured red. Genes confirmed by qPCR are labeled. (**B**) Venn diagram showing the overlap between genes strongly downregulated in edelfosine (< -2 ln(FC)) and genes known to be bound by Rap1 (Lieb et al., 2001), including ribosomal protein genes. (**C**) qPCR of genes identified in (A) show strong agreement with the RNA-seq values. Bars represent mean ± SD for three independent experiments while circles represent individual experiments. (**D**) Growth analysis of Rap1 mutants on defined solid medium containing 20µM edelfosine or vehicle after two days of growth. The first dilution of *rap1-17* cells was A_{600} ~ 1, while the other two strains were A_{600} ~ 0.1, which was experimentally determined to equilibrate strain growth on control plates.

Figure 5 – Lipid circuits activated in response to edelfosine. (**A**) Lipid transport and metabolic pathway map highlighting upregulated (green, Spt23/Mga2 circuit) and downregulated genes (red, Opi1/Ino2/4 circuit) detected by RNA-seq. Color code reflects the fold change as indicated in the figure. (**B**) Volcano plot of transcripts changed in response to edelfosine as identified by the RNA-Seq transcriptome analysis. Targets of Opi1/Ino2/4 are coloured red. Targets of Spt23 are coloured green. Genes confirmed by qPCR (inset) are labeled. For the inset, bars represent mean ± SD for three independent experiments while circles represent individual experiments.

Figure 6 – Spt23 and Mga2 sense changes in the membrane lipid environment induced by edelfosine. (**A**) Schematic created with BioRender.com, adapted from (Ballweg et al., 2020) illustrating the cleavage and translocation of the N-terminal domain of Mga2 and Spt23 in response to changes in membrane environment. (**B**) Representative images of ^{GFP}Spt23 expressed from a centromeric plasmid under the constitutive GPD promoter in *spt23*Δ cells or (**C**) ^{GFP}Mga2 expressed from a centromeric plasmid under the constitutive GPD promoter in *mga2*Δ cells were imaged after growth in SD-Leu+Ade and 30 minutes in the presence of edelfosine (EDLF), the vehicle (Ctrl), or 0.1% methyl methane sulfonate (MMS). Scale bars represent 2 µm. Quantification of cells displaying nucleoplasmic signal is shown beside the microscopy images. Circles represent the percentage of cells displaying nucleoplasmic signal in each experiment [Chi-square test gave a p-value < 0.0001 for each experiment (n ≥ 65 cells per treatment)]. The bar represents the mean of the three experiments ± SD. ** indicates a p-value < 0.01 and **** indicates a p-value < 0.0001 as determined by standard one-way ANOVA with Tukey's multiple comparisons test (N=3). Schematics illustrate what was classified as nucleoplasmic signal for each construct. Western blot of cells expressing ^{GFP}Spt23 (**C**) or ^{GFP}Mga2 (**D**) in wildtype (W303) cells after 60 minutes with edelfosine or control. Bottom panels show protein loading visualized by TCE. P indicates precursor species (~120kDa + EGFP), while N indicates nucleoplasmic species (~90kDa + EGFP).

Figure 7 – NE deformation and Spt23/Mga2 activation are independent of Sir4. (**A**) Representative images of wild-type and *sir4*Δ cells expressing the ER marker Sec63^{GFP} from a centromeric plasmid under the constitutive GPD promoter. Cells were imaged using live fluorescence microscopy after growth in SD-Leu+Ade and 90 minutes in the presence of edelfosine (EDLF) or control (Ctrl). Scale bar represents 2 µm. Quantification of non-round nuclei is shown beside the microscopy images. Representative images of ^{GFP}Spt23 (**B**) or ^{GFP}Mga2 (**C**) expressed from a centromeric plasmid under the constitutive GPD promoter in wild type or *sir4*Δ cells imaged after growth in SD-Leu+Ade and 30 minutes in the presence of edelfosine or control. Scale bars represent 2 µm. Quantification of nucleoplasmic signal is shown beside microscopy images.
For all microscopy quantifications (**A, B, C**), circles represent the percentage of cells displaying the indicated phenotype in each experiment (n=100 cells per treatment), while the bar represents the mean of all independent experiments ± SD. Differences between wild-type and *sir4*Δ were found to be non-significant in both control and edelfosine treatments as determined by 2-way ANOVA with Šídák's multiple comparisons test (N=4 for NE, N=3 for Spt23 and Mga2). (**D**) qPCR of Spt23 and Opi1 targets in wild-type and *sir4*Δ cells treated for 60 minutes with edelfosine (EDLF) or vehicle (Ctrl) expressed as ln(EDLF/Ctrl). * indicates p-value <0.05 as determined by unpaired t tests with Holm-Šídák correction for multiple comparisons.

Figure 8 – Lipotoxicity response in SIR mutants. (**A**) Wild type or *sir4*Δ cells were treated with edelfosine (EDLF) or the vehicle (Ctrl) for 60 minutes and lipid extractions were performed as described in Materials and Methods. Neutral lipids were separated using thin layer chromatography with a solvent system composed of 80:20:1 petroleum ether/diethyl ether/acetic acid. Diacylglycerol (DAG); ergosterol (Erg); oleic acid (OA); triacylglycerol (TAG) and sterol esters (SE). (**B**) Growth analysis of *SIR* and H4^{K16} mutants on plates containing 12 mM oleic acid or vehicle (2% Tergitol, 5% ethanol) and incubated at 30 °C for three days before imaging. (**C**) Representative images of wildtype or *sir4*Δ cells expressing ^{DsRed}HDEL treated with edelfosine

(EDLF) or the vehicle (Ctrl) for 90 minutes and stained with BODIPY™ to visualize lipid droplets (LDs) as described in Materials and Methods. Scale bar represents 2 µm. (**D**) The distribution of values from the quantification of the average number of LDs per cell. Values cumulative from three independent experiments ($n \geq 145$ cells per treatment) are shown as small circles, with the means of each experiment shown as large circles. Bars represent the mean with 95% confidence interval from the pooled values. * indicates a p-value < 0.05 and ** indicates a p-values < 0.01 as determined by nested one-way ANOVA with Šídák's multiple comparisons test (N=3). (**E**) Quantification of cells displaying nuclear lipid droplets (nLDs). Circles represent the percentage of cells displaying nLDs in each experiment ($n \geq 45$ cells per treatment)). The bar represents the mean of the three experiments ± SD. * indicates a p-value < 0.05 as determined by 2-way ANOVA with Šídák's multiple comparisons test (N=3).

Supplementary Figure 1- Edelfosine does not elicit a DNA damage response. (**A**) Anti-Rad53 western blot of whole cell lysate from cells treated with edelfosine, the vehicle or 0.01% MMS for 60 minutes. Bottom panel shows protein loading by Red Ponceau. (**B**) Anti-gammaH2A and anti-H2A blot of whole cell lysate from cells treated with edelfosine, the vehicle or 0.01% MMS for 60 minutes.

Supplementary Figure 2 – Telomere interactions with the nuclear membrane are preserved in edelfosine. (**A**) Representative cells expressing the Tel06R constructs described in Figure 2B were imaged using live fluorescence microscopy after 90 minutes in edelfosine (EDLF) or the vehicle (Ctrl). Scale bar represents 2 µm. (**B**) Membrane and soluble fractions were collected from cells treated with 20 µM edelfosine or vehicle for 60 minutes as described in Materials and Methods, then blotted for endogenous Rap1 and Pgk1 levels.

Supplementary Figure 3 – Only mutation of lysine 16 of histone H4 confers sensitivity to edelfosine. Growth on synthetic solid medium containing 20 µM edelfosine (EDLF) or vehicle (Ctrl) for mutants of (**A**) lysine 16 of histone H4 (**B**) lysine 56 of histone H3 and (**C**) lysine 79 of histone H3 after two (top panels) and three (bottom panels) days of growth. (**D)** Growth on synthetic solid medium containing 20 µM edelfosine or vehicle of cells expressing GFP tagged Sir4 after two days of growth. (**E**) Growth on synthetic solid SD-Leu+Ade plates containing 20 µM edelfosine or vehicle of *sir4*Δ cells expressing Sir4 from a plasmid or the empty vector after three days of growth.

Supplementary Figure 4 – Genetic background and gene ontology analysis of RNA-sequencing hits. (**A**) Scatter plots of the first three principal component analysis scores for the transcriptional landscape of each sample submitted for RNA-sequencing. W303 samples are represented as circles while BY4741 samples are represented as triangles. Large circles indicate the mean PC score for each treatment. (**B**) Molecular function GO terms enriched in genes downregulated in edelfosine more than ln(1.5-fold). (**C**) Molecular function GO terms enriched in genes upregulated in edelfosine more than ln(1.5-fold). (**D**) Legend demonstrating size and colour correlation with number of genes per term and significance of enrichment, respectively.

Supplementary Figure 5 – ^{GFP}Spt23 colocalizes with the ER and translocates to the nucleus in response to edelfosine. Representative images of wildtype cells expressing ^{GFP}Spt23 from a centromeric plasmid under the constitutive GPD promoter and ^{DsRed}HDEL that were imaged after

growth in SD-Leu+Ade and 60 minutes in the presence of edelfosine (EDLF), the vehicle (Ctrl), or 0.1% methyl methane sulfonate (MMS).

Acknowledgments

The authors would like to thank CM Unger and G Bertolesi for their helpful discussions, and S. Gasser for sharing microarray data of internal SIR targets. This work was supported by a Discovery Grant and a Discovery Accelerator Supplement from the Natural Sciences and Engineering Research Council (NSERC) to VZ NSERC 303585 and by operating grants from CIHR MOP-82736; MOP-137062 and NSERC 418122 awarded to JAC. MLSP is funded by a doctoral fellowship from NSERC and Alberta Innovates.

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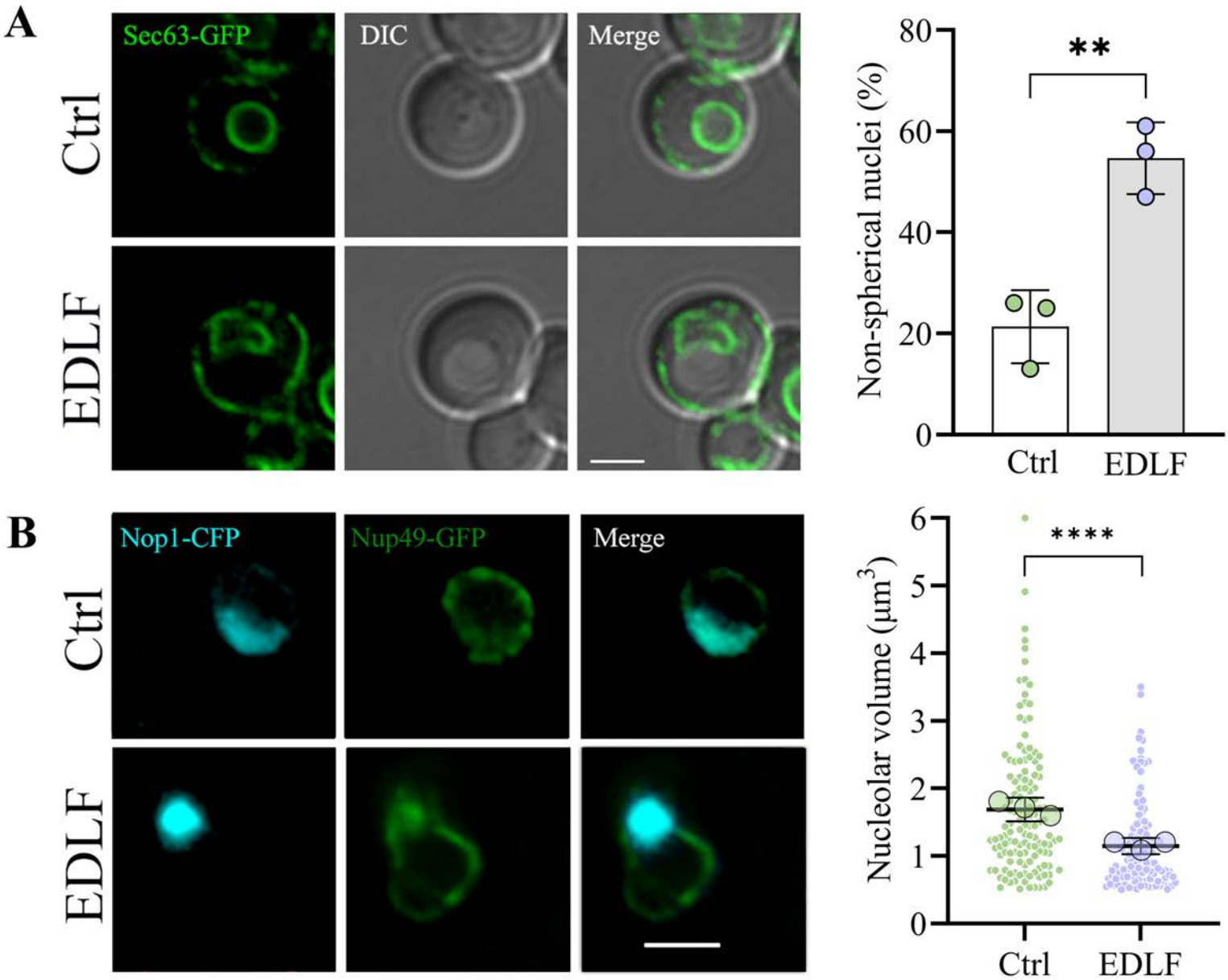
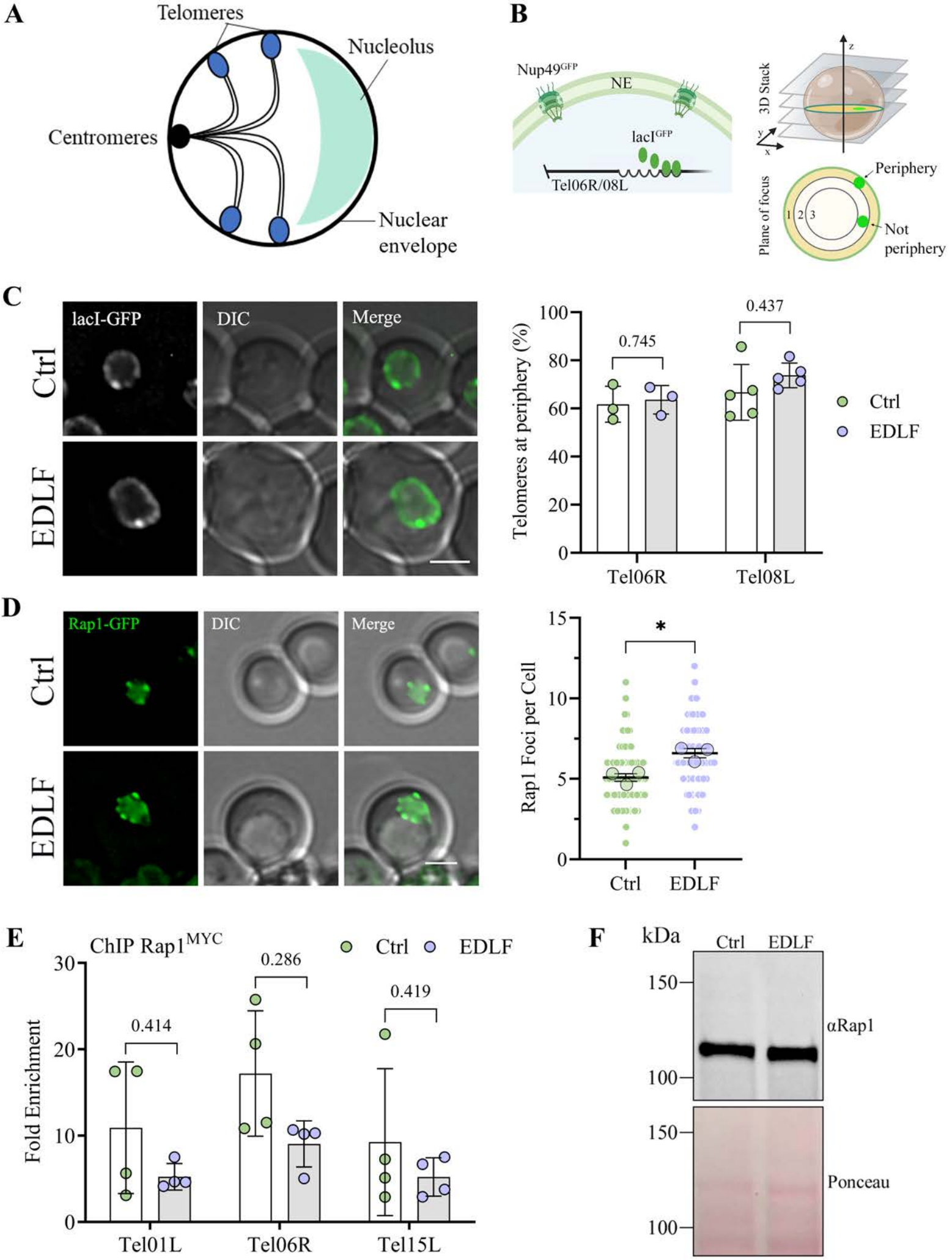
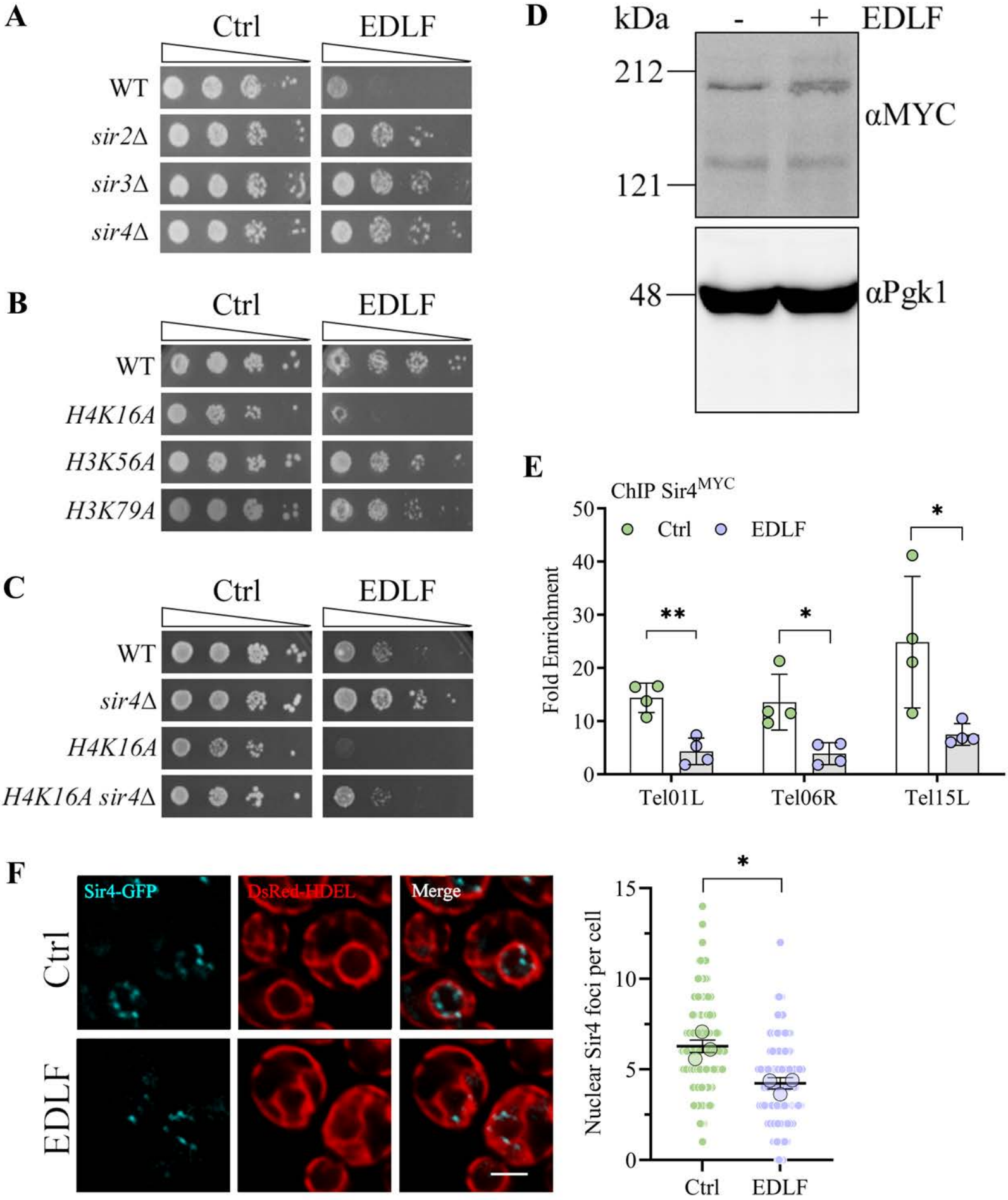
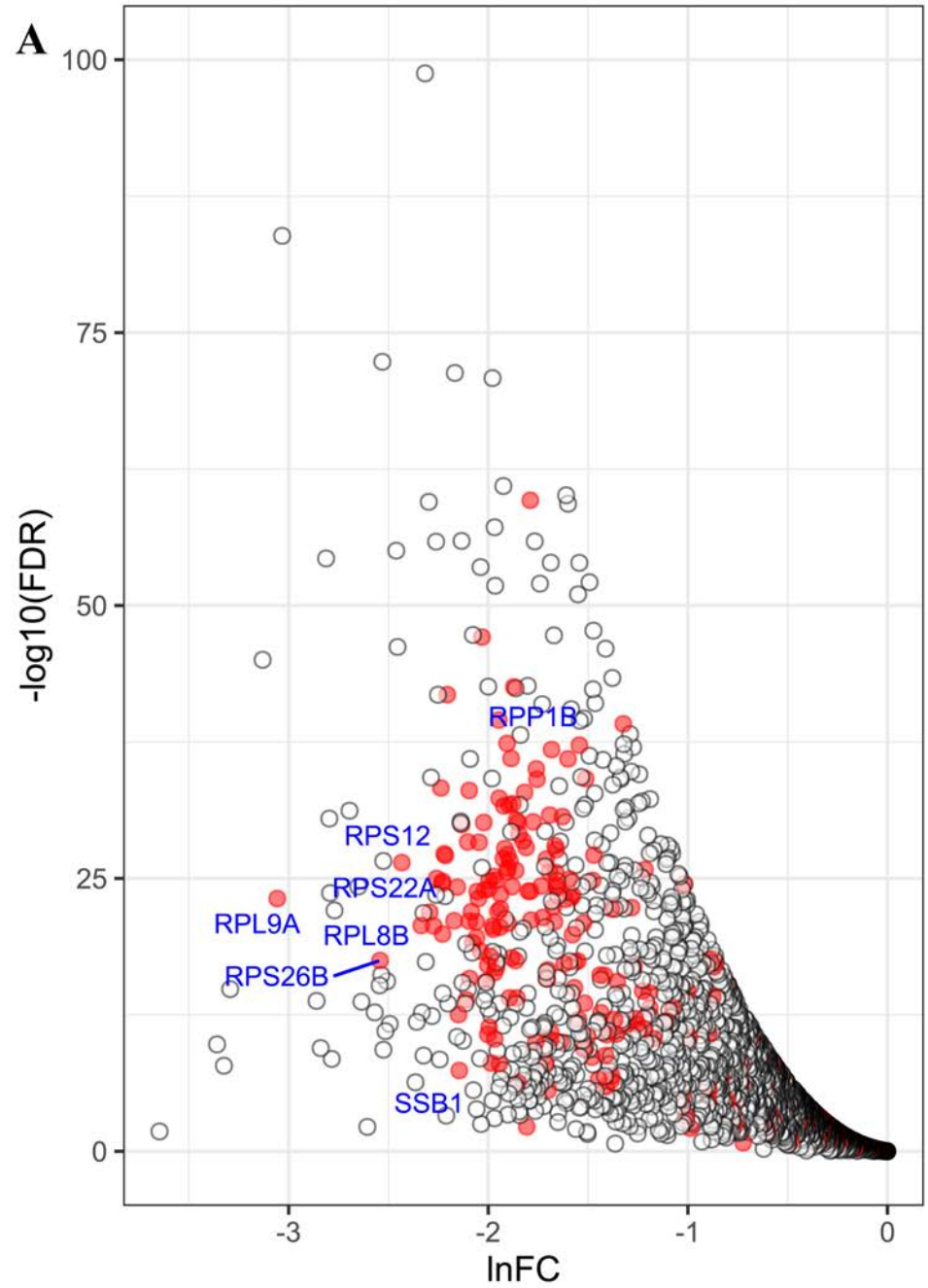
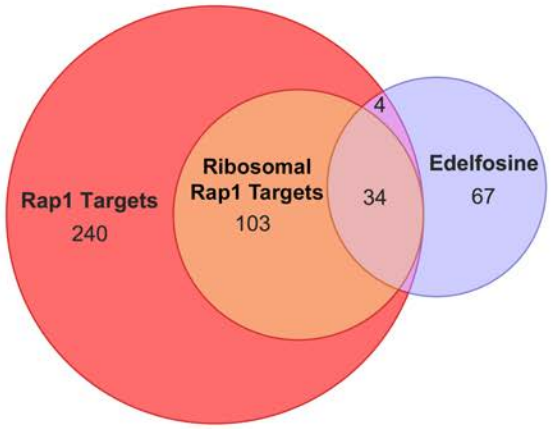
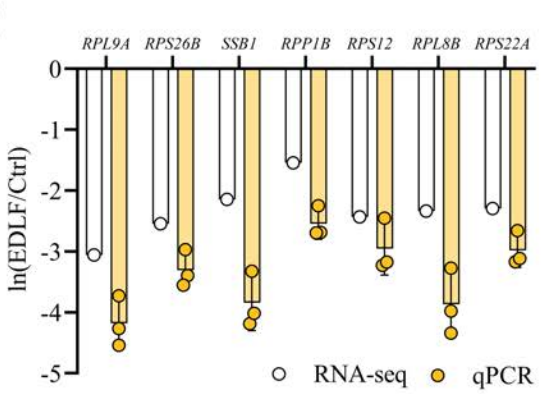

A
Sec63-GFP
DIC
Merge
Ctrl
EDLF
Non-spherical nuclei (%)
80
60
40
20
0
**
Ctrl
EDLF
B
Nop1-CFP
Nup49-GFP
Merge
Ctrl
EDLF
Nucleolar volume (μm3)
6
5
4
3
2
1
0
****
Ctrl
EDLF

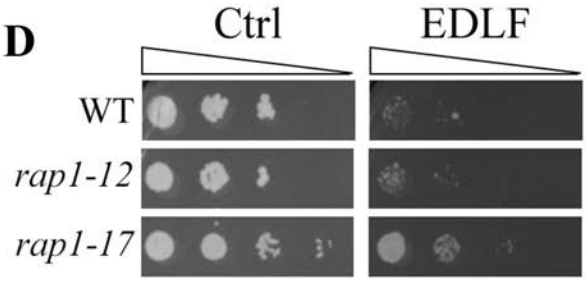

A
Telomeres
Nucleolus
Centromeres
Nuclear envelope
B
Nup49GFP
NE
lacIGFP
Tel06R/08L
3D Stack
z
y
x
Plane of focus
Periphery
Not periphery
1 2 3
C
lacI-GFP
DIC
Merge
Ctrl
EDLF
Telomeres at periphery (%)
0.745
0.437
Tel06R
Tel08L
Ctrl
EDLF
D
Rap1-GFP
DIC
Merge
Ctrl
EDLF
Rap1 Foci per Cell
*
Ctrl
EDLF
E
ChIP Rap1MYC
Ctrl
EDLF
Fold Enrichment
0.414
0.286
0.419
Tel01L
Tel06R
Tel15L
F
kDa
Ctrl
EDLF
150
100
αRap1
150
100
Ponceau

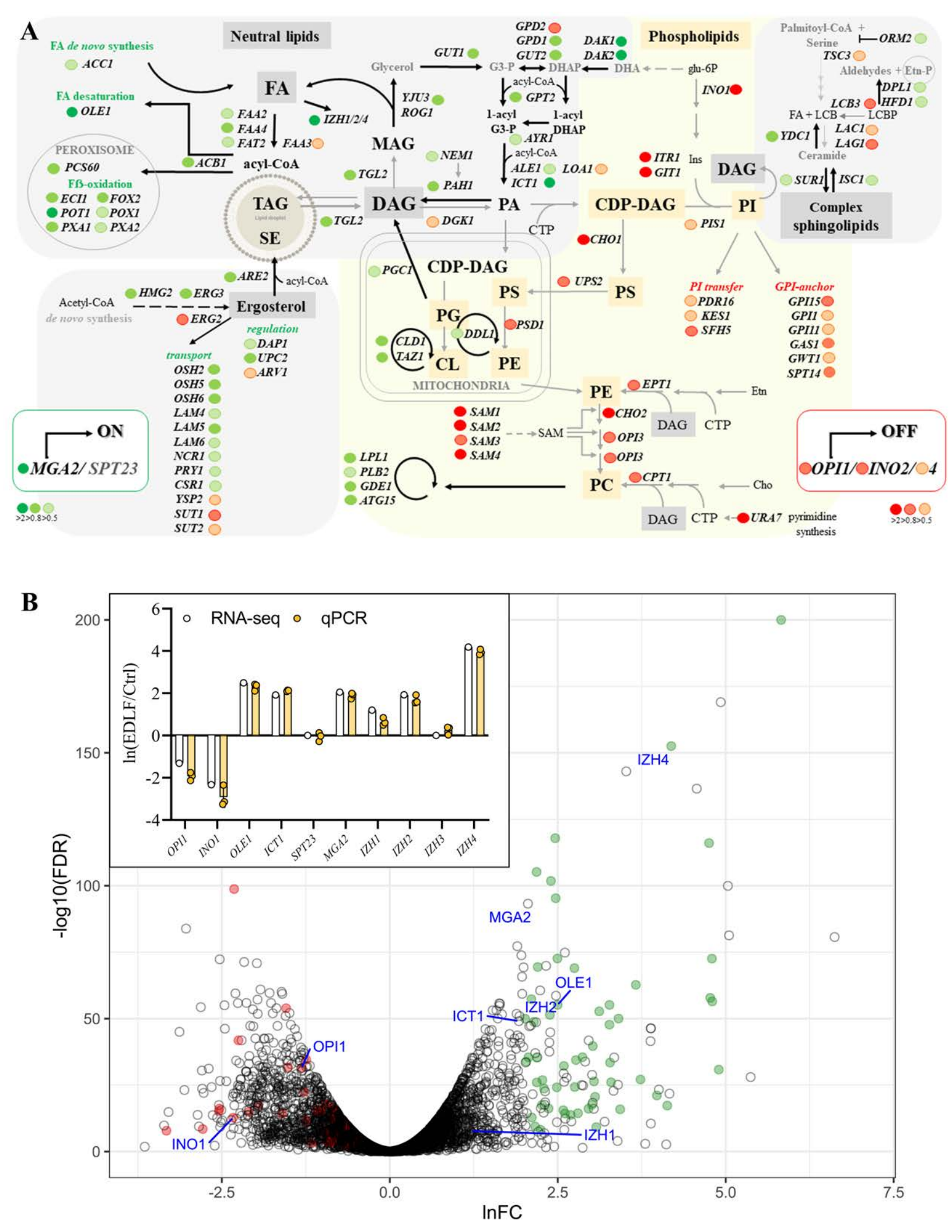

A
Ctrl
EDLF
WT
sir2Δ
sir3Δ
sir4Δ
B
Ctrl
EDLF
WT
H4K16A
H3K56A
H3K79A
C
Ctrl
EDLF
WT
sir4Δ
H4K16A
H4K16A sir4Δ
D
kDa
-
+
EDLF
212
121
αMYC
48
αPgk1
E
ChIP Sir4MYC
Ctrl
EDLF
Fold Enrichment
50
40
30
20
10
0
**
*
*
Tel01L
Tel06R
Tel15L
F
Sir4-GFP
DsRed-HDEL
Merge
Ctrl
EDLF
Nuclear Sir4 foci per cell
15
10
5
0
*
Ctrl
EDLF

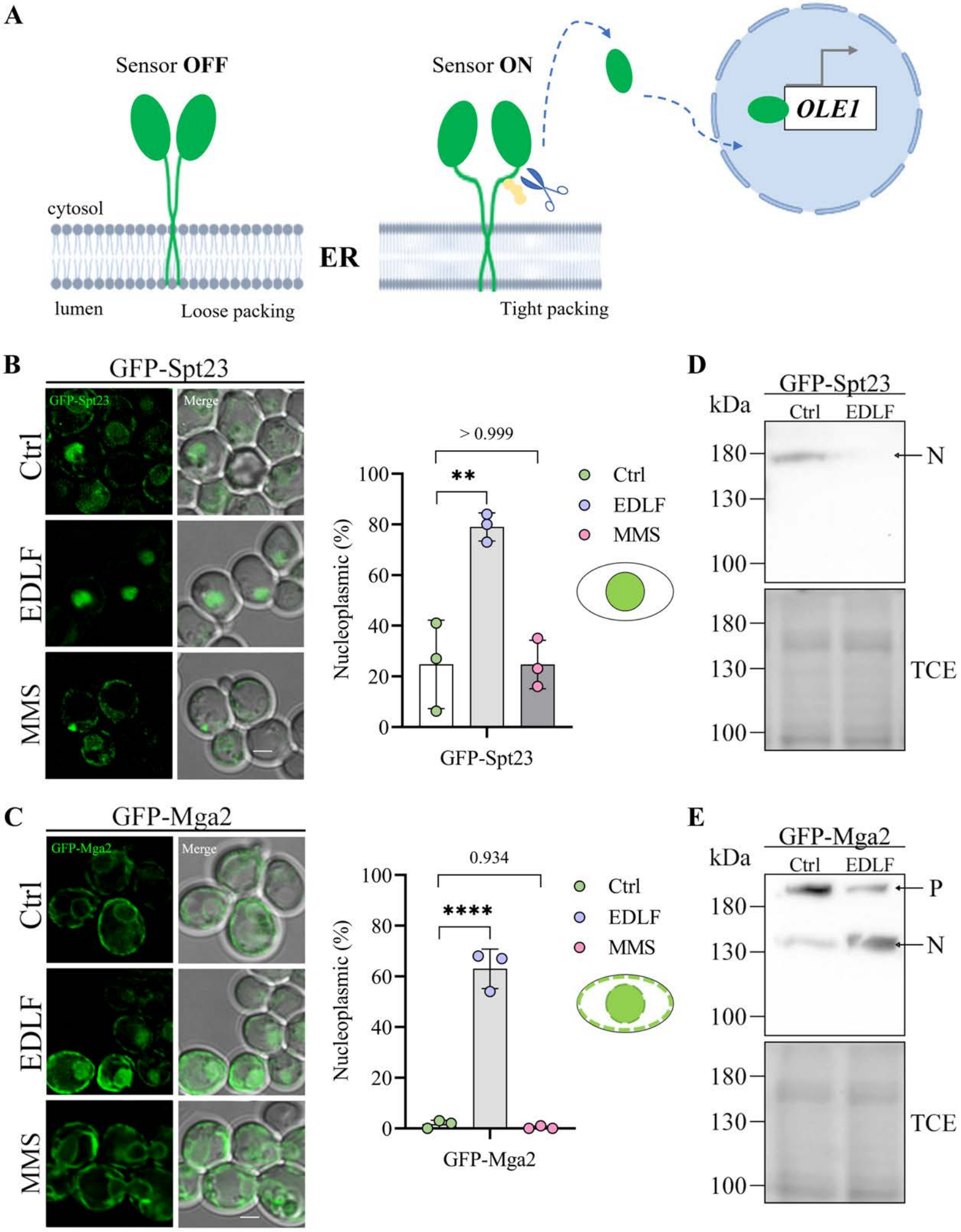

A
-log10(FDR)
lnFC
RPP1B
RPS12
RPS22A
RPL9A
RPL8B
RPS26B
SSB1

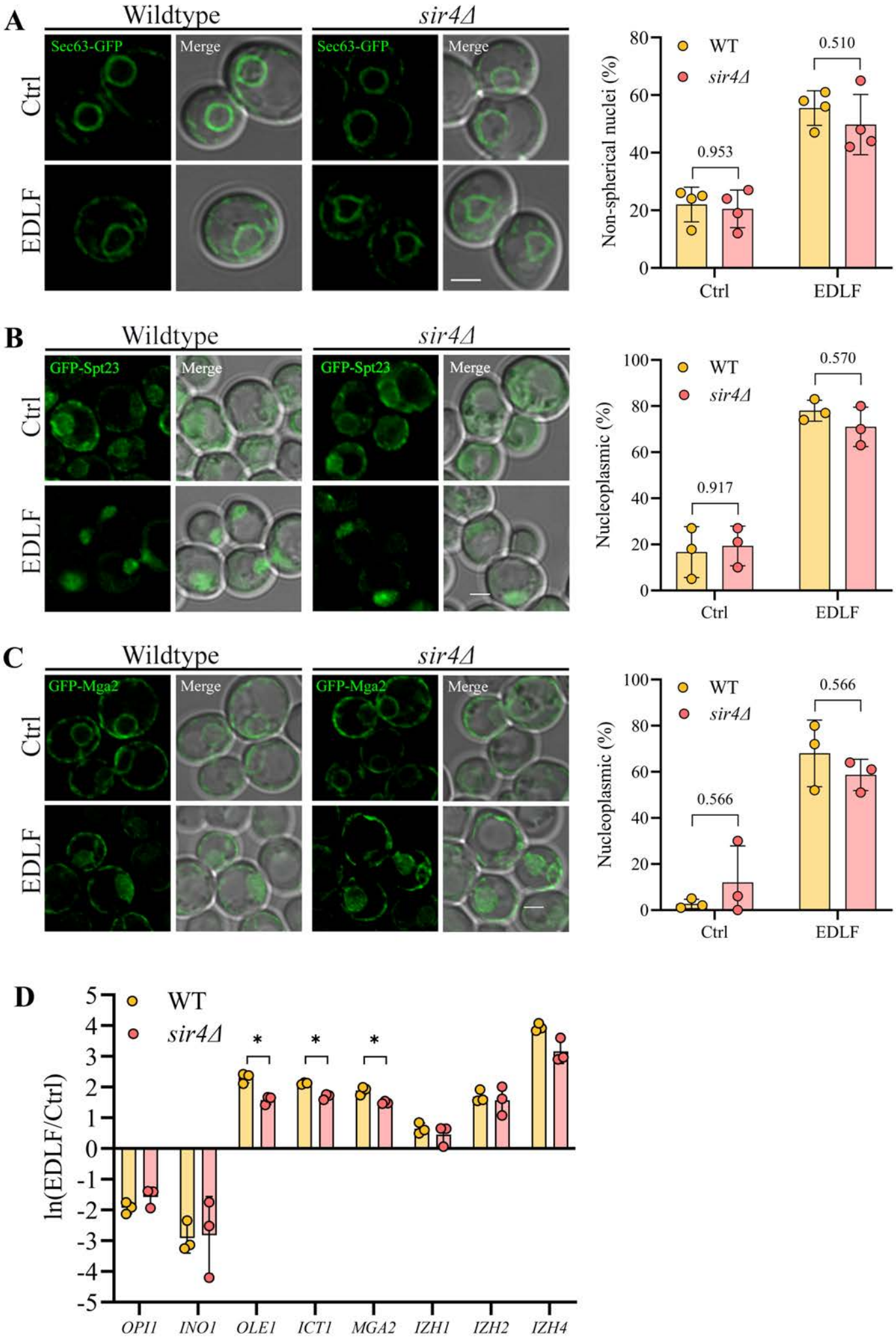

B
Rap1 Targets
240
Ribosomal
Rap1 Targets
103
4
34
Edelfosine
67

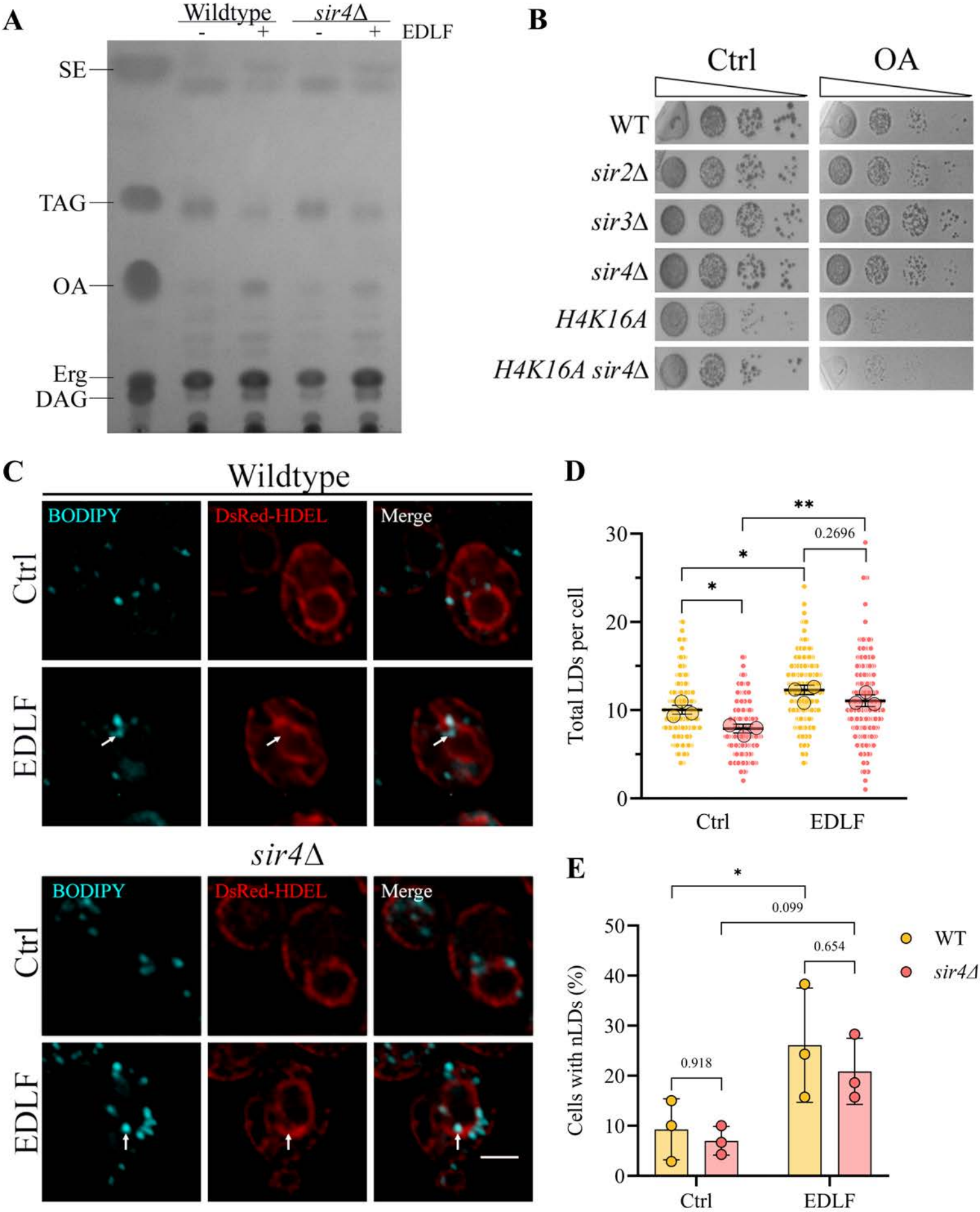

C
RPL9A
RPS26B
SSB1
RPP1B
RPS12
RPL8B
RPS22A
ln(EDLF/Ctrl)
RNA-seq
qPCR

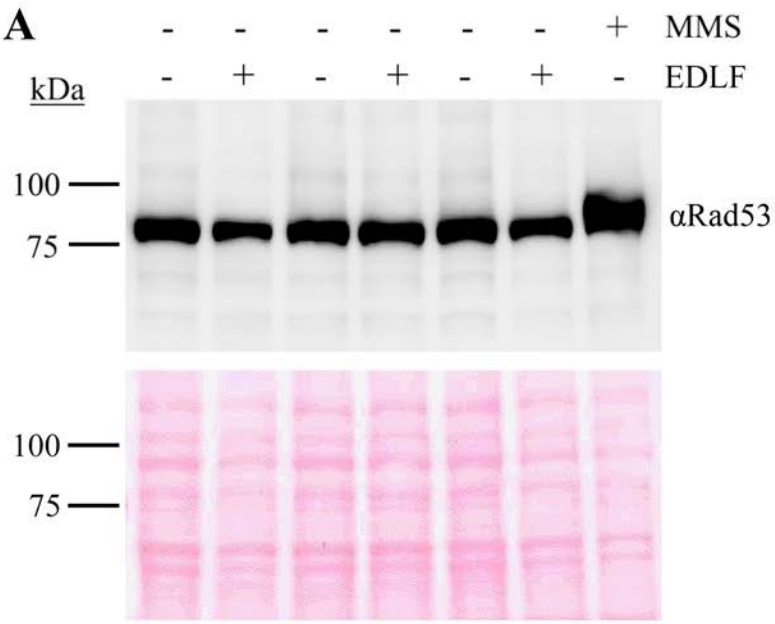

D
Ctrl
EDLF
WT
rap1-12
rap1-17

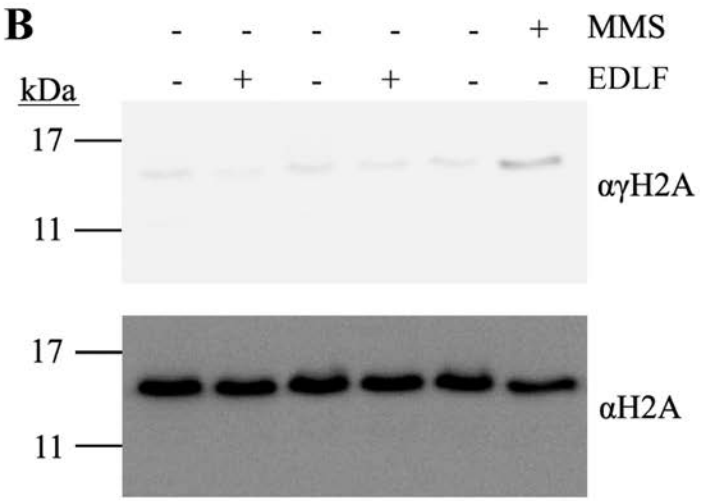

A
Neutral lipids
Phospholipids
FA de novo synthesis
ACC1
FA desaturation
OLE1
FA
FAA2
FAA4
FAT2
FAA3
IZH1/2/4
PEROXISOME
PCS60
Fß-oxidation
ECI1
FOX2
POT1
POX1
PXA1
PXA2
ACB1
acyl-CoA
TAG
SE
Glycerol
GUT1
YJU3
ROG1
MAG
TGL2
DAG
NEM1
PAH1
DGK1
GPD2
GPD1
GUT2
G3-P
DHAP
DAK1
DAK2
DHA
glu-6P
acyl-CoA
GPT2
1-acyl G3-P
1-acyl DHAP
AYR1
ALE1
ICT1
LOA1
PA
CTP
CDP-DAG
CHO1
INO1
ITR1
GIT1
Ins
DAG
PI
PIS1
Palmitoyl-CoA + Serine
ORM2
TSC3
Aldehydes + Etn-P
DPL1
HFD1
LCB3
FA + LCB
LCBP
YDC1
LAC1
LAG1
Ceramide
SUR1
ISC1
Complex sphingolipids
PGC1
CDP-DAG
PS
UPS2
PS
PG
DDL1
PSD1
CLD1
TAZ1
CL
PE
MITOCHONDRIA
PI transfer
PDR16
KES1
SFH5
GPI-anchor
GPI15
GPI1
GPI11
GAS1
GWT1
SPT14
ARE2
acyl-CoA
Acetyl-CoA
de novo synthesis
HMG2
ERG3
ERG2
Ergosterol
regulation
DAP1
UPC2
ARV1
transport
OSH2
OSH5
OSH6
LAM4
LAM5
LAM6
NCR1
PRY1
CSR1
YSP2
SUT1
SUT2
ON
MGA2/ SPT23
>2>0.8>0.5
PE
EPT1
Etn
CHO2
DAG
CTP
SAM1
SAM2
SAM3
SAM4
SAM
OPI3
OPI3
PC
CPT1
Cho
DAG
CTP
URA7
pyrimidine synthesis
LPL1
PLB2
GDE1
ATG15
OFF
OPI1/ INO2/ 4
>2>0.8>0.5
B
RNA-seq
qPCR
ln(EDLF/Ctrl)
OPI1
INO1
OLE1
ICT1
SPT23
MGA2
IZH1
IZH2
IZH3
IZH4
-log10(FDR)
lnFC
IZH4
MGA2
OLE1
IZH2
ICT1
OPI1
INO1
IZH1

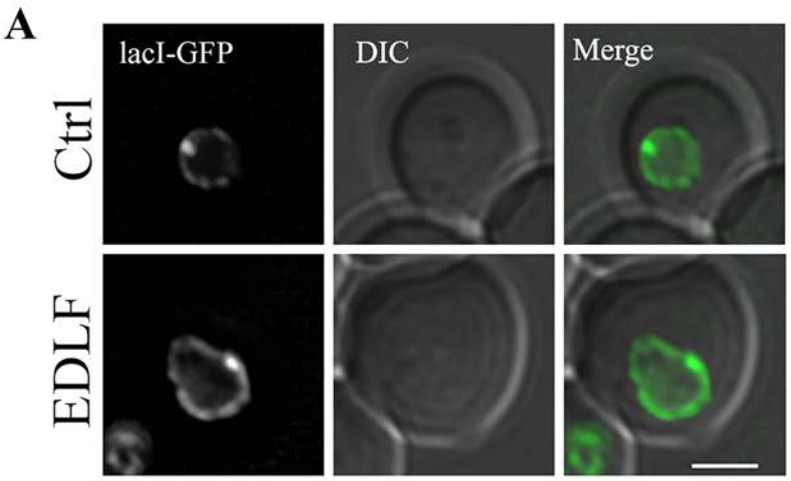

A
Sensor OFF
Sensor ON
OLE1
cytosol
ER
lumen
Loose packing
Tight packing
B
GFP-Spt23
GFP-Spt23
Merge
Ctrl
EDLF
MMS
> 0.999
**
Nucleoplasmic (%)
100
80
60
40
20
0
Ctrl
EDLF
MMS
GFP-Spt23
D
GFP-Spt23
kDa
Ctrl
EDLF
180
130
100
N
TCE
C
GFP-Mga2
GFP-Mga2
Merge
Ctrl
EDLF
MMS
0.934
****
Nucleoplasmic (%)
GFP-Mga2
E
GFP-Mga2
P
N
TCE

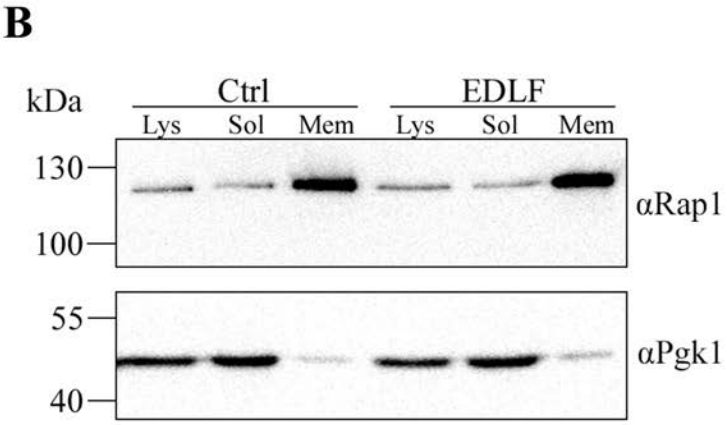

A
Wildtype
sir4Δ
Sec63-GFP
Merge
Ctrl
EDLF
Non-spherical nuclei (%)
WT
sir4Δ
0.953
0.510
B
GFP-Spt23
Nucleoplasmic (%)
0.917
0.570
C
GFP-Mga2
0.566
0.566
D
ln(EDLF/Ctrl)
OPI1
INO1
OLE1
ICT1
MGA2
IZH1
IZH2
IZH4

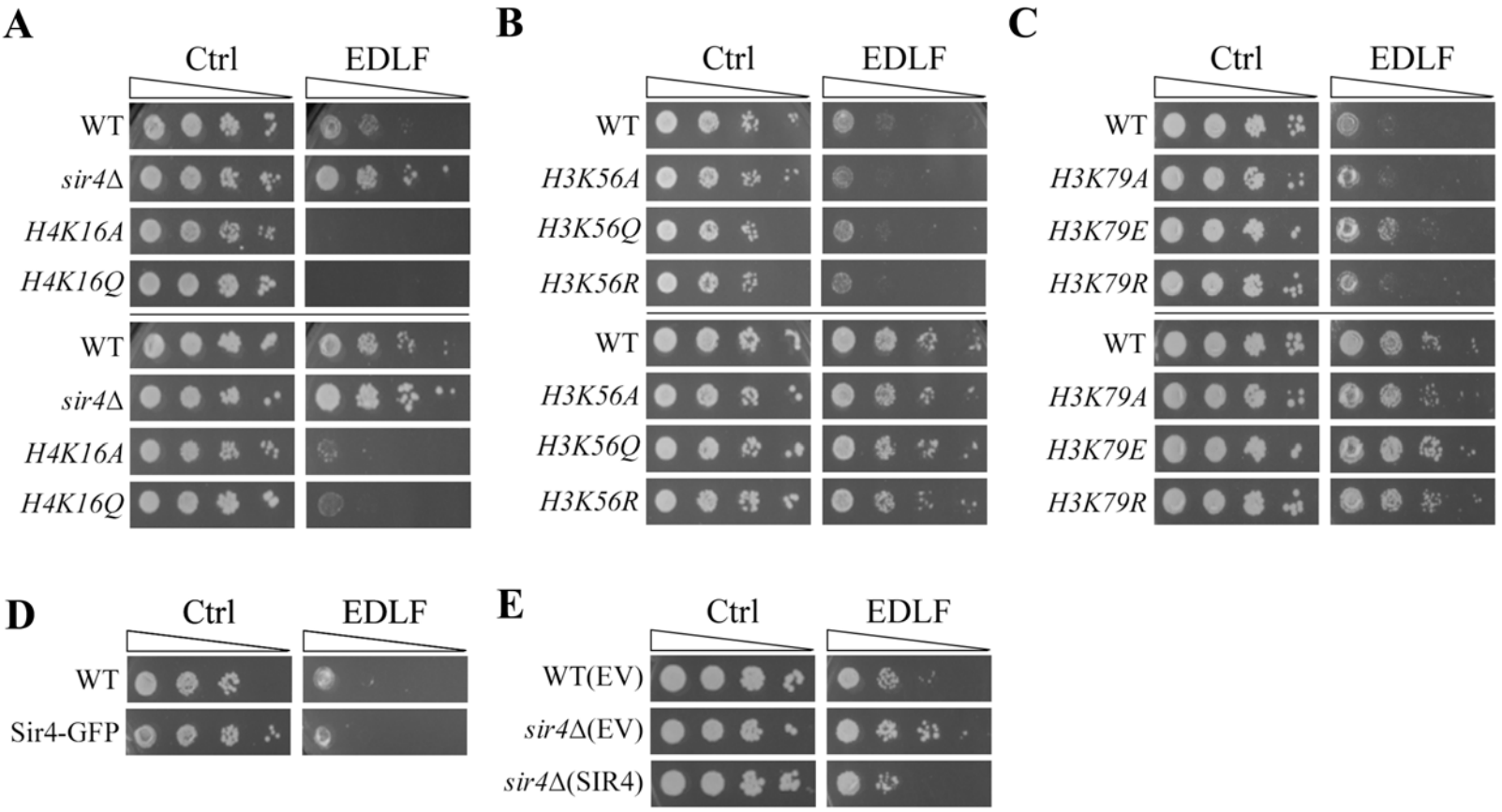

A
Wildtype
sir4Δ
-
+
-
+
EDLF
SE
TAG
OA
Erg
DAG
B
Ctrl
OA
WT
sir2Δ
sir3Δ
sir4Δ
H4K16A
H4K16A sir4Δ
C
Wildtype
BODIPY
DsRed-HDEL
Merge
Ctrl
EDLF
sir4Δ
BODIPY
DsRed-HDEL
Merge
Ctrl
EDLF
D
Total LDs per cell
30
20
10
0
**
0.2696
*
*
Ctrl
EDLF
E
Cells with nLDs (%)
50
40
30
20
10
0
*
0.099
0.654
0.918
WT
sir4Δ
Ctrl
EDLF

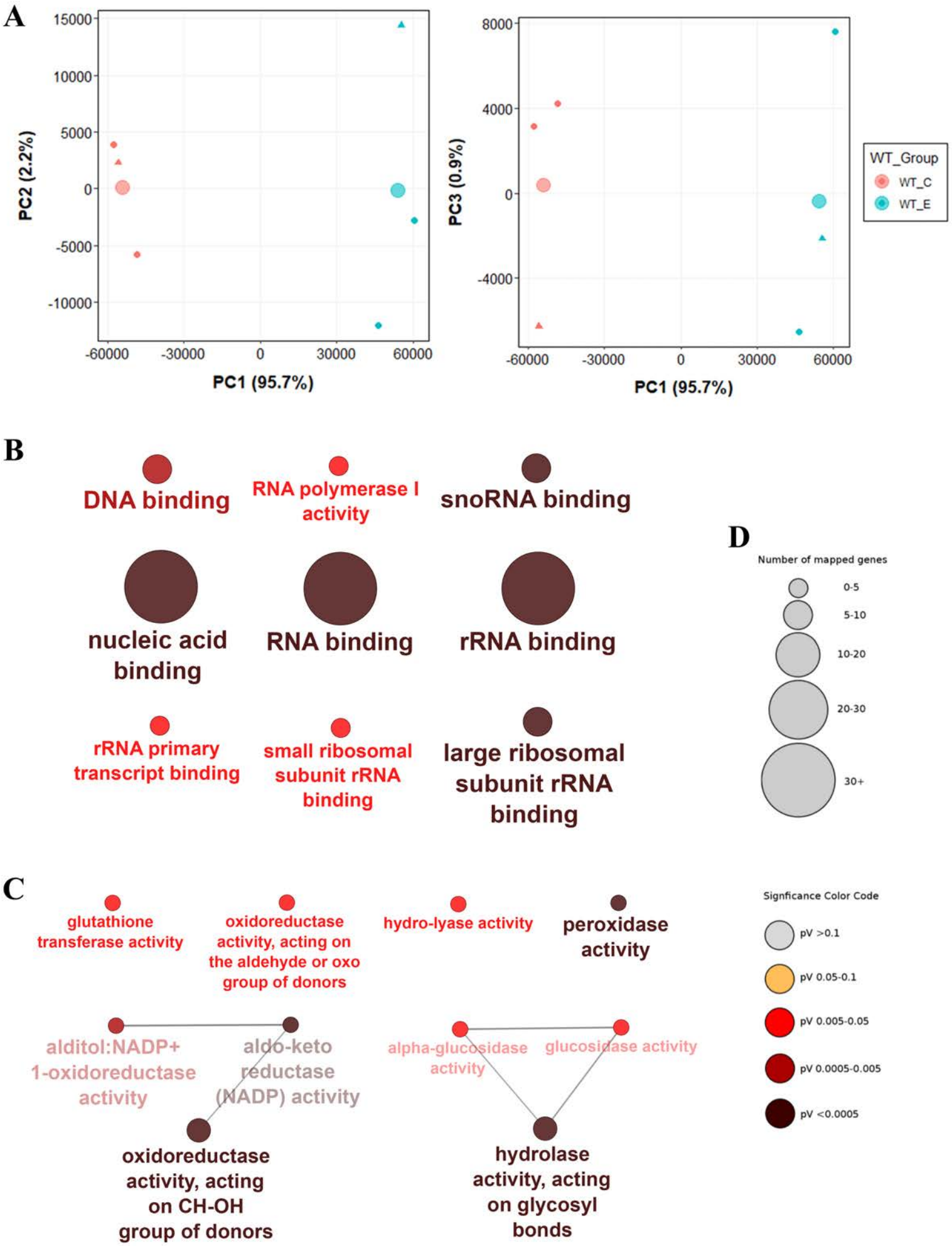

A
- - - - - - + MMS
kDa
- + - + - + - EDLF
100
75
αRad53
100
75

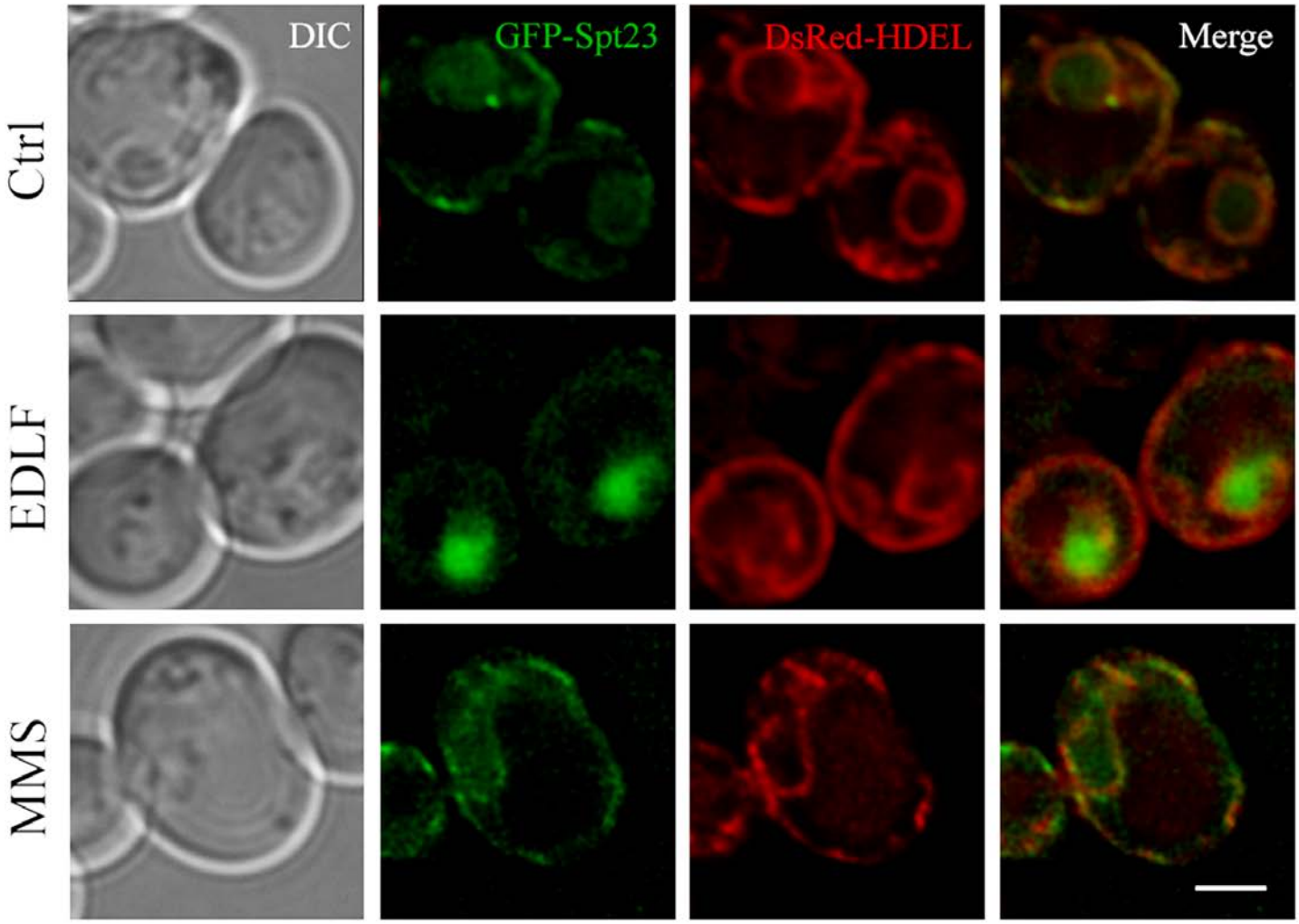

B
- - - - - + MMS
kDa
- + - + - - EDLF
17
11
αγH2A
17
11
αH2A


A
lacI-GFP
DIC
Merge
Ctrl
EDLF


B
kDa
Ctrl
EDLF
Lys
Sol
Mem
Lys
Sol
Mem
130
100
αRap1
55
40
αPgk1


A
Ctrl
EDLF
WT
sir4Δ
H4K16A
H4K16Q
WT
sir4Δ
H4K16A
H4K16Q
B
Ctrl
EDLF
WT
H3K56A
H3K56Q
H3K56R
WT
H3K56A
H3K56Q
H3K56R
C
Ctrl
EDLF
WT
H3K79A
H3K79E
H3K79R
WT
H3K79A
H3K79E
H3K79R
D
Ctrl
EDLF
WT
Sir4-GFP
E
Ctrl
EDLF
WT(EV)
sir4Δ(EV)
sir4Δ(SIR4)


A
15000
10000
5000
0
-5000
-10000
PC2 (2.2%)
-60000
-30000
0
30000
60000
PC1 (95.7%)
8000
4000
0
-4000
PC3 (0.9%)
-60000
-30000
0
30000
60000
PC1 (95.7%)
WT_Group
WT_C
WT_E
B
DNA binding
RNA polymerase I activity
snoRNA binding
nucleic acid binding
RNA binding
rRNA binding
rRNA primary transcript binding
small ribosomal subunit rRNA binding
large ribosomal subunit rRNA binding
D
Number of mapped genes
0-5
5-10
10-20
20-30
30+
C
glutathione transferase activity
oxidoreductase activity, acting on the aldehyde or oxo group of donors
hydro-lyase activity
peroxidase activity
alditol:NADP+ 1-oxidoreductase activity
aldo-keto reductase (NADP) activity
oxidoreductase activity, acting on CH-OH group of donors
alpha-glucosidase activity
glucosidase activity
hydrolase activity, acting on glycosyl bonds
Signficance Color Code
pV >0.1
pV 0.05-0.1
pV 0.005-0.05
pV 0.0005-0.005
pV <0.0005


DIC
GFP-Spt23
DsRed-HDEL
Merge
Ctrl
EDLF
MMS