Uptake mechanisms and physiological effects of furanic compounds from the Maillard reaction in budding yeast

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ABSTRACT

Maillard Reaction Products (MRPs) are formed during the thermal processing of foods and exhibit important sensory attributes. Furanic compounds are a subset of MRPs are commonly found in food products but have been shown to exhibit many toxic effects. Despite this broad issue, the molecular mechanisms by which MRPs interact with eukaryotic cells and inhibit cellular processes is poorly understood. To remedy this, we used budding yeast as a genetic model to explore uptake mechanisms of several common furanic compounds: 5-hydroxymethylfurfural (HMF), furfural (FUR), and 2-Furyl methyl ketone (FMK). Titrations were used to identify sub-lethal doses of each furanic compound for downstream experiments. We then revealed HMF is a potential substrate of the Pdr5 multidrug resistance pump and that both HMF and FUR toxicity correlates with surface nutrient transporters levels. Live cell imaging shows that HMF disrupts mitochondria whilst FUR affects the endolysosomal system. Collectively, this work suggests that the uptake and efflux of even these related furanic compounds have different mechanisms, and exert their toxic effects via distinct intracellular pathways. As many of these cellular components are conserved throughout evolution, this work sheds light on the metabolism of toxic compounds commonly found within animal food sources.

Keywords: Budding yeast, Maillard Reaction products, furanic compounds, transporters, endocytosis

INTRODUCTION

The Maillard reaction is a complex cascade of non-enzymatic browning processes between reducing sugars and amino acids, and is fundamental to the development of flavour, aroma, and colour in thermally processed foods (Liu et al., 2022). The array of molecules produced, collectively termed as Maillard Reaction Products (MRPs), have distinct sensory implications for the food industry (Hosry et al., 2025; Tamanna and Mahmood, 2015). The interplay of various factors, such as chemical composition, temperature, time, humidity, and pH, along with the presence of glycation agents or oxidants, dictates the progression of this chemical cascade and the specific MRPs generated (Nie et al., 2013; Shakoor et al., 2022). Various MRPs have been identified in foods, common examples include acrylamide and furanic compounds (Agcam, 2022; Sun et al., 2022). Humans are frequently exposed to furanic compounds, such as 5-hydroxymethylfurfural (HMF), furfural (FUR), and 2-Furyl methyl ketone (FMK), which are commonly associated with sugar-rich food products (Program, 2010). However, beyond sensory appeal, this reaction also generates a broad spectrum of potentially harmful by-products (Kathuria et al., 2023).

The toxicity of MRPs has been extensively debated, with examples like acrylamide being correlated with various diseases and negative effects but also suggested the range of experimental variation has created ambiguous conclusions (Başaran et al., 2023). Furanic compounds are known to be carcinogenic (Program, 1993). Studies using rodent species have shown that ingestion of furanic compounds induces DNA damage on spleen/liver cells, triggering chromosomal aberrations and cell death (Leopardi et al., 2010; Neuwirth et al., 2012; Yilmaz et al., 2023). In addition to genotoxic effects, furanic compounds are known to induce oxidative stress and inflammation ((CONTAM) et al., 2017). For example, HMF increases reactive oxygen species (ROS) in both fly larvae and cultured human cell models, with the latter example showing ROS triggers apoptosis via mitochondrial pathways (Chen et al., 2024; Qiu et al., 2022). Furanic compounds are known to be produced in substrates used for microorganism conversion to biofuel, which hinders the fermentation process (Wang et al.,

2016). Curiously, the addition to the yeast *Saccharomyces cerevisiae* is toxic and elevates ROS (Allen et al., 2010), which might be explained by mitochondrial mechanisms, since directed evolution efforts to increase tolerance was enriched for mutations with altered mitochondrial function (Ren et al., 2024). Despite their prevalence in the modern diet and evidence of their cytotoxic potential, the molecular mechanisms by which furanic compounds are taken up by cells and trigger physiological stress responses remain poorly understood. Questions persist regarding their transport pathways, intracellular fate, and the genetic factors influencing susceptibility or resistance to furanic-induced stress.

MRPs are present in food in free form or conjugated to proteins, peptides or polyphenols (Nowak et al., 2021; Zhu et al., 2020). The evaluation of the effects of these compounds on human health is mainly carried out in isolated form, as these MRPs are more readily absorbed in the gastrointestinal tract and are, therefore, more representative of the bioavailable fraction immediately after consumption (Pagare et al., 2024). Regulatory agencies use measurements of compounds such as HMF, acrylamide and furfurals in free form to estimate dietary exposure, construct safety limits and compare with the acceptable daily intake ((CONTAM) et al., 2017; Eisenbrand, 2020). The ready conversion of bound to free MRP compounds after acid and enzymatic digestion has already been demonstrated in cells (Zeng et al., 2020). Furthermore, the use of isolated analytical standards also allows the evaluation of toxicity and cellular mechanisms with standardized and reproducible protocols (Qiu et al., 2022), meeting the requirements of toxicological evaluation. Thus, the free form of MRPs is more directly related to acute systemic effects, such as cytotoxicity, genotoxicity, and metabolic alterations, which are biologically more relevant.

Eukaryotic cells adjust their uptake and export of nutrients in response to environmental conditions. *Saccharomyces cerevisiae* is a powerful platform for dissecting these mechanisms at the molecular level. Yeast utilizes a broad array of surface transporters for amino acids, sugars, and metal ions (Bianchi et al., 2019; Donzella et al., 2023). Yeast surface proteins are routinely internalized to endosomes and follow conserved recycling pathways back to the plasma membrane (Laidlaw et al., 2022; MacDonald and Piper, 2017, 2016). Alternatively, internalized surface proteins can be ubiquitinated and sent through the Endosomal Sorting Complexes Required for Transport (ESCRT) mediated pathway to the yeast lysosome (or vacuole) for degradation (Laidlaw and MacDonald, 2018). This downregulation can be triggered in response to substrate or stress, and is mediated upstream of ESCRTs by the arrestin related trafficking adaptors, which specifically unite transporters with the E3 ubiquitin ligase Rsp5 to mediate ubiquitination (Lin et al., 2008; MacDonald et al., 2020; Nikko et al., 2008). Beyond these traditional nutrient uptake mechanisms, yeast also express multidrug resistance transporter proteins, that transport various substances and toxic compounds, out of the cell (Piecuch and Obłąk, 2014). Amongst these, the Pdr5 multidrug transporter is the best characterized, which has remarkable promiscuity for diverse substrates, and therefore of intense interest in clinical and biotechnological applications (Golin and Ambudkar, 2015; Rogers et al., 2001). Whether furanic compounds are substrates of any of these transporters, in yeast or other eukaryotes, has not been directly tested and is not well understood. However, recent transcriptome analyses did suggest expression of pumps like Pdr5 might correlate with toxicity of furanic compounds added to anaerobic yeast cultures of a xylose-utilizing yeast strain (Ask et al., 2013)

In this study, we hypothesise furanic compounds are actively transported across the plasma membrane, and that uptake and downstream effects are evolutionarily conserved. Using yeast, we use different genetic systems to explore transporter regulation of furanic compounds, and cell biological approaches to assess disruption of cellular processes. This work sets out to define fundamental and conserved mechanisms contributing to furanic-induced toxicity and the physiological responses of eukaryotic cells, ultimately contributing to a broader understanding of food-derived toxicants and their impact on human health.

RESULTS

MRPs induce toxic effects in yeast cells

To determine if the budding yeast *Saccharomyces cerevisiae* responded to different furanic compounds, we selected three representatives commonly found in food: HMF, FUR and FMK (**Figure 1**). For initial yeast experiments, we optimised a 96-well plate end-point growth assay, where cell density at the start of the experiment was compared to later time points. Using this assay, we tested titrations of HMF, FUR and FMK using concentration ranges predicted to exert a physiological effect based on previous studies in mammalian cells (Kong et al., 2019; Qiu et al., 2022; Zhao et al., 2017). These experiments showed that yeast exposed to HMF at concentrations of 250 µl/ml or less had no significant defect in growth after 24 hours (**Figure 2A**). However, at higher HMF concentrations, growth was inhibited in a concentration dependent manner. To validate these results, we employed a recently optimised continuous measurement growth assay, which also showed a HMF concentration dependent inhibition of yeast growth, with sub-lethal doses observed for 500 - 1000 µg/ml and almost complete growth inhibition at concentrations of 1000-8000 µg/ml (**Figure 2B**). We performed similar analysis with other MRPs. Both assays revealed a concentration dependent effect on yeast

growth in media containing FUR, with concentrations of ~500-1000 µg/mL having potent effects (**Figures 2C - 2D**). Similarly, titrations of FMK showed a range of growth effects, with subtle inhibition in the range 125-250 µg/mL and much more potent effects at concentrations of 500 µg/mL and above (**Figures 2E - 2F**).

Collectively, this work shows that all three compounds from the Maillard reaction have potent effects on the model yeast. We therefore set out to use the yeast system to test the hypothesis that transporters regulate furanic compounds, which are related to sugar and amino acid molecules that are common transporter substrates (Ask et al., 2013; Cong et al., 2021). We reasoned transporters were less likely to be specific for furanic compounds so sought genetic approaches to broadly test the hypothesis (**Figure 3**).

The Pdr5 efflux pump selectively regulates furanic compounds

Pdr5 is a multidrug transporter surface transporter that drives efflux of many toxic molecules to confer resistance (Balzi et al., 1994; Leppert et al., 1990). We reasoned that the harmful effects of high doses of different furanic compounds could be ameliorated by Pdr5, predicting *pdr5*Δ cells would be hypersensitive to MRPs (**Figure 3B**). To test this hypothesis, we compared growth of wild-type yeast and *pdr5*Δ deletion mutants in the presence of previously identified toxic concentrations of HMF, FUR and FMK. We discovered that *pdr5*Δ mutants are hypersensitive to HMF, with wild-type cells relatively similar to DMSO controls in the presence of 250 µg/mL HMF but *pdr5*Δ mutants growing to only 68% ± 9 (**Figure 4A**). At the higher concentration of 500 µg/mL HMF, wild-type cells grow to 88% ± 10 but *pdr5*Δ cells 37% ± 7. Experiments with sub-lethal doses of FUR inhibited yeast growth, there was no significant difference in effects between wild-type cells and mutants with *PDR5* deleted (**Figures 4B**). 125 µg/mL FUR reduced growth of wild-type cells and *pdr5*Δ cells to 60% ± 11 and 54% ± 16, respectively. 250 µg/mL FUR did exert a more potent effect in wild-type cells 21% ± 5, but was very similar to *pdr5*Δ cells that retained only 19% ± 10. In much the same way, both 125 - 250 µg/mL FMK inhibited growth of wild-type and *pdr5*Δ cells in a similar, albeit concentration dependent manner (**Figures 4C**). We speculate that wild-type cells efflux HMF via Pdr5, resulting in the growth benefit compared to *pdr5*Δ mutants. This leads to the intriguingly possibility that the physiological effect of furanic compounds on yeast cells is enacted through distinct mechanisms, depending on the compound in question and that these molecules are selectively regulated by the host cell. This further suggests that FUR and FMK, which also elicit toxic effects in yeast, are not efficient substrates of the Pdr5 efflux pump. Internal control experiments were included in all experiments, showing that wild-type and *pdr5*Δ strains grew very similar to one another in media treated with only DMSO.

Modulation of surface transporters correlates with furanic compound sensitivity

As furanic compounds are relatively similar to natural substrates of yeast transporters, such as amino acids, we considered that nutrient transporters might uptake furanic compounds. To broadly test this idea, we employed a haploid mutant strain lacking 9 different alpha-arrestin (*9xart*Δ) or arrestin-related trafficking (ART) adaptors (Nikko and Pelham, 2009). These proteins act to bridge an array of nutrient transporters with the promiscuous E3 ubiquitin ligase, Rsp5 (Kahlhofer et al., 2021). Deletion of arrestins therefore elevate surface levels of nutrient transporters, allowing increased uptake of exogenous molecules, potentially including furanic compounds (**Figure 3C**). There was almost no difference in growth between wild-type and *9xart*Δ cells upon addition of HMF after 48 hours at either concentration, besides a very subtle hypersensitive phenotype at 500 µg/ml HMF in *9xart*Δ (**Figure 5A**). Much more strikingly, the *9xart*Δ strain was hypersensitive to FUR, with potent, concentration dependent, growth inhibition at both 250 and 500 µg/mL concentrations (**Figure 5B**). Similar to HMF, different doses of FMK were equally inhibitory between wild-type and *9xart*Δ strains (**Figure 5C**). These data suggest FUR, and potentially HMF when administered at higher doses, may require surface localisation of nutrient transporters to exert toxic effects on yeast cells.

To further test the idea that nutrient transporter residence at the plasma membrane could explain these MRP induced phenotypes in *9xart*Δ cells, we sought alternative experimental avenue. If the arrestin mutants were perturbing nutrient transporter degradation and increasing transporter residence at the surface, we considered that strains lacking nutrient transporters would have the opposite effect (**Figure 3D**). We employed a strain that lacks 10 different nutrient transporters, termed *10xaa*Δ, which is extremely deficient in uptake of most proteinogenic amino acids (Besnard et al., 2016).

These experiments revealed that the *10xaa*Δ strain is more resistant to HMF and FUR than wild-type cells (**Figure 6A - 6B**), but grows similar in DMSO controlled cells. This is supportive of the notion that HMF and FUR cannot inhibit growth in cells lacking surface transporters, which is further bolstered by the observation that these compounds trigger hypersensitive phenotypes in mutants with elevated surface transporter activity (**Figure 5**). In further support of this, FMK, which had no arrestin-related phenotype, also does not have any significant changes in effects in wild-type cells compared with the *10xaa*Δ strain (**Figure 6C**). Taken together, this strongly suggests that furanic compounds are actively uptake and processed in a selective manner by nutrient transporters at the yeast plasma membrane.

Furanic compounds perturb distinct cellular processes

We have uncovered evidence supporting the idea that yeast is a useful model to determine MRP effects on eukaryotic cells, and that despite shared biogenesis, MRPs like HMF, FUR, and FMK are recognised by eukaryotic cells through distinct mechanisms. To support this idea, we surveyed broad biological processes through organelle maintenance in response to furanic compounds, to indicate if specific pathways were involved in recognising or responding to these MRPs. Firstly, as the Maillard reaction is associated with oxidative stress and mitochondrial dysfunction (Allen et al., 2010; Kim and Hahn, 2013; Qiu et al., 2022) , we assessed mitochondrial morphology using a GFP tagged version of Tom6, which localises to a ribbon morphology in wild-type cells (Shaw and Nunnari, 2002). We observed a striking defect in mitochondrial morphology following exposure to HMF for 4 hours, with GFP signal restricted to small bright puncta within the cell (**Figure 7A**). There were different patterns of mitochondrial mis-localisations, but all appear fragmented compared to wild-type and were not observed in DMSO treated cells (**Figure 7B - 7C**). Mitochondria span large regions of the yeast cells, so we performed 3D confocal microscopy to better appreciate the morphology in control cells, which show contiguous ribbons (**Supplemental Movie S1**). 3D imaging of the same GFP-Tom6 expressing cells treated with HMF revealed punctate disparate localisations spread throughout the cytoplasm of the cell (**Supplemental Movie S2**).

Secondly, as our genetic perturbation of nutrient transporters and arrestins correlated with sensitivity to furanic compounds, we considered the endolysosomal system as a likely target organelle. We chose the vacuole as a representative organellular marker, as it is also known to respond to different stress conditions (Kohler and Büttner, 2021; Li and Kane, 2009). Cells expressing a GFP tagged version of the vacuolar ATPase subunit Vph1 were grown to mid-log phase and then exposed to sub-lethal concentrations of different furanic compounds for 4 hours. As expected, the vacuoles of wild-type cells exposed to DMSO showed no defects, which was also true of addition of HMF or FMK (**Figure 8A**). However, FUR induced a variety of abnormal phenotypes never observed in control cells, including accumulations of GFP-Vph1 at the periphery of the vacuole, alongside other non-vacuolar puncta (**Figure 8B - 8C**). This physiological analysis further supports the idea that individual MRPs have cell specific responses and sensitivities sin eukaryotic cells and that many layers of regulation are likely involved in any cellular response to even individual compounds.

DISCUSSION

The intermediate Maillard reaction products classed as furanic compounds are chemical contaminants created during industrial processes or cooking, commonly identified in food following excessive heating (e.g. honey and caramelised syrups) or on the surface of fried or baked foods (Alsafra et al., 2022; Conceição et al., 2024; Santos et al., 2025; Shapla et al., 2018; Sun et al., 2022). These compounds can form through sugar degradation, lipid peroxidation, or thermal decomposition of amino acids, indicating their diverse origins and potential variability in concentration depending on food matrix and processing method (Hosry et al., 2025). EFSA CONTAM Panel identified a worrying range of furan exposure, particularly consumption of ready-to-eat meals and cereals for infants and coffee for adults ((CONTAM) et al., 2017). Given their presence in foods of habitual consumption and their constant exposure in the human diet, it is crucial to evaluate the dietary exposure to these compounds and to understand the mechanisms associated with their uptake, efflux, and impact on processes at the cellular level. Several studies have also highlighted the potential for furanic compounds to induce oxidative stress and mitochondrial dysfunction, which can contribute to cytotoxicity and metabolic disruption in exposed cells (Allen et al., 2010; Aydin et al., 2023; Batool et al., 2025, 2021; Zhang et al., 2015). In this study we focussed on three furanic products from the Maillard reaction commonly found in food: HMF, FUR, and FMK, which present different functional groups, such as hydroxyl, methyl, and ketone, in the molecular rearrangement linked to the structure of a furan ring (**Figure 1**). Although these compounds share molecular features, it is important to consider their differences, which might influence cytotoxic potential, mechanisms and specificity of transport in/out of cells, and mode of action that perturbs cellular processes. Moreover, differential interactions with membrane transporters and detoxification enzymes may underlie the distinct cellular responses observed for each compound, emphasising the need for compound-specific toxicokinetic studies (Quan et al., 2022) . Understanding these nuances is key to assessing the risk associated with dietary intake of Maillard reaction products and developing strategies to mitigate their impact on human health.

Although previous studies have reported toxic effects of furanic compounds on the growth of eukaryotic cells, including yeast (Ask et al., 2013; Lam et al., 2021), our documentation of a dose response inhibition on growth in response to HMF, FUR and FMK suggests regulatory mechanisms are triggered by this stress, and provide optimised concentrations to explore these physiological responses. As mentioned, although MRPs are complex and often conjugated to other molecules, studying MRPs in isolation best represents the common products encountered by cells (Pagare et al., 2024). Curiously only HMF showed clear phenotypes suggesting

clearance via the Pdr5 multidrug resistance pump, with *pdr5*Δ being hypersensitive to different concentrations (**Figure 4**). This specificity may be related to the higher polarity or redox activity of HMF compared to FUR and FMK, which could enhance recognition or affinity to the substrate-binding cavity for efficient Pdr5 efflux (Egner et al., 1998). As a fungal protein, much of the work on Pdr5 is focussed on biomedical and agricultural applications (Golin and Schmitt, 2023). However, this family of ATP-binding cassette (ABC) proteins are conserved from bacteria to humans (Balzi et al., 1994), with various human homologues shown to transport a range of structurally and functionally distinct substrates across the plasma membrane (Lin and Yamazaki, 2002; Sodani et al., 2012). Therefore, we suggest that multidrug resistance pumps are worthy of consideration for regulation of MRPs consumed in human diets.

We do note that the genetic systems we have used to understand the tolerance of yeast to furanic compounds rely on indirect assumptions. It is possible that, although we know more/fewer nutrient transporters localise and function at the plasma membrane in *9xart*Δ and *10xaa*Δ cells, respectively (Besnard et al., 2016; Lin et al., 2008; Nikko et al., 2008; Nikko and Pelham, 2009). Alternative explanations could be through the intracellular targets of furanic compounds being mis-localised in these mutant cells, or other indirect consequences, such as membrane destabilisation under stress (Teixeira et al., 2008) . However, given results such as *9xart*Δ cells are hypersensitive to FUR and *10xaa*Δ mutants are resistant, we believe the most logical explanation is transport via a surface localised transporter facilitating cellular entry of FUR. The nature of what transporter(s) are responsible is not yet known, and yeast transporters are known to vary in their substrate specificity (Bianchi et al., 2019) Furthermore, environmental pressure can modify yeast transporters, either modulating specificity or creating new activity (Karapanagioti et al., 2024). Therefore, it is reasonable to assume that these furanic compounds, which are chemically similar to natural yeast transporter substrates, might utilise transporter activity to enter cells. Changes in exogenous substrate levels, such as glucose or nitrogen depletion, indirectly regulates surface transporters via post-translational modification of arrestins (Kahlhofer et al., 2021; Megarioti et al., 2021; O'Donnell and Schmidt, 2019). Therefore, as proteins like amino acid transporters and alpha arrestins are highly conserved with an array of human orthologues that perform analogous functions in animal cells (Gauthier-Coles et al., 2021; Zbieralski and Wawrzycka, 2022), these uptake mechanisms of furanic compounds may be conserved in other eukaryotic systems.

The intracellular targets of internalised furanic compounds are not well understood, but physiological effects reported in animal cell models have been proposed in the context of mitochondrial dysfunction (Qiu et al., 2022). To test this in yeast, we expressed a GFP-tagged version of Tom6, a component of the Translocase of the Outer Mitochondrial membrane complex (Pfanner et al., 1996). Under control conditions with DMSO, or even with addition of FUR and FMK at concentrations that inhibit growth, we observed no obvious defects in mitochondria morphology, even using 3D confocal microscopy. In contrast, addition of HMF induced fragmentation of mitochondria, with foci dispersed throughout the 3D volume of the cytoplasm (**Figure7**). This suggests furanic compounds drive increased mitochondrial fission, an adaptive stress response in eukaryotic cells associated with starvation and stress conditions that can lead to cell death (Pagliuso et al., 2018; Wang et al., 2022; Westermann, 2012). Thus, changes in mitochondrial morphology in yeast may correlate with conserved oxidative stress and metabolic and inflammatory responses following furanic compound exposure in animal cells (Lee et al., 2019; Qiu et al., 2022; Shapla et al., 2018). Curiously, no obvious mitochondrial defects of HMF and FMK were observed. To further explore potential targets of these other furanic compounds, we considered the vacuole a potential target given the data implicating transporters and endolysosomal trafficking (**Figure 5 & 6**). Furthermore, the vacuole is associated with downregulation of transporters in response to other stresses, like different nutrient starvation conditions (Laidlaw et al., 2021; Müller et al., 2015). Although HMF had no effect on vacuolar morphology, FUR induced a series of mis-localisation phenotypes, that may be directly or indirectly related to the stress induced by FUR leading to cell death (**Figure 8**).

Although FMK concentrations that effectively inhibit yeast growth in a concentration manner were established, our genetic models did not identify conditions where cellular sensitivity or resistance to FMK were altered compared to wild-type controls, and FMK did not exhibit morphological defects in mitochondria or vacuoles. This suggests that FMK may provide toxicity by an entirely distinct mechanism, or a redundant combination of mechanisms. Less is known about FMK in the literature but this compound has already been identified in several thermally processed foods and can inhibit microbial driven production of biofuel ((CONTAM) et al., 2017; Guarnieri et al., 2017; Sayre et al., 1993). Beyond this, structurally related methylfuran compounds have been shown to form adducts with essential cytosolic proteins and perturb NAD^+/NADH balance in yeast (Jilani and Olson, 2023) , indicating FMK might similarly disrupt central metabolism rather than organellar integrity.

Having identified concentrations to study FMK in yeast, future work may provide insight as to its mode of action, which would be relevant for understanding toxic effects in humans. In conclusion, MRPs are complex and diverse, so concentrating our study on not just one but three furanic compounds allowed the specificity and modes of action to be compared. Strikingly, even across relatively similar compounds HMF, FUR and FMK, when studied in isolation we find each elicits differences: in effective concentrations, specificity for uptake/efflux, and perturbation of distinct organelles. The extensive complexity of these physiological responses will be

difficult to unravel, but our work provides a framework to do so in model organisms that can reveal novel and relevant mechanistic models that can be tested in animal cell models. Beyond this, as such MRPs are inhibitory to biotechnological processes, such as the production of biofuel (Allen et al., 2010; Ask et al., 2013; Ren et al., 2024; Wang et al., 2016), this study can guide applications to modulate yeast tolerance for improved processing. Our findings contribute to a better understanding of how even relatively similar furanic compounds elicit different organelle-specific effects. This complexity that should be considered when trying to define the effects of MRPs in the context of human health. Elucidating these mechanisms in yeast not only facilitates screening for dietary safety thresholds but also aids in identifying biomarkers of exposure or damage that could be relevant for human toxicology. Mechanistic knowledge strengthens food risk assessment frameworks and supports evidence-based regulation of processing contaminants.

MATERIALS & METHODS

Yeast strains used in this study

Parental yeast strain BY4742 (Brachmann et al., 1998) was used for experimental work throughout the manuscript. BY4742 was the parental strain to test the role of Pdr5 (BY4742 *pdr5Δ::Kan*^{r} (Giaever et al., 2002) and alpha arrestins (*9xartΔ*: BY4742 *ecm21Δ csr2Δ bsd2Δ rog3Δ rod1Δ ygr068cΔ aly2Δ aly1Δ ldb19Δ ylr392cΔ* (Nikko and Pelham, 2009). BY4741 (Brachmann et al., 1998) was the parent for the *NOP1*-GFP-Tom6 and *NOP1*-GFP-Vph1 strains for confocal microscopy (Weill et al., 2018; Yofe et al., 2016). To assess the role of yeast lacking nutrient transporters, the Σ22574d parental strain (Jauniaux and Grenson, 1990) was used following deletion of ten distinct transporters (*10xaaΔ*: *gap1Δ put4Δ uga4Δ can1Δ lyp1Δ apl1Δ hip1Δ dip5Δ gnp1Δ agp1Δ* (Besnard et al., 2016).

Cell culture

Prior to culturing, yeast strains were grown on yeast extract peptone dextrose (YPD) agar medium plates (2% peptone, 2% glucose, 1% yeast extract, 2% agar) (Formedium, Norfolk, UK) overnight at 30°C. Yeast cultures were grown in synthetic complete (SC) medium (2% glucose, yeast nitrogen base supplemented with a complete mixture of required amino acid, bases and vitamins) (Formedium, Norfolk, UK) overnight at 30°C with shaking to early/mid-log phase ($OD_{600} \leq 1.0$) prior to experimentation.

Steady state growth assays

WT yeast cultures were grown overnight to saturation and 1.5 µl of culture was used to inoculate 200 µl SC medium with a stated titration of three furfural compounds (FC): 5-Hydroxymethylfurfural (HMF)), Furyl methyl ketone (FMK) or Furfual (FUR) (Sigma-Aldrich, USA) or Dimethyl sulfoxide (DMSO) was used as a control in a 96-well plate format. Plates were incubated at room temperature and subjected to readings at OD_{600} at 1hr and 24hrs (*pdr5Δ and 10xaaΔ*), and 1hr and 48hrs (*9xartΔ*) using a microplate spectrophotometer (ThermoMultiskan Go 1510 Sky, Thermo Fisher Scientific Inc., Massachusetts, US). Data was normalized to % cell viability, considering the average growth of each cell strain (without any exposure) as 100% and plotted in GraphPad Prism (version 8) to compare the statistical significance between experimental conditions, with *P*-values included. All end point assays are presented from at least 6 technical and 3 biological repeats. An asterisk (*) is used to denote significance $p < 0.05$.

Continuous growth assays

96-well plates were set up following the same method as above. Growth experiments were monitored continuously for 24 hrs using a Stratus microplate reader (Cerillo, Charlottesville, VA, US) at 30°C. Growth curves presented are averages of at least six technical replicates.

Confocal microscopy

GFP-labelled yeast strains were grown overnight to mid-log phase, cells were pelleted and resuspended in media supplemented with the three different FC compounds at stated concentrations. Cells were imaged at room temperature using a 63x 1.40 oil immersion Plan Apochromat objective lens on a laser scanning confocal microscope (Zeiss LSM980). GFP fluorescence was excited using a 488 nm Argon laser and 495–550 nm emission collected. Processed images were adjusted in Fiji/ImageJ software (NIH).

Statistical analyses

One-Way ANOVA followed by Sidak's multiple comparisons test was utilized to compare wild-type (WT) cells with deletion strains under the same conditions to determine significant differences ($p < 0.05$). Additionally, an unpaired *t*-test was performed to compare normal organelles from GFP-exposed cells with control cells (GraphPad Prism v8, USA).

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DECLARATION OF INTERESTS

The authors declare no competing interests.

FIGURES AND LEGENDS

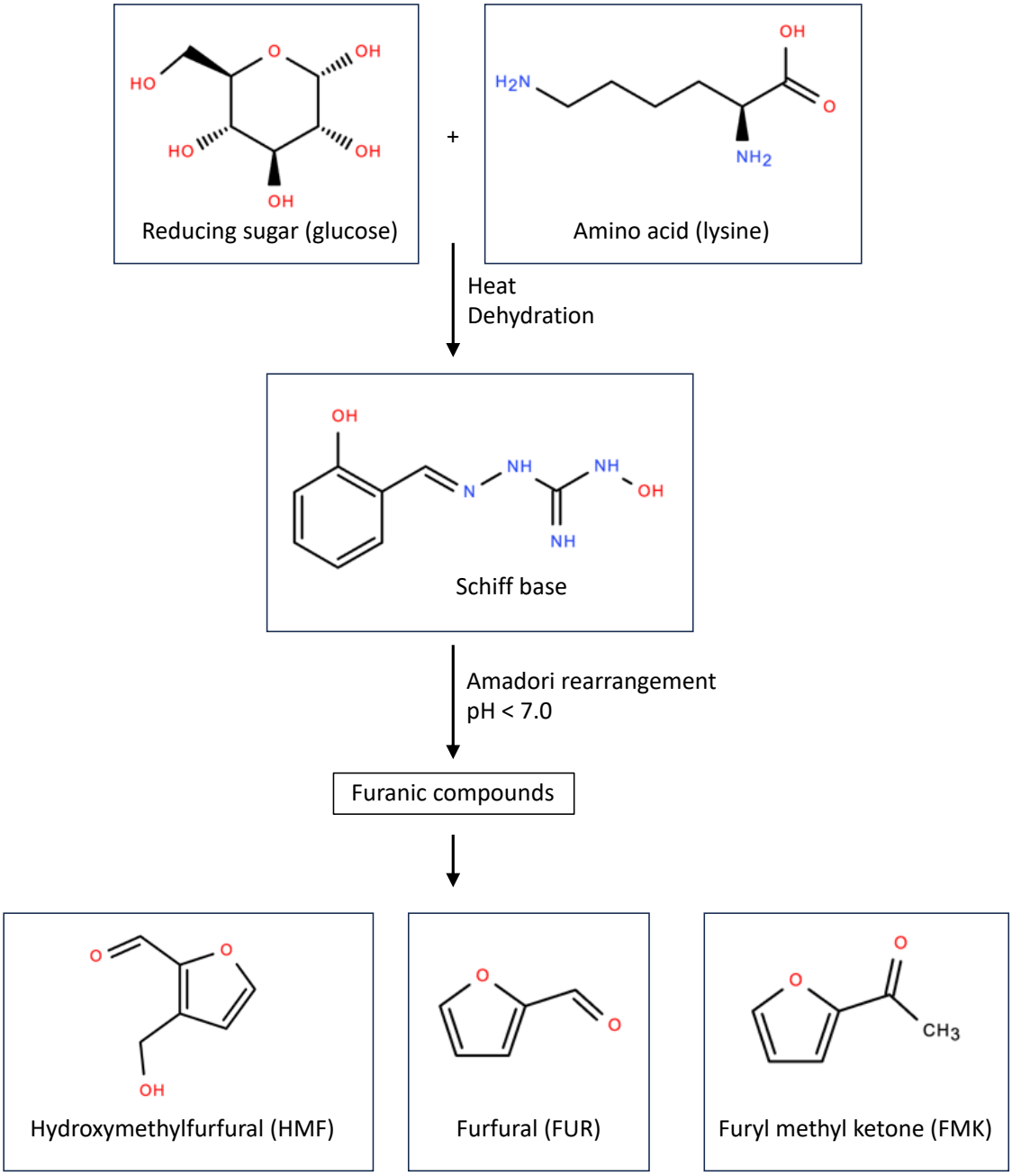

Figure 1: Chemical cascade of Maillard Reaction
The non-enzymatic reaction between carbonyl groups of reducing sugars and an amine group triggers a cascade with many products. Those specific to the production of furfurals are depicted.

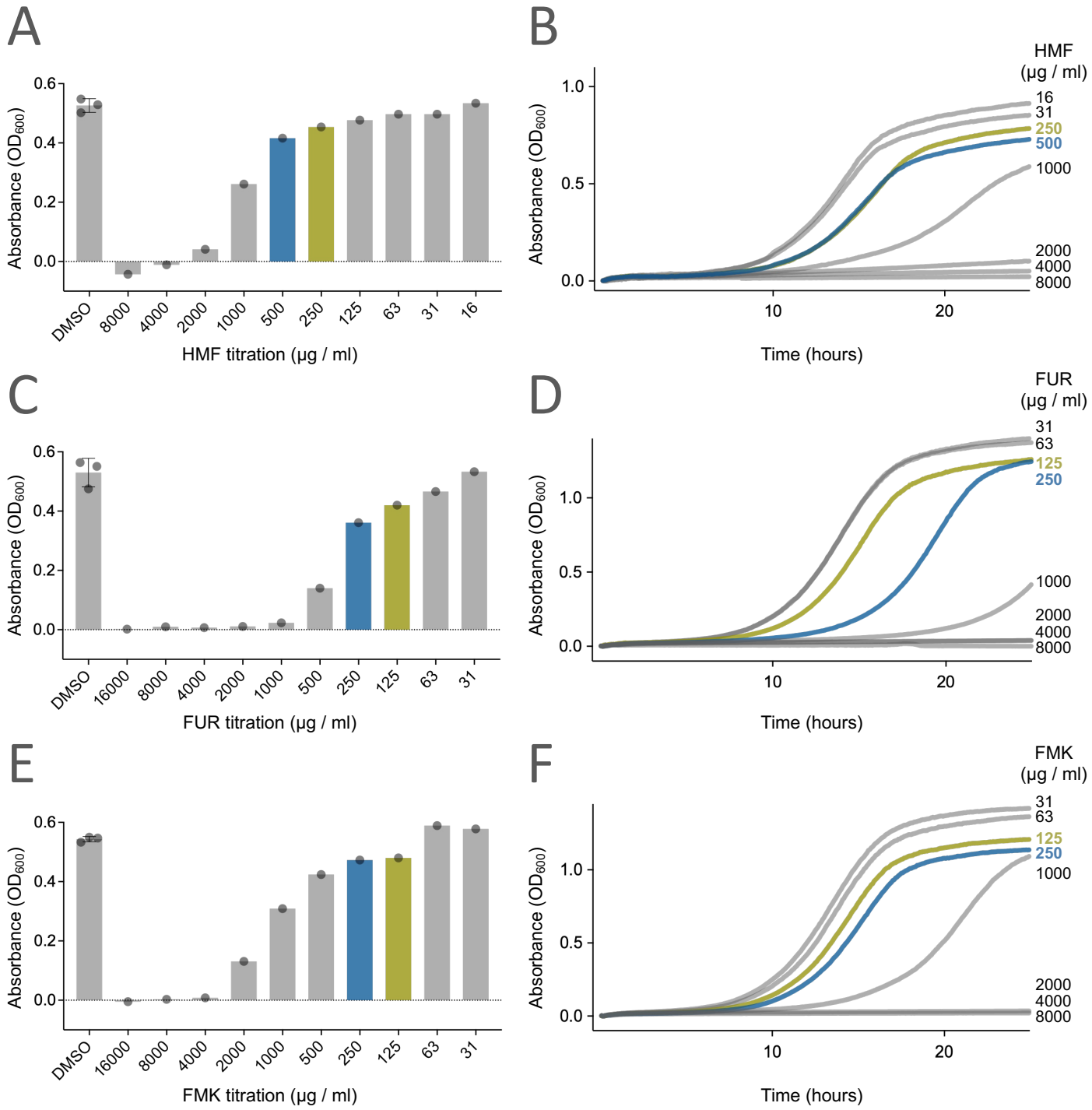

Figure 2: Furanic compounds exert toxic effects on yeast cells
A, C, E) Endpoint assays were performed with wild-type cells were exposed to varying concentrations of Hydroxymethylfurfural (HMF), Furfural (FUR) and 2-Furyl methyl ketone (FMK). The OD_{600} values were measured of 200 µl cultures grown in 96-well plates immediately after inoculation and then again following 24 hours growth at 30°C. **B, D, F)** Yeast growth assays across a titration of HMF, FUR and FMK were performed in liquid cultures with continuous measurements of OD_{600} every 5 minutes. In each experiment, concentrations of furanic compounds which are used for downstream experiments are indicated (blue and green).

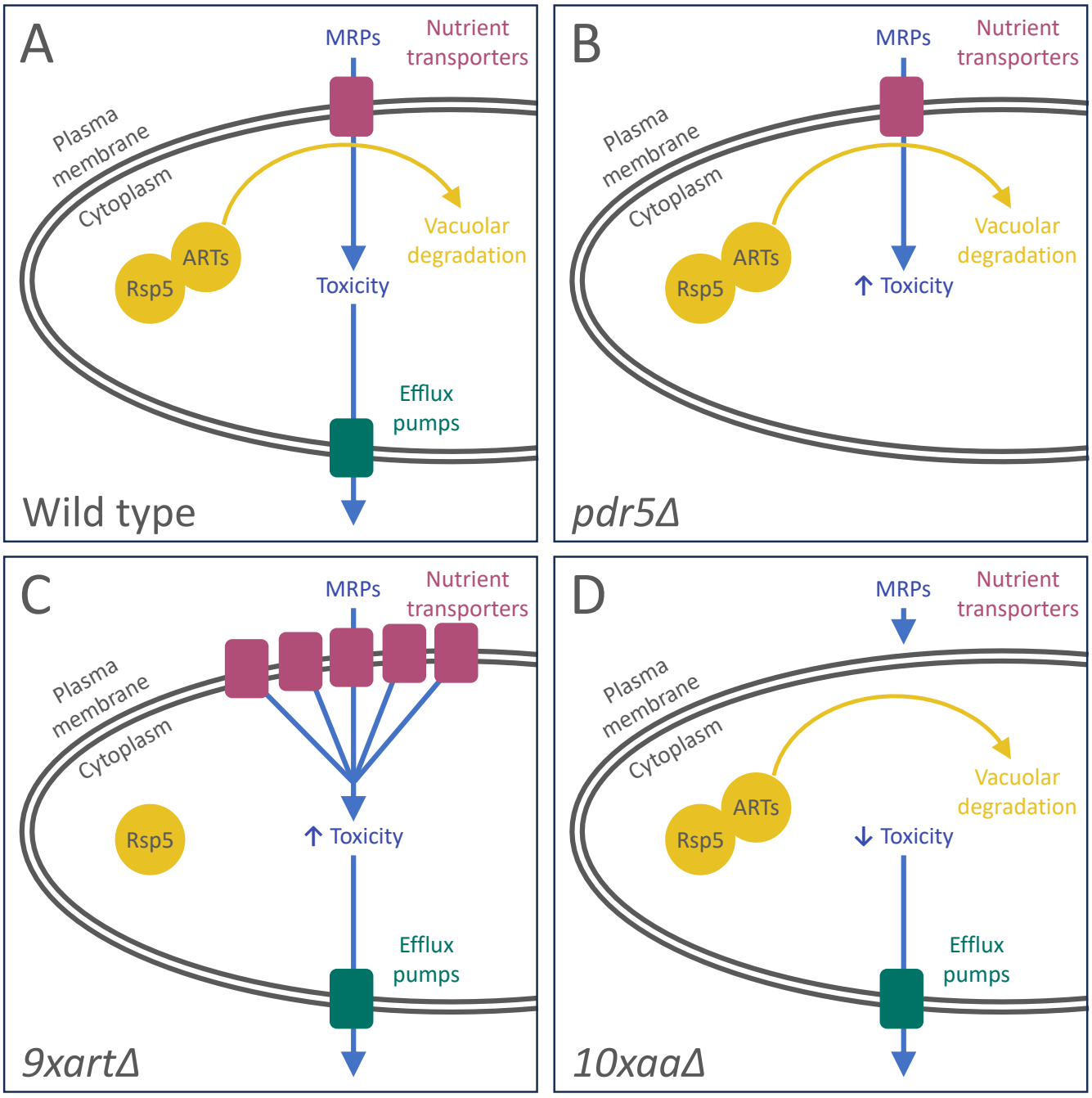

Figure 3: Genetic systems to test sensitivity of furanic compounds in yeast
A) Wild-type yeast expressing nutrient transporters uptake a range of nutrients across the plasma membrane, with toxic compounds being driven out of the cell through the action of efflux pumps like Pdr5. **B)** If harmful compounds, such as furanic compounds, are substrates of the Pdr5-efflux pump, an increase in toxicity would be predicted in *pdr5*Δ deletion mutants. **C)** Nutrient transporters are routinely downregulated by the vacuolar degradation pathway, where the E3 ubiquitin ligase Rsp5 is recruited to specific transporters via ART adaptors to ubiquitinate and trigger their degradation. A strain lacking nine of these ART adaptors (*9xart*Δ) has higher levels of different transporters at the plasma membrane, which we hypothesize would elevate toxicity of any compounds actively uptaken by transporters. **D)** Conversely, if toxic compounds rely on transporters for uptake into the cell, a strain lacking many different amino acid transporters (*10xaa*Δ) would be protected from the effects.

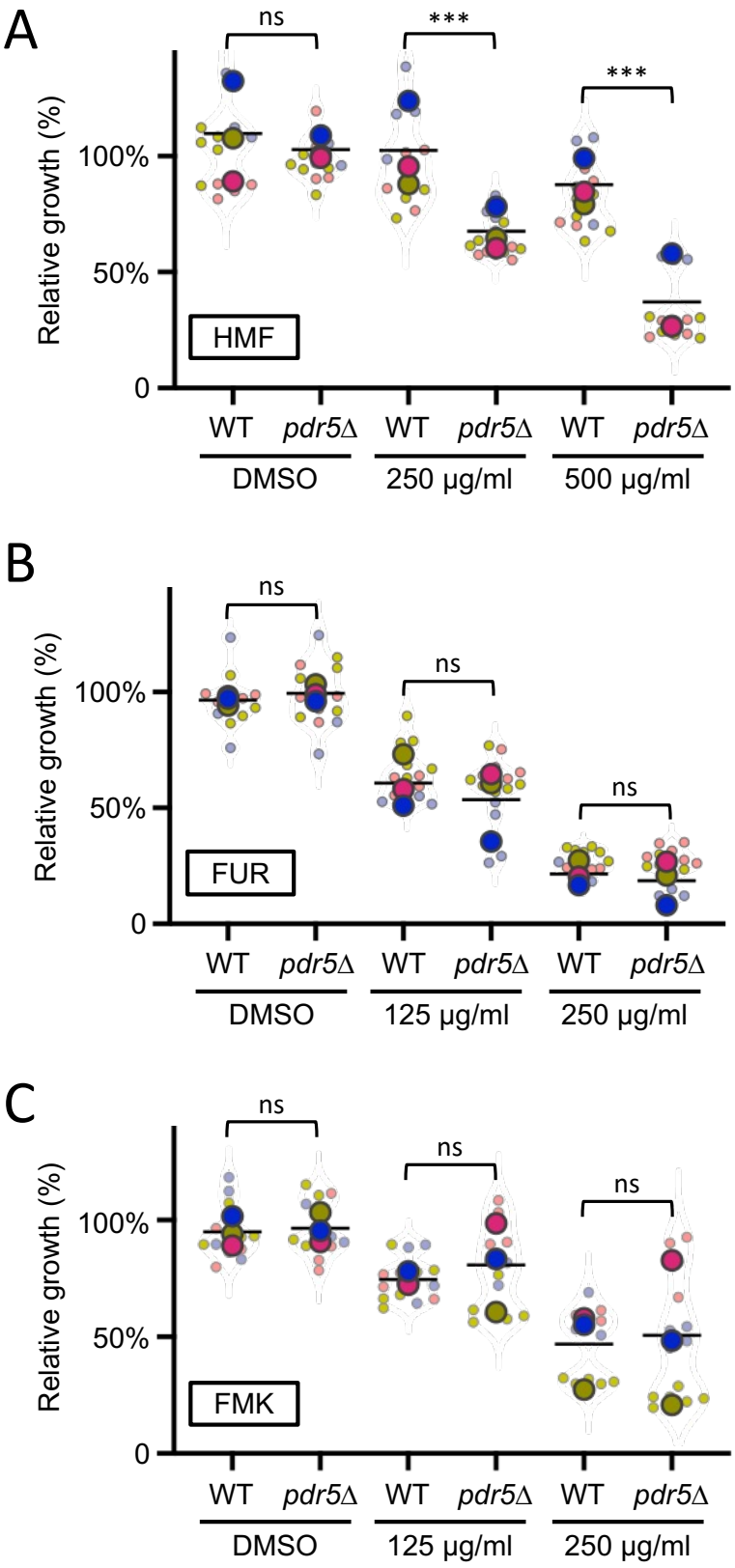

Figure 4: *pdr5*Δ mutants are hypersensitive to exposure of HMF
A, B, C) Relative growth of wild-type (WT) and *pdr5*Δ cells was assessed following exposure to furanic compounds, with DMSO as a control. Cultures were used to inoculate media containing indicated concentrations of HMF **(A)**, FUR **(B)**, and FMK **(C)** followed by growth measurements after 24 hours at room temperature. One-Way ANOVA and Sidak's multiple comparisons tests were performed.
(ns) not significant, (***) $p<0.001$.

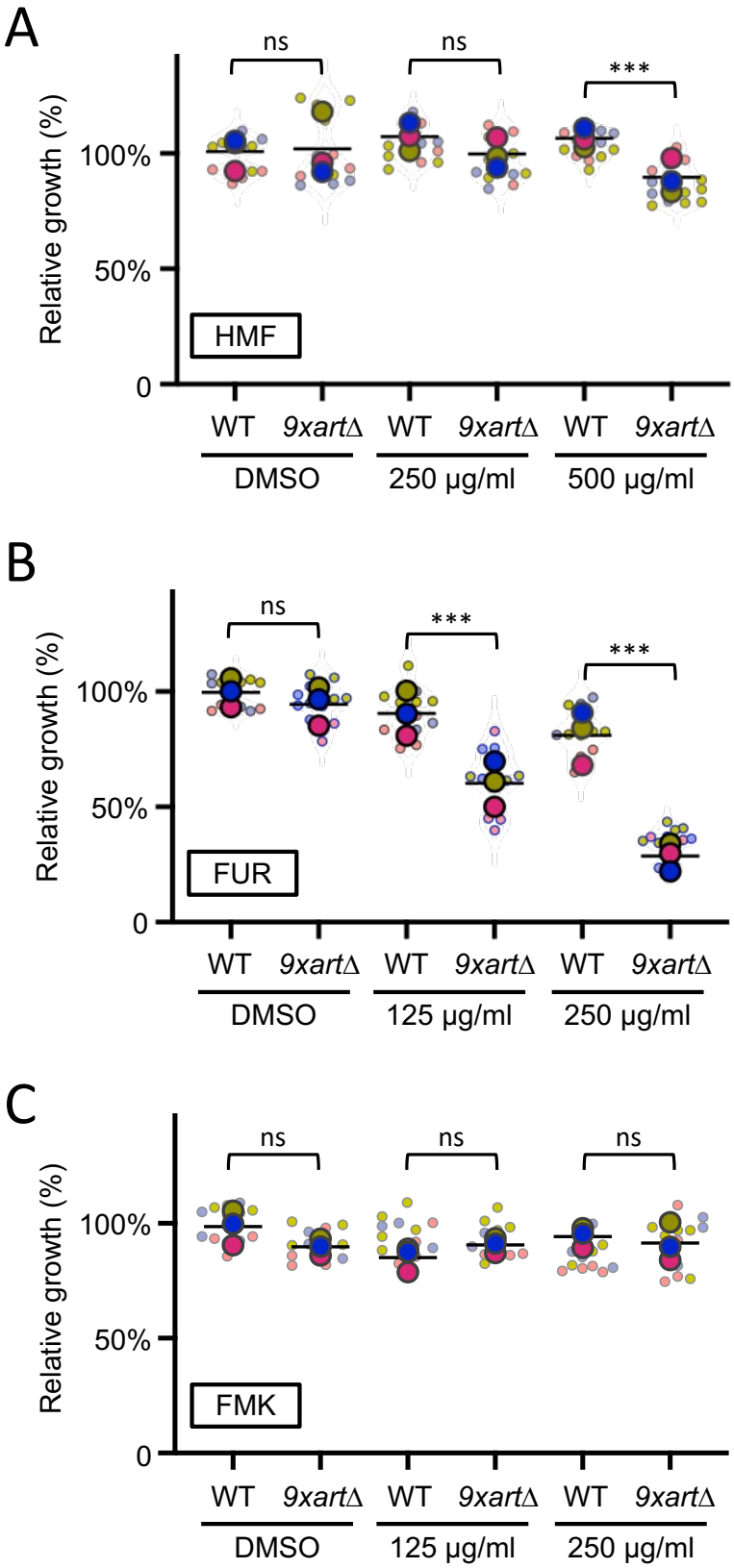

Figure 5: Perturbation of endosomal trafficking adaptors sensitizes cells to furanic compounds
A, B, C) End point growth assays were used to compare growth in DMSO controls or following exposure to furanic compounds: HMF **(A)**, FUR **(B)**, and FMK **(C)** after 48 hours. Wild-type cells were compared to a *9xart*Δ mutant strain across all conditions. One-Way ANOVA and Sidak's multiple comparisons tests are indicated: (ns) not significant, (***) $p<0.001$.

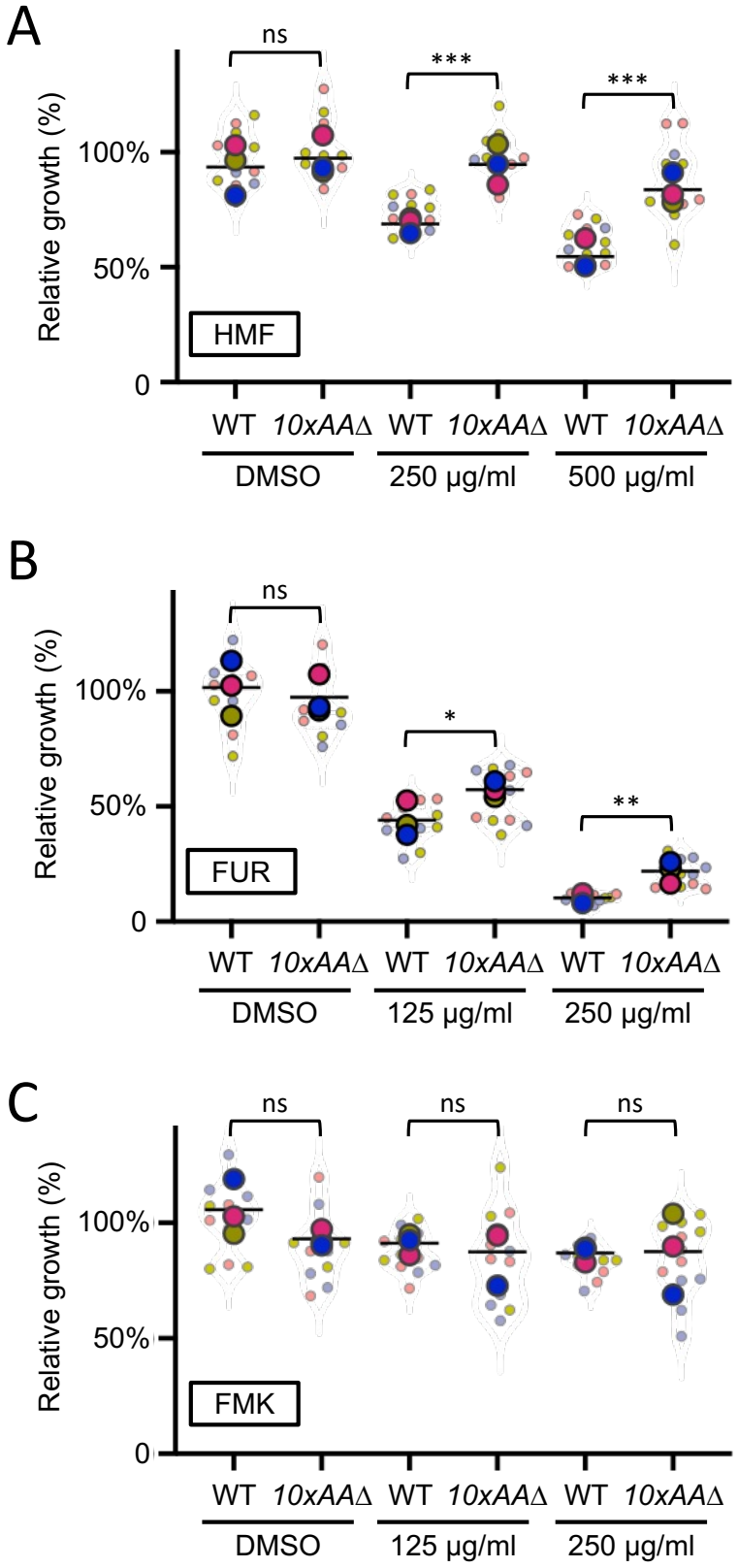

Figure 6: Cells lacking nutrient transporters tolerate furanic compounds
A, B, C) Relative growth of wild-type (WT) cells was compared to a mutant lacking 10 different amnio acid transporters (*10xaa*Δ). Cells were exposed to HMF **(A)**, FUR **(B)**, and FMK **(C)**, with DMSO added as a control for 24 hours porior to measurements. One-Way ANOVA and Sidak's multiple comparisons tests were performed. (ns) not significant, (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$.

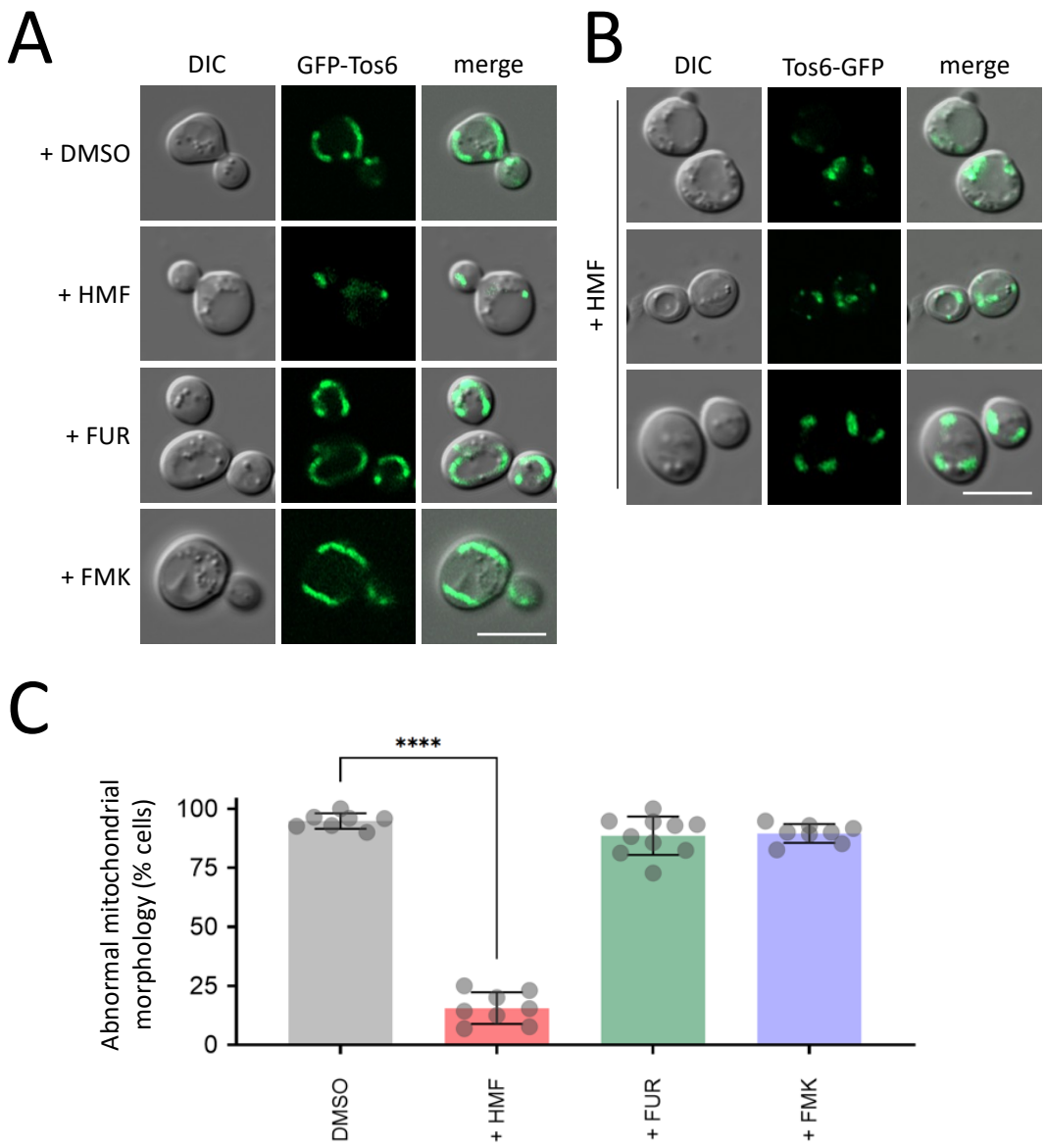

Figure 7: HMF exposure induces morphological defects of the mitochondra
A, B) Wild-type cells expressing a GFP tagged version of Tom6 (GFP-Tom6) were grown to mid-log phase and imaged by fluorescence microscopy following 4 hours exposure to HMF, FUR, FMK and DMSO as a control.
C) The average number of cells (n = 150 - 200, per condition) across replicates (n > 6) that exhibit defective mitochondrial morphology are presented. (****) $p < 0.0001$ shown by unpaired Holm–Sidak *t*-test. Scale bars: 5 µm.

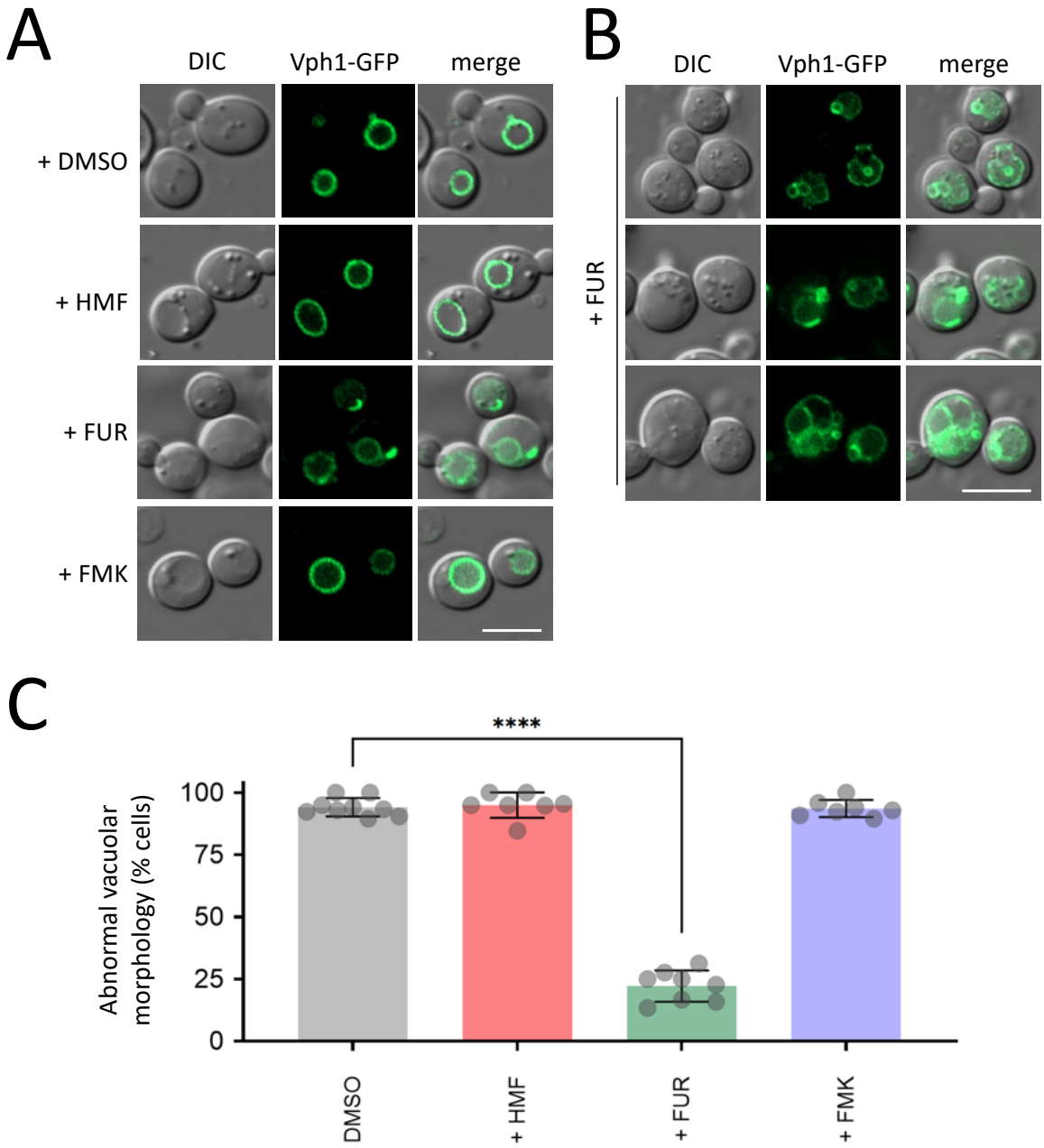

Figure 8: Abnormal vacuolar morphology is induced following FUR treatment
A, B) Cells expressing GFP-Vph1 were grown to mid-log phase prior to DMSO or furanic compounds (HMF, FUR and FMK) being added to the media for 4 hours prior to confocal microscopy.
C) The average number of cells (n > 152 per condition) across replicates (n > 5) that exhibit abnormal vacuolar localisations are presented in the histogram as a percentage. (****) $p < 0.0001$ as determined by unpaired Holm–Sidak *t*-test comparing treatments to DMSO control. Scale bars: 5 µm.

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