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# Lysine harvesting is an antioxidant strategy and triggers underground polyamine metabolism

**Viridiana Olin-Sandoval**[1,4], **Jason Shu Lim Yu**[2], **Leonor Miller-Fleming**[1,8], **Mohammad Tauqeer Alam**[5], **Stephan Kamrad**[2,10], **Clara Correia-Melo**[2], **Robert Haas**[1,2], **Joanna Segal**[2], **David Alejandro Peña Navarro**[9], **Lucia Herrera-Dominguez**[2], **Oscar Méndez-Lucio**[7], **Jakob Vowinckel**[1,6], **Michael Mülleder**[1,2,3], **Markus Ralser**[1,2,3,*]

[1]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

[2]The Molecular Biology of Metabolism Laboratory, The Francis Crick Institute, London, United Kingdom

[3]Department of Biochemistry, Charité University Medicine, Berlin, Germany

[5]Warwick Medical School, University of Warwick, Warwick, United Kingdom

[7]Facultad de Química, Departamento de Farmacia, Universidad Nacional Autonóma de México, Mexico City, Mexico

[9]Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences, Vienna, Austria

[10]Department of Genetics, Evolution & Environment, University College London, London, United Kingdom

## Summary



**Correspondence and requests for materials** should be addressed to MR.
[4]Present address: Department of Nutrition Physiology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[6]Present address: Biognosys AG, Schlieren, Switzerland
[8]Present address: Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom



**Competing interests** The authors declare no competing interests.

**Author Contributions**

V. O-S. identified cadaverine and the enzyme responsible for lysine decarboxylation, cloned *SPE1* and carried out the expression, purification and kinetic characterization of recombinant Spe1p, the quantification of lysine in wild-type and *Δtpo1* strains, the lysine uptake rate experiment in auxotroph and prototroph strains, the growth curves experiment of *Δzwf1* in media supplemented with lysine and methionine, the quantification of GSH-NEM and polyamines and several experiments to identify the function of cadaverine. J.S.L.Y performed the $H_2O_2$ stress response experiments in yeast exposed to D/L-lysine and, together with D.A.P.N., sourced the materials and performed the NADPH sensor experiments. L.M.F performed experiments to uncover a function for cadaverine, cloned *ldc*C, performed the stress tests with Δ*zwf1* and analysed ROS levels by flow cytometry. M.T.A performed metabolic modelling and FBA analysis. S.K. conducted the experiment that assessed the extent of harvesting for all amino acids and $H_2O_2$ resistance in wild yeasts; C.C-M tested interactions between $H_2O_2$ with lysine and methionine; R.H. addressed the effect of cadaverine on growth; J.S. addressed the role of lysine harvesting in mammalian cells; D.A.P.N assessed $H_2O_2$ resistance in *P. pastoris*; L. H-D. conducted bacterial stress test experiments; O.M-L. the molecular docking experiments; J.V. conducted proteomic data analysis; M.M. developed metabolomics method and conducted LC-MS measurements; and M.R. conceived and supervised the study and wrote the first draft of the paper. All authors contributed to the experimental planning and writing up of the study.


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Both single and multicellular organisms depend on anti-stress mechanisms that enable them to deal with sudden changes in the environment, including exposure to heat and oxidants. Central to the stress response are dynamic changes in metabolism, such as the transition from the glycolysis to the pentose phosphate pathway- a conserved first-line response to oxidative insults[1,2]. Here we report a second metabolic adaptation that protects microbial cells in stress situations. The role of the yeast polyamine transporter Tpo1[3–5] in maintaining oxidant resistance is unknown[6]. However, a proteomic time-course experiment suggests a link to lysine metabolism. We reveal a connection between polyamine and lysine metabolism during stress situations, in the form of a promiscuous enzymatic reaction in which the first enzyme of the polyamine pathway, Spe1p, decarboxylates lysine and forms an alternative polyamine, cadaverine. The reaction proceeds in the presence of extracellular lysine, which is taken up by cells to reach concentrations up to one hundred times higher than those required for growth. Such extensive harvest is not observed for the other amino acids, is dependent on the polyamine pathway and triggers a reprogramming of redox metabolism. As a result, NADPH- which would otherwise be required for lysine biosynthesis- is channelled into glutathione metabolism, leading to a large increase in glutathione concentrations, lower levels of reactive oxygen species and increased oxidant tolerance. Our results show that nutrient uptake occurs not only to enable cell growth, but when the nutrient availability is favourable it also enables cells to reconfigure their metabolism to preventatively mount stress protection.

Tpo1p is a polyamine exporter that is found in *Saccharomyces* species [3–5] and is important for their tolerance to oxidants [6]. We re-analysed a proteomic time-course experiment that was recorded with sequential window acquisition of all theoretical mass spectra (SWATH-MS)[6] using a recent workflow[7]. Differentially expressed proteins were enriched for the following Gene Ontology terms: proliferation and cell cycle, nucleic acid biosynthesis, redox metabolism and stress response (Extended data Fig. 1). Moreover, several enzymes of the lysine biosynthetic pathway (Extended data Fig. 2), Lys20p, Lys21p, Aco2p, Lys9p and Lys12p- showed a faster and more pronounced response to $H_2O_2$ exposure upon deletion of the *TPO1* gene (*Δtpo1*) (Fig. 1a). A potential link between the polyamine and the lysine biosynthetic pathways was provided by their chemistry: the decarboxylation of lysine forms cadaverine, a close structural analogue of putrescine, which is the canonical precursor for spermine and spermidine, substrates of Tpo1p[3–5] (Fig. 1b). Notably, although lysine decarboxylation has been reported in bacteria and plants, *Saccharomyces* lacks an enzyme to carry out this transformation[8–11]. To address the possibility that a non-enzymatic or a promiscuous reaction could generate cadaverine, we quantified its intracellular levels. Trace amounts (0.26 ± 0.052 pmol per biomass unit ($OD_{600nm}$)) were detected in minimal medium but increased 22-fold upon supplementation of a physiological amount (25 μg $mL^{-1}$) of lysine (Fig. 1c).

The search for the reaction responsible for the production of cadaverine was guided by the structural similarity of lysine and the putrescine precursor ornithine, and led us to test the enzyme ornithine decarboxylase (Spe1p) (Fig. 1d). Deletion of the gene that encodes this enzyme (Δ*spe1*) did not deplete trace amounts of cadaverine, but did eliminate any increase upon lysine supplementation (Fig. 1c). We then conducted an isotope tracer experiment, which involved feeding cells with [$^{13}C_6$ $^{15}N_2$]lysine. Wild-type cells formed large amounts of [$^{13}C_5$ $^{15}N_2$]cadaverine; however, in Δ*spe1* cells, none of the tracer was converted into

cadaverine (Fig. 1e). Experiments at the pathway level substantiated the role of Spe1p. First, the concentration of cadaverine was found to be lower in Δ*spe2*, Δ*spe3* and Δ*spe4* strains, in which Spe1p is feedback-inhibited as a consequence of the polyamine supplementation necessary to grow these strains [12] (Fig. 1c). Conversely, exposure to lysine increased the levels of putrescine in these strains, which indicates inhibition of the downstream pathway (Extended data Fig. 3a).

We then obtained a 3D structure of Spe1p (UniProt ID: P08432 [13]) by homology modelling, using *Leishmania donovani* (PDB ID: 2OO0[14]) ornithine decarboxylase as a template. Molecular docking suggested that both ornithine and lysine can bind to the catalytic site (Fig. 1f). Both compounds are stabilised by hydrogen bonding between the amino group in the ligand and Asp363 and Tyr354, along with a structural water molecule. However, although lysine fits in the binding site, it needs to overcome steric hindrance in order to share the catalytic site with pyridoxal phosphate. This indicates that Spe1p should have a much lower affinity for lysine than for ornithine. To test this prediction, we cloned, expressed and purified Spe1p, and analysed its catalytic properties (Table 1). Spe1p was found to decarboxylate both ornithine and lysine (Fig. 1g, h), although its affinity for ornithine and the catalytic efficiency were much higher than for lysine. Indeed, the Michaelis-Menten constant (*Km*) for lysine decarboxylation was only in the millimolar range (Fig. 1h, Table 1). However, Spe1p produced considerable amounts of cadaverine in vivo (Fig. 1 c, e), and so we concluded that lysine must have been present at very high concentrations.

We continued to ask whether- and if so, under what conditions- high lysine concentrations can emerge in vivo. The lysine biosynthetic capacity is sufficient for cell growth, and supplementing lysine did not accelerate yeast growth (Extended data Fig. 3b). Under exponential growth in minimal medium, we measured lysine concentrations of 4.93 ± 1.82 nmol per $OD_{600nm}$. Assuming a cell volume of 45.54 fl and a concentration of 3.2 x $10^7$ cells per $OD_{600nm}$ [15], this corresponds to average concentrations of 3.01 ± 1.12 mM and classes lysine as a highly concentrated metabolite[16]. However, this concentration would be insufficient to explain cadaverine formation given the catalytic properties determined for Spe1p (Table 1). We then noted that the main lysine transporter, Lyp1p, was reported to have low affinity for intracellular lysine, leading to unidirectional import [17]. Upon supplementation of 25 μg $mL^{-1}$ lysine we observed a 71.2-fold increase in intracellular lysine levels to 322.75 nmol per $OD_{600nm}$. Corresponding to an approximated average lysine concentration of 215.2 mM, such levels fully suffice to explain cadaverine formation from Spe1p. As such high concentrations (Fig. 2a, Table 1) are, however, not required for growth, we refer to this phenomenon as ‘lysine harvesting’. When *TPO1* was deleted, the harvesting was less effective. Upon supplementation of the Δ*tpo1* strain with 25 μg $mL^{-1}$ lysine we observed a 42.5-fold increase in intracellular lysine concentration, which is less than half the increase seen with the wild-type strain (71.2-fold) (Fig. 2a).

Yeast cells take up a range of amino acids [18], and we next tested whether they are harvested to a similar extent. Regardless of whether amino acids were supplemented individually or as a complex mixture (yeast extract), only lysine concentrations increased to such marked levels (Fig. 2b).

The lysine biosynthetic pathway is prone to feedback inhibition [19–21]. Indeed, we found that prototrophs consumed lysine at the same rate as auxotrophs (Fig. 2c), indicating that harvesters fully switch from self-synthesis to consumption. To determine the metabolic changes induced, we expanded a yeast metabolic network reconstruction [22] to include the new reactions discovered and performed flux balance analysis (FBA) (Fig. 3a). FBA predicted major changes within NADPH-forming reactions- reflecting the fact that when cells harvest lysine, less NADPH is required for growth (Extended data Fig. 4). The growth-optimized model consequently predicted that there would be a reduced flux to NADPH-producing pathways, including the pentose phosphate pathway (PPP) [23] (Extended data Fig. 4). However, a second biological possibility was that- rather than diminishing the production od NADPH- cells could instead channel NADPH into other metabolic processes. We used phenotypic assays to test which of the two scenarios applied in this case. Cells that lacked glucose 6-phosphate dehydrogenase (Δ*zwf1*) are auxotrophic for methionine, as the high demand for NADPH in methionine biosynthesis cannot be met [24,25]. Lysine harvesting complemented the auxotrophy, indicating that there was a greater availability of NADPH in lysine harvesters (Fig. 3b). Next, we used an oxidant-dependent sensor assay[26] to measure the NADPH-mediated reducing power upon exposure to $H_2O_2$. We found that lysine harvesters increased the redox potential of NADPH (Fig. 3c, d).

Increased NADPH levels stimulate glutathione biosynthesis by increasing the activity of γ-glutamylcysteine ligase [27]. We therefore constrained the FBA model in the second iteration, optimising the NADPH consuming glutathione oxidoreductase reaction. This FBA agreed with our experimental results, which suggested that, during harvesting, excessive lysine would be channelled towards polyamine metabolism. The model also predicted that flux through the glutathione oxidoreductase would increase sevenfold (Fig. 3a). We therefore quantified glutathione, and found that lysine harvesting promoted a 7.85-fold increase in its concentration (Fig. 3e).

To assess the resistance of the yeast cells to oxidants, we first applied diamide, a thiol oxidant to which resistance is known to increase with NADPH availability [28–30]. Lysine harvesting increased resistance to diamide (Fig. 3f). We compared this treatment to methionine supplementation, which is also reported to increase tolerance to diamide [30]. Lysine harvesting provided a higher degree of resistance to diamide that did methionine supplementation (Fig. 3f). Furthermore, we tested Δ*zwf1* cells, in which less NADPH is available for antioxidant enzymes. Consistently, we found that, although lysine harvesting restored prototrophy, the stress tolerance of these cells did not reach wild-type levels (Fig. 3f). In parallel, lysine harvesting substantially increased resistance to $H_2O_2$ (Fig. 3g). Furthermore, lysine harvesters maintained much lower levels of reactive oxygen species in the presence of 10 mM $H_2O_2$ (Fig. 3h).

Control experiments confirmed that the metabolic reconfiguration, and not a direct antioxidant action of lysine, explained the protection against stress. First, when instead of L-lysine we supplied D-lysine- which has identical redox properties- cells became oxidant-sensitive (Fig. 3g). Furthermore, pre-incubation of $H_2O_2$ with lysine did not mitigate the cellular response (Extended data Fig. 5). Additionally, we studied a panel of mammalian cell lines (HA1E, HeLa, A549 and BJhTERT). We confirmed that these cells also take up lysine

(Extended data Fig. 6); however, because they are native lysine auxotrophs, they cannot benefit from the re-channelling of NADPH flux and were not stress protected (Extended data Fig. 7a). We next tested whether lysine harvesting protects other prototrophs. Separated by billions of years of evolution, yeast strains isolated from palm wine in Cameroon and from a bakery in Korea, respectively (OS473 and OS265) [31], *Pichia pastoris* and *Candida tropicalis* (Extended data Fig. 7b, c), as well as *Bacillus subtilis* (Extended data Fig. 7d), all gained oxidant resistance. This demonstrates the conservation of this antioxidant strategy across several major clades of life.

In conclusion we have shown that lysine is harvested by cells in large quantities, rendering them resistant to oxidants. On the molecular level, the harvest has at least two consequences. First, a promiscuous reaction of ornithine decarboxylase reaches relevant rates and forms an underground polyamine metabolite, cadaverine. We could not confirm a chemical antioxidant role for cadaverine in mediating stress protection (Supplementary Notes 1 and 2, Extended Data Figs. 8, 9, Extended Data Table 1). However, the polyamine pathway is implicated in the regulation of expression of lysine biosynthetic enzymes and is required to import the concentrations of lysine that are found in wild-type cells. Future studies will be required to investigate how the changes in polyamine levels lead to the observed changes in enzyme expression and lysine concentration. We speculate that these could be a direct consequence of the role of polyamines in transcription and translation [32]. Second, the lysine harvest results in a metabolic reconfiguration that channels more NADPH into glutathione metabolism, reducing levels of reactive oxygen species and increasing tolerance to stress. Therefore, lysine harvesting adds to the series of mechanisms that stimulate NADPH production to prevent imbalances in the redox state under oxidative conditions [1,2,33]. However, to our knowledge, none of the previously reported mechanisms act to preventatively induce an approximately eightfold increase in the pool of reduced glutathione- already one of the most concentrated cellular metabolites. Quantitatively speaking, lysine harvesting may be one of the most powerful preventative metabolic antioxidant strategies available to microbial cells. Cells sense and take up metabolites not only to enable cell growth, but also- when the metabolic environment is favourable- to preventatively reconfigure their metabolism to become more robust.

## Methods

### Yeast cultivation and growth analyses

Haploid wild-type (WT), Δ*spe1*, Δ*spe2*, Δ*spe3,* Δ*spe4* strains of the S288c background (BY4741 and BY4742 derivatives [34]) transformed with the plasmid pHLUM (to repair auxotrophic markers [35]) were grown in synthetic minimal (SM) media (6.8 g $l^{-1}$ yeast nitrogen base (Sigma-Aldrich)), supplemented with 2% glucose as carbon source. In Δ*zwf1* and Δ*tpo1* strains of the *MAT*a yeast knockout collection we detected secondary mutations and recreated the strains by using homologous recombination [30,36,37]. The Δ*spe1*-Δ*spe4* strains are auxotrophic for spermidine and were hence were grown in the presence of 0.1 mM spermidine and with or without 25 μg $ml^{-1}$ lysine as indicated. Wild-type and Δ*tpo1* cells were grown in SM media with or without 25 μg $ml^{-1}$ lysine, as indicated. Δ*zwf1* was grown in SM supplemented with 40 μg $ml^{-1}$ methionine or 100 μg $ml^{-1}$ lysine. The *MAT*α

wild-type strain of the S288c background BY4742 transformed with the plasmids pHLU or pHLUK, to repair auxotrophic markers [35], were grown in SM or synthetic complete (SC) media (0.56 g $l^{-1}$ complete supplement mixture (MP Biomedicals) and 6.8 g $l^{-1}$ yeast nitrogen base (Sigma-Aldrich)) supplemented with 2% glucose, with or without lysine. To complement the polyamine auxotrophy of Δ*spe1* cells, the cells were pre-grown in SM media supplemented with 2 μM of spermidine. Subsequently, they were diluted to $OD_{600nm}$ = 0.2 in SM media supplemented or not with 0.1 mM spermidine and/or 250 mM cadaverine.

For measuring growth rates, wild-type and Δ*zwf1* cells were transferred from cryostock to solid SM media supplemented with 40 μg $ml^{-1}$ methionine or 100 μg $ml^{-1}$ lysine. Then 2 mL pre-cultures were grown in SM, SM + Met (colonies from SM + Met solid media) and SM + lysine (colonies from SM + lysine solid media), overnight at 30°C. The cultures were diluted to $OD_{600nm}$ = 0.2 and incubated at 30°C. The growth was monitored in a multimode detector (Tecan Infinite 200 Pro) and $OD_{600nm}$ was recorded until the cultures reached a stationary phase. Data were analysed with the "grofit" R package [38] and illustrated using Origin Pro 9.0.

To determine the effect of lysine supplementation on yeast growth, pre-cultures of the wild-type (BY4741) were grown overnight in SM media with or without lysine (25 μg $ml^{-1}$) supplementation, diluted to an $OD_{600nm}$= 0.2-0.3 and incubated at 30°C. The growth was monitored in a multimode detector (Tecan Infinite 200 Pro) and recorded until the cultures reached the stationary phase. Data were analysed with the "grofit" R package and illustrated using Origin Pro 9.0.

To determine the effect of the presence of *Escherichia coli* lysine decarboxylase (LdcC) on growth, overnight pre-cultures from prototrophic yeast were transformed with the plasmid pYX212 or with pYX212 to express the gene encoding *E. coli* lysine decarboxylase (*LdcC*) grown in SM medium, and were diluted to an $OD_{600nm}$ = 0.2 in SM media supplemented or not with 250 μg $ml^{-1}$ lysine and incubated at 30°C until the cultures reached the stationary phase.

## Gene amplification and cloning

Genomic DNA from *S. cerevisiae* was isolated, and the *SPE1* coding sequence was amplified by PCR using primers CGTACCATGGATGTCTAGTACTCAAGTAGG (sense) and CATCCTCGAGATCGAGTTCAGAGTCTATG (antisense) to introduce a NcoI and XhoI restriction site, respectively. The PCR product was cloned into the pGEM-T Easy vector (Promega) and *E. coli* DH5α was transformed with the plasmid. Plasmids were verified by sequencing. Then, the *SPE1* coding sequence was subcloned into the NcoI and XhoI sites of the pET20 plasmid (Novagen) in order to express a His-tagged protein.

For the amplification and cloning of *E. coli* LdcC, the coding sequence was amplified from genomic DNA using the primers AAAACACATACAGGAATTCACCATGGATCCTAGGGCCCACATGAACATCATTGCCATTAT and CGTAGTCAGGCACATCATACGGATACCCGGGTCGACGCGTTTATCCCGCCATTTTT

AGGA. The vector pYX212 (Ingenius, R&D systems) was linearized with HindIII. Both PCR products and linearized vector were transformed into yeast for homologous recombination. The construct was verified by sequencing.

### Recombinant protein expression and purification

*E. coli* BL21 was transformed with the plasmid for expression of *S. cerevisiae* 6xHIS-Spe1p. A 5 mL pre-culture was grown in Luria Bertani medium with 100 μg $ml^{-1}$ ampicillin overnight at 37°C. The next day, 10 ml of medium was inoculated with the overnight culture, cells were grown until an $OD_{600nm}$= 0.6 and protein expression was induced with 1 mM isopropyl-β-D-thiogalactoside, upon which the cells were grown for 4 h at 20°C. The cells were collected and frozen at -80°C. Then the frozen pellet was resuspended in 200 mL of lysis buffer (Tris-Cl 50 mM pH 7.4, 300 mM NaCl, 10 mM imidazole, 1 mM DTT, 1 tablet of EDTA-free protease inhibitors). The cells were lysed in a cell disruptor (CF Range, Constant Systems) by passing the cell suspension three times at 30,000 psi. The lysate was then ultracentrifuged at 185,511*g* at 4°C for 30 min. Spe1p was purified by IMAC on a HisTrap (Nickel) column (5 mL; GE Healthcare). The fractions with the peak of the protein of interest were combined and concentrated by centrifugation (2939g, 5°C) through an Amicon Ultra-15 (molecular cut-off 30 kDa, Millipore). The concentrated protein (500 μL) was injected to a Superdex S200 gel filtration column (10 x 300 mm; GE Healthcare) equilibrated with a buffer containing Tris-Cl 50 mM pH 7.4, NaCl 300 mM, 1 mM DTT and EDTA-free protease inhibitor tablets (1 tablet per 100 mL). The fractions with the purest Spe1p were identified by SDS-PAGE, combined and concentrated as mentioned above. 10% glycerol was added for preservation and the protein was stored at -80°C.

### Proteomics data analysis

The SWATH-MS data were previously reported [6], and are available via ProteomeXchange with identifier PXD013373. Raw data were analysed using Spectronaut Pulsar X software (Biognosys) with a spectral library generated previously (ProteomeXchange ID PXD013373). Protein quantities were calculated from all precursor intensities, and protein inference was performed using a protein database obtained from SGD (2018-08-28). Gene Ontology (GO) enrichment was performed with the Spectronaut software using a gene annotation file obtained from SGD (2019-01-01). Data were analysed in R, with function “prcomp” for principal component analysis.

### Metabolite quantification

**Amino acids—**For measuring lysine levels, the yeast cells were pre-cultured overnight in 50 mL of SM media with or without lysine (25 μg $ml^{-1}$) supplementation, and main cultures were initiated by diluting to $OD_{600nm}$ = 0.2-0.3 in 100 mL of SM media with or without lysine (25 μg $ml^{-1}$) supplementation. Cultures were grown until the mid-log phase ($OD_{600nm}$ approximately 1.0), samples for amino acid profiling (1 $ODU_{600nm}$) were collected and the pellet was frozen in dry ice. The samples were stored at -80°C.

For the profiling experiments, the strain BY4741 with auxotrophic loci repaired (HLUM knock-in) was pre-grown on YPD agar medium. Four colonies were picked and grown in 10 ml overnight SM pre-cultures. In a 96-deep-well plate, 1.4 ml cultures with a starting

$OD_{600nm}$ of 0.2, unsupplemented or supplemented with individual proteinogenic amino acids (except cysteine, which inhibits growth, and tyrosine, which is of low solubility) at a concentration of 0.334 mM or yeast extract (2%), were grown at 30°C for 6h with shaking.

The amino acid extraction, separation and detection protocols were adapted from a previous study[15]. 80% ethanol (100 µl) at 80°C was added to the frozen yeast pellet (1 $ODU_{600nm}$) from a mid-log phase culture, and the sample was incubated at 80°C for 2 min. Then, the sample was vortexed. The last two steps were repeated twice. The samples were centrifuged at 12,000*g* for 5 min. The supernatants were collected and 1 µL was injected onto an analytical column (Waters ACQUITY UPLC BEH amide 1.7mm 2.1x100 mm) at 25°C. Buffer A consisted of 50:50 acetonitrile/water, 10 mM ammonium formate, 0.176% formic acid and buffer B was composed of 95:5:5 acetonitrile/methanol/water, 10 mM ammonium formate and 0.176% formic acid. The separation was performed at 0.9 mL/min isocratic flow with a gradient consisting of the following steps: 0.7 min 85% Buffer B followed by a 1.85 min ramping to 5% B, and keeping constant for 0.05 min before returning to the equilibrating conditions of 85% B. The total running time was 3.25 min. The quantification was carried out using a triple quadrupole mass spectrometer (Agilent 6460).

**Polyamines—**For the dansylation derivatization method, polyamines were quantified upon a derivatization with dansyl chloride (dansylation), following a protocol adapted from previous work[39]. Glass beads (200 mg) were added to the frozen cell pellets and were resuspended in 500 µL of 0.2 M $HClO_4$. Then, the cells were lysed in a homogenizer (FastPrep-24 5G MP Biomedicals) using 2 cycles of 20 s, 6.5 m $s^{-1}$ at 4°C. The cell debris was removed by centrifugation at 12,000*g* for 10 minutes at 4 °C and 100 µl were neutralized with 3M sodium carbonate (10 µl). Then, 200 µl of 1,3-diaminopropane was added as an internal standard. The polyamines were dansylated by adding 400 µl of dansyl chloride in acetone (27 mM) to 100 µL of the sample and incubated at 60°C for 20 min in the dark. Then, 120 µl of a 0.8 M proline solution was added to the sample, vortexed and incubated at 25°C for 30 min in the dark. Then, an extraction with 500 µL of toluene was performed. The organic phase was taken and the solvent was removed under a $N_2$ stream. Then, pellets were resuspended in 100 µl acetonitrile. One microlitre of the polyamines sample was separated using chromatography (Zorbax Eclipse Plus C18, RRHD, 2.1 x 50 mm, 1.8 µm) at 20°C. Buffer A consisted of 0.1% formic acid in water, and buffer B was acetonitrile. The separation gradient consisted of an isocratic flow (0.5 mL $min^{-1}$) for 0.4 min of 60% B, followed by a ramp of 3.1 min to 100% B, then washing 0.3 min with 100% B, then returning to starting conditions (60% B) in 0.01 min with 1.19 min for re-equilibration, resulting in a 5-min cycle time. The quantification was carried out using a triple quadrupole mass spectrometer (Agilent 6460).

In the non-derivatised method, for enzyme assays, 1 µl of the supernatant obtained from the kinetic assay was injected onto an analytical column (ACQUITY UPLC BEH amide 1.7 mm 2.1 x 100 mm), and putrescine and cadaverine were separated by HPLC. Buffer A consisted of 2:98 acetonitrile:water, 0.176% formic acid and buffer B was composed of 95:5 acetonitrile:water, 0.176% formic acid. The separation gradient consisted of a 0.5 mL $min^{-1}$ isocratic flow with the following steps: 0.25 min 100% Buffer B followed by a 0.25 min ramping to 72% B and keeping constant for 0.5 min. Then, a 1.5 min ramp to 30% B was

done and kept constant for 0.5 min. This was followed by a 1.5-min ramp to 30%B, which was kept constant for 0.5 min. Next there was a third 0.15 min ramp to 20% B which was kept constant for 0.45 min before returning to the equilibrating conditions (100% B). The total running time was 5 min. The quantification was carried out using a triple quadrupole mass spectrometer (Agilent 6460).

**GSH—**For GSH determination, 5 $OD_{600nm}$ of mid-log phase wild-type cell cultures were collected and quenched in one volume of 4 mM N-ethylmaleimide (NEM) in methanol previously cooled in dry ice [40]. The pellet was resuspended in 200 µl of a buffer composed of 75:25 (v/v) acetonitrile:methanol, 0.2% formic acid and lysed in a homogenizer (FastPrep-24 5G MP Biomedicals) using three cycles for 20 s at 6.5 m $s^{-1}$. Then, the sample was centrifuged for 5 min at 16,000*g*. The pellet was extracted again with 200 µl of UHPLC water and both supernatants were combined and evaporated in a concentrator plus speedvac (Eppendorf), resuspended in 100 µl of 7% acetonitrile and centrifuged. A 1 µl of the sample was injected onto a C8 column (ZORBAX SB-C8 Rapid Resolution HD, 2.1 x 100 mm, 1.8 µm (Agilent)) at 20°C. Buffer A was composed of 10% acetonitrile and buffer B of 50% acetonitrile (v/v). Both buffers contained 750 mg $l^{-1}$ octylammonium acetate as ion-pairing reagent. The gradient consisted of 3.5 min of isocratic flow at 12% acetonitrile, followed by a 2.5-min gradient to 38% acetonitrile and a 0.5-min washing step to 42% acetonitrile and 1 min of re-equilibration to starting conditions with a total cycle time of 7.5 min. The quantification was carried out using a triple quadrupole mass spectrometer (Agilent 6470).

## Nutrient uptake rates

Nutrient uptake rates were determined as described previously[37]. *S. cerevisiae* (BY4742) was transformed with the pHLU and pHLUK [35] to compensate for auxotrophies including or excluding lysine, and were streaked on SM agar media with or without lysine supplementation, respectively. Then pre-cultures inoculated in SM or SM + Lys media were grown overnight at 30°C. The main cultures were initiated with $OD_{600nm}$ = 0.125 in 850 µl of SC or SM + Lys media in 96-deep-well plates and incubated for 30h at 950 r.p.m. and 30°C with a 4-mm stirring bead per well. Then, 20 µl of cells were sampled every 3h and diluted 1:10 in water for $OD_{600nm}$ measurement. The cells were collected at 3,000*g* for 5 min and the supernatant was diluted 1:2 in absolute ethanol. A 1-µL portion of the supernatant was used for quantification of extracellular lysine by LC/MS-MS.

## Diamide stress assays

Cells were grown overnight in liquid minimal media with lysine (50 µg $m^{-1}$) or methionine (40 µg $ml^{-1}$). The following day, the cultures were diluted to an $OD_{600nm}$ = 1 in water, serial diluted (1:10) and spotted onto SM media containing either lysine (50 µg $ml^{-1}$) or methionine (40 µg $ml^{-1}$) and different concentrations of diamide (0-1.5 mM). Plates were incubated for 2-3 days.

## Hydrogen peroxide stress assays

Single wild-type colonies were isolated and resuspended in 70 µl of SM medium. A portion of the cell suspension (20 µl) was then transferred into the 3 conditions (SM, SM + L-Lys, SM + D-Lys, (L/D-Lys at 25 µg $ml^{-1}$)) and cultured overnight in a 3 ml culture volume at

30oC with shaking. The following day, the pre-cultures were diluted to $OD_{600nm}$ = 0.1 and 25 µl was used to inoculate 450 µl of respective media in a deep-well plate (450 µl media, 25 µl preculture, 25 µl $H_2O_2$) for a final $OD_{600nm}$ of 0.005 and incubated for 16 h with $H_2O_2$ (0, 0.125, 0.25 and 0.5 mM). The resultant $OD_{600nm}$ was measured to assess the tolerance of the cells to oxidant stress. For NADPH sensor experiments, wild-type auxotrophic yeast strains (BY4741 and BY4742) were transformed with a centromeric plasmid that permits constitutive expression of mKate2 in conjunction with Yap1p-ox dependent eGFP [26] selectable with *URA3* alone or in conjunction with the set of centromeric plasmids (pH, pL, pM or pK [35]) that permit the restoration of prototrophy. After selection on SM + HLM/K or SM plates respectively, a single colony was isolated and used to inoculate pre-cultures under the three conditions listed above. After exposure to $H_2O_2$, 200 µl of the cultures were transferred to a 96-well plate and subjected to high-throughput flow cytometry (BD Fortessa X20B/HTS) and analysed by FlowJo v9.6.2 (for gating strategy information, see Supplementary Figure 1). Median fluorescence intensity (MFI) was then obtained for both mKate2 and eGFP. mKate MFI values were then used to normalize the eGFP values. Each condition was set up and analysed in quadruplicate.

### ROS quantification by dihydrorhodamine staining

Wild-type cells were grown in SM medium until mid-log phase. Then, cells were incubated with $H_2O_2$ (10 mM) for 1h. Then, cells were collected, washed three times in SM medium, and incubated for 90 minutes in the dark at 30°C with 15 µM dihydrorhodamine 123 (DHR 123). Cells were washed once with media and stained for 5 min with propidium iodide (500 µg $ml^{-1}$) to exclude dead cells, and subsequently washed three times with PBS. Cells were analysed by flow cytometry (BD Fortessa X20B/HTS) and quantified using FlowJo v9.6.2.

### Direct effect of lysine (Lys) and methionine (Met) supplementation on the oxidative capacity of media containing $H_2O_2$

To test for a direct chemical reaction of L-lysine, D-Lysine and L-methionine with $H_2O_2$, these metabolites were supplemented into either $H_2O$ or SM medium at a concentration of 25 µg $ml^{-1}$ for lysine and 40 µg $ml^{-1}$ for methionine. Samples were buffered to pH 7.2 and treated with 2.5 mM of $H_2O_2$ for 0 or 30 min. $H_2O_2$ levels were measured using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (ThermoFisher Scientific, Cat no. A22188). Just before analysis, samples were diluted 1:1000 in Amplex Red reaction buffer to keep within the limits of detection of Amplex Red. Amplex Red analysis was performed according to the manufacturer's instructions. Fluorescence detection was performed using a multimode detector (Tecan Infinite 200 Pro). For testing the direct effect of lysine and methionine supplementation on cell growth in medium containing $H_2O_2$, pre-cultures of the wild-type (BY4741-pHLUM) strain-grown overnight in SM media with or without supplementation with lysine (25 µg $ml^{-1}$) and methionine (40 µg $ml^{-1}$), were diluted to $OD_{600}$= 0.1 and incubated at 30°C and 180 rpm. Cells were grown until the mid-log phase ($OD_{600nm}$~ 0.5), washed, and treated with 2.5 mM of $H_2O_2$ that had previously been incubated in the respective fresh culture medium for 0 or 30 minutes. The growth was monitored in a multimode detector (Tecan Infinite 200 Pro) and recorded until the cultures reached the stationary phase. Data were analysed with the "grofit" R package and illustrated using Origin Pro 9.0.

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### Protective effect of lysine supplementation against $H_2O_2$ in different yeast and bacteria species

The protective effect of lysine against $H_2O_2$ was confirmed in two non-laboratory isolates of *S. cerevisiae*, OS473 (from palm wine, Cameroon) and OS265 (from a bakery in South Korea) [31], as well as *C. tropicalis* (from the National Collection of Yeast Cultures, accession NCYC4). Strains were pre-grown on YPD media and a single colony was suspended in 1 ml of SM media and grown for 6 h with shaking. A 10-μL portion was transferred to a fresh 1.8-ml culture in SM and SM + lysine (25 μg $ml^{-1}$) and grown overnight with shaking. Culture density was measured, and 200 μl of SM culture with a starting $OD_{600nm}$ of 0.005- with or without lysine (25 μg $ml^{-1}$, using matching pre-culture) and with or without 1.5 mM $H_2O_2$- was set in a 96-well plate. The $OD_{600nm}$ was measured every 15 min, with 15 s of shaking just beforehand, in a Tecan Infinite M200 Pro at 30°C.

In addition, we also tested the protective effect of lysine in the Gram-positive bacteria *B. subtilis* 168 trpC2. The strain was pre-cultured at 37°C in S7 minimal media supplemented with 40 μg $ml^{-1}$ tryptophan. Next, the culture was back-diluted to an $OD_{578nm}$ of 0.01 in fresh minimal media and 65 μl was placed in 384-well plates supplemented with 0 or 40 μg $ml^{-1}$ of lysine. $H_2O_2$ was added at the mid-log phase to reach a final concentration of 1.5 mM; the equivalent volume of water was added to the control. The $OD_{578nm}$ was measured every 10 min with cycles of orbital and linear shaking in between measurements in a Tecan Infinite M200 Pro at 37°C (n = 6).

### Effect of the supplementation of lysine on growth and response to oxidative stress in mammalian cells

The mammalian cell lines used were HA1E, HeLa, A549 and BJhTERT, which were supplied by the Crick cell services. Cell authentication was performed using short tandem repeat (STR) profiling. In addition, the species were confirmed using a primer system based on the cytochrome C oxidase subunit 1 gene from mitochondria. For mycoplasma screening, two different tests were used: agar culture (which involves culturing any mycoplasma that may be present in the cell culture on specialised agar) and fluorescent staining (using the Hoechst Stain). For all experiments, cells were maintained in DMEM for stable isotope labelling by amino acids in cel culture (SILAC) (Thermo Scientific) supplemented with 0.4 mM L-arginine HCl (Sigma-Aldrich), 10% dialysed serum (Gibco), and L-lysine HCl (Sigma-Aldrich) at various concentrations. For cell growth assays, cells were seeded at 4,000 cells per well in a 96-well plate in seven different lysine concentrations ranging from 0 mM to 10 mM, in triplicate. The percentage confluence was calculated using an IncuCyte imaging system (Essen Bioscience).

For intracellular lysine quantification, cells were seeded at 0.5 x $10^6$ cells per 6-cm plate at four lysine concentrations (0.01, 0.1, 1 or 10 mM), in triplicate. Cells were grown for 48h before collection. To begin amino acid extraction, the plates were washed once with 37°C PBS and then the cells were trypsinized until they were detached from the plate. The detached cells were collected in 4°C PBS then spun at 300g for 2 min at 4°C. 80% ethanol (100 μl) was added to the pellet and the tubes vortexed to resuspend. The samples were incubated at 95°C for 2 min followed by vortexing. The last two steps were repeated once

more. The tubes were then spun at 20,000g at 4°C for 2 min and the supernatant was analysed.

For oxidant resistance experiments, cells were seeded at 15,000 cells per well in a 96-well plate at four different lysine concentrations (0.1 mM, 1 mM, 5 mM or 10 mM), in triplicate. After 24 h, $H_2O_2$ (Sigma-Aldrich) was diluted in water to a range of concentrations and added directly to the culture media. After 24 h of incubation, the plates were fixed for 5 min in 10% neutral buffered formalin (Sigma-Aldrich) and stained with crystal violet staining solution (Sigma-Aldrich) for 10 min. After washing, the cells were lysed in a citrate buffer (pH 4) and the absorbance at 595 nm was recorded using a spectrophotometer (Tecan Life Sciences).

## Identification of the candidate enzymes catalysing the biosynthesis of cadaverine, $^{13}C$ carbon tracing

Wild-type, Δ*spe1,* Δ*spe2,* Δ*spe3* and Δ*spe4* yeast were grown overnight in 10 ml of SM media with or without 0.1 mM spermidine in the presence and absence of 25 μg $ml^{-1}$ of lysine. Then, 10 $ODU_{600nm}$ were collected and cadaverine was quantified by LC/MS-MS. For the carbon-tracing experiment, wild-type and Δ*spe1* cells were grown overnight in 100 ml of SM media with 0.1 mM spermidine. Then, [$^{13}C_6$ $^{15}N_2$]L-lysine was added to a final concentration of 0.12 mM and samples (10 $OD_{600nm}$) were collected after 0 and 60 min. [$^{13}C_5$ $^{15}N_2$]cadaverine was quantified by LC-MS/MS protocol that involves dansylation (see the ‘Metabolite quantification’ section in Methods).

## Molecular docking

The 3D structure of ornithine decarboxylase from *S. cerevisiae* (Spe1p; UniProt ID: P08432 [13]) was obtained by homology modelling. The homology model was generated using Prime from the Schrödinger suite (v. 2015-3) and the structure of *L. donovani* ornithine decarboxylases (PDB ID: 2OO0 [14]) as template. The cofactor (vitamin B6 phosphate) and structural waters in the binding site were kept in the next steps. Ligand and protein structures were prepared using LigPrep and the Protein Preparation Wizard, respectively. The docking calculation was performed with Glide form Schrödinger Suite (v. 2015-3) using GlideXP settings and the GlideScore version XP5.0 scoring function. The grid size used for calculation was 20 × 20 × 20 Å. The conformation with the best score was reported.

## Kinetic characterisation of Spe1p

The activity of the recombinant Spe1p was determined by measuring the production of cadaverine or putrescine by LC/MS-MS. For measuring enzyme activities, the 0.25-ml standard reaction contained a mix of 40 mM HEPES pH 7.5, 2 mM ornithine, 0.1 mM EDTA, 1 mM DTT, 0.1 mM pyridoxal phosphate (PLP) and 0.1 μg $ml^{-1}$enzyme. The standard reaction for lysine decarboxylase activity was similar except for the addition of 20 mM lysine instead of ornithine. The *Km* for each substrate was determined by varying the concentration of ornithine from 0-2 mM or lysine from 0-20 mM. All kinetic parameters were recorded at 30°C. For determining enzyme activities, 30 μl of the reaction was sampled every minute and 120 μl of cold methanol was added to quench the reaction. Samples were

kept on dry ice for 20 min and then centrifuged at high speed for 10 min. Supernatants were collected and putrescine and cadaverine were quantified by LC-MS/MS.

### Modelling the metabolic reconfiguration caused by lysine harvesting

To investigate the metabolic changes required to adapt for an excess of lysine in the media, a revised version of the *S. cerevisiae* genome-scale metabolic model (iMM904_NADcorrected [22]) was used. In total, six additional reactions related to cadaverine biosynthesis were added: two metabolic reactions for ornithine decarboxylase (H + lys_L --> $CO_2$ + cadaverine) and spermine synthase (S-adenosyl 3-(methylthio) propylamine + cadaverine --> S-methyl-5'-thioadenosine + aminopropylcadaverine), and four reactions for transporting and exchanging cadaverine and aminopropylcadaverine to and from the cell. In silico SM constraints were used according to the original model (iMM904 [41]). For the excess lysine uptake condition, in addition to the minimal media setting, the constraint of L-lysine exchange reaction (EX_lys_L(e)) was fixed to an arbitrarily large value 1, which is far more than the rate required for growth. The model simulations, using the FBA approach, were performed in two steps. First, the maximum growth was predicted in the minimal media condition (we call it a naive model; Extended data Figure 4). Then, the predicted growth rate was fixed, and the rate of glutathione oxidoreductase reaction was maximised in both the minimal and lysine-supplemented media conditions. The COBRA toolbox [42] was used for all the model simulation.

### Statistical Analysis

The metabolite concentration data, the results from the growth-curve analysis and the kinetic parameters are expressed as mean ± sd. The statistical significance was obtained using unpaired two-tailed Student's *t-test* using a confidence level of 95%. Other statistical tests are indicated in the Figure legends.




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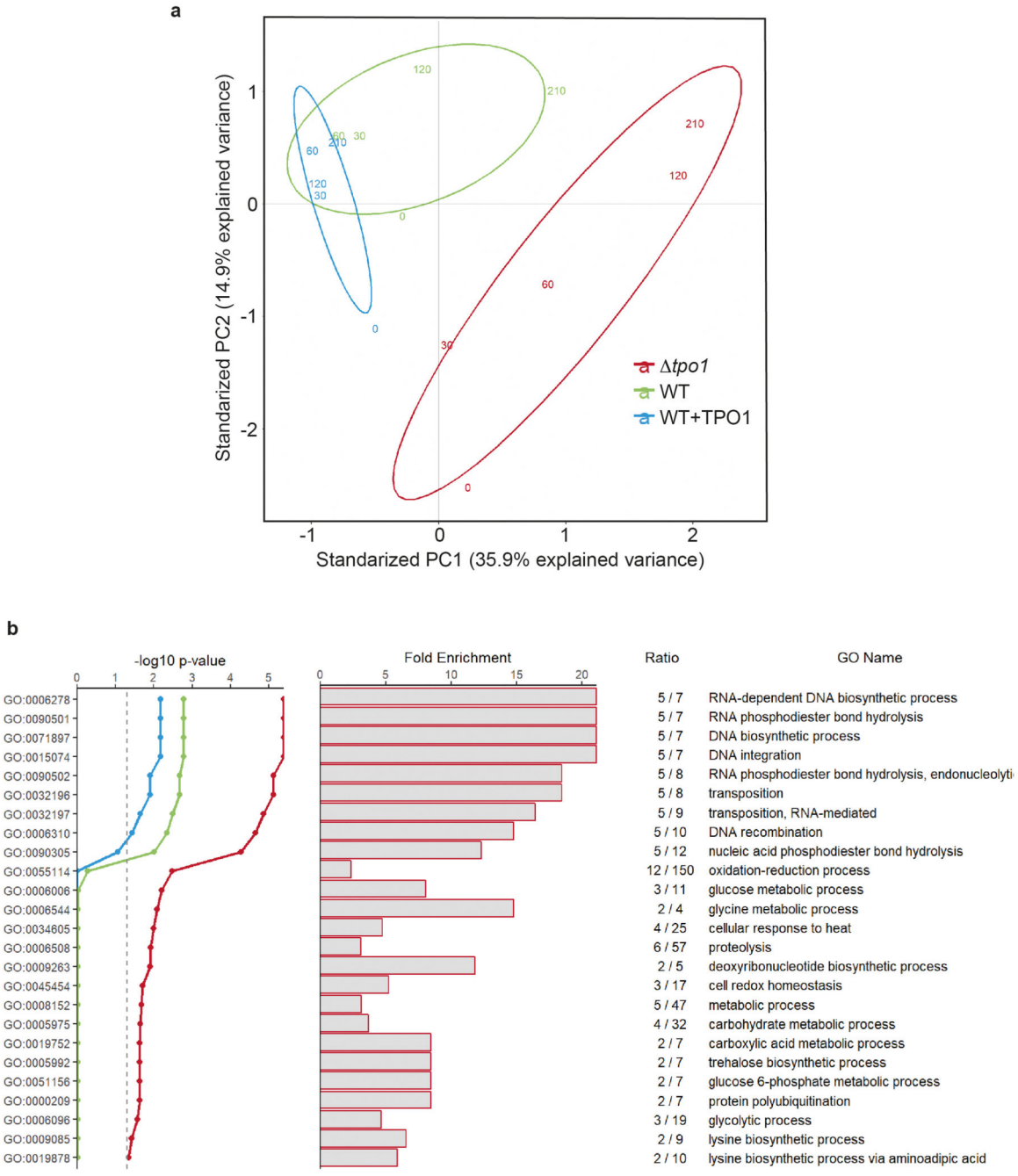
## Extended Data




**Extended data Figure 1. Proteomic analysis.**

A proteomic time-course experiment recorded by SWATH-MS [6], was re-analysed with a new spectral library and a recent version of Spectronaut (Biognosys) software, increasing the depth of the proteomic analysis. **a,** Principal component analysis shows that proteome profiles of $H_2O_2$-exposed Δ*tpo1* yeast [6] are more different from the wild type than that of a Tpo1p-overexpressing strain and that there is a metabolic adaptation in the lysine pathway.

Numbers indicate time points (in minutes) after the oxidative insult. **b,** Gene Ontology (GO) enrichment analysis shows that a number of biosynthetic processes are significantly enriched in proteins differentially expressed between wild-type and Δ*tpo1* yeast upon exposure to $H_2O_2$. Most of the GO terms that are significantly enriched belong to processes that are typically seen to be activated upon treatment with $H_2O_2$, including oxidation-reduction process, cell cycle, nucleotide synthesis and ribosome [43]. Metabolic processes affected include carbohydrate- and amino acid metabolism, including two GO terms specific for the lysine pathway. Significance of enrichment over base frequency was calculated using a binominal test [44], and *P* values (red line) were corrected for false discovery rate using Bonferroni (red) or Benjamini-Hochberg (green) correction. n = 5.

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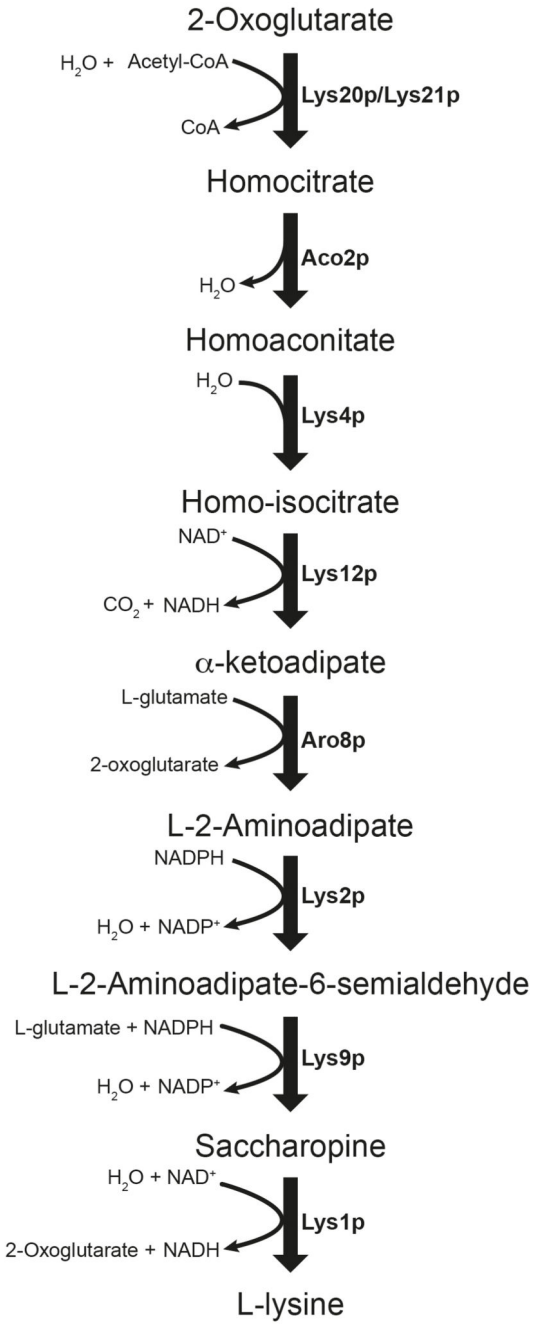


**Extended data Figure 2. The *S. cerevisiae* lysine biosynthesis pathway via aminoadipate.**
Lys20p/Lys21p, homocitrate synthase; Aco2p, aconitase; Lys4p, homoaconitase; Lys12p, homo-isocitrate dehydrogenase; Aro8p, 2-aminoadipate transaminase; Lys2p, α-aminoadipate reductase; Lys9p, saccharopine dehydrogenase ($NADP^+$ and L-glutamate forming); Lys1p, saccharopine dehydrogenase ($NAD^+$ and L-lysine forming).

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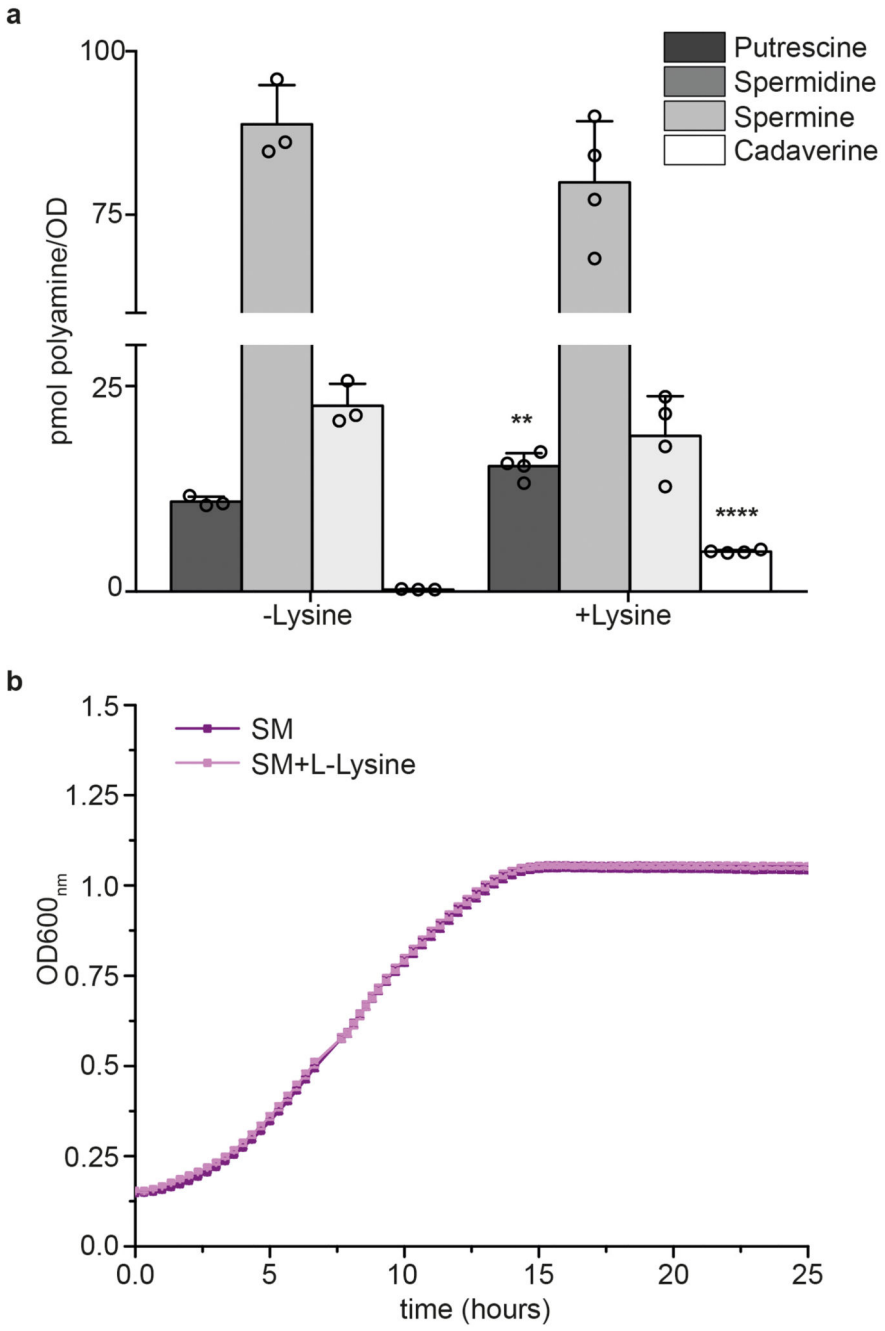
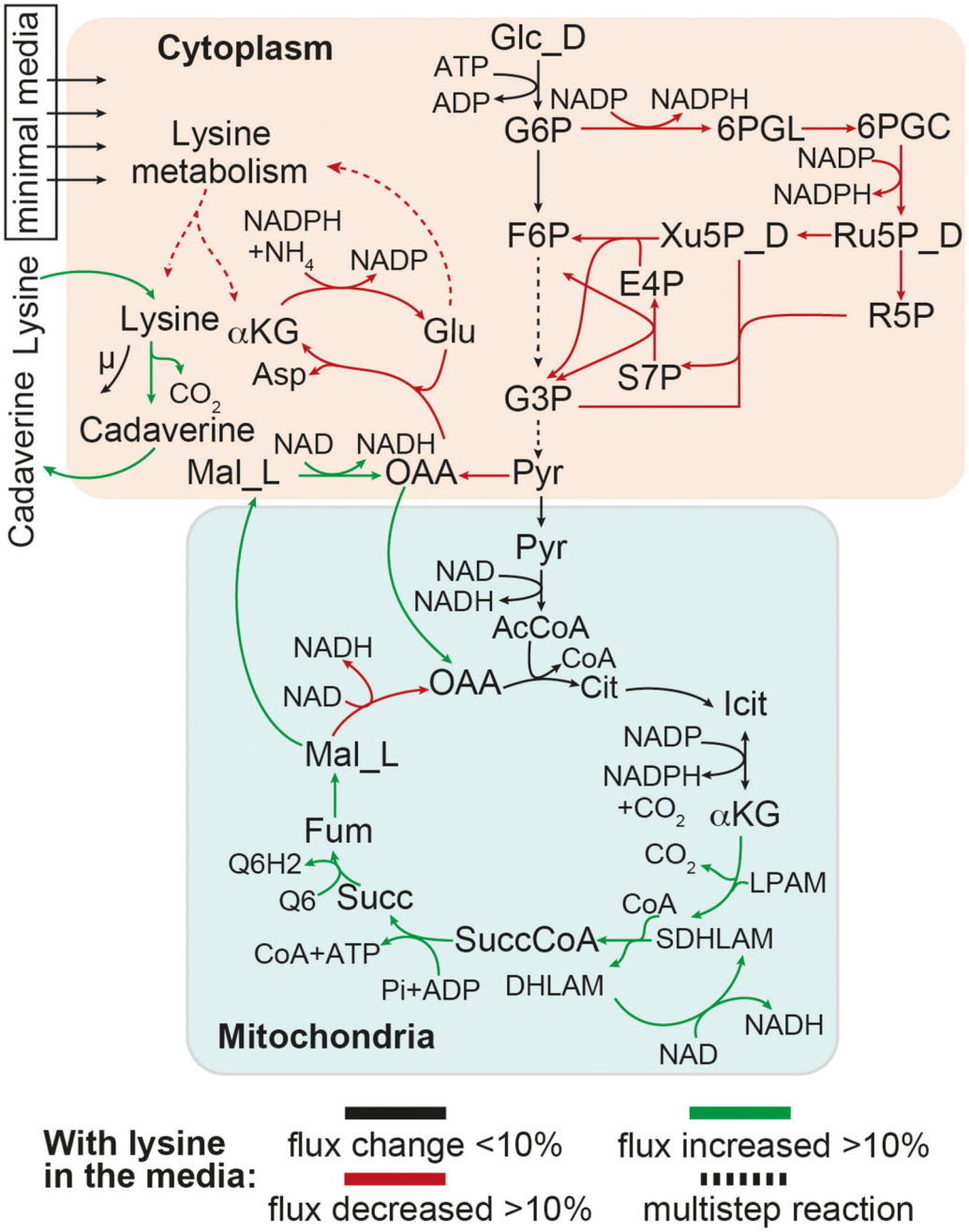



**Extended data Figure 3. Effect of lysine supplementation on the intracellular concentration of the canonical polyamines and growth.**

**a,** Polyamine content in yeast cells in the mid-log phase grown in SM media with and without 25 μg $ml^{-1}$ of lysine, as quantified by LC-MS/MS using selected reaction monitoring. Bar charts represent the mean ± s.d.; n = 3 biologically independent samples for non-supplemented conditions and n=4 for supplemented conditions. Unpaired two-tailed Student's *t*-test, wild-type cells supplemented versus non-supplemented with lysine: **** $P \leq 0.0001$, ** $P = 0.0066$. **b,** Growth curves of wild-type cells grown in SM media with or

without lysine (25 μg $ml^{-1}$) supplementation. The data represent mean ± s.d.; n = 3 biologically independent samples.




**Extended data Figure 4. Predictions of the growth-optimized (naive) model for flux balance analysis of lysine harvesting.**

We used a revised version of the *S. cerevisiae* genome-scale metabolic model (iMM904_NADcorrected [22]). Six additional reactions related to cadaverine biosynthesis were added: two metabolic reactions for ornithine decarboxylase (H + lys_L --> $CO_2$ + cadaverine) and spermine synthase (S-adenosyl 3-(methylthio) propylamine + cadaverine -->

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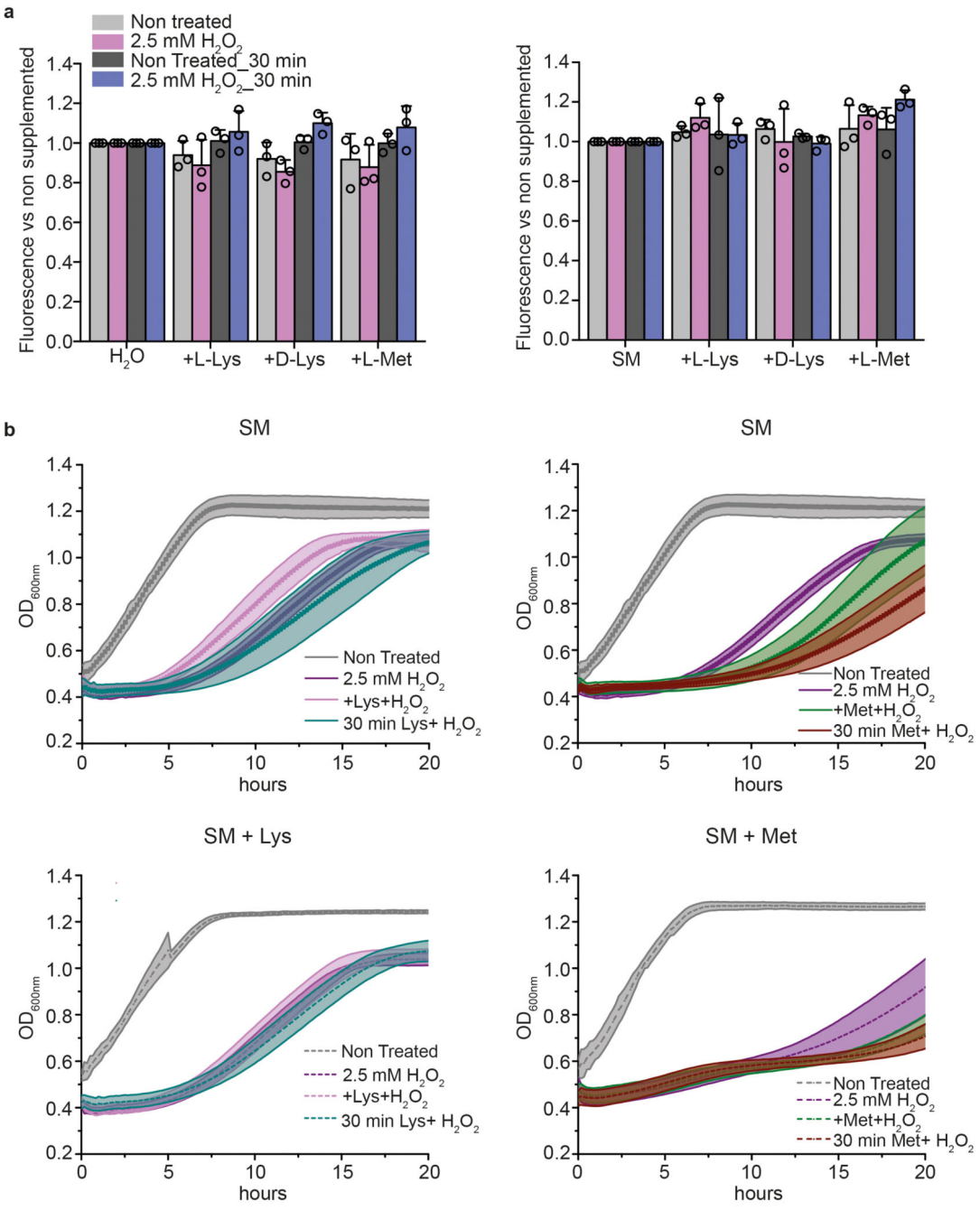
S-methyl-5'-thioadenosine + aminopropyl-cadaverine), and four reactions for transporting and exchanging cadaverine and aminopropylcadaverine. In silico synthetic minimal media constraints were used according to the original model (iMM904 [41]). For excess lysine uptake, the constraint of L-lysine exchange reaction was fixed to value of 1. Model simulation (FBA) was performed using the COBRA toolbox for maximum growth in both media conditions.




**Extended data Figure 5. Lysine and methionine supplementation have no growth-relevant effect on the oxidative capacity of media containing $H_2O_2$.**

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**a,** $H_2O_2$ levels measured by Amplex Red fluorescence in water (left) or SM medium (right) supplemented with L-lysine (25 μg $ml^{-1}$), D-lysine (25 μg $ml^{-1}$) or L-methionine (40 μg $ml^{-1}$) with or without 30 min pre-incubation with 2.5 mM $H_2O_2$. The data represent mean ± s.d.; n = 3 independent experiments. **b,** Growth curves of cells pre-cultured in SM, SM + lysine (SM + Lys, 25 μg $ml^{-1}$) or SM + methionine (SM + Met, μg $ml^{-1}$). At mid-log phase ($OD_{600nm}$ ~0.5) cells were pre-incubated for 0 or 30 minutes with 2.5 mM of $H_2O_2$ in the respective pre-culture medium. The data represent mean ± s.d.; n = 3 biologically independent samples.

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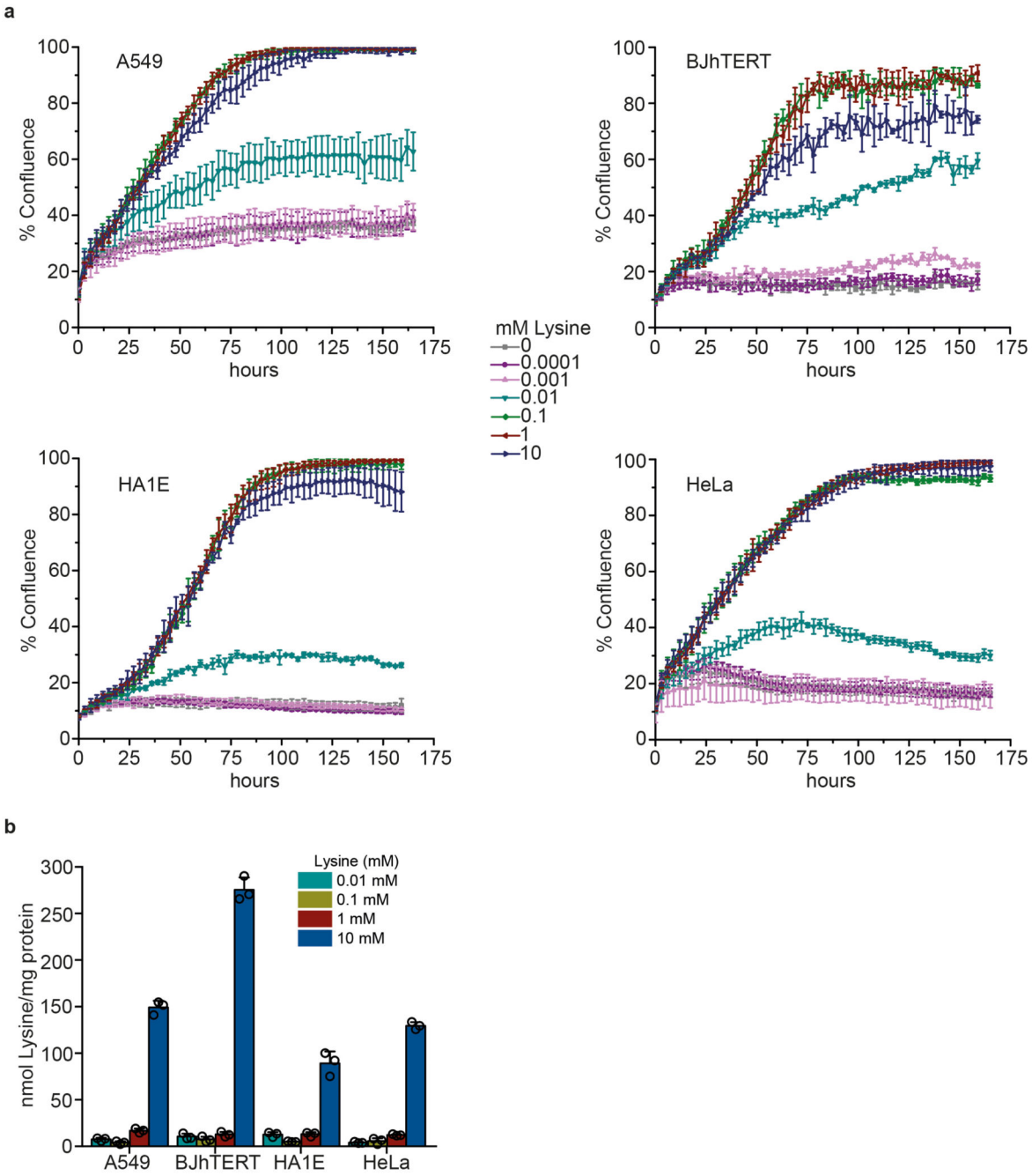
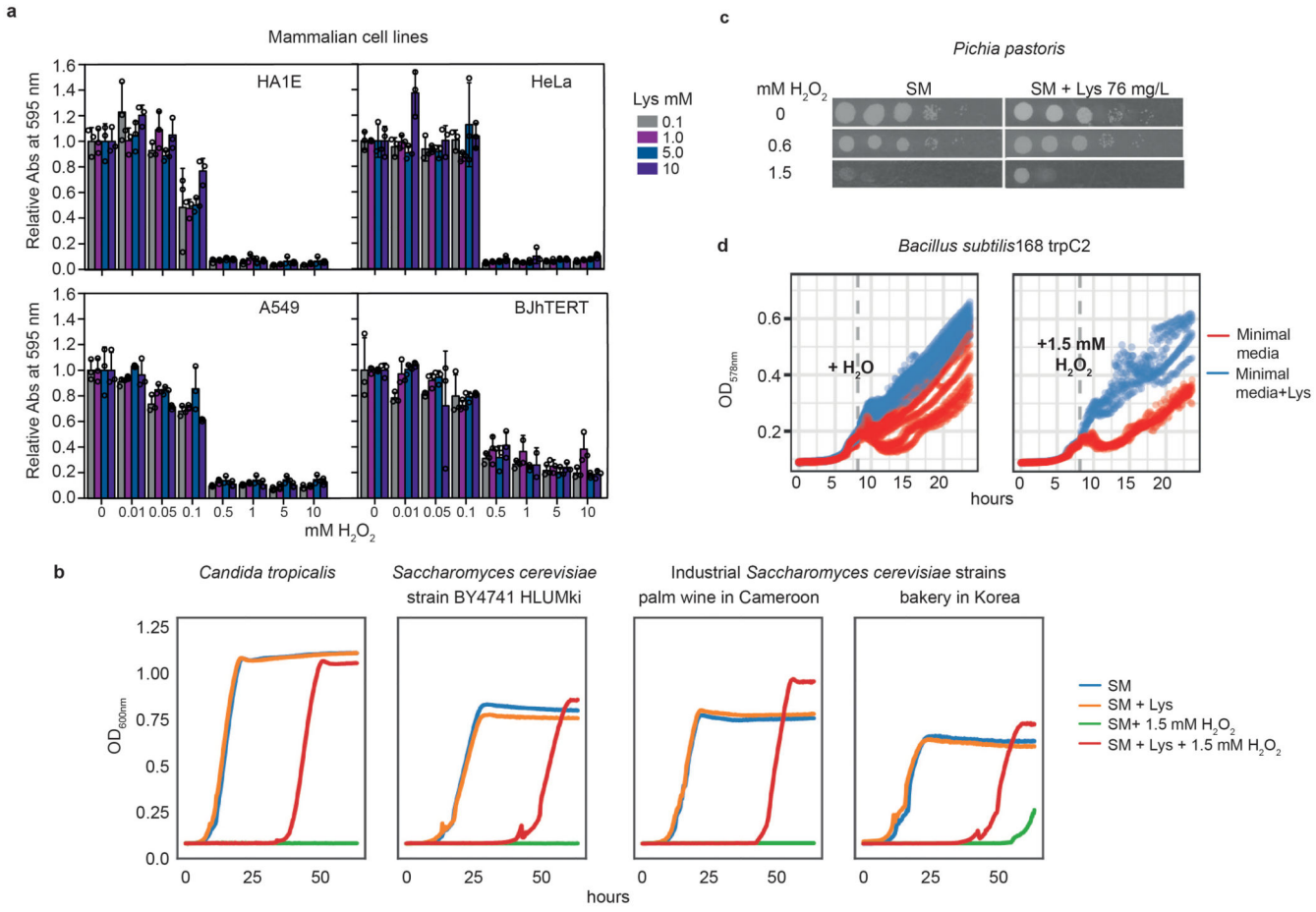



**Extended data Figure 6. Effect of lysine supplementation on mammalian cell lines that are auxotrophic for lysine.**

**a,** Growth curves showing the effect of different concentrations of lysine (0.01-10 mM) on mammalian cell growth. Percentage confluence was calculated using IncuCyte imaging system (Essen Bioscience). The data represent mean ± s.d.; n= 3 biologically independent samples. **b,** Intracellular lysine concentrations in different mammalian cell lines grown in DMEM with a range of concentrations of lysine (0.01-10 mM). Cells were seeded at 0.5 x

$10^6$ cells per 6- cm plate and grown for 48 h before collection. The data represent mean ± s.d.; n = 3 biologically independent samples.




**Extended data Figure 7. Lysine harvesting protects against $H_2O_2$ in different yeast and bacteria species.**

**a,** Effect of the supplementation of lysine on the resistance of mammalian cells to $H_2O_2$. Cell biomass was quantified by crystal-violet staining following 24 h treatment with $H_2O_2$ at a range of concentrations in cultures supplemented with 0.1-10 mM lysine. The data represent mean ± s.d.; n = 3 biologically independent samples. **b,** Growth curves of *C. tropicalis,* the *S. cerevisiae* laboratory strain BY4741 with repaired auxotrophic loci and two non-laboratory isolates of *S. cerevisiae* in SM media with or without 25 μg $ml^{-1}$ lysine. When challenged with 1.5 mM $H_2O_2$, cultures supplemented with lysine (pre-cultures were also supplemented) can survive and eventually grow (red versus green curves). **c,** Spot test of the resistance of *P. pastoris* to $H_2O_2$. Overnight cultures were grown in SM media (potassium phosphate-buffered, pH 6.0) with or without lysine (76 mg $l^{-1}$) supplementation and were diluted to $OD_{600nm}$= 1 in water, serial diluted and spotted onto SM media (potassium phosphate-buffered pH 6.0) with or without lysine supplementation and $H_2O_2$. The experiment was repeated with similar results. **d,** Growth curves of *B. subtilis* 168 trpC2 when challenged with 1.5 mM $H_2O_2$ versus a water control, at mid-log phase in S7 minimal media with or without lysine (40 μg $ml^{-1}$) supplementation.

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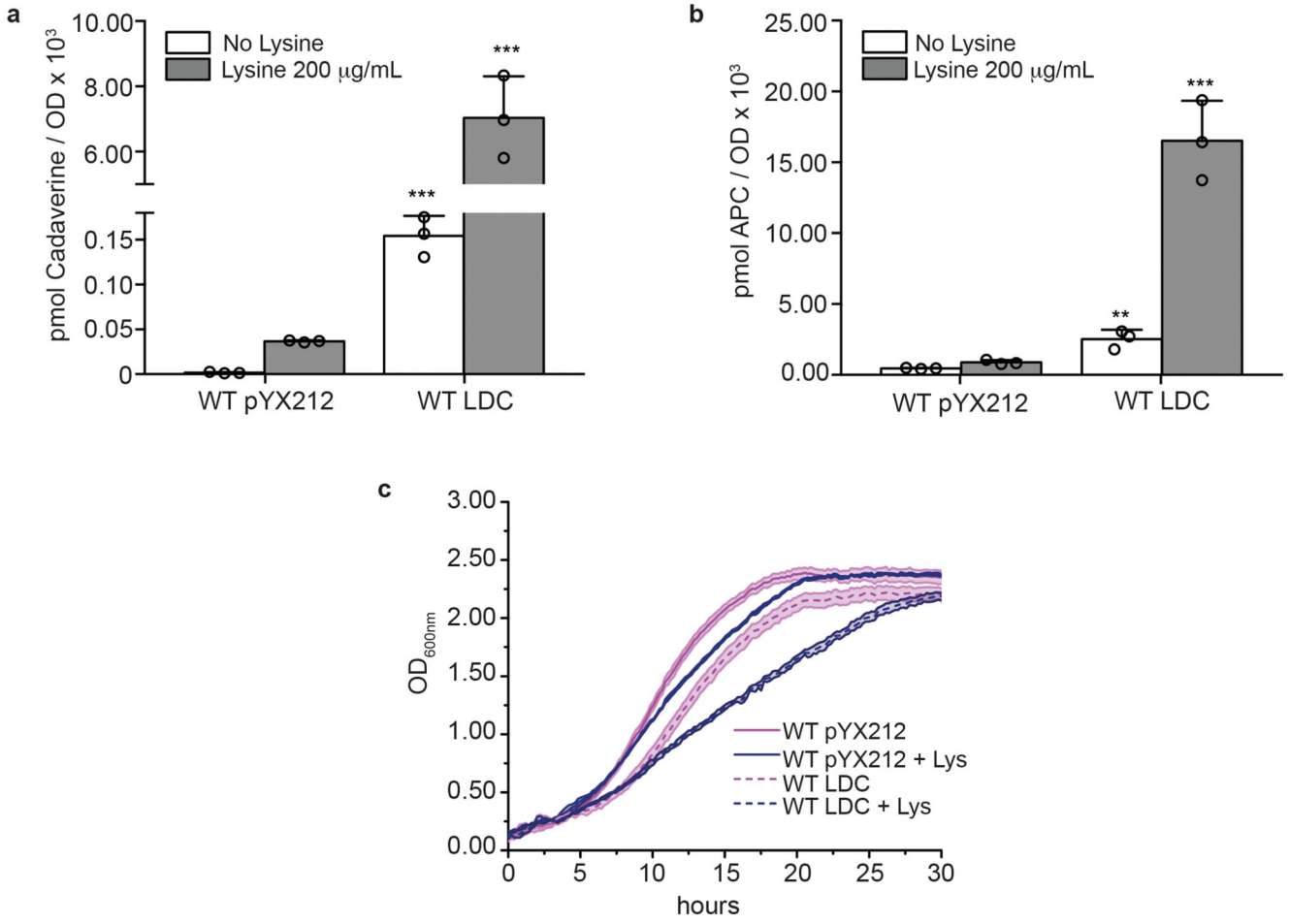


**Extended data Figure 8. Yeast can accumulate high levels of cadaverine and aminopropylcadaverine that are not toxic.**

**a,b,** Cadaverine **(a)** and aminopropylcadaverine (APC) **(b)** concentrations in *S. cerevisiae* wild-type strain overexpressing *E. coli LdcC* (WT LDC) or carrying the empty plasmid (WT pYX212). Polyamines were derivatized with dansyl chloride and quantified by LC-MS/MS. The data represent mean ± s.d.; n = 3 biologically independent samples. Unpaired two-tailed Student's *t*-test: wild-type pYX212 versus WT LDC in non-supplemented conditions *** $P = 0.0003$ and supplemented conditions *** $P = 0.0007$ for **a**; and ** $P = 0.0054$ and *** $P = 0.0007$ for non-supplemented and supplemented conditions for **b**. **c,** Growth curves showing the effect of lysine (250 μg $ml^{-1}$) on growth when wild-type strain is overexpressing *E. coli LdcC*. The data represent mean ± s.d.; n = 3 biologically independent samples.

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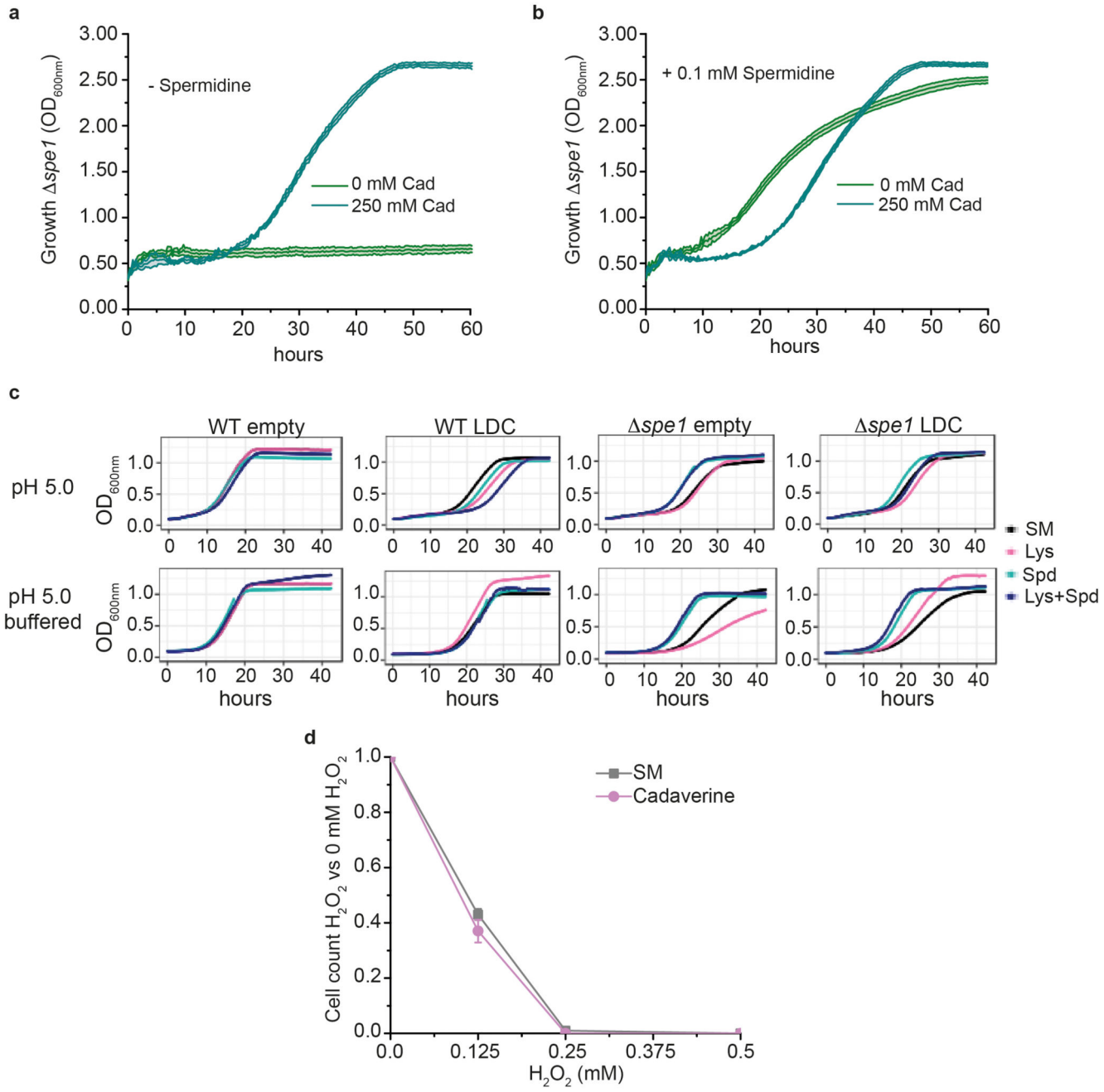


**Extended data Figure 9. Cadaverine can partially substitute for canonical polyamines, but only at non-physiologically high concentrations and at a certain pH, and does not protect *S. cerevisiae* against $H_2O_2$ stress.**

**a,b,** Growth curves of Δ*spe1* strain depleted from spermidine grown in SM media alone or supplemented with 250 mM cadaverine in the absence **(a)** or presence of 0.1 mM spermidine **(b)**. The data represent mean ± s.d.; n = 3 biologically independent samples. **c,** Growth curves showing the effect of lysine supplementation on wild-type and Δ*spe1* strains carrying a control plasmid (empty) or overexpressing *E. coli LdcC* (LDC), when grown in SM media buffered or not buffered at pH 5.0. The data represent mean ± s.d.; n = 4 biologically

independent samples per experiment. The experiment was repeated twice. **d,** $H_2O_2$ tolerances were determined as described above, but substituting lysine with 5 mM cadaverine. The data represent mean ± s.d.; n = 4 independent experiments. Even in the presence of this high level of cadaverine, the yeast cells to not tolerate higher levels of $H_2O_2$.

**Extended data Table 1**

**Effect of cadaverine on abiotic stress and stationary phase survival**

| Experiment | Method | Results |
|---|---|---|
| Cumene hydroperoxide (CumOOH) | Growth curves of WT empty strain, WT *LdcC+* in SM ± lysine (25 μg/mL) with CumOOH (0-1 mM).<br>Growth curves Δ*spe1* empty/Δ*spe1 LdcC+* in SM + spermidine (0.1 mM), ± lysine (25 μg/mL) with CumOOH (0-1 mM). | No difference |
| Paraquat | Growth curves and spot test WT empty strain, WT *LdcC+* in SM, ±lysine (25 μg/mL) with paraquat (0-1 mM). | No difference |
| $H_2O_2$ | Spot test WT and empty strain, WT *LdcC+* in SM with $H_2O_2$ (0-0.5 mM).<br>Overnight incubation of WT with $H_2O_2$ (0-0.5 mM) in SM and SM + cadaverine (5 mM), cell count by flow cytometry. | No difference |
| NaCl | Spot test WT and empty, WT *LdcC+* in SM ± lysine (25 μg/mL) with NaCI (0-1 M). | No difference |
| Chronological ageing | Survival plating for 20 days of WT empty and WT *LdcC+* cultures grown in SM and WT in SM Supplemented with cadaverine. | No difference |

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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## Data availability statement

Proteomic data are accessible through (ProteomeXchange PXD013373). The kinetic data is available in Table 1, and the raw data used to generate the figures is provided in the Supplementary Tables.

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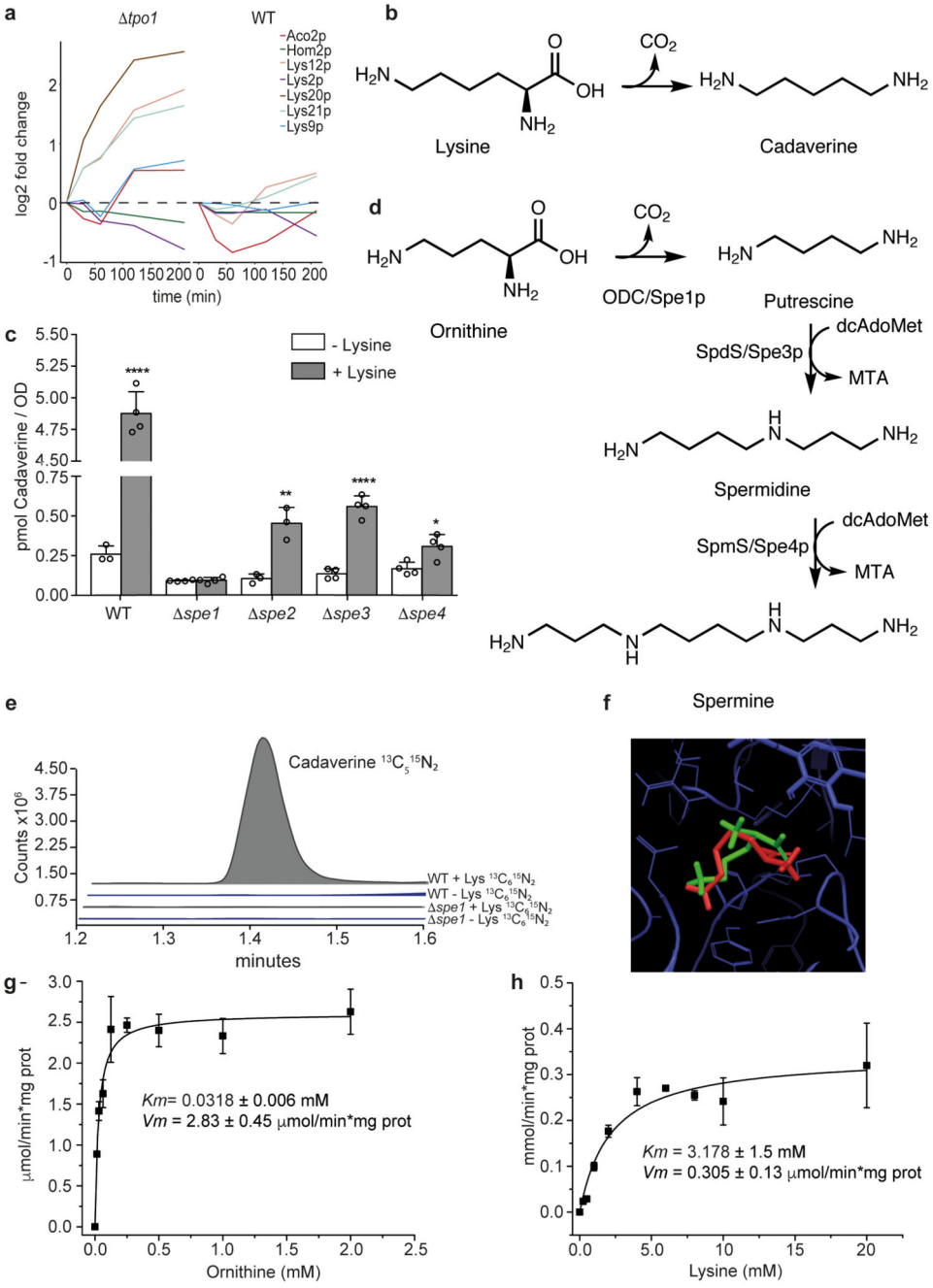


**Figure 1. A promiscuous reaction of Spe1p forms cadaverine from lysine in yeast**

**a.** Lysine biosynthetic enzymes are differentially induced upon exposure to 1.5 mM $H_2O_2$ and deletion of *TPO1* (*Δtpo1*). Shown is a 210-min times series [6] recorded by SWATH-MS and re-processed with Spectronaut Pulsar X. Expression levels are shown as $\log_2$ (differentially expressed enzymes). **b,** The decarboxylation of lysine leads to the formation of cadaverine **c,** Cadaverine concentrations in overnight wild-type (WT) and Δ*spe1*-Δ*spe4* yeast cultures with or without lysine (25 μg $ml^{-1}$) supplementation. Mean ± s.d., n = 4 biologically independent samples except for wild-type non-supplemented and *Δspe2*

supplemented, for which n = 3. Unpaired two-tailed Student's *t*-test versus the non-supplemented control: **P* = 0.0163, ***P* = 0.0045, *****P* ≤ 0.0001. **d,** Schematic illustration of the canonical yeast polyamine biosynthesis pathway. ODC, ornithine decasboxylase **e,** $^{13}C_5$ $^{15}N_2$ cadaverine is formed from isotope-labelled lysine ([$^{13}C_6$ $^{15}N_2$] lysine) after a 1-h incubation of wild-type strains, but is not formed in Δ*spe1* cells. This experiment was repeated three times with similar results. **f**, Results of docking lysine (red) and ornithine (green) to the same site of Spe1p using Glide from Schrödinger suite (v. 2015-3) and a homology model. **g**, **h**, In vitro activity of Spe1p on metabolizing ornithine (**g**) and lysine (**h**) (mean ± s.d.; n = 3 independent experiments). The saturation curves were fitted to a Michaelis-Menten equation.



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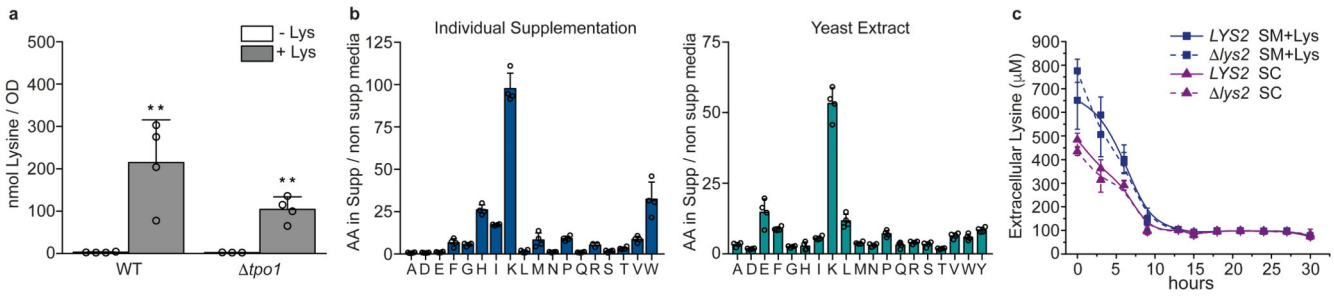


**Figure 2. Lysine harvest in *Saccharomyces cerevisiae*.**

**a,** Lysine concentrations in wild-type and *Δtpo1* yeast grown in synthetic media supplemented with or without lysine (25 μg $ml^{-1}$). The cells were grown until the mid-log phase and analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (mean ± s.d.; n = 4 biologically independent samples except for *Δtpo1*, for which n = 3). Unpaired two-tailed Student's *t*-test versus the non-supplemented control: $^{**}P = 0.0056$ for wild-type and $P = 0.0022$ for *Δtpo1*. **b,** Harvesting to reach extreme concentrations is specific for lysine. Amino acid concentrations in wild-type yeast grown in synthetical minimal (SM) media supplemented with individual amino acids (left) or rich media (yeast extract; right) plotted as fold change compared to the average profile of seven replicates in unsupplemented synthetic media (mean ± s.d., n = 4 biologically independent samples). **c,** Quantification of extracellular lysine during the growth of prototrophic (*LYS2*) and auxotrophic (Δ*lys2*) yeast in SM media supplemented with lysine (SM+Lys) or synthetic complete media (SC). Cultures were initiated with an $OD_{600nm}$ of 0.125 and grown for 30 h. Samples were taken every 3 h, collected and the supernatant was used for quantification. Mean ± s.d.; n = 4 biologically independent samples.




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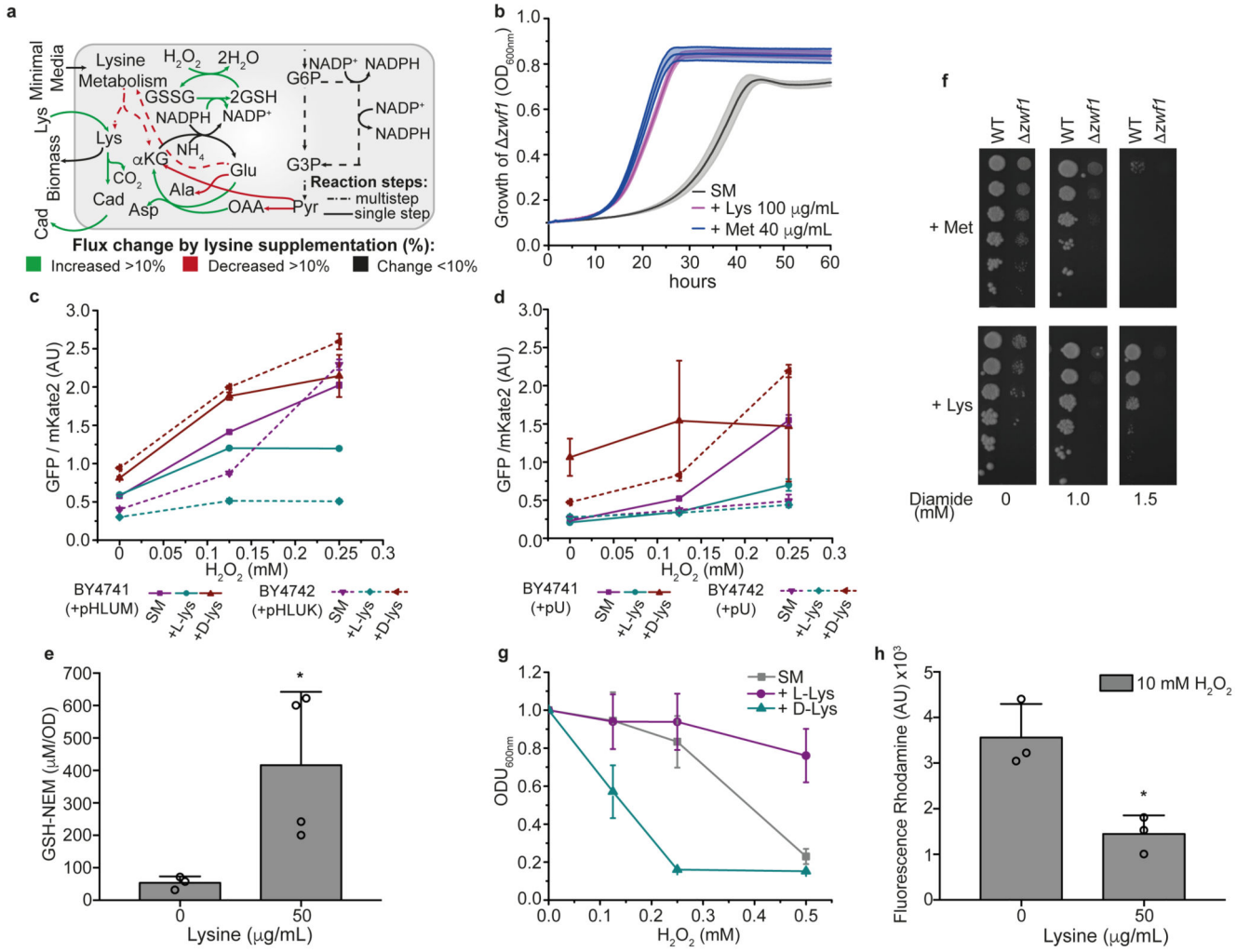


**Figure 3. Lysine harvesting increases the tolerance of yeast to oxidants by replenishing NADPH and increasing glutathione levels.**

**a**, Lysine harvesting replenishes the pools of NADPH and reduced glutathione (GSH). The scheme shows the flux balance analysis upon maximizing the glutathione oxidoreductase reaction **b,** Lysine harvesting complements the methionine auxotrophy of Δ*zwf1* cells. Cells were grown with or without lysine (100 $\mu$g $ml^{-1}$) or methionine (40 $\mu$g $ml^{-1}$) (mean ± s.d.; n = 4 biologically independent samples). **c, d,** The availability of NADPH increases in lysine harvesters. The redox state of NADPH/$NADP^+$ was assessed by a fluorescence biosensor assay[26], using the fluorescent proteins GFP and mKate2, in plasmid-complemented prototrophs (c) and auxotrophs (d) supplemented with histidine, leucine and methionine. Mean ± s.d.; n = 4 independent experiments. **e,** GSH concentrations, measured as a conjugate with N-ethylmaleimide (GSH-NEM) to prevent oxidation, increase in lysine harvesters. Mean ± s.d. (n = 3 biologically independent samples for wild type and n = 4 for lysine-supplemented wild-type). Unpaired two-tailed Student's *t*-test **P* = 0.0426. **f,** Spot test assessing the diamide resistance (0-1.5 mM) of wild-type and Δ*zwf1* cells supplemented with methionine (40 μg $ml^{-1}$) or lysine (50 μg $ml^{-1}$) (n=3) **g,** L-Lysine harvesting increases tolerance to $H_2O_2$, but exposure to D-lysine has adverse effects. Cells were pre-cultured in




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the presence of L-lysine and D-lysine and exposed to $H_2O_2$ for 16 h. The tolerance to $H_2O_2$ stress was assayed by density ($OD_{600nm}$). Mean ± s.d.; n = 4 independent experiments. **h,** Quantification of reactive oxygen species (as assessed by dihydrorhodamine staining) in wild-type cells and lysine harvesters (50 μg $ml^{-1}$) incubated with 10 mM $H_2O_2$ for 1 h (mean ± s.d.; n = 3 independent experiments). Unpaired two-tailed Student's *t*-test **P* = 0.0123.



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## Table 1
## Kinetic parameters of ornithine decarboxylase Spe1p

| Substrate | *Km* (mM) | *Vm*/*Km* (ml $min^{-1}$ $mg^{-1}$) | Concentration (mM) SM | Concentration (mM) SM+lysine |
|---|---|---|---|---|
| Ornithine | 0.0318 ± 0.006 | 88.9 | 2.04 ± 0.18 (wild type)<br>1.6 ± 0.25 (*Δtpo1*) | 2.2 ± 0.12 (wild type)<br>2.2 ± 0.095 (*Δtpo1*) |
| Lysine | 3.178 ± 1.5 | 0.095 | 3.02 ± 1.12 (wild type)<br>2.45 ± 0.23 (*Δtpo1*) | 215.2 ± 100.5 (wild type)<br>104.2 ± 29.8 (*Δtpo1*) |

The kinetic parameters and the concentrations of amino acids represent the mean ± sd, n = 3 independent experiments and biological samples, respectively. SM, synthetic minimal medium; *Vmax*, maximum rate of reaction.