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On demand utilization of phosphoribosyl pyrophosphate by downstream anabolic pathways

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ABSTRACT

The pentose phosphate pathway (PPP) is critical for anabolism and biomass production. Here we show that the essential function of PPP in yeast is the synthesis of phosphoribosyl pyrophosphate (PRPP) catalyzed by PRPP-synthetase. Using combinations of yeast mutants, we found that a mildly decreased synthesis of PRPP affects biomass production, resulting in reduced cell size, while a more severe decrease ends up affecting yeast doubling time. We establish that it is PRPP itself that is limiting in invalid PRPP-synthetase mutants and that the resulting metabolic and growth defect can be bypassed by proper supplementation of the medium with ribose-containing precursors or by the expression of bacterial or human PRPP-synthetase. In addition, using documented pathologic human hyperactive forms of PRPP-synthetase, we show that intracellular PRPP as well as its derived products can be increased in both human and yeast cells and we describe the ensuing metabolic and physiological consequences. Finally, we found that PRPP consumption appears to take place “on demand” by the various PRPP-utilizing pathways, as shown by blocking or increasing the flux in specific PRPP-consuming metabolic routes. Overall, our work reveals important similarity between human and yeast for both synthesis and consumption of PRPP.

INTRODUCTION

The pentose phosphate pathway (Fig. 1A) has two key functions (1). At first, it contributes to a large part of NADP reduction through its oxidative branch and, secondly, it provides ribose-5-phosphate, an obligatory precursor of the synthesis of all nucleotides and derivatives, through the non-oxidative branch. In addition, the PPP produces C3 to C7 metabolites, of which erythrose-4-phosphate is used in the synthesis of aromatic amino acids. Hence, the PPP is central to anabolism, notably through synthesis of pentose for nucleotides and supply of NADP(H) which is involved in most anabolic oxydo-reductive reactions. Therefore, under proliferation conditions, a portion of the carbon flux is routed to PPP and used for anabolism and *de novo* biomass formation.

Synthesis of NADPH by the PPP is particularly important for human erythrocytes and a defect in glucose-6-phosphate dehydrogenase (G6PDH), the first step of the oxidative branch of PPP from glucose-6-phosphate, results in hemolytic anemia (2). In yeast cells, NADPH synthesis *via* the PPP is also important but essentially under oxidative stress conditions or when a significant amount of NADPH is needed for sulfur assimilation. Accordingly, the yeast *zwf1* mutant lacking G6PDH activity is hyper-sensitive to oxidizing agents and is auxotroph for methionine (3). Importantly, while most of the genes encoding enzymes of the PPP are not essential in yeast (4–8), *RKI1* encoding ribose-5-phosphate ketol-isomerase is indispensable for yeast survival (9), presumably due to its critical role in synthesizing ribose-5-phosphate, which is an essential metabolite required for nucleotide biosynthesis. In fact, ribose-5-phosphate is never used as such in biosynthesis reactions; it first has to be activated to phosphoribosyl pyrophosphate (PRPP), the final product of PPP, by a family of enzymes named PRPP-synthetases (Fig. 1A).

In budding yeast, PRPP-synthetases are multimeric enzymes. There are five genes, *PRS1-5*, encoding PRPP synthetase subunits. Based on specific activity measurements from single and combined knockout (KO) (10, 11), as well as single and combined expression of the yeast subunits in *E. coli* (12), it was concluded that Prs1 with Prs3 form the major enzyme complex, while Prs2-Prs5 and Prs4-Prs5 form two minor complexes. Of note, Prs1 and Prs5 carry additional non-homologous regions which interact with the cell wall integrity pathway (13, 14). Importantly, *prs1* and *prs3* individual KO resulted in a severe growth defect while only combinations of KO comprising members of both the Prs1-Prs3 and the Prs2-Prs5 complexes were synthetic lethal (10). Finally, *prs1* and *prs3* mutants had low intracellular nucleotides (10). However, it is not yet established whether the slow growth of these mutants is the direct consequence of limiting amounts of PRPP, or whether there are unrelated effects of the deletion of the *PRS1* or *PRS3* genes. In yeast, PRPP can be used in enzymatic reactions, in 5 different synthesis pathways, catalyzed by 11 phosphoribosyl transferases (Fig.1A) (15): purine nucleotide biosynthesis (Ade4, Apt1, Hpt1, Xpt1); pyrimidine nucleotide biosynthesis (Ura5, Ura10, Fur1); pyridine cofactor biosynthesis (Bna6, Npt1); histidine biosynthesis (His1) and tryptophan biosynthesis (Trp4). Whether some of these pathways are more limiting than others, when PRPP synthesis is low, is not known.

In human, there are three monomeric PRPP synthetase isoforms (PRPS1-3). The PRPS1 and PRPS2 enzymes are 95% identical and are expressed in all tissues examined, while PRPS3 is specifically expressed in testis (16, 17). PRPS1 is the most highly expressed isoform and is mutated in a whole range of genetic diseases (18). Interestingly, several distinct syndromes are associated with specific mutations in PRPS1, either decreasing or increasing its activity (18). It hence appears that in humans, the level of PRPP synthetase must be finely tuned to a given

physiological level. In addition, several lines of evidence support a central role of PRPS1 and PRPS2 in tumor progression and resistance to anti-cancer drugs. Increased activity of PRPS1 was shown responsible for thiopurine resistance in relapsed childhood Acute Lymphoblastic Leukemia (ALL) (19), as well as cisplatin resistance in breast cancer cells (20). Beside the drug resistance effects, increased *PRPS1* expression was found to result in an anti-apoptotic effect in B-ALL cells lines and was associated with a poor prognosis for disease progression (21). Finally, activation of PRPS1 by ketohexokinase-A drives hepatocellular carcinoma formation (22). PRPS2, although it is dispensable in mice, is required for Myc-driven tumorigenesis (23) leading to an interesting alternative strategy to indirectly target the “undruggable” Myc protein. More recently, PRPS2 overexpression was found to increase cell migration and invasion and proposed to drive colorectal cancer metastasis (24). The requirement for PRPP for all the nucleotide synthesis pathways makes synthesis of PRPP an interesting metabolic bottleneck that could be targeted. PRPS1 and PRPS2 are thus generating a growing attention for their roles in cancer cells. However, the physiological fine tuning of PRPS activity, affected in several diseases, makes it challenging to target PRPS. Therefore, a better understanding of PRPP synthesis and utilization is required.

In this work, we first established that synthesis of ribose-5-phosphate and PRPP is the essential function of PPP in yeast. We then aimed at deciphering how PRPP synthesis and consumption affect metabolism and proliferation in yeast cells. We report that PRPP synthetase, but not ribose-5-phosphate, is limiting PRPP synthesis. We also show that PRPP-utilizing enzymes compete for PRPP and that, under physiological conditions, PRPP is limiting for nucleotide synthesis but not for proliferation, and that it is competitively consumed by the downstream pathways. Finally, we investigated PRPP metabolism in human cells and found that the main conclusions drawn from yeast apply quite similarly to human cells.

Results

Synthesis of ribose-5-phosphate and PRPP is the essential function of PPP in yeast

As mentioned above, *RKI1* is the only essential gene in the PPP. This is most likely due to the fact that the PPP can be fed by the glycolysis pathway via several intermediates, namely glucose-6-phosphate, fructose-6-phosphate and glyceraldehyde-3-phosphate (Fig. 1A). It ensues that there is a redundancy in the synthesis of PPP intermediates with a possible exception for ribose-5-phosphate synthesized by Rki1 and for its derived product, PRPP, synthesized by Prs1-5 which are also essential for yeast cells in combinations (10). We thus hypothesized that the lethality associated to *rki1* knock-out should be bypassed if essential metabolites requiring PRPP for their synthesis were provided as supplements to the growth medium. These metabolites are purines, pyrimidines, pyridines and amino acids (Fig. 1B). The supplementation could not simply be done by feeding the cells with preformed nucleotides since nucleotides are not taken up by yeast cells. We hence fed the cells with nucleosides, which are nucleotide precursors carrying the ribose moiety but no phosphate. The nucleosides provided to cells were adenosine, uridine and nicotinamide riboside (Nr) for purines, pyrimidines and pyridines, respectively. Of note, since Nr is chemically unstable, cells were fed with nicotinamide mononucleotide that is metabolized to Nr by the Pho5 phosphatase enzymatic activity and immediately taken up by the Nrt1 permease, as shown previously (25). Since adenosine is not taken up efficiently in *S. cerevisiae*, we expressed a human nucleoside transporter (hENT1) to

facilitate uptake. In addition, tryptophan and histidine were supplied to bypass the requirement of PRPP for the synthesis of these amino acids. This combination of nucleoside and amino-acid supplements clearly bypassed the inviable phenotype of the *rki1* mutant (Fig. 1B) lacking ribose-5-phosphate ketol-isomerase and hence unable to synthesize ribose-5-phosphate from ribulose-5-phosphate (Fig. 1A). We conclude that the main function of the non-oxidative branch of the PPP, in yeast, is to provide ribose-5-phosphate that is used for nucleotide and amino acid synthesis *via* synthesis of PRPP by Prs1-5. PRPP synthesis from ribose-5-phosphate hence appears to be the essential role of the PPP in yeast.

Metabolic and phenotypic effects of alterations in the yeast PRPP synthetase genes

We then evaluated the metabolic and phenotypic contributions of the various yeast PRPP-synthetase isoforms in an isogenic prototrophic background. Prototrophic strains were used to ensure that the observed effects did not result from metabolic interferences with the auxotrophic-markers commonly used to facilitate genetic studies in yeast and which often affect PRPP requiring pathways. Two mutant strains lacking either the major enzyme (*prs1 prs3* double knock-out mutant) or the minor forms (*prs2 prs4 prs5* triple knock-out mutant) were compared with an isogenic wild-type strain. Intracellular PRPP was in the 0.1 mM range for the wild-type strain, lowered by 25% in the *prs2 prs4 prs5* triple mutant and was decreased almost 10 folds in the *prs1 prs3* mutant (Fig. 2A). This result is in good agreement with previous results showing a much more severe defect in PRPP synthetase activity in the *prs1 prs3* mutant than in the *prs2 prs4 prs5* triple mutant (10). Of note, the concentration of PRPP in yeast cells is way below the concentration of nucleotides which is around 4 mM for the sum of adenylic nucleotides (AXP) alone (Fig. 2B). This suggests that PRPP is not stored but rather utilized on demand.

The effects of the mutations on intracellular PRPP (Fig. 2A) were associated with a significant decrease of the ability of the mutant cells to produce biomass, although the effect was much stronger in the case of the *prs1 prs3* mutant (Fig. 2C). Quantifying biomass production rate does not allow, in a single measurement, to differentiate the effects on cell division from those on cell growth. We hence appraised these two parameters separately, by measuring cell volume (Fig. 2D) and population doubling time (Fig. 2E). Clearly, in the triple *prs2 prs4 prs5* mutant, the lower biomass production was associated to a lower cell volume, while doubling time was not increased (Fig. 2D and E). By contrast, in the *prs1 prs3* mutant both factors were severely affected (Fig. 2D and E). Finally, metabolic profiling of the three strains revealed that the *prs1 prs3* mutant was the most affected strain for all four nucleotides as well as the sum of NAD⁺ and NADH (hereafter referred as NAD(H)), while the *prs2 prs4 prs5* mutant was less severely, although significantly, affected (Fig. 2B, Fig. 3A-E, Fig. S1). Of note, by contrast to previously published data (10), we found that AXP, GXP, UXP and CXP were all significantly affected in the *prs2 prs4 prs5* mutant when compared to the isogenic wild-type strain (Fig. 2B, Fig. 3A-C). This discrepancy could be due to the fact that the strains used in our work are prototrophic, while in the previous work the used strains were mutated in four PRPP utilizing pathways (purine, pyrimidine, tryptophan and histidine). Interestingly, uracil, adenine and hypoxanthine, three pyrimidine and purine precursors, were higher in the *prs1 prs3* mutant than in the control strain (Fig. 3E-G), suggesting that the low PRPP level in the mutant limited their consumption by the cognate phosphoribosyl transferases (Fig. 1A).

We conclude that decreasing intracellular PRPP (Fig. 2A), and subsequently intracellular nucleotides (Fig. 2B, Fig. 3A-D), severely affected biomass production rate in both *prs* mutants

(Fig. 2C), while division, as revealed by population doubling time, was only affected in the *prs1prs3* mutant but not significantly in the *prs2 prs4 prs5* mutant (Fig. 2E). Hence, PRPP shortage, apparently, first affected cell growth (size decrease, Fig. 2D) before slowing down cell division (doubling time increase, Fig. 2E).

We then overexpressed the two major isoform genes (*PRS1* and *PRS3*) concomitantly on multicopy yeast plasmids and evaluated the metabolic and phenotypic effects (Fig. 4). Of note, due to the presence of the two plasmids, the yeast strains and the growth media used in Fig. 3 and Fig. 4 are different. The metabolic effects of *PRS1/PRS3* overexpression, increased PRPP (Fig. 4A), as well as nucleotide and NAD(H) concentrations (Fig. 4B-F). This result shows that increasing PRPP synthesis in yeast can significantly increase intracellular nucleotides. Accordingly, the purine intermediate metabolites IMP and inosine were also very significantly increased (Fig. 4G and H), as previously reported for purine overproducing *ADE4* dominant mutants (25). However, nucleotide overexpression did not improve yeast growth and division. Indeed, when considering physiological consequences of *PRS1/PRS3* overexpression, we observed that it negatively increased the population doubling time (Fig. 4I) and diminished the biomass production rate (Fig. 4J), while it had no significant effect on cell size (Fig. 4K). This establishes that there is no strict proportional relationship between PRPP concentration and generation time or cell size and that the connection between metabolism and these traits is probably more complex. The metabolic burden imposed by increased synthesis of nucleotides and accumulation of intermediates such as IMP and inosine in the mM range (Fig. 4G and H), could be responsible for the higher doubling time by diverting a significant part of glucose from glycolysis to nucleotide synthesis *via* the PPP.

Phenotypic and metabolic rescue of the yeast *prs1prs3* mutant by exogenous supplementation or expression of human and bacterial PRPS genes

It should be stressed that the results on doubling time and biomass do not allow to conclude whether these effects are consequences of the decreased intracellular nucleotide levels. It could in part be due to lower intracellular PRPP, independently of its effect on nucleotides, or alternatively to a possible moonlighting role of PRPS independently of its catalytic activity.

First, to establish that PRPP synthesis is indeed the limiting factor for proliferation in the *prs1 prs3* mutant, we expressed the monomeric human PRPP synthetases, PRPS1 and PRPS2, as well as the *E. coli* enzyme (PrsA) under control of a yeast promoter. Expression of the human or bacterial enzymes restored colony growth on plates (Fig. 5A) and doubling time in liquid cultures (Fig. 5B). Importantly, expression of the exogenous PRPS in yeast restored intracellular PRPP, although only partially (Fig. 5C). Similarly, expression of exogenous PRPS in yeast only partially, but significantly, restored nucleotide levels, although to various extents, higher for purines than for pyrimidines for unknown reasons (Fig. 5D-H). We conclude that synthesis of PRPP derivatives is apparently limiting for proliferation in the *prs1 prs3* mutant and that partial restoration of the intracellular pools, by expression of exogenous PRPS, is likely sufficient to recover doubling times not very different from that of the wild-type control. Of note, this result is highly consistent with the one observed previously for the *prs2 prs4 prs5* mutant (Fig. 2E). It should be noted that the phenotypic and metabolic rescue by expression of bacterial PrsA or human PRPS in the *prs1 prs3* mutant is probably contributed, in part, by the Prs2 Prs4 and Prs5 isoforms that are still present in this mutant (11).

We then asked whether it is PRPP itself or its derived products that is limiting growth of the *prs1 prs3* mutant. We intended to address this question by restoring higher intracellular

nucleotides without restoring PRPP-synthetase activity. This was done as above for *rki1* by feeding the cells with nucleosides and amino acids. Of note, histidine is contained in the casamino mixture added to the medium and is present in all conditions. Addition of each alone of the ribose-containing precursors or final products had little effect on colony growth of a *prs3* mutant (Fig. S2), however combined addition of the supplements resulted in a significant improvement of colony growth (Fig. 6A). Furthermore, the rescue of doubling time by the combination of supplements was total in liquid cultures (Fig. 6B), suggesting that the partial rescue effect observed on plates (Fig. 6A) could be due to poor diffusion of the substrates in the agar plate, as previously reported (27). Importantly, addition of the supplements to the *prs1 prs3* mutant did not increase intracellular PRPP concentration (Fig. 6C) but rather lowered it for a yet ununderstood reason, while, as expected, it significantly increased all intracellular NTPs (Fig. 6D-G) to levels comparable to those of the wild-type isogenic strain. Meanwhile, NAD(H) was only partially restored (Fig. 6H), the metabolism of nicotinamide mononucleotide to Nr, and/or the uptake of Nr by Nrt1 being possibly limiting. We conclude that most of the growth defect of the *prs1 prs3* mutant is due to its inability to synthesize a sufficient amount of PRPP-derived metabolites.

Altogether, our results show that low synthesis of PRPP and more specifically of its derived products, such as nucleotides, is the principal cause of the slow growth phenotype of the *prs1 prs3* mutant.

Isolation and characterization of genetic suppressors of the prs1 prs3 mutant

Having shown that supplementation or exogenous PRPS activity could rescue the *prs1 prs3* mutant slow growth, we then asked whether this proliferation phenotype could be suppressed genetically. Starting from a *prs1 prs3* double KO mutant, we searched for suppressors that would alleviate the severe colony growth defect of the mutant and thereby possibly reveal metabolic processes limiting proliferation. Because PRPP is a central molecule in metabolism, the experiment was done on prototrophic yeast to avoid interference with auxotrophy markers. After UV mutagenesis, rapid growers were isolated from the *prs1 prs3* slow growers and further characterized. The experiment was run in parallel on ‘rich’ (SDcasaWAU, containing amino-acids and nucleobases) and ‘poor’ (SD, lacking amino-acids and nucleobases) media to establish whether different types of suppressors would be found when the salvage or *de novo* pathways are mostly challenged (Fig. 1), respectively. A total of 69 and 76 suppressors were respectively isolated from SD and SDcasaWAU medium, growth phenotypes of a subset of these mutants is shown in Fig. 7A. Two mutants (supp3 and supp48) were backcrossed to a *prs1 prs3* parental strain and in both cases, the suppressor mutation was found to segregate as a single locus in the meiotic progeny. Sets of pooled segregants (28) carrying or not the suppressors were sequenced and, for both suppressors, a mutation at the *PRS5* locus was found in the suppressed segregant-pools but not in the non-suppressed segregant-pools. The *PRS5-C328T* (Pro110Ser) and *PRS5-G932T* (Arg311Ile) mutations were confirmed by resequencing the *PRS5* locus in the original supp3 and supp48 mutants, respectively, while none of these changes were found in the parental strains. Sequencing of the *PRS5* locus from 15 suppressor mutants obtained from SD medium and 15 from SDcasaWAU medium revealed that they all carry a mutation in *PRS5* (Table S1). Finally, the *PRS5-supp3*, *PRS5-supp11* and *PRS5-supp48* mutant alleles suppressed *prs1 prs3* when carried on a either centromeric or multicopy plasmids, while the wild-type allele did not (Fig. 7B), further confirming that they are necessary and sufficient suppressor mutations and revealing their dominance over the wild-type *PRS5*

allele. We conclude that several mutations in *PRS5* can efficiently suppress the growth defect of the *prs1 prs3* double mutant. All the tested *PRS5* suppressor mutations were found dominant. A subset of 3 suppressors (P110S, R311I and D401N) was further analyzed revealing that the *PRS5* suppressors decreased the population doubling time (Fig. 7C) and had a significant effect on intracellular PRPP (Fig. 7D) as well as nucleotide content (Fig. 7E-I). Finally, we observed that overexpression of the mutated forms of *PRS5* on multicopy plasmids only slightly improved suppression compared to that obtained with centromeric plasmids (Fig. 7B). We hypothesized that this could be due to limitation of one or both of Prs5 partners, namely Prs2 and/or Prs4. We hence overexpressed *PRS2* or *PRS4* together with *PRS5*^{P110S} and found that indeed the co-overexpression of either *PRS2* or *PRS4* strongly stimulated growth (Fig. 7J), indicating that the *PRS5* suppressor acts through its interaction with its partners Prs2 or Prs4. Hence, suppressors of *prs1 prs3* most likely act by increasing PRPP synthesis by the Prs2/4/5 enzyme rather than modulating downstream consumption.

The results presented in this section and the previous one allow us to conclude that in the *prs1 prs3* mutant, PRPP is limiting for proliferation and that these effects can be alleviated by increasing PRPP (through *PRPS1*, *PRPS2* or *PrsA* expression or *PRS5* suppressors) or by bypassing its requirement (*via* supplementation with nucleosides and amino acids).

PRPP is limiting for nucleotide synthesis but not for proliferation in wild-type yeast

We then asked whether PRPP is present in excess in yeast cells or whether it could be limiting for nucleotide synthesis even in wild-type cells. To address this question, we first expressed in wild-type yeast cells the *PRPS1* human enzyme that was able to suppress the growth defect of the *prs1 prs3* mutant (Fig. 5A). Expression of the human enzyme did not increase intracellular PRPP nor NTPs or NAD(H) in the wild-type cells (Fig. 8A-F). This result suggested that ribose-5-phosphate, the substrate of PRPP-synthetase, could be limiting for the reaction to proceed or, alternatively, that the human enzyme is feedback inhibited by some end-product(s)(16). To settle this question we expressed in yeast two previously described hyperactive variants of *PRPS1* (V142L and A190V) (29). Clearly, expression of these two *PRPS1* mutants resulted in a very significant increase of intracellular PRPP (Fig. 8A) as well as of all NTPs and NAD(H) when expressed in wild-type yeast (Fig. 8B-F). We conclude that PRPP-synthetase activity, rather than the ribose-5-phosphate substrate, is limiting PRPP synthesis and downstream reactions in a wild-type prototroph yeast strain. Furthermore, expression of these hyperactive forms of PRPP-synthetase significantly affected the doubling time (Fig. 8G). Of note, the accumulation of purine intermediates, such as SZMP, IMP and inosine (Fig. 8H-J) when the *PRPS1* mutants are expressed in yeast, indicate that the flux in the purine *de novo* pathway is overloaded. This metabolic burden could be responsible for the higher doubling time by diverting a significant part of glucose from glycolysis to the PPP and nucleotide synthesis in the form of an unused excess of purines.

The increase of all NTPs and NAD(H) in yeast strains expressing the two *PRPS1* mutants (Fig. 8B-F) suggested that, in wild-type yeast cells, PRPP-utilizing enzymes compete for PRPP as a limiting substrate. To address this question more directly, we asked whether affecting one PRPP-consuming pathways results in an effect on the products of other PRPP-consuming pathways. This was first done by blocking *de novo* synthesis of purine nucleotides, using a *ade4* knockout mutation that blocks the first step of the pathway (Fig. 9A and B). This mutant strain can grow and proliferate as long as it is fed with a purine precursor such as adenine, utilizable through the salvage pathway. When shifted for 3 hours in a medium lacking adenine, the *ade4*

mutant showed a strong decrease of adenylic and guanylic nucleotides (Fig. 9D-E) as expected but also CTP and NAD(H) (Fig. 9F and G), most probably because their synthesis is affected at low ATP concentration (30, 31). By contrast to CTP, intracellular UTP showed a strong increase, about four fold, in the *ade4* mutant (Fig. 9H), suggesting that the PRPP that was not used for purine, CTP and pyridine synthesis due to the *ade4* block in the purine pathway could be used to make more UTP. In addition, the expression of an *ADE4* hyperactive dominant allele (Fig. 9A and C) (26, 32) resulted in decreased intracellular UTP and CTP (Fig. 9I-J). Of note, here UTP and CTP both decreased reflecting the fact that under these experimental conditions CTP is synthesized *de novo* from UTP. In the same experiment, ATP and GTP were not significantly affected (Fig. 9K and L), while the flux in the *de novo* purine pathway was high as attested by the accumulation of the metabolic intermediate ZMP and of the degradation product inosine (Fig. 9M and N). These results indicate that the flux in the purine pathway has a strong impact on intracellular UTP. We conclude that, in wild-type yeast, PRPP-utilizing enzymes are competing for PRPP as a substrate which is limiting for synthesis of downstream products.

This competition between PRPP-utilizing enzymes was further confirmed using a *ura2* block in the pyrimidine pathway (Fig. S3A). When these cells were grown in the absence of uracil, we observed a severe decrease of UTP and CTP and a small but significant increase of intracellular ATP (Fig. S3 B-F)). Due to the different intracellular abundance of ATP and UTP (3.8 and 1.1 mM, respectively. Fig. 9A and E) it could be expected that a variation of intracellular ATP would much more severely affect UTP levels than the reverse. Finally, intracellular NAD(H) was tightly correlated to intracellular ATP as reported before (31) and intracellular CTP was correlated to intracellular ATP and UTP which are both required for its synthesis from UTP by CTP-synthase (suggesting that the K_M for ATP of this enzyme might be high).

Altogether, our results allow two conclusions. First, decreasing intracellular PRPP leads to a reduction of all downstream products, while increasing intracellular PRPP had the opposite effect, establishing that in wild-type yeast cells PRPP is limiting nucleotide synthesis. Second, when PRPP is over- or under-used in one pathway, it is compensated by its usage in other PRPP-utilizing pathways, indicating that phosphoribosyl transferases are competing for PRPP. We conclude that, in yeast, PRPP synthesis limits nucleotide synthesis but not cell proliferation. In addition, PRPP utilization appears to directly result from competition between the enzymes of the downstream pathways.

Metabolic and proliferation consequences of PRPS hyperactivity in human cells.

Based on the conclusions obtained with yeast cells, we then questioned several aspects of the PRPP metabolism in human cells. We first aimed at evaluating the metabolic consequences of PRPS hyperactivity in the glioblastoma U-87 MG human cell line. *PRPS1* (wild-type, and the hyperactive mutants V142L and A190V) or *PRPS2* were expressed in U-87 MG cells using lentiviruses and metabolic profiles were established. As in yeast cells, expression of the V142L and A190V *PRPS1* mutants in U-87 MG resulted in a significant increase of PRPP (Fig. 10A) as well as of all NTPs, from 1.6 fold for ATP to more than 2-fold for the other NTPs (Fig. 10B-E) and of 1.2 fold for NAD(H) (Fig. 10F). A significant increase, although slightly lower, was also observed when these mutant *PRPS1* alleles were expressed in the colon tumor cell line HCT116 (Fig. S4), indicating that this effect is not cell line specific. Interestingly, expression of wild-type *PRPS1* or *PRPS2* increased intracellular PRPP in the U-

87 MG cell line (Fig. 10A), while such an effect was not observed in the HCT116 cell line (Fig. S4A) as well as in yeast cells (Fig. 8A), the reasons for this discrepancy are not known. However, while PRPS1 expression had no significant effect on NTPs (Fig. 10B-E) or NAD(H) (Fig. 10F), expression of PRPS2 resulted in a significant increase of all four NTPs and NAD(H) (Fig. 10B-F).

In all experiments, cell proliferation was not accelerated by conditions resulting in higher intracellular NTP content (Fig. 10G), indicating that, as observed in yeast, PRPP is limiting for nucleotide synthesis but not for cell proliferation. Actually, in all cases where intracellular NTPs were high, cell proliferation was severely slowed down (Fig. 10G), indicating that more NTPs is not ‘better’ for human cells, although the precise reasons for this inhibitory effect are not known.

We then investigated whether increasing the flux in one PRPP consumption pathway affects the others, as observed in yeast cells. This was done by growing the HCT116 or U-87 MG cells in dialyzed serum supplemented or not with adenine, which combined to PRPP can be metabolized to AMP by adenine phosphoribosyl transferase and then to ATP. Addition of adenine indeed increased intracellular ATP (Fig. 11A and E) and conjointly decreased all other triphosphate nucleotides (Fig. 11B-D and F-H), suggesting that the PRPP utilized to synthesize ATP from adenine in excess, is limiting the synthesis of other PRPP derived metabolites. We conclude that PRPP utilization in human cells (Fig. 10- Fig.11), similarly to what was obtained in yeast (Fig. 9), is the direct result of a competition between several downstream pathways.

Discussion

Our work on PRPP synthesis and consumption in yeast gives important clues on upstream and downstream pathways. In particular it allowed to establish that ribose-5-phosphate synthesis by Rki1 is essential because of the role of ribose-5-phosphate in PRPP synthesis. Interestingly, this indicates that the alternative synthesis of ribose-5-phosphate from G3P and sedoheptulose-7-phosphate cannot fully compensate for the loss of Rki1 activity to support growth of yeast cells (Fig. 1).

Clearly, *PRPS1* hyperactive mutants expressed in yeast or human cells increase both intracellular PRPP and downstream products such as nucleotides. These results establish that ribose-5-phosphate, just upstream of PRPP synthesis in the PPP (Fig. 1), is not limiting for PRPP synthesis. From these experiments, we propose that ribose-5-phosphate is always in excess and that the portion not used for PRPP synthesis is recycled in the glycolysis. Accordingly, experiments with wild-type PRPS1 suggest that PRPP synthesis is limited by PRPP synthetase regulation. Indeed, by contrast to the hyperactive mutant forms of PRPS1, expression of wild-type PRPS1 has no effect on the concentration of PRPP (except in the U-87 MG cell line Fig. 10), and no effect either on intracellular nucleotides (Fig. 8 B-F; Fig. 10 B-F; Figure S4 B-F). Importantly, overproduction of PRPP decreased proliferation of both the human cells (Fig. 10G, Figure S4G) and the yeast cells (Fig. 8G). The reason for this defect is not established, but it could reflect the metabolic burden associated with production of PRPP, and most importantly nucleotides, that are not further used for growth but degraded and excreted in the form of inosine in yeast and uric acid in humans.

Additionally, our results in yeast show that PRPP is synthesized at the minimal flow allowing the maximal biomass production. Slightly decreasing PRPP concentration (*prs2 prs4 prs5* triple mutant, Fig. 2A) results in lower biomass production rate (Fig. 2C), while doubling

time is not affected (Fig. 2E), indicating that in this condition, maintaining the doubling time is preferred over maintaining cell size. However, further decreasing intracellular PRPP (*prs1 prs3*) double mutant (Fig. 2A) affected both biomass production rate (Fig. 2C) and population doubling time (Fig. 2E). Strikingly, overexpression of *PRS1/PRS3* resulting in increased PRPP concentration also diminished the biomass production rate and increased doubling time (Fig. 4F and H). These results parallel the fact that both gain and loss of function, in *PRPS1*, are associated with human diseases (18). We interpret these results as an indication that, under standard conditions in wild-type yeast, the PRPP concentration is optimal for growth and division and that an increase or decrease of PRPP synthesis capacity affects these parameters (with doubling time taking precedence over cell volume when PRPP is only slightly limiting, see Fig. 2). These results fit well with the central role of PPP in anabolism and with the key role of PRPP as a product of PPP.

Most importantly, PRPP consumption, in yeast and human cells, appears to take place “on demand” by the various PRPP-utilizing pathways. This was shown by blocking or increasing the flux in specific PRPP consuming metabolic routes (Fig. 9, Fig. 11 and Fig. S3). The results show that there is apparently no regulation of the utilization of PRPP between the various pathways using it, just competition for a common substrate.

Together, our results show that PRPP metabolism responds to an action mass law where the ribose-5-phosphate provided by the PPP is not limiting for PRPP synthesis and where PRPP utilization is an on demand process depending most probably on the amount and kinetic parameters of the various involved PRPP-utilizing enzymes.

Experimental procedures

Yeast Media and Strains

SD is a synthetic minimal medium containing 0.5% ammonium sulfate, 0.67% yeast nitrogen base (Difco), 2% glucose. SDcasaW is SD medium supplemented with 0.2% casamino acids (Difco) and tryptophan (0.2 mM). When indicated, adenine (0.3 mM) and/or uracil (0.3 mM) were added in SDcasaW medium, resulting in a medium named SDcasaWA (+ adenine), SDcasaWU (+ uracil) and SDcasaWAU (+ adenine + uracil). SC -Ura -Leu medium is SC medium supplemented with adenine (0.3 mM), histidine (0.06 mM), lysine (0.06 mM), and tryptophan (0.2 mM). The YPD medium contained 1% yeast extract, 2% peptone and 2% glucose. Yeast cells were grown in liquid or solid media at 30°C. Yeast strains (listed in Table S2) belong to, or are derived from, FY4 and FY5 prototrophic strains (33) or disrupted strains isogenic to BY4741 or BY4742 purchased from Euroscarf (Frankfurt, Germany). Multi-mutant strains were obtained by crossing, sporulation and micromanipulation of meiosis progeny.

Human cell culture, viral infection and proliferation assays

The human tumor cell lines HCT116 (CCL-247) and U-87 MG (HTB-14) were obtained from the ATCC. Cells were grown at 37°C, 5% CO₂ in complete DMEM medium containing 4.5 g/L glucose and supplemented with 10% foetal bovine serum (FBS; DUTSCHER #S1810), L-glutamine, penicillin and streptomycin. Dialyzed serum (same FBS as above) was obtained after dialysis in PBS (3 kDa-molecular weight cutoff) and was filtered at 0.1 µm. Lentiviruses expressing *PRPS1*, *PRPS2* and mutants were obtained at the Vect'UB platform (TBMCore Bordeaux University, see Table S3). Titers were given in Transduction Units (TU) and were obtained by qPCR. Cells in suspension in culture medium were incubated with lentiviruses at 0, 1, 3, 5, 10 and 20 TU/cell and then inoculated at the cell density of 10,000 cells/cm² in 6-

well culture dishes. Cells were allowed to settle down at 37°C for 24 hours and 0.5 µg/ml puromycin was then added for 7 days with a medium change every two days. TU-values retained for following analyses were in the range of 5 to 20 depending on the cell type and transduction experiment. Transduction with the different constructs were performed in paralleled experiments, for the sake of better comparisons. Two independent productions of lentivirus were used and gave similar results.

For metabolites quantifications, cells were seeded in 6-well plates and grown up to half confluency. Cells were then washed and incubated for 24 h in the complete medium before metabolic extractions performed as described in (34). Tri-, tetra- or penta-plicate conditions were used. For proliferation experiments, cells were seeded in 24-well plates at 7,000 cells/cm² and grown 4 days at 37 °C with medium changes every 2 days. Triplicate or tetraplicate conditions were used. Cells were harvested by trypsinization and were counted using a Multisizer 4 Coulter counter (Beckman).

Plasmids

All plasmids and oligonucleotides are listed in Table S3 and Table S4, respectively. Plasmids allowing expression of human wild-type *PRPS1* and *PRPS2* in yeast were obtained by PCR amplification of the open reading frames (ORF) with the following pairs of oligonucleotides (5562 + 5563 for *PRPS1* and 5763 + 5764 for *PRPS2*) using respectively cDNA IRAUp969H0616D and IRCMp5012H0142D from Source Biosciences as templates. *PRPS1* Hyperactive mutants were obtained by site directed mutagenesis. PCR products were cloned in the pCM189 or its derivative p2714 plasmids in which gene expression is under the control of tetracycline-repressible promoter. For expression of the same genes in mammalian cells, ORF were amplified with the 5678 + 5563 (*PRPS1*) and 5763 + 5764 (*PRPS2*) and cloned in the pLenti-MND-IRES-Puro^R-WPRES. The *PrsA* expression plasmid was obtained by PCR amplification of *PrsA* ORF with oligonucleotides 2278 + 2279 on *E. coli* genomic DNA as template and cloning in pCM189. The *hENT1* encoding plasmid was a generous gift from Drs. Cass, Damaraju, and Sawyer. The plasmid expressing the hyperactive form of *ADE4* gene (*ADE4-1* mutant R328K) was obtained by cloning in pCM189 the PCR fragment amplified with oligonucleotides 48 + 429 on genomic DNA of the m131 *ADE4* mutant described in (26, 32). All plasmids used to express *PRS1*, *PRS2*, *PRS3*, *PRS4* and *PRS5* (either wild-type or mutated alleles) genes were obtained by PCR amplification of ORFs using yeast genomic DNA as template (FY4 for wild-type genes and *PRS5* suppressors indicated in Table S1) and the following couples of oligonucleotides 2032 + 2033, 5847 + 5848, 5082 + 5083, 5849 + 5850 and 2040 + 2041 for *PRS1*, *PRS2*, *PRS3*, *PRS4* and *PRS5*, respectively. PCR products were then clones in either centromeric (YCplac33) or multicopy (Yeplac181 or Yeplac195) plasmids as indicated in Table S3. For all plasmids, further construction details are available upon request.

Yeast population doubling time

Cells were kept in exponential growing phase ($< 1.5 \cdot 10^7$ cells/ml) for at least 24 h hours by successive dilutions before any measurement. Population doubling time was then determined by following cell number during 8 hours on at least four independent exponential growth culture using the Multisizer 4 Coulter counter (Beckman). Population doubling time was then determined by exponential growth regression of the proliferation curves using GraphPad Prism 5.

Biomass production rate

Cells were exponentially ($< 1.5 \times 10^7$ cells/ml) grown in indicated medium for at least 24 h hours before measurement. Biomass was determined by filtration of aliquots of the cell culture (starting at comparable biomass for each strain around 10 mg corresponding to 50-100 ml at $1-2 \times 10^6$ cells /ml) on 0.45 μ m polyamid pre-weighted filters (25006-47-N; Sartorius) followed by two 10 ml washes with room temperature milliQ water and drying of the filter with successive 30 s cycles (until the mass of filter + cells is constant) in a microwave oven at 800 W. Dried biomass was measured in exponential growth conditions during 4 hours and exponential biomass production rate was determined by linear regression of the experimental points (see Figure S5 as an example).

Metabolic analyses

Metabolic extractions of yeast or mammalian cells were performed using the EtOH boiling methodology as described in (34). Metabolite separation by ionic chromatography was either performed on an ICS3000 chromatography station (Dionex, Sunnyvale, CA) using a CarboPac PA1 column (250 x 2 mm; Dionex) as described in (35), or using the Integrion chromatography station (Thermo Electron) equipped with RFIC eluant generator and eluant suppressor (ADRS 600) and coupled to both a Vanquish diode array detector and a conductivity detector. Metabolites separation was achieved at 0.38 ml/min and 30°C on an AS11-HC-4 μ m (250 x 2 mm, Thermo Electron) with a Potassium hydroxide discontinuous gradient, provided by the eluant generator, starting at 1 mM for 7 min, rising up to 15 mM in 9 min, 30 mM in 9 min and 60 mM in 13 min, followed by a step at 60 mM for 7 min and finally increasing up to 80 mM in 8 min and stay at 80 mM for 29 min. The resin was then re-equilibrated at 1 mM KOH for 10 min before injection of a new sample. Metabolites were detected by conductimetry (PRPP) and by UV absorbance (nucleotides and derivatives) and identified by their UV spectrum signature and/or by injection with standards. For each yeast strain or human cell line, samples normalization was achieved on the basis of cell number and median cell volume using a Multisizer 4 (Beckman coulter). Peak area quantification was done on conductimetric recording for PRPP and with UV absorbance at 260 nm for all nucleotides and derivatives except for Cytidylic nucleotides and NADH that were quantified at 280 nm et 340 nm, respectively. The intracellular concentration of metabolites has been determined using standard curves obtained with pure compounds. The sum of each type of purine (Adenylic and Guanylic) and pyrimidine (Uridylic and Cytidylic) nucleotides is defined as $[NXP] = [NMP] + [NDP] + [NTP]$. All metabolic raw data are presented in Tables S5-S17.

Statistical analyses

For yeast strains and/or human cell lines, growth and metabolites extraction were performed on 3 to 8 biologically independent samples, as detailed in each Figure legend. Statistics, given as p-values noted in each Figures, were determined by a Welch's unpaired t-test. Welch's t-test is more robust than Student's t-test and maintains type I error (rejection of the true null hypothesis) rates close to nominal for unequal variances.

Supporting informations- This article contains supporting informations.

Data availability- All data are contained within the manuscript.

Author contributions- **Benoît Pinson:** conceptualization, writing reviewing and editing, investigation, formal analyses, validation. **Michel Moenner:** conceptualization, writing reviewing and editing, investigation, formal analyses, validation. **Christelle Saint-Marc:** investigation. **Alexandra Granger-Farbos:** investigation. **Bertrand Daignan-Fornier:** conceptualization, writing reviewing and editing, fundings, formal analyses, validation.

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Conflict of Interest

The authors declare that they have no conflicts of interest with the content of this article.

Abbreviations

The abreviations used are: CTP: Cytidine triphosphate; G6PDH: Glucose-6-phosphate dehydrogenase; NAD⁺: Nicotinamide adenine dinucleotide; NADH: NAD⁺ reduced form; NADP⁺: Nicotinamide adenine dinucleotide monophosphate; NADPH: NADP reduced form; NMN : nicotinamide mononucleotide; Nr: Nicotinamide riboside; NTP: Nucleotide triphosphate; PPP: pentose phosphate pathway; PRPP: 5-phosphoribosyl-1-pyrophosphate; UTP: Uridine triphosphate; SZMP: succinyl aminoimidazole carboxamide nucleotide monophosphate and ZMP: aminoimidazole carboxamide nucleotide monophosphate.

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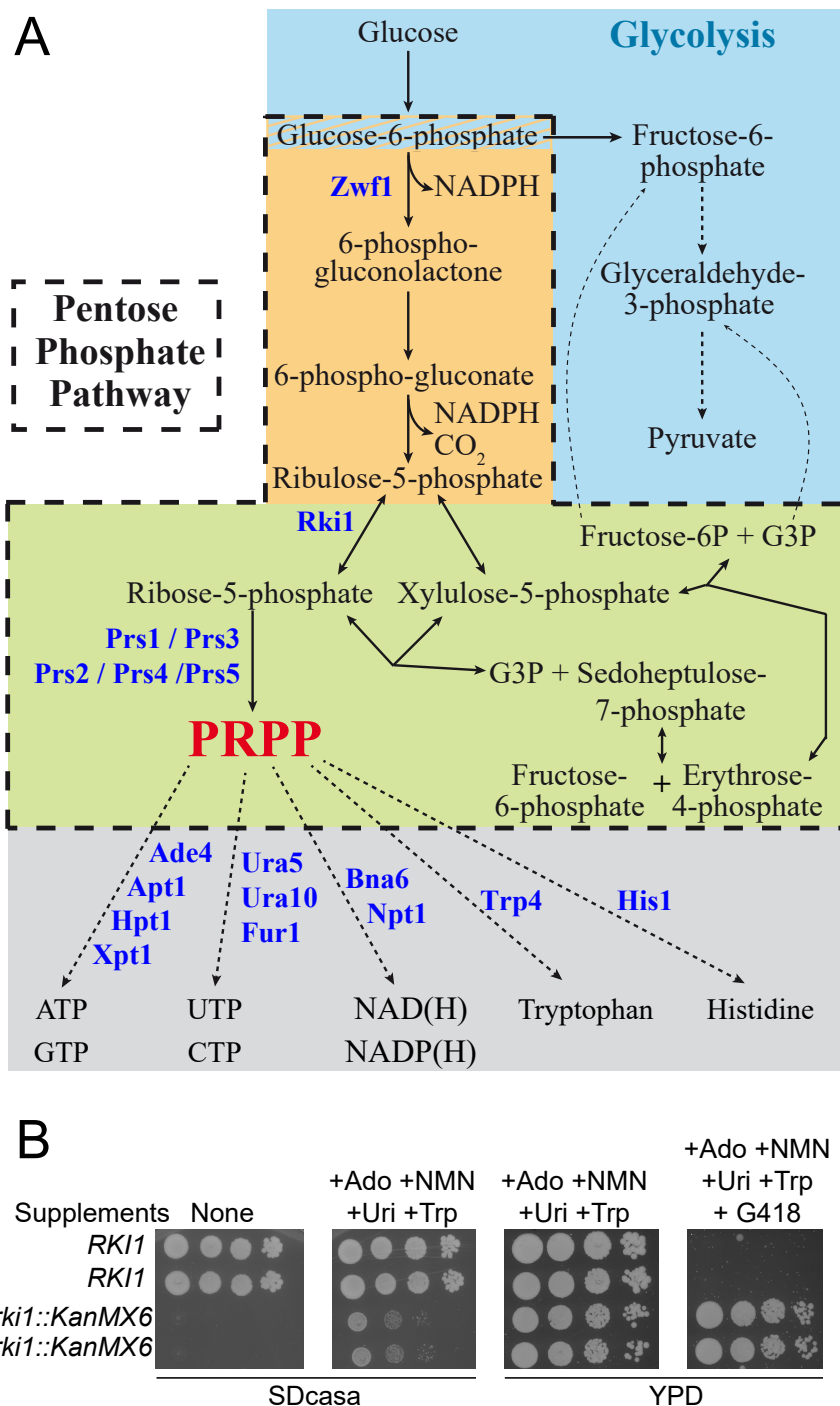


Figure 1. Schematic representation of PRPP synthesis and consumption in *Saccharomyces cerevisiae*. *A*, ATP: adenosine triphosphate; CTP: Cytidine triphosphate; G3P: glyceraldehyde-3-phosphate; GTP: Guanosine triphosphate; NAD(H): Nicotinamide adenine dinucleotide (reduced form); NADP(H): Nicotinamide adenine dinucleotide phosphate (reduced form); PRPP: 5-phosphoribosyl-1-pyrophosphate and UTP: Uridine triphosphate. Proteins mentioned in the text are shown in blue. Glycolysis, pentose phosphate pathway oxidative and non-oxidative branches and PRPP consumption pathways are boxed in blue, orange, green and grey, respectively. *B*, Essential synthesis of ribose-5-phosphate by the ribose-5-phosphate transketolase *Rki1* can be rescued by supplementation bypassing PRPP requirement. The heterozygote diploid *RKI1/rki1Δ* (Y5217) was transformed with a plasmid allowing expression of the human nucleoside carrier hENT1 (p4491). Transformants were sporulated and the meiotic progeny was dissected by micromanipulation on YPDA medium supplemented with Adenosine (Ado), Uridine (Uri) and Tryptophan at 300 μ M each and Nicotinamide mononucleotide (NMN) at 100 μ M. Serial dilutions (1/10) of four spores from a representative tetrad were spotted on indicated media and plates were imaged after 2 days at 30°C.

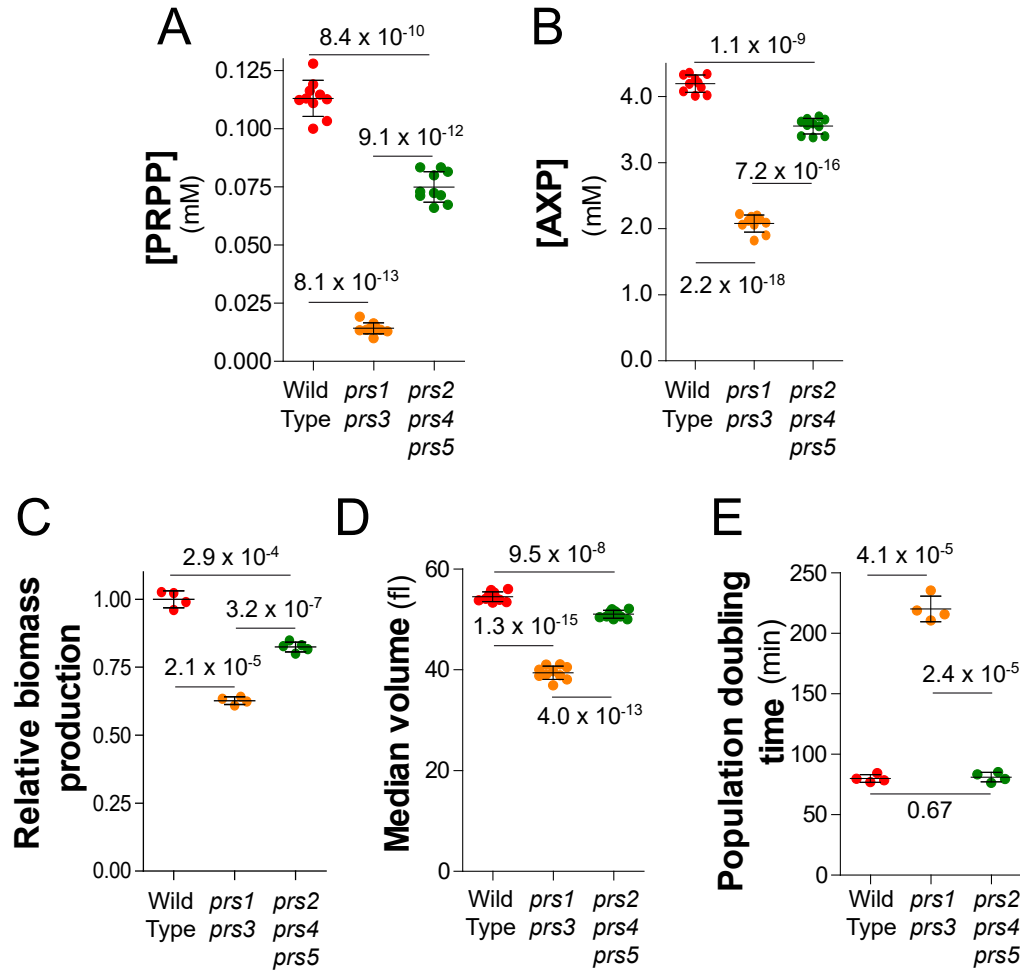


Figure 2. Phenotypic and metabolic consequences of PRPP decrease in yeast. (A-E) Wild-type (Y12325), *prs1*Δ *prs3*Δ (Y12562) and *prs2*Δ *prs4*Δ *prs5*Δ (Y12649) were exponentially grown in SDcasaWAU medium for 24 h before measurement of biomass production rate (C), median volume (D), population doubling time (E), or metabolic extraction, separation and quantification (A-B), done as described in experimental procedures. [AXP] = [ATP] + [ADP] + [AMP] (individually presented in Fig. S1A, E, I). Each parameter was determined on 4 to 8 independent measurements. Numbers on each panels correspond to p-values calculated from a Welch's unpaired t-test.

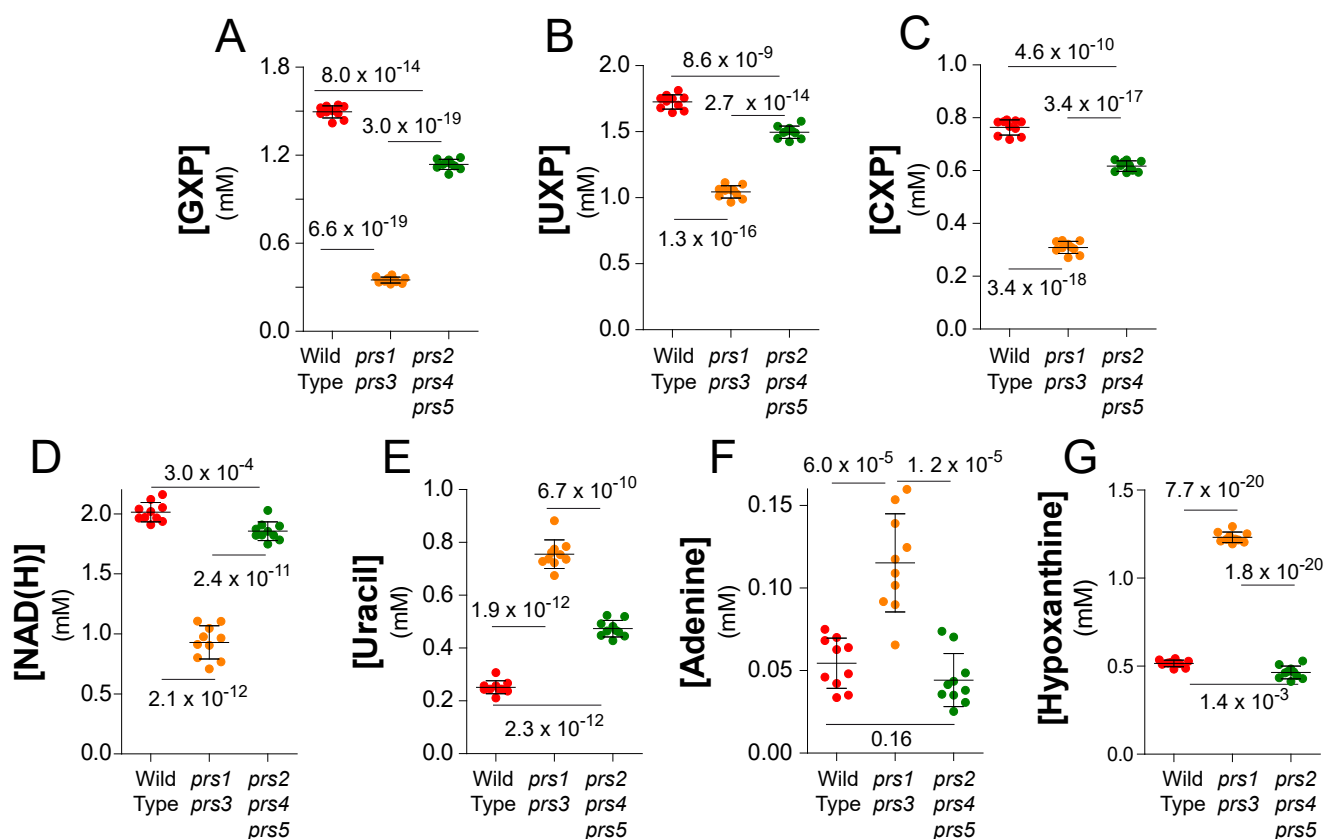


Figure 3. Consequences of PRPP variations on nucleotide synthesis and precursor accumulation. Metabolic extractions and quantification were performed on wild-type (Y12325), *prs1*Δ *prs3*Δ (Y12562) and *prs2*Δ *prs4*Δ *prs5*Δ (Y12649) exponentially grown for 24 h in SDcasaWAU medium, as in Fig. 2. For each nucleotide (N), [NXP] = [NTP] + [NDP] + [NMP] (presented in Fig. S1). NAD(H) corresponds to the sum of the oxidized (NAD⁺) and reduced (NADH) form of the pyridine nucleotide. Each metabolite, nucleotide (A-D) and precursor (E-G), was quantified from 8 independent yeast cultures and metabolite extractions. Numbers on each panels correspond to p-values calculated from a Welch's unpaired t-test.

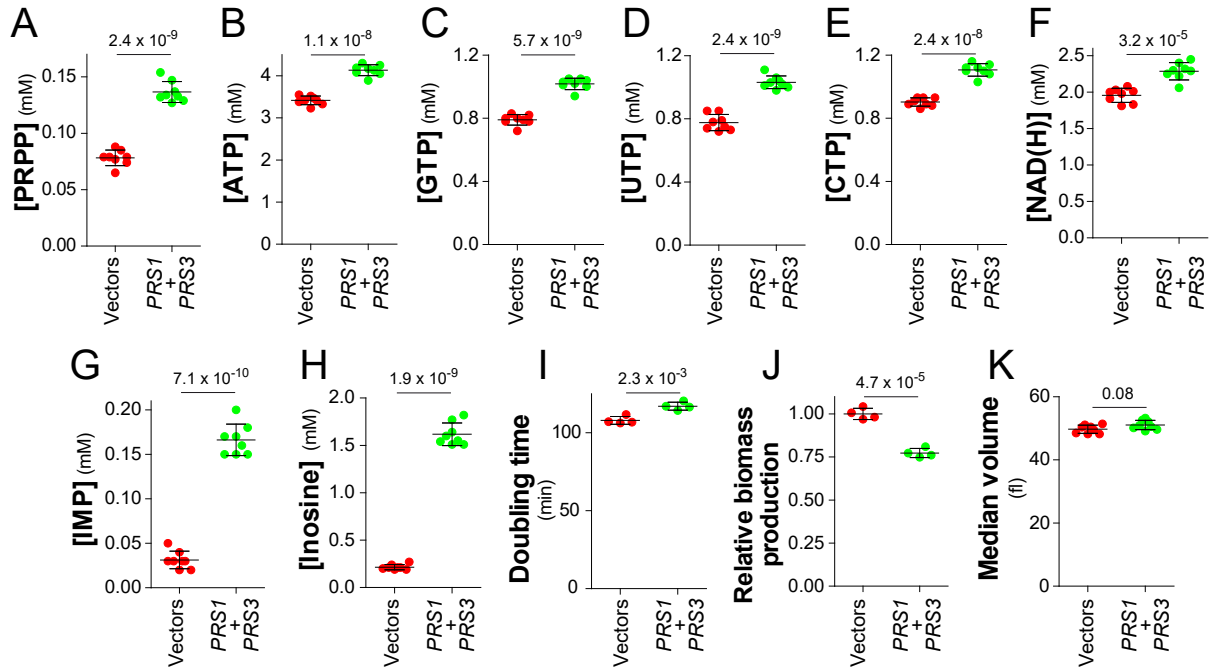


Figure 4. Metabolic and phenotypic consequences of *PRS1-PRS3* overexpression. Wild-type (Y11451) strain was transformed with either the empty vectors (vectors, YepLac181 and YepLac195) or plasmids allowing overexpression of *PRS1* (p6160) and *PRPS3* (p5671) (*PRS1* + *PRPS3*). Transformants selected on SC -Ura -Leu medium were exponentially grown for 24 h in the same liquid medium before extractions and quantification of metabolites from 8 independent cultures (A-H) or determination of either the population doubling time (I), the relative biomass production rate (J) or the median volume (K). The p-values, calculated from a Welch's unpaired t-test are presented by numbers.

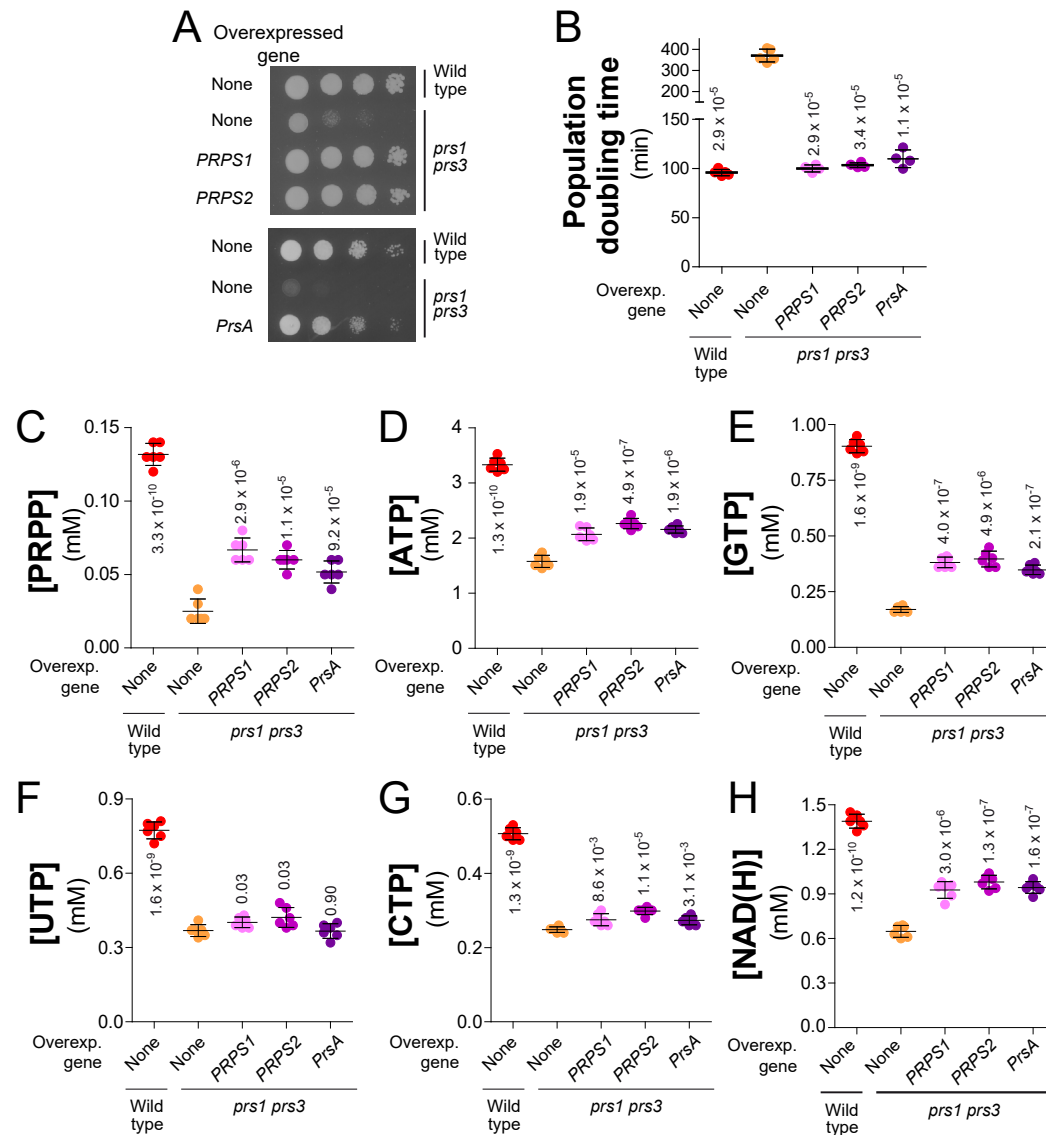


Figure 5. PRPP content is limiting for yeast proliferation and nucleotide synthesis. *A*, The growth defect of the *prs1 prs3* mutant is restored by expression of either the human or bacterial PRPP synthetases. Wild-type (Y11418) and *prs1Δ prs3Δ* (Y11983) strains were transformed with either an empty vector (None) or plasmids allowing expression of human (*PRPS1* (p6001) or *PRPS2* (p6020)) or bacterial (*PrsA*; p5909) PRPP-synthetases. Transformants were selected on SDcasaWA medium and serial dilutions (1/10) of transformants were spotted on SDcasaWA and plates were imaged after 36 h (*PrsA*) or 48 h (*PRPS*) at 30°C. *B-I*, Transformants were exponentially grown for 24 h in liquid SDcasaWA before determination of the population doubling time (*B*) or extractions and quantification of metabolites (*C-H*). Each metabolite was quantified from 6 independent cultures. The p-values, calculated from a Welch's unpaired t-test are presented by numbers. Overexp. stands for overexpressed.

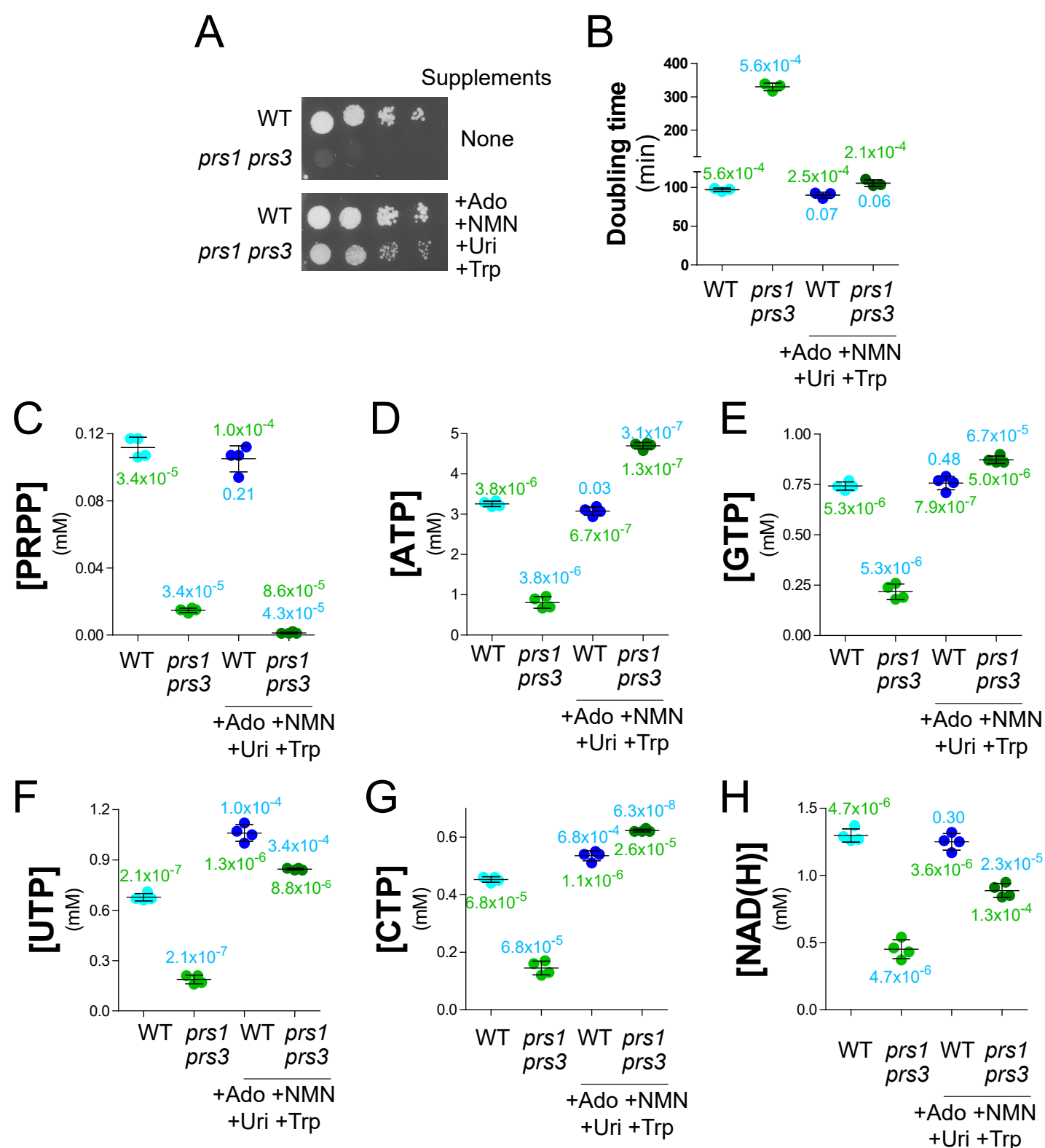


Figure 6. Growth and metabolic defects of the *prs1 prs3* mutant are rescued by supplementation with PRPP-not-requiring precursors. *A*, Addition of nucleoside precursors partially restores the *prs1 prs3* mutant growth defect. Wild-type (Y11418) and *prs1* Δ *prs3* Δ (Y11985) strains were transformed with the *hENT1* expressing plasmid (p4991). Transformants were selected on SDcasaW medium supplemented with adenosine (Ado, 300 μ M), Uridine (Uri, 300 μ M), Tryptophan (300 μ M) and Nicotinamide mononucleotide (NMN, 100 μ M). Transformants were serial diluted (1/10) and spotted on SDcasaW supplemented or not with Ado, Uri, Trp and NMN. Plates were imaged after 36 h at 30°C. *B-H*, Transformants were exponentially grown for 24 h in SDcasaW liquid medium supplemented or not with the indicated precursors prior to population doubling time measurement (*B*) or metabolic extraction, separation and quantification (*C-H*). The p-values, calculated from a Welch's unpaired t-test, are presented by either blue (comparison with wild-type cells grown without precursors) or green numbers (comparison with *prs1* Δ *prs3* Δ cells grown without precursors).

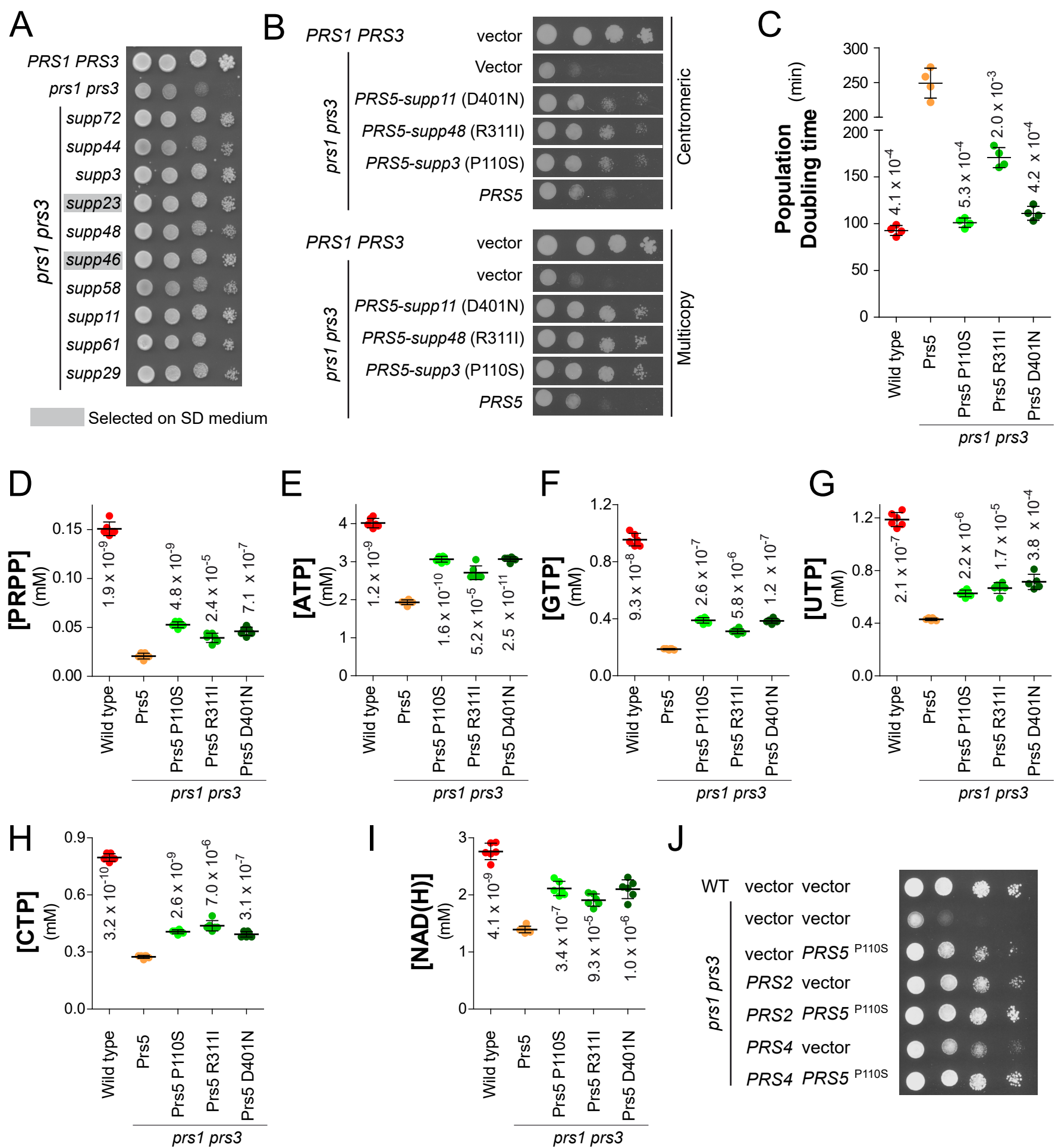


Figure 7. Suppression of *prs1 prs3* growth and metabolic defects by mutations in the *PRS5* gene. *A*, Suppressors were isolated from *prs1Δ prs3Δ* strains as described in the experimental procedure section. Wild-type (FY4), *prs1Δ prs3Δ* (Y12562) and suppressors strains (Table S1) were serially diluted (1/10), spotted on SDcasaW medium and imaged after 48 h at 30°C. *B*, Plasmid driven expression of *PRS5* suppressor alleles is sufficient to alleviate the *prs1Δ prs3Δ* growth defect. Wild-type (Y11418) and *prs1Δ prs3Δ* (Y11983) strains were transformed with empty vectors (YCplac33 (top) or YEplac195 (bottom)), or plasmids expressing the *PRS5* gene either wild-type (p6017 or p6019) or suppressing alleles (*supp11* (p6009 or p 6008); *supp48* (p6014 or p 6011) and *supp3* (p6016 or p 6015)). Transformants were serially diluted (1/10), spotted on SDcasaW medium and imaged after 48 h at 30°C. *C-I*, Wild-type (FY4) and *prs1Δ prs3Δ* strains expressing the *PRS5* gene either wild-type (Y12563) or mutated ((Y12214; Y12271; Y12272) were exponentially grown for 24 h in SDcasaW before determination of population doubling time (*C*) or nucleotides content (*D-I*). Numbers correspond to the p-values calculated from a Welch's unpaired t-test. *J*, Overexpression of *PRS5* in combination with either *PRS2* or *PRS4* is sufficient to restore a wild-type growth in *prs1Δ prs3Δ* genetic background. Wild-type (BY4741) or *prs1Δ prs3Δ* (Y2850) were co-transformed with empty vectors (YEplac195; YEplac181) or plasmids expressing *PRS2* (p6070), *PRS4* (p6094) or *PRS5^{P110S}* (p6015). Transformants, selected on SC-Ura-Leu were serially diluted (1/10), spotted on the same medium and imaged after 48 h at 30°C.

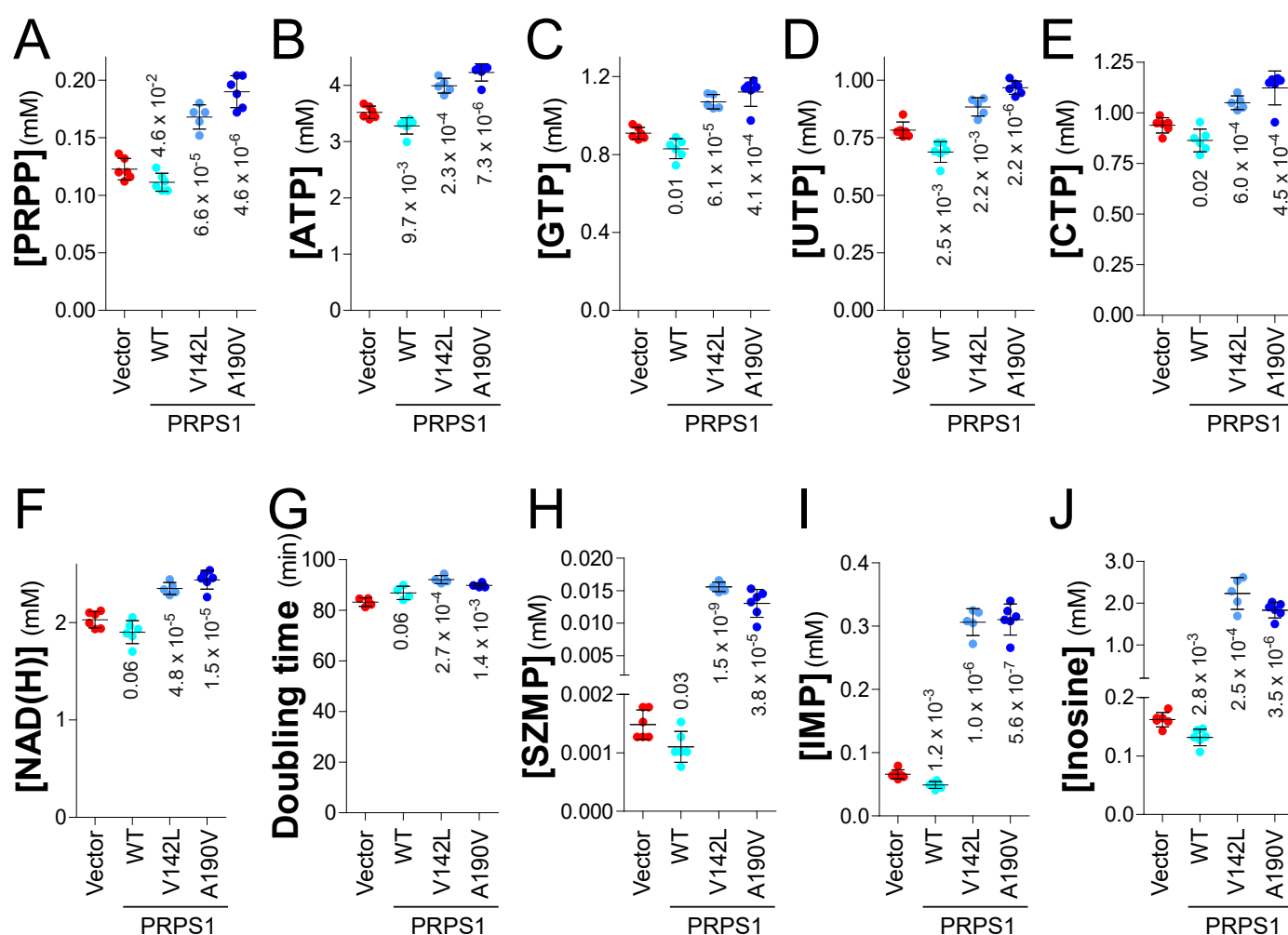


Figure 8. PRPP and nucleotide content is increased by expression of human *PRPS1* hyperactive variants. *A*, The wild-type strain (Y12599) was transformed with an empty vector (p2714) or plasmids expressing the *PRPS1* gene either wild-type (p5949) or hyperactive mutants (P5994; p5995). Transformants were exponentially grown for 24 h in SD_{case}WA liquid medium before metabolite extraction and quantification. Each metabolite was quantified on at least 5 independent cultures. Numbers correspond to the p-values calculated from a Welch's unpaired t-test.

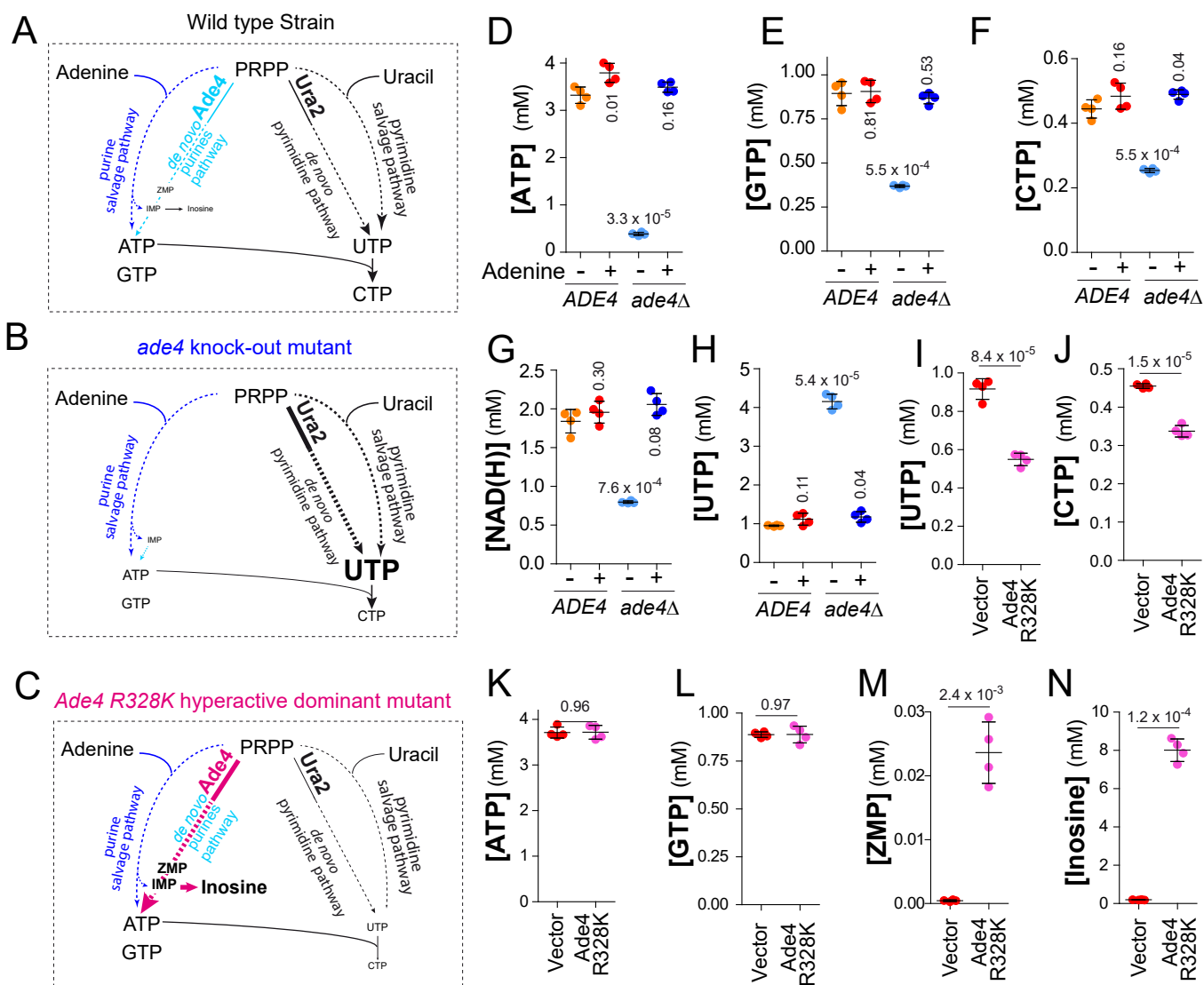


Figure 9. Impairment of PRPP utilization via the purine *de novo* pathway reveals that PRPP is limiting for nucleotide synthesis. A-E, Wild-type (FY4) and *ade4Δ* (Y12258) yeast strains were exponentially grown for 24 h in SDcasaWAU liquid medium. Cells were then harvested by filtration and grown for 3 more hours in SDcasaWU liquid medium supplemented (+) or not (-) with adenine before metabolite extraction, separation and quantification. F-K, The wild-type strain (Y11418) was transformed with an empty vector (pCM189) or a plasmid carrying the *ADE4* hyperactive R328K dominant mutant (p2048). Transformants were exponentially grown for 24 h in SDcasaWA medium before extraction, separation and quantification of metabolites. A-K, Metabolite measurements were performed on 4 independent cultures and numbers correspond to the p-values calculated from a Welch's unpaired t-test.

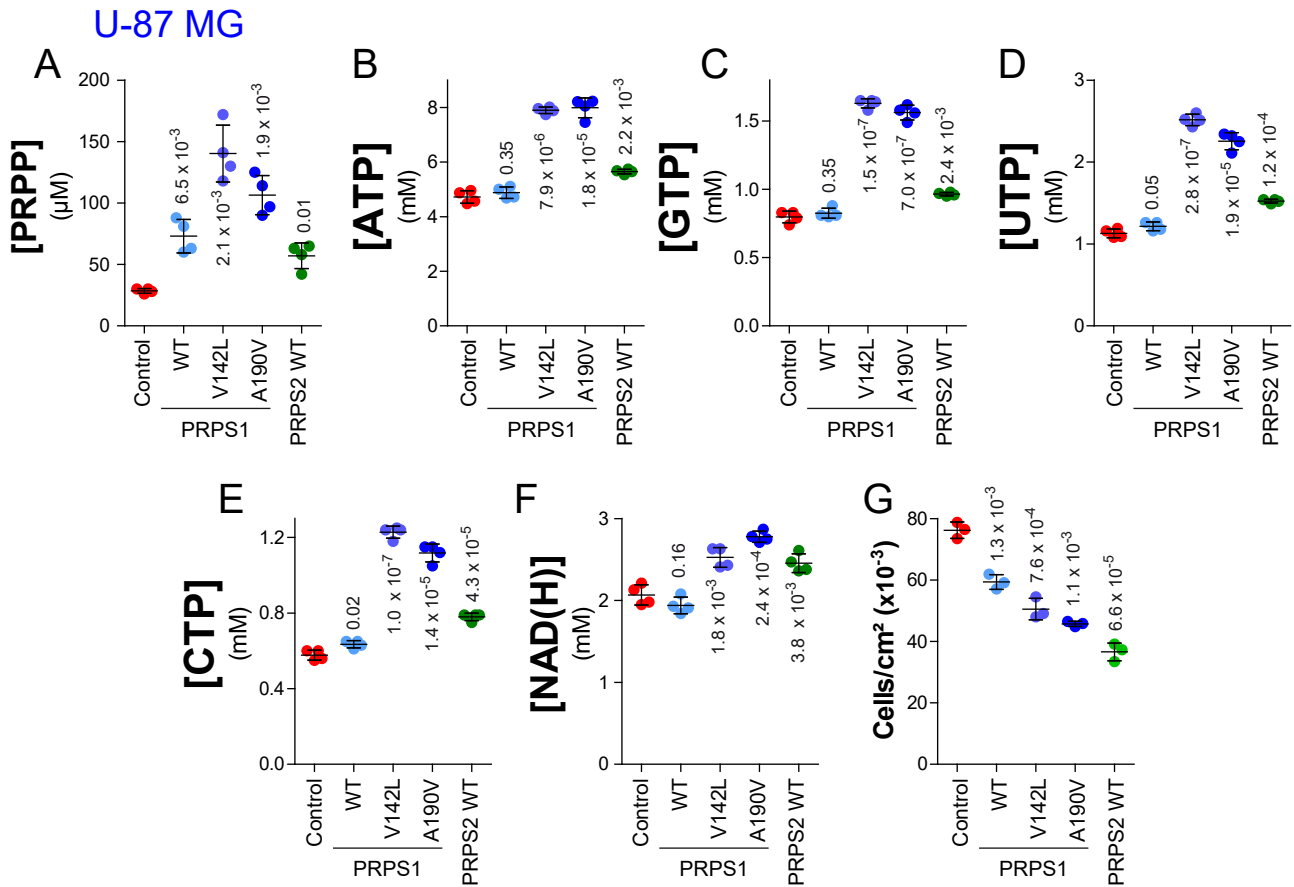


Figure 10. Expression of human *PRPS1* hyperactive variants in human cells increases PRPP and nucleotide content and affects cell proliferation. Lentiviral infection was used to express wild-type human *PRPS1* gene (WT, p6033), its two hyperactive mutants (V142L, p6034 and A190V, p6035), or wild-type human *PRPS2* gene (PRPS2 WT, p6030) in U-87 MG human cells. Infection with the pLenti-MND-IRES-PuroR-WPRE (p5769) empty vector was used as control. Metabolite content (A-F) was determined as described in “Experimental Procedures” and measurements were performed on 4 independent extractions. In cell proliferation experiments (G), cells were seeded at the density of 7,000 cells/cm² and grown for 4 days in complete medium before trypsinization and counting. Numbers correspond to the p-values calculated from a Welch’s unpaired t-test.

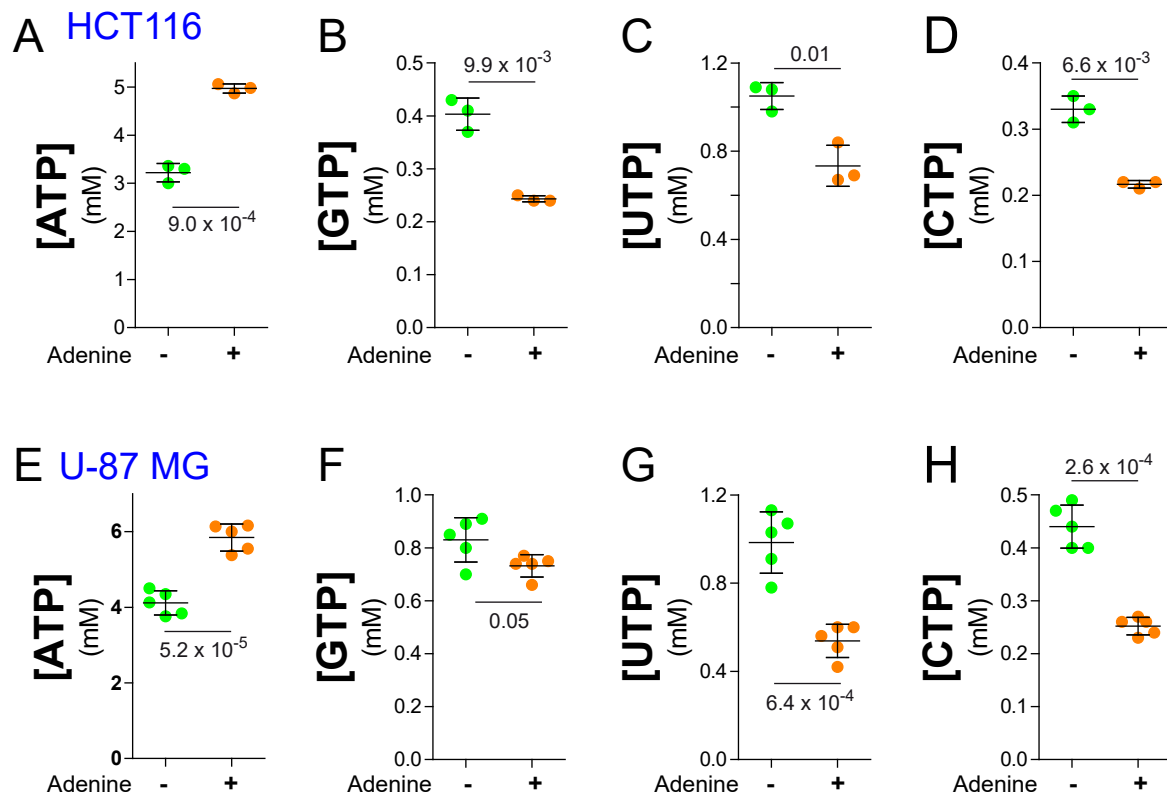


Figure 11. Addition of a purine precursor reveals that PRPP is limiting for synthesis of nucleotides in human cells. The human HCT116 (A-D) and U-87 MG (E-H) cell lines were seeded in 6-well plates and grown up to half-confluency in the complete medium. Cells were then washed and incubated for 2 hours in DMEM without serum, and incubated for 24 hours in DMEM containing 10% dialyzed serum supplemented (+) or not (-) with 50 μ M adenine. Nucleotides content was determined from 3 (A-D) to 5 (E-H) independent extractions and numbers correspond to the p-values calculated from a Welch's unpaired t-test.

Supporting Information

On demand utilization of phosphoribosyl pyrophosphate by downstream anabolic pathways

Pinson, B., Moenner, M., Saint-Marc, C., Granger-Farbos, A and Daignan-Fornier B.

Supplementary Figures

- Figure S1:** Consequences of PRPP variations on mono-, di- and tri-phosphate nucleotide content. p. 2
- Figure S2:** Addition of all nucleotide precursors is required to suppress the growth defect of the *prs1 prs3* double mutant. p. 3
- Figure S3:** Modulation of PRPP utilization by the pyrimidine *de novo* pathway reveals that PRPP is limiting for nucleotide synthesis. p. 4
- Figure S4:** Expression of human *PRPS1* hyperactive variants in HCT116 human cells increases both PRPP and nucleotides content and affects cell proliferation. p. 5
- Figure S5:** Determination of biomass production rate in yeast. p. 6

Supplementary Tables

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- Table S5:** Raw data for Figure 2: phenotypic and metabolic consequences of PRPP decrease in yeast. p. 11
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- Table S8:** Raw data for Figure 5: PRPP content is limiting for yeast proliferation and nucleotide synthesis. p. 15
- Table S9:** Raw data for Figure 6: Growth and metabolic defects of the *prs1 prs3* mutant are rescued by supplementation with PRPP-not-requiring precursors. p. 16
- Table S10:** Raw data for Figure 7: Suppression of *prs1 prs3* growth and metabolic defects by mutations in the *PRS5* gene. p. 17
- Table S11:** Raw data for Figure 8: PRPP and nucleotide content is increased by expression of human *PRPS1* hyperactive variants. p. 18-19
- Table S12:** Raw data for Figure 9: Impairment of PRPP utilization by the purine *de novo* pathway reveals that PRPP is limiting for nucleotide synthesis. p. 20
- Table S13:** Raw data for Figure 10: Expression of human *PRPS1* hyperactive variants in human cells increases PRPP and nucleotide content and affects cell proliferation. p. 21
- Table S14:** Raw data for Figure 11: Addition of a purine precursor reveals that PRPP is limiting for synthesis of nucleotides in human cells. p. 22
- Table S15:** Raw data for Figure S1: Consequences of PRPP variations on mono-, di- and tri-phosphate nucleotide content. p. 23-25
- Table S16:** Raw data for Figure S3: Modulation of PRPP utilization by the pyrimidine *de novo* pathway reveals that PRPP is limiting for nucleotide synthesis. p. 26
- Table S17:** Raw data for Figure S4: Expression of human *PRPS1* hyperactive variants in HCT116 human cells increases PRPP and nucleotide content and affects cell proliferation. p. 27

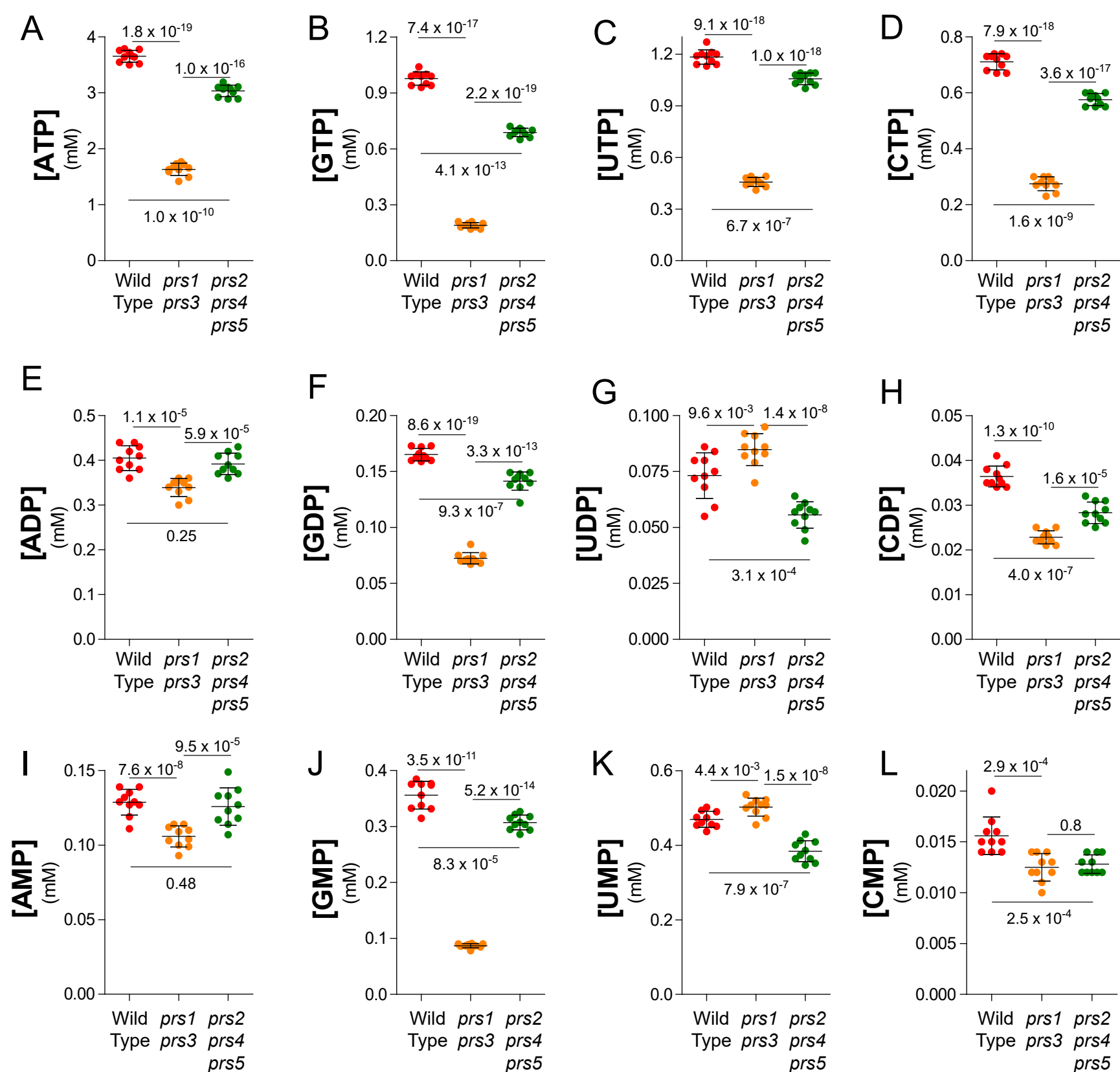


Figure S1. Consequences of PRPP variations on mono-, di- and tri-phosphate nucleotide content. Nucleotide content determination on metabolites extractions from Fig. 2 on the wild-type (Y12325), *prs1* Δ *prs3* Δ (Y12562) and *prs2* Δ *prs4* Δ *prs5* Δ (Y12649). These mono-, di- and triphosphate nucleotides content determinations were used to calculate the NXP content presented in Fig. 3. Each metabolite was quantified from 8 independent metabolite extractions. Numbers on each panels correspond to p-values calculated from a Welch's unpaired t-test.

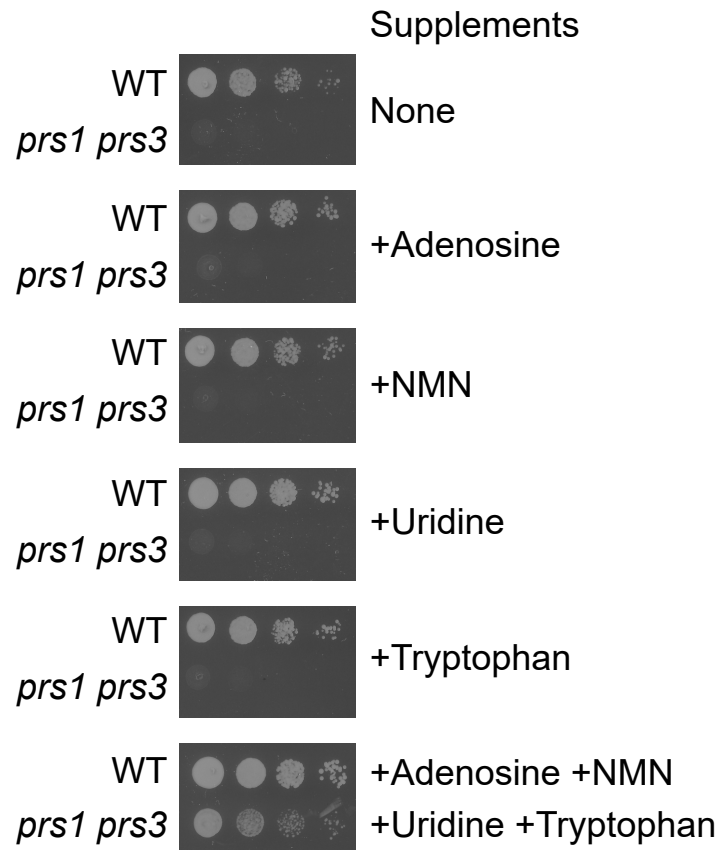


Figure S2. Addition of all nucleotide precursors is required to suppress the growth defect of *prs1 prs3* double mutant. Wild-type (Y11418) and *prs1Δ prs3Δ* (Y11985) strains were transformed with the *hENT1* expressing plasmid (p4991). Transformants were selected on SDcasaW medium supplemented with adenosine (Ado, 300 μ M), Uridine (Uri, 300 μ M), Tryptophan (300 μ M) and Nicotinamide mononucleotide (NMN, 100 μ M). Transformants were serially diluted (1/10) and spotted on SDcasaW supplemented or not (None) with either Ado, Uri, Trp and NMN or the mix of all precursors. Plates were imaged after 36 h at 30°C.

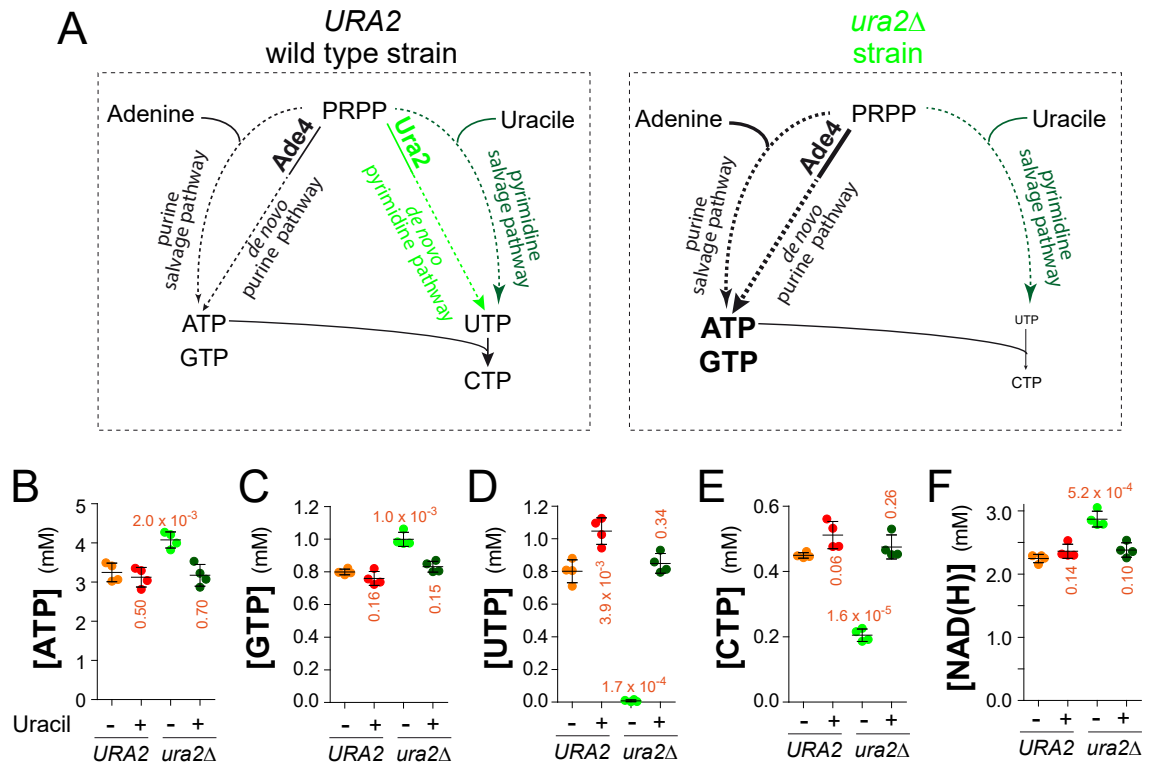


Figure S3. Modulation of PRPP utilization by the pyrimidine *de novo* pathway reveals that PRPP is limiting for nucleotide synthesis. *A*, Schematic representation of the *URA2* gene deletion on purine and pyrimidine nucleotides content. Ade4 and Ura2 correspond to the PRPP-dependent enzymes in the *de novo* purine and pyrimidine pathways, respectively. *B-F*, Determination of nucleotides content in yeast cells expressing or not *URA2* gene. Wild-type (FY4) and *ura2Δ* (Y12253) strains were exponentially grown for 24 h in SDcasaWU liquid medium. Cells were then harvested by filtration and grown for 3 more hours in SDcasaWA liquid medium supplemented (+) or not (-) with uracil before metabolite extraction, separation and quantification. Metabolite measurements were performed on 4 independent extractions and red numbers correspond to the p-values calculated from a Welch's unpaired t-test.

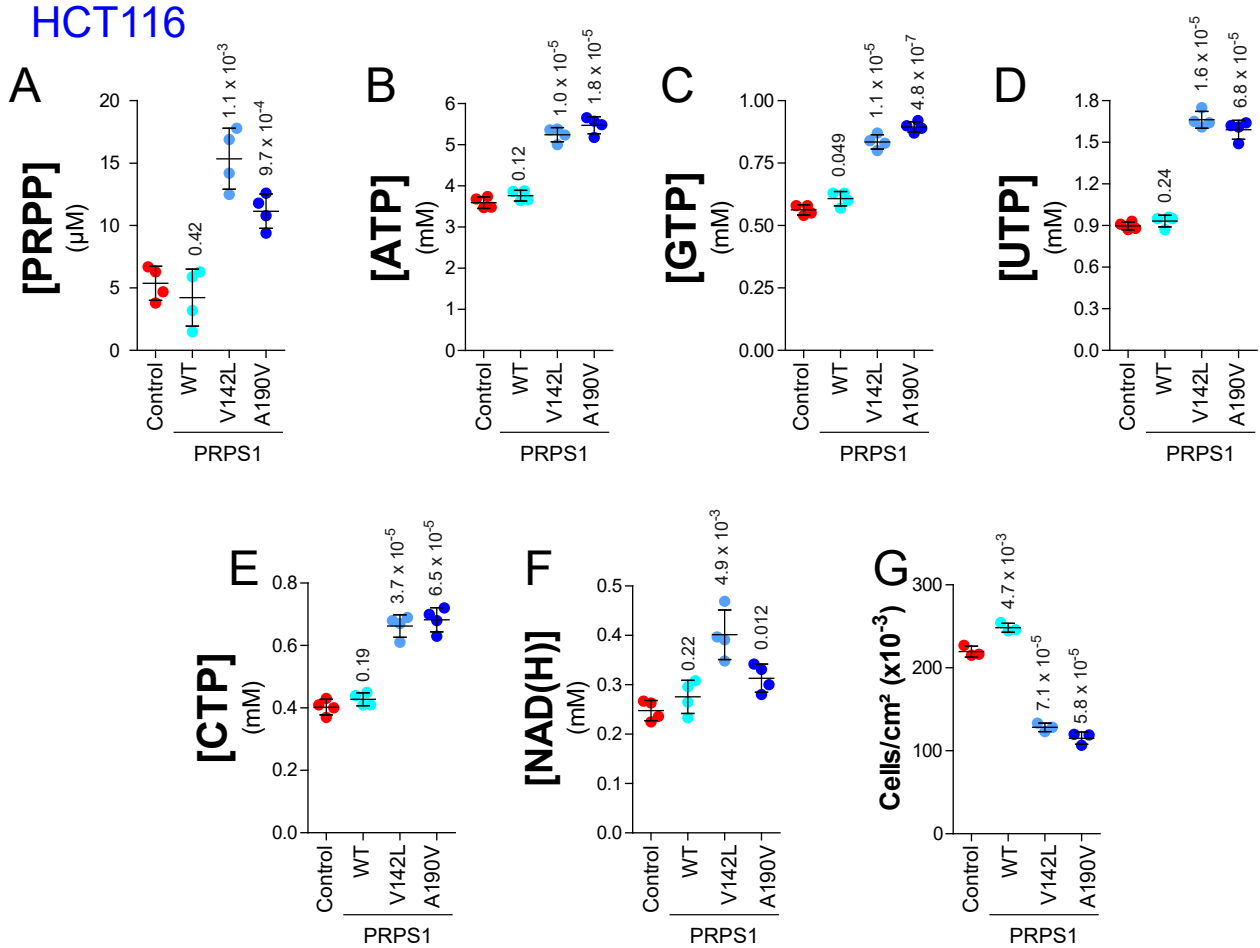


Figure S4. Expression of human *PRPS1* hyperactive variants in HCT116 human cells increases both PRPP and nucleotide content and affects cell proliferation. Lentiviral infection was used to express either wild-type human *PRPS1* (WT, p6033) or its hyperactive mutants (V142L, p6034 and A190V, p6035) in the human HCT116 cell line. Infection with the pLenti-MND-IRES-PuroR-WPRE (p5769) empty vector was used as control. Cells were grown in the complete medium. Metabolite content (A-F) was determined as described in “Experimental Procedures” and measurements were performed on 4 independent extractions. In cell proliferation experiments (G), cells were seeded at the density of 7,000 cells/cm² and grown for 4 days in complete medium before trypsinization and counting. Measurements were performed on 4 independent extractions and numbers correspond to the p-values calculated from a Welch’s unpaired t-test.

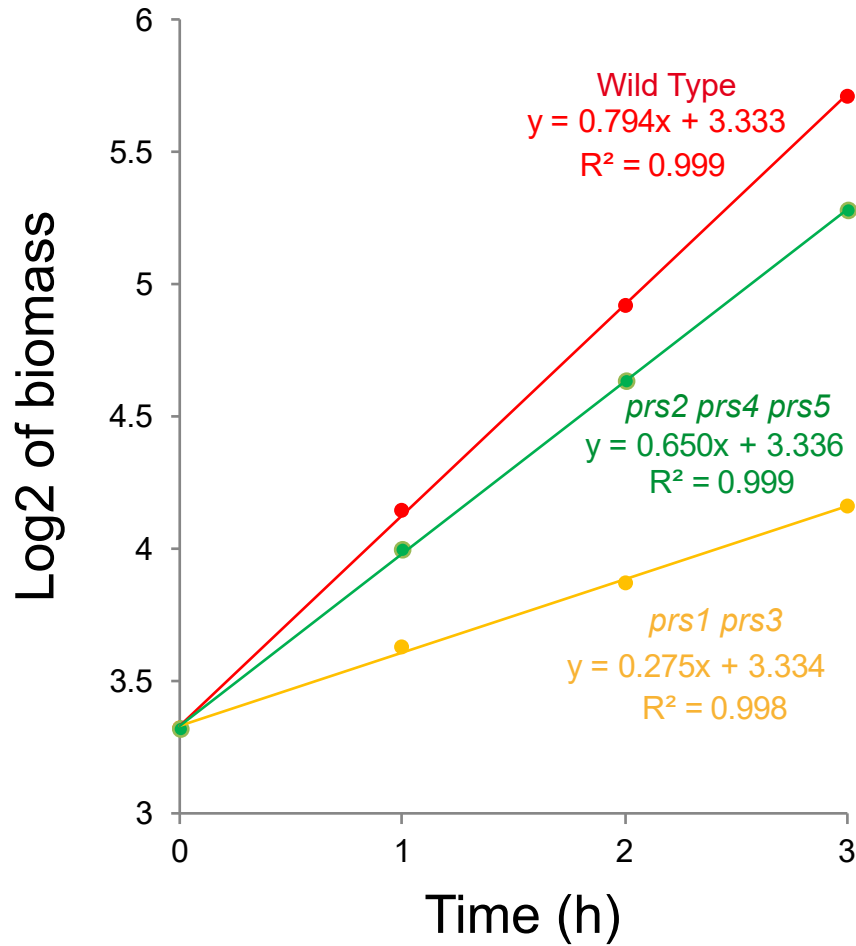


Figure S5. Determination of biomass production rate in yeast. Wild-type (Y12325), *prs1* Δ *prs3* Δ (12562) and *prs2* Δ *prs4* Δ *prs5* Δ (12649) were exponentially grown in SDcasaWUA medium for 24 h. Dry biomass was measured during 4 hours as described in “Experimental Procedures”. This figure shows a representative measurement obtained for each strain. Exponential biomass production was determined by linear regression of the experimental points. Relative biomass production presented in Figure 2 corresponds to the ratio of the slopes obtained for four independent cultures for each strain.

Table S1: List of the *prs1prs3* suppressors either obtained on SD minimal medium or on SDcasaWAU rich medium

Suppressor name	Nucleotide change in <i>PRS5</i>	Amino acid change in Prs5	Selection Medium
supp48	C299T	Pro100Leu	SD minimal medium
supp72	C298T	Pro100Ser	SDcasaWAU
supp44	G322A	Gly108Ser	SDcasaWAU
supp9	C328T	Pro110Ser	SDcasaWAU
supp3	C328T	Pro110Ser	SDcasaWAU
supp23	G655A-T656A	Val219Lys	SD minimal medium
supp16	A920T-T921C	His307Leu	SD minimal medium
supp36	A923C	Lys308Thr	SDcasaWAU
supp24	A922G	Lys308Glu	SD minimal medium
supp47	A924C	Lys317Asn	SD minimal medium
supp48	G932T	Arg311Ile	SDcasaWAU
supp56	G932T	Arg311Ile	SDcasaWAU
supp22	T934	Ser312Pro	SD minimal medium
supp46	T941C	Leu314Ser	SD minimal medium
supp22	A942T	Leu314Phe	SDcasaWAU
supp26	C953T	Pro318Leu	SD minimal medium
supp58	G1192A	Asp398Asn	SDcasaWAU
supp12	G1201A	Asp401Asn	SDcasaWAU
supp11	G1201A	Asp401Asn	SDcasaWAU
supp27	G1201A	Asp401Asn	SD minimal medium
supp8	T1207C	Ser403Pro	SDcasaWAU
supp6	T1207C	Ser403Pro	SDcasaWAU
supp24	T1207C	Ser403Pro	SDcasaWAU
supp27	C1214T	Thr405Ile	SDcasaWAU
supp61	T1291C	Ser431Pro	SDcasaWAU
supp34	T1291C	Ser431Pro	SD minimal medium
supp32	A1490G-G1491T	End497Cys	SD minimal medium
supp33	A1490G-G1491T	End497Cys	SD minimal medium
supp38	A1490G-G1491T	End497Cys	SD minimal medium
supp39	A1490G-G1491T	End497Cys	SD minimal medium

Table S2: Yeast strains

Strain Name	Genotype
BY4741	<i>MAT a ura3Δ0 leu2Δ0 his3Δ1 met15Δ0</i>
BY4742	<i>MAT alpha ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0</i>
FY4	<i>MAT a</i>
FY5	<i>MAT alpha</i>
Y2850	<i>MAT a prs1::KanMX4 prs3::KanMX4 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0</i>
Y5217	<i>MAT a/ Mat alpha rki1::kanMX4/RKI1 ura3Δ0/ ura3Δ0 leu2Δ0/ leu2Δ0 his3Δ1/ his3Δ1 lys2Δ0/LYS2 met15Δ0/MET15</i>
Y9469	<i>pYPhENT1 fui1::TRP1 gal, ura3-52, trp1, lys2, ade2,hisΔ2000</i>
Y11418	<i>MAT alpha ura3Δ0</i>
Y11451	<i>MAT alpha ura3Δ0 leu2Δ0</i>
Y11983	<i>MAT a ura3Δ0 prs1::KanMX4 prs3::KanMX4</i>
Y11985	<i>MAT alpha ura3Δ0 prs1::KanMX4 prs3::KanMX4</i>
Y12149	<i>MAT alpha prs1::KanMX4 prs3::KanMX4</i>
Y12204	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp44 (G322A : Gly108Ser)</i>
Y12206	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp58 (G1192A : Asp398Asn)</i>
Y12207	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp61 (T1291C : Ser431Pro)</i>
Y12211	<i>MAT alpha prs1::KanMX4 prs3::KanMX4 PRS5supp29 (C1369T : Pro457Ser)</i>
Y12214	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp3 (C328T : Pro110Ser)</i>
Y12253	<i>MAT alpha ura2 ::KanMX4 ura3Δ0</i>
Y12258	<i>MAT alpha ade4 ::KanMX4</i>
Y12269	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp72 (C298T : Pro100Ser)</i>
Y12271	<i>MAT alpha prs1::KanMX4 prs3::KanMX4 PRS5supp48 (G932T : Arg311Ile)</i>
Y12272	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp11 (G1201A : Asp401Asn)</i>
Y12325	<i>MAT a</i>
Y12469	<i>MAT a prs2::KanMX4 prs4::KanMX4 prs5::KanMX4</i>
Y12558	<i>MAT a prs1::KanMX4 prs3::KanMX4 PRS5supp23 (G665A T656A : Val219Lys)</i>
Y12560	<i>MAT alpha prs1::KanMX4 prs3::KanMX4 PRS5supp46 (T941C : Leu314Ser)</i>
Y12562	<i>MAT a prs1::KanMX4 prs3::KanMX4</i>
Y12563	<i>MAT a prs1::KanMX4 prs3::KanMX4</i>
Y12599	<i>MAT a ura3Δ0</i>

Table S3: Plasmids

Plasmid Name	Relevant description	Source
pCM189	<i>tetO₇-promoter</i> URA3 CEN AmpR	Gari <i>et al</i>
YCpLac33	URA3 CEN AmpR	Gietz and Sugino
YEpLac181	LEU2 2μ AmpR	Gietz and Sugino
YEPlac195	URA3 2μ AmpR	Gietz and Sugino
P2048	<i>tetO₇-promoter-ADE4-1(</i> Arg328Lys <i>)</i> URA3 CEN AmpR	This study
P2714	<i>tetO₇-promoter-HA</i> URA3 CEN AmpR	This study
P4991	<i>hENT1</i> URA3 2μ	This study
P5671	PRS3 in YEPlac195	This study
P5769	<i>pLenti-MND-IRES-PuroR-WPRE</i>	Vect'UB
P5909	<i>tetO₇-promoter-PrsA</i> URA3 CEN AmpR	This study
P5949	<i>tetO₇-promoter-HA-PRPS1</i> URA3 CEN AmpR	This study
P5994	<i>tetO₇-promoter-HA-PRPS1</i> Val142Leu URA3 CEN AmpR	This study
P5995	<i>tetO₇-promoter-HA-PRPS1</i> Ala190Val URA3 CEN AmpR	This study
P6001	<i>tetO₇-promoter-PRPS1</i> URA3 CEN AmpR	This study
P6008	PRS5supp11 (D401N) in YEPlac195	This study
P6009	PRS5supp11 (D401N) in YCpLac33	This study
P6011	PRS5supp48 (R311I) in YEPlac195	This study
P6014	PRS5supp48 (R311I) in YCpLac33	This study
P6015	PRS5supp3 (P110S) in YEPlac195	This study
P6016	PRS5supp3 (P110S) in YCpLac33	This study
P6017	PRPS5 in YCpLac33	This study
P6019	PRPS5 in YEPlac195	This study
P6020	<i>tetO₇-promoter-PRPS2</i> URA3 CEN AmpR	This study
P6030	PRPS2 in P5769	This study
P6033	PRPS1 in P5769	This study
P6034	PRPS1 Val142Leu in P5769	This study
P6035	PRPS1 Ala190Val in P5769	This study
P6070	PRS2 in YEpLac181	This study
P6094	PRS4 in YEpLac181	This study
P6160	PRS1 in YEpLac181	This study

E Garí, L Piedrafita, M Aldea and E Herrero A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in *Saccharomyces cerevisiae*. (1997) *Yeast* . **13**(9):837-48. doi: 10.1002/(SICI)1097-0061(199707)13:9<837::AID-YEA145>3.0.CO;2-T.

R D Gietz, A Sugino New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. (1988) *Gene* **74**(2):527-34. doi: 10.1016/0378-1119(88)90185-0

Vect'UB: Vectorology and virus production platform from TBMCORE Bordeaux University, CNRS UAR 3427, INSERM US005

Table S4: Oligonucleotides

Oligonucleotide	5'- 3' Sequence
48	CGCGGATCCAAATGTGTGGTATTTTAG
429	AAACTGCAGTCGATAATGTGCACAATTATATAATC
2032	TCTTTGGAAGAAGTTGAAGG
2033	GATCGAATTCGGATGTTGTAATGTGTTTGG
2040	CGCGGATCCTTATATCCAGCATCTCATGC
2041	GATCGGTACCCCAACGGCTCCTCGTCGACC
2278	CTCAATGCGGCCGCTTAGTGTTTCGAACATGGCAG
2279	CGCGGATCCAAGATGCCTGATATGAAGCTTTTTTGC
5082	CGCGGATCCGGCCCAGAAATCTAAGCTTGAGG
5083	AAAACTGCAGGTTCCCGATGGCTCTATTTTGG
5562	CGCGGATCCAGGATGCCGAATATCAAAATCTTCAG
5563	CCAATGCATCTATTATAAAGGGACATGGCTGAATAGG
5678	CGCGGATCCATGCCGAATATCAAAATCTTCAGC
5763	GCCGGATCCACGCGTATGCCCAACATCGTGCTGTTC
5764	GGCCATATGATGCATTTATAGCGGGACATGGCTGAAC
5847	GCGGGATCCTTGCAATTTGCGAATGCGG
5848	AAAACTGCAGCATATTTTCCAAAAGCAAGGGCG
5849	GCGGGATCCGTACTGTAAAATGTGTCGTCGAC
5850	AAAACTGCAGGTAAAGGTTACCGTCTCCT

Table S5: Raw data Figure 2

A: [PRPP]

	CD	CD	CD			
	Peak area	Peak area	Peak area	μM in cells	μM in cells	μM in cells
Metabolite	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	0,030	0,004	0,025	0,100	0,013	0,083
sample 2	0,031	0,003	0,024	0,103	0,010	0,080
sample 3	0,036	0,005	0,025	0,119	0,016	0,083
sample 4	0,034	0,005	0,021	0,115	0,015	0,071
sample 5	0,035	0,004	0,020	0,116	0,014	0,067
sample 6	0,033	0,006	0,022	0,111	0,019	0,073
sample 7	0,038	0,004	0,021	0,128	0,014	0,071
sample 8	0,034	0,004	0,024	0,113	0,014	0,082
sample 9	0,034	0,004	0,020	0,112	0,013	0,066
sample 10	0,034	0,004	0,022	0,113	0,014	0,072
Mean	0,034	0,004	0,022	0,113	0,014	0,075
SD	0,002	0,001	0,002	0,008	0,002	0,007
Unpaired t-test vsWT		8,1E-13	8,4E-10		8,1E-13	8,4E-10
Unpaired t-test vs <i>prs1prs3</i>	8,1E-13		9,1E-12	8,1E-13		9,1E-12

B: Adenylic nucleotides content: [AXP] = [ATP] + [ADP] + [AMP]

	mM in cells	mM in cells	mM in cells
Metabolite	AXP	AXP	AXP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	4,08	1,90	3,40
sample 2	4,01	1,82	3,40
sample 3	4,02	2,06	3,70
sample 4	4,14	2,12	3,38
sample 5	4,26	2,18	3,65
sample 6	4,21	2,06	3,62
sample 7	4,33	2,20	3,57
sample 8	4,36	2,09	3,59
sample 9	4,34	2,22	3,66
sample 10	4,19	2,14	3,55
Mean	4,19	2,08	3,55
SD	0,13	0,13	0,12
Unpaired t-test vsWT		2,2E-18	1,1E-09
Unpaired t-test vs <i>prs1prs3</i>	2,2E-18		7,2E-16

C: Relative biomass production rate

	Relative	Relative	Relative
	Biomass	Biomass	Biomass
	Production	Production	Production
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	1,023	0,609	0,848
sample 2	1,026	0,641	0,816
sample 3	0,960	0,624	0,800
sample 4	0,990	0,634	0,827
sample 5			0,831
Unpaired t-test vsWT		2,1E-05	2,9E-04
Unpaired t-test vs <i>prs1prs3</i>	2,1E-05		3,2E-07

D: Median volume

	Median	Median	Median
	volume (fl)	volume (fl)	volume (fl)
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	53,8	40	51,5
sample 2	53,4	41	51,3
sample 3	54,1	40,5	52,1
sample 4	55,8	41,1	52
sample 5	53,3	38,8	50,4
sample 6	53,9	39,7	50
sample 7	55,3	38,9	50
sample 8	54,2	39,02	51,6
sample 9	55	36,9	50,6
sample 10	56	38,1	50,6
Mean	54,5	39,4	51,0
SD	1,0	1,3	0,8
Unpaired t-test vsWT		1,3E-15	9,5E-08
Unpaired t-test vs <i>prs1prs3</i>	1,3E-15		4,0E-13

E: Population doubling Time

	Doubling	Doubling	Doubling
	Time (min)	Time (min)	Time (min)
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	78,5	235,2	85,1
sample 2	79,6	215,7	83,3
sample 3	84,4	210,6	76,3
sample 4	77,0	218,8	79,4
Unpaired t-test vsWT		4,1E-05	0,67
Unpaired t-test vs <i>prs1prs3</i>	4,1E-05		2,4E-05

Table S6: Raw data Figure 3 (part 1/2)

A: [GXP] in mM

[GXP]= [GTP] + [GDP] + [GMP]						
Metabolite	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Strain	GXP	GXP	GXP	GXP	GXP	GXP
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	49.47	10.89	37.27	1.48	0.33	1.12
sample 2	49.51	10.73	36.93	1.49	0.32	1.11
sample 3	47.31	11.17	39.49	1.42	0.34	1.18
sample 4	47.93	11.82	35.70	1.44	0.35	1.07
sample 5	51.17	11.36	39.17	1.54	0.34	1.18
sample 6	50.75	11.30	38.34	1.52	0.34	1.15
sample 7	51.10	12.81	37.60	1.53	0.38	1.13
sample 8	49.83	12.41	37.93	1.50	0.37	1.14
sample 9	51.45	12.09	38.92	1.54	0.36	1.17
sample 10	50.13	11.97	37.97	1.50	0.36	1.14
Mean	49.865	11.657	37.931	1.50	0.35	1.14
SD	1.380	0.677	1.137	0.04	0.02	0.03
Unpaired t-test vs WT		6.6E-19	8.0E-14		6.6E-19	8.0E-14
Unpaired t-test vs <i>prs1 prs3</i>	6.6E-19		3.0E-19	6.6E-19		3.0E-19

B: [UXP] in mM

[UXP]= [UTP] + [UDP] + [UMP]						
Metabolite	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Strain	UXP	UXP	UXP	UXP	UXP	UXP
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	56.040	32.973	48.196	1.681	0.989	1.446
sample 2	55.197	32.181	49.817	1.656	0.965	1.495
sample 3	54.798	33.728	52.625	1.644	1.012	1.579
sample 4	57.683	35.312	47.454	1.730	1.059	1.424
sample 5	58.523	35.217	51.373	1.756	1.057	1.541
sample 6	58.443	34.272	49.669	1.753	1.028	1.490
sample 7	60.400	36.739	50.148	1.812	1.102	1.504
sample 8	56.683	35.184	50.153	1.700	1.056	1.505
sample 9	59.199	37.132	50.857	1.776	1.114	1.526
sample 10	58.292	35.478	48.329	1.749	1.064	1.450
Mean	57.526	34.822	49.862	1.73	1.04	1.50
SD	1.804	1.559	1.565	0.05	0.05	0.05
Unpaired t-test vs WT		1.3E-16	8.6E-09		1.3E-16	8.6E-09
Unpaired t-test vs <i>prs1 prs3</i>	1.3E-16		2.7E-14	1.3E-16		2.7E-14

C: [CXP] in mM

[CXP]= [CTP] + [CDP] + [CMP]						
Metabolite	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Strain	CXP	CXP	CXP	CXP	CXP	CXP
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	24.212	9.268	19.723	0.726	0.278	0.592
sample 2	24.354	9.000	19.873	0.731	0.270	0.596
sample 3	23.919	9.949	21.362	0.718	0.298	0.641
sample 4	26.286	11.194	21.187	0.789	0.336	0.636
sample 5	26.182	10.262	21.354	0.785	0.308	0.641
sample 6	25.569	10.136	20.033	0.767	0.304	0.601
sample 7	26.416	11.044	20.666	0.792	0.331	0.620
sample 8	25.329	10.340	20.680	0.760	0.310	0.620
sample 9	26.152	11.146	21.103	0.785	0.334	0.633
sample 10	26.135	10.696	19.788	0.784	0.321	0.594
Mean	25.455	10.304	20.577	0.76	0.31	0.62
SD	0.957	0.755	0.669	0.03	0.02	0.02
Unpaired t-test vs WT		3.4E-18	4.6E-10		3.4E-18	4.6E-10
Unpaired t-test vs <i>prs1 prs3</i>	3.4E-18		3.4E-17	3.4E-18		3.4E-17

D: [NAD(H)] in mM

[NAD(H)]= [NAD ⁺] + [NADH]						
Metabolite	μM in extracts	μM in extracts	μM in extracts	mM in cells	mM in cells	mM in cells
Strain	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	65.569	25.579	58.267	1.967	0.767	1.748
sample 2	64.564	23.622	59.418	1.937	0.709	1.783
sample 3	63.677	26.726	67.591	1.910	0.802	2.028
sample 4	68.003	30.409	60.689	2.040	0.912	1.821
sample 5	72.037	32.555	61.811	2.161	0.977	1.854
sample 6	65.645	30.129	63.264	1.969	0.904	1.898
sample 7	70.663	34.888	62.450	2.120	1.047	1.873
sample 8	67.335	32.168	60.773	2.020	0.965	1.823
sample 9	65.470	36.792	63.601	1.964	1.104	1.908
sample 10	68.388	36.886	60.739	2.052	1.107	1.822
Mean	67.135	30.975	61.861	2.01	0.93	1.86
SD	2.684	4.598	2.601	0.08	0.14	0.08
Unpaired t-test vs WT		2.1E-12	3.0E-04		2.1E-12	3.0E-04
Unpaired t-test vs <i>prs1 prs3</i>	2.1E-12		2.4E-11	2.1E-12		2.4E-11

E: [Uracil] in mM

Metabolite	μM in extracts	μM in extracts	μM in extracts	mM in cells	mM in cells	mM in cells
Strain	Uracil	Uracil	Uracil	Uracil	Uracil	Uracil
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	8.148	25.407	15.385	0.244	0.762	0.462
sample 2	8.148	22.481	14.944	0.244	0.674	0.448
sample 3	8.185	24.093	17.470	0.246	0.723	0.524
sample 4	8.578	26.156	15.495	0.257	0.785	0.465
sample 5	8.900	25.828	17.319	0.267	0.775	0.520
sample 6	10.226	24.568	16.080	0.307	0.737	0.482
sample 7	7.037	25.132	15.565	0.211	0.754	0.467
sample 8	8.137	24.253	14.857	0.244	0.728	0.446
sample 9	7.893	29.397	16.402	0.237	0.882	0.492
sample 10	8.574	24.532	14.260	0.257	0.736	0.428
Mean	8.383	25.185	15.778	0.25	0.76	0.47
SD	0.817	1.805	1.046	0.02	0.05	0.03
Unpaired t-test vs WT		1.9E-12	2.3E-12		1.9E-12	2.3E-12
Unpaired t-test vs <i>prs1 prs3</i>	1.9E-12		6.7E-10	1.9E-12		6.7E-10

Table S6: Raw data Figure 3 (part 2/2)

F: [Adenine]in mM

Metabolite	μM in extracts	μM in extracts	μM in extracts	mM in cells	mM in cells	mM in cells
Strain	Adenine	Adenine	Adenine	Adenine	Adenine	Adenine
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	1,172	5,325	1,169	0,035	0,160	0,035
sample 2	2,272	3,059	1,384	0,068	0,092	0,042
sample 3	1,602	2,185	1,189	0,048	0,066	0,036
sample 4	1,123	4,156	1,267	0,034	0,125	0,038
sample 5	1,405	3,635	1,026	0,042	0,109	0,031
sample 6	2,092	2,999	0,844	0,063	0,090	0,025
sample 7	1,534	3,920	1,622	0,046	0,118	0,049
sample 8	2,334	3,391	2,461	0,070	0,102	0,074
sample 9	2,131	5,116	2,343	0,064	0,153	0,070
sample 10	2,501	4,636	1,450	0,075	0,139	0,043
Mean	1,817	3,842	1,476	0,05	0,12	0,04
SD	0,507	0,993	0,535	0,02	0,03	0,02
Unpaired t-test vs WT		6,0E-05	1,6E-01		6,0E-05	0,16
Unpaired t-test vs <i>prs1 prs3</i>	6,0E-05		1,2E-05	6,0E-05		1,2E-05

G: [Hypoxanthine] in mM

Metabolite	μM in extracts	μM in extracts	μM in extracts	mM in cells	mM in cells	mM in cells
Strain	Hypoxanthine	Hypoxanthine	Hypoxanthine	Hypoxanthine	Hypoxanthine	Hypoxanthine
	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	18,085	41,274	15,389	0,543	1,238	0,462
sample 2	17,604	41,718	15,674	0,528	1,252	0,470
sample 3	17,005	40,155	13,765	0,510	1,205	0,413
sample 4	17,273	40,680	16,997	0,518	1,220	0,510
sample 5	17,411	42,033	17,643	0,522	1,261	0,529
sample 6	17,315	40,724	14,890	0,519	1,222	0,447
sample 7	16,266	43,120	14,311	0,488	1,294	0,429
sample 8	16,103	40,569	14,498	0,483	1,217	0,435
sample 9	17,841	39,830	16,133	0,535	1,195	0,484
sample 10	17,137	40,368	15,575	0,514	1,211	0,467
Mean	17,204	41,047	15,487	0,52	1,23	0,46
SD	0,627	0,999	1,206	0,02	0,03	0,04
Unpaired t-test vs WT		7,7E-20	1,4E-03		7,7E-20	1,4E-03
Unpaired t-test vs <i>prs1 prs3</i>	7,7E-20		1,8E-20	7,7E-20		1,8E-20

Table S7: Raw data Figure 4

A: [PRPP] in mM

	Peak area	Peak area	mM	mM
Metabolite	PRPP	PRPP	PRPP	PRPP
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	0.014	0.024	0.074	0.129
sample 2	0.015	0.029	0.079	0.154
sample 3	0.017	0.028	0.088	0.147
sample 4	0.015	0.025	0.079	0.134
sample 5	0.014	0.025	0.077	0.132
sample 6	0.012	0.026	0.065	0.137
sample 7	0.016	0.025	0.085	0.133
sample 8	0.015	0.024	0.079	0.127
Mean	0.015	0.026	0.007	0.137
SD	0.001	0.002	0.008	0.009
Unpaired t-test	2,4E-09		2,4E-09	

C: [GTP] in mM

	Peak area	Peak area	mM	mM
Metabolite	GTP	GTP	GTP	GTP
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	3.09	3.51	0.82	0.94
sample 2	2.70	3.91	0.72	1.04
sample 3	3.00	3.86	0.80	1.03
sample 4	2.95	3.93	0.78	1.05
sample 5	3.02	3.81	0.80	1.02
sample 6	2.95	3.81	0.79	1.01
sample 7	2.92	3.77	0.78	1.00
sample 8	3.13	3.94	0.83	1.05
Mean	2.97	3.82	0.79	1.02
SD	0.13	0.14	0.04	0.04
Unpaired t-test	5,7E-09		5,7E-09	

E: [CTP] in mM

	Peak area	Peak area	mM	mM
Metabolite	CTP	CTP	CTP	CTP
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	2.08	2.31	0.93	1.03
sample 2	1.92	2.58	0.86	1.16
sample 3	1.97	2.49	0.88	1.12
sample 4	2.03	2.49	0.91	1.12
sample 5	2.08	2.42	0.93	1.08
sample 6	1.98	2.45	0.89	1.10
sample 7	1.99	2.45	0.89	1.10
sample 8	2.08	2.55	0.93	1.14
Mean	2.02	2.47	0.91	1.11
SD	0.06	0.09	0.03	0.04
Unpaired t-test	2,4E-08		2,4E-08	

G: [IMP] in mM

	Peak area	Peak area	mM	mM
Metabolite	IMP	IMP	IMP	IMP
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	0.09	0.29	0.05	0.17
sample 2	0.06	0.34	0.04	0.20
sample 3	0.05	0.26	0.03	0.15
sample 4	0.05	0.26	0.03	0.15
sample 5	0.06	0.26	0.03	0.15
sample 6	0.04	0.30	0.03	0.18
sample 7	0.04	0.27	0.02	0.16
sample 8	0.04	0.29	0.02	0.17
Mean	0.05	0.28	0.03	0.17
SD	0.02	0.03	0.01	0.02
Unpaired t-test	7,1E-10		7,1E-10	

I: Population doubling Time (min)

	Doubling Time (min)	Doubling Time (min)
	WT/vectors	WT/PRS1 + PRS3
sample 1	106.3	120.5
sample 2	107.1	117.0
sample 3	111.7	114.3
sample 4	106.8	116.5
Mean	108.0	117.1
SD	2.5	2.6
Unpaired t-test	2,3E-03	

K: Median volume (fl)

	Median Volume (fl)	Median Volume (fl)
	WT/vectors	WT/PRS1 + PRS3
sample 1	48.2	52.1
sample 2	49.5	50.1
sample 3	50.4	53.2
sample 4	48.2	50.9
sample 5	51.4	49.6
sample 6	51.1	50.5
sample 7	50.3	52.6
sample 8	48.5	49.1
Mean	49.7	51.0
SD	1.3	1.5
Unpaired t-test	7,9E-02	

B: [ATP] in mM

	Peak area	Peak area	mM	mM
Metabolite	ATP	ATP	ATP	ATP
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	17.96	19.84	3.52	3.89
sample 2	16.45	21.93	3.23	4.30
sample 3	17.44	21.29	3.42	4.17
sample 4	17.43	21.38	3.42	4.19
sample 5	17.68	20.64	3.47	4.05
sample 6	16.97	21.08	3.33	4.13
sample 7	17.21	20.78	3.38	4.08
sample 8	18.13	21.68	3.55	4.25
Mean	17.41	21.08	3.41	4.13
SD	0.54	0.66	0.11	0.13
Unpaired t-test	1,1E-08		1,1E-08	

D: [UTP] in mM

	Peak area	Peak area	mM	mM
Metabolite	UTP	UTP	UTP	UTP
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	2.11	2.42	0.85	0.98
sample 2	1.78	2.58	0.72	1.04
sample 3	1.93	2.52	0.78	1.02
sample 4	1.81	2.63	0.73	1.06
sample 5	2.11	2.51	0.85	1.01
sample 6	1.82	2.74	0.74	1.11
sample 7	1.85	2.48	0.75	1.00
sample 8	1.96	2.55	0.79	1.03
Mean	1.92	2.55	0.78	1.03
SD	0.13	0.10	0.05	0.04
Unpaired t-test	6,8E-08		6,8E-08	

F: [NAD(H)] in mM

	μM in sample	μM in sample	mM	mM
Metabolite	NAD(H)	NAD(H)	NAD(H)	NAD(H)
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	45.31	51.44	1.81	2.06
sample 2	45.80	57.23	1.83	2.29
sample 3	47.71	56.28	1.91	2.25
sample 4	49.40	57.54	1.98	2.30
sample 5	49.89	55.49	2.00	2.22
sample 6	50.85	59.97	2.03	2.40
sample 7	50.31	58.10	2.01	2.32
sample 8	51.96	61.24	2.08	2.45
Mean	48.90	57.16	1.96	2.29
SD	2.40	2.97	0.10	0.12
Unpaired t-test	3,2E-05		3,2E-05	

H: [Inosine] in mM

	Peak area	Peak area	mM	mM
Metabolite	Inosine	Inosine	Inosine	Inosine
Strain	WT/vectors	WT/PRS1 + PRS3	WT/vectors	WT/PRS1 + PRS3
sample 1	0.79	5.83	0.20	1.51
sample 2	0.83	7.04	0.21	1.82
sample 3	0.83	5.94	0.21	1.54
sample 4	0.93	6.16	0.24	1.60
sample 5	0.75	5.83	0.19	1.51
sample 6	0.75	6.83	0.19	1.77
sample 7	0.82	5.99	0.21	1.55
sample 8	1.04	6.34	0.27	1.64
Mean	0.84	6.25	0.22	1.62
SD	0.10	0.46	0.03	0.12
Unpaired t-test	1,9E-09		1,9E-09	

J: Relative biomass production

	Relative biomass production rate	Relative biomass production rate
	WT/vectors	WT/PRS1 + PRS3
sample 1	0.998	0.922
sample 2	1.017	0.941
sample 3	0.998	0.941
sample 4	0.988	0.941
Mean	1.000	0.936
SD	0.012	0.010
Unpaired t-test	2,0E-04	

Table S8: Raw data Figure 5

B: Population doubling Time (min)

	Doubling Time (min)	Doubling Time (min)	Doubling Time (min)	Doubling Time (min)	Doubling Time (min)
	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
Strain 1	100.5	405.1	95.4	101.3	99.6
Strain 2	96.1	335.6	103.8	106.6	121.4
Strain 3	92.3	357.5	101.2	101.6	107.9
Strain 4	96.3	355.3	99.6	103.8	110.1
Strain 5	93.7	398.7			
Unpaired t-test vs prs1 prs3	2.9E-05		2.9E-05	3.4E-05	1.1E-05

C: [PRPP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM	mM
Metabolite	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP
Strain	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
sample 1	0.034	0.004	0.017	0.016	0.012	0.14	0.02	0.07	0.06	0.05
sample 2	0.031	0.006	0.015	0.015	0.013	0.12	0.02	0.06	0.06	0.05
sample 3	0.033	0.007	0.016	0.017	0.013	0.13	0.03	0.06	0.07	0.05
sample 4	0.033	0.006	0.019	0.013	0.011	0.13	0.02	0.08	0.05	0.04
sample 5	0.036	0.010	0.017	0.015	0.015	0.14	0.04	0.07	0.06	0.06
sample 6	0.032	0.006	0.016	0.015	0.015	0.13	0.02	0.06	0.06	0.06
Mean	0.03	0.01	0.02	0.02	0.01	0.13	0.03	0.07	0.06	0.05
SD	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01
Unpaired t-test vs prs1 prs3	3.3E-10		2.9E-06	1.1E-05	9.2E-05	3.3E-10		2.9E-06	1.1E-05	9.2E-05

D: [ATP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM	mM
Metabolite	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP
Strain	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
sample 1	22.82	11.32	14.98	16.46	14.61	3.36	1.66	2.20	2.42	2.15
sample 2	21.79	10.26	13.41	14.53	14.19	3.20	1.51	1.97	2.14	2.09
sample 3	22.07	11.85	15.09	15.30	14.20	3.25	1.74	2.22	2.25	2.09
sample 4	22.25	10.30	13.27	15.00	14.63	3.27	1.51	1.95	2.21	2.15
sample 5	23.05	10.89	14.02	15.64	14.99	3.39	1.60	2.06	2.30	2.20
sample 6	23.98	9.89	13.75	15.42	15.34	3.53	1.45	2.02	2.27	2.26
Mean	22.66	10.75	14.09	15.39	14.66	3.33	1.58	2.07	2.26	2.16
SD	0.80	0.74	0.79	0.65	0.45	0.12	0.11	0.11	0.10	0.07
Unpaired t-test vs prs1 prs3	1.3E-10		1.9E-05	4.9E-07	3.1E-06	1.3E-10		1.9E-05	4.9E-07	3.1E-06

E: [GTP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM	mM
Metabolite	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP
Strain	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA	WT	prs1 prs3	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
sample 1	4.74	0.89	2.04	2.25	1.67	0.95	0.18	0.41	0.45	0.33
sample 2	4.44	0.81	1.81	1.82	1.68	0.89	0.16	0.36	0.36	0.34
sample 3	4.40	0.93	2.02	2.10	1.63	0.88	0.19	0.40	0.42	0.33
sample 4	4.35	0.80	1.98	1.81	1.69	0.87	0.16	0.40	0.36	0.34
sample 5	4.63	0.85	1.78	2.02	1.83	0.92	0.17	0.36	0.40	0.37
sample 6	4.58	0.82	1.79	1.95	1.89	0.91	0.16	0.36	0.39	0.38
Mean	4.52	0.85	1.90	1.99	1.73	0.90	0.17	0.38	0.40	0.35
SD	0.15	0.05	0.12	0.17	0.10	0.03	0.01	0.02	0.03	0.02
Unpaired t-test vs prs1 prs3	1.6E-09		4.0E-07	4.9E-06	2.1E-07	1.6E-09		4.0E-07	4.9E-06	2.1E-07

E: [UTP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM	mM
Metabolite	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP
Strain	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
sample 1	2.61	1.23	1.35	1.57	1.28	0.79	0.37	0.41	0.48	0.39
sample 2	2.65	1.22	1.28	1.28	1.18	0.80	0.37	0.39	0.39	0.36
sample 3	2.49	1.34	1.40	1.52	1.07	0.75	0.41	0.42	0.46	0.32
sample 4	2.39	1.23	1.43	1.24	1.14	0.72	0.37	0.43	0.38	0.35
sample 5	2.68	1.15	1.25	1.38	1.26	0.81	0.35	0.38	0.42	0.38
sample 6	2.55	1.13	1.26	1.31	1.33	0.77	0.34	0.38	0.40	0.40
Mean	2.56	1.22	1.32	1.38	1.21	0.78	0.37	0.40	0.42	0.37
SD	0.11	0.07	0.08	0.13	0.10	0.03	0.02	0.02	0.04	0.03
Unpaired t-test vs prs1 prs3	1.6E-09		0.03	0.03	0.90	1.6E-09		0.03	0.03	0.90

E: [CTP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM	mM
Metabolite	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP
Strain	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
sample 1	1.51	0.73	0.85	0.93	0.81	0.51	0.25	0.29	0.31	0.27
sample 2	1.45	0.72	0.76	0.82	0.76	0.49	0.24	0.26	0.28	0.26
sample 3	1.47	0.78	0.89	0.88	0.82	0.49	0.26	0.30	0.30	0.28
sample 4	1.49	0.75	0.78	0.89	0.78	0.50	0.25	0.26	0.30	0.26
sample 5	1.53	0.74	0.80	0.89	0.84	0.52	0.25	0.27	0.30	0.28
sample 6	1.59	0.70	0.81	0.89	0.86	0.53	0.24	0.27	0.30	0.29
Mean	1.51	0.74	0.81	0.89	0.81	0.51	0.25	0.27	0.30	0.27
SD	0.05	0.03	0.05	0.03	0.04	0.02	0.01	0.02	0.01	0.01
Unpaired t-test vs prs1 prs3	1.3E-09		8.6E-03	1.1E-05	3.1E-03	1.3E-09		8.6E-03	1.1E-05	3.1E-03

H: [NAD⁺] + [NADH] in mM

	μM in sample	μM in sample	μM in sample	μM in sample	μM in sample	mM	mM	mM	mM	mM
Metabolite	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)
Strain	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA	WT/vector	prs1 prs3/vector	prs1 prs3/PRPS1	prs1 prs3/PRPS2	prs1 prs3/PrsA
sample 1	46.28	20.05	27.82	33.60	29.32	1.39	0.60	0.83	1.01	0.88
sample 2	46.33	20.33	29.81	30.55	31.08	1.39	0.61	0.89	0.92	0.93
sample 3	45.29	22.70	31.95	34.64	31.34	1.36	0.68	0.96	1.04	0.94
sample 4	43.84	20.97	31.74	31.31	32.05	1.32	0.63	0.95	0.94	0.96
sample 5	47.69	22.62	31.83	32.36	31.63	1.43	0.68	0.95	0.97	0.95
sample 6	48.35	23.10	32.81	33.30	33.38	1.45	0.69	0.98	1.00	1.00
Mean	46.30	21.63	30.99	32.63	31.47	1.39	0.65	0.93	0.98	0.94
SD	1.63	1.34	1.84	1.52	1.33	0.05	0.04	0.06	0.05	0.04
Unpaired t-test vs prs1 prs3	1.2E-10		3.0E-06	1.3E-07	1.6E-07	1.2E-10		3.0E-06	1.3E-07	1.6E-07

Table S9: Raw data Figure 6

B : Doubling Time

	Doubling Time (min)	Doubling Time (min)	Doubling Time (min)	Doubling Time (min)
	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	98.7	333.7	85.8	103.4
sample 2	94.6	317.2	92.7	110.4
sample 3	97.5	339.1	91.9	102.4
Unpaired t-test vs WT		5.6E-04	0.07	0.06
Unpaired t-test vs <i>prs1 prs3</i>	5.6E-04		2.5E-04	2.1E-04

C: [PRPP] in mM

	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP
Strain	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	0.0220	0.0030	0.0200	0.0002	0.117	0.016	0.107	0.001
sample 2	0.0220	0.0024	0.0210	0.0003	0.117	0.013	0.112	0.002
sample 3	0.0199	0.0029	0.0201	0.0001	0.106	0.015	0.107	0.001
sample 4	0.0201	0.0029	0.0177	0.0001	0.107	0.015	0.094	0.001
Mean	0.0210	0.0028	0.0197	0.0002	0.112	0.015	0.105	0.001
SD	0.0012	0.0003	0.0014	0.0001	0.006	0.001	0.008	0.001
Unpaired t-test vsWT		3.4E-05	0.21	4.3E-05		3.4E-05	0.21	4.3E-05
Unpaired t-test vs <i>prs1 prs3</i>	3.4E-05		1.0E-04	8.6E-05	3.4E-05		1.0E-04	8.6E-05

C: [ATP] in mM

	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP
Strain	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	14.6	3.2	13.9	21.5	3.21	0.70	3.07	4.74
sample 2	14.5	3.0	14.5	21.6	3.19	0.67	3.19	4.76
sample 3	14.9	4.3	14.1	21.4	3.29	0.96	3.11	4.72
sample 4	15.1	4.1	13.3	20.8	3.33	0.90	2.94	4.58
Mean	14.8	3.6	14.0	21.3	3.26	0.80	3.08	4.70
SD	0.3	0.6	0.5	0.4	0.07	0.14	0.10	0.08
Unpaired t-test vsWT		3.8E-06	0.03	3.1E-07		3.8E-06	3.2E-02	3.1E-07
Unpaired t-test vs <i>prs1 prs3</i>	3.8E-06		6.7E-07	1.3E-07	3.8E-06		6.7E-07	1.3E-07

E: [GTP] in mM

	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP
Strain	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	2.87	0.71	2.90	3.24	0.77	0.19	0.77	0.86
sample 2	2.79	0.69	2.96	3.36	0.74	0.18	0.79	0.90
sample 3	2.80	0.98	2.85	3.28	0.74	0.26	0.76	0.87
sample 4	2.69	0.91	2.67	3.23	0.72	0.24	0.71	0.86
Mean	2.8	0.8	2.8	3.3	0.74	0.22	0.76	0.87
SD	0.1	0.1	0.1	0.1	0.02	0.04	0.03	0.02
Unpaired t-test vsWT		5.3E-06	0.48	6.7E-05		5.3E-06	0.48	6.7E-05
Unpaired t-test vs <i>prs1 prs3</i>	5.3E-06		7.9E-07	5.0E-06	5.3E-06		7.9E-07	5.0E-06

F: [UTP] in mM

	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP
Strain	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	1.75	0.42	2.77	2.07	0.71	0.17	1.12	0.84
sample 2	1.63	0.39	2.64	2.10	0.66	0.16	1.07	0.85
sample 3	1.66	0.53	2.60	2.10	0.67	0.21	1.05	0.85
sample 4	1.66	0.52	2.48	2.08	0.67	0.21	1.00	0.84
Mean	1.7	0.5	2.6	2.1	0.68	0.19	1.06	0.84
SD	0.1	0.1	0.1	0.0	0.02	0.03	0.05	0.01
Unpaired t-test vsWT		2.1E-07	1.0E-04	3.4E-04		2.1E-07	1.0E-04	3.4E-04
Unpaired t-test vs <i>prs1 prs3</i>	2.1E-07		1.3E-06	8.8E-06	2.1E-07		1.3E-06	8.8E-06

G: [CTP] in mM

	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP
Strain	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	1.00	0.28	1.21	1.39	0.45	0.13	0.54	0.62
sample 2	0.99	0.27	1.23	1.39	0.44	0.12	0.55	0.62
sample 3	1.02	0.39	1.21	1.39	0.46	0.17	0.54	0.63
sample 4	1.02	0.35	1.14	1.37	0.46	0.16	0.51	0.62
Mean	1.0	0.3	1.2	1.4	0.45	0.14	0.54	0.62
SD	0.0	0.1	0.0	0.0	0.01	0.03	0.02	0.00
Unpaired t-test vsWT		6.8E-05	6.8E-04	6.3E-08		6.8E-05	6.8E-04	6.3E-08
Unpaired t-test vs <i>prs1 prs3</i>	6.8E-05		1.1E-06	2.6E-05	6.8E-05		1.1E-06	2.6E-05

H: [NAD⁺] + [NADH] in mM

	μM in extract	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)
Strain	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements	WT	<i>prs1 prs3</i>	WT + Supplements	<i>prs1 prs3</i> + supplements
sample 1	63.12	18.54	63.09	42.42	1.26	0.37	1.26	0.85
sample 2	63.36	21.63	66.20	42.15	1.27	0.43	1.32	0.84
sample 3	64.56	27.24	58.57	47.46	1.29	0.54	1.17	0.95
sample 4	68.28	23.25	62.40	45.30	1.37	0.46	1.25	0.91
Mean	64.8	22.7	62.6	44.3	1.30	0.45	1.25	0.89
SD	2.4	3.6	3.1	2.5	0.05	0.07	0.06	0.05
Unpaired t-test vsWT		4.7E-06	0.30	2.3E-05		4.7E-06	0.30	2.3E-05
Unpaired t-test vs <i>prs1 prs3</i>	4.7E-06		3.6E-06	1.3E-04	4.7E-06		3.6E-06	1.3E-04

Table S10: Raw data Figure 7

C: Population doubling time (min)	Doubling	Doubling	Doubling	Doubling	Doubling
	Time (min)	Time (min)	Time (min)	Time (min)	Time (min)
	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
Strain 1	85.9	221.6	94.6	160.2	103.1
Strain 2	98.8	272.1	106.2	183.4	120.8
Strain 3	91.9	244.2	100.1	175.3	111.3
Strain 4	94.1	258.5	103.4	163.4	108.9
Unpaired t-test vs <i>prs1 prs3</i>	4.1E-04		5.3E-04	2.0E-03	4.2E-04

E: [PRPP] in mM						mM	mM	mM	mM	mM
	Peak area	Peak area	Peak area	Peak area	Peak area	in cells	in cells	in cells	in cells	in cells
Metabolite	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP
Strain	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
sample 1	0.037	0.006	0.014	0.008	0.012	0.1480	0.0240	0.0560	0.0320	0.0480
sample 2	0.041	0.005	0.013	0.011	0.011	0.1640	0.0200	0.0520	0.0440	0.0440
sample 3	0.036	0.004	0.013	0.009	0.010	0.1440	0.0160	0.0520	0.0360	0.0400
sample 4	0.037	0.005	0.014	0.010	0.012	0.1480	0.0200	0.0560	0.0400	0.0480
sample 5	0.038	0.006	0.012	0.010	0.011	0.1520	0.0240	0.0480	0.0400	0.0440
sample 6	0.037	0.005	0.013	0.011	0.013	0.1480	0.0200	0.0520	0.0440	0.0520
Mean	0.038	0.005	0.013	0.010	0.012	0.15	0.02	0.05	0.04	0.05
SD	0.002	0.001	0.001	0.001	0.001	0.01	0.00	0.00	0.00	0.00
Unpaired t-test vs <i>prs1 prs3</i>	1.9E-09		4.8E-09	2.4E-05	7.1E-07	1.9E-09		4.8E-09	2.4E-05	7.1E-07

F: [ATP] in mM						mM	mM	mM	mM	mM
	Peak area	Peak area	Peak area	Peak area	Peak area	in cells	in cells	in cells	in cells	in cells
Metabolite	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP
Strain	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
sample 1	20.10	9.88	15.47	13.24	15.90	3.94	1.94	3.03	2.60	3.12
sample 2	20.32	9.92	16.00	15.57	15.75	3.98	1.94	3.14	3.05	3.09
sample 3	20.23	10.26	15.98	14.19	15.78	3.97	2.01	3.13	2.78	3.09
sample 4	21.43	9.84	15.91	13.26	15.74	4.20	1.93	3.12	2.60	3.09
sample 5	19.74	9.91	15.29	13.38	15.02	3.87	1.94	3.00	2.62	2.95
sample 6	21.05	9.27	15.09	13.28	15.56	4.13	1.82	2.96	2.60	3.05
Mean	20.48	9.85	15.62	13.82	15.63	4.02	1.93	3.06	2.71	3.06
SD	0.63	0.32	0.39	0.93	0.32	0.12	0.06	0.08	0.18	0.06
Unpaired t-test vs <i>prs1 prs3</i>	1.2E-09		1.6E-10	5.2E-05	2.5E-11	1.2E-09		1.6E-10	5.2E-05	2.5E-11

G: [GTP] in mM						mM	mM	mM	mM	mM
	Peak area	Peak area	Peak area	Peak area	Peak area	in cells	in cells	in cells	in cells	in cells
Metabolite	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP
Strain	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
sample 1	3.89	0.81	1.59	1.31	1.67	0.91	0.19	0.37	0.31	0.39
sample 2	4.01	0.82	1.71	1.47	1.64	0.94	0.19	0.40	0.34	0.38
sample 3	4.07	0.84	1.72	1.34	1.69	0.95	0.19	0.40	0.31	0.39
sample 4	4.36	0.79	1.74	1.38	1.64	1.02	0.18	0.41	0.32	0.38
sample 5	3.91	0.81	1.68	1.27	1.54	0.91	0.19	0.39	0.30	0.36
sample 6	4.23	0.78	1.57	1.26	1.74	0.99	0.18	0.36	0.29	0.41
Mean	4.08	0.81	1.67	1.34	1.65	0.95	0.19	0.39	0.31	0.39
SD	0.18	0.02	0.07	0.08	0.07	0.04	0.00	0.02	0.02	0.02
Unpaired t-test vs <i>prs1 prs3</i>	9.3E-08		2.6E-07	5.8E-06	1.2E-07	9.3E-08		2.6E-07	5.8E-06	1.2E-07

H: [UTP] in mM						mM	mM	mM	mM	mM
	Peak area	Peak area	Peak area	Peak area	Peak area	in cells	in cells	in cells	in cells	in cells
Metabolite	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP
Strain	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
sample 1	3.16	1.26	1.71	2.00	1.85	1.12	0.44	0.61	0.71	0.66
sample 2	3.29	1.25	1.82	1.96	1.97	1.16	0.44	0.64	0.69	0.70
sample 3	3.26	1.20	1.87	1.89	1.97	1.15	0.42	0.66	0.67	
sample 4	3.49	1.23	1.80	1.90	2.29	1.23	0.43	0.64	0.67	0.81
sample 5	3.39	1.20	1.75	1.67	1.93	1.20	0.42	0.62	0.59	0.68
sample 6	3.56	1.20	1.67	1.90	2.04	1.26	0.43	0.59	0.67	0.72
Mean	3.36	1.22	1.77	1.89	2.02	1.19	0.43	0.63	0.67	0.71
SD	0.15	0.03	0.08	0.11	0.17	0.05	0.01	0.03	0.04	0.06
Unpaired t-test vs <i>prs1 prs3</i>	2.1E-07		2.2E-06	1.7E-05	3.8E-04	2.1E-07		2.2E-06	1.7E-05	3.8E-04

I: [CTP] in mM						mM	mM	mM	mM	mM
	Peak area	Peak area	Peak area	Peak area	Peak area	in cells	in cells	in cells	in cells	in cells
Metabolite	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP
Strain	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
sample 1	2.81	1.01	1.43	1.47	1.46	0.79	0.28	0.40	0.41	0.41
sample 2	2.82	1.00	1.46	1.73	1.48	0.79	0.28	0.41	0.49	0.41
sample 3	2.81	0.99	1.50	1.58	1.37	0.79	0.28	0.42	0.44	0.38
sample 4	2.91	0.98	1.45	1.55	1.41	0.82	0.27	0.41	0.43	0.40
sample 5	2.73	0.99	1.38	1.53	1.35	0.77	0.28	0.39	0.43	0.38
sample 6	2.91	0.93	1.45	1.52	1.37	0.82	0.26	0.41	0.43	0.38
Mean	2.83	0.98	1.44	1.56	1.41	0.79	0.28	0.41	0.44	0.39
SD	0.07	0.03	0.04	0.09	0.05	0.02	0.01	0.01	0.03	0.01
Unpaired t-test vs <i>prs1 prs3</i>	3.2E-10		2.6E-09	7.0E-06	3.1E-07	3.2E-10		2.6E-09	7.0E-06	3.1E-07

J: [NAD(H)] in mM						mM	mM	mM	mM	mM
	μM in sample	μM in sample	μM in sample	μM in sample	μM in sample	in cells	in cells	in cells	in cells	in cells
Metabolite	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)
Strain	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>	<i>prs1 prs3</i>
	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N	Wild Type	PRSS5 WT	PRSS5 P110S	PRSS5 R311I	PRSS5 D401N
sample 1	88.4	47.3	70.5	64.7	64.2	2.53	1.35	2.01	1.85	1.83
sample 2	95.8	52.4	72.8	71.4	77.0	2.74	1.50	2.08	2.04	2.20
sample 3	101.7	46.5	80.4	70.7	74.9	2.91	1.33	2.30	2.02	2.14
sample 4	102.1	48.5	77.9	67.4	74.1	2.92	1.39	2.23	1.93	2.12
sample 5	96.1	49.2	72.8	65.0	70.4	2.75	1.41	2.08	1.86	2.01
sample 6	95.3	48.8	69.3	61.6	81.0	2.72	1.39	1.98	1.76	2.31
Mean	96.56	48.80	73.95	66.81	73.61	2.76	1.39	2.11	1.91	2.10
SD	5.01	2.03	4.32	3.77	5.79	0.14	0.06	0.12	0.11	0.17
Unpaired t-test vs <i>prs1 prs3</i>	2.3E-07		3.4E-06	9.2E-06	4.9E-05	2.3E-07		3.4E-06	9.2E-06	4.9E-05

Table S11: Raw data Figure 8 (Part 1/2)

A: [PRPP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	PRPP	PRPP	PRPP	PRPP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0,028	0,027		0,044	0,112	0,108		0,176
sample 2	0,030	0,026	0,038	0,043	0,120	0,104	0,152	0,172
sample 3	0,030	0,026	0,043	0,051	0,120	0,104	0,172	0,204
sample 4	0,033	0,031	0,045	0,049	0,132	0,124	0,180	0,196
sample 5	0,029	0,028	0,041	0,047	0,116	0,112	0,164	0,188
sample 6	0,034	0,029	0,043	0,051	0,136	0,116	0,172	0,204
Mean	0,031	0,028	0,042	0,048	0,123	0,111	0,168	0,190
SD	0,002	0,002	0,003	0,003	0,009	0,008	0,011	0,014
Unpaired t-test vs empty vector		4,6E-02	6,6E-05	4,6E-06		4,6E-02	6,6E-05	4,6E-06

B: [ATP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	ATP	ATP	ATP	ATP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	24,6390	22,5630		28,8390	3,623	3,318		4,241
sample 2	23,3730	23,1000	27,0610	29,3410	3,437	3,397	3,980	4,315
sample 3	24,9750	20,3550	26,7010	26,6500	3,673	2,993	3,927	3,919
sample 4	23,4690	22,7800	28,3900	29,3420	3,451	3,350	4,175	4,315
sample 5	23,0900	22,7100	26,0100	29,0590	3,396	3,340	3,825	4,273
sample 6	24,0700	22,2500	27,5400	29,3300	3,540	3,272	4,050	4,313
Mean	23,94	22,29	27,14	28,76	3,520	3,278	3,991	4,229
SD	0,75	0,89	0,89	1,05	0,111	0,145	0,131	0,155
Unpaired t-test vs empty vector		9,7E-03	2,3E-04	7,3E-06		9,7E-03	2,3E-04	7,3E-06

C: [GTP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	GTP	GTP	GTP	GTP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	4,6890	4,0090		5,6600	0,937	0,801		1,130
sample 2	4,3900	4,1480	5,2100	5,7100	0,877	0,828	1,041	1,140
sample 3	4,7800	3,7300	5,2800	4,8800	0,955	0,745	1,055	0,975
sample 4	4,4390	4,3300	5,5500	5,7800	0,887	0,865	1,109	1,154
sample 5	4,4700	4,2500	5,1900	5,7400	0,893	0,849	1,037	1,146
sample 6	4,5400	4,4500	5,5700	5,9100	0,907	0,889	1,113	1,180
Mean	4,55	4,15	5,36	5,61	0,909	0,829	1,071	1,121
SD	0,15	0,26	0,19	0,37	0,031	0,051	0,037	0,074
Unpaired t-test vs empty vector		0,01	6,1E-05	4,1E-04		0,01	6,1E-05	4,1E-04

D: [UTP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	UTP	UTP	UTP	UTP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	2,5800	2,2500		3,1200	0,782	0,682		0,945
sample 2	2,4900	2,2900	2,9600	3,2200	0,755	0,694	0,897	0,976
sample 3	2,8080	2,0000	2,7310	3,0630	0,851	0,606	0,828	0,928
sample 4	2,5600	2,4010	3,0380	3,1770	0,776	0,728	0,921	0,963
sample 5	2,5020	2,2790	2,8300	3,2470	0,758	0,691	0,858	0,984
sample 6	2,5600	2,4040	3,0200	3,3300	0,776	0,728	0,915	1,009
Mean	2,58	2,27	2,92	3,19	0,783	0,688	0,884	0,968
SD	0,12	0,15	0,13	0,09	0,035	0,045	0,040	0,029
Unpaired t-test vs empty vector		2,5E-03	2,2E-03	2,2E-06		2,5E-03	2,2E-03	2,2E-06

E: [CTP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	CTP	CTP	CTP	CTP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	2,8200	2,5300	2,8090	3,3900	0,949	0,852		1,141
sample 2	2,7400	2,6300	3,1120	3,4100	0,923	0,886	1,048	1,148
sample 3	2,9300	2,3500	3,1440	2,9310	0,987	0,791	1,059	0,987
sample 4	2,8220	2,4460	3,2680	3,4760	0,950	0,824	1,100	1,170
sample 5	2,5950	2,5970	3,0070	3,4560	0,874	0,874	1,012	1,164
sample 6	2,8100	2,8330	3,0510	3,4440	0,946	0,954	1,027	1,160
Mean	2,79	2,56	3,07	3,35	0,938	0,863	1,049	1,128
SD	0,11	0,17	0,15	0,21	0,038	0,056	0,034	0,070
Unpaired t-test vs empty vector		0,02	5,7E-03	4,5E-04		0,02	6,0E-04	4,5E-04

F: [NAD(H)] in mM

	mM	mM	mM	mM
Metabolite	in cells	in cells	in cells	in cells
Strain	NAD(H)	NAD(H)	NAD(H)	NAD(H)
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	1,9385	1,8617		2,4532
sample 2	1,9335	1,8906	2,3750	2,4179
sample 3	2,1209	1,7032	2,3389	2,2621
sample 4	2,1006	1,9662	2,4430	2,4590
sample 5	1,9955	1,9338	2,2827	2,5381
sample 6	2,0840	2,0527	2,3121	2,5029
Mean	2,03	1,90	2,35	2,44
SD	0,08	0,12	0,06	0,10
Unpaired t-test vs empty vector		0,06	4,8E-05	1,5E-05

Table S11: Raw data Figure 8 (Part 2/2)

G: Doubling time (min)

Metabolite	AEC	AEC	AEC	AEC
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	81,330	84,140	90,740	89,890
sample 2	82,300	89,980	92,040	89,160
sample 3	84,660	85,500	94,490	91,150
sample 4	84,700	87,988	91,470	89,200
Mean	83,25	86,90	92,19	89,85
SD	1,70	2,60	1,63	0,93
Unpaired t-test vs empty vector		0,06	2,7E-04	1,4E-03

H: [SZMP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	S-ZMP	S-ZMP	S-ZMP	S-ZMP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0,0050	0,0030	0,0610	0,0460	0,00127	0,00076		0,01172
sample 2	0,0050	0,0040	0,0650	0,0520	0,00127	0,00102	0,01657	0,01325
sample 3	0,0050	0,0040	0,0610	0,0370	0,00127	0,00102	0,01555	0,00943
sample 4	0,0070	0,0060	0,0590	0,0550	0,00178	0,00153	0,01504	0,01402
sample 5	0,0060	0,0040	0,0580	0,0600	0,00153	0,00102	0,01478	0,01529
sample 6	0,0070	0,0050	0,0630	0,0570	0,00178	0,00127	0,01606	0,01453
Mean	0,0058	0,0043	0,0612	0,0512	0,001	0,001	0,016	0,013
SD	0,0010	0,0010	0,0026	0,0084	0,000	0,000	0,001	0,002
Unpaired t-test vs empty vector		0,03	1,5E-09	3,8E-05		0,03	2,7E-07	3,8E-05

I: [IMP] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	IMP	IMP	IMP	IMP	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0,139	0,092	0,591	0,693	0,062	0,041		0,310
sample 2	0,129	0,101	0,683	0,704	0,058	0,045	0,305	0,315
sample 3	0,145	0,110	0,721	0,596	0,065	0,049	0,322	0,266
sample 4	0,176	0,113	0,728	0,725	0,079	0,051	0,325	0,324
sample 5	0,150	0,126	0,690	0,761	0,067	0,056	0,308	0,340
sample 6	0,144	0,117	0,609	0,690	0,064	0,052	0,272	0,308
Mean	0,147	0,110	0,670	0,695	0,066	0,049	0,307	0,311
SD	0,016	0,012	0,057	0,055	0,007	0,005	0,021	0,025
Unpaired t-test vs empty vector		1,2E-03	1,0E-06	5,6E-07		1,2E-03	3,6E-06	5,6E-07

J: [Inosine] in mM

	Peak area	Peak area	Peak area	Peak area	mM	mM	mM	mM
Metabolite	Inosine	Inosine	Inosine	Inosine	in cells	in cells	in cells	in cells
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0,819	0,686		9,787	0,159	0,133		1,902
sample 2	0,734	0,659	11,740	9,470	0,143	0,128	2,281	1,840
sample 3	0,827	0,553	13,050	7,730	0,161	0,107	2,536	1,502
sample 4	0,848	0,677	13,460	9,070	0,165	0,132	2,615	1,762
sample 5	0,931	0,742	10,480	10,440	0,181	0,144	2,036	2,028
sample 6	0,847	0,757	8,710	10,130	0,165	0,147	1,692	1,968
Mean	0,834	0,679	11,488	9,438	0,162	0,132	2,232	1,834
SD	0,063	0,073	1,945	0,965	0,012	0,014	0,378	0,188
Unpaired t-test vs empty vector		2,8E-03	2,5E-04	3,5E-06		2,8E-03	2,5E-04	3,5E-06

Table S12: Raw data Figure 9

D: [ATP] in mM

	Intracellular concentration	Intracellular concentration	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[ATP]	[ATP]	[ATP]	[ATP]
Strain	ADE4 - adenine	ade4 Δ - adenine	ADE4 + adenine	ade4 Δ + adenine
sample 1	3,500	0,390	3,581	3,390
sample 2	3,275	0,380	3,663	3,403
sample 3	3,405	0,350	4,003	3,568
sample 4	3,103	0,430	3,922	3,599
Mean	3,32	0,39	3,79	3,49
SD	0,17	0,03	0,20	0,11
Unpaired t-test vs ADE4 -adenine		3,3E-05	1,3E-02	1,6E-01

E: [GTP] in mM

	Intracellular concentration	Intracellular concentration	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[GTP]	[GTP]	[GTP]	[GTP]
Strain	ADE4 - adenine	ade4 Δ - adenine	ADE4 + adenine	ade4 Δ + adenine
sample 1	0,955	0,374	0,841	0,823
sample 2	0,936	0,373	0,863	0,897
sample 3	0,884	0,359	0,964	0,881
sample 4	0,802	0,368	0,957	0,871
Mean	0,89	0,37	0,91	0,87
SD	0,07	0,01	0,06	0,03
Unpaired t-test vs ADE4 -adenine		5,5E-04	8,1E-01	5,3E-01

F: [CTP] in mM

	Intracellular concentration	Intracellular concentration	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[CTP]	[CTP]	[CTP]	[CTP]
Strain	ADE4 - adenine	ade4 Δ - adenine	ADE4 + adenine	ade4 Δ + adenine
sample 1	0,475	0,246	0,453	0,467
sample 2	0,445	0,257	0,446	0,493
sample 3	0,449	0,251	0,526	0,495
sample 4	0,407	0,260	0,510	0,500
Mean	0,44	0,25	0,48	0,49
SD	0,03	0,01	0,04	0,01
Unpaired t-test vs ADE4 -adenine		5,5E-04	1,6E-01	4,1E-02

G: [NAD⁺ + NADH] in mM

	Intracellular concentration	Intracellular concentration	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[NAD(H)]	[NAD(H)]	[NAD(H)]	[NAD(H)]
Strain	ADE4 - adenine	ade4 Δ - adenine	ADE4 + adenine	ade4 Δ + adenine
sample 1	1,623	0,789	1,775	1,917
sample 2	1,935	0,785	1,940	1,974
sample 3	1,848	0,793	1,993	2,098
sample 4	1,952	0,818	2,114	2,233
Mean	1,84	0,80	1,96	2,06
SD	0,15	0,01	0,14	0,14
Unpaired t-test vs ADE4 -adenine		7,6E-04	3,0E-01	8,1E-02

H: [UTP] in mM

	Intracellular concentration	Intracellular concentration	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[UTP]	[UTP]	[UTP]	[UTP]
Strain	ADE4 - adenine	ade4 Δ - adenine	ADE4 + adenine	ade4 Δ + adenine
sample 1	0,964	3,931	0,941	1,024
sample 2	0,928	4,052	1,042	1,116
sample 3	0,946	4,344	1,218	1,336
sample 4	0,958	4,276	1,269	1,228
Mean	0,95	4,15	1,12	1,18
SD	0,02	0,19	0,15	0,14
Unpaired t-test vs ADE4 -adenine		5,4E-05	1,1E-01	4,3E-02

I: [UTP] in mM

	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[UTP]	[UTP]
	vector	Ade4 R328K
sample 1	0,955	0,504
sample 2	0,929	0,572
sample 3	0,838	0,574
sample 4	0,944	0,545
Mean	0,92	0,55
SD	0,05	0,03
Unpaired t-test vs vector		8,4E-05

J: [CTP] in mM

	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[CTP]	[CTP]
	vector	Ade4 R328K
sample 1	0,461	0,325
sample 2	0,451	0,358
sample 3	0,459	0,338
sample 4	0,449	0,327
Mean	0,45	0,34
SD	0,01	0,02
Unpaired t-test vs vector		1,5E-04

K: [ATP] in mM

	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[ATP]	[ATP]
	vector	Ade4 R328K
sample 1	3,889	3,557
sample 2	3,664	3,856
sample 3	3,671	3,834
sample 4	3,623	3,619
Mean	3,71	3,72
SD	0,12	0,15
Unpaired t-test vs vector		9,6E-01

L: [GTP] in mM

	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[GTP]	[GTP]
	vector	Ade4 R328K
sample 1	0,903	0,835
sample 2	0,896	0,911
sample 3	0,880	0,934
sample 4	0,871	0,874
Mean	0,89	0,89
SD	0,01	0,04
Unpaired t-test vs vector		9,7E-01

M: [ZMP] in mM

	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[ZMP]	[ZMP]
	vector	Ade4 R328K
sample 1	0,00059	0,01824
sample 2	0,00047	0,02136
sample 3	0,00044	0,02918
sample 4	0,00028	0,02572
Mean	0,00044	0,02363
SD	0,00013	0,00481
Unpaired t-test vs vector		2,4E-03

N: [Inosine] in mM

	Intracellular concentration	Intracellular concentration
Metabolite (mM)	[Inosine]	[Inosine]
	vector	Ade4 R328K
sample 1	0,211	7,257
sample 2	0,186	7,850
sample 3	0,199	8,622
sample 4	0,183	8,295
Mean	0,19	8,00
SD	0,01	0,59
Unpaired t-test vs vector		1,2E-04

Table S13: Raw data Figure 10

A: [PRPP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
sample 1	0.0011	0.0035	0.0056	0.0039	0.0028	0.026	0.081	0.130	0.090	0.065
sample 2	0.0013	0.0038	0.0074	0.0054	0.0027	0.030	0.088	0.172	0.125	0.063
sample 3	0.0013	0.0026	0.0061	0.0049	0.0025	0.030	0.060	0.141	0.114	0.058
sample 4	0.0012	0.0027	0.0051	0.0042	0.0018	0.028	0.063	0.118	0.097	0.042
Mean	0.0012	0.0032	0.0061	0.0046	0.0025	0.028	0.073	0.140	0.107	0.057
SD	0.0001	0.0006	0.0010	0.0007	0.0005	0.002	0.014	0.023	0.016	0.010
Unpaired t-test vs control		6.5E-03	2.1E-03	1.9E-03	0.01		6.5E-03	2.1E-03	1.9E-03	0.01

B: [ATP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP	ATP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
sample 1	5.250	5.487	9.080	8.749	6.660	4.48	4.68	7.74	7.46	5.68
sample 2	5.820	5.865	9.240	9.650	6.730	4.96	5.00	7.88	8.23	5.74
sample 3	5.357	5.537	9.329	9.435	6.630	4.57	4.72	7.96	8.05	5.85
sample 4	5.712	5.996	9.388	9.633	6.497	4.87	5.11	8.01	8.21	5.54
Mean	5.53	5.72	9.26	9.37	6.63	4.72	4.88	7.90	7.99	5.65
SD	0.27	0.25	0.13	0.42	0.10	0.23	0.21	0.11	0.36	0.08
Unpaired t-test vs control		0.35	7.9E-06	1.8E-05	2.2E-03		0.35	7.9E-06	1.8E-05	2.2E-03

C: [GTP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP	GTP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
sample 1	0.716	0.698	1.366	1.288	0.833	0.83	0.81	1.58	1.49	0.96
sample 2	0.638	0.702	1.420	1.395	0.848	0.74	0.81	1.64	1.62	0.98
sample 3	0.679	0.762	1.421	1.345	0.841	0.79	0.88	1.65	1.56	0.97
sample 4	0.714	0.687	1.428	1.361	0.822	0.83	0.80	1.65	1.58	0.95
Mean	0.69	0.71	1.41	1.35	0.84	0.80	0.82	1.63	1.56	0.97
SD	0.04	0.03	0.03	0.04	0.01	0.04	0.04	0.03	0.05	0.01
Unpaired t-test vs control		3.5E-01	1.5E-07	7.0E-07	2.4E-03		3.5E-01	1.5E-07	7.0E-07	2.4E-03

D: [UTP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP	UTP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
sample 1	0.6746	0.6628	1.3801	1.2011	0.8751	1.19	1.16	2.43	2.11	1.54
sample 2	0.6142	0.6725	1.4262	1.3295	0.8663	1.08	1.18	2.51	2.34	1.52
sample 3	0.6203	0.7168	1.4325	1.2780	0.8759	1.09	1.26	2.52	2.25	1.54
sample 4	0.6580	0.7145	1.4818	1.3214	0.8476	1.16	1.26	2.60	2.32	1.49
Mean	0.64	0.69	1.43	1.28	0.87	1.13	1.22	2.51	2.25	1.52
SD	0.03	0.03	0.04	0.06	0.01	0.05	0.05	0.07	0.10	0.02
Unpaired t-test vs control		0.05	2.8E-07	1.9E-05	1.2E-04		0.05	2.8E-07	1.9E-05	1.2E-04

E: [CTP] in mM

	Peak area	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP	CTP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
sample 1	0.3081	0.3101	0.6045	0.5360	0.4052	0.60	0.61	1.18	1.05	0.79
sample 2	0.2803	0.3202	0.6372	0.5904	0.4051	0.55	0.63	1.24	1.15	0.79
sample 3	0.2888	0.3327	0.6358	0.5715	0.4033	0.56	0.65	1.24	1.12	0.79
sample 4	0.3084	0.3347	0.6383	0.5908	0.3844	0.60	0.65	1.25	1.15	0.75
Mean	0.30	0.32	0.63	0.57	0.40	0.58	0.63	1.23	1.12	0.78
SD	0.01	0.01	0.02	0.03	0.01	0.03	0.02	0.03	0.05	0.02
Unpaired t-test vs control		0.02	1.0E-07	1.4E-05	4.3E-05		0.02	1.0E-07	1.4E-05	4.3E-05

F: [NAD(H)] in mM

	µM in sample	µM in sample	µM in sample	µM in sample	µM in sample	mM in cells	mM in cells	mM in cells	mM in cells	mM in cells
Metabolite	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)	NAD(H)
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
sample 1	6.3536	5.2928	7.5551	7.8023	7.5133	2.21	1.84	2.63	2.71	2.61
sample 2	5.6084	5.4943	6.9772	7.9049	6.7833	1.95	1.91	2.43	2.75	2.36
sample 3	5.6958	5.9810	6.9392	8.0038	6.8289	1.98	2.08	2.41	2.78	2.38
sample 4	6.1217	5.5475	7.5475	8.2510	7.1027	2.13	1.93	2.63	2.87	2.47
Mean	5.94	5.58	7.25	7.99	7.06	2.07	1.94	2.52	2.78	2.46
SD	0.35	0.29	0.34	0.19	0.34	0.12	0.10	0.12	0.07	0.12
Unpaired t-test vs control		1.6E-01	1.8E-03	2.4E-04	3.8E-03		1.6E-01	1.8E-03	2.4E-04	3.8E-03

G: Cell proliferation

	Cells/cm²	Cells/cm²	Cells/cm²	Cells/cm²	Cells/cm²
	x10-3	x10-3	x10-3	x10-3	x10-3
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	PRPS2 WT
Strain 1	76.4	61.9	54.5	46.5	33.4
Strain 2	78.7	59.1	48.0	45.8	37.3
Strain 3	73.4	57.1	49.1	44.8	39.1
Unpaired t-test vs control		1.3E-03	7.6E-04	1.1E-03	6.6E-05

Table S14: Raw data Figure 11

HCT116

A: [ATP] in mM

	Peak area	Peak area	mM in cells	mM in cells
Metabolite	ATP	ATP	ATP	ATP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	60,12	97,43	3,00	4,87
sample 2	67,33	101,23	3,36	5,06
sample 3	66,13	99,63	3,30	4,98
Mean	64,53	99,43	3,22	4,97
SD	3,87	1,91	0,19	0,10
Unpaired t-test vs control		9,0E-04		9,0E-04

B: GTP relative content

	Peak area	Peak area	mM in cells	mM in cells
Metabolite	GTP	GTP	GTP	GTP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	5,48	3,43	0,37	0,24
sample 2	6,37	3,48	0,43	0,24
sample 3	6,07	3,51	0,41	0,25
Mean	5,97	3,47	0,40	0,24
SD	0,45	0,04	0,03	0,01
Unpaired t-test vs control		9,9E-03		9,9E-03

C: UTP relative content

	Peak area	Peak area	mM in cells	mM in cells
Metabolite	UTP	UTP	UTP	UTP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	9,48	6,29	0,98	0,67
sample 2	10,54	7,84	1,09	0,84
sample 3	10,52	6,47	1,08	0,69
Mean	10,18	6,87	1,05	0,73
SD	0,61	0,85	0,06	0,09
Unpaired t-test vs control		0,01		0,01

D: CTP relative content

	Peak area	Peak area	mM in cells	mM in cells
Metabolite	CTP	CTP	CTP	CTP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	2,70	1,79	0,31	0,21
sample 2	3,06	1,84	0,35	0,22
sample 3	2,85	1,82	0,33	0,22
Mean	2,87	1,82	0,33	0,22
SD	0,18	0,03	0,02	0,01
Unpaired t-test vs control		6,6E-03		6,6E-03

U-87 MG

E: ATP relative content

	Peak area	Peak area	mM in cells	mM in cells
Metabolite	ATP	ATP	ATP	ATP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	30,09	43,10	3,95	5,66
sample 2	35,23	47,62	4,63	6,25
sample 3	29,49	41,75	3,87	5,48
sample 4	32,35	46,56	4,25	6,11
sample 5	34,08	47,82	4,48	6,28
Mean	32,25	45,37	4,24	5,96
SD	2,48	2,77	0,33	0,36
Unpaired t-test vs control		5,2E-05		5,2E-05

F: GTP relative content

	Peak area	Peak area	content	content
Metabolite	GTP	GTP	GTP	GTP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	4,90	4,23	1,03	0,89
sample 2	5,12	4,42	1,07	0,93
sample 3	4,04	3,79	0,85	0,79
sample 4	4,59	4,25	0,96	0,89
sample 5	5,24	4,28	1,10	0,90
Mean	4,78	4,19	1,00	0,88
SD	0,48	0,24	0,10	0,05
Unpaired t-test vs control		0,05		0,05

G: UTP relative content

	Peak area	Peak area	content	content
Metabolite	UTP	UTP	UTP	UTP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	2,95	1,58	0,79	0,42
sample 2	4,06	2,12	1,09	0,57
sample 3	3,48	1,93	0,93	0,52
sample 4	3,93	2,24	1,05	0,60
sample 5	4,28	2,25	1,14	0,60
Mean	3,74	2,02	1,00	0,54
SD	0,53	0,28	0,14	0,07
Unpaired t-test vs control		6,4E-04		6,4E-04

H: CTP relative content

	Peak area	Peak area	mM in cells	mM in cells
Metabolite	CTP	CTP	CTP	CTP
Extracellular Purine	None	Adenine	None	Adenine
sample 1	1,35	0,80	0,90	0,53
sample 2	1,60	0,90	1,06	0,60
sample 3	1,36	0,80	0,90	0,53
sample 4	1,52	0,88	1,01	0,59
sample 5	1,69	0,89	1,12	0,59
Mean	1,50	0,85	1,00	0,57
SD	0,15	0,05	0,10	0,03
Unpaired t-test vs control		2,6E-04		2,6E-04

Table S15: Raw data Figure S1 (Part 1/3)

A: [ATP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	ATP	ATP	ATP	ATP	ATP	ATP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	118.48	49.71	97.25	3.55	1.49	2.92
sample 2	117.40	47.30	96.23	3.52	1.42	2.89
sample 3	116.72	54.50	106.28	3.50	1.63	3.19
sample 4	121.18	55.48	96.35	3.64	1.66	2.89
sample 5	123.87	57.21	103.25	3.72	1.72	3.10
sample 6	121.42	52.97	103.75	3.64	1.59	3.11
sample 7	125.24	57.51	102.18	3.76	1.73	3.07
sample 8	126.49	54.25	102.28	3.79	1.63	3.07
sample 9	126.11	58.67	103.38	3.78	1.77	3.10
sample 10	121.90	56.09	100.27	3.66	1.68	3.01
Mean	121.88	54.39	101.12	3.66	1.63	3.03
SD	3.55	3.59	3.46	0.11	0.11	0.10
Unpaired t-test vs WT		1.8E-19	1.0E-10		1.8E-19	1.0E-10
Unpaired t-test vs <i>prs1 prs3</i>	1.8E-19		1.0E-16	1.8E-19		1.0E-16

B: [GTP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	GTP	GTP	GTP	GTP	GTP	GTP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	30.892	5.732	22.304	0.93	0.17	0.67
sample 2	31.656	5.599	21.904	0.95	0.17	0.66
sample 3	31.270	6.075	23.973	0.94	0.18	0.72
sample 4	31.391	6.595	21.800	0.94	0.20	0.65
sample 5	32.874	6.186	23.476	0.99	0.19	0.70
sample 6	32.874	6.063	22.830	0.99	0.18	0.68
sample 7	34.583	6.933	22.949	1.04	0.21	0.69
sample 8	33.254	6.998	23.461	1.00	0.21	0.70
sample 9	33.231	6.717	23.466	1.00	0.20	0.70
sample 10	32.854	6.476	23.650	0.99	0.19	0.71
Mean	32.488	6.337	22.981	0.97	0.19	0.69
SD	1.149	0.483	0.759	0.03	0.01	0.02
Unpaired t-test vs WT		7.4E-17	4.1E-13		7.4E-17	4.1E-13
Unpaired t-test vs <i>prs1 prs3</i>	7.4E-17		2.2E-19	7.4E-17		2.2E-19

C: [UTP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	UTP	UTP	UTP	UTP	UTP	UTP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	37.980	14.949	34.040	1.14	0.45	1.02
sample 2	38.081	13.778	34.232	1.14	0.41	1.03
sample 3	37.768	14.461	36.399	1.13	0.43	1.09
sample 4	40.475	15.301	33.355	1.21	0.46	1.00
sample 5	39.535	14.727	35.860	1.19	0.44	1.08
sample 6	39.596	15.117	34.542	1.19	0.45	1.04
sample 7	42.230	16.486	36.031	1.27	0.49	1.08
sample 8	38.794	15.539	36.498	1.16	0.47	1.09
sample 9	40.037	16.225	36.253	1.20	0.49	1.09
sample 10	39.974	16.014	34.871	1.20	0.48	1.05
Mean	39.447	15.260	35.208	1.18	0.46	1.06
SD	1.363	0.838	1.134	0.04	0.03	0.03
Unpaired t-test vs WT		9.1E-18	6.7E-07		9.1E-18	6.7E-07
Unpaired t-test vs <i>prs1 prs3</i>	9.1E-18		1.0E-18	9.1E-18		1.0E-18

D: [CTP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	CTP	CTP	CTP	CTP	CTP	CTP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	22.458	8.082	18.425	0.67	0.24	0.55
sample 2	22.597	7.789	18.437	0.68	0.23	0.55
sample 3	22.276	8.895	19.930	0.67	0.27	0.60
sample 4	24.490	9.957	19.915	0.73	0.30	0.60
sample 5	24.305	9.115	19.974	0.73	0.27	0.60
sample 6	23.910	8.928	18.816	0.72	0.27	0.56
sample 7	24.795	9.891	19.299	0.74	0.30	0.58
sample 8	23.423	9.203	19.240	0.70	0.28	0.58
sample 9	24.558	9.851	19.750	0.74	0.30	0.59
sample 10	24.391	9.544	18.332	0.73	0.29	0.55
Mean	23.720	9.125	19.212	0.71	0.27	0.58
SD	0.961	0.742	0.670	0.03	0.02	0.02
Unpaired t-test vs WT		7.9E-18	1.6E-09		7.9E-18	1.6E-09
Unpaired t-test vs <i>prs1 prs3</i>	7.9E-18		3.6E-17	7.9E-18		3.6E-17

E: [ADP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	ADP	ADP	ADP	ADP	ADP	ADP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	12.77	10.26	11.92	0.38	0.31	0.36
sample 2	11.92	9.96	13.12	0.36	0.30	0.39
sample 3	12.57	10.87	12.21	0.38	0.33	0.37
sample 4	12.97	11.32	12.20	0.39	0.34	0.37
sample 5	13.75	11.68	13.75	0.41	0.35	0.41
sample 6	14.27	11.86	12.74	0.43	0.36	0.38
sample 7	14.58	11.89	12.52	0.44	0.36	0.38
sample 8	14.51	11.63	13.61	0.44	0.35	0.41
sample 9	14.11	11.72	14.22	0.42	0.35	0.43
sample 10	13.33	11.36	13.89	0.40	0.34	0.42
Mean	13.478	11.255	13.019	0.40	0.34	0.39
SD	0.908	0.679	0.814	0.03	0.02	0.02
Unpaired t-test vs WT		1.1E-05	2.5E-01		1.1E-05	2.5E-01
Unpaired t-test vs <i>prs1 prs3</i>	1.1E-05		5.9E-05	1.1E-05		5.9E-05

Table S15: Raw data Figure S1 (Part 2/3)

F: [GDP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	GDP	GDP	GDP	GDP	GDP	GDP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	5.744	2.318	4.827	0.172	0.070	0.145
sample 2	5.301	2.224	4.758	0.159	0.067	0.143
sample 3	5.535	2.509	4.921	0.166	0.075	0.148
sample 4	5.441	2.362	4.066	0.163	0.071	0.122
sample 5	5.751	2.270	4.962	0.173	0.068	0.149
sample 6	5.343	2.330	4.615	0.160	0.070	0.138
sample 7	5.458	2.833	4.532	0.164	0.085	0.136
sample 8	5.325	2.398	4.657	0.160	0.072	0.140
sample 9	5.753	2.408	4.970	0.173	0.072	0.149
sample 10	5.403	2.515	4.784	0.162	0.075	0.144
Mean	5.505	2.417	4.709	0.165	0.072	0.141
SD	0.182	0.173	0.270	0.005	0.005	0.008
Unpaired t-test vs WT		8,6E-19	9,3E-07		8,6E-19	9,3E-07
Unpaired t-test vs <i>prs1 prs3</i>	8,6E-19		3,3E-13	8,6E-19		3,3E-13

G: [UDP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	UDP	UDP	UDP	UDP	UDP	UDP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	2.261	2.856	2.036	0.068	0.086	0.061
sample 2	1.972	2.649	1.824	0.059	0.079	0.055
sample 3	1.841	2.322	1.905	0.055	0.070	0.057
sample 4	2.655	3.053	1.634	0.080	0.092	0.049
sample 5	2.662	3.159	2.140	0.080	0.095	0.064
sample 6	2.387	2.780	1.481	0.072	0.083	0.044
sample 7	2.815	2.864	1.980	0.084	0.086	0.059
sample 8	2.866	2.720	1.937	0.086	0.082	0.058
sample 9	2.431	3.033	1.723	0.073	0.091	0.052
sample 10	2.493	2.789	1.899	0.075	0.084	0.057
Mean	2.438	2.823	1.856	0.073	0.085	0.056
SD	0.339	0.237	0.197	0.010	0.007	0.006
Unpaired t-test vs WT		9,6E-03	3,1E-04		9,6E-03	3,1E-04
Unpaired t-test vs <i>prs1 prs3</i>	9,6E-03		1,4E-08	9,6E-03		1,4E-08

H: [CDP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	CDP	CDP	CDP	CDP	CDP	CDP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	1.182	0.815	0.963	0.035	0.024	0.026
sample 2	1.211	0.820	0.983	0.036	0.025	0.029
sample 3	1.132	0.717	1.019	0.034	0.021	0.031
sample 4	1.287	0.766	0.867	0.039	0.023	0.026
sample 5	1.223	0.698	0.920	0.037	0.021	0.028
sample 6	1.163	0.746	0.833	0.035	0.022	0.025
sample 7	1.155	0.737	0.906	0.035	0.022	0.027
sample 8	1.372	0.735	1.019	0.041	0.022	0.031
sample 9	1.133	0.824	0.950	0.034	0.025	0.028
sample 10	1.265	0.753	1.071	0.038	0.023	0.032
Mean	1.212	0.761	0.943	0.036	0.023	0.028
SD	0.077	0.045	0.079	0.002	0.001	0.002
Unpaired t-test vs WT		1,3E-10	4,0E-07		1,3E-10	4,0E-07
Unpaired t-test vs <i>prs1 prs3</i>	1,3E-10		1,6E-05	1,3E-10		1,6E-05

I: [AMP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	AMP	AMP	AMP	AMP	AMP	AMP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	4.619	3.320	4.217	0.139	0.100	0.127
sample 2	4.227	3.456	3.897	0.127	0.104	0.117
sample 3	4.624	3.303	4.962	0.139	0.099	0.149
sample 4	3.950	3.747	3.947	0.119	0.112	0.118
sample 5	4.302	3.654	4.582	0.129	0.110	0.137
sample 6	4.516	3.792	4.112	0.135	0.114	0.123
sample 7	4.397	3.785	4.428	0.132	0.114	0.133
sample 8	4.222	3.647	3.815	0.127	0.109	0.114
sample 9	4.299	3.424	4.425	0.129	0.103	0.133
sample 10	4.456	3.728	4.284	0.111	0.093	0.107
Mean	4.361	3.586	4.267	0.129	0.106	0.126
SD	0.207	0.192	0.350	0.009	0.007	0.012
Unpaired t-test vs WT		7,6E-08	4,8E-01		4,9E-06	5,7E-01
Unpaired t-test vs <i>prs1 prs3</i>	7,6E-08		9,5E-05	4,9E-06		5,1E-04

J: [GMP] in mM

	μM in extract	μM in extract	μM in extract	mM in cells	mM in cells	mM in cells
Metabolite	GMP	GMP	GMP	GMP	GMP	GMP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	12.831	2.837	10.141	0.385	0.085	0.304
sample 2	12.555	2.908	10.263	0.377	0.087	0.308
sample 3	10.503	2.585	10.591	0.315	0.078	0.318
sample 4	11.098	2.866	9.832	0.333	0.086	0.295
sample 5	12.545	2.908	10.729	0.376	0.087	0.322
sample 6	12.538	2.906	10.892	0.376	0.087	0.327
sample 7	11.054	3.049	10.117	0.332	0.091	0.304
sample 8	11.255	3.014	9.816	0.338	0.090	0.294
sample 9	12.461	2.969	10.488	0.374	0.089	0.315
sample 10	11.876	2.984	9.541	0.356	0.090	0.286
Mean	11.872	2.903	10.241	0.356	0.087	0.307
SD	0.827	0.130	0.436	0.025	0.004	0.013
Unpaired t-test vs WT		3,5E-11	8,3E-05		3,5E-11	8,3E-05
Unpaired t-test vs <i>prs1 prs3</i>	3,5E-11		5,2E-14	3,5E-11		5,2E-14

Table S15: Raw data Figure S1 (Part 3/3)

K: [UMP] in mM

	μM in extract	μM in extract	μM in extract	mM	mM	mM
Metabolite	UMP	UMP	UMP	UMP	UMP	UMP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	15,799	15,168	12,120	0,474	0,455	0,364
sample 2	15,144	15,754	13,760	0,454	0,473	0,413
sample 3	15,190	16,945	14,321	0,456	0,508	0,430
sample 4	14,553	16,958	12,465	0,437	0,509	0,374
sample 5	16,325	17,331	13,373	0,490	0,520	0,401
sample 6	16,460	16,376	13,647	0,494	0,491	0,409
sample 7	15,355	17,389	12,137	0,461	0,522	0,364
sample 8	15,023	16,925	11,718	0,451	0,508	0,352
sample 9	16,731	17,874	12,881	0,502	0,536	0,386
sample 10	15,825	16,675	11,559	0,475	0,500	0,347
Mean	15,641	16,739	12,798	0,469	0,502	0,384
SD	0,706	0,800	0,944	0,021	0,024	0,028
Unpaired t-test vs WT		4,4E-03	7,9E-07		4,4E-03	7,9E-07
Unpaired t-test vs <i>prs1 prs3</i>	4,4E-03		1,0E-08	4,4E-03		1,0E-08

L: [CMP] in mM

	μM in extract	μM in extract	μM in extract	mM	mM	mM
Metabolite	CMP	CMP	CMP	CMP	CMP	CMP
Strain	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>	WT	<i>prs1 prs3</i>	<i>prs2 prs4 prs5</i>
sample 1	0,572	0,371	0,434	0,017	0,011	0,013
sample 2	0,546	0,391	0,454	0,016	0,012	0,014
sample 3	0,511	0,337	0,413	0,015	0,010	0,012
sample 4	0,509	0,471	0,404	0,015	0,014	0,012
sample 5	0,654	0,449	0,459	0,020	0,013	0,014
sample 6	0,496	0,463	0,384	0,015	0,014	0,012
sample 7	0,466	0,417	0,461	0,014	0,013	0,014
sample 8	0,535	0,402	0,420	0,016	0,012	0,013
sample 9	0,461	0,471	0,404	0,014	0,014	0,012
sample 10	0,479	0,400	0,384	0,014	0,012	0,012
Mean	0,523	0,417	0,422	0,016	0,013	0,013
SD	0,058	0,046	0,029	0,002	0,001	0,001
Unpaired t-test vs WT		2,9E-04	2,5E-04		2,9E-04	2,5E-04
Unpaired t-test vs <i>prs1 prs3</i>	2,9E-04		8,0E-01	2,9E-04		8,0E-01

Table S16 Figure S3

B: [ATP] in mM

	mM	mM	mM	mM
	in cells	in cells	in cells	in cells
Metabolite (mM)	[ATP]	[ATP]	[ATP]	[ATP]
Strain	<i>URA2 - uracil</i>	<i>URA2 + uracil</i>	<i>ura2Δ - uracil</i>	<i>ura2Δ + uracil</i>
sample 1	3,064	3,353	3,994	3,072
sample 2	3,490	2,811	4,210	2,869
sample 3	3,020	3,036	3,820	3,223
sample 4	3,409	3,292	4,270	3,527
Mean	3,25	3,12	4,07	3,17
SD	0,24	0,25	0,21	0,28
Unpaired t-test vs <i>URA2 -uracil</i>		0,50	2,0E-03	0,70

C: [GTP] in mM

	mM	mM	mM	mM
	in cells	in cells	in cells	in cells
Metabolite (mM)	[GTP]	[GTP]	[GTP]	[GTP]
Strain	<i>URA2 - uracil</i>	<i>URA2 + uracil</i>	<i>ura2Δ - uracil</i>	<i>ura2Δ + uracil</i>
sample 1	0,795	0,820	0,976	0,806
sample 2	0,797	0,738	1,063	0,802
sample 3	0,823	0,723	0,984	0,870
sample 4	0,782	0,756	0,973	0,848
Mean	0,80	0,76	1,00	0,83
SD	0,02	0,04	0,04	0,03
Unpaired t-test vs <i>URA2 -uracil</i>		0,16	1,0E-03	0,15

D: [UTP] in mM

	mM	mM	mM	mM
	in cells	in cells	in cells	in cells
Metabolite (mM)	[UTP]	[UTP]	[UTP]	[UTP]
Strain	<i>URA2 - uracil</i>	<i>URA2 + uracil</i>	<i>ura2Δ - uracil</i>	<i>ura2Δ + uracil</i>
sample 1	0,709	1,025	0,010	0,795
sample 2	0,809	0,943	0,003	0,813
sample 3	0,805	1,095	0,004	0,931
sample 4	0,890	1,126	0,015	0,858
Mean	0,88	1,13	0,02	0,86
SD	0,07	0,08	0,01	0,06
Unpaired t-test vs <i>URA2 -uracil</i>		3,9E-03	1,7E-04	0,34

E: [CTP] in mM

	mM	mM	mM	mM
	in cells	in cells	in cells	in cells
Metabolite (mM)	[CTP]	[CTP]	[CTP]	[CTP]
Strain	<i>URA2 - uracil</i>	<i>URA2 + uracil</i>	<i>ura2Δ - uracil</i>	<i>ura2Δ + uracil</i>
sample 1	0,446	0,532	0,186	0,453
sample 2	0,442	0,477	0,226	0,451
sample 3	0,461	0,476	0,192	0,466
sample 4	0,448	0,560	0,216	0,530
Mean	0,88	1,13	0,02	0,86
SD	0,01	0,04	0,02	0,04
Unpaired t-test vs <i>URA2 -uracil</i>		0,06	1,6E-05	0,26

F: [NAD⁺ + NADH] in mM

	mM	mM	mM	mM
	in cells	in cells	in cells	in cells
Metabolite (mM)	[NAD(H)]	[NAD(H)]	[NAD(H)]	[NAD(H)]
Strain	<i>URA2 - uracil</i>	<i>URA2 + uracil</i>	<i>ura2Δ - uracil</i>	<i>ura2Δ + uracil</i>
sample 1	2,152	2,298	2,789	2,377
sample 2	2,289	2,298	3,051	2,256
sample 3	2,290	2,316	2,852	2,534
sample 4	2,252	2,528	2,783	2,355
Mean	0,88	1,13	0,02	0,86
SD	0,07	0,11	0,13	0,12
Unpaired t-test vs <i>URA2 -uracil</i>		0,14	5,2E-04	0,10

Table S17: Raw data Figure S4

A: [PRPP] in μM

Metabolite	Peak area	Peak area	Peak area	Peak area	μM in cells	μM in cells	μM in cells	μM in cells
Strain	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0.00045	0.00040	0.00120	0.00080	6.6651	5.9245	17.7736	11.8491
sample 2	0.00032	0.00021	0.00096	0.00085	4.7259	3.1506	14.1778	12.6025
sample 3	0.00042	0.00042	0.00084	0.00063	6.2537	6.2537	12.5073	9.3805
sample 4	0.00026	0.00010	0.00114	0.00073	3.8443	1.5377	16.9148	10.7639
Mean	0.0004	0.0003	0.0010	0.0008	5.3722	4.2166	15.3434	11.1490
SD	0.0001	0.0002	0.0002	0.0001	1.3167	2.2642	2.4343	1.3998
Unpaired t-test vs control		0,42	1,1E-03	9,7E-04		0,42	1,1E-03	9,7E-04

B: [ATP] in mM

Metabolite	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Strain	ATP	ATP	ATP	ATP	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	6.398	6.726	9.175	10.075	3.48	3.66	5.00	5.49
sample 2	6.865	7.131	9.632	10.392	3.74	3.88	5.24	5.66
sample 3	6.760	7.110	9.888	10.224	3.68	3.87	5.38	5.57
sample 4	6.376	6.677	9.845	9.511	3.47	3.64	5.36	5.18
Mean	6.60	6.91	9.64	10.05	3.59	3.76	5.25	5.47
SD	0.25	0.24	0.33	0.38	0.14	0.13	0.18	0.21
Unpaired t-test vs control		0,12	1,0E-05	1,8E-05		0,12	1,0E-05	1,8E-05

C: [GTP] in mM

Metabolite	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Strain	GTP	GTP	GTP	GTP	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0.743	0.810	1.119	1.182	0.55	0.60	0.83	0.87
sample 2	0.790	0.855	1.142	1.248	0.58	0.63	0.84	0.92
sample 3	0.782	0.856	1.171	1.216	0.58	0.63	0.87	0.90
sample 4	0.731	0.773	1.079	1.203	0.54	0.57	0.80	0.89
Mean	0.76	0.82	1.13	1.21	0.56	0.61	0.83	0.90
SD	0.03	0.04	0.04	0.03	0.02	0.03	0.03	0.02
Unpaired t-test vs control		0,05	1,1E-05	4,8E-07		0,05	1,1E-05	4,8E-07

D: [UTP] in mM

Metabolite	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Strain	UTP	UTP	UTP	UTP	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0.788	0.773	1.557	1.435	0.88	0.87	1.75	1.61
sample 2	0.813	0.846	1.432	1.463	0.91	0.95	1.61	1.64
sample 3	0.825	0.850	1.461	1.442	0.93	0.95	1.64	1.62
sample 4	0.773	0.852	1.468	1.324	0.87	0.96	1.65	1.49
Mean	0.80	0.83	1.48	1.42	0.90	0.93	1.66	1.59
SD	0.02	0.04	0.05	0.06	0.03	0.04	0.06	0.07
Unpaired t-test vs control		0,24	1,6E-05	6,8E-05		0,24	1,6E-05	6,8E-05

E: [CTP] in mM

Metabolite	Peak area	Peak area	Peak area	Peak area	mM in cells	mM in cells	mM in cells	mM in cells
Strain	CTP	CTP	CTP	CTP	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	0.319	0.329	0.491	0.544	0.40	0.41	0.61	0.68
sample 2	0.344	0.359	0.551	0.578	0.43	0.45	0.69	0.72
sample 3	0.329	0.353	0.547	0.558	0.41	0.44	0.68	0.70
sample 4	0.297	0.325	0.539	0.504	0.37	0.41	0.67	0.63
Mean	0.32	0.34	0.53	0.55	0.40	0.43	0.66	0.68
SD	0.02	0.02	0.03	0.03	0.02	0.02	0.03	0.04
Unpaired t-test vs control		0,19	3,7E-05	6,5E-05		0,19	3,7E-05	6,5E-05

F: [NAD(H)] in mM

Metabolite	μM in sample	μM in sample	μM in sample	μM in sample	mM in cells	mM in cells	mM in cells	mM in cells
Strain	NAD(H)	NAD(H)	NAD(H)	NAD(H)	PRPP	PRPP	PRPP	PRPP
Strain	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
sample 1	1.182	1.192	1.788	1.350	0.263	0.265	0.397	0.300
sample 2	0.998	1.253	1.983	1.399	0.236	0.296	0.469	0.331
sample 3	0.961	1.314	1.667	1.192	0.225	0.308	0.391	0.280
sample 4	1.156	1.010	1.509	1.484	0.267	0.233	0.348	0.342
Mean	1.07	1.19	1.74	1.36	0.248	0.275	0.401	0.313
SD	0.11	0.13	0.20	0.12	0.020	0.034	0.050	0.029
Unpaired t-test vs control		0,22	4,9E-03	0,01		0,22	4,9E-03	0,01

G: Cell proliferation

	Cell/cm ²	Cell/cm ²	Cell/cm ²	Cell/cm ²
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-3}$
	Control	PRPS1 WT	PRPS1 V142L	PRPS1 A190V
Strain 1	227.1	254.5	128.3	119.1
Strain 2	215.2	245.8	133.3	119.9
Strain 3	216.4	244.7	123.2	106.7
Unpaired t-test vs control		4,7E-03	7,1E-05	5,8E-05