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Phenotypic Consequences of Purine Nucleotide Imbalance in *Saccharomyces cerevisiae*

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Hs AMPD2 ----MRNRGQGLFRLRRCFLHQSLPLGAGRRKGLDVAEPGPSRCRSDSPAVAAVVPAMASYPGSGKPKAKYPPFKRASLQASTAAPEARGLGAPPLQ
 At FAC1 MEPNIYQLAALAFGASFVAVSGFFMHFKALNLVLERGKERKENPDGEPQNPFLVRRRSQVRRKVNVDQYGRSPASLDPATPFTDGGGGGGDTRSGNH
 Sc Amd1p ----MDNQATQRLNLDLSLEPAPSHDEQDGLVIDIDQRKIGDEQAGVVVDETPPLEQQDSHESLAADSRNANFSYHENQQLLENGTKQALDEHDSHA
 Sc Yj1070p -----MQAVERRPSLLFDEYQNSVTKPNETKNEARVLSENDGDVSPSVLKQKEISVDDMMIISLPTFEDRQM
 Sc Ybr284p -----MVQNNSEVFFVECDYSKESPSSTSPIR-----LDDLGDNDVAUSDQGLAFDGDVGTISQARVNRPA

Hs AMPD2 SARSPLGPAPCLKHFLDLRLTSMGKCKEIAEELFTRSLAESELRSAPYEFPEESPIEQLEERRQRLERQISQDVKLEPDIILLRAKQDFLTKDSDSLQL
 At FAC1 VYVDEIIPGLPRLHPTSEGRASVHGASSIRKTGSFVRPISP-----KSPVASASAFESVEESDDDDNLTNSEGLDASVYLAQANGNEMPADANE
 Sc Amd1p ILEQPSHSTNCSSSNIAMNKGHDSADHASQNSGGKP-----RTLSASAQHILPETLKSFAGAPVVKQVRTSASYKMGMLADDASQQF
 Sc Yj1070p VLGSPMFFDLEDEENKIDPLPSVSHHYGNGESDSFVSSYTP-----SNLKTGEETKDLFINPPELVSQMRKRYIAASKQDGIISNKNDEKW
 Sc Ybr284p QIFEDSNTDVLHLDDLDMVP-----LNTKFDMMEMGSPMAMPAPETPPPVEPLKTKDLA

Hs AMPD2 YKEQGEQGGDRSLRERDVLEREFQRVTISGEEKCVPTDLDLDAKSVVRLFIREKYMALSLQSFCTPTTRRYLQQLAEKPLETRTYEQGPDTPVSADAP
 At FAC1 EQISMAASSMIRSHSVSGDLHGVPDPIAADILRKEPEQETFFVRLNVPLEVPTSDVEVEAYKCLQCELELRKRYVQETVAPWEKEVISDPSPTPKNTEPF
 Sc Amd1p LDDPSSSELIDLYSKVAECRNLRAKYQTSVQNDQNPKNKPGWVYPPPPKPSYNSDTKTVPVTKNPDAEVDFPTKCEIPGEDPDWEFTLNDDSDYVHV
 Sc Yj1070p FLYPKPLPKFWRFEEDKRFQDPSDSLNDGDSTGTGAAPPHRGGYYPSTYFDHYYYTSGKLGKGNIKVYPTGEYFDLEDYKQYIYHLSNQENTQN
 Sc Ybr284p YSSLAHLPSYFFEQTHFRID-----RKCLLEMSKLRRLNYLTISKQDALSCPQLHSRVAGGYLKPVKEKLFGRHFLDLEESNT

Hs AMPD2 VHPPALEQHPYEHCEPSTMPGDGLGLRMVGRVHVYTRREPDEHCSEVELPYDLDQEFVADVNVLMALIINGPKSFCYRRLQYLSKFKQMHVLLNEMK
 At FAC1 AHYPQKSD-----HCFEMQDGVVHVFN-----KDAKEDLFPVADATAFFTDLHHVLKVIAGNIRTLCHRRLVLELQKFNHLMLNADK
 Sc Amd1p RSG-----KTDELAIQIPTLRDYYLLEKMSISSDGPAKSFAYRRLQYLEARWNLYYLLNEYQ
 Sc Yj1070p PLSPYSKE-----ESLEEEFLTDVPTFQEFRDDFAYIIELIQSHKFNVEYRRLSYLLDKFELFQYLNYSKK
 Sc Ybr284p VNLQDQNY-----MTELFNSQINIPTFKEFREDFEWCLKIIRDRLSRFSEKRLQYLVNKFVFPQHLHSKE

Hs AMPD2 ELAAQKKVPHRDFYNIKRVDTTHIASSCMNQKHLRFTKRAMKRHLEEIVHVE--QGREQTLREVFEEMNLTAYDLSVDTLDVHADRNTPHRFDKFNKY
 At FAC1 EFLAQKSAHRDFYNVKRVDTTHVHSACMNQKHLRFTKSKLRKEPEVVIIFR--DGYLTLREVFEESLDLTGYDLNVDDLVDVHADKSTFHRFDKFNLYK
 Sc Amd1p ETSVSKRNPHRDFYNVKRVDTTHVHSACMNQKHLRFTKHKLRHRSKDEKVIIFR--DGKLLTLDEVFRSLHLTGYDLSIDTDMHAHKDTPHRFDKFNLYK
 Sc Yj1070p EILANKNVYRDFYNSRKVDRDLSLSCISQRLQSEYIWEKINLEPERIVYQDPETSRKLSLRFIDFQFGCSSNDQPIAIGLKLIDDEFDLDWYRNLYLIDY
 Sc Ybr284p EMRQSKKVPKDFYNCRIKIDLNLLSGCFSQOWLTFEITWTKLRKEPDRVIHQAFNGS--HITLSQLFKVNFEETG--QFFNGKIIDDSFLEWKVYIYLAKY

Hs AMPD2 NPIG-----ESVLEEIFIKTDNRVSGKYFAHIIKEVMSD--LEESKYQNAELRSLIYGRSRDE-----WDKLARWAVMHRVHSPNVRWL
 At FAC1 NPCG-----QSRLREIFLQDNLIQGRFLGEITKQVFS--LEASKYQMAEYRISYIYGRKMS--WDQLASWIVNNDLYSENVVWL
 Sc Amd1p NPIG-----ESLREIFLKTNNYIKCTYLADITKQVIFD--LENSKYQNCYRISVYGRSLDE-----WDKLASWIVDNKVISHNVRWL
 Sc Yj1070p HLLPNKVAKLVG---KEMRFYLLAKVLEFDNFIEGEYLAEIFIKYVIHILEKSKYLAQVSVNFPQYSSGED-----WYKFKSQWLLRKLKLVSYNIRWN
 Sc Ybr284p HLVNDEMEIHTGSHGQLRYLYIAKTFLEFDNYINGEYLAELKTLFKLQKQESKYQLCQLSVDFQFYLYHDNSVDVNDWVFNANLHNHYNIFSNNIRWN

Hs AMPD2 VQVPRFLFDVYRTKQLANFQEMLENIIFLPLFEATVHPASHP-----ELHFLFLEHVDGFDSDVDES--KPNHVFNLESPLPEAWVEE--DNPPYAYVLY
 At FAC1 IQLPRLYNIYKDMGIVTSFQNILDNIFLPLFEATVDPDHP-----QLHVFLKQVVGFDLVDDES--KPE--RRPTKHMPTAQWNTNA--FNPAFSYVYK
 Sc Amd1p VQIPRLYDIYKKTGIVQSFQDICKNLPQPLFEVTKNPQSHP-----KLHVFLQRVIGFSDVDES--K--VDRRFRHKYKPKSLWEAP--QNPYSYVLY
 Sc Yj1070p IQIARIFPKLFKENVVSNFQEFLDLIFNPLFTLEKEQLPIDSSVNTDIIIGLQFLSNVCSMDLVIKESDEYWKFEFTDMNCKPKFWTAQGDNPVVAHYMY
 Sc Ybr284p IRISRIYPELYHTGKVKNFQEYLNLIIFKPLFNAENYLHKSGLG-----PILLLKFLSVSSIDLICIQDNDYIWNFTAVSCLPKDWTSGGDNPTISQYMY

Hs AMPD2 YTFANMAMNHLRQRGFHTFVLRPHCEAGP-----IHHLVSFAFLAEN--ISHGLLLRKAPVLQYLYLQIIGIAMSPLS-----
 At FAC1 YCYANLYVLNKLRESKGMTITLRLPHSAGEAGD-----IDLHAAATFLTCHS--IAHGINLRKSPVLQYLYLQIIGIAMSPLS-----
 Sc Amd1p YLYSNVASLNQWRAKRGFTLVLRLPHCEAGD-----PEHLVSAYLLAHG--ISHGILLRKVPVQYLYLQVGIAMSPLS-----
 Sc Yj1070p YIYKSLAKNFLRSQNLQNTITLRLNYCSPSS--RTSQFGVDLYFTDQVESLVNLLCNGGLLQVEPLWDATMIQYLYLQIIPILAAAPLSSVSLNSQ
 Sc Ybr284p YVYVNLTKLNHRIQALHQNTFTLRSSCSPTSMNRSTSQFNTLNFTEHTAILNLFLLACGGFLNAENLWNAPPVSLVLYLYLQIIPMVVAPLN-----

Hs AMPD2 -----NNSLFLSYHRNPLPEYLSRGLMVSLSLTDDBLQFH--FTKEPLMEEYSIATQVWKLSSCDMCELARNSVLM
 At FAC1 -----NNSFLDYHRNPFVFFLRGLNVSLSLTDDBLQIH--LTKPELVEEYSIAASVWKL SACDLCEIARNSVYQ
 Sc Amd1p -----NNALFLTYDKNPPFRYFKRGLNVSLSLTDDBLQFS--YTREPLIEEYSVAAQIYKLSNVDMCELARNSVLQ
 Sc Yj1070p KSTFLKNKNVLEHLDYKQETAKINPSRDI TVGEQRSYETNPFMKMFKMGLKISLSKSLYNSSYTLEPLIEEYSVAASIYLLNPTDLCELSRTSVLS
 Sc Ybr284p --SIVDSKPTMQEQAPTG-----LVLEPSKYKKNPFMKMFKMFKISLSSESILYNSSYTKEPIIEEYSVAASIYLRHSADLCELLRNSVIT

Hs AMPD2 SGFSHKVKSHWLGPNYTKE-----GPEGNDIRRTNVDPDIRVGYRYETLQCELALITQAVQSEMLETIPEEAGITMSPGPQ
 At FAC1 SGFSHALKSHWIKDYK-----GPDGNDIHKTNVPHIRVEFRDTIWKEEMQVYVYLGKAVISDEVVP-----
 Sc Amd1p SGWEAQIKKHWIKDFDKS-----GVEGNDVVRTNVDPDIRINRYRDTLSTLELNVHNFANFKRTIEEK-----
 Sc Yj1070p SGYEGWYKAHWIYGVVKKAPYFEENVGGINDWYDTARDTSIKHNVPDIRRYYRRETLDQEWNFVRDHFVGNISW-----
 Sc Ybr284p SGFSSTLKNKWLGVSLASHDYFVENTGFVDKWDYCKPNTSLEHNVPIIRRYRSSTLAGEWRLIIA-----

FIGURE S1.—Sequence comparison of AMP deaminase homologs. Sequences of human AMP deaminase isoform 2 (AMPD2, NP_004028), *Arabidopsis thaliana* FAC1 (NP_850294) and yeast Amd1p (NP_013677), Yj1070p (NP_012465) and Ybr284p (NP_009843) were aligned using ClustalW. Residues involved in all five proteins are highlighted in yellow. Dashes indicate the gaps created for alignment. The four histidine residues involved in zinc binding and the SL(S/N)TDDP motif highly conserved in adenosine deaminases are highlighted in red.

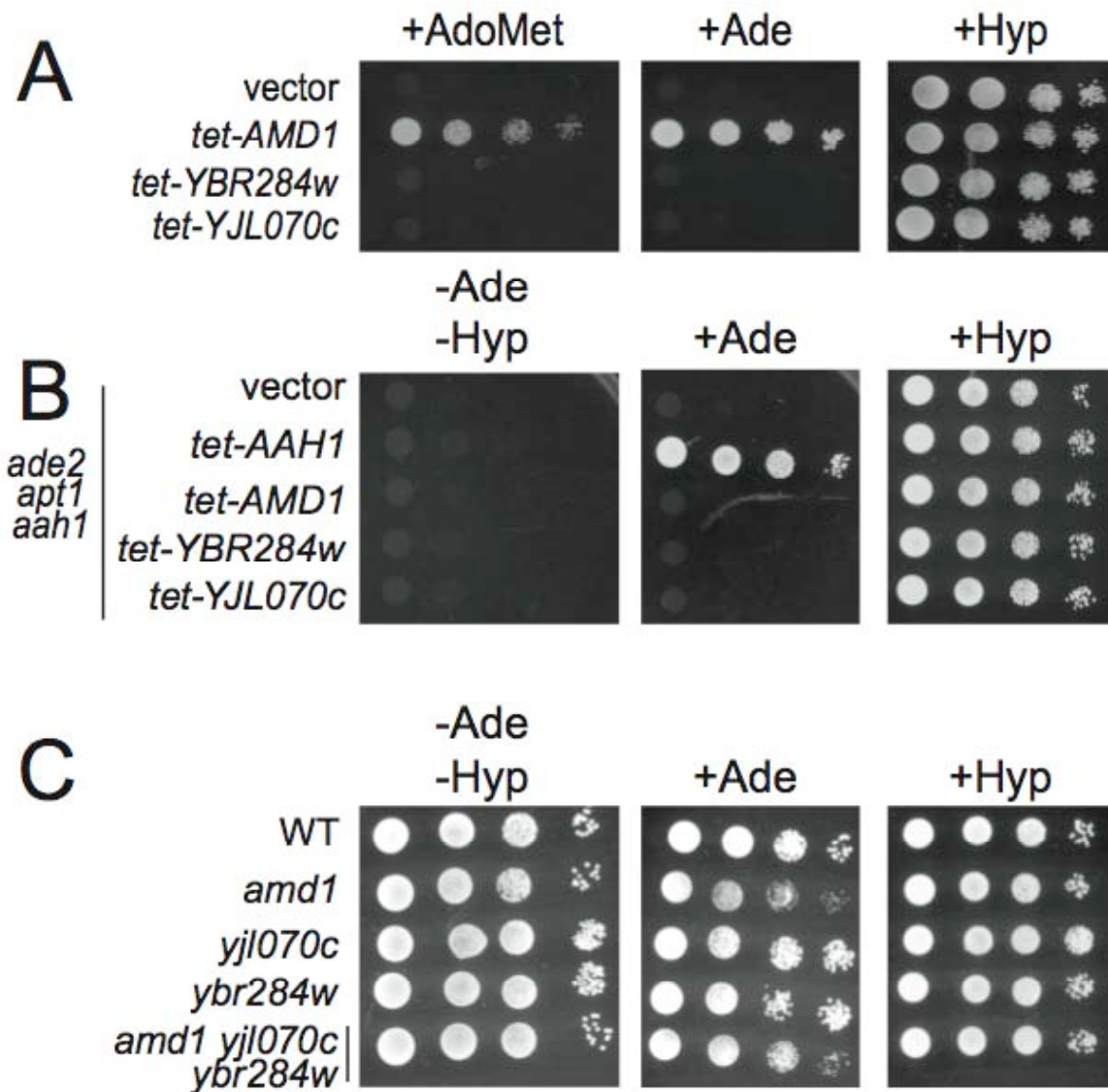
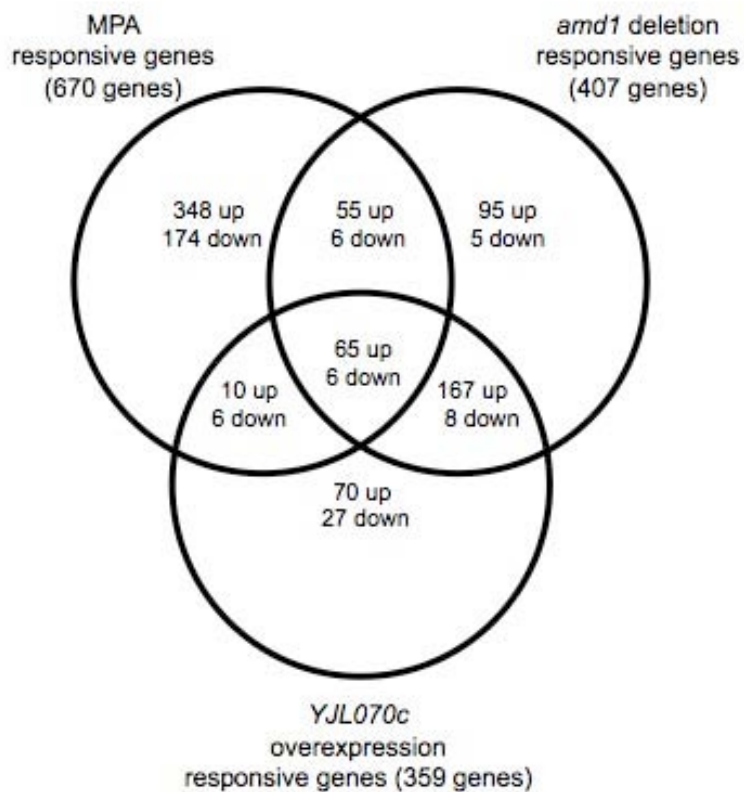


FIGURE S2.—Overexpression of *YBR284w* and *YJL070c* cannot bypass the lack of AMP-, adenosine-, or adenine-deaminase activities *in vivo*. (A) Overexpression of *YJL070c* or *YBR284w* does not allow utilization of S-adenosyl- methionine (AdoMet) or adenine as a purine source in the quadruple *amd1 aah1 ade8 his1*. To be utilized as a purine source, AdoMet requires adenosine deaminase (question mark in Figure 1) or adenosine kinase (ADO1 figure 1) + AMP deaminase activities. Clearly, overexpression of *YJL070c* or *YBR284w* does not provide activities required for AdoMet utilization, while *AMD1* does. (B) Overexpression of *YJL070c* or *YBR284w* does not allow utilization of adenine by an *aah1* mutant lacking adenine deaminase activity. (C) Growth of wild-type, *amd1*, *yjl070c* and *ybr284w* mutant strains in the presence of external purine. Serial dilutions of yeast cells in exponential growth-phase were spotted on SD casaW medium containing uracil and supplemented or not with adenine (+Ade) or hypoxanthine (+Hyp).

A



B

AAD10; ADE3; ARG1; ARG3; ARG4; ARG5,6; ARG8; ARO9; ATF2; BNA1; BOP2; BSC5; CLG1; CPA1; CPA2; DDR48; DLD3; ECM13; ECM17; ECM40; GCV2; GGC1; HIS4; HIS5; HIS7; HO; HOM3; HSP78; IDH2; ILV2; IMD1; IMD2; INO1; KRS1; LYS1; LYS20; MET1; MET10; MET13; MET16; MET17; MET2; MET28; MF(ALPHA)2; NCE103; PCL5; PTR2; PYC1; PYC2; Q0017; RIB3; SAM2; SER3; SNO1; SNX41; SNZ1; STE2; THI20; THI7; TMT1; URA10; VHT1; YAR075W; YAT2; YDL025C; YGL059W; YGL117W; YGR110W; YJL213W; YMR173W-A; YOR302W

FIGURE S3.—(A) Distribution of differentially expressed genes (by a factor 2 or more) in wild-type cells compared to wild-type cells treated with MPA 0.1 mg/l for 60 min (left), to *amd1* deleted mutant (right) or to *YJL070c* overexpression (bottom). (B) List of genes for which expression is up- or down-regulated in all three conditions cited in (A). Gene names in bold correspond to down-regulated genes and gray boxed genes correspond to genes regulated by the Gcn4p transcription factor.

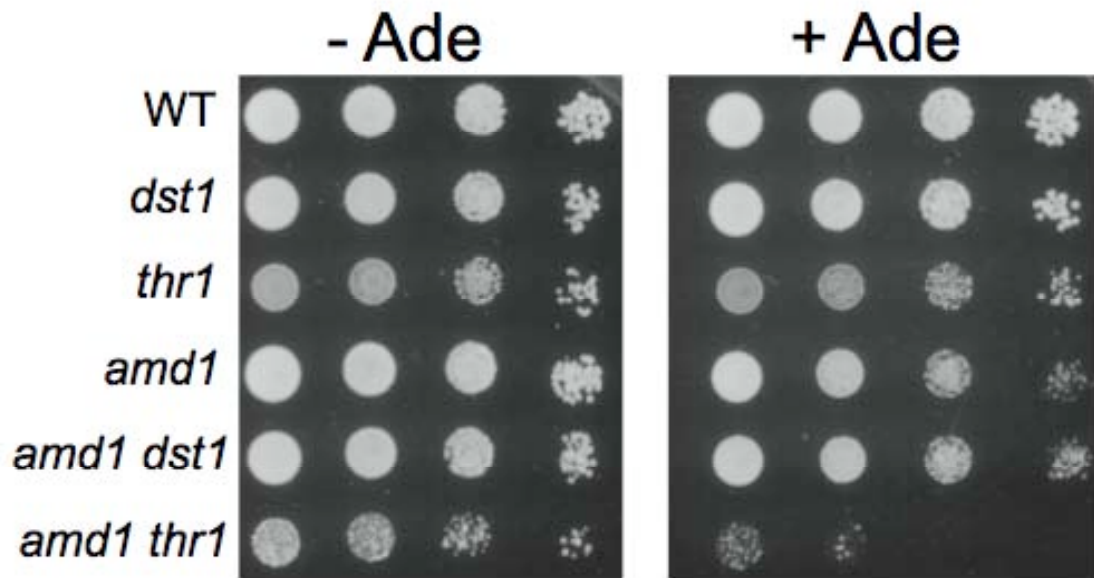


FIGURE S4.—Growth of various mutant strains in the presence or absence of adenine.

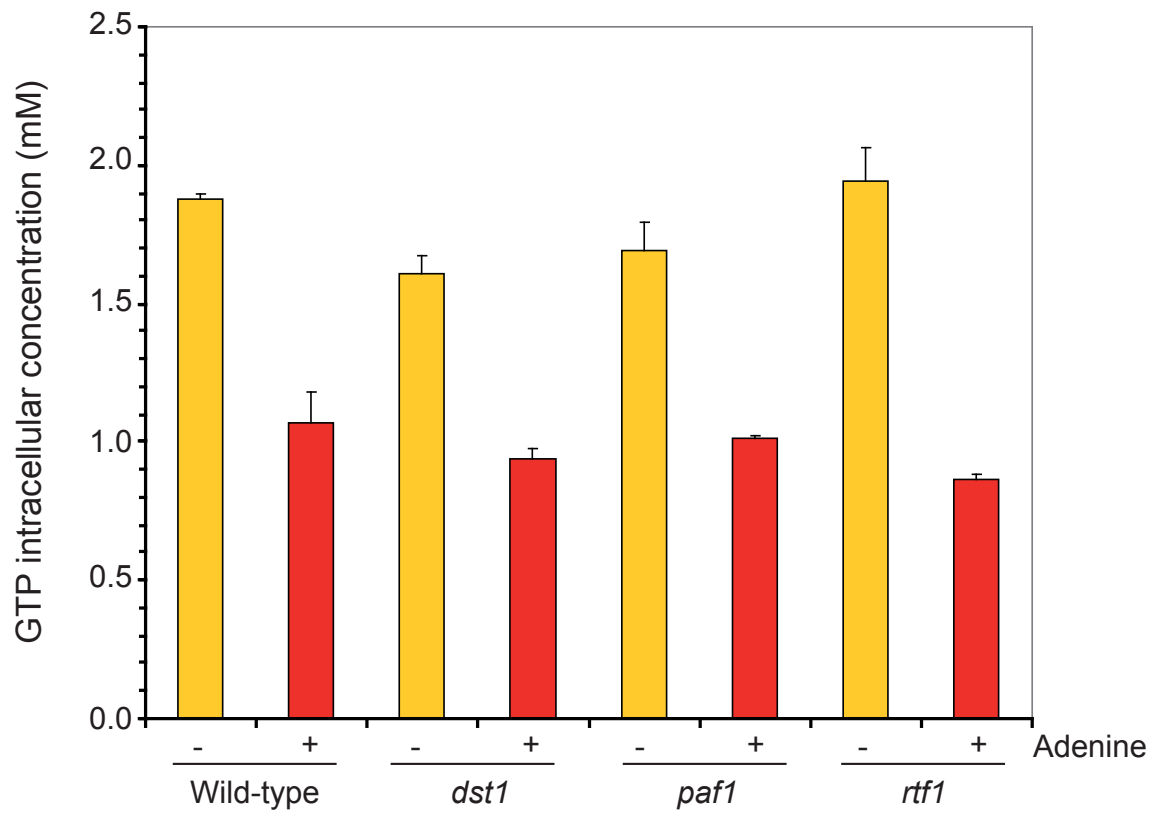


FIGURE S5.—GTP Intracellular concentration in wild-type and mutant strains. Cells were transformed with the plasmid allowing *17L070c* overexpression. Transformants were grown in SD_{caseW} medium supplemented (+) or not (-) with external adenine and intracellular GTP content was measured as previously described (BRETON *et al.* 2008).

TABLE S1

Table S1 is available for download as an Excel file at <http://www.genetics.org/cgi/content/full/genetics.109.105858/DC1>.

All results presented in this file correspond to genes up-regulated (Ratio > 2) or down-regulated (Ratio < 0,5) by at least a factor 2. For all experiments, the entire data sheets are available at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=xvkthqaquiemkji&acc=GSE9557>

Legend of column titles presented in tables of the different data sheets is:

Ratio R = Cy5/Cy3

Mnorm is the log base 2 of the ratio : $M = \log_2(\text{Cy5}/\text{Cy3})$

SD Mnorm is the variation

A is the average of the log base 2 of the intensities : $A = (\log_2(\text{Cy3 intensity}) + \log_2(\text{Cy5 intensity})) / 2$