Supporting figures and tables

Mutations in a single signaling pathway allow cell growth in heavy water

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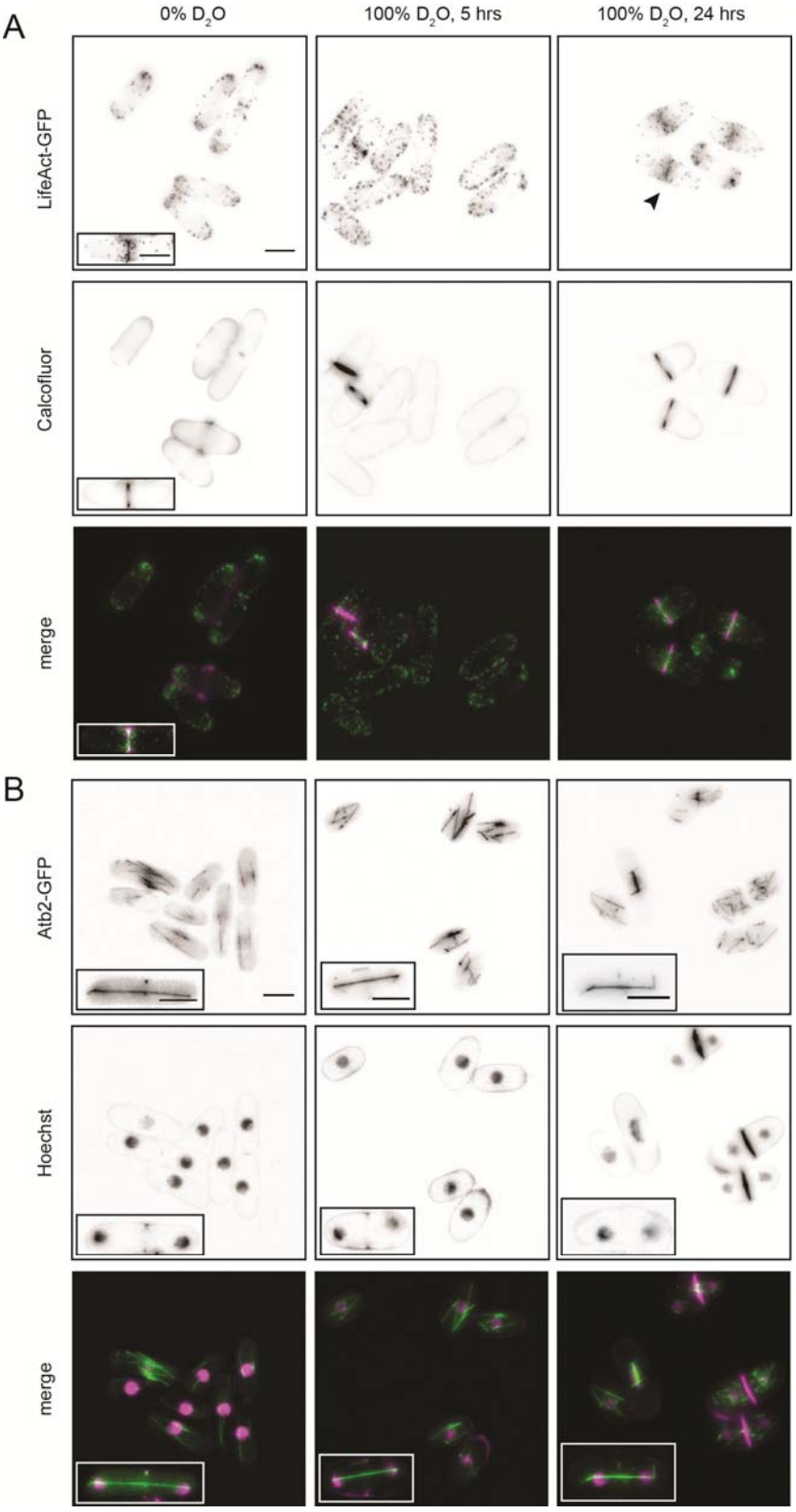
A
0% D2O
100% D2O, 5 hrs
100% D2O, 24 hrs
LifeAct-GFP
Calcofluor
merge
B
Atb2-GFP
Hoechst
merge

Figure S1. *Effects of D_2O on the cytoskeleton.* (A) Wild type cells carrying the LifeAct-GFP actin marker cultured in 0% and 100% D_2O EMM medium for the indicated times were stained with calcofluor (to mark the septa) and analyzed by microscopy. Quantification revealed that the uniform distribution of actin patches was evident in 92±3 (standard deviation) % and 96±4 % of the cells at 5 and 24 hours in D_2O, respectively, while this was only observed in 11±2 % of the cells in H_2O (n=3). Note that the contractile ring appears normal (insert and arrow head). Bar = 5 μm. (B) Wild type cells carrying GFP-tagged α-tubulin Atb2 cultured in 0% and 100% D_2O EMM medium for the indicated times were stained with Hoechst (to mark the nucleus) and analyzed by microscopy. The shorter and/or non-parallel interphase microtubules were observed in at least 30% of the cells at both 5 and 24 hours in D_2O (n=3). In some cells Hoechst also stained the septa. Note that the mitotic spindle appears normal (inserts). Bar = 5 μm.

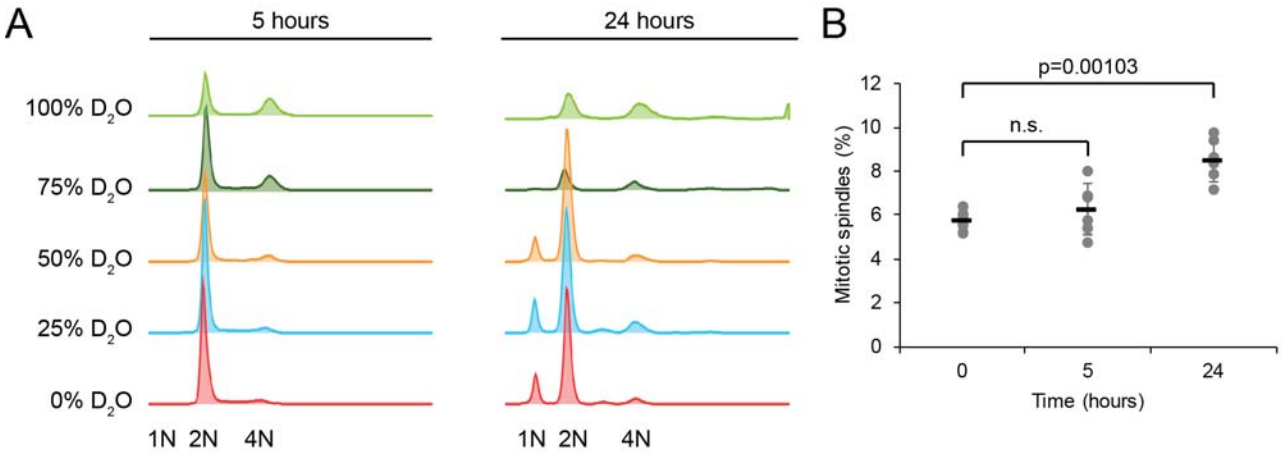

Figure S2. *The effect of D_2O on mitosis.* (A) DNA content analyses. Wild type cells grown at the indicated concentrations of D_2O were DNA stained and analyzed by automated fluorescence microscopy. The positions of the 1N and 2N DNA content peaks are marked. (B) The percentage of Atb2-GFP cells in displaying spindles was determined by fluorescence microscopy after 0, 5 and 24 hours in 100% D_2O by counting cells. The error bars indicate the standard deviation (n=6). Student's t-test p-value is shown; n.s.. not significant.

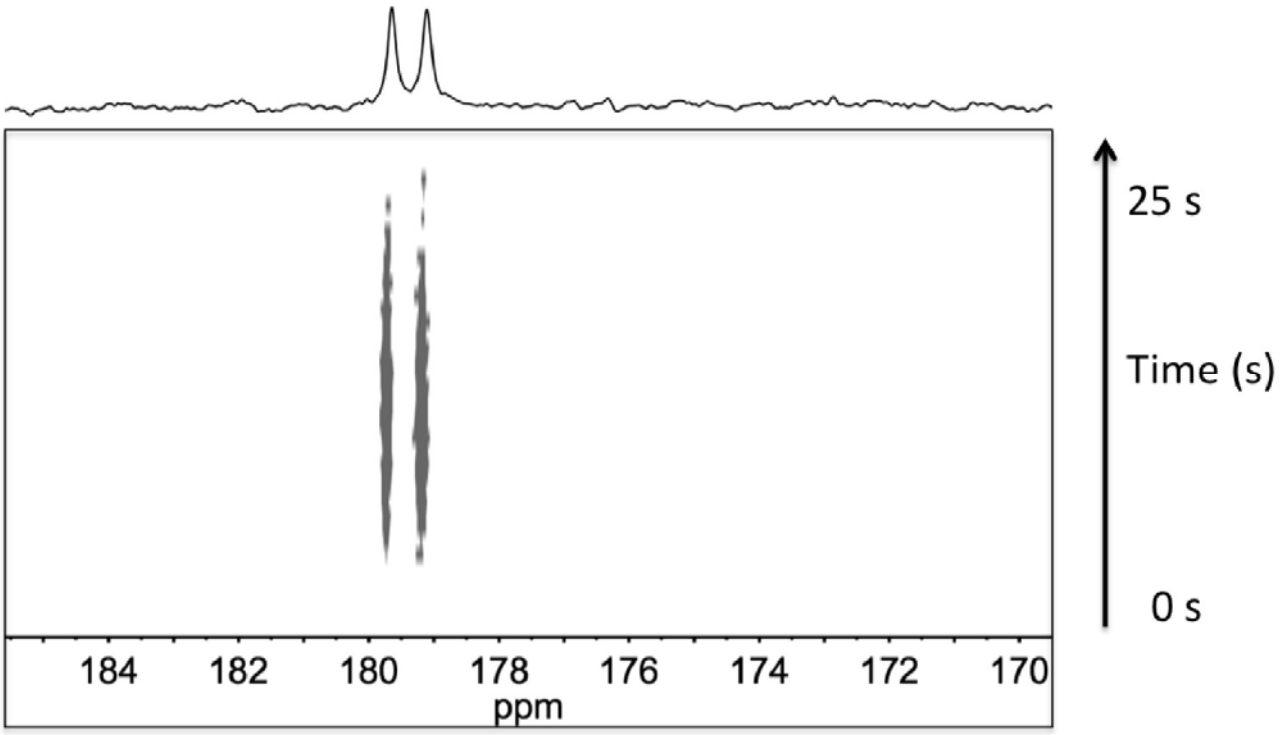

Figure S3. *Hyperpolarized time-resolved ^{13}C-NMR spectra of 1-^{13}C-gluconate*. A hyperpolarized NMR signal is seen developing over 25 seconds at 179.4 ppm. In the top panel a sum of 50 spectra in the dynamic series is shown. Since 1-^{13}C-gluconate originates from uniformly ^{13}C labelled glucose the signal is split due to J-coupling with neighbouring ^{13}C-enriched carbon atom.

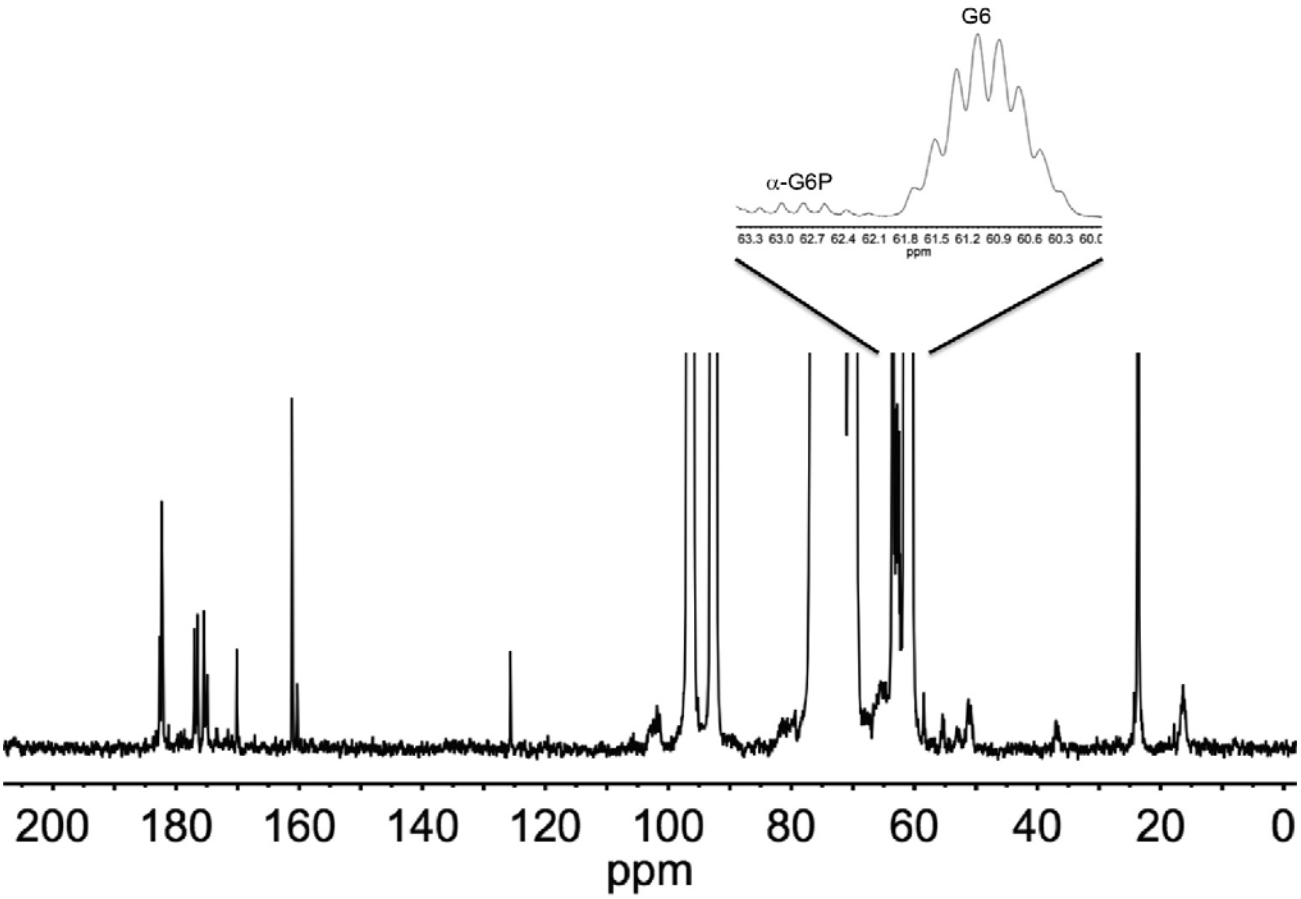

Figure S4. *Single ^{13}C NMR spectrum of cellular metabolite extract following incubation with isotope labelled glucose for 2 minutes in D_2O buffer*. The insert shows a zoom-in on 6-^{13}C-glucose (G6) and the α-form of glucose-6-phosphate (α-G6P).

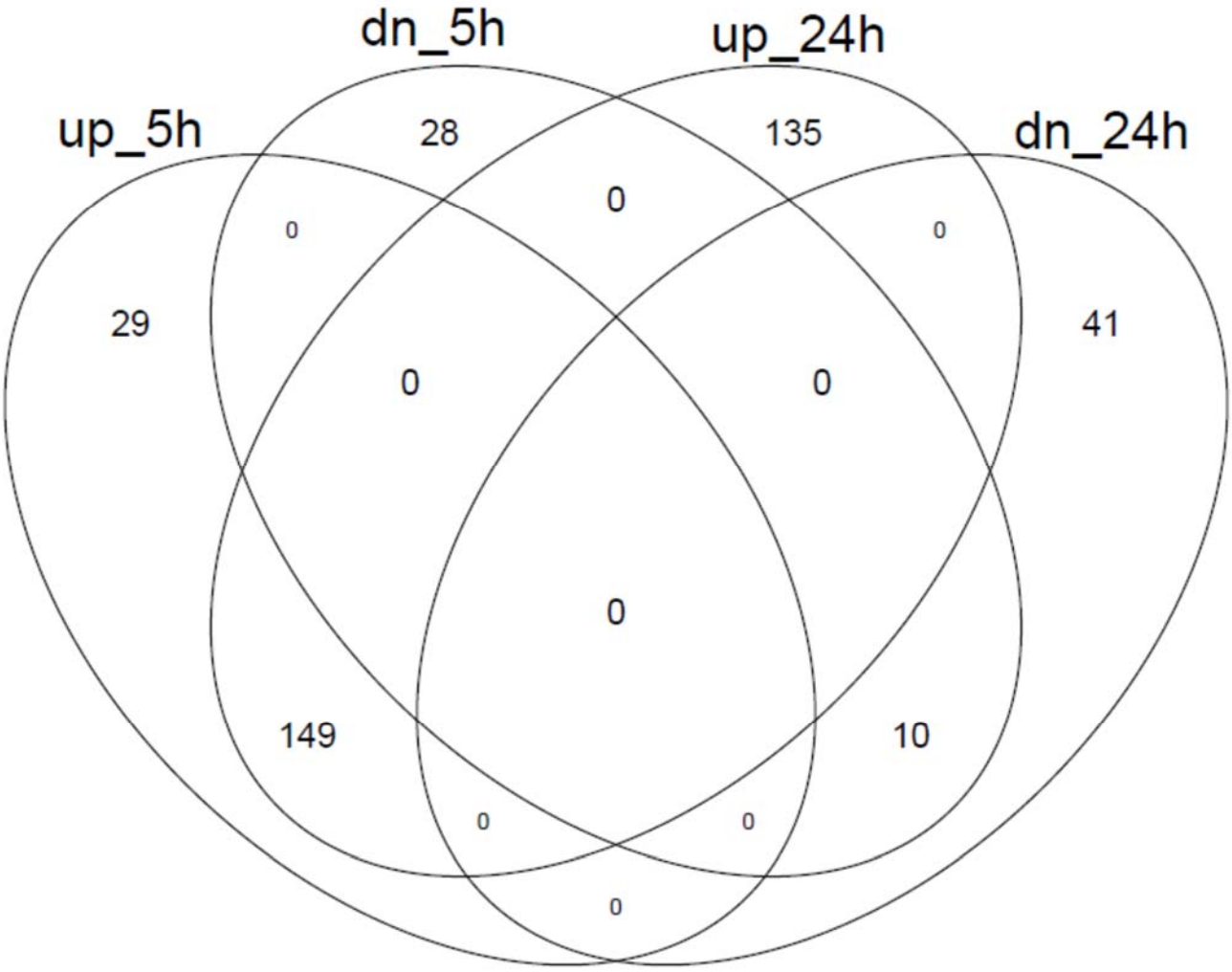

Figure S5 *Differentially expressed genes in response to D_2O.* Venn diagram displaying the number of differentially upregulated (up) genes and downregulated (dn) genes after 5 and 24 hours in response to 100% D_2O. Note that several appeared differentially expressed at both time points.

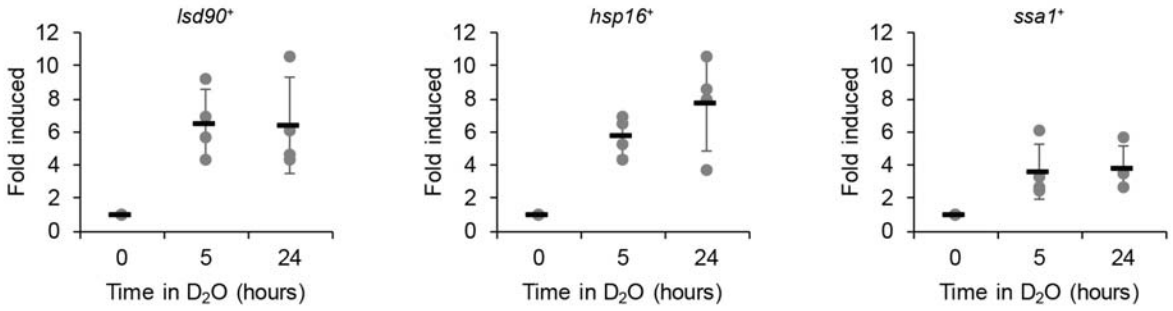

Figure S6 *Real-time PCR on selected differentially expressed genes*. The amounts of *lsd90*$^{+}$, *hsp16*$^{+}$, and *ssa1*$^{+}$ mRNA were compared by qPCR between cells cultured with 100% D_2O for 0, 5, or 24 hours. Error bars indicate the standard deviation (n=4).

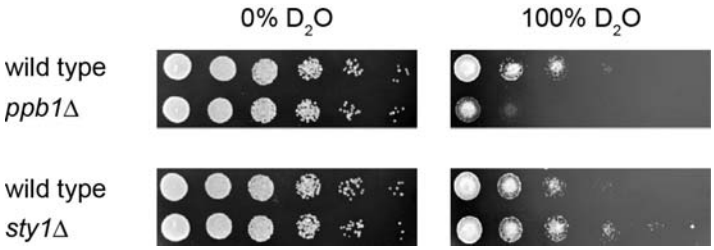

Figure S7 *Growth assays for ppb1 and sty1 mutants*. The growth at 30℃ of the indicated strains was compared on rich media prepared with D_2O or H_2O.

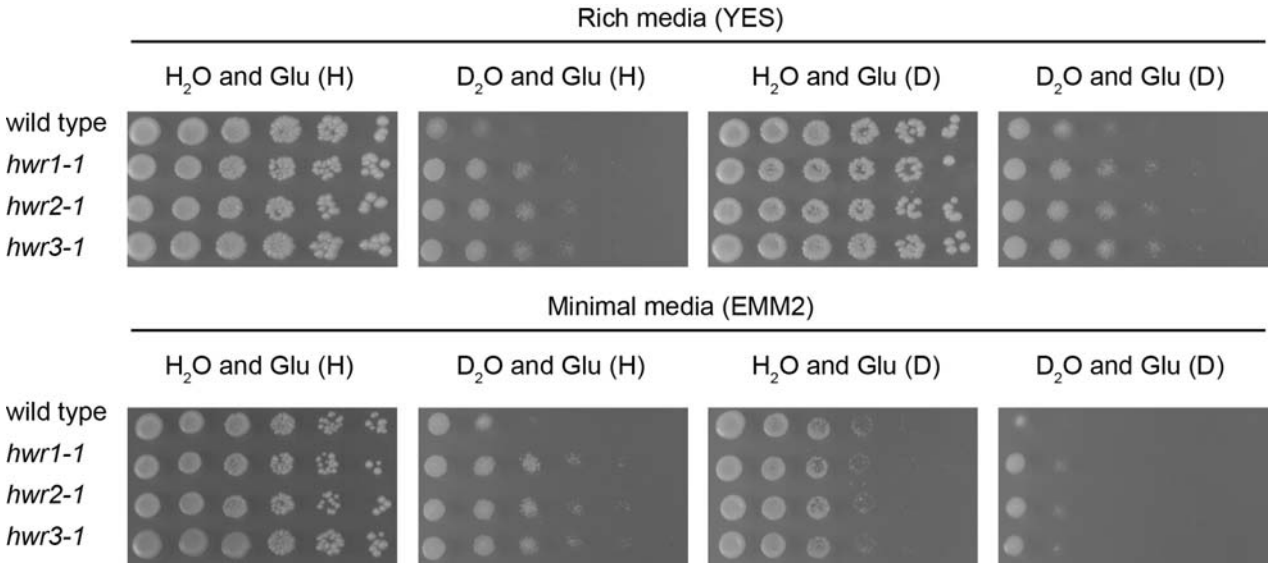

Figure S8. *Growth assays of hwr strains on D_2O and deuterated glucose*. The growth of the indicated strains was compared on media prepared with D_2O or H_2O and with either hydrogenated glucose (Glu (H)) or deuterated glucose (Glu (D)) by serial diluting and spotting onto solid rich (YES) media or minimal media (EMM2) agar plates at 30℃.

```
pek1            CTCGCTGGCACATTCACTGGAACTTCGTATTACATGGCGCCTGAACGAATTTCT 756
wt PCR          CTCGCTGGCACATTCACTGGAACTTCGTATTACATGGCGCCTGAACGAATTTCT
hwr1-1 PCR      CTCGCTGGCACATTCACTGGAACTTCGTATTACATGGCGCCTGAACGAATTTCT

pek1            GGGGGATCTTATACTATATCGTCAGATATATGGTCTTTGGGTTTAACATTGATG 810
wt PCR          GGGGGATCTTATACTATATCGTCAGATATATGGTCTTTGGGTTTAACATTGATG
hwr1-1 PCR      GGGGGATCTTATACTATATCGTCAGATATATGGTCTTTGGGTTTAACATTGATG

pek1            GAGGTCGCATTAAACCGGTTTCCATTCCCTCCCGAGGGTAGCCCCCCACCAATG 864
wt PCR          GAGGTCGCATTAAACCGGTTTCCATTCCCTCCCGAGGGTAGCCCCCCACCAATG
hwr1-1 PCR      GAGGTCGCATTAAACCGGTTTCCATTCCCTCCCGAGGGTAGCCCCCCACCAATG

pek1            CCTATTGAATTGCTCTCGTATATAATAAATATGCCACCTCCCCTTTTACCTCAA 918
wt PCR          CCTATTGAATTGCTCTCGTATATAATAAATATGCCACCTCCCCTTTTACCTCAA
hwr1-1 PCR      CCTATTGAATTGCTCTCGTATATAATAAATATGCCACCTCCCCTTTTACCTCAA

pek1            GAACCCGGTATTAAATGGTCGAAATCCTTTCAACATTTTCTATGCGTGTGTCTG 972
wt PCR          GAACCCGGTATTAAATGGTCGAAATCCTTTCAACATTTTCTATGCGTGTGTCTG
hwr1-1 PCR      GAACCCGGTATTAAATGGTCGAAATCCTTTCAACATTTTCTATGCGTGTGTCTG

pek1            GATAAAGACAAAACTCGTCGTCCTGGACCCCAAAAAATGCTTACCCATCCTTGG 1026
wt PCR          GATAAAGACAAAACTCGTCGTCCTGGACCCCAAAAAATGCTTACCCATCCTTGG
hwr1-1 PCR      GATAAAGACAAAACTCGTCGTCCTGGACCCCAAAAAATGCTT-CCCATCCTTGG

pek1            GTTAAAGCGTTTGAGAGAATTCATGTGGACATGGAAGAGTTCCTTCGTCAAGTC 1080
wt PCR          GTTAAAGCGTTTGAGAGAATTCATGTGGACATGGAAGAGTTCCTTCGTCAAGTC
hwr1-1 PCR      GTTAAAGCGTTTGAGAGAATTCATGTGGACATGGAAGAGTTCCTTCGTCAAGTC
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Figure S9. *PCR sequence confirmation of whole genome sequencing on hwr1-1.* Multiple sequence alignment of a segment of the *pek1* gene, and the PCR sequencing results on a wild type (*wt*) strain and the *hwr1-1* strain. Mutation sites are marked in red.

```
mkh1            CAAGGATATAGTGCTAAGGTCGACGTCTGGTCCTTGGGATGTGTAGTGTTGGAA 3078
wt PCR          CAAGGATATAGTGCTAAGGTCGACGTCTGGTCCTTGGGATGTGTAGTGTTGGAA
hwr2-1 PCR      CAAGGATATAGTGCTAAGGTCGACGTCTGGTCCTTGGGATGTGTAGTGTTGGAA

mkh1            ATGTTAGCTGGTCGTAGACCGTGGTCTACAGATGAGGCTATCCAAGCTATGTTC 3132
wt PCR          ATGTTAGCTGGTCGTAGACCGTGGTCTACAGATGAGGCTATCCAAGCTATGTTC
hwr2-1 PCR      ATGTTAGCTGGTCGTAGACCGTGGTCTACAGATGAGGCTATCCAAGCTATGTTC

mkh1            AAG----------TTAGGTACCGAGAAAAAGGCGCCTCCTATTCCTAGTGAAT 3186
wt PCR          AAG----------TTAGGTACCGAGAAAAAGGCGCCTCCTATTCCTAGTGAAT
hwr2-1 PCR      AAGCTATGTTCAAGTTAGGTACCGAGAAAAAGGCGCCTCCTATTCCTAGTGAAT

mkh1            TGGTGTCTCAGGTATCACCCGAAGCGATTCAATTTTTGAATGCATGCTTTACTG 3240
wt PCR          TGGTGTCTCAGGTATCACCCGAAGCGATTCAATTTTTGAATGCATGCTTTACTG
hwr2-1 PCR      TGGTGTCTCAGGTATCACCCGAAGCGATTCAATTTTTGAATGCATGCTTTACTG

mkh1            TGAATGCTGATGTAAGGCCAACCGCAGAGGAATTATTAAATCACCCGTTTATGA 3294
wt PCR          TGAATGCTGATGTAAGGCCAACCGCAGAGGAATTATTAAATCACCCGTTTATGA
hwr2-1 PCR      TGAATGCTGATGTAAGGCCAACCGCAGAGGAATTATTAAATCACCCGTTTATGA
```

Figure S10. *PCR sequence confirmation of whole genome sequencing on hwr2-1.* Multiple sequence alignment of a segment of the *mkh1* gene, and the PCR sequencing results on a wild type (*wt*) strain and the *hwr2-1* strain. Mutation sites are marked in red.

```
pck2            CCTGAATTTATGGCACCGGAAATCTTATTAGAACAGCAATATACGAGAAGCGTT 2592
wt PCR          CCTGAATTTATGGCACCGGAAATCTTATTAGAACAGCAATATACGAGAAGCGTT
hwr3-1 PCR      CCTGAATTTATGGCACCGGAAATCTTATTAGAACAGCAATATACGAGAAGCGTT

pck2            GACTGGTGGGCTTTTGGTGTACTAATTTACCAAATGCTGCTTGGTCAATCTCCA 2646
wt PCR          GACTGGTGGGCTTTTGGTGTACTAATTTACCAAATGCTGCTTGGTCAATCTCCA
hwr3-1 PCR      GACTGGTGGGCTTTTAGTGTACTAATTTACCAAATGCTGCTTGGTCAATCTCCA

pck2            TTTAGAGGAGAAGACGAAGAAGAAATTTTTGATGCAATTTTATCTGATGAACCT 2700
wt PCR          TTTAGAGGAGAAGACGAAGAAGAAATTTTTGATGCAATTTTATCTGATGAACCT
hwr3-1 PCR      TTTAGAGGAGAAGACGAAGAAGAAATTTTTGATGCAATTTTATCTGATGAACCT

pck2            TTGTATCCTATTCATATGCCAAGGGATTCCGTTTCTATATTACAACAACTTTTG 2754
wt PCR          TTGTATCCTATTCATATGCCAAGGGATTCCGTTTCTATATTACAACAACTTTTG
hwr3-1 PCR      TTGTATCCTATTCATATGCCAAGGGATTCCGTTTCTATATTACAACAACTTTTG

pck2            ACTCGCGATCCTAAAAAAACGACTTGGATCTGGCCCTAACGATGCAGAAGATGTC 2808
wt PCR          ACTCGCGATCCTAAAAAAACGACTTGGATCTGGCCCTAACGATGCAGAAGATGTC
hwr3-1 PCR      ACTCGCGATCCTAAAAAAACGACTTGGATCTGGCCCTAACGATGCAGAAGATGTC
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Figure S11. *PCR sequence confirmation of whole genome sequencing on hwr3-1.* Multiple sequence alignment of a segment of the *pck2* gene, and the PCR sequencing results on a wild type (*wt*) strain and the *hwr3-1* strain. Mutation sites are marked in red.

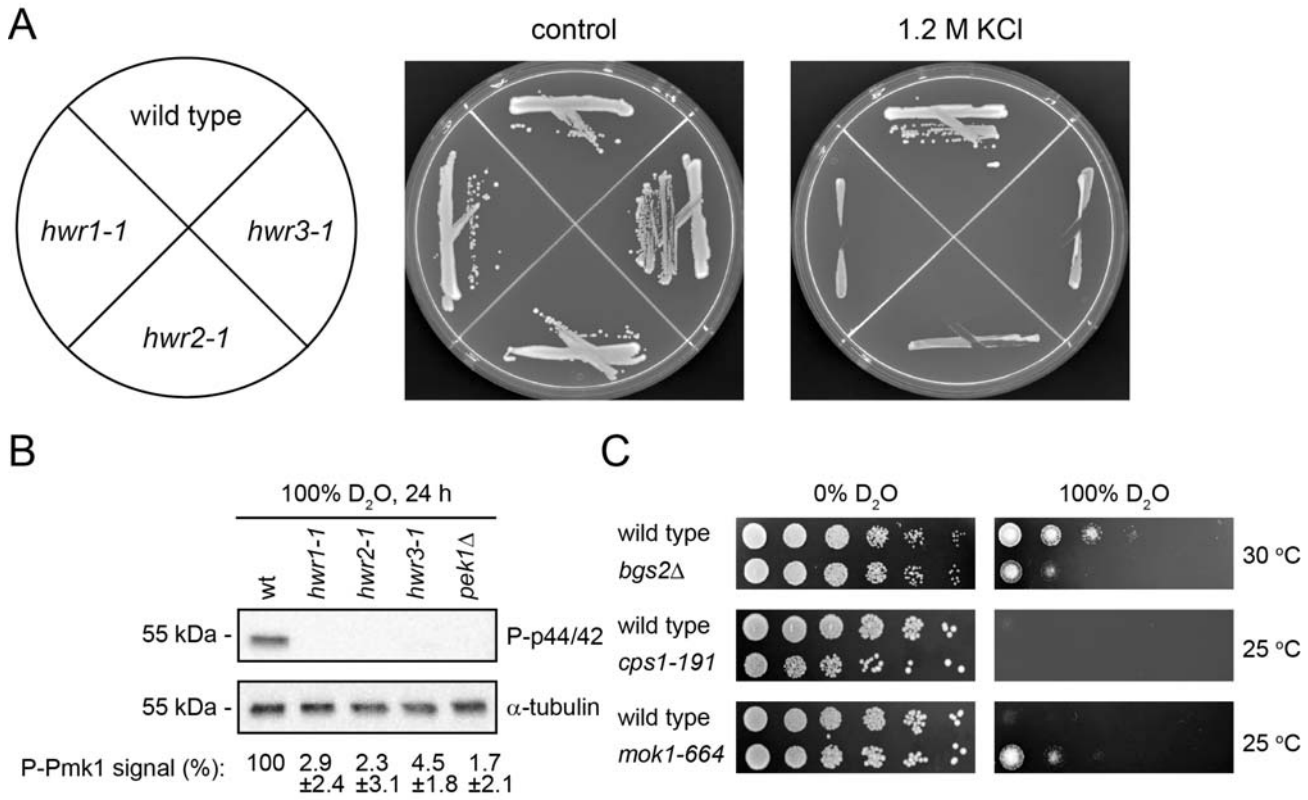

Figure S12. *Additional experiments on the hwr strains.* (A) The indicated strains were streaked on rich solid YES media (control) or YES with 1.2 M KCl and incubated for 48 hours at 30 °C. (B) Whole cell lysates of the indicated strains cultured for 24 hours in D_2O medium were analyzed by SDS-PAGE and Western blotting using antibodies to phosphorylated Pmk1 (P-p44/42). Blotting for α-tubulin served as a loading control. Note that the level of phosphorylated Pmk1 is decreased (% signal ± standard deviation, n=3) in the mutant strains. (C) The growth of the indicated glucan-synthase mutants was compared by serial diluting and spotting onto solid rich (YES) media prepared with D_2O or H_2O. Note for the temperature sensitive *cps1-191* (*bgs1*) and *mok1-664* (*ags1*) strains the experiment was performed at 25 °C.

Table S1

Differentially expressed tRNAs after 24 hours in D_2O

Systematic ID	tRNA	Expression change (fold)	FDR*
SPATRNASER.01	Ser, cytosolic	0.14	0.0087
SPATRNASER.03	Ser, cytosolic	0.16	0.0087
SPATRNAMET.01	Met, cytosolic	0.16	0.0087
SPMITTRNALEU.01	Leu, mitochondrial	0.17	0.0087
SPMITTRNAPHE.01	Phe, mitochondrial	0.18	0.0087
SPMITTRNAVAL.01	Val, mitochondrial	0.18	0.0087
SPMITTRNAARG.02	Arg, mitochondrial	0.18	0.0087
SPMITTRNAGLY.01	Gly, mitochondrial	0.19	0.0087
SPMITTRNAMET.02	Met, mitochondrial	0.20	0.0087
SPMITTRNATYR.01	Tyr, mitochondrial	0.20	0.0087
SPMITTRNAILE.02	Ile, mitochondrial	0.21	0.0087
SPCTRNAMET.07	Met, cytosolic	0.21	0.0087
SPMITTRNAHIS.01	His, mitochondrial	0.22	0.0087
SPMITTRNAPRO.01	Pro, mitochondrial	0.23	0.0087
SPMITTRNASER.02	Ser, mitochondrial	0.23	0.0087
SPATRNAMET.03	Met, cytosolic	0.24	0.0087
SPMITTRNAASP.01	Asp, mitochondrial	0.24	0.0087
SPMITTRNALEU.02	Leu, mitochondrial	0.25	0.0087
SPBTRNAMET.05	Met, cytosolic	0.25	0.0087
SPMITTRNACYS.01	Cys, mitochondrial	0.25	0.0087
SPMITTRNATRP.01	Trp, mitochondrial	0.25	0.0087
SPMITTRNAGLN.01	Gln, mitochondrial	0.26	0.0087
SPATRNATYR.01	Tyr, cytosolic	0.34	0.0203
SPBTRNALYS.07	Lys, cytosolic	0.37	0.0267
SPMITTRNAARG.01	Arg, mitochondrial	0.39	0.0087
SPATRNAPRO.02	Pro, cytosolic	0.40	0.0092
SPMITTRNAILE.01	Ile, mitochondrial	0.42	0.0088
SPMITTRNAALA.01	Ala, mitochondrial	0.43	0.0087
SPATRNALEU.01	Leu, cytosolic	0.44	0.0393
SPATRNALEU.03	Leu, cytosolic	5.28	0.0087

*False discovery rate.

Table S2

Gene deletions scored as D_2O hypersensitive in high-throughput screen

Systematic ID	Gene name	Function
SPAC13G7.03	*upf3*	up-frameshift suppressor 3 family protein
SPBC1539.08	*arf6*	ADP-ribosylation factor, Arf family Arf6
SPAC2F3.02	-	ER protein translocation subcomplex subunit
SPAC328.01c	*msn5*	karyopherin/importin beta family nuclear import/export signal receptor
SPAPB2B4.02	*grx5*	mitochondrial [2Fe-2S] cluster assembly & transfer glutaredoxin Grx5
SPAC4D7.03	*pop2*	F-box/WD repeat protein Pop2
SPCC970.10c	*brl2*	ubiquitin-protein ligase E3 Brl2
SPBC18H10.06c	*swd2*	Set1C complex subunit
SPBC19C7.02	*ubr1*	N-end-recognizing protein E3 Ubr1
SPAC17H9.10c	*ddb1*	Cul4-RING E3 adaptor Ddb1
SPAC26H5.05	*mga2*	IPT/TIG ankyrin repeat transcription regulator of fatty acid synthesis
SPBC106.10	*pka1*	cAMP-dependent protein kinase catalytic subunit Pka1
SPAC6F6.01	*cch1*	plasma membrane calcium ion import channel Cch1
SPAC3H8.02	*csr102*	Sec14 family, phospholipid-intermembrane transfer protein Csr102
SPBC13G1.08c	*ash2*	Ash2-trithorax family protein
SPCP1E11.06	*apl4*	AP-1 adaptor complex gamma subunit Apl4
SPAC10F6.13c	*caa1*	cytoplasmic aspartate aminotransferase Caa1
SPBC23G7.08c	*rga7*	RhoGAP, GTPase activating protein Rga7
SPBC27B12.08	*sip1*	Pof6 interacting protein Sip1, AP-1 accessory protein
SPBC21C3.20c	*git1*	C2 domain protein Git1
SPAC806.07	*ndk1*	nucleoside diphosphate kinase Ndk1
SPAC644.06c	*cdr1*	NIM1 family serine/threonine protein kinase Cdr1/Nim1
SPBC16E9.13	*ksp1*	serine/threonine protein kinase Ksp1
SPCC1739.14	*npp106*	nucleoporin Npp106
SPAC7D4.06c	*alg3*	dolichol-P-Man-dependent alpha(1-3) mannosyltransferase Alg3
SPBC3B9.11c	*ctf1*	mRNA cleavage and polyadenylation specificity factor subunit Ctf1
SPAC2F7.07c	*cph2*	Clr6 histone deacetylase associated PHD protein Cph2
SPBC16E9.08	*mcp4*	prospore membrane protein Mcp4/Mug101
SPAC926.03	*rlc1*	myosin II regulatory light chain Rlc1
SPBC13G1.03c	*pex14*	peroxisomal docking protein Pex14
SPBC776.15c	*kdg2*	dihydrolipoamide S-succinyltransferase Kdg2
SPAC513.03	*mfm2*	M-factor precursor Mfm2
SPAC26F1.04c	*etr1**	enoyl-[acyl-carrier protein] reductase
SPBP8B7.11	*nxt3*	ubiquitin protease cofactor Nxt3
SPAC12B10.12c	*rhp41*	DNA repair protein Rhp41
SPCC777.10c	*ubc12*	NEDD8-conjugating enzyme Ubc12
SPAC4H3.13	*pcc1*	EKC/KEOPS complex subunit Pcc1
SPAC5D6.05	*med18*	mediator complex subunit Med18
SPCC970.06	*erv29*	COP II adaptor Erv29

*: Upregulated in RNA seq. at 24 hours in D_2O.

Table S3

Amino acid changes in affected kinases in the hwr strains

Mutant	Protein	aa change
hwr1-1	Pek1	L338LPILGLKRLREFMWTWKSSFVKSGLIRRNSVICstop
hwr2-1	Mkh1	L1046LCSSstop
hwr3-1	Pck2	G870S