

## Files S1-S2

Available for download at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156984/-/DC1>

### File S1

#### Supplemental Dataset 1

**Sheet 1:** Genes identified that are regulated by PP-1 either positively (at least 1.5-fold down in  $\Delta pp-1$ ) or negatively (at least 1.5-fold up in  $\Delta pp-1$ ) using RNAseq analysis of cells grown for five hours

**Sheet 2:** Functional categories present among the genes that are positively or negatively regulated by PP-1 as given in Sheet 1 (categories overrepresented,  $p < 0.05$ , are given in bold)

### File S2

#### Supplemental Dataset 2

**Sheet 1:** Genes identified that are regulated by MAK-2 either positively (down in  $\Delta mak-2q100G$ ) or negatively (up in  $\Delta mak-2q100G$ ) after addition of 1NM-PP1 compared to treatment with DMSO alone

**Sheet 2:** Functional categories present among the genes that are positively or negatively regulated by MAK-2 as given in sheet 1 (categories overrepresented,  $p < 0.05$ , are given in bold)

**Table S1 Primers Used In This Study**

Primer Name	Sequence	Primer Name	Sequence
Pp-1-Paci-R06	CTTTAATTAATACTAGCTCGTTTCGC A	NCU00995R2	GCGGATAACAATTTACACAGGAAACAG CACGACCATCGAATTCCTACC
Pp-1-Spei-F06	CGACTAAGTATGTATTCTTCGCAGCATGC	NCU01121F1	GTAACGCCAGGGTTTTCCAGTCACGAC GGAAAGACTCTCCAGATCAGC
Pp-1p-Noti-F06	GCGGCCGCGACCATAGTGGTGTAGACTA CT	NCU01121R1	ATCCACTTAACGTTACTGAAATCTCCAAC GGTATTGTTGGGAGTACTGG
Pp-1p-Spei-R06	CGACTAGTTCCAAGCTTAAAAACCTCCTT GATA	NCU01121F2	CTCCTTCAATATCATCTTCTGTCTCCGACC TAGGAAATACGAAGGACGG
Pp-1 Sgra1-F06	TCTACCCACCGCGAGTATG	NCU01121R2	GCGGATAACAATTTACACAGGAAACAG CAGTTACGACGAAACACCCAC
Pp-1-Sfii-R06	AAAGAGGACGAGTTGGCCTGCTGGTGG GCCTTG	NCU01380F1	GTAACGCCAGGGTTTTCCAGTCACGAC GATATGCGGAGGCACTTCAAG
Pp1-Smtrpr06	CGCTGTAACAGAAGAACACCTT	NCU01380R1	ATCCACTTAACGTTACTGAAATCTCCAAC TTGTGCTGAGGCTATCCGTT
Pp1-Smtrp-F06	AAGGTGTTCTTCTGTTACAGCG	NCU01380F2	CTCCTTCAATATCATCTTCTGTCTCCGACA CCCTGAGCTTTATGTGACC
Pp-1-Smc-F06	TACGTCACTCATAACAGCCAGAAG	NCU01380R2	GCGGATAACAATTTACACAGGAAACAG CAAGCTCTATTGGCATGGAGG
Pp-1-Smc-R06	CTTCTGGCTGTATGAGCTGACGTA	F00811	GCGCGCTTACTATTGAAG
Pp-1-Afiii-F06	CGTTTGGAGCACCTTAAGAG	R00811	GATAGATGGCGCAAAGGATG
Pp-1-Nsii-R06	GTAGATTGGAAGGGTGAATG	F00995	GAGGCAAGACGGTAAGTTGG
Fqpcr00881	TGCTCACATCAACTCC	R00995	TCCGGTTGGTATGTTGGTTT
Rqpcr00881	CCGAGGTTGACGTAGATA	F01121	CAGGGGAAAGCAATCAAGAG
Fqpcr04732	CAAGTATAACTCGGCTG	R01121	TCGTCAATCTCCTCCTCGTT
Rqpcr04732	TAGCACCTCGTGACAATG	F01380	CCATCGTCCAACCTTCTCCTC
Fqpcr02613	GCTGGTTTGATTGGGGTAGA	R01380	TTCATGAGCATGTGTGCTTG
Rqpcr02613	AAGTAGGGGCGGAGTTTGAT	F09693	GCAGTCCAGTGGTGAAGGAT
Fqpcr02246	GCTTGCCTCTCACGACTACC	R09693	CCGTTGACCCAGTAGGTTTG
Rqpcr02246	CGGAGCTAAAGCTCAAGGTG	Hygrupflk	GAATAGAGTAGATGCCGACC
Fqpcrprm1	TCTGCTCCAACAACCTCTTC	Ccg-4_F	CTTCTCCGTGACACCACCTT
Rqpcrprm1	CCGCGATATTCAATGTAGCC	Ccg-4_R	TCATCGGAGTGAGCAGTGAG
NCU09693F1	GTAACGCCAGGGTTTTCCAGTCACGAC GGTGCCTGGTTTTCTCGACTT	Mfa-1_F	CGGTATCTCGCCTCTCAACGT
NCU09693R1	ATCCACTTAACGTTACTGAAATCTCCAAC TCTACGGACATAGTCGAGGT	Mfa-1_R	GGTGCTCCCATTGTGCAGA
NCU09693F2	CTCCTTCAATATCATCTTCTGTCTCCGACT TGGTCGACCACTATTTCCC	Pre-1_F	GAATGCTTCCACGTCCCTA
NCU09693R2	GCGGATAACAATTTACACAGGAAACAG CCGTCATAATGGAAGACGGGA	Pre-1_R	AAGGCGGTAGGATTGTTGTG
NCU00811F1	GTAACGCCAGGGTTTTCCAGTCACGAC GCAGTCTACCAAGGAACGACC	Pre-2_F	CACCAACTTTTCTCCTCCA
NCU00811R1	ATCCACTTAACGTTACTGAAATCTCCAAC CCAAGGAGTGTGGTGTAGC	Pre-2_R	GTCGTCGTCGTGGTGTATG
NCU00811F2	CTCCTTCAATATCATCTTCTGTCTCCGACT ACGGGCTACGTTATCTAGG	04732F	AGATCTAGACATGCCCTTGGTATCAGG A
NCU00811R2	GCGGATAACAATTTACACAGGAAACAG CAGTGCTTTCCTACTACCAGACC	04732R	GGGCCCTCATTCAATTAATTAACACCAA ATCTCCTTCTCACTCC
NCU00995F1	GTAACGCCAGGGTTTTCCAGTCACGAC GTTGGTTCCTAGTACTCCTCG	Fgfp+8xgly	TTTGGATCCCGCGCGGTTGGTGGCGGC GGTGGTATGGTGTGAGCAAGGGCGAG
NCU00995R1	ATCCACTTAACGTTACTGAAATCTCCAAC CTAGTCCGTCACATTGAAGC	Rgfp+8xgly	AAAGGATCCCAACCGCCACCACCCGC CGCCCTTGACAGCTCGTCCATGC
NCU00995F2	CTCCTTCAATATCATCTTCTGTCTCCGACG GGATGGGTTTCGTTGAGATA		

**Table S2 Clusters Of Genes Showing Temporal Expression During 3-8 Hr Time Germination Time Course and Genes Found Uniquely Higher Or Lower Expressed In The 4-6 Hr Loop. Genes ID Numbers In Bold Are Genes Up Regulated In The  $\Delta Pp-1$  Mutant Compared To Wild Type.**

Gene ID	Annotation	Cluster	Gene ID	Annotation	Cluster
<b>NCU00566</b>	Synaptobrevin	1	NCU00601	Nf	2
NCU00881	<i>Ham-7</i>	1	NCU00746	<i>Crp-68</i>	2
<b>NCU00906</b>	Hypothetical	1	NCU01380	Hypothetical	2
<b>NCU01183</b>	<i>Gtp-1</i>	1	NCU01697	Hypothetical	2
<b>NCU01772</b>	DNA-Directed RNA Polymerase III	1	NCU02176	Hypothetical	2
<b>NCU02500</b>	<i>Ccg-4</i>	1	NCU02458	Hypothetical	2
<b>NCU02635</b>	Mannan Polymerase Complexes MNN9 Subunit	1	NCU03196	Cyclin Pch1	2
<b>NCU02644</b>	Hypothetical	1	NCU03868	Hypothetical	2
NCU03276	<i>Bem46</i>	1	NCU04429	Mitochondrial Import Protein Mmp37	2
<b>NCU03669</b>	Adomet-Dependent Rrna Methyltransferase Spb1	1	NCU04729	Hypothetical	2
NCU03708	Hypothetical	1	NCU05178	Hypothetical	2
<b>NCU03794</b>	Periodic Tryptophan Protein 2	1	NCU05357	Predicted	2
<b>NCU03830</b>	Hypothetical	1	NCU06373	Hypothetical	2
<b>NCU03837</b>	Snf1 Kinase Complex Beta-Subunit Gal83	1	<b>NCU08457</b>	Eas	2
NCU03960	Hypothetical	1	NCU08833	Hypothetical	2
NCU04122	Malate Dehydrogenase	1	NCU10052	Hypothetical	2
<b>NCU04344</b>	Eif2b-Alpha	1	<b>NCU00209</b>	Hypothetical	3
<b>NCU04611</b>	Transcription Elongation Factor Spt-6	1	NCU00265	Hypothetical	3
NCU04732	<i>Ham-11</i>	1	<b>NCU00309</b>	WSC Domain-Containing Protein	3
NCU04834	<i>Phy-1</i>	1	<b>NCU01121</b>	Hypothetical	3
<b>NCU05194</b>	DNA Replication Licensing Factor Mcm6	1	NCU01618	Predicted	3
NCU05337	Predicted	1	<b>NCU04047</b>	Hypothetical	3
NCU05400	Hypothetical	1	NCU04349	<i>Stk-58 (CHECK)</i>	3
NCU05814	Hypothetical	1	<b>NCU04452</b>	<i>Mig-3</i>	3
NCU06115	Hypothetical	1	NCU05127	D-Alanyl-Alanine Synthetase A	3
NCU06919	Hypothetical	1	NCU05495	<i>Ccg-16</i>	3
NCU07029	Hypothetical	1	NCU05502	Hypothetical	3
<b>NCU07313</b>	Chromosome Transmission Fidelity Protein 18	1	NCU05831	Hypothetical	3
<b>NCU07503</b>	Hypothetical	1	NCU06514	Hypothetical	3
<b>NCU07528</b>	Trna Pseudouridine Synthase	1	NCU07439	Hypothetical	3
NCU07609	MFS Transporter	1	NCU07802	Hypothetical	3
NCU07610	Taurine Dioxygenase	1	NCU09562	Hypothetical	3
<b>NCU07719</b>	Isopentenyl-Diphosphate Delta-Isomerase	1	NCU09693	Hypothetical	3
NCU08332	<i>Hex-1</i>	1	NCU09802	Hypothetical	3
NCU08656	Hypothetical	1	<b>NCU09894</b>	CCAAT-Box-Binding Transcription Factor	3
<b>NCU08824</b>	Molybdopterin Binding Domain-Containing Protein	1	NCU00811	Hypothetical	4

NCU09427	<i>Gpr-3</i>	1	NCU00995	Hypothetical	4
<b>NCU09498</b>	Hypothetical	1	<b>NCU02361</b>	Formamidase	4
<b>NCU09560</b>	Superoxide Dismutase	1	<b>NCU02727</b>	Glycine Cleavage System T Protein	4
<b>NCU10292</b>	Porphobilinogen Deaminase	1	<b>NCU06360</b>	Histidinol-Phosphate Aminotransferase	4

Genes Uniquely Down Regulated In The 4-6 Hour Loop

Genes Uniquely Up Regulated In The 4-6 Hour Loop

Gene ID	Annotation	Gene ID	Annotation
1nc380_040	Hypothetical	<b>NCU00973</b>	Hypothetical
NCU00354	Hypothetical	<b>NCU01124</b>	Hypothetical
NCU00556	RNA Binding Protein	<b>NCU02729</b>	Transducin Family Protein
NCU01189	Hypothetical	<b>NCU03153</b>	Hypothetical
NCU01191	Hypothetical	<b>NCU03192</b>	Hypothetical
NCU01459	Tan Spore <i>Asl-2</i>	<b>NCU04966</b>	Predicted Protein
NCU01545	<i>Atg-8</i>	<b>NCU09300</b>	RNA Polymerase II CTD Phosphatase Fcp1
NCU02298	Hypothetical	<b>NCU09992</b>	Serine Peptidase
NCU02465	Hypothetical		
NCU02488	Hypothetical		
NCU03013	Anchored Cell Wall Protein-10, <i>Acw-10</i>		
NCU03344	Hypothetical		
NCU04192	Vacuolar Aspartyl Aminopeptidase <i>Lap4</i>		
NCU04278	Hypothetical		
NCU04542	Hypothetical		
NCU05233	P60 Domain-Containing Protein		
NCU05395	Hypothetical		
NCU05408	Predicted Protein		
NCU05593	Topogenesis Of Mitochondrial Outer Membrane Beta Barrel Proteins 55, <i>Tob-55</i>		
NCU05856	Hypothetical		
NCU05959	Vesicle Transport V-SNARE Protein VTI1		
NCU06698	Glycogenin <i>Gnn</i>		
NCU07091	Hypothetical		
NCU07817	Non-Anchored Cell Wall Protein-3, <i>Ncw-3</i>		
NCU08037	Hypothetical		
NCU08257	Hypothetical		

**Table S3** (A) functional categories overrepresented ( $P < 0.05$ ) present among the genes identified that are positively regulated by pp-1 (down in  $\Delta pp-1$ ) in at least one time point during the three to eight or the four to six hour time course. (B) functional categories overrepresented ( $P < 0.05$ ) present among the genes identified that are negatively regulated by pp-1 (up in  $\Delta pp-1$ ) in at least one time point during the three to eight or the four to six hour time course.

A.

Functional category	Abs Set	Rel Set	Genes Set	Abs Genome	Rel Genome	Rel Set/Rel Genome	P-Value
14.13.04 Lysosomal and vacuolar protein degradation	2	2.85	NCU01545 NCU04192	13	0.12	23.75	0.004
30.05.02.24 G-protein coupled receptor signalling pathway	2	2.85	NCU01545 NCU09427	17	0.16	17.812	0.006
30.05.02.24.04 Gamma-aminobutyric acid signalling pathway	1	1.42	NCU01545	1	0	0	0.007
30.05 transmembrane signal transduction	3	4.28	NCU01545 NCU09427 NCU04834	63	0.62	6.90	0.009
99 Unclassified proteins	50	71.4	NCU05178 NCU05395 NCU00746 NCU05593 NCU01697 NCU06373 NCU05856 NCU00354 NCU03708 NCU07817 NCU03344 NCU05814 NCU02176 NCU05495 NCU00881 NCU02298 NCU21668 NCU04122 NCU06115 NCU07802 NCU04278 NCU07439 NCU21253 NCU01189 NCU05408 NCU06919 NCU02465 NCU04542 NCU08257 NCU08037 NCU00995 NCU01191 NCU08833 NCU10052 NCU06514 NCU02488 NCU05502 NCU09562 NCU00265 NCU03960 NCU05831 NCU04429 NCU04732 NCU01380 NCU02432 NCU00811 NCU07091 NCU09693 NCU21678 NCU09802	5853	58.1	1.23	0.015
30.05.02 Non-enzymatic receptor mediated signalling	2	2.85	NCU09427 NCU01545	27	0.26	10.96153846	0.01495 7492
14.07.11.01 Autoproteolytic processing	1	1.42	NCU01545	7	0.06	23.66666667	0.04768 8778
14.13.04.02 Vacuolar protein degradation	1	1.42	NCU04192	7	0.06	23.66666667	0.04768 8778
16.01.01 Receptor binding	1	1.42	NCU01545	7	0.06	23.66666667	0.04768 8778

B.

Functional category	Abs Set	Rel Set	Genes Set	Abs Genome	Rel Genome	Rel Set/Rel Genome	P-Value
11 Transcription	8	20.5	NCU04611 NCU02729 NCU03794 NCU05194 NCU07528 NCU01772 NCU03669 NCU01183	725	7.2	2.85	0.006
11.06 rna modification	2	5.12	NCU03669 NCU07528	42	0.41	12.49	0.011
01.06.06.13 Tetraterpenes (carotenoids) metabolism	1	2.56	NCU07719	3	0.02	128.00	0.012
11.02 RNA synthesis	6	15.3	NCU01772 NCU02729 NCU04611 NCU01183 NCU03669 NCU05194	533	5.29	2.89	0.016
01.01.09.05.01 Biosynthesis of tyrosine	1	2.56	NCU06360	5	0.04	64.00	0.019
32.07.07.07 Superoxide metabolism	1	2.56	NCU09560	5	0.04	64.00	0.019
11.02.03 mRNA Synthesis	5	12.8	NCU05194 NCU01772 NCU04611 NCU02729 NCU03669	465	4.61	2.78	0.032
01.01.09.01.02 Degradation of glycine	1	2.56	NCU02727	10	0.09	28.44	0.038
01.05.05.07 C-1 compound catabolism	1	2.56	NCU02727	10	0.09	28.44	0.038
11.06.01 rRNA modification	1	2.56	NCU03669	10	0.09	28.44	0.038
11.02.03.01 General transcription activities	2	5.12	NCU04611 NCU01772	84	0.83	6.17	0.042
11.02.03.04 Transcriptional control	4	10.2	NCU02729 NCU05194 NCU04611 NCU03669	350	3.47	2.94	0.045
01.05.05 C-1 compound metabolism	1	2.56	NCU02727	12	0.11	23.27	0.046
34.11.11 Rhythm (e.g. circadian, ultradian)	1	2.56	NCU02500	12	0.11	23.27	0.046
01.01.09.01.01 Biosynthesis of glycine	1	2.56	NCU02727	13	0.12	21.33	0.049
10.01.03.03 ORI recognition and priming complex formation	1	2.56	NCU05194	13	0.12	21.33	0.049
40.01.05 Growth regulators / regulation of cell size	1	2.56	NCU02729	13	0.12	21.33	0.049

**Table S4 Overlapping Gene Sets That Showed Decreased (Dn) Or Increased (Up) Expression Levels In  $\Delta Pp-1$  And  $Mak-2^{Q100G}$  Datasets**

Dn $\Delta Pp-1$ Microarray And Rnaseq	Annotation	Dn $\Delta Pp-1$ Rnaseq And $Mak-2^{Q100G}$ Microarray	Annotation	Dn $\Delta Pp-1$ Microarray And $Mak-2^{Q100G}$ Microarray	Annotation
NCU00811	Hypothetical	<b>NCU00881</b>	<i>Ham-7</i>	<b>NCU00881</b>	<i>Ham-7</i>
<b>NCU00881</b>	<i>Ham-7</i>	<b>NCU00995</b>	Hypothetical	<b>NCU00995</b>	Hypothetical
<b>NCU00995</b>	Hypothetical	NCU01328	Transketolase	<b>NCU01697</b>	Hypothetical
NCU01380	Hypothetical	NCU01509	Hypothetical	<b>NCU03960</b>	<i>Ham-12</i>
<b>NCU01697</b>	Hypothetical	<b>NCU01697</b>	Hypothetical	<b>NCU04122</b>	Malate Dehydrogenase
NCU03013	<i>Acw-10</i>	NCU02110	<i>Nox-1</i>	<b>NCU04732</b>	<i>Ham-11</i>
<b>NCU03960</b>	<i>Ham-12</i>	NCU02209	Fatty Acid Desaturase	NCU07439	Hypothetical
<b>NCU04122</b>	Malate Dehydrogenase	NCU02767	<i>Ham-6</i>	<b>NCU09693</b>	Hypothetical
NCU04192	Vacuolar Aspartyl Aminopeptidase	NCU03132	Hypothetical		
<b>NCU04732</b>	<i>Ham-11</i>	NCU03192	Hypothetical		
NCU05814	Hypothetical	NCU03396	<i>Nop-58</i>		
NCU06698	Glycogenin	<b>NCU03960</b>	<i>Ham-12</i>		
NCU07802	Hypothetical	<b>NCU04122</b>	Malate Dehydrogenase		
NCU08332	<i>Hex-1</i>	NCU04527	Hypothetical		
NCU09562	Hypothetical	NCU04647	Actin-Binding Protein		
<b>NCU09693</b>	Hypothetical	<b>NCU04732</b>	<i>Ham-11</i>		
		NCU05789	Secreted Glucosidase		
		NCU05835	Hypothetical		
		NCU06034	Hypothetical		
		NCU06117	Hypothetical		
		NCU06989	Hypothetical		
		NCU07192	Hypothetical		
		NCU07392	<i>Adv-1</i>		
		NCU07850	<i>Nor-1</i>		
		NCU09108	Hypothetical		
		NCU09307	<i>Lfd-1</i>		
		NCU09337	<i>Prm-1</i>		
		<b>NCU09693</b>	Hypothetical		

Up $\Delta Pp-1$ Microarray And Rnaseq	Annotation	Up $\Delta Pp-1$ Rnaseq And $Mak-2^{Q100G}$ Microarray		Up $\Delta Pp-1$ Microarray And $Mak-2^{Q100G}$ Microarray	
NCU00309	WSC Domain- Containing Protein	NCU03152	DUF1348 Domain- Containing Protein	NCU02727	Glycine Cleavage System T Protein
NCU02361	Formamidase	NCU03222	Hypothetical	NCU06360	Histidinol-Phosphate Aminotransferase
NCU02500	<i>Ccg-4</i>	NCU04479	Lap2		
NCU07503	Hypothetical	NCU05686	Cell Wall Glucanase		
NCU08824	Molybdopterin	NCU07953	<i>Aod-1</i>		

NCU09560	Binding Domain- Containing Protein Superoxide Dismutase	NCU09116	Aromatic Aminotransferas e Aro8
		NCU09141	<i>Mig-9</i>

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