

## Supplementary information

Analysis of polygenic mutants suggests a role for Mediator in regulating transcriptional activation distance in *Saccharomyces cerevisiae*

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File S1 – PERL script to calculate the frequency of all SNPs in two matched pools

Table S1 – *S. cerevisiae* strains

Table S2 – Oligonucleotides

Table S3 – Crosses for bulk segregant analysis

Table S4 – Sequencing coverage of segregant pools

Table S5 – Causal mutant candidates

Table S6 – Number of mutants with >2-fold change in RNA levels in the polygenic mutants

Table S7 – Adjacent gene pairs enrichment

Figure S1 – Evidence for disomy in two of the polygenic mutants

Figure S2 – Western analysis of Med3

## File S1

PERL script to calculate the frequency of all SNPs in two matched pools

```
# Usage: cerevisiae_VCF_diff <vcf-file> <min-counts> <min-fraction>
# Input file must be a VCF file created from two samples (e.g., WT and mutant).
# When calling the function, the user must specify the minimum reads needed in
each sample
# (this study used 10) and the minimum fraction of an alternative allele
observed in either
# of the two samples (this study used 0.5).
# This program will ask which column is the WT and which column is the mutant.
# Output file is a tab-delimited file with same name as input file, plus the
date.
# Output file contains the allele descriptions and their counts.
# "FRACTION_DIFF_REF" shows the fraction difference of the reference allele: WT
minus mutant.
# The closer this value is to 1 (or -1), the greater the difference between WT
and mutant.

#!/usr/bin/perl
use warnings;
use strict;
use List::Util qw( min max );

my $input = shift;
my $date = ((localtime)[5]+1900)."_" . ((localtime)[4]+1)."_" . ((localtime)[3]);
my $output = $input.$date.".txt";

open IN, $input or die $!;
open OUT, ">$output" or die $!;
select OUT;

my $min_num = shift;
my $min_fraction = shift;

my
%chr=(chrI=>1,chrII=>2,chrIII=>3,chrIV=>4,chrV=>5,chrVI=>6,chrVII=>7,chrVIII=>8,
      chrIX=>9,chrX=>10,chrXI=>11,chrXII=>12,chrXIII=>13,chrXIV=>14,chrXV=>15,chr
rXVI=>16,chrMito=>17,"2-micron"=>18);
my %column;

$/="\n";

my $labels = join "\t", qw/CHROM POS REF_ALLELE ALT_ALLELE1 ALT_ALLELE2
ALT_ALLELE3 NUM_REF_WT NUM_ALT1_WT NUM_ALT2_WT NUM_ALT3_WT NUM_REF_MUT
NUM_ALT1_MUT NUM_ALT2_MUT NUM_ALT3_MUT FRACTION_DIFF_REF/;
print $labels,"\n";

my $WT_column = "-1";
my $mutant_column = "-1";

while (<IN>) {
    my @row = split /\t/, $_;
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my @new_row;

# Meta-information lines
if ($row[0] =~ /^##/) {next;}

# header line: determine order of columns
elsif ($row[0] =~ /^#[A-Za-z0-9]/) {
    my $count=0;

    for (@row) {
        print STDOUT "$count: $_\n";
        $column{$_} = $count;
        $count++;
    }
    print STDOUT "Which column is the WT sample? ";
    chomp ($WT_column = <STDIN>);
    print STDOUT "Which column is the mutant sample? ";
    chomp ($mutant_column = <STDIN>);
    next;
}

# data line (i.e., with mutation)
else {
    $new_row[0] = $chr{$row[$column{"#CHROM"}]};
    $new_row[1] = $row[$column{POS}];
    $new_row[2] = $row[$column{REF}];

    my @MUTATIONS = split /,/, $row[$column{ALT}];
    if (!defined$MUTATIONS[0] || ($MUTATIONS[0] eq "."))
    {$new_row[3] = "";}
    else {$new_row[3]=$MUTATIONS[0];}
    if (!defined$MUTATIONS[1] || ($MUTATIONS[1] eq "."))
    {$new_row[4] = "";}
    else {$new_row[4]=$MUTATIONS[1];}
    if (!defined$MUTATIONS[2] || ($MUTATIONS[2] eq "."))
    {$new_row[5] = "";}
    else {$new_row[5]=$MUTATIONS[2];}

    my %format_column;
    my @format = split /:/, $row[$column{FORMAT}];
    my $count=0;
    my $RA="RA"; my $AA="AA";
    for (@format) {
        $format_column{$_} = $count;
        if ($_ eq "RO") { $RA = "RO"; }
        if ($_ eq "AO") { $AA = "AO"; }
        $count++;
    }

    if ($row[$WT_column] =~ /:/) {
        my @WT = split /:/, $row[$WT_column];
        chomp (@WT);
        $new_row[6] = $WT[$format_column{$RA}];
        my @AA1 = split /,/, $WT[$format_column{$AA}];

```

```

        if (!defined$AA1[0] || ($AA1[0] eq "."))      {$new_row[7] =
0;}
        else {$new_row[7]=$AA1[0];}
        if (!defined$AA1[1] || ($AA1[1] eq "."))      {$new_row[8] =
0;}
        else {$new_row[8]=$AA1[1];}
        if (!defined$AA1[2] || ($AA1[2] eq "."))      {$new_row[9] =
0;}
        else {$new_row[9]=$AA1[2];}
    }
    else {
        @new_row[6..9] = ("?", "?", "?", "?");
    }

    if ($row[$mutant_column] =~ /:/) {
        my @MUT = split /:/, $row[$mutant_column];
        chomp (@MUT);
        $new_row[10] = $MUT[$format_column{$RA}];
        my @AA2 = split /,/, $MUT[$format_column{$AA}];
0;}
        if (!defined$AA2[0] || ($AA2[0] eq "."))      {$new_row[11] =
0;}
        else {$new_row[11]=$AA2[0];}
        if (!defined$AA2[1] || ($AA2[1] eq "."))      {$new_row[12] =
0;}
        else {$new_row[12]=$AA2[1];}
        if (!defined$AA2[2] || ($AA2[2] eq "."))      {$new_row[13] =
0;}
        else {$new_row[13]=$AA2[2];}
    }
    else {
        @new_row[10..13] = ("?", "?", "?", "?");
    }
}

my $WT_sum = 0;
my $mut_sum = 0;

for (@new_row[6..9]) {
    if ($_ ne "?") {$WT_sum=$WT_sum+$_;}
}
for (@new_row[10..13]) {
    if ($_ ne "?") {$mut_sum=$mut_sum+$_;}
}

if ( $WT_sum >= $min_num && $mut_sum >= $min_num ) {
    if ( ( ( max(@new_row[7..9]) / $WT_sum) >= $min_fraction) ||
        ( ( max(@new_row[11..13]) / $mut_sum) >= $min_fraction) ) {
        $new_row[14] = ($new_row[6]/$WT_sum)-($new_row[10]/$mut_sum);
        my $line = join "\t", @new_row;
        print $line, "\n";
    }
}
}
}

```

**Table S1. *S. cerevisiae* strains**

Strain	Genotype
FY76	<i>MATa lys2-128δ</i>
FY3045	<i>MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX</i>
FY3046 (2.0)	<i>MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX</i>
FY3047 (1.0)	<i>MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX</i>
FY3048 (1.1)	<i>MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX grr1-1</i>
FY3049 (1.2)	<i>MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX mot3-1 grr1-1 sgm1-1 rim8-1 tma108-1 sgf73-1</i>
FY3050 (2.1)	<i>MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX yor019w-1</i>
FY3051 (2.2)	<i>MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX ptr3-1 yor019w-1</i>
FY3052 (2.3a)	<i>MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX mit1-1 ptr3-1 yor019w-1</i>
FY3053 (2.3b)	<i>MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3-natMX msn2-1 ptr3-1</i>

*yor019w-1*

- FY3054 *MATa his3Δ200 ura3Δ0 leu2Δ0 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806*
- FY3055 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3*
- FY3056 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1 sgm1-1 rim8-1 tma108-1 sgf73-1*
- FY3057 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 ptr3-1 yor019w-1*
- FY3058 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 ptr3-1 yor019w-1*
- FY3059 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3- 1*
- FY3060 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 grr1-531*
- FY3061 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1*
- FY3062 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1*
- FY3063 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 msn2-1*
- FY3064 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 sin4Δ0*

FY3065 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UASGAL1 799-HIS3 dug2 $\Delta$ ::TRP1-UASGAL1806 mit1-1 msn2-1*

FY3066 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UASGAL1 799-HIS3 dug2 $\Delta$ ::TRP1-UASGAL1806 mit1-1 sin4 $\Delta$ 0*

FY3067 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UASGAL1 799-HIS3 dug2 $\Delta$ ::TRP1-UASGAL1806 mot3-1 mit1-1*

FY3068 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UASGAL1 799-HIS3 dug2 $\Delta$ ::TRP1-UASGAL1806 mot3-1 mit1-1 msn2-1*

FY3069 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 MIT1 ptr3-1 yor019w-1*

FY3070 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 PTR3 yor019w-1*

FY3071 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 ptr3-1 YOR019W*

FY3072 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2  $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 MSN2 ptr3-1 yor019w-1*

FY3073 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2  $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 PTR3 yor019w-1*

FY3074 *MATa his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2  $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 ptr3-1 YOR019W*

FY3075 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mot3 $\Delta$ 0::URA3 grr1-1*

FY3076 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1 $\Delta$ 0::URA3*

FY3077 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mit1Δ0::URA3 ptr3-1 yor019w-1*

FY3078 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 ptr3Δ0::URA3 yor019w-1*

FY3079 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 msn2Δ0::URA3 ptr3-1 yor019w-1*

FY3080 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 ptr3-Δ0::URA3 yor019w-1*

FY3081 *MATa his3Δ200 ura3Δ0 leu2Δ0 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub>280-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806*

FY3082 *MATa his3Δ200 ura3Δ0 leu2Δ0 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub>1397-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806*

FY3083 *MATa his3Δ200 ura3Δ0 leu2Δ0 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub>2027-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806*

FY3084 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub>280-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3*

FY3085 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub>1397-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3*

FY3086 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub>2027-HIS3 dug2 Δ::TRP1-UAS<sub>GAL1</sub>806-URA3*

FY3087 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub>280-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1*

FY3088 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-UAS<sub>GAL1</sub>1397-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1*



FY3089 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>2027-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1*

FY3090 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>280-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 ptr3-1 yor019w-1*

FY3091 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>1397-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 ptr3-1 yor019w-1*

FY3092 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>2027-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 mit1-1 ptr3-1 yor019w-1*

FY3093 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>280-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 ptr3-1 yor019w-1*

FY3094 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>1397-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 ptr3-1 yor019w-1*

FY3095 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 trp1 $\Delta$ 63 lys2-128 $\delta$  sin4 $\Delta$ 0::LEU2 bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub>2027-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 msn2-1 ptr3-1 yor019w-1*

FY3096 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 lys2-128 $\delta$  bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 UAS<sub>GAL1</sub>-10 $\Delta$ ::hphMX*

FY3097 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 lys2-128 $\delta$  bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 UAS<sub>GAL1</sub>-10 $\Delta$ ::hphMX MED3-FLAG::natMX*

FY3098 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 lys2-128 $\delta$  bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 UAS<sub>GAL1</sub>-10 $\Delta$ ::hphMX sin4 $\Delta$ 0::LEU2*

FY3099 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 lys2-128 $\delta$  bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3 dug2 $\Delta$ ::TRP1-UAS<sub>GAL1</sub>806 UAS<sub>GAL1</sub>-10 $\Delta$ ::hphMX sin4 $\Delta$ 0::LEU2 MED3-FLAG::NatMX*

FY3100 *MAT $\alpha$  his3 $\Delta$ 200 ura3 $\Delta$ 0 leu2 $\Delta$ 0 lys2-128 $\delta$  bph1 $\Delta$ ::kanMX-UAS<sub>GAL1</sub> 799-HIS3*

*dug2Δ::TRP1-UAS<sub>GAL1</sub>806 UAS<sub>GAL1</sub>-10Δ::hphMX sin4Δ0::LEU2 mot3-1 grr1-1*  
 FY3101 *MATa his3Δ200 ura3Δ0 leu2Δ0 lys2-128δ bph1Δ::kanMX-UAS<sub>GAL1</sub>799 dug2*  
*Δ::TRP1-UAS<sub>GAL1</sub>806 UAS<sub>GAL1</sub>-10Δ::hphMX sin4Δ0::LEU2 mot3-1 grr1-1 MED3-*  
*FLAG::NatMX*  
 FY3102 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 MOT3 grr1-1 sgm1-1 rim8-1 tma108-*  
*1 sgf73-1*  
 FY3103 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub>799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 GRR1 sgm1-1 rim8-1 tma108-*  
*1 sgf73-1*  
 FY3104 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806 mot3-1 grr1-1 SGM1 rim8-1 tma108-*  
*1 sgf73-1*  
 FY3105 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub>799 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3 ptr3-1*  
 FY3106 *MATa his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3 yor019w-1-1*  
 FY3107 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub>799 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3 ptr3-1 yor019w-1*  
 FY3108 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3 mit1-1 ptr3-1 yor019w-1*  
 FY3109 *MATα his3Δ200 ura3Δ0 leu2Δ0 trp1Δ63 lys2-128δ sin4Δ0::LEU2 bph1Δ::kanMX-*  
*UAS<sub>GAL1</sub> 799-HIS3 dug2Δ::TRP1-UAS<sub>GAL1</sub>806-URA3 msn2-1 ptr3-1 yor019w-1*  
 FY2650 *MATa his3Δ200 lys2-128d leu2Δ0 ura3Δ0 trp1Δ63 sin4Δ0::LEU2 med3Δ0::LEU2*

*bph1Δ::kanMX-UAS<sub>GAL1</sub>799-HIS3*

FY2651 *MATa ade8-140 his3Δ200 lys2-128d leu2Δ0 ura3Δ0 trp1Δ63 sin4Δ0::LEU2*

*med2Δ0::URA3 bph1Δ::kanMX-UAS<sub>GAL1</sub>799-HIS3*

FY2657 *MATα ade8-140 his3Δ200 lys2-128d leu2Δ0 ura3Δ0 trp1Δ63 sin4Δ0::LEU2*

*cdk8Δ0::URA3 bph1Δ::kanMX-UAS<sub>GAL1</sub>799-HIS3*

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Strains isolated during the mutant isolation are indicated by additional numbers in parentheses which indicate the stage when they were isolated. Lineage 1 has strains 1.0, 1.1, and 1.2, and lineage 2 has strains 2.0, 2.1, 2.2, 2.3a, and 2.3b.

**Table S2. Oligonucleotides**

Oligo	Purpose	Sequence
FO8064	replace <i>HIS3</i> ORF with <i>URA3</i> ORF in <i>dug2</i> reporter	TATACTAAAAAATGAGCAGGCAAGATAAACGAAGGCAAAGATGTCGA AAGCTACATATAA
FO8065	replace <i>HIS3</i> ORF with <i>URA3</i> ORF at <i>dug2</i> reporter	TATATATATCGTATGCTGCAGCTTTAATAATCGGTGTCATTAGTTTT GCTGGCCGCATC
FO6834	Northern probe of <i>HIS3</i>	GATCTTTCGAACAGGCCGTA
FO4995	Northern probe of <i>HIS3</i>	ACCTTTGGTGGAGGGAACAT
FO481	Northern probe of <i>URA3</i>	CCATGGAGGGCACAGTTAAGCCGC
F0483	Northern probe of <i>URA3</i>	CCCTTCCCTTTGCAAATAGTCCTC
FO1324	Northern probe of <i>SNR190</i>	GGCCCTGATGATAATG
FO1325	Northern probe of <i>SNR190</i>	GGCTCAGATCTGCATG
FO3662	delete <i>URA3</i> from <i>dug2</i> reporter	GTTGGTTGGGTGACCCAACAAATCAT
FO3663	delete <i>URA3</i> from	CTATTGAATACTTTAGACAAAATCTCA

*dug2* reporter

FO10433 integrate *URA3* at TCGCAACAAAGACATTTTCTATGCCCTTGGTGCTTAAGCAAGATTGT  
site of *mot3* ACTGAGAGTGCAC  
mutation

FO10434 integrate *URA3* at AATTGTTGTAGTTAAAGATGATGTTGTTTTTCTTGAGTTCCTGTGCGG  
site of *mot3* TATTTACACCG  
mutation

FO10277 replace *URA3* with ACGACAGCACCTAACCATCC  
*mot3* allele

FO10278 replace *URA3* with TCTTCATTTTCGGGAGCTGT  
*mot3* allele

FO10271 integrate *URA3* at AAAAAAATCCAAGAGTTTCTGGTTGTTATAGAGAAACGTAAGATTGTA  
site of *grr1* CTGAGAGTGAC  
mutation

FO10272 integrate *URA3* at TTTAAGGTTGTCTAGCTCAATTCGTTTCAGTATTTTTTTTTCTGTGCGG  
site of *grr1* TATTTACACCG  
mutation

FO10273 replace *URA3* with CGCTGAACGGGATTGACATA  
WT *GRR1* allele

FO10274 replace *URA3* with GCTTGGAATGGCAGTATGCA  
WT *GRR1* allele

FO10435 integrate *URA3* at AAAGAAAAAATGATATTGAAGAAAAATATCAAACCTGCCTAGATTGTA  
site of *sgm1* CTGAGAGTGAC  
mutation

FO10436 integrate *URA3* at GTCTCTCCAAGTCTTTCACTTCCAAAAGTGCCTTCGAATGCTGTGCG  
site of *sgm1* GTATTTACACCG  
mutation

FO10281 replace *URA3* with TCAAAGGCCAACTGGGATAG  
*sgm1* allele

FO10282 replace *URA3* with TCGCCAAGTTTTGACATTGA  
*sgm1* allele

FO10263 integrate *URA3* at AAGCTGAAGAGACCTCCATTCAACTCGATTGAAAATTTACAGATTGTA  
site of *mit1* CTGAGAGTGCAC  
mutation

FO10264 integrate at site of ATTTTGTTTTATATCTTTTACAGAATAATAAGAGACAATACTGTGCGG  
*mit1* mutation TATTTACACCG

FO10265 replace *URA3* with GTA CTGATTCCGCCGTCATT  
*mit1* allele

FO10266 replace *URA3* with TCAGGGGAGTGGAAGAGTTG  
*mit1* allele

FO10431 integrate *URA3* at AACTTCAACCAACTAACAGAGCAATCGTCATCTTCACTCTAGATTGTA  
site of *ptr3* CTGAGAGTGCAC  
mutation

FO10432 integrate *URA3* at TATCAAGAAATCATTGGAAAGTTTGCAAAAACGTTGGCTCCTGTGCG  
site of *ptr3* GTATTTACACCG  
mutation

FO10269 replace *URA3* with GCACATGATCTGGACGAAGA  
*ptr3* allele

FO10270	replace <i>URA3</i> with <i>ptr3</i> allele	ATGGGGAATCTCGACACGTA
FO10397	integrate <i>URA3</i> at site of <i>yor019w</i> mutation	CCAGCTTTAAGAATGCTTTGATAGGCAATGGGTGCGAAAAAAGATTGT ACTGAGAGTGAC
FO10398	integrate <i>URA3</i> at site of <i>yor019w</i> mutation	CTGAGGAAGAATATGGTATTAAAGATTTTCTAAACTTTGTCTGTGCGG TATTTACACCG
FO10363	replace <i>URA3</i> with <i>yor019w</i> allele	CCCAGCATTCAAGAAGGAAG
FO10364	replace <i>URA3</i> with <i>yor019w</i> allele	GCACCGGCACTTTTAACTTT
FO10453	integrate <i>URA3</i> at site of <i>msn2</i> mutation	AAGTGTCGTAATAGAATCAACAAAGGAACTCGAGGAGAAAAGATTGT ACTGAGAGTGAC
FO10454	integrate <i>URA3</i> at site of <i>msn2</i> mutation	GGTCGTTTCGTTAGAGTGAACAGATCTCACATGCCTTTTCACTGTGCG GTATTTACACCG
FO10451	replace <i>URA3</i> with <i>msn2</i> allele	TATCACCATTTCCACAGCA
FO10452	replace <i>URA3</i> with <i>msn2</i> allele	TGACAAGCAAATGGTCGTTT
FO10323	delete <i>MOT3</i> with <i>URA3</i>	TAGGCAAATAGTAAAGGGACATATCATATTTGAGCAATGAAGATTGT ACTGAGAGTGAC

FO10324	delete <i>MOT3</i> with <i>URA3</i>	ATGAGTGGGAAGGGATATTTTGTGTGTCTATAAAGTCTATCTGTGCG GTATTTACACCCG
FO10317	delete <i>GRR1</i> with <i>URA3</i>	AAACAGTTTTGCGTTTTCTTTATACTAAGAAGGTCTATAAGATTGTA CTGAGAGTGCAC
FO10318	delete <i>GRR1</i> with <i>URA3</i>	AAAGGTGTAGTAGGACAGTAAGTATTCAATGAAATACAACCTGTGCGG TATTTACACCCG
FO10519	delete <i>MIT1</i> with <i>URA3</i>	CAACATTTCAACTAGACACAAGGACAACGTAAATTTCTAAAGATTGTA CTGAGAGTGCAC
FO10520	delete <i>MIT1</i> with <i>URA3</i>	AAGAAAAGAAAATCGGAGTACTTTTTTAAAATATATTTACCTGTGCGG TATTTACACCCG
FO10521	delete <i>PTR3</i> with <i>URA3</i>	ACACATACATAGGTACGAAATACACAACCTGATAGGCGTTCAGATTGT ACTGAGAGTGCAC
FO10522	delete <i>PTR3</i> with <i>URA3</i>	GTATACCAGAACCTTAAACATACGTATATATTTAGATGCACTGTGCG GTATTTACACCCG
FO7014	delete <i>MSN2</i> with <i>URA3</i>	TTTTTCAACTTTTATTGCTCATAGAAGAACTAGATCTAAAAGATTGTAC TGAGAGTGCAC
FO7015	delete <i>MSN2</i> with <i>URA3</i>	TTATGAAGAAAGATCTATCGAATTAATAAATAATGGGGTCTACTGTGCG GTATTTACACCCG
FO7018	delete <i>MSN4</i> with <i>URA3</i>	TTCGGCTTTTTTTCTTTTCTTCTTATTAATAAACAATATAAGATTGTAC TGAGAGTGCAC
FO7019	delete <i>MSN4</i> with <i>URA3</i>	TAGCTTGTCTTGCTTTTATTTGCTTTTGACCTTATTTTTCTGTGCGGT ATTTACACCCG
FO10461	replace <i>kanMX-</i> <i>GAL1</i> with <i>URA3</i>	ATTCACAACCTTTGGTCAAACGCCTTTACAAATATTTTCAGGAGATTGTA CTGAGAGTGCAC



FO10462	replace <i>kanMX-</i> <i>GAL1</i> with <i>URA3</i>	AAGATTGTCTTCTCAAATATTGGCTTCATTGGAACCTTACCTGTGCG GTATTTACACCCG
FO6828	replace <i>URA3</i> with endogenous <i>BPH1</i> sequence	TTACCCAGGCGCTGTAAATC
FO6829	replace <i>URA3</i> with endogenous <i>BPH1</i> sequence	GGTTACCTGAAACCGAATGC
FO2143	integrate <i>kanMX-</i> <i>GAL1</i> at 280 bp	TCTGGGACATGATTAATTTTGAAGTGAATTCGAGCTCGTTTAAAC
FO2054	integrate <i>kanMX-</i> <i>GAL1</i> at 280 bp	AAATATCGTAAAGATAAGCATTTCGGGAATTCGAGCTCGTTTAAAC
FO3085	integrate <i>kanMX-</i> <i>GAL1</i> at 1397 bp	TATGAAGCTTTGGCTTCCCTGGAAAATGCTGAATTCGAGCTCGTTTA AAC
FO3086	integrate <i>kanMX-</i> <i>GAL1</i> at 1397 bp	AGATATGAAGATACTATCATAGCTGAGGAATCATCGCTTCGCTGATT AATTACCC
FO6666	integrate <i>kanMX-</i> <i>GAL1</i> at 2027 bp	CGCAAGAATCACGGGGATATGACGGTTAGCTGAATTCGAGCTCGTT TAAAC
FO6667	integrate <i>kanMX-</i> <i>GAL1</i> at 2027 bp	AGTTTCCAAACAAAGACTTCGTGCTTTAGG TCATCGCTTCGCTGATTAATTACCC
FO10659	Flag-tag MED3	GAACAATCTGGAATTAGGTGGTCTGAACATGGATTTCTTGCGGATCC CCGGGTAAATTA
FO10660	Flag-tag MED3	TATACAGATAATTACTATCTTGGATACATAGATGCACCAGGAATTCGA GCTCGTTTAAAC

FO10241	delete	TATACATATCCATATCTAATCTTACTTATATGTTGTGGAAGGGAACAA
	endogenous	AAGCTGG
	UAS <sub>GAL1-10</sub> with	
	hphMX	
FO10242	delete	GCAGCTTTTCCATTTATATATCTGTTAATAGATCAAAAATTATAGGGC
	endogenous	GAATTGG
	UAS <sub>GAL1-10</sub> with	
	hphMX	
FO10663	ChIP qPCR	GGAAATCAGACGCCAATAGC
	CCW12 UAS	
FO10664	ChIP qPCR	GCCACCCTCACCTCACTAAC
	CCW12 UAS	
F02947	ChIP qPCR	AGGCACATCTGCGTTTCAG
	reporter UAS	
FO10665	ChIP qPCR	ACGCTTAACTGCTCATTGCT
	reporter UAS	
FO10679	ChIP qPCR	GCGAAGCGATGAGTAAGGTT
	<i>BPH1</i> spacer 1	
FO10680	ChIP qPCR	GCGAAGCCCCTCCAGTATAA
	<i>BPH1</i> spacer 1	
FO2295	ChIP qPCR	ATCGTAAAGATAAGCATTCCGGT
	<i>BPH1</i> spacer 2	
FO4216	ChIP qPCR	TCGTGATAACAGCGCATCTC
	<i>BPH1</i> spacer 2	

FO4993	ChIP qPCR	GGGCTTTCTGCTCTGTCATC
	<i>HIS3</i> promoter	
FO5062	ChIP qPCR	CGCAAAAGGTCGCAATATCT
	<i>HIS3</i> promoter	
FO10671	ChIP qPCR	GCTAGGTGAGAGAAAGCAAAGG
	Chr V untranscribed	
	control	
FO10672	ChIP qPCR	TACGATCTTAGTTCCAATGGTGA
	Chr V untranscribed	
	control	

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**Table S3. Crosses for bulk segregant analysis**

Cross	Total tetrads	Segregants with strong phenotype	Complete tetrads
FY3049 (1.2) x FY3046	282	66 (6.8%)	172 (61%)
FY3052 (2.3a) x FY3047	297	59 (6.4%)	134 (45%)
FY3053 (2.3b) x FY3047	340	42 (4.1%)	129 (38%)

**Table S4. Sequencing coverage of segregant pools**

<b>Cross</b>	<b>Segregants</b>	<b>Pool size (segregants)</b>	<b>Number of mapped reads</b>	<b>Fold coverage</b>
FY3049 (1.2) x FY3046	Wild type	45	12,115,678	89
	Mutant	45	12,364,877	85
FY3052 (2.3a) x FY3047	Wild type	48	13,097,764	101
	Mutant	48	14,174,495	97
FY3053 (2.3b) x FY3047	Wild type	42	11,391,104	80
	Mutant	42	38,280,078	260

In each cross, wild-type segregants were those that were sensitive to 1 mM 3AT and mutant segregants were those resistant to 10 mM 3AT.

**Table S5. Causal mutant candidates**

Mutant strain	Candidate	Amino acid	Causative?	Mutant reads/total read
	gene	change		
FY3049 (1.2)	<i>MOT3</i>	N388H	Yes	73/74
	<i>GRR1</i>	L181stop	Yes	67/79
	<i>SGM1</i>	L407S	No	79/95
	<i>SGF73</i>	P84L	No	46/69
	<i>TMA108</i>	A458V	No	42/67
	<i>RIM8</i>	P52L	No	46/68
FY3052 (2.3a)	<i>PTR3</i>	S363stop	Yes	68/68
	<i>YOR019W</i>	N553K	Weakly	48/97
	<i>MIT1</i>	H187R	Yes	81/81
FY3053 (2.3b)	<i>PTR3</i>	N553K	Yes	231/235
	<i>YOR019W</i>	N553K	Weakly	62/259
	<i>MSN2</i>	C652stop	Yes	252/252

Causality was determined by allele replacement for most of the listed mutations as described in Materials and Methods. For three mutations, in *SGF73*, *TMA108*, and *RIM8*, allele replacement was not done. Instead, these mutations were tested during reconstruction of the polygenic mutant and were shown to not confer any mutant phenotype.

**Table S6. Number of genes with a two-fold or greater change in RNA levels in the polygenic mutants**

		Strain			
Carbon source	Genes affected	<i>sin4Δ0 mot3-1</i>			
		1.2	<i>grr1-1</i>	2.3a	2.3b
Glucose	Up	416	182	145	167
	Down	158	79	62	82
Galactose	Up	474	133	146	184
	Down	426	139	78	133
Raffinose	Up	313	152	180	172
	Down	135	84	123	141

Shown for each strain is the number of genes whose RNA levels change by two-fold or more when the strains are grown in YP medium with the indicated carbon sources. The actual strains used are as follows: 1.2, FY3056; *sin4Δ0 mot3-1 grr1-1*, FY3061; 2.3a, FY3057; 2.3b, FY3058.

**Table S7. Adjacent gene pairs enrichment**

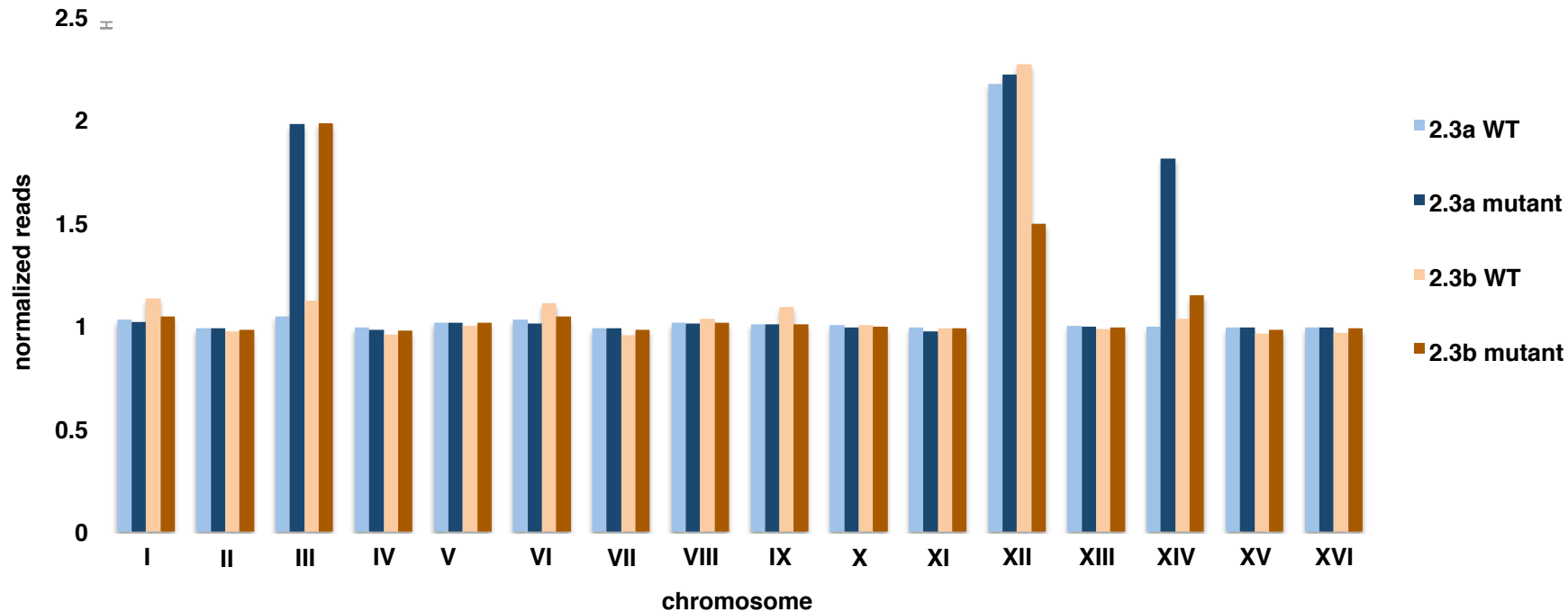
		Promoter Orientation								
		Convergent			Divergent			Tandem		
		E	O	p-value	E	O	p-value	E	O	p-value
Raffinose	1.2	2.75	8	2.10E-03	1.87	15	1.90E-10	6.07	22	1.20E-07
	<i>sin4Δ0</i> <i>mot3-1 grr1-1</i>	0.613	4	4.30E-04	0.344	4	3.00E-05	0.943	5	4.40E-04
	2.3a	1.08	2	9.50E-02	0.636	5	5.40E-05	1.85	11	6.30E-07
	2.3b	0.873	4	2.10E-03	0.514	8	4.30E-09	1.91	9	3.20E-05
Glucose	1.2	5.77	19	3.00E-06	3.63	18	1.10E-08	4.87	23	4.80E-10
	<i>sin4Δ0</i> <i>mot3-1 grr1-1</i>	0.760	5	1.40E-04	0.488	3	1.60E-03	0.340	4	2.90E-05
	2.3a	0.726	5	1.10E-04	0.323	3	3.50E-04	0.468	8	2.00E-09
	2.3b	1.02	7	1.20E-05	0.424	5	5.70E-06	0.726	8	8.00E-08
Galactose	1.2	6.07	22	1.20E-07	11.89	24	6.00E-04	14.99	35	2.90E-06
	<i>sin4Δ0</i> <i>mot3-1 grr1-1</i>	0.943	5	4.40E-04	2.238	9	1.20E-04	0.754	4	1.10E-03
	2.3a	1.85	11	6.30E-07	1.40	8	1.60E-05	1.40	11	3.20E-08
	2.3b	1.91	9	3.20E-05	1.77	10	2.70E-06	2.08	16	1.00E-10

Shown for each strains are the expected number (E) of adjacent gene pairs upregulated given the overall number of upregulated genes, the observed (O) number of upregulated gene pairs, and the p-value for the significance in difference between observed and expected. Analysis was performed for convergent, divergent, and tandem promoter orientation when the strains are grown in YP medium with the indicated carbon sources. The actual strains used are as follows: 1.2, FY3056; *sin4Δ0 mot3-1 grr1-1*, FY3061; 2.3a, FY3057; 2.3b, FY3058.



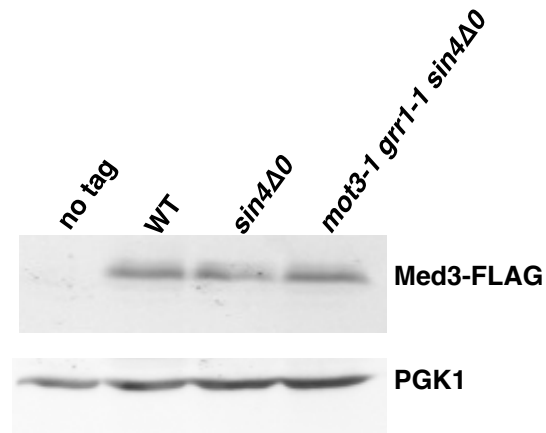
## Supplemental Figure 1

A



**Figure S1.** Evidence for disomy in two of the polygenic mutants. (A) Shown are the normalized levels of sequencing reads across each *S. cerevisiae* chromosome. The elevated reads for chromosomes III and XIV are likely caused by disomy for those chromosomes. The elevated reads for chromosome XII are likely an artifact of the repeated rDNA.

## Supplemental Figure 2



**Figure S2.** Western analysis of Med3. Shown is a representative western looking at levels of Med3-FLAG. Quantitation of three experiments is shown in Figure 9C.