Supplemental Tables and Figures

Table S1: Deletion strains sensitive (n=135) to polygodial ranked as 0 and 1. In bold are hypersensitive mutants (n=66). Gene functions and cellular locations were taken from the SGD database.

Biological process	Genes
Mitochondrial genome maintenance and normal morphology	*GET1*, *PCP1*, ***YME2***
Mitochondria electron transport chain	***QCR10***
Mitochondria ATPase	***ATP2***
Mitochondrial protein maturation	***ISA1***
Mitochondrial import,folding, maturation, degradation	*YME1*
Mitochondrial TCA cycle	*KGD2*
Mitochondrial NADP biosynthesis	*POS5*
Mitochondrial riboflavin biosynthesis	***RIB4***
Peroxisome protein deubiquitination	***UBP15***
Vacuolar acidification, V-ATPase	***VMA1***, ***VMA3***, ***VMA4***, ***VMA7***, ***VMA8***, ***VMA13***, ***VMA16***, ***VPH2***
Vacuolar degradation autophagy	*ATG8*, *ATG12*, *ATG10*, *ATG13*
Vesicular trafficking to vacuole, vacuolar protein sorting	***VAM6***, ***VAM3***, ***GYP1***, ***VAM7***, ***VPS16***, ***VPS54***, *VPS61*, *VPS63*, *VPS65*
Golgi P-type ATPase	***PMR1***
ER Golgi endosome trafficking	*CHS5*, *RGP1*, ***YPT6***, *VPS35*, ***VPS52***, ***VPS54,*** *SEC22*, *SEC28*, *YSP1*
ER protein targeting to membrane, translocation	*SEC66*, *SGT2*, *GET1*, ***GET4***
ER Golgi protein N-linked glycosylation	***OST4***, *OCH1*
ER ergosterol biosynthesis	***ERG3***, *ERG2*, *ERG28*
ER sterol transport	***SIP3***
ER lipid droplet formation and triacelglyceride homeostasis	*VPS66/LOA1*
ER glycosylphosphatidylinositol biosynthesis	***LAS21***
ER-associated degradation (ERAD)	***SHP1***
Nuclear enveloppe phospholipid biosynthesis, lipid droplets	*NEM1*
Chromatin organization, structure, remodelling	*EAF1*, *ULS1*, *RTF1*, ***ARP5***
Plasma membrane transport	***FUR4,*** *TRK1*
Cell wall synthesis	*SMI1*, *FKS1*
Bud site selection & axial budding	***BUD25***
Cytoskeleton organization and cellular morphogenesis	*BEM2*
Actin stabilization	***TPM1***
Transcription regulation	*SWI3*, ***TAF14***, ***SSN3***, *GAL11*, *CSE2*, ***SIP3***, ***BUR2***, ***GON7***, ***ROX3***, ***SRB2***, *SNF6*, ***SNF12***, *SRB5*, *RTF1*, ***ADA2***, *UME6*, ***RPN4***, *MED2*, *TUP1*, *RPA14*

Mitosis, meiosis	*DCC1, CTF4, CNM67, VPS54,* ***HTL1***, ***CIK1***, *MRE11*
mRNA export from nucleus	*SAC3,* ***NPL3***
Nuclear protein import	***MOG1***
DNA repair	*FYV6*
Amino acid synthesis	***TYR1***, ***CPA1***, ***ADE3***, ***PRO1***, ***PRO2***, ***SER1***, ***SER2***, ***TRP1***, ***TRP2***, ***TRP5, CPR2***, ***ARG2***
Ribosomal protein; translation and protein synthesis	*RPL13B,* ***MRPL23***, ***TIF3***, *CPA1 uORF*
Ribosome biogenesis	*SAC3, BMT5*
Signal transduction	*TPK1,* ***IRA2, PHO85***, *CLA4, NBP2, STE11*
Glycolysis	***PFK2***, *ADH1*
Glutathione biosynthesis, binding	***GSH1***, *URE2*
Cyanamide metabolism	***DDI3***
Trehalose synthesis	*TPS2*
Nucleotide metabolism	*ADE3, ADK1*
Intracellular copper transport	*CCS1*
Mating	*SCP160*
Unknown function	*ECM33, BOP2, JJJ2, YGL024W, YML012C-A, YER091C-A, YDR433W, YDR157W, YMR102C, YOR331C, YNL296W*

Table S2: Mutants resistant to polygodial ranked as 5 and 6 (n=233). In bold are hyper-resistant mutants (n=47). Gene functions and cellular locations were taken from the SGD database.

Biological process	Genes
Mitochondrial transcription and translation	*PET494, MSK1, MEF1, MEF2, MTF2, MRM2, MRPL38,* ***MRPL35***, ***SLS1***, *TUF1,* ***MSE1***, ***RSM27,*** *MRX5*
Mitochondrial genome maintenance, fusion, fission and normal morphology	***MCP2***, *CCE1, RIM1, ACO1,* ***MGM1***, ***DNM1***, *UGO1*
Mitochondria ATPase	*ATP15,* ***ATP17***, ***AFG3***
Mitochondrial inner membrane electron transport chain	***QCR9***
Mitochondrial protein folding	*CPR3, MDJ1*
Mitochondrial TCA cycle	***FUM1***, *PDX1, CIT1, SDH5, ACO1, ACO2*
Mitochondrial amino acid biosynthesis	***GDH3***, ***ALT1***, *THI4, AAT1*
Mitochondrial transporter activity	*MDL1, HOT13, FLX1, MRS2*
Mitochondrial lipid homeostasis	*UPS2, MCP2*
Mitochondrial unknown function	*MRP8, FMP33, FMP48, FMP49*
Peroxisome biogenesis	*PEX30*
Vacuolar protein sorting, MVB, ESCRT, CORVET	*SNA3, MIL1, CPS1, YCK3,* ***VPS8***, ***VPS9***, *VPS21, VPS36, VPS60, VPS25, VPS27, MVB12, SSH4, LST4, ENV7*
Vacuolar transport	*AVT3,* ***BPT1,*** *CCC1*
Vacuolar carboxypeptidases	*ATG42, CPS1*
Golgi glycosylation	***MNN4***
ER Golgi transport	*COY1*

ER enzymes and transport	*ICE2, ERV2, YBR137W*
ER cargo exit	*TED1*
ER lipid homeostasis	***OSH2***, *APQ12*
ER inheritance	*SHE3*
Actin cytoskeleton reorganization, microtubules and endocytosis	***RVS167***, ***RVS161***, ***SLA1***, ***END3***, ***ACF2***, ***SRV2***, *GVP36, TPM2, PAC11, CIN1*
Plasma membrane transport	*QDR1, AQR1, HXT10, ALR2, FRE7, BOR1, AMF1,*
Cell wall	*FIT3, KDX1, PRY3, TOS1*
Bud site selection & axial budding	*BUD2*, ***BUD20***, *BUD21, BUD26, BUD28, AXL2*
Mating	*FUS3, KDX1, PRY3*
Mitosis and Meiosis	*HOP1, CLG1, IRC8, RMR1, HOS4, REC8, SMA2, GMC1*
Nuclear protein, protein quality control	*CMR1*
DNA repair and excision	*RAD7, RAD16, EAF7, MMS4, REV7, UBC13, YNK1*
Transcription regulators	*MSS11, TEA1, RFX1, MRK1,* ***HAP4***, *CSI1, URC2, SPT7, HAA1,* ***EAF7***, ***HAP2***, *NOT3,* ***HAP5***, *YAP7, TYE7, ACM1, HOS2, LAG2*
RNA processing	*NNF2, MUD1, CAF40, PES4*
Ribosomal protein; translation and protein synthesis	*RPP2A, RPL24B,* ***RPL37A***, ***RPS28B***, *RPL29, RPL24A, RPL23A, RPL23B, RPL16A, RPL41B,* ***RPL13A***, ***RPS16B***, *RPS17B, RPS16A, RPL27B, RPL19A, RPS25A,* ***RPL1B***, *RPS30A, RPS1B*
Ribosome biogenesis	*SYO1, NOP12, GVP36*
Signal transduction, GSE/EGO complex	***MDS3, GTR1, GTR2,*** *LTV1, PIB2,* ***CKA2,*** *RCN1, CNB1,* ***SIP5,*** *GIS4*
Ubiquitination & proteasome complex activity	***UBI4***, ***UBR2***, ***UBC13***, *PRE9, MMS2,* ***MUB1***, ***NPL4***, *UBR1, HUL5, ELA1*
Nucleotide biosynthesis	*YNK1, URA4, AMD1*
Cytoplasmic L-methionine salvage	*UTR4*
Glycogen biosynthesis, glycolysis and gluconeogenesis	***TDH3***, *GSY2*
Glycerol metabolism	*GPD2*
NADH-dependent aldehyde reductase	*YNL134C*
NAD biosynthesis	*YEF1*
Undefined/ unclear/unknown	***IRC19***, *OCA4,* ***OCA5***, *GDS1, FHN1, STB6, NQM1, PBP4, FMP33, FMP48, BSC4, CMR3, CMG1, SDD2,* YPR078C, YOR333C, YOL079W, YOR309C, YNR005C, YML084W, YMR141C, YML083C, YKL147W, YLR407W, YLR169W, YLR125W, YLR184W, YLL047W, YKL075C, YJR056C, YIL161W, YJL028W, YIL141W, YGR219W, YGR137W, YGR122C-A, YFL032W, YFL015C, YER038W-A, YDR442W, YBR013C, YDL094C, YDL062W, YBR287W, YML095C-A, YBR144C, YBR138C, YJL017W, YJL206C-A, YBR096W, YGR153W

Figure S1: An example of a read-out from the chemogenomic screen. The same mutant strains from a 96-well plate were spotted on these three agar plates. The top agar plate contains DMSO (diluent of polygodial) and is a control plate. The middle agar plate contains 2 μg/mL polygodial (to detect hypersensitive strains) while the bottom plate contains 6 μg/mL polygodial (to detect resistant strains). Ranking was based on the growth of the mutants relative to the WT BY4742. Lower ranks (0-2) represent poor growth, 3 represents WT and more growth than WT ranks from 4-6.

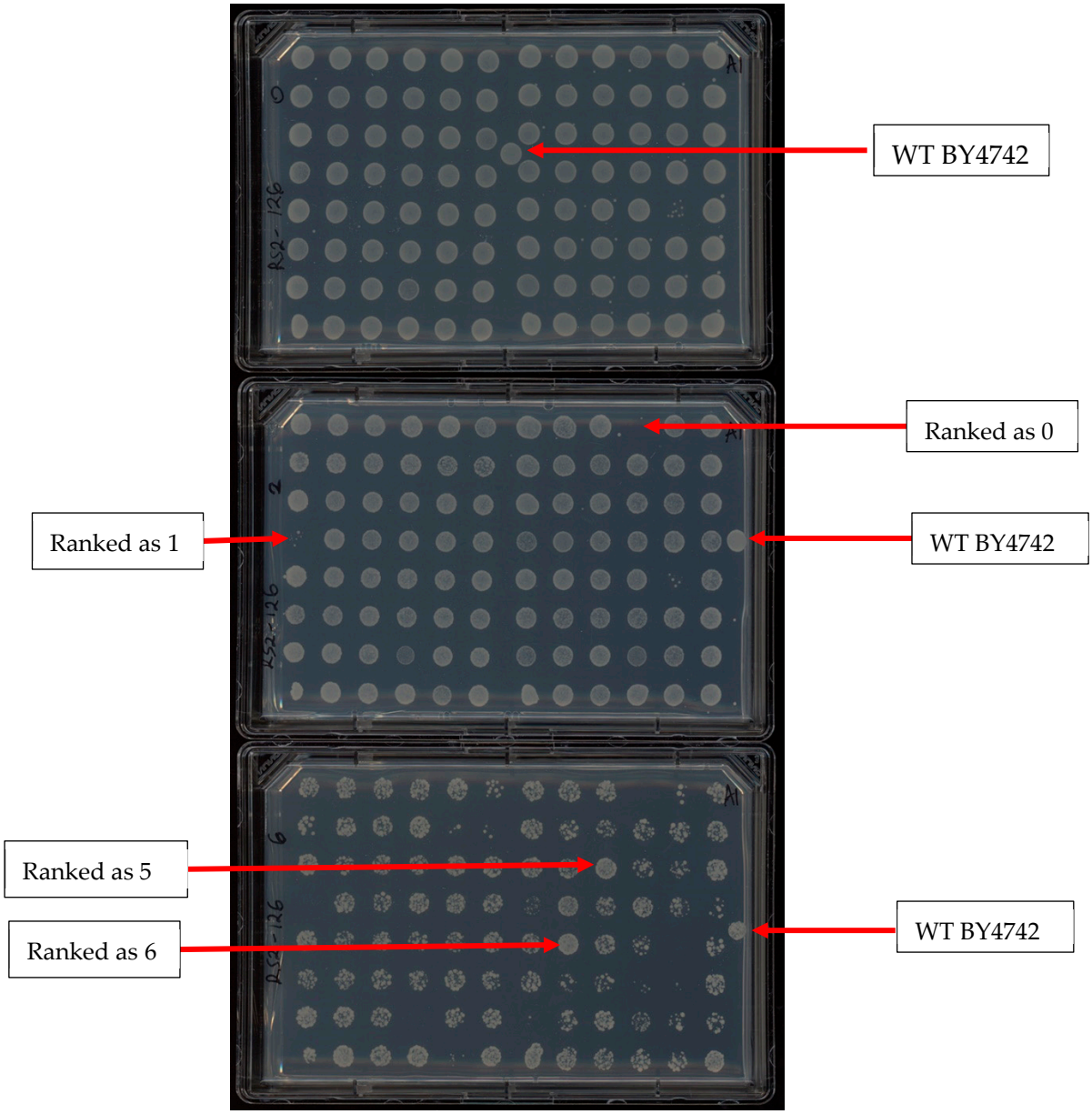

Figure S2: A clustering visualization of all polygodial sensitive strains using the STRING database. The colored circular nodes represent proteins, while the edges joining the nodes represent interactions (protein-protein), either established known interactions or predicted interactions.

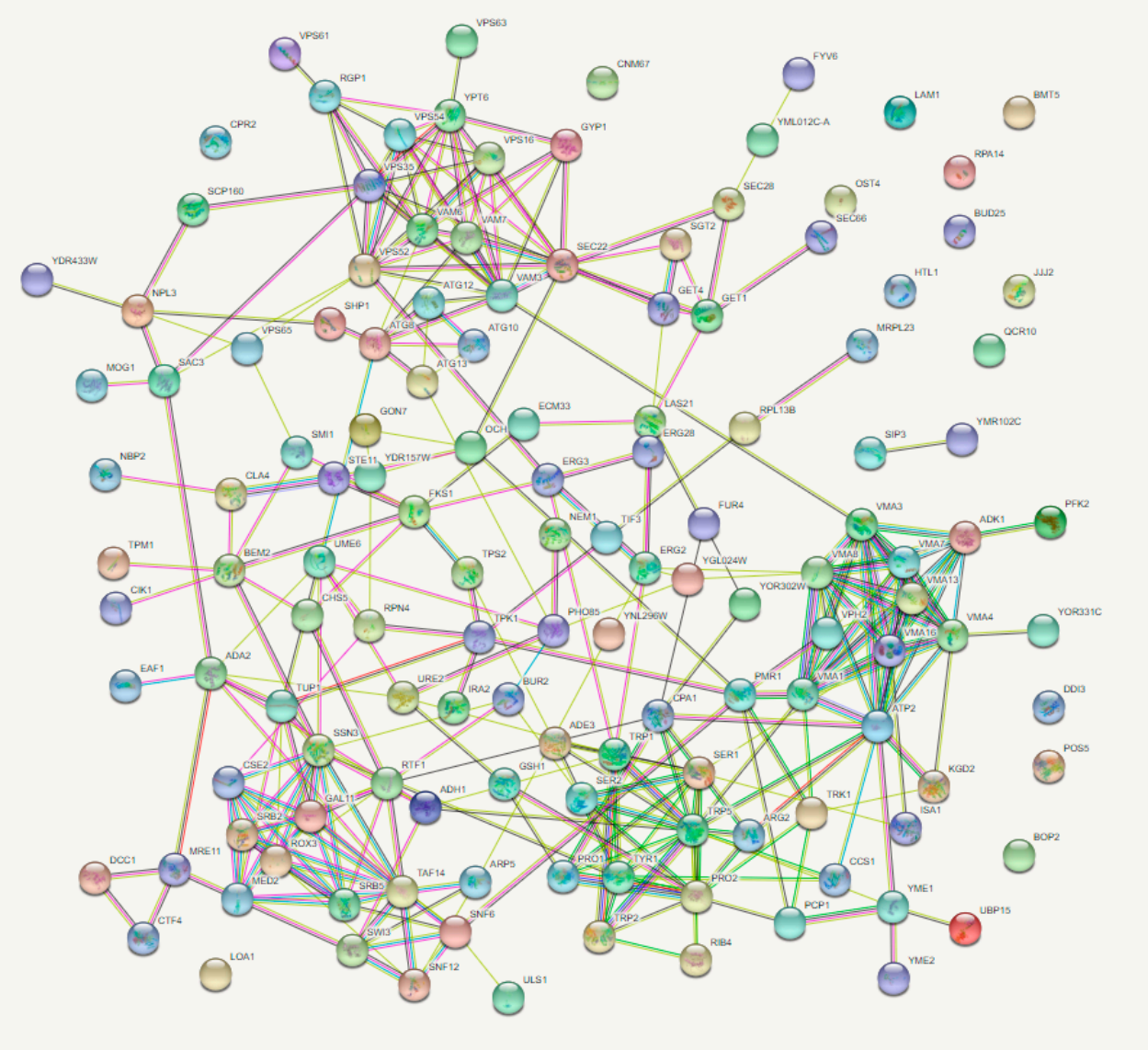

Figure S3: A clustering visualization of all polygodial resistant strains using the STRING database. The colored circular nodes represent proteins, while the edges joining the nodes represent interactions (protein-protein), either established known interactions or predicted interactions.