Additional file 1 – Supplementary figures S1 - S11 and their corresponding figure legends (PDF, This file can be viewed with: Adobe Acrobat Reader).

Figure S1. Chitosan oligosaccharide (COS-5.44) has higher antifungal activity than higher molecular weight chitosan (T8s). Chitosan oligosaccharide (5.44 kDa) and chitosan (70KDa) were screened in wild type yeast (BY4743) in 0.5X YPD pH 5. Five concentrations of COS-5.44 (91.1 – 112.5 µg/ml) and 4 of chitosan (125 – 162.5 µg/ml) were tested. Optical density readings were taken every 15 minutes over 20 hrs using a Tecan Genios reader (see Methods).

Figure S2. Multicopy suppression profile assay (MSP) of yeast growing in 0.5X YPD pH 5 with 112.5 µg/ml of chitosan oligosaccharide (COS-5.44). The scatter plot shows the correlation between the two biological replicates of the multicopy suppression profile assay (MSP). Sixty-eight genes are potential suppressors to COS-5.44 sensitivity. Potential suppressors identified by replicate 1 are shown in red, replicate 2, blue and in both replicates, purple. The twenty-one strains confirmed as suppressors are indicated by their gene name. The ARL1 suppressor strain was also found in the HIP-HOP assay as a sensitive deletion strain (highlighted).

Figure S3. Enriched biological processes from transcript changes in COS and Chitosan treated cells. The GSEA enrichment map displays a comparison of enriched terms from the transcriptional profile of COS treated wild type cells from our study with transcript data from chitosan treated cells from Zakrzewska et al. (2005). Enrichments were mapped to the inner node area and to the node border, respectively. A node represents a biological process significantly enriched (FDR < 0.001, see Methods). The node size correlates to the number of genes annotated to that functional category. Red and blue node border colours indicate the biological processes that are enriched in the up- and down-regulated gene transcripts respectively. The thickness of the edge correlates to the degree of gene overlap between the two connected categories. If the overlap coefficient is less than 0.5 edges are not shown (see Methods). Cluster relationship is shown by edge colour, where blue edges represent “gene overlap/relationship among biological processes” in our study and green in the Zakrzewska et al. (2005) data set. Clustering of terms is based on degree of overlap of genes among the categories.

Figure S4. Hierarchical clustering of the expression profiles of COS-5.44 resistant yeast overexpressing strains compared with the wild type in the absence of COS-5.44. One hundred and eighty four genes showed a significant change in expression (P-value ≤ 0.05 and log2 fold change ≥ 1 or < -1) in at least one of the overexpression strains compared with the wild type basal expression, clustering was based in similarity (see Methods). A) Significantly up-regulated genes in Arl1 and Rba50 overexpressing strains. B) RBA50 gene is up-regulated in the corresponding overexpressing strain. C) Cluster of down-regulated genes among the 5 overexpressing strains, except that MSG5 is up-regulated in the corresponding overexpressing strain. D) Cluster of up-regulated genes among the 5 overexpressing strains.
Figure S5. Biological processes associated with differentially expressed genes in an *Arl1* overexpression strain when exposed to chitosan oligosaccharide (COS). A node represents a biological process significantly enriched (FDR ≤ 0.1, see Methods). Boxes on the side show summary of the main biological process found in a cluster. The node size correlates to the number of genes annotated to that functional category. Red and blue node border colours indicate the biological processes that are enriched in the up- and down-regulated gene transcripts respectively. The width of the edge correlates to the degree of gene overlap between the two connected categories. If the overlap coefficient is less than 0.5, edges are not shown (see Methods). Cluster of terms is shown by node color, where clustering is based on degree of overlap of genes among the categories. Bar plots show log fold change of the genes that contributed to the functional enrichment of the cluster, with the border color surrounding the plots correlating with the nodes in the cluster. The top 10 genes in each cluster category are shown in each plot.

Figure S6. Biological process associated with differentially expressed genes on *Bck2* overexpression strain when exposed to chitosan oligosaccharide (COS). For details in annotation refer to Figure S5.

Figure S7. Biological process associated with differentially expressed genes on *Erg24* overexpression strain when exposed to chitosan oligosaccharide (COS). For details in annotation refer to Figure S5.

Figure S8. Biological process associated with differentially expressed genes on *Msg5* overexpression strain when exposed to chitosan oligosaccharide (COS). For details in annotation refer to Figure S5.

Figure S9. Biological process associated with differentially expressed genes on *Rba50* overexpression strain when exposed to chitosan oligosaccharide (COS). For details in annotation refer to Figure S5.

Figure S10. Quantitative RT-PCR verification of the microarray data for 4 up-regulated (*VBA5, MUC1, YJU2* and *FIG2*) and 5 down-regulated genes (*CMD1, COX5b, UBI4, RCR1* and *HSP30*) yields similar results. Two biological replicates from the wild type (vector control BY4743) and the *Arl1* overexpressing strain that were either untreated or exposed to COS-5.44 for 60 min were used for qRT-PCR. Expressed as log2 fold change as relative expression to the wild type samples.

Figure S11. Effect of yeast environmental stress response agents on yeast WT (BY4743) grown in 0.5X YPD pH 5, amended with chitosan oligosaccharide (COS-4.55). Wild type cells do not acquire resistance to COS-4.55 treatment after exposure to sub lethal doses of thermal (B), salt (C), osmotic (D) and oxidative (E-F) stresses. A) Mock Control (normal growth conditions).
**Figure S1**

### COS 5.44 kDa

- 1% DMSO control
- 82 µg/ml
- 91.1 µg/ml
- 96 µg/ml
- 101.25 µg/ml
- 106.8 µg/ml

### Chitosan 70kDa screen

- HCL pH5.7 control
- 125 µg/ml
- 137.5 µg/ml
- 150 µg/ml
- 162.5 µg/ml
Confirmed suppressors of COS sensitivity

Figure S2
Glucose metabolism
Protein catabolism
Response to temperature
Proteosomal ubiquitin catabolism
Trehalose biosynthesis

Mitochondrion organization
Mitochondrial translation
Mitochondrial ribosome subunits
Respiratory chain complex assembly

Oxidation reduction
Cellular respiration
ATP production

Nucleotide metabolism
Proton transport
Nitrogen compound biosynthesis
Regulation of ATPase activity

Protein folding
Ribosomal large subunit

Figure S5
qRT-PCR confirmation of microarray results

- **Gene**: VDJ-1, MUC1, Y-1,2, PHE1, UCA1, UBI4, RCR1, HSPA1A

- **Y-axis**: Expression level

- **Legend**:
  - **microarray** (black bars)
  - **qRT-PCR** (gray bars)

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**Figure S10**
