# Supplementary Information (SI)

This file contains supplementary information for manuscript entitled “Yeast mannan-rich fraction modulates endogenous reactive oxygen species generation and antibiotic sensitivity in resistant *E. coli*”.

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**This file includes:**

Figures S1, S2 and S3
Tables S1 and S2

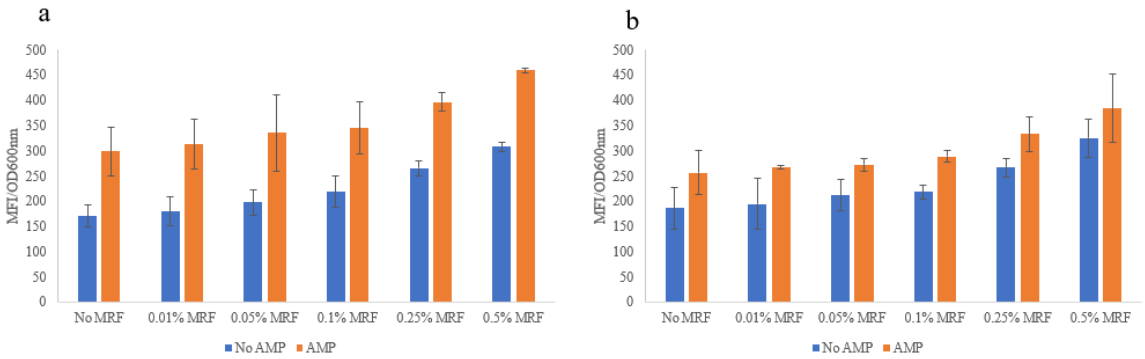


**Figure S1. The effect of MRF (0.01 – 0.5%) on ROS (MFI) combined with and without ampicillin.** Diagram displays mean fluorescent intensity (MFI/OD600nm) of [a] AMP susceptible *E. coli* treated with AMP (5μgmL$^{-1}$) compared to no antibiotic treatment and [b] AMP resistant *E. coli* treated with AMP (5mgmL$^{-1}$) compared to no antibiotic treatment. Standard deviation is represented by error bars (n=6).

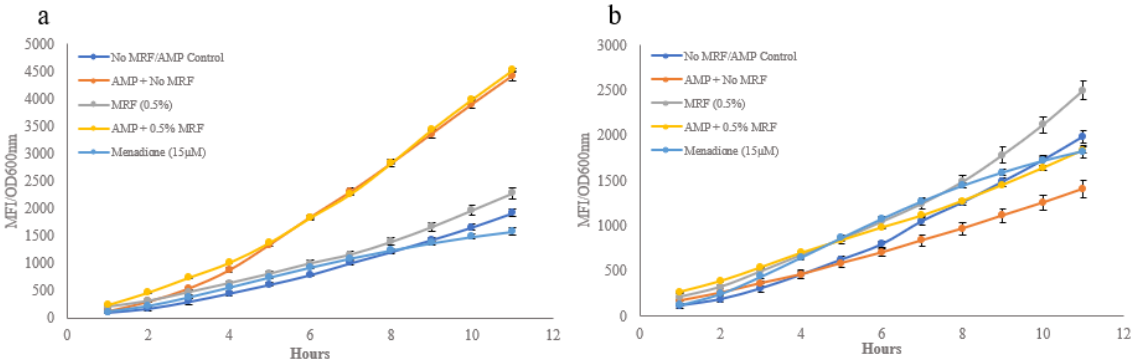


**Figure S2. The effect of MRF on ROS (MFI) with and without ampicillin treatment, over time.** Diagram displays mean fluorescent intensity (MFI/OD600nm) of [a] AMP susceptible *E. coli* over time; control (No MRF/AMP)] compared to AMP (5μgmL$^{-1}$), MRF (0.5%, w/v) and MRF (0.5%, w/v) combined with AMP (5μgmL$^{-1}$); [b] AMP resistant *E. coli* over time; control (No MRF/AMP) compared to AMP (5mgmL$^{-1}$), MRF (0.5%, w/v) and MRF (0.5%, w/v) combined with AMP (5mgmL$^{-1}$). Standard deviation is represented by error bars (n=6). Means that are significantly different to the respective control conditions are marked with an asterisk [*] ($p \leq 0.05$, ANOVA, Fisher-LSD).

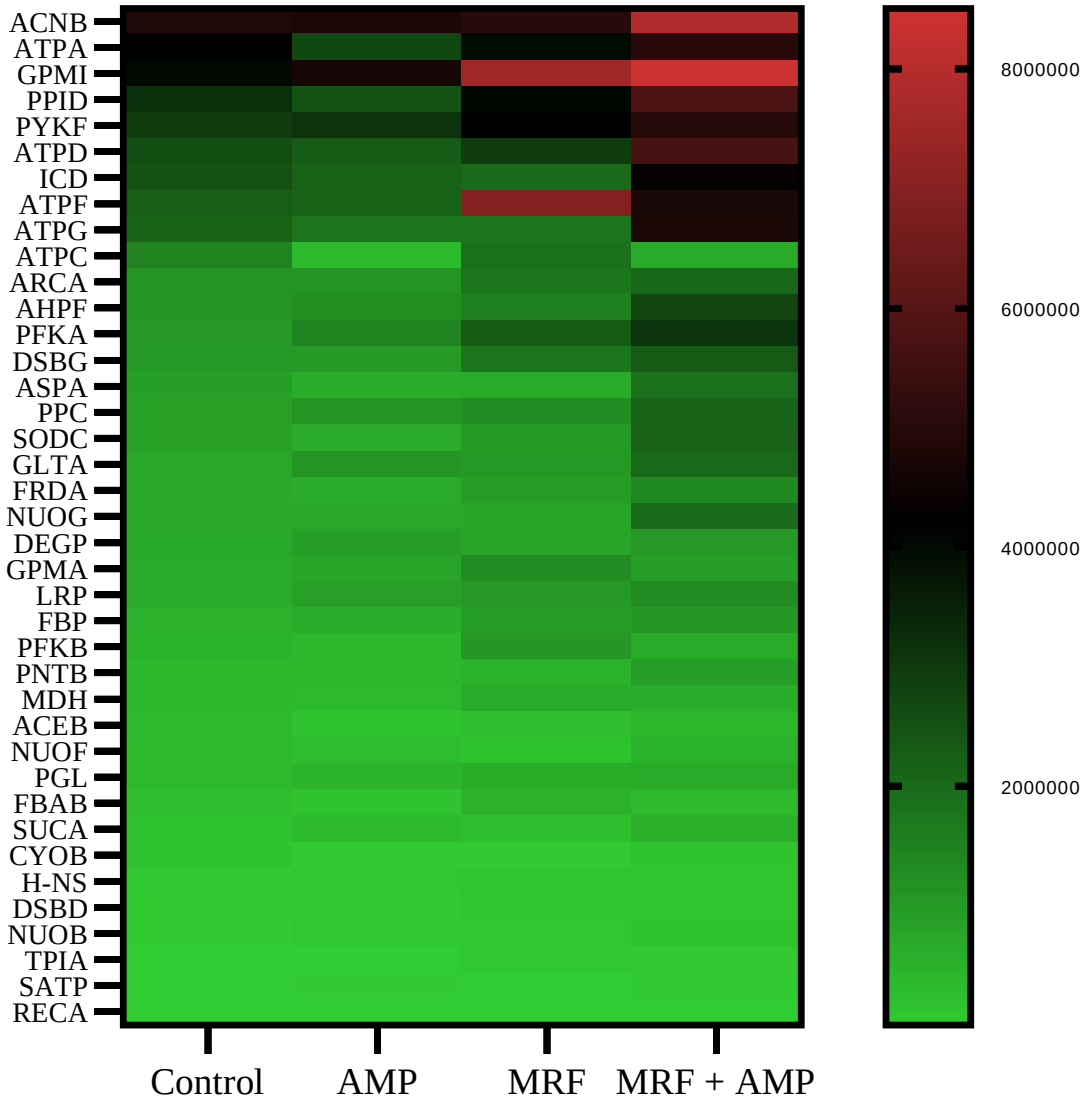


**Figure S3. The influence of MRF and antibiotic treatment on normalized peptide abundance of relevant proteins of interest.** Control culture treated with Amp (0.1 mgmL$^{-1}$ Amp), MRF (0.1%, w/v), or combination of Amp (0.1mgmL$^{-1}$) + MRF (0.1%, w/v).

**Table S1.** The influence of MRF (1 mgmL$^{-1}$) and antibiotic treatment (0.1 mgmL$^{-1}$ AMP) on fold change expression (normalized peptide abundance) of other detected intermediates of the citric acid cycle compared to control.

| | | | | | | MRF | | AMP | | MRF + AMP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accession | Protein | PC | UP | PSM | SC (%) | Fold change[a] | *p*-value | Fold change[a] | *p*-value | Fold change[a] | *p*-value | Highest Mean Condition |
| Q8X7C7 | ACNA | 9 | 9 | 71 | 12 | -0.15 | 0.14 | -0.21 | 0.09 | +0.44 | 0.28 | MRF + AMP |
| P0A838 | SUCC | 8 | 8 | 73 | 24 | +0.43 | 0.20 | -0.18 | 0.21 | +0.17 | 0.21 | MRF |
| P0AGF1 | SUCD | 6 | 6 | 48 | 24 | +0.09 | 0.56 | -0.40 | 0.17 | -0.57 | 0.12 | MRF |
| P0AC43 | SDHA | 2 | 2 | 12 | 3 | +0.55 | 0.10 | +0.22 | 0.40 | +0.19 | 0.55 | MRF |
| A0A0H3JRL7 | FUMA | 4 | 2 | 10 | 10 | +2.06 | 0.25 | +1.61 | 0.30 | +3.96 | 0.08 | MRF + AMP |
| P0A8Q2 | FRDC | 1 | 1 | 6 | 8 | +0.45 | 0.28 | +0.09 | 0.71 | -0.32 | 0.29 | MRF |
| Q8XDS0 | ASPA | 7 | 7 | 31 | 12 | -0.27 | 0.23 | -0.31 | 0.15 | +1.02 | 0.06 | MRF + AMP |
| Q8X743 | PPC | 14 | 14 | 102 | 18 | +0.52 | 0.17 | +0.31 | 0.21 | +0.45 | 0.12 | MRF |

[a]Fold change increase symbolized with a [+]. Fold change decrease symbolized with a [-]. Unequal variances T-test comparison of each treatment group to control was performed, ANOVA ($p \leq 0.05$). Abbreviations: PC; Peptide count, UP; Unique peptides, SC (%); PSM; Peptide spectrum match; Sequence coverage (%); ACNA; Aconitate hydratase, SUCC; Succinate--CoA ligase [ADP-forming] subunit beta, SUCD; Succinate-CoA ligase [ADP-forming] subunit alpha, SDHA; Succinate dehydrogenase flavoprotein subunit, FUMA; Fumarate hydratase class I, FRDC; Fumarate reductase subunit C, ASPA; Aspartate ammonia-lyase, PPC; Phosphoenolpyruvate carboxylase.

**Table S2.** The influence of MRF (1 mgmL$^{-1}$) and antibiotic treatment (0.1 mgmL$^{-1}$ AMP) on fold change expression (normalized peptide abundance) of other detected key components of central metabolic pathways compared to control.

| | | | | | | | MRF | | AMP | | MRF + AMP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Accession | Protein | PC | UP | PSM | SC (%) | Fold change[a] | *p*-value | Fold change[a] | *p*-value | Fold change[a] | *p*-value | Highest Mean Condition |
| **Acetyl-coenzyme A** | Q8XCU8 | PTA | 10 | 10 | 56 | 18 | +0.12 | 0.62 | -0.25 | 0.39 | +0.32 | 0.23 | MRF + AMP |
| | P0A6A5 | ACKA | 2 | 2 | 9 | 8 | +0.19 | 0.52 | +0.21 | 0.51 | +0.81 | 0.35 | MRF + AMP |
| | Q8X5T5 | ACS | 1 | 1 | 3 | 2 | -0.42 | 0.27 | -0.17 | 0.54 | +0.52 | 0.23 | MRF + AMP |
| | Q8X6L4 | POXB | 6 | 6 | 31 | 10 | +0.04 | 0.75 | +0.01 | 0.94 | +0.57 | 0.09 | MRF + AMP |
| **EMP pathway** | P0A8A6 | PPSR | 1 | 1 | 4 | 3 | -0.24 | 0.12 | -0.17 | 0.10 | +0.27 | 0.33 | MRF + AMP |
| | P0A9B4 | GAPA | 9 | 9 | 70 | 30 | +0.95 | 0.07 | +0.05 | 0.82 | +0.09 | 0.68 | MRF |
| | Q8XD03 | PGK | 11 | 11 | 67 | 26 | -0.01 | 0.86 | -0.12 | 0.35 | +1.27 | 0.06 | MRF + AMP |
| | P0A6Q1 | ENO | 13 | 13 | 71 | 46 | +3.62 | 0.12 | +0.30 | 0.36 | +3.92 | 0.06 | MRF + AMP |
| | P0ADF7 | EDD | 2 | 2 | 10 | 7 | +0.91 | 0.18 | -0.18 | 0.62 | +0.47 | 0.33 | MRF |
| | Q8X774 | PNTA | 1 | 1 | 4 | 2 | -0.44 | 0.06 | -0.25 | 0.17 | +0.34 | 0.17 | MRF + AMP |
| | Q7DBF8 | GND | 9 | 9 | 61 | 28 | +0.28 | 0.24 | -0.18 | 0.37 | +0.65 | 0.23 | MRF + AMP |
| | P0A8A6 | PPSR | 1 | 1 | 4 | 3 | -0.24 | 0.12 | -0.17 | 0.11 | +0.27 | 0.33 | MRF + AMP |
| **OxPhos** | P0ABJ0 | CYOB | 1 | 1 | 7 | 1 | -0.70 | 0.06 | -0.57 | 0.02 | +0.01 | 0.86 | MRF + AMP |
| **Bacterial stress signalling and response** | P66828 | SODA | 4 | 4 | 46 | 18 | +0.17 | 0.47 | -0.31 | 0.33 | +0.28 | 0.54 | MRF + AMP |
| | P0AGD5 | SODB | 1 | 1 | 5 | 8 | +0.81 | 0.39 | +0.42 | 0.61 | +0.81 | 0.36 | MRF + AMP |
| | A0A0H3JFP5 | KATE | 18 | 18 | 110 | 24 | +0.08 | 0.86 | -0.29 | 0.56 | +0.32 | 0.51 | MRF + AMP |
| | Q7A978 | KATG1 | 10 | 10 | 53 | 13 | +0.66 | 0.09 | +0.05 | 0.55 | +0.35 | 0.45 | MRF |
| | Q7ABA5 | RPOS | 1 | 1 | 11 | 3 | +0.07 | 0.36 | +0.33 | 0.35 | +0.79 | 0.10 | MRF + AMP |
| | P0ACK0 | CRP | 6 | 6 | 38 | 33 | +0.39 | 0.12 | -0.40 | 0.16 | +0.05 | 0.81 | MRF |
| | P0AE89 | CPXR | 3 | 3 | 33 | 13 | +0.06 | 0.75 | -0.49 | 0.13 | -0.29 | 0.22 | MRF |
| | P66798 | UVRY | 1 | 1 | 7 | 6 | +0.43 | 0.26 | -0.20 | 0.46 | +0.87 | 0.06 | MRF + AMP |
| | Q8XDH7 | TREA | 7 | 7 | 59 | 14 | -0.26 | 0.18 | -0.21 | 0.22 | +0.09 | 0.44 | MRF + AMP |
| | P0A6Y3 | IHF | 1 | 1 | 6 | 10 | +1.32 | 0.15 | -0.40 | 0.19 | +1.77 | 0.16 | MRF + AMP |
| | P0A974 | CSPE | 2 | 2 | 6 | 39 | -0.33 | 0.13 | -0.11 | 0.63 | +0.10 | 0.56 | MRF + AMP |
| | P0AEG5 | DSBA | 4 | 4 | 23 | 24 | +0.55 | 0.24 | -0.01 | 0.96 | -0.58 | 0.22 | MRF |
| | P0AEG7 | DSBC | 2 | 2 | 14 | 7 | +0.51 | 0.49 | +0.65 | 0.41 | +3.63 | 0.17 | MRF + AMP |
| | P0AFL5 | PPIA | 2 | 2 | 18 | 15 | +0.07 | 0.27 | +0.01 | 0.77 | +1.71 | 0.20 | MRF + AMP |

[a]Fold change increase symbolized with a [+]. Fold change decrease symbolized with a [-]. Unequal variances T-test comparison of each treatment group to control was performed, ANOVA ($p \leq 0.05$). Abbreviations: PC; Peptide count, UP; Unique peptides, SC (%); PSM; Peptide spectrum match; Sequence coverage (%); PTA; Phosphate acetyltransferase, ACKA; Acetate kinase, ACS; Acetyl-coenzyme A synthetase, POXB; Pyruvate dehydrogenase, PPSR; Phosphoenolpyruvate synthase regulatory protein, GAPA; Glyceraldehyde-3-phosphate dehydrogenase A, PGK; Phosphoglycerate kinase, ENO; Enolase, EDD; Phosphogluconate dehydratase, PNTA; NAD(P) transhydrogenase subunit alpha, GND; 6-phosphogluconate dehydrogenase, decarboxylating, PPSR; Phosphoenolpyruvate synthase regulatory protein, CYOB; Cytochrome bo(3) ubiquinol oxidase subunit 1, SODA; Superoxide dismutase [Mn], SODB; Superoxide dismutase [Fe], KATE; Catalase, KATG1; Catalase-peroxidase 1, RPOS; RNA polymerase sigma factor RpoS, CRP; cAMP-activated global transcriptional regulator, CPXR; Transcriptional regulatory protein, UVRY; Response regulator, TREA; Putative periplasmic trehalase, IHF; Integration host factor subunit beta, CSPE; Cold shock-like protein, DSBA; Thiol:disulfide interchange protein, DSBC; Thiol:disulfide interchange protein, PPIA; Peptidyl-prolyl cis-trans isomerase A.