# Lactobacilli modulate epithelial cytoprotection through the Nrf2 pathway

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## Experimental procedures

### Fly culture and strains

Flies were maintained on standard media at 25°C. The *gstD-gfp* reporter line*, UAS-cncC, UAS-keap1, UAS-cncC*$^{RNAi}$ and *UAS-keap1*$^{RNAi}$ was a gift from Dirk Bohmann (Sykiotis and Bohmann, 2008). *MyoIA-GAL4*; was a gift from Shigeo Takashima (Takashima et al., 2008).

### Microarray analysis of Drosophila midgut tissue

RNA from forty dissected *Drosophila* midguts per sample was prepared using TRIzol reagent (Invitrogen, Carlsbad, California, USA), and cleaned using RNeasy kit (Qiagen) total RNA clean up protocol. Microarray analysis was undertaken by Vanderbilt University Medical Center Genome Sciences Resource (Vanderbilt University, TN) using a Drosophila Gene Expression Microarray platform**,** 4x44K (Agilent Technologies Cat# G2519F). Genes with >1.8 fold change were further analyzed for the presence of a Antioxidant Response Element (ARE) with the Scope Motif Finder (Dartmouth College) (Chakravarty et al., 2007) (http://genie.dartmouth.edu/SCOPE/) using the DNA sequence 'tkaynnngcr', which is a 10 bp core element that lies at the center of the 20 bp ARE consensus sequence (Nioi et al., 2003) (**Table S1)**. These data have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE70715 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70715).

### Generation of germ-free *Drosophila* and Gnotobiotic colonization of *Drosophila* larvae or adult flies.

*Drosophila* embryos were collected and transferred to a cell strainer. Under a sterile hood, embryos were washed three times with sterile PBS, soaked in 50% bleach for 5 minutes, before washing again with sterile PBS. The mesh of the cell strainer was cut with a sterile blade, and transferred into a sterile Perti dish containing sterilize *Drosophila* food, and incubated for 24 hours at 25°C. For microarray analysis for the detection of genes upregulated in response to Paraquat challenge, germ-free larvae were grown until third instar stage of life before being transferred into another Petri dish containing 2ml liquefied sterile *Drosophila* food containing a total of $1x10^6$ cfu pure bacterial culture. After 4 hours, the midgut of the third-instar larvae were dissected, and total RNA isolated as described above. To determine that the larvae ingested *L*. *plantarum*, five larval intestines were dissected into 1ml sterile PBS, and cfu calculated by plate count method. We

detected $5.5\times10^5$ total cfu of *L. plantarum* per dissected instar larval intestine (Std. dev. = $4\times10^4$, *n*=5). For cytoprotection assays in adult *Drosophila*, germ-free flies of assayed phenotype were raised until 5-days-old. Then, flies were transferred in groups of 10 into sterile vials containing 2ml liquefied sterile *Drosophila* food containing a total of $1x10^6$ cfu pure bacterial culture for 6 hours before addition of Paraquat solution to a final concentration of 25mM in 2ml. Survivors were scored for up to 5 days, or until 100% lethality. Percent surviving flies were recorded and compared by log-rank Martel–Cox test using Graphpad Prism 5 Software (GraphPad Software, Inc. La Jolla, CA). To determine that the adult Drosophila ingested similar numbers of bacterial monoculture, adult intestines were dissected into 1ml sterile PBS, and cfu calculated by plate count method. In experiments described in figure 1 and supplementary figure 1, we detected $1\times10^6$ cfu *L. plantarum* per adult intestine (Std. dev. = $5\times10^4$, *n*=5), $1.3\times10^5$ cfu *B. cereus* per intestine (Std. dev. = $1.2\times10^4$, *n*=5), $8\times10^5$ cfu *S. capitis* per intestine (Std. dev. = $4\times10^4$, *n*=5), $3\times10^6$ cfu *A. piechaudii* per intestine (Std. dev. = $5\times10^4$, *n*=5), $7\times10^5$ cfu *A. xylosoxidans* per intestine (Std. dev. = $8\times10^4$, *n*=5) respectively. In experiments described in figure 2E and 2F, we detected $2.2\times10^6$ cfu *L. plantarum* per *myoIA*-gal4 UAS-*gal4*$^{IR}$ adult intestine (Std. dev. = $3\times10^4$, *n*=5), and $1.6\times10^6$ cfu *L. plantarum* per *myoIA*-gal4 UAS-*cncC*$^{IR}$ adult intestine (Std. dev. = $1\times10^5$, *n*=5). In experiments described in figure 4A, we detected $5.8\times10^6$ cfu *L. plantarum* per *myoIA*-gal4 UAS-*gal4*$^{IR}$ adult intestine (Std. dev. = $2.4\times10^4$, *n*=5), and $5.6\times10^6$ cfu *L. plantarum* per *myoIA*-gal4 UAS-*dnox*$^{IR}$ adult intestine (Std. dev. = $6\times10^4$, *n*=5).

**Quantitative (q) PCR Confirmation of Gene Expression Fold Change detected by Microarray.**

RNA from three independent replicates (each containing 10 intestines per assay) was extracted using TRIzol reagent (Invitrogen, Carlsbad, California, USA), and cleaned using RNeasy kit (Qiagen) total RNA clean up protocol. Transcript levels were measured for each replicate in duplicate by qRT-PCR. *cyp6a18* transcript levels were amplified using primers Cyp6a18-RT-F 5'-ACATACTTTCTCTTCCAAGTGGC-3', and Cyp6a18-RT-R 5'-TGGGGTTTATCATACGGAATGC-3', and *gstZ2* transcripts amplified using primers *gstZ2*-RT-F,5'-CCGCGAGGTGAATCCAATG-3', and *gstZ2*-RT-R 5'- CTGGGGACGTGTTTCCTCC-3'. *Cyp6a18* and *gstZ2* transcript levels were normalized against *rp49* transcript levels measured using Rp49-F, 5'- AGCATACAGGCCCAAGATCG-3' and Rp49-R, 5'-TGTTGTCGATACCCTTGGGC-3'. The data generated by qPCR assays were normalized using the average value of the PBS treatment control group.

**Quantitative (q)PCR for the detection of transcript depletion in Drosophila expressing enterocyte-specific RNA$^{IR}$ against CncC or Keap1**.

RNA from three independent replicates of dissected midgut per genotype (each containing 10 intestines per assay) was extracted using TRIzol reagent (Invitrogen, Carlsbad, California, USA), and cleaned using RNeasy kit (Qiagen) total RNA clean up protocol. Transcript levels were measured for each replicate in duplicate by qRT-PCR. Primers used in include CncC-F 5'-CTGCATCGTCATGTCTTCCAGT-3' and CncC-R 5'-AGCAAGTAGACGGAGCCAT-3', Keap1-F 5'-AGGCCAATGTGTTTATTGAGCG-3', and Keap1-R 5'- GCAATCAACTGATATGCCGAAAG-3'. Transcript levels were normalized against *rp49* transcript levels measured using Rp49-F, 5'-AGCATACAGGCCCAAGATCG-3' and Rp49-R, 5'- TGTTGTCGATACCCTTGGGC-3'. The data generated by qPCR were normalized using the average value of the isogenic non-specific RNA$^{IR}$ controls (**Fig S3**).

**RNA-Seq analysis of Murine colonic tissues**

Germ-free mice (7 weeks old) purchased from (Taconic farms Inc., NY, USA) were divided into three groups of treatment (n=4) that received an oral gavage of (2 X $10^9$) cfu/mL of *L. rhamnosus* GG, *E. coli* and PBS respectively. Four hours after respective treatments mice were euthanized and colon tissues (4 cm) were excised and immediately frozen on dry ice in microcentrifuge tubes containing in 1 mL *TRIzol*® Reagent (Invitrogen, 15596-018). Colonic tissues from respective treatment groups were homogenized in Trizol reagent, followed by chloroform/isopropyl alcohol extraction to generate crude RNA preparations. Crude RNA extracts were quantified using a nanodrop ND-1000 (Thermo Scientific, USA) and further cleaned up using on column RNase-free/DNase set (Qiagen, Cat #79254) treatment and RNAeasy spin column purification (Qiagen, Cat #74104). Purified RNA were subjected to QC analysis using Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) using a cut-off RNA Integrity Number (RIN) of 7, samples from each treatment group were pooled together (n=4) to make three samples each of (1) PBS (2) LGG (3) *E.coli* treatment groups. Purified RNA pools (2 µg) were further processed by Emory EIGC (Emory Integrated Genomics Core) and Hudson Alpha (AL, USA) to generate RNAseq libraries. These libraries were then subjected to 50 million paired-end reads sequencing using the Illumina HiSeq2000 platform.These data have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE70715 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70715).

**Bioinformatics analysis**

FASTQ files generated from the Illumina HiSeq2000 were analyzed by Emory Integrated Genomics Core Computing Division. RNA-Seq Data Analysis Pipeline used by EIGC computing division subjected raw data for quality filtering and trimming using FastQC (v.0.52) and FASTQ Quality Trimmer (v1.0.0). RNA-Seq reads were mapped to *Mus musculus* reference genome using a splice junction mapper TopHat2 (v0.5) (Kim et al., 2013). Transcripts assembly and relative abundances were estimated using Cufflinks (v2.1.1). Differentially expressed genes and transcripts between each treatment groups (PBS, *E.coli*, LGG) were calculated using Cuffmerge/Cuffdiff (v.2.0.2) (Trapnell et al., 2013). Cufdiff outputs were further screened for differentially expressed genes with a $\log_2$-fold change of 0.5 to 1 and a cut-off p-value of ($p<0.005$). Genes sorted for significant differential expression between treatment groups, (E.coli vs LGG) and (PBS vs. LGG) were further screened to identify ARE (antioxidant response elements) motifs using SCOPE version 2.1.0 (Suite for Computational identification Of Promoter Elements). Selected genes containing ARE motifs in their promoters were then validated using qPCR analysis from RNA samples (n=4) biological replicates of respective treatment groups **(Fig. S4 and Table S2)**.

**Murine subjects and γ-irradiation**

All experiments were done using 8-week-old $Nrf2^{-/-}$ mice were generated as described in Chan et al. (Chan et al., 1996) and C57BL/6 background littermates raised in the Emory University Department of Animal Resources. For irradiation insults, mice whole bodies were exposed to 12 Gy of γ-radiation using a γ-Cell 40 $^{137}Cs$ irradiator at a dose rate of 75 rads/min. *L. rhamnosus* GG ($2x10^9$ cfu total) was administered by oral gavage daily for 4 days before irradiation, and body weights and mortality were monitored. Animal experiments were approved by the Emory University institutional ethical committee and performed according to the legal requirements. Histological sections of the colon were prepared from four irradiated animals per treatment. Sections were assessed by TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assay (Roche, Indianapolis, Indiana, USA). TUNEL-positive cells were counted and the average number of positive cells in forty 200× fields per treated animal was determined.

**Confirm changes in Gene Expression in bacterial-fed B6 and $Nrf2^{-/-}$ mice by rtPCR**

The total mRNA (1 µg) was reverse transcribed into cDNA using QuantiTect® Reverse Transcription Kit (cat # 205311, Qiagen). Relative quantification of mRNA expression was performed using QuantiFast SYBR® Green PCR Kit (cat # 204054, Qiagen) on a *MyiQ*™ Real

time PCR system (Biorad). Delta-delta Ct analysis (ΔΔCT) method was used to quantify relative gene expression compared with Actin controls, using following primers: Cyp2c65-F,5'-GAGTTTGCTGGAAGAGGAGTT-3', Cyp2c65-R,5'-CGCAGAGTCATGAGTGAGAAG-3', Cyp2c55-F,5'-GCTGTTGCTATGCTGGTATCT-3', Cyp2c55-R,5'-GACTGGATTGTGGGAGAATGAA-3', Cyp4b1-F, 5'-TCTACTGCATGGCCCTTTATC-3', Cyp4b1-R, 5'-CACATGGTCAGGTAGGTCATC-3', β-Actin-F,5'-AATGTGGCTGAGGACTTTGT-3', β-Actin,5'-GGGACTTCCTGTAACCACTTATT-3'. The data generated by qPCR assays were normalized using the average value of the PBS treatment control group.

## References


Chakravarty, A., Carlson, J.M., Khetani, R.S., and Gross, R.H. (2007). A novel ensemble learning method for de novo computational identification of DNA binding sites. BMC Bioinformatics *8*, 249.

Chan, K., Lu, R., Chang, J.C., and Kan, Y.W. (1996). NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. Proceedings of the National Academy of Sciences of the United States of America *93*, 13943-13948.

Edgar, R., Domrachev, M., and Lash, A.E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res *30*, 207-210.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol *14*, R36.

Nioi, P., McMahon, M., Itoh, K., Yamamoto, M., and Hayes, J.D. (2003). Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. Biochem J *374*, 337-348.

Sykiotis, G.P., and Bohmann, D. (2008). Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Dev Cell *14*, 76-85.

Takashima, S., Mkrtchyan, M., Younossi-Hartenstein, A., Merriam, J.R., and Hartenstein, V. (2008). The behaviour of Drosophila adult hindgut stem cells is controlled by Wnt and Hh signalling. Nature *454*, 651-655.

Trapnell, C., Hendrickson, D.G., Sauvageau, M., Goff, L., Rinn, J.L., and Pachter, L. (2013). Differential analysis of gene regulation at transcript resolution with RNA-seq. Nat Biotechnol *31*, 46-53.

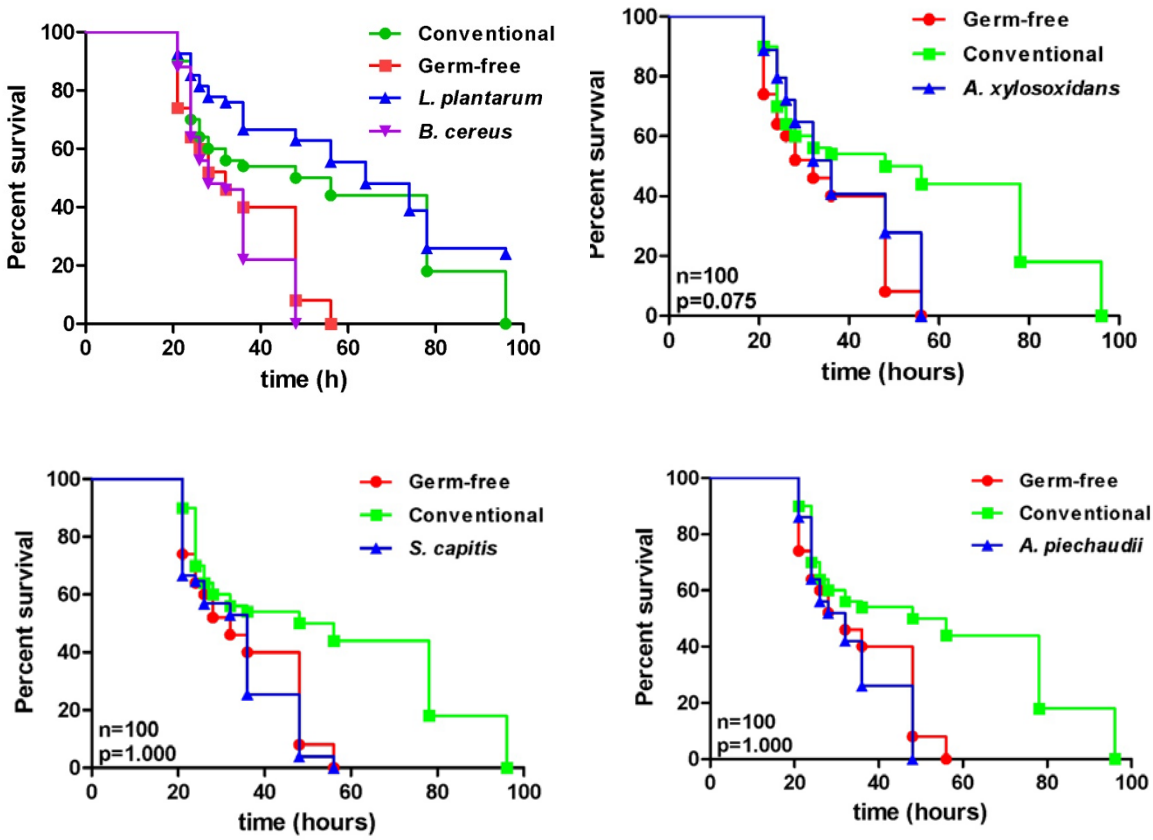



**Figure S1. Relative survival of germ-free adult *Drosophila* gnotobiotically monocolonized with *S. capitis, A. xylosoxidans, or A. piechaudii* in response to Paraquat challenge (*Related to Figure 1*).** Note no measurable increased survival of flies monocolonized with any of the three tested bacteria. Each experiment was carried concurrently with experiments detailed in Figure 1D (shown again here for comparison). Statistics notes within the survival curve represent Log-rank (Mantel-Cox) Test of germ-free survival curve compared to flies fed survival curve of monocolonized adult flies. In addition, Log-rank (Mantel-Cox) Test of *L. plantarum* fed flies against other monocolonized flies are are follows: (Germ-free + *L. plantarum* vs. germ-free + *A. xylosoxidans*, P=<0.0001, n=100). (Germ-free + *L. plantarum* vs. germ-free + *S. capitis*, P=<0.0001, n=100), (Germ-free + *L. plantarum* vs. germ-free + *A. piechaudii*, P=<0.0001, n=100).

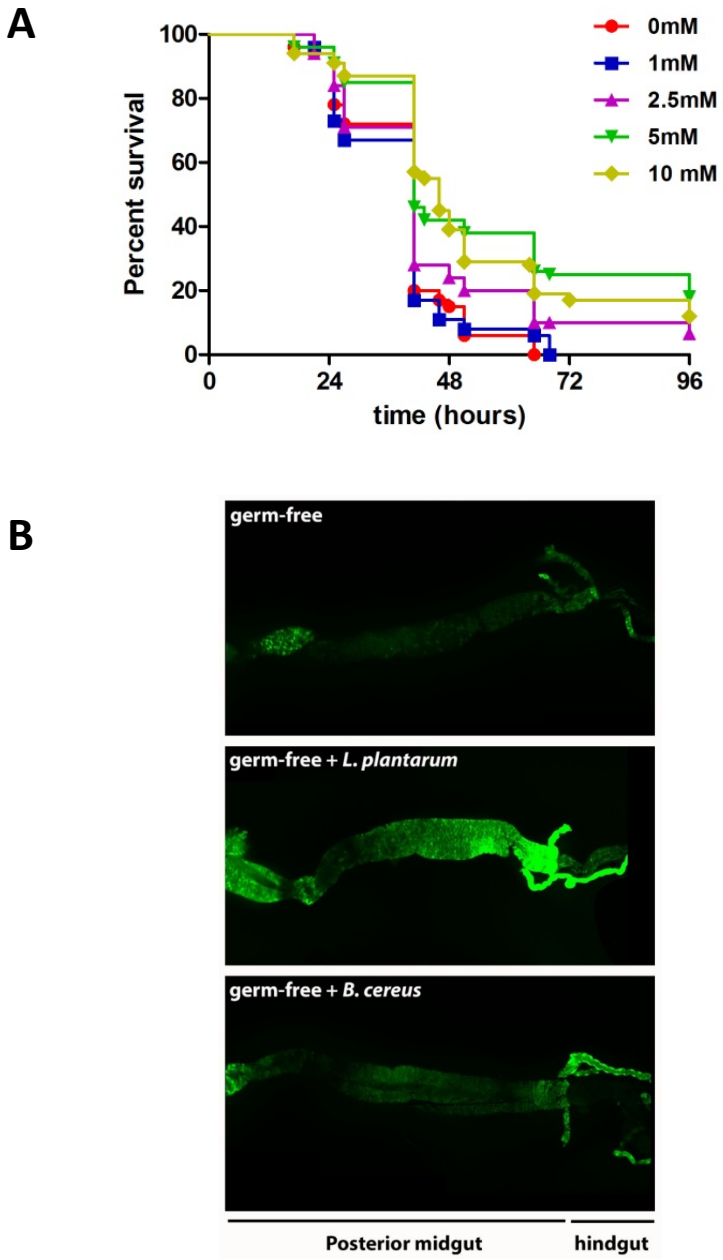



**Figure S2. Response of the Drosophila to exogenously-induced ROS (*Related to Figure 1*). (A)** Relative survival of germ-free adult *Drosophila* exposed to defined amounts of Paraquat (between 1mM and 10mM) for 24 hours, before exposure to 25mM Paraquat. Survival was recorded upon contact with 25mM Paraquat. Note that exposure to between 2.5mM and 10mM paraquat for 24 hours prior to the lethal higher dose significantly increased survival (Log-Rank test for 10mM pre-exposure vs. no pre-exposure P=<0.001, n=100), (5mM pre-exposure vs. no pre-exposure P=<0.001, n=100), (2.5mM pre-exposure vs. no pre-exposure P=0.0424, n=100), but no increase survival of flies pre-exposed to 1mM paraquot (Log-Rank test for 1mM vs. no pre-exposure P=0.7882, n=100). **(B)** Detection of ARE dependent GFP expression in the dissected adult midgut of germ-free $P_{gstD1}$-*gfp* third instar larvae following ingestion of *L. plantarum* or *B. cereus.* Data presented are mosaics of multiple 10X magnification images of one representative adult midgut.

| GENE SYMBOL | GENE FUNCTION | FOLD CHANGE | UPSTREAM ARE (tkaynnngcr) |
|---|---|---|---|
| **RESPONSE TO STRESS** | | | |
| Cyp6a18 | Cytochrome P450 E-Class Group1 | 5.21 | + |
| Uro | Antioxidant | 4.88 | |
| GstZ2 | Glutathione transferase | 2.92 | + |
| GstZ1 | Glutathione transferase | 2.76 | |
| Cyp4p3 | Cytochrome P450 E-Class Group1 | 2.52 | + |
| Cyp4p1 | Cytochrome P450 E-Class Group1 | 2.51 | + |
| Cyp4ac3 | Cytochrome P450 E-Class Group1 | 2.16 | + |
| Cyp4p2 | Cytochrome P450 E-Class Group1 | 2.05 | + |
| Cyp4ac1 | Cytochrome P450 E-Class Group1 | 1.92 | + |
| GstE10 | Glutathione transferase | 1.88 | + |
| **IMMUNE SYSTEM** | | | |
| AttD | Anti-Microbial Peptide | 8.08 | |
| Pirk | Peptidoglycan Recognition | 5.51 | |
| Muc11A | Mucin production | 3.68 | + |
| PGRP-SC1b | Peptidoglycan Recognition | 2.79 | + |
| PGRP-LF | Peptidoglycan Recognition | 2.51 | |
| Dpt | Anti-Microbial Peptide | 1.85 | + |
| **UNKNOWN** | | | |
| CG13075 | Unknown function | 7.48 | + |
| CG16762 | Unknown function | 5.77 | |
| CG34043 | Unknown function | 5.32 | + |
| CG13324 | Unknown function | 4.99 | |
| CG34026 | Unknown function | 4.70 | |
| CG14302 | Unknown function | 4.59 | + |
| CG10560 | Unknown function | 3.71 | + |
| CG3604 | Unknown function | 3.60 | + |

**Table S1. Microarray analysis for the detection of transcript enrichment in the midgut of germ-free third instar larvae that have ingested food containing *L. plantarum* for 4 hours, compared to non-supplemented germ-free food (*Related to Figure 1*).** Antioxidant response element (ARE) sequences were detected by sequence homology to the 10 bp core element that lies at the center of the 20 bp sequence ARE consensus sequence using SCOPE motif finder (Dartmouth College).

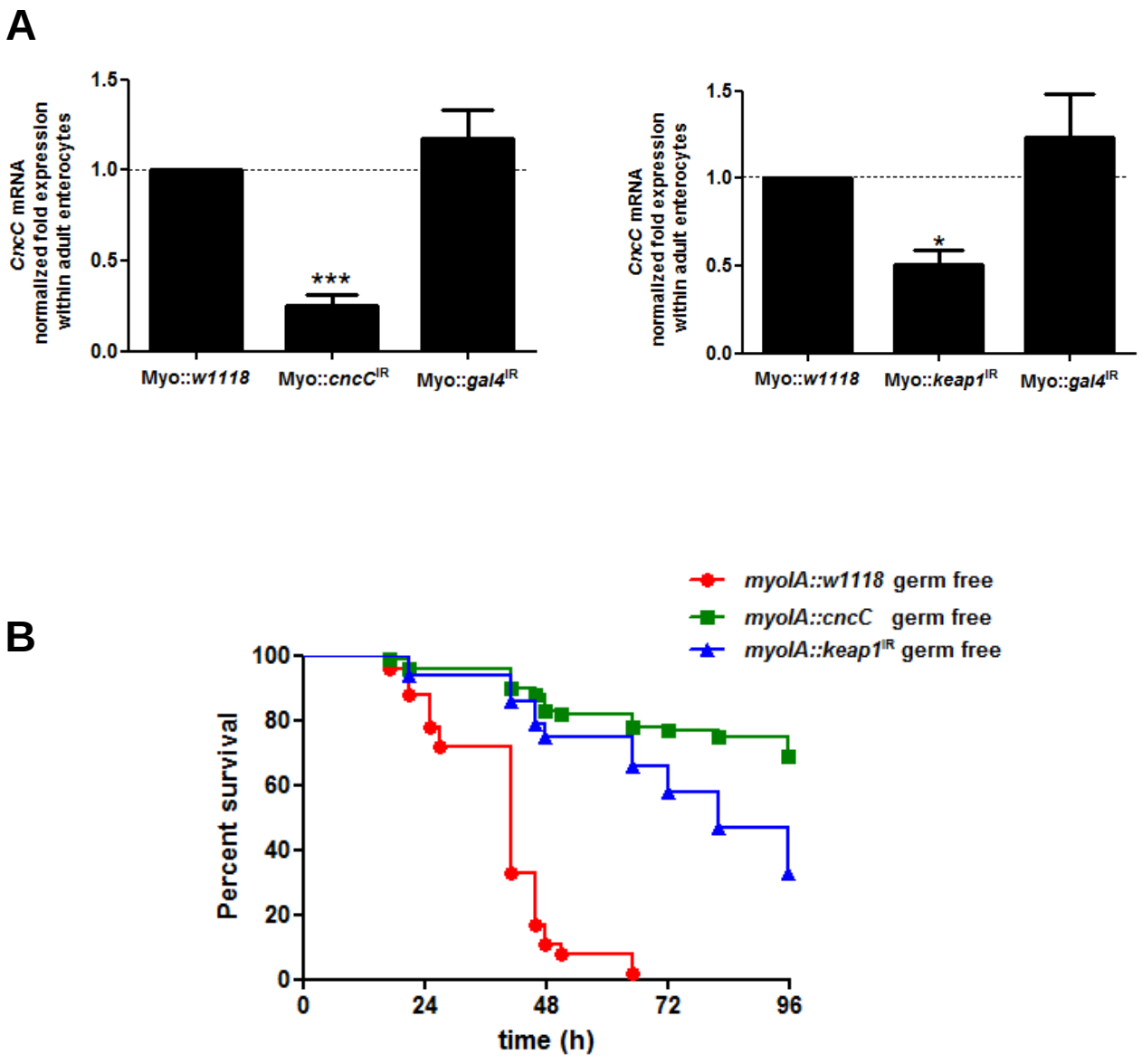
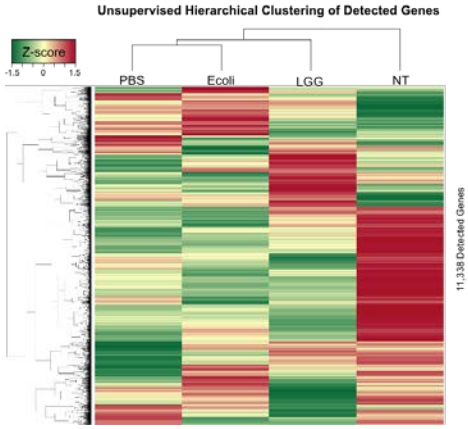
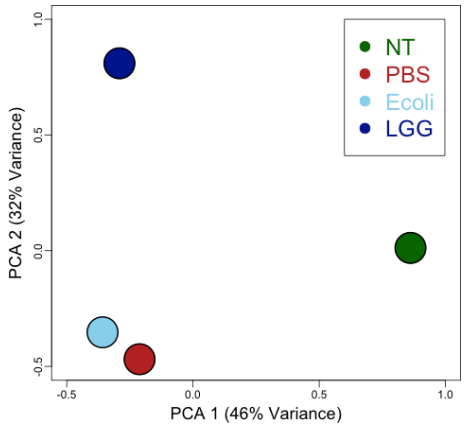



**Figure S3. Relative survival of adult *Drosophila* in response to Paraquat challenge (*Related to Figure 2*). (A)** Detection of *cncC* or *keap1* transcript levels in the midgut of adult *Drosophila* of genotypes MyoIA-GAL4 UAS-cncC$^{IR}$ and MyoIA-GAL4 UAS-keap1$^{IR}$ which harbor RNAi constructs under the control of UAS promoter that target against *cncC* and *keap1* respectively (Sykioitis and Bohmann, 2008) expressed under enterocytes-specific MyoIA-GAL4. Midguts were dissected from 5-day-old adult Drosophila, and transcript levels measured as outlined in materials and methods section. *=P<0.05, ***=P<0.001, n=10. **(B)** Paraquat challenge of germ-free adult *Drosophila* either constitutively expressing *cncC* (UAS-CncC), or in where the levels of Keap1 (UAS-*keap1*$^{IR}$) are diminished. (Log-Rank test for *myoIA-GAL4 w1118* vs. *myoIA*-GAL4 UAS-*cncC*, P=<0.0001, n=100) and (Log-Rank test for *myoIA-GAL4 w1118* vs. *myoIA*-GAL4 UAS-*keap1*$^{IR}$, P=<0.0001, n=100).

**A**



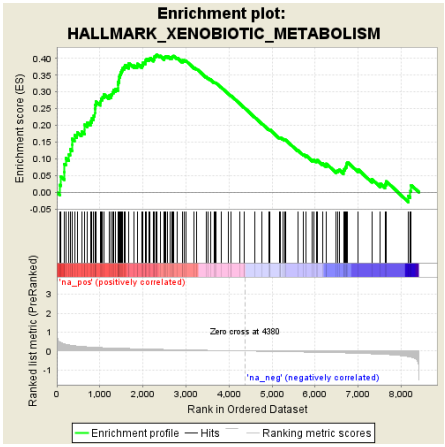

**B**




**C**

| GSEA Hallmark Gene Set | Gene Set Size | Enrichment Score | NOM p-value | FDR q-value |
|---|---|---|---|---|
| OXIDATIVE PHOSPHORYLATION | 181 | 0.559 | < 0.001 | < 0.001 |
| BILE ACID METABOLISM | 72 | 0.482 | 0.002 | 0.015 |
| ADIPOGENESIS | 173 | 0.411 | < 0.001 | 0.019 |
| XENOBIOTIC METABOLISM | 128 | 0.411 | 0.003 | 0.026 |
| FATTY ACID METABOLISM | 126 | 0.392 | 0.001 | 0.040 |
| P53 PATHWAY | 157 | 0.367 | 0.005 | 0.045 |
| PEROXISOME | 82 | 0.408 | 0.019 | 0.050 |
| REACTIVE OXIGEN SPECIES PATHWAY | 39 | 0.454 | 0.034 | 0.062 |
| UV RESPONSE UP | 120 | 0.328 | 0.055 | 0.211 |
| KRAS SIGNALING UP | 129 | 0.287 | 0.169 | 0.475 |

**D**



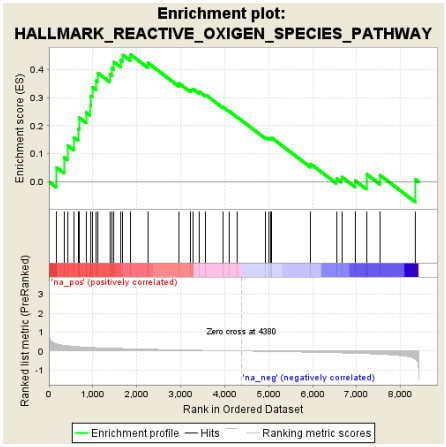




**Figure S4**. **RNAseq analysis fot the detection of transcript enrichment in colonic tissue from germ-free mice administered either *L. rhamnosus* GG (LGG), E.coli or PBS by oral gavage for 4 hours. n=4 (*Related to Figure 3*). (A)** Heat map representation of transcript enrichment in assayed tissues. Note that LGG-fed mice up regulate a unique set of genes compared to PBS and E.coli treated samples. **(B)** Principal components analysis (PCA) of enriched genes identified in (A). Note LGG colons display a unique expression signature compared to PBS or E. coli treated mice. In addition, LGG, E.coli and PBS treated mice all have unique expression signatures from No Treatment mice. **(C)** Gene Set Enrichment Analysis (GSEA) of transcript enrichment detected tissue by RNAseq in colonic in colonic tissue from germ-free mice administered either *L. rhamnosus* GG (LGG), E.coli or PBS by oral gavage for 4 hours. n=4. Table represents of Hallmark gene sets enriched in LGG treated mice compared to PBS- treated mice. **(D)** Representative enrichment plots of gene sets related to investigations in this manuscript. http://www.broadinstitute.org/gsea/index.jsp

| GENE SYMBOL | GENE FUNCTION | LGG/*E.coli* log2 (fold_change) | P value | UPSTREAM ARE |
|---|---|---|---|---|
| **RESPONSE TO STRESS** | | | | |
| Cyp2e1 | Cytochrome P450 2E1 | 0.97 | 0.00185 | + |
| Cyp2c65 | Cytochrome P450 2C65 | 0.87 | 0.00005 | + |
| Cyp2c55 | Cytochrome P450 2C55 | 0.80 | 0.00055 | + |
| Cyp4b1 | Cytochrome P450 4b1 | 0.79 | 0.00045 | + |
| **IMMUNE/DEFENCE** | | | | |
| C9 | Membrane Attack Complex | 1.63 | 0.03215 | |
| Orm1 | Orosomucoid 1 | 1.49 | 0.02565 | |
| Xlr3a | lymphocyte-regulated 3A | 1.34 | 0.00935 | |
| Cidec | cell death-inducing effector c | 1.30 | 0.00005 | |
| Saa3 | Serum amyloid A | 1.10 | 0.0474 | |
| Reg3g | Regenerating islet-derived protein 3 gamma | 0.99 | 0.03215 | |
| G0s2 | Lymphocyte G0/G1 Switch | 0.86 | 0.0089 | |
| **METABOLISM/ENDOCRINE** | | | | |
| Apoa4 | Apolipoprotein | 4.35 | 0.00045 | |
| Apoa1 | Apolipoprotein | 2.24 | 0.00005 | |
| Lep | Leptin | 2.21 | 0.00405 | + |
| Plin1 | Perilipin 1 | 1.80 | 0.00035 | |
| Hp | Haptoglobin | 1.71 | 0.0006 | |
| Mrap | Melanocortin receptor | 1.68 | 0.0044 | |
| Mogat2 | Glycerol transferase | 1.27 | 0.00295 | |
| Nat8l | N-acetyltransferase | 1.20 | 0.00375 | |
| Adipoq | adiponectin | 1.09 | 0.0003 | |
| Car3 | Carbonic anhydrase 3 | 1.05 | 0.00005 | + |
| Pnpla3 | Phospholipase | 0.98 | 0.0079 | |
| Acaa1b | acyltransferase 1B | 0.94 | 0.0062 | + |
| Inmt | methyltransferase | 0.94 | 0.0466 | + |
| Retn | Resistin | 0.94 | 0.00205 | |
| Lpl | lipoprotein lipase | 0.94 | 0.0001 | |
| Ggt1 | γ-glutamyltransferase 1 | 0.93 | 0.0229 | |
| Nts | neurotensin | 0.85 | 0.0051 | |
| Me1 | Malic Enzyme 1 | 0.84 | 0.0001 | + |
| Sptssb | Serine palmitoyltransferase | 0.83 | 0.00055 | |
| Sct | Secretin | 0.82 | 0.0025 | |
| Abca12 | ATP- transporter 12 | 0.78 | 0.00705 | |
| Fabp4 | fatty acid binding protein 4 | 0.78 | 0.00065 | |
| Btn1a1 | Butyrophilin | 0.77 | 0.01705 | |

**Table S2. RNAseq analysis for the detection of transcript enrichment in the colon of 6-week-old germ-free mice fed *L. rhamnosus* GG for 4 hours, compared to 6-week-old germ-free mice fed *E.coli* for 4 hours. (*Related to Figure 3*).**

| GENE SYMBOL | GENE FUNCTION | LGG/*E.coli* log2 (fold_change) | P value | UPSTREAM ARE |
|---|---|---|---|---|
| **DEVELOPMENT/STRUCTURAL** | | | | |
| Asb16 | SOCS box-containing | 2.11 | 0.0307 | |
| Prap1 | p53-response element | 1.67 | 0.00005 | |
| Fgl1 | Fibrinogen-like 1 | 1.39 | 0.0432 | + |
| Zmat4 | Matrin-type 4 | 1.33 | 0.0136 | |
| Tlx2 | T-cell leukemia homeobox | 1.17 | 0.0512 | |
| Hoxb6 | Homeobox B6 | 1.15 | 0.00025 | |
| Hoxb7 | Homeobox B7 | 1.05 | 0.0027 | |
| Pou3f3 | Homeobox 3 | 0.86 | 0.0324 | |
| Creb3l3 | Transcription factor | 0.84 | 0.01405 | |
| Hoxb8 | Homeobox B8 | 0.81 | 0.0054 | |
| Prkg2 | protein kinase family | 0.80 | 0.00085 | |
| Hoxd4 | Homeobox D4 | 0.77 | 0.0327 | |
| Isx | intestine-specific homeobox | 0.76 | 0.0009 | |
| **UNKNOWN** | | | | |
| Serpina3c | Peptidase inhibitor | 1.95 | 0.01605 | |
| Cml5 | Camello-like protein 5 | 1.42 | 0.0174 | |
| Thrsp | thyroid hormone responsive | 1.32 | 0.00005 | |
| Hapln4 | Hyaluronan-Link Protein | 1.12 | 0.0396 | |
| Slc22a3 | Solute carrier family 22 | 1.05 | 0.0371 | + |
| Nr2e3 | Nuclear receptor family 2 | 1.02 | 0.05105 | |
| Nrtn | Neurturin | 1.01 | 0.0062 | |
| Sprr2a1 | small proline-rich protein 2A1 | 0.99 | 0.00005 | |
| Ugt2b5 | glucuronosyltransferase 2 | 0.95 | 0.0004 | |
| Oprk1 | Opioid receptor-like | 0.91 | 0.0471 | |
| Hsd17b13 | hydroxysteroid dehydrogenase | 0.88 | 0.00265 | |
| Cxxc4 | Dvl-Binding Protein IDAX | 0.86 | 0.0538 | |
| Osr2 | Odd-skipped-related 2 | 0.85 | 0.00065 | |
| Tusc5 | tumor suppress candidate 5 | 0.84 | 0.04685 | |
| Tmigd1 | Immunoglobulin domain containing 1 | 0.78 | 0.00035 | |
| Grin3a | Glutamate receptor | 0.77 | 0.00445 | |
| Retnlb | Resistin-like beta | 0.76 | 0.00075 | |

**Table S2 Continued. RNAseq analysis for the detection of transcript enrichment in the colon of 6-week-old germ-free mice fed *L. rhamnosus* GG for 4 hours, compared to 6-week-old germ-free mice fed *E.coli* for 4 hours (*Related to Figure 3*).**