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Lactobacilli modulate epithelial cytoprotection through the Nrf2 pathway

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Summary

An optimal gut microbiota influences many beneficial processes in the metazoan host. However, the molecular mechanisms that mediate and function in symbiont-induced host responses have not yet been fully characterized. Here, we report that cellular ROS enzymatically generated in response to contact with lactobacilli in both mice and *Drosophila* has salutary effects against exogenous insults to the intestinal epithelium via the activation of Nrf2 responsive cytoprotective genes. These data show that the xenobiotic inducible Nrf2 pathway participates as a signaling conduit between the prokaryotic symbiont and the eukaryotic host. Indeed, our data imply that the capacity of lactobacilli to induce redox signaling in epithelial cells is a highly conserved hormetic adaptation to impel cellular conditioning to exogenous biotic stimuli. These data also highlight the role the microbiota plays in eukaryotic cytoprotective pathways, and may have significant implications in the characterization of a eubiotic microbiota.

Graphical Abstract

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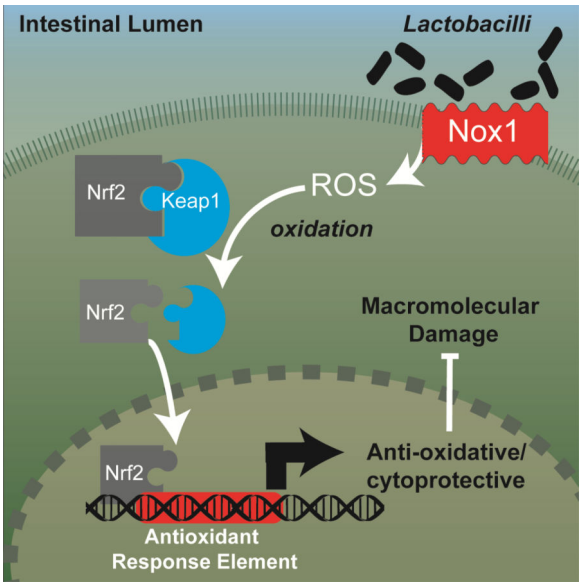
Contributions

RMJ and ASN conceived and designed the experiments. RMJ, LL and ESK performed experiments on the *Drosophila* animal model. RMJ, CD and CSA performed experiments using the Nrf2 null murine model, and TMD performed experiments using the Nox1 null murine model. RMJ, CD, CDS, ESK and AAW analyzed the data. RMJ and ASN wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

See Supplemental Information on other Experimental Procedures



Keywords

Nrf2; lactobacillus; Reactive oxygen species; microbiota; cytoprotection; hormesis

Introduction

The prokaryotic microbiota of virtually all metazoans participate in many symbiotic functions, including regulation of cellular growth and survival (Neish and Jones, 2014). However, the host intrinsic mechanisms that mediate non-immune symbiont-induced effects are largely unknown. A well-studied and evolutionarily highly conserved system for transducing exogenous stimuli into eukaryotic transcriptional responses is the Nrf2 pathway. Nrf2 activation upregulates a regulon of genes including those involved in xenobiotic and reactive oxygen species (ROS) detoxification, as well as pro-restitutive function. This pathway has attracted considerable attention because small molecule inducers of Nrf2 have cytoprotective effects against oxidant and electrophilic environmental stressors (Kobayashi et al., 2009). Our research group has recently reported that lactobacilli, which are constituents of the microbiota in many metazoans and well-known probiotic agents, are highly adapted to induce the generation of physiological levels of ROS in cells of the intestinal epithelium and consequent induction of cell proliferation (Jones et al., 2013).

Gut bacteria stimulate reactive oxygen species (ROS) production in epithelial cells by an enzymatic mechanism analogous to the pathogen-induced respiratory burst in phagocytes (Alam et al., 2013; Jones et al., 2013). Cellular ROS are generated by the catalytic action of NADPH oxidases. The first and exemplary member of this family of enzymes, Nox2, was characterized in phagocytes, and is well known to function in neutrophil microbicidal ROS generation in response to pathogens. Thereafter, isozymes of Nox2 were identified in nonphagocytic tissues, including Nox1, which is highly expressed in colonic enterocytes of both flies and mice (Lambeth and Neish, 2014). Interestingly, enzymatically generated ROS

in the epithelia is stimulated not only by potential pathogens, but also by symbiotic bacteria, especially members of the lactobacilli taxon, and promotes cell proliferation and migration (Wentworth et al., 2011; Wentworth et al., 2010), accelerates restitution post injury (Swanson et al., 2011), and modifies epithelial NF-κB signaling (Kumar et al., 2007).

The Nrf2 system is a well-characterized ubiquitin dependent signaling pathway that responds to oxidative stress and electrophilic xenobiotics, and is recognized as a major regulator of cytoprotective responses against such environmental cellular stresses (Jones et al., 2012). Components of the Nrf2 signaling are functionally conserved even in lower metazoans such as *C. elegans*, and are fully developed in *Drosophila* and mammals. Disruption of Nrf2 signaling results in increased sensitivity to oxidative-stress in flies (Sykiotis and Bohmann, 2008), and increased sensitivity to radiological insult in mice (McDonald et al., 2010). The Nrf2 pathway has been implicated in proliferative control of gut stem cells (Hochmuth et al., 2011). At low levels of ROS, Nrf2 is bound to its cytoplasmic inhibitor Keap1 which suppresses the activity of Nrf2 by targeting it for constitutive polyubiquitination by a Cullin3-based E3 ligase complex and consequent proteasomal degradation. Keap1 contains redox-sensitive cysteine residues that under oxidative stresses conditions react and alter the functional conformation of Keap1, thereby abolishing the inactivation of Nrf2. Thereafter, Nrf2 translocates to the nucleus, binds to Antioxidant Response Element (ARE) sequences and initiates the transcription of a battery of antioxidant enzymes and detoxifying proteins, known as the “Phase 2 detoxification response”. We hypothesized that symbiont-induced ROS may activate epithelial Nrf2 pathway signaling, and thereby mechanistically mediate the beneficial influences of a eubiotic microbiota.

Results

Lactobacillus plantarum induces cytoprotection against oxidative insult and potentiates upregulation of CncC pathway responsive genes in *Drosophila*

We first implicated a role for the indigenous microbiota in cytoprotection against oxidative injury in the intestine by showing that adult germ-free *Drosophila*, compared to conventionally raised controls, are significantly more sensitive to Paraquat-induced toxicity (**Fig. 1A),** and exhibit elevated numbers of apoptotic cells in the highly proliferative Posterior 3 (P3) (Marianes and Spradling, 2013) section of the midgut **(Fig. 1B and 1C**). To identify if, or which, specific bacterial genera contribute to cytoprotection, we studied germ-free *Drosophila* gnotobiotically colonized with monocultures of bacterial species that we previously isolated from the luminal content of adult *Drosophila* (Jones et al., 2013), and examined the extent to which individual members of the microbiota influenced cytoprotection. Midguts were dissected and comparable levels of bacterial ingestion confirmed by plate count. Of those tested, only flies gnotobiotically colonized with pure *Lactobacillus plantarum* cultures significantly protected *Drosophila* from oxidative injury (**Fig. 1D**) and (**Fig. S1**). To demonstrate the beneficial hormetic effect of pre-exposure of germ-free adult Drosophila to lower levels of ROS, we show that lower doses of paraquat, with 5mM for 24 hours being optimal, can protect against ensuing severe insults triggered by 25mM paraquat feeding (**Fig S2**). Because we also previous showed that only *L*

plantarum possessed the capacity to induce ROS generation in *Drosophila* enterocytes following colonization (Jones et al., 2013), these data suggested the possibility of redox signaling in mediation of commensal mediated cytoprotection. To examine this notion we used a GFP reporter fly bearing an antioxidant response element (ARE) dependent promoter (*gstD1*-GFP) that responds to Nrf2 (known as CncC in *Drosophila)* (Sykiotis and Bohmann, 2008). Third instar larva were utilized as their feeding behavior allows rapid colonization and synchronous induction of signaling events. We observed that colonization of either germ-free larvae or adult *Drosophila* harboring a CncC-responsive reporter was activated following *L. plantarum*, but not *B. cereus* ingestion (**Fig. 1E and Fig S2**). To assess gene expression associated with *L. plantarum*–induced cytoprotection, we employed microarray analysis on dissected larval midgut tissue following *L. plantarum* ingestion by germ-free *Drosophila*. Gene ontology analysis identified highly represented transcripts in *L. plantarum* fed larva (compared to germ-free) (**Table S1**), many of which were found to be located downstream of a promoter region harboring a putative antioxidant response element (ARE) homologous to the 10 bp core element that lies at the center of the 20 bp sequence ARE consensus sequence (Nioi et al., 2003). Many of these genes have a known cytoprotective function, including glutathione S-transferase zeta 1 GstZ1, GstZ2 and GstD10, as well as a number of Cytochrome P450, E-class, group I enzymes, including Cyp4p1, Cyp4ac1 and Cyp4ac3. Consistently, upregulation of Cytochrome P450 family of genes was detected in midgut sections P2, P3 and P4 in other recent investigations (Marianes and Spradling, 2013). Real time quantitative PCR (RT-qPCR) analysis corroborated the detection of the increased representation of transcripts for the aforementioned proteins in midgut tissues of larva fed *L. plantarum*, but not in the midgut regions of larvae colonized with other, non-ROS inducing bacteria (**Fig. 1F**). These data indicate that the transcriptional response is specific to *L. plantarum* ingestion, and based on transcriptional profile analysis, that the mechanism of *L. plantarum*-induced cytoprotection may by as a result of the activation of genes under the regulatory control of an ARE promoter sequence.

CncC pathway signaling mediates *Lactobacillus plantarum* induced cytoprotection against oxidative insult in *Drosophila*

To mechanistically substantiate that CncC signaling is mediating *L. plantarum*-induced cytoprotection, we employed the bipartite –GAL4/UAS- system to modulate CncC pathway activity by constitutively driving the expression of Keap1 or CncC, or by using RNAi constructs against CncC as previously described (Sykiotis and Bohmann, 2008), under the enterocyte specific *myoIA*-GAL4 driver fly. Depletion of transcript levels in the midgut of these genotypes were confirmed by qPCR **(Fig S3)**. Constitutive enterocyte specific expression of CncC (**Fig. 2A**), or depletion of Keap1 levels (**Fig. 2B**), both of which result in Nrf2 pathway activation, significantly enhanced survival of adult *Drosophila* in response to Paraquat-induced toxicity, correlating with the extent of apoptotic cells observed in segments P3 and P4 of the midgut (Marianes and Spradling, 2013) (**Fig. 2C and 2D**). Conversely, constitutive activation of Keap1 (**Fig. 2A**), or depletion of CncC transcripts (UAS-*cncC*-RNAi) (**Fig. 2B**) (both of which inhibit pathway activation) in the midgut rendered flies significantly more susceptible to Paraquat-induced toxicity, again with correlative apoptotic damage to the midgut (**Fig. 2E and 2F**). The cytoprotective function of CncC in enterocytes was also detected in germ-free adult *Drosophila* (**Fig. S3**). Importantly,

depletion of CncC, while having no effect on bacterial numbers (**see materials and methods**), essentially abolished *L. plantarum*-induced cytoprotection against Paraquat to levels observed for germ-free adult *Drosophila* (**Fig. 2G**). Indeed, enterocyte-specific depletion of CncC-levels significantly reduced *L. plantarum*-induced activation of *cyp6a18* and *gstZ2* in the adult *Drosophila* midgut (**Fig. 2H**) together indicating that *L. plantarum*-induced protection against oxidative insult was dependent on CncC pathway signaling.

Colonization of the murine intestine with *Lactobacillus rhamnosus* GG induces Nrf2-dependent cytoprotection

Nrf2 signaling is highly conserved across metazoans. To determine whether lactobacilli-induced and Nrf2 pathway mediated cytoprotection occurs in mammals, we recapitulated our investigations in the murine model. Transcriptional profiling and gene ontology analysis identified highly represented transcripts in the colonic tissues of germ-free mice orally gavaged with *L. rhamnosus* GG, a mammalian adapted lactobacillus, when compared to transcripts in the colonic tissues of germ-free mice orally gavaged with equivalent inoculum of *E. coli*, which in our previous studies could not induce detectable generation of ROS in intestinal epithelial cells (Jones et al., 2013). Interestingly, *L. rhamnosus* GG induced transcripts cluster distinct from transcripts induced by *E. coli* or vehicle (**Fig. S4**). Indeed, comparison of *L. rhamnosus* GG-fed to *E. coli*-fed tissues identified a set of enriched transcripts from the Cytochrome P450 family of proteins which have conserved upstream ARE promoter sequences, consistent with our observations in the analogous *Drosophila* experiments (**Table S2**). Specifically, increased transcript representation of Cyp2c65, Cyp2c55 and Cyp4b1 was detected, and confirmed in these samples by qPCR (**Fig. 3A**). Importantly, qPCR analysis also confirmed that transcripts for these three Cytochrome P450 genes were not enriched in colonic tissues of *E. coli* fed mice, further supporting our observation that of the bacteria tested, the upregulation of these genes are specifically induced in response to lactobacilli (**Fig. 3A**). Previous reports have described the cytoprotective effects of *L. rhamnosus* GG in mice against radiological insult (Ciorba et al., 2012). We corroborate these data by confirming that mice fed *L. rhamnosus* GG have significantly enhanced survival, are protected against weight loss, and have fewer apoptotic cells at within colonic crypts following irradiation (**Fig. 3B to 3E**). Strikingly, each of the measured cytoprotective influences of *L. rhamnosus* GG against irradiation were completely abolished in $Nrf2^{-/-}$ mice (**Fig. 3B to 3E**). Note also that we did not detect any changes in the response to irradiation insult between untreated wild type and Nrf2−/− mice. Together, these data show that Nrf2 is required for the optimal cytoprotective influences of *L. rhamnosus* GG in the intestine in conventionally raised mice.

Nox1 is required for optimal lactobacilli-induced cytoprotection

We previously showed that Nox1 in mice and the orthologous dNox in *Drosophila* are required for lactobacilli-induced ROS generation in intestinal enterocytes (Jones et al., 2013). To show that Nox1-generated ROS function in lactobacilli-induced cytoprotection, we employed the same bipartite –GAL4/UAS- system as above to expressed RNAi constructs against dNox under the enterocyte specific *myoIA*-GAL4 driver fly. Depletion of transcript levels in the midgut of these genotypes were confirmed in (Jones et al., 2013).

Constitutive depletion of d*nox* transcripts (UAS-d*nox*-RNAi), but not d*duox*, while again having no effect on bacterial numbers (see materials and methods), significantly reduced *L. plantarum*-induced cytoprotection against Paraquat (**Fig. 4A**) with correlative apoptotic damage to the midgut (**Fig. 4B and 4C**). These data corroborate our previous observations in the fly that symbiotic lactobacilli-induced ROS generation is mediated via the enzymatic activity of dNox, and not by dDuox, which by contrast is reported to function in the *Drosophila* anti-microbial response to pathogens (Ha et al., 2005). To show that Nox1 is also required for *L. rhamnosus* GG –induced cytoprotection against irradiation in mice, we used the intestinal epithelial cell-specific Nox1-deficient knockout (B6.Nox1$^{\Delta IEC}$) (Leoni et al., 2013). Consistent with our previous data, *L. rhamnosus* GG – induced enhanced survival and reduced loss of body weight was significantly abrogated in the B6.Nox1$^{\Delta IEC}$ compared to wild type littermates (**Fig 4D to 4G**). In summary, these data are compelling evidence showing that enterocyte-expressed ROS generation catalyzed by Nox1 is required for lactobacilli-potentiated cytoprotection.

Discussion

In this study, we identified the Nrf2 pathway as a distinct system for the non-immune perception and response to specific members of the microbiota, namely lactobacilli. In addition, we show that this ROS-sensitive signal transduction system mediates cytoprotection, consistent with our previous observations of lactobacilli-induced enzymatic generation of ROS in the gut in mammals and *Drosophila* (Jones et al., 2013). Members of the diverse Lactobacillus taxon have long been exploited by humans in the production of fermented dairy products and are commonly employed as candidate probiotic agents. They are characteristic early colonists of the neonatal mammalian gut and are fructose fermenters present in decaying fruit that form the major energy source of *Drosophila*. Lactobacilli-specific symbiotic functions include cytoprotective in the mouse (Ciorba et al., 2012), and effects in *Drosophila* ranging from mate selection (Sharon et al., 2010), to metabolic regulation (Storelli et al., 2011). These effects likely involve the mucus binding adhesive properties of these bacteria (Ardita et al., 2014). Thus, members of the genus lactobacilli may have evolved symbiotic relationships where microbial induced generation of ROS functions as a transducer of bacterial signals into host gene regulatory events that potentiate multiple effects in disparate biological systems. We describe a molecular mechanism by which lactobacilli can elicit their beneficial influences on host gut tissues, wherein ROS generated by Nox following bacterial contact activates downstream cytoprotective signaling. These observations were particularly striking in germ-free *Drosophila* mono-associated with *L. plantarum*. Importantly, this influence of lactobacilli was also observed in conventionally raised mice indicating that animals do not necessarily have to be germ-free to benefit from lactobacilli-induced cytoprotection.

One intriguing observation is that flies depleted of d*nox* transcripts and monoassociated with *L. plantarum* have a similar sensitivity as germ-free flies over the first half of the experimental time assayed, and thereafter exhibit a degree of resistance to oxidative stress over the second half of the assay **(Fig. 4A)**. This infers that lactobacilli-Nox-Nrf2 signaling may not be the only evolved mechanism that lactobacilli exploited to elicit cytoprotective influences on the host. Indeed, other pathways reported to be activated in response to

symbiotic bacteria include the TOR pathway in *Drosophila* (Storelli et al., 2011). Consistent with our study Storelli et al. 2011 concluded that mono-ingestion of *L. plantarum* is sufficient to recapitulate the effects of the normal microbiota. In addition, lactobacilli were reported to protect the murine intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner (Ciorba et al., 2012). Going forward, it will be interesting to determine the extent to which both TOR signaling, and TLR-MAPK signaling synergistically interacts with ROS generated by Nox in cytoprotection. Lactobacilli's impact on host physiology has been found to be strain-specific (Storelli et al., 2011). In our experiments, we utilized a single *L. plantarum* strain isolated from the intestine of *Drosophila* from our fly stock. Whether the influence of *L. plantarum* in *Drosophila*, (or the influence of *L. rhamnosus* GG in mice) is strain and species specific, or a more general phenomenon of lactobacilli is a subject of intense focus within research group.

Taken together, these observations are consistent with the concept of “hormesis”, wherein low, near threshold levels of a stressor is protective against more intense or prolonged stimuli, which is explicitly demonstrated in figure 1E. This concept has been implicated in ROS induced, Nrf2 mediated protection against irradiation injury (in the bone marrow) (Kim et al., 2014) and cytotoxic mucosal injury (in the airway) (Paul et al., 2014). The microbiota, despite having a beneficial relationship with the host, nevertheless is an extrinsic influence, and ROS stimulated by microbes is a potent signaling stressor. Thus, hormesis, as a response to xenobiotic and environmental stimuli, evidently extends to the acquisition of and adaption to exogenous microbiota, illustrating a mechanism of co-evolution and symbiosis between host cells and microbes.

Experimental Procedures

Drosophila Paraquat resistance assays

Whole animal cytoprotection in *Drosophila* was measured in response to Methyl viologen dichloride (Paraquat™) -induced oxidative stress. Groups of 10 per vial, of 5-day-old adult *Drosophila* of assayed genotypes were starved for 3 hours and then fed a solution of 5% sucrose containing a semi-lethal dose of Paraquat (25 mM). 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. From each genotype and gender, triplicate assays of initial surviving 100 flies were scored. Gut tissue cytoprotection in response (Paraquat) -induced oxidative stress was analyzed following dissection of the fly midgut, fixing the tissue in 4% paraformaldehyde, followed by TUNEL assay analysis using In Situ Cell Death Detection Kit (Roche).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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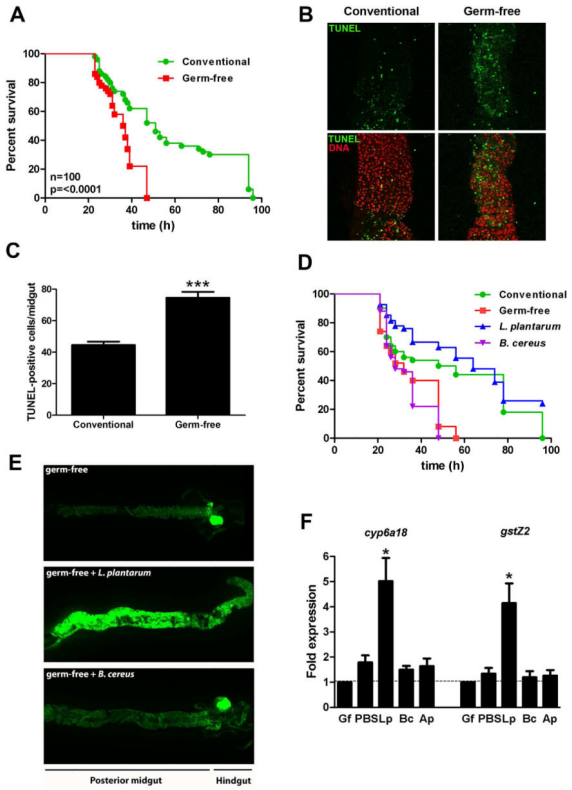

Fig. 1. *Lactobacillus plantarum* induces cytoprotection against oxidative insult and potentiates upregulation of CncC pathway responsive genes in *Drosophila*

(A) Relative survival of 5-day-old germ-free and conventionally raised adult *Drosophila* in response to Paraquat challenge. Log-Rank test P=<0.001, n=100. **(B)** TUNEL analysis of posterior midgut tissues dissected from *Drosophila* described in (A) at 24 hours following Paraquat challenge. Note the high concentration of TUNEL positive cells in the Posterior 3 (P3) section of the midgut. **(C)** Numeration of TUNEL-positive cells per midgut examined in 1B. *** = P<0.001, n=10. **(D)** Relative survival of germ-free adult *Drosophila* gnotobiotically monocolonized with *L. plantarum* or *B. cereus* in response to Paraquat challenge. Note increased survival of flies monocolonized with *L. plantarum* (Log-Rank test for g-f vs. g-f + *L. plantarum*, P=<0.0001, n=100). Note also significantly increased survival of germ-free fed *L. plantarum* compared to *B. cereus* fed flies. (Log-Rank for g-f + *L. plantarum* vs. g-f + *B. cereus*, P=<0.0001, n=100). **(E)** Detection of ARE dependent GFP expression in the midgut of germ-free P_{gstD1}-*gfp* third instar larvae following ingestion of *L. plantarum*. **(F)** Real time quantitative PCR (RT-qPCR) analysis for the detection of *cyp6a18*, and *gstZ2* transcript enrichment in the midgut of germ-free third instar larvae at 4 hours following ingestion of either *L. plantarum* (Lp), *B. cereus* (Bc), *A. piechaudii* (Ap). *= P<0.05, n=30.

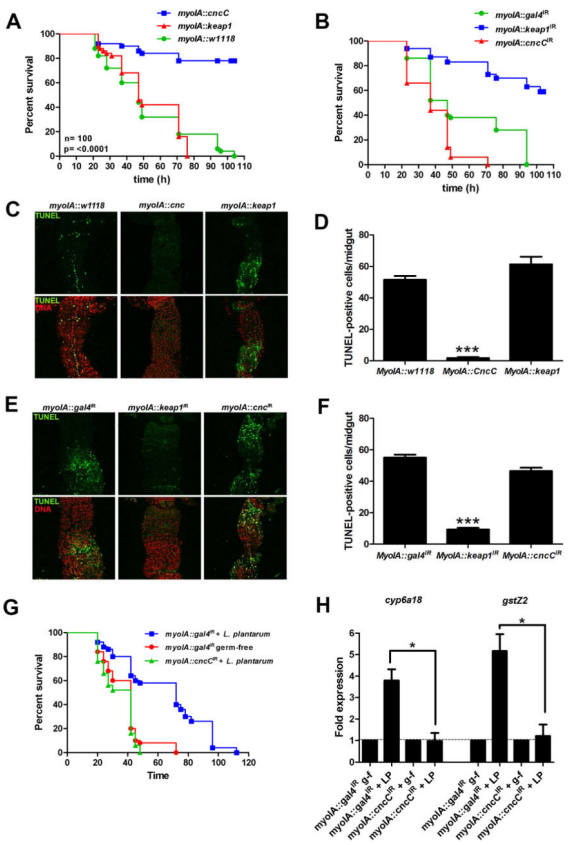

Fig. 2. CncC pathway signaling mediates *Lactobacillus plantarum* induced cytoprotection against oxidative insult in *Drosophila*

(A) Relative survival in response to Paraquat challenge of conventionally raised adult *Drosophila* either constitutively expressing *cncC* (UAS-CncC) or constitutive expressing *keap1* under the enterocyte specific *myoIA-GAL4* driver. (Log-Rank test for *myoIA-GAL4 w1118* vs. *myoIA*-GAL4 UAS-*cncC*, P=<0.0001, n=100). **(B)** Survival in response to Paraquat challenge of 5-day-old conventionally raised adult *Drosophila* where the levels of *cncC* (UAS-*cncC*^{IR}) or Keap1 (UAS-*keap1*^{IR}) are diminished. (Log-Rank test for *myoIA-GAL4 w1118* vs. *myoIA*-GAL4 UAS-*cncC*^{IR}, P=<0.0001, n=100). **(C)** TUNEL analysis of posterior midgut dissected from adult *Drosophila* listed in (A), and exposed to Paraquat challenge for 48 hours. **(D)** Numeration of TUNEL-positive cells per midgut examined in 2C. *** = P<0.001, n=10. **(E)** TUNEL analysis of posterior midgut tissues dissected from adult *Drosophila* described in (C), following 48 hours exposure to Paraquat challenge. **(F)** Numeration of TUNEL-positive cells per midgut examined in 2E. *** = P<0.001, n=10. **(G)** Survival in response to Paraquat challenge of germ-free adult *Drosophila* monocolonized with *L. plantarum* where the levels of CncC (UAS-*cncC*^{IR}) are diminished under the enterocyte specific *myoIA-GAL4* driver, (Log-Rank for *myoIA-GAL4;*UAS-*gal4*^{IR} + *L. plantarum* vs. *myoIA*-GAL4;UAS-*cncC*^{IR} + *L. plantarum*, P=<0.0001, n=100). **(H)** Real time quantitative PCR (RT-qPCR) analysis for the detection of *cyp6a18* and *gstZ2*, transcript enrichment in the midgut of adult Drosophila treated as described in (G). *= P<0.05, n=30.

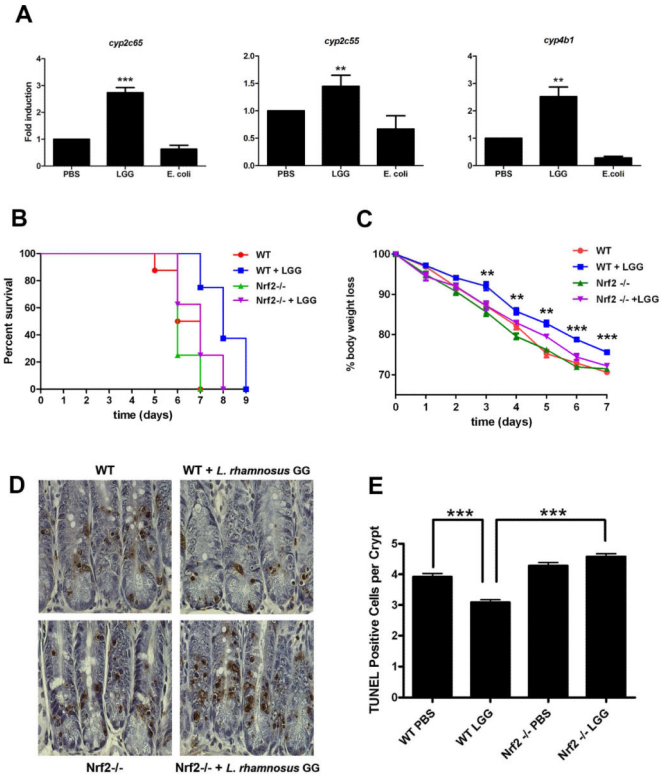

Fig. 3. Colonization of the murine intestine with *Lactobacillus rhamnosus* GG induces Nrf2-dependent cytoprotection

(A) Quantitative (q)PCR analysis to measure the abundance of mRNA transcripts expressed from *cyp2c65*, *cyp2c55* and *cyp4b1* in colonic tissues of wild type B6 germ-free mice fed either 2×10^9 cfu total *L. rhamnosus* GG, or *E.coli* by oral gavage for 4 hours. **(B)** Survival of 7-week-old $Nrf2^{-/-}$ or and wild type littermates fed *L. rhamnosus* GG (LGG) following 12 Gy irradiation insult. Note significantly increased survival of irradiated WT mice fed LGG in response to irradiation (Log-Rank test for WT vs. WT+LGG, P=0.0013, n=8). Also note significantly deceased survival rate of $Nrf2^{-/-}$ mice fed LGG compared to WT mice fed LGG (Log-Rank test for WT+LGG vs. $Nrf2^{-/-}$ +LGG, P=0.0142, n=8), and no significant increase in survival of $Nrf2^{-/-}$ mice fed LGG compared to unfed WT (Log-Rank test for WT vs. $Nrf2^{-/-}$ +LGG, P=0.2150, n=8), **(C)** Percent body weight loss of mice described in (B). Statistical analysis represents comparison of WT+LGG vs. Nrf2−/− +LGG on each respective day. *=P<0.05, **=P<0.01, n=8. **(D)** Detection of TUNEL-positive cells within colonic tissues harvested from mice described in (A) and (B). **(E)** Quantification of TUNEL-positive cells in (c) ***=P<0.001, n=5.

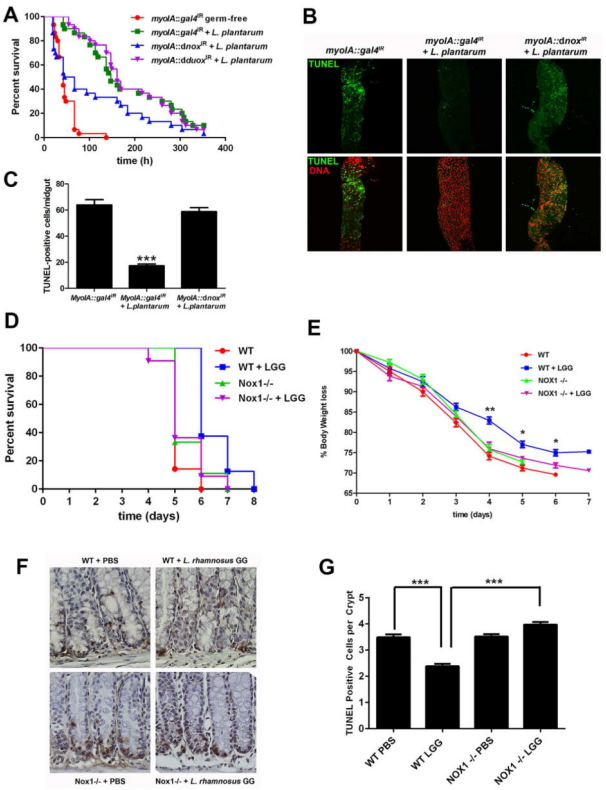

Fig. 4. Nox1 is required for optimal lactobacilli-induced cytoprotection

(A) Survival in response to Paraquat challenge of 5-day-old conventionally raised adult *Drosophila* where the levels of d*nox* (UAS-d*nox*^{IR}) are diminished, under the enterocyte specific *myoIA-GAL4* driver. (Log-Rank test for *myoIA-GAL4;* UAS-*gal4*^{IR} + *L. plantarum* vs. *myoIA*-GAL4;UAS-d*nox*^{IR} + *L. plantarum*, P=0.0185, n=100). **(B)** TUNEL analysis of posterior midgut tissues dissected fromadult *Drosophila* listed in (A), and exposed to Paraquat challenge for 48 hours. **(C)** Numeration of TUNEL-positive cells per midgut examined in 4B. *** = P<0.001, n=10. **(D)** Survival of 7-week-old intestinal epithelial cell-specific Nox1-deficient (B6.Nox1$^{\Delta IEC}$) and wild type littermates fed *L. rhamnosus* GG (LGG) following 12 Gy irradiation insult. Note significantly increased survival of irradiated WT mice fed LGG in response to irradiation (Log-Rank test for WT vs. WT+LGG, P=0.0012, n=8). Also note significantly deceased survival rate of B6.Nox1$^{\Delta IEC}$ mice fed LGG compared to WT mice fed LGG (Log-Rank test for WT+LGG vs. B6.Nox1$^{\Delta IEC}$ + LGG, P=0.0118, n=8). Finally, note no significantly increased survival rates of irradiated WT unfed mice and B6.Nox1$^{\Delta IEC}$ mice fed LGG compared (Log-Rank test for WT vs. B6.Nox1$^{\Delta IEC}$ + LGG, P=0.4465, n=8). **(E)** Percent body weight loss of mice described in (D). Statistical analysis represents comparison of WT+LGG vs. B6.Nox1$^{\Delta IEC}$+LGG on each day, *=P<0.05, **=P<0.01, n=8. **(F)** Detection of TUNEL-positive cells within colonic tissues harvested from mice described in (C) and (D). **(G)** Quantification of TUNEL-positive cells in (E) ***=P<0.001, n=5.