Mitochondrial protein Prohibitin-1 as a promising candidate to counteract *Salmonella* Typhimurium infections in the gastrointestinal tract

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Introduction

- Annually, **gastroenteritis (GE)** causes ~2 million deaths globally.
- Enteropathogens, particularly *Salmonella* Typhimurium (STm), are primary etiological agents causing GE. Typically self-limiting in humans, 5% of GE cases by STm progress to severe complications like bacteremia.
- The intestinal epithelium is a key regulator of innate immunity, functioning as a physical, semi-permeable barrier between the luminal contents and the underlying intestinal tissue. STm employs a Type 3 Secretion System to invade intestinal epithelial cells (IECs).
- Mitochondria within IECs act as signaling hubs in the host defense against bacterial pathogens, both as targets of bacterial toxins and as inhibitors of immune responses. **Prohibitin-1 (PHB1)** is a chaperone protein of the mitochondrial electron transport chain complex subunits.
- Our previous studies demonstrated that **PHB1 is protective against intestinal inflammation and tumorigenesis**, but its role in bacterial infections is unknown.
- This study investigates the **role of PHB1 *in vivo* and *in vitro*** as a novel strategy to combat pathogenic infection.

Hypothesis

Mitochondrial protein PHB1 confers protection to the host against STm invasion *in vivo* and *in vitro*.

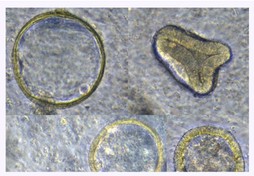
Methods

PHB1 overexpression

WT (B6)

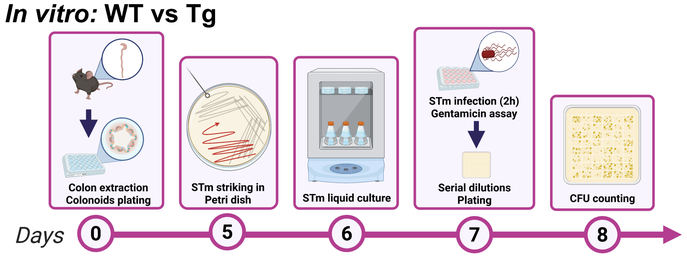
Villin PHB1 - Tg

- 3-fold increase of PHB1 constitutive expression.

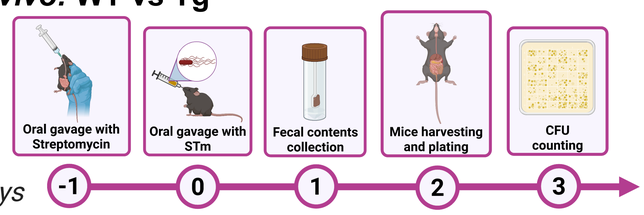


Specific overexpression in IECs

***In vitro:* WT vs Tg**



***In vivo:* WT vs Tg**



Results

Fig. 1 Overexpression of PHB1 in the IECs confers a protective effect to the host against STm invasion *in vitro*.

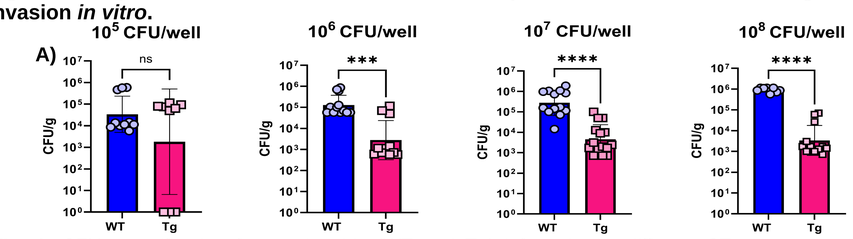


Fig. 1. CFU quantification from colonoids (WT and Tg) infected with different STm concentrations, 2h post-infection (A). ***Statistical analysis:*** Unpaired t test. **≤0.01, ***≤0.001, ****≤0.0001. N=3 independent experiments.

Fig. 2. Overexpression of PHB1 in the IECs confers a protective effect to the host against STm invasion *in vivo*.

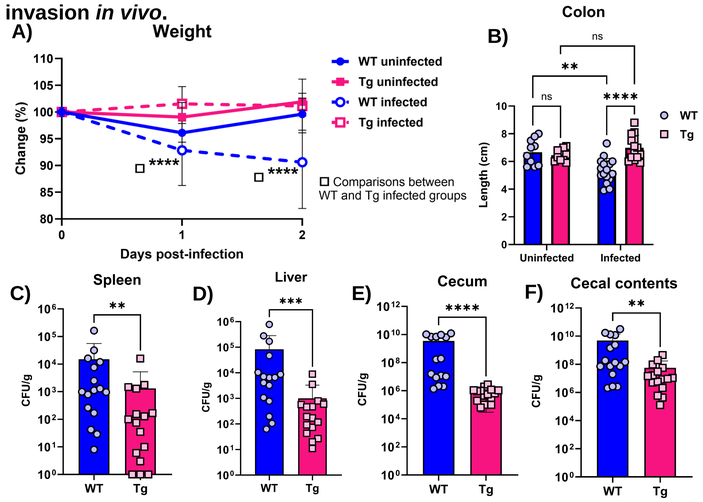


Fig. 2. Mice were euthanized after losing ≥15% of their body weight (2 days post-infection). In contrast to WT mice, Tg mice did not lose body weight (A) nor suffered significant colon shrinkage (B). ***Statistical analysis:*** *2way ANOVA.* Tg mice showed reduced bacterial loads in different organs and cecal contents (C-F). ***Statistical analysis:*** *Mann-Whitney.* N=16 biological replicates per infected group, N=10 biological replicates per uninfected group. **≤0.01, ***≤0.001, ****≤0.0001.

Fig. 3. Overexpression of PHB1 in the IECs reduces inflammation against STm invasion *in vivo* and protects mice for longer periods of time.

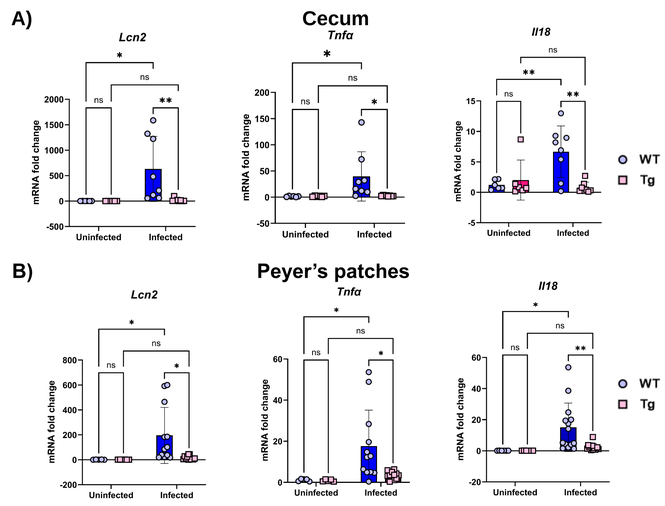


Fig. 3. Inflammation was reduced in Tg mice compared to WT after STm infection by q-RT-PCR quantification of inflammatory genes from cecum (A) and Peyer's patches (B). ***Statistical analysis:*** *2way ANOVA.* N=6 biological replicates per uninfected group, N=9-12 biological replicates per infected group. Tg mice survived longer than WT mice (C) ***Statistical analysis:*** *Survival curve.* N= 19 biological replicates (WT), N=21 biological replicates (Tg). Uninfected and infected H&E-stained cecal sections (D). *p<0.05, **≤0.01.

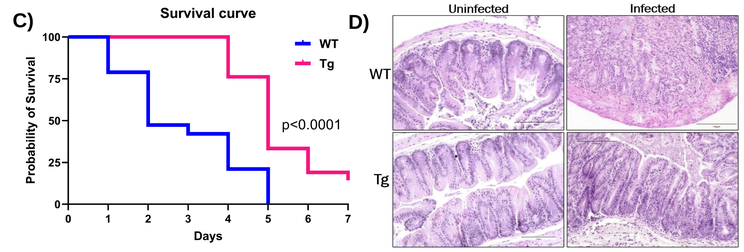


Fig. 4. Protective effect of PHB1 is extended against *E. coli* LF82 *in vitro*.

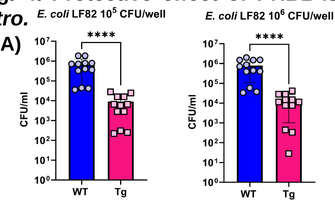


Fig. 4. CFU quantification from colonoids (WT and Tg) infected with different *E. coli* LF82 concentrations, 2h post-infection (A). ***Statistical analysis:*** Unpaired t test. ****≤0.0001. N=3 independent experiments.

Fig. 5. Microfold (M) cells increase might contribute to STm infection.

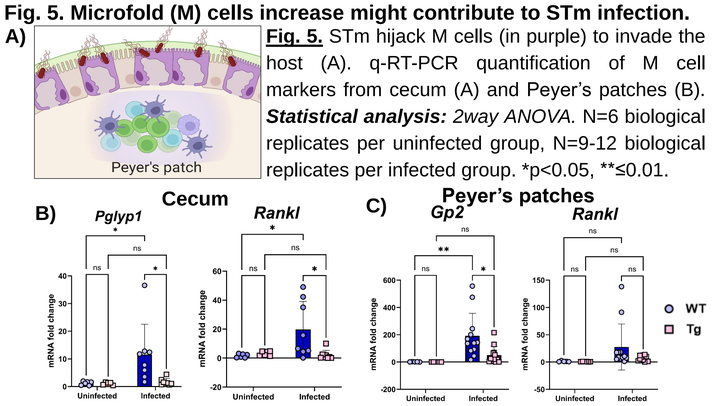


Fig. 5. STm hijack M cells (in purple) to invade the host (A). q-RT-PCR quantification of M cell markers from cecum (A) and Peyer's patches (B). ***Statistical analysis:*** *2way ANOVA.* N=6 biological replicates per uninfected group, N=9-12 biological replicates per infected group. *p<0.05, **≤0.01.

Fig. 6. Downregulation of cytoskeleton and actin remodeling genes in the KEGG "*Salmonella* infection" pathway of uninfected Tg mice.

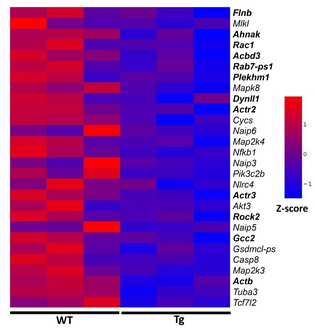


Fig. 5. RNA-seq shows downregulation of the KEGG 'Salmonella infection' pathway in uninfected Tg mice; reduced cytoskeleton gene expression may underlie decreased STm invasion and protection in comparison to WT. ***Statistical analysis:*** Z-score, 3 biological replicates per group.

Conclusions

- Both ***in vitro* and *in vivo*** overexpression of **PHB1 in IECs conferred protection** to the host against STm invasion.
- After STm infection, **PHB1 in IECs reduces inflammation** in the GI tract. This protective effect is extended to additional enteropathogens.
- **PHB1 reduces cytoskeletal genes** at basal level in the host, potentially altering the ability of STm ability to hijack them and **limiting invasion.**
- **PHB1 might** contribute to control **reduce M cells availability and proliferation** to limit STm invasion.