Supplementary Materials

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Mouse generation. Phb1$^{fl/fl}$ mice on the C57Bl/6 genetic background were generated by flanking exons 4 and 5 of the *Phb1* alleles with *loxP* sites as previously described.[1] Cre-mediated excision of exons 4 and 5 generates a frame shift mutation and early STOP codon at amino acid 117, resulting in a truncated, non-functional PHB1 protein lacking the C-terminus from Leu85 to Gln272.[1] *Phb1$^{fl/fl}$* mice were crossed with either *Villin-Cre-ER^{T2}* mice (kindly provided by Dr. Sylvie Robine, Institut Curie-CNRS, Paris), *Defα6-Cre* mice (described previously[2]), or *Mist1-Cre-ER^{T2}* mice (described previously[3]) (all C57Bl/6). The resulting offspring were genotyped by PCR analysis of tail genomic DNA obtained at weaning for expression of the floxed *Phb1* allele and *Villin-Cre-ER^{T2}*, *Defα6-Cre*, or *Mist1-Cre-ER^{T2}* transgenes using primer sequences previously described.[1-4] Experiments were performed with age- and gender-matched littermate mice. All mice were grouped-housed in standard cages under SPF conditions and were allowed standard chow and tap water *ad libitum*. All experiments were approved by the Baylor Scott & White Research Institute Institutional Animal Care and Use Committee.

Reagents and Antibodies. The following antibodies were used for western immunoblotting: PHB1 (70R-5543, polyclonal, Fitzgerald), PHB2 (14085, E1Z5A, Cell Signaling), Keratin 17 (4543, D73C7, Cell Signaling), CD3 (4443, CD3-12, Cell Signaling), Vimentin (49636, V9, Cell Signaling), PKR (sc136038, 13, Santa Cruz), ClpP (sc271284, B-12, Santa Cruz), Hsp60 (sc136291, 24, Cell Signaling), PCNA (ab18197, polyclonal, Abcam), Cleaved Caspase 3 (9661, Asp175, Cell Signaling), Cleaved Caspase 1 (sc56036, 14F468, Santa Cruz), CHOP (2895, L63F7, Cell Signaling), sXBP1 (12782, D2C1F, Cell Signaling), BiP (3177, C50B12, Cell

Signaling), β-actin (A1978, AC-15, Sigma-Aldrich). Antibodies were validated by western blot using the respective recombinant protein as positive control. The following antibodies were used for immunostaining: PHB1 (70R-5543, polyclonal, Fitzgerald), Lysozyme (sc27956, polyclonal, Santa Cruz), Muc2 (ab134119, EPR6145, Abcam), Ki67 (ACK02, K-2, Leica), Fabp6 (sc23994, polyclonal, Santa Cruz), Chromogranin A (ab45179, polyclonal, Abcam), 4-HNE (ab46545, polyclonal, Abcam). Isotype controls were included to validate immunostaining. The following secondary antibodies were used: donkey anti-goat IgG (Rhodamine Red X AffiniPure F(ab')$_2$ Fragment, 705-296-147, polyclonal, Jackson Immuno), donkey anti-rabbit IgG (FITC AffiniPure F(ab')$_2$ Fragment, 711-096-152, polyclonal, Jackson Immuno), Horseradish peroxidase-labeled goat anti-rabbit IgG (111-036-003, polyclonal, Jackson Immuno).

Inducible deletion of Phb1 from intestinal epithelial cells. Tamoxifen (Sigma Aldrich, St. Louis, MO) was prepared as described previously.[4] 8-week old *Phb1*$^{i\Delta IEC}$ mice and *Phb1*$^{iMist1\Delta PC}$ male and female mice were i.p. injected with 100 µl of 10 mg/ml tamoxifen for 4 consecutive days to induce deletion of *Phb1*. Mice were sacrificed 1, 3, 6 or 12 weeks after initial tamoxifen injection. Tamoxifen injections were repeated every 3-4 weeks to ensure continuity of PHB1 deletion.[4] Body weight was measured weekly. Of the 85 *Phb1*$^{i\Delta IEC}$ mice studied, 77 survived to the experimental endpoint; 6 mice died between weeks 3-6, 2 mice between weeks 9-12.

Treatment of mice with Mito-TEMPO. Concurrent with tamoxifen injection, 8-week old mice were i.p. injected with 500 µg/kg body weight Mito-TEMPO (Enzo Life Sciences) or vehicle (sterile saline) daily for 3 weeks. A subset of mice were administered Mito-Tempo (500 µg/kg body weight every other day) for an additional 9 weeks to assess severity of ileitis by histology.

Histology and Alcian Blue Periodic Acid-Schiff staining. Whole stomach and cecum and Swiss-rolls of small intestine and colon were fixed in 10% formalin and embedded in paraffin. To assess histology, 5-μm sections were stained with H&E. The entire GI tract was examined by trained GI pathologists (W.L.N. and K.T.) for alterations to tissue structure or inflammation. Sections of ileum were histologically scored by trained GI pathologists (W.L.N. and K.T.) in a blinded fashion for severity of villous distortion, active inflammation, and chronic inflammation as previously described.[5] Sections were stained with Alcian Blue (AB; Sigma Aldrich) solution, pH 2.5, for 5 minutes and washed in running tap water for 5 minutes. Sections were then stained with Periodic Acid and Schiff's (PAS) reagent according to PAS kit (Sigma Aldrich) protocol and mounted using xylene-based mounting media. Area of cytoplasmic mucin/cell for AB^+ cells above the crypt base was measured using Image J software (National Institutes of Health).

Transmission electron microscopy. Distal ileum was fixed in 2% gluteraldehyde in PBS, dehydrated and embedded in epoxy resin for electron microscopy. Ultrathin 70 nm sections were examined on a transmission electron microscope (Hitachi BioMedical TEM).

Immunohistochemistry. 7 μM paraffin-embedded sections of ileum were dehydrated in xylene and ethyl alcohol gradient, incubated in 0.3% H_2O_2 for 30 min, washed, incubated in 10mM sodium citrate for antigen epitope retrieval, blocked in 5% normal goat serum, and exposed to the appropriate antibody at 4ºC overnight. Peroxidase-labeled anti-rabbit immunoglobulin G secondary antibody and ABC reagent were added using the peroxidase-conjugated avidin ABC kit (Vector Laboratories, Burlingame, CA). Sections were counterstained with hematoxylin to visualize histology.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining.

Immunofluorescent TUNEL staining was performed to measure apoptosis from paraffin-embedded sections using the In Situ Cell Death Detection kit as described by the manufacturer (Roche, Indianapolis, IN). Nuclei were stained with 4, 6'diamidino-2-phenylindole (DAPI) to assess total cells per crypt.

Isolation of intestinal epithelial cells (IECs). IEC isolation was performed as previously described.[6]

Detection of mitochondrial Complex I, II and IV activities, ATP, and 8-OHdG. The activities of Complexes I, II, and IV were measured in whole cell lysates of freshly isolated IECs using Complex I, II, and IV Microplate Assay Kits (Abcam) according to the manufacturer's instructions. 50 μg of protein extracted from ileum IECs was used. The concentration of adenosine triphosphate (ATP) in isolated IECs was determined using the ENLIGHTEN ATP Assay Bioluminescence Detection kit (Promega) according to the manufacturer's protocol. To measure 8-OHdG, DNA was extracted (DNeasy Kit, Qiagen) from homogenized ileal samples, digested using nuclease P1 (New England Biolabs), and measured for 8-OHdG by ELISA (Abcam).

Flow cytometry. Mesenteric lymph nodes from the ileum were removed using a dissecting microscope. Lamina propria immune cells were isolated using the Mouse Lamina Propria Dissociation Kit (Miltenyi) and counted using the Countess II Automated Cell Counter. The following antibodies were used for flow cytometry (BD FACSAria III): violetFluor™ 450 Anti-Mouse CD45 (75-0451-U025, Tonbo), APC-Cyanine7 Anti-Mouse CD4 (25-0042, Tonbo), FITC Anti-Mouse TCR beta (35-5961, Tonbo), PE-Cyanine5 Anti-Human/Mouse CD11b (55-

0112, Tonbo), APC Anti-Mouse CD11c (20-0114-U025, Tonbo), PE-Cyanine7 Anti-Mouse F4/80 Antigen (60-4801, Tonbo), 7-AAC (13-6993, Tonbo).

Western blot analysis. Total protein was isolated from whole ileum or isolated IECs and separated by SDS-PAGE and analyzed by western blotting as described previously.[7]

Cytokine ELISA. Whole ileum was homogenized and analyzed using the Mouse Inflammatory Cytokines Multi-Analyte ELISArray Kit and the Mouse Common Chemokines Multi-Analyte ELISArray Kit (Qiagen) as described by the manufacturer. IL-1β and IL-18 were assayed using ELISA kits from Abcam.

RNA isolation and quantitative real-time PCR analysis. Total RNA was isolated from ileum using the RNeasy kit (Qiagen, Valencia, CA). Quantitative real-time PCR was performed as described previously.[7] For graphical representation of quantitative PCR data, the $\Delta\Delta C_T$ was calculated as follows: $\Delta\Delta C_T = (Ct,_{\text{target}} - Ct,_{\beta\text{-actin}})_{Phb1i\Delta IEC\ or\ Phb1\Delta PC} - (Ct,_{\text{target}} - Ct,_{\beta\text{-actin}})_{Phb1fl/fl}$, with the final graphical data derived from $2^{-\Delta\Delta CT}$. Primers sequences used for quantitative RT-PCR are provided in Supplemental Table 2.

16S rRNA gene sequencing. Stool was collected from $Phb1^{fl/fl}$ and $Phb1^{i\Delta IEC}$ F2 generation littermates at baseline prior to tamoxifen injection (week 0, two separate collections 1 day apart) and 1, 3, and 12 weeks after *Phb1* deletion. Mice were co-housed throughout the experiment to standardize the gut microbiota for isolating the contribution of genetics to phenotype.[8] DNA extraction, 16S ribosomal RNA gene PCR amplication, and sequencing on an Illumina MiSeq platform was performed by Diversigen, Houston, TX. MiSeq Reagent kit (v3) was used with 2 ×

300 bp read lengths. Mothur software was used to analyze α and β diversity of taxonomic classification and principle coordinates analysis.

Ileal enteroid culturing. Mouse ileal crypts were isolated and plated in Reduced Growth Factor Matrigel (356230, BD Biosciences) using the method by Sato et. al.[9] Enteroids were cultured in Advanced DMEM/F-12 (12634-010, Gibco, ThermoFisher) supplemented with GlutaMAX (Gibco 35050-061, ThermoFisher), Pen/Strep (P4458, Sigma Aldrich), HEPES (Gibco 15630-080, ThermoFisher), 1× N2 supplement (Gibco 17502-048, ThermoFisher), 1 ng/μl EGF (2028-EG, R&D Systems, Minneapolis, MN), 2 ng/μl Noggin (250-38, PeproTech, Rocky Hill, NJ), 10 ng/μl murine R-spondin (3474-RS-050, R&D Systems), and Y27632 (ALX-270-333-M025, Enzo Life Sciences, Farmingdale, NY). Enteroids were counted at day 1, and bud formation and death by visualizing altered morphology as described[10] were assessed at day 2, 3, 4, and 7 using an Zeiss Axioskope Plus Inverted Microscope. Enteroids were harvested at day 7 and RNA was isolated using RNeasy kits (Qiagen). 100 nM Mito-Tempo (Enzo Life Sciences) or vehicle were added daily with fresh media from days 0-7 of culture. MitoSOX fluorescence was measured on day 7 by incubating enteroids in with Hank's balanced salt solution (HBSS) with 5 μM MitoSOX Red Mitochondrial Superioxide Indicator dye (Invitrogen) for 10 min at 37°C. Enteroids were washed twice with warm HBSS and fluorescent intensity was measured at 510 nm excitation/580 nm emission. Fluorescent intensity was normalized to number of enteroids.

Statistical analysis. Values are expressed as mean ± SEM or as individual data points ± SEM. Statistical analysis was performed using unpaired 2-tailed Student's *t* test was used for single comparisons and 1-way ANOVA with Bonferroni post-hoc test for multiple comparisons (PRISM 6.0, GraphPad Software). For experiments using Mito-TEMPO treatment, statistical

analysis was performing using 2-way ANOVA and subsequent Bonferroni post-hoc tests. $P < 0.05$ was considered significant.

Patient and public involvement. Patients/the public were not involved in the design of this study.

Supplementary Materials References

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