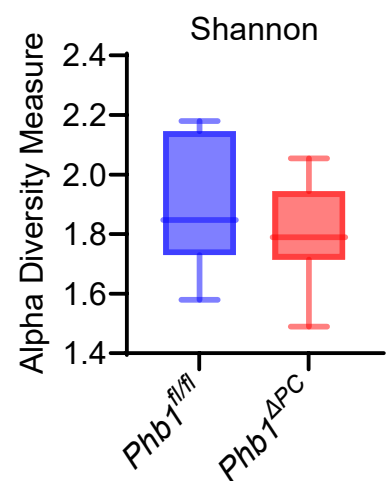


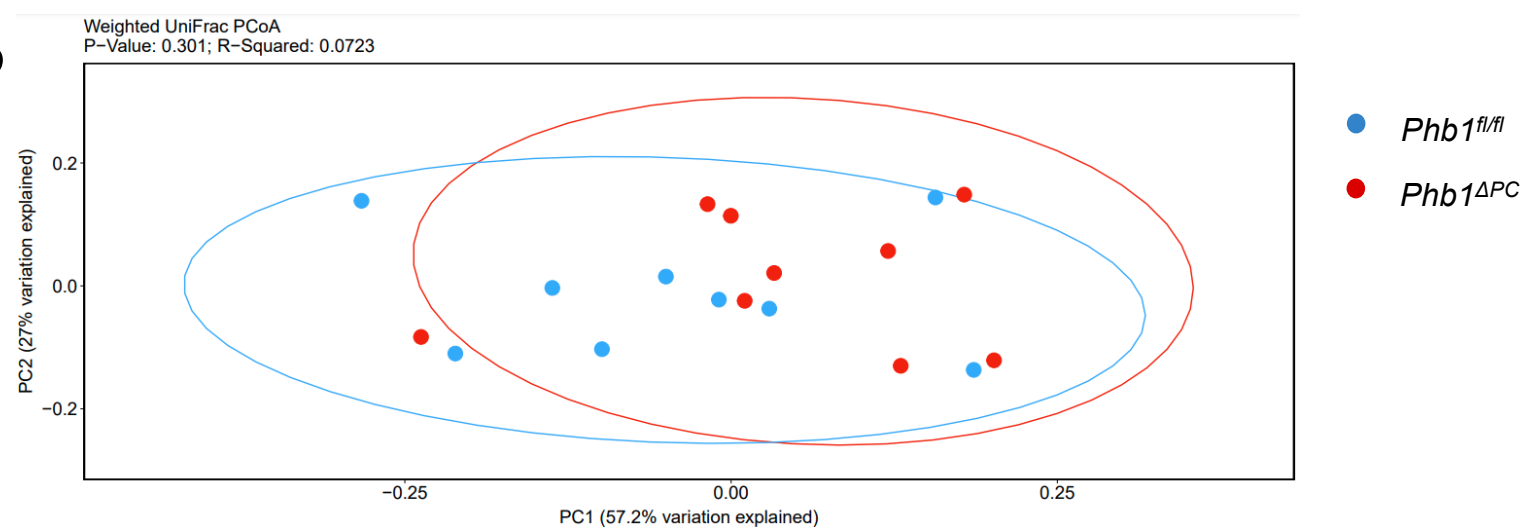
Supplemental Figure S1. Elimination of gut microbiota by broad-spectrum antibiotics (ABX) prevents the manifestation of crypt base AB-PAS⁺ cells in *Phb1* deficient mice. (A) Bacterial elimination by ABX shown by reads per sample by 16S rRNA sequencing. (B) AB-PAS staining of ileum. Bar = 100 μ m. Dashed line denotes crypt base. (C) Number of AB-PAS⁺ cells/crypt base across 50 crypts. AB-PAS, alcian blue-periodic acid Schiff. $n = 7-13$ each group. **** $P < 0.0001$.

Co-housed across genotype

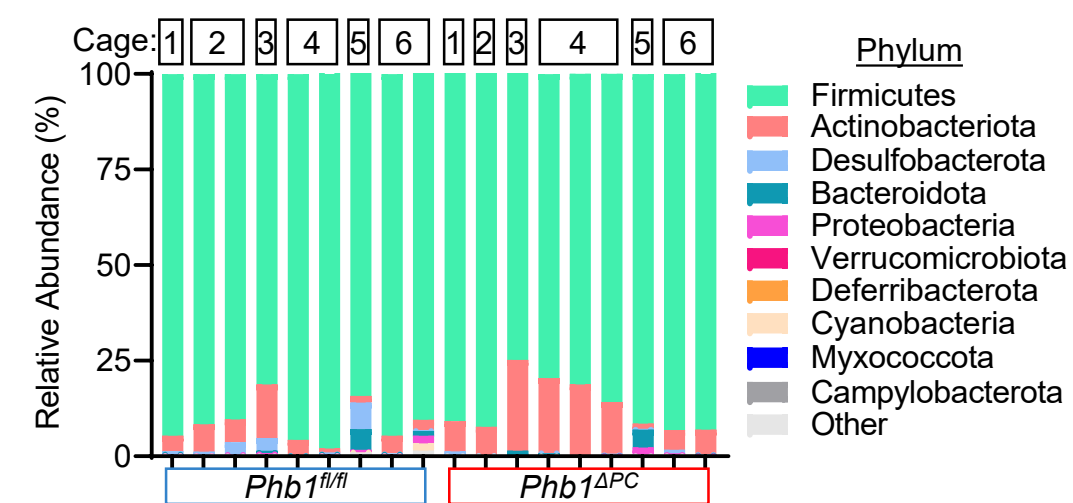
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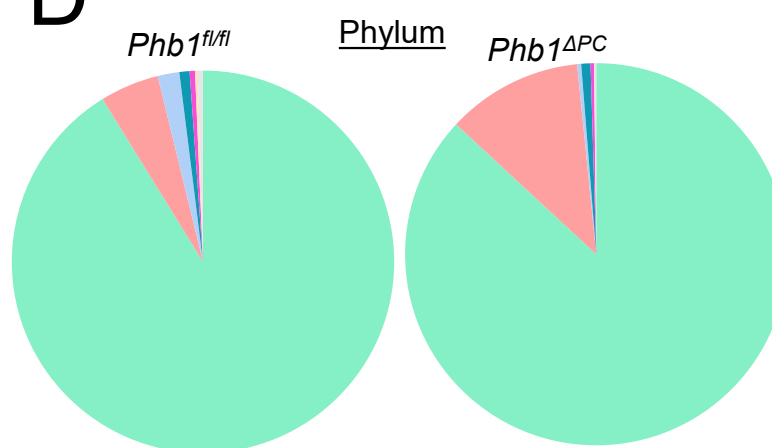
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C

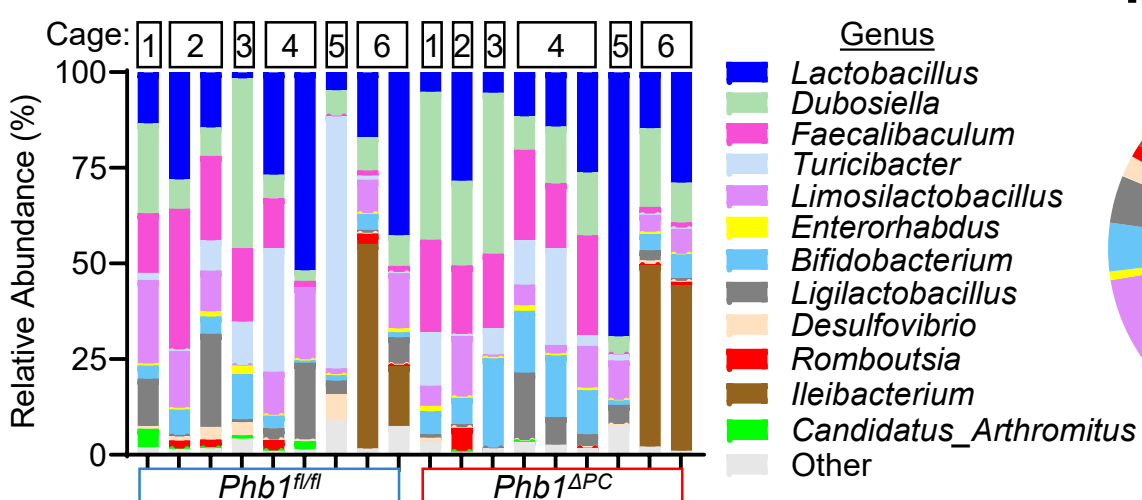


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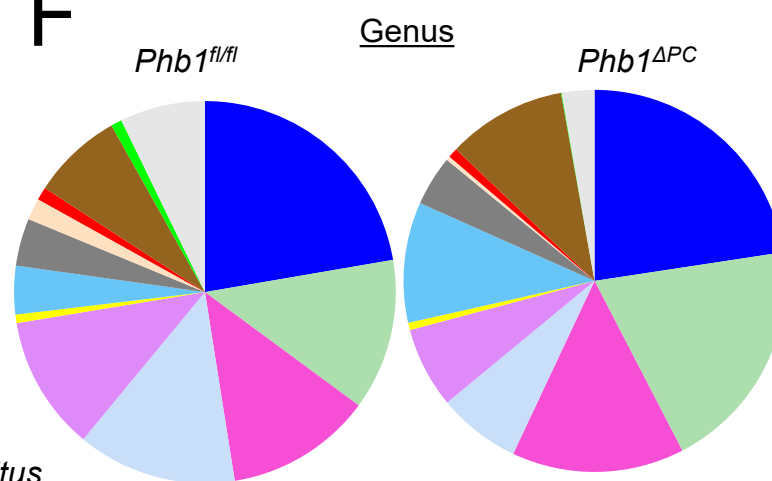


Phylum (%)	<i>Phb1^{fl/fl}</i>	<i>Phb1^{ΔPC}</i>
Firmicutes	91.2	86.9
Actinobacteriota	5	11.5
Desulfobacterota	1.8	0.3
Bacteroidota	0.9	0.8
Proteobacteria	0.5	0.3
Verrucomicrobiota	0	0
Defferibacterota	0	0
Cyanobacteria	0.2	0
Myxococcota	0	0
Campylobacterota	0	0
Other	0.4	0.2

E



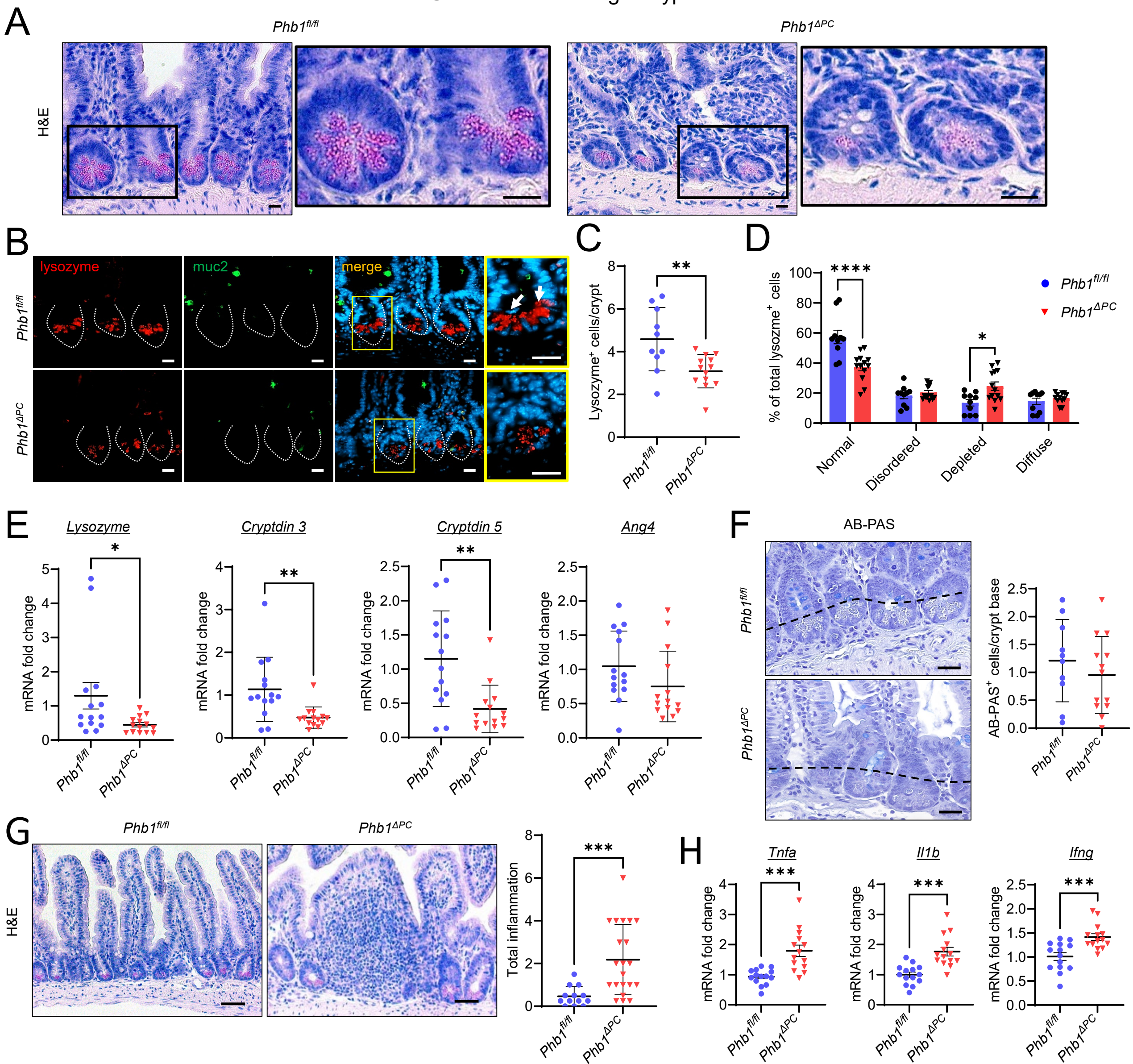
F



Genus (%)	<i>Phb1^{fl/fl}</i>	<i>Phb1^{ΔPC}</i>
<i>Lactobacillus</i>	22.3	22.6
<i>Dubosiella</i>	12.8	19.8
<i>Faecalibaculum</i>	12.4	14.6
<i>Turicibacter</i>	13.5	7.0
<i>Limosilactobacillus</i>	11.4	6.8
<i>Enterorhabdus</i>	0.7	0.6
<i>Bifidobacterium</i>	4.1	10.2
<i>Ligilactobacillus</i>	4.0	4.3
<i>Desulfovibrio</i>	1.8	0.3
<i>Romboutsia</i>	1.1	0.8
<i>Ileibacterium</i>	7.7	10.1
<i>Candidatus_Arthromitus</i>	0.9	0.06
Other	7.2	2.8

Supplemental Figure S2. Co-housed *Phb1^{ΔPC}* mice exhibit similar ileal microbiota composition as *Phb1^{fl/fl}* littermates. 16S RNA sequencing of luminal ileal content of co-housed *Phb1^{fl/fl}* and *Phb1^{ΔPC}* mice. (A) Alpha diversity measure by Shannon index. (B) Weighted UniFrac PCoA plot of 16S rRNA gene sequences. (C-D) Relative abundance of bacteria at phylum level (C) in individual mice and (D) combined mice by genotype. (E-F) Relative abundance of bacteria at genus level (E) in individual mice and (F) combined mice by genotype. *n* = 9 each genotype.

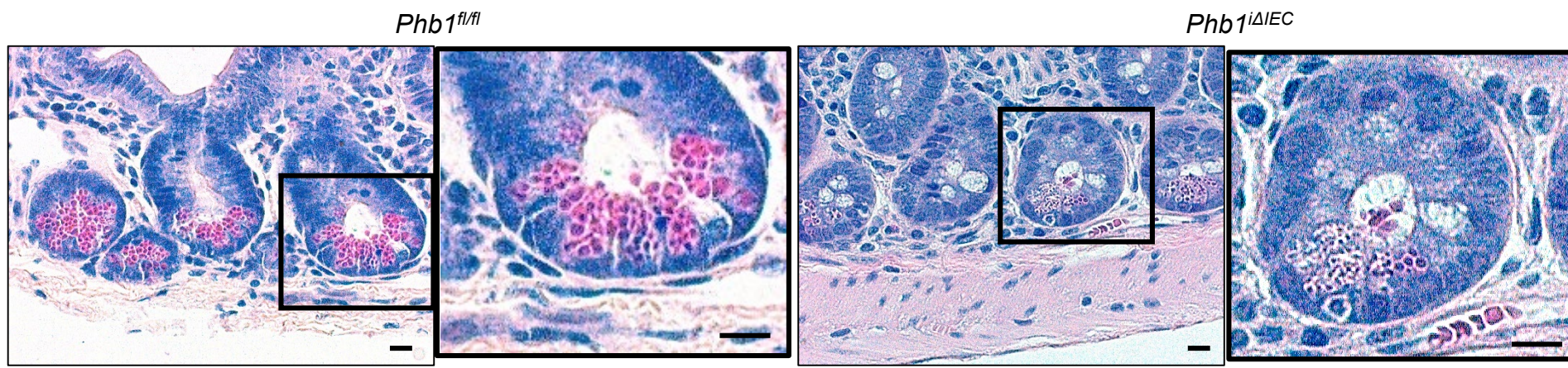
Co-housed across genotype



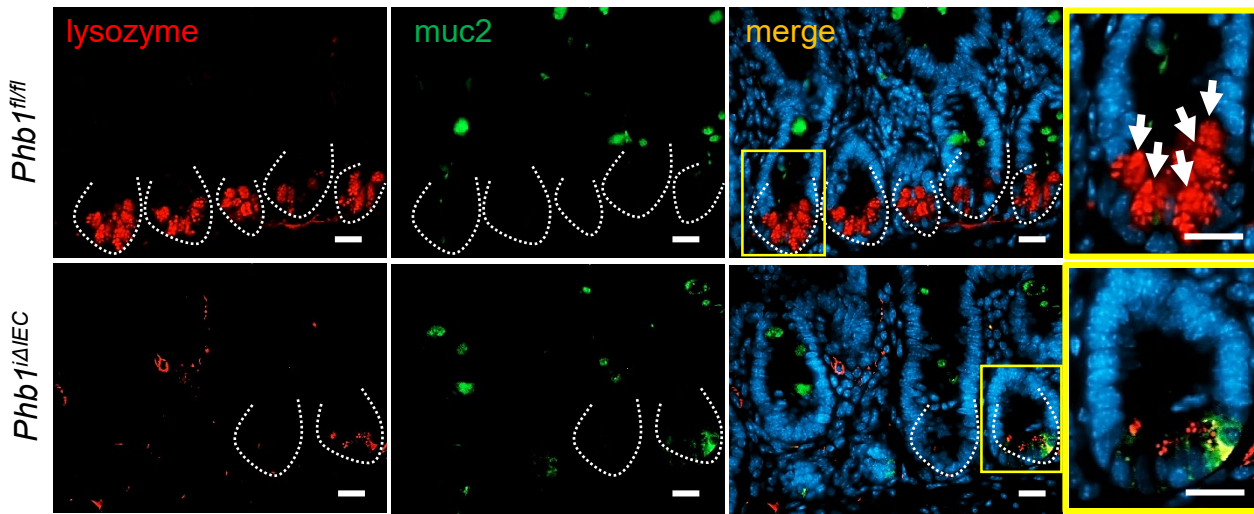
Supplemental Figure S3. Co-housed *Phb1^{ΔPC}* mice manifest PC abnormalities and ileitis. (A) H&E staining showing Paneth cells (pink granules) in the ileal crypts. Bar = 50 μm; boxed pullout bar = 200 μm. (B) Immunofluorescent-staining for lysozyme (red), muc2 (green), and DAPI (nucleus, blue) in ileal crypts (dashed line). Arrows denote Paneth cells with normal lysozyme packaging into granules. Bar = 50 μm. (C) Average number of lysozyme⁺ cells per crypt per mouse. A minimum of 50 crypts per mouse were quantitated. *n* = 13 *Phb1^{ΔPC}* or 10 *Phb1^{fl/fl}* littermates. (D) Paneth cell lysozyme allocation patterns. A minimum of 50 crypts per mouse were quantitated. *n* = 13 *Phb1^{ΔPC}* or 10 *Phb1^{fl/fl}* littermates. (E) mRNA quantification in ileum by qRT-PCR. *n* = 14 each genotype. (F) AB-PAS staining of ileum and number of AB-PAS⁺ cells/crypt base. Bar = 100 μm. Dashed line denotes crypt base. *n* = 13 *Phb1^{ΔPC}* or 10 *Phb1^{fl/fl}* littermates. (G) Representative H&E-stained ileum and histological inflammation scoring of ileum. Bar = 100 μm. *n* = 21 *Phb1^{ΔPC}* or 10 *Phb1^{fl/fl}* littermates. (H) mRNA quantification in ileum by qRT-PCR. *n* = 14 each genotype. AB-PAS, alcian blue-periodic acid Schiff; Ang4, angiogenin 4; H&E, hematoxylin & eosin; Ifng, interferon gamma; Il1b, interleukin 1 beta; muc2, mucin2; Tnfa, tumor necrosis factor alpha. Results are presented as individual mice ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.. *****P* < 0.0001.

Co-housed across genotype

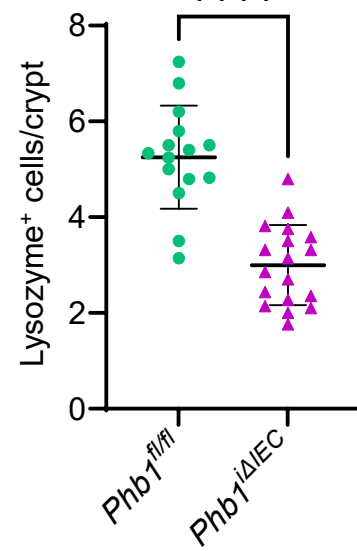
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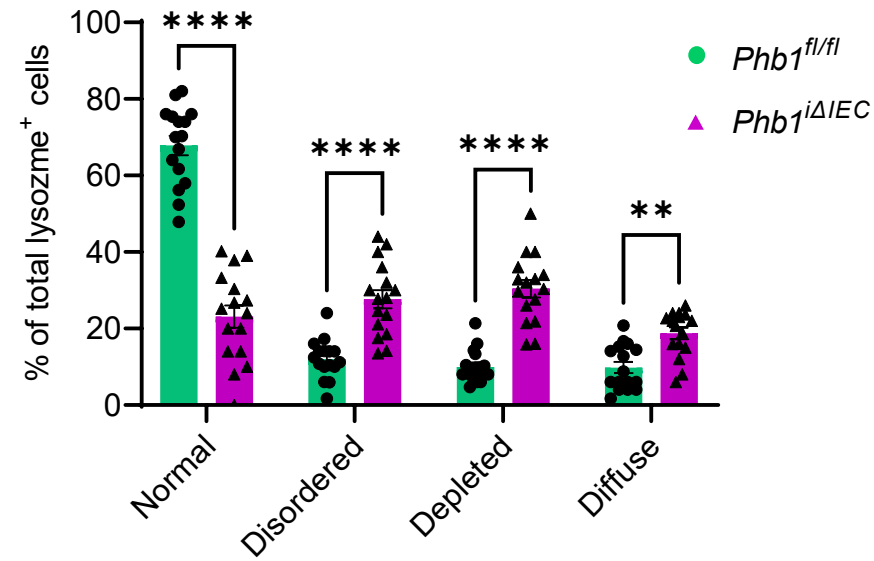
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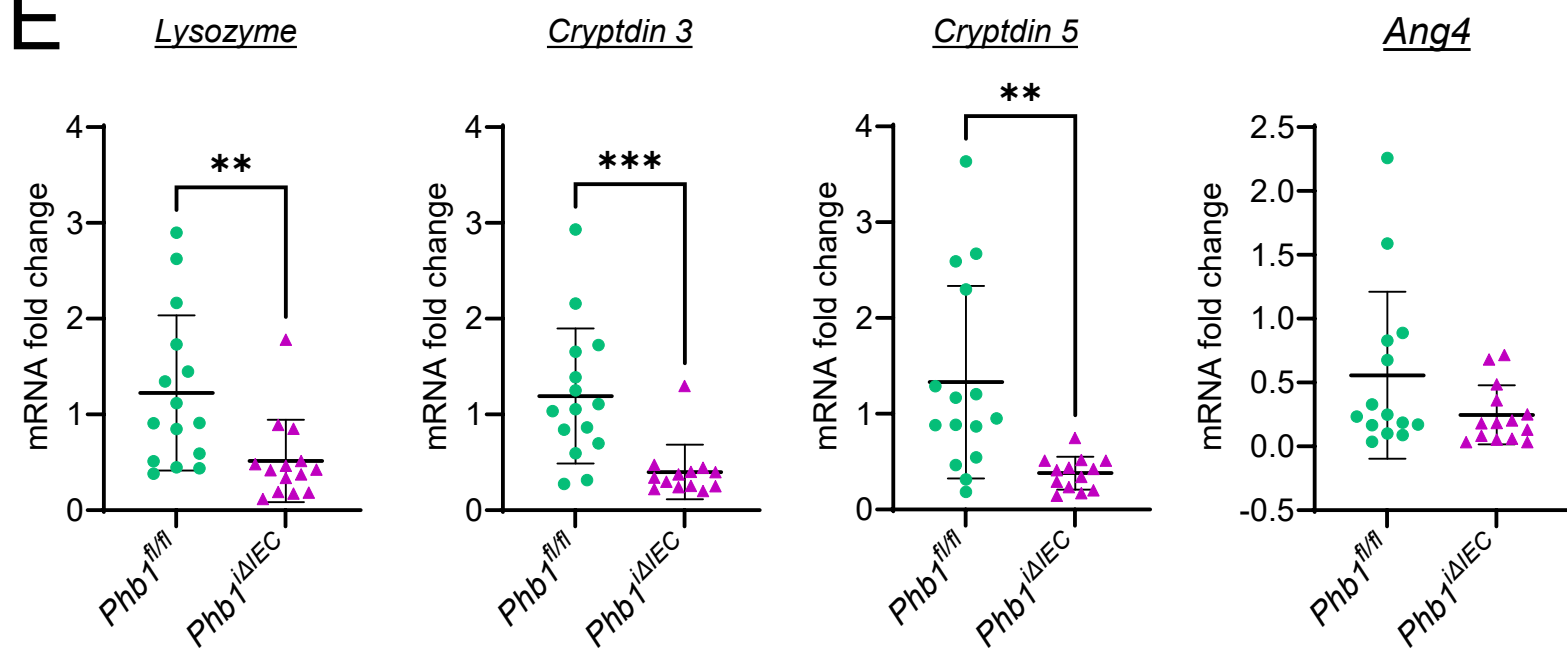
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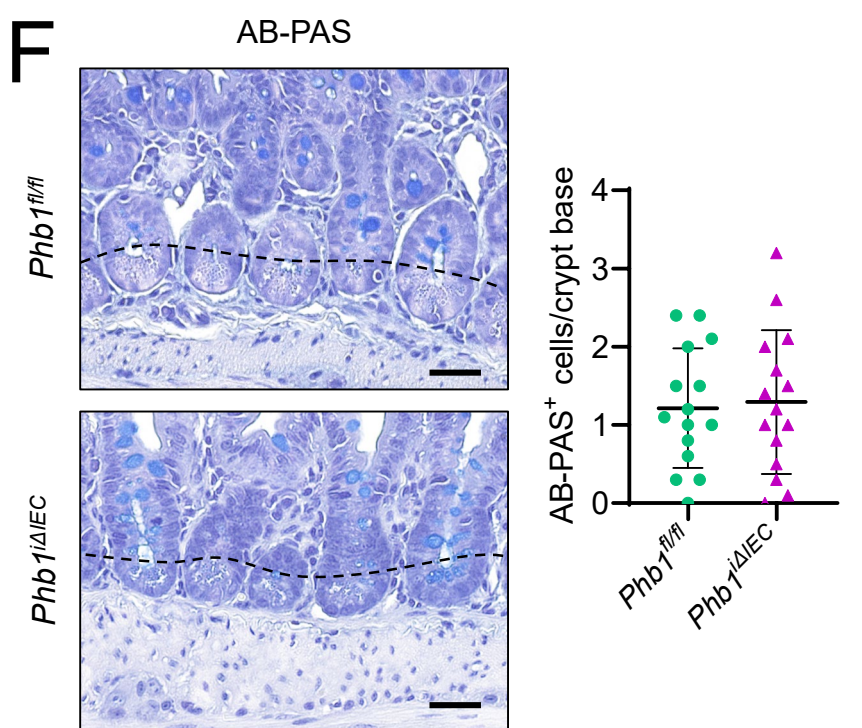
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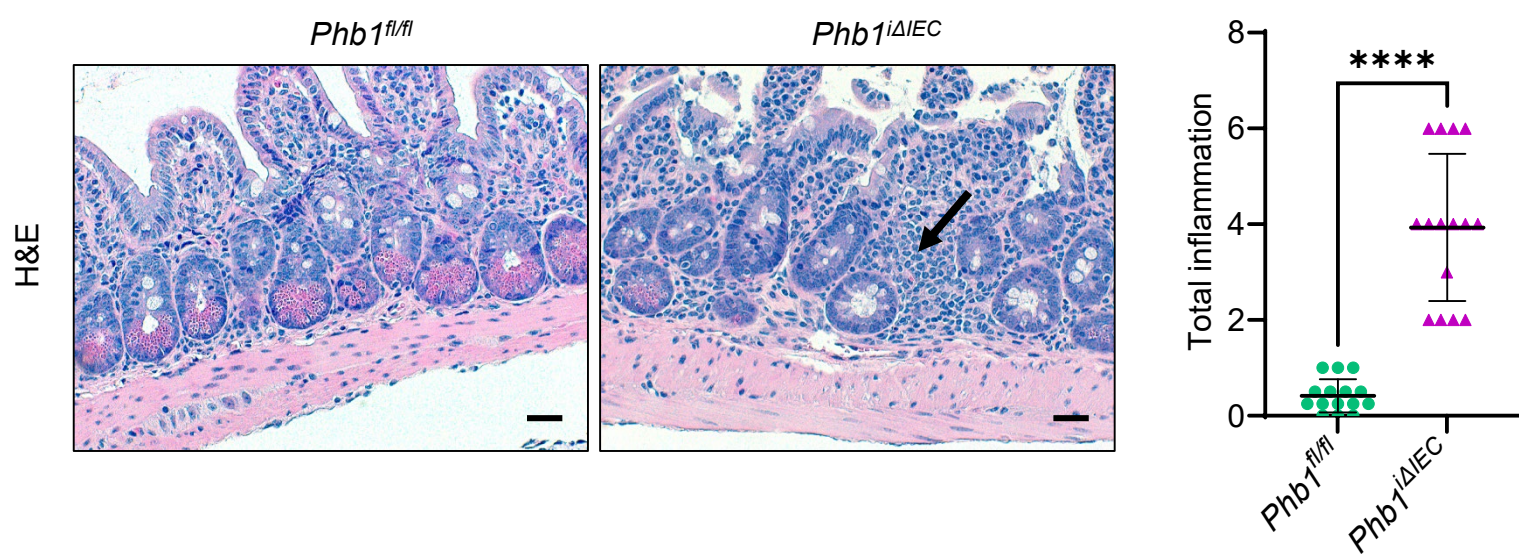
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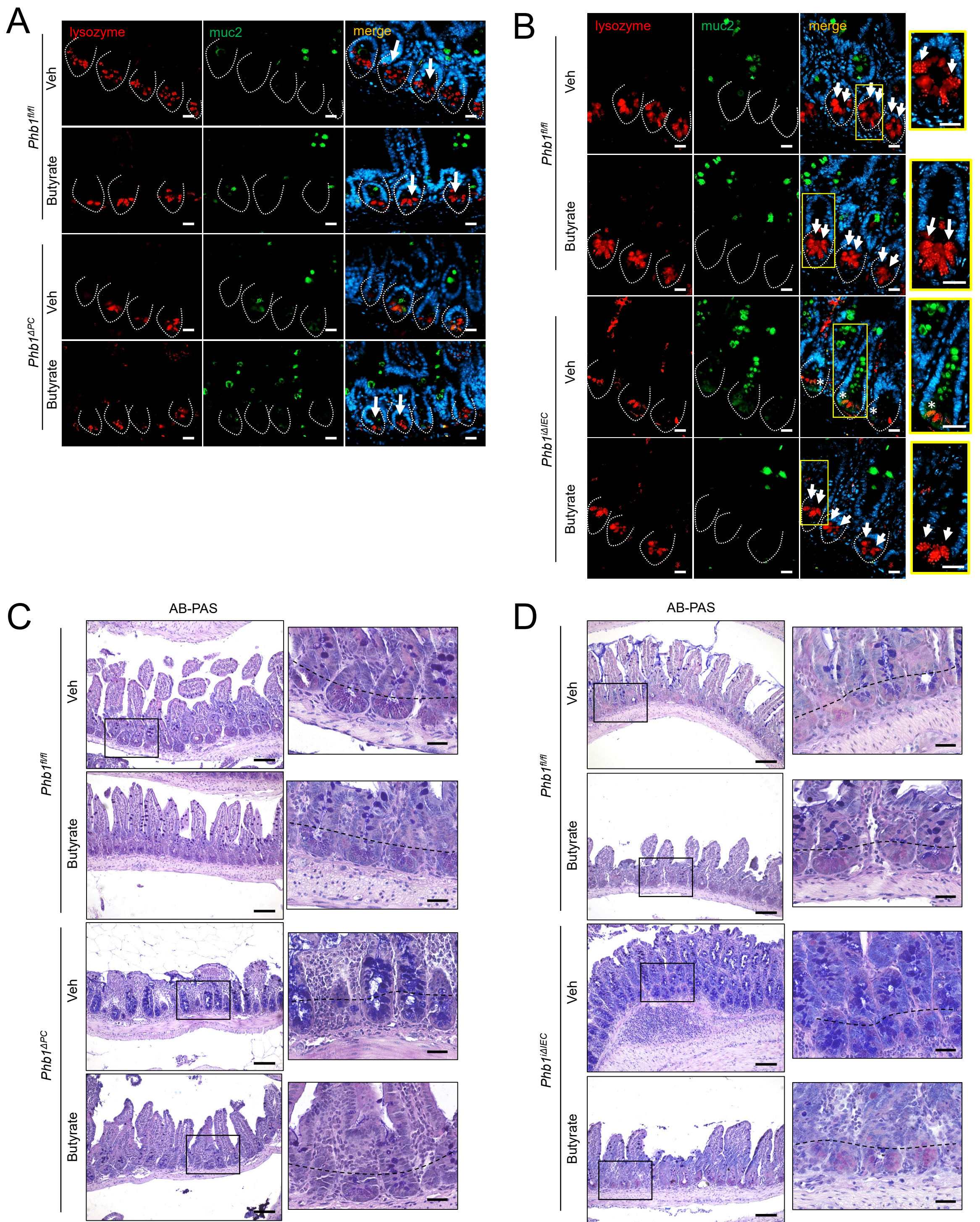
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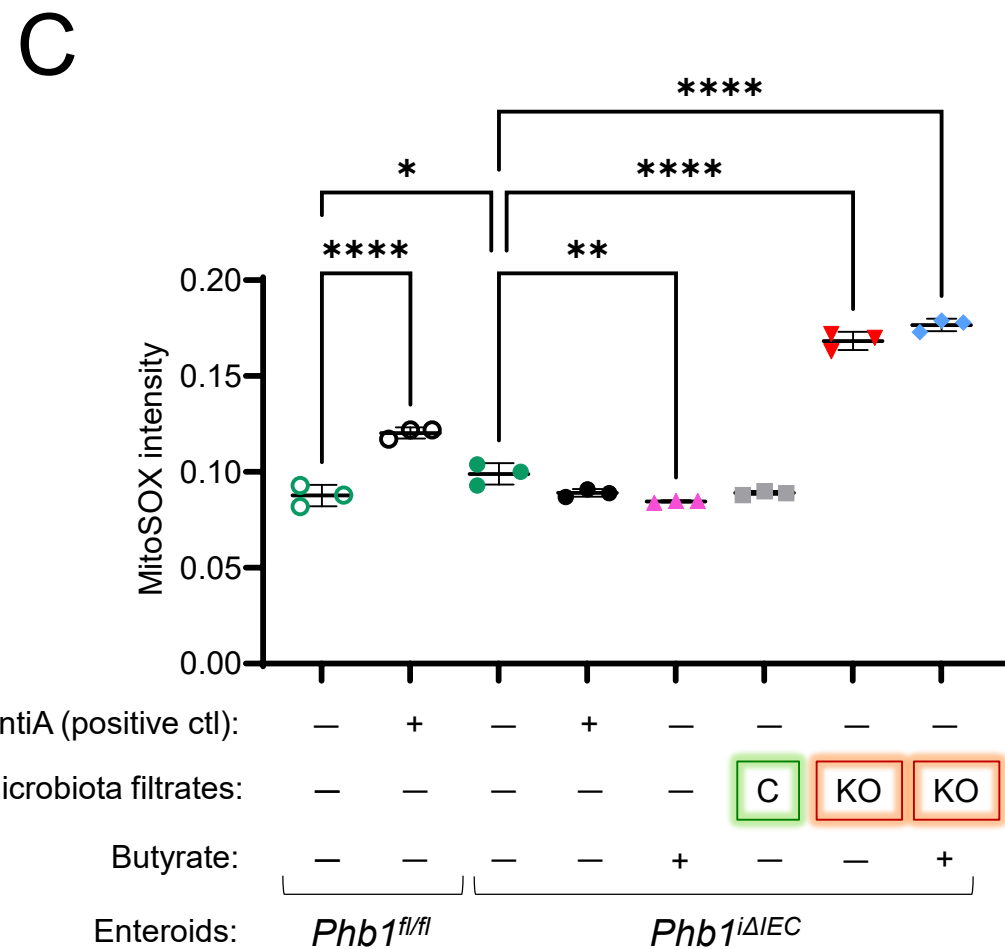
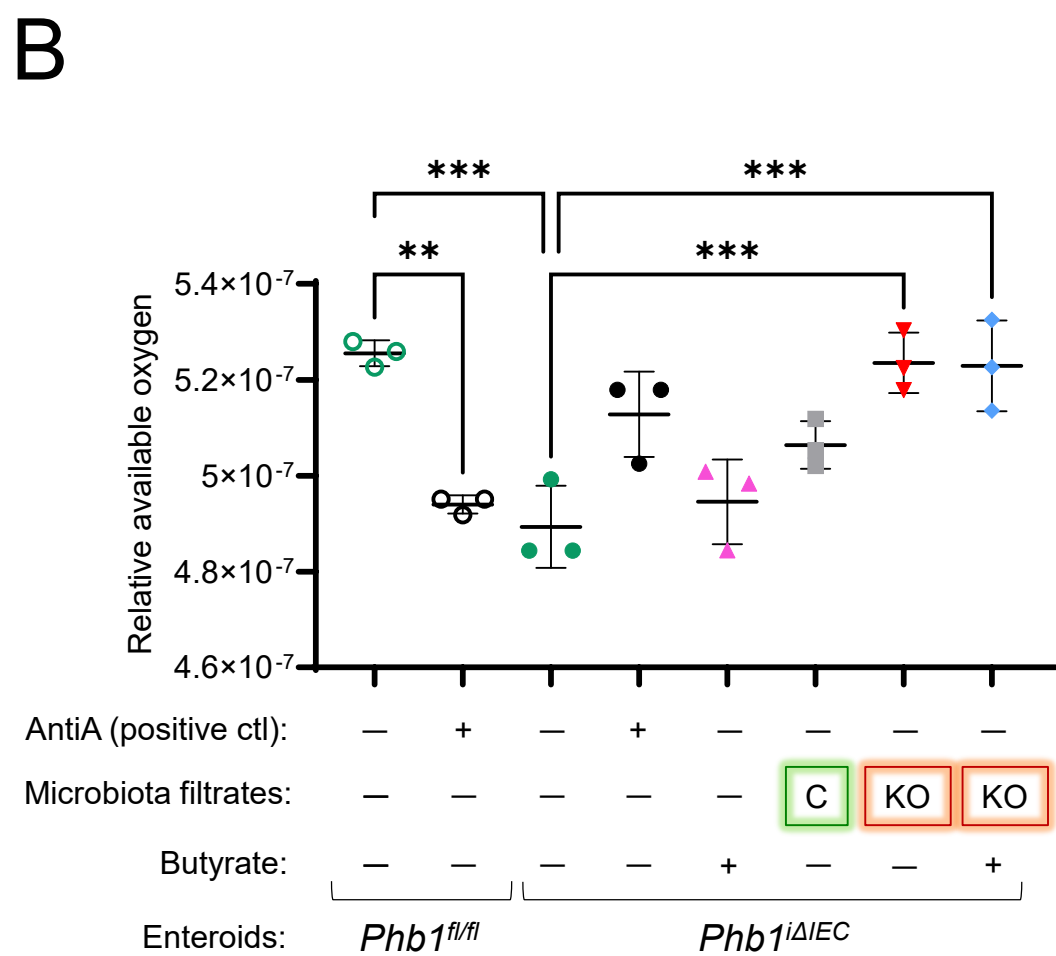
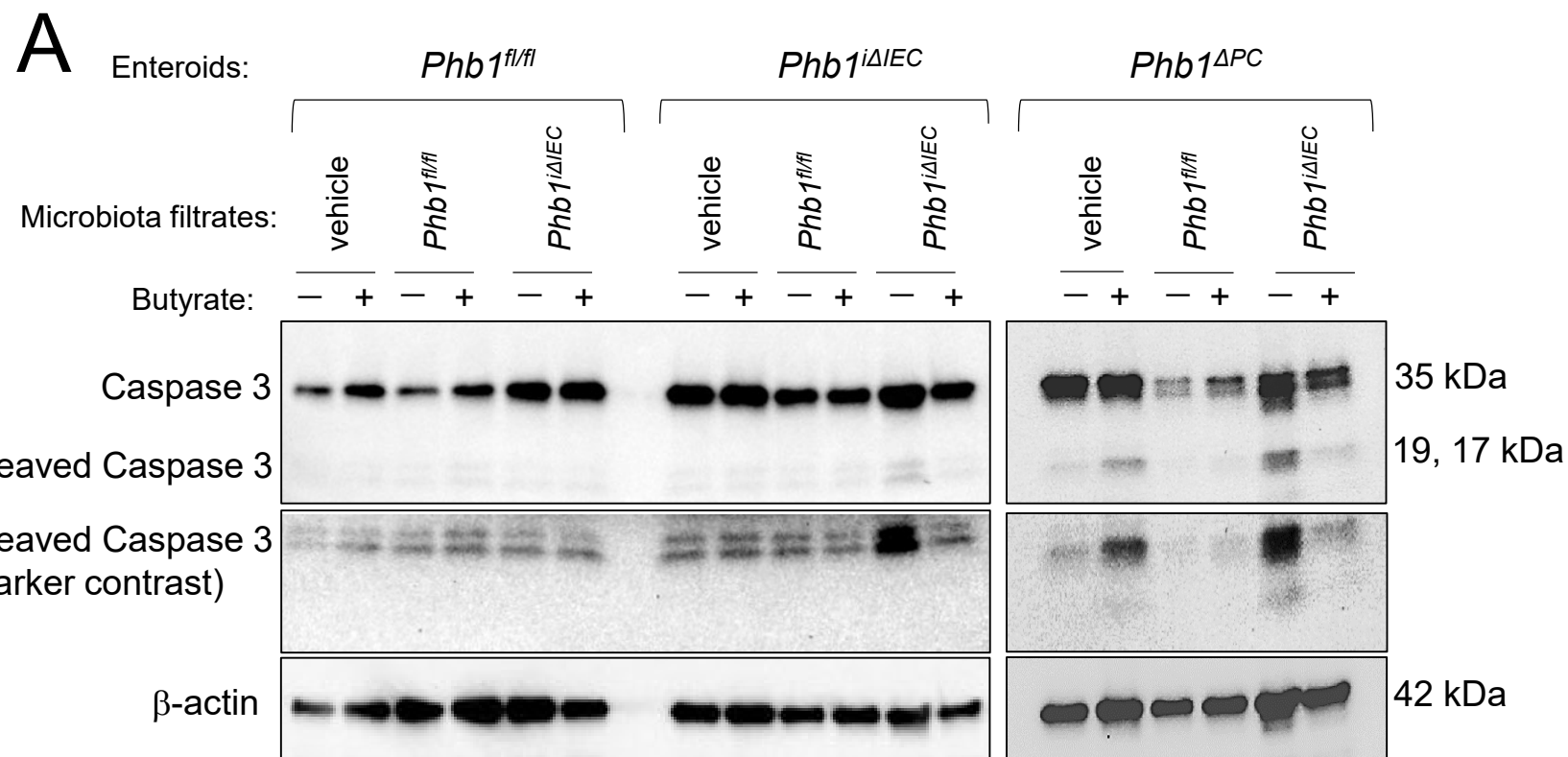
G



Supplemental Figure S4. *Phb1^{ΔIEC}* mice co-housed with *Phb1^{fl/fl}* littermates exhibit Paneth cell defects and spontaneous ileitis. (A) H&E staining showing Paneth cells (pink granules) in the ileal crypts. Bar = 50 μm. (B) Immunofluorescent-staining for lysozyme (red), muc2 (green), and DAPI (nucleus, blue) in ileal crypts (dashed line). Arrows denote Paneth cells with normal lysozyme packaging into granules. Bar = 50 μm. (C) Average number of lysozyme⁺ cells per crypt per mouse. *n* = 14 *Phb1^{fl/fl}* and 18 *Phb1^{ΔPC}* mice. (D) Paneth cell lysozyme allocation patterns. *n* = 16 each genotype. (E) mRNA quantification in ileum by qRT-PCR. *n* = 15 *Phb1^{fl/fl}* and 14 *Phb1^{ΔPC}* mice. Outliers were identified by ROUT test (*Q* = 1%) and were removed from *Phb1^{fl/fl}* *Ang4* and *Phb1^{ΔPC}* *Cryptdin3*, *Cryptdin 5*. (F) AB-PAS staining of ileum and number of AB-PAS⁺ cells/crypt base. Bar = 100 μm. Dashed line denotes crypt base. *n* = 15 each genotype. (G) Histological inflammation scoring of ileum. Arrow denotes infiltrating immune cells. Bar = 100 μm. *n* = 15 each genotype. AB-PAS, calcein blue-periodic acid Schiff; Ang4, angiogenin 4; H&E, hematoxylin & eosin; muc2, mucin2. Results are presented as individual mice ± SD. ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.



Supplemental Figure S5. Butyrate supplementation prevents Paneth cell defects in *Phb1*-deficient mice. (A-B) Immunofluorescent-staining for lysozyme (red), muc2 (green), and DAPI (nucleus, blue) in ileal crypts (white outline) of *Phb1*^{ΔPC} mice and *Phb1*^{fl/fl} littermates (A) or *Phb1*^{ΔIEC} mice and *Phb1*^{fl/fl} littermates (B). Arrows denote Paneth cells with normal Lysozyme packaging into granules. Asterisk denotes Muc2 colocalization with Lysozyme. Bar = 50 μm. (C-D) AB-PAS staining of ileum. Bar = 100 μm. Dashed line denotes crypt base. AB-PAS, alcian blue-periodic acid Schiff; muc2, mucin2; Veh, vehicle



Supplemental Figure S6. Butyrate protects *Phb1*-deficient ileal enteroids from *Phb1^{iΔIEC}* microbiota-induced death. (A) Western blots for total and cleaved Caspase 3 indicating apoptosis. (B) Oxygen consumption relative to *Phb1^{fl/fl}* vehicle enteroids. As a positive control, enteroids were treated with 0.1 μM Antimycin A 15 min prior to oxygen measurements. $n = 3$ for all treatments. (C) Mitochondrial superoxide level as measured by MitoSOX fluorescence intensity. $n = 3$ for all treatments, results are representative of 2 separate experiments. C: *Phb1^{fl/fl}* microbiota filtrate, KO: *Phb1^{iΔIEC}* microbiota filtrate. $n = 3$ for all treatments. AntiA, Antimycin A. Results are presented as individual mice \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.0001$.