Supplementary Information

Genomic characterisation of *Lactiplantibacillus plantarum* HCD07 and stage-resolved host–microbiota responses in ETEC O8-challenged mice

Scientific Reports

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References cited in this Supplementary Information are listed in the main manuscript.

This file contains Supplementary Tables S1–S10 and Supplementary Figs. S1–S3.

Supplementary Table S1. Genome assembly, taxonomic assignment, safety-related genomic screening and predicted functional features of HCD07.

Section	Analysis or metric	Result	Interpretation
Genome assembly and annotation	Genome size	3,360,189 bp	Draft-assembly metric.
Genome assembly and annotation	Scaffolds	31	Draft-assembly metric.
Genome assembly and annotation	N50	376,432 bp	Draft-assembly metric.
Genome assembly and annotation	GC content	44.32%	Draft-assembly metric.
Genome quality	CheckM completeness	99.07%	Genome-quality estimate.
Genome quality	Strain heterogeneity	0.00%	CheckM estimate.
Gene annotation	Protein-coding sequences	3,194	Predicted coding sequences.
Gene annotation	tRNA genes	60	Predicted tRNA genes.
Gene annotation	rRNA genes	One annotated copy each of 5S, 16S and 23S rRNA	Draft-assembly annotation.
Taxonomic assignment	GTDB-Tk	Lactiplantibacillus plantarum	Supports species-level assignment.
Taxonomic assignment	FastANI reference	GCF_014131735.1	Reference genome used for ANI analysis.
Taxonomic assignment	Average nucleotide identity	98.91%	Above the conventional 95% species-level threshold.
Taxonomic assignment	Alignment fraction	0.911	Above the prespecified 0.5 criterion.
Safety-related genomic screening	Acquired AMR genes (ResFinder)	Not detected	Does not replace phenotypic susceptibility testing.
Safety-related genomic screening	CARD homology hits	33	Sequence-homology annotations; not direct resistance evidence.
Safety-related genomic screening	VFDB homology hits	16	Sequence-homology annotations; not direct virulence evidence.
Safety-related genomic screening	Explicitly annotated toxin genes	Not identified in the reported screen	Sequence-level observation.
Safety-related genomic screening	Complete integrons / In0 / CALIN	Not detected	Sequence-level screen only.
Safety-related genomic screening	Complete secretion systems	No complete system predicted	MacSyFinder/TXSScan prediction.
Predicted mobile elements	Prophage-associated regions	19 predicted regions	Completeness was not established.
Predicted mobile elements	Genomic islands	18 predicted islands	Computational prediction.
Predicted mobile elements	Plasmid carriage	Not systematically assessed	Plasmid carriage cannot be excluded on the basis of the reported analysis.
Predicted functional potential	Carbohydrate transport and metabolism genes (COG)	247	Predicted carbohydrate-use potential.
Predicted functional potential	Glycoside hydrolases (CAZy)	52	CAZy annotation.
Predicted functional potential	Glycosyltransferases (CAZy)	39	CAZy annotation.
Predicted functional potential	Histidine kinase-associated proteins	13	Predicted regulatory potential.
Predicted functional potential	Response regulator-associated proteins	13	Predicted regulatory potential.
Predicted functional potential	Candidate biosynthetic gene clusters	5: arylpolyene, terpene, RiPP-like, cyclic-lactone-autoinducer and type III polyketide synthase	Predicted potential; expression and metabolites were not tested.

Note: CARD and VFDB results are sequence-homology annotations and do not constitute direct evidence of antimicrobial resistance or virulence phenotypes. Functional annotations indicate predicted genomic potential and do not demonstrate gene expression, metabolite production or in vivo activity.

Supplementary Table S2. Growth, fermentation-related, antagonistic and haemolytic phenotypes of HCD07.

A. Growth under salt and acid stress

Assay	Condition	OD600, mean ± SD	Relative growth (%)
Normal growth	MRS control	2.446 ± 0.024	100.00
NaCl stress	2% NaCl	2.857 ± 0.029	116.80
NaCl stress	4% NaCl	2.376 ± 0.024	97.14
NaCl stress	6% NaCl	2.289 ± 0.023	93.58

Assay	Condition	OD600, mean ± SD	Relative growth (%)
NaCl stress	8% NaCl	2.034 ± 0.020	83.16
NaCl stress	10% NaCl	1.061 ± 0.011	43.38
Acid stress	pH 2	0.519 ± 0.005	21.22
Acid stress	pH 3	0.761 ± 0.008	31.11
Acid stress	pH 4	1.609 ± 0.016	65.78

Note: Relative growth was calculated from OD600 measurements relative to the untreated MRS control and does not represent viable-cell survival or colony-forming units.

B. Skimmed-milk coagulation

HCD07:skimmed milk ratio	Coagulation	Approximate endpoint pH
1:5	3/3 positive	approximately 4.5-5.0
1:10	3/3 positive	approximately 5.0
1:15	3/3 positive	approximately 5.5
1:20	3/3 positive	approximately 6.0
1:25	3/3 positive	approximately 6.5

Note: Endpoint pH was estimated using broad-range pH strips with a minimum scale interval of 0.5 pH unit. Samples were incubated at 37 °C for 14 h.

C. Agar-well diffusion and haemolysis

Assay	Test organism or medium	Result
Agar-well diffusion	Escherichia coli ATCC 25922	8.00 ± 0.00 mm
Agar-well diffusion	Staphylococcus aureus ATCC 29213	11.45 ± 0.18 mm
Haemolysis	5% sheep-blood agar	No visible haemolytic zone in 3/3 cultures

Note: Total agar-well diffusion diameters included the 8-mm well diameter. A total diameter of 8 mm indicated that no visible inhibition extended beyond the well edge.

Supplementary Table S3. Disk-diffusion phenotype of HCD07 against the verified antimicrobial agents.

Antimicrobial agent	Abbreviation	Class	Disk content	Recorded zone measure, mm	Operational category
Amoxicillin	AMX	β-lactam	25 µg	0.00 ± 0.00	R
Ceftriaxone	CRO	β-lactam	30 µg	0.00 ± 0.00	R
Cefoperazone	CFP	β-lactam	75 µg	11.20 ± 0.18	I
Ceftazidime	CAZ	β-lactam	30 µg	14.32 ± 0.18	I
Cefalexin	CEX	β-lactam	30 µg	17.48 ± 0.18	I
Penicillin G	PEN-G	β-lactam	10 U	18.33 ± 0.18	I
Cefradine	CED	β-lactam	30 µg	21.02 ± 0.18	S
Cefazolin	CZO	β-lactam	30 µg	23.21 ± 0.18	S
Ampicillin	AMP	β-lactam	10 µg	29.80 ± 0.18	S
Cefotaxime	CTX	β-lactam	30 µg	35.25 ± 0.18	S
Levofloxacin	LVX	Fluoroquinolone	5 µg	0.00 ± 0.00	R
Norfloxacin	NOR	Fluoroquinolone	10 µg	0.00 ± 0.00	R
Ofloxacin	OFX	Fluoroquinolone	5 µg	13.87 ± 0.18	I
Enrofloxacin	ENR	Fluoroquinolone	10 µg	14.65 ± 0.18	I
Gentamicin	GEN	Aminoglycoside	120 µg	0.00 ± 0.00	R
Doxycycline	DOX	Tetracycline	30 µg	0.00 ± 0.00	R
Minocycline	MIN	Tetracycline	30 µg	16.13 ± 0.18	I
Azithromycin	AZM	Macrolide	15 µg	2.74 ± 0.18	R
Chloramphenicol	CHL	Phenicols	30 µg	25.59 ± 0.18	S

Note: Values follow the laboratory measurement convention used in the source dataset; absence of a visible zone was recorded as 0 mm. Operational categories were assigned using the study-specific criteria of >20 mm for S, 10-20 mm for I and <10 mm for R. These categories are descriptive and should not be interpreted as standardised clinical susceptibility breakpoints for Lactiplantibacillus plantarum.

Supplementary Table S4. Cage-level diarrhoea scores across the experimental phases.

Day	Group	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Median [range]
0	Control	0	0	0	0	0	0 [0-0]
0	O8	0	0	1	0	0	0 [0-1]
0	HCD07 prevention	0	0	0	0	0	0 [0-0]
0	Chloramphenicol	0	0	0	0	0	0 [0-0]
0	HCD07 treatment	0	0	0	0	0	0 [0-0]
0	HCD07-only	0	0	0	0	0	0 [0-0]
3	Control	0	0	1	0	0	0 [0-1]
3	O8	1	1	2	2	2	2 [1-2]
3	HCD07 prevention	1	1	1	2	1	1 [1-2]
3	Chloramphenicol	1	2	2	1	2	2 [1-2]
3	HCD07 treatment	2	1	2	2	1	2 [1-2]
3	HCD07-only	0	0	0	1	0	0 [0-1]
7	Control	0	0	0	0	0	0 [0-0]
7	O8	2	1	2	2	1	2 [1-2]
7	HCD07 prevention	0	1	1	0	1	1 [0-1]
7	Chloramphenicol	0	1	0	1	0	0 [0-1]
7	HCD07 treatment	1	0	1	1	0	1 [0-1]
7	HCD07-only	0	0	0	0	0	0 [0-0]

Note: Each value represents one independent cage (n = 5 cages per group and time point). Scores were summarised descriptively using the median and range and were not subjected to inferential statistical testing.

Supplementary Table S5. Mouse-level jejunal ELISA summaries and stage-specific statistical results.

Stage	Analyte	Control	O8	HCD07-Pre	CHL-Tx	HCD07-Tx	HCD07-only	p value
Pre-challenge	IL-6	0.51 [0.41-0.54]	—	17.07 [14.67-18.27]	—	—	—	0.1000
Pre-challenge	IL-10	0.70 [0.51-0.80]	—	64.48 [60.58-74.17]	—	—	—	0.1000
Pre-challenge	sIgA	3.01 [2.10-3.11]	—	86.94 [79.74-101.70]	—	—	—	0.1000
Pre-challenge	TNF-α	0.50 [0.48-0.70]	—	11.91 [8.34-13.86]	—	—	—	0.1000
Late challenge	IL-6	24.81 [22.94-27.97]	17.54 [13.65-20.26]	16.73 [13.34-17.69]	—	—	22.94 [19.54-27.66]	0.0499
Late challenge	IL-10	28.80 [25.89-31.11]	28.86 [25.85-30.19]	16.37 [15.47-18.02]	—	—	33.51 [26.84-35.44]	0.0656
Late challenge	sIgA	36.99 [29.26-41.12]	35.52 [31.01-40.03]	69.33 [67.80-70.92]	—	—	49.72 [25.53-52.11]	0.0862
Late challenge	TNF-α	15.17 [14.12-16.77]	11.78 [8.31-17.17]	11.41 [10.61-15.85]	—	—	14.71 [9.48-16.70]	0.7641
Post-treatment	IL-6	16.97 [16.03-27.00]	31.68 [24.68-47.17]	13.80 [10.29-19.38]	22.20 [17.07-22.83]	27.45 [25.37-48.01]	29.92 [23.61-56.18]	0.0372
Post-treatment	IL-10	8.80 [6.96-9.14]	16.76 [14.21-18.89]	6.16 [6.06-7.09]	8.54 [8.07-9.94]	13.73 [11.87-17.31]	19.69 [15.62-21.27]	0.0114
Post-treatment	sIgA	96.07 [89.76-161.33]	51.57 [45.04-63.38]	51.44 [44.50-74.07]	92.85 [70.40-174.85]	50.99 [41.41-81.90]	23.54 [7.68-24.49]	0.0211
Post-treatment	TNF-α	11.11 [9.38-11.13]	18.33 [14.76-21.99]	7.90 [3.78-7.94]	4.19 [3.47-4.23]	4.79 [4.64-7.79]	10.14 [7.42-12.45]	0.0159

Note: Values are median [minimum-maximum] from n = 3 mice per group. Cytokines are expressed in pg/mL and sIgA in μg/mL. Pre-challenge p values are from exact two-sided Mann-Whitney U tests; late-challenge and post-treatment p values are Kruskal-Wallis omnibus results. Full mouse-level values, O8-referenced pairwise comparisons and assay-range flags are provided in the accompanying spreadsheet Supplementary_Table_S5_ELISA_mouse_level_and_statistics.xlsx.

Supplementary Table S6. Cage-linked 16S sample structure and stage-specific alpha- and beta-diversity summary.

Stage	Day	Group	Sample IDs	n cages	Mean Shannon	Stage summary
Pre-challenge	0	Control	A1_1_1-A1_1_3	3	5.672	Kruskal-Wallis p = 0.0054; NMDS stress = 0.0209
Pre-challenge	0	O8	A1_3_1-A1_3_3	3	5.255	
Pre-challenge	0	HCD07-Pre	A1_5_1-A1_5_3	3	5.143	
Pre-challenge	0	CHL-Tx	A1_6_1-A1_6_3	3	4.750	
Pre-challenge	0	HCD07-Tx	A1_4_1-A1_4_3	3	4.558	
Pre-challenge	0	HCD07-only	A1_2_1-A1_2_3	3	6.046	
Late challenge	3	Control	B2_1_1-B2_1_3	3	5.810	Kruskal-Wallis p = 0.0076; NMDS stress = 0.1500
Late challenge	3	O8	B2_3_1-B2_3_3	3	6.751	
Late challenge	3	HCD07-Pre	B2_5_1-B2_5_3	3	7.481	
Late challenge	3	CHL-Tx	B2_6_1-B2_6_3	3	7.310	
Late challenge	3	HCD07-Tx	B2_4_1-B2_4_3	3	7.244	
Late challenge	3	HCD07-only	B2_2_1-B2_2_3	3	5.807	
Post-treatment	7	Control	C3_1_1-C3_1_3	3	5.997	Kruskal-Wallis p = 0.0064; NMDS stress = 0.0579
Post-treatment	7	O8	C3_3_1-C3_3_3	3	6.205	
Post-treatment	7	HCD07-Pre	C3_5_1-C3_5_3	3	7.134	
Post-treatment	7	CHL-Tx	C3_6_1-C3_6_3	3	7.175	
Post-treatment	7	HCD07-Tx	C3_4_1-C3_4_3	3	7.532	
Post-treatment	7	HCD07-only	C3_2_1-C3_2_3	3	6.962	

Note: The same three cages were linked across phases, but a different mouse was sampled from each cage at each terminal phase. Stage-specific Kruskal-Wallis tests were cross-sectional; the connecting trajectories in Fig. 6B were descriptive and were not analysed as repeated measurements.

Supplementary Table S7. Highest-scoring non-redundant exploratory LEfSe features displayed in Fig. 6F.

Rank	Feature	Stage	Associated condition	LDA score	p value
1	Lactobacillus	Pre-challenge	CHL-Tx	5.4069	1.68e-05
2	S24-7	Late challenge	HCD07-only	5.2414	1.86e-05
3	Akkermansia muciniphila	Pre-challenge	Control	5.0220	1.97e-05
4	Akkermansia	Pre-challenge	Control	5.0133	1.97e-05
5	Lactobacillus helveticus	Late challenge	HCD07-only	4.9194	1.81e-05
6	Helicobacter	Post-treatment	HCD07-only	4.8298	1.69e-05
7	Allobaculum	Late challenge	O8	4.6546	2.80e-05
8	Enterobacteriaceae	Late challenge	O8	4.5741	2.04e-05
9	Rikenellaceae	Post-treatment	HCD07-Pre	4.5459	1.67e-05
10	Oscillospira	Post-treatment	HCD07-Pre	4.5170	1.87e-05
11	Bifidobacterium pseudolongum	Late challenge	O8	4.5169	1.64e-05
12	Bacteroides acidifaciens	Late challenge	HCD07-only	4.4862	1.79e-05

Note: Features were selected from the supplied LEfSe output across all 18 phase-by-group conditions by ranking LDA scores and retaining non-duplicated terminal taxon labels. These unadjusted exploratory features may reflect baseline, phase or intervention effects and do not establish causal taxon roles.

Supplementary Table S8. Prespecified stage-specific qPCR contrasts calculated from mouse-level ΔCq values.

A. Pre-challenge comparison

Gene	HCD07-Pre / Control fold ratio	Stage-wise BH-adjusted p
Tnf	31.52	<0.001
Il6	50.84	<0.001
Muc2	3.94	<0.001
Tjp1	4.28	0.0025
Ocln	1.15	0.250
Bax	0.54	0.0184
Bcl2	2.81	0.0073
Casp3	1.15	0.162

B. Late-challenge contrasts

Gene	O8 / Control fold ratio; stage-wise BH-adjusted p	HCD07-Pre / O8 fold ratio; stage-wise BH-adjusted p
Tnf	1.07; 0.947	11.84; <0.001
Il6	79.38; <0.001	4.53; <0.001
Muc2	0.89; 0.852	14.00; <0.001
Tjp1	0.56; 0.0029	28.51; <0.001
Ocln	0.19; <0.001	16.12; <0.001
Bax	8.71; <0.001	0.56; 0.0100
Bcl2	0.20; <0.001	61.52; <0.001
Casp3	0.52; <0.001	24.41; <0.001

C. Post-treatment contrasts

Gene	O8 / Control fold ratio; stage-wise BH-adjusted p	HCD07-Pre / O8 fold ratio; stage-wise BH-adjusted p	CHL-Tx / O8 fold ratio; stage-wise BH-adjusted p	HCD07-Tx / O8 fold ratio; stage-wise BH-adjusted p
Tnf	6.27; <0.001	0.87; 0.603	0.47; <0.001	2.71; <0.001
Il6	17.86; <0.001	1.65; <0.001	2.34; <0.001	1.24; 0.0861
Muc2	0.29; <0.001	7.34; <0.001	15.35; <0.001	7.36; <0.001
Tjp1	0.65; 0.0053	3.66; <0.001	0.17; <0.001	1.80; <0.001
Ocln	0.32; <0.001	5.28; <0.001	2.63; <0.001	3.59; <0.001
Bax	0.97; 0.999	0.31; <0.001	13.26; <0.001	0.25; <0.001
Bcl2	0.35; <0.001	4.67; <0.001	11.68; <0.001	6.46; <0.001
Casp3	0.57; 0.0042	3.12; <0.001	3.01; <0.001	3.12; <0.001

Note: Fold ratios were calculated as 2^[−(mean ΔCq of numerator − mean ΔCq of denominator)]. Values >1 indicate higher transcript abundance in the numerator group and values <1 indicate lower abundance. Pre-challenge within-gene p values were obtained using two-sided Welch t tests. Late-challenge and post-treatment within-gene p values were Tukey-adjusted pairwise results following gene-specific one-way ANOVA. The prespecified contrast p values were then adjusted by the Benjamini-Hochberg procedure across all prespecified contrasts within each experimental phase: eight at pre-challenge, 16 at late challenge and 32 after treatment. Each condition included three mice, and each mouse value was the mean of three technical reactions. Full mouse-level data, omnibus ANOVA results, within-gene p values, stage-wise adjusted p values and all Tukey contrasts are provided in the accompanying qPCR source-data spreadsheet.

Supplementary Table S9. qPCR quality-control and interpretation summary.

Quality-control item	Result
Biological replication	Three mice per condition
Technical replication	Three qPCR reactions per mouse
Primary inferential unit	Mouse; technical reactions were averaged
Median technical-replicate SD	0.055 Cq
Maximum technical-replicate SD	0.241 Cq
Low-abundance measurements	Post-treatment HCD07-only Bax: 3/3 mouse mean Cq values ≥35
Mouse-level raw Gapdh Cq range	22.246-26.725
Reference-gene design	Gapdh was the sole reference gene; raw-Cq variation was evaluated together with target-gene shifts
Interpretive status	Exploratory transcriptional evidence; not protein-level or mechanistic proof
Within-condition centred target-Gapdh raw-Cq correlation	r = 0.720
Median target raw-Cq SD versus ΔCq SD	0.187 Cq versus 0.135 Cq
Median dispersion reduction after ΔCq normalisation	25.5%
Blocks with lower dispersion after ΔCq normalisation	76/112

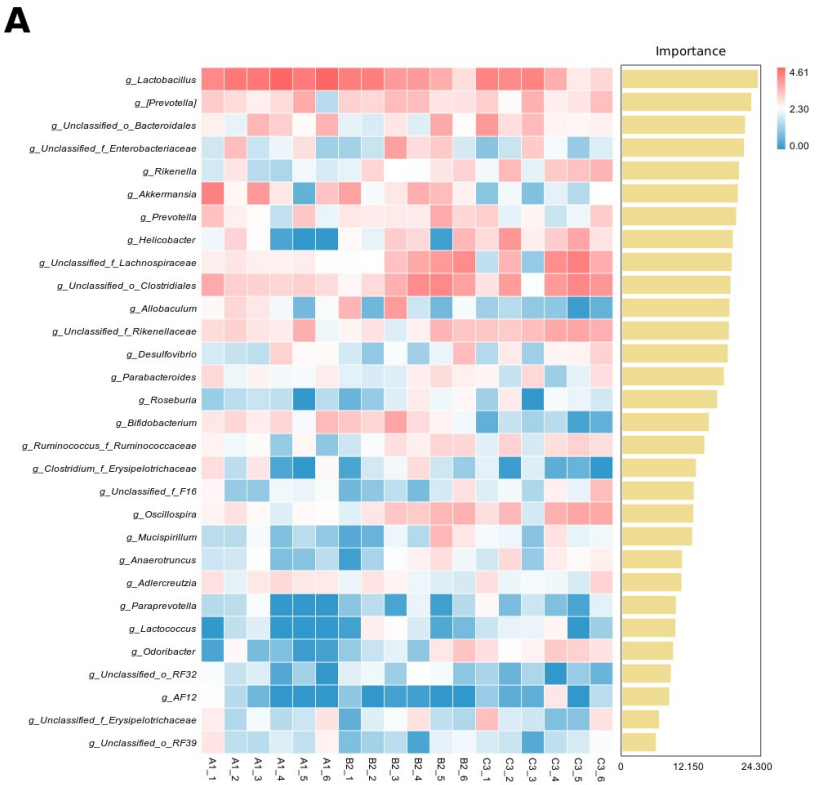
Supplementary Table S10. Primer sequences used for jejunal quantitative PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Verified published source
Tnf	CGCTCTTCTGTCTACTGAACTTCGG	GTGGTTTGTGAGTGTGAGGGTCTG	[41]
Il6	CTTCTTGGGACTGATGCTGGTGAC	AGTGGTATCCTCTGTGAAGTCTCCTC	[41]
Muc2	CAACTGAATCCTCGACGCCT	GGGAGGAGGAGAGAGGAGTGGA	—
Tjp1	CTTTTCCTCCTTGACCTCCCT	TGATTCTACAATGCGGCGATA	—
Ocln	GTGAATGGGTCACCGAGGG	AAGATAAGCGAACCTGCCGAG	[59]
Bax	CCAGGATGCGTCCACCAAGA	GCAAAGTAGAAGAGGGCAACCAC	[60]
Bcl2	GTCGCTACCGTCGTGACTTC	CAGACATGCACCTACCCAGC	[60]
Casp3	TGGTGATGAAGGGGTCATTTATG	TTCGGCTTTCCAGTCAGACTC	[60]
Gapdh	CATGTTCCAGTATGACTCCACTC	GGCCTCACCCCATTTGATGT	[61]

Note: Primer sequences are shown in the 5′-to-3′ orientation. Source citations are reported only when both primer sequences exactly matched a primer pair previously used in a published mouse study.

Supplementary figure legends and figures

Supplementary Fig. S1. Exploratory random-forest classification and PICRUSt2 functional-category summaries from the 16S data. (A) Random-forest feature heatmap and importance ranking supplied by the GenesCloud analysis. The reported confusion matrix yielded an overall accuracy of 0.9259 across 18 phase-by-group conditions, but each condition contained only three samples and the model was not externally validated. (B) Broad COG functional-category summary predicted by PICRUSt2. (C) Broad KEGG functional-category summary predicted by PICRUSt2, with KEGG cited in the main manuscript [43-45]. Panels B and C are aggregate predicted-category summaries, not direct measurements or confirmatory between-group tests. Panel C is a category-summary visualisation generated from the PICRUSt2 output and does not reproduce or adapt a KEGG pathway map image. Condition codes are A1, pre-challenge; B2, late challenge; C3, post-treatment; and group codes 1, Control; 2, HCD07-only; 3, O8; 4, HCD07-Tx; 5, HCD07-Pre; 6, CHL-Tx.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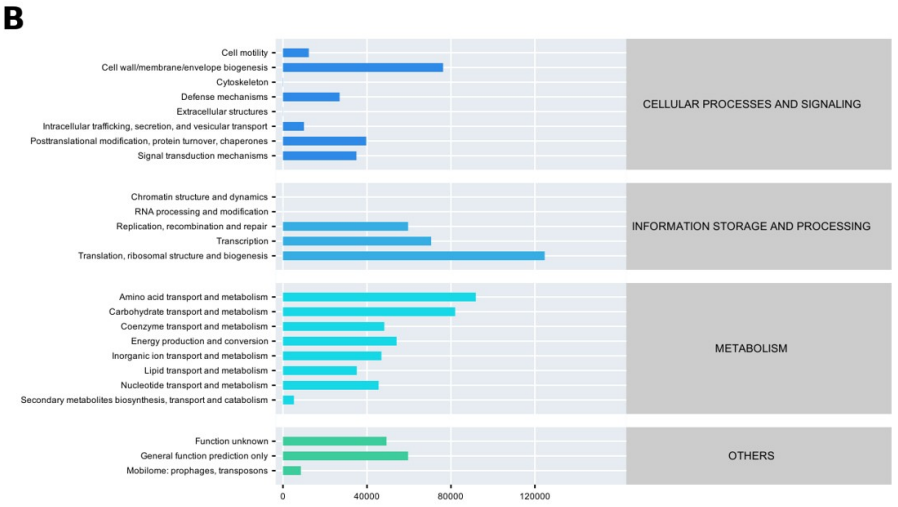
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Cell wall/membrane/envelope biogenesis
Cytoskeleton
Defense mechanisms
Extracellular structures
Intracellular trafficking, secretion, and vesicular transport
Posttranslational modification, protein turnover, chaperones
Signal transduction mechanisms
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Chromatin structure and dynamics
RNA processing and modification
Replication, recombination and repair
Transcription
Translation, ribosomal structure and biogenesis
INFORMATION STORAGE AND PROCESSING
Amino acid transport and metabolism
Carbohydrate transport and metabolism
Coenzyme transport and metabolism
Energy production and conversion
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Lipid transport and metabolism
Nucleotide transport and metabolism
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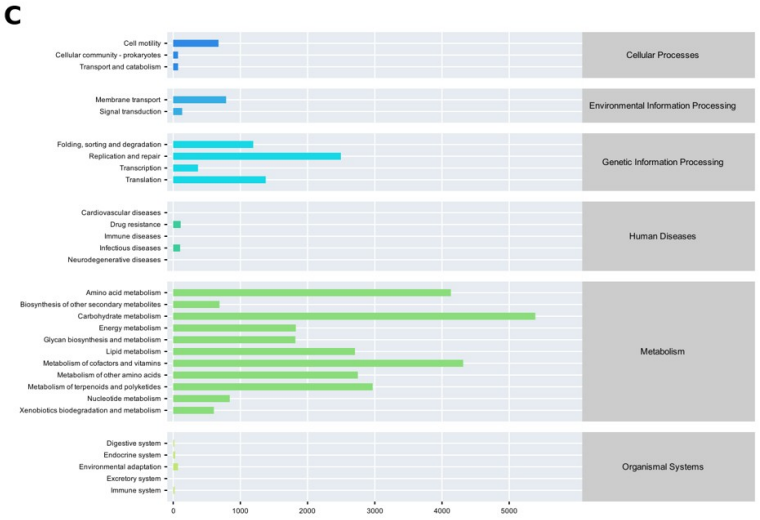
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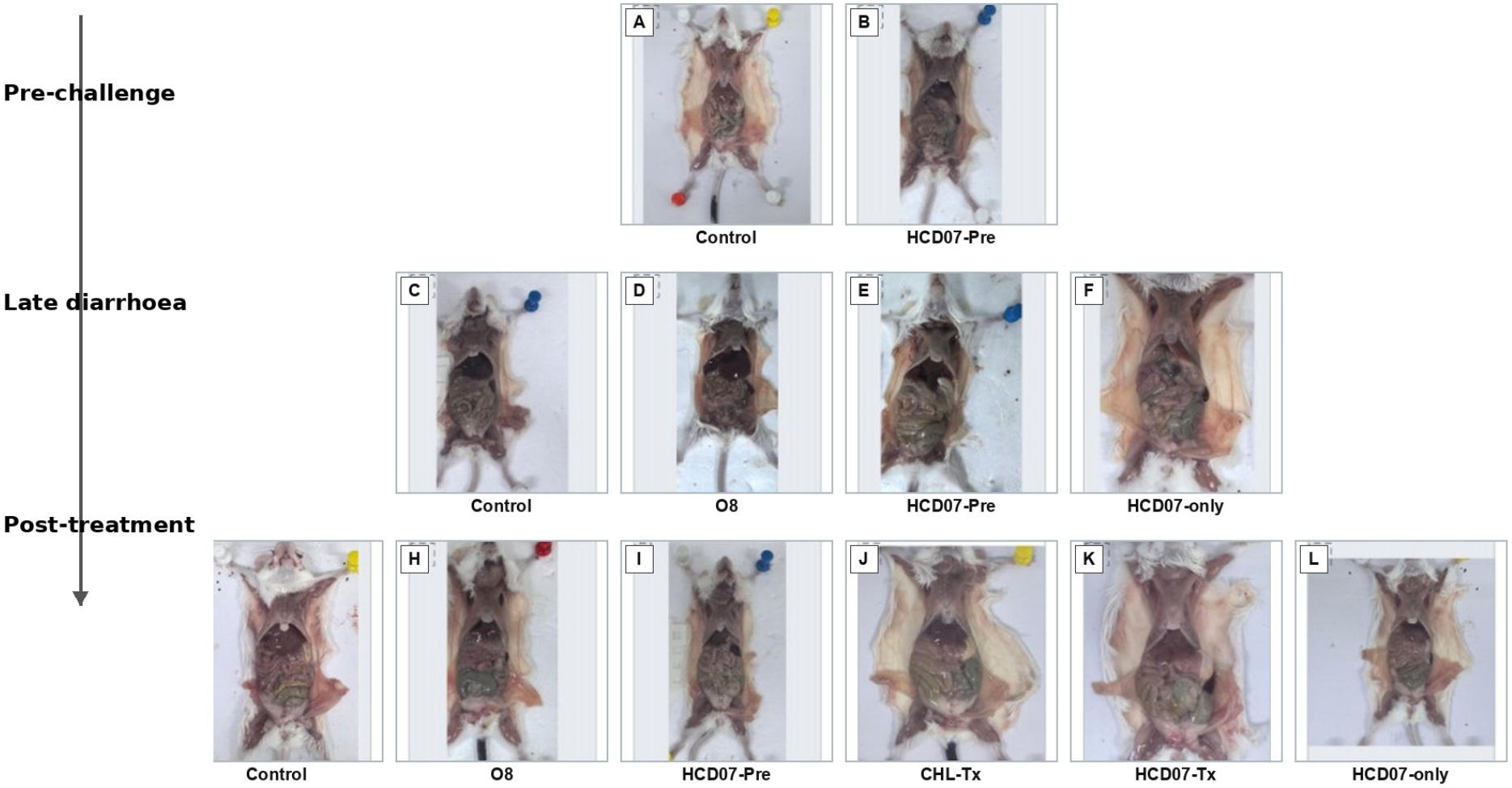
Membrane transport
Signal transduction
Environmental Information Processing
Folding, sorting and degradation
Replication and repair
Transcription
Translation
Genetic Information Processing
Cardiovascular diseases
Drug resistance
Immune diseases
Infectious diseases
Neurodegenerative diseases
Human Diseases
Amino acid metabolism
Biosynthesis of other secondary metabolites
Carbohydrate metabolism
Energy metabolism
Glycan biosynthesis and metabolism
Lipid metabolism
Metabolism of cofactors and vitamins
Metabolism of other amino acids
Metabolism of terpenoids and polyketides
Nucleotide metabolism
Xenobiotics biodegradation and metabolism
Metabolism
Digestive system
Endocrine system
Environmental adaptation
Excretory system
Immune system
Organismal Systems
0
1000
2000
3000
4000
5000

Supplementary Fig. S2. Representative gross abdominal and gastrointestinal appearance and loose-faeces observations across the experimental timeline. Panels A-B show the pre-challenge stage (A, Control; B, HCD07-Pre). Panels C-F show the late-challenge stage (C, Control; D, O8; E, HCD07-Pre; F, HCD07-only). Panels G-L show the post-treatment stage in the fixed group order Control, O8, HCD07-Pre, CHL-Tx, HCD07-Tx and HCD07-only. Panels M-O show representative loose-faeces observations from the late-challenge stage. The images provide descriptive gross phenotypic context only and were not subjected to blinded quantitative stool-consistency or gross-lesion scoring. Original biological content was retained. Images were cropped and globally adjusted for presentation where necessary, and the unedited source images are retained for verification.


Pre-challenge
Late diarrhoea
Post-treatment
A
B
Control
HCD07-Pre
C
D
E
F
Control
O8
HCD07-Pre
HCD07-only
H
I
J
K
L
Control
O8
HCD07-Pre
CHL-Tx
HCD07-Tx
HCD07-only
Representative loose faeces during the late diarrhoeal stage
Three representative observations
se-faeces observations
M
N
O

Supplementary Fig. S3. Stage-resolved representative jejunal H&E image set at low magnification (10× objective). (A, B) Pre-challenge day 0: Control and HCD07-Pre. (C-F) Late challenge day 3: Control, O8, HCD07-Pre and HCD07-only. (G-L) Post-treatment day 7: Control, O8, HCD07-Pre, CHL-Tx, HCD07-Tx and HCD07-only. The low-magnification images provide an overview of villus orientation, mucosal continuity and the distribution of structural alterations across the sampled fields. Control sections retained relatively intact mucosa, whereas representative O8 sections showed more marked villus injury. Some prevention and treatment fields appeared less severely altered, while residual abnormalities remained. These observations were qualitative and were not supported by blinded scoring, morphometry or statistical comparison. Scale bars, 200 µm.