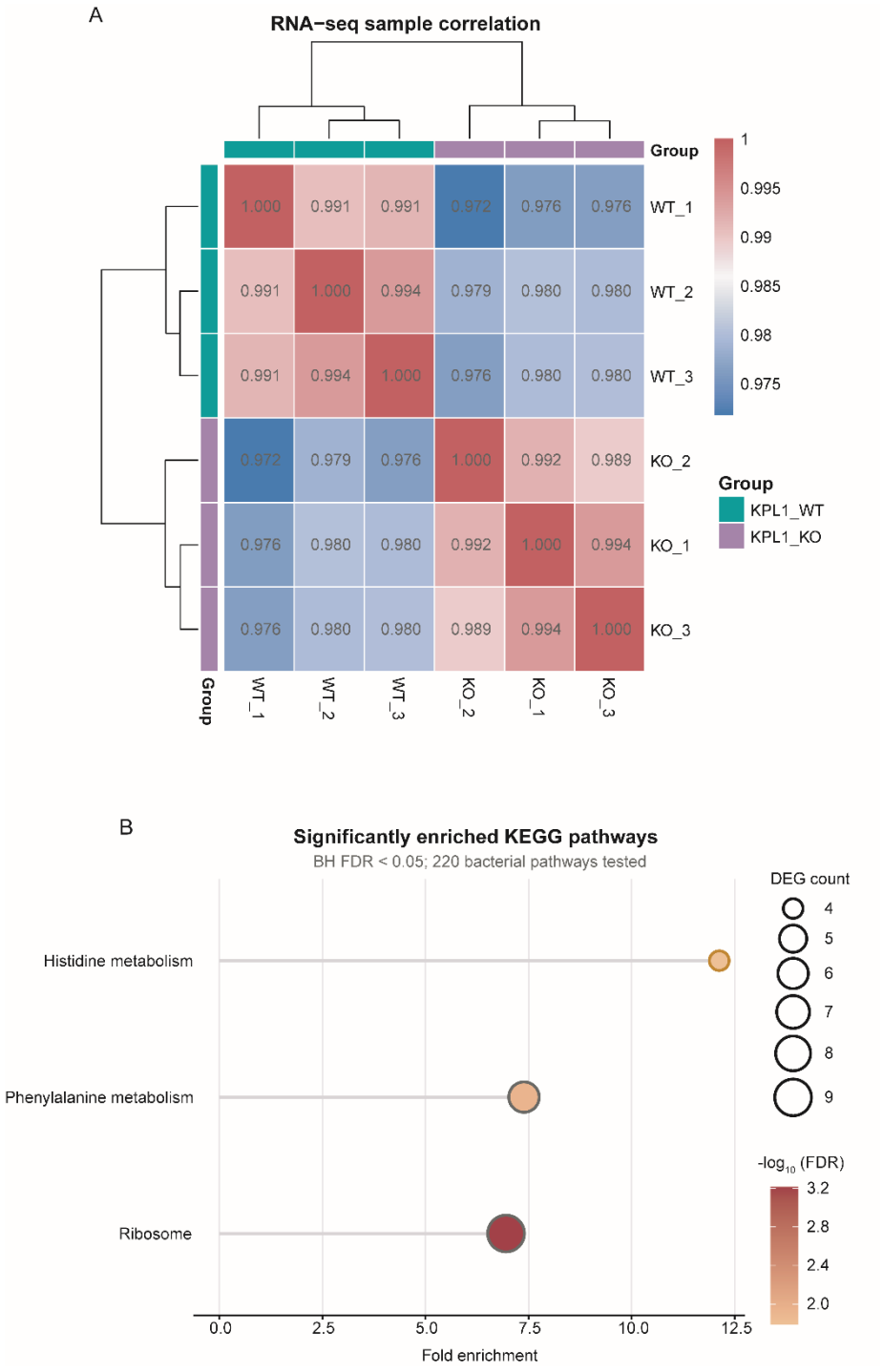
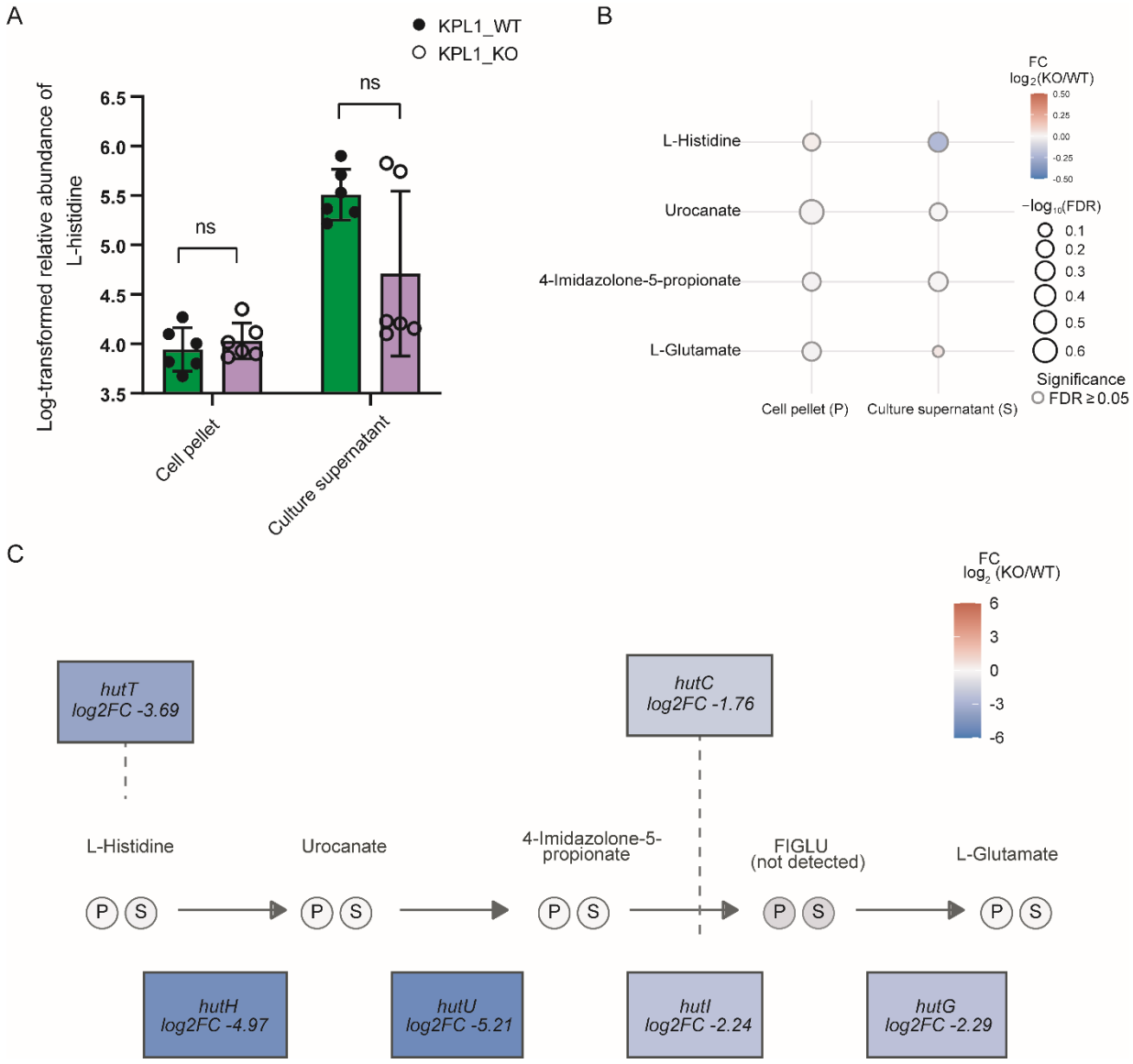
Supplementary Materials

Supplementary figures



Supplementary Figure S1. RNA-seq reproducibility and KEGG enrichment. (**A**) Pearson correlation matrix and hierarchical clustering of three KPL1_WT and three KPL1_KO replicates. (**B**) FDR-significant KEGG pathways. Bubble position indicates fold enrichment, size indicates the DEG count, and color indicates −log10(FDR).



Supplementary Figure S2. Histidine-related metabolites and pathway-level integration. (**A**) Untargeted relative abundance of L-histidine in pellets and supernatants (n = 6/group). ns, not significant after FDR correction. (**B**) Effect-size plot for L-histidine, urocanate, 4-imidazolone-5-propionate, and L-glutamate. Color indicates log2 fold change, size indicates −log10(FDR), and open outlines indicate FDR ≥ 0.05. (**C**) Exploratory mapping of HUT transcript changes and histidine-pathway metabolites. P and S denote pellet and supernatant; FIGLU was not reliably detected. Omics samples were independently cultured and non-matched.

Supplementary Table S1. Antimicrobial susceptibility profile of KPL1

Antimicrobial agent	Method	MIC or inhibition-zone diameter	Interpretation
Ampicillin	Disk diffusion	6 mm	R
Ampicillin–sulbactam	Disk diffusion	6 mm	R
Piperacillin–tazobactam	MIC	≥128 μg/mL	R
Ticarcillin–clavulanate	MIC	≥128 μg/mL	R
Cefoperazone–sulbactam	MIC	≥64 μg/mL	R
Cefazolin	Disk diffusion	6 mm	R
Cefuroxime, parenteral	Disk diffusion	6 mm	R
Ceftriaxone	Disk diffusion	6 mm	R
Ceftazidime	MIC	≥64 μg/mL	R
Cefepime	MIC	≥32 μg/mL	R
Aztreonam	MIC	≥64 μg/mL	R
Imipenem	MIC	≥16 μg/mL	R
Meropenem	MIC	≥16 μg/mL	R
Tobramycin	MIC	≤1 μg/mL	S
Amikacin	MIC	≤2 μg/mL	S
Ciprofloxacin	MIC	≥4 μg/mL	R
Levofloxacin	MIC	≥8 μg/mL	R
Doxycycline	MIC	≥16 μg/mL	R
Minocycline	MIC	≥16 μg/mL	R
Trimethoprim–sulfamethoxazole	MIC	≤20 μg/mL[1]	S
Tigecycline	MIC	1 μg/mL	S
Colistin	MIC	≤0.5 μg/mL	S

Susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI M100-S34 and M45-A3), except for tigecycline and colistin, which were interpreted using the corresponding US Food and Drug Administration and EUCAST version 10.0 breakpoints, respectively.

Supplementary Table S2. Characteristics of the plasmids identified in KPL1

Plasmid	Size (bp)	GC content (%)	Predicted CDSs	Representative genetic determinants	Proposed function
pKPL1-KPC	124,809	53.41	168	*bla*$_{KPC}$, *bla*$_{SHV-2}$, copper-, silver- and mercury-resistance loci	Carbapenem resistance and heavy-metal tolerance
pKPL1-Vir	185,389	50.48	210	*peg-344*, *rmpA*, *rmpA2*, *iucABCD-iutA* and iron-acquisition-associated genes	Virulence-associated plasmid
pKPL1-TEM-Tet	81,347	54.18	97	*blaTEM*, *tetA* and *tetR*	Accessory antimicrobial-resistance plasmid

Supplementary Table S3. Primers used for construction of the KPL1 Δpeg-344 mutant

Primer name	Sequence (5′–3′)
Cas-F	ATGGATAAGAAATACTC
Cas-R	GTCACCTCCTAGCTGACTC
M13-R	CAGGAAACAGCTATGAC
Spacer-F	TAGTCCCTCTAATTGCAGTAAGCT
Spacer-R	AAACAGCTTACTGCAATTAGAGGG
peg344_Up_F	GCTTTTTTTGATATCCTAATTCAAACGTAATC
peg344_Up_R	CTACACTATATTTTCATTCCCCTTCATTA
peg344_Down_F	GAAGGGGAATGAAAATATAGTGTAGATAAC
peg344_Down_R	CCGCTCTAGAACTAGTCGAACTTCAGAAGGTGC

Supplementary Table S4. Genomic positions and RNA-seq expression profiles of genes neighboring *peg-344* in KPL1_WT and KPL1_KO.

Gene ID	Gene name	Gene description	KPL1_pKPL1-Vir position (bp)	Strand	WT mean expression	KO mean expression	log2FC (source)	Padjust
C2D63_RS18475	*cysS*	cysteine--tRNA ligase	112502-113812	-	61.200	43.540	-0.129	0.420815098
C2D63_RS17270	*fur*	ferric iron uptake transcriptional regulator	113963-114373	-	134.233	118.513	0.169	0.282166576
C2D63_RS26775	C2D63_RS26775	RES domain-containing protein	115273-115743	-	0.483	0.120	-1.665	1.000000000
C2D63_RS25970	*peg-344*	DMT family inner membrane transporter PEG344	119775-120674	-	0.393	0.000	-4.965	1.000000000
C2D63_RS08750	*rcsA*	transcriptional regulator RcsA	120747-121298	-	4.083	2.513	-0.327	0.433232495
C2D63_RS25985	*rmpA*	mucoid phenotype regulator RmpA	121679-122311	-	5.457	4.580	0.120	0.821486102
C2D63_RS16630	*dinG*	ATP-dependent DNA helicase DinG	126357-127319	-	11.480	6.540	-0.445	0.006377894
C2D63_RS26010	C2D63_RS26010	type I-E CRISPR-associated protein Cas6/Cse3/CasE	127322-127888	-	0.683	0.547	0.052	1.000000000

Gene IDs correspond to annotations in the RNA-seq reference genome, whereas genomic coordinates refer to the pKPL1-Vir virulence plasmid of KPL1. Expression values represent the mean of three biological replicates per group. Log_2fold changes are expressed as KPL1_KO relative to KPL1_WT, and adjusted P values were obtained using DESeq2. Genes with ambiguous or non-unique annotation correspondence were excluded from this analysis.

Supplementary Table S5. Primers used for qRT-PCR validation of HUT-associated genes

Primer name	Sequence (5′–3′)	Amplicon size (bp)
hutH-qF	TGATTGGCCTGGTGAATGCCGGC	97 bp
hutH-qR	AACGACATATGCGCCAGTGGCGC	
hutG-qF	ACGTACTGCCGGCGCGAGAAATG	112 bp
hutG-qR	TGCAGCTTACCGTTGCGGCACAG	
hutI-qF	AAGCTGAACTACTGGCGCTGGCC	111 bp
hutI-qR	TGCGTTCGTTTGGCAGGTCGAGG	
hutT-qF	ATTCGCCACGGTATGGGTGTGGC	99 bp
hutT-qR	ATGGGAAAGGCGATCTGGCTGCG	
yciH-qF	CTGATTAGCGGGATTGATGC	108 bp
yciH-qR	GATCTCGATCACGCCATCT	
recA-qF	ATGCGCCTGGGTGAAGACCGCA	168 bp
recA-qR	TGCGGCGATAACCTGCAGCGTCA	

Supplementary Table S6. Amplification efficiency of primers used for qRT-PCR analysis

Gene	cDNA template	Slope	R^2	Efficiency (%)
yciH	Pooled cDNA	-2.739	0.9951	131.78
recA	Pooled cDNA	-3.3640	0.9946	98.27
hutH	WT-2 cDNA	-3.3418	0.9976	99.18
hutG	Pooled cDNA	-3.3516	0.9980	98.77
hutI	WT-2 cDNA	-3.2028	0.9990	105.23
hutT	WT-2 cDNA	-3.2549	0.9967	102.88

Primer amplification efficiencies were evaluated using serial dilutions of cDNA. Pooled cDNA was initially used for standard-curve assessment. For *hutH*, *hutI*, and *hutT*, cDNA from the WT-2 biological replicate was used because the pooled template did not yield a sufficiently reliable dilution series. Amplification efficiency was calculated as $E = (10^{(-1/\text{slope})} - 1) \times 100\%$. The *yciH* primer pair exhibited an amplification efficiency of 131.78% and was excluded from the final normalization strategy.

Supplementary Table S7. Ct distribution of candidate reference genes in KPL1_WT and KPL1_KO

Reference gene	WT Ct (Mean ± SD)	KO Ct (Mean ± SD)	Ct difference	P value
recA	21.185 ± 0.088	21.460 ± 0.104	+0.275	0.0261
yciH	26.714 ± 0.232	26.662 ± 0.052	−0.052	0.7372

Ct values are presented as mean ± SD from three biological replicates per group. Differences between KPL1_WT and KPL1_KO were assessed using two-sided Welch's t-tests. These comparisons describe Ct variability and between-group differences but do not constitute a comprehensive validation of reference-gene stability.

Supplementary Table S8. Phenotypic characterization of carbapenemase production in KPL1

Phenotypic assay	Result	Interpretation
Modified carbapenem inactivation method (mCIM)	Positive	Carbapenemase production detected
EDTA-modified carbapenem inactivation method (eCIM)	Negative	Metallo-β-lactamase phenotype not detected
Serine carbapenemase assay	Positive	Serine carbapenemase phenotype detected
Metallo-β-lactamase assay	Negative	Metallo-β-lactamase phenotype not detected