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SUPPLEMENTAL DATA

Leukocyte telomere length and gut microbiome profiles in pancreatic ductal adenocarcinoma and at-risk conditions

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Full article is available at the following link: Leukocyte telomere length and gut microbiome profiles in pancreatic ductal adenocarcinoma and at-risk conditions

Supplemental file 1.

Table S1. Primers for relative telomere length measurement

	Primer sequence
Tel G	5′ ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT 3′
Tel C	5′ TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA 3′
Alb U	5′ CGGCGGCGGGCGGCGCGGGCTGGGCGGCCATGCTTTTCAGCTCTGCAAG TC 3′
Alb D	5′ GCCCGGCCCGCCGCGCCCGTCCCGCCGAGCATTAAGCTCTTTGGCAACG TAGGTTTC 3′

Abbreviations: Alb D, albumin downstream primer; Alb U, albumin upstream primer; Tel C, telomere C-rich primer; Tel G, telomere G-rich primer.

Table S2. Mastermix preparation for relative telomere length analysis

Parameter	***Description***
qPCR plate format	384-well plate
Total reaction volume	9.75 µL (8.0 µL master mix + 1.75 µL DNA sample)
DNA input	1.75 ng of genomic DNA (1 ng/µL) per reaction
Technical replicates	Triplicates for all samples
Master mix (per 384-well plate)	2262 µL double-distilled H_2O, 740 µL 5× HOT FIREPol® Probe qPCR Mix Plus (ROX), 5.5 µL SYTO® 9 (1 mM stock; final concentration 1.5 µM)
Primer concentrations (final per reaction)	TelG and AlbU: 200 nM; TelC and AlbG: 400 nM
Standard curve	Two-fold serial dilution of pooled genomic DNA starting at 29.75 ng/µL
Standard curve points	8 dilution points
Standard curve replicates	Hexaplicates

Abbreviations: AlbD, albumin downstream primer; AlbU, albumin upstream primer; DNA, deoxyribonucleic acid; qPCR, quantitative polymerase chain reaction; ROX, carboxy-X-rhodamine; TelC, telomere C-rich primer; TelG, telomere G-rich primer.

Table S3. PCR thermal profile and cycle conditions for telomere detection

PCR Stage	*Step*	*Temperature (°C)*	*Time (mm:ss)*	*Number of cycles*	*Signal detection*
Initial denaturation	1	95	15:00	1	No
Pre-amplification	1	95	00:20	2	No
	2	49	01:00	2	No
Amplification	1	85	00:20	25	No
	2	59	00:30	25	Yes (telomere detection)

Abbreviation: PCR, polymerase chain reaction.

Table S4. PCR thermal profile and cycle conditions for albumin detection

PCR Stage	*Step*	*Temperature (°C)*	*Time (mm:ss)*	*Number of cycles*	*Signal detection*
Initial denaturation	1	95	00:15	1	No
Amplification	1	95	00:15	35	No
	2	85	00:30	35	No
	3	84	00:30	35	Yes (Albumin Detection)
Melt curve stage	1	95	00:15	1	No
	2	60	01:00	1	No
	3	95	00:15	1	Yes

Abbreviation: PCR, polymerase chain reaction.

Table S5. Diagnostic performance of adjusted relative leukocyte telomere length in distinguishing PDAC from chronic pancreatitis and healthy controls

ROC	n_cases	n_controls	AUC	AUC_CI	Youden index	ROC operating point	sensitivity	specificity	ppv	npv
roc_PDACvsCP_LTLage	37	56	0,663	0.549 - 0.776	0,302	-0,074	0,784	0,518	0,518	0,784
roc_PDACvsCP_LTLall	33	52	0,593	0.469 - 0.717	0,198	-0,142	0,909	0,288	0,448	0,833
roc_PDACvsCTRL_LTLage	37	52	0,63	0.512 - 0.749	0,236	0,053	0,486	0,75	0,581	0,672
roc_PDACvsCTRL_LTLall	33	51	0,651	0.53- 0.771	0,273	-0,024	0,606	0,667	0,541	0,723

Abbreviations: AUC, area under the curve; CI, confidence interval; CP, chronic pancreatitis; LTL, leukocyte telomere length; NPV, negative predictive value; PDAC, pancreatic ductal adenocarcinoma; PPV, positive predictive value; ROC, receiver operating characteristic.

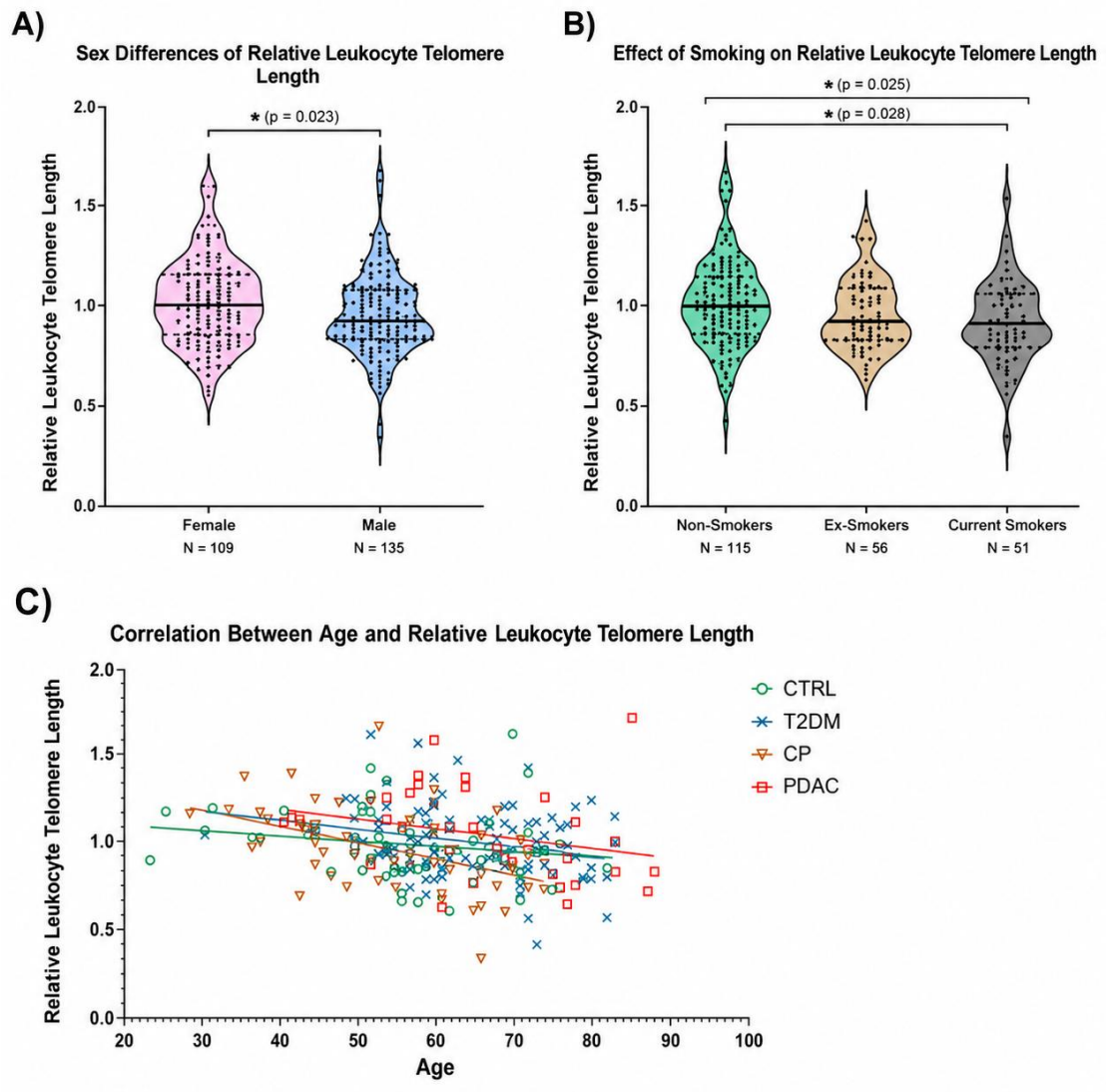


Figure S1. Associations of relative leukocyte telomere length with sex, smoking status, and age. (A) Distribution of LTL according to sex, showing significantly longer telomeres in females than in males (p = 0.023). **(B)** Distribution of LTL according to smoking status. LTL differed significantly across smoking-status groups (p = 0.025), with current smokers exhibiting significantly shorter telomeres than non-smokers in pairwise analysis (p = 0.028). **(C)** Association between age and LTL across the study groups. LTL was inversely correlated with age in all groups, with statistically significant correlations observed in patients with CP (r = −0.471, P < 0.001) and T2DM (r = −0.250, p = 0.013), whereas the inverse trends in patients with PDAC and controls were not statistically significant. **Abbreviations**: CP, chronic pancreatitis; CTRL, controls; LTL, leukocyte telomere length; PDAC, pancreatic ductal adenocarcinoma; T2DM, type 2 diabetes mellitus.

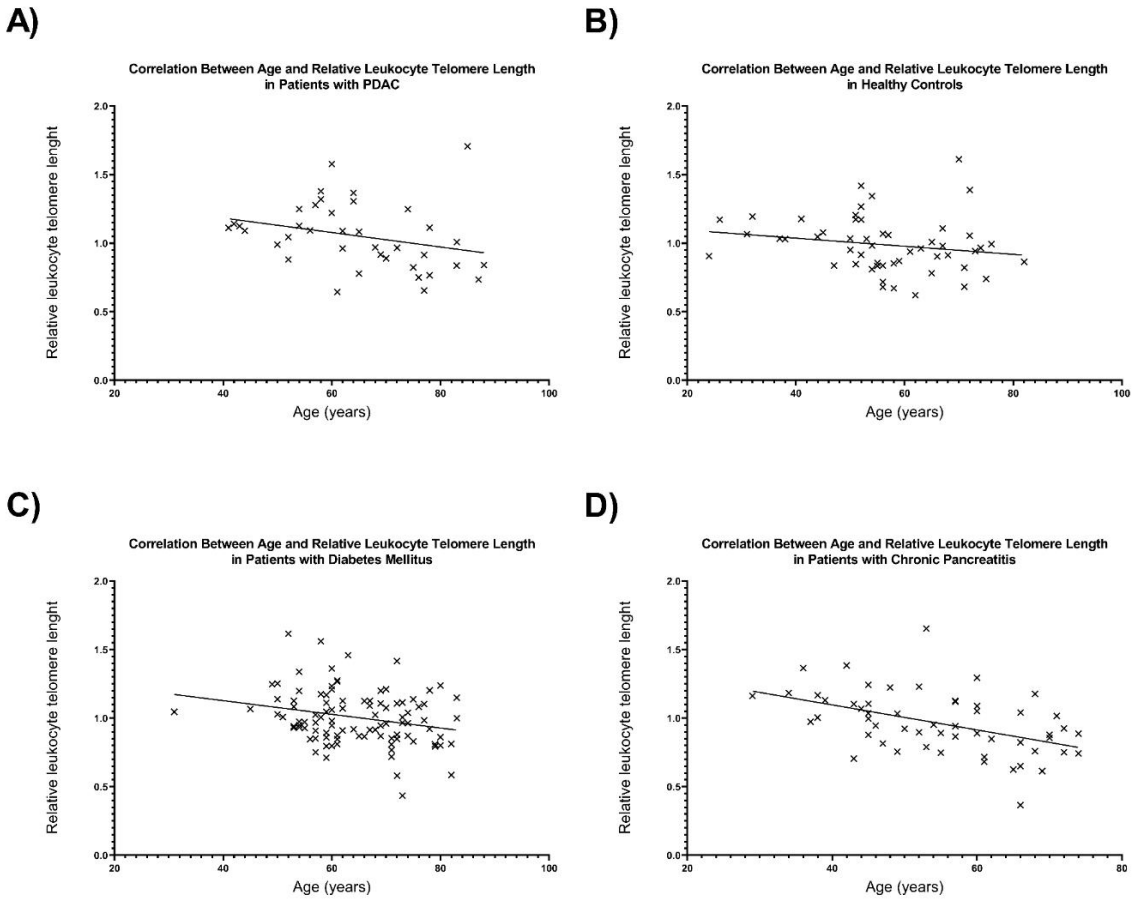


Figure S2. Association between age and relative leukocyte telomere length across study groups. (A) Association between age and LTL in patients with PDAC. **(B)** Association between age and LTL in healthy controls. **(C)** Association between age and LTL in patients with T2DM, showing a significant inverse correlation ($r = -0.250$, $p = 0.013$). **(D)** Association between age and LTL in patients with CP, showing the strongest inverse correlation among the study groups ($r = -0.471$, $p < 0.001$). Negative but nonsignificant trends were observed in patients with PDAC and healthy controls. **Abbreviations**: CP, chronic pancreatitis; LTL, leukocyte telomere length; PDAC, pancreatic ductal adenocarcinoma; T2DM, type 2 diabetes mellitus.

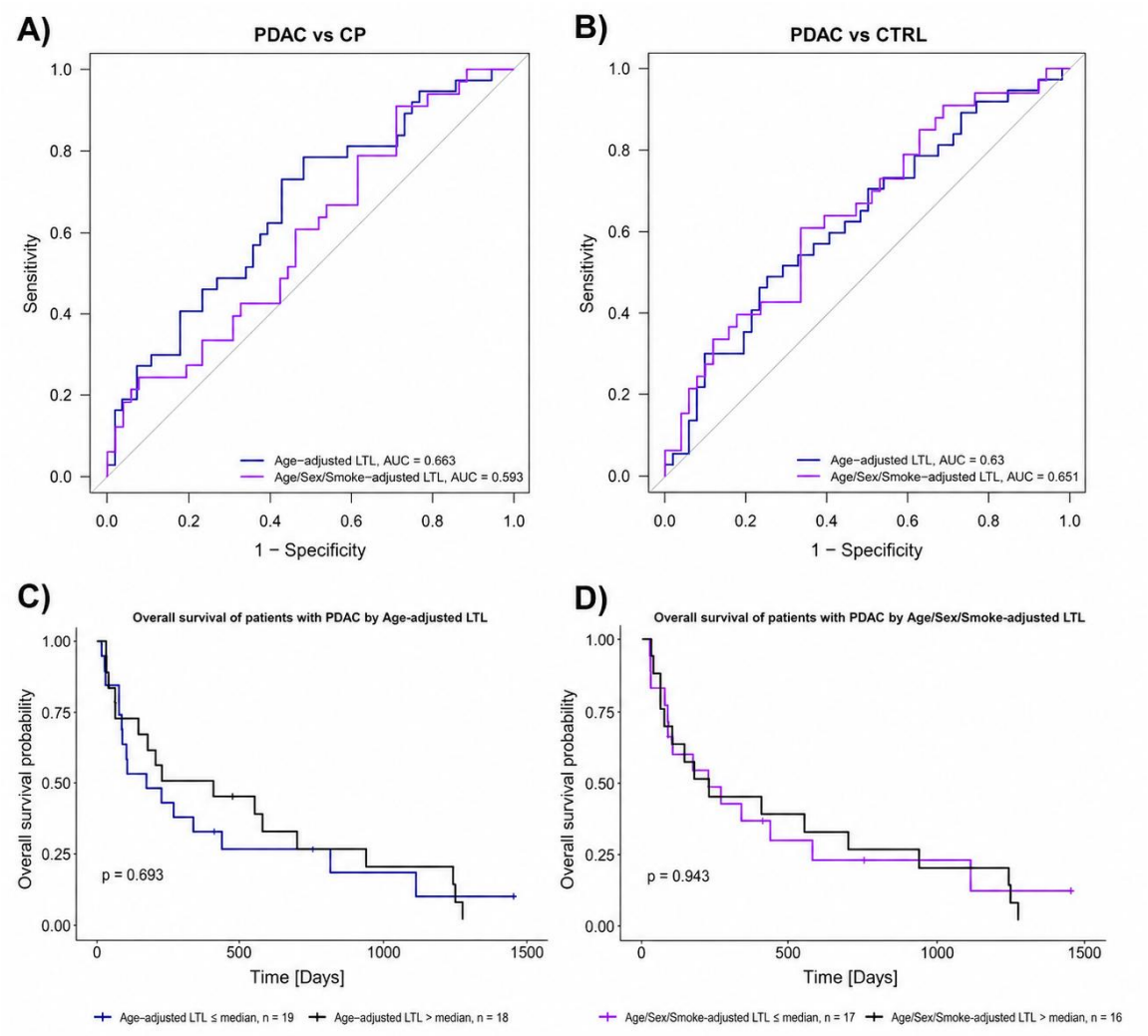
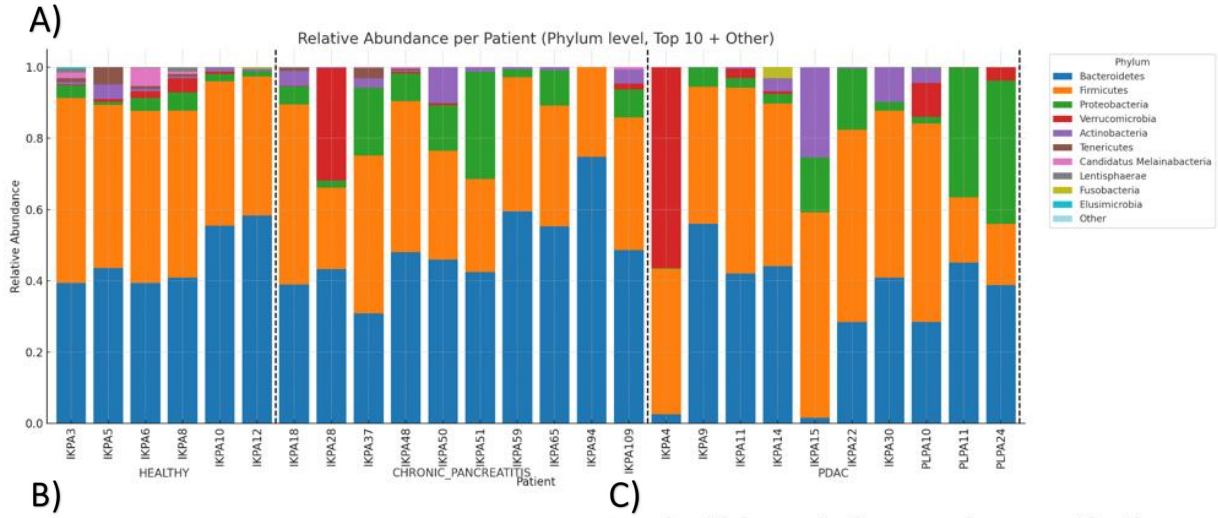


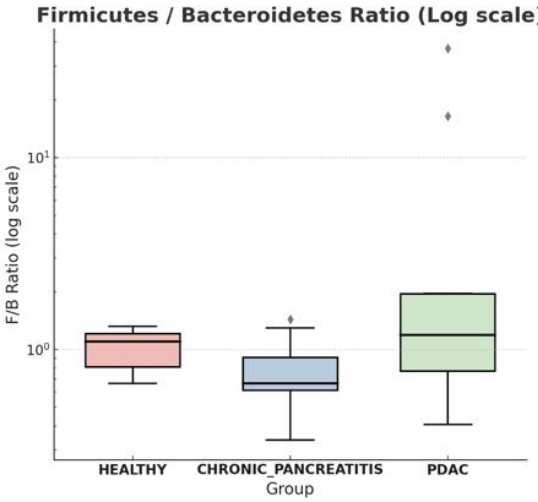
Figure S3. Diagnostic and prognostic performance of adjusted relative leukocyte telomere length in PDAC. (A) ROC curves for discrimination between PDAC and CP based on age-adjusted LTL and LTL adjusted for age, sex, and smoking status. The age-adjusted model showed limited discriminatory performance (AUC = 0.663), which decreased after additional adjustment for sex and smoking status (AUC = 0.593). **(B)** ROC curves for discrimination between PDAC and healthy controls based on age-adjusted LTL and LTL adjusted for age, sex, and smoking status. The age-adjusted model yielded an AUC of 0.630, whereas the fully adjusted model showed a slight increase in discriminatory performance (AUC = 0.651). Sensitivity, specificity, PPV, and NPV were calculated at the ROC operating point defined by Youden's index and are presented as descriptive exploratory metrics. **(C)** Kaplan–Meier curves for overall survival in patients with PDAC stratified by the median age-adjusted LTL, showing no significant association between LTL and survival (p = 0.693). **(D)**

Kaplan–Meier curves for overall survival in patients with PDAC stratified by the median LTL adjusted for age, sex, and smoking status, likewise showing no significant association with survival ($p = 0.943$). **Abbreviations**: AUC, area under the curve; CP, chronic pancreatitis; CTRL, controls; LTL, leukocyte telomere length; NPV, negative predictive value; PDAC, pancreatic ductal adenocarcinoma; PPV, positive predictive value; ROC, receiver operating characteristic.

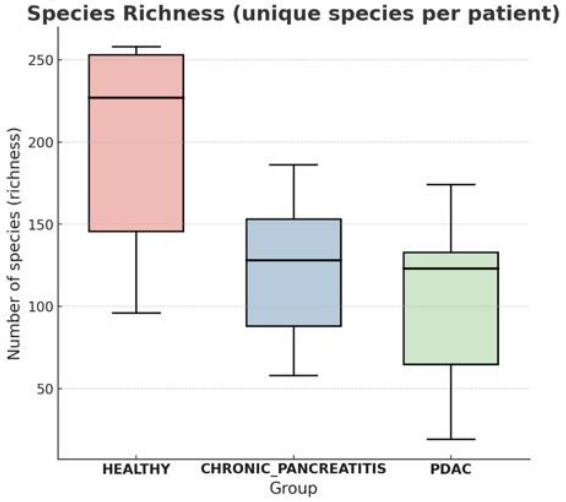
A)



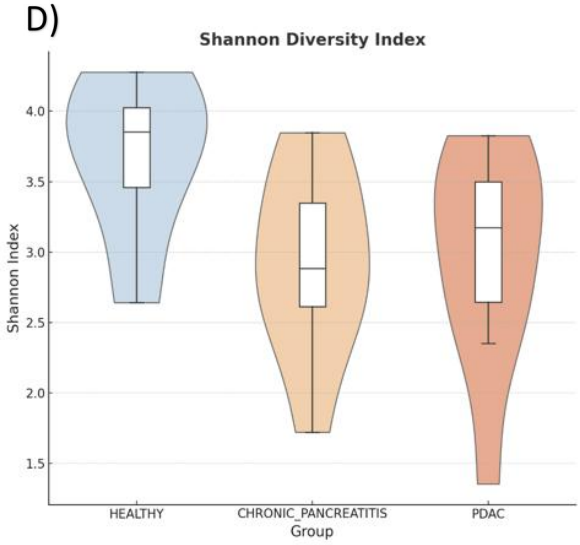
B)



C)



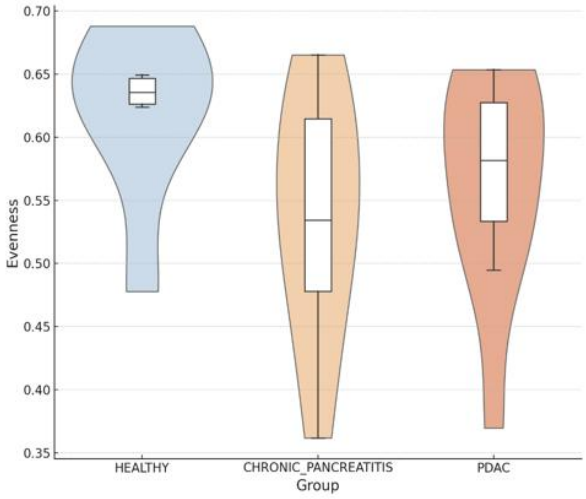
D)



Shannon Index (Mann–Whitney, Bonferroni):
- HEALTHY vs CHRONIC_PANCREATITIS: p = 0.126
- HEALTHY vs PDAC: p = 0.280
- CHRONIC_PANCREATITIS vs PDAC: p = 1.000

- Kruskal-Wallis p = 0.098
- ANOVA p = 0.095

E)



Evenness Index (Mann–Whitney, Bonferroni):
- HEALTHY vs CHRONIC_PANCREATITIS: p = 0.168
- HEALTHY vs PDAC: p = 0.542
- CHRONIC_PANCREATITIS vs PDAC: p = 1.000

- Kruskal-Wallis p = 0.135
- ANOVA p = 0.218

Figure S4. Gut microbiome composition and alpha-diversity across study groups. **(A)** Relative abundances of bacterial taxa at the phylum level for individual participants in the healthy control, CP, and PDAC groups. *Firmicutes* and *Bacteroidetes* were the predominant phyla across all groups. **(B)** *Firmicutes*/*Bacteroidetes* ratio across the three study groups. No significant overall difference was observed (Kruskal–Wallis $p = 0.138$; ANOVA $p = 0.202$), and no pairwise comparison remained significant after Bonferroni correction. **(C)** Species richness, expressed as the number of unique species per participant, was highest in healthy controls and lower in CP and PDAC groups. The overall ANOVA indicated a significant difference ($p = 0.005$), whereas the Kruskal–Wallis test did not reach statistical significance ($p = 0.056$); no pairwise comparison remained significant after Bonferroni correction. **(D)** Shannon diversity index across the study groups, with no significant overall differences (Kruskal–Wallis $P = 0.098$; ANOVA $P = 0.095$). **(E)** Pielou's evenness index across the study groups, likewise showing no significant overall differences (Kruskal–Wallis $p = 0.135$; ANOVA $p = 0.218$). **Abbreviations**: ANOVA, analysis of variance; CP, chronic pancreatitis; PDAC, pancreatic ductal adenocarcinoma.