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Supplemental information

**Immunoactive signatures of circulating
tRNA- and rRNA-derived RNAs
in chronic obstructive pulmonary disease**

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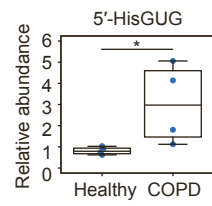


Figure S1. The levels of 5'-HisGUG in plasma samples

Plasma RNAs (Healthy: $n = 4$; COPD: $n = 4$) were subjected to TaqMan qPCR for specific quantification of 5'-HisGUG. Spike-in RNA was added during RNA extraction, and its levels were used for normalization. The value of one of the healthy samples was set as 1, and the relative values for the other samples are shown. $*p < 0.05$.

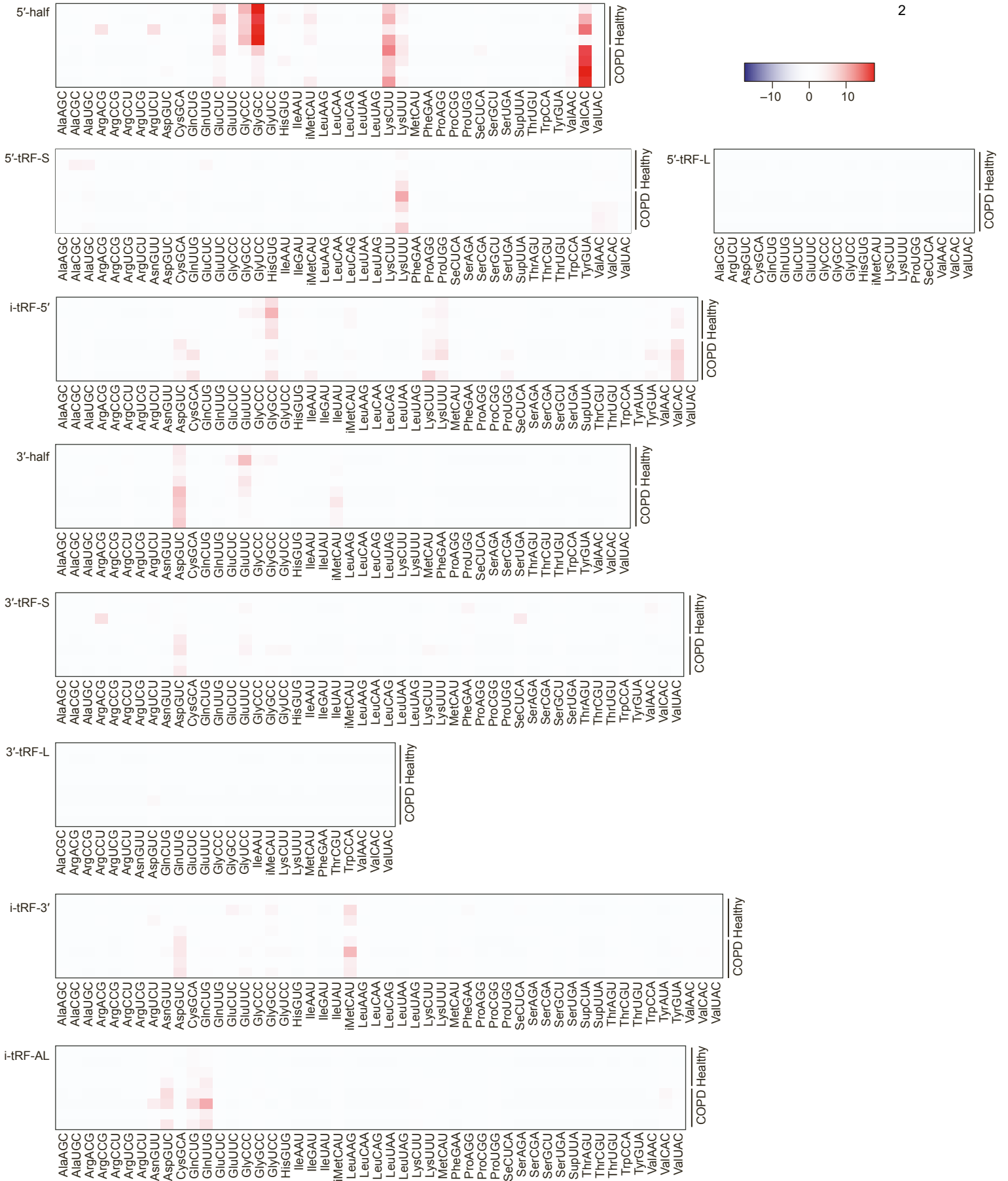


Figure S2. Heatmap of each subclass of tRNA-derived snRNAs

The expression profiling was performed across all subclasses of tRNA-derived snRNAs, and the results are presented in alphabetical order of tRNA isoacceptors.

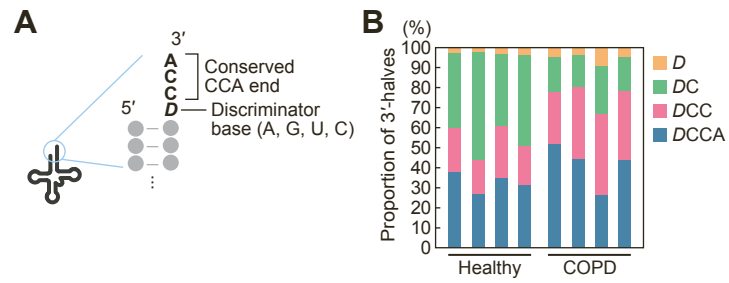


Figure S3. Nucleotide analysis of the 3'-ends of 3'-halves

(A) Schematic representation of tRNA termini. Mature tRNA commonly contains a 4-nt protruding 3'-end consisting of a discriminator base and conserved CCA trinucleotides.

(B) Proportions of each 3'-terminal variant of 3'-halves.

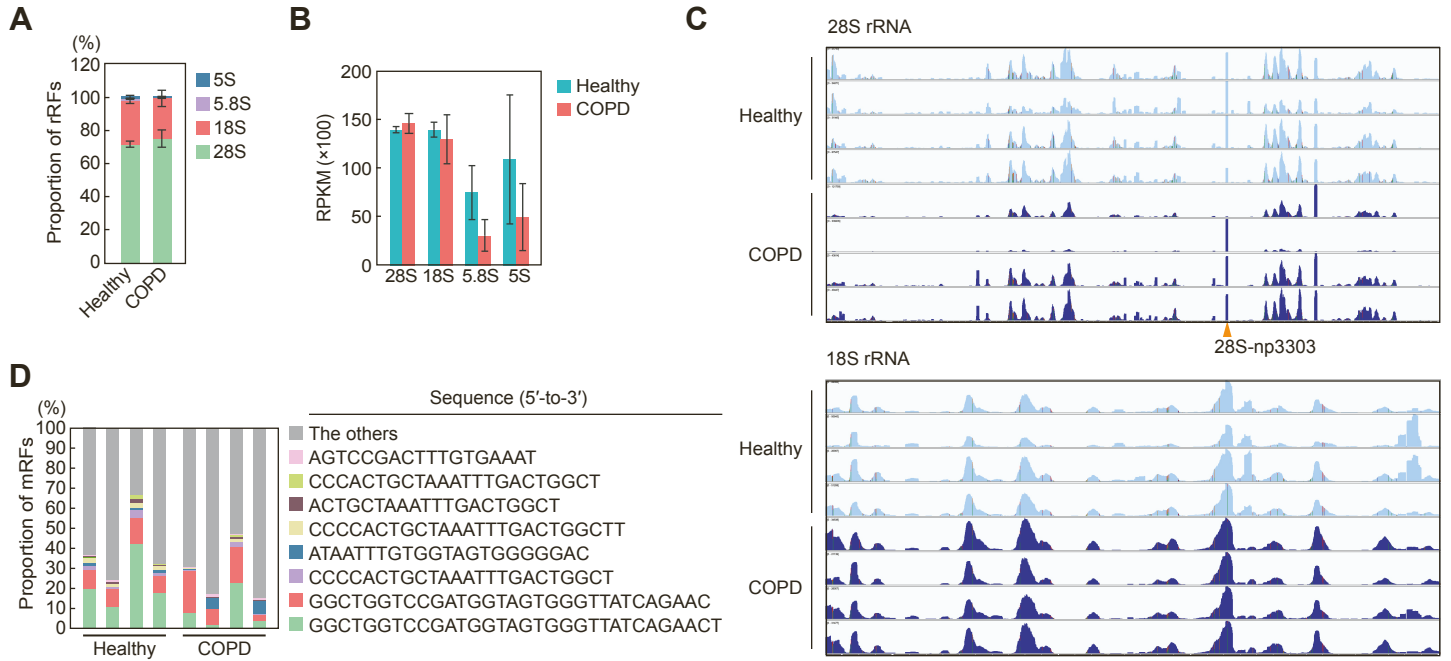


Figure S5. Analysis of rRFs and mRFs

(A) Proportion of rRFs for each rRNA gene. The error bar indicates the SD from four replicates.

(B) Abundance of rRFs is shown as RPKM of each rRNA gene. The error bar indicates the S.D. from four replicates.

(C) Read alignment of rRFs visualized by Integrative Genomics Viewer.

(D) Proportion of the 8 most abundant mRFs.

Table S1. Unique ID for the sncRNAs investigated in this study

Name	Sequence (5'-to-3')	License plate	tDRname
5'-HisGUG	GCCGUGAUCGUUAGUGGUUAGUACUCUGCGUUG	tRF-34-PW5SVP9N15WV2P	tDR-1:34-His-GTG-1
5'-GlyGCC	GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCCUGC	tRF-34-PNR8YP9LON4VHM	tDR-1:35-Gly-GCC-2-M3
5'-ValCAC	GUUCCGUAGUGUAGUGGUUACACGUUCGCCUC	tRF-34-79MP9P9NH57S15	tDR-1:34-Val-CAC-1-M3
18S-np22	AGCAUAUGCUUGUCUCAAGAUUAAGCCAUGCAUGUCUG	rRF-39-FQI19720IOPQP4JP	(N/A)
28S-np4533	AGACCGUCGUGAGACAGGUUAGUUUJACC	rRF-29-F1SW5D1N1Z2V	(N/A)

Table S2. Plasma donor information

Category	ID	Exp # in Fig. 1H	Bio/VT Lot ID	Experiments	5'-Val value	Age	Gender	BMI	Race	Diagnosis (year)	Severity/ GOLD Stage	Smoking status	Smoking history	Smoking frequency	Medications	Previous Treatments
Healthy	1	Set 1	HMN664977	Val qPCR, RNA-seq	0.537	56	Male	28	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	2	Set 1	HMN826748	Val qPCR, RNA-seq, Gly qPCR	1.433	61	Female	28	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	3	Set 1	HMN829012	Val qPCR, RNA-seq, Gly qPCR	1.006	64	Male	28	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	4	Set 1	HMN826749	Val qPCR, Gly qPCR	1.586	68	Male	32	Caucasian	Normal donor -		N/A	N/A	N/A	None	None or N/A
	5	Set 1	HMN690294	Val qPCR, RNA-seq, Gly qPCR	0.818	70	Male	28	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	6	Set 2	133-0488	Val qPCR, Gly qPCR	0.895	56	Female	31	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	7	Set 2	133-0533	Val qPCR, Gly qPCR	2.167	56	Female	26	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	8	Set 2	HMN715572	Val qPCR, Gly qPCR	1.000	61	Male	34	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	9	Set 2	133-0531	Val qPCR, Gly qPCR	0.555	67	Female	29	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
	10	Set 2	HMN664941	Val qPCR, Gly qPCR	0.556	56	Female	30	Caucasian	Normal Donor -		N/A	N/A	N/A	None	None or N/A
COPD	1	Set 1	HMN811067	Val qPCR, RNA-seq, Gly qPCR	1.394	66	Female	26	Caucasian	COPD (N/A)	Mild	N/A	N/A	N/A	Albuterol sulfate HFA	None or N/A
	2	Set 1	HMN811069	Val qPCR, Gly qPCR	1.365	63	Male	35	Caucasian	COPD (N/A)	Severe	N/A	N/A	N/A	Trelegy, Prednisone, Naproxen	Albuterol
	3	Set 1	HMN811070	Val qPCR, Gly qPCR	9.995	57	Male	32	Caucasian	COPD (N/A)	Moderate	N/A	N/A	N/A	Advair, ProAir HFA, Albuterol and Ipratropium	Spiriva (2018-2019), Prednisone (11/27/2019-12/02/2019)
	4	Set 1	HMN811071	Val qPCR, RNA-seq, Gly qPCR	3.824	53	Female	27	Caucasian	COPD (N/A)	Stage 3	N/A	N/A	N/A	Pulmicort, Flonase, Albuterol, Ventolin	None or N/A
	5	Set 1	HMN811072	Val qPCR, Gly qPCR	1.022	69	Male	31	Caucasian	COPD (N/A)	Severe, Stage 3	N/A	N/A	N/A	Advair Diskus, Levofloxacin, Prednisone, ProAir, Trelegy Ellipta, Tudorza Pressai	None or N/A
	6	Set 1	HMN811073	Val qPCR, RNA-seq, Gly qPCR	9.969	51	Female	33	Caucasian	COPD (N/A)	Mild, Stage 1	N/A	N/A	N/A	Ipratropium-Albuterol	None or N/A
	7	Set 1	HMN811074	Val qPCR, RNA-seq, Gly qPCR	3.262	62	Male	27	Caucasian	COPD (N/A)	Mild	N/A	N/A	N/A	Symbicort, Ventolin HFA	None or N/A
	8	Set 2	HMN875976	Val qPCR, Gly qPCR	1.213	64	Male	33	Caucasian	COPD (N/A)	N/A	N/A	N/A	N/A	None	Cardiac Stent Placement (2008)
	9	Set 2	259155A2	Val qPCR, Gly qPCR	3.801	57	Male	31	Caucasian	COPD (1994)	N/A	Never Used	-	-	Prednisone	None or N/A
	10	Set 2	259157A3	Val qPCR, Gly qPCR	6.098	69	Female	30	Caucasian	COPD (2000)	N/A	Current Use	For 54 years	20 smoke(s)/day	Advair Diskus, Oxygen, Ipratropium bromide, Singulair	None or N/A
	11	Set 2	286170A1	Val qPCR, Gly qPCR	2.976	60	Male	31	Caucasian	COPD (2006)	N/A	Previous Use	For 32 years	10 smoke(s)/day	Albuterol, Asmanex	None or N/A
	12	Set 2	286193A2	Val qPCR, Gly qPCR	8.542	66	Female	29	Caucasian	COPD (2000)	N/A	Previous Use	For 25 years	20 smoke(s)/day	Theophylline, Spiriva, Advair, Fluciclonide ointment	None or N/A
	13	Set 2	338854A2	Val qPCR, Gly qPCR	1.833	60	Male	27	Caucasian	COPD (2013)	N/A	Current Use	For >5 years	5 smoke(s)/day	Symbicort, Salbutamol	None or N/A
	14	Set 2	338845A2	Val qPCR, Gly qPCR	1.727	61	Male	29	Caucasian	COPD (2013)	N/A	Current Use	For >20 years	10 smoke(s)/day	Fluticasone and salmeterol, Salbutamol	None or N/A
	15	Set 2	485043A1	Val qPCR, Gly qPCR	0.494	55	Male	23	Caucasian	COPD (2015)	N/A	Previous Use	25 years	20 smoke(s)/day	Albuterol, Symbicort	None or N/A

N/A: Not available

Healthy: N = 10 (average 61.5 ± 5.5), 50% female

COPD: N = 15 (average 60.9 ± 5.5), 33% female

Table S3. Proportion of mapped reads (%)

Group	Library	tRNA	rRNA	mRNA	Mitochondria transcript	Genome
Healthy	1	1.677	59.816	3.866	0.794	33.847
	2	2.382	69.006	3.212	0.670	24.730
	3	1.480	51.871	10.619	0.876	35.153
	4	1.587	68.192	2.778	0.552	26.890
COPD	1	1.143	70.956	2.647	1.343	23.910
	2	0.589	61.756	2.904	0.097	34.654
	3	2.188	52.714	6.475	2.634	35.989
	4	1.103	49.072	3.829	0.292	45.704

Table S4. Sequences of the oligos used in this study

Experiment	Target	Type	Sequence (5'-to-3')
TaqMan qPCR	5'-HisGUG	Forward primer	GCTCGCCGTGATCGTATAGT
		TaqMan probe	/5HEX/TAGTACTCT/ZEN/GCGTTGGAACACTGCGTTTGC/3IABkFQ/
	5'-GlyGCC	Forward primer	GCATTGGTGGTTCAGTGGT
		TaqMan probe	6-FAM/ATTCTCGCC/ZEN/TGCGAACACTGCG/3IABkFQ/
	5'-ValCAC	Forward primer	GCTCGTTCCGTAGTGTAGTGGT
		TaqMan probe	/56-FAM/ACGTTTCGCC/ZEN/TCGAACACTGCGTT/3IABkFQ/
	R-Luc	Forward primer	CAGTGGTGGGCCAGATGT
		TaqMan probe	/5HEX/TTCTTGAA/ZEN/CACTGCGTTTG/3IABkFQ/
<i>In vitro</i> transcription	5'-ValCAC	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGACTACGGAAACCTGATGAGTCCGTGAGGAC
		Reverse primer	mGmAGGCGAACGTGATAACCACTACACTACGGAAACGACGGTACCGGGTACCGTTTCGTCCTCACGGACT
	18S-np22	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGAAGCATATGCTCTGATGAGTCCGTGAGGAC
		Reverse primer	mAmGACATGCATGGCTTAATCTTTGAGACAAGCATATGCTGACGGTACCGGGTACCGTTTCGTCCTCACGGACT
	28S-np4533	Forward primer	CCTGCAGTAATACGACTCACTATAGGGAGAACGACGGTCTCTGATGAGTCCGTGAGGAC
		Reverse primer	mGmGTAATAACTAACCTGTCTCACGACGGTCTGACGGTACCGGGTACCGTTTCGTCCTCACGGACT

Table S5. Sequences of the synthetic RNAs used in this study

Name	Sequence (5'-to-3')
5'-ValCAC	GUUCCGUAGUGUAGUGGUUAUCACGUUCGCCUC
18S-np22	AGCAUAUGCUUGUCUCAAGAUUAAGCCAUGCAUGUCUG
28S-np4533	AGACCGUCGUGAGACAGGUUAGUUUUACC
ssRNA40	GCCCGUCUGUUGUGUGACUC
ssRNA41	GCCCGACAGAAGAGAGACAC