Supplemental Materials:

Tissue-specific reprogramming of host tRNA transcriptome by the microbiome

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Contents:

Table S1. Sequencing read counts and mapping results of this study. B: brain; I: intestine; K: kidney; L: liver; SPF: specific pathogen-free; GF: germ-free; −DM: non-demethylase-treated (untreated, UT); +DM: demethylase-treated.

Sample	Tissue	Mouse	Treatment	Replicate No.	Index sequence	Total reads	Mapped tRNA reads		Mapped tRNA molecules	
							Cytosolic	Mitochondrial	Cytosolic	Mitochondrial
B_SPF_UT_1	Brain	SPF	−DM	1	TACAAG	41,776,556	10,288,214	2,493,350	10,061,721	2,192,345
B_SPF_UT_2	Brain	SPF	−DM	2	TTGACT	40,245,850	9,618,666	2,082,170	9,421,661	1,803,392
B_SPF_UT_3	Brain	SPF	−DM	3	GGAACT	45,487,286	9,828,949	2,393,392	9,566,131	1,950,487
B_SPF_DM_1	Brain	SPF	+DM	1	CGTACG	28,690,654	4,515,183	368,684	4,391,993	438,202
B_SPF_DM_2	Brain	SPF	+DM	2	CCACTC	30,895,820	4,999,269	414,130	4,819,861	486,015
B_SPF_DM_3	Brain	SPF	+DM	3	GCTACC	18,428,290	628,480	279,745	603,691	258,358
B_GF_UT_1	Brain	GF	−DM	1	GATCTG	45,789,834	7,171,919	3,175,379	7,119,765	3,017,019
B_GF_UT_2	Brain	GF	−DM	2	AAGCTA	56,495,774	10,427,943	6,722,929	10,280,203	6,237,586
B_GF_UT_3	Brain	GF	−DM	3	GTAGCC	26,463,226	2,700,375	1,339,527	2,682,343	1,290,821
B_GF_DM_1	Brain	GF	+DM	1	CTCTAC	38,436,276	9,797,868	2,354,362	9,169,156	2,222,744
B_GF_DM_2	Brain	GF	+DM	2	GGCCAC	32,213,624	6,130,858	1,214,328	6,048,139	1,286,908
B_GF_DM_3	Brain	GF	+DM	3	CGAAAC	24,513,364	5,780,616	875,133	5,636,868	914,144
I_SPF_UT_1	Intestine	SPF	−DM	1	CGTGAT	35,290,352	15,784,305	6,654,051	11,669,277	4,245,910
I_SPF_UT_2	Intestine	SPF	−DM	2	ACATCG	40,053,616	15,546,029	9,942,546	11,537,828	6,223,704
I_SPF_UT_3	Intestine	SPF	−DM	3	GCCTAA	13,803,180	3,120,132	1,662,684	2,447,626	1,034,974
I_SPF_DM_1	Intestine	SPF	+DM	1	GGACGG	4,063,776	198,778	102,387	194,896	98,055
I_SPF_DM_2	Intestine	SPF	+DM	2	GCGGAC	5,853,226	57,299	14,209	56,372	13,679
I_SPF_DM_3	Intestine	SPF	+DM	3	TTTCAC	21,972,878	411,494	146,222	402,414	142,019
I_GF_UT_1	Intestine	GF	−DM	1	TCAAGT	38,808,280	10,372,997	10,878,163	8,663,283	7,668,357
I_GF_UT_2	Intestine	GF	−DM	2	CTGATC	17,139,612	1,364,089	1,166,342	1,176,612	897,828
I_GF_UT_3	Intestine	GF	−DM	3	TGACAT	2,436,316	157,445	96,748	145,071	78,702
I_GF_DM_1	Intestine	GF	+DM	1	TGGTCA	31,261,850	8,238,700	1,948,200	8,138,896	1,970,474
I_GF_DM_2	Intestine	GF	+DM	2	CACTGT	22,475,862	6,115,784	1,032,464	6,063,262	1,034,103
I_GF_DM_3	Intestine	GF	+DM	3	ATTGGC	26,426,170	5,230,280	1,635,730	5,207,303	1,632,411
K_SPF_UT_1	Kidney	SPF	−DM	1	TACAAG	26,945,166	6,269,173	2,062,844	6,215,392	1,928,002
K_SPF_UT_2	Kidney	SPF	−DM	2	TTGACT	46,481,358	8,265,553	2,615,604	8,200,538	2,455,749
K_SPF_UT_3	Kidney	SPF	−DM	3	GGAACT	36,558,894	8,467,000	3,827,220	8,314,357	3,413,918
K_SPF_DM_1	Kidney	SPF	+DM	1	CGTACG	21,368,410	1,916,927	1,149,365	1,866,821	1,067,150
K_SPF_DM_2	Kidney	SPF	+DM	2	CCACTC	34,976,618	7,839,574	1,313,265	7,687,736	1,359,780
K_SPF_DM_3	Kidney	SPF	+DM	3	GCTACC	35,467,018	8,338,385	1,157,392	8,235,346	1,242,683

K_GF_UT_1	Kidney	GF	−DM	1	GATCTG	40,041,188	11,110,591	5,519,137	10,949,868	5,080,446
K_GF_UT_2	Kidney	GF	−DM	2	AAGCTA	45,459,158	12,220,255	4,812,856	12,159,498	4,637,940
K_GF_UT_3	Kidney	GF	−DM	3	GTAGCC	34,859,812	8,519,777	4,538,068	8,398,367	4,222,622
K_GF_DM_1	Kidney	GF	+DM	1	CTCTAC	32,110,612	8,610,315	1,416,894	8,315,714	766,797
K_GF_DM_2	Kidney	GF	+DM	2	GGCCAC	22,976,416	3,679,101	821,885	3,631,681	846,641
K_GF_DM_3	Kidney	GF	+DM	3	CGAAAC	17,795,592	4,253,423	443,397	4,221,607	489,822
L_SPF_UT_1	Liver	SPF	−DM	1	TGGTCA	18,550,428	2,443,125	3,512,336	2,229,020	3,175,596
L_SPF_UT_2	Liver	SPF	−DM	2	CACTGT	20,233,708	3,097,641	3,586,976	2,586,057	2,857,784
L_SPF_UT_3	Liver	SPF	−DM	3	ATTGGC	52,170,314	6,672,730	8,797,669	6,051,539	7,923,271
L_SPF_DM_1	Liver	SPF	+DM	1	GGACGG	2,494,822	17,711	9,164	17,248	8,550
L_SPF_DM_2	Liver	SPF	+DM	2	GCGGAC	3,983,208	121,175	37,051	117,283	34,789
L_SPF_DM_3	Liver	SPF	+DM	3	TTTCAC	1,836,760	161,817	37,006	159,760	37,608
L_GF_UT_1	Liver	GF	−DM	1	CGTGAT	8,359,028	188,414	163,533	156,955	125,270
L_GF_UT_2	Liver	GF	−DM	2	ACATCG	151,618,438	34,707,525	28,900,278	32,782,836	26,056,018
L_GF_UT_3	Liver	GF	−DM	3	GCCTAA	3,145,434	345,026	377,362	337,981	363,434
L_GF_DM_1	Liver	GF	+DM	1	TCAAGT	3,835,010	292,951	45,484	272,678	40,748
L_GF_DM_2	Liver	GF	+DM	2	CTGATC	14,980,484	1,913,525	2,036,529	1,884,314	1,992,343
L_GF_DM_3	Liver	GF	+DM	3	TGACAT	7,325,582	919,646	180,265	908,068	186,341

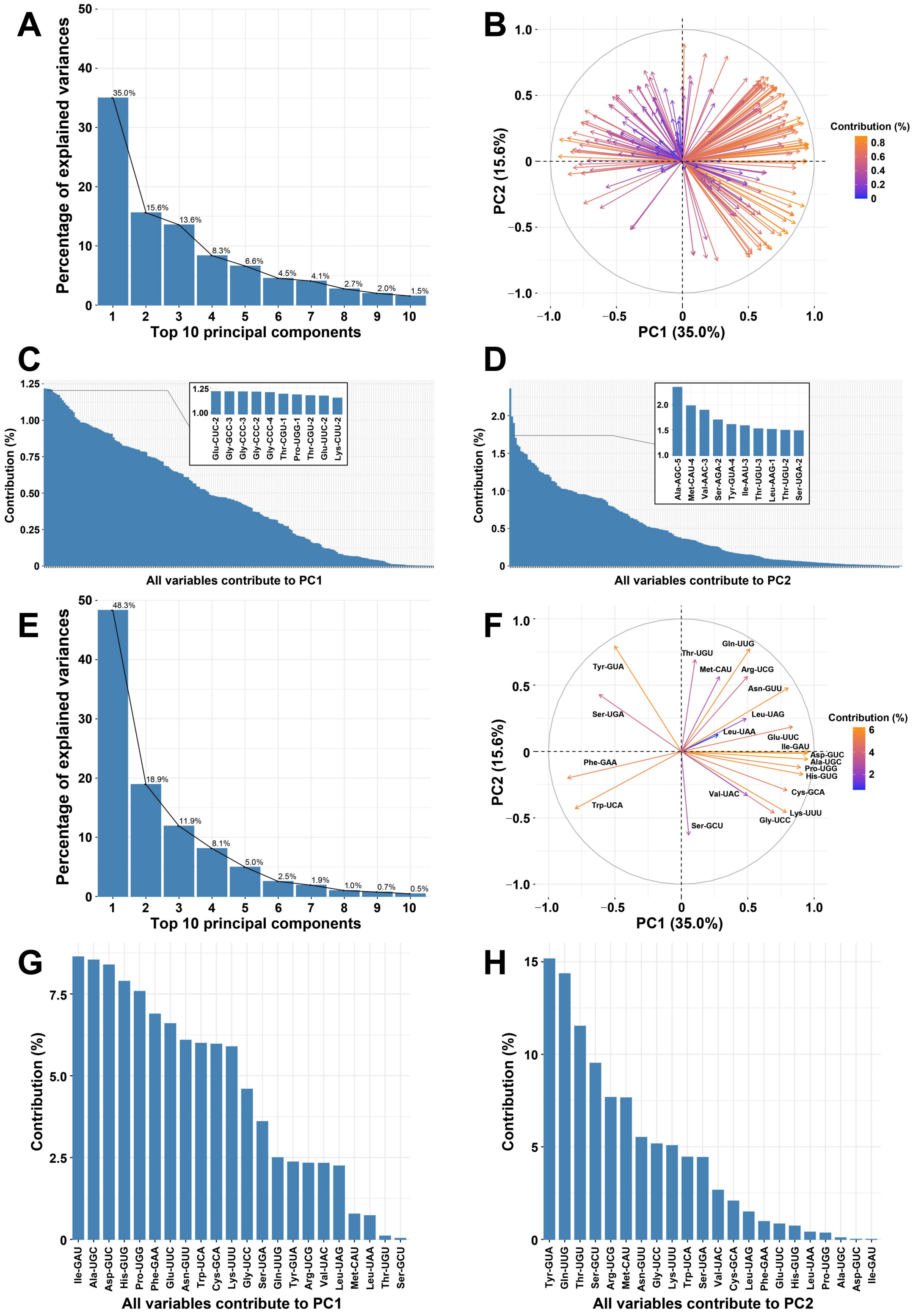

Figure S1. Supplemental data for the principal component analyses (PCAs) of the cytosolic (Fig. 2A) and mitochondrial tRNAs (Fig. 2B), respectively. (*A*) Top 10 PCs of Fig. 2A. (*B*) All the 216 cytosolic tRNA isodecoders contributed to the PC1 and

PC2 of Fig. 2A. (*C*) All the 216 cytosolic tRNA isodecoders and their contributions to the PC1 of Fig. 2A in descending order. (*D*) All the 216 cytosolic tRNA isodecoders and their contributions to the PC2 of Fig. 2A in descending order. (*E*) Top 10 PCs of Fig. 2B. (*F*) All the 22 mitochondrial tRNA isoacceptors contributed to the PC1 and PC2 of Fig. 2B. (*G*) All the 22 mitochondrial tRNA isoacceptors and their contributions to the PC1 of Fig. 2B in descending order. (*H*) All the 22 mitochondrial tRNA isoacceptors and their contributions to the PC2 of Fig. 2A in descending order.

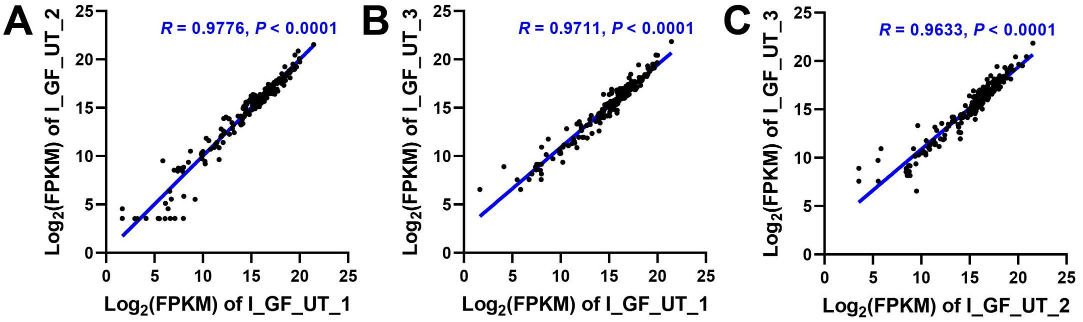

Figure S2. Sample correlation analyses of the tRNA expression. These analyses were based on the fragments per kilobase per million (FPKM) values of 216 cytosolic and 22 mitochondrial tRNAs from the three intestinal biological replicates of the GF mice. (*A*) I_GF_UT_1 versus I_GF_UT_2. (*B*) I_GF_UT_1 versus I_GF_UT_3. (*C*) I_GF_UT_2 versus I_GF_UT_3. Sample names follow those in the Supplemental Tables S1.

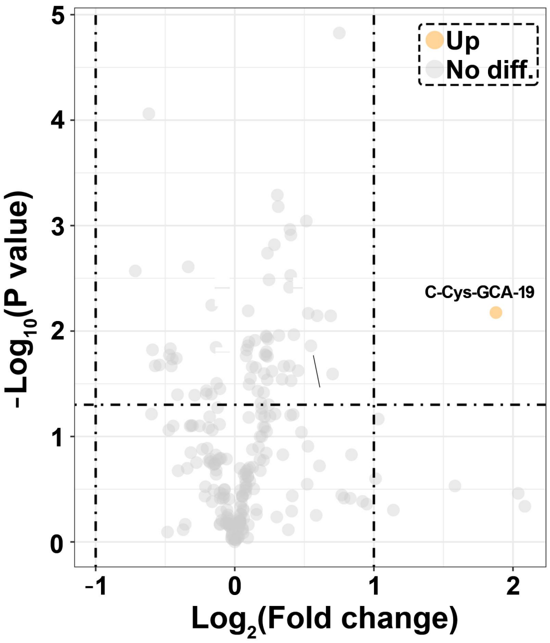

Figure S3. Volcano plot for the kidneys at the tRNA isodecoder level. This analysis was based on the tRNA expression of FPKM values of 216 cytosolic and 22 mitochondrial tRNAs from the kidneys (*n* = 3, SPF versus GF mice). $P < 0.05$ and $\log_2$ (fold change) > 1 were set as the thresholds of significance, and the name of the significant cytosolic tRNA isodecoder follows the name in the Genomic tRNA Database (GtRNAdb, http://gtrnadb.ucsc.edu/genomes/eukaryota/Mmusc10/). C: cytosolic tRNA.

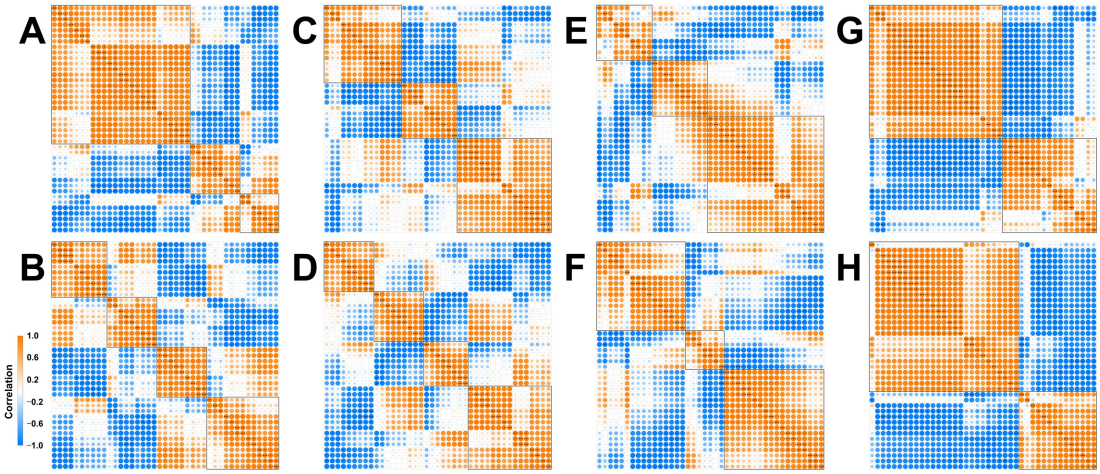

Figure S4. tRNA co-expression correlation plots at the amino acid isotype level. The analyses were based on the FPKM values of 21 cytosolic and 20 mitochondrial tRNA isotypes from four tissue types (brain, intestine, kidney, and liver; $n = 3$ for both GF and SPF mice). (*A*) Brains of the GF mice. (*B*) Brains of the SPF mice. (*C*) Intestines of the GF mice. (*D*) Intestines of the SPF mice. (*E*) Kidneys of the GF mice. (*F*) Kidneys of the SPF mice. (*G*) Livers of the GF mice. (*H*) Livers of the SPF mice. The squares indicate hierarchical clusters. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

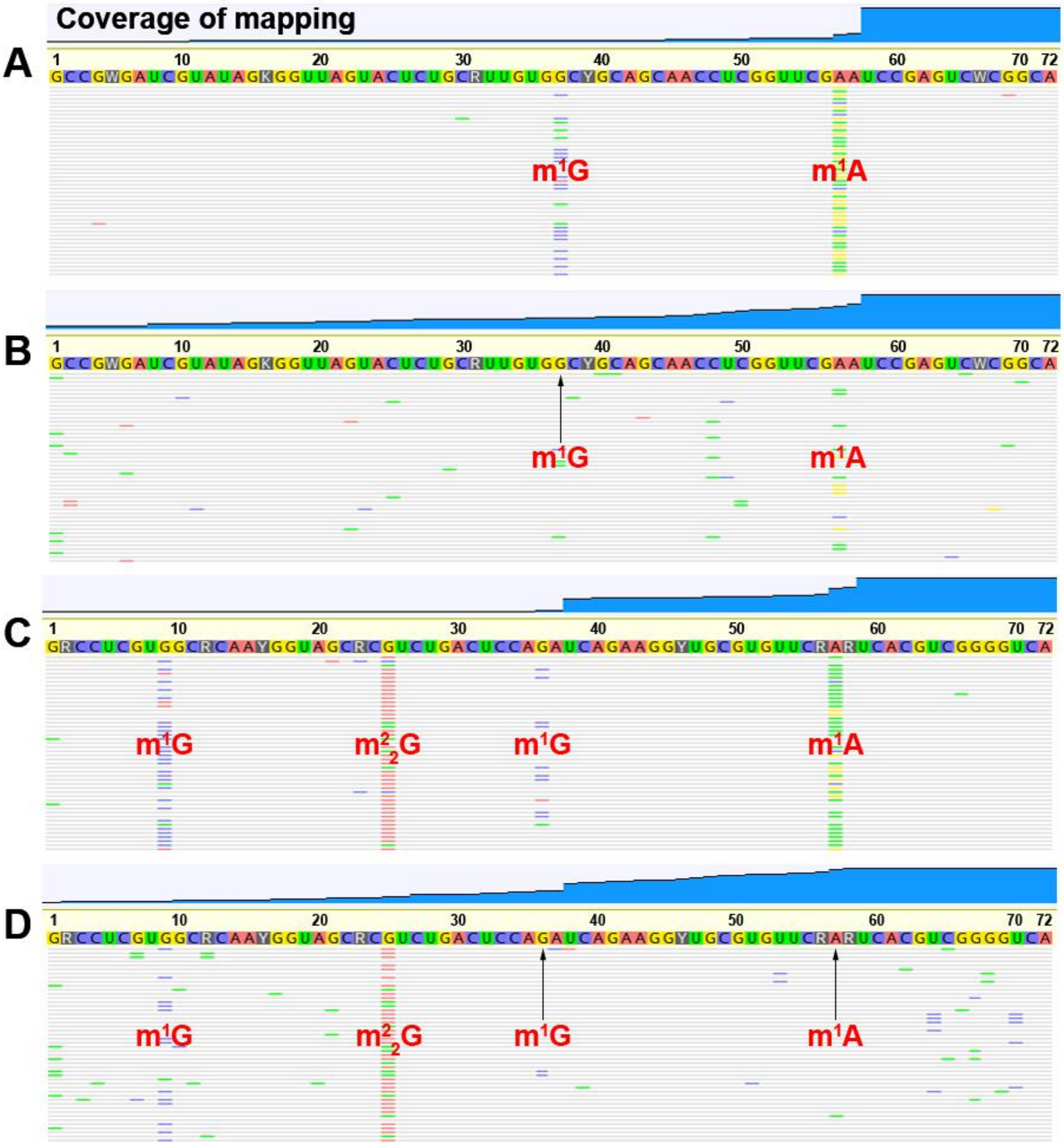

Figure S5. Mapping alignments of two cytosolic amino acid isotypes (tRNA^{His} and tRNA^{Phe}) in the brains. The data are from the −DM or +DM libraries (n = 6, combining the data of GF and SPF mice), respectively. (*A*) tRNA^{His} in the −DM libraries. (*B*) tRNA^{His} in the +DM libraries. (*C*) tRNA^{Phe} in the −DM libraries. (*D*) tRNA^{Phe} in the +DM libraries. m^{1}G: N^1-methylguanosine; m^{1}A: N^1-methyladenosine; m$^2_2$G: N^2,N^2-dimethylguanosine.

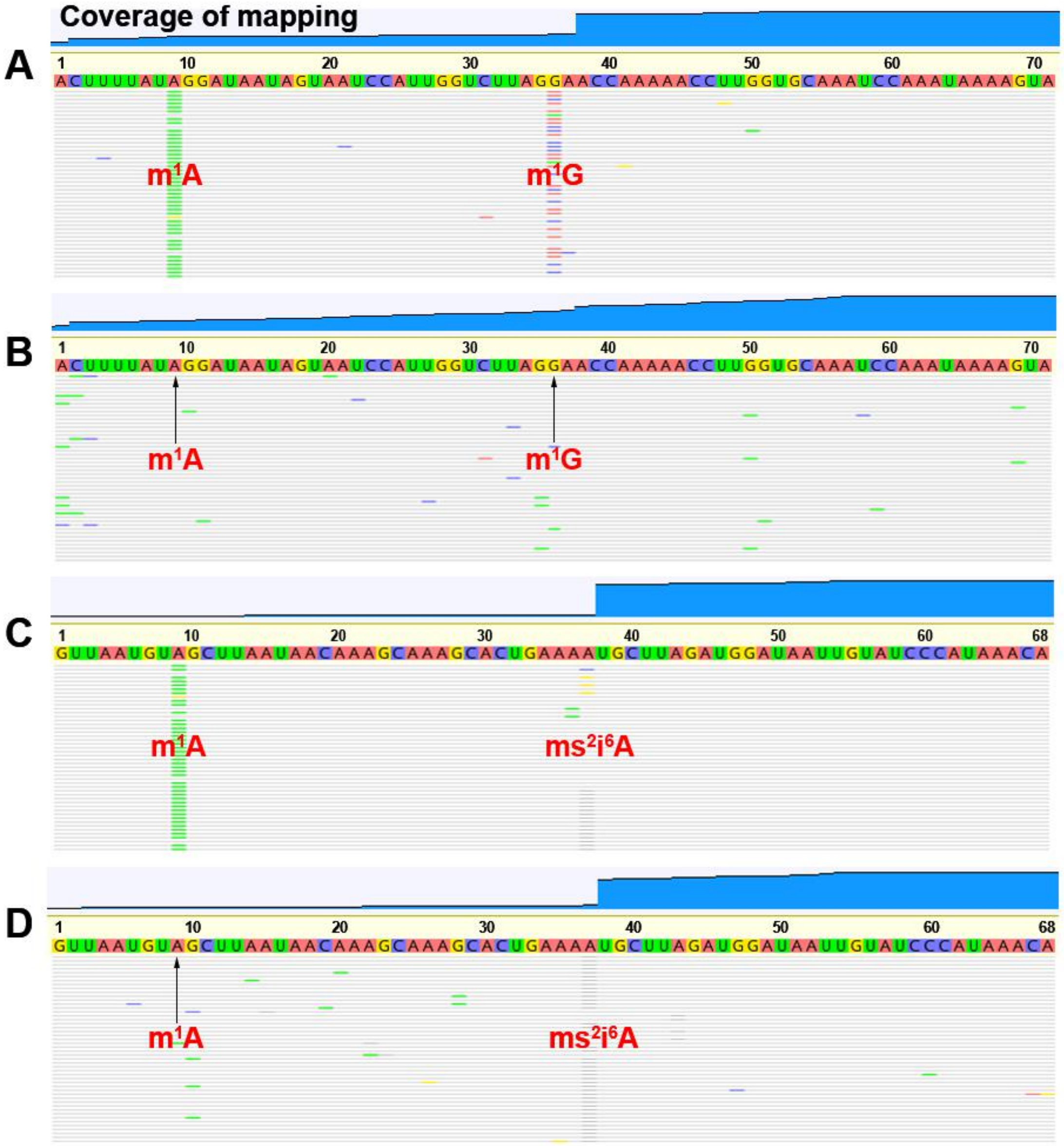

Figure S6. Mapping alignments of two mitochondrial isoacceptors (tRNA$^{\text{Leu-UAG}}$ and tRNA$^{\text{Phe}}$) in the brains. The data are from the −DM or +DM libraries (n = 6, combining the data of GF and SPF mice), respectively. (*A*) tRNA$^{\text{Leu-UAG}}$ in the −DM libraries. (*B*) tRNA$^{\text{Leu-UAG}}$ in the +DM libraries. (*C*) tRNA$^{\text{Phe}}$ in the −DM libraries. (*D*) tRNA$^{\text{Phe}}$ in the +DM libraries. ms^{2}i^{6}A: 2-methylthio-N^6-isopentenyladenosine.

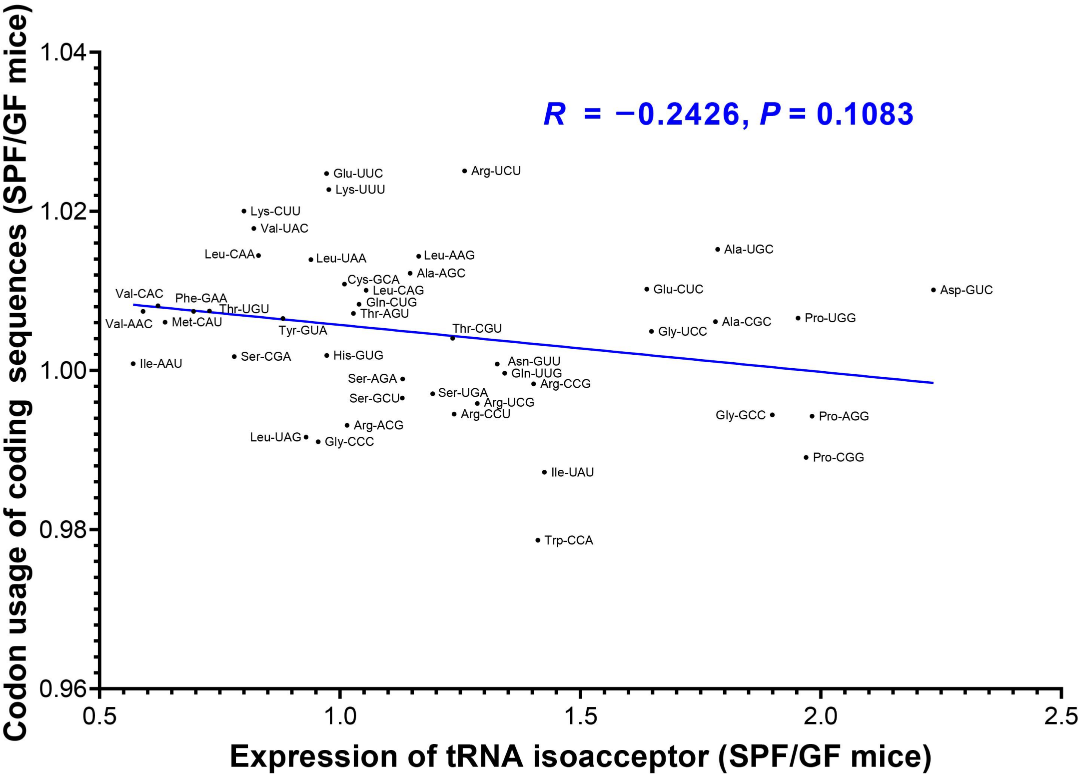

Figure S7. Correlation plot between the mammalian anticodons and codons. The anticodons are from 45 tRNA isoacceptors based on the FPKM values and the codons are from codon usage of 15,901 coding sequences (CDSs) in the livers (*n* = 3, SPF/GF mice) from the −DM libraries. The dots represent tRNA isoacceptor types.