



Figure S1. Supporting data figure for generation and characterization of transgenic mice with an expanded genetic code. (A) Genotyping results of 34 F0 pups (two negative F0, No. 2 and 19, were not included). The red arrowheads mark the DNA band amplified by PCR primers against the AzFRS gene indicating the AzFRS/tRNA genomic integration. M denotes size marker lane. (B) mRNA level of AzFRS was determined by RT-PCR using fibroblasts isolated from tails of founder F0-1, F0-10,

F0-20, F0-33, and wild type control mouse (wt). (C) Hematoxylin and eosin (H&E) staining of tissues from 4-5 weeks old RS(tRNA)^{4×} and wild type mice. Scale bar, 100 μm. (D) Genes that exhibited stable relative expression levels (FPKM/the sum of FPKM across all samples) among genes involved in liver metabolic activity were extracted. (E) Serum aspartate transaminase (AST), alanine aminotransferase (ALT), albumin, total bile acid (TBA) and total bilirubin (TB), low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) were determined in transgenic RS(tRNA)^{4×} and wildtype mice (n=4). Data are presented as the mean of four independent measurements. Error bar represents the standard deviation. P-value was determined using two-tailed unpaired Student's t-test. (F) The blood chemical indexes as described in (E) were determined in transgenic mice after feeding with or without 30mg/ml AzF dissolved in grape-flavored water for 10 days. (G) The ratios of mCherry and eGFP positive cells of differentiated neurons (a) and bone marrow cells (b) and fibroblasts (c) incubated in the presence or absence of AzF.