Appendix

Queuosine-tRNA promotes sex-dependent learning and memory formation by maintaining codon-biased translation speed.

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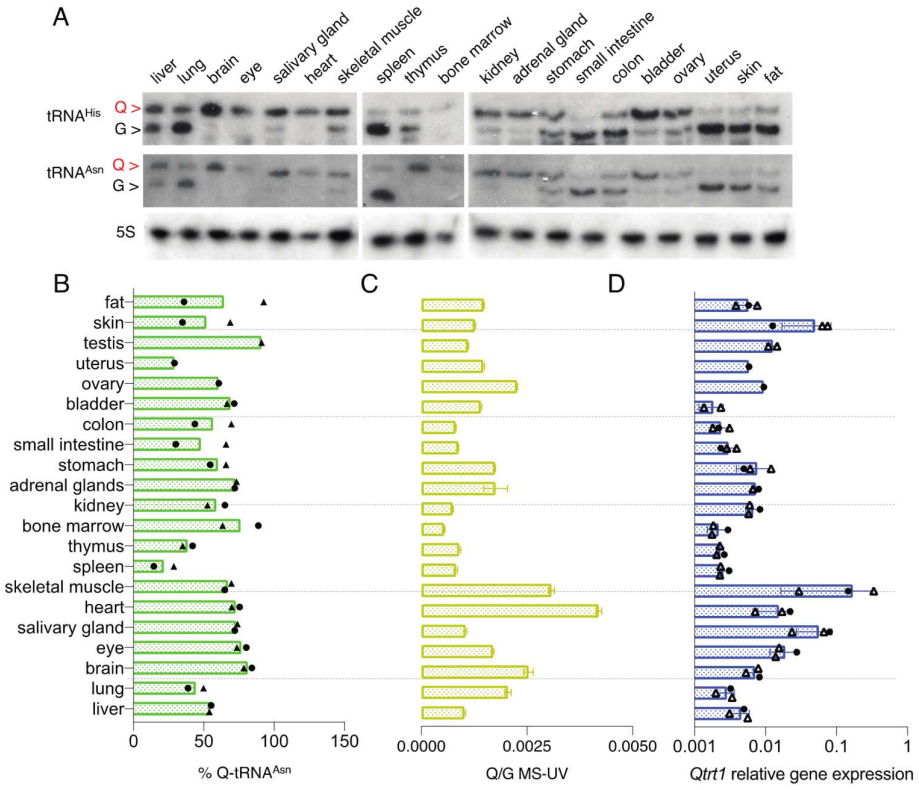
Supplementary Methods

cDNA synthesis and qRT-PCR.
Reverse transcription was performed according to manufacturer's instructions using up to 1 μg RNA (Quantitect, Qiagen). Synthesis was followed by qPCR using Sybr Green (Mesa Green) in LightCycler 480 (Roche). The PCR conditions were 1 cycle of 95°C for 15 min, 40 cycles of [95°C 15 sec; 60°C 40 sec reading], and a final melting curve from 65°C to 95°C, read every 1°C.
Sequences of mouse *Qtrt1* gene-specific primers:
forward: GGTACACACAGTCGAACATGTCA,
reverse: TTGTCTGAAAGAGATGACCAAGA
Dark/light compartment test.
The dark–light box is an open white rectangle (40 × 40 × 33 cm) attached to an 8 × 10 cm opening to a dark compartment, a dark box with a lid (20 × 40 × 33 cm). The light compartment was illuminated with 500 lx. Each mouse was put in the dark chamber and the number of visits, duration and distance to the light compartment within 10 min, were measured. The behavior was video recorded with a digital camera and tracked using the SYGNIS tracker software (SYGNIS).
Grip strength.
The muscle strength was determined by measuring the forelimb muscle tension of each animal with a grip strength meter apparatus (Ugo Basile, Comerio, Italy). Horizontally positioned animals were instructed to hold the platform exclusively with their forepaws. Following to gentle pullback by the animal's tail, the maximal grip strength was measured three times per session for three weeks at two-week intervals. Only animals with grasping power more than 800 mN were recorded; otherwise, the test was repeated. The total strength was calculated as the mean of three measurements.
Rotarod
Every two weeks for over 4 months, animals were tested for motor coordination on a rotarod (Ugo Basile, Comerio, Italy). Each session was performed as 3 trials per session using a constant acceleration protocol (4-40 rpm for 480 sec). Between each trial, the animals were rested for at least 30 min. Whenever an animal fell before 10 sec, the training was repeated. 5 seconds after placing the animals on the rod, the acceleration was started if the animal faced forward. Fall-off latency was recorded as the first time an animal passively rotated on the rod or fell.

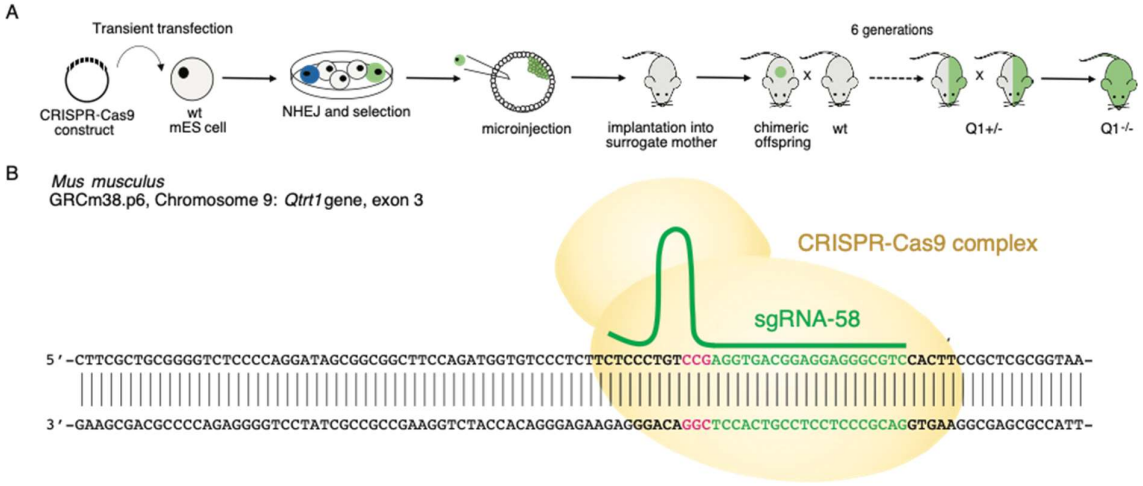
I. cohort of mice	**Behavior test**
6 female wt 6 female Q1 6 male wt 6 male Q1	Open field Home-cage Grip strength Rotarod MWM Fear conditioning
Littermates of Q1+/- x Q1+/- after 6 generations of backcrossing with C57BL/6J. Tested for behavior at the age of 4 to 6 months. Afterwards selected tissues were used for molecular analyses: RNA-Seq, Ribo-Seq, polysome profiling, proteomics, and Western blot. Additional littermates were used for *in situ* hybridization and immunostainings.	
II. cohort of mice	**Behavior test**
8 female wt 10 female Q1 8 male wt 8 male Q1	Dark/light Home-cage IntelliCage Fear conditioning
Littermates of wt x wt and Q1 x Q1 after 7 generations of backcrossing with C57BL/6J. Tested for behavior at the age of 4 to 6 months. Afterwards, selected tissues were used for molecular analyses: Western blot, *in situ* hybridization and immunohistochemistry.	

Appendix Table 1
Description of mice used in behavior and molecular analyses.

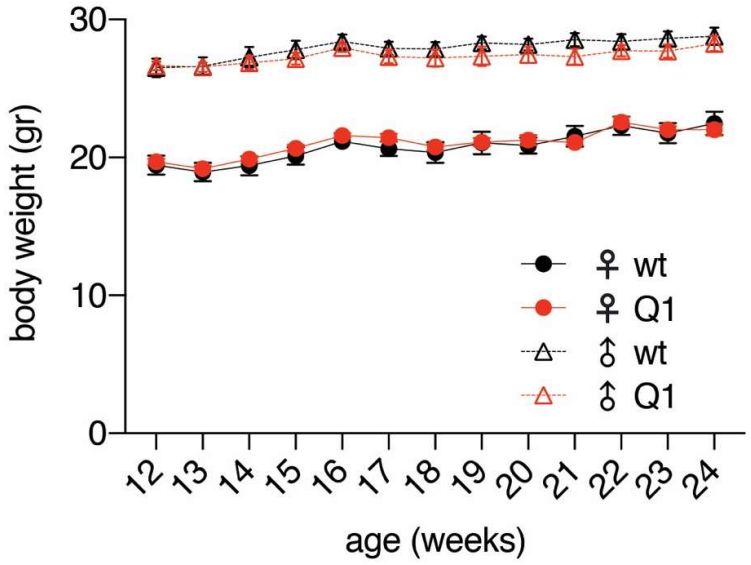
Apeendix Figures



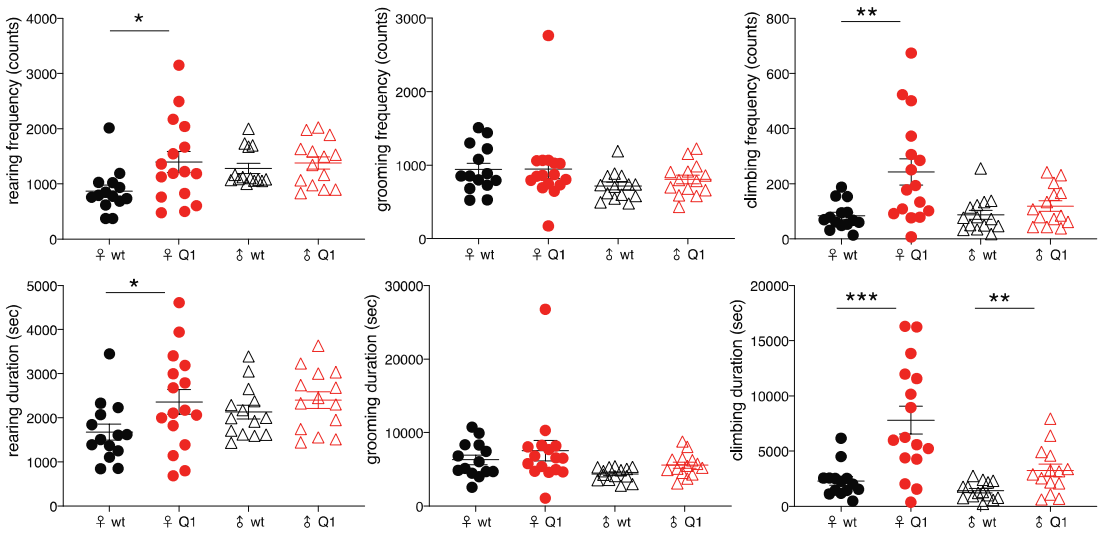
Appendix Figure 1. Q modification atlas.
A. APB Northern blots showing the distribution of Q modification in $tRNA^{His}$ and $tRNA^{Asn}$ in 20 tissues from a 3-month-old female mouse. 5S rRNA was used as a loading control.
B. The level of $Q\text{-}tRNA^{Asn}$ was calculated by quantifying the Q and G signals. Data are represented as mean (n=2, female (dot) and male (triangle)).
C. Mass spectrometry quantification of Q, Galactosyl-Q or Mannosyl-Q levels in total RNA from the indicated tissue. Data are represented as mean ±SD; (n=3 technical replicates).
D. *Qtrt1* gene expression patterns by qPCR. Relative gene expression was obtained by normalising the CT values of *Qtrt1* to that of *Actb*. Data are represented as mean ±SD; (n=3 biological replicates with 3 technical replicates; female (dot) and male (triangle)).
Q: Queuosine-tRNA, G: Guanosine-tRNA.



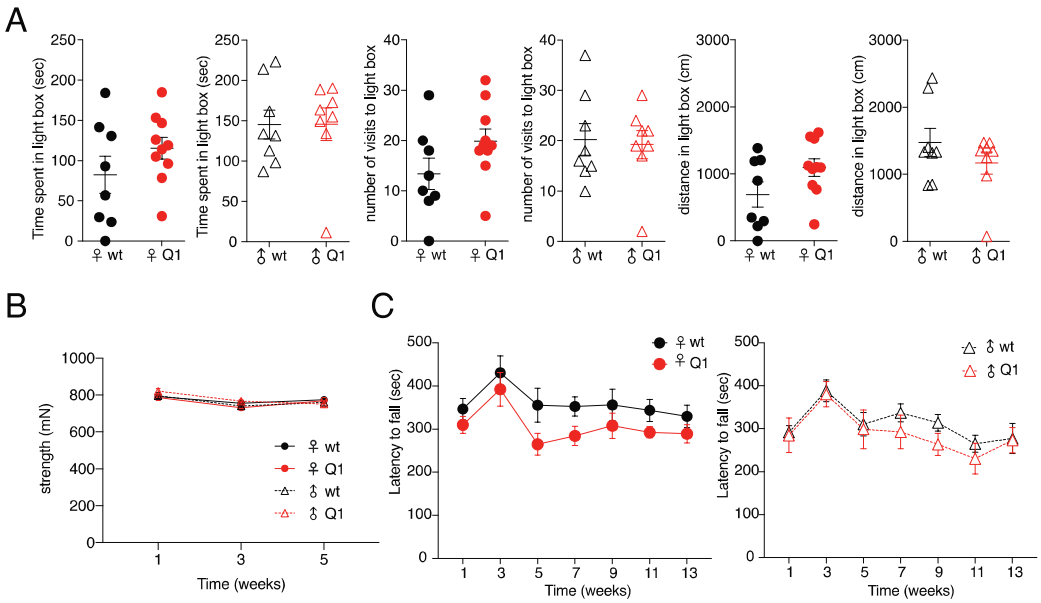
Appendix Figure 2. Generation of Q1 transgenic mouse line using CRISPR-Cas9 technology.
A. Workflow of CRISPR-Cas9 engineered mouse line generation.
B. Scheme of the genome editing event in the third exon of the *Qtrt1* gene. sgRNA-58: GACGCCCTCCTCCGTCACCT was used for producing the transgenic mouse line.



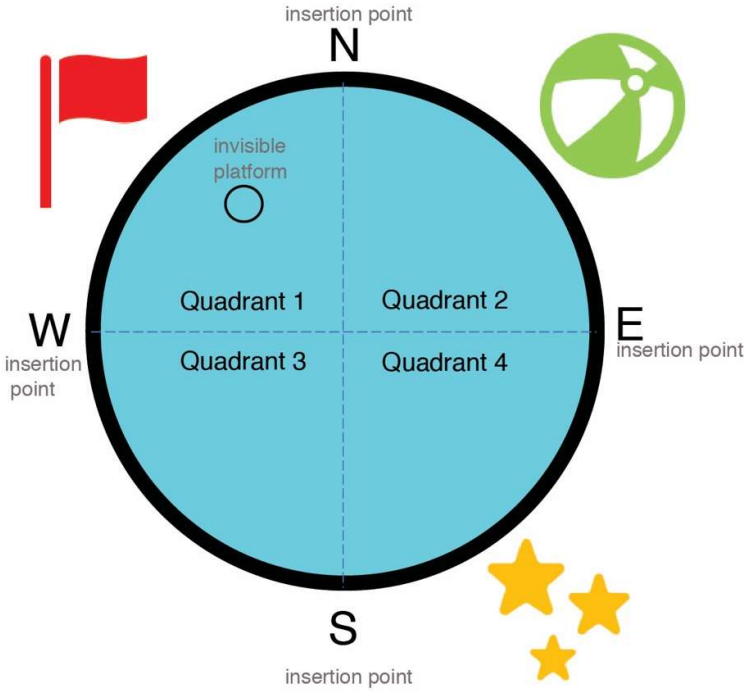
Appendix Figure 3. Body weight
Body weight measurement. Data points are shown as ±SEM; (n=6).



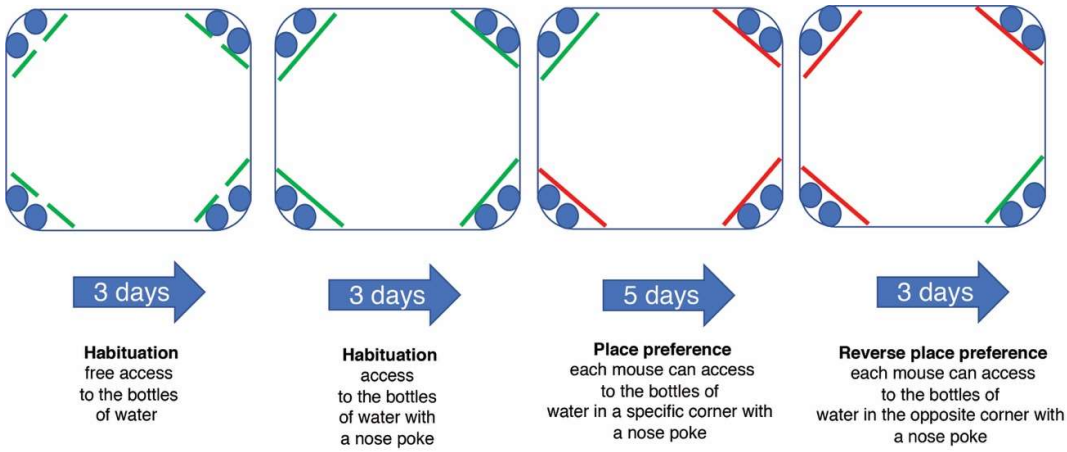
Appendix Figure 4. Home-cage (LABORAS) test.
The graphs show the rearing, grooming and climbing behaviors measured in Home-cage observation test. Statistical significance was analyzed by two-tailed unpaired t-test. Data points are shown as ±SEM; (n≥14). *=*p*-value < 0.05, **=*p*-value < 0.01, ***=*p*-value < 0.001.



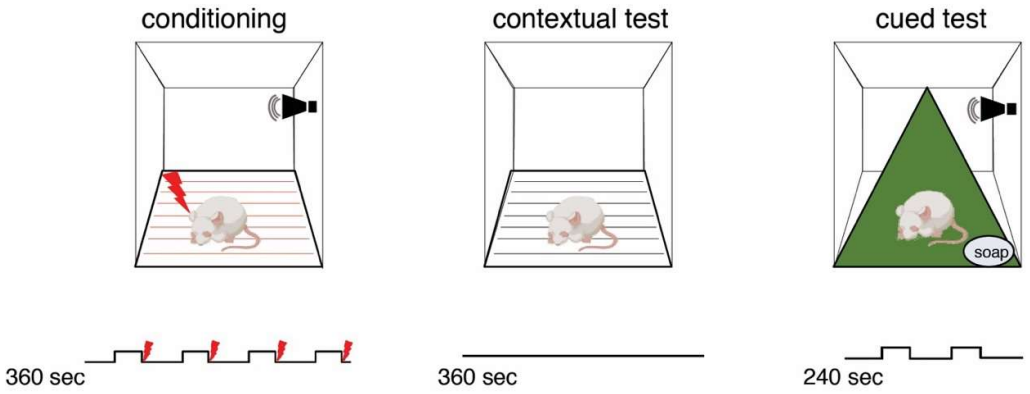
Appendix Figure 5. Dark/light, Grip strength and Rotarod tests.
A. Q1 mice exhibited similar behavior in terms of number of visits, time spent and distance travelled to the light compartment compared to wild type mice in the dark/light compartment test. Data are represented as ±SEM; (n≥8).
B. Grip strength analysis illustrating no difference in muscle strength between wt and Q1 mice. Data points are shown as ±SEM; (n=6)
C. Rotarod performance. A standard constant acceleration protocol (4-40 rpm for 480 sec) was used to monitor the motor coordination of 4-month-old Q1 mice for 13 weeks. Each animal was tested in three trials per week. Latency to fall (seconds) was recorded as the first time an animal passively rotates on the rod or falls. Data are represented as ±SEM; (n=6).



Appendix Figure 6. MWM experimental setting.
Illustration of the pool and experimental setup for the MWM test indicating the position of the invisible platform, the division in quadrants and the insertion point of the mice. During visual test, the platform is visible and highlighted with flags. During the 10 days of trial, the platform is under the water and invisible. The mice can orient themselves during swimming using the labeled objects in the environment. During the probe trial, the invisible platform is removed.



Appendix Figure 7. IntelliCage experimental setup.
Mice with micro-transponders were housed in IntelliCages and habituated for 6 days with free access to two water bottles at each corner for 3 days and with nose-poke for another 3 days. Next, the water access was randomly limited to one of the four cage corners for 5 days to assess place preference. For the assessment of reversal place preference in the last 3 days, each mouse was assigned to the opposite drinking corner. The number of visits and nose pokes and leak in each corner were recorded.



Appendix Figure 8. Illustration of the experimental setup for the fear conditioning test. At day 1, after 90 s of habituation, the mice were conditioned to tone and environment after four pairs of the tone (at 90–120 s, 150–180 s, 210–240 s, and 270–300 s), followed by a mild electric foot shock (0.6 mA, 1s). Freezing was registered for the entire trial of 360 s. After 24 h, contextual freezing behavior was registered in the same environment without tone and shock for 360 s. 48 h after the conditioning, the cued test was performed, in a new environment where surfaces and space, including the smell, were changed. Freezing was registered for 240 s during which the 30 s conditioned stimulus was presented twice, terminating at 60 and 180 s, respectively.