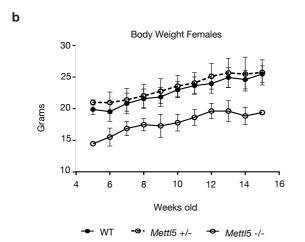
а



ATGAAGAAGTTAAAGCTTAAGGAACTAGAGAG.......TCGAAAAGCCCAAGTTACTTCTAGAACAGTATCCCACCAGGCCGCACATTGCAG

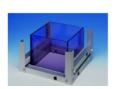
С



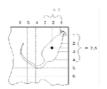
100 Threshold (db SPL) 80 60 40 20 0 Click 6 12 18 24 30 Frequncy (kHz) -**□**- Mettl5 -/-· WT

KO

d



Test arena for Open Field test



Calculating the center of gravity



ravity Detecting rearings

Locomotion: distance travelled (cm)

Exploration: vertical = rearings; horizontal = distance

Anxiety-related behaviour: time spent in the center (%)

Habituation: time course

Conditions: lenght: 45.5 x 45.5 cm, walls: 39.5 cm

150 - 200 lx, Center: 42%

Time: 20 min Infrared beam breaks

Figure S6. Mettl5 KO mice show multiple phenotypes.

- a. Schematic representation of the CRISPR/Cas9 gene editing strategy to create *Mettl5* KO mice. The KO was created by a 23bp deletion in exon 1. The nucleotide sequences of wt and KO alleles are shown. The conventional start codon (ATG) is indicated in red. The excised fragment of 23 bp is indicated in blue and causes a frameshift mutation resulting in a stop codon in exon 2. See full description in the Material and Methods. The scheme was generated in Benchling (https://benchling.com).
- b. Weight monitoring of mice from the age of 5 to 15 weeks showed significant differences (p-value 0,007) between female Mettl5 KO and control animals (data are means \pm SD, n= 22 wt +/+, n=10 Mettl5 +/-, n=3 Mettl5 -/-).
- c. Auditory Brainstem Response (ABR) was performed as previously described (Fuchs et al. 2018) by applying different sound stimuli to anesthetized mice and measuring sound pressure thresholds for eliciting a detectable ABR response. ABR curves of 14-week-old mice show increased thresholds of mutants indicative of reduced hearing sensitivity. ** p<0.01 by Wilcoxon rank-sum test; Data are means \pm SD, males and females pooled, n = 36 wt, n = 11 *Mettl5* KO.
- d. Scheme of the open field test used in the experiment presented in Figure 5f. For details see material and methods and (Garrett et al. 2012).