Mary Lyon Centre at MRC Harwell

FINAL REPORT

Phenotyping Final Report

Project Reference: Tmem233-Cre-b6j

Contents

Report date: 26/05/2022

1. COMPLIANCE AND QUALITY STATEMENT

The project was conducted in accordance with the MLC Standard Operating Procedures and protocols described herein. All deviations are documented and the report represents an accurate and complete records of the results obtained.

Any relevant SOPs are listed in the appendix.

2. RESPONSIBLE PERSONNEL

a)	**Testing Facility**	MRC Harwell Mary Lyon Centre Harwell Science and Innovation Campus Oxfordshire OX11 0RD UK
b)	**Project Manager**	R Sonia Bains

3. INTRODUCTION

To breed cohorts of mice heterozygous from the stock Tmem233^{Cre} and to screen controls and mutants, male and female, through both an early adult and a late adult high-throughput phenotype pipeline.

4. EXPERIMENTAL ANIMALS

a) Background strain: C57BL/6J

b) Age range: 4 – 58 weeks

5. HOUSING AND HUSBANDRY

a) Husbandry and animal welfare
All procedures conducted are done so in accordance with the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (SI 4 2012/3039)

b) Type of cage
Tecniplast – 1284L and 1285L Individually Ventilated Cages (IVCs)

c) Bedding material
Datesand Aspen bedding autoclaved (134oC for 3 minutes) in cage base and cage bases changed weekly

d) Food/water
- Special Diet Services (Dietex) RM3 (E) irradiated to 2.5 Mrads
- Mains water through reverse osmosis (RO) filter and then chlorinated to 9-13 ppm. Bottles changed weekly with autoclaved bottle and cap.

e) Light/dark cycle
12 hours light: 12 hours dark
30 minute dusk to dawn, dawn to dusk period- except for Home Cage Analyser protocol

Lights off at 19:00 hours and on at 07:00 hours

f) Environmental enrichment

It is an important requirement to ensure that the mice have access to an enriched environment without comprising the scientific data collected. We comply with our MLC Policy on the Use of Environmental Enrichment.

g) Health checks

Daily

h) Number of cage companions

A maximum number of 3 animals per cage

Due to the Home Cage Analyser system protocol being optimised for 3 animals per cage (Bains RS, 2016).

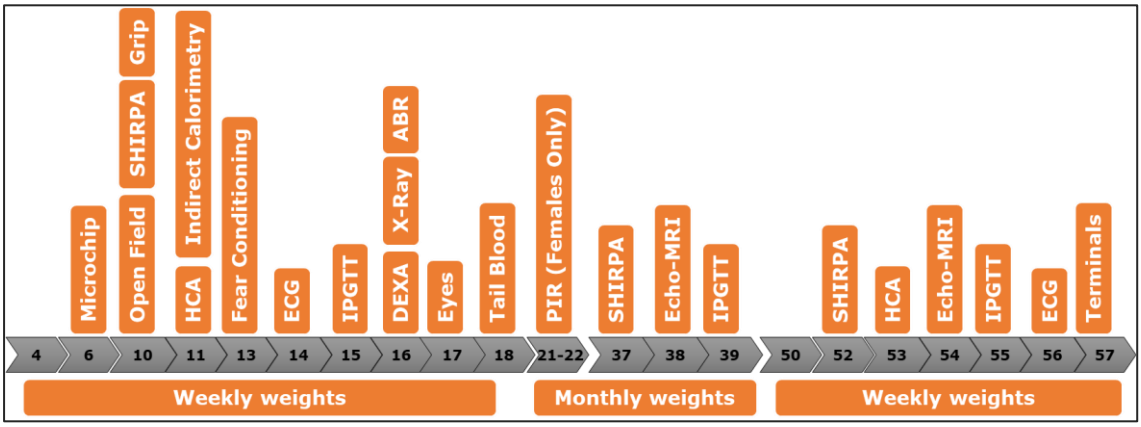
6. VIABILITY

From 16 matings, we obtained 54 female Tmem233^{Cre} hets, 23 female Tmem233^{Cre} homs, 30 Tmem233^{Cre} WT controls, 51 male Tmem233^{Cre} hets, 21 Tmem233^{Cre} homs and 45 Tmem233^{Cre} WT controls. 16 mice did not survive to weaning. These pups were not genotyped but Mendelian ratios at weaning suggest that they were a mixed genotype and did not die due to the genetic alteration.

7. SAMPLE SIZE

The sample size is based on analysis run on previous data and power analysis run on tests where the experimental unit is the home cage, e.g. HCA.

8. PIPELINE OF WORK



For SOPs refer to Appendix 1

9. ALLOCATING ANIMALS TO EXPERIMENTAL GROUPS

Mice were genotyped at 14 days of age and then randomised at weaning.

10. METHOD OF EUTHANASIA

As part of the terminal procedure, mice were exsanguinated under isoflurane anaesthesia and then a second confirmation of death was used, either by cervical dislocation or cessation of heartbeat.

11. UNSCHEDULED DEATHS

Mice that died before the end of the pipeline have all been noted in the file AnimalWeights_TMem233Cre.xlsx, in the sheet called 'Welfare comments'.

12. RETENTION OF RECORDS, SAMPLES AND SPECIMENS

All project specific raw data, documentation and Final Report generated from this project will be archived at the MLC for a period of 10 years.

13. RESULTS

Results from the quantitative data has been represented in graphical format below, all raw data will be shared as a part of this report as attachments.

Body Weights

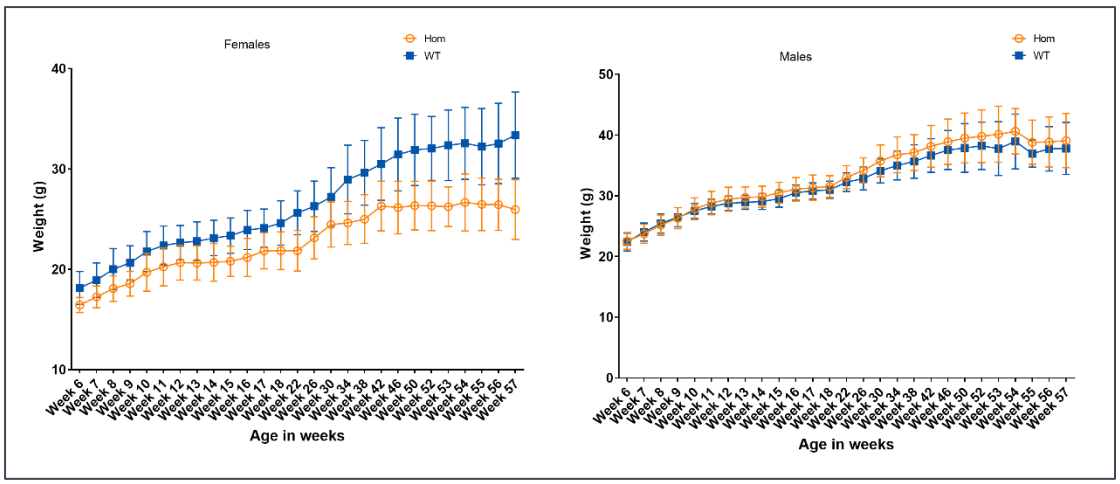


Figure1. Data shown are average body weight (g) per week split by sex and genotype, T bars represent the standard error of mean.

Open Field

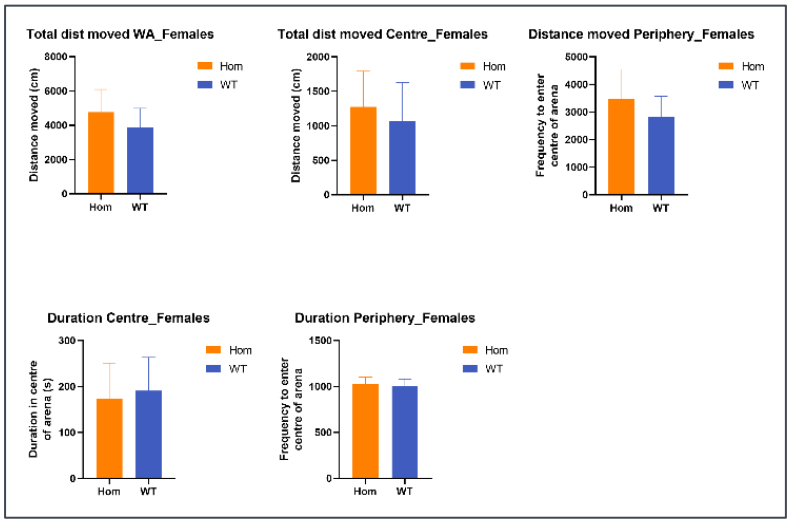


Figure2. Data shown are Open field parameters for females split by genotype, T bars represent the standard error of mean.

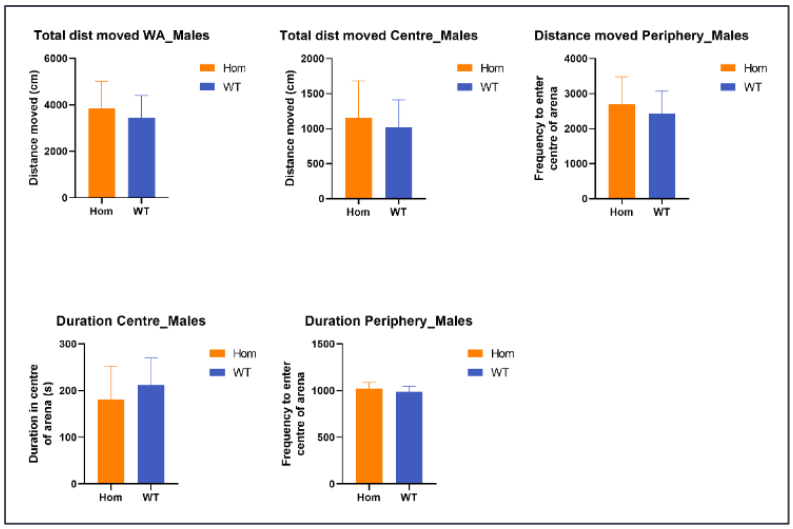


Figure3. Data shown are Open field parameters for males split by genotype, T bars represent the standard error of mean.

Grip

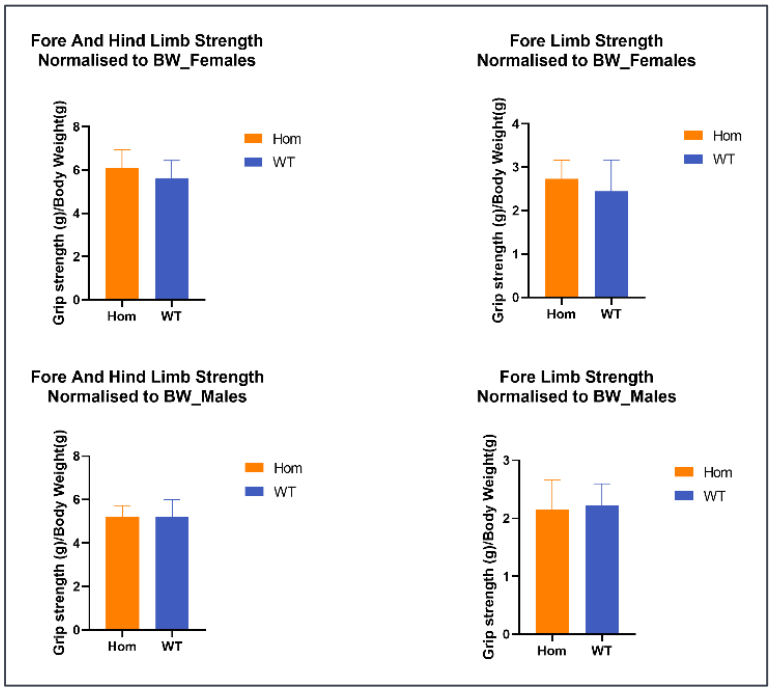


Figure4. Data shown are grip strength parameters split by sex and genotype, T bars represent the standard error of mean.

HCA

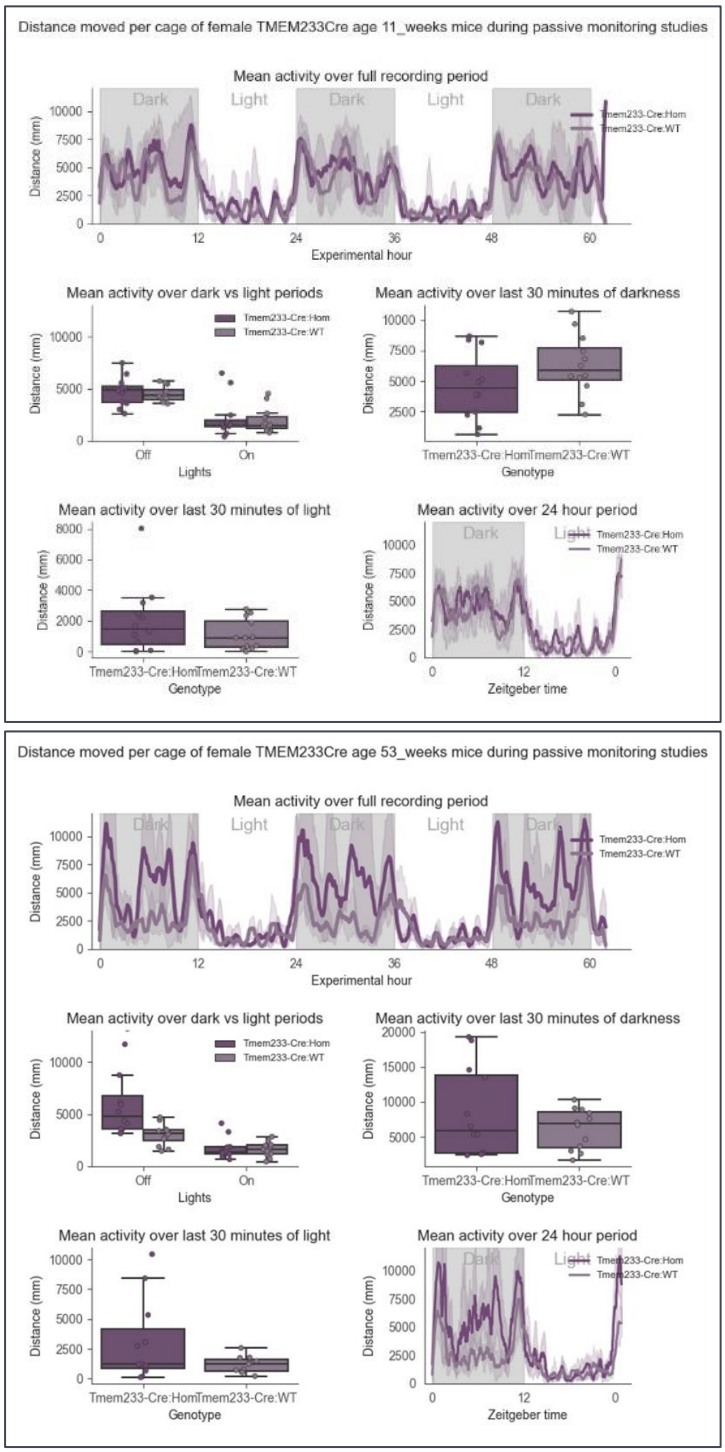


Figure 5. The figure panels represent the Distance in mm moved in Home Cage Analyser for Female Tmem233^{Cre} mice at 11 and 53 weeks of age.

Distance moved (mm) over experimental hour of recording session, binned into six minute time bins. Line represents mean distance over time within cages of a sex group, error shaded area represents 95% confidence interval. Data from three individual mice within a cage was summed to produce one time-series per cage. Shaded areas on plot represent periods of darkness.

Boxplot of mean distance moved within a cage per six minute time bin during periods of either light or dark, for one sex group and split by genotype. One data point represents one cage.

Boxplot of mean distance moved within a cage per six minute time bin during 30 minute period preceding dark phase, for one sex group. One data point represents one cage.

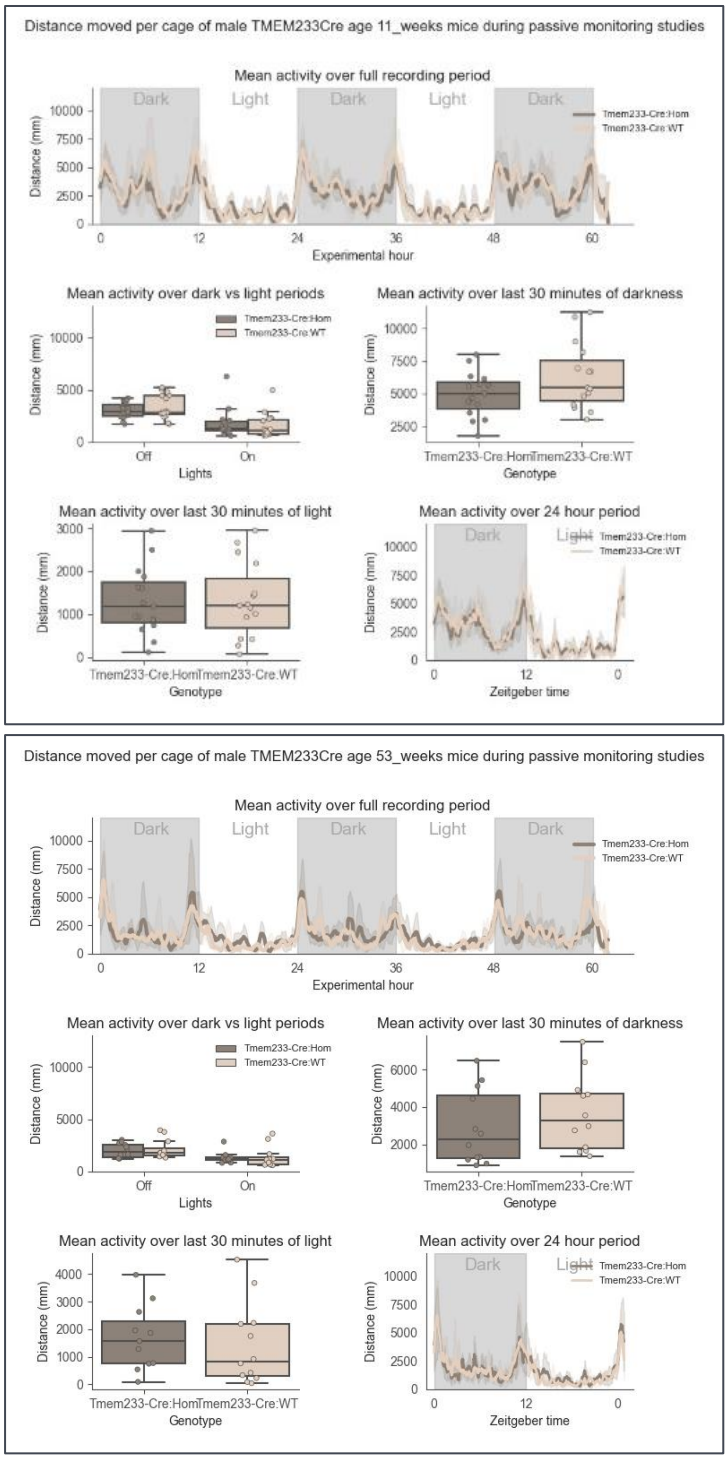


Figure6. The figure panels represent the Distance in mm moved in Home Cage Analyser for Male Tmem233^{Cre} mice at 11 and 53 weeks of age.

Distance moved (mm) over experimental hour of recording session, binned into six minute time bins. Line represents mean distance over time within cages of a sex group, error shaded area represents 95% confidence interval. Data from three individual mice within a cage was summed to produce one time-series per cage. Shaded areas on plot represent periods of darkness.

Boxplot of mean distance moved within a cage per six minute time bin during periods of either light or dark, for one sex group and split by genotype. One data point represents one cage.

Boxplot of mean distance moved within a cage per six minute time bin during 30 minute period preceding dark phase, for one sex group. One data point represents one cage.

Indirect Calorimetry

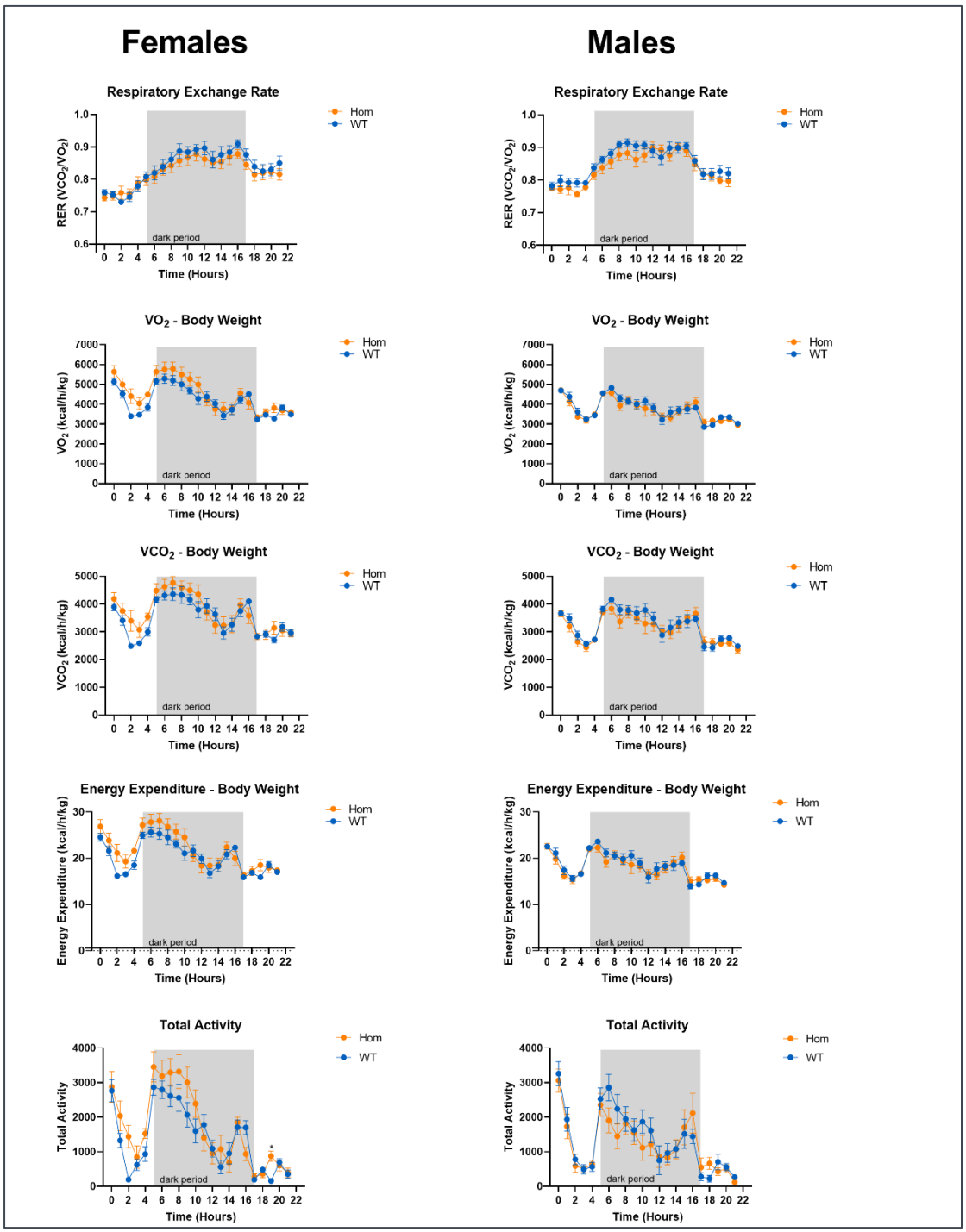


Figure7. Data shown in average/hour. Data were collected for 21 hours starting at 14:00 and finishing at 11:00 the following day. VO_2, VCO_2, and Energy Expenditure data is normalised to body weight. Data were analysed by repeated measures 2way ANOVA with Šídák correction. *$p<0.05$*.

Fear Conditioning

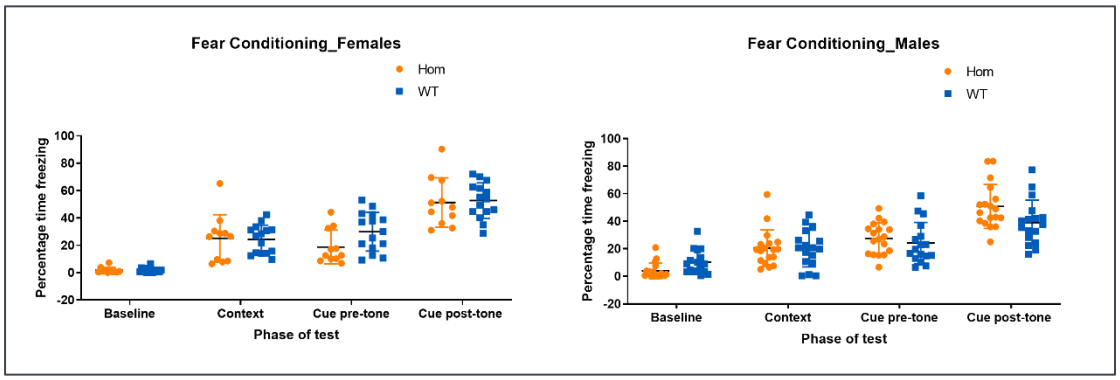


Figure8. Data shown are average percent time spent freezing split by sex and genotype, T bars represent the standard error of mean.

ECG

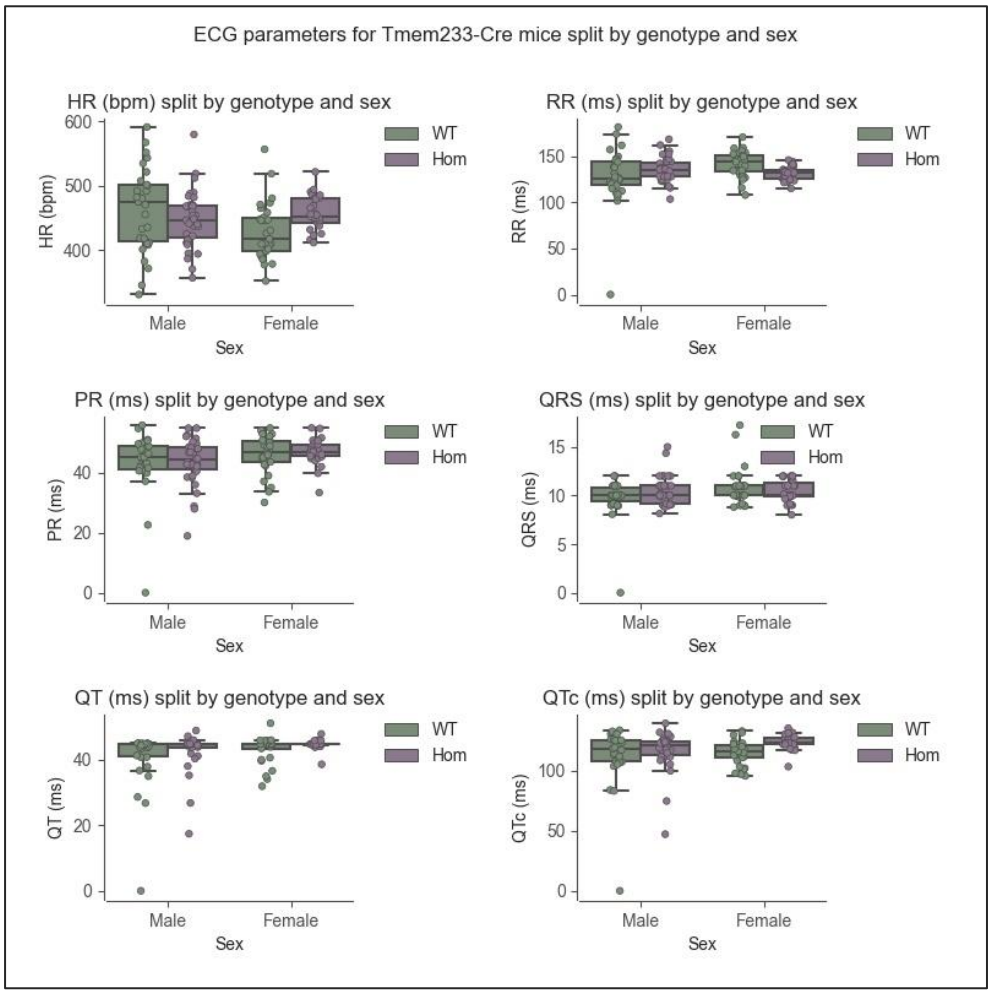


Figure9. Box Plots represent mean ECG parameter and the upper and lower quartile, whiskers represent 1.5 standard deviations from the mean.

IPGTT

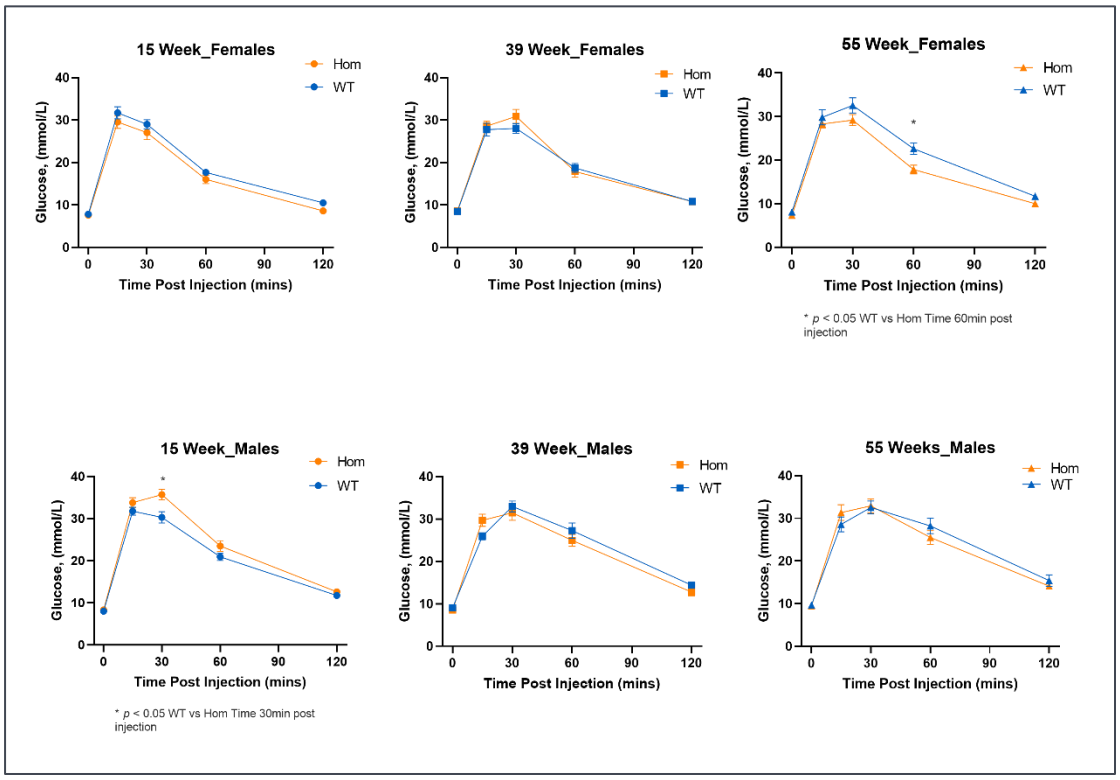


Figure9. Data shown are average per genotype per time point. Data analysed by repeated measures 2way ANOVA with Šídák correction. **p<0.05*.

ABR

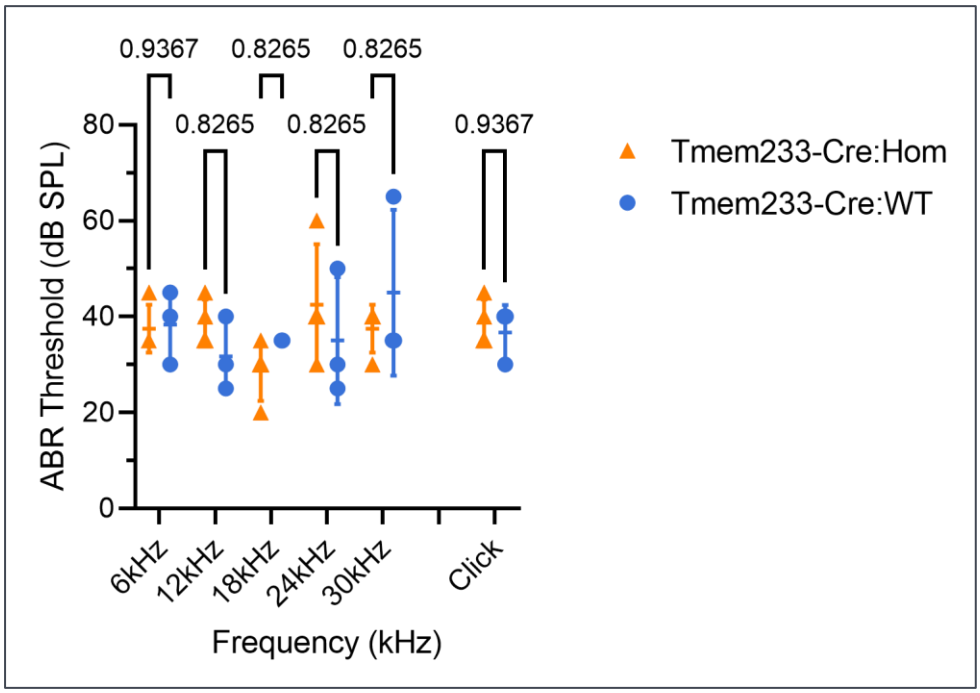


Figure10. X-axis represents the range of stimuli measured for each frequency, while the Y-axis represents the evoked frequency. Click covers a broad range of frequencies and is therefore represented as "unattached". The data in the graphs represents the mean ±SD. Data analysed by Two way ANOVA with Holm-Šídák's multiple comparisons test.

PIR

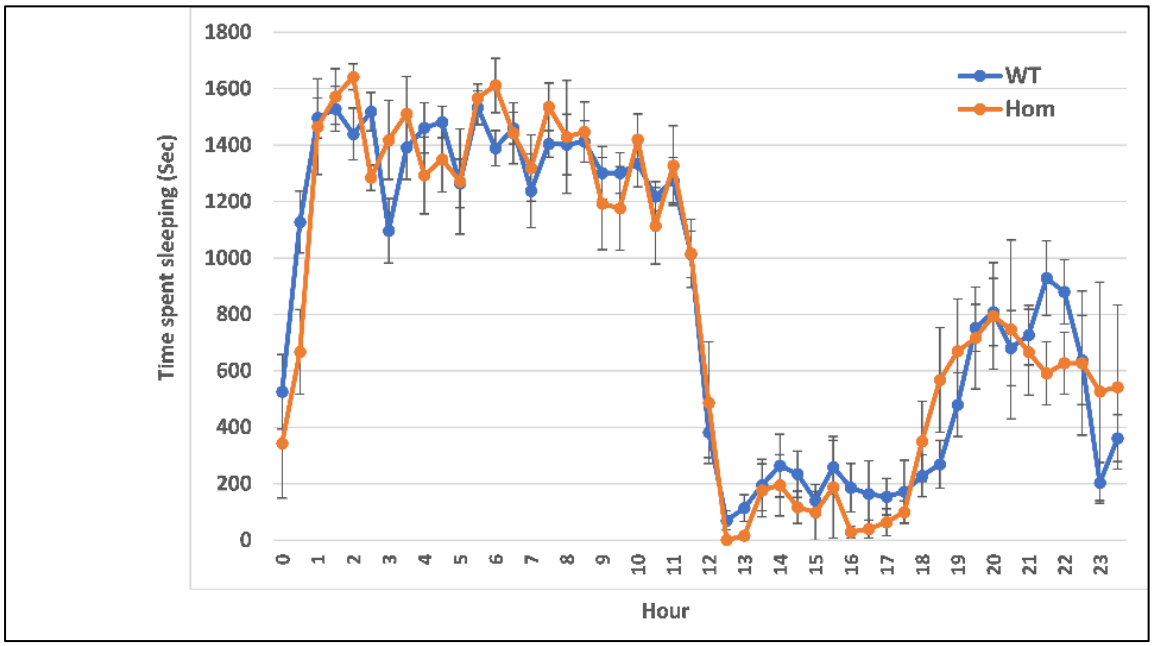


Figure11. Data shown are the average time spent sleeping (Sec) per hour in LD cycle.

Echo-MRI

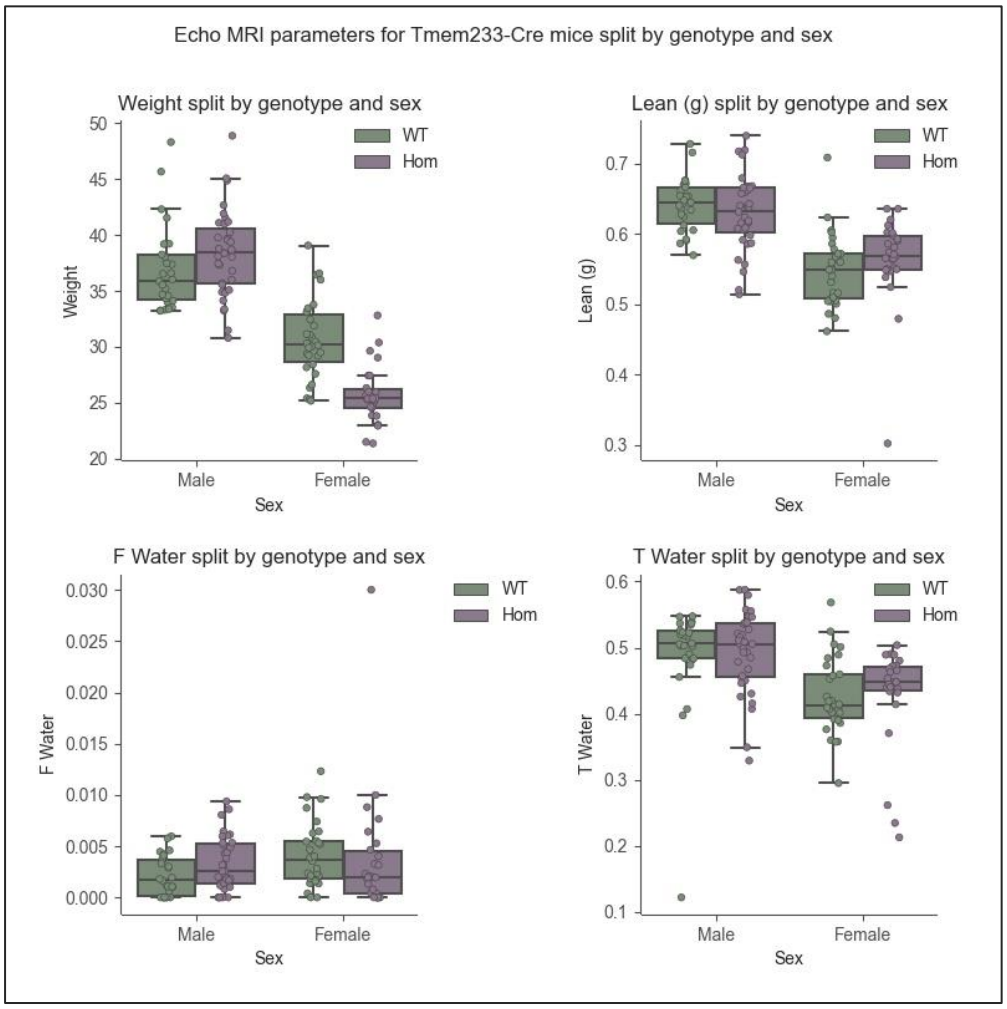


Figure12. Data shown are normalised against body weight. Box Plots represent mean Echo-MRI parameter and the upper and lower quartile, whiskers represent 1.5 standard deviations from the mean.

14. DEVIATIONS

PIR test for Cohort 3 was delayed from week 21 to week 25 due for operational needs arising from the pandemic.

15. REPORT APPROVAL

This report and the data it contains have been reviewed and approved for release by the Phenotyping Manager.

Name: Sara Wells

Signature:

Date: 26/05/2022

16. APPENDICES

Methods:

Weights

The mice were weighed using a dynamic weighing balance (Ohaus) and were always weighed in the morning to limit the effect of the circadian rhythm on body weight.

Microchipping

RFID microchips were injected subcutaneously into the lower left or right quadrant of the abdomen of each mouse at 6 weeks of age. These microchips were contained in standard ISO biocompatible glass capsule (11.5x2mm, PeddyMark Ltd. UK). The procedure was performed on sedated mice (Isoflo, Abbott, UK) after topical application of local anaesthetic cream on the injection site prior to the procedure (EMLA Cream 5%, AstraZeneca, UK). The entry wound was sealed using a topical tissue adhesive, GLUture (Zoetis). The protocol is fully described in https://doi.org/10.1002/cpmo.80

Open field

The open field test is used to assess anxiety and exploratory behaviour. Mice were placed in well-lit arenas (150-200 lux, 44 x 44 cm) for 20minutes. The activity of the mice in the centre zone (40% of the total area), periphery (area 8 cm towards the centre from the wall) and whole arena was captured in 5 minute bins.

Equipment details: Omnitech Electronics Inc. SuperFlex

SHIRPA

The SHIRPA test identifies physical and behavioural abnormalities through observation. Mice were individually observed in a series of environments to test a range of attributes including hearing, visual placement, activity, motor coordination, righting ability as well as morphological features. The protocol is fully described on IMPRESS (www.mousephenotype.org/impress).

Grip strength

Grip strength was measured using a grip strength meter (Bioseb, France), recording the maximum force generated by a mouse using just its forelimbs or all four limbs. Grip strength measures were carried out in triplicate for each mouse and for both groups of limbs. More details available at IMPRESS (www.mousephenotype.org/impress).

Indirect Calorimetry

The metabolic rate of the mice was assessed using indirect calorimetry. Mice were individually housed overnight for a period of 21 hours in Phenomaster cages (TSE Systems, Germany) with standard bedding and igloos. The air content in each cage was sampled for 1 minute, inturn and a reference cage with no mice in it was also sampled for comparison. The oxygen consumption and carbon dioxide production for each mouse was then determined as the difference between the reference amount of gas and the level in the mouse cage. VO2 and VCO2 are calculated based on the flow rate of air (litres per minute), the percentage change in gas mixture (dO2 or dCO2) and accounts for the temperature in each chamber as well. VO2[1], VCO2[1] and Heat[1] are values normalised to body weight, VO2[2], VCO2[2] and Heat[2] are values normalised to an expected lean mass of 0.75 x body weight and VO2[3], VCO2[3] and Heat[3] are values that are not normalised. These last values are usually more pertinent and can be normalised to the lean mass as measured in Echo-MRI. RER is defined as VCO2/VO2.

The data is represented as relative to a 0 hour time point which is 7pm when the lights in the centre go off. The plots are average line plots for each experimental group. 0 – 12 represents the night-time period and the period of highest metabolic activity.

For the activity values, 'A' represents ambulatory movement and 'F' represents fine movement. These are determined by the program depending on how the mouse breaks consecutive beams. The directions are given in the X and Y axis with the Z axis equating to rearing. The activity is also split into zones – Central (Cen) and Periphery (Per). Activity measurements are continuous for the time bin and are not measurements per minute.

Fear conditioning

Fear conditioning assesses the memory of an aversive experience and determines if it is based on the cue, the context or both {Crawley, 1999 #3370}. Mice were placed into square chambers (17 cm^2) on day one and allowed to explore for 120 s during which the amount of freezing behaviour was measured (baseline). An auditory stimulus (70 dB) was presented for 20 s and was followed by one footshock (0.5 mA, 0.5 s). After a total of 261 s, the mouse was returned to its home cage. On day 2, the mice were placed into the same chambers and the amount of freezing behaviour was measured over a 5 min period (context). Four hours later, the mice were placed into circular chambers (20 cm in diameter) with additional vanillin essence to reinforce the novel environment setting. Freezing in the new arena was measured for 120 s (pre-cue), after which the auditory stimulus was presented alone and again the amount of freezing behaviour was measured for another 120 s (post-cue). The comparison of baseline v context or pre-cue v post-cue percentage freezing behaviour was used to evaluate associative memory to context or cue respectively. More details available at IMPRESS (www.mousephenotype.org/impress).

Electrocardiogram

The cardiac phenotype of the mice was assessed using electrocardiogram (ECG). Mice were anaesthetised under isoflurane and the ECG was recorded using BioAmp

(AD Instruments, Australia) and LabChart Pro software (AD Instruments). More details available at IMPRESS (www.mousephenotype.org/impress).

Glucose tolerance test

Mice were fasted overnight for 18 h. A sample of blood was analysed from the tail, to determine the fasted blood glucose concentration using the Accu-Chek glucose meter (Abbott, UK). The mice were injected intraperitoneally with 20% glucose solution (2 g glucose/kg body weight). Blood glucose measurements were taken again at 15, 30, 60 and 120 min after injection of glucose. More details available at IMPRESS (www.mousephenotype.org/impress).

DEXA

The body composition of the mice was assessed using the Faxitron Ultrafocus machine (GE Medical Systems, USA). Mice were anaesthetised with ketamine/xylazine or with isoflurane. High energy X-Ray images were automatically analysed for fat tissue content, lean tissue content, bone mineral density and bone mineral content. More details available at IMPRESS (www.mousephenotype.org/impress). If mice were anaesthetised with ketamine and xylazine, the mice also underwent the auditory brain stem response test (see below). Mice anaesthetised with isoflurane also had X-Ray images captured.

X-Ray Annotations

X-ray images of the mice were collected whilst the mice were anaesthetised (isoflurane) and done under the same anaesthetic regime as for DEXA. The X-rays were captured using the Faxitron Ultrafocus X-Ray machine (US). A lateral view, a dorsal-ventral view and a skull image were all taken to enable a full qualitative assessment of the integrity of the skeleton (www.mousephenotype.org/impress). A 2cm lead bar was positioned to provide a calibration scale for the measurement of the tibia length, achieved using the ImageJ program.

Auditory brain stem response

To determine their hearing range, mice were anaesthetised with ketamine/xylazine and the auditory brain stem response was recorded in response to either a click sound or to tones at 6 kHz, 12 kHz, 18 kHz, 24 kHz and 30 kHz, using subdermal electrodes placed on the vertex and the left and right bulla. Intensity of the sounds was increased from 0 dB to 85dB sound pressure level (SPL) and the threshold of the response was determined as the lowest sound intensity that gave a recognisable ABR waveform response (Parker A et al., 2015). More details available at IMPRESS (www.mousephenotype.org/impress).

Opthalmoscopy

Eye morphology and visual response of the mice was assessed using a slit lamp and an opthalmascope. Tropicamide was used to dilate the pupils and observations were manually scored for morphological or response abnormalities. More details available at IMPRESS (www.mousephenotype.org/impress).

Echo-MRI

Test performed at 38 and 54 weeks on all carrier and WT mice in the behavioural pipeline. The Echo-MRI equipment measures the relative fat and lean mass components of the body composition. Due to insertion of the microchip artificially

increasing the fat mas of the mouse as a result of fatty fibrous tissue surrounding the microchip, only the lean mass is included in this report.

HCA

Mouse home cages were placed individually into one of the Home Cage Analyzer rigs (HCA; Actual Analytics, Edinburgh) on Day 0 of the study time point and left undisturbed for 72 hours. The HCA rigs comprising of high-definition infra-red camera and a baseplate with RfiD antennae on known locations, occupied two spaces on a standard IVC rack. Where one half of the rig housed the camera and the electronics, while the other half housed the mouse home cage with the RfiD baseplate, forming the floor of the rig on which the home cage was placed. The data were recorded continuously in 30 minute segments the roof of the rig housed the infra-red light source. On day3 of the test the recording stopped automatically at the end of 72 hours and video data were stored as .flv files and the baseplate data in HDF5 (hierarchical data format 5) format. The home cages were removed from the HCA rig at the end of the testing period and returned to a standard IVC rack. The data were analysed using the home cage as the experimental unit. The protocol is fully described in https://doi.org/10.1002/cpmo.80

PIR

Mice were individual placed into open top cages with the area under the hopper blocked from access. The cages were placed under passive infrared cameras to record activity. The cameras and cages were housed inside cabinets with individual light controls. The first five days of the experiment were carried out under 12:12 Light:Dark cycle, followed by 14 days of continuous darkness. The activity of the mice was recorded by the passive infrared camera, 40 consecutive seconds of inactivity were defined as sleep. More details are available at https://dx.doi.org/10.12688%2Fwellcomeopenres.9892.2

Terminal Blood collection

Mice were anaesthetised with isoflurane and blood collected under anaesthesia from the retro-orbital sinus into either Lithium-Heparin coated tubes or into EDTA-coated tubes.

Clinical chemistry

Lithium heparin samples from both free-fed and fasted mice were kept on wet ice and centrifuged within 1 h of collection for 10 min at 5,000 x g in a refrigerated centrifuge set to 8°C. The resulting plasma samples were frozen until analysis. Clinical chemistry of free-fed plasma was analysed with a Beckman Coulter AU680 clinical chemistry analyser using reagents and settings recommended by the manufacturer. Insulin, glucagon, leptin and adiponectin were measured in plasma from fasted mice and glucose and triglycerides were measured in plasma from both fasted and free-fed mice using ELISA kits from Millipore (EZRMI-13K), Mercodia (10-1281-01), Biovendor (RD291001200R) and R&D Systems (MRP300) respectively. More details on Clinical Chemistry analysis are available at IMPRESS (www.mousephenotype.org/impress).

Haematology

A full blood count and differential analysis of whole blood samples collected from free-fed mice (cohort 1) in EDTA-coated tubes were performed with a Siemens Advia 2120 analyser using reagents and settings recommended by the manufacturer. More details available at IMPRESS (www.mousephenotype.org/impress).