

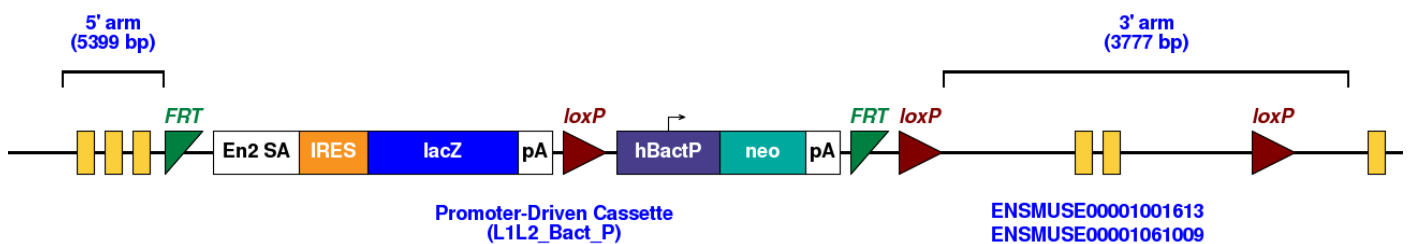
Knockout mouse lines presenting with welfare issues affecting their survival (abnormal survival [MP:0010769]) are processed through a bespoke sub-pipeline known as the “sick mouse procedure” (SMP) to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify phenotypic abnormalities arising from the targeted allele.

Oxr1^{tm1a(EUCOMM)Wtsi}

oxidation resistance 1

Genetic Background: C57BL/6N;C57BL/6NTac

ES Cell Clone: EPD0600_1_B09



Affected genotypes

Homozygous (*Oxr1*^{tm1a(EUCOMM)Wtsi/ tm1a(EUCOMM)Wtsi}).

Alternative breeding strategy

Following initial welfare observations, a wild-type x heterozygous mating strategy was employed to complete phenotyping work in standard pipeline using heterozygous mice only.

Heterozygous mice have been phenotyped on the primary screen.

Welfare observations

Homozygous mice exhibit:

- Uncoordinated movements (female 100% n=4/4, male 66.7% n=8/12 vs. WT female 0% n=0/94, male 0% n=0/74)
- Tremors (6/13)
- Loss of ability to right (2/13)
- Seizures whilst under anaesthesia (2/13)

Homozygous Viability:

All genotyped mice from het x het intercross considered. When 28 mice are available, viability is calculated. [$> 13\%$ = *Homozygous viable*; $>0\%$ and $<13\%$ = *Sub-viable*; 0% = *Lethal*]

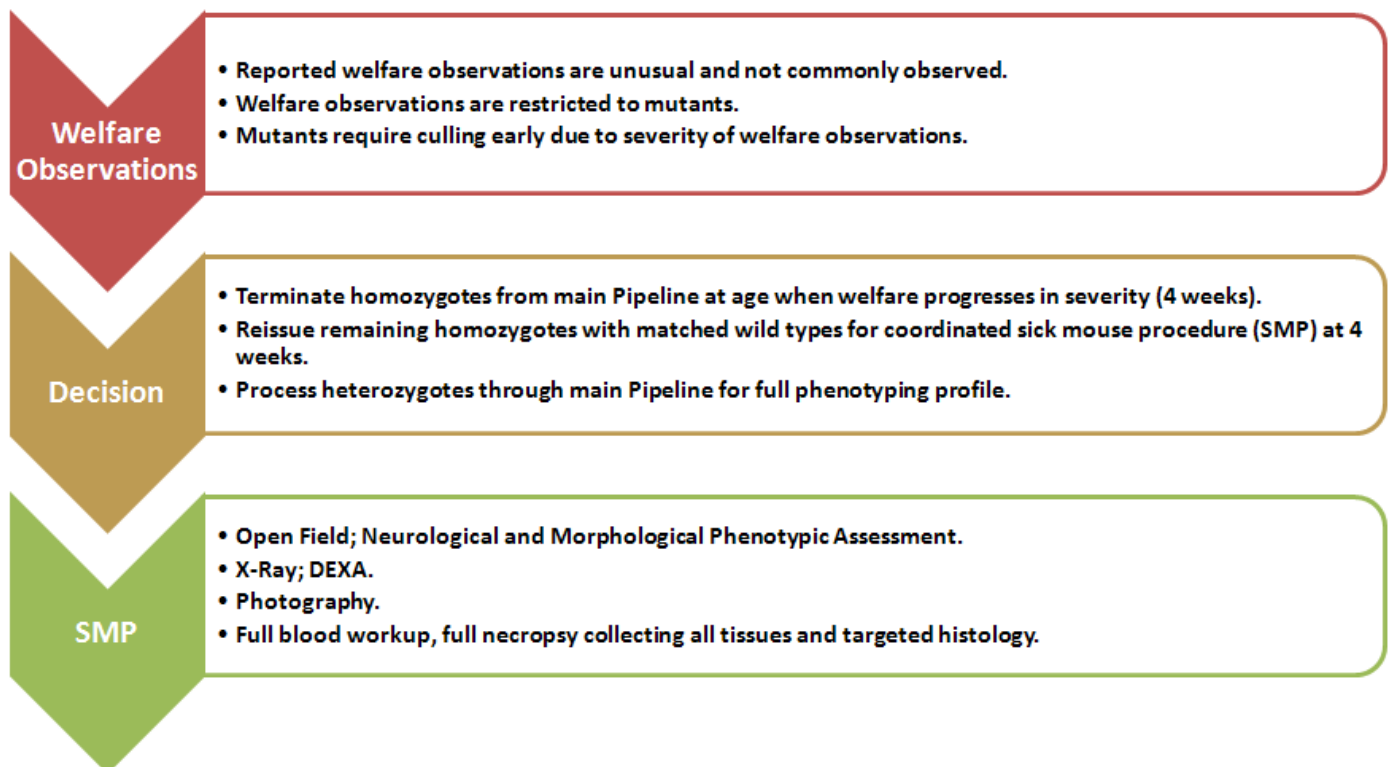
- **Viable** : 15Homs / 61 Total = 24.6%

Sick Mouse Procedure (SMP)

Initial welfare observations were reported when the first homozygotes were born during the breeding and expansion stage. Homozygotes were still viable when issued to the phenotyping pipelines (4 weeks) but severity progressed rapidly.

Welfare observations in homozygotes described above progressed to moderate severity upon which SMP (see schematic below) was initiated on mice between 3 and 4 weeks old. 9 male and 4 female homozygotes were processed alongside 9 male and 4 female matched wild-types. No further homozygotes were phenotyped due to the aforementioned alternative breeding strategy employed to reduce further welfare implications.

Schematic Outline of Bespoke SMP Pipeline



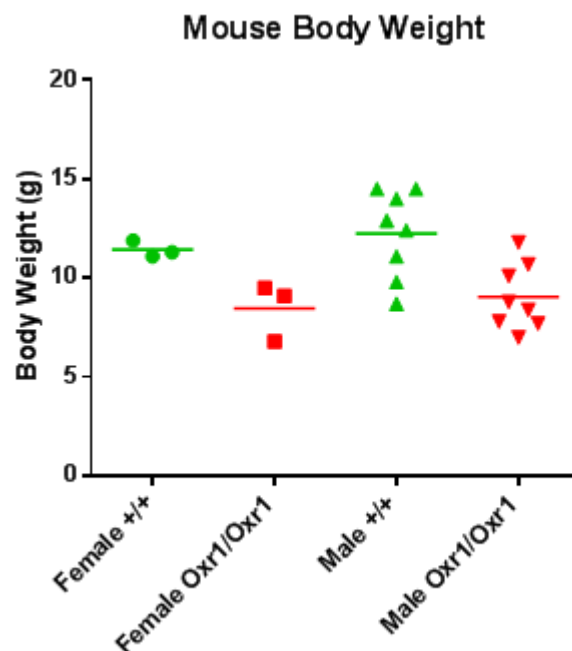
Phenotyping Heat Map

Colony Prefix	Allele Name	Genotype	Weight Curves	Open Field	NaMPA	Body Composition (DEXA)	X-ray Imaging	Plasma Chemistry	Haematology (CBC)	Peripheral Blood Leukocyte	Tissue Biobank
MEBW	Oxr1<tm1a(EUCOMM)Wtsi>	Homozygous									

- Significant / Interesting
- Not significant
- Resources available

Phenotyping data of interest (significant changes)

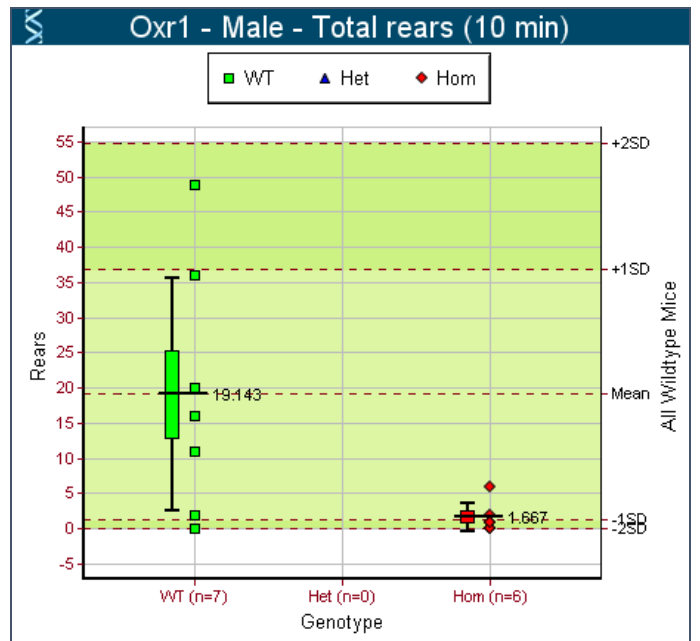
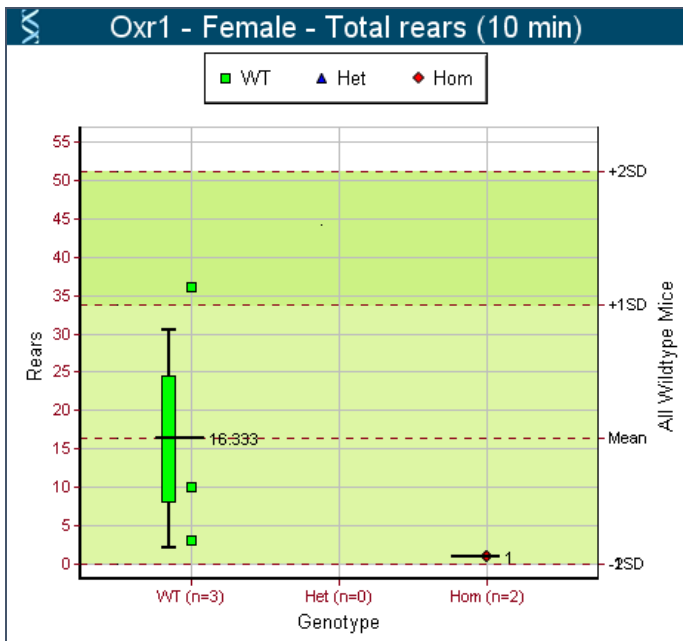
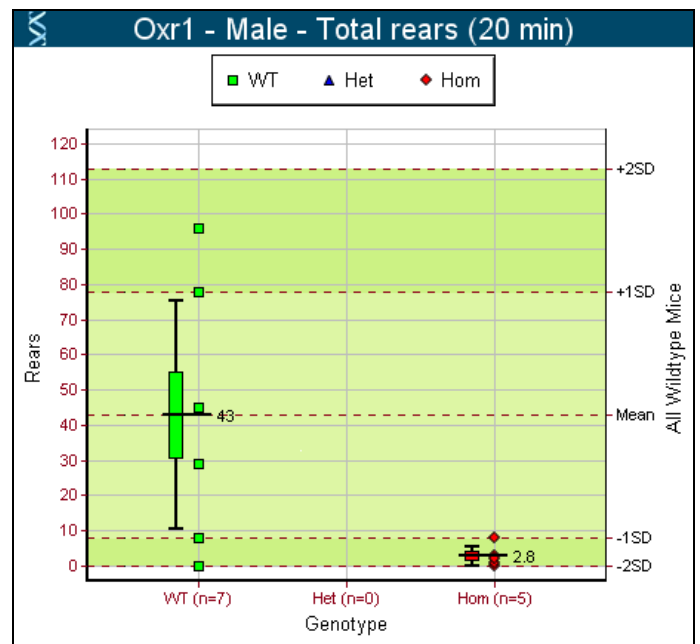
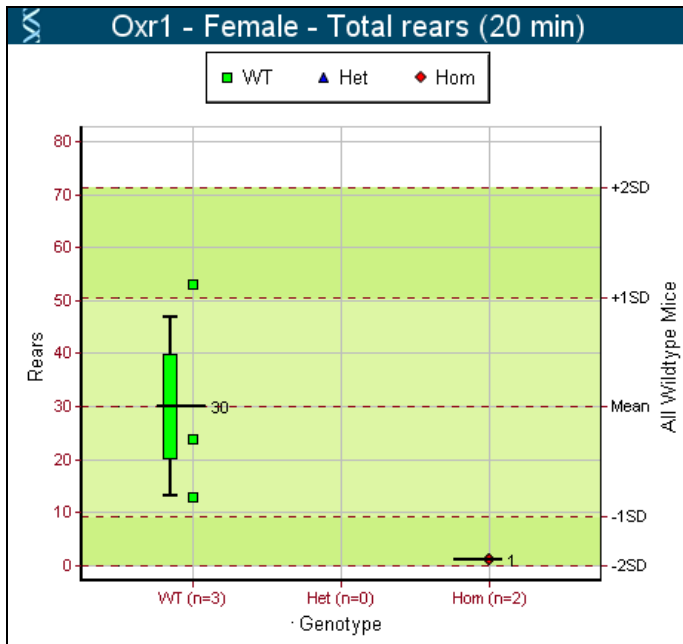
In life phenotyping



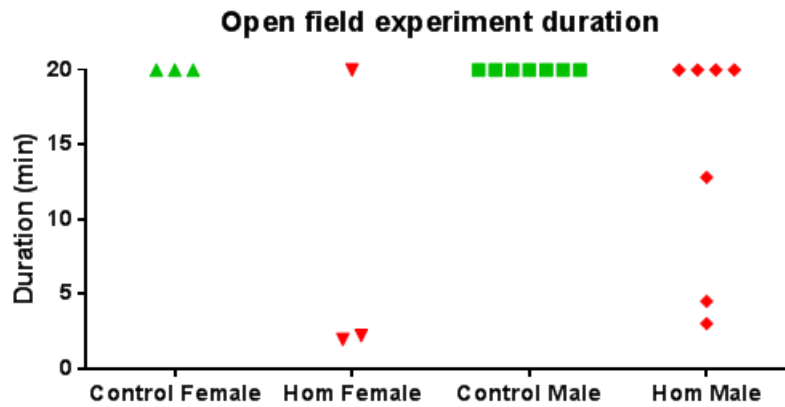
Body weight was collected between 3.2 and 3.6 weeks of age.

Males and Females – Decreased Body Weight [MP:0001262]

Open Field



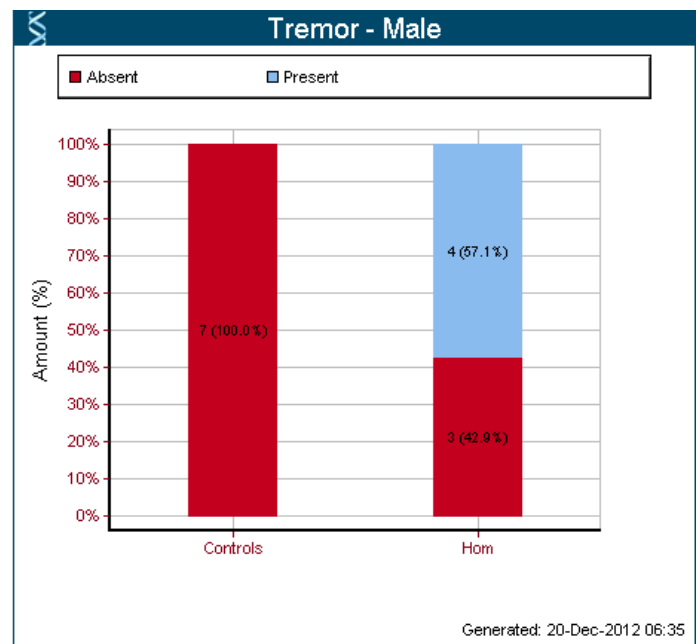
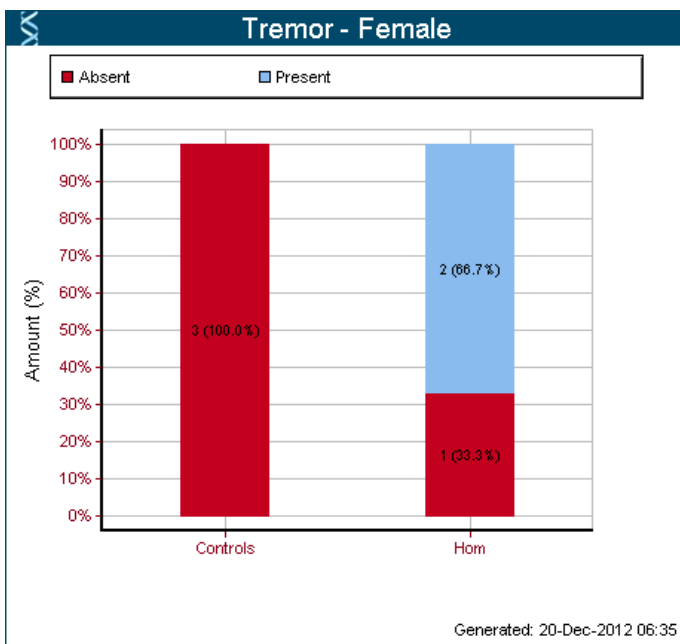
Males and Females - decreased vertical activity [MP:0002757]



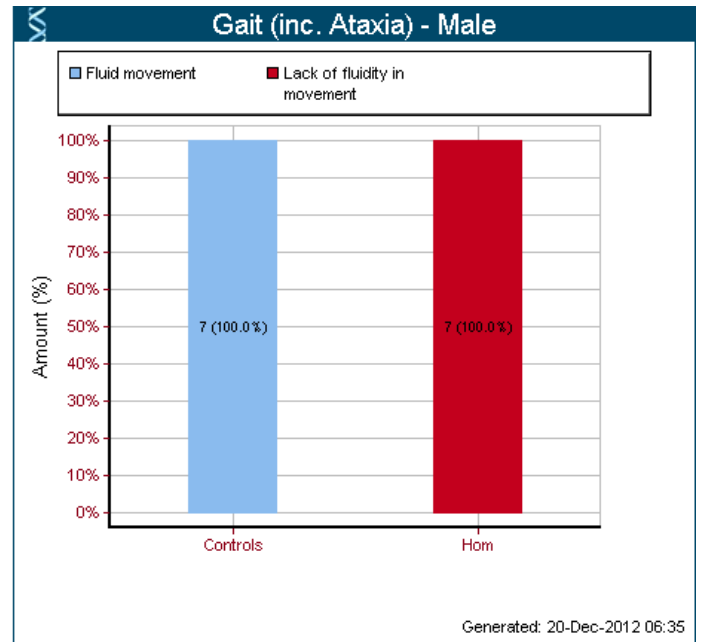
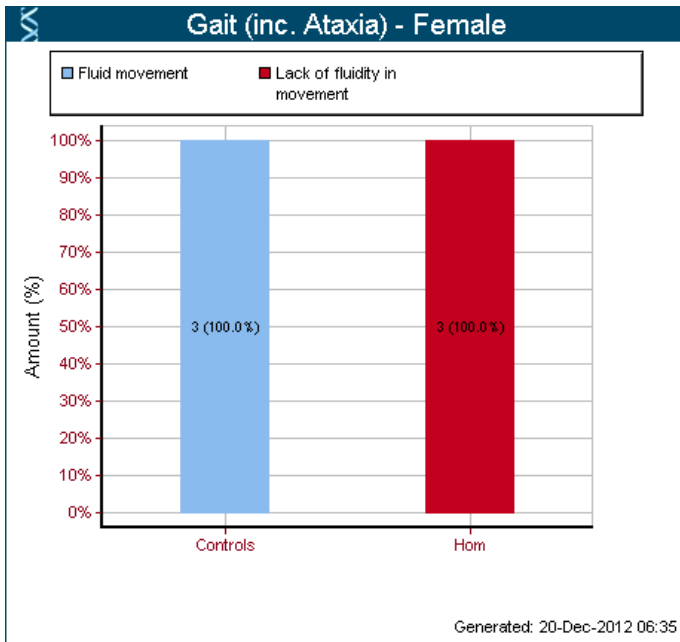
3 hom males and 2 hom females were removed from the OF arena prior to the 20 minute test period due to welfare issues.

Males and Females - Abnormal motor coordination/balance [MP:0001516]

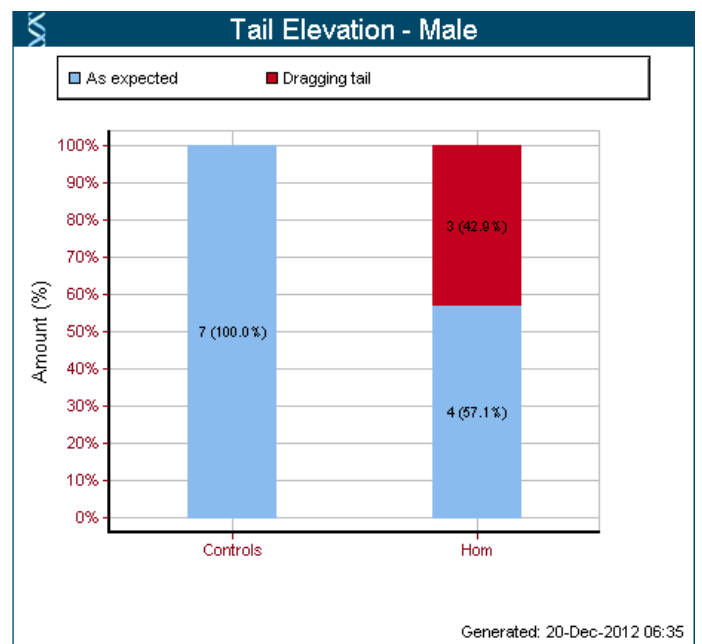
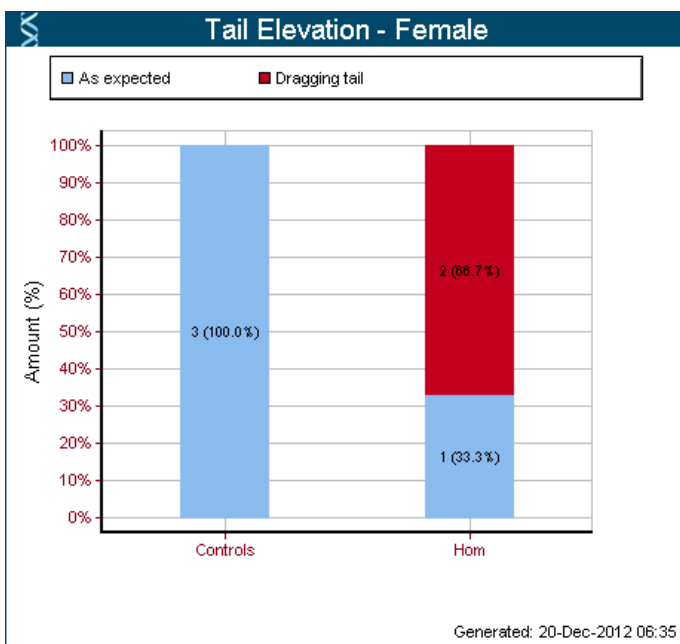
Modified SHIRPA



Males and Females - Tremors [MP:0000745]

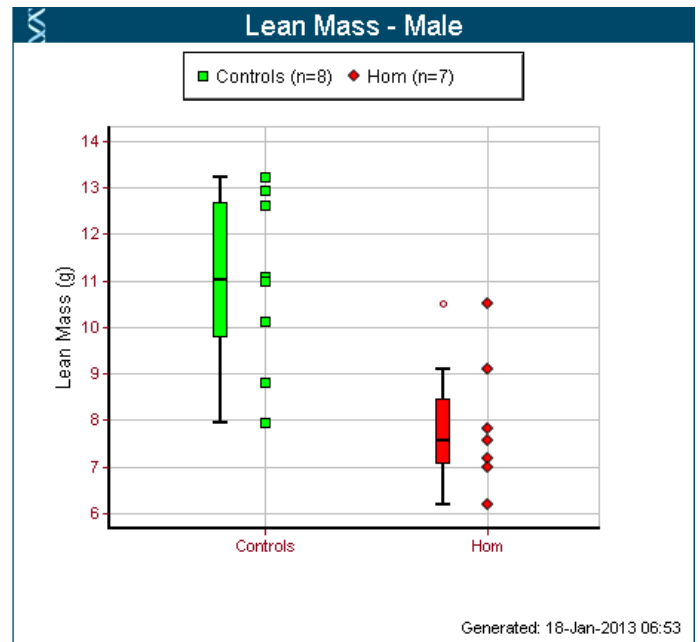
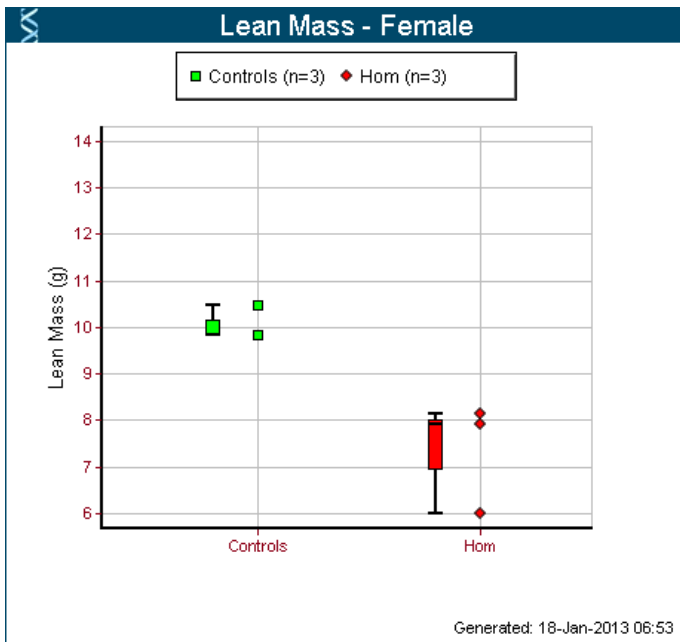


Males and Females - Abnormal gait [MP:0001406]



Males and Females - Tail dragging [MP:0005507]

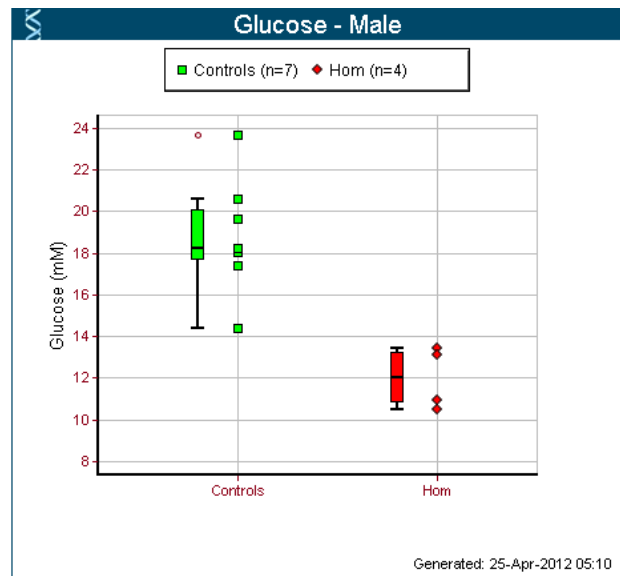
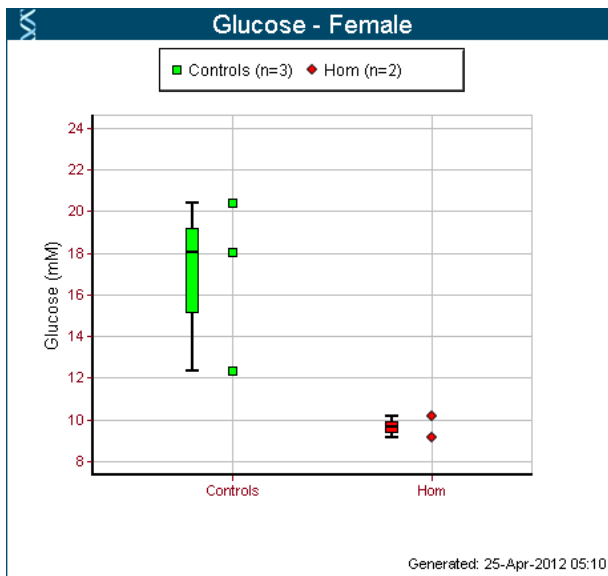
Body Composition (DEXA)



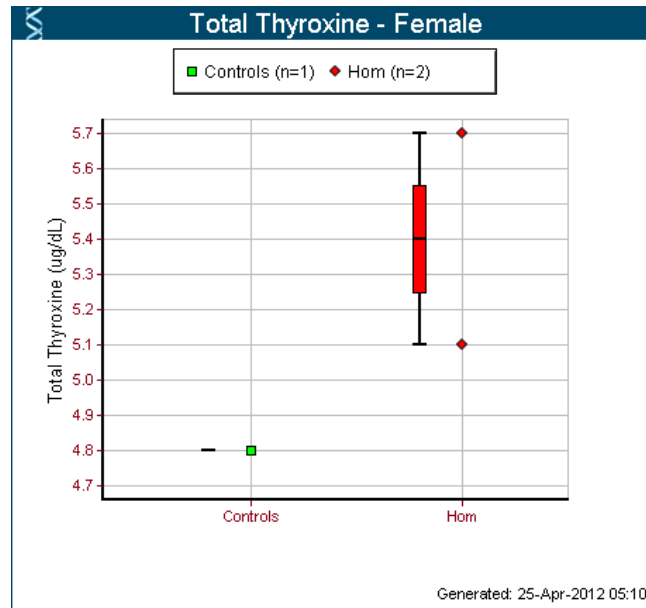
Males and Females – Decreased Lean Body Mass [MP:0003961]

Ex vivo phenotyping

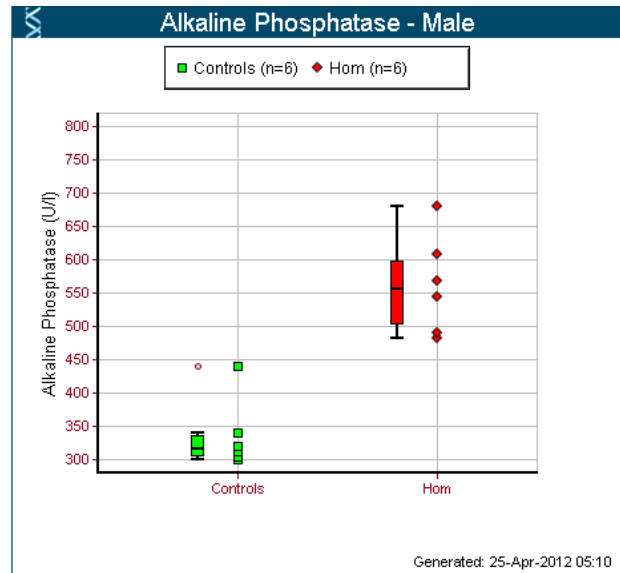
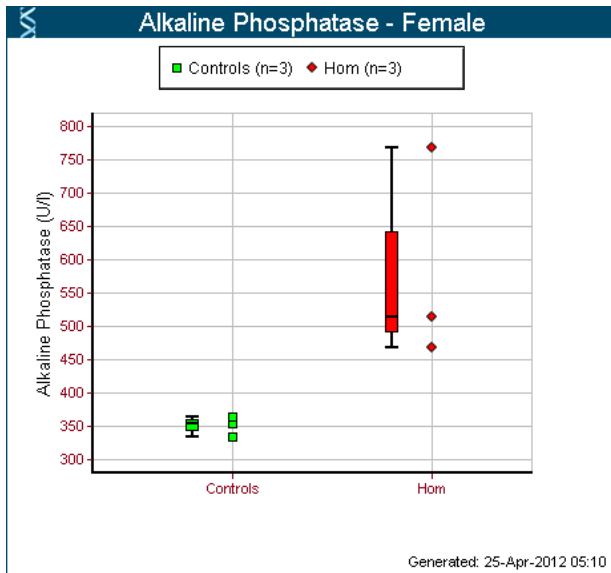
Plasma Chemistry



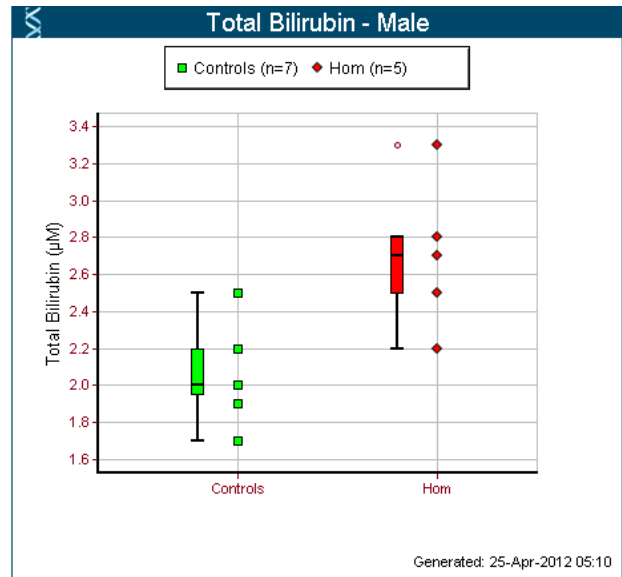
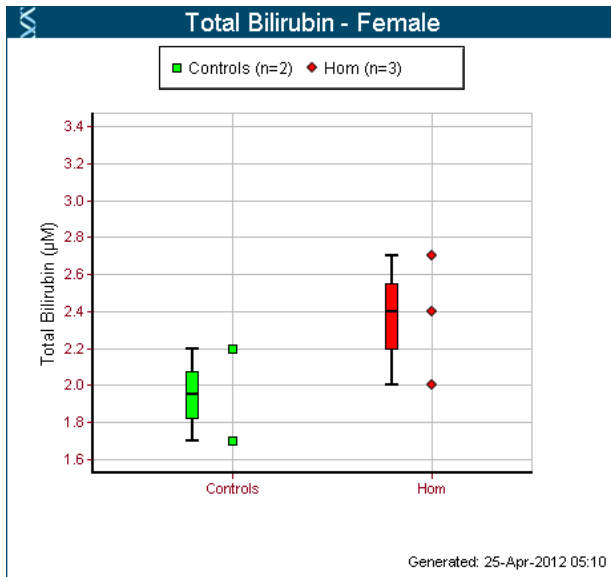
Males and Females - Decreased circulating glucose level [MP:0005560]



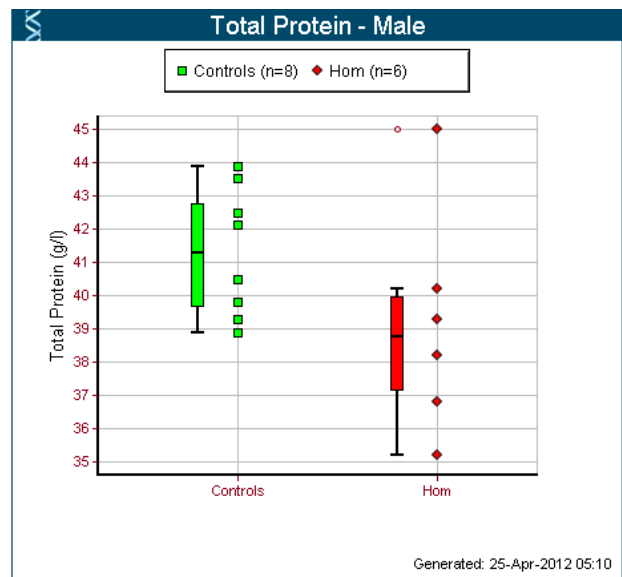
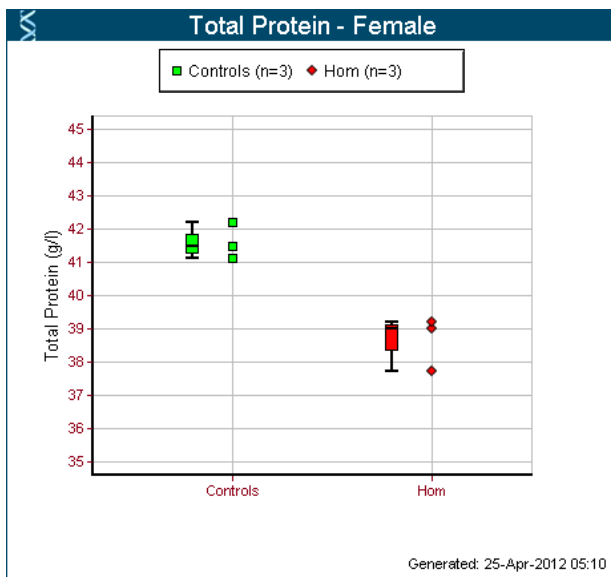
Females - Increased circulating thyroxine level [MP:0005477]



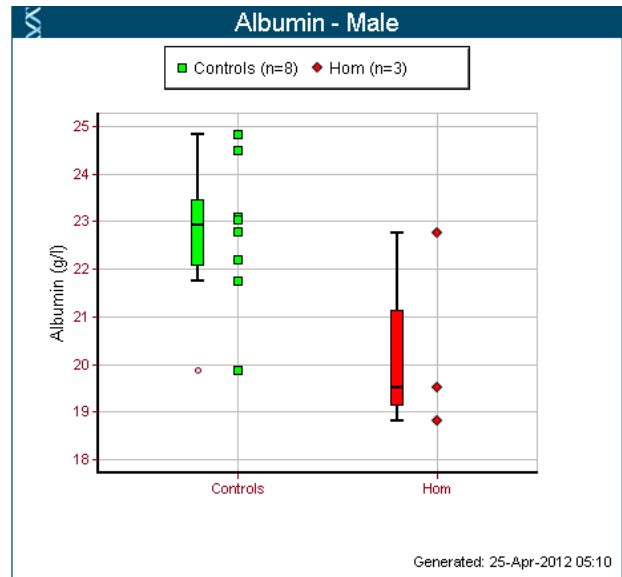
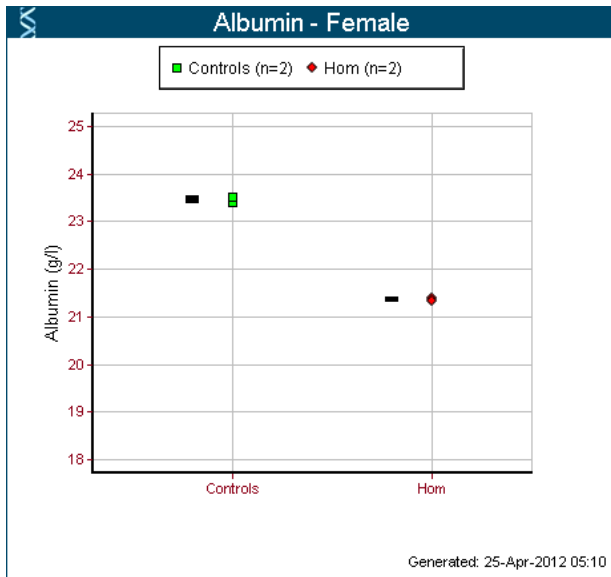
Males and Females - Increased circulating alkaline phosphatase level [MP:0002968]



Males and Females - Increased circulating bilirubin level [MP:0005344]

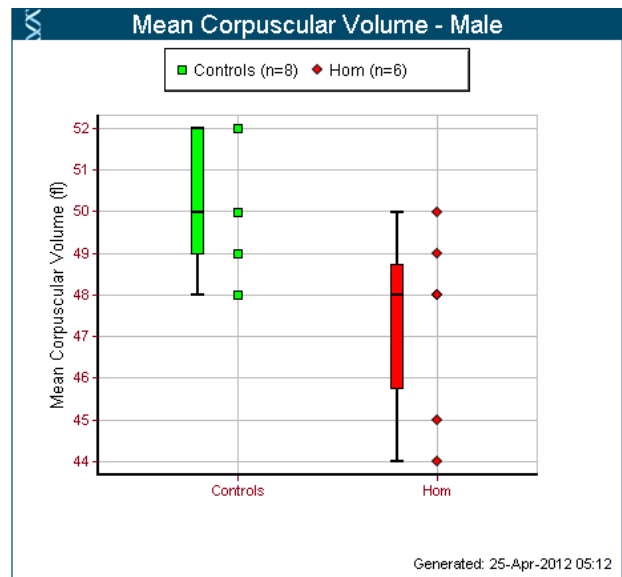
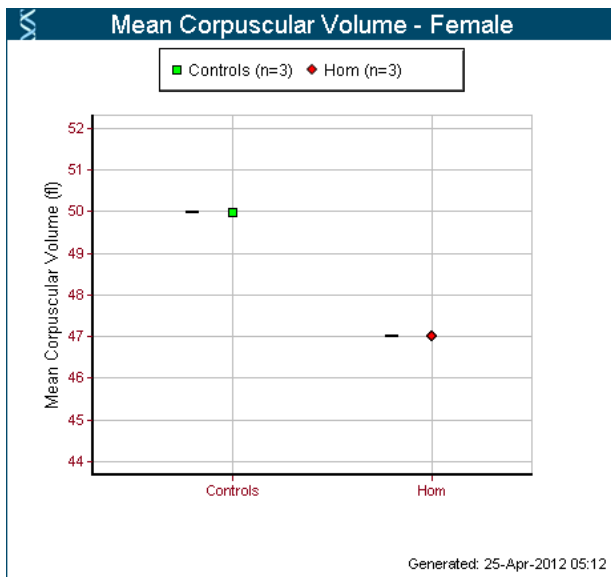


Males and Females - Decreased circulating total protein level [MP:0005567]

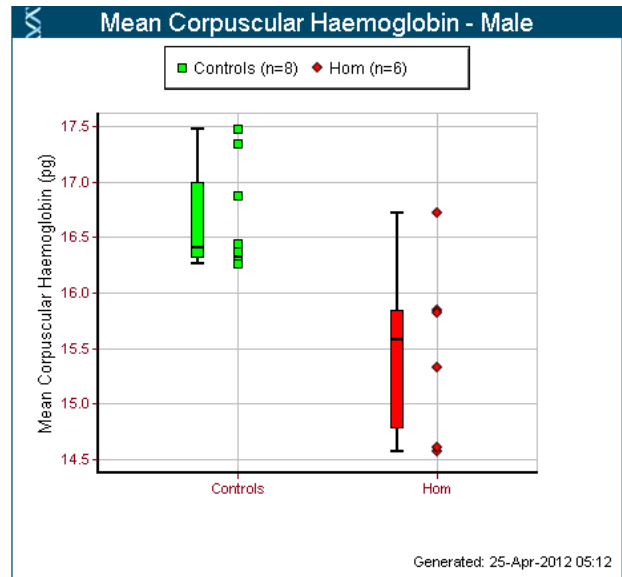
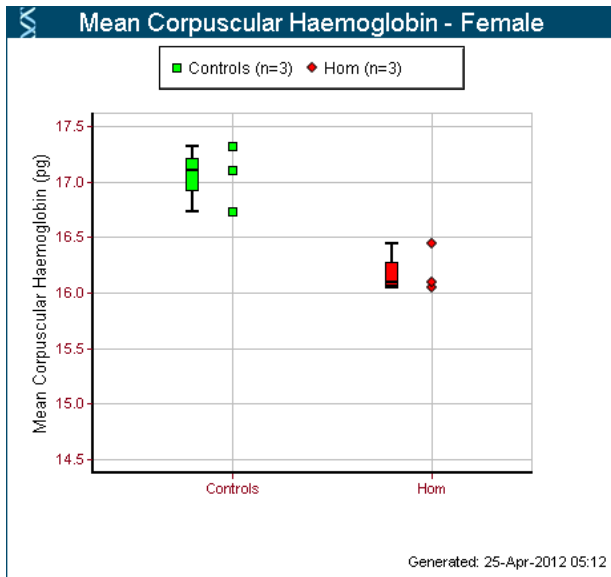


Males and Females - Hypoalbuminemia [MP:0005419]

Haematology

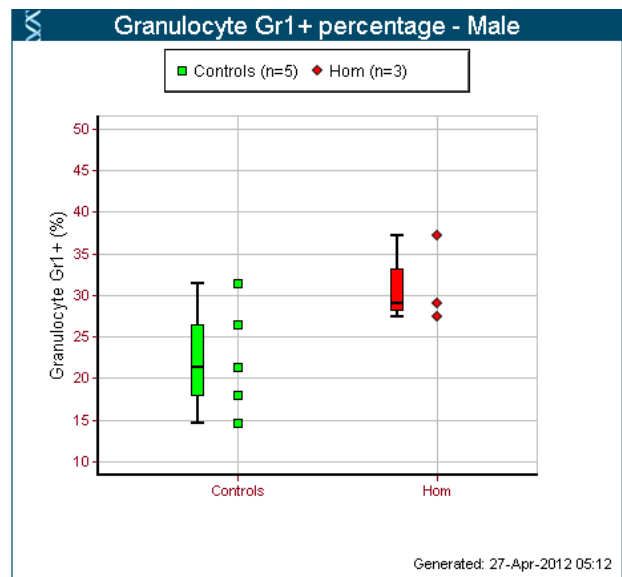
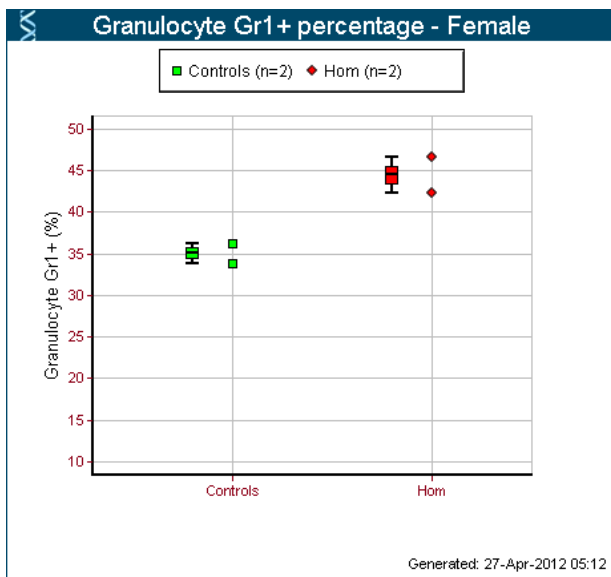


Males and Females - Decreased mean corpuscular volume [MP:0002591]

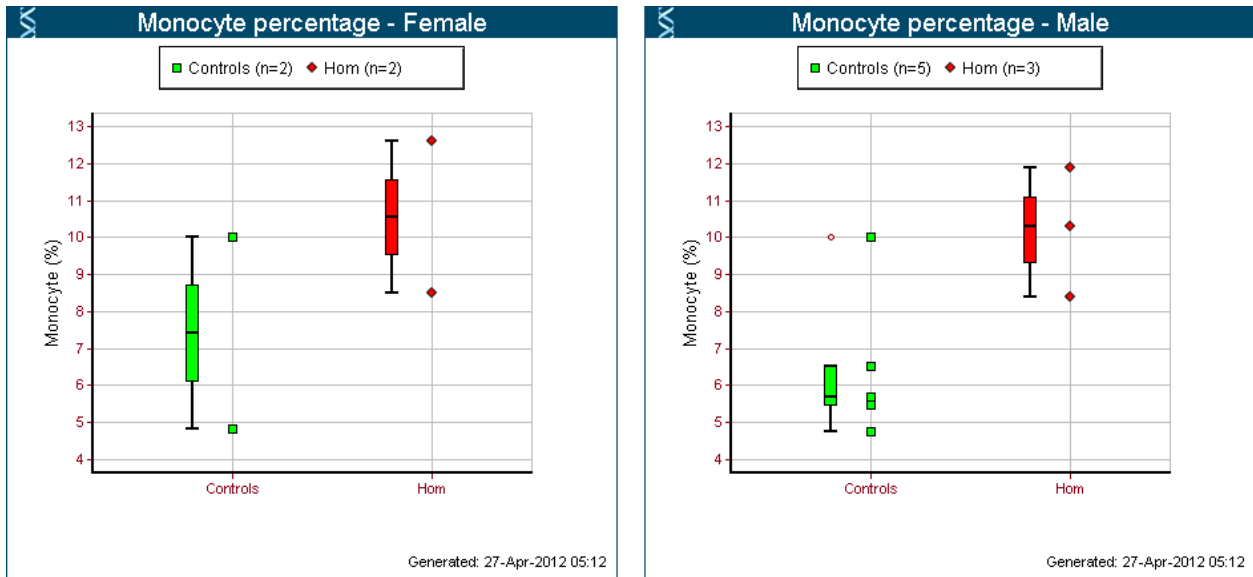


Males and Females - Decreased mean corpuscular hemoglobin [MP:0005562]

PBL Flow Cytometry Analysis



Males and Females - Increased granulocyte number [MP:0000322]



Males and Females - Increased monocyte cell number [MP: 0000220]

Targeted Histology

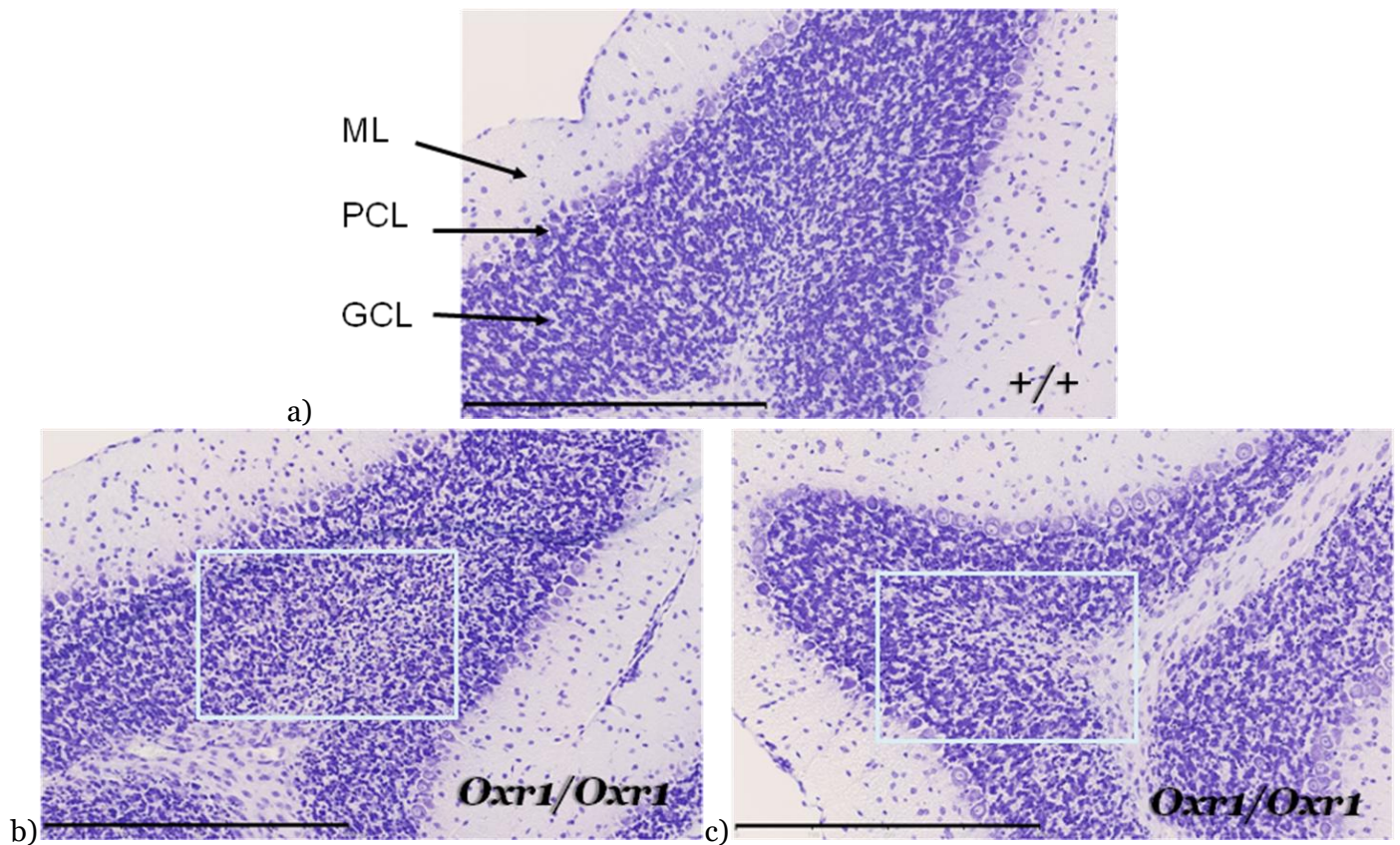


Figure 1. Coronal section microscopy image of (a) wild-type alongside (b) and (c) homozygous cerebellum using Nissl's staining. ML: molecular layer, PCL: Purkinje cell layer, GCL: Granular cell layer. ROI boxes show condensed nuclei in GCL in Homs which are indicative of cell death. Scale bar is equal to 400 micron.

Images

Videos displaying abnormal gait are available for this line.