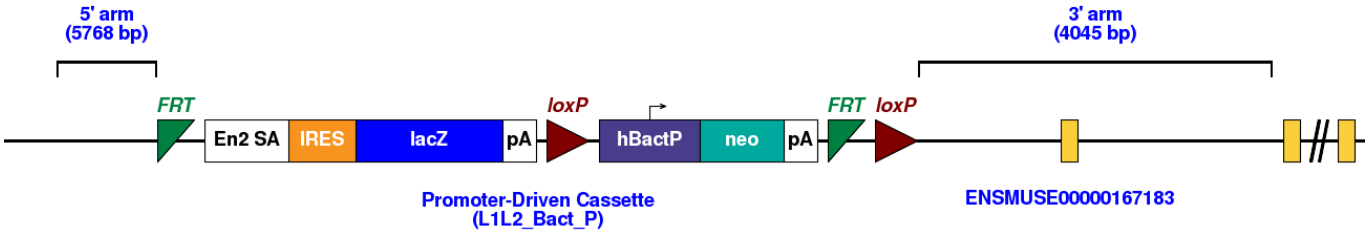
Knockout mouse lines presenting with welfare issues affecting their survival (abnormal survival [MP:0010769]) are processed through a bespoke sub-pipeline known as the "sick mouse procedure" (SMP) to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify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

Creb3l1$^{tm1e(EUCOMM)Wtsi}$

cAMP responsive element binding protein 3-like 1

Genetic Background: C57BL/6NTac

ES Cell Clone: EPD0651_2_A12



Affected genotypes

Homozygous (*Creb3l1* $^{tm1e(EUCOMM)Wtsi/tm1e(EUCOMM)Wtsi}$).

Alternative breeding strategy

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

Heterozygous mice are currently being phenotyped on the primary screen.

Welfare observations

Homozygous mice exhibit:

- Runted; Homozygous = 14% (8/57), Heterozygous = 1% (1/99), Wild-Type = 2.2% (2/90)
- Abnormal gait; Homozygous = 17.5% (10/57), Heterozygous = 0% (0/99), Wild-Type = 0% (0/90)
- Malformed paws; Homozygous = 15.8% (9/57), Heterozygous = 0% (0/99), Wild-Type = 0% (0/90)

Homozygous Viability:

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

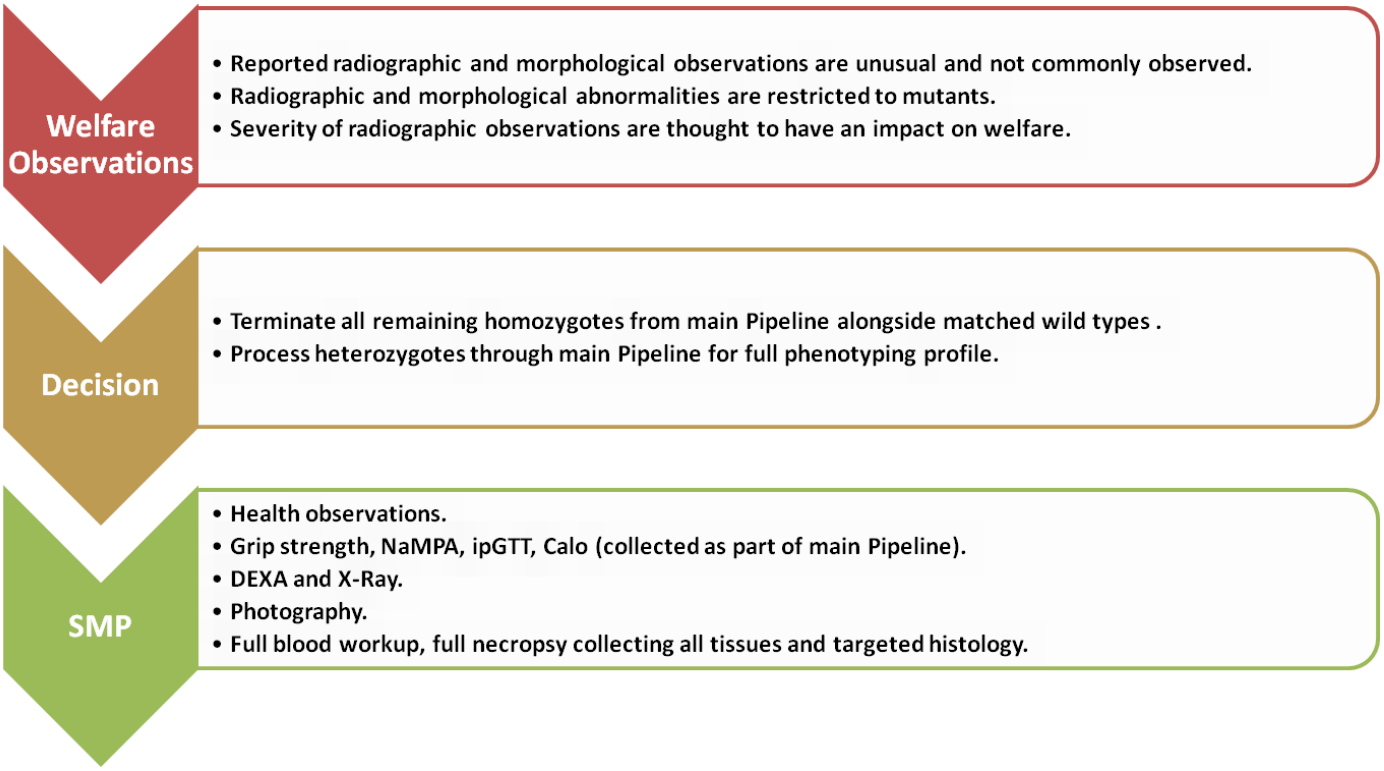
Viable : 24 Homs / 100 Total = 24%

Sick Mouse Procedure (SMP)

Initial welfare observations (abnormal gait; malformed paws) were reported when the first homozygotes were issued to the phenotyping pipelines (4 weeks). The first cohorts of mice progressed through the Pipeline with no external signs of adverse welfare. During analysis of X-ray data, multiple fractures and bone abnormalities were observed and thus all remaining homozygotes were terminated from the Pipeline and processed through SMP.

Cohorts of mice were 7 weeks, 13 weeks, 14 weeks and 16 weeks of age at the time at which they were terminated from the Pipeline and SMP (see schematic below) was initiated. 8 male and 8 female homozygotes were processed and data from wild-type animals of each sex and same genetic background that were processed at the same age are used as controls. The numbers on the graphs reflect the number of animals that went through each of the phenotyping tests. No further homozygotes were phenotyped due to the aforementioned alternative breeding strategy employed to minimize welfare concerns.

Schematic Outline of Bespoke SMP Pipeline



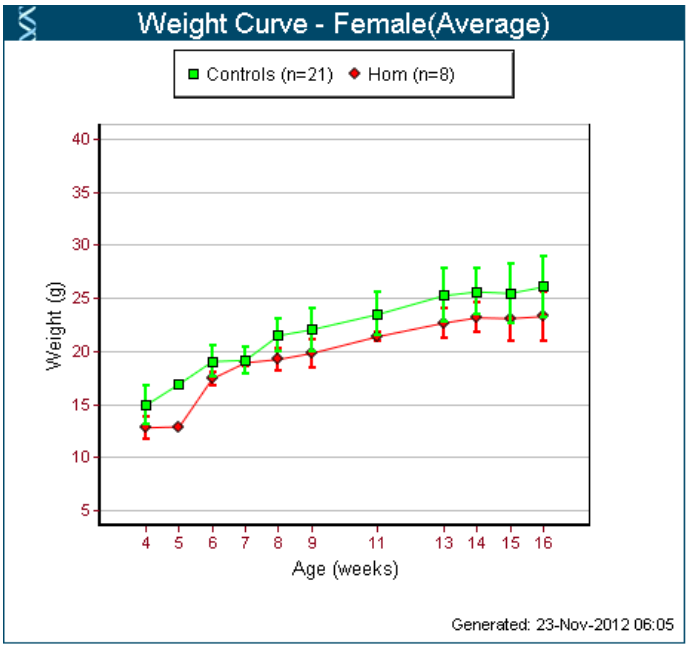
Phenotyping Heat Map

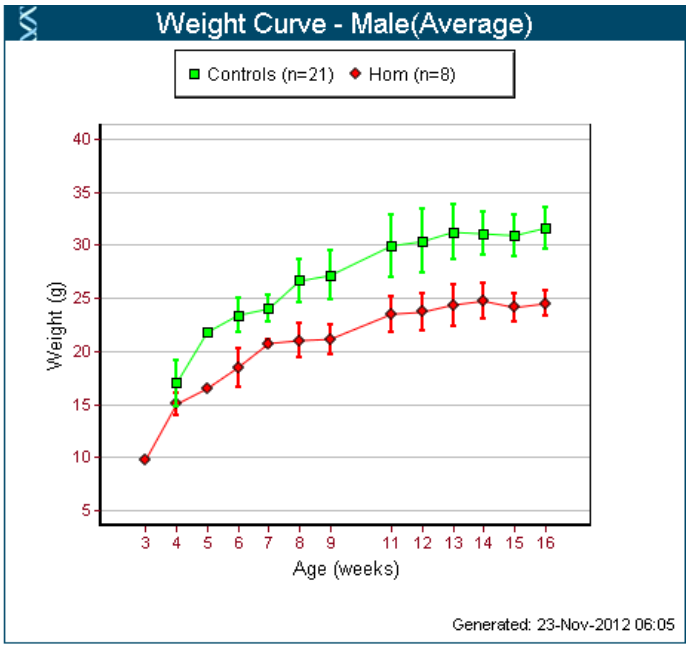
Colony Prefix	Allele Name	Genotype	Weight Curves	Grip Strength	NaMPA	Glucose Tolerence (ip)	Indirect Calorimetry	Body Composition (DEXA)	X-ray Imaging	Plasma Chemistry	Haematology (CBC)	Peripheral Blood Leukocytes	Heart Weight	Tail Epidermis Wholemount	Tissue Biobank
MEEN	Creb3l1<tm1e(EUCOMM)Wtsi>	Homozygous													

Significant / Interesting
Not significant
Resources available

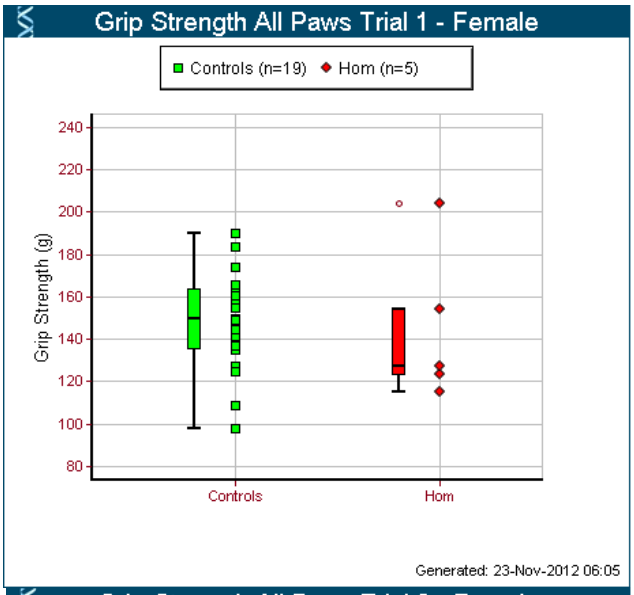
Phenotyping data of interest (significant changes)

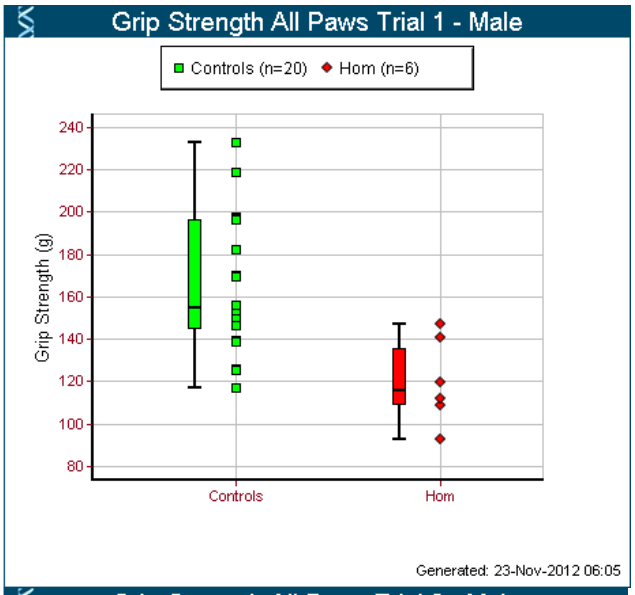
In life phenotyping

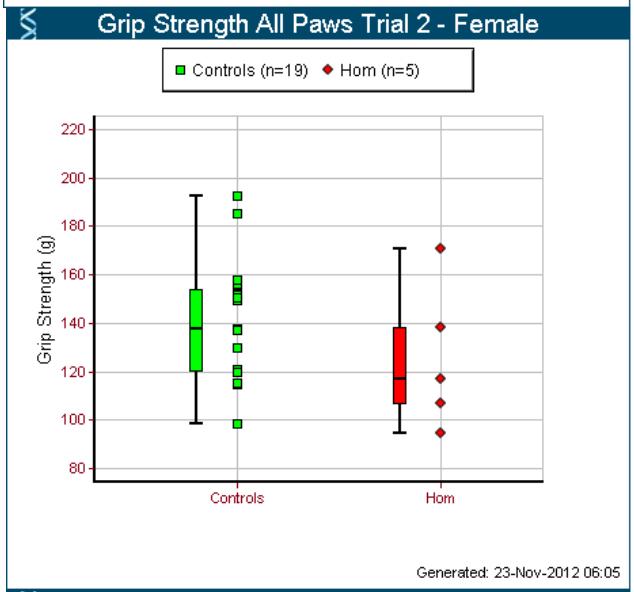


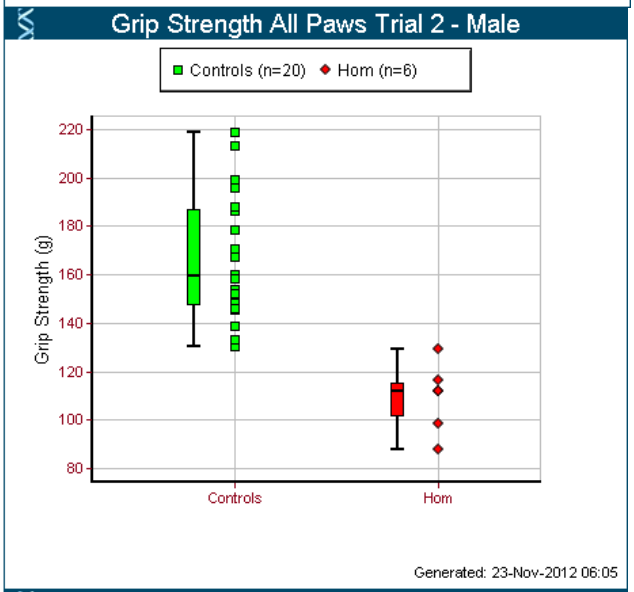


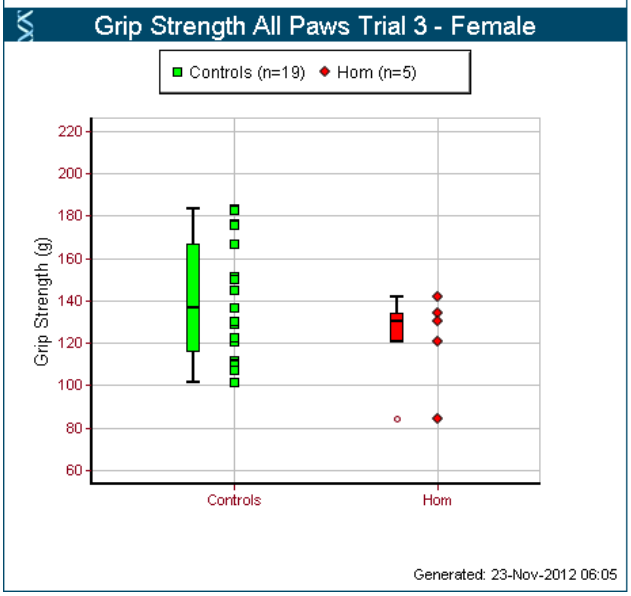
Males - Reduced body weight [MP:0001262]

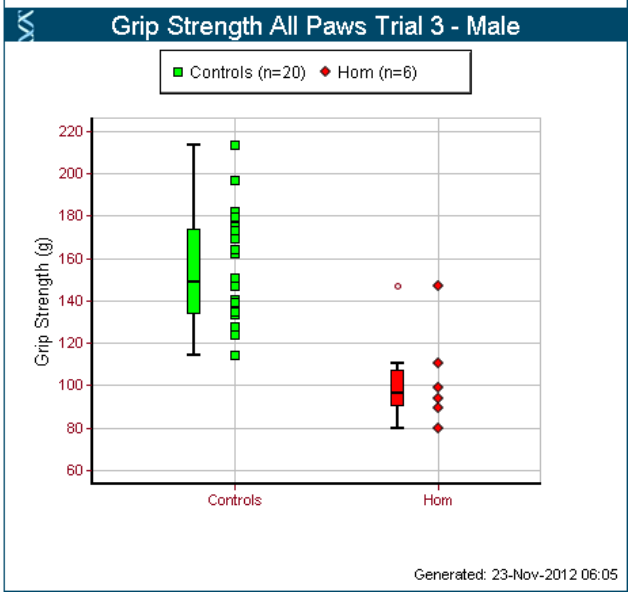




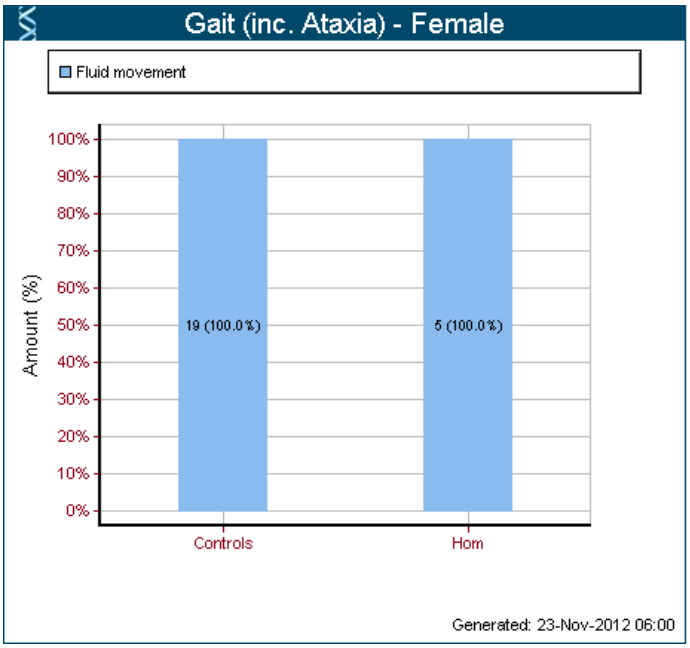


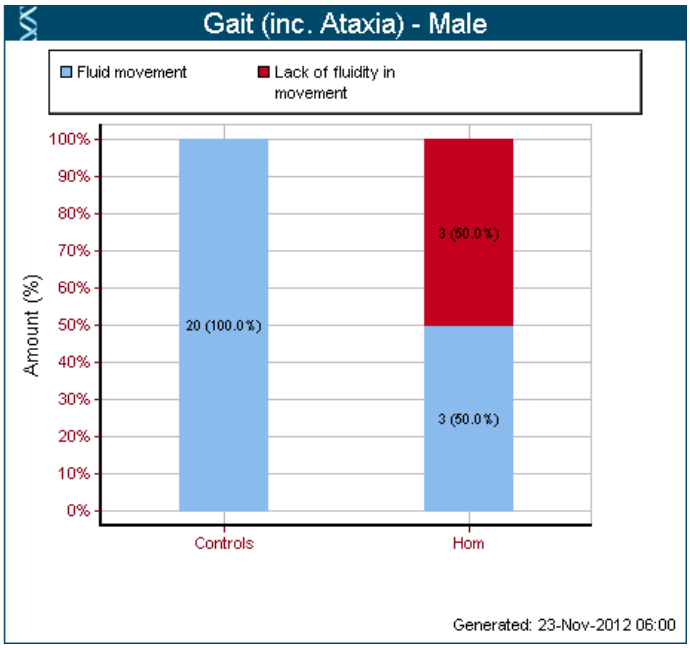




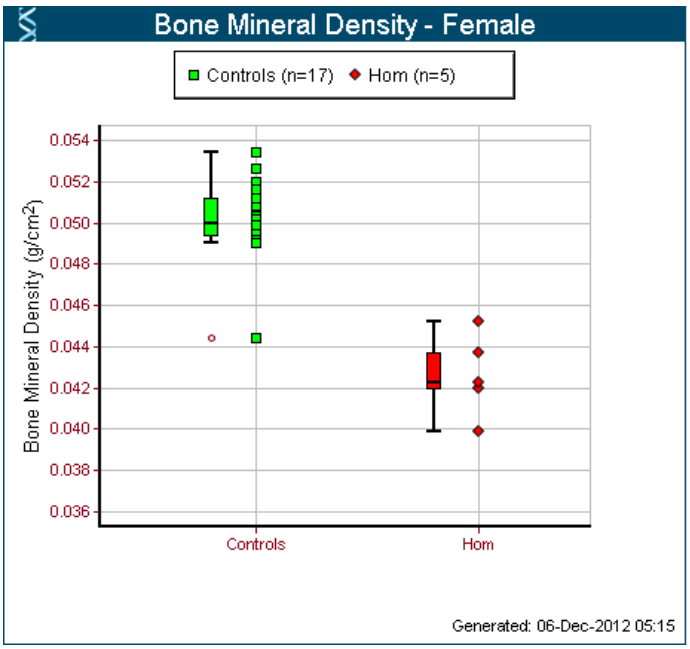


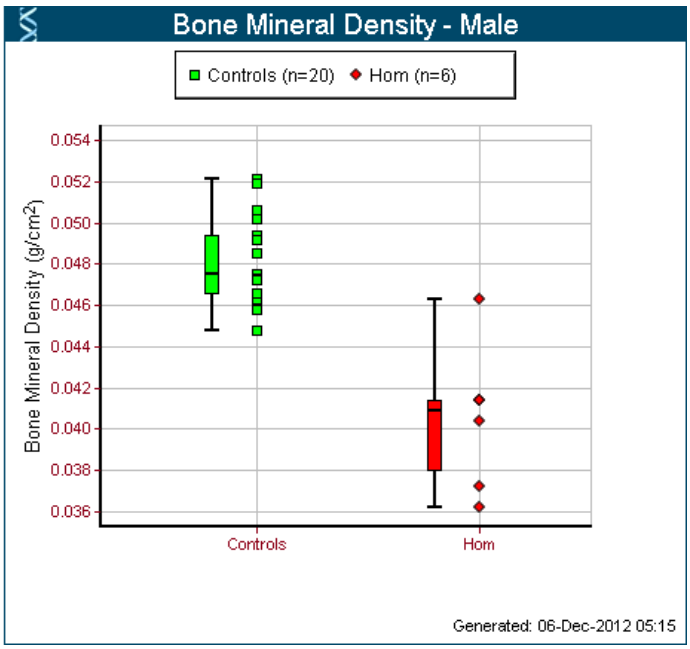
Males - Decreased grip strength [MP:0010053]



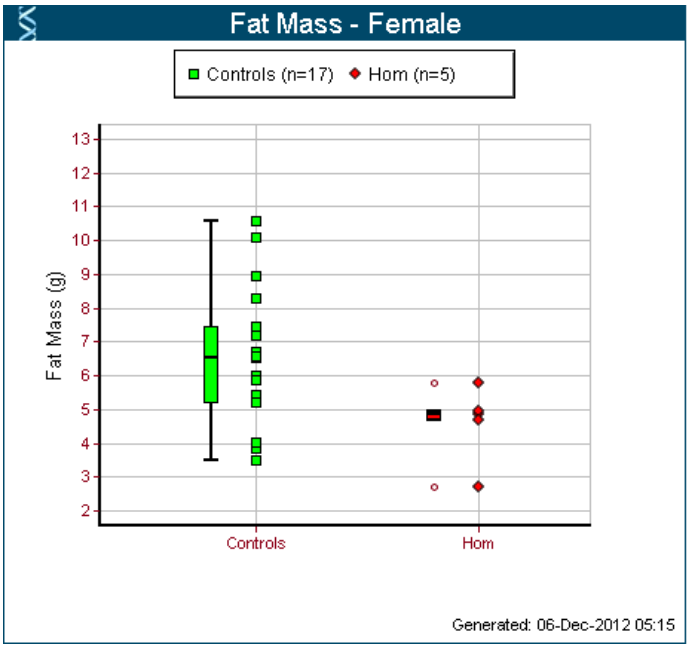


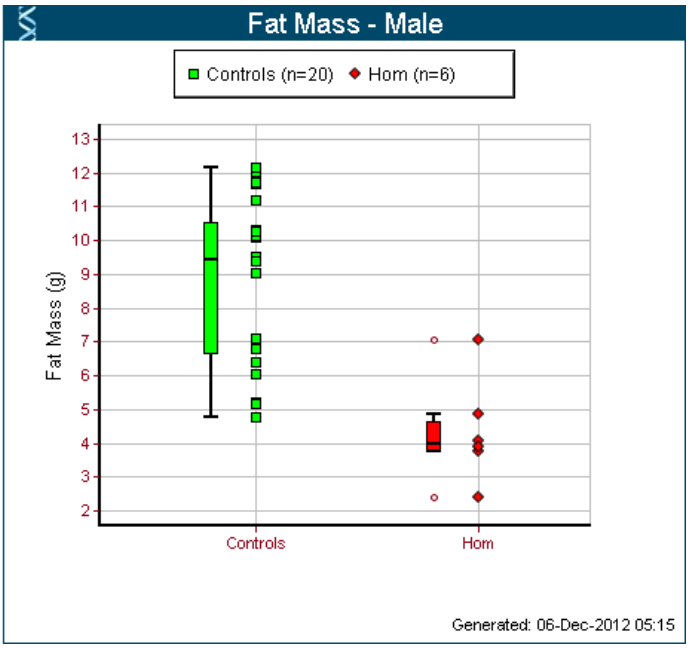
Males - Abnormal gait [MP:0001406]



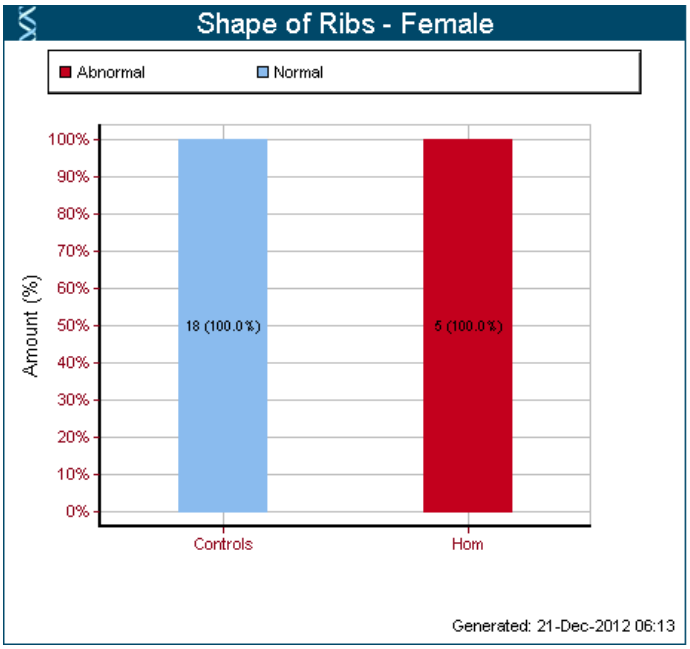


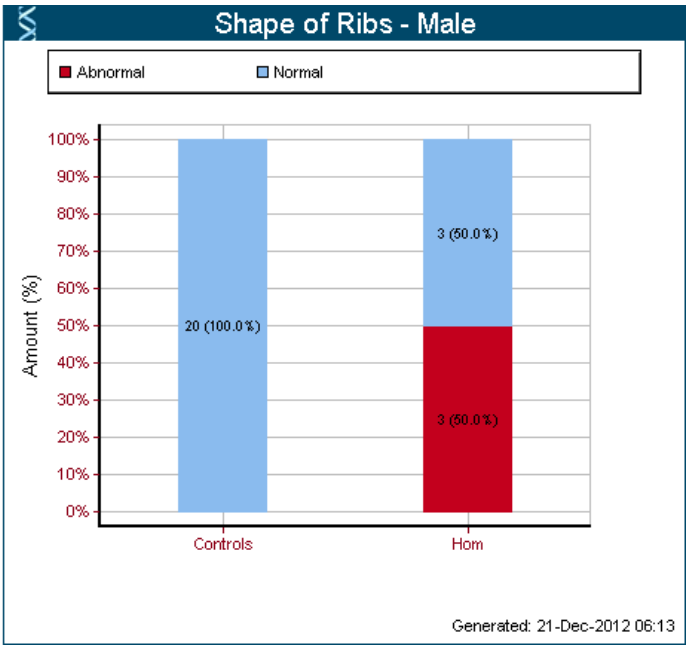
Males and Females - Decreased bone mineral density [MP:0000063]



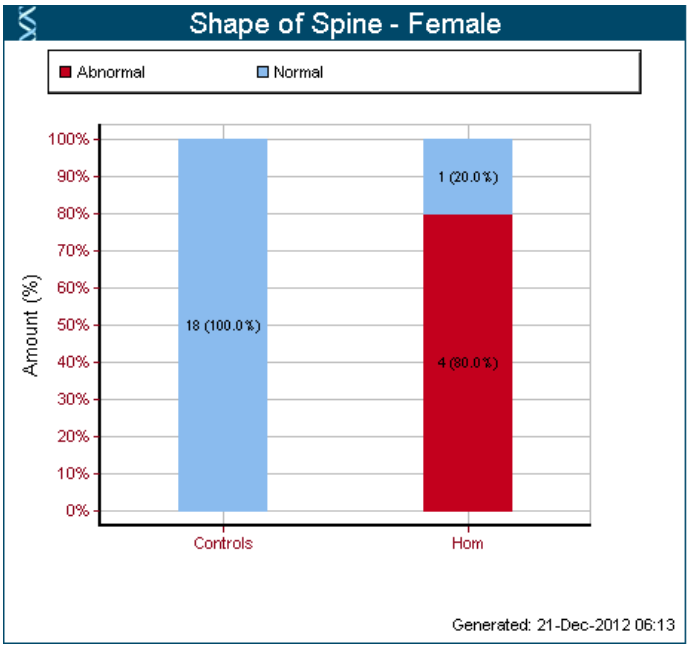


Males - Decreased total body fat amount [MP:0010025]

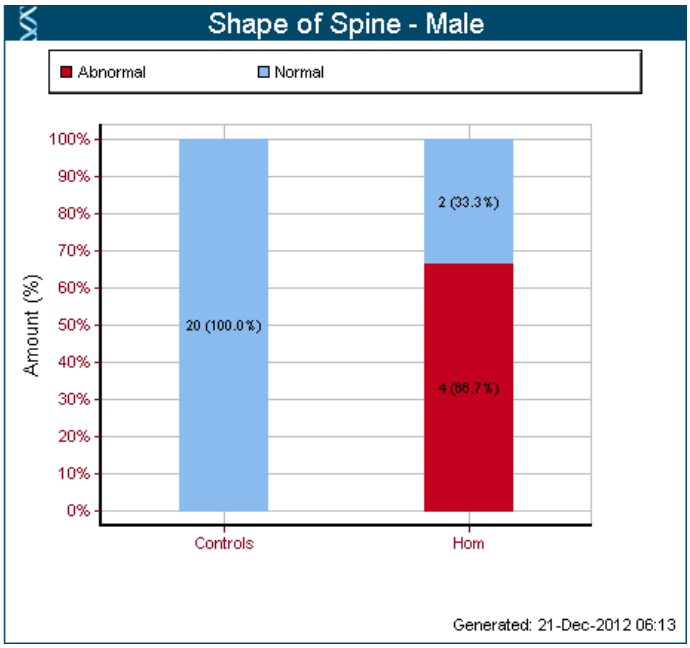




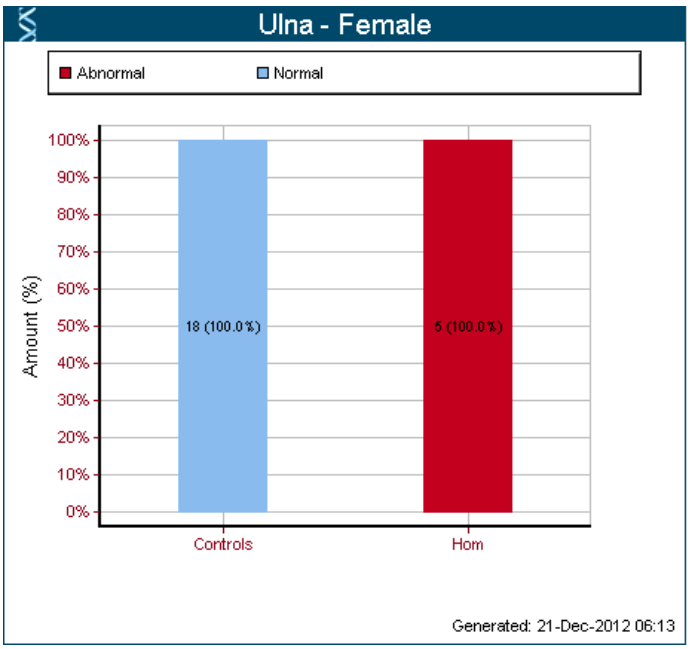
Males and Females- Abnormal rib morphology [MP:0000150]

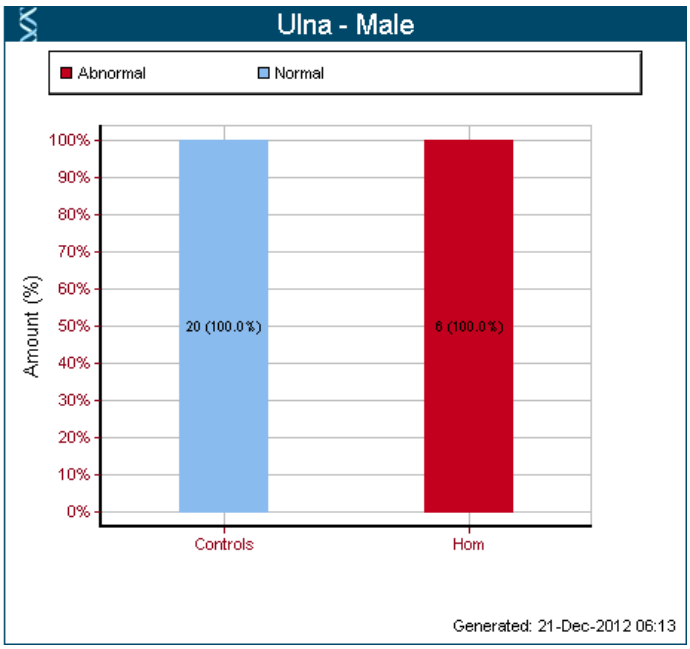


Females - Scoliosis [MP:0000161]

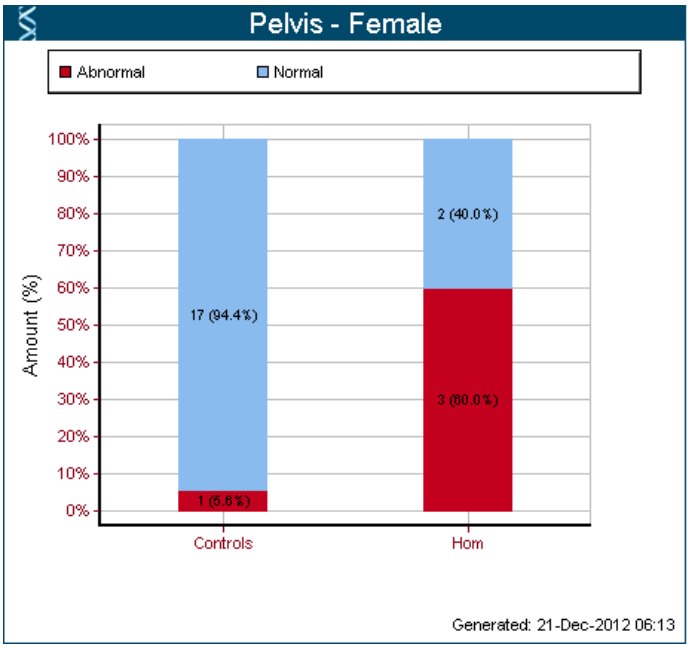


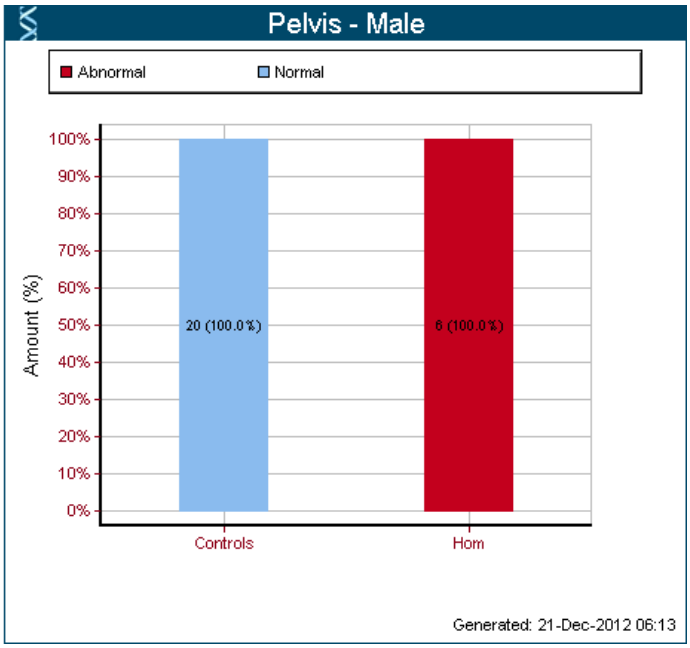
Males - Kyphoscoliosis [MP:0000069]



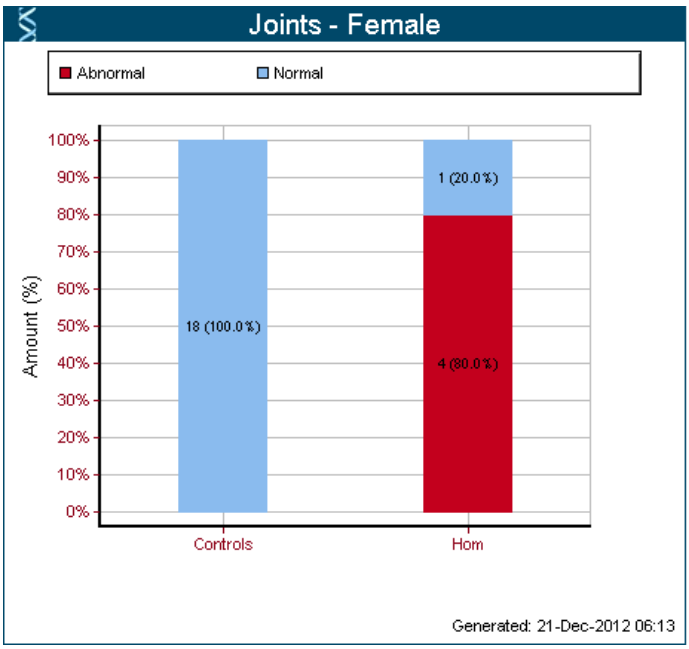


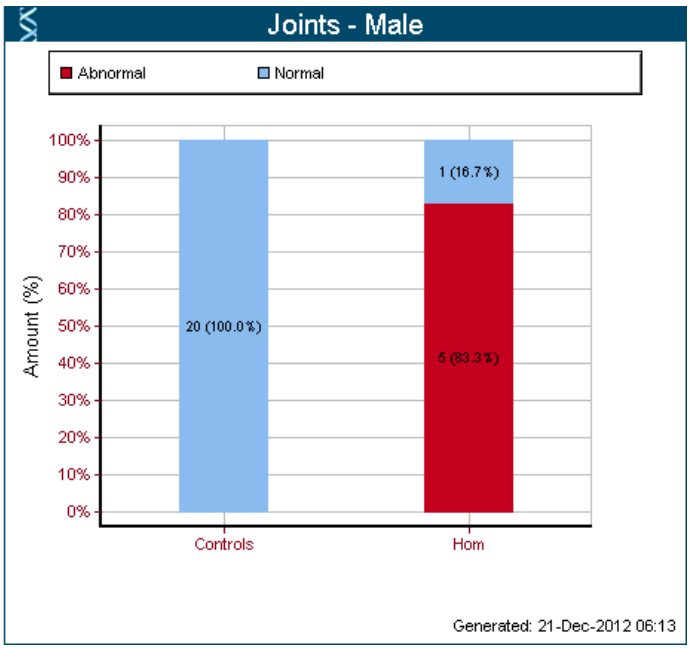
Males and Females - Abnormal ulna morphology [MP:0005108]



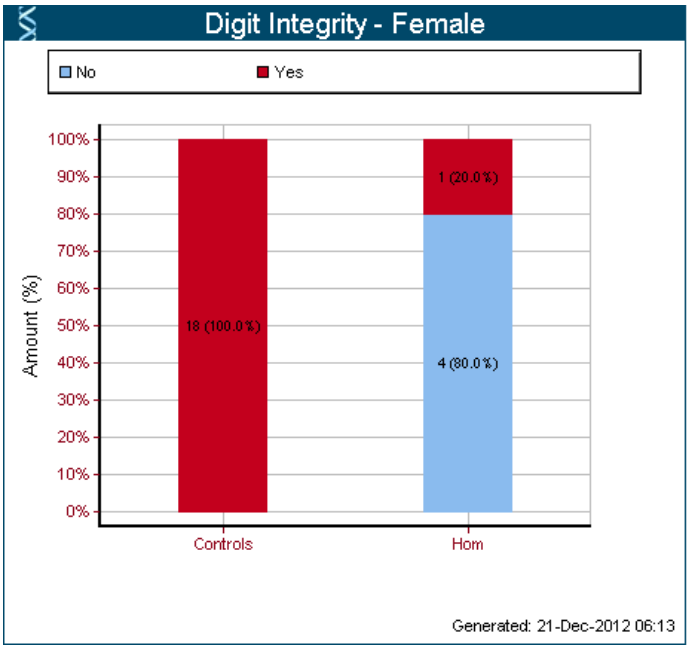


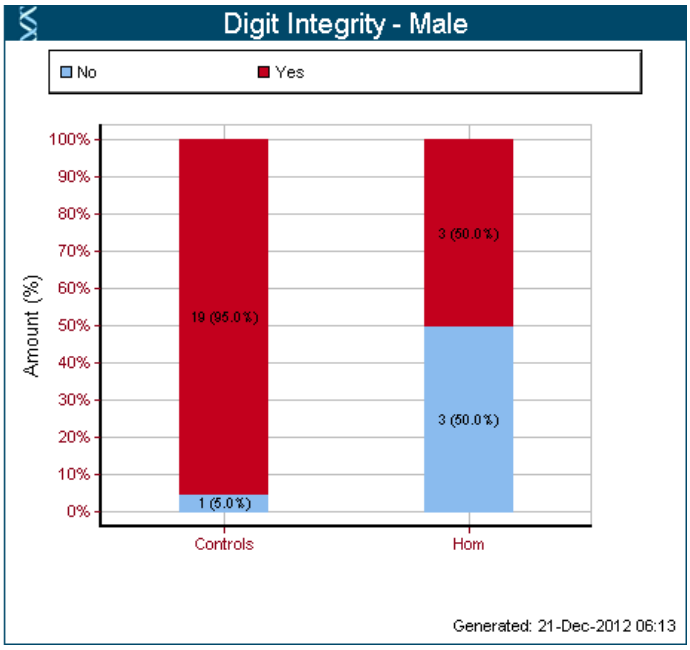
Males and Females - Abnormal ischium morphology [MP:0004507]



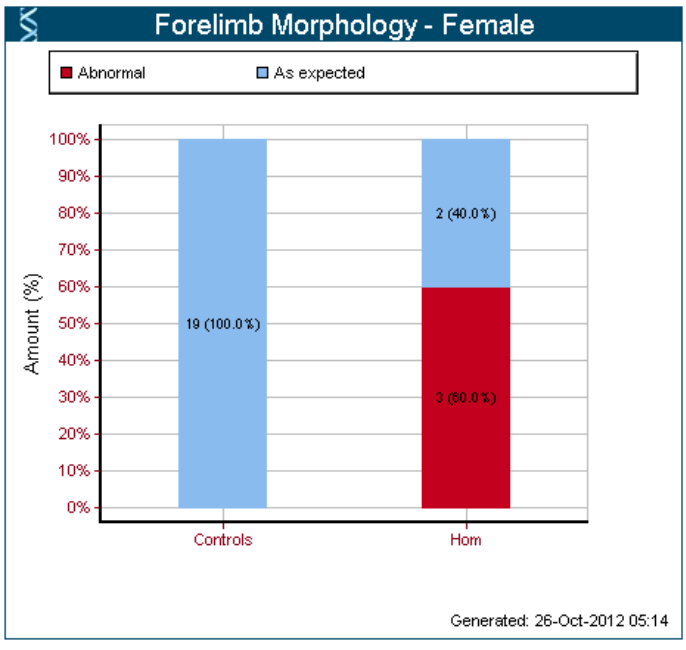


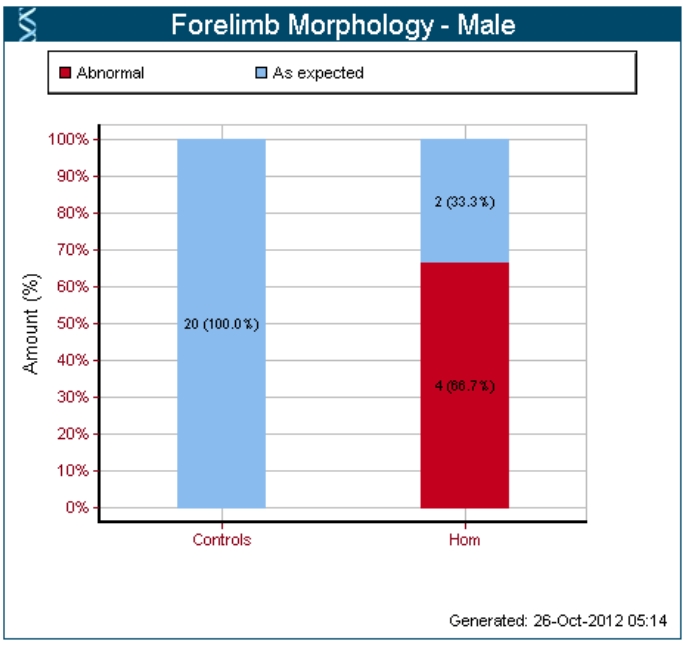
Males and Females - Abnormal joint morphology [MP:0002932]
Males and Females - Abnormal calcaneum morphology [MP:0009728]



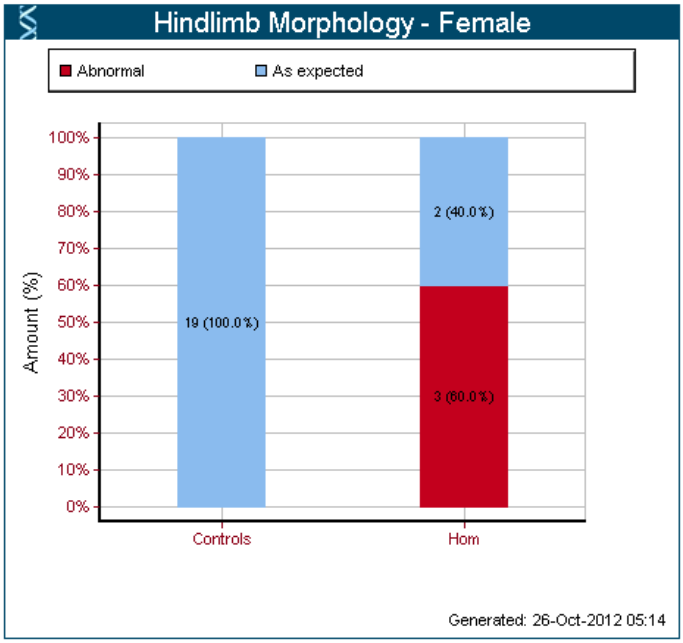


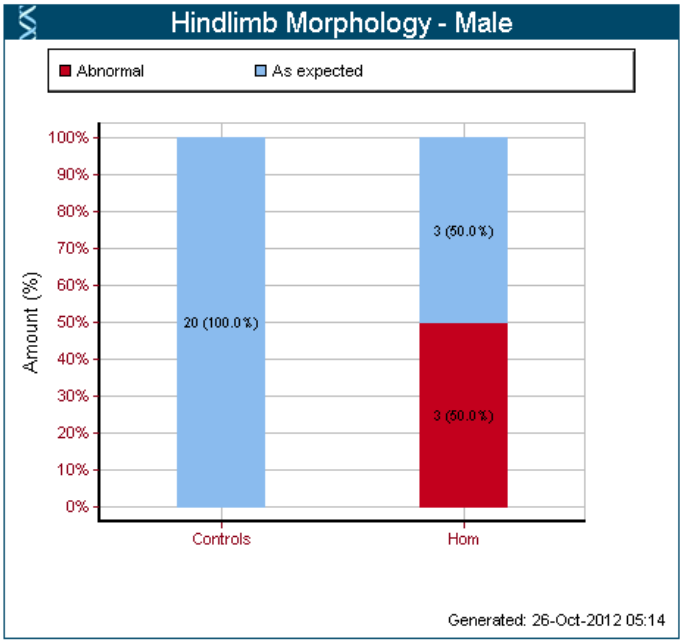
Males and Females - Abnormal digit morphology [MP:0002110]



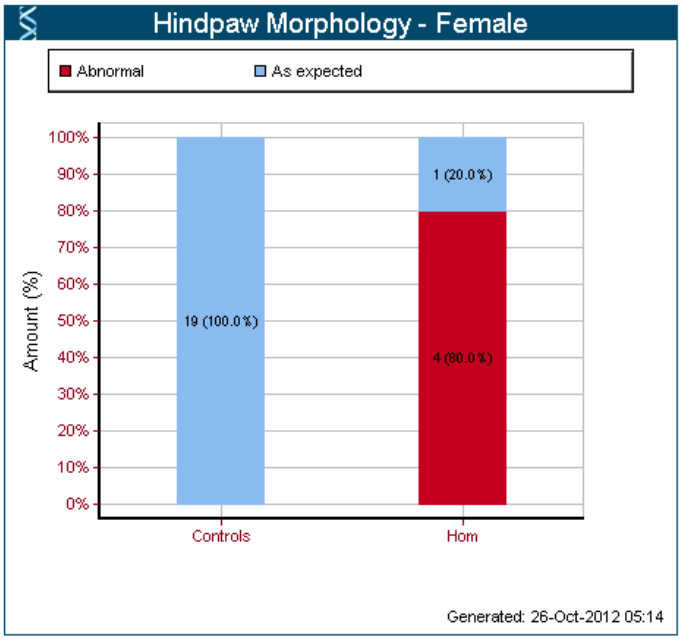


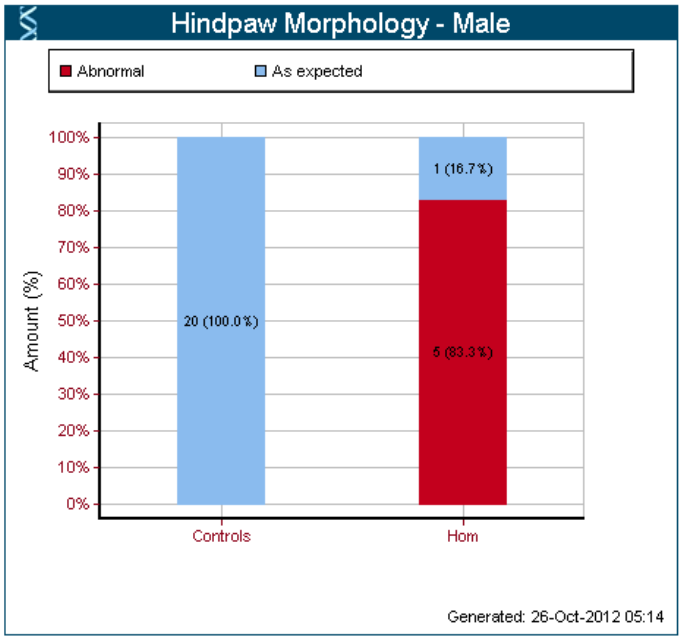
Males and Females - Abnormal forelimb morphology [MP:0000550]





Males and Females - Abnormal hindlimb morphology [MP:0000556]





Males and Females - Abnormal autopod morphology [MP:0000572]

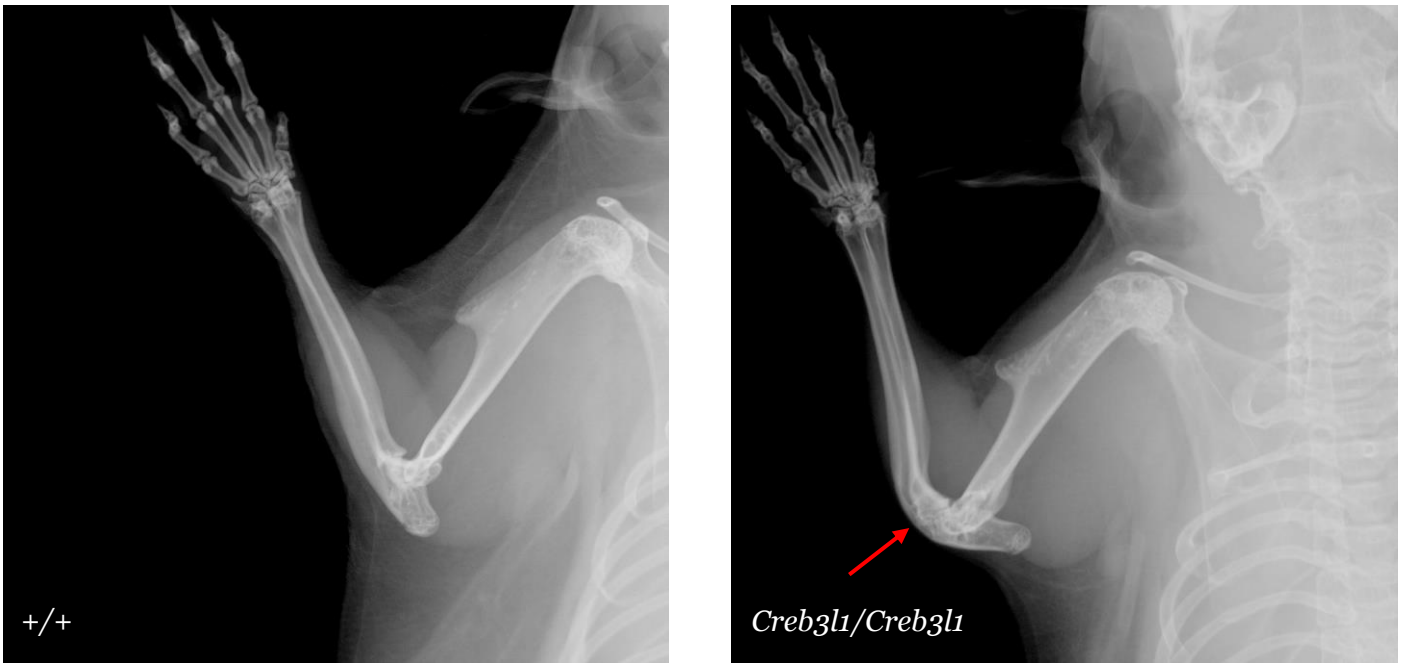
a b

Figure 1. Dorsal view of wild-type (a) and homozygous (b) mice showing abnormality in hindpaws (abnormal limb morphology [MP:0002109])

a b

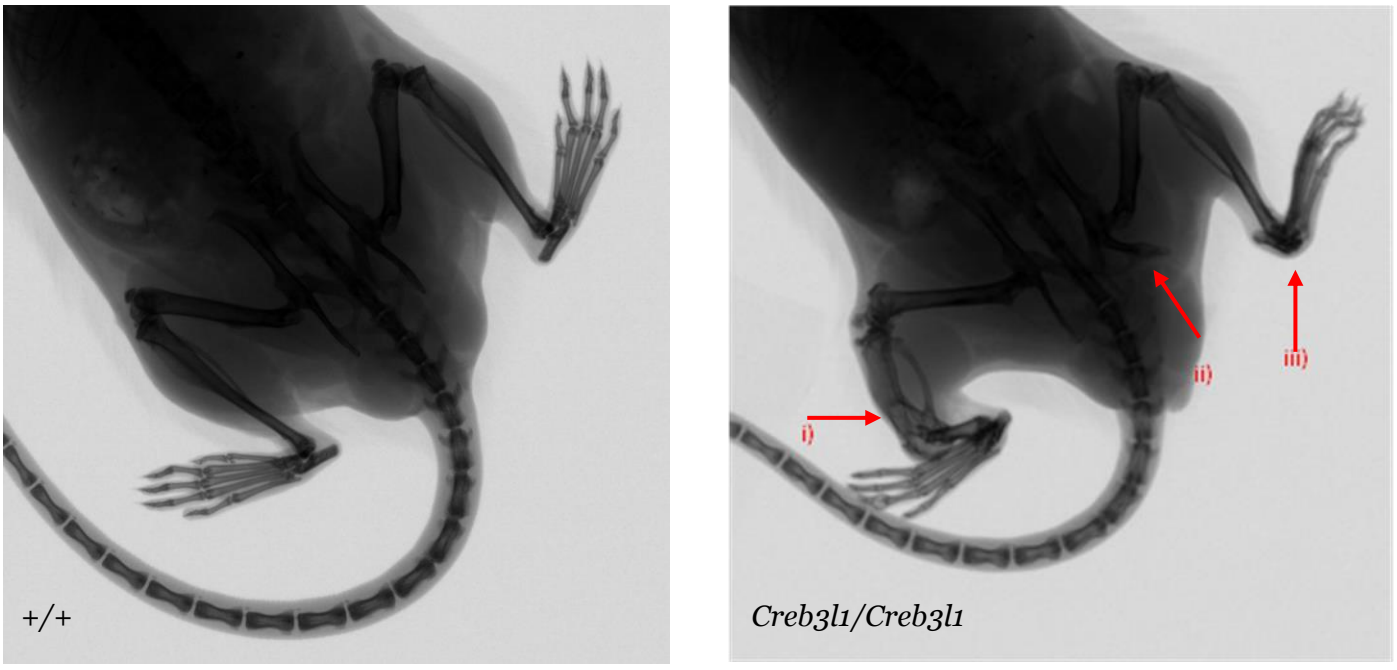
Figure 2. (a) Ventral view of wild-type (a) and homozygous (b) mice showing abnormality in hindlimb (abnormal limb morphology [MP:0002109]).

The homozygous mouse shown here is an example of a mouse with a very severe manifestation of the internal bone phenotype, as shown in figure 4b. The internal bone phenotype determined by radiography in less severe cases was not clearly observed by visual inspection or on the photographic images.



a b

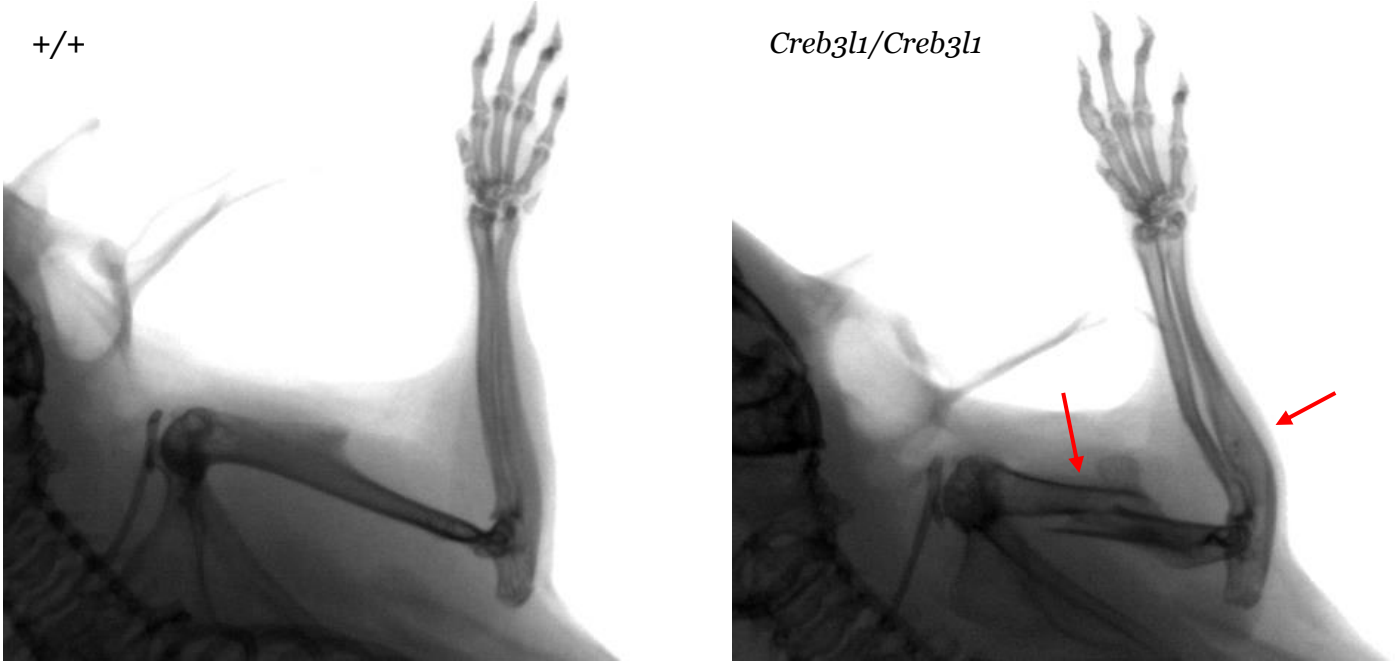
Figure 3. Dorsoventral radiograph of (a) wild-type and (b) homozygous mouse left forelimb displaying bowing of the ulna towards the olecranon (abnormal ulna morphology [MP:0005108])



a b

Figure 4. Dorsoventral radiograph of the hindquarters of (a) wild-type and (b) homozygous mouse. The pelvis appears fractured at the right ischium (i) (abnormal ischium morphology [MP:0004507]). The limbs displayed different abnormalities between mice due to fracture site(s) (abnormal limb morphology [MP:0002109]). In this mouse the left hind limb display bowing of the left tibia with abnormal ossification around a site of fracture (ii). The right calcaneus is abnormal in morphology affecting the talocalcaneal joint and hind paw (iii) (abnormal joint morphology [MP:0002932]; abnormal calcaneum morphology [MP:0009728]).

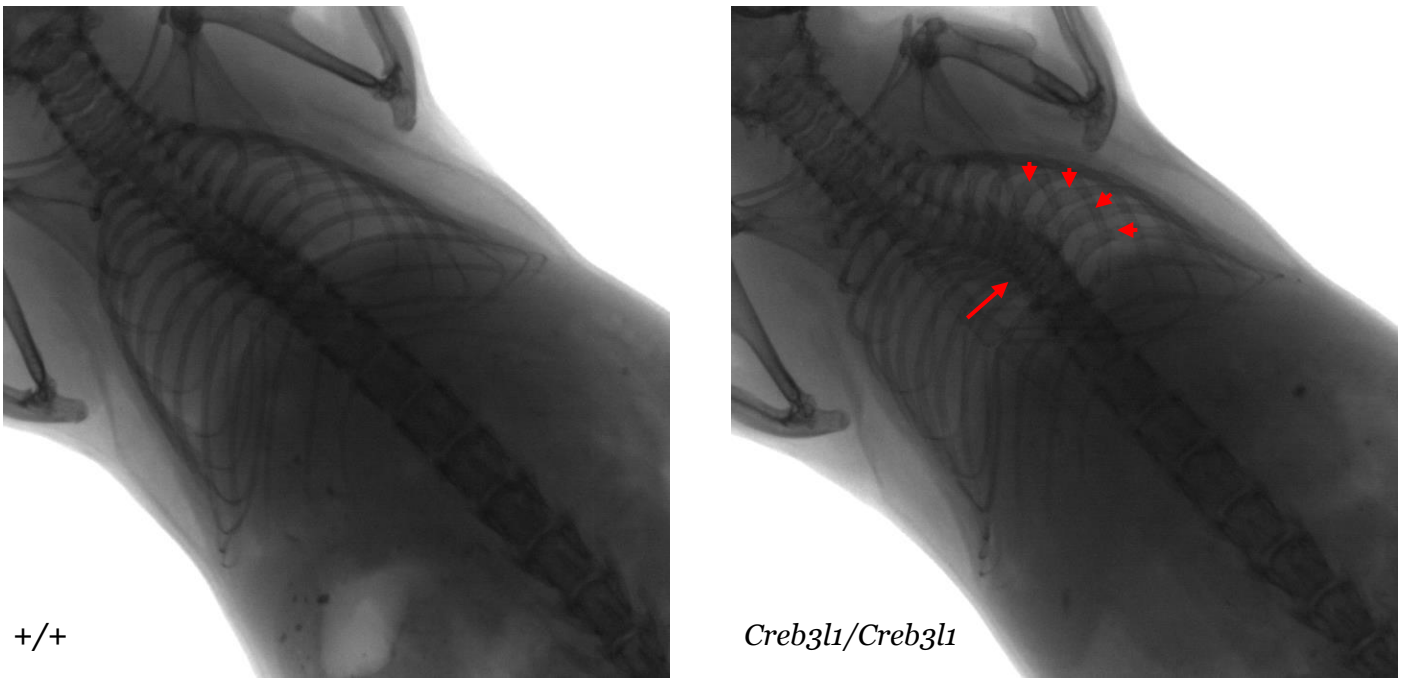
The homozygous mouse shown here is an example of a mouse with a very severe bone abnormality and is the same mouse displayed in the photograph in figure 2b.



a b

Figure 5. Dorsoventral radiograph of (a) wild-type and (b) homozygous mouse right forelimb displaying bowing of the right radius (abnormal radius morphology [MP:0000552]) and ulna (abnormal ulna morphology [MP:0005108]), fracture of right humerus (abnormal humerus morphology [MP:0005296]) and abnormal reossification. The limbs displayed different abnormalities between mice due to fracture site(s) (abnormal limb morphology [MP:0002109]).

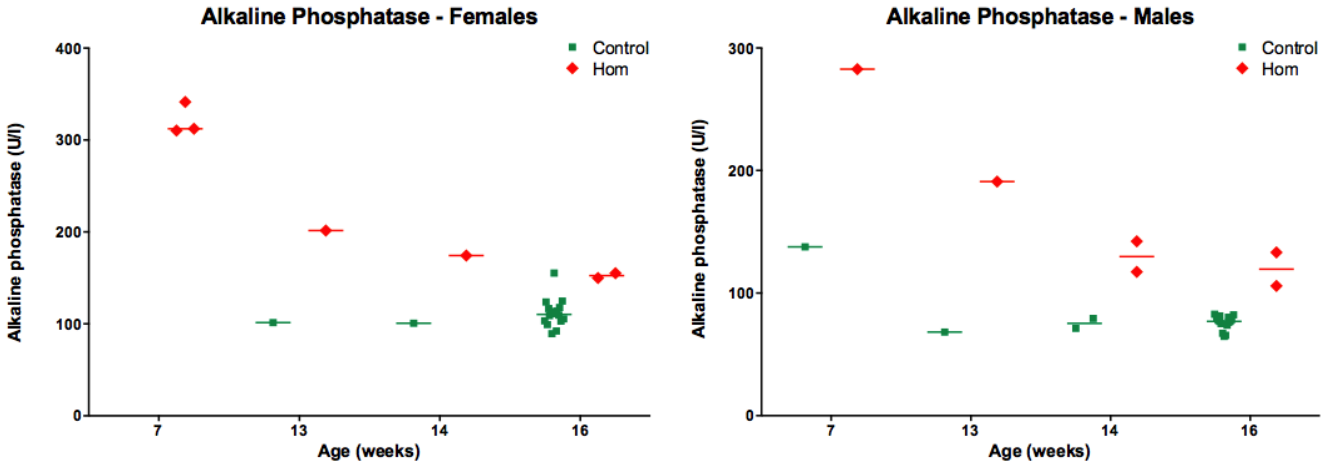
The homozygous mouse shown here is another example of a mouse with a very severe bone abnormality.



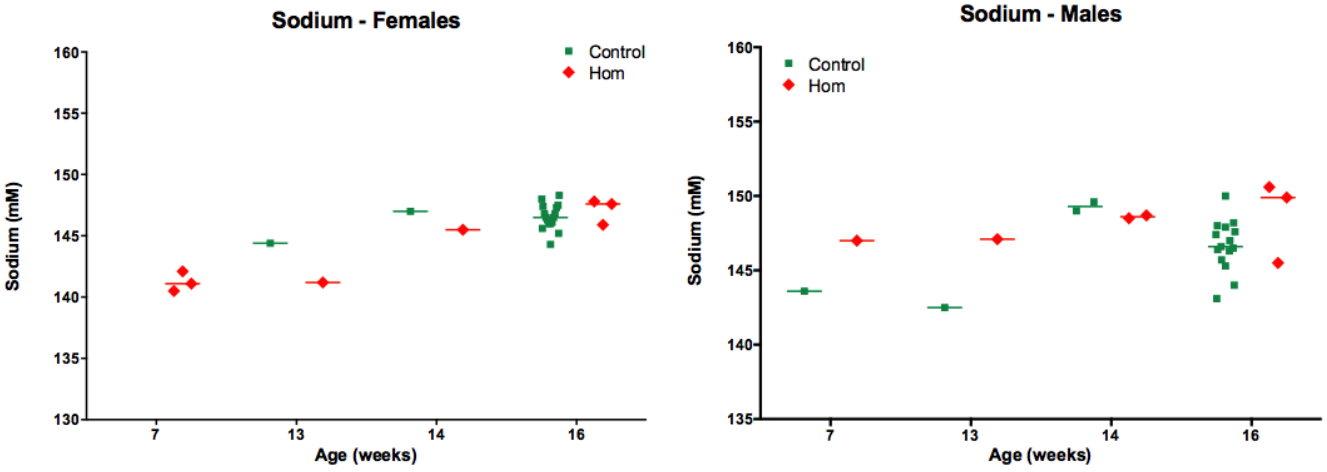
a b

Figure 6. Dorsoventral radiograph of (a) wild-type and (b) homozygous mouse thorax displaying thoracic scoliosis (scoliosis [MP:0000161]) and abnormal ribs (abnormal rib morphology [MP:0000150]).

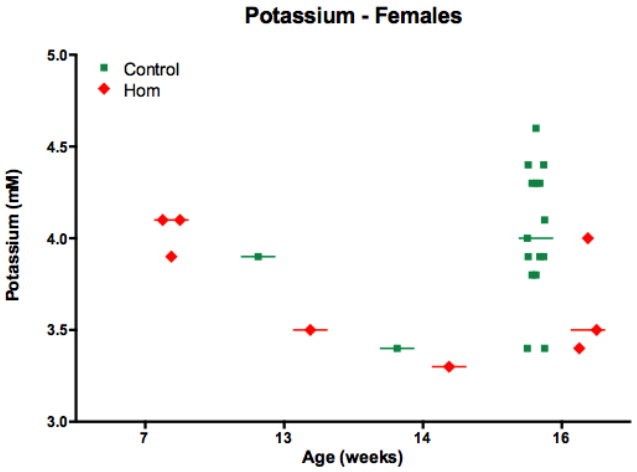
Ex-vivo phenotyping

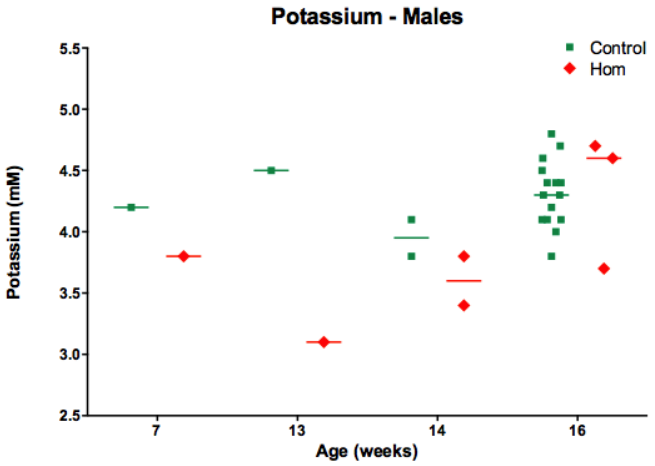


Males and Females - Increased circulating alkaline phosphatase level [MP:0002968]

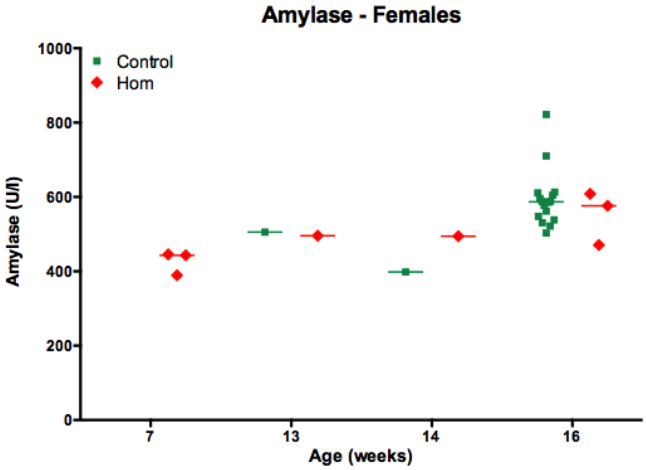


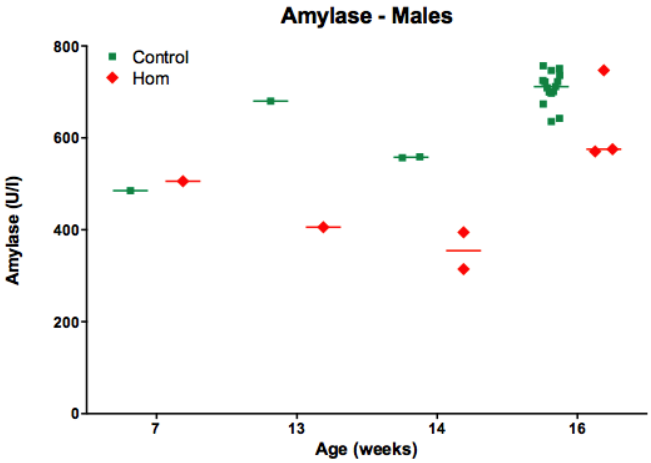
Females - decreased circulating sodium level [MP:0005634]



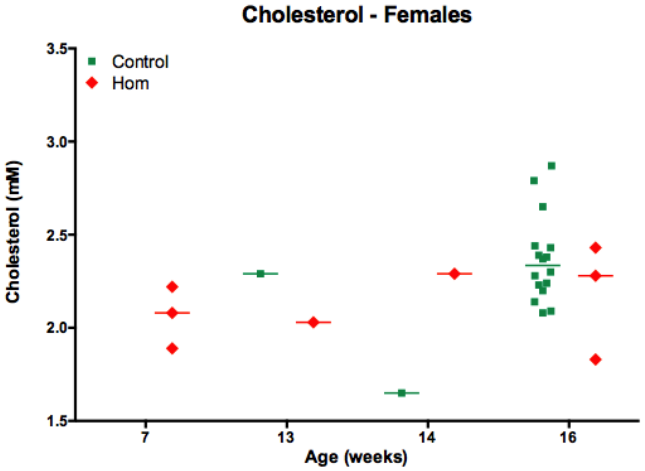


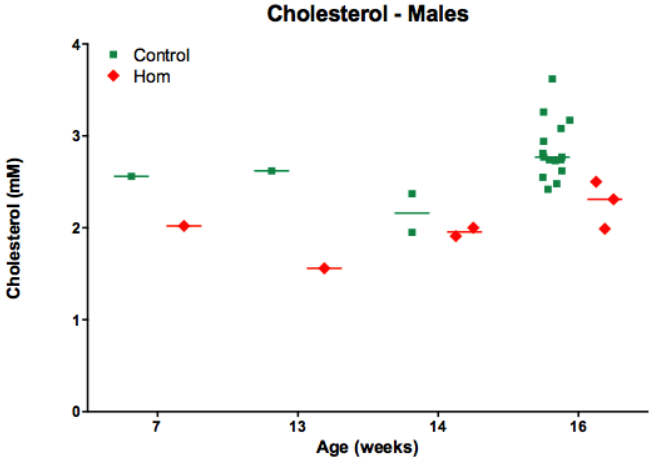
Males - decreased circulating potassium level [MP:0005628]



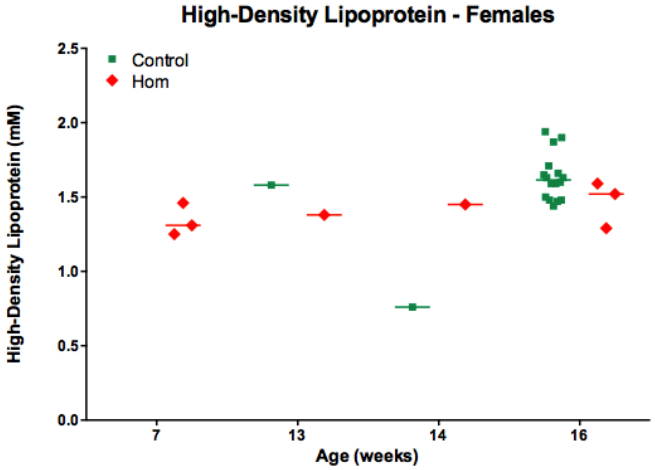


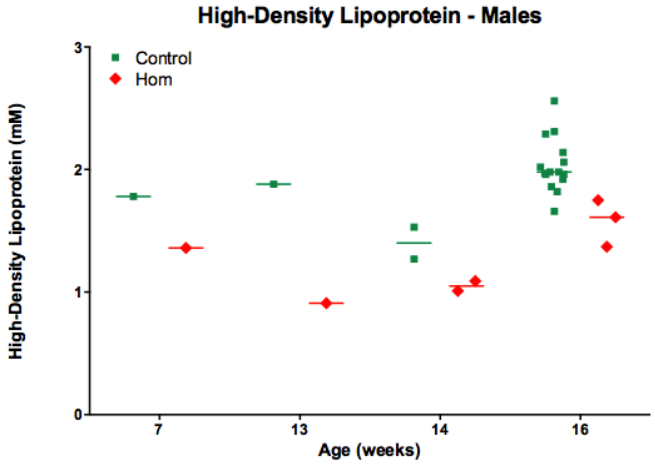
Males - decreased circulating amylase level [MP:0008805]





Males - Decreased circulating cholesterol level [MP:0005179]





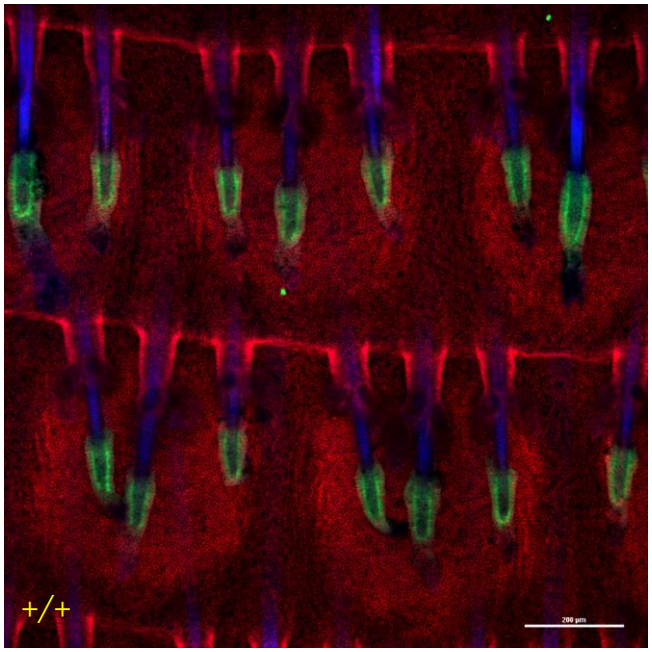
Males - Decreased circulating HDL cholesterol level [MP:0000186]

Necropsy observations

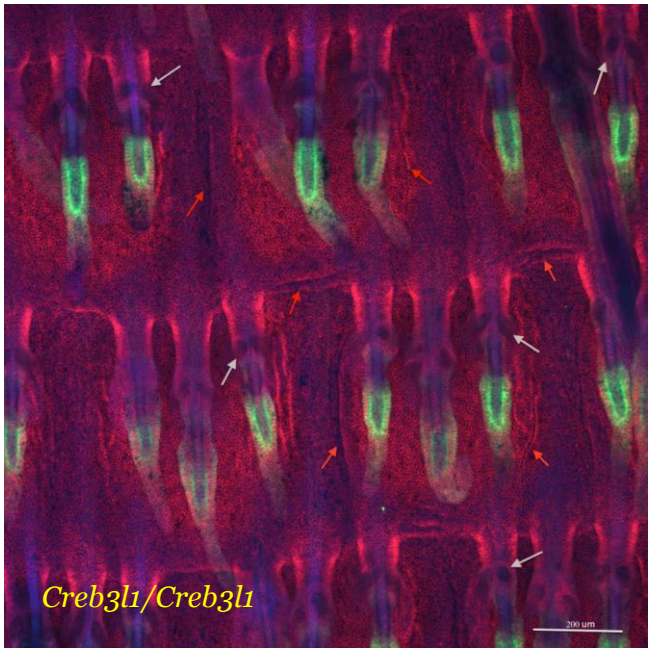
1 male homozygote displayed haemorrhages around joints. No further significant observations are reported at necropsy.

Tail Epidermis Wholemount

2 female homozygotes analysed showed fragile and thin skin (thin skin [MP:0001199]), narrow hair follicles (small hair follicles [MP:0000380]) and small sebaceous glands (abnormal sebaceous gland morphology [MP:0000647]).



a



b

Figure 6. Confocal microscopy image of (a) wild-type and (b) homozygous mouse tail epidermis at 100x magnification (*immunofluorescence staining with keratin 14 (red), keratin 15 (green) & DAPI (blue);*

200µm scale bar). The arrows in grey are pointing to the smaller Sebaceous glands and the arrows in red indicate abnormal epidermal stratum basale morphology [MP:0001231].

Imaging and analysis performed by Kif Liakath-Ali and Emma Heath from the Watt lab at King's College London.