

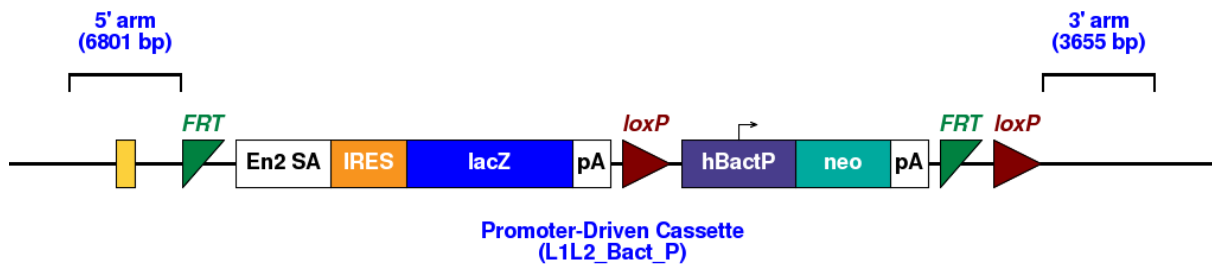
Knockout mouse lines presenting with welfare issues affecting their survival (abnormal survival [MP:0010769]) are processed through a bespoke sub-pipeline known as the “sick mouse procedure” (SMP) to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify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

Cox6a1^{tm1(KOMP)Wtsi}

Cytochrome c oxidase, subunit VI a, polypeptide 1

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

Allele: Deletion (Promotor Driven Cassette)



Affected genotypes

Homozygous (*Cox6a1*^{tm1(KOMP)Wtsi/ tm1(KOMP)Wtsi}).

Alternative breeding strategy

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

Heterozygous mice have been phenotyped through the primary screen.

Welfare observations

Homozygous mice exhibit:

- Failure to survive past sexual maturity (100% n=11) [MP:0008770 - decreased survivor rate].
- Small body size (hom female 57% n=4/7, male 50% n=2/4 vs WT female 0% n=0/62, male 2% 1/55) [MP:0000452 - abnormal body weight].
- Abnormal gait described as shuffle and stumble prone movements (hom female 43% n=3/7, male 25% n=1/4 vs WT female 0% n=0/62, male 0% 0/55) [MP:0001259 - abnormal gait].

- Swelling to the inner mouth (hom female 100% n=7/7, male 50% n=2/4 vs WT female 0% n=0/62, male 0% 0/55) [MP:0001406 - abnormal mouth morphology].

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*]

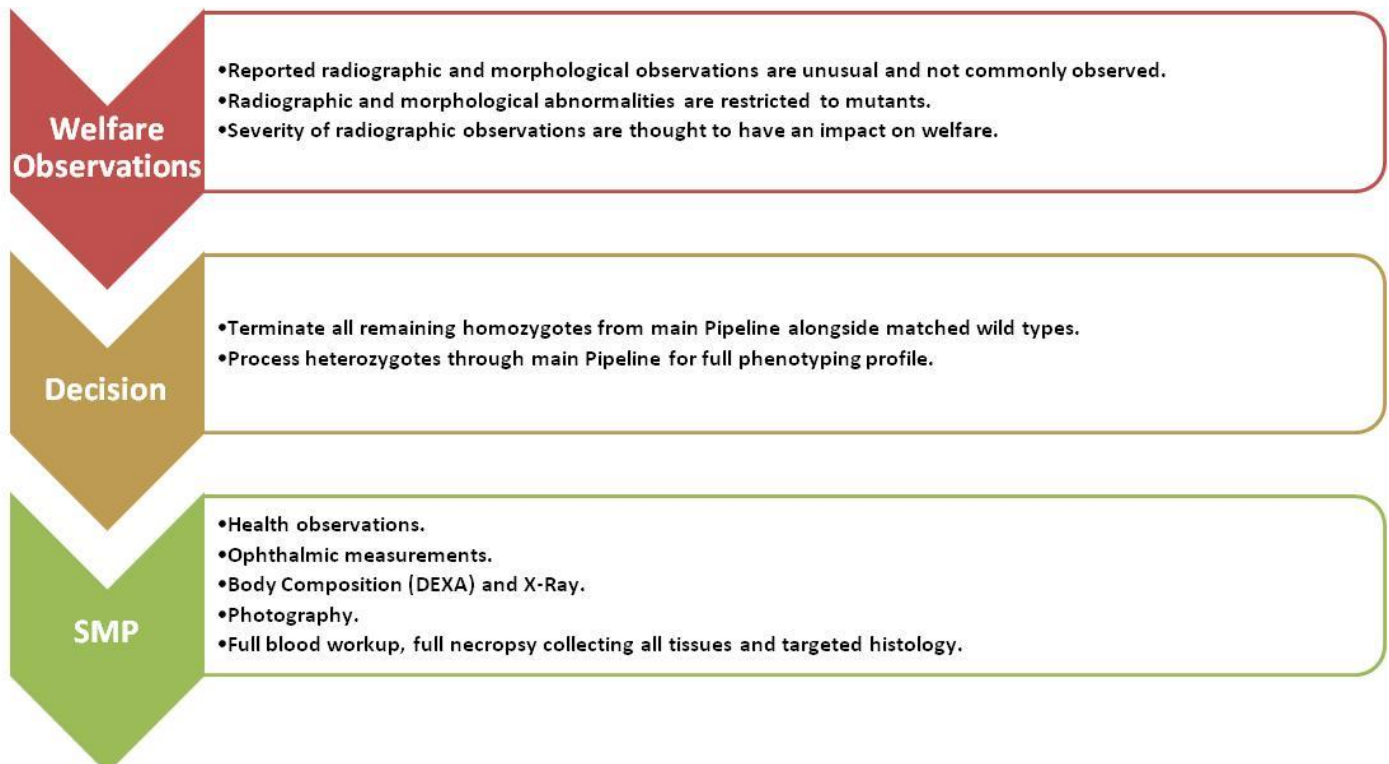
- **Viable** : 11 Homs / 35 Total = 31.4%

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 following weaning.

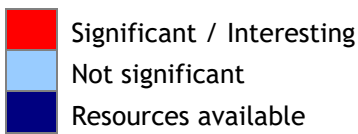
Welfare observations in homozygotes described above progressed to moderate severity following weaning upon which Comprehensive SMP (see schematic below) was initiated. Three female homozygotes were processed alongside 3 matched wild-types at 4 weeks of age. No further homozygotes were phenotyped due to the aforementioned alternative breeding strategy employed to reduce further welfare implications.

Schematic Outline of Bespoke SMP Pipeline



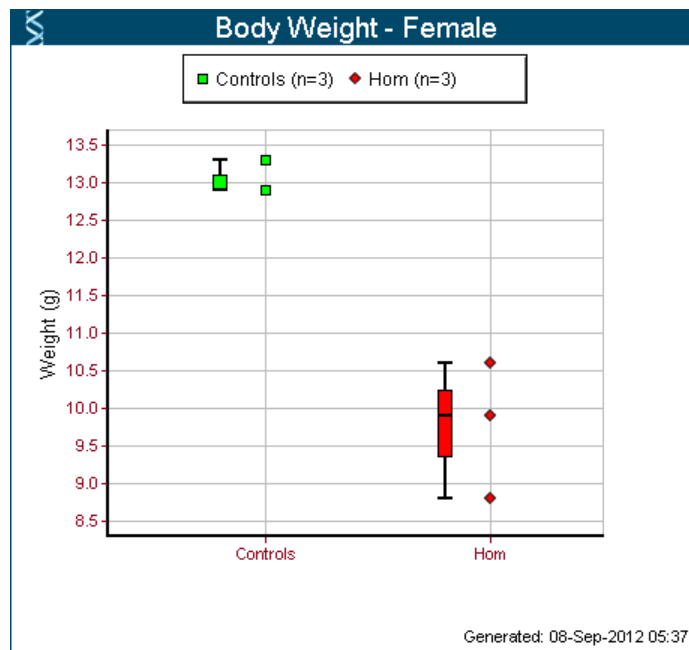
Phenotyping Heat Map

| Colony Prefix | Allele Name | Genotype | Weight Curves | Stress Induced Hypothermia | Ophthalmic Measurements | Body Composition (DEXA) | X-ray Imaging | Plasma Chemistry | Haematology (CBC) | Peripheral Blood Leukocytes | Tissue Biobank |
|---------------|---------------------|------------|---------------|----------------------------|-------------------------|-------------------------|---------------|------------------|-------------------|-----------------------------|----------------|
| MCQW | Cox6a1tm1(KOMP)Wtsi | Homozygous | Red | Red | Light Blue | Red | Red | Red | Red | Red | Dark Blue |

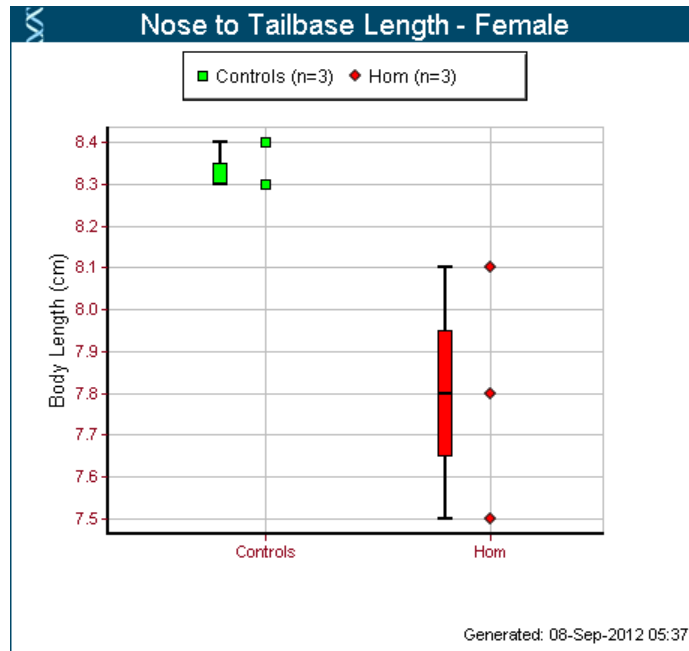


Phenotyping data of interest (significant changes)

In life phenotyping

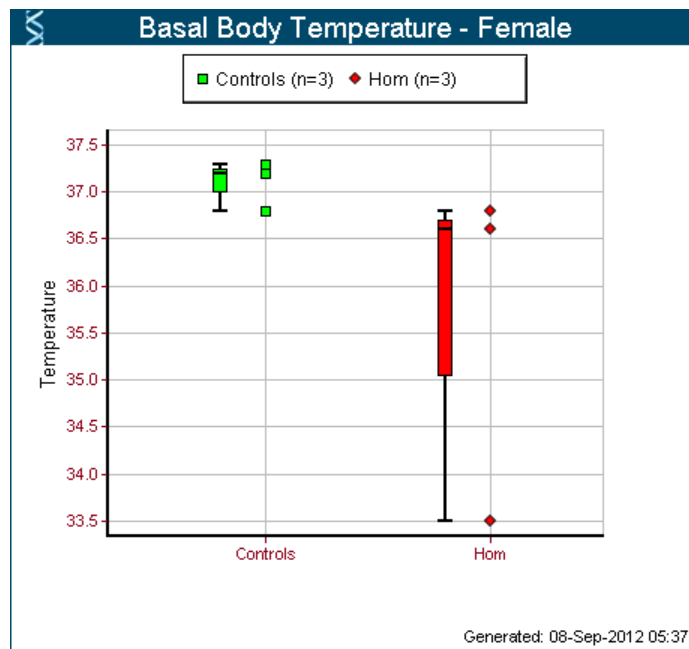


Female - Decreased Body Weight [MP:0001262]



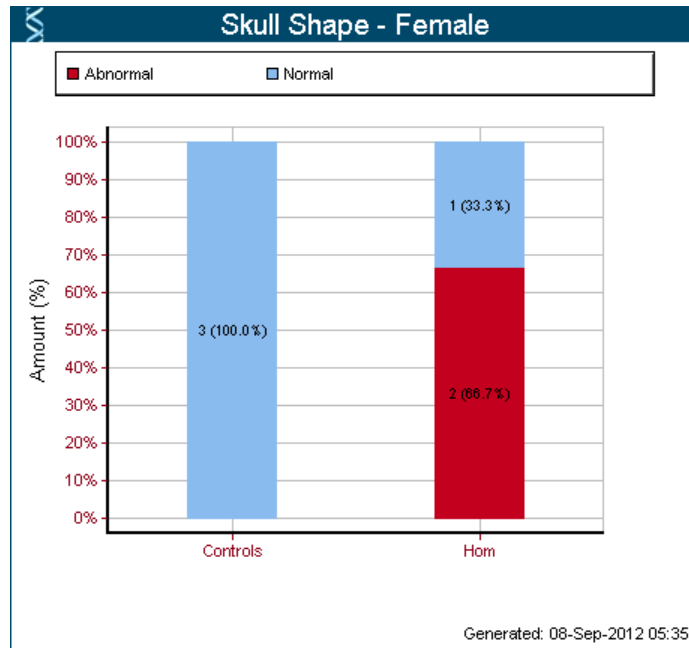
Female - Decreased Body Length [MP:0001258]

Stress-Induced Hypothermia



Female - Decreased Body Temperature [MP:0005534]

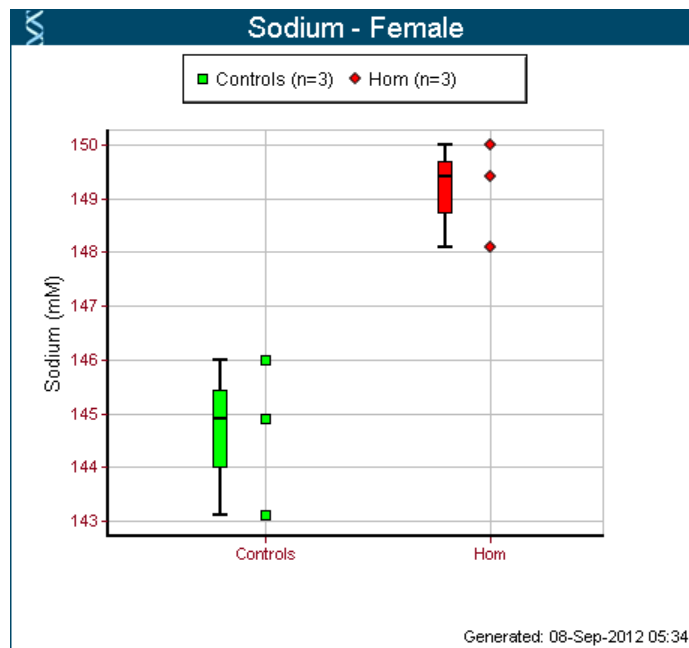
X-Ray Imaging



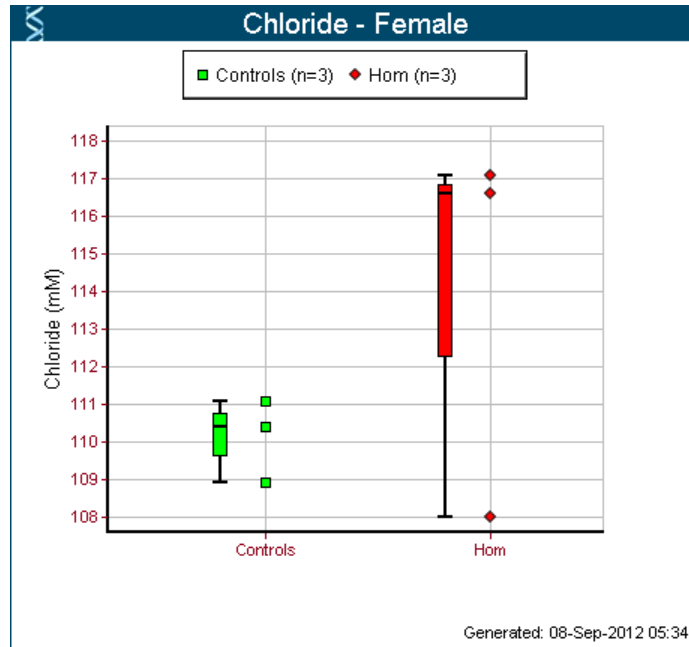
Female - Abnormal Cranium Morphology [MP:0000438].

Ex-vivo phenotyping

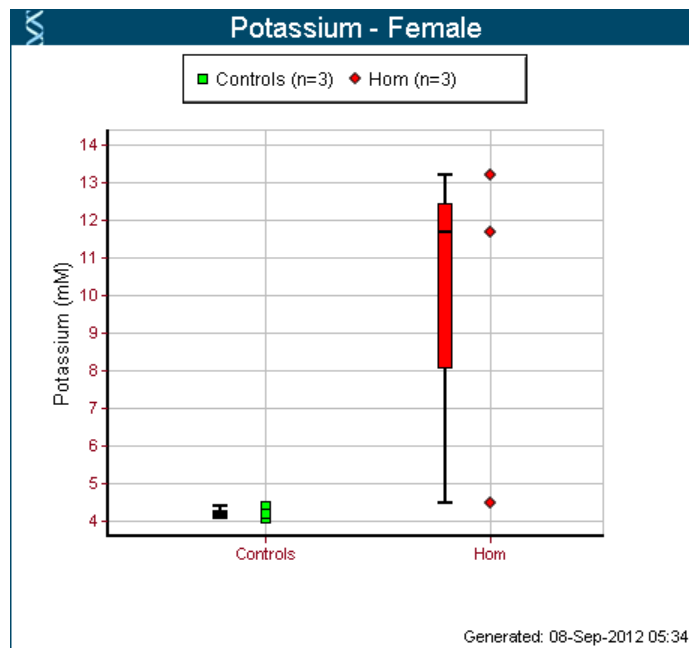
Plasma Chemistry



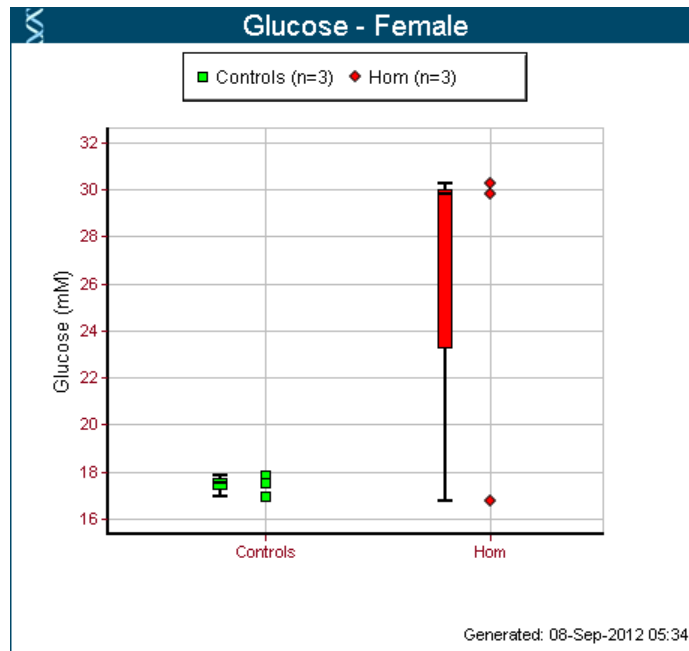
Female - Increased circulating sodium level [MP:0005633]



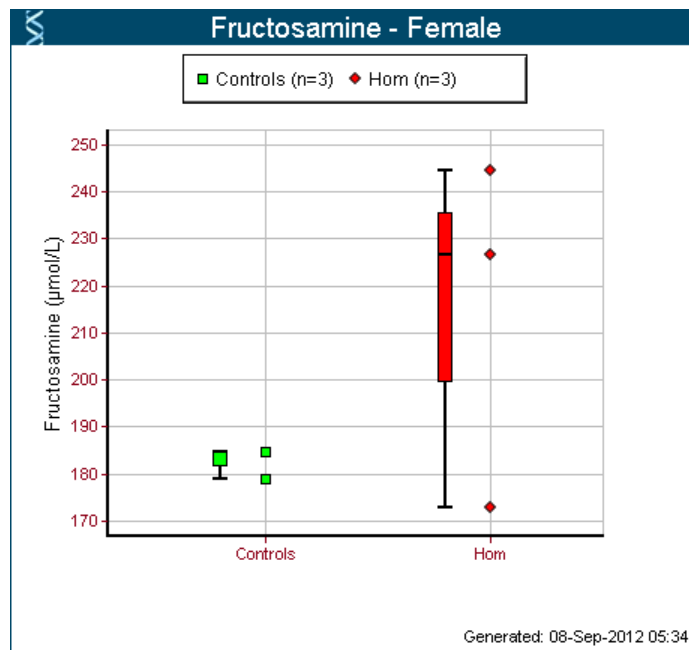
Female - Increased circulating chloride level [MP:0003019]



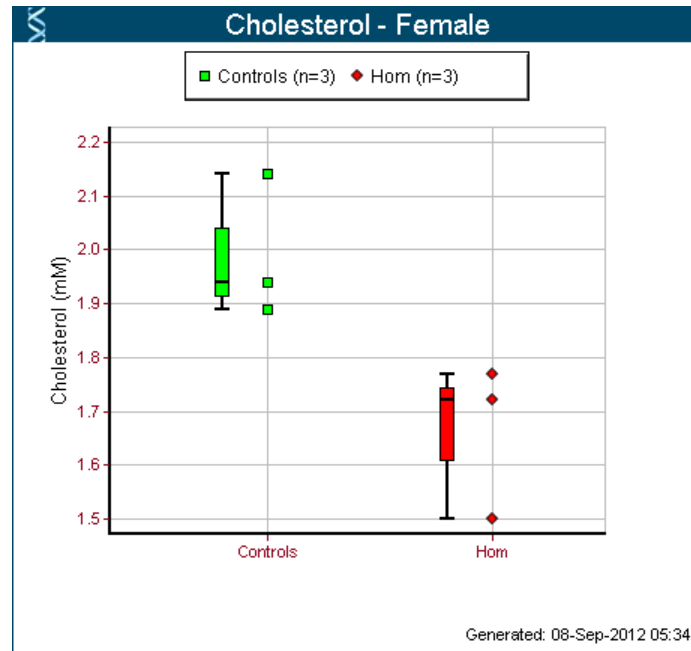
Female - Increased circulating potassium level [MP:0005627]



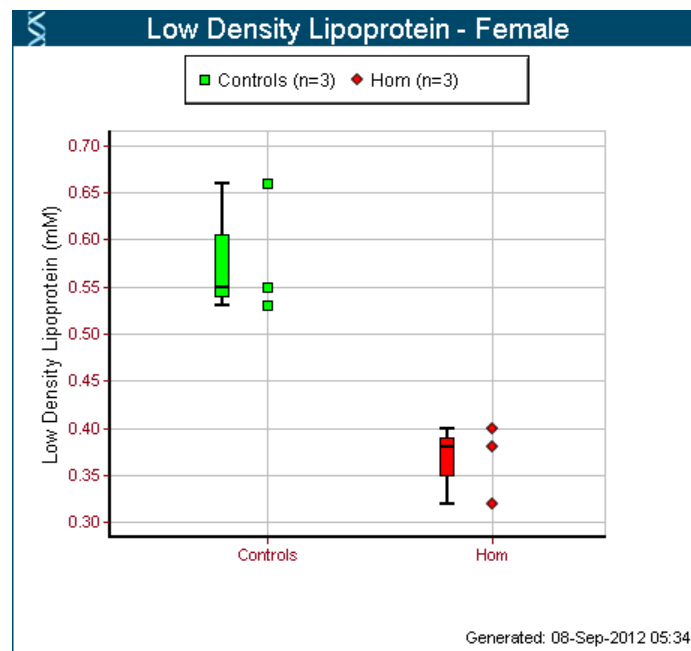
Female - Increased circulating glucose level [MP:0005559]



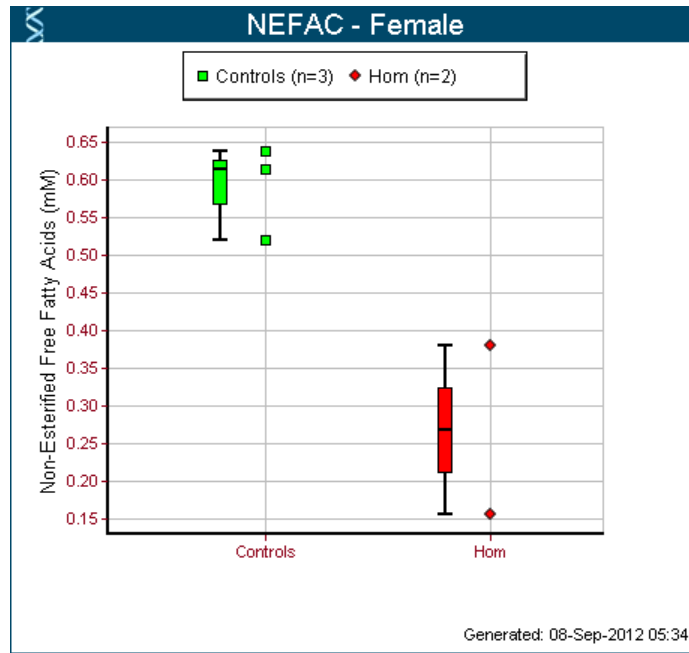
Female - Increased circulating fructosamine level [MP:0010087]



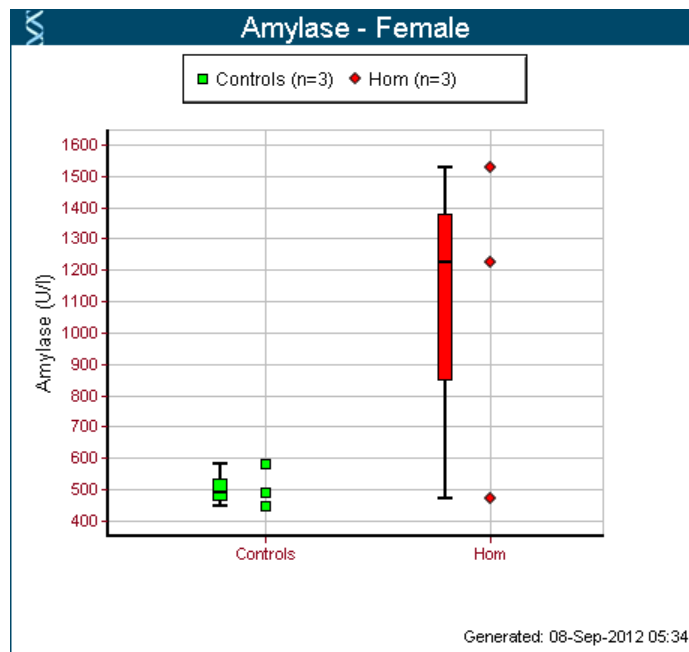
Female - Decreased circulating cholesterol level [MP:0005179]



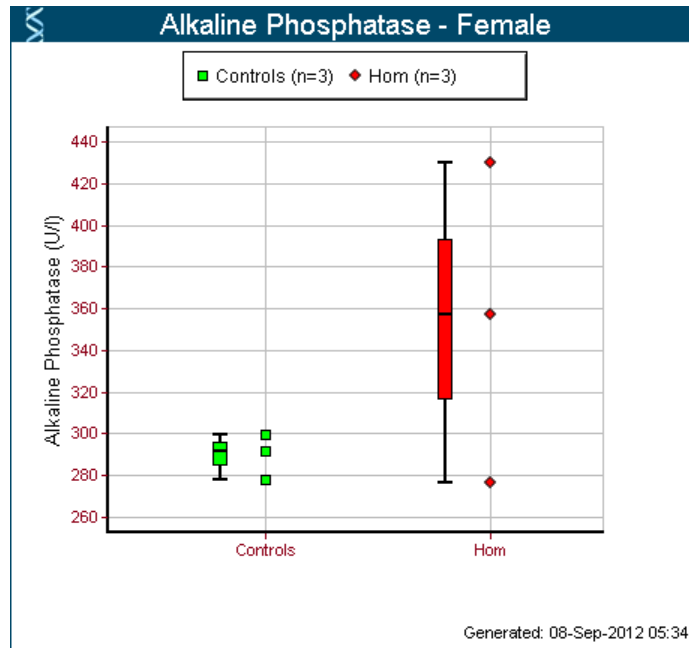
Female - Decreased circulating LDL cholesterol level [MP:0000183]



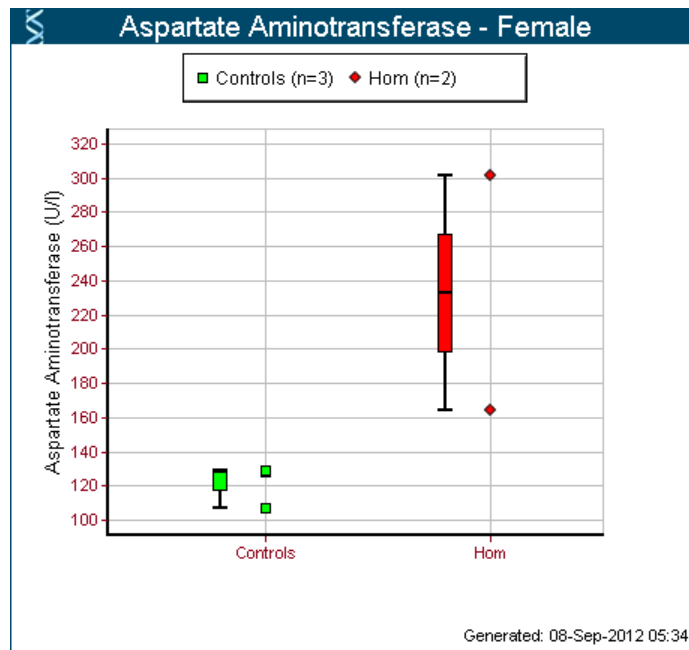
Female - Decreased circulating free fatty acid level [MP:0002702]



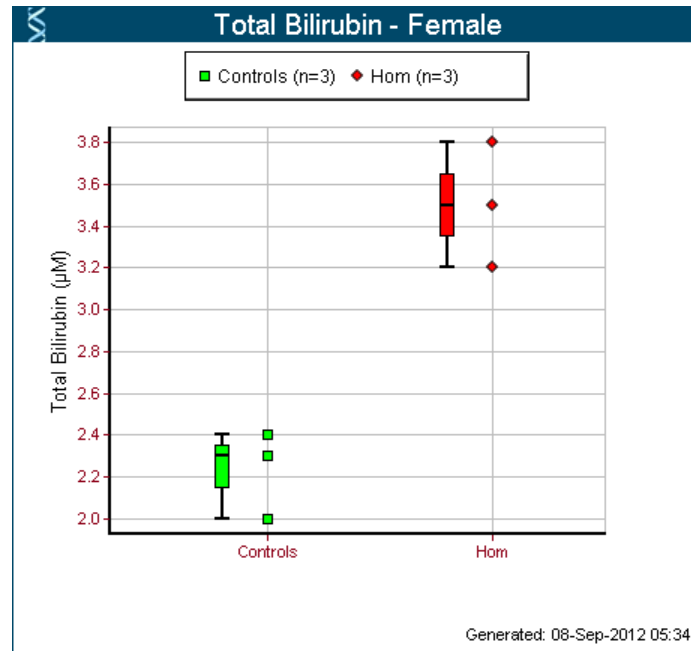
Female - Increased circulating amylase level [MP:0008806]



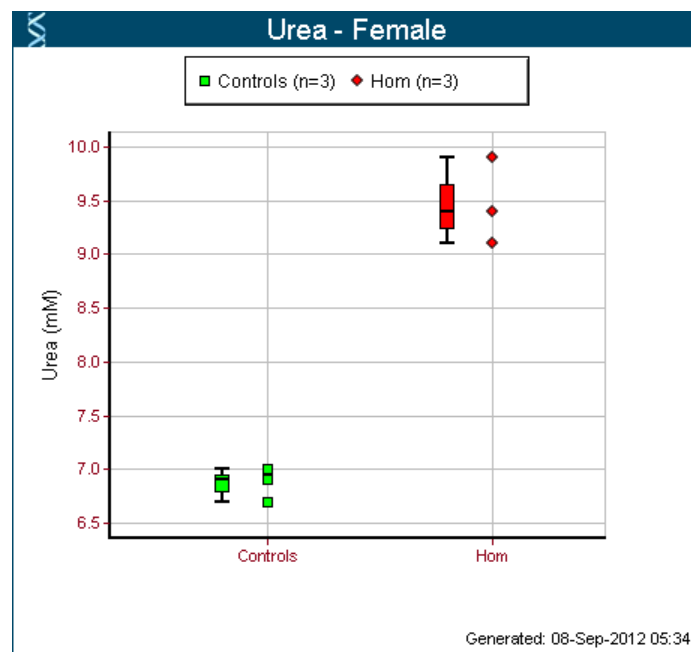
Female - Increased circulating alkaline phosphatase level [MP:0002968]



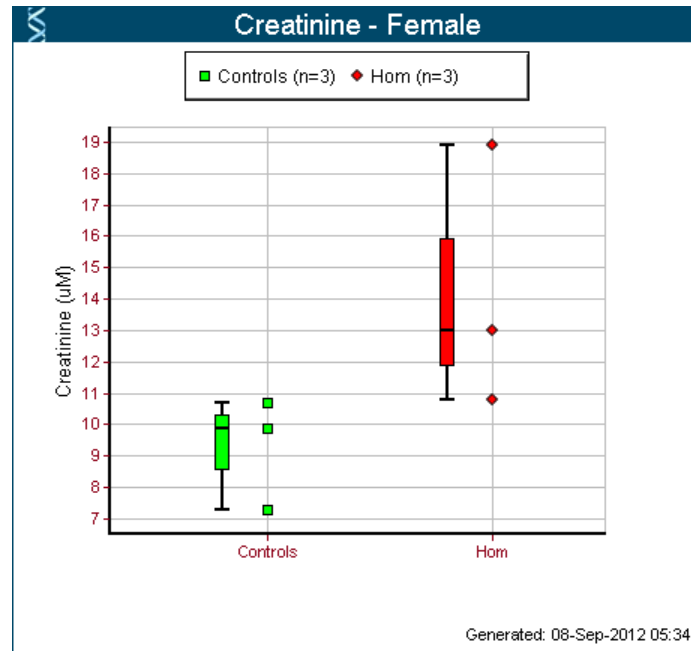
Female - Increased circulating aspartate transaminase level [MP:0005343]



Female - Increased circulating bilirubin level [MP:0005344]

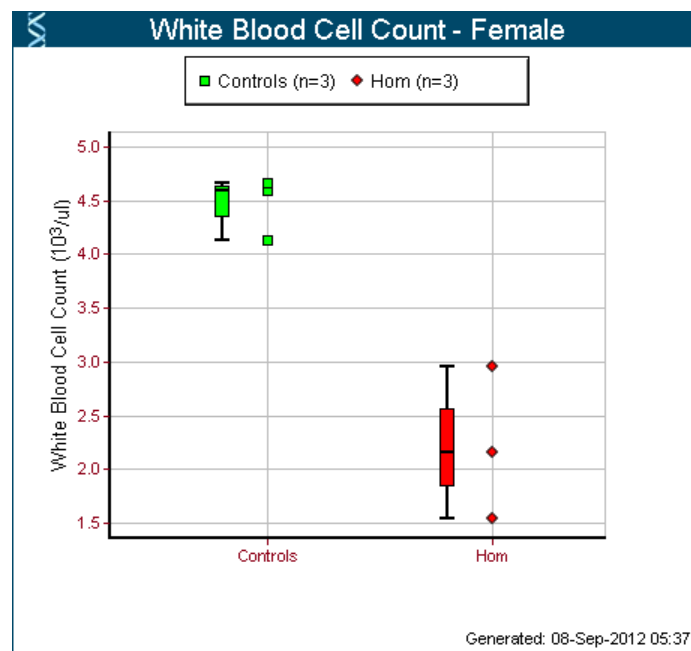


Female - Increased blood urea nitrogen level [MP:0005565]

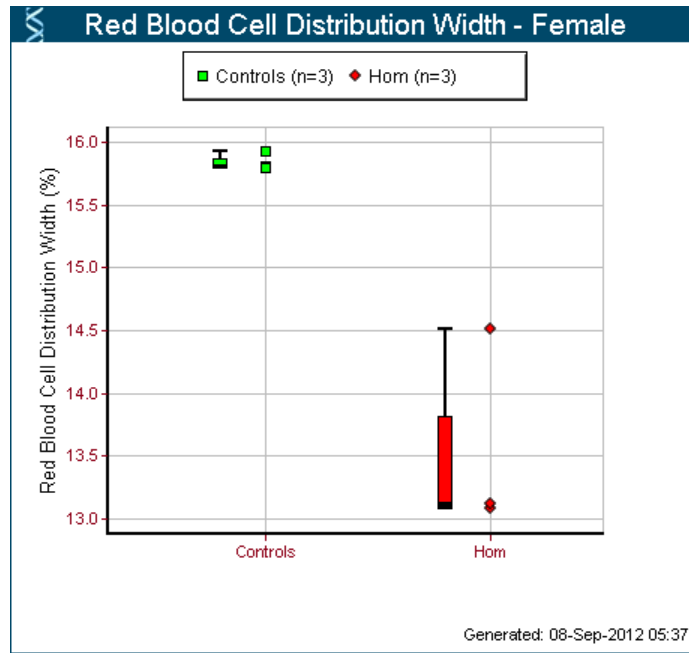


Female - Increased circulating creatinine level [MP:0005553]

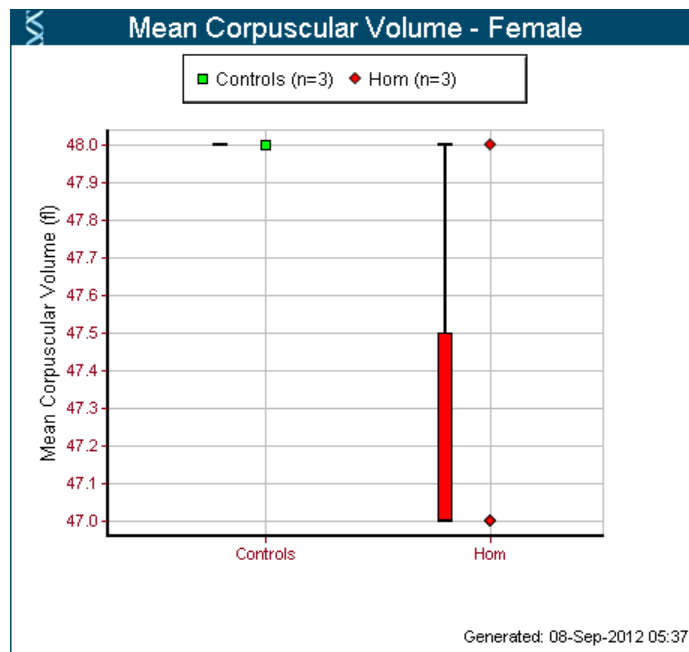
Haematology



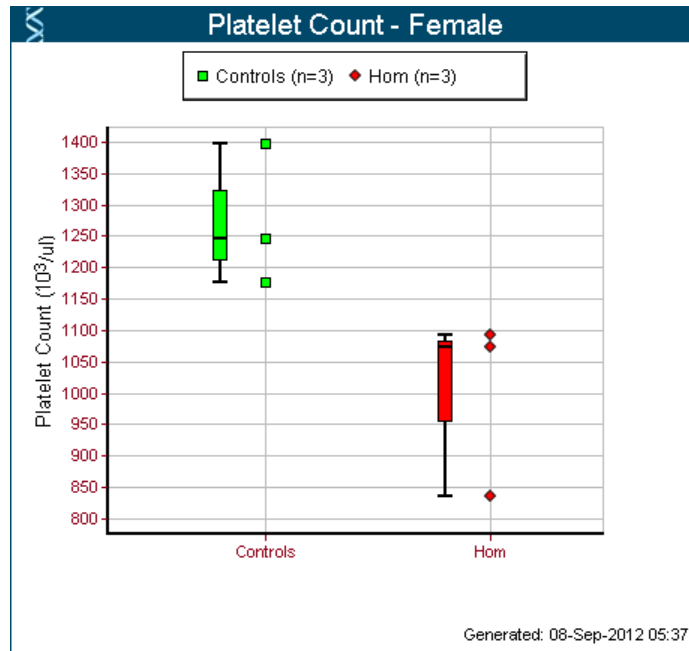
Female - Decreased leukocyte cell number [MP:0000221]



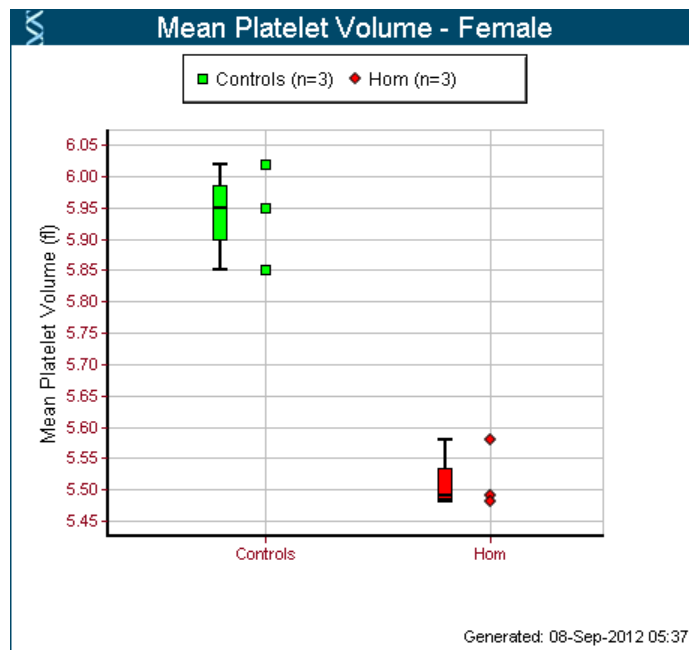
Female - Decreased red blood cell distribution width [MP:0010068]



Female - Decreased mean corpuscular volume [MP:0002591]

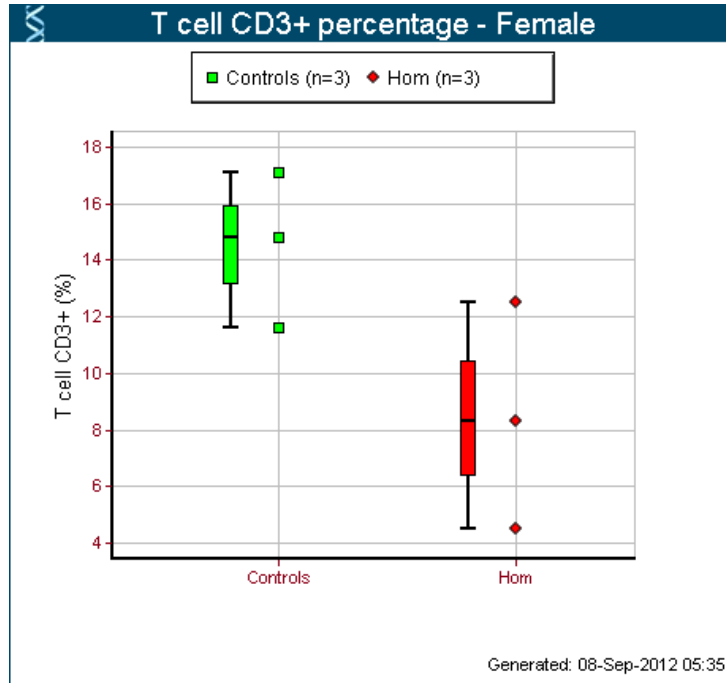


Female - Decreased platelet cell number [MP:0003179]

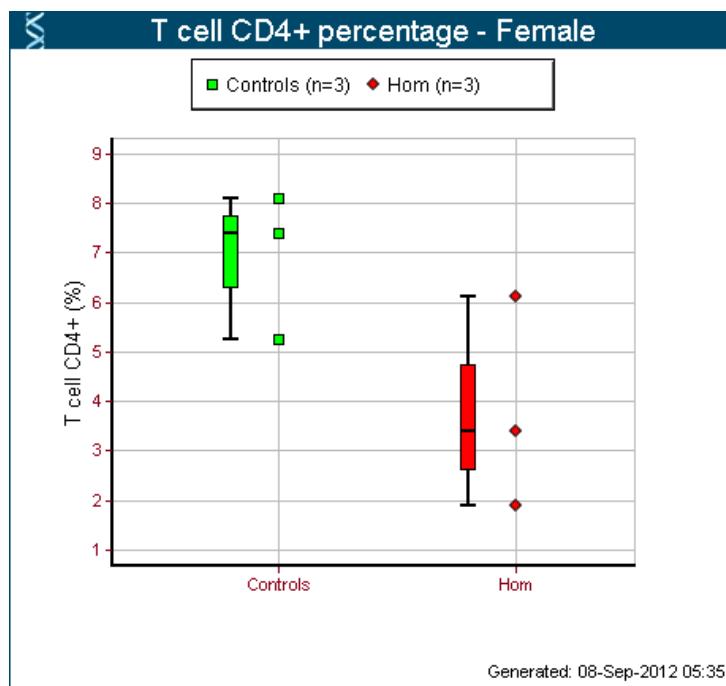


Female - Decreased mean platelet volume [MP:0008935]

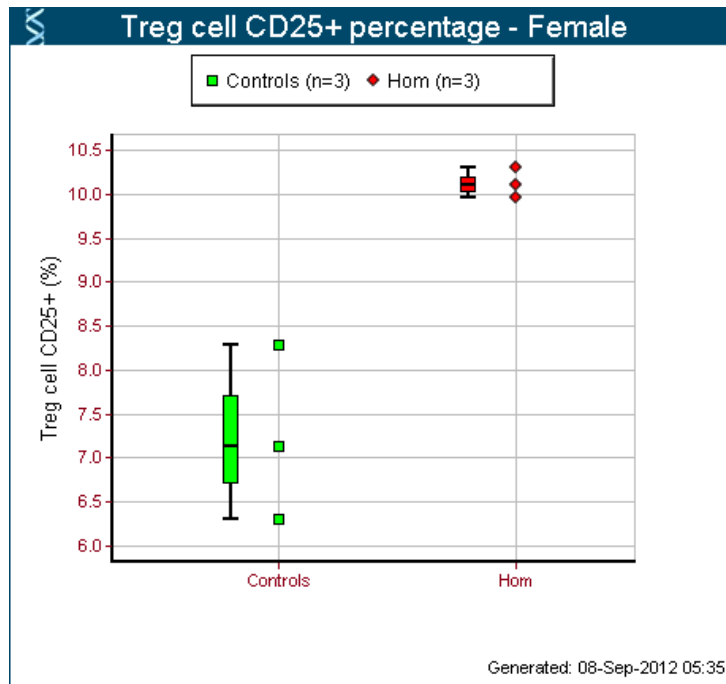
PBL Flow Cytometry Analysis



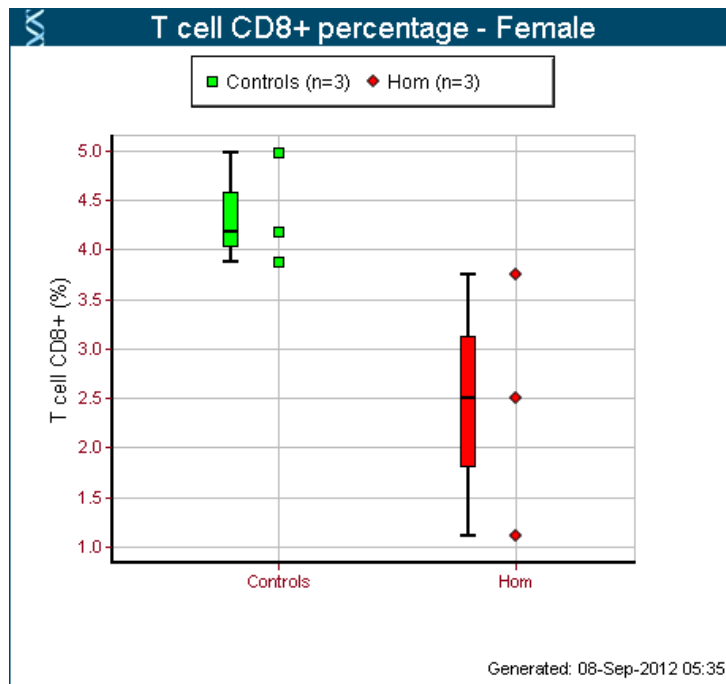
Female - Decreased T cell number [MP:0005018]



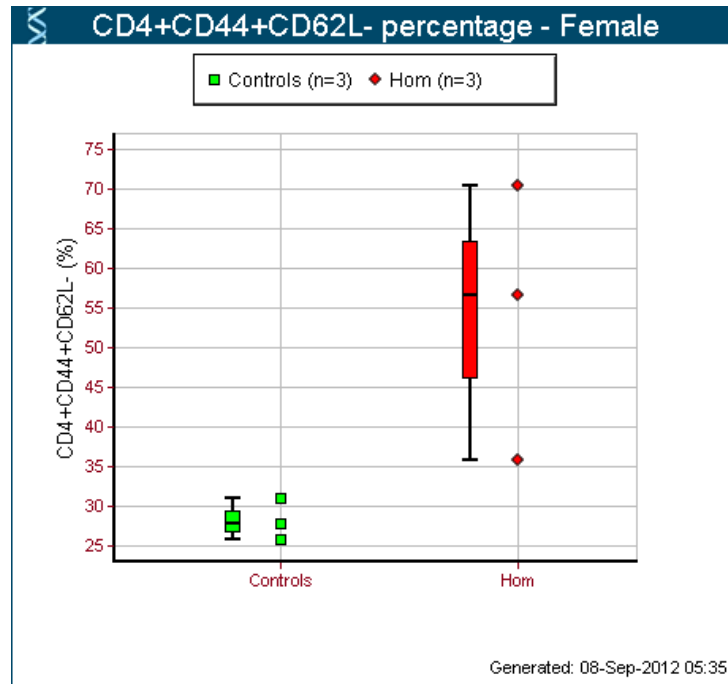
Female - Decreased CD4+ T cell number [MP:0008075]



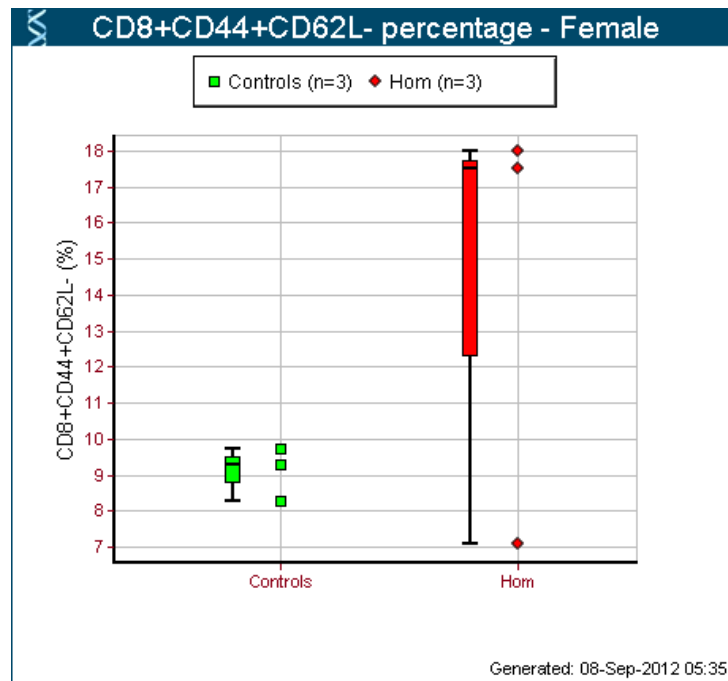
Female - Increased regulatory T cell number [MP:0010168]



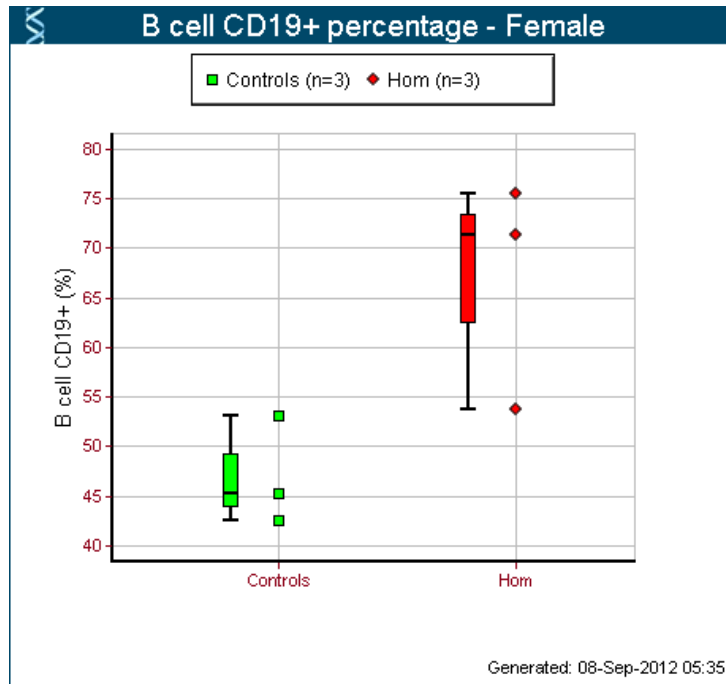
Female - Decreased CD8+ T cell number [MP:0008079]



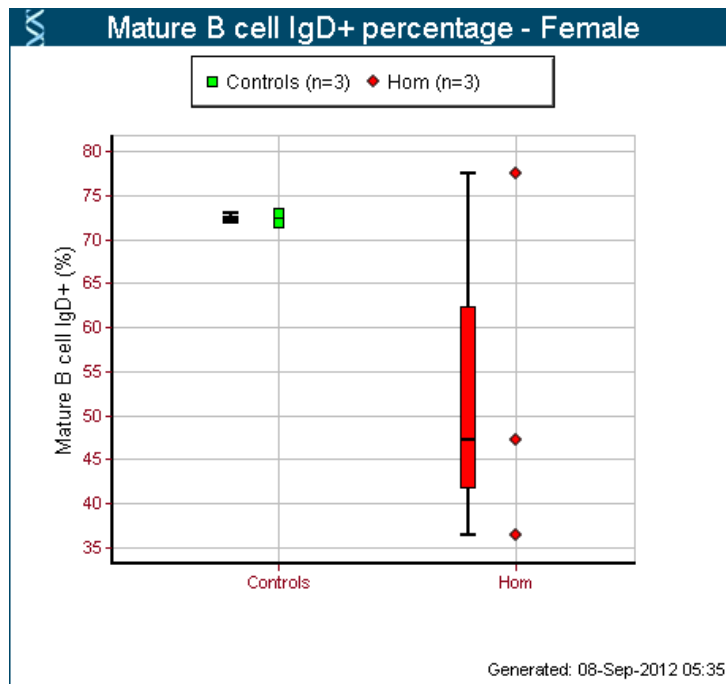
Female - Increased CD4+ alpha-beta memory T cell number [MP:0010835]



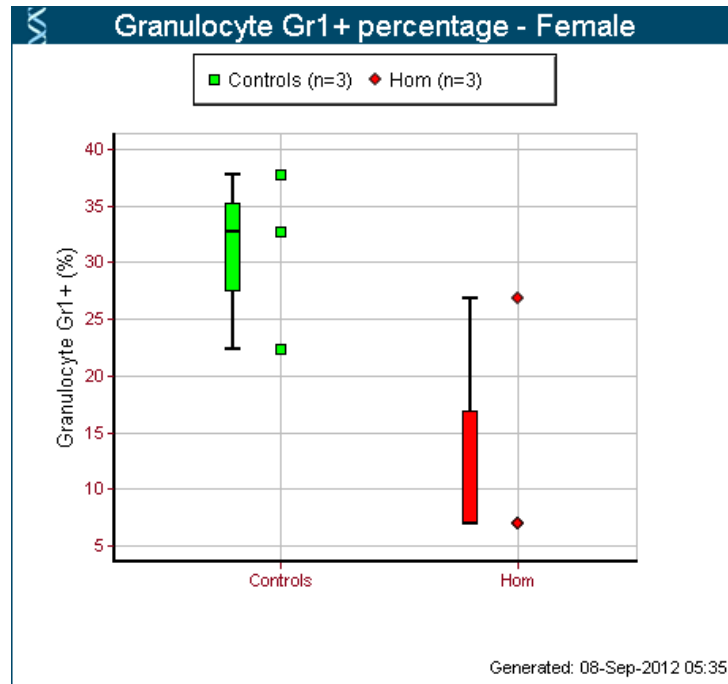
Female - Increased CD8+ alpha-beta memory T cell number [MP:0010838]



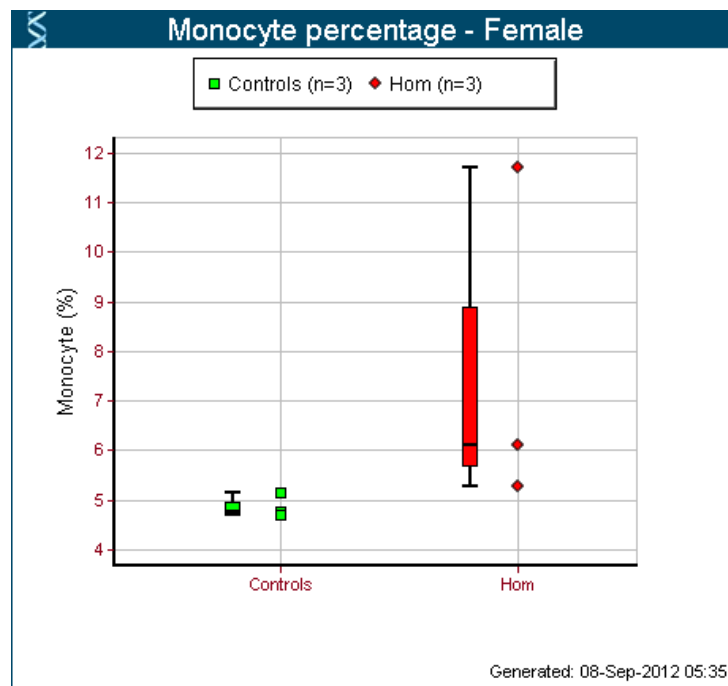
Female - Increased B cell number [MP:0005014]



Female - Decreased mature B cell number [MP:0008211]

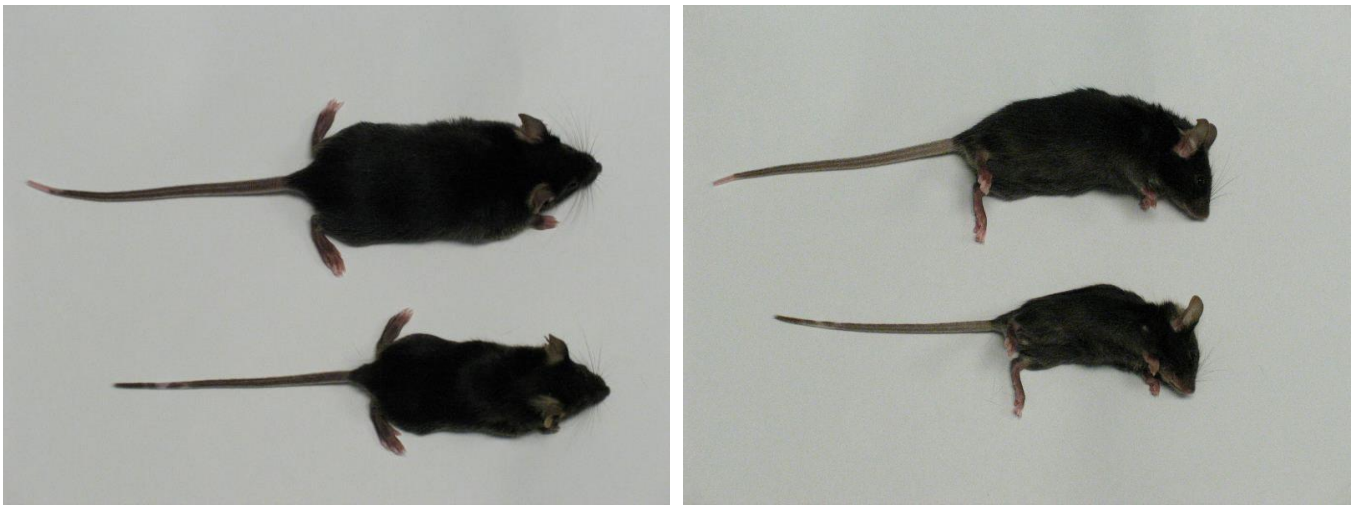


Female - Decreased Granulocyte cell number [MP:0000334]



Female - Increased monocyte cell number [MP:0000220]

Images



a b
Figure 1. (a) Dorsal view of wild-type (above) and homozygous (below) mice showing difference in body size. (B) Lateral view of wild-type (above) and homozygous (below) mice showing difference in body size. [MP:0001265]



a b
Figure 2. (a) Lateral view of wild-type and (b) lateral view of homozygous mouse skull displaying abnormal nasal bone. [MP:0000102]