

### 3i Tests

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<http://www.immunophenotype.org/threei/home>

This was a collaboration with the 3i (infection, immunity, immunophenotyping) project:

At the end of the pipeline at 16 weeks of age, a series of tissues and blood were collected from mutant and wild-type mice of both sexes at necropsy, stored and shipped to the different specialist collaborators in the consortium.

Flow Cytometric Analysis: of spleen, mesenteric lymph nodes and bone marrow of 3 male and 3 female mice. The flow-cytometric analysis comprised all major immune cell compartments of these organs.

Cytotoxic T Cell Function: the cytotoxicity of splenic CD8 T cells from 2 male and 2 female mice were assessed in a kill assay using P14 cells.

Anti-nuclear Antibody Assay: the plasma of 3 male and 3 female mice was tested for the presence of antinuclear IgG antibodies.

Microscopic Analysis: of the immunological composition of the ear epidermis in 2 male and 2 female mice, focussing on number, size and shape of Langerhans cells and dendritic epidermal T cells (DETC).

Mice were fed on Mouse Breeder Diet (5021, Labdiet) from weaning and anaesthetized with Ketamine/Xylazine at the end of the pipeline.

Organ/Test	Number of Mice	Readout
Spleen, mesenteric lymph node, bone marrow	3M, 3F	Flow cytometry
Antinuclear antibodies	3M, 3F	Microscopy
Ear epidermis	2M, 2F	Microscopy
Cytotoxic T lymphocytes	2M, 2F	Kill assay