

The

# GERMAN MOUSE CLINIC

## Report for Miz1

Helmut Fuchs, Valérie Gailus-Durner, Christoph Lengger, Beatrix Naton, Lore Becker, Ines Bolle, Markus Brielmeier, Julia Calzada-Wack, Claudia Dalke, Nicole Ehrhardt, Ralf Elvert, Tobias Franz, Elisabeth Grundner-Culemann, Sabine M. Hölter, Gabriele Hölzlwimmer, Marion Horsch, Anahita Javaheri, Svetoslav Kalaydjiev, Martina Klempt, Eva Kling, Sandra Kunder, Thomas Lisse, Holger Maier, Ildiko Racz, Claudia Reinhard, Ilka Schneider, Ralf Steinkamp, Johannes Beckers, Heidrun Behrendt, Dirk H. Busch, Jochen Graw, Gerhard Heldmaier, Heinz Höfler, Jack Favor, Thilo Jacob, Martin Klingenspor, Thomas Klopstock, Markus Ollert, Leticia Quintanilla-Fend, Jörg Schmidt, Holger Schulz, Eckhard Wolf, Wolfgang Wurst, Andreas Zimmer, and Martin Hrabé de Angelis

# The German Mouse Clinic



The German Mouse Clinic (GMC) was founded January 2002 at the GSF research center in Munich/Neuherberg to provide an open access platform for standardized mouse phenotyping. The GMC is supported by the National Genome Research Network (NGFN, <http://www.ngfn.de/>) and is a partner of the EUMORPHIA research program (<http://www.eumorphia.org/>).

In the GMC, experts from various fields of mouse genetics, physiology and pathology in close collaboration with clinicians work side by side at one location. We offer a primary phenotypic analysis of mouse mutants (more than 240 parameters/mouse) in the areas of allergy, behavior, bone and cartilage, cardiovascular diseases, clinical chemistry, energy metabolism, eye development and vision, immunology, lung function, molecular phenotyping, neurology, nociception, and pathology. Additional screens for host-pathogen interaction can be performed at the GBF Braunschweig. Secondary and tertiary screening for in depth analysis is offered by the different screens and is available on demand.

## Director

Prof. Dr. Martin Hrabé de Angelis  
Institute of Experimental Genetics  
GSF National Research Center for  
Environment and Health  
Ingolstädter Landstraße 1  
D-85764 Neuherberg / Munich  
Tel.: 089-3187-3302  
Fax: 089-3187-3500



*institute of*  
*experimental genetics* **ieg**

## Content

1	Summary.....	1
1.1	Primary Screening .....	1
1.2	Secondary Screening.....	2
2	General Part.....	3
2.1	The Role of the Gene.....	3
2.2	Known Phenotypes .....	3
2.3	Expected Phenotypes .....	3
2.4	Mice .....	4
2.4.1	Number and kind of mice .....	4
2.4.2	Housing conditions .....	4
2.5	Workflow .....	4
2.5.1	Standardized workflow for the primary screen in the German Mouse Clinic.....	4
2.5.2	Applied screens .....	6
2.5.3	Quality Management.....	6
2.6	Statistical Analysis of Data.....	6
2.7	References.....	6
3	Specific part .....	9
3.1	Behavior Screen .....	9
3.1.1	Summary .....	9
3.1.2	Mice .....	9
3.1.3	Material and Methods .....	9
3.1.4	Parameters .....	10
3.1.5	Results.....	10
3.1.6	Discussion .....	11
3.1.7	Reference .....	11
3.2	Dysmorphology, Bone and Cartilage .....	17
3.2.1	Summary .....	17
3.2.2	Mice .....	17
3.2.3	Material and Methods .....	17
3.2.4	Parameters .....	19
3.2.5	Results and Discussion.....	20
3.2.6	References .....	20
3.3	Neurology Screen .....	24
3.3.1	Summary .....	24
3.3.2	Mice .....	24
3.3.3	Material and Methods .....	24
3.3.4	Parameters .....	25
3.3.5	Results.....	26
3.3.6	Discussion .....	26
3.3.7	References .....	26

3.4	Eye Screen .....	33
3.4.1	Summary .....	33
3.4.2	Mice .....	33
3.4.3	Materials and Methods.....	33
3.4.4	Parameters .....	34
3.4.5	Results and Discussion.....	34
3.4.6	References .....	35
3.5	Clinical-Chemical Screen .....	37
3.5.1	Summary .....	37
3.5.2	Mice .....	37
3.5.3	Materials and Methods.....	37
3.5.4	Parameters .....	38
3.5.5	Results.....	39
3.5.6	Discussion .....	39
3.5.7	References .....	39
3.6	Immunology Screen .....	43
3.6.1	Summary .....	43
3.6.2	Mice .....	43
3.6.3	Material and Methods .....	43
3.6.4	Parameters .....	44
3.6.5	Results.....	44
3.6.6	Discussion .....	44
3.6.7	References .....	45
3.7	Allergy Screen.....	47
3.7.1	Summary .....	47
3.7.2	Mice .....	47
3.7.3	Material and Methods .....	47
3.7.4	Results and Discussion.....	47
3.7.5	References .....	48
3.8	Nociceptive Screen .....	49
3.8.1	Summary .....	49
3.8.2	Mice .....	49
3.8.3	Material and Methods .....	49
3.8.4	Parameters .....	50
3.8.5	Results and Discussion.....	50
3.8.6	References .....	50
3.9	Lung Function Screen.....	52
3.9.1	Summary .....	52
3.9.2	Mice .....	52
3.9.3	Material and Methods .....	52
3.9.4	Parameters .....	54
3.9.5	Results and Discussion.....	54
3.9.6	References .....	55
3.10	Expression Profiling .....	58
3.10.1	Summary .....	58
3.10.2	Mice .....	58
3.10.3	Material and Methods .....	59
3.10.4	Discussion .....	61
3.10.5	References .....	61

3.11	Metabolic Screen .....	62
3.11.1	Summary .....	62
3.11.2	Mice .....	62
3.11.3	Material and Methods .....	62
3.11.4	Parameters .....	63
3.11.5	Results and Discussion.....	63
3.11.6	References .....	63
3.12	Pathology Screen.....	66
3.12.1	Summary .....	66
3.12.2	Mice .....	66
3.12.3	Materials and Methods.....	66
3.12.4	Results.....	67
3.12.5	Discussion .....	69
3.12.6	References .....	69

# 1 Summary

## 1.1 Primary Screening

In a primary screen 54 mice (25 *Miz1*<sup>-/-</sup> mutants and 29 *Miz1*<sup>+/+</sup> wild-type littermate controls) were analyzed in the German Mouse Clinic (GMC) in the screens Behavior, Dymorphology, Bone and Cartilage, Neurology, Eye, Clinical Chemistry, Immunology, Allergy, Nociception, Lung Function, Metabolism, Expression Profiling and Pathology.

**Behavior:** Mutant females presented increased vertical exploratory (rearing) activity and increased social affinity. No significant behavioral alterations were detected in mutant males. These results confirm the results obtained with a first, smaller batch of *Miz1* mice received for initial behavioral overview analysis prior to introduction of this line to the GMC. At present, it is unclear whether the observed behavioral pattern is related to the middle ear phenotype.

**Neurology:** The comparison of mutant to control mice showed that mutant mice had a significantly retarded body constitution which was displayed in their reduced body weight and body length in 10-week-old mice. Furthermore, we detected an altered pelvic elevation, tail elevation, and wire maneuver behavior in male mutant mice.

**Immunology:** Under standard screen conditions, mutant mice showed significant changes in the percentage of B cells and NK cells. The lower numbers of B cells probably reflect a real phenotype, since this was confirmed for two different B-cell markers. However, under baseline conditions those changes did not lead to altered Ig production - similar isotype levels were found in mutants and controls (with the exception of IgM, which seemed to be higher in mutant males). Further immunological investigations in a varied setting could be interesting.

**Lung Function Screen:** Comparing control to mutant mice, the mutants showed reduced tidal volumes resulting in a reduced breathing pattern in both sexes. Further investigations are needed to determine whether these results are secondary effects of, e.g. reduced muscle function or are primarily related to impaired lung function.

**Pathology:** We confirmed a minor difference in the testis weight, i.e. slight reduction in mutant males. However, no morphological differences between wild-type and mutant mice were observed. We did not find additional genotype-specific alterations.

In the screens **Dymorphology, Eye, Allergy, Nociception, Clinical Chemistry, Metabolism, and Expression Profiling** no genotype-specific differences could be found.

## 1.2 Secondary Screening

Secondary screening is suggested from the screens Neurology and Behavior.

Behavior secondary screens in acoustic startle reflex (ASR) have already been performed in accordance with the mouse provider (see 3.1.5). In addition to these experiments we would recommend analyzing :

**Neurology Screen:** In order to confirm a neurological / muscular phenotype, further neurological examinations have to be done. We suggest secondary screening of the mice with particular emphasis on grip strength and Rotarod testing.

Please contact Valérie Gailus-Durner to discuss further steps and details.

## 2 General Part

### 2.1 The Role of the Gene

PIAS $\alpha$  and PIAS $\beta$  are splicing variants of the *Piasx/Miz1* gene. PIAS means “Protein Inhibitor of Activated STAT”, *Miz1* means “Msx2 interacting zinc finger 1”. PIAS Proteins function as SUMO-1 E3 ligases (Jackson, 2001). SUMO-1 (Small Ubiquitin-like Modifier) is a small protein of 101 amino acids which can be covalently linked to other proteins. In contrast to ubiquitination, sumolation does not necessarily result in degradation of the target protein. Instead, SUMO-1 attachment regulates the stability, protein-protein interaction properties, transcriptional activity and/or cellular localization of the target protein. In addition to the ligase activity, PIASx has been shown to be a transcriptional corepressor of *Stat4*-dependent transcription and to coregulate steroid receptor dependent transcription (Müller *et al.*, 2001).

### 2.2 Known Phenotypes

The mutation of the *Miz1* gene was generated by integration of a genetrapp vector into Intron 2. No wild-type mRNA is produced in homozygous mice. It could be shown that *Miz1* is ubiquitously expressed, especially in the adult brain. Since PIAS proteins regulate steroid-dependent transcription, it would be well possible that memory function and behavior of mutant animals is impaired. In a primary behavioral screen, **behavioral changes** could be observed. In detail, male mutants showed an increased hole exploration (directed exploration behavior) and reduced object recognition ability. Female mutants showed increased number of rearings (activity exploration) and stayed longer in a group (increased social behavior). *Miz1*<sup>-/-</sup> animals are **viable** and **fertile**; they do not show gross morphological alterations. However, subtle morphological phenotypes could be observed, namely, a **reduction of testis weight** and a **reduced sperm count** (published later by Santti *et al.*, 2005).

All further findings we consider as new.

### 2.3 Expected Phenotypes

PIASx is a SUMO ligase that was shown to modify steroid receptor signalling (Müller *et al.*, 2001). This suggests that PIASx

- might influence the functions of gonads, sex organs, and various "nonreproductive" organs and systems, including muscles, liver, skin, nervous system, and the immune system via modification of androgen receptor action.
- might modify HPA axis function via modification of glucocorticoid receptor action.

## 2.4 Mice

### 2.4.1 Number and kind of mice

Fifty-seven *Miz1*-deficient and their littermate controls arrived in week 39 in 2003. Additionally 20 heterozygous littermates were delivered. As described by the sender, the mice analyzed were a 6<sup>th</sup> backcross generation to C57BL/6 originating from gene-trapped ES-cell line TBV-2.

### 2.4.2 Housing conditions

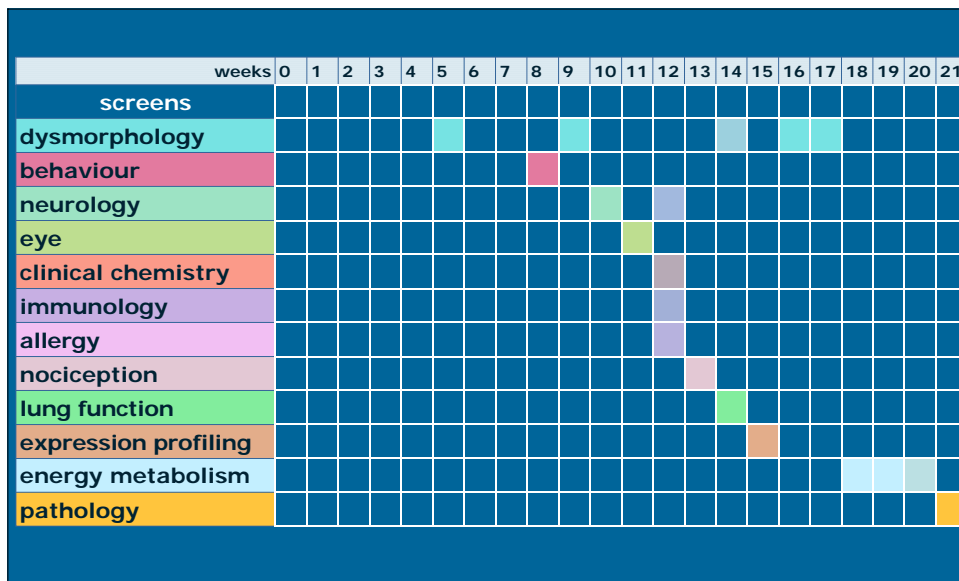
In the GMC mice are housed in type II polycarbonate cages in individually ventilated caging (IVC) systems (VentiRack Bioscreen TM, Biozone, Margate, UK) on wood fiber (Altromin, Lage, Germany). The IVCs operate with positive pressure. Mice are transferred in weekly intervals to new cages with forceps in Laminar Flow Class II changing stations. Mice are fed with irradiated standard rodent high energy breeding diet (Altromin 1314) and given semidemineralized filtered (0.2 µm) water *ad libitum*. Light is adjusted to a 12h/12h light/dark cycle; temperature and relative humidity are regulated to  $22 \pm 1^\circ\text{C}$  and  $55 \pm 5\%$ , respectively. In specified modules husbandry conditions are adjusted according to the experiment requirements (See corresponding sections). All people attending the facility completely change their garment (jackets and trousers autoclaved) and shoes and wear caps and masks before entering the GMC (Brielmeier *et al.*, 2002).

Outbred 8-week-old male SPF Swiss mice are used as sentinels and kept on a mixture of new bedding and aliquots of soiled bedding (50:50) from all cages of the IVC rack. In addition, the sentinels were also exposed to soiled air from all “upstream” cages of the IVC rack. Health monitoring is carried out by on-site examination of the sentinel mice by certified laboratories according to FELASA recommendations ([www.felasa.org](http://www.felasa.org)). Mice are kept according to the German laws. Tests were carried out by authority of the Regierung von Oberbayern.

## 2.5 Workflow

### 2.5.1 Standardized workflow for the primary screen in the German Mouse Clinic

Mouse mutants entering the GMC are examined in a primary screen according to the following standard workflow (Fig. 1). Analyzed parameters are listed in Table 1.



**Figure 1: Workflow of the primary screen**

Explanation below,  Analysis of blood-based parameters.

After the mice arrive at the GMC, they are acclimatized in the new environment for one week. The males then start in the Behavior Screen. There they stay for three weeks. Directly after the Behavior Tests, the anatomical inspection of the Dysmorphology Screen is performed. In the next week, the Neurology Screen is applied. One week later the mice go through the tests of the Eye Screen. When the mice were 12 weeks old, blood is taken, and samples are distributed to the blood-based screens for Clinical Chemistry, Immunology, Allergy and the Lactate test. One week later, the animals are tested in the Nociceptive Screen. Two weeks after testing of the first blood sample, a second sample is taken to confirm outliers, and to supply the Dysmorphology Screen with material for determination of blood-based bone-related parameters. In parallel, 10 mutant animals (5 males / 5 females) and 10 controls (5 males / 5 females) leave the animal facility for the Lung Function Analysis, which for technical reasons is located elsewhere. These animals are, for hygienic reasons, not allowed to re-enter the German Mouse Clinic. The females go directly to Pathology. The males are used to freeze organs for future expression profiling on demand (remaining organs from those animals are analyzed by the Pathology). All other animals go through the bone and cartilage tests of the Dysmorphology Screen, and then stay three weeks in the Metabolic Screen. After completion of the primary screen, all animals end up in the Pathology.

The screening of female animals starts one week later and follows the same workflow (with the exception of Expression Profiling sampling). Deviations from our Standard operation procedure (SOP) are listed below; please take the specific number of analyzed animals from the sections of the applied screen.

### **2.5.2 Applied screens**

The GMC standard workflow for the primary screen as described above was applied to analyze the Miz1 mice. As the demanded number of 60 animals (15 mice per sex per genotype) could not be delivered, the workflow was adapted to the available number of animals. Some parameters from the blood based screens could not be determined in all animals, as it was not possible to get the needed amount of blood from these animals. A few animals died during the primary screen and thus they could not be analyzed for all parameters.

### **2.5.3 Quality Management**

As a routine quality control, we take blood samples from for serological tests of the sanitary status of all mice after they went through the GMC primary screen. When indicated, the serum is tested for MHV (BioDoc, Hannover). We chose MHV as a "sentinel" pathogen, as it is one of the most common viruses in mouse facilities worldwide and it is transmitted easily. To be open for collaboration for as many partners as possible, we allow MHV positive animals to enter our facility.

Microgranulomas in the liver are observed commonly in mice on a C57BL/6 genetic background. In those cases the results of the MHV tests are used to exclude MHV as one possible reason for these infiltrates (See chapter 3.12 Pathology Screen).

## **2.6 Statistical Analysis of Data**

If not otherwise stated, data of males and females was analyzed separately comparing mutant and control data using a Student's t-test. Sex differences within the mutant or the control group also were determined with a t-test. Tables summarizing the data will show mean  $\pm$  standard error of the mean. Significant differences are indicated stepwise from 0.05, 0.02, 0.01, 0.001 to 0.0001.

## **2.7 References**

Brielmeier M., H. Fuchs, G. Przemeck, V. Gailus-Durner, M. Hrabé de Angelis, J. Schmidt (2002) The GSF – Phenotype Analysis Center (German Mouse Clinic, GMC): A sentinel-based health-monitoring concept in a multi-user unit for standardized characterization of mouse mutants. In: J. Guenet and C. Herweg (Eds.) Laboratory Animals Science - Basis and Strategy for Animal Experimentation Vol. 11, Proceedings of the 8th FE-LASA Symposium, Laboratory Animals Ltd., Aachen, pp. 19-22.

Jackson P.K. (2001): A new RING for SUMO: wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. *Genes Dev.* 15: 3053-3058.

Müller S. *et al.*, (2001): SUMO, ubiquitin's mysterious cousin. *Nat. Rev. Mol. Cell Biol.* 2, 202-210.

Santti H, Mikkonen L, Anand A, Hirvonen-Santti S, Toppari J, Panhuysen M, Vauti F, Perera M, Corte G, Wurst W, Janne OA, Palvimo JJ. (2005) Disruption of the murine PIASx gene results in reduced testis weight. *J Mo Endocrinol.* 34 (3): 645-54.

### Abbreviations and Wording

Miz1	<i>Msx2</i> interacting zinc finger 1, synonym to PIASx- $\beta$ (protein) and mutant mouse line <i>italics: Miz1</i> gene
GMC	German Mouse Clinic
IVC	individually ventilated cage
+/+	homozygous wild type, control
-/-	homozygous mutant
FELASA	European Laboratory Animal Science Associations, 25 Shaftesbury Avenue, London W1D 7EG, UK, <a href="http://www.felasa.org">www.felasa.org</a>

**Table 1: Primary Screen at GMC**

<b>Screens</b>	<b>Goal</b>	<b>Methods</b>
<b>Dysmorphology, Bone and Cartilage</b>	morphological analysis of body, skeleton, bone and cartilage	morphological observation, bone densitometry, X-ray, AVL analyzer, micro-computer tomography
<b>Behavior</b>	locomotor, exploratory, emotional and social behavior, object recognition memory	modified hole board
<b>Neurology</b>	assessment of muscle, spinocerebellar, sensory, and autonomic function	modified SHIRPA protocol
<b>Eye</b>	assessment of morphological and functional alterations of the eye	electroretinography, slit lamp biomicroscopy
<b>Clinical Chemistry</b>	determination of clinical-chemical and hematological parameters in blood	blood autoanalyzer, ABC-animal blood counter
<b>Immunology</b>	analysis of peripheral blood samples for immunological parameters	flow cytometry, ELISA
<b>Allergy</b>	analysis of total plasma IgE	ELISA
<b>Nociception</b>	detection of altered pain response	hot plate assay
<b>Lung function</b>	assessment of alterations in breathing patterns	whole body plethysmography (Buxco®)
<b>Expression Profiling</b>	RNA expression profiling	DNA-chip technology
<b>Energy Metabolism</b>	measurement of altered body weight regulation, body temperature and energy balance	bomb calorimetry
<b>Pathology</b>	microscopic and macroscopic examination	histology, immunochemistry

# 3 Specific part

## 3.1 Behavior Screen

### 3.1.1 Summary

The modified hole board test is used as primary screen in the behavioral phenotyping module of the GMC, because it allows the comprehensive analysis of a range of behavioral parameters known to be indicative of behavioral dimensions such as locomotor activity, exploratory behavior, arousal, emotionality, memory and social affinity in a single short test (See Ohl *et al.*, 2001).

Mutant females presented with increased vertical exploratory (rearing) activity and increased social affinity. No significant behavioral alterations were detected in mutant males. These results confirm the results obtained with a first, smaller batch of mice received for initial behavioral overview analysis prior to introduction of this line to the GMC. At present, it is unclear whether the observed behavioral pattern is related to the middle ear phenotype (see discussion).

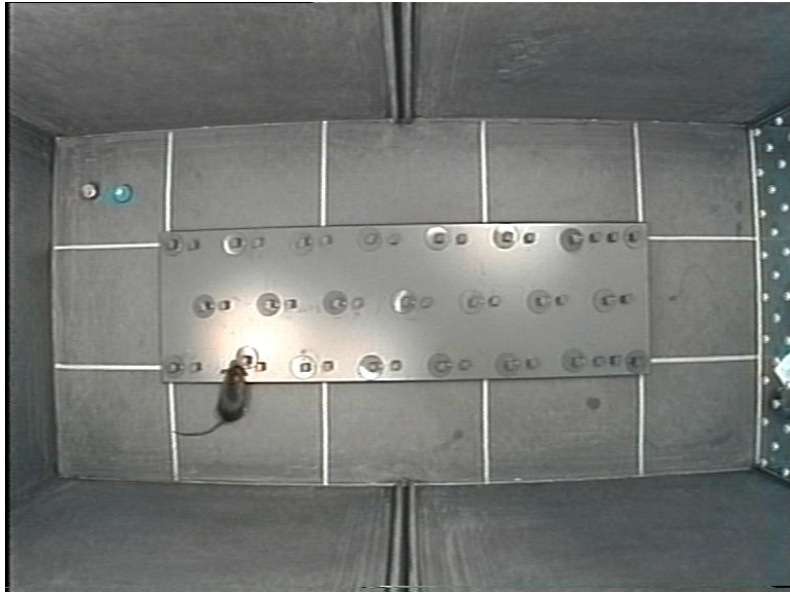
### 3.1.2 Mice

Mice were housed with food and water *ad libitum* under standard laboratory conditions. Animals were separated based on sex, but not genotype. They entered the laboratory at the age of six weeks, were given two weeks for acclimatization and were tested at the age of eight weeks. Three days before testing, an object (metal cube) was placed into the home cage and removed one day before testing.

In this screen, 25 female mice (13 wt, 12 mutants) and 28 male mice (15 wt, 13 mutants) were available for analysis.

### 3.1.3 Material and Methods

**The modified hole board test** was carried out according to the procedures described by Ohl *et al.*, 2001. The test apparatus consisted of a test arena (100 x 50 cm), in the middle of which a board (60 x 20 x 2 cm) with 23 holes (1.5 x 0.5 cm) staggered in three lines with all holes covered by movable lids was placed, thus representing the central area of the test arena as an open field. The area around the board was divided into 12 similarly sized quadrants by lines taped onto the floor of the box (See Ohl *et al.*, 2001). Both box and board were made of dark grey PVC. All lids were closed before the start of a trial. For each trial, an unfamiliar object (a blue plastic tube lid, similar in size to the metal cube) and the familiar object (metal cube) were placed into the test arena with a distance of 2 cm between them. The illumination levels were set at approximately 150 lux in the corners and 200 lux in the middle of the test arena.



**Figure 2: Test Arena for modified hole board test.**

For testing, each animal was placed individually into the test arena and allowed to explore it freely for 5 min. The animals were always placed into the test arena in the same corner next to the partition, facing the board diagonally. The two objects were placed in the corner quadrant diametrical to the starting point. During the 5 min trial, the animal's behavior was recorded by a trained observer with a hand-held computer. Data were analyzed by using the Observer 4.1 Software (Noldus, Wageningen). Additionally, a camera was mounted 1.20 m above the center of the test arena, and the animal's track was videotaped and its locomotor path analyzed with a video-tracking system (Ethovision 2.3, Noldus, Wageningen). After each trial, the test arena was cleaned carefully with a disinfectant.

**Data were statistically analyzed** using SPSS software (SPSS Inc, Chicago, USA). The chosen level of significance was  $p < 0.05$ .

### 3.1.4 Parameters

<b>Manually recorded behavior</b>
Line crossings, rearings, board entries, hole explorations, hole visits, stretched attends (risk assessment), partition (group contact), grooming, defecation, unfamiliar and familiar object exploration
<b>Video-track analysis</b>
Total distance moved, mean velocity, maximum velocity

### 3.1.5 Results

Female mutants demonstrated increased vertical exploratory behavior (rearing), and increased social affinity as indicated by percentage time spent in

group contact. These parameters were altered in the same direction in male mutants, but not significantly (Table 2). In addition, female mutants demonstrated a shorter maximum stay on the board and an increased distance to the board, which might be related to the increased time spent at the partition to the group compartment (Table 3). No other parameters were significantly altered, and no significant alterations were detected in male mutants.

### 3.1.6 Discussion

These results confirm the results obtained with a first, smaller batch of Miz1 mice received for initial behavioral overview analysis prior to introduction of this line to the GMC.

In a **secondary screen**, a third batch of Miz1 mice was analyzed for acoustic startle response (ASR) at the age of 9-11 weeks, which indicated a strong reduction of ASR in female mutants, but only a tendency in the same direction in males (Figs. 3 and 4). These results are not shown in detail here because they are part of the secondary screen and the mouse provider has already got all original data files. In the meantime, there is histological evidence that the middle ear of mutants is filled with tissue and that they may suffer from a chronic otitis, which might, at least partially, explain the ASR phenotype.

It might be interesting to discuss with the Dymorphology screen, whether the chronic otitis could be due to an involvement of *Miz1* in craniofacial development.

At present, it is unclear whether the behavioral alterations (increased vertical exploratory activity and social affinity) are a consequence of the middle ear phenotype, or an additional, independent phenotype related to disruption of *Miz1* function in the brain. It is equally unclear whether the differences in the extent of the observed alterations between males and females reflect sex-specific differences in the *behavioral outcome* of the same underlying physiological processes, or whether both sexes are differentially physiologically (and maybe also morphologically) affected by *Miz1* dysfunction.

Comprehensive information about steroid hormone levels in these mice would be interesting - and highly appreciated.

### 3.1.7 Reference

Ohl, F., Sillaber, I., Binder, E., Keck, M.E. & Holsboer, F. (2001) Differential analysis of behavior and diazepam-induced alterations in C57BL/6N and BALB/c mice using the modified hole board test. *J. Psychiatr. Res.* 35: 147-154.

**Table 2: Results of Behavioral Observation in the Modified Hole Board Test**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=15)	(n=13)	<i>p - value</i>	(n=13)	(n=12)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Line crossing [frequency]	135.73 $\pm$ 4.83	131 $\pm$ 8.28	N.A.	131.08 $\pm$ 9.24	128.75 $\pm$ 5.67	N.A.	n.s.	n.s.
Line crossing [latency]	0.99 $\pm$ 0.15	0.64 $\pm$ 0.06	N.A.	1.21 $\pm$ 0.51	0.83 $\pm$ 0.12	N.A.	n.s.	n.s.
Rearings in box [frequency]	28.67 $\pm$ 0.8	27.92 $\pm$ 1.08	N.A.	31.77 $\pm$ 1.61	34.75 $\pm$ 2.33	N.A.	n.s.	<b>p&lt;0.05</b>
Rearings in box [latency]	31.81 $\pm$ 3.5	24.16 $\pm$ 3.19	N.A.	26.25 $\pm$ 3.86	29.18 $\pm$ 4.55	N.A.	n.s.	n.s.
Hole exploration [frequency]	28.6 $\pm$ 1.43	26.77 $\pm$ 2.47	N.A.	29 $\pm$ 2.29	24.83 $\pm$ 2.69	N.A.	n.s.	n.s.
Hole exploration [latency]	32.81 $\pm$ 5.95	19.05 $\pm$ 3.64	N.A.	30.52 $\pm$ 7.07	30.18 $\pm$ 6.99	N.A.	n.s.	n.s.
Hole visit [frequency]	0 $\pm$ 0	0 $\pm$ 0	N.A.	0 $\pm$ 0	0 $\pm$ 0	N.A.	n.s.	n.s.
Hole visit [latency]	300 $\pm$ 0	300 $\pm$ 0	N.A.	300 $\pm$ 0	300 $\pm$ 0	N.A.	n.s.	n.s.
Board entry [frequency]	8.53 $\pm$ 0.64	8.31 $\pm$ 0.85	N.A.	8.08 $\pm$ 1.02	7.5 $\pm$ 1.05	N.A.	n.s.	n.s.
Board entry [latency]	60.99 $\pm$ 8.78	65.95 $\pm$ 12.46	N.A.	64.64 $\pm$ 10.88	81.33 $\pm$ 22.2	N.A.	n.s.	n.s.
Board entry [total duration %]	9.41 $\pm$ 0.8	11.01 $\pm$ 1.43	N.A.	9.36 $\pm$ 1.19	7.84 $\pm$ 1.3	N.A.	n.s.	n.s.

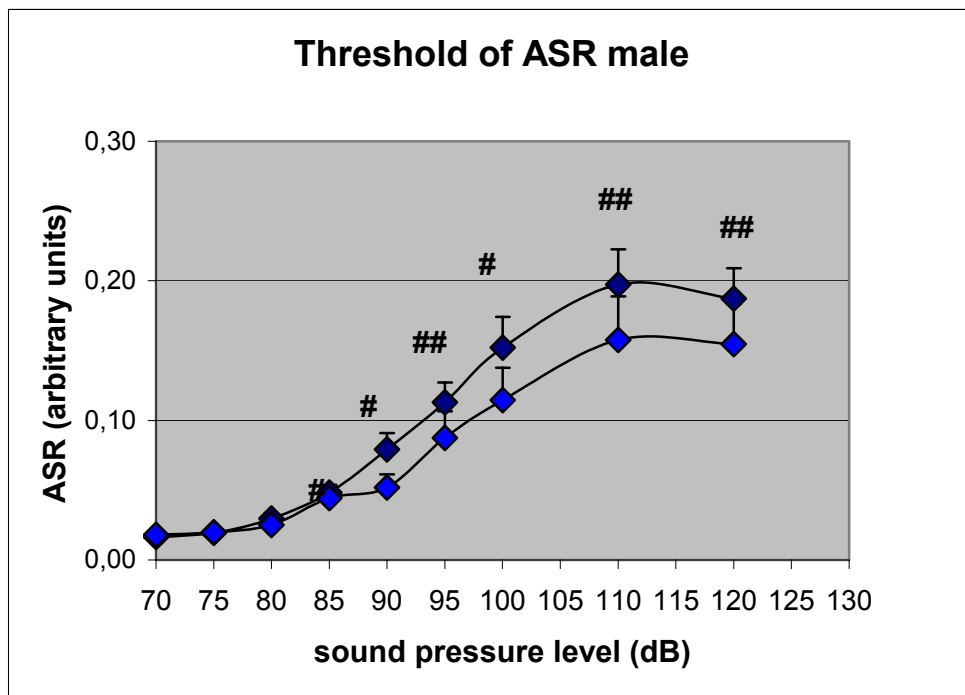
Rearing on board [frequency]	0.47 ± 0.19	0.77 ± 0.34	N.A.	0.54 ± 0.31	0.17 ± 0.17	N.A.	n.s.	n.s.
Rearing on board [latency]	265.08 ± 14.22	270.34 ± 14.81	N.A.	276.34 ± 12.13	284.34 ± 15.66	N.A.	n.s.	n.s.
Risk assessment [frequency]	0 ± 0	0 ± 0	N.A.	0 ± 0	0 ± 0	N.A.	N.A.	n.s.
Risk assessment [latency]	300 ± 0	300 ± 0	N.A.	300 ± 0	300 ± 0	N.A.	N.A.	n.s.
Group contact [frequency]	12.73 ± 0.78	11.77 ± 0.94	N.A.	11.54 ± 1	13.5 ± 0.62	N.A.	n.s.	n.s.
Group contact [latency]	15.35 ± 2.51	17.91 ± 3.19	N.A.	20.36 ± 4.79	16.86 ± 2.75	N.A.	n.s.	n.s.
Group contact [total duration %]	16.37 ± 1.16	20.51 ± 1.7	N.A.	20.55 ± 1.97	33.45 ± 1.8	N.A.	n.s.	p<0.05
Grooming [frequency]	0.33 ± 0.13	0.77 ± 0.23	N.A.	0.62 ± 0.21	0.25 ± 0.13	N.A.	n.s.	n.s.
Grooming [latency]	267.37 ± 15.73	248.23 ± 20.08	N.A.	247.85 ± 19.28	275.61 ± 16.59	N.A.	n.s.	n.s.
Grooming [total duration %]	0.35 ± 0.19	0.59 ± 0.23	N.A.	0.82 ± 0.28	0.38 ± 0.21	N.A.	n.s.	n.s.
Defecation [frequency]	0.27 ± 0.12	0.08 ± 0.08	N.A.	0.77 ± 0.28	0 ± 0	N.A.	n.s.	n.s.
Defecation [latency]	230.71 ± 31.45	290.31 ± 9.69	N.A.	237.95 ± 22.09	300 ± 0	N.A.	n.s.	n.s.
Unfamiliar object exploration [frequency]	6.33 ± 0.69	6.77 ± 0.53	N.A.	6.77 ± 0.68	6 ± 0.62	N.A.	n.s.	n.s.
Familiar object exploration [frequency]	7.53 ± 0.59	6.31 ± 0.63	N.A.	7.38 ± 0.65	6.17 ± 0.53	N.A.	n.s.	n.s.
Unfamiliar object exploration [latency]	38.57 ± 10.33	34.45 ± 10.42	N.A.	31.48 ± 5.9	23.59 ± 4.54	N.A.	n.s.	n.s.

<b>Familiar object exploration [latency]</b>	29.57 ± 5.14	33.8 ± 6.16	<b>N.A.</b>	35.89 ± 8.39	38.09 ± 8.14	<b>N.A.</b>	<b>n.s.</b>	<b>n.s.</b>
<b>Unfamiliar object exploration [total duration %]</b>	1.49 ± 0.14	1.85 ± 0.23	<b>N.A.</b>	1.77 ± 0.2	1.71 ± 0.18	<b>N.A.</b>	<b>n.s.</b>	<b>n.s.</b>
<b>Familiar object exploration [total duration %]</b>	1.38 ± 0.1	1.35 ± 0.12	<b>N.A.</b>	1.39 ± 0.15	1.31 ± 0.12	<b>N.A.</b>	<b>n.s.</b>	<b>n.s.</b>
<b>Object Index</b>	0.02 ± 0.04	0.12 ± 0.04	<b>N.A.</b>	0.11 ± 0.06	0.12 ± 0.06	<b>N.A.</b>	<b>n.s.</b>	<b>n.s.</b>

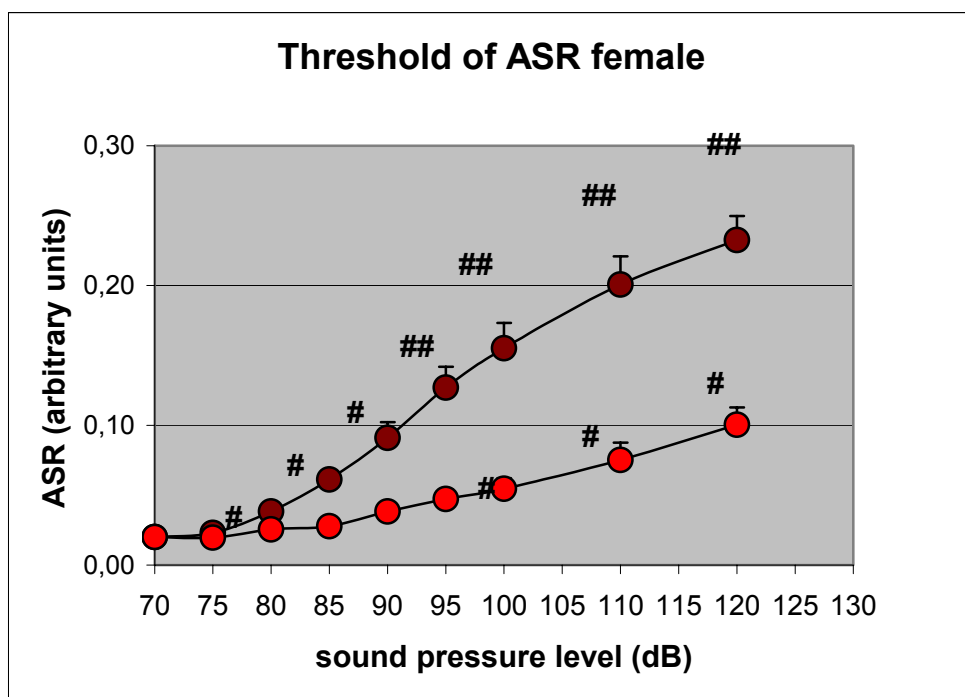
**Table 3: Video-Tracking Results Regarding Locomotor Behavior**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=15)	(n=13)	<i>p</i> -value	(n=13)	(n=12)	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
Total Distance Moved [cm]	3513.24 $\pm$ 117.18	3426.6 $\pm$ 151.24	N.A.	3460.98 $\pm$ 193.24	3304.37 $\pm$ 145.46	N.A.	n.s.	n.s.
Mean Velocity [cm/sec]	19.79 $\pm$ 0.5	19.89 $\pm$ 0.76	N.A.	19.46 $\pm$ 0.78	19.7 $\pm$ 0.63	N.A.	n.s.	n.s.
Maximum velocity [cm/sec]	56.95 $\pm$ 2.27	54.71 $\pm$ 2.02	N.A.	58.92 $\pm$ 3.18	54.6 $\pm$ 1.95	N.A.	n.s.	n.s.
Turns [Frequency]	1870.81 $\pm$ 32.18	1817.77 $\pm$ 45.17	N.A.	1856.69 $\pm$ 61.37	1761.83 $\pm$ 46.4	N.A.	n.s.	n.s.
Distance to Wall [cm]	7.21 $\pm$ 0.23	7.26 $\pm$ 0.31	N.A.	7.27 $\pm$ 0.28	6.42 $\pm$ 0.29	N.A.	n.s.	n.s.
Distance to Board [cm]	8.6 $\pm$ 0.16	8.75 $\pm$ 0.2	N.A.	8.59 $\pm$ 0.2	9.5 $\pm$ 0.19	N.A.	n.s.	<b>p&lt;0.05</b>
Board entry, maximum duration [sec]	7.45 $\pm$ 0.72	10.26 $\pm$ 1.73	N.A.	7.92 $\pm$ 1.18	5.32 $\pm$ 0.67	N.A.	n.s.	<b>p&lt;0.05</b>



**Figure 3: Acoustic startle response of males (normalized for body weight).**  
 (black: male controls, blue: male mutants)



**Figure 4: Acoustic startle response of females (normalized for body weight).**  
 (dark red: female controls, light red: female mutants)

## 3.2 Dysmorphology, Bone and Cartilage

### 3.2.1 Summary

In the Dysmorphology, Bone and Cartilage Screen of the German Mouse Clinic mice are analyzed for morphological abnormalities in different organ systems with special focus on bone and cartilage development and homeostasis. We adapted the successful dysmorphology screening protocol from the Munich ENU-Mutagenesis Screen (Hrabé de Angelis *et al.* 2000) for use in the German Mouse Clinic. The nomenclature of the parameters was adapted according to the Mammalian Phenotype Ontology wording ([www.informatics.jax.org/searches/MP\\_form.shtml](http://www.informatics.jax.org/searches/MP_form.shtml)). Further tests for defects in bone development and homeostasis were taken over from human diagnosis, and were adapted for the use in mice analysis. Such tests include: X-ray analysis, bone densitometry and, in a limited number of animals, micro-computer tomography.

A total of 54 animals of the Miz1 mutant mouse line were analyzed in the Dysmorphology, Bone and Cartilage module of the German Mouse Clinic. In the morphological investigation via visual inspection and X-ray analysis some minor phenotypes were found. No correlation of any phenotype with a certain genotype was detected. In the quantitative, bone-related parameters, no significant differences between mutant mice and controls were detected.

### 3.2.2 Mice

Twenty-nine male (16 +/+, 13 -/-) and 25 female (13 +/+, 12 -/-) mice were analyzed by morphological inspection at the age of 9 weeks. Blood was taken at the age of 14 weeks for determination of ionic calcium from 19 knockout (KO) and 23 control animals, and 16-week-old KOs (14 animals) and controls (17 animals) entered the bone density and X-ray analysis. As the analysis in the clickbox-test indicated that there might be reduced hearing in the mutants, these animals were also tested again for the clickbox-test.

### 3.2.3 Material and Methods

The Dysmorphology, Bone and Cartilage module of the German Mouse Clinic analyzed the mice in different phases:

1. At the age of 5 weeks, i.e. when the mice entered the facility, the general physical condition and health were checked;
2. At the age of 9 weeks, a morphological observation as a whole-body checkup was performed;
3. The ionized fraction of calcium in blood was analyzed in 14-week-old mice, and
4. At the age of 16 to 17 weeks, X-ray analysis and bone densitometry were performed.

#### Morphological Observation

The animals were screened using the protocol for morphological analysis from Fuchs *et al.* (2000) as adapted for the German Mouse Clinic.

Using a clickbox (supplied by the MRC Institute of Hearing Research, Nottingham, UK) we tested the mice's ability to hear a sound of 20 kHz. The reaction of the animals was classified into six categories (0=no reaction at all, 1=no Preyer reflex, 2= retarded reaction, 3= normal reaction, 4= strong reaction, 5= particularly strong reaction).

### **Ionized calcium Analysis**

*Equipment:* AVL 9180 Electrolyte Analyzer (distributed by Roche Diagnostics GmbH, Mannheim, Germany)

cleaning solution and conditioning solution (Roche),

ISEtrol Quality Control Solutions (Roche),

lithium-heparin polypropylen tubes,

glass capillary (0.8 mm diameter, 32 mm length, without heparin; special product of Laborteam K+K, Munich).

*Quality control:* Calibration of the system and quality control were performed at intervals recommended and with solutions provided by the manufacturer. The results from the quality control were recorded by the system. Before blood measurement, daily cleaning, conditioning and calibration of the analyzer were performed.

*Procedure:* Blood (100 µl) was collected from anesthetized mice in lithium heparin tubes and transferred directly to the analyzer. Values were transferred directly to the database.

### **X-ray Images**

*Equipment:* Faxitron X-ray Model MX-20 (Specimen Radiography System, Illinois, USA),

NTB Digital X-ray Scanner EZ 40 (NTB GmbH, Diepholz, Germany),

*Quality control:* Calibration of the system is done in monthly intervals,

*Settings:* Voltage 25 kV, integration time 40 ms,

*Procedure:* The anesthetized mouse was fixed on an X-ray-permeable plate and placed in the machine. Using iX-Pect software supplied by the manufacturer of the X-ray scanner, the image was taken and analyzed. Analysis was done qualitatively by visual inspection of the images as well as quantitatively by using the ruler tool of iX-Pect software.

### **Bone density analysis**

*Equipment:* pDEXA Sabre X-ray Bone Densitometer (Norland Medical Systems. Inc., Basingstoke, Hampshire, UK; distributed by Stratec Medizintechnik GmbH, Pforzheim, Germany),

*Quality control:* Calibration of the system was done in daily intervals using the QC and the QA phantoms delivered by the manufacturer. Results from the quality control were recorded by the system.

*Settings:* Scan speed 20 mm/s, Resolution 0.5 mm x 1.0 mm, HAW 0.020

*Procedure:* After anesthesia, the weight and length of the mouse were recorded, and the mouse was placed in the analyzer. After a scout scan, the area of interest was optimized and the measure scan started.

*Data-analysis:* For analysis of the data, regions have to be defined. The standard analysis comprises a whole body analysis as well as a whole body analysis excluding the skull.

### **Statistical analysis of data**

Analysis of quantitative data sets was carried out using StatView software package (SAS corporation).

### **3.2.4 Parameters**

<p><b>Morphological inspection</b></p> <p><i>Growth/weight/body size:</i> abnormality  <i>Eye:</i> dysmorphology, corneal or lens defect  <i>Coat:</i> hair growth defects, hair texture defects, color anomalies, hair follicle, structure/orientation anomalies  <i>Skin:</i> pigmentation anomalies, texture/condition, anomalies  <i>Vibrissae:</i> dysmorphology  <i>Extremities:</i> limb dysmorphology, digit dysmorphology, tail dysmorphology  <i>Teeth:</i> tooth dysmorphology  <i>Ears:</i> auditory defects/deafness, dysmorphology  <i>Musculature:</i> muscle dysmorphology,  <i>Skeletal:</i> osteogenesis/developmental anomalies, axial defects, extremities defects, craniofacial defects  <i>Neurological / behavioral:</i> seizures/epilepsy, motor capabilities / coordination / movement anomalies, feeding / drinking anomalies  <i>Respiratory system:</i> dysmorphology  <i>Reproductive system:</i> dysmorphology  <i>Other aberrant phenotype</i></p>
<p><b>X-ray analysis</b></p> <p>Skull shape, mandibles, maxilla, teeth, orbit, number of vertebrae (cervical, thoracic, lumbar, pelvic, sacral), vertebrae shape, number of ribs, rib shape, scapulas, clavicle, pelvis, femur diameter, femur shape, tibia, fibula, humerus, ulna, radius, number of digits, completeness of digits, subcutaneous fat, joints</p>
<p><b>Dual energy X-ray absorption</b></p> <p>Bone mineral density (BMD), partial bone mineral density (pBMD, whole body excluding skull), specific bone mineral density (sBMD), bone mineral content (BMC), lean mass, fat mass, bone content, lean content, fat content</p>
<p><b>AVL analyzer</b></p> <p>Free ionic calcium</p>
<p><b>Computer tomography</b></p> <p>3D-visualization of whole skeleton, 2D-examination of inner organs and soft tissue, high-resolution analysis of regions of interest</p>

### 3.2.5 Results and Discussion

In the morphological inspection and X-ray analysis of the mice, some phenotypes were discovered, however, they were found in mutants and controls (Tables 4 and 6). The clickbox analysis to test the hearing of the mice, which is part of the morphological observation in nine-week-old mice, gave a subtle hint at reduced hearing in the mutants. We tried to confirm the reduced hearing in 17-week-old mice, but at that age also many control mice showed retarded reaction to the clickbox. We expect that the slow reaction of aged mice to the clickbox might be an effect of the genetic background (Johnson *et al.* 2000). Thus, the clickbox test can only contribute in a limited way to the findings in the Behaviour Screen (3.1.5).

In the X-ray analysis it was confirmed that in three mutant males the snout was not straight (Table 6), in two of these three animals also long teeth were found (Table 4). We do not expect that this phenotype is related to the mutation in *Miz1*.

No quantitative parameter was significantly different between the genotypes mutant and control in males and in females (Tables 8) with one exception of fat tissue in females. We do not expect, that this finding is related to *Miz1* since the other fat related parameters (fat mass and fat content) were not elevated.

Raw data will be available on demand.

### 3.2.6 References

Fuchs H, Schughart K, Wolf E, Balling R, Hrabé de Angelis M. (2000): Screening for dysmorphological abnormalities - a powerful tool to isolate new mouse mutants. *Mammalian Genome* 11(7): 528-30.

Hrabé de Angelis, M., H. Flaswinkel, H. Fuchs, B. Rathkolb, D. Soewarto, S. Marschall, S. Heffner, W. Pargent, K. Wuensch, M. Jung, A. Reis, T. Richter, F. Alessandrini, T. Jakob, E. Fuchs, H. Kolb, E. Kremmer, K. Schaeble, B. Rollinski, A. Roscher, C. Peters, T. Meitinger, T. Strom, T. Steckler, F. Holsboer, T. Klopstock, F. Gekeler, C. Schindewolf, T. Jung, K. Avraham, H. Behrendt, J. Ring, A. Zimmer, K. Schughart, K. Pfeffer, E. Wolf and R. Balling (2000): Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nature Genetics* 25: 444 – 447

Johnson KR, Zheng QY, Erway LC. (2000): A major gene affecting age-related hearing loss is common to at least ten inbred strains of mice. *Genomics*. 70(2): 171-80.

### Abbreviations

BMC	bone mineral content
BMD	bone mineral density
pBMD	partial bone mineral density (excluding skull)
sBMD	specific bone density

<b>Table 4: Results from the Morphological Inspection</b>				
Data obtained from nine-week-old mice				
Phenotype	Male		Female	
	-/-	+/+	-/-	+/+
<b>Clickbox score</b>				
(1)	5	1	3	-
(2)	1	-	-	-
(2.5)	-	-	2	-
<b>Growth/weight/body size</b>				
Smaller body length	-	-	5	-
Body more compact	1	-	-	-
<b>Reproductive system</b>				
Protrusion of penis	1	-	-	-
<b>Teeth</b>				
Long teeth	2	-	1	-
White tooth in maxilla	-	1	-	-
<b>Coat</b>				
Dark skin	-	-	-	2
<b>Neurological / behavioral</b>				
Trembling	1	-	-	-
<b>Animals analyzed</b>	<b>13</b>	<b>16</b>	<b>12</b>	<b>13</b>

<b>Table 5: Results from the second Clickbox Testing</b>				
Data obtained from 17-week-old mice				
Score	Males		Females	
	-/-	+/+	-/-	+/+
(0)	2	3	4	-
(1)	4	8	6	1
(2)	3	4	3	13
(3)				
<b>Animals analyzed</b>	<b>9</b>	<b>15</b>	<b>13</b>	<b>14</b>

0: no reaction at all  
1: very slow reaction  
2: retarded reaction  
3: normal reaction  
4: strong reaction

<b>Table 6: Results from the X-Ray Analysis</b>				
Phenotype	Male		Female	
	-/-	+/+	-/-	+/+
Back slightly bent	1	1	-	2
Snout not straight	3	-	-	-
<b>Animals analyzed</b>	<b>7</b>	<b>10</b>	<b>7</b>	<b>7</b>

**Table 7: Bone-Related Quantitative Parameters**

Data are presented as mean ± standard error of mean.

Parameter	Control (A)			Mutant (B)			Male A~B	Female A~B
	Male (n=10)	Female (n=7)	p-value	Male (n=7)	Female (n=7)	p-value	p-value	p-value
BMD [mg/ cm <sup>2</sup> ]	60.0 ±0.001	59.0 ±3.0	n.s.	56.0 ±1.0	60.0 ±2.0	n.s.	n.s.	n.s.
BMC [mg]	556.0 ±36.0	443 ±33	< 0.05	470 ±86	450.0 ±39.0	n.s.	n.s.	n.s.
Lean mass [g]	21.63 ±0.77	16.56 ±0.90	< 0.001	20.88 ±0.33	15.82 ±0.86	< 0.001	n.s.	n.s.
Fat mass [g]	3.81 ±0.61	1.74 ±0.58	< 0.05	3.12 ±0.89	2.86 ±0.93	n.s.	n.s.	n.s.
Body Length [cm]	8.6 ±0.1	8.1 ±0.1	< 0.001	8.6 ±0.1	8.1 ±0.1	< 0.01	n.s.	n.s.
Weight [g]	28.45 ±0.69	21.00 ±0.50	< 0.001	26.69 ±1.21	21.64 ±0.26	< 0.01	n.s.	n.s.
sBMD [10 <sup>-3</sup> x cm <sup>-2</sup> ]	2.11 ±0.08	2.82 ±0.11	< 0.001	2.13 ±0.11	2.78 ±0.11	< 0.01	n.s.	n.s.
Bone Content [%]	1.96 ±0.12	2.12 ±0.17	n.s.	1.70 ±0.28	2.09 ±0.17	n.s.	n.s.	n.s.
Lean Content [%]	76.08 ±2.12	78.69 ±3.06	n.s.	78.96 ±2.81	73.28 ±4.36	n.s.	n.s.	n.s.
Fat Content [%]	13.26 ±1.98	8.45 ±2.82	n.s.	10.96 ±2.92	13.03 ±4.10	n.s.	n.s.	n.s.
pBMD [mg/ cm <sup>2</sup> ]	480.0 ±1.0	460.0 ±2.0	n.s.	450.0 ±1.0	470.0 ±2.0	n.s.	n.s.	n.s.
Lumbar vertebra height [mm]	6.0 ±0.1	5.7 ±0.1	n.s.	5.8 ±0.1	5.7 ±0.1	n.s.	n.s.	n.s.
Lumbar vertebra width [mm]	5.1 ±0.1	5.0 ±0.1	n.s.	5.0 ±0.1	4.8 ±0.1	n.s.	n.s.	n.s.

<b>Fat tissue [mm ]</b>	6.0 ±0.3	5.9 ±0.2	n.s.	6.2 ±0.3	7.0 ±0.4	n.s.	n.s.	< 0.05
	<b>Male (n=15)</b>	<b>Female (n=8)</b>	<b>p - value</b>	<b>Male (n=12)</b>	<b>Female (n=7)</b>	<b>p - value</b>	<b>p - value</b>	<b>p - value</b>
<b>Ionized Calcium [mM]</b>	1.16 ±0.02	1.17 ±0.02	n.s.	1.14 ±0.02	1.19 ±0.03	n.s.	n.s.	n.s.

## 3.3 Neurology Screen

### 3.3.1 Summary

In the primary neurological screen 25 *Miz1*-deficient mice (13 males/12 females), 29 *Miz1*-control mice (16 males/13 females) were screened. Animals were analyzed according to our modified SHIRPA protocol where a battery of behavioral tests is carried out. This primary observation screen is a modification of the Irwin procedure (Irwin, 1968) and was proposed as a rapid, comprehensive and semi-quantitative screening method for qualitative analysis of abnormal phenotypes in a mouse strain (Rogers *et al.*, 1994). We carried out 37 of 40 designed test parameters (See web page: [http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shirpa\\_summary.html](http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shirpa_summary.html)) to detect phenotypic differences between mutant and control mice. Each test parameter contributes to an overall assessment in muscle, lower motor neuron, spinocerebellar, sensory and autonomic function. The primary neurological screen is focused on investigating neurological reflexes to determine the neurological functioning of a mouse. We also examine lactate levels in the blood of mice to draw conclusions about energy metabolism.

The comparison of mutant to control mice showed that mutant mice had a significantly retarded body constitution which was displayed in their reduced body weight and body length. Furthermore, we detected an altered pelvic elevation, tail elevation, and wire maneuver behavior in male mutant mice. These observations might point towards a muscular alteration.

### 3.3.2 Mice

Thirteen 10-week-old male mutant and sixteen 10-week-old male control mice entered the neurological screen at the beginning of the 42<sup>nd</sup> calendar week in 2003. Twelve 10-week-old female mutant and thirteen 10-week-old female control mice entered the neurological laboratory one week later. All animals were fed *ad libitum* for a period of one week during their stay in the neurological screen.

### 3.3.3 Material and Methods

At the age of 10 weeks assessment of each animal started with observation of undisturbed behavior (*Viewing Jar Behavior*) in a glass cylinder (11 cm in diameter). The mice were then transferred to an arena consisting of a clear Perspex box (420 x 260 x 180 mm) in which a Perspex sheet on the floor is marked with 15 squares. In this arena, locomotor activity and motor behavior was observed (*Behavior recorded in the Arena*). This was followed by a sequence of manipulations testing reflexes, grip strength, toe pinch and wire maneuver (*Behavior recorded on or above the arena*). For the wire maneuver test, a rigid horizontal wire (3 mm in diameter) is secured across the rear right corner of the arena. For grip strength testing, a grid (270 x 275 mm) is secured across the width of the arena. In the last part of the observation (*Behavior recorded during Supine Restraint*), the animals were restrained in a supine position to record autonomic responses such as salivation. Measurements

were completed with the recording of limb tone, provoked biting, and body length. The last part of the primary screen also involves the analysis of righting reflex, negative geotaxis and contact righting reflex. A glass cylinder (35 mm diameter, 135 mm length) is used for testing the contact-righting reflex. Throughout the entire procedure, abnormal behavior, irritability, fear, aggression and vocalization were recorded. Between testing of each mouse, faecal pellets and urination were removed from the viewing jar and arena. All experimental equipment is thoroughly cleaned with Pursept-A and dried prior to testing.

Values for body length, body weight and locomotor activity are presented as means  $\pm$  SEM. Kruskal-Wallis-test (S-PLUS, Insightful) was used to test for effects of genotype and gender factors on these parameters. The Chi-Squared test was applied for all other parameters.

### 3.3.4 Parameters

<b>Muscle/lower motor neuron function</b>
Body position, gait, Positional passivity, wire maneuver, tail elevation, limb tone, body tone, abdominal tone, grip strength, urination, defecation
<b>Spinocerebellar function</b>
Body position, gait, righting reflex, tail elevation, visual placing, limb tone, body tone, abdominal tone, grip strength
<b>Sensory function</b>
Transfer arousal, touch escape, gait, visual placing, toe pinch, pinna reflex, righting reflex
<b>Autonomic function</b>
Palpebral closure, urination, salivation, respiration rate, defecation
<b>Neurological reflexes</b>
Righting reflex (pons), contact righting reflex, visual placing, toe pinch/flexion reflex (cerebellar/spinal cord), negative geotaxis, corneal reflex (medulla), pinna reflex (hearing test)
<b>Physiological parameters</b>
Body weight, body length
<b>General appearance</b>
Body weight, body length, body position, transfer arousal, fear, touch escape, irritability, vocalization, positional passivity, aggression, spontaneous activity, locomotor activity, skin color

### 3.3.5 Results

Female mutant mice showed a significantly increased body weight as compared to control mice (Table 8). In contrast to females, male mutant mice showed a significantly reduced body weight and reduced body length. Other parameters with significant findings were only found in male mutant mice. In detail, male mutant mice displayed an altered pelvic elevation and tail elevation (Table 10). In addition, these mice showed an impaired wire maneuver test (Table 11). All other parameters measured were without significant differences.

Raw data for each individual are available on demand in Excel sheets.

### 3.3.6 Discussion

In our neurological screen, only male mutant mice displayed markedly changed behavior as compared to control mice. Since *Miz1* is most abundantly expressed in the subventricular zones of the ventricles, in the pyramidal cell layer of the hippocampus, and in the granule cells of the cerebellum, it would be possible that memory function and cerebellar controlled motor behavior is impaired in *Miz1*-mutant animals. Screening of male mutant mice revealed an changed tail elevation. These results might not necessarily demonstrate a pathological change because tail elevation is a parameter that is also influenced by behavioral aspects (e.g., marking of territory). Parameters that might indicate a pathological change in the male mutant mice are the **impaired wire maneuver test** and the **altered pelvic elevation**. These observations might point to a muscular alteration. A reduced muscle function might also contribute to the reduced breathing pattern observed in the Lung screen (3.9.5).

In order to confirm a neurological / muscular phenotype, further neurological examinations have to be done. We suggest **secondary screening** of the mice with particular emphasis on **grip strength** and **Rotarod** testing.

### 3.3.7 References

- Irwin S. (1968) Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia*. 13(3): 222-257.
- Rogers D. C., E.M. Fisher, S.D. Brown, J. Peters, A.J. Hunter, J.E. Martin (1997) Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome*. 8(10): 711-713.

## Abbreviations

- SHIRPA **S**mithKline Beecham Pharmazeuticals; **H**arwell, MRC Mouse Genome Centre and Mammalian Genetics Unit; **I**mperial Col-  
legeschool of Medicine at St. Mary`s; **R**oyal London Hospital, **P**he-  
notype **A**ssessment  
[http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shir  
pa\\_summary.html](http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/shirpa_summary.html)
- s.a. Sub-maxillary area

**Table 8: Recording of Body Length and Body Weight**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Male			Female		
	Control (n=16)	Mutant (n=13)	<i>p-value</i>	Control (n=13)	Mutant (n=12)	<i>p-value</i>
<b>Body Length [g]</b>	8.5 $\pm 0.01$	8.2 $\pm 0.03$	<b>0.01</b>	7.4 $\pm 0.01$	7.3 $\pm 0.02$	<b>n.s.</b>
<b>Body Weight [g]</b>	26.3 $\pm 0.1$	23.65 $\pm 0.2$	<b>0.007</b>	18.8 $\pm 0.1$	20 $\pm 0.08$	<b>0.04</b>

**Table 9: Behavior Recorded in Viewing Jar**Data shown represents the results of test parameters from major tests where a behavioral response was observed. Test parameters which did not elicit any response were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0.05$ 

Parameter	Male			Female		
	Control (n=16)	Mutant (n=13)	<i>p-value</i>	Control (n=13)	Mutant (n=12)	<i>p-value</i>
<b>Body Position</b>						
Sitting or standing	6	7		11	9	
Rearing on hind legs	8	5		1	0	
Repeating vertical leaping	2	1	<b>n.s.</b>	1	3	<b>n.s.</b>
<b>Spontaneous Behavior</b>						
Slow movement	0	0		0	1	
Moderate movement	11	11		7	9	
Vigorous movement	5	2	<b>n.s.</b>	6	2	<b>n.s.</b>
<b>Respiration rate</b>						
Normal	16	13	<b>n.s.</b>	13	12	<b>n.s.</b>
<b>Tremor</b>						
None	16	13	<b>n.s.</b>	13	12	<b>n.s.</b>

**Table 10: Recording of Locomotor Activity and Behavior in the Arena**

Locomotor activity data are shown as mean ( $\pm$  SEM). Data from behavior recorded in the Arena represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0.05$

Parameter	Male			Female		
	Control (n=16)	Mutant (n=13)	<i>p</i> -value	Control (n=13)	Mutant (n=12)	<i>p</i> -value
<b>Locomotor Activity</b>	13 $\pm 1.1$	14.6 $\pm 2.5$	<i>n.s.</i>	14 $\pm 0.37$	16.2 $\pm 0.37$	<i>n.s.</i>
<b>Transfer arousal</b>						
Brief freeze, then active movement	1	1	<i>n.s.</i>	13	12	<i>n.s.</i>
No freeze, immediate movement	15	12		0	0	
<b>Palpebral Closure</b>						
Eyes wide open	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Piloerection</b>						
None	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Gait</b>						
Normal	15	13		13	12	
Fluid but abnormal	1	0	<i>n.s.</i>	0	0	<i>n.s.</i>
<b>Pelvic Elevation</b>						
Normal	15	6		13	12	
Elevated	1	7	<b>0.014</b>	0	0	<i>n.s.</i>
<b>Tail Elevation</b>						
Horizontally extended	14	6		0	1	
Elevated/Straub tail	2	7	<b>0.046</b>	13	11	<i>n.s.</i>
<b>Touch Escape</b>						
No response	0	1		0	0	
Mild	9	8		13	12	
Moderate	7	4	<i>n.s.</i>	0	0	<i>n.s.</i>
<b>Positional Passivity</b>						
Struggles when held by tail	16	13		13	11	
Struggles when hold by neck	0	0	<i>n.s.</i>	0	1	<i>n.s.</i>

**Table 11: Behavior Recorded in or above the Arena**  
 Data shown represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0.05$

Parameter	Male			Female		
	Control (n=16)	Mutant (n=13)	<i>p-value</i>	Control (n=13)	Mutant (n=12)	<i>p-value</i>
<b>Trunk Curl</b>						
Absent	0	0	<i>n.s.</i>	13	12	<i>n.s.</i>
Present	16	13		0	0	
<b>Limb Grasping</b>						
Absent	16	13	<i>n.s.</i>	11	12	<i>n.s.</i>
Present	0	0		2	0	
<b>Visual Placing</b>						
Upon vibrassee contact	8	10	<i>n.s.</i>	5	5	<i>n.s.</i>
Before vibrassee contact	7	3		8	7	
<b>Grip strength</b>						
Moderate grip	0	0	<i>n.s.</i>	13	12	<i>n.s.</i>
Active grip	16	13		0	0	
<b>Body Tone</b>						
Flaccid	0	2	<i>n.s.</i>	0	0	<i>n.s.</i>
Slight resistance	16	11		13	12	
<b>Pinna reflex</b>						
None	1	0	<i>n.s.</i>	0	0	<i>n.s.</i>
Active retraction	15	13		13	12	
<b>Corneal Reflex</b>						
None	0	0	<i>n.s.</i>	3	2	<i>n.s.</i>
Active single eye blink	16	13		10	10	
<b>Toe Pinch</b>						
None	0	1	<i>n.s.</i>	0	0	<i>n.s.</i>
Moderate withdrawal	4	3		13	12	
Brisk withdrawal	12	9		0	0	
<b>Wire maneuver</b>						
Active grip	8	2	<i>0.017</i>	2	4	<i>n.s.</i>
Difficulty to grasp	5	14		8	8	
Unable to grasp	0	0		3	0	

**Table 12: Behavior during Supine Restraint**

Data shown represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0.05$ .

Parameter	Male			Female		
	Control (n=16)	Mutant (n=13)	<i>p-value</i>	Control (n=13)	Mutant (n=12)	<i>p-value</i>
<b>Skin Color</b>						
Pink	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Limb Tone</b>						
No resistance	16	13	<i>n.s.</i>	0	0	<i>n.s.</i>
Slight resistance	0	0		13	12	
<b>Abdominal Tone</b>						
Slight resistance	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Lacrimation</b>						
None	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Salivation</b>						
None	1	0	<i>n.s.</i>	0	1	<i>n.s.</i>
Slight margin of s.a	8	4		13	11	
Wet zone entire of s.a.	7	9		0	0	
<b>Provoked biting</b>						
Absent	14	13	<i>n.s.</i>	6	7	<i>n.s.</i>
Present	2	0		7	5	
<b>Righting reflex</b>						
No impairment	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Contact righting reflex</b>						
Present	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Negative Geotaxis</b>						
Turns and climbs the grid	16	12	<i>n.s.</i>	13	12	<i>n.s.</i>
Turns but then freezes	0	1		0	0	
<b>Fear</b>						
None	16	13	<i>n.s.</i>	13	12	<i>n.s.</i>
<b>Irritability</b>						
None			<i>n.s.</i>			<i>n.s.</i>
Struggles during supine restraint	8	4		13	12	
	8	9	0	0		
<b>Aggression</b>						
None	14	13	<i>n.s.</i>	7	7	<i>n.s.</i>
Provoked biting or attack	2	0		6	5	
<b>Vocalization</b>						
None	0	1	<i>n.s.</i>	0	1	<i>n.s.</i>
Provoked during handling	16	12		13	11	

**Table 13: Lactate Levels**

Data shown represent the results of the mean blood lactate concentrations, value ( $\pm$  SEM). Statistical analysis: Chi-squared test; significance  $p < 0.05$ .

	Male			Female		
	Control (n=16)	Mutant (n=13)	<i>p-value</i>	Control (n=13)	Mutant (n=12)	<i>p-value</i>
<b>Lactate [mmo/l]</b>	5.5 $\pm$ 0.36	4.6 $\pm$ 0.4	<i>n.s.</i>	4.0 $\pm$ 0.32	5.0 $\pm$ 0.33	<i>n.s.</i>

## 3.4 Eye Screen

### 3.4.1 Summary

In the Eye Screen, a high throughput electroretinography method (ERG) was employed to examine mice for retinal impairment (Dalke *et al.*, 2004). Furthermore, mice were examined for anterior segment abnormalities by slitlamp biomicroscopy (Favor, 1983).

In humans blindness is caused by several different ocular diseases. Among these, the cataracts are responsible for half of all cases (Johnson and Foster, 2003). The retinal disorders cover a broad variety of clinical symptoms and many different genes are involved in the corresponding pathological conditions in humans. The two most important groups are retinitis pigmentosa (RP) and age-related-macular-degeneration (ARMD; for recent reviews, see Rivolta *et al.*, 2002 and Stone *et al.*, 2001). Mouse models are appropriate tools to understand the genetic and biochemical mechanisms of ocular disorders. There is a rapid increasing number of mouse mutants available suffering from various types of eye diseases (for a recent review see Graw, 2003).

No significant differences between *Miz1* mutant mice and their littermate controls were detected.

### 3.4.2 Mice

Twenty-nine *Miz1* wild-type littermate control (16 male, 13 female) and twenty-five *Miz1*-mutant mice (13 male, 12 female) entered the Eye Screen at the age of 11 weeks. Mice were first examined by slitlamp biomicroscopy and on the following day, an ERG was performed. Mice were kept under standard laboratory conditions with food and water *ad libitum*.

### 3.4.3 Materials and Methods

**Electroretinography (ERG)** was used to examine the retinal function as described (Dalke *et al.*, 2004). Mice were dark-adapted for at least 12 hours and anaesthetized with 137 mg Ketamine and 6.6 mg Xylazine per kg body weight. After pupil dilation (1 drop Atropine 1%), individual mice were fixed on a sled with Velcro straps. Gold wires (as active electrodes) were placed on the cornea; care was taken not to obstruct the pupillary opening. The ground electrode was a subcutaneous needle in the tail; a reference electrode was placed subcutaneously between the eyes. The mice were introduced into an ESPION ColorBurst Handheld Ganzfeld LED stimulator (Diagnosys LLC, Littleton, MA, USA) on a rail to guide the sled (High-Throughput Mouse-ERG, STZ for Biomedical Optics and Function Testing, Tübingen, Germany). To minimize temperature influences on the ERG, body temperature was kept at 37°C using a warming plate. 10 ms light pulses were delivered at a frequency of 0.48 Hz in two steps at 500 and 12,500 cd/m<sup>2</sup>. Bandpass filter was set ranging from 0.15 to 1000 Hz. Responses were recorded simultaneously from both eyes with an ESPION Console (Diagnosys LLC, Littleton, MA, USA) and stored for offline analysis after averaging 10-40 individual measurements at each step.

**Slit Lamp Biomicroscopy:** Mice were examined biomicroscopically for eye abnormalities as previously described (Favor, 1983). Briefly, pupils were dilated with a 1% atropine solution applied to the eyes at least 10 min prior to examination. Both eyes of the mice were examined by slit lamp biomicroscopy (Zeiss SLM30) at 48x magnification with a narrow beam slit lamp illumination at 25-30° angle from the direction of observation. Observed phenotypic variants of the eyes were carefully documented.

**Statistical Analysis:** ERG data were statistically analyzed using MS-Excel. Differences between mouse groups were evaluated with the Student's t-test. Statistical significance was set at  $p < 0.05$ . Data are presented as mean values  $\pm$  standard error of the mean (SEM).

### 3.4.4 Parameters

<b>Electroretinography (ERG)</b>
a/b-wave, left/right eye at 500/12.50 cd/m <sup>2</sup>
<b>Slit lamp biomicroscopy</b>
(qualitative) abnormalities of lens and cornea like opacity and development disorders
<b>Histology</b>
(qualitative) retinal lamination and morphology of cell layers and lens
<b>Morphology</b>
(qualitative) like size and degree of closure

### 3.4.5 Results and Discussion

**ERG responses** were recorded from the groups of *Miz1* (control – mutant) mice with light pulses at two different light intensities. These two luminance levels were chosen because at 500 cd/m<sup>2</sup> a well discernable b-wave amplitude (nearly no a-wave) mainly stemming from the rod system is induced, while light pulses at 12,500 cd/m<sup>2</sup> induce a maximally developed b-wave response and an a-wave, coming presumably from rods and cones. At first, a comparison of the left and right eyes for each group was performed on the amplitudes of a- and b-wave for both luminance intensities (data not shown).

ERG screening is a quick, robust and reproducible *in-vivo* method to detect functional retinal impairment in mice. For the analysis of ERG data, the average amplitudes from left and right eyes was used, as no major differences between the eyes were detected in the ERG response (Table 16). Although the comparison of a- and b-wave amplitudes of males and females revealed significant differences, all ERG amplitudes varied in normal, non pathologic ranges and were observed in the group of wild-type and homozygous *Miz1*-deficient mice. No consistent differences were found between mutant and wild-type mice. No effect due to loss of function of the *Miz1* gene could be observed in ERG.

All animals were examined by **slit lamp biomicroscopy**. There were no major eye abnormalities and the minor opacities did not correlate with the genotype of animals. We conclude that there are no effects due to mutation of the *Miz1* gene.

### 3.4.6 References

- Dalke C., J. Löster, H. Fuchs, V. Gailus-Durner, D. Soewarto, J. Favor, A. Neuhäuser-Klaus, W. Pretsch, F. Gekeler, K. Shinoda, E. Zrenner, T. Meitinger, M. Hrabé de Angelis and J. Graw (2004): Electroretinography as a screening method for mutations causing retinal dysfunction in mice. *IOVS* 45: 601-609.
- Favor, J. (1983): A comparison of the dominant cataract and recessive specific-locus mutation rates induced by treatment of male mice with ethylnitrosourea. *Mutation Research* 110: 367-382.
- Graw J. (2003): The genetic and molecular basis of congenital eye defects. *Nat. Rev. Genet.* 4: 876-888.
- Johnson G.J. and A. Foster (2003): Prevalence, incidence and distribution of visual impairment. In: G.J. Johnson, D.C. Minassian, R.A. Weale, S.K. West (eds.): *The epidemiology of the eye disease*. Arnold, London, UK, 2003, 3-28.
- Rivolta C., D. Sharon, M. Hrabé de Angelis and T.P. Dryja (2002): Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. *Hum. Mol. Genet.* 11: 1219-1227.
- Stone E.M., V.C. Sheffield and G.S. Hageman (2001): Molecular genetics of age-related macular degeneration. *Hum. Mol. Genet.* 10: 2285-2292.

### Abbreviations

cd/m <sup>2</sup>	candela per square meter
ERG	electroretinography
Hz	hertz
n.s.	not significant

<b>Table 14: Comparison of ERG-Responses at Illumination Levels of 500 and 12,500 cd/m<sup>2</sup>.</b>								
Mean ± standard error is calculated for a- and b-wave amplitudes.								
Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=9)	(n=10)	<i>p - value</i>	(n=14)	(n=13)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
<b>a-wave</b> 500 cd/m <sup>2</sup>	-12 ± 1.1	-11 ± 1.5	n.s.	-18 ± 2.2	-11 ± 1.3	<0.01	<0.02	n.s.
<b>b-wave</b> 500 cd/m <sup>2</sup>	192 ± 10.7	164 ± 6.2	<0.05	196 ± 8.8	152 ± 7.2	<0.001	n.s.	n.s.
<b>a-wave</b> 12.500 cd/m <sup>2</sup>	-46 ± 2.6	-32 ± 4.1	<0.001	-41 ± 3.1	-40 ± 2.4	n.s.	n.s.	n.s.
<b>b-wave</b> 12.500 cd/m <sup>2</sup>	249 ± 11.6	182 ± 10.0	<0.001	236 ± 12.9	184 ± 8.5	<0.01	n.s.	n.s.

## 3.5 Clinical-Chemical Screen

### 3.5.1 Summary

The aim of the Clinical-Chemical Screen is the detection of hematological changes, defects of various organ systems, and changes in metabolic pathways and electrolyte homeostasis by means of suitable laboratory diagnostic tools. Since most inherited metabolic disorders are known to lead directly or indirectly, via altered organ function, to changes in the parameters investigated, this screening process provides a comprehensive investigation of clinical phenotypes with counterparts in humans and animal species (Rathkolb *et al.*, 2000). The methods used are routine procedures, allowing the appropriate screen of large numbers of mice for a broad spectrum of clinical-chemical and hematological parameters (Champy *et al.*, 2004; Hough *et al.*, 2002).

In the primary clinical chemical screen, twenty-eight (16 males/12 females) control mice and twenty-three (12 males /11 females) *Miz1*-deficient mice were analyzed. Twenty different clinical-chemical parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes. Additionally, we measured eight basic hematological parameters.

All parameters of both mutants and controls were in normal ranges.

### 3.5.2 Mice

Sixteen 12-week-old control and twelve 12-week-old mutant males entered the clinical-chemical screen at the beginning of the 44<sup>th</sup> calendar week in 2003. Twelve 12-week-old control and eleven 12-week-old mutant females entered the screen at the beginning of the 45<sup>th</sup> calendar week in 2003.

### 3.5.3 Materials and Methods

#### Blood Withdrawal and Storage

The Clinical-chemical Screen of the German Mouse Clinic routinely analyzed 12-week-old mice. A blood sample was taken from an ether-anesthetized mouse by puncturing the retro-orbital sinus with a non-heparinized capillary (0.8 mm in diameter; Laborteam K&K; Munich, Germany; Art.No. 1.28.13.1.2). The time for sample taking was recorded in a work list. Blood was collected in a heparinized tube (Li-heparin, KABE; Nümbrecht, Germany; Art.No. 078028). An additional smaller sample was collected (using the same capillary) in EDTA-coated tubes (KABE, Art.No 078035). The tube was immediately inverted five times to achieve a homogeneous distribution of the anticoagulant.

After removal of 40  $\mu$ l blood for the Neurology Screen, the Li-heparin-coated tubes were stored in a rack at room temperature for two hours. Afterwards, cells and plasma were separated by a centrifugation step (10 min, 4656 x g; Biofuge, Heraeus; Hanau, Germany). Plasma was distributed between the Immunology Screen (30  $\mu$ l), the Allergy Screen (30  $\mu$ l), the Clinical Chemical Screen (130  $\mu$ l) and the Steroid Screen (residual), while the cell pellet was given to the Immunology Screen for FACS-analysis. The plasma sample for the clinical chemical analysis was transferred into an Eppendorf tube

and diluted 1:2 with aqua dest. The solution was mixed for a few seconds (Vortex genie, Scientific Industries, New York, America) to prevent clotting and then centrifuged again for 10 min at 4656 x g. Additionally the Clinical Chemical Screen received the EDTA-blood sample for hematological investigations.

### **Clinical Chemistry**

The high-throughput of the screen was insured by the use of an Olympus AU 400 autoanalyzer and adapted reagents from Olympus (Hamburg, Germany) and Roche (Mannheim, Germany). In the primary screen, 20 different parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes.

### **Hematology**

A volume of 50 µl EDTA-blood was used to measure basic hematological parameters with a blood analyzer, which has been carefully validated for the analysis of mouse blood (ABC-Blutbild-Analyzer, Scil Animal Care Company GmbH, Viernheim). Red blood cells, white blood cells, and platelets are measured by electrical impedance, and hemoglobin by spectrophotometry. Mean corpuscular volume (MCV) is calculated directly from the cell volume measurements, the hematocrit (HCT) from  $MCV \times \text{red blood cell count}$ . Mean corpuscular hemoglobin (MCH) and mean concentration of corpuscular hemoglobin (MCHC) are calculated from hemoglobin/red blood cells count (MCH) and hemoglobin/hematocrit (MCHC).

### **Analysis of Data**

Data were statistically analyzed using Excel and Sigma Stat 2.0 with the level of significance set at  $p < 0.05$ .

## **3.5.4 Parameters**

<b>Proteins and plasma enzyme activities</b>
Alkaline phosphatase (EC 3.1.3.1), $\alpha$ -Amylase (EC 3.2.1.1), Creatine kinase (EC 2.7.3.2), Aspartate-aminotransferase (AST/GOT; EC 2.6.1.1), Alanine-aminotransferase (ALT/GPT; EC 2.6.1.2), Ferritin, Transferrin, Lipase (EC 3.1.1.3), Total protein
<b>Plasma concentrations of specific substrates</b>
Glucose, Cholesterol, Triglycerides, Uric acid, Urea, Creatinine
<b>Plasma concentrations of electrolytes</b>
Potassium, Sodium, Chloride, Calcium, Inorganic phosphate
<b>Basic hematology</b>
White blood cell count (WBC), Red blood cell count (RBC) Hematocrit (HCT), Hemoglobin (HGB), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Platelet count (PLT)

### **3.5.5 Results**

#### **Clinical Chemistry**

Most values obtained for the clinical chemical parameters were within the normal ranges usually found in C57BL/6 mice at the age of three months and were supported by previously published data (Table 15; Suckow *et al*, 2001; Quimby, 1999 and publications cited therein). In the control animals, we saw sex-related differences in several parameters (chloride, creatinine, uric acid, cholesterol, triglyceride, alanin-aminotransferase, alkaline phosphatase and amylase concentrations) like in *Miz1*-deficient mice (in sodium, urea, uric acid, cholesterol, triglyceride, alanin-aminotransferase, alkaline phosphatase, amylase and ferritin concentrations). Slight differences between mutants and controls were uncovered in ferritin level only in males and in cholesterol level only in females.

#### **Hematology**

In the primary screen for hematological parameters all parameters of both wild type and mutants were in normal ranges (Table 16). We found no remarkable differences between *Miz1*-deficient mice and their littermate controls.

Raw data for each individual are available on demand in Excel sheets.

### **3.5.6 Discussion**

All clinical chemical and haematological parameters were within the normal ranges. We found a lower ferritin level in male mutant mice. Serum ferritin is especially useful in distinguishing iron deficiency from the anaemia of chronic disorders. A low serum ferritin level almost always indicates iron deficiency.

Additionally we saw an elevated cholesterol level in female mutant mice. High cholesterol levels usually correlate with high body fat content (compare to the slightly but not statistically significant increase in body fat; Table 6, 3.2.5 Dysmorphology screen). However, the differences are quite small and the results for all groups for these parameters are still within the physiologic ranges. Therefore these findings are judged to be without pathological meaning.

Raw data for each individual are available on demand in Excel sheets.

### **3.5.7 References**

Champy, M.-F., M. Selloum, L. Piard, V. Zeitler, C. Caradec, P. Chambon and J. Auwerx (2004): Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mammalian Genome* 15: 768-783

Hough T.A., P. Nolan, V. Tshipouri, A., Toye, I. Gray, M. Goldsworthy, L. Moir, R. Cox, S. Clements, P. Glenister, J. Wood, R. Selley, M. Strivens, L.

Vizor, S. McCormack, J. Peters, E. Fisher, N. Spurr, S. Rastan, J. Martin, S. Brown and A. Hunter (2002): Novel phenotypes identified by plasma biochemical screening in the mouse. *Mammalian Genome* 13: 595-602

Quimby, F. (1999) The Mouse. In: *The clinical chemistry of laboratory animals*, ed. by W. F. Loeb and F. W. Quimby. Taylor and Francis, New York, pp. 3-31

Rathkolb B, Decker T, Fuchs E, Soewarto D, Fella C, Heffner S, Pargent W, Wanke R, Balling R, Hrabe de Angelis M, Kolb HJ and E. Wolf (2000): The clinical-chemical screen in the Munich ENU Mouse Mutagenesis Project: screening for clinically relevant phenotypes. *Mammalian Genome* 11: 543-546

Suckow, M.A., P. Dannemann and C. Brayton (2001) *The laboratory mouse* CRC Press, Boca Raton, London, New York, Washington

<b>Table 15: Clinical-Chemical Parameters.</b>								
Data are presented as mean $\pm$ standard error of mean.								
Parameter	Mutant (A)			Control (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=12)	(n=11)	<i>p- value</i>	(n=16)	(n=13)	<i>p-value</i>	<i>p-value</i>	<i>p-value</i>
<b>Sodium [mmol/l]</b>	157 $\pm 0.67$	154 $\pm 0.72$	<b>&lt;0.01</b>	157 $\pm 0.57$	157 $\pm 1.54$	n.s.	n.s.	n.s.
<b>Potassium [mmol/l]</b>	4.6 $\pm 0.48$	3.7 $\pm 0.07$	n.s.	4.0 $\pm 0.07$	3.9 $\pm 0.08$	n.s.	n.s.	n.s.
<b>Calcium [mmol/l]</b>	1.9 $\pm 0.15$	2.0 $\pm 0.00$	n.s.	1.9 $\pm 0.02$	2.0 $\pm 0.03$	n.s.	n.s.	n.s.
<b>Chloride [mmol/l]</b>	112.5 $\pm 0.33$	113.5 $\pm 0.56$	n.s.	112.5 $\pm 0.51$	115.5 $\pm 1.19$	<b>&lt;0.05</b>	n.s.	n.s.
<b>Inorganic Phosphate [mmol/l]</b>	1.8 $\pm 0.09$	1.6 $\pm 0.07$	n.s.	1.6 $\pm 0.05$	1.6 $\pm 0.17$	n.s.	n.s.	n.s.
<b>Total Protein [g/dl]</b>	4.8 $\pm 0.09$	4.8 $\pm 0.11$	n.s.	4.7 $\pm 0.08$	4.8 $\pm 0.08$	n.s.	n.s.	n.s.
<b>Creatinine [mg/dl]</b>	0.357 $\pm 0.01$	0.337 $\pm 0.01$	n.s.	0.373 $\pm 0.01$	0.351 $\pm 0.00$	<b>&lt;0.05</b>	n.s.	n.s.
<b>Urea [mg/dl]</b>	63.7 $\pm 1.50$	56.1 $\pm 2.33$	<b>&lt;0.02</b>	60.3 $\pm 3.1$	58.1 $\pm 2.9$	n.s.	n.s.	n.s.
<b>Uric acid [mg/dl]</b>	3.0 $\pm 0.28$	4.4 $\pm 0.29$	<b>&lt;0.01</b>	2.7 $\pm 0.4$	3.8 $\pm 0.2$	<b>&lt;0.05</b>	n.s.	n.s.
<b>Cholesterol [mg/dl]</b>	95.4 $\pm 3.85$	81.5 $\pm 2.79$	<b>&lt;0.01</b>	86.9 $\pm 4.1$	73.1 $\pm 2.5$	<b>&lt;0.01</b>	n.s.	<b>&lt;0.05</b>
<b>Triglyceride [mg/dl]</b>	136.0 $\pm 12.10$	101.4 $\pm 8.42$	<b>&lt;0.03</b>	131.5 $\pm 13.3$	89.8 $\pm 5.2$	<b>&lt;0.01</b>	n.s.	n.s.
<b>Creatine Kinase [U/l]</b>	121 $\pm 44.89$	69 $\pm 22.20$	n.s.	101 $\pm 28$	52 $\pm 10.00$	n.s.	n.s.	n.s.
<b>Alanine-Amino-transferase (ALAT,GPT) [ U/l]</b>	32 $\pm 5.17$	17 $\pm 2.18$	<b>&lt;0.02</b>	24 $\pm 3.00$	16 $\pm 1.00$	<b>&lt;0.02</b>	n.s.	n.s.
<b>Aspartate-Amino-transferase (AST,GOT) [U/l]</b>	36 $\pm 4.84$	31 $\pm 3.15$	n.s.	32 $\pm 3.00$	30 $\pm 2.00$	n.s.	n.s.	n.s.
<b>Alkaline Phosphatase [U/l]</b>	171 $\pm 12.46$	220 $\pm 7.08$	<b>&lt;0.01</b>	164 $\pm 8.00$	226 $\pm 5.00$	<b>&lt;0.001</b>	n.s.	n.s.
<b><math>\alpha</math>-Amylase [U/l]</b>	2328 $\pm 86.24$	1885 $\pm 53.73$	<b>&lt;0.001</b>	2413 $\pm 76.00$	2020 $\pm 52.00$	<b>&lt;0.001</b>	n.s.	n.s.
<b>Glucose [mg/dl]</b>	194.3 $\pm 9.93$	186.9 $\pm 12.31$	n.s.	198.8 $\pm 7.3$	202.6 $\pm 13.2$	n.s.	n.s.	n.s.
<b>Ferritin [ng/ml]</b>	22.3 $\pm 1.27$	27.1 $\pm 0.96$	<b>&lt;0.01</b>	28.9 $\pm 2.8$	29.2 $\pm 1.6$	n.s.	<b>&lt;0.05</b>	n.s.
<b>Transferrin [mg/dl]</b>	125.2 $\pm 2.46$	119.6 $\pm 5.01$	n.s.	121.5 $\pm 1.1$	109.5 $\pm 8.4$	n.s.	n.s.	n.s.

**Table 16: Hematological Parameters.**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Mutant (A)			Control (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=12)	(n=12)	<i>p</i> - value	(n=16)	(n=13)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
White blood cell count [10 <sup>3</sup> /μl]	6.72 ±0.44	6.18 ±0.54	n.s.	6.54 ±0.51	6.84 ±0.48	n.s.	n.s.	n.s.
Red blood cell count [10 <sup>3</sup> /μl]	9.97 ±0.21	10.22 ±0.13	n.s.	10.25 ±0.30	10.48 ±0.27	n.s.	n.s.	n.s.
Hemoglobin [g/dl]	15.64 ±0.29	15.83 ±0.17	n.s.	16.12 ±0.41	16.07 ±0.35	n.s.	n.s.	n.s.
Hematocrit [%]	45 ±0.93	47 ±0.64	n.s.	47 ±1.36	48 ±1.12	n.s.	n.s.	n.s.
Mean corpuscular volume [fl]	45.33 ±0.22	46.00 ±0.17	<0.05	46.38 ±0.15	46.00 ±0.28	n.s.	<0.01	n.s.
Mean corpuscular hemoglobin [pg]	15.71 ±0.17	15.49 ±0.08	n.s.	15.76 ±0.09	15.35 ±0.09	n.s.	n.s.	n.s.
Mean corpuscular hemoglobin concentration [g/dl]	34.62 ±0.28	33.74 ±0.18	<0.02	34.06 ±0.19	33.41 ±0.13	<0.01	n.s.	n.s.
Platelet count [10 <sup>3</sup> /μl]	827 ±34.69	786 ±23.06	n.s.	771 ±29.05	746 ±23.44	<0.01	n.s.	n.s.

## 3.6 Immunology Screen

### 3.6.1 Summary

Mouse models have been a primary source of information for understanding the intricate mechanisms of the immune system (Blüethmann and Ohashi, 1994; Mak *et al.*, 2001; Fischer 2002; Rogner and Avner, 2003). The Immunology Screen at the GMC was set up to conduct a broad immunological phenotyping of mouse mutant lines with the intention of identifying distinct gene functions, which play key roles in the immune defenses of the organism through a complex network of cellular and soluble components (Janeway *et al.*, 2004).

According to the data summary presented to the phenotyping center, no immunological phenotype was known in *Miz1*-deficient mice. Their analysis in the Immunology screen revealed certain differences between mutants and their littermate controls.

### 3.6.2 Mice

We analyzed twenty-three mutant animals (11 females and 12 males) and the 29 age- and sex-matched littermate controls (13 females and 16 males).

### 3.6.3 Material and Methods

Peripheral blood leukocytes (PBLs) were isolated from 500  $\mu$ l blood by erythrocyte lysis with  $\text{NH}_4\text{Cl}$  (0.17M) - Tris buffer (pH 7.45) directly in 96-well microtiter plates. After subsequent washing with FACS staining buffer (PBS, 0.5% BSA, 0.02% sodium azide, pH 7.45), PBLs were incubated for 20 min with 1  $\mu$ M ethidium monazide bromide (EMA, Molecular Probes, The Netherlands) and Fc block (clone 2.4G2, PharMingen, San Diego, USA). EMA bound to the DNA of dead cells was photocrosslinked by brief light exposure. Cells were then stained with fluorescence-conjugated monoclonal antibodies (PharMingen).

The following main cell populations were analyzed: B cells (CD19<sup>+</sup> clone 1D3), B1 B cells (CD19<sup>+</sup>CD5<sup>+</sup>, clone 53-7.3), B2 B cells (CD19<sup>+</sup>CD5<sup>-</sup>), T cells (CD3<sup>+</sup>, clone 145-2C11), CD4<sup>+</sup> T cells (clone RM4-5), CD8<sup>+</sup> T cells (CD8 $\alpha$ , clone 53-6.7; CD8 $\beta$ , clone H35-17.2),  $\gamma/\delta$ T cells (clone GL3), granulocytes (Gr-1<sup>+</sup>, clone RB6-8C5), and NK cells (CD49b<sup>+</sup>, clone DX5). We also analyzed additional subpopulations based on the following surface antigens: IgD (clone 11-26c.2a), B220 (clone RA3-6B2), CD11b (clone M1/70), CD103 (clone 2E7), CD25 (clone PC61), CD62L (clone MEL-14), CD45RA (clone 14.8), Ly-6C (clone AL-21), and CD44 (clone IM7). Data were acquired on a FACS Calibur (Becton Dickinson, San Diego, USA) and were analyzed using FlowJo software (TreeStar Inc, USA). All samples were acquired until a total number of 25,000 cells was reached.

The plasma levels of IgM, IgG<sub>1</sub>, IgG<sub>2a</sub>, IgG<sub>2b</sub>, IgG<sub>3</sub>, and IgA were determined by standard sandwich ELISAs using goat anti-mouse immunoglobulin antibodies and alkaline phosphatase (AP) conjugates (SouthernBiotech, Birmingham, USA). The presence of rheumatoid factor and anti-DNA antibodies was evaluated by indirect ELISA with rabbit IgG (Sigma-Aldrich, Steinheim,

Germany) and calf thymus DNA (Sigma-Aldrich), respectively, as antigens and AP-conjugated goat anti-mouse secondary antibody (Sigma-Aldrich). Serum samples from MRL/MpJ-Tnfrsf6<sup>lpr</sup> mice (Jackson Laboratory, Bar Harbor, USA) were used as positive controls in the autoantibody assays.

### 3.6.4 Parameters

<b>Flow cytometry</b>
B cells (CD19 <sup>+</sup> ), B1 B cells (CD19 <sup>+</sup> CD5 <sup>+</sup> ), B2 B cells (CD19 <sup>+</sup> CD5 <sup>-</sup> ), T cells (CD3 <sup>+</sup> ), CD4 <sup>+</sup> T cells, CD8 <sup>+</sup> T cells, $\gamma/\delta$ T cells, granulocytes (Gr-1 <sup>+</sup> ), and NK cells (CD49b <sup>+</sup> ). Furthermore, all potential subpopulations which can be identified by co-staining for other surface markers (IgD, B220, CD11b, MHC II, I-A <sup>k</sup> , CD25, CD8 $\beta$ , CD62L, CD45RA, Ly-6C, CD44) using 6 parameter/5 color flow cytometry were analyzed.
<b>ELISA</b>
IgM, IgG <sub>1</sub> , IgG <sub>2a</sub> , IgG <sub>2b</sub> , IgG <sub>3</sub> , IgA; anti-DNA antibodies, rheumatoid factor

### 3.6.5 Results

The analysis of standard immunological parameters measured in the primary screen (Table 14) revealed significant differences between *Miz1*-deficient mice and their littermate controls. The percentage of CD19<sup>+</sup> cells (B cells) was significantly lower in the mutants (also found for another B-cell marker, B220). CD49b (pan-NK marker), was increased in mutant females. No significant genotype differences could be observed with regard to other cell surface markers included in the screen.

We also found a number of sex differences in both mutants and controls, but they represented a well established phenomenon in inbred mouse strains.

### 3.6.6 Discussion

Under standard screen conditions, *Miz1*-deficient mice showed significant changes in the percentage of B cells and NK cells. The lower numbers of B cells probably reflect a real phenotype, since this was confirmed for two different B-cell markers. However, under baseline conditions those changes did not lead to altered Ig production - similar isotype levels were found in mutants and controls (with the exception of IgM, which seemed to be higher in mutant males). Further immunological investigations in a varied setting could be interesting.

Raw data will be available on demand.

### 3.6.7 References

Bluethmann, H., and P. S. Ohashi (Eds.) (1994): Transgenesis and targeted mutagenesis in immunology. Academic Press, San Diego.

Fischer, A. (2002): Natural mutants of the immune system: a lot to learn! *Eur J Immunol* 32: 1519-1523.

Janeway C, Travers P, Walport M, Shlomchik M and M.J. Shlomchik (2004) *Immunobiology: The Immune System in Health and Disease*. 6th edition, Garland Publishing, London.

Mak, T. W., J. M. Penninger and P. S. Ohashi (2001): Knockout mice: a paradigm shift in modern immunology. *Nat Rev Immunol* 1: 11-19.

Rogner, U. C., and P. Avner (2003): Congenic mice: cutting tools for complex immune disorders. *Nat Rev Immunol* 3: 243-252.

**Table 17: Basic Parameters Analyzed in the Immunology Screen.**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Mutants (A)			Control (B)			A - B	
	Male	Female		Male	Female		Male	Female
	(n=12)	(n=11)	<i>p</i> - value	(n=16)	(n=13)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
<b>CD19<sup>+</sup></b> [%]	37.4 $\pm$ 1.6	25.3 $\pm$ 2.9	<b>&lt;0.01</b>	44.1 $\pm$ 1.3	33.1 $\pm$ 2.4	<b>&lt;0.001</b>	<b>&lt;0.01</b>	<b>&lt;0.05</b>
<b>CD19<sup>+</sup>CD5<sup>-</sup></b> [%]	91.9 $\pm$ 0.5	84.7 $\pm$ 3.4	<b>&lt;0.05</b>	91.5 $\pm$ 0.3	89.4 $\pm$ 2.3	n.s.	n.s.	n.s.
<b>CD19<sup>+</sup>CD5<sup>+</sup></b> [%]	8.1 $\pm$ 0.5	10.1 $\pm$ 2.0	<b>&lt;0.05</b>	8.5 $\pm$ 0.3	14.3 $\pm$ 2.9	n.s.	n.s.	n.s.
<b>CD3<sup>+</sup></b> [%]	28.3 $\pm$ 1.6	36.0 $\pm$ 3.2	<b>&lt;0.05</b>	27.1 $\pm$ 1.1	35.8 $\pm$ 2.1	<b>&lt;0.001</b>	n.s.	n.s.
<b><math>\gamma/\delta</math> TCR<sup>+</sup></b> [%]	3.0 $\pm$ 0.2	3.6 $\pm$ 0.4	n.s.	3.0 $\pm$ 0.2	3.3 $\pm$ 0.4	n.s.	n.s.	n.s.
<b>Gr-1<sup>+</sup></b> [%]	29.9 $\pm$ 2.8	21.6 $\pm$ 3.9	n.s.	27.7 $\pm$ 1.7	16.0 $\pm$ 1.1	<b>&lt;0.001</b>	n.s.	n.s.
<b>CD49b<sup>+</sup></b> [%]	18.3 $\pm$ 1.9	31.2 $\pm$ 1.9	<b>&lt;0.001</b>	19.4 $\pm$ 1.2	24.8 $\pm$ 1.8	<b>&lt;0.02</b>	n.s.	<b>&lt;0.05</b>
<b>CD4<sup>+</sup></b> [%]	17.3 $\pm$ 0.7	19.3 $\pm$ 2.5	n.s.	17.5 $\pm$ 1.2	18.1 $\pm$ 2.0	n.s.	n.s.	n.s.
<b>CD8<math>\beta</math><sup>+</sup></b> [%]	11.4 $\pm$ 0.7	12.6 $\pm$ 1.3	n.s.	11.2 $\pm$ 0.5	13.1 $\pm$ 1.2	n.s.	n.s.	n.s.
<b>IgG<sub>1</sub></b> [ $\mu$ g/ml]	185.9 $\pm$ 27.6	225.2 $\pm$ 31.9	n.s.	184.6 $\pm$ 24.2	226.5 $\pm$ 20.5	n.s.	n.s.	n.s.
<b>IgG<sub>2a</sub></b> [ $\mu$ g/ml]	94.8 $\pm$ 7.3	106.9 $\pm$ 9.2	<b>&lt;0.02</b>	80.3 $\pm$ 7.9	119.8 $\pm$ 13.1	n.s.	n.s.	n.s.
<b>IgG<sub>2b</sub></b> [ $\mu$ g/ml]	24.6 $\pm$ 3.2	265.0 $\pm$ 46.3	<b>&lt;0.001</b>	27.3 $\pm$ 3.8	200.1 $\pm$ 35.0	<b>&lt;0.001</b>	n.s.	n.s.
<b>IgG<sub>3</sub></b> [ $\mu$ g/ml]	300.7 $\pm$ 49.7	293.6 $\pm$ 40.6	n.s.	190.7 $\pm$ 38.2	265.4 $\pm$ 34.8	n.s.	n.s.	n.s.
<b>IgM</b> [ $\mu$ g/ml]	119.0 $\pm$ 19.1	1093 $\pm$ 197	<b>&lt;0.001</b>	72.9 $\pm$ 6.4	1319 $\pm$ 109	<b>&lt;0.001</b>	<b>&lt;0.02</b>	n.s.
<b>IgA</b> [ $\mu$ g/ml]	15.4 $\pm$ 3.2	137.6 $\pm$ 16.4	<b>&lt;0.001</b>	15.0 $\pm$ 2.3	124.4 $\pm$ 16.7	<b>&lt;0.001</b>	n.s.	n.s.
<b>Anti-DNA Ab</b> [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.
<b>Rheumatoid factor</b> [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.

Raw data will be available on demand.

## 3.7 Allergy Screen

### 3.7.1 Summary

The goal of the Allergy screen within the German Mouse Clinic (GMC) is to search for IgE mutants in order to establish mouse models for allergic diseases and to find new strategies for antiallergic therapy. The increased production of IgE in response to common environmental antigens is the hallmark of atopic diseases in man (Hamelmann *et al.* 1999). Mouse mutants with phenotypic alterations in IgE production represent a valuable tool to study and characterize the molecular mechanisms of IgE-mediated allergic hypersensitivity (Zhang *et al.* 1997).

In the primary Allergy screen of *Miz1* mice, 24 control and 21 mutant animals were screened. Their analysis did not reveal any profound differences between mutant and control mice.

### 3.7.2 Mice

An age- and sex-matched group of 24 control (14 females, 10 males) and 21 mutant (10 females, 11 males) mice aged 12 weeks was analyzed in Allergy screen.

### 3.7.3 Material and Methods

Twelve-week-old male and female mice were screened for alterations in plasma total IgE concentrations. Blood samples were taken from animals by puncturing the retroorbital plexus under ether anesthesia. Plasma IgE concentrations were measured by isotype-specific sandwich ELISA technique with a lower detection limit of 1 ng/ml. briefly, microtiter plates were coated with the IgG fraction of sheep anti-mouse IgE in sodium bicarbonate buffer (pH 9.6). After incubation, plates were washed with Tris buffer (pH 7.4) and blocked with 3% (w/v) bovine serum albumin at room temperature. Diluted plasma samples and standard were added to the plates. After overnight incubation biotinylated rat anti-mouse IgE was added and plates were incubated at room temperature for 2 h. Then plates were incubated in the presence of peroxidase-labeled streptavidin. After washing, tetramethylbenzidine (TMB) substrate solution was added and after an appropriate incubation time the stop solution (sulphuric acid, 2M) was added. The plates were read in a standard microplate reader at a wavelength of 450 nm. Total murine IgE data are reported in ng/ml, based on a standard curve of purified murine IgE. (Alessandrini *et al.*, 2001)

### 3.7.4 Results and Discussion

The analysis of total IgE levels in plasma of *Miz1*-deficient mice and their sex- and age-matched littermate controls revealed higher mean IgE concentration in female control animals comparing to male mice. The total IgE concentration in male KO mice was higher than in female animals. However, this difference was statistically not significant (Table 18).

**Table 18: Total plasma IgE in Miz1 mice**Data are presented as mean  $\pm$  standard error of mean.

	Control (A)			Mutant (B)			A~B	A~B
	Female	Male		Female	Male		Female	Male
	(n=14)	(n=10)	<i>p</i> - value	(n=10)	(n=11)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
<b>Total IgE [ng/ml]</b>	13.5 $\pm$ 6	6.1 $\pm$ 5.2	n.s.	9.3 $\pm$ 2.2	17.1 $\pm$ 6.7	n.s.	n.s.	n.s.

Raw data will be available on demand.

### 3.7.5 References

Alessandrini, F., Jakob, T., Wolf, A., Wolf, E., Balling, R., Hrabé de Angelis, M., Ring, J., Behrendt, H. (2001): ENU mouse mutagenesis: Generation of mouse mutants with aberrant plasma IgE levels. *Int Arch Allergy Immunol.* 124: 25-28

Hamelmann, E., K. Takeda, A. Oshiba and E.W. Gelfand (1999): Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness – a murine model. *Allergy* 54: 297-305

Zhang, Y., W.J.E. Lamm, R.K. Albert, E.Y. Chi, W.R.Henderson and D.B. Lewis (1997) Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Respir Crit Care Med.* 155: 661-669

## 3.8 Nociceptive Screen

### 3.8.1 Summary

Pain is the perception of an aversive or unpleasant sensation that originates from a specific region of the body. The highly subjective nature of pain is one of the factors that make it difficult to define and to treat clinically. Pain is more than a conspicuous sensory experience that warns of danger.

Nociceptors are activated by tissue injury but also by mechanical, thermal, or chemical stimuli. Harmful stimuli applied to the skin or to subcutaneous tissue, activate nociceptors, the peripheral endings of primary sensory neurons whose cell bodies are located in the dorsal root or in the trigeminal ganglia.

A noxious stimulus activates the nociceptor by depolarizing the membrane of the sensory ending. When peripheral tissues are damaged, the sensation of pain in response to subsequent stimuli is enhanced. This phenomenon termed hyperalgesia, may involve a lowering of threshold of the nociceptors or an increase in the magnitude of pain evoked by suprathreshold stimuli. Hyperalgesia can occur both at the site of tissue damage (primary hyperalgesia) and in the surrounding undamaged areas (secondary hyperalgesia; Wall and Melzak, 1984). By means of different inbred mouse strains it could be demonstrated that rodents display large and heritable differences in both nociceptive and analgesic sensitivity (Mogil, 1999; Mogil *et al.*, 1999)

In the primary screen the responsiveness of the intact somatosensory system to thermal pain was tested in the *Miz1* mice by means of the hot plate test (nociceptive pain). We did not find any differences in pain responsiveness between female and male animals as well as between mutants and controls.

### 3.8.2 Mice

Nineteen *Miz1*-mutant mice (12 male, seven female), and 23 control animals (16 male, seven female) were tested in our first screen.

### 3.8.3 Material and Methods

#### Hot plate test

The mice were placed on a metal surface maintained at  $52 \pm 0.2^\circ\text{C}$  (Hot plate system was made by TSE GMBH, Germany; Eddy and Leimbach, 1953). Locomotion of the mouse on the hot plate was constrained by 20 cm high plexiglas wall to a circular area with a diameter of 28 cm. Mice remained on the plate until they performed one of three behaviors regarded as indicative of nociception: hind paw lick (h.p. licking), hind paw shake/flutter (h.p. shaking) or jumping.

We evaluated only hind paw but not the front paw responses, because fore paw licking and lifting are components of normal grooming behavior. Each mouse was tested only once since repeated testing leads to profound changes in response latencies. The latency was recorded to the nearest 0.1 s. To avoid tissue injury 60 s cut-off time was used. The data values are given in seconds.

### Statistical analysis

Statistical analysis was performed using a statistical package Statgraphics® (Statistical Graphics Corporation, Rockville, MD). The differences between the groups were compared with ANOVA, LSD test was used as *post hoc*. Statistical significance was assumed at  $p < 0.05$ .

### 3.8.4 Parameters

<b>Hind paw licking</b>
Reaction with licking of hind paw to the thermal pain
<b>Hind paw shaking</b>
Reaction with shaking of hind paw to the thermal pain
<b>Jumping</b>
Jumping reaction to the thermal pain

### 3.8.5 Results and Discussion

Typically, the first nociceptive response observed in mice was hind paw shaking, followed by hind paw licking. The third examined response was jumping of the animals.

It was no sex difference in response to thermal pain either wild-type or mutant animals detectable, both sexes reacted uniformly, as well as no genotype-specific difference was observed (Table 19).

Raw data will be available on demand.

### 3.8.6 References

Eddy, N.B. Leimbach, D. (1953): Synthetic analgesics II. Diethienylbutenyl – and dithienylbutylamines. *J. Pharmacol. Exp. Ther.* 107: 385-393

Mogil J.S. (1999): The genetic mediation of individual differences in sensitivity to pain and its inhibition. *Proc. Nat. Acad. Sci.* 96: 7744-7751

Mogil J.S., S.G. Wilson, K. Bon, S.E. Lee, K. Chung, P. Raber, J.O. Pieper, H.S. Hain, J.K. Belknap, L. Hubert, G.I. Elmerl, J.M. Chung and M. Devor (1999): Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain.* 80:67-82.

Wall P.D. and R. Melzack (Eds.) *Textbook of Pain*, Churchill Livingstone, London, 1984

### Abbreviations

h.p. hind paw

**Table 19: Nociceptive Screen**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Mutant (A)			Control (B)			A~B	A~B
	Female	Male		Female	Male		Female	Male
	(n=7)	(n=12)	<i>p-value</i>	(n=7)	(n=16)	<i>p-value</i>	<i>p-value</i>	<i>p-value</i>
<b>H.p. licking</b>	13.8 $\pm$ 1.51	13.8 $\pm$ 1.15	n.s.	15.3 $\pm$ 1.51	16.7 $\pm$ 0.99	n.s.	n.s.	n.s.
<b>H.p. shaking</b>	13.1 $\pm$ 1.6	10.3 $\pm$ 1.21	n.s.	14.5 $\pm$ 1.59	11.6 $\pm$ 1.06	n.s.	n.s.	n.s.
<b>Jumping</b>	42.4 $\pm$ 3.52	51.3 $\pm$ 2.59	n.s.	55.2 $\pm$ 3.52	55.6 $\pm$ 2.33	n.s.	n.s.	n.s.

## 3.9 Lung Function Screen

### 3.9.1 Summary

Neural and mechanical processes that control breathing frequency have been investigated in man for a long time (Mead, 1960; Otis *et al.*, 1959), but only with the availability of mouse inbred strains the contribution of genetic determinants to differential baseline breathing patterns could be elucidated (Tankersley *et al.*, 1997; Tankersley, 1999). By use of genetically engineered mice, candidate genes for human developmental disorders of breathing have been identified (Katz, 2003).

Spontaneous breathing patterns during rest and activity were studied in 15 weeks old male and female *Miz1*-mutant and wild-type mice. Concerning the absolute parameters of the spontaneous breathing pattern, statistically relevant sex differences were not pronounced in wild-type mice, neither in mutants. For the specific values, which take the body weight into account, females demonstrated higher specific tidal volumes and, thus, higher specific minute ventilation than males in both wild-type and mutant mice. These differences might be due to the differences in body weight between male and female mice.

Comparing control to mutant mice, the mutants showed reduced tidal volumes resulting in a reduced breathing pattern in both sexes. Further investigations are needed to determine whether these results are secondary effects of, e.g. reduced muscle function or are primarily related to impaired lung function. A first hint that the muscle strength could be reduced in the *Miz1*-deficient mice was also seen in the “wire maneuver test” performed within the primary tests of the Neurology screen.

### 3.9.2 Mice

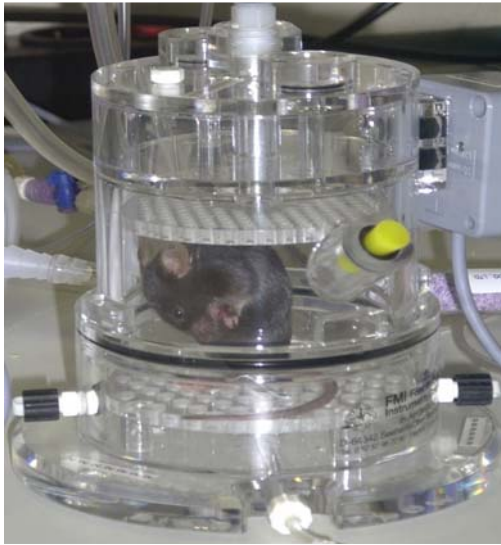
Control and mutant mice of both sexes were studied at the age of 15 weeks. Mean body weights were listed in Table 21. As to be expected, body weight in female mice was significantly lower.

### 3.9.3 Material and Methods

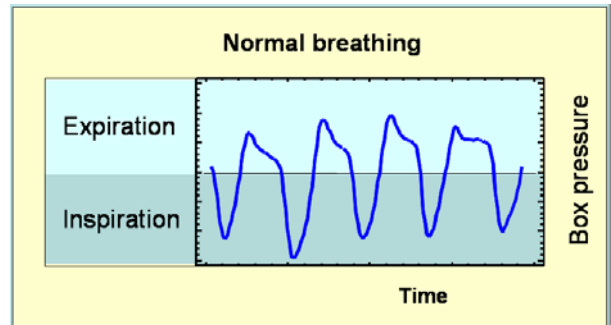
#### Whole Body Plethysmography

A commercially available system from Buxco<sup>®</sup> Electronics (Sharon, Connecticut) was used to assess breathing patterns in unrestrained animals according to the principle described by Drorbaugh and Fenn (1955). It measures the pressure changes which arise from inspiratory and expiratory temperature and humidity fluctuations during breathing (Fig. 5 and 6).

Calibration of the system allows to transform these pressure swings into flow and volume signals so that automated data analysis provides tidal volumes (TV), respiratory rates (f), minute ventilation (MV), inspiratory and expiratory times (Ti, Te), as well as peak inspiratory and peak expiratory flow rates (PIF, PEF). These data were stored online as mean values at 10 s intervals.



**Figure 5: System used at GMC to assess breathing patterns.**



**Figure 6: Recorded data used to calculate the breathing parameters.**

Measurements were always performed between 8 a.m. and 11 a.m. to account for potential diurnal variations in breathing. The system was set up in a quiet room where temperature and humidity were kept constant throughout the measurements. Before each measurement, the system was calibrated and the actual barometric pressure, temperature, and humidity were supplied to warrant adequate calculations of flow rates and volumes. After placing the animals into the chamber, data recording was immediately started and was continued for 40 min. Mice underwent typical phases during the measuring period. Primarily, the animals were stressed so that the respiratory rate was highest at the beginning. Usually after 5 min. the animals became calmer, they slightly reduced their respiratory rate, and began to explore the chamber and start cleaning themselves – *phase of activity*. Later activity was more and more interrupted by phases of rest or even short periods of snoozing – *resting phase*. Some of the animals even went to *phases of sleep*, which resulted in a further marked decrease in respiratory rate. The frequency histogram of the respiratory rates was determined for each individual, and breathing was analyzed for the above mentioned parameters during the phases of activity and rest. In addition to the directly recorded parameters, mean inspiratory and expiratory flow rates (MEF, MIF) were calculated offline from the ratio of tidal volume and the respective time interval. The relative duration of inspiration ( $T_i/TT$ ) was determined from the ratio of inspiratory time to total time required for the breathing cycle. Specific tidal volumes and minute ventilations (sTV, sMV) were calculated by relating the absolute values to the body weight of the animal. Furthermore, the mean of all breathing frequencies (mean\_f) measured during the 40-minute-period was calculated as a rough and ready pa-

parameter to assess whether the duration of rest and activity was similar in all mouse strains.

### Statistical Analysis of Data

Statistical analyses were performed using a commercially available statistics package (Statgraphics®, Statistical Graphics Corporation, Rockville, MD). Differences between strains were evaluated by Student's t-test. Statistical significance was assumed at  $p < 0.05$ . Data are presented as mean values  $\pm$  standard error of the mean (SEM).

### 3.9.4 Parameters

<b>Directly recorded data</b>
Tidal volumes (TV), respiratory rates (f), minute ventilation (MV), inspiratory and expiratory times (Ti, Te), as well as peak inspiratory and peak expiratory flow rates (PIF, PEF).
<b>Calculated data</b>
mean inspiratory flow rates (MEF), expiratory flow rates (MIF), relative duration of inspiration (Ti/TT), specific tidal volumes (sTV), minute ventilations (sMV), mean of all breathing frequencies (mean_f)

### 3.9.5 Results and Discussion

Table 21 summarizes the results obtained for spontaneous breathing under resting and active conditions.

**Sex-related differences:** No significant sex differences were observed for the absolute values. During rest and activity females demonstrated higher specific tidal volumes and, thus, higher specific minute ventilation than males in both wild-type and mutant mice. Since these specific parameters take the body weight into account and the absolute values primarily were not affected, the observed sex differences are likely due to the significantly higher body weight in males compared to females. Additionally, it was found that mutant females displayed significantly higher breathing rates and, thus, smaller inspiratory and expiratory timing during activity phase. This tendency was also seen in wild-type females. In conclusion, these sex-related differences are common observations within various mouse inbred strains.

**Differences between mutant and control mice:** Control and mutant mice breathed at comparable rates but the mutants used lower tidal volumes and minute ventilation. In males, mainly the absolute parameters were affected and in females rather the specific values were decreased, but in both sexes, mutants showed a reduced breathing pattern compared to control mice. Possible explanations for that finding are a reduced ventilatory drive, reduced muscle function or impaired lung function. A first hint that the muscle strength could be reduced in the *Miz1*-deficient mice was also seen in the “wire manoeuvre test” performed within the primary tests of the Neurology screen (3.3.5) and will be investigated more closely in the secondary Neurology screen by use of the “grip strength test” (3.3.6). Considering the fact that the breathing pattern in the mutants was not dramatically altered, it is unlikely that

the lung is primarily affected. To determine the underlying cause, a larger number of mice and continuative investigations are needed.

Raw data are available on demand.

### **3.9.6 References**

Drorbaugh J.E. and W.O. Fenn (1955): A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16: 81-87

Katz D.M. (2003): Neuronal growth factors and development of respiratory control. *Respir. Physiol. Neurobiol.* 135: 155-165

Mead, J. (1960): Control of respiratory frequency. *J. Appl. Physiol.* 15: 325-336

Otis, A.B., W.O. Fenn and H. Rahn (1950): Mechanics of breathing in man. *J. Appl. Physiol.* 2: 592-607

Tankersley, C.G. (1999): Genetic control of ventilation: What are we learning from murine models? *Current Opinion in Pulmonary Medicine* 5: 344-348

Tankersley, C.G., Fitzgerald R.S., Levitt R.C., Mitzner W.A., Ewart S.L. and S.R. Kleeberger (1997): Genetic control of differential baseline breathing pattern. *J. Appl. Physiol.* 82: 874-81

## Abbreviations

bw	body weight (g)
mean_f	mean of all respiratory rates (1/min)
f	respiratory rate (1/min)
TV	tidal volume (ml)
sTV	specific tidal volume ( $\mu\text{l/g}$ )
MV	minute ventilation (ml/min)
sMV	specific ventilation (ml/min/g)
Ti	inspiratory time (ms)
Te	expiratory time (ms)
Ti/TT	relative duration of inspiration
PIF	peak inspiratory flow rate (ml/s)
PEF	peak expiratory flow rate (ml/s)
MIF	mean inspiratory flow rate (ml/s)
MEF	mean expiratory flow rate (ml/s).

**Table 20: Characterization of Studied Mice**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Control (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=5)	(n=4)	<i>p-value</i>	(n=5)	(n=5)	<i>p-value</i>	<i>p-value</i>	<i>p-value</i>
<b>bw</b>	27.3 $\pm$ 0.4	19.7 $\pm$ 0.5	< 0.001	25.0 $\pm$ 0.9	19.7 $\pm$ 0.7	< 0.01	n.s.	n.s.
<b>mean_f</b>	447.0 $\pm$ 9.4	395.3 $\pm$ 10.8	n.s.	457.5 $\pm$ 15.5	421.1 $\pm$ 29.4	n.s.	n.s.	n.s.

**Table 21: Spontaneous Breathing Pattern during Rest and Activity**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Control (A)			Mutant (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=5)	(n=4)	<i>p</i> - value	(n=5)	(n=5)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
<b>Rest</b>								
<b>f</b>	393.1 $\pm$ 1.2	386.6 $\pm$ 2.7	n.s.	395.4 $\pm$ 6.5	388.6 $\pm$ 2.0	n.s.	n.s.	n.s.
<b>TV</b>	0.27 $\pm$ 0.01	0.27 $\pm$ 0.02	n.s.	0.22 $\pm$ 0.004	0.23 $\pm$ 0.009	n.s.	< 0.02	< 0.05
<b>sTV</b>	9.7 $\pm$ 0.4	13.7 $\pm$ 0.6	< 0.001	9.0 $\pm$ 0.5	11.4 $\pm$ 0.4	< 0.01	n.s.	< 0.02
<b>MV</b>	102.8 $\pm$ 4.9	101.4 $\pm$ 6.2	n.s.	87.7 $\pm$ 1.1	86.7 $\pm$ 4.2	n.s.	< 0.02	0.0822
<b>sMV</b>	3.8 $\pm$ 0.2	5.2 $\pm$ 0.3	< 0.01	3.5 $\pm$ 0.2	4.4 $\pm$ 0.2	< 0.01	n.s.	< 0.05
<b>Ti</b>	49.7 $\pm$ 1.3	48.9 $\pm$ 1.5	n.s.	47.3 $\pm$ 1.6	45.6 $\pm$ 0.2	n.s.	n.s.	n.s.
<b>Te</b>	103.0 $\pm$ 0.8	106.3 $\pm$ 1.2	n.s.	104.6 $\pm$ 2.2	108.9 $\pm$ 0.7	n.s.	n.s.	n.s.
<b>Ti/TT</b>	0.33 $\pm$ 0.01	0.31 $\pm$ 0.009	n.s.	0.31 $\pm$ 0.01	0.30 $\pm$ 0.002	n.s.	n.s.	n.s.
<b>PIF</b>	9.5 $\pm$ 0.6	10.0 $\pm$ 0.8	n.s.	8.7 $\pm$ 0.3	9.0 $\pm$ 0.5	n.s.	n.s.	n.s.
<b>PEF</b>	5.9 $\pm$ 0.3	5.8 $\pm$ 0.5	n.s.	5.7 $\pm$ 0.2	5.3 $\pm$ 0.3	n.s.	n.s.	n.s.
<b>MIF</b>	5.4 $\pm$ 0.3	5.5 $\pm$ 0.4	n.s.	4.7 $\pm$ 0.2	4.9 $\pm$ 0.2	n.s.	n.s.	n.s.
<b>MEF</b>	2.6 $\pm$ 0.1	2.5 $\pm$ 0.2	n.s.	2.1 $\pm$ 0.02	2.1 $\pm$ 0.1	n.s.	< 0.01	< 0.05
<b>Activity</b>								
<b>f</b>	509.5 $\pm$ 6.4	527.0 $\pm$ 3.1	n.s.	506.6 $\pm$ 8.7	532.8 $\pm$ 3.8	< 0.05	n.s.	n.s.
<b>TV</b>	0.27 $\pm$ 0.01	0.28 $\pm$ 0.01	n.s.	0.24 $\pm$ 0.005	0.24 $\pm$ 0.008	n.s.	n.s.	n.s.
<b>sTV</b>	10.0 $\pm$ 0.6	14.3 $\pm$ 0.5	< 0.01	9.5 $\pm$ 0.4	12.3 $\pm$ 0.3	< 0.001	n.s.	< 0.02
<b>MV</b>	137.2 $\pm$ 6.5	146.1 $\pm$ 6.9	n.s.	119.0 $\pm$ 1.4	128.8 $\pm$ 4.9	n.s.	< 0.05	n.s.
<b>sMV</b>	5.0 $\pm$ 0.2	7.4 $\pm$ 0.3	< 0.001	4.8 $\pm$ 0.2	6.5 $\pm$ 0.2	< 0.001	n.s.	< 0.05
<b>Ti</b>	40.8 $\pm$ 0.3	39.4 $\pm$ 0.8	< 0.02	40.3 $\pm$ 0.6	38.5 $\pm$ 0.3	< 0.01	n.s.	n.s.
<b>Te</b>	77.0 $\pm$ 1.7	74.5 $\pm$ 0.3	n.s.	78.3 $\pm$ 1.9	74.1 $\pm$ 0.9	< 0.05	n.s.	n.s.
<b>Ti/TT</b>	0.35 $\pm$ 0.006	0.35 $\pm$ 0.005	n.s.	0.34 $\pm$ 0.007	0.34 $\pm$ 0.004	n.s.	n.s.	n.s.
<b>PIF</b>	11.6 $\pm$ 0.7	12.7 $\pm$ 0.8	n.s.	10.6 $\pm$ 0.3	11.1 $\pm$ 0.5	n.s.	n.s.	n.s.
<b>PEF</b>	7.8 $\pm$ 0.4	8.3 $\pm$ 0.7	n.s.	7.3 $\pm$ 0.3	7.4 $\pm$ 0.3	n.s.	n.s.	n.s.
<b>MIF</b>	6.7 $\pm$ 0.4	7.1 $\pm$ 0.3	n.s.	5.9 $\pm$ 0.1	6.3 $\pm$ 0.2	n.s.	n.s.	n.s.
<b>MEF</b>	3.5 $\pm$ 0.1	3.8 $\pm$ 0.2	n.s.	3.0 $\pm$ 0.1	3.3 $\pm$ 0.1	n.s.	< 0.01	0.062

## 3.10 Expression Profiling

### 3.10.1 Summary

In this report, we describe the results of using close to genome-wide 21K cDNA microarrays for the RNA expression profiling of **testis** tissue of ten animals of the Miz1 mutant mouse line. The data analysis and various statistical methods detected only the mutated gene differentially regulated between mutant and control tissue.

### 3.10.2 Mice

The molecular phenotyping screen archives organs of mutant mice for subsequent DNA-chip expression profiling analysis. Ten male mice of the Miz1 mouse mutant line were provided to the molecular phenotyping screen (Table 23).

Organs were collected at the age of 105-110 days. To minimize the influence of circadian rhythm on gene expression, mice were killed between 9 a.m. and 12 a.m. by carbon dioxide asphyxiation. The following 17 organs were collected and archived in liquid nitrogen following our established standard operating protocols: bulbourethral gland, spleen, kidney, seminal vesicles, testis, white fat, liver, heart, lung, thymus, skin/cartilage (outer ear), bone (femur), skeletal muscle, salivary gland, brain, brown fat, and eye.

<b>Mouse ID</b>	<b>Strain</b>	<b>Sex</b>	<b>Date of Birth</b>	<b>Genotype</b>	<b>Date of Collection</b>
30012699	Miz1	m	31.07.2003	-/-	17.11.2003
30012705	Miz1	m	31.07.2003	-/-	17.11.2003
30012735	Miz1	m	31.07.2003	-/-	17.11.2003
30012736	Miz1	m	31.07.2003	-/-	17.11.2003
30012732	Miz1	m	02.08.2003	-/-	17.11.2003
30012676	Miz1	m	30.07.2003	+/+	17.11.2003
30012701	Miz1	m	31.07.2003	+/+	17.11.2003
30012702	Miz1	m	31.07.2003	+/+	17.11.2003
30012704	Miz1	m	31.07.2003	+/+	17.11.2003
30012675	Miz1	m	01.08.2003	+/+	17.11.2003

### 3.10.3 Material and Methods

#### Isolation of total RNA

Total RNA was isolated just before processing for expression profiling. For preparation of total RNA individual organs were thawed in buffer containing chaotropic salt (RLT buffer, Qiagen) and homogenized using a Polytron homogenizer. Total RNA from individual samples was obtained according to manufacturer's protocols using RNeasy Midi kits (Qiagen). 2µg RNA aliquots were run on a formaldehyde agarose gel to check for RNA integrity and the concentration was calculated from OD<sub>260/280</sub> measurement. The RNA was stored at -80°C in RNase free water (Qiagen).

#### Chip design

We use a glass-surface DNA-chip containing ≈ 21000 probes. About 20200 of these probes are from the commercial Lion mouse array-TAG clone set, which is mostly derived from 3'UTRs. All Lion probes have been sequenced. The remaining probes are genes associated with immune response. Mouse array-TAG clones have the general ID MG-VW-XYZ (e.g. MG-3-1a5, MG-12-190m5,...) and the other probes are named s0-geneID (e.g. s0-birk, s0-mark1...).

#### DNA Microarrays

PCR products with 5'-aminogroup were amplified from the mouse arrayTAG library from Lion Bioscience comprising approximately 20.200 clones (Heidelberg, Germany). PCR products were dissolved in 3-fold SSC buffer and spotted on aldehyde-coated slides (Telechem, USA) using a Microgrid TAS II spotter (Biorobotics) with 48 Stealth™ SMP3 pins (Telechem). Spotted slides were rehydrated overnight in a humid chamber containing 50-70% aqueous solution of glycerol. Rehydrated slides were immersed in blocking solution (0,1M sodium borohydride in 0,75 fold PBS with 25% ethanol) for 5 minutes, boiled in water for 2 minutes, briefly immersed in 100% ethanol and air-dried. Slides were pre-hybridized for 1 hour in pre-hybridization buffer (6-fold SSC, 1%BSA, 0,5%SDS) rinsed in water, dried and hybridized the same day (Seltmann et al, 2005).

#### Reverse Transcription and Fluorescent Labeling

For labeling 20µg of total RNA were used for reverse transcription and indirectly labeled with Cy3 or Cy5 fluorescent dye according the TIGR protocol ([http://pga.tigr.org/sop/M004\\_1a.pdf](http://pga.tigr.org/sop/M004_1a.pdf)). Labeled cDNA was dissolved in 30µl hybridisation buffer (6x SSC, 0,5% SDS 5 fold Denhardt's solution and 50% formamide) and mixed with 30µl of reference cDNA solution (pool from 5 wt animals) labeled with the second dye. This hybridization mixture was placed on a pre-hybridized microarray, under a cover slip, placed into a hybridization chamber (Genetix) and immersed in a thermostatic bath at 42°C for at least 16 hours. After hybridization slides were washed in 40ml of 3x SSC, 40ml of 1x SSC and 40ml of 0,25x SSC at room temperature. For drying slides were placed in an empty 50ml Falcon tube (Becton Dickinson, USA) and centrifuged at 4000 m/s<sup>2</sup>. Dried slides were scanned with a GenePix 4000A microarray scanner and the images were analyzed using the GenePix Pro3.0 image processing software (Axon Instruments, USA). All data were normalized by adjusting the median of log-ratios of Cy5 to Cy3 intensities to 0. For

data analysis Pattern Analysis of Microarrays = PAM ([http://www.gsf.de/ieg/groups/exppro\\_cpt.html#PAM](http://www.gsf.de/ieg/groups/exppro_cpt.html#PAM)) was used.

### Chip Hybridization

Depending on the amount of RNA available for hybridization, in general four chip hybridizations were performed with RNA from all organs of each four individual mutant mice (in total 16 hybridizations). Each chip hybridization was performed against the identical pool of each organ of wt RNAs (reference RNA pool; wt). For each individual the chip experiments included two color-flip experiments. Results

### Selected Organs and Isolated RNA

Testis was selected as organ for expression profiling analysis based on data from GMC-screens. We isolated total RNA of testis from five *Miz1* mutant mice and five control individuals.

Table 23: Amount of Total RNA [ $\mu$ g] Isolated from Testis.	
Mouse ID	Testis
30008284	32
30008285	48
30008307	44
30008308	72
30008309	143
30008253	187
30008089	236
30008090	43
30008091	82

### Chip hybridization

Four chip hybridizations were performed with RNA from testis of all five individual mutant mice. Each chip hybridization was performed against the identical pool of control RNAs (reference RNA pool). For each individual the chip experiments included 2 color-flip experiments. Twenty chip hybridizations were used for data analysis.

### Data analysis

The data analysis and various statistical methods did not detect any gene differentially regulated between mutant and control testis tissue in all experiments.

Inspection of expression data from individual mice revealed a stronger correlation of up- and down-regulated genes in samples 732, 732 and 699. Expression patterns of sample 705 and 735 show anti-correlation to these three samples.

In ten experiments belonging to samples 732, 736 and 699 *Miz1* was detected as being down-regulated with a fold induction range between 1.69 – 5.27 ( $\pm$  1.45). Signals on these spots in the other experiments were below detection thresholds (signal intensity above 200).

### 3.10.4 Discussion

The data analysis detected no genes differentially regulated between mutant and control testis tissue in all experiments. Inspection of expression data from individual mice revealed stronger correlation of differentially expressed genes in animals 732, 736 and 699. An anti-correlation in expression patterns for some genes of 705 and 735 was found (data not shown). Maybe biological variability in genes oscillating in a circadian rhythm and stress-responsive genes (organs were taken directly after lung-function-test) are the reason for anti-correlation in the expression patterns between single individuals. Also, several recent publications have provided evidence for biological variability of expression levels for particular genes (Oishi *et al.*, 2003; Pritchard *et al.*, 2001; Drobyshev *et al.*, 2003a; Drobyshev *et al.*, 2003b; Churchill *et al.*, 2002).

The mutated gene *Miz1* was detected as being down-regulated in ten experiments belonging to three different individual samples. In the other experiments the signals on these spots were below detection thresholds. The 3' part of *Miz1* is not effected in the *Miz1* mutation. Due to the existing exons 3-14 of the *Miz1* gene, a shortened transcript may be possible. These findings are in agreement with the positioning of the probe sequence in relation to the mutation in the *Miz1* mutant mouse line.

Using different selection criteria, we could identify only the *Miz1* gene that is significantly differentially expressed in testis of three individuals of *Miz1*-mutant mice.

### 3.10.5 References

- Churchill G.A. (2002): Fundamentals of experimental design for cDNA microarrays. *Nat Genet* 32 Suppl.490-495
- Drobyshev A., M. Hrabé de Angelis and J. Beckers (2003a): Artefacts and reliability of DNA microarrays expression profiling data. *Current Genomics* 4: 615-621
- Drobyshev A., C. Machka, M. Horsch, M. Seltmann, V. Liebscher, M. Hrabé de Angelis and J. Beckers (2003b): Specificity assessment from fractionation experiments (SAFE): a novel method to evaluate microarray probe specificity base on hybridisation stringencies. *Nucleic Acids Res.* 31(2):E1-1.
- Seltmann M., M. Horsch, A. Drobyshev, Y. Chen, M. Hrabé de Angelis and J. Beckers (2005): Assessment of a systematic expression profiling approach in ENU-induced mouse mutant lines. *Mammalian Genome* 16: 1-10

## 3.11 Metabolic Screen

### 3.11.1 Summary

The metabolic screening provides a comparative analysis of bioenergetic parameters in mice. Mechanisms which lead to disturbances in body weight regulation and energy metabolism are determined. Hence, the basal energetic demands are monitored during *ad libitum* feeding and under food restricted conditions. In humans unbalanced energy uptake and energy expenditure cause the development of obesity (Spiegelman and Flier, 2001) or anorexia nervosa with severe weight loss (Hebebrand *et al.*, 2003). Some rodent and other species tend to increase activity upon food restriction leading to weight loss when given access to an activity wheel (Exner *et al.*, 2000). Several studies described that fasting in mice results in transient depression of metabolic rate, heart rate, body temperature and locomotor activity (Duffy *et al.*, 1990; Williams *et al.*, 2002). Therefore the primary Metabolic Screening focused on the determination of food and energy uptake under *ad libitum* conditions and metabolic adaptations during food restriction and serves as the origin for further investigations in the Secondary and Tertiary screening which go into details of energy expenditure and energy storage.

In the primary metabolic screen 13 control mice and 11 *Miz1*-deficient mice were analyzed. They were first fed under *ad libitum* conditions for two weeks, followed by one week of food restriction to 60% of *ad libitum*. The primary metabolic screen focuses on investigation of metabolic demands of mice determining daily body weight, energy uptake, metabolizable energy and body temperature and adaptive capacity of metabolic processes.

While males and females showed the common differences in body weight, also body temperature was elevated in females of both genotypes. But any further major differences between males and females could not be found. Also genotype-specific differences were not detectable, indicating no metabolic phenotype caused by the mutation in the *Miz1* gene.

### 3.11.2 Mice

Seven adult control males and four adult mutant males entered the Metabolic screen at the beginning of calendar week 50 in 2003. The females (six control and seven mutant) entered the metabolic laboratory one week later.

The mice were single caged on grid panels (0.5 cm grid hole diameter). They were fed *ad libitum* for a period of 14 days, followed by a period of food restriction to 60% of *ad libitum* for seven days to analyze adaptive responses of metabolism.

### 3.11.3 Material and Methods

#### Recorded Data

During the different feeding regimes body weight, food consumption ( $F_{\text{con}}$ ), rectal temperature ( $T_{\text{re}}$ ), daily feces production ( $F_{\text{fec}}$ ), energy uptake ( $E_{\text{up}}$ ), energy content of the feces ( $E_{\text{fec}}$ ), metabolizable energy ( $E_{\text{met}}$ ) and the food assimilation coefficient ( $F_{\text{ass}}$ ) were recorded.

### Analysis of Feces

The separation of mice in single cages allowed collection of feces in three day intervals. Samples of lab chow and feces (~1 g) were dried at 60°C for two days, homogenized in a coffee grinder and squeezed to a pill for determination of energy content in a bomb calorimeter (IKA Calorimeter C7000) based on dry measurement principle. Energy uptake is determined as the product of food consumed and the caloric value of the food. To obtain metabolizable energy ( $E_{met}$ ) the energy content of feces and urine (2% of  $E_{up}$ ; Drozd 1975) were subtracted from energy uptake.

### Statistical Analysis

All values are presented as means  $\pm$  SEM. Two-way-ANOVA (SigmaStat, Jandel Scientific) was used to test for effects of the factors genotype and gender. The Tukey test was applied for post hoc multiple comparison. The Mann-Whitney-Test for paired samples was used to analyze the effect of nutritional status on parameters of energy metabolism.

### 3.11.4 Parameters

Recorded Data during the different feeding regimes
--

body weight, food consumption ( $F_{con}$ ), rectal temperature ( $T_{re}$ ), daily feces production (Fec), energy uptake ( $E_{up}$ ), energy content of the feces ( $E_{fec}$ ), metabolizable energy ( $E_{met}$ ), food assimilation coefficient ( $F_{ass}$ )
--

### 3.11.5 Results and Discussion

No information about metabolic parameters were available prior the metabolic screening of *Miz1*-deficient mice. Mutant and control mice showed common sex-related differences in body weight, while males were heavier than females (Table 24). But no differences between the genotypes could be detected. Also in body temperature we measured higher values in females, however in both genotypes. In energetic parameters we could not confirm any genotype-specific difference. Sex-specific differences could only be determined in controls concerning body weight related energy uptake and ratio of metabolized energy. The food assimilation of mutant females was slightly increased compared to control females due to reduced feces production and lowered caloric value of feces samples. Parameters investigated in the primary metabolic screen could not confirm any metabolic phenotype.

Raw data for each individual are available on demand in Excel sheets.

### 3.11.6 References

Drozd M. (1975) A Food habits and food assimilation in mammals. In: Methods for Ecological Bioenergetics, edited by W. Grodzinski, R.Z. Klekowski and A Duncan. Oxford, UK: Blackwell, p: 23-47

Duffy, P.H., R. J. Feuers and R. W. Hart (1990): Effect of chronic caloric restriction on the circadian regulation of physiological and behavioral variables in old male B6C3F1 mice. Chronobiol Int 7: 291-303

Exner, C., J. Hebebrand, H. Remschmidt, C. Wewetzer, A. Ziegler, S. Herpertz, U. Schweiger, W. F. Blum, G. Preibisch, G. Heldmaier and M. Klingenspor (2000): Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. *Mol Psychiatry* 5: 476-481.

Hebebrand J., C. Exner, K. Hebebrand, C. Holtcamp, R.C. Casper, H. Remschmidt, B. Herpertz-Dahlmann, M. Klingenspor (2003): Hyperactivity in patients with anorexia nervosa and in semistarved rats: Evidence for a pivotal role of hypoleptinemia. *Physiology and Behavior* 79: 25-37

Spiegelman B.M. and J.S. Flier (2001): Obesity and the regulation of energy balance. *Cell* 104: 531-543

Williams T. D., J.B. Chambers, R.P. Henderson, M.E. Rashotte and J.M. Overton (2002): Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. *Am J Physiol Regul Integr Comp Physiol* 282: R1459-67

## Abbreviations

$F_{con}$	Food consumption
$T_{re}$	rectal temperature
Fec	daily feces production
$E_{up}$	energy uptake
$E_{fec}$	energy content of the feces
$E_{met}$	metabolizable energy
$F_{ass}$	food assimilation coefficient

**Table 24: Metabolic Parameters Recorded in the Primary Screen**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Control (A)						Mutant (B)					A~B	
	<i>ad libitum</i>			food reduction, 7 days to 60%			<i>ad libitum</i>			food reduction, 7 days to 60%			
	Male	Female		Male	Female		Male	Female		Male	Female	Male	Female
	(n=7)	(n=6)	<i>p</i> - value	(n=7)	(n=6)		(n=4)	(n=7)	<i>p</i> - value	(n=4)	(n=7)	<i>p</i> - value	<i>p</i> - value
Body weight [g]	28.6 $\pm$ 0.63	22.2 $\pm$ 0.81	< 0.001	23.2 $\pm$ 0.96	18.9 $\pm$ 0.78		28.1 $\pm$ 1.42	22.0 $\pm$ 0.74	< 0.01	22.5 $\pm$ 1.11	17.8 $\pm$ 0.54	n.s.	n.s.
Rectal body temperature [°C]	36.06 $\pm$ 0.11	36.8 $\pm$ 0.05	= 0.001	34.75 $\pm$ 0.25	34.9 $\pm$ 0.32		36.37 $\pm$ 0.14	36.9 $\pm$ 0.09	< 0.01	35.42 $\pm$ 0.24	35.2 $\pm$ 0.33	n.s.	n.s.
Food consumption [g day <sup>-1</sup> ]	3.34 $\pm$ 0.29	3.19 $\pm$ 0.25	n.s.	60% of <i>ad libi-</i> <i>tum</i>			3.48 $\pm$ 0.29	3.31 $\pm$ 0.19	n.s.	60% of <i>ad libi-</i> <i>tum</i>		n.s.	n.s.
Energy uptake [kJ day <sup>-1</sup> ]	61.8 $\pm$ 2.49	59.1 $\pm$ 4.71	n.s.	37.1 $\pm$ 1.49	35.5 $\pm$ 2.83		64.4 $\pm$ 5.29	61.2 $\pm$ 3.47	n.s.	38.6 $\pm$ 3.17	36.7 $\pm$ 2.08	n.s.	n.s.
Energy uptake BW <sup>-1</sup> [kJ g <sup>-1</sup> day <sup>-1</sup> ]	2.16 $\pm$ 0.08	2.67 $\pm$ 0.2	< 0.05	1.61 $\pm$ 0.07	1.87 $\pm$ 0.12		2.35 $\pm$ 0.33	2.82 $\pm$ 0.21	n.s.	1.75 $\pm$ 0.24	2.07 $\pm$ 0.13	n.s.	n.s.
Feces production [g day <sup>-1</sup> ]	0.56 $\pm$ 0.03	0.52 $\pm$ 0.03	n.s.	0.29 $\pm$ 0.03	0.33 $\pm$ 0.02		0.52 $\pm$ 0.03	0.45 $\pm$ 0.02	n.s.	0.35 $\pm$ 0.01	0.32 $\pm$ 0.02	n.s.	n.s.
Energy content feces [kJ g <sup>-1</sup> ]	16.2 $\pm$ 0.06	16.1 $\pm$ 0.08	n.s.	16.5 $\pm$ 0.08	15.6 $\pm$ 0.11		16.3 $\pm$ 0.05	15.9 $\pm$ 0.11	n.s.	16.4 $\pm$ 0.16	15.8 $\pm$ 0.11	n.s.	n.s.
Metabolized energy [kJ day <sup>-1</sup> ]	52.87 $\pm$ 2.16	50.85 $\pm$ 4.28	n.s.	32.43 $\pm$ 1.2	30.51 $\pm$ 2.53		56.11 $\pm$ 5.09	54.24 $\pm$ 3.47	n.s.	33.12 $\pm$ 3.1	31.8 $\pm$ 1.76	n.s.	n.s.
Metabolized energy [kJ g <sup>-1</sup> day <sup>-1</sup> ]	1.85 $\pm$ 0.07	2.29 $\pm$ 0.19	< 0.05	1.40 $\pm$ 0.07	1.61 $\pm$ 0.10		2.05 $\pm$ 0.31	2.49 $\pm$ 0.20	n.s.	1.50 $\pm$ 0.22	1.78 $\pm$ 0.10	n.s.	n.s.
Food assimilation coefficient [%]	85.5 $\pm$ 0.4	85.8 $\pm$ 0.62	n.s.	87.5 $\pm$ 0.81	85.8 $\pm$ 0.57		86.9 $\pm$ 0.91	88.3 $\pm$ 0.88	n.s.	85.4 $\pm$ 0.91	86.5 $\pm$ 0.28	n.s.	< 0.05

## 3.12 Pathology Screen

### 3.12.1 Summary

The Pathology screen performed a complete morphological analysis with standard stains. Due to the possibility of finding a phenotype in testes, testes of ten animals (five controls and five mutants) were carefully weight and examined. The ratio between the testis weight and the body weight was on average 0.33% in the *Miz1*-deficient mice, and 0.37% in the control mice. In conclusion, we confirmed a minor difference in the testis weight. However, no morphological differences between wild-type control and mutant mice were observed. We did not find any additional genotype-specific alterations.

### 3.12.2 Mice

A total of 51 mice, 25 *Miz1*-deficient mice (13 males, 12 females) and 26 control mice (14 males, 12 females) were analyzed. Due to the workflow in the GMC, mice of different ages were received from different screens (Table 25).

Table 25: <i>Miz1</i> -mutant mice and their control littermates analyzed.						
	Control		Mutant		Number of Animals	Age (weeks)
	Male	Female	Male	Female		
Lung Screen	0	4	0	5	9	15
Expression profiling Screen	5	0	4	0	9	14 -15
Dysmorphology Screen	2	1	2	0	5	21 - 22
Metabolic Screen	7	7	7	7	28	21 - 22
Total Number of Animals	14	12	13	12	51	

### 3.12.3 Materials and Methods

Mice received in the laboratory of pathology were sacrificed with CO<sub>2</sub>. The animals were analyzed macroscopically and weighed ([www.eulep.org/Necropsy\\_of\\_the\\_Mouse/index\\_2004.php](http://www.eulep.org/Necropsy_of_the_Mouse/index_2004.php)). The thymus and left lobe of the liver were measured. Blood samples were taken, centrifuged and the serum was saved at -20°C. Tails were preserved at -70°C for further genetic analysis. Following a complete dissection, an x-ray of the complete bone structure was taken, when indicated (Hewlett Packard, Cabinet X-Ray System Faxitron Series). All organs were fixed in 4% buffered formalin and embedded in paraffin for histological examination. Two-µm-thick sections from skin, heart, muscle, lung, brain, cerebellum, thymus, spleen, cervical lymph

nodes, thyroid, parathyroid, adrenal gland, stomach, intestine, liver, pancreas, kidney, reproductive organs, and urinary bladder were cut and stained with haematoxylin and eosin (H&E). Prussian's Blue staining was performed when indicated. The presence of Mouse Hepatitis Virus (MHV) was serologically investigated using an immunofluorescence assay (1:20) by BioDoc (Hannover).

### 3.12.4 Results

Overview on genotype-specific results:

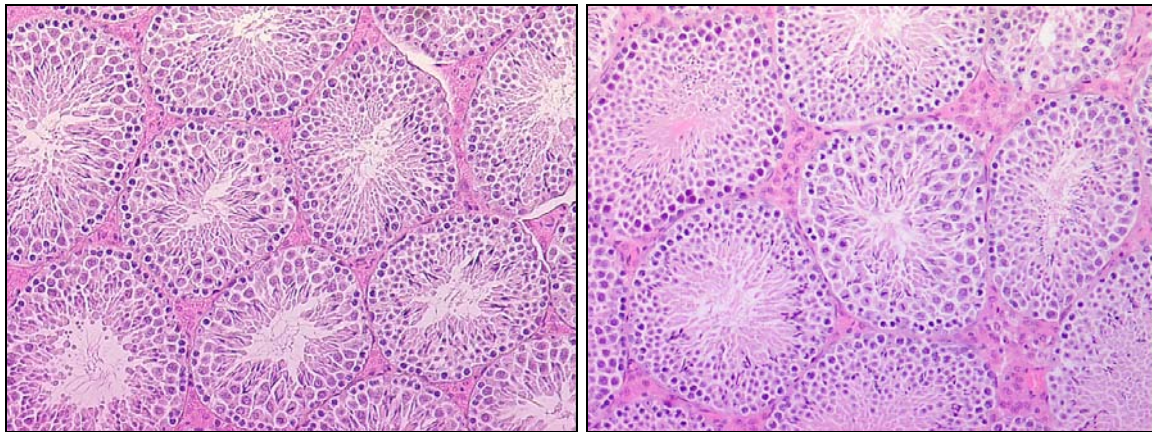
Table 26: Genotype-specific morphological alterations					
<b>Organ</b>	<b>Skin</b>	<b>Musculoskeletal System</b>	<b>Eyes</b>	<b>Brain</b>	<b>Cerebellum</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Heart</b>	<b>Trachea</b>	<b>Lung</b>	<b>Teeth</b>	<b>Salivary glands</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Esophagus</b>	<b>Stomach</b>	<b>Small Intestine</b>	<b>Large Intestine</b>	<b>Liver</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Pancreas</b>	<b>Cervical Lymph Nodes</b>	<b>Thymus</b>	<b>Spleen</b>	<b>Thyroid</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Parathyroid</b>	<b>Adrenal Gland</b>	<b>Kidneys</b>	<b>Urinary Bladder</b>	<b>Testes</b>
<b>Alteration</b>	no	no	no	no	yes
<b>Organ</b>	<b>Epididymis</b>	<b>Funiculus spermaticus</b>	<b>Ovaries</b>	<b>Uterus</b>	<b>Vagina</b>
<b>Alteration</b>	no	no	no	no	no

#### Testes

On average, in the mutant mice the ratio between the right testis weight and the body weight was 0.33% and 0.37% in the control mice. In conclusion, we confirmed a minor difference in testes weight (see Table 27 for more details), however, the testes of the mutants did not show morphological alterations (Fig. 7).

**Table 27: Testis weight (g) of mutant mice and their control littermates.**

	Control		Mutant	
	Testis weight	Body weight	Testis weight	Body weight
	0.111	27.3	0.094	27.1
	0.098	26.1	0.080	21.9
	0.113	28.4	0.098	26.5
	0.110	28.1	0.072	24.9
	0.086	26.7	0.075	24.8
<b>Mean weight</b>	0.104	27.3	0.084	25.0
<b>Ratio average testis w./ body w.</b>	0.37%		0.33%	



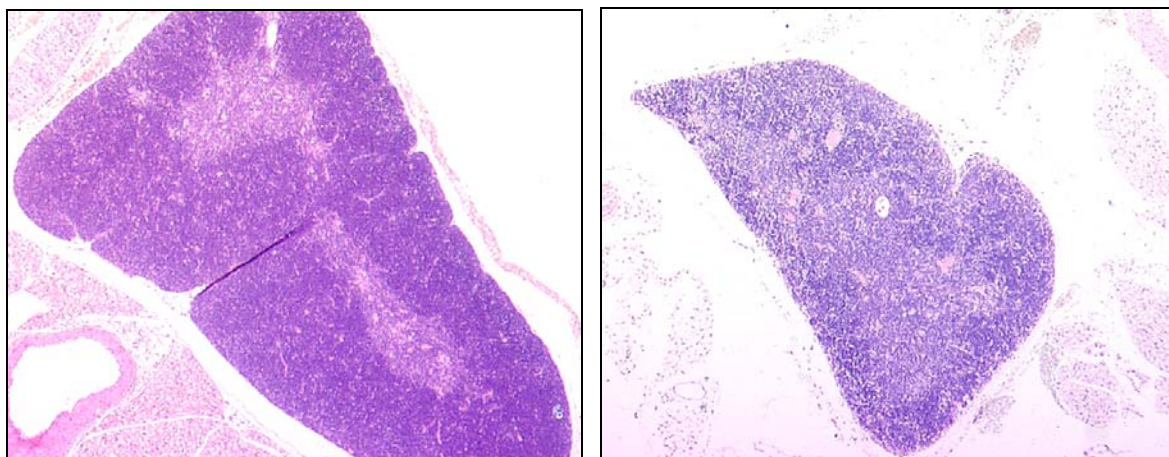
**Figure 7: Histological analysis of testis tissue.**

The left panel depicts a picture of the normal seminiferous tubules from a control mouse compared to a mutant mouse (right picture). H&E, 200x. Note, that in both animals the germ cell maturation is normal.

**Secondary results**

In both, the mutant mice and their control littermates, secondary lesions were identified.

**Thymus:** In 12 of 13 female mice (six controls and six mutant mice), received from the metabolic screen, cortical thymus atrophy was observed (Fig. 8).



**Figure 8: Histological analysis of thymus tissue.**

The left panel shows a normal thymus of a control mouse from the metabolic screen. Note the thick, dense, dark blue coloured cortex given by the presence of normal number of lymphocytes (H&E, 25x). In contrast, the right panel demonstrates the atrophy of the cortex in a mutant mouse from the metabolic screen. Note the loss of cortex density due to the decreased number of lymphocytes (H&E, 25x).

### 3.12.5 Discussion

According to the preliminary information received from the provider, the *Piasx/Miz1* gene is highly expressed in the testes, suggesting a role in the spermatogenesis. In our screen, a subtle reduction of the testis weight was observed, as published by Santti and coworkers (2005). The testis and epididymis from the mutant mice and their littermate controls do not show morphological alterations.

Thymic atrophy is a frequent finding in C57BL/6J strain in mice received from the metabolic screen. We do believe that the thymic atrophy is secondary to food restriction and/or the stress induced during this analysis (unpublished observation). The atrophy usually correlates with the amount of fat loss during the food restriction period.

### 3.12.6 References

Santti H, Mikkonen L, Anand A, Hirvonen-Santti S, Toppari J, Panhuysen M, Vauti F, Perera M, Corte G, Wurst W, Janne OA, Palvimo JJ. (2005) Disruption of the murine PIASx gene results in reduced testis weight. *J Mo Endocrinol.* 34 (3): 645-54.

## **Acknowledgements**

A large team consisting of scientists, technicians and animal caretakers all contribute to the success of the German Mouse Clinic. We want to thank Reinhard Seeliger, Elfi Holupirek, Christine Fürmann, Kerstin Kutzner, Mareike Maurer, Susanne Sommer, Rose Austin, Florian Schleicher, Gregor Pahnke, Susanne Wittich, Martin Taube, Claudia Zeller, Sandra Schädler, Elenore Samson, Nadine Kink, Claudia Kloss, Jaqueline Müller, Sabine Holthaus for expert technical help and Daniela Kißling, Monika Katzbach, Uwe Drescher, Heiko Engelniederhammer, Manuela Krug, Tina Kohler, Petra Thalmeier, Daniela Elvert, Aline Weingärtner and Sven Korb for the care of the mice.

## Appendix: Tables

Table	1: Primary Screen at GMC .....	8
Table	2: Results of Behavioral Observation in the Modified Hole Board Test.....	12
Table	3: Video-Tracking Results Regarding Locomotor Behavior.....	15
Table	4: Results from the Morphological Inspection .....	21
Table	5: Results from the second Clickbox Testing .....	21
Table	6: Results from the X-Ray Analysis.....	21
Table	7: Bone-Related Quantitative Parameters.....	22
Table	8: Recording of Body Length and Body Weight .....	28
Table	9: Behavior Recorded in Viewing Jar .....	28
Table	10: Recording of Locomotor Activity and Behavior in the Arena .....	29
Table	11: Behavior Recorded in or above the Arena .....	30
Table	12: Behavior during Supine Restraint .....	31
Table	13: Lactate Levels .....	32
Table	14: Comparison of ERG-Responses at Illumination Levels of 500 and 12,500 cd/m <sup>2</sup> .....	36
Table	15: Clinical-Chemical Parameters.....	41
Table	16: Hematological Parameters.....	42
Table	17: Basic Parameters Analyzed in the Immunology Screen. ....	46
Table	18: Total plasma IgE in Miz1 mice .....	48
Table	19: Nociceptive Screen .....	51
Table	20: Characterization of Studied Mice .....	56
Table	21: Spontaneous Breathing Pattern during Rest and Activity .....	57
Table	22: <i>Miz1</i> -deficient and Control Mice Stored for Expression Profiling.....	58
Table	23: Amount of Total RNA [µg] Isolated from Testis.....	60
Table	24: Metabolic Parameters Recorded in the Primary Screen .....	65
Table	25: <i>Miz1</i> -mutant mice and their control littermates analyzed. ....	66
Table	26: Genotype-specific morphological alterations.....	67
Table	27: Testis weight (g) of mutant mice and their control littermates. ....	68

## Figures

Figure	1: Workflow of the primary screen.....	5
Figure	2: Test Arena for modified hole board test. ....	10
Figure	3: Acoustic startle response of males (normalized for body weight). ....	16
Figure	4: Acoustic startle response of females (normalized for body weight). ....	16
Figure	5: System used at GMC to assess breathing patterns. ....	53
Figure	6: Recorded data used to calculate the breathing parameters.....	53
Figure	7: Histological analysis of testis tissue. ....	68
Figure	8: Histological analysis of thymus tissue.....	69

## Addresses of screeners and modules

### Coordinators

Dr. Valérie Gailus-Durner  
Dr. Helmut Fuchs  
Dr. Christoph Lengger  
Dr. Beatrix Naton  
Prof. Dr. Martin Hrabé de Angelis  
Institute of Experimental Genetics  
GSF National Research Center for Environment and Health  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3613  
Fax: 089-3187-3500  
Email: [gailus@gsf.de](mailto:gailus@gsf.de)

### Behavior Screen

Dr. Vera Pedersen  
Dr. Sabine M. Hölter  
Institute of Developmental Genetics  
GSF National Research Center for Environment and Health  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3674  
Fax: 089-3187-3099  
Email: [hoelter@gsf.de](mailto:hoelter@gsf.de)

### Dysmorphology Screen,

Dr. Helmut Fuchs  
Dr. Elisabeth Grundner-Culemann  
Thomas Lisse  
Prof. Dr. Martin Hrabé de Angelis  
GSF National Research Center for Environment and Health  
Institute of Experimental Genetics  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3151  
Fax 089-3187-3500  
Emai [hfuchs@gsf.de](mailto:hfuchs@gsf.de)

## Neurology Screen

Dr. Ilka Schneider  
Dr. Lore Becker  
Eva Kling  
GSF National Research Center for Environment and Health  
Institute of Experimental Genetics  
German Mouse Clinic (GMC)/Neurology  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3654  
Fax: 089-3187-3500  
Email: [Ilka.Schneider@gsf.de](mailto:Ilka.Schneider@gsf.de)

PD Dr. Thomas Klopstock  
Department of Neurology  
Klinikum Großhadern  
LMU Ludwig-Maximilians-University  
Marchioninistraße 15  
D-81377 München  
Tel.: 089-7095-5920  
Fax: 089-7095-3677  
Email: [Thomas.Klopstock@nro.med.uni-muenchen.de](mailto:Thomas.Klopstock@nro.med.uni-muenchen.de)

## Eye Screen

Dr. Claudia Dalke  
GSF-National Research Center for Environment and Health  
Institute of Developmental Genetics  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-2910  
Fax: 089-3187-2210  
Email: [dalke@gsf.de](mailto:dalke@gsf.de)

## Clinical-Chemical Screen

Dr. Martina Klempt  
Institute of Experimental Genetics  
GMC - German Mouse Clinic  
Clinical-Chemical Screen  
Institute for Experimental Genetics  
GSF - National Research Center for Environment and Health  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3282  
Email: [klempt@gsf.de](mailto:klempt@gsf.de)

Prof. Dr. Eckhard Wolf  
Dr. Birgit Rathkolb  
Institute of Molecular Animal Breeding and Biotechnology  
Genecenter  
LMU München  
Feodor Lynen-Straße 25  
D-81377 München  
Tel.: 089-21807-6800  
Email: [ewolf@lmb.uni-muenchen.de](mailto:ewolf@lmb.uni-muenchen.de)  
Email: [b.rathkolb@gen.vetmed.uni-muenchen.de](mailto:b.rathkolb@gen.vetmed.uni-muenchen.de)

## **Immunology Screen**

Dr. Svetoslav Kalaydjiev  
Tobias Franz  
Prof. Dr. Dirk Busch  
German Mouse Clinic  
Institute for Experimental Genetics  
GSF National Research Center for Environment and Health  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3656  
Fax: 089-3187-3500  
Email: [svetoslav.kalaydjiev@lrz.tum.de](mailto:svetoslav.kalaydjiev@lrz.tum.de)  
Email: [tobias.franz@gsf.de](mailto:tobias.franz@gsf.de)

Prof. Dr. Dirk Busch  
Institute for Medical Microbiology, Immunology and Hygiene  
Technische Universität München (TUM)  
Trogerstr. 9  
D-81675 München  
Tel.: 089-4140-6191  
Fax: 089-4140-4139  
Email: [dirk.busch@lrz.tum.de](mailto:dirk.busch@lrz.tum.de)

## **Allergy Screen**

Anahita Javaheri, MSc  
Prof. Dr. Markus Ollert  
Klinik und Poliklinik für Dermatologie und Allergologie am Biederstein  
Technische Universität München (TUM)  
Biedersteinerstraße 29  
D-80802 München  
Tel.: 089-4140-3551 (M.O.)  
Tel.: 089-3187-2554 (A.J.)  
Fax: 089-4140-3552  
E.-mail: [ollert@lrz.tum.de](mailto:ollert@lrz.tum.de)

## **Nociceptive Screen**

Dr. Ildiko Racz  
Laboratory of Molecular Neurobiology,  
Department of Psychiatry  
University of Bonn  
Sigmund-Freud-Straße 25,  
D-53105 Bonn  
Tel.: 0228-287-9578  
Fax: 0228-287-9125  
E.-mail: [iracz@uni-bonn.de](mailto:iracz@uni-bonn.de)

Prof. Dr. Andreas Zimmer  
Laboratory of Molecular Neurobiology,  
Department of Psychiatry,  
University of Bonn  
Sigmund-Freud-Straße 25,  
D-53105 Bonn. Germany  
Tel.: 0228-287-9124  
Fax.: 0228-287-9125

## **Lung Function Screen**

Prof. Dr. Holger Schulz  
Dr. Claudia Reinhard  
Dr. Ines Bolle  
GSF – National Research Center for Environment and Health  
Institut für Inhalationsbiologie  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-4119  
Fax.: 089-3187-2400  
Email: [schulz@gsf.de](mailto:schulz@gsf.de)

## **Expression Profiling**

Dr. Johannes Beckers  
Dr. Marion Horsch  
GSF – National Research Center for Environment and Health  
Institute of Experimental Genetics  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3513  
Fax: 089-3187-4085  
Email: [beckers@gsf.de](mailto:beckers@gsf.de)

## **Metabolic Screen**

Dr. Ralf Elvert  
Nicole Ehrhardt  
Institute of Experimental Genetics  
GMC - German Mouse Clinic  
Metabolic Screen  
GSF - National Research Center for Environment and Health  
Ingolstädter Landstraße 1  
D-85764 Neuherberg  
Tel.: 089-3187-3648 or 3151  
Fax: 089-3187-3500  
Email: [elvert@gsf.de](mailto:elvert@gsf.de)

## **Pathology Screen**

Dr. Julia Calzada-Wack

Sandra Kunder

Gabriele Hölzswimmer

PD Dr. Leticia Quintanilla-Fend

GSF - National Research Center for Environment and Health

Institute of Pathology

Ingolstädter Landstraße 1

D-85764 Neuherberg

Tel.: 089-3187-2312

089-3187-3241

Fax 089-3187-3360

Email: [calzada@gsf.de](mailto:calzada@gsf.de)

[sandra.kunder@gsf.de](mailto:sandra.kunder@gsf.de)