BKS-db

Strain Name: BKS-*Lepr*^{em2Cd479}*Dock7*^{misty}/Gpt
Strain Number: T056480
Strain Type: Cas9-KO
Background: C57BLKS/JGpt

Description

The Obese Gene Receptor (ob-R) also known as LEPR gene is encoding a Type I cytokine receptor, a protein that in humans have a large relationship with obesity, hypertension, diabetes, lipid metabolism disorders, etc. [1,2]. The db/db mouse is a model of obesity, diabetes, and dyslipidemia wherein leptin receptor activity is deficient because leptin mutation are performed by CRISPR/Cas9 technology. The disease course is greatly affected by the genetic background, *Lepr (db)* defects in the BKS background are often accompanied by higher levels of blood sugar and body weight. Higher levels of blood glucose and gluconeogenesis enzyme activity cannot be controlled by insulin treatment on *Lepr (db)* defects mouse, in which accompanied by phenotypes of peripheral neuropathy, myocardial disease, delayed wound healing, accelerated metabolic efficiency [3], hypothalamic lesions [4]. In addition, female db/db mice were observed infertility together with reduced uterine and ovarian weight and decreased estrogen secretion [5-6].
The *Lepr* gene mutant mouse (BKS-db v2) was constructed on the background of BKS-misty heterozygous mice by GemPharmatech using gene editing technology. The *misty* mutation and the *Lepr* mutation in this mouse model are located on two distinct chromosome 4, resulting in segregation during the breeding process. By monitoring the blood glucose levels for this strain, it was found that BKS-db v2 mouse have significantly higher level of blood glucose than that of the wild control. This strain is an ideal model for type II diabetes research.

Application

1. Metabolic research (diabetes);
2. Endocrine disorder research;
3. Reproductive biology research.

Data support

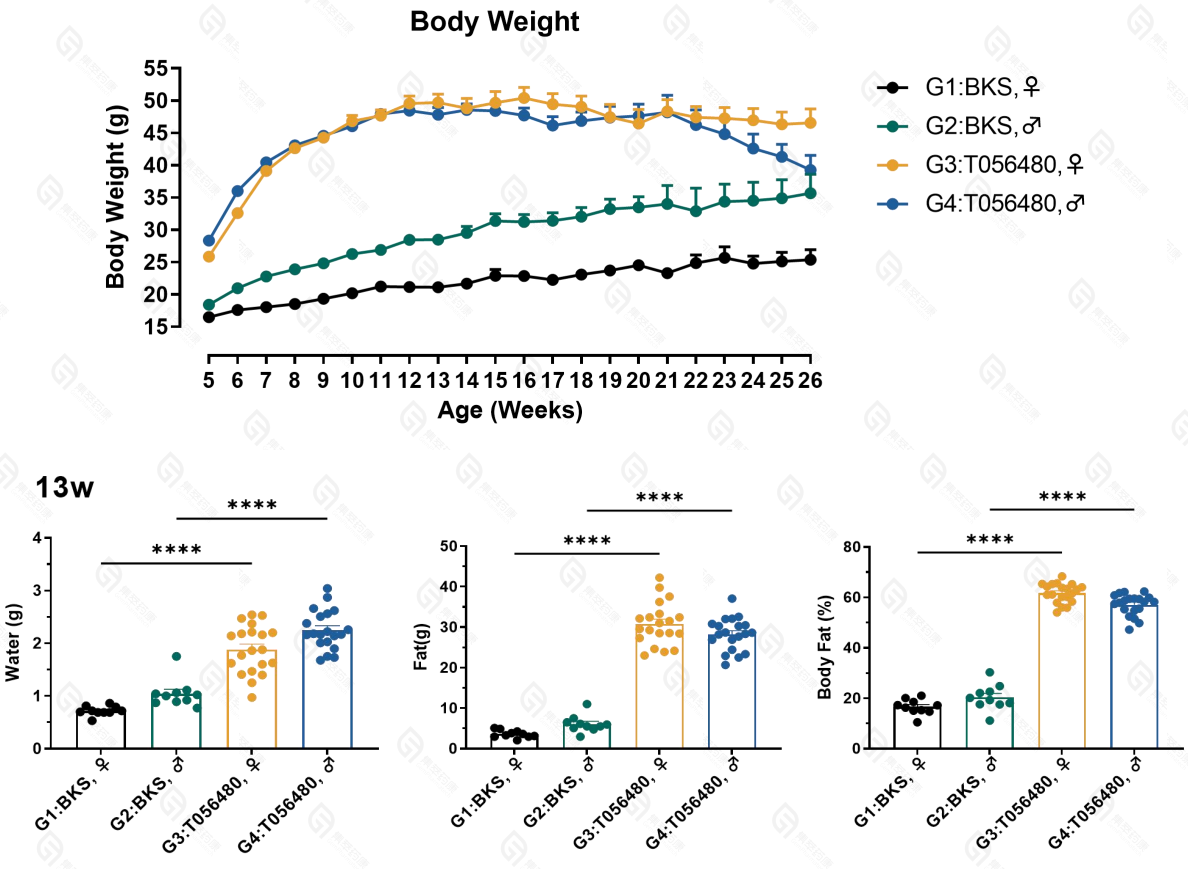

Fig 1. Weight gain and body fat data in BKS-db v2 mice

Monitoring the weight gain data of mice aged 5 to 26 weeks, while also assessing the body fat data of mice at 13 weeks. The results indicate that T056480 mice exhibit significantly higher body weight starting from 5 weeks of age compared to wild-type mice. Additionally, at 13 weeks of age, the body fat percentage of T056480 mice is significantly higher than that of wild-type mice, demonstrating an obese phenotype. (Data were presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20)

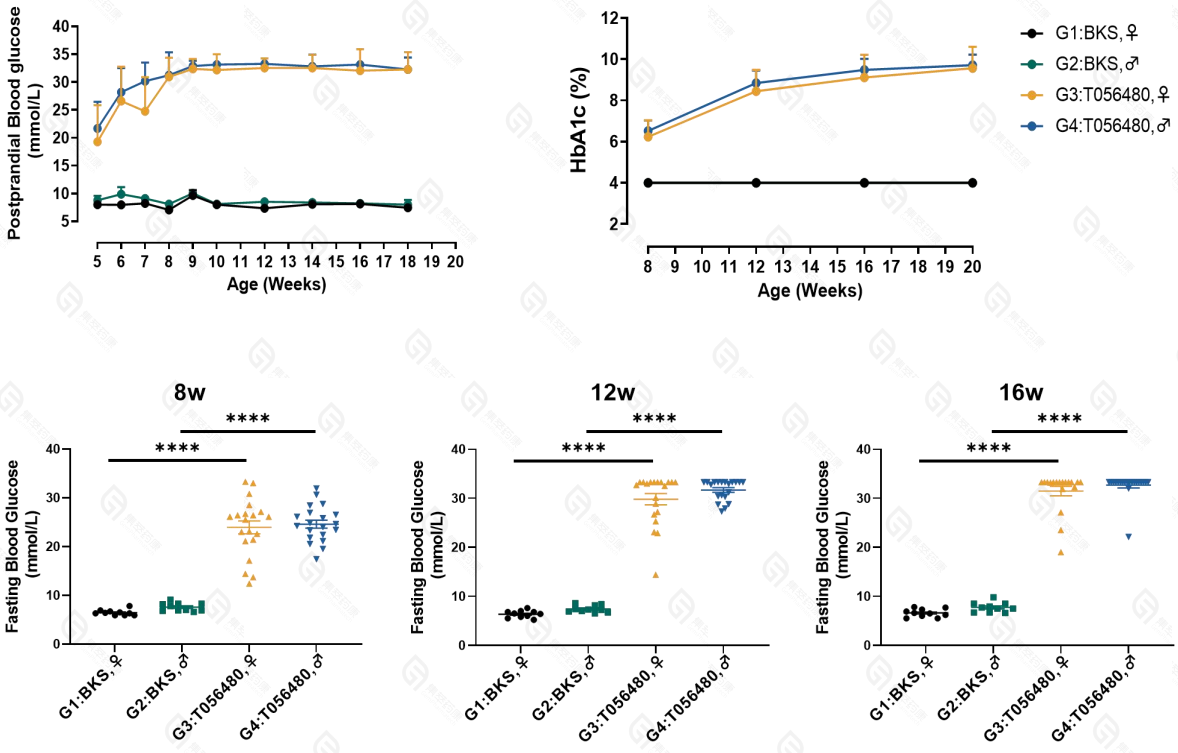

Fig 2. Increased blood glucose levels in BKS-db v2 mice

Measuring blood glucose and glycated hemoglobin (HbA1c) data of mice aged 5 to 20 weeks, with fasting blood glucose measurements taken after a 5-hour at 8, 12, and 16 weeks.

The blood glucose and HbA1c levels in T056480 mice were significantly increased compared to the control at all time points. (Data were presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20)

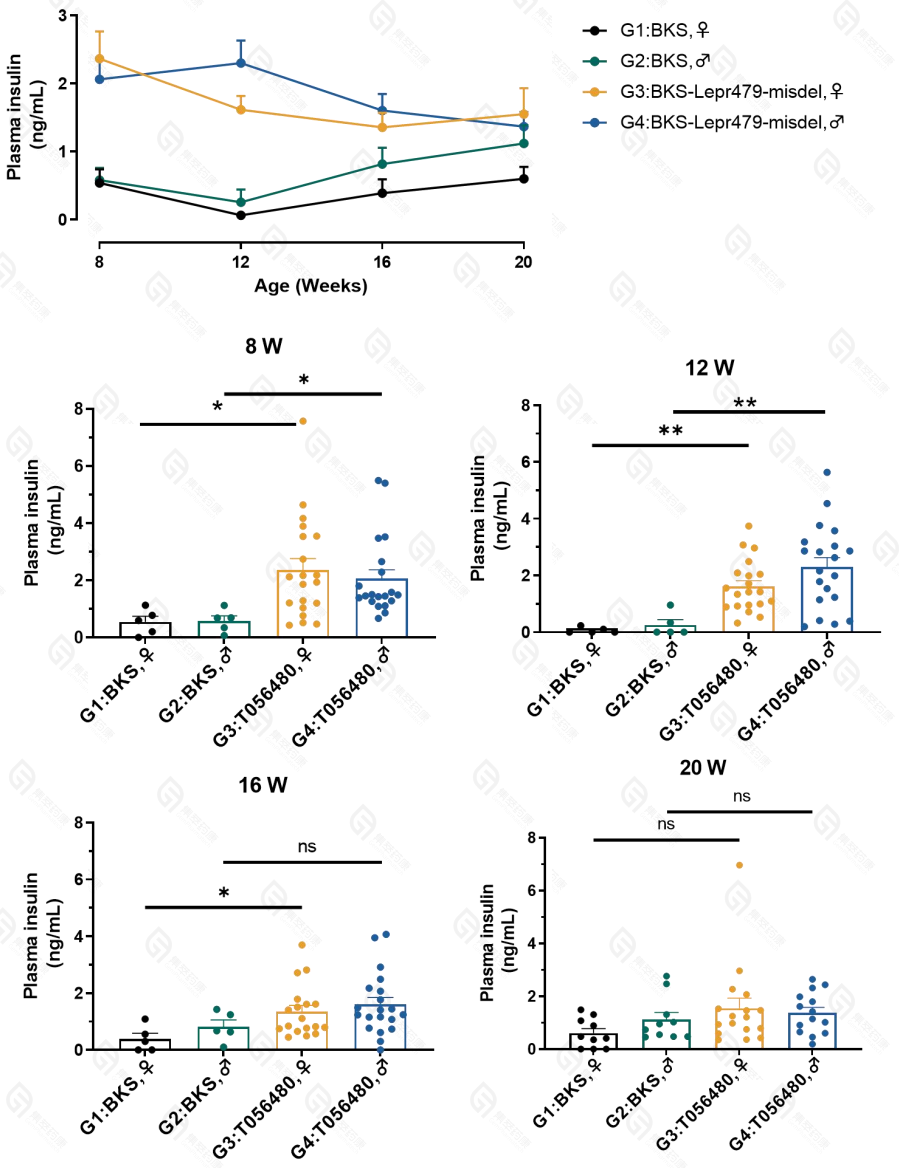

Fig 3. Increased insulin levels in BKS-db v2 mice

Testing the insulin levels in the blood of mice at different ages: 8 weeks, 12 weeks, 16 weeks, and 20 weeks. The results indicate a significant hyperinsulinemia in T056480 mice at 8 weeks and 12 weeks. As the age increases(at 20 weeks), diabetic mice show a decrease in insulin secretion, suggesting a gradual transition into a decompensatory phase of pancreatic function. (Data were presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20)

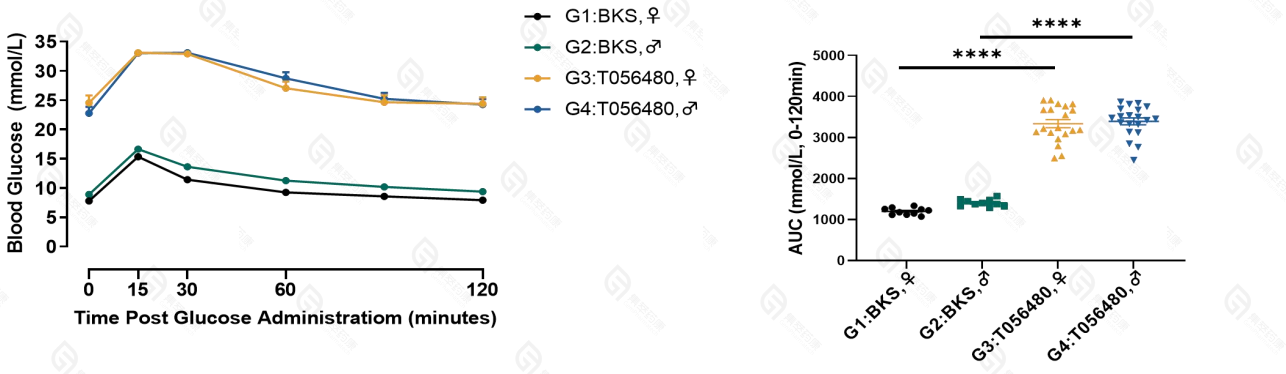

Fig 4. Glucose Tolerance Test in BKS-db v2 Mice

A glucose tolerance test (OGTT) was conducted on 7-week-old mice, and the area under the curve (AUC) was calculated. The results demonstrate that T056480 mice exhibit significantly reduced tolerance to orally administered glucose compared to wild-type mice. (Data presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20)

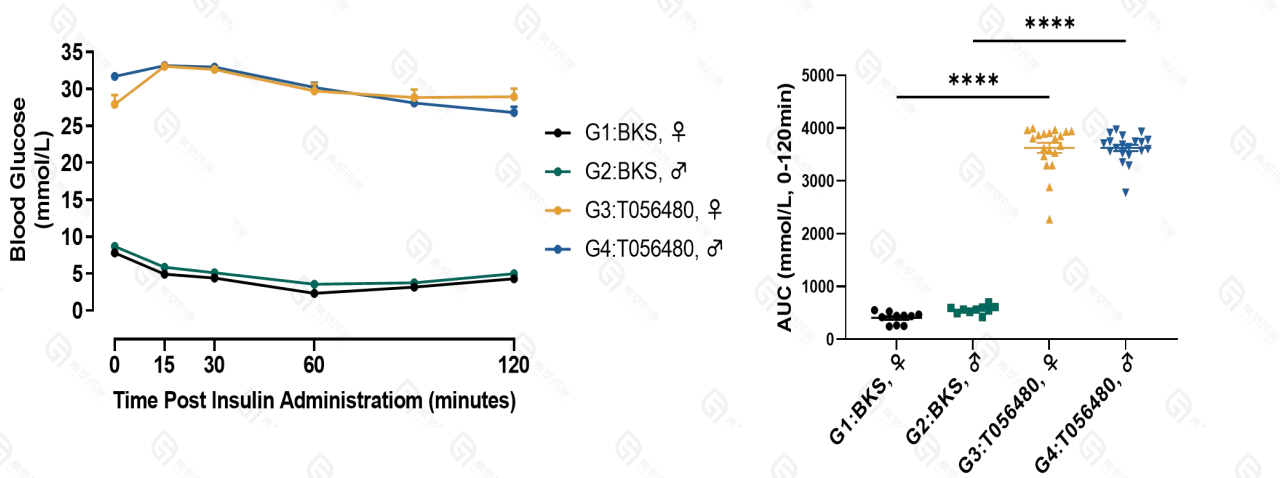

Fig 5. Insulin Tolerance Test in BKS-db v2 Mice

An insulin tolerance test (ITT) was performed on 9-week-old mice, and the area under the curve (AUC) was calculated. The results indicate a significant reduction in insulin sensitivity in T056480 mice compared to wild-type mice. (Data presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20)

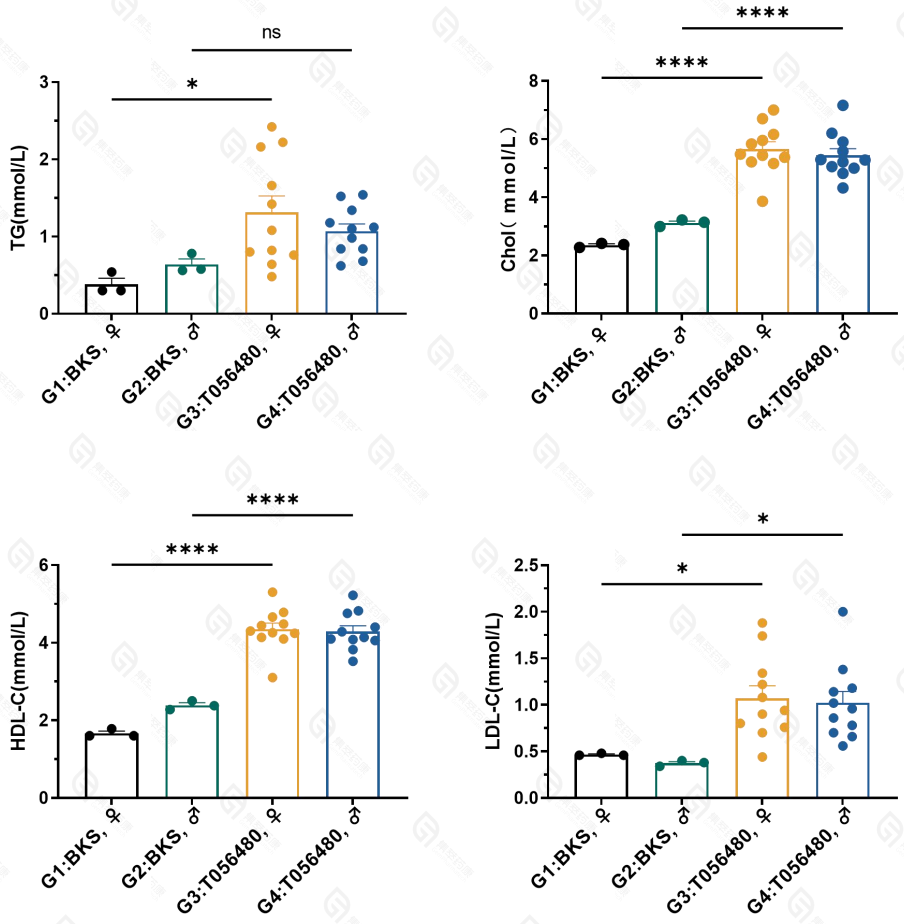

Fig 6. Increased Blood Lipid Levels in BKS-db v2 Mice

Blood lipid parameters including triglycerides (TG), cholesterol (Chol), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were measured in the blood of mice at 8 weeks. The results indicate a significant hyperlipidemia in T056480 mice. (Data presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20)

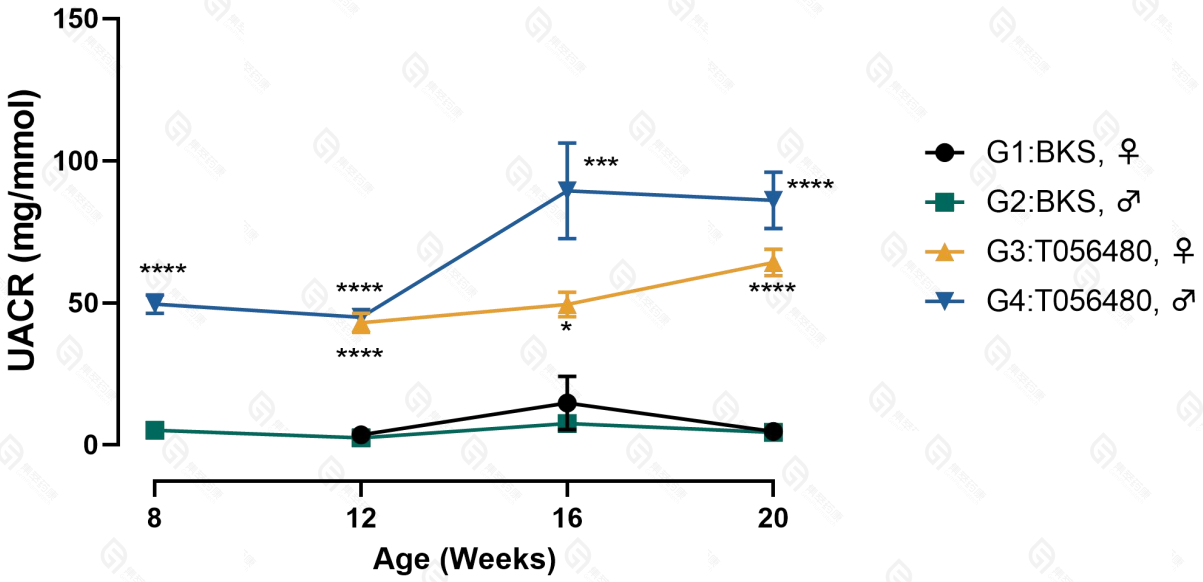

Fig 7. Early Onset of Diabetic Nephropathy in BKS-db v2 Mice

Urinary levels of creatinine (CREA) and microalbumin (mAlb) were measured in mice at different ages (8w, 12w, 16w, 20w), and the urine albumin-to-creatinine ratio (UACR) was calculated. The results indicate that T056480 mice start exhibiting proteinuria as early as 8 weeks of age, suggesting early signs of diabetic nephropathy. (Data presented as Mean±SEM, G1-G2: n=10, G3-G4: n=20.)

Precautions

1. Characteristics of blood glucose

BKS-db v2 is a period of rapid blood glucose rise at 4-7 weeks of age. During this period, the blood glucose of mice has a wide range, and the blood glucose between different individuals is also very different. Blood glucose groupings were strictly measured before, and the experimental results may have certain errors. After 8 weeks of age, the blood glucose levels gradually tend to be consistent. Considering possible changes in the mouse feeding environment, it is recommended to increase the margin by 10%. Onset criteria: fasting blood glucose>11.1mmol/l, random blood glucose>16.7mmol/l[7-8]. Environmental stimuli, mouse state, measurement time, and satiety (fasting time) can all affect blood glucose measurements. It is recommended to be raised in a barrier facility, ensure adequate drinking water and diet, change bedding frequently, reduce stress, and unify the time and blood collection method for blood glucose measurement. After receiving the mice, adaptive feeding was performed for 1 to 2 weeks for the experiment. Fasting blood sugar fasting generally does not exceed 6h.

2. Characteristics of diabetic nephropathy

Under normal circumstances, some mice began to develop severe renal complications at the age of 16 weeks, manifested as adhesion of renal tissue, unable to remove the

intact kidney for pathological examination or death of the mouse. Some mice with higher blood glucose may develop similar severe kidney complications around 12 weeks of age. For blood glucose-related research, it is recommended to add an additional 20% to 30% of the surplus on the basis of the actual number of participants, or to select materials from an earlier age. Generally, the evaluation indicators for renal lesions include pathological sections, blood biochemistry and urine biochemistry, among which the urine biochemistry data is the most unstable, and it is recommended to combine the other two tests for judgment. For studies related to diabetic nephropathy, it is necessary to strictly set the entry indicators and select appropriate mice for the experiment. In addition, batch-to-batch differences are prone to occur, and intra-batch comparisons are recommended.

3. Feeding related

BKS-db v2 homozygous mice, the phenotype can be seen at approximately three weeks of age. Homozygous mice gain weight rapidly, up to three times the normal weight of wild-type control mice. Homozygous mice are obese, with enlarged buttocks, wider legs, and short legs. Homozygous male mice are prone to genital atrophy over 8 weeks of age. This strain is a diabetic model mouse. With age, blood glucose and body weight will gradually increase. The blood glucose of 5-9 weeks old mice will increase rapidly, and it will reach a critical value after 9 weeks of age. Later, mice will lose weight, etc. Diabetic complication phenotype, the body weight of mice of the same age varies greatly. Note that 5-9 weeks of age is the period of rising blood glucose and body weight of mice. During this period, the mouse cage is more likely to be dirty and needs to be changed once a week, and twice a week. The blood glucose will reach the critical value around 9 weeks of age. Rat cage boxes are particularly prone to moisture, and often need to be replaced every two days, and even some older stock cages need to be replaced once a day. Therefore, in the case of sufficient cage space, it is necessary to reduce the cage density of older stocks.

References

1. Chen, Hong, et al. "Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice." Cell 84.3 (1996): 491-495.
2. Yoon, Ji-Won, et al. "Genetic control of organ-reactive autoantibody production in mice by obesity (ob) diabetes (db) genes." Diabetes 37.9 (1988): 1287-1293.
3. Kämpfer, Heiko, et al. "Lack of interferon-gamma production despite the presence of interleukin-18 during cutaneous wound healing." Molecular Medicine 6.12 (2000): 1016.
4. Garris, Bryan L., et al. "Hypophyseal lipoapoptosis: diabetes (db/db) mutation-

associated cytolipidemia promotes pituitary cellular disruption and dysfunction." Pituitary 7.1 (2004): 5-14.
5. Garris, David R. "Estrogenic stimulation of ovarian follicular maturation in diabetes (db/db) mutant mice: restoration of euglycemia prevents hyperlipidemic cytoatrophy." Cell and tissue research 318.2 (2004): 365-373.
6. Garris, David R., and Bryan L. Garris. "Genomic modulation of diabetes (db/db) and obese (ob/ob) mutation-induced hypercytolipidemia: cytochemical basis of female reproductive tract involution." Cell and tissue research 316.2 (2004): 233-241.
7. Edirs S, Jiang L, Lei X, et al. "XinKursi Wufarikun Ziyabit Improves the Physiological Changes by Regulating Endoplasmic Reticulum Stress in the Type 2 Diabetes db/db Mice." Evid Based Complement Alternat Med. 2021 Aug 16;2021:2100128.
8. Huang CZ, Xu JH, Zhong W, et al. "Sox9 transcriptionally regulates Wnt signaling in intestinal epithelial stem cells in hypomethylated crypts in the diabetic state. " Stem Cell Res Ther. 2017 Mar 9;8(1):60.