B6 Trex1-KO

Strain Name: B6/JGpt-*Trex1*^{em1Cd1194}/Gpt
Strain Type: Knock-out
Strain Number: T013987
Background: B6/JGpt

Description

TREX1 (DNase III) is the major 3′-5′ DNA exonuclease of mammalian cells, which has been proposed to have a major role in cell death and genomic DNA degradation [1]. It has been suggested that dysfunction of *Trex1* may activate immune response by self DNA as suggested by the TREX1 null mice which develop an inflammatory myocarditis similar to autoimmune cardiomyopathy and produce type 1 IFN [2]. Moreover, TREX1 D18N mutation causes a monogenic, cutaneous form of lupus called familial chilblain lupus, and the TREX1 D18N enzyme exhibits dysfunctioned dsDNA-degrading activity, providing a link between dsDNA degradation and nucleic acid-mediated autoimmune disease.

We generated the *Trex1*$^{-/-}$ mice at GemPharmatech and performed comprehensive phenotyping to evaluate the loss of function of TREX1 and its potential application in systemic lupus erythematosus (SLE) study. This model exhibited lupus-like inflammatory autoimmune response with increased anti-dsDNA levels and inflammation in multiple organs, providing a useful tool to study TREX1-mediated autoimmunity and to evaluate potential treatments for lupus.

Strategy

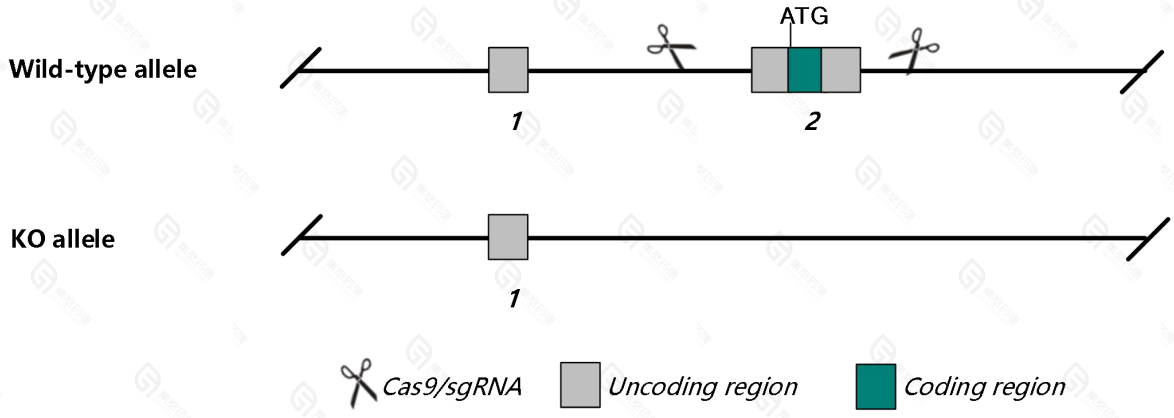


Fig.1 Schematic diagram of B6 *Trex1*-KO model strategy.

Applications

1. Study of mechanism involved in systemic lupus erythematosus (SLE)
2. Anti-SLE drug screening and efficacy test
3. Immune system-related research

Supporting Data

1. Detection of TREX1 expression

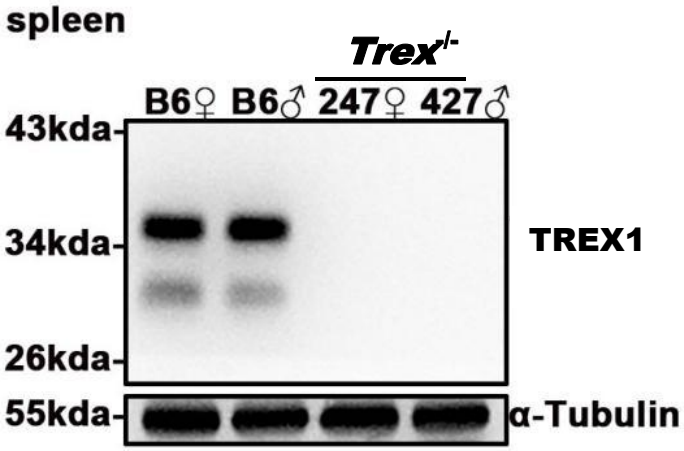


Fig 2. Detection of TREX1 expression on B6 *Trex1*-KO mice.

The knock out of *Trex1* was confirmed by western blot analysis. Spleen was harvested from wild type B6 mice and B6 *Trex1*-KO mice , and no TREX1 were detected in protein level in *Trex1*-KO mice.

2. Survival rate of TREX1 null mice

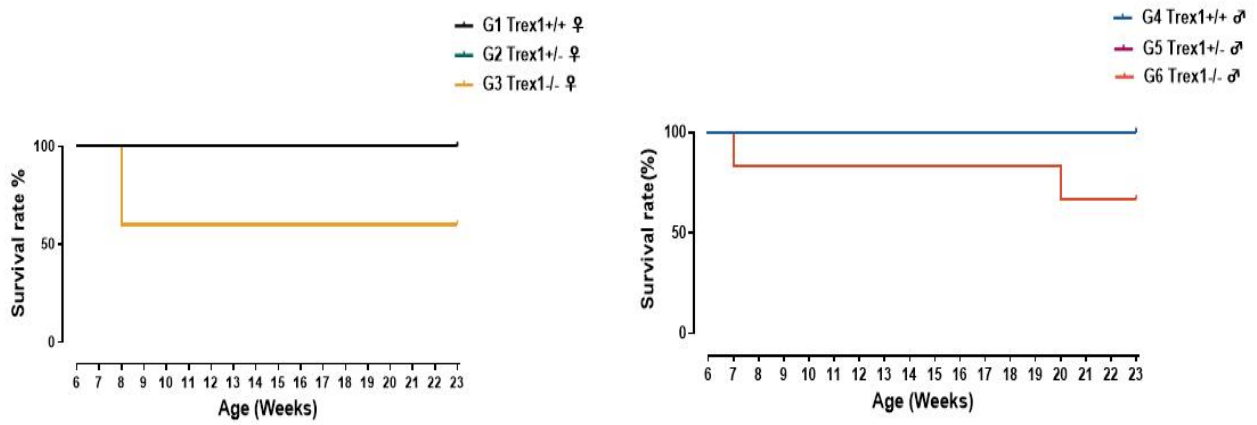
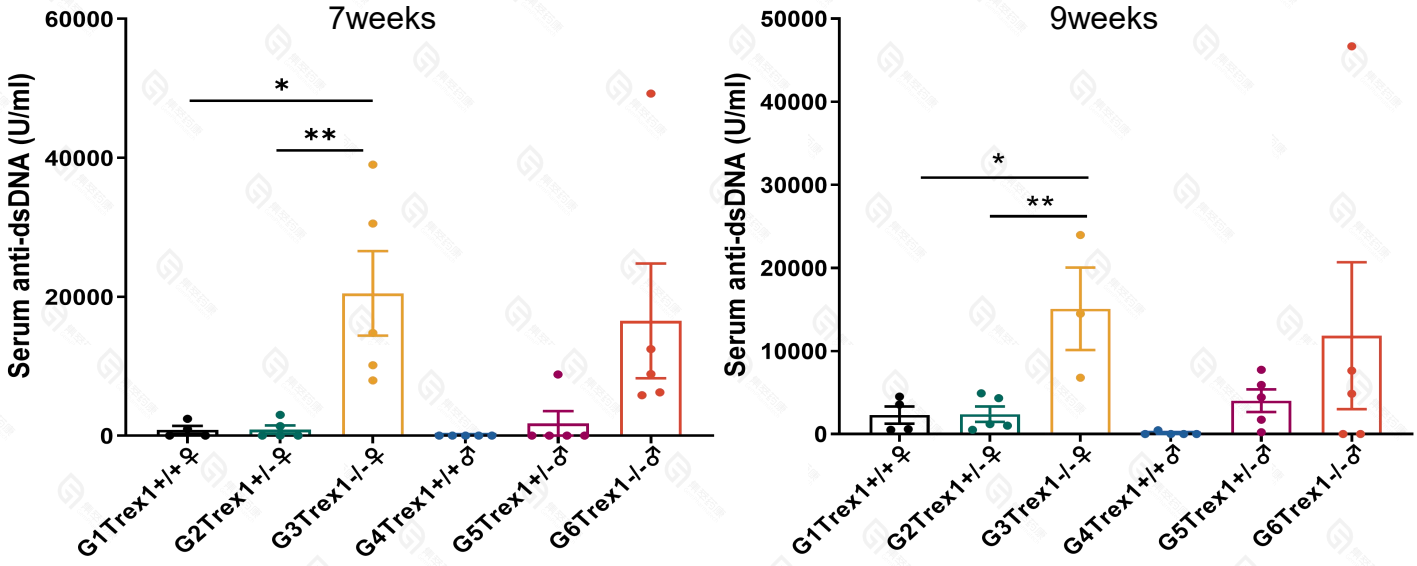


Fig 3. Survival rate of B6 *Trex1*-KO mice

Reduced survival of B6 *Trex1*-KO mice were observed during study.

3. Anti-dsDNA level evaluation



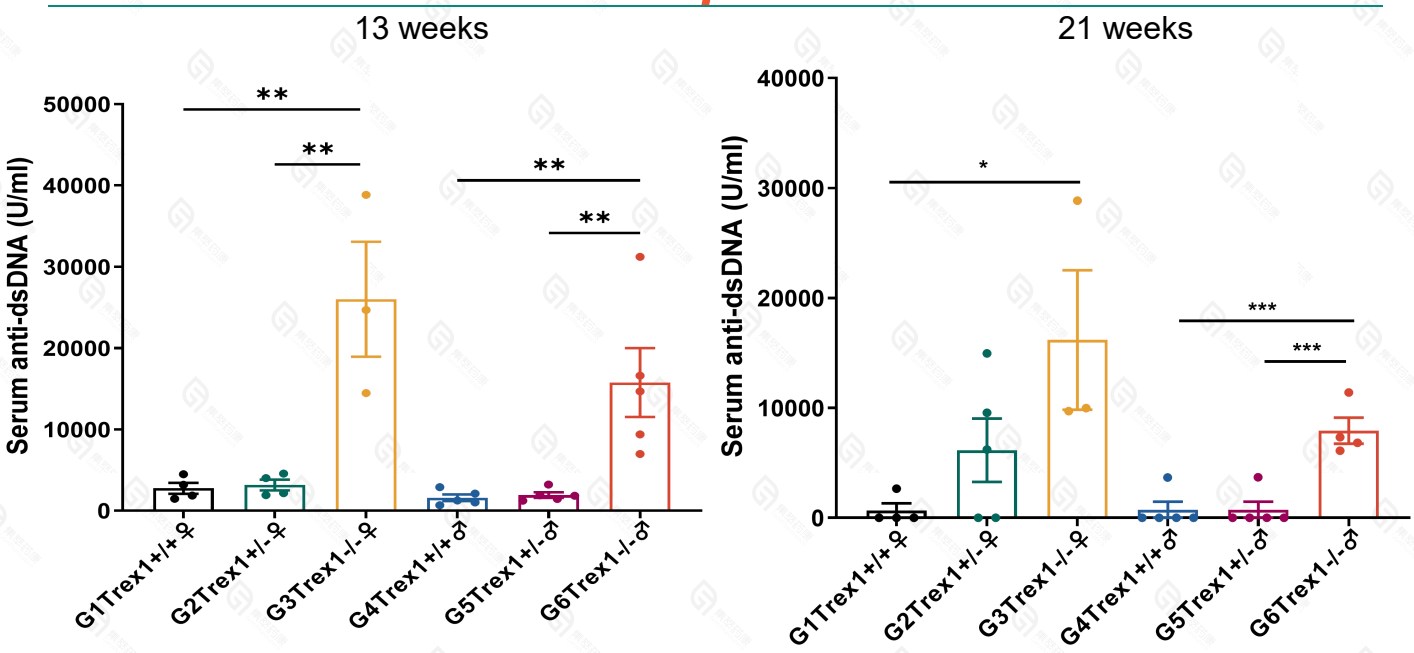
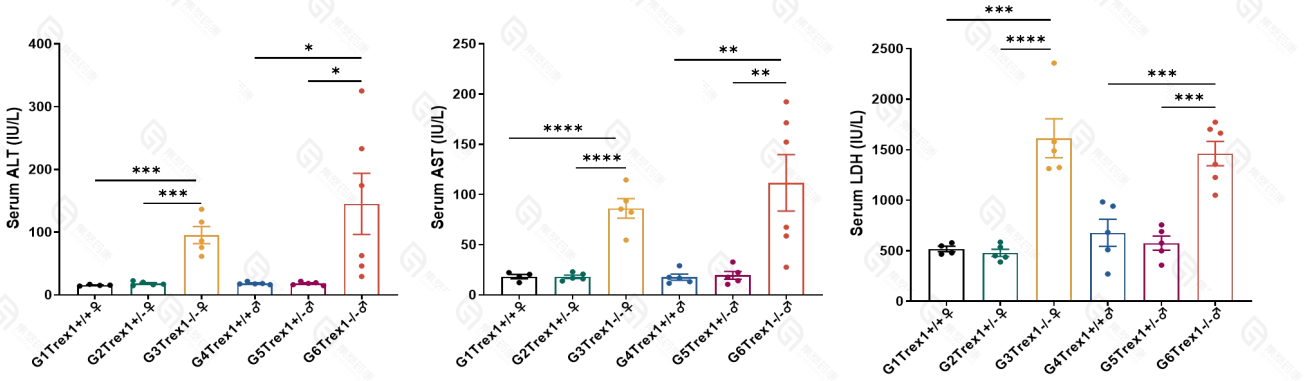


Fig 4. Detection of anti-dsDNA level on B6 *Trex1*-KO mice

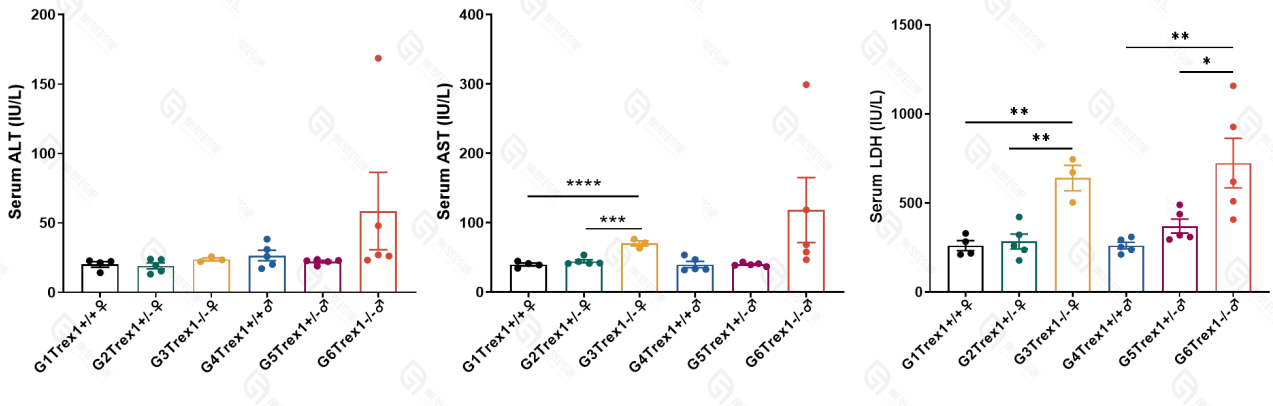
The serum anti-dsDNA IgG antibody levels was detected in both *Trex1*$^{-/-}$ male and female mice . The level of anti-dsDNA IgG were significantly increased in *Trex1*$^{-/-}$ male and female mice compared with wildtype mice, which was kept high from 7 to 21 weeks of age.

4. Blood biochemistry analysis

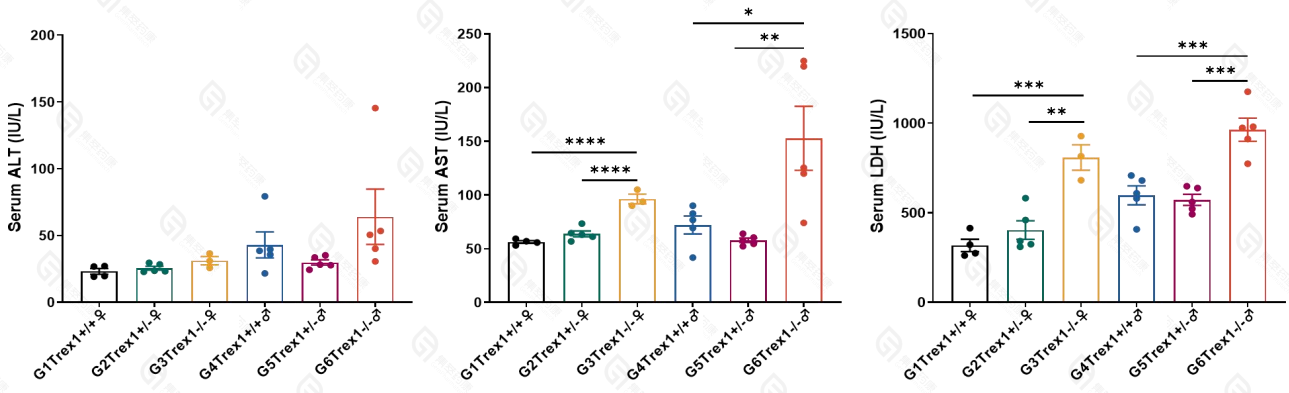
Blood biochemistry in 6-week-old mice



Blood biochemistry in 10-week-old mice



Blood biochemistry in 14-week-old mice



Blood biochemistry in 18-week-old mice

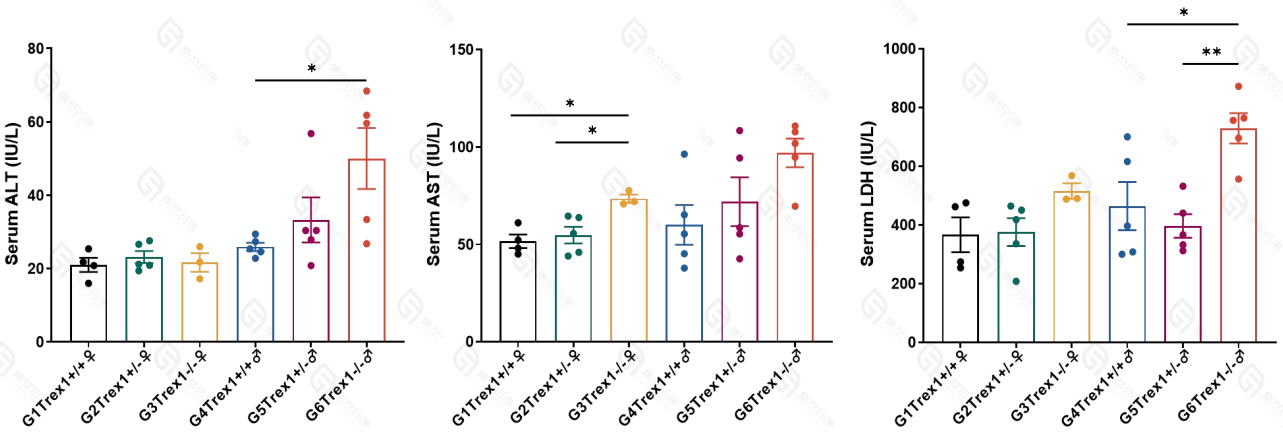
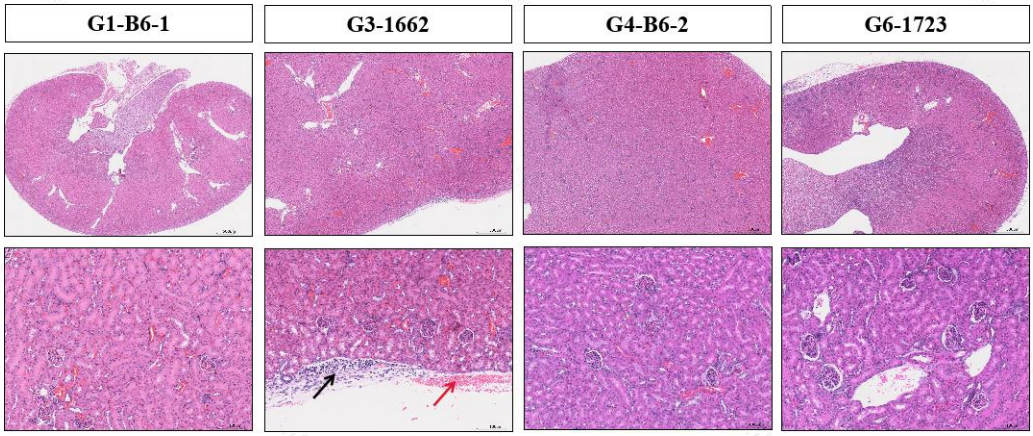


Fig 5. Blood chemistry analysis

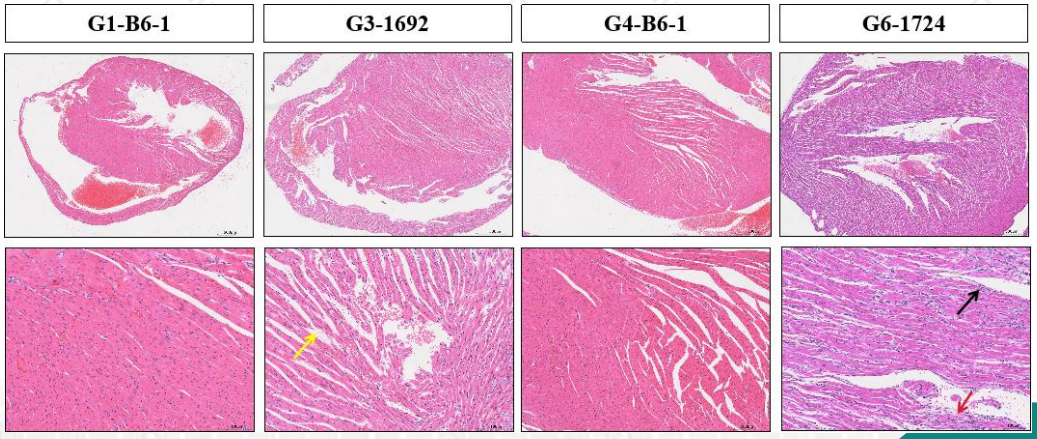
Serum ALT, AST and LDH levels were dramatically increased in both male and female *Trex1*$^{-/-}$ mice compared with wild type and *Trex1*$^{+/-}$ mice from 6-18 weeks of age, indicating injuries of liver and heart.

5. Histopathological analysis

A.



B.



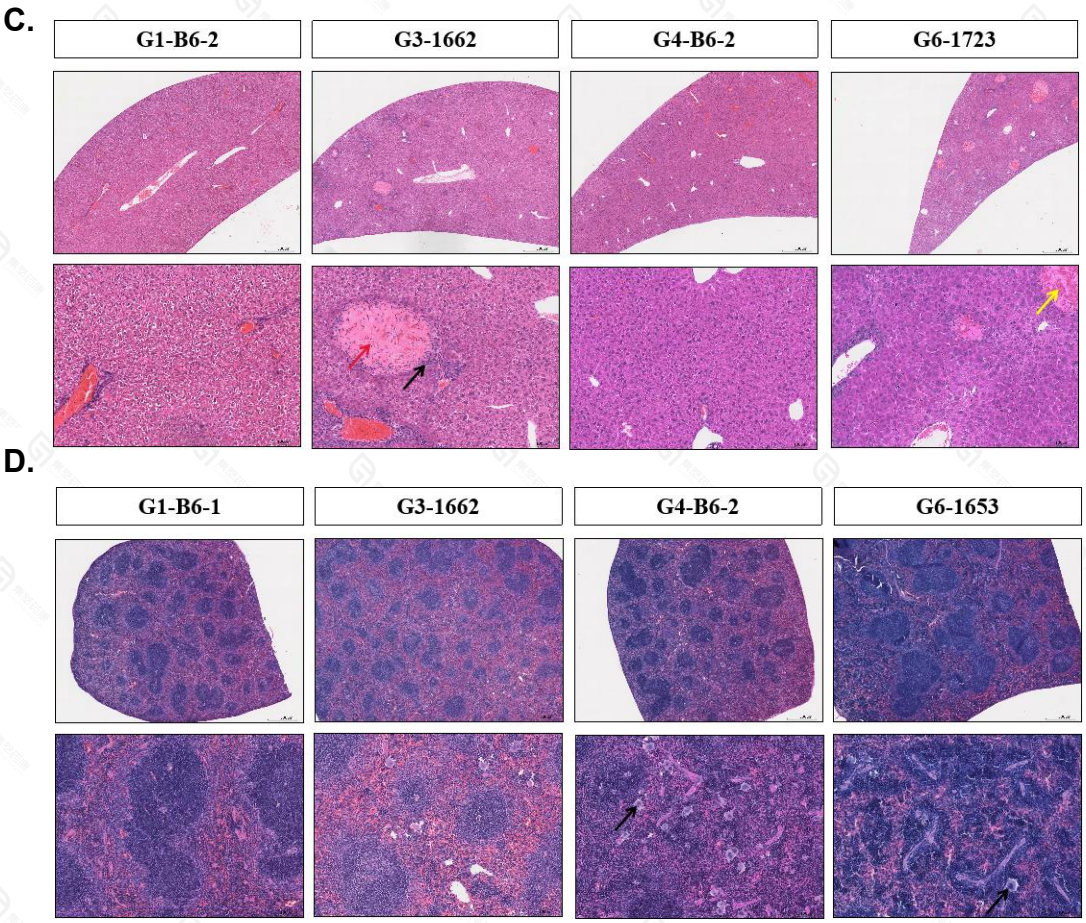
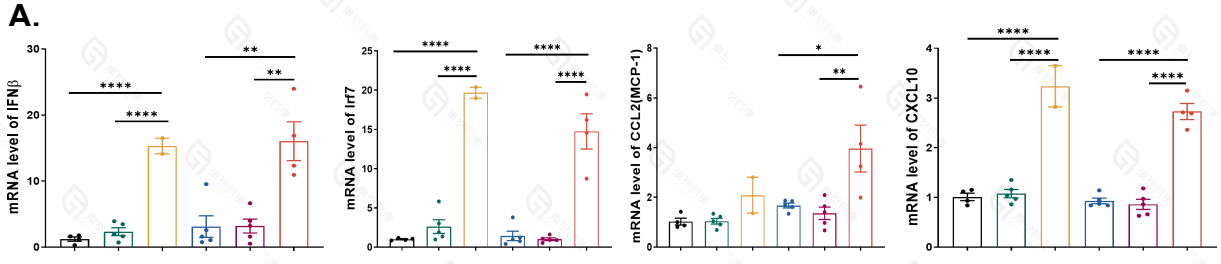


Fig 6. Histopathological analysis of inflammation in B6 *Trex1*-KO mice

Infiltration of inflammatory immune cells in the kidney (A), heart (B), liver (C) and spleen (D) of 8-week-old mice. G1: wildtype female, G3: *Trex1*$^{-/-}$ female, G4: wildtype male, G6: *Trex1*$^{-/-}$ male. Upper panel, Scale Bar=500 μm；lower panel, Scale Bar=100 μm.

6.Interferon-stimulatory DNA (ISD) response 32-week-old mice



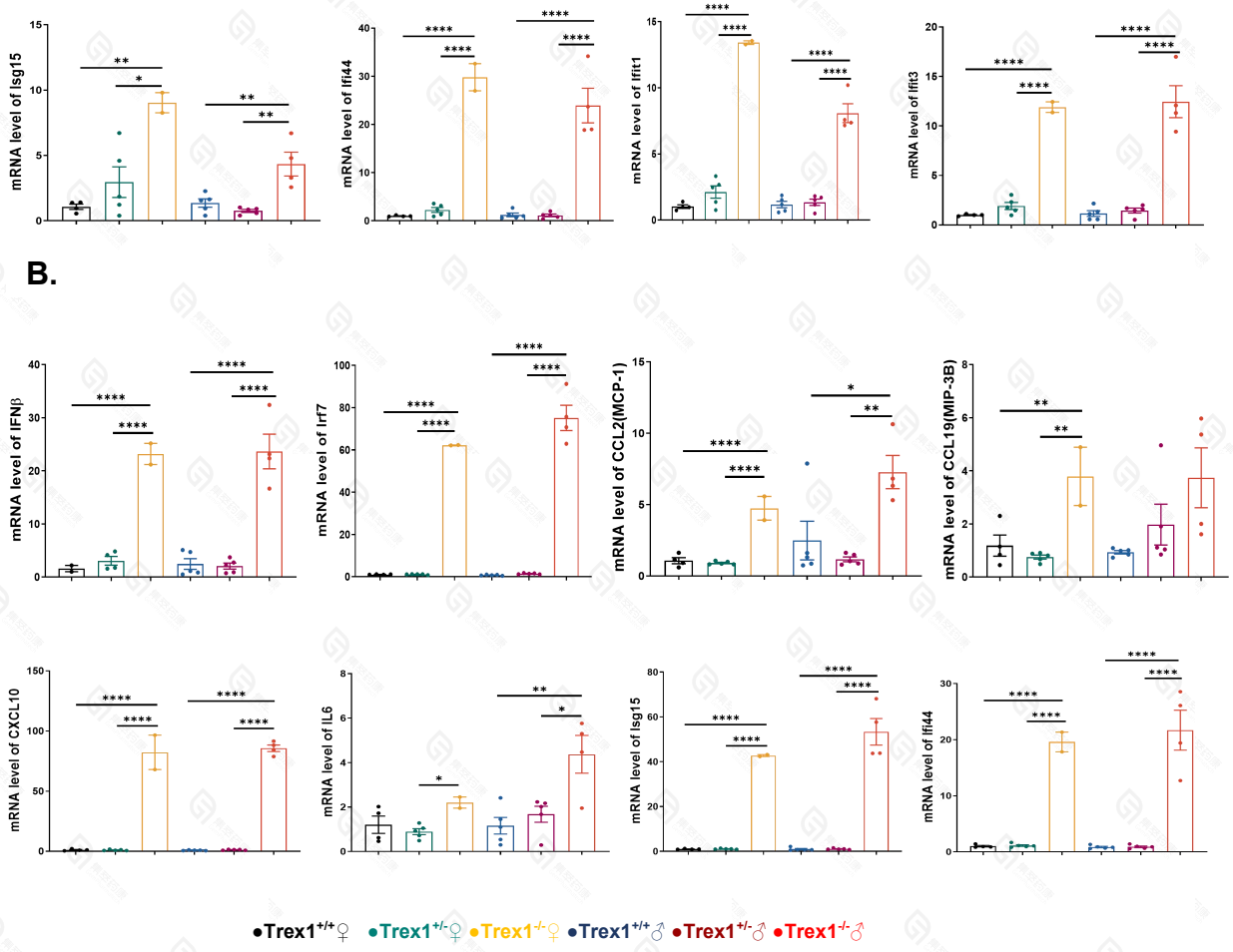


Fig 7. Interferon-stimulatory DNA (ISD) response in sepleen and heart tissue
Spleen (A) and hearts (B) were collected at 32 weeks and performed the Q-PCR tests to evaluate the interferon-stimulatory DNA (ISD) response.The ISD were dramatically increased in both male and female *Trex1*$^{-/-}$ mice compared with wild type and *Trex1*$^{+/-}$ mice.

7.*In vivo* drug efficacy study

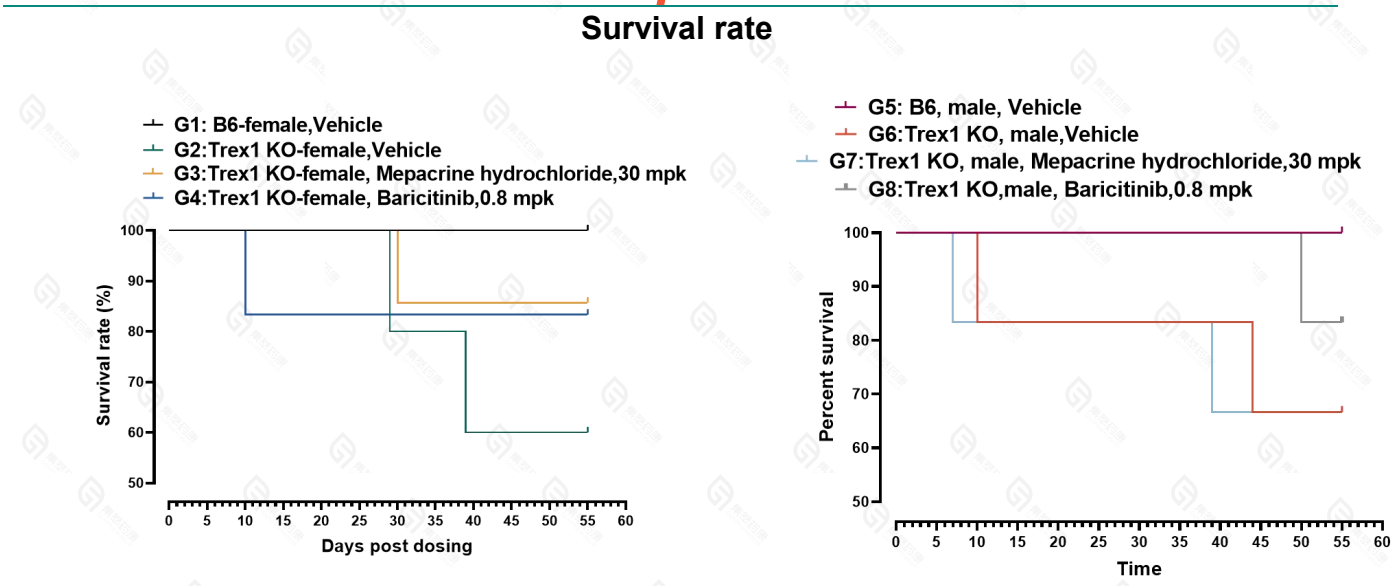


Fig 8. Survival curve after drug treatment

Trex1$^{-/-}$ mice were treated with mapacrine(antimalarials) and baricitinib (JAK1/2 inhibitors). Spontaneous mortality was observed in *Trex 1*-KO mice and drug treatment improved survival rate.

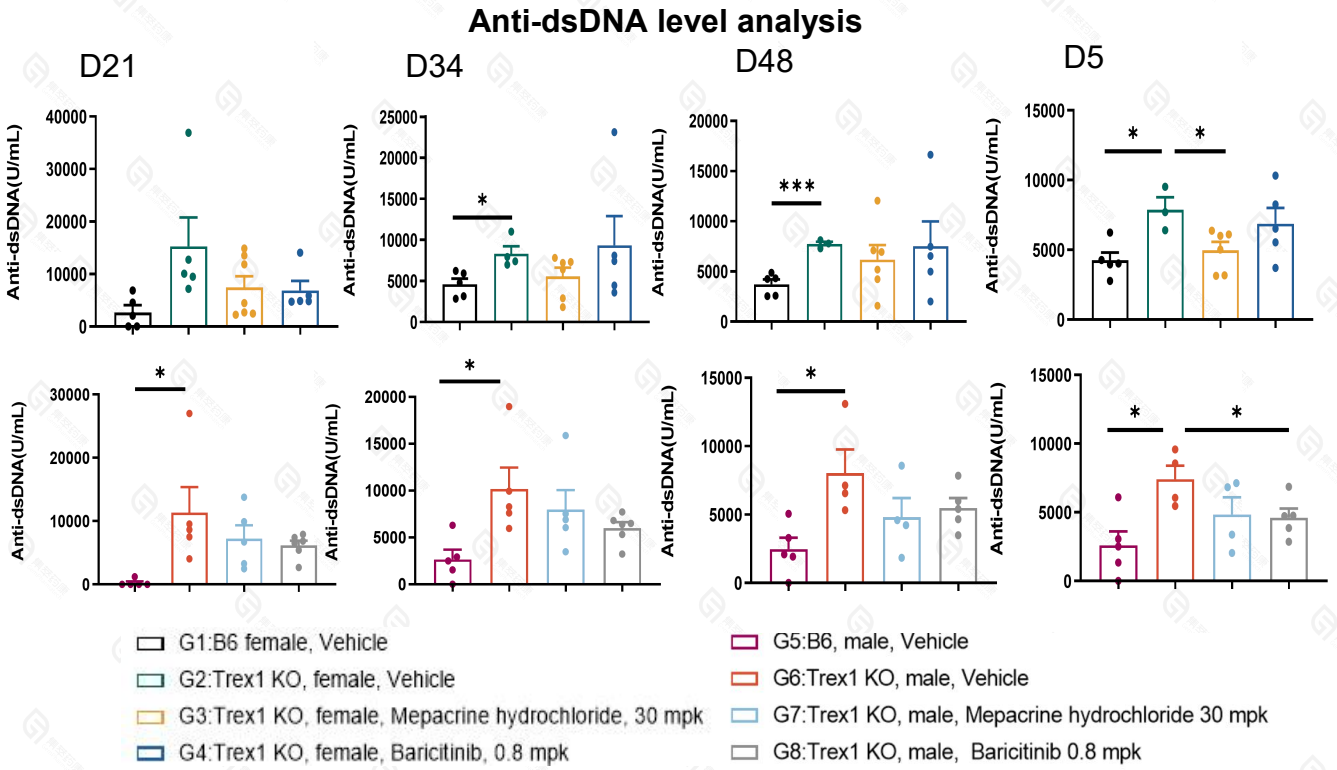


Fig 9. Serum assay for anti-dsDNA level

The serum anti-dsDNA levels were reduced with baricitinib treatment in *Trex 1*-KO mice. The mepacrine also showed a therapeutic effect in the *Trex 1*-KO model.

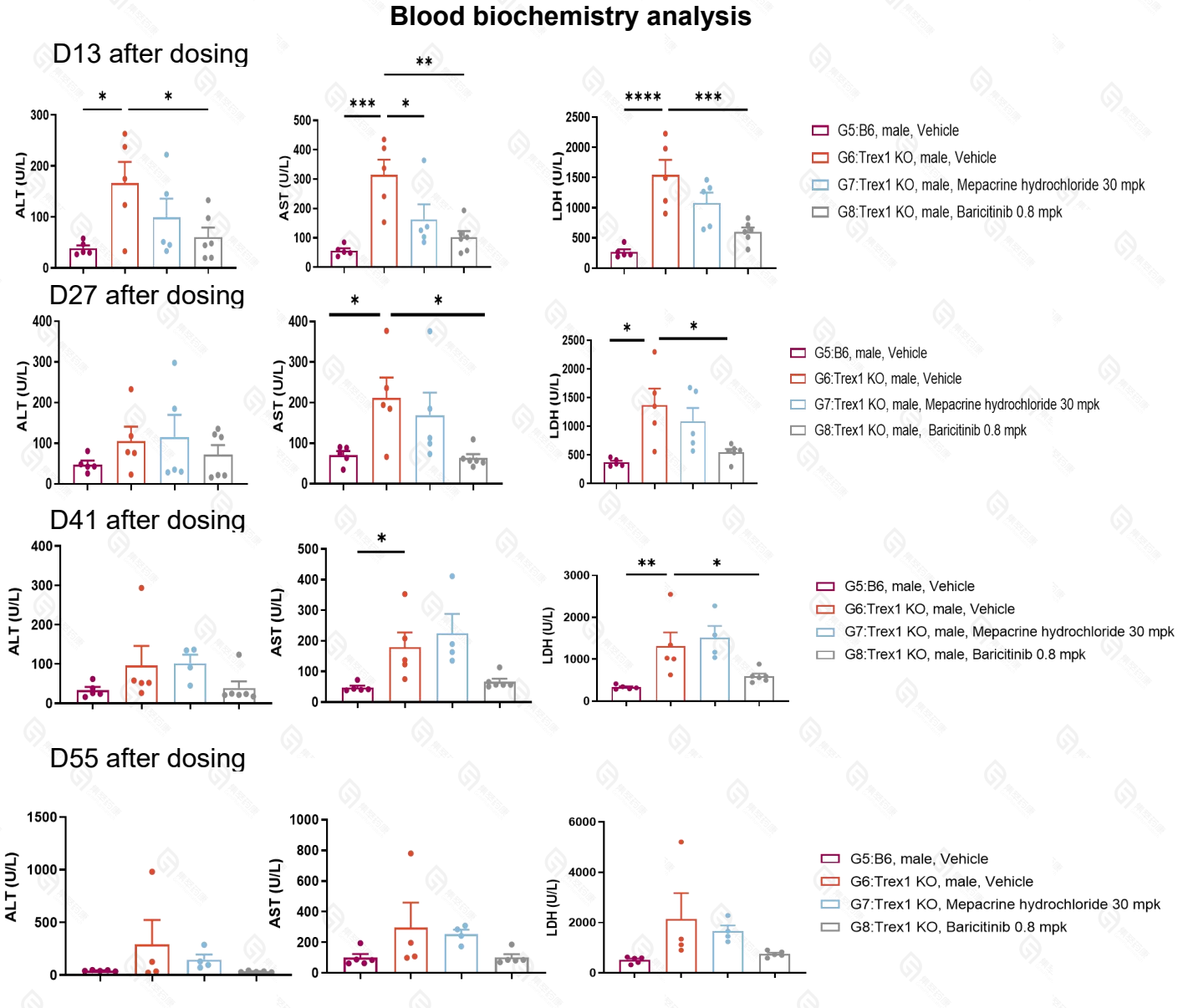


Fig 10. Blood biochemistry analysis

ALT, AST and LDH were tested in blood biochemistry analysis. The serum levels of ALT, AST, and LDH were reduced with baricitinib treatment in *Trex 1*-KO mice.The mepacrine also showed a therapeutic effect in the *Trex 1*-KO model.

Histopathological analysis

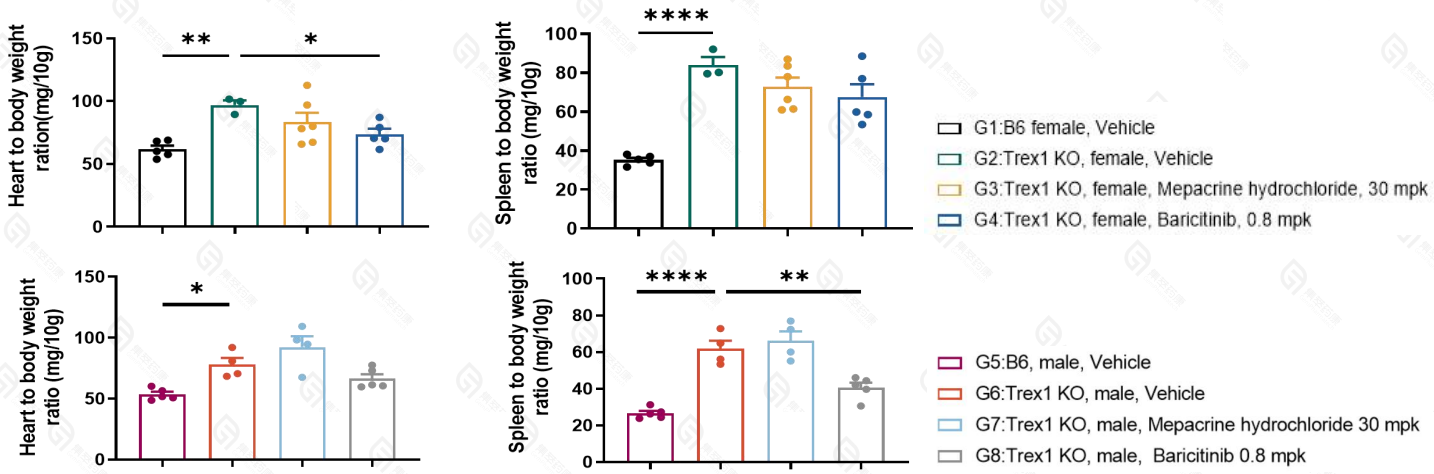


Fig 11. The organ-to-body weight ratios of heart and spleen

The *Trex 1*-KO mice displayed heart and spleen enlarged and drug treatment could alleviate it.

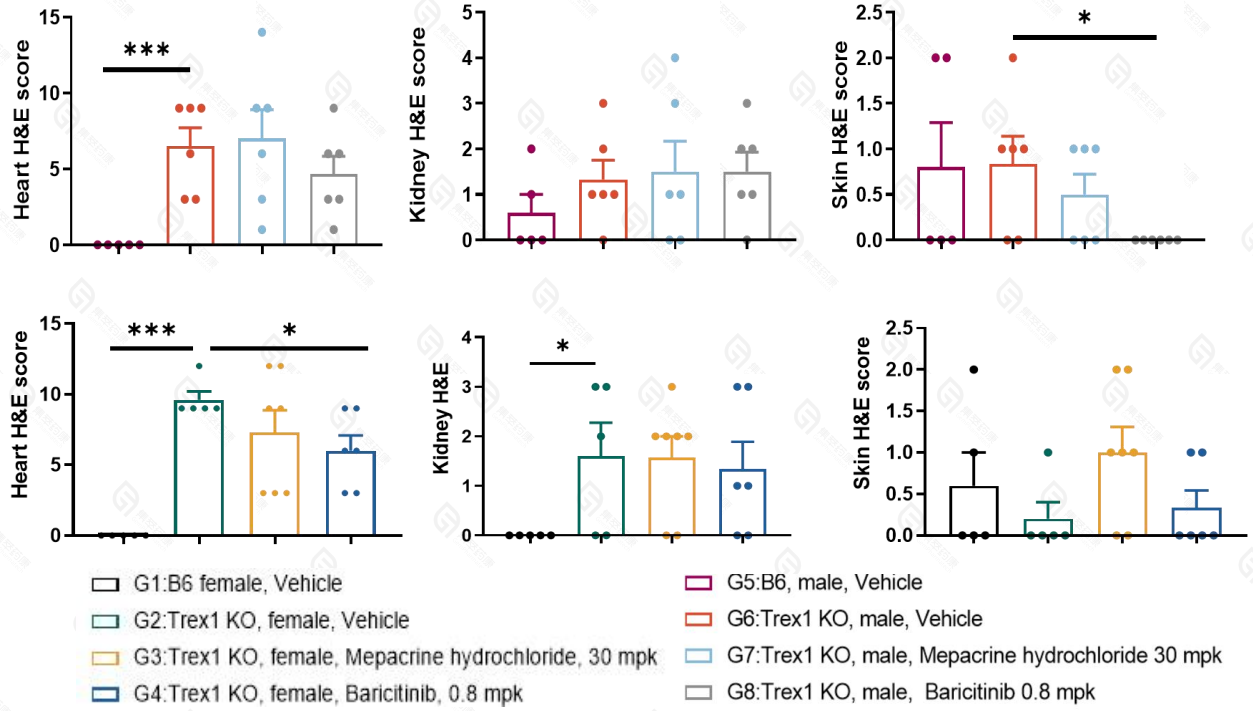


Fig 12. The pathological sores of heart, kidney and skin

Obvious pathological changes were observed in the heart, kidney and skin of *Trex 1*-KO mice; baricitinib treatment alleviated the pathological changes.

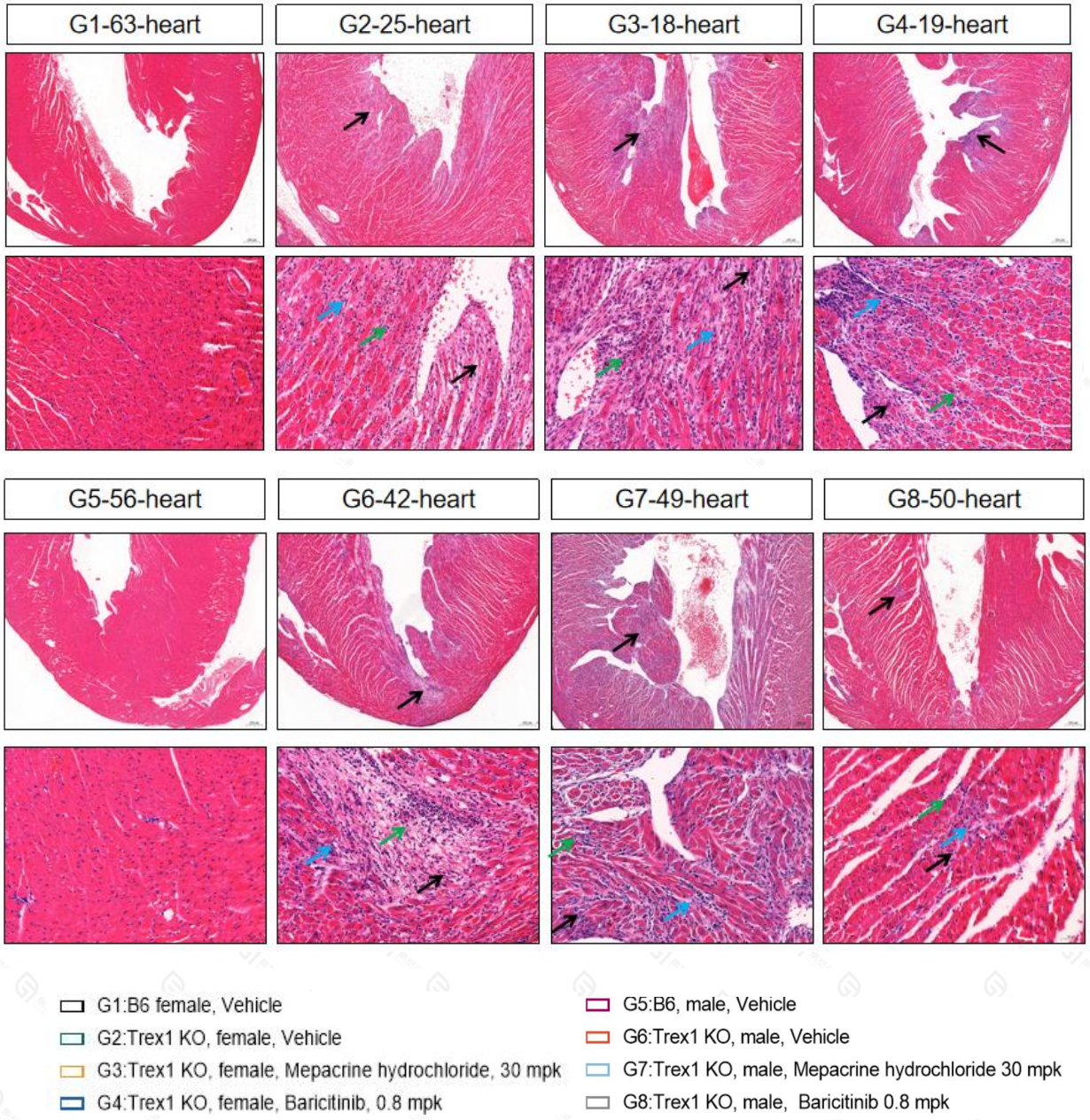


Fig 13. Histopathology of heart

Heart tissues were collected and performed histological study. Myocardial fibrosis and necrosis (black arrow); Fibrous tissue hyperplasia (blue arrow); Inflammatory cell infiltration (green arrow).

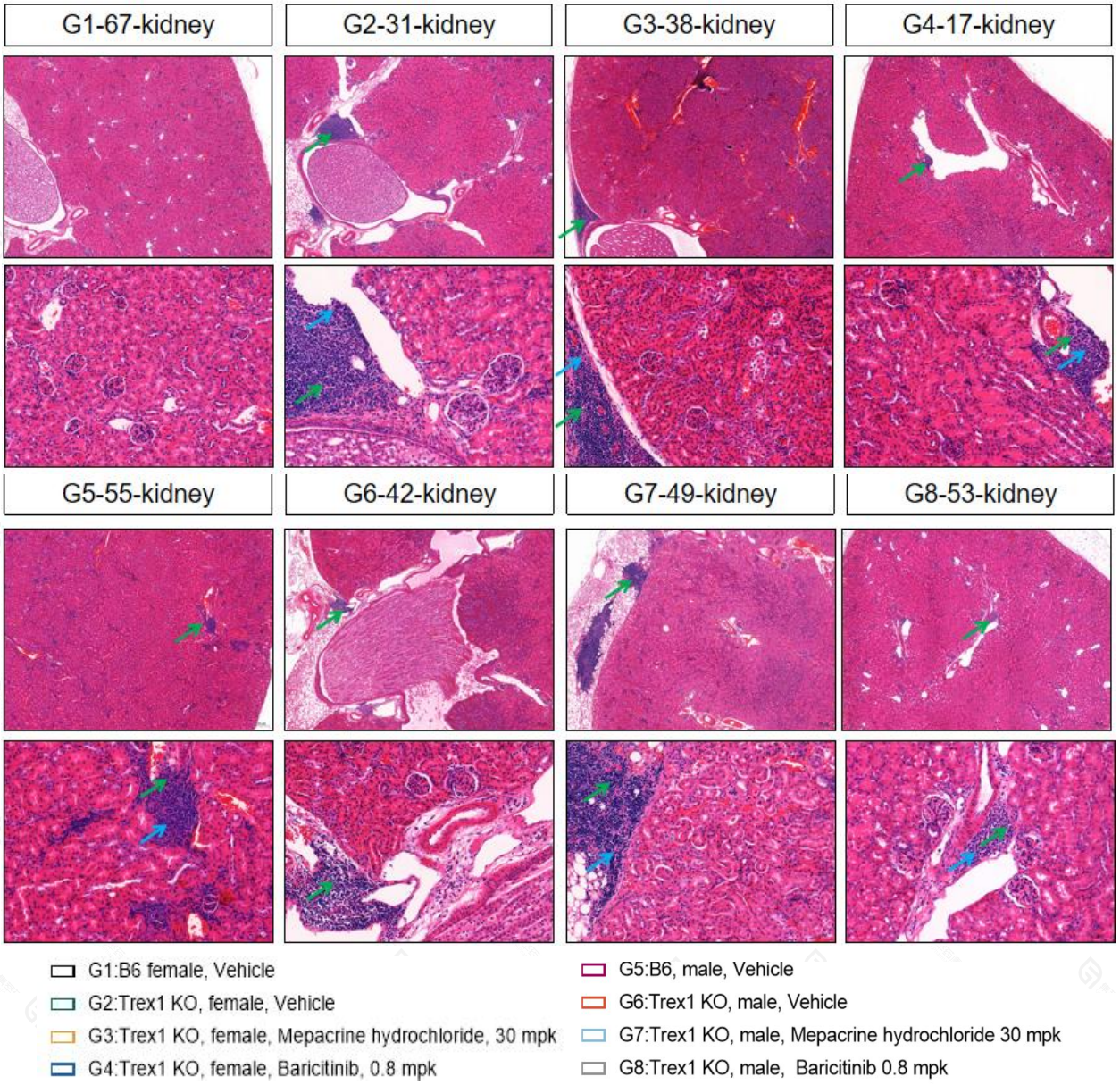


Fig 14. Histopathology of kidney

Kidney tissues were collected and performed histological study. Fibrous tissue hyperplasia (blue arrow);Inflammatory cell infiltration (green arrow).

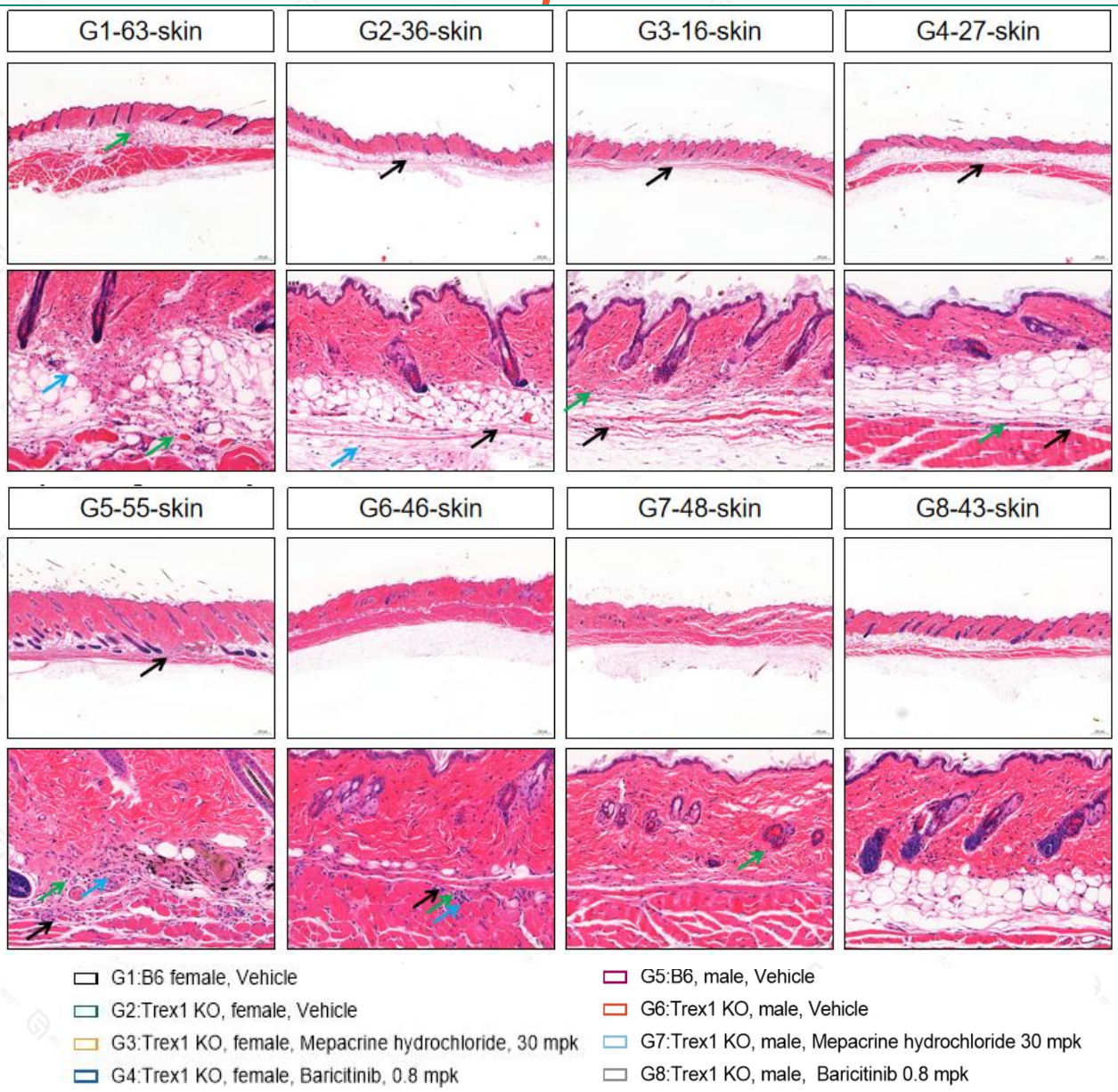


Fig 15. Histopathology of skin

Skin tissues were collected and performed histological study. Fibrous tissue hyperplasia (blue arrow);Inflammatory cell infiltration (green arrow); Muscle fiber degeneration and necrosis (black arrow).

References

1. Mazur DJ, Perrino FW. Structure and expression of the TREX1 and TREX2 3' --> 5' exonuclease genes. J Biol Chem. 2001 May 4;276(18):14718-27.
2. Lee-Kirsch MA, et al.Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. Nat Genet. 2007 Sep;39(9):1065-7.
3. Grieves JL, Fye JM, Harvey S, Grayson JM, Hollis T, Perrino FW. Exonuclease TREX1 degrades double-stranded DNA to prevent spontaneous lupus-like inflammatory disease. Proc Natl Acad Sci U S A. 2015 Apr 21;112(16):5117-22.