B6-Mybpc3-KO

Strain Name: B6/JGpt-*Mybpc3*^{em19Cd10922}/Gpt
Strain Type: Knock-out
Strain ID: T027708
Background: C57BL/6JGpt

Description

Cardiac myosin-binding protein C, also known as cMyBP-C, is encoded by the MYBPC3 gene[1]. cMyBP-C is a component of the A-band of the sarcome, which specifically interacts with myosin, actin and titin to regulate cardiac contraction[2]. Previous research indicated that mutations in MYBPC3 gene caused hypertrophic cardiomyopathy (HCM), HCM-associated dilation, or dilated cardiomyopathy (DCM) in human[3]. The main mutation types of MYBPC3 gene are nonsense mutations and frameshift mutations, which usually result in complete or partial MYBPC3 loss-of-function. MYBPC3 mutations account for about 30-40% of the causing mutations among all HCM patients[4].

Homozygous mice bearing cardiac MYBPC3 gene mutations develop dilated cardiomyopathy[5]. Therefore, we created a *Mybpc3* gene knockout mouse model via gene editing technology to simulate HCM and DCM in human. B6-Mybpc3-KO strain was created on the C57BL/6JGpt background in Gempharmatech. Cardiac dysfunction and histopathology in this homozygous strain shows parallel phenotypes to human DCM, including marked left ventricular (LV) dilation, myocardial hypertrophy, myofibrillar disarray and fibrosis, as expected. Therefore, B6-Mybpc3-KO strain is an ideal model for preclinical anti-HCM or DCM drug evaluation.

The B6-Mybpc3-KO strain was created at GemPharmatech using gene editing technology whereby exon 2-exon 22 of *Mybpc3* was knocked out, leading to loss of MYPBC3 protein.

Strategy

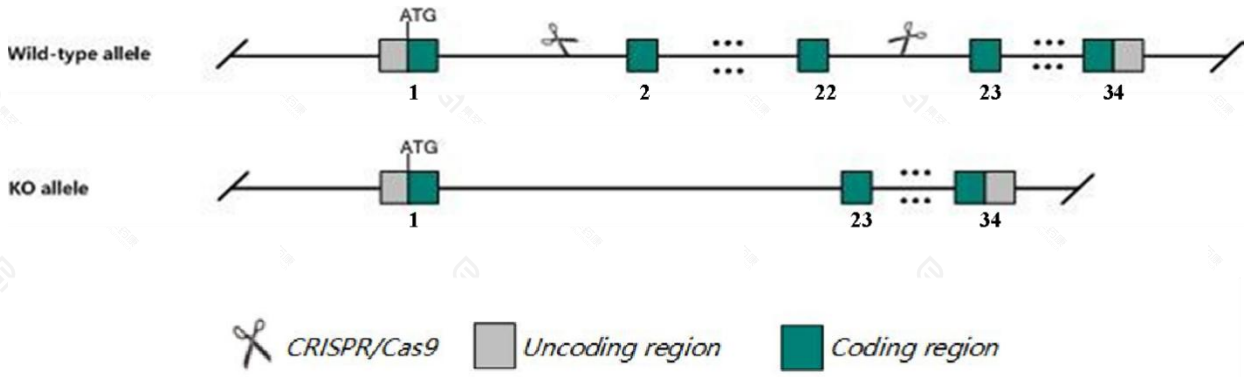


Fig 1. The B6-Mybpc3-KO strain strategy

The exon 2-exon 22 of *Mybpc3* gene was knocked out via CRISPR technology.

Applications

1.Anti-HCM or DCM drug screening and efficacy test.

2.Research on related diseases (such as myocardial hypertrophy, ventricular dilation and fibrosis) caused by defects of *Mybpc3*.

Data support

1. Expression analysis of MYBPC3 protein of WT and *Mybpc3* $^{-/-}$ mice

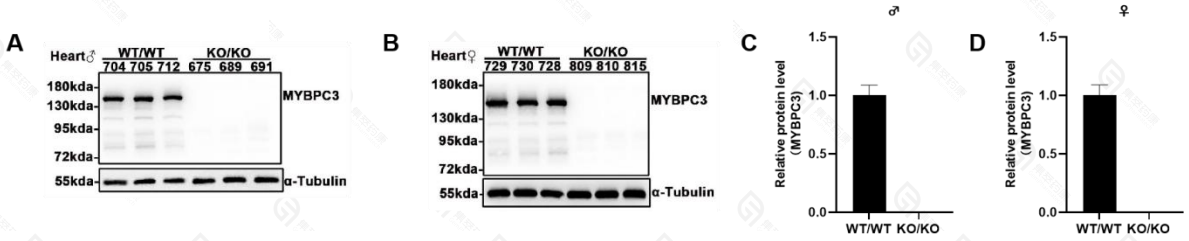


Fig 2. Expression analysis of MYBPC3 protein of WT and *Mybpc3*$^{-/-}$ mice

Compared with WT mice, western blotting analysis confirmed complete deletion of *Mybpc3* gene in *Mybpc3* $^{-/-}$ mice hearts. (A, B) Immunoblot results of MYBPC3 in WT and *Mybpc3* $^{-/-}$ mice hearts. (C, D) Quantitative gray scale analysis of protein level in A and B. Values are expressed as mean ± SEM. Comparison between groups involved unpaired two-tailed Student's t test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

2. Echocardiography analysis of *Mybpc3* knockout mice at 2 months of age

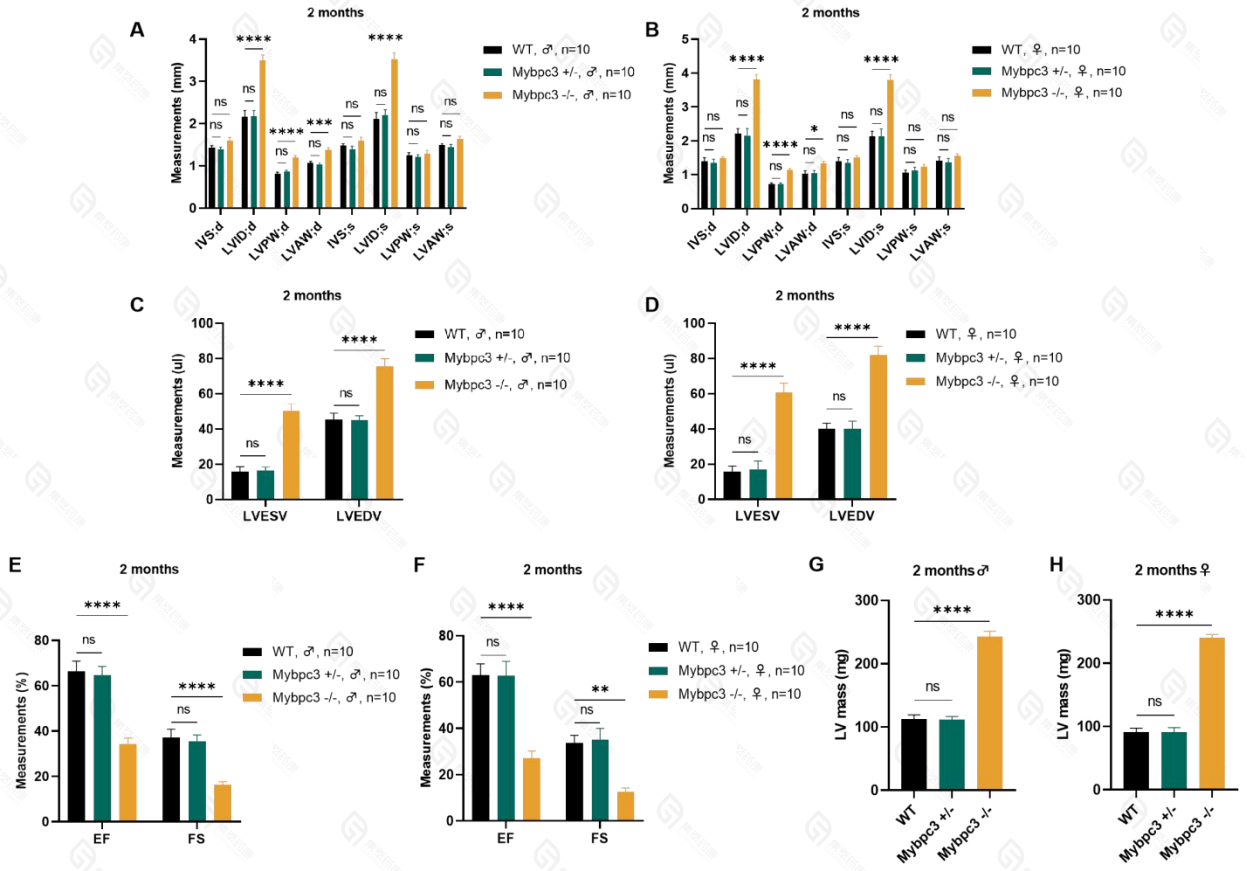
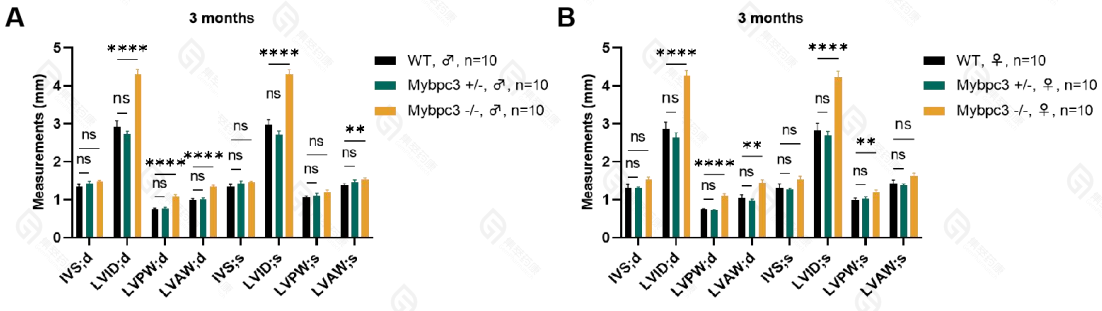


Fig 3. Echocardiography analysis of *Mybpc3* knockout mice at 2 months of age

A-H: Echocardiography data for WT, *Mybpc3* $^{+/-}$, *Mybpc3* $^{-/-}$ mice at the age of 2 months (n=10). Compared with WT mice, LVID, LVPW, LVAW, LVESV, LVEDV and LVM of *Mybpc3* $^{-/-}$ mice were significantly increased at 2 months of age, whereas EF and FS were markedly decreased, which indicated LV dilation, myocardial hypertrophy, impaired relaxation, depressed systolic contractility in *Mybpc3* $^{-/-}$ mice at 2 months of age. No significant differences were found between *Mybpc3* $^{+/-}$ and WT mice. Values are expressed as mean ± SEM. Comparison between groups involved two-way ANOVA test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

3. Echocardiography analysis of *Mybpc3* knockout mice at 3 months of age



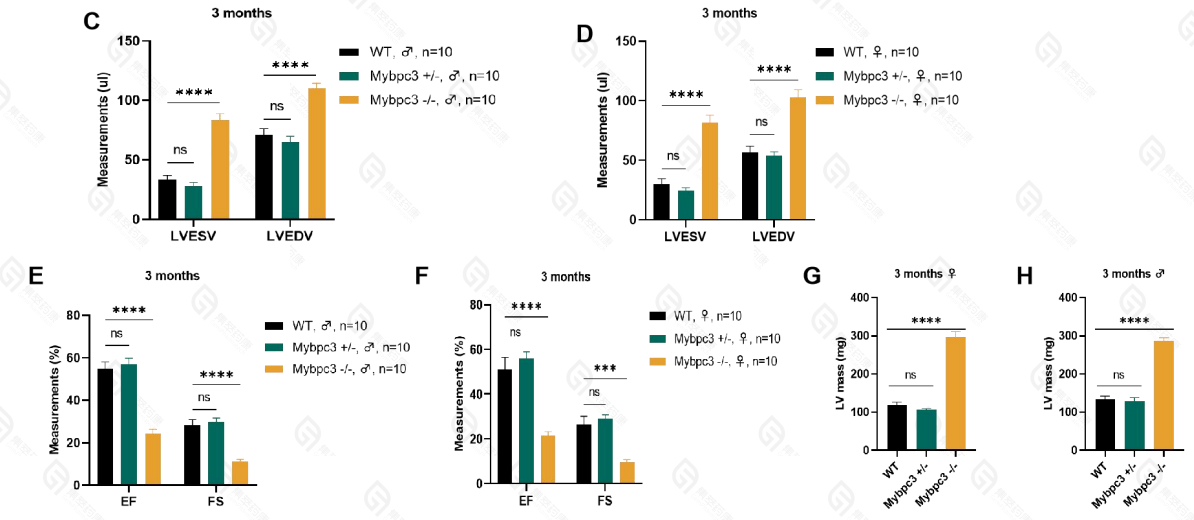


Fig 4. Echocardiography analysis of *Mybpc3* knockout mice at 3 months of age

A-H: Echocardiography data for WT, *Mybpc3* $^{+/-}$, *Mybpc3* $^{-/-}$ mice at the age of 3 months (n=10). Compared with WT mice, LVID, LVPW, LVAW, LVESV, LVEDV and LVM of *Mybpc3* $^{-/-}$ mice were significantly increased at 3 months of age, whereas EF and FS were markedly decreased, which indicated LV dilation, myocardial hypertrophy, impaired relaxation, depressed systolic contractility in *Mybpc3* $^{-/-}$ mice at 3 months of age. No significant differences were found between *Mybpc3* $^{+/-}$ and WT mice. Values are expressed as mean ± SEM. Comparison between groups involved two-way ANOVA test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

4. Analysis of Hypertrophy and fibrosis associated gene expression in WT and *Mybpc3* $^{-/-}$ mice

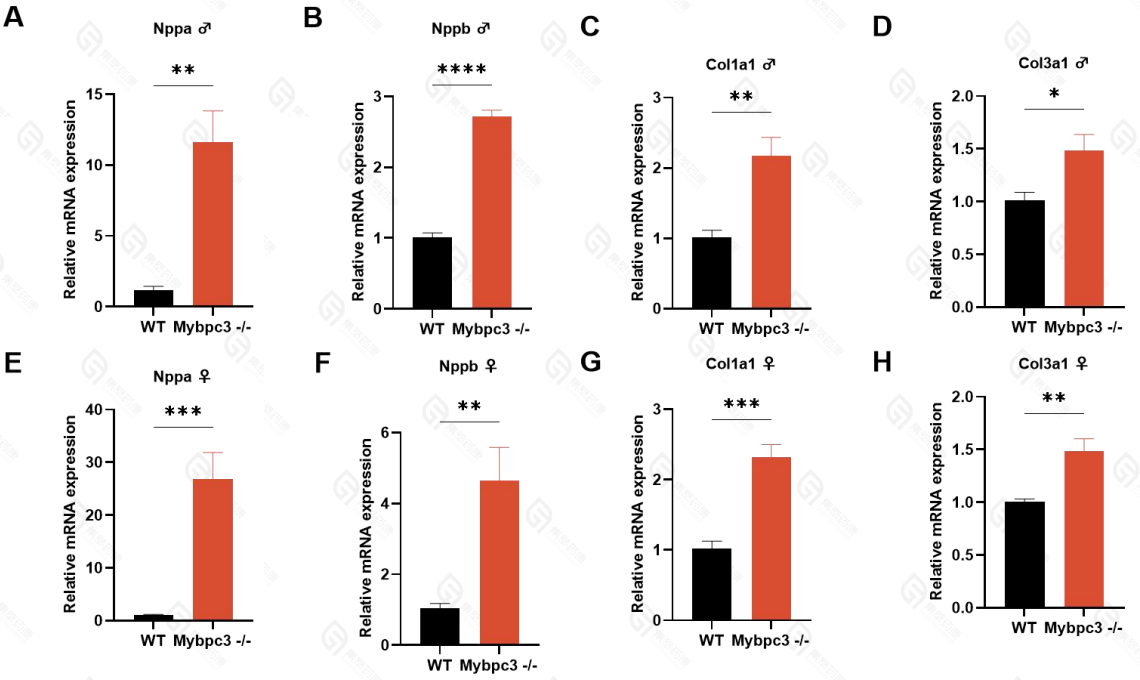


Fig 5. Hypertrophy and fibrosis associated gene expression analysis in the hearts of *Mybpc3* knockout mice

Compared with WT mice, mRNAs for markers of hypertrophy (Nppa, Nppb) and fibrosis (Col1a1, Col3a1) were significantly up-regulated in *Mybpc3* $^{-/-}$ mice. (A-B, E-F) Analysis of hypertrophy-associated gene expression in WT and *Mybpc3* $^{-/-}$ mice by quantitative RT-PCR (n=5). (C-D, G-H) Analysis of fibrosis-associated gene expression in WT and *Mybpc3* $^{-/-}$ mice by quantitative RT-PCR (n=5). All data represent as mean ± SEM. Comparison between groups involved unpaired two-tailed Student's t test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

5. Cardiac morphological and histological analysis of WT and *Mybpc3* $^{-/-}$ mice

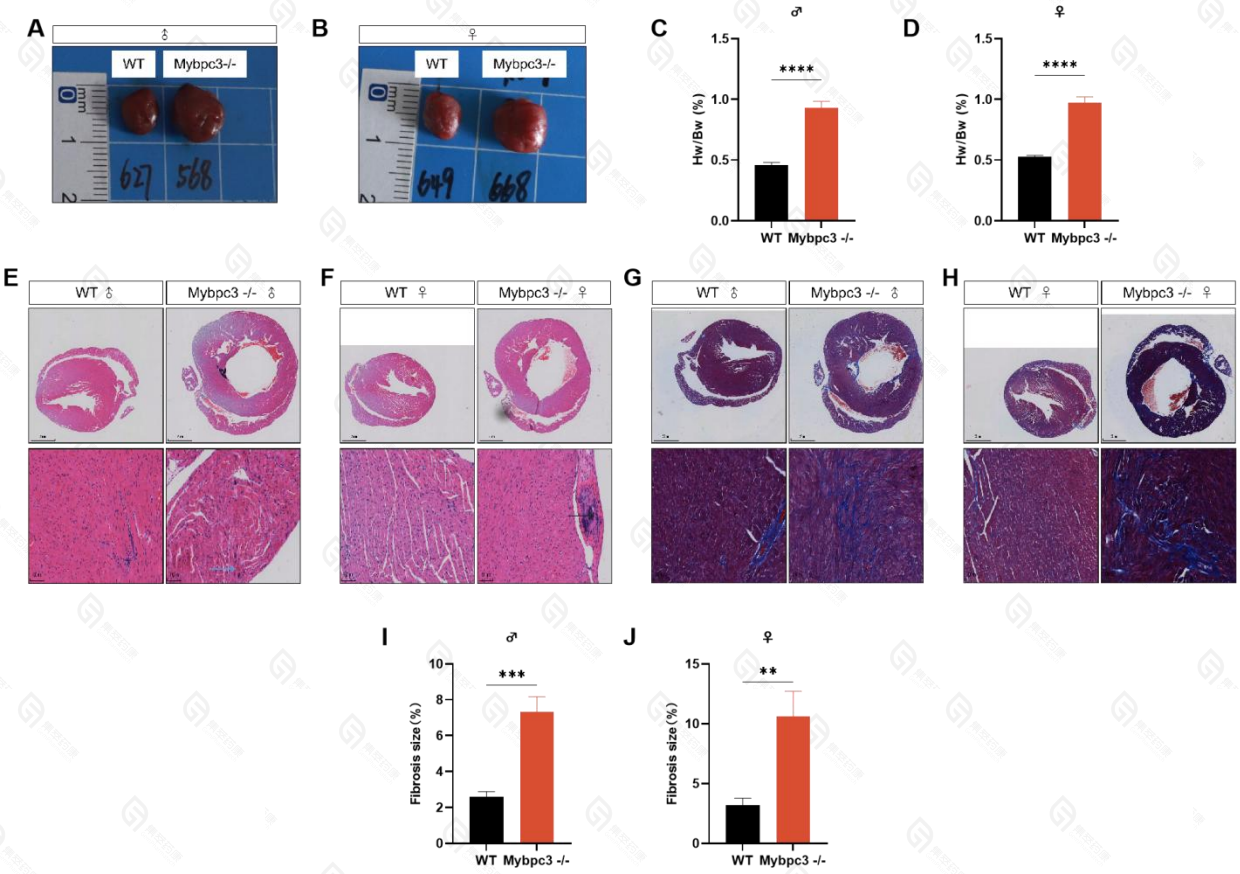


Fig 6. Cardiac morphological and histological analysis in WT and *Mybpc3* $^{-/-}$ mice

(A-B) Compared with WT mice, cardiac morphological in *Mybpc3* $^{-/-}$ mice at 3 months of age indicated significant cardiac enlargement. (C-D) Compared with WT mice, cardiac weight/body weight ratio significantly increased in *Mybpc3* $^{-/-}$ mice at 3 months of age (n=5). (E-F) Hematoxylin-eosin staining in WT and *Mybpc3* $^{-/-}$ mice at 3 months of age (n=5), the results showed cardiomyocyte eosinophilic enhancement (blue arrow) and necrosis (black arrow) in *Mybpc3* $^{-/-}$ mice when compared to WT mice. (G-H) Masson's trichrome staining in WT and *Mybpc3* $^{-/-}$ mice at 3 months of age (n=5), (I-J) quantification results of fibrosis size analyzed by ImageJ (n=5), the results showed significantly increased myocardial interstitial fibrosis in *Mybpc3* $^{-/-}$ mice. All data represent as mean ± SEM. Comparison between groups involved unpaired two-tailed Student's t test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

References

1. Ehlermann P, Weichenhan D, Zehelein J, Steen H, Pribe R, Zeller R, Lehrke S, Zugck C, Ivandic BT, Katus HA. Adverse events in families with hypertrophic or dilated cardiomyopathy and mutations in the MYBPC3 gene. BMC Med Genet. 2008 Oct 28; 9:95.
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3. Chen PP, Patel JR, Powers PA, Fitzsimons DP, Moss RL. Dissociation of structural and functional phenotypes in cardiac myosin-binding protein C conditional knockout mice. Circulation. 2012 Sep 4; 126(10):1194-205.
4. Kuster DW, Sadayappan S. MYBPC3's alternate ending: consequences and therapeutic implications of a highly prevalent 25 bp deletion mutation. Pflugers Arch. 2014 Feb; 466(2):207-13.
5. McConnell BK, Jones KA, Fatkin D, Arroyo LH, Lee RT, Aristizabal O, Turnbull DH, Georgakopoulos D, Kass D, Bond M, Niimura H, Schoen FJ, Conner D, Fischman DA, Seidman CE, Seidman JG. Dilated cardiomyopathy in homozygous myosin-binding protein-C mutant mice. J Clin Invest. 1999 Nov; 104(9):1235-44.