## Supplemental Information

**Supplemental Table S1：** List of putative off-target sites homologous to sgRNA.

PAM sequences are labeled in blue. Base substitutions are shown in red.

| | |
|---|---|
| **Mouse-mybpc3-sgRNA** | GGAGCCTGCAATGACCGCGT AGG |
| **OTS1** | GGAGCCTGGAATGACAGCTT TGG |
| **OTS2** | AGAGCCTGCACTGGCCGCGT TGG |
| **OTS3** | GGAGCCTGTAATGACCCAGT GGG |
| **OTS4** | GGAGCCTGCTATGTCCCCGT GGG |
| **OTS5** | TGAGCCTGCAATGATCACGT GGG |
| **Pig-*mybpc3*-sgRNA** | CTCCCGGGTCAGCTCCACCC CGT |
| **OTS1** | ATCCCAGCACAGCTCCACCC TGG |
| **OTS2** | CTCCCGAGCTAGCACCACCC GGG |
| **OTS3** | CCCACTGGCCAGCTCCACCC TGG |
| **OTS4** | CATTCGGGACAGCTCCACCC TGG |
| **OTS5** | TTCCCGCAGCAGCTCCACCC CGG |
| **OTS6** | CCCGCAGGCCAGCTCCACCC TGG |

**Supplemental Table S2**：List of primers for PCR amplification of off-target sites.

| Primers | Sequences (5' to 3') | Amplicon (bp) |
|---|---|---|
| **Mouse-MYBPC3- sgRNA-OTS1** | AACGTGCTCATGCCTCTCAG | 624 |
| | GCAGACAGAATGCACACTGG | |
| **Mouse-MYBPC3- sgRNA-OTS2** | AATCCCAGAACGAAGGCCTG | 783 |
| | GCAAAGAGGACACTGAGGCT | |
| **Mouse-MYBPC3 - sgRNA-OTS3** | CTCAACCTGTGGGTCGAGAC | 686 |
| | CAGCAGGACCCATGGAAAGT | |
| **Mouse-MYBPC3 - sgRNA-OTS4** | GTTGGCTCCCTTGCTTCTCT | 754 |
| | CATGGAGAGATCCGAGCCAC | |
| **Mouse-MYBPC3 - sgRNA-OTS5** | CCCTGTTCACGGCATCTCAT | 713 |
| | CCGCCGCCATCAGTATAACT | |
| **Pig-MYBPC3- sgRNA-OTS1** | GTCCCAGGGAAGAAACCAGG | 863 |
| | TTGTCCAGTGTGGCTTGGTT | |
| **Pig-MYBPC3- sgRNA-OTS2** | GGTTACAGACACGGCTTGGA | 936 |
| | GGAGGCACCAAGGAAGTTGA | |
| **Pig-MYBPC3- sgRNA-OTS3** | CAGAGATCCTCCATCACCGC | 811 |
| | AGCTCTGAGTCACCTCCCTT | |
| **Pig-MYBPC3- sgRNA-OTS4** | GCCCTCTCTCATAGCCACAC | 698 |
| | GGTCGTGGAAACAGGCTACA | |
| **Pig-MYBPC3- sgRNA-OTS5** | AGTGAGGATGCAGCGTTTGA | 923 |
| | AACCTATCCCTGCCCTTTGC | |

| | | |
|---|---|---|
| **Pig-MYBPC3- sgRNA-OTS6** | CTGGCCTCTCTGAGCATCTG | 840 |
| | TCCACACAGCTCTGGAAACC | |

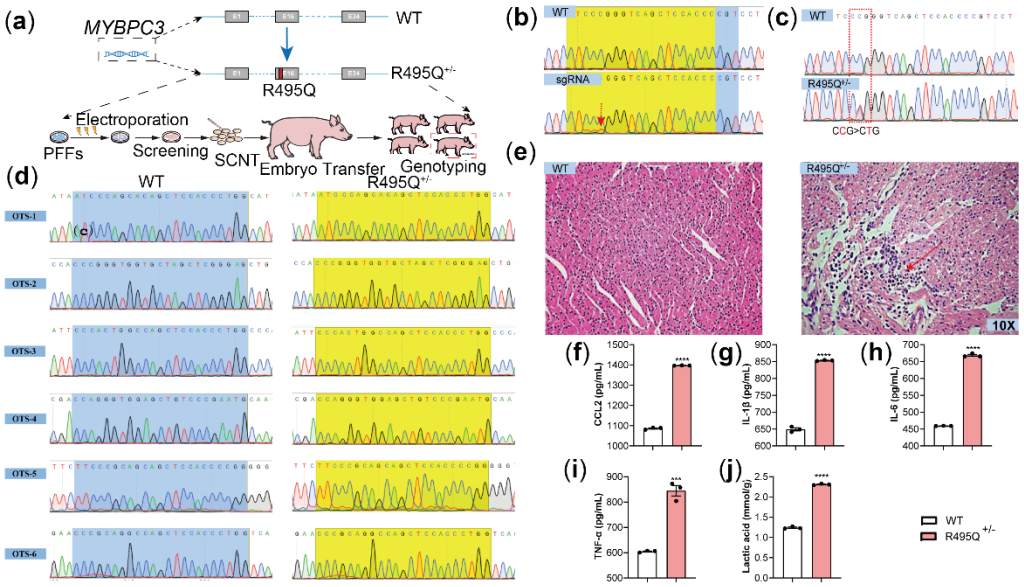
**Supplemental Table S3：** List of the primers used in RT-PCR.

| Primers | Sequences (5' to 3') | Amplicon (bp) |
|---|---|---|
| **Mouse-MYBPC3-RT** | GAACACTACCGACGCACTCA | 176 |
| | AGGGCCTTGTATTTGGGTGG | |
| **Mouse-HIF-1α-RT** | CCAGCCTAACAGTCCCAGTG | 240 |
| | GGAGGGCTTGGAGAATTGCT | |
| **Mouse-TGF-β1-RT** | ACTGGAGTTGTACGGCAGTG | 123 |
| | GGGGCTGATCCCGTTGATTT | |
| **Mouse-GCK-RT** | AGGTAGAGCAGATCCTGGCA | 143 |
| | CACGTAGGTGGATGAGAGCTC | |
| **Mouse-PFKM-RT** | TTGGAGCTGATGGAAGGCAG | 297 |
| | ATCGCGAATGGTGAAGGGTT | |
| **Mouse-LDHA-RT** | GGAGCAGTGGAAGGAGGTTC | 293 |
| | TGTCTGCGCTCTTCTTCAGG | |
| **Mouse-COL1A1-RT** | CCCAGTGGCGGTTATGACTT | 117 |
| | CTCAAGGTCACGGTCACGAA | |
| **Mouse-α-SMA-RT** | CCCAATGGTGAGACGTGGAA | 198 |
| | CTTGGGTCCCTCGACTCCTA | |
| **Mouse-ACTB-RT** | TCGAGACCTTCAACACCCCA | 101 |
| | GTCTCCGGAGTCCATCACG | |
| **Pig-MYBPC3-RT** | CTGTGGTGCCAATCGCTCTT | 177 |
| | GGACGTCCAGACGTAGCTTG | |

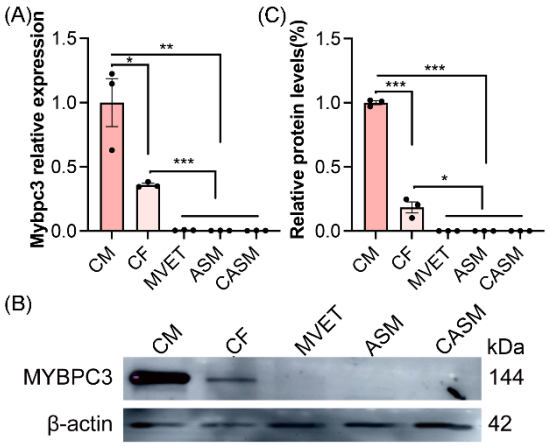
| | | |
|---|---|---|
| **Pig-HIF-1α-RT** | TACTCATCCGTGCGACCATG | 257 |
| | CCAAGCACGTCATAGGTGGT | |
| **Pig-TGF-β1-RT** | GGCCAGATTCTGTCCAAGCT | 209 |
| | ATTTGGTTGCCGCTTTCCAC | |
| **Pig-GCK-RT** | CCGACTTCCTGGACAAGCAT | 181 |
| | CGTTTGATGGCATCTCGCAG | |
| **Pig-PFKM-RT** | AGGGCCTTGTGATCATTGGG | 202 |
| | TGCTTAATGCGGTCACAGGT | |
| **Pig-LDHA-RT** | GTCATGGGTGGATCCTTGGG | 141 |
| | TGTGAACCGCTTTCCAGTGT | |
| **Pig-COL1A1-RT** | CCTGGACGCCATCAAAGTCT | 125 |
| | AGACGTGCCTCTTGTCCTTG | |
| **Pig-α-SMA-RT** | AAGATCAGGACAGGCTTGCC | 226 |
| | GCATTAGCTGGGGTGACTGT | |
| **Pig-GAPDH-RT** | ATCCTGGGCTACACTGAGGA | 130 |
| | TGTCGTACCAGGAAATGAGCT | |

**Supplemental Table S4：** List of the primary and secondary antibodies used in this study.

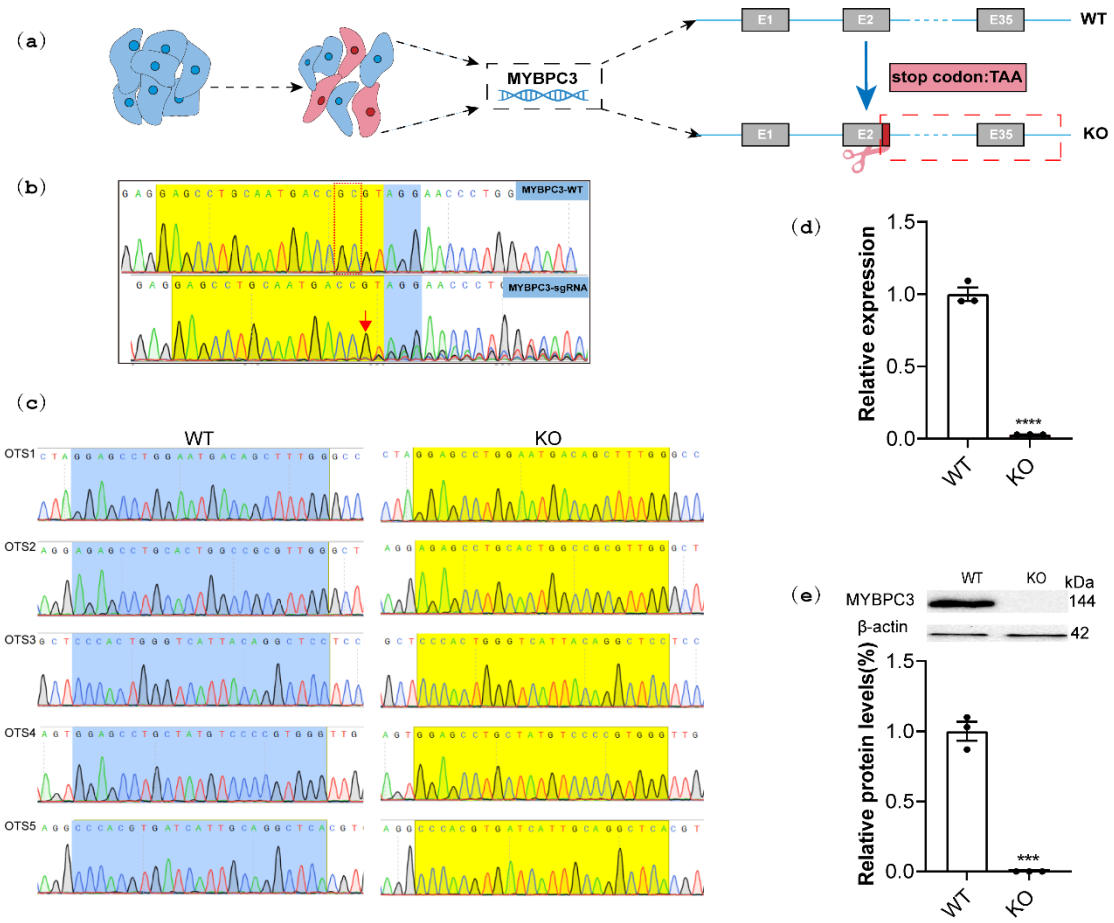
| Antibodies | Host | Dilutions | Supplier |
|---|---|---|---|
| Anti-MYBPC3 Antibody (E-7) | Mouse | 1:1000 | Santa Cruz Biotechnology |
| MYL (F-5) | Mouse | 1:1000 | Santa Cruz Biotechnology |
| Anti-VIM Antibody | Mouse | 1:1000 | BOSTER |
| Rabbit Anti-Troponin T/TNNT2 | Rabbit | 1:1000 | BOSTER |
| Anti-TGF beta 1 Antibody | Rabbit | 1:1000 | BOSTER |
| Anti-COL1A1 Antibody | Rabbit | 1:1000 | BOSTER |
| a-SMA Antibody (monoclonal,1A4) | Mouse | 1:1000 | BOSTER |
| Anti-GCK Antibody | Rabbit | 1:1000 | BOSTER |
| PFKM Polyclonal antibody | Rabbit | 1:1000 | Proteintech |
| Anti-LDHA Antibody | Rabbit | 1:1000 | BOSTER |
| Anti-HIF1A Antibody | Rabbit | 1:1000 | BOSTER |
| Phospho-NF-κB p65 (Ser536) (93H1) | Rabbit | 1:1000 | Cell Signaling Technology |
| NF-κB p65 (D14E12) | Rabbit | 1:1000 | Cell Signaling Technology |
| β-Actin Mouse Monoclonal Antibody | Mouse | 1:1000 | Beyotime |
| HRP-labeled Goat Anti-Mouse IgG(H+L) | Goat | 1:1000 | Beyotime |
| HRP-labeled Goat Anti-Rabbit IgG(H+L) | Goat | 1:1000 | Beyotime |
| Alexa Fluor 488 Goat anti-Mouse -IgG(L+H) | Goat | 1:500 | Proteintech |




**Supplemental Figure S1. Sequence analysis of pig *MYBPC3* gene and design of CBEs-targeting strategy.** (a) The flowchart to generate MYBPC3-R495Q mutant pigs. (b) Sanger sequencing traces of PCR amplicons from WT and electrotransfected PFFs. The target base is labeled with a red arrow. (c) Gene targeting analysis by T-cloning and Sanger sequencing in all piglets. (d) Analysis of off-target activities at the top predicted off-target sites. (e) The H&E-stained images of WT and R495Q$^{+/-}$ pig heart sections. (f-i) Serum levels of CCL2, IL-1β, IL-6 and TNF-α in WT and R495Q$^{+/-}$ pigs (n=3). ***P<0.001, ****P<0.0001. (j) Lactic acid measurements in heart tissues of WT and R495Q$^{+/-}$ pigs (n=3). ****P<0.0001.

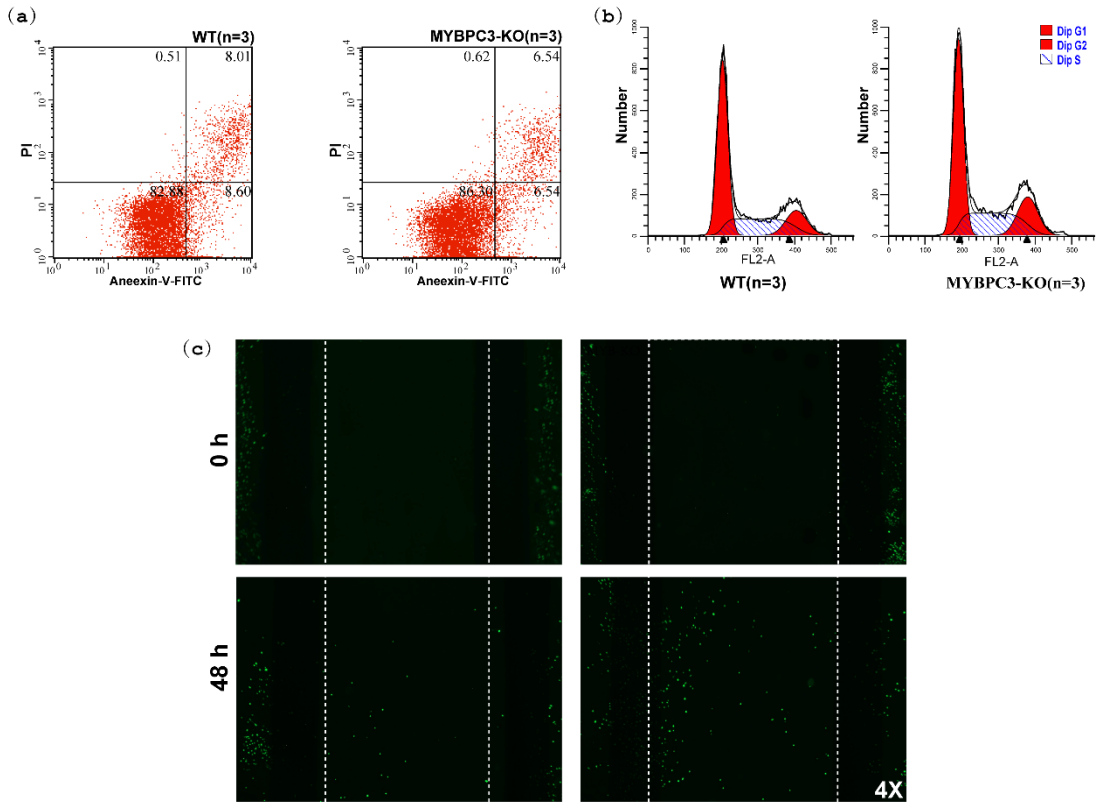



**Supplemental Figure S2. Expression of MYBPC3 in major cell types in mouse heart.** (A) The relative expression of *Mybpc3* transcript in mouse cardiac myocytes (CM), fibroblasts (CF), microvascular endothelial cells (MVET), aortic smooth muscle cells (ASM) and coronary artery smooth muscle cells (CASM). *P<0.05, **P<0.01, ***P<0.001, n=3. (B, C) Western blot and densitometry analysis of MYBPC3 expression in mouse heart cells.

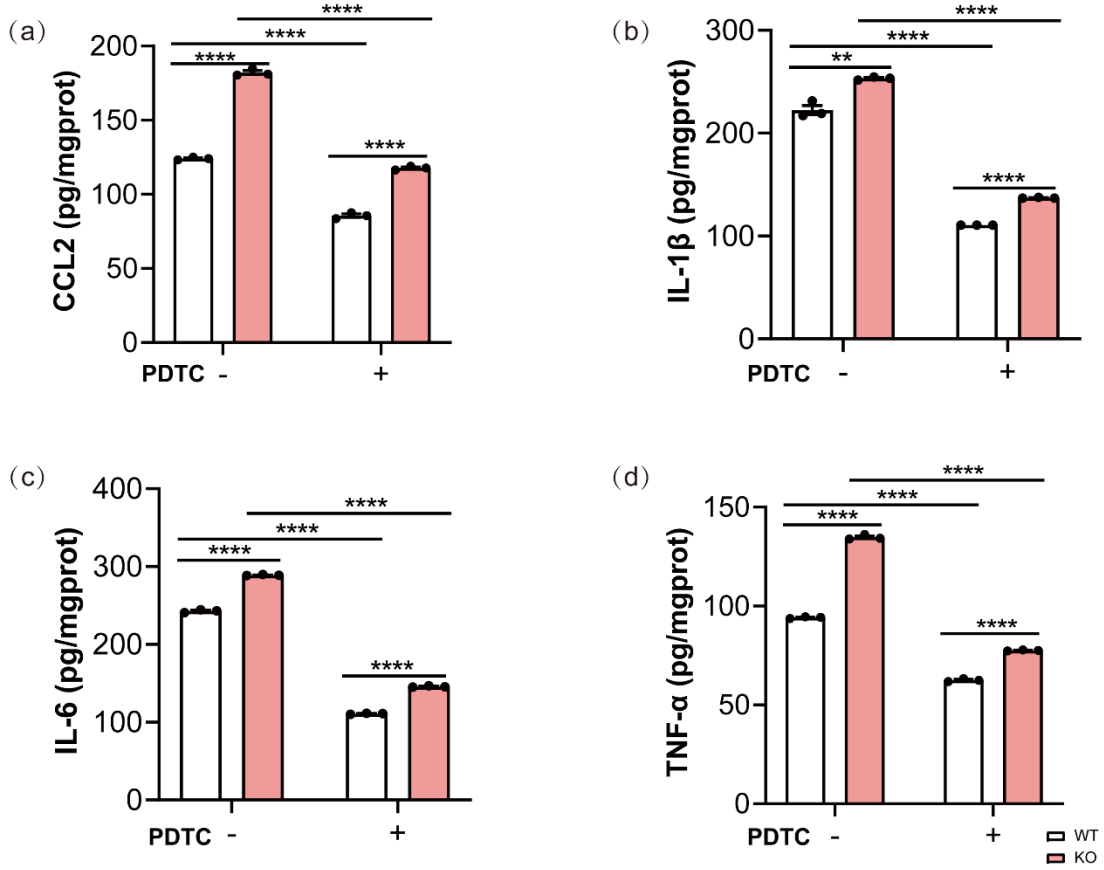



**Supplemental Figure S3. The design of CRISPR-targeting strategy in fibroblasts.**

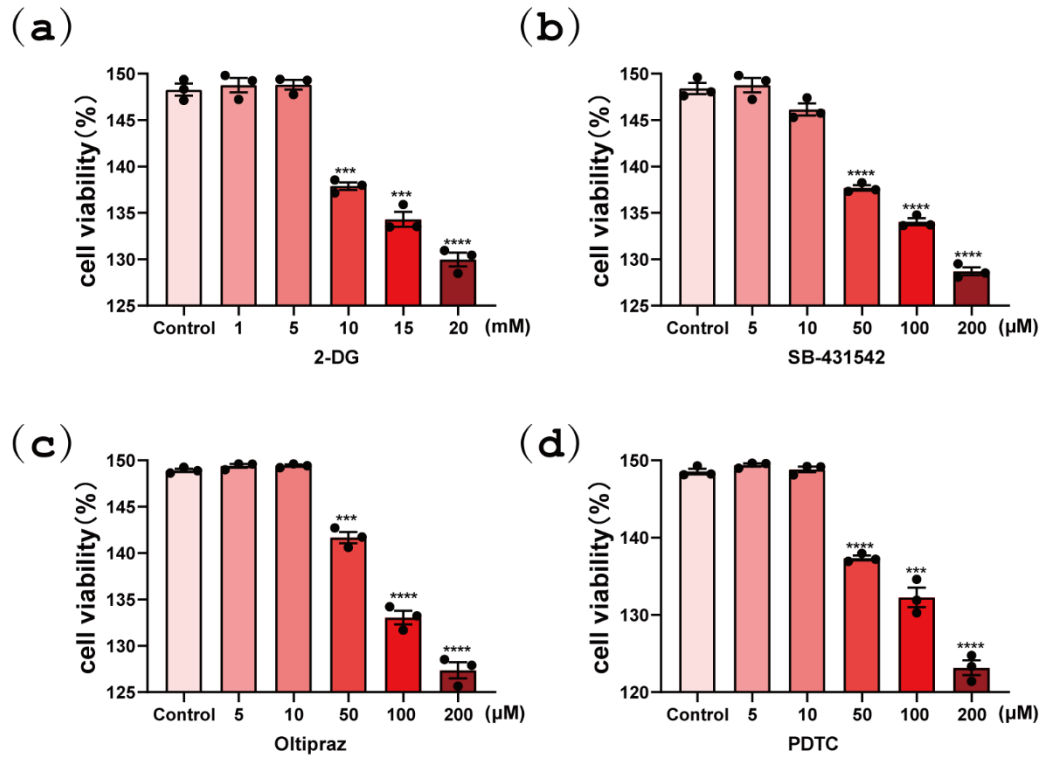
(a) Schematic showing the targeting strategy to generate MYBPC3-KO NIH3T3 fibroblasts. (b) Sanger sequencing traces showing the CRISPR/Cas9-engineered indels in mouse *Mybpc3* gene. PAM is highlighted in blue. (c) The off-target activity analysis. (d, e) Quantitative RT-PCR and Western blot analysis of *Mybpc3* expression in WT and MYBPC3-KO NIH3T3 cells (n=3). ***P<0.001, ****P<0.0001.



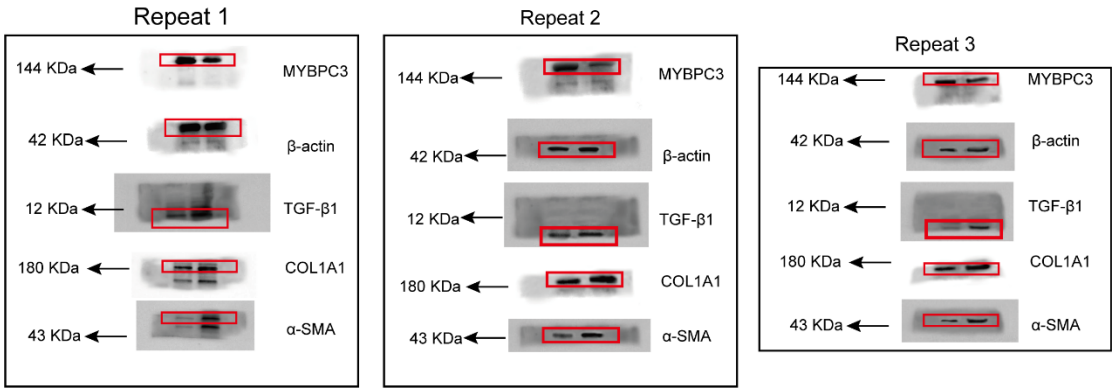

**Supplemental Figure S4. The impact of MYBPC3 disruption on apoptosis, cell cycle and migration of NIH3T3 fibroblasts.** (a-b) Analysis of apoptosis (a) and cell cycle (b) in WT and MYBPC3-KO NIH3T3 fibroblasts by fluorescence-activated cell sorting (FACS). (c) Cell migration analysis in WT and MYBPC3-KO fibroblasts by the wound scratch assay.



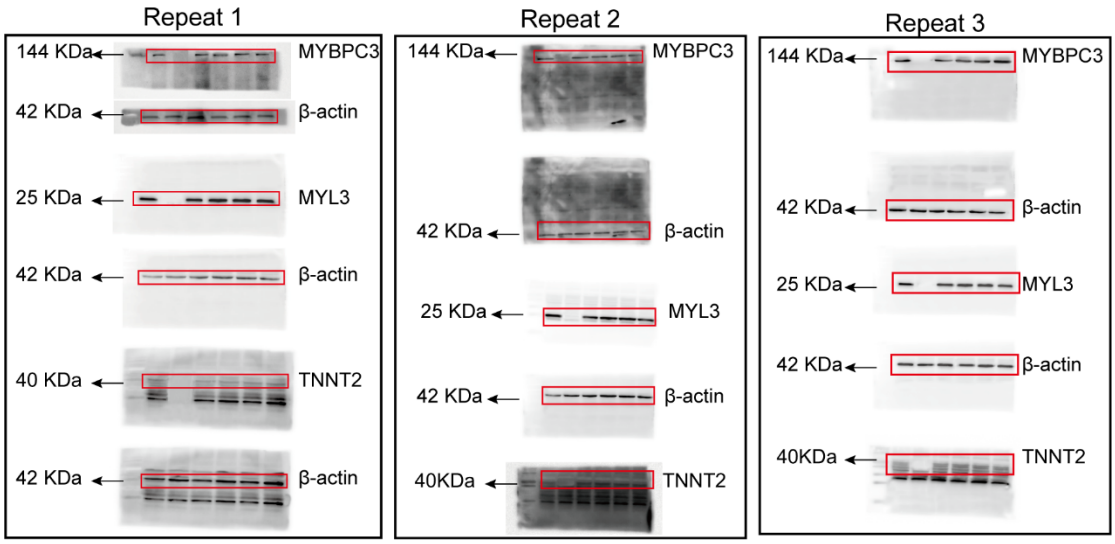

**Supplemental Figure S5. Activated NF-κB signaling pathway in MYBPC3-KO fibroblasts.** Measurements of CCL2, IL-1β, IL-6 and TNF-α in cell lysates of WT and MYBPC3-KO NIH3T3 fibroblasts with or without PDTC treatment (n=3). **P<0.01, ****P<0.0001.



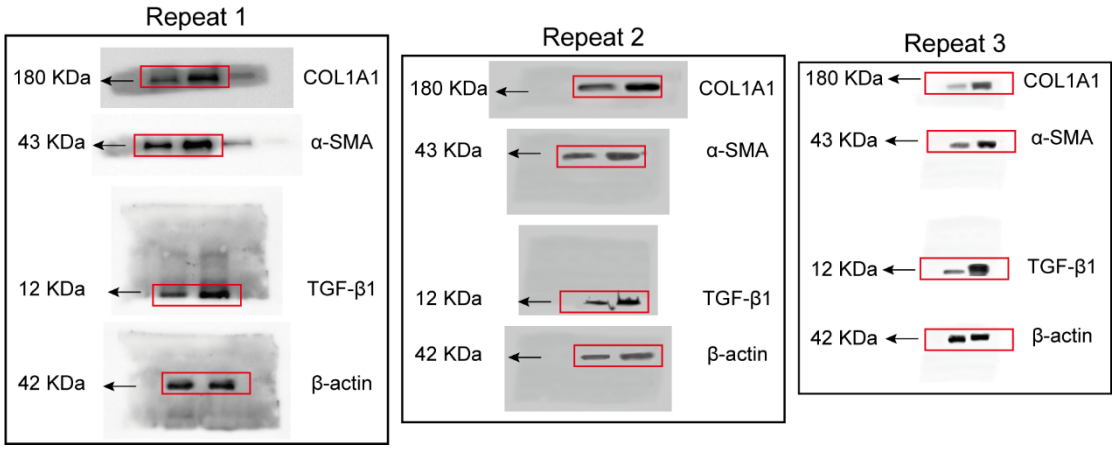

**Supplemental Figure S6. Concentration-dependent cell toxicity of 2-DG, SB-431542, Oltipraz and PDTC.** The cell toxicity of 2-DG, SB-431542, Oltipraz and PDTC was analyzed by CCK8 assays (n=3). ***P<0.001, ****P<0.0001.



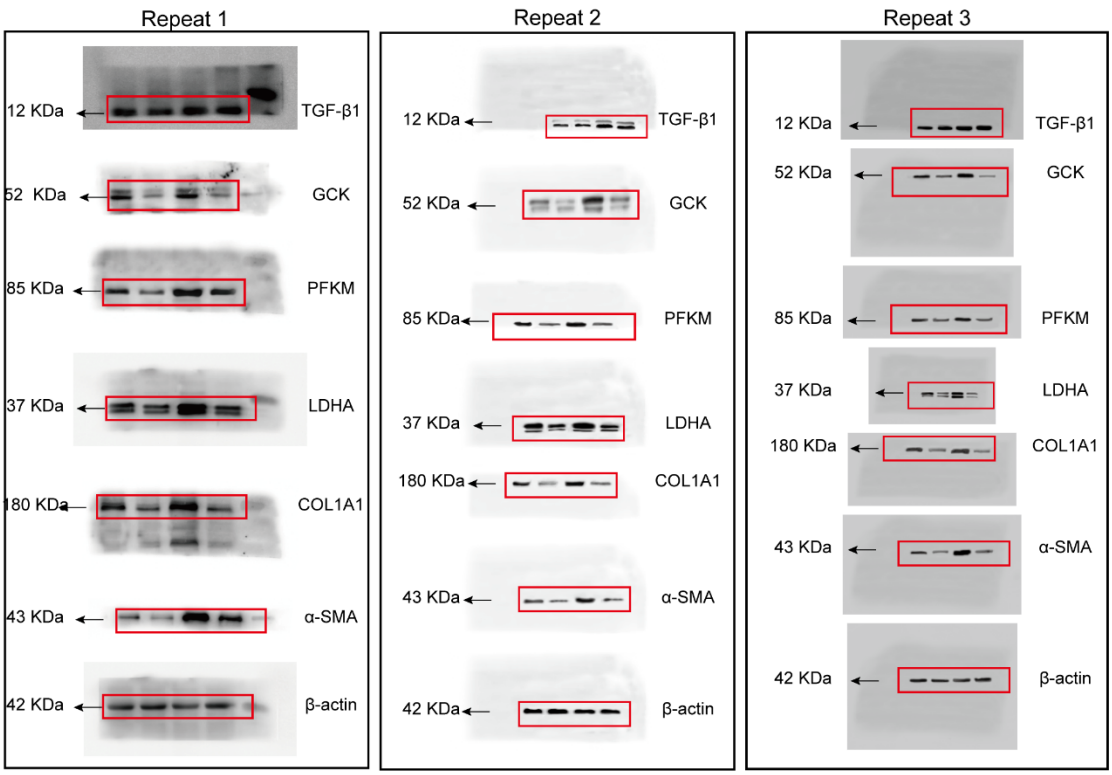

**Supplemental Figure S7 related to Figure 1B.** Uncropped immunoblots of MYBPC3, TGF-β1, COL1A1 and α-SMA with β-actin obtained from heart tissue lysate. All experiments were performed three times.



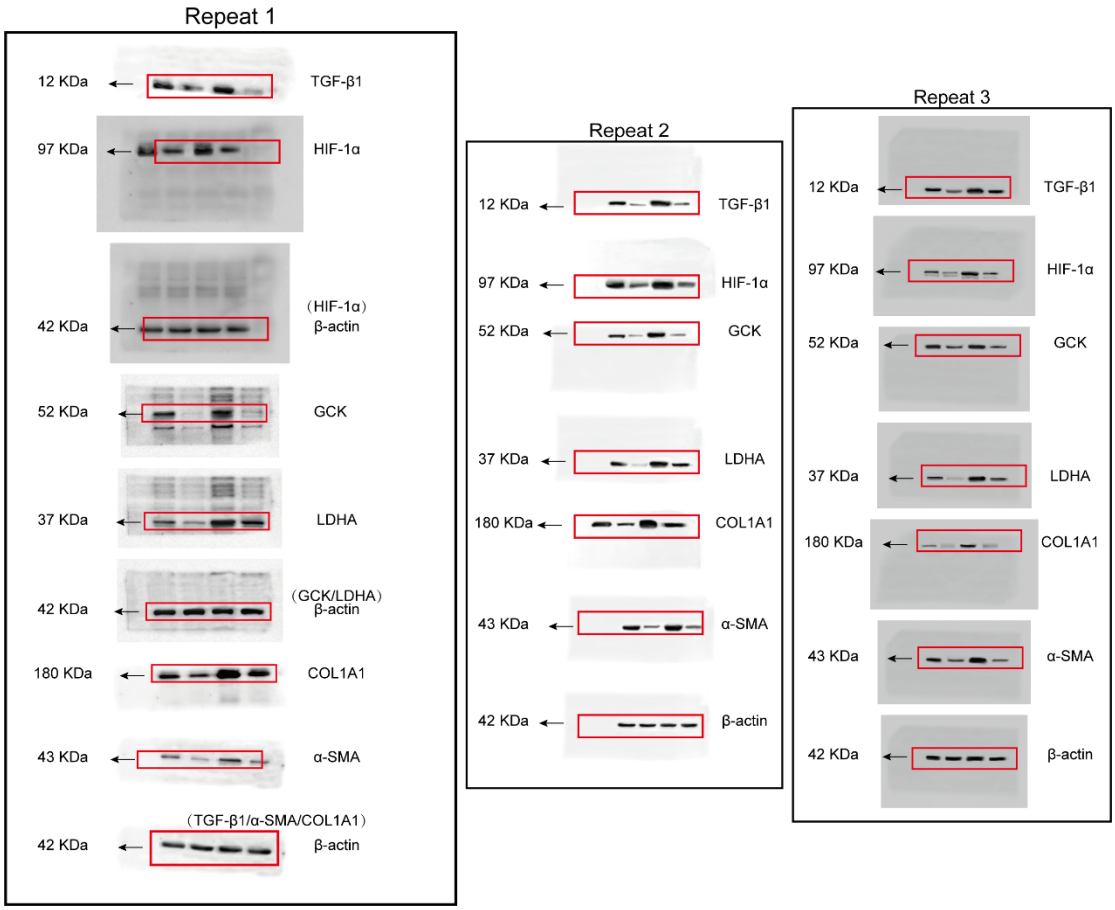

**Supplemental Figure S8 related to Figure 2B.** Uncropped immunoblots of MYBPC3, MYL3, TNNT2 with β-actin obtained from fibroblasts cell lysate. All experiments were performed three times.



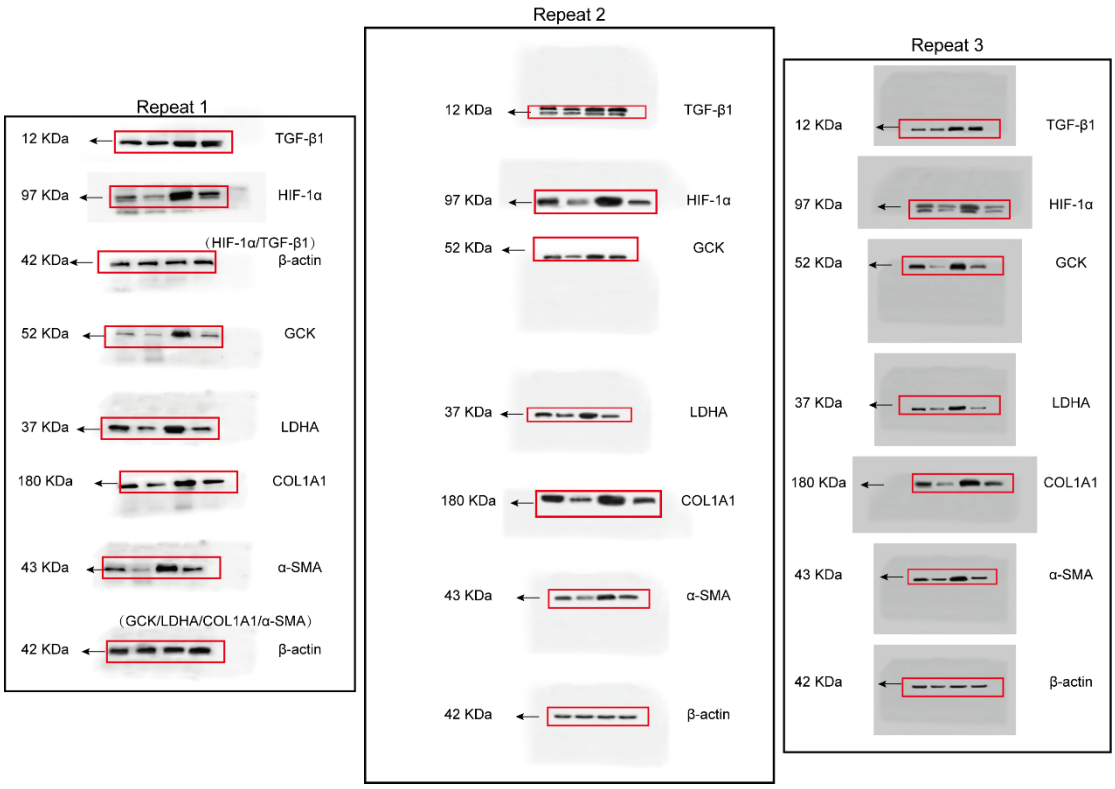

**Supplemental Figure S9 related to Figure 3B.** Uncropped immunoblots of COL1A1, α-SMA and TGF-β1 with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.



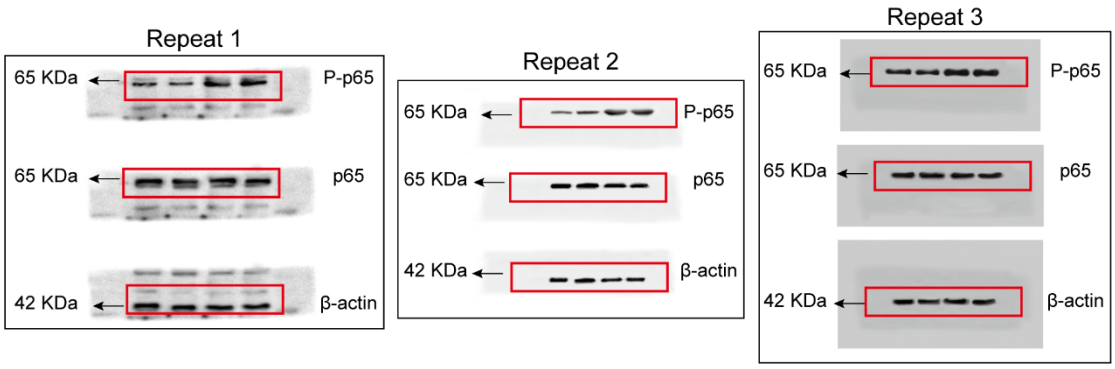

**Supplemental Figure S10 related to Figure 4G.** Uncropped immunoblots of TGF-β1, GCK, PFKM, LDHA, COL1A1, α-SMA and with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.



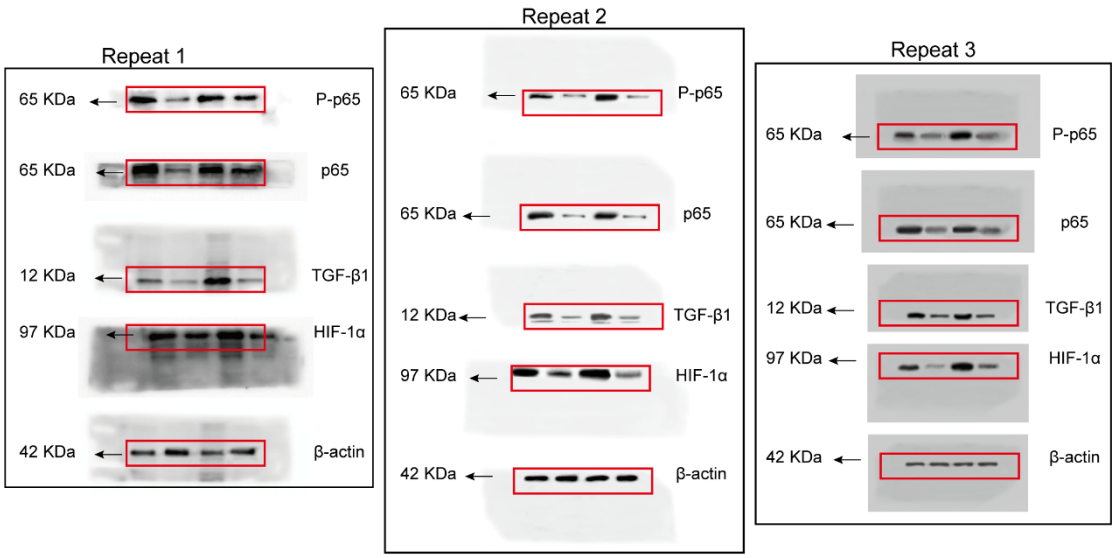

**Supplemental Figure S11 related to Figure 5A.** Uncropped immunoblots of TGF-β1, HIF-1α, GCK, LDHA, COL1A1, α-SMA and with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.



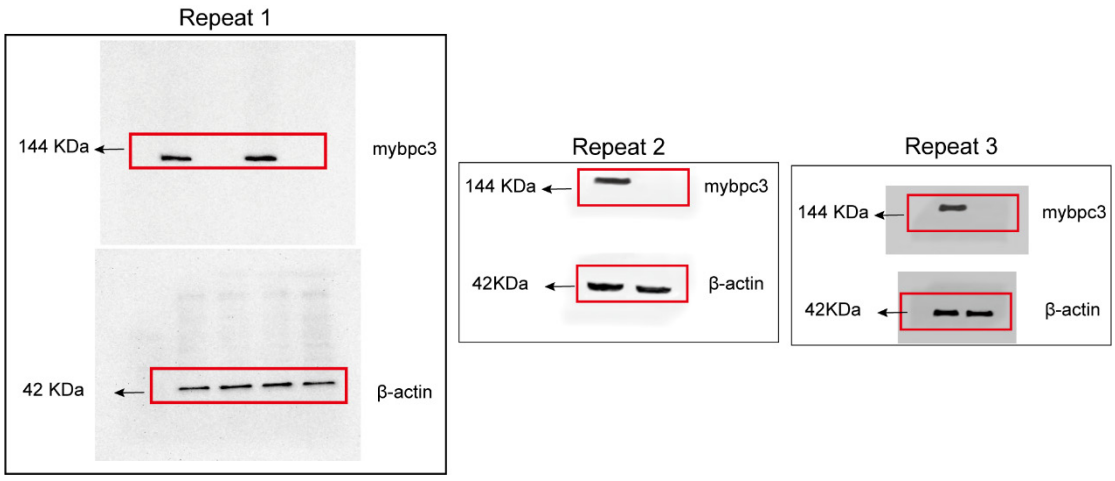

**Supplemental Figure S12 related to Figure 5B.** Uncropped immunoblots of TGF-β1, HIF-1α, GCK, LDHA, COL1A1, α-SMA and with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.




**Supplemental Figure S13 related to Figure 6A.** Uncropped immunoblots of P-p65 and p65 with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.




**Supplemental Figure S14 related to Figure 6G.** Uncropped immunoblots of P-p65, p65, TGF-β1 and HIF-1α with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.




**Supplemental Figure S15 related to Supplemental Figure S3e.** Uncropped immunoblots of MYBPC3 with β-actin obtained from NIH-3T3 cell lysate. All experiments were performed three times.