**Immunofluorescence staining**

OCT-embedded heart tissue was cut into 5-μm sections for staining studies. Tissue sections were fixed in 4 % polyformaldehyde for 10-15 minutes at room temperature and washed three times with PBS for 5 min each. After blocking with 5% BSA for 1 hour at room temperature, washing with PBS was repeated three times for 5 minutes each time. The sections were incubated with the primary antibody at 4 ° C overnight. After the unbound primary antibody was washed with PBS, the sections were incubated in the secondary antibody (1: 1,000, Alexa Fluor 488/594, Thermo Fisher Scientific, Inc.) at 37 ° C for 1 hour and then were washed with PBS for three times. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, 1:5000 Sigma) at room temperature for 5 min. Finally tissue sections were mounted with coverslips. The confocal imaging systems (Zeiss, Germany) were used to capture images for analysis. Goat serum was used instead of primary antibody to stain section as control.

**Table S1. Primers used in real-time PCR analysis.**

| **Gene name** | **Forward primer, 5'-3'** | **Reverse primer, 3'-5'** |
|---|---|---|
| Wnt5a (mouse) | CTGCGGAGACAACATCGACTA | CGTGGATTCGTTCCCTTTCTCTA |
| Wnt11 (mouse) | GCACTGAATCAGACGCAACAC | CGACAGGGCATACACGAAGG |
| GAPDH (mouse) | ACCACAGTCCATGCCATCAC | TCCACCACCCTGTTGCTGTA |
| MMP2 (mouse) | CAGGGAATGAGTACTGGGTCTATT | ACTCCAGTTAAAGGCAGCATCTAC |
| MMP9 (mouse) | AATCTCTTCTAGAGACTGGGAAGGAG | AGCTGATTGACTAAAGTAGCTGGA |
| Col1α1 (mouse) | CCTCAAGGGCTCCAACGAG | TCAATCACTGTCTTGCCCCA |
| Col3α1(mouse) | ACGTAGATGAATTGGGATGCAG | GGGTTGGGGCAGTCTAGTC |
| GAPDH (rat) | AACAAGCAACTGTCCCTGAGC | GTAGACAGAAGGTGGCACAGA |
| CTSD (rat) | CATCGCAGCCAAGTTTGATG | CCGGGAGCACATTGTTAACA |

**Table S2. All the primary antibodies used in the Western blot analysis**.

| Antibody | Company | Catalog# | Size(kDa) | 2° |
|---|---|---|---|---|
| LRP6(C47E12)Rabbit mAb (Mouse tissue) | Cell Signaling Technology | 3395 | 180 | Rabbit |
| LRP6 (C5C7) Rabbit mAb (Rat tissue) | Cell Signaling Technology | 2560 | 180 | Rabbit |
| β-Catenin(6B3)Rabbit mAb | Cell Signaling Technology | 9582 | 102 | Rabbit |
| p-Smad2/3(C47E12)Rabbit mAb | Cell Signaling Technology | 8828 | 52，60 | Rabbit |
| Active β-Catenin(C47E12)Rabbit mAb | Cell Signaling Technology | 8814 | 102 | Rabbit |
| Rabbit(DA1E) mAb IgG | Cell Signaling Technology, | 3900 | 50 | Rabbit |
| Collagen I Rabbit pAb | Millipore | 3072327 | 130 | Rabbit |
| Wnt5a Rabbit pAb | GeneTex，Inc. | GTX111187 | 42 | Rabbit |
| Wnt11 Rabbit pAb | GeneTex，Inc. | GTX105971 | 43 | Rabbit |
| Collagen III Rabbit pAb | Proteintech | 22734-1-AP | 139 | Rabbit |
| MMP2 Rabbit pAb | Proteintech | 10373-2-AP | 72 | Rabbit |
| MMP9 Rabbit pAb | Proteintech | 10375-2-AP | 92 | Rabbit |
| TGF-β1 Rabbit pAb | Abcam | ab92486 | 44 | Rabbit |
| Alpha-SMA Rabbit mA | Abcam | ab124964 | 42 | Rabbit |
| Anti-flag tag | Abcam | Ab205606 | - | Rabbit |
| Anti-His tag | Abcam | Ab213204 | - | Rabbit |
| Cathepsin D Antibody | Proteintech | 21327-1-AP | 46 | Rabbit |
| Anti-Myc tag | Abcam | Ab32 | - | Mouse |
| p-LRP6 Rabbit pAb | Cell Signaling Technology | 2568 | 180 | Rabbit |

**Table S3. Basic echocardiographic index of MCM, LRP6$^{CAG}$/MCM and LRP6$^{CAG}$ male mice after tamoxifen treatment.**

| | MCM | LRP6$^{CAG}$/MCM | LRP6$^{CAG}$ |
|---|---|---|---|
| Number | 13 | 13 | 6 |
| HR (bpm) | 425.4±11.11 | 437.8±11.33 | 407.3±14.53 |
| LVAW;d (mm) | 0.77±0.02 | 0.80±0.03 | 0.76±0.02 |
| LVPW;d (mm) | 0.75±0.02 | 0.79±0.03 | 0.73±0.02 |
| LVID;d (mm) | 4.02±0.06 | 3.95±0.08 | 4.07±0.04 |
| LVAW;s (mm) | 1.17±0.03 | 1.18±0.06 | 1.12±0.06 |
| LVPW;s (mm) | 1.15±0.04 | 1.13±0.05 | 1.12±0.03 |
| LVID;s (mm) | 2.62±0.09 | 2.52±0.09 | 2.58±0.07 |
| EF (%) | 62.90±2.29 | 66.36±2.42 | 65.91±2.68 |
| FS (%) | 33.96±1.64 | 36.61±1.91 | 36.15±2.07 |

7-8 weeks male mice were injected tamoxifen for three days, after 1 week, parameters were analyzed. The data were expressed by mean±SEM, HR: Heart rate; LVAW;d; left ventricular diastolic anterior wall thickness; LVPW;d: left ventricular diastolic posterior wall thickness; LVAW;s: left ventricular systolic anterior wall thickness; LVPW;s: left ventricular diastolic posterior wall thickness; LVID;d: left ventricular diastolic inner dimension; LVID;s: left ventricular systolic inner dimension. EF: ejection fraction; FS: fraction shortening.

**Table S4. HW/BW in MCM, LRP6$^{CAG}$/MCM and LRP6$^{CAG}$ mice after tamoxifen treatment.**

| | MCM | LRP6$^{CAG}$/MCM | LRP6$^{CAG}$ |
|---|---|---|---|
| Number | 5 | 5 | 5 |
| BW(g) | 28.83±0.66 | 26.96±0.86 | 28.80±1.17 |
| HW(mg) | 137.3±4.78 | 131.3±7.00 | 137.3±6.00 |
| HW/BW(mg/g) | 4.76±0.14 | 4.86±0.14 | 4.77±0.11 |

7-8 weeks male mice were injected tamoxifen for three days, after 1 week, parameters were analyzed. The data were expressed by mean±SEM, BW: body weight; HW: heart

weight; HW/BW: the ratio of heart weight to body weight.

**Table S5. Nano-HPLC-MS/MS analysis of the proteins interacted with LRP6 in stretched-cardiomyocytes (The unique peptide number ≥3).**

| | Accession | Protein | Description | #Unique peptide |
|---|---|---|---|---|
| 1 | P00507 | AATM | Aspartate aminotransferase | 12 |
| 2 | Q8VHF5 | CISY | Citrate synthase | 7 |
| 3 | P56574 | IDHP | Isocitrate dehydrogenase [NADP] | 7 |
| 4 | P32551 | QCR2 | Cytochrome b-c1 complex subunit 2 | 7 |
| 5 | D4AE41 | RMXL1 | RNA binding motif protein, X-linked-like-1 | 6 |
| 6 | P10760 | SAHH | Adenosylhomocysteinase | 5 |
| 7 | P15650 | ACADL | Long-chain specific acyl-CoA dehydrogenase | 5 |
| 8 | P17764 | THIL | Acetyl-CoA acetyltransferase | 5 |
| 9 | P15999 | ATPA | ATP synthase subunit alpha | 5 |
| 10 | P08503 | ACADM | Medium-chain specific acyl-CoA dehydrogenase | 5 |
| 11 | P13437 | THIM | 3-ketoacyl-CoA thiolase | 4 |
| 12 | Q9EPH2 | MRP | MARCKS-related protein | 4 |
| 13 | P51635 | AK1A1 | Alcohol dehydrogenase [NADP(+)] | 4 |
| 14 | P12007 | IVD | Isovaleryl-CoA dehydrogenase | 4 |
| 15 | P24268 | CTSD | Cathepsin D | 3 |
| 16 | P60711 | ACTB | Actin, cytoplasmic 1 | 3 |
| 17 | P62738 | ACTA | Actin, aortic smooth muscle | 3 |
| 18 | P50753 | TNNT2 | Troponin T, cardiac muscle | 3 |
| 19 | A1A5P2 | RRS1 | Ribosome biogenesis regulatory protein homolog | 3 |
| 20 | P05065 | ALDOA | Fructose-bisphosphate aldolase A | 3 |
| 21 | P63018 | HSP7C | Heat shock cognate 71 kDa protein | 3 |
| 22 | P16617 | PGK1 | Phosphoglycerate kinase 1 | 3 |

**Table S6. The peptide sequences of Cathepsin D were identified by Nano-HPLC-MS/MS analysis.**

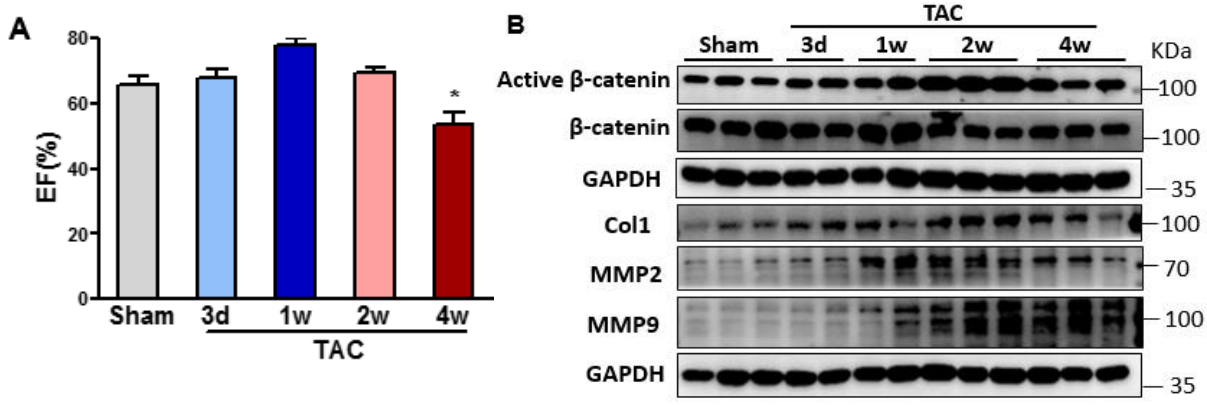
| Accession | Protein | Sequence |
|---|---|---|
| P24268 | Cathepsin D | QPGVVFIAAK |
| | | EPVSELLK |
| | | SDLGGIKVEK |

**Table S7. Genotype and clinical phenotypes of Dilated cardiomyopathy (DCM) patients carrying the missense mutation of LRP6 or CTSD that is absent in controls.**

| Gene | LRP6 | CTSD |
|---|---|---|
| Mutation | c.C3310T | c.G1138A |
| Amino acid | p.P1104S | p.G316R |
| Patient Number | A101 | A27 |
| Sex | Male | Male |
| Age | 31 | 36 |
| SBP | 140 | 142 |
| DBP | 100 | 91 |
| HR | 70 | 87 |
| EF | 33% | 29% |
| Diagnosis | DCM | DCM |

LPR6:Low-Density Lipoprotein Receptor-Related Protein 6; CTSD: cathepsin D; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; EF: ejection fraction;

**Supplemental figures and figure legends**



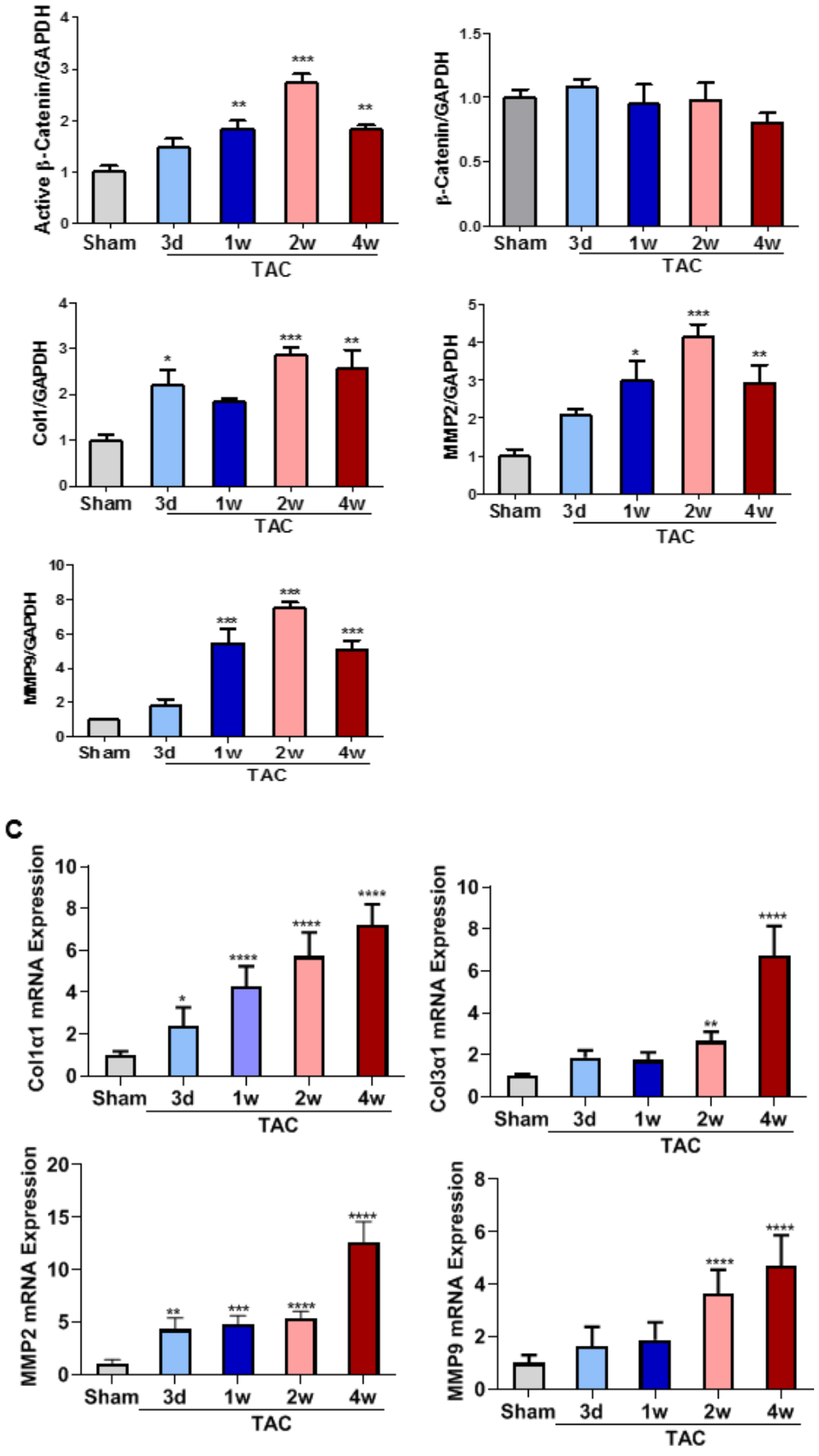



Figure S1. Cardiac function and fibrotic-related proteins or genes are evaluated in mice

at different time-points (3d, 1w, 2w and 4w) after TAC. A, Echocardiographic analysis of ejection fraction (EF). *p < 0.05 *vs* Sham group; n=6-10 mice/each group. B, Western blot analysis of active β-catenin, β-catenin, Col1, MMP2 and MMP9 expression. *p < 0.05; **p < 0.01; ***p < 0.001 vs sham group; n = 3 mice/each group. C, Real-time-PCR analysis of the mRNA level of Col1, Col3, MMP2 and MMP9. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*vs* sham group; n = 3 mice/each group.

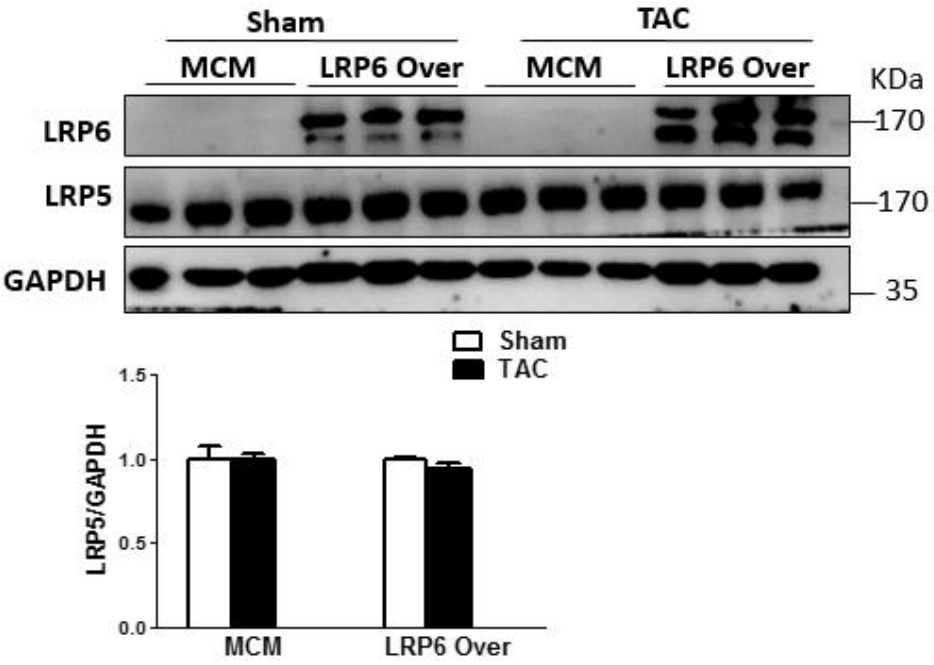



Figure S2. Western blot analysis of LRP5 expression in heart tissue from cardiac LRP6 overexpressing (LRP6 Over) or tamoxifen-injected-MCM (MCM) mice at 4 weeks after TAC or sham operation. n=3/each group.

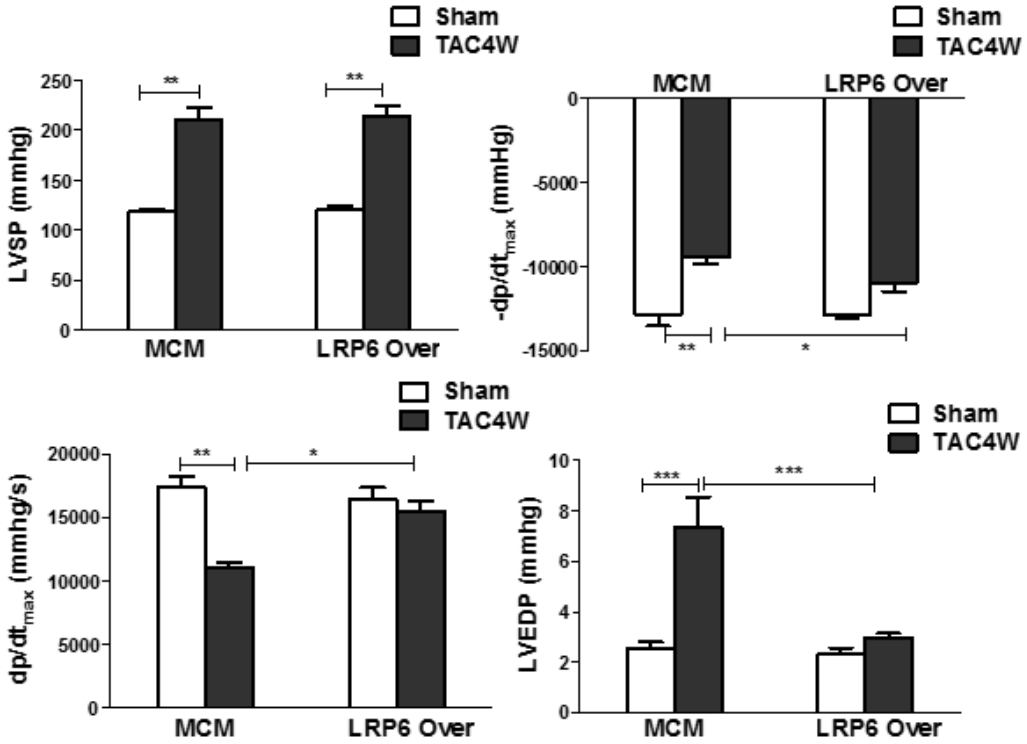



Figure S3. Hemodynamic analysis of LVSP, LVEDP, $dp/dt_{max}$, and $-dp/dt_{max}$ in tamoxifen-injected-LRP6$^{CAG}$/MCM (LRP6 Over) or –MCM (MCM) mice at 4 weeks after TAC or sham operation. LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure; *p<0.05; **p<0.01; ***p<0.001; n=6-7/each group.

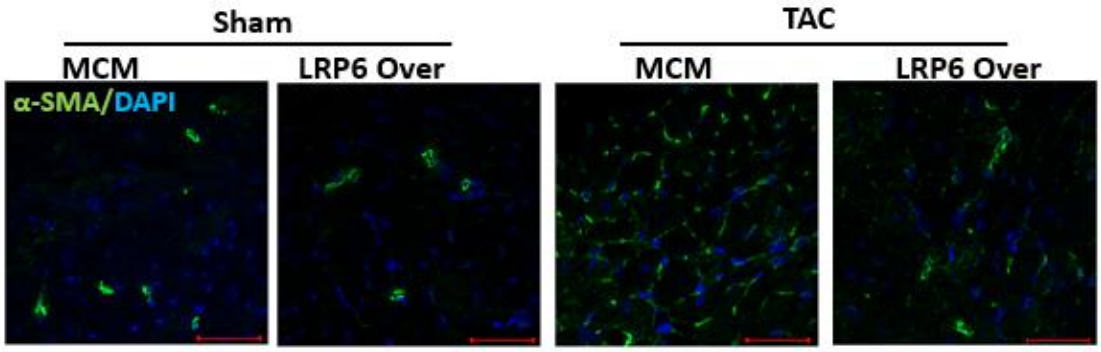



Figure S4. α-SMA immunofluorescence staining of heart tissue, Scale bars, 50um. Tamoxifen-injected-LRP6$^{CAG}$/MCM (LRP6 Over) or –MCM (MCM) mice were analyzed at 4 weeks after TAC or sham operation.

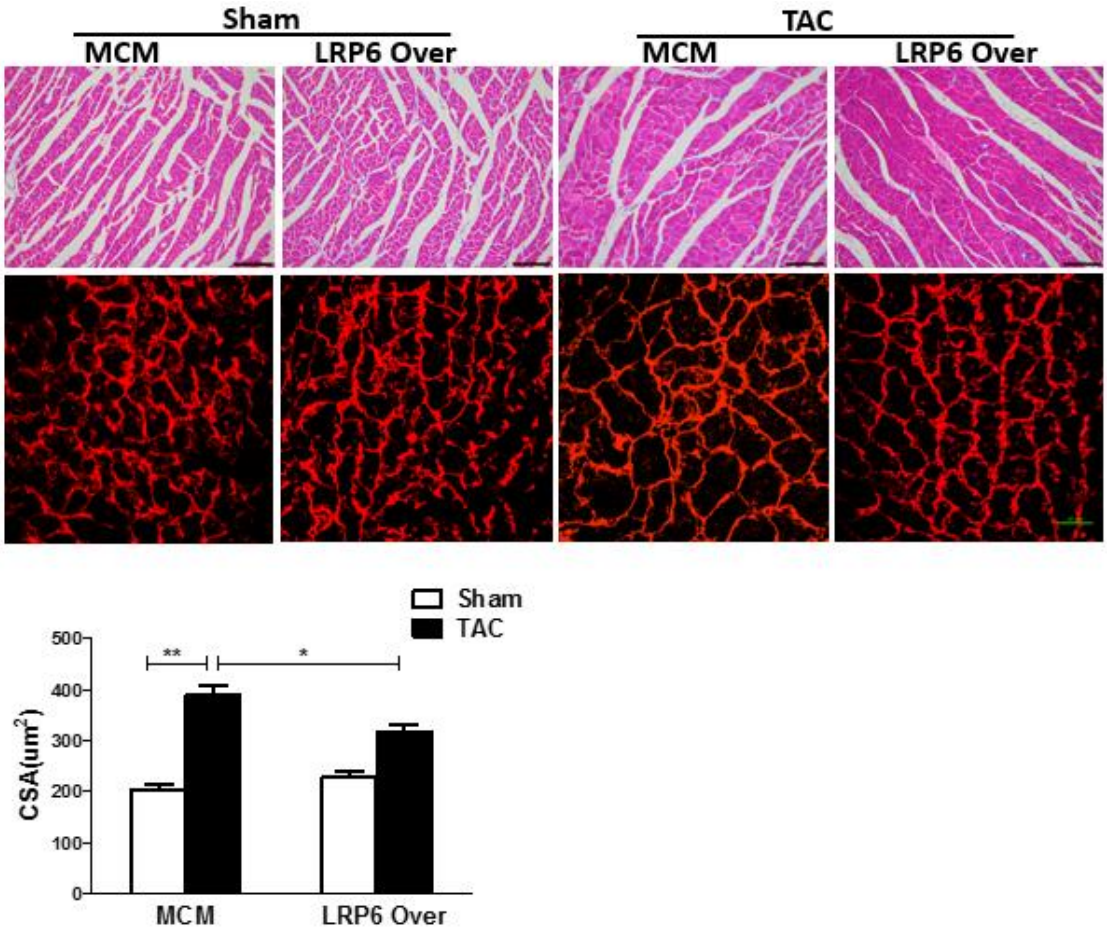
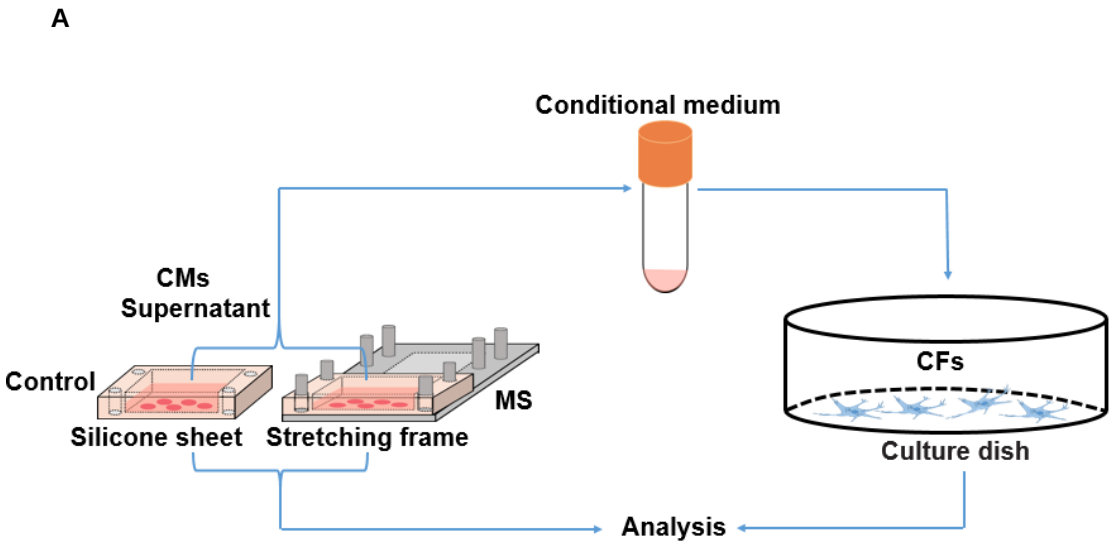



Figure S5. Cardiac specific LRP6 overexpression inhibits cardiac hypertrophy induced by pressure overload. HE and *Wheat germ agglutinin* (WGA) staining of heart tissues. Upper lane: HE staining; Scale bars,100um. Down lane: WGA staining. Scale bars, 50μm. Cross section area (CSA) of cardiomyocytes was quantitatively analyzed. *p<0.05; **p<0.01; n=7-11/each group.



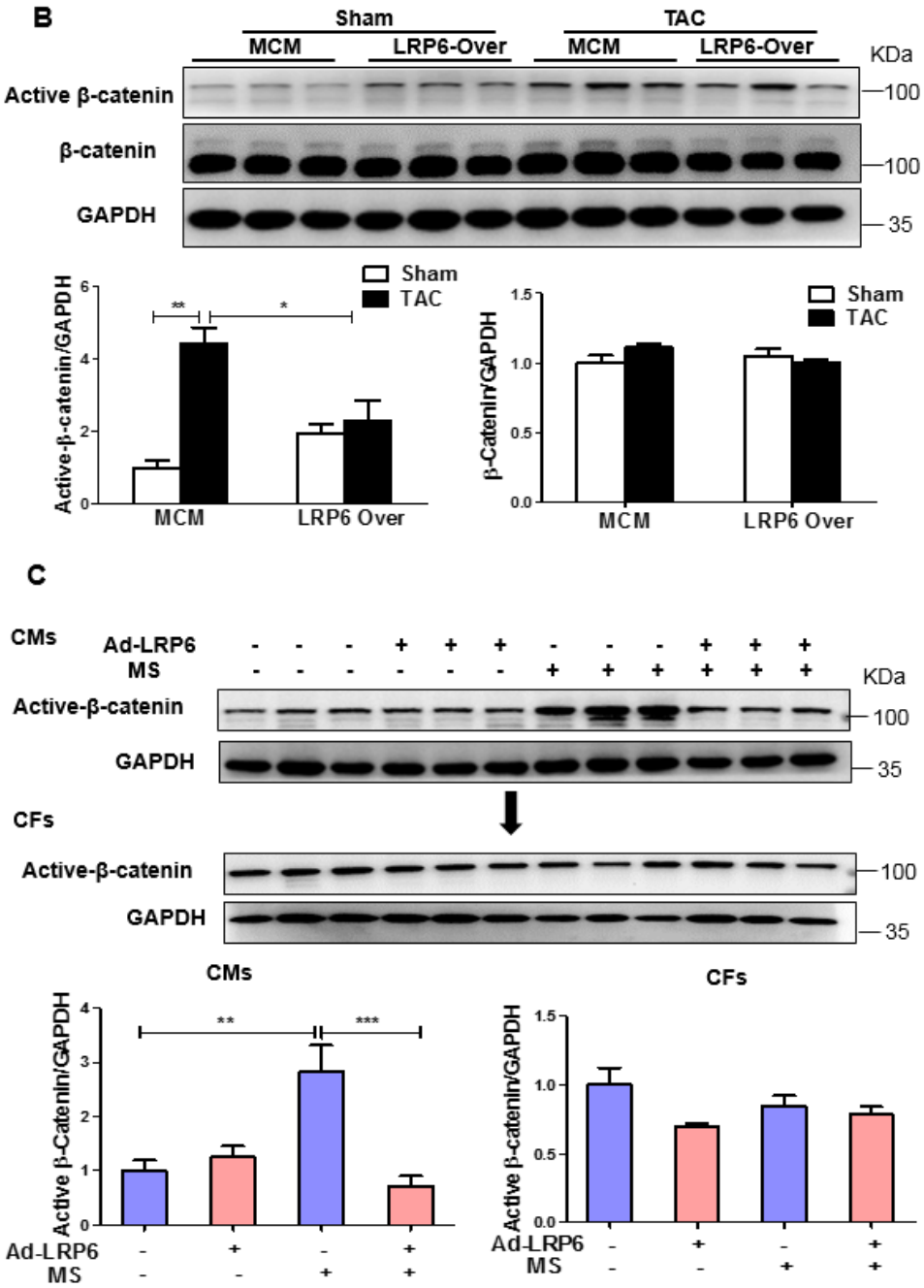



Figure S6. Cardiac specific LRP6 overexpression inhibits the activation of β-catenin. (A), Diagram of conditioned medium from stretched CMs or control CMs stimulating CFs. (B), Western blot analysis of active-β-catenin and β-catenin expression in tamoxifen-injected-LRP6$^{CAG}$/MCM (LRP6 Over) or MCM (MCM) mice after TAC or sham operation. *$p < 0.05$; **$p < 0.01$; n = 3/each group. (C), Western blot analysis of active-β-catenin expression in cultured CMs and CFs. Stretched (MS) or control CMs were pre-transfected with LRP6 adenovirus (Ad-LRP6) or control adenovirus (Ad-CON), and the conditioned medium from these CMs was used to stimulate CFs. **$p < 0.01$; ***$p < 0.001$; n = 6/each group.

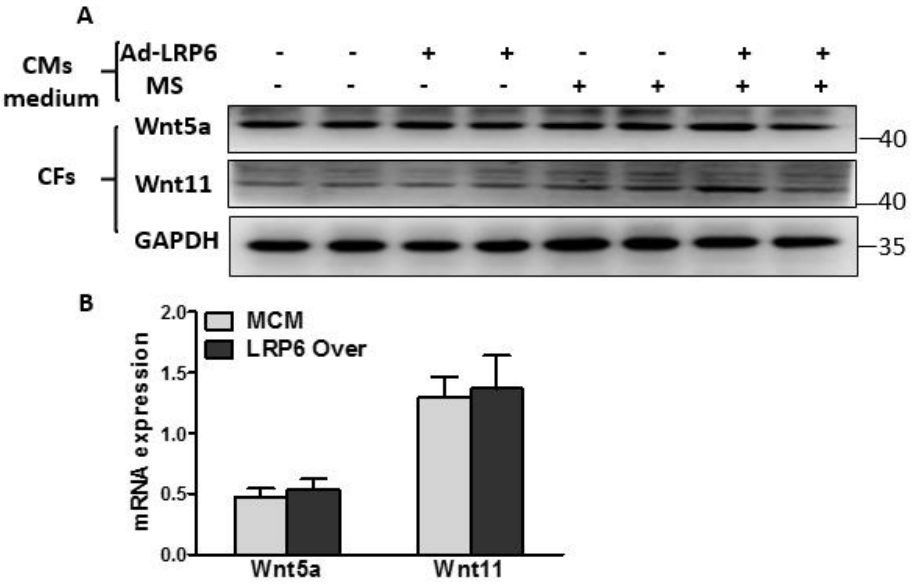



Figure S7. The effects of cardiac LRP6 overexpression on the expression of Wnt5a and Wnt11 under pressure overload. (A), The representative images of Wnt5a and Wnt11 expression (western blot analysis) in cultured CFs stimulated with the culture medium from stretched (MS) or control CMs transfected with LRP6 adenovirus (Ad-LRP6) or control adenovirus (Ad-CON). (B), Real-time PCR analysis of Wnt5a and Wnt11 mRNA expression in cardiac LRP6 overexpressing (LRP6 Over) or tamoxifen-injected-MCM (MCM) mice at 4 weeks after TAC. n=6/each group.

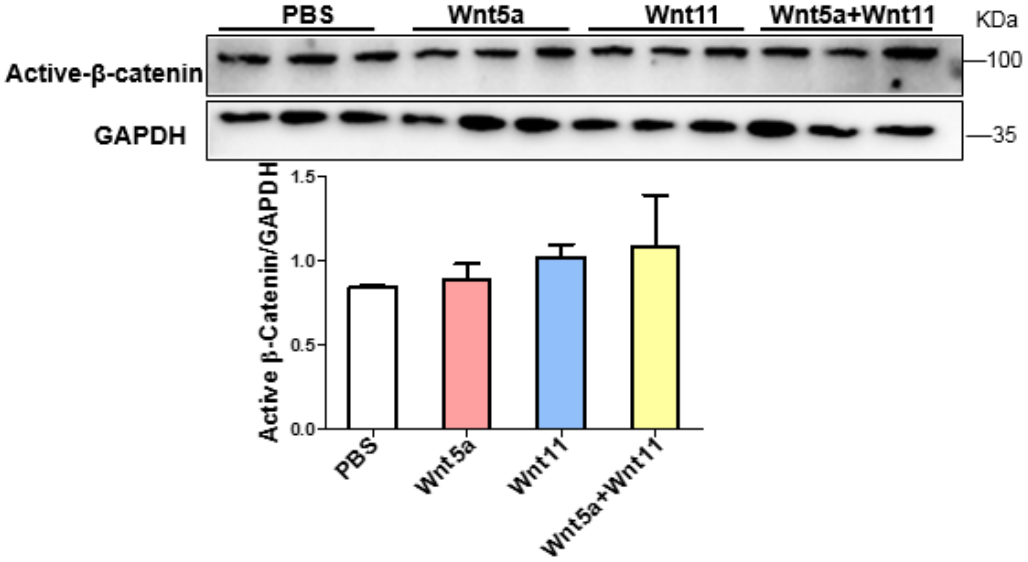



Figure S8, Western blot analysis of active β-catenin expression in CFs treated with Wnt5a, Wnt11, or Wnt5a+Wnt11, at 10 ng/mL. n=3/each group.

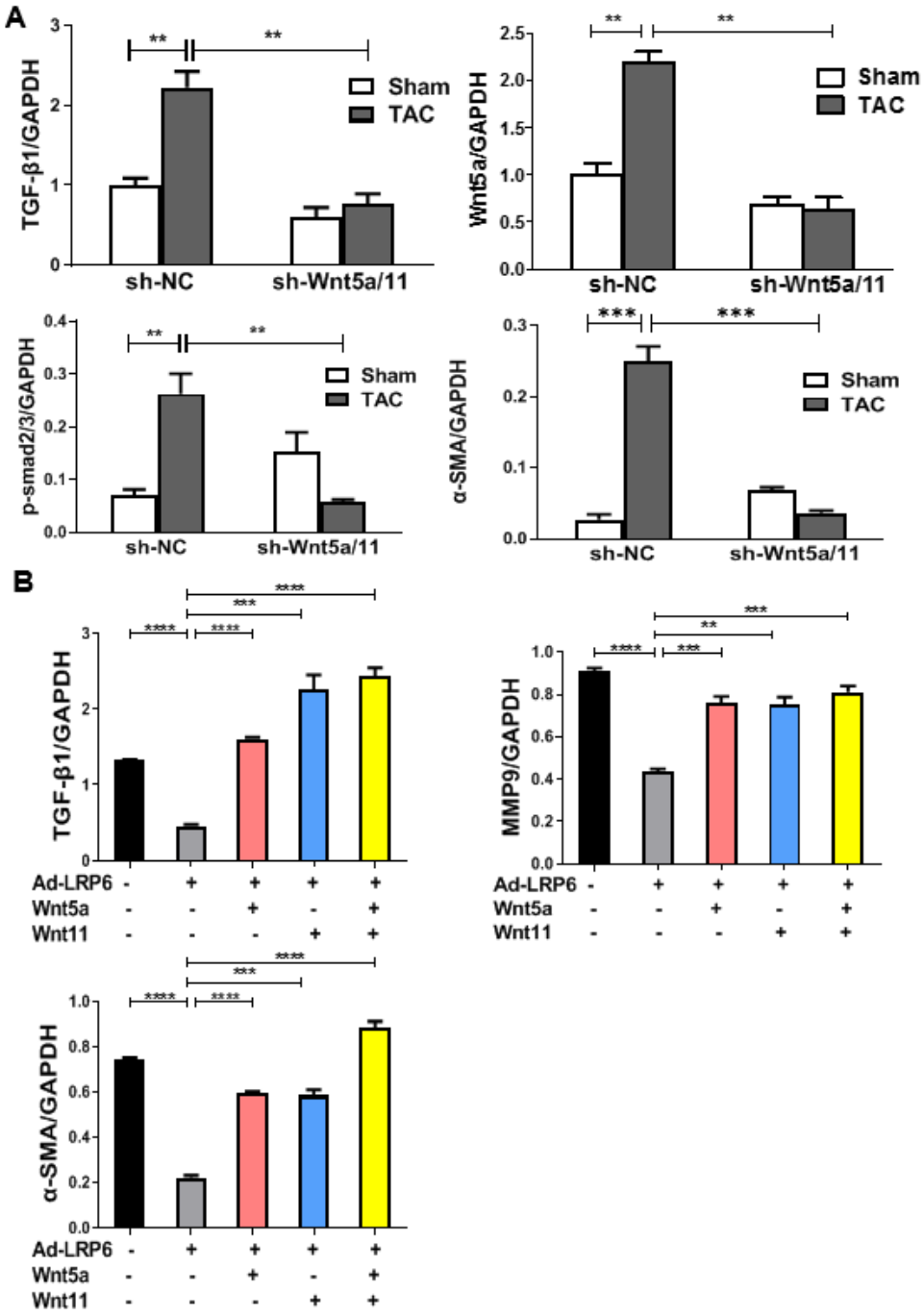



Figure S9. LRP6 overexpression inhibits cardiac fibrosis by promoting the degradation of Wnt5a and Wnt11 under pressure overload. (A), Quantitative analysis of the expression of Wnt5a, TGF-β1, α-SMA, and p-smad2/3 expression in heart tissue from TAC or sham mice pre-injected with sh-Wnt5a/Wnt11-AAV9 (sh-Wnt5a/Wnt11) or sh-scramble-AAV9 (sh-NC) by tail vein. **p < 0.01; ***p < 0.001. n=4-6 mice/each group. (B), Quantitative analysis of TGF-β1, α-SMA and MMP9 expression in CFs stimulated with the culture medium from control (Ad-CON) or LRP6 overexpressing CMs (Ad-LRP6), Wnt5a, Wnt11, Wnt5a+Wnt11 at 10 ng/mL, or PBS were supplemented with the culture medium from Ad-LRP6-CMs under MS and treated with CFs. **p < 0.01; ***p < 0.001; ****p < 0.0001. n=3/each group.

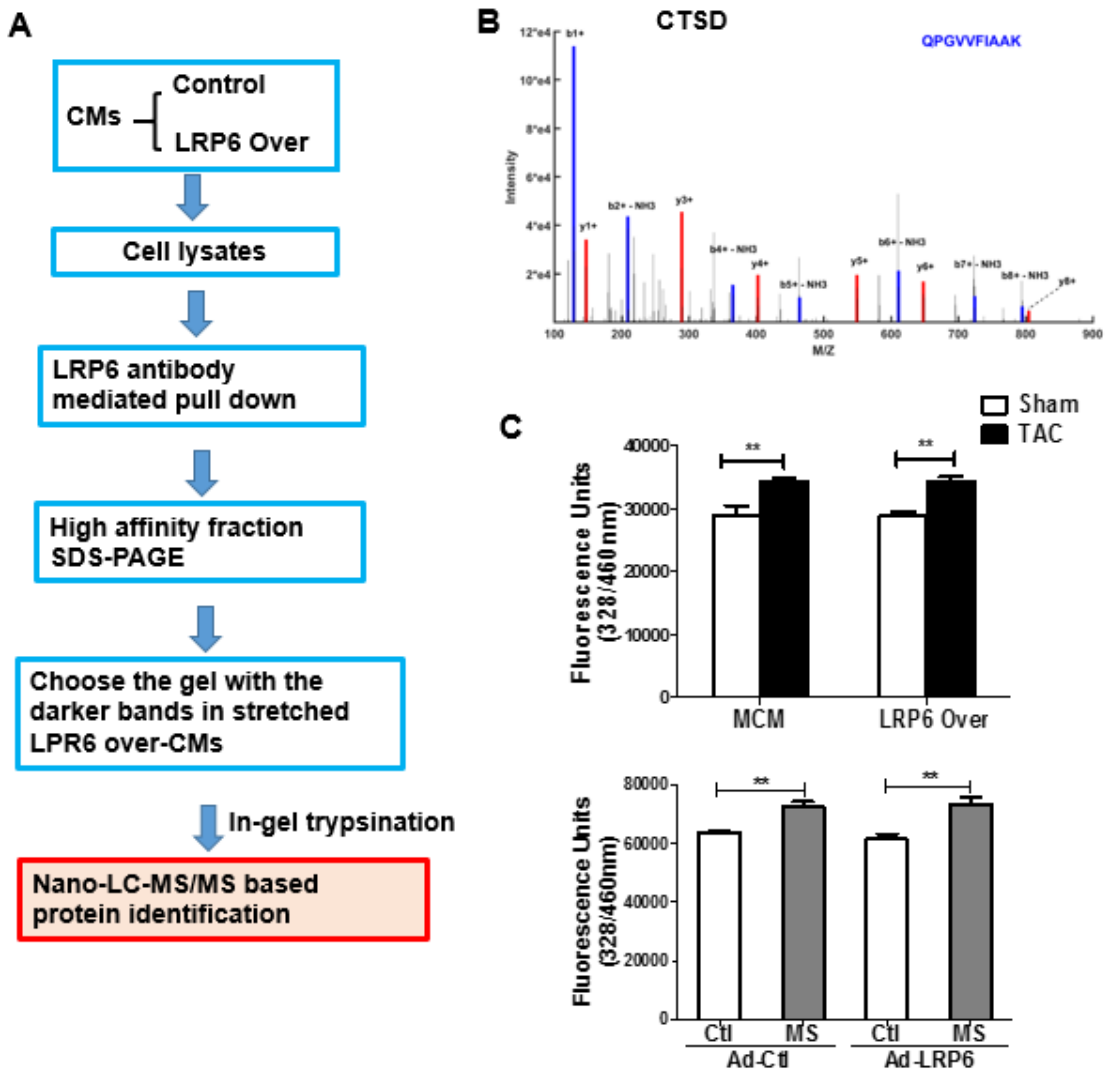



Figure S10. LRP6 overexpression enhances the interaction of LRP6 and CTSD but doesn't alter the activity of CTSD. (A), Summary of Nano-HPLC-MS/MS analysis of the proteins interacting with LRP6 in CMs under MS. (B), CTSD was identified to be interact with LRP6 by Nano-HPLC-MS/MS analysis. (C), CTSD activity was analyzed in the heart tissue from tamoxifen-injected-LRP6$^{CAG}$/MCM (LRP6 Over) or –MCM (MCM) mice at 4 weeks after TAC or sham operation (Upper lane) and in control (Ad-ctl) or LRP6-overexpressing CMs (Ad-LRP6) with or without MS (Ctl) (Down lane), respectively. **$p < 0.01$; n = 4-6/each group.

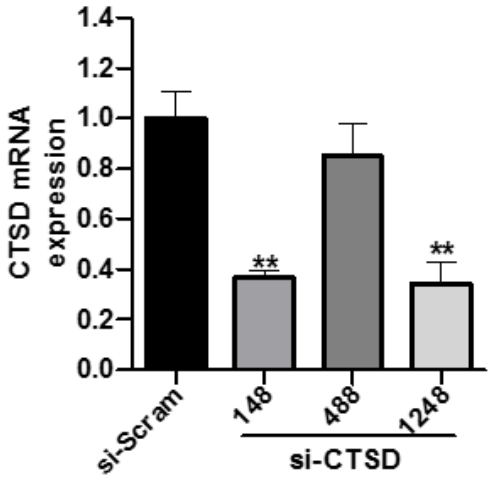
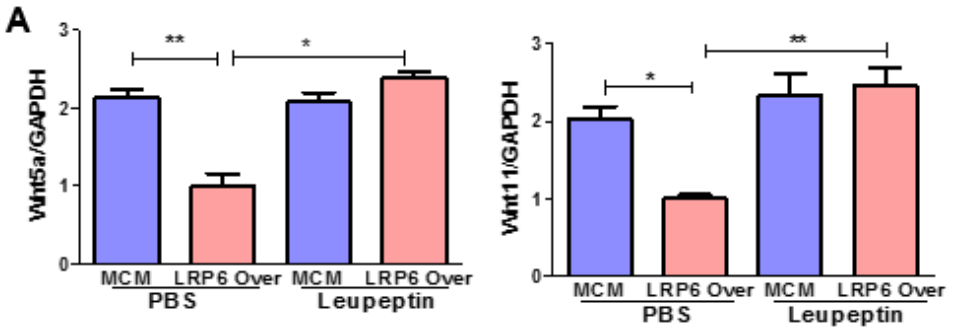
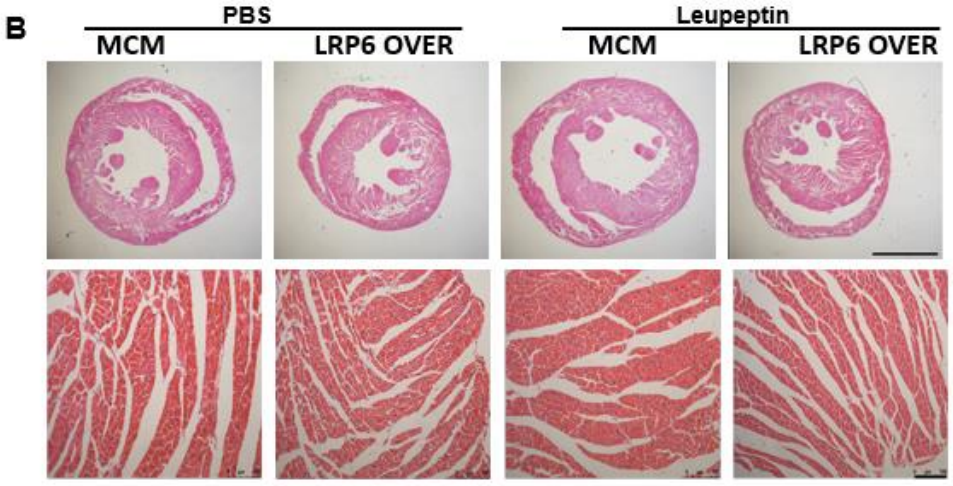



Figure S11. CTSD mRNA analysis in cardiomyocytes transfected into si-CTSD (number: 148, 488, 1248) or si-Scramble (si-Scram). **p<0.01; n=5/each group.



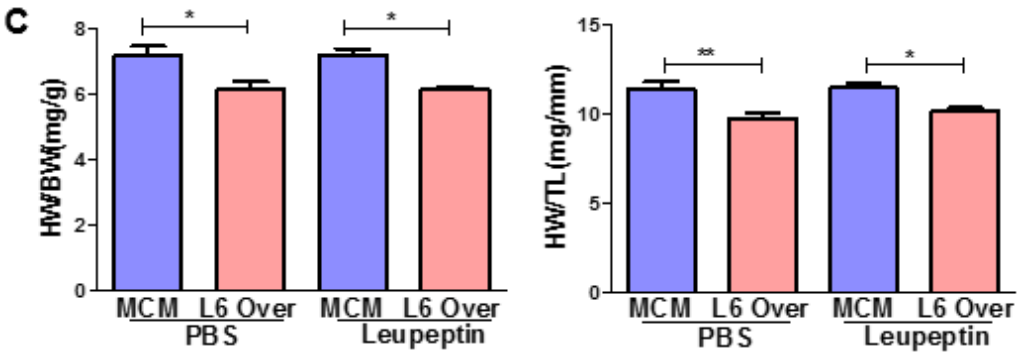



Figure S12. Protease inhibitor doesn't attenuate the inhibition of cardiac hypertrophy in LRP6 overexpressing mice after TAC. (A), Quantitative analysis of Wnt5a and Wnt11 expression. n = 3/each group. (B), HE staining of heart tissue. The upper lane: bar=2mm; The down lane: bar=100μm. (C), Quantitative analysis of heart weight/body weight ratio (HW/BW) and heart weigh/tibia length ratio (HW/TL). Protease inhibitor, Leupeptin (40mg/kg), was intraperitoneally injected to LRP6 Over and MCM mice from 2 weeks to 4 weeks (Every other day) after TAC, PBS treatment was as control. *$p < 0.05$; **$p < 0.001$; n = 7-11/each group.