

Supporting Data

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Supporting table S1 related to Experimental Procedures

Primers used in the direction of 5'-3' orientation for qPCR.

Name of the genes	Sequence	Comments/Use
HUMAN		
<i>AXIN2</i> forward (F)	GTGTGAGGTCCACGGAAACT	qPCR
<i>AXIN2</i> reverse (R)	AATCATCCGTCAGCGCATCA	qPCR
<i>GAPDH</i> F	AAGGCTGTGGGCAAGGTCATC	qPCR
<i>GAPDH</i> R	GCGTCAAAGGTGGAGGAGTGG	qPCR
<i>TCF7L2</i> F	CAGACCTGAGCGCTCCTAAG	qPCR
<i>TCF7L2</i> R	TCAGTCTGTGACTTGGCGTC	qPCR
MOUSE		
<i>Axin2</i> intron F	CAGGCTGGGACTGAATGAAG	ChIP-qPCR
<i>Axin2</i> intron R	AAACAAGCATGGGACCACACC	ChIP-qPCR
<i>Axin2</i> promoter F	GAGCGCCTCTGTGATTGGC	ChIP-qPCR
<i>Axin2</i> promoter R	ACAGCAAAGCTCTCCTTTGGG	ChIP-qPCR
<i>Axin2</i> F	AGCCGCCATAGTC	qPCR
<i>Axin2</i> R	GGTCCTCTTCATAGC	qPCR
<i>Cacna1g</i> F	AAAGAGAAGGAGTAAGGAGAAGCAG	qPCR
<i>Cacna1g</i> R	ATCTGGGGCTGCTGGTAATG	qPCR
Cre (Hgh) F	GTCTGACTAGGTGTCCTTCT	Genotyping
Cre (Hgh) R	CGTCCTCCTGCTGGTATAG	Genotyping
<i>Dstn</i> F	ACCCATGCACTCCCCTTAAC	qPCR
<i>Dstn</i> R	CTGAACTCCTGAGGCCATGTT	qPCR
<i>Gapdh</i> F	ATGTTCCAGTATGACTCCACTCACG	qPCR
<i>Gapdh</i> R	GAAGACACCAGTAGACTCCACGACA	qPCR
<i>Hand2</i> F	GCCGACACCAAACCTCTCCAAG	qPCR

<i>Hand2</i> R	CCTCCGCCTCTCCGTTCTG	qPCR
<i>Hand2</i> peak F	TCATCTTTCAGTCGTGCCGT	ChIP-qPCR
<i>Hand2</i> peak R	CTGAGTAGTGACATGACAGC	ChIP-qPCR
<i>Myh7</i> F	ATGTGCCGGACCTTGGA	qPCR
<i>Myh7</i> R	CCTCGGGTTAGCTGAGAGATCA	qPCR
<i>Nppa</i> promoter F	CGATGAATCAGGTGTGAAGC	qPCR
<i>Nppa</i> promoter R	TGTCAGGGGCTCCAAATAAG	qPCR
<i>Rock2</i> F	AGAGTCTGCTGGATGGCTTAAA	qPCR
<i>Rock2</i> R	TTCACCAAAGCACCTCTTCC	qPCR
<i>Shisa3</i> F	CAGGGCAACTATCACGAGGG	qPCR
<i>Shisa3</i> R	GACATAGACAGGTTGCGCGG	qPCR
<i>Sox4</i> F	GTGTTGAGCTTAGGGGAGCA	qPCR
<i>Sox4</i> R	ATGTCCATTTCCGAGGCTGG	qPCR
<i>Tbp</i> F	CCAGAACAACAGCCTTCCACC	qPCR
<i>Tbp</i> R	CAACGGTGCAGTGGTCAGAGT	qPCR
<i>Tbx20</i> F	AGAAGGAGGCAGCAGAGAACAC	qPCR
<i>Tbx20</i> R	GCACAGAGAGGATGAGGATGGG	qPCR
<i>Tbx3</i> peak F	TCGGGGTTAGGCCTTGATAAC	ChIP-qPCR
<i>Tbx3</i> peak R	GTCTTGGCTGCCTCTCAGTT	ChIP-qPCR
<i>Tcf7l2</i> F (Exons7-8)	GGGAAATCCACCTCCGCACT	qPCR
<i>Tcf7l2</i> R (Exons7-8)	TCCTCCTGTCGTGATTGGGTACAC	qPCR
Δ <i>NCat</i> F	GCTGCTGTGACACCGCTGCGTGGAC	Genotyping
Δ <i>NCat</i> R	CACGTGTGGCAAGTTCCGCGTCATCC	Genotyping

Supporting table S2 related to Experimental Procedures

Anesthetics and preanesthetics used in this study.

Intervention	Drug	Applications- route	Dosis	Applications- time
TAC	Metamizole	Oral	1,33 mg/ml in Drink water	2 days before and 7 days after operation (OP)
	Buprinorphine	Subcutaneous injection	0.05-0.1 mg/kg Body weight	30 min before and after OP
	Midazolame	Subcutaneous injection	5 mg/kg body weight in 0,9% NaCl	During OP
	Fentanyl	Subcutaneous injection	0,05 mg/ body weight in 0,9% NaCl	
	Medetomidin	Subcutaneous injection	0,5 mg/ body weight in 0,9% NaCl	
	Atipamezole	Subcutaneous injection	2,5 mg/kg body weight in 0,9% NaCl	After OP
	Flumazenil	Subcutaneous injection	0,5 mg/kg body weight in 0,9% NaCl	
Echocardiography	Isoflurane	Inhalation	2% [vol/vol] Isoflurane	During measurements

Supporting Experimental Procedures

DNA, RNA isolation and quantitative real-time PCR

Ventricular tissue was macrodissected and used for DNA and RNA isolation. DNA and RNA were isolated using NucleoSpin Tissue genomic DNA and RNA kit (Macherey-Nagel), respectively, as described elsewhere (1). Tissue samples were immediately snap frozen and stored at -80°C till RNA or DNA preparation. Nucleic acid quantification was assessed using Nanodrop photometer (Thermo Scientific). 500ng RNA was used for cDNA synthesis using 0.5 µg Oligo(dT)20 primer and 100 U M-MLV reverse transcriptase (Promega) for 1h 42°C. Quantitative real-time PCR (qPCR) analyses were performed with SYBR Green (Promega) on a 7900-HT Real-time cycler (Applied Biosystems) using the primers listed in Supporting table S1. Gene expression was normalized to the indicated housekeeper in every experiment. Copy numbers were calculated using the SDS2.4 software with a relative standard curve obtained using the log dilutions of cDNA of gene of interest. All reactions were run in triplicates and normalized to reference control genes. Primers are listed in Supplemental Table S1.

Cellular fractionation

Cardiac tissue was homogenized in a hypotonic buffer containing 10 mM HEPES, 10 mM KCl, 2 mM MgCl₂, 0.1 mM EDTA and 1 mM DTT with protease inhibitors. After 10 min ice incubation, NP-40 was added to a final concentration of 1% followed by 5 min ice incubation. Lysates were centrifuged at 4000rpm for 5 min at 4°C. Supernatants were cytosol-enriched fractions. To the pellets, a hypertonic buffer containing 50 mM HEPES, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT and 10% glycerol with protease inhibitors was added and incubated on ice for 25 min. NP-40 was added to a final concentration of 1% followed by 10 min ice incubation. Lysates were centrifuged at 13000rpm for 5 min at 4°C. Supernatants were nuclei-enriched fractions.

Epifluorescence microscopy and calcium measurements

CM was isolated as described above. Cells were plated on laminin-coated recording chambers and left to settle for 20 min, followed by incubation with a Fura-2 AM loading buffer (10 µmol/L, Molecular Probes) for 15 min. After staining, the CMs were superfused with experimental solution for 5 min before measurements were started to enable complete de-esterification of intracellular Fura-2 and allow cellular rebalance of Ca²⁺ cycling properties. Experimental solution contained (in mmol/L): KCl 4, NaCl 140, MgCl₂ 1, HEPES 5, glucose 10, 5 CaCl₂ 1 (pH 7.4, NaOH, room temperature). During measurements, CMs were continuously superfused with experimental solution. Measurements were performed with a Motic AE32 microscope (Speed Fair Co. Ltd, Hong Kong) provided with a fluorescence detection system (ION OPTIX Corp., Milton MA). Cells were excited at 340 and 380 nm, and the emitted fluorescence was

collected at 510 nm. The intracellular Ca²⁺ level was measured as the ratio of fluorescence at 340 and 380 nm (F340 / F380, in ratio units). Systolic Ca²⁺ transients were recorded at steady-state conditions under constant field stimulation (1, 2, and 3 Hz). To assess the SR Ca²⁺ content, the amplitude of caffeine-induced Ca²⁺ transients was measured. After stopping the stimulation during steady-state conditions at 1 Hz, caffeine (10 mmol/L, Sigma-Aldrich, St Louis, MO, USA) was applied directly onto the cell, leading to immediate and complete SR Ca²⁺ release. The recorded Ca²⁺ transients were analyzed with the software IONWizard® (ION OPTIX Corp.). As a measure of SERCA2a-dependent Ca²⁺ reuptake into the SR, the decay constant *k* (reciprocal of Tau) of caffeine-induced Ca²⁺ transients was subtracted from the decay constant *k* of systolic Ca²⁺ transients at 1 Hz (*k*_{sys} - *k*_{caff}).

Chromatin immunoprecipitation (ChIP-seq) and data analyses (extended)

ChIP was optimized for the cardiac ventricular tissue. TCF7L2 and H3K27ac ChIPs in cardiac ventricular tissue from 3 weeks post-induced β -cat ^{Δ ex3} mice were performed by 20 minutes crosslinking with 1.3% formaldehyde and first sonicated for 20 cycles with the buffer containing 150 mM NaCl, 20 mM EDTA (pH 8.0), 0.5 % sodium deoxycholate, 50 mM Tris-HCl (pH 8.0), 1% (v/v) NP-40, 20 mM Sodium Fluoride, 0.1% SDS and protease inhibitors. The lysates were centrifuged at 12000 x g at 4°C for 2 min and supernatants were collected and stored. To the pellets the above buffer was added again and sonication was repeated for 25 cycles. These supernatants from the second centrifugation per sample were pooled and pre-cleared with sepharose beads for 45 min to reduce unspecific binding. For IP, 2 μ g of anti-TCF7L2, anti-IgG (17-10109, Millipore), anti-GATA4 (sc-25310 X, SantaCruz) or anti-H3K27ac (C15410196, Diagenode) was added to the nuclear extracts and incubated O/N at 4°C on a rotor. Antibodies were pulled down using protein-A-sepharose beads followed by washing and DNA extraction. For sequencing, the DNA was isolated using phenol chloroform extraction. For this purpose, 50 μ l of 10 mM Tris HCl pH 8.0 containing 10 μ g of RNAse A was added to the already washed chromatin-bound beads as well as the input samples and incubated for 30 min at 37°C. Then, 50 μ l of buffer containing 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 2% SDS and 20 μ g of Proteinase K were added and the samples were incubated overnight at 65°C with a subsequent centrifugation step at 2,000g for 2 min at RT. The supernatant was collected, and the beads were rinsed again with 100 μ l of 10 mM Tris pH 8.0. The samples were centrifuged and the supernatant was added to the first one. For extraction, 10 μ l of 8 M LiCl, 4 μ l co-precipitant (linear polyacrylamide) and 200 μ l phenol/chloroform/isoamyl alcohol (25:24:1) were added, samples were vortexed for 30 sec and centrifuged for 2 min at full speed. The aqueous phase was collected and the phenol phase was back extracted with 200 μ l 10 mM Tris HCl pH 8.0 and 400 mM LiCl. After vortexing and centrifugation, the second aqueous phase was pooled with the first one and precipitation was performed by addition of 100% ethanol and incubation for 2 h at -80°C. After that, samples were centrifuged at maximal speed for 30 min (4°C), pellets were washed with 70% ethanol, dried and re-dissolved in 40 μ l of water. 5 μ l of the DNA was used for qRT-PCR to confirm efficient

chromatin immunoprecipitation and 35 µl for sequencing. DNA concentration was measured using a Qubit dsDNA HS assay on a Qubit® 2.0 Fluorometer. To identify protein complexes bound to the chromatin, proteins were extracted from protein-A-sepharose beads by directly adding protein lysis buffer to the beads, incubating at 95°C for 10 min with constant shaking. Samples were centrifuged and supernatants were used for immunoblotting and/or analysed with mass spectrometry. The efficiency of sonication was determined by performing a shearing check. Briefly, 10 µl of sheared chromatin was used for phenol chloroform extraction. After re-suspension of the DNA in 15 µl of 10 mM Tris HCl pH 8.0, 100 µg/ml RNase A was added and the mixture was incubated for 1 h at 37°C (700 rpm). The DNA was then run on a 1.5% agarose gel and analyzed on the gel documentation. Efficient shearing consisted of a smear from about 150bp to 1 kb with a maximum around 150- 300 bp. ChIP-seq library preparation was performed using NEBNext Ultra DNA library prep kit for Illumina (E7370) as per manual's instructions. 5 - 50 ng of fragmented DNA from ChIP was re-sonicated for 15 min in Bioruptor to ensure small fragments suitable for sequencing. End preparation was performed by adding end prep enzyme mix and end repair reaction buffer (10X) to a final reaction volume of 65 µl. Samples were placed on a thermocycler with cycles of 20°C for 30 min and 65°C for 30 min followed by adaptor ligation with blunt/TA ligase master mix, NEBNext adaptor and ligation enhancer to a final volume of 83.5 µl. Samples were incubated at 20°C for 15 min, then 3 µl of User enzyme was added and placed at 37°C for 15 min. Adaptor – ligated DNA was cleaned up using 0.9x AMPure XP beads on magnetic stand and finally DNA was resuspended in 28 µl of 10 mM Tris pH 8.0. From this, 23 µl of DNA was used for PCR amplification followed by clean-up of PCR amplified product using AMPure XP beads. The DNA was resuspended in 33 µl of 10 mM Tris pH 8.0 and analyzed on Bioanalyzer. Quantitation of DNA libraries was done on an Invitrogen Qubit 2.0 Fluorometer and the size range of DNA libraries was performed on an Agilent Bioanalyzer 2100 (High Sensitivity DNA Assay). DNA libraries were amplified and sequenced by using the cBot and HiSeq2500 from Illumina (20-25 million reads per sample). Sequence reads were aligned to the mouse reference assembly (UCSC version mm9) using Bowtie (2). Peak calling was performed with Model Based Analysis of ChIPseq (MACS2) version 2.1.0.20140616.0, which is the updated version of MACS (3). Genes proximal to the bound chromatin regions were identified by GREAT analyses (4) using 'Basal plus extension' method where each genomic region is overlapped with genes which are 5kb upstream and 1 kb downstream (proximal), plus up to 1000 kb (distal). To integrate ChIP-seq with differential gene regulation i.e RNA-seq, BioVenn web application was used to compare, create and analyse Venn diagrams showing commonly or differently bound genes between two or more datasets (5). Gene ontology/pathway analyses for gene lists were performed using default parameters and stringency in 'ClueGO': a Cytoscape plug-in (6) and the significant 'Gene Ontology Biological Processes' were shown with $p \leq 0.05$. Published/public ChIP-seq datasets were used from the following sources: TCF7L2 liver: GSE32513, GATA4, NKX2-5 and TBX3: GSM862697-(7) DNase-seq: GSM1014166, H3K4me1: GSM769025 and RNAPII: GSM918723.

For own data, the following secure token has been created to allow review of record GSE97763 while it remains in private status: yhcviyemxdivbqj.

CM isolation and immunocytochemistry

For CM (CM) isolation, hearts were retrogradely perfused by a modified Langendorff solution (NaCl 120.4 mM, KCl 14.7 mM, KH₂PO₄ 0.6 mM, Na₂HPO₄ 0.6 mM, MgSO₄ 1.2 mM, Na-HEPES 10 mM, NaHCO₃ 4.6 mM, taurine 30 mM, 2, 3-butanedione-monoxime 10 mM, collagenase type II (600 U/ml), glucose 5.5 mM, pH 7.4) for 7 min at 37°C at a flow rate of 4 ml/min. The residual tissue was removed by using a 100- μ m cell strainer (BD Falcon, 352360). Bovine calf serum (10%) and 12.5 μ M CaCl₂ in perfusion buffer was used to inhibit collagenase activity. For immunofluorescence, isolated myocytes were plated on laminin (L2020, Sigma)- 2 coated glass coverslips, fixed with 4% PFA, followed by PBS washing and permeabilization with 0.2%BSA and 0.3%Triton in PBS for 10 min. CM were then blocked with 5%BSA and 0.1%Triton at RT. Primary and secondary antibodies (listed in Supplementary methods) were diluted in 2%BSA and 0.1%Triton in PBS. Coverslips were mounted with ProLong Gold medium containing DAPI (Invitrogen) and imaged in Zeiss LSM 710 NLO confocal microscope.

Histology and immunohistochemistry

Immunohistochemistry was performed as described previously (8). Hearts were dissected, rinsed in PBS, fixed in 4% PFA O/N at 4°C, embedded in paraffin and sectioned at 3 μ m thickness in Leica RM2255 microtome. Sections were deparaffinized, rehydrated and antigen was unmasked by microwaving sections for 10min in 10mM sodium citrate buffer at pH 6.0. For immunofluorescence (IF), sections were blocked at RT for 1hr with 5%BSA in PBS + 0.1% Triton. For IF, primary antibodies were incubated O/N at 4°C as follows: anti-Cardiac Troponin T (ab8295, Abcam, 1:200), anti- β -catenin (610153, BD Transduction labs, 1:120), anti-Ki67 (ab15580, Abcam, 1:50), anti-acTub (ab24610, Abcam, 1:500), anti-Caveolin3 (ab30750, Abcam, 1:500), anti-TCF7L2 (ab76151, Abcam, 1:50), anti-Shisa3 (HPA054754, Sigma, 1:20), anti-N-cadherin (sc-7939, SantaCruz, 1:100) in 1% BSA in PBS+0.1% Triton. Next, sections were washed in PBS and incubated with secondary anti-rabbit IgG-Alexa 594 or anti-mouse IgG-Alexa 488(1:200; Molecular Probes) antibodies. For assessing proliferation in vivo, 200 μ g of 5-ethynyl-2'-deoxyuridine (EdU) EdU per mouse was applied by a single i.p. injection on the same day as TX induction. 3 weeks post-induction, hearts were analyzed by immunofluorescence (IF) with Click-it EdU Assay kit (Invitrogen) according to the manufacturer's instructions. For assessing cross-sectional area (CSA) of CM, sections were stained with 20 mg/ml lectin wheat germ agglutinin (WGA) FITC (Sigma–Aldrich) and mounted with Prolong Gold (Invitrogen), random fields were photographed and 150 cells were counted to calculate CSA using semiautomatic AxioVision software (Zeiss). Microscopic images were captured with a digital microscope (IX70, Olympus). All sections were stained with Hoechst 55532 (Sigma-Aldrich) to visualize

nuclei. Sections were stained with DirectRed80 for 1 h (Sigma–Aldrich) for Sirius Red staining. Fibrosis was quantified using ImageJ.

Analysis of the microtubule network

To analyze the density and complexity of microtubule networks as well as the orientation of individual network components, a published protocol (9) for the analysis of membrane networks in CMs was adapted using the image processing program Fiji. Probe preparation and data analysis was done with the help of SFB 1002 service unit (S02 High resolution fluorescence microscopy and integrative data analysis). Confocal images of fixed, α -tubulin stained ventricular myocytes were used for the microtubule network analysis. Initially, ROIs that enclosed the microtubule network were defined. After background subtraction and smoothing, a statistical region merging algorithm (10) was applied to these ROIs. Next, the ROIs were converted into binary images by application of a defined threshold. Binary images of the processed ROIs were then skeletonized using the Fiji plugin "Skeletonize (2D/3D)". Successful skeletonization was confirmed by superposition of the original α -tubulin images and the skeletonized image as shown in Fig. 3G. Quantitative skeleton properties like length and the number of network junctions were automatically analyzed with the plugin "Analyze Skeleton (2D/3D)" (11) and normalized to the area of each ROI.

Luciferase reporter assay

Genomic regions of interest were amplified and cloned upstream of a minimal promoter in KpnI and XhoI digested pGL4.25 (Promega). A fragment located ~150 kb upstream of the Tbx20 gene and of ~120 kb upstream of the Hand2 gene (containing two and four TCF/LEF consensus sites, respectively) was cloned for luciferase assay into the pGL4.25 vector system. TSA201 cells were co-transfected with pGL4.25-Hand2 enhancers (enh) and -Tbx20enh, pCDNA3.1- β -catenin Δ ex3 and pBabeX-Gata4 expression vectors with Turbofect (Thermo Scientific) transfection reagent. Empty plasmids were used for adjustment of equal DNA content per transfection. 40 h after transfection the dual luciferase assay were performed and measured in a GloMax-96 Microplate Luminometer (Promega). Luciferase activity was normalized to Renilla luciferase activity and expressed as fold change against the empty vector pGL4.25. Every experiment was done in technical and biological triplicates.

SUPPORTING REFERENCES

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Supporting figures and figures legends

Figure S1: Regulation of Wnt nuclear components upon cardiac pressure-overload. (A) Base mean values, representing the normalized reads count for each transcript by DESeq2, of TCF/LEF factors in the murine and human normal adult heart. (B) Validation of the TAC-induced mouse model. Trans-aortic gradient measurements for controlling successful banding; echocardiography analysis showing heart-to-body weight (HW/BW) ratio and fractional area shortening (FAS) in 3-days, 2-weeks and 8-weeks post-TAC mice compared to sham controls, $n \geq 5$. (C,D) Additional immunoblots depicting protein expression of β -catenin and TCF7L2 in 3 days and 8 weeks-post TAC heart tissue and their densitometric quantification ($n \geq 4$). (E) Immunoblots depicting protein expression of β -catenin and TCF7L2 in 2 weeks-post TAC heart tissue ($n \geq 3$). (F) Immunoblots depicting nuclear and whole lysates protein expression of β -catenin and pSer⁶⁷⁵- β -catenin in 6 weeks-post TAC heart tissue ($n \geq 3$). (G) Densitometric quantification of β -catenin and pSer⁶⁷⁵- β -catenin and TCF7L2 protein normalized to GAPDH in human ventricular biopsies for Figure 1G (NF: $n=2$; DCM: $n=6$; ICM: $n=6$). GAPDH was used as protein loading control in C,D and E. TPT1 was used additionally as a loading control in E. TBX5 and GAPDH were used for nuclear and cytosolic fractions as controls respectively, in F.

Figure S2: Validation of β -cat ^{Δ ex3} model. (A) Scheme showing inducible CM-specific β -catenin stabilization by crossing a mouse possessing a *Ctnnb1* allele with *loxP*-flanked exon 3 (β -catenin^{flxed-ex3}), with a *Myh6*-promoter driven tamoxifen (TX)-inducible-Cre expressing line. The recombined allele (β -cat ^{Δ ex3}) produces a stabilized truncated GSK3 β -degradation-resistant β -catenin. (B) PCR indicating successful recombination 3 and 21 days post-TX induction in Cre^{pos} and β -cat ^{Δ ex3} cardiac ventricles. 900 bp: WT β -catenin; 700 bp: truncated β -catenin. Confocal image of β -catenin (magenta) and DAPI (blue) in Cre^{pos} and β -cat ^{Δ ex3} isolated CM showing perinuclear/nuclear accumulation, $n=3$. (C) Scheme illustrating the time-points of analyses post-tamoxifen (TX) administration in *Myh6*-merCREmer/ β -cat wild-type (WT) (Cre^{pos}), β -catflox-exon3 (Cre^{neg}) controls and *Myh6*-merCREmer/ β -catflox-exon3 (β -cat ^{Δ ex3}) mice. (D) Systolic, caffeine-induced Ca²⁺ transients and systolic half-times intracellular calcium relaxation (RT50%) in β -cat ^{Δ ex3} and Crepos cardiomyocytes. Data are mean \pm SEM; ANOVA, Bonferroni's multiple comparison test. Scale bar: 10 μ m.

Figure S3: Cell cycle activity and cytoskeletal structure analyses in β -cat ^{Δ ex3} CM. (A) Gene Ontology (GO) biological processes of downregulated genes ($\log_2FC < -0.5$, $p < 0.05$) in β -cat ^{Δ ex3} ventricles. All enriched pathways depicted are significant with $p \leq 0.05$. (B) Representative immunofluorescence images of cardiac Troponin T (CTNT, green) and Ki67 (magenta) in isolated CM in β -cat ^{Δ ex3} and Cre^{pos} control. (C) Table showing cell cycling genes upregulated in ventricles of β -cat ^{Δ ex3} and TAC-induced hearts along with immunofluorescence staining of CTNT (green), Ki67 (magenta) in 6-weeks post-TAC myocardium. (D) CTNT (green) and N-cadherin (NCADH, magenta) in Cre^{pos} and β -cat ^{Δ ex3} cardiac ventricular tissue

and (E) Alpha-ACTININ (ACTN2) (magenta) and NCADH (green) in isolated CM from β -cat ^{Δ ex3} and Cre^{pos} control. DAPI was used as a nuclear stain (blue). Scale bar (b, c and d) 20 μ m; (E) 10 μ m.

Figure S4: TCF7L2 occupancy profiles and qPCR validations in the adult heart. (A) Occupancy profiles of TCF7L2 on *Axin2* and *SP5* classical Wnt target genes in the normal (green) and β -cat ^{Δ ex3} hearts (pink). (B) Fold enrichment of TCF7L2 at *Axin2* binding site in comparison to IgG in adult hearts validating the TCF7L2-ChIP protocol. (C) Occupancy profiles of TCF7L2 and H3K27ac on *Tcf7l2* gene in the normal and β -cat ^{Δ ex3} hearts.

Figure S5: Downregulated genes not directly bound to TCF7L2 in β -cat ^{Δ ex3} ventricles. (A) Venn diagram of genes bound by TCF7L2 (orange) with upregulated (violet) or downregulated (green) genes with log₂FC \geq 0.5, p \leq 0.05 in β -cat ^{Δ ex3} ventricles. (B) Venn diagram depicting genes bound by TCF7L2 (green, 977) and upregulated with log₂FC \geq 0.5, p \leq 0.05 (red, 376) in β -cat ^{Δ ex3} ventricles. Black arrow represents upregulated genes but not bound by TCF7L2, which annotated to mitotic and cell cycle processes.

Figure S6: GATA4 co-occupancy and co-regulation with TCF7L2. (A) CentriMo motif density search on TCF7L2-bound regions in β -cat ^{Δ ex3} hearts showing GATA motif significantly enriched (p=5.4E-4). (B) GO biological processes of GATA4-specific, TCF7L2-specific and GATA4-TCF7L2 commonly bound genes. (C) Scheme illustrating ChIP-protein isolation and validating the GATA4-ChIP. Enrichment of GATA4 on its known target promoter *Nppa* is shown, n=2. Validation of anti-GATA4 IP depicted by a correct pull-down as detected by GATA4 immunoblot is shown (*heavy chain). (D) Immunoblot showing β -catenin expression in both nuclear and cytosolic fractions of healthy cardiac ventricular tissue, n=2. GAPDH and TBX5 were used as cytosolic and nuclear fraction controls respectively. (E) ChIP-qPCR analyses for GATA4 binding to *Tbx3* enhancer locus in β -cat ^{Δ ex3} and 6 weeks post-TAC (Wnt-active) hearts (n=3). Relative fold enrichment was calculated with respect to IgG control, normalized to 10% input chromatin.

Figure S7: β -catenin loss of function inducible model. (A) Scheme of the CM-specific β -catenin loss of function (β -cat ^{Δ ex2-6}) mouse model, depicting the locus truncation and primer binding sites for genotyping with corresponding recombination PCR. (B) Immunoblot showing reduction in total β -catenin in β -cat ^{Δ ex2-6} ventricular tissue compared to Cre^{pos} controls. (C) Trans-aortic gradient measurements post-TAC confirming a homogenous induced-pressure overload in all groups for validation of functional data. (D) Immunoblots showing expression of β -catenin and pSer⁶⁷⁵- β -catenin in 6 weeks post-TAC and sham controls in Cre^{pos} and β -cat ^{Δ ex2-6} heart lysates. (E) Relative (Rel) transcript levels of *Axin2*, *Hand2* and *Dstn* in β -catenin downregulation and control cardiac ventricles, n \geq 7. (F) Relative transcript levels of *Hand2* and *Rock2* upon β -catenin downregulation in late stages post-TAC (6-weeks) and corresponding

controls, $n \geq 7$. **(G)** CHIP-qPCR analyses for GATA4 binding to *Tbx3* enhancer locus in normal (WT), 6 weeks post-TAC (WT) and β -cat ^{Δ ex2-6} TAC hearts ($n=3$). Relative fold enrichment was calculated with respect to IgG control, normalized to 10% input chromatin. *Tbp* was used for transcript normalization in E and F. GAPDH was used as loading control in B and D.

Figure S1

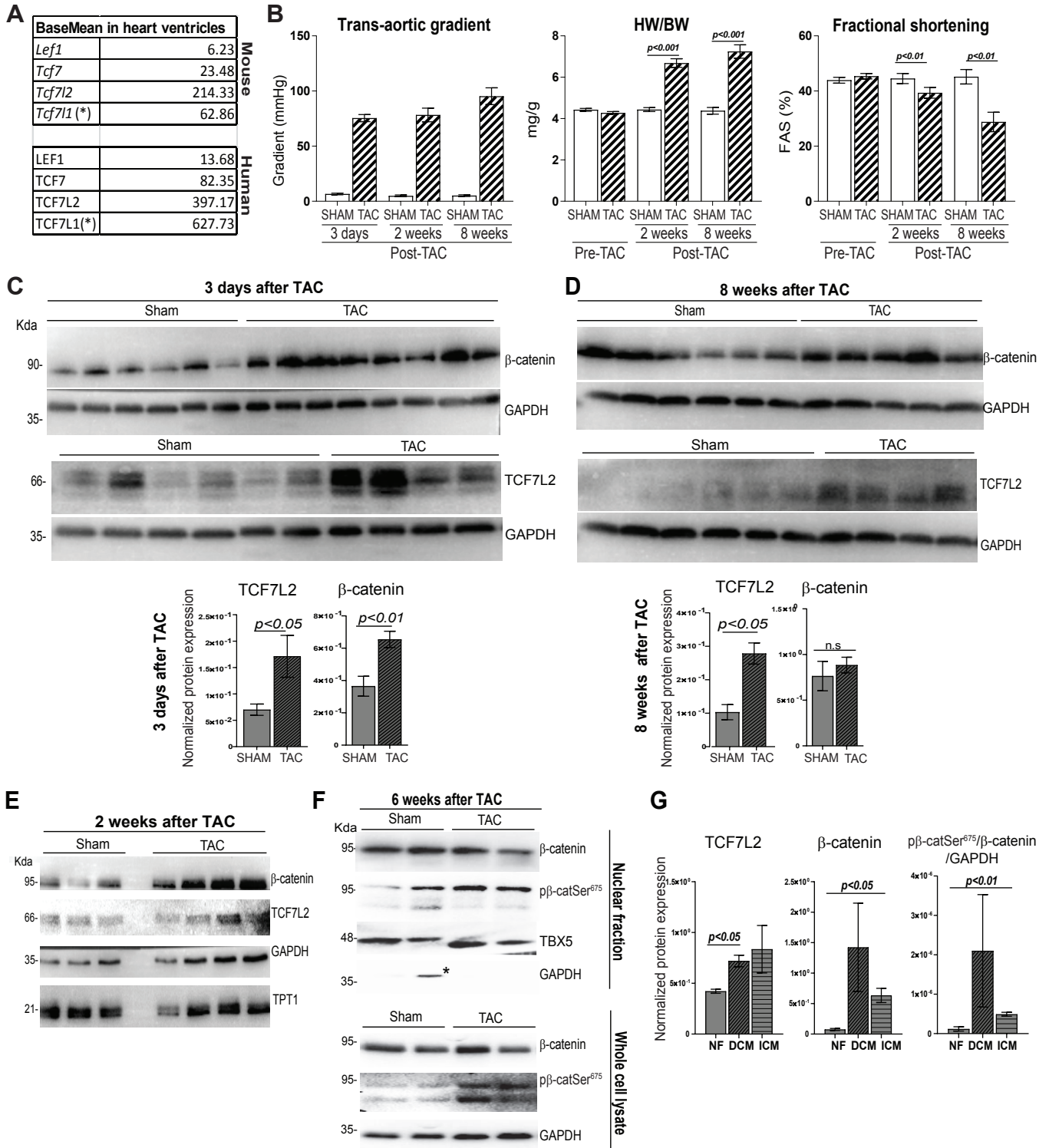


Figure S2

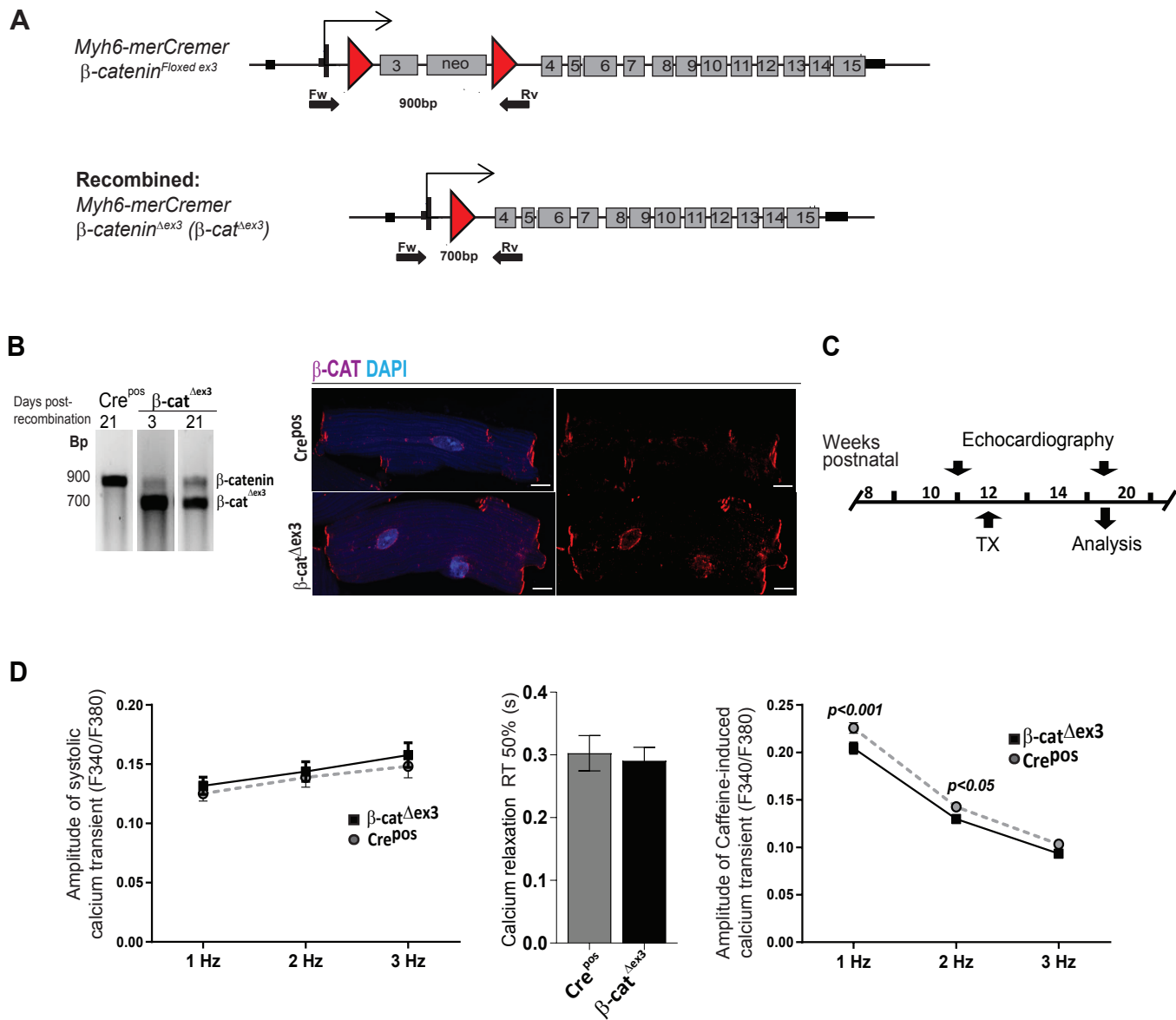
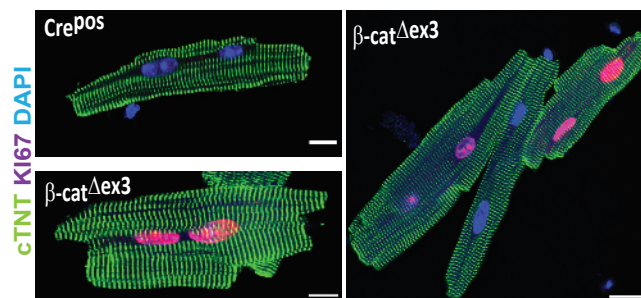


Figure S3

A

GOID	GO Term (Downregulated genes log ₂ fold>1.0)	Term P Value
GO:0086043	action potential	9.20E-05
GO:0050433	regulation of catecholamine secretion	6.00E-04
GO:0060078	regulation of postsynaptic membrane potential	4.30E-03
GO:0086010	membrane depolarization during action potential	9.60E-03
GO:0055117	regulation of cardiac muscle contraction	1.60E-02
GO:0090280	positive regulation of calcium ion import	2.70E-02
GO:0031644	regulation of neurological system process	3.90E-02
GO:0017158	regulation of calcium ion-dependent exocytosis	4.20E-02
GO:0042312	regulation of vasodilation	4.30E-02

B

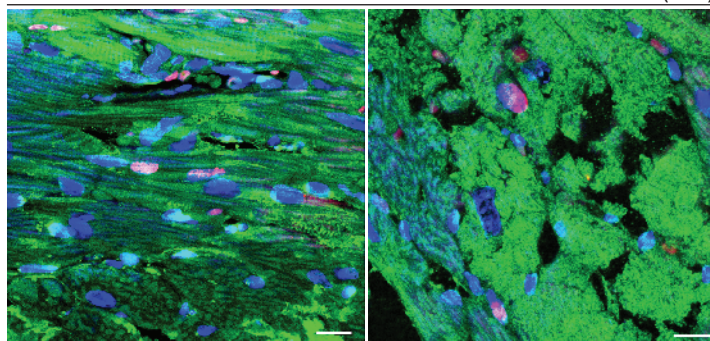


C

	log ₂ FC	padj	
β-cat ^{Δex3}	<i>Ccna2</i>	1.75	2.73E-14
	<i>Ccnb1</i>	1.36	1.27E-05
	<i>Ccnb2</i>	1.36	1.04E-05
post-TAC	<i>Ccna2</i>	3.04	5.62E-42
	<i>Ccnb2</i>	3.17	3.75E-36
	<i>Ccnb1</i>	3.10	7.36E-17

cTNT KI67 DAPI

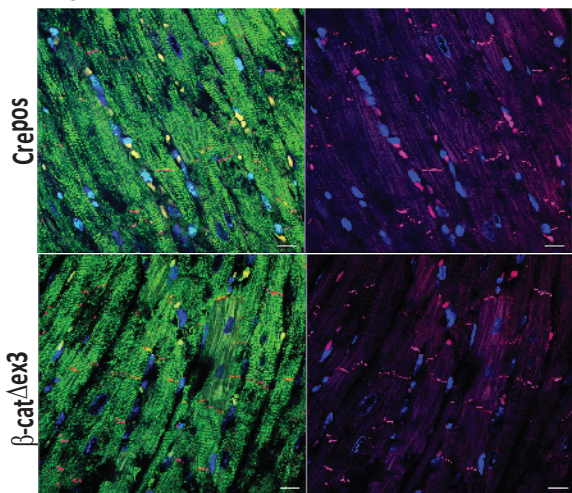
Post-TAC LV (WT)



D

cTNT NCAD DAPI

NCAD DAPI



E

ACTN2 NCAD

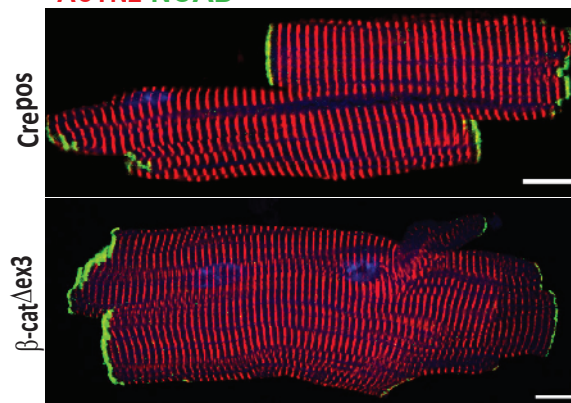


Figure S4

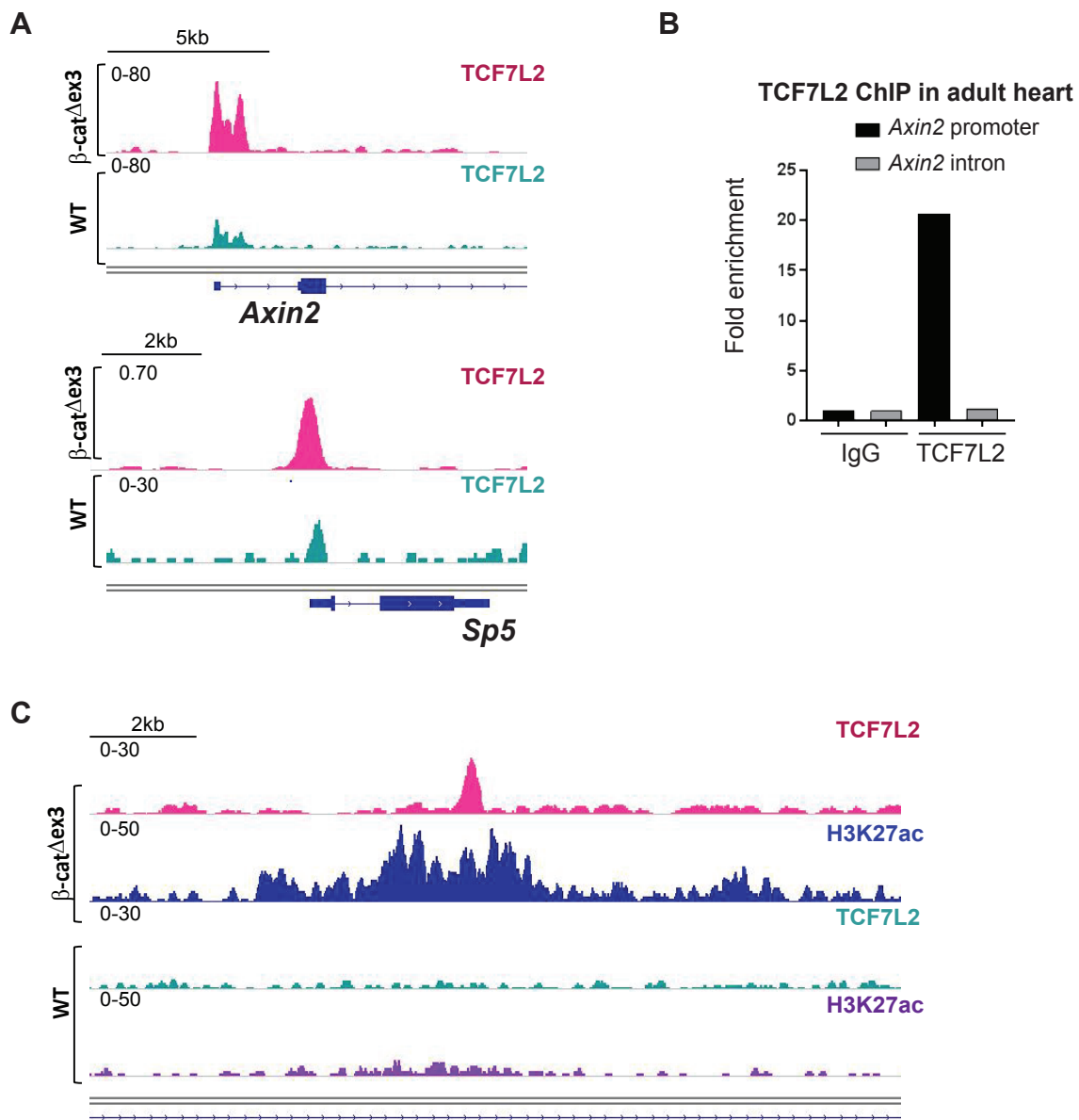
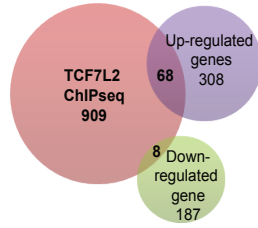
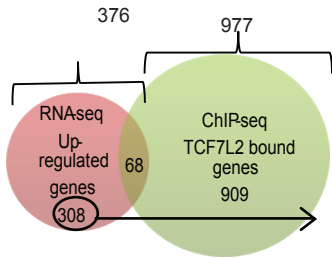


Figure S5

A



B



GO: Biological processes

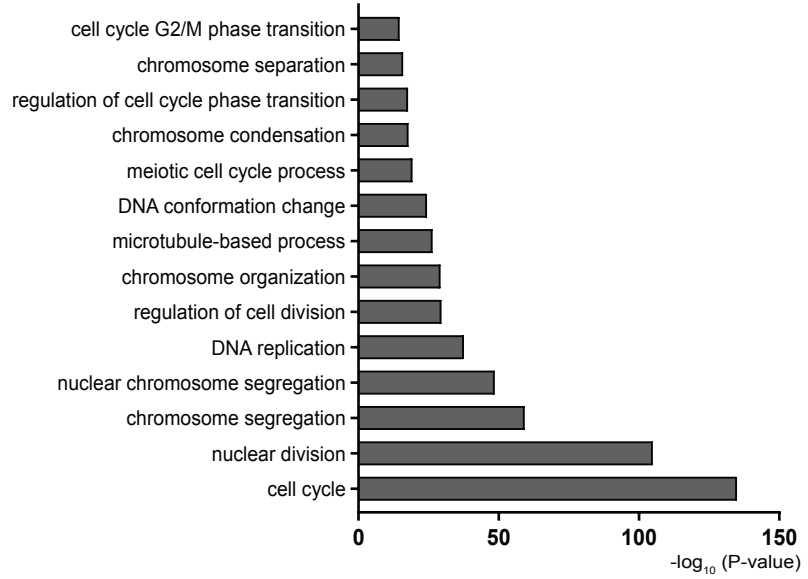


Figure S6

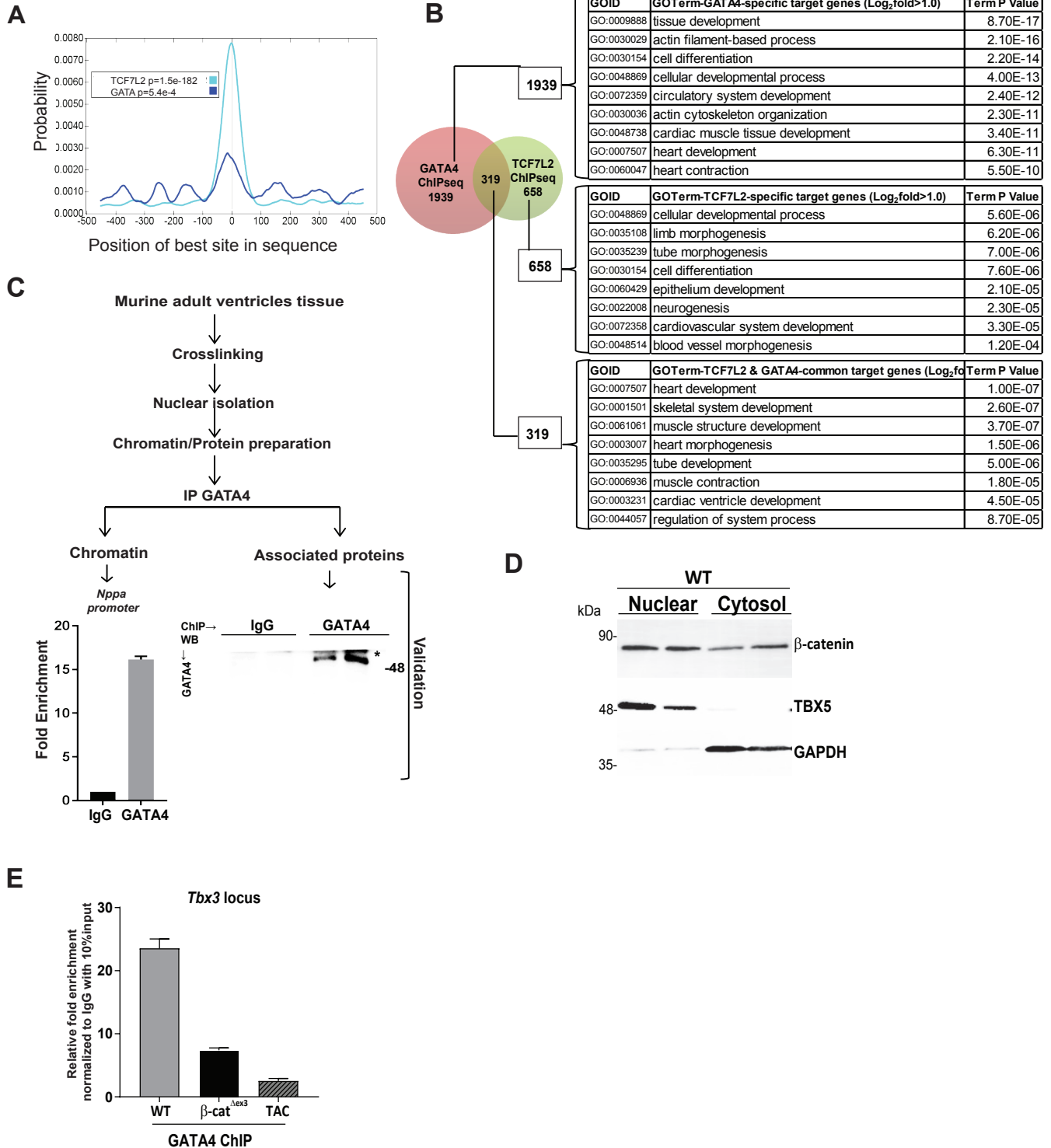


Figure S7

