

Supplementary Materials for

Enhancing KLF15 Activity in Cardiomyocytes: A Novel Approach to Prevent Pathological Reprogramming and Fibrosis via Nuclease-Deficient dCas9VPR

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Materials and Methods

CRISPR/Cas9 gene editing in human induced pluripotent stem cells for KLF15 loss of function generation. *KLF15* loss of function in CRISPRa-human induced pluripotent stem cells (hiPSCs) (RUCDRi002-A-15 clone 8) was generated by ribonucleoprotein-based CRISPR/Cas9 targeting exon 2 of the *KLF15* gene, as described previously.¹ Following nucleofection, hiPSCs were singularized, and individual clones were screened for successful editing by Sanger sequencing. One hiPSC line with a heterozygous 31bp out-of-frame deletion (RUCDRi002-A-35 clone 2) and one hiPSC line with a homozygous 31bp out-of-frame deletion (RUCDRi002-A-35 clone 11) were selected for further characterization. For molecular karyotyping, genomic DNA of hiPSC clones was sent for genome-wide analysis via Illumina BeadArray (Life&Brain). Digital karyotypes were analyzed in GenomeStudio v2.0 (Illumina) with the CNV partition algorithm 3.2.0 (default settings). Copy number events were reported if larger than 3.5×10^5 bps and 1×10^6 bps for loss of heterozygosity. Pluripotency analysis was performed via immunocytochemistry and flow cytometry, as previously described.¹

Cell culture. HEK293T cells (Takara) were cultured in DMEM (Gibco) supplemented with 10 % v/v fetal bovine serum (FBS) (Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco). For passaging, cells were washed with DPBS (Gibco) and incubated in TrypLE Express (Gibco) for 5 min at 37 °C. Cells were resuspended in culture medium and centrifuged at 300 *g* for 5 min at 4 °C. Cells were counted with a Casy Cell Counter (OmniLabs). The day before transfections, 4×10^5 cells were plated in a 12-well dish and transfected the following day with a total of 2 µg of plasmid DNA except otherwise stated. Cells were cultured for 48 h until further downstream analyses.

Engineered human myocardium (EHM) and *in vitro* stress model. EHM were generated as described previously² by combining hiPSC-cardiomyocytes with human foreskin fibroblasts and mixing in a bovine collagen matrix (Collagen Solutions). Each EHM contained 3.85×10^5 hiPSC-cardiomyocytes and 1.65×10^5 human foreskin fibroblasts. Tissues were cast into a 48 EHM multi-well plate (myriamed GmbH), and EHM medium² with 5 ng/mL TGFβ1 (Peprotech) was added 1 hour after casting. EHM were cultured for 3 days in IMDM (Gibco) based EHM medium supplemented with 5 ng/mL TGFβ1. From day 3 onwards, EHM were cultured in IMDM (Gibco) based EHM medium without TGFβ1. From day 14 onwards, EHM were cultured in alpha-MEM based EHM medium supplemented with 4 % v/v B27 no insulin (Thermo Fisher Scientific), 1 % MEM non-essential amino acid solution (Gibco), 100 ng/mL IGF-1, 5 ng/mL VEGF165, 10 ng/mL FGF-2 (all Peprotech), 100 U/mL penicillin, and 100 µg/mL streptomycin. On day 28, EHM were divided into the flexible pole culture group (Flex) or transferred to metal pole holders to induce mechanical stress (Fix) and cultured under the same conditions for an additional 7 days.

Contractile force assessment of EHM was performed as described previously³. Isometric force assessment of EHM was performed in a 37 °C thermo-regulated organ bath (Fohr Medical Instruments) containing carbogen (95 % O₂, 5 % CO₂) saturated Tyrode's solution (119.8 mM NaCl, 5.4 mM KCl, 1.05 mM MgCl₂, 0.2 mM CaCl₂, 0.42 mM NaH₂PO₄, 22.6 mM NaHCO₃, 5.05

mM glucose, and 0.28 mM ascorbic acid). EHM were electrically field stimulated with monophasic pulses of 200 mA, 5 ms, and 1.5 Hz. Baths were initially adjusted to 1.8 mM Ca^{2+} and each EHM incrementally lengthened according to the Frank-Starling principle to achieve the maximum contractile force (F_{max}). A Ca^{2+} concentration response (0.2 - 4 mM) was performed to determine the $[\text{Ca}^{2+}] \text{EC}_{50}$, and assessment of the force-frequency response (1, 1.5, 2 and 3 Hz) was performed at this Ca^{2+} concentration.

Tissue dimensions were assessed with a Stereo Lumar.V12 stereomicroscope (Carl Zeiss) and cross-sectional areas (CSA [mm^2]) were estimated based on the tissues' diameters. Volumes (mm^3) were calculated by multiplying the CSA by the length of the tissues. Material properties of EHM were assessed by destructive tensile strength measurements with an RSA-G2 rheometer (TA Instruments) at 37 °C in DPBS (Gibco) under a constant linear strain rate of 0.03 mm/s. The force values obtained (F [mN]) were divided by the CSA to obtain stress values (s [kPa]), which were plotted against the strain values (e [DL/ L_0]) using GraphPad Prism8 (GraphPad Software, Inc.). Important points in the stress-strain curves were identified, including the yield strain (e_{yield} , end of elastic region, elasticity) and ultimate strain (e_{ultimate} , strain at rupture point, extensibility). The following parameters were additionally obtained: Young's modulus representing EHM stiffness (slope of the curve in the elastic region identified by linear regression) and ductility ($e_{\text{ultimate}} - e_{\text{yield}}$, plasticity) ⁴. EHM contractility was determined using an isolated organ bath apparatus ⁴ (IOA – 5301; Föhr Medical Instruments GmbH) under isometric contractions in Tyrode's buffer (120 mM NaCl, 1 mM MgCl_2 , 0.2 mM CaCl_2 , 5.4 mM KCl, 22.6 mM NaHCO_3 , 4.2 mM NaH_2PO_4 , 5.6 mM glucose, and 0.56 mM L-ascorbic acid) at 37 °C and 5% CO_2 / 95% O_2 , and EHM were stretched according to Frank-Starling-Mechanism to exert maximum force at 1.8 mM Ca^{2+} and electrical pacing at 1.5 Hz and 5 ms square pulses of 200 mA. The Ca^{2+} response was measured by increasing the concentration in 0.4 mM steps and 1.5 Hz and 5 ms square pulses of 200 mA electrical pacing. Response to an adrenergic stimulus was tested with 1 μM isoprenaline at the $\text{Ca}^{2+} \text{EC}_{50}$ and electrical pacing at 1.5 Hz and 5 ms square pulses of 200 mA.

RNA isolation and quantitative transcript analysis. Cells and tissue were lysed in 1 mL Trizol (Thermo Fisher Scientific) using a Tissue Lyser II (Qiagen). Chloroform (200 μL) was added and samples were mixed vigorously. Samples were centrifuged at 12,000 g for 15 min at 4 °C. The aqueous layer was collected and added to 500 μL isopropanol and 1 μL GlycoBlue (Thermo Fisher Scientific). RNA was precipitated by centrifugation at 12,000 g for 10 min at 4 °C. The RNA pellet was washed with 1 mL 75 % ethanol and centrifuged for 5 min at 7,600 g for 5 min at 4 °C. RNA was resuspended in nuclease-free H_2O . RNA concentrations were determined using a Nanodrop (Implen). For quantitative PCR, RNA was transcribed into cDNA using an MMLV Reverse Transcription Kit (Promega). Quantitative PCR was performed using the Takyon Mix (Takyon) and a 7900 HT (Applied Biosystems). A list of primers is provided in **Table 2**. When indicated, RNA counts were determined using a Nanostring (Nanostring Technologies) with 100 ng of total RNA and according to manufacturer's protocol.

The NanoString nCounter platform was used for parallel transcript quantification using 60 ng of total RNA per sample as input and analyzed using the nSolver 4.0 software (NanoString Technologies, Inc.). The threshold for gene expression analyses was set to > 50 transcript counts. Transcripts were normalized to TBP expression.

Bulk RNA sequencing. RNA quality was assessed by measuring the RNA integrity number (RIN) using a Fragment Analyzer HS Total RNA Kit (Advanced Analytical Technologies, Inc.). Library preparation for RNA-Seq was performed in the STAR Hamilton NGS automation using the Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (#N° 20040525) and the ID for Illumina RNA UD Indexes Set A, Ligation with 96 Indexes (#N° 20040553) starting from 300 ng of total RNA. The size range of the final cDNA libraries was determined by applying the SS NGS Fragment 1- to 6000-bp Kit on the Fragment Analyzer (average 340 bp). Accurate quantification of cDNA libraries was performed by using the DeNovix DS-Series System. cDNA libraries were sequenced by using an S2 flow cell NovaSeq6000; 100 cycles, 25 Mio reads/sample (Illumina). Sequence images were transformed with BaseCaller Illumina software to BCL files and demultiplexed to fastq files with bcl2fastq v2.20.0.422. Sequencing quality was determined using FastQC v. 0.11.5 software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Sequences were aligned to the reference genome Homo sapiens (hg38 version 96, https://www.ensembl.org/Homo_sapiens/Info/Index) using STAR aligner v. 2.5.2a, allowing for 2 mismatches within 50 bases. Subsequently, read counting was performed using featureCounts v. 1.5.0-p1.

GWAS analysis. The 29 cardiovascular GWAS were downloaded from the GWAS catalog, including all available child traits (GWAS catalog, version 2020-12-02, <https://www.ebi.ac.uk/gwas/docs/file-downloads>). Indels, duplicated SNVs, and all SNVs from GWAS not based on a European cohort were excluded. Next, the SNV in linkage disequilibrium based on the data provided from SNI⁵ using the European cohort as population and an Linkage Disequilibrium (LD) threshold of 0.8 were determined. We aimed to identify SNVs that have a regulatory potential (rSNVs), meaning that these SNVs affect at least one TF binding site and thus may change target gene expression. To do so, the software SNEEP⁶ (link GitHub: <https://github.com/SchulzLab/SNEEP>) was applied. As TF motifs, 817 non-redundant human motifs from the JASPAR (version 2022)⁷, HOCOMOCO⁸, and Kellis ENCODE motif database⁹ were used. The ‘wildtype’ allele, the allele protecting against the disease, was compared against the risk allele. Within the version of the GWAS Catalog used in this study, alleles were provided in the assumed order; however, this ordering had to be verified for proxy SNPs. Therefore, available summary statistics for the analyzed diseases were downloaded, and the direction of effect of proxy SNPs was determined based on the beta coefficient, with negative beta coefficients indicating a protective effect and positive beta coefficients indicating support for the phenotype.

SNEEP returns regulatory SNVs (rSNVs) and provides information on whether an rSNV increases (gain of function) or decreases (loss of function) the binding behavior of a transcription factor (TF) at its binding site. For each disease, the frequency with which a TF was associated with gain or

loss of function was quantified. A two-sided binomial test was applied to assess whether a TF was significantly more frequently associated with either loss or gain of function. For the bidirectional bar plot, only TFs that showed a significant association with either loss or gain of function in at least three analyzed diseases were considered.

Flow cytometry. For flow cytometry (Flow), cells were fixed in ice-cold 70% v/v ethanol, permeabilized for 10 min at room temperature, and blocked with DPBS with 5% v/v FBS, 1% w/v BSA and 0.5% v/v Triton X-100 for 1 hour at room temperature. Cells were incubated with the primary antibodies against ACTN2, OCT3/4, NANOG and TRA1-60 (**Table 3**) followed by incubation with secondary antibody. Nuclei were stained with 10 µg/ml Hoechst 33342 (Thermo Fisher Scientific) for 10 min at room temperature. Cells (10,000 events per sample) were analyzed with a BD LSR II Flow Cytometer and BD FACSDiva Software (both Becton Dickinson) or Flowing Software 2.5.1 (Turku Centre for Biotechnology, University of Turku, Finland).

Immunoblotting whole cell lysates. For preparing samples for immunoblotting (IB), cells and tissues were lysed in 350 mM NaCl, 20 mM HEPES, 1 mM MgCl₂, 0.5 mM EDTA, 0.1 mM EGTA, 1 % v/v Igepal CA-630, 20 % v/v glycerol including PhosSTOP (Sigma Aldrich) and cOmplete Mini Protease Inhibitor (Sigma Aldrich) and centrifuged for 20 min at 14,000 g, 4 °C. Supernatant was collected and protein concentration was determined with RotiQuant (Carl Roth).

For nuclear and cytoplasm fractionation, cells were swollen in Hypotonic Buffer (10 mM KCl, 10 mM HEPES, 2 mM MgCl₂, 100 µM EDTA, 1 mM DTT) including PhosSTOP and cOmplete Mini Protease Inhibitor for 15 min on ice. Igepal CA-630 was added at a final concentration of 1 % v/v and incubated another 5 min on ice. Samples were centrifuged for 5 min, 1,500 g, 4 °C. The supernatant was collected and kept as the cytoplasm fraction. The pellet was washed ones in Hypotonic Buffer and centrifuged again for 5 min at 1,500 g, 4 °C. Hypertonic Buffer (300 mM NaCl, 50 mM KCl, 50 mM HEPES, 100 µM EDTA, 1 mM DTT, 10 % v/v Glycerol) including PhosSTOP and cOmplete Mini Protease Inhibitor was added to the pellet and incubated for 25 min on ice before addition of 1 % v/v Igepal CA-630 and further incubation for 5 min on ice. Samples were centrifuged for 5 min at 16,000 g, 4 °C. The collected supernatant was kept as the nucleoplasm fraction. Protein concentration was quantified with RotiQuant (Carl Roth) using a FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices). An amount of 1-20 µg of total protein was prepared using 4x concentrated loading dye (100 mM TRIS-HCl, 0.025 % v/v bromophenol blue, 8 % w/v SDS, 10 % v/v glycerol, 10 % v/v β-mercaptoethanol) and loaded on Mini-PROTEAN TGX Precast Gels (Bio-Rad). Electrophoresis was performed in 25 mM TRIS ultrapure, 190 mM glycine, 0.1 % w/v SDS at 110 V for 2 h. Transfer of proteins onto methanol activated PVDF membranes was performed in 25 mM TRIS ultrapure, 190 mM glycine, 20 % v/v methanol) at 100 V for 2.5 h. Membranes were blocked and incubated in antibodies (see **Table 3**) in 5 % w/v non-fat dry milk (AppliChem) or 5 % w/v BSA in TBST (20 mM TRIS ultrapure, 150 mM NaCl, 0.1 % v/v Tween (Carl Roth), pH 7.6). Membranes were analyzed using femtoLUCENT PLUS-HRP Kit (G-Biosciences) and a ChemiDoc MP Imaging System with Image Lab Version 5.1 (Bio-Rad).

Chromatin Immunoprecipitation and sequencing. For Chromatin Immunoprecipitation (ChIP), hiPSC-cardiomyocytes were transduced with FLAG-HA-KLF15 open reading frame (ORF) or FLAG-HA-EGFP expressing lentivirus 5 days prior to the sample collections. Cells were fixed with 1 % v/v Formaldehyde/PBS for 15 min at room temperature. Cells were washed twice with PBS and centrifuged for 2 min at 12,000 g, 4 °C. Nuclei isolation was performed using 1 ml NucleiPreparation Buffer (0.15 M NaCl, 0.02 M NaF, 0.02 M EDTA, 0.05 M TRIS-HCl, 0.5 % v/v Igepal CA-630, 1 % v/v Triton X-100, cOmplete Mini protease inhibitor (SigmaAldrich), PhosSTOP (Sigma Aldrich)) and mechanical disruption. Nuclei were pelleted and washed again in 500 µl Nuclei Preparation Buffer. Sonication Buffer I (0.01 M EDTA, 0.05 M TRIS-HCl, 1% w/v sodium dodecyl sulfate, cOmplete Mini protease inhibitor, PhosSTOP) was added to the nuclei and incubated for 10min on ice. Sonication Buffer II (0.15 M NaCl, 0.02 M NaF, 0.02 M EDTA, 0.05 M TRIS-HCl, 1 % v/v Igepal CA-630, cOmplete Mini protease inhibitor, PhosSTOP) was added to samples and sonicated in 1.5 ml Bioruptor PlusTPX microtubes (Diagenode) in a Bioruptor sonication device (Diagenode) for 13 cycles (30 s On/Off per cycle). Samples were centrifuged for 2 min at 12,000 g, 4 °C. Supernatant was pre-cleared by incubation with Protein G Sepharose beads (cyvita) for 45 min at 4 °C. Beads were pelleted by centrifugation for 5 min at 2000 g, 4 °C and 10 % of each sample was kept as input. The remaining sample was divided and either incubated with 2 µg of anti-FLAG or anti-HA or normal mouse IgG and supplemented with 500 µl Immunoprecipitation Buffer (0.15 M NaCl, 0.02 M NaF, 0.02 M EDTA, 0.05 M TRIS-HCl, 1% v/v Igepal CA-630, 0.5 % w/v sodium deoxycholate, 0.1 % w/v sodium dodecyl sulfate, cOmplete Mini protease inhibitor, PhosSTOP) for 16 hours at 4 °C. Afterwards, Protein G Sepharose beads (cyvita) were added to samples and incubated for 2 hours at 4 °C. Samples were centrifuged for 2 min at 12,000 g, 4 °C and supernatant was removed. Bead-antibody-chromatin complexes were washed with Immunoprecipitation Buffer twice (without cOmplete Mini protease inhibitor and PhosSTOP), with IP Wash Buffer (0.5 M LiCl, 0.02 M NaF, 0.02 M EDTA, 0.1 M TRIS-HCl, 1 % v/v Igepal CA-630, 1 % w/v sodium deoxycholate) twice, again with Immunoprecipitation Buffer four times (without cOmplete Mini protease inhibitor and PhosSTOP) and with TE Buffer (0.01 M TRIS-HCl, 0.001 M EDTA). To Bead-antibody-chromatin complexes pellet, 400 µl of Elution Buffer (50 mM NaCl, 50 mM TRIS-HCl, 5 mM EDTA, 1 % w/v sodium dodecyl sulfate, pH 7.5) was added and incubated at 65 °C for 90 min. Beads were pelleted for 2 min at 6,000 g and supernatant was collected. Inputs were supplemented with 400 µl Elution Buffer. To all samples 24 µl of 5 M NaCl and 8 µl of 20 mg/ml Proteinase K (Macherey Nagel) incubated for 16 hours at 62 °C. DNA was purified using NucleoSpin Gel and PCR clean-up (Buffer NTB Protocol) according to manufacturer's instructions (Macherey Nagel).

For sequencing, ChIP-seq libraries were performed using the DNA SMART CHIP-Seq Kit- 48 rxn (Takara bio) as manual's instructions and were sequenced on the NovaSeq6000 (Illumina) with 100 cycles, 25 Mio reads/sample. Adapter and quality trimming were performed with Trim Galore (v0.6; wrapper for Cutadapt and FastQC) using paired-end mode and NextSeq-specific quality trimming, followed by standard QC with FastQC. Trimmed reads were aligned to the human reference genome GRCh38 (Ensembl release 114 annotation) using STAR v2.7.10, and alignments

were processed with samtools v1.20 and bedtools v2.31.1 to retain high-quality, properly paired, non-duplicate reads for downstream analysis. Peak calling was performed with MACS3 v3.0.3 in paired-end mode using the corresponding Input or IgG as control to obtain enrichment tracks and narrowPeak peak sets for each comparison. For relative enrichment between treatment and control, additional MACS3 pileup tracks were generated and differential enrichment was quantified from treatment vs control using MACS3 bedGraph-based comparisons, from which differential peak sets were derived. Genomic annotation and enrichment relative to genes and transcription start sites were obtained with HOMER v4.11 using the hg38 annotation, and de novo motif discovery was performed with HOMER on MACS3 peak summits. Normalized coverage tracks were generated from filtered BAM files and MACS3 signal tracks and converted to bigWig format with UCSC utilities for visualization in IGV.

Immunoprecipitation and Mass spectrometry. For immunoprecipitation (IP), hiPSC-cardiomyocytes were transduced with FLAG-HA-KLF15 open reading frame (ORF) or FLAG-HA-EGFP expressing lentivirus 5 days prior to the sample collections. To crosslink interacting proteins, cells were incubated for 30 min at 37 °C in 100 µM DSP/PBS (Thermo Scientific) that was pre-dissolved before in DMSO accordingly to the product manual. The cells were then washed three times with PBS and Stop buffer (20 mM TRIS-HCl in PBS, pH 7.4) was added and incubated for 5 min at room temperature. The cells were washed twice with PBS and 1mL Pulldown buffer (20 mM NaPO₄, 150 mM NaCl, 2 mM MgCl₂ × 6 H₂O, 0.1 % v/v Igepal CA-630, 10 % v/v Glycerol, 10 mM NaF, 0.1 mM Na-Orthovanadate, 10 mM Na-Pyrophosphate, 1 mM DTT, cOmplete Mini protease inhibitor (SigmaAldrich)) was added to lysis the cells. The lysate was passed through 26G syringe 6 times and then 27G syringe 4 times. Lysate were incubated on ice for 30 min. Lysate was centrifuged at 14,000 g for 15 min at 4°C. To avoid unspecific precipitation, lysate was pre-cleaned with Protein G Sepharose beads (cyvita). For each sample, 100 µl beads were added and incubated for 1 hour at 4°C by rotating. To remove beads, lysate was centrifuged at 8,200 g for 1 min at 4°C. The lysate was collected and incubated with 1 µg of anti-FLAG or anti-HA or normal mouse IgG for 16 hours at 4 °C. Afterwards, Protein G Sepharose beads (cyvita) were added to samples and incubated for 2 hours at 4 °C. Finally, sepharose beads were carefully washed 3 times in Pulldown buffer by centrifugation at 8,200 g for 1 min. After removing the excess Pulldown buffer, the Sepharose beads containing the trapped proteins were shock-frozen in liquid nitrogen.

For Mass spectrometry (MS), the Sepharose beads were resuspended in 50 µl LYSE buffer (iST kit, PreOmics) and vortexed thoroughly. The proteins were eluted and denatured at 95 °C for 10 min with shaking (1000 rpm) and then cooled to room temperature. Protein digestion and peptide clean-up were performed using the iST kit (PreOmics) according to the manufacturer's instructions. Briefly, the proteins were digested with Trypsin/LysC for 1.5 hours at 37 °C with gentle shaking (500 rpm). The resulting peptides were cleaned up using iST cartridges and eluted into a 96-well microtiter plate. After complete drying at 45 °C in a Concentrator Plus (Eppendorf), the peptides were resuspended in 15 µl LC-LOAD buffer by vortexing for 5 min.

Peptides were analyzed by LC–MS/MS using an UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) coupled to an Orbitrap Eclipse Tribrid mass spectrometer. For each sample, 5 µl of peptide solution was loaded onto a PepMap Neo trap column (Thermo Fisher Scientific) for on-line concentration and desalting, followed by separation on an Aeros C18 column (1.9 µm, 25 cm × 150 µm; ProtumLink) using an Easy-Spray adapter. The analytical column was maintained at 60 °C. Peptide separation was achieved using a linear gradient of solvent A (0.15 % formic acid in HPLC-grade water) and solvent B (0.15 % formic acid in HPLC-grade acetonitrile) at a flow rate of 0.5 µl/min as follows: 0 – 5 min (5 % B), 5 – 7.5 min (5 – 10 % B), 7.5 – 45 min (10 – 35 % B), and 45 – 48 min (35 – 90 % B). The column was then rinsed with 90 % B for 5 min at 0.8 µl/min and re-equilibrated with 5 % B for 4 min at 0.8 µl/min and 3 min at 0.5 µl/min. The total run time per sample was 60 min.

MS analysis was performed in positive ion mode using electrospray ionization at 2.1 kV, with the ion transfer tube temperature set to 275 °C. Data were acquired in data-dependent acquisition (DDA) mode using collision-induced dissociation (CID) and a Top20 method. MS1 spectra were acquired in the Orbitrap at a resolution of 75,000 (at m/z 200) over a 375 – 1500 m/z range, with an AGC target of 3×10^6 and automatic maximum injection time. The RF lens was set to 30 %, and MS1 spectra were recorded in profile mode. For MS2, precursor ions were isolated in the quadrupole (isolation window = 1.6 m/z) and analyzed in the ion trap in rapid scan mode. CID was performed with a normalized collision energy of 35 %. The normalized AGC target was set to 250 %, and the maximum injection time was dynamic.

Immunohistochemistry and histology. For immunofluorescence (IF), cells were fixed in buffered 4 % paraformaldehyde for 15 min at room temperature, subsequently washed 3 times with DPBS, and permeabilized with 0.2 % w/v BSA, 0.3 % v/v Triton X-100 in PBS. Next, they were blocked in 5 % w/v BSA, 0.1 % Triton X-100 in PBS, and incubated with antibodies (see **Table 3**) in 2 % BSA, 0.1 % v/v Triton X-100 in DPBS. Murine heart tissue was dissected, rinsed in PBS, fixed in 4 % paraformaldehyde overnight at 4 °C, embedded in paraffin, and sectioned at 3-µm thickness using a Leica microtome. Paraffin-embedded sections were de-paraffinized and rehydrated. For immunostaining, the antigen was unmasked by microwaving (180 Watt) sections for 10 min in 10 mM sodium citrate buffer, pH 6. Sections were blocked at room temperature for 1 h with 5 % w/v BSA and 0.1% v/v Triton X-100 in DPBS. Primary antibodies (see **Table 3**) were incubated overnight at 4 °C. Next, sections were washed in DPBS and incubated with the corresponding secondary antibodies (Molecular Probes)¹⁷. Sections were stained with DirectRed80 for 1 hour (Sigma–Aldrich) presented as Sirius Red staining to evaluate fibrosis¹⁰. Images were taken with a Zeiss LSM 710 NLO confocal microscope and analyzed with ZEN lite 2012 software.

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Samples were reconstituted in 1× NuPAGE LDS Sample Buffer (Invitrogen) and applied to 4 – 12 % NuPAGE Novex Bis-Tris Minigels (Invitrogen). Samples were run 1 cardiomyocyte into the gel for purification and stained with Coomassie Blue for visualization purposes. After washing, gel slices

were reduced with dithiothreitol (DTT), alkylated with 2-iodoacetamide and digested with trypsin (Gibco) overnight. The resulting peptide mixtures were then extracted, dried in a SpeedVac, reconstituted in 2 % acetonitrile/0.1 % formic acid/ (v/v) and prepared for nanoLC-MS/MS. All samples were spiked with a synthetic peptide standard used for retention time alignment (iRT Standard). Protein digests were analyzed on a nanoflow chromatography system (Eksigent nanoLC425) hyphenated to a hybrid triple quadrupole-TOF mass spectrometer (TripleTOF 5600+) equipped with a Nanospray III ion source (Ionspray Voltage 2400 V, Interface Heater Temperature 150 °C, Sheath Gas Setting 12) and controlled by Analyst TF 1.7.1 software build 1163 (all AB Sciex). In brief, peptides were dissolved in loading buffer (2 % acetonitrile, 0.1 % formic acid in water) to a concentration of 0.3 µg/µl. For each analysis, 1.5 µg of digested protein were enriched on a precolumn (0.18 mm ID × 20 mm, Symmetry C18, 5 µm, Waters) and separated on an analytical RP-C18 column (0.075 mm ID × 250 mm, HSS T3, 1.8 µm, Waters) using a 90 min linear gradient of 5 – 35 % acetonitrile/0.1 % formic acid (v/v) at 300 nl min⁻¹. Qualitative LC/MS/MS analysis was performed using a Top25 data-dependent acquisition (DDA) method with an MS survey scan of m/z 350 – 1250 accumulated for 350 ms at a resolution of 30,000 full width at half maximum (FWHM). MS/MS scans of m/z 180 – 1,600 were accumulated for 100 ms at a resolution of 17,500 FWHM and a precursor isolation width of 0.7 FWHM, resulting in a total cycle time of 2.9 s. Precursors above a threshold MS intensity of 125 cps with charge states 2⁺, 3⁺, and 4⁺ were selected for MS/MS, the dynamic exclusion time was set to 30 s. MS/MS activation was achieved by CID using nitrogen as a collision gas and the manufacturer's default rolling collision energy settings. Two technical replicates per sample were analyzed to construct a spectral library. For quantitative analysis, MS/MS data were acquired using data-independent acquisition (DIA) with 65 variable-size windows across the 400–1050 m/z range. Fragments were produced using rolling collision energy settings for charge state 2⁺ and were acquired over an m/z range of 350 – 1400 for 40 ms per segment. Including a 100 ms survey scan, this resulted in an overall cycle time of 2.75 s. 3 technical replicates were acquired for each sample. Protein identification was achieved using ProteinPilot Software version 5.0 build 4769 (AB Sciex) at “thorough” settings. The combined qualitative analyses were searched against the UniProtKB mouse reference proteome (revision 12-2017, 60717 entries) augmented with a set of 52 known common laboratory contaminants to identify proteins at a False Discovery Rate (FDR) of 1 %. Spectral library generation and SWATH peak extraction were achieved in PeakView Software version 2.1 build 11041 (AB Sciex) using the SWATH quantitation microApp version 2.0 build 2003. Following retention time correction using the iRT standard, peak areas were extracted using information from the MS/MS library at an FDR of 1 %. The resulting peak areas were then summed to peptide and finally protein area values per injection, which were used for further statistical analysis in Perseus 1.5.6.0 software (Max Planck Institute for Biochemistry, Martinsried, Germany).

Seahorse measurement. The hiPSC-cardiomyocytes were seeded in 24-well Seahorse assay plates at a density of 1×10⁵ cells/well, 7 days before to the experiment day. Mitochondrial respiration was assessed using a Mito Stress Test. The cell culture medium was replaced with

Agilent (Santa Clara) XF assay medium containing 10 mM glucose and 1 mM sodium pyruvate one hour before assay initiation. The hiPSC-cardiomyocytes were then incubated for 60 min at 37 °C in the absence of CO₂. After three baseline respiration records, the ATP synthase inhibitor oligomycin A (final concentration: 1 μM, Sigma-Aldrich) was injected, followed by subsequent injection of the uncoupler FCCP (final concentration: 0.5 μM, Sigma-Aldrich), and complex I and III inhibitors rotenone (final concentration: 1 μM, Sigma-Aldrich) and antimycin A (final concentration: 1 μM, Sigma-Aldrich), respectively. Measurements of cellular respiration were obtained via a Seahorse XFe24 Analyzer. Total protein content served as the normalization factor for oxygen consumption rate (OCR) in each well. Respiration linked to ATP synthase activity was quantified as the difference in oxygen consumption rate (OCR) before and after the addition of oligomycin A. Maximal respiration was determined by subtracting post-rotenone/antimycin respiration from the post-FCCP oxygen consumption rate.

Epifluorescence Ca²⁺ measurements. Ca²⁺ imaging and epifluorescence microscopy were performed by seeding hiPSC-cardiomyocytes on Matrigel (Corning) coated 35mm glass bottom dishes (MatTek) in a density of 1×10⁵ and 1.5×10⁵ cells per dish. 7 days after plating, the cells were incubated in 1.5 ml Normal Tyrode's solution (NT) (140 mM NaCl, 5.4 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM glucose, and 1.8 mM CaCl₂ (pH 7.4)) and Indo-1 AM (final concentration: 1 μmol/L, Invitrogen) for 20 min at 37 °C. Following the IonOptix Calcium and Contractility System with fluorescence detection software program, 30 cells were selected, while being paced at 0.5 Hz and continuously perfused with NT containing the 1.8 mM Ca²⁺. After the selection process a protocol with the following steps was started: 3× 0.25 Hz, 3× 0.5 Hz, 3× 0.75 Hz, 3× 1.0 Hz, 1× 0.5 Hz, 3× 0.5 Hz. Fluorescence was recorded for 4 sec to 1 sec per step. For each frequency the mean of each cell was taken for the analysis with the IonWizard 6.3 software by IonOptix. Measurements were performed using the CYTOCYPHER inverted microscope. Basal measurements were conducted in 1× Tyrode's solution, and for β-adrenergic stimulation, cells were incubated with 1 μM isoprenaline for 3 min.

Sarcomere regularity and mitochondrial measurements. Measurements of sarcomere regularity were performed as described previously.¹¹ A total of 5×10⁴ hiPSC-cardiomyocytes were seeded on 18 mm round coverslips coated with Matrigel (Corning). Before fixation, the cells were washed twice with DPBS and incubated with 250 nmol/L MitoSpy Orange CMTMRos (BioLegend), a fluorogenic mitochondrial probe, for 30 min at 37 °C. The cells were then fixed with 4 % Roti-Histofix solution (Carl Roth) for 20 min at room temperature and subsequently blocked overnight at 4 °C in 1 % w/v BSA in PBS. To visualize the sarcomere structure, the hiPSC-cardiomyocytes were stained with antibodies targeting the Z disk and M line with anti-α-actinin and anti-Titin-M8/M9, respectively. Secondary antibodies were applied afterwards. Fluorescence images were captured using a Leica DMi8 microscope. 20 images of iPSC-CMs stained for α-actinin and titin were processed with a python script using Fast Fourier Transformation to determine the regularity of sarcomere organization.

For mitochondrial branch analysis, cells were stained with TOMM20 and TNNT2-FITC as described above. DAPI was used to counter stain nuclei at a final concentration of 10 µg/mL for 10 min at room temperature. High-resolution Z-stack images were acquired with a LSM880 Airy Scan (Carl Zeiss AG). Quantitative mitochondria network analyses were performed with Mitochondria Analyser (3D analyses)¹² for Fiji.¹³

Data and code availability. Single-cell sequencing data are available at NCBI GEO under accession: GSE315590 for scRNA-seq of mouse hearts following TAC or sham surgery (Dataset-1); GSE120064 for publicly available scRNA-seq data of mouse hearts following TAC (Dataset-2); and GSE273656 for scRNA-seq of mouse hearts treated with rAAV2/9-TRISPR-Klf15Klf15 or control following TAC or sham surgery (Dataset-3). The bulk RNA-seq data from human iPSC-derived cardiomyocytes EHM are available at NCBI GEO under accession GSE273827. Publicly available single-cell nuclei RNA-seq datasets from human heart analyzed in this study were downloaded from Single Cell Portal (https://singlecell.broadinstitute.org/single_cell) project ID SCP1303. The full code used for single-cell analysis is available at https://github.com/fblec/KLF15_SCS.git. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE¹⁴ partner repository with the dataset identifier PXD072456. Material, mouse and cell lines are available upon request from the authors with corresponding material transfer agreement. **Statistical.** GraphPad Prism 11 was used for statistical analyses unless otherwise specified. Differences with $P < 0.05$ were considered statistically significant. Datasets were checked for normal distribution using the Shapiro-Wilk test. Two-group comparisons were made with unpaired Student's t-test Wilcoxon rank sum test. For multiple group analyses, one-way ANOVA and Tukey's post hoc test were used. If more than one variable was investigated, two-way ANOVA and Tukey's post hoc test were used unless otherwise specified.

Supplementary Table 1. Guide RNAs sequences.

gRNA ID	Target organism	Cas9 variant	Sequence (5' - 3')
KLF15 A	Homo sapiens	SpyCas9	GCGCCGCGAAGGCTCGCAGG
KLF15 B	Homo sapiens	SpyCas9	GACGCGCAGCGCGGGCCAAT
KLF15 C	Homo sapiens	SpyCas9	GCTCAAGTCCGGGAGCCGCG
KLF15 D	Homo sapiens	SpyCas9	GCGTGCGCGTCTGGCAGCTC
KLF15 E	Homo sapiens	SpyCas9	GGACCAGGCAGCGTGTTGGG
KLF15 F	Homo sapiens	SpyCas9	GCTGTCTCGCGGCGTCCACG
KLF15 G	Homo sapiens	SpyCas9	GGGAACCGAGCTTTAGAAGC
KLF15 H	Homo sapiens	SpyCas9	GTGATTGAGCGGGAAGCGCT
Klf15 A	Mus musculus	SpyCas9	GGGACTCTGCGGGCTTTCAG
Klf15 B	Mus musculus	SpyCas9	GTGCAGATCCACGAAGAGAC
Klf15 C	Mus musculus	SpyCas9	GACACGGTCCCTACTAGATC
KLF15 A	Homo sapiens	Sauri/SlugCas9	GGCGCCGCGAAGGCTCGCAG
KLF15 B	Homo sapiens	Sauri/SlugCas9	GACGCGCAGCGCGGGCCAAT
KLF15 C	Homo sapiens	Sauri/SlugCas9	GCTCAAGTCCGGGAGCCGCG
KLF15 D	Homo sapiens	Sauri/SlugCas9	GCGTGCGCGTCTGGCAGCTC
NT1	all-purpose	all-purpose	GTCCAGCGGATAGAATGGCG
NT2	all-purpose	all-purpose	GGAGCGGTTTTGGATATTAG
NT3	all-purpose	all-purpose	GTATGAGCGCGATGAAGGTG

Supplementary Table 2. RT-qPCR Primers

Gene	Target organism	Sequence (5' - 3')
<i>KLF15</i>	<i>Homo sapiens</i>	AGCAAGGACTTGGATGCCTG
		AGGGCAGGTTCAAGTTGGAG
<i>ACTA2</i>	<i>Homo sapiens</i>	GGAAAAGATCTGGCACCCTC
		GCGTCCAGAGGCATAGAGAG
<i>TBP</i>	<i>Homo sapiens</i>	GCACAGGAGCCAAGAGTGAA
		TTGTTGGTGGGTGAGCACAA
<i>BMP7</i>	<i>Homo sapiens</i>	TTCGTCAACCTCGTGAACA
		AGCTTCCCCTTCTGGGATCT
<i>AZGP1</i>	<i>Homo sapiens</i>	ATGGGACTCTGGAGACAGGT
		GCAATACGTGAGACCCGTTAC
<i>ACTN2</i>	<i>Homo sapiens</i>	TCGTGAACACCCCTAAACCC
		TCCATTCCAAAAGCTCACTCG
<i>CCND1</i>	<i>Homo sapiens</i>	CCCTCGGTGTCCTACTTCAA
		AGGAAGCGGTCCAGGTAGTT
<i>ALDH2</i>	<i>Homo sapiens</i>	TGCTGACCGTGGTTACTTCA
		ACGTGGAATTGTTGGCTCTC
<i>Klf15</i>	<i>Mus musculus</i>	CAACTCATCTGAGCGGGAA
		CAAGAGCAGCCACCTCAAG
<i>Aldh2</i>	<i>Mus musculus</i>	ACGGCAAGCCTTATGTCATC
		ACCTTCATCACCACCAGTT
<i>Nppa</i>	<i>Mus musculus</i>	CATCACCCTGGGCTTCTTCCT
		TGGGCTCCAATCCTGTCAATC
<i>Tbp</i>	<i>Mus musculus</i>	GCCTTCCACCTTATGCTCAG
		TGTGTGGGTTGCTGAGATGT
Sauri-dCas9VPR	-	GGAGTGAACAGCGACATCAA
		AGTTCTCCCCGCTTGAAGAT

Supplementary Table 3. Antibodies

Target	Antibody	Dilution
Anti-Spy-dCas9VPR	Diagenode, #C15310258	1:1,000 (IB)
Anti-HA-HRP	Sigma Aldrich, #H6533	1:2,000 (IB)
Anti-GAPDH	Proteintech, #60004-1-Ig	1:10,000 (IB)
Anti-Lamin A/C	Santa Cruz Biotechnology, #sc-6215	1:1000 (IB)

Anti-c-Myc	Cell Signaling, #9402	1:500 (IB)
Anti- α SMA	Sigma Aldrich, #A2547	1:1,000 (IB), 1:200 (IF)
Anti-ACTN2	Sigma Aldrich #A7811	1:2,000 (IB), 1:200 (IF)
Anti-cTnT	Abcam, #ab45932	1:200 (IF)
Anti-cTnT-FITC	Miltenyi Biotec, # 130-119-575,	1:400 (IF)
Anti-Titin-M8/M9	Myomedix, # TTN-9	1:500 (IF)
Anti-TOMM20	Thermo Fisher Scientific, # MA5-55630	1:400 (IF)
Anti-TurboGFP	Thermo Fisher Scientific, #PA5- 22688	1:5,000 (IB)
Anti-OCT4	Abcam, #19857	1:50 (IF/Flow)
Anti-TRA1-60	Thermo Fisher Scientific, #MA1- 023	1:50 (IF/Flow)
Anti-NANOG	Thermo Fisher Scientific, #MA1- 017	1:100 (IF)
Anti-Vimentin	Abcam, #ab92547	1:1000 (IF)
Anti-KLF15	Proteintech, #66185-1-Ig	1:200 (IF)
Anti-AZGP1	Proteintech, #13399-1-AP	1:1000 (IB)
Anti-Phospho- SMAD2/3	Cell signaling Technology, #8828S	1:1000 (IB)
Anti-SMAD2/3	Cell signaling Technology, #5678S	1:1000 (IB)
Anti-Phospho-p38	Cell Signaling Technology, #9211S	1:1000 (IB)
Anti-p38	Cell Signaling Technology, #9212S	1:1000 (IB)
Anti-Phospho-AKT	Cell signaling Technology, #9271	1:1000 (IB)
Anti-AKT	Cell Signaling Technology, #9272	1:1000 (IB)
Anti-Phospho- ERK1/2	Cell Signaling Technology, #9101	1:1000 (IB)
Anti-ERK1/2	Cell Signaling Technology, ##9102	1:1000 (IB)
Anti-Phospho-HSP27	Cell signaling Technology, #2405T	1:1000 (IB)
Anti-HSP27	Cell signaling Technology, #95357T	1:1000 (IB)
Anti-Vinculin	Cell Signaling Technology, #4650	1:1000 (IB)

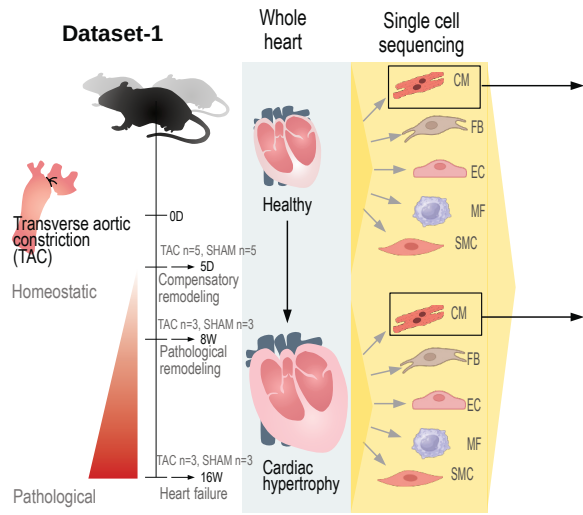
Anti-Alpha-tubulin	Proteintech, #66031-1	1:1000 (IB)
Anti-POSTN	Cell signaling Technology, #27371S	1:1000 (IB)
Anti-CTGF	Santa Cruz, #sc-25440	1:1000 (IB)
Anti-TAGLN	Abcam, #ab14106	1:1000 (IB)
Anti-HA	Sigma Aldrich, #H3663	1:1000 (IB/IP), 1:500 (CHIP)
Anti-Flag	Sigma Aldrich, #F1804	1:1000 (IB/IP), 1:500 (CHIP)
Anti-mouse-HRP	Dako, #P0260	1:10,000 (IB)
Anti-rabbit-HRP	Dako, #P0448	1:5,000 (IB)
Anti-goat-HRP	Dako, #P0049	1:2,000 (IB)
Anti-rabbit IgG Alexa Fluor488	Thermo Fisher Scientific, #A11008	1:400 (IF)
Anti-mouse IgG Alexa Fluor555	Thermo Fisher Scientific, #A21424	1:400 (IF)
Anti-mouse IgG Alexa Fluor633	Thermo Fisher Scientific, #A21052	1:400 (IF)
AF488 donkey anti- mouse	Thermo Fisher Scientific, # A31572	1:700 (IF)
AF555 donkey anti- mouse	Thermo Fisher Scientific, # A21432	1:700 (IF)
Normal Mouse IgG	Sigma Aldrich, #12-371	1:500 (IP)

Data S1. Study design and RPKM (Reads per kilobase of transcript per Million mapped reads) of the transcriptomic data of control/stressed EHM with either NT gRNA or *KLF15* activation in CRISPRa-hiPSC cardiomyocytes.

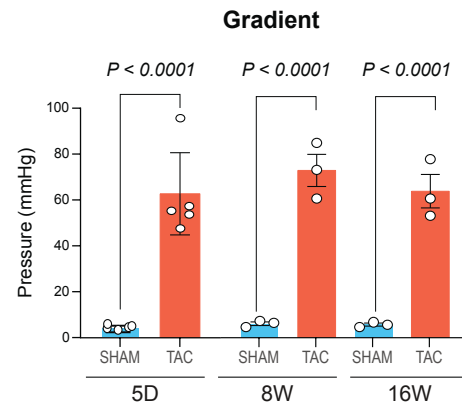
SUPPLEMENTARY FIGURE AND LEGENDS

Figure S1

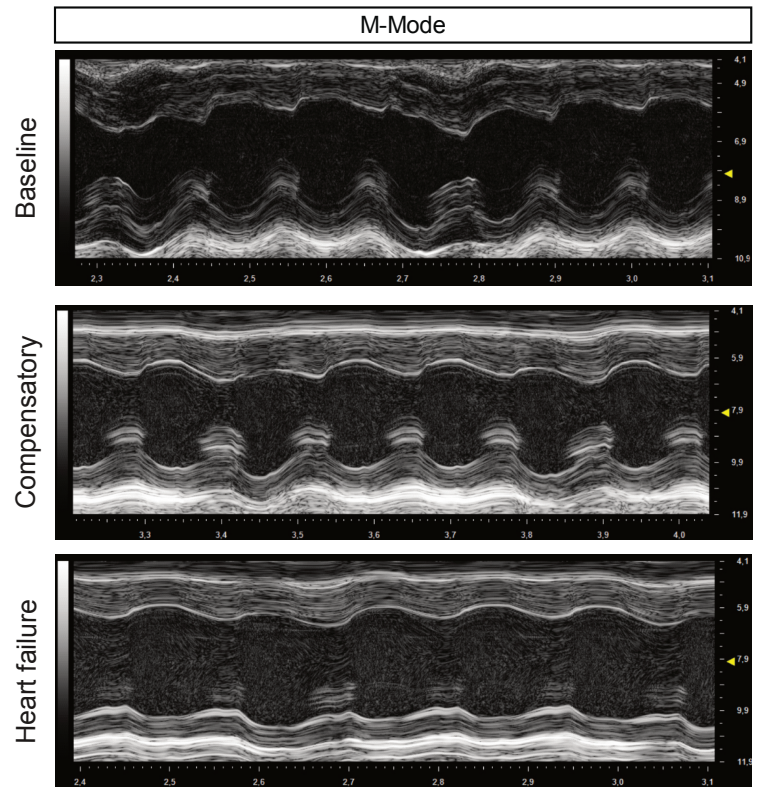
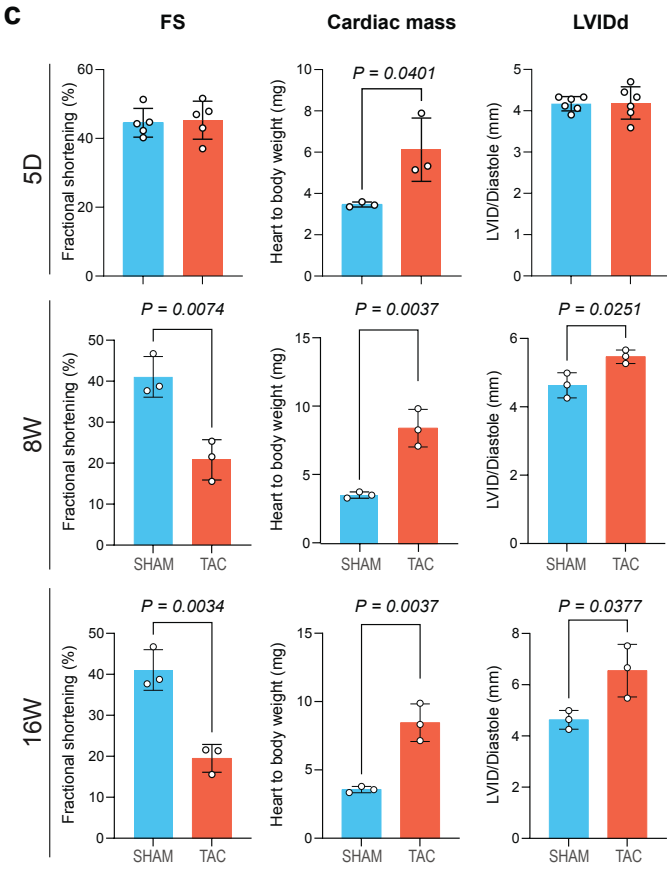
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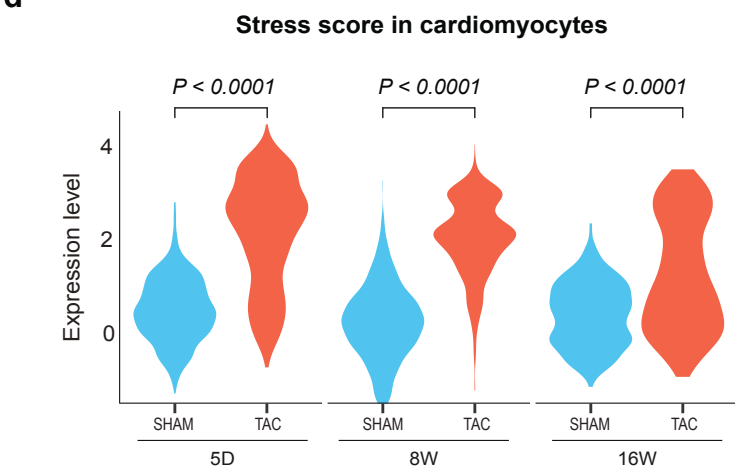
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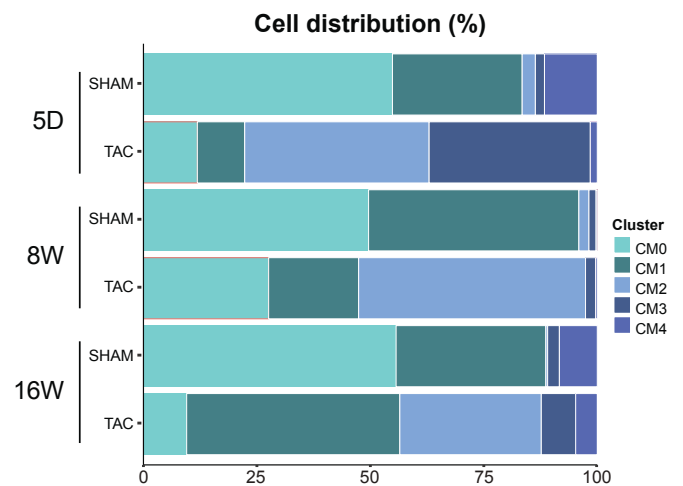
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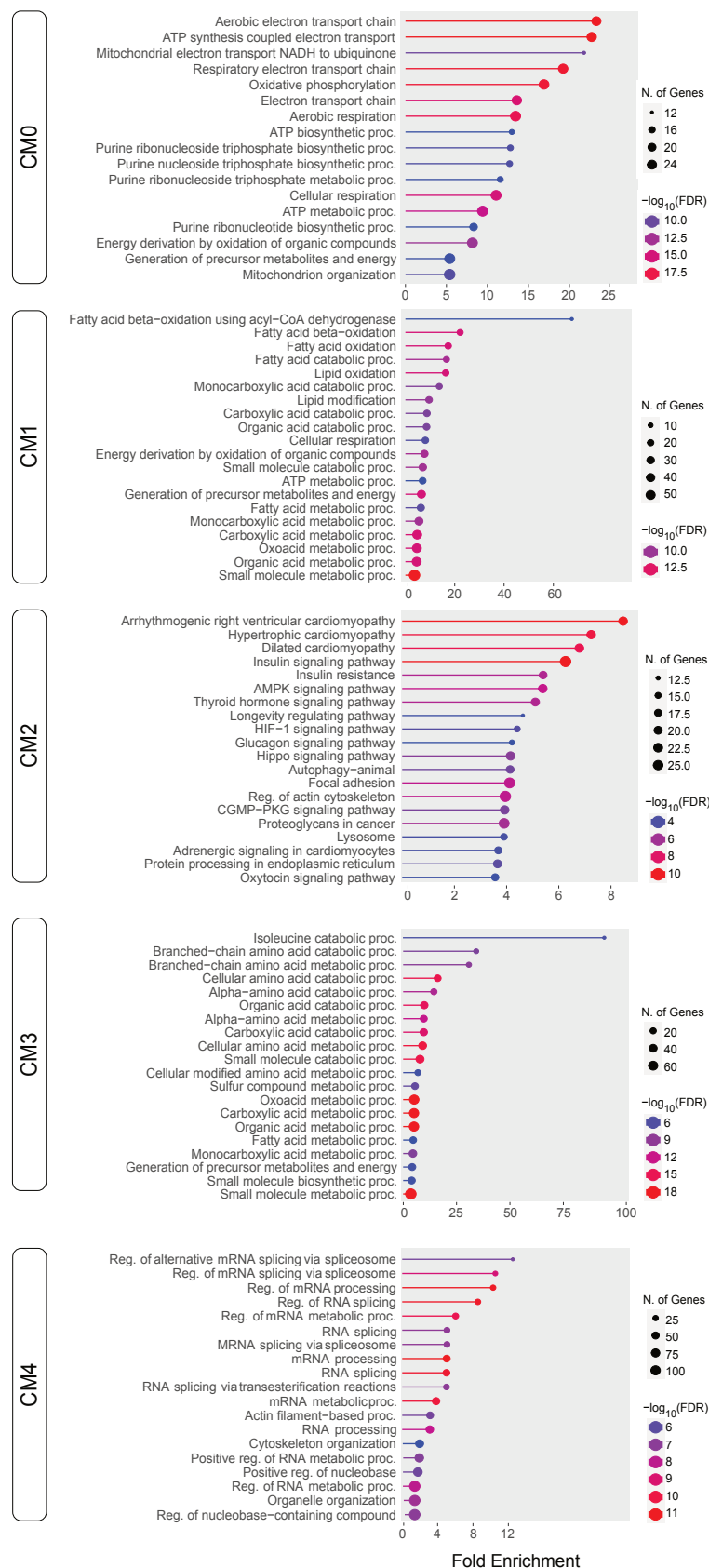
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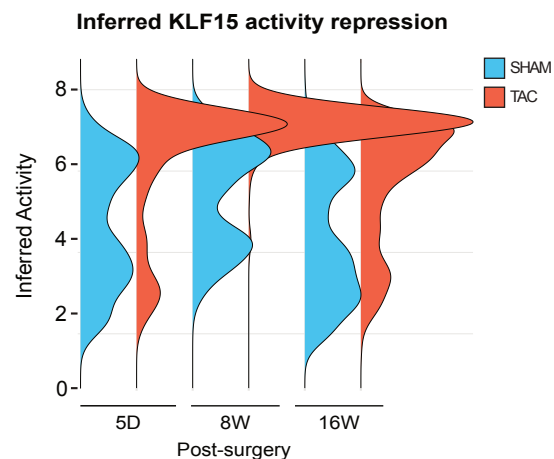
Supplementary Fig. 1: (a) Experimental design scheme showing transverse aortic constriction (TAC) surgery timeline (Dataset-1). Mice underwent either SHAM or TAC surgery and hearts were harvested at 5 days (5D), 8 weeks (8W), and 16 weeks (16W) post-surgery for single-cell RNA sequencing (scRNA-seq). Sample sizes: 5D ($n=5$ TAC, $n=5$ SHAM), 8W ($n=3$ TAC, $n=3$ SHAM), 16W ($n=3$ TAC, $n=3$ SHAM). Different cell types isolated: cardiomyocytes (CM), endothelial cells (EC), fibroblasts (FB), macrophages (MF) and smooth muscle cells (SMC). (b) Gradient pressure measurement (mmHg) after TAC induction showing comparable pressure increase for all 3 time points (5D, 8W, and 16W post-surgery). (c) Echocardiographic measurements of cardiac function in TAC and SHAM mice at different timepoints post-surgery: 5D, 8W, and 16W ($n=3-6$) along with representative M mode images at compensatory and failing stages. Parameters measured include fractional shortening (FS), cardiac mass (heart to body weight), and left ventricular internal diameter in diastole (LVIDd). (d) Violin plots showing stress score distribution in cardiomyocytes, calculated based on expression of *Nppa*, *Nppb*, *Acta1*, and *Ankrd1*. (e) Relative distribution of cardiomyocyte subclusters (CM0-CM4) across conditions showing cluster shifts during disease progression. Data shown as percentage of total cardiomyocytes at 5D, 8W, and 16W. Mean $\pm$ standard error of the mean (SEM) in (b) and (c). Statistical comparisons between SHAM and TAC groups at each timepoint using Wilcoxon rank-sum test with Benjamini-Hochberg correction for multiple testing in (d), Student's t-test was used to determine statistical significance in (b) and (c).

Figure S2

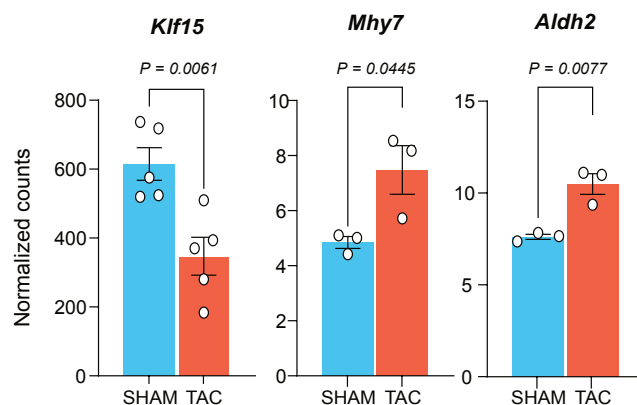
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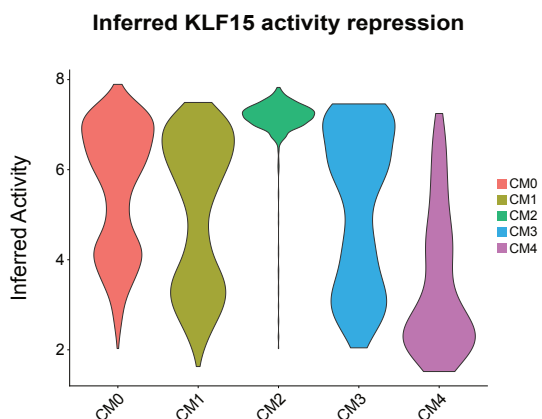
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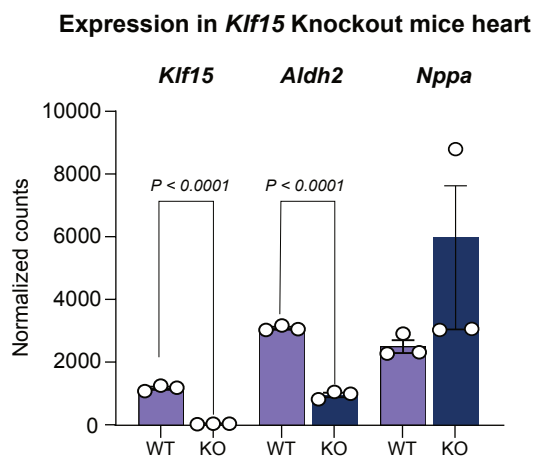
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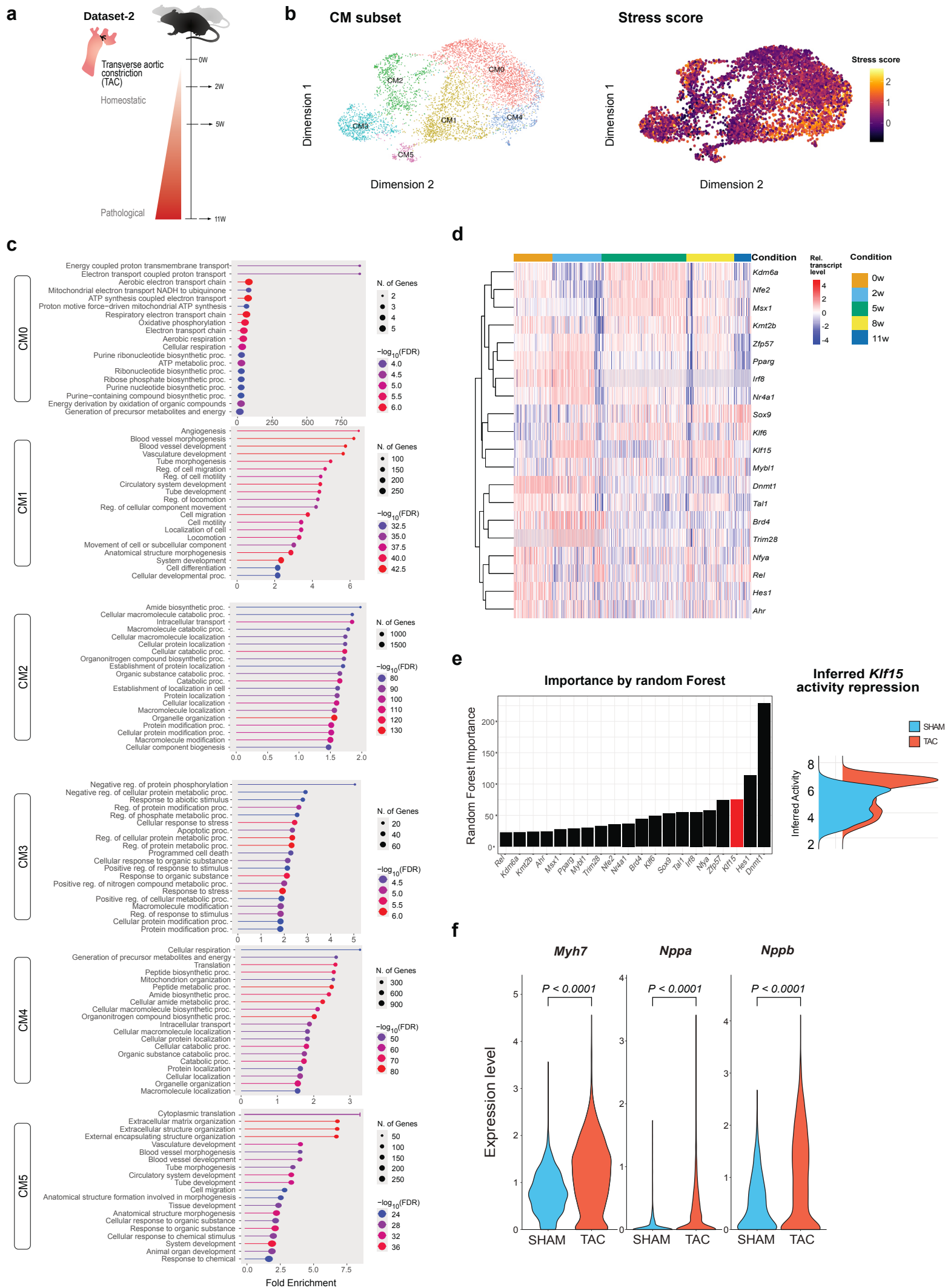


e



Supplementary Fig. 2: (a) Gene ontology (GO) enrichment analysis of biological processes for cardiomyocyte subclusters CM0-CM4. Significantly enriched genes ($\text{Log}_2\text{FC} \geq 0.25$, adjusted P -value ≤ 0.05) were identified for each subcluster and analyzed using over-representation analysis via ShinyGO 0.82 and Enrichr. Dot size represents number of genes; color indicates statistical significance ($-\log_{10}$ FDR). (b) IStream plot showing inferred KLF15 transcription factor activity in cardiomyocytes at different disease stages. Decreased activity indicates loss of KLF15 repressive function during TAC progression. (c) Expression level of *Klf15*, *Myh7* and *Aldh2* in TAC and SHAM mouse heart as derived from RNA-seq data analyses. $n=3$ per group. (d) Inferred KLF15 activity across cardiomyocyte subclusters CM0-CM4. (e) Expression level of *Klf15*, *Aldh2*, and *Nppa* in *Klf15*^{-/-} knock-out mouse hearts as derived from RNA-seq data analyses. $n=3$ per group. Mean $\pm$ standard error of the mean (SEM) in (c) and (e). Student's t-test was used to determine statistical significance in (c) and (e).

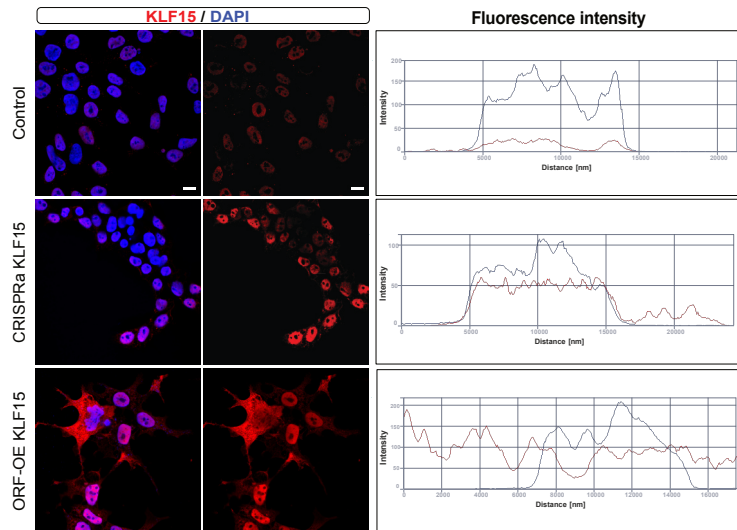
Figure S3



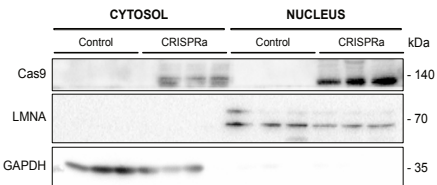
Supplementary Fig. 3: (a) Schematic overview of time course analysis of TAC-induced pressure overload followed by single cell (sc) RNA sequencing (seq) from publicly available dataset-2 from Wang et al.¹⁵ Mice underwent TAC surgery with heart collection at 0W (baseline), 2W, 5W and 11W post-surgery for scRNA-seq. (b) Uniform Manifold Approximation and Projection (UMAP) of the cardiomyocyte subclusters (CM0-CM5) (left) and stress score distribution (right) from the Wang et al. dataset, showing progressive cardiomyocyte stress activation. Stress score calculated from *Nppa*, *Nppb*, *Myh7* and *Ankrd1* expression. (c) Gene ontology (GO) of biological processes analysis of the different cardiomyocyte subclusters (CM0-CM5) based on the significantly enriched genes in each subcluster. Significantly enriched genes were identified based on $\text{Log}_2\text{FC} \geq 0.25$, adjusted $P\text{-value} \leq 0.05$. Dot size represents number of genes; color indicates statistical significance ($-\log_{10} \text{FDR}$). (d) Heatmap showing inferred transcription factor (TF) activity across disease progression timepoints (0W, 2W, 5W, 8W and 11W) in TAC versus SHAM hearts. TF activity inferred using BITFAM. Each row represents one TF; columns represent individual samples. (e) Random Forest feature importance analysis identifying transcription factors that best classify TAC versus SHAM cardiomyocytes. Bar height indicates feature importance score. KLF15 ranks among top predicted TFs. Inset shows density plot of KLF15 activity distribution in TAC (red) versus SHAM (blue) conditions. (f) Expression levels of *Myh7*, *Nppa*, and *Nppb* used as stress score genes comparison between SHAM and TAC mice as derived from scRNA-seq data. Statistical significance was tested by Wilcoxon test with Benjamini-Hochberg correction for multiple testing in (f).

Figure S4

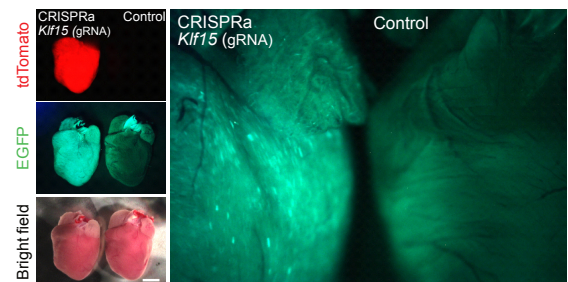
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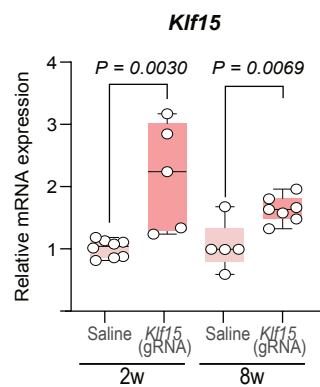
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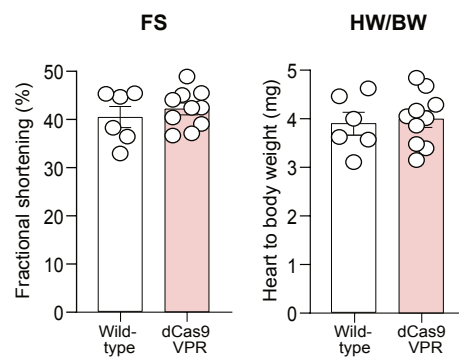
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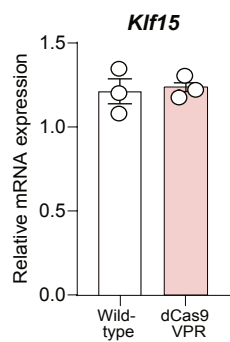
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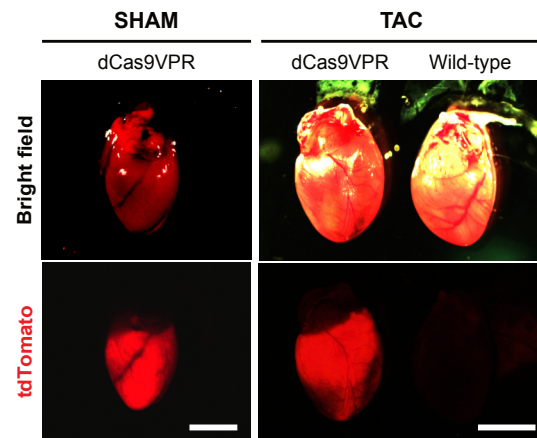
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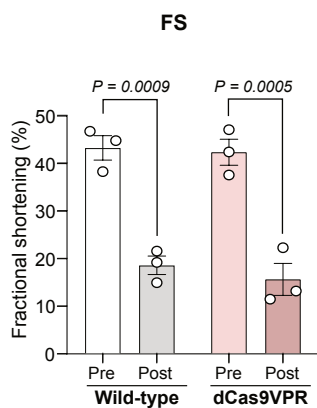
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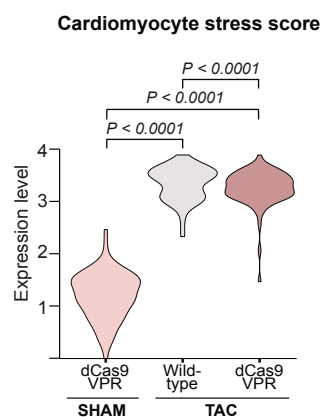
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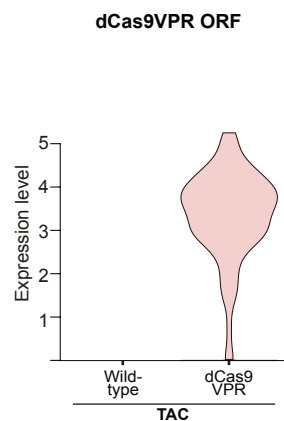
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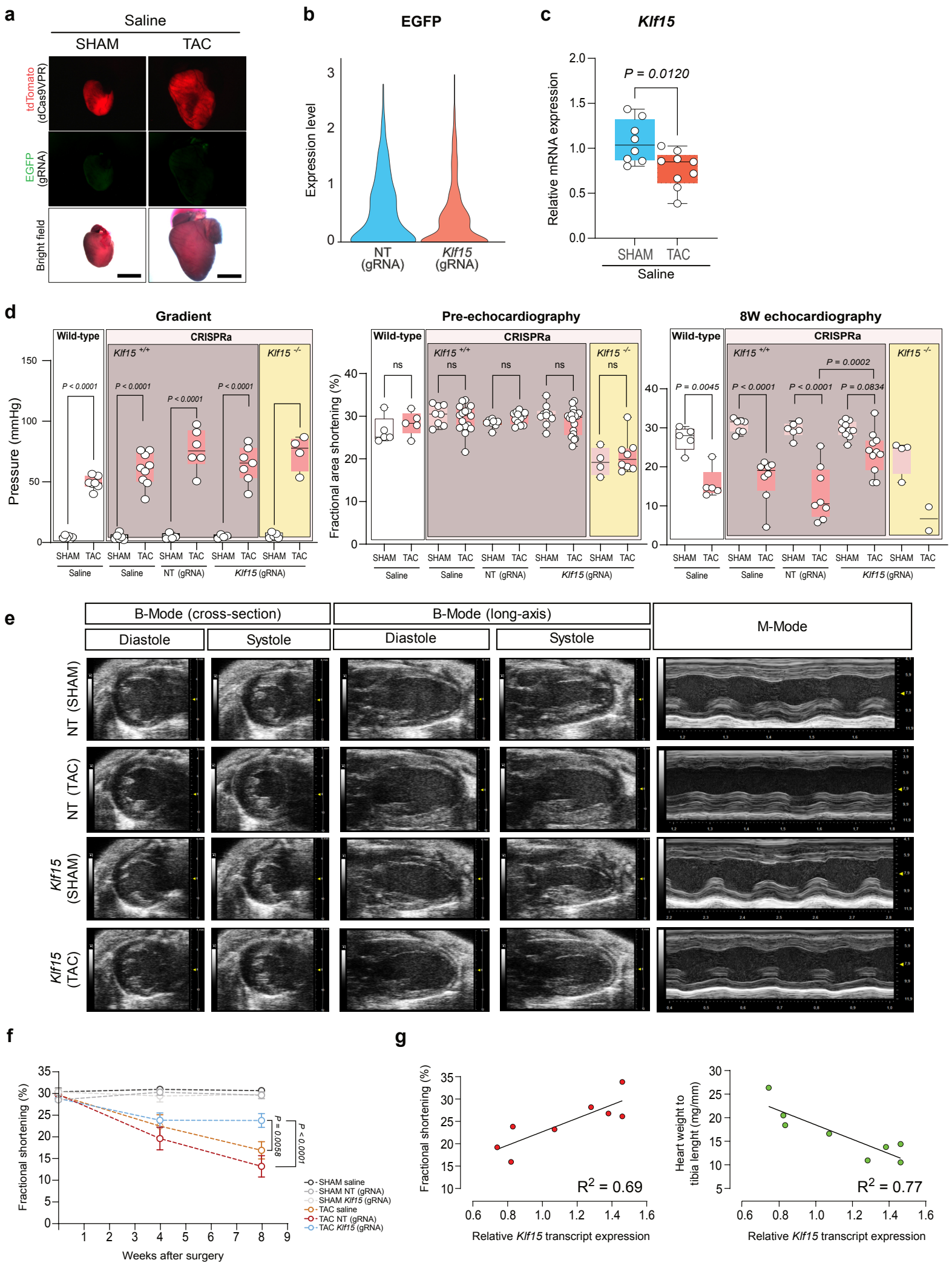


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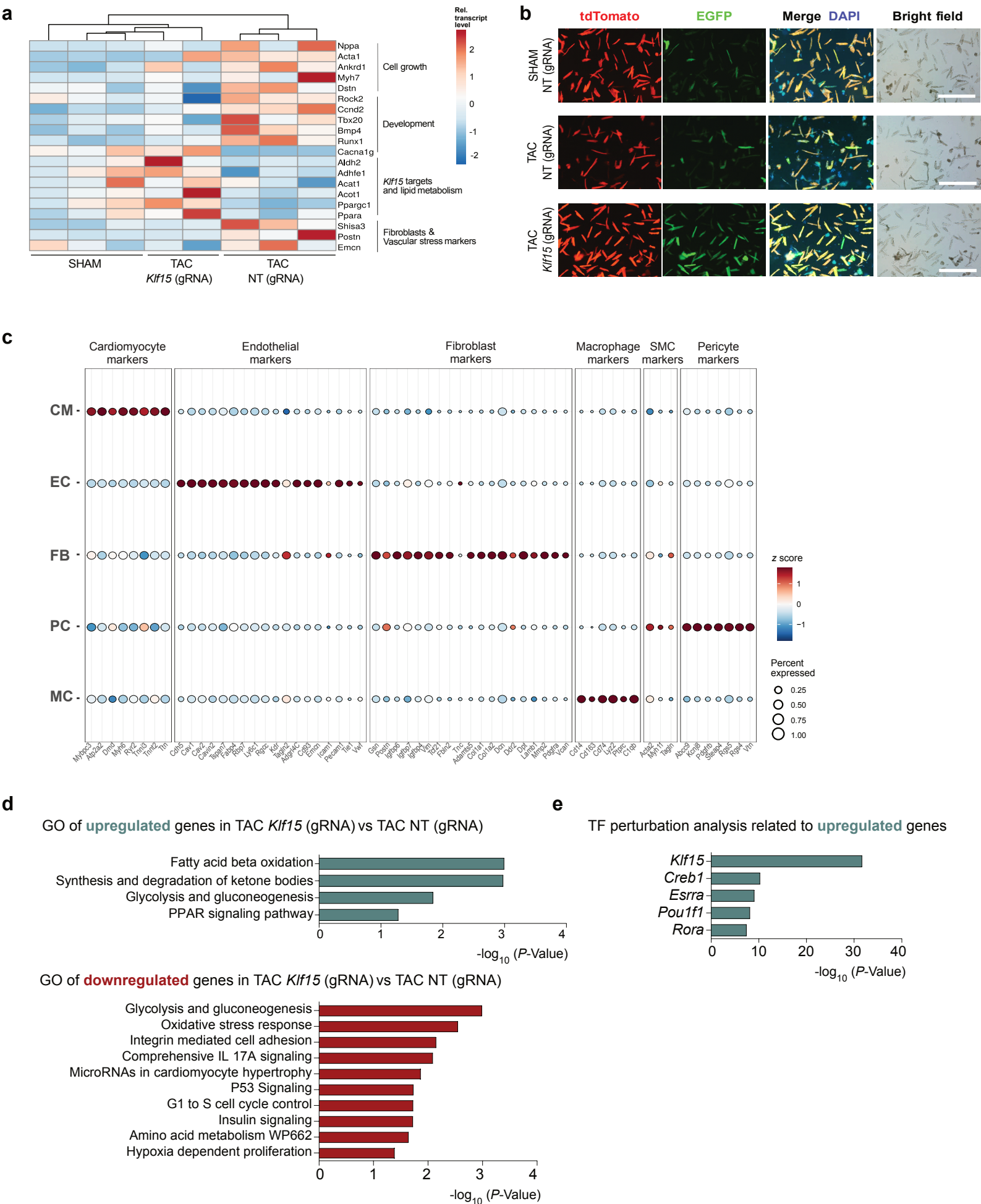
Supplementary Fig. 4: (a) Subcellular localization of KLF15 as indicated by immunofluorescence images together with the fluorescence intensity plots comparing KLF15 open reading frame overexpression (ORF-OE) and CRISPRa-mediated induction in HEK293T cells. Scale bar = 20 μ m, KLF15 (red), nuclei (DAPI, blue). (b) Subcellular localization and abundance of constitutively expressed dCas9VPR protein in hearts of CRISPRa mice confirmed by immunoblotting. GAPDH and LAMIN A/C (LMNA) were used as loading controls for cytoplasm and nuclear extracts, respectively. $n = 3$ per group. (c) Adult CRISPRa mice were systemically injected with 1×10^{12} AAV9 vg per mouse. Delivery of *Klf15* gRNA containing the EGFP cassette was tested in six-month-old CRISPRa mice, confirming expression of dCas9VPR (tdTomato) and gRNA (EGFP) eight weeks post-injection. Saline injected mice served as negative control. Scale bar = 2 mm. (d) Enhanced expression of *Klf15* was confirmed by RT-qPCR at 2 and 8 weeks (W) in *Klf15* (gRNA) compared to saline injected hearts ($n=5-8$ per group). (e-f) Comparison of CRISPRa-expressing mice to wild-type littermates by echocardiography analysis (Fractional shortening (FS) and heart to body weight ratio ($n=6-10$ per group) and expression of *Klf15* demonstrated by RT-qPCR ($n=3$ per group). (g) Representative images of whole hearts of CRISPRa and wild-type mice eight weeks after TAC and SHAM surgery showing expression of tdTomato only in dCas9VPR hearts. Scale bar = 5 mm. (h) Fractional shortening (FS) prior (Pre) to and 8 weeks post TAC surgery (Post) in CRISPRa (red bars) and wild-type mice (white and gray bars, $n = 3$ per group). (i-j) Violin plots depicting stress score characterized by the expression of hypertrophy-associated genes, including *Nppa*, *Nppb*, *Ankrd1* and *Acta1* in the cardiomyocyte population of the different groups in response to pressure overload along with dCas9VPR expression. Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (d). Mean $\pm$ standard error of the mean (SEM) in (e) (f) and (h). Student's t-test was used to determine statistical significance in (d), (e), (f) and (h). Statistical significance by Wilcoxon test in (i).

Figure S5



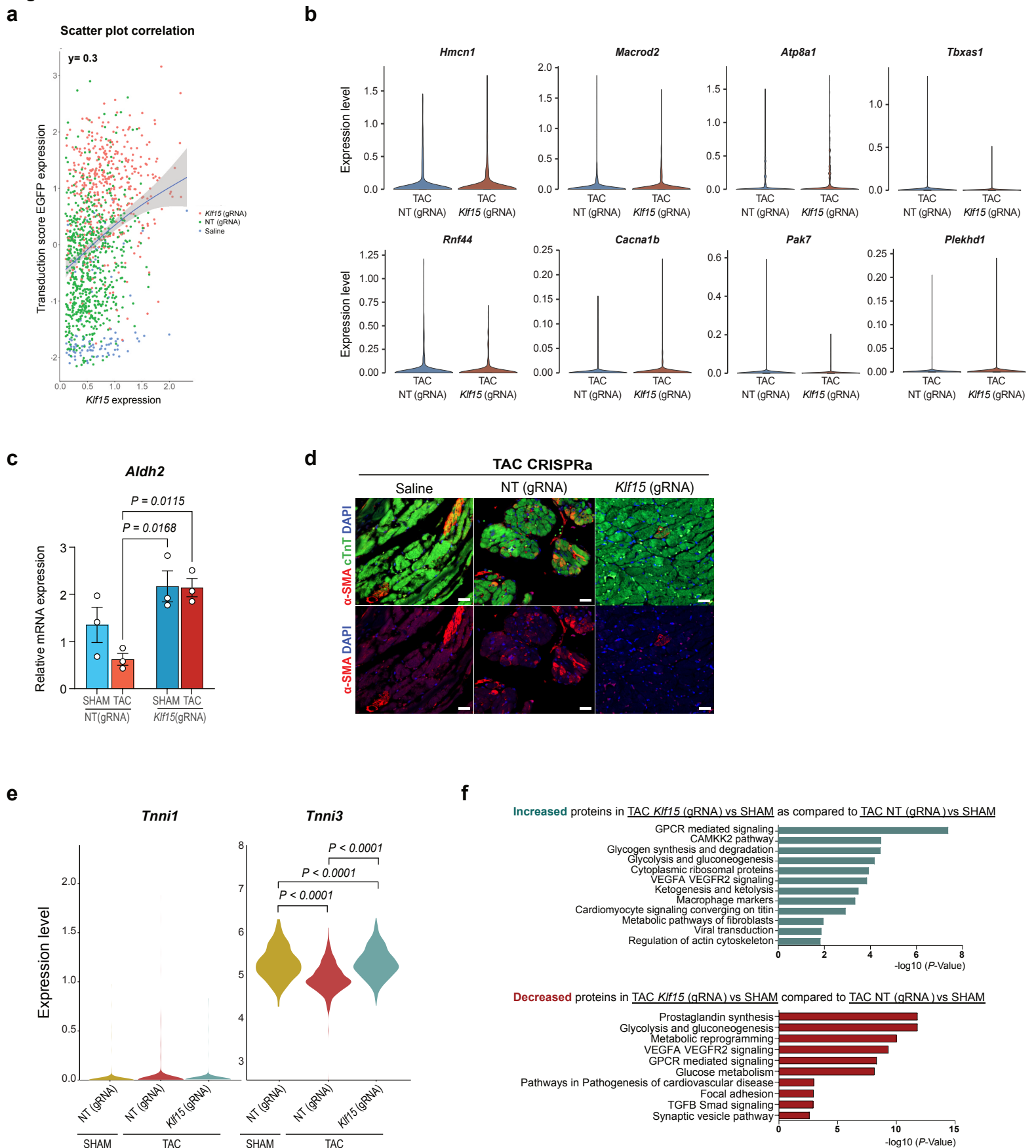
Supplementary Fig. 5: (a) Representative images of whole hearts of the SHAM and TAC conditions in saline CRISPRa mice showing heart dimensions as well as expression of tdTomato (dCas9VPR) and negative for EGFP (gRNA). Control for the data presented in Figure 1k. Scale bar = 5 mm. (b) Violin plot showing no statistical differences in expression of EGFP indicating AAV9 delivery, comparing NT (gRNA) and *Klf15* (gRNA) groups. (c) *Klf15* expression in whole hearts as measured by RT-qPCR in CRISPRa saline injected mice with either SHAM or TAC surgery ($n=8$ (SHAM saline), $n=9$ (TAC saline)). (d-e) Gradient (mmHg) and fractional shortening (FS) measurements of the different experimental groups before and 8 weeks after TAC and SHAM surgery along with representative M-mode image of the main four groups CRISPRa TAC and SHAM with either NT or *Klf15* (gRNA). $n=2-18$ per group. (f) Fractional shortening showing the time course of functional performance eight weeks after TAC induction in the different groups as indicated in the graph. $n=8-18$ per group. (g) Correlation plots comparing relative *Klf15* mRNA levels with fractional shortening (left) and with heart weight to tibia length (right) in TAC CRISPRa hearts of mice injected with *Klf15* gRNA ($n=8$). Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (c) and (d). Wilcoxon rank sum test in (b), Student's t-test in (c) and (d), One-way ANOVA in (d), Two-way ANOVA and Tukey's post hoc test in (f) were used to test for statistical significance. Linear regression model was used to test for correlation in (g) and respective R^2 values are depicted.

Figure S6



Supplementary Fig. 6: (a) Heatmap of indicated genes expressed after 16 weeks post TAC surgery in mice injected with NT or *Klf15* (gRNA) as compared to SHAM condition derived from RNA bulk sequencing ($n=2-3$ per group). Expression of genes related to cell growth, development, KLF15 targets/lipid metabolism, and fibroblast/vascular stress markers across experimental conditions are depicted. Z-scored normalized expression values are shown. (b) Representative images after cell dissociation via modified Langendorff perfusion subjected to scRNA-seq of CRISPRa mice for indicated conditions. Expression of dCas9VPR and gRNA are indicated by tdTomato and EGFP, respectively, in the isolated cardiomyocytes. Scale bar = 100 μm , nuclei (DAPI, blue). (c) Dot plot with the corresponding genes used for clustering to specifically select cell populations. Dot size indicates the proportion of cells expressing the respective gene and color reflects average z-scored expression level. Marker genes confirm robust clustering and annotation of major cardiac cell populations. (d) Gene ontology (GO) biological processes of up- and downregulated genes in TAC *Klf15* (gRNA) versus TAC NT (gRNA) groups. (e) Bioinformatic TF perturbation analysis related to upregulated genes in TAC CRISPRa-*Klf15*. This analysis combined regulated genes with TF perturbation data identifying over-represented GO terms.

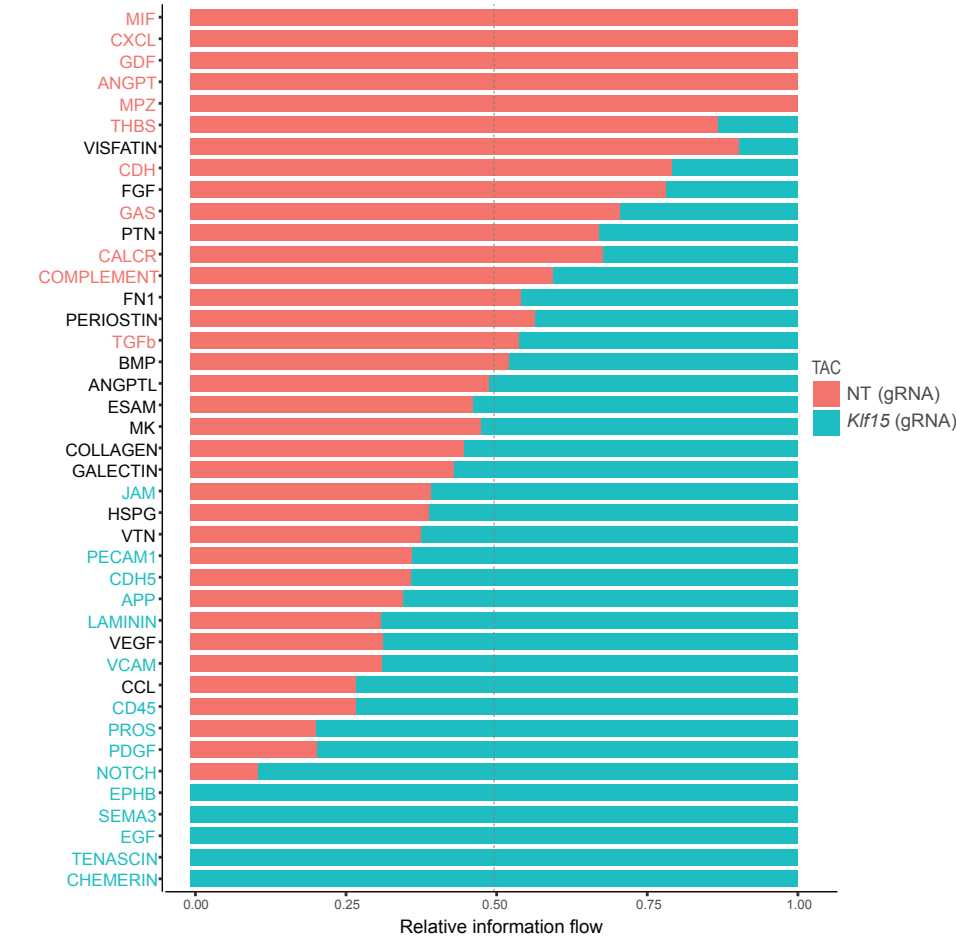
Figure S7



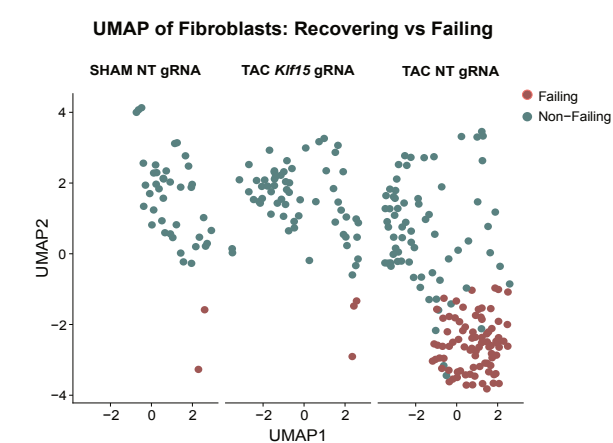
Supplementary Fig. 7: (a) Scatter plot showing correlation between AAV9 transduction efficiency (EGFP expression, y-axis) and *Klf15* transcript levels (x-axis) in cardiomyocytes of experimental groups *Klf15* (gRNA) - (green), NT (gRNA) - (red), and saline (gray). (b) Violin plots showing expression of *Hmcn1*, *Macrod2*, *Atp8a1*, *Tbxas1*, *Rnf44*, *Cacna1b*, *Pak7*, *Plekhd1*. No significant differences observed between TAC NT gRNA and TAC *Klf15* gRNA groups. (c) Expression of the *Klf15* target gene *Aldh2* analyzed by RT-qPCR in NT and *Klf15* (gRNA) SHAM and TAC groups ($n=3$ per group). (d) Representative images of immunofluorescence staining depicting α -SMA expression in cTnT positive cells in different TAC groups (saline, NT and *Klf15* (gRNA)). Scale bar = 20 μ m, α -SMA (red), cTnT (green), nuclei (DAPI, blue). (e) Violin plots depicting expression of *Tnni1* and *Tnni3* characterizing fetal and mature cardiomyocytes, respectively, in SHAM NT (gRNA), TAC NT (gRNA) and TAC *Klf15* (gRNA) groups. (f) Gene ontology (GO) biological processes of increased and decreased proteins in TAC *Klf15* (gRNA) versus SHAM as compared to TAC NT (gRNA) versus SHAM. Mean $\pm$ standard error of the mean (SEM) in (c). Statistical significance was determined using the Pearson correlation test in (a). Wilcoxon rank sum test was used for statistical significance in (b) and (e). Two-way ANOVA and Tukey's post-hoc test were used for group comparison in (c).

Figure S8

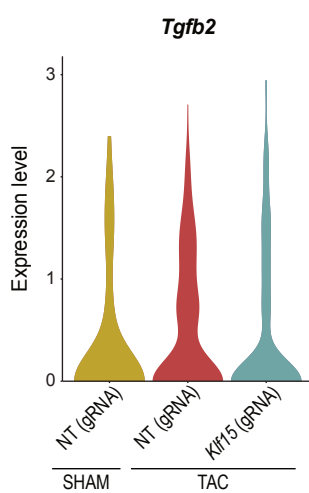
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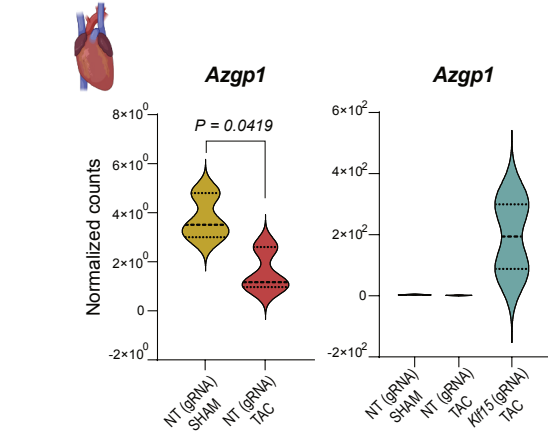
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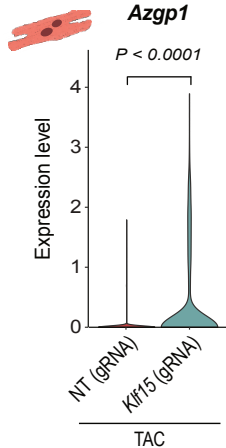
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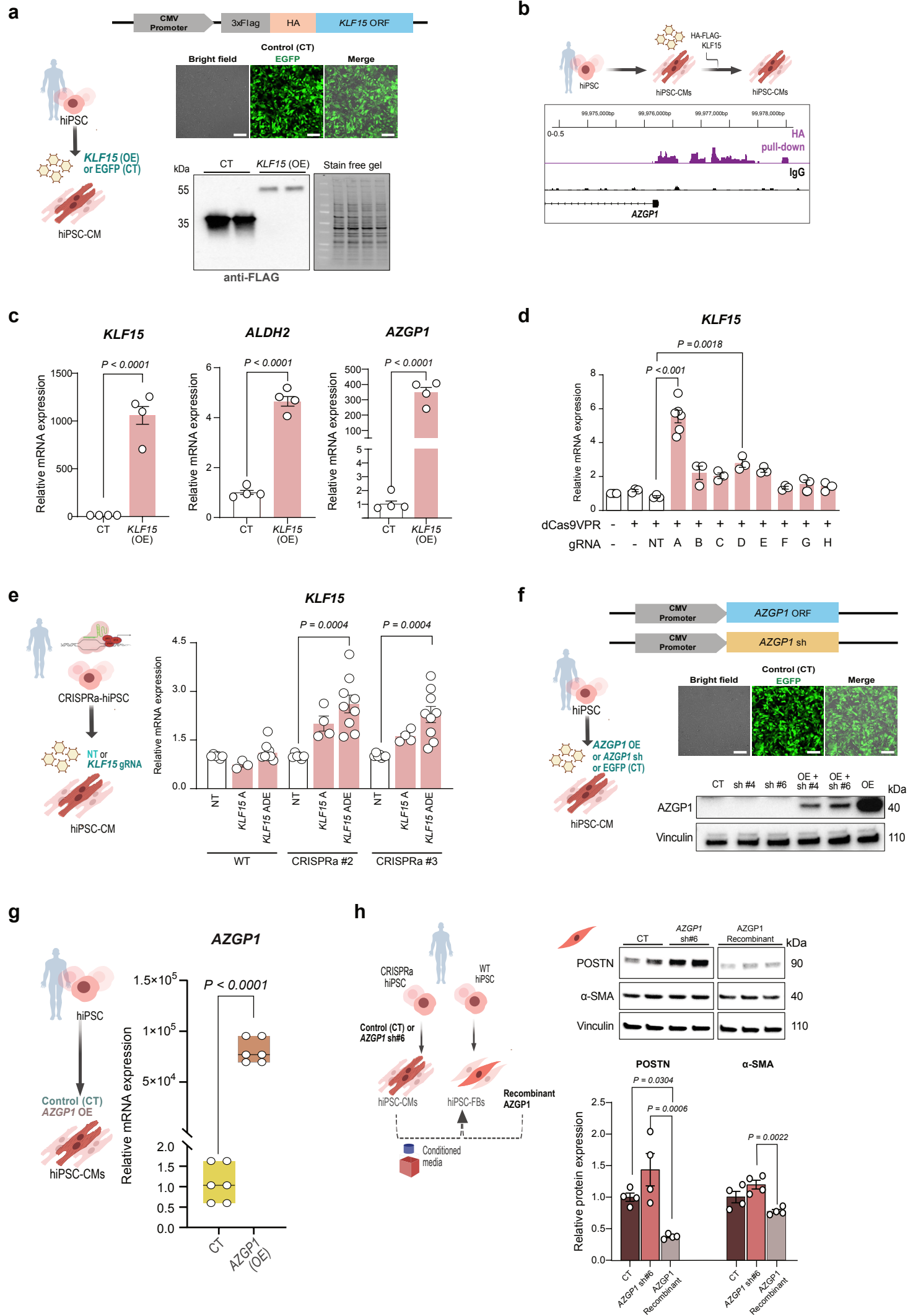
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	2,37	1,45E-11	Serpinb1c
	2,08	4,35E-20	Gm15743
	2,01	6,99E-41	Uchl1
	1,95	4,26E-65	Cdkn1a
	1,94	1,10E-02	Vgll2
	1,94	4,36E-109	Nppa
	1,91	2,68E-05	Arhgdig
CM1	2,32	1,06E-11	Dbh
	1,89	1,40E-05	Sbk2
	1,70	5,94E-09	Slc22a1
	1,67	4,01E-07	Snai3
	1,51	1,67E-03	Hspa12a
	1,41	5,09E-10	Fos
	1,33	2,22E-15	Ces1d
	1,32	2,35E-03	Rbfox1
	1,28	6,84E-09	Smco1
CM2	5,24	1,05E-18	Ucma
	4,42	2,58E-02	Pon1
	4,40	1,81E-04	Necab3
	4,02	7,20E-04	Gm6166
	3,75	2,81E-35	Nrn1l
	3,59	8,20E-19	Tac2
	3,42	1,77E-31	Azgp1
	3,32	1,21E-02	Serpina12
	3,27	2,39E-30	Tceal7
CM3	6,53	1,66E-03	Gm31462
	6,33	1,60E-02	Gm44093
	6,01	1,38E-02	2610028D06Rik
	5,95	2,03E-09	9330162B11Rik
	5,74	4,97E-03	Gm10384
	5,74	5,36E-11	Gm45809
	5,73	8,62E-04	Ddi1
	5,72	6,45E-17	Gm48302
	5,70	8,91E-05	Gm43858
TAC Klf15 (gRNA) vs TAC NT (gRNA)	5,65	2,80E-18	Tac2
	4,96	1,12E-14	Azgp1
	4,78	6,26E-05	Cbs
	4,10	4,45E-11	Cda
	4,06	2,49E-02	Nlrc3
TAC Klf15 (gRNA) vs SHAM	5,12	2,28E-14	Ucma
	4,82	1,39E-27	Azgp1
	4,62	1,04E-30	Tac2
	4,58	6,13E-79	Aldob
	4,51	3,03E-82	Cox6b2

f



Supplementary Fig. 8: (a) CellChat analysis showing relative information flow of ligand-receptor signaling pathways in TAC hearts. Comparison between NT (gRNA) (red) and *Klf15* (gRNA) (blue) conditions. Bar length indicates total signaling strength for each pathway calculated from ligand-receptor expression patterns. Analysis performed using CellChat's computeCommunProb and aggregateNet functions. (b) UMAP visualization of fibroblast subclustering showing Failing (red) and Non-failing (blue) fibroblast populations in three conditions: SHAM NT (gRNA), TAC *Klf15* (gRNA) and TAC NT (gRNA). Fibroblast state classification based on expression of activation markers (*Postn*, *Acta2*, *Cdh11*). (c) Violin plot showing *Tgfb2* expression across cardiac cell types including cardiomyocytes, endothelial cells, fibroblasts and pericytes. (d) Top list of differential gene expression analysis between conditions across CM0, CM1, CM2 and CM3 as well as total cardiomyocyte population in TAC *Klf15* (gRNA) versus TAC NT (gRNA) and TAC *Klf15* (gRNA) versus SHAM. Average log₂ fold-change values (log2FC), adjusted p-values (p_val_adj) and associated gene names for the top significantly differentially expressed genes in each comparison are shown. Differential expression analysis was performed using Seurat's FindMarkers function with Wilcoxon rank-sum test. (e) Violin plot showing the expression level of *Azgp1* in SHAM NT (gRNA), TAC NT (gRNA) and TAC *Klf15* (gRNA) based on normalized counts ($n=3-4$). (f) Violin plot of *Azgp1* expression levels in TAC NT (gRNA) and TAC *Klf15* (gRNA) condition in different cell types including cardiomyocytes, endothelial cells, fibroblasts and pericytes. Wilcoxon test with Benjamini-Hochberg correction for multiple testing in (c), (e) and (f), Wilcoxon rank-sum test in (d) were used for statistical significance.

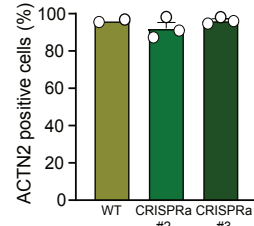
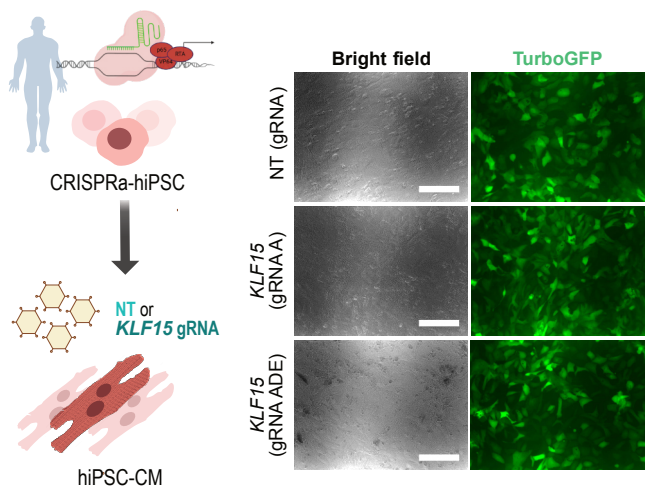
Figure S9



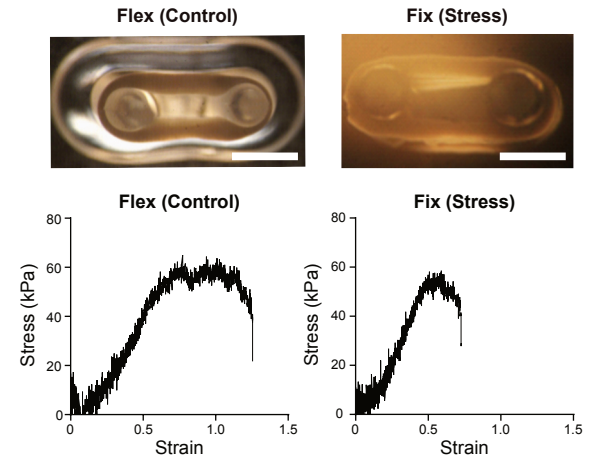
Supplementary Fig. 9: (a) Schematic representation of the *KLF15* open reading frame (ORF) overexpression (OE) experiments in hiPSC-cardiomyocytes including scheme of the lentiviral construct and immunoblot validation detecting FLAG-tag sequence in *KLF15* OE ($n=2$) as compared to EGFP control (CT, $n=2$). The transduction efficiency was checked by EGFP reporter expression using fluorescence microscopy. Scale bar = 100 μm . (b) Chromatin Immunoprecipitation (ChIP)-sequencing (seq) gene tracks (FLAG-HA-KLF15 HA pull-down, pink tracks) at the *AZGP1* locus from hiPSC-cardiomyocytes ($n=1$). (c) Validation of increased expression of *KLF15* via *KLF15* OE in hiPSC-cardiomyocytes as well as its known and novel target genes (*ALDH2* and *AZGP1*) compared to control (CT) by RT-qPCR ($n=4$). (d) Validation of *KLF15* gRNAs in HEK293T cells using RT-qPCR ($n=3-6$ per gRNA) identified gRNA A as a most effective single gRNA used for experiments with *Streptococcus pyogenes* (Spy)dCas9VPR. (e) Schematic representation of the experiments for CRISPRa in hiPSC-cardiomyocytes using established cell lines (CRISPRa cell lines #2 and #3^{15,16}). CRISPRa hiPSC-cardiomyocytes were transduced with gRNA (either NT gRNA, a single *KLF15* (A) gRNA or a combination of three *KLF15* gRNAs (ADE)) expressing lentivirus. The use of ADE *KLF15* gRNAs resulted in enhanced *KLF15* activation in hiPSC-cardiomyocytes compared to the single gRNA condition confirmed by RT-qPCR ($n=3-9$ per condition). (f) Schematic representation of the *AZGP1* ORF-OE or *AZGP1* knock down using short hairpin RNA (sh) experiments in hiPSC-cardiomyocytes including scheme of the lentiviral construct. Immunoblot validating increased protein level of *AZGP1* using OE and decreased protein level of *AZGP1* using sh compared to EGFP control (CT). Vinculin was used as a loading control. Transduction efficiency was assessed by EGFP reporter expression using fluorescent microscopy. Scale bar = 100 μm . (g) Validation of *AZGP1* OE in hiPSC-cardiomyocytes as confirmed by RT-qPCR ($n=6$). (h) Schematic representation of the conditioned medium from hiPSC-cardiomyocytes transduced with *AZGP1* sh and human recombinant *AZGP1* protein experiments in hiPSC-fibroblasts. Immunoblot validated increased POSTN and α -SMA expression in hiPSC-fibroblasts exposed to *AZGP1* sh transduced hiPSC-cardiomyocytes conditioned medium as well as decreased POSTN and α -SMA in hiPSC-fibroblasts exposed to *AZGP1* recombinant protein (final concentration: 1 $\mu\text{g}/\text{ml}$, $n=4$, 5days) compared to control (CT). Vinculin was used as a loading control. Mean $\pm$ standard error of the mean (SEM) in (c), (d), (e), and (h). Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (g). Student's t-test was used to test for statistical significance in (c) and (g). One-ANOVA and Tukey's post hoc test were used for group comparison in (d), (e), and (h).

Figure S10

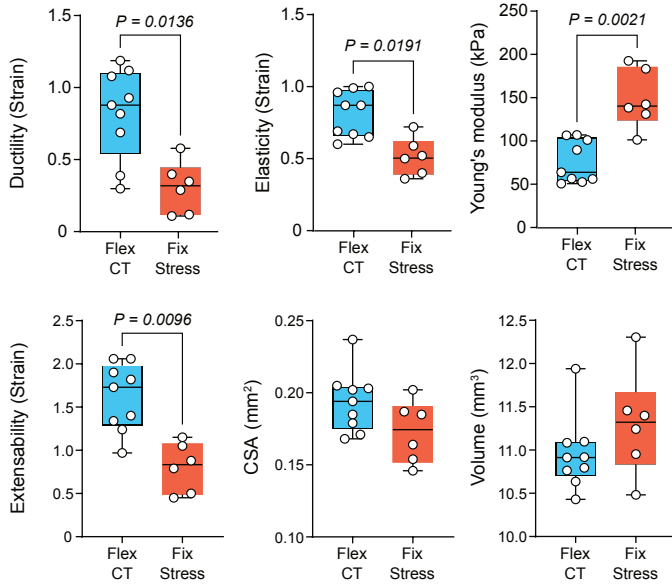
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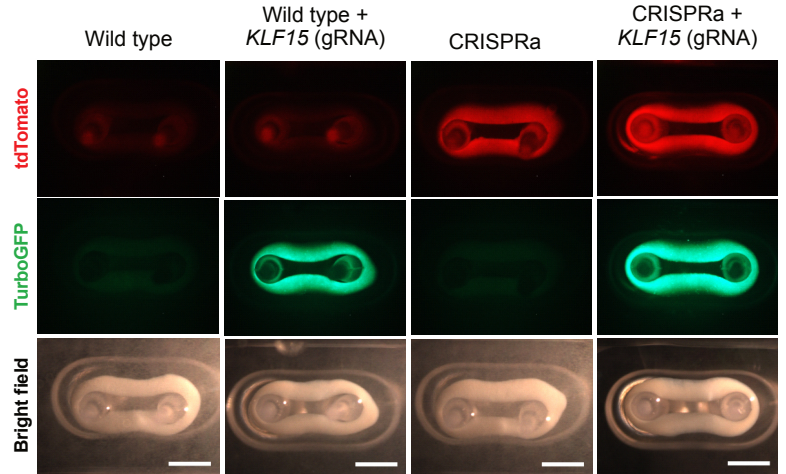
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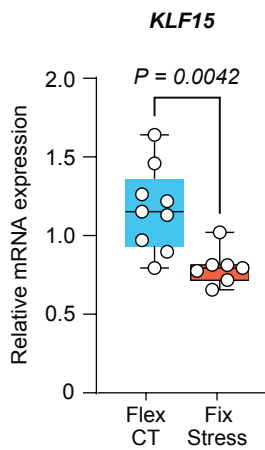
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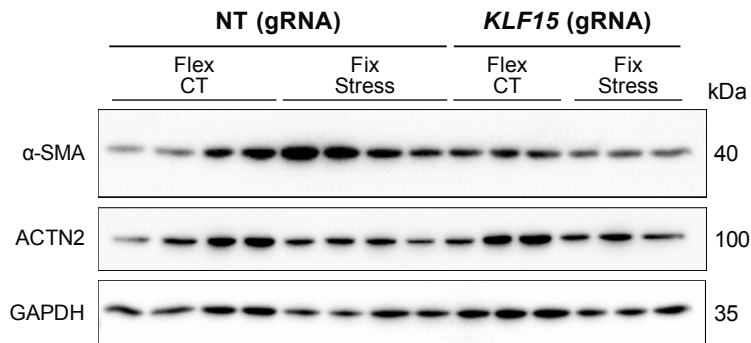
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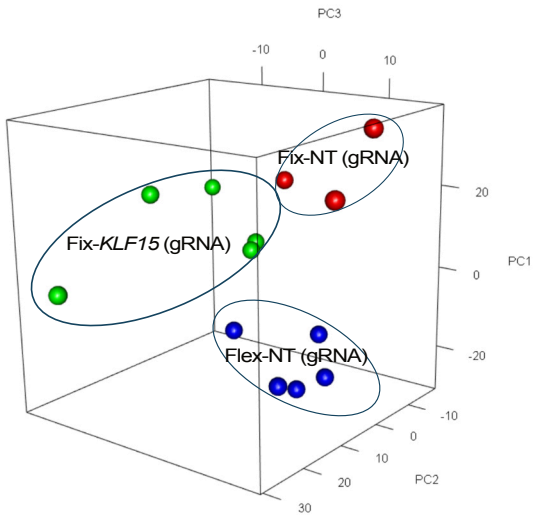
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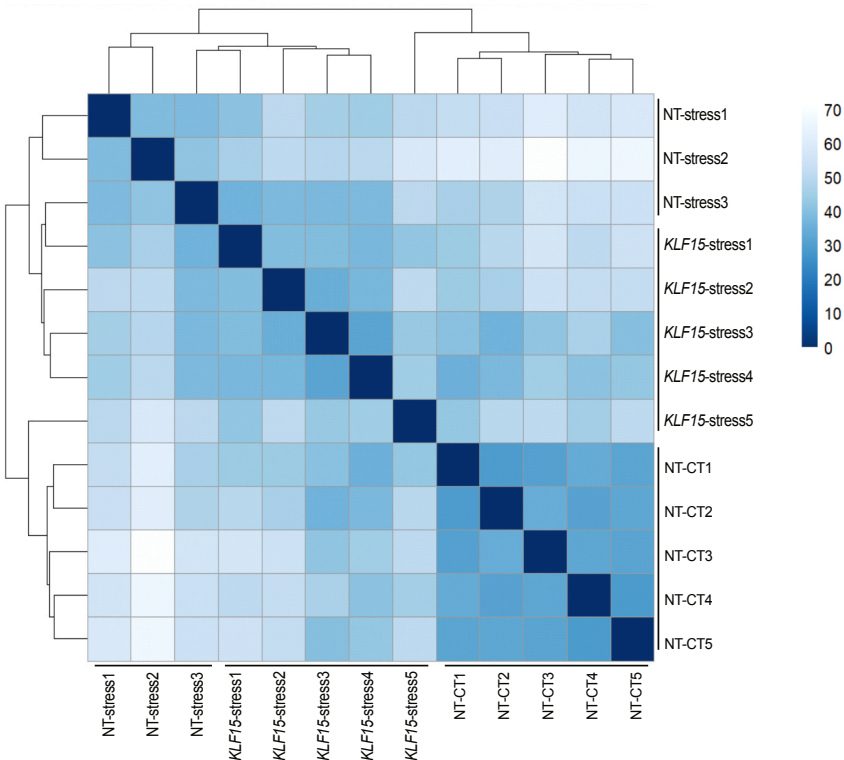
Supplementary Fig. 10: (a) Validation of hiPSC-cardiomyocytes used for generation of engineered human myocardium (EHM). The lentiviral vector co-expressed TurboGFP confirming comparable transduction efficiencies across conditions. Directed differentiation of CRISPRa hiPSC-cardiomyocytes yielded in > 95 % alpha-actinin-2 (ACTN2)-positive cardiomyocytes used for subsequent experiments ($n=2-3$ per group). (b) Exemplary bright field images of EHM cultured on flexible (Flex (control), auxotonic contraction) and non-flexible (Fix (Stress), isometric contraction) poles and representative stress-strain curves of destructive tensile measurements. Scale bar = 2 mm. (c) Rheological tissue property characterization of EHM cultured under control (Flex CT, $n=9$) and stress (Fix Stress, $n=6$) conditions. Derived tissue parameters were: Ductility, elasticity, extensability, Young's modulus as measures for EHM brittleness and stiffness as well as overall tissue cross sectional area (CSA) and volume. (d) Representative overview images taken one week after EHM generation expressing tdTomato (dCas9VPR) and gRNAs (TurboGFP) including non-transduced and non-transgenic control (Wild type) groups. Scale bar = 2 mm. (e) Relative *KLF15* expression in non-transduced, non-transgenic hiPSC-cardiomyocyte EHM cultured under control (Flex CT, $n=9$) and stress (Fix Stress, $n=7$) conditions as evaluated by RT-qPCR. (f) Immunoblot showing increased α -SMA expression in stress (Fix) NT (gRNA) EHM as compared to control tissues (Flex NT (gRNA)) and reduced α -SMA activation response upon enhanced expression of *KLF15* in stress *KLF15* (gRNA) EHM ($n=3-4$ per condition). GAPDH was used as a loading control. Mean $\pm$ standard error of the mean (SEM) in (a). Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (c) and (e). Student's t-test was used to test for statistical significance in (c) and (e).

Figure S11

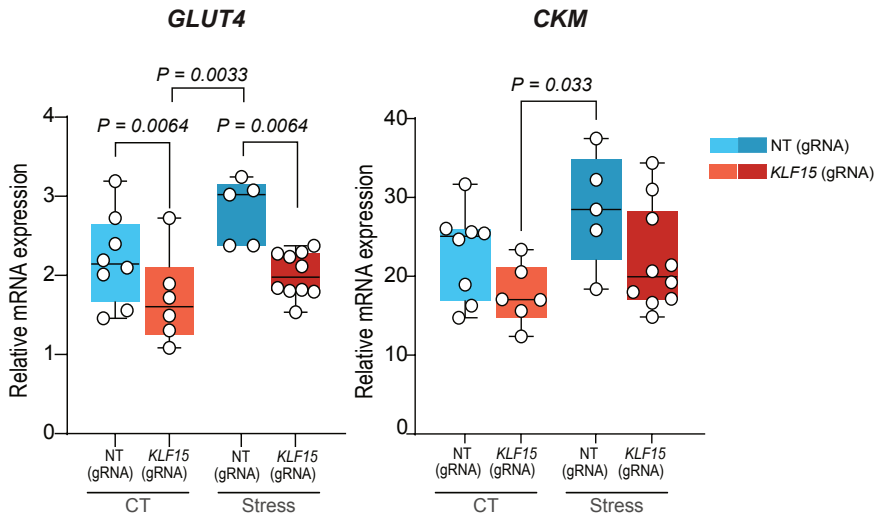
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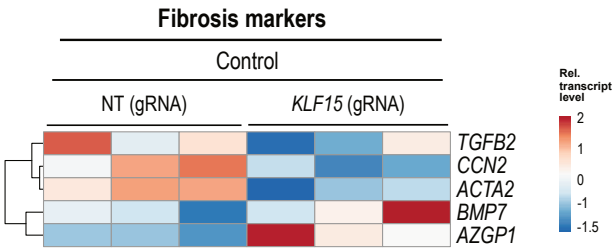
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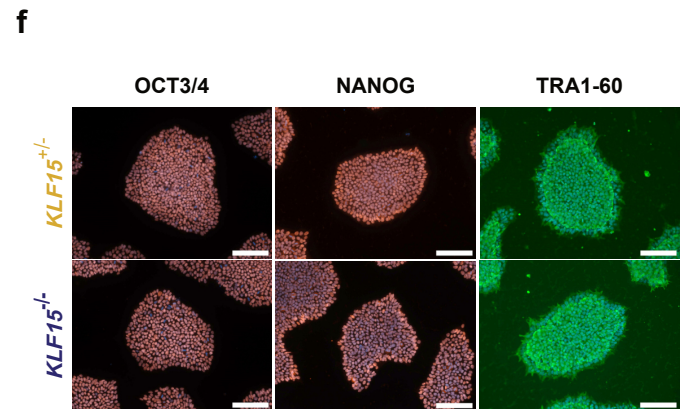
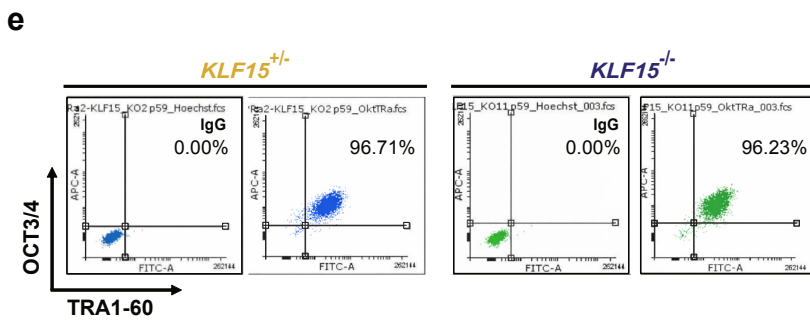
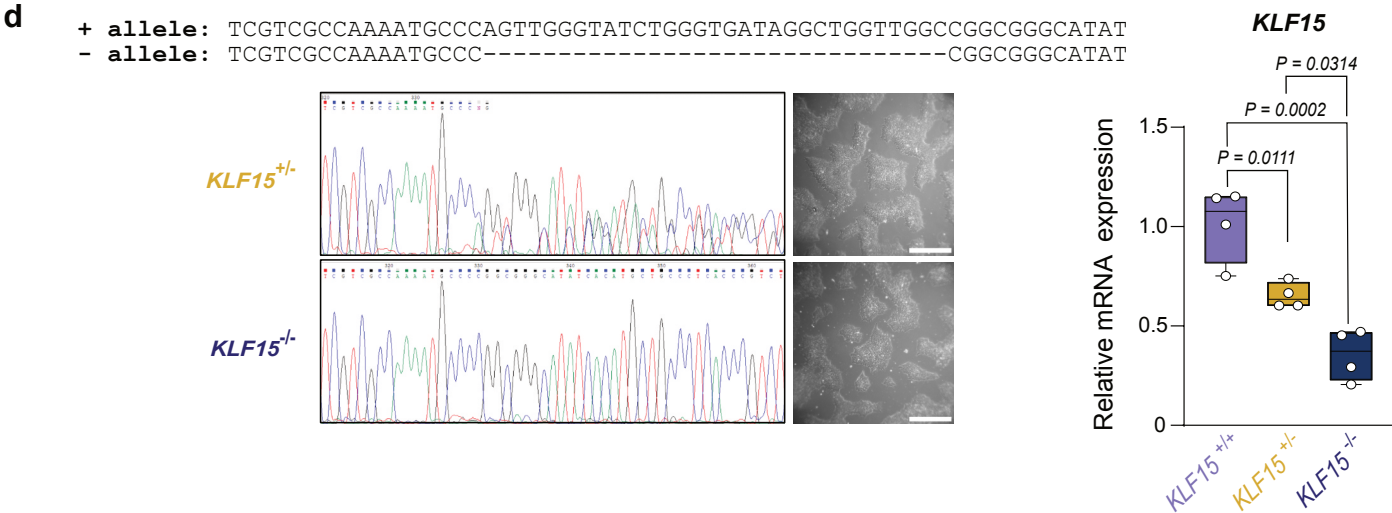
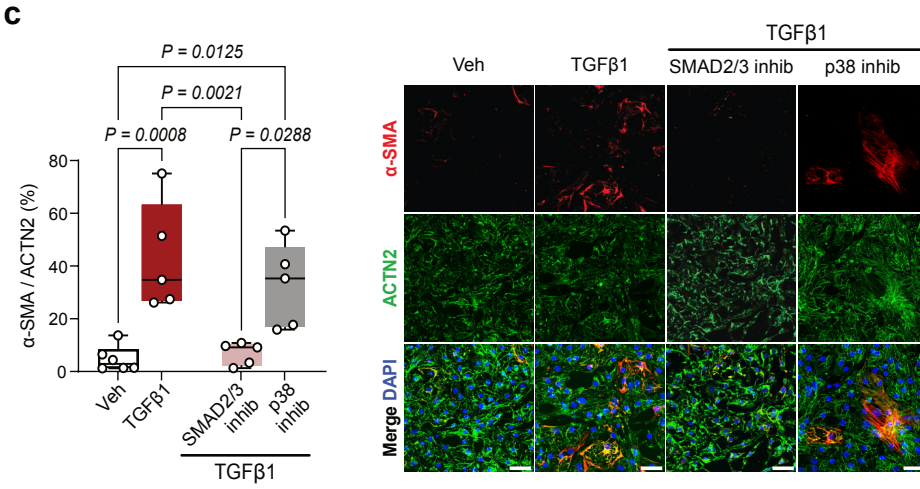
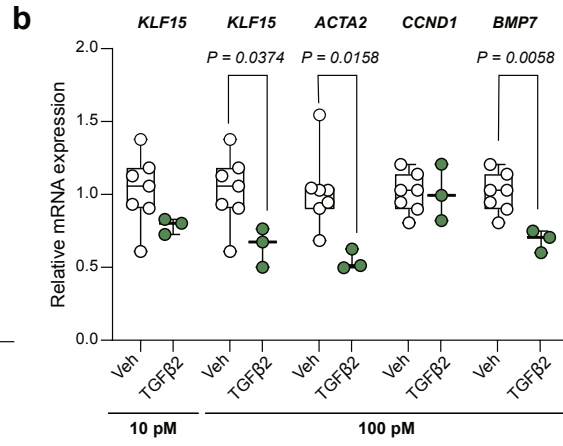
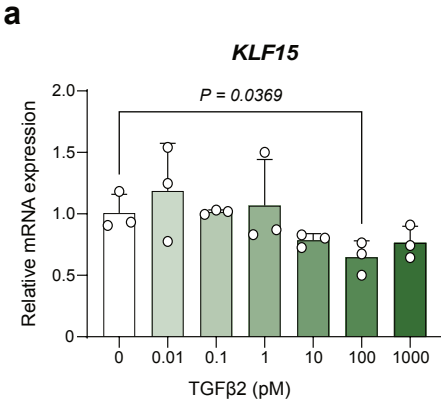


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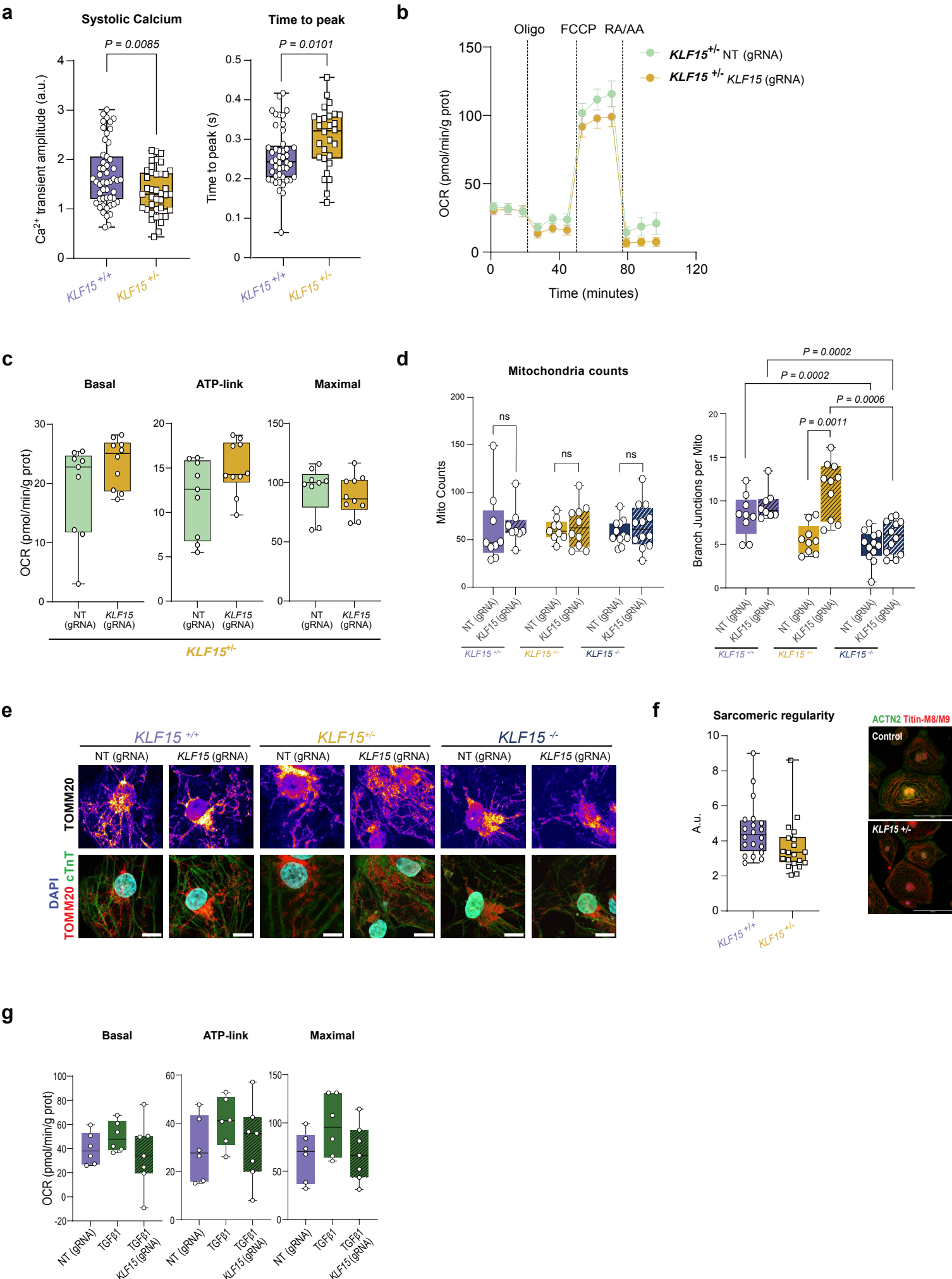
Supplementary Fig. 11: (a) Three-dimensional principal component (PC) analysis of control (Flex) NT (gRNA) and stress (Fix) NT (gRNA) as well as stress *KLF15* (gRNA) EHM based on RNA-seq expression profiles (Flex-NT (gRNA) $n=5$; Fix-NT (gRNA) $n=3$; Fix-*KLF15* (gRNA) $n=5$). (b) Hierarchical clustering of control (CT) NT (gRNA) ($n=5$) and stress NT (gRNA) ($n=3$) as well as stress *KLF15* (gRNA) EHM ($n=5$) derived from RNA-seq data. (c) Further validation of transcriptional regulation assessed by NanoString transcript analyses of control versus stressed EHM with NT and *KLF15* (gRNA) (*GLUT4*, *CKM4*, $n=5-10$). (d) Heatmap panel of fibrosis markers as well as newly identified *KLF15* target gene *AZGP1* as derived from RNA-seq analyses of control EHM transduced with NT (gRNA) or *KLF15* (gRNA). $n=3$ per group. Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (c). Two-way ANOVA and Tukey's post-hoc test were used to test for statistical significance in (c).

Figure S12



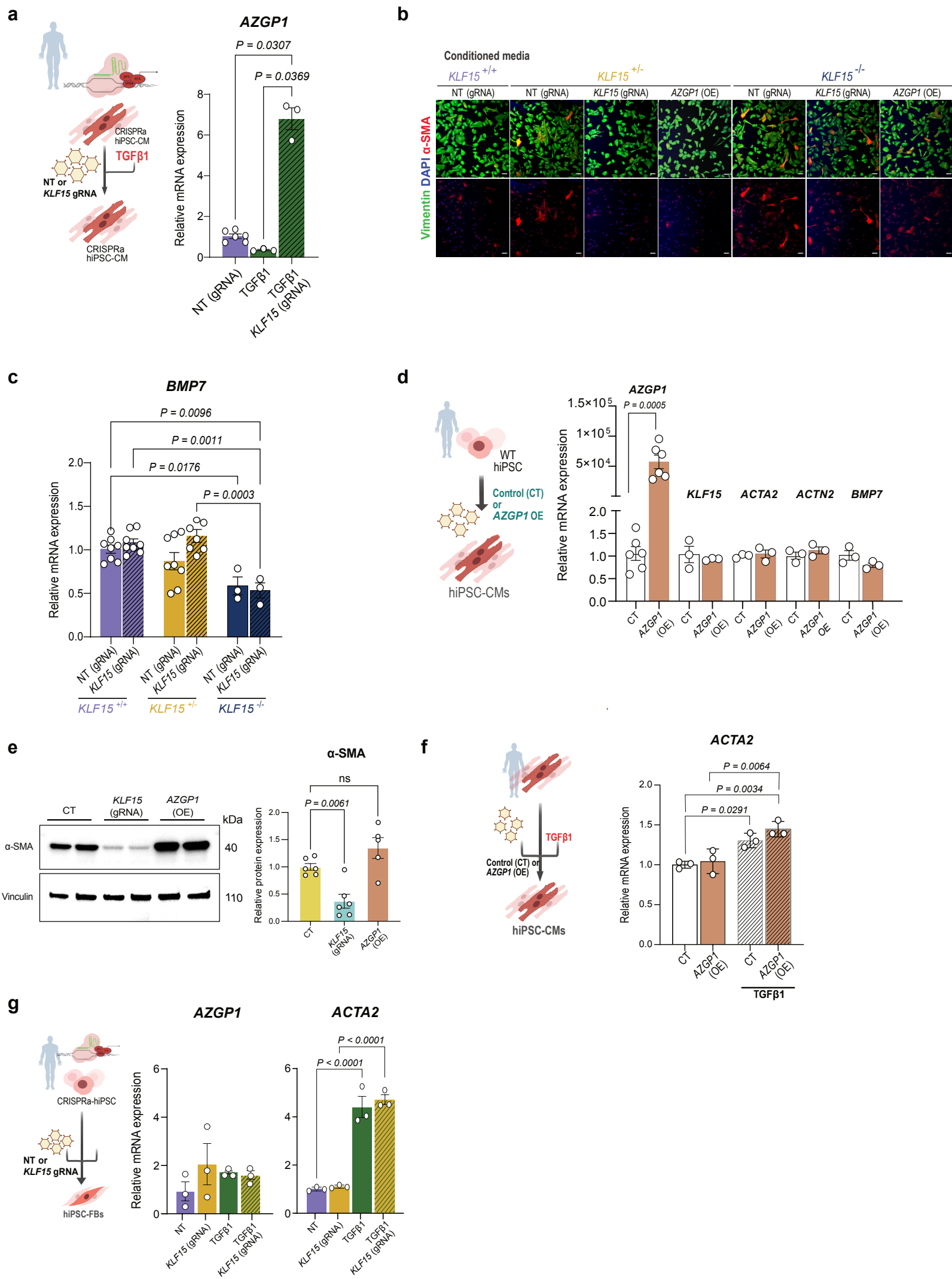
Supplementary Fig. 12: (a) RT-qPCR analysis showing *KLF15* expression in hiPSC-cardiomyocytes upon TGFβ2 exposure with the indicated concentrations of TGFβ2 (5 days, *n*=3). (b) *KLF15* expression in hiPSC-cardiomyocytes measured by RT-qPCR after TGFβ2 exposure (final concentration: 10 and 100 pM, 5 days). Expression of *ACTA2*, *CCND1* and *BMP7* were evaluated upon 100 pM TGFβ2, 5 days exposure. Vehicle (Veh) exposure served as control group (Veh; *n*=7, TGFβ2; *n*=3). (c) Representative immunofluorescence images and quantification of α-SMA expression normalized to ACTN2 expression in hiPSC-cardiomyocytes upon TGFβ1 exposure (final concentration: 10 pM, 5 days) with respective inhibitors: SB431542 (SMAD2/3 inhibitor (inhib, final concentration: 10 μM) and SB203580 (p38 inhibitor (inhib), final concentration: 10 μM). α-SMA (red), ACTN2 (green), nuclei (DAPI, blue), scale bar = 40 μm. *n*=5-6 field of views per condition. (d) Sanger sequencing of PCR products from wild-type: *KLF15*^{+/+}, heterozygous: *KLF15*^{+/-} and homozygous: *KLF15*^{-/-} knock out (KO) hiPSC mutants with the representative bright field images showing normal hiPSC colony morphology. Validation of *KLF15* expression in respective hiPSC lines as assessed by RT-qPCR (*n*=4). Scale bar = 200 μm. (e) Pluripotency markers assessed by flow cytometry (FC) and (f) immunofluorescence (IF) microscopy for OCT4, TRA-160 (FC and IF) and NANOG (IF) expression. IgG control was used for FC negative control. Scale bar = 500 μm. Mean ± standard error of the mean (SEM) in (a). Mean ± 95% confidence interval (box) and minimum/maximum (whiskers) in (b), (c), and (d). One-way ANOVA and Tukey's post-hoc test were used to test for statistical significance in (a), (c) and (d). Student's t-test was used to test for statistical significance in (b).

Figure S13



Supplementary Fig. 13: (a) Amplitudes of systolic Ca^{2+} transients and rates of Ca^{2+} transient upstroke velocity (time to peak) were measured in *KLF15*^{+/+} (*n*=45 cells) and *KLF15*^{+/-} KO (*n*=39 cells) hiPSC-cardiomyocytes supporting the data in Figure 5d. (b-c) Mitochondrial stress test in CRISPRa-hiPSC-cardiomyocytes upon NT or *KLF15* gRNA transduction in *KLF15* heterozygous knock out (KO) cells (*KLF15*^{+/-}). Basal and maximal oxygen consumption rate (OCR) as well as ATP-link respiration were calculated (c). No significant differences were observed between the groups (*n*=9-10). All the measurements were normalized by total protein level. (d) Total mitochondria (Mito) counts and branch junctions per mitochondria in *KLF15*^{+/+}, *KLF15*^{+/-} and *KLF15*^{-/-} CRISPRa-hiPSC-cardiomyocytes upon NT or *KLF15* gRNA transduction. *n*=8-12 field of views per condition. (e) Representative maximum intensity projection images of AiryScan confocal data used for mitochondria network analyses (Figure 5f and S13d). Depicted are indicated groups of hiPSC-cardiomyocytes stained for the mitochondria outer membrane protein TOMM20 (radiant fire color palette - upper panel and red - lower panel), cTnT (green) and nuclei (DAPI, blue), scale bar = 10 μm . (f) Sarcomeric regularity was quantified and assessed by ACTN2 and Titin-M8/M9 staining as surrogate of sarcomere organization shown with representative confocal images. No difference was observed between control (*KLF15*^{+/+}) and *KLF15*^{+/-} hiPSC-cardiomyocytes. ACTN2 (Green), Titine-M8/M9 (red), scale bar = 10 μm , *n*=20 per condition. (g) Mito-stress evaluated in hiPSC-cardiomyocytes transduced with NT and *KLF15* gRNA upon TGF- β 1 exposure (final concentration: 10 pM, 5 days). Basal and maximal oxygen consumption rate (OCR) as well as ATP-link respiration were calculated (*n*=6-7). All the measurements were normalized by total protein level. Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (a), (c), (d), (f) and (g). Student's t-test was used to test for statistical significance in (a), (c) and (f). Two-way ANOVA and Tukey's post-hoc test were used to test for statistical significance in (d) and One-way ANOVA and Tukey's post-hoc test in (g).

Figure S14

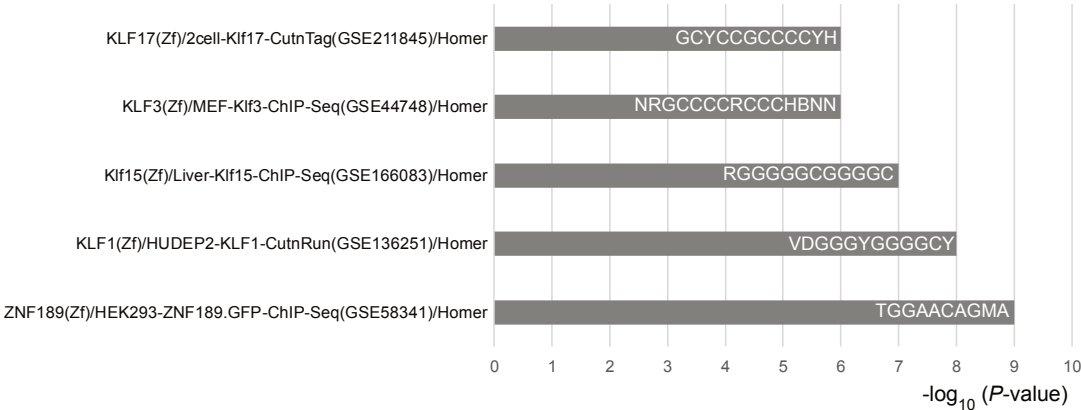


Supplementary Fig. 14: (a) RT-qPCR validation of *AZGP1* expression upon NT or *KLF15* gRNA transduction in CRISPRa-hiPSC-cardiomyocytes and TGFβ1 exposure (final concentration: 10 pM, 5 days, NT (gRNA); $n=6$, TGFβ1; $n=3$, *KLF15* (gRNA); $n=3$). (b) Representative immunofluorescence images of the conditioned media treated hiPSC-fibroblasts. Conditioned media from hiPSC-cardiomyocytes (conditions presented in Figure 5a) along with conditioned media from *AZGP1* overexpressing (OE) hiPSC-cardiomyocytes. α-SMA (red), Vimentin (green), nuclei (DAPI, blue), scale bar = 50 μm. (c) Analysis of *BMP7* transcript expression by RT-qPCR in CRISPRa-hiPSC-cardiomyocytes with different genetic doses of *KLF15* upon NT or *KLF15* gRNA transduction (*KLF15*^{+/+}; $n=8$, *KLF15*^{+/-}; $n=6-8$, *KLF15*^{-/-}; $n=3$). (d) RT-qPCR analysis of *AZGP1*, *KLF15*, *ACTA2*, *ACTN2* and *BMP7* expression in lentivirally mediated overexpression of *AZGP1* (*AZGP1* OE) in hiPSC-cardiomyocytes compared to control (CT) ($n=3-6$). (e) Immunoblot of α-SMA protein expression and quantification in hiPSC-cardiomyocytes transduced with *KLF15* (gRNA), *AZGP1* (OE) and control (CT). Vinculin was used as a loading control. $n=5-6$ per group. (f) RT-qPCR validation of *ACTA2* expression upon Control (CT) or *AZGP1* lentiviral induced overexpression (OE) in hiPSC-cardiomyocytes and TGFβ1 exposure (final concentration: 10 pM, 5 days, $n=3$). (g) RT-qPCR analysis of *AZGP1* and *ACTA2* expression in CRISPRa-hiPSC-fibroblasts upon NT or *KLF15* gRNA transduction and TGFβ1 exposure (final concentration: 10 pM, $n=3$). Mean ± standard error of the mean (SEM) in (a), (c), (e), (f) and (g). One-way ANOVA and Tukey's post-hoc test were used to test for statistical significance in (a), (e), (f) and (g). Two-way ANOVA and Tukey's post-hoc test were used to test for statistical significance in (c). Student's t-test was used to test for statistical significance in (d).

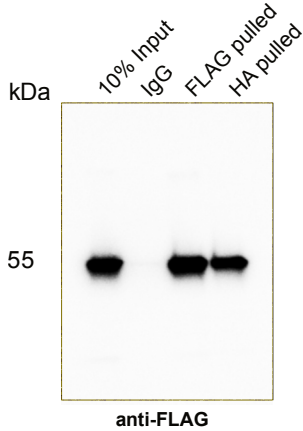
Figure S15

a

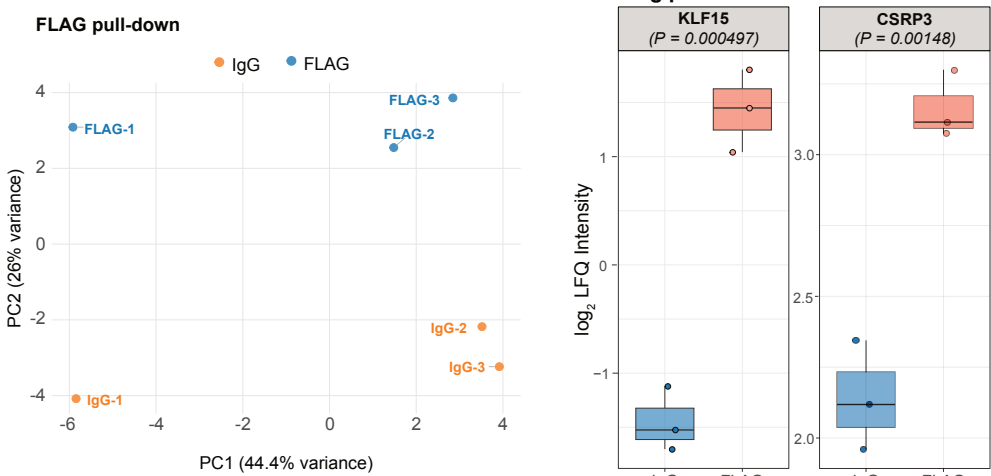
Motif analysis in FLAG-HA-KLF15 enriched sequences



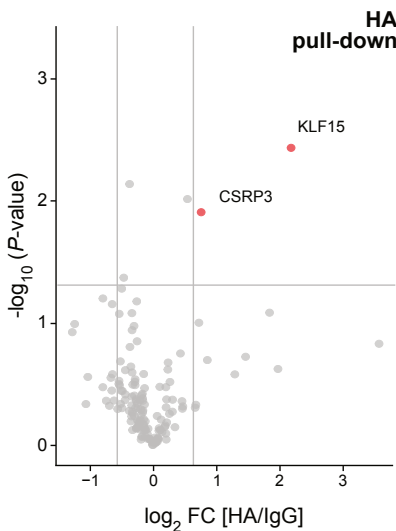
b



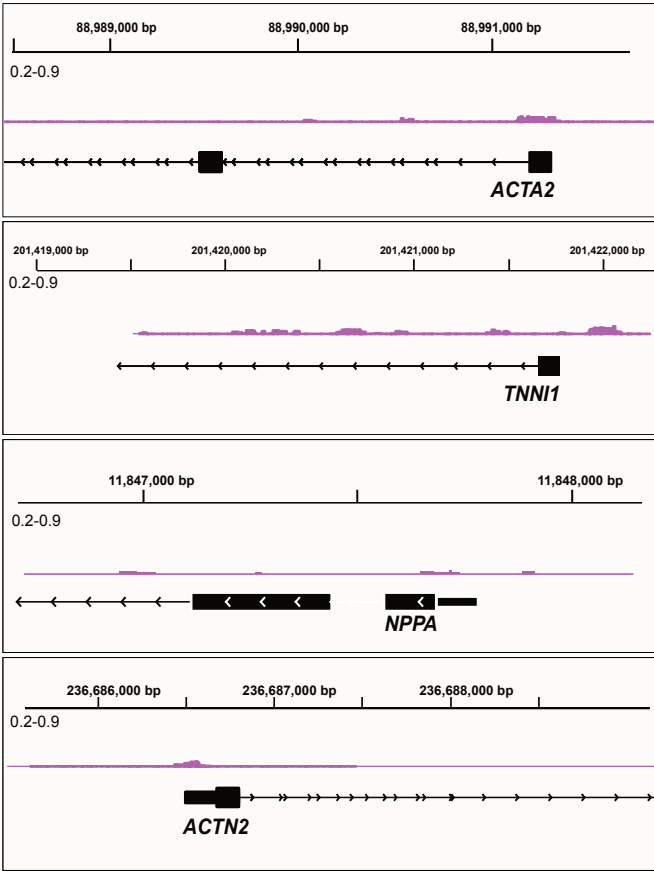
c



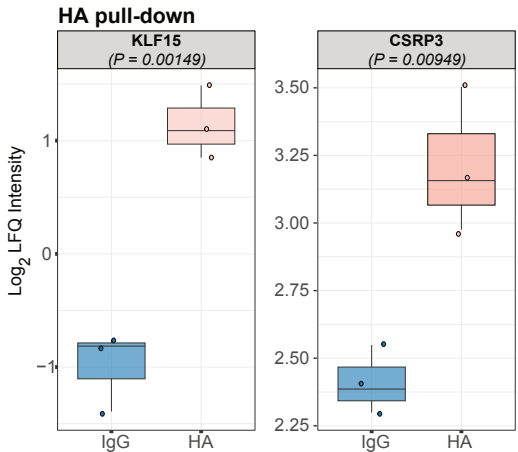
d



f



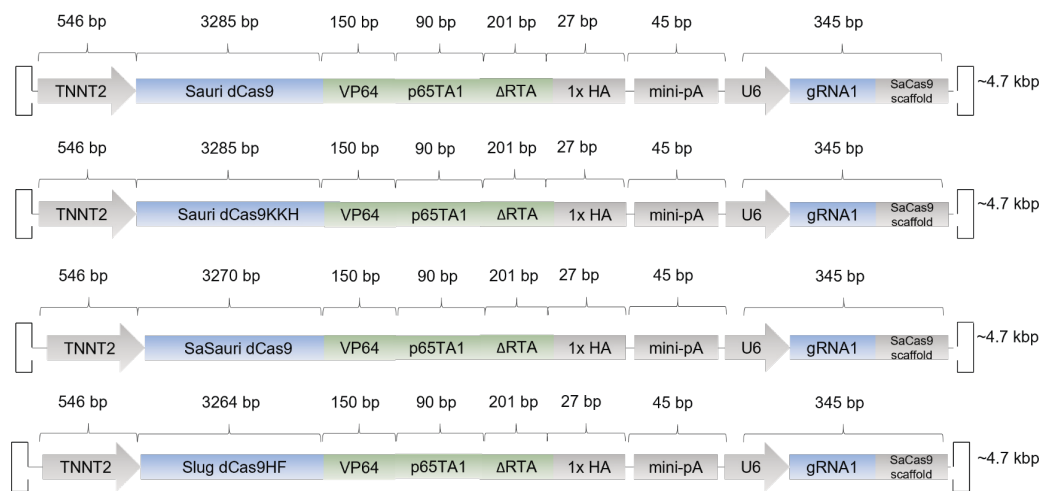
e



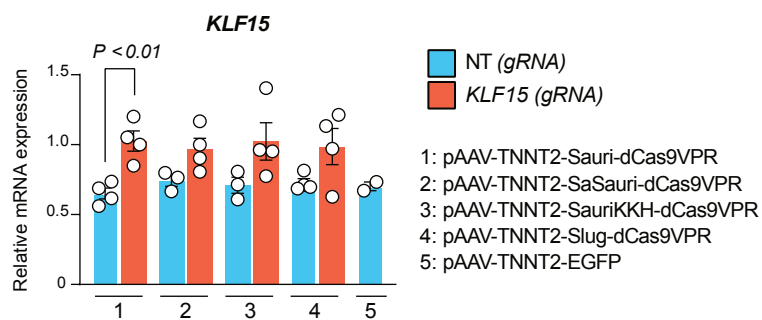
Supplementary Fig. 15: (a) Enriched KLF15 target motifs derived from Chromatin Immunoprecipitation (ChIP)-sequencing (seq) analyses. (b) Confirmation of Flag and HA pull-down by probing for the Flag-tag in hiPSC-cardiomyocytes expressing Flag-HA-KLF15 open reading frame by immunoblot. IgG was used as a pull-down control. (c) Principal component (PC) analyses of KLF15 co-immunoprecipitation in hiPSC-cardiomyocytes along with confirmation of KLF15 pull-down by Flag tag and identification of CSRP3 as a novel KLF15 binding partner in hiPSC-cardiomyocytes. $n=3$ per condition. IgG was used as a pull-down control. LFQ; Label-free quantification. (d) Volcano plot depicting significantly enriched proteins from KLF15 pull-down experiments (HA pull-down). FC; fold change. (e) Confirmation of KLF15 pull-down by HA tag and identification of CSRP3 as a novel KLF15 binding partner in hiPSC-cardiomyocytes ($n=3$ per group). LFQ; Label-free quantification. (f) KLF15 ChIP-seq traces identified occupancy at the *ACTA2*, *TNNI1*, *NPPA*, and *ACTN2* promoter in hiPSC-cardiomyocytes (pink traces, $n=1$). Mean $\pm$ 95% confidence interval (box) and minimum/maximum (whiskers) in (c) and (e). Student's t-test was used to test for statistical significance in (c) and (e).

Figure S16

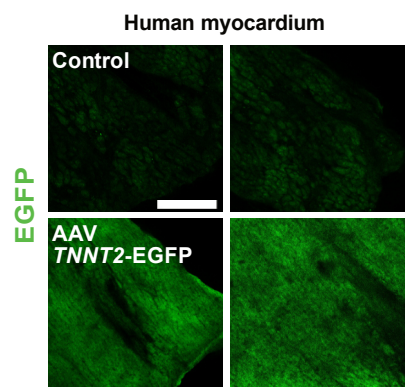
a



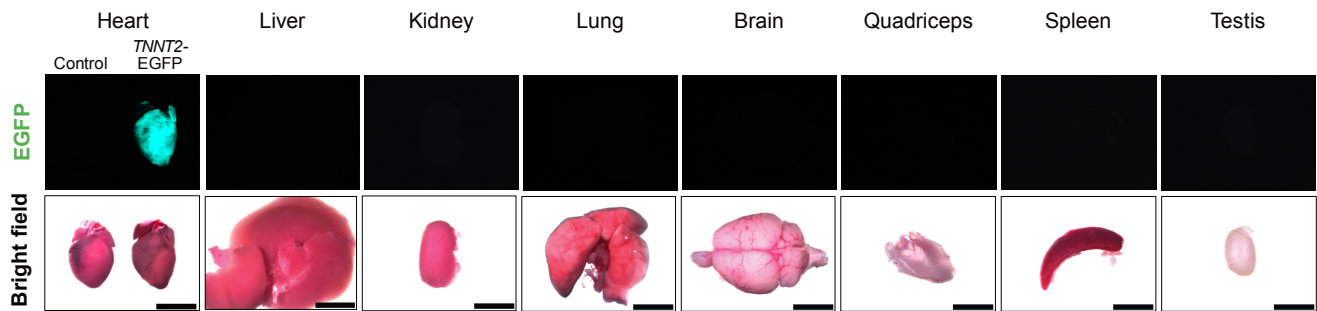
b



c



d



Supplementary Fig. 16: (a) Overview of all-in-one AAV vector designs for the expression using different Cas9 variants. (b) Transfected hiPSC-cardiomyocytes with respective dCas9VPR variants and *KLF15* (gRNA) significantly induced *KLF15* expression compared to NT (gRNA). Vector backbone encoding EGFP under *TNNT2* promoter (pAAV-TNNT2-EGFP) served as control. $n=2-4$ per group. (c) Biomimetic cultures of human myocardial tissue transduced with AAV9 encoding for EGFP under *TNNT2* promoter indicated successful transduction of myocardial tissue in human cardiac slice cultures after 11 days. Scale bar = 20 μm . (d) Delivery and expression of pAAV-TNNT2-EGFP in the heart in comparison to a saline-injected mouse (control) together with EGFP expression across different organs in a mouse following injection of AAV9 particles delivering the *TNNT2* promoter driven EGFP vector, which served as the backbone for the all-in-one CRISPRa system developed in this study. Scale bar = 5 mm. Mean $\pm$ standard error of the mean (SEM) in (b). Student's t-test was used to test for statistical significance in (b).

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