

SUPPLEMENTARY MATERIALS

An alternative mechanism of subcellular iron uptake deficiency in cardiomyocytes

Yuanyuan Dai, Nadezda Ignatyeva, Hang Xu, Ruheen Wali, Karl Toischer,
Sören Brandenburg, Christof Lenz, Julius Pronto, Funsho E. Fakuade, Samuel Sossalla,
Elisabeth M. Zeisberg, Andreas Janshoff, Ingo Kutschka, Niels Voigt, Henning Urlaub,
Torsten Bloch Rasmussen, Jens Mogensen, Stephan E. Lehnart, Gerd Hasenfuss, Antje Ebert

DETAILED METHODS

Study design

The objective of the study was to investigate molecular patho-mechanisms in presence of inherited DCM mutations causing disturbed sarcomere organization and function. We employed a combined model of patient-derived and CRISPR/Cas-edited induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) as well as patient-derived heart tissue. We used an integrated platform of MS-DIA-based proteomics and signaling pathway interrogation and identified from this analysis a molecular patho-mechanism, impaired subcellular iron deficiency. We next confirmed this molecular disease mechanism in both DCM iPSC-CMs and clinical specimens from DCM patients with end-stage HF. To rescue the molecular disease pathway, we employed correction of the TPM1-L185F mutation in DCM patient-derived iPSCs or treatment with a small compound, RhoA activator II (3 $\mu\text{g/mL}$ for 3 h or overnight), recovering PM-localized PIP2 levels, uptake of transferrin-bound iron, endosome distribution, mitochondrial iron levels, sarcomere length, and/or contractility. Moreover, we pheno-copied the effects of the TPM1-L185F mutation (abnormal F/G actin ratio, reduced PIP2 levels, endosome distribution defects) into WT iPSC-CMs by treatment with Lat-A (3 μM for 20 min) or UNC3230 (10 μM for 24 h). Biochemical and cell biology experiments (Western blotting, immunoprecipitation, uptake assay, image analysis) were conducted in an unblinded fashion for ≥ 2 independent experiments to ensure reproducibility, and end points were determined from the literature and pilot studies, respectively. The numbers of independent experiments performed for each figure are listed in Supplementary Table 5. We employed an iPSC model covering $n=2$ inherited mutations in different proteins that lead to dilated cardiomyopathy

(DCM), troponin T (TnT-R141W) and tropomyosin (TPM1-L185F). Clinical specimens were employed from n=5 patients with DCM and end-stage heart failure and n=4 patients with preserved LV function that underwent aortic valve replacement (severe valve stenosis) and coronary artery bypass graft surgery in the control group.

Human iPSC reprogramming and culture. The protocols for the studies with iPSC were approved by the Goettingen University Ethical Board (15/2/20 and 20/9/16An) and the Odense University Ethical Board (Projekt ID S-20140073HLP). Informed consent was obtained from all participants and all research was performed in accordance with relevant guidelines and regulations. Approval for the study of human myocardial samples was granted by the Goettingen University Ethical Board (No. 21/10/00 and 31/9/00), and written informed consent was obtained from all patients. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. A TPM1-L185F family cohort was recruited for this study and five mutation carriers as well as four healthy individuals without the mutation donated a skin biopsy. From all skin biopsies, fibroblasts were derived and employed for iPSC reprogramming. The skin punch biopsies were dissociated, and isolated skin fibroblasts were kept in Dulbecco's modified Eagle's medium (Life Technologies) supplied with 10% fetal bovine serum (Gibco) under sterile culture conditions at 37°C. For generation of the human iPSC lines, human fibroblasts from skin biopsies were reprogrammed to hiPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) and colonies with stem cell-like morphology were picked and expanded^{21,38,106,107}. Individuals II-4 and III-5 and the corresponding human iPSC lines control WT 1 (WT/WT) and DCM patient-specific TPM1-

L185F (WT/MUT) were selected for this study. Individual II-4 is a 55-year-old male who presented with no phenotype or symptoms and was negative for the TPM1-L185F mutation (WT/WT). Individual III-5 is a 27-year-old female patient with severe DCM who presented with non-sustained ventricular tachycardia and received a heart transplantation when 15 years old due to end-stage heart failure. Patient III-5 genotyped positive for the TPM1-L185F mutation (WT/MUT). WT/WT iPSCs from control WT 1 and DCM patient-specific TPM1-L185F iPSCs (WT/MUT) were utilized to generate a set of isogenic control lines that diverge from the respective parental line only in the site of the TPM1-L185F mutation. WT/WT iPSCs were used as parental line to generate TPM1-L185F MUT-introduced iPSCs (MUT-int/MUT-int). WT/MUT patient-specific iPSCs were employed to generate TPM1-L185F MUT-corrected iPSCs (WT-cor/WT-cor). The iPSC line control WT 2 was reported and characterized previously^{21,26,108}. The DCM patient-specific iPSC line carrying the mutation TnT-R173W was employed as a control and has been reported and characterized before^{21,23}. Both the iPSC lines WT2 and DCM TnT-R173W were kind gifts by Joseph C. Wu (Stanford University, CA) The derivation of the TnT-R141W CRISPR/Cas9 mutation-introduced iPSC line, TnT-R141W MUT-int/MUT-int, has been described before²⁶. The isogenic parental line for TnT-R141W MUT-int/MUT-int is the iPSC line control WT 2²⁶. The iPSC lines have been deposited at the Goettingen University Medical Center Biobank (control WT 1, DCM patient-specific TPM1-L185F) or the Stanford Cardiovascular Institute Biobank (control WT 2, DCM patient-specific TnT-R173W). Human iPSCs were grown as described previously on Matrigel-coated plates (ES qualified, BD Biosciences) using chemically defined E8 medium^{23,37,38}. The culture

medium was changed daily, and iPSCs were passaged every four days using EDTA (Life Technologies).

Cardiac differentiation of human iPSC. Human iPSCs were grown to 80–90% confluence using matrigel-coating and chemically defined E8 medium³⁷. Subsequently, iPSCs were differentiated into beating cardiomyocytes with a small molecule–based monolayer method described previously^{39,40}. Briefly, a GSK inhibitor, Chir (Selleckchem) was applied for 24–48 h, followed by addition of a canonical Wnt-signaling inhibitor, IWR1 (Selleckchem). From day 7–10 onward, beating iPSC-derived cardiomyocytes (iPSC-CMs) were observed. Subsequently (from day 7 onward), human iPSC-CMs were cultured in RPMI medium plus B-27 Supplement (Life Technologies). Following 20–22 days of cardiac differentiation, beating iPSC-CM monolayers were dissociated using TrypLE (Life Technologies) and plated in suitable assay formats.

CRISPR/Cas9 genetic engineering, molecular cloning, and plasmid sources.

CRISPR/Cas9-mediated gene editing via homology-directed repair to introduce the TPM1-L185F mutation into WT/WT iPSCs or correct this mutation in the DCM patient-specific TPM1-L185F WT/MUT iPSCs was performed as described before^{26,109–111}. In brief, the sgRNA sequence (5'-CCTGGAACGTGCAGAGGAGC-3') was subcloned into the pSpCas9(BB)-2A-Puro plasmid (a kind gift from Matthew Porteus, Stanford University). Subsequently, to produce TPM1-L185F MUT-introduced iPSCs (MUT-int/MUT-int), sgRNA and the new sequence (5'-CTAGAGCGCGCTGAAGAACGTGCAGAATTTC-3' in a pUC vector

backbone) containing the single nucleotide exchange C to T, which results in TPM1-L185F, were transfected into iPSCs via electroporation. Likewise, to generate TPM1-L185F MUT-corrected iPSCs (WT-cor/WT-cor), the sgRNA and respective new sequence (5'-CTAGAGCGCGCTGAAGAACGTGCAGAACTC-3' in a pUC vector backbone available at Addgene), were transfected into iPSCs. Single clones were isolated and sequence verified.

Nanon CardioExcyte 96 measurements. Human iPSC-CMs on day 17-19 of differentiation were washed with PBS and dissociated using TrypLE (Life Technologies). After spinning iPSC-CMs for 5 min at 200 g, 37 °C, cells were resuspended in culture medium supplemented with 5% FBS and seeded on Nanion CardioExcyte 96 Plates. Cells were allowed to recover from the passage for 6-7 days. Plates were recorded using a Nanion CardioExcyte 96 instrument with temperature control. Data were acquired and analyzed via CardioExcyte Control software.

Immunoprecipitation and immunoblotting. A vinculin-specific antibody (Thermo Fisher Scientific) was immobilized on Dyna beads (Thermo Fisher Scientific) according to the manufacturer's instructions. Lysates from WT and patient iPSC-CMs were prepared for immunoprecipitation binding with was buffer (25 mM Tris, NaCl 150 mM, EDTA 1 mM, pH=7.5). Eluted protein solutions as well as lysate input were subjected to SDS-PAGE and transferred to PVDF membranes. Following after incubation with primary and secondary antibodies (LI-COR), membranes were scanned in a LI-COR infrared imaging system. Quantification was performed using an Image Studio Lite Ver 5.2. Bound TnT signal was

normalized for the corresponding amount of bound vinculin by dividing the bound TnT signal by the bound vinculin signal for the respective groups. Bar graph shows normalized signal intensities.

PIP2 quantification. Human iPSC-CMs were plated on coverslips and allowed to recover for 2-3 days. Fixed cells were stained with a PIP2-specific antibody (mouse, Echelon Biosciences) overnight at 4 °C and stained with secondary antibodies as described before⁴⁵. Images were acquired under a 63x objective at a confocal LSM710 microscope for the relevant channels. Line ROIs were drawn on the edge of cells using ImageJ, and mean fluorescence of the ROIs was quantified.

Immunohistochemistry of adult human heart tissue. The investigation conforms to the principles outlined in the Declaration of Helsinki. The institutional ethics committee approved the study, and all patients provided written informed consent for the use of cardiac tissue samples. Clinical data for patients are presented in Tab. S2 and Tab. S3. Heart tissues from patients with dilated cardiomyopathy who received a heart transplantation due to end-stage heart failure (indicated as “heart failure”, HF, or heart transplantation) were analyzed. In comparison, LV heart tissue obtained during open-heart surgery from patients with preserved LV function that underwent aortic valve replacement (severe valve stenosis) and coronary artery bypass grafting was used in the control group (“control”). Explanted hearts or LV myocardial biopsies were directly put in pre-cooled cardioprotective solution (Custodiol®, Dr. Franz Köhler Chemie, Bensheim, Germany; in mmol/L: NaCl 15, KCl 9, MgCl₂ 4, histidine

hydrochloride monohydrate 18, histidine 180, tryptophan 2, mannitol 30, CaCl₂ 0.015, and potassium hydrogen 2-oxopentandiate 1). Subsequently, LV tissue samples were excised with scalpels and fixed in 4% paraformaldehyde overnight (Roti-Histofix 4 %, Carl Roth, Germany) prior to embedding in paraffin for immunohistology, or snap-frozen in liquid nitrogen before tissue lysis for immunoblotting (please see below). Human heart tissue slides were prepared and stained equally based on a previously published protocol ^{46,47}. Slides were incubated with primary antibodies overnight, such as TnT (rabbit, Thermo Fisher Scientific), vinculin (mouse, Sigma-Aldrich) or CCDC53 (rabbit, ProteinTech). Wheat germ agglutinin (WGA, Alexa Fluor™ 594 Conjugate, Thermo Fisher Scientific), DAPI (Thermo Fisher Scientific), and secondary antibodies such as Alexa Fluor 488 goat anti-Rabbit antibody (Thermo Fisher Scientific), Alexa Fluor 568 goat anti-Rabbit antibody (Thermo Fisher Scientific), Alexa Fluor 488 goat anti-mouse antibody (Thermo Fisher Scientific), were applied afterwards. Slides were mounted with FluroMount mounting medium (Thermo Fisher Scientific). Images were taken with 63x (plan apochromat oil) objectives from a confocal microscope (Carl Zeiss, LSM 710, Göttingen, Germany) and ZEN software (Carl Zeiss). Controls for staining specificity were performed (Fig. S14A-E). ImageJ-based analysis was used for image analysis. ImageJ was used to calculate the regularity of orientation for vinculin-positive structures. Data display the standard error of mean of angles from vinculin-positive areas in each image. To analyze the location of CCDC53, automated threshold setting in WGA staining was used to define PM-localized ROIs. Data were normalized by the total CCDC53 signal per image.

Immunoblotting of adult human heart tissue. The investigation conforms to the principles outlined in the Declaration of Helsinki. The institutional ethics committee approved the study, and all patients provided written informed consent for the use of cardiac tissue samples. Human LV heart tissue (healthy controls) was obtained from freshly explanted hearts of five donor hearts that could not be transplanted due to clinical reasons⁴⁸. Clinical data for 8 DCM patients are presented in Tab. S2 and Tab. S3. Lysates were obtained by homogenizing tissues at 4 °C for 5 min at 50 Hz in RIPA buffer (Thermo Fisher Scientific) with a metal bead (QIAGEN). Subsequently, lysates were incubated for 1 h at 4 °C and supernatant was boiled at 100 °C for 10 min in sample buffer followed by SDS-PAGE and immunoblot analysis for WASL (Rabbit, ProteinTech).

LC/MS/MS DIA analysis. 50 µg protein per sample were loaded onto a 4-12% NuPAGE Novex Bis-Tris Minigels (Invitrogen) and run into the gel for 1.5 cm. Following Coomassie staining, the protein areas were cut out, diced and subjected to reduction with dithiothreitol, alkylation with iodoacetamide and finally overnight digestion with trypsin. Tryptic peptides were extracted from the gel, the solution dried in a Speedvac and kept at -20 °C for further analysis⁴¹.

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 ug/ul. For each analysis 1.5 ug protein were enriched on a self-packed precolumn (0.15 mm ID x 20 mm, Reprosil-Pur120 C18-AQ 5 μ m, Dr. Maisch, Ammerbuch-Entringen, Germany) and separated on an analytical RP-C18 column (0.075 mm ID x 200 mm, Reprosil-Pur 120 C18-AQ, 3 μ m, Dr. Maisch) using a 100 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 Top30 data-dependent acquisition method with an MS survey scan of m/z 380–1250 accumulated for 250 ms at a resolution of 35 000 full width at half maximum (FWHM). MS/MS scans of m/z 180–1500 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 3.4 s. Precursors above a threshold MS intensity of 200 cps with charge states 2+, 3+, and 4+ were selected for MS/MS, the dynamic exclusion time was set to 15 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 WT and MUT each were analysed to construct a spectral library.

For quantitative data-independent acquisition (DIA) analysis, MS/MS data were acquired using 100 variable size windows⁴² across the 400-1200 m/z range. Fragments were produced using rolling collision energy settings for charge state 2+, and fragments acquired over an m/z range of 180–1500 for 40 ms per segment. Including a 250 ms survey scan this resulted in an overall cycle time of 4.3 s. For WT and MUT groups, two technical replicates each were processed, and 2 replicated injection per sample were acquired, resulting 4 samples per group.

Protein identification was achieved using ProteinPilot Software version 5.0 build 4304 (AB Sciex) at “thorough” settings. A total of 436,865 MS/MS spectra from the combined qualitative

analyses were searched against the UniProtKB Homo sapiens reference proteome (revision 04-2016) augmented with a set of 51 known common laboratory contaminants.

DIA peak extraction was achieved in PeakView Software version 2.1 build 11041 (AB Sciex) using the DIA quantitation microApp version 2.0 build 2003. Following retention time correction on endogenous peptides spanning the entire retention time range, peak areas were extracted using information from the MS/MS library⁴³. The resulting peak areas were then summed to peptide and finally protein area values, which were used for further statistical analysis. A non-parametric Wilcoxon test was performed to establish statistical significance.

Confocal live imaging, Fe measurements, continuous transferrin uptake, and LDL uptake. Human iPSC-CMs were plated into imaging chambers 2-3 days before the experiments and with Mitotracker Green (Cell Signaling Technology) at 37 °C as by the manufacturer's instructions, then incubated cells with 100 µM ammonium Fe²⁺ (Santa Cruz) and subsequently incubated with FerroOrange (Dojindo EU GmbH) as by the manufacturer's instructions. Images were acquired using a confocal microscope (Carl Zeiss, LSM 710 Meta, Göttingen, Germany). To measure transferrin uptake, iPSC-CMs were starved for 1 h at 37 °C in RPMI medium containing human albumin and ascorbic acid as described before¹⁰⁷. Cells were incubated with 20 µg/mL transferrin (Alexa Fluor™ 488 Conjugate, Thermo Fisher Scientific) for indicated times at 37 °C. Following a PBS wash, cells were fixed for 20 min at room temperature for each individual time point. Z-stack images were taken under a 20x (plan apochromat) confocal objective (Carl Zeiss, LSM 710 Meta, Göttingen, Germany). Mean fluorescence from each time point is normalized by the data acquired at 0 min. To measure

LDL uptake, cells were incubated with LDL-BODIPY (Thermo Fisher Scientific) as by the manufacturer's instructions for 0 min or 10 min at 37°C. Subsequently, cells were washed with PBS and fixed for 20 min at room temperature. Images were acquired under a 63x (plan apochromat oil) objective from a confocal microscope (Carl Zeiss, LSM 710 Meta, Göttingen, Germany) and ZEN software (Carl Zeiss) as well as ICY software were used for data analysis.

Immunohistochemistry (IHC) staining and confocal microscopy. Cells were grown on glass coverslips (Thermo Fisher Scientific), fixed with 4% glyoxal¹¹² or 4% PFA, permeabilized in 0.01% digitonin 20 min or 0.2% TritonX-100 60 min at room temperature (RT). Afterwards cells were incubated with primary antibodies: POU5F1 (Cell Signaling Technology), TRA-1-60 (Chemicon), SSEA4 (R&D systems), EEA1 (Cell signaling Technology), TnT (Thermo Fisher Scientific), SAA (Sigma), TPM (Abcam), vinculin (Sigma), PIP2 (Echelon Biosciences), CCDC53 (ProteinTech) overnight at 4 °C and secondary antibodies (Alexa Fluor dyes, Thermo Fisher Scientific) at room temperature for 2 h. Coverslips were mounted onto a glass slide in FluroMount mounting medium (Thermo Fisher Scientific). Images were taken with 63x (plan apochromat oil) objectives from a confocal microscope (Carl Zeiss, LSM 710 Meta, Göttingen, Germany) and ZEN software (Carl Zeiss).

In-Cell-Western. Cells were seeded in a 96-well plate and allowed to recover for 2-3 days. In-Cell-Western was performed as reported previously⁵⁰. Briefly, cells were then fixed with 3 % PFA, treated with 0.2% Triton for 1 h and subsequently blocked with 1% BSA for 1.5 h at room temperature. Next, samples were incubated overnight at 4 °C with primary antibodies

and, after a PBS wash, incubated with secondary antibodies (IRD680 goat anti-mouse antibodies and IRD800 goat anti-mouse antibodies) for 1 h at room temperature. Fluorescence signals were acquired by a LI-COR infrared imaging system.

nanoLC-MS/MS analysis. Samples were reconstituted in 1× NuPAGE LDS Sample Buffer (Invitrogen), separated on 4-12 % NuPAGE Novex Bis-Tris Minigels (Invitrogen) and stained with Coomassie Blue. Lanes were cut into 23 equidistant slices irrespective of staining. After washing, gel slices were reduced with DTT, alkylated with 2-iodoacetamide and digested with Endopeptidase Trypsin 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 as described previously⁴¹. For mass spectrometric analysis, samples were enriched on a self-packed reversed phase-C18 precolumn (0.15 mm ID x 20 mm, Reprosil-Pur120 C18-AQ 5 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) and separated on an analytical reversed phase-C18 column (0.075 mm ID x 200 mm, Reprosil-Pur 120 C18-AQ, 1.9 µm, Dr. Maisch) using a 46 min linear gradient of 4-35 % acetonitrile/0.1% formic acid (v:v) at 300 nl min⁻¹. The eluent was analyzed on either Q Exactive or Q Exactive Plus hybrid quadrupole/orbitrap mass spectrometer (ThermoFisher Scientific, Dreieich, Germany) equipped with a FlexIon nanoSpray source and operated under Excalibur 2.5 software using Top20 and Top30 data-dependent acquisition methods, respectively. One full MS scan across the 350-1600 *m/z* range was acquired at a resolution setting of 70,000 or 60,000 FWHM, respectively, and AGC target of 1*10⁶ and a maximum fill time of 50 ms. Precursors of charge states 2 to 6 above a 4*10⁴ intensity threshold were then sequentially isolated at 2.0 FWHM isolation width, fragmented

with nitrogen at a normalized collision energy setting of 28%, and the resulting product ion spectra recorded at a resolution setting of 17,500 or 15,000 FWHM, AGC targets of 1×10^5 and a maximum fill time of 60 ms. Selected precursor m/z values were then excluded for the following 30 s. Two technical replicates per sample were acquired. Raw data were processed using MaxQuant Software version 1.6.0.1 (Max Planck Institute for Biochemistry, Martinsried, Germany) or Mascot 2.3.0.2 (Matrixscience, London, UK) using standard search parameters (Trypsin/P, maximum 2 missed cleavages, fixed modification C-carbamidomethyl, variable modification protein N-terminal acetylation and M-oxidation, 20 ppm precursor and fragment tolerance, 1% FDR as established by a target/decoy search against a forward/reverse concatenated database). Data were searched against the UniProtKB human reference proteome v12.2016 (77995 entries) and analysed for normalized spectral counting quantification in Scaffold 5.0.0 software (Proteome Software, London, UK)

Tissue procurement and human atrial myocyte isolation. For all patient samples, all experimental protocols were authorized by the ethics committee of the University Medical Center Göttingen (No. 4/11/18) and performed following the Declaration of Helsinki. Right atrial appendages were resected during routine cannulation of the right atrium (for extracorporeal circulation) of patients undergoing open-heart coronary bypass cardiac surgery. Excised tissues were immediately transported in a cardioplegic-based sterile transport solution (30 mM 2,3-butanedione monoxime, 20 mM glucose, 10 mM KCl, 1.2 mM KH_2PO_4 , 5 mM MgSO_4 , 5 mM MOPS, 100 mM NaCl, 50 mM taurine; pH 7.0) to the laboratory for atrial myocyte isolation using a previously described protocol ⁴⁹. Briefly, obtained atrial tissues were

carefully trimmed of fat and minced into small pieces of approximately 1 mm³ in pre-chilled Ca²⁺-free solution (20 mM glucose, 10 mM KCl, 1.2 mM KH₂PO₄, 5 mM MgSO₄, 5 mM MOPS, 100 mM NaCl, 50 mM taurine; pH 7.4). Minced tissues were then digested in Ca²⁺-free solution containing 286 U/ml collagenase type I (Worthington, USA) and 5 U/ml protease XXIV (Sigma-Aldrich) enzymes for 45 min in a jacketed beaker with constant 100% O₂ bubbling at 37 °C. After 10 min of digestion, CaCl₂ was introduced to attain a final concentration of 20 µM. After 45 min, a second digestion step was initiated using a new 20 mL Ca²⁺-free solution containing 286 U/ml collagenase type I (Worthington, USA) and 20 µM CaCl₂. 5 min into tissue digestion, the supernatant was visualized under the microscope to detect dissociated myocytes. This process was repeated every 3 min until dissociated myocytes were detected. Upon detection of myocytes, tissue chunks were retrieved and subjected to gentle mechanical trituration in a storage solution to dislodge myocytes. The resulting supernatant containing dissociated myocytes was strained with a nylon mesh and centrifugated at 90 *g* for 7 min to pellet the cells. Cell pellets were resuspended in fresh storage solution (1% w/v albumin, 10 mM glucose, 10 mM β-hydroxybutyric acid, 70 mM L-glutamic acid, 20 mM KCl, 10 mM KH₂PO₄, 10 mM taurine; pH 7.4 adjusted with 1 M KOH) and used for subsequent downstream experiments.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Clinical data of individuals from the recruited family carrying the TPM1-L185F variant

(A) Clinical data from family carrying TPM1-L185F mutation. BSA, body surface area; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; ECG, electrocardiogram; NYHA, New York Heart Association. ARB, angiotensin receptor blocker; BB, beta receptor blocker; MRA, mineralocorticoid receptor blocker; D, diuretic; DI, digoxin; ICD, implantable cardioverter defibrillator; SR, sinus rhythm; LVH, left ventricular hypertrophy; nsVT, non-sustained ventricular tachycardia; PVC, premature ventricular contractions; HTx, heart transplantation. §, died nine months old of dilated cardiomyopathy. #, ICD for primary prevention. No therapies for ventricular tachycardia or -fibrillation delivered at follow up. □, underwent subacute heart transplantation following diagnosis. (B-C) Electrocardiograms (ECGs) for a healthy sibling of II-4 (healthy donor, HD) (B) and patient III-5, a 15-year-old male carrier who received a heart transplantation. ECG displayed non-sustained ventricular tachycardia and poor precordial R-wave progression (C).

Fig. S2. Characterization of iPSCs

(A-E) iPSCs showed regular expression of stem cell marker, such as POU5F1, TRA-1-81, SOX2 and SSEA-4. (A-B) Representative 20x confocal images of human iPSC following immuno-staining for pluripotency markers (A) 20x confocal images of human iPSCs clones stained with POU5F1 and TRA-1-81 antibodies; scale bar, 40 μ m. (B) Representative 20x confocal images of human iPSCs clones stained with SOX2 and SSEA-4 antibodies. Scale bar, 40 μ m. (C-E) iPSCs showed regular expression of pluripotency markers at mRNA levels, such

as Nanog (C), Pou5f1 (D) and Sox2 (E). (C) ***, $P < 0.001$ for WT control (WT1, WT/WT) vs. DCM patient-derived (Pat) iPSC-CMs (Adjusted P Value= 9.41×10^{-4}); ns, not significant for WT1 vs. TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1, MUT-int/MUT-int) iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). (D) ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test). (E) ns, not significant for WT1 vs. patient and WT1 vs. Mut-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). Data are shown as mean $\pm$ sem. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares, #4 as diamonds.

Fig. S3. Expression levels of cardiac markers are comparable in iPSC-CMs carrying the TPM1-L185F mutation versus WT

(A-D) Human iPSC-CMs displayed regular expression of cardiac markers at mRNA levels, such as (A) TPM, (B) TnT, (C) MYH6 and (D) MYH7. ns, not significant for WT1 vs. patient and WT1 vs. TPM1-L185F Mut-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). (E-G) iPSC-CMs displayed regular expression of cardiac markers. (E) Representative 63x confocal images of iPSC-CMs following immunostaining with MLC2a and TnT antibodies; scale bar, 20 μ m. (F) Representative 63x confocal images of iPSC-CMs following immunostaining with MYH7 and TnT antibodies; scale bar, 20 μ m. (G) Representative 63x confocal images of iPSC-CMs following immunostaining with MYBPC3 and TnT antibodies; scale bar, 20 μ m. (H-K) iPSC-CMs display regular expression of cardiac

markers at protein levels. Representative scans of immunoblots and quantification from cardiac markers, such as (H) TPM, (I) TnT, (J) TnC and (K) SAA. Bargraphs displayed averages of n=3 experiments and representative immunoblots are shown below; ns, not significant for WT1 vs. patient and WT1 vs. Mut-int 1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). (L-M) Protein expression levels of apolipoprotein A1 (APOA1) were decreased in DCM (MUT) iPSC-CMs compared to the WT group. (L) Representative images of immunoblots were shown. (M) Protein expression levels of APOA1 were decreased in DCM iPSC-CMs compared to WT controls. *, $P < 0.05$ for WT vs. DCM (P value=0.0206) (Mann Whitney test). (N and O) Increased protein expression of AP1B1 and DNML1 were detected in Mut-int1 iPSC-CMs compared to WT1 iPSC-CMs as analyzed by In-Cell-Western using a LI-COR infrared imaging system. Data were normalized by GAPDH. **, $P < 0.01$ for WT1 vs. Mut-int1 (P value= 0.00216) (Mann Whitney test) in AP1B1. ***, $P < 0.001$ for WT1 vs. Mut-int1 (P value= 0.00216) (Mann Whitney test) in DNML1. Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares, #4 as diamonds.

Fig. S4. The TPM1-L185F mutation results in defective contractility as well as reduced field potential duration and amplitude

(A-E) Reduced contractility in DCM patient-derived (Pat, WT/MUT) and TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1, MUT-int/MUT-int). (A-C) Representative

recordings from each group. TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1) showed reduced time to maximum (D) and maximal decay (E). (D) ns, not significant for WT1 vs. Pat; **, $P < 0.01$ WT1 vs. MUT-int1 (Adjusted P Value= 1.36×10^{-3}) (Kruskal-Wallis test and Dunn's multiple comparisons test). (E) ns, not significant for WT1 vs. Pat; ***, $P < 0.001$ WT1 vs. MUT-int1 (Adjusted P Value= 5.81×10^{-6}) (Kruskal-Wallis test and Dunn's multiple comparisons test). (F-G) Analysis of electrophysiological parameters using a Nanion CardioExcyte 96 instrument. (F) Reduced amplitude in patient and TPM1-L185F MUT-int1 iPSC-CMs. ***, $P < 0.001$ for WT1 vs. patient (Adjusted P Value= 1.20×10^{-6}) and WT1 vs. Mut-int1 (Adjusted P Value= $< 1 \times 10^{-15}$) (Kruskal-Wallis test and Dunn's multiple comparisons test). (G) Reduced field potential duration in patient and TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1). ***, $P < 0.001$ for WT1 vs. patient (Adjusted P Value= 1×10^{-14}) and WT1 vs. Mut-int1 (Adjusted P Value= 1.28×10^{-8}) (Kruskal-Wallis test and Dunn's multiple comparisons test). Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares, #4 as diamonds.

Fig. S5. Relative controls for immunoprecipitation of vinculin for interactions with sarcomeric proteins (relating to Fig. 2F-P)

(A) Protein expression levels for vinculin were not significantly altered among groups. Representative membrane scans of blots and quantification are shown. Data were normalized by GAPDH. ns, not significant for WT1 vs. DCM patient-derived (Pat) t and WT control (WT1,

WT/WT) vs. TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1, MUT-int1/MUT-int) (Kruskal-Wallis test and Dunn's multiple comparisons test). (B-C) IgG control showed reduced binding ability with vinculin compared to WT groups. (B) Representative membrane scans are shown. (C) Quantification of (B). **, $P < 0.01$ for WT vs. IgG (P value= 7.94×10^{-3}) (Mann Whitney test). (D-F) The negative control, IgG, showed the background signal for this experiment regarding detection of vinculin and TnT, which was decreased, compared to the WT group. (D) Representative membrane scans were shown. (E and F) Quantification of (D). ns, not significant (P value=0.100) for WT vs. igg in bound vinculin (Mann Whitney test); ns, not significant (P value=0.100) for WT vs. igg in bound TnT (Mann Whitney test). Per group, $n=3$ experiments ($n=1$ independent cardiac differentiation). (G-I) Protein levels for vinculin (H) and TnT (I) were not significantly different between groups. (G) Representative membrane scans of input samples are shown. (H) ns, not significant for WT vs. patient, WT vs. Mut-int1 and WT vs IgG (Kruskal-Wallis test and Dunn's multiple comparisons test). (I) ns, not significant for WT vs. patient, WT vs. Mut-int1 and WT vs igg (Kruskal-Wallis test and Dunn's multiple comparisons test). Data were normalized by GAPDH. Per group, $n=3$ experiments ($n=1$ independent cardiac differentiation). (J-K) Immunoprecipitation of TnT and its interaction partner, vinculin, from cell lysates of DCM (MUT) TnT-R173W iPSC-CMs vs. WT controls. Eluted fractions were analyzed by nanoLC-MS/MS analysis, showing that TnT interacts with vinculin. The interaction of TnT with vinculin was reduced in DCM iPSC-CMs compared to WT. Cell lysates from DCM and WT iPSC-CMs were incubated with dynabeads decorated with anti-TnT antibody. As a negative control, WT iPSC-CM cell lysates were incubated with dynabeads that had not been decorated with antibody (“no-antibody” control). As an additional

control for specificity, iPSC-CMs from a previously generated CRISPR/Cas9-based TnT knock-out iPSC line⁸ were used. (J) NanoLC/LC mass spectrometry data indicated binding of WT TnT to vinculin, which was reduced in presence of the DCM mutation (TnT-R173W), compared to WT control. The amount of vinculin pulled down in the negative controls, TnT KO and no-antibody, was comparable with the amount detected in the DCM TnT-R173W group. (K) The amount of TnT protein immobilized on TnT-antibody-decorated dynabeads is comparable between the WT and DCM groups. No TnT protein was detected in the CRISPR/Cas9 edited TnT KO iPSC-CM control cell line. The amount of WT-TnT that binds to the no-antibody negative control presents a reference for the background signal in this experiment. Due to limited capacity of the highly sensitive LC/LC mass spectrometry readout, only n=1 sample per group was processed. Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares, #4 as diamonds.

Fig. S6. Plasma membrane-PIP2 levels are critical for F-actin polymerization

(A) Quantification of protein levels for beta-actin showed no significant difference between groups. Representative images of immunoblots are shown. ns, not significant for WT1 vs. DCM patient-derived (Pat) and WT1 vs. Mut-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). (B-C) Reduction of PIP2 levels around the PM in WT2 iPSC-CMs treated with UNC3230, a PIP5K inhibitor. (B) 63x confocal images of WT2 iPSC-CMs treated

with CV and UNC3230 (10 μ M, 24 h) followed by a PIP2-specific immunostaining. Scale bar, 20 μ m. Enlarged regions were zoomed in 5.5 times. (C) Quantification of (B). ***, $P < 0.001$ for CV vs. UNC323 WT2 iPSC-CMs (P value $< 1 \times 10^{-15}$) (Mann-Whitney test). (D) Reduction of F-actin content in UNC3230 treated WT1 iPSC-CMs compared with CV. $P < 0.01$ (P value $= 2.94 \times 10^{-3}$) for CV WT1 vs. UNC323 WT1 (Mann-Whitney test). (E) Reduction of F-actin content in UNC3230-treated WT2 iPSC-CMs compared with CV. ***, $P < 0.001$ (Adjusted P Value $= 2.22 \times 10^{-5}$) for CV vs. UNC3230 WT2 iPSC-CMs (Mann-Whitney test). (F) Kinetics of continuous transferrin uptake. Quantification of 20x confocal Z stack images acquired from individual time point. Data were normalized by number from 0 min. (G) Protein levels of transferrin receptor were not significantly different among groups. Representative membrane scans of blots and quantification are shown. ns, not significant for WT1 vs. patient and WT1 vs. Mut-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares, #4 as diamonds.

Fig. S7. TPM1-L185F mutation-introduced iPSC-CMs are characterized by impaired LDL uptake

(A-B) LDL-BODIPY uptake was impaired in TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1, MUT-int1/MUT-int) vs. WT control (WT1). (A) Representative 63x confocal images of continuous LDL-BODIPY uptake. Scale bar, 20 μ m. (B) Quantification of (A),

quantification is done using spot detection plugin in ICY software with 63x confocal images.

******, $P < 0.01$ for WT1 vs. Mut-int1 iPSC-CMs (P value = 1.31×10^{-3}) (Mann Whitney test). (C) LDL receptor protein expression levels were not significantly different between groups. Representative membrane scans of blots and quantification are shown. Ns, not significant for WT1 vs. DCM patient-derived (Pat) and WT1 vs. Mut-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). (D) Phagocytosis is impaired in CRISPR/Cas9 mutation-introduced (Mut-int1) iPSC-CMs, compared to WT controls. Phagocytosis was measured by in live cells using a pHrodo™ Red E. coli Bioparticle assay followed by a multi-well plate readout for fluorescence at 560/590 nm. ******, $P < 0.01$ for WT1 vs. Mut-int1 iPSC-CMs (P value = 0.00649) (Mann Whitney test). (E) Differential expression of phagocytosis pathway proteins was shown for DCM iPSC-CM vs. WT controls, analyzing the DIA-MS data set shown in Fig. 1C-E. Volcano plot indicating downregulated- vs. upregulated proteins for phagocytosis signaling pathways (Ingenuity Pathway Analysis (IPA) software annotation, “Fcγ receptor-mediated phagocytosis in macrophages and monocytes” in DCM iPSC-CMs vs. WT controls. A non-parametric, Wilcoxon signed rank test was employed to assess statistical significance. (F) Bar graph for data shown in (E) indicating individual upregulated and downregulated proteins involved in phagocytosis signaling pathways for DCM vs. WT control iPSC-CMs (annotated by IPA software). Fold change (FC) was calculated as the ratio between the DCM and WT groups. Protein IDs corresponding to the phagocytosis signaling pathway as described above were derived from the Uniprot database. (G) Protein expression levels of EEA1 were not significantly different between groups. Representative membrane scans of blots and quantification were shown. NS, not significant for WT1 vs. DCM patient-derived (Pat) and

WT1 vs. Mut-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares.

Fig. S8. Actin polymerization is critical for proper distribution of early endosomes.

(A) Reduction of F-actin content in Lat-A treated WT control (WT1, WT/WT) iPSC-CMs compared with CV. *, $P < 0.05$ (P value = 1.89×10^{-2}) for CV vs. Lat-A WT1 (Mann-Whitney test).

(B-C) Inhibition of actin polymerization via Lat-A treatment results in abnormal EE distribution in WT control (WT2, WT/WT) iPSC-CMs. Confocal images (B) and quantification (C) of WT2 cells treated with Lat-A (3 μ M, 30 min) followed by immunostaining with an anti-EEA1 antibody. Scale bar, 20 μ m. Enlarged regions were zoomed in 6 times. ***, $P < 0.001$ for CV WT2 vs. Lat-A WT2 (P value = 6.02×10^{-8}). Statistics were calculated by a Mann-Whitney test. Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares.

Fig. S9. Mitochondrial dysfunction and increased oxidative stress in DCM patient-derived and TPM1-L185F mutation-introduced iPSC-CMs

(A) Protein expression levels of a mitochondrial marker, Tom20, were not significantly altered

between groups. Representative membrane scans of blots and quantification are shown. ns, not significant for WT1 vs. DCM patient-derived (Pat) and WT control (WT1, WT/WT) vs. TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1, MUT-int1/MUT-int) (Kruskal-Wallis test and Dunn's multiple comparisons test). (B) Mitochondrial membrane potential (MMP) was measured via JC-1 staining using a multi-well plate reader. Data were calculated as the red/green JC-1 fluorescence intensity ratio. In patient and TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1), a reduced MMP was detected. ***, $P < 0.001$ for WT1 vs. Patient (Adjusted P Value = 8.40×10^{-4}), **, $P < 0.01$ for WT1 vs Mut-int1 iPSC-CMs (Adjusted P Value = 0.00469) (Kruskal-Wallis test and Dunn's multiple comparisons test). (C-D) In presence of mutations in sarcomere proteins, iPSC-CMs display reduced basal oxygen consumption rate (OCR, pmol/min). (C) Mut-int2 iPSC-CMs showed defective mitochondrial respiration. (D) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown. ***, $P < 0.001$ for WT2 vs. Mut-int2 iPSC-CMs (P value $< 1 \times 10^{-15}$) (Mann Whitney test). (E-F) Transferrin uptake in Mut-int1 iPSC-CMs treated with CV, Fe(II) (100 μ M, 30 min) (II)+ebselen (5 μ M, 1.5 h). (E) Representative 63x confocal images of fixed cells after incubation with Alexa Fluor™ 488-conjugated transferrin for 5 min followed by an EEA1-specific antibody staining; scale bar, 20 μ m. (F) Quantification of 20X confocal images. Ns, not significant for CV vs. Fe(II), CV vs. Fe(II)+ebselen, and Fe(II) vs. Fe(II)+ebselen (Kruskal-Wallis test and Dunn's multiple comparison test). (G-H) Fe(II) supplementation improved mitochondrial iron levels in Mut-int1 iPSC-CMs. Ebselen treatment in presence of Fe(II) supplementation blocked the increase of mitochondrial iron levels in Mut-int1 iPSC-CMs delivered by Fe(II) supplementation. (G) Representative 63x confocal images of live cells

stained with Mitotracker Green and FerroOrange; scale bar, 20 μ m. (H) Quantification of (G). ***, $P < 0.001$ for CV vs. Fe(II) (Adjusted P Value= 1.87×10^{-13}) and Fe(II) vs. Fe(II)+ebselen (Adjusted P Value= 1.18×10^{-8}); ns, not significant for CV vs. Fe(II)+ebselen (Kruskal-Wallis test and Dunn's multiple comparisons test). (I) Fe(II) supplementation recovered the contraction amplitude in Mut-int1 iPSC-CMs. Ebselen treatment in presence of Fe(II) supplementation blocked the improvement of the contraction amplitude mediated by Fe(II) treatment in Mut-int1 iPSC-CMs. ***, $P < 0.001$ for CV vs. Fe(II) (Adjusted P Value= $< 1 \times 10^{-15}$), Fe(II) vs. Fe(II)+ebselen (Adjusted P Value= 6.48×10^{-10}) and CV vs. Fe(II)+ebselen (Adjusted P Value= 4.05×10^{-4}) (Kruskal-Wallis test and Dunn's multiple comparisons test). (J-K) LDL uptake was not affected by Fe(II) treatment. (J) Representative 63x confocal images are shown; scale bar, 20 μ m. (K) Quantification of (J); ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (Mann Whitney test). (L) Fe(II) treatment did not alter impaired phagocytosis in Mut-int1 iPSC-CMs. ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (Mann Whitney test). (M) Fe(II) treatment rescued reduced mitochondrial Fe(II) levels induced by Lat-A treatment (1 μ M, 1 h) in WT1 iPSC-CMs. ***, $P < 0.001$ for CV vs. Lat-A (Adjusted P Value= $< 1 \times 10^{-15}$) and Lat-A vs. Lat-A+ Fe(II) (Adjusted P Value= $< 1 \times 10^{-15}$); ns, not significant for CV vs. Lat-A + Fe(II) (Kruskal-Wallis test and Dunn's multiple comparisons test). (N) Mitochondrial superoxide levels were increased in patient (WT/MUT) and TPM1-L185F mutation-introduced iPSC-CMs (Mut-int1) measured via MitoSox staining in a high-content plate reader. **, $P < 0.01$ for WT1 vs. patient (Adjusted P Value=0.00596) and WT1 vs. Mut-int1 iPSC-CMs (Adjusted P Value=0.00538) (Kruskal-Wallis test and Dunn's multiple comparisons test). (O-P) Fe(II) treatment reduced mitochondrial superoxide levels in patient (WT/MUT) and TPM1-L185F

mutation-introduced iPSC-CMs (Mut-int1). **, $P < 0.01$ for CV vs. Fe(II) patient iPSC-CMs (P value = 0.0015) (Mann Whitney test) and ***, $P < 0.001$ for CV vs. Fe(II) Mut-int1 iPSC-CMs (P value = 1.06×10^{-4}) (Mann-Whitney test). (Q-R) UNC3230 treatment (10 μ M, 24 h) reduced mitochondrial respiration in WT1 iPSC-CMs. (Q) UNC3230 treatment reduced mitochondrial respiration in WT1 iPSC-CMs. (R) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown. ***, $P < 0.001$ for WT1 CV vs. WT1 UNC3230 (P value = 7.01×10^{-4}) (Mann Whitney test). (S-T) Lat-A treatment (3 μ M, 30 min) reduced mitochondrial respiration in WT1 iPSC-CMs. (S) Lat-A treatment reduced mitochondrial respiration in WT1 iPSC-CMs. (T) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown. ***, $P < 0.001$ for WT1 CV vs. WT1 Lat-A (P value $< 1 \times 10^{-15}$) (Mann Whitney test). Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: #1 as circles, #2 as triangles, #3 as squares, #4 as diamonds, #5 as crosses, #6 as stars, #7 as circles with crosses inside, #8 as squares with crosses inside, #9 as diamonds with crosses inside.

Fig. S10. Recovering actin polymerization via RhoA activation rescues PM-localized PIP2 levels and sarcomere connections with the plasma membrane

(A) Increased F-actin content in RhoA II treated Mut-int1 iPSC-CMs compared with CV. **, $P < 0.01$ (P value = 0.0051) for CV vs. RhoA II Mut-int1 iPSC-CMs (Mann-Whitney test). (B-D) RhoA II treatment facilitates TnT-vinculin interaction in patient and TPM1-L185F

mutation-introduced (Mut-int1) iPSC-CMs. (B) Representative 63X confocal images of TPM1-L185F Mut-int1 iPSC-CMs treated with CV and RhoA II (3 $\mu\text{g/mL}$, 16 h) followed by immunostaining with vinculin and TnT antibodies. Scale bar, 20 μm . Enlarged regions were zoomed in 5 times. (C-D) ROI based quantification of (B). **, $P < 0.01$ for CV vs. RhoA II Patient ($P \text{ value} = 6.36 \times 10^{-3}$) and ***, $P < 0.001$ for CV vs. RhoA II Mut-int1 ($P \text{ value} = 6.68 \times 10^{-5}$) iPSC-CMs (Mann-Whitney test). (E-G) Rho activator II treatment improved the contractility in TPM1-L185F mutation-introduced (Mut-int1) iPSC-CMs. (E-F) Representative recordings were shown for each group. Maximal decay (G) improvement following Rho activator II treatment (3 $\mu\text{g/mL}$, 3 h). ***, $P < 0.001$ for CV vs. RhoA II Mut-int 1 iPSC-CMs ($P \text{ value} = 1.32 \times 10^{-4}$) (Mann-Whitney test). Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: #1 as circles, #2 as triangles, #3 as squares, #4 as diamonds, #5 as crosses, #6 as stars, #7 as circles with crosses inside, #8 as squares with crosses inside, #9 as diamonds with crosses inside.

Fig. S11. Characterization of TPM1-L185F MUT-corrected iPSCs and iPSC-CMs

(A-E) DCM patient-derived (Pat) and mutation-corrected (mut-cor, WT-cor/WT-cor) iPSCs showed regular expression of stem cell marker, such as OCT3/4, TRA-1-81, Sox2 and SSEA4. (A-B) Representative 20x images of human iPSC clones immuno-stained with pluripotency markers. (A) Representative 20x confocal images of human iPSCs co-stained with POU5F1 and TRA-1-81 antibodies; scale bar, 40 μm . (B) Representative 20x confocal images of DCM

patient-derived (Pat) and mutation-corrected (Mut-cor) iPSCs clones co-stained with Sox2 and SSEA4 antibodies; scale bar, 40 μ m. (C-E) DCM patient-derived (Pat) and mutation-corrected (Mut-cor) iPSCs showed regular expression of pluripotency markers at mRNA levels, such as Nanog (C), Pou5f1 (D) and Sox2 (E). ns, not significant for Pat vs. mut-cor iPSC-CMs as calculated by Mann Whitney test. (F-I) Patient and Mut-cor iPSC-CMs display regular expression of cardiac markers at mRNA levels, such as (F) TPM, (G) TnT, (H) MYH6 and (I) MYH7. (F, I) Ns, not significant for Patient vs. Mut-cor (Mann Whitney test). (G) *, $P < 0.05$ for Patient vs. Mut-cor iPSC-CMs (P value=0.0400) (Mann Whitney test). (H) ***, $P < 0.001$ for Patient vs. Mut-cor iPSC-CMs (P value= 4.11×10^{-5}) (Mann Whitney test). (J-L) DCM patient-derived (Pat) and mutation-corrected (mut-cor) iPSC-CMs display regular expression of cardiac markers. (J) Representative 63x confocal images of iPSC-CMs stained with MLC2a and TnT antibodies; scale bar, 20 μ m. (K) Representative 63x confocal images of iPSC-CMs stained with MYH7 and TnT antibodies; scale bar, 20 μ m. (L) Representative 63x confocal images of iPSC-CMs stained with MYBPC3 and TnT antibodies; scale bar, 20 μ m. (M-P) DCM patient-derived (Pat) and mutation-corrected (Mut-cor) iPSC-CMs displayed regular expression of cardiac markers at protein levels, such as (M) TPM, (N) TnT, (O) TnC and (P) SAA. Representative scans of immunoblots and quantification were shown. (M, N, O, P) ns, not significant for patient vs. Mut-cor iPSC-CMs (Mann Whitney test). (Q-R) UNC3230 treatment (10 μ M, 24 h) reduced mitochondrial respiration in Mut-cor iPSC-CMs. (Q) UNC3230 treatment reduced mitochondrial respiration in Mut-cor iPSC-CMs. (R) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown. *, $P < 0.05$ for Mut-cor CV vs. Mut-cor UNC3230 (P value= 7.01×10^{-4}) (Mann Whitney test). (S-T) Lat-A

treatment (3 μ M, 30 min) reduced mitochondrial respiration in Mut-cor iPSC-CMs. (S) Lat-A treatment reduced mitochondrial respiration in Mut-cor iPSC-CMs. (T) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown. ***, $P < 0.001$ for Mut-cor CV vs. Mut-cor Lat-A (P value = 1.62×10^{-2}) (Mann Whitney test). Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations. Data points per experiment are shown as different shapes: # 1 as circles, #2 as triangles, #3 as squares.

Fig. S12. TFR Ab treatment results in reduced CME-based uptake of transferrin uptake, impaired mitochondrial function and contractility

(A-B) Transferrin uptake in Mut-cor iPSC-CMs treated with CV, TFR Ab (20 μ g/mL or 30 μ g/mL for 1 h, Thermo Fisher Scientific, catalog #14-0711-82). (A) Representative 63x confocal images of fixed cells after incubation with Alexa Fluor™ 488 conjugated transferrin for 5 min followed by an EEA1-specific antibody-based staining; scale bar, 20 μ m. (B) Quantification of 20X confocal images. (C) Treatment with the TFR Ab reduced mitochondrial respiration in WT1 iPSC-CMs. (D) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown, displaying averages of 3 independent experiments per group. ***, $P < 0.001$ for WT1 CV vs. WT1 TFR Ab (P value = 1.28×10^{-4}) (Mann Whitney test). (E) Treatment with the TFR Ab reduced mitochondrial respiration in Mut-cor iPSC-CMs. (F) A complete diagram of mitochondrial respiration recorded via a Seahorse XF analyzer is shown, displaying averages of 3 independent experiments per group. ***, $P < 0.001$ for Mut-

cor CV vs. Mut-cor TFR Ab (P value= 1.72×10^{-8}) (Mann Whitney test). (G) Contractility measurements in presence of higher concentrations of TFR Ab (R and D Systems, catalog #AF2474) blocked more transferrin receptor sites on the PM of Mut-cor iPSC-CMs, limiting the rescue delivered by a parallel RhoA II treatment. Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group. Unless indicated otherwise, the number of independent experiments equals the number of independent iPSC cardiac differentiations.

Fig. S13. Heart tissues show regular expression of CCDC53 and TnT

(Corresponding to Figure 7) (A-B) Heart tissues show regular expression of CCDC53 and TnT. Representative 20X confocal images of tissues from controls and HF patients stained with TnT and CCDC53 antibodies. control, n=4 patients; HF, n=5 patients; scale bar, 40 μ m. Images shown in figures are representative of the average value of each group.

Fig. S14. Relative controls in human heart tissue staining and characterization of protein expression levels of critical proteins within the CME pathway

(Corresponding to Figure 7) (A-E) Relative controls in human heart tissue staining. (A) Representative 63x confocal images from slides stained with CCDC53 (rabbit) and Alexa Fluor 488 goat anti-mouse antibody; scale bar, 20 μ m. (B) Representative 63x confocal images from slides stained with TnT (mouse) and Alexa Fluor 561 goat anti-rabbit antibody; scale bar, 20 μ m. (C) Representative 63x confocal images from slides stained with Alexa Fluor 488 goat anti-rabbit antibody only; scale bar, 20 μ m. (D) Representative 63x confocal images from slides

stained with Alexa Fluor 488 goat anti-mouse antibody only; scale bar, 20 μ m. (E) Representative 63x confocal images from slides stained with Alexa Fluor 561 goat anti-rabbit antibody only; scale bar, 20 μ m. (F-H) Characterization of protein levels for WASL in adult human heart tissues by immunoblots. (F) Representative images of immunoblots were shown. (G-H) Quantification of protein expression levels of WASL. *, $P < 0.05$ for donor (healthy control) vs. DCM (P Value=0.0473) (Mann Whitney test). Healthy control, $n=5$ individuals; DCM, $n=8$ individuals. Data are shown as mean $\pm$ sem. Images shown in figures are representative of the average value of each group.

SUPPLEMENTAL TABLES

Supplementary Table 1:

Raw data (peptide count) for LC/LC mass spectrometry corresponding to Fig. S5J-K.

Supplementary Table 2:

Patient information corresponding to Fig. 7, Fig. S13, and Fig. S14A-E

Conditions of patients in the control and HF groups in immunohistochemistry LV heart tissue from patients with dilated cardiomyopathy who received a heart transplantation due to end-stage heart failure (indicated as “heart failure”, HF, or heart transplantation) are presented, corresponding to data shown in Figure 7. In comparison, LV heart tissue from patients with preserved LV function that underwent aortic valve replacement (severe valve stenosis) and coronary artery bypass graft surgery was used in the control group (“control”).

Supplementary Table 3:

Patient information corresponding to Fig. 7, Fig. S13, and Fig. S14A-H

Clinical data corresponding to controls and HF patients. Data are represented as mean $\pm$ sem.

ACE, angiotensin-converting enzyme; AT1, angiotensin II receptor type 1; CABG, coronary artery bypass grafting; ICD, implantable cardioverter defibrillator; LV, left-ventricular.

Supplementary Table 4:

Clinical characteristics of patients used for human cardiomyocyte isolation

corresponding to Fig. 8A and B

Clinical characteristics of patients whose samples were used for measurements of CME-dependent transferrin uptake after CV and RhoA II treatment. ACE, angiotensin-converting enzyme; AT, angiotensin receptor; CC, clinical chemistry; CAD, coronary artery disease; ECC, extracorporeal circulation; IVSd, interventricular septum thickness at end-diastole; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVPWd, left ventricular posterior wall thickness at end-diastole; NYHA, New York Heart Association Functional Classification; MVD/AVD, mitral/aortic valve disease; TIA, transient ischemic attack. Continuous and categorical data are presented as actual values. “+” and “-” signifies presence or absence of selected parameters, respectively.

Supplementary Table 5:

Number of experiments and statistics for each figure

Fig. S1

A

		At diagnosis				At latest follow up					
ID	BSA (m2)	Age(year)	LVEDD (mm)	LVEF (%)	ECG	Age(year)	LVEDD (mm)	LVEF (mm)	ECG	Medical therapy	NYHA class
I-1	n/a	62	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
II-2	1.81	47	61	50	SR, LVH	57	61	45	nsVT	ARB	2
II-5	1.95	53#	60	52	SR	63	67	27	nsVT, PVC (10%)	ARB, BB, MRA, D, ICD#	3
II-7	2.42	39	65	50	SR	50	63	50	SR	ARB, BB	1
III-1	n/a	<1§	n/a	n/a	n/a	-	-	-	-	-	-
III-3	1.82	15	74	15	SR	28	68	40	SR	ACEI, BB, MRA, DI, D	2
III-4	2.08	23	61	52	SR	30	60	51	SR	ACEI	1
III-5	n/a	15	73	15	SR, nsVT	-	-	-	-	HTx▯	-

B

Normal ECG
Healthy sibling of II-4



C

Severe DCM
III-5

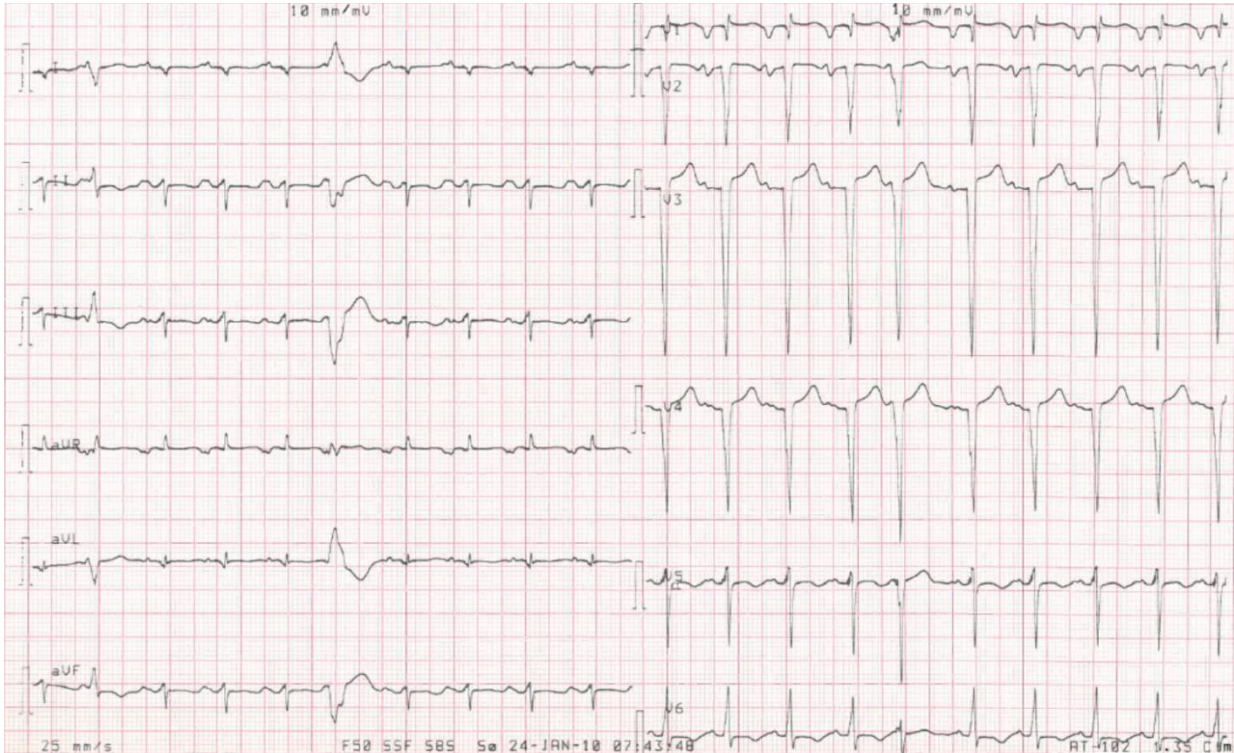


Fig. S2

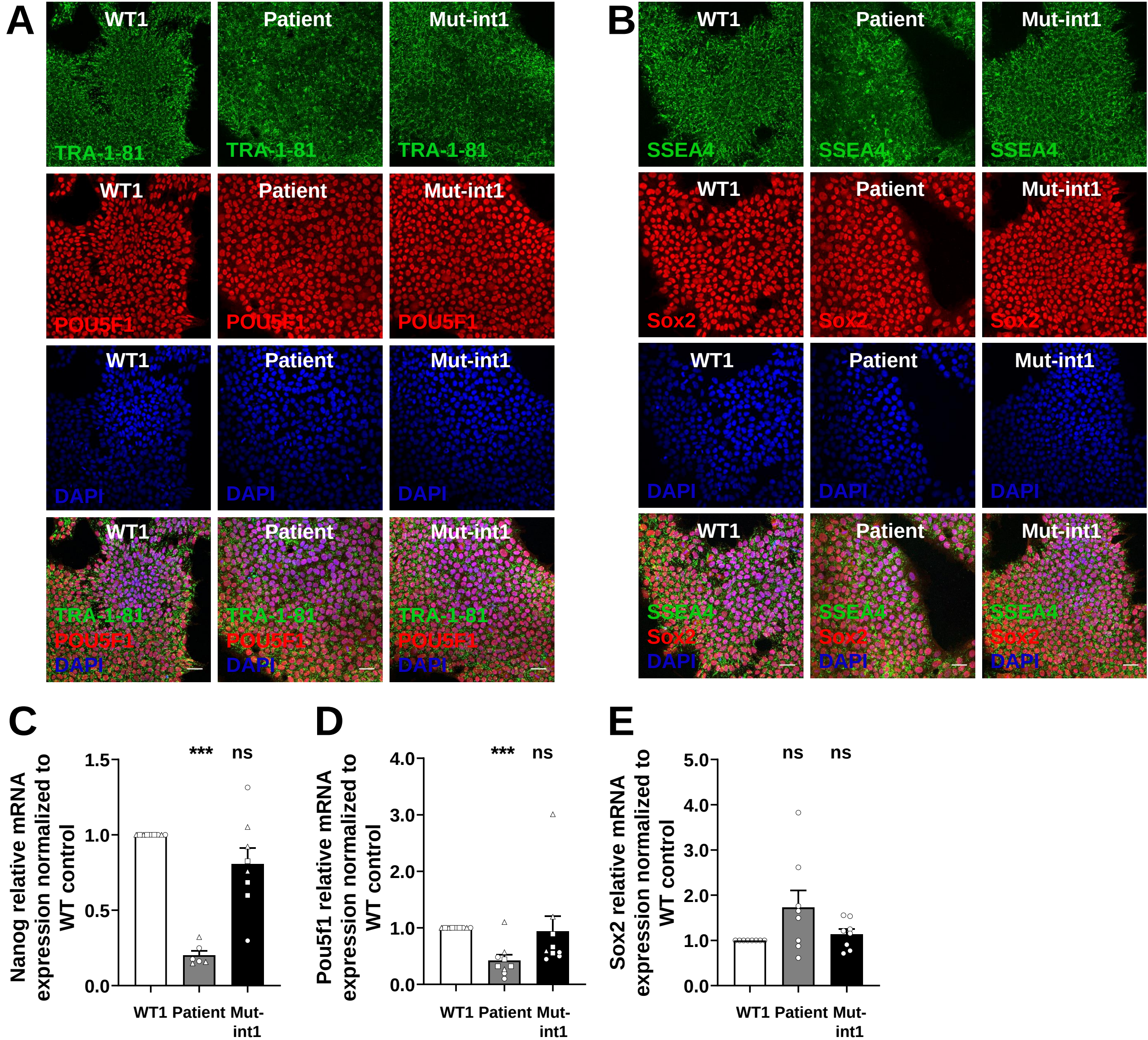


Fig. S3

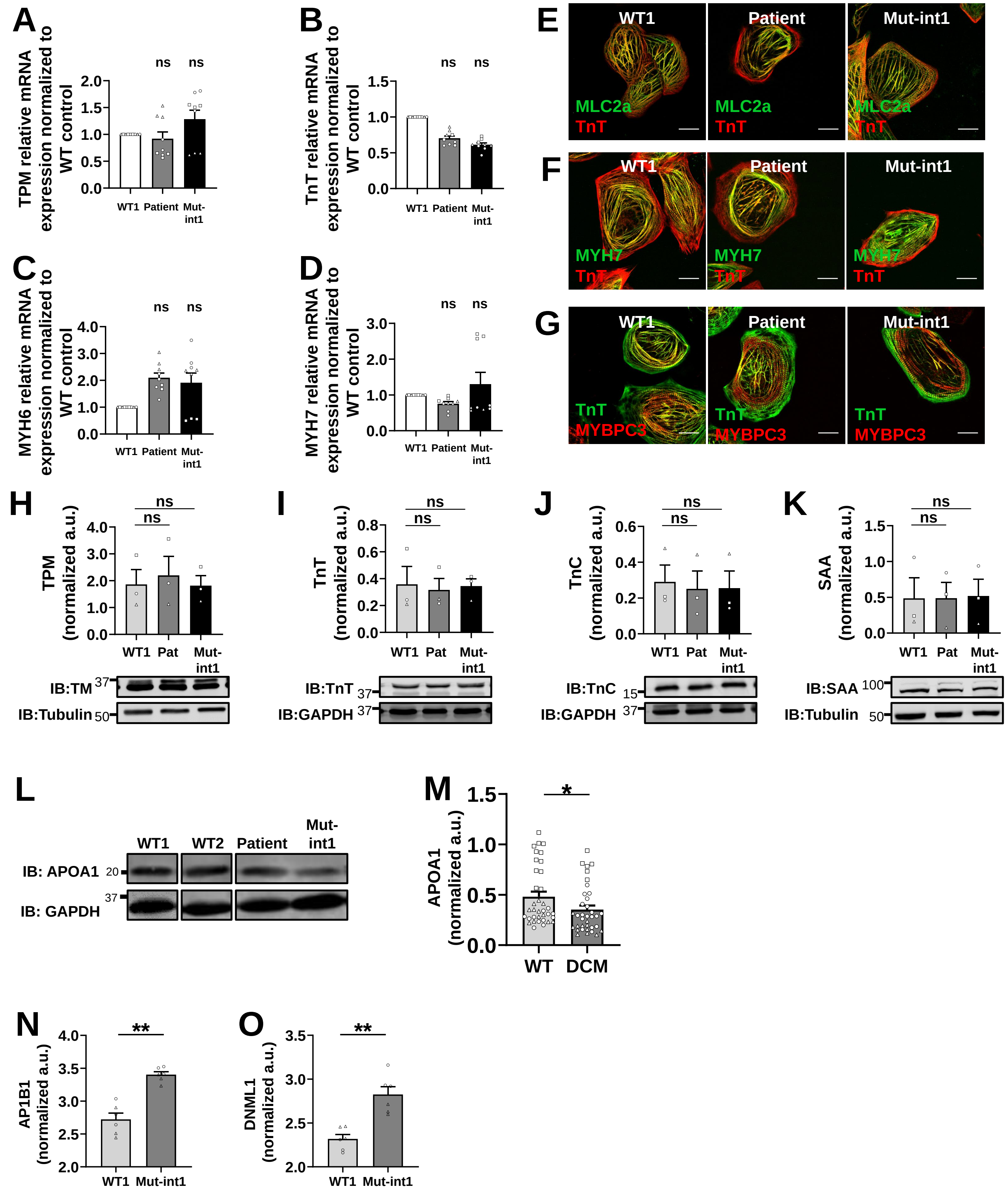


Fig. S4

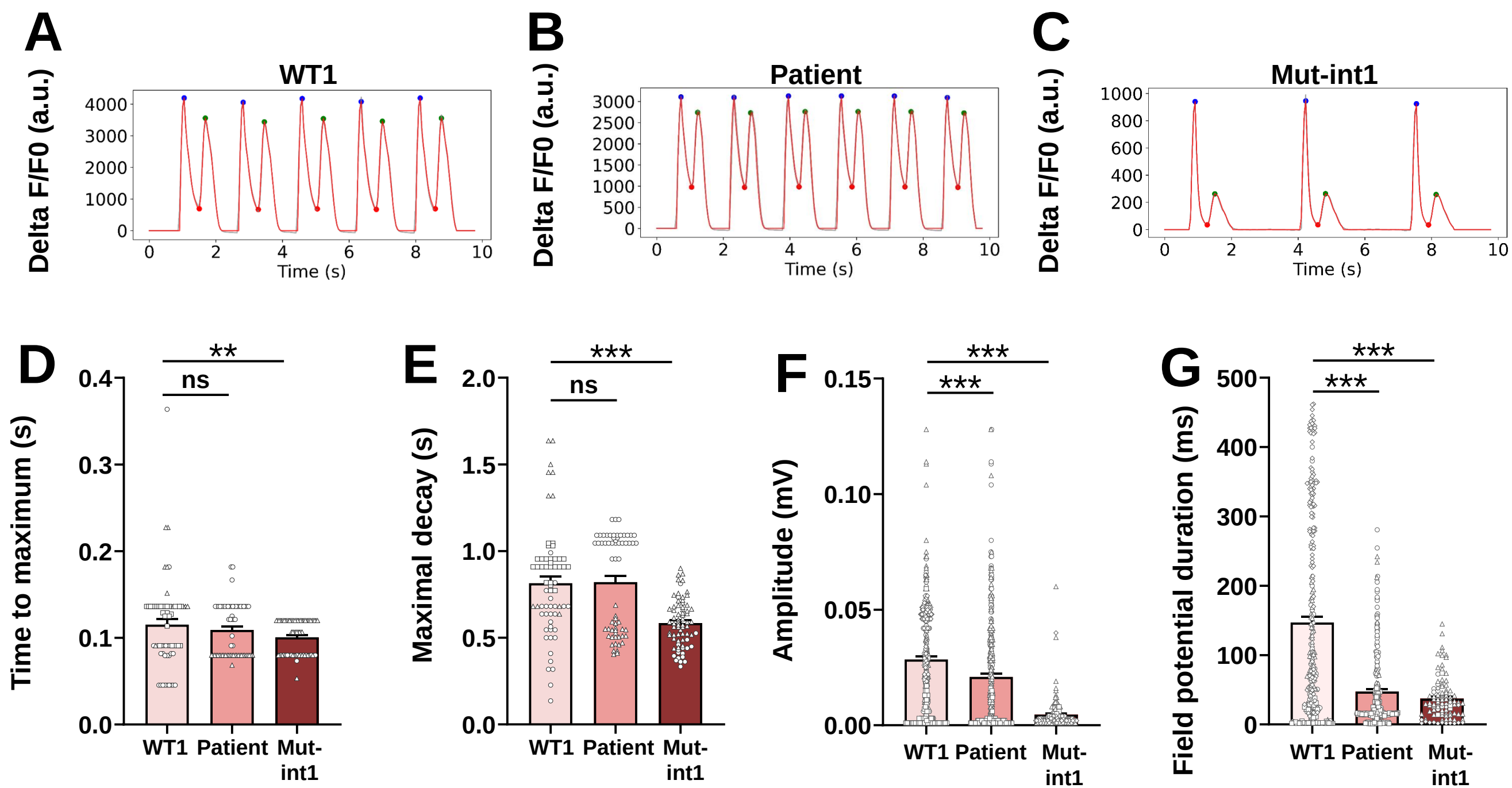
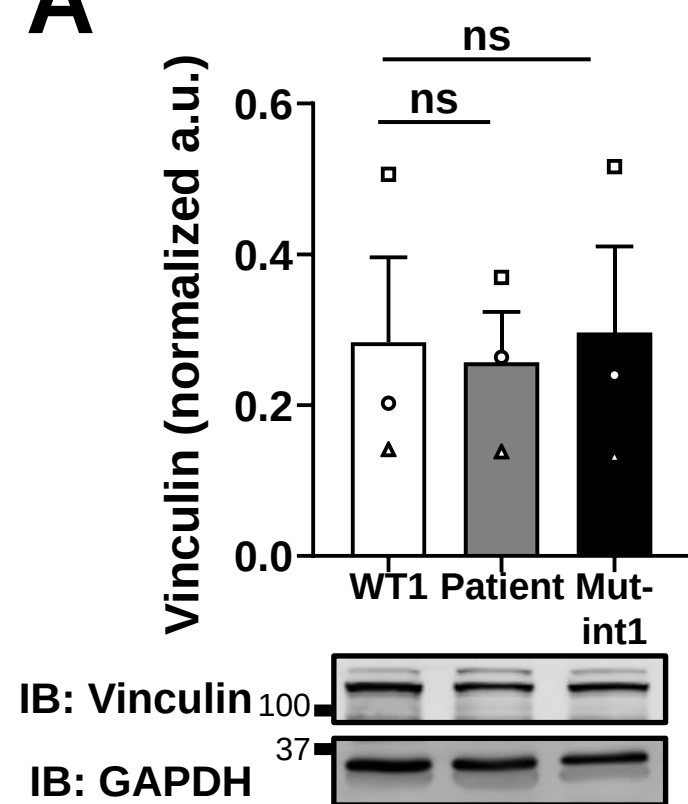
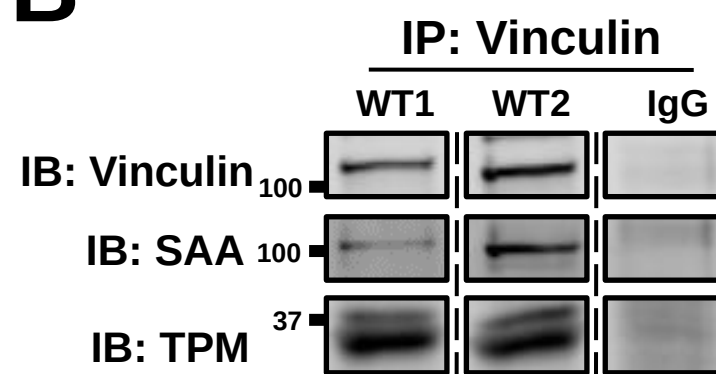


Fig. S5

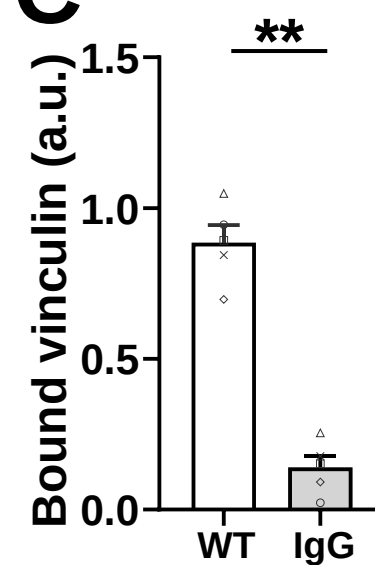
A



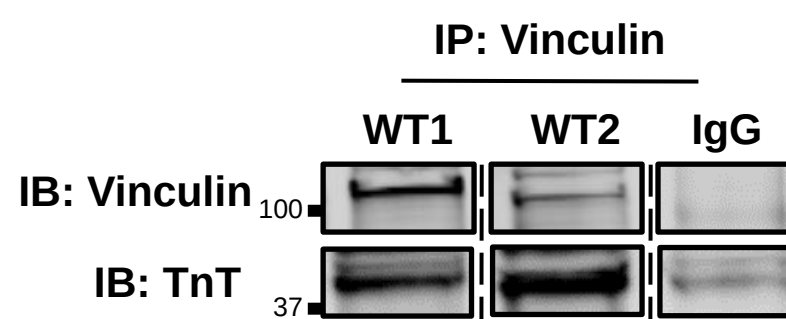
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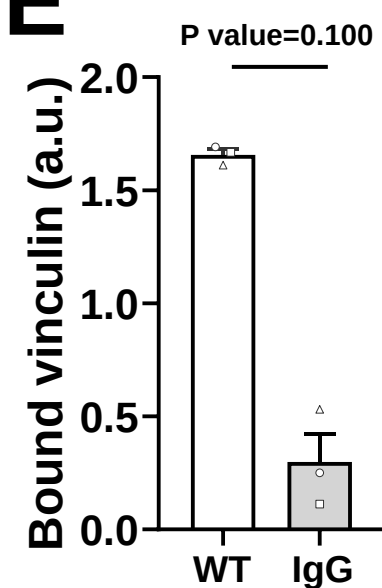
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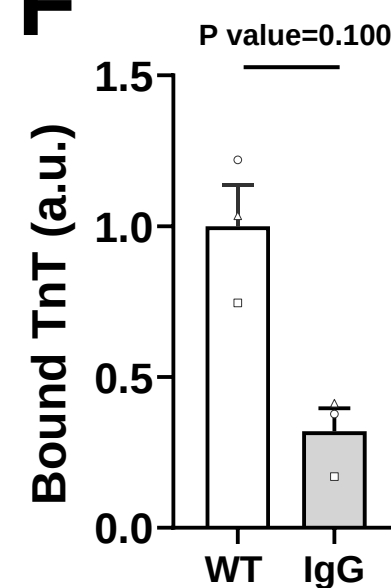
D



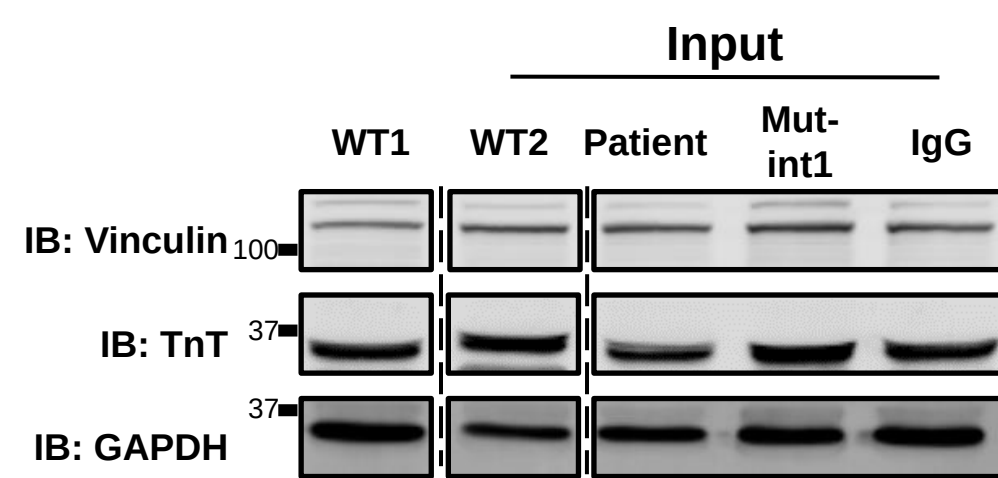
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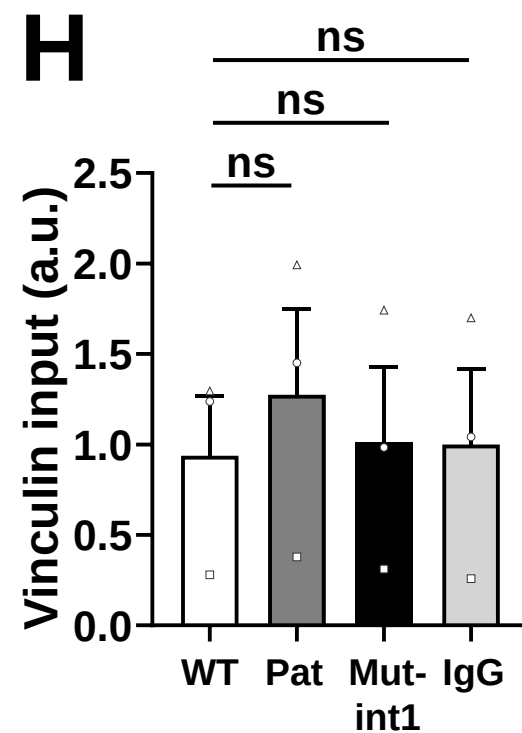
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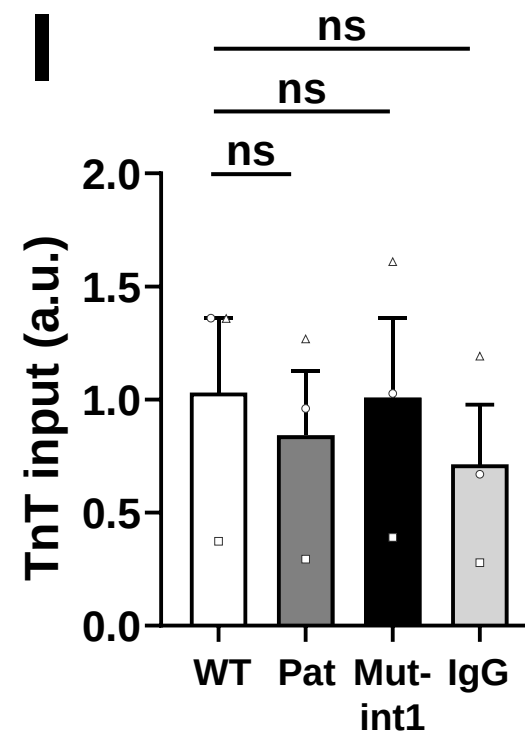
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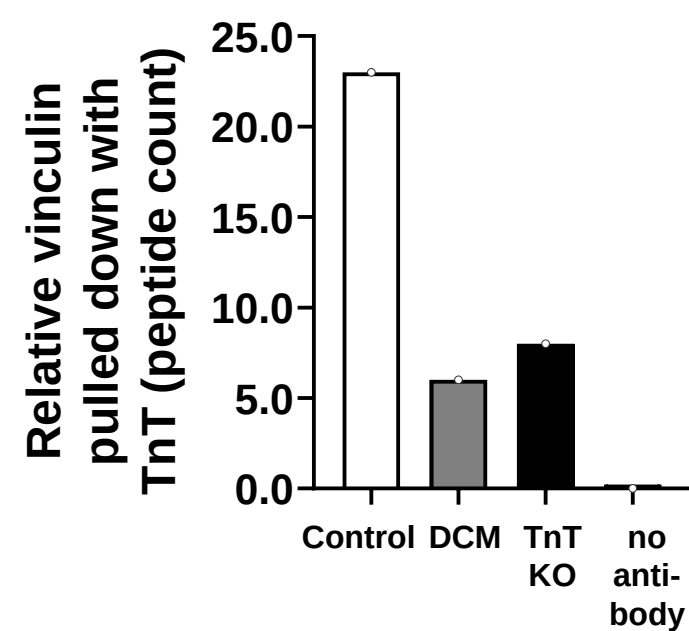
H



I



J



K

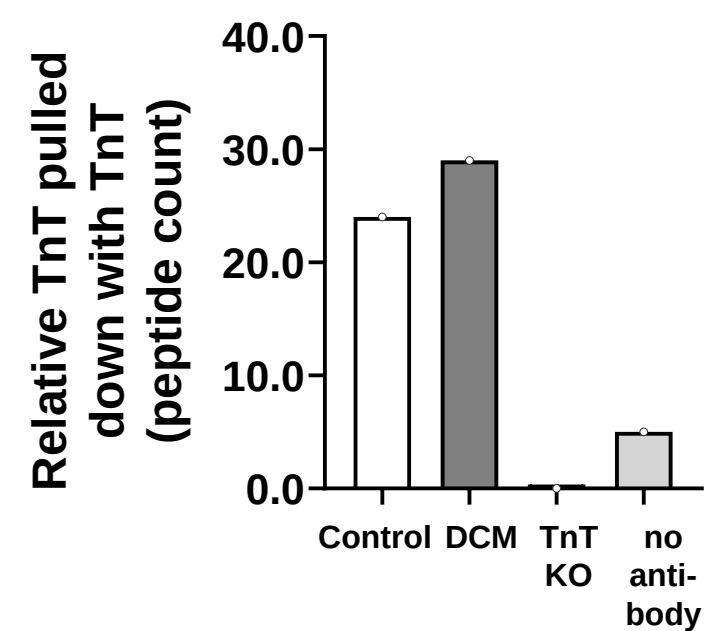


Fig. S6

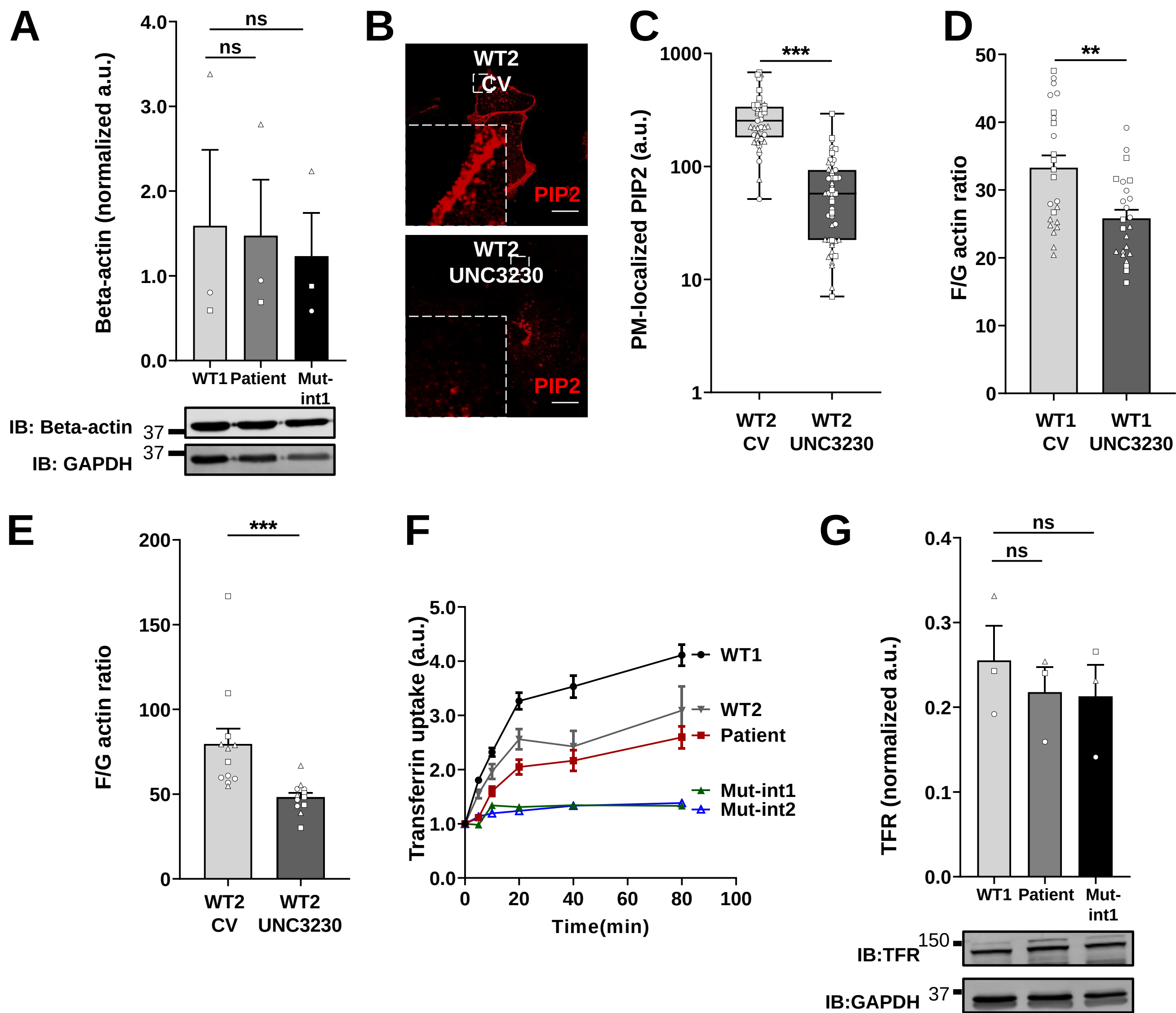


Fig. S7

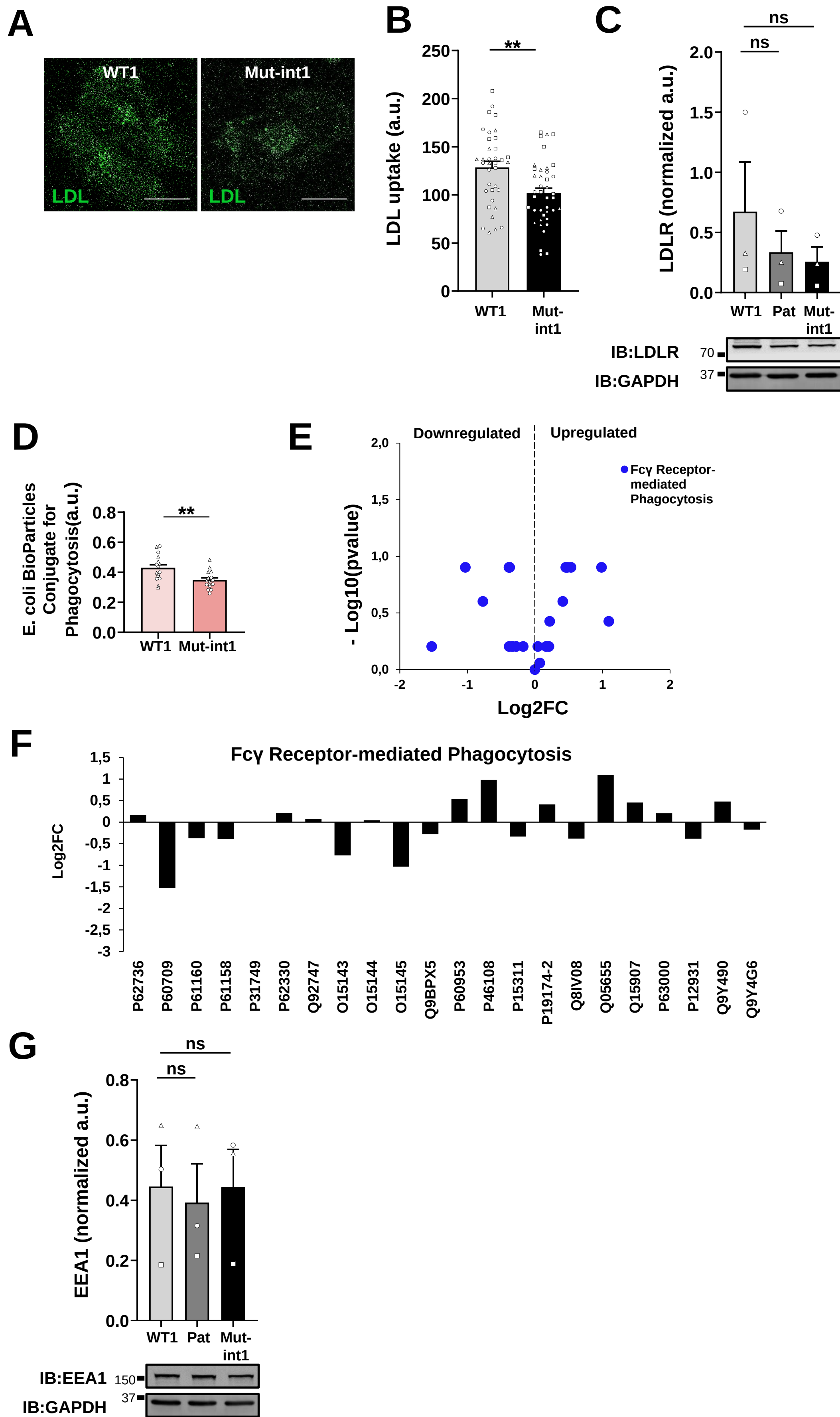


Fig. S8

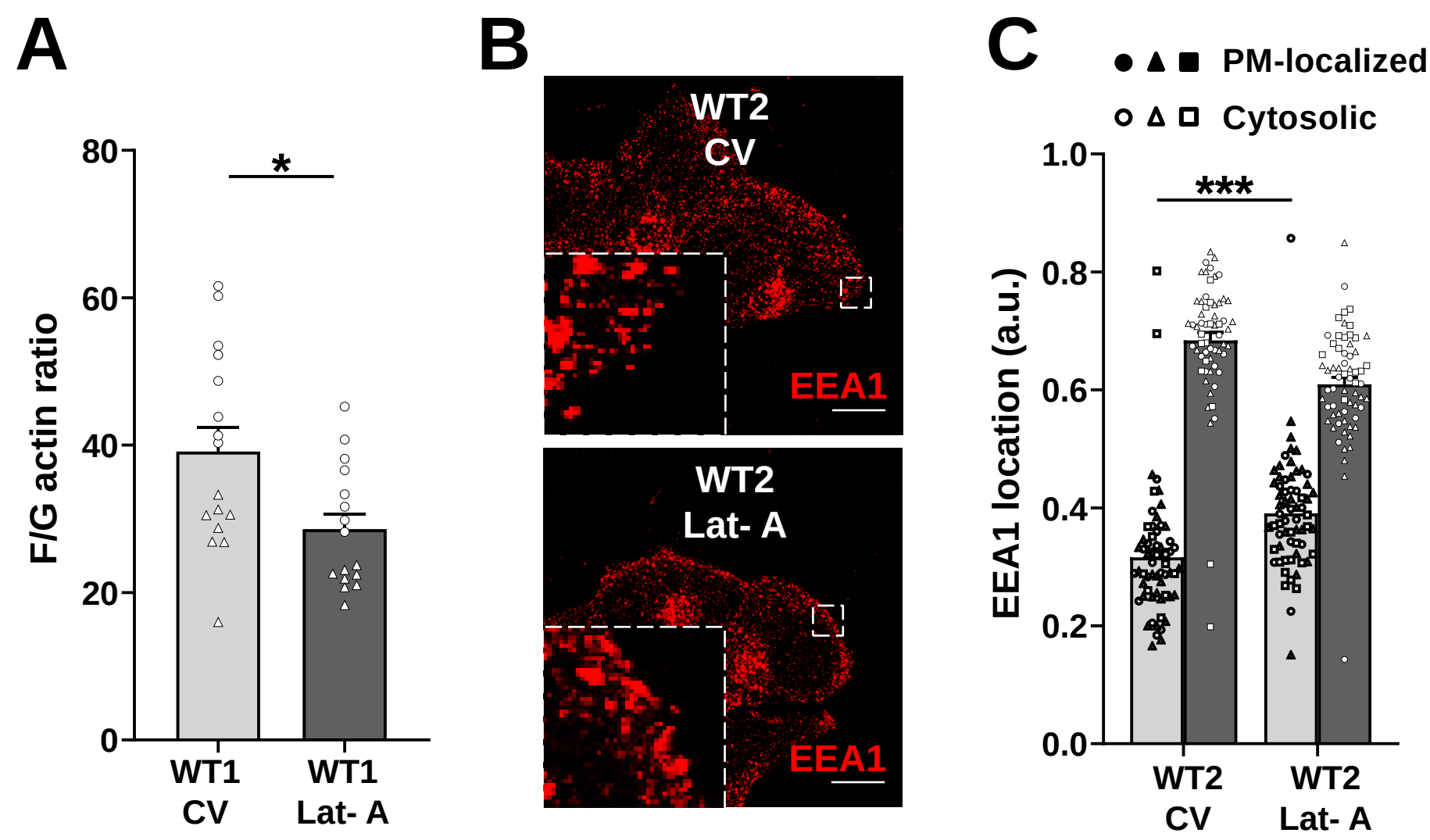


Fig. S9

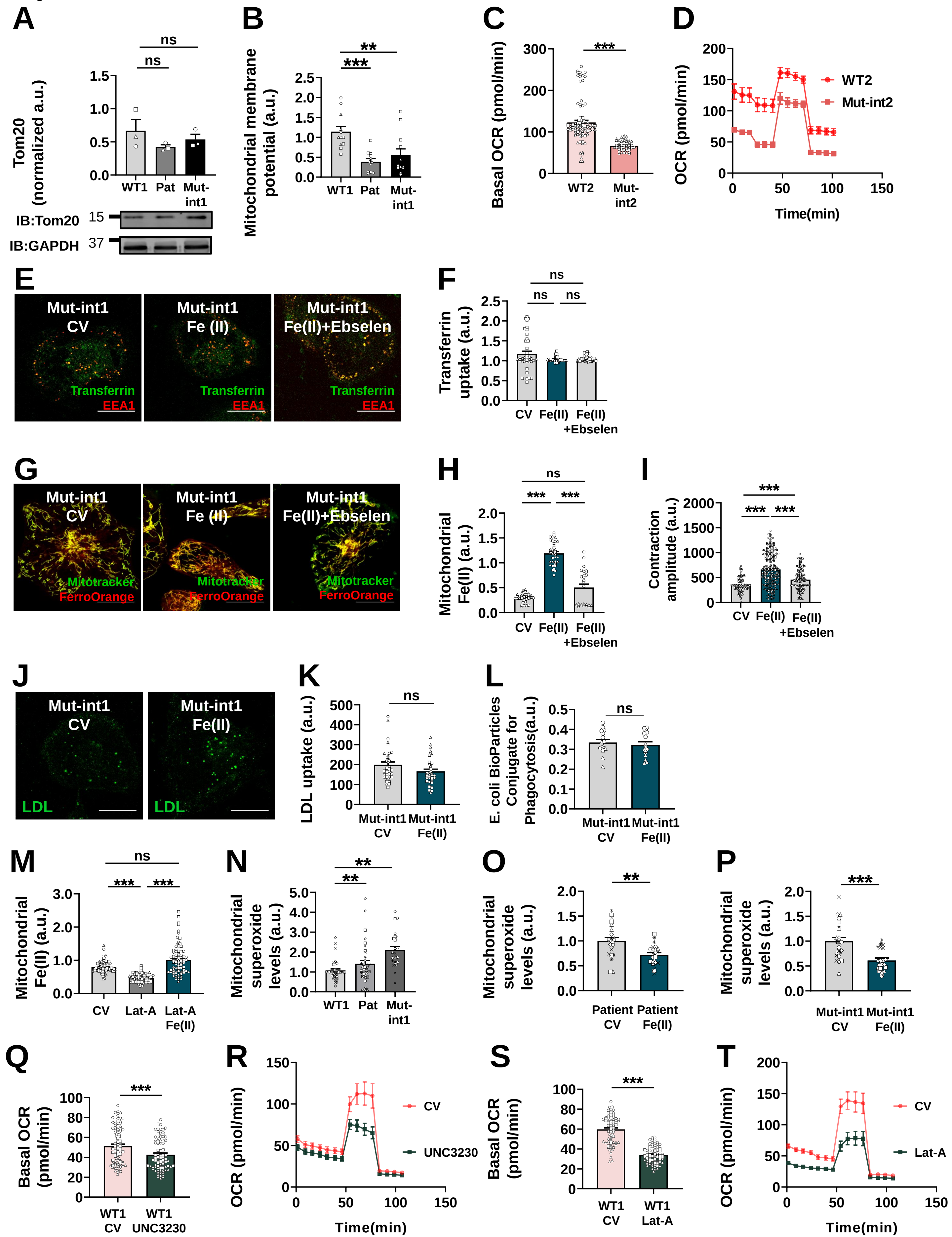


Fig. S10

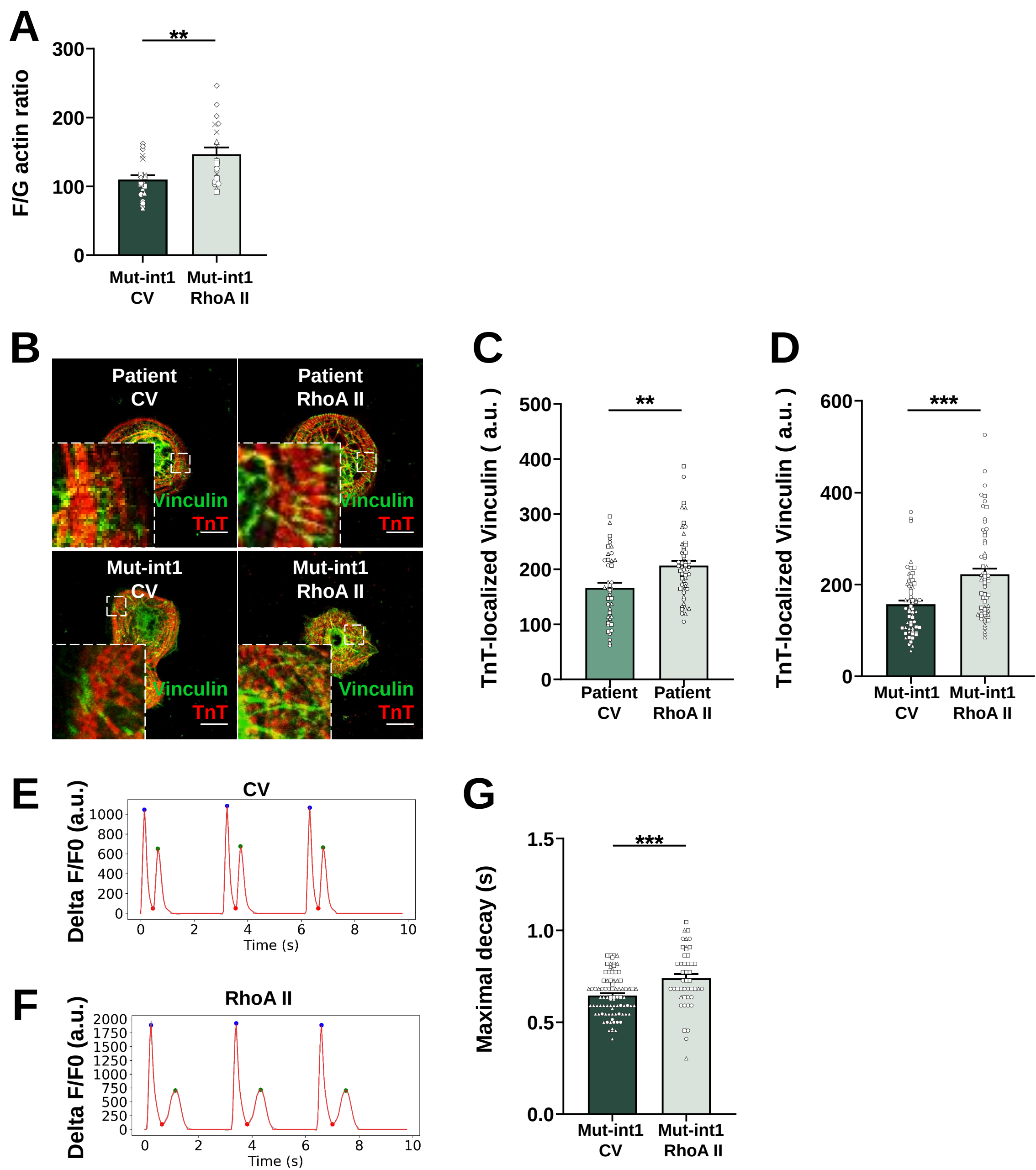


Fig. S11

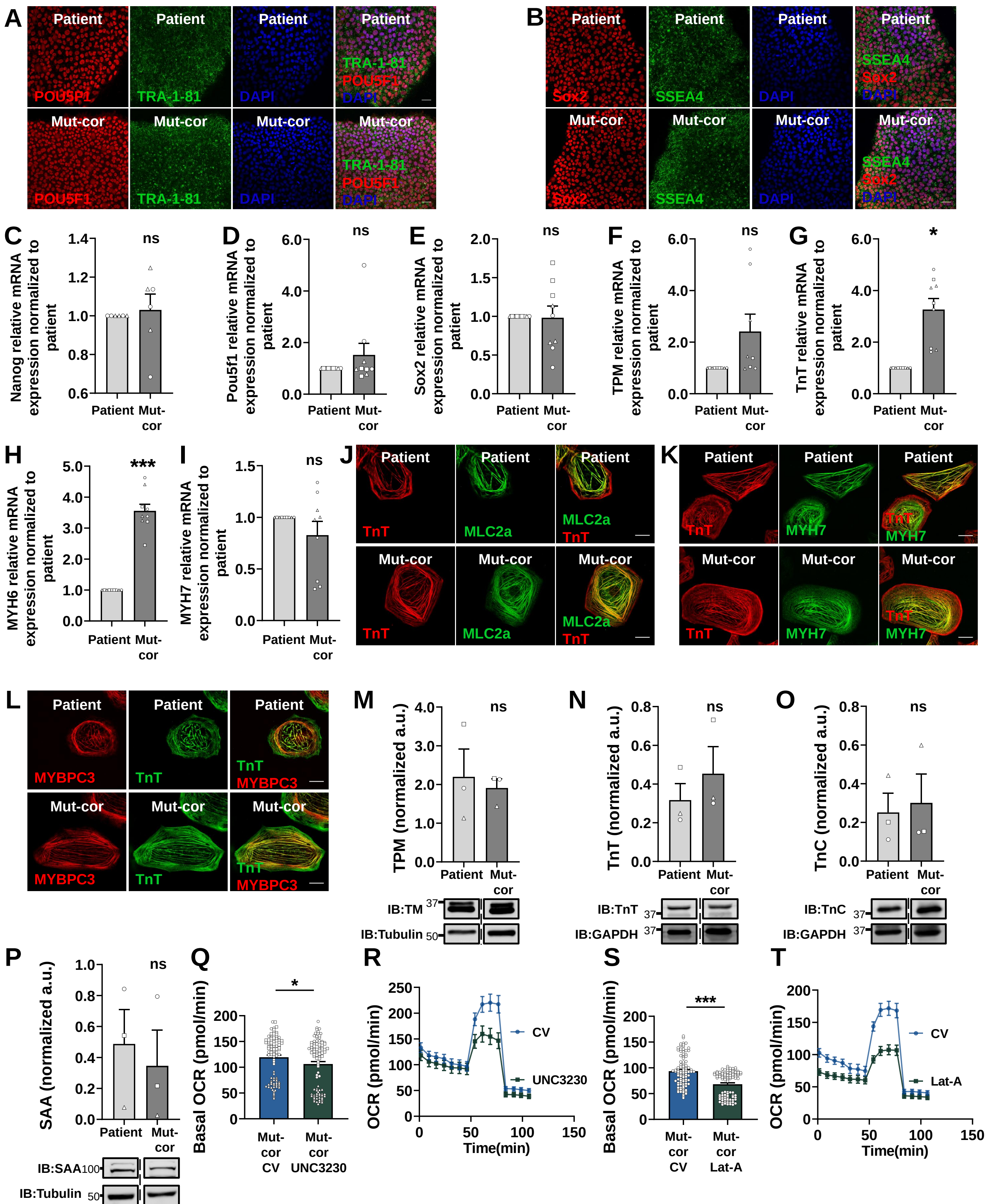


Fig. S12

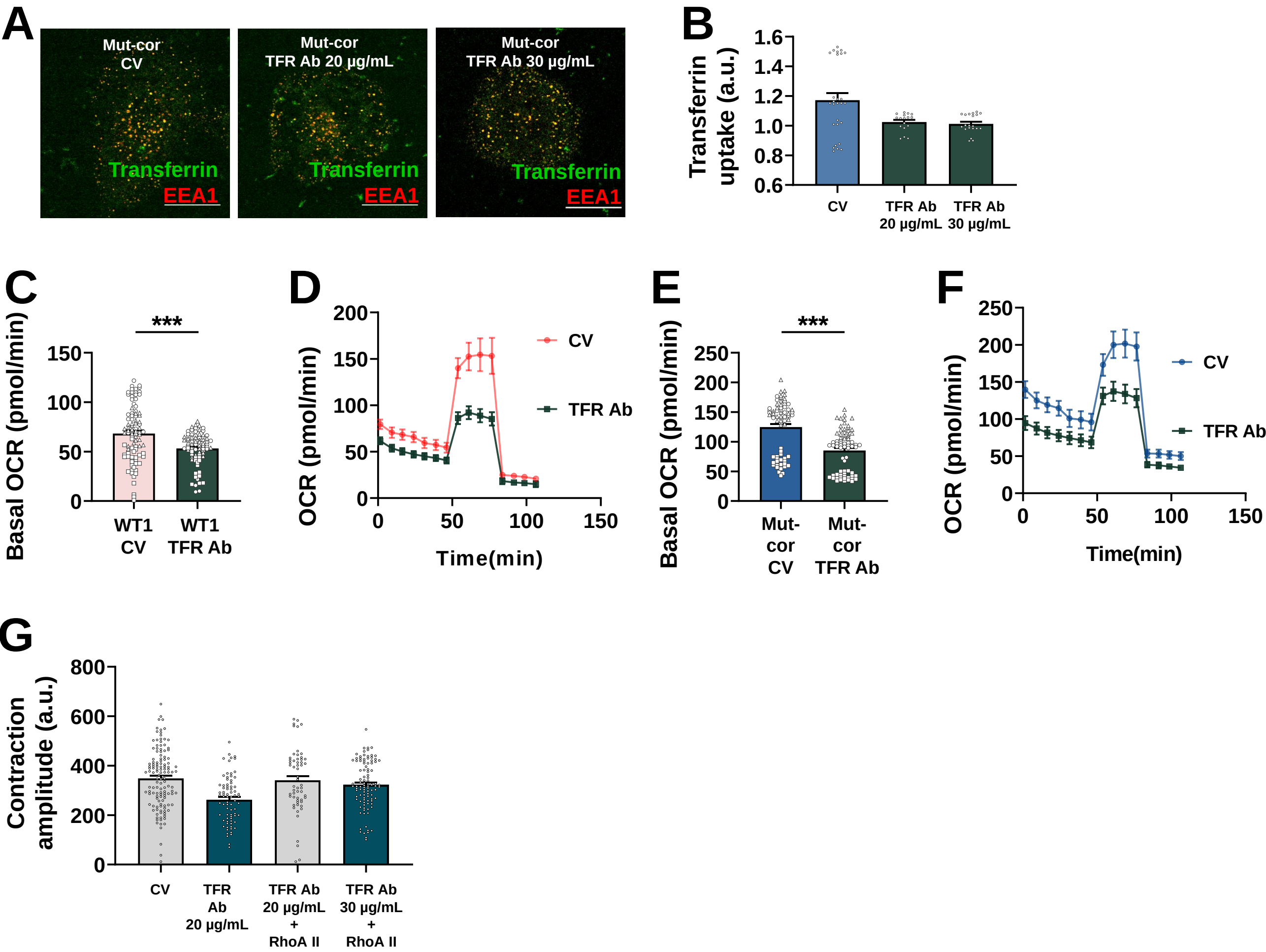


Fig. S13

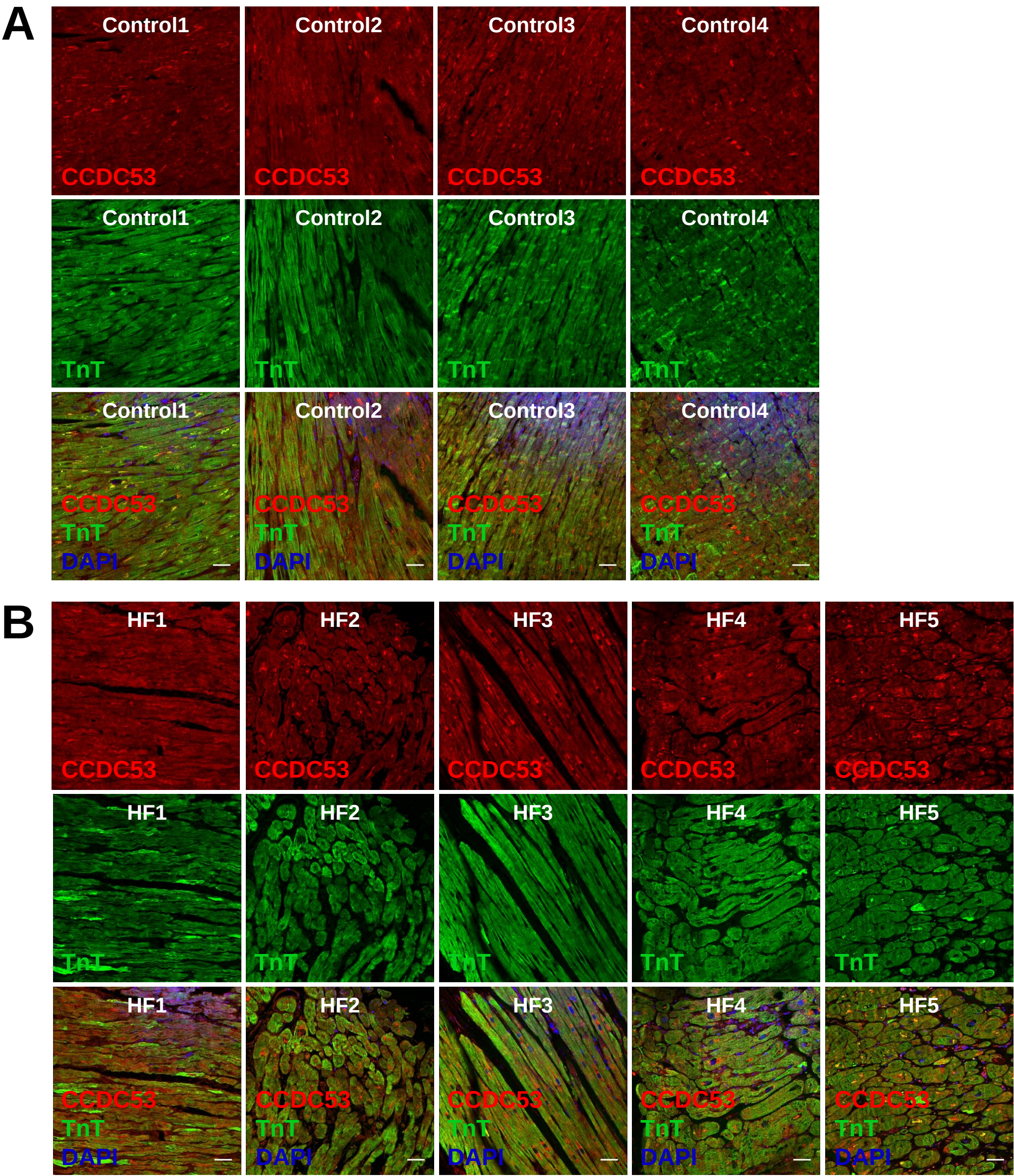
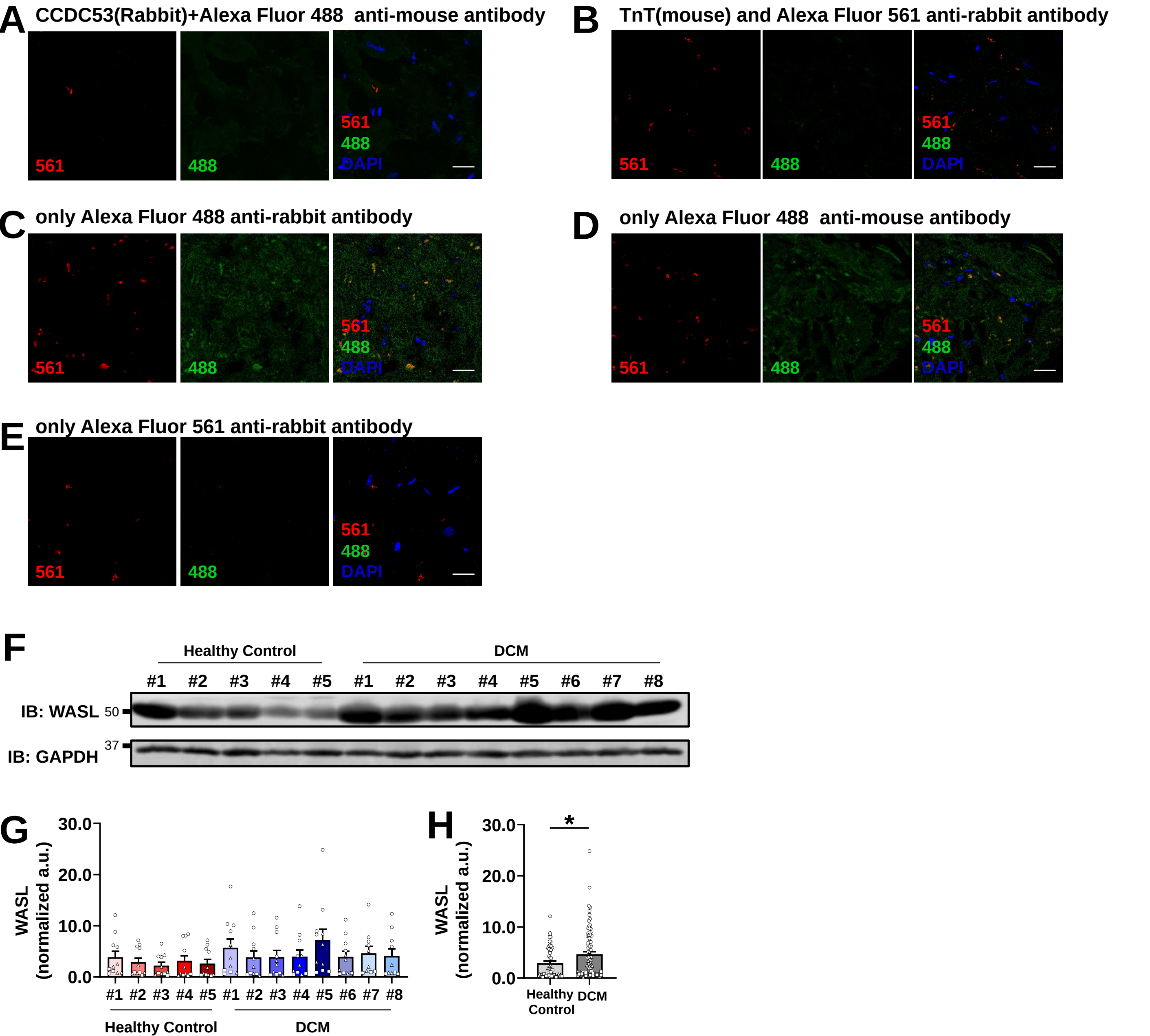


Fig. S14



Tab. S1

	Gene names	Number of proteins	Peptides	Razor + unique peptides	Unique peptides	Control	DCM	TnT KO	no antibody
Troponin T, cardiac muscle	TNNT2;HNTN1	18	34	34	20	24	29	0	5
Vinculin	VCL;HEL114	8	29	29	29	23	6	8	0

Tab. S2

	ID	Conditions of patients
Control 1	Nr.19	severe aortic valve stenosis with compensated LV function that underwent surgical valve replacement
Control 2	Nr.19-3	severe aortic valve stenosis with compensated LV function that underwent surgical valve replacement
Control 3	Nr.20	severe aortic valve stenosis with compensated LV function that underwent surgical valve replacement
Control 4	Nr.31	severe aortic valve stenosis with compensated LV function that underwent surgical valve replacement
HF 1	Nr.60-4	heart transplantation because of severe heart failure due to dilated cardiomyopathy
HF 2	Nr.62-1	heart transplantation because of severe heart failure due to dilated cardiomyopathy
HF 3	Nr.73-2	heart transplantation because of severe heart failure due to dilated cardiomyopathy
HF 4	Nr.74-2	heart transplantation because of severe heart failure due to dilated cardiomyopathy
HF 5	Nr.68-2	heart transplantation because of severe heart failure due to dilated cardiomyopathy

Tab. S3

	Compensated LV hypertrophy	Dilated cardiomyopathy (Immunohistology)	Dilated cardiomyopathy (Immunoblotting)
Indication for heart surgery	Aortic valve implantation + CABG	Heart transplantation	Heart transplantation
Count (n)	4	5	8
Clinical characteristics			
Age (years)	73.8 ± 3.0	58.6 ± 6.9	58.3 ± 6.8
Sex, female, n (%)	1 (25)	1 (20)	2 (25)
Body mass index (kg/m²)	24.3 ± 0.7	24.2 ± 3.9	26.6 ± 3.8
LV ejection fraction (%)	58.5 ± 7.4	19.3 ± 9.3	18.0 ± 4.7
LV assist device, n (%)	0 (0)	2 (40)	5 (62.5)
ICD, n (%)	0 (0)	3 (60)	7 (87.5)
Coronary artery disease, n (%)	4 (100)	0 (0)	1 (12.5)
Prior myocardial infarction, n (%)	2 (50)	0 (0)	0 (0)
Prior CABG, n (%)	0 (0)	0 (0)	0 (0)
Hypertension, n (%)	3 (75)	1 (20)	1 (12.5)
Hyperlipidemia, n (%)	3 (75)	0 (0)	3 (37.5)
Diabetes, n (%)	1 (25)	0 (0)	2 (25)
Atrial fibrillation, n (%)	2 (50)	4 (80)	5 (62.5)
Peripheral vascular disease, n (%)	0 (0)	0 (0)	0 (0)
Prior cerebral ischemia event, n (%)	0 (0)	0 (0)	1 (12.5)
Chronic pulmonary disease, n (%)	1 (25)	1 (20)	0 (0)
Medication			
ACE inhibitors	2 (50)	3 (60)	3 (37.5)
AT1 blockers	2 (50)	1 (20)	2 (25)
Beta-blockers	2 (50)	5 (100)	5 (62.5)
Calcium channel inhibitors	2 (50)	0 (0)	0 (0)
Digitalis	0 (0)	0 (0)	3 (37.5)
Lipid-lowering drugs	3 (75)	0 (0)	1 (12.5)
Mineralocorticoid-receptor antagonists	0 (0)	4 (80)	4 (50)
Other diuretics	1 (25)	4 (80)	7 (87.5)

Tab. S4

		Patient 1	Patient 2	Patient 3
General	Sex	male	male	male
	Age, y	63	55	43
	Body mass index, kg/m²	29.4	27.1	40.3
Intervention	CAD	+	+	+
	MVD/AVD	-	-	-
	CAD+MVD/AVD	-	-	-
	Intervention time, min	245	254	434
	ECC time, min	117	107	217
Anamnesis	Hypertension	+	-	+
	Diabetes	+	-	-
	Hyperlipidemia	+	-	+
	NYHA (I/II/III)	III	I	III
	Stroke	-	-	-
	TIA	-	-	-
CC	Creatinin, µmol/L	0.87	0.85	0.86
Echocardiography	LAD, mm	42	32	45
	LVEDD, mm	59	47	51
	IVSd, mm	11	11	11
	LVPWd, mm	11	8	-
	LVEF, %	48	65	60
	Diastolic dysfunction, Grade I/II/III	I	I	II
Medication	Digitalis	-	-	-
	ACE inhibitors	+	-	+
	AT1 blockers	-	-	-
	β-Blockers	+	+	+
	Dihydropyridines	-	-	-
	Diuretics	-	-	+
	Nitrates	-	-	-
	Lipid-lowering drugs	+	-	+

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Troponin T	Abcam	ab45932	1/100 or 1/200		https://www.abcam.com/cardiac-troponin-t-antibody-ab45932.html
EEA1	Cell signaling Technology	#2411	1/100 or 1/200		https://www.cellsignal.de/products/primary-antibodies/eea1-antibody/2411
MLC-2A	SYSY	311011AT1	1/100		https://sysy.com/product/311011AT1
sarcomeric alpha actinin	Sigma	A7811	1/100 or 1/200		https://www.sigmaaldrich.com/DE/en/product/sigma/a7811
PI(4,5)P2	Echelon Biosciences	Z-P045	1/100		https://www.echelon-inc.com/product/purified-anti-ptdins-45p2-igm/
MYBPC3	Abcam	ab108522	1/100		https://www.abcam.com/mybpc3-antibody-epr30082-ab108522.html
Vinculin	Sigma	V9131	1/100 or 1/200		https://www.sigmaaldrich.com/DE/en/product/sigma/v9131
Transferrin Receptor	DSHB	G1/22/12-s	1/200		https://dshb.biology.uiowa.edu/G1-221-12
LDLR	NSJ Bioreagents	F51451	1/200		https://www.nsjbio.com/tds/ldlr-antibody-f51451
CCDC53	Protein-tech	24445-1-AP	1/100		https://www.ptglab.com/products/CCDC53-Antibody-24445-1-AP.htm
Anti-Active RhoA	Biomol	NB-26904	1/100		https://www.biomol.com/products/antibodies/primary-antibodies/general/anti-active-rhoa-monoclonal-nb-26904
β-Actin	Santa Cruz	SC-47778	1/200		https://www.scbt.com/p/beta-actin-antibody-c4
AP1B1	Proteintech	16932-1-AP	1/200		https://www.ptglab.com/products/AP1B1-Antibody-16932-1-AP.htm
DNML1	Proteintech	12957-1-AP	1/200		https://www.ptglab.com/products/DNM1L,DLP1-Antibody-12957-1-AP.htm
Apolipoprotein A1	Proteintech	14427-1-AP	1/200		https://www.ptglab.com/products/APOA1-Antibody-14427-1-AP.htm
WASL	Proteintech	14306-1-AP	1/200		https://www.ptglab.com/products/WASL-Antibody-14306-1-AP.htm
Cardiac Troponin C	Santa Cruz	Sc52263	1/200		https://www.scbt.com/p/troponin-c-slow-skeletal-cardiac-antibody-12g3
Troponin T	Invitrogen	13-11	1/100 or 1/200		https://www.thermofisher.com/antibody/product/Cardiac-Troponin-T-Antibody-clone-13-11-Monoclonal/MA5-12960

GAPDH	Santa Cruz	Sc-32233	1/500		https://www.scbt.com/p/gapdh-antibody-6c5
Tom20	Santa Cruz	Sc-11415	1/200		https://www.scbt.com/p/tom20-antibody-fl-145
MYH7	Proteintech	22280-1-AP	1/200		https://www.ptglab.com/products/MYH7-specific-Antibody-22280-1-AP.htm
Sox2	Novus	NB110-37235S	1/100		https://www.novusbio.com/products/sox2-antibody_nb110-37235
Oct-3/4	R&D biosystems	AF1759-SP	1/100		https://www.rndsystems.com/products/human-mouse-oct-3-4-antibody_af1759
SSEA4	R&D systems	MAB1435	1/100		https://www.rndsystems.com/products/human-mouse-ssea-4-antibody-mc-813-70_mab1435
TRA-1-81	Stemgent Pelobiotech	09-0011	1/100 (IF)		https://www.citeab.com/antibodies/350414-4-09-0069-stemgent-stainalive-tra-1-81-antibody-dyl
Transferrin Receptor	R and D Systems	AF2474	15-30 µg/mL		https://www.rndsystems.com/products/human-tfr-transferrin-r-antibody_af2474
Alexa Fluor 488 goat anti-mouse IgG	Invitrogen	A-21121	1/400		https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG1-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21121
Alexa Fluor 568 goat anti-rabbit IgG	Invitrogen	A-11011	1/40 (IF)		https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11011
Alexa Fluor 488 rat anti-mouse IgM	Biolegend	406521	1/400		https://www.biolegend.com/nl-be/products/alexa-fluor-488-anti-mouse-igm-9095
Alexa Fluor 568 donkey anti-goat IgG	Invitrogen	A-11057	1/400		https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11057
IRDye® 680RD Goat anti-Mouse IgG	Licor	925-68070	1/10000		https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-mouse-igg-secondary-antibody
IRDye® 680RD Donkey anti-Rabbit	Licor	926-68073	1/10000		https://www.licor.com/bio/reagents/irdye-680rd-donkey-anti-rabbit-igg-secondary-antibody
IRDye® 800CW Donkey anti-Mouse IgG (H+ L)	Licor	925-32212	1/10000		https://www.licor.com/bio/reagents/irdye-800cw-donkey-anti-mouse-igg-secondary-antibody

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
Contractility Measurements	Circ Res. 2018 Feb 2;122(3):e5-e16.	DOI: 10.1161/CIRCRESAHA.117.312067
	Circ Res. 2019 Jun 21;125(1):90-103.	DOI: 10.1161/CIRCRESAHA.118.313973
	Sci Rep. 2020 Jan 14;10(1):209.	DOI: 10.1038/s41598-019-56597-3
Sarcomere length analysis	FEBS Lett. 2021 Oct;595(20):2544-2557.	DOI: 10.1002/1873-3468.14189
Atomic force microscopy (AFM) and contractility measurements	FEBS Lett. 2021 Oct;595(20):2544-2557.	DOI: 10.1002/1873-3468.14189

Fig. 2

	WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F18L, MUT-int/MUT-int)	WT2 (WT/WT)	MUT-int2 (TnT-R141W, MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56?	sensitivity analysis
C	n=84 cells (348 ROIs); n=3 experiments (n=3 independent cardiac differentiation)	n= 92 cells (421 ROIs); n=3 experiments (n=3 independent cardiac differentiation)	n=95 cells (329 ROIs); n=3 experiments (n=3 independent cardiac differentiation)			ROIs	no	***, P < 0.001 for WT1 vs. Pat (Adjusted P value <1 × 10 ⁻⁶ ; Yes 15) and WT1 vs. MUT-int1 (Adjusted P value=1.56 × 10 ⁻⁹) (Kruskal-Wallis test and Dunn's multiple comparisons test).		ns, not significant for WT1 vs. Pat (Adjusted P Value=0.0507) and WT1 vs. MUT-int1 (Adjusted P Value=0.147) (Kruskal-Wallis test and Dunn's multiple comparisons test).
D	n=76 cells (n=3 experiments/n=3 independent cardiac differentiation)	n= 44 cells (n=4 experiments/n=4 independent cardiac differentiation)	n=80 cells (n=3 experiments/n=3 independent cardiac differentiation).			cells	no	***, P < 0.01 for WT1 vs. Pat (Adjusted P Value=1.35 × 10 ⁻³); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=1.36 × 10 ⁻⁴) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	*, P < 0.05 for WT1 vs. Pat (Adjusted P Value=0.0511); *, P < 0.05 for WT1 vs. MUT-int1 (Adjusted P Value=0.052) (Kruskal-Wallis test and Dunn's multiple comparisons test).
E	n=65 movies (n=3 experiments/n=3 independent cardiac differentiation);	n=62 movies (n=2 experiments/n=2 independent cardiac differentiation);	n=72 movies (n=2 experiments/n=2 independent cardiac differentiation)			movies	no	*, P < 0.05 for WT1 vs. Pat (Adjusted P Value=2.65 × 10 ⁻⁶ ; Yes 2); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=1 × 10 ⁻¹⁵) (Kruskal-Wallis test and Dunn's multiple comparisons test).		ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.104) (Kruskal-Wallis test and Dunn's multiple comparisons test).
G	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)		independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for WT vs. Pat and WT vs. MUT-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.929) and WT vs. MUT-int1 iPSC-CMs (Adjusted P Value=0.657) (Kruskal-Wallis test and Dunn's multiple comparisons test).
H	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)		independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for WT vs. Pat (Adjusted P Value=0.208); *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0178) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.208); *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0178) (Kruskal-Wallis test and Dunn's multiple comparisons test).
I	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)		independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	*, P < 0.05 for WT vs. Pat (Adjusted P Value=0.0267) and ***, P < 0.01 for WT vs. MUT-int1 (Adjusted P Value=9.36 × 10 ⁻³) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	*, P < 0.05 for WT vs. Pat (Adjusted P Value=0.0267) and ***, P < 0.01 for WT vs. MUT-int1 (Adjusted P Value=9.36 × 10 ⁻³) (Kruskal-Wallis test and Dunn's multiple comparisons test).
K	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)		independent experiments	no	ns, not significant for WT vs. Pat (Adjusted P Value=0.363); WT vs. MUT-int1 (Adjusted P Value=0.999) and WT vs. igg (Adjusted P Value=0.929) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.363); WT vs. MUT-int1 (Adjusted P Value=0.999) and WT vs. igg (Adjusted P Value=0.929) (Kruskal-Wallis test and Dunn's multiple comparisons test).
L	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)		independent experiments	no	ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.999) iPSC-CMs. *, P < 0.05 for WT vs. Igg (Adjusted P Value=0.0418) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.999) iPSC-CMs. *, P < 0.05 for WT vs. Igg (Adjusted P Value=0.0418) (Kruskal-Wallis test and Dunn's multiple comparisons test).
M	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)	n=5 experiments (n=4 independent cardiac differentiation)		independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for WT vs. Pat (Adjusted P Value=0.326); WT vs. MUT-int1 (Adjusted P Value=0.599) iPSC-CMs and WT vs. igg (Adjusted P Value=0.855) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.326); WT vs. MUT-int1 (Adjusted P Value=0.599) iPSC-CMs and WT vs. igg (Adjusted P Value=0.855) (Kruskal-Wallis test and Dunn's multiple comparisons test).
O	n=3 experiments (n=1 independent cardiac differentiation).	n=3 experiments (n=1 independent cardiac differentiation).	n=3 experiments (n=1 independent cardiac differentiation).	n=3 experiments (n=1 independent cardiac differentiation).		independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.423) iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.423) iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).
P	n=3 experiments (n=1 independent cardiac differentiation).	n=3 experiments (n=1 independent cardiac differentiation).	n=3 experiments (n=1 independent cardiac differentiation).	n=3 experiments (n=1 independent cardiac differentiation).		independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant (Adjusted P value=0.202) for WT vs. Pat; *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0341) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant (Adjusted P value=0.202) for WT vs. Pat; *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0341) (Kruskal-Wallis test and Dunn's multiple comparisons test).
R	n= 46 cells (n=3 experiments (n=3 independent cardiac differentiation)	n=63 cells; n=3 experiments (n=3 independent cardiac differentiation)	n=54 cells; n=3 experiments (n=3 independent cardiac differentiation)	n=43 cells; n=3 experiments (n=3 independent cardiac differentiation)	n=42 cells; n=3 experiments (n=3 independent cardiac differentiation)	cells	no	***, P < 0.01 for WT1 vs. Pat (Adjusted P Value=3.58 × 10 ⁻³); *, P < 0.05 for WT1 vs. MUT-int1 (Adjusted P Value=0.0122); ***, P < 0.001 for WT2 vs. MUT-int2 (Adjusted P Value=8.47 × 10 ⁻⁸) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.604); WT1 vs. MUT-int1 (Adjusted P Value=0.946) and WT2 vs. MUT-int2 (Adjusted P Value=0.166) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. 3									
WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-FHSL MUT- int/MUT-int)	WT2 (WT/WT)	MUT-int2 (Tst-RE141W MUT- int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n50?	sensitivity analysis
A n=4 experiments /4 independent cardiac differentiation	n=4 experiments /4 independent cardiac differentiation	n=4 experiments /4 independent cardiac differentiation	n=3 experiments /3 independent cardiac differentiation	n=4 experiments /4 independent cardiac differentiation	Technical replicates from independent experiments (Each experiment contains 4 technical replicates)	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	**, P < 0.01 for WT1 vs. Pat (Adjusted P Value=1.25 × 10 ⁻³); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=4.77 × 10 ⁻⁸); ns, not significant for WT2 vs. MUT-int2 (Adjusted P Value=0.061) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.235); *, P < 0.05 for WT1 vs. MUT- int1 (Adjusted P Value=0.0115); ns, not significant for WT2 vs. MUT-int2 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
C n=78 cells (n=3 experiments/3 independent cardiac differentiation)	n=50 cells (n=3 experiments/3 independent cardiac differentiation);	n=101 cells (n=3 experiments/3 independent cardiac differentiation)	n=39 cells (n=3 experiments/3 independent cardiac differentiation);	n=35 cells (n=2 experiments/2 independent cardiac differentiation).	cells	no	***, P < 0.001 for WT1 vs. Pat (Adjusted P Value=4.23 × 10 ⁻¹²), WT1 vs. MUT-int1 (Adjusted P Value=1.23 × 10 ⁻⁴) and WT2 vs. MUT-int2 (Adjusted P Value=6.92 × 10 ⁻⁷) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.291), WT1 vs. MUT-int1 (Adjusted P Value=0.999) and WT2 vs. MUT-int2 (Adjusted P Value=0.380) (Kruskal-Wallis test and Dunn's multiple comparisons test).
E n=23 cells (n=2 experiments/2 independent cardiac differentiation);	n=23 cells (n=2 experiments/2 independent cardiac differentiation);	n=28 cells (n=2 experiments/2 independent cardiac differentiation)	n=42 cells (n=3 experiments/3 independent cardiac differentiation)	n=28 cells (n=3 experiments/3 independent cardiac differentiation)	cells	no	***, P < 0.001 for WT1 vs. Pat (Adjusted P Value=2 × 10 ⁻¹⁵), WT1 vs. MUT-int1 (Adjusted P Value=5.88 × 10 ⁻⁶), and WT2 vs. MUT-int2 (Adjusted P Value=2.24 × 10 ⁻⁴) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.497), WT1 vs. MUT-int1 (Adjusted P Value=0.075) and WT2 vs. MUT-int2 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
G n=52 cells (n=3 experiments/3 independent cardiac differentiation)	n=49 cells (n=3 experiments/3 independent cardiac differentiation)	n=59 cells (n=3 experiments/3 independent cardiac differentiation)	n=26 cells (n=2 experiments/2 independent cardiac differentiation)	n=31 cells (n=2 experiments/2 independent cardiac differentiation)	cells	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	**, P < 0.01 for WT1 vs. Pat (Adjusted P Value=1.347 × 10 ⁻³); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=8.16 × 10 ⁻¹¹); **, P < 0.01 for WT2 vs. MUT-int2 (Adjusted P Value=1.105 × 10 ⁻³) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.224), WT1 vs. MUT-int1 (Adjusted P Value=0.139) and WT2 vs. MUT-int2 (Adjusted P Value=0.598) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. 4										
	WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F185L MUT-int/MUT-int)	WT2 (WT/WT)	MUT-int2 (TaT-R141W MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n567	sensitivity analysis
B	n=87 cells (n=3 experiments/3 independent cardiac differentiation)	n=60 cells (n=3 experiments/3 independent cardiac differentiation)	n=120 cells (n=3 experiments/3 independent cardiac differentiation)	n=55 cells (n=3 experiments/3 independent cardiac differentiation)	n=50 cells (n=2 experiments/2 independent cardiac differentiation).	cells	no	*, P<0.05 for WT1 vs. Pat (Adjusted P Value=1.86 × 10 ⁻²); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=9.88 × 10 ⁻⁶) and WT2 vs. MUT-int2 (Adjusted P Value=2.15 × 10 ⁻¹⁰) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value<0.999), WT1 vs.MUT-int1 (Adjusted P Value<0.999) and WT2 vs. MUT-int2 (Adjusted P Value<0.528) (Kruskal-Wallis test and Dunn's multiple comparisons test).
C	n=4 experiments/4 independent cardiac differentiation	n=4 experiments/4 independent cardiac differentiation	n=4 experiments/4 independent cardiac differentiation			Technical replicates from independent experiments	no	***, P<0.001 for WT1 vs. patient (Adjusted P Value=8.17 × 10 ⁻⁶) and WT1 vs. mut-int1 (Adjusted P Value=1 × 10 ⁻¹⁵) iPSC-CMs, (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value<0.999) and WT1 vs.MUT-int1 (Adjusted P Value<0.232) (Kruskal-Wallis test and Dunn's multiple comparisons test).
F		CV, n=47 cells; Fe(II), n=50 cells n=2 experiments/2 independent cardiac differentiation				cells	no	***, P < 0.001 for CV vs. Fe(II) Pat iPSC-CMs (P value=1.65 × 10 ⁻⁸) (Mann-Whitney test).	Yes	ns, not significant for CV vs. Fe(II) Pat iPSC-CMs (P Value=0.667) (Mann-Whitney test).
H			CV, n=48 cells; Fe(II), n=45 cells n=2 experiments/2 independent cardiac differentiation			cells	no	***, P < 0.001 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P value<1 × 10 ⁻¹⁵) (Mann-Whitney test).	Yes	ns, not significant for CV vs. Fe(II) MUT-int1 iPSC- CMs (P Value=0.333) (Mann-Whitney test).
I		CV, n=21 movies; Fe(II), n=21 movies n=2 experiments/2 independent cardiac differentiation				Technical replicates from independent experiments	no	***, P < 0.001 for CV vs. Fe (II) in Pat iPSC-CMs (P value=8.80 × 10 ⁻⁴) (Mann-Whitney test).	Yes	ns, not significant for CV vs. Fe(II) Pat iPSC-CMs (P Value=0.333) (Mann-Whitney test).
J			CV, n=41 movies; Fe (II), n=115 movies n=6 experiments/6 independent cardiac differentiation			Technical replicates from independent experiments	no	***, P < 0.001 for CV vs. Fe(II) in MUT-int1 iPSC-CMs (P value<1 × 10 ⁻¹⁵) (Mann-Whitney test).	Yes	*, P < 0.05 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P value=0.0411) (Mann-Whitney test).
K					CV, n=29 movies; Fe(II), n=63 movies n=5 experiments/5 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P<0.001 for CV vs. Fe(II) MUT-int2 (P value=1.60 × 10 ⁻⁴) (Mann-Whitney test).	Yes	ns, not significant for CV vs. Fe(II) MUT-int2 iPSC- CMs (P Value=0.1508) (Mann-Whitney test).

Fig. 5

	Patient (WT/MUT)	MUT-int1 (TPM1-F18SL MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56?	sensitivity analysis
A		n=3 experiments/3 independent cardiac differentiation	Values from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns. for CV vs. RhoA II (P value=0.100) (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.100) (Mann-Whitney test).
C	CV, n=39 cells; RhoA II, n=38 cells; n=2 experiments/2 independent cardiac differentiation		cells	no	***, P < 0.001 (P value=6.36 × 10 ⁻³) for CV vs. RhoA II Patient (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.200) (Mann-Whitney test).
D		CV, n=19 cells; RhoA II, n=26 cells; n=2 experiments/2 independent cardiac differentiation	cells	no	***, P < 0.001 (P value=6.68 × 10 ⁻⁵) for CV vs. RhoA II MUT-int1 (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.333) (Mann-Whitney test).
F		CV, n=37 cells; RhoA II, n=87 cells; n=3 experiments/3 independent cardiac differentiation	cells	no	** P < 0.01 (P value=2.30 × 10 ⁻³) for CV vs. RhoA II in MUT-int1 (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.400) (Mann-Whitney test).
H		CV, n=40 cells; RhoA II, n=37 cells; n=3 experiments/3 independent cardiac differentiation	cells	Yes (pass Shapiro-Wilk test)	***, P < 0.001 for CV vs. Rho activator II in PM-localized EEA1 (P value=2.51 × 10 ⁻⁵) (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.100) (Mann-Whitney test).
J	CV, n=45 cells; RhoA II, n=36 cells; n=2 experiments/2 independent cardiac differentiation		cells	no	***, P < 0.001 for CV Pat vs. RhoA II Pat (P value=1.19 × 10 ⁻⁵) (Mann-Whitney test)	Yes	ns, not significant for CV vs. RhoA II (P Value=0.667) (Mann-Whitney test).
L		CV, n=50 cells; RhoA II, n=59 cells; n=3 experiments/3 independent cardiac differentiation	cells	no	***, P < 0.001 for CV vs. RhoA II MUT-int1 (P value=6.34 × 10 ⁻⁴) (Mann-Whitney test)	Yes	ns, not significant for CV vs. RhoA II (P Value=0.400) (Mann-Whitney test).
M	n=2 experiments/2 independent cardiac differentiation		Technical replicates from independent experiments	no	** P<0.01 for patient CV vs. patient RhoA II (P value=2.44 × 10 ⁻⁴) (Mann Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.667) (Mann-Whitney test).
O		n=2 experiments/2 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P<0.001 for mut-int1 CV vs. mut-int1 RhoA II (P value<1 × 10 ⁻¹⁵) (Mann Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.333) (Mann-Whitney test).
Q		CV, n=88 movies; RhoA II, n=46 movies; n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P < 0.001 CV vs. Rho activator II MUT-int1 (P value=5.07 × 10 ⁻⁷) (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.400) (Mann-Whitney test).
S	CV, n=56 cells, n=287 ROIs; RhoA II, n= 67 cells, n=292 ROIs; n=3 experiments/3 independent cardiac differentiation		ROIs	no	*, P < 0.05 (P value=0.0363) CV vs. RhoA II Pat (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value>0.999) (Mann-Whitney test).
T		CV, n=52 cells, n=142 ROIs; RhoA II, n=51 cells, n=165 ROIs; n=3 experiments/3 independent cardiac differentiation	ROIs	no	*, P < 0.05 (P value=0.0231) CV vs. RhoA II MUT-int1 (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II (P Value=0.100) (Mann-Whitney test).

Fig. 6

	Patient (WT/MUT)	Mut-corrected (WT-cor/WT-cor)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n567	sensitivity analysis
B	n=4 experiments/ 4 independent cardiac differentiation	n=5 experiments/ 5 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	***, $P < 0.001$ (P value= 9.011×10^{-4}) for Pat vs. mut-corrected (Mann-Whitney test).	Yes	ns, not significant for Pat vs. mut-corrected (P Value=0.111) (Mann-Whitney test).
D	n=23 cells; n=2 experiments/ 2 independent cardiac differentiation	n=52 cells; n=3 experiments/ 3 independent cardiac differentiation	cells	no	***, $P < 0.001$ (P value= 1.63×10^{-4}) for Pat vs. mut-corrected (Mann-Whitney test).	Yes	ns, not significant for Pat vs. mut-corrected (P Value=0.800) (Mann-Whitney test).
F	n=49 cells; n=3 experiments/ 3 independent cardiac differentiation	n=41 cells; n=3 experiments/ 3 independent cardiac differentiation	cells	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	***, $P < 0.001$ for Pat vs. mut-corrected in PM-localized EEA1 (P value= 1.308×10^{-5}) (Mann-Whitney test).	Yes	ns, not significant for Pat vs. mut-corrected (P Value=0.200) (Mann-Whitney test).
H	n=44 cells; n=2 experiments/ 2 independent cardiac differentiation	n=43 cells; n=2 experiments/ 2 independent cardiac differentiation	cells	no	***, $P < 0.001$ for Pat vs. mut-corrected (P value= 1×10^{-15}) (Mann-Whitney test).	Yes	ns, not significant for Pat vs. mut-corrected (P Value=0.333) (Mann-Whitney test).
I	n=4 experiments/ 4 independent cardiac differentiation	n=2 experiments/ 2 independent cardiac differentiation	values from independent experiments	no	***, $P < 0.001$ for patient vs. mut-cor iPSC-CMs (P value= 3.54×10^{-6}) (Mann-Whitney test).	Yes	ns, not significant for Pat vs. mut-corrected (P Value=0.800) (Mann-Whitney test).
L	n=92 cells; n=421 ROI; n=3 experiments/ 3 independent cardiac differentiation	n=36 cells; n=299 ROI; n=2 experiments/ 2 independent cardiac differentiation	ROIs	no	P value= 6.62×10^{-3} , **, $P < 0.01$ for Pat vs. mut-corrected (Mann-Whitney test).	Yes	ns, not significant for Pat vs. mut-corrected (P Value=0.400) (Mann-Whitney test).
M	n=44 cells; n=4 experiments/ 4 independent cardiac differentiation	n=58 cells; n=3 experiments/ 3 independent cardiac differentiation	cells	no	***, $P < 0.001$ for Pat vs. mut-corrected (P value= 5.36×10^{-5}) (Mann-Whitney test).	Yes	*, $P < 0.05$ for Pat vs. mut-corrected (P value=0.0286) (Mann-Whitney test).
O		CV, n=33 cells; DFO, n=34 cells; DFO+RhoA II, n=38 cells; n=2 experiments/2 independent cardiac differentiation	cells	no	***, $P < 0.001$ for CV vs. DFO (P value= 1.47×10^{-8}) and DFO vs. DFO+RhoA II (P value= 8.41×10^{-11}). ns, not significant for CV vs. DFO +RhoA II (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for CV vs. DFO (Adjusted P Value=0.326), DFO vs. DFO+RhoA II (Adjusted P Value=0.326) and CV vs. DFO +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
P		CV, n=24 movies; DFO, n=23 movies; DFO+RhoA II, n=24 movies; n=2 experiments/2 independent cardiac differentiation	Technical replicates from independent experiments	no	***, $P < 0.001$ for CV vs. DFO (Adjusted P Value= 3.47×10^{-12}) and DFO vs. DFO+RhoA II (Adjusted P Value= 1×10^{-15}). ns, not significant for CV vs. DFO+RhoA II (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for CV vs. DFO (Adjusted P Value=0.999), DFO vs. DFO+RhoA II (Adjusted P Value=0.326) and CV vs. DFO +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
R		CV, n=34 cells; TFR Ab, n=33 cells; TFR Ab+RhoA II, n=39 cells; n=2 experiments/2 independent cardiac differentiation	cells	no	***, $P < 0.001$ for CV vs. TFR Ab (Adjusted P Value= 1.19×10^{-4}) and TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value= 9×10^{-15}); **, $P < 0.01$ for CV vs. TFR Ab+RhoA II (Adjusted P Value= 7.05×10^{-4}) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for CV vs. TFR Ab (Adjusted P Value=0.999), TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value=0.326) and CV vs. TFR Ab +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
S		CV, n=29 movies; TFR Ab, n=30 movies; TFR Ab+RhoA II, n=29 movies; n=2 experiments/2 independent cardiac differentiation	Technical replicates from independent experiments	no	***, $P < 0.001$ for CV vs. TFR Ab (Adjusted P Value= 1×10^{-15}) and TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value= 1×10^{-15}); *, $P < 0.05$ for CV vs. TFR Ab +RhoA II (Adjusted P Value=0.047) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for CV vs. TFR Ab (Adjusted P Value=0.184), TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value=0.544) and CV vs. TFR Ab +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. 7						
	Control	HF	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6? sensitivity analysis
C	n=45 images, n=4 patients	n=52 images, n=5 patients	images	no	** $P<0.01$ for control vs. HF (P value=0.00187) (Mann-Whitney test).	Yes ** $P<0.01$ for control vs. HF (P value=0.00187) (Mann-Whitney test).
F	n=61 images, n=4 patients	n=76 images, n=5 patients	images	no	** $P<0.01$ for control vs. HF (P value=0.00121) (Mann-Whitney test).	Yes ** $P<0.01$ for control vs. HF (P value=0.00121) (Mann-Whitney test).

Fig. 8						
	CV	RhoAII	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6? sensitivity analysis
B	n=42 cells; n=3 patients	n=47 cells; n=3 patients	cells	no	***, P<0.001 for CV vs. RhoA II (P value= 1.21×10^{-5}) (Mann Whitney test).	Yes ***, P<0.001 for CV vs. RhoA II (P value= 1.21×10^{-5}) (Mann Whitney test).

Fig. S2							
WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F18SL MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6?	sensitivity analysis
C n=2 experiments/2 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	no	***, $P < 0.001$ for WT1 vs. Pat (Adjusted P Value= 9.41×10^{-4}); ns, not significant for WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.0883) and WT1 vs. MUT-int1 (Adjusted P Value<0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
D n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro- Wilk test and Kolmogorov-Smirnov test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.0507) and WT1 vs. MUT-int1 (Adjusted P Value=0.742) (Kruskal-Wallis test and Dunn's multiple comparisons test).
E n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	no	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value<0.999) and WT1 vs. MUT-int1 (Adjusted P Value>0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. S3							
	WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F18SL MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56? sensitivity analysis
A	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	no	ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
B	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.912) (Kruskal-Wallis test and Dunn's multiple comparisons test).
C	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	no	ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.202) and WT1 vs.MUT-int1 (Adjusted P Value=0.593) (Kruskal-Wallis test and Dunn's multiple comparisons test).
D	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.912) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
H	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
I	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
J	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.742) (Kruskal-Wallis test and Dunn's multiple comparisons test).
K	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	n=3 experiments/3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 β SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
M	WT (WT1+WT2) n=2 cell lines, n=3 independent experiments/3 independent cardiac differentiation (each experiment contains 6 replicates)	DCM (Patient+Mut-int1) n=2 cell lines, n=3 independent experiments/3 independent cardiac differentiation (each experiment contains 6 replicates)		Technical replicates from independent experiments	no	*, P<0.05 for WT vs. DCM (P value=0.0206) (Mann Whitney test).	Yes ns, not significant for WT vs. DCM (P Value=0.400)(Mann Whitney test).
N	WT1 (WT/WT) n=2 independent experiments were performed, each experiment contained n=3 technical replicates.	Mut-int1 (MUT/MUT) n=2 independent experiments were performed, each experiment contained n=3 technical replicates.		Technical replicates from independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	**, P<0.01 for WT1 vs. Mut-int1 (P value=0.00216) (Mann Whitney test).	Yes ns, not significant for WT1 vs. Mut-int1 (P Value=0.333) (Mann Whitney test).
O	n=2 independent experiments (2 independent cardiac differentiation) were performed, each experiment contained n=3 technical replicates.	n=2 independent experiments (2 independent cardiac differentiation) were performed, each experiment contained n=3 technical replicates.		Technical replicates from independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	**, P<0.01 for WT1 vs. Mut-int1 (P value=0.00216) (Mann Whitney test).	Yes ns, not significant for WT1 vs. Mut-int1 (P Value=0.333) (Mann Whitney test).

Fig. S4							
WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F18SL MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56?	sensitivity analysis
D n=65 movies (n=3 experiments/n=3 independent cardiac differentiation)	n=62 movies (n=2 experiments/n=2 independent cardiac differentiation)	n=72 movies (n=2 experiments/n=2 independent cardiac differentiation)	movies	no	ns, not significant for WT1 vs. Pat; **, $P < 0.01$ WT1 vs. MUT-int1 (Adjusted P Value= 1.36×10^{-3}) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.894) and WT1 vs.MUT-int1 (Adjusted P Value=0.621) (Kruskal-Wallis test and Dunn's multiple comparisons test).
E n=65 movies (n=3 experiments/n=3 independent cardiac differentiation)	n=62 movies (n=2 experiments/n=2 independent cardiac differentiation)	n=72 movies (n=2 experiments/n=2 independent cardiac differentiation)	movies	no	ns, not significant for WT1 vs. Pat; ***, $P < 0.001$ WT1 vs. MUT-int1 (Adjusted P Value= 5.81×10^{-6}) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.410) (Kruskal-Wallis test and Dunn's multiple comparisons test).
F n=4 experiments/n=4 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=4 experiments/n=4 independent cardiac differentiation	Technical replicates from independent experiments	no	***, $P < 0.001$ for WT1 vs. Pat (Adjusted P Value= 1.20×10^{-6}) and WT1 vs. Mut-int1 (Adjusted P Value= 1×10^{-15}) (Kruskal-Wallis test and Dunn's multiple comparisons test)	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.859) and WT1 vs.MUT-int1 (Adjusted P Value=0.176) (Kruskal-Wallis test and Dunn's multiple comparisons test).
G n=4 experiments/n=4 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=4 experiments/n=4 independent cardiac differentiation	Technical replicates from independent experiments	no	***, $P < 0.001$ for WT1 vs. Pat (Adjusted P Value= 1×10^{-14}) and WT1 vs. Mut-int1 (Adjusted P Value= 1.28×10^{-8}) (Kruskal-Wallis test and Dunn's multiple comparisons test)	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.482) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. S5									
	WT (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F185L) MUT-int/MUT-int	WT2 (WT/WT)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56?	sensitivity analysis
A	WT1, n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation		independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
B-C	WT (WT1+WT2), n=5 experiments/n=4 independent cardiac differentiation				independent experiments	Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)	** P < 0.01 for WT vs. Igg (P value=7.94 × 10 ⁻³) (Mann Whitney test).	Yes	** P < 0.01 for WT vs. Igg (P value=7.94 × 10 ⁻³) (Mann Whitney test).
E	WT (WT1+WT2), n=3 experiments/n=1 independent cardiac differentiation				independent experiments	Yes (pass Shapiro-Wilk test)	P value=0.100. ns, not significant for WT vs. igg (Mann Whitney test).	Yes	P value=0.100. ns, not significant for WT vs. igg (Mann Whitney test).
F	WT (WT1+WT2), n=3 experiments/n=1 independent cardiac differentiation				independent experiments	Yes (pass Shapiro-Wilk test)	P value=0.100. ns, not significant for WT vs. igg (Mann Whitney test).	Yes	P value=0.100. ns, not significant for WT vs. igg (Mann Whitney test).
H	WT (WT1+WT2), n=3 experiments/n=1 independent cardiac differentiation	n=3 experiments/n=1 independent cardiac differentiation	n=3 experiments/n=1 independent cardiac differentiation	n=3 experiments/n=1 independent cardiac differentiation	independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT vs. Pat, WT vs. MUT-int1 and WT vs. igg (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.999), WT vs. MUT-int1 (Adjusted P Value=0.999) and WT vs. igg (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
I	WT (WT1+WT2), n=3 experiments/n=1 independent cardiac differentiation	n=3 experiments/n=1 independent cardiac differentiation	n=3 experiments/n=1 independent cardiac differentiation	n=3 experiments/n=1 independent cardiac differentiation	independent experiments	no	ns, not significant for WT vs. Pat, WT vs. MUT-int1 and WT vs. igg (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT vs. Pat (Adjusted P Value=0.999), WT vs. MUT-int1 (Adjusted P Value=0.999) and WT vs. igg (Adjusted P Value=0.773) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. S6

	WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F18SL MUT-int/MUT-int)	WT2 (WT/WT)	MUT-int2 (TnT-R141W MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56?	sensitivity analysis
A	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation			independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value>0.999) and WT1 vs. MUT-int1 (Adjusted P Value>0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
C				CV, n=45 cells; UNC3230, n=51 cells; n=3 experiments/n=3 independent cardiac differentiation		cells	no	***, P < 0.001 (P value<1 × 10 ⁻¹⁵) for CV WT2 vs. UNC3230 WT2 (Mann-Whitney test).	Yes	ns, not significant for CV WT2 vs. UNC3230 WT2 (P Value=0.100) (Mann-Whitney test).
D	n=3 experiments/n=3 independent cardiac differentiation					Technical replicates from independent experiments	no	**, P<0.01 (P value=2.94 × 10 ⁻³) for CV WT1 vs. UNC3230 WT1 (Mann-Whitney test).	Yes	ns, not significant for CV WT1 vs. UNC3230 WT1 (P Value=0.400) (Mann-Whitney test).
E				n=3 experiments/n=3 independent cardiac differentiation		Technical replicates from independent experiments	no	***, P<0.001 (Adjusted P Value=2.22 × 10 ⁻⁵) for CV vs. UNC3230 WT2 iPSC-CMs (Mann-Whitney test).	Yes	ns, not significant for CV WT2 vs. UNC3230 WT2 (P Value=0.100) (Mann-Whitney test).
F	n=2 experiments/n=2 independent cardiac differentiation	n=2 experiments/n=2 independent cardiac differentiation	n=2 experiments/n=2 independent cardiac differentiation	n=2 experiments/n=2 independent cardiac differentiation	n=2 experiments/n=2 independent cardiac differentiation					
G	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation			independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value>0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.912) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. S7							
WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (TPM1-F18SL MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6?	sensitivity analysis
B n=36 cells;n=3 experiments/n=3 independent cardiac differentiation		n=40 cells;n=3 experiments/n=3 independent cardiac differentiation	cells	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro Wilk test and Kolmogorov- Smirnov test)	** , P < 0.01 for WT1 vs. MUT-int1 iPSC- CMs (P value= 1.31×10^{-3}) (Mann Whitney test).	Yes	ns, not significant for WT1 vs. MUT-int1 iPSC- CMs (P Value=0.100) (Mann Whitney test).
C n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value>0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.742) (Kruskal-Wallis test and Dunn's multiple comparisons test).
D n=2 experiments/n=2 independent cardiac differentiation		n=2 experiments/n=2 independent cardiac differentiation	cells	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro Wilk test and Kolmogorov- Smirnov test)	** , P<0.01 for WT1 vs. Mut-int1 iPSC- CMs (P value=0.00649) (Mann Whitney test).	Yes	ns, not significant for WT1 vs. MUT-int1 iPSC- CMs (P Value=0.333) (Mann Whitney test).
G n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value>0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).

Fig. S8

	WT1 (WT/WT)	WT2 (WT/WT)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6?	sensitivity analysis
A	n=2 experiments/n=2 independent cardiac differentiation		Technical replicates from independent experiments	no	*, $P < 0.05$ (P value = 1.89×10^{-2}) for CV WT1 vs. Lat-A WT1 (Mann-Whitney test).	Yes	ns, not significant for CV WT1 vs. Lat-A WT1 (P Value = 0.667) (Mann-Whitney test).
C		CV, n=61 cells; Lat-A, n=65 cells; n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	***, $P < 0.001$ for CV WT2 vs. Lat-A WT2 in PM-localized EEA1 (P value = 6.02×10^{-8}) (Mann-Whitney test).	Yes	ns, not significant for CV WT2 vs. Lat-A WT2 in PM-localized EEA1 (P Value = 0.200) (Mann-Whitney test).

Fig. S9									
WT1 (WT/WT)	Patient (WT/MUT)	MUT-int1 (CPM-F185L MUT-intMUT-int)	WT2 (WT/WT)	MUT-int2 (TnT-R141W MUT-intMUT-int)	what data points were used in analysis	Passed normality test (alpha=0.05)?	Statistics method	n567	sensitivity analysis
A	n=3 experiments/w=3 independent cardiac differentiation	n=3 experiments/w=3 independent cardiac differentiation	n=3 experiments/w=3 independent cardiac differentiation		independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.272) and WT1 vs. MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.272) and WT1 vs. MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
B	n=3 experiments/w=3 independent cardiac differentiation	n=3 experiments/w=3 independent cardiac differentiation	n=3 experiments/w=3 independent cardiac differentiation		Technical replicates from independent experiments	no	***, P<0.001 for WT1 vs. Pat (Adjusted P Value=8.40 × 10 ⁻⁴). ***, P<0.01 for WT1 vs. MUT-int1 (Adjusted P Value=0.00469) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.147) and WT1 vs. MUT-int1 (Adjusted P Value=0.359) (Kruskal-Wallis test and Dunn's multiple comparisons test).
C			n=3 experiments/w=3 independent cardiac differentiation	n=2 experiments/w=2 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P<0.001 for WT2 vs. Mut-int2 iPSC-CMs (P value<1 × 10 ⁻⁵). Yes 15) (Mann Whitney test).		ns, not significant for WT2 vs. Mut-int2 iPSC-CMs (P Value=0.133) (Mann Whitney test).
F			n=3 experiments/w=3 independent cardiac differentiation were performed; CV, n=42 cells; Fe(II), n=32 cells; Fe(II)-chicken, n=39 cells.		cells	no	ns, not significant for CV vs. Fe(II), CV vs. Fe(II)-chicken, and Fe(II) vs. Fe(II)-chicken (Kruskal-Wallis test and Dunn's multiple comparison test)	Yes	ns, not significant for CV vs. Fe(II) (Adjusted P Value=0.999), CV vs. Fe(II)-chicken (Adjusted P Value=0.890), and Fe(II) vs. Fe(II)-chicken (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparison test)
H			n=3 experiments/w=3 independent cardiac differentiation were performed; CV, n=37 cells; Fe(II), n=36 cells; Fe(II)-chicken, n=26 cells.		cells	no	***, P<0.001 for CV vs. Fe(II) (Adjusted P Value=1.87 × 10 ⁻¹³). Yes and Fe(II) vs. Fe(II)-chicken (Adjusted P Value=1.18 × 10 ⁻⁸). ns, not significant for CV vs. Fe(II)-chicken (Kruskal-Wallis test and Dunn's multiple comparisons test)	Yes	ns, not significant for CV vs. Fe(II) (Adjusted P Value=0.326), Fe(II) vs. Fe(II)-chicken (Adjusted P Value=0.326) and CV vs. Fe(II)-chicken (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test)
I			n=2 experiments/w=2 independent cardiac differentiation were performed; CV, n=28 movies; Fe(II), n=55 movies; Fe(II)-chicken, n=37 movies.		movies	no	***, P<0.001 for CV vs. Fe(II) (Adjusted P Value=1 × 10 ⁻¹⁵). Yes Fe(II) vs. Fe(II)-chicken (Adjusted P Value=4.48 × 10 ⁻¹⁰) and CV vs. Fe(II)-chicken (Adjusted P Value=4.05 × 10 ⁻⁴) (Kruskal-Wallis test and Dunn's multiple comparisons test)	Yes	ns, not significant for CV vs. Fe(II) (Adjusted P Value=0.699), Fe(II) vs. Fe(II)-chicken (Adjusted P Value=0.999) and CV vs. Fe(II)-chicken (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test)
K			n=3 experiments/w=3 independent cardiac differentiation were performed; CV, n=35 cells; Fe(II), n=37 cells.		cells	no	ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (Mann Whitney test).	Yes	ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (P Value=0.400) (Mann Whitney test).
L			n=2 experiments/w=2 independent cardiac differentiation		Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (Mann Whitney test);	Yes	ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (P Value=0.667) (Mann Whitney test);
M	CV=78 cells; Lat-A=72 cells; Lat-A+Fe(II)=78 cells; n=3 experiments/w=3 independent cardiac differentiation				cells	no	***, P<0.001 for CV vs. Lat-A (Adjusted P Value=1 × 10 ⁻¹⁵) and Lat-A vs. Lat-A+Fe(II) (Adjusted P Value=1 × 10 ⁻¹⁵); ns, not significant for CV vs. Lat-A+Fe(II) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for CV vs. Lat-A (Adjusted P Value=0.2309), Lat-A vs. Lat-A+Fe(II) (Adjusted P Value=0.760) and CV vs. Lat-A+Fe(II) (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).
N	n=9 experiments/w=9 independent cardiac differentiation; n=36 technical replicates	n=9 experiments/w=9 independent cardiac differentiation; n=36 technical replicates	n=6 experiments/w=6 independent cardiac differentiation; n=28 technical replicates		Technical replicates from independent experiments	no	** P<0.01 for WT1 vs. Pat (Adjusted P Value=0.00596) and WT1 vs. MUT-int1 (Adjusted P Value=0.00538) (Kruskal-Wallis test and Dunn's multiple comparisons test).	Yes	ns, not significant for WT1 vs. Pat (Adjusted P Value=0.572) and *, P<0.05 for WT1 vs. MUT-int1 (Adjusted P Value=0.021) (Kruskal-Wallis test and Dunn's multiple comparisons test)
O		n=6 experiments/w=6 independent cardiac differentiation; n=23 technical replicates.			Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	**, P<0.01 for CV vs. Fe(II) Par iPSC-CMs (P value=0.0015) (Mann Whitney test);	Yes	**, P<0.01 for CV vs. Fe(II) Par iPSC-CMs (P value=0.00216) (Mann Whitney test).
P			n=6 experiments/w=6 independent cardiac differentiation; n=24 technical replicates		Technical replicates from independent experiments	no	***, P<0.001 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P value=1.06 × 10 ⁻⁴) (Mann Whitney test).	Yes	**, P<0.01 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P value=0.00216) (Mann Whitney test).
Q	n=3 experiments/w=3 independent cardiac differentiation				Technical replicates from independent experiments	no	***, P<0.001 for WT1 CV vs. WT1 UNC3230 (P value=7.01 × 10 ⁻⁴) (Mann Whitney test).	Yes	ns, not significant for WT1 CV vs. WT1 UNC3230 (P Value=0.700) (Mann Whitney test).
S	n=3 experiments/w=3 independent cardiac differentiation				Technical replicates from independent experiments	no	***, P<0.001 for WT1 CV vs. WT1 Lat-A (P value<1 × 10 ⁻¹⁵) (Mann Whitney test).	Yes	ns, not significant for WT1 CV vs. WT1 Lat-A (P Value=0.100) (Mann Whitney test).

Fig. S10

Patient (WT/MUT)	MUT-int1 (TPM1-F185L MUT-int/MUT-int)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6?	sensitivity analysis
A	n=5 experiments/n=5 independent cardiac differentiation;each experiment contains 4 technical replicates	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	** , P<0.01 (P value=0.0051) for CV vs. RhoA II Mut-int1 iPSC-CMs (Mann-Whitney test).	Yes	ns, not significant for CV vs. RhoA II Mut-int1 iPSC-CMs (P Value=0.1508) (Mann- Whitney test).
C	CV, n=42 cells; RhoA II, n= 53 cells; n=3 experiments/n=3 independent cardiac differentiation	cells	no	** , P < 0.01 (P value=6.36×10 ⁻³) CV vs. RhoAII Patient (Mann- Whitney test).	Yes	ns, not significant for CV vs. RhoAII Patient (P Value=0.1000) (Mann-Whitney test).
D	CV, n=64 cells; RhoA II, n= 61 cells; n=3 experiments/n=3 independent cardiac differentiation	cells	no	*** , P < 0.001 (P value=6.68×10 ⁻⁵) CV vs. RhoA II MUT-int1 (Mann- Whitney test).	Yes	ns, not significant for CV vs. RhoA II MUT- int1 (P Value=0.200) (Mann-Whitney test).
G	CV, n=88 movies; RhoA II, n=46 movies; n=3 experiments/n=3 independent cardiac differentiation	movies	no	*** , P < 0.001 for CV vs. RhoA II MUT-int1 (P value=1.32 × 10 ⁻⁴) (Mann- Whitney test).	Yes	ns, not significant for CV vs. RhoA II MUT- int1 (P Value=0.400) (Mann-Whitney test).

Fig. S11

	Patient (WT/MUT)	Mut-corrected (WT-cor/WT-cor)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6?	sensitivity analysis
C	n=2 experiments/n=2 independent cardiac differentiation	n=2 experiments/n=2 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for Patient vs. Mut-cor (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor (P Value=0.800) (Mann Whitney test).
D	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for Patient vs. Mut-cor (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor (P Value>0.999) (Mann Whitney test).
E	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test)	ns, not significant for Patient vs. Mut-cor (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor (P Value>0.999) (Mann Whitney test).
F	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	ns, not significant for Patient vs. Mut-cor iPSC-CMs (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.200) (Mann Whitney test).
G	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	*, P < 0.05 for Patient vs. Mut-cor iPSC-CMs (P value=0.0400) (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.400) (Mann Whitney test).
H	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P < 0.001 for Patient vs. Mut-cor iPSC-CMs (P value=4.11 × 10 ⁻⁵) (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.100) (Mann Whitney test).
I	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	ns, not significant for Patient vs. Mut-cor iPSC-CMs (Mann Whitney test).	Yes	ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.700) (Mann Whitney test).
M	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).	Yes	ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value>0.999) (Mann Whitney test).
N	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).	Yes	ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value=0.400) (Mann Whitney test).
O	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Data from independent experiments	no	ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).	Yes	ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value>0.999) (Mann Whitney test).
P	n=3 experiments/n=3 independent cardiac differentiation	n=3 experiments/n=3 independent cardiac differentiation	Data from independent experiments	Yes (pass Shapiro-Wilk test)	ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).	Yes	ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value=0.700) (Mann Whitney test).
Q		n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	*, P<0.05 for Mut-cor CV vs. Mut-cor UNC3230 (P value=7.01 × 10 ⁻⁴) (Mann Whitney test).	Yes	ns, not significant for Mut-cor CV vs. Mut-cor UNC3230 (P Value=0.800) (Mann Whitney test).
S		n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P<0.001 for Mut-cor CV vs. Mut-cor Lat-A (P value=1.62 × 10 ⁻²) (Mann Whitney test).	Yes	ns, not significant for Mut-cor CV vs. Mut-cor Lat-A (P Value=0.400) (Mann Whitney test).

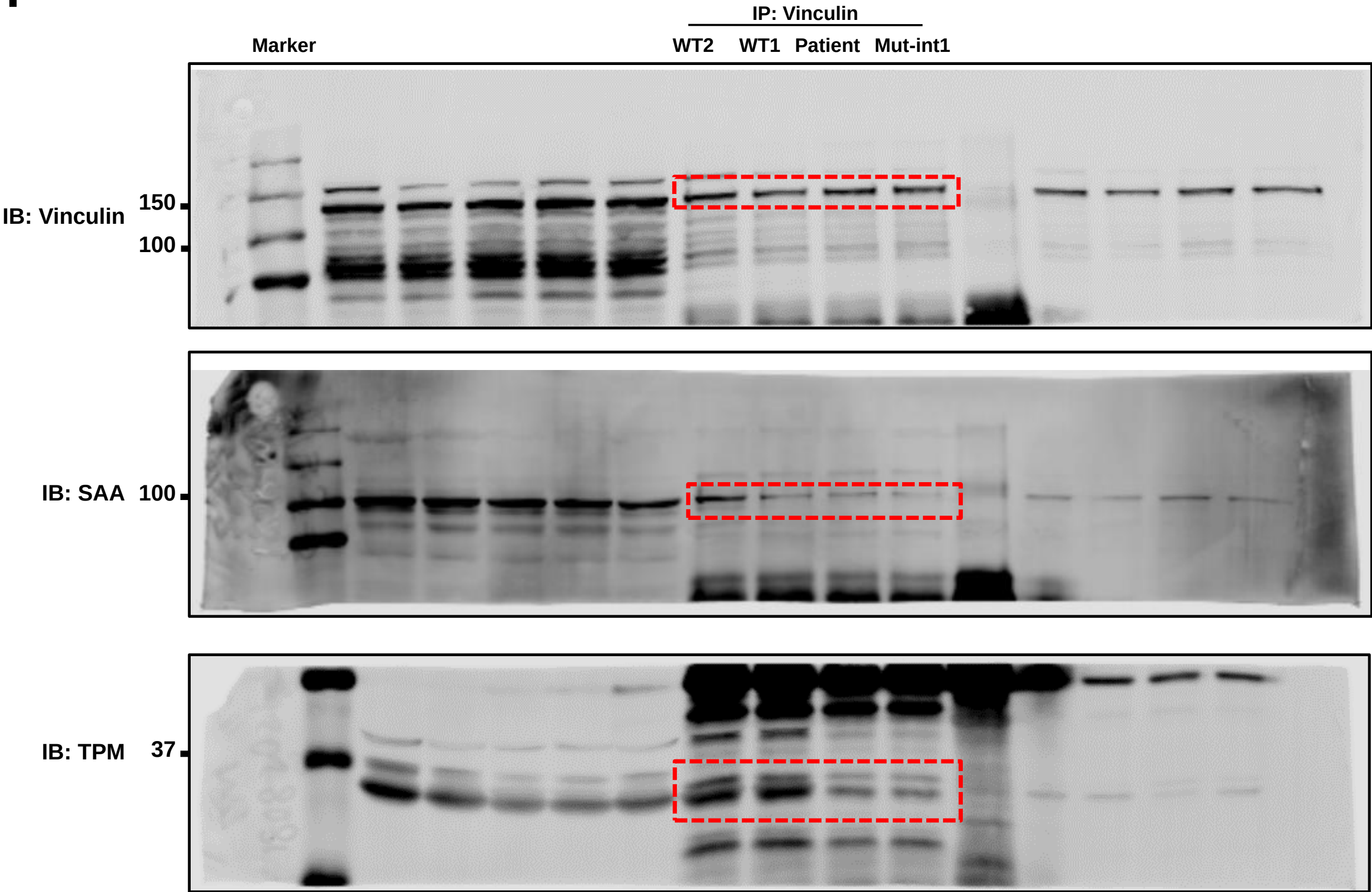
Fig. S12

	WT1 (WT/WT)	Mut-corrected (WT-cor/WT-cor)	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n56?	sensitivity analysis
B		n=1 experiment/n=1 independent cardiac differentiation; CV, n=27 cells; TFR Ab 20 µg/mL, n=17 cells; TFR Ab 30 µg/mL, n=20 cells.	cells				
C	n=3 experiments/n=3 independent cardiac differentiation		Technical replicates from independent experiments	no	***, P<0.001 for WT1 CV vs. WT1 TFR Ab (P value=1.28×10 ⁻⁴) (Mann Whitney test).	Yes	ns, not significant for WT1 CV vs. WT1 TFR Ab (P Value=0.400) (Mann Whitney test).
E		n=3 experiments/n=3 independent cardiac differentiation	Technical replicates from independent experiments	no	***, P<0.001 for Mut-cor CV vs. Mut-cor TFR Ab (P value=1.72×10 ⁻⁸) (Mann Whitney test).	Yes	ns, not significant for Mut-cor CV vs. Mut-cor TFR Ab (P Value=0.400) (Mann Whitney test).
G		CV, n=2 experiments, n=29 movies; TFR Ab 20 µg/mL, n=1 experiment, n=15 movies; TFR Ab 20 µg/mL + RhoA II, n=1 experiment, n=11 movies; TFR Ab 30 µg/mL + RhoA II, n=1 experiment, n=14 movies.	movies	no			

Fig. S14						
	Healthy control	DCM	what data points were used in analyses	Passed normality test (alpha=0.05)?	Statistics method	n≤6? sensitivity analysis
G and H	n=3 independent experiments;n=5 individuals	n=3 independent experiments;n=8 individuals	Technical replicates from independent experiments	no	*, P<0.05 for donor (healthy control) vs. DCM (P Value=0.0473) (Mann Whitney test).	Yes **, P<0.01 for donor (healthy control) vs. DCM (P Value=0.0031) (Mann Whitney test).

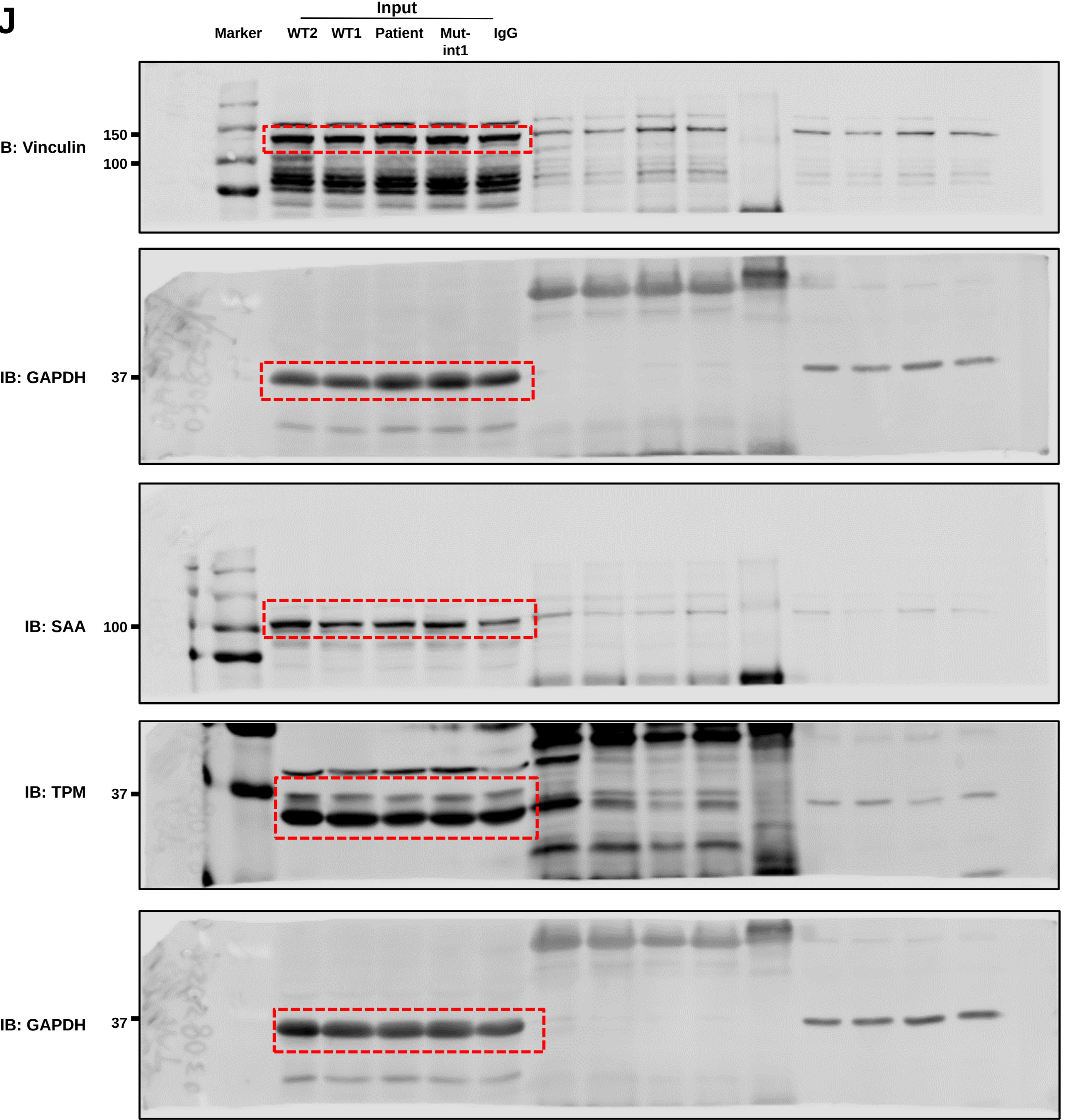
Full unedited membrane scan for Fig. 2

F



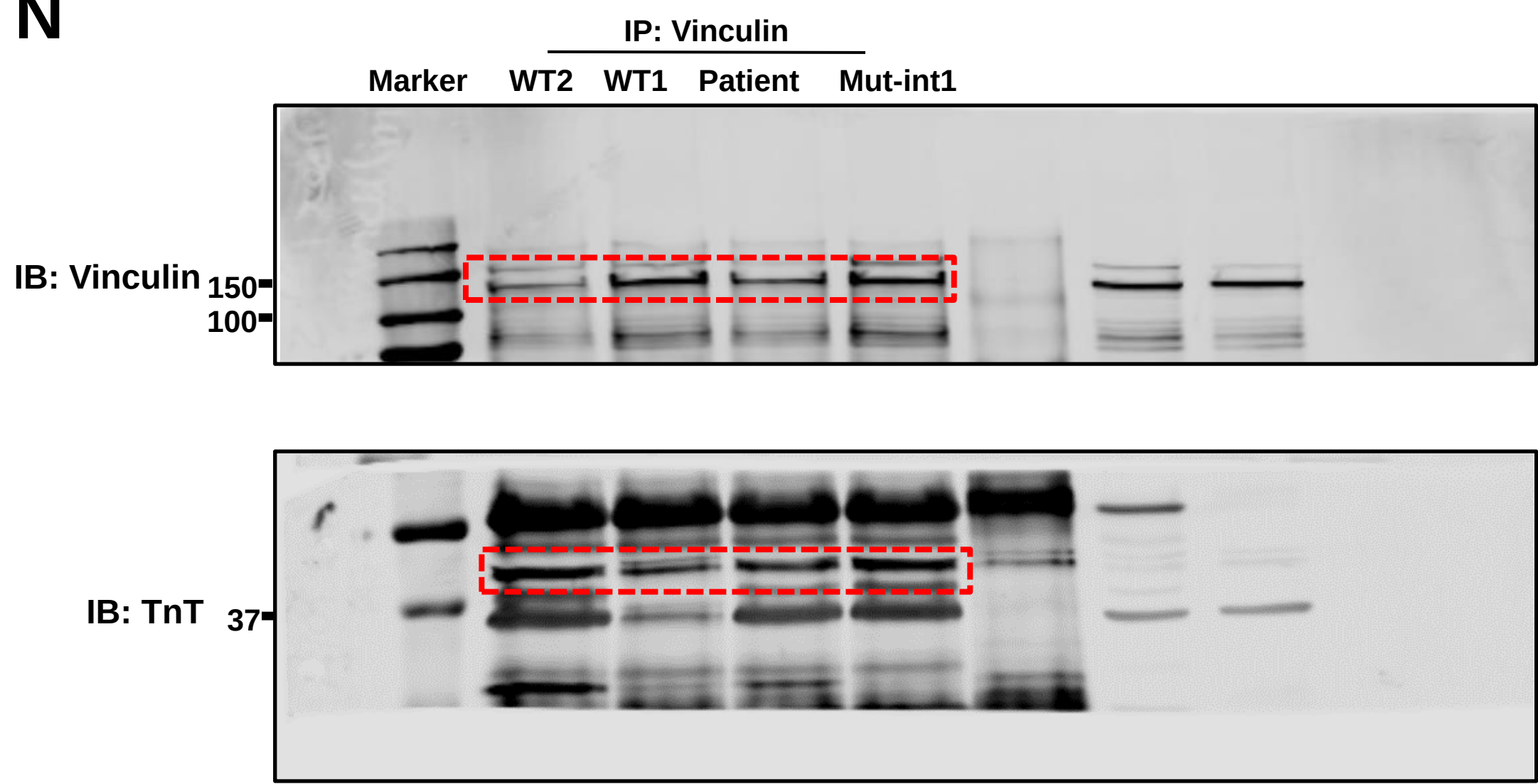
Full unedited membrane scan for Fig. 2

J



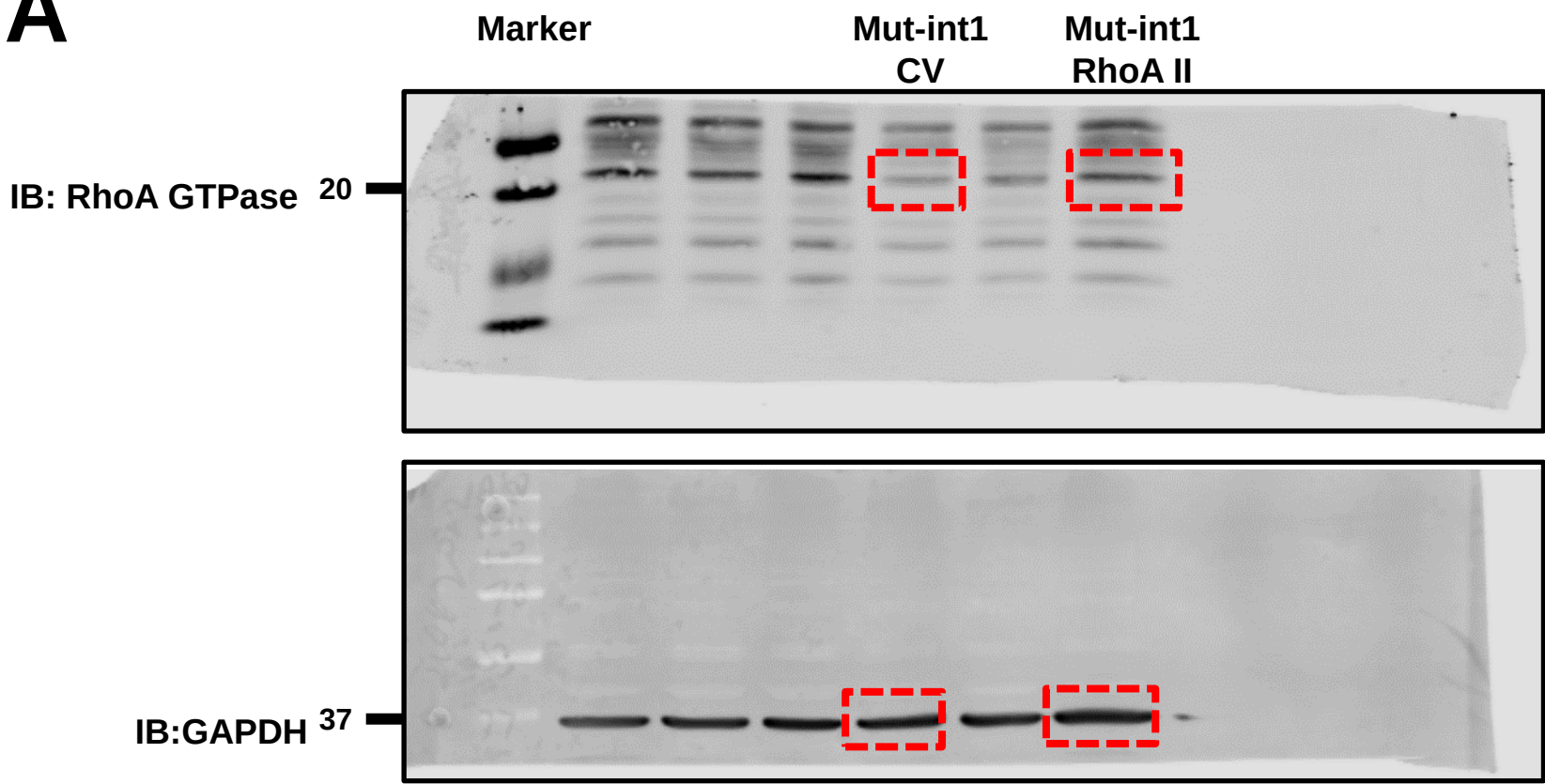
Full unedited membrane scan for Fig. 2

N

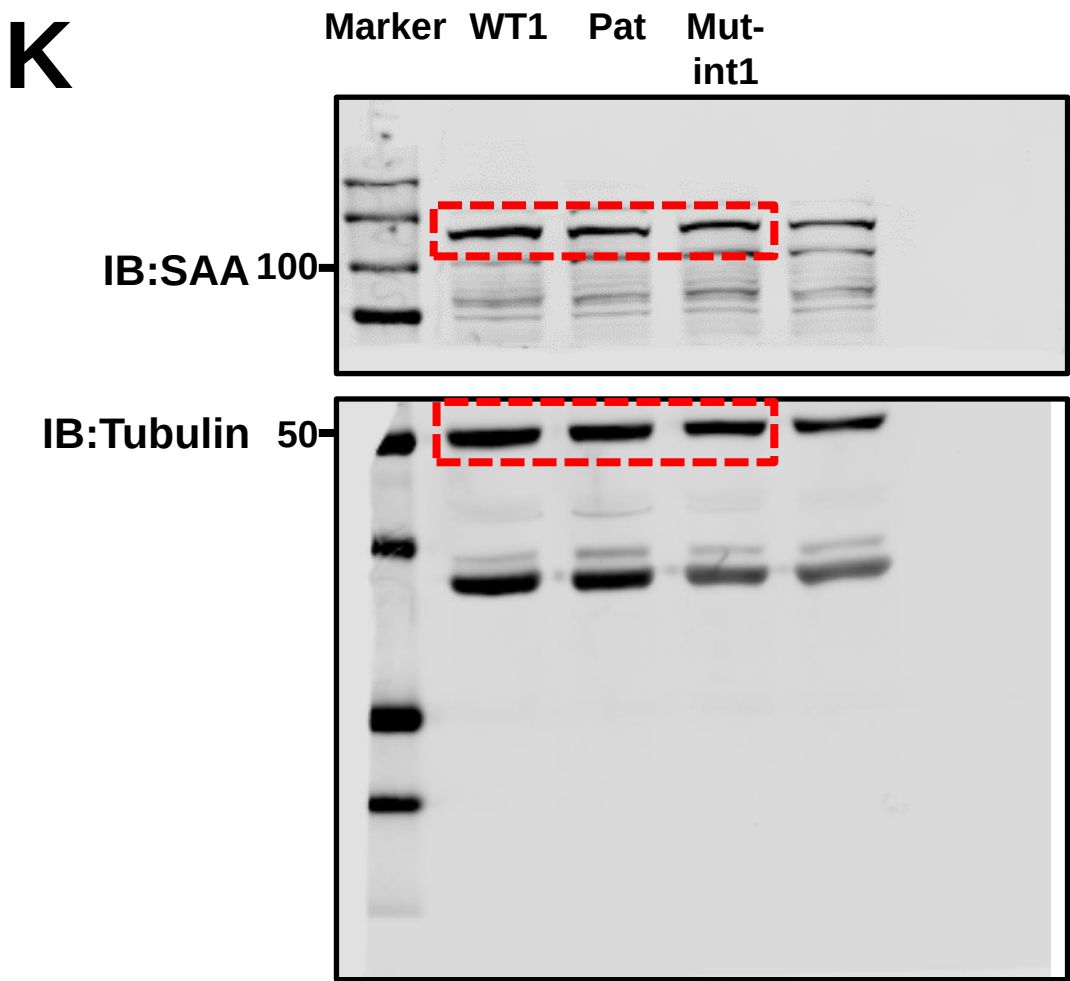
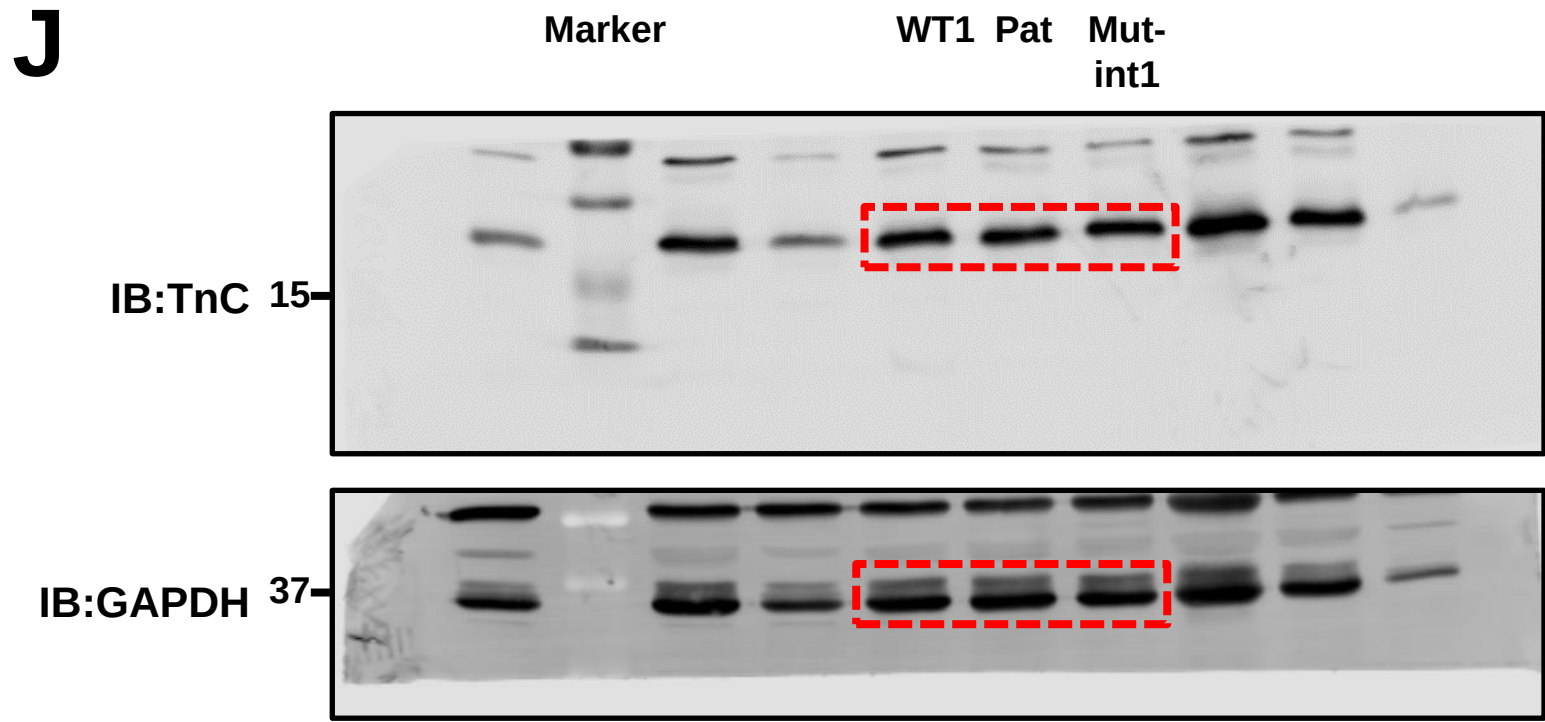
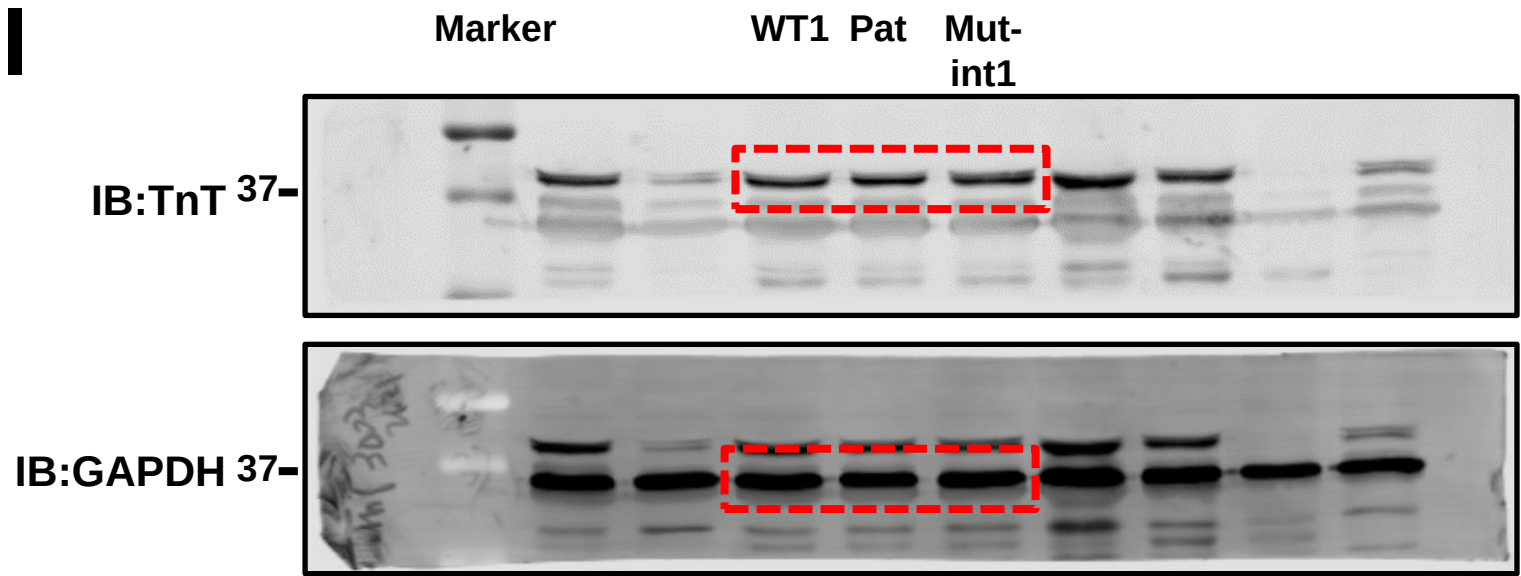
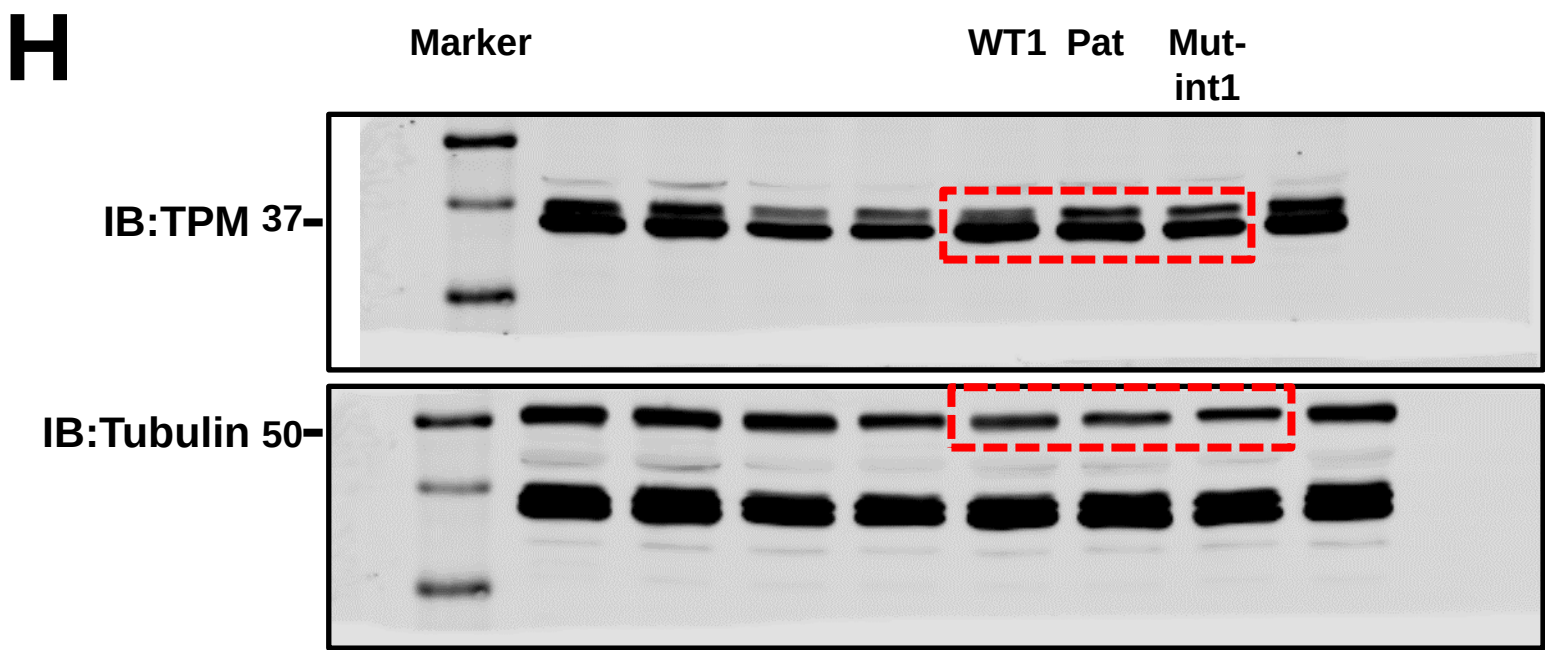


Full unedited membrane scan for Fig. 5

A

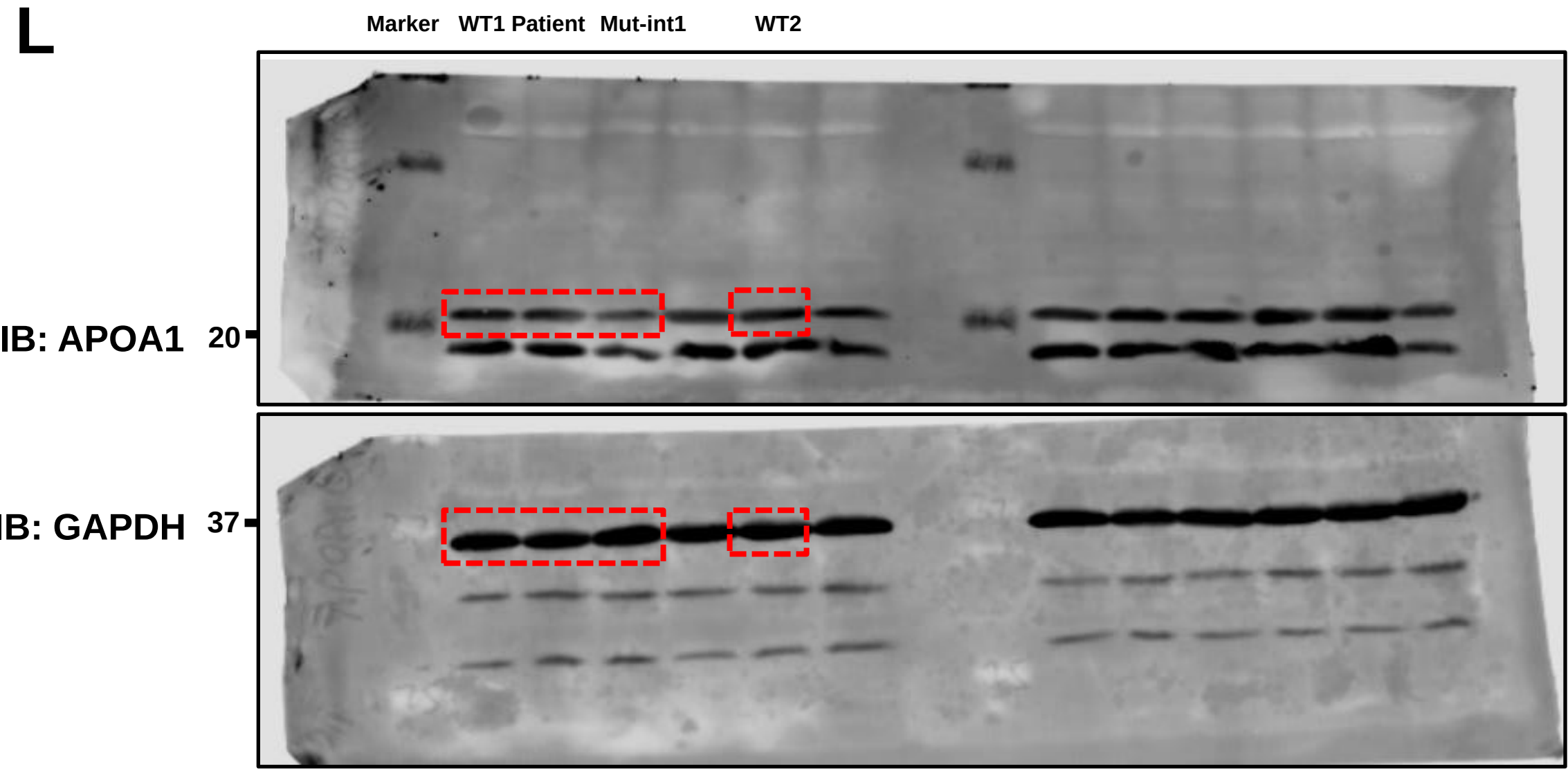


Full unedited membrane scan for Fig. S3



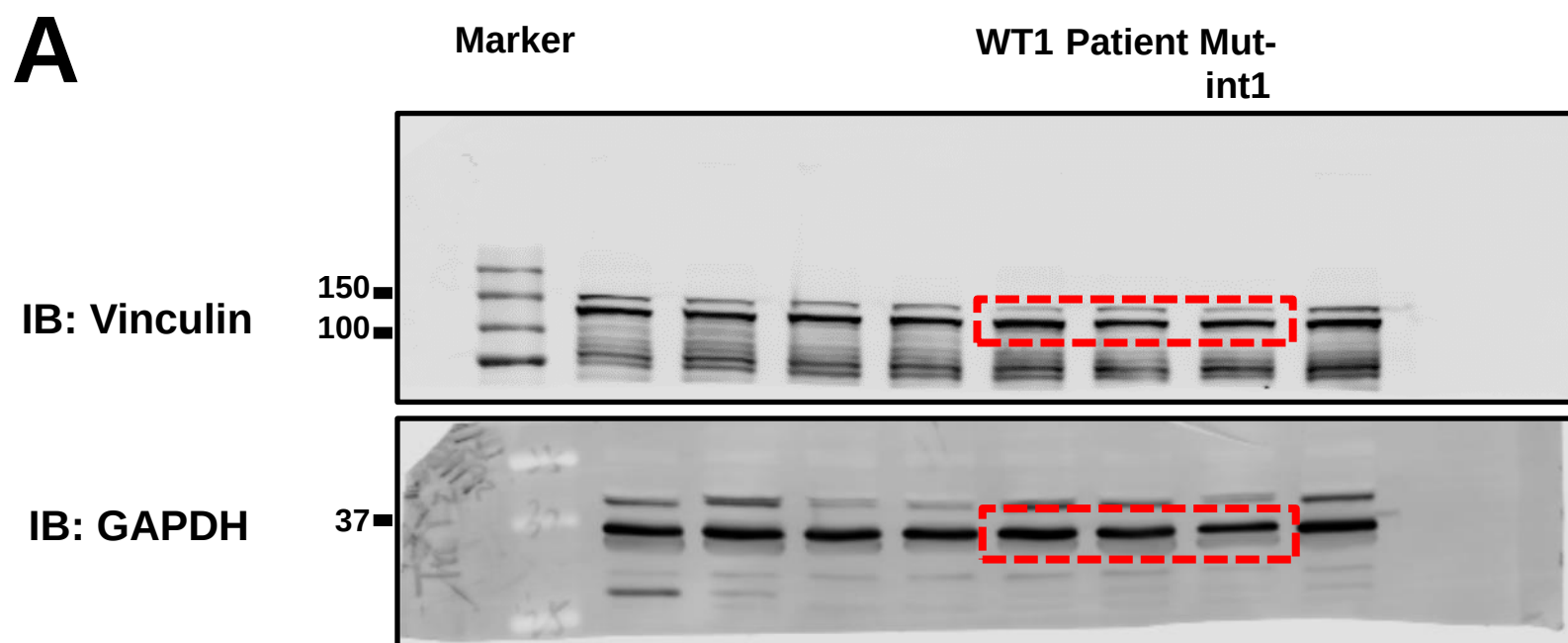
Full unedited membrane scan for Fig. S3

L

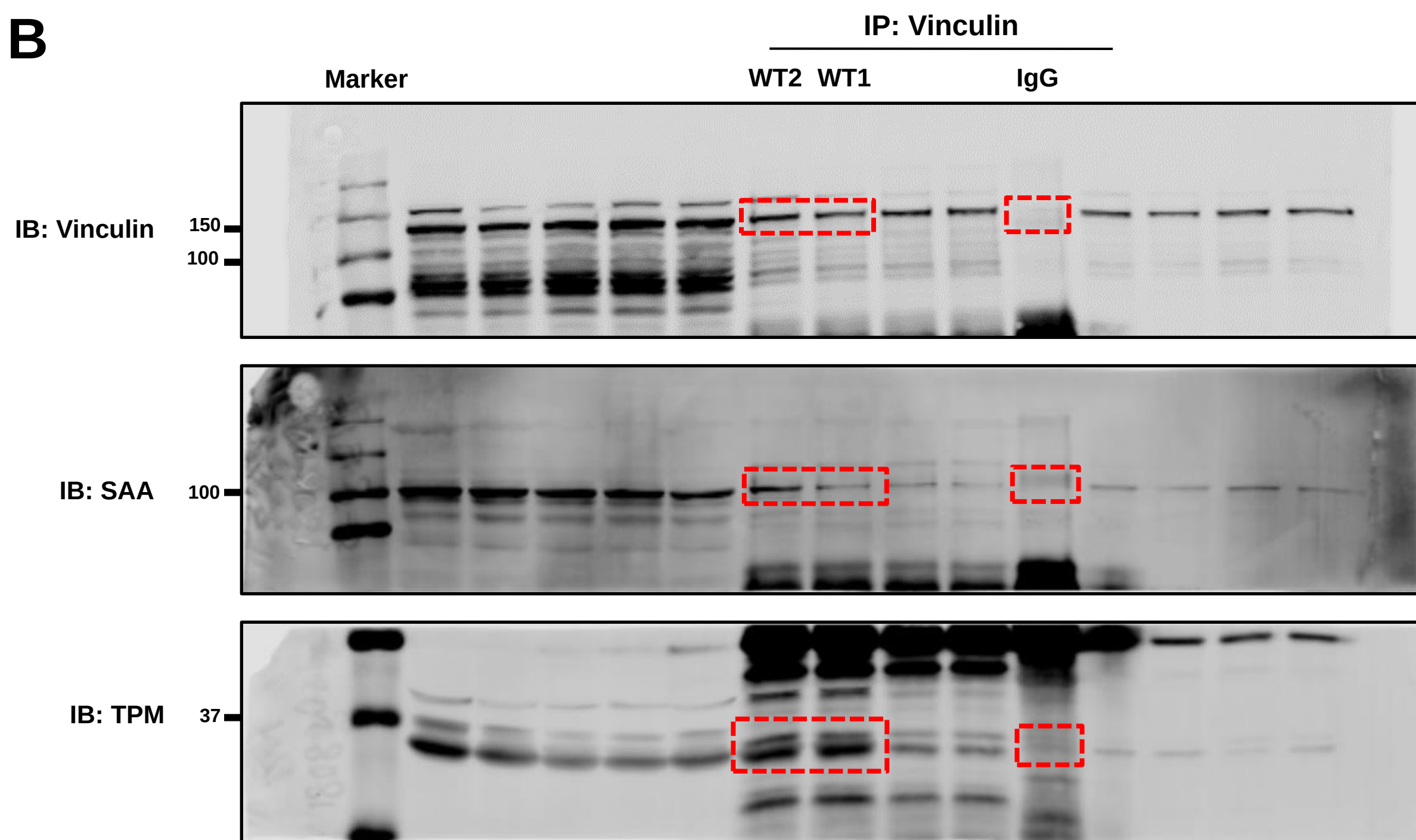


Full unedited membrane scan for Fig. S5

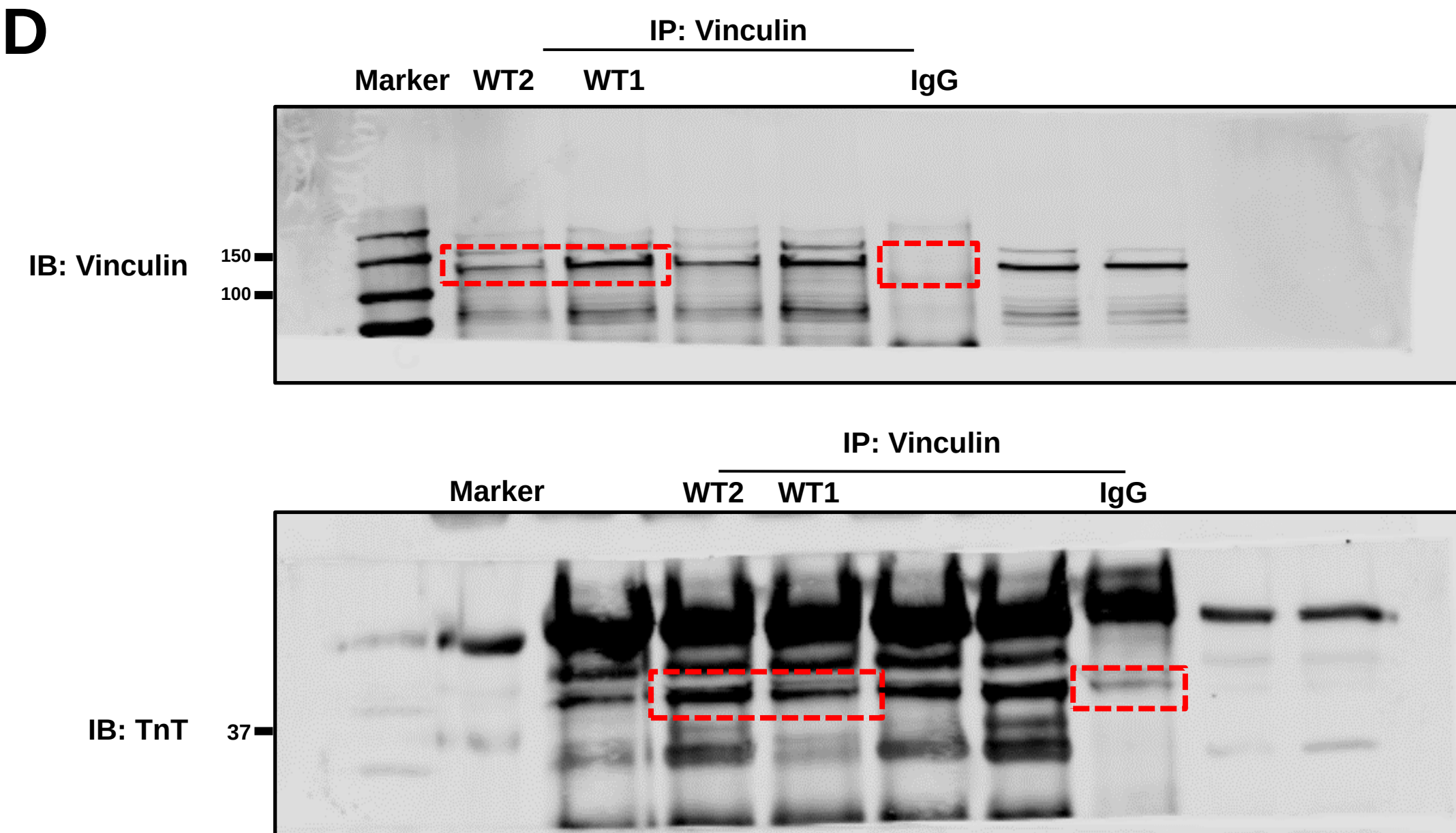
A



B

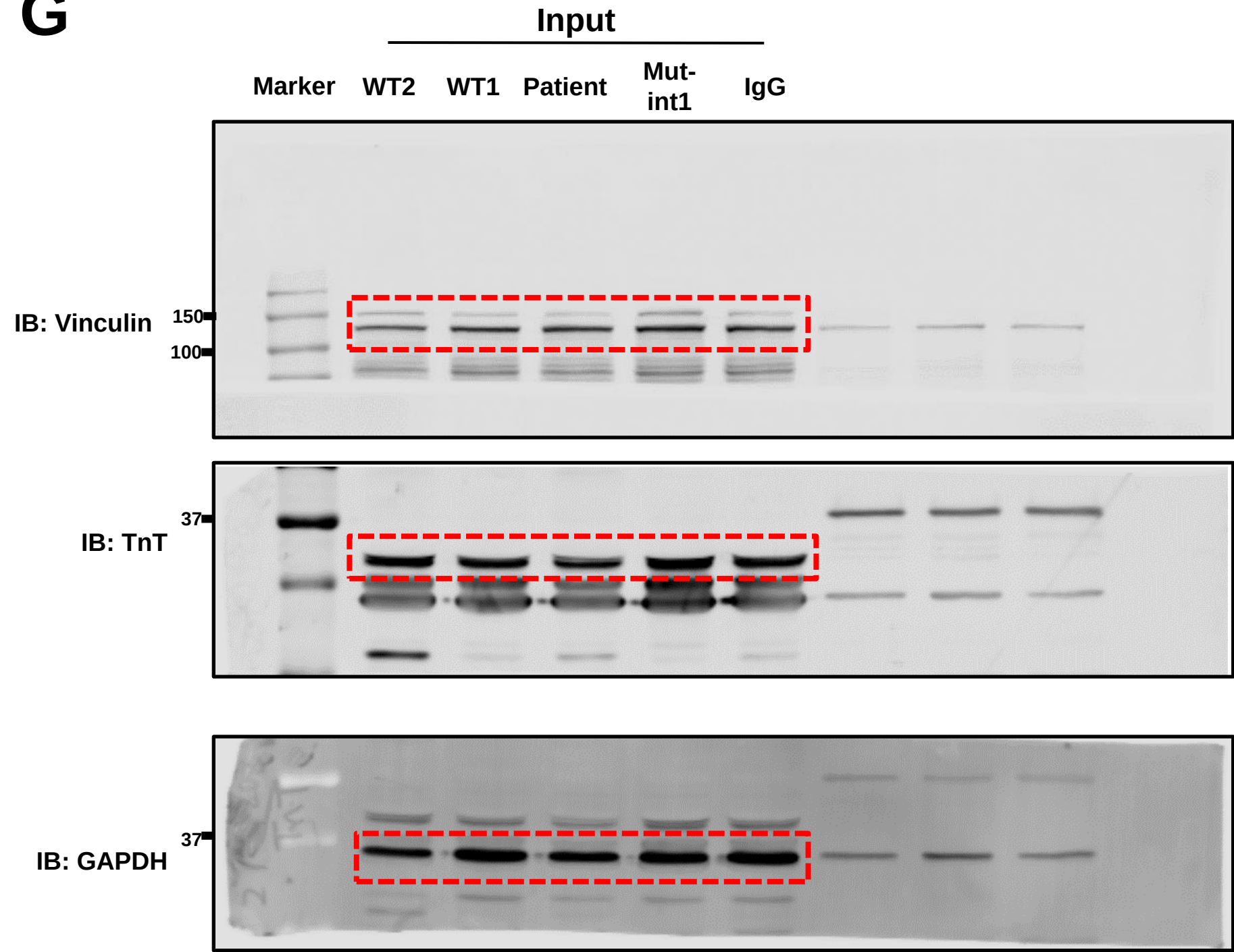


D

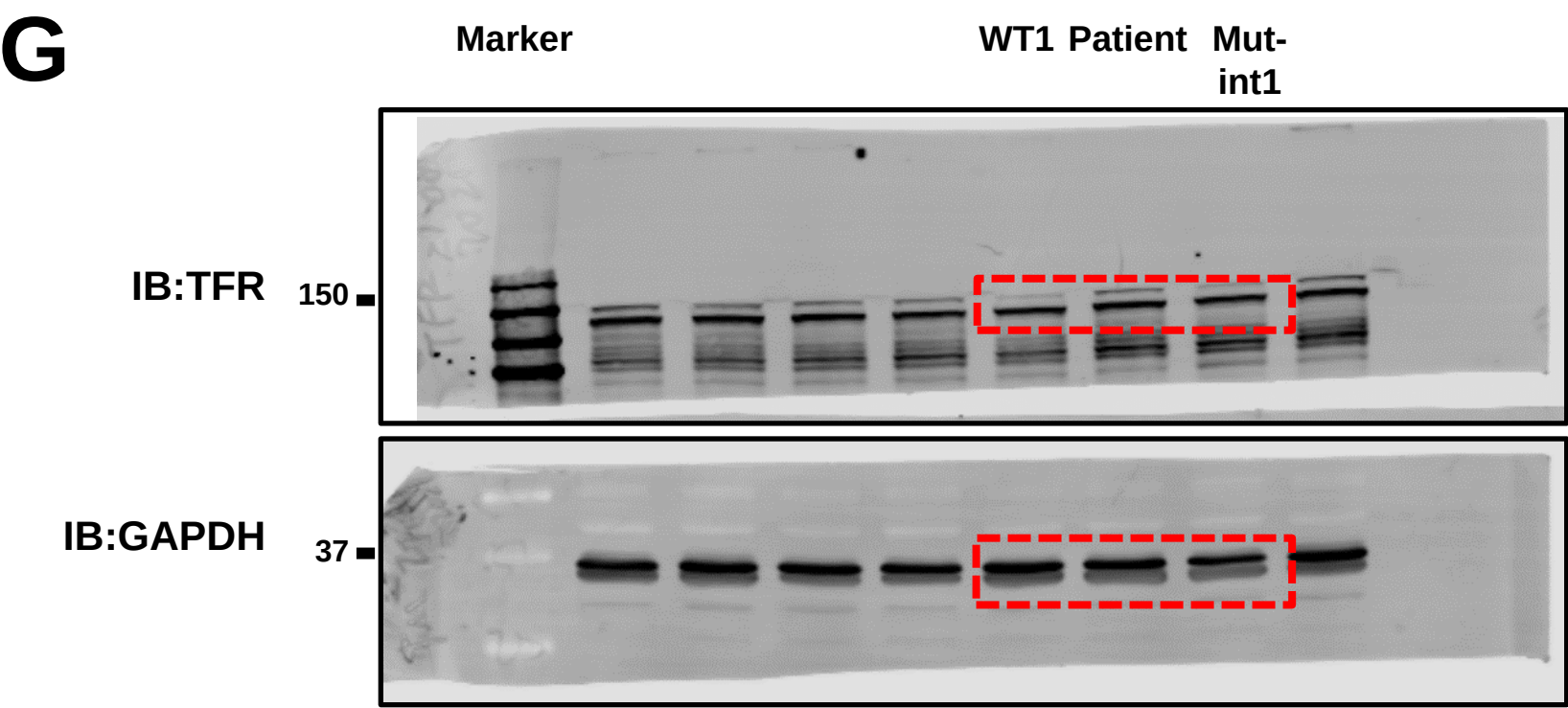
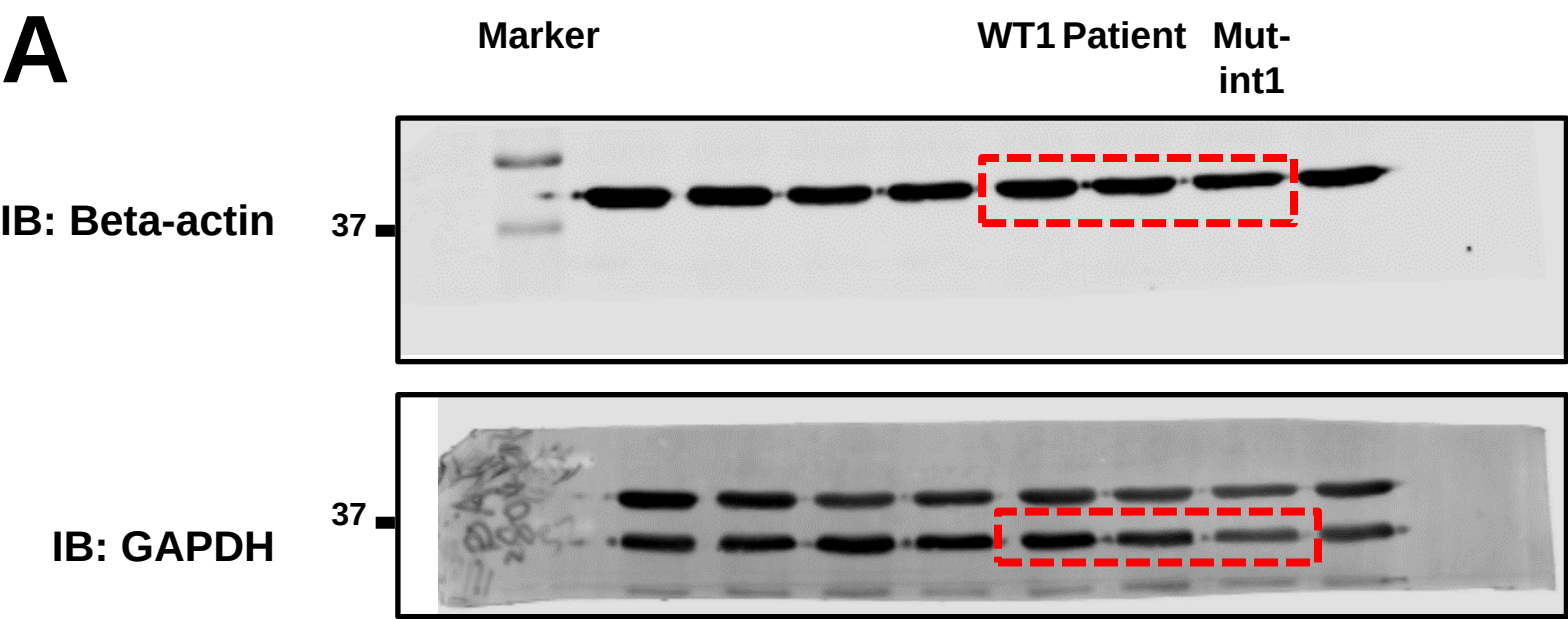


Full unedited membrane scan for Fig. S5

G

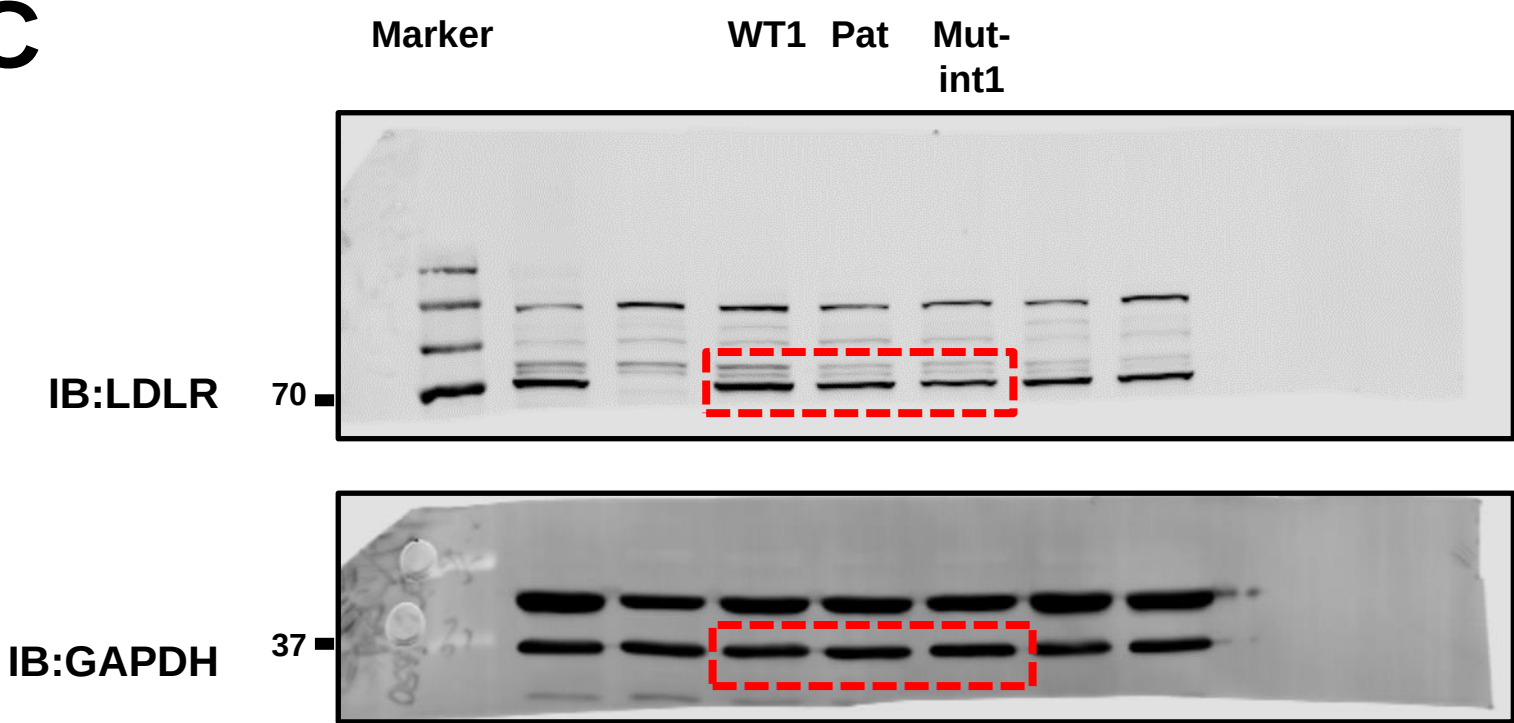


Full unedited membrane scan for Fig. S6

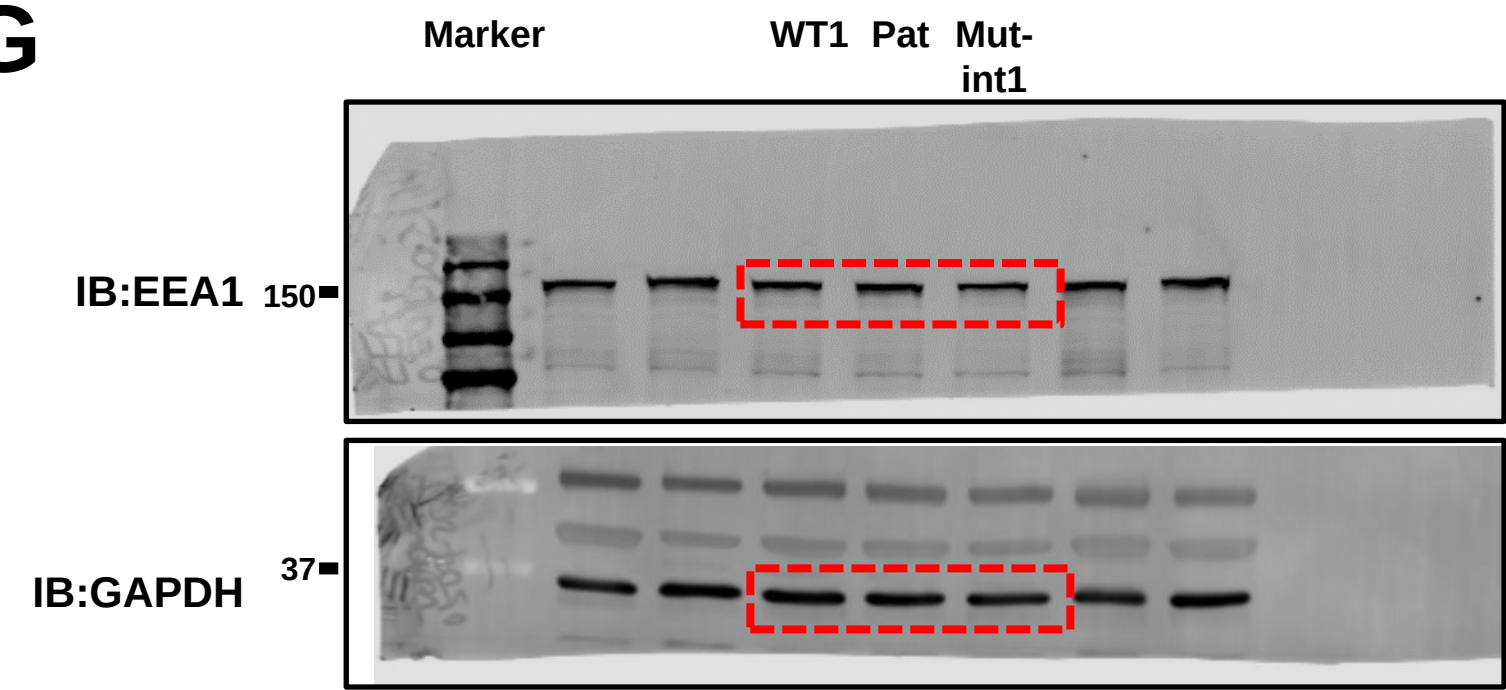


Full unedited membrane scan for Fig. S7

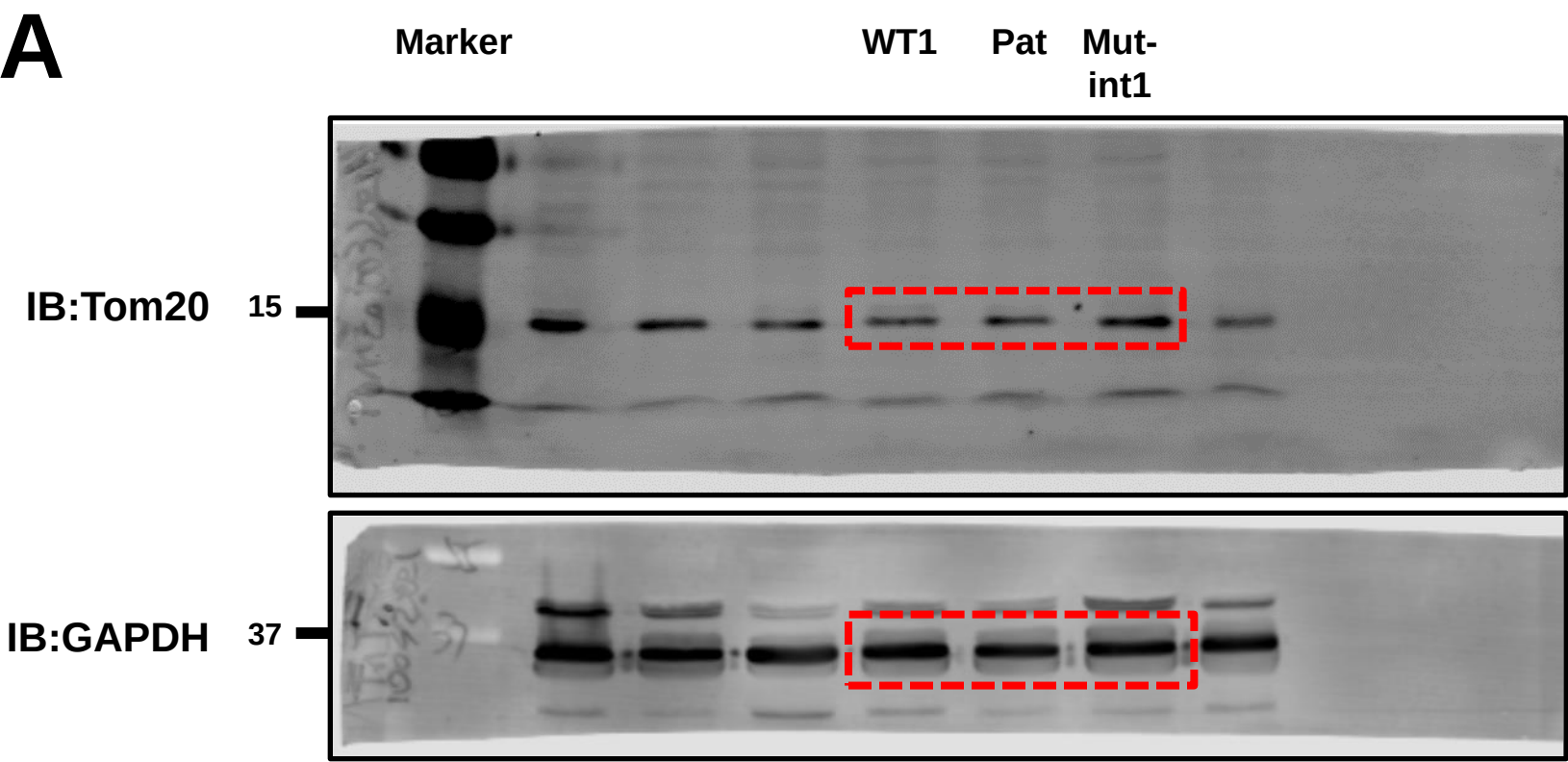
C



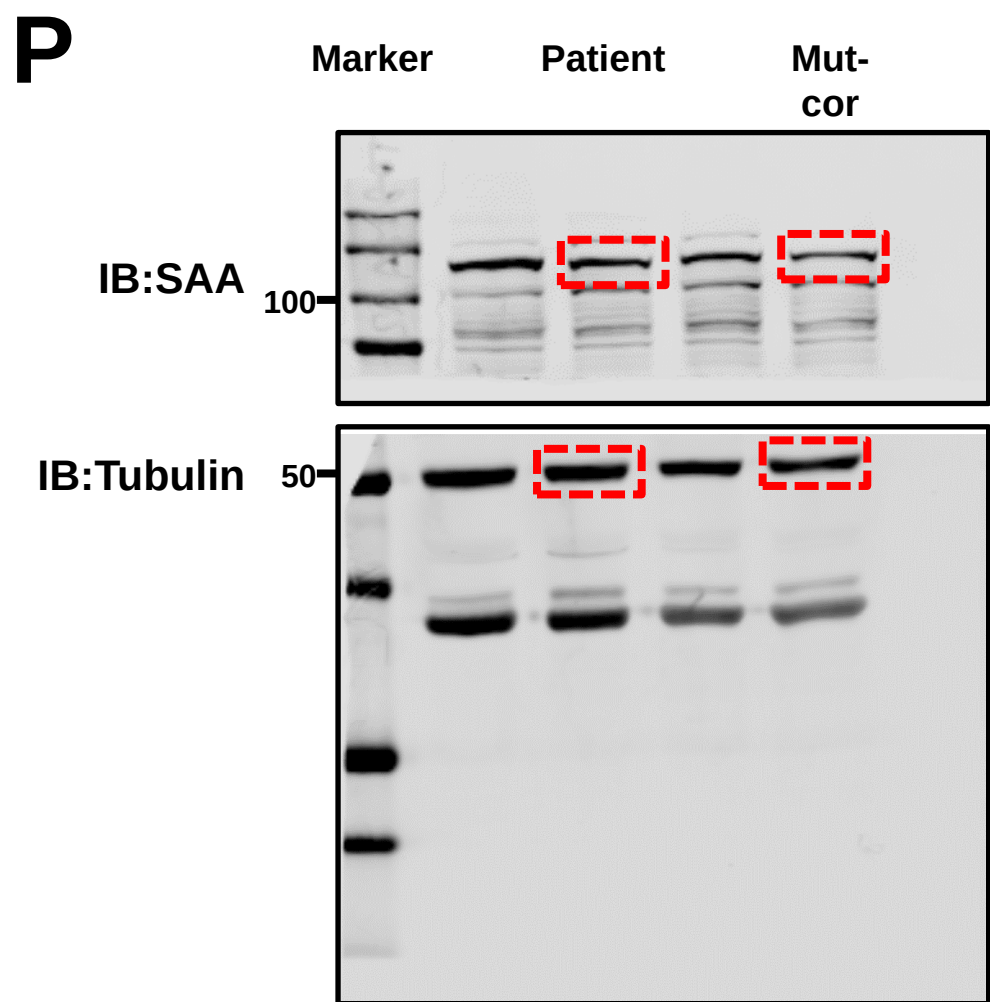
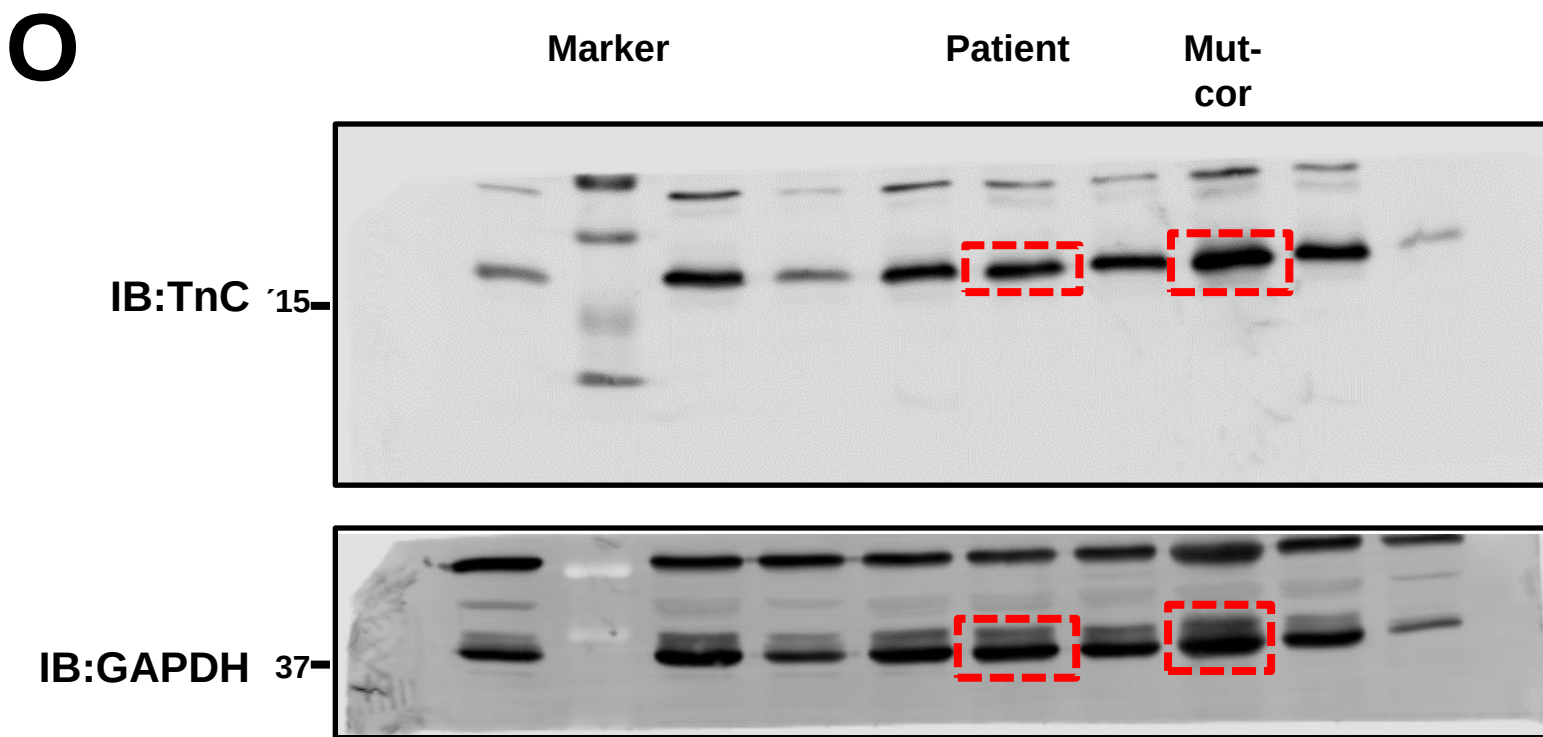
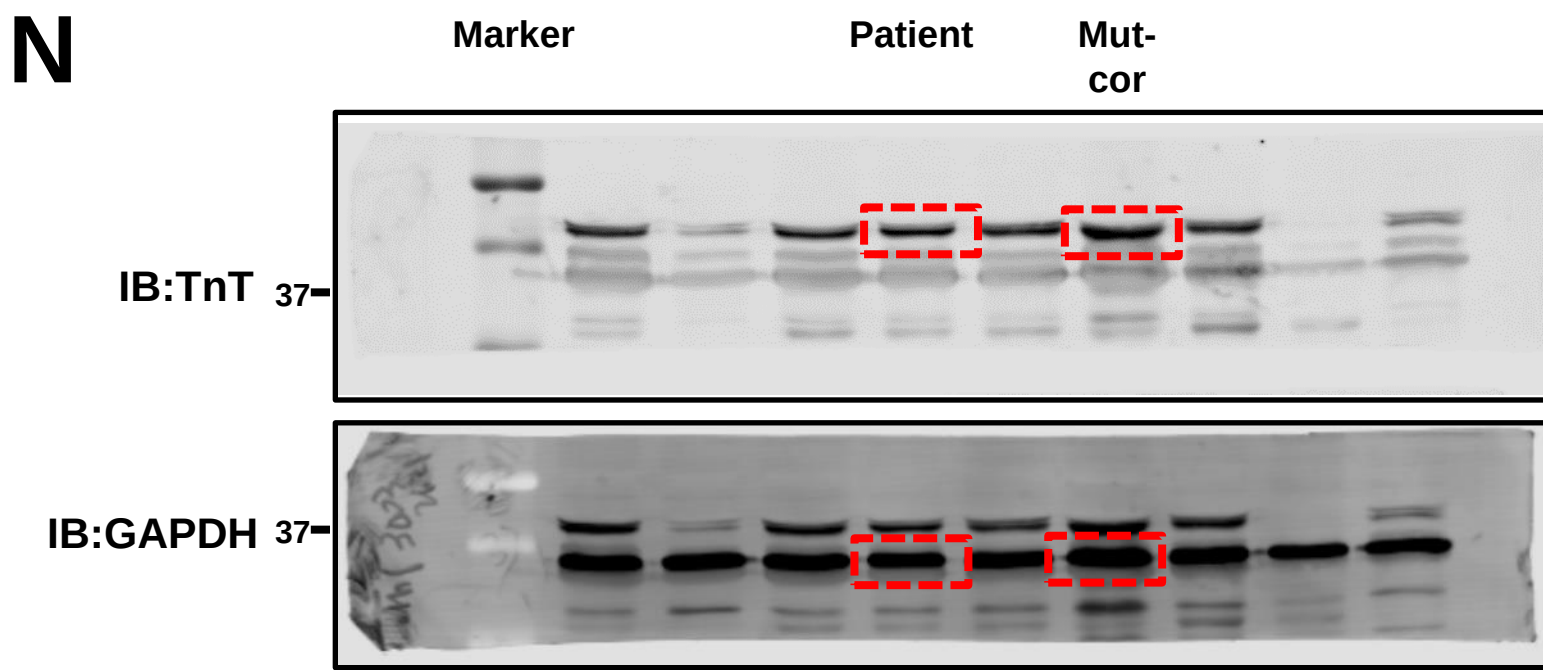
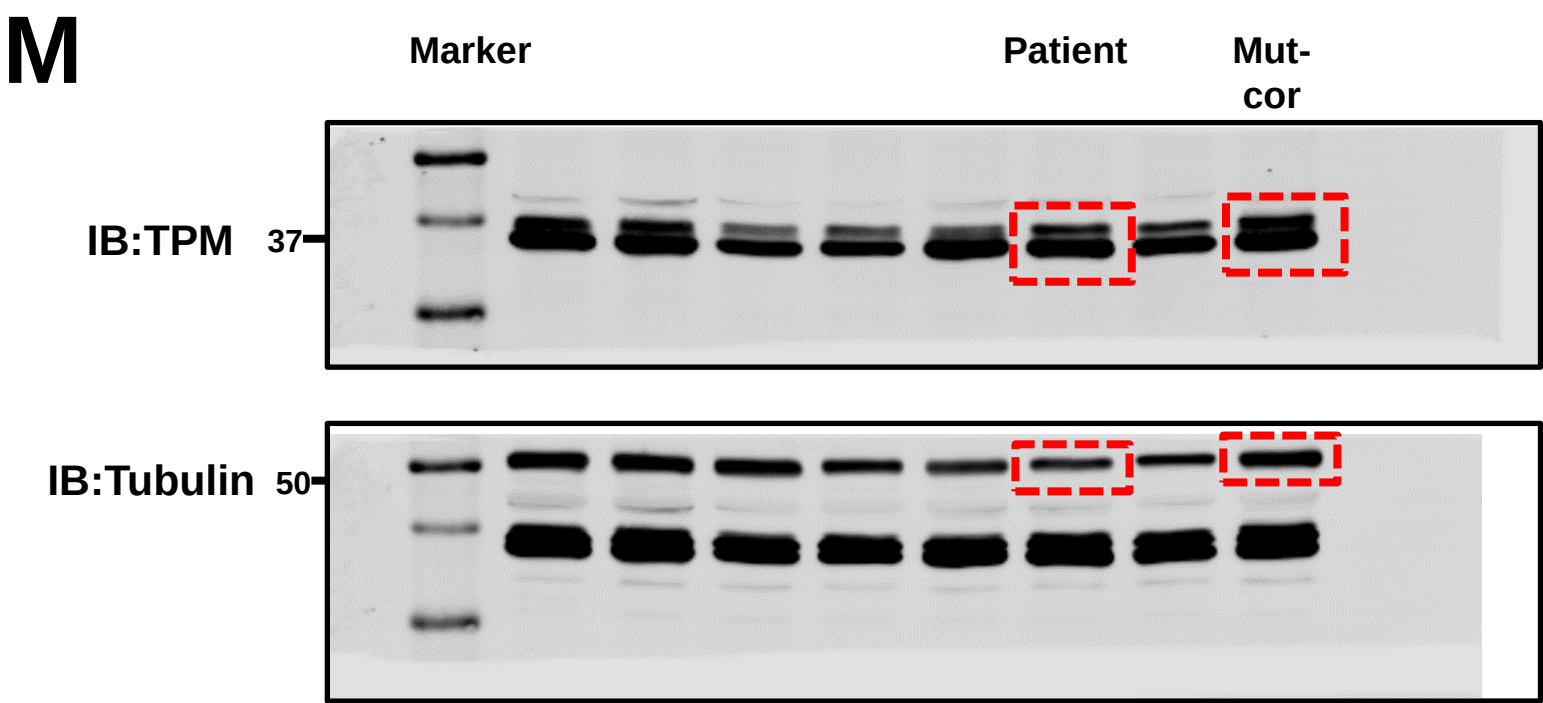
G



Full unedited membrane scan for Fig. S9



Full unedited membrane scan for Fig. S11



F

