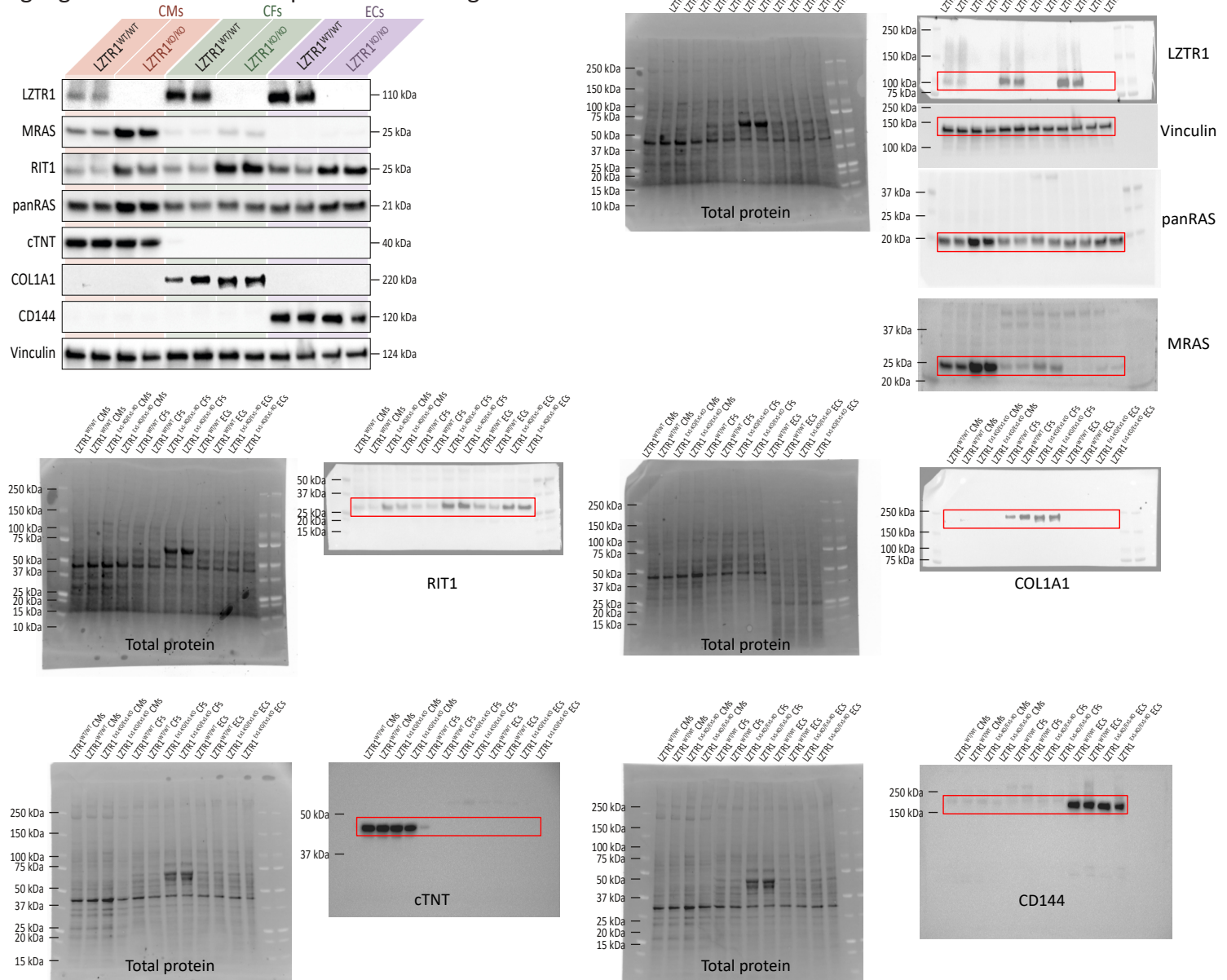
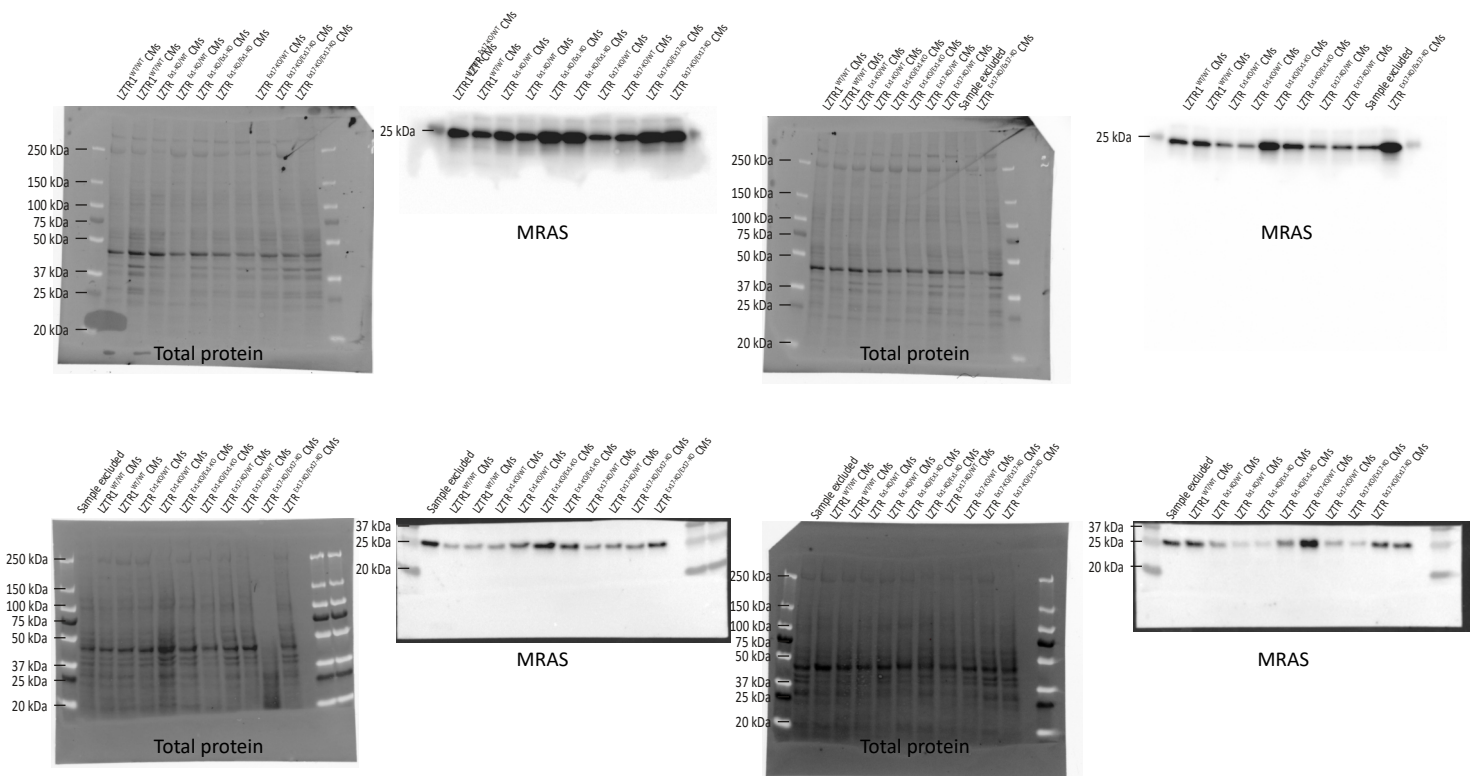


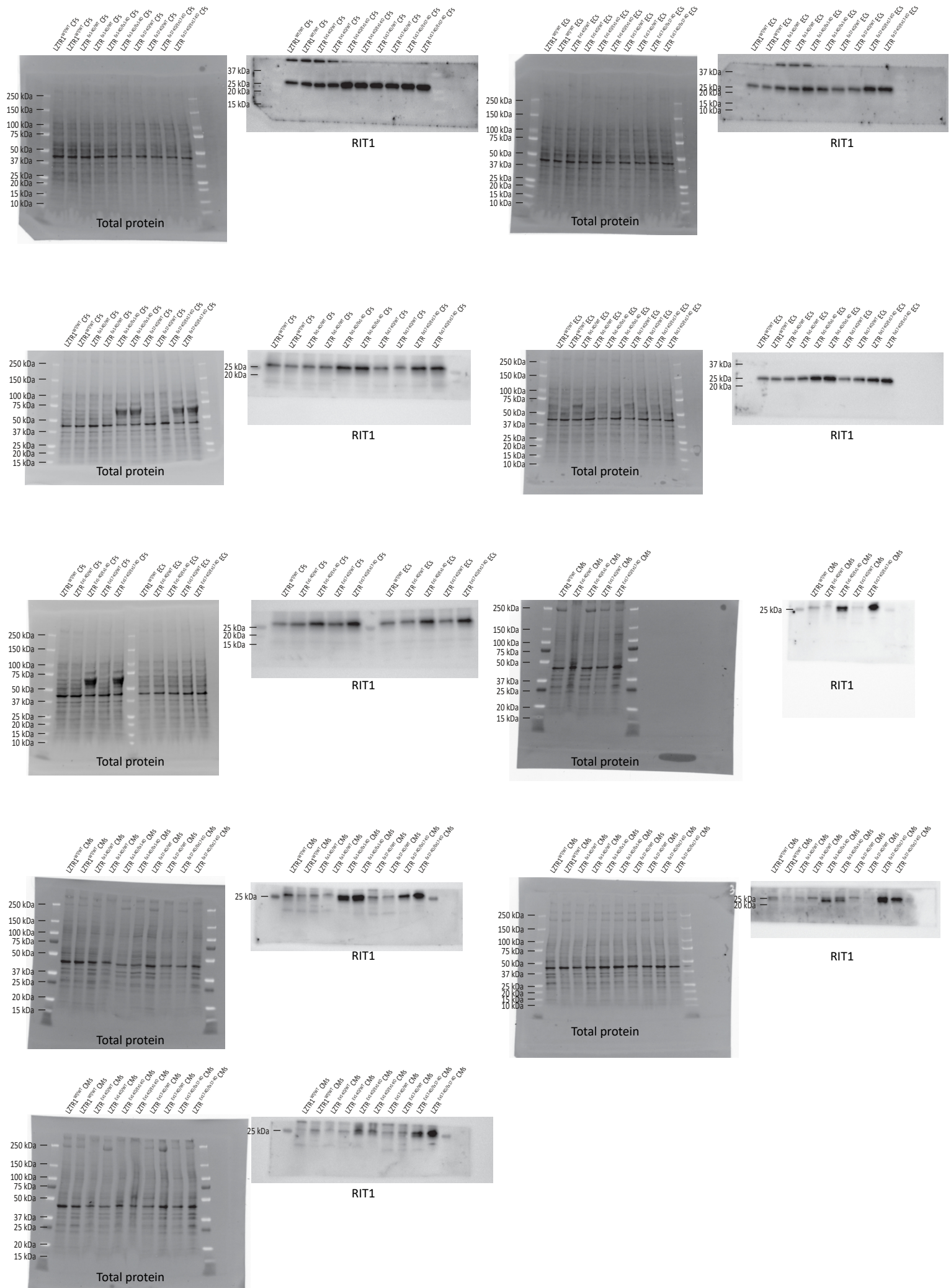
Highlighted blots used as representative images



Blots used for quantification

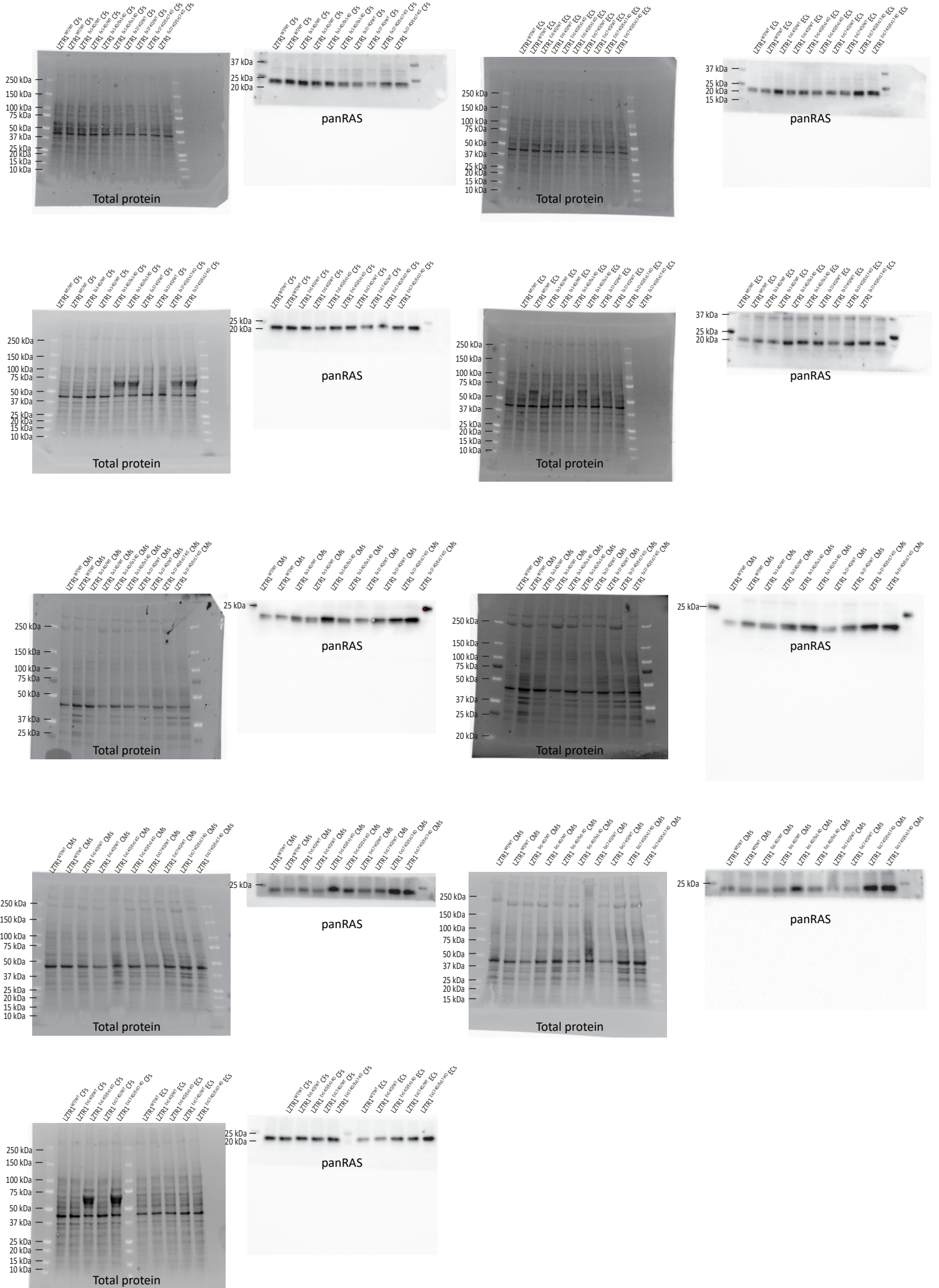


Blots used for quantification



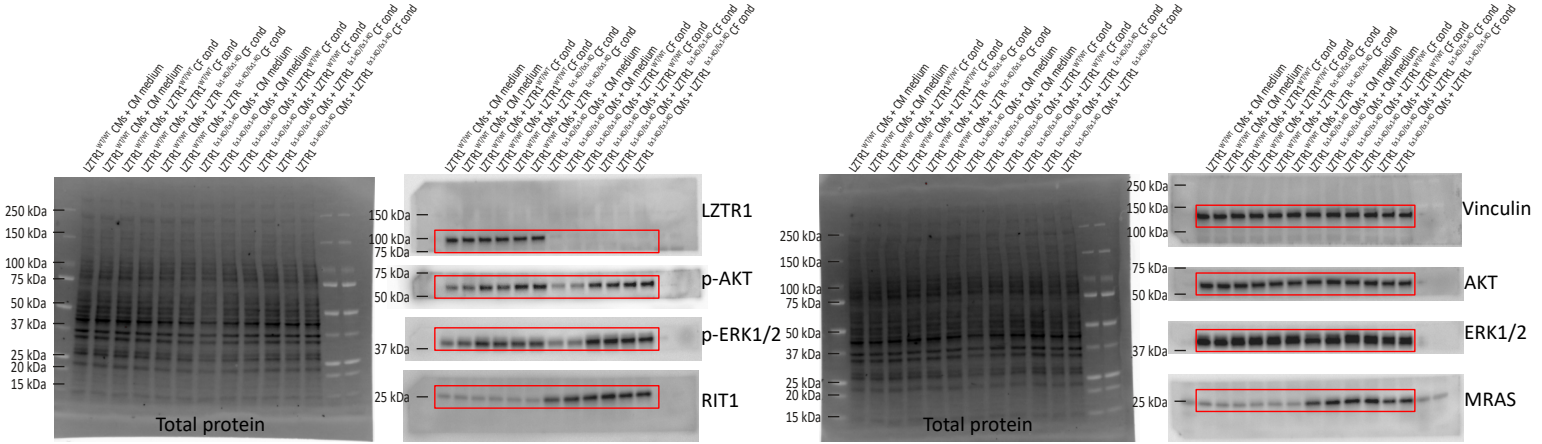
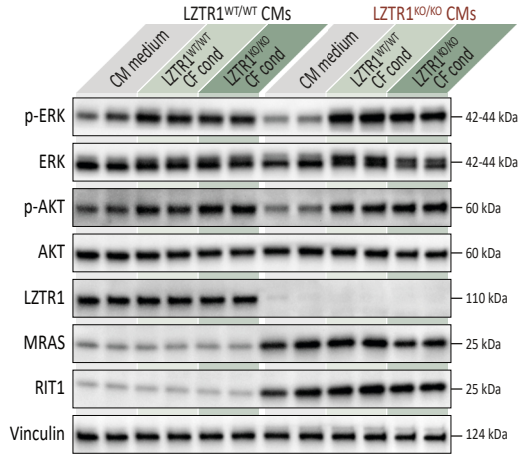
Full unedited gels for Figure 1H and Supplementary Figure 3

Blots used for quantification



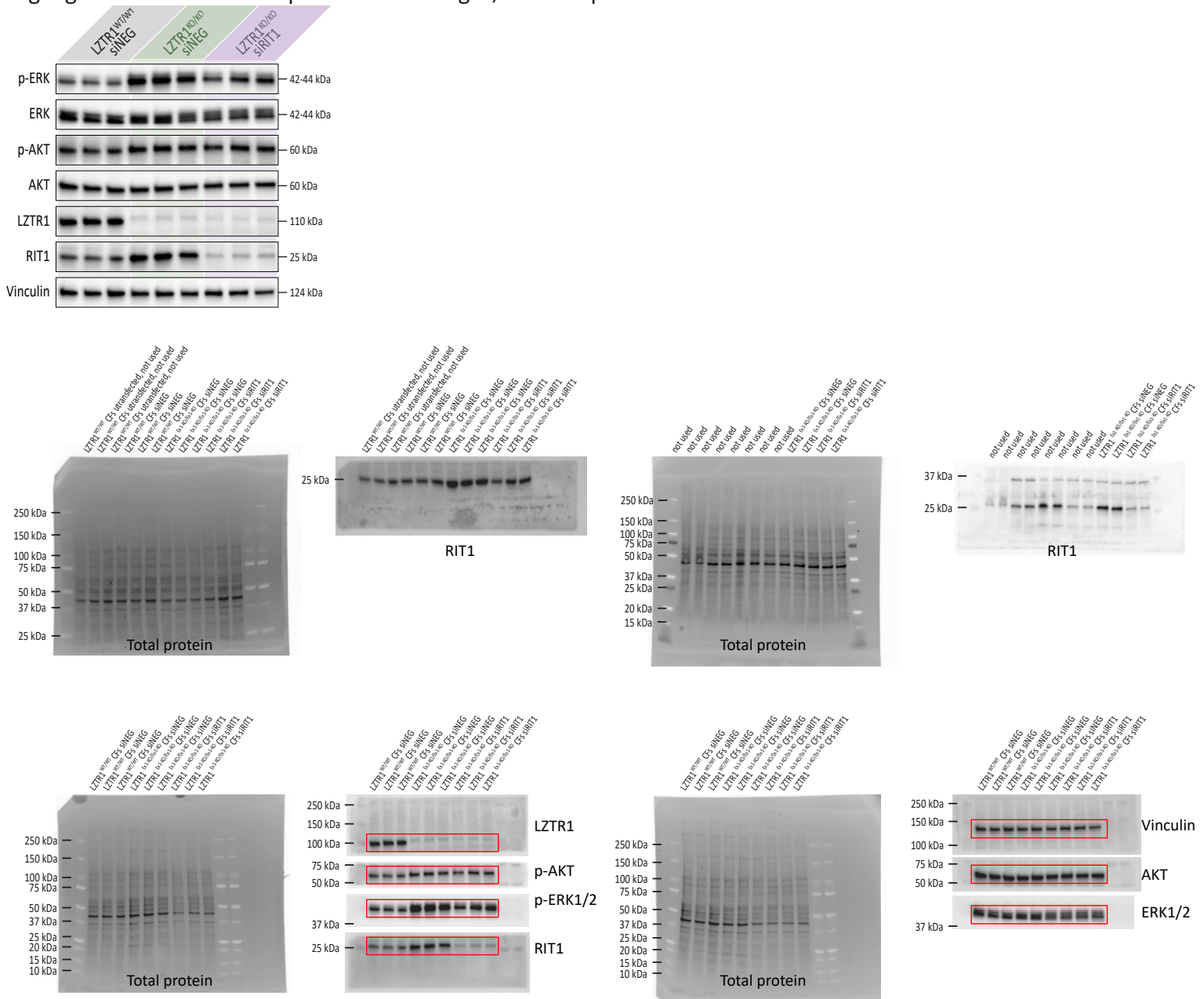
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Highlighted blots used as representative images



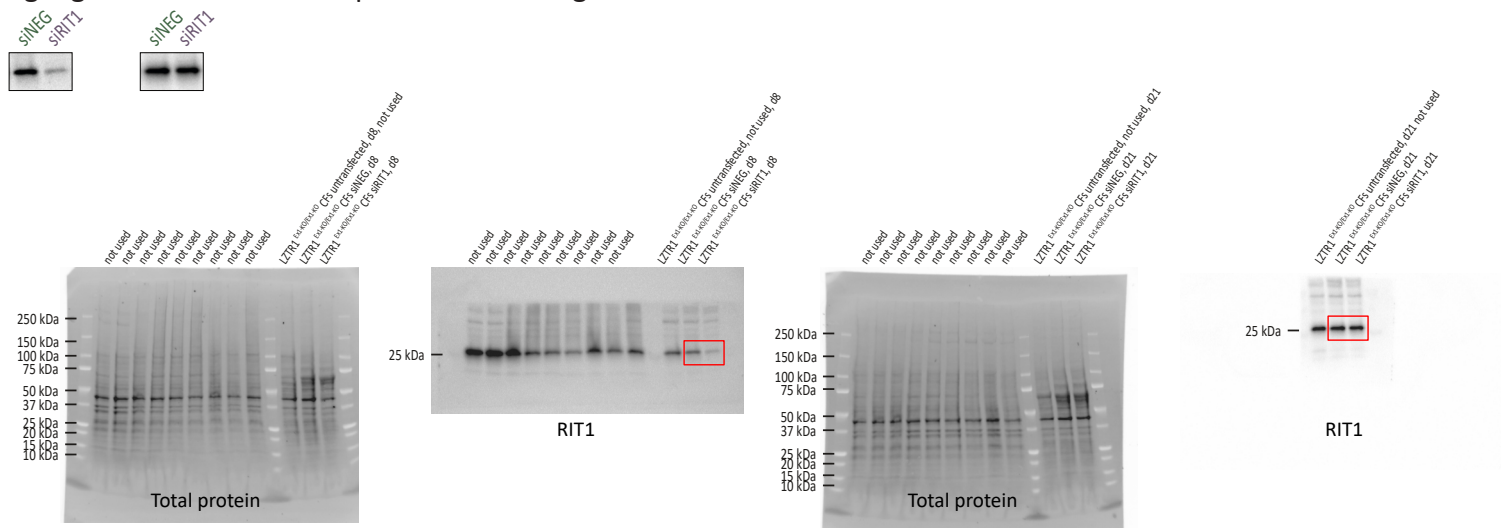
Highlighted blots used as representative images, rest for quantification

Highlighted blots used as representative images, rest for quantification



Full unedited gels for Figure 3L

Highlighted blots used as representative images



Highlighted blots used as representative images

A

LZTR1^{WT/WT} CMs LZTR1^{KO/KO} CMs

CM medium LZTR1^{WT/WT} CF cond LZTR1^{KO/KO} CF cond CM medium LZTR1^{WT/WT} CF cond LZTR1^{KO/KO} CF cond

RAS-MAPK

p-ERK1/2 42-44 kDa

ERK1/2 42-44 kDa

p-MEK1/2 45 kDa

MEK1/2 45 kDa

PI3K-AKT

p-AKT 60 kDa

AKT 60 kDa

p-S6K 70 kDa

S6K 70 kDa

p-S6 32 kDa

S6 32 kDa

p-4E-BP1 20 kDa

4E-BP1 20 kDa

JNK

p-JNK 46-54 kDa

JNK 46-54 kDa

p38

p-p38 40 kDa

p38 40 kDa

ERK5

p-ERK5 115 kDa

ERK5 115 kDa

LZTR1 110 kDa

MRAS 25 kDa

RIT1 25 kDa

Vinculin 124 kDa

250 kDa 150 kDa 100 kDa 75 kDa 50 kDa 37 kDa 25 kDa 20 kDa 15 kDa

Total protein

100 kDa 75 kDa 50 kDa 20 kDa 15 kDa

p-S6K

p-ERK1/2

p-4E-BP1

250 kDa 150 kDa 100 kDa 75 kDa 50 kDa 37 kDa 25 kDa 20 kDa 15 kDa

Total protein

100 kDa 75 kDa 50 kDa 37 kDa 20 kDa

S6K

ERK1/2

4E-BP1

50 kDa 37 kDa 20 kDa

p-MEK1/2

250 kDa 150 kDa 100 kDa 75 kDa 50 kDa 37 kDa 25 kDa 20 kDa 15 kDa

Total protein

75 kDa 50 kDa

p-AKT

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Total protein

50 kDa 37 kDa

MEK1/2

250 kDa 150 kDa 100 kDa 75 kDa 50 kDa 37 kDa 25 kDa 20 kDa 15 kDa

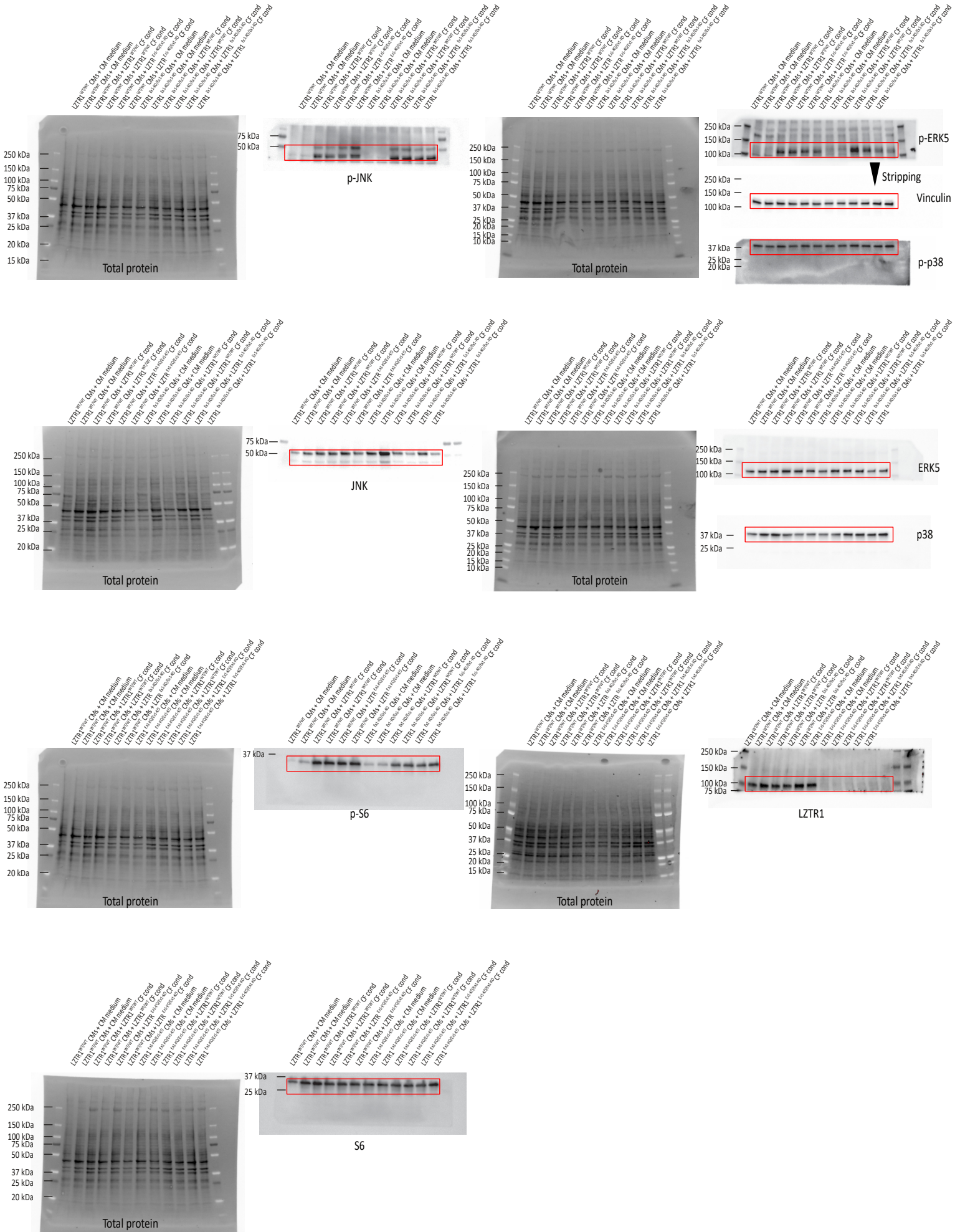
Total protein

75 kDa 50 kDa

AKT

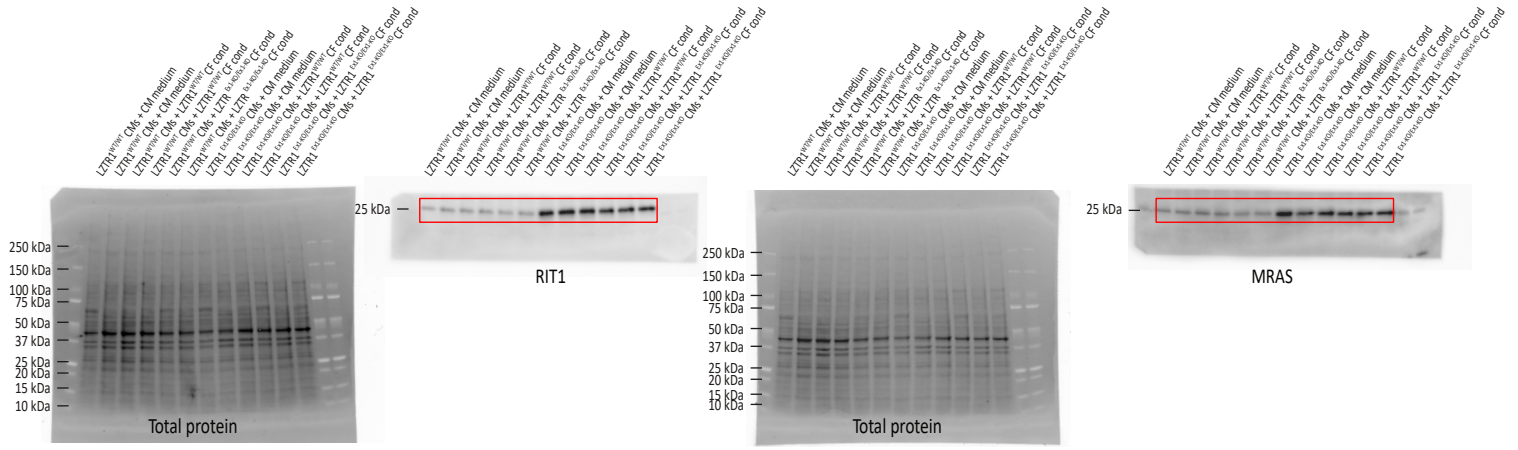
Full unedited gels for Supplementary Figure 5A

Highlighted blots used as representative images



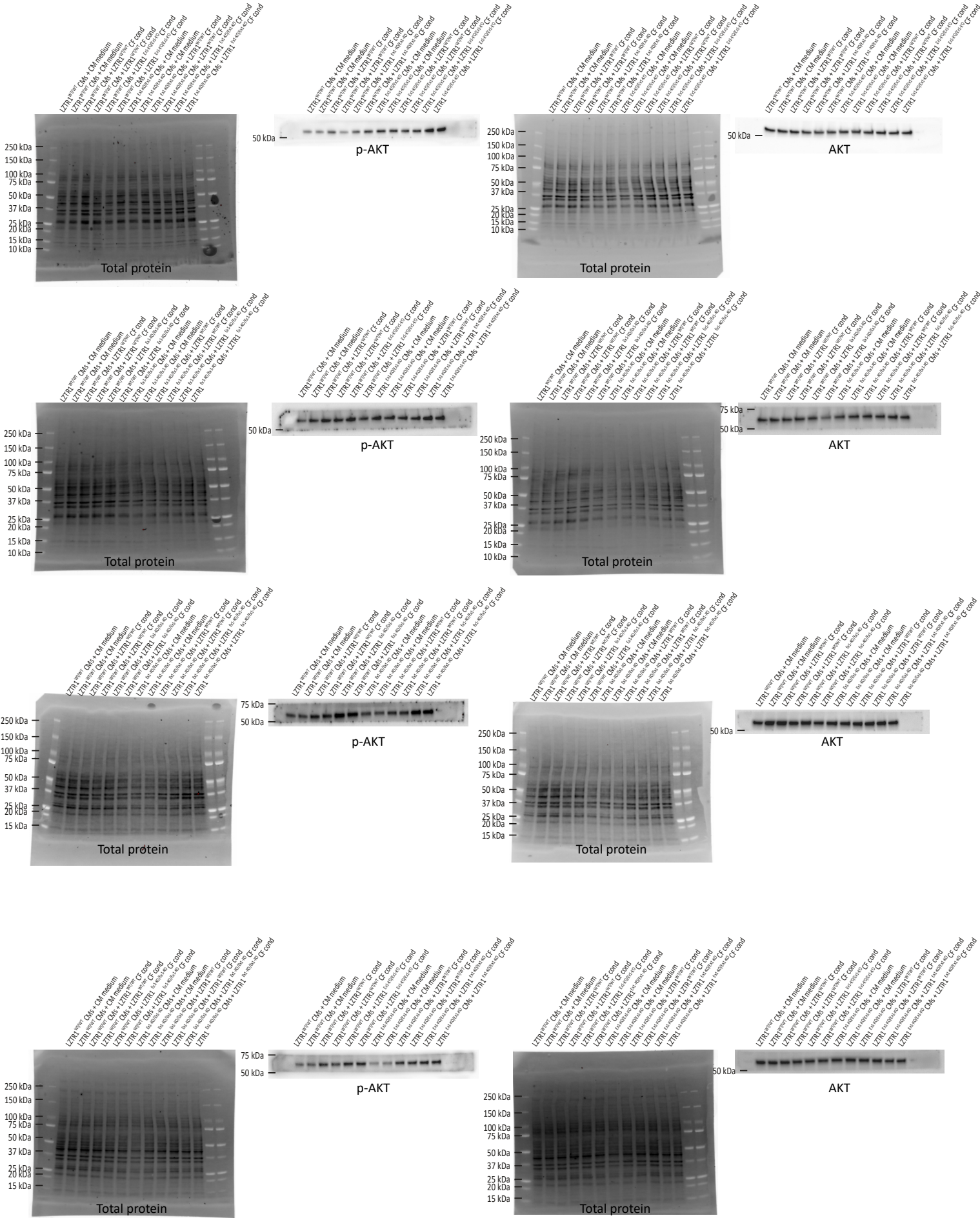
Full unedited gels for Supplementary Figure 5A

Highlighted blots used as representative images



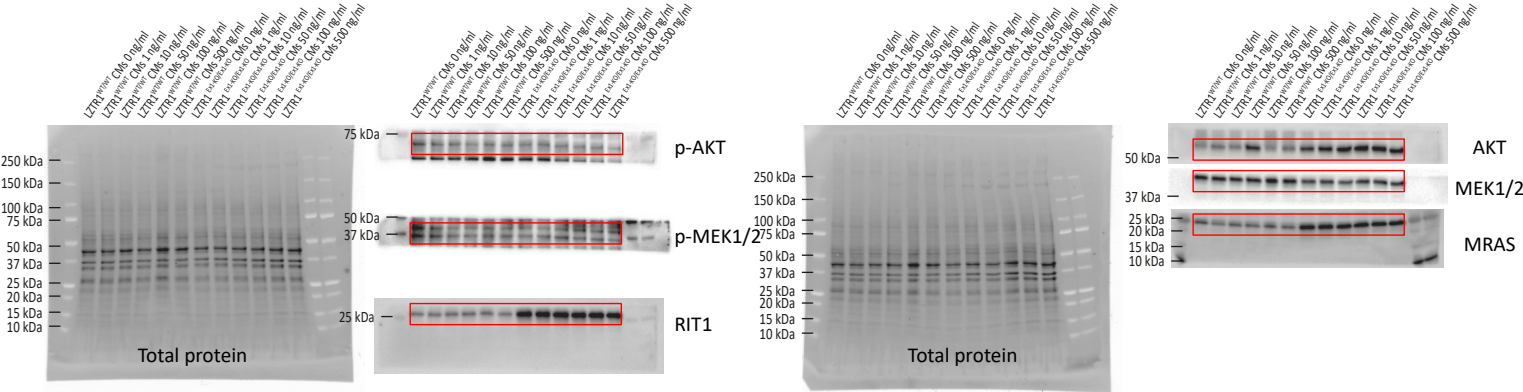
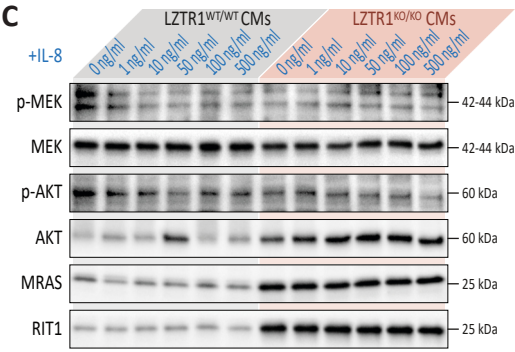
Full unedited gels for Supplementary Figure 5C

Blots used for quantification



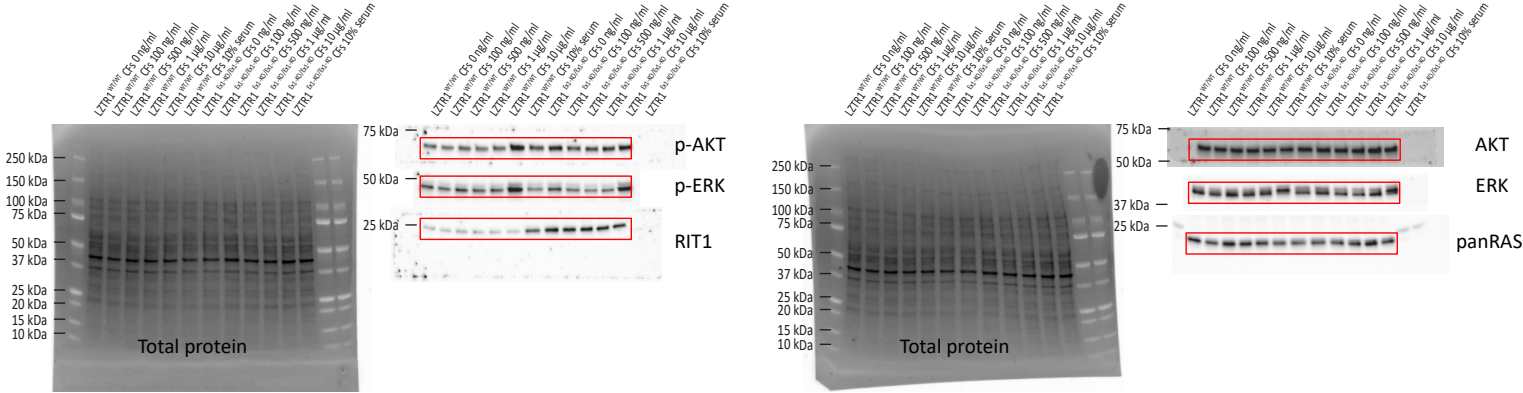
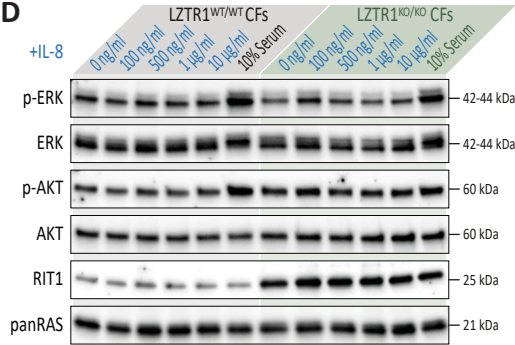
Full unedited gels for Supplementary Figure 11C

Highlighted blots used as representative images



Full unedited gels for Supplementary Figure 11D

Highlighted blots used as representative images



Targeting Interleukin-8 mediated cellular crosstalk reverses hypertrophic cardiomyopathy and cardiac fibrosis in Noonan syndrome

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SUPPLEMENTAL MATERIAL

Materials and Methods

Ethical approval

The study was approved by the Ethics Committee of the University Medical Center Göttingen (approval number: 10/9/15) and carried out in accordance with the approved guidelines. Written informed consent was obtained from all participants or their legal representatives prior to the participation in the study.

Generation and culture of human iPSCs

WT iPSC line UMGi014-C clone 14 (referred to as WT) was generated from dermal fibroblasts from a healthy male donor using integration-free Sendai virus and was described previously.⁵¹ Patient-derived iPSC lines UMGi030-A clone 14 (referred to as LZTR1^{biallelic}),⁸ UMGi177-A clone 1 (referred to as RIT1^{F99L}), and UMGi034-A clone 4 (referred to as RAF1^{S257L}) were generated from patient dermal fibroblasts using integration-free Sendai virus according to manufacturer's instructions. Genome editing in early passage iPSCs was performed using ribonucleoprotein-based CRISPR-Cas9 with crRNA/tracrRNA and Hifi SpCas9. 300 pmol Alt-R CRISPR-Cas9 crRNA and 300 pmol Alt-R CRISPR-Cas9 tracrRNA were pre-assembled with 122 pmol Alt-R Hifi SpCas9 Nuclease 3NLS (all IDT DNA Technologies) to form the ribonucleoprotein complex. Nucleofection was performed with 2×10⁶ iPSCs using the 4D Amaxa Nucleofector system (Lonza; program CA-137) and the P3 Primary Cell 4D-Nucleofector X Kit (Lonza) according to manufacturer's instructions. Following nucleofection, iPSCs were replated into a Matrigel-coated (growth factor reduced, BD Biosciences) 6-well plate containing StemFlex medium (Thermo Fisher Scientific) supplemented with 2 μM thiazovivin (Merck) and 100 U/ml penicillin and 100 μg/ml streptomycin (Thermo Fisher Scientific). After 3 days, transfected iPSCs were singularized using the CellenOne single cell dispenser (Cellenion/Scienion) in StemFlex medium on Matrigel-coated 96-well plates. Successful genome editing was identified by Sanger sequencing. For *LZTR1* knockout generation, WT iPSCs were genome-edited by CRISPR-Cas9, targeting *LZTR1* exon 1 using guide RNA target sequence 5'- AGCACGGGGGGGCAGATCG**GGG**-3' (PAM in bold) or exon 17 using guide RNA target sequence 5'-CCAGGTATGCCTTCATGTCCT**TGG**-3' (PAM in bold), respectively, and the CRISPR-edited isogenic iPSC lines UMGi014-C-15 clone 3 (referred to as LZTR1^{Ex1-KO/Ex1-KO}), UMGi014-C-15 clone 41 (referred to as LZTR1^{Ex1-KO/WT}), UMGi014-C-16 clone 1 (referred to as LZTR1^{Ex17-KO/WT}), and UMGi014-C-16 clone 7 (referred to as LZTR1^{Ex17-KO/Ex17-KO}) were established. For gene correction of LZTR1^{biallelic}, patient iPSC line UMGi030-A clone 14 was genome-edited by CRISPR-Cas9, targeting *LZTR1* intron 16 using guide RNA target sequence 5'- CTTGATCATGAGGTCAGTGAG**GGG**-3' (PAM in bold), in combination with a single-stranded oligonucleotide for homology-directed repair, and the CRISPR-edited isogenic iPSC line UMGi030-A-1 clone 34 (referred to as LZTR1^{biallelic}-corr) was established and described previously.⁸ For gene correction of RIT1^{F99L}, patient iPSC line UMGi177-A clone 1 was genome-edited by CRISPR-Cas9, targeting *RIT1* exon 5 using guide RNA target sequence 5'- GGCAGAGTTGACAGCCATGCG**GGG**-3' (PAM in bold), in combination with a single-stranded oligonucleotide for homology-directed repair, and the CRISPR-edited isogenic iPSC line UMGi177-A-1 clone 8 (referred to as RIT1^{F99L}-corr) was established. For gene correction of RAF1^{S257L}, patient iPSC line UMGi034-A clone 4 was genome-edited by CRISPR-Cas9, targeting *RAF1* exon 7 using guide RNA target sequence 5'- TGGATGTCAACCTCTGCCTCT**TGG**-3' (PAM in bold), in combination with a single-stranded oligonucleotide for homology-directed

repair, and the CRISPR-edited isogenic iPSC line UMGi034-A-1 clone D8 (referred to as RAF1^{S257L}-corr) was established. For generation of MRAS^{G23V}, WT iPSCs were genome-edited by CRISPR-Cas9, targeting *MRAS* exon 2 using guide RNA target sequence 5'-TCTGGAAAACTGGATGGT**GAGG**-3' (PAM in bold), in combination with a single-stranded oligonucleotide for homology-directed repair, and the CRISPR-edited isogenic iPSC line UMGi014-C-17 clone G2 (referred to as MRAS^{G23V}) was established and described previously.¹⁸ The collagen reporter line was generated using CRISPR-Cas9 in WT line RUCDRi002-A⁵² as described above by C-terminal tagging of *COL1A2* with a monomeric citrine fluorescent protein using guide RNA target sequence 5'-GCTGCTCAGTATGATGGAAA**AAGG**-3' (PAM in bold). 2 µg of the homology-directed repair plasmid DNA was added to the nucleofector solution. A homozygous-tagged iPSC line RUCDRi002-A-36 clone 2 (referred to as collagen reporter) was selected. Generated iPSC lines were maintained on Matrigel-coated plates, passaged every 4-6 days with Versene solution (Thermo Fisher Scientific) and cultured in StemMACS iPS-Brew XF medium (Miltenyi Biotec) supplemented with 2 µM thiazovivin on the first day after passaging with daily medium changes. Cultures were maintained in a humidified atmosphere (95% air, 5% CO₂) at 37 °C. Pluripotency analysis via immunocytochemistry and flow cytometry was performed as previously described.⁸ For molecular karyotyping, genomic DNA from iPSC clones was sent for genome-wide analysis using Illumina BeadArray (Life&Brain, Germany). Digital karyotypes were analyzed in GenomeStudio v2.0 software (Illumina) and visualized using Circa (OMGenomics Labs). Copy number events were reported if larger than 3.5×10⁵ bps and 1×10⁶ bps for loss of heterozygosity. All iPSC lines, antibodies, guide RNAs and genomic primers are listed in the Major Resources Table.

Differentiation of human iPSCs into CMs and non-CMs

Human iPSC lines were differentiated into functional ventricular iPSC-CMs via WNT signaling modulation and subsequent metabolic selection, as previously described.¹⁹ Briefly, iPSC-CM differentiation was initiated at a confluence of 80-90% in Matrigel-coated plates with cardio differentiation medium composed of RPMI 1640 with GlutaMAX and HEPES (Thermo Fisher Scientific), 0.5 mg/ml human recombinant albumin (Merck) and 0.2 mg/ml L-ascorbic acid 2-phosphate (Merck), and sequential treatment with 4 µM CHIR99021 (Merck) for 48 h and 5 µM IWP2 (Merck) for further 48 h. At day 8, medium was changed to cardio culture medium composed of RPMI 1640 with GlutaMAX and HEPES, and B27 (Thermo Fisher Scientific), with medium changed every other day. Differentiated cultures around day 15 were digested with 0.25% Trypsin/EDTA (Thermo Fisher Scientific) and replated at lower density (1:2-1:3) in Matrigel-coated plates. Metabolic selection of iPSC-CMs was performed using cardio selection medium composed of RPMI 1640 without glucose (Thermo Fisher Scientific), 0.5 mg/ml human recombinant albumin, 0.2 mg/ml L-ascorbic acid 2-phosphate and 4 mM lactate (Merck) for 5 days. iPSC-CMs were maintained in feeder-free and serum-free culture conditions until day 60 post-differentiation before being used for molecular and cellular experiments. Differentiation of human iPSCs into iPSC-CFs was performed according to Zhang et al. with modifications.²⁰ Briefly, iPSCs in confluent monolayer were treated with 4-6 µM CHIR99021 for 2 days in cardio differentiation medium. After 48 h, the cells were treated with 5 µM of IWP2 in cardio differentiation medium for 48 h and recovered in cardio differentiation medium for another 48 h. On day 6, differentiating cells were replated at a density of 2-5×10⁴ cells/cm² in cardio culture medium, supplemented with 2 µM thiazovivin. On day 7, differentiating cells were treated with 5 µM CHIR99021 and 2 µM retinoic acid (Merck) and allowed to recover in cardio culture

medium for 3 days. Subsequently, cultures were replated in Fibrolife (Cellsystems) and 10 ng/ml FGF-basic (PeproTech) and cultured for another 8 days with media changes every other day. Optionally, iPSC-CFs were treated with 10 μ M SB431542 (Miltenyi Biotec) for 6 days followed by two medium changes with Fibrolife, before being used for molecular and cellular experiments. Differentiation of human iPSCs into iPSC-ECs was performed according to Natividad-Diaz et al. with modifications.²¹ Briefly, iPSCs in confluent monolayer were treated with 6 μ M CHIR99021 for 48 h in StemMACS iPS-Brew XF. After 48 h, the cultures were treated with 50 ng/ml VEGF (Miltenyi Biotec), 10 μ M SB431542 (Miltenyi Biotec), and 10 ng/ml BMP4 (Miltenyi Biotec) for another 3 days. On day 5, medium was changed to Endothelial Growth Medium 2 (Lonza). On day 6 or 7, CD31⁺ magnetic-activated cell sorting of iPSC-ECs was performed using Dynabeads M280 (Thermo Fisher Scientific), anti-CD31 Antibody (Thermo Fisher Scientific), DynaMag2-Magnet (Thermo Fisher Scientific) in Endothelial Growth Medium 2 and 2 μ M thiazovivin. CD31⁺ cultures were cultured for 5-7 days with media changes every other day, before being used for molecular and cellular experiments. All cultures were maintained in a humidified atmosphere (95% air, 5% CO₂) at 37 °C.

Generation of ECTs

ECTs were generated as previously described.²² Briefly, fully differentiated iPSC-CFs were digested with TrypLE Express (Thermo Fisher Scientific) at day 20 of differentiation and centrifuged at 200 $\times$ g for 5 min. Acid-solubilized bovine collagen type 1 (Collagen Solutions, ~6 mg/ml) was added 1:1 to 2 $\times$ DMEM (Thermo Fisher Scientific) containing 20% fetal calf serum (Promocell), 100 U/ml penicillin, and 100 μ g/ml streptomycin. The pH of the collagen mixture was neutralized with 0.2 N NaOH. The collagen mixture and cell suspension were mixed to generate ECTs composed of 0.3 mg collagen and 2 $\times$ 10⁵ iPSC-CFs per ECT. 180 μ l of the mixture was carefully dispensed into each mold of a 48-well mold plate with two flexible poles (myrPlate TM5, myriamed, Germany). ECTs were allowed to solidify for 1 h before Fibrolife was added. ECTs were cultured for 9-13 days after casting in a humidified atmosphere (95% air, 5% CO₂) at 37 °C with daily media changes using Fibrolife. For treatment, culture medium was supplemented with 100 ng/ml IL-8 (Miltenyi Biotec), 16.6 nM Latrunculin A (Enzo Life Sciences), 3 μ M H1152P (Biomol), 10 μ M reparixin (Merck) or vehicle control (0.1% DMSO for drug compounds; 0.1% BSA in PBS for IL-8). Pole deflection was employed to determine contraction in the 48-well plate format as previously described.²² The plate was imaged daily under UV-A light to detect the poles containing an optical brightener. ImageJ (National Institutes of Health) was used to analyze the measured pole distances compared to the day of casting to quantify relative pole deflection over time. Cross-sectional area (CSA) was assessed to determine ECT compaction by imaging top and side views of ECTs using a Lumar.V12 stereomicroscope (Zeiss). The diameter was determined by measuring 4-10 different positions in each image plane, and CSA was calculated by using the following formula: $CSA = \pi \times (\text{diameter mean top view}/2) \times (\text{diameter mean side view}/2)$. Destructive tensile strength measurements were performed with an RSA-G2 rheometer (TA Instruments). ECTs were attached to two custom-made hooks in a 37°C tempered organ bath with PBS (Thermo Fisher Scientific) and stretched at a constant linear rate of 0.03 mm/s until rupture. The recorded forces [mN] were divided by the CSA to obtain stress values [Pa]. The beginning of the toe region (L0) was determined and the length deformation, given by the distance between the two hooks (L), was converted to strain by (L-L0)/L0. Linear regression analysis within the elastic region of the stress-strain curve provided the Young's modulus. Yield point strain (end of the linear elastic phase), ultimate strength (point of maximum stress), and ultimate strain (point

of sudden drop in stress) were analyzed. Tissue resilience and toughness were calculated from the area under the stress-strain curve up to the yield and failure points, respectively, using GraphPad Prism 10. For RNA isolation, ECTs were digested in 2 mg/ml collagenase I (Merck) with 20% fetal bovine serum (FBS, Thermo Fisher Scientific) in calcium-containing PBS at 37°C for 2 h. Supernatants were kept on ice. Remaining tissue fragments were washed with PBS without calcium and incubated in accutase (Merck), 0.025% trypsin-EDTA (Thermo Fisher Scientific), and 20 µg/ml DNase (Calbiochem) at 37°C for 30 min. Remaining fragments were mechanically dispersed and the enzymatic activities were inhibited by addition of 5% FBS-containing PBS. Cells were pooled with the initial supernatant, centrifuged, and used for RNA isolation using the NucleoSpin RNA kit (Macherey-Nagel) according to manufacturer's instructions.

Generation of BCTs

Bioartificial cardiac tissues (BCTs) were generated as previously described.^{15,23} Briefly, iPSCs were differentiated into iPSC-CMs and metabolically purified in dynamic suspension culture at 60 rpm. On day 21 of differentiation, the resulting cardiac bodies were dissociated and used for tissue preparation. For one BCT, 1×10^6 iPSC-CMs were combined with 2.5×10^5 iPSC-CFs around day 20 in an extracellular matrix mix composed of 230 µg Cultrex rat tail collagen type I (R&D Systems) and 10% Matrigel (Thermo Fisher Scientific) with a final volume of 250 µl. The cell-matrix mix was cast into silicone molds (5×5×10 mm) containing two horizontally placed titanium rods spaced 6 mm apart to support and suspend the developing BCT. Culture medium, composed of DMEM with 25 mM glucose, 10% horse serum, 2 mM L-glutamine (all Thermo Fisher Scientific), 10 µg/mL insulin, 100 U/mL penicillin, and 100 µg/mL streptomycin (all Merck), was exchanged every other day. For treatment, culture medium was supplemented with 10 µM reparixin or vehicle control (0.1% DMSO). Physiological measurements were performed weekly between weeks 2 and 6 after casting using a custom-made bioreactor system.²³ At the end of each experimental round, BCTs were snap-frozen and lysed in 100 µl lysis buffer, composed of 50 mM Tris/HCl (Carl Roth), 100 mM NaCl, 2 mM MgCl₂, 1% IGEPAL CA-630, 10% Glycerol (all Merck), and protease and phosphatase inhibitor (Thermo Fisher Scientific), using 5 mm stainless steel beads (Qiagen) and a TissueLyser (Qiagen) set to 30 Hz for 30 sec. RNA was extracted from the pellets using a commercial kit (Promega) according to the manufacturer's instructions.

Immunofluorescence

Cells were plated on Matrigel-coated coverslips in cell-type specific culture conditions and fixed in Roti-Histofix 4% (Carl Roth) at room temperature for 20 min. Fixed cells were blocked with 1% Bovine Serum Albumin (BSA; Merck) in PBS at 4°C overnight. Primary antibodies were applied in 1% BSA in PBS at 37°C for 1 h or at 4°C overnight. Secondary antibodies with minimal cross reactivity were administered in 1% BSA in PBS at room temperature for 1 h. For nuclear or cytosolic proteins, cells were permeabilized with 0.1% Triton-X100 (Carl Roth) in staining solution. Nuclei were stained with 8.1 µM Hoechst 333424 (Thermo Fisher Scientific) at room temperature for 10 min. Samples were mounted in Fluoromount-G (Thermo Fisher Scientific). Images were collected using the Axio Imager M2 microscope (Carl Zeiss). For Ki-67 analysis, 0.15×10^5 iPSC-CFs were seeded in a 24-well plate. After 4 days, the cells were immunostained for Ki-67, and 19 predefined image fields per well were acquired using the 20× objective of the CQ1 confocal image cytometer and analyzed with CellPathfinder software (both Yokogawa Electric Corporation). Unbiased quantification was performed using CellProfiler, including

images with nuclei counts within the defined range of 30-90 nuclei per image.

Western blot, proteomics and kinase assay analysis

Cell lysates were extracted from cultured iPSC-derivatives using M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific) or RIPA buffer (Thermo Fisher Scientific) and phosphatase inhibitor cocktail 3 (Merck) and kept at -80°C for at least 10 min. Pellets were collected by scraping and protein containing supernatant was collected by centrifugation and stored at -80°C. Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Lysates were subjected to Western blot analysis, proteomics, and kinase assay. For proteomics, protein samples were purified by running them short distance into a 4-12% NuPAGE Novex Bis-Tris Minigel (Thermo Fisher Scientific), Coomassie staining and in-gel digestion with trypsin.⁵³ For generation of cell-type specific libraries, equal amount aliquots from each cell type were pooled to a total amount of 200 µg, and separated into twelve fractions using a basic pH reversed phase C18 separation on an FPLC system (äktä pure, Cytiva) and a staggered pooling scheme. All samples were spiked with a synthetic peptide standard used for retention time alignment (iRT Standard, Switzerland). Protein digests were analyzed on a nanoflow chromatography system (Dionex Ultimate nanoRSLC) hyphenated to a hybrid quadrupole-orbitrap mass spectrometer (Exploris 480, Thermo Fisher Scientific). In brief, 400 ng equivalents of peptides were dissolved in loading buffer (2% acetonitrile, 0.1% trifluoroacetic acid in water), enriched on a self-packed reversed phase-C18 precolumn (0.15 mm ID × 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, 3 µm, Dr. Maisch) using a 90 min linear gradient of 5-35% acetonitrile/0.1% formic acid (v:v) at 300 nl/min). DDA analysis was using a Top25 method using HCD fragmentation as described elsewhere. Two technical replicates per C18 fraction were acquired. DIA analysis was performed in using a 40-variable window DIA method. Two technical replicates per biological replicate were acquired. Protein identification was achieved with the Pulsar algorithm in Spectronaut Software 14.2 (Biognosys) using default settings against the UniProtKB *Homo sapiens* reference proteome (revision 09-2021) augmented with a set of 51 known common laboratory contaminants. For quantitation of the DIA-MS data, up to the 6 most abundant fragment ion traces per peptide, and up to the 10 most abundant peptides per protein were integrated and summed up to provide protein area values. Mass and retention time calibration as well as the corresponding extraction tolerances were dynamically determined. Both identification and quantification results were trimmed to a False Discovery Rate of 1% using a forward-and-reverse decoy database strategy. Quantitative data were median-normalized. Following export of the data matrix, data were log₂-transformed, and missing values imputed using a downshifted, randomized distribution of values in Perseus software 1.6.15 (Max Planck Institute for Biochemistry, Martinsried, Germany). Following averaging of technical replicates, differential abundance was performed using Student's t-test with a Benjamini-Hochberg correction, with significance at adjusted/corrected p-values ≤ 0.05. Reactome pathway enrichment analysis of differential abundant proteins (adjusted p-value ≤ 0.05; log₂ fold change ≥ 0.5) was performed using the ClueGo v2.5.10 plugin in Cytoscape v3.10.0. Kinase activity profiling was performed as previously described.⁵⁴ In short, protein tyrosine (PTK) and serine-threonine kinase (STK) profiles were determined using the PamChip microarray platform with the PamStation12 (PamGene International). Per array, 10 µg and 2 µg (PTK and STK, respectively) of protein and 400 µM ATP were added. Phosphorylation intensity was quantified (and corrected for local background) using the BioNavigator software (version 6.3,

PamGene International). Upstream kinase analysis was performed using the functional scoring method implemented in PamGene. For each kinase, a kinase statistic was calculated from the phosphorylation levels of associated substrate peptides. Specificity and sensitivity scores were subsequently derived using permutation-based testing. The specificity score was obtained by permuting peptide–kinase associations and recalculating the kinase statistic to estimate deviation from random peptide assignment. The sensitivity score was obtained by permuting sample labels (treatment versus control) to estimate differential kinase activity between conditions. The final kinase score was defined as the sum of specificity and sensitivity scores and used to rank kinases for putative involvement in the observed experimental differences. Kinases with a final score >1.2 (corresponding to a nominal significance level of 0.05 based on the permutation distribution) and an adjusted false discovery rate < 0.05 were considered significant and visualized in the heatmap. For Western blot, samples were diluted to 1 µg/µl in sample buffer (final solution contained 10% glycerol, 50 mM DTT and bromophenol blue). Samples were heated to 99°C for 5 min. Proteins were separated in Mini-PROTEAN TGX Stain-Free Precast Gels (Bio Rad) using 1× tris/glycine/SDS buffer (Bio-Rad). Total protein was visualized after UV activation for 5 min using a ChemiDoc imaging system (Bio-Rad). Next, proteins were transferred to nitrocellulose membranes using the Trans-Blot Turbo RTA Transfer kit (Bio-Rad). Total protein on the membrane was subsequently imaged with the ChemiDoc system. Membranes were then blocked with a polyvinylpyrrolidone-based solution (Serva Electrophoresis), incubated with primary antibodies overnight at 4°C and secondary HRP-labeled antibodies for 1 h at room temperature, and developed using enhanced chemiluminescence. Band intensities were quantified by densitometry and normalized to the total protein of the corresponding lane from the post-transfer membrane image using Image Lab (Bio-Rad). Antibodies are listed in the Major Resources Table.

Cytokine assay

Conditioned media were collected, filtered using a 0.22 µm Steriflip (Merck), snap-frozen in liquid nitrogen and stored at -80°C. Detection of cytokine presence in conditioned media was performed using Human XL Cytokine Array Kit (R&D Systems) according to the manufacturer's instructions using 500 µl of supernatant. For signal quantification, ImageLab (Bio-Rad) was used.

RNA sequencing

RNA-seq libraries were prepared using a modified, strand-specific, massively parallel cDNA sequencing protocol using the TruSeq Stranded Total RNA Kit (Illumina). For cDNA library quantification, the QuantiFluo dsDNA System (Promega) was utilized. Final cDNA library sizes were determined with the dsDNA 905 Reagent Kit (Fragment Analyzer, Advanced Bioanalytical), averaging 300 bp. Libraries were pooled and sequenced on an Illumina HiSeq 4000, producing 50 bp single-end reads (30-40 million reads per sample). Sequence images were processed into BCL files using the Illumina BaseCaller and then demultiplexed into fastq files with bcl2fastq v2.17.1.14. Sequencing quality was verified using FastQC (version 0.11.5). Sequences were aligned to the *Homo sapiens* reference genome hg38 (Ensembl version 89) using the STAR aligner version 2.5.2a, allowing for 2 mismatches within 50 bases. Read counting was conducted with featureCounts version 1.5.0-p1. Read counts were analyzed in the R Bioconductor environment (version 3.4.2) using the DESeq2 package version 1.16.1. Gene annotation was performed using the *Homo sapiens* entries via the biomaRt R package version 2.32.1. Read counts were converted into reads per kilobase million values. Differentially expressed genes were identified with an

absolute log₂-fold change of ≥ 0.5 and a false discovery rate-corrected p-value (padj) of ≤ 0.05 . KEGG pathway enrichment analysis was conducted using the ClueGo v2.5.10 plugin in Cytoscape v3.10.0, and string interaction analysis was conducted using the string-db online tool.

Quantitative real-time PCR analysis

Pellets of iPSC-derivatives were collected, snap-frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel) according to the manufacturer's instructions. 200 ng RNA was used for the first-strand cDNA synthesis by using the M-MLV Reverse Transcriptase and Oligo d(T)16 (Thermo Fisher Scientific). Subsequently, cDNA was diluted 1:1 with nuclease-free water (Promega). Quantitative real-time PCR reactions were carried out using the SYBR Green PCR master mix and ROX Passive Reference Dye (Bio-Rad) with Micro-Amp Optical 384-well plates, and the 7900HT fast real-time PCR system (Applied Biosystems) according to the manufacturer's instructions with the following parameters: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Samples were measured in triplicates. Analysis was conducted using the $\Delta\Delta CT$ method and values were normalized to *GAPDH*, *TUBB5*, and *RPL37A* gene expression and to WT and/or vehicle-treated controls. BCTs were further normalized to cardiomyocyte content (*TNNT2*). Primer sequences are listed in the Major Resources Table.

Nanostring gene expression assay

For gene panel analysis, a customized nCounter Elements TagSet panel designed by NanoString Technologies was utilized. 200 ng of RNA per sample were hybridized to target-specific capture and reporter probes at 67°C for 16-20 h according to the manufacturer's instructions. Samples were then loaded into the NanoString cartridge and the nCounter gene expression assay was initiated. Raw reads were analyzed with nSolver™ Data Analysis Software. For background subtraction the geometric means of negative controls was used. RNA counts were normalized to four housekeeping genes (*TBP*, *HPRT1*, *POL2RA*, and *GAPDH*). TagSets are listed in the Major Resources Table.

Analysis of CM cell size

To determine cell size of iPSC-CMs in suspension, 2.5×10^4 cells at day 50-60 of differentiation were seeded in a 12-well plate. After 7 days of recovery, cells were stimulated and/or treated with conditioned media, 100 ng/ml IL-8, 10 μ M reparixin or the vehicle control (0.1% DMSO for reparixin; 0.1% BSA in PBS for IL-8) for additional 7 days, administered during media change every other day. Subsequently, cells were singularized with StemPro Accutase Cell Dissociation Reagent (Thermo Fisher Scientific) and 0.025% trypsin-EDTA for at 37°C for 15 min. Enzymatic reaction was stopped using FBS. Cells were centrifuged at 100 $\times$ g for 10 min. The supernatant was aspirated and the pellet was resuspended in 50% CM medium and 50% FBS for measurement of the cell diameter using the CASY cell counter system (OMNI Life Science). For cell size analysis in adherent culture, three representative images per well were acquired and cell area of each cell was manually evaluated using the ROI Manager plugin in ImageJ. Cell size was only determined in cells with clearly visible cell boundaries.

RNA knockdown experiments

For transient knockdown of *RIT1*, 2×10^5 iPSC-CFs were plated, depending on the experiment setting, in a 6-well plate or 12-well plate. RNA interference was performed by using TriFECTa RNAi Kit (IDT DNA). Briefly, three different pre-designed Dicer-Substrate Short Interfering RNAs (DsiRNAs) were mixed to obtain 20 μM in buffer containing 100 mM Na^+ or K^+ . A negative DsiRNA sequence was used as a control. From the DsiRNA solution, 25 μL was added to 125 μL of Opti-MEM (Thermo Fisher Scientific). In parallel, 25 μL of Lipofectamine Stem Transfection Reagent (Thermo Fisher Scientific) was added to 125 μL Opti-MEM. Both mixtures were combined and incubated for 10 min. Finally, 100 μL of transfection solution was added into each well and cultures were analyzed at different time points after transfection.

Cell proliferation, collagen secretion and scratch assay

For analysis of proliferation, 2.5×10^4 iPSC-CFs were plated in a 12-well plate. If required, iPSC-CFs were transfected either with the described DsiRNA-RIT1 or DsiRNA-NEG 24 h after plating with media changes every other day. Similarly, stimulation and/or treatment with 100 ng/ml IL-8, 1-100 μM reparixin (Merck), ladarixin, navarixin, danirixin, and AZD5069 (all Hycultec), or the vehicle control (0.1% DMSO for drug compounds; 0.1% BSA in PBS for IL-8) were started 24 h after cell plating and administered during media change every other day. Image acquisition was performed using the IncuCyte S3 System (Sartorius) in 8 h intervals for the duration of the experiments. Cell proliferation was derived by automated analysis of occupied area (% confluence) over time. For analysis of collagen deposition, 2×10^5 iPSC-CFs were plated in a 12-well plate and imaged using the IncuCyte S3 System. Fluorescent signal was derived by automated analysis over time. The scratch assay was performed according to the 96-well Kinetic Cell Migration & Invasion Assay (Sartorius). Briefly, 2.5×10^4 iPSC-ECs were seeded on Imagelock 96-well plates (Sartorius). After 24 h, the wound was created and wound closing was monitored using the IncuCyte S3 System. Wound confluence represented the fractional area of the wound occupied by cells. Proliferation curves were calculated based on Gompertz growth or fourth order polynomial equations using GraphPad Prism 10.

Biosensor-based analysis of ERK signaling dynamics in iPSC-CMs

To measure ERK dynamics over time in live cells, genetically-encoded kinase translocation reporters (KTRs) were used that translate ERK activity into quantifiably nucleus-cytoplasm shuttling.⁵⁵ Briefly, ERK-KTR encoding lentiviral particles were produced in HEK293T cells transfected with 6 μg transfer plasmid (pLentiPGKPuroDESTERKKTR was a gift from Markus Covert; RRID:Addgene_90227), 4 μg envelope plasmid (pMD2.G was a gift from Didier Trono; RRID:Addgene_12259), and 5 μg packaging plasmid (psPAX2 was a gift from Didier Trono; RRID:Addgene_12260) using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's instruction. The virus was harvested from day 2 to day 5 post-transfection by medium collection (DMEM + 10% FBS, 1% L-glutamine and 1% non-essential amino acids) and centrifugation at $500 \times g$ at 4°C for 5 min. The harvested virus was filtered using a 0.45 μm filter and a syringe. For transduction, 1.5×10^4 iPSC-CMs were seeded per well of a 96-well plate and lentiviral transduction was performed 7 days after cell digestion. Lentivirus was diluted in CM medium supplemented with 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 10 $\mu\text{g}/\text{ml}$ Polybrene Transfection Reagent (Merck). 24 h after transduction, medium was replaced with CM medium

and cultures were maintained for further 7 days. The medium was exchanged 24 h before live cell imaging was performed with ERK-KTR transduced iPSC-CMs at day 60 of differentiation. First, cells were imaged at basal conditions followed by stimulation with iPSC-CF conditioned media. In case of 10 μ M reparixin treatment, drug was administered 1 h before addition of conditioned media. DMSO (0.1%) served as a vehicle control for reparixin. IL-8 was administered at a concentration of 100 ng/ml (diluted in 0.1% BSA in PBS). Live cell imaging experiments were acquired using the CQ1 confocal image cytometer (Yokogawa Electric Corporation) with a 20 $\times$ long working distance objective under environmental control (37°C, 5% CO₂) and 24 h recording time with 10 min scanning intervals. Exported images (CellPathfinder software, Yokogawa Electric Corporation) were further processed using StarDist for nucleus segmentation.⁵⁶ The StarDist network was retrained on 60 images from our dataset with annotations manually produced using napari.⁵⁷ For a new image, the nuclei were then segmented with StarDist, and a ring element around each nucleus was computed to approximate the cytosol. Mean fluorescence of both compartments was measured for each cell individually.

Cell viability assay

To analyze cell viability, 1×10^5 iPSC-CMs were seeded onto a 24-well plate. After 7 days of recovery, cells were treated with different concentrations of reparixin or the vehicle control (0.1% DMSO) for additional 7 days, administered during media change every other day. Before imaging acquisition, 1 μ M Calcein AM Viability Dye (Thermo Fisher Scientific) in RPMI medium without phenol red was administrated to the cultures for 30 min at 37°C. Subsequently, cells were washed twice with PBS, incubated with 500 nM propidium iodide (Merck) in RPMI medium without phenol red, and analyzed using the IncuCyte S3 System to discriminate between live and dead cells.

Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean (SEM) unless otherwise specified. Statistical comparisons were performed using the D'Agostino-Pearson normality test. Subsequently, the unpaired t test or the nonparametric Mann-Whitney was used to compare two groups, and the ordinary one-way ANOVA followed by Holm-Sidak's multiple comparisons test or the nonparametric Kruskal-Wallis test followed by Dunn's multiple comparisons test was used to compare three or more groups using GraphPad Prism 10. Results were considered statistically significant when the p-value was ≤ 0.05 .

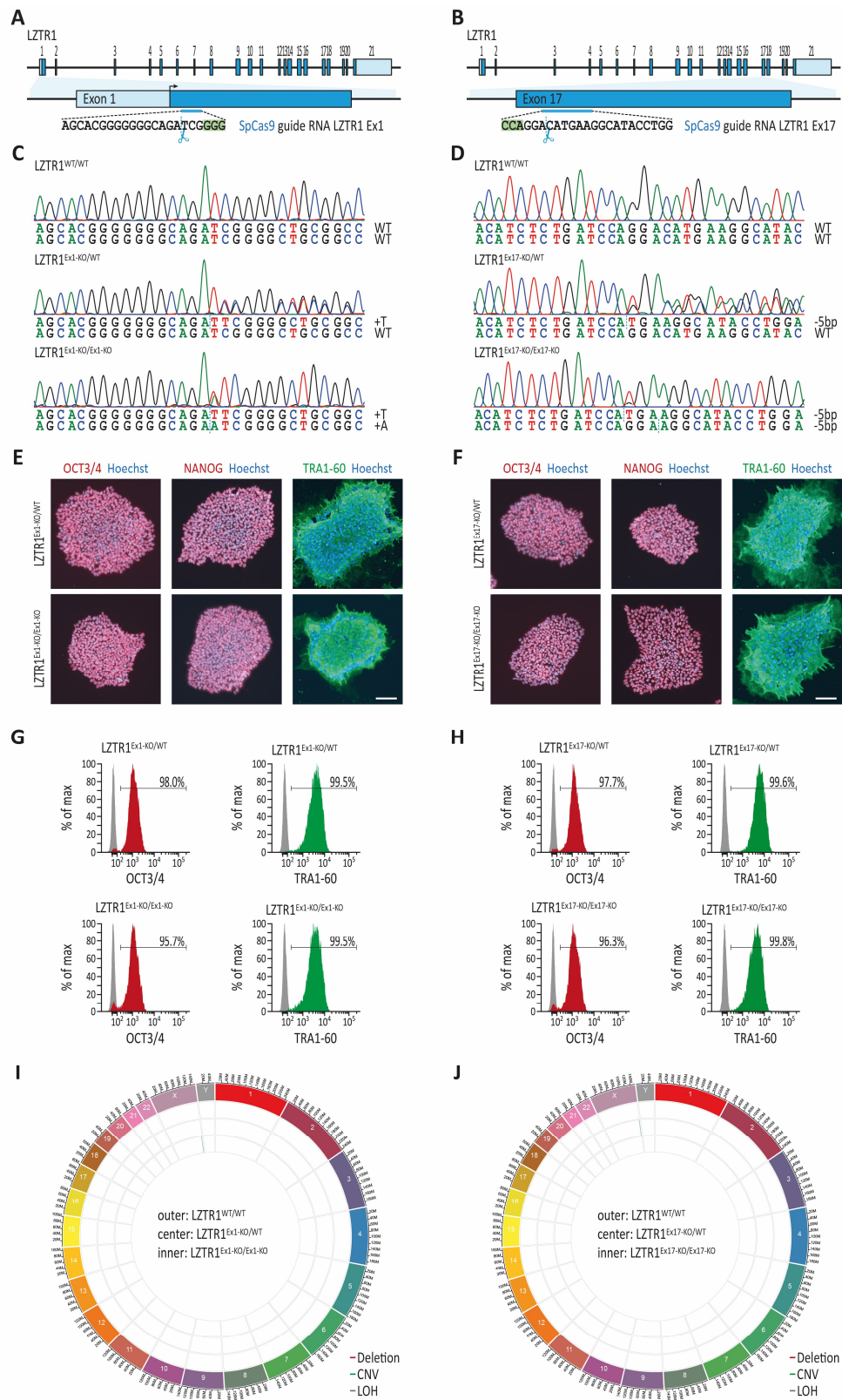


Figure S1: Generation of *LZTR1*-deficient iPSCs by CRISPR-Cas9. (A,B) Depiction of CRISPR-Cas9 genome editing approach for non-homologous end joining-based introduction of

frameshift variants in *LZTR1* exon 1 (A) and *LZTR1* exon 17 (B) in WT iPSCs. **(C,D)** Sanger sequencing of unedited and CRISPR-edited iPSCs with heterozygous and homozygous *LZTR1* frameshift variants. **(E,F)** Expression of key pluripotency markers OCT3/4, NANOG, and TRA-1-60 in CRISPR-edited iPSCs, assessed by immunocytochemistry, nuclei were counter-stained with Hoechst 33342 (blue); scale bar: 200 μ m. **(G,H)** Flow cytometry analysis of the pluripotency marker OCT3/4 and TRA-1-60 revealing homogeneous populations of pluripotent cells in CRISPR-edited iPSCs; gray peaks represent negative controls. **(I,J)** Molecular karyotyping using a genome-wide microarray demonstrated chromosomal stability of CRISPR-edited iPSCs; CNV, copy number variants; LOH, loss of heterozygosity.

Figure S2: Cell type-specific gene expression of RAS GTPases in cardiomyocytes and non-cardiomyocytes. (A-D) Relative transcript levels of cell type-specific markers, including pluripotency marker *SOX2* (A), CM marker *ACTN2* (B), CF marker *COL1A1* (C), and EC marker *PECAM1* (D), assessed by RT-qPCR in *LZTR1*^{WT/WT}, *LZTR1*^{KO/WT}, and *LZTR1*^{KO/KO} iPSCs, iPSC-CMs, iPSC-CFs, and iPSC-ECs; data were normalized to housekeepers (*GAPDH*, *TUBB5*, *RPL37A*) and corresponding WT; n=3 independent differentiations per iPSC line from *LZTR1*^{WT/WT}, *LZTR1*^{Ex1-KO/WT}, *LZTR1*^{Ex1-KO/Ex1-KO}, *LZTR1*^{Ex17-KO/WT}, and *LZTR1*^{Ex17-KO/Ex17-KO}, n=3-6 independent passages for iPSCs. **(E-J)** Relative transcript levels of *LZTR1* (E) and RAS GTPases, including *MRAS* (F), *RIT1* (G), *HRAS* (H), *KRAS* (I), and *NRAS* (J), assessed by RT-qPCR in *LZTR1*^{WT/WT}, *LZTR1*^{KO/WT}, and *LZTR1*^{KO/KO} iPSCs, iPSC-CMs, iPSC-CFs, and iPSC-ECs revealed cell type-specific expression differences; data were normalized to housekeepers (*GAPDH*, *TUBB5*, *RPL37A*) and WT iPSCs; n=3 independent differentiations per iPSC line from *LZTR1*^{WT/WT}, *LZTR1*^{Ex1-KO/WT}, *LZTR1*^{Ex1-KO/Ex1-KO}, *LZTR1*^{Ex17-KO/WT}, and *LZTR1*^{Ex17-KO/Ex17-KO}, n=3-6 independent passages for iPSCs.

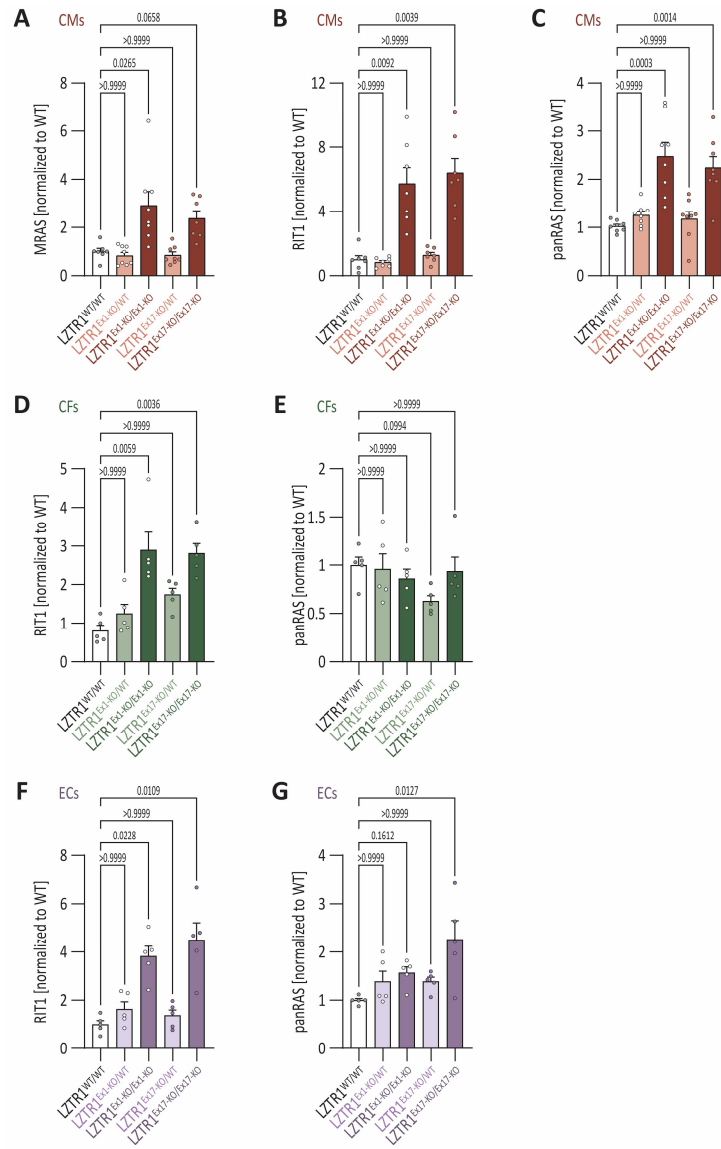


Figure S3: RAS GTPase protein levels in *LZTR1*-deficient cardiomyocytes and non-cardiomyocytes. (A-C) Quantitative analysis of Western blots for MRAS (A), RIT1 (B), and pan-RAS recognizing HRAS, KRAS, and NRAS (C) in *LZTR1*^{WT/WT}, *LZTR1*^{KO/WT} and *LZTR1*^{KO/KO} iPSC-CMs; data were normalized to stain-free total protein and corresponding WT; n=7-8 samples from 6 independent differentiations per iPSC line from *LZTR1*^{WT/WT}, *LZTR1*^{Ex1-KO/WT}, *LZTR1*^{Ex1-KO/Ex1-KO}, *LZTR1*^{Ex17-KO/WT}, *LZTR1*^{Ex17-KO/Ex17-KO}. (D,E) Quantitative analysis of Western blots for RIT1 (D) and pan-RAS (E) in *LZTR1*^{WT/WT}, *LZTR1*^{KO/WT} and *LZTR1*^{KO/KO} iPSC-CFs; data were normalized to stain-free total protein and corresponding WT; n=5 samples from 5 independent differentiations per iPSC line from *LZTR1*^{WT/WT}, *LZTR1*^{Ex1-KO/WT}, *LZTR1*^{Ex1-KO/Ex1-KO}, *LZTR1*^{Ex17-KO/WT}, *LZTR1*^{Ex17-KO/Ex17-KO}. (F,G) Quantitative analysis of Western blots for RIT1 (F) and pan-RAS (G) in *LZTR1*^{WT/WT}, *LZTR1*^{KO/WT} and *LZTR1*^{KO/KO} iPSC-ECs; data were normalized to stain-free total protein and corresponding WT; n=5 samples from 5 independent differentiations per iPSC line from *LZTR1*^{WT/WT}, *LZTR1*^{Ex1-KO/WT}, *LZTR1*^{Ex1-KO/Ex1-KO}, *LZTR1*^{Ex17-KO/WT}, *LZTR1*^{Ex17-KO/Ex17-KO}. Data were analyzed by

nonparametric Kruskal-Wallis test with Dunn's post hoc correction (A-G) and are presented as mean $\pm$ SEM.

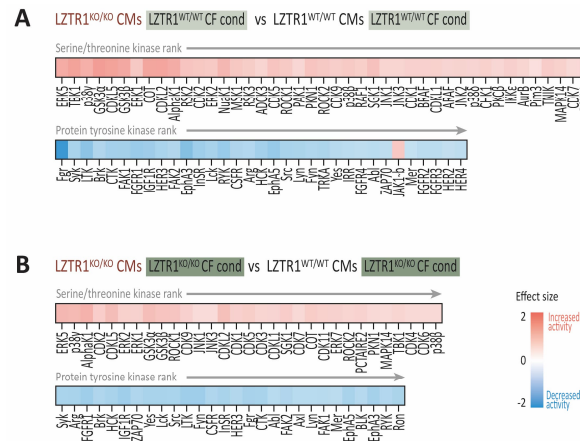


Figure S4: Kinase activity in *LZTR1*-deficient cardiomyocytes treated with conditioned media from fibroblasts. (A,B) Heatmaps showing effect size (mean kinase statistic) of significantly differentially regulated kinases in $LZTR1^{KO/KO}$ and $LZTR1^{WT/WT}$ iPSC-CMs under stimulation with conditioned medium collected from $LZTR1^{WT/WT}$ (A) and $LZTR1^{KO/KO}$ (B) iPSC-CFs using the PamChip microarray platform; $n=3-4$ samples per condition from 2 independent differentiations per iPSC line from $LZTR1^{Ex1-KO/Ex1-KO}$.

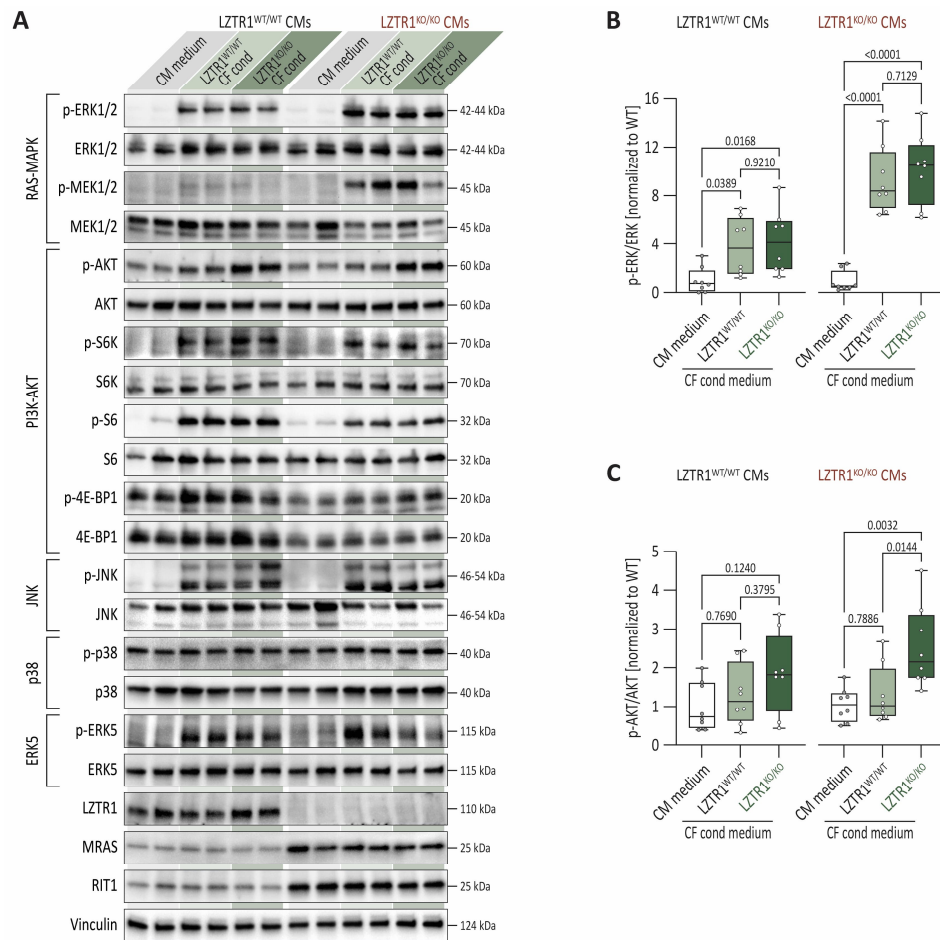


Figure S5: Signaling pathway analysis in *LZTR1*-deficient cardiomyocytes. (A) Representative Western blot images of ERK, AKT, JNK, p38 and ERK5 signaling activity in *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CMs under basal conditions and under stimulation for 30 min with conditioned medium from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CFs; *LZTR1* and RAS GTPases were used to confirm genotype; vinculin is shown as housekeeper. (B,C) Quantitative analysis of Western blots for p-ERK/ERK (B) and p-AKT/AKT (C) ratios in unstimulated and stimulated *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CMs; data were normalized to stain-free total protein and corresponding WT; n=8 samples per condition from 4 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}. Data were analyzed by ordinary one-way ANOVA with Holm-Sidak post hoc correction (B,C) and are presented as mean ± SEM.

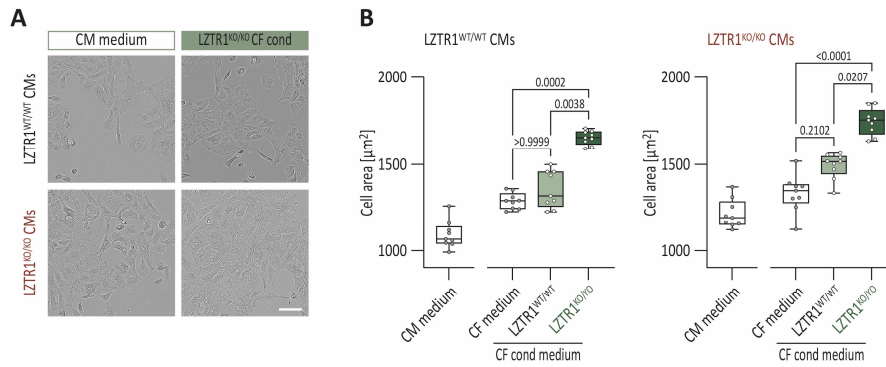


Figure S6: Cell size analysis of *LZTR1*-deficient cardiomyocytes. (A) Representative microscopic images of LZTR1^{WT/WT} and LZTR1^{KO/KO} iPSC-CMs under unstimulated conditions and under stimulation with conditioned medium from LZTR1^{KO/KO} iPSC-CFs for 7 days; scale bar: 100 μm. **(B)** Quantitative cell size analysis of cells in adherent culture of LZTR1^{WT/WT} and LZTR1^{KO/KO} iPSC-CMs under unstimulated conditions, under exposure to unconditioned CF medium, and under stimulation with conditioned medium from LZTR1^{WT/WT} and LZTR1^{KO/KO} iPSC-CFs for 7 days; each dot represents the mean cell area per well (with 16 images per well); n=9 samples per condition from 3 independent differentiations per iPSC line from LZTR1^{WT/WT} and LZTR1^{Ex1-KO/Ex1-KO}. Data were analyzed by nonparametric Kruskal-Wallis test with Dunn's post hoc correction (B) and are presented as mean ± SEM.

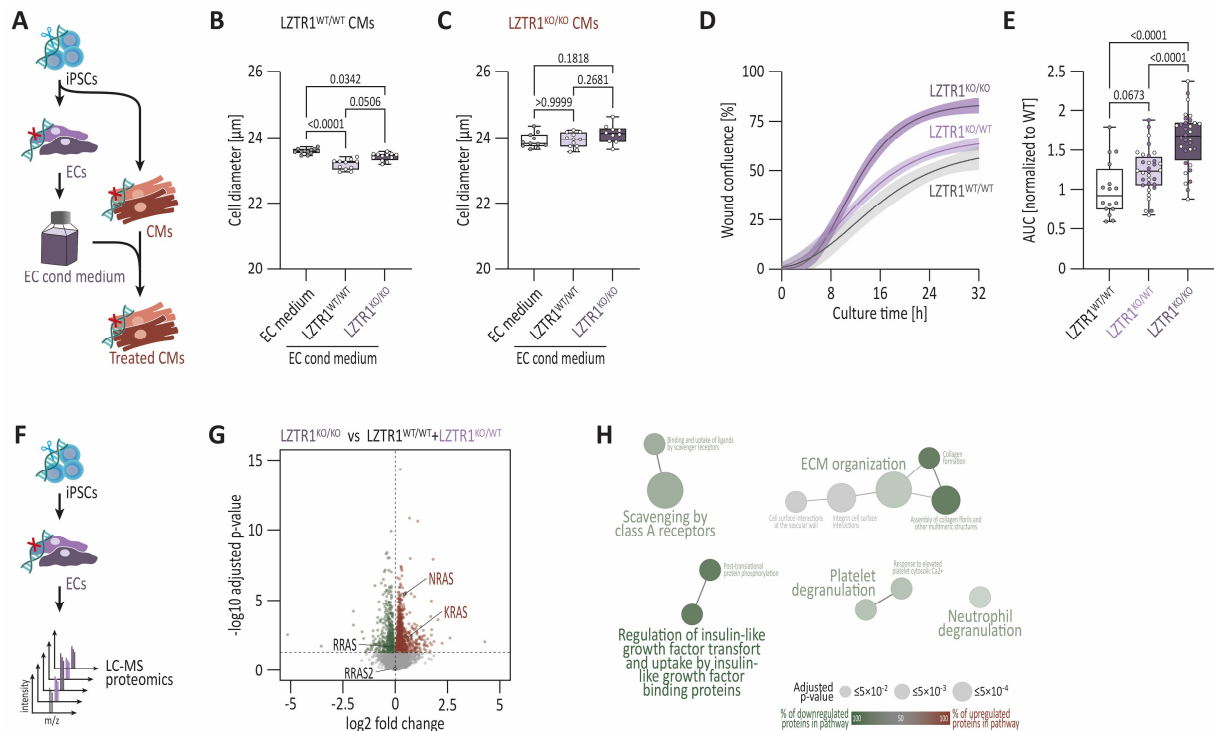


Figure S7: Molecular and cellular phenotyping of LZTR1-deficient endothelial cells. (A) LZTR1^{WT/WT} and LZTR1^{KO/KO} iPS-CMs were stimulated with conditioned medium collected from LZTR1^{WT/WT} and LZTR1^{KO/KO} iPS-ECs and subsequently analyzed for cellular hypertrophy. (B,C) Quantitative cell size analysis of singularized cells in suspension of LZTR1^{WT/WT} (B) and LZTR1^{KO/KO} iPS-CMs (C) under exposure to unconditioned EC medium and under stimulation with conditioned medium from LZTR1^{WT/WT} and LZTR1^{KO/KO} iPS-ECs for 7 days; n=10-12 samples per condition from 4 independent differentiations per iPSC line from LZTR1^{WT/WT} and LZTR1^{Ex1-KO/Ex1-KO}. (D,E) Proliferation curves after scratch wound (solid lines, mean; shaded areas, $\pm$ SEM) (D) and quantitative analysis (E) for LZTR1^{WT/WT}, LZTR1^{KO/WT} and LZTR1^{KO/KO} iPS-ECs; quantification of proliferation was determined by area under the curve analysis and normalized to WT; n=16 samples from 4 independent differentiations per iPSC line from LZTR1^{WT/WT}, LZTR1^{Ex1-KO/WT}, LZTR1^{Ex1-KO/Ex1-KO}, LZTR1^{Ex17-KO/WT}, and LZTR1^{Ex17-KO/Ex17-KO}. (F) LZTR1^{WT/WT}, LZTR1^{KO/WT} and LZTR1^{KO/KO} iPS-ECs were analyzed by quantitative global proteomics via LC-MS/MS at day 12 of differentiation; n=3 independent differentiations per iPSC line from LZTR1^{WT/WT}, LZTR1^{Ex1-KO/WT}, LZTR1^{Ex1-KO/Ex1-KO}, LZTR1^{Ex17-KO/WT}, LZTR1^{Ex17-KO/Ex17-KO}, measured in technical duplicates. (G) Volcano plot comparing the global proteome of LZTR1-deficient iPS-ECs (LZTR1^{Ex1-KO/Ex1-KO} and LZTR1^{Ex17-KO/Ex17-KO}) with functional LZTR1 samples (LZTR1^{WT/WT}, LZTR1^{Ex1-KO/WT} and LZTR1^{Ex17-KO/WT}); RAS proteins are highlighted; differential abundance was assessed using Student's t-test with Benjamini-Hochberg multiple testing correction; dotted line indicates adjusted p-value of 0.05. (H) Reactome pathway enrichment analysis in LZTR1-deficient versus functional LZTR1 iPS-ECs; node color indicates up- and downregulated proteins within each pathway; node size reflects adjusted p-value. Data were analyzed by ordinary one-way ANOVA with Holm-Sidak post hoc correction (E) or nonparametric Kruskal-Wallis test with Dunn's post hoc correction (B,C) and are presented as mean $\pm$ SEM.

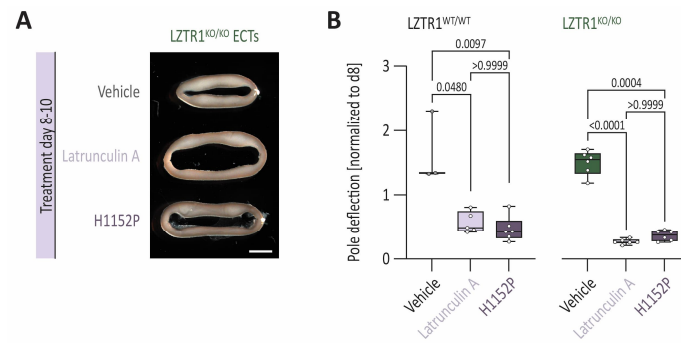


Figure S8: Analysis of cytoskeletal dynamics in *LZTR1*-deficient engineered connective tissues. (A,B) LZTR1^{WT/WT} and LZTR1^{KO/KO} ECTs were treated with 16.6 nM Latrunculin A (inhibition of F-actin polymerization), 3 μ M H1152P (inhibition of ROCK-dependent contractility), or vehicle control from day 8 to day 10 after casting. (A) Representative macroscopic images of LZTR1^{KO/KO} ECTs after 2 days of treatment with vehicle control, Latrunculin A, or H1152P; scale bar: 1 mm. (B) Quantification of pole deflection at day 10 following 2 days of treatment; pole distances were normalized to day 8 of tissue culture (treatment start); n=3-6 tissues per condition per iPSC line from LZTR1^{WT/WT} and LZTR1^{Ex1-KO/Ex1-KO}. Data were analyzed by nonparametric Kruskal-Wallis test with Dunn's post hoc correction (B) and are presented as mean $\pm$ SEM.

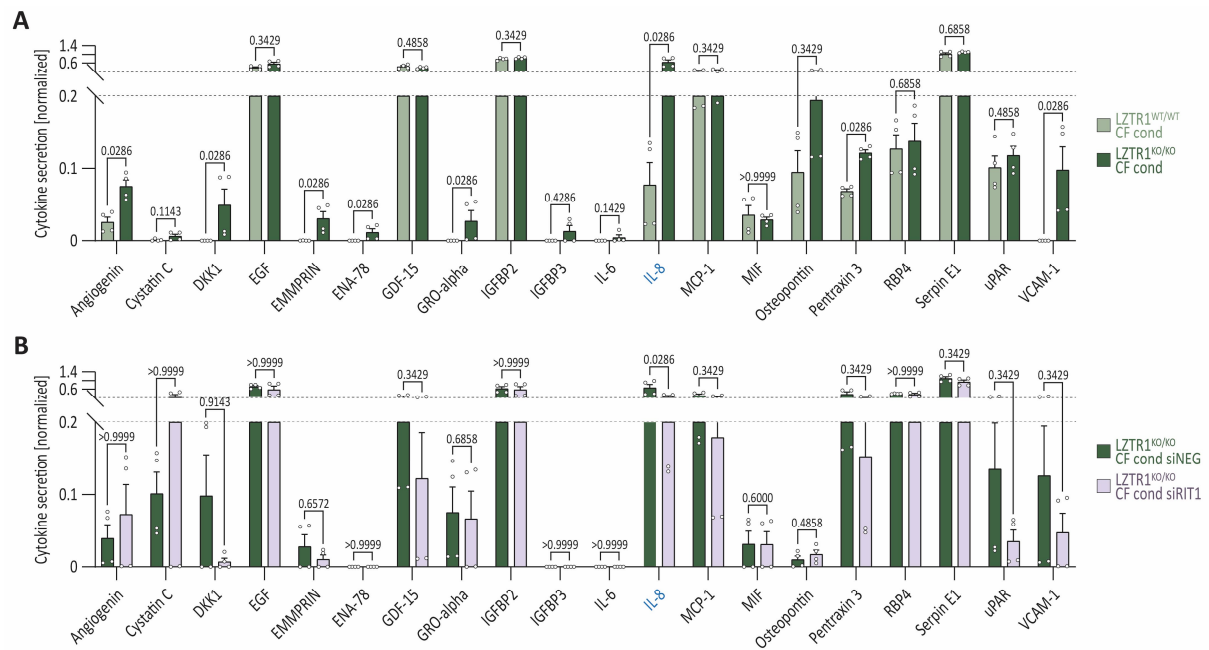


Figure S9: Detection of paracrine factors in *LZTR1*-deficient cardiac fibroblasts. (A) Quantitative analysis of highly and low-secreted cytokines in conditioned media assessed by cytokine arrays from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CFs; data were normalized to assay's positive controls; n=2 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}, measured in technical duplicates. **(B)** Quantitative analysis of highly and low-secreted cytokines in conditioned media assessed by cytokine arrays from *LZTR1*^{KO/KO} iPSC-CFs transfected with siNEG or siRIT1; data were normalized to assay's positive controls; n=2 samples per condition from 2 independent differentiations from *LZTR1*^{Ex1-KO/Ex1-KO}, measured in technical duplicates. Data were analyzed by Mann-Whitney test (A,B) and are presented as mean ± SEM.

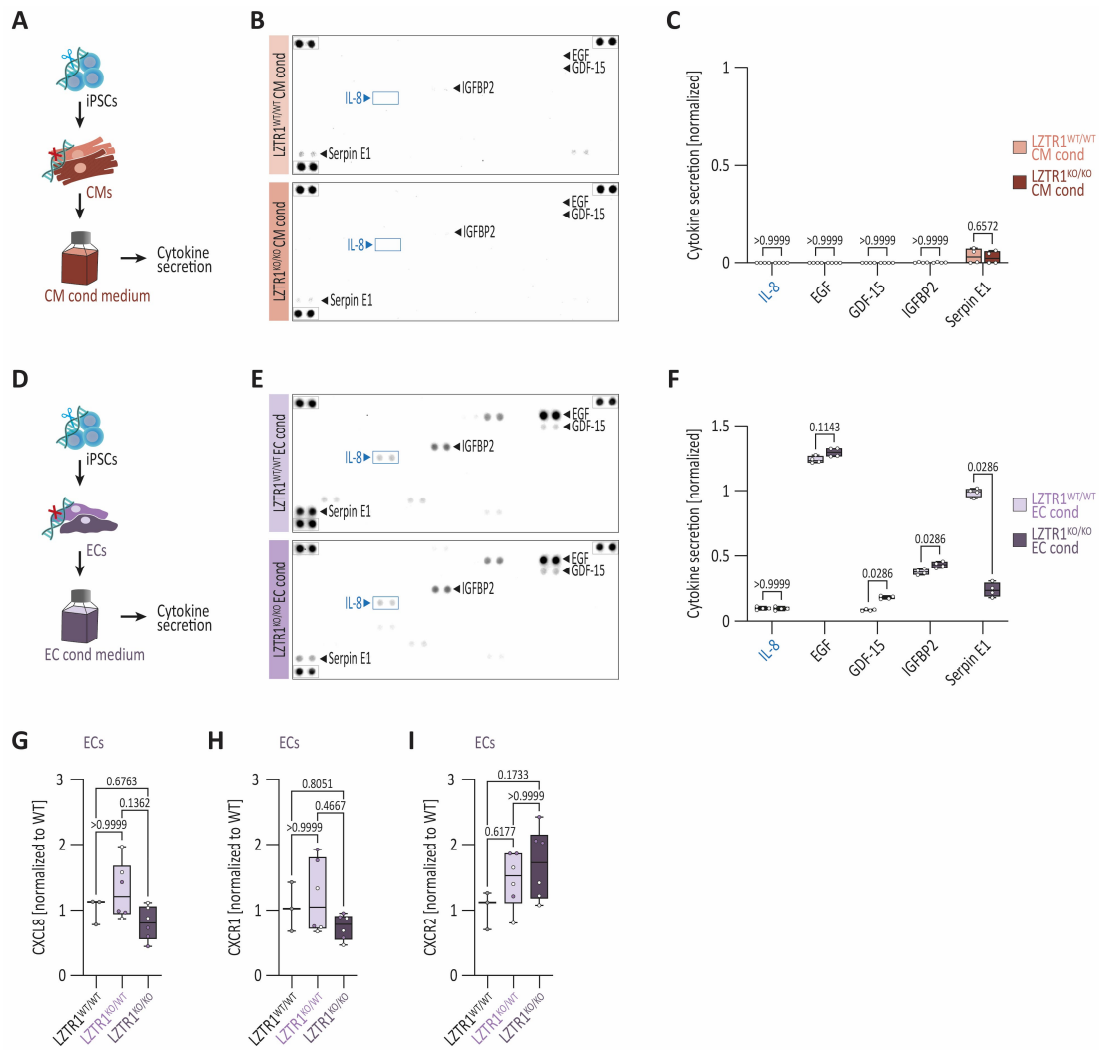


Figure S10: Detection of paracrine factors in *LZTR1*-deficient cardiomyocytes and endothelial cells. (A) Conditioned media from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CMs were collected and analyzed using a cytokine array. (B) Representative cytokine array membranes incubated with conditioned media from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CMs; selected cytokines are highlighted. (C) Quantitative analysis of selected cytokines in conditioned media from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CMs; data were normalized to assay's positive controls; n=2 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}, measured in technical duplicates. (D) Conditioned media from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-ECs were collected and analyzed using a cytokine array. (E) Representative cytokine array membranes incubated with conditioned media from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-ECs; selected cytokines are highlighted. (F) Quantitative analysis of selected cytokines in conditioned media from *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-ECs; data were normalized to assay's positive controls; n=2 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}, measured in technical duplicates. (G-I) Relative transcript levels of *CXCL8* (G) and its receptors *CXCR1* (H) and *CXCR2* (I) assessed by RT-qPCR in *LZTR1*^{WT/WT}, *LZTR1*^{KO/WT}, and *LZTR1*^{KO/KO} iPSC-ECs; data were normalized to housekeepers (*GAPDH*, *TUBB5*, *RPL37A*) and corresponding WT; n=3 independent differentiations per iPSC line from *LZTR1*^{WT/WT}, *LZTR1*^{Ex1-KO/WT}, *LZTR1*^{Ex1-KO/Ex1-KO}, *LZTR1*^{Ex17-KO/WT}, and *LZTR1*^{Ex17-KO/Ex17-KO}.

Data were analyzed by Mann-Whitney test (C,F) or nonparametric Kruskal-Wallis test with Dunn's post hoc correction (G,H,I) and are presented as mean $\pm$ SEM.

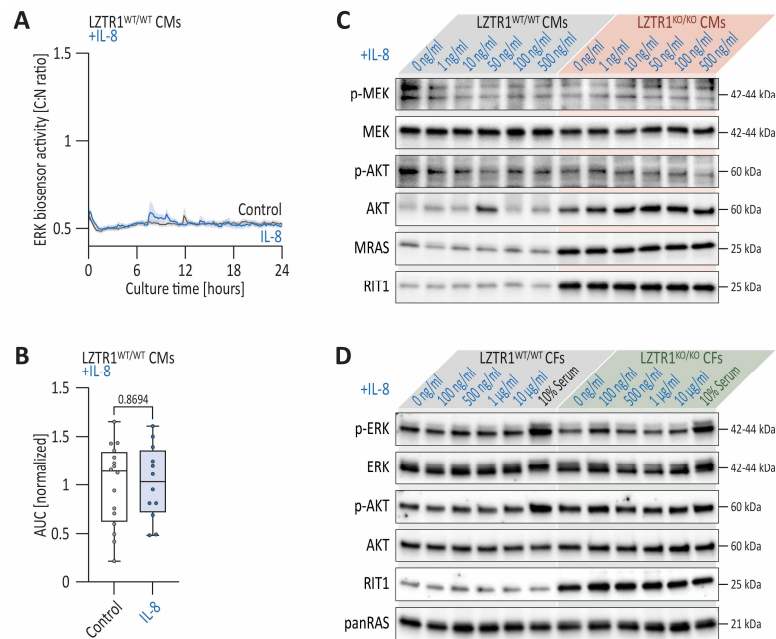


Figure S11: Influence of IL-8 on ERK signaling in cardiomyocytes and cardiac fibroblasts. (A,B) ERK dynamics in lentivirally transduced LZTR1^{WT/WT} iPSC-CMs under stimulation with 100 ng/ml IL-8 or vehicle control (A); quantification of signaling activity (B) was determined as the cytosolic-to-nuclear (C:N) ratio (solid lines, mean; shaded areas, $\pm$ SEM) and area under the curve analysis, normalized to untreated samples; n=12-16 samples per condition from 4 independent differentiations from LZTR1^{WT/WT}; control condition is replicated from Fig. 2I. (C) Representative Western blot images of MEK and AKT signaling activity in LZTR1^{WT/WT} and LZTR1^{KO/KO} iPSC-CMs under stimulation with increasing concentrations of IL-8 for 30 min; RAS GTPases were used to confirm genotype. (D) Representative Western blot images of ERK and AKT signaling activity in LZTR1^{WT/WT} and LZTR1^{KO/KO} iPSC-CFs under stimulation with increasing concentrations of IL-8 for 30 min versus 20% serum; RAS GTPases were used to confirm genotype. Data were analyzed by unpaired t test (B) and are presented as mean $\pm$ SEM.

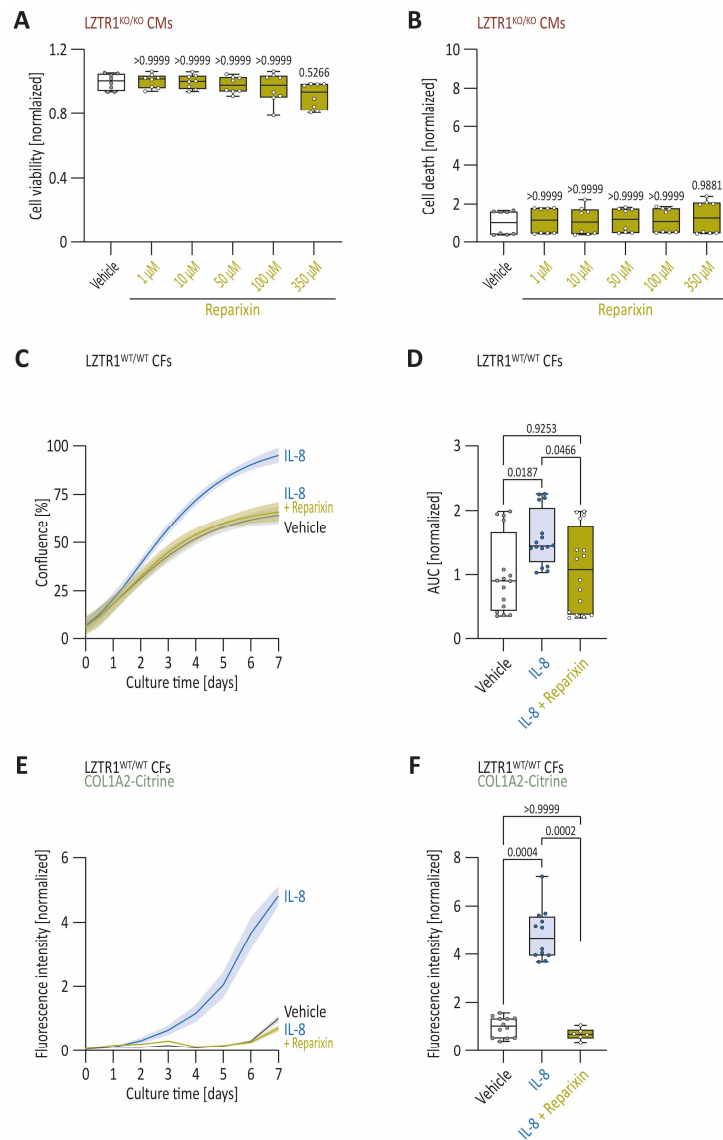


Figure S12: Reparixin inhibits IL-8–CXCR1 mediated signaling. (A,B) Cytotoxicity assay in LZTR1^{KO/KO} iPSC-CMs treated with increasing concentrations of reparixin for 7 days and analysis of cell viability using a calcein dye (A) and cell death using propidium iodide staining (B); data were normalized to vehicle-treated samples; n=8 samples per condition from 2 independent differentiations from LZTR1^{Ex1-KO/Ex1-KO}. (C,D) Proliferation growth curves (solid lines, mean; shaded areas, $\pm$ SEM) (C) and quantitative analysis (D) for LZTR1^{WT/WT} iPSC-CFs under stimulation with 100 ng/ml IL-8 either treated with 10 μ M reparixin or vehicle control; quantification of proliferation was determined by area under the curve analysis and normalized to vehicle-treated samples; n=16 samples per condition from 4 independent differentiations from LZTR1^{WT/WT}; IL-8 and vehicle control conditions are replicated from Figure 6B and 6C. (E,F) Collagen deposition over time (solid lines, mean; shaded areas, $\pm$ SEM) (E) and quantitative analysis of collagen deposition at day 7 (F) for LZTR1^{WT/WT} iPSC-CFs under stimulation with 100 ng/ml IL-8 either treated with 10 μ M reparixin or vehicle control; quantification of collagen deposition was based on fluorescence intensity and normalized to vehicle-treated samples; n=6-12 samples per condition from 2-4 independent differentiations from collagen reporter line; IL-8 and

vehicle control conditions are replicated from Figure 6F and 6G. Data were analyzed by ordinary one-way ANOVA with Holm-Sidak post hoc correction (D) or nonparametric Kruskal-Wallis test with Dunn's post hoc correction (A,B,F) and are presented as mean $\pm$ SEM.

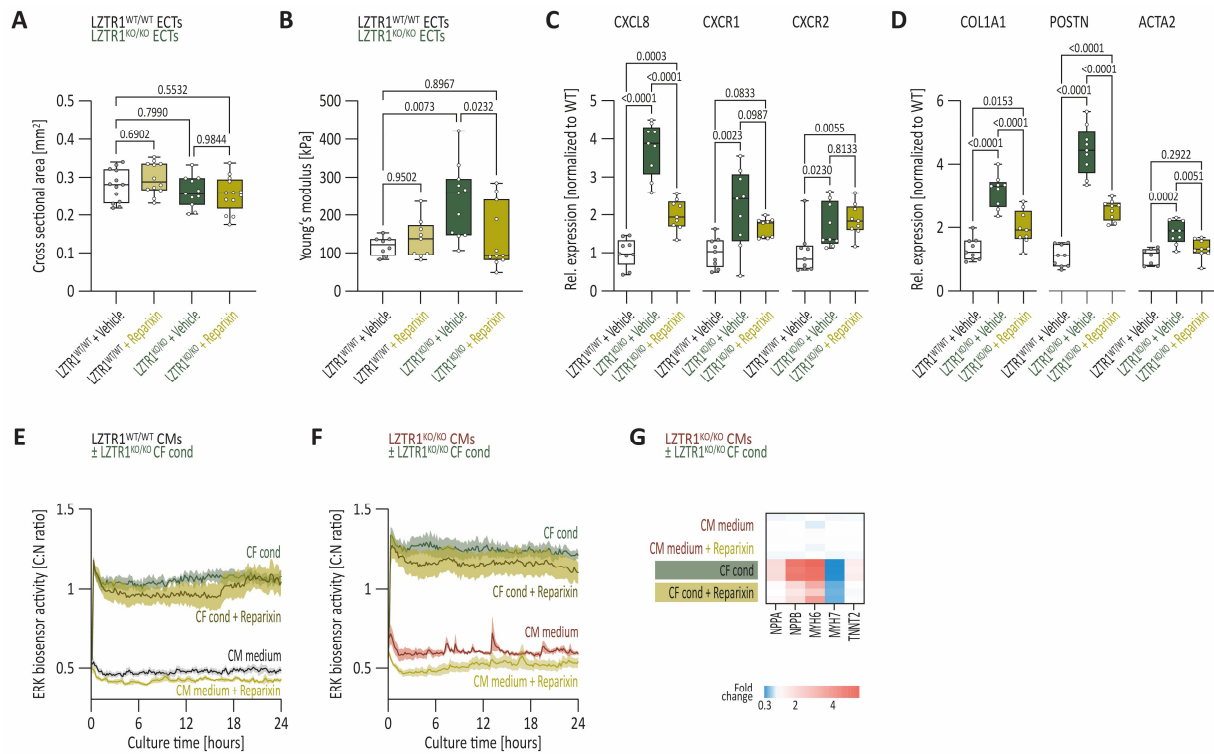


Figure S13: Effects of IL-8–CXCR1 signaling inhibition on *LZTR1*-deficient cardiac fibroblasts and cardiomyocytes. (A) Cross-sectional area of *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} ECTs under treatment with 10 μM reparixin or vehicle control at day 11 following 6 days of treatment was calculated based on imaging top and side views; n=11-13 tissues per condition from 2 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}. (B) Biomechanical properties, represented by Young's modulus, of *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} ECTs under treatment with 10 μM reparixin or vehicle control at day 11 following 6 days of treatment were determined by stress-strain analysis; n=11-13 tissues per condition from 2 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}. (C,D) Relative transcript levels of *CXCL8*, its receptors *CXCR1* and *CXCR2* (C), and fibrosis-associated genes *COL1A1*, *POSTN*, and *ACTA2* (D) assessed by RT-qPCR in *LZTR1*^{WT/WT} and *LZTR1*^{KO/KO} iPSC-CFs under treatment with 10 μM reparixin or vehicle control; data were normalized to housekeepers (*GAPDH*, *TUBB5*, *RPL37A*) and vehicle-treated WT; n=8-9 samples per condition from 3 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}. (E,F) ERK dynamics in lentivirally transduced *LZTR1*^{WT/WT} (E) and *LZTR1*^{KO/KO} iPSC-CMs (F) under unstimulated conditions (CM medium) and under stimulation with conditioned medium from *LZTR1*^{KO/KO} iPSC-CFs either treated with 10 μM reparixin or vehicle control; signaling activity was determined as the cytosolic-to-nuclear (C:N) ratio (solid lines, mean; shaded areas, ± SEM); n=6 samples per condition from 2 independent differentiations per iPSC line from *LZTR1*^{WT/WT} and *LZTR1*^{Ex1-KO/Ex1-KO}. (G) Nanostring nCounter gene expression analysis of hypertrophy-related genes in *LZTR1*^{KO/KO} iPSC-CMs under unstimulated conditions and under stimulation with conditioned medium from *LZTR1*^{KO/KO} iPSC-CFs either treated with 10 μM reparixin or vehicle control; data were normalized to housekeepers (*TBP*, *HPRT1*, *POL2RA*, *GAPDH*) and WT basal medium; n=3 samples per condition from 3 independent differentiations from *LZTR1*^{Ex1-KO/Ex1-KO}. Data were analyzed by ordinary one-way ANOVA with Holm-Sidak post hoc correction (A-D) and are presented as mean ± SEM.

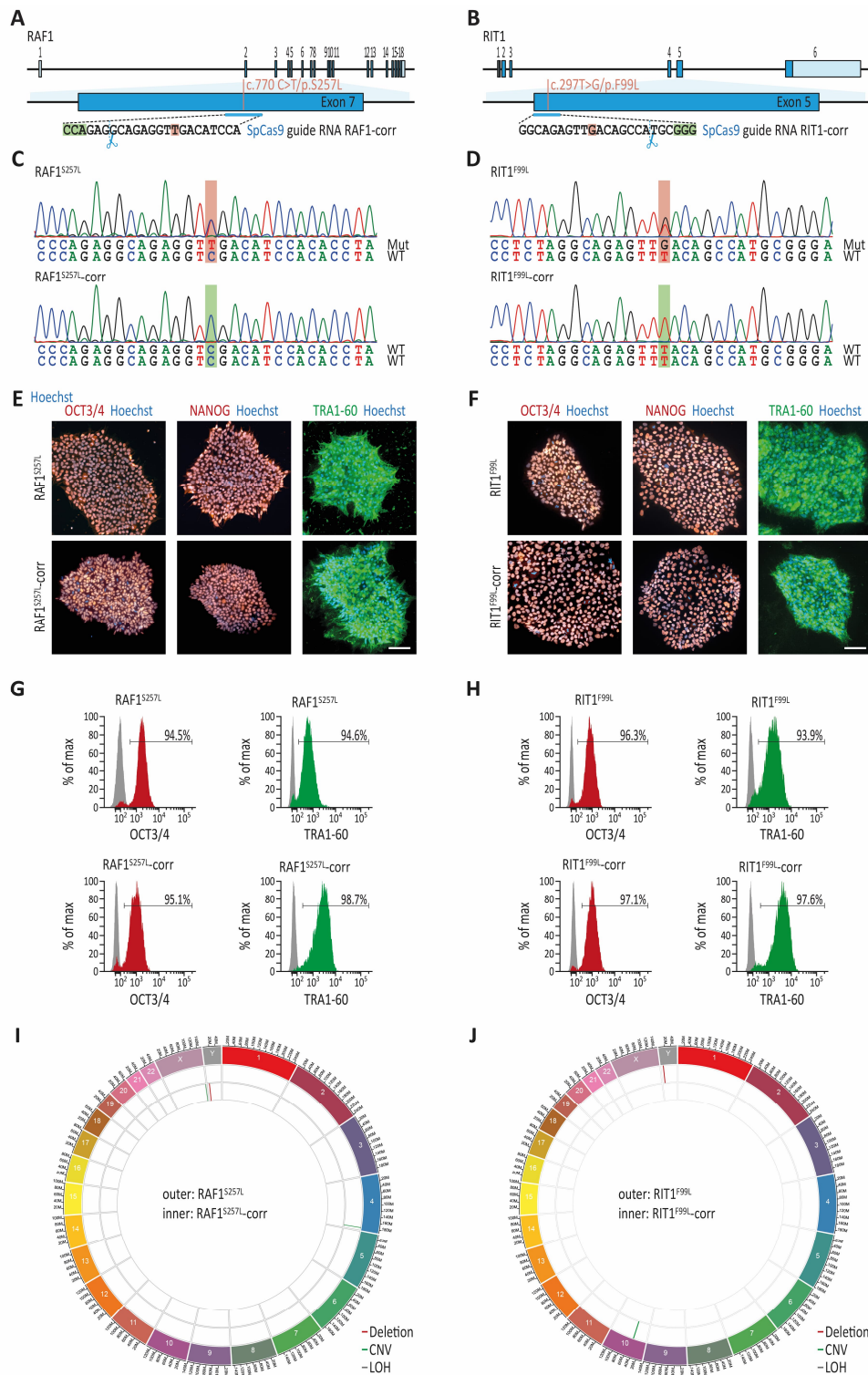


Figure S14: Generation of patient-specific and CRISPR-corrected iPSCs. (A,B) Depiction of CRISPR-Cas9 genome editing approach for SNP correction in *RAF1* exon 7 (A) and *RIT1* exon 5 (B) in patient-derived iPSCs. **(C,D)** Sanger sequencing of patient-derived and CRISPR-corrected iPSCs *RAF1*^{S257L} and *RAF1*^{S257L-corr} (C), and *RIT1*^{F99L} and *RIT1*^{F99L-corr} (D). **(E,F)** Expression of key pluripotency markers OCT3/4, NANOG, and TRA-1-60 assessed by immunocytochemistry

in of patient-derived and CRISPR-corrected iPSCs RAF1^{S257L} and RAF1^{S257L}-corr (E), and RIT1^{F99L} and RIT1^{F99L}-corr (F); nuclei were counter-stained with Hoechst 33342 (blue); scale bar: 200 μ m. **(G,H)** Flow cytometry analysis of the pluripotency marker OCT3/4 and TRA-1-60 in of patient-derived and CRISPR-corrected iPSCs RAF1^{S257L} and RAF1^{S257L}-corr (G), and RIT1^{F99L} and RIT1^{F99L}-corr (H); gray peaks represent negative controls. **(I,J)** Molecular karyotyping using a genome-wide microarray demonstrated chromosomal stability of patient-derived and CRISPR-corrected iPSCs RAF1^{S257L} and RAF1^{S257L}-corr (I), and RIT1^{F99L} and RIT1^{F99L}-corr (J); CNV, copy number variants; LOH, loss of heterozygosity.

Major Resources Table

iPSC lines

Description	iPSC line	Source	Identifier
WT iPSC line	WT/ LZTR1 ^{WT/WT}	UMG	UMGi014-C clone 14
LZTR1 knockout line, homozygous	LZTR1 ^{Ex1-KO/Ex1-KO}	UMG	UMGi014-C-15 clone 3
LZTR1 knockout line, heterozygous	LZTR1 ^{Ex1-KO/WT}	UMG	UMGi014-C-15 clone 41
LZTR1 knockout line, heterozygous	LZTR1 ^{Ex17-KO/WT}	UMG	UMGi014-C-16 clone 1
LZTR1 knockout line, homozygous	LZTR1 ^{Ex17-KO/Ex17-KO}	UMG	UMGi014-C-16 clone 7
Noonan syndrome patient iPSC line	LZTR1 ^{biallelic}	UMG	UMGi030-A clone 14
Noonan syndrome patient iPSC line, CRISPR-Cas9 corrected	LZTR1 ^{biallelic} -corr	UMG	UMGi030-A-1 clone 34
Noonan syndrome patient iPSC line	RAF1 ^{S257L}	UMG	UMGi034-A clone 4
Noonan syndrome patient iPSC line, CRISPR-Cas9 corrected	RAF1 ^{S257L} -corr	UMG	UMGi034-A-1 clone D8
Noonan syndrome patient iPSC line	RIT1 ^{F99L}	UMG	UMGi177-A clone 1
Noonan syndrome patient iPSC line, CRISPR-Cas9 corrected	RIT1 ^{F99L} -corr	UMG	UMGi177-A-1 clone 8
Collagen reporter line	Collagen reporter	UMG	RUCDRi002-A-36 clone 2
MRAS variant line	MRAS ^{G23V}	UMG	UMGi014-C-17 clone G2

Antibodies for Western blot (WB), immunocytochemistry (ICC), and flow cytometry (FC)

Primary antibody	Vendor	Identifier	Dilution
4E-BP1 monoclonal rabbit	Cell Signaling	AB_2097841	1:1000 (WB)
Phospho-4E-BP1 monoclonal rabbit	Cell Signaling	AB_560835	1:1000 (WB)
α -actinin monoclonal mouse	Merck	AB_476766	1:1000 (WB)
AKT polyclonal rabbit	Cell Signaling	AB_329827	1:1000 (WB)
Phospho-AKT polyclonal rabbit	Cell Signaling	AB_329825	1:1000 (WB)
CD144 monoclonal mouse	Santa Cruz Biotechnology	AB_2077957	1:1000 (WB)
COL1A1 monoclonal mouse	Cell Signaling	AB_2920541	1:1000 (WB, ICC)
cTNT monoclonal mouse	Thermo Fisher Scientific	AB_11000742	1:1000 (WB) (1:500 ICC)
ERK1/2 polyclonal rabbit	Cell Signaling	AB_330744	1:1000 (WB)
Phospho-ERK1/2 polyclonal rabbit	Cell Signaling	AB_331646	1:1000 (WB)
ERK5 polyclonal rabbit	Cell Signaling	AB_330491	1:1000 (WB)
Phospho-ERK5 polyclonal rabbit	Cell Signaling	AB_2140424	1:1000 (WB)
JNK polyclonal rabbit	Cell Signaling	AB_2250373	1:1000 (WB)
Phospho-JNK polyclonal rabbit	Cell Signaling	AB_331659	1:1000 (WB)
Ki-67 monoclonal rabbit	Cell Signaling	AB_2687446	1:400 (ICC)
LZTR1 monoclonal rabbit	Abcam	AB_3076250	1:1000 (WB)
MEK1/2 polyclonal rabbit	Cell Signaling	AB_823567	1:1000 (WB)
Phospho-MEK1/2 polyclonal rabbit	Cell Signaling	AB_331648	1:1000 (WB)
MRAS polyclonal rabbit	Proteintech	AB_10950895	1:800 (WB)
NANOG monoclonal mouse	Thermo Fisher Scientific	AB_2536677	1:200 (ICC), 1:50 (FC)
OCT3/4-PE monoclonal human	Miltenyi Biotec	AB_2784442	1:200 (ICC), 1:50 (FC)
p38 MAPK polyclonal rabbit	Cell Signaling	AB_330713	1:1000 (WB)
Phospho-p38 MAPK monoclonal rabbit	Cell Signaling	AB_2139682	1:1000 (WB)
pan-RAS monoclonal mouse	Merck	AB_2121151	1:800 (WB)
PECAM1 monoclonal mouse	Thermo Fisher Scientific	AB_779679	1:400 (ICC)
RIT1 polyclonal rabbit	Abcam	AB_882379	1:1000 (WB)

S6 monoclonal rabbit	Cell Signaling	AB_331355	1:1000 (WB)
Phospho-S6 monoclonal rabbit	Cell Signaling	AB_916156	1:1000 (WB)
S6K monoclonal rabbit	Cell Signaling	AB_390722	1:1000 (WB)
Phospho-S6K monoclonal rabbit	Cell Signaling	AB_2269803	1:1000 (WB)
TRA-1-60 monoclonal mouse	Abcam	AB_778563	1:200 (ICC)
TRA-1-60-Alexa488 monoclonal mouse	BD Biosciences	AB_1645379	1:50 (FC)
Vinculin monoclonal mouse	Merck	AB_477629	1:1000 (WB)
Secondary antibody	Vendor	Identifier	Dilution
Alexa488 polyclonal goat anti-rabbit	Thermo Fisher Scientific	AB_143165	1:1000 (ICC)
Alexa555 polyclonal donkey anti-mouse	Thermo Fisher Scientific	AB_2536180	1:1000 (ICC)
HRP polyclonal donkey anti-rabbit	Merck	AB_2722659	1:2000 (WB)
HRP polyclonal donkey anti-mouse	Merck	AB_772210	1:2000 (WB)

Primer sequences for PCR and RT-qPCR

Gene (gDNA)	Primer (5'-3')
<i>LZTR1 Ex1</i>	GGTAGGCTTGTCGGGAAGAG / CTCAGGACCCCATGAAGCC
<i>LZTR1 Ex17</i>	CAACATGGGCAGATATGCAG / ACACAAAGCAATGACCCAC
<i>RIT1</i>	TCAGGATAGCAGGTAATCGAC / AAGCCAAGCCAAGAATCTGT
<i>RAF1</i>	GGTTGTACAGGTAGAGTTTGCCC / TCCTTGATCAGATTGAAACCC
Gene (RT-qPCR)	Primer (5'-3')
<i>ACTA2</i>	TGTCCACCGCAAATGCTTCT / AGCTTTGGCTAGGAATGATTGGA
<i>ACTN2</i>	GCCAGAGAGAAGGATGCAATCAC / AAGCATGGGAACCTGGAATCAA
<i>COL1A1</i>	GAGGGCCAAGACGAAGACATC / CAGATCAGTCATCGCACAAC
<i>CXCL8</i>	GAGAGTGATTGAGAGTGGACCAC / CACAACCCTCTGCACCCAGTTT
<i>CXCR1</i>	TCCTTTTCCGCCAGGCTTACCA / GGCACGATGAAGCCAAAGGTGT
<i>CXCR2</i>	TCCGTCACGTGATGTCTACCTGC / TCCTTCAGGAGTGAGACCACCT
<i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT / GGCTGTTGTCATACCTTCTCATGG
<i>HRAS</i>	ACGCACTGTGGAATCTCGGCAG / TCACGCACCAACGTGTAGAAGG
<i>KRAS</i>	AGTGCCTTGACGATACAG / GCATCATCAACACCCTGTCTT
<i>LZTR1</i>	GAGCCAACTCAAGGAGCACT / CAATGTCCACTGGCTGGTCC
<i>MRAS</i>	CCACCATTGAAGACTCCTACCTG / ACGGAGTAGACGATGAGGAAGC
<i>MYH6</i>	ACCTGGTGGACAAGCTGCAA / CTCCTTCCTTGTCATCGGGCA
<i>MYH7</i>	TGTAGACACACTTGAGTAGCCC / CACCTTGCGGAACCTGGACA
<i>NPPA</i>	CAACGCAGACCTGATGGATTT / AGCCCCGCTTCTTCATTC
<i>NPPB</i>	TGGAAACGTCCGGGTACAG / CTGATCCGGTCCATCTTCCT
<i>NRAS</i>	GGCAATCCCATAACAACCCTGAG / GAAACCTCAGCCAAGACCAGAC
<i>PECAM1</i>	GCACCGCCTGTGAAATACCA / CATCTGTGCTTGTCCACCTTCA
<i>POSTN</i>	CACGCAAGCCATTATCTCTCCA / ACCCACTCATATAGAAATGTGCAAAGC
<i>RIT1</i>	TTCATCAGCCACCGATTCCC / GCAGGCTCATCATCAATACGG
<i>RPL37A</i>	GTGGTTTCCTGCATGAAGACAGTG / TTCTGATGGCGGACTTTACCG
<i>SOX2</i>	TGGCGAACCATCTCTGTGGT / CCAACGGTGTCAACCTGCAT
<i>TNNT2</i>	GGCAGCTCCTGTTTGAAATG / TTATTACTGGTGTGGAGTGGGTGTG
<i>TUBB5</i>	CTGGACCGCATCTCTGTGTACT / GCCAAAAGGACCTGAGCGAACA

Nanostring nCounter Elements TagSet panel

Gene	Identifier	Gene	Identifier
<i>GAPDH</i>	NM_001256799.1	<i>NR4A1</i>	NM_173157.1
<i>POL2RA</i>	NM_000937.2	<i>ADRB1</i>	NM_000684.1
<i>TBP</i>	NM_001172085.1	<i>ADRB2</i>	NM_000024.3
<i>HPRT</i>	NM_000194.1	<i>CAMK2A</i>	NM_171825.1
<i>NKX2-5</i>	NM_004387.3	<i>CAMK2D</i>	NM_172127.1
<i>NR2F2</i>	NM_021005.2	<i>ATP5F1A</i>	NM_001001937.1
<i>GATA4</i>	NM_002052.3	<i>SLC2A1</i>	NM_006516.2
<i>HEY1</i>	NM_012258.3	<i>SLC2A4</i>	NM_001042.2
<i>HEY2</i>	NM_012259.2	<i>SLC27A1</i>	NM_198580.1
<i>HCN4</i>	NM_005477.2	<i>MYOM1</i>	NM_003803.3
<i>TNNT2</i>	NM_001276346.1	<i>CRYAB</i>	NM_001885.1
<i>TNNI1</i>	NM_003281.3	<i>CKM</i>	NM_001824.4
<i>TNNI3</i>	NM_000363.4	<i>GATA6</i>	NM_005257.3
<i>TTN</i>	NM_133432.1	<i>NFATC1</i>	NM_172389.1
<i>TTN-N2B</i>	NM_003319.4	<i>NFATC3</i>	NM_004555.2
<i>TTN-N2BA</i>	NM_001256850.1	<i>JUN</i>	NM_002228.3
<i>MYH6</i>	NM_002471.3	<i>JUND</i>	NM_005354.4
<i>MYH7</i>	NM_000257.2	<i>FOS</i>	NM_005252.2
<i>MYL7</i>	NM_021223.2	<i>MYC</i>	NM_002467.3
<i>MYL2</i>	NM_000432.3	<i>ELK1</i>	NM_005229.3
<i>RYR2</i>	NM_001035.2	<i>ELK4</i>	NM_001973.3
<i>CASQ2</i>	NM_001232.3	<i>ETS1</i>	NM_005238.3
<i>ATP2A2</i>	NM_001681.3	<i>CCND1</i>	NM_053056.2
<i>PLN</i>	NM_002667.3	<i>TP53</i>	NM_000546.2
<i>SLC8A1</i>	NM_021097.1	<i>MRAS</i>	NM_012219.3
<i>SCN5A</i>	NM_198056.2	<i>HRAS</i>	NM_005343.2
<i>CACNA1C</i>	NM_199460.2	<i>KRAS</i>	NM_004985.3
<i>KCNA5</i>	NM_002234.2	<i>NRAS</i>	NM_002524.3
<i>KCNQ1</i>	NM_181798.1	<i>RIT1</i>	NM_006912.4
<i>KCNH2</i>	NM_172057.2	<i>RRAS</i>	NM_006270.3
<i>NPPA</i>	NM_006172.2	<i>RRAS2</i>	NM_001102669.2
<i>NPPB</i>	NM_002521.2	<i>RAF1</i>	NM_002880.3
<i>MEF2C</i>	NM_002397.3	<i>MAP2K1</i>	NM_002755.2
<i>MEF2D</i>	NM_001271629.1	<i>MAP2K2</i>	NM_030662.2
<i>RBM20</i>	NM_001134363.1	<i>MAPK3</i>	NM_001040056.1
<i>MYBPC3</i>	NM_000256.3	<i>MAPK1</i>	NM_138957.2

Guide RNA target sequences

guideRNA	Sequence (5'-3', PAM in bold)
<i>COL1A2</i> -tagging	GCTGCTCAGTATGATGGAAA AGG
<i>LZTR1</i> exon 1	AGCACGGGGGGGGCAGATCG GGG
<i>LZTR1</i> exon 17	CCAGGTATGCCTTCATGTC CTGG
<i>LZTR1</i> intron 16	CTTGATCATGAGGTCAGTG AGG
<i>MRAS</i> exon 2	TCTGGAAAACTGGATGGTG AGG
<i>RAF1</i> exon 7	TGGATGTCAACCTCTGCCT CTGG
<i>RIT1</i> exon 5	GGCAGAGTTGACAGCCATG CGGG

siRNA sequences

siRNA	Sequence (5'-3')
siNEG	IDT, 51-01-14-04
siRIT1	AGAACAGUAUUCUCAGUACAAGCTT
siRIT1	AUACCGCUACUAUAUUGAUGAUGTT
siRIT1	GCUAAGACAGGUCACCAAGGAAGAA

Critical kits

Kit name	Vendor	Identifier
BCA Protein Assay Kit	Thermo Fisher Scientific	23225
dsDNA 905 Reagent Kit	Fragment Analyzer, Advanced Bioanalytical	NC1923347
Human XL Cytokine Array Kit	Bio-technie R&D systems	ARY022B
NucleoSpin RNA kit	Machery-Nagel	740955.50
P3 Primary Cell 4D-Nucleofector X Kit	Lonza	V4XP-9096
PamChip array	PamGene	87102
RNA isolation kit	Promega	Z3105
Trans-Blot Turbo RTA Transfer kit	Bio-Rad	1704272
TriFECTa RNAi Kit hs.Ri.RIT1.13	IDT	51-01-08-03
TruSeq Stranded Total RNA Kit	Illumina	NRS-122-2001

Software used for analysis

Description	Vendor	Version number	RRID/URL
biomaRt	Bioconductor	v2.32.1	RRID:SCR_019214
BioNavigator software	PamGene	v6.3	https://pamgene.com/technology/
CellPathfinder	Yokogawa Electric Corporation	v3.07.01.06	https://www.yokogawa.com/solutions/product-s-and-services/life-science/high-content-analysis/analysis-software/cellpathfinder/#Details
Circa	OMGenomics Labs	v1.0	https://circa.omgenomics.com
ClueGO	INSERM UMRS 1138, Paris, France	v2.5.10	RRID:SCR_005748
Cytoscape	Cytoscape Consortium	v3.10.0	RRID:SCR_003032
FastQC	Babraham Bioinformatics	0.11.5	RRID:SCR_014583
GenomeStudio software	Illumina	v2.0	https://www.illumina.com/products/by-type/informatics-products/microarray-software/genomestudio.html
GraphPad Prism	GraphPad Software, San Diego, CA, USA	v10	https://www.graphpad.com
ImageJ	NIH, Bethesda, MD, USA	v1.53	https://imagej.net (RRID:SCR_003070)
Incucyte Analysis Software	Sartorius, Göttingen, Germany	v2022A	https://downloads.essenbioscience.com/get/incucyte-2022a-gui
nSolver Analysis Software	NanoString Technologies	v 4.0	RRID:SCR_003420

Perseus software	Max Planck Institute of Biochemistry, Germany	v1.6.15	RRID:SCR_015753
Spectronaut Software	Biognosys	v14.2	https://biognosys.com/software/spectronaut/

Critical reagents used in experiments

CRISPR-Cas9 and Transfection		
Reagent	Vendor	Identifier
Alt-R CRISPR-Cas9 tracrRNA	IDT DNA Technologies	1075927
Alt-R Hifi SpCas9 Nuclease 3NLS	IDT DNA Technologies	1081060
Lipofectamine 3000	Thermo Fisher Scientific	L3000008
Lipofectamine Stem Transfection Reagent	Thermo Fisher Scientific	STEM00008
P3 Primary Cell 4D-Nucleofector X Kit	Lonza	V4XP-3024
Polybrene Transfection Reagent	Merck	TR-1003
Cell Culture Media and Supplements		
Reagent	Vendor	Identifier
2× DMEM	Thermo Fisher Scientific	12800017
B27 supplement	Thermo Fisher Scientific	12587010
DMEM with 25 mM glucose	Thermo Fisher Scientific	11965092
Endothelial Growth Medium 2	Lonza	CC-3162
Fibrolife	Cellsystems	LL-0001
Opti-MEM	Thermo Fisher Scientific	31985070
RPMI 1640 with GlutaMAX and HEPES	Thermo Fisher Scientific	72400047
RPMI 1640 without glucose	Thermo Fisher Scientific	11879020
StemFlex medium	Thermo Fisher Scientific	A3349401
StemMACS iPS-Brew XF medium	Miltenyi Biotec	130-104-368
Proteins, Growth Factors and Cytokines		
Reagent	Vendor	Identifier
BMP4 (10 ng/ml)	Miltenyi Biotec	130-111-165
Bovine Serum Albumin (BSA)	Merck	A7906
Fetal bovine serum (FBS)	Thermo Fisher Scientific	10270106
FGF-basic	PepproTech	100-18B
Horse serum	Thermo Fisher Scientific	16050122
Human recombinant albumin	Merck	A9731
IL-8	Miltenyi Biotec	130-093-943
VEGF (50 ng/ml)	Miltenyi Biotec	130-109-385
Small Molecules and Inhibitors		
Reagent	Vendor	Identifier
AZD5069	Hycultec	HY-19855
CHIR99021	Merck	SML1046
Danirixin	Hycultec	HY-19768
H1152P	Biomol	SYN-1221-M001
IWP2	Merck	681671
Lactate	Merck	L7900
Ladarixin	Hycultec	HY-19519
L-ascorbic acid 2-phosphate	Merck	A8960
Latrunculin A	Enzo Life Sciences	BML-T119-0100
Navarixin	Hycultec	HY-10198
Reparixin	Merck	SML2655

Retinoic acid	Merck	R2625
SB431542	Miltenyi Biotec	130-106-543
Thiazovivin	Merck	SML1045
Cell Processing Reagents		
Reagent	Vendor	Identifier
0.25% Trypsin/EDTA	Thermo Fisher Scientific	25200072
Accutase	Merck	A6964
Cultrex rat tail collagen type I	R&D Systems	3440-100-01
Matrigel (growth factor reduced)	BD Biosciences	354230
Collagenase I	Merck	C0130
DNase	Calbiochem	260913
StemPro Accutase	Thermo Fisher Scientific	A1110501
TrypLE Express	Thermo Fisher Scientific	12605028
Versene solution	Thermo Fisher Scientific	15040066
Staining		
Reagent	Vendor	Identifier
Roti-Histofix 4%	Carl Roth	P087.3
Fluoromount-G	Thermo Fisher Scientific	00-4958-02
Triton X-100	Carl Roth	3051.3
Hoechst 33342	Thermo Fisher Scientific	H1399
Viability and Cell Analysis		
Reagent	Vendor	Identifier
Calcein AM	Thermo Fisher Scientific	C1430
Propidium iodide	Merck	P4170
Protein work		
Reagent	Vendor	Identifier
4-12% NuPAGE Novex Bis-Tris Minigel	Thermo Fisher Scientific	NP0321BOX
Glycerol	Merck	G5516
IGEPAL CA-630	Merck	I8896
Mini-PROTEAN TGX Precast Gels	Bio-Rad	4561083
M-PER Mammalian Protein Extraction Reagent	Thermo Fisher Scientific	78501
Phosphatase inhibitor cocktail 3	Merck	P0044
Protease and phosphatase inhibitor	Thermo Fisher Scientific	78440
RIPA buffer	Thermo Fisher Scientific	89900
Tris/glycine/SDS buffer (1×)	Bio-Rad	1610732
Tris/HCl	Carl Roth	4855.2
RNA and cDNA work		
Reagent	Vendor	Identifier
M-MLV Reverse Transcriptase	Thermo Fisher Scientific	28025013
Oligo d(T)16	Thermo Fisher Scientific	N8080128
QuantiFluor dsDNA System	Promega	E2670
ROX Passive Reference Dye	Bio-Rad	1725858
SYBR Green PCR master mix	Bio-Rad	1725271