

Supplemental Methods

In vivo mouse studies – A subset of WT and qv^{4J} animals were administered the STAT3 inhibitor S3I-201 *in-vivo* at 20mg/kg daily intraperitoneal for 14 days (1). Transaortic constriction (TAC) was performed on a subset of WT mice for 6 weeks to induce chronic pressure-overload and heart failure, as described (1, 2). Cardiac performance was assessed prior to surgery and at 6 weeks post TAC using Vevo 2100 (Visualsonics). Specifically, cardiac function was determined using the MS-400 transducer in the short axis M-mode. For heart extractions, mice were anesthetized with 2.5% gaseous isoflurane and oxygen and sacrificed with confirmation by the absence of response to probing of the lower extremities.

Immunoblot analysis – Primary ventricular cardiac fibroblast lysate was analyzed using SDS-PAGE and immunoblotting, as described (1, 3, 4). Briefly, equal protein loading was achieved using standard BCA protein assay protocols and verified by Ponceau staining of immunoblots. The following antibodies were used for immunoblotting: Total STAT3 (1:1000, Cell Signaling, Catalog #: 4904), vimentin (1:1000, Abcam, Catalog # ab92547:) and GAPDH (1:5000, Fitzgerald, Catalog #: 10R-G109A).

Immunofluorescence – Cardiac fibroblasts were seeded in 35 mm Matex glass bottom dishes until ~50-70% confluency. Cells were then fixed in 4% paraformaldehyde and permeabilized with TritonX-100 for 10 mins. Non-specific binding was blocked using blocking buffer comprised of 1.5% bovine serum albumin (BSA), 3% goat serum in 1xPBS (Invitrogen) overnight at 4°C. Primary antibodies [β_{IV} -spectrin (1:100, Millipore, Clone# N393/76), total STAT3 (1:100, Cell Signaling, Catalog#: 4904), plakoglobin/anti-gamma catenin (1:400, Abcam, Catalog #: ab15153)] were prepared in blocking buffer and added for overnight incubation at 4°C. After washing, secondary Alexa Flour 568 anti-mouse (1:200, Invitrogen, Catalog #: A11004), 466 anti-rabbit (1:200, Invitrogen, Catalog #: A11008), and 633 phalloidin (1:200, Invitrogen, Catalog #: A22284) antibodies were prepared in blocking buffer and incubated for 2 hrs at 4°C. After washing, DAPI (Vecta Laboratories) was applied and dishes were stored at 4°C in the dark until they were imaged using confocal microscopy. Multiple random cells were selected from each field/preparation for analysis. STAT3 localization analysis was conducted by extracting color bands (blue and green) from images and removing background noise with a 3x3 median filter in MATLAB. Threshold values were set, and binary images were generated, as described (3).

Histology – Whole hearts were fixed in 10% formalin, trimmed to reveal ventricles, processed into paraffin, and serially sectioned using microtome into 5 microns. Longitudinal heart sections were then stained using Masson's Trichrome to evaluate amounts of interstitial and perivascular fibrosis. Percent fibrosis was quantified across the entire longitudinal section (not including atria) using custom software in MATLAB.

Quantitative real-time PCR (qPCR) – Total RNA from mouse or human primary cardiac fibroblasts was extracted with TRIzol Reagent following manufacturer's instructions. RNA

concentration was measured using Agilent 2100 Bioanalyzer (Agilent Technologies). SuperScript III reverse transcriptase VILO cDNA Synthesis kit (Invitrogen) was used for the first-strand complementary DNA synthesis (20ng/ μ L) (primers for mouse and human targets provided in Tables S4 and S5). qPCR reactions were performed in triplicate on cDNA samples in 96-well optical plates with SYBR Green Gene Expression Assays and SYBR Green Universal PCR Master Mix (Invitrogen). qPCR was performed at 95°C for 3 mins., 40 cycles of 95°C for 15 s. and 60°C for 1 min. on Applied Biosystems 7900HT Fast Real-Time PCR System or StepOnePlus Real-Time PCR System (Life Technologies). qPCR data were analyzed using relative standard curve method and 2 delta Ct was used to calculate fold changes in relative gene expression. qPCR products were confirmed by melt-curve analysis, amplicon length, and DNA sequencing. *Rpl-7* levels were used as a normalization control. Experiments were conducted in technical triplicates.

RNA sequencing - RNA was isolated from male and female wildtype (WT) and *qv^{AJ}* mouse primary cardiac fibroblasts and sequenced with Ocean Ridge BioSciences (Deerfield Beach, FL). Mice were sacrificed using isoflurane overdose, hearts were immediately excised, and cardiac fibroblasts were isolated and cultured at passage 1 conditions as previously described. Total RNA was isolated from the cardiac fibroblasts using the TRI Reagent® (Molecular Research Center). Total RNA was quantified by O.D. measurement and assessed for quality on a 1% agarose – 2% formaldehyde gel. The RNA was then treated with RNase free DNase I (Epicentre) and re-purified on RNeasy MinElute columns (Qiagen). The newly digested RNA samples were then assessed for quality by chip-based capillary electrophoresis on Agilent 2100 Bioanalyzer RNA 6000 Pico assays (Agilent Technologies). Amplified cDNA libraries suitable for sequencing were prepared from 200 ng of DNA-free total RNA using the Universal Plus mRNA-Seq Library Prep Kit (NuGEN Technologies, Inc.). The quality and size distribution of the amplified libraries were determined by chip-based capillary electrophoresis on Agilent 2100 Bioanalyzer High Sensitivity DNA assays (Agilent Technologies). Libraries were quantified using the Takara Library Quantification Kit. The libraries were pooled at equimolar concentrations and diluted prior to loading onto a flow-cell on an Illumina cBot. The libraries were extended and bridge amplified to create sequence clusters. The flow cell was transferred to the Illumina HiSeq 4000 instrument and sequenced using 150 nt paired-end reads plus a single index read. Quality-filtered and base-trimmed reads were used for alignment. Sequence alignment was performed using HISAT2 version 2.0.5. The read summarization program featureCounts2 version 1.5.1 was used for exon- and gene-level counting. Normalized RPKM values were calculated from the raw featureCounts read, and were then filtered to retain a list of genes with a minimum of approximately 50 mapped reads in 25% or more samples. The threshold of 50 mapped reads is considered the Reliable Quantification Threshold. The resulting gene list with calculated fold changes was analyzed using Ingenuity Pathway Analysis [QIAGEN (5)]. The software was used to identify highly indicated upstream regulators and disease and function networks.

Supplemental References

1. Unudurthi SD, et al. betaIV-Spectrin regulates STAT3 targeting to tune cardiac response to pressure overload. *J Clin Invest.* 2018;128(12):5561-72.
2. Glynn P, et al. Voltage-Gated Sodium Channel Phosphorylation at Ser571 Regulates Late Current, Arrhythmia, and Cardiac Function In Vivo. *Circulation.* 2015;132(7):567-77.
3. Hund TJ, et al. A betaIV spectrin/CaMKII signaling complex is essential for membrane excitability in mice. *J Clin Invest.* 2010;120(10):3508-19.
4. Hund TJ, et al. beta(IV)-Spectrin regulates TREK-1 membrane targeting in the heart. *Cardiovasc Res.* 2014;102(1):166-75.
5. Kramer A, Green J, Pollard J, Jr., and Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics.* 2014;30(4):523-30.

Supplemental Table 1: Echocardiographic features in wildtype (WT) and qv^{AJ} mice at baseline and WT mice subjected to 6 weeks of transaortic constriction (TAC)

	WT (N=5)	qv^{AJ} (N=5)	WT TAC (N=5)
HR (bpm)	446.71±11.83	392.14±23.24	448.46±8.78
EF (%)	63.24±0.60	49.49±0.85	34.89±2.20
LVID,d (mm)	2.94±0.03	3.34± 0.08*	4.28±0.12*#
LVID,s (mm)	2.02±0.07	2.53±0.07*	3.58±0.13*#
FS (%)	33.24±0.37	24.34±0.47*	16.55±1.19*#
LVAW,d (mm)	0.75±0.02	0.62±0.03	0.95±0.07*#
LVAW,s (mm)	1.19±0.04	0.83±0.05*	1.16±0.09#
LVPW,d (mm)	0.78±0.17	0.632±0.06	0.80±0.10
LVPW,s (mm)	0.96±0.07	0.71±0.03	0.91±0.09

HR = heart rate;

EF = ejection fraction;

LVID,d = left ventricular inner chamber diameter in diastole;

LVID,s = left ventricular inner chamber diameter in systole;

FS = fractional shortening;

LVAW,d = LV anterior wall thickness in diastole;

LVAW,s = LV anterior wall thickness in systole;

LVPW,d = LV posterior wall thickness in diastole;

LVPW,s = LV posterior wall thickness in systole;

*P<0.05 vs. WT

#P<0.05 vs. qv^{AJ}

Supplemental Table 2: Differentially regulated genes in qv^{AJ} fibroblasts with more than twofold difference from wildtype (WT)

Gene name	Description	Entrez gene ID	fold change from WT
<i>Serpina3n</i>	serine (or cysteine) peptidase inhibitor, clade A, member 3N	20716	14.74341
<i>Ang2</i>	angiogenin, ribonuclease A family, member 2	11731	14.61122**
<i>Lrrm4</i>	leucine rich repeat neuronal 4	320974	12.29352
<i>Igll1</i>	immunoglobulin lambda joining 1	404737	11.31642
<i>Angptl7</i>	angiopoietin-like 7	654812	10.29877
<i>Upk3b</i>	uroplakin 3B	100647	8.24076

Rnase2a	ribonuclease, RNase A family, 2A (liver, eosinophil-derived neurotoxin)	93726	7.21412*
<i>Atp10d</i>	ATPase, class V, type 10D	231287	5.85188*
<i>Traj32</i>	T cell receptor alpha joining 32	100124357	5.80643*
<i>Erdr1</i>	erythroid differentiation regulator 1	170942	5.74261**
<i>Col28a1</i>	collagen, type XXVIII, alpha 1	213945	5.49104*
<i>Tagap</i>	T cell activation Rho GTPase activating protein	72536	5.37396*
<i>Rtn4rl2</i>	reticulon 4 receptor-like 2	269295	5.06487*
<i>Ighd1-1</i>	immunoglobulin heavy diversity 1-1	777657	5.00956
<i>Rbp4</i>	retinol binding protein 4, plasma	19662	4.927054
C3	complement component 3	12266	4.688854
Serpina3g	serine (or cysteine) peptidase inhibitor, clade A, member 3G	20715	4.572000
<i>Scn7a</i>	sodium channel, voltage-gated, type VII, alpha	20272	4.526735*
<i>Msln</i>	mesothelin	56047	4.477639
<i>Scin</i>	Scinderin	20259	4.411967
C4b	complement component 4B (Chido blood group)	12268	4.271997*
Alpl	alkaline phosphatase, liver/bone/kidney	11647	4.25337*
<i>Hddc3</i>	HD domain containing 3	68695	4.23735
Grb14	growth factor receptor bound protein 14	50915	4.18844
<i>Wfdc18</i>	WAP four-disulfide core domain 18	14038	4.15534
<i>Olfml1</i>	olfactomedin-like 1	244198	4.1226*
Grem1	gremlin 1	23892	3.989100
<i>Myoz3</i>	myozenin 3	170947	3.969423
Steap4	STEAP family member 4	117167	3.859898
Gda	guanine deaminase	14544	3.853971*
Lbp	lipopolysaccharide binding protein	16803	3.781387*
Des	Desmin	13346	3.720956
Gpm6a	glycoprotein m6a	234267	3.594891
Wt1	Wilms tumor 1 homolog	22431	3.582742
<i>Hlf</i>	hepatic leukemia factor	217082	3.54016*
<i>Hist1h2af</i>	histone cluster 1, H2af	319173	3.536802*
<i>Prelp</i>	proline arginine-rich end leucine-rich repeat	116847	3.382093**
Slit3	slit homolog 3	20564	3.260345
<i>Diras2</i>	DIRAS family, GTP-binding RAS-like 2	68203	3.25526*
<i>Pdlim3</i>	PDZ and LIM domain 3	53318	3.208513
<i>Slc16a2</i>	solute carrier family 16 (monocarboxylic acid transporters), member 2	20502	3.192105*
Cck	Cholecystokinin	12424	3.169123
<i>Olfml3</i>	olfactomedin-like 3	99543	3.118997*
<i>C1s1</i>	complement component 1, s subcomponent 1	50908	3.068953
Ptn	Pleiotrophin	19242	3.045265
<i>Upk1b</i>	uroplakin 1B	22268	3.036640
<i>Hpgd</i>	hydroxyprostaglandin dehydrogenase 15 (NAD)	15446	2.988462*
Trf	transferrin	22041	2.984101**

Stc1	stanniocalcin 1	20855	2.984044
Eef1a2	eukaryotic translation elongation factor 1 alpha 2	13628	2.974607**
Agt	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	11606	2.973397*
Aldh11l1	aldehyde dehydrogenase 1 family, member L1	107747	2.948904*
Bmx	BMX non-receptor tyrosine kinase	12169	2.932555
Lrrc17	leucine rich repeat containing 17	74511	2.903739*
L1cam	L1 cell adhesion molecule	16728	2.821812
Fmod	fibromodulin	14264	2.818407*
Sod3	superoxide dismutase 3, extracellular	20657	2.795713***
Abca8a	ATP-binding cassette, sub-family A (ABC1), member 8a	217258	2.784457**
Kif26b	kinesin family member 26B	269152	2.751576
Egr3	early growth response 3	13655	2.728689*
Ugt1a6b	UDP glucuronosyltransferase 1 family, polypeptide A6B	394435	2.697063*
Cpxm1	carboxypeptidase X 1 (M14 family)	56264	2.679917*
Cp	ceruloplasmin	12870	2.661656*
Galnt16	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 16	108760	2.658979*
Sh2d4a	SH2 domain containing 4A	72281	2.627055
Dpt	dermatopontin	56429	2.588892**
Cfi	complement component factor i	12630	2.582797**
Ugt1a9	UDP glucuronosyltransferase 1 family, polypeptide A9	394434	2.582534*
Rai2	retinoic acid induced 2	24004	2.578047
Sfrp1	secreted frizzled-related protein 1	20377	2.56157*
Ugt1a6a	UDP glucuronosyltransferase 1 family, polypeptide A6A	94284	2.551891*
Ugt1a8	UDP glucuronosyltransferase 1 family, polypeptide A8		2.532961
Sost	Sclerostin	74499	2.532539
Gpc3	glypican 3	14734	2.529329*
Srpx	sushi-repeat-containing protein	51795	2.523656
Ugt1a10	UDP glycosyltransferase 1 family, polypeptide A10	394430	2.511275*
Ugt1a2	UDP glucuronosyltransferase 1 family, polypeptide A2	22236	2.511275*
Ugt1a1	UDP glucuronosyltransferase 1 family, polypeptide A1	394436	2.501692*
Dpep1	dipeptidase 1 (renal)	13479	2.495092*
Ugt1a5	UDP glucuronosyltransferase 1 family, polypeptide A5	394433	2.492987*
Usp51	ubiquitin specific protease 51	635253	2.474067*
Slc1a3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	20512	2.473677*
Cxcl14	chemokine (C-X-C motif) ligand 14	57266	2.449264**
Ccl8	chemokine (C-C motif) ligand 8	20307	2.448558
Ly6k	lymphocyte antigen 6 complex, locus K	76486	2.399223*
Mest	mesoderm specific transcript	17294	2.381802
Nr4a1	nuclear receptor subfamily 4, group A, member 1	15370	2.378512*
Lppr3	lipid phosphate phosphatase-related protein type 3	216152	2.336948
Dcn	decorin	13179	2.311674**
Gabra3	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3	14396	2.311499*

Myo7a	myosin VIIA	17921	2.303092
Heyl	hairy/enhancer-of-split related with YRPW motif-like	56198	2.293864*
Gas7	growth arrest specific 7	14457	2.276496**
Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	17132	2.273172
Fam167b	family with sequence similarity 167, member B	230766	2.263419*
Sned1	sushi, nidogen and EGF-like domains 1	208777	2.26305*
Pde1a	phosphodiesterase 1A, calmodulin-dependent	18573	2.256116**
Plekha4	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 4	69217	2.256055*
Lum	lumican	17022	2.243506**
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1	13123	2.239900
Mmp19	matrix metalloproteinase 19	58223	2.23726*
Cox4i2	cytochrome c oxidase subunit IV isoform 2	84682	2.234068**
Wisp2	WNT1 inducible signaling pathway protein 2	22403	2.232019*
Dact1	dapper homolog 1, antagonist of beta-catenin (xenopus)	59036	2.229825*
Dnmt3l	DNA (cytosine-5-)-methyltransferase 3-like	54427	2.213772**
Hs3st1	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	15476	2.212726**
Acot1	acyl-CoA thioesterase 1	26897	2.204452*
Ifi205	interferon activated gene 205	226695	2.204013
Ly6c1	lymphocyte antigen 6 complex, locus C1	17067	2.198477*
Itgb8	integrin beta 8	320910	2.194193*
Bcl3	B cell leukemia/lymphoma 3	12051	2.179287*
Samd5	sterile alpha motif domain containing 5	320825	2.178835**
Timp3	tissue inhibitor of metalloproteinase 3	21859	2.172083**
Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	2.165344***
Ugt1a7c	UDP glucuronosyltransferase 1 family, polypeptide A7C	394432	2.162046*
Rtn1	reticulon 1	104001	2.160099*
Prss35	protease, serine 35	244954	2.158280
Rbp1	retinol binding protein 1, cellular	19659	2.150435*
Ms4a4d	membrane-spanning 4-domains, subfamily A, member 4D	66607	2.131072**
Cpxm2	carboxypeptidase X 2 (M14 family)	55987	2.129651
Nkd2	naked cuticle 2 homolog (Drosophila)	72293	2.129614*
Shroom3	shroom family member 3	27428	2.122467**
Icam1	intercellular adhesion molecule 1	15894	2.104725**
Abca8b	ATP-binding cassette, sub-family A (ABC1), member 8b	27404	2.098532**
Nov	nephroblastoma overexpressed gene	18133	2.09235*
F3	coagulation factor III	14066	2.077147**
Vmn1r53	vomer nasal 1 receptor 53	113853	2.070386
Mmp3	matrix metalloproteinase 3	17392	2.069885**
Pcdhb5	protocadherin beta 5	93876	2.046458***
Cxcl5	chemokine (C-X-C motif) ligand 5	20311	2.045955
Mfap2	microfibrillar-associated protein 2	17150	2.042179
Fads6	fatty acid desaturase domain family, member 6	328035	2.034038**

<i>Prickle1</i>	prickle homolog 1 (Drosophila)	106042	2.023104**
<i>Gpx3</i>	glutathione peroxidase 3	14778	2.019856***
<i>Homez</i>	homeodomain leucine zipper-encoding gene	239099	2.015131***
<i>Epha1</i>	Eph receptor A1	13835	2.014361***
<i>Eva1c</i>	eva-1 homolog C (C. elegans)	70967	2.011877**
<i>Adarb1</i>	adenosine deaminase, RNA-specific, B1	110532	2.004542*
<i>Rnls</i>	renalase, FAD-dependent amine oxidase	67795	2.004139
<i>Islr</i>	immunoglobulin superfamily containing leucine-rich repeat	26968	2.003491
<i>Igfbp2</i>	insulin-like growth factor binding protein 2	16008	0.497039*
<i>Eps8l2</i>	EPS8-like 2	98845	0.494132*
<i>Prl8a9</i>	prolactin family8, subfamily a, member 9	67310	0.490824*
<i>Sema3a</i>	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	20346	0.488818**
<i>Col2a1</i>	collagen, type II, alpha 1	12824	0.487204**
<i>Tmem254c</i>	transmembrane protein 254c	100039192	0.486374*
<i>Rspo2</i>	R-spondin 2	239405	0.486292*
<i>Pop1</i>	processing of precursor 1, ribonuclease P/MRP family, (S. cerevisiae)	67724	0.485328***
<i>Tiam2</i>	T cell lymphoma invasion and metastasis 2	24001	0.472904***
<i>Krt80</i>	keratin 80	74127	0.469719**
<i>Coch</i>	coagulation factor C homolog (Limulus polyphemus)	12810	0.457576**
<i>Cspg4</i>	chondroitin sulfate proteoglycan 4	121021	0.441943**
<i>Nppb</i>	natriuretic peptide type B	18158	0.438516
<i>Adcy4</i>	adenylate cyclase 4	104110	0.435801***
<i>Cyp1a1</i>	cytochrome P450, family 1, subfamily a, polypeptide 1	13076	0.433803
<i>Mycl</i>	v-myc myelocytomatosis viral oncogene homolog, lung carcinoma derived (avian)	16918	0.428823*
<i>Ccl5</i>	chemokine (C-C motif) ligand 5	20304	0.418392*
<i>Tll1</i>	tolloid-like	21892	0.417471*
<i>Plac9a</i>	placenta specific 9a	211623	0.414677**
<i>Plac9b</i>	placenta specific 9b	100039246	0.393378
<i>Cdh26</i>	cadherin-like 26	381409	0.400763**
<i>Rnase10</i>	ribonuclease, RNase A family, 10 (non-active)	75019	0.381905***
<i>Nptx2</i>	neuronal pentraxin 2	53324	0.378102**
<i>Trim30a</i>	tripartite motif-containing 30A	20128	0.344244
<i>Hist1h4m</i>	histone cluster 1, H4m	100041230	0.342009**
<i>Peli2</i>	pellino 2	93834	0.337204***
<i>Tmem171</i>	transmembrane protein 171	380863	0.308158*
<i>Ppp1r3e</i>	protein phosphatase 1, regulatory (inhibitor) subunit 3E	105651	0.240354***
<i>Traj11</i>	T cell receptor alpha joining 11	100124392	0.235494
<i>Cd200</i>	CD200 antigen	17470	0.234058

N=4 independent hearts per genotype; *P<0.05, **P<0.01, ***P<0.001 between *qv^{AJ}* and WT fibroblasts; Bolded gene names indicate predicted gene function related to cell quantity/proliferation, cell motility/migration, and/or cell development/differentiation.

Supplemental Table 3: Echocardiographic features in inducible fibroblast-specific β_{IV} -spectrin knockout (β_{IV} -ifKO) and control (floxed, Cre-negative) mice at baseline and following 2 weeks of tamoxifen diet + angiotensin II (Tam+AngII)

	Control Baseline (N=7)	Control Tam+AngII (N=6)	β_{IV} -ifKO Baseline (N=10)	β_{IV} -ifKO Tam+AngII (N=8)
HR (bpm)	375.58±14.41	352.17±11.87	390.86±9.85	353.78±18.12
EF (%)	64.17±1.02	55.25±1.52*	64.97±1.26	44.87±2.19*#
LVID,d (mm)	3.49±0.08	3.73±0.07*	3.47±0.065	3.92± 0.06*
LVID,s (mm)	2.29±0.06	2.77±0.07	2.27±0.06	2.98±0.08
FS (%)	34.25±0.74	28.33±1.01*	34.84±0.91	21.97±1.25*#
LVAW,d (mm)	0.84±0.04	0.77±0.07	0.87±0.04	0.68±0.03*
LVAW,s (mm)	1.27±0.03	1.09±0.07	1.24±0.03	0.95±0.02
LVPW,d (mm)	0.81±0.05	0.66±0.06	0.77±0.02	0.62±0.02
LVPW,s (mm)	1.08±0.06	5.28±2.86	7.30±2.10	5.85±1.96

HR = heart rate;

EF = ejection fraction;

LVID,d = left ventricular inner chamber diameter in diastole;

LVID,s = left ventricular inner chamber diameter in systole;

FS = fractional shortening;

LVAW,d = LV anterior wall thickness in diastole;

LVAW,s = LV anterior wall thickness in systole;

LVPW,d = LV posterior wall thickness in diastole;

LVPW,s = LV posterior wall thickness in systole;

*P<0.05 vs. Baseline

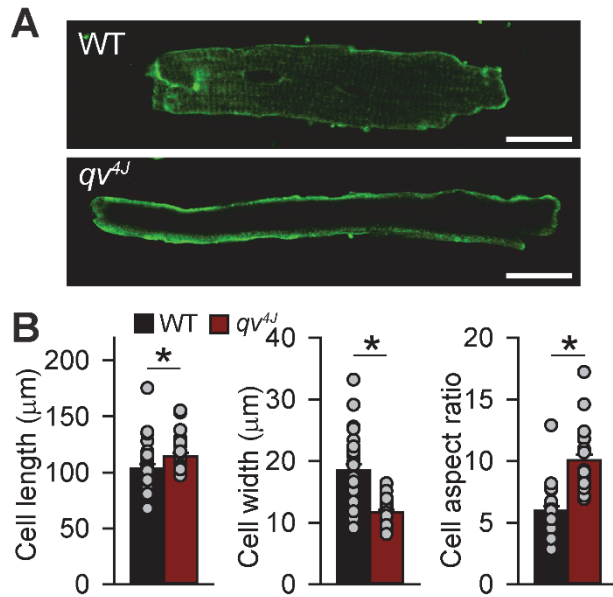
#P<0.05 vs. Control

Supplemental Table 4: Primer sequences for quantitative PCR experiments on mouse fibroblasts

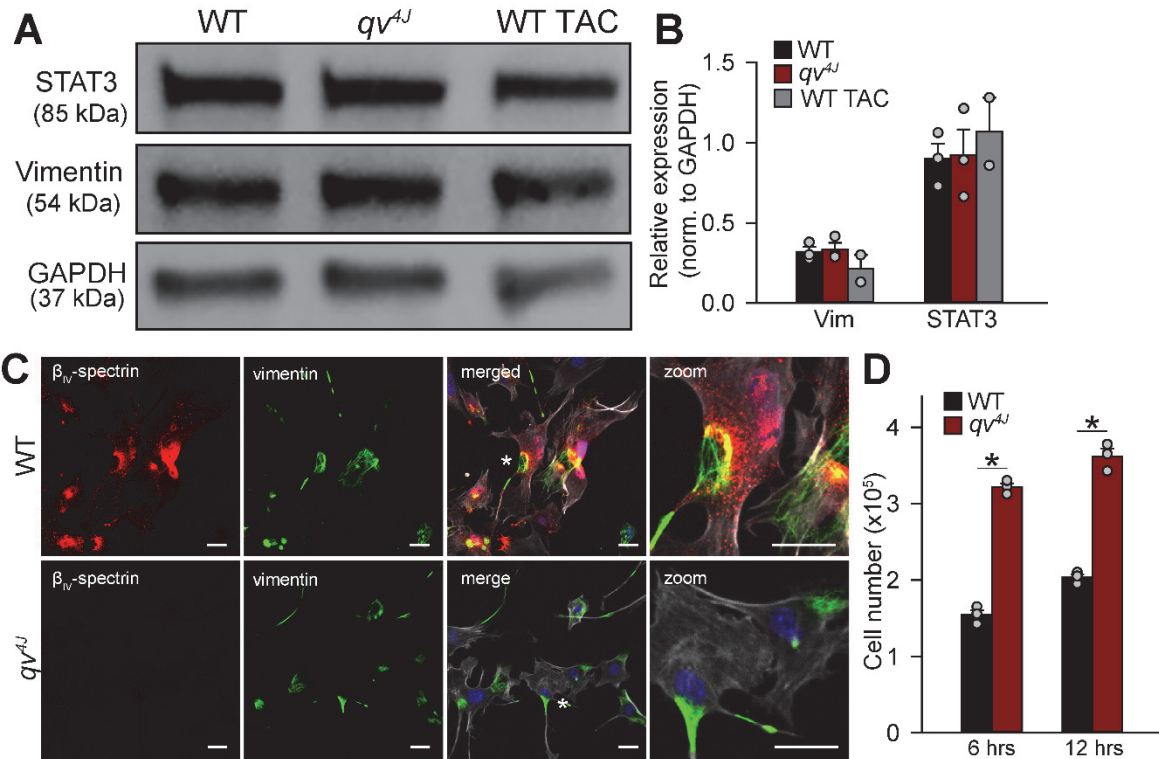
Gene name	Primer direction	Sequence
<i>Agt</i>	Forward	TGGATAGAGAACCCGCCTCC
	Reverse	TTGTTCAAGATTGCCTCCGC
<i>Fmod</i>	Forward	AGAAGATCCCTCCTGTCAACAC
	Reverse	GCAGCTTGGAGAAGTTCATGAC
<i>Icam1</i>	Forward	TTTCTCATGCCGCACAGAAC
	Reverse	TGTCGAGCTTTGGGATGGTAG
<i>Il6</i>	Forward	CCAGAAACCGCTATGAAGTTCC
	Reverse	TCACCAGCATCAGTCCCAAG
<i>Mmp3</i>	Forward	AAGGGTGGATGCTGTCTTTG
	Reverse	GGGTCAAATTCCTCAACTGCGAAG
<i>Rpl7</i>	Forward	TAAGCACCTCCACCGATGAC
	Reverse	CACAGCGGGAACCTTTTTTC
<i>Serpina3n</i>	Forward	GGACATTGATGGTCTGGTGA
	Reverse	CTCTTGCCCGCGTAGAACTC
<i>Postn</i>	Forward	AGTAACGAGGCTTGGGAGAAC
	Reverse	CCGTGTTTCAGGTCCTTGGT

Supplemental Table 5: Primer sequences for quantitative PCR experiments on human fibroblasts

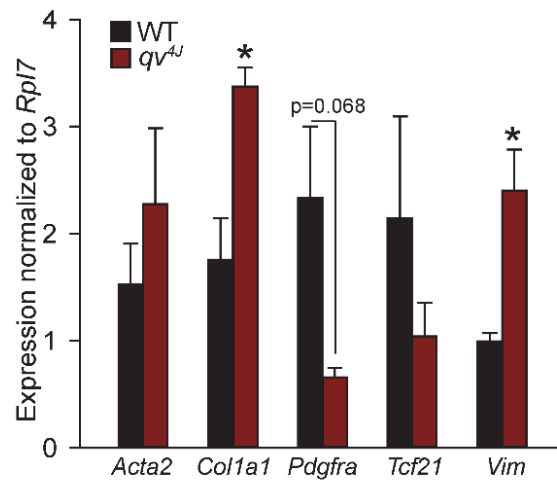
Gene name	Primer direction	Sequence
<i>AGT</i>	Forward	TCCAGCAAAACTCCCTCAAC
	Reverse	GGTCATAAGATCCTTGCAGCAC
<i>FMOD</i>	Forward	CCCCAGTCAACACCAACCTG
	Reverse	GCTGCGCTTGATCTCGTTC
<i>ICAM1</i>	Forward	TGACCGTGAATGTGCTCTCC
	Reverse	TGGCGGTTATAGAGGTACGTG
<i>IL6</i>	Forward	TGAGAGTAGTGAGGAACAAGCC
	Reverse	TTGGGTCAGGGGTGGTTATTG
<i>MMP3</i>	Forward	TCAGAACCCTTTCCTGGCATCC
	Reverse	TCACCTCTTCCCAGACTTTCAG
<i>RPL7</i>	Forward	GGCTTCGATTAACATGCTGAGG
	Reverse	TGCCATAACCACGCTTGTAG
<i>SPTBN4</i>	Forward	ACAACAGAGAGACACGCTCAG
	Reverse	TGAATGAGGGCATTGAAGGC
<i>SERPINA3</i>	Forward	CCTGAAGCGGTGGAGAGACT
	Reverse	CCTCGAGATGGAAAACCTTGGC



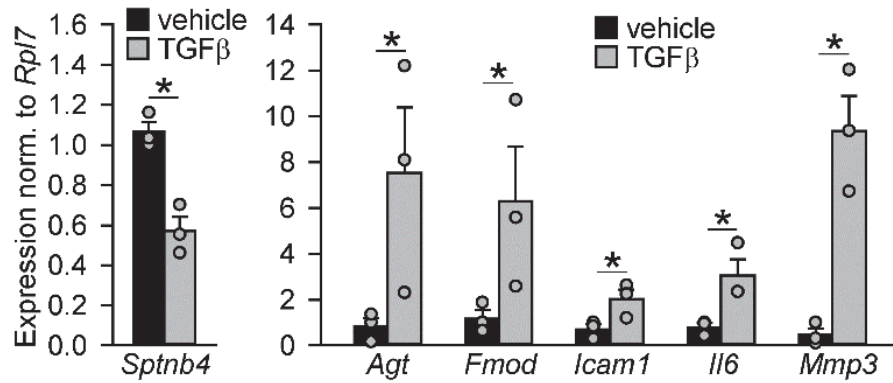
Supplemental Figure 1: Changes in myocyte dimension in *qv^{4J}* myocytes. **(A)** Representative confocal images of isolated WT and *qv^{4J}* cardiomyocytes stained for wheat germ agglutinin (scale bar = 20 μm); and **(B)** summary data (mean±SEM) for myocyte length, width, and aspect ratio measurements. N=29 cells for each genotype. *P<0.05 by two-tailed t-test.



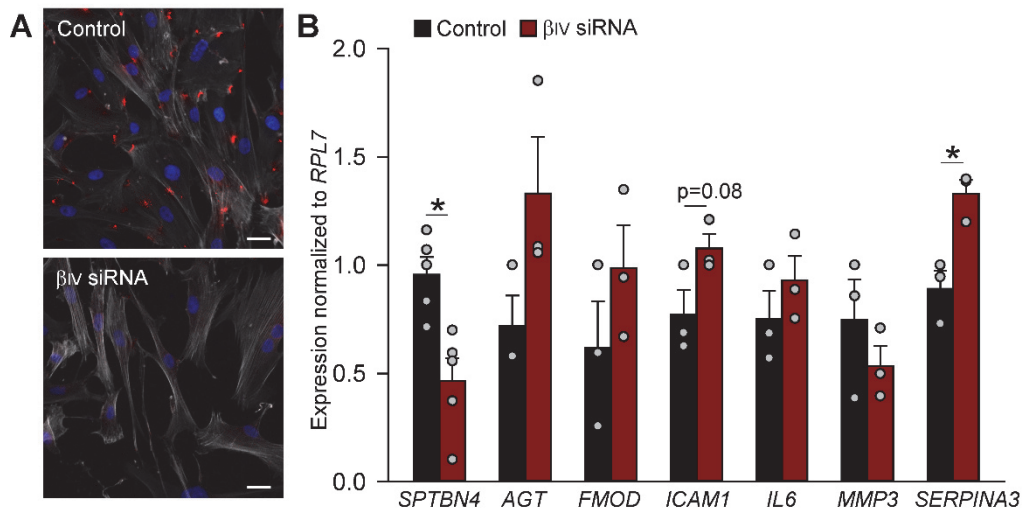
Supplemental Figure 2: Validation of successful fibroblast isolation and proliferation assay. **(A)** Representative immunoblots and **(B)** densitometric measurements for STAT3 and vimentin (Vim) in WT, qv^{4J} and WT TAC cardiac fibroblasts. GAPDH was used as loading control. P=NS by one-way ANOVA; N = 3 independent preparations for WT and qv^{4J} and 2 for WT TAC. **(C)** Representative confocal microscopy images (10x magnification) of permeabilized adult WT and qv^{4J} cardiac fibroblasts immunostained for β_{IV} -spectrin (red), vimentin (green), phalloidin (gray in merged image) and DAPI (blue in merged image). White asterisk in merged image indicates region of zoom shown in far right panel. Scale bar = 20 μ m. **(D)** Numbers of WT and qv^{4J} fibroblasts at 2, 6 and 12 hours following plating at initial cell density of 1×10^5 . N=3 independent preparations for each genotype/time point; *P<0.05 by two-tailed t-test.



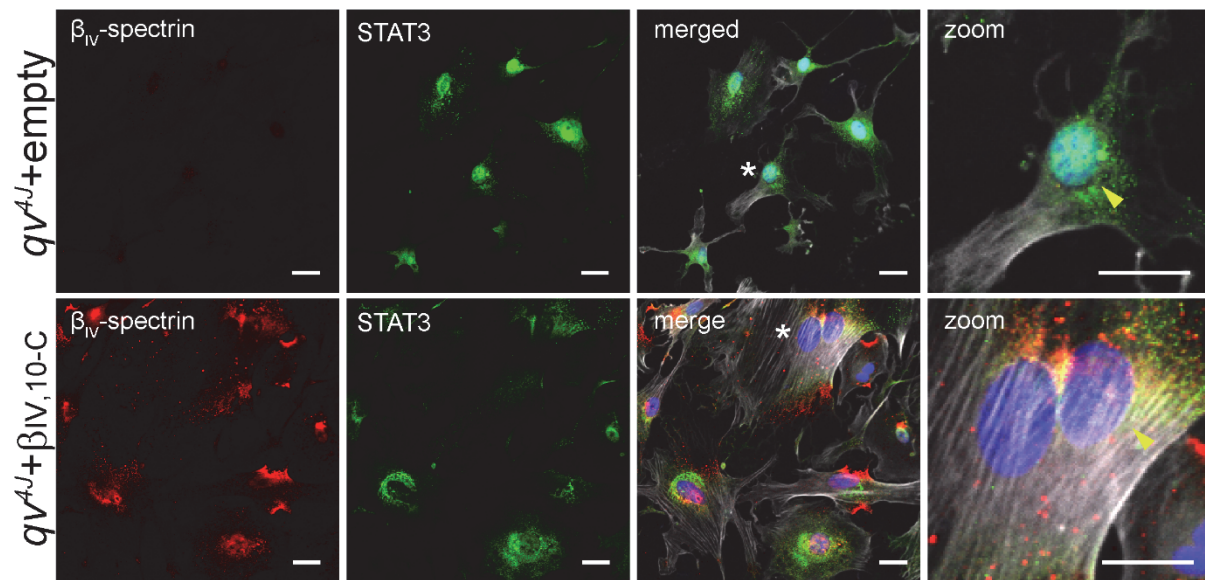
Supplemental Figure 3: Effects of β_{IV} -spectrin deficiency on myofibroblast markers. Expression of select myofibroblast genes (relative to *Rpl7*) determined by quantitative PCR in CFs from WT and *qv^{4J}* mice. Data presented as mean \pm SEM; N=3 for WT, N=4 for *qv^{4J}*, where N is the number of independent preparations; *P<0.05 vs. WT by two-tailed t-test.



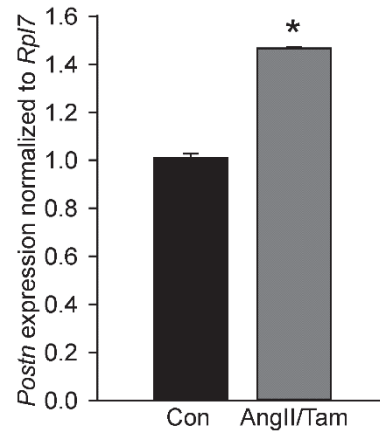
Supplemental Figure 4: TGF- β induces downregulation of β_{IV} -spectrin. Expression of β_{IV} -spectrin and select STAT3 differentially regulated genes with TGF- β and vehicle treatment (relative to *Rpl7*) determined by quantitative PCR in CFs from WT mice. Data presented as mean \pm SEM; N=3, where N is the number of independent preparations; *P<0.05 by two-tailed t-test.



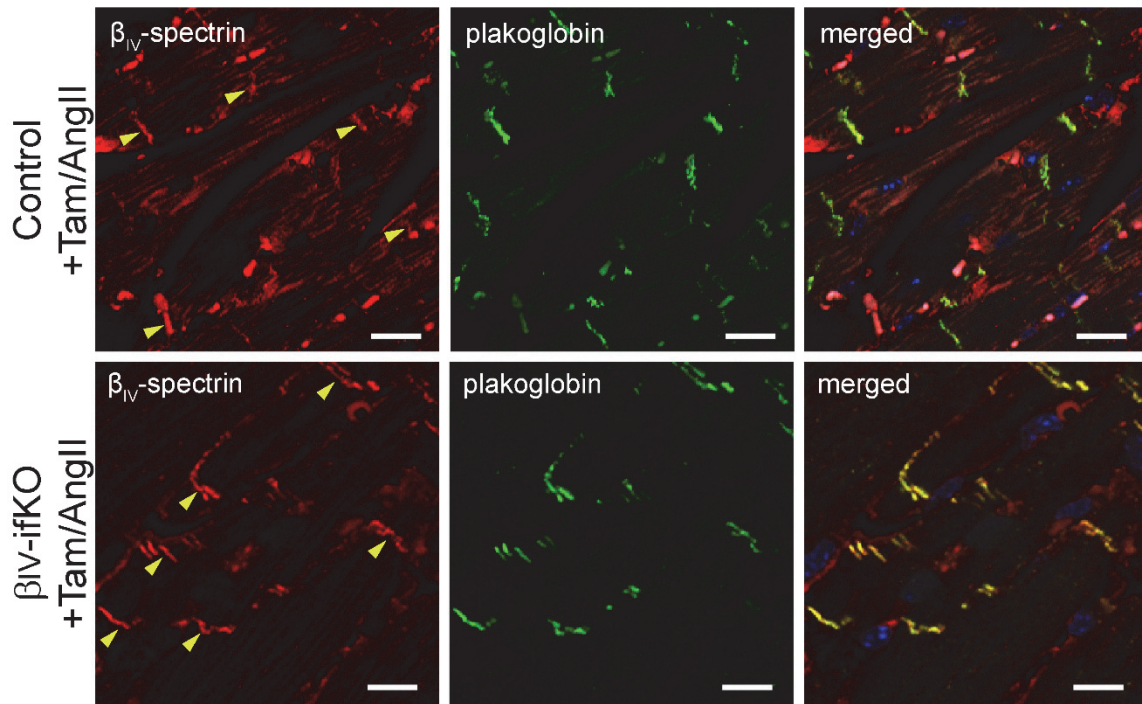
Supplemental Figure 5: Effects of β_{IV} -spectrin deficiency on gene expression in human cardiac fibroblasts. **(A)** Representative confocal microscopy images (10x) of human cardiac fibroblasts (CF) transfected with a combination of two human β_{IV} -spectrin siRNAs or control siRNA. Immunostained for β_{IV} -spectrin (*red*) and phalloidin (*gray*). Scale bar = 20 μ m. **(B)** Expression of select genes (relative to *Rpl-7*) determined by quantitative PCR. Data presented as mean \pm SEM; N=5 for *SPTBN4* and N=3 for other genes for both groups (control and β_{IV} -siRNA) where N is the number of independent preparations; *P<0.05 by two-tailed t-test.



Supplemental Figure 6: Rescue of STAT3 localization in β_{IV} -spectrin-deficient cardiac fibroblasts. Representative confocal microscopy images (10x magnification) of permeabilized adult qv^{4J} cardiac fibroblasts immunostained for β_{IV} -spectrin (*red*), STAT3 (*green*), phalloidin (*grey* in merged image) and DAPI (*blue* in merged image) following transfection of empty vector plasmid or $\beta_{IV,10-C}$. *White asterisk* in merged image indicates region of zoom shown in far right panel. *Yellow arrow* in zoom image indicates nucleus border. Scale bar = 20 mm.



Supplemental Figure 7: Expression of periostin (relative to *Rpl-7*) following angiotensin II and tamoxifen treatment determined by quantitative PCR in CFs from control mice. Data presented as mean \pm SEM; N=3, where N is the number of independent preparations; *P<0.05 by two-tailed t-test.



Supplemental Figure 8: Myocyte β_{IV} -spectrin expression is unaffected in fibroblast-specific β_{IV} -spectrin knockout mouse. Representative confocal microscopy images (40x magnification) of ventricular tissue sections immunostained for β_{IV} -spectrin (*red*), plakoglobin (*green*), and DAPI (*blue* in merged image) from control (β_{IV} -spectrin floxed, Cre-) and inducible fibroblast-specific β_{IV} -spectrin knockout mice (β_{IV} -iffKO, β_{IV} -spectrin floxed x periostin^{MerCreMer}) following 2 weeks of tamoxifen/angiotensin II treatment. Scale bar = 20 μ m.