SUPPLEMENTAL MATERIAL

Supplemental Methods

Evans blue staining

Evans blue staining of cardiomyocytes was used as an indicator of necrosis as previously described[1]. 1% Evans blue dye (in PBS) at a volume of 1% of body mass was injected intraperitoneally 24h prior to sacrifice. Cardiac cryosections from CSILK-KO and control mice were then stained with α-actinin and DAPI.

MMP activity

Enzymatic activity of MMP-2 and MMP-9 was determined by gelatin zymography of cardiac tissue homogenate, as described[2]. In brief, 40 µg of protein from heart homogenate were electrophoresed through a 10% polyacrylamide gel copolymerized with gelatin (Bio-Rad). The gels were washed with renaturing buffer and incubated for 30 minutes at room temperature, and incubated overnight at 37°C in and developing buffer (Bio-Rad). After incubation, the gels were stained with 0.5% Coomassie Blue R-250 (Bio-Rad) for 30 minutes, then de-stain the gel. Gelatinolytic activities were detected as transparent bands against the dark blue background. Zymograms were digitally scanned, and band intensities were quantified using NIH image J.

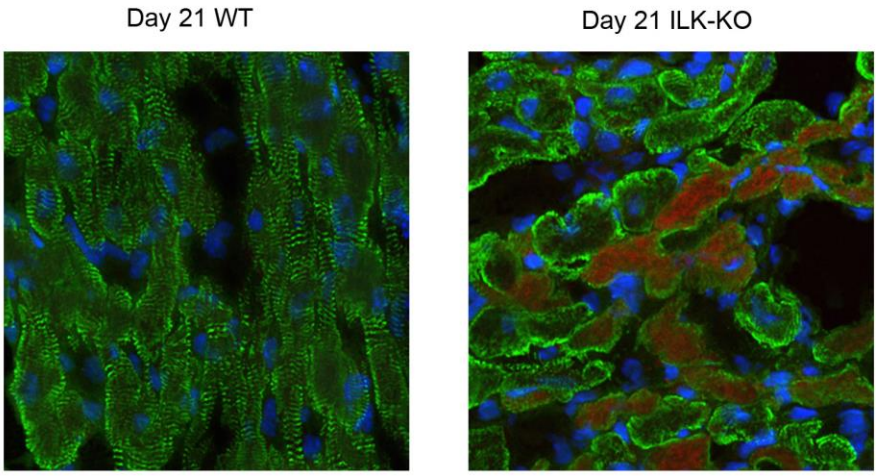
Supplemental Table 1. Primer sequence of QRT-PCR

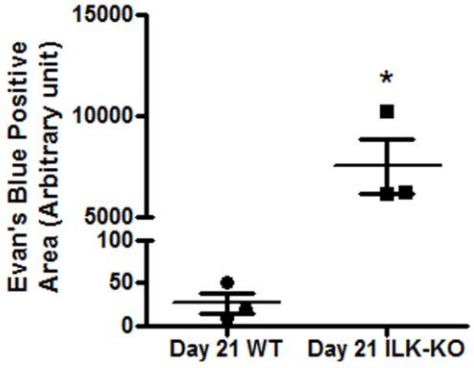
Symbol	Full name		Sequence
Acta1	Actin, alpha 1, skeletal muscle	Forward	AGTGCGACATCGACATCAGG
		Reverse	GGAGCCAGAGCTGTGATCT
Ankrd1	Ankyrin repeat domain 1 (cardiac muscle)	Forward	ATGCCAAGGACAGAGAAGGA
		Reverse	TCTCCTTGAGGCTGTCGAAT
Clu	Clusterin	Forward	AGCAGGAGGTCTCTGACAATG
		Reverse	GGCTTCCTCTAAACTGTTGAGC
Col1a2	Col1a2	Forward	CTGGAACAAATGGGCTCACTG
		Reverse	CAGGCTCACCAACAAGTCCTC
Ctgf	Connective tissue growth factor	Forward	GGGCCTCTTCTGCGATTTC
		Reverse	ATCCAGGCAAGTGCATTGGTA
Cxcl4	Chemokine (C-X-C motif) ligand 4	Forward	GTTGTTTCTGCCAGCGGTGGTT
		Reverse	TTCACACACACACAGCTAAGA
Igf1	Igf1	Forward	CTGGACCAGAGACCCTTTGC
		Reverse	GGACGGGGACTTCTGAGTCTT
Mgp	Matrix Gla protein	Forward	GGCAACCCTGTGCTACGAAT
		Reverse	CCTGGACTCTCTTTTGGGCTTTA
Nppa	Natriuretic peptide precursor type A	Forward	GCTTCCAGGCCATATTGGAG
		Reverse	GGGGGCATGACCTCATCTT
Nppb	Natriuretic peptide precursor type B	Forward	CCCAAAAAGAGTCCTTCGGTC
		Reverse	CGGTCTATCTTGTGCCCAAAG

Nr4a1	Nuclear receptor subfamily 4, group A, member 1	Forward	CCTCATCACTGATCGACA
		Reverse	AGCCATGTGCTCCTTCAGACAG
Perp	PERP, TP53 apoptosis effector	Forward	ATCGCCTTCGACATCATCGC
		Reverse	CCCCATGCGTACTCCATGAG
Plxdc1	Plxdc1	Forward	AGTTCTCACCGACAGGCTTCAA
		Reverse	GTTGCTATGGTGATCTGC
Postn	Periostin, osteoblast specific factor	Forward	CCTGCCCTTATATGCTCTGCT
		Reverse	AAACATGGTCAATAGGCATCACT
Ppara	Ppara	Forward	AGAGCCCCATCTGTCCTCTC
		Reverse	ACTGGTAGTCTGCAAAACCAAA
Ppm1k	Protein phosphatase 1K	Forward	CTTTGTCAACCAGTGCCACGAT
		Reverse	TATTGCCTGCTCAGTCAC
Ptgds	Prostaglandin D2 synthase (brain)	Forward	GCTCCTGGACACTACACCTAC
		Reverse	CTTGGTGCCTCTGCTGAATAG
Spp1	Secreted phosphoprotein 1	Forward	ATCTCACCATTCGGATGAGTCT
		Reverse	TCAGTCCATAAGCCAAGCTATCA
Sqstm1	Sequestosome 1	Forward	ATGTGGAACATGGAGGGAAGA
		Reverse	GGAGTTCACCTGTAGATGGGT
TaoK2	TAO kinase 2	Forward	GCATCCTAATACCATTCAGTAC
		Reverse	AAGCTGAGCCCAGGCAATACTC
Thbs4	Thrombospondin 4	Forward	CTGCCACAAGCACAGGAGA
		Reverse	TGACCTGCTGCCTCAGAAGA
Timp1	Tissue inhibitor of metalloproteinase 1	Forward	GCAACTCGGACCTGGTCATAA
		Reverse	CGGCCCGTGATGAGAAACT

Tnc	Tenascin C	Forward	ACGGCTACCACAGAAGCTG
		Reverse	ATGGCTGTTGTTGCTATGGCA
Tnf	Tumor necrosis factor	Forward	CCCTCACACTCAGATCATCTTCT
		Reverse	GCTACGACGTGGGCTACAG
Wisp1	WNT1 inducible signaling pathway protein 1	Forward	CAGCACCACTAGAGGAAACGA
		Reverse	CTGGGCACATATCTTACAGCATT
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Forward	TGGTGAAGCAGGCATCTGAG
		Reverse	TGCTGTTGAAGTCGCAGGAG

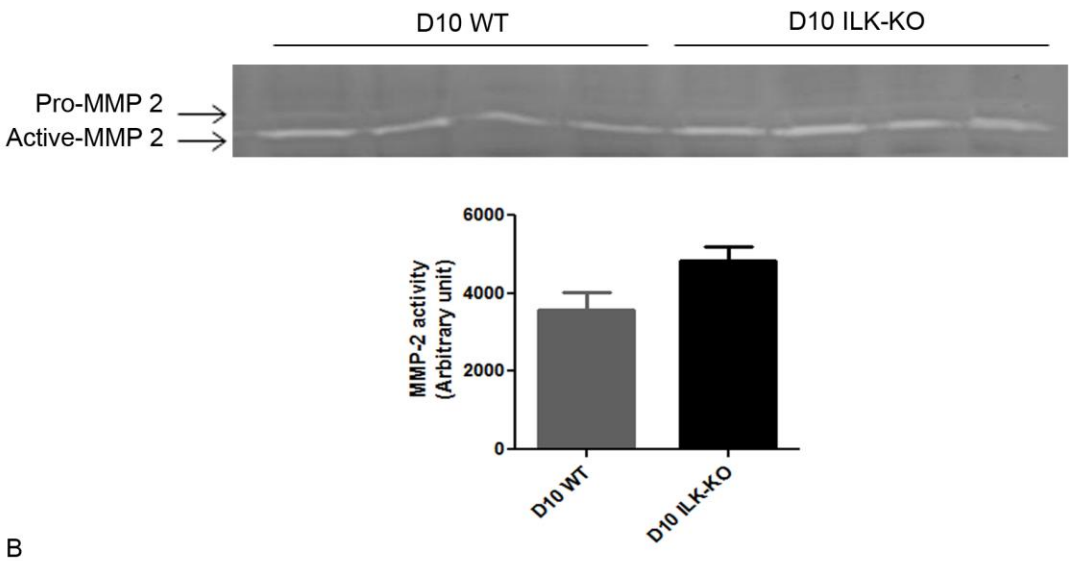
Supplemental Figure 1



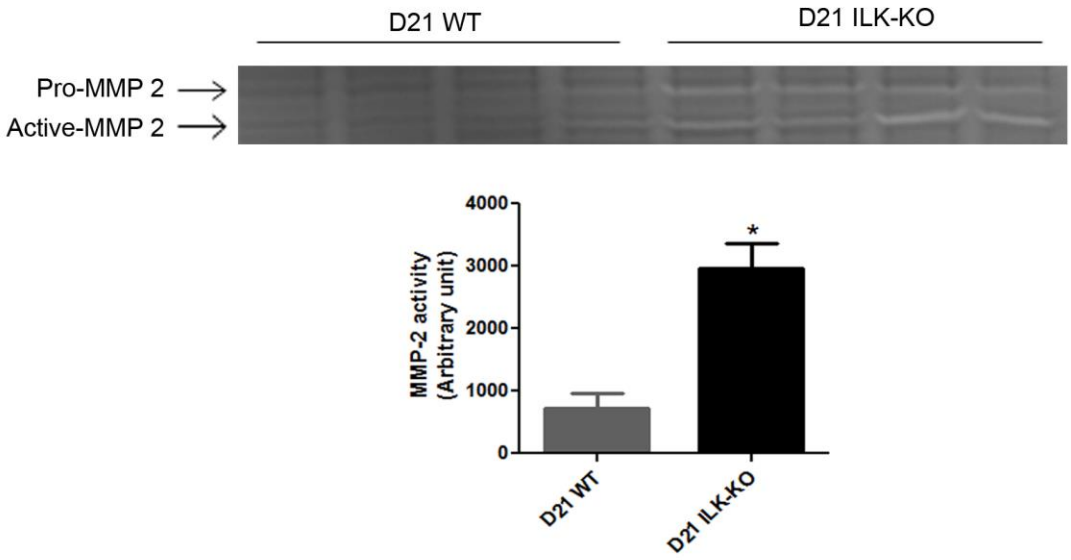


Supplemental Figure 2

A



B



Supplemental Figure Legends

Supplemental Figure 1. Increased necrosis in CSILK-KO hearts. Co-localization of Evans blue (red auto-fluorescence) and α-actinin staining (green) in cardiomyocytes of 21 day old CSILK-KO 24 h after i.p. injection of 1% Evans blue indicates an increase in cardiomyocyte necrosis. Representative results from three independent experiments are shown in upper panel with corresponding quantitation shown in the lower panel (*p < 0.05 vs. littermate controls).

Supplemental Figure 2. Increased MMP activity in CSILK-KO hearts. Gelatin zymography showed increased pro-MMP2 and active MMP2 levels in hearts of 10 (A) and 21 day old (B) CSILK-KO mice compared with littermate controls. Quantitation shown on the lower panel, *p < 0.05 vs. WT, n=6.

References

1. Miller DL, Li P, Dou C, Armstrong WF, Gordon D. Evans blue staining of cardiomyocytes induced by myocardial contrast echocardiography in rats: evidence for necrosis instead of apoptosis. *Ultrasound Med Biol.* 2007;33:1988-1996.

2. Hu X, Beeton C. Detection of functional matrix metalloproteinases by zymography. *J Vis Exp.* 2010;45:2445.