

## Supplemental Material

### **Fibronectin contributes to pathological cardiac hypertrophy but not physiological growth**

Mathias H. Konstandin, MD<sup>1</sup>; Mirko Völkens, MD<sup>1</sup>; Brett Collins, BS<sup>1</sup>; Pearl Quijada, MS<sup>1</sup>; Mercedes Quintana<sup>1</sup>; Andrea De La Torre<sup>1</sup>; Lucy Ormachea<sup>1</sup>; Shabana Din, BS<sup>1</sup>; Natalie Gude, PhD<sup>1</sup>; Haruhiro Toko, MD<sup>1</sup>; Mark A. Sussman, PhD<sup>1</sup>

Short title: Konstandin, Fibronectin in cardiac hypertrophy

### Supplemental Figure 1

Schematic depicting the experimental design of the study for wheel running **(a)** or transaortic constriction **(b)**. **(c)** Heart rate assessment during echocardiography. **(d)** Efficiency of the global knockdown is depicted for the listed organs measured by RT-PCR. \*\*\*: p<0.001; \*\*: p<0.01; \*: p<0.05 compared to control.

### Supplemental Figure 2

**(a)** Immunoblot depicting Fn expression in control and KO mice after sham and TAC procedure. **(b)** Immunohistochemistry from control (left) and KO animals (right) after sham (top panel), or 3 weeks (middle panel) and 8 weeks after TAC procedure (bottom panel) depicting Fn expression (white), cardiomyocytes stained for sarcomeric actin (red) and nuclei (blue).

### Supplemental Figure 3

**(a)** Low magnification images for NRCMs plated on collagen (left) or Fn (right) after transfection with Ad-NFAT-GFP. Please see also Fig. 4c (scale 50µm). **(b)** Low magnification images of mice, which received Ad-NFAT-GFP via intra-myocardial injection 2 days before surgery. Hearts were harvested 2 days after TAC. Control mouse (left) and KO animal (right) after TAC are depicted (scale: 150µm). Please see also Fig. 4d. **(c)** Treatment with CyclosporinA (1µM) inhibits Fn induced activation of the pathological gene program.

### Supplemental Table 1

Application	Antibody	Dilution	Amplification	Company
IHC	GFP	1:100	no	Rockland, 600-101-215
IHC	Desmin	1:200	no	Abcam, 15200
IHC	sarcomeric actin	1:200	no	Sigma, A2172
IHC	Fn	1:100	no	Sigma, F3684
immunoblot	Fn	1:1000	no	Sigma, F3684

Antibodies used in the study. Application, dilution, amplification procedure as well as order information are provided.

### Supplementary Table 2

	Forward	Reverse
S18	CGAGCCGCCTGGATACC	CATGGCCTCAGTTCCGAAAA
HPRT	AAGGACCTCTCGAAGTGTGGATA	CATTTAAAAGGAACTGTTGACAACG
bActin	CATGAAGATCAAGATCATTGCTCCT	GCTGATCCACATCTGCTGGAA
ANP	TCTGATGGATTTCAAGAACCTGC	CTGCTTCCTCAGTCTGCTCACTC
BNP	GCAATTCAAGATGCAGAAGCTG	CTGCCTTGAGACCGAAGGACT
Fibronectin	ACCGAAGCCGGGAAGAGCAA	GGTCCGTTCCCACTGCTGATTTATC
aSkeleton Actin	CGCCAGCCTCTGAAACTAGA	AGCCGTTGTACACACAAGA
bMHC	GAGCCTTGGATTCTCAAACG	GTGGCTCCGAGAAAGGAAG
RCAN1.4	TCCAGCTTGGGCTTGACTGAG	ACTGGAAGGTGGTGTCTTGT
c/EBPβ	ACGACTTCCTCTCCGACCTCT	AGGCTCACGTAACCGTAGTCG

CITED4	TGCCAGATGACAGTTGGGTC	GGAATCCGAAGGCTGGTTCA
$\alpha$ MHC	GCAGCTGTGCATCAACTTCAC	CACTCAATGCCCTCCTTCTTG
Mef2c	GATGAAGTGAAGCGTGGGAAGG	CACAGCTCAGTTCCCAAATCC
Nkx2.5	ACCTTTAGGAGAAGGGCGATG	GAGGGTGGGTGTGAAATCTGA
PGC1 $\alpha$	AATCAGACCTGACACAACGC	GCATTCCTCCTCAATTTACCAA
VEGF $\alpha$	TGAGCTTCCTACAGCACAGCAG	TTACACGTCTGCGGATCTTGGA
Col 1a1	ACGCCATCAAGGTCTACTGC	ACTCGAACGGGAATCCATCG
Col 3a1	CCCTGGACCTCAGGGTATCA	GGGTTTCCATCCCTTCCAGG

Mouse primer sequences applied in the study are depicted.

### Supplementary Table 3

	<b>Forward</b>	<b>Reverse</b>
S18	CGAGCCGCCTGGATACC	CATGGCCTCAGTTCCGAAAA
HPRT	AAGGACCTCTCGAAGTGTGGATA	CATTTAAAAGGAACTGTTGACAACG
bActin	CATGAAGATCAAGATCATTGCTCCT	GCTGATCCACATCTGCTGGAA
ANP	TACAGTGCGGTGTCCAACACAGAT	TGGGCTCCAATCCTGTCAATCCTA
BNP	GTTCAAGCTGCTTTGGGCAGAAGA	ACTGTGGCAAGTTTGTGCTGGAAG
$\alpha$ Skeleton Actin	AGCAGCAGAACTAGACACCA	CCACGATGGATGGGAACACA
$\beta$ MHC	GTTTGGCCACACCAAGGTGTTCTT	AGTAGAGCTTCATCCACGGCCAAT
RCAN1.4	CGGAGGCCAGAGTACACACC	GGTCAGTGTGCCTGTTGAGCT
c/EBP $\beta$	GGGTTGTTGCTGTTGATGT	GCTCGAAACGGAAAAGGTTT
CITED4	ACGAGGGTGGTTTTGCAGTCT	CAACTCAGCCAGACAGAGGAA
$\alpha$ MHC	GTGACAGTGGGAAAGGCAAAG	AAAGTGAGGATGGGTGGTCCT
Mef2c	AAGGAATGGATACGGCAAC	TCCTAGATTCATAGGGGGAGGA
Nkx2.5	CTCGGATTTACACCCACACT	CTCCGGGTCTGATATGGAAT
PGC1 $\alpha$	TCCCACGACTCCTCCTCATAA	TGCCTTGGGTACCAGAACAT
VEGF $\alpha$	TGAAAGACTCCGGTGTGGTCT	GTTTCTGGAAGTGAGCCAACG

Rat primer sequences applied in the study are depicted.