

Figure Legends supplemental figures and tables

Supplemental Figure 1 (a) Heart weights of sedentary and trained rats. The heart weight of trained rats is significantly increased compared to the hearts of untrained animals. Sedentary hearts was 1.53 ± 0.14 vs 1.22 ± 0.11 gram ($P=0.0159$) **(b and c)**. Cardiomyocyte physiological remodelling. cells were measured for length and midpoint width on an inverted microscope. Cardiomyocyte length was increased in trained rats compared the the sedentary animals. $119.1 \pm 3.1 \mu\text{m}$ vs $131.4 \pm 2.9 \mu\text{m}$, ($p < 0.01$) Cardiomyocyte width remained unchanged. $25.9 \pm 1.4 \mu\text{m}$ in sedentary animals and $26.1 \pm 1.2 \mu\text{m}$ in trained animals. **(d)**. Fractional shortening (FS) is increased in exercise induced hypertrophy. The FS of isolated cardiomyocytes was $13.1 \pm 3 \%$ in sedentary animals and 19.7 ± 6.5 in endurance trained cells ($P=0.036$).

Supplemental Figure 2. Lentiviral mediated overexpression of shRNA against rat ALK5 in cultured neonatal cardiomyocytes results in knockdown of ALK5. Cardiomyocytes were infected with a lentivirus containing shRNA against ALK5 or with a control lentivirus. ALK5 mRNA levels were significantly increased in the shALK5 transduced group compared to the control group. (§: $p < 0.05$ compared to TGF β treated control cells, $n=3$ per group)

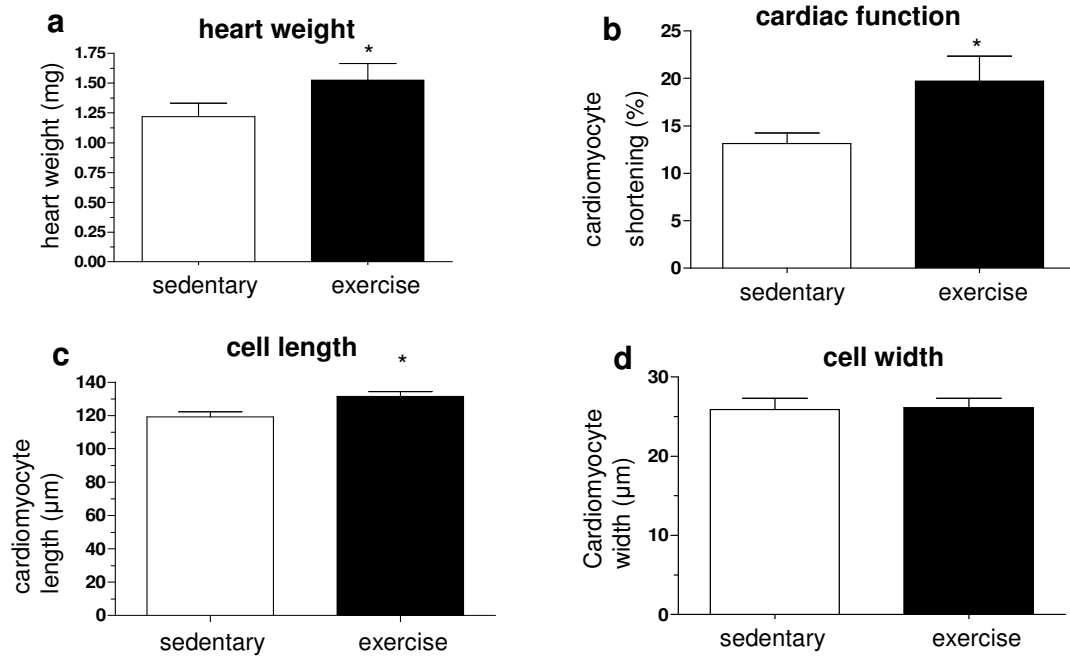
Supplemental Figure 3. Repression of KLF15 mRNA in cultured cardiomyocytes is mediated by p38-TGF β signalling. TGF β -induced downregulation of KLF15 is completely prevented by two independent p38 inhibitors (SB203580 and SB202190). The expression of KLF15 inversely correlates with ANF expression in these cells.

Supplemental Figure 4. Knockdown of KLF15 results in increased cell size. A) Knockdown of KLF15 in cultured neonatal mouse cardiomyocytes by using an siRNA smartpool results in a significant downregulation of KLF15, an 28% increase in cell surface area. ($n=3/\text{group}$ *: $p=0.03$) and an increase in ANF mRNA. The same result was observed in four other independent experiments. B) Cultured neonatal mouse cardiomyocytes transfected with a control siRNA smartpool or an siRNA smartpool targeting KLF15 were stained with phalloidin to demonstrate the increase in cell size. C) KLF15 knockdown in rat neonatal cardiomyocytes shows an increase in cell size of about 16%. D) Lentiviral overexpression of KLF15 in cultured neonatal rat cardiomyocytes represses ANF expression, as compared to cells that were transduced with a control virus. *: $p < 0.05$, $n=3$ per group.

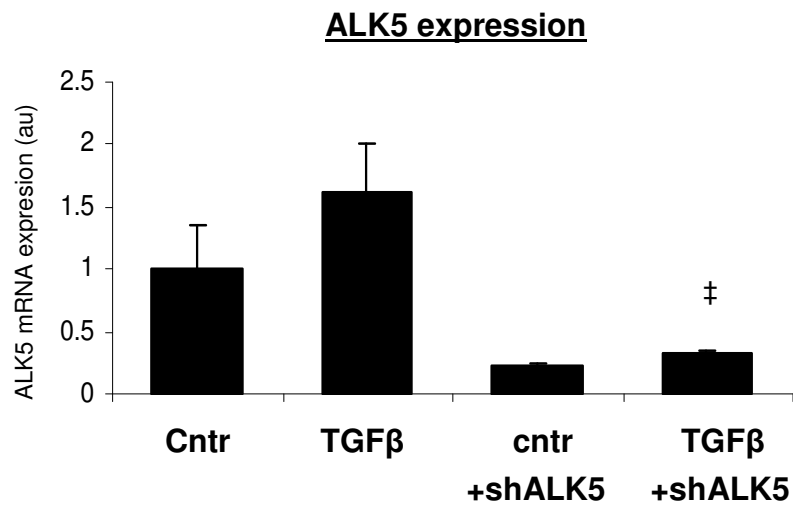
Supplemental Figure 5. KLF15 does not directly interact with SRF and MEF2a and does not have a direct effect on reporter constructs. **(a)** KLF15 does not repress the ANF, SM22 and the 3xCARG reporter in the absence of myocardin. COS cells were transfected with the indicated luciferase reporters and KLF15. Luciferase activity is expressed as fold change over the empty expression vector, pcDNA3.1 ($n=3$, mean \pm SD). **(b)** GST pull-down assays show that KLF15 physically associates with myocardin but not with SRF and MEF2A. 35S-labeled SRF, MEF2A and myocardin proteins were translated *in vitro* and incubated with GST-KLF15 fusion protein. Proteins were captured on glutathione agarose beads and analyzed by SDS-PAGE. The input lanes contain 10% of the amount of 35S-labeled SRF, MEF2A and myocardin proteins in the pull-down lanes. GST-KLF15 fusion protein is shown on a Coomassie gel on the right.

Supplemental tables. (a) Oligos used for creating pll3.7-shALK5 **(b)** Oligos used for quantitative Real-Time PCR

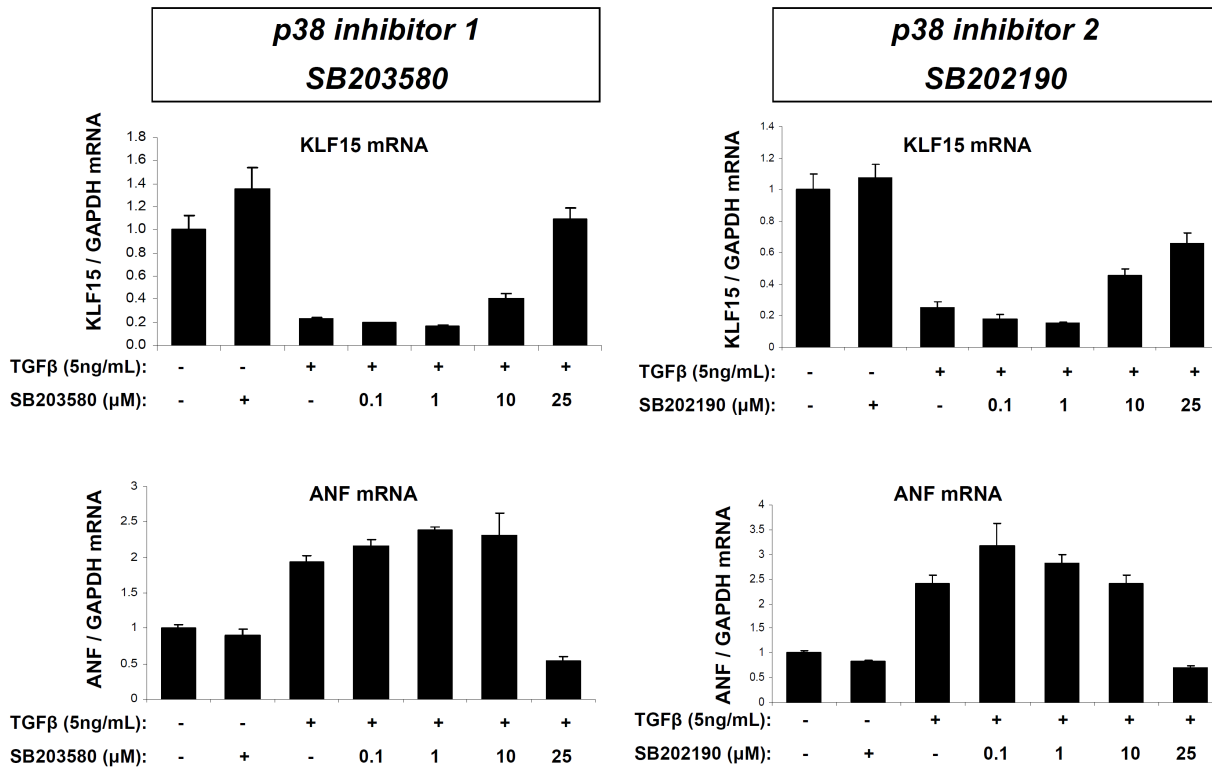
Supplemental Figure 1



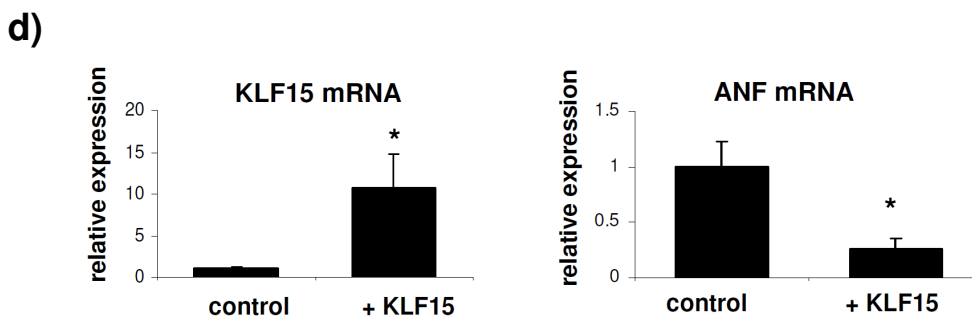
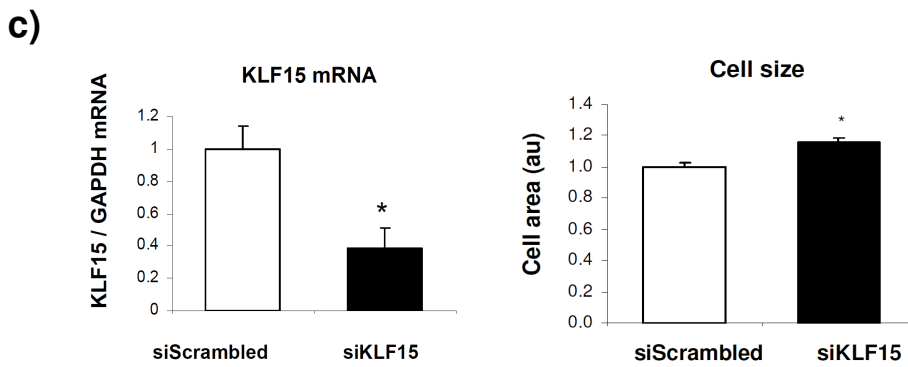
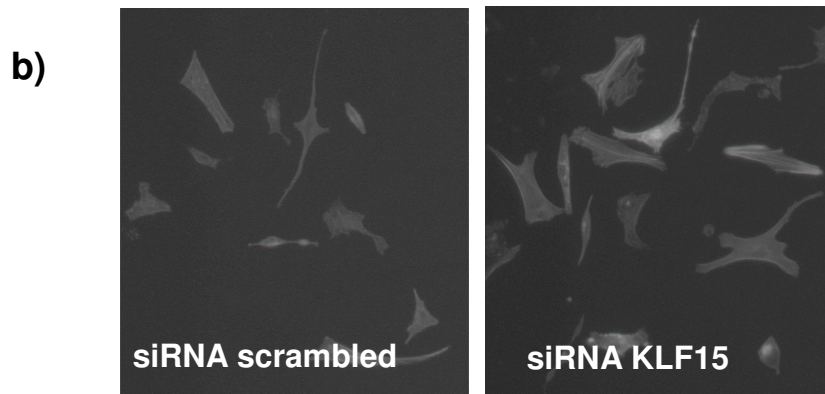
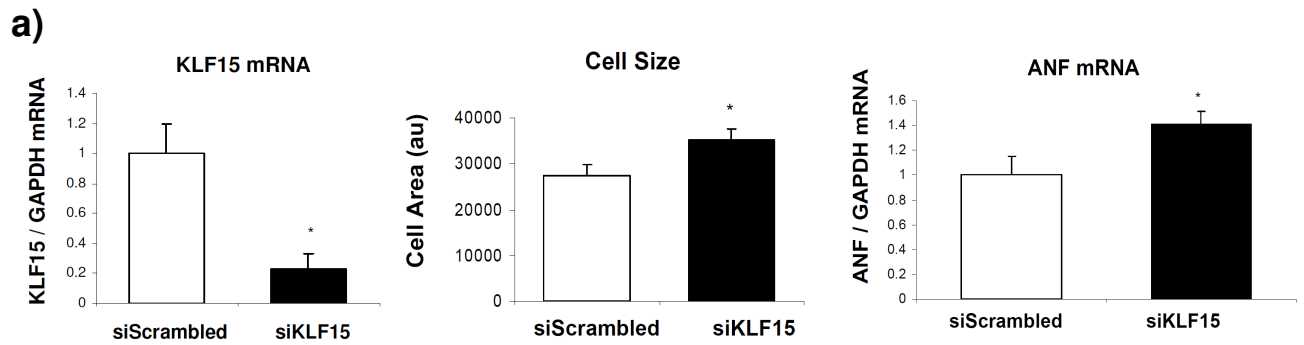
Supplemental Figure 2



Supplemental Figure 3

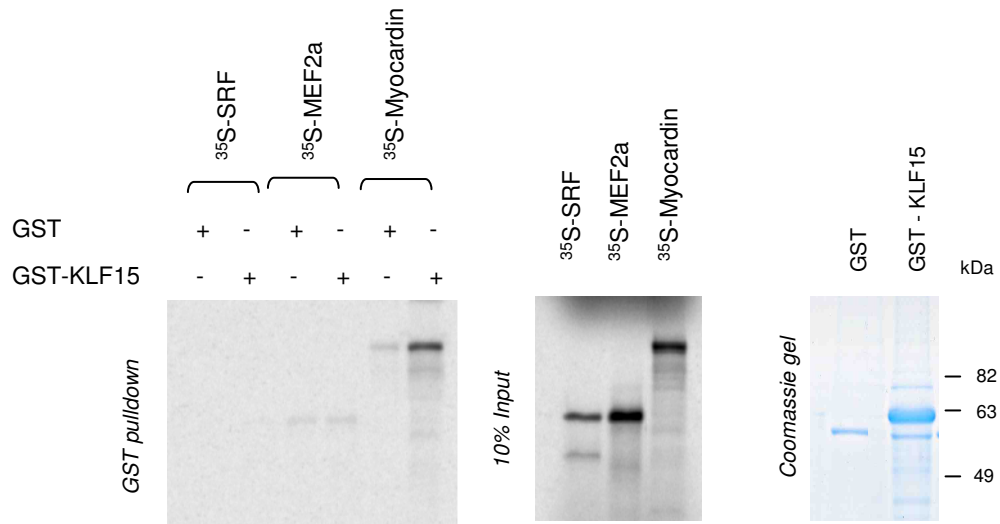


Supplemental Figure 4

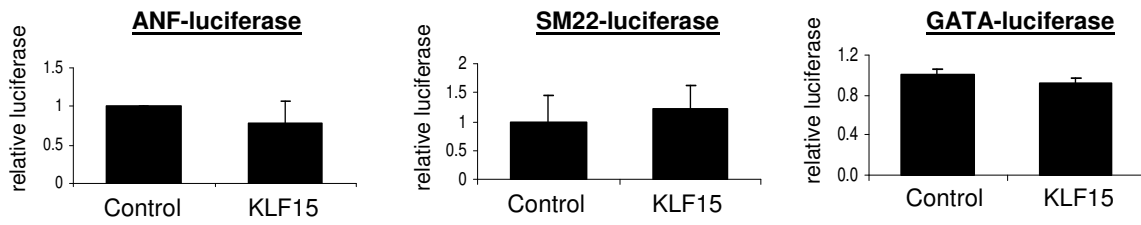


Supplemental Figure 5

a



b



Supplemental tables 1

a

Gene	NCBI Accession	Forward 5' → 3'	Reverse 5' → 3'
BNP (rat)	NM_008726	GCTGCTTTGGGCAGAAGATAGA	GCCAGGAGGTCTTCCTAAAACA
KLF15 (rat)	NM_023184	CCAAGAGCAGCCACCTCAAG	TCGCATACGGGACACTGGTA
ANF (rat)	NM_012612	ATCACCAAGGGCTTCTTCCT	TGTTGGACACCGCACTGTAT
SM22 (rat)	NM_031549	ACGATGGACACTACCGTGG	TGACTGTCTGTGAACTCCC
ASKA (rat)	NM_019212	TCGCTGACCGCATGCA	CCGCCGATCCACACTGA
ALK5 (rat)	NM_012775	GGATTATAGCAGCAGACAAC	CATTCCTTCCACAGTAACAGT

b

shALK5		Sequence
Oligo 1	Sense	CCACCGCGTACCAAATGAAAttcaagagaTTCATTTGGTACGCGGTGGTTTTTC
	Antisense	TCGAGAAAAACCACCGCGTACCAAATGAAAtctcttgaaTTCATTTGGTACGCGGTGG
Oligo 2	Sense	GGACCATTGTGCTACAAGAttcaagagaTCTTGTAGCACAATGGTCCTTTTTTC
	Antisense	TCGAGAAAAAGGACCATTGTGCTACAAGAtctcttgaaTCTTGTAGCACAATGGTCC
Oligo 3	Sense	GACTACCAGTTGCCTTACTtcaagagaAGTAAGGCAACTGGTAGTCTTTTTTC
	Antisense	TCGAGAAAAAGACTACCAGTTGCCTTACTtctcttgaaAGTAAGGCAACTGGTAGTC