Supplemental information

Heterozygous loss of Rbm24 in the adult mouse heart increases sarcomere slack length but does not affect function

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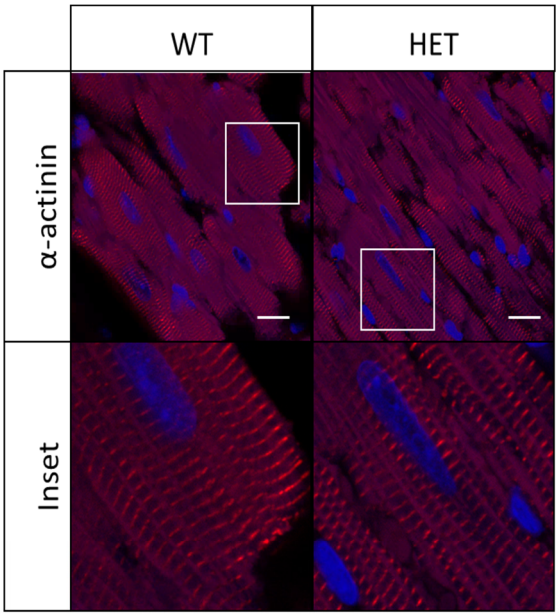
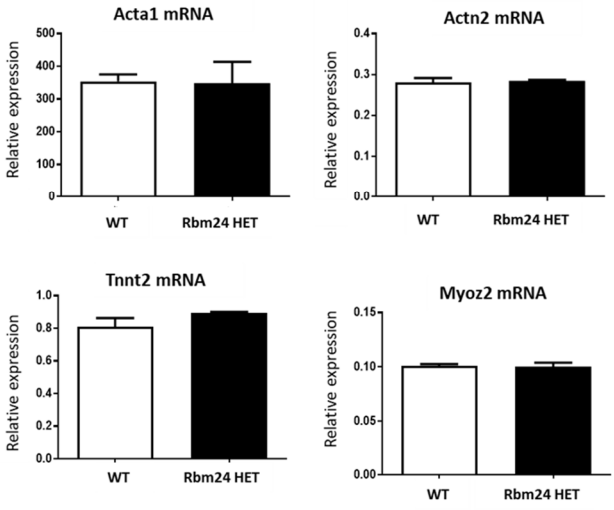


Supplemental Figure 1: α-actinin immunohistochemistry hearts of adult WT and Rbm24 HET mice.

Representative images are shown and scale bar represents 20μm.

A



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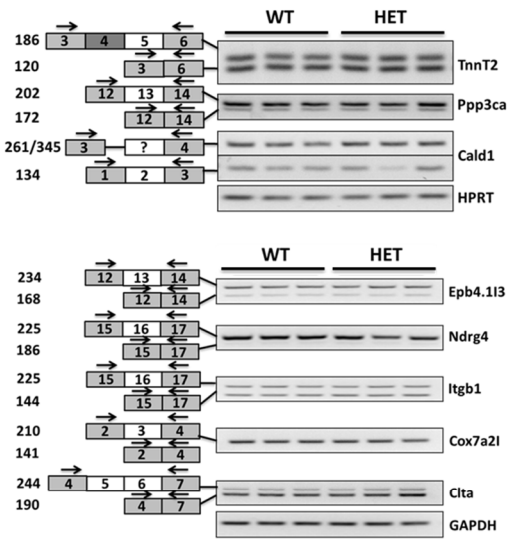
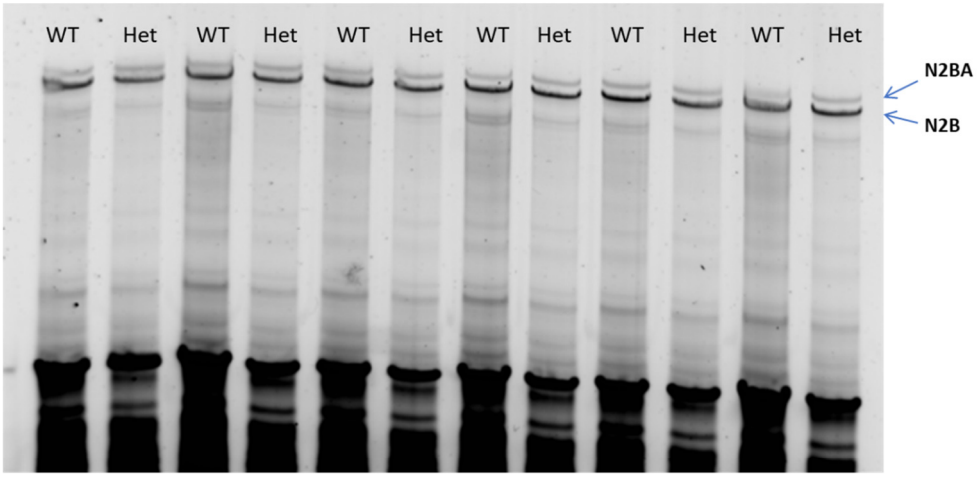
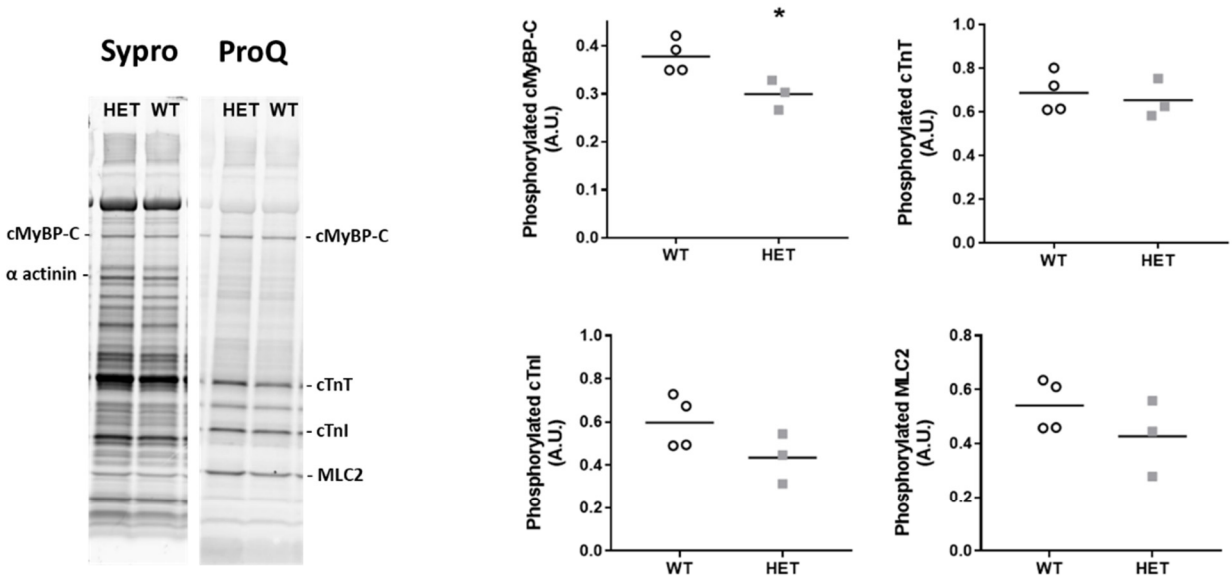


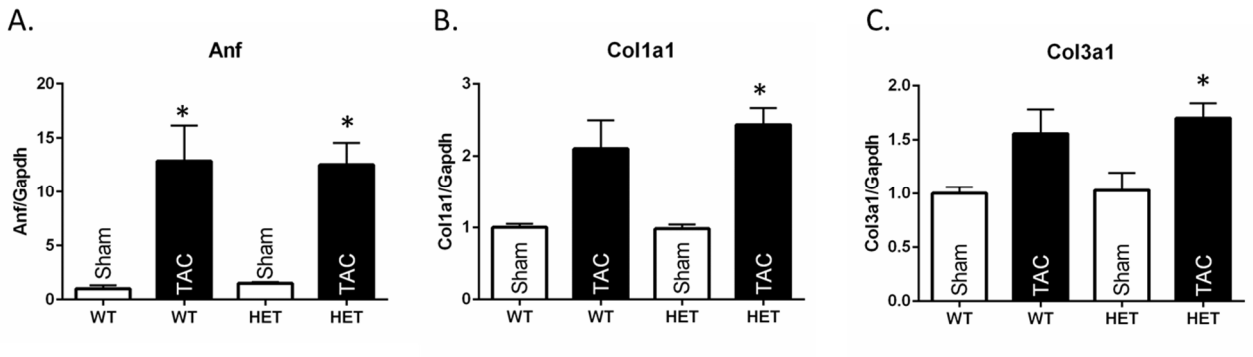
Supplemental Figure 2: Alternative splicing and mRNA expression in adult Rbm24 HET hearts. A. qRT-PCR for mRNA of Acta1, Actn2, Tnnt2, Myoz2 and Ryr2 in adult Rbm24 HET hearts (n=3) and in WT controls (n=3). Expression is normalized by GAPDH expression. Student's t-test did not reveal significant changes. **B.** RT-PCR results of previously identified Rbm24 target genes[14] in adult hearts of WT (n=3) and Rbm24 HET (n=3) mice show that alternative splicing is not affected.



Supplemental Figure 3: Titin isoforms analysis by SDS-agarose gelelectrophoresis. SDS-agarose gel electrophoresis of protein extracts of WT and Rbm24 HET hearts shows the expression of two TTN isoforms: N2BA and N2B.



Supplemental Figure 4: Proteins separated by gel electrophoresis were stained with SYPRO Ruby and Pro-Q Diamond Phosphoprotein Stain and enables the simultaneous analyses of expression levels and phosphorylation of myofilament proteins cardiac myosin-binding protein C (cMyBP-C), cardiac troponin T (cTnT), cardiac troponin I (cTnI) and myosin light chain 2 (MLC2). 3 WT and 3 Rbm24 HET hearts were analyzed. A slightly lower phosphorylation of cMyBP-C was observed in HET compared to WT. Student's T-test *P<0.05.



Supplemental Figure 5: qRT-PCR analysis of WT and Rbm24 HET hearts after 5 weeks of TAC. qRT-PCR analysis of Anf, Col1a1, and Col3a1 in sham-operated (n=4) and TAC- operated (n=6) WT hearts and sham-operated (n=5) and TAC-operated (n=12) Rbm24 HET hearts. * $p<0.05$ compared to sham animals of the same genotype. No significant interactions were found between the intervention and the genotype by 2-way ANOVA.

Supplemental Table 1: Primers used for genotyping, qRT-PCR and RT-PCR

Gene	Primer sequence
Genotyping Rbm24 fw	gcacaccacccagaaggacacg
Genotyping Rbm24 rv	ggtctgtgatgaccaccgct
Rbm24 fw	gccagcctgcgcaagtacttt
Rbm24 rv	gttgggatccttgcaggccctt
skNAC fw	tacagagcaggagttgccac
skNAC rv	gcagtttcagctgttatggg
Coro6 fw	tcatcatctggaatgtgggc
Coro6 rv	gtaccgaatgctactgtcac
α-NAC fw	tacagagcaggagttgccac
α-NAC rv	ctaactgtgcttgctgagac
Col3a1 fw	tcaaggctgaaggaaacagca
Col3a1 rv	gatgggtagtctcattgcc
ANF fw	attgacaggattggagcccagagt
ANF rv	tgacacaccacaagggcttaggat
GAPDH fw	ggtggacctcatggcctaca
GAPDH rv	ctctcttgctcagtgtccttgct
HPRT fw	cctaagatgagcgcaagttgaa
HPRT rv	ccacaggactagaacacctgctaa

Supplemental Table 2: Viable embryos collected from Rbm24 heterozygous intercrosses

Embryonic stage	Genotype	# of embryos
E11.5	WT	6 (22%)
	HET	15 (56%)
	KO	6 (22%)
E12.5/E13.5	WT	11 (40%)
	HET	14 (52%)
	KO	2 (7%)

Full gels

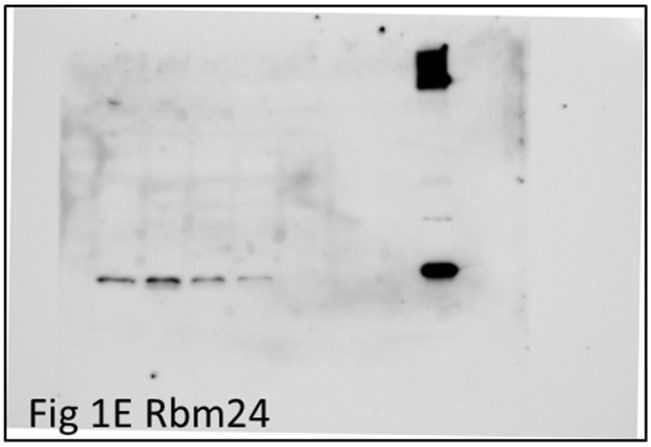


Fig 1E Rbm24

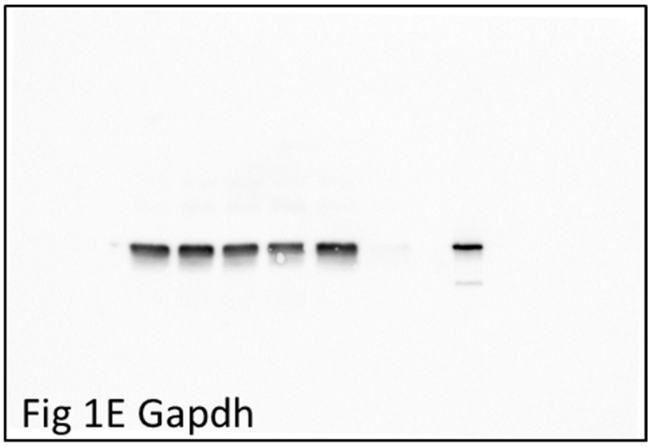


Fig 1E Gapdh

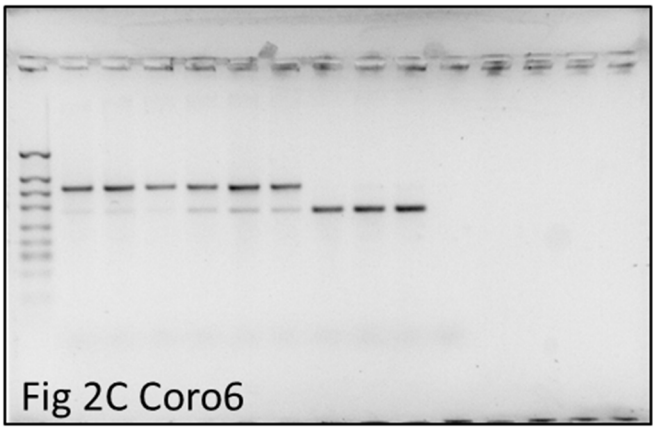


Fig 2C Coro6

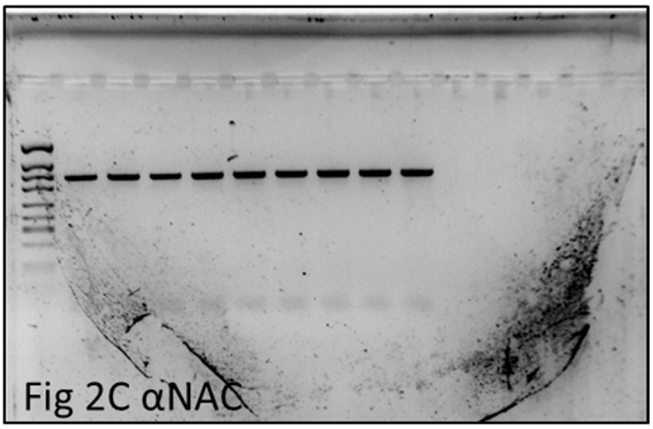


Fig 2C αNAC

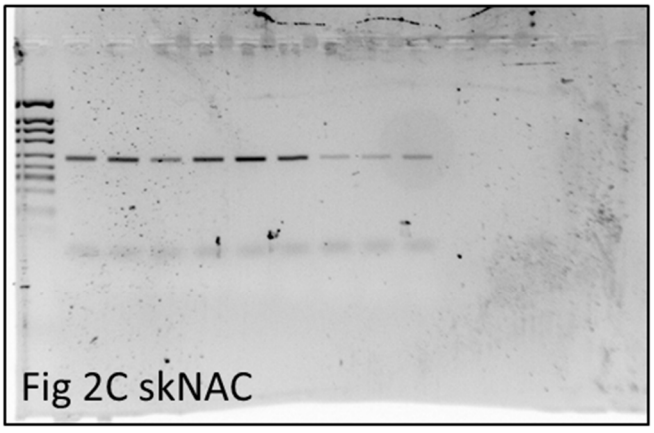


Fig 2C skNAC

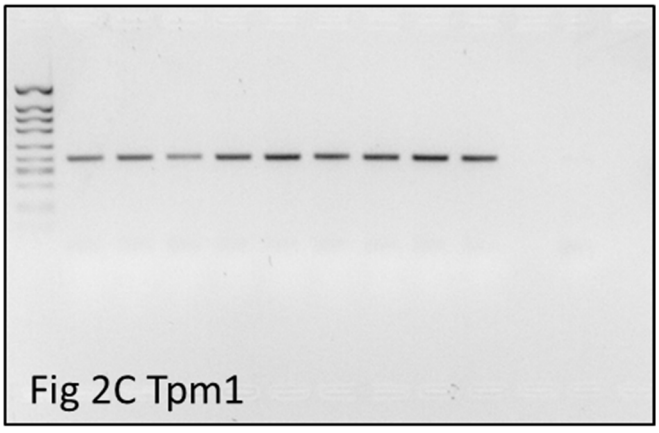
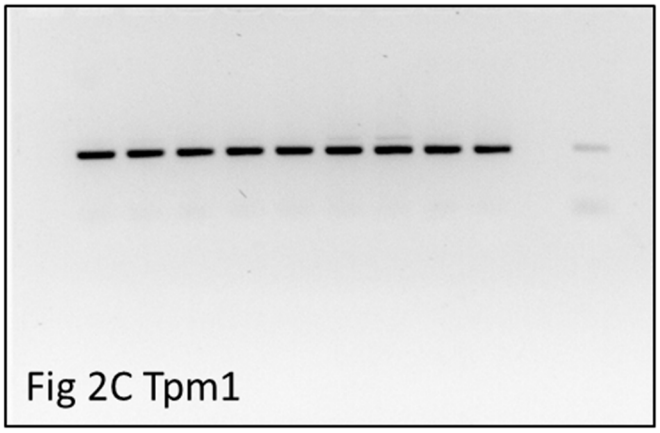


Fig 2C Tpm1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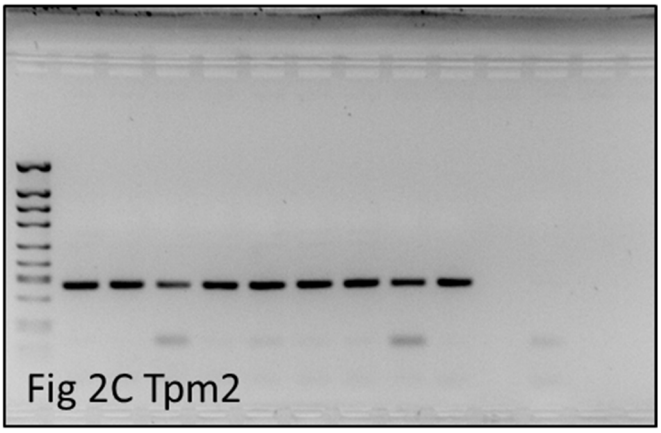
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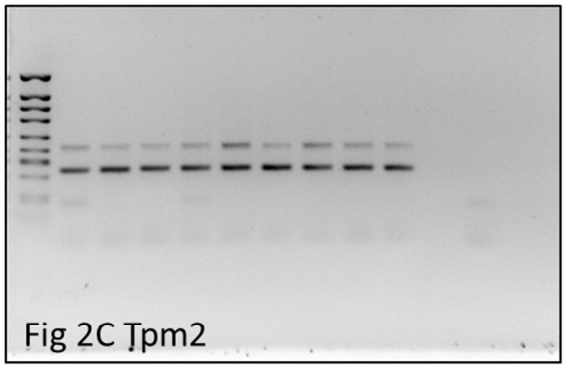

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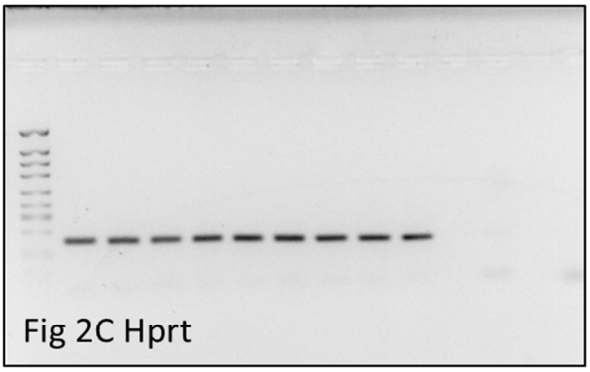


Fig 2C Hprt

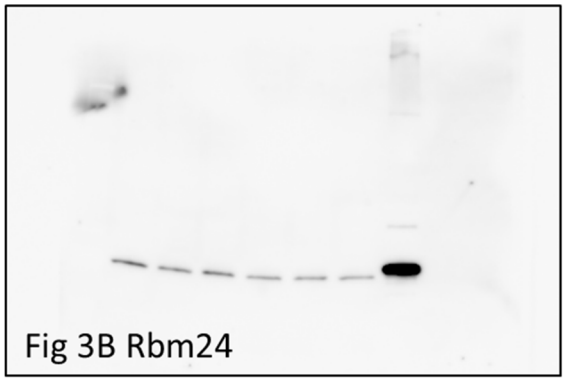


Fig 3B Rbm24

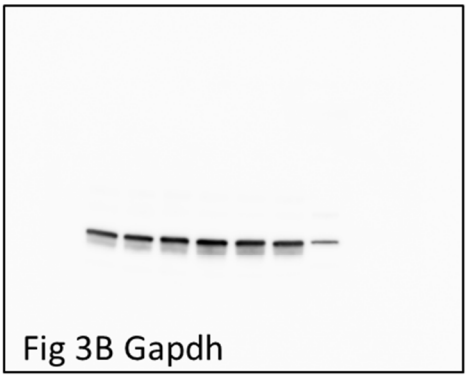


Fig 3B Gapdh

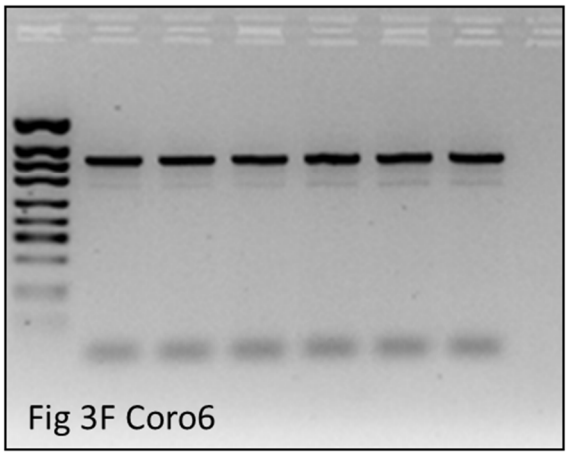


Fig 3F Coro6

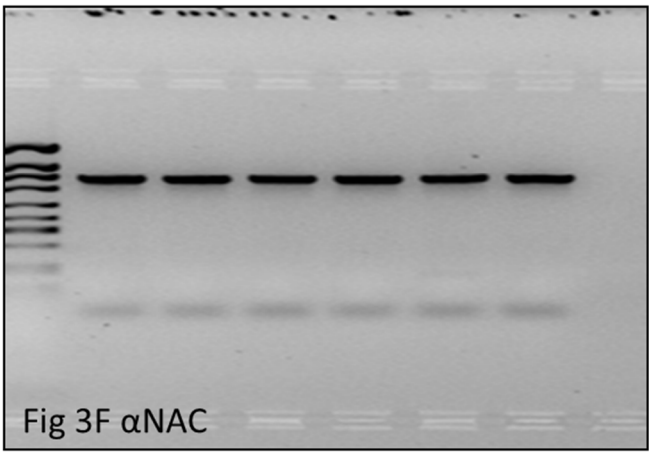
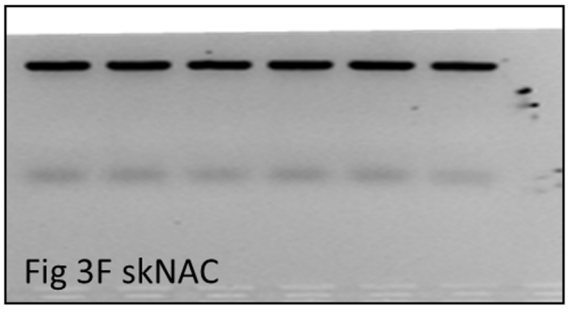


Fig 3F αNAC



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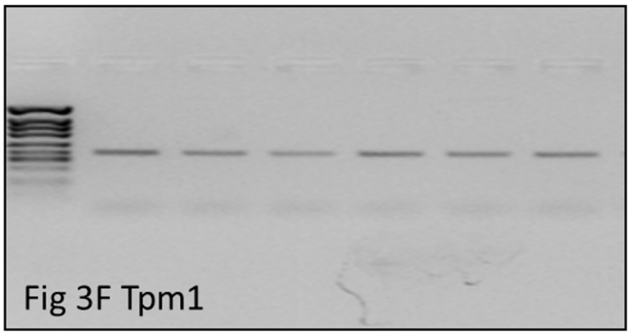

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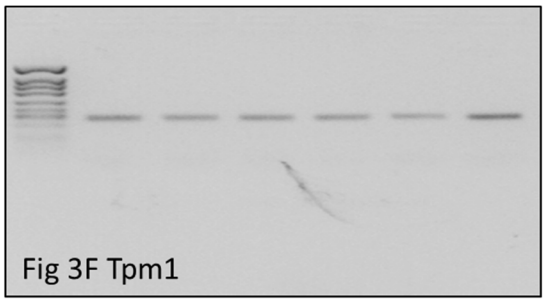
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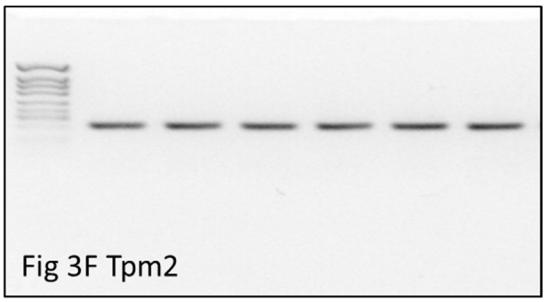
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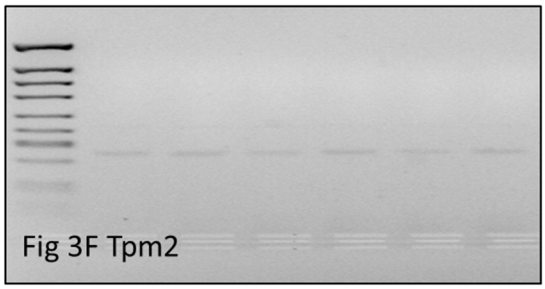
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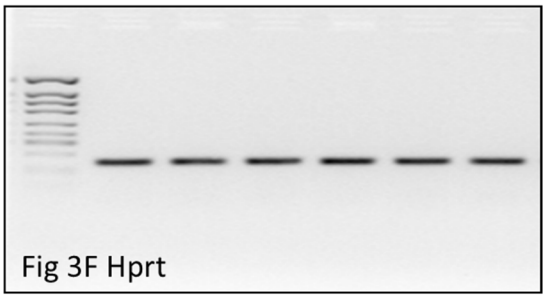
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