

III. Index

I. Zusammenfassung	IV
II. Abstract	V
III. Index	VI
IV. Figure Index.....	IX
V. Abbreviations	XII
1. Introduction.....	1
1.1 Muscle tissue.....	1
1.1.1 Adult muscle regeneration	1
1.1.1.1 Adult myogenesis	2
1.1.1.2 Muscle specific transcription factors.....	3
1.1.2 Muscle fiber types	5
1.1.2.1 Transcriptional control of myofiber type plasticity	7
1.2 LIM-domain proteins	9
1.2.1 The LIM-domain protein Trip6	9
1.2.2 nTrip6	10
1.3 Aim of this project	12
2. Material & Methods.....	13
2.1 Material.....	13
2.1.1 Chemicals	13
2.1.2 Kits	14
2.1.3 Hardware and consumables	14
2.1.4 Enzymes	15
2.1.5 Bacterial strains and growth media	15
2.1.5.1 Bacterial strains	15
2.1.5.2 Bacterial growth media	15
2.1.6 Cell lines and cell culture media.....	15
2.1.6.1 Cell lines	15
2.1.6.2 Cell culture media.....	16
2.1.7 Plasmids	16
2.1.7.1 Mammalian expression vectors	16
2.1.7.2 Expression vectors for BiFC-Assay	19

Index

2.1.7.3 Reporter gene vectors	20
2.1.8 Oligonucleotids.....	21
2.1.9 siRNA.....	23
2.1.10 Antibodies	24
2.1.11 Fluorescent dyes.....	25
2.2 Methods.....	26
2.2.1 Animal handling.....	26
2.2.1.2 Degeneration of murine <i>M soleus</i>	26
2.2.1.3 <i>In vivo</i> Transfection.....	26
2.2.2 Tissue culture methods	27
2.2.2.1 Preparing and sectioning skeletal muscle tissue	27
2.2.2.2 Immunofluorescence staining of muscle tissue sections	27
2.2.2.3 Haematoxilin/Eosin Staining	27
2.2.2.4 Metachromatic dye-ATPase staining	28
2.2.3 Cell culture methods	28
2.2.3.1 Cell culture conditions.....	28
2.2.3.2 Thawing cells.....	29
2.2.3.3 Passaging and seeding of cells	29
2.2.3.4 Freezing cells	29
2.2.4 Transfection methods.....	30
2.2.4.1 Transfection of C2C12 cells (Amaxa)	30
2.2.4.2 Transfection of C2C12 cells (Promofectin)	30
2.2.4.3 Transfection of HeLa cells (Lipofectamin).....	30
2.2.5 DNA methods.....	31
2.2.5.1 DNA digestion.....	31
2.2.5.2 Ligation of DNA-fragments	31
2.2.5.3 In Fusion™ PCR cloning	31
2.2.5.4 PCR (Pfu-DNA-Polymerase)	31
2.2.5.5 Genotyping PCR (Taq-DNA-Polymerase)	32
2.2.5.6 Colony PCR.....	32
2.2.5.7 qRT-PCR	32
2.2.5.8 Electrophoresis.....	33
2.2.5.9 Isolation of DNA fragments out of agarose gels	33
2.2.5.10 Small scale plasmid DNA purification	34

Index

2.2.5.11 Large scale plasmid DNA purification.....	34
2.2.6 Transformation of chemically competent <i>E. coli</i>	35
2.2.6.1 Establishing chemically competent <i>E. coli</i>	35
2.2.6.2 Transformation of <i>E. coli</i> (DH5α)	35
2.2.7 RNA methods.....	35
2.2.7.1 RNA isolation.....	35
2.2.7.2 cDNA synthesis (RT-PCR)	36
2.2.8 Protein methods	36
2.2.8.1 Protein isolation (in RIPA buffer).....	36
2.2.8.2 Measurement of total protein concentration according to Lowry.....	37
2.2.8.3 Separation of proteins via SDS-Page	37
2.2.8.4 Western Blotting	38
2.2.8.5 BiFC Assay.....	38
2.2.8.6 ChIP Assay.....	39
2.2.8.7 Luciferase Assay	40
2.3 Software and statistical analysis	41
3. Results.....	42
3.1 nTrip6 acts as a co-repressor for Mef2c	42
3.2 nTrip6 mediates the recruitment of HDAC5 to Mef2c target promoters	50
3.3 nTrip6 maintains fast fiber type identity	53
3.4 nTrip6 expression is regulated through eIF2α phosphorylation	58
3.5 Trip6/nTrip6 are involved in adult muscle regeneration	61
4. Discussion	66
4.1 nTrip6: nTrip6 acts as a co-repressor for Mef2c	66
4.2 nTrip6 regulates myofiber type identity	68
4.3 A translational regulation determines fiber type identity.....	69
4.4 Trip6 and nTrip6 in adult muscle myogenesis	71
4.5 Conclusion.....	73
5. Literature	75
6. Acknowledgments	91