Myonuclear identification and epigenetics in skeletal muscle

By

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SUMMARY

Skeletal muscle is a heterogeneous tissue comprised of a variety of different cell types, including the multinucleated skeletal myofiber. Although myofibers are post-mitotic, they hold a remarkable ability to adapt new functional characteristics. One of the mechanisms used to achieve phenotypical plasticity is by structural reorganization of the genome. Commonly referred to as epigenetics, these adaptations occur through different processes such as methylation and acetylation of histones. In this thesis, we wanted to study the epigenetic landscape to understand more about the mechanisms of muscle physiology.

In paper I, we investigate if myonuclei are lost from the myofiber during cancer-induced atrophy. We induced cachexia by transplanting PC3 prostate cancer cells in nu/nu mice. Using in-vivo imaging techniques and histochemical analysis, we found no loss of myofibers or myonuclei from tissue that had undergone severe atrophy.

In paper II, we address the issue of cellular heterogeneity and show that an antibody against Pericentriolar material-1 (PCM1) can be used to identify myonuclei on tissue cross-sections from mice, rats, and humans. The labeling allows us to distinguish myonuclei from other nuclei residing in the tissues.

In paper III, we used the PCM1 antibody to enrich myonuclei for epigenetic analysis. By comparing data from sorted and unsorted nuclei, we show that myonuclear enrichment reduces interference from stromal cells. The method was furthermore used to generate global maps of histone modifications in the fast/glycolytic extensor digitorum longus (EDL) and slow/oxidative soleus (SOL) muscles and found that the differences in phenotype are reflected in the epigenetic landscape.

The work presented in this thesis make it possible to study skeletal muscle fibers more specifically; in regards to physiological and epigenetic responses to a changing milieu without complicating responses from other cell types. By showing that myonuclei are preserved during cachexia, our model can be used to study the underlying causes of atrophy.

LIST OF PAPERS

Paper I

Cachexia does not induce loss of muscle fibers or muscle cell nuclei during xenografted prostate cancer in mice

Submitted manuscript

Winje IM, Sheng X, Hansson K-A, Solbrå A, Tennøe S, Saatcioglu F, Bruusgaard JC, Gundersen K

Paper II

Specific labeling of myonuclei by an antibody against Pericentriolar material 1 on skeletal muscle tissue sections

Acta Physiologica (2018)

Winje IM, Bengtsen M, Eftestøl E, Juvkam I, Bruusgaard JC, Gundersen K

Paper III

Comparing the epigenetic landscape of fast/glycolytic and slow/oxidative muscle (working title)

Manuscript

Winje IM, Bengtsen M, Landskron J, Meza-Zepeda L, Gundersen K

ABBREVIATIONS

ACTA1	Gene encoding alpha-skeletal actin
AMPK	AMP-activated kinase
C26	Colon-26
ChIP	Chromatin immunoprecipitation
ChIP-seq	Sequenced ChIP material
CS	Centriolar satellites
CSA	Cross-sectional area
EDL	Extensor digitorum longus
H3K27ac	Acetylated lysine-27 on histone 3
H3k27me3	Tri-methylated lysine-27 on histone 3
H3K4me1	Mono-methylated lysine-4 on histone 3
H3K4me3	Tri-methylated lysine-4 on histone
HDAC	Histone deacetylase
IGF1	Insulin-like growth factor 1
IL6	Interleukin-6
MACS	Magnetic associated cell sorting
mATPase	Myosin ATPase
mTOC	Microtubule-organizing center
mTOR	Mammalian target of rapamycin
MyHC	Myosin heavy chain
PCM1	Pericentriolar material-1
PI3K	Phosphatidylinositol-3 kinase
PTM	Post-translational modification
S6K	Ribosomal kinase S6
SOL	Soleus
TNFα	Tumor necrosis factor alpha
TSS	Transcriptional start site

INTRODUCTION

VERTEBRATE SKELETAL MUSCLE

A central theme in the survival of an organism is adaptation. Although some adaptations are extreme and present at the macroscopic level; all cells continuously monitor their environment and adapt their expression profile accordingly. Nutritional availability, cytokines and cell-to-cell communication are among extracellular features that determine the cellular response to a given signal. In concert with this monitoring, intracellular attributes in the form of protein modifications, regulation of gene expression and structural reorganization of DNA enables a swift response in accordance with the cellular profile. A typical skeletal muscle fiber (myofiber) may reach up to 20 cm in length (Heron et al., 1993), a feature possible due to the presence of multiple nuclei spread along the length of the myofiber just beneath the sarcolemma.

In adult animals, it is well established that myofibers are post-mitotic, meaning that the cell and its constituent nuclei (myonuclei) have stopped dividing (Moss et al., 1971). As a consequence, phenotypical remodeling is accomplished by regulating the abundance and expression profile of various structural and metabolic protein isoforms, providing a way for adaptation without apoptosis or regeneration of the tissue. During a lifetime, the system is confronted as the organism experiences periods of exercise, disuse, aging and diseases, forcing the cell to adapt in a manner refined through evolution.

One of these mechanisms is commonly referred to as epigenetics and refers to changes in the structural organization of DNA. Using various epigenetic mechanisms, such as DNA methylation and covalent modifications of histone tails, the cell can control gene expression by regulating the accessibility and stability of the genome (Lawrence et al., 2016). These mechanisms will be discussed further in later sections. However, from a physiological perspective, it is the pattern of the action potentials delivered by the motor neurons that have received the most attention related to phenotype.

THE PHENOTYPICAL INFLUENCE OF THE NERVE

Skeletal muscles are controlled by motor neurons originating from the central nervous system. Within the muscle, a single motor neuron innervates multiple myofibers forming a motor unit; a set of myofibers under the same neuronal influence (Edstrom et al., 1968). Historically, motor units are divided into two groups, fast-twitch, and slow-twitch (Close, 1967). The fast-twitch motor units contain neurons exerting short bursts of high-frequency activity, while slow-twitch motor units receive a high amount of impulses with low frequency (Hennig et al., 1985). The manner in which the nerves influence the phenotype of the muscle is complex and depends on many integrated signaling pathways (Gundersen, 2011; Schiaffino et al., 1996). A central hypothesis is that the transients are decoded according to their peak amplitude and temporal patterns (Chin, 2005; Crabtree, 1999), thus culminating as different phenotypes.

From a functional perspective, the simple explanation is that fast-twitch motor units contain fast-contracting myofibers with a large cross-sectional area for rapid force production, while the slow-twitch units are smaller and are recruited in low force movements (Harridge et al., 1996; Schiaffino & Reggiani, 1996). By varying the combination of different motor units, the organism can fine-tune each muscle and muscle group according to their functional demands (Schiaffino et al., 2011). Relevant for this thesis, the hind-limb muscle *extensor digitorum longus* (EDL) is composed almost entirely of fast motor units and is among the most studied of the fast twitch muscles. Compared to the slow-twitch *soleus* (SOL) muscle, the twitch time to peak duration is approximately three times faster in EDL, making force production in the latter more rapid upon stimulation (Barany et al., 1971; Close, 1967; Gundersen, 1985).

The functional relationship between mechanical and biochemical properties was established with the discovery of the myosin ATPase (mATPase) domain, which provided a relationship between shortening velocity and the muscle's ability to hydrolyze adenosine triphosphate (ATP) (Barany, 1967). With the notion that myofibers within the same motor units were constrained to the same pattern of enzymatic activity (Barnard et

al., 1971; Edstrom & Kugelberg, 1968), investigation of myosin ATPase activity on muscle cross-sections led to the current definition of fiber types based on their expression of myosin heavy chain (MyHC) isoforms (Reiser et al., 1985; Schiaffino et al., 1989).

In rodent limbs, there are four different myosin heavy chain (MyHC) isoforms; MyHC1; 2a; 2x; 2b. Being the most important factor in determining contractile speed, classification of myofibers into fiber-types give indications of other metabolic properties such as oxidative capacity and fatigability (Figure 1) (Schiaffino & Reggiani, 1996). Myofibers expressing the MyHC1 isoform are referred to as slow-twitch or type 1 fibers, and are highly specialized in ATP production through oxidative phosphorylation. Slow-twitch muscles, such as SOL, contain a high percentage of MyHC1 fibers and are often highly vascularized, allowing a steady supply of ATP for prolonged activity (Murakami et al., 2010). Myofibers expressing MyHC 2a, MyHC 2x and MyHC 2b are referred to as fast-twitch or type 2 fibers. In the listed order they show increased contractile speed and cross-sectional area (CSA), accompanied by a decrease in mitochondrial content, making them rely to a greater extent on glycolysis for ATP production (Barnard et al., 1971).

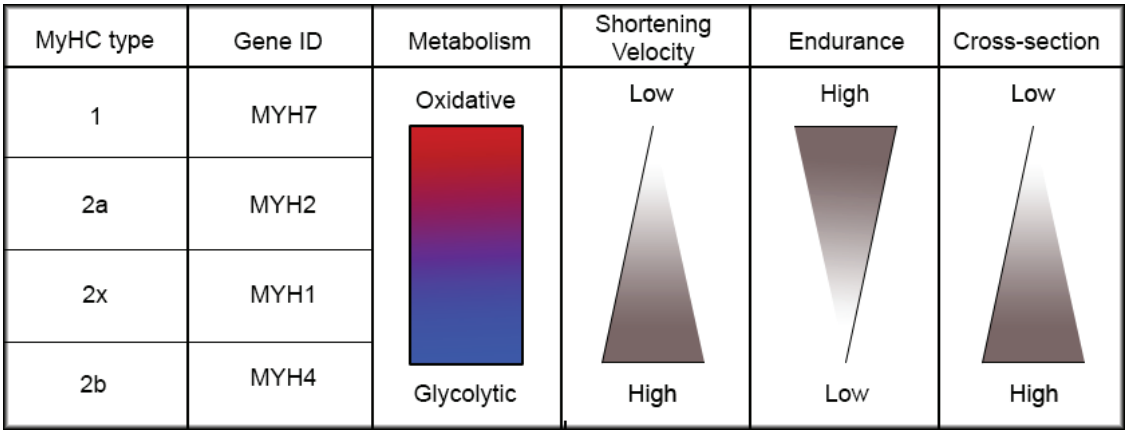

Figure 1. General overview showing the properties of the different fiber-types in rodent skeletal muscle. Figure adapted from Gundersen (2011).

REGULATION OF MUSCLE VOLUME

Since skeletal muscle cells are post-mitotic, changes in muscle size are ascribed to synthesis and degradation of contractile proteins in pre-existing fibers. To achieve hypertrophy and increased myofibrillar volume, the rate of protein synthesis must exceed protein breakdown (V. Kumar et al., 2009). Conversely, a reduction of the myofibrillar volume (atrophy) occurs when muscle protein is degraded at a rate higher than it is synthesized. Regulation of the two processes take place at multiple levels and include numerous signaling pathways (Bolster et al., 2004).

A potent activator of the hypertrophic program is insulin-like growth factor-1 (IGF1) (Philippou et al., 2007). Being primarily a liver-derived factor, IGF1 is produced locally in muscle tissue where it works in an autocrine and paracrine fashion (Tonkin et al., 2015). When IGF1 binds to its cell surface receptor, it causes activation of the serine/threonine kinase Akt, which regulates the activity of various downstream proteins (Bodine et al., 2001). One important target is the mammalian target of rapamycin (mTOR), which stimulates protein synthesis through activation of ribosomal kinase S6 (S6K). Thus causing upregulation of mRNA translation efficiency (Manning et al., 2007). Another essential feature of mTOR is the ability to integrate growth signals from the IGF1/Akt pathway with intracellular signals that reflect the energy and nutritional status of the cell (Moberg et al., 2016). Conversely, mTOR can be inhibited by the AMP-activated kinase (AMPK), an energy sensor activated by ATP depletion (Gwinn et al., 2008), thus limiting protein translation when the energy status of the cell is low. An additional anabolic effect of Akt is its ability to constrain protein degradation by inhibiting transcription factors in the FoxO family (Calnan et al., 2008). In skeletal muscles, the FoxO family regulates the expression of the E3 ubiquitin-ligases atrogin-1 and MuRF1 (Sanchez et al., 2014; Sandri, 2013). These two are a part of the ubiquitin-proteasome pathway which has been implicated in various atrophic conditions such as disuse, denervation, starvation, and cancer (Sanchez et al., 2014). Starvation-induced AMPK signaling is shown to antagonize Akt-mediated inhibition of the FoxO-family (Greer et al., 2007).

MYONUCLEI AND MUSCLE SATELLITE CELLS

Because of the large cytoplasmic volume, myofiber homeostasis is maintained by the presence of multiple myonuclei that are distributed along the sarcolemma of the myofiber (Bruusgaard et al., 2003). A central concept within skeletal muscle physiology is the existence of a myonuclear domain, where each myonucleus governs a confined area of cytoplasm, maintaining the protein production within its local domain (Ralston et al., 1989; Ralston et al., 1997). A hypothetical upper limit for the myonuclear domain means that in order to grow larger, the myofiber must acquire additional nuclei (Figure 2). It is well established that an increase in fiber size is correlated with an increase in myonuclear number (Allen et al., 1999; Bruusgaard et al., 2010; Schiaffino et al., 1976).

Using synergist ablation, it has been demonstrated that an increase in myonuclear number precedes hypertrophy (Bruusgaard et al., 2010). A possible explanation is that a diffusion constraint of nuclear products is suboptimal for maintaining cellular homeostasis/function as the myonuclear domain increases (Papadopoulos et al., 2000; Russell et al., 1992). The acquisition of additional myonuclei occurs through the recruitment from a population of muscle stem cells called satellite cells (Moss & Leblond, 1971; Snow, 1978). Under normal circumstances, the satellite cells are dormant but are activated in response to various stimuli such as hypertrophic growth (Schiaffino et al., 1976), hormones (Egner et al., 2013) or muscle injury (Hill et al., 2003). Ablation of the satellite cell population using γ- (Adams et al., 2002), x-irradiation (Gulati, 1987) or genetic alterations (Egner et al., 2016) is shown to ablate overload-induced hypertrophy and inhibiting the regenerative capacity of the muscle.

During atrophic conditions, the myonuclear domain decreases (Allen et al., 1999). In response to disuse, it has been demonstrated that nuclei do not disappear from the myofiber following hind-limb suspension induced atrophy (Bruusgaard et al., 2012; Jackson et al., 2012). Furthermore, after testosterone-induced increase in myonuclear number, newly acquired myonuclei remain in the cytosol after drug withdrawal, even though the myofibers returned to their original size (Egner et al., 2013). In aged mice,

there is reported to be little or no apoptosis of myonuclei, even though the fiber cross-section and myonuclear domain have significantly decreased (H. Wang et al., 2014). No loss of myonuclei is reported following atrophy induced by long-term denervation (Bruusgaard et al., 2008; Wada et al., 2002).

Together these findings suggest that "excess" nuclei remain in the cytosol during various atrophic conditions. Several studies of cancer survivors have shown resistance training to be a valuable asset for regaining muscle mass (De Backer et al., 2009; Dos Santos et al., 2017). However, it is not clear if myonuclei are preserved during cancer-induced atrophy.

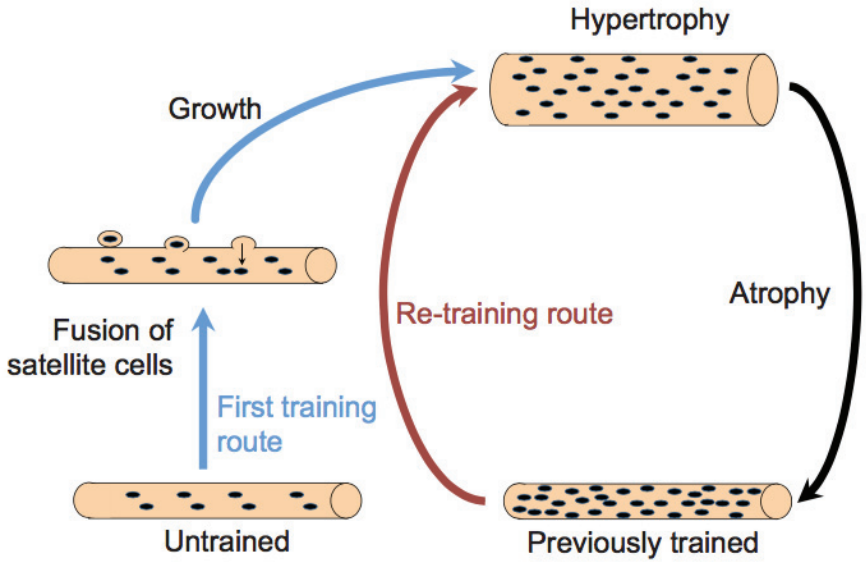

Figure 2. Changes in myonuclear number during hypertrophy and atrophy. For naive myofibers that have not previously undergone hypertrophy, myonuclei must first be recruited from satellite cells to allow subsequent hypertrophic growth. This temporarily reduces the myonuclear domain, thus allowing the fiber to acquire a larger volume. During atrophy, several models suggest that the additional nuclei remain in the cytosol, providing a "memory" of the previous state by allowing hypertrophic growth without the need to recruit new nuclei. (For additional info see main text). Figure adapted from Bruusgaard et al. (2010).

CANCER-INDUCED MUSCLE WASTING AND CACHEXIA

Cachexia is a metabolic wasting syndrome where loss of muscle and adipose tissue cannot be reversed by nutritional support (Porporato, 2016). If sustained the disease state cause physiological impairment and in worst-case death by cardiac and respiratory failure (K. Fearon et al., 2011). Being prevalent in several pathologies such as chronic heart failure, obstructive pulmonary disease and renal failure, the disease state prevails in cancer where it affects up to 60 % of all patients (Lok, 2015; Tsoli et al., 2013). In some cancer types, such as pancreatic and gastric cancer up to 80 % of patients are affected (Tisdale, 2010), and it is believed that the condition is responsible for up to 20 % of all cancer-related deaths (K. C. Fearon, 2008).

In the clinic, cancer cachexia is defined as an involuntary weight loss of more than 5 % over six months in the absence of starvation; or a weight loss higher than 2% in individuals already showing symptoms or having a body mass index less than 20 (K. Fearon et al., 2011). Being prevalent in terminal cancer, the degree of weight loss at the time of diagnosis is a strong predictor of survival in the later stages of the disease (Vigano et al., 2000). Patients suffering from an involuntary weight loss of more than 5% over the last three months before being diagnosed are associated with low survival rates (Vigano et al., 2000).

Though inactivity and decreased caloric intake contributes to cancer cachexia (Porporato, 2016), the decrease in body weight is accompanied by metabolic abnormalities, including alterations in carbohydrate, protein, lipid metabolism and development of insulin resistance (Baracos, 2002). At the cellular level, cachexia is characterized by two common features; a hyperactivation of the cell degradation pathways (Yuan et al., 2015), and a reduction in mRNA translation efficacy (Mueller et al., 2016). Although the exact mechanisms behind cancer cachexia are still not completely understood, several studies suggest the condition to be a result of a systemic inflammation propelled by blood-borne factors released from tumors, immune cells and tissues actively undergoing wasting (de Matos-Neto et al., 2015). The hypothesis is that the disease state is initiated as a tumor begins to secrete pro-inflammatory cytokines into its microenvironment, causing activation of stromal/immune cells in the tumors vicinity (de Matos-Neto et al., 2015).

Once activated, the cells start to emit their own cytokines triggering a cascade of adverse signaling pathways where the target tissue is wedged into an inflammatory-like disease state (Porporato, 2016). Although the pathogenesis of cachexia is multifactorial, many of the effects are due to aberrant signaling within the mechanisms controlling energy homeostasis (Porporato, 2016). Through clinical sampling and various experimental cachexia models, the pro-inflammatory cytokines; Interleukin 6 (IL6) and tumor necrosis factor-α (TNFα) has been revealed to be among the most important mediators of the cachectic signal (Grivennikov et al., 2011; Munoz-Canoves et al., 2013).

In the widely used murine colon-26 (C26) allograft, a high IL-6 secreting tumor model, administration of antibodies targeting the IL-6 receptor is shown to attenuate development of cachectic phenotype (Fujita et al., 1996; Tsujinaka et al., 1996). The importance of IL6 has also been demonstrated by crossing the spontaneous tumor producing $Apc^{min/+}$ mice, with an IL6 knockout strain, showing that the resulting $Apc^{Min/+}/IL\text{-}6^{-/-}$ mice do not develop cachexia (Baltgalvis et al., 2008). IL6 production and the development of cachexia have also been confirmed in a human prostate PC3 xenograft model (Giri et al., 2001; Malinowska et al., 2009; Waning et al., 2015).

The development of a cachectic phenotype is not solely due to the direct effect of the cytokines on myofiber metabolism. Another layer of complexity arises as systemic effects triggering changes in the global homeostasis. IL6 administration to humans and mice is shown to reduce hepatic production of IGF1, having suppressive effects on growth signaling through the IGF1/Akt/mTOR pathway (De Benedetti et al., 1997; Nemet et al., 2006).

Being a multifactorial condition, variability in experimental models and heterogeneity in the clinic makes it difficult to decipher the causality behind cachexia (Dagogo-Jack et al., 2018). Emerging evidence points towards epigenetic mechanisms playing an important role in muscle wasting (Alamdari et al., 2013; Carr et al., 2017; Segatto et al., 2017). Further advances in the field will possibly provide insight into how the dysregulation occurs at a genomic level.

EPIGENETIC REGULATION OF GENE TRANSCRIPTION

The word epigenetics was coined in 1942 by Conrad H. Waddington in an attempt to address the importance of investigating the developmental processes underlying the transition from genotype to phenotype (Waddington, 2012). However, being phrased in an era before the discovery of the DNA structure; the complicated manners required for studying these mechanisms resulted in the field receiving little attention at the time. Not until three decades later, with the discovery of mechanisms where genes could be switched on and off due to the presence of methylation groups on DNA (Holliday et al., 1975; Riggs, 1975), epigenetics slowly grew into a discipline. Today, the development of sequencing technologies has enabled us insight into the interactions between DNA and the dynamic cell. The term epigenetics is widely being used to describe research exploring DNA methylation, chromatin dynamics and regulation of gene transcription. Different elements of gene regulation used by the cell to canalize cellular differentiation, as well as stabilization of gene expression programs (Atlasi et al., 2017).

THE IMPORTANCE OF METHYLATION IN REGULATING PHENOTYPE

Conceptually, DNA methylation is the simplest of the epigenetic mechanisms, and refers to the covalent addition of methyl groups to the nucleotide cytosine (Moore et al., 2013). Commonly, methylated cytosines are positioned next to a guanine (CpG methylation), creating a dinucleotide found at a higher frequency in regulatory regions, such as promoters and enhancers (Deaton et al., 2011). Traditionally, CpG methylation is associated with transcriptional repression, and it is believed that the presence of methyl-groups inhibits transcription by preventing the binding of transcription factors to DNA (Gopalakrishnan et al., 2008; Kass et al., 1997).

In skeletal muscle, tissue-specific methylation patterns are thought to be one of the mechanisms behind stabilization of the myogenic lineage (Terragni et al., 2014). Analysis of methylation patterns in isolated myofibers has provided evidence suggesting that DNA

methylation might be an influential factor in maintaining fiber type specificity, as methylation patterns are inversely correlated with transcription of fiber type-specific genes (Begue et al., 2017). Studies of muscle tissue biopsies have demonstrated changes in the methylation profile of genes involved in oxidative metabolism in response to exercise (Kanzleiter et al., 2015; Lindholm et al., 2014). These findings suggest that DNA methylation can be important for long- and short-term adaption of skeletal muscle phenotype.

CHROMATIN AND HISTONE MODIFICATION

Chromatin is the DNA/protein complex found in the nucleus of all eukaryotic cells. One of the key functions of chromatin is to allow partitioning of DNA into transcriptionally active and repressed regions (Cutter et al., 2015). At the highest structural order, this partitioning can be divided into euchromatin and heterochromatin, representing an open and a closed chromatin structure, respectively (Saksouk et al., 2015). Traditionally, euchromatin is thought of as the active part of the genome where an open structure allows assembly of transcriptional complexes, while heterochromatin designates a condensed chromatin state with DNA being in a transcriptionally inert configuration (Eymery et al., 2009).

The main component of chromatin is the nucleosome, a protein octamer composed of two copies of each of the four core histone proteins (H2A, H2B, H3, and H4), comprising a globular structure that DNA wraps around 1.67 times (Cutter & Hayes, 2015). To achieve the high level of control required for modulating local chromatin structure eukaryotic cells has developed a variety of measures to regulate chromatin dynamics. This can involve the incorporation of specific histone variants at specific loci and non-covalent remodeling of local nucleosome structure by ATP-dependent remodeling enzymes (Harada et al., 2015; P. B. Talbert et al., 2017).

POST-TRANSLATIONAL MODIFICATION OF THE HISTONE TAIL

Central to this thesis is the covalent modifications of the N-terminal histone tails that protrude out from the globular core of the nucleosome (Cutter & Hayes, 2015). On each tail there are specific residues that are recognized and covalently modified by different classes of histone modifying enzymes (Bannister et al., 2011). The post-translational modifications (PTMs) transferred to the histones include methylation, acetylation, phosphorylation, ubiquitination etc. (Lawrence et al., 2016). There are a few main hypotheses explaining how histone modifications affect nucleosome function. One hypothesis proposes that different combinations of PTMs constitute a "histone code" that creates epitopes recognized by transcriptional modulators (Hake et al., 2004). It has also been suggested that the various modifications affect the electrostatic interaction between histones and DNA (Ettig et al., 2011).

By combining chromatin immunoprecipitation (ChIP) and sequencing technologies, genome-wide association studies of sequenced ChIP material (ChIP-seq) has revealed how different histone modifications are distributed across the genome. The technique allows identification of characteristic patterns that are used to identify transcriptionally active and repressed regions of the genome. The technique also allows the identification of regulatory elements such as insulators, enhancers and promoters (Cuddapah et al., 2009).

When referring to histone modifications in the literature, denotations follow a specific nomenclature that begins with the name of the histone (e.g. H3) followed by the modified residue relative to the N-terminus (e.g. K27, lysine at position 27) ending with the specific modification of the residue (Me; methyl, Ac: acetyl, P: phosphate). With over 50 different post-translational changes identified (Figure 4) (Lawrence et al., 2016), not all will be covered in this thesis. However, I will address the most relevant to my project.

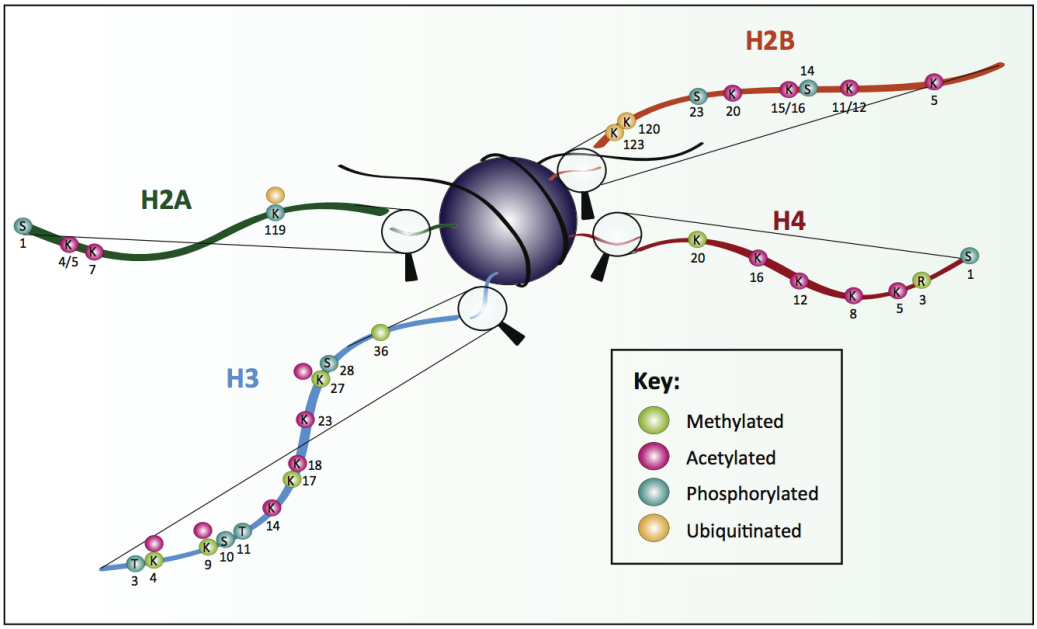

Figure 4. Overview of different post-translational modification depositions along histone tails This overview shows the location and species of individual histone modification found along the different histone tails. Color identifies how each residue is modified (green = methylated, pink = acetylated, turquoise = phosphorylated, beige = ubiquitinated). Location of each residue relative to the N-terminal is shown in black. Figure from Lawrence et al. (2016).

The histone mark H3K4me3 show open chromatin

Due to the high correlation with actively transcribed genes, Tri-methylation of lysine four at histone H3 (H3K4me3) has become one of the most studied histone marks (Lawrence et al., 2016). The modification is found enriched at the transcription start site (TSS), where it peaks at a narrow band of approximately 1000 base pairs up/down-stream of the transcriptional start site of actively transcribed genes (Z. Zhang et al., 2016). Due to its strong correlation with transcription, H3K4me3 has commonly been referred to as a prerequisite for gene transcription (Santos-Rosa et al., 2002). However, recent research suggests that the modification is deposited at nucleosomes in response to gene transcription (Howe et al., 2017). Here it is believed to maintain transcriptional stability through the recruitment of chromatin remodelers (Flanagan et al., 2005; Li et al., 2006; Mosesson et al., 2014) and by preventing binding of negative transcriptional regulators to nucleosomes (Nishioka et al., 2002).

Active and repressive effects of H3K27 modification

Genome-wide analysis of tri-methylated lysine-27 on histone H3 (H3K27me3) has shown that there is an inverse relationship between transcriptional activity and genes enriched for the particular modification (Barski et al., 2007; Boyer et al., 2006). Unlike H3K4me3 modified histones that are distributed in a narrow band around TSS, repressive histone marks such as H3K27me3 usually have a broad distribution across the gene body or intergenic regions (Pauler et al., 2009). The modification has received much attention due to its involvement in maintaining pluripotency of stem cells, where one of the functions is to orchestrate time-dependent activation of developmentally critical genes, thus preventing premature expression and cellular differentiation (Figure 5) (Boyer et al., 2006; Juan et al., 2016).

At some loci H3K27me3 is found enriched together with H3K4me3, a state of chromatin that is commonly referred to as bivalent chromatin. When this occurs at promoter regions, the genes are referred to as poised (Voigt et al., 2013). Although poised genes have an open profile, the combination of epigenetic marks keeps the gene repressed or expressed at low levels, and the genes are often found pre-loaded with RNA polymerase II to allow rapid transcription activation (Voigt et al., 2013).

Another feature of H3K27 is its ability to become acetylated (H3K27ac), providing an antagonistic effect on H3K27me3 (Tie et al., 2009). Because of this, H3K27 acetylated genes are associated with an open chromatin configuration and active transcription (Figure 5) (Lawrence et al., 2016). Similar to H3K4me3, H3K27ac is found enriched at the promoters of actively transcribed genes (Heintzman et al., 2007). Histone acetylation is regulated by a balance between two classes of antagonistic enzymes called histone deacetylases (HDACs) and acetyl-transferases, acting as transcriptional repressors and activator, respectively (Peserico et al., 2011). The interplay between the two classes of enzymes is of particular importance in skeletal muscle, as several of the members are targeted by transcription factors know to regulate myofiber phenotype (McKinsey et al., 2001).

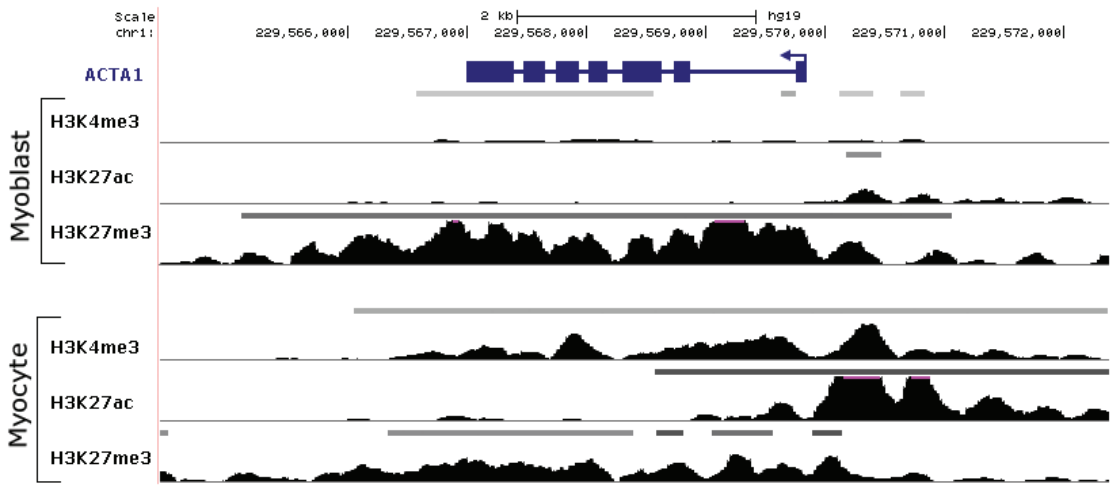

Figure. 5. Dynamic changes in histone profile at the skeletal muscle actin gene during differentiation of myoblast to myocytes. Image of the UCSC genome browser window showing the distribution of histone modifications across an 8 kb region of human chromosome 1 ([GRCh37/hg19 assembly], February 2009). The displayed region contains the gene encoding skeletal actin (ACTA1), represented as a blue line with thin and thick segments indicating introns and exons, respectively. The figure displays the ChIP-seq signal for two active histone modifications (H3K4me3 and H3K27ac) representing open chromatin, and repressive H3K27me3, shown as black peaks along the X-axis. Gray lines above the peaks correspond to regions where the local signal/enrichment of a particular modification is higher than the average background. The data is obtained from human myoblasts, before and after differentiation into myocytes (GSE29611, Bernstein lab, Jan 2011). In myoblasts, the low enrichment of H3K4me3, H3K27ac and a high relative enrichment of H3K27me3 are associated with a low transcriptional activity. When comparing the two differentiation states, it becomes visually apparent that the gene attains an active/open profile upon differentiation due to an increased enrichment of H3K4me3 and H3K27ac. These findings are in accordance with studies showing ACTA1 expression levels and protein content to be lower in undifferentiated C2C12 myoblasts (Juretic et al., 2007; Kislinger et al., 2005).

HISTONE MARKS ON ENHANCERS

Enhancers are non-coding DNA sequences that contain binding motifs for sequence-specific transcription factors (Shlyueva et al., 2014). Being targeted by various co-activators and co-repressors the combined cues determine the activity of the enhancer (Calo et al., 2013). A functional characteristic of enhancers is their ability to regulate the expression of genes independently of the distance to their target gene (Amano et al., 2009). Being implicated in both cellular differentiation and maintenance of cellular identity (Bonn et al., 2012), evidence points to enhancer activity as an essential regulator of gene expression in response to physiological changes (Gray et al., 2015).

As with regions containing protein-coding DNA, there is a correlation between enhancer activity and histone modifications (Creyghton et al., 2010; Heintzman et al., 2007). Similar to promoters, active enhancers are enriched for H3K27ac. However, they do not display high levels of H3K4me3 but the closely related H3K4me1, providing a stereotypical pattern allowing genome-wide identification of enhancers and promoters (Heintzman et al., 2007; Rada-Iglesias et al., 2011). Thus, the PTMs are not necessarily found on the enhancer, due to them being devoid of nucleosomes, but instead located to the nucleosomes flanking the element (Boyle et al., 2008).

In skeletal muscle, there are several enhancers involved in the differentiation of myoblasts to myocytes (Blum et al., 2012; Yu et al., 2017). With regard to MyHC phenotype, a putative enhancer element required for maintenance of the fast transcriptional program has been identified (Sakakibara et al., 2014). A similar regulatory region has been identified for the MYH7 gene, encoding MyHC 1 (Greco et al., 2016). However, the *in vivo* physiological function and chromatin status of the various enhancers remains to be explored.

STUDIES OF HISTONE MODIFICATIONS IN MUSCLE TISSUE

Although several studies has preformed ChIP-seq on satellite cells derived from skeletal muscle (Lilja et al., 2017; Liu et al., 2013; Woodhouse et al., 2013), only a limited number have examined histone PTMs in chromatin from whole tissue. To our knowledge, only one paper has preformed global-sequencing of whole tissue chromatin, demonstrating broad H3K27me3 enrichment at stem-cell genes in chicken skeletal muscle (David et al., 2017).

Using ChIP-qPCR, a method allowing investigation of histone deposition in 50-200 bp regions, Pandorf et al. (2009), show that H3K4me3 and H3 acetylation at the MyHC genes correlates with RNA expression in fast and slow muscle. Following disuse atrophy by hind-limb suspension, the same study shows that RNA expression and histone enrichment at MyHC 1 gene decrease in SOL, while there is increased expression and enrichment at the MyHC 2x and 2b genes. This type of slow to fast transition of phenotype is also observed at the protein level (Oishi, 1993). In response to exercise, it is increased nuclear export of HDAC4 (McGee et al., 2009), a protein that is upregulated in denervated muscles (Beharry et al., 2015). These findings demonstrate that the histone landscape in skeletal muscle is under active regulation of histone modifying enzymes (McGee et al., 2017).

An important issue that has been addressed in studies of heart tissue is the importance of performing ChIP analysis on purified cell populations, as a mix between different cell types causes an increase in the number of false negatives and positives (Gilsbach et al., 2014; Preissl et al., 2015). Being composed of approximately 40–50 % myonuclei (Schmalbruch et al., 1977), isolation of single myofibers preceding transcriptome (Chemello et al., 2011) and methylome analysis (Begue et al., 2017) is shown to be a valuable approach for increasing the resolution power and reduce interference from stromal cells. Concerning histone modifications, the impact of tissue heterogeneity has not been addressed in skeletal muscle.

THE CENTROSOME AND THE PERICENTRIOLAR MATERIAL-1

In an animal cell, the centrosome is best known for its function as a microtubule-organizing center (MTOC), where it during cell cycle coordinates assembly of the spindle-poles required for separation of chromosomes during mitosis (Nigg et al., 2009). In non-dividing cells, the centrosome has additional roles such as regulating cell polarity and cytoskeletal architecture by functioning as a primary nucleation site for microtubule formation, cilia formation and cell adhesion (Bartolini et al., 2006). In addition to the structural functions, the centrosome serves an additional role as a binding site for many cellular signaling pathways, such as the G1/S cyclins, making it a critical checkpoint for progression through the cell cycle (Meraldi et al., 1999; Pascreau et al., 2011). Due to its prominent role in the cell cycle, incomplete replication, structural alteration and changes in centrosomal proteostasis lead to cell cycle arrest or apoptosis (Mikule et al., 2007). On the structural level, centrosomes are composed of two tubulin-based centrioles surrounded by an amorphous mass of proteins called the pericentriolar material, containing most of the proteins required for MTOC function (Mennella et al., 2014). Affiliated with the centrosome and the pericentriolar material are small microtubule anchored granules of approximately 70-100 nm in diameter called centriolar satellites (CS) (Balczon et al., 1994). Although information about their function remains scarce, CS is believed to regulate centrosomal protein composition by facilitating active transport along the microtubule or sequestering of centrosomal proteins for later stages of the cell cycle (Bugnard et al., 2005; Kubo et al., 2003).

Being only present during the cellular interphase, CS are found scattered around the cytoplasm with an increasing concentration toward the centrosome (Kubo et al., 1999). Upon entry of the mitotic phase, the structures congregate around the centrosomes where they gradually disappear (Balczon et al., 1994). Following cytokinesis the granules reappear, regaining their original localization (Kubo et al 1999, 2003).

Being the first discovered CS protein, the 228 kD Pericentriolar material-1 (PCM1) is viewed as one of its core components (Balczon et al., 1994; Dammermann et al., 2002). Commonly used as a marker for identification of CS, PCM1 is believed to serve as a protein scaffold and binding site for CS proteins (Tollenaere et al., 2015). Mapping studies have resulted in the identification of 30 CS proteins, most of which are also present at the centrosomes (Hori et al., 2017), strengthening the hypothesis that centriolar satellites are involved in regulation and maintenance of centrosomal proteostasis (Tollenaere et al., 2015). Knockdown of PCM1 is shown to cause failed microtubular anchoring and a reduction in the number of CS proteins at the centrosome (Dammermann & Merdes, 2002; Hori et al., 2014). These alterations will eventually cause cell cycle arrest by p38 mediated phosphorylation of the tumor suppressors p53 and p21 (Mikule et al., 2007).

BECOMING A POST-MITOTIC CELL

Although the mechanisms leading to cell cycle arrest probably have evolved to prevent the development of cancer cells (DeGregori, 2011), many of the same mechanisms are being used to confine a cell to a post-mitotic state (Mal et al., 2000). To differentiate and fuse into myotubes, muscle progenitor cells must first withdraw from the cell cycle (Lassar et al., 1994). Evidence suggests that withdrawal from the cell cycle initiates increased activity of the CDK-inhibitors p21, p38 and the tumor suppressor Rb which in turn lock the cell in a state where it is unresponsive to cell cycle promoting factors and unable to divide (Andres et al., 1996; Cabane et al., 2003; Mal et al., 2000; Thorburn et al., 1993). Simultaneously there is an extensive re-localization of centrosomal proteins to the nuclear envelope (Espigat-Georger et al., 2016). The centrosome disappears, and several of the MTOC associated proteins (including PCM1) translocate to the nuclear membrane of the developing myotubes (Figure 6) (Fant et al., 2009; Srsen et al., 2009), resulting in the myonuclear envelope adopting some of the core MTOC function as a foundation for cytoskeletal architecture (Bartolini & Gundersen, 2006; Tassin et al., 1985). As the evidence supports the requirement of a functional centrosome for cellular proliferation, changes in the distribution of centrosomal proteins combined with

increased tumor suppressor activity may be an efficient way to achieve a post-mitotic state. Centrosomal disassembly and the establishment of a nuclear envelope based MTOC is also hypothesized to be an efficient way of strengthening the cytoskeletal architecture in response to physical strain (Zebrowski et al., 2015). In the heart, PCM1 is commonly being used as a marker for identifying cardiomyocytes, the post-mitotic myofiber equivalent in the heart (Bergmann et al., 2012; Gilsbach et al., 2014; Richardson, 2016). Since skeletal myofibers share many of the same features as myocytes PCM1 could also be a valuable addition to skeletal muscle research.

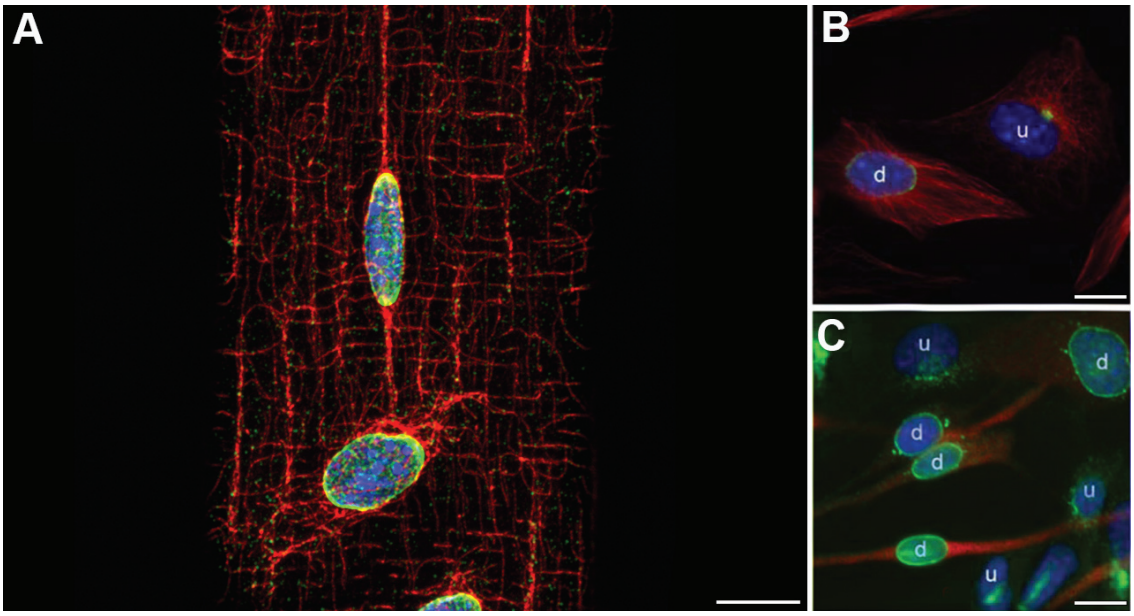

Figure 6. PCM1 is localized to the nuclear envelope in mouse skeletal myofibers. (A) The figure shows two myonuclei in a collagenase-isolated myofiber, labeled with antibodies against microtubule (alpha-Tubulin, red), pericentriolar material 1 (PCM1, green) and nuclei (DAPI, blue). The PCM1-antibody labels the nuclear envelope, with microtubular projection originating out of the same area, demonstrating a relocalization of MTOC proteins in differentiated cells. *Image A: I.M.Winje, K.A Hansson, Unpublished* **(B)** Image show an undifferentiated C2C12 myoblast (denoted u). As a myoblast starts to differentiate (d), MTOC function and PCM1 (green) re-localizes to the nuclear envelope. **(C)** After differentiation and formation of myotubes, PCM1 (green) strongly labels the nuclear envelope. Cells are also labeled with an antibody against embryonic myosin (red) to visualize the cytosol. Image B and C are from Srsen et al. (2009). Scale bars are 10 um.

AIMS OF THE THESIS

Research aim paper I:

1) Investigate if myonuclei are lost from the myofiber syncytium during cancer-induced atrophy.

Research aim paper II:

1) Identify whether an antibody against Pericentriolar material-1 can be used to selectively label myonuclei on tissue cross sections from humans, mice, and rats.

Research aims paper III:

1) Establish a method for labeling and sorting myonuclei from tissue homogenate using the antibody against Pericentriolar material-1.

2) Perform chromatin immunoprecipitation and global sequencing of myonuclear DNA from EDL and Soleus muscle.

3) Investigate differences in H3K4me3 and H3K27ac enrichment patterns between the EDL and Soleus muscle.

METHODOLOGICAL CONSIDERATIONS

In this thesis we have utilized various techniques to study and characterize myonuclei in adult skeletal muscle. In this section, I will discuss methodological considerations related to the central experiments.

BRIEF OUTLINE OF EXPERIMENTAL PROCEDURES

In paper I, we wanted to investigate if myonuclei are lost from the myofiber syncytium following cancer-induced atrophy. To induce cachexia, human prostate PC3 cells were xenografted into nude mice. Six weeks after inoculation and the development of a cachectic phenotype, the *in vivo* myonuclear number of EDL was determined by microscopic analysis of myofibers microinjected with a fluorescent oligonucleotide that is actively transported into the nuclei (Bruusgaard et al., 2003; Utvik et al., 1999). As this technique only allows the study of superficial myofibers, muscles were cryosectioned and studied by immunohistological techniques to determine myonuclear number and CSA on tissue cross-sections. The histopathological status of the muscles was determined by Hematoxylin and Eosin staining to identify signs of degeneration or infiltrating immune-cells cells. Muscles were also analyzed with TUNEL assay to detect apoptosis.

In paper II, we investigated whether an antibody against the Pericentriolar material-1 (PCM1) could be used to selectively label myonuclei. This was done by triple-labeling cryosections from mice, rat and human muscle for anti-PCM1, DNA and anti-dystrophin. Samples were imaged in different focal planes, and each z-plane was manually assessed to determine whether the PCM1 positive nuclei were positioned inside or outside the dystrophin ring, representing myo- and stromal nuclei, respectively. To validate whether the PCM1 antibody selectively labels myonuclei, muscles were transduced with a myonuclei specific marker to investigate its colocalization with PCM1. We also used the antibody in an experimental setting, to investigate if the antibody labels newly inserted myonuclei in response to overload-induced hypertrophy.

In paper III, we established a method for investigating the chromatin landscape in skeletal muscle. Because the tissue is made up of a heterogeneous cell population, we used the PCM1 antibody to enrich myonuclei from crude muscle homogenate. To determine whether myonuclear enrichment enhance the myogenic signal, chromatin from sorted and unsorted fractions were immunoprecipitated with an antibody against H3K27ac and analyzed with ChIP-qPCR. We then used the method to generate an epigenetic map of the H3K27ac and H3K4me3 distribution in the fast EDL and slow SOL muscle. Although the ultimate analyses are not fully completed, the distribution of the two modifications follow a characteristic enrichment pattern with fast and slow genes being enriched in EDL and SOL, respectively.

ETHICS AND APPROVAL

The work in this thesis involves studies of living animals and animal tissues. All experiments involving living animals were approved by the National Animal Research Authority of Norway before initiation (Project ID: 8336,7626,7431 and 6666). The Norwegian Animal Research Authority provided governance to ensure that facilities and experiments were performed in accordance with the National Regulations of January 15th, 1996, and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of March 18th, 1986. Human studies were approved by the Regional Committees for Medical and Health Research Ethics in Southeast Norway (Project ID: 2014/834), which were performed in accordance with the Declaration of Helsinki. All human subjects signed a written informed consent before participation. Generation of the custom adeno-associated virus was approved by The Norwegian Labor Inspection Authority (Arbeidstilsynet) and the Norwegian Food Safety Authority (Mattilsynet). The virus used is replication defective and classified as Biosafety class 2.

CHOOSING A MODEL SYSTEM FOR STUDYING CACHEXIA

The use of animal models in cancer research is essential for bridging the gap between cell culture assays and human disease. When designing an *in vivo* tumor study, researchers may choose between various tumor-models that differ in their clinical relevance and experimental robustness. Hence, critical evaluation of the different models is important as they represent imperfect models of the heterogenic and multifactorial mechanisms behind human disease (Day et al., 2015). Two common models for studying tumors *in vivo* are the murine allografts and the cancer cell line-derived xenografts. The main difference between the two models is that the allograft uses cancer-cells derived from an animal with the same genetic background, while the xenograft use human-derived cells transplanted into a host.

In a xenograft, rejection of the foreign cells is prevented by the use of mouse strains with various degrees of immunodeficiency (Romano et al., 2012), providing an advantage to the allograft system because it accounts for potential interaction between the tumor and the hosts' immune system. Although the xenograft model system lacks this interaction, it is more representative of human cancers and has a higher predictive value when assessing the clinical utility of cancer therapy agents (Jung, 2014; Voskoglou-Nomikos et al., 2003).

In the recent years, the development of new models such as patient cancer-derived xenograft and genetically engineered mice is emerging (Dobrolecki et al., 2016). These models may prove advantageous over the traditional xenograft/allograft models. However, they often require a prominent infrastructure and are more challenging to work with (Morton et al., 2016; Ruggeri et al., 2014).

For our experimental setup, we conducted a pilot study in collaboration with a research group working with prostate xenografts model and investigated the cachectic potential of three different human cell lines (22rv1, Du145, and PC3). From these experiments, only the PC3 and the Du145 grafted mice developed a cachectic phenotype, but due to a rapid tumor growth and the development of ulcers, the Du145 group was euthanized to prevent

any unnecessary suffering for the animals. The PC3 group had a high reproducibility within the group, and the cachectic properties of the cell-line have also been well described in the literature (Marchildon et al., 2015; Z. Wang et al., 2003; Waning et al., 2015).

As the immune system is an important component in cachexia, we considered using one of the more established allographic cell-lines (e.g., C26). But due to lack of experience with the relevant model system and cell-lines, we chose to use the PC3 cells, as the primary aim of the experiment was to determine myonuclear dynamics. The experiments were performed using Balb/c nu/nu mice; while these mice lack a thymus and are unable to produce T-cells, other components of the immune system are present (Budzynski et al., 1994).

ISOLATION OF MYONUCLEI FOR EPIGENETIC ANALYSIS

The preparation of nuclei from skeletal muscle tissue presents several problems compared to that of other tissues. Firstly, the tissue is especially difficult to homogenize due to the amount of connective tissue. This causes the conventional methods applied on cell-culture and soft tissues to be unsuccessfully applied. As a consequence, the homogenization procedures are often vigorous and must account for a careful balance between tissue disruption and nuclei integrity. Secondly, the DNA content within skeletal muscle is particularly low compared to that of other tissues, being approximately one-fourth of that in the heart and one-sixth of that in the liver (Kuehl, 1975). Thirdly, homogenization of the tissue causes release of myofibrillar components that tend to co-purify with the nuclei and make it difficult to separate the various fractions (Hahn et al., 1990).

Another common method for isolating myogenic cells is by digesting the extracellular matrix with collagenase, allowing dissociation of the tissue into mononuclear cells and single myofibers (Ohkawa et al., 2012). Mononuclear cells such as satellite cells can then

be isolated, cultured or analyzed by flow cytometry, and the isolated myofibers can be harvested for transcriptomics and methylome analysis (Begue et al., 2017). Although this method may provide pure myonuclear fractions for epigenetic analyses, the procedure requires prolonged digestion times of up to 45 minutes when digesting a small muscle such as EDL (Chemello et al., 2011). Such long tissue preparation times could possibly expose the muscle to an hypoxic environment (Chang et al., 2015) and mechanical stimulation (Wozniak et al., 2005) that may alter the expression profile of the cells. For analysis of histone PTMs, the prolonged preparation time may also be problematic as histone modifications and nucleosomes can be modified or displaced within minutes (Katan-Khaykovich et al., 2002; Riffo-Campos et al., 2015).

As no studies have adequately discussed and investigated these problems in skeletal muscle, our method in paper III is based on snap frozen tissue to limit the potential influence of tissue preparation. An overview of the experimental procedures is shown in figure 7. Muscles were obtained from sedated animals by first removing the skin and connective tissue above and around the respective muscles of interest before cutting the nerve and blood supply. Muscles were then excised and frozen down in liquid nitrogen within half a minute and further stored at -80 degrees. For isolating total nuclei, muscles were cut into tiny pieces of 1-2 mm3 at -20 degrees before being homogenized in ice-cold buffer containing chelating agents, protease-, RNase- and acetylase-inhibitors to halt biological activity.

Using the gentleMACS dissociator, we can isolate approximately $3x10^6$ nuclei from 100mg tissue; a yield is similar to that of other protocols using sucrose (Cutler et al., 2017) or Percoll (Hahn & Covault, 1990) based density fractionation. After PCM1 labeling and MACS sorting, the final yield is around $0.6–0.8x10^6$ PCM1 positive nuclei.

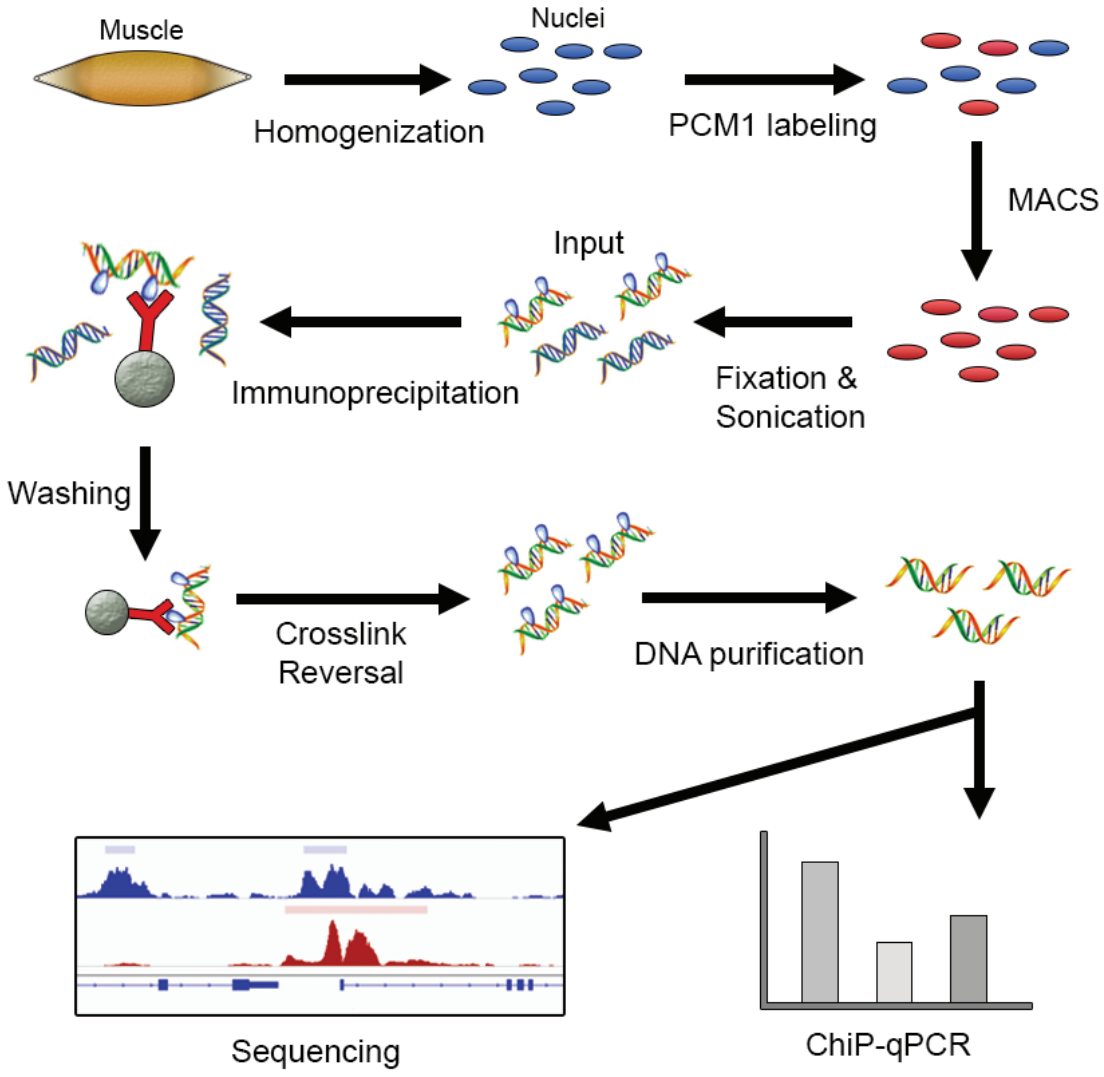

Figure 7. Outline of the main experimental procedures for analysis of histone PTMs from skeletal muscle nuclei. Crude nuclei prepared from flash frozen muscle tissue were labeled with an antibody against PCM1 and sorted using magnetic associated cell sorting (MACS). Using MACS, debree and PCM1 negative cells do not bind to the column. Nuclei were then fixated and sonicated before being immunoprecipitated with antibodies against specific post-translational modifications. After immunoprecipitation, the enriched chromatin was purified into dsDNA, which can be analyzed with various techniques to investigate DNA enrichment. In ChIP-qPCR experiments, single loci of 50 – 200bp can be amplified and compared. In our experiments, ChIP-qPCR was used for optimization of the protocol and comparison of sorted and unsorted nuclei. Sequencing was used for the comparison of the histone landscape in EDL and SOL.

CHROMATIN IMMUNOPRECIPITATION AND SEQUENCING (CHIP-SEQ)

The combination of sequencing and chromatin immunoprecipitation (ChIP-seq) is a technique used to determine whether a specific protein or post-translational modification is associated with a particular genomic region. In our experimental setup ChIP was performed by cross-linking the isolated nuclei to preserve protein/DNA interaction, followed by sonication to yield short DNA-protein fragments of approximately 200-300 bp. Fragments were then immunoprecipitated with antibody-Dynabead complexes to capture the specific PTMs of interest. After enrichment, cross-linking was reversed and the DNA purified, resulting in DNA that can either be quantified by qPCR or sequenced to reveal information about global enrichment.

As each step of the experimental protocol is prone to artifacts, an essential part of ChIP-seq experimental designs is to determine appropriate controls. First, regarding antibody specificity, antibodies may have cross-reactivity and show binding towards structurally similar modification (Rothbart et al., 2015). This was accounted for by using ChIP-grade antibodies quality checked and tested for cross-reactivity (e.g., H3K4me3 vs. H3K4me2). Secondly, actively transcribed regions of DNA usually have an open configuration and are more accessible to shearing during sonication (Kidder et al., 2011). Consequently, these regions may potentially have a higher background signal (Kidder et al., 2011). This was accounted for by comparing our immunoprecipitated DNA with an input-control sample containing non-immunoprecipitated chromatin from our starting ChIP-material (input). In the ChIP-qPCR experiments where we compared PCM1 sorted and unsorted nuclei. The input sample was used as a standard for normalizing our experimental qPCR data as percent/input, referring to the relative enrichment of the specific loci before and after immunoprecipitation. In our sequencing data, the signal from the input sample was subtracted from our experimental data. Thirdly, parallel experiments were performed with a non-specific IgG antibody to account for signals arising from non-specific binding to antibodies or Dynabeads.

From our sequencing of H3K4me3, H3K27ac and H3K27me3 immunoprecipitated chromatin from purified myonuclei from EDL and SOL, we see a differential distribution across the genome (Figure 8). The description and functional implications of the differences in the epigenetic landscape between EDL and soleus is still ongoing. Currently, we are testing different peak calling algorithms to resolve an optimal ratio between enrichment and noise, to limit false negatives and positives. Due to high variability in the H3K27me3 track, it is currently not included in the work presented in Paper III.

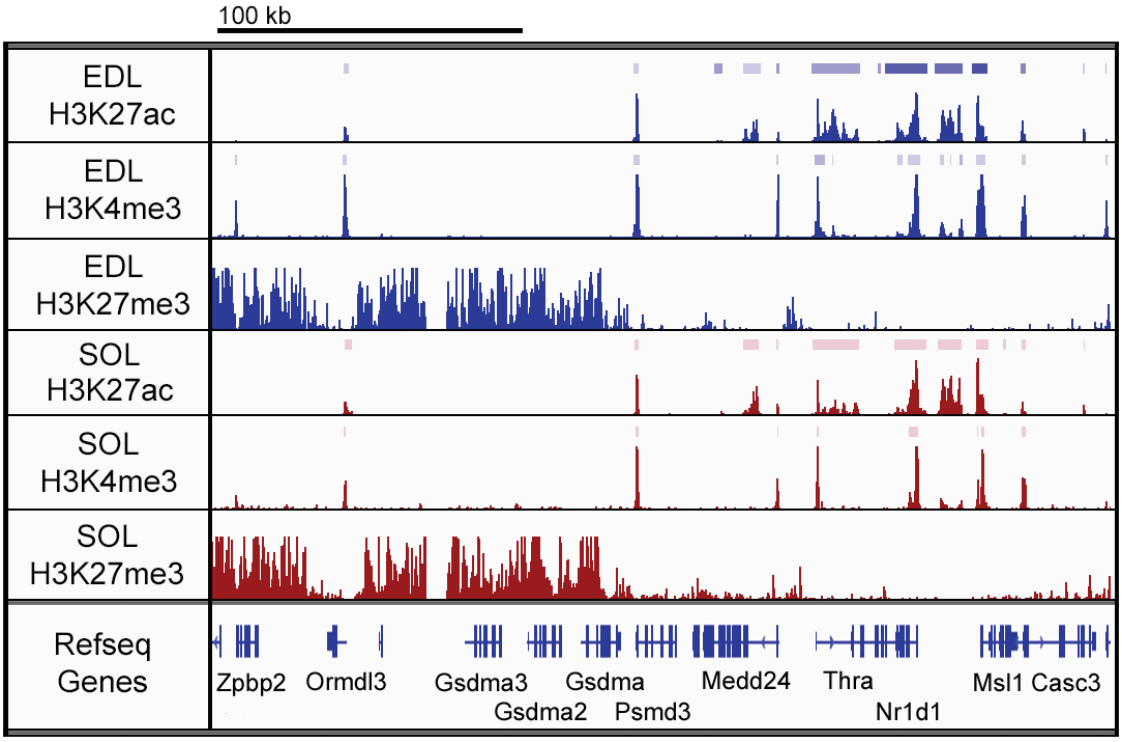

Figure 8. Histone modifications localize to distinct genomic regions in EDL and SOL. IGV browser tracks are showing enrichment of H3K4me3, H3K27ac and H3K27me3 in EDL (blue) and SOL (red) across a 244 kb segment of chromosome 11 (chr11:98,400,746-98,645,010). Sequenced DNA was aligned against the house mouse ([NCBI37/mm9 assembly] July 2007). Colored lines above the peaks correspond to regions of DNA where the local signal/enrichment is higher than the average background. The panel displays the average signal from three biological replicates.

SYNOPSIS OF RESULTS

PAPER I

Cachexia is a wasting disorder that involves the loss of body- and muscle mass. It is not clear whether the condition is merely a result of a disturbance in the protein balance or if it involves a degenerative loss of myofibers or myonuclei from the tissue. Based on literature from non-disease atrophy models, we hypothesized that myonuclei would remain in the myofiber syncytium following cancer-induced cachexia.

Using the PC3 xenograft model, we induced a 16% loss in whole-body weight over a 6-week period. The loss of body weight was accompanied by a decrease in heart mass and an increased spleen, two phenotypical features associated with cachexia. Using *in vivo* microinjections, we found no loss of myonuclei in the cachectic group. This was verified by analysis of muscle cross-sections from EDL, where we found a 21% reduction in the mean cross-sectional area and no loss of myonuclei. From our histopathological analysis, there was no sign of regeneration or apoptosis in the tissue. However, there was a non-significant 8 % reduction in the total number of fibers in the cachectic group. Considering that most of the body-weight was lost during the last two weeks of the experiment, we find it likely that signs of fiber degeneration or TUNEL positive nuclei would be present in our histopathological analysis if fibers were truly lost during this period, and thus interpret the 8% reduction to be spurious.

This paper suggests that the cachectic phenotype caused by xenografted prostate tumor PC3 cells is not caused by degenerative processes, and could be attributed to an imbalance in protein synthesis and degradation.

PAPER II

The assessment of myonuclei is important in many areas of muscle research. In most of the literature concerning myonuclear numbers, the identification of myonuclei relies on determining the position of the individual nuclei relative to the dystrophin ring surrounding the cell. This provides an element of subjectivity as both operators and equipment vary between labs. Hence, the lack of a non-standardized method for identifying myonuclei, could probably explain some of the conflicting findings in the literature.

In this paper, we addressed these problems and showed that an antibody against pericentriolar material-1 (PCM1) can be used to specifically label myonuclei on cross-sections from rat, mouse and human. Using confocal stack-analysis, we show that the PCM1 antibody labels all nuclei residing within the dystrophin ring. To verify that the antibody labels myonuclei, we used viral-transduction and found that PCM1 labeling co-localizes with a myonuclear specific reporter. To investigate if the antibody label newly acquired nuclei, a synergist-ablation model were used and we found an increase in the number of PCM1 positive nuclei in hypertrophic muscles.

Our results suggest that the PCM1 labeling technique could be a valuable tool for myonuclear identification as it provides a standardized method for detecting myonuclei on muscle cross-sections.

PAPER III

In recent years, covalent modification of histone tails have emerged as important regulatory mechanism of phenotypic plasticity and cellular identity. In the field of skeletal muscle research, these mechanisms have not received much attention, possibly due to the tissue being made up of a heterogeneous cell population and a syncytial architecture that makes tissue dissociation and myonuclear enrichment challenging.

In this paper, we present a novel method for studying histone modifications in skeletal muscle. Using the PCM1 labeling technique from paper II, we demonstrate that myonuclei can be labeled and enriched from crude nuclei homogenate. From our flow-cytometric analysis of crude and PCM1 sorted nuclei, we show that our purified fraction makes up 97% myonuclei, as opposed to 60% in the unsorted fraction. To investigate the effect of myonuclear enrichment, we performed ChIP-qPCR analysis, showing increased H4K27ac enrichment at skeletal muscle-specific genes in the PCM1 sorted fraction, accompanied by reduced signal from genes associated with the stromal population. We further used this method to create a map over the H3K4me3 and H3K27ac distribution in the slow oxidative soleus (SOL) and the fast glycolytic extensor digitorum longus (EDL). This map indicates that the physiological phenotype of the two muscles is reflected in their histone landscape.

The methods presented in this paper could be used to study skeletal muscle fibers more specifically, and would be a great tool for investigating physiological and epigenetic responses to a changing milieu without influence from other cell types.

GENERAL DISCUSSION

MUSCLE MEMORY IN CACHEXIA

In paper I, we found no signs of muscle degeneration in cachectic animals following a 16% reduction in body weight. From our analysis of EDL, there was a 21 % reduction in muscle cross-sectional area (CSA) and no significant reduction in the number of muscle fibers. Analysis of myonuclear number on cross-sections and by *in vivo* microinjections showed no loss of myonuclei, suggesting that they are resistant to apoptosis under the atrophic and systemic conditions present in our experimental setup.

In the literature, there is a discrepancy regarding whether or not myonuclei are lost during muscle atrophy (Gundersen et al., 2008). In most non-disease animal models, there is acceptance towards myonuclei being resilient towards apoptosis (Bruusgaard et al., 2012; Jackson et al., 2012; Wada et al., 2002). However, during more severe conditions such as critical illness myopathy (Barnes et al., 2015), burn damage (Fry et al., 2016); regeneration, apoptosis, and loss of fibers is observed in the tissue, illustrating that the degree and nature of the atrophic signal is influence the outcome. As cancer cachexia is a multifactorial disease where the nature of the phenotype develops over time, there is no clear consensus on myonuclear behavior. This has been demonstrated in a study by Baltgalvis et al. (2010), finding no signs of apoptosis on cross-section from $Apc^{min/+}$ mice following a 6% reduction in body-mass and CSA, and the presence of apoptosis after a 12 % and 37% reduction in body-mass and CSA, respectively. Unfortunately, the study did not determine the identity of the apoptotic nuclei, however it suggests that degeneration might occur at more advanced stages of cachexia. Furthermore, molecular markers of apoptosis have been found in studies of muscle homogenate in both cachectic patients (Busquets et al., 2007) and animal models (Belizario et al., 2001; Busquets et al., 2004), but the source of the apoptotic cells has not been identified.

As discussed in paper I & II, identification of myonuclei is critical for this type of analysis. Furthermore the use of different inclusion criteria has probably led to a lot of conflicting results in literature. An example illustrating the importance of inclusion criteria is found in Berardi et al. (2008), where the authors define myonuclei as nuclei

residing at inside of the extracellular molecule laminin and report a 25% reduction in myonuclear number following a 25% reduction in body weight. In their control material, there is approximately 75% more nuclei per myofibers than in our study (Paper I, Figure 4c), and we suggest that the differences are caused by inclusion of stromal-cell in their analysis.

Another factor that contributes to conflicting literature is differences in study design. In a study by Iwata et al. (2016), the authors report no signs of regeneration following 46% reduction in CSA, and few signs of infiltrating immune cells or TUNEL positive nuclei. Although this study supports our hypothesis, they have performed analysis on a different hind-limb muscle, causing the cross-sectional area of their control group to be approximately 300% higher than in our study, making it difficult to make a direct comparison between the two studies.

Although we have not investigated the causal relationship between the PC3 tumor and the observed phenotype, it has been reported that the PC3 cell-line secretes IL6 (Giri et al., 2001; Malinowska et al., 2009), suggesting that the mechanisms behind the PC3 induced phenotype might be similar to the C-26 model (Bonetto et al., 2012; Strassmann et al., 1992). Similarly to our findings, little or no signs of apoptosis or degeneration are present in the skeletal muscles of mice grafted with the C-26 tumor following a 24-46% reduction in CSA (Acharyya et al., 2005; Iwata et al., 2016).

Compared to our study where the body weight started to decrease four weeks after inoculation (Paper I, Figure 1a), C-26 grafted tumor-cells are more aggressive, causing a decrease in body weight two week after inoculation (Acharyya et al., 2004; Bonetto et al., 2016). As the disease progresses, the mice may experience a typical loss of up to 25-46% reduction in muscle mass three weeks post-inoculation (Strassmann et al., 1992; E. E. Talbert et al., 2014). Compared to our study where the muscle mass was reduced by 14 % (Paper I, Figure 2a), it can be stated that the PC3 xenograft used in our experimental setup is less aggressive than C-26, so whether our findings on the conservation of myonuclear number can be translated to models or later stages where the atrophy is more pronounced remains to be investigated.

IDENTIFICATION OF MYONUCLEI WITH ANTI-PCM1

As mentioned in the last section, the lack of standardized methods for identifying cell types and nuclei on tissue-sections has probably led to a lot of conflicting results in the literature. To address this problem, we in paper II show that an antibody against PCM1 can be used to label and identify myonuclei on cryo-sections from rats, mice, and humans. The protein was chosen as a candidate myonuclear marker as it is shown to relocate to the nuclear envelope in differentiating muscle cells in culture (Fant et al., 2009; Srsen et al., 2009). In cardiac research, labeling of PCM1 is commonly used to identify cardiomyocyte nuclei on cross-sections and in suspension (Bergmann et al., 2011; Gilsbach et al., 2014), making this type of labeling suited for myonuclear enrichment.

Before our study, the best methods for detecting myonuclei on cross-sections has been to label all nuclei with a DNA labeling dye and determine their identity by their relative position to the dystrophin ring. This can be problematic as the quality of the labeling is dependent on the sample quality, microscopic and labeling techniques used by the individual researcher. This will still be an issue when applying the PCM1 labeling technique, but it will eliminate some of the variability, such as the subjectivity in assessing the position of the nuclei.

Our confocal analysis demonstrates that local folds and small deviations in the angle of the nuclei relative to the section plane may result in individual nuclei appearing to be positioned in, on or outside of the fiber boundary (Paper II, Figure 2B). We demonstrate this by comparing the dystrophin inclusion criteria with that of the PCM1 based method and show that the dystrophin inclusion criterion is prone to false negatives (Paper II, Figure 3A). While false negatives might distort absolute numbers, they probably represent a random subpopulation of nuclei when comparing groups with similar morphology. However, as structural changes, e.g., hypertrophy or atrophy is to be

expected in many areas of muscle research, we recommend the use of a myonuclear marker such as PCM1.

Though the use of anti-PCM1 could be of great aid when assessing troublesome samples with histopathological differences between groups, e.g., muscular dystrophies (Bell et al., 1968), nuclear envelope proteins have been demonstrated to be inconsistent markers in pathological samples (Le Thanh et al., 2017). Therefore, the staining pattern of PCM1 should be validated in diseased tissue to ensure that the PCM1 staining patterns are consistent.

In the cachexia study (Paper I), the PCM1 antibody was not used because the study was performed before paper II. Attempts to label the cachectic muscles with the PCM1 antibody show similar labeling as in paper II (Figure 9). However, as mentioned in the previous paragraph, the antibody should be appropriately validated before use, due to the pathologic nature of cachexia. Based on findings from paper II, we can expect the presence of false negatives in our analysis of the myonuclear number on tissue cross sections, but considering that our findings are supported by single fiber injections (paper I, figure 4B) the conclusion that there is no loss of myonuclear number should still be valid.

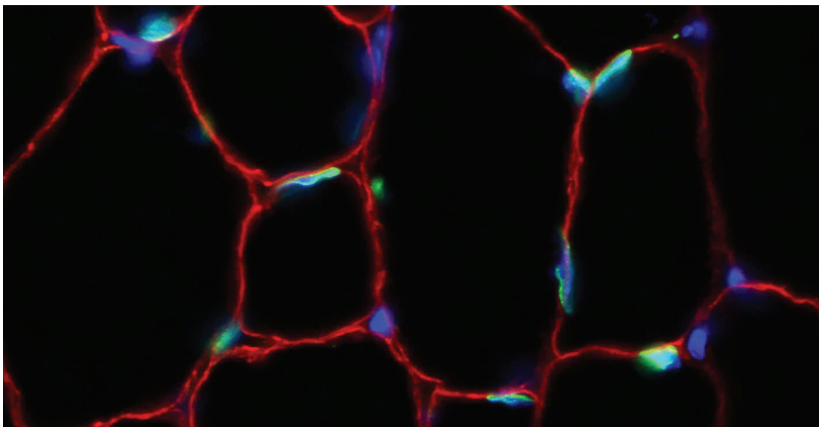

Figure 9. Labeling of cachectic muscles with anti PCM1. Cross section from a cachectic mouse EDL labeled with antibodies against PCM1 (green), anti-dystrophin (red) and DAPI (blue).

ALTERNATIVE METHODS FOR LABELING AND ISOLATION OF MYONUCLEI

In addition to PCM1, several proteins have been shown to relocate or be expressed on the nuclear envelope of differentiated cells *in vitro* (Bleunven et al., 2008; Bugnard et al., 2005; Le Thanh et al., 2017; Wilkie et al., 2011). Several of which could potentially serve the same purpose as anti-PCM1. However, it has not been investigated whether these proteins can be used for identification of myonuclei on tissue samples.

In a recent paper by Cutler et al. (2017), the authors present an alternative method for isolating myonuclei without the need for active sorting, e.g., MACS, collagenase, etc. Their approach is based on gentle homogenization of the tissue followed by ultracentrifugation of crude nuclei isolates over a two-step sucrose cushion to separate the nuclei from cellular debris. The percentage of myonuclei is determined by labeling with antibodies against TMEM38A, a nuclear envelope protein in excitatory cells (Bleunven et al., 2008) and Mab414, a ubiquitously expressed component of the nuclear pore complex.

In their flow-cytometric analysis, the authors report their nuclear isolate to contain 96.4 % myonuclei, defined as TMEM38A and Mab414 positive events. Compared to our results from paper III the purity of their myonuclear fraction is the same as our MACS sorted fraction (Paper III, Figure 2). These results suggest that PCM1 based enrichment is redundant. However, the difference could be explained by their gating strategy. When comparing flow-data from paper III to that from PCM1 labeled cardiomyocyte nuclei (Bergmann et al., 2011; Gilsbach et al., 2014; Richardson et al., 2015), PCM1 labeling is similar in that positive and negative events cluster into two distinct populations separated by an approximate 10-fold labeling intensity (Paper III, Figure 2). Interestingly, the two TMEM38A populations in Cutler et al. (2017) follow a similar pattern, which the author's claim is a result of residual endoplasmic reticulum-derived TMEM38A associated with the nuclei. However, our interpretation is that their dim population might be non-myonuclei interpreted as a positive signal due to non-specific labeling of the primary antibody.

Since their article does not show labeling of muscle cross sections, we performed immuno-labeling with anti-TMEM38A, revealing a staining pattern similar to that presented in paper II (Figure 10). Although the intensity of the TMEM38A nuclei in SOL is higher, these findings are suggesting that the TMEM38A antibody may indeed be used to label myonuclei. Hence, the protocols and the relevant antibodies should be compared.

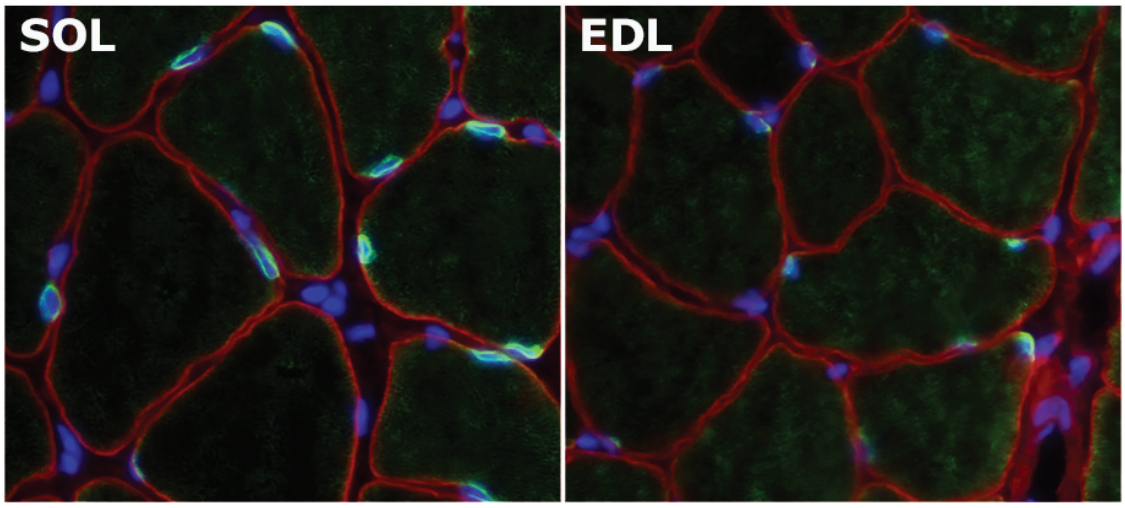

Figure 10. Labeling of TMEM38A is similar to that of PCM1. Cross section from mice SOL and EDL muscles labeled with TMEM38A (green), dystrophin (red) and DAPI (blue). Due to antibody-isotype specificity, sections could not be co-labeled with anti-PCM1 to investigate colocalization.

EPIGENETICS IN SKELETAL MUSCLE

In paper III, we present a novel method for analyzing the myo-specific epigenome. Prior to our study, the investigation of histones in skeletal muscle has mainly been performed using *in vitro* models (Jin et al., 2016), with much attention devoted to the differentiation of myoblasts into myocytes (Asp et al., 2011; Cui et al., 2017). Although these models provide valuable information on the epigenetic mechanisms behind differentiation of the myogenic lineage, the development of myofibrillar phenotype is dependent on neuronal input (Hennig & Lomo, 1985).

Of the studies that have studied tissue-derived chromatin most have focused on DNA methylation in complex tissue samples (David et al., 2017; Kanzleiter et al., 2015; Lindholm et al., 2014). As the epigenetic signatures of individual genes are highly cell-type specific, the use of whole tissue samples may lead to ambiguous results (Michels et al., 2013). This point should not be neglected as several conditions may alter the cellular composition in the tissue. For example, exercise may cause infiltration of immune-cells (Malm et al., 2000) and increase the capillary density (Laughlin et al., 2008). Lindholm et al. (2014), confirmed this by observing differences in the expression of genes encoding endothelial marker proteins in trained individuals. The importance of studying purified cell populations has also been addressed in cardiac research where comparison of epigenetic profiles from whole tissue and isolated cardiomyocytes show that mixed-cell populations are prone to an increased number of false negatives and positives (Gilsbach et al., 2014; Preissl et al., 2015).

As no other study has performed genome-wide analysis of histone modifications in purified myonuclei or myofibers, our method is a significant contribution to the field as it allows detection of subtle changes in histone enrichment. Furthermore, the technique could be used to investigate permanent alterations in the histone landscape. An example of a potential application would be to examine the long-term effects of testosterone abuse in athletes. In skeletal muscle, the androgen receptor is an important mediator of testosterone-induced activation of mTOR (Basualto-Alarcon et al., 2013). The methylation and expression pattern of the androgen receptor gene is inversely regulated by testosterone (R. C. Kumar et al., 2004a, 2004b). However, it has not been examined if the methylation profile of the gene return to “normal” after ended treatment.

In skeletal muscle, long-term effect on DNA methylation has been demonstrated following a five days exposure to a high-fat diet, showing genome-wide changes in the methylation profile of 6500 genes in the following 6-8 weeks after treatment (Jacobsen et al., 2012). In a similar study of liver tissue, rats exposed to a high-fat diet display decreased methylation and increased transcriptional activity of pro-inflammatory genes connected to the development of liver disease (Yoon et al., 2017). In humans, it has been demonstrated to be differences in the methylation profile between people with and

without an inherited predisposition to diabetes (Nitert et al., 2012). Some of the differentially methylated genes have important metabolic functions, such as members of the IGF1 pathway and AMPK. These findings demonstrate that characterization of the epigenetic landscape could prove important for understanding the pathology and development disease.

In atrophying skeletal muscle, it is an altered expression of histone deacetylases (HDACs) (Beharry & Judge, 2015). Interestingly, the expression patterns of the various HDACs are dependent on the specific atrophy model (Beharry & Judge, 2015). In response to denervation or hind limb suspension, there is an increased expression of HDAC1, whose activity is required for FoxO mediated upregulation of Atrogin-1 (Beharry et al., 2014). While nutrient deprived animals also express HDAC1 they express high levels of SIRT1 (Beharry & Judge, 2015). Being a class III HDAC, SIRT1 requires NAD+ as a cofactor and is activated when the energy status of the cell is low (Canto et al., 2015; T. Zhang et al., 2010). Upregulation of SIRT1 has been found in skeletal muscles of cachectic tumor bearing rats (Toledo et al., 2011), suggesting that the mechanisms cancer-induced atrophy might be similar to nutrient deprivation.

The involvement of histone modifying enzymes in the development of cachexia is supported by preclinical trials (Sun et al., 2016; Tseng et al., 2015) and animal studies (Segatto et al., 2017), showing that inhibition of HDACs can counteract muscle loss. These findings suggest that changes in the histone landscape could be an influential factor in the development of cancer cachexia. By showing that myonuclei or myofibers are conserved during cachexia, our model can be used to investigate how the epigenome is altered in cachectic tissue

As much evidence points towards epigenetics playing a vital role in maintenance and regulation of phenotype, the work presented in this thesis could provide a valuable tool for studying the epigenetic mechanisms regulating muscle phenotype.

REFERENCES

Acharyya, S., Butchbach, M. E., Sahenk, Z., Wang, H., Saji, M., Carathers, M., . . . Guttridge, D. C. (2005). Dystrophin glycoprotein complex dysfunction: a regulatory link between muscular dystrophy and cancer cachexia. *Cancer Cell, 8*(5), 421-432.

Acharyya, S., Ladner, K. J., Nelsen, L. L., Damrauer, J., Reiser, P. J., Swoap, S., & Guttridge, D. C. (2004). Cancer cachexia is regulated by selective targeting of skeletal muscle gene products. *J Clin Invest, 114*(3), 370-378.

Adams, G. R., Caiozzo, V. J., Haddad, F., & Baldwin, K. M. (2002). Cellular and molecular responses to increased skeletal muscle loading after irradiation. *Am J Physiol Cell Physiol, 283*(4), C1182-1195.

Alamdari, N., Aversa, Z., Castillero, E., & Hasselgren, P. O. (2013). Acetylation and deacetylation--novel factors in muscle wasting. *Metabolism, 62*(1), 1-11.

Allen, D. L., Roy, R. R., & Edgerton, V. R. (1999). Myonuclear domains in muscle adaptation and disease. *Muscle Nerve, 22*(10), 1350-1360.

Amano, T., Sagai, T., Tanabe, H., Mizushina, Y., Nakazawa, H., & Shiroishi, T. (2009). Chromosomal dynamics at the Shh locus: limb bud-specific differential regulation of competence and active transcription. *Dev Cell, 16*(1), 47-57.

Andres, V., & Walsh, K. (1996). Myogenin expression, cell cycle withdrawal, and phenotypic differentiation are temporally separable events that precede cell fusion upon myogenesis. *J Cell Biol, 132*(4), 657-666.

Asp, P., Blum, R., Vethantham, V., Parisi, F., Micsinai, M., Cheng, J., . . . Dynlacht, B. D. (2011). Genome-wide remodeling of the epigenetic landscape during myogenic differentiation. *Proc Natl Acad Sci U S A, 108*(22), E149-158.

Atlasi, Y., & Stunnenberg, H. G. (2017). The interplay of epigenetic marks during stem cell differentiation and development. *Nat Rev Genet, 18*(11), 643-658.

Balczon, R., Bao, L., & Zimmer, W. E. (1994). PCM-1, A 228-kD centrosome autoantigen with a distinct cell cycle distribution. *J Cell Biol, 124*(5), 783-793.

Baltgalvis, K. A., Berger, F. G., Pena, M. M., Davis, J. M., Muga, S. J., & Carson, J. A. (2008). Interleukin-6 and cachexia in ApcMin/+ mice. *Am J Physiol Regul Integr Comp Physiol, 294*(2), R393-401.

Baltgalvis, K. A., Berger, F. G., Pena, M. M., Mark Davis, J., White, J. P., & Carson, J. A. (2010). Activity level, apoptosis, and development of cachexia in Apc(Min/+) mice. *J Appl Physiol (1985), 109*(4), 1155-1161.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Res, 21*(3), 381-395. doi:10.1038/cr.2011.22

Baracos, V. E. (2002). Hypercatabolism and hypermetabolism in wasting states. *Curr Opin Clin Nutr Metab Care, 5*(3), 237-239.

Barany, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J Gen Physiol, 50*(6), Suppl:197-218.

Barany, M., & Close, R. I. (1971). The transformation of myosin in cross-innervated rat muscles. *J Physiol, 213*(2), 455-474.

Barnard, R. J., Edgerton, V. R., Furukawa, T., & Peter, J. B. (1971). Histochemical, biochemical, and contractile properties of red, white, and intermediate fibers. *Am J Physiol, 220*(2), 410-414.

Barnes, B. T., Confides, A. L., Rich, M. M., & Dupont-Versteegden, E. E. (2015). Distinct muscle apoptotic pathways are activated in muscles with different fiber types in a rat model of critical illness myopathy. *J Muscle Res Cell Motil, 36*(3), 243-253.

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., . . . Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. *Cell, 129*(4), 823-837.

Bartolini, F., & Gundersen, G. G. (2006). Generation of noncentrosomal microtubule arrays. *J Cell Sci, 119*(Pt 20), 4155-4163.

Basualto-Alarcon, C., Jorquera, G., Altamirano, F., Jaimovich, E., & Estrada, M. (2013). Testosterone signals through mTOR and androgen receptor to induce muscle hypertrophy. *Med Sci Sports Exerc, 45*(9), 1712-1720.

Begue, G., Raue, U., Jemiolo, B., & Trappe, S. (2017). DNA methylation assessment from human slow- and fast-twitch skeletal muscle fibers. *J Appl Physiol (1985), 122*(4), 952-967.

Beharry, A. W., & Judge, A. R. (2015). Differential expression of HDAC and HAT genes in atrophying skeletal muscle. *Muscle Nerve, 52*(6), 1098-1101.

Beharry, A. W., Sandesara, P. B., Roberts, B. M., Ferreira, L. F., Senf, S. M., & Judge, A. R. (2014). HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. *J Cell Sci, 127*(Pt 7), 1441-1453.

Belizario, J. E., Lorite, M. J., & Tisdale, M. J. (2001). Cleavage of caspases-1, -3, -6, -8 and -9 substrates by proteases in skeletal muscles from mice undergoing cancer cachexia. *Br J Cancer, 84*(8), 1135-1140.

Bell, C. D., & Conen, P. E. (1968). Histopathological changes in Duchenne muscular dystrophy. *J Neurol Sci, 7*(3), 529-544.

Berardi, E., Aulino, P., Murfuni, I., Toschi, A., Padula, F., Scicchitano, B. M., . . . Adamo, S. (2008). Skeletal muscle is enriched in hematopoietic stem cells and not inflammatory cells in cachectic mice. *Neurol Res, 30*(2), 160-169.

Bergmann, O., & Jovinge, S. (2012). Isolation of cardiomyocyte nuclei from post-mortem tissue. *J Vis Exp*(65). doi:10.3791/4205

Bergmann, O., Zdunek, S., Alkass, K., Druid, H., Bernard, S., & Frisen, J. (2011). Identification of cardiomyocyte nuclei and assessment of ploidy for the analysis of cell turnover. *Exp Cell Res, 317*(2), 188-194.

Bleunven, C., Treves, S., Jinyu, X., Leo, E., Ronjat, M., De Waard, M., . . . Zorzato, F. (2008). SRP-27 is a novel component of the supramolecular signalling complex involved in skeletal muscle excitation-contraction coupling. *Biochem J, 411*(2), 343-349.

Blum, R., Vethantham, V., Bowman, C., Rudnicki, M., & Dynlacht, B. D. (2012). Genome-wide identification of enhancers in skeletal muscle: the role of MyoD1. *Genes Dev, 26*(24), 2763-2779.

Bodine, S. C., Stitt, T. N., Gonzalez, M., Kline, W. O., Stover, G. L., Bauerlein, R., . . . Yancopoulos, G. D. (2001). Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol, 3*(11), 1014-1019.

Bolster, D. R., Jefferson, L. S., & Kimball, S. R. (2004). Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. *Proc Nutr Soc, 63*(2), 351-356.

Bonetto, A., Aydogdu, T., Jin, X., Zhang, Z., Zhan, R., Puzis, L., . . . Zimmers, T. A. (2012). JAK/STAT3 pathway inhibition blocks skeletal muscle wasting downstream of IL-6 and in experimental cancer cachexia. *Am J Physiol Endocrinol Metab, 303*(3), E410-421.

Bonetto, A., Rupert, J. E., Barreto, R., & Zimmers, T. A. (2016). The Colon-26 Carcinoma Tumor-bearing Mouse as a Model for the Study of Cancer Cachexia. *J Vis Exp*(117).

Bonn, S., Zinzen, R. P., Girardot, C., Gustafson, E. H., Perez-Gonzalez, A., Delhomme, N., . . . Furlong, E. E. (2012). Tissue-specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development. *Nat Genet, 44*(2), 148-156.

Boyer, L. A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L. A., Lee, T. I., . . . Jaenisch, R. (2006). Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature, 441*(7091), 349-353.

Boyle, A. P., Davis, S., Shulha, H. P., Meltzer, P., Margulies, E. H., Weng, Z., . . . Crawford, G. E. (2008). High-resolution mapping and characterization of open chromatin across the genome. *Cell, 132*(2), 311-322.

Bruusgaard, J. C., Egner, I. M., Larsen, T. K., Dupre-Aucouturier, S., Desplanches, D., & Gundersen, K. (2012). No change in myonuclear number during muscle unloading and reloading. *J Appl Physiol (1985), 113*(2), 290-296.

Bruusgaard, J. C., & Gundersen, K. (2008). In vivo time-lapse microscopy reveals no loss of murine myonuclei during weeks of muscle atrophy. *J Clin Invest, 118*(4), 1450-1457.

Bruusgaard, J. C., Johansen, I. B., Egner, I. M., Rana, Z. A., & Gundersen, K. (2010). Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining. *Proc Natl Acad Sci U S A, 107*(34), 15111-15116.

Bruusgaard, J. C., Liestol, K., Ekmark, M., Kollstad, K., & Gundersen, K. (2003). Number and spatial distribution of nuclei in the muscle fibres of normal mice studied in vivo. *J Physiol, 551*(Pt 2), 467-478.

Budzynski, W., & Radzikowski, C. (1994). Cytotoxic cells in immunodeficient athymic mice. *Immunopharmacol Immunotoxicol, 16*(3), 319-346.

Bugnard, E., Zaal, K. J., & Ralston, E. (2005). Reorganization of microtubule nucleation during muscle differentiation. *Cell Motil Cytoskeleton, 60*(1), 1-13.

Busquets, S., Deans, C., Figueras, M., Moore-Carrasco, R., Lopez-Soriano, F. J., Fearon, K. C., & Argiles, J. M. (2007). Apoptosis is present in skeletal muscle of cachectic gastro-intestinal cancer patients. *Clin Nutr, 26*(5), 614-618.

Busquets, S., Figueras, M. T., Fuster, G., Almendro, V., Moore-Carrasco, R., Ametller, E., . . . Lopez-Soriano, F. J. (2004). Anticachectic effects of formoterol: a drug for potential treatment of muscle wasting. *Cancer Res, 64*(18), 6725-6731.

Cabane, C., Englaro, W., Yeow, K., Ragno, M., & Derijard, B. (2003). Regulation of C2C12 myogenic terminal differentiation by MKK3/p38alpha pathway. *Am J Physiol Cell Physiol, 284*(3), C658-666.

Calnan, D. R., & Brunet, A. (2008). The FoxO code. *Oncogene, 27*(16), 2276-2288.

Calo, E., & Wysocka, J. (2013). Modification of enhancer chromatin: what, how, and why? *Mol Cell, 49*(5),

Canto, C., Menzies, K. J., & Auwerx, J. (2015). NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. *Cell Metab, 22*(1), 31-53.

Carr, R. M., Enriquez-Hesles, E., Olson, R. L., Jatoi, A., Doles, J., & Fernandez-Zapico, M. E. (2017). Epigenetics of cancer-associated muscle catabolism. *Epigenomics*.

Chang, N. J., Weng, W. H., Chang, K. H., Liu, E. K., Chuang, C. K., Luo, C. C., . . . Pang, S. T. (2015). Genome-wide gene expression profiling of ischemia-reperfusion injury in rat kidney, intestine and skeletal muscle implicate a common involvement of MAPK signaling pathway. *Mol Med Rep, 11*(5), 3786-3793.

Chemello, F., Bean, C., Cancellara, P., Laveder, P., Reggiani, C., & Lanfranchi, G. (2011). Microgenomic analysis in skeletal muscle: expression signatures of individual fast and slow myofibers. *PLoS One, 6*(2), e16807.

Chin, E. R. (2005). Role of Ca2+/calmodulin-dependent kinases in skeletal muscle plasticity. *J Appl Physiol (1985), 99*(2), 414-423.

Close, R. (1967). Properties of motor units in fast and slow skeletal muscles of the rat. *J Physiol, 193*(1), 45-55.

Crabtree, G. R. (1999). Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. *Cell, 96*(5), 611-614.

Creyghton, M. P., Cheng, A. W., Welstead, G. G., Kooistra, T., Carey, B. W., Steine, E. J., . . . Jaenisch, R. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U S A, 107*(50), 21931-21936.

Cuddapah, S., Jothi, R., Schones, D. E., Roh, T. Y., Cui, K., & Zhao, K. (2009). Global analysis of the insulator binding protein CTCF in chromatin barrier regions reveals demarcation of active and repressive domains. *Genome Res, 19*(1), 24-32.

Cui, H., Bansal, V., Grunert, M., Malecova, B., Dall'Agnese, A., Latella, L., . . . Sperling, S. R. (2017). Muscle-relevant genes marked by stable H3K4me2/3 profiles and enriched MyoD binding during myogenic differentiation. *PLoS One, 12*(6),

Cutler, A. A., Dammer, E. B., Doung, D. M., Seyfried, N. T., Corbett, A. H., & Pavlath, G. K. (2017). Biochemical isolation of myonuclei employed to define changes to the myonuclear proteome that occur with aging. *Aging Cell, 16*(4), 738-749. doi:10.1111/acel.12604

Cutter, A. R., & Hayes, J. J. (2015). A brief review of nucleosome structure. *FEBS Lett, 589*(20 Pt A), 2914-2922.

Dagogo-Jack, I., & Shaw, A. T. (2018). Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol, 15*(2), 81-94.

Dammermann, A., & Merdes, A. (2002). Assembly of centrosomal proteins and microtubule organization depends on PCM-1. *J Cell Biol, 159*(2), 255-266.

David, S. A., Piegu, B., Hennequet-Antier, C., Pannetier, M., Aguirre-Lavin, T., Crochet, S., . . . Coustham, V. (2017). An Assessment of Fixed and Native Chromatin Preparation Methods to Study Histone Post-Translational Modifications at a Whole Genome Scale in Skeletal Muscle Tissue. *Biol Proced Online, 19*, 10.

Day, C. P., Merlino, G., & Van Dyke, T. (2015). Preclinical mouse cancer models: a maze of opportunities and challenges. *Cell, 163*(1), 39-53.

De Backer, I. C., Schep, G., Backx, F. J., Vreugdenhil, G., & Kuipers, H. (2009). Resistance training in cancer survivors: a systematic review. *Int J Sports Med, 30*(10), 703-712.

De Benedetti, F., Alonzi, T., Moretta, A., Lazzaro, D., Costa, P., Poli, V., . . . Fattori, E. (1997). Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. *J Clin Invest, 99*(4), 643-650.

de Matos-Neto, E. M., Lima, J. D., de Pereira, W. O., Figueredo, R. G., Riccardi, D. M., Radloff, K., . . . Seelaender, M. (2015). Systemic Inflammation in Cachexia - Is Tumor Cytokine Expression Profile the Culprit? *Front Immunol, 6*, 629.

Deaton, A. M., & Bird, A. (2011). CpG islands and the regulation of transcription. *Genes Dev, 25*(10), 1010-1022. doi:10.1101/gad.2037511

DeGregori, J. (2011). Evolved tumor suppression: why are we so good at not getting cancer? *Cancer Res, 71*(11), 3739-3744.

Dobrolecki, L. E., Airhart, S. D., Alferez, D. G., Aparicio, S., Behbod, F., Bentires-Alj, M., . . . Lewis, M. T. (2016). Patient-derived xenograft (PDX) models in basic and translational breast cancer research. *Cancer Metastasis Rev, 35*(4), 547-573.

Dos Santos, W. D. N., Gentil, P., de Moraes, R. F., Ferreira Junior, J. B., Campos, M. H., de Lira, C. A. B., . . . Vieira, C. A. (2017). Chronic Effects of Resistance Training in Breast Cancer Survivors. *Biomed Res Int, 2017*, 8367803.

Edstrom, L., & Kugelberg, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. *J Neurol Neurosurg Psychiatry, 31*(5), 424-433.

Egner, I. M., Bruusgaard, J. C., Eftestol, E., & Gundersen, K. (2013). A cellular memory mechanism aids overload hypertrophy in muscle long after an episodic exposure to anabolic steroids. *J Physiol, 591*(Pt 24), 6221-6230.

Egner, I. M., Bruusgaard, J. C., & Gundersen, K. (2016). Satellite cell depletion prevents fiber hypertrophy in skeletal muscle. *Development, 143*(16), 2898-2906.

Espigat-Georger, A., Dyachuk, V., Chemin, C., Emorine, L., & Merdes, A. (2016). Nuclear alignment in myotubes requires centrosome proteins recruited by nesprin-1. *Journal of Cell Science, 129*(22), 4227-4237.

Ettig, R., Kepper, N., Stehr, R., Wedemann, G., & Rippe, K. (2011). Dissecting DNA-histone interactions in the nucleosome by molecular dynamics simulations of DNA unwrapping. *Biophys J, 101*(8), 1999-2008.

Eymery, A., Callanan, M., & Vourc'h, C. (2009). The secret message of heterochromatin: new insights into the mechanisms and function of centromeric and pericentric repeat sequence transcription. *Int J Dev Biol, 53*(2-3), 259-268.

Fant, X., Srsen, V., Espigat-Georger, A., & Merdes, A. (2009). Nuclei of non-muscle cells bind centrosome proteins upon fusion with differentiating myoblasts. *PLoS One, 4*(12), e8303.

Fearon, K., Strasser, F., Anker, S. D., Bosaeus, I., Bruera, E., Fainsinger, R. L., . . . Baracos, V. E. (2011). Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol, 12*(5), 489-495.

Fearon, K. C. (2008). Cancer cachexia: developing multimodal therapy for a multidimensional problem. *Eur J Cancer, 44*(8), 1124-1132.

Flanagan, J. F., Mi, L. Z., Chruszcz, M., Cymborowski, M., Clines, K. L., Kim, Y., . . . Khorasanizadeh, S. (2005). Double chromodomains cooperate to recognize the methylated histone H3 tail. *Nature, 438*(7071), 1181-1185.

Fry, C. S., Porter, C., Sidossis, L. S., Nieten, C., Reidy, P. T., Hundeshagen, G., . . . Finnerty, C. C. (2016). Satellite cell activation and apoptosis in skeletal muscle from severely burned children. *J Physiol, 594*(18), 5223-5236.

Fujita, J., Tsujinaka, T., Yano, M., Ebisui, C., Saito, H., Katsume, A., . . . Monden, M. (1996). Anti-interleukin-6 receptor antibody prevents muscle atrophy in colon-26 adenocarcinoma-bearing mice with modulation of lysosomal and ATP-ubiquitin-dependent proteolytic pathways. *Int J Cancer, 68*(5), 637-643.

Gilsbach, R., Preissl, S., Gruning, B. A., Schnick, T., Burger, L., Benes, V., . . . Hein, L. (2014). Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. *Nat Commun, 5*, 5288.

Giri, D., Ozen, M., & Ittmann, M. (2001). Interleukin-6 is an autocrine growth factor in human prostate cancer. *Am J Pathol, 159*(6), 2159-2165.

Gopalakrishnan, S., Van Emburgh, B. O., & Robertson, K. D. (2008). DNA methylation in development and human disease. *Mutat Res, 647*(1-2), 30-38.

Gray, J. M., Kim, T. K., West, A. E., Nord, A. S., Markenscoff-Papadimitriou, E., & Lomvardas, S. (2015). Genomic Views of Transcriptional Enhancers: Essential Determinants of Cellular Identity and Activity-Dependent Responses in the CNS. *J Neurosci, 35*(41), 13819-13826.

Greco, C. M., Kunderfranco, P., Rubino, M., Larcher, V., Carullo, P., Anselmo, A., . . . Condorelli, G. (2016). DNA hydroxymethylation controls cardiomyocyte gene expression in development and hypertrophy. *Nat Commun, 7*, 12418.

Greer, E. L., Oskoui, P. R., Banko, M. R., Maniar, J. M., Gygi, M. P., Gygi, S. P., & Brunet, A. (2007). The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem, 282*(41), 30107-30119.

Grivennikov, S. I., & Karin, M. (2011). Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. *Ann Rheum Dis, 70 Suppl 1*, i104-108.

Gulati, A. K. (1987). The effect of X-irradiation on skeletal muscle regeneration in the adult rat. *J Neurol Sci, 78*(1), 111-120.

Gundersen, K. (1985). Early effects of denervation on isometric and isotonic contractile properties of rat skeletal muscles. *Acta Physiol Scand, 124*(4), 549-555.

Gundersen, K. (2011). Excitation-transcription coupling in skeletal muscle: the molecular pathways of exercise. *Biol Rev Camb Philos Soc, 86*(3), 564-600.

Gundersen, K., & Bruusgaard, J. C. (2008). Nuclear domains during muscle atrophy: nuclei lost or paradigm lost? *J Physiol, 586*(11), 2675-2681.

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., . . . Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell, 30*(2), 214-226.

Hahn, C. G., & Covault, J. (1990). Isolation of transcriptionally active nuclei from striated muscle using Percoll density gradients. *Anal Biochem, 190*(2), 193-197.

Hake, S. B., Xiao, A., & Allis, C. D. (2004). Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br J Cancer, 90*(4), 761-769.

Harada, A., Maehara, K., Sato, Y., Konno, D., Tachibana, T., Kimura, H., & Ohkawa, Y. (2015). Incorporation of histone H3.1 suppresses the lineage potential of skeletal muscle. *Nucleic Acids Res, 43*(2), 775-786.

Harridge, S. D., Bottinelli, R., Canepari, M., Pellegrino, M. A., Reggiani, C., Esbjornsson, M., & Saltin, B. (1996). Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans. *Pflugers Arch, 432*(5), 913-920.

Heintzman, N. D., Stuart, R. K., Hon, G., Fu, Y., Ching, C. W., Hawkins, R. D., . . . Ren, B. (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet, 39*(3), 311-318.

Hennig, R., & Lomo, T. (1985). Firing patterns of motor units in normal rats. *Nature, 314*(6007), 164-166.

Heron, M. I., & Richmond, F. J. (1993). In-series fiber architecture in long human muscles. *J Morphol, 216*(1), 35-45.

Hill, M., Wernig, A., & Goldspink, G. (2003). Muscle satellite (stem) cell activation during local tissue injury and repair. *J Anat, 203*(1), 89-99.

Holliday, R., & Pugh, J. E. (1975). DNA modification mechanisms and gene activity during development. *Science, 187*(4173), 226-232.

Hori, A., Ikebe, C., Tada, M., & Toda, T. (2014). Msd1/SSX2IP-dependent microtubule anchorage ensures spindle orientation and primary cilia formation. *EMBO Rep, 15*(2), 175-184.

Hori, A., & Toda, T. (2017). Regulation of centriolar satellite integrity and its physiology. *Cell Mol Life Sci, 74*(2), 213-229.

Howe, F. S., Fischl, H., Murray, S. C., & Mellor, J. (2017). Is H3K4me3 instructive for transcription activation? *Bioessays, 39*(1), 1-12.

Iwata, Y., Suzuki, N., Ohtake, H., Kamauchi, S., Hashimoto, N., Kiyono, T., & Wakabayashi, S. (2016). Cancer cachexia causes skeletal muscle damage via transient receptor potential vanilloid 2-independent mechanisms, unlike muscular dystrophy. *J Cachexia Sarcopenia Muscle, 7*(3), 366-376.

Jackson, J. R., Mula, J., Kirby, T. J., Fry, C. S., Lee, J. D., Ubele, M. F., . . . Dupont-Versteegden, E. E. (2012). Satellite cell depletion does not inhibit adult skeletal muscle regrowth following unloading-induced atrophy. *Am J Physiol Cell Physiol, 303*(8), C854-861.

Jacobsen, S. C., Brons, C., Bork-Jensen, J., Ribel-Madsen, R., Yang, B., Lara, E., . . . Vaag, A. (2012). Effects of short-term high-fat overfeeding on genome-wide DNA methylation in the skeletal muscle of healthy young men. *Diabetologia, 55*(12), 3341-3349.

Jin, W., Peng, J., & Jiang, S. (2016). The epigenetic regulation of embryonic myogenesis and adult muscle regeneration by histone methylation modification. *Biochem Biophys Rep, 6*, 209-219.

Juan, A. H., Wang, S., Ko, K. D., Zare, H., Tsai, P. F., Feng, X., . . . Sartorelli, V. (2016). Roles of H3K27me2 and H3K27me3 Examined during Fate Specification of Embryonic Stem Cells. *Cell Rep, 17*(5), 1369-1382.

Jung, J. (2014). Human tumor xenograft models for preclinical assessment of anticancer drug development. *Toxicol Res, 30*(1), 1-5.

Juretic, N., Urzua, U., Munroe, D. J., Jaimovich, E., & Riveros, N. (2007). Differential gene expression in skeletal muscle cells after membrane depolarization. *J Cell Physiol, 210*(3), 819-830.

Kanzleiter, T., Jahnert, M., Schulze, G., Selbig, J., Hallahan, N., Schwenk, R. W., & Schurmann, A. (2015). Exercise training alters DNA methylation patterns in genes related to muscle growth and differentiation in mice. *Am J Physiol Endocrinol Metab, 308*(10), E912-920.

Kass, S. U., Pruss, D., & Wolffe, A. P. (1997). How does DNA methylation repress transcription? *Trends Genet, 13*(11), 444-449.

Katan-Khaykovich, Y., & Struhl, K. (2002). Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes Dev, 16*(6), 743-752.

Kidder, B. L., Hu, G., & Zhao, K. (2011). ChIP-Seq: technical considerations for obtaining high-quality data. *Nat Immunol, 12*(10), 918-922.

Kislinger, T., Gramolini, A. O., Pan, Y., Rahman, K., MacLennan, D. H., & Emili, A. (2005). Proteome dynamics during C2C12 myoblast differentiation. *Mol Cell Proteomics, 4*(7), 887-901.

Kubo, A., Sasaki, H., Yuba-Kubo, A., Tsukita, S., & Shiina, N. (1999). Centriolar satellites: molecular characterization, ATP-dependent movement toward centrioles and possible involvement in ciliogenesis. *J Cell Biol, 147*(5), 969-980.

Kubo, A., & Tsukita, S. (2003). Non-membranous granular organelle consisting of PCM-1: subcellular distribution and cell-cycle-dependent assembly/disassembly. *J Cell Sci, 116*(Pt 5), 919-928.

Kuehl, L. (1975). Isolation of skeletal muscle nuclei. *Exp Cell Res, 91*(2), 441-448.

Kumar, R. C., & Thakur, M. K. (2004a). Androgen receptor mRNA is inversely regulated by testosterone and estradiol in adult mouse brain. *Neurobiol Aging, 25*(7), 925-933.

Kumar, R. C., & Thakur, M. K. (2004b). Sex steroids reduce DNaseI accessibility of androgen receptor promoter in adult male mice brain. *Brain Res Mol Brain Res, 131*(1-2), 1-7.

Kumar, V., Atherton, P., Smith, K., & Rennie, M. J. (2009). Human muscle protein synthesis and breakdown during and after exercise. *J Appl Physiol (1985), 106*(6), 2026-2039.

Lassar, A. B., Skapek, S. X., & Novitch, B. (1994). Regulatory mechanisms that coordinate skeletal muscle differentiation and cell cycle withdrawal. *Curr Opin Cell Biol, 6*(6), 788-794.

Laughlin, M. H., & Roseguini, B. (2008). Mechanisms for exercise training-induced increases in skeletal muscle blood flow capacity: differences with interval sprint training versus aerobic endurance training. *J Physiol Pharmacol, 59 Suppl 7*, 71-88.

Lawrence, M., Daujat, S., & Schneider, R. (2016). Lateral Thinking: How Histone Modifications Regulate Gene Expression. *Trends Genet, 32*(1), 42-56.

Le Thanh, P., Meinke, P., Korfali, N., Srsen, V., Robson, M. I., Wehnert, M., . . . Schirmer, E. C. (2017). Immunohistochemistry on a panel of Emery-Dreifuss muscular dystrophy samples reveals nuclear envelope proteins as inconsistent markers for pathology. *Neuromuscul Disord, 27*(4), 338-351.

Li, H., Ilin, S., Wang, W., Duncan, E. M., Wysocka, J., Allis, C. D., & Patel, D. J. (2006). Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature, 442*(7098), 91-95.

Lilja, K. C., Zhang, N., Magli, A., Gunduz, V., Bowman, C. J., Arpke, R. W., . . . Dynlacht, B. D. (2017). Pax7 remodels the chromatin landscape in skeletal muscle stem cells. *PLoS One, 12*(4), e0176190.

Lindholm, M. E., Marabita, F., Gomez-Cabrero, D., Rundqvist, H., Ekstrom, T. J., Tegner, J., & Sundberg, C. J. (2014). An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics, 9*(12), 1557-1569.

Liu, L., Cheung, T. H., Charville, G. W., Hurgo, B. M., Leavitt, T., Shih, J., . . . Rando, T. A. (2013). Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. *Cell Rep, 4*(1), 189-204.

Lok, C. (2015). Cachexia: The last illness. *Nature, 528*(7581), 182-183.

Mal, A., Chattopadhyay, D., Ghosh, M. K., Poon, R. Y., Hunter, T., & Harter, M. L. (2000). p21 and retinoblastoma protein control the absence of DNA replication in terminally differentiated muscle cells. *J Cell Biol, 149*(2), 281-292.

Malinowska, K., Neuwirt, H., Cavarretta, I. T., Bektic, J., Steiner, H., Dietrich, H., . . . Culig, Z. (2009). Interleukin-6 stimulation of growth of prostate cancer in vitro and in vivo through activation of the androgen receptor. *Endocr Relat Cancer, 16*(1), 155-169.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B., & Lundberg, I. (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol, 529 Pt 1*, 243-262.

Manning, B. D., & Cantley, L. C. (2007). AKT/PKB signaling: navigating downstream. *Cell, 129*(7), 1261-1274.

Marchildon, F., Lamarche, E., Lala-Tabbert, N., St-Louis, C., & Wiper-Bergeron, N. (2015). Expression of CCAAT/Enhancer Binding Protein Beta in Muscle Satellite Cells Inhibits Myogenesis in Cancer Cachexia. *PLoS One, 10*(12), e0145583.

McGee, S. L., Fairlie, E., Garnham, A. P., & Hargreaves, M. (2009). Exercise-induced histone modifications in human skeletal muscle. *J Physiol, 587*(Pt 24), 5951-5958.

McGee, S. L., & Walder, K. R. (2017). Exercise and the Skeletal Muscle Epigenome. *Cold Spring Harb Perspect Med, 7*(9).

McKinsey, T. A., Zhang, C. L., & Olson, E. N. (2001). Control of muscle development by dueling HATs and HDACs. *Curr Opin Genet Dev, 11*(5), 497-504.

Mennella, V., Agard, D. A., Huang, B., & Pelletier, L. (2014). Amorphous no more: subdiffraction view of the pericentriolar material architecture. *Trends Cell Biol, 24*(3), 188-197.

Meraldi, P., Lukas, J., Fry, A. M., Bartek, J., & Nigg, E. A. (1999). Centrosome duplication in mammalian somatic cells requires E2F and Cdk2-cyclin A. *Nat Cell Biol, 1*(2), 88-93.

Michels, K. B., Binder, A. M., Dedeurwaerder, S., Epstein, C. B., Greally, J. M., Gut, I., . . . Irizarry, R. A. (2013). Recommendations for the design and analysis of epigenome-wide association studies. *Nat Methods, 10*(10), 949-955.

Mikule, K., Delaval, B., Kaldis, P., Jurcyzk, A., Hergert, P., & Doxsey, S. (2007). Loss of centrosome integrity induces p38-p53-p21-dependent G1-S arrest. *Nat Cell Biol, 9*(2), 160-170.

Moberg, M., Apro, W., Ekblom, B., van Hall, G., Holmberg, H. C., & Blomstrand, E. (2016). Activation of mTORC1 by leucine is potentiated by branched-chain amino acids and even more so by essential amino acids following resistance exercise. *Am J Physiol Cell Physiol, 310*(11), C874-884.

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology, 38*(1), 23-38.

Morton, J. J., Bird, G., Refaeli, Y., & Jimeno, A. (2016). Humanized Mouse Xenograft Models: Narrowing the Tumor-Microenvironment Gap. *Cancer Res, 76*(21), 6153-6158.

Mosesson, Y., Voichek, Y., & Barkai, N. (2014). Divergence and selectivity of expression-coupled histone modifications in budding yeasts. *PLoS One, 9*(7), e101538.

Moss, F. P., & Leblond, C. P. (1971). Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec, 170*(4), 421-435.

Mueller, T. C., Bachmann, J., Prokopchuk, O., Friess, H., & Martignoni, M. E. (2016). Molecular pathways leading to loss of skeletal muscle mass in cancer cachexia--can findings from animal models be translated to humans? *BMC Cancer, 16*, 75.

Munoz-Canoves, P., Scheele, C., Pedersen, B. K., & Serrano, A. L. (2013). Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? *FEBS J, 280*(17), 4131-4148.

Murakami, S., Fujino, H., Takeda, I., Momota, R., Kumagishi, K., & Ohtsuka, A. (2010). Comparison of capillary architecture between slow and fast muscles in rats using a confocal laser scanning microscope. *Acta Med Okayama, 64*(1), 11-18.

Nemet, D., Eliakim, A., Zaldivar, F., & Cooper, D. M. (2006). Effect of rhIL-6 infusion on GH-->IGF-I axis mediators in humans. *Am J Physiol Regul Integr Comp Physiol, 291*(6), R1663-1668.

Nigg, E. A., & Raff, J. W. (2009). Centrioles, centrosomes, and cilia in health and disease. *Cell, 139*(4), 663-678.

Nishioka, K., Chuikov, S., Sarma, K., Erdjument-Bromage, H., Allis, C. D., Tempst, P., & Reinberg, D. (2002). Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. *Genes Dev, 16*(4), 479-489.

Nitert, M. D., Dayeh, T., Volkov, P., Elgzyri, T., Hall, E., Nilsson, E., . . . Ling, C. (2012). Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes, 61*(12), 3322-3332.

Ohkawa, Y., Mallappa, C., Vallaster, C. S., & Imbalzano, A. N. (2012). Isolation of nuclei from skeletal muscle satellite cells and myofibers for use in chromatin immunoprecipitation assays. *Methods Mol Biol, 798*, 517-530.

Oishi, Y. (1993). Relationship between myosin heavy chain IId isoform and fibre types in soleus muscle of the rat after hindlimb suspension. *Eur J Appl Physiol Occup Physiol, 66*(5), 451-454.

Pandorf, C. E., Haddad, F., Wright, C., Bodell, P. W., & Baldwin, K. M. (2009). Differential epigenetic modifications of histones at the myosin heavy chain genes in fast and slow skeletal muscle fibers and in response to muscle unloading. *Am J Physiol Cell Physiol, 297*(1), C6-16.

Papadopoulos, S., Jurgens, K. D., & Gros, G. (2000). Protein diffusion in living skeletal muscle fibers: dependence on protein size, fiber type, and contraction. *Biophys J, 79*(4), 2084-2094.

Pascreau, G., Churchill, M. E., & Maller, J. L. (2011). Centrosomal localization of cyclins E and A: structural similarities and functional differences. *Cell Cycle, 10*(2), 199-205.

Pauler, F. M., Sloane, M. A., Huang, R., Regha, K., Koerner, M. V., Tamir, I., . . . Barlow, D. P. (2009). H3K27me3 forms BLOCs over silent genes and intergenic regions and specifies a histone banding pattern on a mouse autosomal chromosome. *Genome Res, 19*(2), 221-233.

Peserico, A., & Simone, C. (2011). Physical and functional HAT/HDAC interplay regulates protein acetylation balance. *J Biomed Biotechnol, 2011*, 371832.

Philippou, A., Maridaki, M., Halapas, A., & Koutsilieris, M. (2007). The role of the insulin-like growth factor 1 (IGF-1) in skeletal muscle physiology. *In Vivo, 21*(1), 45-54.

Porporato, P. E. (2016). Understanding cachexia as a cancer metabolism syndrome. *Oncogenesis, 5*, e200.

Preissl, S., Schwaderer, M., Raulf, A., Hesse, M., Gruning, B. A., Kobele, C., . . . Gilsbach, R. (2015). Deciphering the Epigenetic Code of Cardiac Myocyte Transcription. *Circ Res, 117*(5), 413-423.

Rada-Iglesias, A., Bajpai, R., Swigut, T., Brugmann, S. A., Flynn, R. A., & Wysocka, J. (2011). A unique chromatin signature uncovers early developmental enhancers in humans. *Nature, 470*(7333), 279-283.

Ralston, E., & Hall, Z. W. (1989). Intracellular and surface distribution of a membrane protein (CD8) derived from a single nucleus in multinucleated myotubes. *J Cell Biol, 109*(5), 2345-2352.

Ralston, E., McLaren, R. S., & Horowitz, J. A. (1997). Nuclear domains in skeletal myotubes: the localization of transferrin receptor mRNA is independent of its half-life and restricted by binding to ribosomes. *Exp Cell Res, 236*(2), 453-462.

Reiser, P. J., Moss, R. L., Giulian, G. G., & Greaser, M. L. (1985). Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. *J Biol Chem, 260*(16), 9077-9080.

Richardson, G. D. (2016). Simultaneous Assessment of Cardiomyocyte DNA Synthesis and Ploidy: A Method to Assist Quantification of Cardiomyocyte Regeneration and Turnover. *J Vis Exp*(111).

Richardson, G. D., Laval, S., & Owens, W. A. (2015). Cardiomyocyte Regeneration in the mdx Mouse Model of Nonischemic Cardiomyopathy. *Stem Cells Dev, 24*(14), 1672-1679.

Riffo-Campos, A. L., Castillo, J., Tur, G., Gonzalez-Figueroa, P., Georgieva, E. I., Rodriguez, J. L., . . . Franco, L. (2015). Nucleosome-specific, time-dependent changes in histone modifications during activation of the early growth response 1 (Egr1) gene. *J Biol Chem, 290*(1), 197-208.

Riggs, A. D. (1975). X inactivation, differentiation, and DNA methylation. *Cytogenet Cell Genet, 14*(1), 9-25.

Romano, R., Palamaro, L., Fusco, A., Iannace, L., Maio, S., Vigliano, I., . . . Pignata, C. (2012). From murine to human nude/SCID: the thymus, T-cell development and the missing link. *Clin Dev Immunol, 2012*, 467101.

Rothbart, S. B., Dickson, B. M., Raab, J. R., Grzybowski, A. T., Krajewski, K., Guo, A. H., . . . Strahl, B. D. (2015). An Interactive Database for the Assessment of Histone Antibody Specificity. *Mol Cell, 59*(3), 502-511.

Ruggeri, B. A., Camp, F., & Miknyoczki, S. (2014). Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery. *Biochem Pharmacol, 87*(1), 150-161.

Russell, B., & Dix, D. J. (1992). Mechanisms for intracellular distribution of mRNA: in situ hybridization studies in muscle. *Am J Physiol, 262*(1 Pt 1), C1-8.

Sakakibara, I., Santolini, M., Ferry, A., Hakim, V., & Maire, P. (2014). Six homeoproteins and a Iinc-RNA at the fast MYH locus lock fast myofiber terminal phenotype. *PLoS Genet, 10*(5), e1004386.

Saksouk, N., Simboeck, E., & Dejardin, J. (2015). Constitutive heterochromatin formation and transcription in mammals. *Epigenetics Chromatin, 8*, 3.

Sanchez, A. M., Candau, R. B., & Bernardi, H. (2014). FoxO transcription factors: their roles in the maintenance of skeletal muscle homeostasis. *Cell Mol Life Sci, 71*(9), 1657-1671.

Sandri, M. (2013). Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. *Int J Biochem Cell Biol, 45*(10), 2121-2129.

Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Emre, N. C., . . . Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature, 419*(6905), 407-411.

Schiaffino, S., Bormioli, S. P., & Aloisi, M. (1976). The fate of newly formed satellite cells during compensatory muscle hypertrophy. *Virchows Arch B Cell Pathol, 21*(2), 113-118.

Schiaffino, S., Gorza, L., Sartore, S., Saggin, L., Ausoni, S., Vianello, M., . . . Lomo, T. (1989). Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *J Muscle Res Cell Motil, 10*(3), 197-205.

Schiaffino, S., & Reggiani, C. (1996). Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol Rev, 76*(2), 371-423.

Schiaffino, S., & Reggiani, C. (2011). Fiber types in mammalian skeletal muscles. *Physiol Rev, 91*(4), 1447-1531.

Schmalbruch, H., & Hellhammer, U. (1977). The number of nuclei in adult rat muscles with special reference to satellite cells. *The Anatomical record, 189*(2), 169-175.

Segatto, M., Fittipaldi, R., Pin, F., Sartori, R., Dae Ko, K., Zare, H., . . . Caretti, G. (2017). Epigenetic targeting of bromodomain protein BRD4 counteracts cancer cachexia and prolongs survival. *Nat Commun, 8*(1), 1707.

Shlyueva, D., Stampfel, G., & Stark, A. (2014). Transcriptional enhancers: from properties to genome-wide predictions. *Nat Rev Genet, 15*(4), 272-286.

Snow, M. H. (1978). An autoradiographic study of satellite cell differentiation into regenerating myotubes following transplantation of muscles in young rats. *Cell Tissue Res, 186*(3), 535-540.

Srsen, V., Fant, X., Heald, R., Rabouille, C., & Merdes, A. (2009). Centrosome proteins form an insoluble perinuclear matrix during muscle cell differentiation. *BMC Cell Biol, 10*, 28.

Strassmann, G., Fong, M., Kenney, J. S., & Jacob, C. O. (1992). Evidence for the involvement of interleukin 6 in experimental cancer cachexia. *J Clin Invest, 89*(5), 1681-1684.

Sun, R., Zhang, S., Hu, W., Lu, X., Lou, N., Yang, Z., . . . Yang, H. (2016). Valproic acid attenuates skeletal muscle wasting by inhibiting C/EBPbeta-regulated atrogin1 expression in cancer cachexia. *Am J Physiol Cell Physiol, 311*(1), C101-115.

Talbert, E. E., Metzger, G. A., He, W. A., & Guttridge, D. C. (2014). Modeling human cancer cachexia in colon 26 tumor-bearing adult mice. *J Cachexia Sarcopenia Muscle, 5*(4), 321-328.

Talbert, P. B., & Henikoff, S. (2017). Histone variants on the move: substrates for chromatin dynamics. *Nat Rev Mol Cell Biol, 18*(2), 115-126.

Tassin, A. M., Maro, B., & Bornens, M. (1985). Fate of microtubule-organizing centers during myogenesis in vitro. *J Cell Biol, 100*(1), 35-46.

Terragni, J., Zhang, G., Sun, Z., Pradhan, S., Song, L., Crawford, G. E., . . . Ehrlich, M. (2014). Notch signaling genes: myogenic DNA hypomethylation and 5-hydroxymethylcytosine. *Epigenetics, 9*(6), 842-850.

Thorburn, A. M., Walton, P. A., & Feramisco, J. R. (1993). MyoD induced cell cycle arrest is associated with increased nuclear affinity of the Rb protein. *Mol Biol Cell, 4*(7), 705-713.

Tie, F., Banerjee, R., Stratton, C. A., Prasad-Sinha, J., Stepanik, V., Zlobin, A., . . . Harte, P. J. (2009). CBP-mediated acetylation of histone H3 lysine 27 antagonizes Drosophila Polycomb silencing. *Development, 136*(18), 3131-3141.

Tisdale, M. J. (2010). Reversing cachexia. *Cell, 142*(4), 511-512.

Toledo, M., Busquets, S., Ametller, E., Lopez-Soriano, F. J., & Argiles, J. M. (2011). Sirtuin 1 in skeletal muscle of cachectic tumour-bearing rats: a role in impaired regeneration? *J Cachexia Sarcopenia Muscle, 2*(1), 57-62.

Tollenaere, M. A., Mailand, N., & Bekker-Jensen, S. (2015). Centriolar satellites: key mediators of centrosome functions. *Cell Mol Life Sci, 72*(1), 11-23.

Tonkin, J., Temmerman, L., Sampson, R. D., Gallego-Colon, E., Barberi, L., Bilbao, D., . . . Rosenthal, N. (2015). Monocyte/Macrophage-derived IGF-1 Orchestrates Murine Skeletal Muscle Regeneration and Modulates Autocrine Polarization. *Mol Ther, 23*(7), 1189-1200.

Tseng, Y. C., Kulp, S. K., Lai, I. L., Hsu, E. C., He, W. A., Frankhouser, D. E., . . . Chen, C. S. (2015). Preclinical Investigation of the Novel Histone Deacetylase Inhibitor AR-42 in the Treatment of Cancer-Induced Cachexia. *J Natl Cancer Inst, 107*(12), djv274.

Tsoli, M., & Robertson, G. (2013). Cancer cachexia: malignant inflammation, tumorkines, and metabolic mayhem. *Trends Endocrinol Metab, 24*(4), 174-183.

Tsujinaka, T., Fujita, J., Ebisui, C., Yano, M., Kominami, E., Suzuki, K., . . . Monden, M. (1996). Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. *J Clin Invest, 97*(1), 244-249.

Utvik, J. K., Nja, A., & Gundersen, K. (1999). DNA injection into single cells of intact mice. *Hum Gene Ther, 10*(2), 291-300.

Vigano, A., Dorgan, M., Buckingham, J., Bruera, E., & Suarez-Almazor, M. E. (2000). Survival prediction in terminal cancer patients: a systematic review of the medical literature. *Palliat Med, 14*(5), 363-374.

Voigt, P., Tee, W. W., & Reinberg, D. (2013). A double take on bivalent promoters. *Genes Dev, 27*(12), 1318-1338.

Voskoglou-Nomikos, T., Pater, J. L., & Seymour, L. (2003). Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. *Clin Cancer Res, 9*(11), 4227-4239.

Wada, K. I., Takahashi, H., Katsuta, S., & Soya, H. (2002). No decrease in myonuclear number after long-term denervation in mature mice. *American Journal of Physiology-Cell Physiology, 283*(2), C484-C488.

Waddington, C. H. (2012). The epigenotype. 1942. *Int J Epidemiol, 41*(1), 10-13.

Wang, H., Listrat, A., Meunier, B., Gueugneau, M., Coudy-Gandilhon, C., Combaret, L., . . . Bechet, D. (2014). Apoptosis in capillary endothelial cells in ageing skeletal muscle. *Aging Cell, 13*(2), 254-262.

Wang, Z., Corey, E., Hass, G. M., Higano, C. S., True, L. D., Wallace, D., Jr., . . . Vessella, R. L. (2003). Expression of the human cachexia-associated protein (HCAP) in prostate cancer and in a prostate cancer animal model of cachexia. *Int J Cancer, 105*(1), 123-129.

Waning, D. L., Mohammad, K. S., Reiken, S., Xie, W., Andersson, D. C., John, S., . . . Guise, T. A. (2015). Excess TGF-beta mediates muscle weakness associated with bone metastases in mice. *Nat Med, 21*(11), 1262-1271.

Wilkie, G. S., Korfali, N., Swanson, S. K., Malik, P., Srsen, V., Batrakou, D. G., . . . Schirmer, E. C. (2011). Several novel nuclear envelope transmembrane proteins identified in skeletal muscle have cytoskeletal associations. *Mol Cell Proteomics, 10*(1), M110 003129.

Woodhouse, S., Pugazhendhi, D., Brien, P., & Pell, J. M. (2013). Ezh2 maintains a key phase of muscle satellite cell expansion but does not regulate terminal differentiation. *J Cell Sci, 126*(Pt 2), 565-579.

Wozniak, A. C., & Anderson, J. E. (2005). Single-fiber isolation and maintenance of satellite cell quiescence. *Biochem Cell Biol, 83*(5), 674-676.

Yoon, A., Tammen, S. A., Park, S., Han, S. N., & Choi, S. W. (2017). Genome-wide hepatic DNA methylation changes in high-fat diet-induced obese mice. *Nutr Res Pract, 11*(2), 105-113.

Yu, X., Zhang, Y., Li, T., Ma, Z., Jia, H., Chen, Q., . . . Zhu, D. (2017). Long non-coding RNA Linc-RAM enhances myogenic differentiation by interacting with MyoD. *Nat Commun, 8*, 14016. doi:10.1038/ncomms14016

Yuan, L., Han, J., Meng, Q., Xi, Q., Zhuang, Q., Jiang, Y., . . . Wu, G. (2015). Muscle-specific E3 ubiquitin ligases are involved in muscle atrophy of cancer cachexia: an in vitro and in vivo study. *Oncol Rep, 33*(5), 2261-2268.

Zebrowski, D. C., Vergarajauregui, S., Wu, C. C., Piatkowski, T., Becker, R., Leone, M., . . . Engel, F. B. (2015). Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes. *Elife, 4*.

Zhang, T., & Kraus, W. L. (2010). SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. *Biochim Biophys Acta, 1804*(8), 1666-1675.

Zhang, Z., Shi, L., Dawany, N., Kelsen, J., Petri, M. A., & Sullivan, K. E. (2016). H3K4 tri-methylation breadth at transcription start sites impacts the transcriptome of systemic lupus erythematosus. *Clin Epigenetics, 8*, 14.