THE TAZ PROTEIN INTERACTOME IN STRIATED MUSCLE

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Abstract

Hippo signalling is a prominent regulator of cell proliferation, differentiation, and death. Previously, we characterized the Hippo transcriptional effector TAZ as a repressor of the myogenic differentiation program. Without DNA-binding ability, TAZ function exclusively depends on its protein:protein interaction network. This prompted us to undertake a proteomic-based study aimed at identifying the TAZ interactome in striated muscle cells. Using a novel GFP-Nanotrap based affinity purification approach coupled with LC-MS/MS protein identification, we document a comprehensive list of known and novel TAZ interactome components in myogenic cells. TAZ interacting proteins include components of Hippo signalling: TEAD1-4, LATS1, 14-3-3 proteins. Epigenetic regulators were also represented: NuRD complex, FACT complex, and SWI/SNF complex. We focused on characterizing the TAZ interaction with the Wnt co-repressor TLE3 in myogenic cells. In myogenic cells, TAZ and TLE3 interact and co-localize within the nucleus. Functionally, TAZ and TLE3 repress β-catenin activation, as indicated by the Wnt-responsive TOP FLASH reporter gene system. Depletion of TAZ reduced the degree of TLE3-mediated repression of β-catenin activation. TAZ and TLE3 repressed MyoD-driven activation of the *myogenin* promoter. Overall, these data demonstrate a role for a TAZ/TLE3 complex in repressing the myogenic differentiation machinery having implications for muscle development and regeneration.

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List of Abbreviations

AMOT – Angiomotin

AMOTL1/2 – Angiomotin-like 1/2

AMPK – 5’ AMP-activated protein kinase

APC – adenomatois spolyposis coli

β-TrCP – Beta-transducin repeat containing E3 ubiquitin protein ligase

BMD – Becker muscular dystrophy

BMP – Bone morphogenic protein

BRD4 – Bromodomain-containing protein 4

CAMK – $Ca^{2+/}$calmodulin-dependent protein kinase

CDK9 – Cyclin-dependent kinase 9

CK1 – Casein kinase 1

CTGF – Connective tissue growth factor

CYR61 – Cysteine-rich angiogenic inducer 61

DMD – Duchenne muscular dystrophy

DUX4 – Double homeobox 4

DVL – Disheveled

EMT – Epithelial-to-mesenchymal transition

ERK5 – Extracellular-signal-regulated kinase 5

F-actin – Filamentous actin

FGF2 – Fibroblast growth factor 2

FSHD – Facioscapulohumeral muscular dystrophy

GSK3 – Glycogen synthase kinase-3

HGH – Human growth hormone

HM – Hydrophobic motif

IDR – Intrinsically disordered region

IFG-1 – Insulin-like growth factor 1

JAK1 – Janus kinase 1

LATS1/2 – Large tumor suppressor kinase 1/2

LLPS – Liquid-liquid phase separation

MAP4K – Mitogen-activated protein kinase 4

MED1 – Mediator complex subunit 1

MEF2 – Myocyte enhancer factor 2

MMP – Matrix metallopeptidase

MOB1 – Mps1-binder-related 1

MRFs – Myogenic regulatory factor

MST1/2 – Mammalian STE20-like protein kinase 1/2

mTOR – Mammalian target of rapamycin

MyoD – Myoblast determination protein 1

MyoG – Myogenin

NF2 – Neurofibromatosis type 2

NO – Nitrous oxide

NuRD – Nucleosome Remodeling Deacetylase

P73 – Protein 73

Pax3/7 – Paired pox gene 3/7

Runx1/2 – Runt-related transcription factor 1

SARAH – Sav/Rassf/Hpo

SAV1 – Salvador 1

SC – Satellite cell

Shh – Sonic hedgehog

SMAD1/2/3/7 – Suppressor of mothers against decapentaplegic

STAT1 – Signal transducer and activator of transcription 1

STK25 – Serine-Threonine kinase 25

TAO – Thousand and one

TAZ – Transcriptional activator with PDZ-binding motif

TCF/LEF – T-cell factor/lymphoid enhancer factor

TEAD1-4 – TEA domain family domain 1-4

TRAIL – TNF-related apoptosis-inducing ligand

Wnt – Wingless integration site signaling pathway

YAP – Yes-associated protein

ZO-1/2 – Zonula occludens 1/2

Chapter I: Literature review

Overview of skeletal muscle

Skeletal muscle is a form of striated muscle that represents the largest body component in humans. While predominantly involved in generating movement and maintaining an upright posture, skeletal muscle also contributes significantly to other bodily functions, including respiratory mechanics, nutrient storage, metabolic regulation, and thermoregulation (Frontera & Ochala, 2015). Hence, proper operation of the skeletal musculature is essential for healthy aging and participation in everyday social and occupational settings in a functionally independent manner. Muscle is subject to atrophy, which results in a loss of skeletal muscle mass and can lead to muscle weakness and possible physical disability. Sarcopenia is age-related muscle atrophy in older individuals, causing systemic muscle wasting and a progressive decline in skeletal muscle quality (McLeod et al., 2016). Certain diseases, including cancer and heart failure, underlie a muscle wasting syndrome known as cachexia, also triggering muscle atrophy (Baracos et al., 2018). Severe neuromuscular disorders can arise from genetic mutations, including Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and facioscapulohumeral muscular dystrophy (FSHD). DMD and BMD result from a mutated dystrophin protein (Wilson et al., 2017), while the aberrant production of DUX4 protein causes FSHD (Hamel & Tawil, 2018). Ultimately, given how skeletal muscle dysfunction manifests in debilitating and sometimes lethal diseases, it remains crucial to investigate the molecular pathways occurring during muscle development, known as myogenesis. Improving our knowledge in this domain could potentially lead to advancements in developing therapies aimed at reversing muscle aging and disease.

Skeletal myogenesis

Skeletal muscle possesses the remarkable ability to regenerate adult tissue in response to injury. Muscle regeneration hinges on the activation of a resident stem cell population known as satellite cells (SCs, also referred to as muscle stem cells, MuSCs) (Tedesco et al., 2010). Post-natal muscle regeneration is considered to operate similarly to embryonic muscle development in that they are both governed by a hierarchal network of transcription factors (TFs) featuring the paired-box TFs Pax3/Pax7 and the four myogenic regulatory factors (MRFs): Myf5, MyoD, MyoG, and MRF4 (Wang & Conboy, 2010). Despite differences between the processes, particularly in the micro-environment, we must consider this "regeneration recapitulates development" concept and, thus, explore the mechanisms governing embryonic myogenesis before fully appreciating the repair mechanisms present in skeletal muscle generation.

Embryonic myogenesis

The genesis of skeletal muscle during embryonic development is initiated during somitogenesis, the process where segmentation of paraxial mesoderm produces spherical masses called somites. Somites are bilaterally positioned on either side of the neural tube along the anterior-posterior axis of the embryo (Buckingham et al., 2003; Musumeci et al., 2015). Cells in the ventromedial region of the somites, under the influence of morphogen gradients generated by wingless-related integration site (Wnt), sonic hedgehog (Shh), and bone morphogenetic protein (BMP) signaling, undergo an epithelial-to-mesenchymal transition (EMT) to form the sclerotome, which contains precursors to cartilage and bone (Fan & Tessier-Lavigne, 1994; Johnson et al., 1994; Tabata & Takei, 2004). The remaining dorsolateral region of the somite, now the dermomyotome, remains epithelial and contains the earliest myogenic progenitor cells marked by the expression of the

paired-box transcription factor Pax3 and Pax7 (Buckingham & Relaix, 2015). Mice homozygous mutant for Pax3 lack the hypaxial dermomyotome and fail to develop organized body muscle (Relaix et al., 2004). Under control of the sine oculis-related homeobox family members Six1 and Six4 (Grifone et al., 2005), Pax3 expression is vital for upregulating Myf5, the first MRF to be expressed in myogenesis, allowing cells located in the epaxial (dorsomedial) and hypaxial (ventrolateral) lips of the dermomyotome to undergo an EMT and delaminate underneath the dermomyotome, forming a myogenic mesenchyme called the myotome (Buckingham & Relaix, 2015). Furthermore, Pax3 is responsible for upregulating the expression of c-Met, a tyrosine kinase receptor that is required for migration of myogenic precursors (Epstein et al., 1996). The myotome is marked by the downregulation of Pax3 and upregulation of Myf5. The epaxial myotome will give rise to the back muscles, and the hypaxial myotome gives rise to the limb and ventral trunk muscles (Buckingham & Relaix, 2015; Deries & Thorsteinsdottir, 2016).

Pax3 is genetically upstream of the four MRFs, the highly conserved class II basic helix-loop-helix (bHLH) TFs that lay the foundation of the genetic hierarchy governing myogenic progression (Rudnicki & Jaenisch, 1995). MRFs contain a conserved DNA-binding basic domain and a helix-loop-helix domain that are collectively required for binding onto genomic E-box motif sequences, a motif found upstream of many muscle-specific gene promoters (Massari & Murre, 2000). Under the control of Pax3, elevation of Myf5 expression signals the rapid induction of MyoD expression, a chief marker of myogenic commitment, followed by MyoG, a differentiation factor preceding the expression of the proteins of the contractile apparatus. MyoG knockout mice contain myoblasts; however, they cannot differentiate and fuse, highlighting the importance of MyoG for the terminal differentiation of myoblasts (Hasty et al., 1993). In general, the genetic hierarchy during myogenic progression in the limb proceeds from Six1/4 and Pax3 expression to

Myf5 expression, to MyoD expression, and lastly, MyoG expression. A schematic illustrating embryonic development is shown in Figure 1.

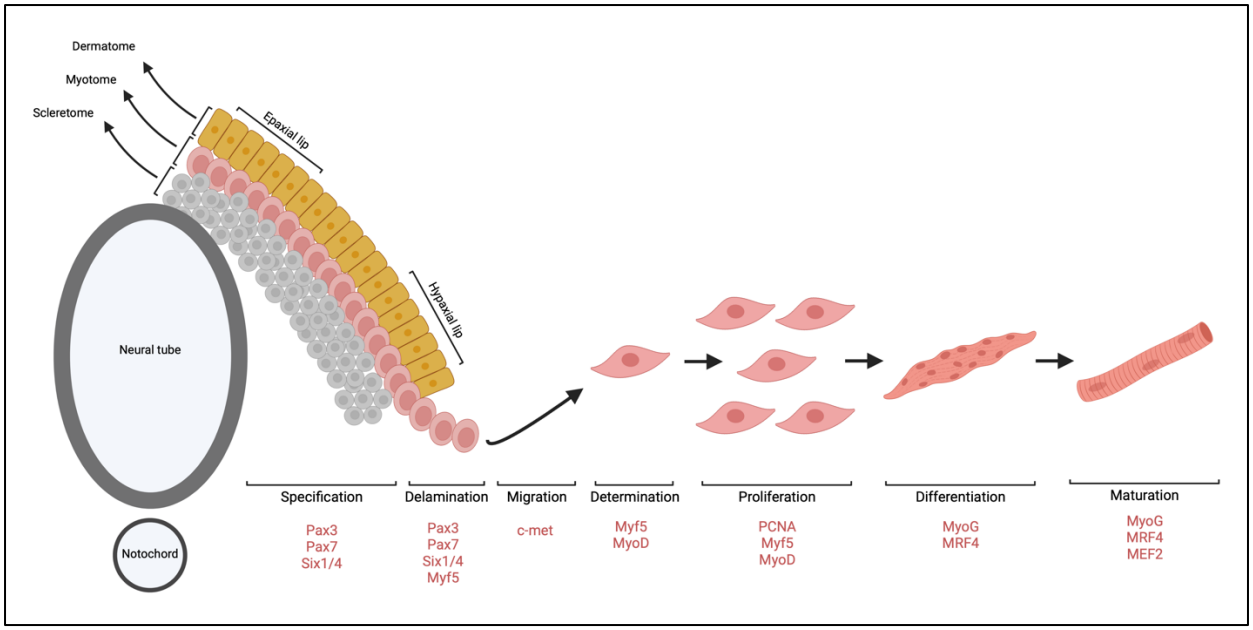

Figure 1. Somitogenesis and muscle formation in the limb. Specification begins with the differentiation of the somite to form the sclerotome and dermomyotome, marked by Pax 3, Pax7, and Six1/4 expression. The dermomyotome then differentiates into the dermatome and myotome, of which the latter begins expressing Myf5, causing delamination and migration into the limb field.

Adult myogenesis

Skeletal muscle regeneration is mediated by SCs, which constitute a reservoir of adult somatic stem cells located in niches between the myofiber plasmalemma and the basal lamina (Mauro, 1961). While most Pax3/7^{+} myogenic progenitors from the dermomyotome ultimately express MRFs and commit towards a myogenic lineage, a subset of Pax3/7^{+} cells from the central dermomyotome do not express MRFs, instead proliferate and adopt the SC position. While Pax3 is critical during embryonic myogenesis, SCs primed for regeneration are internally marked and dominated by the expression of Pax7. In the absence of injury, inactive SCs remain quiescent, expressing Pax7 and Myf5 (besides a small subset of Myf5neg SCs cells). The quiescent stage is considered an active process, revealed by gene expression analysis showing the upregulation of over 500 genes compared to activated stem cells (Fukada et al., 2007). Quiescence is maintained through various mechanisms, including repression of cell cycle and regulation of intracellular signaling pathways. One such example is Notch signalling, whose activity is important in maintaining SC quiescence. This is demonstrated by Notch receptor Syndecan-3 knockout SCs, which exhibit impaired Notch signaling, resulting in premature exit from quiescence and depletion of the SC pool (Pisconti et al., 2010). Premature SCs activation is also regulated at the level of mRNA sequestration, where mRNP granules have been shown to sequester and prevent the accumulation of Myf5 mRNA in quiescent SCs (Crist et al., 2012).

Following muscle injury, stimulation of extracellular signals from the damaged environment triggers multiple signaling pathways that promote the migration of SCs toward the injury site and re-entrance into the cell cycle for proliferation. Examples of signaling molecules that trigger the exit from quiescence into a proliferative state include IGF-1, which is known to stimulate the Akt/mTOR pathway (Musaro et al., 2001), and NO, which has been demonstrated to stimulate

MMP expression (Tatsumi, 2010), both resulting in SC activation. Following activation, stimulation of cell division is facilitated by many of the same growth factors that promote SC activation, including IFG-1, FGF2, and HGH (Chakravarthy et al., 2000; Yablonka-Reuveni & Rivera, 1997; Yamada et al., 2010). An additional means of encouraging cell proliferation at this stage is through the repression of premature differentiation. For instance, p38gamma has been shown to phosphorylate MyoD directly, thus forming a repressive complex on the myogenin promoter (Gillespie et al., 2009) meanwhile, MEF2 gene expression is known to be repressed by JAK1/STAT1 signaling (Sun et al., 2007). A subset of SCs remain Myf5neg and are responsible for self-renewing the stem cell pool. Upon entry into the cell cycle, these SCs can undergo asymmetric or symmetric division. Symmetric division occurs in an apical-basal orientation, producing one daughter SC and one committed daughter SC. The daughter SC will maintain the SC pool, while the committed daughter SC will participate in myogenesis. Alternatively, SCs can undergo symmetric division in a planar orientation, producing two identical daughter SCs, favoring SC expansion. In contrast, a committed mother SCs can produce two identical daughter committed SCs, favoring myogenesis.

Upon activation, the proliferative phase of SCs is initiated and committed $Myf5^{pos}$ SCs begin to express MyoD to become myoblasts, further continuing to proliferate. MyoD is key in facilitating the switch from myoblast proliferation to differentiation. This is achieved chiefly by downstream induction of myogenin and MRF4, which contribute to the expression of genes related to muscle contractility (Davie et al., 2007; Rawls et al., 1998). MyoD and myogenin are synergistically activated at muscle gene promoters by the MEF2 family of TFs (Molkentin et al., 1995). Moreover, increased intracellular calcium concentrations contribute to myogenic differentiation by activating calcineurin and calcium/calmodulin-dependent protein kinase (CaMK) pathways. MEF2 activities

are promoted by calcineurin and CAMK, thus activating MyoD and consequently resulting in increased myogenin expression (Friday et al., 2003; Lu et al., 2000). The final stages of differentiation involve a cell fusion process triggered by myoblast-myoblast contact. Fusion is primarily mediated by the muscle-specific membrane protein myomaker, whose expression is induced by MyoD and myogenin and involves rearrangement of the actin cytoskeleton (Millay et al., 2013). Fusion is followed by myotube maturation and is primarily supported by the anabolic effects of Akt-1/mTOR pathway activity (Park & Chen, 2005; Park et al., 2005). A schematic summarizing the stages of adult myogenesis is shown in Figure 2.

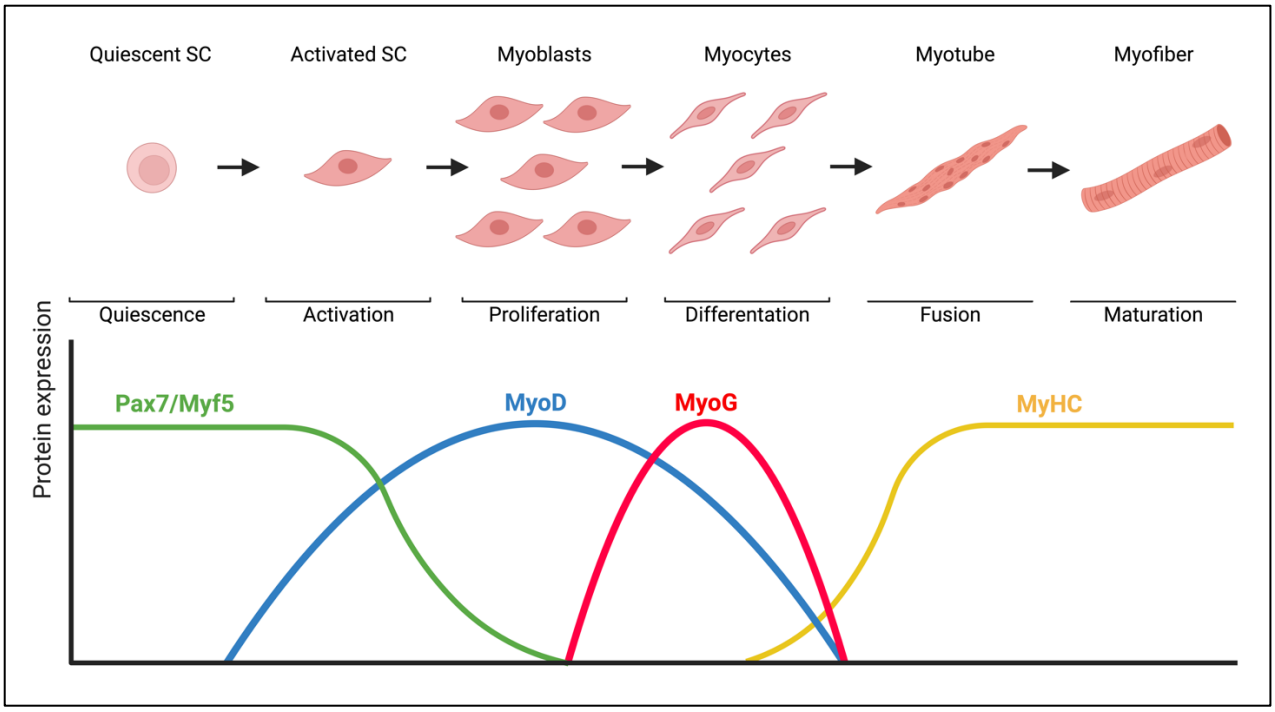

Figure 2. Adult regeneration in skeletal muscle. Upon muscle injury, quiescent SCs expressing Pax7 activate and proliferate into myoblasts under the expression of Myf5 and MyoD. An increase in MyoD and MyoG results in myoblast differentiation into myocytes, which further differentiate into a myofiber.

The Hippo signaling pathway

Overview of Hippo signaling

The Hippo signalling pathway is an evolutionarily conserved signalling pathway that is a master regulator of cell fate and organ growth in animals. The discovery of Hippo signalling originates with genetic screens in Drosophila aimed at identifying tumor suppressor genes. The first four components of the pathway discovered, tumor suppressors genes *hippo* (Mst1-2 in humans) (Wu et al., 2003), *salvador* (Sav1 in humans) (Tapon et al., 2002), *warts* (Lats1-2 in humans) (Justice et al., 1995), and *mats* (Mob1 in humans) (Lai et al., 2005), all display an overgrowth phenotype when inactivated by mutation. Biochemically, it was revealed that these four genes form the serine/threonine Hippo kinase cascade, with their prime target being the transcriptional co-activator Yorkie (YAP/TAZ in humans) (Wei et al., 2007; Wu et al., 2003). Hippo kinases suppress downstream Yorkie via phosphorylation and trigger its retention in the cytoplasm and eventual degradation. When localized in the nucleus, Yorkie partners with the Scalloped (TEAD in humans) family of TFs to mediate tissue overgrowth (Goulev et al., 2008; Wu et al., 2008). Intense research has followed the discovery of the hippo kinase cascade, establishing Hippo signaling as a key determinant of organ size in animals. The pathway remains extensively studied, with current sights on exploiting the pathway for applications in tissue engineering and regenerative medicine.

The core Hippo kinase cascade

The core feature of the canonical Hippo signalling pathway is a cytoplasmic serine/threonine kinase cascade that negatively regulates the activity of downstream transcriptional co-regulators YAP/TAZ. Mammals have two Hpo homologs (MST1 and MST2), one Sav homolog (SAV1), two Wts homologs (LATS1 and LATS2), and two Mats homologs (MOBKL1A and MOBKL1B,

referred collectively to as MOB1). MST kinases, initiators of the cascade, undergo auto-activation through auto-phosphorylation on Thr183 in MST1 and Thr180 in MST2 (Praskova et al., 2004). Additionally, MST1/2 form homo- and heterodimers mediated by their distinctive coiled-coil structure called the SARAH domain (Scheel & Hofmann, 2003). SAV1 forms a complex with the MST1/2 heterodimer, assisting its stabilization and phosphorylation of downstream LATS1 and LATS2 at Thr1079 and Thr1041, respectively (Callus et al., 2006; Chan et al., 2005). MST1/2 proteins also phosphorylate MOB1A/MOB1B at Thr35 and Thr12, respectively (Praskova et al., 2008), thereby promoting the interaction of MOB1A/MOB1B to LATS1/2, which in turn leads to stabilization and auto-phosphorylation of LATS1/LATS2 at Ser909 and Ser872, respectively (Chan et al., 2005; Hergovich, Schmitz, et al., 2006).

Upstream regulation of YAP/TAZ

The hippo kinase cascade culminates with the activation of the LATS kinases which phosphorylate and subsequently inactivate Yorkie homologs YAP and TAZ, hereon collectively referred to as YAP/TAZ. LATS kinases recognize and phosphorylate their substrates on HxRxxS sequences (Hergovich, Stegert, et al., 2006), of which YAP has five (Ser61, Ser109, Ser127, Ser164, and Ser381) and its paralog TAZ has four (Ser66, Ser89, Ser117, and Ser311) (Hao et al., 2008; Lei et al., 2008). YAP/TAZ each have one HxRxxs motif (Ser127 in YAP and S89 in TAZ) that generates a binding site for phosphoserine/phosphothreonine-binding 14-3-3 proteins following phosphorylation by LATS1/2 (Zhao et al., 2007) (Kanai et al., 2000). 14-3-3 protein binding results in cytoplasmic retention of YAP/TAZ. LATS1/2-mediated phosphorylation of YAP/TAZ at other HxRxxS motifs similarly promotes the inactivation of YAP/TAZ, either through cytoplasmic sequestration or ubiquitin-mediated protein degradation (Zhao et al., 2010). Moreover, YAP/TAZ can be phosphorylated and inactivated by AMPK, linking the activity of YAP/TAZ to cellular

energy status (Mo et al., 2015; Wang et al., 2015). On the other hand, several kinases can activate YAP/TAZ by phosphorylation. Nemo kinases block LATS phosphorylation by immediately phosphorylating YAP/TAZ on sites adjacent to the LATS1 site in the 14-3-3 binding region, resulting in enhanced nuclear localization and activity of YAP/TAZ (Moon et al., 2017). Alternatively, the Src family of kinases can phosphorylate and activate YAP on Tyr residues (Li et al., 2016; Taniguchi et al., 2015). The schematic of the Hippo signalling pathway is summarized shown in Figure 3.

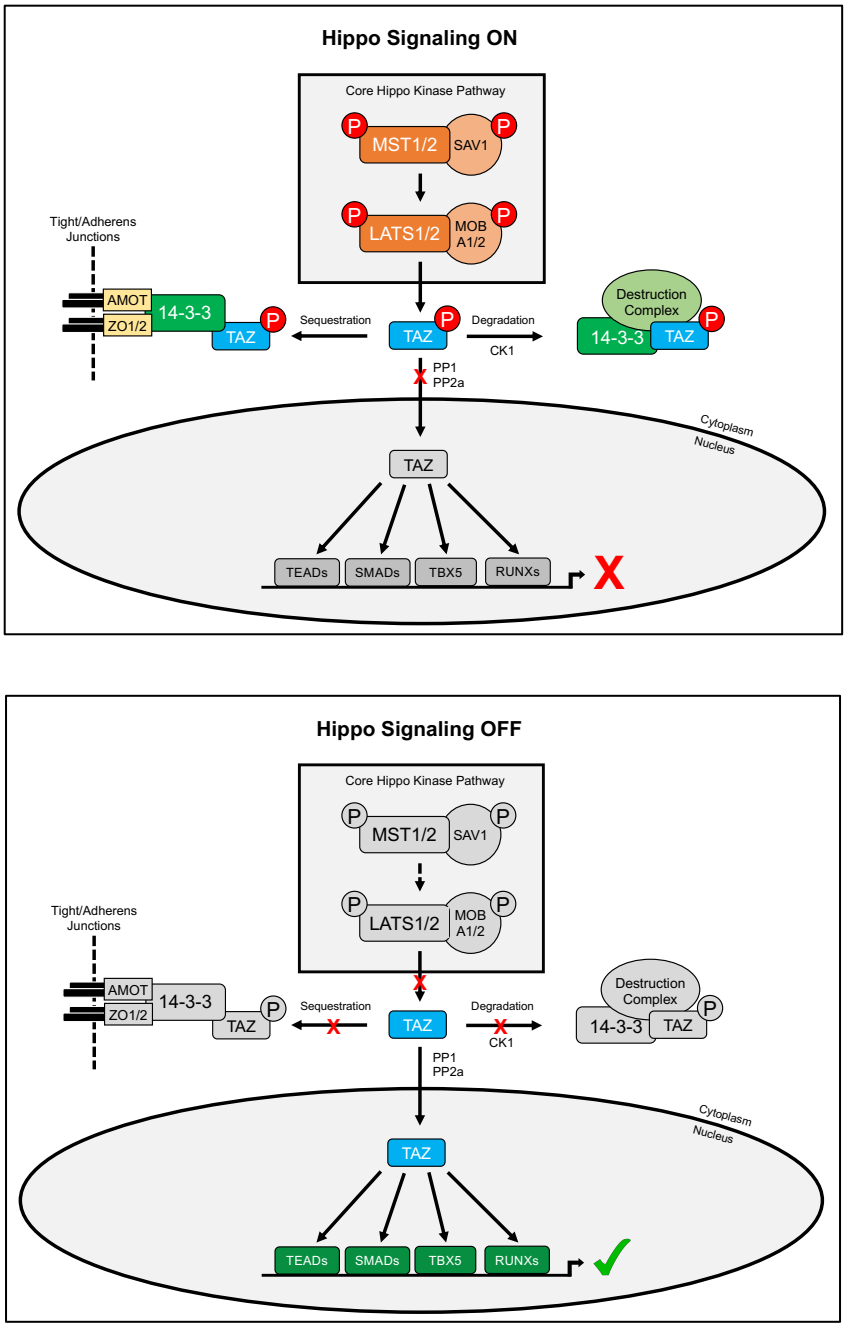

Figure 3. The Hippo signalling pathway. When Hippo signalling is ON, upstream signals activate the core Hippo kinase cascade, resulting in phosphorylation and activation of LATS1/2 kinase. Transcriptional co-regulators YAP/TAZ are phosphorylated by LATS kinases, stimulating the recruitment of 14-3-3 proteins to sequester YAP/TAZ in the cytoplasm for proteolytic degradation. When Hippo signalling is OFF, the Hippo kinase cascade remains inactivated and can no longer phosphorylate YAP/TAZ. YAP/TAZ localizes to the nucleus when not phosphorylated, forms complexes with various TFs, and regulates genes required for proliferation and survival.

Mechanoregulation of YAP/TAZ

Cells functionally respond to changes in their mechanical microenvironment in a process known as mechanotransduction. YAP/TAZ are known sensors of mechanical stimuli generated by the actin cytoskeletal, focal adhesions, and adherens junctions (Dasgupta & McCollum, 2019). Disruption of filamentous actin (F-actin) results in the activation of kinases MST1/2 (Chan et al., 2005), MAP4K-family (Li et al., 2014; Meng et al., 2015; Zheng et al., 2015), STK25 (Lim et al., 2019), TAO (Plouffe et al., 2016), and NF2 (Yin et al., 2013), which all subsequently phosphorylate LATS1/2 on its hydrophobic motif (HM), triggering LATS1/2 to activate by autophosphorylation at its activation loop (AL) and phosphorylate YAP/TAZ. In the presence of assembled F-actin, the Hippo kinase cascade generally remains inactive, and YAP/TAZ are permitted to enter the nucleus. Angiomotin proteins (AMOT, AMOTL1, AMOTL2) are key players that negatively regulate YAP/TAZ in response to alterations in the actin cytoskeleton and cell-cell contact. At low F-actin levels, angiomotins directly bind and sequester YAP/TAZ in the cytoplasm while independently activating LATS1/2 in conjunction with NF2 (Li et al., 2015; Mana-Capelli & McCollum, 2018). At high F-actin levels, angiomotins bind to F-actin, become sequestered, and can no longer retain YAP/TAZ in the cytoplasm (Mana-Capelli & McCollum, 2018). F-actin has additionally been shown to regulate YAP/TAZ activity in the nucleus. At low levels of nuclear F-actin, SWI/SNF complex co-factor ARID1A binds to YAP/TAZ and represses its association with TEAD TFs (Chang et al., 2018). A rise in nuclear F-actin sequesters ARID1A in the nucleus, favouring the formation of the YAP/TAZ-TEAD complex (Chang et al., 2018). Moreover, density-dependent cell-cell contact triggers the recruitment of angiomotin-YAP/TAZ complexes at tight junctions (TJs) (Zhao et al., 2011) and α-catenin-YAP/TAZ complexes at adherens junctions (Schlegelmilch et al., 2011). Therefore, under the control of

mechanotransduction, YAP/TAZ activity is inhibited by disruption of the F-actin cytoskeleton and cell-cell contact-induced junctional localization of angiomotins and α-catenin. A schematic demonstrating the mechanoregulation of YAP/TAZ is summarized in Figure 4.

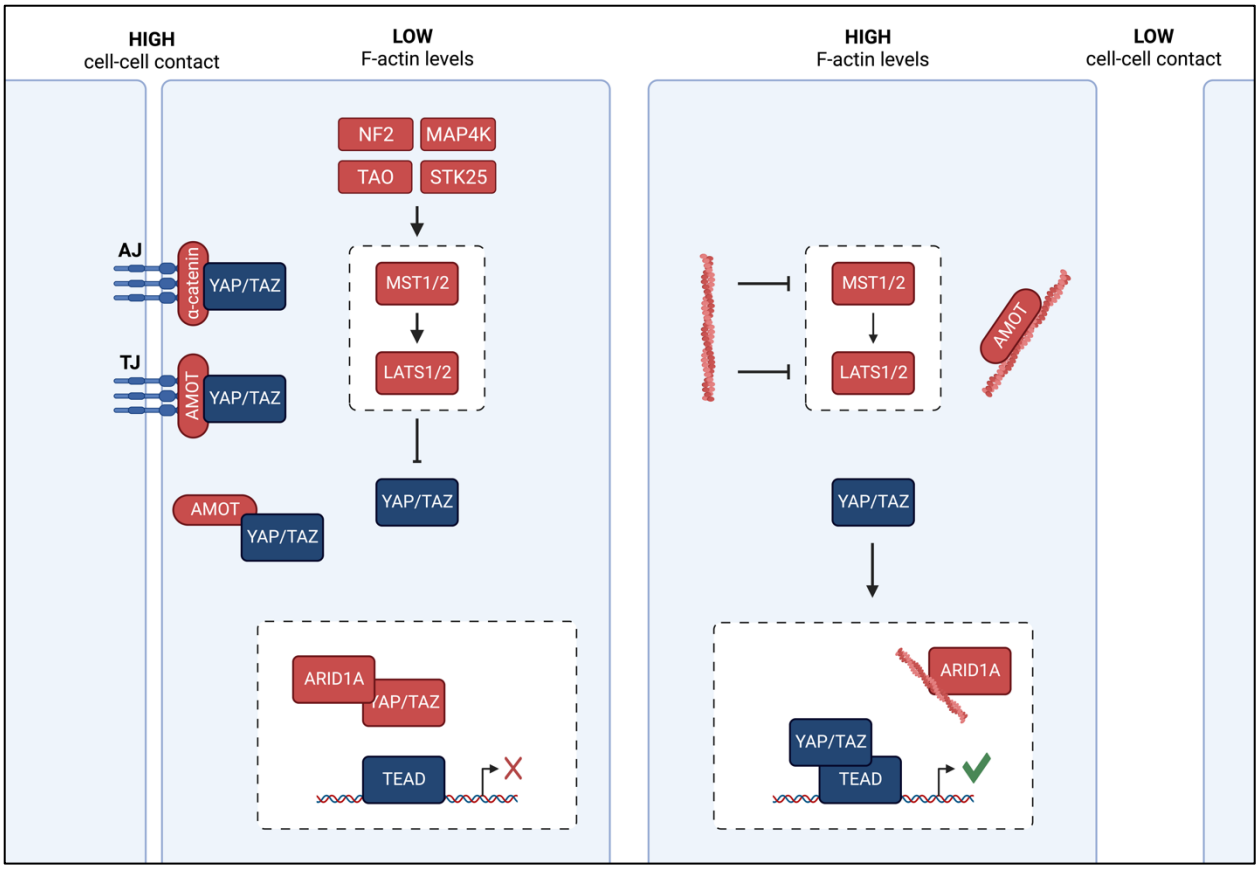

Figure 4. Mechanoregulation of YAP/TAZ. Under low F-actin levels, upstream kinases NF2, TAO, STK25, and MAP4K activate the core Hippo kinases, resulting in cytoplasmic retention of YAP/TAZ. Low nuclear F-actin levels enable ARID1A-mediated inhibition of YAP/TAZ in the nucleus. Cell-cell contact results in the junctional localization of angiomotins and alpha-catenin, sequestering YAP/TAZ in the cytoplasm. High F-actin levels trigger inhibition of the core Hippo kinase, sequestration of angiomotin in the cytoplasm, and ARID1A in the nucleus, all resulting in nuclear localization and increased activity of YAP/TAZ.

Downstream regulation by YAP/TAZ

YAP/TAZ, despite being unable to bind to DNA, are potent co-regulators of transcription. During the inactive state of Hippo signaling, LATS1/2 are inactive, providing YAP/TAZ unrestricted access to the nucleus. Given the absence of a DNA-binding domain, YAP/TAZ elicit their biological functions mainly through association with a variety of TFs in the nucleus. Most notably, YAP/TAZ interact with the TEAD TFs (TEAD1-4) to mediate gene expression of genes related to cell growth and proliferation, including connective tissue growth factor (*ctgf*) and cysteine-rich angiogenic inducer 61 (*cyr61*) (Lei et al., 2008; Pobbati et al., 2012; Stein et al., 2015; Zhao et al., 2008). TEAD TFs share a DNA-binding domain, the TEA domain, which binds to the MCAT elements 5'-CATTCCA/T-3' on DNA (Jacquemin et al., 1996; Jiang et al., 2000). Interestingly, the majority of YAP/TAZ-TEAD associations occur at distant enhancers rather than at promoter regions of YAP/TAZ target genes. Enhancers bound with YAP/TAZ-TEAD associate with their cognate promoters through DNA looping to promote p300-dependent acetylation of lysine 27 of histone H3 (H3K27ac) (Galli et al., 2015; Stein et al., 2015). In addition, AP-1 TFs are recruited to YAP/TAZ-TEAD-bound enhancers for synergistic activation of genes that promote tumour proliferation, migration, and invasion (Liu et al., 2016; Zanconato et al., 2015). Other transcription factors YAP/TAZ that have been shown to cooperate with include Smad1/2/3/7 (Alarcon et al., 2009; Ferrigno et al., 2002; Tripathi et al., 2022; Varelas et al., 2008; Varelas, Samavarchi-Tehrani, et al., 2010), RUNX1/2 (Brusgard et al., 2015; Zaidi et al., 2004), p73 (Strano et al., 2001), and myc (Croci et al., 2017). The YAP/TAZ-TEAD complex can also engage in transcriptional co-repression by recruiting the NuRD complex to deacetylase histones at tumour-suppressor genes encoding DDIT4 (DNA-damage-inducible transcript 4) and TRAIL (TNF-related apoptosis-inducing ligand) (Kim et al., 2015). dis

Structure of YAP/TAZ

YAP and TAZ are structurally similar paralogs that share a TEAD-binding region, a WW domain, a coiled-coil domain, a transactivation domain, and a C-terminal PDZ-binding motif (Figure 5). In addition to these domains, YAP uniquely possesses an N-terminal proline-rich area, two WW domains as opposed to one in TAZ, and an SH3-binding motif. TEAD transcription factors recognize and bind to the TEAD-binding region of YAP/TAZ. This region holds the 14-3-3 motif (S127 in YAP and S89 in TAZ) targeted by LATS1/2 for cytoplasmic sequestration and subsequent degradation (Freeman & Morrison, 2011). In addition, the 14-3-3 site is targeted for dephosphorylation by protein phosphatase 2A (PP2A) (Hein et al., 2019; Jiang et al., 2021). The tryptophan residue-containing WW domains of YAP/TAZ mediate interactions with proline-rich "PPXY motifs" in proteins, including Angiomotin (Varelas, 2014). Lastly, the C-terminal PDZ-binding motif recognizes and binds to PDZ domain-containing proteins such as ZO-1/2 (Lee & Zheng, 2010).

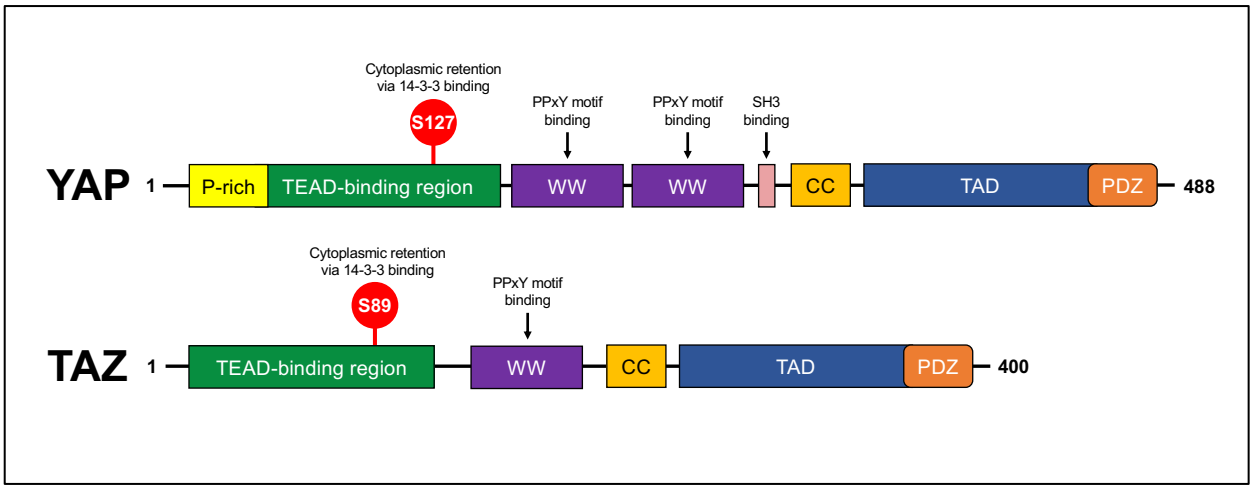

Figure 5. Structure of YAP/TAZ proteins. Structurally, YAP and TAZ share a TEAD-binding region, a central WW domain, a coiled-coiled (CC) domain, a transcriptional activation domain, and a PDZ-domain. YAP differs from TAZ in that it has a second WW domain and SH3-binding domain. LATS1/2 kinase target YAP and TAZ at serine 127 and 89, respectively, to YAP/TAZ trigger cytoplasmic retention via 14-3-3 protein binding.

Hippo signalling in myogenesis and muscle growth

Generally speaking, Hippo kinase activity during myogenesis is reduced during the myoblast proliferation stage and heightened during the differentiation stage. Inversely, YAP mRNA and protein levels are highest during proliferation and lowest during differentiation (Judson et al., 2012; Watt et al., 2010). Moreover, YAP S127 phosphorylation dramatically increases upon differentiation, and overexpression of constitutively active mutant YAP (YAP S127A), which is resistant to LATS1/2 phosphorylation, in C2C12 myoblasts downregulates *MyoD* and *Mef2* and impairs myotube formation. Similar results were replicated in satellite cells, where YAP was found to be highly expressed in activated SCs. Overexpression of YAP 127A significantly expands the pool of activated SCs and myoblasts but prevents their differentiation (Judson et al., 2012). These studies indicate that activating hippo kinases is essential to reducing nuclear YAP levels and promoting myogenic differentiation (Watt et al., 2010). Conversely, a later study reported YAP as a positive regulator of myogenesis in C2C12 through activation of the MEK5/ERK5 pathway (Chen et al., 2017). Similar discrepancies in the literature are observed with TAZ. Although TAZ is a well-established enhancer of myoblast proliferation (Mohamed et al., 2016; Sun et al., 2017), it remains unclear if TAZ represses myogenic differentiation similar to its paralog YAP. Several early studies showed TAZ enhanced MyoD-mediated myogenesis (Jeong et al., 2010; Mohamed et al., 2016; Sun et al., 2017). This was followed by more recent work implicating TAZ as a repressor of myogenesis via sequestration of the myogenic machinery and antagonizing β-catenin-driven transcription (Tripathi et al., 2022). Interestingly, mRNA levels of TAZ, but not YAP1, sharply rise in response to cardiotoxin-induced muscle injury, perhaps reflecting a TAZ-only repair mechanism (Sun et al., 2017). In all, though it is well established that YAP/TAZ are potent

enhancers of myoblast proliferation, whether or not YAP/TAZ have distinct roles during myogenesis remains unclear and must be further studied.

The role of YAP/TAZ in post-natal muscle growth is not entirely understood either. Following overexpression/activation of YAP in postnatal mice, one group reported muscle atrophy (Judson et al., 2013), while others reported hypertrophy (Goodman et al., 2015; Watt et al., 2015). Despite these contradictory reports, solid evidence points to YAP and TEADs as promoters of gene expression associated with anabolic muscle growth during the postnatal stage (Watt et al., 2015). It should be noted, however that skeletal muscle-specific deletion of YAP in mice does not alter muscle fiber size compared to controls (Zhao et al., 2017). On the other hand, the role of TAZ in post-natal skeletal muscle fibers remains mostly unexplored. TAZ may potentially mirror the function of YAP in promoting muscle mass, considering pharmacological activation of TAZ prevents dexamethasone-induced muscle atrophy in mice (Yang et al., 2014).

YAP/TAZ in phase separation

The nucleus is organized in part through the formation of a wide array of biomolecular condensates. These membraneless intracellular compartments are dynamically assembled by liquid-liquid phase separation (LLPS). LLPS is a thermodynamic phenomenon that permits the enclosure and concentration of macromolecules inside a restricted space for improved rates of biochemical reactions (Wang et al., 2021). Accumulating evidence suggests that YAP/TAZ play a role in regulating transcription via LLPS condensates (Franklin & Guan, 2020). Though both proteins participate in LLPS, YAP fails to form LLPS condensates under conditions when TAZ readily does. YAP and TAZ both contain IDRs and CC domains, which are known drivers of LLPS formation, yet the differential condensate-forming ability of YAP and TAZ lies in the unique properties of the TAZ CC domain, which unlike in YAP, allows TAZ to engage in homo-

oligomerization within LLPS condensates (Lu et al., 2020). The formation of TAZ LLPS condensates is correlated to the transcriptional activity of TAZ, mediated by TAZ-dependant recruitment of transcription factor TEAD4 along with co-factors CDK9, BRD4, and MED1 to its condensates (Lu et al., 2020). Furthermore, TAZ condensates have been reported in myonuclear speckles cells, where they have been proposed to sequester pro-differentiation machinery to facilitate repression of myogenic differentiation (Tripathi et al., 2022).

Cross-talk between Hippo and Wnt signalling pathways

The Wnt signalling pathway is a prominent and well-studied pathway with widespread roles in development, regeneration, and disease (MacDonald et al., 2009). In the absence of Wnt signalling, β-catenin is degraded in the cytoplasm through a β-catenin destruction complex consisting of APC/Axin/GSK3 and CK1. Wnt ligand activation of the pathway disrupts the destruction complex, causing β-catenin to stabilize and subsequently accumulate in the nucleus. In the nucleus, β-catenin primarily partners with members of the transcription factor T-cell factor/lymphoid enhancer-binding factor 1 (TCF/LEF1) family to activate TCF/LEF gene expression (Clevers, 2006). Moreover, β-catenin is a known regulator of myogenic differentiation by interacting with MyoD and enhancing its binding to E-box elements (Tanaka et al., 2011). A schematic summarizing canonical Wnt signalling is shown in Figure 6.

YAP/TAZ are well-documented players in canonical Wnt signalling and known components of the β-catenin destruction complex. During the Wnt OFF state, YAP/TAZ interacts with Axin to recruit ubiquitin ligase β-TrCP to the destruction complex for subsequent β-catenin degradation (Azzolin et al., 2014). Inversely, β-catenin bridges TAZ, but not YAP, to β-TrCP to promote TAZ degradation (Azzolin et al., 2012). In addition, independent of the destruction complex, TAZ restricts Wnt activation by interacting with and limiting the activation of upstream Disheveled

(DVL) (Barry et al., 2013; Varelas, Miller, et al., 2010). These findings demonstrate a model of reciprocal antagonism whereby YAP/TAZ and β-catenin promote degradation of each other through the same ubiquitin ligase. Wnt pathway activation results in both TAZ and β-catenin stabilization and nuclear translocation. Therefore, the biological response of canonical Wnt signaling is mediated by β-catenin-TCF/LEF and YAP/TAZ-TEAD activity.

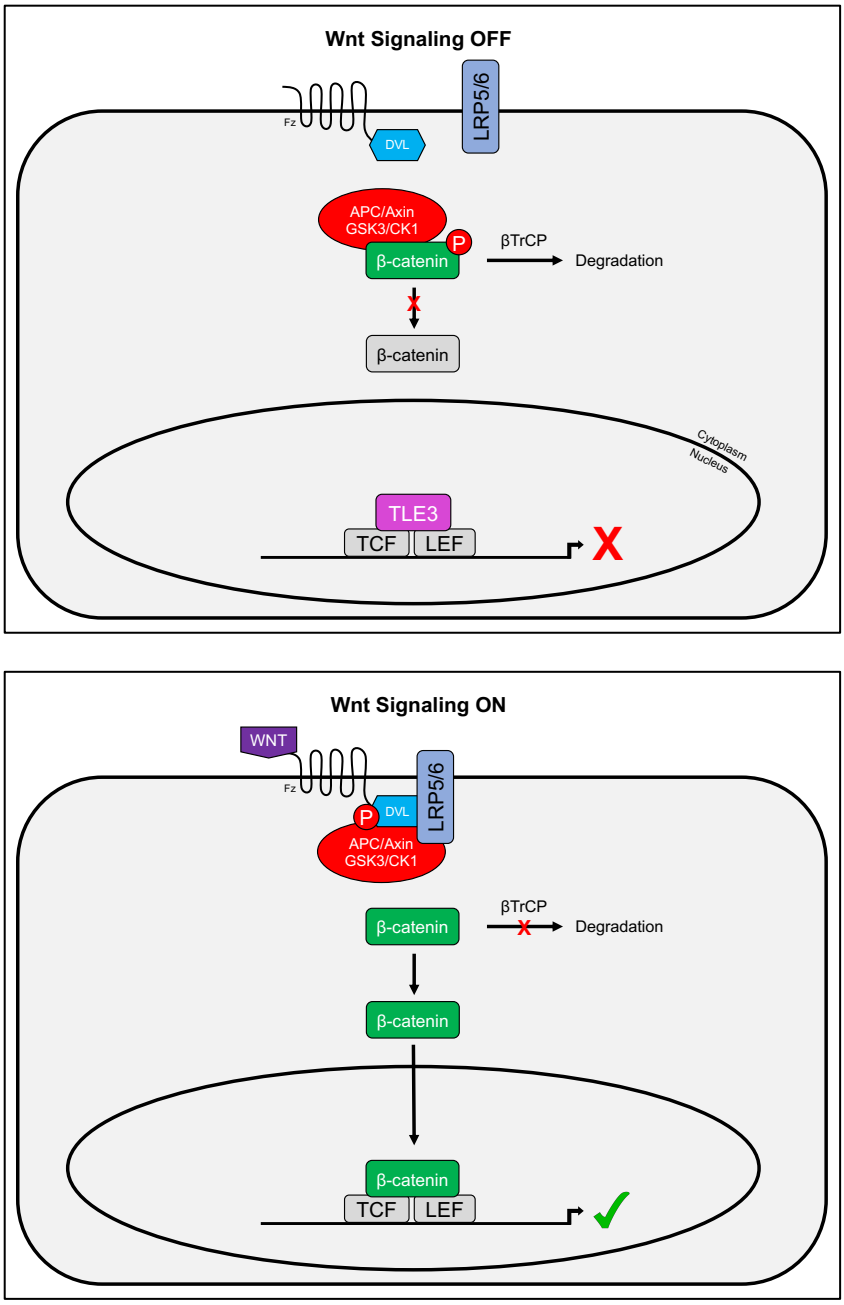

Figure 6. The Wnt signalling pathway. When Hippo signalling is OFF, β-catenin is recruited into a destruction complex comprising of APC, Axin, GSK3, and CK1. Phosphorylation by CK1 primes for GSK3β phosphorylation results in ubiquitination and proteasomal degradation of β-catenin. In the absence of nuclear β-catenin, Wnt co-repressor TLE3 binds to and represses TCF/LEF gene expression. When Hippo signalling is ON, upstream Wnt ligands bind to the Frizzled (Fz) receptor and deactivate the destruction complex, allowing β-catenin to accumulate in the nucleus and activate TCF/LEF gene expression.

YAP/TAZ in Chromatin Remodeling

The process of transcription is tightly regulated by chromatin remodeling, whereby alterations to chromatin structure through histone modifications or nucleosome repositioning modifies gene accessibility to transcription machinery proteins. It is known that YAP/TAZ associates with chromatin remodeling complexes, with one well-studied example being the Switch/sucrose nonfermentable (SWI/SNF) complex (Chang et al., 2018; Skibinski et al., 2014). Mechanistically, the SWI/SNF complex facilitates chromatin access by repositioning nucleosomes, ejecting octamers, or histone eviction (Tang et al., 2010). As mentioned earlier, SWI/SNF complex co-factor ARID1A represses YAP/TAZ-TEAD complex formation in the nucleus dependent on nuclear levels of F-actin (Chang et al., 2018). This finding indicates that in addition to hippo kinase inactivation and subsequent YAP/TAZ nuclear accumulation, activation of YAP/TAZ-TEAD also requires the mechanical inhibition of ARID1A–SWI/SNF in the nucleus. In contrast, one study concluded that SWI/SNF positively cooperates with TAZ to drive TEAD target gene transcription (Skibinski et al., 2014). SWI/SNF ATPase subunit BRM was shown to interact with TAZ, and in addition, ChIP experiments showed an enrichment of BRM at genomic regions containing TEAD binding motifs (Skibinski et al., 2014). YAP/TAZ are also documented to interact with the nucleosome-remodeling and deacetylase (NuRD) complex. The NuRD complex represses transcription by coupling ATP-dependent chromatin remodeling with histone deacetylase activity, resulting in the compaction of nucleosomes and reduced genomic accessibility to target gene loci (Torchy et al., 2015). Although initially regarded to function solely as a transcriptional co-activator, a repressive role of YAP/TAZ was discovered and shown to involve recruitment of the NuRD complex to target gene loci in a TEAD-dependent manner (Kim et al., 2015).

In summary, the YAP/TAZ-TEAD complex is dynamically regulated, according to the mechanical environment and the recruitment of chromatin remodelers, such as the SWI/SNF and NuRD complexes.

Statement of Purpose

The Hippo signaling transcriptional effector TAZ is an important factor in shaping the transcriptome in numerous contexts. When not targeted downstream of the core Hippo kinase cascade, TAZ translocates to the nucleus, where it functions as a transcriptional co-regulator and chromatin remodeler. Deregulation of TAZ, and its paralog YAP, is frequently associated with the progression of various human cancers, tissue fibrosis, chronic inflammation, cardiac hypertrophy, and muscular dystrophy (Chen et al., 2020; Thompson, 2020; Vita et al., 2018; Wang et al., 2016). Previous studies have shown that TAZ enhances the proliferation of myoblasts (Sun et al., 2017). Moreover, we recently documented that TAZ represses myogenesis by antagonizing β-catenin and MRFs in the myogenic transcription complex (Tripathi et al., 2022). TAZ does not bind to DNA and functions as a co-activator or co-repressor depending on the context, leading us to speculate that its differential function during myoblast proliferation and differentiation is highly linked to its protein:protein network. Therefore, ***the purpose of the study was to identify and characterize protein interactions with TAZ in myogenic cells.*** To address this objective, we have undertaken a proteomic-based study directed at identifying the TAZ interactome in skeletal and cardiac muscle cells. Using a novel GFP-Nanotrap based affinity purification approach followed by subsequent LC-MS/MS protein identification, we report a comprehensive list of known and novel TAZ interactome components in myogenic cells.

Chapter II: The TAZ protein interactome in striated muscle

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In preparation for submission to the Journal of Biochemistry

Author contributions

Experimental Design

Jonathan Kelebeev, Tetsuaki Miyake, and John C. McDermott

Drafting manuscript

Jonathan Kelebeev

Editing manuscript

Jonathan Kelebeev, Tetsuaki Miyake, and John C. McDermott

Conducting experiments

Jonathan Kelebeev:	Figure 7A-E
	Figure 8A-D
	Figure 9A-E
	Figure 10A-D
	Figure 11A-G

Abstract

Hippo signalling is a prominent regulator of cell proliferation, differentiation, and death. Previously, we characterized the Hippo transcriptional effector TAZ as a repressor of the myogenic differentiation program. Without DNA-binding ability, TAZ function exclusively depends on its protein:protein interaction network. This prompted us to undertake a proteomic-based study aimed at identifying the TAZ interactome in striated muscle cells. Using a novel GFP-Nanotrap based affinity purification approach coupled with LC-MS/MS protein identification, we document a comprehensive list of known and novel TAZ interactome components in myogenic cells. TAZ interacting proteins include components of Hippo signalling: TEAD1-4, LATS1, 14-3-3 proteins. Epigenetic regulators were also represented: NuRD complex, FACT complex, and SWI/SNF complex. We focused on characterizing the TAZ interaction with the Wnt co-repressor TLE3 in myogenic cells. In myogenic cells, TAZ and TLE3 interact and co-localize within the nucleus. Functionally, TAZ and TLE3 repress β-catenin activation, as indicated by the Wnt-responsive TOP FLASH reporter gene system. Depletion of TAZ reduced the degree of TLE3-mediated repression of β-catenin activation. TAZ and TLE3 repressed MyoD-driven activation of the *myogenin* promoter. Overall, these data demonstrate a role for a TAZ/TLE3 complex in repressing the myogenic differentiation machinery having implications for muscle development and regeneration.

Introduction

The evolutionarily conserved Hippo signalling pathway is a central regulator of organ growth and size by controlling cell proliferation and apoptosis (Ma et al., 2019). At the molecular level, a canonical Hippo signaling pathway has been elucidated in which cell proliferation is restricted by LATS1/2-mediated phosphorylation of downstream transcriptional effectors Yes1-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) at conserved serine residues (Ser127 in YAP; Ser89 in TAZ) (Basu et al., 2003; Zhao et al., 2007). Phosphorylated YAP/TAZ is sequestered in the cytoplasm in complexes with 14-3-3 proteins and directed for proteasome-mediated degradation (Kanai et al., 2000; Tripathi et al., 2022; Zhao et al., 2007). In contrast, when Hippo signaling is reduced or absent, YAP/TAZ remain unphosphorylated at Ser 127/ Ser 89 and translocate to the nucleus, where they regulate gene expression in concert with DNA-binding transcription factors. At present, the most documented nuclear interactions are with members of the TEAD family and some epigenetic regulators (Chan et al., 2009; Chang et al., 2018; Murakami et al., 2005; Zhang et al., 2009).

To date, some studies have implicated YAP/TAZ as positive regulators of myoblast proliferation (Judson et al., 2012; Mohamed et al., 2016). Ectopic expression of mutant hyperactive YAP/TAZ (YAP127A, TAZ89A) proteins that evade LATS1/2-mediated phosphorylation and subsequent degradation enhance the rate of myoblast proliferation (Mohamed et al., 2016). In addition, in response to elevated muscle stiffness following muscle injury, YAP/TAZ localizes to the nuclei of muscle stem cells (MuSCs) and promotes proliferation and migration by activating genes associated with proliferation (Silver et al., 2021). While YAP/TAZ functions are similar under proliferative conditions in myoblasts, the role of YAP/TAZ during terminal differentiation of myogenic cells remains unclear. Sustained expression of YAP127A in C2C12 myoblasts has been

shown to prevent myoblast differentiation into multinucleated myotubes, highlighting the possibility that post translational regulation of YAP Ser127 by phosphorylation may be a critical step in the transition from a pro-proliferative to a post-mitotic state allowing differentiation to proceed (Watt et al., 2010). Congruently, we recently characterized the Hippo downstream effector TAZ as a repressor of the myogenic transcription complex containing SMAD7 and β-catenin at muscle gene promoters (Tripathi et al., 2022). TAZ-mediated repression was documented on the *myogenin* and *myod1* enhancer regions while also potently repressing β-catenin-driven transcription on Wnt-responsive promoters (Tripathi et al., 2022). These studies indicate that when Hippo signaling is inactive under growth conditions, TAZ primarily localizes to the nucleus to enhance the proliferation of myoblasts while concurrently supressing the onset of myogenic differentiation by repressing β-catenin and MRFs in the myogenic transcription complex (Tripathi et al., 2022).

While YAP and TAZ are often grouped together as Hippo effectors, there are notable differences in their properties and biological roles (Reggiani et al., 2021). Moreover, the capability of TAZ to function as either a potent co-activator or co-repressor of transcription depending on the context, prompted us to speculate that that TAZ function is largely determined by its protein:protein interaction network. To address this idea, we have undertaken a proteomic based study aimed at identifying the TAZ interactome in striated muscle cells. Using a novel nanotrap based affinity purification approach coupled with robust LC MS/MS protein identification we document a comprehensive list of both known and novel TAZ interactome components in myogenic cells. Subsequently, we focused on the TAZ interaction with the Wnt co-repressor TLE3, documenting its role in converting TAZ to a co-repressor function in muscle cells. Collectively, we document

the TAZ interactome in myogenic cells and provide evidence of a functional interaction between TAZ and the TLE3 co-repressor.

Materials and methods

Cell line culture

C2C12 myoblasts, HEK293T cells, and H9C2 myoblasts were obtained from the American Type Culture Collection (ATCC). Cell lines were cultured in growth medium (GM) consisting of high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco), 10% fetal bovine serum (FBS) supplemented with 1% penicillin-streptomycin (Invitrogen, ThermoFisher). C2C12 myotube formation was induced by replacing GM with differentiation medium (DM), consisting of DMEM supplemented with 2% FBS and 1% penicillin-streptomycin. Cells were maintained in an incubator at 95% humidity, 5% CO_2, and 37 °C and replenished with fresh medium every 48 hours.

Primary cardiomyocyte isolation

Neonatal rat cardiomyocytes (NRCMs) were prepared from 1 to 3 days old Sprague Dawley rats using the Neonatal Cardiomyocyte Isolation system (Worthington Biochemical Corp, Lakewood, NJ, USA). Briefly, whole hearts (8-16) were dissociated with trypsin (Promega, Madison, WI, USA) and collagenase (Worthington Biochemical Corp). The cells were re-suspended in Dulbecco's Modified Eagle's medium F12 (Gibco, Burlington, ON, Canada) supplemented with 10 % fetal bovine serum, 1% penicillin/streptomycin and 50 mg/l gentamycin sulfate (Invitrogen, Burlington, ON, Canada). The isolated cells were plated for 60 min in 37 °C humidified incubator with a 5% CO_2 in air, allowing differential attachment of non-myocardial cells. Cardiomyocytes were counted using a hemocytometer, then transferred to gelatin-coated plates. The following day, the culture media was removed and replaced with fresh media. The following day, the media was replaced with fresh, serum-free media before subsequent experimentation.

Transfections

For ectopic protein expression in HEK293T, cells were transfected using polyethyleneimine at a PEI:DNA ratio of three. C2C12 and H9C2 myoblasts were transfected with lipofectamine 2000 (Life Technologies) at a lipofectamine:DNA ratio of three. All cells were re-fed 16 hours post-transfection and harvested the next day.

Antibodies

Antibodies for YAP/TAZ (rabbit, polyclonal, #D24E24), HA (rabbit, polyclonal, #C29F4), Myc (mouse, monoclonal, #9B11), alpha-Tubulin (rabbit, polyclonal, #2144S) and Histone H3 (rabbit, polyclonal, #9715S) was purchased from Cell Signaling. MyoG (mouse, monoclonal, #F5D) and MyoD (mouse, monoclonal, #2A5) antibodies were purchased from Developmental Studies Hybridoma Bank (DSHB). MCK (mouse, monoclonal, #sc-365046) and β-Actin (mouse, monoclonal, #sc-47778) antibodies were purchased from Santa Cruz Biotechnology. Antibodies for FLAG (Mouse, monoclonal, #F3165) was from Sigma-Aldrich, TLE3 (Rabbit, polyclonal, #22094-1-AP) was from Proteintech, and GFP (Rat, monoclonal, #3H9) was from ChromoTek.

Plasmids

Myc-TEAD1 and Myc-TEAD4 were gift from Kunliang Guan (Addgene plasmid #33109 & #24638) (Zhao et al., 2008). FLAG-TAZ was a gift from Jeff Wrana (Addgene plasmid #24809) (Varelas et al., 2008). HA-TAZ was a gift from Kunliang Guan (Addgene plasmid #32839) (Lei et al., 2008). FLAG-MTA2 was a gift from Brian Hendrich (Addgene plasmid #140964) (Burgold et al., 2019). pcDNA3 was a gift from William Sellers (Addgene plasmid #10792). HA-CARM1 was a gift from Sung Hee Baek (Addgene plasmid #81118) (Shin et al., 2016). Myc-TLE1 was a gift from Ramesh Shivdasani (Addgene plasmid #11067) (Lepourcelet & Shivdasani, 2002). HOP-

Flash and HIP-Flash (HOP-flash mutant) were gifts from Barry Gumbiner (Addgene plasmid #83467 & #83466) (Kim & Gumbiner, 2015). TOP-Flash and HOP-Flash were gifts from Randall Moon (Addgene plasmid #12456 & # 12457) (Veeman et al., 2003). Myogenin core promoter was a gift from Michael Chin (Addgene plasmid #134722) (Sun et al., 2001). MyoD-pCLBabe was a gift from Stephen Tapscott (Addgene plasmid # 20917) (Yang et al., 2009). Renilla (pRL-Renilla) plasmid was purchased from Promega. TLE3 open reading frame (ORF) was amplified by PCR using cDNA derived from C2C12 cells and digested by BamHI and Xho1 and inserted into pcDNA3 (Invitrogen). EYFP-TAZ and GFP constructs were described previously (Pagiatakis et al., 2017). β-catenin open reading frame was amplified by PCR using cDNA derived from C2C12 cells and inserted at EcoRI and Xho1 sites into pcDNA3 (Invitrogen). Myc epitope was inserted into pcDNA3 at HindIII and EcoRI sites into the β-catenin ORF. GFP ORF was subcloned by PCR into pcDNA3 at HindIII and BamHI sites.

Cell harvesting

C2C12, H9C2, and NRCM cells were harvested using NP-40 lysis buffer (Table 1) or, if destined for gene reporter assay, with 1x reporter lysis buffer (Promega, #E4030). Cells were washed three times with cold PBS before adding in lysis buffer. Lysates were collected and vortexed at 4 °C for 15 minutes. Lysates were then centrifuged at 4 °C, 10 000 RPM for 10 minutes, followed by the collection of the supernatant constituting the soluble protein.

GFP-Trap sample preparation for mass spectrometry

Bait protein (EYFP-TAZ and GFP) constructs were transfected in HEK293T cells and harvested the next day with NP40 lysis buffer. 100 μg of each bait protein were incubated with GFP-Trap magnetic agarose beads (ChromoTek, gtma-100) at 4 ◦C on a rotator for 1h. Beads were washed

twice with 500 μL RIPA buffer (Table) at 4 ◦C for 5 minutes, followed by another wash with 800 μL NP40 lysis buffer at 4 ◦C for 10 minutes. Prey proteins (C2C12 and NRCMs) were harvested as described above. GFP-Trap beads, now with immobilized bait protein, were incubated with 1 mg of prey protein at 4 ◦C on a rotator overnight. Beads were washed three times with 1 mL of NP40 lysis buffer, rotating at 4 ◦C for 5 mins, followed by a final wash with 1X PBS, rotating at 4 ◦C for 5 minutes. Mass spectrometry was performed by SPARC BioCentre (Molecular Analysis), The Hospital for Sick Children, Toronto, Canada.

Western blot analysis

Protein samples were denatured in 3x SDS loading buffer (Table 1) at 100°C for 10 min. Samples were then loaded onto 10% SDS-PAGE gels and ran at 100V for approximately 1 hr. The gel was transferred to a PVDF membrane (Millipore) in 1X transfer buffer (Table 1) and blocked in 5% blocking buffer (Table 1) for 1 hr on an orbital shaker at room temperature. Primary antibodies were prepared by diluting the indicated antibody (Table 2) in 1% blocking buffer (Table 1) (1:1000). Membranes were probed with primary antibody solutions overnight at 4°C on a rocker. After brief washing with TBS supplemented with 0.1% Tween® 20 (TBS-T), blots were incubated with corresponding HRP-conjugated secondary antibody in 1% blocking buffer (1:2000) for 1 hr at room temperature. After three washes with TBS-T, protein/antibody immune-complexes were detected by incubating blots in HRP substrate (Bio-Rad) and exposing them to an iBright CL1500 Imaging System (Thermo Fisher Scientific).

Co-immunoprecipitation (co-IP)

For endogenous co-IP, Dynabeads Protein G (Invitrogen, #10003D) were washed three times with PBS before incubation with YAP/TAZ antibody (Cell Signaling, #D24E4; diluted to 1:2000 with

1X PBS) or Rabbit IgG (Cell Signaling; #2729; diluted to 1:10000 with 1X PBS) on a rotator at 4 °C overnight. Beads were washed three times with PBS to remove unbound antibody. Dynabeads were incubated with 1 mg of C2C12 myoblast extracts on a rotator at 4 °C overnight. Beads were then washed three times with PBS to remove unspecific protein binding. Samples were eluted by heating with SDS loading buffer (Table 1) for 10 mins at 95 °C and analyzed by western blot. For FLAG co-IPs, anti-FLAG M2 magnetic beads (Sigma, #M8823) were washed three times with PBS before incubation with 500 ug of HEK293T cell extracts on a rotator at 4 °C overnight. Beads were then washed three times with PBS to remove unspecific protein binding. Immunoprecipitates were eluted in 500 μg/ml FLAG 3x peptide solution (Sigma, #4799) on a rotator at room temperature for 30 minutes and analyzed by western blot.

Cell fractionation

C2C12 cells were grown in GM until 90% confluency and then in DM for 4 days. Myotubes were isolated by incubating the cells with 1% cold trypsin for 30 seconds and removing the detached myotubes. Remaining reserve cells were removed using a cell scraper. Nuclear and cytoplasmic fractions of the myotube and reserve cells fractions were harvested using the NE-PER kit (Thermo Scientific, #78833), according to manufacture instructions.

Immunofluorescence

C2C12 cells were seeded on glass-bottom dishes (MatTek) and washed with cold PBS three times before fixation with 4% paraformaldehyde at room temperature for 10 minutes. Cells were washed with PBS and permeabilized with ice-cold 90% methanol on ice for 5 minutes. Cells were washed three times with PBS and incubated with IF blocking buffer (5% FBS in PBS) for 1 hour and 30 minutes at room temperature, then incubated with indicated primary antibody in IF blocking buffer

at 4 ◦C overnight. Cells were washed the next day to remove unbound antibody and incubated with Alexa fluor conjugated secondary antibody (Life Technologies) in IF blocking buffer for 1 hour and 30 minutes. Cells were washed three times with PBS and counter-stained with Hoechst 33342 for 10 minutes. Cells were subjected to imaging with a Zeiss Observer Z1 confocal fluorescent microscope equipped with Yokogawa CSU-XI spinning disk. Images were recorded by AxioCam MRm camera (Zeiss) and processed by Zen 2.5 (blue edition) software (Zeiss).

Gene silencing

Depletion of TAZ by small interfering RNA (siRNA) was done using mission siRNA (Sigma-Aldrich). Using lipofectamine 2000, H9C2 cells were transfected with siTAZ #1 (SASI_Rn01_0012_0450/WWTR1), siTAZ #2 (SASI_Rn02_0020_2747/WWTR1), siTAZ #3 (SASI_Rn01_0012_0448/WWTR1), siTAZ #4 (SASI_Rn01_0012_0445/WWTR1), siTAZ #5 (SASI_Rn01_0012_0449/WWTR1), siTAZ #6 (SASI_Rn01_0012_0446/WWTR1) and universal siControl (SIC001) at a final concentration of 50 nM.

Gene reporter assays

Transcriptional reporter assays were performed using luciferase reporter plasmids along with expression constructs (indicated in figure legends) and a Renilla plasmid (Promega, pRL-Renilla), as an internal control. Cells were washed with cold PBS and harvested in Luciferase Lysis Buffer with 1X reporter lysis buffer (Promega, #E4030). Enzymatic activity was measured in each sample on a luminometer (Berthold, Lumat LB) using Luciferase assay substrate (Promega, #E1501) or Renilla assay substrate (Promega, #E2820). Luciferase activity values were normalized to Renilla activity in the same cell extracts and expressed as fold activation to the control.

Results

Affinity purification- LC MS/MS analysis of the TAZ interactome in striated muscle cells

TAZ is a potent co-regulator of gene transcription despite its lack of direct DNA binding activity. Thus, TAZ function and target gene selectivity are determined by protein:protein interactions with sequence specific DNA binding factors and other transcription complex components. Therefore, we reasoned that identifying TAZ interacting partners would further our understanding of the transcriptional mechanisms by which TAZ operates in the nucleus. Based on our previous identification of TAZ as a novel repressor of skeletal muscle gene expression, our initial aim was to characterize the TAZ interactome in skeletal myogenic cells and cardiomyocytes. For this we used a proteomic strategy utilizing the power of NanoTrap technology to trap TAZ interacting proteins from cellular lysates. Initially, we immobilized our 'bait' protein EYFP-TAZ and the corresponding EYFP alone control on beads. These 'baits' were generated in HEK293T cells and captured using GFP-Nanotrap beads conjugated with GFP-binding protein (GBP) nanobodies. This step was followed by incubation with myogenic cell extracts (nuclear C2C12 and whole-cell NRCM). The advantage of this affinity capture method is that, unlike other proteomic interactome workflows, there is no ectopic expression of the bait protein in the cells of interest which can lead to stoichiometry problems thus maintaining the normal unaltered and native state of the myogenic proteomes. The workflow of the incubation affinity purification mass spectrometry (AP- LC MS/MS) approach is illustrated in Figure 7A. Efficient expression and subsequent immunoprecipitation of the GFP-tagged bait proteins for the GBP-Nanotrap is shown in Figure 1B. Datasets were processed by filtering out non-specific interactions with less than 3 fold enrichment (EYFP-$TAZ_{spectra}$/$GFP_{spectra}$), a threshold selected based on known, well characterized TAZ interacting proteins being identified in the screen around this threshold value. Unique

proteins co-immunoprecipitated with EYFP-TAZ meeting the threshold criteria were automatically included in the list of candidates. After processing, the C2C12 and NRCM datasets featured 250 and 220 protein candidates, respectively (Figure 7C), while 97 proteins were identified in both the C2C12 and NRCM datasets (Figure 7D). A summary of selected proteins identified from the C2C12 (blue circle) and NCRM (red circle) interactome lists is shown in Figure 7E, notably featuring well known TAZ interactions with components of the SWI/SNF complex (SMARCC1, SMARCA4) (Chang et al., 2018), membrane-associated proteins (AMOT, SCRIB) (Chan et al., 2011; Cordenonsi et al., 2011), and components of the Hippo pathway (TEAD transcription factors, 14-3-3 proteins, LATS1 kinase) (Kanai et al., 2000; Lei et al., 2008; Zhang et al., 2009). In addition to the SWI/SNF complex, other components belonging to chromatin remodeling complexes with TAZ are represented in the interactomes, such as the FACT (SSRP1) and NuRD (MTA2, CHD4, HDAC1) complexes.

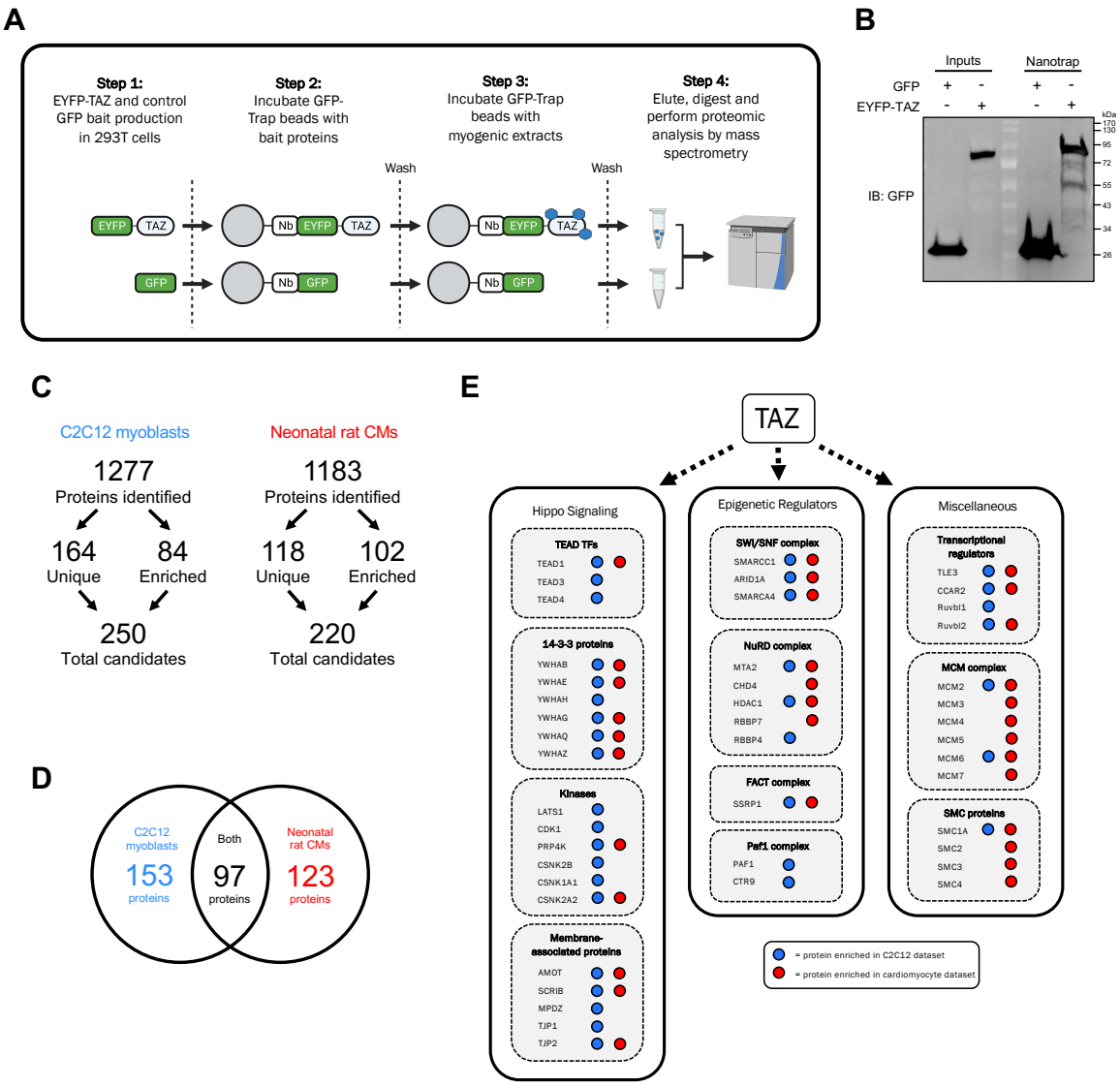

Figure 7. Interactome study of TAZ in striated muscle. **(A)** Schematic overview of GFP-nanoTrap co-immunoprecipitation method to identify TAZ protein partners with nuclear C2C12 and whole-cell neonatal rat cardiomyocyte (NRCM) extracts. **(B)** Western blot analysis confirming immunoprecipitation of GFP bait proteins by GFP-Trap co-IP in HEK293T. (C) Flow chart indicating the number of total and enriched TAZ protein candidates identified in the C2C12 myoblast and neonatal rat CM datasets. (D) Venn diagram indicating the number of unique and overlapping TAZ proteins candidates between the C2C12 myoblast and neonatal rat CM datasets. **(E)** Summary of notable TAZ protein partners identified in the C2C12 and NRCM extracts. Blue circles indicate the protein was enriched in the C2C12 dataset, while red circles indicate enrichment in the NRCM dataset.

Bioinformatic analysis of the TAZ interactomes in myogenic cells and cardiomyocytes

Bioinformatic analysis of the C2C12 (blue) and NRCM (red) datasets was performed to identify signaling pathways relevant to the set of proteins that were found to interact with TAZ. Ingenuity pathway analysis revealed Hippo signaling as a top 10 associated pathway (Figure 8A) in both datasets, highlighting the fidelity of the approach to capture proteins found along the canonical signaling pathway of TAZ. Top 40 Ingenuity canonical pathways, along with their respective proteins, are listed in the Supplemental Tables 1-2. Notable inclusions are 14-3-3-mediated signaling (#11 in C2C12, #30 in NRCM), Wnt/β-catenin signaling (#36 in C2C12), tight junction signaling (#31 in C2C12) and DNA methylation and transcriptional repression signalling (#26 in NRCM). Additional pathway analysis was carried out using KEGG pathway and Reactome pathway analysis to further gather insight for downstream experimental direction. The Hippo signaling pathway was again identified by KEGG pathway analysis (#3 in C2C12, #10 in NRCM) (Figure 8B) and the Wnt signaling pathway was identified by Reactome pathway analysis featuring Signaling by WNT (#8 in C2C12) and TCF-dependent signaling in response to WNT (#13 in C2C12) (Figure 8C). Top 20 pathways KEGG and Reactome pathways enriched in the C2C12 and NRCM datasets along with their respective associated proteins are listed in the Supplemental Tables 3-6. A schematic highlighting previously documented TAZ interacting proteins placed in the context of Hippo signaling, as well as some novel interactions identified in our interactome, are highlighted in Figure 8D.

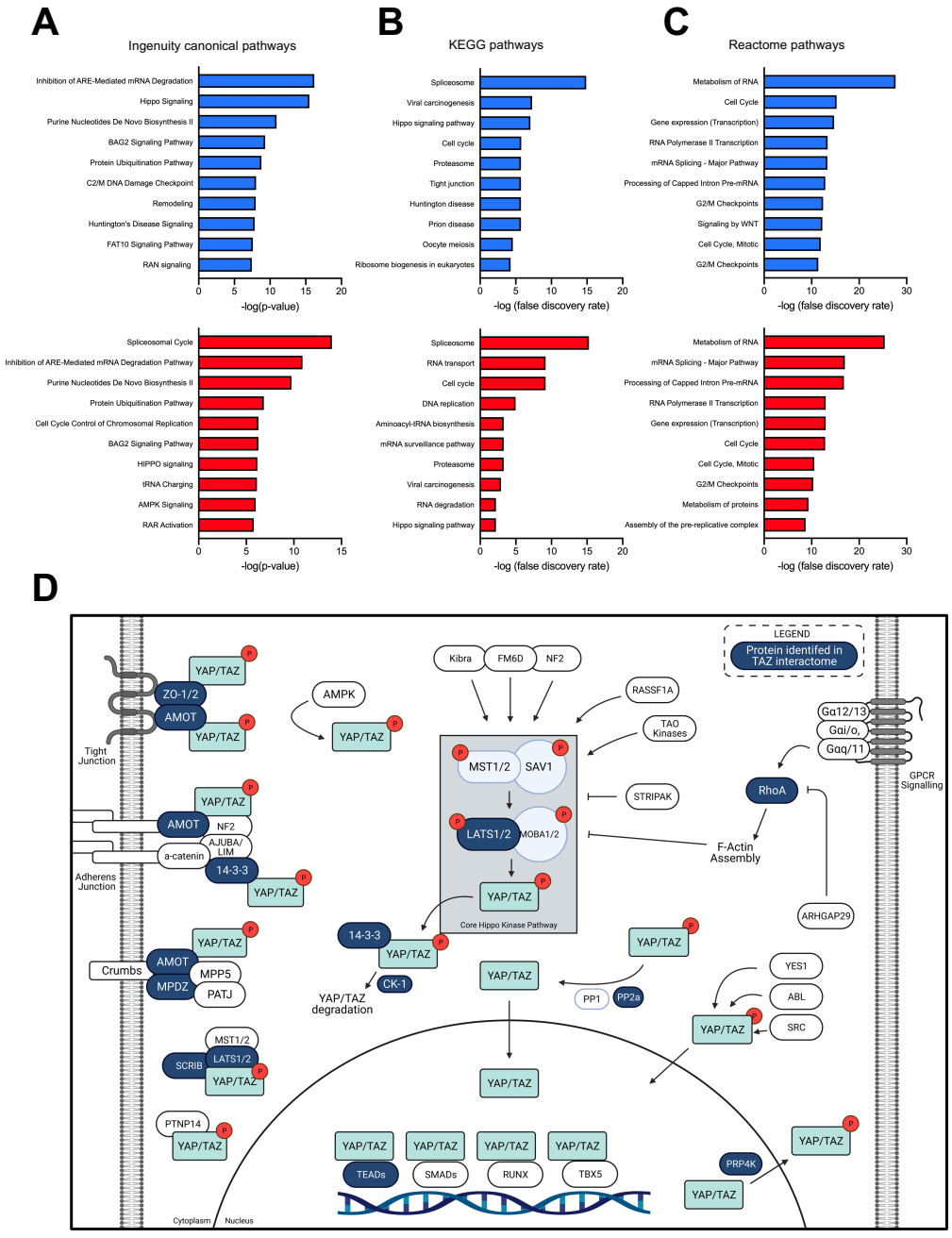

Figure 8. Bioinformatic analysis of the myogenic TAZ interactome. (A) Top 10 canonical pathway analysis by QIAGEN Ingenuity Pathway Analysis software associated with the C2C12 (blue) and NRCM (red) TAZ interactomes. (B) Top 10 KEGG pathways associated with the C2C12 (blue) and NRCM (red) TAZ interactomes. (C) Top 10 Reactome pathways associated with the C2C12 (blue) and NRCM (red) TAZ interactomes. (D) Schematic showing proteins (colored in navy) representing known components of Hippo signaling pathway that were identified in the C2C12 TAZ interactome.

TAZ interacts with TLE3 in the nuclei of proliferating myoblasts

To further validate novel protein-protein interactions identified in the interactomes biochemically, we performed FLAG co-immunoprecipitation in HEK293T. Positive controls were established using the known interaction between TAZ and transcription factors TEAD1 and TEAD4 (Figure 9A). Among the list of proteins in the interactome, we were particularly interested in the interaction between TAZ and the co-repressor TLE3 due to our prevcious report of TAZ fulfilling a repressive role in a myogenic context (Tripathi et al., 2022). TLE3 has been previously shown to repress both canonical Wnt signalling, through binding to TCF/LEF transcription factors (Villanueva et al., 2011), and skeletal myogenesis, by interacting and inhibiting MyoD-driven transcription (Kokabu et al., 2017). Moreover, TAZ has a well-documented role in canonical Wnt signalling due to antagonistic interactions with β-catenin (Azzolin et al., 2012). We first confirmed that ectopic TAZ interacts with ectopic TLE3 (Figure 9B) in HEK293T cells by FLAG co-IP analysis. An interaction between TAZ and the NuRD complex component MTA2 was also validated although not pursued further (Figure 9C), indicating the veracity of the approach to identify interactions featuring low-abundance nuclear proteins. Next, we further confirmed the interaction between endogenous TAZ and TLE3 in C2C12 myoblasts by co-IP analysis (Figure 3D). Subsequent immunofluorescence analysis to document cellular localization indicated that TAZ and TLE3 co-localize in the nuclei of C2C12 myoblasts (Figure 9E).

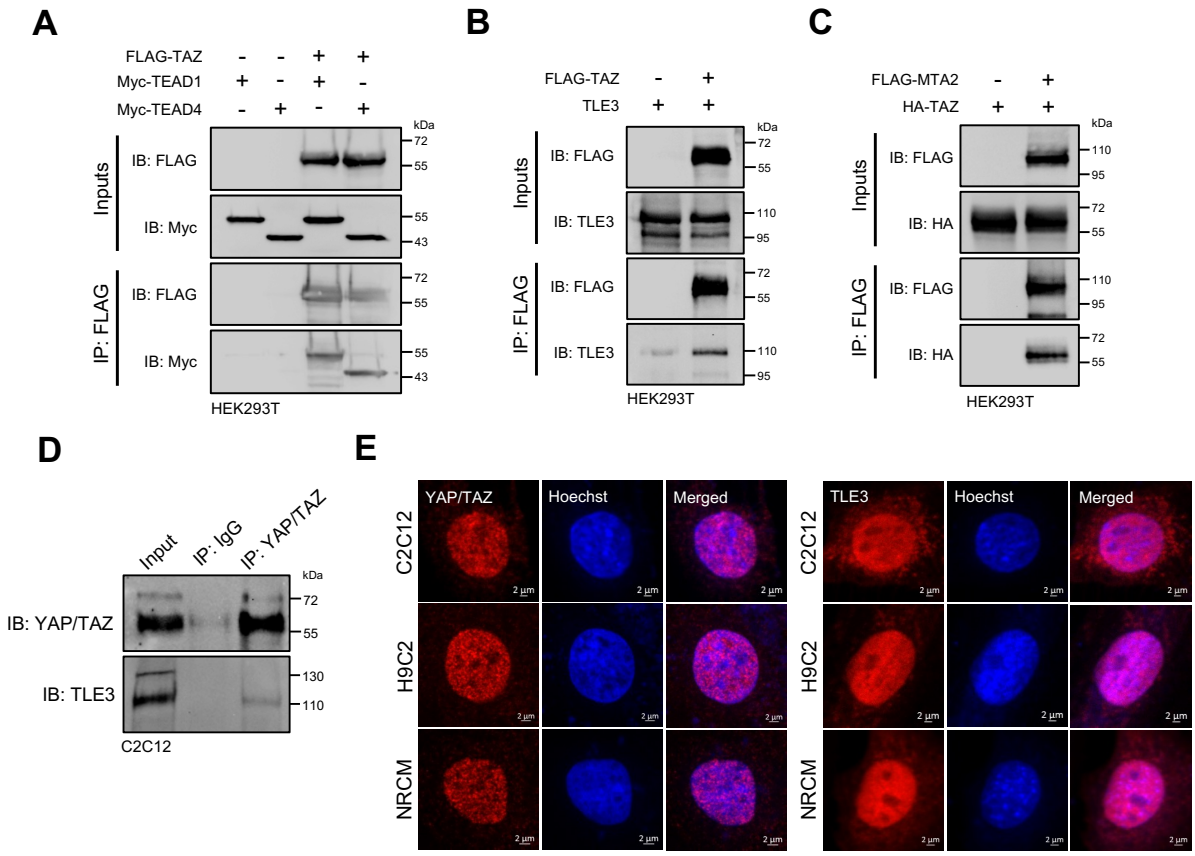

Figure 9. TAZ interacts with TLE3 in the nuclei of proliferating myoblasts. (A) HEK293T cells were transfected with Myc-TEAD1 or Myc-TEAD4 with/without FLAG-TAZ and subject to FLAG immunoprecipitation with anti-FLAG beads. Eluates were analyzed using immunoblotting technique for contents of co-immunoprecipitated with Myc-TEAD1 and Myc-TEAD4 to serve as positive controls for the co-immunoprecipitation analysis. **(B)** HEK293T cells were transfected with TLE3 with/without FLAG-TAZ, and subject to FLAG immunoprecipitation with anti-FLAG beads. Eluates were analyzed using immunoblotting for contents of co-immunoprecipitated with TLE3. **(C)** HEK293T cells were transfected with HA-TAZ with/without FLAG-MTA2, and subject to FLAG immunoprecipitation with anti-FLAG beads. Eluates were analyzed using immunoblotting for contents of co-immunoprecipitated with HA-TAZ. **(D)** C2C12 myoblasts extracts were subject to YAP/TAZ immunoprecipitation. Eluate contents were analyzed using immunoblotting for contents of co-immunoprecipitated with endogenous TLE3. **(E)** Immunofluorescence analysis of fixed C2C12 myoblast, H9C2, and NRCM cells individually immunostained for YAP/TAZ (red) and TLE3 (red) and counterstained with Hoechst 33342 to indicate nuclei (blue).

TLE3 and TAZ are expressed in the nuclei of C2C12 myoblast and myotubes

To further characterize the localization and expression of TAZ and TLE3 during myogenesis, C2C12 cells were fixed in growth media (GM) and differentiation media (DM) (24 and 48 hours) and assessed for TAZ (Figure 10A) and TLE3 (Figure 10B) sub-cellular localization by immunofluorescence analysis. Both proteins mainly exhibited localization in the nucleus during proliferative and differentiating conditions. Upon induction of MyoG gene expression, a marker for the onset of differentiation, TLE3 and TAZ localization was retained in the nucleus at DM 24h. A decline in TAZ and TLE3 protein levels was detected in differentiating C2C12 cells, as verified quantitatively by normalizing TAZ and TLE3 band intensity to Actin (Figure 10C). Collectively, these data indicate that TAZ and TLE3 interact in the nuclei under growth conditions. TLE3 nuclear localization was confirmed by fractionating the myotube and reserve cells subsequently followed by nuclear and cytoplasmic fractionation (Figure 10D).

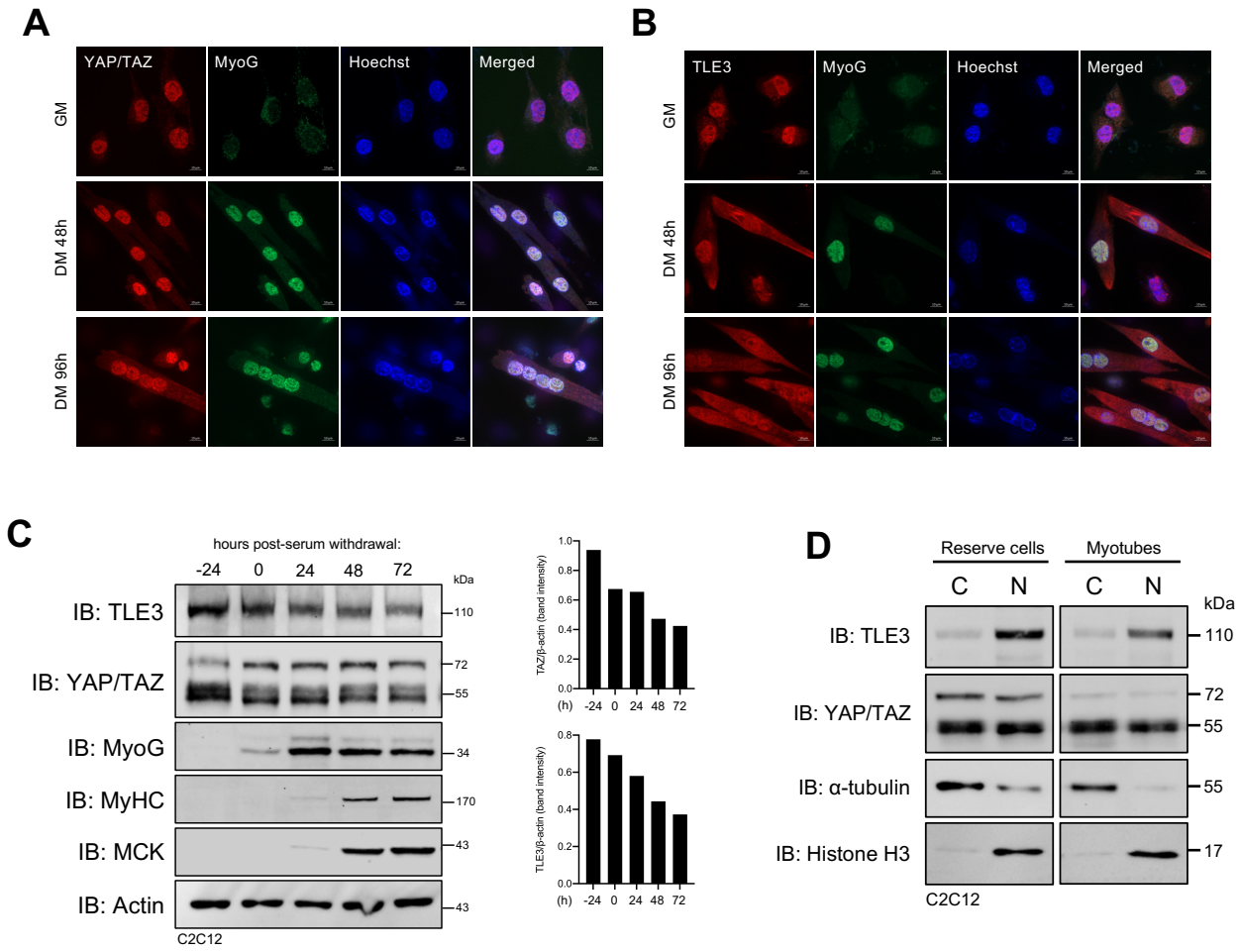

Figure 10. Localization and expression of TAZ and TLE3 in myogenic cells.
Immunofluorescence analysis of endogenous YAP/TAZ (red) (A) and TLE3 (red) (B) in C2C12 cells during myoblast (GM), early (DM 48 h), and late myotube (DM 96 h) stages. Cells were immunostained for MyoG (green) to label cells entering myogenic differentiation and counterstained with Hoechst 33342 to indicate nuclei (blue). (C) C2C12 myoblasts were cultured in GM before transfer into DM. Lysates were collected once prior to media transfer and daily at four timepoints. Protein levels of TLE3, YAP/TAZ, myogenic markers (MyoG, MyHC, MCK) and β-actin were assessed immunoblot analysis. (D) Myotube-reserve cell fractionation of C2C12 cells cultured for 96 h in DM followed by nucleus/cytoplasm subcellular fractionation. Protein levels of TLE3 and YAP/TAZ in the cytoplasmic (C) and nuclear (N) fractions were assessed by immunoblot analysis. Alpha-tubulin and Histone H3 were used as cytoplasmic and nuclear markers, respectively.

Depletion of TAZ reduces TLE3-mediated repression

To assess the functionality of the TAZ-TLE3 interaction, we employed luciferase reporter gene assays aimed at uncovering the potential co-operativity between TAZ and TLE3 as transcriptional regulators. We initially tested whether TLE3, along with another Gro/TLE family member TLE1 and other novel interactors identified in this study, influences the *trans*activation potential of TAZ on a TEAD-responsive HOP reporter gene assay, using HIP as a corresponding negative control. We observed that TLE3 and TLE1 significantly repressed TAZ-driven activation of HOP/HIP in HEK293T cells, while protein arginine methyltransferase CARM1 potentiated TAZ activation (Figure 11A). The NuRD complex component MTA2 did not considerably alter reporter gene activity (Figure 11A). Given that both TAZ and TLE3 are well-established negative regulators of TCF/LEF transcription (Tripathi et al., 2022; Villanueva et al., 2011), we investigated the functionality of the TAZ-TLE3 interaction on the TCF/LEF binding site-containing TOP flash reporter gene system, which serves as a measure of β-catenin mediated transcriptional activation. The TOP flash reporter assay utilizes FOP flash, which contains mutated TCF/LEF binding sites, as a negative control. In agreement with previous observations from our group and others, we confirmed that ectopic TAZ and TLE3 potently repressed β-catenin-driven activation of TOP/FOP flash activity in HEK293T cells (Figure 11B). Combined expression of ectopic TAZ and TLE3 did not further repress TOP/FOP activity indicating saturated repression by TLE3 alone (Figure 11B). We then investigated the functional aspects of the TAZ:TLE3 interaction in the context of myogenesis using the well-characterized *myogenin* promoter driven luciferase reporter gene assay. Similar to the TOP/FOP assay outcome, TAZ and TLE3 individually repressed MyoD-driven *myogenin* promoter reporter gene activation in HEK293T and, when combined, did not further repress the reporter gene activity (Figure 11C). We also carried out a parallel analysis in the H9C2

cell line, which is derived from embryonic BD1X rat heart tissue and displays both a cardiomyocyte and skeletal muscle like phenotype (Watkins et al., 2011). To assess if TAZ is required for TLE3-mediated repression, we utilized siRNAs to deplete the expression of endogenous TAZ and then performed TOP/FOP and *myogenin* promoter reporter assays. Specific depletion of endogenous TAZ in H9C2 cells is shown in Figure 11D. Depletion of TAZ in the presence of β-catenin did not alter the activity of TOP/FOP flash compared to β-catenin alone; however, the level of TLE3 mediated repression on TOP/FOP flash was reduced in TAZ-depleted cells compared to control cells (Figure 11D). When assessed on the *myogenin* promoter reporter gene assay, TAZ depletion enhanced MyoD-driven reporter gene activation and this was unaffected by TLE3 manipulation suggesting a different mechanism of repression on this promoter (Figure 11E). All corresponding immunoblots for Figures 11A-E are displayed in Supplemental Figure 1 showing ectopic expression levels of indicated proteins. Depleted TAZ protein levels by siTAZ in H9C2 cells are displayed in Figure 11F. Collectively, these data indicate the role of TAZ in promoting TLE3-mediated repression of β-catenin driven TCF/LEF transcription in myogenic cells, as illustrated in our working model displayed in Figure 11G.

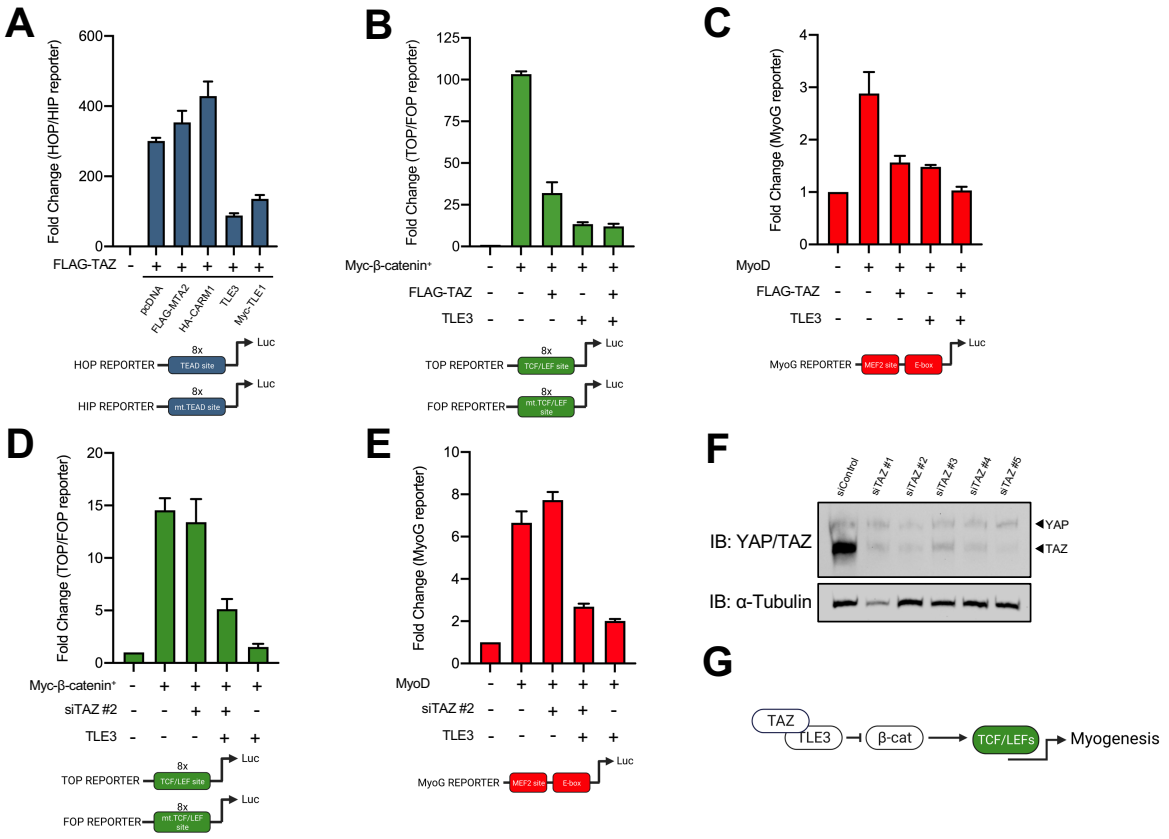

Figure 11. Depletion of TAZ reduces the repressive capacity of TLE3. FLAG-TAZ alone and combined with either one of FLAG-MTA2, HA-CARM1, TLE3, and Myc-TLE1 were ectopically expressed with the HOP reporter gene (normalized to HIP reporter as a negative control). (B) Myc-β-catenin alone and in varying combinations with FLAG-TAZ and TLE3 were ectopically expressed with a TOP flash reporter gene (normalized to FOP flash reporter as a negative control). (C) MyoD alone and in varying combinations with FLAG-TAZ and TLE3 were ectopically expressed with a *myogenin* reporter gene. *Renilla* luciferase served as a transfection control. Transfection with an empty vector (pcDNA) served as a control for endogenous activity. Cells were harvested 24 hours post-transfection. Normalized luciferase activity was compared to the pcDNA control to determine fold changes. (D) immunoblot analysis of H9C2 cells transfected with siControl and TAZ-directed siRNAs. (E) Myc-β-catenin alone and in varying combinations with siTAZ and TLE3 were ectopically expressed with a TOP flash reporter gene (normalized to mutant FOP flash reporter as a negative control). (F) MyoD alone and in varying combinations with siTAZ and TLE3 were ectopically expressed with *myogenin* promoter reporter gene. *Renilla* luciferase served as a transfection control. Transfection with an empty vector (pcDNA) served as a control for endogenous activity.

Transfection with siControl served to control for potential off-target effects of siTAZ. Cells were harvested 24 hours post-transfection. Normalized luciferase activity was compared to the pcDNA control to determine fold changes. Each bar represents the mean of three technical replicates. The error bars represent standard error of the mean (SEM). (G) Working model for TAZ-TLE3 repression of myogenesis.

Discussion

The Hippo signaling pathway has garnered considerable interest of late as a critical regulator of organ size control and potential modulator of stem cell regenerative potential (Heng et al., 2020). The canonical Hippo pathway culminates with the downstream regulation of transcriptional co-regulators YAP/TAZ, which serve to regulate Hippo dependent gene expression and also integrate the Hippo pathway with Wnt, TGF-β and Notch signalling pathways (Attisano & Wrana, 2013; Kim & Jho, 2014; Totaro et al., 2018). Without the ability to directly bind to DNA, TAZ co-operates extensively with sequence specific DNA-binding proteins to exert control over gene regulation. Thus, TAZ function is quite dependent on its cohort of interacting proteins. Using a nanobody-mediated two-step affinity purification approach coupled with tandem mass spectrometry, we report interactome datasets for TAZ in striated muscle cells. Among the identified interactome components in muscle cells, we further characterized a TAZ interaction with the co-repressor molecule TLE3, owing to its involvement in both skeletal myogenesis and Wnt signalling. Functionally, we document a role for TAZ in promoting TLE3-mediated repression of β-catenin driven-TCF/LEF transcription, unveiling a mechanism in which TAZ can mediate transcriptional repression as well as activation.

Comparing our interactome findings with previous TAZ interactome studies from different cell types reveals a core interactome highlighted by TEAD transcription factors, LATS kinases, PDZ proteins, 14-3-3 chaperone proteins and SWI/SNF complex components (Kohli et al., 2014; Li et al., 2020; Wang et al., 2014). Several candidates identified in our study merit future characterization in a myogenic context are the components of the chromatin remodeling NuRD complex. The NuRD complex decreases chromatin accessibility and transcriptional activity by coupling ATP-dependent chromatin remodeling with histone deacetylase (HDAC)-mediated

histone deacetylase activity (Xue et al., 1998). The repressor function of YAP/TAZ has, in some contexts, been shown to involve the recruitment of the NuRD complex through interactions between YAP/TAZ and MTA subunits (Kim et al., 2015). Interestingly, a NuRD-TLE-TBX20 repressor complex has also been discovered to function during embryonic heart development (Kaltenbrun et al., 2013). This possibility is supported in our studies since the NuRD subunits and TLE3 were identified in our TAZ interactome profile.

Bioinformatic analysis of the interactome datasets revealed strong associations with Wnt signalling and TCF/LEF-dependent transcription. Accordingly, we chose to pursue the Wnt co-repressor TLE3 as the focal candidate in this study. An interaction between TAZ and TLE3 was previously identified from a BioID proximity-ligation screen in HEK293T cells (Li et al., 2020). The interaction was shown to require the *trans*activation domain of TAZ and the C-terminal WD40 domain of TLE3. Mechanistically, the data indicate that TAZ reduced the proteasomal degradation of TLE3, thus stabilizing TLE3 and enhancing its repression on TCF/LEF sites. β-catenin is a known positive regulator of myogenic differentiation and has been shown to interact with MyoD and enhance its binding to E-box cis elements on myogenic promoter/enhancer regions (Kim et al., 2008; Tanaka et al., 2011). During post-natal myogenesis, the switch from the proliferative phase to the differentiation phase is accompanied by a switch from Notch to Wnt signalling (Brack et al., 2008). Moreover, an intact β-catenin-TCF complex was found to be essential for efficient human myogenic cell fusion and differentiation (Agley et al., 2017). Taking these findings into consideration along with the data presented here, we propose a mechanism in which TAZ represses myogenesis through the enhancement of TLE3-mediated repression of β-catenin.

In this study we identified TAZ interactors in skeletal muscle myoblasts and primary cardiomyocytes. While we focused most of our attention on the downstream role of TAZ

interacting proteins in myogenesis, the role of interactome components in cardiomyocytes may also prove to be important for understanding cardiac gene regulation. The Hippo pathway plays a pivotal role in the development, disease, and regeneration of the heart (Chen et al., 2020; Heallen et al., 2011). YAP is a well-known stimulator of cardiomyocyte proliferation, presenting an intriguing experimental approach to overcoming the limited potential for heart regeneration and repair following injury (Monroe et al., 2019; Xin et al., 2013). Cardiac-specific YAP deletion impedes embryonic heart development while, conversely, mice with cardiac-specific TAZ deletion are viable, suggesting that the roles of YAP and TAZ in heart may differ (Xin et al., 2013). A conditional deletion of TAZ in zebrafish results in reduced cardiomyocyte proliferation and cardiac trabeculation (Lai et al., 2018). The only TEAD member appearing in the cardiomyocyte TAZ interactome was TEAD1, the most abundant TEAD family member with nonredundant roles in cardiac development (Chen et al., 1994; Liu et al., 2017). TEAD1 has been shown to enhance the reprogramming efficiency of cardiomyocytes from cardiac fibroblasts (Singh et al., 2021). Furthermore, TAZ and TEAD1 co-migrate to the nucleus in cardiomyocytes derived from hearts with desmin mutation (Hou et al., 2017). These observations indicate potential association between TAZ and TEAD1 in the heart. Other interesting inclusions in the cardiomyocyte interactome are the cohesin (SMC1A, SMC3) and condensin (SMC2, SMC4) subunits, along with minichromosomal maintenance (MCM) proteins MCM2-7. MCM proteins were shown recently to extrude cohesin-mediated DNA loops (Dequeker et al., 2022), suggesting TAZ might also be involved in regulating DNA looping in the heart.

Overall, this study points to TAZ as a modulator of the myogenic transcription complex. Our data indicate that TAZ dependent recruitment of TLE3 under growth conditions might maintain the cells in a proliferative, undifferentiated state by repressing the myogenic differentiation machinery.

These observations may have implications for our understanding of the switch between proliferation and differentiation since this is a crucial determinant of skeletal muscle ontogeny and also post-natal muscle regeneration.

Future directions and implications

This study has identified numerous novel and known TAZ interactome components in striated muscle. Our interactome screens in skeletal and cardiac muscle revealed TAZ interactions with multiple chromatin remodeling complex subunits and many proteins native to canonical Hippo signaling. Among the proteins identified in the datasets, we focused on characterizing a TAZ:TLE3 interaction which led us to reveal their roles in the repression of skeletal myogenesis. While these findings are important for advancing our understanding of the precise role of TAZ during myogenesis, future work must focus on further characterizing the in-vivo consequences of the interaction.

Studying mice harboring an inducible TAZ-knockout gene driven by the expression of HSA and Pax7, to be conditionally knocked out in myofiber and satellite cells, respectively, could be of great utility in examining the impact TAZ deletion has on TCF/LEF transcription and regeneration. Further, the interaction can be investigated as part of a greater co-repressor complex that features the NuRD complex. It has been reported that the NuRD complex assembles with YAP/TAZ-TEAD to form a co-repressor complex (Kim et al., 2015) while it has been independently reported that the NuRD complex forms interactions with TLE co-repressors (Kaltenbrun et al., 2013). Given these findings, TAZ could play a role in recruiting co-repressor complexes and altering the chromatin landscape to prevent inappropriate differentiation-associated gene activation during myoblast proliferation.

Another TAZ interaction identified in this study we believe merit future characterization in a myogenic context is with CARM1. CARM1 (coactivtor-associated arginine methyltransferase 1) catalyzes the methylation of arginine residues of histone and non-histone proteins and has recently

emerged as an important regulator of skeletal muscle biology (Stouth et al., 2020; Suresh et al., 2021). CARM1 methylates multiple arginine residues of Pax7 resulting in the epigenetic induction of Myf5 during satellite stem cell asymmetric division (Kawabe et al., 2012). TAZ hypermethylation has been previously reported in patient cases of multiple myeloma (MM), although the upstream mechanism remains unknown (Grieve et al., 2019). It would be worthy to examine if TAZ is targeted for methylation by CARM1 and its potential consequences for myogenesis.

Ultimately our interactome datasets strongly indicate that TAZ engages with chromatin remodeling factors to alter the chromatin landscape. The Hippo community would benefit significantly from a TEAD-based reporter gene that is integrated into the genome. Integrated reporter genes are a powerful tool to reveal how remodeling factors operate within a local chromatin context (Akhtar et al., 2013). With the proper methods available, TAZ interactions with chromatin remodelers identified in this study can be properly studied and placed in the context of myogenesis.

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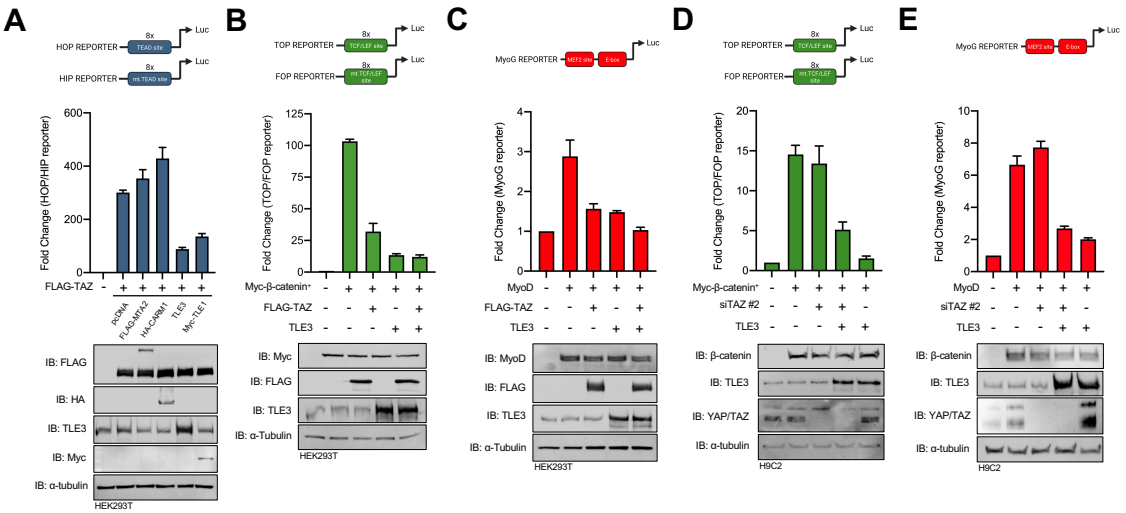
Supplementary data



Supplementary Figure 1. Western blot analysis of cellular extracts corresponding to the luciferase reporter assays presented in Figure 11.

Supplemental Table 1. Top 40 Ingenuity canonical pathways enriched in the C2C12 TAZ interactome dataset.

#	Ingenuity Canonical Pathway – C2C12	-log(p-value)	Proteins
1	Inhibition of ARE-Mediated mRNA Degradation Pathway	16.2	Cnot9, Exosc10, Exosc4, Ppp2ca, Ppp2r1a, Ppp2r2a, Psmc1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd11, Psmd2, Psmd3, Xrn1, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
2	HIPPO signaling	15.5	Amot, Lats1, Llgl1, Ppp2ca, Ppp2r1a, Ppp2r2a, Scrib, Stk4, Tead1, Tead3, Tjp2, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
3	Purine Nucleotides De Novo Biosynthesis II	10.9	Adsl, Adss2, Gart, Gmps, Impdh2, Paics, Pfas
4	BAG2 Signaling Pathway	9.31	Hsp90aa1, Hspa1b, Hspa1l, Psmc1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd11, Psmd2, Psmd3
5	Protein Ubiquitination Pathway	8.79	Dnajb1, Eloc, Hsp90aa1, Hspa1l, Hspa4l, Hsph1, Psmc1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd11, Psmd2, Psmd3, Ube2m, Ube2o, Usp7, Usp9x
6	Cell Cycle: G2/M DNA Damage Checkpoint Regulation	8.04	Cdk1, Ep300, Prkdc, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
7	Remodeling of Epithelial Adherens Junctions	7.98	Actr2, Arpc2, Dnm2, Nme1, Rab5a, Rab5c, Tuba1c, Tubb2b, Tubb6, Tubg1
8	Huntington's Disease Signaling	7.84	Clta, Dnajb1, Dnm2, Ep300, Hdac1, Hspa1b, Hspa1l, Polr2b, Polr2l, Psmc1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd11, Psmd2, Psmd3
9	FAT10 Signaling Pathway	7.59	Psmc1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd11, Psmd2, Psmd3
10	RAN Signaling	7.44	Cse1l, Kpna1, Kpnb1, Ran, Ranbp1, Xpo1
11	14-3-3-mediated Signaling	7.23	Pdcd6ip, Rap1b, Tuba1c, Tubb2b, Tubb6, Tubg1, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
12	Polyamine Regulation in Colon Cancer	7.19	Psmc1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd11, Psmd2, Psmd3
13	Spliceosomal Cycle	6.86	Ddx39b, Dhx38, Eftud2, Sf3b1, Sf3b3, Sf3b4, Snrnp200, U2af2
14	Hereditary Breast Cancer Signaling	6.7	Arid1a, Cdk1, Ep300, Hdac1, Mre11, Polr2b, Polr2l, Rad50, Rap1b, Smarca4, Smarcc1, Tubg1
15	DNA Double-Strand Break Repair by Non-Homologous End Joining	6.3	Lig3, Mre11, Prkdc, Rad50, Xrcc1
16	p70S6K Signaling	6.13	Eef2, Ppp2ca, Ppp2r1a, Ppp2r2a, Rap1b, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz

17	ATM Signaling	5.5	Cdk1, Mre11, Ppp2ca, Ppp2r1a, Ppp2r2a, Rad50, Smc1a, Smc3, Usp7
18	Clathrin-mediated Endocytosis Signaling	5.28	Actr2, Ap1m1, Ap3m1, Arpc2, Clta, Csnk2b, Dnm2, Rab5a, Rab5c, Rac1, Tfrc, Usp9x
19	PI3K/AKT Signaling	5.15	Elp1, Hsp90aa1, Ppp2ca, Ppp2r1a, Ppp2r2a, Rap1b, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
20	BER pathway	4.99	Lig3, Pnkp, Polb, Xrcc1
21	AMPK Signaling	4.98	Arid1a, Eef2, Ep300, Fasn, Pfkl, Pfkm, Ppp2ca, Ppp2r1a, Ppp2r2a, Rab1a, Rab8a, Smarca4, Smarcc1
22	Glycolysis I	4.84	Aldoa, Eno1, Pfkl, Pfkm, Pkm
23	Mitotic Roles of Polo-Like Kinase	4.78	Cdk1, Hsp90aa1, Ppp2ca, Ppp2r1a, Ppp2r2a, Smc1a, Smc3
24	Coronavirus Replication Pathway	4.75	Arcn1, Copb1, Copz1, Tuba1c, Tubb2b, Tubb6
25	ERK5 Signaling	4.53	Rap1b, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
26	NER (Nucleotide Excision Repair, Enhanced Pathway)	4.39	Cops2, Ep300, Lig3, Polr2B, Polr2l, RpaA3, Usp7, Xrcc1
27	IGF-1 Signaling	4.36	Csnk2b, Rap1b, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
28	Telomerase Signaling	4.27	Dkc1, Hdac1, Hsp90aa1, Ppp2ca, Ppp2r1a, Ppp2r2a, Ptges3, Rap1b
29	HIF1α Signaling	4.24	Eloc, Ep300, Hsp90aa1, Hspa1b, Hspa1l, Ldha, Ldhb, Pkm, Rac1, Ran, Rap1b
30	Role of CHK Proteins in Cell Cycle Checkpoint Control	4.15	Cdk1, Mre11, Ppp2ca, Ppp2r1a, Ppp2r2a, Rad50
31	Tight Junction Signaling	4.12	Cpsf3, Llgl1, Mpdz, Ppp2ca, Ppp2r1a, Ppp2r2a, Rac1, Rhoa, Tjp1, Tjp2
32	Protein Kinase A Signaling	3.82	ACP1, Calm1, Ptpn23, Pygl, Pygm, Rap1b, Rhoa, Timm50, Ttn, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaq, Ywhaz
33	Epithelial Adherens Junction Signaling	3.81	Actr2, Arpc2, Rac1, Rap1b, Rhoa, Tuba1c, Tubb2b, Tubb6, Tubg1
34	Phagosome Maturation	3.79	Dync1h1, Prdx6, Rab5a, Rab5c, Rac1, Tuba1c, Tubb2b, Tubb6, Tubg1
35	Virus Entry via Endocytic Pathways	3.52	Ap1m1, Ap3m1, Clta, Dnm2, Rac1, Rap1b, Tfrc
36	Wnt/β-catenin Signaling	3.52	Csnk1a1, Csnk2b, Ep300, Hdac1, Ppp2ca, Ppp2r1a, Ppp2r2a, Ruvbl2, Tle3
37	Caveolar-mediated Endocytosis Signaling	3.49	Arcn1, Copb1, Copz1, Dnm2, Rab5a, Rab5c
38	ERK/MAPK Signaling	3.47	Ppp2ca, Ppp2r1a, Ppp2r2a, Rac1, Rap1b, Ywhab, Ywhag, Ywhah, Ywhaq, Ywhaz
39	5-aminoimidazole Ribonucleotide Biosynthesis I	3.35	Gart, Pfas
40	Inosine-5'-phosphate Biosynthesis II	3.35	Adsl, Paics

Supplemental Table 2. Top 40 Ingenuity canonical pathways enriched in the NRCM TAZ interactome dataset.

#	Ingenuity Canonical Pathway	-log(p-value)	Proteins
1	Spliceosomal Cycle	14	Bcas2, Ddx39b, Dhx15, Dhx38, Eftud2, Eif4a3, Plrg1, Prpf19, Sf3b1, Sf3b2, Snrnp200, U2af2
2	Inhibition of ARE-Mediated mRNA Degradation Pathway	10.9	Cnot1, Dis3, Edc4, Prkar2b, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd2, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
3	Purine Nucleotides De Novo Biosynthesis II	9.75	Adsl, Adss2, Gart, Impdh1, Impdh2, Paics
4	Protein Ubiquitination Pathway	6.85	Dnaja1, Hsp90aa1, Hspa1l, Hsph1, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd2, Ube2m, Ube2o, Usp10, Usp7
5	Cell Cycle Control of Chromosomal Replication	6.27	Mcm2, Mcm3, Mcm4, Mcm5, Mcm6, Mcm7, Pcna
6	BAG2 Signaling Pathway	6.16	Hsp90aa1, Hspa1l, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd2
7	HIPPO signaling	6.12	Amot, Scrib, Tjp2, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
8	tRNA Charging	5.99	Farsa, Gars1, Iars1, Lars1, Nars1, Tars1
9	AMPK Signaling	5.79	Arid1a, Fasn, Gnb2, Gys1, Pfkl, Pfkm, Prkar2b, Rab1a, Rab7a, Smarca4, Smarcc1, Smarcd1
10	RAR Activation	5.74	Arid1a, Carm1, Csnk2a2, Csnk2b, Parp1, Prkar2b, Prmt1, Psmc5, Smarca4, Smarcc1, Smarcd1
11	IGF-1 Signaling	5.45	Csnk2a2, Csnk2b, Prkar2b, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
12	Cell Cycle: G2/M DNA Damage Checkpoint Regulation	5.34	Prkdc, Ywhab , Ywhae, Ywhag, Ywhah, Ywhaz
13	Huntington's Disease Signaling	5.13	Capn1, Dnm2, Gnb2, Hdac1l, Hspa1l, Polr2b, Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd2
14	FAT10 Signaling Pathway	5.05	Psmc2, Psmc3, Psmc4, Psmc5, Psmc6, Psmd2
15	Caveolar-mediated Endocytosis Signaling	4.31	Arcn1, Copb1, Copg1, Dnm2, Flnb, Rab5c
16	Methionine Degradation I (to Homocysteine)	4.29	Ahcy, Ahcyl1, Prmt1, Prmt5
17	Cysteine Biosynthesis III (mammalia)	4.15	Ahcy, Ahcyl1, Prmt1, Prmt5
18	Epithelial Adherens Junction Signaling	4.15	Afdn, Iqgap1, Rhoa, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
19	Amyloid Processing	4.1	Capn1, Csnk1a1, Csnk2a2, Csnk2b, Prkar2b
20	EIF2 Signaling	3.81	Eif3a, Eif3c, Eif3d, Eif3e, Eif3f, Eif4a3, Eif4g2, Rpl39, Rplp1
21	Inosine-5'-phosphate Biosynthesis II	3.65	Adsl, Paics
22	ATM Signaling	3.64	Cbx3, Smc1a, Smc2, Smc3, Trim28, Usp7

23	Clathrin-mediated Endocytosis Signaling	3.53	Ap1m1, Ap2b1, Csnk2a2, Csnk2b, Cttn, Dnm2, Rab5c, Rab7a
24	Superpathway of Methionine Degradation	3.45	Ahcy, Ahcyl1, Prmt1, Prmt5
25	RAN Signaling	3.4	Ipo5, Kpnb1, Xpo1
26	DNA Methylation and Transcriptional Repression Signaling	3.36	Chd4, Hdac1l, Mta2, Rbbp7
27	ERK5 Signaling	3.33	Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
28	mTOR Signaling	3.26	Eif3a, Eif3c, Eif3d, Eif3e, Eif3f, Eif4a3, Eif4g2, Rhoa
29	Tetrahydrofolate Salvage from 5, 10-methenyltetrahydrofolate	3.13	Gart, Mthfd1l
30	14-3-3-mediated Signaling	3.07	Pdcd6ip, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
31	Protein Kinase A Signaling	3.03	Flnb, Gnb2, Gys1, Prkar2b, Pygl, Rhoa, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
32	Regulation of eIF4 and p70S6K Signaling	3.01	Eif3a, Eif3c, Eif3d, Eif3e, Eif3f, Eif4a3, Eif4g2
33	CSDE1 Signaling Pathway	2.85	Csde1, Cttn, Edc4, Pum1
34	Hereditary Breast Cancer Signaling	2.82	Arid1a, Hdac1l, Polr2b, Smarca4, Smarcc1, Smarcd1
35	PI3K/AKT Signaling	2.74	Gys1, Hsp90aa1, Ywhab, Ywhae, Ywhag, Ywhah, Ywhaz
36	Mitotic Roles of Polo-Like Kinase	2.58	Capn1, Hsp90aa1, Smc1a, Smc3
37	Remodeling of Epithelial Adherens Junctions	2.54	Dnm2, Iqgap1, Rab5c, Rab7a
38	Pyrimidine Ribonucleotides De Novo Biosynthesis	2.36	Cad, Ctps1, Dhx9
39	Glycogen Degradation II	2.33	Agl, Pygl
40	Role of BRCA1 in DNA Damage Response	2.28	Arid1a, Smarca4, Smarcc1, Smarcd1

Supplemental Table 3. Top 20 KEGG pathways enriched in the C2C12 TAZ interactome dataset.

#	KEGG pathway	-log(FDR)	Proteins
1	Spliceosome	14.90	Prpf6, U2af2, Hspa1l, Prpf8, Snrpf, Eftud2, Sf3b1, Srsf9, Rbm17, Sf3b3, Dhx38, Rbm25, Snrnp70, Prpf40a, Sf3b4, Prpf38a, Cherp, U2surp, Snrnp200, Ppih, Snrpa1, Hspa1b, Ddx39b
2	Viral carcinogenesis	7.30	Scrib, Rhoa, Ywhab, Ywhah, Cdk1, Psmc1, Ywhaz, Polb, Pkm, Ywhag, Ep300, Ywhae, Rac1, Ubr4, Hdac1, Ywhaq, Ranbp1, Usp7
3	Hippo signaling pathway	7.06	Scrib, Ppp2r1a, Ywhab, Ywhah, Ppp2ca, Ywhaz, Ywhag, Tead1, Llgl1, Ywhae, Ppp2r2a, Ywhaq, Amot, Tead3, Lats1
4	Cell cycle	5.77	Ywhab, Ywhah, Cdk1, Ywhaz, Prkdc, Smc3, Smc1a, Ywhag, Mcm2, Ep300, Ywhae, Hdac1, Ywhaq
5	Proteasome	5.74	Psmd2, Psmd3, Psmd11, Psmc5, Psmc1, Psmc6, Psmc2, Psmc4, Psmc3
6	Tight junction	5.74	Actr2, Scrib, Rab8a, Ppp2r1a, Rhoa, Ppp2ca, Tuba1c, Llgl1, Rac1, Ppp2r2a, Tjp2, Tjp1, Mpdz, Amot
7	Huntington disease	5.74	Tubb6, Psmd2, Psmd3, Psmd11, Ap2b1, Psmc5, Psmc1, Psmc6, Cyc1, Ndufa10, Psmc2, Polr2b, Psmc4, Polr2l, Tuba1c, Ep300, Psmc3, Tubb2b, Hdac1
8	Prion disease	5.74	Tubb6, Psmd2, Hspa1l, Psmd3, Psmd11, Psmc5, Psmc1, Psmc6, Cyc1, Csnk2b, Ndufa10, Psmc2, Psmc4, Tuba1c, Psmc3, Tubb2b, Rac1, Hspa1b
9	Oocyte meiosis	4.59	Ppp2r1a, Ywhab, Ywhah, Cdk1, Ppp2ca, Ywhaz, Smc3, Smc1a, Ywhag, Ywhae, Ywhaq
10	Ribosome biogenesis in eukaryotes	4.28	Csnk2b, Nat10, Gnl2, Ran, Dkc1, Xrn1, Fbl, Mdn1, Xpo1
11	Amyotrophic lateral sclerosis	4.14	Tubb6, Rab8a, Psmd2, Psmd3, Psmd11, Rab5a, Psmc5, Psmc1, Psmc6, Cyc1, Ndufa10, Psmc2, Psmc4, Tuba1c, Psmc3, Tubb2b, Rac1, Rab1
12	Purine metabolism	4.11	Adss, Pfas, Adsl, Gart, Hprt, Gmps, Paics, Pkm, Impdh2, Nme1, Prps1l3
13	Parkinson disease	3.85	Tubb6, Psmd2, Psmd3, Psmd11, Psmc5, Psmc1, Psmc6, Cyc1, Ndufa10, Psmc2, Psmc4, Tuba1c, Psmc3, Tubb2b
14	mRNA surveillance pathway	3.70	Ppp2r1a, Wdr82, Ppp2ca, Pnn, Etf1, Cpsf3, Gspt1, Ppp2r2a, Ddx39b
15	Glucagon signaling pathway	3.64	Pfkl, Ldhb, Pdha1, Pkm, Pygm, Pfkm, Ep300, Pygl, Ldha
16	HIF-1 signaling pathway	3.29	Pfkl, Tfrc, Ldhb, Pdha1, Pfkm, Ep300, Aldoa, Ldha, Tceb1
17	Spinocerebellar ataxia	3.29	Psmd2, Psmd3, Psmd11, Psmc5, Psmc1, Psmc6, Pum1, Psmc2, Psmc4, Psmc3
18	Glycolysis / Gluconeogenesis	3.17	Pfkl, Ldhb, Pdha1, Pkm, Pfkm, Aldoa, Ldha
19	Endocytosis	3.17	Rab8a, Arpc2, Hspa1l, Rhoa, Rab5a, Ap2b1, Tfrc, Ehd1, Vps35, Arf1, Pdcd6ip, Dnm2, Hspa1b
20	Epstein-Barr virus infection	3.17	Psmd2, Psmd3, Psmd11, Psmc5, Psmc1, Psmc6, Psmc2, Psmc4, Psmc3, Rac1, Hdac1, Usp7

Supplemental Table 4. Top KEGG pathways enriched in the NRCM TAZ interactome dataset.

#	KEGG pathway	-log(FDR)	Proteins
1	Spliceosome	15.27	Prpf6, U2af2, Bcas2, Hspa1l, U2af1, Prpf8, Eftud2, Sf3b2, Alyref, Eif4a3, Sf3b1, Dhx15, Rbm17, Dhx38, Srsf7, U2surp, Snrnp200, Rbmx, Plrg1, Ddx39b, Prpf19
2	RNA transport	9.19	Kpnb1, Eif3e, Prmt5, Eif3a, Alyref, Eif4a3, Cyfip1, Eif3c, Eif3f, Strap, Tardbp, Fmr1, Eif3d, Xpo1, Eif4g2, Rnps1, Ddx39b
3	Cell cycle	9.19	Mcm7, Ywhab, Ywhah, Ywhaz, Prkdc, Mcm4, Smc3, Mcm6, Pcna, Smc1a, Ywhag, Mcm3, Mcm2, Ywhae, Mcm5
4	DNA replication	4.94	Mcm7, Mcm4, Mcm6, Pcna, Mcm3, Mcm2, Mcm5
4	Aminoacyl-tRNA biosynthesis	3.30	Gars, Farsa, Tars, Nars, Lars, Iars
5	mRNA surveillance pathway	3.30	Alyref, Eif4a3, Cpsf1, Gspt1, Tardbp, Fip1l1, Rnps1, Ddx39b
6	Proteasome	3.30	Psmd2, Psmc5, Psmc6, Psmc2, Psmc4, Psmc3
7	Viral carcinogenesis	2.92	Scrib, Rhoa, Ywhab, Ywhah, Ywhaz, Ywhag, Chd4, Ywhae, Ubr4, Usp7
8	RNA degradation	2.20	Pfkl, Skiv2l2, Edc4, Dis3, Pfkm, Cnot1
9	Oocyte meiosis	2.20	Ywhab, Ywhah, Ywhaz, Smc3, Smc1a, Ywhag, Ywhae
10	Hippo signaling pathway	2.20	Scrib, Ywhab, Ywhah, Ywhaz, Ywhag, Ywhae, Amot,
11	Amyotrophic lateral sclerosis	2.08	Psmd2, Psmc5, Psmc6, Alyref, Psmc2, Psmc4, Srsf7, Psmc3, Tubb4a, Tardbp, Matr3, Rab1
12	Prion disease	2.07	Psmd2, Hspa1l, Psmc5, Psmc6, Csnk2b, Psmc2, Psmc4, Csnk2a2, Psmc3, Tubb4a
13	Purine metabolism	1.98	Adss, Adsl, Gart, Hprt, Paics, Impdh1, Impdh2
14	Spinocerebellar ataxia	1.91	Psmd2, Psmc5, Psmc6, Pum1, Psmc2, Psmc4, Psmc3
15	Alanine, aspartate and glutamate metabolism	1.88	Cad, Adss, Adsl, Asns
16	Adherens junction	1.84	Rhoa, Csnk2b, Csnk2a2, Mllt4, Iqgap1
17	Tight junction	1.75	Scrib, Rhoa, Pcna, Tjp2, Cttn, Amot, Mllt4
18	Biosynthesis of amino acids	1.69	Pfkl, Aldh18a1, Asns, Pfkm, Phgdh
19	Ribosome biogenesis in eukaryotes	1.69	Csnk2b, Fbl, Csnk2a2, Xpo1, Nop58
20	Metabolic pathways	1.45	Ckb, Gys1, Atp6v1b2, P4ha1, Cad, Adss, Pfkl, Adsl, Gart, Aldh18a1, Hprt, Ahcyl1, Ctps, Paics, Asns, Agl, Ptges3, Fasn, Pfkm, Ahcy, Phgdh, Pygl, Prdx6, Impdh1, Impdh2, Acly, Mthfd1l

Supplemental Table 5. Top 20 Reactome pathways enriched in the C2C12 TAZ interactome dataset.

#	Reactome pathway – C2C12	-log(FDR)	Proteins
1	Metabolism of RNA	27.66	Chtop, Prpf6, U2af2, Psmd2, Ppp2r1a, Khsrp, Psmd3, Exosc10, Psmd11, Prpf8, Ywhab, Pelp1, Ltv1, Snrpf, Ppp2ca, Psmc5, Eftud2, Psmc1, Psmc6, Ywhaz, Prmt5, Etf1, Sf3b1, Psmc2, Polr2b, Srsf9, Psmc4, Xrn1, Fbl, Dhx9, Nop14, Rbm17, Srrt, Polr2l, Sf3b3, Dhx38, Exosc4, Patl1, Cpsf3, Psmc3, Snrnp70, Prpf40a, Sf3b4, Prpf38a, Cherp, U2surp, Gspt1, Rqcd1, Ppp2r2a, Snrnp200, Xpo1, Ppih, Adar, Snrpa1, Hspa1b, Ddx39b
2	Cell Cycle	15.25	Kpnb1, Tubb6, Rab8a, Psmd2, Ppp2r1a, Rpa3, Psmd3, Psmd11, Ywhab, Dync1h1, Ywhah, Cdk1, Ppp2ca, Rad50, Psmc5, Psmc1, Psmc6, Ywhaz, Csnk2b, Smc3, Psmc2, Ran, Ruvbl1, Psmc4, Dkc1, Mre11a, Smc1a, Tubg1, Nek9, Tuba1c, Ywhag, Mcm2, Ywhae, Psmc3, Tubb2b, Ppp2r2a, Hsp90aa1, Hdac1, Rbbp4, Xpo1, Ywhaq, Rab1
3	Gene expression (Transcription)	14.74	Chtop, Paf1, U2af2, Ctr9, Psmd2, Ppp2r1a, Rpa3, Psmd3, Psmd11, Ywhab, Ywhah, Cdk1, Snrpf, Ppp2ca, Rad50, Psmc5, Psmc1, Psmc6, Ywhaz, Prmt5, Csnk2b, Sf3b1, Psmc2, Polr2b, Ran, Srsf9, Psmc4, Mre11a, Smarca4, Gtf3c3, Srrt, Polr2l, Dhx38, Ywhag, Gtf3c1, Tead1, Ep300, Cpsf3, Ywhae, Psmc3, Ssrp1, Polr1c, Rqcd1, Smarcc1, Usp9x, Mta2, Hdac1, Rbbp4, Ywhaq, Tead3, Arid1a, Usp7, Ddx39b, Tceb1
4	RNA Polymerase II Transcription	13.35	Chtop, Paf1, U2af2, Ctr9, Psmd2, Ppp2r1a, Rpa3, Psmd3, Psmd11, Ywhab, Ywhah, Cdk1, Snrpf, Ppp2ca, Rad50, Psmc5, Psmc1, Psmc6, Ywhaz, Prmt5, Csnk2b, Psmc2, Polr2b, Srsf9, Psmc4, Mre11a, Smarca4, Srrt, Polr2l, Dhx38, Ywhag, Tead1, Ep300, Cpsf3, Ywhae, Psmc3, Ssrp1, Rqcd1, Smarcc1, Usp9x, Mta2, Hdac1, Rbbp4, Ywhaq, Tead3, Arid1a, Usp7, Ddx39b, Tceb1
5	mRNA Splicing - Major Pathway	13.30	Prpf6, U2af2, Prpf8, Snrpf, Eftud2, Sf3b1, Polr2b, Srsf9, Dhx9, Rbm17, Srrt, Polr2l, Sf3b3, Dhx38, Cpsf3, Snrnp70, Prpf40a, Sf3b4, Prpf38a, Cherp, U2surp, Snrnp200, Ppih, Snrpa1
6	Processing of Capped Intron-Containing Pre-mRNA	12.87	Chtop, Prpf6, U2af2, Prpf8, Snrpf, Eftud2, Sf3b1, Polr2b, Srsf9, Dhx9, Rbm17, Srrt, Polr2l, Sf3b3, Dhx38, Cpsf3, Snrnp70, Prpf40a, Sf3b4, Prpf38a, Cherp, U2surp, Snrnp200, Ppih, Snrpa1, Ddx39b
7	G2/M Transition	12.41	Tubb6, Rab8a, Psmd2, Ppp2r1a, Psmd3, Psmd11, Dync1h1, Cdk1, Ppp2ca, Psmc5, Psmc1, Psmc6, Psmc2, Psmc4, Tubg1, Tuba1c, Ywhag, Ywhae, Psmc3, Tubb2b, Ppp2r2a, Hsp90aa1, Xpo1
8	Signaling by WNT	12.26	Psmd2, Ppp2r1a, Rhoa, Psmd3, Psmd11, Ap2b1, Ppp2ca, Psmc5, Psmc1, Psmc6, Ywhaz, Csnk2b, Psmc2, Psmc4, Vps35, Smarca4, Ep300, Psmc3, Rac1, Chd8, Hdac1, Xpo1, Clta, Tle3, Csnk1a1
9	Cell Cycle, Mitotic	11.91	Kpnb1, Tubb6, Rab8a, Psmd2, Ppp2r1a, Rpa3, Psmd3, Psmd11, Dync1h1, Cdk1, Ppp2ca, Psmc5, Psmc1, Psmc6, Csnk2b, Smc3, Psmc2, Ran, Psmc4, Smc1a, Tubg1, Nek9, Tuba1c, Ywhag, Mcm2, Ywhae, Psmc3, Tubb2b, Ppp2r2a, Hsp90aa1, Hdac1, Rbbp4, Xpo1, Rab1

10	G2/M Checkpoints	11.39	Psmd2, Rpa3, Psmd3, Psmd11, Ywhab, Ywhah, Cdk1, Rad50, Psmc5, Psmc1, Psmc6, Ywhaz, Psmc2, Psmc4, Mre11a, Ywhag, Mcm2, Ywhae, Psmc3, Ywhaq
11	M Phase	11.37	Kpnb1, Tubb6, Psmd2, Ppp2r1a, Psmd3, Psmd11, Dync1h1, Cdk1, Ppp2ca, Psmc5, Psmc1, Psmc6, Csnk2b, Smc3, Psmc2, Ran, Psmc4, Smc1a, Tubg1, Nek9, Tuba1c, Ywhag, Ywhae, Psmc3, Tubb2b, Ppp2r2a, Hsp90aa1, Xpo1, Rab1
12	Cellular responses to stress	11.35	Tubb6, Dnajb1, Psmd2, Hspa1l, Rpa3, Psmd3, Psmd11, Dync1h1, Rad50, Psmc5, Psmc1, Psmc6, Psmc2, Psmc4, Mre11a, Ccar2, Ptges3, Tuba1c, Ep300, Ywhae, Psmc3, Prdx6, Hsph1, Tubb2b, Hspa4l, Hsp90aa1, Rbbp4, St13, Hspa1b, Tceb1
13	TCF-dependent signaling in response to WNT	11.26	Psmd2, Ppp2r1a, Psmd3, Psmd11, Ppp2ca, Psmc5, Psmc1, Psmc6, Ywhaz, Csnk2b, Psmc2, Psmc4, Smarca4, Ep300, Psmc3, Chd8, Hdac1, Xpo1, Tle3, Csnk1a1
14	Regulation of mRNA stability by proteins that bind AU-rich elements	10.77	Psmd2, Khsrp, Psmd3, Psmd11, Ywhab, Psmc5, Psmc1, Psmc6, Ywhaz, Psmc2, Psmc4, Xrn1, Exosc4, Psmc3, Xpo1, Hspa1b
15	Immune System	10.62	Actr2, Ilf2, Kpnb1, Npepps, Tubb6, Ap1m1, Ube2m, Arpc2, Psmd2, Ppp2r1a, Rhoa, Psmd3, Psmd11, Ywhab, Dync1h1, Ap2b1, Rab5c, Cand1, Pfkl, Ppp2ca, Psmc5, Sec23a, Psmc1, Ddx41, Psmc6, Ywhaz, Csnk2b, Huwe1, Sec22b, Psmc2, Cyfip1, Psmc4, Copb1, Mre11a, Smarca4, Pkm, Dhx9, Sec24c, Eef2, Tuba1c, Rap1b, Ep300, Psmc3, Pygl, Prdx6, Tubb2b, Rac1, Impdh2, Arf1, Ube2o, Aldoa, Hsp90aa1, Sec31a, Ubr4, Acly, Clta, Eif4g2, Dnm2, Hspa1b, Tceb1
16	Cell Cycle Checkpoints	10.38	Psmd2, Ppp2r1a, Rpa3, Psmd3, Psmd11, Ywhab, Dync1h1, Ywhah, Cdk1, Ppp2ca, Rad50, Psmc5, Psmc1, Psmc6, Ywhaz, Psmc2, Psmc4, Mre11a, Ywhag, Mcm2, Ywhae, Psmc3, Xpo1, Ywhaq
17	Mitotic Anaphase	10.17	Kpnb1, Tubb6, Psmd2, Ppp2r1a, Psmd3, Psmd11, Dync1h1, Cdk1, Ppp2ca, Psmc5, Psmc1, Psmc6, Smc3, Psmc2, Ran, Psmc4, Smc1a, Tuba1c, Psmc3, Tubb2b, Ppp2r2a, Xpo1
18	Innate Immune System	9.77	Actr2, Ilf2, Kpnb1, Ap1m1, Ube2m, Arpc2, Psmd2, Ppp2r1a, Rhoa, Psmd3, Psmd11, Dync1h1, Rab5c, Cand1, Pfkl, Ppp2ca, Psmc5, Psmc1, Ddx41, Psmc6, Csnk2b, Huwe1, Psmc2, Cyfip1, Psmc4, Copb1, Mre11a, Pkm, Dhx9, Eef2, Rap1b, Ep300, Psmc3, Pygl, Prdx6, Rac1, Impdh2, Aldoa, Hsp90aa1, Ubr4, Acly, Dnm2, Hspa1b
19	Generic Transcription Pathway	9.71	Psmd2, Ppp2r1a, Rpa3, Psmd3, Psmd11, Ywhab, Ywhah, Cdk1, Ppp2ca, Rad50, Psmc5, Psmc1, Psmc6, Ywhaz, Prmt5, Csnk2b, Psmc2, Polr2b, Psmc4, Mre11a, Smarca4, Polr2l, Ywhag, Tead1, Ep300, Ywhae, Psmc3, Ssrp1, Rqcd1, Smarcc1, Usp9x, Mta2, Hdac1, Rbbp4, Ywhaq, Tead3, Arid1a, Usp7, Tceb1
20	Transcriptional Regulation by TP53	9.53	Ppp2r1a, Rpa3, Ywhab, Ywhah, Cdk1, Ppp2ca, Rad50, Ywhaz, Prmt5, Csnk2b, Polr2b, Mre11a, Polr2l, Ywhag, Ep300, Ywhae, Ssrp1, Rqcd1, Mta2, Hdac1, Rbbp4, Ywhaq, Usp7, Tceb1

Supplemental Table 6. Top 20 Reactome pathways enriched in the NRCM TAZ interactome dataset.

#	Reactome pathways – NRVM	-log(FDR)	Proteins
1	Metabolism of RNA	25.39	Prpf6, U2af2, Bcas2, Psmd2, Rplp1, U2af1, Prpf8, Ywhab, Ftsj3, Psmc5, Eftud2, Skiv2l2, Psmc6, Ywhaz, Prmt5, Sf3b2, Alyref, Eif4a3, Sf3b1, Psmc2, Dhx15, Polr2b, Ddx47, Psmc4, Fbl, Dhx9, Edc4, Rbm17, Dis3, Dhx38, Poldip3, Psmc3, Cpsf1, U2surp, Gspt1, Srrm2, Cnot1, Snrnp200, Xpo1, Fip1l1, Rpl39, Plrg1, Rnps1, Ddx39b, Prpf19, Hnrnpr, Nop58
2	mRNA Splicing - Major Pathway	17.01	Prpf6, U2af2, Bcas2, U2af1, Prpf8, Eftud2, Skiv2l2, Sf3b2, Alyref, Eif4a3, Sf3b1, Dhx15, Polr2b, Dhx9, Rbm17, Dhx38, Cpsf1, U2surp, Srrm2, Snrnp200, Fip1l1, Plrg1, Rnps1, Prpf19, Hnrnpr
3	Processing of Capped Intron-Containing Pre-mRNA	16.82	Prpf6, U2af2, Bcas2, U2af1, Prpf8, Eftud2, Skiv2l2, Sf3b2, Alyref, Eif4a3, Sf3b1, Dhx15, Polr2b, Dhx9, Rbm17, Dhx38, Poldip3, Cpsf1, U2surp, Srrm2, Snrnp200, Fip1l1, Plrg1, Rnps1, Ddx39b, Prpf19, Hnrnpr
4	RNA Polymerase II Transcription	12.98	U2af2, Trim28, Psmd2, U2af1, Ywhab, Ywhah, Psmc5, Psmc6, Ywhaz, Smarcd1, Prmt5, Csnk2b, Alyref, Eif4a3, Parp1, Pcna, Psmc2, Polr2b, Cbx3, Psmc4, Rbbp7, Smarca4, Supt16, Zfhx3, Dhx38, Ywhag, Poldip3, Csnk2a2, Chd4, Ywhae, Psmc3, Cpsf1, Ssrp1, Smarcc1, Mta2, Cnot1, Prmt1, Fip1l1, Arid1a, Usp7, Rnps1, Ddx39b
5	Gene expression (Transcription)	12.96	U2af2, Trim28, Psmd2, U2af1, Ywhab, Ywhah, Psmc5, Psmc6, Ywhaz, Smarcd1, Prmt5, Csnk2b, Alyref, Eif4a3, Sf3b1, Parp1, Pcna, Psmc2, Polr2b, Cbx3, Psmc4, Rbbp7, Smarca4, Supt16, Smarca5, Zfhx3, Dhx38, Ywhag, Poldip3, Csnk2a2, Chd4, Ywhae, Psmc3, Cpsf1, Ssrp1, Smarcc1, Mta2, Cnot1, Prmt1, Fip1l1, Arid1a, Usp7, Rnps1, Ddx39b
6	Cell Cycle	12.86	Mcm7, Kpnb1, Psmd2, Ywhab, Ywhah, Psmc5, Psmc6, Ywhaz, Mcm4, Csnk2b, Smc3, Mcm6, Pcna, Psmc2, Psmc4, Rbbp7, Prkar2b, Smarca5, Smc1a, Smc4, Ywhag, Csnk2a2, Mcm3, Mcm2, Ywhae, Psmc3, Tubb4a, Rcc2, Hsp90aa1, Xpo1, Smc2, Mcm5, Rab1
7	Cell Cycle, Mitotic	10.57	Mcm7, Kpnb1, Psmd2, Psmc5, Psmc6, Mcm4, Csnk2b, Smc3, Mcm6, Pcna, Psmc2, Psmc4, Prkar2b, Smc1a, Smc4, Ywhag, Csnk2a2, Mcm3, Mcm2, Ywhae, Psmc3, Tubb4a, Rcc2, Hsp90aa1, Xpo1, Smc2, Mcm5, Rab1
8	G2/M Checkpoints	10.34	Mcm7, Psmd2, Ywhab, Ywhah, Psmc5, Psmc6, Ywhaz, Mcm4, Mcm6, Psmc2, Psmc4, Ywhag, Mcm3, Mcm2, Ywhae, Psmc3, Mcm5
9	Metabolism of proteins	9.32	Ube2m, Psmd2, Rhoa, Rplp1, Srp14, Rab5c, Cand1, Psmc5, Scfd1, Psmc6, Eif3e, Prkdc, Mrpl40, Csnk2b, Otub1, Smc3, Eif3a, Parp1, Fbn1, Pcna, Psmc2, Cct6a, Gnb2, Psmc4, Eif3c, Copb1, Eif3f, Rbbp7, Arcn1, Mrps22, Sec23ip, Smc1a, Mrps7, Csnk2a2, Psmc3, Tubb4a, Gfm1, Gspt1, Eif3d, Rab7, Copg1, Rpl39, Usp10, Usp7, Pcmt1, Rab1, Mrpl16, Nop58
10	Assembly of the pre-replicative complex	8.73	Mcm7, Psmd2, Psmc5, Psmc6, Mcm4, Mcm6, Psmc2, Psmc4, Mcm3, Mcm2, Psmc3, Mcm5
11	Orc1 removal from chromatin	8.57	Mcm7, Psmd2, Psmc5, Psmc6, Mcm4, Mcm6, Psmc2, Psmc4, Mcm3, Mcm2, Psmc3, Mcm5

12	Cell Cycle Checkpoints	8.32	Mcm7, Psmd2, Ywhab, Ywhah, Psmc5, Psmc6, Ywhaz, Mcm4, Mcm6, Psmc2, Psmc4, Ywhag, Mcm3, Mcm2, Ywhae, Psmc3, Rcc2, Xpo1, Mcm5
13	Metabolism of RNA	8.24	Mcm7, Psmd2, Psmc5, Psmc6, Mcm4, Smc3, Mcm6, Pcna, Psmc2, Psmc4, Smc1a, Mcm3, Mcm2, Psmc3, Mcm5
14	mRNA Splicing - Major Pathway	8.22	Trim28, Psmd2, Ywhab, Ywhah, Psmc5, Psmc6, Ywhaz, Smarcd1, Prmt5, Csnk2b, Parp1, Pcna, Psmc2, Polr2b, Cbx3, Psmc4, Rbbp7, Smarca4, Supt16, Zfhx3, Ywhag, Csnk2a2, Chd4, Ywhae, Psmc3, Ssrp1, Smarcc1, Mta2, Cnot1, Prmt1, Arid1a, Usp7
15	Processing of Capped Intron-Containing Pre-mRNA	7.83	Kpnb1, Psmd2, Psmc5, Psmc6, Csnk2b, Smc3, Psmc2, Psmc4, Prkar2b, Smc1a, Smc4, Ywhag, Csnk2a2, Ywhae, Psmc3, Tubb4a, Rcc2, Hsp90aa1, Xpo1, Smc2, Rab1
16	RNA Polymerase II Transcription	7.45	Mcm7, Psmd2, Psmc5, Psmc6, Mcm4, Mcm6, Pcna, Psmc2, Psmc4, Mcm3, Mcm2, Psmc3, Mcm5
17	Gene expression (Transcription)	7.39	Kpnb1, Ap1m1, Psmd2, Rhoa, Srp14, Rab5c, Cand1, Pfkl, Csnk2b, Capn1, Psmc2, Cyfip1, Copb1, Agl, Psmc3, Pygl, Prdx6, Impdh1, Impdh2, Hsp90aa1, Ubr4, Acly, Rab7, Iqgap1
18	Cell Cycle	7.39	U2af2, U2af1, Alyref, Eif4a3, Dhx38, Poldip3, Cpsf1, Fip1l1, Rnps1, Ddx39b
19	Cell Cycle, Mitotic	7.15	Cad, Adss, Adsl, Gart, Paics, Impdh1, Impdh2
20	G2/M Checkpoints	6.99	Mcm7, Psmd2, Psmc5, Psmc6, Mcm4, Mcm6, Psmc2, Psmc4, Mcm3, Mcm2, Psmc3, Mcm5

Extended list of materials

Table 1. List of plasmids used in this study.

Plasmids	**Source**
EYFP-TAZ	See materials and methods section
GFP	See materials and methods section
Myc-TEAD1	Addgene (#33109)
Myc-TEAD4	Addgene (#24638)
FLAG-TAZ	Addgene (#24809)
HA-TAZ	Addgene (#32839)
FLAG-MTA2	Addgene (#140964)
TLE3	See materials and methods section
pcDNA	Addgene (#10792)
HA-CARM1	Addgene (#81118)
Myc-TLE1	Addgene (#11067)
Myc-B-catenin	See materials and methods section
MyoD	(Addgene #20917)
HOP Flash	Addgene (#83467)
HIP Flash	Addgene (#83466)
TOP Flash	Addgene (#12456)
FOP Flash	Addgene (#12457)
Myogenin reporter	Addgene (#134722)
Renilla	Promtega (pRL-Renilla)

Table 2. List of antibodies used in this study.

Antibodies	**Source**
YAP/TAZ	Cell Signaling (#D24E24)
TLE3	Proteintech (#22094-1-AP)
FLAG	Sigma-Aldrich (#F3165)
HA	Cell Signaling (#C29F4)
Myc	Cell Signaling (#9B11)
GFP	ChromoTek (#3H9)
MyoG	DSHB (#F5D)
MCK	Santa Cruz (#sc-365046)
β-Actin	Santa Cruz (#sc-47778)
Alpha-tubulin	Cell Signaling (#2144S)
Histone H3	Cell Signaling (#9715S)
MyoD	DSHB (#2A5)

Table 3. List of reagents used in this study.

Reagents	Constituents/Source
NP-40 Lysis Buffer	0.5 % NP-40 50 mM Tris-HCL pH 7.6 150 mM NaCl 100 mM NaF 10 mM Sodium pyrophosphate 2 mM EDTA 1 mM of Na3VO4 1 mM PMSO
3X SDS Loading Buffer	Per 8 ml buffer: 0.6 ml of 1 M Tris-HCl (pH = 8) 2.4 ml of glycerol 2.4 ml of 10% SDS 0.6 ml of Beta-Mercaptoethanol Bromo blue dye
1X Transfer Buffer	10% 10X Transfer Buffer 30.3g Tris-HCL 144.2 g Glycine 10 g SDS Equilibrated to 1L with ddH_20 80% ddH_20 10% of methanol
5% Blocking buffer	5% nonfat dry milk in 1X TBS, 0.1% Tween® 20
1% Blocking buffer	1% nonfat dry milk in 1X TBS, 0.1% Tween® 20
Protein G Dynabeads	Invitrogen (#10003D)
Anti-FLAG M2 magnetic beads	Sigma Aldrich (#M8823)
FLAG 3X peptide	Sigma Aldrich (#SAE0194)
Luciferase assay substrate	Promega (#E1910)
Renilla assay reagent	Promega (#E2820)
GFP-Trap Magnetic Beads	ChromoTek (#gtma)