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Article Title (within 20 words without abbreviations)	Stage-specific gene expression and MEK-dependent ECM regulation in Cornish and Ogye chicken muscle
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Abstract

This study aimed to explore the conserved regulatory mechanism underlying muscle development by performing a comparative transcriptomic analysis of breast muscles at 5 and 10 weeks of age in Cornish (broiler) and Ogye (Korean indigenous) chickens. Using RNA sequencing, we identified 199 DEGs between the two time points, with 135 upregulated and 64 downregulated genes ($P<0.05$, ($|\log_2 FC| \geq 1$)). Gene set enrichment analysis showed activation of pathways involved in cell multiplication and receptor signaling in 10-week-old chickens. For example, "cell differentiation," "regulation of response to stimulus," and "cellular developmental process" were enriched. On the other hand, pathways related to energy metabolism, such as "ATP biosynthetic process" and "proton-transporting synthase complex," were suppressed, indicating a metabolic shift as the birds grow. KEGG pathway analysis demonstrated significant enrichment of extracellular matrix (ECM)-receptor interaction and focal adhesion pathways. Key structural genes, including collagen (*COL6A1*, *COL6A2*, *COL6A3*), integrin (*ITGA11*), and laminin (*LAMA1*, *LAMB2*), were upregulated. We also confirmed increased expression of myogenic regulatory factors *PAX7* and *MyoD* in the 10-week-old Cornish and Ogye chickens. Functional analysis using primary chicken myoblasts treated with MAPK pathway inhibitors (p38, JNK, MEK) revealed that all three pathways regulated *PAX7* and *MyoD* expression. Notably, MEK inhibition specifically reduced the expression of *collagen type VI alpha 3 chain* (*COL6A3*) and *coiled-coil domain-containing protein 80* (*CCDC80*) in a dose-dependent manner. These findings reveal that MEK signaling critically regulates myogenic factors and ECM-related genes during muscle development. Thus, our findings offer new insights that could contribute to the regulation of muscle growth in commercial poultry.

Keywords: Cornish; Ogye; breast muscle; differentially expressed genes; ECM-receptor interaction; MAPK signaling pathways

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Introduction

The poultry industry is an important contributor to animal-source protein globally but faces increasing challenges in meeting the growing demand for food security amid a growing world population [1]. Because skeletal muscle accounts for about 50-70% of carcass weight in livestock species, its growth is closely related to meat productivity in the livestock industry [2]. Therefore, increasing skeletal muscle mass and promoting muscle development are essential for meeting rising meat consumption demands [3].

Skeletal muscle is a complex tissue organized in cylindrical multinucleated muscle fibers (myofibers), connective tissues, and intramuscular adipose tissues [3, 4]. Besides muscle cells, skeletal muscle tissue contains various cell types, such as adipocytes and fibroblasts, which play important roles in muscular anatomy and physiology [5]. Fibroblasts are part of connective tissue, comprise a small proportion, but play important roles in maintaining muscle structure and producing the extracellular matrix (ECM) [6]. The development of intramuscular fat (IMF) tissues is closely related to meat quality, including flavor, tenderness, and juiciness, which depend on the number and volume of adipocytes [7, 8]. ECM-receptor interaction pathway and focal adhesion signaling are important mechanisms that regulate fibroblast and intramuscular fat tissue development [9, 10]. Based on these facts, research into genes regulating muscle development pathways and their expression can significantly improve muscle growth and meat quality.

Previous studies have investigated the role of the mitogen-activated protein kinase (MAPK) signaling pathway in regulating the activity of myogenic transcription factors [11, 12]. As MAPK signaling pathways are major mediators of cell communication during the development of different tissues and organs, they also regulate muscle stem cell differentiation and muscle mass [13, 14]. Previous studies elucidated the specific roles of p38 MAP kinases (p38 MAPK) and C-Jun N-terminal kinases (JNK) pathways during myogenesis, noting that p38 MAPK promotes muscle differentiation, while the JNK pathway can negatively regulate skeletal muscle differentiation. In addition, the extracellular signal-regulated kinase (ERK) signaling pathway also plays an important role in muscle stem cell differentiation, in which ERK activity is induced during terminal differentiation, and nuclear-to-cytoplasmic shuttling of ERK promotes this differentiation [11, 12].

Insights from MAPK signaling and its role in muscle differentiation are generally important for understanding muscle development and how breed-related factors may influence these processes. Korean native chicken breeds are valuable genetic resources that have been adapted to local conditions over generations [15]; their commercial potential remains under-realized. These breeds have unique genetic traits that support sustainable production and the preservation of genetic diversity [16]. They showed distinct genetic characteristics, with genetic distances of 0.11-0.18 from commercial breeds, and superior meat quality due to high arachidonic acid content [16]. However, growth performance studies revealed

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divergent growth performances between these indigenous breeds and high-yield commercial broilers. Cornish chickens consistently weighed more than Ogye chickens at 4 and 8 weeks. By 8 weeks of age, Cornish males achieve 2.3-fold heavier body weight (1,783 g) compared to Ogye males (764g). A similar growth pattern was observed in females by 8 weeks of age; the body weight of Cornish females was 1,491 g, while that of Ogye females was 602 g [17]. In addition to preserving the genetic resources of Korean native chicken breeds, a comprehensive understanding of the transcriptional programs governing muscle development is still needed to enhance meat production [17].

RNA sequencing, also known as transcriptomic analysis, is an effective tool for identifying novel genes involved in muscle development and metabolism. To date, transcriptomic studies of indigenous chickens have primarily focused on discovering breed-specific expression signatures to delineate the unique genomic features [18, 19]. While comprehensive transcriptome analyses have been conducted on 20 tissues from the Ogye breed, an indigenous Korean chicken breed [20, 21], research focused specifically on conserved mechanisms of muscle growth across divergent breeds at different developmental stages remains limited. A comparative transcriptomic approach using phenotypically divergent chicken breeds provides a unique opportunity to identify stage-specific expression signatures that constitute the conserved regulatory mechanisms for chicken muscle development.

In this study, we performed transcriptomic analysis of the *pectoralis major* at 5 and 10 weeks in the Cornish and Ogye chickens to uncover conserved regulatory molecular signatures shared by the two breeds, despite their divergent growth kinetics. The breast muscle (*pectoralis major*) was selected for its high commercial value in the chicken meat industry and its role as an indicator of overall chicken growth [22, 23]. We carried out Gene Set Enrichment Analysis (GSEA) and KEGG pathway analysis to explore the conserved regulatory program by investigating the genes and pathways involved in muscle formation. Additionally, we employed the pharmacological inhibition of the p38, JNK, and MEK signaling pathways to investigate their specific roles in modulating the transcription of extracellular matrix (ECM)-associated genes.

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Materials and Methods

Experimental animals and sampling

The experimental animals used in this study were pure-line Cornish (S line) and Ogye (O line) breeds, maintained at the Poultry Research Center, National Institute of Animal Science, The Rural Development Administration, Republic of Korea. The chickens were raised in group-housing cages (0.65 m²) from hatch to 17 weeks of age in windowless poultry houses equipped with ventilation and temperature control systems. The ambient conditions were set at 34°C and 50% relative humidity for the first 3 days of life, and thereafter,

95 the ambient temperatures were gradually decreased by 2°C per week until 24°C. Seven birds per cage were
96 raised for the Cornish chickens, while 10 birds per cage were raised for the Ogye chickens. Feed was
97 provided *ad libitum* with starter feed (ME 2,850 kcal/kg, CP 19.0%) from hatch to 6 weeks of age and
98 grower feed (ME 2,820 kcal/kg, CP 15.0%) from 7 to 16 weeks of age. The lighting program used step-
99 down and step-up methods, decreasing light to 8 hours from 10 days of age until 16 weeks of age. Animal
100 management and experimental procedures in this study were conducted according to the regulations and
101 approval of the Experimental Animal Care and Research Ethics Committee at the Poultry Research Center,
102 National Institute of Animal Science, The Rural Development Administration, Republic of Korea
103 (NIAS2022-0568).

104 For the Cornish chickens, experimental animals were randomly selected from a progeny population of
105 2,000 birds; for the Ogye chickens, they were randomly selected from a progeny population of 600 birds.
106 A total of 10 female birds (5 Cornish and 5 Ogye) were sampled at 5 weeks of age, and then, at 10 weeks
107 of age, another 10 female birds (5 Cornish and 5 Ogye) were sampled. The average body weight of the
108 sampled Cornish and Ogye birds was 746 g and 283 g at 5 weeks and 1,840 g and 658 g at 10 weeks,
109 respectively. Birds were euthanized by cervical dislocation, and their breast muscles were sampled for
110 downstream RNA-sequencing analysis. The collected breast muscle tissues were stored at −80 °C till
111 further analysis.

112 ***RNA-Sequencing Data Pre-Processing***

113 RNA sequencing was carried out by eGenomes Inc. (Seoul, Korea). Total RNAs were purified using
114 Trizol™ according to the manufacturer's instructions. RNA quality was analyzed using a BioAnalyzer 2100
115 (Agilent Technologies, Santa Clara, CA, USA). The sequencing libraries were prepared using the Illumina
116 TruSeq RNA Library Prep kit (Illumina Inc., San Diego, CA, USA) with 1 µg of total RNA having an RNA
117 Integrity Number (RIN) > 7 and sequenced on an Illumina NovaSeq 6000 platform. On average, a total of
118 21,506,563.65 reads were obtained per sample (range of 14,361,799 to 29,178,509 reads). Before mapping,
119 the average input read length was 202 base pairs (bp), except for two samples, which used single-end reads
120 (101 bp). The quality of raw sequence reads was assessed using FastQC (Galaxy Version 0.74+galaxy0)
121 [24]. and MultiQC (Galaxy Version 1.11+galaxy1) [25]. The Cutadapt tool (Galaxy Version 4.9+galaxy1)
122 [26] was used to remove potential sequencing adaptors and low-quality score bases from raw reads using a
123 phred score threshold of 20. After removing low-quality nucleotides and sequencing adaptors, the high-
124 quality reads were mapped to the *Gallus gallus* reference genome "bGalGal1.mat.broiler.GRCg7b"
125 available in Ensembl (https://www.ensembl.org) [27] using STAR software (Galaxy Version
126 2.7.11a+galaxy0) [28]. Uniquely mapped reads were then used in downstream analysis. These were 81.57%
127 (range = 62.68% – 91.87%) of the reads per sample (**Supplementary Table S1**). RNA sequencing raw data

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128 have been deposited into the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/)
129 under accession number GSE308328.

130 ***Identification of Differentially Expressed Genes (DEGs)***

131 Differential expression analysis was performed using the DESeq2 tool available in Galaxy (Galaxy Version
132 2.11.40.8+galaxy0) [29]. Differentially expressed genes (DEGs) were identified with the following criteria:
133 FDR-adjusted p-values < 0.05 and their log fold change ($|\log_2(FC)| \geq 1$). We then used the 'Annotate
134 DESeq2/DEXSeq output tables' tool in Galaxy (Galaxy Version 1.1.0) to annotate DEGs using the
135 "bGalGal1.mat.broiler.GRCg7b" genome reference model. The primary analysis focused on age-related
136 changes (10-week vs 5-week) across both breeds, and these DEGs were visualized by a volcano plot using
137 Galaxy (Galaxy Version 0.0.6).

138 ***Functional Analysis of Differentially Expressed Genes***

139 The DEGs identified from RNA-sequencing analysis in Galaxy software were imported into R for
140 functional analysis. Gene set enrichment analysis (GSEA) and Kyoto Encyclopedia of Genes and Genomes
141 (KEGG) pathway analysis were performed to investigate the functions of DEGs. GSEA was conducted
142 using the R packages AnnotationDbi (version 1.66.0) [30], GO.db (version 3.19.1)[31], and the org.
143 Gg.eg.db (version 3.21.0) [32]. Both GSEA and KEGG analyses were implemented using the
144 clusterProfiler package [33], and results were visualized as dotplots using the enrichplot package [34]. For
145 both GSEA and KEGG analyses, pathways were considered significant if their false discovery rate (FDR)
146 was < 0.05 and |normalized enrichment score (NES)| ≥ 1.5.

147 ***Primary Chicken Myoblast Cell Culture***

148 The chicken myoblast (pCM) cells were cultured as described in a previous study [35]. The pCM cells were
149 maintained in Medium 199, purchased from Gibco (Riverside, MO, USA), supplemented with 10% fetal
150 bovine serum (FBS), 1% chicken serum, and 100 U/mL each of penicillin and streptomycin (Thermo Fisher
151 Scientific, Waltham, MA, USA) at 37°C in 60-70% relative humidity and 5% CO_2.

152 ***Treatment of Mitogen-Activated Protein Kinase Signaling Pathway Inhibitors***

153 For MAPK pathway inhibition, SB203580 (p38 inhibitor) and SP600125 (JNK inhibitor) were purchased
154 from Invivogen (San Diego, CA, USA), and PD98059 (MEK inhibitor) was obtained from Medchem
155 Express (Monmouth Junction, NJ, USA). These inhibitors were stored following the manufacturer's
156 instructions. The inhibitors were applied to pCM cells at the following concentrations as previously
157 described: 5 μM and 50 μM [12, 36]. All inhibitors were applied at 25 μM and 50 μM for the specified

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158 exposure durations: p38 and JNK inhibitors for 1 h and the MEK inhibitor for 18 h, as per established
159 protocols for optimal pathway inhibition. Vehicle controls using DMSO (Dimethyl Sulfoxide) at equivalent
160 concentrations were included in all experiments.

161 ***RNA Extraction and cDNA Synthesis***

162 Total RNA from pCM cells treated with MAPK signaling pathway inhibitors was isolated using the Pure-
163 link RNA Mini Extraction Kit (Invitrogen, Carlsbad, CA, USA). The quality and quantity of the isolated
164 RNA were evaluated using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Illkirch, France).
165 In preparation for real-time quantitative polymerase chain reaction (RT-qPCR), 1 μg of total RNA was
166 utilized for cDNA synthesis, which was carried out using the ReverTra Ace-α-first strand cDNA synthesis
167 kit (Toyobo, Osaka, Japan).

168 ***Quantitative Real-Time PCR (Qrt-PCR)***

169 For the analysis of chicken muscle DEG expression, quantitative real-time PCR (qRT-PCR) was performed
170 using SYBR green supermix using the CFX96™ IVD Real-time PCR System (Bio-Rad, Hercules, CA,
171 USA). Primer sequences for the DEGs are listed in **Table 1**. The PCR conditions were as follows: an initial
172 step at 94°C for 3 minutes, followed by 39 cycles at 94°C for 10 seconds, 60°C for 30 seconds, and 72°C
173 for 30 seconds, then a final step at 72°C for 10 minutes. Dissociation was performed at 0.5°C increments
174 from 55°C to 95°C for over 25 minutes. Relative quantification analysis was performed using the
175 comparative Ct ($2^{-\Delta\Delta CT}$) method [37]. *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) expression
176 was used as an endogenous control to detect mRNA levels.

177 ***Statistical Analysis***

178 The data were analyzed using appropriate statistical methods in R. For tissue samples, a two-way ANOVA
179 was performed to assess the effects of breed, age, and their interaction on gene expression, followed by
180 Tukey's post hoc test for multiple comparisons. For cell culture experiments, one-way ANOVA was used
181 to compare treatment groups, followed by Dunnett's test to compare each treatment to the vehicle control.
182 The means of at least three independent biological replicates were reported with mean ± SD (n = 3-5) for
183 each group. Statistical significance was set at $P < 0.05$.

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Results

Differentially expressed genes (DEGs) in muscle development

Breast muscle tissue samples were collected from Cornish and Ogye chickens at 5 and 10 weeks of age (n = 5 per group) and subjected to RNA sequencing analysis. Read mapping rates are presented in **Supplementary Table S1**. DESeq2 analysis in the Galaxy software identified stage-specific differentially expressed genes (DEGs) by comparing 10-week-old against 5-week-old chickens, using combined mapped data from both breeds, grouped by age. This analysis revealed 135 up-regulated and 64 down-regulated DEGs ($|\log_2FC| \geq 1$, adjusted $P < 0.05$), representing common molecular signatures of muscle growth progression (**Fig. 1; Supplementary table S2**).

The volcano plot displays up- and down-regulated DEGs ($|\log_2FC| \geq 0.5$, adjusted $P < 0.05$) in Cornish and Ogye 10-week muscle, including up-regulated *gremlin 1* (*GREM1*), *contactin-associated protein family member 5* (*CNTNAP5*), *C-C prostaglandin D2 synthase (PTGDS)*, *G-protein coupled receptor 42-like 5* (*GR42L5*), *netrin-5-like* (*NTN4L*), *5-hydroxytryptamine receptor 1E* (*HTR1E)*, and down-regulated *collagen type X alpha 1 chain* (*COL10A1*), *17-beta-hydroxysteroid dehydrogenase type 2* (*HSD17B2*), *purkinje cell protein 4 like 1* (*PCP4L1*) and *interferon alpha-inducible protein 6* (*IFI6*) (Fig 1). These genes mediate fundamental cellular functions in muscle, including cytoskeletal regulation, extracellular matrix organization, secretory and membrane receptor signaling, and ion channel regulation. To validate these results, 12 representative DEGs were selected for qRT-PCR analysis (**Table 2**).

Gene set enrichment analysis (GSEA) and KEGG pathway enrichment analysis of chicken muscle tissue DEGs

To understand the biological functions of DEGs between 10-week-old and 5-week-old muscle tissues, we performed GSEA and KEGG pathway enrichment analysis of DEGs in the muscle tissues of Ogye and Cornish chickens at 10 weeks compared to 5 weeks of age. GSEA results showed that gene sets for cell development, cell differentiation, signaling receptor activity, and DNA-binding were enriched in 10-week-old chickens ($FDR < 0.05$ and $|NES| \geq 1.5$; **Fig. 2A; Supplementary tables S3**). However, energy metabolism pathways were suppressed, including ATP synthesis, mitochondrial processes, and fatty acid oxidation. This suggests a metabolic shift from energy production to growth processes during muscle development.

KEGG pathway analysis showed that most enriched DEGs were related to muscle development pathways, especially ECM-receptor interaction and focal adhesion, as well as PI3K-Akt signaling and protein digestion pathways ($FDR < 0.05$ and $|NES| \geq 1.5$; **Fig. 2B; Supplementary tables S4**). Analysis of specific pathways identified important genes for muscle structure, including collagen genes (*COL6A1*,

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216 *COL6A2*, *COL6A3*), integrin (*ITGA11*), laminin (*LAMA1*, *LAMB2*), *CHAD*, and *tenascin* (*TNN*) in both
217 ECM-receptor interaction and focal adhesion pathways (**Fig. 2C**). These results indicate that muscle
218 development processes become more active between 5 and 10 weeks of age, with ECM remodeling and
219 cell adhesion being important mechanisms for muscle growth.

220 ***Validation of myogenic regulatory factors and DEGs in Cornish and Ogye muscle tissues***

221 We performed qRT-PCR analysis to confirm the expressions of the myogenic factor genes and 12 selected
222 DEGs from RNA-seq analysis (**Fig. 3**). Expression levels of *Paired box 7* (*PAX7*) and *myogenic*
223 *differentiation 1* (*MyoD*), which encode key transcription factors for muscle development, were
224 significantly higher in 10-week-old chickens compared to 5-week-old chickens in both breeds ($P < 0.05$),
225 confirming that muscle development remained active during this growth period. For up-regulated DEGs,
226 the expression of c*oiled-coil domain containing 80* (*CCDC80*) and *CCL15* was increased in both 10-week-
227 old chickens from both breeds, as shown by RNA-seq analysis. It is worth noting that adenosine A1 receptor
228 (ADOR12B), COL6A3, and ephrin type-A receptor 13 (EPH13) increased mainly in Cornish chickens,
229 suggesting a discrepancy between RNA-seq and qRT-PCR analyses.

230 For down-regulated DEGs, including *actin alpha cardiac muscle 1* (*ACTC1*), *carboxypeptidase Z* (CPZ),
231 *glycine amidinotransferase* (*GATM*), *interferon alpha inducible protein 6* (*IFI6*), *N-terminal EF-hand*
232 *calcium binding protein 1* (*NECAB1*), and *POU class 2 homeobox 1* (POU2F1), the expression of DEGs
233 was the same as shown by RNA-seq analysis. These results highlight dynamic transcriptional regulation of
234 structural, metabolic, and signaling genes across developmental stages.

235 ***Effects of MAPK signaling pathway inhibition on myogenic regulatory factors in chicken myoblasts***

236 Before analyzing muscle growth-related DEG expression levels following MAPK signaling pathway
237 inhibition, qRT-PCR was performed to investigate the roles of different MAPK signaling pathways on
238 *PAX7* and *MyoD* transcription, which are key myogenic regulatory factors (**Fig. 4**). The qRT-PCR results
239 showed that *PAX7* and *MyoD* expressions were down-regulated following p38, JNK, and MEK inhibition
240 compared to vehicle controls. *PAX7* transcription was significantly downregulated 50 μM concentrations
241 of JNK and MEK inhibitors ($P < 0.05$). *MyoD* transcription was significantly downregulated at 5 μM and
242 50 μM concentrations of p38 and MEK inhibitors ($P < 0.05$). These results suggest that JNK and MEK
243 signaling cascades, in addition to the well-established p38 pathway, may play important roles in the
244 transcription of myogenic regulatory factors.

245 ***Effects of MAPK signaling pathway inhibition on muscle development-related gene expression in***
246 ***myoblast cells***

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Based on GSEA results showing enrichment of the ECM-receptor interaction pathway in 10-week-old chicken muscle, we investigated how MAPK pathways might regulate muscle development genes in myoblast cells. Since ECM-receptor interaction is important for muscle development, we selected *CCDC80* and *COL6A3* genes, which are involved in cell adhesion and ECM structure, for pathway inhibition analysis.

During pCM cell culture, both *CCDC80* and *COL6A3* showed increased expression from day 0 to day 1, confirming their involvement in early myoblast development (**Fig. 5A**). When cells were treated with different MAPK pathway inhibitors, *CCDC80* expression showed variable responses to p38 inhibitors at different concentrations, while JNK inhibitors caused modest reductions. However, MEK inhibitors significantly decreased *CCDC80* expression in a dose-dependent manner compared to vehicle controls ($P < 0.05$) (**Fig. 5B**).

Similarly, *COL6A3* expression was not significantly affected by p38 and JNK inhibitors but was significantly reduced by MEK inhibitor treatment in a dose-dependent manner ($P < 0.05$) (**Fig. 5B**). These results suggest that the MEK signaling pathway may play a role in regulating both *CCDC80* and *COL6A3* expression during muscle cell development, though further validation is needed to confirm direct pathway involvement.

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Discussion

The present study identified ECM-related genes as common DEGs that showed distinct mRNA transcriptomic patterns in the muscle tissue of 10-week-old Cornish and Ogye chickens and validated their differential expression at the transcriptional level as well as transcriptional regulation by MAPK pathways. GSEA revealed activation of pathways associated with cell development, differentiation, and receptor signaling, while energy metabolism pathways were suppressed in older birds. KEGG pathway analysis demonstrated that ECM-receptor interaction, focal adhesion, cell adhesion, and MAPK signaling pathways are enriched during this muscle growth period.

We observed the concurrent upregulation of *PAX7* and *MyoD* in 10-week-old chickens, indicating that muscle development remains active during this growth period. PAX7 serves as a marker for satellite cell population and is required for muscle growth, whereas MyoD functions as a key regulator of myogenic differentiation [38, 39].

Transcriptomic analysis indicated increased expression of ECM-related genes, including *COL6A1*, *COL6A2*, *COL6A3*, *ITGA11*, *LAMA1*, *LAMB2*, *CHAD*, and *TNN*, in both 10-week-old Cornish and Ogye muscle tissues, suggesting enhanced ECM remodeling during this growth phase. Our findings agree with previous studies on comparative transcriptome studies examining chickens with divergent growth rates, which showed comparable expression profiles for a subset of genes essential to myogenesis, specifically

279 those encoding ECM components (COL6A3, COL6A2, LAMA1), and contractile proteins (myosin heavy
280 chain 15 (MYH15)), demonstrating that distinct gene expression profiles are associated with muscle
281 development across different growth statuses, and the ECM-receptor interaction and MAPK signaling
282 pathway may constitute a crucial axis in chicken muscle development [40, 41]. These data support the
283 observed age-dependent transcriptional shifts, suggesting a conserved regulatory program governing avian
284 skeletal muscle development. Collectively, our results strengthen the established importance of these
285 pathways and underscore the need to investigate the specific mechanistic relationship between MAPK
286 signaling and ECM-mediated muscle maturation [9].

287 Analysis of MAPK signaling pathways revealed that pharmacological inhibition of p38, JNK, and MEK
288 reduced mRNA expression of *PAX7* and *MyoD*, with JNK and MEK inhibitors exhibiting effects
289 comparable to those of p38 inhibition. Although previous studies have established the p38 MAPK pathway
290 as a primary regulator of myogenic transcription factors [11, 40], our data suggest that JNK and MEK
291 pathways may also contribute to the transcriptional regulation of myogenic factor expression. Specifically,
292 these findings imply that JNK and MEK signaling cascades also contribute to maintaining satellite cell
293 stemness and stabilizing myogenic regulatory networks during postnatal muscle development [12, 14].
294 Although p38 MAPK has traditionally been the focus of myogenesis research, our results indicate that an
295 integrated MAPK signaling environment might be required for optimal myogenic factor expression in these
296 breeds. Nevertheless, as these conclusions are derived from pharmacological inhibition, future
297 investigations employing targeted genetic approaches, such as CRISPR-based modulation, are warranted
298 to rule out potential off-target effects and further delineate the direct vs. indirect cross-talk between these
299 cascades. Among DEGs, MEK signaling inhibition resulted in reduced *CCDC80* and *COL6A3* expression
300 in myoblast cultures. *COL6A3* encodes a subunit of collagen VI that is essential for maintaining ECM-
301 myofiber structural integrity [6, 42]. Mutations in collagen VI genes are associated with muscular
302 dystrophies [43], and *COL6A3* upregulation has been linked to myopathic conditions in chickens, including
303 wooden breast and white striping syndromes [44, 45]. *CCDC80* was initially identified as an estrogen-
304 induced gene but has since been found to play roles in energy metabolism, extracellular matrix organization,
305 and muscle formation [46-50]. A limitation of our study is that the differential expression of these proteins
306 was not verified, mainly due to the lack of specific antibodies. In order to establish the role of these proteins
307 in avian myogenesis, i.e., myoblast proliferation and differentiation, functional assays, such as gain-of-
308 function by overexpressing these proteins or loss-of-function using siRNA or CRISPR-based knockout
309 experiments, are warranted. The apparent regulation of both genes by MEK pathway inhibition suggests
310 that this pathway may coordinate ECM assembly and cellular adhesion during muscle growth, though direct
311 pathway validation experiments would be needed to confirm these relationships.

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312 This study builds upon previous transcriptomic analyses of Korean indigenous chickens, including
313 comprehensive tissue-specific expression profiling of Ogye chickens [20, 21]. Our work extends this
314 foundation by providing the first comparative analysis of muscle development, aiming to elucidate
315 conserved transcriptional signatures shared across the Cornish and Ogye chickens at different growth stages.
316 Previous research on the muscles of these birds has primarily focused on whole-genome sequencing (WGS)
317 or genome-wide association (GWAS) studies that connect genotypes to meat quality traits [51-53]. Our
318 study provides a genome-wide transcriptional landscape that enhances understanding of the mechanisms
319 underlying muscle development in chickens.

320 The observed metabolic shift from energy production to growth-related processes in 10-week-old chickens
321 reflects metabolic remodeling during muscle development. Downregulation of ATP synthesis,
322 mitochondrial processes, and fatty acid oxidation pathways indicates that cellular resources are redirected
323 toward biosynthetic processes and ECM deposition. This metabolic transition is consistent with established
324 energy requirements for muscle development in growing poultry [3, 5].

325 Even though extensive analysis was conducted in this study, we acknowledge the following limitations.
326 First, MAPK pathway inhibition experiments rely on pharmacological approaches that may have off-target
327 effects or indirect interactions with the pathway. Second, the study focuses on mRNA expression levels
328 without protein-level validation. Third, the cell culture system, while informative, may not fully recapitulate
329 the complex tissue environment of developing muscle. Future studies should, if possible, validate these
330 transcriptional changes at the protein level, investigate the temporal patterns of MAPK pathway activation
331 across different stages of chicken muscle development, and employ more direct approaches, such as genetic
332 knockdown or overexpression, to confirm pathway relationships.

333 In conclusion, by leveraging the phenotypic contrast between these two distinct chicken breeds across
334 critical growth stages, this study will offer opportunities to identify growth-stage-specific genes and
335 regulatory pathways involved in muscle development, potentially revealing new targets for genetic
336 improvement of meat production traits in chickens.

337

338 Competing interests

339 The authors declare that they have no conflict of interest.

340

341 Authors' contributions

342 The authors made the following contributions: JRS, BRM, HT, and KDS conceived and designed the

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343 experiments; IS, TSP, WA, HT, HC, and KH provided the reagents, materials, and analysis tools; JRS and
344 AWB performed the experiments; JRS, IS, and AWB analyzed and interpreted the data; and JRS, AWB,
345 BRM, HT, and KDS wrote the manuscript.

346

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354

355 Declaration of AI and AI-assisted technologies in the writing process.

356 During the preparation of this work, the author(s) used [Chatgpt / Open AI], [Claude / Anthropic],
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358 tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility
359 for the content of the publication

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Tables

515 ***Table 1***: *The DEGs and myogenic factor genes primer used in the RNA-sequencing DEGs*
516 *validation and in in-vitro analysis.*

Gene name	Symbol	GeneBank ID	Primer sequence (5’ → 3’)
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	NM_204305.1	F: TGCTGCCCAGAACATCATCC R: ACGGCAGGTCAGGTCAACAA
Actin Alpha Cardiac Muscle 1	*ACTC1*	NM_001079481.2	F: TGTCCTGTCCCTGTATGCCT R: GTAGTCGGTCAGATCCCTGC
Adenosine A2b Receptor	*ADORA2B*	NM_205087.2	F: GACCGGTACTTGGCCATCAA R: AGCCCGATCACAAAGGACAG
Coiled-coil domain-containing protein 80	*CCDC80*	NM_204431.2	F: CGCTCACTTCATCCCGCTT R: TTCGCCTTCCAGAGATGAGG
C-C motif chemokine ligand 15	*CCL15*	NM_001321492.1	F: CGAGCTGTCGTCTTCAAGGT R: GCTGGTACCTCTTCACCCAC
Collagen type VI alpha 3 chain	*COL6A3*	NM_205534.3	F: CCAGCCCAAAGTGACCTACA R: CAGATGTCCATGACATTTTCAGGC
Carboxypeptidase Z	*CPZ*	NM_204578.5	F: AGCCTTGCAGGCATGTATGT R: TAGGGCCAGGCCATGTCTAT
Ephrin type-A receptor 3	*EPHA3*	NM_205430.3	F: GCCCCGGCAACGAAGTTA R: GGGTAGGAGATCCAGCCCA
Glycine amidinotransferase	*GATM*	NM_001397432.1	F: GGCGCATTTCATCGGCTC R: TCCCACTCGTTGTAGGAGCA
Myogenic differentiation 1	*MyoD1*	NM_204214.3	F: GCCGCCGATGACTTCTATGA R: CAGGTCCTCGAAGAAGTGCAT
Paired Box 7	*Pax7*	NM_001011688.2	F: ATCCTGTTAGCAATGGCCTCT R: TCCACCTGGAGCACTGCAT
POU Class 2 Homeobox 1	*POU2F1*	NM_205472.1	F: TTACAGGCTGCTGCTCAGTC R: ATGAGCTGGGTCTGGGGTAT
Pancreatic Progenitor Cell Differentiation and Proliferation Factor	*PPDPF*	NM_001197037.2	F: ATGGCCACGCACAACTATCA R: TGACCTCCCCAGCATACTCA
Interferon Alpha Inducible Protein 6	*IFI6*	NM_00 1001296.6	F: GCCGGTTTCACTTCCTCTGG R : CCCCCAAAGGATTTTGCCTC
N-Terminal EF-Hand Calcium Binding Protein 1	*NECAB1*	NM_001277413.2	F: TGCTGATGGAGTTCTGAGTGG R: TGTCAAGATTGTCTGTATTGTGTGT

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Figure legends

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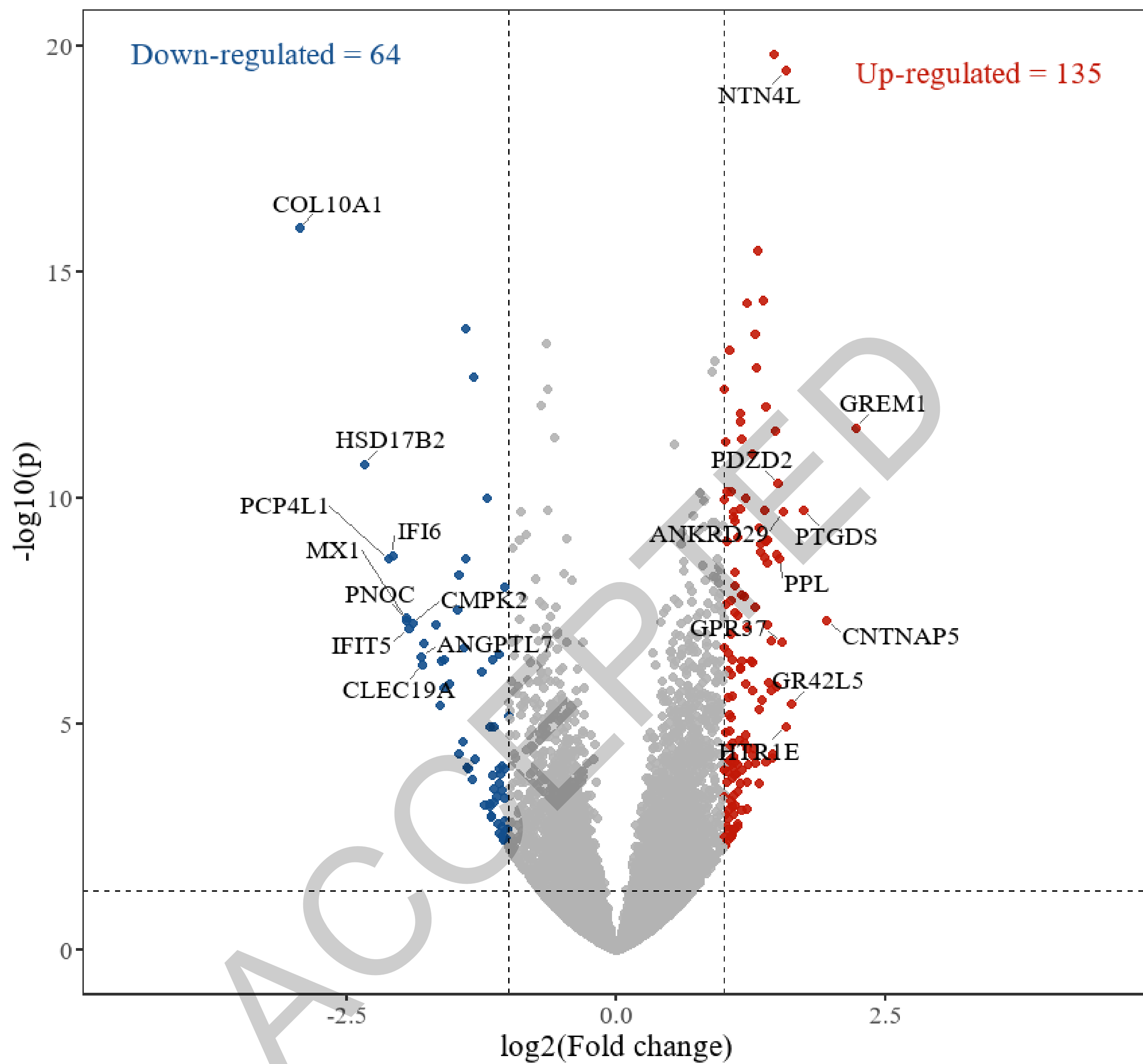

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522 o **Fig. 1**. Volcano plot of differentially expressed genes (DEGs) in 10-week-old chickens
523 compared with their 5-week-old counterparts, based on mapped data aggregated across
524 Cornish and Ogye breeds for each growth stage. The names of the top 10 up- and down-
525 regulated DEGs were labeled in the plot. Blue, red and gray dots indicated significantly down-
526 regulated DEGs, significantly up-regulated DEGs, and genes with no significant differences
527 between the two groups, respectively

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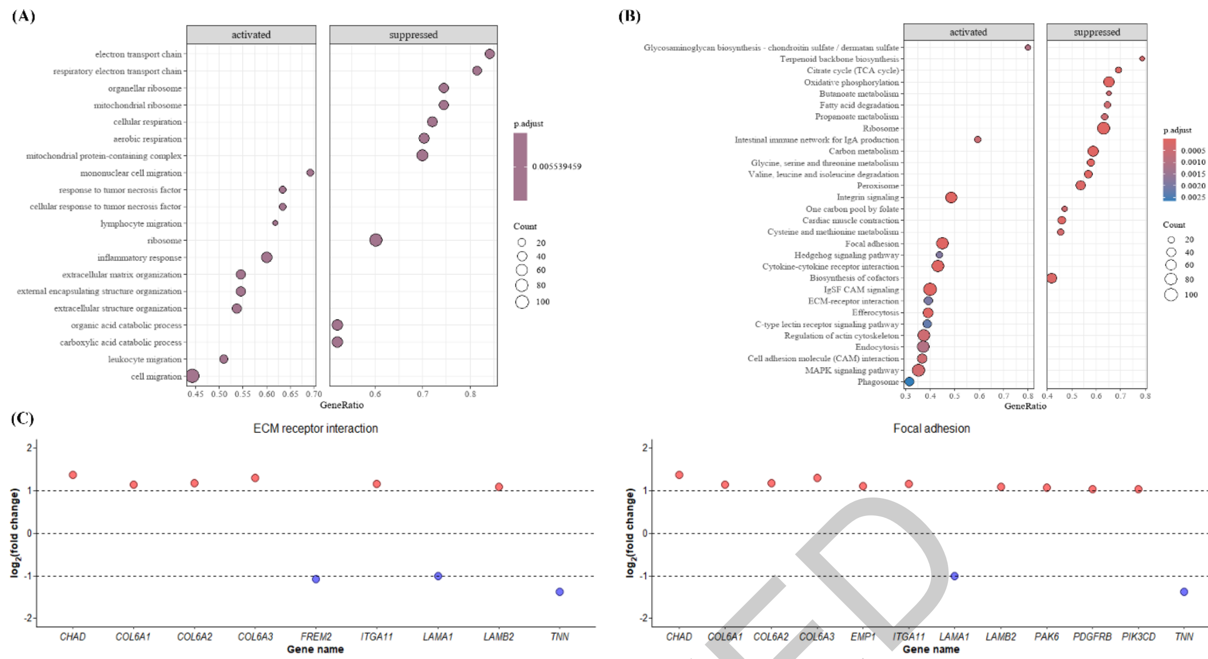

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530 ○ **Fig. 2**. GSEA and KEGG pathway enrichment analysis of differentially expressed genes
531 (DEGs) in the muscle tissues of Ogye and Cornish chickens at 10 weeks compared to 5 weeks
532 of age. (A) Activated and Suppressed gene sets in the GSEA analysis, (B) Significantly
533 enriched pathways in the KEGG pathway enrichment analysis, and (C) The log2 fold change
534 (log2(FC)) values of the significantly enriched pathways

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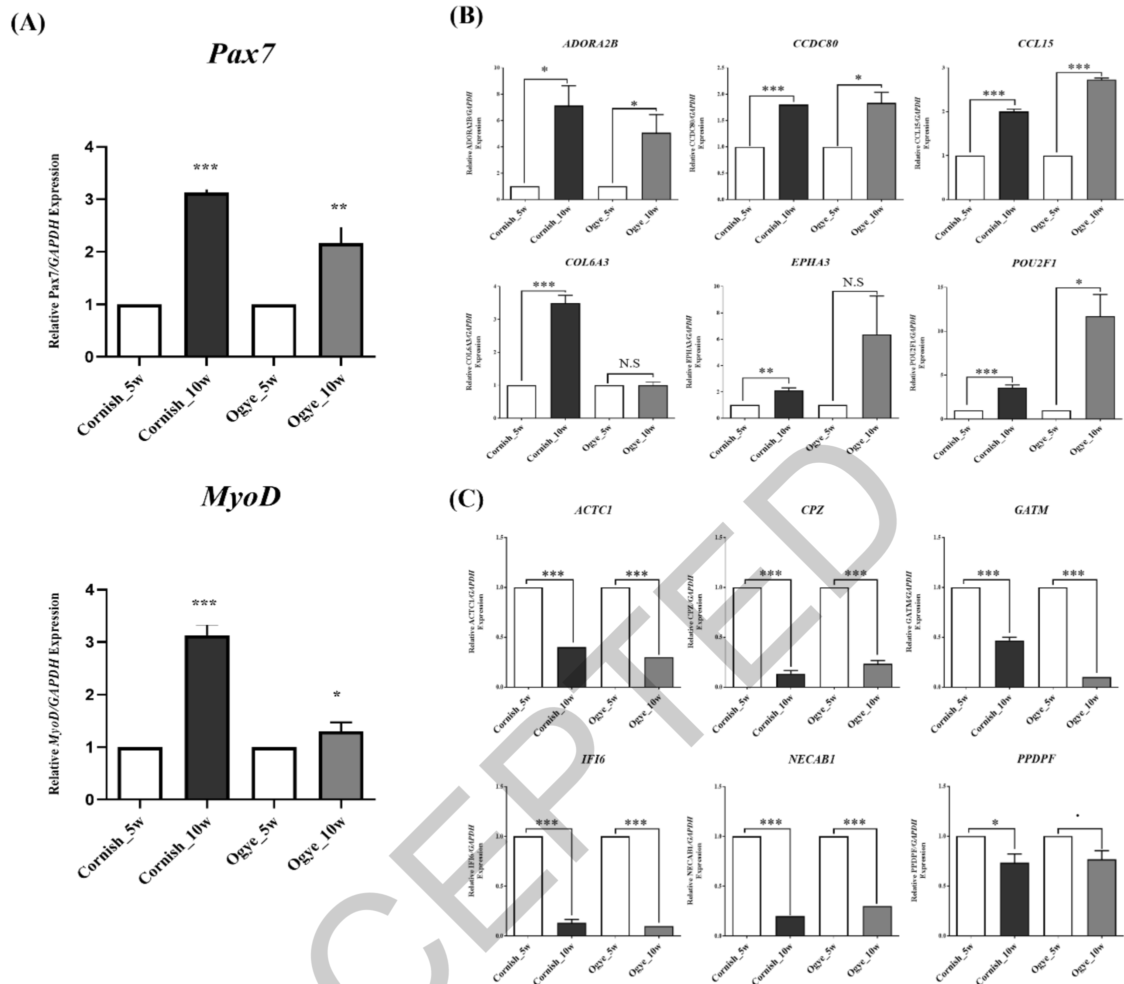

Fig. 3. The expression levels of the myogenic transcription factor, *Pax7* and *MyoD*, in the muscle tissues of 5-week and 10-week-old chicken. (A) Validation of differentially expressed genes (DEGs), (B) Up-regulated DEGs, and (C) Down-regulated DEGs. Data is expressed as the mean ± SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, calculated using a one-way ANOVA test.

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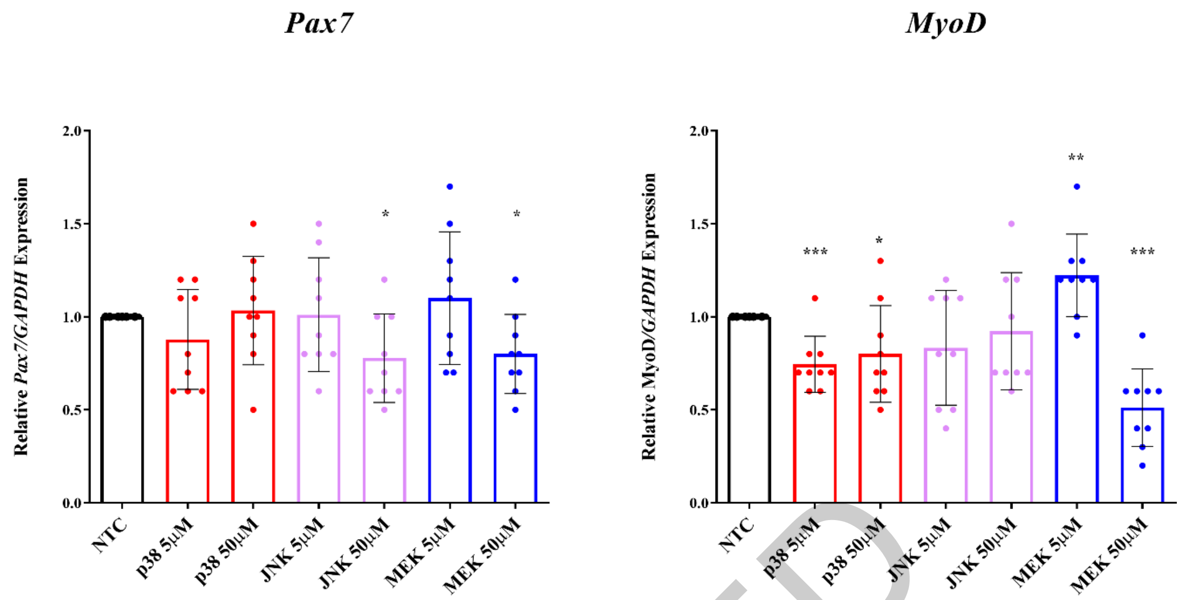

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544 ○ **Fig. 4**. The expression levels of *Pax7* and *MyoD* in pCM cells treated with MAPK inhibitors.
545 Data is expressed as the mean ± SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p <$
546 0.0001.

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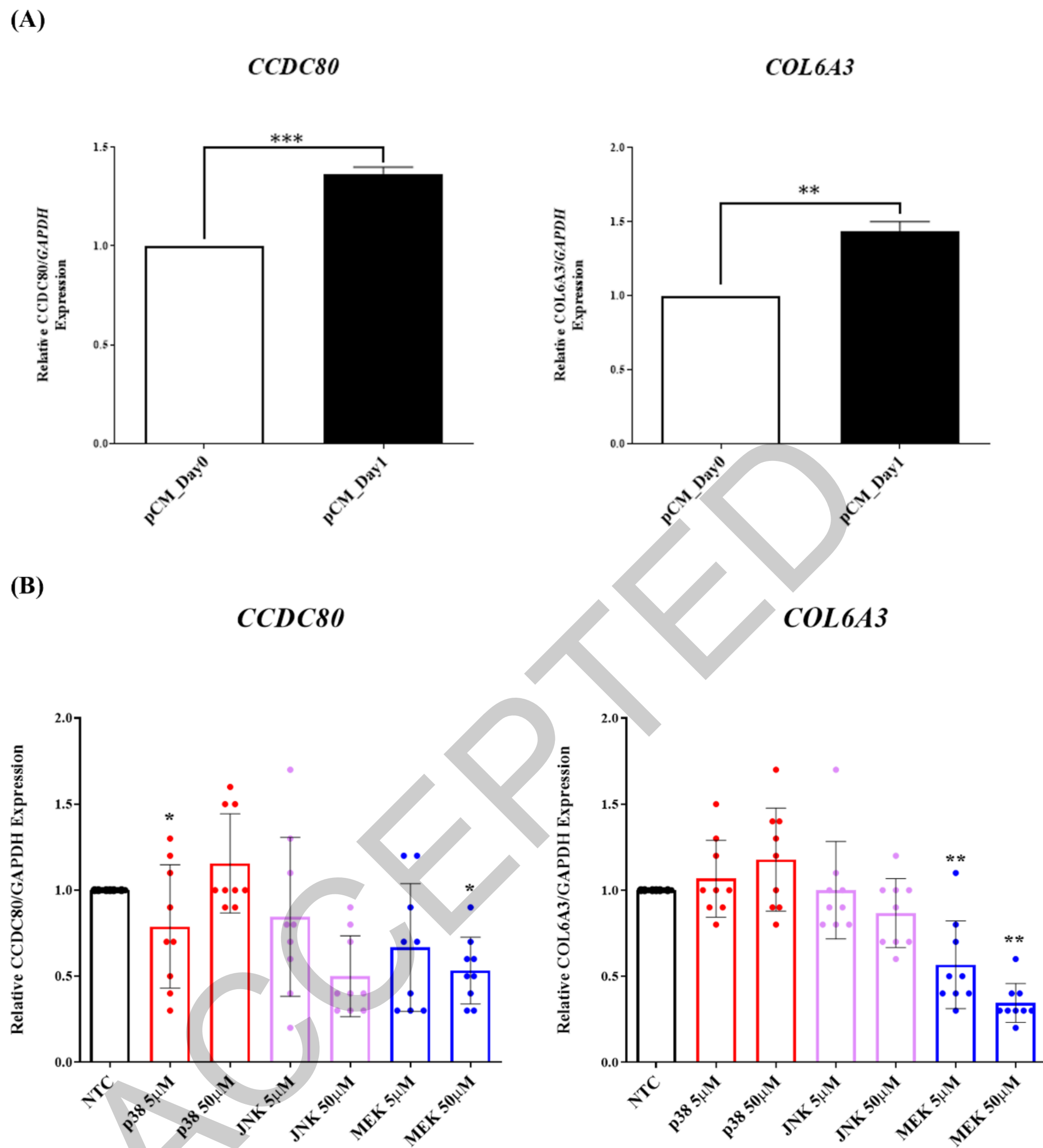

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549 o **Fig. 5**. The expression level of muscle growth-related DEGs in pCM cell proliferation (A) and
550 the regulation of muscle growth-related DEGs in MAPK inhibitors treated pCM cells (B).
551 *CCDC80* and *COL6A3* expression patterns showed down-regulation in MEK inhibition (B).
552 Data is expressed as the mean ± SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p <$
553 0.0001, calculated using a one-way ANOVA test.