



Sirolimus as a repurposed drug for tendinopathy: A systems biology approach combining computational and experimental methods



Zetao Wang ^{a,b,c,d,e,f,1}, Junchao Luo ^{b,c,d,e,f,1}, Luyong Jiang ^{c,d},
^{e,f,1}, Chenqi Tang ^{b,c,d,e,f,g}, Yangwu Chen ^{b,c,d,e,f}, Kun Yang ^c,
^{d,e,f}, Zicheng Wang ^a, Jiabao Dong ^a, Xiao Chen ^{c,d,e,f}, Zi Yin ^{c,d,e,f}, Jianyou Li ^a,
Weiliang Shen ^{a,b,c,d,e,f,*}

^a Department of Orthopedics, Affiliated Huzhou Hospital & Liangzhu Laboratory, Zhejiang University School of Medicine, Huzhou, China

^b Department of Sports Medicine & Orthopedic Surgery, the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

^c Institute of sports medicine, Zhejiang University, Hangzhou, China

^d Orthopedics Research Institute of Zhejiang University, Hangzhou, China

^e Key Laboratory of Motor System Disease Research and Precision Therapy of Zhejiang Province, Hangzhou, China

^f Clinical Research Center of Motor System Disease of Zhejiang Province, Hangzhou, China

^g Binjiang Institute of Zhejiang University, Hangzhou, China

ARTICLE INFO

Keywords:

Tendinopathy
Drug repositioning
Systems biology
Functional genomics
Mendelian randomization

ABSTRACT

Background: Effective drugs for tendinopathy are lacking, resulting in significant morbidity and re-tearing rate after operation. Applying systems biology to identify new applications for current pharmaceuticals can decrease the duration, expenses, and likelihood of failure associated with the development of new drugs.

Methods: We identify tendinopathy signature genes employing a transcriptomics database encompassing 154 clinical tendon samples. We then proposed a systems biology based drug prediction strategy that encompassed multiplex transcriptional drug prediction, systematic review assessment, deep learning based efficacy prediction and Mendelian randomization (MR). Finally, we evaluated the effects of drug target using gene knockout mice.

Results: We demonstrate that sirolimus is a repurposable drug for tendinopathy, supported by: 1) Sirolimus achieves top ranking in drug-gene signature-based multiplex transcriptional drug efficacy prediction, 2) Consistent evidence from systematic review substantiates the efficacy of sirolimus in the management of tendinopathy, 3) Genetic prediction indicates that plasma proteins inhibited by mTOR (the target of sirolimus) are associated with increased tendinopathy risk. The effectiveness of sirolimus is further corroborated through in vivo testing utilizing tendon tissue-specific mTOR gene knockout mice. Integrative pathway enrichment analysis suggests that mTOR inhibition can regulate heterotopic ossification-related pathways to ameliorate clinical tendinopathy.

Conclusions: Our study assimilates knowledge of system-level responses to identify potential drugs for tendinopathy, and suggests sirolimus as a viable candidate. A systems biology approach could expedite the repurposing of drugs for human diseases that do not have well-defined targets.

1. Background

Tendinopathy is a prevalent musculoskeletal condition, representing around 30 % of musculoskeletal appointments in general practice [1]. The rotator cuff is the area most prone to injury, with a prevalence rate as high as 5.5 % in the whole population [1]. Each year, more than 200,

000 surgical procedures are carried out in the United States to repair the rotator cuff in cases of severe rotator cuff tendinopathy (RCT). These treatments contribute to healthcare expenses amounting to around 474 million dollars [2]. At now, arthroscopic rotator cuff repair is the main method used to treat RCT. It enhances the functional scores of shoulder joint and reduces pain symptoms in patients [3]. Nevertheless, patients

* Corresponding author. Department of Orthopedics, Affiliated Huzhou Hospital & Liangzhu Laboratory, Zhejiang University School of Medicine, Huzhou, China.
E-mail address: wlsen@zju.edu.cn (W. Shen).

¹ These authors contributed equally.

frequently encounter the peril of postoperative non-healing and re-tearing [4]. Over the years, corticosteroid injections have been the mainstay pharmacological treatment for tendinopathy. Nevertheless, the effectiveness of corticosteroid injections remains controversial [5]. In conclusion, there is an urgent need for effective pharmacological treatment for tendinopathy.

The process of drug discovery is typically inefficient, marked by escalating expenses, lengthy duration, and a high percentage of wastage [6]. Drug repurposing is a strategy that involves using existing pharmaceuticals that have already been thoroughly investigated for their safety and pharmacokinetic characteristics to treat different medical conditions [7]. Sildenafil, initially an antihypertensive, was repurposed by Pfizer for erectile dysfunction. By 2012, it held a 47 % market share, with global sales of \$2.05 billion [8]. The typical cost of introducing a repurposed medicine to the market is projected to be around US\$300 million, while a novel chemical entity is anticipated to cost between US \$2–3 billion [7]. However, even in the case of medications that already exist, the procedure of conducting experimental preclinical screening followed by human safety and efficacy studies is arduous and time-consuming. Hence, it is imperative to prioritize the candidate medications.

Over the past few decades, there has been substantial advancement and extensive adoption of high-throughput technologies, leading to a dramatic surge in the volume of omics data. There is a wealth of publicly available research that extensively examines the relationship between genetic variation and the effectiveness of treatment. The intricacy of disease biology and the necessity for customized treatment can be addressed by a systematic approach that relies on high-throughput data to confront significant obstacles in drug repurposing. In this regard, the application of systems biology might accelerate the process of prioritizing candidate drugs by utilizing various strategies to thoroughly examine and integrate the existing information on a specific drug [8]. The goal of systems biology is to understand physiology and disease from the levels of molecular pathways, regulatory networks, cells, tissues, organs, and ultimately the entire organism [9]. Drug repositioning based on systems biology can be defined as: in drug repositioning under specific drug conditions, the use of high-throughput genomic data from humans and various model organisms. These different types of data are integrated using systems biology approaches, including network-based and signature-based methods; the predicted outputs are validated in different model organisms [10].

This work presents a systems biology approach to identify prospective treatment candidates for tendinopathy by 1) applying multiplex transcriptional drug prediction based on a clinical tendon transcriptomics database, 2) screening candidates through systematic review and evidence level assessment, 3) evaluating high-potential drugs through deep learning integrating drug structure information, and 4) exploring genetic evidence of drugs for tendinopathy treatment in Mendelian randomization (MR) analyses. Finally, we validated therapeutic potential of the sirolimus target against clinical tendinopathy based on animal models.

2. Methods

2.1. Key resources table

The table offers an easy overview of the key reagents and resources used in the study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Tendon samples from patients	The Second Affiliated Hospital Zhejiang University School of Medicine (Hangzhou, China)	This paper

(continued on next column)

(continued)

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Tendon samples from collagenase model	The Second Affiliated Hospital Zhejiang University School of Medicine (Hangzhou, China)	This paper
Chemicals, peptides, and recombinant proteins		
TRIzol Reagent	Invitrogen	15596026
Collagenase	Gibco	17100017
Critical commercial assays		
DNF-471 STANDARD SENSITIVITY RNA ANALYSIS KIT	AATI	DNF-471-0500
Optimal Dual-mode mRNA Library Prep Kit	BGI	LR00R96
BGI Plug-In Adapter Kit	BGI	LA00R04
Magnetic beads	BGI	LB00V60
Qubit® ssDNA Assay Kit	Invitrogen	Q10212
MGISEQ-2000RS High-throughput Sequencing Set(FCL PE150)	MGI	1000012555
Deposited data		
RNAseq for human tendon samples	This paper	GSA: HRA006534
RNAseq for collagenase model samples	This paper	GSA: CRA014406
RNAseq for mTOR-knockout mice	Chen et al. [11]	PMID: 32494667
Rotator cuff syndrome GWAS	FinnGen Consortium	https://r9.finngen.fi/
LDL cholesterol GWAS	Global Lipids Genetics Consortium	http://csg.sph.umich.edu/willer/public/lipids2013/ https://www.phpc.cam.ac.uk/ceu/proteins/
mTORC1-dependent circulating protein GWAS	The INTERVAL study	
Experimental models: Organisms/strains		
Mouse: <i>Scx</i> ^{Cre}	Dr. R. Schweitzer (Oregon Health & Science University, Oregon, USA)	N/A
Mouse: mTOR ^{f/f}	Dr. Yiting Zhou (Zhejiang University)	N/A
Mouse: <i>Scx</i> ^{Cre} , mTOR ^{f/f}	Dr. Xiao Chen (Zhejiang University)	N/A
Rat (Sprague Dawley)	Hangzhou Medical College	N/A
Software and algorithms		
R v4.3.1	R Core Team	https://www.r-project.org/ https://cytoscape.org/
Cytoscape v3.10.0	Shannon et al. [12]	https://clue.io/ http://www.dreimt.org
Connectivity Map DREIMT	Lamb et al. [13] Troulé et al. [14]	http://www.spied.org.uk http://bioinformatic.s.cing.ac.cy/codres
SPIED v3	Williams [15]	https://www.dleps.tech/dleps/submit
CoDReS	Karatzas et al. [16]	http://cspade.fimm.fi/
DLEPS	Zhu et al. [17]	
C-SPADE	Ravikumar et al. [18]	
STRING	Szklarczyk et al. [19]	https://string-db.org/
RRHO	Plaisier et al. [20]	http://systems.crump.ucla.edu/rankrank/
ActivePathways v2.0.0	Paczkowska et al. [21]	https://github.com/reimanidlab/ActivePathways
TwoSampleMR	Hemani et al. [22]	https://github.com/MRCIEU/TwoSampleMR

2.2. Experimental model and study participant details

2.2.1. Human tendon tissue acquisition

Tissue samples of tendinopathy were obtained from 126 patients with rotator cuff damage during arthroscopic shoulder surgery. A total

of 28 individuals undergoing anterior cruciate ligament surgery reconstruction provided healthy tendon samples from their hamstring tendons. The patient's consent and clearance from the local ethics committee were acquired (Ethics Committee of the second Affiliated Hospital, School of Medicine, Zhejiang University, code: 2019-168, 2020-080).

2.2.2. Experimental model

The Zhejiang University Experimental Animal Welfare and Ethics Committee approved animal studies in accordance with the guidelines of Institutional Animal Care and Use Committee (ZJU20220006).

In the *in vivo* drug experiment, 12 8-week-old rats (equal numbers of males and females) were induced with tendinopathy by injecting 30U Type I collagenase (Gibco, 17100017) into the midportion of the Achilles tendon of 8-week-old rats. The same procedure was repeated after 3 days to stabilize the effect. Divide the 12 rats into two groups, with each rat receiving four subcutaneous injections (between the Achilles tendon and the skin, every 3 days) one week after modeling: the control group (sterile PBS, 20 μ L) and the sirolimus group (sirolimus, 70uM, 20 μ L). Weekly assessments of motor function were conducted on the rats after modeling, followed by euthanasia for transcriptomic sequencing and histological evaluation.

The effectiveness of sirolimus is further corroborated through *in vivo* testing utilizing tendon tissue-specific mTOR gene knockout mice. The tendinopathy was generated by injecting 12.5 U of type 1 collagenase (Gibco, 17100017) into the middle of the right Achilles tendons of 8-week-old mice. The same operation was performed 3 days later to stabilize the effect. At 6 weeks following modeling, 8 wild-type (WT) mice and 8 mTOR tendon knockout (mTOR-TKO) mice were euthanized. The legs were then excised for histopathology.

2.3. Method details

2.3.1. RNA sequence

The tissue was subjected to RNA extraction using TRIzol® Reagent following the directions provided by the manufacturer (Invitrogen). Subsequently, DNase I (TaKara) was used to eliminate genomic DNA. The RNA-seq transcriptome libraries were generated using the TruSeqTM RNA sample preparation Kit from Illumina (San Diego, CA). A total of 1 μ g of RNA was used for this process. In brief, messenger RNA was extracted using polyA selection through oligo(dT) beads and subsequently fragmented using a fragmentation buffer. The process involved in this study included cDNA synthesis, end repair, A-base addition, and ligation of the Illumina-indexed adaptors, following the methodology provided by Illumina. The libraries were subsequently chosen based on their size, specifically cDNA target fragments of 200–300 bp, using 2 % Low Range Ultra Agarose. These selected libraries were then subjected to PCR amplification using Phusion DNA polymerase (NEB) for a total of 15 PCR cycles. Following quantification using the TBS380, the paired-end libraries were sequenced using the Illumina HiSeq with a run length of 2X150bp.

2.3.2. Reads quality control and mapping

The unprocessed paired end readings underwent trimming and quality checking using Trimmomatic with the default parameters (<http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic>). The clean reads were aligned to the reference genome using the hisat2 software in orientation mode [23]. Tophat is a software application designed to match RNA-Seq reads to a genome. Its purpose is to discover gene expression and exon-exon splice junctions. It is constructed using the high-speed short read mapping software Bowtie2. This software was utilized to create a map using the default settings.

2.3.3. Tendinopathy signature genes identification

DESeq2 is used to identify the differential genes between tendinopathy samples and healthy tendon samples [24]. After filtering the p

value with a threshold of 0.05, the differential genes were sorted by Log₂(Foldchange) value. Ultimately, we identified the top 150 genes that were up-regulated and the top 150 genes that were down-regulated as the definitive genes associated with tendinopathy.

2.3.4. Multi-pipeline transcriptomic drug prediction and reranking

Tendinopathy signature genes were used as an input in three complementary signature-based medication repurposing tools: Connectivity Map, DREIMT and SPIED [13–15]. We obtained four candidate drug lists, with Connectivity Map and DREIMT providing one each, and SPIED's two databases providing one each. In Connectivity Map, drugs were ranked according to connectivity scores. In DREIMT, 100 drugs are initially screened based on Drug specificity score (DSS) and then drugs are ranked by prioritization score (tau). In SPIED, drugs with negative correlation were selected and ranked according to significance. Every drug list consists of 80 drugs with the highest potential in certain method for reversing tendinopathy at the molecular level (Supplementary Table 1).

The Computational Drug Repositioning Score (CoDReS) tool was employed to reevaluate the potential drugs by considering (a) a functional score that combines the relevance of drug targets to the disease and the strength of their binding to target genes, (b) an a-priori score that is determined by the initial ranking of drugs in each list, and (c) a structural score that indicates violations in drugability [16]. We conducted CoDReS reranking on the top 80 medications from 4 lists. The normalized a-priori repurposing score was calculated by dividing the original ranking score of each drug from the 4 lists by the absolute maximum ranking score. The top 30 drugs based on CoDReS aggregate score were selected for further analysis (Supplementary Table 2).

2.3.5. Systematic review

A search on the Medline database using PubMed as the search engine was conducted reviewing a combination of 36 different keywords, targeting articles related to using the candidate drugs for tendinopathy treatment released between 2013 and 2022. The following records were excluded: 1. Titles and abstracts with no data of interest; 2. Studies written in non-English; 3. Studies that do not belong to: *in vitro* or lab tests, animal studies, editorials, case reports, case series, case-control studies, cohort studies, randomized controlled trials (RCTs), systematic reviews (SRs) and meta-analyses (MAs). The keywords and studies included in systematic review are listed in the Supplementary Table s 3 and 4.

2.3.6. Chemical structure diversity analysis and clustering

We conducted a thorough search and obtained the molecular structures of the potential pharmaceutical compounds from DrugBank [25]. Next, we obtained the Simplified Molecular Input Line Entry System (SMILES) format of the compounds from PubChem. This format was then utilized as input in the C-SPADE tool to calculate the distance matrix, which measures the chemical and structural similarities of the compounds [18,26]. The structural similarity was calculated based on Dice similarity coefficient.

2.3.7. Deep learning-based efficacy prediction system

The Deep Learning based Efficacy Prediction System (DLEPS) employed a variational autoencoder to encode small molecules in SMILES format. It then utilized a dense network to predict changes in transcriptional profiles based on the latent vectors. Tendinopathy transcriptomic signatures and potentially reusable drugs SMILES were used as input for Online DLEPS system(<https://www.dleps.tech/dlep/s/submit>) [17].

2.3.8. Mendelian randomization (MR)

MR was employed to examine causal associations between drug targets and tendinopathy using the MR-Base TwoSampleMR package in R (<https://github.com/MRCIEU/TwoSampleMR>) [22]. The two-sample

MR studies utilized publicly accessible summary-level data obtained from genome-wide association studies (GWAS) ([Supplementary Table 5](#)). Each of these investigations had obtained approval from the appropriate institutional review boards, and participants had given their informed permission.

GWAS data of RCT was obtained by extracting summary statistics from 299,273 individuals of European ancestry from the FinnGen consortium (R9 release) [27]. The FinnGen Study is a comprehensive genetic study conducted in Finland, involving a nationwide meta-analysis of 9 biobanks. Importantly, the samples used in this study were distinct and did not overlap with those used in the plasma proteome GWAS. The GWAS conducted in the FinnGen Study involved a total of 24,061 individuals diagnosed with rotator cuff syndrome and 275,212 persons without the condition who served as controls. The rotator cuff syndrome was defined according to the international classification of diseases-10 (ICD-10): Rotator cuff or supraspinatus tear or rupture (complete or incomplete), not specified as traumatic supraspinatus syndrome.

We have suggested a tool that involves the selection of single nucleotide polymorphisms (SNPs) within 100 kb windows from the target gene HMGCR. These SNPs are associated with low-density lipoprotein (LDL) cholesterol levels at a genome-wide significance level (P-value $< 1 \times 10^{-6}$). The purpose of this tool is to serve as a proxy for the exposure to statins. In order to optimize the effectiveness of the instrument, SNPs employed as instruments were permitted to have a low level of weak linkage disequilibrium ($r^2 < 0.30$) with one another. A GWAS summary data of LDL cholesterol levels obtained from the Global Lipids Genetics Consortium (GLGC) was utilized to identify SNPs [28]. The data on exposure to mTOR-related genes were collected from the proteomics-GWAS data, which is publicly accessible, in a total of 3301 people from the INTERVAL research [29]. SNPs reaching genome-wide significance (P-value $< 1 \times 10^{-6}$) and linkage disequilibrium-pruned ($r^2 < 0.05$) were included.

2.3.9. Treadmill assessment

Following two consecutive days of acclimatization (15 m/min, 30 min/day, 0°), the rats' running performance was tested by measuring their ability to run until exhaustion. In the functional evaluation, the rats ran on a treadmill (SANS, SA101) at an initial speed of 2 m/min, followed by incremental speeds of 2 m/min² until they reached a pre-defined exhaustion point (defined as when the animal's hind limbs remained on the grid for over 10 s, indicating exhaustion).

2.3.10. Histological evaluation

Tissue specimens were harvested and fixed in 4% (v/v) PFA for 24 h at room temperature. After dehydration with gradient alcohol, the samples were embedded in paraffin and sectioned at 6 µm, and performed hematoxylin & eosin (HE) and Masson staining. Adapted from a modified version of the Movin grading system [30], the histological evaluation was scored by a blinded scorer using a semi-quantitative scoring system based on H&E staining results.

2.3.11. Protein-protein interaction network construction and visualization

Using the STRING database (v.12.0), we constructed a protein-protein interaction network with a medium confidence score of 0.4 using differentially expressed genes as input. We downloaded a tab-separated value format file and imported it into Cytoscape (v.3.9.1) for subsequent interactive analysis. The largest clusters were selected based on the number of nodes to build the visual network. Functional enrichments of the network were performed using gene ontology and TISSUES.

2.3.12. Integrative pathway enrichment analysis

Integrative pathway enrichment analysis was instructed to excavate the potential of mTOR inhibition in clinical tendinopathy treatment. Analysis of differential genes from tendinopathy patients versus healthy controls and WT mice versus mTOR-TKO mice were conducted using the

ActivePathways R package [21]. ActivePathways utilized the Brown's version of the Fisher's combined probability test to generate an integrated gene list. This list combines the significance (P-values) from many datasets for each input gene [31]. An integrated gene was subjected to a pathway enrichment study utilizing a ranked hypergeometric test and a collection of gene sets (c5.all.v7.5.1.symbols from Human Molecular Signatures Database (MSigDB)) [32]. Subsequently, the family-wise multiple testing correction method was employed to identify the pathways that were significantly enriched in the integrated gene list ($Q_{pathway} < 0.05$). Fold-change in gene expression was used as directional assumptions for prioritizing negatively-associated pairs of tendinopathy/mTOR-inhibition pairs. Significantly enriched pathways ($p < 0.05$) were visualized using Cytoscape with standard protocols [33].

2.3.13. Transcriptional overlap analysis

We employed rank-rank hypergeometric overlap (RRHO) analysis to determine transcriptional overlap between tendinopathy patients and the collagenase model [20]. The gene lists were ordered and signed based on their level of differential expression in tendinopathy patients and the collagenase model compared to healthy controls, respectively (measured as $\text{Log}_2(\text{Foldchange})$). A hypergeometric P-value matrix was generated using repeated statistical tests to assess the proportion of ranking genes that vary between different conditions. The Benjamini and Yekutieli approach was used to accomplish multiple testing correction [34]. The adjusted P-values were visualized using a heat map, where each pixel represents the adjusted $-\log_{10}$ hypergeometric P-values. These values indicate the level of transcriptional overlap between tendinopathy patients and the collagenase model.

2.4. Quantification and statistical analysis

2.4.1. Statistical analysis

The primary method of MR analysis employed was inverse variance weighted (IVW) analysis. The weighted median and MR Egger regression methods were employed in additional analysis [35]. The Cochrane's Q test was employed to ascertain the presence of heterogeneity among the SNPs [36]. A directional pleiotropy measure was calculated based on the intercept obtained from MR Egger regression [37]. A significance level of $p < 0.05$ was utilized to determine statistical significance. Additionally, MR-PRESSO was employed to identify outlier SNPs [38]. If the presence of horizontal pleiotropy is detected but no heterogeneity is seen, the MR Egger approach will be chosen. If heterogeneity was detected without evidence of pleiotropy, the weight median technique was chosen. A positive result can be considered if the IVW approach yields a significant result ($p < 0.05$), even if the findings of other methods are not significant, and there is no evidence of pleiotropy and heterogeneity. This is contingent upon the beta values of the other methods being in the same direction [39]. We conducted "leave-one-out" studies, where we systematically excluded one SNP at a time, in order to assess the robustness of our findings. The numerical data were displayed as the average value plus or minus the standard deviation. A Student's t-test was conducted to determine if there were any significant statistical differences between the groups. Statistical significance was attributed to P values less than 0.05. The significance level is denoted as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

3. Results

3.1. Multiplex transcriptional prediction and drug reranking

Having a sophisticated candidate drug ranking is essential in order to choose the most promising medications for testing. During the first part of the analysis, we collected 154 clinical tendon samples from patients to acquire highly informed tendinopathy transcriptional signatures. After filtering the p-values with a threshold of 0.05, differentially expressed

genes were ranked based on $\text{Log}_2(\text{Foldchange})$ values. Ultimately, we identified the top 150 upregulated genes and the top 150 downregulated genes as the key genes associated with tendinopathy. Secondly, we applied three discrete prediction approaches based on (i) Connectivity Map, (ii) DREIMT and (iii) SPIED. These three drug repurposing tools are complementary in terms of prediction methods and databases. We aim to obtain reliable and comprehensive lists of candidate drugs through their synergistic application (Fig. 1). All medicines listed can be found in [Supplementary Table 1](#).

During the last part of the analysis, the Computational Drug Repositioning Score (CoDReS) tool was used to rerank the candidate drugs from multiple lists [16]. In addition to incorporating drug prioritization information from drug repurposing tools, CoDReS also examined other types of drug information. We conducted CoDReS reranking on the top 80 medications based on their weighted normalized score from each of the four drug lists. Fig. 2 illustrates the performance of the top 30 drugs based on the CoDReS aggregate score across different drug prediction pipelines.

Step 1: we identified a set of robust tendinopathy signature genes based on a transcriptomics database encompassing 154 clinical tendon samples. Step 2: we conducted multi-pipeline transcriptomic drug prediction in three complementary signature-based drug repurposing tools. The connectivity score in Connectivity Map is the proportion of reference gene sets that have a higher resemblance to the perturbagen compared to the current query. The DREIMT Drug specificity score (DSS) summarizes the replicability of a given drug profile across multiple cell lines. The prioritization score (τ) indicates the degree of specificity for a given drug profile-immune signature association pair compared to a large set of drug-immune signatures association pairs in DREIMT's database. The tendinopathy signature genes in SPIED3 are rated based on their connection with the particular SPIED3 entry using a Z-score derived from a Pearson regression analysis. Step 3: we reranked drugs using CoDReS based on a combination of functional and structural criteria.

3.2. Filtering drugs with systematic review and evidence level assessment

We conducted a systematic review on the top 30 candidate drugs' efficacy for tendinopathy (Fig. 3). Information on the related drug, title, publication year, research category, major findings and conclusion are extracted ([Supplementary Table 4](#)). Subsequently, we narrowed down the selection of prospective medications for tendinopathy treatment by considering their established evidence. This resulted in a total of 8 candidate drugs, as depicted in Fig. 4. Some preclinical evidence suggests the potential effectiveness of diethylstilbestrol, calpeptin, raloxifene, and progesterone. We will discuss the statins and sirolimus in greater detail below due to their abundant and high-level evidence.

The effectiveness of statins is supported by the highest level of evidence. A systematic review included three cohort studies and one case-control study showed that simvastatin can reduce the risk of RCT [40]. A nationwide 11-year follow-up study showed that hyperlipidemia alone was an independent risk factor for RCT. Statins use might provide protection against RCT in patients with hyperlipidemia [41]. However, some studies demonstrated that statins are not beneficial to tendinopathy, and even lead to tendinopathy. A combined cohort and experimental investigation shown that the use of statins raises the clinical risk of tendinopathy by causing the release of matrix metalloproteinases [42]. A animal study evaluated the biomechanical and histopathological effects of statins on the Achilles tendon in rats [43]. The results showed that statins are associated with calcific tendinopathy risk. Overall, the effects of statins are controversial.

Consistent evidence from 1 case series, 2 animal studies, and 1 in vitro test indicated the effectiveness of sirolimus. Clinical date indicated that sirolimus is efficient in systemic lupus erythematosus induced tendinopathy [44]. Preclinical studies have shown that sirolimus can alleviate tendon ossification and reverse tendon aging. In our previous

study, we found that targeted pathological collagen delivery of sustained release sirolimus can prevent heterotopic tendon ossification [11]. Another study provided evidence that the intake of sirolimus through diet effectively decelerated or halted the natural progression of age-related tendon stiffness and the decline in energy storage capability [45]. Furthermore, a study shown that sirolimus has a partial inhibitory effect on senescence-associated β -gal activity and morphological changes. This suggests that sirolimus can reverse senescence in rat tendon stem/progenitor cells at both the molecular and cellular levels [46].

A, Flow diagram for the selection of articles with data of candidate drugs against tendinopathy published from 2013 up to 2022. The 36 search terms are listed in the [Supplementary Table 3](#). B, Assessment of candidate drug in evidence pyramid. SRs, systematic reviews; MAs, meta-analyses; RCTs, randomized controlled trials.

Score refers to the CoDReS aggregate score. Each grid in the heat map represents a study included in systematic review. For studies hypothesizing that drugs aid in tendinopathy treatment, evidence was categorized as effective (green) or ineffective (blue); for studies hypothesizing that drugs may cause tendinopathy, evidence was categorized as harmful (red) or harmless (yellow).

3.3. Deep learning-based drug efficacy prediction

In this section, we conducted a re-evaluation of high-potential drugs by integrating drug structure information. Firstly, we constructed and visualized a network depicting structural similarities among drugs to assess structural diversity. We computed the structural distance matrix by utilizing the Dice similarity coefficient to compare all possible pairs of candidate medicines. The hierarchical clustering analysis demonstrated that the input medications exhibited a wide range of chemical structural diversity, as shown in Fig. 5A. Afterwards, we utilized DLEPS to evaluate the effectiveness of medicines. DLEPS is a neural network that use linear representation of chemical structures as input to accurately model transcriptional profiles obtained from the L1000 project. This enables researchers to make predictions on the relationship between chemical structure and transcriptional features [17]. DLEPS analysis showed that all eight medications exhibited promising potential in alleviating tendinopathy (Fig. 5B). Statins and sirolimus, which are ranked high in multiplex transcriptional prediction and supported by abundant literature evidence, continue to perform well in DLEPS.

A, Drugs structural similarity dendrogram. The candidate drugs are the nodes and the distance between two nodes is an estimate of their similarity. B, Enrichment scores of drug candidates from DLEPS based on tendinopathy gene signatures as the genetic input.

Using MR to identify genetic evidence of drugs for tendinopathy treatment.

We further explored genetic evidence of statins and sirolimus for tendinopathy treatment (Fig. 6). This study includes two pharmacological targets as exposures: mTOR inhibitors and HMGCR inhibitors. We performed a MR analysis using the summary statistics from a European population-based GWAS dataset. The analysis focused on mTORC1-related proteins in plasma, specifically RP-S6K, EIF4EBP, and EIF-4F components. In addition, we suggested a method in which we chose certain SNPs within 100 kilobase windows from the HMGCR gene, which was linked to LDL cholesterol levels at a significant level across the entire genome. This method serves as a proxy for measuring the effects of statins. GWAS data of RCT was obtained by extracting summary statistics from 299,273 individuals in the FinnGen consortium.

This study utilises the connections of the chosen genetic tools to represent the pharmacological adjustment of the drug's target protein of interest. Genetic variations originating from the gene responsible for producing the specific protein targeted by statins are chosen as instruments. The main downstream effect of statins is decreased circulating LDL cholesterol. Genetic variants from mTORC1 downstream target proteins of sirolimus are selected as instruments. Sirolimus can

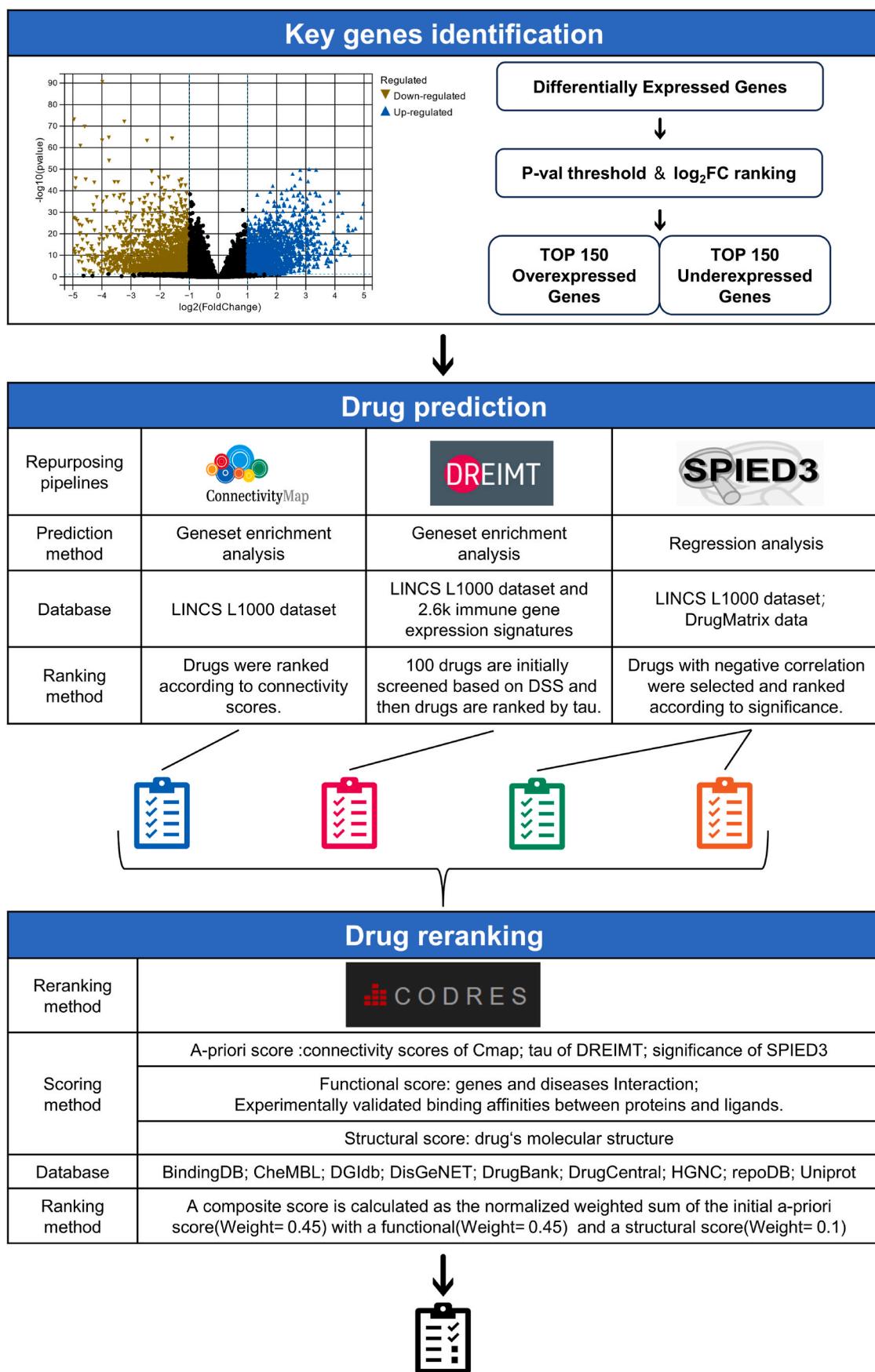


Fig. 1. Study design of multiplex transcriptional drug prediction and drug reranking.

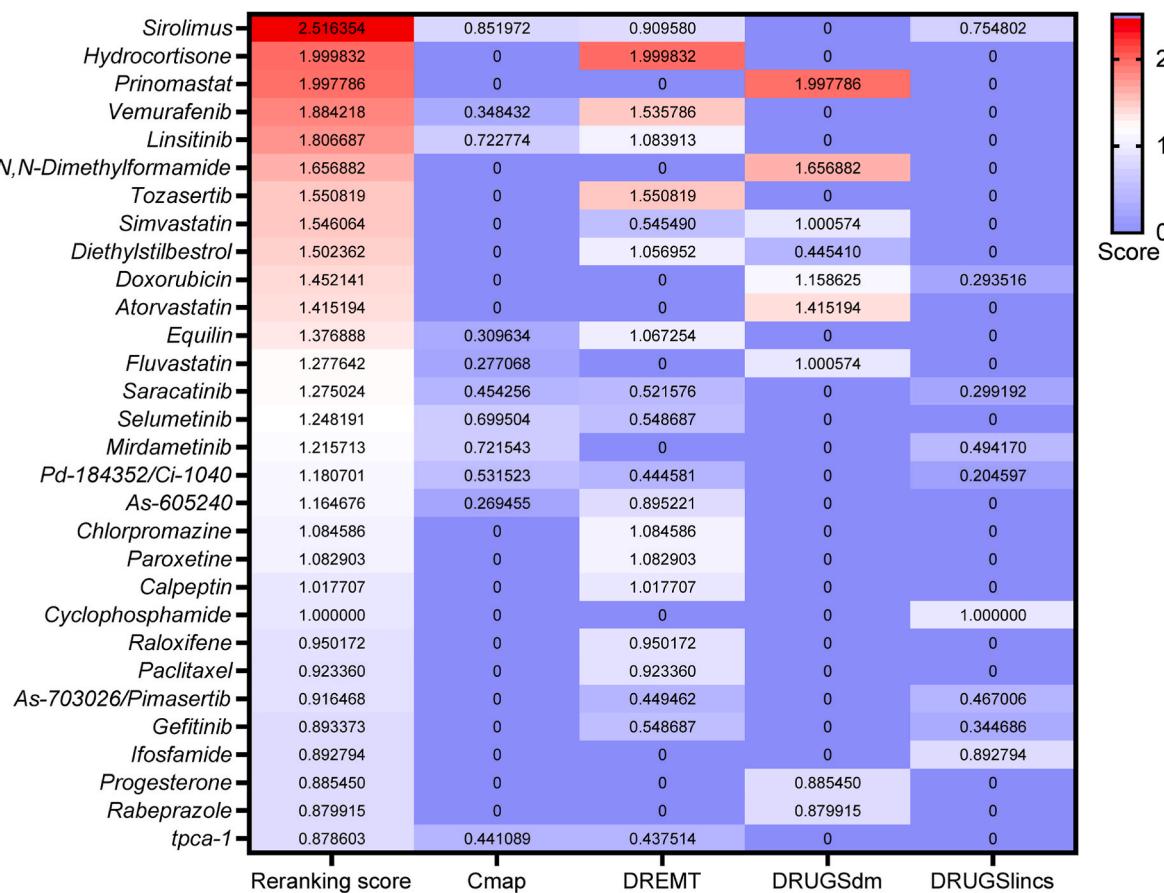


Fig. 2. The performance of the top 30 reranking drugs in four drug prediction pipelines.
Reranking score refers to the CoDReS aggregate score.

bind to mTOR and cause a conformational shift in mTORC1. mTORC1 controls cellular function by phosphorylating proteins downstream. Following phosphorylation by mTORC1, PR-S6K triggers the activation of EIF-4B, which in turn exerts a positive regulatory effect on EIF-4F. EIF4E-BP functions as a suppressor of EIF-4E. When phosphorylated by mTORC1, it separates from EIF-4E and subsequently facilitates the formation of the EIF-4F complex. The EIF-4F complex consists of EIF-4E, EIF-4G, and EIF-4A. The outcome of MR is rotator cuff syndrome extracted from the FinnGen consortium. The primary MR analysis utilized inverse variance weighted analysis. The study employed weighted median and MR Egger regression methods in supplementary analysis.

A proteome-wide MR approach is employed to ascertain the correlation between circulating protein levels reliant on mTORC1 and RCT. After removing SNPs that exhibited linkage disequilibrium, outline, or palindromic sequence, a total of 55 SNPs were selected for genetic prediction of mTORC1 downstream proteins (Supplementary Table 6). We evaluated the potency of SNPs employed as the instrument by utilizing the F-statistic. To mitigate any potential bias from weak instruments, we only considered SNPs with an F-statistic greater than 10 [47]. The results indicate that there is evidence linking the higher expression of mTORC1 downstream proteins in blood with an increased risk of RCT in IVW (odds ratio (OR) = 1.03, 95 % confidence interval (CI) = 1.01–1.08; P-value = 1.11×10^{-3}), Weighted median (OR = 1.04, CI = 1.01–1.06; P-value = 0.01) and MR Egger (OR = 1.05, CI = 1.01–1.08; P-value = 4.11×10^{-3}), indicating that sirolimus might lower the risk of RCT (Fig. 7A–C). In addition, the analyses that excluded each SNP showed that no one SNP was responsible for these results. Instead, the results were indicative of a combined pattern (Fig. 7B). The funnel plot displayed no evidence of heterogeneity (Fig. 7D).

Next, we examined the causality between HMGCR-mediated LDL

cholesterol levels and RCT. From the GWAS summary data of LDL cholesterol levels, a total of 7 SNPs located within or near the HMGCR gene were chosen (Supplementary Table 6). There was no notable association observed between the levels of HMGCR-mediated LDL cholesterol and the risk of RCT. Despite suggestive evidence found by IVW (OR = 0.70, CI = 0.60–0.81; P-value = 4.42×10^{-6}) and Weighted median (OR = 0.76, 95 % CI = 0.63–0.91; P-value = 3.94×10^{-3}) analyses, the beta value of MR Egger analysis does not align with the findings of IVW and Weighted median (Fig. 7A).

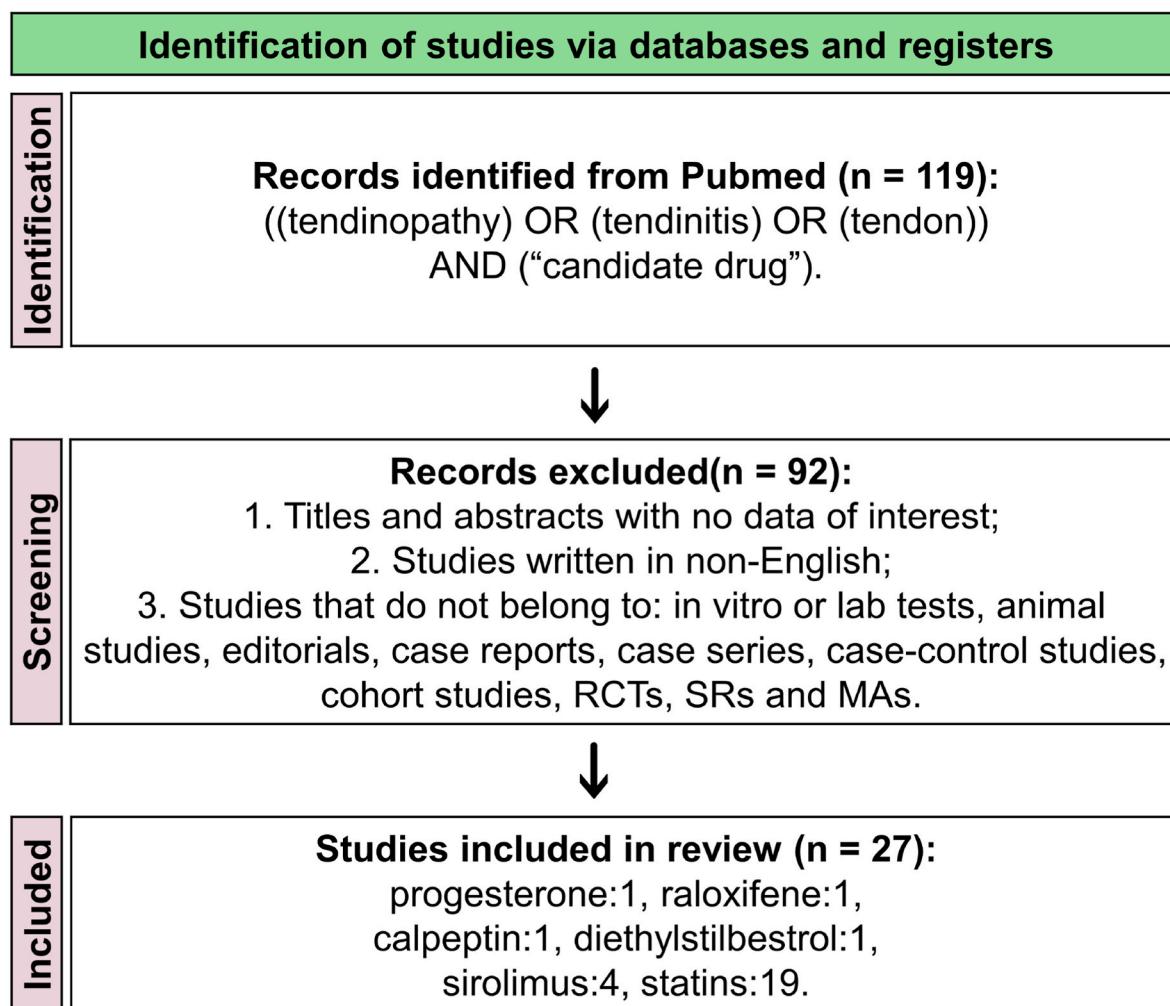
The Cochran Q test did not detect any heterogeneity in any presented results for IVW analysis (all p > 0.05; Supplementary Table 7). Both the intercept term in MR Egger regression and MR-PRESSO analysis indicated no statistically significant horizontal pleiotropy generally (all p > 0.05; Supplementary Table 7).

3.4. Validating the therapeutic potential of sirolimus target against clinical tendinopathy

Thus far, sirolimus's potential effectiveness for tendinopathy is supported by evidence from multiple levels. Sirolimus is a highly effective drug that suppresses the immune system. It works by attaching to FK-binding protein-12 and preventing the activation of the protein kinase called mammalian target of sirolimus mycin [48]. In 1999, the US Food and Drug Administration granted approval for sirolimus as a means to avoid the rejection of solid organ transplants. Since then, over the course of the previous two decades, sirolimus has been discovered to be a medicine that is well-tolerated by patients and had an outstanding safety record [48].

We studied the therapeutic efficacy of sirolimus in a tendinopathy model. Treadmill running experiments showed that the sirolimus

A



B

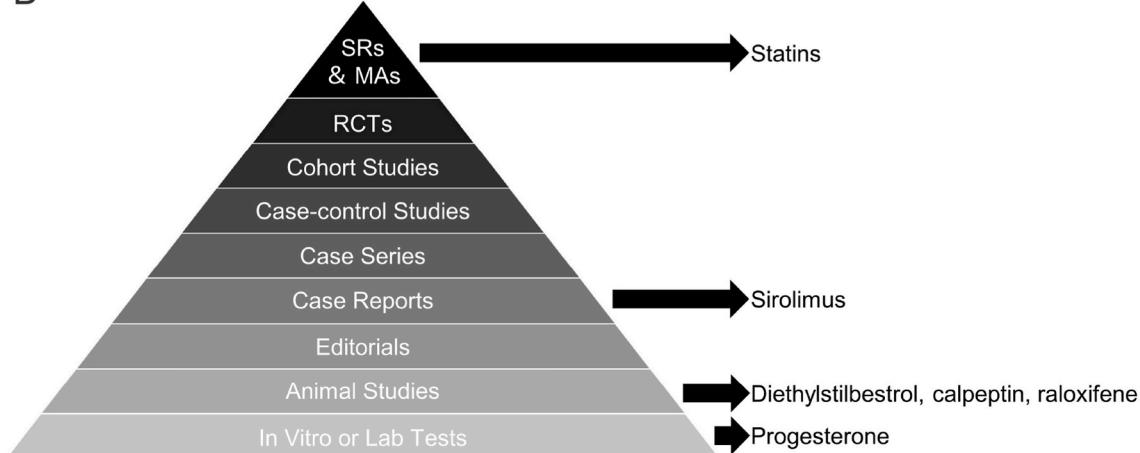


Fig. 3. Systematic review and evidence level assessment of candidate drug.

treatment group outperformed the PBS group at three time points: after two doses, after four doses, and before sacrifice. In the assessment after four doses, the functional improvement in the sirolimus treatment group compared to the PBS group was statistically significant (Fig. 8B). Histology indicated that sirolimus treated the tendon calcification induced by collagenase injection and significantly improved histological scores

(Fig. 8C and D). Subsequently, Gene Ontology enrichment analysis was performed on the gene expression differences between the sirolimus treatment group and the PBS group, revealing that the sirolimus treatment group attenuated skeletal system development and osteoblast differentiation (Fig. 8E). We constructed the protein-protein interaction network and identified key proteins in sirolimus treatment using the

Drug	Score	Target gene	Major Findings	Conclusion
Sirolimus	2.52	MTOR	1. Sirolimus is efficient in patients with systemic lupus erythematosus induced tendinopathy.(Case Series, PMID: 30787878) 2. Long-term administration of sirolimus can resist tendon aging in adult mice.(Animal Studies, PMID: 26809496) 3. Sirolimus can prevent tendon heterotopic ossification. (Animal Studies, PMID: 32494667) 4. Sirolimus treatment of tendon stem/progenitor cells reduces cellular senescence by upregulating autophagy.(In Vitro or Lab tests, PMID: 33603790)	Case Series
				Animal Studies
				In Vitro or Lab tests
Statins	1.55	HMGR ITGAL HDAC2	1. There is a paucity of evidence to implicate statin therapy as a well established risk factor or causal mechanism for tendon rupture in the general population. There is strong evidence that simvastatin reduces the risk of tendinopathy.(Systematic review, PMID: 26866552) 2. Statin treatment increases the clinical risk of tendinopathy through matrix metalloproteinase release.(Cohort study, PMID: 31784541) 3. Statin use might provide protection against rotator cuff disease in patients with hyperlipidemia.(Cohort study, PMID: 26085191) 4. There is no association between statin use and the prevalence of tendon-related injuries.(Cross-Sectional Survey, PMID: 28138920) 5. This case attributed less responsibility for the patient's tendonopathy to the statin/ezetimibe treatment.(Case Reports, PMID: 32682502)	Systematic Review
			6. The co-administration of statin and gemfibrozil should warrant prescribers' awareness of tendon-related complications of statin use. (Case Reports, PMID: 31624609)	Cohort Study
			7. Simvastatin-loaded nanofibers demonstrated effectiveness and sustainable capability for the repair of Achilles tendons. (Animal Studies, PMID: 35321025) 8. Simvastatin with PRP promotes wounded rat achilles tendon-bone interface healing In Vivo. (Animal Studies, PMID: 30668918)	Cross-Sectional Survey
	1.42	HMGR DPP4 AHR HDAC2 NR1I3	9. Statins affected the organization of the collagen fibers and decreased the biomechanical strength of the tendons, making them more predisposed to ruptures. (Animal Studies, PMID: 25544391) 10. Statins tested are associated with calcific tendinopathy risk. (Animal Studies, PMID: 26275370) 11. Simvastatin treatment does not negatively affect tendon properties. (Animal Studies, PMID: 26970227) 12. Simvastatin offered no benefit over control groups in rotator cuff repair. (Animal Studies, PMID: 27925641)	Case Reports
			13. Surface modification of the simvastatin factor-loaded silk fibroin promotes the healing of rotator cuff injury through β-catenin signaling. (Animal Studies, PMID: 33779364) 14. Statins make the tendons more prone to microdamage and ruptures. (Animal Studies, PMID: 28112540) 15. Simvastatin-loaded porous microspheres have great potential for tendon healing and restoration in Achilles tendinitis. (Animal Studies, PMID: 29534523) 16. Geranylgeranyl pyrophosphate was shown to prevent the adverse effect of simvastatin in tendon cells. (In Vitro or Lab tests, PMID: 26577051) 17. Statin administration disturbed balance in matrix production of tendon. (In Vitro or Lab tests, PMID: 28264197) 18. Statins in a dose-dependent manner decrease migration of human tendon cells, alter their expression profile and impair the functional network, but do not inhibit gap junction function. (In Vitro or Lab tests, PMID: 25846724) 19. Statins enhance rotator cuff healing by stimulating the COX2/PGE2/EP4 pathway. (In Vitro or Lab tests, PMID: 25184246)	Animal Studies
	1.30	HMGR HDAC2		In Vitro or Lab tests
Diethylstilbestrol	1.50	ESR2; ESR1 ESRRG; ESRRA ESRRB; NCOA2 NR1I2; AR; SHBG	Diethylstilbestrol improves the quality of rotator cuff repair. (Animal Studies, PMID: 33533088)	Animal Studies
Calpeptin	1.02	/	Calpain is a potential target to extend the temporal window for reconstruction of the ruptured rotator cuff tendon before recovery turns impossible. (Animal Studies, PMID: 30393967)	Animal Studies
Raloxifene	0.95	ESR1 ESR2 SERPINB9 TFF1	A combination treatment of raloxifene and vitamin D enhances bone-to-tendon healing of the rotator cuff in a rat model. (Animal Studies, PMID: 32574070)	Animal Studies
Progesterone	0.89	PGR; NR3C2 ESR1; CYP17A1 OPRK1; ORM1 NR3C1; AR SHBG; ESR2	Estrogen receptor and progesterone receptor are present in the supraspinatus tendon of patients with rotator cuff tendinopathy.(In Vitro or Lab tests, PMID: 34670550)	In Vitro or Lab tests

Research perspective			
Drug treats tendinopathy		Drug causes tendinopathy	
Effective	Ineffective	Harmless	Harmful

Fig. 4. Summary of candidate drugs with existing research evidence.

STRING database. Sirolimus can mitigate endochondral ossification through Col2a1, Col10a1, Col1a1, Dlx5. The TISSUES enrichment analysis indicates a significant association between the proteins down-regulated in the sirolimus treatment group and cartilage as well as the skeletal system (Fig. 8F).

Next, we established a connection between the models and patients using data generated from high-throughput analysis, aiming to investigate the effectiveness of sirolimus target in clinical tendinopathy. Firstly, we conducted an integrative pathway enrichment analysis to explore the potentially reversible human tendinopathy molecular

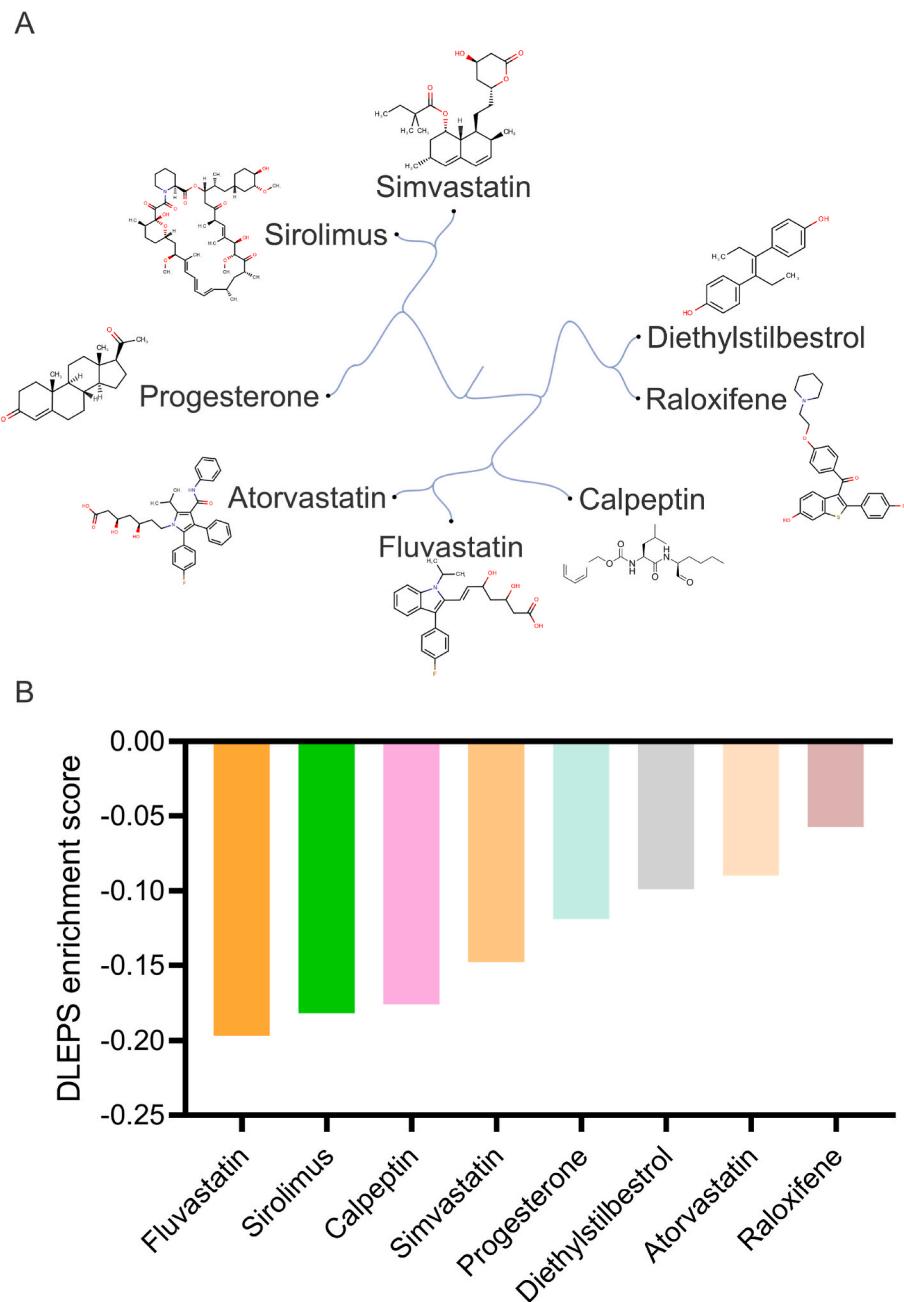


Fig. 5. Prediction of candidate drug efficacy with deep learning.

signature through mTOR inhibition. Additionally, we tried to replicate human tendinopathy biological system to observe the impact of mTOR inhibition on the occurrence and progression of tendinopathy. Additionally, we investigated the reliability of the collagenase model in replicating the human tendinopathy biological system to observe the impact of mTOR inhibition on the occurrence and progression of clinical tendinopathy.

The therapeutic impact of inhibiting mTOR was examined in a transgenic mouse model, where the mTOR gene was selectively deactivated in the tendon lineage [49]. An enrichment analysis was conducted to examine the gene expression changes between the WT and mTOR-TKO mice. The analysis confirmed the validity of mTOR tendon knockout (Fig. 9A).

To explore whether mTOR inhibition can treat clinical tendinopathy, we performed an integrative transcriptional analysis across species. We leveraged differential expression analysis data in tendinopathy patients

and mTOR-TKO mice versus healthy control, respectively. The pathway analysis was instructed to prioritize tendinopathy/mTOR-inhibition opposed feature pairs. The major findings involved the processes and pathways of cytoplasmic translation, cytoskeletal protein binding and bone morphology (Fig. 9B).

We further observed the effect of mTOR inhibition on the occurrence and development of tendinopathy *in vivo*. Firstly, we demonstrated that the collagenase injection model faithfully replicates the molecular signature of clinical tendinopathy. In order to determine the extent of similarity in gene expression between patients with tendinopathy and the collagenase model, we employed RRHO analysis to compare their transcriptional signatures. Our findings showed a substantial overlap (P -value = 1.9×10^{-109}) in the genes that were typically upregulated or downregulated, as depicted in Fig. 9C. Furthermore, our research revealed common transcriptional alterations across individuals with tendinopathy and the collagenase model ($\log_2\text{Foldchange} > 2$ & $p <$

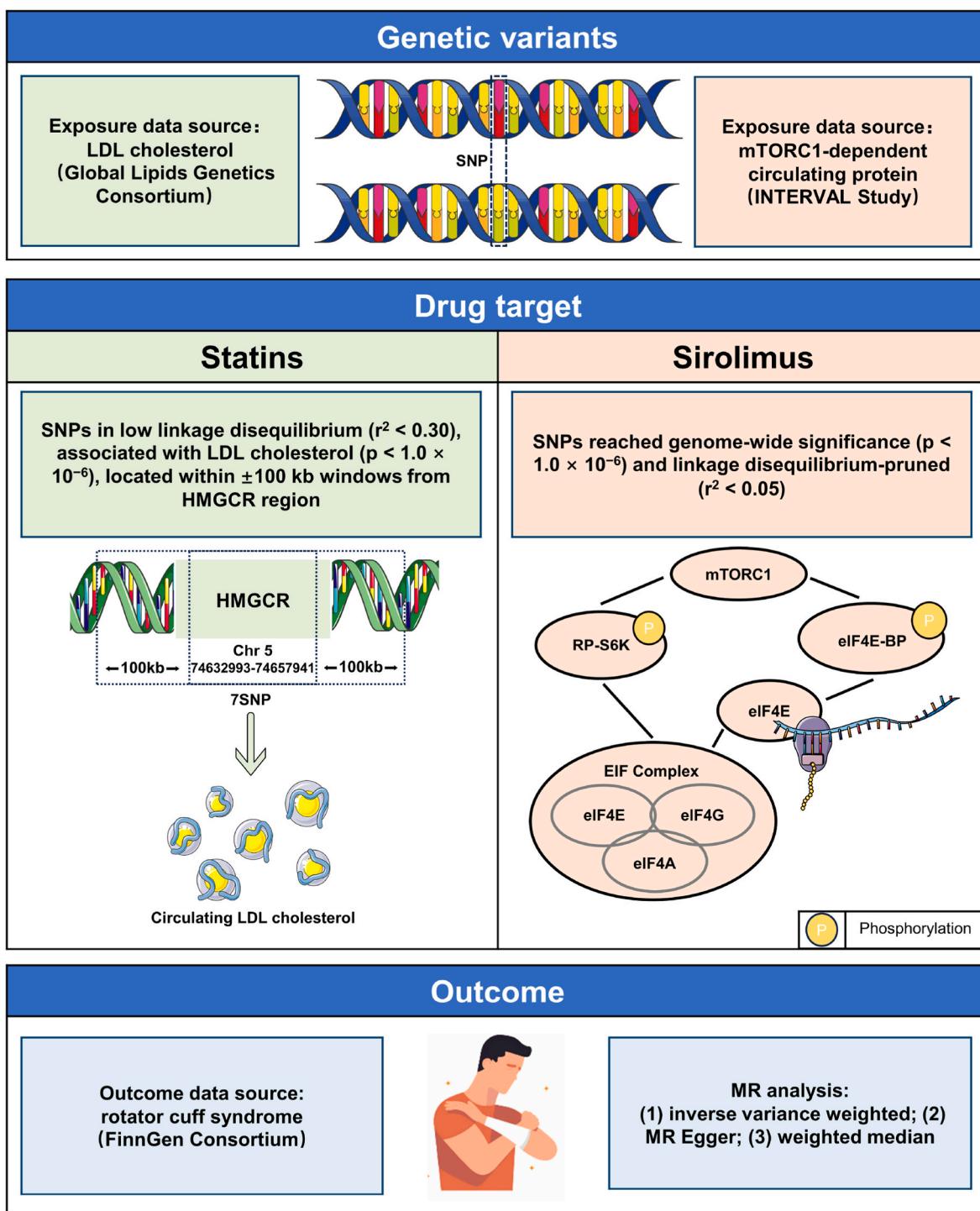
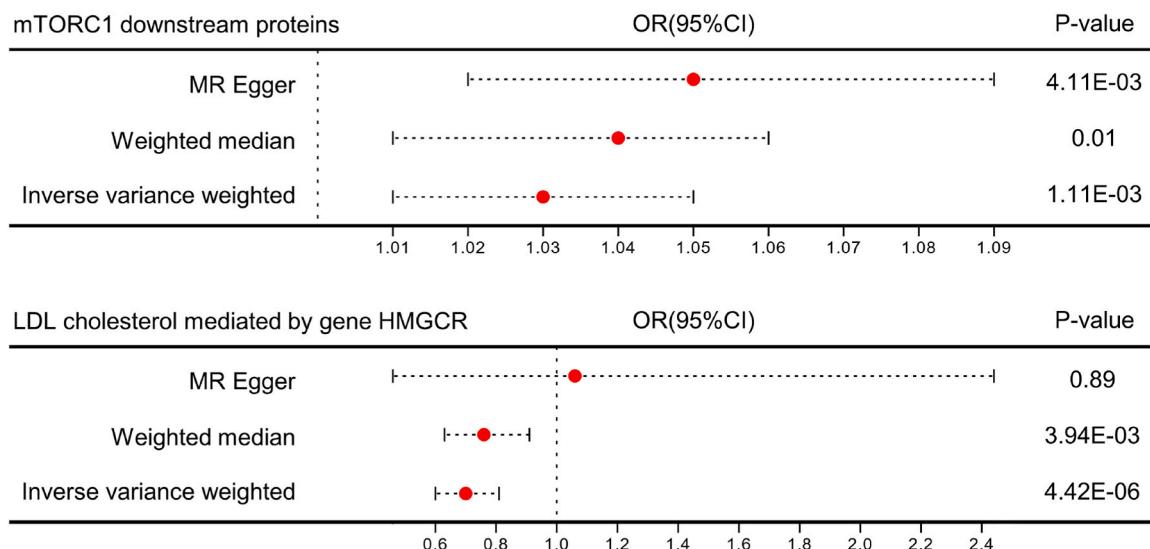
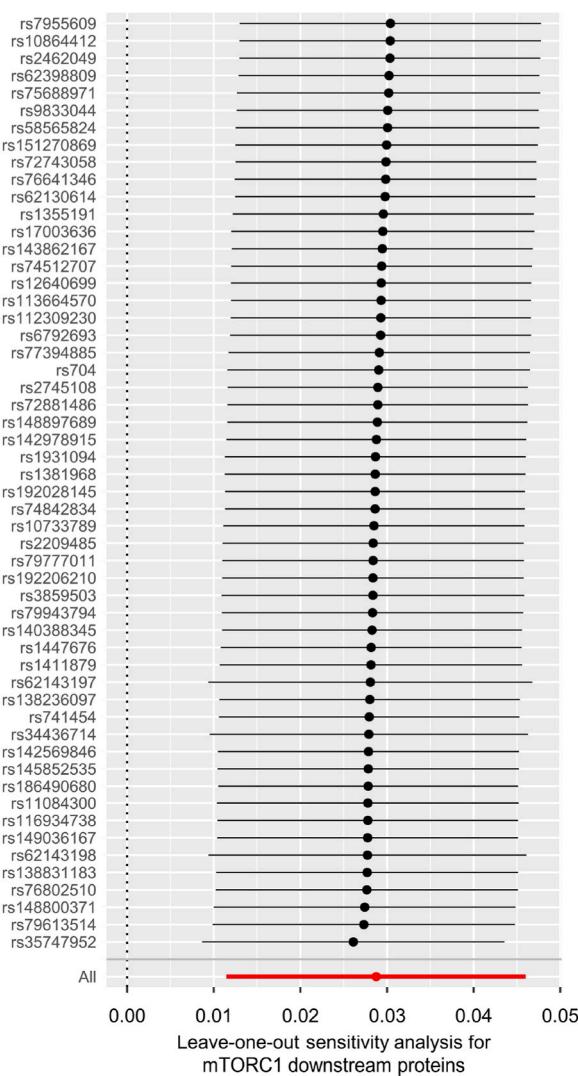
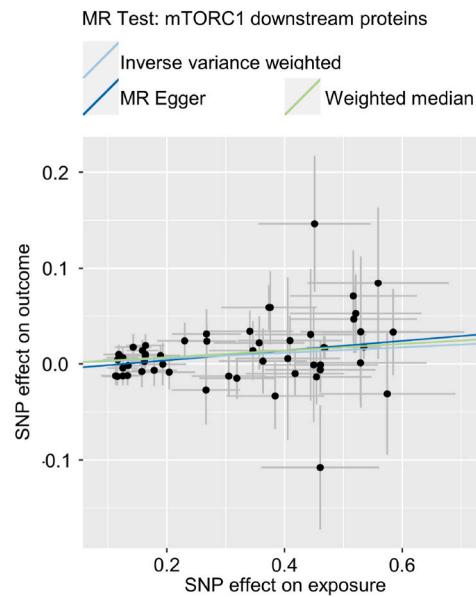
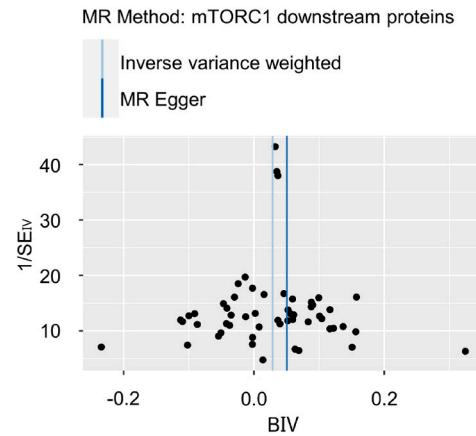


Fig. 6. Study design of MR based drug target evaluation.

0.05). The enrichment of overlap differential genes involved “Extracellular matrix organization”, “Endochondral ossification” and “Bone mineralization” (Fig. 9D). Subsequently, collagenase was administered by injection into the Achilles tendon of both wild-type (WT) mice and mTOR-TKO mice. Histological staining results showed mTOR-TKO mice exhibited enhanced resistance to tendinopathy. These findings confirmed that sirolimus had strong potential for reversing clinical tendinopathy.

4. Discussion

Our study proposed a systems biology based drug repurposing strategy by leveraging the power of tendon and drug-perturbed transcriptomics data database, pre-existing research evidence, and contemporary statistical methods for causal inference. We have proven that sirolimus is an effective medicine for treating tendinopathy, as supported by evidence from all aspects of our work. Firstly, in the transcriptional multiplex drug prediction and drug reranking, sirolimus achieved the top ranking. Secondly, consistent evidence from 1 case series, 2 animal studies, and 1 in vitro test indicated the effectiveness of

A**B****C****D**

(caption on next page)

Fig. 7. Genetic association of potential drug targets with RCT.

A, MR results for the relationship between potential drug targets and RCT. B, A sensitivity analysis was conducted to explore the potential influence of a specific SNP on the relationship between mTORC1-dependent circulating protein levels and RCT. C, Scatter graphs comparing the genetic associations between mTORC1-dependent circulating protein levels and RCT. The gradients of each line indicate the causal relationship for each approach. The blue line corresponds to the estimate obtained through inverse-variance weighting, the green line corresponds to the estimate obtained through weighted median, and the dark blue line corresponds to the estimate obtained by MR Egger method. D, Funnel plot used to evaluate heterogeneity. The blue line shows the estimate calculated using inverse variance weighting, whereas the dark blue line reflects the estimate calculated using the MR Egger method. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sirolimus. Thirdly, sirolimus performed excellently in deep learning based drug efficacy prediction. Fourthly, genetically predicted plasma levels of downstream proteins inhibited by sirolimus increased tendinopathy risk. Fifthly, after sirolimus treatment, the motor function and histological score of animal models were significantly improved. Sixthly, mTOR-TKO mice exhibited enhanced resistance to tendinopathy. The significance of our findings is especially relevant for patients suffering from calcific tendinopathy. Through integrative pathway enrichment analysis, we identified the clinical tendinopathy pathways that can be reversed by mTOR inhibition. A substantial enrichment of the bone morphology-related pathways indicated that sirolimus may provide potential therapeutic benefits in ameliorating heterotopic ossification in clinical tendinopathy.

Despite the high prevalence and incidence of tendinopathy, effective methods for disease relief are currently lacking. Severe tendinopathy can be treated with surgical interventions, but the outcomes are often unsatisfactory [50]. Conventional drug treatments are limited to symptomatic relief, with uncertain efficacy [51]. Our results highlight the potential clinical application of currently available FDA-approved mTOR inhibitors, which could potentially be repurposed for conservative treatment of rotator cuff tendinopathy, fundamentally slowing the progression of tendinopathy.

We employed many methodologies to offer a holistic outlook on pharmacological repurposing. Transcriptomics drug prediction is based on the principle of signature reversion (SRP), which assumes that if a treatment can reverse the expression pattern of genes that are characteristic of a specific disease phenotype, then it may have the ability to reverse the disease phenotype itself [8]. SRP has effectively been utilized to discover new possibilities for repurposing drugs across several fields of therapy [52–54]. However, the drug-perturbed transcriptomics profiles are obtained from cells [55]. This is perhaps the most fundamental limitation in transcriptomics drug prediction, as much of physiology and pharmacology is non-cell-autonomous. We applied systematic review to supplement the evidence of drugs at the level of tissues and humans. The focused attention on drugs supported by clinical evidence allowed us to efficiently identify candidate drugs. However, it inevitably led to the omission of certain drugs. MR provides a rapid and affordable approach to evaluating causal questions [56]. Genetic variants in MR are used as instrumental variables to examine the possible impact of drug target on disease outcomes in large observational datasets. These variants can be seen as proxies for an intervention on the drug target [57]. However, there is a difference between genetic effect and pharmacological effect. Genetic variants that represent the effects of drugs are the result of tiny changes in drug targets over a person's lifetime. This is different from a pharmacological intervention in later life, which usually has a larger effect but for a shorter duration [35]. It is for exactly this reason that MR estimates of genetically proxied drug effects are typically greater in magnitude than those observed in clinical practice [58]. Although these restraints make the effectiveness of sirolimus warrants further investigation, the consistency between findings supports the robustness of our conclusions.

The goal of systems biology is to comprehend physiology and disease from the level of molecules, pathways, cells, tissues, organs and ultimately the whole organism [10]. Our drug repurposing strategy employs diverse approaches that span multiple levels to adequately consider the biological complexity of tendinopathy. Three principal approaches in systems biology are plenarily employed in this study: large-scale data

generation and mining; in silico simulation; and the use of complex biological systems to assay and model biology [9]. Firstly, the generated profiling data from high-throughput analysis was utilized to: 1) define molecular characteristics of tendinopathy, 2) acquire genetic association data and predict the causal relationship between tendinopathy and drug targets, and 3) establish comprehensive connections between animal models and patients. Secondly, complementary in silico simulations enabled us to integrate diverse information, leading to a sophisticated candidate drug list. Thirdly, we took biological responses as a cornerstone in drug prediction (drug-perturbed transcriptomics profiles from cells) and drug verification (biological system recapitulation, cross-species integration analysis).

Nevertheless, this study has some limitations. Our tendinopathy samples were from the rotator cuff, while healthy samples were from the Achilles tendon. The ideal control for this study would have been a matched number of healthy supraspinatus tendon samples; however, in clinical practice, opportunities to collect these samples without impacting patient health are extremely rare. This study conducted a large-scale ($n > 100$) transcriptome analysis of clinical tendinopathy patients, involving only a single-center cohort of RCT patients. Therefore, the lack of reliable RNA-seq datasets from other sources limits the reproducibility of tendinopathy gene signatures. Besides, we did not consider the disease heterogeneity of tendinopathy. Additional investigation is necessary to examine the clinical characteristics and molecular classifications of patients who may experience positive outcomes from sirolimus treatment.

5. Conclusions

Our study assimilates knowledge of system-level responses to identify potential drugs for tendinopathy. Sirolimus, a commonly used mTOR inhibitor with a recognized safety profile, has the potential to be repurposed for the treatment of tendinopathy. This could offer a quick road to clinical application. Drug repositioning based on systems biology identifies and targets biologically significant pathways that are essential and can be effectively addressed with pharmacological interventions. We have presented a approach for the repositioning of medications and the development of therapies that are tailored to specific diseases based on their characteristics.

CRediT authorship contribution statement

Zetao Wang: Writing – original draft, Resources, Methodology, Data curation, Conceptualization. **Junchao Luo:** Methodology. **Luyong Jiang:** Investigation. **Chenqi Tang:** Resources. **Yangwu Chen:** Resources. **Kun Yang:** Investigation. **Zicheng Wang:** Investigation. **Jiabao Dong:** Investigation. **Xiao Chen:** Resources. **Zi Yin:** Writing – review & editing. **Jianyou Li:** Resources. **Weiliang Shen:** Writing – review & editing, Supervision, Conceptualization.

Availability of data and materials

Lead contact: Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Weiliang Shen (wlschen@zju.edu.cn).

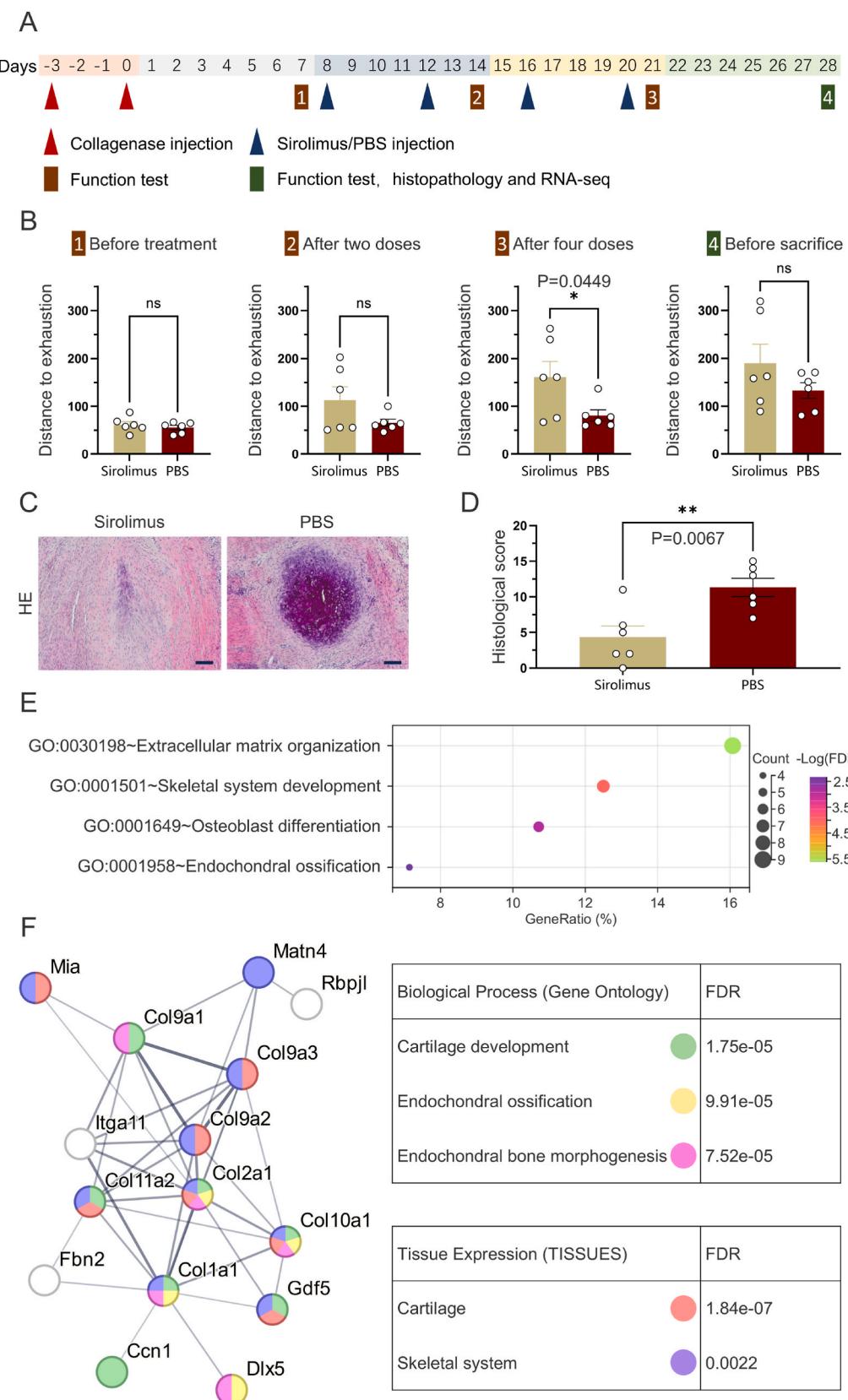
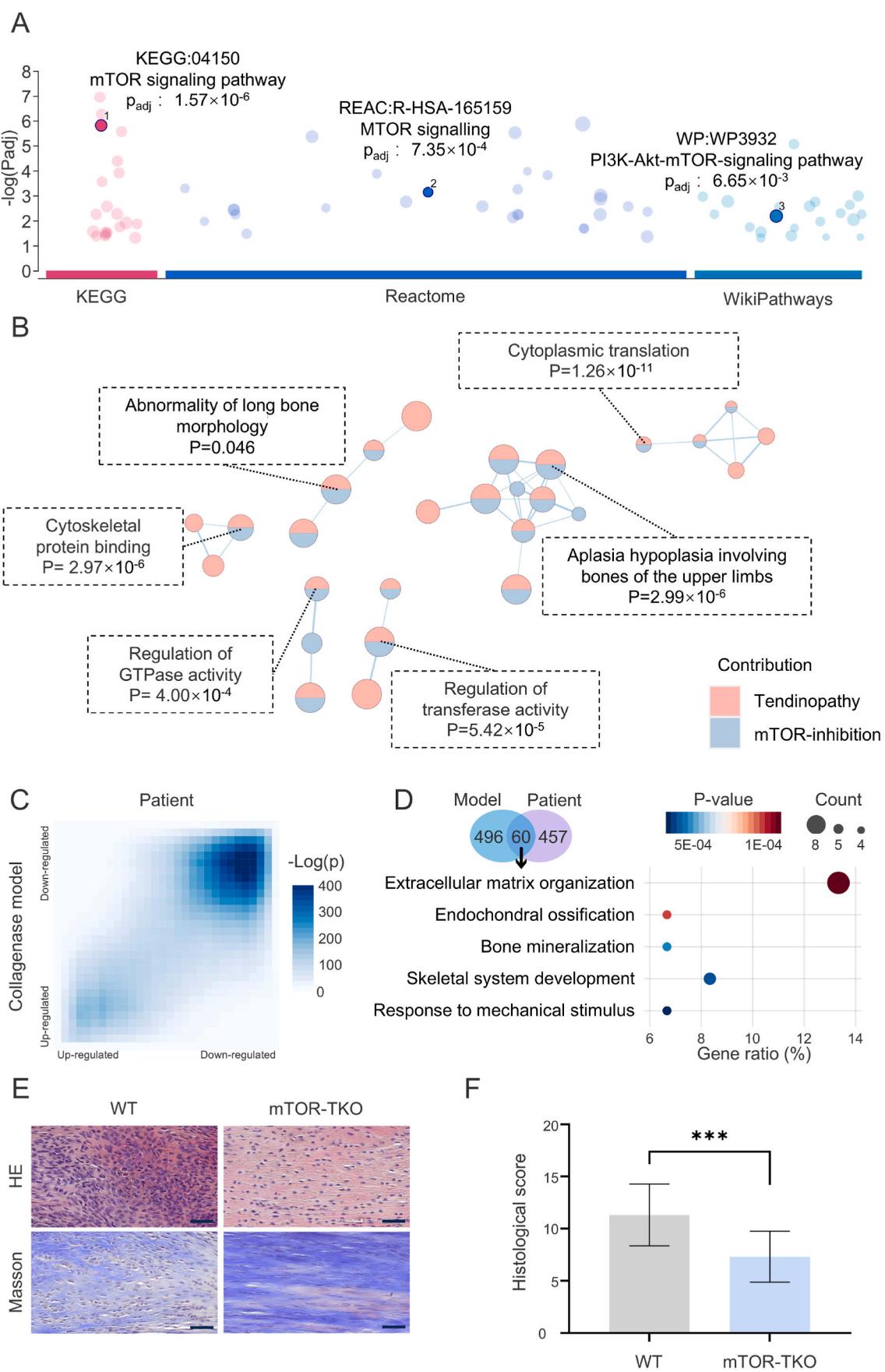


Fig. 8. In vivo assessment of sirolimus in a tendinopathy rat model. A, Overview of the In vivo study design. The rats were divided into 2 groups, receiving either sirolimus or PBS injection. B, Running distance of each group during the run-to-exhaustion test. C and D, HE staining and histological score evaluation were performed on sirolimus and PBS group. n = 6. Scale bar, 100 μ m. E, GO analysis of differentially expressed genes ($P_{adj} < 0.05$; $\log_2\text{Foldchange}$ (sirolimus/PBS) < -1). F, Defining protein-protein interaction network of differentially expressed genes. Edges indicated both functional and physical protein associations, and their thickness indicated the strength of data support.



(caption on next page)

Fig. 9. mTOR-TKO mice exhibited enhanced resistance to tendinopathy.

A, Enrichment results for the differential genes between mTOR-TKO mice and WT mice. B, The enrichment map displays the pathways that are more abundant in tendinopathy patients compared to healthy control individuals, as well as in wild-type mice compared to mTOR-TKO mice. The nodes in the network symbolize pathways, and routes that share numerous genes are linked together. The nodes are assigned colors based on the supporting data from tendinopathy and mTOR-inhibition characteristics. Multicolored nodes indicate opposed pathways between tendinopathy/mTOR-inhibition. C, RRHO map displays transcriptional overlaps between tendinopathy patients and the collagenase model. The signals in the bottom left and upper right quadrants indicate an overlap for genes that are typically upregulated and commonly downregulated, respectively. The color bar indicates the level of statistical significance (-log (Padjust -value); FDR adjusted; two-tailed) of the overlap between transcriptional signatures in patients with tendinopathy and the collagenase model. D, The intersecting representative differential genes of tendinopathy patients versus healthy controls and the collagenase model versus healthy controls ($P < 0.05$, $\text{Log}_2(\text{Foldchange}) > 2$). The top five GO annotations for overlap genes are displayed. E and F, HE, Masson staining and histological score evaluation were performed on WT and mTOR-TKO mice. $n = 8$. Scale bar, 50 μm . WT, wild-type; mTOR-TKO mTOR tendon knockout. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Materials availability

This study did not generate new unique reagents.

Data and code availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: [CRA014406](#), GSA: [HRA006534](#)) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Ethics statement

The patient's consent and clearance from the local ethics committee were acquired (Ethics Committee of the second Affiliated Hospital, School of Medicine, Zhejiang University, code: 2019-168, 2020-080). The Zhejiang University Experimental Animal Welfare and Ethics Committee approved animal studies in accordance with the guidelines of Institutional Animal Care and Use Committee (ZJU20220006).

Funding

This work was supported by the National key research and development program of China (2022YFA1106800), NSFC grants (T2121004, 82072463, 82222044, 82372376), Medical Health Science and Technology Project of Zhejiang Provincial Health Commission (2022RC161, 2024KY409), Zhejiang Provincial Program for the Cultivation of High-level Innovative Health talents, Zhejiang Lingyan project (2024C03207), Science and Technology Project of Hu Zhou City (2022GZ65), Dr Li Dak Sum & Yip Yio Chin Regeneration Medicine Foundation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors thank Global Lipids Genetics Consortium, INTERVAL study and FinnGen consortium for sharing the summary-level data. Figures created with smart.servier.com.

Abbreviations

RCT: Rotator cuff tendinopathy; MR: Mendelian randomization; WT: Wild-type; mTOR-TKO: mTOR tendon knockout; HE: hematoxylin and eosin; DSS: Drug specificity score; CoDReS: Computational Drug

Repositioning Score; RCTs: Randomized controlled trials; SRs: Systematic reviews; MAs: Meta-analyses; SMILES: Simplified Molecular Input Line Entry System; DLEPS: Deep Learning based Efficacy Prediction System; GWAS: Genome-wide association; ICD-10: International classification of diseases-10; SNPs: single nucleotide polymorphisms; LDL: low-density lipoprotein; GLGC: Global Lipids Genetics Consortium; RRHO: rank-rank hypergeometric overlap; IVW: Inverse variance weighted; OR: odds ratio; CI: 95 % confidence interval; SRP: signature reversion principle.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.combiomed.2025.109665>.

References

- [1] N.L. Millar, K.G. Silbernagel, K. Thorborg, P.D. Kirwan, L.M. Galatz, G.D. Abrams, et al., Tendinopathy, *Nat Rev Dis Primers* 7 (1) (2021) 1.
- [2] R.A. Pedowitz, K. Yamaguchi, C.S. Ahmad, R.T. Burks, E.L. Flatow, A. Green, J. P. Iannotti, B.S. Miller, R.Z. Tashjian, W.C. 3rd Watters, et al., Optimizing the management of rotator cuff problems, *J. Am. Acad. Orthop. Surg.* 19 (2011) 368–379.
- [3] S. Cederqvist, T. Flinkkilä, M. Sormala, J. Ylinen, H. Kautiainen, T. Irmola, H. Lehtokangas, J. Litakonen, K. Pamilo, T. Ridanpää, et al., Non-surgical and surgical treatments for rotator cuff disease: a pragmatic randomised clinical trial with 2-year follow-up after initial rehabilitation, *Ann. Rheum. Dis.* 80 (2021) 796–802.
- [4] M.C. Ranebo, H.C. Björnsson Hallgren, T. Holmgren, L.E. Adolfsson, Surgery and physiotherapy were both successful in the treatment of small, acute, traumatic rotator cuff tears: a prospective randomized trial, *J. Shoulder Elbow Surg.* 29 (2020) 459–470.
- [5] E.A. Malavolta, M. Gracitelli, J.H. Assunção, A.A. Ferreira Neto, M. Bordalo-Rodrigues, O.P. de Camargo, Clinical and structural evaluations of rotator cuff repair with and without added platelet-rich plasma at 5-year follow-up: a prospective randomized study, *Am. J. Sports Med.* 46 (2018) 3134–3141.
- [6] T.T. Ashburn, K.B. Thor, Drug repositioning: identifying and developing new uses for existing drugs, *Nat. Rev. Drug Discov.* 3 (2004) 673–683.
- [7] N. Nosengo, Can you teach old drugs new tricks, *Nature* 534 (2016) 314–316.
- [8] S. Pushpakom, F. Iorio, P.A. Eyers, et al., Drug repurposing: progress, challenges and recommendations, *Nat. Rev. Drug Discov.* 18 (1) (2019) 41–58.
- [9] E.C. Butcher, E.L. Berg, E.J. Kunkel, Systems biology in drug discovery, *Nat. Biotechnol.* 22 (10) (2004) 1253–1259.
- [10] B. Turanli, O. Altay, J. Borén, et al., Systems biology based drug repositioning for development of cancer therapy, *Semin. Cancer Biol.* 68 (2021) 47–58.
- [11] Y. Chen, et al., Targeted pathological collagen delivery of sustained-release rapamycin to prevent heterotopic ossification, *Sci. Adv.* 6 (2020) eaay9526.
- [12] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks, *Genome Res.* 13 (2003) 2498–2504.
- [13] J. Lamb, E.D. Crawford, D. Peck, J.W. Modell, I.C. Blat, M.J. Wrobel, J. Lerner, J. P. Brunet, A. Subramanian, K.N. Ross, et al., The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease, *Science* 313 (2006) 1929–1935.
- [14] K. Troulé, H. López-Fernández, S. García-Martín, M. Reboiro-Jato, C. Carretero-Puche, J. Martorell-Marugán, G. Martín-Serrano, P. Carmona-Sáez, D. Glez-Peña, F. Al-Shahrour, et al., DREIMT: a drug repositioning database and prioritization tool for immunomodulation, *Bioinformatics* 37 (2021) 578–579.
- [15] G. Williams, SPIEDw: a searchable platform-independent expression database web tool, *BMC Genom.* 14 (2013) 765.
- [16] E. Karatzas, G. Minadakis, G. Kolios, A. Delis, G.M. Spyrou, A web tool for ranking candidate drugs against a selected disease based on a combination of functional and structural criteria, *Comput. Struct. Biotechnol. J.* 17 (2019) 939–945.

- [17] J. Zhu, J. Wang, X. Wang, M. Gao, B. Guo, M. Gao, J. Liu, Y. Yu, L. Wang, W. Kong, et al., Prediction of drug efficacy from transcriptional profiles with deep learning, *Nat. Biotechnol.* 39 (2021) 1444–1452.
- [18] B. Ravikumar, Z. Alam, G. Peddinti, T. Aittokallio, C-SPADE: a web-tool for interactive analysis and visualization of drug screening experiments through compound-specific bioactivity dendograms, *Nucleic Acids Res.* 45 (2017) W495–W500.
- [19] D. Szklarczyk, et al., The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest, *Nucleic Acids Res.* 51 (2023) D638–D646.
- [20] S.B. Plaisier, R. Taschereau, J.A. Wong, T.G. Graeber, Rank-rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures, *Nucleic Acids Res.* 38 (2010) e169.
- [21] M. Paczkowska, J. Barenboim, N. Sintupisut, N.S. Fox, H. Zhu, D. Abd-Rabbo, M. W. Mee, P.C. Boutros, J. Reimand, Integrative pathway enrichment analysis of multivariate omics data, *Nat. Commun.* 11 (2020) 735.
- [22] G. Hemani, J. Zheng, B. Elsworth, K.H. Wade, V. Haberland, D. Baird, C. Laurin, S. Burgess, J. Bowden, R. Langdon, et al., The MR-Base platform supports systematic causal inference across the human genome, *Elife* 7 (2018) e34408.
- [23] C. Trapnell, L. Pachter, S.L. Salzberg, TopHat: discovering splice junctions with RNA-Seq, *Bioinformatics* 25 (2009) 1105–1111.
- [24] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, *Genome Biol.* 15 (2014) 550.
- [25] C. Knox, M. Wilson, C.M. Klinger, M. Franklin, E. Oler, A. Wilson, J. Cox, N. Chin, S.A. Strawbridge, et al., DrugBank 6.0: the DrugBank knowledgebase for 2024, *Nucleic Acids Res.* (2023) gkad976 [pii].
- [26] S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P. A. Thiessen, B. Yu, et al., PubChem in 2021: new data content and improved web interfaces, *Nucleic Acids Res.* 49 (2021) D1388–D1395.
- [27] M.I. Kurki, J. Karjalainen, P. Palta, T.P. Sipilä, K. Kristiansson, K.M. Donner, M. P. Reeve, H. Laivuori, M. Aavikko, M.A. Kaunisto, et al., FinnGen provides genetic insights from a well-phenotyped isolated population, *Nature* 613 (2023) 508–518.
- [28] C.J. Willer, E.M. Schmidt, S. Sengupta, G.M. Peloso, S. Gustafsson, S. Kanoni, A. Ganna, J. Chen, M.L. Buchkovich, S. Mora, et al., Discovery and refinement of loci associated with lipid levels, *Nat. Genet.* 45 (2013) 1274–1283.
- [29] B.B. Sun, J.C. Maranville, J.E. Peters, D. Stacey, J.R. Staley, J. Blackshaw, S. Burgess, T. Jiang, E. Paige, P. Surendran, et al., Genomic atlas of the human plasma proteome, *Nature* 558 (2018) 73–79.
- [30] D. Ruan, Y. Fei, S. Qian, Z. Huang, W. Chen, C. Tang, et al., Early-stage primary anti-inflammatory therapy enhances the regenerative efficacy of platelet-rich plasma in a rabbit Achilles tendinopathy model, *Am. J. Sports Med.* 49 (12) (2021) 3357–3371.
- [31] M.B. Brown, 400: a method for combining non-independent, one-sided tests of significance, *Biometrics* 31 (1975) 987–992.
- [32] J. Reimand, M. Kull, H. Peterson, J. Hansen, J. Vilo, g:Profiler—a web-based toolset for functional profiling of gene lists from large-scale experiments, *Nucleic Acids Res.* 35 (2007) W193–W200.
- [33] J. Reimand, R. Isserlin, V. Voisin, M. Kucera, C. Tannus-Lopes, A. Rostamianfar, L. Wadi, M. Meyer, J. Wong, C. Xu, et al., Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap, *Nat. Protoc.* 14 (2019) 482–517.
- [34] L. He, J.F. Heyse, Improved power of familywise error rate procedures for discrete data under dependency, *Biom. J.* 61 (2019) 101–114.
- [35] D. Gill, M.K. Georgakis, V.M. Walker, A.F. Schmidt, A. Gkatzionis, D.F. Freitag, C. Finan, A.D. Hingorani, J. Howson, S. Burgess, et al., Mendelian randomization for studying the effects of perturbing drug targets, *Wellcome open research* 6 (2021) 16.
- [36] J. Song, A. Li, Y. Qian, B. Liu, L. Lv, D. Ye, X. Sun, Y. Mao, Genetically predicted circulating levels of cytokines and the risk of cancer, *Front. Immunol.* 13 (2022) 886144.
- [37] J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression, *Int. J. Epidemiol.* 44 (2015) 512–525.
- [38] M. Verbanck, C.Y. Chen, B. Neale, R. Do, Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases, *Nat. Genet.* 50 (2018) 693–698.
- [39] X. Chen, J. Kong, X. Diao, J. Cai, J. Zheng, W. Xie, H. Qin, J. Huang, T. Lin, Depression and prostate cancer risk: a Mendelian randomization study, *Cancer Med.* 9 (2020) 9160–9167.
- [40] A.J. Teichtahl, S.R. Brady, D.M. Urquhart, A.E. Wluka, Y. Wang, J.E. Shaw, F. M. Cicuttini, Statins and tendinopathy: a systematic review, *Med. J. Aust.* 204 (2016) 115–121.e1.
- [41] T.T. Lin, C.H. Lin, C.L. Chang, C.H. Chi, S.T. Chang, W.H. Sheu, The effect of diabetes, hyperlipidemia, and statins on the development of rotator cuff disease: a nationwide, 11-year, longitudinal, population-based follow-up study, *Am. J. Sports Med.* 43 (2015) 2126–2132.
- [42] P. Eliasson, F. Dietrich-Zagone, A.C. Lundin, P. Aspenberg, A. Wolk, K. Michaelsson, Statin treatment increases the clinical risk of tendinopathy through matrix metalloproteinase release - a cohort study design combined with an experimental study, *Sci. Rep.* 9 (2019) 17958.
- [43] F. Kaleağasıoğlu, E. Olcay, V. Olgaç, Statin-induced calcific Achilles tendinopathy in rats: comparison of biomechanical and histopathological effects of simvastatin, atorvastatin and rosuvastatin, *Knee Surg. Sports Traumatol. Arthrosc.* 25 (2017) 1884–1891.
- [44] P. Eriksson, P. Wallin, C. Sjöwall, Clinical experience of sirolimus regarding efficacy and safety in systemic lupus erythematosus, *Front. Pharmacol.* 10 (2019) 82.
- [45] L.W. Zaseck, R.A. Miller, S.V. Brooks, Rapamycin attenuates age-associated changes in tibialis anterior tendon viscoelastic properties, *J. Gerontol. A Biol. Sci. Med. Sci.* 71 (2016) 858–865.
- [46] D. Nie, J. Zhang, Y. Zhou, J. Sun, W. Wang, J.H. Wang, Rapamycin treatment of tendon stem/progenitor cells reduces cellular senescence by upregulating autophagy, *Stem Cells Int.* 2021 (2021) 6638249.
- [47] S. Burgess, S.G. Thompson, Avoiding bias from weak instruments in Mendelian randomization studies, *Int. J. Epidemiol.* 40 (2011) 755–764.
- [48] S.N. Sehgal, Sirolimus: its discovery, biological properties, and mechanism of action, *Transplant. Proc.* 35 (2003) 7S–14S.
- [49] X.X. Cong, X.S. Rao, J.X. Lin, X.C. Liu, G.A. Zhang, X.K. Gao, M.Y. He, W.L. Shen, W. Fan, D. Pioletti, et al., Activation of AKT-mTOR signaling directs tenogenesis of mesenchymal stem cells, *Stem Cell.* 36 (2018) 527–539.
- [50] C. Zhang, Y.Z. Cai, Y. Wang, Injection of leukocyte-poor platelet-rich plasma for moderate-to-large rotator cuff tears does not improve clinical outcomes but reduces retear rates and fatty infiltration: a prospective, single-blinded randomized study, *Arthroscopy* 38 (2022) 2381–2388.e1.
- [51] T. Cook, C. Minns Lowe, M. Maybury, J.S. Lewis, Are corticosteroid injections more beneficial than anaesthetic injections alone in the management of rotator cuff-related shoulder pain? A systematic review, *Br. J. Sports Med.* 52 (2018) 497–504.
- [52] S.D. Kunkel, M. Suneja, S.M. Ebert, K.S. Bongers, D.K. Fox, S.E. Malmberg, F. Alipour, R.K. Shields, C.M. Adams, mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass, *Cell Metab.* 13 (2011) 627–638.
- [53] Y.Y. Hsieh, C.J. Chou, H.L. Lo, P.M. Yang, Repositioning of a cyclin-dependent kinase inhibitor GW8510 as a ribonucleotide reductase M2 inhibitor to treat human colorectal cancer, *Cell Death Discov.* 2 (2016) 16027.
- [54] B. Malcomson, H. Wilson, E. Veglia, G. Thillaiyampalam, R. Barsden, S. Donegan, A. El Banna, J.S. Elborn, M. Ennis, C. Kelly, et al., Connectivity mapping (ssCMap) to predict A20-inducing drugs and their antiinflammatory action in cystic fibrosis, *Proc. Natl. Acad. Sci. U.S.A.* 113 (2016) E3725–E3734.
- [55] A. Subramanian, R. Narayan, S.M. Corsello, D.D. Peck, T.E. Natoli, X. Lu, J. Gould, J.F. Davis, A.A. Tubelli, J.K. Asiedu, et al., A Next generation connectivity map: L1000 platform and the first 1,000,000 profiles, *Cell* 171 (2017) 1437–1452.e17.
- [56] C.A. Emdin, A.V. Khera, S. Kathiresan, Mendelian randomization, *JAMA* 318 (2017) 1925–1926.
- [57] B.A. Ference, Interpreting the clinical implications of drug-target mendelian randomization studies, *J. Am. Coll. Cardiol.* 80 (2022) 663–665.
- [58] S. Burgess, A. Butterworth, A. Malarstig, S.G. Thompson, Use of Mendelian randomisation to assess potential benefit of clinical intervention, *BMJ (Clinical research ed.)* 345 (2012) e7325.