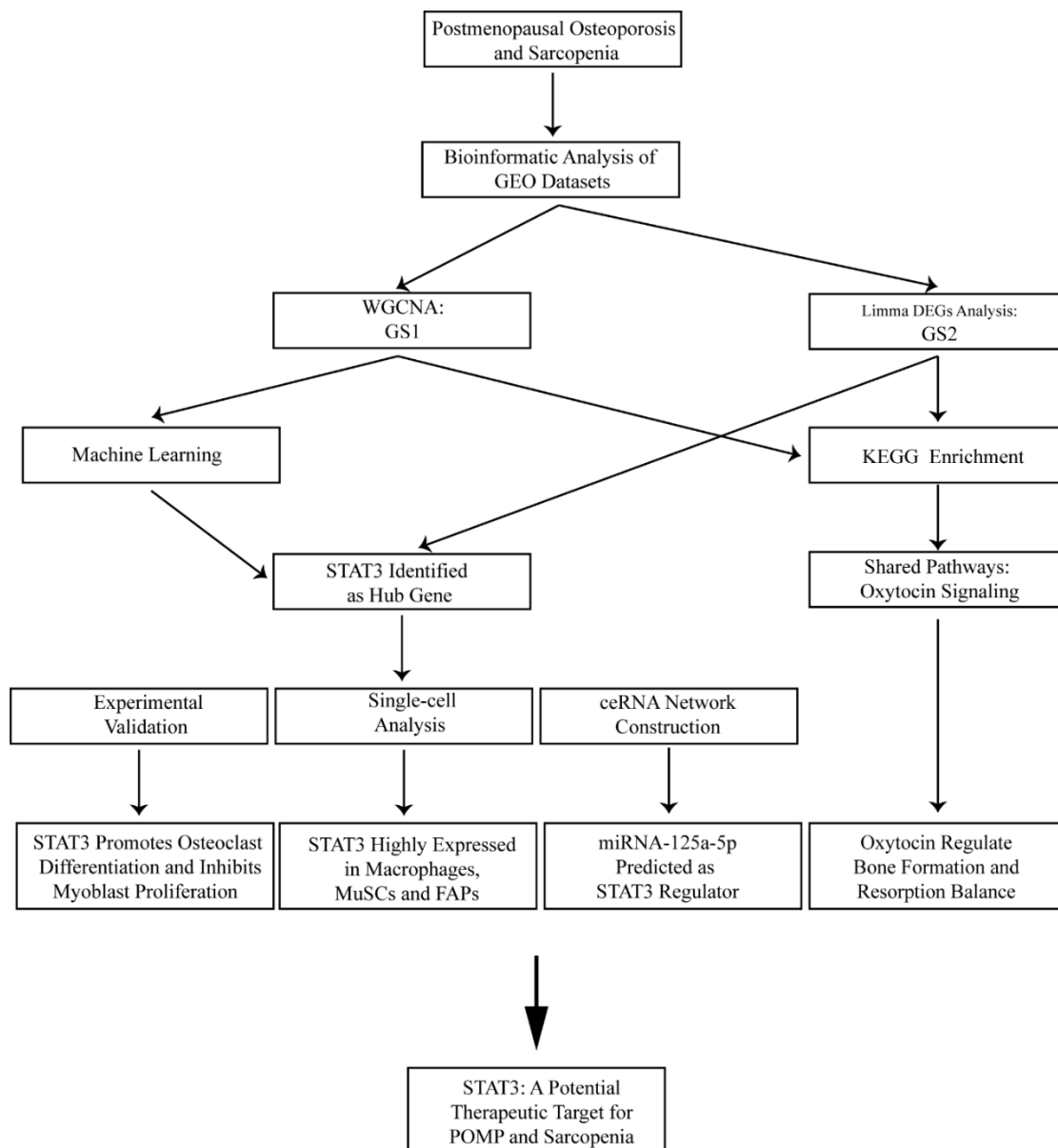# Bone & Joint Research

Supplementary Material

Fig a. Workflow diagram summarizing the study design and bioinformatic analyses. The study began with the clinical problem of postmenopausal osteoporosis and sarcopenia comorbidity. Datasets

from the Gene Expression Omnibus (GEO) database were analyzed using weighted gene co-expression network analysis (WGCNA) to identify gene co-expression modules related to the two conditions (GS1). Machine learning and differential expression analysis in validation datasets further narrowed down the gene list (GS2). The hub gene signal transducer and activator of transcription 3 (*STAT3*) was identified from the overlap. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment highlighted shared pathways like oxytocin signalling. Single-cell RNA sequencing revealed cell types with high *STAT3* expression in each disease. A ceRNA network predicted miRNA-125a-5p as a potential regulator of *STAT3*. Finally, in vitro experiments confirmed the effects of *STAT3* on osteoclast differentiation and myoblast proliferation. The results implicate *STAT3* as a promising therapeutic target for the comorbidity. DEG, differentially expressed gene; FAP, fibro-adipogenic progenitor; miRNA, microRNA; MuSC, muscle stem cell.
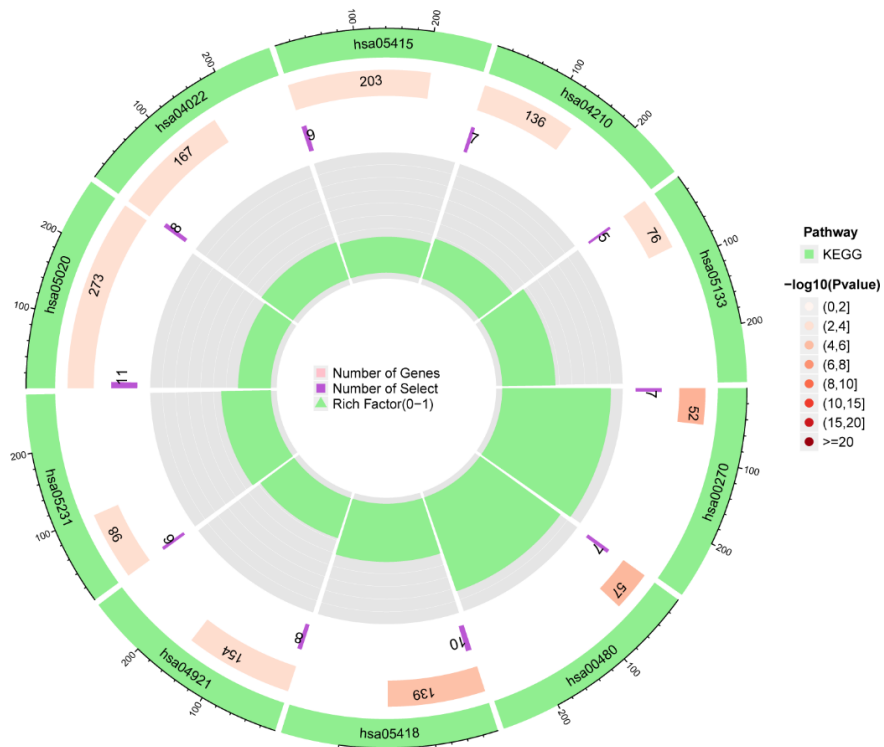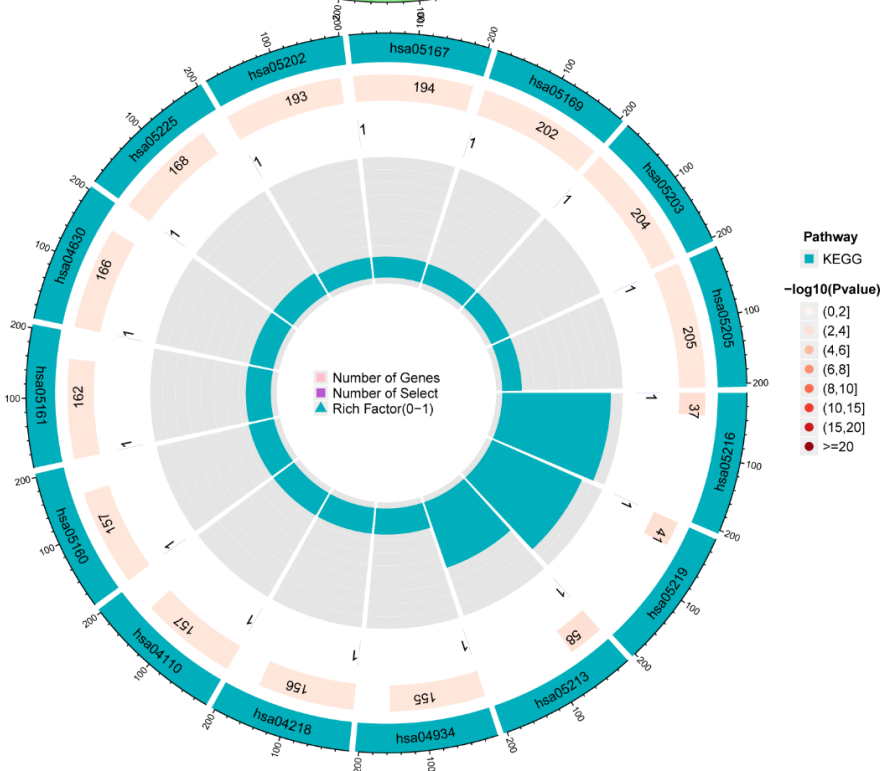
Fig b. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for a) gene set 1 (GS1) and b) gene set 2 (GS2). The number of genes and gene ratio for each enriched pathway

are shown in the dot plot, with colour indicating adjusted p-value (hypergeometric test). a) Pathways enriched in the weighted gene co-expression network analysis (WGCNA) module genes overlapping between postmenopausal osteoporosis (POMP) and sarcopenia (GS1) datasets. The oxytocin signalling pathway is notably enriched, suggesting its involvement in the pathogenesis of both conditions. b) Pathways enriched in the differentially expressed genes common to the validation POMP and sarcopenia datasets (GS2). The oxytocin signalling pathway is again enriched, confirming its relevance. Other pathways such as thyroid cancer and bladder cancer may reflect general disease processes. The shared enrichment of the oxytocin pathway in both gene sets supports its potential mechanistic role linking the two diseases.
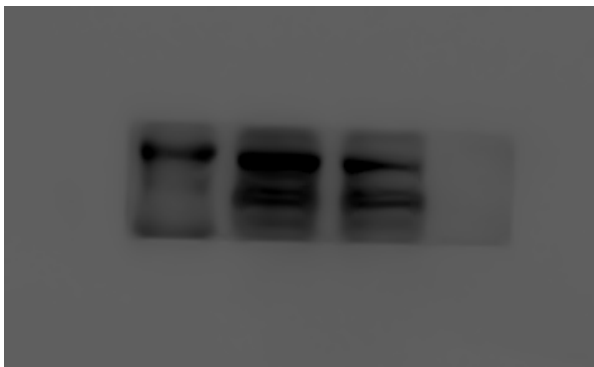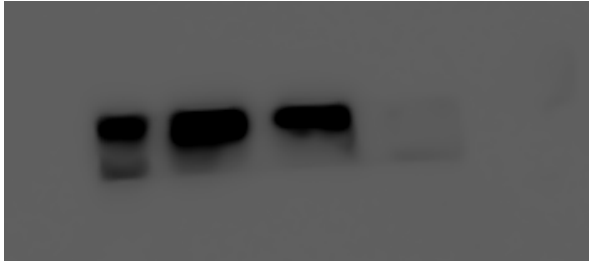
Fig c.

STAT3-88KDa
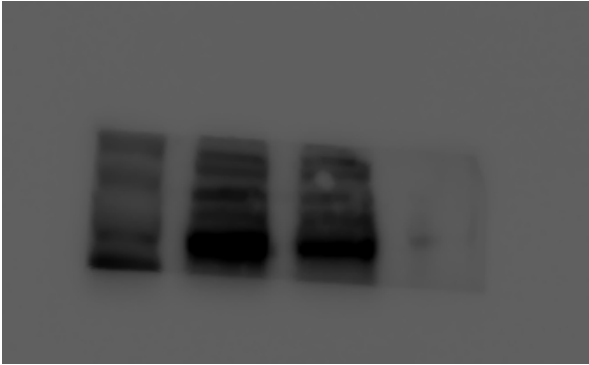

TRAP1-80KDa
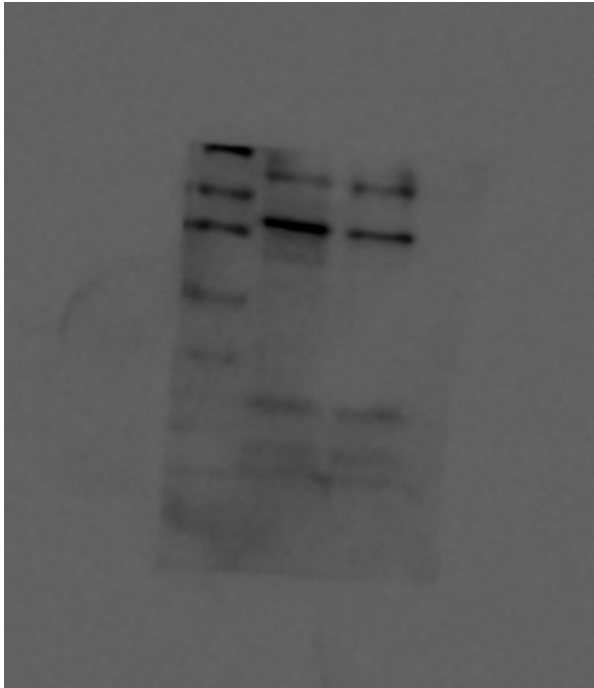
MMP9-40KDa

CTSK-37KDa

β-Actin-43KDa

Fig d.

STAT3-88KDa

FBXO32-42KDa

TRIM63-40KDa

MyoG-25KDa
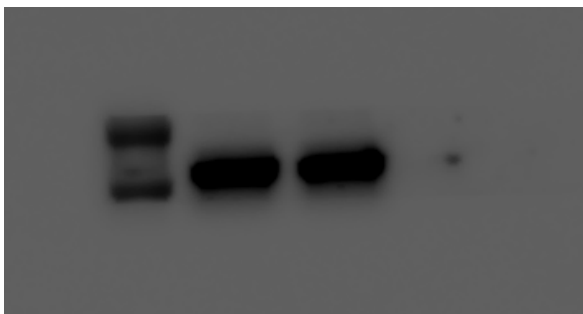
β-Actin-43KDa

Fig e.

STAT3-88KDa

TRAP1-80KDa

MMP9-40KDa

CTSK-37KDa

β-Actin-43KDa
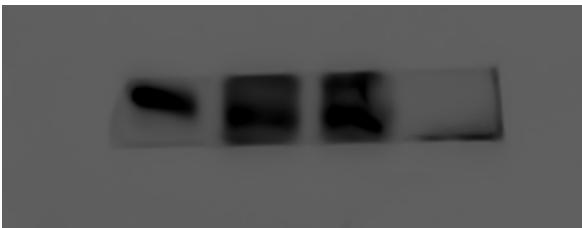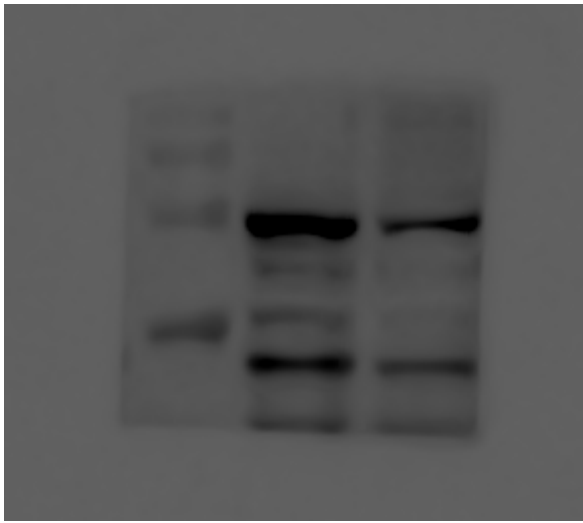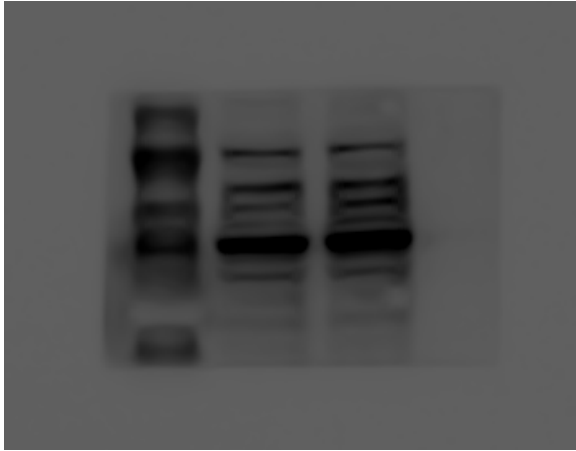
Fig f.

STAT3-88KDa

FBXO32-42KDa

TRIM63-40KDa

MyoG-25KDa

β-Actin-43KDa
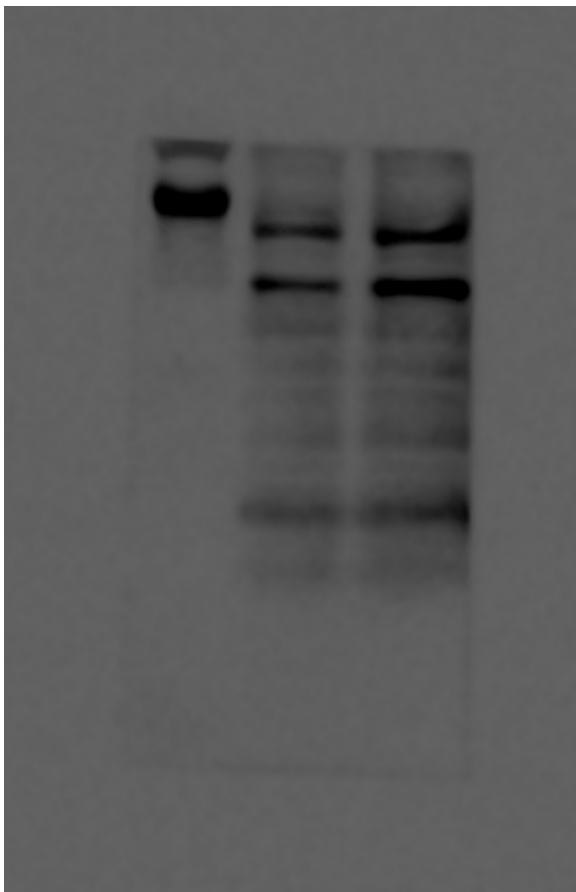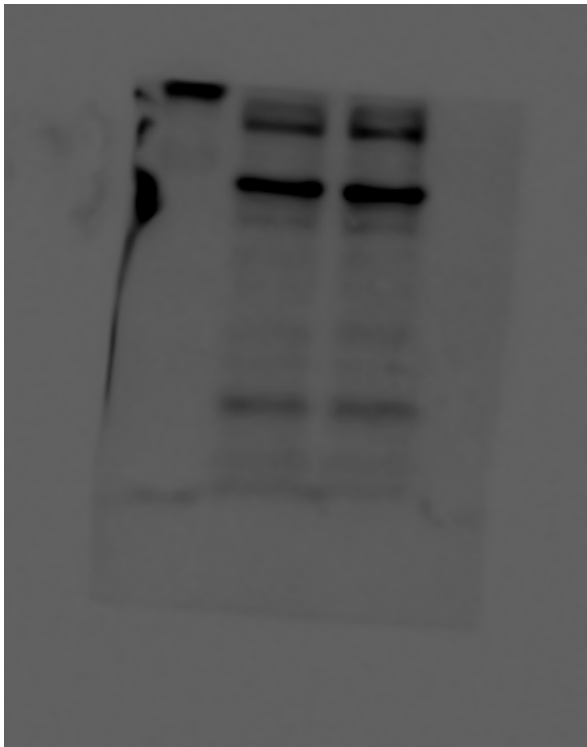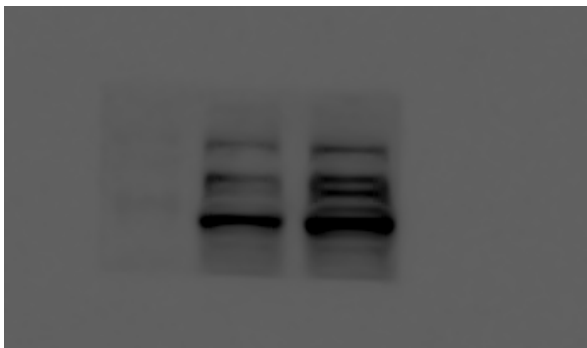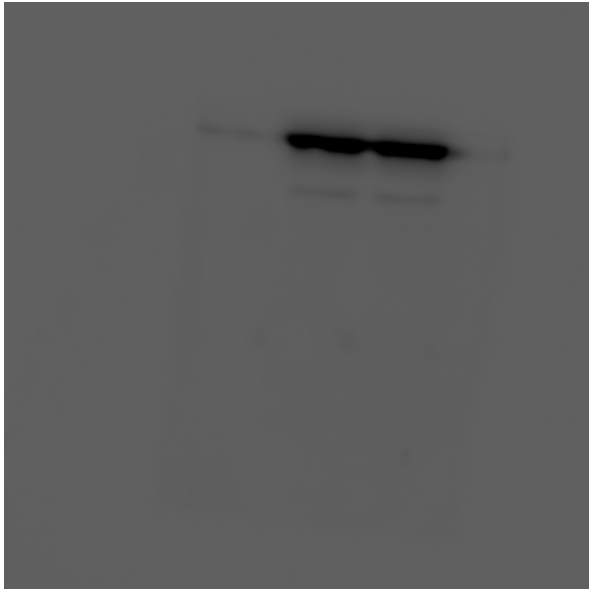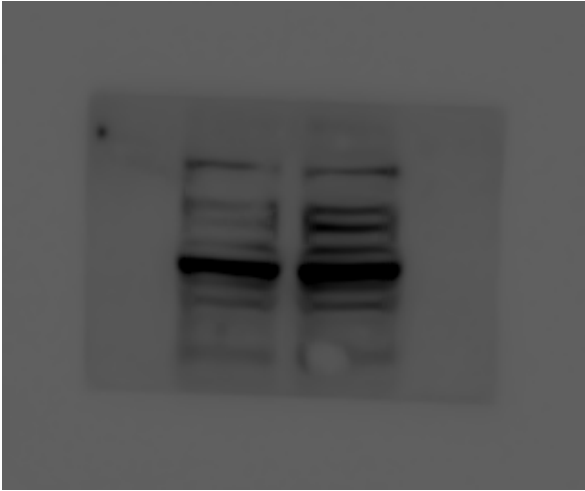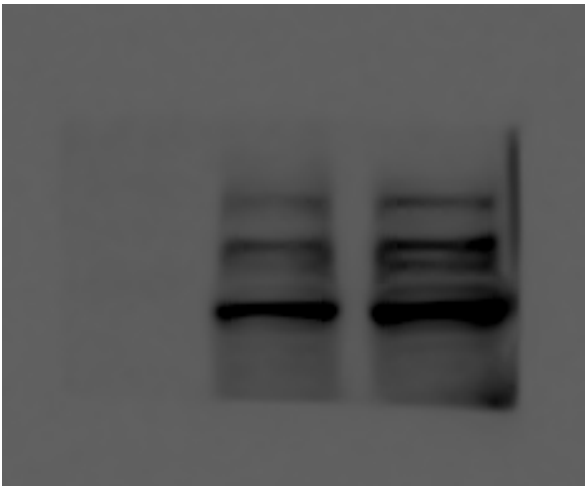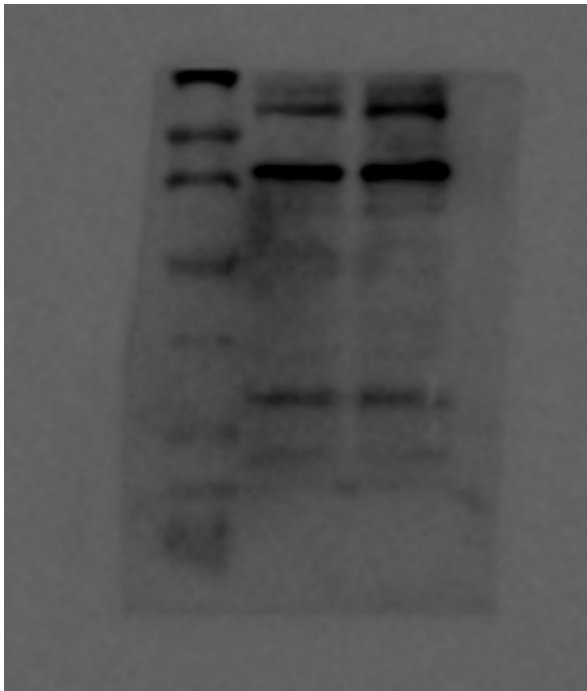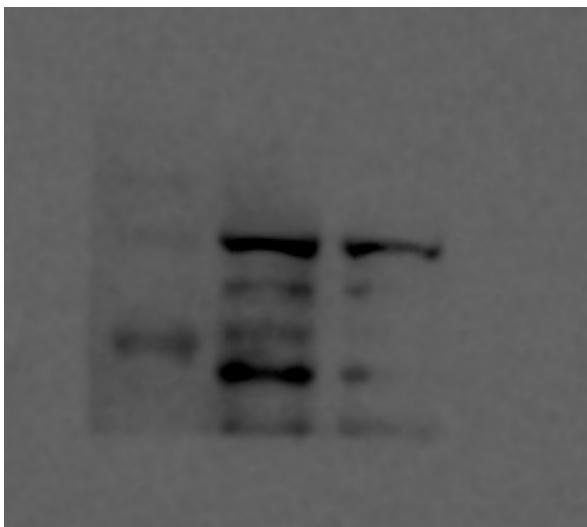
Table i. Details of the data sources used in our study.

ID	Organism	Type	Sexual	Tissue	Control, n	Treat, n
GSE1428	Homo sapiens	Bulk RNA-seq	Mixed	Skeletal muscle	10	12
GSE136344	Homo sapiens	Bulk RNA-seq	Mixed	Skeletal muscle	11	12
GSE7158	Homo sapiens	Bulk RNA-seq	Female	Blood monocytes	14	12
GSE56814	Homo sapiens	Bulk RNA-seq	Female	Blood monocytes	17	11
GSE147287	Homo sapiens	scRNA-seq	Mixed	Mesenchymal stem cells	1	1
GSE172410	Mus musculus	scRNA-seq	Mixed	Skeletal muscle	3	3

scRNA-seq, single-cell RNA sequencing.

Table ii. Sequences of primer used.

Gene	Nucleic acid sequence
mouse-actin-F	CGTTGACATCCGTAAAGACC
mouse-actin-R	AACAGTCCGCCTAGAAGCAC
mouse-STAT3-F	CTTGGCCCTTTGGAATGAAG
mouse-STAT3-R	CAAGTGAAAGTGACCCCTCC
mouse-MMP9-F	TCCTTGCAATGTGGATGT
mouse-MMP9-R	CTTCCAGTACCAACCGTCCT
mouse-CSTK-F	CCTGGAAAATGCTTCTGTCCGTTTGCC
mouse-CSTK-R	GAGTTGATAGCTGGCTGGTGGCAGATG
mouse-TRAP1-F	AGGACGACTGTTCAGCACG
mouse-TRAP1-R	CCGGGCAACAATGTCCAAAAG
mouse-TRIM63-F	CCTGCAGAGTGACCAAGGA
mouse-TRIM63-R	GGCGTAGAGGGTGTCAAACT
mouse-FBXO32-F	AGTGAGGACCGGCTACTGTG
mouse-FBXO32-R	GATCAAACGCTTGCGAATCT
mouse-Myogenin-F	CCTTGCTCAGCTCCCTCA
mouse-Myogenin-R	TGGGAGTTGCATTCACTGG

CSTK, cathepsin K; FBXO32, F-box only protein 32; MMP9, matrix metallopeptidase 9; STAT3, signal transducer and activator of transcription 3; TRAP1, tumour necrosis factor receptor associated protein 1; TRIM63, tripartite motif containing 63.

Table iii. Specific details of gene set 1.

Gene set 1							
SERPINB10	PEX2	PADI2	ZNF302	TUBA8	DDX42	FNTA	DAZAP1
LUC7L3	PPARG	HSPB1	STAT3	CCR4	RTCB	HAT1	ZNF721
MRPS17	UEVLD	TMCC2	YTHDC1	MOB4	NAE1	PLN	EPM2AIP1

GMPR	AHCY	ATG7	MAPK1	DYNLL1	PRDM1	APIP	MRPS33
CTSO	MCM6	UBQLN4	BARD1	SLC25A32	P2RY2	RPS18	HSPE1
ODC1	NTSR1	CLK4	RGL1	CAV2	MDH2	DENR	ZG16
ZNF91	HNRNPM	UBE2D1	DPYSL2	CD79A	STARD7	LITAF	GJA1
MGST3	PPP1CB	ME1	PAGE1	SDHC	RGS2	COPB1	SPEN
IFIT1	CHCHD7	ACTA2	SRRM1	RPL11	DAAM1	SRM	HSPB3
TUBA4A	C1QA	LY86	NDUFS4	WASF3	RASGRP3	PDGFC	DPM3
MYL9	ADI1	SND1-IT1	TBCA	HCAR3	PMP22	OAZ1	CTNNBIP1
TPPP3	GOT1	PAM	GYS1	MAP3K7CL	FDFT1	YWHAB	VWF
RPL35	SIDT2	HCLS1	CSNK2B	NDUFS6	UCN	AMIGO2	MYCBP2
ERH	CLEC10A	TPI1	ACTR6	CROCCP2	DYNLT3	ADRA2A	NIF3L1
RPL8	LSM8	PHTF2	SMPDL3A	BNIP3	SLC20A1	LRPAP1	NDUFA8
C3AR1	ACYP1	IMPA1	NARS2	LACTB2	GPX3	BCL2L2	APOLD1
ORMDL2	IRS1	EIF3L	CCDC7	PDPR	APOL6	GTPBP2	ROCK1
PRSS3P2	DIP2A	FOS	FLJ20712	CD3D	ZNF146	ILF2	ANKRD46
NDUFB5	RNF38	TTC33	FASTKD1	EPS8	LILRB1	TGFB1I1	PYY2
CLU	RIOK3	IFRD1	ANG	TRDN	MT1X	LRIF1	SERINC1
SPX	WDR76	OAT	TSPAN32	SH3BGRL	HBD	MEA1	ETFA
ENDOD1	TAF1D	RHEB	PER3	BANF1	IER3	ANKZF1	CD2
ACTB	CMPK1	PYROXD1	AMD1	APEX1	MC3R	PSMB9	GSTT1
CRYZ	KRT18	NAP1L1	DEDD	GCA	PCM1	KPNA3	CS
JUN	HMOX1	OR1D5	GPX4	CREG1	POMZP3	KTN1	HTATSF1
EIF4B	SYP	PARVB	SEMA3C	TMBIM6	CD93	CSGALNACT1	MAN1A1
UBQLN2	TGFBR3	HERC1	PPIA	METTL7A	PPP2CB	CCT6A	DPM2
SLTM	TAS2R8	ERICH1	PNMT	UBE2W	SUMO1	CYBRD1	TRIM22
COPS4	TJP2	ELP5	UBL4A	IFITM2	FHL3	SPINT3	BLCAP
TECR	THBD	AHNAK	MTIF2	LRRFIP1	CCT5	ATP6AP1	YTHDF1
LGALS3BP	CRAT	DHRS7	RIT1	EMC7	CDIPT	VSIG4	ATIC
PFDN5	TCERG1	NDUFAF1	VPREB3	SLC24A3	VWA1	MYH1	MRPS2
OPHN1	DUSP22	BTN2A1	BDH2	C3	TAP1	LAMP2	NIPSNAP3B
FOXN3	ACTN1	GSTP1	NBR2	STAG3L4	GCC2	ABCC5	GABARAP
DUSP11							