SUPPLEMENTARY INFORMATION

METHODS

Materials

Details of primary antibodies used:

Antigen	Supplier	Clone or Catalogue number
Phosphorylated p38 (T180/Y182)	R&D Systems, Minneapolis, USA	Catalogue number AF869
Pan p38	R&D Systems, Minneapolis, USA	Catalogue number AF8691
Sirtuin 1	Novus Biologicals, Cambridge, USA	Clone E54
Phosphorylated p53 (S6)	Cell Signaling Technologies, Danvers, MA, USA	Catalogue number 9285
Phosphorylated p53 (S15)	Cell Signaling Technologies, Danvers, MA, USA	Catalogue number 9284
Phosphorylated p53 (S20)	Cell Signaling Technologies, Danvers, MA, USA	Catalogue number 9287
Phosphorylated p53 (S37)	Cell Signaling Technologies, Danvers, MA, USA	Catalogue number 9289
Phosphorylated p53 (S392)	Cell Signaling Technologies, Danvers, MA, USA	Catalogue number 9281
Phosphorylated p53 (Thr81)	Cell Signaling Technologies, Danvers, MA, USA	Catalogue number 2676
Acetylated p53 (K320)	Millipore, Temecula, CA, USA	Catalogue number 06-1283
Pan p53 *	Life Technologies, Paisley, UK	Clone DO-1

$p16^{INK4a}$	Cell Signaling Technologies, Danvers, MA, USA	Clone EP4353Y
$p21^{cip}$ *	Cell Signaling Technologies, Danvers, MA, USA	Clone 12D1
Phosphorylated p70 S6 kinase (Thr389)	Cell Signaling Technologies, Danvers, MA, USA	Clone 108D2
Pan p70 S6 kinase	Cell Signaling Technologies, Danvers, MA, USA	Clone 49D7

* Antibodies used for immunohistochemistry were tested for target specificity by Western Blotting and produced a single band at the appropriate size (data not shown).

The adSirt1 adenoviral vector was purchased from Applied Biological Materials Inc., Canada (catalogue number 131079A). The adGFP adenoviral vector was a gift from Prof Jillian Cornish, University of Auckland, New Zealand). DMEM-F12 was purchased from Lonza Group Ltd (Cambrex), Wokingham, UK. Glucose-free DMEM-F12 was purchased from BioSera, Ringmer, UK. Cells-to-cDNA lysis buffer, DNase I and DNase buffer were purchased from Ambion, Austin, USA. Random primers, Reverse Transcriptase (RT) buffer and MMLV Reverse transcriptase were purchased from Invitrogen, Paisley, UK. Quantitect primer assays for p53 and sirtuin 1 were purchased from Qiagen Ltd, Crawley, UK. Dexamethasone, pifithrin-α, glucose, mannitol and EX527 were purchased from Sigma, Poole, UK. Unless otherwise stated, all other chemicals were purchased from Sigma, Poole, UK and were of the highest purity available.

Cell Culture

Bone, tendon and cartilage tissue was obtained from the Oxford Musculoskeletal BioBank and were collected with informed donor consent in full compliance with National and Institutional ethical requirements, the United Kingdom Human Tissue Act, and the

Declaration of Helsinki. Previous studies demonstrate that primary tenocytes remain phenotypically stable until passage 5 when passaged subconfluence (21). In the following experiments, cells were passaged at 70% confluence and used up until passage 3. Cells were routinely cultured in DMEM/F12 (containing 17.5mM glucose) supplemented with 5% FBS without antibiotics.

Primary human chondrocytes were cultured in antibiotic-free DMEM-F12 with 10% FBS and used without passage in experiments. Primary human osteoblasts were also used without passage in experiments. Osteoblasts were cultured in α-MEM with 10% FBS.

Low glucose media was defined as 2mM glucose as this is representative of blood glucose levels in fasted patients. Mannitol was added to low glucose media to ensure the osmolarity of the culture medium was constant across the various glucose concentrations used. Pifithrin-α and EX527 were used at concentrations that are double the IC50 indicated by the supplier. Resveratrol was used at 10 and 30μM, a concentration range previously been shown to activate sirtuin 1 (22).

RNAi-mediated gene silencing

Cells were cultured for 18 hours in serum-free DMEM-F12 with lipofectamine RNAimax (1.64μl/ml) and 36pM siRNA (either non-targeting control catalogue number D-001810-01-05, ThermoScientific, Rockford, IL, USA or p53 catalogue number SI02655170, Qiagen, Crawley, UK). Lipofectamine-containing media was subsequently removed and replaced with standard growth media (DMEM-F12 containing 5% FBS). Success of gene knockdown was confirmed by Western Blotting 48h post-transfection.

Senescence-Associated β-Galactosidase Staining

Tenocytes were plated at 3000 cells/ml on glass coverslips in 24-well dishes and treated as specified in individual experiments. Cells were fixed in 1% formaldehyde/0.25%

glutaraldehyde then incubated overnight with β-galactosidase stain solution containing 1 mg/mL 5-bromo- 4-chloro-3-indolyl-b-D-galactoside, 5 mM ferrocyanide, 2 mM magnesium chloride, and 150 mM sodium chloride in citric acid/sodium phosphate buffer at pH 6.