Obesity is a major risk factor underlying the development of metabolic disease and a growing public health concern globally. Strategies to promote skeletal muscle metabolism can be effective to limit the progression of metabolic disease. Here, we demonstrate that the levels of the Hippo pathway transcriptional co-activator YAP are decreased in muscle biopsies from obese, insulin-resistant humans and mice. Targeted disruption of Yap in adult skeletal muscle resulted in incomplete oxidation of fatty acids and lipotoxicity. Integrated ‘omics analysis from isolated adult muscle nuclei revealed that Yap regulates a transcriptional profile associated with metabolic substrate utilisation. In line with these findings, increasing Yap abundance in the striated muscle of obese (db/db) mice enhanced energy expenditure and attenuated adiposity. Our results demonstrate a vital role for Yap as a mediator of skeletal muscle metabolism. Strategies to enhance Yap activity in skeletal muscle warrant consideration as part of comprehensive approaches to treat metabolic disease.
Obesity is a major healthcare issue affecting more than 600 million individuals worldwide, with 350 million individuals currently suffering from obesity-driven type 2 diabetes and associated diabetic complications1–2. Obese humans and mice demonstrate features of skeletal muscle dysfunction including muscle fibre atrophy, insulin resistance and altered substrate metabolism3–5. Importantly, stimulating skeletal muscle metabolism via interventions such as physical activity is associated with improved whole-body metabolic capacity6,7. However, adherence to the required amounts of physical activity in obese individuals remains low8. The rising incidence of obesity-driven pathologies highlights the urgent requirement to develop effective interventions to treat metabolic dysfunction. A deeper understanding of the signalling networks that impact skeletal muscle metabolism, and their contribution to metabolic dysfunction, may help to identify new pharmacological and lifestyle interventions that can be exploited to combat metabolic disease.

The Hippo signalling pathway is a critical mediator of organ development across species that functions by limiting the activity of the transcriptional co-activators Yap and Taz9,10. Yap and Taz, orthologue Yorkie, shuttle dynamically between the cytoplasm and nucleus11–13. When nuclear, Yap and Taz can physically associate with the TEA-domain (Tead) transcription factors to control transcriptional programmes that influence cell fate, metabolism, and survival12,14–17. In skeletal muscle, Yap–Tead and Taz–Tead complexes operate as important post-mitotic regulators of cell size18–21. Activation of Yap–Tead and Taz–Tead complexes in adult mammalian muscles can enhance skeletal muscle size18,19,22. Conversely, inhibition of Yap activity in adult mammalian skeletal muscle can cause a reduction in muscle fibre volume by inhibiting anabolic processes19. Furthermore, changes in Hippo pathway activity have been reported in muscles undergoing atrophy associated with aging, disruption of the neuromuscular junction, or corticosteroid administration, and in muscles undergoing hypertrophy associated with increased loading18–20,23,24. Combined, these observations identify the transcriptional regulators Yap and Taz as important post-natal effectors of skeletal muscle biology in health and disease, yet the biological processes controlled by the Hippo pathway in skeletal muscle remain unclear.

Here, we demonstrate that Yap regulates fatty acid oxidation and a transcriptional programme associated with metabolic substrate utilisation in adult skeletal muscle. Yap levels are reduced in the muscles of obese insulin-resistant humans and mice, with knockdown of Yap in mouse limb muscles resulting in incomplete oxidation of fatty acids. Yap knockdown impacts a programme of gene expression associated with metabolism that includes the TCA cycle enzyme, Idh2. Importantly, increasing Yap levels in the muscles of obese db/db mice attenuates adiposity by increased energy expenditure, independent of changes in lean mass, food intake or activity. Collectively, these findings provide evidence for an important role of the Hippo pathway as a postnatal regulator of metabolism in skeletal muscle.

**Results**

Yap abundance is reduced in the skeletal muscles of insulin-resistant humans and mice. To explore a potential metabolic role for the Hippo pathway in skeletal muscle, we first sought to establish if the abundance of Yap, the core effector of the Hippo pathway, was altered in the muscles of insulin-resistant humans. Yap levels were assessed in vastus lateralis muscle biopsies obtained from obese, non-diabetic humans that were stratified according to skeletal muscle insulin sensitivity following a hyperinsulinemic-euglycemic clamp (Fig. 1A). Consistent with the hypothesis that Yap functions as a positive mediator of metabolism in skeletal muscle, we found that Yap protein levels were reduced in the muscles of obese, insulin-resistant individuals compared to obese, insulin-sensitive individuals (Fig. 1B, Supplementary Fig. 1A). We also identified a positive correlation between Yap levels in human muscle biopsies and the glucose infusion rate relative to fat free mass (FFM) during a hyperinsulinemic-euglycemic clamp; a gold standard measure of skeletal muscle insulin sensitivity (Fig. 1C). Further, we observed a positive correlation between skeletal muscle Yap levels and the difference in respiratory quotient (RQ) from fasted to glucose-infused states (ΔRQ), demonstrating impaired whole-body metabolic flexibility is associated with reduced Yap abundance in the skeletal muscle of obese humans (Fig. 1D).

We next assessed the levels of Yap in the skeletal muscles of mouse models of genetically-induced hyperphagic obesity (GIO; leptin receptor null; db/db strain) and diet-induced obesity (DIO; 6 weeks 43% high-fat diet feeding of C57BL/6J). Consistent with our observations in insulin-resistant humans, Yap levels were markedly reduced in the predominantly glycolytic tibialis anterior (TA) muscles of db/db mice at 6 weeks of age compared to littermate db/+ controls (Fig. 1E–G). In contrast, we found that Yap levels were increased in the predominantly oxidative soleus muscle (Fig. 1F, H, Supplementary Fig. 1B, C). No differences were observed in Yap mRNA expression between db/db and db/+ TA muscles indicating that the reduced levels of Yap protein were due to post-transcriptional mechanisms (Supplementary Fig. 2A). The abundance of Yap was similar in the TA and Soleus muscles of mice fed standard lab and high-fat diets for 6 weeks (Supplementary Fig. 2B). Collectively, these observations demonstrate dysregulation of the Hippo pathway in the skeletal muscles of insulin-resistant humans and a GIO mouse model.

Yap regulates adult skeletal muscle mass regardless of metabolic phenotype. Previously, we showed that Yap is a positive regulator of adult skeletal muscle mass19. Considering the differential levels of Yap that we observed in obese human and mouse skeletal muscle groups, we sought to determine if the anabolic effects of Yap were dependent on the intrinsic metabolic properties of a muscle group. Cohorts of 4-week-old C57BL/6J mice were treated with AAV6:Yap-shRNA or AAV6:lacZ-shRNA vectors by intravenous administration to knockdown Yap in muscles throughout the body, including the TA, Gastrocnemius (Gastroc) and Soleus hindlimb muscles which exhibit phenotypically distinct metabolic and fibre type properties25. Body composition analysis conducted over 8 weeks following treatment demonstrated that mice expressing Yap-shRNA exhibited a reduced accumulation of body mass and lean mass compared with mice administered AAV6:lacZ-shRNA (Supplementary Fig. 3A, B). No difference was observed in total fat mass or average daily food intake between groups (Supplementary Fig. 3C, D). Western blot analysis performed on lysates of all muscle groups excised at experimental endpoint confirmed knockdown of Yap protein in muscles following treatment, but no difference in Yap protein abundance in the livers of treated mice (Supplementary Fig. 3E–H). Consistent with previous reports in the TA muscle of adult mice19, Yap knockdown elicited atrophy in the TA (−11 ± 3%), Gastroc (−17 ± 3%), and Soleus (−22 ± 5% of mean values for mice administered control lacZ-shRNA vector) muscles (Supplementary Fig. 4A). In contrast, no evidence of atrophy was observed in the heart (Supplementary Fig. 4A). The reduction in mass observed in all limb muscles assessed following Yap knockdown was due to a diminished muscle fibre cross-sectional area (Supplementary Fig. 4B). No evidence of muscle fibre degeneration or centrally located nuclei (indicative of ongoing muscle regeneration) was observed on histological
sections in any muscle group (Supplementary Fig. 4C). Together, these findings demonstrate that reductions in the levels of Yap in adult skeletal muscle fibres impacts muscle fibre size independently of the muscles’ intrinsic metabolic phenotype.

**Inhibition of Yap impairs fatty acid oxidation and leads to lipotoxicity in adult skeletal muscle.** To explore the functional consequences of inhibiting Yap on the metabolic properties of skeletal muscle, we first performed steady state-polar metabolomic analysis of Soleus muscles collected 28 days after treatment with AAV6:Yap-shRNA or AAV6:lacZ-shRNA vectors. In total, we detected 297 metabolite profiles (Supplementary Table 1). Hierarchical clustering revealed robust changes in metabolites between conditions (Supplementary Fig. 5A). Metabolites that were decreased in response to Yap knockdown included fatty acids (Undecanoic acid, Capric acid, 2-Octenoic acid) and 2-oxoglutaric acid (alpha-ketoglutarate), a key metabolite of the TCA cycle, suggesting impaired fatty acid metabolism occurs in adult skeletal muscles with reduced Yap abundance (Fig. 2a, Supplementary Fig. 5A, Supplementary Table 1). Soleus muscles administered AAV6:Yap-shRNA also displayed reduced levels of a number of amino acids (L-Lysine, L-Serine, L-Proline and Aspartic Acid), Ureidopropionic acid and Carnosine, indicating alterations in amino acid, uracil and carnosine metabolism pathways (Fig. 2a, Supplementary Fig. 5A, Supplementary Table 1).
Based on these observations, we profiled changes to the skeletal muscle lipidome. Muscles treated with AAV6:Yap-shRNA accumulated increased quantities of long-chain acylcarnitines and select triglyceride species demonstrating that sustained reductions in Yap in adult skeletal muscle leads to an accumulation of neutral lipids and fatty acid derived-intermediates (Fig. 2b, c, Supplementary Fig. 5B, Supplementary Table 1).

To establish the functional significance of Yap in the context of lipid metabolism, we submitted intact EDL muscles of 8-week-old C57BL/6J male mice treated with AAV6:Yap-shRNA or AAV6: lacZ-shRNA to ex vivo fatty acid oxidation assays using 14C labelled oleate. Using this approach, we were able to assess the effect of modulating Yap abundance on fatty acid metabolism specifically in skeletal muscles, thus mitigating potential secondary effects from other organs. The knockdown of Yap in EDL muscles increased total fatty acid oxidation rates, which were determined to be a product of higher rates of incomplete oxidation to β-oxidation intermediates, with no difference in the rates of complete oxidation to CO2 (Fig. 2d–f). No differences...
were observed to the rates of fatty acid uptake or triglyceride esterification (Supplementary Fig. 6A, B). Incomplete fatty acid oxidation and the accumulation of lipid intermediates has been reported to lead to lipotoxicity and is associated with DNA fragmentation and increased reactive oxygen species (ROS)4,27–34. Consistent with these reports, the sustained inhibition of Yap in adult skeletal muscle fibres led to a robust increase in the numbers of muscle fibre nuclei with fragmented DNA and ROS, as determined by the presence of TUNEL and Dihydroethidium reactivity respectively (Fig. 2g, Supplementary Fig. 6C).

Skeletal muscle from obese individuals is characterised by perturbed mitochondrial function due to a disconnect between the rates of beta-oxidation and the capacity of the TCA cycle, rather than defects in the electron transport chain directly4,29,35. To confirm that the knockdown of Yap in adult limb muscles did not impact mitochondria function directly, we isolated mitochondria from Gastroc muscles 8 weeks after administration of AAV6:Yap-shRNA or AAV6:lacZ-shRNA treatments to measure mitochondrial oxygen consumption rates. No differences were observed in the rates of basal or stimulated oxygen consumption in isolated mitochondria from muscles treated with AAV6:Yap-shRNA or AAV6:lacZ-shRNA vectors (Supplementary Fig. 6D).

To validate these findings by an independent methodology, we also assessed respiratory capacity in intact EDL limb muscles in which mitochondria remain within their biological niche. Consistent with our findings in isolated mitochondria, no difference was observed in mitochondrial respiratory capacity between intact EDL muscles administered AAV6:Yap-shRNA or AAV6:lacZ-shRNA suggesting that Yap knockdown is unlikely to directly influence electron transport function or respiratory capacity in mitochondria (Supplementary Fig. 6E). Together, these findings support the conclusion that Yap is required for efficient fatty acid oxidation in adult skeletal muscle fibres, such that loss of Yap in skeletal muscle results in presentation of critical features of lipotoxicity and metabolic disease.

Yap regulates a transriptional programme that impacts skeletal muscle metabolic substrate utilisation. To gain insight into the molecular changes that occur following Yap knockdown in adult skeletal muscle fibres, we administered AAV6:Yap-shRNA and AAV6:lacZ-shRNA vectors to the contralateral Gastroc and TA muscles of 8-week-old male C57BL/6J mice. At 28 days post-administration of AAV6:LacZ shRNA or AAV6:Yap shRNA vector (n = 6, mean ± SEM, * indicates p < 0.05 with exact p values provided in source data, two-sided t test without adjustment for multiple comparisons), (c) selected triglyceride species in Soleus muscles treated as in (b) (n = 6 biologically independent animals, mean ± SEM, * indicates p < 0.05 with exact p values provided in source data, two-sided t test without adjustment for multiple comparisons), (d) Total fatty acid oxidation (tracer present in cell and media acid-soluble metabolites and CO2 gas phase) in pmol/h/mg tissue (n = 12 biologically independent animals, mean ± SEM, * shows significant difference, p = 0.03, two-sided t test), (e) Incomplete fatty acid oxidation (tracer in cell and media acid-soluble metabolites) and (f) complete fatty acid oxidation to CO2 (tracer present in CO2 gas phase only) in pmol/h/mg tissue in EDL muscles analysed 2 weeks after injection of AAV6:lacZ-shRNA or AAV6:Yap-shRNA vectors (n = 12 biologically independent animals, mean ± SEM, * shows significant difference, p = 0.03 and 0.71 respectively, two-sided t test), (g) Representative images of Dihydroethidium (red) and DAPI (blue) staining in Soleus muscles treated with AAV6:lacZ-shRNA and AAV6:Yap-shRNA and assessed 8 weeks after treatment (n = 10 biologically independent animals, scale bars indicate 50 μm).

In parallel to our investigation of the effects of depressing Yap activity on the transcriptome, we measured the impact of Yap knockdown on the skeletal muscle proteome. Applying label-free quantitative proteomics to mouse Gastroc muscles treated with AAV6:Yap-shRNA or AAV6:lacZ-shRNA, we quantified 1512 distinct proteins (Fig. 3F, Supplementary Table 5). The abundance of 150 proteins was significantly different following Yap knockdown (Fig. 3F, Supplementary Table 5; adjusted p value < 0.05). In total, 119 proteins were increased in abundance that included metabolic enzymes (Succ2, Ampd1) and components of the Polyamine signalling pathway (Amd1, Smox), and 31 proteins were reduced in abundance including the enzymes Pdk4 and Acdsb that play key roles in substrate utilisation and lipid metabolism, respectively (Fig. 3F, Supplementary Table 5; adjusted p value < 0.05). GSEA analyses of differentially expressed proteins in limb muscles expressing Yap-shRNA supported our transcriptomics data and revealed an increase in the abundance of proteins associated with the catabolism of carbohydrates and a decrease in the abundance of proteins associated with the sequencing libraries for Assay for Transposable-Accessible Chromatin (ATAc) and RNA-sequencing to assess global chromatin accessibility and gene expression respectively38. In parallel, the Gastroc muscles of a separate cohort of mice treated in the same manner were isolated for label-free proteomics (Fig. 3A).

ATAc-sequencing in PCM1+ve nuclei from limb muscles mapped a total of 36,097 peaks across samples representing regions of accessible chromatin that were detected in at least two independent samples. Of these, 275 peaks were different between muscles treated with AAV6:lacZ-shRNA and AAV6:Yap-shRNA, with 42 regions more accessible in limb muscles administered AAV6:Yap-shRNA and 233 more accessible in limb muscles administered AAV6:lacZ-shRNA (Fig. 3B, Supplementary Table 2; FDR < 0.05). Regions of chromatin that became less accessible following Yap knockdown were located largely within intergenic regions (30.1% vs. 40.1% of peaks in YAP shRNA and lacZ-shRNA muscles respectively; Supplementary Fig. 7B). In contrast to the reduced accessibility in intergenic regions, chromatin accessibility at promoters (1 kb ± TSS) was increased in limb muscles expressing YAP shRNA compared to muscles expressing lacZ-shRNA (16.3% vs. 10.5% of ATAC-peaks: Supplementary Fig. 7B).

RNA-sequencing from the same pool of isolated muscle nuclei detected 6269 transcripts that were differentially expressed following Yap knockdown, comprising 3851 upregulated and 2418 downregulated genes (Fig. 3C, Supplementary Table 3; FDR < 0.05). To assess the impact of global changes in gene expression, we performed Gene-set enrichment analysis (GSEA) of RNA-sequencing data39. GSEA identified an increase in gene expression associated with immune response and inflammatory pathways (Fig. 3D, Supplementary Table 4) and a downregulation in gene expression associated with actin binding with CcT/TriC and the TCA cycle in limb muscles expressing Yap shRNA (Fig. 3E, Supplementary Table 4).

In parallel to our investigation of the effects of depressing Yap activity on the transcriptome, we measured the impact of Yap knockdown on the skeletal muscle proteome. Applying label-free quantitative proteomics to mouse Gastroc muscles treated with AAV6:Yap-shRNA or AAV6:lacZ-shRNA, we quantified 1512 distinct proteins (Fig. 3F, Supplementary Table 5). The abundance of 150 proteins was significantly different following Yap knockdown (Fig. 3F, Supplementary Table 5; adjusted p value < 0.05). In total, 119 proteins were increased in abundance that included metabolic enzymes (Succ2, Ampd1) and components of the Polyamine signalling pathway (Amd1, Smox), and 31 proteins were reduced in abundance including the enzymes Pdk4 and Acdsb that play key roles in substrate utilisation and lipid metabolism, respectively (Fig. 3F, Supplementary Table 5; adjusted p value < 0.05). GSEA analyses of differentially expressed proteins in limb muscles expressing Yap-shRNA supported our transcriptomics data and revealed an increase in the abundance of proteins associated with the catabolism of carbohydrates and a decrease in the abundance of proteins associated with the

Fig. 2 Inhibition of Yap impairs fatty acid oxidation and leads to lipotoxicity in adult skeletal muscle. a Top 20 downregulated metabolites in muscles treated with AAV6:Yap shRNA ranked by −log10 p value, (b) Acylcarnitine levels in Soleus muscles examined 4 weeks after administration of AAV6:LacZ shRNA or AAV6:Yap shRNA vector (n = 6, mean ± SEM, * indicates p < 0.05 with exact p values provided in source data, two-sided t test without adjustment for multiple comparisons), (c) selected triglyceride species in Soleus muscles treated as in (b) (n = 6 biologically independent animals, mean ± SEM, * indicates p < 0.05 with exact p values provided in source data, two-sided t test without adjustment for multiple comparisons), (d) Total fatty acid oxidation (tracer present in cell and media acid-soluble metabolites and CO2 gas phase) in pmol/h/mg tissue (n = 12 biologically independent animals, mean ± SEM, * shows significant difference, p = 0.03, two-sided t test), (e) Incomplete fatty acid oxidation (tracer in cell and media acid-soluble metabolites) and (f) complete fatty acid oxidation to CO2 (tracer present in CO2 gas phase only) in pmol/h/mg tissue in EDL muscles analysed 2 weeks after injection of AAV6:lacZ-shRNA or AAV6:Yap-shRNA vectors (n = 12 biologically independent animals, mean ± SEM, * shows significant difference, p = 0.03 and 0.71 respectively, two-sided t test), (g) Representative images of Dihydroethidium (red) and DAPI (blue) staining in Soleus muscles treated with AAV6:lacZ-shRNA and AAV6:Yap-shRNA and assessed 8 weeks after treatment (n = 10 biologically independent animals, scale bars indicate 50 μm).
catabolism of fatty acids and cellular lipids (Fig. 3G, Supplementary Table 6). Together, these findings demonstrate that Yap regulates a transcriptional programme that governs metabolic substrate utilisation in adult skeletal muscle.

Idh2 upregulation prevents impaired fatty acid oxidation associated with Yap knockdown. To further explore how the observed differences in gene expression impact fatty acid metabolism in adult muscles expressing Yap-shRNA, we mapped the following:

**Fig. 3** Yap influences a transcriptional programme that impacts skeletal muscle metabolic substrate utilisation. A Schematic demonstrating integrated transcriptomics and proteomics experimental design including PCM1 cell sorting strategy to isolate adult myonuclei from 8-week-old C57BL/6 male mouse limb muscles at 28 days following treatment with AAV6: lacZ-shRNA or AAV6: Yap-shRNA. (B) MA plot of ATAC-sequencing showing more and less accessible regions of chromatin following Yap knockdown. Differential peaks are shown in red (n = 3 biologically independent animals; adjusted p value < 0.01, multiple comparisons were corrected using Benjamini–Hochberg procedure). (C) MA plot of RNA-sequencing showing up and downregulated transcripts following Yap knockdown. Differential transcripts are shown in red (n = 3 biologically independent animals; adjusted p value < 0.01, multiple comparisons were corrected using Benjamini–Hochberg procedure). (D) top five upregulated and (E) top five downregulated GSEA terms from RNA-seq data generated in (C). (F) volcano plot of proteins detected using label-free proteomics in muscles treated as in (A). Grey dots show proteins that were not significantly different, blue dots show proteins that were significantly downregulated and red dots show proteins that were significantly upregulated (n = 5 biologically independent animals; adjusted p value < 0.01, t test with correction for multiple comparisons using Benjamini–Hochberg procedure). (G) GSEA analysis of proteomics datasets. Top five upregulated terms are shown in red, top five downregulated terms in blue.
changes in chromatin accessibility identified by ATAC-sequencing at enhancers or promoters to target gene transcript abundance. Using this approach, we identified 141 regions of chromatin identified as enhancers that became less accessible and were associated with a reduction in target gene expression, and 88 enhancers that were more accessible with an increase in target gene expression following Yap knockdown (Fig. 4a, Supplementary Table 3, FDR < 0.05). A total of 112 promoters (1 kb ± transcriptional start site) were less accessible, and 94 promoters more accessible, with a corresponding directional change in target gene expression in Yap-shRNA expressing muscle nuclei (Fig. 4a, Supplementary Table 3). YAP predominately binds the TEAD transcription factors to interact with the transcriptome at distal enhancers. Consequently, we focussed our analysis on the 141 genes that were downregulated and associated with enhancers that displayed reduced accessibility following Yap knockdown. The top downregulated genes included established mediators of substrate metabolism and the TCA cycle (Slc2a3/Glut3, Gpt2, Idh2 and Glul) glucocorticoid signalling (Nr3c1, Sgk1) and G-Protein coupled receptor signalling (Adrb2, Arrdc2 and Prkce) (Supplementary Table 3). Of these genes, 28 were associated with enhancers that contain a TEAD binding motif including the enzyme, Idh2 (Fig. 4b, Supplementary Table 3).

**Fig. 4 Idh2 upregulation prevents impaired fatty acid oxidation associated with Yap knockdown.** a Graph showing the changes in chromatin accessibility and transcript abundance at enhancers or promoters (TSS). Numbers within bars indicate the number of genes detected in each comparison (FDR < 0.05, rank-MANOVA), (b) sequencing tracks of Idh2 transcript in AAV6:lacZ-shRNA (blue) and AAV:Yap shRNA (red). Each track represent a biological replicate from an individual animal (n = 3), (c) incomplete fatty acid oxidation in EDL muscles analysed 2 weeks after injection of control, AAV6:Yap-shRNA, AAV6:Idh2 vectors or both (n = 10 biologically independent animals, mean ± SEM, * shows significant difference, one-way ANOVA with Tukey’s multiple comparison test with exact p values provided in source data), (d) representative western blots of Yap, Idh2, and total protein levels in TA muscles from mice treated as in (c) (n = 5 biologically independent animals, scale bars indicate 50 µm).
Increasing Yap in the musculature of obese genic db/db mice enhances energy expenditure to reduce adiposity and hepatic steatosis. In light of the reduced levels of Yap in the muscles of obese insulin resistant humans and mice, and the deleterious metabolic effects of experimentally reducing Yap protein in mouse muscles, we sought to test the functional importance of skeletal muscle Yap during the progression of metabolic disease. A single systemic administration of AAV6 vectors encoding mouse Yap (AAV6:Yap), or AAV6:empty vector was delivered via tail vein injection to 4-week-old db/db mice to achieve body-wide transduction of skeletal musculature. Following treatment, we observed that db/db mice administered AAV6:empty vector that failed to accumulate body mass at the same rate as mice administered AAV6:empty vector (Fig. 5a, b). The observed differences in body mass as a consequence of treatment with AAV6:Yap were driven exclusively by a failure to accumulate additional fat mass (Fig. 5c), as no differences in lean mass were observed between db/db mice treated with AAV6:Yap and AAV6:empty vector (Supplementary Fig. 9A). Expression of exogenous Yap in the Gastroc hindlimb muscles of db/db mice administered AAV6:Yap was confirmed by Western blotting (Supplementary Fig. 9B). No increase in Yap levels was observed in the livers of treated mice, demonstrating specific transduction of the striated musculature following treatment (Supplementary Fig. 9C). Of note, Yap mRNA expression in treated mice correlated with the expression of established target genes Cyr61, Ctgf and Amotl2, as well as the expression of Idh2, in line with our findings in non-obese C57BL/6j mice (Supplementary Fig. 10–12). No difference in the mass of limb muscles, or the heart was detectable at 14 weeks after vector administration (Supplementary Fig. 10E–G). Average daily food intake for db/db mice treated with AAV6:Yap was similar between db/db mice administered AAV6:empty vector indicating that the restoration of Yap in skeletal muscle limited the accumulation of adiposity independent of changes in lean mass or food intake, and in spite of continued excess energy intake (Fig. 5d).

While persistently hyperphagic db/db mice that overexpressed Yap in striated muscles continued to exhibit fasting hyperglycaemia and impaired whole-body glucose tolerance (Supplementary Fig. 11A, B), fasting plasma concentrations of insulin (Fig. 5e) and c-peptide levels (Supplementary Fig. 11C) were lower indicative of a reduction in insulin secretion. To ascertain if the upregulation of Yap in skeletal muscles improved glucose-mediated insulin secretion, we administered a physiologically relevant bolus of glucose via oral gavage to db/db mice treated with AAV6:Yap or AAV6:empty vector and assessed plasma insulin secretion. Following glucose challenge, plasma insulin concentrations increased in db/db mice previously treated with AAV6:Yap, but not in db/db mice administered AAV6:empty vector (Fig. 5f), demonstrating that glucose-stimulated insulin secretion was enhanced in db/db mice expressing increased levels of Yap protein in the striated musculature.

To gain further insight as to how Yap over-expression in the skeletal muscles of db/db mice limited adiposity, we performed metabolic caging studies to determine whole-body metabolic function. While no significant differences were observed in total activity in active or inactive phases (Supplementary Fig. 11D, E), db/db mice treated with AAV6:Yap displayed a marked increase in energy expenditure at the onset of the active phase that was independent of total lean mass (Fig. 5g, h). Histological analysis of peripheral tissues 14 weeks after systemic administration of AAV6:Yap to db/db mice confirmed a reduction in adipocyte size, in line with body composition data (Fig. 5i). Strikingly, we found that inguinal adipose tissues of db/db mice treated with AAV6:Yap contained beige adipocytes as indicated by the presence of multilocular adipocytes that exhibited immunoreactivity to the beige adipocyte marker, Ucp1 (Fig. 5i, j). Elevated Ucp1 expression in white adipose tissue was confirmed by qPCR (Supplementary Fig. 11F). Importantly, no increase in Yap mRNA expression was detected in adipose tissue further confirming that the expression of AAV6:Yap was restricted to the striated musculature (Supplementary Fig. 11G). Additionally, muscle-directed Yap gene delivery was accompanied by an increased intensity of histochemical reaction for succinate dehydrogenase activity in TA limb muscles (Supplementary Fig. 11H) and protection from the development of liver steatosis (Fig. 5k). Collectively, these findings demonstrate that the observed reduction in Yap protein in the skeletal muscles of obese insulin-resistant db/db mice contributes to whole-body and organ-specific alterations in lipid homeostasis. Activating Yap in striated muscle is associated with an increase in energy expenditure, burning of white adipose tissue and increased skeletal muscle oxidative potential. Together, these effects result in an attenuation of the progression of pathological features of hyperphagia-induced obesity.

Discussion

While the Hippo signalling pathway has been defined as a regulator of cell fate, apoptosis and proliferation in many tissues, several studies have now demonstrated that the Hippo signalling pathway also regulates cell size in adult skeletal muscle. Seeking to further define the role of the Hippo pathway in mature skeletal muscle, we report herein a metabolic function for Yap in adult skeletal muscle fibres, such that sustained inhibition of Yap alters metabolic substrate utilisation leading to incomplete fatty acid oxidation.

A key finding of our study is that Yap influences the rate of oxidation of fatty acids in skeletal muscle, where inhibition of Yap results in elevated, but incomplete, fatty acid oxidation. While initially thought to be associated with reduced rates of β-oxidation, a growing body of evidence supports the concept that elevated rates of incomplete β-oxidation without concurrent increases in Krebs/TCA cycle intermediates or mitochondrial respiratory capacity is a critical event in the development and progression of metabolic stress. Recent studies have demonstrated causal interactions between fatty acid metabolism and skeletal muscle mass. However, in obesity, anabolic signalling in skeletal muscle is perturbed. Our observations...
support these conclusions; while Yap is sufficient, and required, for muscle growth in healthy adult mice\(^{19,20}\), Yap over-expression in obese mice led to improved metabolic function without changes in lean mass. These findings suggest that the metabolic and anabolic consequences of modulating the Hippo pathway in adult skeletal muscle are distinct processes. Further work exploring the essential genes that regulate the anabolic and metabolic programmes controlled by YAP in skeletal muscle may provide new insight into how the response to anabolic interventions is impaired by obesity. Our study identifies a previously unappreciated link between the Hippo pathway and metabolism in the post-mitotic cell. Our findings demonstrating Yap
functions as a mediator of substrate utilisation and lipid metabolism in adult skeletal muscle contrast with those conducted in mitotically-active epithelial cells where the Hippo pathway is responsive to, and a mediator of, glycolysis and glucose metabolism. 48–53. The differences between our findings and these studies likely reflect the substrate preferences and metabolic flexibility of the different cells/tissues studied, as many groups have examined Hippo pathway effects in cancer cell lines, where metabolic reprogramming towards anaerobic glycolysis is an established feature. 54. While our findings demonstrate that sustained inhibition of Yap in adult skeletal muscle leads to negative metabolic consequences, our observations are also consistent with a model where transient inhibition of Yap may be a necessary feature of a concerted programme of adaptive events to partition substrate utilisation and tune anabolic/catabolic activity during episodes of energy stress. Assessing Yap activity during conditions of energy stress such as caloric restriction or intense bouts of exercise may provide further insight into the physiological role of the Hippo pathway in adult skeletal muscle fibres. While the beneficial metabolic effects of Yap over-expression in obese mice are likely due to the reported increases in energy expenditure, the precise mechanisms for how YAP activation impacts energy expenditure and leads to browning of adipose tissue remain elusive. Future studies exploring skeletal muscle intrinsic and extrinsic factors known to mediate tissue cross-talk in obesity such as myokines are required. 55.

In conclusion, we demonstrate that, in addition to the anabolic function of Yap in healthy adult skeletal muscle, the Hippo pathway regulates the expression of genes required for efficient substrate utilisation and fatty acid oxidation. Our studies show that the Hippo pathway is perturbed in the skeletal muscle of humans and mice with metabolic disease. Our studies show that increasing skeletal muscle Yap activity in pre-clinical models of metabolic disease limits adiposity and hepatic steatosis by increasing energy expenditure. Our findings identify the Hippo pathway effector Yap as a regulator of skeletal muscle attributes with consequences on whole-body and organ-specific lipid homeostasis. Interventions that target Yap may offer opportunities to enhance skeletal muscle lipid metabolism with implications for the treatment of metabolic disease.

Methods

Ethical approval. Animal experiments were conducted in accordance with the relevant codes of practice for the care and use of animals for scientific purposes as stipulated by the National Health and Medical Research Council of Australia and with approval from the respective Animal Ethics Committees of the Alfred Medical Research and Education Precinct (AMREP, E/1659/2015/B), and the University of Melbourne (Melbourne, Australia, 1714353 and 1814680). Human muscle biopsies were procured with ethical consent provided by the St Vincent’s Hospital Human Ethics Research Ethics Committee (HREC/10/SVH/133. Sydney, Australia). All relevant ethical regulations were complied with and informed consent was provided by study participants.

Reagents. Reagents were purchased from Sigma-Aldrich, Australia unless stated otherwise.

Generation of serotype 6 recombinant adeno-associated viral (AAV) vectors. DNA sequences that encode mouse Yap or Yap-shRNA were synthesised with Genscript and sub-cloned into AAV6-CMV-MCS-SV40pA or AAV6-CMV-eGFP-MCS-SV40pA plasmids respectively. DNA sequences that encode mouse Idh2 were synthesised by Vectorbuilder and sub-cloned into AAV6-CMV-MCS-SV40pA. Empty vector (AAAV6-CMV-MCS-SV40pA; called AAV6empty vector), or shRNA targeting lacZ (AAV6:lacZ-shRNA) were used as controls. Recombinant adeno-associated viral vectors were generated by co-transfection of 10 µg of plasmids containing cDNA constructs with 20 µg of pDGM6 packaging plasmid into HEK293 cells (seeded 16 h prior at a density of 3.2 × 106 cells) using the calcium phosphate precipitate method to generate type-6 pseudotyped viral vectors. After 72 h, cells and culture medium were collected, and homogenised, before clarification using a 0.22 µm filter (EMD Millipore). Vectors were purified by affinity chromatography using a HiTrap heparin affinity column (GE Healthcare), ultra-centrifuged overnight and resuspended in sterile Ringer’s solution. Vector concentration was determined using a customised quantitative PCR reaction (Applied Biosystems).

Intramuscular and intravenous injections of AAV vectors. Intramuscular injections of AAV vectors were performed in male C57BL/6J mice at 8–10 weeks of age. Intravenous injections of AAV vectors (for systemic delivery of AAV vectors to striated muscles body-wide) were performed via tail vein in male C57BL/6J or B6.BKS(D)-Leprdb/db (db/db) mice at 4 weeks of age. C57BL/6J mice were bred at AMREP animal services, Melbourne, Victoria. All db/db mice were obtained from Jackson laboratories, USA (stock number 009697). All mice were maintained in a 12:12 h light:dark cycle at a temperature of 22 ± 1 °C, a humidity of 40–70%, and provided ad libitum access to food and water during the study duration. For intramuscular injection studies, mice were placed under general anaesthesia (2% isoflurane in O2), then administered a subcutaneous injection of carprofen (5 mg/kg) for post-operative analgesia, followed by hindlimb muscle injections of AAV6 vectors diluted in 30 µl of Hank’s buffered salt solution (HBSS) to a dosage of 1 × 1010 vectors genomes. For systemic muscle transduction studies, conscious mice were administered a tail vein injection of AAV6 vectors diluted in 100 µl of HBSS to a dosage of 1 × 1011 vg. At experimental endpoints, C57BL/6J mice were killed by cervical dislocation in the fed state (within 3 h of the initiation of the light phase) unless indicated. All db/db mice were fasted for 5 h commencing within 3 h of the initiation of the light phase. Tissue samples were rapidly excised and snap-frozen for biochemical assessment or processed for histology as described below.

PC1M cell sorting of skeletal muscle nuclei for ATAC/RNA-sequencing. Skeletal muscles were pooled to obtain enough material for sequencing (4 × Gastrocnemius and 4 × tibialis anterior muscles per replicates; n = 3 per condition). The samples were homogenised in 15 ml lysis buffer (320 mMol/L sucrose, 15 mMol/L Tris-HCl (pH = 8), 5 mMol/L CaCl2, 5 mMol/L magnesium acetate, 2 mMol/L EDTA, 0.5 mMol/L EGTA, 1 mMol/L DTT and 1× complete protease inhibitor, all chemical reagents from Sigma-Aldrich) using an electrical homogeniser (T10 basic homogeniser workcentre, IKA 4095500). The lysate was combined with another 15 ml lysis buffer and subjected to homogenisation using a dounce tissue grinder (Wheaton, #357546, Millville, NJ, USA) for 10–15 strokes. The cell lysate was sequentially filtered through 100, 70, and 40 µm cell strainers (Becton Dickinson,
Franklin Lakes, NJ, 10 USA) and centrifuged to pellet nuclei at 1000 × g for 5 min. Nuclear pellets were resuspended in 4 mL sucrose buffer (1 mM LiCl, sucrose, 10 mM Tris-Cl (pH 7.5), 0.5 mM EDTA, 0.5% Triton X-100, 0.5% Nonidet P-40), 5 mM MgCl₂, 1.5 mM spermine and 1 × complete protease inhibitor). The nuclei suspension was cushioned on top of a 2× volume of the sucrose buffer, which was centrifuged at 1000 × g for 5 min to pellet nuclei. The nuclei pellets were washed once in 3 mL of nuclei storage buffer (NSB, 440 mM LiCl, sucrose, 10 mM Tris-Cl (pH = 7.2), 70 mM LiCl, KCl, 10 mM MgCl₂, 10% goat serum, 0.4% BSA, 0.2% spermine and 1% protease inhibitor cocktail) and resuspended in Alexa Fluor 633 (1:500, Thermo Fisher Scientific (Molecular Probes)) at 4 °C for 30 min. Nuclei were washed twice in NSB and resuspended in PBS before being processed for cell sorting to obtain PCMI labelled (+ve) and unlabelled (−ve) fractions (via FACS ARIA cell sorter, BD Biosciences). Following sorting, nuclei were pelleted at 1500 × g for 20 min.

Quantitative real-time reverse transcriptase PCR. Total RNA was extracted from 10 to 20 mg of tissue using TRizol (Life Technologies) or from isolated nuclei using TRizol followed by Direct-zol™ RNA MicroPrep kit (R2060, Zymo Research) as per manufacturer’s protocols. Reverse transcription was performed on 1 µg of total RNA using MULTIScribe reverse transcriptase (Life Technologies) as per manufacturer’s protocols. Transcripts were measured using Taqman® fluorogenic primers (Life Technologies, Victoria, Australia) or Syber green (ThermoFisher). Mass spectrometry data were processed using Andromeda/MaxQuant (v1.6.2.2)59 and searched against the mouse UniProt database (June, 2019) using all default settings with peptide spectral matches and protein false-discovery rate (FDR) set to 1%. First search mass tolerances were set to 20 ppm for MS1 and MS2 and following recalibration, a second search was performed with MS1 tolerance of 4.5 ppm and MS2 tolerance of 20 ppm. The data were searched with a maximum of 2 miss-cleavages, and methionine oxidation and protein N-terminal pyroglutamylation were set as variable modifications while carbamidomethylation of cysteine was set as a fixed modification. The MaxLFQ algorithm was enabled including match between runs with default parameters59. Data were post-processed via Log2 transformation and median normalisation.

Proteomic data analysis. Mass spectrometry data were processed using Andromeda/MaxQuant (v1.8.2.1)59 and searched against the mouse UniProt database (June, 2019) using all default settings with peptide spectral matches and protein false-discovery rate (FDR) set to 1%. First search mass tolerances were set to 20 ppm for MS1 and MS2 and following recalibration, a second search was performed with MS1 tolerance of 4.5 ppm and MS2 tolerance of 20 ppm. The data were searched with a maximum of 2 miss-cleavages, and methionine oxidation and protein N-terminal pyroglutamylation were set as variable modifications while carbamidomethylation of cysteine was set as a fixed modification. The MaxLFQ algorithm was enabled including match between runs with default parameters59. Data were post-processed via Log2 transformation and median normalisation.

Histological assessment. Following surgical excision, muscles were quickly blotted, weighed, and transversely sectioned, so that a transverse section of each muscle could be embedded in cryomolds containing Optimum Cutting Temp. compound (Sakura Scientific, Victoria, Australia). Sections were fixed in 4% PFA, −20 °C. Protein pellets were resuspended in 10% trichloroacetic acid in 100 mM HEPES pH 7.5, quantified with BCA and normalised to 20 µg/10 µl. The lysates were digested with 0.4 µg of Sequencing Grade Trypsin (Sigma) overnight at 37 °C. Peptides were acidified with 100 µl of 1% trifluoroacetic acid (TFA) and purified using strong cation exchange reversed phase microcolumns. The columns were washed with 50 µl of 5% acetonitrile containing 0.2% TFA and eluted with 60% acetonitrile containing 1% ammonium hydrogen carbonate then dried by vacuum centrifugation.

Mass spectrometry. Peptides were resuspended in 2% acetonitrile, 0.1% TFA and loaded onto a 30 cm × 75 µm inner diameter column packed in-house with 1.9 µm C18AQ particles (Dr Maisch GmbH HPLC) using Dionex nanoUPLC. Peptides were washed with 100% ACN containing 0.1% TFA for 0.300 nl/min (Buffer A = 0.1% formic acid; Buffer B = 80% acetonitrile, 0.1% formic acid). The column was maintained at 50 °C using a PRSO-V1 ion-source (Sonaton) coupled directly to an Orbitrap Fusion Lumos mass spectrometer (MS). A full-scan MS1 was measured at 60,000 resolution at 200 m/z (350–1850 m/z; 50 ms injection time; 4× automatic gain control target) followed by data-dependent analysis of the most abundant precursor ions for MS/MS by HCD (1.4 m/z isolation; 30 ns normalised collision energy; 15,000 resolution; 28 ms injection time, 4× automatic gain control target, 2× fast cycle time).

Proteomic extraction and western blotting. Mouse and human skeletal muscles were lysed in homogenisation buffer (50 mM Tris-HCl, 1 mM EDTA, 1% (vol/ vol), Triton X-100, 0.1% (vol/vol) 2-mercaptoethanol; pH 7.5) supplemented with protease inhibitors (Roche, 100 µl of 99 µg ethyl acetate containing 1% TFA) followed by 50% acetonitrile containing 0.2% TFA and eluted with 60% acetonitrile containing 1% ammonium hydrogen carbonate then dried by vacuum centrifugation

Bioinformatics. The reference mouse genome was downloaded from Ensembl (Mus musculus.GRCm38.dna.primaryassembly.fai) alongside Ensembl v69 annotation in GTF format58. RNA-seq reads underwent quality trimming using Skewer (Skewer 0.2.2)59 with minimum base quality threshold of 20 and minimum read length of 20 nt. As a quality control measure, we quantified ribosomal RNA in the sequence dataset. Mouse rRNA sequences were obtained from RefSeq for SS, 5.8S, 18S and 28S rRNA. A sample of 1 million reads was mapped to the rRNAs with BWAmem. Separate runs were mapped to the reference genome using STAR aligner (STAR 2.5.3a) for rRNA-seq and BWA (BWA 0.7.17) for ATAC-seq with default settings60,61. Duplicated reads were removed from the ATAC-seq experiment using Samtools (Samtools 0.1.8)52. Peak calling during ATAC-seq sequencing was performed using MACS1.4.2.153. Peaks that were detected in at least 2 samples were used to curate a peak set with individual sample’s peaks counted to the curated peak sets using FeatureCounts (v1.5.4)54. FeatureCounts was used to quantify reads mapped to whole gene with mapping quality ≥10 and otherwise default settings. Genes with fewer than 10 reads per sample on average were discarded prior to statistical analysis. General linear model feature of EdgeR (v3.16.5 and v3.26.8) was used to compare data53. Pathway analysis was performed using GSEA software to contrast data to MsigDB and/or Reactome gene sets after ranking by differential abundance score [sign of fold change divided by log2 (p value)]59. Genes and pathways with FDR < 0.05 were considered significant. To understand Yap directed enhancer regulation, we annotated ATAC-seq regions of interest to their nearby gene regulatory features: TSS and enhancer target gene65. Genes with altered ATAC-seq signal (p value < 0.01) were collected to separate gene sets for enhancer/TSS and up/down directions. These gene sets underwent enrichment analysis with mix using RNA profile data57. From the list of genes with reduced RNA expression and enhancer accessibility, we screened for those containing a YAP/TEAD4 binding site in the enhancer (Jaspar motif MA0809) with a BedTools (v2.28.0) based shell script68. To create sequence coverage plots, the BedTools genomewide tool was used to convert bam files to bedgraph format. Bedgraph data were imported into R v4.0.3 with rtracklayer (v1.30.0), normalised by library size and plotted with a custom R script.

Sample preparation for proteomic analysis. Frozen tissue was tip-pore sonicated in 6 M guanidine chloride containing 10 mM tris(2-carboxyethyl)phosphine and 40 mM chloroacetamide in 100 mM Tris pH 8.5. The lysate was incubated at 95 °C for 5 min and centrifuged at 20,000 × g for 30 min at 4 °C. Lysates were precipitated with 5 volumes of acetone overnight at −20 °C. Protein pellets were centrifuged at 5000 × g, 10 min at 4 °C and washed with 80% acetone. Protein pellets were resuspended in 10% trifluoroacetic acid in 100 mM HEPES pH 7.5, quantified with BCA and normalised to 20 µg/10 µl. The lysates were digested with 0.4 µg of Sequencing Grade Trypsin (Sigma) overnight at 37 °C. Peptides were acidified with 100 µl of 1% trifluoroacetic acid (TFA) and purified using strong cation exchange reverse phase microcolumns. The columns were washed with 50 µl of 5% acetonitrile containing 0.2% TFA and eluted with 60% acetonitrile containing 1% ammonium hydrogen carbonate then dried by vacuum centrifugation.
Assessment of fatty acid metabolism in intact EDL muscles. Fatty acid metabolism was assessed in intact (tendon-to-tendon isolated) EDL muscle examined 14 days after administration of AAV6: lacZ-shRNA or AAV6: Yap-shRNA. Muscle was incubated for 2 h at 37°C. Pre-gassed (95% O2–5% CO2) low-glucose DMEM containing 2% fatty acid-free BSA, 300 μM oleic acid and 1 μCi/mL [1-13C] palmitate. The Incubation medium was replaced at 5 h and 24 h. After 24 h, the samples were centrifuged at 18928 × g for 10 min at 4°C before injecting 7 μL into the Agilent hydrophilic interaction (HILIC) LC and high-resolution mass spectrometry (QTOF-6545). Metabolite peak calling and quality check was performed using QTOF MassHunter Quanti software (Agilent). The remaining 250 μL of extract was used for lipidomics analysis following rigorous vortex agitation to resuspend the pellet. A 100 μL aliquot of this resuspended solution was used for subsequent lipid extraction. Lipids were extracted using 2:1 Methanol-Chloroform containing 2 μM each of internal standards of PC 19/0190, PG 17/17/0, PE-d31, and TG-d5. Samples were then incubated in a Thermomix at 20°C for 15 min, before centrifugation at 25200 × g for 10 min. The supernatant was evaporated in a rotary vacuum concentrator with nitrogen to complete dryness. Finally, the samples were reconstituted in 9:1 Butanol-Methanol before 2 μL injection into the Lipid Liquid Chromatography Mass Spectrometer (QQQ-6590, Agilent). Metabolite peak calling and quality check was performed using QQQ MassHunter Quanti software (Agilent). Samples were normalised according to the soluble protein concentration as determined by DC Protein assay kit (Bio-Rad). Heatmaps were generated using MetaboAnalyst 4.0.

Human muscle biopsies. Human muscle biopsies were obtained from obese, skeletal muscle insulin-sensitive and insulin-resistant individuals. Subjects were fasted overnight and subjected to a 6 h hyperinsulinaemic-euglycemic clamp. A 2 h infusion of low-dose insulin (15 μU/m2/min) and a 2 h infusion of high-dose insulin (80 μU/m2/min) in the euglycemic state were performed and glucose infusion rates (GIR) were calculated over a 90–120 min period then normalised to subject BMI. Whole-body RQ was obtained using indirect calorimetry in the fasted state (Fasted RQ) prior to the clamp and during the final 30 min (glucose-infused RQ). ARQ (a measure of whole-body metabolic flexibility) was determined as the difference between the glucose-infused RQ (Fasted RQ (Fasting Metabolism True Overload)–Muscle insulin sensitivity determined separately for men and women. Study participants were assigned to the muscle-insulin-sensitive group if GIR was in the upper tertile of the cohort, and to the insulin-resistant group when GIR fell in the lower two tertiles. Hepatic glucose output was determined during the low-dose insulin infusion state.

Statistics and reproducibility. Graphpad Prism v8 was used for statistical analysis and generation of figures except for ATAC/RNA-sequencing, Proteomics and Lipidomics datasets that were processed as indicated in the relevant methods section. Images in Figs. 1A, E, 3A were generated using BioRender (https://app.biorender.com). Sample sizes for experiments with animals were determined using previously published work with an alpha value of 0.05 and a beta value of 0.1. Animals were randomly assigned to experimental groups with data presented as mean ± S.E.M., assumed to be normally distributed and are representative of independent biological replicates as indicated in the appropriate figure legends. The investigators were blinded to allocation during experiments or to experimental outcome. Comparisons between changes from control muscles were made using the Student’s unpaired, two-sided t test for two comparisons. One-way analysis of variance (ANOVA) test or two-way ANOVA as appropriate was used to compare multiple conditions with the Tukey’s post-hoc test used for comparisons between specific group means. Significant differences reported are p < 0.05. Transcriptional datasets were corrected for multiple comparisons using the Benjamini–Hochberg FDR method using a false-discovery rate of 5%. Statistical significance was calculated by Student’s unpaired, two-sided t test. Human datasets are presented as mean ± S.E.M and are representative of a total of 54 individuals. Data for human subjects were not normally distributed and so correlations in human datasets were performed using a Spearman rank-order correlation test. Spearman’s correlation co-efficient (r) and p value are reported in the appropriate figure legends.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. ATAC-seq and RNA-seq data reported in this paper have been deposited in the NCBI Gene Expression Omnibus and are accessible through the GEO Series accession number GSE146702 (Gene Expression Atlas—Atlas). The mass spectral datasets were deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD024379. Source data are provided with this paper.
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**Author contributions**

K.I.W., K.F.H. and P.G. designed the studies, K.I.W., D.C.H., M.Z., C.B.S., D.S.-B., B.L.P., M.K.M., T.M.S., G.T.D., R.L.Y., H.Q., R.E.T., A.H., J.R.D., R.K. and S.T.B., performed experimental procedures, K.I.W., D.C.H., M.Z., C.B.S., M.K.M., D.S.-B., T.M.S., B.L.P., R.K., A.H., S.T.B. and A.G.C. performed data analysis, A.E.-O., J.R.G., B.G.D., M.J.W., M.A.F., A.G.C., E.R.P., K.F.H. and P.G., provided resources and supervision, K.I.W., P.G. wrote the original draft, all authors contributed to revisions and approved the final draft, K.I.W., K.F.H. and P.G. acquired funding.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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