MacroH2A1.1 regulates mitochondrial respiration by limiting nuclear NAD⁺ consumption

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Histone variants are structural components of eukaryotic chromatin that can replace replication-coupled histones in the nucleosome. The histone variant macroH2A1.1 contains a macrodomain capable of binding NAD⁺-derived metabolites. Here we report that macroH2A1.1 is rapidly induced during myogenic differentiation through a switch in alternative splicing, and that myotubes that lack macroH2A1.1 have a defect in mitochondrial respiratory capacity. We found that the metabolite-binding macrodomain was essential for sustained optimal mitochondrial function but dispensable for gene regulation. Through direct binding, macroH2A1.1 inhibits basal poly-ADP ribose polymerase 1 (PARP-1) activity and thus reduces nuclear NAD⁺ consumption. The resultant accumulation of the NAD⁺ precursor NMN allows for maintenance of mitochondrial NAD⁺ pools that are critical for respiration. Our data indicate that macroH2A1.1-containing chromatin regulates mitochondrial respiration by limiting nuclear NAD⁺ consumption and establishing a buffer of NAD⁺ precursors in differentiated cells.

NAD⁺ and its reduced and phosphorylated forms have central roles in cellular metabolism1. As donor and acceptor molecules for redox reactions, they are essential for glycolysis and mitochondrial respiration2. In addition to redox metabolism, a number of enzymes consume NAD⁺ by breaking the bond between its nicotinamide (NAM) and ADP ribose moieties. These enzymes include ADP ribose transferases, poly-ADP ribose (PAR) polymerases (PARPs), cADP ribose synthases and sirtuins3.

Among these proteins, PARP-1 is known to be the major NAD⁺-consuming enzyme in the cell3. PARP-1 is an abundant nuclear protein that functions in the cellular stress response. Although it is best known for its role in the DNA damage response, additional, non-stress-related functions, such as transcriptional regulation, have also been described4. Activated PARPs transfer ADP ribose to protein substrates and can generate elongated and branched PAR polymers5. Their negative charge and capacity to recruit effector proteins enable PAR polymers to locally affect chromatin structure and function. Through NAD⁺ consumption, PARP-1 is also linked to NAD⁺-dependent reactions and energy metabolism6.

MacroH2A proteins are histone variants that can alter chromatin structure by replacing the replication-coupled H2A histone in the nucleosome7. Among histone variants, macroH2A proteins are unique in having a tripartite structure that comprises an N-terminal histone fold connected by an unstructured linker to a C-terminal macrodomain8. Macrodomains are ancient, globular folds that contain a binding pocket that recognizes NAD⁺-derived ADP ribose9. MacroH2A-encoding genes appeared at the transition from unicellular to multicellular life10, and in mammals two genes and one mutually exclusive splicing event give rise to three macroH2A proteins—macroH2A1.1, macroH2A1.2 and macroH2A2 (ref. 8)—that differ in the size and hydrophobicity of the macrodomain binding pocket. When a macroH2A protein is incorporated into chromatin, its unstructured linker domain places the macrodomain at an accessible site outside of the nucleosome11. Affinity for ADP ribose is unique to the macroH2A1.1 variant12. By binding ADP ribose in a capping mode, the macrodomain of macroH2A1.1 is further able to interact with PAR chains both when free and when covalently linked to a protein13, and macroH2A1.1 binding to auto-modified PARPs has

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been demonstrated for several members of the PARP family, including PARP-1/ARTD1 (refs. 13,14), PARP-5/tankyrase-1/ARTD5 (ref. 15) and PARP-10/ARTD10 (refs. 16,17).

Loss-of-function studies have established that macroH2A proteins contribute to embryonic development in zebrafish, are tumor suppressors in cancers such as melanoma and act as barriers to somatic cell reprogramming. Mice that lack macroH2A-encoding genes develop normally but display metabolic phenotypes, although the roles of macroH2A proteins in metabolic fitness are controversial. Two studies reported glucose intolerance and female-specific liver steatosis. However, when fed a high-fat diet, macroH2A1-knockout mice were leaner as a result of higher energy expenditure and reduced fat mass compared with wild-type mice. Thus, it remains unclear whether the macrodomain and its ADP-ribose-binding properties are relevant to any of these phenotypes. Here we used a myogenic cell culture model to investigate the role of macroH2A1.1 and its ADP-ribose-binding capacity in cellular metabolism.

RESULTS
MacroH2A1 splicing switches during myogenesis
To identify the most suitable tissue and cell type with which to investigate the function and metabolic-binding capacity of macroH2A1.1, we used restriction-fragment-length polymorphism to determine the relative abundance of alternatively spliced macroH2A1 (H2afy) mRNAs in a panel of mouse tissues (Fig. 1a, Supplementary Data Set 1 and Supplementary Fig. 1a,b). We observed the highest relative levels of macroH2A1.1 in skeletal muscle (Fig. 1a). We also detected macroH2A1.1 protein in skeletal muscle in immunoblots with an isoform-specific antibody. Next, we turned to cultured primary myoblasts and immortal myogenic C2C12 cells. Although we detected both alternatively spliced products in proliferating myoblasts, the mRNA encoding macroH2A1.1 rapidly became predominant during myogenic differentiation (Fig. 1c,d and Supplementary Fig. 1c,d). At the protein level, macroH2A1.1 expression increased with differentiation in C2C12 cells as macroH2A1.2 expression decreased (Fig. 1e). In analogous experiments, mRNA levels of macroH2A1.1 were much more pronounced at the protein level than at the mRNA level (compare Figs. 1c,d and Supplementary Figs. 1c,d). A comparison of immunoblot signals in differentiated C2C12 cells indicated that the macroH2A1.1 and macroH2A1.2 protein isoforms were present at similar levels, whereas macroH2A2 was much less abundant (Supplementary Fig. 1f). As would be expected for a chromatin component, macroH2A1.1 was nuclear in muscle tissue and differentiating C2C12 cells (Supplementary Fig. 2a,b). To determine whether macroH2A1.1 is incorporated into chromatin, we confirmed its presence in the chromatin fraction after cellular fractionation (Supplementary Fig. 2c) and its genome-wide distribution in differentiated C2C12 cells by chromatin immunoprecipitation followed by sequencing (ChIP-seq) (Supplementary Fig. 2d–f).

Taken together, these results suggest that, compared with proliferating myoblasts, differentiated muscle nuclei are characterized by a chromatin state that includes increased levels of macroH2A1.1, and that a switch in alternative splicing of the macroH2A1 transcript provides a rapid way to establish this chromatin state during muscle differentiation.

Depletion of macroH2A1.1 induces metabolic changes but does not affect differentiation
We next investigated the loss-of-function phenotype associated with macroH2A1.1 during myogenic differentiation of C2C12 cells. To specifically and efficiently suppress the expression of macroH2A1.1 and macroH2A1.2, we transfected proliferating C2C12 cells with isoform-specific short interfering RNAs (siRNAs) and induced differentiation the day after transfection (Fig. 2a). During the 4 d of differentiation, macroH2A1.1-depleted and macroH2A1.2-depleted myoblasts were equally efficient as control cells in differentiating into multinucleated myotubes (Fig. 2b). Although we did not observe any influence on differentiation-induced upregulation of the myogenic transcription factors MyoD and myogenin, expression of the late-differentiation gene muscle-specific creatine kinase (Ckm) at day 4 was slightly increased after knockdown of macroH2A1.1 and was reduced after knockdown of macroH2A1.2 compared with expression in cells treated with control siRNA (Fig. 2c). Together, these results show that, in contrast to the reported inhibition of myogenic differentiation by suppression of total macroH2A1 (ref. 26), knockdown of individual macroH2A1 isoforms had only a minor influence on differentiation.

To understand the physiological relevance of macroH2A1.1 expression and ADP-ribose-binding capacity in myotubes, we examined differentiated cells collected at day 4 of differentiation. Knockdown of macroH2A1.1 led to reduced acidification of the cell culture media compared with the control culture (Fig. 2d). When we analyzed the

Figure 1 A splicing switch upregulates macroH2A1.1 during myogenic differentiation. (a) Restriction-fragment-length polymorphism (RFLP) of macroH2A1 (mH2A1)-encoding transcripts in a panel of the indicated mouse tissues. Fragments sizes were 131 bp for macroH2A1.1 and two overlapping fragments of 89 bp for macroH2A2.1.2 (see Supplementary Fig. 1 for details). Sk., skeletal; int., intestine. (b) Immunoblot analysis of mouse skeletal muscle and Flag-tagged reference samples using isoform-specific antibodies to macroH2A1.1 (top) and Flag (bottom). (c) RFLP analysis of primary myoblasts and C2C12 cells differentiated for the indicated number of days. Note that fragment sizes were different in human versus mouse samples (see Supplementary Fig. 1b for details). The zero time point in c–e refers to subconfluent cells cultured in growth medium. DM, differentiation medium. (d) Relative mRNA levels of macroH2A1 transcripts (normalized to equimolar reference samples to allow direct comparison) and the differentiation markers Myog and Ckm from C2C12 cells analyzed by RT-qPCR during a time course of differentiation. Data shown are the mean and s.d. Source data for d are available online.
Medium, we found that both glucose consumption and lactate production were reduced compared with those in control cells (Fig. 2e), indicating a global reduction of cellular metabolism in macroH2A1.1-depleted myotubes. We also measured rates of extracellular acidification and oxygen consumption as indirect readouts for glycolysis and oxidative phosphorylation. In keeping with the reduced metabolic rates of macroH2A1.1-depleted myotubes, these cells showed substantially reduced maximal glycolytic and mitochondrial capacities compared with those of control cells (Fig. 2f,g). Together, these results suggest that specific reduction of macroH2A1.1 in the nucleus provokes a change in cellular metabolism that cannot be explained by a delay in differentiation.

**MacroH2A1.1 is required for optimal mitochondrial capacity**

To characterize the nature of the alteration in mitochondrial metabolism, we analyzed the oxygen-consumption rate in starved cells that had been primed with fatty acids. As fatty acids are direct mitochondrial substrates, this approach allowed us to avoid confounding influences from cytosolic metabolic pathways. Under these conditions, macroH2A1.1-depleted but not macroH2A1.2-depleted myotubes showed substantially impaired basal and maximal mitochondrial activity compared with control cells (Fig. 3a). The amount of mitochondrial DNA did not decrease after knockdown of macroH2A1.1, but rather tended to increase (Fig. 3b). We observed slightly increased expression of Cox4i1 and genes encoding components of the five complexes of the respiratory chain induced during myogenic differentiation in macroH2A1.1-depleted but not macroH2A1.2-depleted myotubes compared with controls (Fig. 3c). We did not observe any changes in the ratio of nuclear-encoded to mitochondrial-encoded mitochondrial ribosomal RNA (Fig. 3d). Together, these results suggest that specific reduction of macroH2A1.1 in the nucleus provokes a change in cellular metabolism that cannot be explained by a delay in differentiation.

**Figure 2** MacroH2A1.1 knockdown results in metabolic changes without affecting myogenic differentiation. (a) Left, a scheme of the experimental setup. Proliferating C2C12 cells were grown in growth medium (GM) and differentiated in differentiation medium (DM). Medium-change intervals and siRNA (si) transfections are indicated. Right, mRNA levels of mH2A1.1 and mH2A1.2 as determined by RT-qPCR. n = 5 independent experiments. *P < 0.05 compared to the control siRNA (si Ctrl)-treated cells; Student’s t-test. (b) Top, C2C12 cells were treated as described in a and analyzed at day 4. Anti-embryonic myosin heavy chain (eMHC) immunostaining was used to mark myotubes, and DAPI was used as a DNA counterstain. Bottom, the total number of nuclei analyzed in a predefined area and as a measure of differentiation (the percentage of nuclei among all eMHC-stained cells). n = 3 independent experiments. ns, not significant by Student’s t-test. (c) Relative RNA levels of the differentiation genes Myod1, Myog and Ckm as determined by RT-qPCR. n = 4 independent experiments. *P < 0.05 compared to the si Ctrl; Student’s t-test. (d) The difference in medium color observed after 4 d of differentiation and 48 h after the last media change. (e) Concentrations of extracellular glucose (n = 3) and lactate (n = 4) and total cellular protein levels (n = 4) in the samples shown in d. *P < 0.05; ns, not significant; Student’s t-test. (f) Results of glycolysis analysis by monitoring of the extracellular acidification rate (ECAR) in siRNA-treated C2C12 cells after overnight glucose starvation (n = 5 independent experiments), glucose (Glc) addition and quenching with 2-deoxyglucose (2-DG), normalized for genomic DNA content. (g) Mitochondrial respiratory capacity as assessed by measurement of the oxygen-consumption rate (OCR) of siRNA-transfected C2C12 cells in medium with normal sugar content normalized for genomic DNA content (n = 5 independent experiments). The ATPase inhibitor oligomycin (O), the uncoupling compound FCCP (F) and the electron transport chain inhibitors rotenone and antimycin A (AA) were subsequently added as indicated. Data in a–e are plotted as the mean ± s.d. Source data for a–g are available online.
compared with that in cells treated with control siRNA (Fig. 3e). Finally, we conducted a gain-of-function experiment by expressing comparable amounts of macroH2A1.1 and macroH2A1.2 in HepG2 cells (Fig. 3f). To avoid any confounding influence of endogenous macroH2A expression, we used cells that were efficiently depleted of all macroH2A isoforms. Expression of macroH2A1.1 but not of macroH2A1.2 increased the maximal cellular respiratory capacity compared with that in control cells (Fig. 3f). Together, these results suggest that the levels of macroH2A1.1 but not of macroH2A1.2 contribute to mitochondrial activity while leaving mitochondrial biogenesis unaffected.

Changes in gene expression are unlikely to account for metabolic phenotype

As structural chromatin components, macroH2A proteins have an ambivalent, probably indirect role in gene expression and can both positively and negatively influence transcription. To determine whether changes in gene expression were responsible for the mitochondrial defect we had observed, we carried out a transcriptomic analysis to compare control and macroH2A1.1-depleted myotubes at day 4 of differentiation, and we identified 797 differentially expressed genes (Supplementary Data Set 2). Most of these changes were subtle, and fewer than 50 genes were up- or downregulated by more than twofold (Supplementary Fig. 3a). Surprisingly, we did not identify any obvious candidates for the observed reduced metabolic activity among the top regulated genes (Fig. 4); indeed, known fatty acid oxidation or glycolysis genes were either not affected or only slightly upregulated (Supplementary Fig. 3b). Moreover, there was no enrichment of Gene Ontology terms related to metabolism among the differentially expressed genes (Supplementary Data Set 2). Thus, the analysis did not provide a clear hypothesis for the cause of the observed metabolic phenotype.

To examine whether the metabolite-binding pocket was required for the influence of macroH2A1.1 on gene expression, we carried out rescue experiments to compare wild-type macroH2A1.1 and the G224E mutant, which is unable to bind ADP ribose. Equal levels of both proteins were stably overexpressed in C2C12 cells that had been depleted of endogenous macroH2A1.1, and rescue was analyzed at day 4 of differentiation (Fig. 4b). We selected the three top regulated genes (Itga11, Cdhr1 and Tmem171) as well as two genes related to muscle growth (Mstr and Igf1) for our analysis, and found that wild-type and mutant constructs were equally efficient in rescuing the transcription of all five genes (Fig. 4c). Thus, the gene-regulatory role of macroH2A1.1 in myotubes does not depend on ADP ribose binding.

**Figure 3** Knockdown of macroH2A1.1 causes defective mitochondrial respiration. (a) Left, starved C2C12 cells were primed with fatty acids, and ATPase inhibitor oligomycin (O), the uncoupling compound FCCP (F) and the electron transport chain inhibitors rotenone and antimycin A (AA) were subsequently added as indicated. C2C12 cells had been treated previously with control siRNA, macroH2A1.1 siRNA or macroH2A1.2 siRNA. Right, the relative maximal respiration (max. resp.). (b) Mitochondrial DNA (mtDNA) content normalized to nuclear DNA (gDNA) with mt-Nd2 and Nduf1. (c) RT-qPCR analysis of mRNAs encoding components of respiratory chain complexes in proliferating (GM) and differentiated (DM; 4 d) cells treated with siRNA targeting macroH2A1.1 or siCtrl (siCtrl set to 1). (d) The mRNA levels of nuclear and mitochondrial genes encoding components of complexes I (Ndufa9 and mt-Nd3) and IV (Cox4i1 and mt-Co1) as measured by Taqman-probed RT-qPCR to assess balanced transcription. (e) The same conditions as in a, except that mitochondria were isolated before the analysis of respiration in cells treated with siCtrl or si mH2A1.1. (f) Left, fatty acid oxidation in HepG2 cells with double-knockdown of macroH2A1 and macroH2A2 (DKD) that stably expressed transgenic Flag-tagged (F-) macroH2A1.1, macroH2A1.2 or none of either (control). Data are the mean ± s.d. of n = 9 measurements of different cultures. Right, immunoblots with antibodies to the indicated proteins, showing the different expression levels. Lanes 3 and 4 are samples of DKD cells expressing YFP-tagged wild-type and G224E mutant macroH2A1.1, respectively (further analyzed in Fig. 5e)). Uncropped blot images are shown in Supplementary Data Set 1. Data in a–e are the mean ± s.d. of n = 6 (a,b,e) or 4 (c,d) independent experiments. *P < 0.05, Student’s t-test.
The binding pocket of the macroH2A1.1 macrodomain accommodates the ADP ribose molecule in a capping mode, which allows macroH2A1.1 to bind ADP ribose that is part of a PAR chain or in the context of a post-translational modification covalently linked to protein, such as PARP-1. Because links to metabolic activity have been reported for PARP-1 (refs. 29,30), we decided to assess the binding of macroH2A1.1 to PARP-1 and the requirement for the binding pocket in our system. We found that, when transfected into HEK293T cells, the macrodomain of wild-type macroH2A1.1, but not of the G224E mutant or the macroH2A1.2 isoform, was able to precipitate PARylated PARP-1 (Fig. 5b). Next, we monitored the activity of recombinant PARP-1 in the presence of various macrodomains. The macrodomain of wild-type macroH2A1.1, but not of the G224E mutant, inhibited auto-PARylation of PARP-1 in vitro (Fig. 5c). We further confirmed that PARP-1 binding depended on the integrity of the binding pocket when macroH2A1.1 was expressed in C2C12 cells (Fig. 5d). To examine the influence of macroH2A1.1 on stress-induced PAR formation in cells, we used quantitative immunofluorescence to analyze HepG2 cells that expressed wild-type macroH2A1.1 or the G224E binding-pocket mutant in a depleted background. After treatment with H2O2, PAR accumulated in the nucleus of the cells (Fig. 5e). This accumulation was largely inhibited when cells expressed wild-type macroH2A1.1, whereas it was not affected by expression of the G224E mutant. Thus, in agreement with an earlier report, macroH2A1.1 bound and inhibited PARP-1 in a manner that was dependent on the integrity of its ADP-ribose-binding pocket.
PARP activity is mediated by the inhibition of nuclear PARP activity, particularly of PARP-1.

The NAD+ precursor NMN mediates macroH2A1.1 influence on mitochondrial function

PARP-1 is one of the major consumers of the nuclear NAD+ pool. To test whether changes in NAD+ metabolism provide a molecular link between macroH2A1.1 and mitochondrial activity, we assessed changes in NAD+ and its precursors. The salvage pathway that regenerates NAD+ from NAM is the main biosynthetic pathway for NAD+. After the conversion of NAM to NAD+ by nicotinamide phosphoribosyltransferase in the cytosol, different NMN adenyl transferases (NMNATs) generate NAD+ from NMN in distinct subcellular compartments. During myogenic differentiation, mRNAs encoding nuclear NMNAT1 and mitochondrial NMNAT3 were upregulated, whereas the mRNA encoding the Golgi/cytosolic enzyme NMNAT2 was downregulated. Knockdown of macroH2A1.1 increased levels of the mRNA encoding the Golgi enzyme but did not affect those of nuclear and mitochondrial enzyme mRNAs. When we directly measured the cellular levels of NAD+, NAM and NMN under the same conditions, we found that knockdown of macroH2A1.1 substantially reduced the level of NMN without altering levels of the other metabolites. NMN was also the only metabolite of the NAD+ salvage pathway for which levels changed during differentiation, and that amount was several-fold higher in differentiated cells. To determine whether altered NMN levels would translate into a specific change in mitochondrial NAD+, we purified mitochondria and repeated the metabolite measurement. We found that levels of both NMN and NAD+ were substantially reduced in mitochondria, whereas the level of NAM was slightly increased. Thus, in myotubes that lacked macroH2A1.1 the reductions in total cellular and mitochondrial NMN correlated with reduced mitochondrial respiratory capacity. The addition of NMN to culture media rescued mitochondrial respiratory capacity in macroH2A1.1-depleted cells, and the same was true when mitochondria were isolated before analysis. Knockdown of the mitochondrial enzyme NMNAT3 reduced mitochondrial activity on its own and blunted the ability of NMN to rescue mitochondrial respiration in macroH2A1.1-depleted cells. Furthermore, neither NMN treatment nor PARP-1 inhibition rescued a panel of genes sensitive to macroH2A1.1 depletion. These results strongly support the idea that the influence of macroH2A1.1 on mitochondrial activity is mediated through NAD+ metabolism, in particular through the levels of the NAD+ precursor NMN.

We conclude that macroH2A1.1 controls mitochondrial metabolism by maintaining mitochondrial levels of NAD+ through inhibition of its nuclear consumption by PARP-1.

**DISCUSSION**

A chromatin component inhibits PARP-1 and affects mitochondrial activity

Our results describe a novel mechanism by which differentiating muscle cells couple a change in chromatin composition with de novo requirements for metabolic activity. We found that during myogenic differentiation, muscle cells altered the splicing of the transcript that encodes histone variant macroH2A1 to increase expression of the ADP-ribose-binding macroH2A1.1 isoform in myotubes. Although myoblasts are highly proliferative and have a high demand for the generation of biomass, differentiating cells exit the cell cycle and shift toward increased use of carbon for mitochondrial ATP production.

**Figure 6** Inhibition of PARP-1 rescues the mitochondrial phenotype. (a) Nuclear PARPs are expressed in differentiated myoblasts. We analyzed mRNA levels for PARP-1 and PARP-2 in proliferating myoblasts in growth medium (GM) and in differentiated myotubes after 4 d in differentiation medium (DM). (b) Western blot signals of PARP-1 and macroH2A1.1 in differentiated C2C12 cells (DM) compared with those of equal amounts of purified reference proteins. (c) Western blot signals of PARP-1 and His-tagged macroH2A1.1 in differentiated C2C12 cells compared with those of equal amounts of purified reference proteins. (d) The NAD+ levels in myoblasts, but not those in myotubes, are sensitive to PARP inhibition. Proliferating (GM) and differentiated (DM) C2C12 cells (DM) were treated with 100 nM PARP inhibitor (inhibit) ABT-888 or left untreated. Untreated controls were set to 1. (e) Rescue of mitochondrial activity by genetic and pharmacologic PARP inhibition. Maximal respiratory capacity in fatty-acid-primed C2C12 cells treated with siRNA for 4 d and rescued by co-administration of 100 nM PARP inhibitor ABT-888 for 16 h (left) or co-transfection of PARP-1 siRNA (right). si Ctrl cells treated or not with DMSO vehicle (left or right, respectively) were set to 100%. (f) The same conditions as in e, except that mitochondria were isolated before the analysis of respiration. Data in a–f are the mean and s.d. of n = 4 independent experiments. *P < 0.05, Student’s t-test. Source data for a are available online.

PARP inhibition rescues the mitochondrial phenotype

We next tested whether nuclear PARPs mediate the mitochondrial phenotype induced by macroH2A1.1 depletion. During myogenic differentiation, PARP-1 was downregulated at the levels of both mRNA and protein (Fig. 6a,b), whereas PARP-2 mRNA levels remained unaltered (Fig. 6a). In differentiated cells, macroH2A1.1 and PARP-1 were expressed at similar levels, but macroH2A1.1 was slightly more abundant than PARP-1 (Fig. 6c). Because we lacked the tools needed to directly measure the basal activity of PARP-1, we instead indirectly assessed cellular PARP activity by monitoring the effect of a PARP inhibitor that targets several PARPs, including PARP-1 and PARP-2, on the cellular levels of the PARP cofactor NAD+. Short-term treatment with the inhibitor increased the levels of NAD+ in proliferating myoblasts but not in differentiated myotubes (Fig. 6d), suggesting that PARPs could have a basal activity in myoblasts that is absent in myotubes.

To test the intriguing hypothesis that a loss of PARP-1 inhibition caused by macroH2A1.1 depletion might underlie the metabolic phenotype, we carried out rescue experiments. Differentiated C2C12 cells depleted for macroH2A1.1 were either treated with PARP inhibitor or siRNA-depleted for PARP-1, and mitochondrial capacity was determined. Both pharmacologic and genetic inhibition of PARP-1 were sufficient to rescue mitochondrial activity in macroH2A1.1-depleted myotubes and isolated mitochondria (Fig. 6c). These observations further substantiate that the influence of macroH2A1.1 on mitochondrial activity is mediated by the inhibition of nuclear PARP activity, particularly of PARP-1.
In our study, knockdown of macroH2A1.1 in myotubes reduced the oxidative capacity of the cells’ mitochondria, thus demonstrating that knockdown of this structural chromatin component affects cellular metabolism. Because knockdown of the individual macroH2A1.1 splice isoform had no apparent effect on differentiation, we conclude that the influence of macroH2A1.1 on mitochondrial metabolism is not the result of a simple delay in differentiation.

The abundance and wide genomic distribution of macroH2A1.1 confer the potential to mediate global nuclear effects. Taken together, our results and previous reports13,14,31 suggest that macroH2A1.1 can bind and inhibit auto-modified PARP-1 and possibly other nuclear PARPs. PARP-1’s basal activity is lost when its endogenous antagonist macroH2A1.1 is expressed during myogenic differentiation. Although it is conceivable that strong stimuli may be able to overcome this level of inhibition, the threshold for PARP-1 activation is higher in fully differentiated cells that express macroH2A1.1. PARP-1 is the major NAD+ consumer in the nucleus3, and its activity affects NAD+-dependent reactions in other organelles and mitochondrial activity29,30. The expression of macroH2A1.1 and its dampening of PARP-1 activity may allow nonproliferating muscle cells to avoid unnecessary, low-stress-induced NAD+ consumption in the nucleus and thus prioritize its use for ATP production in mitochondria and cell survival.

**Figure 7** The influence of macroH2A1.1 on mitochondrial function is mediated by the NAD+ precursor NMN. (a) A schematic representation of the NAD+ recycling pathway and its reported compartmentalization. (b) mRNA levels for genes that encode the enzymes of the NAD+ salvage pathway, as analyzed by RT-qPCR in proliferating (GM) and differentiated C2C12 cells (DM; day 4) treated with siRNA targeting macroH2A1.1 or control siRNA. mRNA levels in differentiated control cells were set to 1. The reported main localization of the encoded enzymes is indicated. (c) Quantification of the metabolites of the NAD+ salvage pathway by LC-MS/MS in proliferating (GM) and differentiated C2C12 cells (DM; day 4) treated with or without siRNA targeting macroH2A1.1. (d) Quantification of the metabolites in mitochondria isolated from C2C12 myotubes at day 4 of differentiation after treatment with siRNA targeting macroH2A1.1 or control siRNA. Data were normalized for mitochondrial NDUFA9 protein levels. (e) NMN rescues mitochondrial capacity. A fatty acid oxidation assay was used to analyze the maximal respiratory capacity in fatty-acid-primed C2C12 cells treated with siRNAs and 500 nM NMN for 24 h at day 4 of differentiation. (f) The same conditions as in e, except that mitochondria were isolated before the analysis of respiration. (g) A schematic summary of the results showing how NMN links nuclear PARP-1 inhibition by macroH2A1.1 to maintenance of the mitochondrial NAD+ pool. Ox. phos., oxidative phosphorylation. Data in b-f are the mean ± s.d. of n = 4 (b-d) or 6 (e,f) independent experiments. *P < 0.05, Student’s t-test. Source data for d are available online.

**Influence on mitochondrial activity is largely independent of gene regulation**

MacroH2A proteins have a reportedly ambivalent role in transcription28 that may be, to some extent, secondary to their role in regulating higher-order chromatin architecture7. Although we cannot fully exclude the influence of gene expression, several observations suggest that the effect of macroH2A1.1 on mitochondrial activity is largely independent of transcriptional regulation. First, we did not identify any obvious candidates among the deregulated genes in macroH2A1.1-depleted myotubes. Second, PARP inhibition rescued the respiratory phenotype of macroH2A1.1-depleted cells (Fig. 6e,f) while mimicking the loss of macroH2A1.1 in gene regulation33. Third, in addition to PARP inhibition, the NAD+ precursor NMN rescued the mitochondrial phenotype but did not alter gene expression (compare Fig. 7e and Supplementary Fig. 4c). Finally, assays with the G224E mutant of macroH2A1.1 suggested that the binding pocket is required to rescue respiratory capacity but not gene expression (compare Figs. 4c and 5a). The requirement for ADP ribose binding by macroH2A1.1 is in line with the observation that macroH2A1.2 did not affect stress-induced PARylation or mitochondrial activity. It is possible that all macroH2A proteins have a common function with regard to chromatin architecture and thus exert shared influence on a subset of genes. The ability to inhibit PARP-1, and possibly other PARPs, however, is unique to macroH2A1.1 and underlies its influence on mitochondrial activity.

**NMN communicates metabolic states between nucleus and mitochondria**

Nuclei and mitochondria are important organelles that control genome usage and energy metabolism, respectively. It is intuitive that cells have evolved mechanisms that allow them to communicate and coordinate these two key activities. As mitochondria and nuclei are spatially separated compartments, shared metabolites have great potential to act as messenger molecules. Our results suggest
that changes in the level of the NAD⁺ precursor NMN mediate the influence of macroH2A1.1 on mitochondrial activity.

NAD⁺ is a key metabolite of central metabolism and is required for metabolic pathways in the nucleus and mitochondria. NAD⁺ is indeed required for proper mitochondrial function, and its reduced form NADH is essential for proper functioning of the respiratory chain2. Because cells are able to replenish NAD⁺ pools primarily through the salvage pathway that regenerates NAD⁺ from NAM through NMN1, we also analyzed the influence of macroH2A1.1 depletion on levels of the precursors NAM and NMN. We found that NMN was depleted in macroH2A1.1-knockdown cells, which suggests that in the absence of macroH2A1.1, NAD⁺ consumption is indeed accelerated, but this consumption is largely compensated by the salvage pathway.

Because NAD⁺ cannot cross the mitochondrial membrane, mitochondrial and nucleo-cytoplasmic NAD⁺ pools are separate. How mitochondria maintain their NAD⁺ pool is a topic of ongoing debate34–37. The results of a study involving novel biosensors suggested that different mechanisms exist and that their relative contributions to the mitochondrial NAD⁺ pool are to some extent cell-type dependent38. Among these mechanisms, the import of the precursor NMN and its subsequent conversion to NAD⁺ by NMNAT3 contribute substantially to the mitochondrial NAD⁺ pool in several cell types39. We found that depletion of the total NMN pool in macroH2A1.1-knockdown cells resulted in a specific reduction of mitochondrial NAD⁺, whereas total NAD⁺ levels were not affected. This suggests that the mitochondria are less effective in regenerating NAD⁺ from NMN than from the nucleo-cytoplasmic compartment. Indeed, nuclear NMMAT1 has a lower Kₘ for NMN than mitochondrial NMMAT3 does32 and thus might be more effective in regenerating NAD⁺, in particular when the concentration of NMN is relatively low. Further, secondary biosynthetic pathways might facilitate the regeneration of NAD⁺ primarily in the cytosol. Independently of the exact mechanism, our results support a model in which NMN is in a central position, communicating changes in nuclear NAD⁺ usage to mitochondria. In strong support of this model, we found that the addition of NMN to culture media was sufficient to rescue the mitochondrial defect in macroH2A1.1-depleted cells (Fig. 7e,f). This rescue was abrogated by the knockdown of mitochondrial NMMAT3, confirming the necessity of the NMN salvage pathway for the restoration of normal mitochondrial activity.

In conclusion, our findings suggest a model in which the expression of macroH2A1.1 links the chromatin state to optimal energy metabolism by limiting nuclear NAD⁺ consumption and buffering the NAD⁺ precursor NMN. Different mammalian tissues express different levels of macroH2A1.1 (Fig. 1a), which provides a mechanism to adjust the threshold of nuclear PARP activation to cell-type-specific nuclear and mitochondrial requirements for NAD⁺. Several studies have related the expression of macroH2A1.1 to the progression of differentiation and reduced proliferation39,40. It will be interesting to evaluate whether a shift in splicing toward more macroH2A1.1 might be a general phenomenon of terminal differentiation processes that are coupled to an exit from the cell cycle. The observation that overexpression of macroH2A1.1 but not of macroH2A1.2 prevents nutrient-enforced fat accumulation in liver cells41,42 supports the idea that such a mechanism would not be restricted to the muscle lineage. Future work will address the exciting question of to what extent macroH2A1.1 controls metabolic homeostasis in vivo, particularly under conditions that challenge mitochondrial function, such as stress, exercise and aging.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.P.M., R.T. and M.B. conceived the project; M.P.M., S.H.-B., M.S., P.B., I.A., A.G.L., R.M.G.-R., O.Y., J.A.P., R.T. and M.B. designed experiments and interpreted data; O.Y. contributed methods; M.P.M., S.H.-B., M.L., V.V., H.D., M.N., D.C., I.G., J.D. and P.G.-P. performed experiments; R.M. analyzed high-content data; and M.P.M., J.A.P., R.T. and M.B. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Cell cultures and isolation of primary myoblasts. C2C12 myoblasts (ATCC; CRL-1772) were grown in growth medium consisting of DMEM (Gibco) containing 20% FBS (Invitrogen) and 1% penicillin-streptomycin (Gibco). Tissues and primary mouse myoblasts were prepared from 2-month-old C57Bl/6 mice as described elsewhere. Animal housing and handling procedures to isolate primary muscle cells were approved by the ethics committee of the Germans Trias i Pujol Research Institute, in accordance with the Catalan Government’s animal care guidelines. Primary myoblasts were maintained in Ham’s F10 (Gibco) medium supplemented with 20% FBS (Invitrogen), 10 ng/ml bFGF (Invitrogen), 0.1% Fungizone (Invitrogen), and penicillin-streptomycin. For the maintenance of primary myoblasts, cell culture plates were coated with rat-tail collagen I (BD Biosciences) for 2 h at 37 °C. For differentiation, cell culture plates were Matrigel-treated (BD Biosciences) for 1 h at 37 °C. The differentiation medium for all cell cultures was DMEM supplemented with 2% horse serum (Gibco) and penicillin-streptomycin. To induce differentiation, we seeded cells at high confluence and, the next day, washed them once with PBS before inducing differentiation by adding DM.

HEK293T cells (ATCC; CRL-3216), GP2 packaging cells (Clontech; 631458) and HepG2 cells (HB-0865) were grown in DMEM containing 10% FBS and penicillin-streptomycin. Primary mouse myoblasts were prepared as described elsewhere. Human primary myoblasts were kindly provided by Eduard Gallardo (Institut de Recerca Hospital de la Santa Creu i Sant Pau) and maintained in DMEM (BioWhittaker) containing 10% FBS (Hyclone), 15 ng/ml bFGF (Invitrogen), 10 ng/ml EGF and 1% insulin (1 mg/ml; Sigma-Aldrich) and on 0.1% gelatin-coated plates. For differentiation, medium containing 1% insulin (1 mg/ml; Sigma-Aldrich) and 1% penicillin-streptomycin. To induce differentiation, we seeded cells at high confluence and, the next day, washed them once with PBS before inducing differentiation by adding DM.

Plasmids, gene transduction and siRNA transfection. For plasmid construction, we used standard cloning techniques. Mouse macroH2A1.1 was amplified from C2C12 cell cDNA and inserted into a retroviral pBABE.puro backbone. The GS24E binding-pocket mutant and the silent mutations at the siRNA recognition site were generated via Stratagene’s site-directed mutagenesis QuikChange protocol. Plasmids encoding GFP and His-tagged macro domains (corresponding to amino acids 155–369 of macroH2A1.1 and 155–372 of macroH2A1.2) were described previously.

Retroviral infections were performed essentially as described, with minor changes in the preparation of the target cells. Briefly, C2C12 myoblasts were seeded the day before infection onto six-well plates at a density of 10,000 cells per well. Cells were exposed to viral supernatant containing 0.3 volumes of fresh medium and 8 µg/ml polybrene (Sigma-Aldrich) for 2 h at 37 °C. For differentiation, the cells were grown at 20 °C after induction with 500 µM IPTG. Bacteria were collected by centrifugation for 3 min at 1,000 g and washed once more in sucrose buffer. The lysates were incubated with anti-macroH2A1.1 (ref. 25); anti-macroH2A1.2 (Cell Signaling; 4827S); anti-ubiquitin (Trevigen, 4336-APC-050); anti-tubulin (Sigma-Aldrich; T6074); anti-NPM1 (Abcam); anti-PAR (AbCam, ab14713; Santa Cruz Biotechnology; sc-12732); anti-GFP (Santa Cruz Biotechnology; sc-145); anti-H3K4me3 (AbCam; ab6097); for 2 h and washed with 10 µM imidazole-containing buffer. Proteins of interest were subsequently eluted with elution buffer containing 200 µM imidazole. The eluted proteins were dialyzed overnight against PBS, 5 mM β-mercaptoethanol and 10% glycogen, and finally stored at −80 °C.

Immunoprecipitation and immunoblotting. For immunoprecipitation, we isolated nuclei and solubilized chromatin by sonication. Specifically, cells were collected by scraping and lysed in sucrose buffer (0.32 M sucrose, 10 mM Tris-HCl, pH 8.0, 3 mM CaCl2, 2 mM MgOAc, 0.1% Triton buffer, 1:100 PMSF and 1:500 leupeptin). Samples were passed through a syringe to facilitate membrane disruption and then further incubated for 8 min on ice. Intact nuclei were collected by centrifugation for 3 min at 1,000 g and washed once more in sucrose buffer. Washed nuclei were resuspended in lysis buffer (50 mM Tris-HCl, pH 7–8, 135 mM NaCl, 0.1% Triton, 1 mM EDTA, 1 mM DTT, 1:1000 PMSF and 1:500 leupeptin), and chromatin was solubilized by progressive sonication with a Bioruptor (Diagenode). For the precipitation of PARylated PARP-1, PARP and PARP inhibitors (1 µM ADP-HPD (CalBioChem) and 1 µM olaparib (SelleckChem), respectively) were added to the lysis buffer. The remaining insoluble material was removed by centrifugation, and lysates were pre-cleared with Sepharose beads (Sigma). At this step, 5% of the total lysate was kept as input material, and the rest of the lysate was incubated with antibody-bound beads for 3 h to overnight. Beads had been previously blocked with 1% BSA in lysis buffer. Precipitates were washed three times with lysis buffer containing 1% Triton X-100. For SDS-PAGE and western blotting analysis, typically 1% input and 20% immunoprecipitated material was loaded. The relative intensity of immunoblot bands was quantified by ImageJ software (version 2.0.0-rc-15/1.49k). For cell fractionations, nuclei were prepared as described above and the supernatant was kept as the cytosolic fraction. Nuclei were then incubated with buffer (20 mM HEPES, pH 7.9, 1.5 mM MgCl2, 0.2 mM EDTA, 25% glycerol, 1% NP-40) containing 410 mM KCl, and ultracentrifugation was used to separate the chromatin (pellet) and nucleosol (supernatant).

Immunofluorescence and immunocytochemistry. For immunofluorescence, cells were grown on Menzel-Gläser slides and fixed in 4% paraformaldehyde (10 min at room temperature). The slides were permeabilized for 10 min with 0.1 M HCl, 0.5% Triton-X 100 in PBS and washed three times with PBST (PBS containing 0.1% Tween 20). Then, slides were pre-blocked with PBST-BSA (5%) for 30 min at room temperature and incubated with a 1:50 to 1/100 dilution of specific primary antibody for 2 h in BSA 5% buffer. After three washes with PBST, the slides were incubated for 1 h at room temperature with a 1/100 dilution of secondary goat anti-rabbit antibody conjugated to Alexa Fluor 488 (Thermo Fisher). After successive washes with PBST, slides were mounted with Vectashield mounting medium with DAPI. Images were obtained with a Leica DMI6000B Advance Fluorescence microscope (Leica) equipped with a 63x/1.4-numerical aperture (NA) plan-apochromat oil-immersion objective. Images were loaded and analyzed in Fiji (ImageJ).

For immunohistochemistry, 10 µM muscle sections were fixed in 100% cold acetone at room temperature. For immunostaining we incubated anti-macroH2A1.1 for 1 h in a Ventana UltraView Universal DAB machine. Nuclei were additionally stained for 1 min in hematoxylin (Sigma-Aldrich). The sections were washed extensively, rinsed in 96% ethanol, dehydrated twice in absolute ethanol and mounted. Additional biopsy of human muscle was obtained from the Department for Pathologic Anatomy at the Hospital Universitari Germans Trias i Pujol (Badalona, Spain).

Bacterial protein expression and purification. BL21 (DE3) chemically competent Escherichia coli were transformed with bacterial expression constructs and grown at 30 °C after induction with 500 µM IPTG. Bacteria were collected and lysed in lysis/wash buffer containing 50 mM Tris-HCl, pH 8, 300 mM NaCl, 10 mM imidazole and protease inhibitors. Afterward, lysates were cleared by centrifugation for 30 min at 4 °C and 20,000g. The lysates were incubated with Ni-NTA beads (Qiagen) for 2 h and washed with 10 mM imidazole-containing buffer. Proteins of interest were subsequently eluted with elution buffer containing 200 mM imidazole. The eluted proteins were dialyzed overnight against PBS, 5 mM β-mercaptoethanol and 10% glycogen, and finally stored at −80 °C.

In vitro PARP-1 activity assay. Auto-PARYlation was performed in the buffer containing 50 mM Tris-HCl, pH 8, 50 mM NaCl and 1 mM MgCl2 at room temperature for 20 min. The reaction volume of 30 µL contained 0.2 units/µL PARP-1 HSA enzyme (Trevigen), 0.3× activated DNA (from 10× activated DNA; Trevigen), 200 µM NAD+ and different concentrations of purified macrodomains. Reactions were stopped by the addition of Laemmli’s sample buffer and boiling at 95 °C, separated on SDS-PAGE and analyzed by immunoblotting. Transferred proteins were counterstained with naphthol blue (Sigma).

RNA analysis including restriction-fragment-length polymorphisms. Total RNA from cells was isolated with the Invitrogen PureLink RNA kit, and cDNA was synthesized with the First Strand cDNA synthesis kit (Fermentas) as described.
Relative cDNA levels were quantified by RT-qPCR. Values were normalized to two or three housekeeping genes (RpL32, Gapdh and Rp167 for mouse samples, and Hprt1 and Riplo for human samples). All oligos were purchased from Invitrogen and are listed in **Supplementary Table 1**. To check for possible mitochondrial imbalance, we tested two mitochondrially encoded (mt-Nd3 and mt-Co1) and two nuclear-genome-encoded (Ndufa9 and Cox4i1) genes belonging to complexes I and IV with TaqMan probes from Applied Biosystems (Life Technologies).

Restriction-fragment-length polymorphism was used to qualitatively assess the amount of macroH2A1 isoforms. Essentially, cDNA obtained from RNA was used directly for the PCR reaction with oligos specifically annealing on exons 5 and 8 on exons 6 and 9 of the mouse or human transcripts encoding H2AFy/ macroH2A1, respectively. Amplicons were digested with HpaII/MspI restriction enzyme (Thermo Scientific) for 2 h at 37 °C. See the scheme in **Supplementary Figure 1b** for further detail and **Supplementary Table 1** for oligo sequences. The digested products were separated by electrophoresis on 2% agarose gel.

**Mitochondrial isolation.** All steps were performed at 4 °C. Freshly collected cell pellets were resuspended in ice-cold hypotonic lysis buffer (10 mM NaCl, 1.5 mM MgCl2, 10 mM Tris-HCl, pH 7.5) containing deactetylases (10 mM NAM, 2 mM TSA) and protease inhibitors (1 µM PMSE, 1 µM leupeptin) and incubated on ice for 2 min to allow swelling. To keep the organelles intact, we added ice-cold 2.5× homogenization buffer (800 mM sucrose, 25 mM Tris-HCl, 2.5 mM EDTA) to the homogenate. Cell membranes were disrupted by 15 strokes of a Teflon pestle in a Potter-Elvehjem homogenizer (Sigma-Aldrich). The nuclear fraction was removed by two successive centrifugations for 10 min at 1,200g. Mitochondria were collected by centrifugation for 20 min at 16,000g and resuspended in 1× homogenization buffer (320 mM sucrose, 10 mM Tris-HCl, 1 mM EDTA) containing protease and deacetylase inhibitors (10 mM NAM, 2 mM TSA, 1 µM PMSE, 1 µM leupeptin).

**Oxygen consumption and extracellular acidification rate measurements.** We analyzed the oxygen consumption rate and the extracellular acidification rate with a Seahorse XF96 flux analyzer (Seahorse Bioscience) as described47. Briefly, 24 h after siRNA transfection, C2C12 cells were seeded onto XF 96-well cell culture microplates, and the next day mitochondrial stress tests (MSTs) and glycolytic stress tests were performed. The MST was performed in minimal medium containing 25 mM glucose with subsequent addition of 1 µM oligomycin, 3 µM FCCP and 2 µM rotenone together with 2 µM antimycin A. The glycolytic stress test was performed in glucose-free medium with 10 mM glucose and 10 mM 2-deoxyglucose. For the fatty acid oxidation MST, cells were additionally starved for 24 h in substrate-limited medium (DMEM containing 0.5 mM glucose, 1 mM Glutamax, 0.5 mM citrate and 1% FBS) before the assay. Palmitate-BSA was added, and the MST was performed. Measurements of the oxygen consumption rate and extracellular acidification rate were normalized for genomic DNA content.

**Lactate and glucose measurements.** To measure lactate, we transferred cell culture supernatant to 5-mm NMR tubes. 1H-NMR spectra were recorded at 300 K on an Avance III 600 spectrometer (Bruker, Germany) operating at a proton frequency of 600.13 MHz. 1H-NMR spectra were referenced to the chemical shift of the residual water peak. The acquired spectral width was 12 kHz (20 ppm). The spectra were baseline corrected using MatLab (MathWorks) and integrated the specific NMR region of lactic acid by using the AMIX 3.9 software package. Glucose concentrations were measured via the glucose hexokinase method (Siemens, Dimension RHL Max Clinical Chemistry System, Siemens Healthcare Diagnostics) at the clinical biochemistry laboratory of the Hospital Universitari Germans Trias i Pujol (Spain).

Targeted metabolomics. Adherent cells were washed three times with PBS, immediately collected by scraping, and shock-frozen. Alternatively, mitochondrial were isolated and frozen as pellets. We extracted metabolites into the extraction solvent by adding 300 µL of cold acetone (ACN):H2O (1:1). Samples were vigorously mixed by vortexing for 30s and stored at −20 °C for 1h to enable protein precipitation. Subsequently, samples were centrifuged for 15 min at 4 °C and 22,600g, and the supernatant was transferred to an LC-MS vial. The samples were analyzed on a UHPLC system (1290 Agilent) coupled to a triple-quadrupole (QqQ) mass spectrometer (6490; Agilent Technologies) with iFunnel technol- ogy operated in multiple reaction monitoring (MRM) and positive electrospray ionization mode. Metabolites were separated by C18-RP (Acquity UPLC BEH, 1.7 µm, Waters) chromatography at flow rate of 0.3 mL/min. The solvent system was A (20 mM ammonium acetate and 15 mM ammonia in water:ACN (97:3)) and ACN was B. The gradient elution started at 100% A (time 0–1 min) and finished at 100% B (8–11 min). The injection volume was 5 µL. Electrospray ionization conditions were as follows: gas temperature, 170 °C; dry gas, 11 L/min; nebulizer, 20 psi; and fragmentor, 380 V. Quality controls consisting of pooled samples were used. Four quality control samples were injected repeatedly during the whole analysis. MRM transitions were as follows: NAM (236.06→170.10, 80.10), NMIN (335.07→123.10, 97.00) and NAD+ (664.12→428.00, 136.00). The peak areas were manually integrated and the data were normalized to total DNA for cell extracts and NDUFA9 protein for isolated mitochondria.

**Transcriptomic analysis.** Cells were collected at day 4 of the differentiation, washed well with 1× PBS and stored as pellets at −80 °C. The experiment was repeated more than six times. Once all the pellets were collected, RNA was extracted at once and aliquots of RNA were made. A small aliquot was used to transcribe the RNA into cDNA with the oligo(dT) (as described), and the knockout of macroH2A1.1 was tested in all biological replicates. The four biological replicates with the most successful macroH2A1.1 knockdown were selected. RNA was quantified, and quality was checked by Eukaryote Total RNA Nano assay by the IMPPC Genomics facility. RNA was amplified and loaded onto an Agilent SurePrint G3 Mouse GE 8×60K microarray slide. Differentially expressed genes were identified with LIMMA48 and selected with a cutoff q-value of 0.05 calculated after false discovery rate correction. The Gene Ontology analysis was done with the ChipPeakAnno R package49 using P < 0.01 and 30 as the minGOTerm (minimum count in a genome for a GO term to be included). We applied a Bonferroni multiple-hypothesis-testing adjustment to adjust the enrichment result. REVIGO online software was used to visualize summaries of the GO analysis50. Medium was chosen for the SimRel parameter.

**ChIP-seq.** We used a described pan-macroH2A1 antibody18 for chromatin immunoprecipitation experiments coupled to massively parallel sequencing (ChIP-seq) that were performed and analyzed essentially as previously described, with minor modifications. After cleaning and trimming, reads were aligned to mouse genome (mm9) with Bowtie 2 version 2.0.6, with a sensitive pre-setting (−D 15 -R 2 -L 22 -s 5,1,1,15)31. To detect genomic regions enriched for multiple overlapping peaks, we used SICER software version 1.1 to identify enriched genomic regions, using the following settings: redundancy threshold, 2; window size, 600; fragment size, 250; effective genome fraction, 0.75; gap, 1,200; false discovery rate, 0.05 (ref. 52).

**Statistical analysis.** If not indicated otherwise, a two-tailed Student's t-test was used to assess statistical significance. The number of technical replicates or independent cell culture experiments is indicated in the relevant figure legend(s).

**Data availability.** Microarray expression and ChIP-seq data have been deposited in GEO under accession number GSE8257. Source data for Figures 1d, 2e, 4c, 6a and 7d are available online. A *Life Sciences Reporting Summary* for this paper is available.

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Experimental design

1. Sample size
   Describe how sample size was determined. Whenever possible statistical methods have been used to determine sample size requirements and experiments have been performed and analyzed accordingly.

2. Data exclusions
   Describe any data exclusions. No data was excluded.

3. Replication
   Describe whether the experimental findings were reliably reproduced. All experiments were reliably reproduced ≥ 3 times. Occasional failures did not follow any consistent patterns.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups. Does not apply here.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis. Image analysis was automatized circumventing for blinding.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - [x] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

   - [x] A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

   - [x] A statement indicating how many times each experiment was replicated

   - [x] The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

   - [x] A description of any assumptions or corrections, such as an adjustment for multiple comparisons

   - [x] The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted

   - [x] A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

   - [x] Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.
Software

7. Software

Describe the software used to analyze the data in this study.

Published software has been cited in the online methods. Small customized scripts for image analysis are available on request.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restrictions beside the limitation of non-renewable stocks such as for antibodies.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

For all antibodies the references for validating papers or data from suppliers has been given.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

C2C12 cell lines have been authenticated by their myogenic differentiation capacity. All other cell lines were authenticated using AmpFISTR Identifiler PlusPCR Amplification Kit (Life Technologies, Ref.4427368) following the supplier’s instructions.

b. Describe the method of cell line authentication used.

Stable HepG2 cell lines (DKD and rescue lines) and used packaging cells lines were tested negative for mycoplasma and grown under controlled mycoplasma free conditions. C2C12 stocks were cultured for a limited number of few weeks and not further tested.

c. Report whether the cell lines were tested for mycoplasma contamination.

HEK293T cells have been used for biochemical experiments. No contaminant was detected using AmpFISTR Identifiler PlusPCR Amplification Kit (Life Technologies, Ref.4427368).

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Primary myoblasts and tissues were isolated from 2 month old male and female C57BL/6 mice. No sex specific differences were observed.

Animals and human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Does not apply.