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Integrative regulation of physiology by histone deacetylase 3

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Abstract

Cell-type-specific gene expression is physiologically modulated by the binding of transcription factors to genomic enhancer sequences, to which chromatin modifiers such as histone deacetylases (HDACs) are recruited. Drugs that inhibit HDACs are in clinical use but lack specificity. HDAC3 is a stoichiometric component of nuclear receptor co-repressor complexes whose enzymatic activity depends on this interaction. HDAC3 is required for many aspects of mammalian development and physiology, for example, for controlling metabolism and circadian rhythms. In this Review, we discuss the mechanisms by which HDAC3 regulates cell type-specific enhancers, the structure of HDAC3 and its function as part of nuclear receptor co-repressors, its enzymatic activity and its post-translational modifications. We then discuss the plethora of tissue-specific physiological functions of HDAC3.

The precise regulation of gene expression is essential for the regulation of mammalian development and physiology to ensure proper growth, function, homeostasis and adaptation to changing conditions. Gene regulation is primarily mediated by sequence-specific transcription factors, which localize at *cis*-regulatory enhancer elements[1–3]. Binding of lineage-determining transcription factors facilitates chromatin opening and binding of signal-dependent transcription factors, including nuclear receptors[4–10]. This leads to the intricate assembly of transcriptional complexes that regulate gene expression, in part through the recruitment of transcription co-regulators that further modulate chromatin structure, including through the reversible acetylation of histone proteins by histone acetyltransferases (HATs) and histone deacetylases (HDACs)[11,12].

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M.J.E. and M.A.L. researched data for the article, made substantial contributions to the writing and content and reviewed and edited the manuscript before submission.

Competing interests

M.A.L. is a consultant to KDAC, a company developing his-tone deacetylase (HDAC) inhibitors, and Novartis, and serves on scientific advisory boards for Pfizer and Eli Lilly and Co.

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HAT activity is an intrinsic property of transcription co-activators, which promote gene expression through the transfer of an acetyl group from acetyl-CoA to the ε-amine of several lysine residues on the amino-terminal tails of histones[13–16]. HDACs antagonize the functions of HATs through the deacetylation of histone tails and thus repress gene expression. Numerous studies have demonstrated that precise control of these opposing enzymatic functions is crucial for development and physiology[17].

The mammalian genome encodes 11 HDAC isoforms that make up the HDAC superfamily, which exert both specific and sometimes overlapping functions in the nucleus and cytosol. The first HDAC to be isolated and characterized was HDAC1 (REFS[18–21]), and the gene encoding it is the founding member of the 11 highly conserved mammalian genes making up the HDAC family[22]. Those sharing the highest sequence homology to HDAC1 are referred to as class I HDACs, which include HDAC1, HDAC2, HDAC3 and HDAC8. The class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10. Class I and class II HDACs all contain a conserved deacetylase domain and require zinc for enzymatic function[19], although catalytic activity has been difficult to demonstrate for class IIa HDACs (HDAC4, HDAC5, HDAC7 and HDAC9)[17,23]. The single class IV HDAC is HDAC11, which demonstrates sequence conservation with class I and class II HDACs within the deacetylase domain. The structurally distinct, nicotinamide adenine dinucleotide-dependent class III HDACs (also known as sirtuins) do not share sequence similarity to class I and class II HDACs and have recently been reviewed elsewhere[24–26], as have HDACs in general[17,19,27].

In this Review, we comprehensively discuss the unique molecular and physiological functions of HDAC3. HDAC inhibitors are increasingly used in medicine, yet the drugs currently used affect the catalytic activity of multiple class I HDACs, including HDAC3 (REF[28]). We therefore focus on the fundamental differences between HDAC3 and other class I HDACs and on the varied, tissue-specific functions of HDAC3.

The structure and function of HDAC3

The recognition that HDAC3 is unique in comparison with other class I HDACs stems from the discovery in 2000 that HDAC3 is the predominant HDAC associated with nuclear receptor co-repressor 1 (NCoR1 or NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT; also known as NCoR2), which are both nuclear receptor co-repressors[29–31]. Studies of these complexes provided valuable insights into the enzymatic activity of HDAC3.

The enzymatic activity of HDAC3 is dependent on its interaction with NCoR or SMRT.

Nuclear receptors function as signal-dependent transcription factors that integrate and deliver developmental, hormonal, environmental and nutrient cues to the genome, thereby acting as genetic switches of gene transcription[32–35]. In the classical model of nuclear receptor function, ligand binding elicits an allosteric change in the structure of the nuclear receptor, which facilitates differential recruitment of co-activators or co-repressors[36,37] Co-activators with HAT activity bind to ligand-bound nuclear receptors, whereas co-repressors bind ligand-free nuclear receptors to directly mediate gene repression or less commonly to

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indirectly mediate gene activation (FIG. 1a). In the absence of activating ligands, nuclear receptors interact on chromatin with NCoR and SMRT, which dampen gene expression[36,38,39].

HDAC3 is a stoichiometric component of both the NCoR[29,30] and the SMRT[31] co-repressor complexes. Core NCoR and SMRT complexes contain the WD40 repeat-containing proteins TBL1X and TBL1XR1[31,40], which can recognize histones and recruit the ubiquitylation machinery and the 19S proteasome. The NCoR and SMRT complexes also include GPS2 (REF.[41]), whose molecular function within these complexes remains unclear. Together with HDAC3, these molecules represent the core stoichiometric components of the NCoR and SMRT complexes. Importantly, although earlier studies had demonstrated in vitro interactions between NCoR or SMRT and other class I HDACs, including HDAC1 (REFs[42–44]), HDAC3 was the only class I HDAC found in endogenous NCoR and SMRT complexes. Class II HDACs also interact with NCoR and SMRT in vitro and when overexpressed in mammalian cells[42,45], but they have not been identified in endogenous NCoR or SMRT complexes.

HDAC3 is not only a stoichiometric component of the NCoR and SMRT complexes; its catalytic function requires physical interaction with a conserved domain of the NCoR and SMRT proteins, which is known as the deacetylase-activating domain (DAD)[41,46–49] (FIG. 1 b). The DAD includes a SANT motif that is 57 amino acids in length, which is found in many chromatin remodellers and transcription regulators[50–52]; in both NCoR and SMRT, the SANT motif is flanked by a unique amino terminus of 36 amino acids in length that is required for interaction with and activation of HDAC3 by NCoR and SMRT[53]. The crystal structure of HDAC3 in complex with the SMRT DAD revealed extensive protein-protein interactions at the surface of the amino terminus of HDAC3 (REF[49]). The crystal structure also unexpectedly revealed the presence of inositol tetraphosphate (Ins(1,4,5,6)P_4 or IP4), which serves as an ‘intermolecular glue’ by forming hydrogen bonds and salt bridges[54] between the two proteins (FIG. 1 b). The atomic-level resolution of the DAD-HDAC3 structure also explained why a conserved tyrosine residue in SMRT (Y470) or NCoR (Y478) is crucial for the activating interaction with HDAC3 (REFS[53,55,56]). Interestingly, in the absence of NCoR or SMRT interactions, HDAC3 is unstable[57] and sequestered into a cytosolic TCP1 ring complex[58], which is a chaperone that facilitates folding of the HDAC3 polypeptide in the cytoplasm and is released upon presentation to NCoR or SMRT in the nucleus, allowing for the formation of an active HDAC3 enzyme complex in a process requiring ATP[47,59].

Non-enzymatic functions of HDAC3.

The potent deacetylase activity of HDAC3 can be abolished by point mutations in the active site of the enzyme (Y298F), by mutation of two catalytically important histidine residues (H134A-H135A; known as the HAHA mutant) or by mutation of a lysine at the interface of HDAC3 with IP4 and DAD (K25A)[59]. Remarkably, these mutant proteins are still able to partially rescue the effect of *Hdac3* deletion in mouse liver[59]. Furthermore, although loss of HDAC3 is lethal owing to gastrulation defects[57,58,60,61], mice with mutations in the DAD of both NCoR and SMRT are born in expected Mendelian ratios, despite lacking detectable

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HDAC3 enzymatic activity[55,62]. These observations strongly suggest that HDAC3 has important non-enzymatic functions. This is consistent with the recent observation that nearly 10% of mammalian enzymes have active site-inactivating mutations yet remain widely conserved, implying selective pressure for non-catalytic functions[56]. The non-enzymatic mechanisms of HDAC3 function remain to be identified, but must be taken into careful consideration when evaluating HDAC3 function in different tissues, and have important implications for the development of drugs targeting the enzymatic activity of HDAC3.

Post-translational modification of HDAC3.

All mammalian HDACs contain putative sites of post-translational modification, including phosphorylation sites that may alter HDAC3 activity, stability or protein complex assembly[63], similar to many nuclear receptor co-regulators[64]. Casein kinase 2 phosphorylates HDAC3 on Ser424 (REF[65]), a site that is not conserved in other class I HDACs, and alters the deacetylase activity of HDAC3 in the NCoR and SMRT complexes[66]. Biochemical co-purification studies also identified the protein serine/threonine phosphatase 4 complex to be associated with the HDAC3 complex[66]. The signalling mediators upstream of casein kinase 2, the functions of Ser424 phosphorylation in vivo and other functional post-translational modifications of HDAC3 in vivo remain to be identified.

Tissue-specific functions of HDAC3

Cell type-specific deletion of the *Hdac3* gene is required to understand its physiological roles and the early embryonic lethality of whole-body deletion of *Hdac3*. The development of genetically engineered transgenic mouse lines expressing Cre recombinase (Cre) under the precise control of tissue-specific promoters has facilitated studies by generating *Hdac3* deletions in an array of tissues and organs. Next, we discuss the consequences of *Hdac3* deletion in different mouse tissues.

HDAC3 controls lipid metabolism and circadian histone deacetylation in the liver.

Hepatocyte-specific deletion of *Hdac3* in a mouse line engineered to express Cre under the control of the albumin promoter (albumin-Cre) revealed considerable changes within liver hepatocytes in vivo in lipid, cholesterol and carbohydrate metabolism[67], which were consistent with early cellular studies[68]. Deletion of *Hdac3* in adult hepatocytes using adeno-associated virus (AAV) serotype 8 expressing Cre under control of the *TBG* promoter (AAV8-*TBG*-Cre) leads to massive hepatosteatosis owing to increased lipogenesis and decreased fatty acid oxidation[69,70]. Despite their hepatosteatosis, mice lacking HDAC3 in the liver are not insulin resistant but rather demonstrate improved glucose tolerance and insulin sensitivity, as intermediary metabolites are shunted away from gluconeogenesis towards de novo lipogenesis, where new lipids are safely stored by derepression of lipid storage pathways[69], which likely prevents lipotoxicity and insulin resistance.

Genome-wide chromatin immunoprecipitation followed by sequencing (ChlP-seq) analysis of hepatic HDAC3-bound enhancers revealed a subset of enhancers exhibiting circadian HDAC3 recruitment[70]. This is mediated through interaction of the NCoR-HDAC3 complex with the circadian nuclear receptor REV-ERBα (also known as NR1D1), which has higher

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affinity for NCoR than for SMRT[71,72]. The rhythmic recruitment of HDAC3 leads to a circadian rhythm of histone acetylation specifically at REV-ERBα binding sites in the liver, which are enriched for lipogenic genes. Consistent with this, REV-ERBα-deficient mice exhibit hepatosteatosis[73]. Mechanistically, the circadian rhythm of genomic histone deacetylation mediated by HDAC3 at REV-ERBα-regulated enhancers opposes chromatin looping between enhancers and promoters and gene activation through eviction of the acetylation reader bromodomain-containing protein 4 and the looping factor mediator complex subunit 1 (REF[74]).

REV-ERBα-dependent HDAC3 recruitment to enhancers in the liver is mediated by two mechanisms. First, during the day, direct binding of REV-ERBα to the DNA motifs RevDR2 and ROR response element (RORE) mediates the recruitment of HDAC3 to repress circadian clock genes[70,73]; during the night, the same enhancers are bound by RAR-related orphan receptor (ROR) with its co-activators, thereby imparting clock gene expression (FIG. 2a). Second, at metabolic genes, HDAC3 is recruited through tethering of REV-ERBα to the liver-specific transcription factor hepatocyte nuclear factor 6 (HNF6)[75]. During the night, when REV-ERBα is not expressed, HDAC3 is released from HNF6 sites, allowing for co-activator recruitment and metabolic gene activation in anticipation of feeding (Fig. 2b).

Livers lacking HDAC3 are considerably more fatty than those lacking REV-ERBα[69,70], suggesting that other transcription factors are involved in the regulation of liver genes by HDAC3. Analysis of the nuclear interactome of HDAC3 in mouse livers identified a novel HDAC3 co-repressor module containing pros-pero homeobox protein 1 (PROX1), which is recruited to chromatin by the nuclear receptor HNF4α independently of REV-ERBα[76] (Fig. 2c). It remains unclear how PROX1 mechanistically influences the HDAC3-containing NCoR and SMRT complexes. Other transcription factors, including nuclear receptors, are also likely to contribute to the overall function of HDAC3 in the liver[76].

HDAC3 primes the thermogenic capacity of brown fat for survival in cold conditions.

In brown adipose tissue (BAT), HDAC3 is required for nonshivering thermogenesis and for the maintenance of core body temperature; thus, mice lacking HDAC3 in BAT fail to survive acute exposure to very low temperatures[77]. In BAT, uncoupling protein 1 (UCP1) generates heat by dissipating energy through the uncoupling of mitochondrial respiration and subsequent increase in metabolic oxidation rates[78]. Mice with BAT-specific deletion of *Hdac3* using *Ucp1*-Cre or adiponectin-Cre exhibit almost no expression of UCP1, as well as transcriptional downregulation of mitochondrial and oxidative phosphorylation genes required for nonshivering thermogenesis and cold survival[77] (Fig. 3a). Bioinformatics interrogation of global run-on sequencing (GRO-seq) data in BAT to map functional enhancers through the identification of enhancer RNAs revealed thousands of sites where enhancer activity was downregulated upon loss of HDAC3 binding. Additional bioinformatics integration of ChlP-seq data sets uncovered a novel mechanism of HDAC3 co-activation at enhancers bound by the thermogenic nuclear receptor oestrogen-related receptor-α (ERRα)[77,79–81].

As exemplified by the *Ucp1* locus, HDAC3 colocalization with ERRα on chromatin is required for enhancer activation and *Ucp1* transcription (FIG. 3b). These *Ucp1* enhancers

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are also bound by peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α)[82], which is essential for ERRα-dependent transcription[77,83]. PGC1α is repressed by acetylation and derepressed by HDAC3-mediated deacetylation (FIG. 3c). Furthermore, HDAC3, PGC1α and ERRα co-bind enhancers of the *Ppargc1a* and *Ppargc1b* genes, thereby initiating a positive transcriptional feedback loop that supports the expression of *Ppargc1a, Ppargc1b, Ucp1* and oxidative phosphorylation genes that are essential for thermogenesis (FIG. 3c). Two subsequent studies demonstrated that ERRα[84] and its closely related family member ERRγ[85] are required for establishing the basal thermogenic and oxidative capacity of BAT; a tissue-specific deletion of these factors causes a phenotype highly similar to the HDAC3 BAT-specific knockout[84,85]. Additionally, the BAT transcription factor PR domain-containing protein 16 (PRDM16)[86] interacts with HDAC3 and NCoR[87], suggesting the existence of additional interactions among several important regulators of BAT thermogenesis. Notably, in the white adipose tissue of aged mice exhibiting impaired induction of brown-like 'beige' or 'brite' adipocytes[88], HDAC3 may modulate futile metabolic cycles[89]. Collectively, these studies demonstrate that HDAC3 is a major regulator of BAT-mediated thermogenesis and energy homeostasis.

HDAC3 influences cardiac development and cardio-myocyte metabolism.

The prevalence of congenital heart defects is nearly 1% of live births[90]. Cardiac progenitor cells (CPCs) making up the first heart field (FHF) and second heart field (SHF) are controlled by distinct gene networks that facilitate the development of the major cell types of the adult heart[91]. Depletion of HDAC3 in CPCs in vivo initiates precocious differentiation of cardiomyocytes, indicating the existence of a HDAC3-dependent coordination of CPC specification[92–94]. In embryonic stem cell models, HDAC3 was shown to tether cardiac lineage genes located within lamina-associated domains (LADs) to the nuclear lamina to silence the genes through the formation of facultative heterochromatin[94] (FIG. 4a). HDAC3 tethering of LADs to the nuclear periphery is independent of its catalytic function and mediated through physical interactions with LAD-associated protein complexes[90,94–96]. During differentiation, the HDAC3-tethered cardiac LADs are released from the nuclear periphery to dissipate the heterochromatin configuration and establish a chromatin configuration that is permissive for the expression of cardiomyocyte genes[97]. How HDAC3-tethered LADs are released from the nuclear lamina during development is unknown[94].

Deletion of *Hdac3* in the FHF using *Nkx2–5-Cre* results in lethality between embryonic day 11.5 (E11.5) and birth owing to severe underdevelopment of ventricular walls and to ventricular septal defects[92]. During FHF cardiogenesis, the transcription factor TBX5 promotes HDAC3 recruitment with nuclear receptors at select enhancers to repress cardiac lineage genes. Notably, a mutation in human *TBX5* (G125R) causes Holt-Oram syndrome, which is characterized by skeletal abnormalities and cardiac malformations, and may affect HDAC3 protein interactions and recruitment to chromatin[92].

Progenitor cells of the SHF give rise to structures that are involved in nearly two-thirds of all human congenital cardiac defects, including those seen in tetralogy of Fallot and DiGeorge syndrome[98]. Deletion of *Hdac3* in the SHF using *Isl1-Cre* and *Mef2c-anterior* heart field (AHF)-Cre activated transforming growth factor-β (TGFβ) signalling pathways and caused

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aberrant secretion of extracellular matrix proteins by myofibroblasts, which precipitated a connective tissue disorder and resulted in perinatal lethality owing to severe aortic, outflow tract, valve, ventricle and septal defects[93]. The phenotype of loss of HDAC3-mediated silencing of TGFβ signalling resembles the pathogenic connective tissue disruption and alteration of TGFβ signalling seen in Marfan syndrome and Loeys-Dietz syndrome[93,99,100]. In the SHF, HDAC3 silences TGFβl through a deacetylase-independent function by acting as a protein scaffold for the recruitment of components of the histone H3 lysine 27 (H3K27) methyltransferase Polycomb repressive complex 2 (PRC2) (FIG. 4b). Deletion of *Hdac3* in neural crest cells (NCCs) using *Wnt1-Cre* or *Pax3-Cre* also causes perinatal cardiovascular abnormalities through impairment of cell-autonomous differentiation into the smooth muscle of the heart owing to altered Notch signalling[101].

Prenatal cardiac-specific deletion of *Hdac3* using *Myhca-Cre* resulted in lethality by 16 weeks of age. Mice with loss of HDAC3 in cardiomyocytes developed severe hypertrophic cardiomyopathy and interstitial fibrosis of the left ventricle and septum, which were caused by pathological ventricular dysfunction owing to severe mitochondrial dysfunction, reduced activity of the electron transport chain and elevated levels of fasting myocardial triglycerides[61]. Postnatal deletion of *Hdac3* using *Mck*-Cre in cardiac and skeletal muscles resulted in a milder phenotype of ventricular and septal thickening, enlarged atria and elevated levels of fasting myocardial triglycerides associated with repression of cardiac oxidative phosphorylation and fatty acid metabolism pathways[102]. Challenging these mice with a high-fat diet caused severe hypertrophic cardiomyopathy and lethal heart failure owing to impaired fatty acid oxidation and myocardial energy starvation[102], suggesting that HDAC3 modulates cardiomyocytes to respond to dynamic environmental and nutrient conditions. The cardiomyopathies of both mouse models are reminiscent of those of mice lacking peroxisome proliferator-activated receptors or ERRs[103], or their co-activators PGC1α and PGC1β[104]. The complex consequences of *Hdac3* deletion in the heart clearly require further characterization of the specific functions of HDAC3 in development and in specific cell populations.

HDAC3 regulates neuronal cell fate and function.

HDAC3 also has an important role in neurodevelopment. Loss of HDAC3 in neural progenitor cells (NPCs) of the central nervous system leads to death within 16 hours of birth, which is attributed to impaired neuronal migration and cortical lamination, possibly owing to loss of HDAC3-dependent expression of the transcription factor T-box brain protein 1 (REF[105]) (FIG. 5a). HDAC3 in NPCs also controls glial cell fate, as mice lacking HDAC3 in NPCs exhibit increased numbers of astrocytes with a concomitant decrease in oligodendrocytes[105] (FIG. 5b). Indeed, deletion of *Hdac3* in primitive oligodendrocyte progenitor cells (OPCs) leads to a significant increase in the number of astrocytes relative to oligodendrocytes, resulting in pathological tremors and optic nerve impairment owing to myelination defects[106]. Neurodevelopmental cell fate mapping in mice demonstrated that HDAC3 guides OPCs towards the oligodendrocyte fate and away from the astrocyte fate[106]. ChlP-seq of HDAC3 in combination with global gene expression profiling in OPCs revealed a mechanism whereby HDAC3 deacetylates and inactivates the transcription factor signal transducer and activator of transcription 3 (STAT3) to inhibit astrogliogenesis; HDAC3 also

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interacts with the HAT p300 to activate enhancers and genes that control oligodendrocyte formation presumably through deacetylation of non-histone proteins[106] (FIG. 5b). Recent studies also revealed that HDAC3 represses pro-myelination expression programmes that include the HIPPO signalling effector TEAD4[107].

Thy1-Cre-mediated and *Camk2a*-Cre-mediated knockout of *Hdac3* in the postnatal forebrain and cerebellum led to lethality by 6 weeks of age. These mice had developed severe locomotor deficits, progressive ataxia and paralysis secondary to the loss of Purkinje neurons and substantial cerebellum abnormalities[105]. As HDAC3 is the most highly expressed class I HDAC in the adult brain, there is great interest in its influence on the transcriptional regulation of learning and memory. Loss of HDAC3 within the adult CA1 hippocampal region through bilateral intracranial injection of AAV-Cre revealed that HDAC3 is an important inhibitor of the formation of long-term memory through repression of the gene nuclear receptor subfamily 4, group A, member 2 *(Nr4a2)*[108] (FIG. 5c). Mice with the Y478A mutation in the NCoR DAD phenocopied the hippocampal deletion of *Hdac3* and thus established the existence of a HDAC3 catalytic-dependent mechanism that restricts the formation of long-term memory. Similarly, cocaine use decreases HDAC3 occupancy at the *Nr4a2* enhancer and promotes *Nr4a2* expression in the nucleus accumbens[109]. Thus, cocaine-induced *Nr4a2* expression may enhance memory formation and promote future drug abuse (Fig. 5c).

HDAC3 has recently been associated with the neurological disorder Rett syndrome. A missense mutation (R306C) in methyl-CpG-binding protein 2 (MECP2) impairs the recruitment of the NCoR-HDAC3 complex to MECP2-bound methylated DNA to repress gene transcription[110,111]. Deletion of *Hdac3* using *Camk2-Cre* in forebrain excitatory neurons causes a phenotype similar to that of the *Mecp2* knockout mouse model of Rett syndrome, including cognitive deficits and impaired sociability and locomotor coordination[112]. HDAC3 cistrome and transcriptome analyses in vivo showed that HDAC3 is a positive transcriptional regulator at a subset of neuronal genes; it is recruited to these genes by MECP2 and deacetylates the neuronal transcription factor FOXO3 (Fig. 5d). Notably, NPCs derived from human induced pluripotent stem cells harbouring the Rett syndrome MECP2-R306C mutation and corrected by CRISPR-Cas gene editing mechanistically re-capitulated and validated the mouse models demonstrating HDAC3 working as a positive transcriptional regulator at enhancers with FOXO3 (REP[112]).

HDAC3 coordinates lung development.

Conditional deletion of *Hdac3* using sonic hedgehog (*Shh*-Cre) in the lung endodermal epithelium at E8.75 revealed a requirement for HDAC3 in lung sacculation and early alveologenesis, resulting in lethality between 2 and 10 days after birth[113]. In lung endodermal epithelium, HDAC3 is recruited to enhancers through an undetermined nuclear receptor to repress the transcription of the miR-17–92 microRNA cluster and many of the nearly 50 miRNAs transcribed from the imprinted *Dlk1-Dio3* gene cluster (FIG. 6a). Expression of miR-17–92 inhibits the cell-autonomous TGFβ signalling that is required for cell flattening and spreading during sacculation of lung alveolar type I cells, thereby ensuring efficient gas exchange[113]. Deletion of *Hdac3* in the developing lung mesenchyme

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by twist basic helix-loop-helix transcription factor 2 *(Twist2)-Cre* also resulted in complete lethality at birth as a result of respiratory failure[114]. Unlike *Hdac3* deletion in lung endoderm, deletion in the underlying lung mesoderm causes lung hypoplasia and loss of alveolar type I cell differentiation owing to decreased WNT-β-catenin signalling from the mesenchyme to the epithelium[114].

HDAC3 governs intestinal homeostasis and host defence.

In intestinal epithelial cells (IECs), HDAC3 maintains intestinal homeostasis by coordinating intestinal lymphocyte-mediated host defences against pathogenic microorganisms, with implications for the course of inflammatory bowel disease (IBD) and bacterial enteritis[115,116]. Deletion of *Hdac3* in IECs using *Vil1*-Cre or inducible *Vil1*-CreER resulted in the downregulation of IEC antimicrobial defence genes, the loss of Paneth cells and impairment in the function of the IEC barrier, causing increased intestinal damage and inflammation[115] (FIG. 6b). Notably, the phenotype of IEC-specific *Hdac3* knockout mice re-derived in germ-free conditions was largely normal, suggesting that HDAC3 is a hub of commensal-bacteria-derived signals that maintains intestinal homeostasis[115]. Furthermore, in IECs, HDAC3 orchestrates a host defence programme that is mediated through cytokine communication from IECs to resident $CD8^+$ T cell lymphocytes in the intestines to protect against pathogenic gut bacteria[116]. Intestinal epithelial HDAC3 has also been shown to contribute to diet-induced obesity in mice[117].

HDAC3 in pancreatic β-cells controls glucose-stimulated insulin secretion.

Loss of *Hdac3* in insulin-producing pancreatic β-cells in the mouse insulin promoter CreERT transgenic mouse line resulted in improved glucose tolerance owing to enhanced insulin secretion without changes in insulin production or β-cell mass[118]. β-Cells deficient for HDAC3 demonstrate increased response to glucose levels through secretion of greater amounts of insulin into the systemic circulation. The phenotype of β-cells lacking HDAC3 is consistent with the use of a selective HDAC3 inhibitor, which improved glucose metabolism in diabetic rats[119]. Moreover, HDAC3 depletion alleviated progressive cytokine-mediated β-cell apoptosis, which was hypothesized to contribute to type 2 diabetes[120,121]. By contrast, the rat insulin II promoter Cre HDAC3 β-cell deletion mouse model exhibited decreased insulin content in the pancreas and impaired glucose-stimulated insulin secretion[122], which was perhaps confounded by aberrant Cre-mediated excision in the central nervous system[123,124]. Future studies of HDAC3 function in the endocrine pancreas will have to target specific endocrine cell types and use more refined Cre deletion strategies in order to explain these conflicting results.

HDAC3 impacts skeletal muscle metabolism and a fuel source switch.

Deletion of *Hdac3* in skeletal muscles using *Mlc-Cre* causes severe systemic and skeletal muscle-specific insulin resistance, impaired insulin and glucose tolerance, and diminished glucose uptake into skeletal muscles, outcomes that are independent of changes in insulin signalling. Paradoxically, deletion of *Hdac3* in skeletal muscles improved exercise endurance despite these metabolic alterations[125]. ChIP-seq, RNA sequencing and quantitative proteomic analyses revealed that, during the daytime, skeletal muscle HDAC3 in concert with REV-ERBα represses the expression of the catabolic enzyme branched-chain

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amino acid aminotransferase, mitochondrial (BCAT2) and AMP deaminase 3 (AMPD3), which is the first enzyme of the purine nucleotide cycle pathway[125] (FIG. 6c). In the absence of HDAC3 in skeletal muscles, elevated expression of BCAT2 and AMPD3 feeds metabolic intermediates into the tricarboxylic acid cycle to promote a fuel source switch favouring protein catabolism and lipid oxidation over glucose utilization[125]. Thus, HDAC3 and REV-ERBα control a gene regulatory circuit that imparts a circadian rhythm of skeletal muscle fuel source usage to anticipate muscle endurance requirements during periods of daily fasting[125].

HDAC3 promotes haematopoietic cell lineages and prevents a lethal autoimmune disease.

HDAC3 orchestrates a remarkable number of processes and functions in immune cells, which influence cell homeostasis and transit through development stages and systemic immune responses. For example, *Lysm*-Cre-mediated depletion of HDAC3 in macrophages promoted an anti-inflammatory histone acetylation profile and gene expression pattern and enhanced interleukin-4-induced alternative macrophage activation, with a protective effect from parasitic inflammation in vivo[126]. Reciprocally, loss of HDAC3 has been shown to impair canonical pro-inflammatory gene expression programmes induced by lipopolysaccharides[127]. Highlighting the importance of HDAC3 in macrophage physiology, transplantation of anti-inflammatory HDAC3-lacking macrophages into atherosclerosis-prone low-density lipoprotein receptor (LDLR)-null mice induced the formation of more stable arterial plaque lesions with decreased lipid accumulation within foam cells and no effect on systemic lipid levels[128]. It remains unclear which nuclear receptor recruits HDAC3 to chromatin in macrophages; however, the lineage-determining transcription factor PU.1, which is enriched at HDAC3 binding sites, likely facilitates adjacent binding of nuclear receptors[126].

Within the haematopoietic compartment, deletion of *Hdac3* using *Vav1-Cre* produced a dysfunctional thymus with significant loss of lymphoid cells, hypocellular bone marrow and anaemia arising from DNA replication defects and genomic instability[129,130]. Furthermore, HDAC3 is required for stem cell proliferation, renewal and repopulation in bone marrow transplantation assays[130]. Deletion of *Hdac3* in early B cell progenitors using *Cd79a-Cre* impedes B cell development owing to a substantial hindrance of distal V_H-DJ_H immunoglobulin heavy chain recombination, which is caused by altered chromatin structure that is thought to impair the formation of long-distance chromatin interactions (chromatin looping), which, notably, requires the catalytic activity of HDAC3 (REF.[131]).

Deletion of *Hdac3* using *Lck-Cre* at the early double-negative ($CD4^-CD8^-$) stage of T cell development impaired the transition to the double-positive ($CD4^+CD8^+$) stage and revealed a downregulation of a majority of genes for T cell development, function and cell cycle by an unclear mechanism[132]. Consistent with the phenotype of Lck-Cre-mediated loss of HDAC3 (REF[132]), deletion of *Hdac3* at the $CD4^+CD8^+$ stage using *Cd2-Cre* or *Cd4-Cre* led to a considerable block in T cell positive selection, maturation and ultimately T cell survival[133,134]. The *Cd2-Cre* mouse model of HDAC3 deletion uniquely exhibited rectal prolapse and predisposition to IBD by 2 months of age, perhaps through altered function of peripheral regulatory T (T_{reg}) cells[133]. Deletion of *Hdac3* in T_{reg} cells using *Foxp3-Cre*

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disrupted the HDAC3-dependent function of immunosuppressive T_{reg} cells, which caused activation of conventional T cells and B cells and initiated lethal autoimmunity in animals by 4–6 weeks of age, perhaps through interactions of FOXP3 and nuclear factor-κB with HDAC3 (REFS[135,136]). Similarly to the *Cd2-Cre* mouse model, T_{reg} cell HDAC3 was required to suppress IBD[133,135].

HDAC3 is required for the development and physiological remodelling of bones.

Following genetic studies in NCCs, mesenchymal progenitors, osteoblasts and osteoclasts, which covered many stages of skeletogenesis, HDAC3 has emerged as a regulator of bone development and growth[137]. *Wnt*-Cre-driven and Pax3-Cre-driven deletion of *Hdac3* in pre-migratory NCCs, which make up the craniofacial mesenchyme, causes severe microcephaly, micrognathia, cleft palate and impaired odontogenesis, ultimately resulting in perinatal lethality[101,138]. *Sp7*-Cre-mediated deletion of *Hdac3* in osteochondral progenitor cells resulted in less-severe craniofacial developmental abnormalities but significantly shortened long bones and caused severe osteopenia affecting bone strength, a phenotype that ultimately decreased lifespan[139]. Specific deletion of *Hdac3* during limb bud development using *Prrx1-Cre* caused severe shortening of both the forelimbs and the hindlimbs and perinatal lethality[140]. Deletion of *Hdac3* in early developing chondrocytes using *Col2a1-Cre* was embryonic lethal[141].

Deletion of *Hdac3* in mature chondrocytes using tamoxifen-inducible *Col2a1-CreERT* delayed secondary ossification, altered growth plate and long bone development and increased osteoclast activity, which caused significant reductions in bone density[141]. In adult osteoblasts, HDAC3 deficiency considerably increased age-related osteopenia, osteoporosis and bone fractures of relevance in human ageing[142]. At bone gene enhancers, HDAC3 may function in part through the transcription factors RUNX2 and zinc-finger protein 521 and yet unknown nuclear receptors, resulting in complex and temporal effects in bone and cartilage[143–145]. The phenotypes caused by loss of HDAC3 may in part explain the axial and cranial skeletal defects and predisposition to bone fractures observed with the use of HDAC inhibitors[146–153].

Conclusions and future perspective

HDAC3 is unique among the HDAC family proteins as the major HDAC that functions as part of nuclear receptor co-repressor complexes and requires them for its catalytic activity[29–31]. HDAC3 is vital to life because it regulates many developmental, physiological and metabolic processes. Throughout development, HDAC3 serves to integrate complex signals from the environment and deliver them onto the genome to influence cellular migration, differentiation, fate and growth. In adult mammals, HDAC3 uniquely regulates major metabolic and energy utilization pathways and circadian, nutrient and environmental challenges, influences memory formation, limits autoimmunity and protects skeletal integrity, among other homeostatic functions.

Transcriptomics analyses of mouse models with conditional HDAC3 loss of function have revealed an extraordinarily diverse set of tissue-specific HDAC3 targets. Indeed, many of the genes regulated by HDAC3 are highly tissue-specific (FIG. 7), which is consistent with cell

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context-dependent functions of, and tissue-specific mechanisms of, gene regulation by HDAC3, reinforcing the notion that the function and gene targets of HDAC3 cannot be predicted a priori. Although less complete, genome-wide analyses of the mechanisms of HDAC3 activity have revealed a remarkably diverse set of nuclear receptors and other transcription factors that recruit HDAC3 to chromatin, histone and non-histone targets and to participate in enzymatic and non-enzymatic functions. Thus, HDAC3 evolved as a dynamic chromatin regulator that is co-opted by many tissue-specific factors to exert biological effects, many of which remain to be discovered.

To date, no human disease-causing mutations have been found in the *HDAC3* gene, likely owing to the lethality of such germline mutations, which are expected to affect multiple tissues. Despite this, enhancers may exist with single nucleotide polymorphisms that fail to recruit HDAC3 owing to impaired binding of transcription factors or nuclear receptors[10,154,155], causing pathological gene expression. Other HDAC3 'enhanceropathies'[156] may occur as a result of mutations in HDAC3-associated proteins.

Many questions remain about the biology of HDAC3. One glaring question is whether all functions of HDAC3 require its presence in the NCoR and SMRT co-repressor complexes. Because interaction with NCoR or SMRT is required for HDAC3 activity in vivo[55], it seems likely that the enzymatic functions of HDAC3 are mediated by these complexes, but this is not the case for the non-enzymatic functions of HDAC3. Indeed, although HDAC3 loss-of-function mouse models have provided great insight into the physiological roles of HDAC3, we currently have little mechanistic understanding of the non-enzymatic functions of HDAC3. This is particularly important given the pharmaceutical interest in targeting the enzymatic activity of HDAC3 (REF.[150]).

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Glossary

Lineage-determining transcription factors
Transcription factors that determine the differentiation fate of cells.

Signal-dependent transcription factors
Transcription factors that associate with or bind to specific DNA sequences near lineage-determining transcription factors in response to environmental or physiological cues.

Nuclear receptors
A superfamily of transcription factors that bind to highly specific DNA motifs in direct response to ligand binding to activate or repress gene transcription.

Stoichiometric component

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A protein whose interaction with another component of a protein complex is based on their equal molarity.

WD40 repeat-containing proteins
Proteins containing a motif of 40 amino acids that assumes a β-propeller structure and functions in establishing protein-protein interactions, signal transduction and transcription regulation.

Cre recombinase
(Cre). A bacteriophage P1 type I topoisomerase that catalyses DNA recombination between site-specific *loxP* sites.

Adeno-associated virus
(AAV). A small, non-enveloped, replication-incompetent virus with a small singlestranded DNA genome, which is maintained extrachromosomally and used for gene delivery.

Hepatosteatosis
Fatty liver, often owing to the pathological accumulation of triglycerides.

Nonshivering thermogenesis
A facultative and adaptive process that protects core body temperature.

Oxidative phosphorylation
The transfer of electrons in mitochondria between energy carriers down the electron transport chain to generate a proton gradient and produce ATP.

Global run-on sequencing
(GRO-seq). A nuclear run-on assay coupled to next-generation sequencing to map all transcription by engaged RNA polymerases throughout the genome.

Futile metabolic cycles
Biochemical pathways that operate simultaneously in opposing directions, often leading to the dissipation of energy.

First heart field
(FHF). A developmental structure that gives rise to the cardiac crescent, early cardiac tube and left ventricle.

Second heart field
(SHF). A developmental structure that gives rise to the right ventricle, atrial myocardium and cardiac outflow tract.

Nuclear lamina
A cellular structure adjacent to the inner nuclear membrane that is composed of lamin polymers and other proteins and forms a skeletal nuclear structure that interacts with nuclear scaffold proteins and chromatin.

Facultative heterochromatin

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Tightly packed, inaccessible chromatin containing inactive genes, which may become accessible and expressed in certain cell types or following certain cellular cues.

Marfan syndrome
A connective tissue disorder caused by mutations in the fibrillin 1 gene, often presenting as tall, slender individuals with arachnodactyly, cardiac valve disease and predisposition to aortic aneurysm and dissection.

Loeys–Dietz syndrome
A connective tissue disorder with five subtypes, each caused by unique mutations in five genes of the TGFβ signalling pathway, which cause congenital cardiac defects and predisposition to aortic aneurysm and dissection.

CA1 hippocampal region
An area of the brain that is important for memory.

Nucleus accumbens
A region of the brain that is involved in cognitive processing of reward, with neurons containing mostly dopamine receptors; the nucleus accumbens is thought to be the major nucleus involved in addiction.

Paneth cells
Epithelial cells of the small intestine that secrete antimicrobial peptides and proteins, mediate host-microorganism interactions and help defend against enteric pathogens in the gut lumen.

Regulatory T cells
(T_{reg} cells). $CD4^+$, $CD25^+$ and $FOXP3^+$ T cells that suppress the activation of the immune system to maintain tolerance to self-antigens and prevent autoimmune disease.

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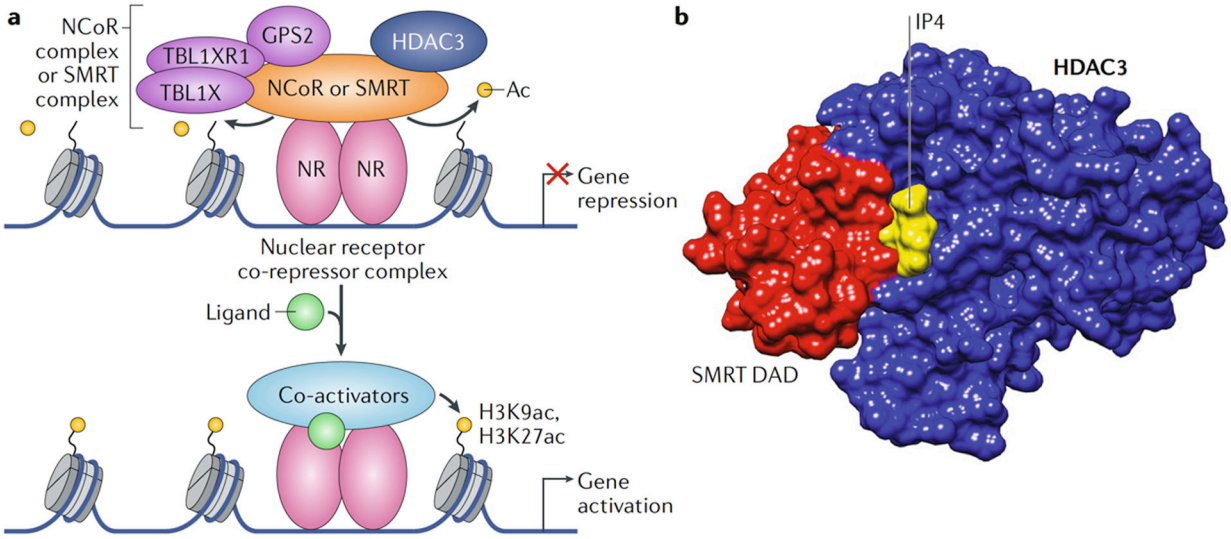

Fig. 1 |. HDAC3 is a core component of nuclear receptor co-repressor complexes that modulate nuclear receptor-mediated transcription.

a | Histone deacetylase 3 (HDAC3)-containing nuclear receptor co-repressors complexes bind to ligand-free nuclear receptors and repress transcription, partly by deacetylating histones. Ligand binding by the nuclear receptors dismisses the co-repressor complex and recruits co-activators that promote gene transcription, partly through histone acetylation. **b** | Crystal structure modelling (Protein Data Bank (PDB) identifier (ID): 4A69) of the HDAC3 interaction with the deacetylase-activating domain (DAD) of silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and inositol tetraphosphate (IP4) is shown[49]. The interaction with the SMRT DAD is required for the enzymatic activity of HDAC3 (REFS[31,47,54]). Ac, acetyl group; GPS2, G protein pathway suppressor 2; H3K27ac, acetylated histone H3 lysine 27; H3K9ac, acetylated histone H3 lysine 9; NCoR, nuclear receptor co-repressor 1; NR, nuclear receptor; TBL1X, transducin β-Like 1, X-Linked; TBL1XR1, TBL1-reLated protein 1.

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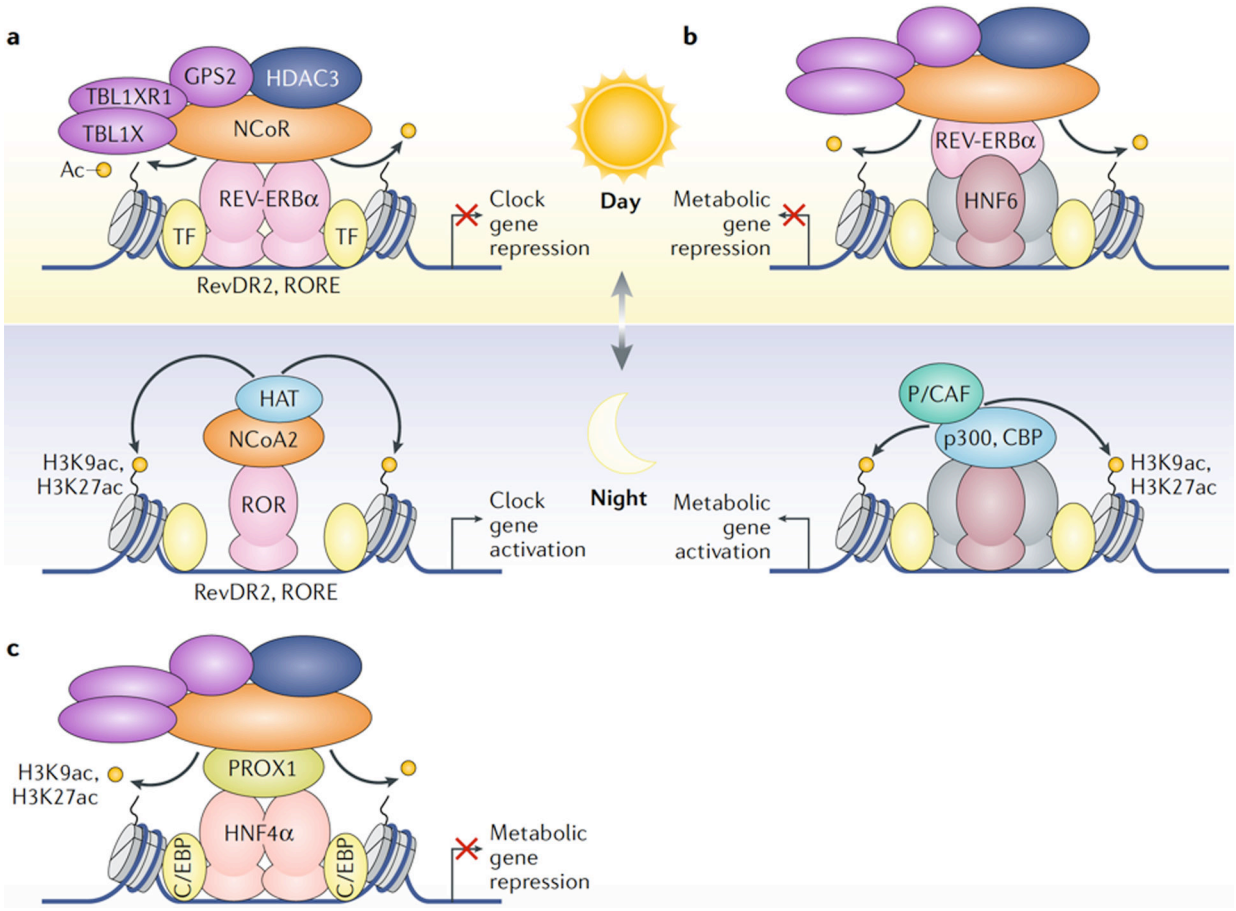

Fig. 2 |. HDAC3 suppresses liver metabolism and circadian clock genes through distinct enhancer complexes.

a | The circadian nuclear receptor REV-ERBα is maximally expressed in mice at Zeitgeber time (ZT) 10, during the light period, and is nearly absent 12 hours later in the dark period (at ZT 22). REV-ERBα recruits histone deacetylase 3 (HDAC3) through the nuclear receptor co-repressor 1 (NCoR) complex to its canonical DNA-binding motifs RevDR2 and ROR response element (RORE) to repress core circadian clock genes[70,73]. When REV-ERBα is not expressed, the nuclear receptor RAR-related orphan receptor (ROR) binds these motifs instead and recruits nuclear receptor co-activator 2 (NCoA2) and histone acetyltransferases (HATs) to activate gene transcription. In the depiction, ZT 0–12 is the light period and ZT 12–24 is the dark period. **b** | REV-ERBα tethered to hepatocyte nuclear factor 6 (HNF6) recruits HDAC3 through binding to the NCoR complex to mediate circadian repression of metabolic genes[75]. In the absence of REV-ERBα during the dark period, the HATs p300, CREB-binding protein (CBP) and p300/CBP-associated factor (P/CAF; also known as KAT2B) acetylate nearby histones and promote metabolic gene expression. **c** | Prospero homeobox protein 1 (PROX1) forms a distinct co-repressor module at enhancers bound by HNF4α independently of REV-ERBα[76]. PROX1 and HDAC3 are co-recruited to HNF4α-bound enhancers enriched with different C/EBP transcription factors. Ac, acetyl group; GPS2, G protein pathway suppressor 2; H3K27ac, acetylated histone H3 lysine 27; H3K9ac,

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acetylated histone H3 Lysine 9; TBL1X, transducin β-Like 1, X-Linked; TBL1XR1, TBL1-reLated protein 1; TF, transcription factor.

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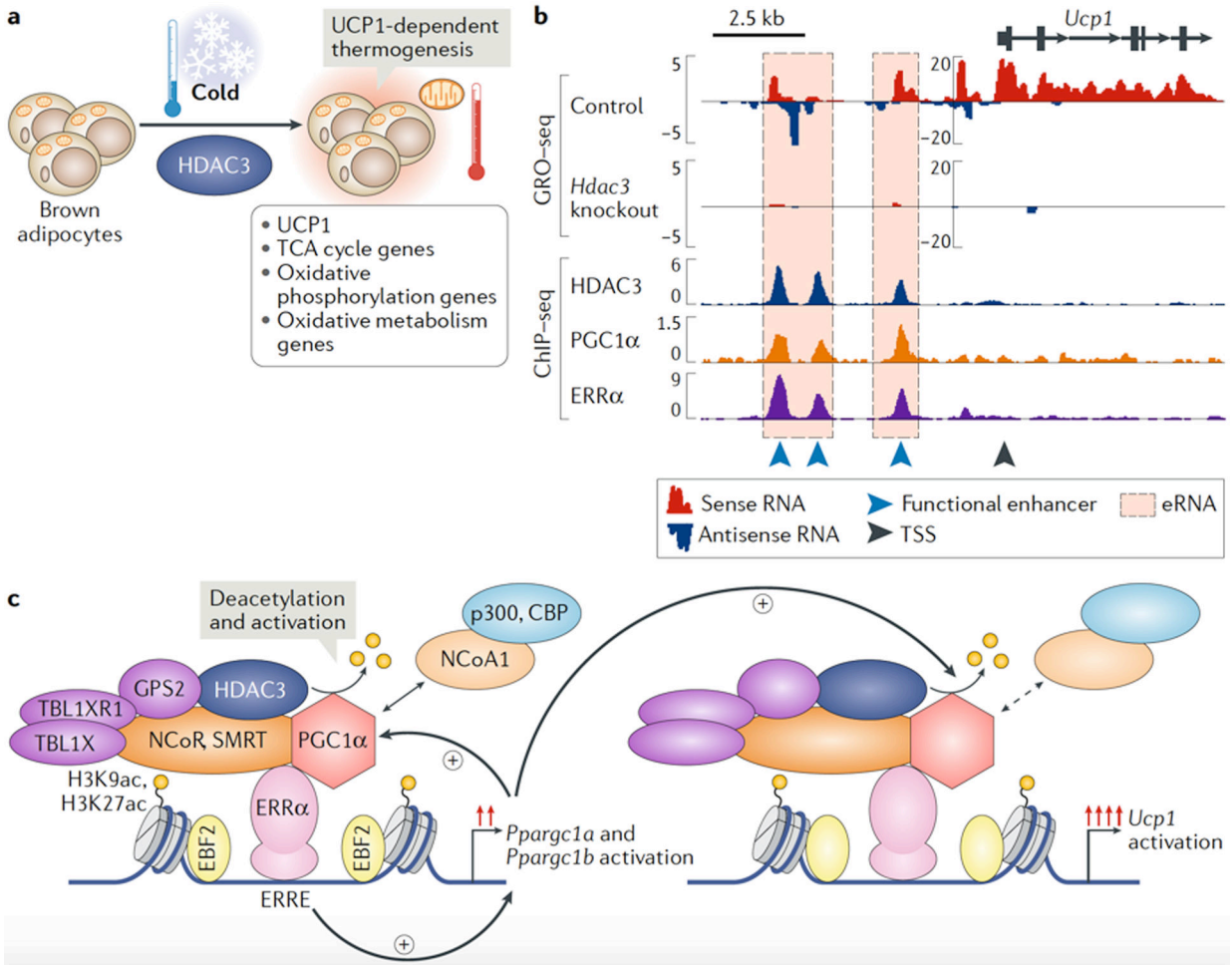

Fig. 3 |. HDAC3 primes thermogenic gene transcription in brown adipose tissue.

a | In brown adipose tissue (BAT), histone deacetylase 3 (HDAC3) primes the expression of *Ucp1* (which encodes mitochondrial brown fat uncoupling protein 1), tricarboxylic acid (TCA) cycle genes, oxidative phosphorylation genes and oxidative metabolism genes to facilitate rapid UCP1-dependent thermogenesis upon exposure to acute cold[77]. **b** | A genomic view of the *Ucp1* locus is shown, highlighting the co-activator activity of HDAC3 at oestrogen-related receptor-α (ERRα)-bound enhancers in BAT. Global run-on sequencing (GRO-seq) data demonstrate strong enhancer RNA (eRNA) transcription at *Ucp1* enhancers and transcription of the *Ucp1* gene in control BAT. Upon loss of HDAC3, eRNA transcription and expression of the *Ucp1* gene are lost at enhancers bound by HDAC3, ERRα and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) (another ERRα co-activator)[77]. The GRO-seq *y* axis represents reads per kilobase per million; the chromatin immunoprecipitation followed by sequencing (ChIP-seq) *y* axis represents reads per million. **c** | In BAT, HDAC3 activates PGC1α by deacetyLating it[77,157,158]. HDAC3 and ERRα bind to enhancers of the *Ppargcla* and *Ppargclb* genes to promote their basal transcription levels. Increased expression of PGC1α facilitates an autoreguLatory loop maintained by HDAC3, ERRα and PGC1α, which drives the transcription of *Ucp1* and oxidative phosphorylation genes to ensure thermogenic aptitude and survival upon exposure

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to a cold environment[77]. Notably, enhancers bound by HDAC3, ERRα and PGC1α are also marked by the brown fat lineage factor early B cell factor 2 (EBF2)[159,160]. CBP, CREB-binding protein; ERRE, ERR response element; GPS2, G protein pathway suppressor 2; H3K27ac, acetylated histone H3 Lysine 27; H3K9ac, acetylated histone H3 Lysine 9; NCoA1, nuclear receptor co-activator l; NCoR, nuclear receptor co-repressor l; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; TBL1X, transducin β-Like l, X-Linked; TBL1XRl, TBL1-reLated protein l; TSS, transcription start site.

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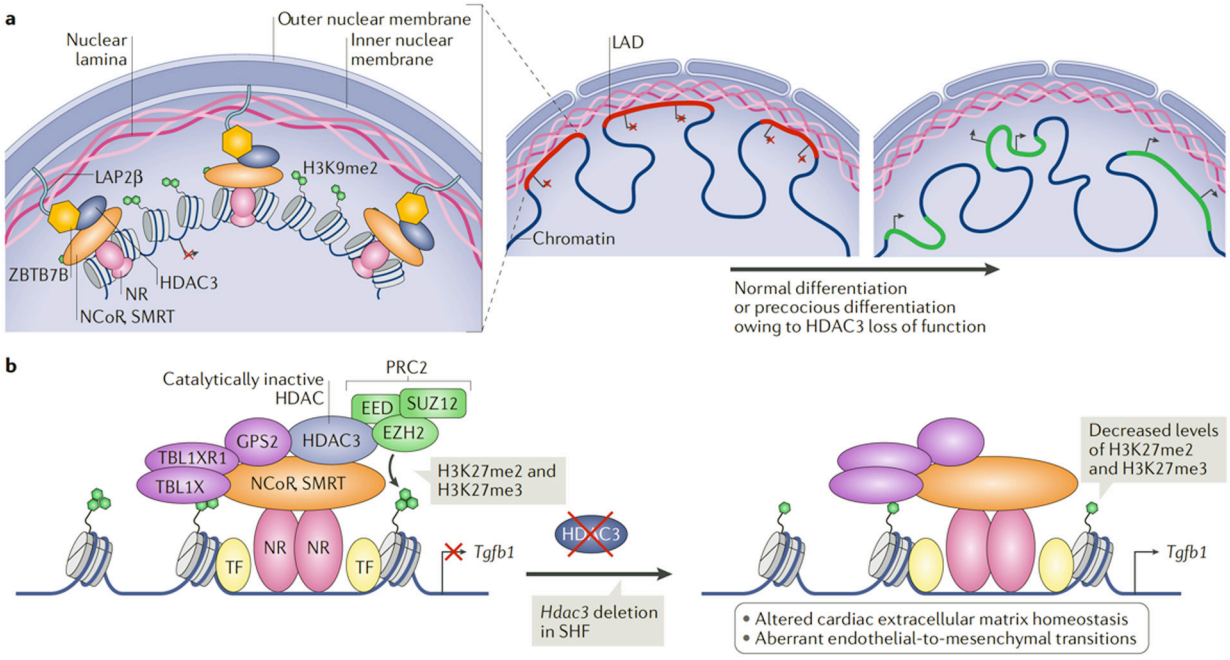

Fig. 4 |. HDAC3 influences cardiac development through deacetylase-independent mechanisms.

a | Histone deacetylase 3 (HDAC3) prevents the precocious differentiation of cardiac progenitor cells and premature expression of cardiomyocyte genes[92–94]. HDAC3 tethers cardiac lineage genes located in lamina-associated domains (LADs) to the nuclear periphery for silencing. HDAC3 bound to the nuclear receptor co-repressor 1 (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) complexes on chromatin interacts with the lamina proteins zinc-finger and BTB domain-containing protein 7B (ZBTB7B) and lamina-associated polypeptide 2, isoform-β (LAP2β) to tether LADs to the nuclear lamina, where they become facultative heterochromatin and enriched in the repressive histone H3 lysine 9 dimethylation (H3K9me2) modification[90–94]. The release of LADs from the nuclear periphery alters their chromatin configuration and facilitates the expression of cardiomyocyte genes[94,97]. **b** | In the second heart field (SHF), the non-enzymatic activity of HDAC3 represses transforming growth factor-β (TGFβ) signalling by recruiting the H3K27 methyltransferase Polycomb repressive complex 2 (PRC2) to the *Tgfb1* gene. EED, embryonic ectoderm development protein; EZH2, enhancer of zeste homologue 2; GPS2, G protein pathway suppressor 2; H3K27me3, histone H3 lysine 27 trimethylation; NR, nuclear receptor; TF, transcription factor; SUZ12, suppressor of zeste 12 protein homologue; TBL1X, transducin β-like 1, X-linked; TBL1XR1, TBL1-related protein 1.

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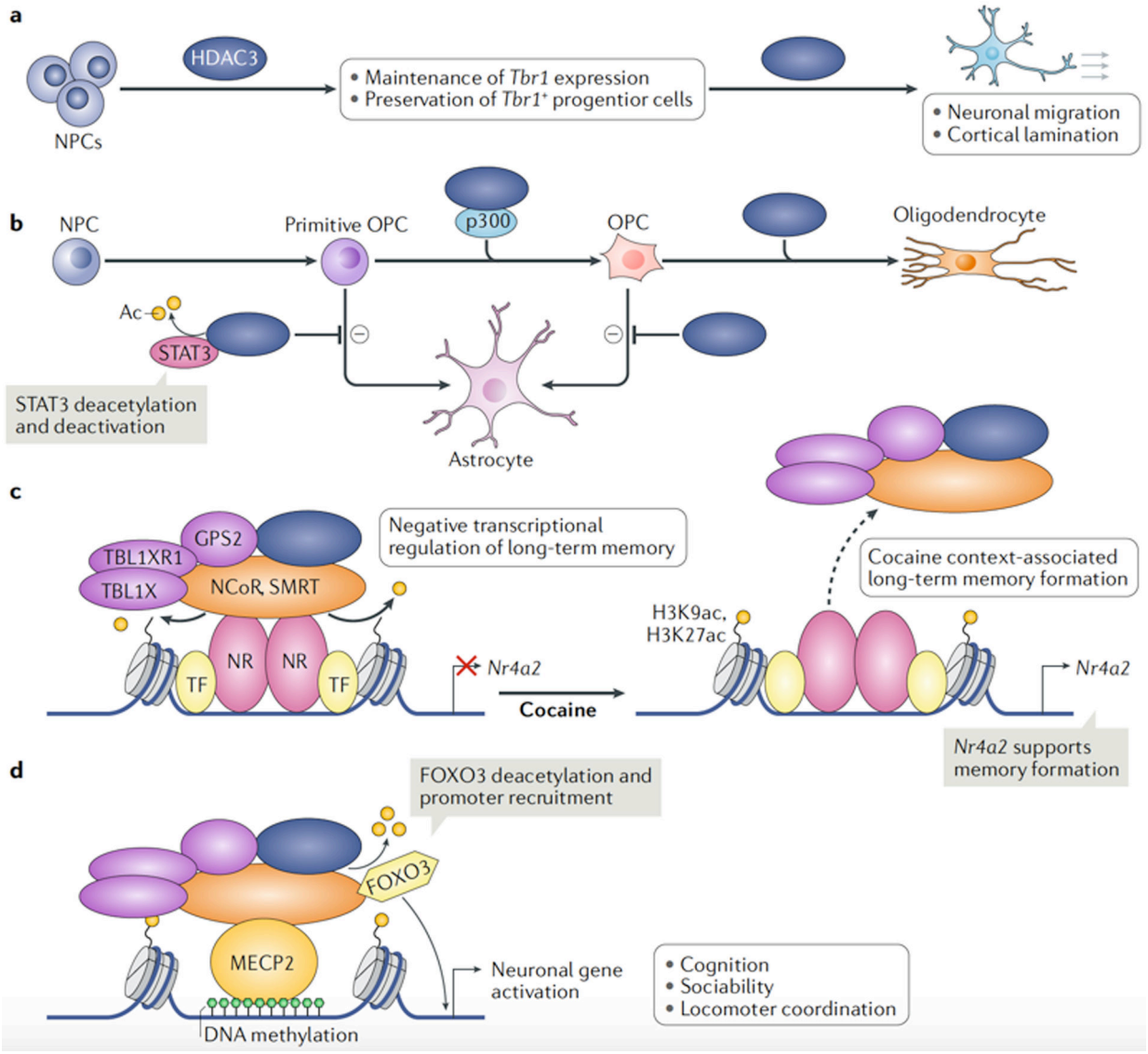

Fig. 5 |. HDAC3 controls brain development, glial cell fate and the formation of long-term memory.

a | Histone deacetylase 3 (HDAC3) controls brain development through maintenance of T-box brain 1 (*Tbr1*) gene expression or preservation of the *Tbr1*$^{+}$ progenitor cell population. Loss of HDAC3 leads to impaired differentiation and migration of neural progenitor cells (NPCs), resulting in severe brain cytoarchitectural defects[105]. **b** | HDAC3 controls glial cell fate. HDAC3 inhibits intermediate primitive oligodendrocyte progenitor cells (OPCs) and OPCs from undergoing astrogliogenesis, thereby promoting the formation of oligodendrocytes. HDAC3 inhibition of astroglial differentiation occurs through deacetylation and inhibition of signal transducer and activator of transcription 3 (STAT3), and by interaction with the histone acetyltransferase p300 to activate oligodendrocyte gene enhancers[105,106]. Loss of HDAC3 decreases oligodendrocyte numbers and increases astrocyte numbers[105,106]. **c** | In the hippocampus, HDAC3 represses nuclear receptor subfamily 4, group A, member 2 *(Nr4a2)* gene, which is important for the formation of long-

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term memory and object recognition[108]. HDAC3 also regulates the *Nr4a2* gene in the nucleus accumbens, where HDAC3 binding at enhancers is attenuated by cocaine use, which may facilitate long-term memories associated with drug abuse and reinforce future use[109]. **d** | HDAC3 is recruited to specific sites of methylated DNA by the nuclear receptor co-repressor 1 (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) complexes through their interaction with methyl-CpG-binding protein 2 (MECP2), which is implicated in Rett syndrome[110,111]. There, HDAC3 deacetylates and activates the transcription factor forkhead box protein O3 (FOXO3) to promote neuronal gene expression[112]. Ac, acetyl group; GPS2, G protein pathway suppressor 2; H3K27ac, acetylated histone H3 lysine 27; H3K9ac, acetylated histone H3 lysine 9; NR, nuclear receptor; TBL1X, transducin β-like 1, X-linked; TBL1XR1, TBL1-related protein 1; TF, transcription factor.

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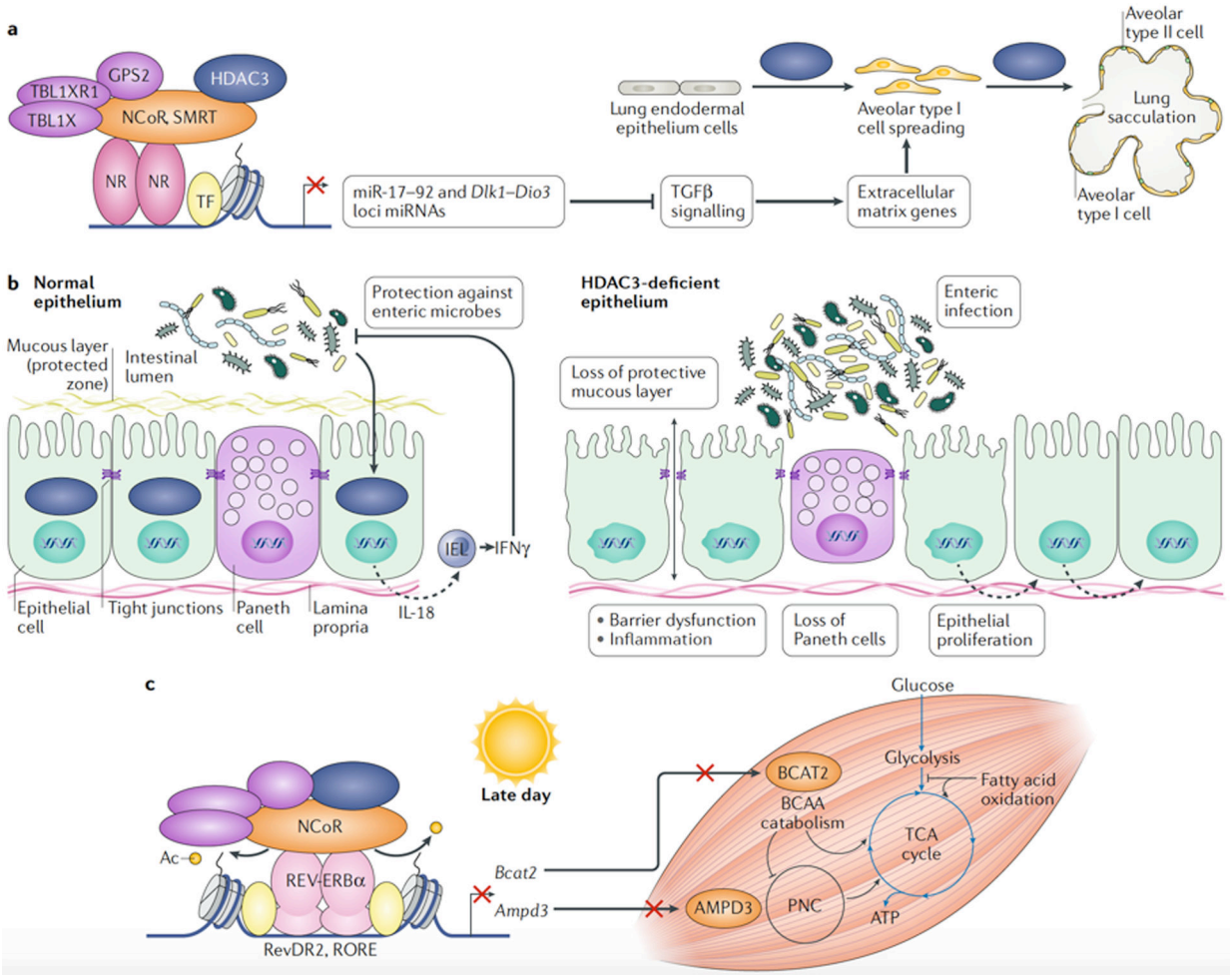

Fig. 6 |. HDAC3 regulates lung development, intestinal homeostasis, pancreatic β-cell insulin secretion and skeletal muscle metabolism.

a | Histone deacetylase 3 (HDAC3) regulates the differentiation of lung epithelial cells through repression of the *Dlk1-Dio3* and miR-17–92 microRNA (miRNA) clusters. Increased expression of miRNAs represses genes in the transforming growth factor-β (TGFβ) signalling pathway, leading to defective extracellular matrix formation and failure of alveolar cell spreading. Defects in cell spreading and flattening impair lung sacculation and alveologenesis[113]. **b** | In intestinal epithelial cells (IECs), HDAC3 is required for maintenance of the epithelial barrier that separates the gut lumen from the systemic circulation, ensures Paneth cell survival, controls cell proliferation and protects against enteric bacteria. HDAC3 in IECs prevents enteric infection by signalling to intraepithelial $CD8^+$ T lymphocytes (IELs) to mount a protective interferon-γ (IFNγ) immune response[116]. **c** | Late in the day, at pre-dusk hours (Zeitgeber time (ZT) 10–12), HDAC3 and REV-ERBα repress branched-chain amino acid (BCAA) catabolism and the purine nucleotide cycle (PNC) pathway through repression of branched-chain amino acid aminotransferase, mitochondrial (BCAT2) and AMP deaminase 3 (AMPD3), respectively, to promote glucose utilization during the upcoming feeding cycle when glucose supply is abundant[125]. At pre-dawn hours (ZT 22–24), when REV-ERBα and HDAC3 do not bind,

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increased BCAT2 and AMPD3 expression promotes the utilization of protein and lipid fuels over glucose to supply metabolic intermediates to the tricarboxylic acid (TCA) cycle. Muscle utilization of protein and lipid fuels during periods of daily fasting preserves glucose supplies for the brain and other vital organs[125]. Ac, acetyl group; GPS2, G protein pathway suppressor 2; IL-18, interleukin-18; NCoR, nuclear receptor co-repressor 1; NR, nuclear receptor; RORE, ROR response element; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; TBL1X, transducin β-like 1, X-linked; TBL1XR1, TBL1-related protein 1; TF, transcription factor.

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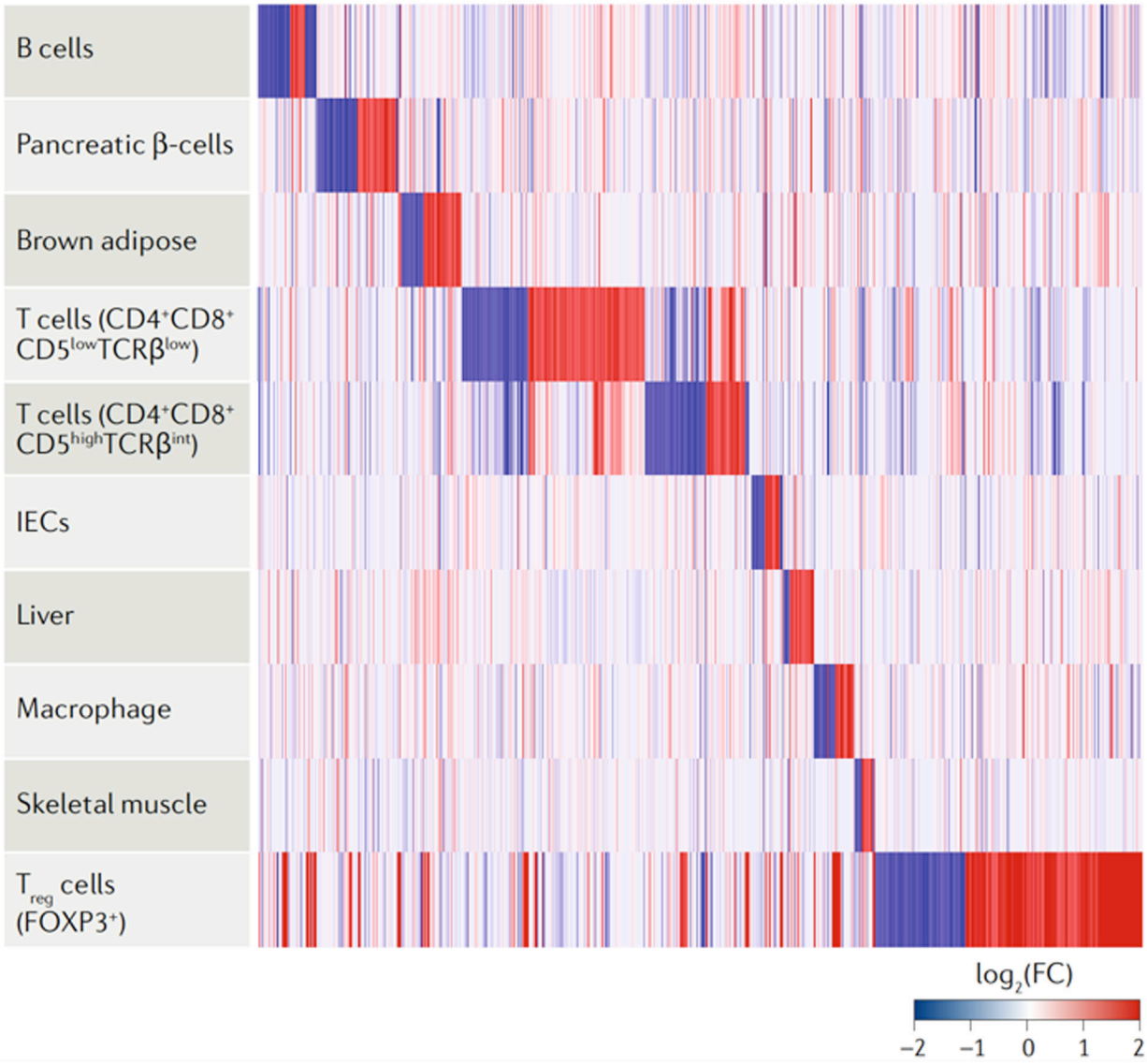

Fig. 7 |. HDAC3 regulates distinct mouse tissue-specific gene expression programmes.
A heat map illustrating the differential gene expression and hierarchical clustering of ten mouse tissues is shown, which characterize tissue-specific histone deacetylase 3 (HDAC3)-dependent gene expression programmes achieved through the numerous and complex tissue-specific regulatory mechanisms highlighted in this Review. We display all the expressed genes in each tissue as log2 of the fold change (FC) of HDAC3 knockout versus controls (false discovery rate <0.05); the rows (tissues) were sorted by hierarchical clustering. Gene expression data were obtained from publicly available Gene Expression Omnibus (GEO) data sets, normalized for cross-platform comparisons[161] and log-transformed; comparisons were made using the R package limma[162]. The GEO data sets used for this analysis are GSE98650, GSE90531, GSE83927, GSE72917, GSE50188, GSE85929, GSE33609, GSE79696 and GSE68991. IEC, intestinal epithelial cell; FOXP3, forkhead box protein P3; TCRβ, T cell receptor-β; T_{reg} cell, regulatory T cell.