SETD3 is an actin histidine methyltransferase that prevents primary dystocia

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For more than 50 years, the methylation of mammalian actin at histidine 73 has been known to occur1. Despite the pervasiveness of His73 methylation, which we find is conserved in several model animals and plants, its function remains unclear and the enzyme that generates this modification is unknown. Here we identify SET domain protein 3 (SETD3) as the physiological actin His73 methyltransferase. Structural studies reveal that an extensive network of interactions clamps the actin peptide onto the surface of SETD3 to orient His73 correctly within the catalytic pocket and to facilitate methyl transfer. His73 methylation reduces the nucleotide–exchange rate on actin monomers and modestly accelerates the assembly of actin filaments. Mice that lack SETD3 show complete loss of actin His73 methylation in several tissues, and quantitative proteomics analysis shows that actin His73 methylation is the only detectable physiological substrate of SETD3. SETD3-deficient female mice have severely decreased litter sizes owing to primary maternal dystocia that is refractory to ecologic induction agents. Furthermore, depletion of SETD3 impairs signal-induced contraction in primary human uterine smooth muscle cells. Together, our results identify a mammalian histidine methyltransferase and uncover a pivotal role for SETD3 and actin His73 methylation in the regulation of smooth muscle contractility. Our data also support the broader hypothesis that protein histidine methylation acts as a common regulatory mechanism.

SETD3, a ubiquitously expressed protein, is epigenetically upregulated in response to muscle loading and hypertrophy2. SETD3 contains an N-terminal SET domain and a C-terminal domain that is homologous to a Rubisco large subunit methyltransferase (LSMT) interaction domain (Extended Data Fig. 1a). Proteins that contain a catalytic lysine methyltransferase SET domain are found in all three kingdoms of life and regulate diverse biological and pathological processes3. In humans, around half of the 55 SET family members methylate lysines on histone and/or non-histone protein substrates3. The enzymatic activities of the remainder of human SET proteins, including SETD3, are unclear. SETD3 has been reported to methylate histone H3 at lysines 4 and 364. However, this conclusion was based on mass spectrometry data for which mass shifts were inconsistent with a bona fide methylation event (see figure 2c of a previous study5). When we tested the activity of SETD3, it did not methylate histones in contrast to two established protein lysine methyltransferases (PKMTs), NSD2 and MLL23 (Fig. 1a and Extended Data Fig. 1b). On the basis of these results and the cytoplasmic localization of SETD3 (Extended Data Fig. 1c), we conclude that SETD3 is not a histone methyltransferase and that it probably targets a non-histone substrate.

Among active PKMTs, SETD3 is most similar to human SETD65. We generated a putative SETD3 catalytic mutant based on this homology (Extended Data Fig. 1a) and performed in vitro methylation assays with 3H-SAM (S-adenosyl methionine) on total cytoplasmic extracts with wild-type or mutant SETD3. An approximately 42-kDa band was more prominent in reactions with wild-type SETD3 relative to the mutant (Fig. 1b). A three-step fractionation protocol was used to partially purify the SETD3-dependent activity for mass spectrometry analysis (Extended Data Fig. 1d–g). The ten most abundant hits in the proteomic dataset with molecular weights close to the candidate substrate (Extended Data Fig. 1h and Supplementary Table 1) were expressed as recombinant proteins and directly tested as SETD3 substrates in vitro (Fig. 1c). Of these proteins and other potential substrates, only β-actin was methylated by SETD3 (Fig. 1c and Extended Data Fig. 1i). SETD6 and 10 additional active PKMTs do not methylate actin (Fig. 1d, e and Extended Data Fig. 1j). In addition, SETD3 does not methylate the SETD6 substrate RelA6 (Fig. 1d). These data identify SETD3 as an enzyme that methylates actin in vitro.

To identify the specific actin residue(s) that are modified by SETD3, in vitro methylated actin was analysed by tandem mass spectrometry; deuterated SAM was used as the methyl donor to distinguish SETD3-catalysed methylation from all other sources. Unexpectedly, no lysine methylation events on actin were observed (Extended Data Fig. 2). We therefore extended the analysis to search for methylation events at other residues (arginine, glutamine, cysteine and histidine) and unambiguously identified incorporation of a single deuterated methyl moiety at His73 of actin (Extended Data Fig. 3a–c). Histidine methylation is known to exist on a limited number of proteins. The only known histidine methyltransferase is the yeast enzyme Hpm1p, which methylates Rpl3 (not actin) and bears no structural resemblance to SETD37,8. However, methylation of actin at His73 (actin-His73me) is a canonical modification present in mammals but not in yeast1,7,9. We identified His73me3 (or equivalent) on actin purified from 10 different model organisms, ranging from worms to plants to humans, that all have a SETD3 homologue, but not in Saccharomyces cerevisiae, which lacks SETD3 (Extended Data Fig. 4). Thus, actin-His73me3 is an evolutionarily conserved modification that is present in a broad range of multicellular eukaryotic organisms.

Mutation of actin His to Ala at position 73 (H73A) prevents methylation by SETD3 (Fig. 2a). Histidine can potentially be methylated on the nitrogen in position 1 (τ, N1) or N3 position (π) of the imidazole ring (Fig. 2b); His(3-me) is the physiological modification that is found on endogenous actin. Methylation assays performed on actin peptides that spanned His73 (residues 66–80 of β-actin) and in which His73 was either unmethylated or methylated at the N1 or N3 position (Extended Data Fig. 3d) showed that SETD3 methylated the unmodified peptide (kcat, 0.4 min−1) and the His73(1-me)-containing peptide (at a much slower rate), but not the His73(3-me)-containing peptide, indicating

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https://doi.org/10.1038/s41586-018-0821-8
that SETD3 catalyses methylation of His73 at the physiologically relevant N3 position (Fig. 2c and Extended Data Fig. 3e–g).

To gain insight into the molecular basis of how SETD3 methylates a histidine substrate, we co-crystallized SETD3 with an actin peptide (residues 66–80) that contains His73 in the presence of S-adenosyl-l-homocysteine (SAH). We obtained three highly similar crystal forms, consisting of an extensive network of charged/polar and hydrophobic interactions that involve both inter- and intramolecular contacts (Extended Data Table 1). SETD3 contains two lobes (SET and LSMT-like) forming a V-shaped cleft (Extended Data Fig. 5a). The 15-residue actin peptide occupies an extended surface groove of the SET domain (Fig. 2d), with His73 inserted into the active-site channel positioned to meet SAH from the other end (Fig. 2e). From the viewpoint of the cofactor–binding pocket, the methyl-accepting His73 N3 atom is seen at the bottom of the channel (Extended Data Fig. 5b). Structural superimposition of SETD3 and SETD6 reveals that for SETD3, the methyl donor group of SAM points towards and is within the distance of 1.8 Å of the target N3 atom of the imidazole ring (Fig. 2f). An intramolecular hydrogen bond between the main-chain carbonyl oxygen of Glu72 and the main-chain amide nitrogen of Ile75 bends the peptide substrate in a sharp turn at His73. This configuration allows His73 and Gly74 (the smallest residue) to enter deep into the channel and positions the target N3 atom in line with the methyl group and sulfur atom of SAM (Fig. 2f and Extended Data Fig. 5c, d), a linear arrangement comprising the nucleophile, the methyl group and the leaving thioester group in the transition state is required for the S$_\text{N2}$ reaction mechanism used by SAM-dependent methyltransferases.

All 15 residues of the actin peptide are bound in the structure (Fig. 2d and Extended Data Fig. 5d), with the SETD3–actin peptide interface consisting of an extensive network of charged/polar and hydrophobic interactions that involve both inter- and intramolecular contacts (Extended Data Fig. 5d–n). For example, Trp79 and Ile71 of actin each fit into hydrophobic pockets on the SETD3 surface (Fig. 2g). This long and ordered peptide–substrate recognition groove for SETD3 is unique compared to other SET domains, suggesting that SETD3 would inefficiently accommodate substrates that diverge from actin.

Structure-guided point mutations in actin that disrupt the hydrophobic interaction (I71A) or are predicted to interfere with proper positioning of His73 in the catalytic pocket (G74I) impair the ability of SETD3 to methylate actin-His73 and the double mutant (I71A/G74I) abolishes activity (Fig. 3a). Furthermore, although a W79E substitution is tolerated (Extended Data Fig. 5e), SETD3 has no activity on actin I71A/W79E double mutations (Fig. 3a). These data highlight the sensitivity of SETD3 activity on actin to changes in the sequence that includes His73.

Indeed, the 15-residue sequence spanning His73 is highly conserved among the six human actin isoforms with the only difference being an Ile/Val variation at position 76 that does not affect SETD3 recognition (Extended Data Fig. 5g, o). Accordingly, all actin isoforms are methylated by SETD3 in vitro (Fig. 3b). Finally, the putative SETD3 catalytic mutant is a conserved tyrosine (Tyr312) that participates in forming the active-site pocket (Extended Data Fig. 5d, i, p). This mutant has residual activity; however, mutation of additional conserved residues that are important for SAM binding abolished SETD3 activity (Fig. 3c and Extended Data Fig. 5p).

We next generated a state-specific actin-His73me antibody that selectively recognized His73(3-me) peptides (Extended Data Fig. 6a). The majority of endogenous actin is marked by His73 methylation in HT1080 cancer cells and the depletion of endogenous SETD3 by two independent single-guide RNAs (sgRNAs) results in almost complete loss of this modification, as determined by western blotting and mass spectrometry (Fig. 3d and Extended Data Fig. 6b). We also observed complete loss of actin-His73me in a clonal HeLa cell line in which the SETD3 alleles were disrupted (Fig. 3e and Extended Data Fig. 6c). Furthermore, complementation of SETD3-depleted HT1080...
cells with CRISPR-resistant wild-type, but not catalytically dead SETD3(NHY), reconstituted actin methylation at His73 (Fig. 3f and Extended Data Fig. 6d). To assess the physiological role of SETD3 in regulating actin His73 methylation, we obtained Setd3+/− mice from the Canadian Mouse Mutant Repository4 and generated Setd3 null homozygote mice (Setd3−/−) that are viable. Analysis of actin purified from nine different tissue types isolated from Setd3+/− and Setd3−/− mice showed that in tissues that expressed SETD3, the majority of actin is methylated; however, this modification is not detected in any tissue obtained from SETD3-deficient mice (Fig. 3g and Extended Data Fig. 7a–d). Finally, quantitative profiling of serum amino acids identified depletion of 3-methyl histidine as the only abnormality in Setd3−/− mice (Extended Data Fig. 7e). Collectively, these results identify SETD3 as the physiological actin-His73 methyltransferase.

Actin is one of the most abundant proteins in the human proteome and His73me is a high stoichiometry event. Our data argue that SETD3 is the principal enzyme tasked with generating actin-His73me; however, it remained unclear whether actin-His73 was the only SETD3 substrate or one of many substrates. There are few known histidine-methylated proteins in addition to actin—one is myosin, which SETD3 does not methylate (Fig. 3h). In addition, SETD3 has no activity on histones and several other proteins (Fig. 1c, d and Extended Data Fig. 1i). To investigate the catalytic specificity of SETD3 in a physiological and unbiased setting, we used quantitative proteomics to compare the methylene of HeLa cells with and without SETD3 (Fig. 3e). Of the more than 900 methylation events detected in the analysis, including around 180 histidine methylated peptides (Supplementary Table 2), actin-His73me was the only modification that was quantitatively altered upon SETD3 depletion (Fig. 3i). Together, these results indicate that the principal physiological enzymatic function of SETD3 is actin-His73 methylation.

On the basis of studies that use actin mutants, His73 and its methylation are suggested to influence actin polymerization dynamics, in part owing to its proximity to the ATP-binding site.12,14 However, a direct side-by-side comparison of the properties of actin with and without methylation has, to our knowledge, not previously been possible. We purified actin with and without His73me (Extended Data Fig. 8a, b) from HeLa cells (Fig. 3e) and found that methylation promoted actin polymerization kinetics in vitro but did not affect depolymerization (Fig. 4a and Extended Data Fig. 8c). Moreover, the rate of ATP nucleotide exchange was faster using the unmethylated species (Fig. 4b). We note that actin-His73me exhibited a subtle increase in polymerization when extending off of phalloidin–actin seeds, and made little difference to the polymerization rates in response to porcine Arp2/3 complex stimulation (Extended Data Fig. 8d, e). To explore the role of His73 methylation during actin polymerization in cells, mouse embryonic fibroblasts were isolated from Setd3−/− mice that were positive for actin-His73me and Setd3−/− mice that lacked methylation (Fig. 4c). Cells that contained actin-His73me were modestly more efficient at migration than cells that were deficient in methylated actin (Fig. 4d and Extended Data Fig. 8f). Together, these data are consistent with a model in which SETD3 methylation of actin positively regulates actin polymerization in cells.

Although Setd3−/− mouse embryonic fibroblasts show a defect in cell migration, Setd3−/− mice are viable. Therefore, actin-His73me must have a specialized physiological role in vivo that is not essential for basal actin functions. The characterization of Setd3-knockout mice by the International Mouse Phenotypic Consortium scored several phenotypes, including moderate skeletal muscle myopathy, abnormal cardiac electrocardiogram and mildly decreased lean mass4,19. We did not observe gross histopathological changes in muscle tissue isolated from young adult Setd3−/− mice (Extended Data Fig. 9a, b). Although we did not perform a comprehensive proteomic analysis, we observed that the litter sizes of female Setd3−/− mice, regardless of the genotype of the sire, were significantly smaller than litters from Setd3+/+ and Setd3+/− females (Extended Data Fig. 9c). To explore this observation further, we analysed parturition in Setd3−/− littermate control females that were all mated with wild-type males. Normal parturition was defined as birth at 19 days post-coitum (d.p.c.) and dystocia (delayed parturition) was defined as either no births (≥20 d.p.c.) or incomplete delivery with fetuses remaining in utero (≥20 d.p.c.). Dystocia is normally a rare phenomenon; however, it was observed in 8 out of 9 Setd3−/− mice, whereas normal parturition was observed for all of the control mice (Fig. 5a). As expected, in control mice, no fetuses were detected in utero at 20 d.p.c. (Fig. 5b). By contrast, Setd3−/− females typically had fetuses remaining in utero at ≥20 d.p.c. (Fig. 5b, c).

The dystocia observed in the Setd3−/− females is independent of the genetic type of the fetus as (1) wild-type males were used as sires; (2) pups successfully born to Setd3−/− mice were viable; and (3) Setd3−/− females had normal parturition. Thus, the cause of dystocia is of maternal origin. Secondary maternal dystocia is unlikely as there were no obvious and consistent anatomical pelvic abnormalities present in Setd3−/− females.
and an anatomical defect would not explain the presence of intact fetuses in utero. Thus, the dystocia is primary, either due to abnormal myometrial contractions or a defect in hormone signalling. To distinguish between these possibilities, early labour was induced in wild-type and Setd3+/− females using oxytocin administration at 18 d.p.c. (see schematic in Fig. 5d). Wild-type mice went into early labour and all pups were born within 24 h of treatment, whereas the dystocia of Setd3−/− mice was refractory to oxytocin (Fig. 5e, f). Similar results were observed with prostaglandin treatment as an independent ebolic agent13 (Extended Data Fig. 9d, e). These data identify a specific requirement for SETD3 in the contraction mechanism of the uterus during labour, a mechanism that fundamentally relies on proper actin behaviour16.

As multiple, unique point mutations in actin are implicated in the aetiology of several human smooth muscle myopathies17,18, we propose that there is a link between actin-His73me and uterine cell contraction in the primary dystocia of Setd3−/− females. To test this idea, we depleted SETD3 and actin-His73me from primary human myometrial cells (Fig. 5g) and measured cellular contraction under normal conditions and in response to two stimulans of uterine smooth muscle contractions. Although depletion of SETD3 had only a small effect on intrinsic contractions, the absence of SETD3 significantly impeded signal–induced contractions by oxytocin and endothelin-1 (Fig. 5h, i). Finally, the loss of endothelin-1–induced cellular contractions upon SETD3 knockdown was rescued in the primary human myometrial cells by complementation with CRISPR-resistant wild-type SETD3, but not by the catalytically inactive Setd3(NHY) (Fig. 5j, k) and Extended Data Fig. 9f). These data suggest a specific role for actin-His73me in signal–induced contraction of smooth muscle.

Here we identify SETD3 as a metazoan protein histidine methyltransferase and the enzyme that is responsible for generating actin-His73me. SETD3 represents a SET domain family member that methylates a residue other than lysine, broadening the current framework of possible chemistries catalysed by SET domains. Our analysis reveals that histidine methylation—previously detected on only a handful of proteins—is surprisingly common, observed with a frequency that is similar to that of protein lysine di- and trimethylation19. Actin mutations are implicated in smooth muscle myopathies17,18, including in the case of a pregnant patient who suffered from uterine muscle atony20. We propose that SETD3 deficiency and loss of His73me presents, similar to intrinsic actin mutations, by altering actin responsiveness to polymerization cues, which leads to defects in smooth muscle contraction. The effect of His73me on the interactions between actin and myosin is marginal (Extended Data Fig. 9g, h), suggesting that the regulation of smooth muscle contractions is mediated by a different mechanism. Specifically, in addition to the canonical actin–myosin pathway, there is a distinct role for dynamic actin polymerization in mediating mechanical force generation of smooth muscle21. In summary, our work identifies a functional role for mammalian histidine methylation in regulating actin and smooth muscle contraction and suggests a broad role for this modification in the regulation of mammalian proteomes.

Online content
Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0821-8.

Received: 24 March 2018; Accepted: 30 November 2018; Published online 10 December 2018.
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8. Credit author ORCID iDs

Acknowledgements We thank J. Drozak and colleagues for sharing their independent identification of SETD3 as the actin-His73 methyltransferase22 and members of the Gozani laboratory for critical reading of the manuscript. This work was supported in part by grants from the NIH to O.G. (RO1 GM079641), J.E.C. (DP2 AI104557 and U19 AI109662), X.C. (RO1 GM114306), J.A.S. (GM33289), and a CPRIT grant to X.C. (RR160029). J.E.E. received support from Stanford ChEM-H. J.E.C. is supported by an AAF Scholar Award.

Reviewer information Nature thanks S. Richard and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions A.W.W. performed biochemical and molecular experiments, and was responsible for experimental design and execution, data analysis and manuscript preparation. A.W.W. performed and analysed mass spectrometry experiments with help from S.L., T.-M.L. and J.E.E. C.M.N. and J.D. performed mouse experiments with help from Y.S.O. K.M.C. and J.G.V.-M. performed histopathology. J.M. and T.C. analysed plasma amino acids. S.D., J.D. performed mouse experiments with help from Y.S.O. K.M.C. and J.G.V.-M. Performed kinetic experiments and determined X-ray structures. D.S., D.V.T. and C.L. provided myosin and analysed actin–myosin interactions, supervised by K.M.R. and J.A.S. O.G., J.E.C. and X.C. supervised the research, interpreted data and prepared the manuscript.

Competing interests O.G. is a co-founder of EpiCypher Inc. and Athelas Therapeutics Inc.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0821-8. Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0821-8. Reprints and permissions information is available at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to X.C., J.E.C. or O.G.

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METHODS

Reagents. Biotinylated peptides were synthesized at the UNC High-Throughput Peptide Synthesis and Array Facility. Fmoc-t-histidine amino acids with either 1-me or 3-me modifications (Sigma-Aldrich) were used in the synthesis of these peptides and synthesized peptides were purified by high-performance liquid chromatography (≥92% purity). The following antibodies were used in this study: SETD3 (Abcam), pan-actin (Cytoskeleton), β-tubulin (Millipore), H3K36me2 (Cell Signaling), H3K36me4 (Cell Signaling), HisDE (see below), GSTP (Invitrogen), Alexa488 anti-antibody (Life Technologies), anti-rabbit and anti-mouse peroxidase-conjugated antibodies (Jackson Immunoresearch). Western blots were visualized by chemiluminescence (GE Healthcare).

Anti-His7(3-me)-specific antibody generation. A peptide that corresponds to amino acids 68–78 of β-actin (KYPIEHGVITV) with His(3-me) at position 73 was synthesized and purified by high-performance liquid chromatography (99% purity). This peptide was conjugated to KLH and used as antigen to immunize rabbits. Rabbit polyclonal antibodies, immunization and antisera purification were performed by Pocono Rabbit Farm & Laboratory. Antiserum was negatively selected against an unmodified actin peptide (amino acids 66–80). Final purification with washes was performed with an immobilized antigenic peptide to select for His7(3-me)-specific antibodies.

Recombinant protein expression for in vitro reactions. SETD3 (Uniprot ID: Q867UT) and actin were cloned into plGEX-6P-1. The MLL2 catalytic complex contained the GST-tagged catalytic SET domain of MLL2, as well as full-length, 6×His-tagged ASH2L, WDR5, DPY30 and RBBP5 proteins that were all individually expressed in bacteria and assembled in stoichiometric proportions to result in a catalytic complex. Lysine methyltransferases that were used as controls were reactivated with previously reported substrates. Candidate substrate cDNAs were cloned from the human ORFeome (Open Biosystems) using an LR recombination reaction (Life Technologies) into pDEST15. Mutations of all proteins were generated using Phusion site-directed mutagenesis (Thermo Scientific) according to the manufacturer’s recommendations. All proteins were expressed as N-terminal GST fusions (except for non-catalytic MLL complex components) in BL21 or BL21 (DE3) codon-plus bacteria. Expression was performed overnight at 16 °C in the presence of 0.1 mM IPTG. Recombinant GST fusions were purified with Glutathione Sepharose 4B (GE Healthcare) and eluted in 100 mM Tris pH 8.0, 10 mg ml⁻¹ reduced glutathione (Sigma-Aldrich). Any cleavage of GST tags was performed with PreScission Protease (GE Healthcare) overnight at 4 °C. Recombinant 6×His-tagged proteins were purified with Ni-NTA agarose (Life Technologies) and eluted in 100 mM Tris pH 8.0 and 250 mM imidazole. Kinetics experiments and crystallography used additional purification steps as described below.

Purification of SETD3 for crystallization. SETD3 was expressed as a GST-fusion protein as described above. Cleared bacterial lysates that contained the soluble GST-tagged protein were loaded on a 1 ml HiTrap Q-HP column (GE Healthcare) with a linear gradient from 50 mM to 1 M NaCl and 500 mM NaCl; SETD3 eluted together with cleaved GST around 200–300 mM NaCl. To remove GST, fractions were loaded onto a fresh glutathione-sepharose column and the flow through was collected and concentrated to around 2 ml. SETD3 was then further purified by size-exclusion chromatography (Superdex 200, GE Healthcare) in 20 mM Tris (pH 8.0), 200 mM NaCl, 5% glycerol, 0.05 M TCEP and 0.05 M TCEP. All purification steps were performed at 4 °C. The chromatography experiments were conducted in a BIO-RAD NGCTM system.

Cellular fractionation. Cytoplasmic cellular extracts were prepared using established protocols. In brief, cells were disrupted by hypotonic lysis (25 mM KCl, 1.5 mM MgCl₂, 10 mM HEPES–KOH pH 7.9 and protease inhibitors) and dounce homogenization. Size-exclusion chromatography (Superose 6) was performed in hypotonic lysis buffer. Ion-exchange chromatography (Sepharose Q HP) columns were equilibrated in hypotonic lysis buffer and proteins were eluted on a linear gradient from 25 to 600 mM KCl. Fractions were concentrated with centrifugal filter units (Amicon, 3,000 Da molecular weight cut-off) before being used as substrate in in vitro reactions with radiolabelled SAM. Chromatography for substrate enrichment was performed with an AKTA FPLC (GE Healthcare).

In vitro methylation reactions. In vitro methyltransferase reactions were performed with 5 μg methyltransferase, 1 μg substrate, and 2 μCi 1-H-S-adenosylmethionine (American Radiolabeled Chemicals) in a buffer that contained 50 mM Tris pH 8.0, 20 mM KCl and 5 mM MgCl₂ and were incubated at 30 °C overnight. Reactions were resolved by SDS–PAGE and gels were stained with Coomassie blue as a loading control. Reactions with deuterated SAM (CDN Isotopes) or normal SAM (Sigma-Aldrich) were performed with SAM at a final concentration of 80 μM.

Steady-state kinetic measurements. Human β-actin peptide (residues 66–80; TLKYPIEHGVITVNDW) was used as a substrate for SETD3. The reaction mixture contained 20 mM Tris (pH 8.0), 50 mM NaCl, 0.1 mg ml⁻¹ BSA, 1 mM DTT and 0.18 μM SETD3. To determine the Michaelis constant (Kₘ) value of the peptide, the concentration of SAM was kept constant at 40 μM, whereas for the determination of the Kₘ of SAM, the peptide concentration was kept constant at 50 μM. Reactions were carried out at room temperature for 20 min with a total volume of 20 μl, and terminated by addition of 2 μl 0.1 M sodium hydroxide. The activity of SETD3 was measured using a bioluminescence assay (MTase-Glo, Promega) in which the reaction by-product SAH is converted into ATP in a two-step reaction and ATP can be detected through a luciferase reaction. In general, 5 μl of reaction mixture was transferred to a low-volume 384-well plate and the luminescence assay was performed according to the manufacturer’s protocol. A Synergy 4 Multi-Mode Microplate Reader (BioTek) was used to measure luminescence signals.

Time course of methylation for three β-actin peptides. Typically, a reaction mixture contained 20 mM Tris (pH 8.0), 50 mM NaCl, 0.1 mg ml⁻¹ BSA, 1 mM DTT, 40 μM SAM and 50 μM peptide. In addition to the unmodified peptide, 1-methyl-His and 3-methyl-His peptides were used in the activity assay for the unmodified peptide, 0.18 μM SETD3 was used, whereas for modified peptides, 80-fold higher amounts of SETD3 (14.4 μM) were used to very low activity on these peptides. For each time point, 10 μl of reaction mixture was taken and trifluoroacetic acid was added to 0.1% to stop the reaction. The luminescence assay was performed as described above.

Mass spectrometry. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was performed using an Orbitrap Elite or Fusion (Thermo Scientific) and data were analysed manually or using MaxQuant software. Searches to identify peptides using MaxQuant were performed with parameters to identify methionine oxidation, N-acetylation, monomethyl lysine, dimethyl lysine, trimethyl lysine, methyl histidine, monomethyl arginine, dimethyl arginine, methyl glutamine and methyl cysteine. In vitro reactions were directly resolved by SDS–PAGE before mass spectrometry analysis was performed as described above and digested with trypsin, chymotrypsin or Glu-C (Promega). Quantitative proteomic analysis was performed with SILAC, light isotopic labels (K0R0) and heavy isotopic labels (K8R10). In the SETD3-dependent methylation experiments, no peptides with a SILAC ratio greater than 1.5 were deemed to be SETD3-dependent methyl events. No peptides (other than actin) with at least a 1.5-fold ratio in the reverse direction and Na/N ratio in the forward direction were identified at all in forward direction. Actin His73 methylation was detected with light (wild-type cells, K0R0) labelling in the forward direction and not with heavy labelling (SETD3 knockout cells, K8R10). We thus concluded that the lack of a calculable ratio (light/heavy) in the forward direction is due to the clonal nature of the cell lines being used. We therefore plotted the forward ratio as infinite (Fig. 3i).

Targeted quantification of actin methylation was performed by manual identification of methylated and unmodified peptides followed by integration of the area under the curve for chromatographic peaks associated with the corresponding m/z. The percentage of methylated peptides was determined as a fraction of the sum of the total area for methylated and unmethylated species. Actin was isolated from cellular or tissue sources using DNase I affinity chromatography. DNase I was immobilized on NHS-activated Sepharose FF (GE Healthcare) overnight in 100 mM HEPES pH 7.5, 150 mM NaCl and 80 mM CaCl₂. Cells and tissues were homogenized using a buffer that contained 1 mM Tris pH 8.0, 600 mM KCl, 0.5 mM MgCl₂, 4% NP-40, 1% Tween-20, 1 mM DTT, 1 mM ATP and protease inhibitors (Roche). Cleared lysates were incubated with immobilized DNase I resin overnight, washed, boiled in SDS sample buffer and purified actin was resolved by SDS–PAGE. Gels were silver-stained (Thermo Scientific) after which actin bands were excised and processed for mass spectrometry by in-gel digestion with sequencing-grade trypsin. The following sources were used for the analysis of actin methylation across evolution: S. cerevisiae, mid-log BY4741; Arabidopsis thaliana, leaf tissue; Nicotiana benthamiana, leaf tissue; Caenorhabditis elegans, mixed population; Drosophila melanogaster, larvae; Spodoptera frugiperda, S9 cells; Danio rerio, five days post-fertilization embryos; Xenopus laevis, egg extract; Gallus gallus, skeletal muscle; Musculus, brain tissue as well as uterine and skeletal muscle tissues; Homo sapiens, cell lines.

Crystallography. Crystalization of the SETD3–SAH–β-actin peptide (residues 66–80; Genscript) complex was carried out using the hanging-drop vapour-diffusion method at 20 °C after mixing SETD3 (approximately 15 mg ml⁻¹) with SAH and peptide at a molar ratio of 1:4.5. This mixture was then mixed with an equal amount of crystallization solution. Three different crystal forms were obtained. Crystals with space group P2₁ were obtained with 0.2 M ammonium acetate, 0.1 M sodium citrate tribasic dihydrate pH 5.6 and 30% (w/v) polyethylene glycol (PEG) 8000. Crystals with space group P2₁ were obtained with 0.2 M ammonium sulfate, 0.2 M sodium chloride, 0.1 M Bis-Tris pH 5.5, 25% (w/v) polyethylene glycol 3350 and 0.1 M Bis-Tris pH 5.5, 25% (w/v) polyethylene glycol 3350. The first form, like the P2₁ crystal, contained
For X-ray diffraction data collection, single crystals were flash-frozen in liquid nitrogen by equilibrating in a cryoprotectant buffer that contained crystallization solution with 25% (v/v) ethylene glycol. All datasets were collected at the SER-CAT beamline 21ID of Advanced Photon Source at Argonne National Laboratory. Crystallographic datasets were processed with HKL2000.27 Molecular replacement was performed with the PHENIX Phaser module41 using the structure of human SETD3 in complex with SAM (Protein Data Bank (PDB) 3SMT) as a search model. PHENIX REFINEM46 was used for refinement with 5% randomly chosen reflections for validation by the Rfree value. COOT was used for peptide building and model corrections between refinement rounds. Structure quality was analysed during PHENIX refinements and later validated by the PDB validation server. Molecular graphics were generated using PyMOL (Schrodinger).

We note that the high-resolution data at 1.69 Å (PDB 6MBK) were the result of merging two datasets that were collected from different parts of the same crystal. This crystal did diffract X-rays to a higher resolution but was susceptible to radiation damage, especially in the higher resolution range. The merge resulted in 98.4% completeness in the highest resolution shell of 1.75–1.69 Å. The slightly higher Rwork and Rfree values of the structure (PDB 6MBK) might be an indication of radiation damage that could not be modelled and further refinement would not lower these R values substantially and the structure and map compared well with the quality of the other two crystal forms.

**Actin purification for biochemical assays.** Actin was purified from wild-type HeLa cells or a clonally derived HeLa cell line in which SETD3 was knocked out. Purification was achieved from established protocols48. In brief, HeLa cells were resuspended in lysis buffer (10 mM Tris–HCl pH 8.0, 11.6% (v/v) sucrose, 1 mM EGTA, 1 mM ATP, 5 mM DTT and protease inhibitor cocktail) and lysed by sonication. Lysates were cleared by ultracentrifugation (100,000 g, 2 h). Cleared cellular extracts were loaded onto an anion-exchange column (Sepharse Q HP, GE Healthcare) equilibrated with column buffer (10 mM Tris–HCl pH 8.0, 0.2 mM CaCl2, 0.5 mM ATP and 0.5 mM DTT). Protein actin was eluted on a linear gradient of column buffer containing 0 mM KCl to column buffer containing 600 mM KCl. Actin-positive fractions were polymerized with 2 mM MgCl2 and 1 mM ATP at room temperature, and polymerized actin was pelleted at 100,000g for 2 h. F-actin was resuspended and depolymerized in 2 mM Tris pH 8.0, 0.2 mM CaCl2, 0.5 mM DTT and 0.2 mM ATP for ≥2 days. Residual F-actin was cleared by ultracentrifugation as described above and the protein in the supernatant was used in biochemical assays.

Pyrene-based polymerization assays were performed according to commercially available kit instructions (Cytoskeleton). Purified HeLa actin was combined in a 10:1 ratio with pyrene-labelled actin from rabbit skeletal muscle (Cytoskeleton). Polymerization was induced with 2 mM MgCl2, 50 mM KCl and 1 mM ATP, and polymerized actin was pelleted at 100,000g for 2 h. F-actin was resuspended and depolymerized in 2 mM Tris pH 8.0, 0.2 mM CaCl2, 0.5 mM DTT and 0.2 mM ATP for ≥2 days. Residual F-actin was cleared by ultracentrifugation as described above and the protein in the supernatant was used in biochemical assays.

**Immunofluorescence imaging.** H1-HeLa cells were seeded overnight onto autoclaved 12-mm circle micro cover glasses (VWR) at 50,000 cells per well in 24-well plates (Greiner Bio-One). Samples were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 30 min at room temperature. Each sample was incubated with anti-GFP antibody (1:300) in immunofluorescence blocking buffer (1 × phosphate-buffered saline (PBS) with 3% BSA, 1% saponin, 1% Triton X-100) overnight at 4 °C. Cells were washed thoroughly with 1 × PBS and then incubated for 1 h at room temperature with Alexa488 anti-rabbit antibody (1:500) and DAPI (Insitus Biotechnologies, 1-300 dilution) in immunofluorescence blocking buffer. Washed micro cover glasses were then mounted onto microscope slides (Fisher Scientific) using Vectashield with DAPI (Vector Laboratories). Images were taken with a Zeiss LSM 700 confocal microscope and processed with Velocity software.

**Generation of the mouse embryonic fibroblast cell line.** Embryos were collected 13.5 d.p.c. and internal organs were removed. The remaining tissues of the embryos were minced and treated with trypsin to dissociate cells for 30 min at 37 °C. Trypsinization was quenched with growth medium (DMEM containing 10% dialyzed fetal bovine serum, 115 mg proline (Applichem), 50 mg lysine (light, lysine HCl (Applichem); heavy, 13 C6,15 N2-l-lysine HCl (Silantes)) and 50 mg arginine (light, arginine HCl (Applichem); heavy, 13 C6,15 N2-arginine HCl (Silantes)). Cells used for immunofluorescence were cultured in SILAC medium for at least one week before analysis.

**Cell migration assays.** Cell migration assays were performed with Radius cell migration kits (Cell Biolabs). Mouse embryonic fibroblasts (1 × 105 cells) were plated in 24-well migration plates and allowed to attach overnight. The Radius gel was removed according to the manufacturer’s protocol. After gel removal, imaging was performed over a 24-h period with a Nikon Eclipse Ti inverted fluorescence microscope and NIS Elements (version 4.60) software. During image acquisition, the environmental conditions were kept at 5% CO2 and 37 °C (Okolab). After the 24-h period, cells were fixed and stained with DAPI according to kit instructions. Genotyping of new cell lines was performed with residual embryonic tissue and confirmed by western blot analysis.

**Cell contraction assays.** Cellular contraction assays were performed on a collagen substrate (Cell Biolabs). In brief, 450 μl collagen mixture made with the provided 5:1 PBS was added to a 24-well plate and allowed to polymerize according to the manufacturer’s protocol. Primary uterine smooth muscle cells (5 × 104 cells) were layered on top of a collagen matrix in vascular basal medium without the growth kit. Cells were allowed to attach to the collagen overnight at 37 °C. The next day, the collagen disks were released from the sides of each well. Induction of contraction was performed with 10 μM oxytocin (Tocris) or 1 μM endothelin-1 (Tocris). Contraction was monitored by imaging after 24 h of contraction. Quantification of collagen disk area was performed in Imagel (NIH). Relative disk area was determined by normalizing to the area of disks that lacked cells. All assays were performed in triplicate.
Maintenance of mouse colonies. Husbandry is performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition (2010) and the 2015 revision of the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Room conditions included a temperature of 23 °C, relative humidity of 30–40% and a 12:12 h light-dark cycle (lights on, 07:00). All mouse colonies are maintained under specific pathogen-free conditions in irradiated disposable individually ventilated cages (Innocage, Innovative). The mice were fed ad libitum a high-fat diet (TD 91043, Harlan Teklad), water was provided ad libitum and ultraviolet-light-irradiated, acidified (pH 2.5–3.0), reverse-osmosis-purified bottle water (Aquavive, Innovative). The mouse colonies are monitored for viral, bacterial and parasitic pathogens using dirty-bedding sentinel that were tested quarterly and were found to be free of mouse parvovirus, minute virus of mice, mouse hepatitis virus, mouse rotavirus, Thiel er's murine encephalomyelitis virus, murine norovirus, Sendai virus, mouse adenosivirus 1 and 2, ectromelia virus, lymphochoriomeningitis virus, pneumonia virus of mice, respiratory enterovirus III, mites, lice and pinworms. Sample size was maximized as much as possible to gain highest confidence in the results. No randomization was necessary as only single variables changed per experiment. Blinding was not necessary as any phenotypic assessment or other measurements was performed using discrete, quantitative measurements.

Mouse strains. Setd3f−/− (INO/COM/mice) or C57BL/6N heterozygous mice were obtained from the Canadian Mouse Mutant Repository (CMMR). The strain was made at the Toronto Centre for Phenogenomics as part of the NorCOMM project using NorCOMM embryonic stem cells46. Progeny were genotyped using either the Setd3-McKerlie PCR assay to identify the 212-bp wild-type band (primers: Setd3 WT F1: AACCACGCTCAGGAGACAC; and/or a 566-bp tm1.1 band (primers: Setd3 tm1 F1: AAAATGCTTGTGCTGTAAACCTGGGC; GH717 CACCAGGCAATCACAAC) following instructions provided by the CMMR or the use of a commercial genotyping service (Transnetyx). For timed-pregnant matings, C57BL/6N male mice were purchased from Charles River Laboratories. Male mice began breeding at 8 weeks and female mice at 6 weeks. Typically, mice no older than 6 months were used in this study.

Quantification of litter size and length of gestation. Quantitation of pregnancies and litters for both trio- or pair-breeding females were tabulated for Setd3−/−, Setd3+/− and Setd3+/+ females. All litters that were observed (confirmed) were counted, even if pups subsequently died. To determine the number of pups per litter, litters that were lost before the pups could be counted were listed as 0 pups for that litter. The length of gestation for Setd3−/−, Setd3+/− and Setd3+/+ females was determined by timed-pregnant matings. Females were mated to wild-type C57BL/6N males (Charles River Laboratories). Whatever the female was a virgin (primigravida) or had a previous pregnancy, (multipravida) was recorded. Presence of a vaginal plug was noted 0 d.p.c. Plugged females were moved to a separate cage and mated at a later time. Fertility of the females that delivered on 19 d.p.c. still exhibited ≥ 20% tolerance. All experiments were conducted at 23 °C.

In vitro motility. Experiments were conducted following previously described methods58–60. Briefly, a truncated version of MYH7 (residues 1–808), corresponding to sS1, with 5–10× molar excess of unlabelled F-actin on ice for 5 min, followed by addition of 2 mM ATP for 3 min, then centrifuged at 350,000 × g for 3 min, then centrifuged at 350,000 × g in a TLA-100 rotor (Beckman Coulter) at 4 °C for 20 min. The supernatant was collected for use in the motility. Flow chamber was built on a nitrocellulose-coated coverslip mounted on a glass slide. Deadhead myosin was immobilized via specific binding to the GFP antibody that non-specifically binds to the coverslip surface, followed by blocking with assay buffer (25 mM imidazole pH 7.5, 25 mM KCl, 4 mM MgCl2, 1 mM EDTA, 10 mM DTT and 1 mg/ml BSA). A final solution containing fluorescently labelled bovine serum albumin (BSA, 2 mg/ml; GE Healthcare), alpha-actinin (1 mg/ml; Sigma), creatine kinase (2 mg/ml; Sigma), ATP (1 mM), glucose (0.11 mg ml−1 glucose oxidase and 0.018 mg ml−1 catalase) was subsequently flowed in before imaging on a total internal reflection fluorescence microscope (Nikon Eclipse Ti-E) equipped with a 561-nm laser (Cobolt) and a 100× 1.11 numerical aperture (Nikon)56. Time-lapse movies were acquired at multiple fields of views for each experimental condition at 1 Hz sampling rate. Movies were analysed using the FAST (fast automated spud trekker) software57 to obtain the mean velocities with 20% tolerance. All experiments were conducted at 23 °C.

Actin-activated myosin ATPase activity. Purified actin by extensive (four times over 4 days) dialysis in ATPase buffer (10 mM imidazole pH 7.5, 50 mM KCl, 1 mM MgCl2, 1 mM EGTA, 10 mM DTT and 1 mg/ml BSA). A final solution containing fluorescently labelled bovine serum albumin (BSA, 2 mg/ml; GE Healthcare), alpha-actinin (1 mg/ml; Sigma), creatine kinase (2 mg/ml; Sigma), ATP (1 mM), glucose (0.11 mg ml−1 glucose oxidase and 0.018 mg ml−1 catalase) was subsequently flowed in before imaging on a total internal reflection fluorescence microscope (Nikon Eclipse Ti-E) equipped with a 561-nm laser (Cobolt) and a 100× 0.11 numerical aperture (Nikon)56. Time-lapse movies were acquired at multiple fields of views for each experimental condition at 1 Hz sampling rate. Movies were analysed using the FAST (fast automated spud trekker) software57 to obtain the mean velocities with 20% tolerance. All experiments were conducted at 23 °C.

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Data availability
The X-ray structures (coordinates and structure factor files) of SETD3 with bound actin peptide have been submitted to the PDB under accession numbers 6MBJ (P2₁), 6MBK (two complexes in P2₁;2,1) and 6MBL (one complex in P2₁;2,1). Source Data for this study are provided included in the online version of the paper for Figs. 4d, 5b, c, e, f, i, k and Extended Data Figs. 7e, 9c, h. Gel source data are provided as Supplementary Fig. 1 for cropped images shown in Fig. 1a–e, 2a, c, 3a–g, 4c, 5g, j and Extended Data Fig. 1b. Mass spectrometry data associated with Fig. 1c and Extended Data Fig. 1h are provided in Supplementary Tables 1. Mass spectrometry data associated with Fig. 3i are provided in Supplementary Tables 2. Additional requests for data can be made to the corresponding authors.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Identification of actin as a SETD3 substrate.

a, Top, domain structure of SETD3 containing an N-terminal SET domain and a C-terminal domain homologous to plant Rubisco LSMT. Bottom, alignment of the homologous methyltransferases human SETD3 and human SETD6. Red box, putative catalytic tyrosine. b, Methylation reactions as in Fig. 1a with non-radiolabelled SAM and analysed by western blot with indicated antibodies. Total histone H3 is shown as a loading control.

c, SETD3 localizes to the cytoplasm. Representative immunofluorescence images of GFP or GFP–SETD3 localization in HeLa cells (left) are merged with DAPI counterstaining (right). Scale bars, 7 μm. d–g, Biochemical enrichment of a candidate SETD3 substrate. d, Schematic of biochemical strategy to identify the methylated band indicated in Fig. 1b. e, In vitro methylation reactions using cell extracts separated by size-exclusion chromatography as a substrate. Reactions were performed with either wild-type (WT) SETD3 or a putative catalytic mutant (Y312A). Reactions were analysed as in Fig. 1. Arrowhead, candidate substrate. f, Ion-exchange chromatography separates candidate substrates. Fractions positive for SETD3-specific methylation by size-exclusion chromatography were further separated by ion-exchange chromatography and either the flow through (FT) or the pooled fractions containing a notable silver-stained band (arrowhead) at the size of the candidate substrate were used as substrate in an in vitro methylation reaction. g, Flow through or pooled eluent from ion-exchange chromatography were used as substrate for methylation reactions as in e. Arrowhead, protein band in eluent that was analysed by mass spectrometry.

h, Top candidate substrates with molecular weights (MW) that were similar to the size of the SETD3-dependent band from in vitro reactions with cell extracts. Candidates identified by mass spectrometry are ranked by abundance determined by MS/MS count (Supplementary Table 1). i, In vitro methylation reactions with SETD3 on actin, recombinant histone H3 (rH3), FOXM1 or a no substrate as a control. Top, 3H-SAM is the methyl donor and methylation visualized by autoradiography. Bottom, Coomassie staining of proteins in the reaction. j, In vitro methylation reactions with the indicated enzymes and substrates. All experiments were repeated at least three times with similar results.
Extended Data Fig. 2 | SETD3 does not methylate β-actin lysines. GST–β-actin was expressed in bacteria, and cleaved using PreScission Protease. Cleaved β-actin was used in an in vitro methylation assay with SETD3 and deuterated SAM. Spectra are representative of experiments independently performed three times with similar results. MS/MS spectra with identified ions for unmethylated lysine residues produced with indicated proteases. a, K18 (trypsin) (identified N-terminal GPLGS amino acids are residual, vector-specific amino acids from the original GST fusion protein). b, Lys50 (trypsin). c, Lys61 (trypsin). d, Lys68 (trypsin). e, Lys84 (chymotrypsin). f, Lys113 (trypsin). g, Lys118 (Glu-C). h, Lys191 (trypsin). i, Lys213 (trypsin). j, Lys215 (Glu-C). k, Lys238 (chymotrypsin). l, Lys284/K291 (chymotrypsin). m, Lys315 and Lys326 (trypsin). n, Lys326 and Lys328 (trypsin). o, Lys336 (trypsin). p, Lys373 (trypsin). © 2019 Springer Nature Limited. All rights reserved.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3  |  SETD3 generates actin-His73(3-me).  a–c, MS/MS identifies His73 of actin as a SETD3-methylated residue.  a, Top, methylated tryptic peptide with indicated b and y ions. Modifications: me, methylation; ox, oxidation. Bottom, m/z for b and y ions identified in MS/MS spectra from methylation reactions with (+SETD3) or without (−SETD3) using deuterated SAM as the methyl donor (+17.03 Da mass shift). Peptides containing His73 are indicated by an asterisk.  b, c, In vitro methylation reactions with (b) and without (c, negative control) SETD3 and β-actin with deuterated SAM were analysed by mass spectrometry. Left, MS/MS spectra of tryptic peptides containing His73. Right, b and y ions identified from oxidized peptides in each spectrum are indicated.  d, Dot blot loading control for Fig. 2c of biotinylated ACTB peptides (amino acids 66–80) that are unmodified at His73 or methylated in the N3 (3-me) or N1 (1-me) position. Serial dilutions of the peptides were visualized by streptavidin–HRP and chemiluminescence.  e–g, Methylation reaction kinetics for SETD3 and the actin peptide.  e, Time course of SETD3 with His(1-me) or His(3-me) modified peptides as substrate. Inset, unmodified peptide using 80-fold lower enzyme concentrations compared to the concentrations used for modified peptides.  f, g, Steady-state kinetics of SETD3 for the human β-actin peptide (residues 66–80) (f) and SAM (g). Kinetic measurements were performed using a bioluminescence methyltransferase assay, MTase-Glo (Promega). Data are mean ± s.d. of biological triplicates for each data point.  a–g, Data are representative of three independent experiments with similar results.
Extended Data Fig. 4 | Methylation of the conserved actin-His73 residue is present in diverse organisms. a, Summary of actin histidine methylation on the conserved His73 residue among model organisms. Abundance of methylation is reported as a percentage of peptide that is methylated. b, Chromatograms of MH3 ions (m/z ± 10 p.p.m.) for methylated and unmethylated versions of the indicated peptides. The unmethylated actin peptide that was analysed for each species is shown above the corresponding chromatograms. When α-actin peptides were detected, associated chromatograms and quantification are provided on the right. The area of indicated peaks was normalized to the sum of the area between peptides (with or without methylation) and the percentage abundance is labelled. Chromatograms that quantify actin histidine methylation in human and mouse can be found in Extended Data Figs. 6, 7. N.D., not detected. Quantification represents data from two independent experiments with similar results.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Structural details of SETD3–actin peptide interactions. a, Overall structure of SETD3 with a V-shaped cleft constructed by the SET domain (green) and an LSMT-like domain (cyan). Helices are shown as cylinders and strands as arrows. The actin peptide is shown as a stick model. b, View of the target histidine through the channel from the SAH-binding pocket. c, Omit electron density of Fo−Fc, contoured at 3.0σ above the mean, is shown for omitting cofactor SAH and the actin peptide used for co-crystallization. d, Details of inter- and intramolecular interactions between SETD3 (green) and actin peptide (yellow). e, Trp79 of actin bound in a hydrophobic surface pocket of SETD3. f, Thr77 and Asn78 of actin form hydrogen bonds with Asn153 and Gln254 of SETD3. g, Val76 of actin is in van der Waals contact with SETD3 residues His323 and Arg315, which in turn interacts with Glu72 of actin. h, Ile75 of actin is in van der Waals contact with main-chain Cα of Asn255 and Gln256 of SETD3. i, Gly74 of actin is located in the amino end of a SETD3 helix. The imidazole ring of His73 (the substrate target) is parallel with the aromatic ring of Tyr312 of SETD3. j, SETD3 Arg315 bridges between the carboxylate oxygen of Glu72 and the main-chain carbonyl oxygen of His73 of actin. k, Ile71 of actin is accommodated in a surface hydrophobic pocket of SETD3. l, Tyr69 and Pro70 of actin interact with a stretch of SETD3 residues from Ile283 to Leu289. m, Tyr69 of actin packs against Pro258 of SETD3 and Lys68 of actin interacts with Glu290 of SETD3. n, Leu67 and Pro70 of actin form an intramolecular interaction and both interact with Ile283 and Thr284 of SETD3. o, Alignment of the amino acids from all six human actin isoforms corresponding to β-actin amino acids 66–80. Variant amino acids are highlighted in yellow. p, The cofactor (SAH) binding site includes residues Arg253, Tyr312, Asn277 and His278 of SETD3.
Extended Data Fig. 6 | SETD3 is required for actin methylation in cells. a, Peptide dot blot spotted with biotinylated β-actin peptides (amino acids 66–80) containing His73, His73(3-me) or His73(1-me). Blots were probed with a His73(3-me)-specific antibody or streptavidin as a loading control. b–d, Chromatograms for quantification of actin-His73me in human cells. Stoichiometry of oxidized β/γ-actin His73 peptide (YPIEHGIVTNWDDM(ox)EK) with and without methylation after purification from cells. MH\textsuperscript{1+} m/z: unmethylated, 654.968 ± 10 p.p.m.; methylated, 659.6401 ± 10 p.p.m. α-Actin peptide was not detected in these cells. Quantification performed as in Extended Data Fig. 4. b, Chromatograms of HT1080 cells treated with CRISPR-Cas9 that is targeted with a control sgRNA or SETD3-specific sgRNAs. c, Chromatograms of actin His73 methylation in wild-type HeLa cells or clonal HeLa SETD3 knockout cells. N.D., not detected. d, Chromatograms of actin His73 methylation in HT1080 cells treated with CRISPR-Cas9 targeted with either a control or SETD3-specific sgRNA and complemented with CRISPR-resistant SETD3(WT), SETD3(NHY) or control plasmids. Experiments were independently performed three times with similar results.
Extended Data Fig. 7 | SETD3 is required for actin-His73 methylation in mice. a–d, Chromatograms for the quantification of actin histidine methylation in mouse tissues. a, Chromatograms to determine the abundance of histidine methylation of the β/γ-actin His73 peptide (YPIEHGTVNWDDM(ox)EK) with and without methylation after purification from brain tissue of mice with the indicated genotypes. MH3 \( \text{m/z} \) unmethylated, 654.968 ± 10 p.p.m.; methylated, 659.640 ± 10 p.p.m. α-Actin peptide was not detected in these cells. Quantification performed as in Extended Data Fig. 4. b, Chromatograms to determine the abundance of histidine methylation on α-actin H75 peptide (YPIEHGTVNWDDM(ox)EK) from skeletal muscle as in a. MH3 \( \text{m/z} \) unmethylated, 659.640 ± 10 p.p.m.; methylated, 664.312 ± 10 p.p.m. The β/γ-actin peptide was not detected in these cells. c, Chromatograms to determine the abundance of β/γ-actin His73 methylation from uterine tissue as in a. d, Chromatograms to determine the abundance of α-actin H75 methylation from uterine tissue as in b. Quantification was independently performed three times with similar results. e, Quantitative amino acid panel from mouse blood serum. Quantitative profiling of amino acids in plasma is used clinically to diagnose metabolic disorders. 3-methyl histidine (indicated by an asterisk) is one of the amino acids measured in the panel, and actin is thought to be the primary source of this metabolite. Amino acid levels from Setd3\(^{-/-}\) (KO, \( n = 8 \)) mice were normalized to levels of amino acids from animals with normal His73 methylation levels (total, \( n = 12 \); Setd3\(^{+/+}\) (WT), \( n = 5 \); Setd3\(^{+/-}\) (HET), \( n = 7 \)). Standard error of the difference between two means is shown for the indicated \( n \).
Extended Data Fig. 8 | SETD3-dependent actin-His73 methylation modestly regulates polymerization. a, b, Purification of actin with and without His73 methylation. a, Coomassie-stained gel of the actin purified from HeLa cell lines described in Fig. 3f for actin(+me) or actin(−me), and used in the biochemical assays described in Fig. 4a, b and in c–e. b, Mass spectrometry quantification of actin His73 methylation from a. Representative data from an experiment performed at least three times with similar results. c, Methylation does not alter actin depolymerization rates. Actin polymerized as in Fig. 4a was diluted to 0.02 μM and depolymerization was monitored by fluorescence normalized to initial values. d, Elongation of the indicated monomeric actin (1 μM, 0.1 μM pyrene–actin) measured in the presence of 2 μM phalloidin–actin seeds made with methylated (circles) or unmethylated (triangles) actin. e, Arp2/3 complex-induced actin polymerization performed in the presence of 100 nM WASP VCA and 5 nM Arp2/3 complex with 1 μM of the indicated monomeric actin and 0.1 μM pyrene–actin. c–e, Mean values plotted with s.e.m. from three independent biological replicates. f, SETD3 promotes cell migration. Representative images of cell migration assays performed three times with similar results using cells from Fig. 4c. A circular void of cells was created at the start of the assay (0 h, dashed red circle). After 24 h of migration, cells were fixed and stained with DAPI. Scale bars, 100 μm.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Analysis of SETD3 and actin-His73me in parturition and uterine smooth muscle contraction. a, b, Histology of muscle tissues from Setd3+/+ and Setd3−/− mice. a, Haematoxylin and eosin staining of aorta, colon, heart, tongue and hind limb muscle from Setd3+/+ and Setd3−/− mice. b, Tongue and hind limb (striated muscle) sections from a were re-imaged without a condenser to highlight the sarcoplasmic striations characteristic of this muscle type. Scale bars, 20 μm. Images from three independent experiments gave similar results. c, Quantification of pups per litter for Setd3+/+ (n = 12), Setd3−/− (n = 43), Setd3−/− (n = 26) mothers. ***P value ≤ 0.001. d, e, Labour induction at 19 d.p.c. does not rescue dystocia of Setd3−/− pregnant mice. d, Schematic of prostaglandin treatment protocol. The PGF2α cocktail was administered at 0, 3 and 8 h on day 19 with euthanasia and quantification of fetuses at 32 h after the first treatment. e, Quantification of the births and post-term fetuses in utero for the indicated genotypes at the time shown in the schematic. Note: controls delivered before treatment commenced. f, Collagen contraction assay as in Fig. 5h with indicated reconstitution cell lines from Fig. 5j performed with three independent biological replicates. g, h, Actin-His73me does not notably alter myosin activity. g, In vitro actin motility assay. Gliding velocities of actin filaments prepared from actin described in Fig. 4 were measured using human β-cardiac sS1 myosin or a hyperactive mutant (H251N). Data are mean ± s.e.m. velocities from four different experiments. h, Actin-activated ATPase of human β-cardiac sS1 using actin as in g. The Michaelis–Menten equation was fitted to the ATPase data for wild-type actin (solid line) and knockout actin (dashed line). Points are means from two independent experiments. For gel source data, see Supplementary Fig. 1.
Extended Data Table 1 | Summary of X-ray data collection from the SER-CAT beamline (22-ID) at a wavelength of 1 Å and refinement statistics

| SETD3              | Actin peptide | Actin peptide | Actin peptide |
|--------------------|---------------|---------------|---------------|
|                    | SAH           | SAH           | SAH           |
| PDB Code           | 6MBJ          | 6MBK          | 6MBL          |

**Data Collection**

|                  | SETD3 | SETD3 | SETD3 |
|------------------|-------|-------|-------|
| Space group       | $P_{2_1}$ | $P_{2_2}2_1$ | $P_{2_2}2_1$ |
| Cell dimensions (Å) | 60.35, 176.17, 66.58 | 60.28, 115.97, 173.59 | 56.56, 92.83, 111.64 |
| $\alpha, \beta, \gamma$ (°) | 90, 92.9, 90 | 90, 90, 90 | 90, 90, 90 |
| Resolution (Å)    | 39.07±1.79 (1.85±1.79) | 39.38±1.69 (1.75±1.69) | 48.30±2.19 (2.27±2.19) |
| $R_{\text{merge}}$ | 0.146 (0.892) | 0.129 (0.800) | 0.125 (0.741) |
| $R_{\text{merge}}$ | 0.041 (0.436) | 0.061 (0.592) | 0.066 (0.548) |
| $R_{\text{merge}}$ | (0.463, 0.795) | (0.720, 0.915) | (0.402, 0.757) |
| $R_{\text{merge}}$ | 14.5 (1.0) | 11.3 (1.7) | 14.1 (1.5) |
| Completeness (%)   | 94.5 (78.9) | 99.2 (98.4) | 99.5 (96.7) |
| Redundancy         | 13.6 (8.0) | 19.2 (8.9) | 10.6 (4.7) |
| Observed reflections | 1,695,347 | 2,597,462 | 323,060 |
| Unique reflections  | 124,387 (10,380) | 135,382 (13,262) | 30,434 (2920) |

**Refinement**

|                  | SETD3 | SETD3 | SETD3 |
|------------------|-------|-------|-------|
| Resolution (Å)    | 1.79  | 1.69  | 2.19  |
| No. reflections   | 123,743 | 135,110 | 30,334 |
| $R_{\text{work}}/R_{\text{free}}$ | 0.199 / 0.223 | 0.228 / 0.243 | 0.182 / 0.227 |
| No. Atoms Protein | Two complexes | Two complexes | One complex |
|                  | 7824  | 7953  | 3923  |
|                  | 248   | 252   | 124   |
|                  | 52    | 52    | 26    |
|                  | 777   | 933   | 179   |
| B-factors (Å²)    | 34.3  | 29.6  | 47.3  |
|                  | 41.4  | 34.5  | 42.3  |
|                  | 18.0  | 16.7  | 32.0  |
|                  | 45.2  | 39.9  | 46.5  |

**R.m.s. deviations**

|                  | SETD3 | SETD3 | SETD3 |
|------------------|-------|-------|-------|
| Bond lengths (Å)  | 0.003 | 0.010 | 0.004 |
| Bond angles (°)   | 0.564 | 0.942 | 0.683 |

Values in parenthesis correspond to highest resolution shell. $R_{\text{merge}} = \frac{\sum |I - \langle I \rangle| |I|}{\sum |\langle I \rangle|}$ where $I$ is the observed intensity and $\langle I \rangle$ is the averaged intensity from multiple observations. $\langle I / \sigma \rangle$ indicates the averaged ratio of the intensity ($I$) to the error of the intensity ($\sigma$). $R_{\text{work}} = \frac{\sum |F_{\text{obs}} - F_{\text{calc}}|}{\sum |F_{\text{obs}}|}$ where $F_{\text{obs}}$ and $F_{\text{calc}}$ are the observed and calculated structure factors, respectively. $R_{\text{free}}$ was calculated using a randomly chosen subset (5%) of reflections that were not used for refinement.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a** Confirmed
- □ **The exact sample size (n)** for each experimental group/condition, given as a discrete number and unit of measurement
- □ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- □ The statistical test(s) used AND whether they are one- or two-sided
  - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- □ A description of all covariates tested
- □ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- □ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- □ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - *Give P values as exact values whenever suitable.*
- □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- □ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- □ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
- □ Clearly defined error bars
  - *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

### Software and code

**Policy information about availability of computer code**

**Data collection**
- X-ray collection: HKL2000. Bio-Tek plate readers: GenS (v3.04.17). Microscopy: NIS elements (v4.60), Volocity 3D analysis (Perkin Elmer).

**Data analysis**
- Structural analysis: PHENIX PHASER, PHENIX REFINE, and COOT. Statistical analysis: GraphPad Prism 7.0, KaleidaGraph 4.5. Mass spectrometry analysis: MaxQuant (version 1.5.5.1). Image analysis: ImageJ 1.51 (NIH). Pymol (Schrodinger, v2.2). Metabolic analysis: Agilent Masshunter B08. Actin motility: FAST (Fast Automated Spud Trekker) software (Askel et al. Cell Reports, 2015).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

### Data

**Policy information about availability of data**

All manuscripts must include a **data availability statement**. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The X-ray structures (coordinates and structure factor files) of SETD3 with bound actin peptide have been submitted to PDB under accession number 6MBJ (P21),...
6MBK (two complexes in P212121) and 6MBL (one complex in P212121). Source data for this study are provided as supplementary information (Fig. 4d, 5b, 5e, 5i, 5k; Extended Data Fig. 7e, 9c, 9h). Source gel data provided for cropped images (Fig. 1a, 1d, 1e, 2a, 2c, 3a-f, 5f; Extended Data Fig. 1b) is provided in Supplementary Figure 1. Mass spectrometry data associated with Fig. 1c/Extended Data Fig. 1h and Fig. 3i are provided as a Supplementary Tables. Additional requests can be made to the corresponding authors.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical measure was used to predetermine sample size. The dystocia phenotypic analysis resulted in an almost binary outcome leading to a strong conclusion with the sample size provided. |
| Data exclusions | No data were excluded |
| Replication | Experiments were replicated as indicated in the figure legends. Generally, all experiments were performed with three independent biological replicates. All results repeated during replication. |
| Randomization | No randomization was necessary as only single variables changed per experiment |
| Blinding | Blinding was not necessary as any phenotypic assessment or other measurements was performed using discrete, quantitative measurements |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|----------------------|
| ☐ | Unique biological materials |
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☐ | Palaeontology |
| ☐ | Animals and other organisms |
| ☐ | Human research participants |

Methods

| n/a | Involved in the study |
|-----|----------------------|
| ☒ | ChIP-seq |
| ☐ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Most materials are freely commercially available from common sources. Anti-actin-H73(3-me) antibody generated in this study is available upon request.

Antibodies

Antibodies used

Primary: SETD3 (Abcam, #176582, Lot GR283420-S); Actin (Cytoskeleton, #AAN01, Lot 123); Beta-tubulin (Millipore, #05-661 clone AA2, Lot 2834905); H3K4me2 (Cell Signaling, #9725); H3K36me2 (Cell Signaling, #2901); GFP (Invitrogen, A-11122). Anti-actin-H73(3-me) polyclonal antibody was made in-house. Secondary: Alexa488 anti-rabbit antibody (Life Technologies, A-11008), anti-rabbit-HRP (Jackson Immunoresearch, 711-035-152), anti-mouse-HRP (715-035-151)

Validation

SETD3 antibody validated by loss of detection after CRISPR/Cas9 knockout with multiple guide RNAs. Actin, beta-tubulin, and secondary antibodies were validated by the vendor across multiple cell types. Anti-actin-H73(3-me) polyclonal antibody was validated by dot blot with unmodified, H73(3-me), and H73(1-me) peptides and western blotting with cell extracts where actin methylation was quantified by mass spectrometry. H3K36me2 and H3K4me2 antibodies were validated by the vendor and independently on a peptide array containing multiple modified histone peptides.
### Eukaryotic cell lines

| Policy information about cell lines |
|-----------------------------------|
| Cell line source(s) | HEK293T, H1-HeLa, HT1080, and primary uterine smooth muscle cells were all acquired from ATCC. Murine embryonic fibroblasts (MEFs) were generated from mice in this study. |
| Authentication | HEK293T, H1-HeLa, HT1080, and primary uterine smooth muscle cells were authenticated by ATCC. MEFs were genotyped from residual tissue after embryonic dissection and the presence of SETD3 was probed by western blot in this study. |
| Mycoplasma contamination | Cell lines were declared free of mycoplasma by ATCC. MEFs were not tested. |
| Commonly misidentified lines | (See [ICLAC register](#)) |
| (See [ICLAC register](#)) | no misidentified lines utilized in this study |

### Animals and other organisms

| Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research |
|--------------------------------------------------|
| Laboratory animals | Mice: Strains derived from C57BL/6N - Setd3tm1.1(NCOM)Mfgc/Tcp heterozygous mice. Timed pregnancy matings were performed with C57BL/6N male mice. Male mice began breeding at 8 weeks and female mice at 6 weeks. Typically, mice no older than 6 months old were used in this study. |
| Wild animals | No wild animals used |
| Field-collected samples | No field-collected samples used. |