The WHHERE coactivator complex is required for retinoic acid-dependent regulation of embryonic symmetry

Gonçalo C. Vilhais-Neto1,2, Marjorie Fournier1, Jean-Luc Plassat1, Mihaela E. Sardiu2, Anita Saraf2, Jean-Marie Garnier1, Mitsuji Maruhashi1,2, Laurence Florens2, Michael P. Washburn2,3 & Olivier Pourquié1,2,4,5,6

Bilateral symmetry is a striking feature of the vertebrate body plan organization. Vertebral precursors, called somites, provide one of the best illustrations of embryonic symmetry. Maintenance of somitogenesis symmetry requires retinoic acid (RA) and its coactivator Rere/Atrophin2. Here, using a proteomic approach we identify a protein complex, containing Wdr5, Hdac1, Hdac2 and Rere (named WHHERE), which regulates RA signaling and controls embryonic symmetry. We demonstrate that Wdr5, Hdac1, and Hdac2 are required for RA signaling in vitro and in vivo. Mouse mutants for Wdr5 and Hdac1 exhibit asymmetrical somite formation characteristic of RA-deficiency. We also identify the Rere-binding histone methyltransferase Ehmt2/G9a, as a RA coactivator controlling somite symmetry. Upon RA treatment, WHHERE and Ehmt2 become enriched at RA target genes to promote RNA polymerase II recruitment. Our work identifies a protein complex linking key epigenetic regulators acting in the molecular control of embryonic bilateral symmetry.
he development of bilaterally symmetrical structures, such as limbs or somites takes place concomitantly with the asymmetric formation of internal organs such as heart, gut, and liver. Whereas the pathway responsible for establishing left–right identity in the embryo begins to be well understood, little is known about the mechanisms controlling embryonic symmetry. Retinoic acid (RA) is a derivative of vitamin A, signaling via a heterodimeric RAR/RXR nuclear receptor transcription factor. In the absence of RA, the heterodimer binds target genes together with the SMRT and NCoR corepressor complexes and histone deacetylases such as Hdac3 to silence gene expression. When the RA ligand binds to RAR, the corepressors are replaced by a set of coactivators including histone acetyltransferases, contributing to active transcription of RA target genes. In the absence of RA signaling in the mouse embryo, somite formation becomes asymmetrical, showing a significant delay on the right side. A similar somite desynchronization phenotype is also observed in mutants for the protein Rere (or Atrophin2) which acts as a coactivator for RA signalling.

Here, we identify and characterize biochemically a previously undescribed retinoic coactivator complex, containing the proteins Wdr5, Hdac1, Hdac2, and Rere (the WHHERE complex). We demonstrate that mouse mutants of the WHHERE complex members down-regulate RA signaling and exhibit somite bilateral symmetry defects. We report that the WHHERE complex also binds the histone methyltransferase Ehm2/G9a. Null mouse mutants for Ehm2 also exhibit RA downregulation and somite symmetry defects indicating that it also acts as an activator of RA signaling. We show that Ehm2 and the WHHERE complex bind to the promoter of RA targets and serve to recruit PolII to trigger gene activation.

Results

Identification of the WHHERE complex. In order to understand the mechanism of action of Rere in the RA pathway controlling somite symmetry, we first set out to identify Rere-interacting proteins in the mouse mesoderm. To that end, we generated a transgenic mouse line, which allows the conditional expression of a tagged version of Rere containing two HA epitopes at the C-terminal end of the protein (Rere-HA). A Rere-HA construct preceded by a LoxP-STOP-LoxP cassette was introduced into the Rosa26 locus by homologous recombination in mouse embryonic stem (ES) cells. We then used these cells to generate a Rosa26-LoxP-STOP-LoxP-Rere-HA mouse line (RS-Rere-HA line). Whereas Rere mutants (Rere<sup>om/om</sup>) die around E9.5 with defects in forebrain, heart and a right side specific delay in somite formation<sup>5</sup>, expression of at least one Rere-HA allele in the mutant Rere<sup>om/om</sup> background led to morphologically normal embryos (Supplementary Fig. 1a–c). Therefore, the tagged Rere-HA protein is functional in vivo.

To direct expression of Rere-HA to the mesoderm, RS-Rere-HA mice were crossed to the T-Cre mouse line<sup>10</sup>. We prepared whole cell protein extracts from ~600 whole cell protein extracts from ~600 NIH3T3 fibroblast cultures with expression vectors coding for either Rere, Wdr5, Hdac1, or Hdac2, together with a RA reporter containing the well-characterized retinoic acid response element (RARE) of the known RA target retinoic Acid Receptor beta (Rarbeta)<sup>12</sup>, driving luciferase expression. In the presence of RA, overexpression of each of the four WHHERE complex proteins increased RA reporter expression (Fig. 2a). This activation was not observed in the absence of RA (Supplementary Fig. 3a). Also overexpression of the WHHERE complex components did not activate either a minimal or a SV40 promoter driving luciferase supporting the specificity of the RA response (Supplementary Fig. 3b, c). Furthermore, co-transfection of Hdac1 and Hdac2 cooperated to activate RA signaling (Fig. 2b). Conversely, treatment of fibroblast cultures with siRNAs against Rere, Hdac1, or Wdr5 in the presence of RA decreased RARE-Luciferase reporter activity (Fig. 2c and Supplementary Fig. 3d–f). Treatment with Hdac2 siRNA led to an increase of RARE-Luciferase reporter activity (Fig. 2c and Supplementary Fig. 3g), which might be explained by a stabilization of Hdac1 (due to the decrease of Hdac2), potentially resulting in an increase in RA signaling.

In vitro characterization of the WHHERE complex. To validate the identification of Wdr5 as an interacting partner of Rere, Hdac1, and Hdac2, we co-expressed tagged versions of the four proteins (Rere-Flag, Flag-Hdac1, Flag-Hdac2, and HA-Wdr5) using a baculovirus-insect cell expression system. After Flag immunoprecipitation of Rere, Hdac1 and Hdac2, HA-Wdr5 was detected in the eluates, as confirmed by LC–MS/MS analysis of the Coomassie stained gel bands (Fig. 1c, Supplementary Figs. 2b and 7). The four proteins still co-purified together at high salt concentration (500 mM KCl) suggesting the existence of a stable protein complex comprising Rere, Hdac1, Hdac2, and Wdr5 (Fig. 1c and Supplementary Fig. 7). By co-immunoprecipitation of baculovirus-expressed Rere-Flag and HA-Wdr5, we could demonstrate that Rere binds directly to Wdr5 (Fig. 1d and Supplementary Fig. 7). Wdr5 does not bind directly to Hdac1 or Hdac2 in high or low salt wash conditions (Supplementary Figs. 2c and 9). Wdr5 binding to Hdac1 and Hdac2 could only be detected when Rere is also co-expressed (Supplementary Figs. 2c and 9), suggesting that Rere acts as a scaffolding component binding Hdac1/Hdac2 and Wdr5. To analyze whether the four co-immunopurified proteins form a stable protein complex, we carried out gel filtration chromatography followed by western blot and mass spectrometry analysis. All four proteins co-eluted together in a fraction corresponding to a high molecular weight complex of 0.5–0.6 MDa (Figs. 1e, f, Supplementary Figs. 2d, e and 7). This molecular weight is consistent with the abundance predicted by the NSAF values of the proteomic analysis. Additionally, Hdac1, Hdac2, and Wdr5 co-immunoprecipitated with the endogenous Rere in NIH3T3 cells further supporting the existence of such a protein complex (Fig. 1g and Supplementary Fig. 7). Altogether, these results suggest that Wdr5, Hdac1, Hdac2 and Rere can form a stable protein complex, which we call the WHHERE complex.

WHHERE complex members act as coactivators of RA signaling. To test the coactivator properties of WHHERE on RA signaling, we transfected NIH3T3 fibroblast cells with expression vectors coding for either Rere, Wdr5, Hdac1, or Hdac2, together with a RA reporter containing the well-characterized retinoic acid response element (RARE) of the known RA target retinoic Acid Receptor beta (Rarbeta), driving luciferase expression. In the presence of RA, overexpression of each of the four WHHERE complex proteins increased RA reporter expression (Fig. 2a). This activation was not observed in the absence of RA (Supplementary Fig. 3a). Also overexpression of the WHHERE complex components did not activate either a minimal or a SV40 promoter driving luciferase supporting the specificity of the RA response (Supplementary Fig. 3b, c). Furthermore, co-transfection of Hdac1 and Hdac2 cooperated to activate RA signaling (Fig. 2b). Conversely, treatment of fibroblast cultures with siRNAs against Rere, Hdac1, or Wdr5 in the presence of RA decreased RARE-Luciferase reporter activity (Fig. 2c and Supplementary Fig. 3d–f). Treatment with Hdac2 siRNA led to an increase of RARE-Luciferase reporter activity (Fig. 2c and Supplementary Fig. 3g), which might be explained by a stabilization of Hdac1 (due to the decrease of Hdac2), potentially resulting in an increase in RA signaling.

**Fig. 1b, Supplementary Figs. 2a and 9). To estimate relative protein levels, we compared the NSAF values for each protein (Supplementary Figs. 2c and 9). Wdr5 binding to Hdac1 and Hdac2 could only be detected when Rere is also co-expressed (Supplementary Figs. 2c and 9), suggesting that Rere acts as a scaffolding component binding Hdac1/Hdac2 and Wdr5.**
signalling.\textsuperscript{18, 19} Consistent with this possibility, the double knockdown of \textit{Hdac1} and \textit{Hdac2} further decreased RA signalling compared to \textit{Hdac1} siRNA alone (Fig. 2d). Furthermore, \textit{Hdac1} or \textit{Hdac1}/\textit{Hdac2} depletion reduced RA activation mediated by \textit{Rere} and \textit{Wdr5} (Fig. 2e, f). Inhibition of deacetylase enzymatic activity with a range of chemical inhibitors decreased RA signalling (Fig. 2g)\textsuperscript{20}. In line with this, overexpression of \textit{Rere} or \textit{Wdr5} did not lead to a significant increase in RA signalling when cells were treated with the HDAC inhibitors Trichostatin A (TSA) or sodium butyrate (SB) (Fig. 2h, i). \textit{Hdac1} and \textit{Hdac2} have been shown to bind Rere N-terminal region\textsuperscript{9, 13, 14}. In the presence of RA, overexpression of the N-terminal region of Rere (N-Rere) strongly increased RA signalling whereas no activation could be seen with Rere C-terminal region (Rere C) (Fig. 2j). The activation by N-Rere was dependent on deacetylase activity since TSA or SB treatment strongly decreased N-Rere-dependent RA signalling (Fig. 2k). Overall, these results show that the WHHERE complex proteins Rere, Wdr5, Hdac1, and Hdac2 act to activate the RA pathway in NIH3T3 cells. Moreover, activation of RA signalling by the WHHERE complex depends on Hdac1 and Hdac2 deacetylase activity.

**WHHERE regulates RA signalling and somite bilateral symmetry.** To analyze WHHERE-dependent RA regulation in vivo, we characterized the phenotype of mouse embryos mutant for the different components of the complex. \textit{Hdac1} mutants exhibit a variety of developmental defects similar to \textit{Rereom/om} embryos\textsuperscript{21}. We introduced the \textit{RARE-LacZ} reporter\textsuperscript{22} in \textit{Hdac1}-null embryos and observed a strong downregulation of LacZ expression, similar to that seen in \textit{Rere} mutants (Fig. 3a–c). \textit{Hdac2} mutants do not show developmental defects and can survive until the perinatal period\textsuperscript{21}. To analyze \textit{Wdr5} function in vivo we first set out to generate a conditional knockout mouse line by introducing \textit{LoxP} sites flanking exons 2 to exon 4 (Supplementary Fig. 4a). No heterozygous embryos were recovered at E8.5 after crossing
**Fig. 2** The WHHERE complex acts as a coactivator for retinoic acid signaling. a-k RARE-Luciferase activity from NIH3T3 cells treated or not with 1 μM RA for 20 h. a Cells transfected with expression plasmids containing Rere, Wdr5, Hdac1, or Hdac2 (n = 4). b Cells transfected with expression plasmids containing Hdac1, Hdac2, or both (n = 4). c Cells treated either with siRNA for Rere, Wdr5, Hdac1, or Hdac2 (n = 4). d Cells treated with siRNA for Hdac1, Hdac2 or both (n = 4). e Cells overexpressing Rere or Wdr5 and treated with siRNA against Hdac1 (n = 4). f Cells overexpressing Rere or Wdr5 and treated with siRNA against both Hdac1 and Hdac2 (n = 4). g Cells treated with the HDAC inhibitors Trichostatin A (TSA) (60 nM), sodium butyrate (SB) (3 mM), apicidin (Api) (300 nM), LAQ824 (LAQ) (60 nM) and Panobinostat (Pano) (30 nM) (n = 4). h Cells overexpressing Rere or Wdr5 and treated with TSA (30 nM) (n = 4). i Cells overexpressing Rere or Wdr5 and treated with SB (1.5 mM) (n = 4). j Cells transfected with expression plasmids containing Rere, N-Rere (Rere N-terminal domain) or Rere C (Rere C-terminal domain) (n = 3). k Cells overexpressing N-Rere (Rere N-terminal domain) and treated with TSA (30 nM) or SB (1.5 mM) (n = 4). l Cells transfected with expression plasmids containing Rere, Wdr5, or both (n = 4). m Cells overexpressing Rere and treated with siRNA for Wdr5 (n = 4). In all graphs data represent mean ± s.e.m. NS—not significant, *P < 0.05 and **P < 0.01. Student’s unpaired two-tailed t-test.

with an ubiquitous Cre suggesting that the Wdr5 mutation is heterozygous lethal at early stages. To circumvent this issue, we crossed mice homozygous for the Wdr5 conditional allele (Wdr5^{B/C}) to the mesoderm-specific T-Cre line^{10}. Removal of a single Wdr5 allele in Wdr5^{B/C};T-Cre;RARE-LacZ embryos was sufficient to strongly decrease β-galactosidase staining in the mesoderm (Fig. 3d). In contrast, no significant downregulation of Notch target genes such as Lng or Hes5 was detected in these mutants suggesting the Notch-dependent somite segmentation clock appears normal (Supplementary Fig. 4b–e). Altogether, these results support the function of the WHHERE complex in the control of RA signaling in the embryo.

We next examined the effect of null mutations of members of the WHHERE complex on somite symmetry. In nearly half of the Hdac1^{−/−} embryos, we observed a right side delay of somite formation resembling the defect observed in Rere^{om/om} and Raldh2^{−/−} mutants^{7, 8} (Fig. 3e–g, i, j and Supplementary Fig. 4f). Similar lateraled somite desynchronization defects were observed in a limited number of Wdr5^{B/C};T-Cre embryos which also exhibited broad somitogenesis defects (Fig. 3h). We next intercrossed Wdr5^{B/C} and Rere;T-Cre mice. Strikingly Rere^{om/om}, Wdr5^{B/C};T-Cre embryos exhibited a stronger phenotype than either Rere^{om/om} or Wdr5^{B/C};T-Cre (Fig. 3i–o) with ~ 80% (7 out of 9) of the embryos with a right side delay in
ChIP experiments performed 1 h after RA treatment of NIH3T3 RARE-LacZ symmetric (Luciferase reporter and of the endogenous Rar gene in NIH3T3 cells treated with RA for 1, 2, and 6 h. At 2 and 6 h, strong transcriptional activation was observed for both RA targets (Fig. 4a, b). In mouse embryos, we observed by ChIP analysis that the retinoic acid receptor alpha (Rarα) and all the WHHERE complex components were present at the Rarβ promoter and at the RARE-containing reporter RARE-LacZ (Fig. 4c–f). In ChIP experiments performed 1 h after RA treatment of NIH3T3 cells, Rere, Wdr5, Hdac1, and Hdac2 but not Rarα increased at the Rarβ promoter (Fig. 4g, h). This effect was specific, as no significant enrichment of WHHERE complex members could be observed in regions upstream of the Rarβ promoter in similar conditions (Fig. 4h, bottom graph). Recruitment of RNA Polymerase II (Pol II) increased following RA treatment paralleling the WHHERE complex recruitment (Fig. 4i). Then we investigated the requirement of Hdac1 deacetylase activity in transcription activation and Pol II recruitment during RA signaling. Decreased RARE-Luciferase and Rarβ expression was observed in TSA-treated cells after RA treatment demonstrating the importance of HDAC activity in early activation of RA target genes (Fig. 4a, b). TSA treatment or siRNA-mediated knockdown of Hdac1 decreased Pol II recruitment at the Rarβ promoter (Fig. 4j, k). In contrast, no change in Pol II occupancy levels could be detected in RA target genes unresponsive to RA in NIH3T3 cells, such as Cyp26a1 and Hoxa1 or in promoters of genes unresponsive to RA signaling following such treatments (Supplementary Fig. 3k–m). In transfected cells expressing Flag-Hdac1 alone or Flag-Hdac1 and Rere-HA, we observed binding of Hdac1 to endogenous Rarβ (Fig. 4j, k). Transfection of Flag-Hdac1 alone or Flag-Hdac1 and Rere-HA, we observed binding of Hdac1 to endogenous Rarβ. This suggests that Hdac1 could bridge the WHHERE complex to Rarα (Fig. 4l–n and Supplementary Fig. 8). Together, these data support a role of the WHHERE complex in the recruitment of Pol II necessary for early activation of RA regulated genes. This role depends on the deacetylation activity of Hdac1 and Hdac2.
Ehmt2 acts together with WHHERE to activate RA signaling. The histone methyltransferase Ehmt2 (G9a) was shown to bind the N-terminal SANT domain of Rere, and together with Hdac1/Hdac2 to regulate the methylation of H3K9 at specific loci leading to the formation of compact heterochromatin and gene silencing\(^1\). In the proteomic experiment, Ehmt2 (and the related protein Ehmt1) were detected with low NSAF values compared to the members of the WHHERE complex suggesting that its binding to Rere might be transient (Supplementary Fig. 5a). In mouse embryos deficient for Ehmt2\(^2\) crossed to the RARE-LacZ reporter\(^2\), LacZ expression is downregulated suggesting that Ehmt2 is also implicated in positive regulation of RA signaling (Fig. 5a, b). Furthermore, half of the Ehmt2 mutant embryos presented a delay in somite formation on the right side.

![Graphs and figures showing the regulation of RARE-luciferase, Rab mRNA, and ChIP experiments involving Rere, Hdac1, Hdac2, and Ehmt2.](image)
resembling mutants of members of the WHHERE complex (Fig. 5c–e; Supplementary Fig. 4f). In NIH3T3 cultures, siRNA-mediated knockdown of Ehmt2 led to a downregulation of the RARE-Luciferase reporter activity (Fig. 5f and Supplementary Fig. 5c), whereas overexpressing Ehmt2 (or Ehmt1) stimulates RA signaling (Fig. 5g and Supplementary Fig. 5b).

Co-transfection of Ehmt2 with Rere increases the RA response more than transfection of either one alone (Fig. 5h). siRNA-mediated knockdown of Ehmt2 inhibited Rere and Hdac1-dependent activation of the RA pathway (Fig. 5i). In NIH3T3 cells, Ehmt2 was recruited at the RARE element of the Rarβ promoter after 1 h of RA treatment while no such enrichment was observed in upstream regions (Fig. 5j and Supplementary Fig. 5c).

In mouse embryos Ehmt2 could also be detected at the RAR element present in both the Rarβ promoter and the RARE-LacZ reporter (Fig. 4a, p). Knockdown of Ehmt2 in NIH3T3 cells reduced the occupancy levels of Rere, Wdr5, Hdac1, Hdac2, and Pol II at the Rarβ promoter (Fig. 5k, l and Supplementary Fig. 5d). In NIH3T3 cells, inhibition of Ehmt2 methyltransferase activity with UNC0638 (U38) or UNC0646 (U46)24, 25 did not alter RARE-Luciferase reporter activity or Rarβ mRNA expression (Supplementary Fig. 5e, f). Also no difference in H3K9me1 nor H3K9me2 levels was observed by ChIP after 1 h of RA treatment suggesting that Ehmt2 function is independent from its methyltransferase activity (Supplementary Fig. 5g, h). This suggests that Ehmt2 could function as a scaffold protein to stabilize the WHHERE complex at RA regulated genes to allow Pol II loading. Thus, these results indicate that Ehmt2 acts together with the WHHERE coactivator complex in the RA-dependent control of somite bilateral symmetry.

**Effect of RA signaling on key histone marks.** Wdr5 is part of complexes such as ATAC, MOF, and MLL, which are involved in histone acetylation and H3K4 methylation, which are chromatin modifications associated with transcriptional activation26. Despite the requirements of HDAC enzymatic activity for WHHERE function, we observed an increase of the levels of acetylated H3 and H4 as well as H3K27ac on the Rarβ promoter upon 1 h of RA treatment of NIH3T3 cells (Fig. 6a–c). This suggests that Hdac1 and Hdac2 might act on non-histone substrates or both could participate to the stability of the WHHERE complex. The H3K36me3 mark, which is associated to transcription elongation, was also increased (Fig. 6d). We also found that in the same conditions, H3K27me3 is absent from the Rarβ promoter (Fig. 6e), whereas H3K4me1 increases while H3K4me2 and H3K4me3 decrease (Fig. 6f–h). MLL3 and MLL4 are complexes which contain Wdr5 and regulate the deposition of the H3K4me1 mark27. The MLL3 and MLL4 complexes have been shown to be involved in RA-dependent transcription and Wdr5 might provide a link with the WHHERE complex28. Whether these complexes are also required for the WHHERE-dependent Pol II recruitment to the promoter of RA targets remains to be investigated.

**Negative regulation of RA signaling by Ep300.** The histone acetyl transferase Ep300 (p300) has been shown to acetylate Hdac1 leading to a decrease of its deacetylase activity29. In the presence of RA, overexpression of Ep300 in NIH3T3 cells decreased RA signaling and inhibited Hdac1-dependent RA activation (Fig. 7a). In line with this, transfection of an Hdac1 mutant form resistant to Ep300 acetylation (H1-6R)30 activated more strongly the RA pathway than wild-type Hdac1 (H1-WT) (Fig. 7b). Moreover, Rara- and Rere-dependent RA activation was inhibited following Ep300 overexpression whereas transfection of the histone acetyl transferase Kat2a (Gen5) together with Rara or Rere increased RA signaling more than transfection of either construct alone (Fig. 7c, d). While treatment of fibroblast cultures with siRNAs against Rere or Kat2a in the presence of RA decreased RA reporter activity, knockdown of Ep300 did not affect the RA pathway (Fig. 7e and Supplementary Fig. 3i, j). Similarly in Ep300 mutant embryos (Ep300+/−)30, the RARE-LacZ reporter25 expression appeared normal (Fig. 7f, g) and somitogenesis progressed symmetrically (Figs. 7h, i). Altogether these results demonstrated that Ep300 negatively regulates Hdac1 activation of RA signaling. Furthermore Kat2a can participate together with the WHHERE complex in the activation of the RA pathway.

**Discussion**

The molecular mechanisms controlling embryonic bilateral symmetry are still poorly understood. In mouse, the only genes shown to act in this process are Raldh2 and Rere which are involved in the control of RA signaling2, 8. Here, we identify a protein complex, called WHHERE which associates key epigenetic regulators such as Wdr5, Hdac1, and Hdac2 to the control of bilateral symmetry downstream of RA signaling. In the background of a situs inversus mutation (a mutation that can reverse left–right identity of the body), Raldh2 and Rere-deficient mouse embryos can show a reversed somite defect, i.e., left-side delay of somitogenesis8, 31. This therefore argues that WHHERE-dependent RA signaling buffers a desynchronizing influence of the left–right determination pathway, involved in asymmetrical development of internal organs. RA signaling was shown to directly antagonize the left identity determinant Fgf8 in the mouse embryo through recruitment of Hdac1 and Prc2, providing a potential explanation for this buffering effect32, 33. Thus, our work identifies a new pathway antagonizing FGF signaling acting downstream of RA to control bilateral symmetry in the mouse embryo.

Strikingly most of the members of the WHHERE complex including Rere, Hdac1, Hdac2, and also Ehmt2 have been
Ehmt2 controls retinoic acid signaling and symmetric somite formation. a, b RARE-LacZ activity in control Ehmt2+/+ a and Ehmt2gt/gt b embryos at E8.75-E9.0 (dorsal views). c, d In situ hybridization for Uncx4.1 in wild-type Ehmt2+/+ c and Ehmt2gt/gt d embryos at E8.75-E9.0 (dorsal views). e Graph representing the number of 7- somite to 15-somite stage Ehmt2gt/gt embryos with left-sided (orange), symmetric (blue), or right-sided (green) delay in somite formation.

Methods

Generation of transgenic mice. The Rere-HA construct was generated by adding two HA tags at the C-terminal extremity of Rere. Rere-HA was cloned downstream of a loxP-STOP-loxP cassette and inserted into the Rosa26 locus by homologous recombination in 129S6/SvEvTac-derived W4 embryonic stem (ES) cells. Animals were maintained on a 50% C57BL/6 genetic background.  

Mice breeding and generation of mutant embryos. To remove the STOP cassette from the RS-Rere-HA mouse line, we crossed this line with ZP3-Cre mice, which express the Cre recombinase specifically in oocytes. Mice in which the STOP cassette was removed were subsequently intercrossed with Rereom/+ mice. The Rere-HA construct rescued the Rereom/+ mutation indicating that the protein Rere-HA is functional. In order to obtain embryos expressing Rere-HA specifically in the mesoderm for proteomic experiments, we generated a homozygous line for RS-Rere-HA and crossed it with homozygous Rereom/om mice. This homozygous line is functional (Supplementary Fig. S1a).

Ehmt2 generally associated to transcriptional repression. However, Rere was shown to act as a coactivator for RA signaling and Hdac1/Hdac2 are required for transcriptional activation of the MMTV promoter downstream of the glucocorticoid receptor. Ehmt2 has also been shown to participate in the positive regulation of RA signaling by co-regulators, which may act as coactivators or corepressors depending on the developmental context and genes involved. The positive role of these proteins in signaling mediated by other nuclear receptors suggests that the WHHERE complex could also have a broader role as a coactivator downstream of nuclear receptor signaling. The positive regulation of RA signaling by acetylation of the retinoic acid receptors decreases their activity. The acetyltransferase Ep300 can reduce Rere-dependent RA signaling. We further show that the acetyl transferase Ep300 can reduce Rere-dependent RA signaling.
The mouse mutant for NATURE COMMUNICATIONS were obtained by mating heterozygous males and females for the mutation. The embryos were maintained on at least 50% C57BL/6 genetic background. Embryos T-Cre animals10. The mouse line with T-Cre mice. The embryos T-Cre animals10. The embryos were obtained in an N-ethyl-N-nitrosourea (ENU) screen9 and the line was maintained on a C57BL/6 genetic background. Heterozygous males and females have one mutant allele in the mesodermal tissues. To generate the mice from Nakatani lab) were used to assay RA signaling in NIH3T3 cells. Ep300 and H3K4me3 were crossed to: \( \text{Ep300}^{\text{fl}} \) and H3K4me3 \( \text{Hdac1}^{\text{fl}} \) were crossed to: \( \text{Hdac1}^{\text{fl}} \) and H3K4me3

Fig. 6 Effect of RA treatment on different histone acetylation and methylation marks. a-h ChIP analysis of the Rarβ promoter from NIH3T3 cells treated with 1µM RA during 1 h. ChIP was performed with specific antibodies to H3ac, H4ac, H3K27ac, and H3K4me3. In all graphs data represent mean ± s.e.m. NS—not significant, *P < 0.05 and **P < 0.01. Student’s unpaired two-tailed t-test was performed according to the protocol described in ref.43. Brieﬂy, formaldehyde-fixed embryos were treated with protease and reﬂuxed with 4% formaldehyde/0.1% glutaraldehyde. Hybridization with DIG-labeled RNA probes was performed under stringent conditions (1.3X SSC, 50% formamide at 65 °C, pH 5) in a buffer containing 0.2% Tween-20 and 0.5% CHAPS. Washed embryos were treated with Boehringer blocking reagent and incubated overnight in alkaline phosphatase-coupled anti-DIG antibody. After extensive washes, embryos were stained from 30 min to 16 h. The probes used are described in the literature: Uncox15, Lfng5, and Hes78.

Whole-mount in situ hybridization. Whole-mount in situ hybridizations were performed according to the protocol described in ref. 43. Brieﬂy, formaldehyde-fixed embryos were treated with protease and reﬂuxed with 4% formaldehyde/0.1% glutaraldehyde. Hybridization with DIG-labeled RNA probes was performed under stringent conditions (1.3X SSC, 50% formamide at 65 °C, pH 5) in a buffer containing 0.2% Tween-20 and 0.5% CHAPS. Washed embryos were treated with Boehringer blocking reagent and incubated overnight in alkaline phosphatase-coupled anti-DIG antibody. After extensive washes, embryos were stained from 30 min to 16 h. The probes used are described in the literature: Uncox15, Lfng5, and Hes78.

Vector construction and baculovirus generation. To assay RA signaling in NIH3T3 cells, we used the RA reporter RARE-Luciferase and mouse Rere-HA subcloned into pMyc-CMV (Sigma) described in ref. 3. The mouse full-length Wdr5, Hdac1, Hdac2, and Rere cDNAs were subcloned into pFlag-CMV (Sigma). Human Flag-Ehmt2 in PCN polymid and Flag-Ehmt2 in pCDNA3 plasmid (both from Nakatani lab) were used to assay RA signaling in NIH3T3 cells. Rere C-terminal region (a.a. 1–571) (N-Rere) and Rere C-terminal region (a.a. 572–1558) (Rere C) were subcloned into pMyc-CMV (Sigma) and pFlag-CMV (Sigma), respectively. Human Hdac1 wild-type (H1-WT) and Hdac1 mutant (H1-6R) in pEGFP-N1 (Clontech) was a gift from Gordon Hager. Human Ep300 in pCMVb was a gift from Richard Eckner and mouse Kat2a (Gen5) in pCMV-sport247 (Invitrogen) (Addgene plasmid 23098) was a gift from Sharon Dent.

For the generation of baculoviruses expressing Flag-Hdac1 and Flag-Hdac2, the full-length cDNA encoding mouse Hdac1 and Hdac2 fused with a N-terminal Flag tag, were cloned into the pVL1393 entry vector at BpmI and XbaI sites. For the generation of baculoviruses expressing Rere-Flag, the full-length cdNA encoding mouse Rere, was cloned into the pVL1392 entry vector at BpiI and KpnI sites. The viruses were then generated from these entry vectors following the transfecting genes protocol (Method I) described in Summers and Smith (1987) “A manual of methods for bacalo vectors and insect cell culture procedures”. The viruses expressing HA-Wdr5 was provided by Dr. Laslo Tora (IGBMC).

Cell culture, RA reporter assay and siRNA experiments. NIH3T3 cells were cultured in DMEM (Invitrogen) containing 4.5 g/l D-glucose, L-glutamine, pyruvate, and 10% newborn calf serum. For RA reporter assay, when cells reached 50% confluence in 24-well plates, they were co-transfected with RARE-pG4.L4.23 (200 ng), pGL3-Promoter (200 ng), PRL-CMV (5 ng) (Promega), TA-Luc (200 ng) (Panomics) and the different expression constructs containing Rere, Wdr5, Hdac1, Hdac2, Ehmt2, N-Rere (N-Rere N-terminal region), Rere C (C-terminal region), Hdac1 wild-type (H1-WT), Hdac1 mutant (H1-6R), Ep300, Rara and Kit2a using Fugene 6 (Roche). For single transfection, we used 200 ng of each construct whereas in co-transfection experiments 130 ng of each plasmid was used. One micro molar RA (Sigma) was added after 24 h of transfection, and 20 h later, cells were collected and lysed. Renilla luciferase and firefly luciferase activity were measured using a dual luciferase assay kit (Promega) and a microplate luminometer (Berthold Centro XS3 LB 960). Five different histone deacetylase inhibitors were used: Trichostatin A (30–100 nM), Sodium butyrate (1.5–3 mM), Apicidin (300 nM) (all from Sigma) and LAQ824 (60 nM), Panobinostat (30 nM) (both from Selleckchem). UNC0638 and UNC0646 (both from Tocris) were used to inhibit Ehmt2 methyltransferase activity.

For siRNA experiments, NIH3T3 cells were transfected with ON-TARGETplus Smartpool mouse siRNA (Dharmacon) or ON-TARGETplus non-targeting control siRNA pool (Dharmacon) using DharmaFECT 1 or DharmaFECT Duo (for luciferase experiments) reagents (Dharmacon) and following the manufacturer’s instructions. The ON-TARGETplus Smartpool mouse siRNA were used against the
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Kat2a but not Ep300 acts as a coactivator for retinoic acid signaling. a–e RARE-Luciferase activity from NIH3T3 cells treated or not with 1 µM RA for 20 h. a Cells transfected with expression plasmids containing Ep300, Hdac1, or both (n = 4), b Cells transfected with expression plasmids containing human Hdac1 (H1-WT) or Hdac1 mutant (H1-6R) (n = 4). c Cells transfected with expression plasmids containing Rara, Rere, and Ep300 or Rara and Kat2a (n = 3). d Cells transfected with expression plasmids containing Rere, Rere and Ep300 or Rara and Kat2a (n = 3). e Cells treated either with siRNA for Rara, Ep300, or Kat2a (n = 4). In all graphs data represent mean ± s.e.m. NS—not significant, *P < 0.05 and **P < 0.01. Student’s unpaired two-tailed t-test. f, g RARE-LacZ expression in wild-type Ep300+/+/f and Ep300+/−g embryos at E8.75–E9.0 (dorsal views). h, i In situ hybridization showing somites labeled with Uncx4.1 in wild-type Ep300+/−h and Ep300+/−i embryos at E8.75–E9.0 (dorsal views). For each genotype at least 5–10 embryos were analyzed. Bar = 100 microns.

Co-immunoprecipitation experiments and western blots. NIH3T3 cells were cultured in 10 cm cell culture plates overnight to 70–80% confluence and then transfected with pFlag-Hdac1 alone or together with pMyc-Rere-HA vectors using Fugene 6 (Roche). NIH3T3 cells were cultured in T-25 flasks overnight to 70–80% confluence and then transfected with the expression vectors using Fugene 6 (Roche). Cells were collected 24 h post-transfection and lysed with a hypotonic gentle lysis buffer (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 10 mM EDTA, 0.5% Triton X-100 and Complete protease inhibitors (Roche)). After the lysis, the NaCl concentration was raised to 150 mM. Soluble extracts, together with either mouse IgG (Upstate), M2 anti-FLAG (Sigma) or anti-HA (Sigma), were incubated with Dynabeads M-280 sheep anti-Mouse IgG (Invitrogen) overnight at 4°C. Beads were washed 10 times with 50 mM Tris-HCl pH 7.5, 150 mM NaCl and 0.05% Triton-X100, and then bound protein was eluted using Laemmli sample buffer (Sigma) and heated at 100°C for 5 min. Elutions were resolved on 7.5% polyacrylamide Tris-HCl gels (BioRad) and transferred to polyvinylidene difluoride membrane (Millipore). Blots were treated according to a standard western blot protocol.

The following reagents and antibodies were used: mouse IgG (Upstate), M2 anti-Flag (Sigma), anti-HA (Sigma), Dynabeads M-280 sheep anti-mouse IgG (Invitrogen), Flag-coupled HRP (Sigma), HA-coupled HRP (Roche) and Rar-C-20 (Santa Cruz).

Chromatin immunoprecipitation assay. NIH3T3 cells were grown in 15 cm dishes and treated with 1 µM of RA (Sigma) for 1 h before collecting. At 70–80% confluence, cells were crosslinked with 1% formaldehyde at room temperature for 10 min. For ChIP experiments with NIH3T3 cells, 50 µg of chromatin were used per immunoprecipitation. F9 or NIH3T3 cells were grown in 15 cm dishes and
Table 1 Antibodies used for ChIP

| Antibody | Catalog number (Company) | Concentration/ChIP |
|----------|--------------------------|-------------------|
| Rere H-113 | sc-98415 (Santa Cruz) | 10 µg |
| Wdr5 | 07-706 (Millipore) | - 5 µg (1:200) |
| Hdac1 C-19 | sc-6298 (Santa Cruz) | 10 µg |
| Hdac2 H-54 | sc-7899 (Santa Cruz) | 10 µg |
| Ranr C-20 | sc-551 (Santa Cruz) | 10 µg |
| Rpb1 (total Pol II) | PB-7C2 (Euromedx) | - 3:1 (1:200) |
| Ehmt2 | 07-551 (Millipore) | 10 µg |
| H3K9me1 | ab9045 (Abcam) | 2 µg |
| H3K12me2 | ab7292 (Abcam) | 2 µg |
| H3K4me2 | ab8895 (Abcam) | 2 µg |
| H3K4me3 | ab32356 (Abcam) | 2 µg |
| H3K4me4 | ab8580 (Abcam) | 2 µg |
| H3K27ac | ab4729 (Abcam) | 2 µg |
| H3ac | 06-599 (Millipore) | 2 µg |
| H3ac | 06-598 (Millipore) | 2 µg |
| H3K36me3 | ab9050 (Abcam) | 2 µg |
| H3K27me3 | ab6602 (Abcam) | 2 µg |

Proteomic approach to identify Rere-associated proteins. To identify proteins binding to Rere, we first generated a mouse colony homozygous for both the T-Cre transgene and the RS-Rere-HA allele. Thus, all the embryos generated by crossing these mice express Rere-HA only in mesodermal tissues. We collected about 600 E10.5 embryos and prepared whole cell extracts using either a low salt (350 mM NaCl) or a high salt (420 mM NaCl) extraction buffer (Supplementary Note 1). For the high salt condition, affinity purification was done at 150 mM NaCl; for the high salt protocol, it was performed at 300 mM NaCl. Each affinity purification was performed on biological replicates of protein extracts from about 50–70 embryos prepared on different days. Two different anti-HA antibodies from Sigma and Roche were used for the affinity purification. For each of the conditions, six different experiments were performed with each antibody and analyzed independently for a total of 12 MudPIT analyses.

Multidimensional protein identification technology. TCA-precipitated protein samples from mouse HA immunoprecipitations were solubilized in either 30 µl or 60 µl (for pooled elutions only) of freshly made 0.1 M Tris-HCl pH 8.5, 8 M urea, 5 mM TCEP (Tris(2-Carboxyethyl)-Phosphine Hydrochloride, Pierce). After 30 min at room temperature, freshly made 0.5 M IAM (Iodoacetamide, Sigma) was added to a final concentration of 10 mM, and the samples were left at room temperature for an additional 30 min. During the course of the experiment, the pH was corrected to 8.0 with 38 mM NaOH. Antibodies (5–10 µg per immunoprecipitation) were pre-bound to a protein A sepharose slurry (0.15 g lyophilized protein A sepharose beads (Sigma) were resuspended in 1.3 mL 1X TE with 300 µg sonicated salmon sperm DNA and 1.5 µg BSA). Forty microliters of the resulting slurry was used per immunoprecipitation. Chromatin was pre-cleared using this slurry without antibody and pre-cleared chromatin was then added to the antibody-bead complexes overnight. Chromatin from ~3 × 106 NIH3T3 or F9 cells was used per immunoprecipitation. Complexes were washed the next morning for 10 min each with Buffer A: 0.1% SDS, 1% Triton-X100, 1 mM EDTA, 20 mM Tris-HCl pH 8.0, 50 mM NaCl; Buffer B (same as Buffer A except contained 500 mM NaCl); Buffer C: 0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, 20 mM Tris-HCl pH 8.0, 8.35 mM NaCl. Antibodies (5–10 µg per immunoprecipitation) were pre-bound to a protein A sepharose slurry (0.15 g lyophilized protein A sepharose beads (Sigma) were resuspended in 1.3 mL 1X TE with 300 µg sonicated salmon sperm DNA and 1.5 µg BSA). Forty microliters of the resulting slurry was used per immunoprecipitation. Chromatin was pre-cleared using this slurry without antibody and pre-cleared chromatin was then added to the antibody-bead complexes overnight. Chromatin from ~3 × 106 NIH3T3 or F9 cells was used per immunoprecipitation. Complexes were washed the next morning for 10 min each with Buffer A: 0.1% SDS, 1% Triton-X100, 1 mM EDTA, 20 mM Tris-HCl pH 8.0, 50 mM NaCl; Buffer B (same as Buffer A except contained 500 mM NaCl); Buffer C: 0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, 20 mM Tris-HCl pH 8.0, followed by two washes in 1X TE). Beads were then eluted at room temperature with 1% SDS and 0.1 M NaHCO3, crosslinks were reversed, proteinase K digestion was performed and DNA was isolated. PCR was performed with the Fast SYBR Green Master Mix (Applied Biosystems) using a 7500 Fast Real-Time PCR System (Applied Biosystems). PCR conditions were as follows: 95 °C (20 s) (40×). Primer pairs used for the RARE-LacZ reporter were: 5′-TGGTGCAGCAGGGAA-3′ and 5′-CCACAACTCCTCATATGAA-3′. Antibodies used for ChIP are listed in Table 1.

qPCR was performed with LightCycler 480 SYBR Green I master mix (Roche) in a LightCycler 480 II System (Roche) using the primers described in Supplementary Table 1.

Results from different runs were compared and merged using CONTRAST52. Three different elution buffers were used: 5% and 10% formic acid (Buffer C). The last two chromatography steps were combined in a high salt wash with 100% Buffer C followed by the acetonitrile gradient. The application of a 2.5 kV distal voltage electrospayed the eluting peptides directly into the mass spectrometer12. Three different elution buffers were used: 5% acetonitrile, 0.1% formic acid (Buffer B); and 0.5 M ammonium acetate, 5% acetonitrile, 0.1% formic acid (Buffer C). The last two chromatography steps were combined in a high salt wash with 100% Buffer C followed by the acetonitrile gradient. The application of a 2.5 kV distal voltage electrospayed the eluting peptides directly into the mass spectrometer12. Three different elution buffers were used: 5% acetonitrile, 0.1% formic acid (Buffer B); and 0.5 M ammonium acetate, 5% acetonitrile, 0.1% formic acid (Buffer C). The last two chromatography steps were combined in a high salt wash with 100% Buffer C followed by the acetonitrile gradient. The application of a 2.5 kV distal voltage electrospayed the eluting peptides directly into the mass spectrometer12. Three different elution buffers were used: 5% acetonitrile, 0.1% formic acid (Buffer B); and 0.5 M ammonium acetate, 5% acetonitrile, 0.1% formic acid (Buffer C). The last two chromatography steps were combined in a high salt wash with 100% Buffer C followed by the acetonitrile gradient. The application of a 2.5 kV distal voltage electrospayed the eluting peptides directly into the mass spectrometer12. Three different elution buffers were used: 5% acetonitrile, 0.1% formic acid (Buffer B); and 0.5 M ammonium acetate, 5% acetonitrile, 0.1% formic acid (Buffer C). The last two chromatography steps were combined in a high salt wash with 100% Buffer C followed by the acetonitrile gradient. The application of a 2.5 kV distal voltage electrospayed the eluting peptides directly into the mass spectrometer12. Three different elution buffers were used: 5% acetonitrile, 0.1% formic acid (Buffer B); and 0.5 M ammonium acetate, 5% acetonitrile, 0.1% formic acid (Buffer C). The last two chromatography steps were combined in a high salt wash with 100% Buffer C followed by the acetonitrile gradient. The application of a 2.5 kV distal voltage electrospayed the eluting peptides directly into the mass spectrometer12.
Spectral Counting system (GE Healthcare) using a buffer containing 25 mM Tris-HCl pH 8.0, Superose 6 PC 3.2/30 (GE Healthcare) mounted on an AKTA micro chromatography system and SEC fractions of 75 µl each were collected throughout the gel filtration run and ed sample was used for western blotting to check for the presence of Rere, Hdad1, Hdad2, and Wdr5. The following antibodies were used for western blot: Rere (Santa Cruz, sc-98415), Hdad1 (Abcam, ab7028), Hdad2 (Abcam, ab7029), and Wdr5 (Abcam, ab22512).

Statistics. Statistics were performed using Excel software (Microsoft). Significance of difference was analyzed with a Student’s unpaired two-tailed t-test. All data are presented as mean ± s.e.m. and a P-value of 0.05 was considered significant. The results were presented as the average of at least three independent experiments unless otherwise stated in the legends.

Data availability. The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files or from the corresponding author upon reasonable request. The mass spectrometry data set has been deposited in the MassIVE/ProteomesXchange under accession codes MSV000089020 and PXD006303.

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References
1. Blum, M., Feistel, K., Thumberger, T. & Schwickert, A. The evolution and conservation of left-right patterning mechanisms. Development 141, 1603–1613 (2014).
2. Vilhas-Neto, G. C. & Pourque, O. Retinoic acid. Curr. Biol. 18, R191–R192 (2008).
3. Rhinn, M. & Dolle, P. Retinoic acid signalling during development. Development 139, 843–858 (2012).
4. Cunningham, T. J. & Duewel, G. Mechanisms of retinoic acid signalling and its roles in organ and limb development. Nat. Rev. Mol. Cell Biol. 16, 110–123 (2015).
5. Xu, L., Glass, C. K. & Rosenfeld, M. G. Coactivator and corepressor complexes in nuclear receptor function. Curr. Opin. Genet. Dev. 9, 140–147 (1999).
6. Rosenfeld, M. G., Lunyak, V. V. & Glass, C. K. Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev. 20, 1405–1428 (2006).
7. Vermot, J. et al. Retinoic acid controls the bilateral symmetry of somite formation in the mouse embryo. Science 308, 563–566 (2005).
8. Vilhas-Neto, G. C. et al. Rere controls retinoic acid signalling and somite bilateral symmetry. Nature 463, 953–957 (2010).
9. Zollewicz, J. S., Stewart, N. J., Leung, R. & Peterson, A. S. Atrophin 2 recruits histone deacetylase and is required for the function of multiple signaling centers during mouse embryogenesis. Development 131, 3–14 (2004).
10. Peranoni, A. O. et al. Inactivation of FGFR8 in early mesoderm reveals an essential role in kidney development. Development 132, 3859–3871 (2005).
11. Washburn, M. P., Wolters, D. & Yates, J. R. 3rd Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat. Biotechnol. 19, 242–247 (2001).
12. Florens, L. & Washburn, M. P. Proteomic analysis by multidimensional protein identification technology. Methods Mol. Biol. 328, 159–175 (2006).
13. Wang, L., Rajan, H., Pitman, J. L., McKeon, M. & Tsai, C. C. Histone deacetylase-associated Atrophin proteins are nuclear receptor corepressors. Genes Dev. 20, 525–530 (2006).
14. Wang, L., Charroux, B., Kerridge, S. & Tsai, C. C. Atrophin recruits HDAC1/2 and G9a to modify histone H3K9 and to determine cell fates. EMBO Rep. 9, 555–562 (2008).
15. Plaster, N., Sonntag, C., Schilling, T. F. & Hammerschmidt, M. REREa/Atrophin-2 interacts with histone deacetylase and Fg8 signaling to regulate multiple processes of zebrafish development. Dev. Dyn. 236, 1819–1827 (2007).
16. Hein, M. Y. et al. A human interactome in three quantitative dimensions organized by subellular compartments and abundances. Cell 163, 712–723 (2015).
17. Shen, S., Kruyt, F. A., den Hertog, J., van der Saag, P. T. & Kruijer, W. Mouse and human retinoic acid receptor beta 2 promoters: sequence comparison and localization of retinoic acid responsiveness. DNA Seq. 2, 111–119 (1991).
18. Luo, Y. et al. Trans-regulation of histone deacetylase activities through acetylation. J. Biol. Chem. 284, 34901–34910 (2009).
19. Wilting, R. H. et al. Overlapping functions of Hdac1 and Hdac2 in cell cycle regulation and haematopoiesis. EMBO J. 29, 2586–2597 (2010).
20. Bantscheff, M. et al. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nat. Biotechnol. 29, 255–265 (2011).
21. Monteilhery, R. L. et al. Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. Genes Dev. 21, 1790–1802 (2007).
22. Rossant, J., Zirnbigl, R., Cado, D., Shago, M. & Giguere, V. Expression of a retinoic acid response element-hspacZ transgene defines specific domains of transcriptional activity during mouse embryogenesis. Genes Dev. 5, 1333–1344 (1991).
23. Weinshilboum, R. A. G9a histone methyltransferase contributes to imprinting in the mouse placenta. Mol. Cell Biol. 28, 1104–1113 (2008).
24. Vedadi, M. et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nat. Chem. Biol. 7, 566–574 (2011).
25. Liu, F. et al. Optimization of cellular activity of G9a inhibitors 7-aminoalkoxyquinazolines. J. Med. Chem. 54, 6139–6150 (2011).
26. Grieve, N. C. & Shilatifard, A. WDR5, a complexed protein. Nat. Struct. Mol. Biol. 16, 678–680 (2009).
27. Hu, D. et al. The ML133/ML14 branches of the COMPASS family function as major histone H3K4 monomethyltransferases at enhancers. Mol. Cell Biol. 33, 4745–4754 (2013).
28. Lee, S. et al. Coactivator as a target gene specificity determinant for histone H3 lysine 4 monomethyltransferase. Nat. Cell Biol. 10, 1539–15397 (2006).
29. Qiu, Y. et al. HDAC1 acetylation is linked to progressive modulation of steroid receptor-induced gene transcription. Mol. Cell 22, 669–679 (2006).
30. Yao, T. P. et al. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Mol. Cell 51, 361–372 (1998).
31. Vermot, J. & Pourque, O. Retinoic acid coordinates somitogenesis and left-right patterning in vertebrate embryos. Nature 435, 215–220 (2005).
32. Meyers, E. N. & Martin, G. R. Differences in left-right axis pathways in mouse and chick: functions of FGFR8 and SHH. Science 285, 403–406 (1999).
33. Kumar, S. & Duester, G. Retinoic acid controls body axis extension by directly repressing Fgf8 transcription. Development 141, 2972–2977 (2014).

34. Moser, M. A., Hagelkuys, A. & Seiser, C. Transcription and beyond: the role of mammalian class I lysine deacetylases. Chromosoma 123, 67–78 (2014).

35. Wang, L. & Tsai, C. A. Atrophin proteins: an overview of a new class of nuclear receptor corepressors. Nucl. Recept. Signal. 6, e009 (2008).

36. Shankar, S. R. et al. G9a, a multipotent regulator of gene expression. Epigenetics 8, 16–22 (2012).

37. Lee, D. Y., Nortrup, J. P., Kuo, M. H. & Stalcup, M. R. Histone H3 lysine 9 methyltransferase G9a is a transcriptional coactivator for nuclear receptors. J. Biol. Chem. 281, 8476–8485 (2006).

38. Bittencourt, D. et al. G9a functions as a molecular scaffold for assembly of transcriptional coactivators on a subset of glucocorticoid receptor target genes. Proc. Natl Acad. Sci. USA 109, 19673–19678 (2012).

39. Tang, X. H. & Gudas, L. J. Retinoids, retinoic acid receptors, and cancer. Annu. Rev. Pathol. 6, 345–364 (2010).

40. Srinivas, S. et al. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev. Biol. 1, 4 (2001).

41. Birling, M. C., Dierich, A., Jacquot, S., Herault, Y. & Pavlovic, G. Highly efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background. Genesis 50, 482–489 (2011).

42. Kemler, R. et al. Stabilization of beta-catenin in the mouse zygote leads to premature epithelial-mesenchymal transition in the epiblast. Development 131, 5817–5824 (2004).

43. Henrique, D. et al. Expression of a Delta homologue in prospective neurons in the chick. Nature 375, 787–790 (1995).

44. Mansouri, A. et al. Paired-related murine homeobox gene expressed in the segmentation. Genes Dev. 19, 207–210 (2005).

45. Aulehla, A. & Johnson, R. L. Dynamic expression of lunatic fringe suggests a link between notch signaling and an autonomous cellular oscillator driving somite segmentation. Dev. Biol. 207, 49–61 (1999).

46. Besho, Y. et al. Dynamic expression and essential functions of Hes7 in somite segmentation. Genes Dev. 15, 2642–2647 (2001).

47. Martinez-Balbas, M. A., Bauer, U. M., Nielsen, S. I., Brehm, A. & Kouzarides, T. Regulation of E2F1 activity by acetylation. EMBO J. 19, 662–671 (2000).

48. Wolters, D. A., Washburn, M. P. & Yates, J. R. 3rd An automated multidimensional protein identification technology for shotgun proteomics. Anal. Chem. 73, 5683–5690 (2001).

49. Ohl, M. D. et al. Proteomics analysis reveals stable multiprotein complexes in both fission and budding yeasts containing Myb-related Cdc5p/CeF1p, novel pre-mRNA splicing factors, and snRNAs. Mol. Cell Biol. 22, 2011–2024 (2002).

50. McDonald, W. H. et al. MS1, MS2, and SQT-three unified, compact, and easily parsed file formats for the storage of shotgun proteomic spectra and identifications. Rapid Commun. Mass Spectrom. 18, 2162–2168 (2004).

51. Eng, J. K., McCormack, A. L. & Yates, J. R. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. J. Am. Soc. Mass Spectrom. 5, 976–989 (1994).

52. Tabb, D. L., McDonald, W. H. & Yates, J. R. 3rd DTASelect and Contrast: tools for assembling and comparing protein identifications from shotgun proteomics. J. Proteome. Res. 1, 21–26 (2002).

53. Pauletti, A. C. et al. Quantitative proteomic analysis of distinct mammalian Mediator complexes using normalized spectral abundance factors. Proc. Natl Acad. Sci. USA 103, 18928–18933 (2006).

54. Zybailov, B. et al. Statistical analysis of membrane proteome expression changes in Saccharomyces cerevisiae. J. Proteome. Res. 5, 2339–2347 (2006).

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Author contributions
G.C.V.-N. designed, performed and analyzed the experiments with O.P. G.C.V.-N., M.F. and I.M.G. performed the molecular biology, biochemistry, and gel filtration experiments. G.C.V.-N., J.-L.P. and M.M. did the qPCR and qChIP experiments. G.C.V.-N., M.E.S., A.S., L.F. and M.P.W. performed and analyzed the proteomic experiment. G.C.V.-N. did the mouse analysis. G.C.V.-N. and O.P. wrote the manuscript and supervised the project. All authors discussed and agreed on the results and commented on the manuscript.

Additional information
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