MLL4-associated condensates counterbalance Polycomb-mediated nuclear mechanical stress in Kabuki syndrome

Alessandra Fasciani1,2,11, Sarah D’Annunzio1,11, Vittoria Poli1,11, Luca Fagnocchi1, Sven Beyes1, Daniela Michelatti1, Francesco Corazza1, Laura Antonelli3, Francesco Gregoretti3, Gennaro Oliva3, Romina Belli1, Daniele Peroni1, Enrico Domenici1,4, Samuel Zambrano5,6, Daniela Intartaglia7, Carmine Settembre7,8, Ivan Conte7,9, Claudia Testi10, Panagiotis Vergyris10, Giancarlo Ruocco10 and Alessio Zippo1,2

The genetic elements required to tune gene expression are partitioned in active and repressive nuclear condensates. Chromatin compartments include transcriptional clusters whose dynamic establishment and functioning depend on multivalent interactions occurring among transcription factors, cofactors and basal transcriptional machinery. However, how chromatin players contribute to the assembly of transcriptional condensates is poorly understood. By interrogating the effect of KMT2D (also known as MLL4) haploinsufficiency in Kabuki syndrome, we found that mixed lineage leukemia 4 (MLL4) contributes to the assembly of transcriptional condensates through liquid–liquid phase separation. MLL4 loss of function impaired Polycomb-dependent chromatin compartmentalization, altering the nuclear architecture. By releasing the nuclear mechanical stress through inhibition of the mechanosensor ATR, we re-established the mechano-signaling of mesenchymal stem cells and their commitment towards chondrocytes both in vitro and in vivo. This study supports the notion that, in Kabuki syndrome, the haploinsufficiency of MLL4 causes an altered functional partitioning of chromatin, which determines the architecture and mechanical properties of the nucleus.

Cellular responses to both mechanical and biochemical stimuli are influenced by the chromatin context, which modulates the accessibility to cis-regulatory elements such as promoters and enhancers12. Defined chromatin patterns, including monomethylated histone H3 lysine 4 (H3K4me1) enrichment and acetylated H3K27 (H3K27ac), mark active enhancers13-15. Besides chromatin modifications, the interaction between transcription factors and genome spatial organization dictate the spatiotemporal control of gene expression16,17.

Recent findings suggest that gene regulation occurs in transcriptional condensates, in which transcription factors, cofactors and transcriptional machinery dynamically cluster at multiple cis-regulatory elements18-21. It has been proposed that liquid–liquid phase separation (LLPS) guides the formation of these membraneless compartments12-14. Multiple weak interactions are promoted by intrinsically disordered regions (IDRs), which favor the assembly of biomolecular condensates15. Among IDRs, prion-like domains (PrLDs) are of particular relevance, as they are characterized by stretches of polar amino acids16,17 and are enriched among transcription factors and chromatin players18. Nevertheless, the contribution of the epigenetic landscape and chromatin-associated factors to transcriptional condensate assembly has not been fully elucidated.

Among the chromatin players, the multi-protein mixed lineage leukemia 4 (MLL4)–complex of proteins associated with Set 1 (COMPASS) complex is involved in depositing H3K4me1 and modulating H3K27ac19-20. MLL4 belongs to the Trithorax (TrxG) group of proteins that functionally antagonize Polycomb (PcG) proteins for the maintenance of active or repressive gene expression states21-22. Recent findings have shown that, apart from the Su(var)3-9, Enhancer of zeste and Trithorax (SET) domain, other MLL4 modules could be relevant for its activity in regulating the chromatin state and gene expression23-25. This possibility is supported by genetic evidence showing that haploinsufficiency of KMT2D (which encodes MLL4) represents, in most cases, the underlying cause of Kabuki syndrome—a rare multi-systemic disorder characterized by craniofacial anomalies, postnatal growth retardation, intellectual disability and various organ malformations26. The retrieved KMT2D mutations in patients with Kabuki syndrome are heterogeneous and widespread along the gene27,28, suggesting diversity in the functional consequences of altering the MLL4 protein and its contribution to chromatin regulation.

Besides biochemical signals, stem cells sense and respond to the mechanical forces required to properly direct their fate29,30. Tissues and cells respond to external mechanical cues by modulating...
Fig. 1 | KMT2D loss of function destabilizes MLL4–COMPASS complex activity. a, Left: western blot analysis of MLL4–COMPASS complex in WT and MLL4Q4092X MSCs using the indicated antibodies. Lamin B1 was used as the loading control. Serial dilutions of protein extracts were loaded. Right: signal quantifications for MLL4 and UTX are reported as bar plots. The data are presented as means ± s.e.m. from four independent experiments. NS, not significant. b, c, Representative images (left) and relative quantifications (right) of immunostaining for MLL4 (b) and UTX (c) in WT and MLL4Q4092X MSCs. Scale bars, 25 µm. d, Relative abundance of H3K4 modifications in WT and MLL4Q4092X MSCs by mass spectrometry analysis. The large pies represent the average abundance of H3K4me1 and unmodified H3K4 with respect to the total histone H3. The small pies represent the average abundance of H3K4me2 and H3K4me3 with respect to the total histone H3. The relative quantifications were determined using four biologically independent samples. e, Abundance of H3K27ac, relative to H327–40, in WT and MLL4Q4092X MSCs, as measured by mass spectrometry analyses. The data are presented as means ± s.e.m. (n = 4 biologically independent samples). f, g, Representative images (left) and relative quantifications (right) of immunostaining for H3K4me1 (f) and H3K27ac (g) in WT and MLL4Q4092X MSCs. Scale bars, 20 µm. In b, c, f and g, the numbers of analyzed nuclei (n) are reported in the figure. The box plots in b, c, f and g indicate median values (middle lines), first and third quartiles (box edges) and 10th and 90th percentiles (error bars) of the fluorescence intensity of analyzed nuclei (n). Statistical significance was determined by one-tailed Student's t-test (a) or unpaired two-tailed Student's t-test (b, c and e–g) (**P < 0.001).
Fig. 2 | Effects of MLL4 LoF on the clustering of transcriptional biomolecular condensates. 

**a, b.** Representative images (left) and relative quantifications (right) of cluster intensities for BRD4 (**a**) and MED1 (**b**) immunostaining in WT and MLL4\textsuperscript{Q4092X} MSCs. Scale bars, 10 µm. The numbers of analyzed nuclei (n) are reported in the figure. **c.** Left: representative STORM images of BRD4 in WT (top) and MLL4\textsuperscript{Q4092X} MSCs (bottom), representative of three independent experiments. Scale bars, 5 µm. Right: magnified images highlighting the clustered distribution of the signal. Scale bars, 1 µm. **d.** Quantification of the number of BRD4 clusters, measured in individual nuclei of WT and MLL4\textsuperscript{Q4092X} MSCs (n = 25 nuclei analyzed per sample over three independent experiments). **e.** Distribution of the area of the BRD4 clusters detected in WT and MLL4\textsuperscript{Q4092X} MSCs. **f.** Representative images of five independent experiments of blue-light-induced clustering of MED1–IDR at the indicated time points, in WT and MLL4\textsuperscript{Q4092X} MSCs. Scale bar, 5 µm. **g.** Quantification of the number of MED1–IDR clusters, measured in individual nuclei of WT (n = 32) and MLL4\textsuperscript{Q4092X} (n = 25) MSCs at the indicated time points. **h.** Distribution of the normalized area of MED1–IDR clusters, measured in individual nuclei of WT and MLL4\textsuperscript{Q4092X} MSCs. The area of each cluster at each time point was normalized versus the corresponding area measured at the first time point post-stimulation (t\textsubscript{0}). The dashed lines represent the corresponding time life of MED1–IDR. In **a, b, d, g** and **h**, box plots indicate median values (middle lines), first and third quartiles (box edges) and 10th and 90th percentiles (error bars). Statistical significance in **a, b, d, g** and **h** was determined by unpaired two-tailed Student’s t-test (****P < 0.0001).
cytoskeletal organization and tension, leading to force transmission to the nucleus and the nuclear translocation of transcription factors such as myocardin-related transcription factor-F and yes-associated protein 1 (YAP)\(^{13,32}\). Importantly, the physical properties of the chromatin shape the nucleus, which is mechanically coupled with the cytoskeleton through the linker of nucleoskeleton and cytoskeleton (LINC) complex and the nuclear lamina\(^{28,36}\). The biological outcome of perturbing these inward nuclear forces, by altering chromatin organization and nuclear mechanical properties, requires further study.

Here, we investigated whether MLL4 loss of function (LoF) can affect chromatin compartmentalization and result in nuclear mechanical stress and impaired mesenchymal stem cell (MSC) lineage commitment. We found that MLL4 drives the assembly of transcriptional condensates through LLPS. Via a stem cell-based Kabuki syndrome disease model, we determined that MLL4 maintains an equilibrium between transcription-associated and PcG condensates, which is required for preserving nuclear mechanical properties. By blocking the nuclear mechanosensor ataxia telangiectasia and Rad3-related protein (ATR), we rescued MLL4-dependent mechanical stress, permitting proper commitment of MSCs towards chondrocytes both in vitro and in vivo.

**Results**

**KMT2D truncating mutations reiterate the effect of MLL4 LoF on patients with Kabuki syndrome.** To address the consequences of MLL4 LoF in Kabuki syndrome, we inserted a frameshift mutation in the coding region (exon 39) of KMT2D in MSCs via CRISPR–Cas9-mediated editing. We generated MSCs carrying a heterozygous frameshift mutation that truncates the MLL4 protein (hereafter termed MLL4\(^{Q4092X}\)) (Extended Data Fig. 1a). Despite the unaltered transcript abundance, the mutation reduced MLL4 protein levels (Fig. 1a and Extended Data Fig. 1b,c), similar to previous observations in patient-derived samples\(^{37,38}\). The mutation did not affect the relative abundance of components of the MLL4–COMPASS complex such as WD repeat-containing protein 5 (WDR5) and PAXIP1-associated glutamate-rich protein 1 (PA1) (Fig. 1a). Ubiquitously transcribed TPR protein on the X chromosome (UTX) protein levels were lower in MLL4\(^{Q4092X}\) MSCs compared with wild-type (WT) MSCs (Fig. 1a and Extended Data Fig. 1d). Single-cell analyses by quantitative immunofluorescence showed that both MLL4 and UTX were uniformly reduced in MLL4\(^{Q4092X}\) MSCs, while PA1 was unchanged (Fig. 1b,c and Extended Data Fig. 1e,f).

Considering that MLL4 has specific monomethyl transferase activity for histone H3, we determined whether its haploinsufficiency could affect H3K4me1 levels. Mass spectrometry profiling of the relative changes in histone modifications showed that MLL4\(^{Q4092X}\) MSCs had relatively lower H3K4me1 levels compared with WT cells, while dimethylated and trimethylated H3K4 (H3K4me2/3) levels were unchanged (Fig. 1d). As the MLL4–COMPASS complex associates with the histone acetyltransferase P300 (ref. \(^{29}\)), we also quantified the relative abundance of H3K27ac. Mass spectrometry showed that MLL4\(^{Q4092X}\) MSCs had reduced H3K27ac compared with WT MSCs (Fig. 1e). Immunofluorescence analyses supported these results (Fig. 1f,g). Importantly, an independent MSC clone carrying a different truncating mutation of MLL4 (encoding MLL4\(^{Q4091X}\)) also exhibited reduced protein abundance of MLL4 and UTX and the associated reduction of H3K4me1 and H3K27ac (Extended Data Fig. 1g,h). To ensure that these alterations were not dependent on the developed in vitro Kabuki syndrome model, we also measured the same alterations in primary fibroblasts derived from patients with Kabuki syndrome. We found that, independent from the mutations causing KMT2D haploinsufficiency, the diminished abundance of MLL4 resulted in decreased H3K4me1 and H3K27ac levels, which were mirrored by reduced UTX abundance (Extended Data Fig. 2a–d). Together, these data show that KMT2D truncating mutations cause MLL4 LoF, impairing H3K4me1 deposition and decreasing H3K27ac levels.

**MLL4 favors the compartmentalization of transcriptional cofactors.** Considering the role of enhancers in transcriptional condensate assembly\(^{4,11,32,34}\), we examined whether MLL4 haploinsufficiency would impair enhancer-associated cofactor clustering. Measurement of mediator complex subunit 1 (MED1) and bromodomain-containing protein 4 (BRD4) nuclear distribution showed that these cofactors were distributed in clusters (Fig. 2a,b). MLL4\(^{Q4092X}\) MSCs had decreased intensity of BRD4 and MED1 condensates compared with the WT MSCs (Fig. 2a,b and Extended Data Fig. 2e,f). An independent MSC clone carrying the MLL4\(^{Q4093X}\) alteration yielded a similar pattern, as did fibroblasts derived from patients with Kabuki syndrome (Extended Data Fig. 2g–i). Determining the number and distribution of BRD4 and MED1 clusters within the nuclear volume confirmed their reduced abundance upon MLL4 LoF (Extended Data Fig. 3a,b).

To better define whether MLL4 haploinsufficiency alters cofactor organization in condensates in MSCs, we performed...
**Figure 1:**

**a** MLL4 and MLL4\_\_\_FID

**b** MLL4 and Pol II

**c** Number of clusters per cell

**d** MLL4 and R1, R2

**e** MLL4 and Pol II

**f** MLL4 and Pol II

**g** MLL4\_\_\_FID

**h** MLL4\_\_\_FID

**i** Condensed protein (relative amount)

**j** Pre-stimulation

**k** Number of puncta per cell

**l** MLL4\_\_\_FID and MLL4\_\_\_FID

**m** MLL4\_\_\_FID and MLL4\_\_\_FID

**n** MLL4\_\_\_FID and MLL4\_\_\_FID

**o** MLL4\_\_\_FID and MLL4\_\_\_FID

**p** MLL4\_\_\_FID and MLL4\_\_\_FID
Fig. 4 | PcG clustering is affected by MLL4 LoF. a–c. Representative images (top) and relative quantifications (bottom) of immunostaining for H3K27me3 (a), BMI (b) and RING1B (c) in WT and MLL4-Q4092X MSCs. Scale bars, 20 µm. d. Left: western blot analysis of BMI1 and RING1B in WT and MLL4-Q4092X MSCs. Histone H3 was used as the loading control. Right: signal quantification for BMI and RING1B. The data are presented as means ± s.e.m. from three independent experiments. e,f. Top: representative images of the distribution of BMI (e) and RING1B clusters (f) within the nuclear volume of WT and MLL4-Q4092X MSCs. Scale bars, 10 µm. Middle: 3D reconstructions of the positioning of the BMI (e) and RING1B clusters (f) with respect to the nuclear centroid. Bottom: quantification of the number (left) and volume (right) of BMI (e) and RING1B clusters (f) with respect to the nuclear centroid. g. Left: representative STORM images of RING1B in WT and MLL4-Q4092X MSCs. Scale bars, 5 µm. Right: magnified images of the portions of the images to the left indicated by a red square, highlighting the clustered distribution of PcG protein. Scale bars, 1 µm. h. Left: analysis of the number of RING1B clusters per nucleus measured in WT and MLL4-Q4092X MSCs (n = 10 analyzed independent nuclei per sample). Right: representative distribution of the area of the RING1B clusters detected in WT and MLL4-Q4092X MSCs. In a–c, e and f, the numbers of analyzed nuclei (n) are reported in the figure. The box plots in a–c, e, f and h indicate median values (middle lines), first and third quartiles (box edges) and 10th and 90th percentiles (error bars). Statistical significance was determined by unpaired two-tailed Student’s t-test (a–c, e, f and h) or unpaired one-tailed Student’s t-test (d) (****P < 0.0001).
super-resolution imaging by stochastic optical reconstruction microscopy (STORM). This revealed that BRD4 is distributed throughout the nuclei in clusters ranging from ∼0.0051 to 0.013 μm² (Fig. 2c,d). Notably, MLL4 LoF reduced BRD4 clustering, resulting in the formation of fewer and smaller condensates (Fig. 2d,e). These results suggest that MLL4 facilitates the assembly of chromatin clusters enriched for transcriptional cofactors.

To strengthen these findings, we measured whether MLL4 LoF affected the clustering dynamics of transcriptional condensates, adopting an optogenetic approach that allows modulation of the clustering of proteins containing self-associating IDRs40,41. We followed the dynamic formation and disassembly of MED1 clusters via live imaging by combining the light-responsive photolysase homology domain of Cry2 with the IDR region of MED1 (Extended Data Fig. 3c). A single pulse of blue light was sufficient to drive MED1 assembly in MSCs (Fig. 2f). Time-lapse imaging showed that MED1–IDR clusters formed immediately after light stimulation and dissolved within 5 min (Extended Data Fig. 3d,e and Supplementary Videos 1 and 2). The formed clusters corresponded to transcriptional condensates, as they co-localized with BRD4 and MLL4 (Extended Data Fig. 3f). Quantitative analyses showed that although the MED1–IDR clusters formed with the same timing in the WT and MLL4ΔKPrLD MSCs, their number and size were reduced upon MLL4 LoF (Fig. 2g,h). WT cells formed transient condensates with a mean lifetime of 119 s, while MLL4ΔKPrLD MSC clusters disassembled more rapidly, with a lifetime of 102 s (Fig. 2i). These results show that MLL4 LoF reduces the nucleation efficacy, as well as the assembly and disassembly kinetics, of transcriptional condensates.

**MLL4 is clustered in biomolecular condensates.** To define the possible mechanism by which the MLL4–COMPASS complex supports transcriptional condensate clustering, we investigated MLL4 distribution by quantitative imaging, and found that it is organized in clusters (Fig. 3a). Dual immunofluorescence showed that both MED1 and BRD4 were enriched within MLL4 clusters that co-localized with RNA polymerase II (Fig. 3b and Extended Data Fig. 4a). Comparative analyses showed that KMT2D haploinsufficiency reduced the number of MLL4 condensates without altering their dimensions (Fig. 3c and Extended Data Fig. 4b). STORM imaging showed that MLL4 exhibited puncta-like distribution, with cluster sizes between 0.0062 and 0.013 μm² (Fig. 3d,e). Notably, a small subset of MLL4 protein was clustered in large condensates, each formed by more than 150 localizations compared with the average distribution of 72.4 localizations per cluster. These results suggest that the MLL4–COMPASS complex is organized in biomolecular condensates that could influence MED1 and BRD4 distribution.

Bioinformatics analyses predicted that MLL4 contains large IDRs that could potentially participate in driving LLPS. Among these, we identified an MLL4-specific PrLD, which is conserved in multiple species and is specifically deleted in patients with Kabuki syndrome (Extended Data Fig. 4c,d). Given that PrLDs are low-complexity regions that promote multivalent interactions42, we determined whether MLL4 IDR harboring the PrLD (MLL4ΔKPrLD (3560–4270)) was sufficient to drive LLPS in vitro. We found that recombinant mCherry (mCh)–MLL4ΔKPrLD (Extended Data Fig. 4e) phase separated in physiological ionic-strength conditions in the presence of a crowding agent (Fig. 3g,h). To determine the saturation concentration (C_sat) at which the MLL4ΔKPrLD phase separated, we quantified the relative intensity of the mCh signal inside the droplets. We found that the C_sat of MLL4ΔKPrLD was ~1.7 μM, behaving similarly to the prototype PrLD-containing protein FUS (Fig. 3i). Importantly, the MLL4ΔKPrLD phase separation was reversible, as the formed droplets were dissolved by increasing the ionic strength or by competing out the hydrophobic interactions by adding 1,6-hexanediol (Extended Data Fig. 4f,g). As the identified MLL4ΔKPrLD contained a polyQ tract (78 amino acids), we deleted this region from the mCh–MLL4ΔKPrLD protein and assessed its contribution to phase separation (Extended Data Fig. 4e). Removing the polyQ stretch was sufficient to reduce the droplet-forming proficiency of MLL4ΔKPrLD, suggesting that this region is required to drive phase separation (Extended Data Fig. 4h).

To determine whether MLL4ΔKPrLD participates in the formation of transcriptional condensates in living cells, we modulated the local protein concentration using the light-activated optoIDR approach40,41. A single pulse of blue light induced clustering in most of the expressing cells, forming spherical droplets (Fig. 3j). The resultant number of formed droplets was proportional to the optoMLL4 expression level (Extended Data Fig. 4i). Time-lapse imaging showed that, following light stimulation, the optoMLL4 nucleated in droplets whose number and dimensions persisted for 300 s before diffusing into the nucleoplasm, reaching the initial distribution (Fig. 3k and Supplementary Video 3). Deleting the polyQ tract affected their assembly, as the light-induced optoMLL4ΔKPrLD did not form detectable droplets, despite its expression level being similar to that of optoMLL4 (Fig. 3j) and Extended Data Fig. 4j).

Co-localization analyses showed that optoMLL4 clustered with BRD4 and MED1 to the same extent as the endogenous proteins (Fig. 3l and Extended Data Fig. 4a). Based on these results, we conclude that MLL4 participates in the formation of transcriptional biomolecular condensates.

**MLL4 LoF unbalances PcG compartments.** Given the genetic and functional antagonism between the TrxG and PcG complexes25, we investigated whether the transcriptional condensate perturbation caused by MLL4 LoF could affect the repressive compartments associated with PcG complexes. Quantitative immunofluorescence analyses showed that, although Polycomb repressive complex 2 component (EED, EZH2 and SUZ12) protein levels were unaltered (Extended Data Fig. 5a), H3K27me3 deposition was increased (Fig. 4a). Analysis of Polycomb repressive complex 1 (PRC1) components...
revealed increased RING finger protein 1B (RING1B) and B lymphoma Mo-MLV insertion region 1 homolog (BMI1) signals, which were distributed in the condensates (Fig. 4b,c). We detected higher levels of both BMI1 and RING1B proteins, while their transcripts were unaltered (Fig. 4d and Extended Data Fig. 5b). Notably, MLL4Q4092X MSCs and patient-derived fibroblasts also had similar enrichment of H3K27me3 and PRC1 components (Extended Data Fig. 5c–f). To determine the spatial organization of PcG foci, we...
measured the number and distribution of BMI and RING1B clusters within the nuclear volume of WT and MLL4Q4092X MSCs and found that MLL4Q4092X MSCs contained a higher number of PcG foci with a similar distribution (Fig. 4e,f). STORM nanoscopy imaging of the PRC1 components supported these results. Quantitative analyses determined that RING1B formed heterogeneous clusters whose abundance and dimensions were increased in MLL4Q4092X MSCs compared with WT MSCs (Fig. 4g,h).

To ascertain whether the increased PcG clustering was dependent on MLL4 abundance, we rescued its expression in MLL4Q4092X MSCs via CRISPR-mediated gene activation (CRISPRa) (Extended Data Fig. 5g–k). Quantitative imaging analyses showed that the transient re-establishment of MLL4 protein levels counteracted the augmented PcG clustering and H3K27me3 deposition (Extended Data Fig. 5k). These data indicate that MLL4 LoF affects repressive compartments, with increased PcG protein clustering.

**MLL4 regulates nuclear mechanics and chromatin compaction.**

The results indicate that MLL4 LoF altered the balance between transcriptional and PcG condensates. Considering that chromatin dynamics exert forces that shape three-dimensional (3D) genome folding and nuclear structure46,47, we analyzed the effects of MLL4 LoF on nuclear architecture and mechanics. The MLL4Q4092X MSCs had altered nuclear morphology compared with the WT cells (Fig. 5a). There was a consistent reduction in nuclear area and volume, which resulted in decreased nuclear flattening (Fig. 5b and Extended Data Fig. 6a,b). Moreover, rescuing MLL4 via CRISPRa re-established the nuclear shape in the MLL4Q4092X MSCs (Extended Data Fig. 6c). These findings suggest that, although MSCs were grown in conditions favoring force transmission to the nucleus48, MLL4 LoF impacted the nuclear architecture. To test this hypothesis, we assessed the relative protein levels of lamin A/C, the abundance of which is modulated in response to changes in tensile forces49. We found that although the lamin A/C transcript levels were unchanged (Extended Data Fig. 6d), MLL4Q4092X MSCs had reduced protein abundance compared with WT cells (Fig. 5c,d). Considering that lamin A/C assembly and protein turnover balance change in mechanical stresses, we determined their phosphorylation state, which is coupled with nuclear lamina organization50. We found that MLL4 LoF caused increased lamin A/C phosphorylation and protein turnover, resembling the pattern observed under low-nuclear-stress conditions45 (Fig. 5c,d and Extended Data Fig. 6e). To directly determine nuclear mechanical properties, we performed all-optical, label-free, non-invasive measurements by Brillouin microscopy51, where the high-frequency longitudinal elastic modulus can be determined in intact cells by measuring the Brillouin frequency shift. Here, the increased Brillouin shift indicated that MLL4Q4092X MSCs had increased nuclear stiffness compared with the WT cells (Fig. 5e,f). We then determined whether the alterations in nuclear architecture were coupled with perturbed cytoskeletal organization. We measured a decrease in the occupied cell area linked to reduced cytoskeletal fibers in the MLL4Q4092X MSCs (Extended Data Fig. 6f). As the LINC complex mediates cell/nucleus mechanical coupling, we investigated whether the altered intranuclear forces could determine the perturbed cytoskeletal organization in MLL4Q4092X MSCs. By overexpressing the dominant negative GFP–Nesprin-1/2–KASH protein that interferes with the formation of a functional LINC complex, we observed partial rescue of cell size and proper actin polymerization (Extended Data Fig. 6g).

We then examined whether the mechanical stresses detected in MLL4Q4092X MSCs were associated with changes in chromatin compaction. We measured the relative level of H4K16ac, which controls chromatin structure by weakening internucleosomal interactions52. Immunofluorescence analyses showed decreased H4K16ac in MLL4Q4092X MSCs and in patient-derived fibroblasts (Fig. 5g and Extended Data Fig. 6h,i). Treatment of MLL4Q4092X MSCs with a histone deacetylase inhibitor (trichostatin A (TSA)) rescued the altered chromatin organization and nuclear architecture (Fig. 5h,i). Specifically, TSA treatment re-established proper nuclear morphology and structure in MLL4Q4092X MSCs, as shown by the relative abundance of lamin A/C (Fig. 5h,i). In summary, these findings show that MLL4 LoF causes nuclear mechanical stress by affecting chromatin organization and nuclear architecture.

**PcG clustering impinges on nuclear architecture.**

Besides altering nuclear morphology, we noted that TSA treatment reduced H3K27me3 and PcG condensate levels in MLL4Q4092X MSCs (Extended Data Fig. 7a,b), suggesting that their relative abundance could be coupled with chromatin compartmentalization and nuclear mechanics. To test this, we rescued PcG clustering in MLL4Q4092X MSCs by overexpressing histone H3.3 carrying a p.Lys27Met alteration (H3.3K27M), which has a dominant negative effect on PcG activity53. H3.3K27M overexpression reduced H3K27me3 levels in the MLL4Q4092X MSCs and re-established BMI1 and RING1B protein abundance (Fig. 6a and Extended Data Fig. 7c). Visualization of PcG protein distribution in the presence of H3.3K27M showed that both BMI1 and RING1B clusters were strongly reduced, with few remaining larger foci marked by H3K27me3 (Fig. 6b). MLL4Q4092X MSCs expressing H3.3K27M showed increased nuclear volume and area, with nuclear flattening similar to that in WT MSCs (Fig. 6c). These changes were coupled with increased lamin A/C protein, re-establishing the nuclear architecture (Fig. 6d). Brillouin frequency shift measurement determined that H3.3K27M expression and TSA treatment rescued the increased nuclear stiffness in MLL4Q4092X MSCs (Extended Data Fig. 7d).

To examine whether PcG-mediated compartmentalization represents a driving force establishing nuclear mechanics, we adopted...
an optogenetic approach to induce BMI1 clustering in living cells. Measurement of the light-induced clustering of BMI1–Cry2 showed that a single pulse of blue light drove the formation of relatively stable BMI1 clusters, with a lifetime of 12 min (Fig. 6e, Extended Data Fig. 8a,b and Supplementary Video 4). The light-induced condensates were enriched at PcG binding sites as BMI1–Cry2 co-localized with RING1B and H3K27me3, similar to that detected for the endogenous proteins (Extended Data Fig. 8c,d). Importantly,
prolonged light stimulation drove consistent PcG clustering, which determined the altered nuclear shape, as measured by the decreased nuclear volume and area (Fig. 6f,g). Taken together, these findings show that PcG-mediated chromatin compartmentalization affects the nuclear architecture.

MLL4 restricts the mechanoresponsiveness of MSCs. We investigated whether the alterations in nuclear architecture driven by the unbalanced transcription-associated and PcG condensates would affect MSC mechanoresponsiveness. We measured the cellular distribution of the mecha-noneffectors yes-associated protein 1 (YAP1; hereafter referred to as YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), whose nuclear accumulation depends on mechanical forces32,46,52. Comparison of the cellular distribution of YAP/TAZ in WT versus MLL4Q4092X MSCs showed that MLL4 LoF was associated with reduced nuclear YAP/TAZ independent of the Hippo pathway (Fig. 7a and Extended data Fig. 9a,b). Notably, the mechanically induced YAP/TAZ nuclear localization was mirrored by augmented YAP/TAZ transcriptional activity, which was reduced in MLL4Q4092X MSCs (Fig. 7b). To ascertain whether the reduced YAP/TAZ nuclear shuttling was caused by the altered nuclear mechanics of MLL4Q4092X MSCs, we blocked the nuclear mechanosensor ATR with a specific inhibitor, VE-822 (Extended Data Fig. 9c)53,54. VE-822 treatment restored YAP/TAZ nuclear localization in MLL4Q4092X MSCs without increasing DNA damage (Fig. 7c and Supplementary Table 2)55,56. Importantly, their expression levels (Extended Data Fig. 10a). In the same setting, we also measured increased expression levels of the chromatin architectural genes (such as TOP2A and TOP2B), condensin factors SMC2/4 and the cohesin component RAD21 (Fig. 7f). To verify whether YAP/TAZ transcriptional regulation of the ATR-responsive genes relies on the nuclear mechanical stress caused by PcG hyperactivity, we rescued its functionality by over-expressing H3.3K27M in MLL4Q4092X MSCs. Besides re-establishing the nuclear abundance of YAP/TAZ (Extended Data Fig. 10c), chromatin architectural gene expression levels were rescued as well (Fig. 7g). These findings indicate that ATR modulates YAP/TAZ nuclear accumulation and the expression of key factors involved in chromatin organization and compaction.

ATRI re-established MSC lineage commitment towards chondrocytes and osteocytes. To establish the biological relevance of these results, we determined whether MLL4 LoF affected MSC mechanoresponsiveness during differentiation. We found that, although the differentiation potential of MLL4Q4092X MSCs towards adipocytes was unaltered, their commitment towards chondrocytes was strongly affected, while osteogenesis was partially impaired (Extended Data Fig. 10d,e). Notably, exogenously expressing MLL4 rescued the altered chondrogenesis (Extended Data Fig. 10f); ATRi partially rescued the chondrogenic differentiation potential of the MLL4Q4092X MSCs (Fig. 8a).

To support the notion that targeting ATR can re-establish the mechanical responsiveness of MSCs in Kabuki syndrome, we tested the capacity of ATRi for rescuing chondrogenesis and skeletogenesis in vivo. We developed an in vivo Kabuki syndrome model by knocking down olKmt2d in medaka fish, with a specific morpholino (MO) directed against the ATG initiation codon within the 5′ untranslated region (MOkmt2d). At the late developmental stages, most of the MOkmt2d embryos had a clearly visible spectrum of morphological craniofacial anomalies (73±5% of 1,600 injected embryos). Cartilage and bone growth were significantly impaired, culminating in an obviously shorter length at developmental stage (St) 40 (Fig. 8b). Similar to that described in zebrafish57,58, the MOkmt2d morphants exhibited smaller heads with dysmorphism, resembling the clinical features of patients with Kabuki syndrome (Fig. 8b,c and Extended Data Fig. 10g). Importantly, ATRi of S34 Kmt2d morphants was sufficient for restoring the chondrogenic and skeletal defects, rescuing proper head morphogenesis without evidence of adverse effects or toxicity (Fig. 8b,c and Extended Data Fig. 10g).

To better characterize the chondrogenesis and skeletogenesis defects in MLL4 morphant larvae, we used a transgenic medaka line (Col10a1:GFP/Osx:mCherry), in which chondrocytes and osteoblasts are detected by green fluorescent protein (GFP) and mCh expression, respectively39. Double-positive cells mark sites of ongoing intramembranous osteogenesis (perichondrial, cleithrum and fifth ceratobranchial cartilage), while perichondrial ossification...
(palatoquadrate and ceratohyal) is marked by GFP-positive chondrocytes surrounded by mCh-positive osteoblasts. In the MOKmt2d morphants, both perichondral and intramembranous ossification were affected, with decreased Osx:mCherry osteoblastic cells (Fig. 8d and Extended Data Fig. 10h–k). Notably, ATRi rescued the altered chondrogenesis and skeletogenesis, re-establishing the pattern of cell lineage commitment (Fig. 8d and Extended Data Fig. 10h–k). Together, these results indicate that targeting ATR in Kabuki syndrome model systems is sufficient for restoring proper chondrogenesis and skeletogenesis both in vitro and in vivo.

**Discussion**

The forces driving genome spatial organization and how chromatin compartmentalization modulates both the genetic and non-genetic functions of the genome represent key questions that have been poorly addressed.
**Figure a:** Absorbance at 650 nm for MLL4WT and MLL4Q4092X with ATRi 0.08 µM and ATRi 0.2 µM. 

**Figure b:** Images showing Ctrl (MLL4WT), MOklzid, and MOklzid + ATRi treatments.

**Figure c:** Graph showing ossification of cartilage for Ctrl (MLL4WT) and MOklzid.

**Figure d:** Images displaying Col10a1:nGFP/Osx:mCherry for Ctrl, MOklzid, and MOklzid + ATRi.

**Figure e:** Diagram illustrating the relationship between enhancer, promoter, and transcriptional condensates with PCG clustering, nuclear mechanical stress, YAP/TAZ, and lineage commitment towards chondrocytes and osteocytes.
By developing a stem cell-based Kabuki syndrome disease model, we show that MLL4 maintains equilibrium between transcription-associated and PcG condensates, which is required for preserving nuclear mechanical properties (Fig. 8e). MLL4 LoF caused a PcG-mediated increase in nuclear mechanical stress, affecting YAP/TAZ nuclear accumulation and consequently the transcriptional regulation of its targets, including the cohesin and condensin genes (Fig. 8e). ATRi rescued the MLL4-dependent mechanical stress, permitting proper MSC commitment towards chondrocytes both in vitro and in vivo. These findings support the notion that nuclear inward forces driven by chromatin compartmentalization contribute to MSC responsiveness to mechanical stimuli, guiding their differentiation program.

Here, we provide evidence that MLL4 plays a central role in establishing transcriptional condensates, which balance the PcG-mediated repressive chromatin context. We showed that MLL4 phase separates in droplets, whose assembly depends on its PrLD. As this domain is codified by a genomic region whose clinical variants associate with a pathogenic setting in Kabuki syndrome, our findings highlight the potential relevance of the PrLD-driven clustering of MLL4 in tuning its biological activity. These results highlight a distinct function of MLL4, which contributes to control of the dynamic composition of transcriptional condensates and their interplay with other chromatin compartments (Fig. 8e). We show that MLL4 abundance is critical for proper transcriptional condensate assembly, as its haplosufficiency impinged on MED1 and BRD4 clustering. These results suggest that MLL4 may work as a scaffold protein for transcriptional condensate nucleation, favoring the dynamic recruitment of cofactors and activators.

We also show that MLL4 LoF was mirrored by the redistribution of PcG proteins, affecting their clustering in PcG bodies. The increased PcG activity could depend on different mechanisms, including the BMI1-mediated increase of RING1B protein stability and enzymatic activity. Besides maintaining a repressive chromatin state, PcG plays a central role in establishing long-range interactions, contributing to the determination of genome architecture and chromatin compaction. Importantly, these PcG-mediated interactions occur within an active chromatin environment, suggesting possible interplay between TrxG and PcG components in shaping chromatin folding. Our findings indicate that part of the antagonistic action of MLL4 and PcG complexes could rely on their function on genome architecture and chromatin compartmentalization. We propose that the frequency of PcG-mediated long-range interactions is modulated by the clustering of transcriptional condensates, which are interspersed within the active chromatin compartments (Fig. 8e).

We found that, besides permitting the establishment of a certain chromatin state, MLL4 is required for modulating the non-genetic function of the genome; namely, exerting mechanical forces that shape nuclear architecture. MLL4 LoF caused increased chromatin compaction and nuclear stiffness, which was counterbalanced by decreased lamin A/C levels. Furthermore, our data indicate that PcG-mediated chromatin clustering represents a driving force for tuning nuclear mechanics. These data shed light on the impact of chromatin compartmentalization on nuclear architecture and its responsiveness to mechanical cues. Although environmental mechanical forces lead to chromatin rearrangement depending on PcG activity, recent evidence supports the notion that inward forces generated by chromatin organization modulate nuclear mechanics and architecture. In this context, it would be relevant to establish whether the proposed therapeutic regime for Kabuki syndrome, which is based on enhancing chromatin opening, may re-establish proper chromatin compartmentalization, thereby decreasing nuclear mechanical stress. Here, we propose that inhibiting ATR could represent a novel therapeutic option for re-establishing nuclear mechanical properties and responsiveness to mechanical stimuli by re-establishing YAP/TAZ activity. Further investigations are required to define the clinical relevance of our findings, with the intent of attenuating some of the pathological conditions affecting patients with Kabuki syndrome.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41588-020-00724-8.

Received: 19 September 2019; Accepted: 22 September 2020; Published online: 9 November 2020

References
1. Fukaya, T., Lim, B. & Levine, M. Enhancer control of transcriptional bursting. Cell 166, 358–368 (2016).
2. Spitz, F. Gene regulation at a distance: from remote enhancers to 3D regulatory ensembles. Semin. Cell Dev. Biol. 57, 57–67 (2016).
3. Rada-Iglesias, A. et al. A unique chromatin signature uncovers early developmental enhancers in humans. Nature 470, 279–283 (2011).
4. Calo, E. & Wysocka, J. Modification of enhancer chromatin: what, how, and why? Mol. Cell 49, 825–837 (2013).
5. Stadhouders, R., Filion, G. J. & Graf, T. Transcription factors and 3D genome conformation in cell-fate decisions. Nature 569, 345–354 (2019).
6. Van Steensel, B. & Furlong, E. E. M. The role of transcription in shaping the spatial organization of the genome. Nat. Rev. Mol. Cell Biol. 20, 327–337 (2019).
7. Chong, S. et al. Imaging dynamic and selective low-complexity domain interactions that control gene transcription. Science 361, eaar2555 (2018).
8. Cho, W. K. et al. Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science 361, 412–415 (2018).
9. Sabari, B. R. et al. Coactivator condensation at super-enhancers links phase separation and gene control. Science 361, eaar9598 (2018).
10. Boija, A. et al. Transcription factors activate genes through the phase-separation capacity of their activation domains. Cell 175, 1842–1855.e16 (2018).
11. Lu, H. et al. Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. Nature 558, 318–323 (2018).
12. Alberti, S. Phase separation in biology. Curr. Biol. 27, R1097–R1102 (2017).
13. Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. 18, 285–298 (2017).

14. Shin, Y. & Brangwynne, C. P. Liquid phase condensation in cell physiology and disease. Science 357, eaaf4382 (2017).

15. Alberti, S., Halfmann, R., King, O., Kapila, A. & Lindquist, S. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. Cell 137, 146–158 (2009).

16. Molllex, A. et al. Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. Cell 163, 123–133 (2015).

17. Franzmann, T. M. & Alberti, S. Prion-like complexity-sequence features: key regulators of protein solubility and phase behavior. J. Biol. Chem. 294, 7128–7136 (2019).

18. Møes, Z. M., Quiros, O. D. & Shorter, J. Prion-like domains as epigenic regulators, scaffolds for subcellular organization, and drivers of neurodegenerative disease. Brain Res. 1647, 9–18 (2016).

19. Herz, H. M. et al. Enhancer-associated H3K4 monomethylation by Trithorax-related, the Drosophila homolog of mammalian MLL3/MLH4. Genes Dev. 26, 2604–2620 (2012).

20. Wang, S. P. et al. A UTX–MLL4–p300 transcriptional regulatory network coordinately shapes active enhancer landscapes for eliciting transcription. Mol. Cell 67, 308–321.e6 (2017).

21. Ringrose, L. & Paro, R. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu. Rev. Genet. 38, 413–443 (2004).

22. Schuettengruber, B., Bourbon, H. M., Di Croce, L. & Cavalli, G. Genome regulation by Polycat and Polyomath: 70 years and counting. Cell 171, 34–57 (2017).

23. Local, A. et al. Identification of H3K4me1-associated proteins at mammalian enhancers. Nat. Genet. 50, 73–82 (2018).

24. Dorigli, K. M. et al. MLL3 and MLL4 facilitate enhancer RNA synthesis and transcription from promoters independently of H3K4 monomethylation. Mol. Cell 66, 568–576.e4 (2017).

25. Rickels, R. et al. Histone H3K4 monomethylation catalyzed by Trr and mammalian COMPASS-like proteins at enhancers is dispensable for development and viability. Nat. Genet. 49, 1647–1653 (2017).

26. Ng, S. B. et al. Exome sequencing identifies ML2 mutations as a cause of Kabuki syndrome. Nat. Genet. 42, 790–793 (2010).

27. Banka, S. et al. How genetically heterogeneous is Kabuki syndrome?: ML2 testing in 116 patients, review and analyses of mutation and phenotypic spectrum. Eur. J. Hum. Genet. 20, 381–388 (2012).

28. Cocciadiferro, D. et al. Dissecting KMT2D missense mutations in Kabuki syndrome patients. Hum. Mol. Genet. 27, 3651–3668 (2018).

29. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. Cell 126, 677–689 (2006).

30. Huebsch, N. et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat. Med. 9, 518–526 (2010).

31. Vanpoucke, M. K., Guerder, S., Larjani, B. & Treisman, R. Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL. Science 316, 1749–1752 (2007).

32. Dupont, S. et al. Role of YAP/TAZ in mechanotransduction. Nature 474, 179–183 (2011).

33. Ulder, C. & Shivasankar, G. V. Regulation of genome organization and gene expression by nuclear mechanotransduction. Nat. Rev. Mol. Cell Biol. 18, 717–727 (2017).

34. Stephens, A. D., Banigian, E. J., Adam, S. A., Goldman, R. D. & Marko, J. F. Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus. Mol. Biol. Cell 28, 1984–1996 (2017).

35. Ghosh, S. et al. Deformation microscopy for dynamic intracellular and intranuclear mapping of mechanics with high spatiotemporal resolution. Cell Rep. 27, 1607–1620.e4 (2019).

36. Heo, S. J. et al. Differentiation alters stem cell nuclear architecture, mechanics, and mechano-sensitivity. elife 5, e18207 (2016).

37. Micali, L. et al. Molecular analysis, pathogenic mechanisms, and readthrough therapy on a large cohort of Kabuki syndrome patients. Hum. Mutat. 35, 841–850 (2014).

38. Zhang, J. et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. Nat. Med. 21, 1190–1198 (2015).

39. Elguero, S. et al. Phase separation of ligand-activated enhancers licenses cooperative chromosomal enhancer assembly. Nat. Struct. Mol. Biol. 26, 193–203 (2019).

40. Shin, Y. et al. Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. Cell 168, 159–171.e14 (2017).

41. Lehman, N. et al. Molecular, clinical and neuropsychological study in 31 patients with Kabuki syndrome and KMT2D mutations. Clin. Genet. 92, 298–305 (2017).

42. Wang, J. et al. A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. Cell 174, 688–699.e16 (2018).

43. Bugai, L. J., Choksi, A. T., Mesuda, C. K., Kane, R. S. & Schaffer, D. V. Optogenetic protein clustering and signaling activation in mammalian cells. Nat. Methods 10, 249–252 (2013).

44. Buxboim, A. et al. Matrix elasticity regulates lamin-A,C phosphorylation and turnover with feedback to actomyosin. Cell Reports 24, 1909–1917 (2014).

45. Antonacci, G., de Turris, V., Rosa, A. & Ruocco, G. Background-deflection Brillouin microscopy reveals altered biomechanics of intracellular stress granules by ALS protein FUS. Commun. Biol. 1, 139 (2018).

46. Shogren-Knaak, M. et al. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 311, 844–847 (2006).

47. Lewis, P. W. et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glblastoma. Science 340, 857–861 (2013).

48. Bustin, M. & Misteli, T. Nongenetic functions of the genome. Dev. Cell 32, 2604–2620 (2012).

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature America, Inc. 2020
Methods

Cell culture conditions. The cell lines used in this study included NIH3T3 cells (American Type Culture Collection), HEK293T cells (American Type Culture Collection), human primary fibroblasts derived from either healthy individuals or patients with Kabuki syndrome (Genomic and Genetic Disorders Biobank-Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy) and iNTERT-immortalized human adipose-derived MSCs (a kind gift from P. Tatrai). Primary fibroblasts and NIH3T3 and HEK293T cells were maintained at 37 °C under 5% CO₂ in DMEM medium supplemented with 10% fetal bovine serum (Euroclone; ECS0180L), while MSCs were cultured in 1:1 DMEM/F-12 medium (Gibco; 11320-074) supplemented with 10% fetal bovine serum (Euroclone; ECS0180L).

For adipocyte differentiation, cells were seeded at a density of 1 x 10⁶ cells per cm² in MSCs medium. The day after, the medium was replaced with adipogenesis medium (Gibco; A10410-01) supplemented with Stem-Pro Adipogenesis supplement (Gibco; 10066-01). For complete differentiation, the cells were maintained in culture for 3 weeks, with regular media changes.

For osteoblast differentiation, they were seeded at a density of 5 x 10⁶ cells per cm² in MSCs medium. The day after, the medium was replaced with osteogenesis medium (Gibco; A10069-01) supplemented with Stem-Pro Osteogenesis supplement (Gibco; 10066-01). For complete differentiation, the cells were maintained in culture for 3 weeks, with regular media changes.

For chondrocyte micromass culture, a cell solution of 1.6 x 10⁶ viable cells per ml was produced. Micromass cultures were generated by seeding 5-μl droplets of cell solution. After cultivating micromass cultures for 2 h under high-humidity conditions, MSCs were added. The day after, the medium was replaced with chondrogenesis medium (Gibco; A10069-01) supplemented with Stem-Pro Chondrogenesis supplement (Gibco; 10064-01). For complete differentiation, the cells were maintained in culture for 3 weeks, with regular media changes.

CRISPR-Cas9 mediated genome editing. Single-guide RNA (sgRNAs) were designed using the online tool E-CRISPR (http://www.e-crispr.org/E-CRISPR/). Once designed, they were cloned in the pLX sgRNA vector (Addgene; 50662). Briefly, new target sequences were cloned into pLX sgRNA between the XhoI and Nhel sites using overlap-extension PCR followed by restriction/ligation into the plx sgRNA vector. MSCs were genome edited by expression of the doxycycline-inducible Cas9 (pCW Cas9; Addgene; 50661) combined with sgRNA construct, followed by puromycin and blasticidin selection. Clonal selection was performed to identify targeted cells. Genomic DNA was collected from different clones and subjected to surveyor assay (using the T7 endonucleases; NEB; M0302). Positive clones were selected and sequenced to determine the insertion of the truncating mutation. The oligonucleotides used in this work for the generation of sgRNAs containing plasmids are listed in Supplementary Table 3.

Epigenetic rescue of MLL4 expression. The putative promoter region was identified through the UCSC genome browser on data coming from human bone marrow-derived MSCs. The region identified was chromosome 12 (49453096–49455679). To identify the sequences recognized by the CRISPR RNAs (crRNAs), we used the CRISPR RNA-guided endonuclease–Cas9 designer with the protospacer-adjacent-motif sequence 5′-TTTTN-3′. These sequences were cloned into the plasmid BPK3082 (Addgene; 78742): for each crRNA, a pair of complementary oligonucleotides, containing the desired sequence, were synthesized. Adapters (described in Supplementary Table 3) were cloned into the plasmid BPK3082. Single LbCpf1 crRNA expression plasmids were constructed by ligating annealed oligo duplexes into Esp3I-digested BPK3082 plasmid. For the transcription activation, we used the dLbCpf1–VPR plasmid (Addgene; 104567).

For each experiment, the cells were subjected to nucleofection (Lonza; Kit LOY-A101032) with the dLbCpf1–VPR and LbCpf1 crRNA expression plasmids at a 3:1 ratio. Then, 48 h after the nucleofection, the cells were re-plated for RNA extraction or immunofluorescence analysis. After another 24 h, the cells were collected for RNA extraction and analysis. After an additional 48 h, the cells were fixed for immunofluorescence analysis.

DNA constructs. The mCh–Cry2 sequence was PCR amplified from pHr–mCh–MED1ex–Cry2, a gift from the Young laboratory (Sabari et al.), and cloned between the Xhol and NotI sites in the pCAG vector with or without insertion of an SV40 nuclear localization sequence (NLS) at the 3′ end of Cry2. The MLL4 promoter region (amino acids 3560–4270) was PCR amplified and cloned between the Xhol and Nhel sites in the expression vector pCAG mCh–Cry2–NLS. The MLL4ΔQ region was obtained by overlap-extension PCR and cloned between the Xhol and Nhel sites in the pCAG mCh–Cry2–NLS vector. The mouse BMI coding sequence was amplified and cloned between the EcoRV and SpeI sites in the expression vector pCAG mCh–Cry2. The oligonucleotides used in this work for cloning are listed in Supplementary Table 3.

Stable cell lines. Mscs expressing mCh–MED1–Cry2 were obtained transducing WT and mutant MSCs with the lentiviral vector pHr–mCh–MED1–Cry2. Mscs expressing histone H3.3K27M were obtained transducing mutant MSCs with the lentiviral vector pCDH–EP1–MSCS–ires–puRO–H3.3K27M (a gift from the Allis laboratory). MSCs overexpressing YAP were obtained transducing mutant MSCs with the lentiviral vector Fuw tetO YAP (Addgene; 84009).

Immunofluorescence. For the immunofluorescence assays, cells were seeded on coverslips coated with 0.1% gelatin (Sigma–Aldrich; G1393). When needed, cells were fixed with 4% paraformaldehyde for 10 min at 4 °C. Coverslips were processed as described: permeabilization and blocking with phosphate-buffered saline (PBS)/1% bovine serum albumin/5% goat serum/0.5% Triton X-100 (blocking solution) for 1 h at room temperature, followed by incubation with primary antibody (diluted in the blocking solution) for 2 h at room temperature (or overnight at 4 °C, depending on the primary antibody used), three to five washes in PBS and incubation with secondary antibodies (diluted in the blocking solution), DRAQ5 for nuclear staining and phalloidin-TRITC for 1 h at room temperature. Images were acquired using a Leica TCS SP5 confocal microscope with a HCX Plan Apo 63×1.0 objective. Confocal z stacks were acquired with sections of 0.5 μm. In cases where image analysis was performed, random sampling was performed while image acquisition settings were kept constant. The antibodies used in this work are listed in Supplementary Table 4.

Confocal imaging data analyses. Confocal imaging data analyses were performed using ImageJ software. For 2D/3D analysis, DRAQ5 DNA dye was used to identify the nucleus and define the region of interest. The fluorescence intensity and physical parameters were determined. The values of the fluorescence intensity were background subtracted and normalized with respect to the DRAQ5 fluorescence intensity and the measure of volume and flatness were performed. For intensity analysis using the 3D plugin suite (an ImageJ plugin). For the co-localization analysis, the ImageJ plugin JACoP was used. For each staining, the DRAQ5 signal was used to identify the nucleus as an area of interest on which the Pearson coefficient was determined.

Quantification of nuclear to cytosolic localization of YAP/TAZ. To quantify the nucleus to cytosolic localization of YAP/TAZ, we adapted a MATLAB routine that is deposited in GitHub (https://github.com/SZambrano/RoutinesNucCytoYAP). In short, images of the Hoechst and YAP/TAZ channels were saved as 16-bit TIFF files. To segment the nuclei, we used the signal from the Hoechst channel. The nuclear masking was performed using a threshold the mean intensity of the image plus twice the standard deviation. After thresholding, segmentation was carried out after a watershed transformation, so most of the few overlapping nuclei could be separated. The segmented nuclei were filtered for their size to exclude artifacts or improperly segmented clusters of nuclei. To estimate the average cytosolic intensity per cell, a ring of 30-pixel width (approximately 7 μm) around each segmented nucleus was found. Pixels of the ring with too low intensity of the YAP/TAZ signal (below twice the value of the background signal) were discarded. The average cytosolic signal for each cell was the average intensity of the remaining pixels. We then calculated for each cell the nuclear to cytosolic intensity as the ratio of the background-corrected nuclear and cytosolic average YAP/TAZ intensities.

2D cluster analyses. 2D cluster analyses were performed using ImageJ software. For the 2D cluster analysis of MED1 and BRD4, background subtraction was applied. To calculate the background level, the mean of the minimum level of intensity of the specific staining was calculated among all of the nuclei analyzed. Two-dimensional sharpening and median filters were applied. The clusters were identified with the Yen automatic threshold and the intensity of each cluster was calculated, redrawing the measurement on the unmodified images. 3D reconstructions of PBs were obtained through the connected components algorithm (bwconncomp MATLAB function, using a connectivity of 6).

The algorithm computes the number of chromatin domains, the volume of any chromatin domain and the centroid distance of each chromatin domain from the nuclear periphery and the nuclear centroid.

Super-resolution microscopy. Super-resolution localization imaging of fixed and immunostained cells was obtained by direct STORM using a ground-state depletion microscope (Leica SR GSD; Leica Microsystems) equipped with two solid-state lasers of 532 and 640 nm, an oil immersion objective lens (HCX Plan Apo x150 1.45 NA) and an electron multiplying charge coupled device camera (Andor iXon Ultra 897). All direct STORM experiments were performed with the Smart kit buffer (Abbelight). To induce the majority of the fluorophores into the dark state, we excited the samples using the laser in a straight configuration. Once the density of fluorescent dye was sufficient, we activated the real-time localization using the laser in an oblique configuration (Hilo). For all recorded images, the integration time and the electron multiplying charge coupled device gain were set to 8 ms and 300, respectively. For each sample, we acquired 35,000 frames. The identification and localization of single events from raw images was run on the Leica software.

STORM image data analysis. The cluster analysis was performed with a custom-written MATLAB script following the routine described by Ricci et al.8. Briefly, for each cell, the localizations list was used to reconstruct a STORM image with a pixel size of 20 nm. This image was used to exclude areas of very low
localization density (density threshold = 0.0025 nm⁻²) and to identify the local maxima in the areas of higher density. Only localizations within high-density regions were analyzed. The number and position of the maxima were used to initialize the centroids of the clusters. The subdivision of the localizations in clusters was performed by a machine-learning k-means algorithm that optimized the grouping of localizations based on their proximity to the centroid of the cluster. The algorithm ran on the raw localization coordinates. The area attributed to the cluster was the convex hull area associated with that set of localizations.

**Live imaging and optogenetics.** Time-lapse video microscopy and single-cell tracking of MSCs and NIH3T3 cells were carried out continuously for the indicated times at 37 °C and 5% CO₂ using an Eclipse TiZ fully automated system (Nikon). Images of fluorescent cells were acquired as indicated in the figure captions, with an ×100 or ×60 Plan Apo i objective (Nikon) using a light-emitting diode illumination system combined with a complementary metal oxide semiconductor camera (Andor) for the detection. Single-cell tracking was performed using the NIH software and videos were assembled using ImageJ software.

**Live imaging data analysis.** For analysis of the optogenetics experiments, the NIH software was used. Regarding the analyses of MED1 clusters, for each nucleus background correction, a Gaussian–Laplace sharpen filter was applied. A threshold was set such that clusters were identified after the illumination. A single-cluster tracking was performed and the area of each cluster was determined. To identify the grouping of localizations based on their proximity to the centroid of the cluster, the area of each cluster was determined by a machine-learning algorithm. The area attributed to the cluster was the convex hull area associated with that set of localizations.

**Medaka fish.** The Cab strain of WT medaka fish (Oryzias latipes) was maintained following standard conditions (that is, 12 h/12 h dark/light conditions at 27 °C). Embryos were staged according to the method proposed by Iwamatsu⁷⁴. All studies on fish were conducted in strict accordance with the institutional guidelines for animal research and approved by the Italian Ministry of Health, Department of Public Health, Animal Health, Nutrition, and Food Safety.

**Kmt2d morpholine injections and drug treatments in medaka fish.** The available medaka olKmt2d (ENSORL00000009505.1) genomic sequences were retrieved from public databases (http://genome.ucsc.edu) from human KMT2D (NM_003682.3) transcript. A morpholine (MO; Gene Tools) was designed against the ATR initiation codon within the 5’ untranslated region of the medaka ortholog MOstart (5’-CCCTGGTCGTCGGTTGTACCTTTTGGTTG-3’), which is orthologous to the KMT2D gene. The specificity and inhibitory efficiencies of morpholinos were determined as previously described⁷⁵. MOstart was injected at a concentration of 0.015 mM into one blastomere at the one- to two-cell stage. Off-target effects of the morpholine injections were excluded by repeated experiments with control morpholinos or by coinjection with a P53 morpholine (MO-P53; 5’-CGGGAGATCGACCGACACACACTACG-3’). For the drug treatment, chonrons from injected and control embryos were removed with the hatchling enzyme at St3. From St34 onward, both morphant and control embryos were grown in 0.15 μM VE-822 diluted in 1% dimethyl sulfoxide and 1× Yamamoto for 5 d until St40. The solution was refreshed every 24 h. For the control experiments, the St34 morphant or control embryos were grown in 1× Yamamoto/1% dimethyl sulfoxide.

**Cartilage and bone staining in medaka fish.** Staining for cartilage (Alcian blue) and bone (Alizarin red) in fixed embryos was performed according to standard medaka skeleton phenotyping protocols (https://stigen.nig.ac.jp/medaka/medakaabook/index.php). Pictures were taken using DM5000 microscopy (Leica Microsystems). Transgenic medaka larvae were subjected to anesthesia before fixation at St40 by 2 h of incubation in 4% paraformaldehyde, 2× PBS and 0.1% Tween 20 at room temperature. After embedding in PBS/50% glycerol, confocal pictures were taken with a Zeiss LSM700 using 400- and 543-nm laser lines for GFP and mCh analysis, respectively. Imaging data were processed using ImageJ.

**Inelastic Brillouin scattering.** Brillouin scattering is an inelastic scattering process taking place when photons exchange energy with thermally excited acoustic waves or phonons⁶⁶. This causes a small red or blue frequency shift (Δν) of the scattered light corresponding to the emission or absorption of a phonon, respectively. This frequency shift is given by Δν = 2πνλ/λ₀, where λ is the incident wavelength, ρ and n are the density and material refractive index of the material, M is the longitudinal elastic modulus and θ is the scattering angle.

**Confocal Brillouin microscopy, data acquisition and analysis.** Brillouin scattering is exploited within Brillouin microscopy for reconstructing samples’ 3D images of mechanical properties in a non-invasive manner. In this work, Brillouin microscopy was combined with a confocal imaging set-up and a virtually imaged phased array (VIPA)-based spectrometer. The source light was emitted from a continuous-wave single-longitudinal mode laser at a wavelength of 532 nm (Oxxius) and focused onto the sample by an oil immersion objective lens (Olympus UPlanApo 100; numerical aperture = 1.4). The same lens was used to collect the backscattered light, providing a theoretical spatial resolution of 0.3 × 0.3 × 1.1 μm³. 3D image acquisition was performed thanks to a programmable motorized stage (Prior; HLD117TX). Finally, the collected light was focused by a single-mode optical fiber, filtered from the undesired elastic scattered light⁶⁷ and delivered to the spectrometer.

The spectrometer consisted of a modified solid Fabry–Pérot etalon with a free spectral range of 30 GHz (VIPA; LightMachinery; OP-6721-3371-2) that provided high (>50%) transmission efficiency thanks to an antireflection-coated entrance window that minimized entrance losses. Generally, in Brillouin microscopy, two or more crossed tandem-mounted VIPAs are used, reaching a contrast of 60 dB (ref. ⁷³). However, multistaged VIPAs mitigate the output efficiency, increasing the acquisition times. Our single-stage VIPA spectrometer allowed the registering of signals with a contrast of 80% higher than 40 dB. This, in combination with our filtering strategy, paved the way for fast-acquisition Brillouin microscopy imaging systems. For Brillouin imaging, cells were seeded at low density (sparse condition) on a µ-slide 4-well ibiTreat (ibidi) and cultured for 24 h. After removal of the medium, the cells were washed twice with PBS (Sigma–Aldrich), fixed with 4% paraformaldehyde (Sigma–Aldrich) for 15 min at room temperature, washed three times with PBS and then left in PBS for the acquisitions on the Brillouin microscope.

During data acquisitions, the stage longitudinal step size on the sample was 400 nm, the acquisition time was 100 ms and the optical power delivered to the sample was lower than 10 mW. For pixels-to-GHz conversion of Brillouin spectra, we acquired before each experiment as a reference the Brillouin spectrum of distilled water, which in our configuration had a Brillouin shift at 7.40 GHz. We acquired Stokes and anti-Stokes lines and fitted them with a sum of Lorentzian functions: the maps of Brillouin shifts reported are the center of Stokes- and anti-Stokes-fitted Lorentzian functions. Brillouin maps were produced by 2x resisting and a 3×3 mean filter. For analysis of the mean Brillouin shift across the nucleus, we used custom-made programs that automatically segmented different regions of the maps. We considered a nucleus to include all of the pixels above 7.70 GHz; the program then connected different parts of the images with the same shift. The data reported as bar graphs show the mean nuclear stiffness in different cells. All data analysis was performed using custom-made programs in MATLAB.

**Code availability** The custom-made code for quantifying the cytoplasmic/nuclear ratio of YAP/TAZ has been deposited in the public repository GitHub (https://github.com/SZambrano/RoutinesNucCytoYAP). The custom-made code for quantifying the Brillouin shift has been deposited in the public repository GitHub (https://github.com/ClaudiaBrill/CodeBrillouinFascian). Additional data supporting the findings of this study are available from the corresponding authors upon reasonable request. Source data are provided with this paper.

**References**
72. Tátrai, P. et al. Combined introduction of Bmi-1 and IERTERT immortalizes human adipose tissue-derived stromal cells with low risk of transformation. Biochem. Biophys. Res. Commun. 422, 28–35 (2012).
73. Ricci, M. A., Manzo, C., Garcia-Parajo, M. F., Lakadamyali, M. & Cosma, M. P. Chromatin fibers are formed by heterogeneous groups of nucleosomes in vivo. Cell 160, 1145–1158 (2015).
74. Iwamatsu, T. Stages of normal development in the medaka Oryzias latipes. Mech. Dev. 121, 605–618 (2004).
75. Conte, I. et al. MiR-204 is responsible for inherited retinal dystrophy associated with ocular coloboma. *Proc. Natl Acad. Sci. USA* **112**, E3236–E3245 (2015).

76. Lepert, G., Gouveia, R. M., Connan, C. J. & Paterson, C. Assessing corneal biomechanics with Brillouin spectro-microscopy. *Faraday Discuss.* **187**, 415–428 (2016).

77. Antonacci, G., Lepert, G., Paterson, C. & Torôk, P. Elastic suppression in Brillouin imaging by destructive interference. *Appl. Phys. Lett.* **107**, 061102 (2015).

Acknowledgements

We thank the Next-Generation Sequencing Facility at the Department of Cellular, Computational and Integrative Biology (CIBIO) for help with RNA sequencing, the Protein Technology Facility at CIBIO for recombinant protein purification, the Advanced Light and Electron Microscopy Bioimaging Center at the San Raffaele Scientific Institute (Milan, Italy) for STORM imaging acquisition and data analyses, the TIGEM Medaka Core Facility and F. G. Salerno for technical assistance and the CrestOptics-IIT JointLab for Advanced Microscopy for financial and technical support. We thank G. Merla (IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy). The Genomic and Genetic Disorders Biobank, a member of the Telethon Network of Genetic Biobanks (project number GTB12001), funded by Telethon Italy and of the EuroBioBank network, provided us with the human primary fibroblasts derived from either healthy donors or donors with Kabuki syndrome. We thank E. Biasini and the members of the Zippo laboratory for helpful discussions and critical reading of the manuscript. We thank D. Allis for sharing the H3.3K27M construct, R. Young for sharing the OptoIDR vectors and Z. Qiuping for the EGFP-Nesprin1/2-KASH vectors. The Mass Spectrometry and Proteomics Core Facility of CIBIO is supported by the European Regional Development Fund 2014–2020. Work in the Zippo group was supported by grants from the Italian Ministry of Health (GR-2011-02351172), Telethon Foundation (GEP13057) and AFM Telethon (AFM 21514). V.P. is the recipient of an AIRC fellowship (21158).

Author contributions

A.F. and A.Z. conceived of the study, designed the experiments and interpreted the data. S.D. performed the phase separation and immunofluorescence experiments and participated in data analyses. V.P. performed the cellular and molecular biology studies and participated in data analyses. L.F. and S.B. performed the RNA sequencing experiments and computational analyses. F.C. and D.M. performed the molecular biology studies. I.C., F.G., G.O., E.D. and S.Z. performed the computational analyses on imaging and genomic data. R.B. and D.P. performed the mass spectrometry experiments. D.I., C.S. and I.C. performed the in vivo assays in medaka fish. C.T., P.V. and G.R. performed the Brillouin microscopy and computational data analysis. A.Z. supervised the work and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41588-020-00724-8.

Supplementary information is available for this paper at https://doi.org/10.1038/s41588-020-00724-8.

Correspondence and requests for materials should be addressed to A.F. or A.Z.

Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
**Extended Data Fig. 1 | KMT2D haploinsufficiency affects MLL4 activity.**

**a.** Schematic representation of KMT2D gene and the corresponding MLL4 protein. The position of the inserted mutations in exon 39 and the relative changes in the coding sequences are highlighted.

**b.** qRT-PCR of KMT2D in WT and MLL4<sup>Q4092X</sup> MSCs, normalized on GAPDH level. Data are means ± SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis.

**c.** Western Blot analysis of MLL4 protein in WT and MLL4<sup>Q4092X</sup> MSCs by using a specific antibody recognizing a central portion of the protein; Lamin B1 was used as loading control.

**d.** qRT-PCR of KDM6A in WT and MLL4<sup>Q4092X</sup> MSCs, normalized on GAPDH level. Data are means ± SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis.

**e.** Representative images and quantifications of immunostaining for MLL4 in WT and MLL4<sup>Q4092X</sup> MSCs grown on the same coverslips. WT MSCs were pre-labelled with CellTrace Violet. Scale bar, 20 μm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; Student’s two-tailed unpaired t-test was applied for statistical analysis.

**f.** Representative images and quantifications of immunostaining for PA1 in WT and MLL4<sup>Q4092X</sup> MSCs. Scale bar, 20 μm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis.

**g.** Western Blot analysis of MLL4 and UTX in WT and MLL4<sup>P4093X</sup> MSCs; Lamin B1 was used as loading control (n=1).

**h.** Quantifications of immunostaining for MLL4, PA1, UTX, H3K4me1 and H3K27ac in WT and MLL4<sup>Q4092X</sup> MSCs. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; Student’s two-tailed unpaired t-test was applied for statistical analysis (**P<0.0001).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | KMT2D truncating mutations cause MLL4 LoF. a–d, Representative images and quantifications of immunostaining for MLL4 (a), H3K4me1 (b), H3K27ac (c) and UTX (d) in primary fibroblasts from healthy donor or Kabuki patients. Scale bar, 20 µm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student's t-test was applied for statistical analysis (***,P<0.0001). e, Quantifications of immunostaining for BRD4 and MED1 in WT and MLL4Q4092X MSCs grown on the same coverslips. WT MSCs were pre-labelled with CellTrace Violet Scale bar, 20 µm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student's t-test was applied for statistical analysis. f, Western Blot analysis of BRD4 and MED1 in WT and MLL4Q4092X MSCs; histone H3 was used as loading control. Signal quantifications are reported as bar plots. Data are means + SEM of 4 independent experiments for BRD4 and 5 independent experiments for MED1; one-tailed Student's t-test was applied for statistical analysis. g–i, Representative images and quantifications of cluster intensity for BRD4 and MED1 immunostaining in WT and MLL4Q4092X MSCs (g) on in primary fibroblasts from healthy donor or Kabuki patients (h, i). Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student's t-test was applied for statistical analysis (***,P<0.0001).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Effects of MLL4 LoF on the clustering of MED1 and BRD4. a, b, Representative images and 3D reconstruction of the distribution of BRD4 (a) and MED1 (b) clusters in WT and MLL4<sup>Q4092X</sup> MSCs; Scale bar, 20 µm. Quantification of the number of BRD4 and MED1 clusters in WT and MLL4<sup>Q4092X</sup> MSCs. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis. c, Graphical representation of the experimental design adopted to measure the clustering of the MED1-IDR tagged with mCherry and fused to the Cry2 module. A single blue light stimulation (488nm, 50% light intensity, 2 seconds) was applied (green bars) followed by acquisition of mCherry signal (red bars). d, Quantification of the fluorescence intensity of MED1-IDR, measured in WT (n=39) and MLL4<sup>Q4092X</sup> (n=47) MSCs at the indicated time points. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis. e, Representative images of light-stimulated MSCs expressing the mCherry-Cry2 construct, at different time points; scale bar, 10 µm. f, Representative images of light-induced clustering of MED1-IDR in MSCs, after a single pulse of stimulation (2 seconds, 488nm wavelength), stained for MLL4 and BRD4; scale bar, 10 µm. Pearson coefficient between MED-1DR and MLL4 or BRD4 was determined. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Characterization of the MLL4-specific PrLD. 

**a**, Representative images of dual immunostaining for MLL4 and BRD4 or MED1 in WT MSCs; scale bar, 10 µm. Asterisks indicate Golgi aspecific signal. Pearson coefficient between MLL4 and BRD4 or MED1 was determined. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n. 

**b**, Representative images of immunostaining for MLL4 in WT MSCs, and in cells carrying KMT2D truncating mutation on one (MLL4Q4092X/WT) or both alleles (MLL4Q4092X/Q4092X). 

**c**, Graphical representation of the Prion-like amino acid composition of MLL4 retrieved by PLAAC analysis. 

**d**, Analyses on the KMT2D gene showing the constrained coding regions (CCR), the known clinical variants (clinVar), the protein changing variants, and the conservation pattern (GERP). The square indicates the genomic regions codifying for the MLL4-PrLD. 

**e**, SDS-PAGE and Coomassie staining of purified MLL4-PrLD and MLL4-PrLDΔQ recombinant proteins. 

**f**, Phase separation of MLL4-PrLD recombinant protein visualized and quantified by fluorescence microscopy, in presence of increasing concentration of NaCl or 1,6-hexanediol; scale bar 5 µm. 

**g**, Phase separation of MLL4-PrLDΔQ at different protein concentrations; scale bar 5 µM. 

**h**, Measurements of the relative amount of condensed MLL4-PrLDΔQ versus protein concentration. Red line represents the regression line. 

**i**, Measurements of the number of formed MLL4-PrLD clusters versus the expression level in NIH3T3 cells. The quantification is the result of three independent experiments. Red line represents the regression line. 

**j**, Measurement of the mean fluorescent intensity in NIH3T3 cells expressing the OptoMLL4-PrLD or the OptoMLL4-PrLDΔQ, respectively. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n. 

Unpaired two-tailed Student’s t-test was applied for statistical analysis.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | KMT2D truncating mutations affect PcG clustering. a, Western Blot analysis of EED, EZH2 and SUZ12 in WT and MLL4<sup>54092X</sup> MSCs; histone H3 was used as loading control. b, qRT-PCR of BMI and RING1B in WT and MLL4<sup>54092X</sup> MSCs, normalized on GAPDH level. Data are means + SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis. c, Quantifications of immunostaining for H3K27me3, BMI, and RING1B in WT and MLL4<sup>54092X</sup> MSCs. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (** **P < 0.0001). d-f, Representative images and quantifications of immunostaining for H3K27me3 (d), BMI (e), and RING1B (f) in primary fibroblasts from healthy donor or Kabuki patients; scale bar, 20 μm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (** **P < 0.0001). g, qRT-PCR of KMT2D in WT and MLL4<sup>54092X</sup> MSCs, expressing CRISPRa with or without crRNA targeting KMT2D promoter. Retrieved data were normalized on GAPDH level. Data are means + SEM (n=4 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis. h, i, Quantifications of immunostaining for MLL4, H3K4me1, BMI, RING1B and H3K27me3 in WT and MLL4<sup>54092X</sup> MSCs expressing CRISPRa with or without crRNA targeting KMT2D promoter. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the normalized fluorescence intensity. The number of analyzed nuclei is reported in figure as n; Student’s two-tailed unpaired t-test was applied for statistical analysis (** **P < 0.0001).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | MLL4 abundance modulates chromatin compaction and nuclear architecture. a, b Reconstructed 3D images of nuclei retrieved from images of WT and MLL4Δ4093X MSCs (a) or primary fibroblasts from healthy donor and Kabuki patients (b). Scale bar, 5 µm. The nuclear area, volume and flattening were determined and represented as box plots indicating the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).

c Measurements of nuclear shape in WT and MLL4Δ4093X MSCs expressing CRISPRa with or without crRNA targeting KMT2D promoter. The nuclear area, volume and flattening were represented as box plots indicating the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).

d qRT-PCR of LMNA in WT and MLL4Δ4093X MSCs, normalized on GAPDH level. Data are means ± SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis. e Quantifications of immunostaining for LMNA and phosphorylated LMNA/C (pLMNA) in WT and MLL4Δ4093X MSCs. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).

f, g Representative images and quantifications of cellular area detected by Phalloidin staining in WT and MLL4Δ4093X MSCs (f) or in the same cells expressing either EGFP or EGFP-Nesprin-KASH (g). Scale bar, 20 µm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the cellular area. The number of analyzed cells is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).

h, i Representative images and quantifications of immunostaining for H4K16ac in WT and MLL4Δ4093X MSCs (h) or in WT primary fibroblasts from healthy donor or Kabuki patients (i). Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the normalized fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).
Extended Data Fig. 7 | Rescuing chromatin compartmentalization re-establishes nuclear mechanical properties. a, b Representative images and quantifications of immunostaining for H3K27me3 (a) and BMI (b) in WT and MLL4Q4092X MSCs untreated or treated with TSA (1.5 µM for 8h). Scale bar, 20 µm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the normalized fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).

Representative images and quantification of immunostaining for H3K27me3 in WT, MLL4Q4092X or MLL4Q4092X MSCs expressing either H3.3WT or H3.3K27M. Scale bar, 20 µm. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the normalized fluorescence intensity. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).

d Representative maps of stiffness distribution in WT, MLL4Q4092X MSCs, MLL4Q4092X MSCs expressing H3.3K27M or MLL4Q4092X MSCs treated with TSA for 24h; Scale bars, 10 µm. Bar plot representing nuclear Brillouin shift in the different conditions. Data are means ± SEM (n=4 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).
Extended Data Fig. 8 | Clustering dynamics of PcG-associated condensates. 

**a**  Quantification of the number and the area of the light-induced droplets of BMI-Cry2 at different time points. First panel shows the distribution changes in the time window of 300 seconds post-stimulation. Second panel shows the formation and disassembly of BMI-Cry2 clusters within a time window of 35 minutes.

**b**  Quantification of the relative frequency of small (<0.1 \(\mu m^2\)) and large (>0.1 \(\mu m^2\)) droplets of BMI-Cry2 at different time points.

**c**  Representative images of dual immunostaining for BMI1 and RING1B (upper panels) or H3K27me3 (middle panels) or BRD4 (lower panels) in WT MSCs; scale bar, 10 \(\mu m\). Pearson coefficient was represented as box plots, indicating the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n.

**d**  Representative images of immunostaining for H3K27me3 and RING1B in NIH3T3 expressing BMI-Cry2, after light-induced clustering. Scale bar, 10 \(\mu m\). Pearson Coefficient between BMI-Cry2 and H3K27me3 or RING1B was represented as box plots indicating the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Targeting ATR rescues the YAP/TAZ activity. **a** Quantifications of immunostaining for YAP/TAZ in WT and MLL4\(^{\text{Q4092X}}\) MSCs. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001). **b** Western Blot analysis of YAP, TAZ and phosphorylated YAP (Ser127) in WT and MLL4\(^{\text{Q4092X}}\) MSCs; GAPDH was used as loading control. **c** Representative images of the distribution of ATR in WT and MLL4\(^{\text{Q4092X}}\) MSCs. Scale bar, 20 \(\mu\)m. **d** Representative images and quantifications of immunostaining for YAP/TAZ in WT and MLL4\(^{\text{Q4092X}}\) MSCs untreated or treated with different concentrations of the ATR inhibitor VE-822. Scale bar, 20 \(\mu\)m. Data are represented as box plots, indicating the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001). **e** Representative images and quantification of phH2A.X in WT and MLL4\(^{\text{Q4092X}}\) MSCs. Scale bar, 20 \(\mu\)m. Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars) of the phH2A.X distribution per cell. The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis. **f** Volcano plot of differentially expressed genes between WT and MLL4\(^{\text{Q4092X}}\) MSCs. Vertical blue lines indicate the chosen cutoff (−2>fold change>2), horizontal red line indicates Wald test-derived p-value cutoff of 0.05. **g** Heat map of k-means clustering analysis of expressed genes in WT MSCs untreated or treated with ATR inhibitor at the indicated time points. **h** Venn diagram showing the overlap between the ATR responsive genes identified in WT and MLL4\(^{\text{Q4092X}}\) MSCs. **i** qRT-PCR of the indicated genes in WT, MLL4\(^{\text{Q4092X}}\) (i) and MLL4\(^{\text{Q4093X}}\) MSCs (k) untreated or treated with ATR inhibitor at the different time points. The expression level was normalized on GAPDH level. Data are means + SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.001).
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Targeting ATR restores chondrogenesis and osteogenesis. a qRT-PCR of chromatin architecture genes in WT and MLL4Q4092X MSCs expressing CRISPRa with or without crRNA targeting KMT2D promoter. Retrieved data were normalized on GAPDH level. Data are means ± SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis. b, c Quantifications of immunostaining for YAP/TAZ in WT and MLL4Q4092X MSCs expressing CRISPRa with or without crRNA targeting KMT2D promoter (b) or in WT and MLL4Q4092X MSCs expressing either H3.3WT or H3.3K27M (c). Box plots indicate the median (middle line), the first and third quartiles (box), and the 10th and 90th percentile (error bars). The number of analyzed nuclei is reported in figure as n; unpaired two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001). d Differentiation of WT and MLL4Q4092X MSCs into adipocytes, osteocytes or chondrocytes was detected by Oil red, Alizarin red or by Alcian blue staining, respectively. Scale bar, 100 μm. e qRT-PCR of the indicated genes during differentiation of WT and MLL4Q4092X MSCs towards chondrocytes. The expression level was normalized on GAPDH level. Data are means ± SEM (n=3 independent experiments); unpaired two-tailed Student’s t-test was applied for statistical analysis. f Chondrogenic differentiation of WT and MLL4Q4092X MSCs or MLL4Q4092X MSCs expressing exogenous MLL4 was detected by Alcian blue staining. Scale bar, 500 μm. g Bar plot of the relative quantification of Ethmoid plate (EP), Palatoquadrate (PQ), Ceratohyal (CH) and Ceretobranchials 1 to 5 (CBs) cartilage length in Ctrl, KMT2D 5'UTR-morpholino and KMT2D 5'UTR-morpholino-treated with ATRi inhibitor. Data are means ± SEM (n=10 animals; n=12 animals for CH quantification in the KMT2D morphants); two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001). h Bar plot of the relative quantification of Palatoquadrate (PQ), Ceratohyal (CH), Operculum (OP), Cleithrum (CL) and fifth ceratobranchial (CB) cartilage length in KMT2D 5'UTR-morpholino and ATRi-treated KMT2D 5'UTR-morpholino medaka fish. Data are means ± SEM (n=10 animals); two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001). k Bar plot of the relative quantification of Palatoquadrate (PQ), Ceratohyal (CH), Operculum (OP), Cleithrum (CL) and fifth ceratobranchial (CB) mineralization in KMT2D 5'UTR-morpholino and ATRi-treated KMT2D 5'UTR-morpholino medaka fish. Data are means ± SEM (n=10 animals); two-tailed Student’s t-test was applied for statistical analysis (****P<0.0001).
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.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- [ ] A statement on 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
- [ ] 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- LAS AF Software 2.6.0 was used for confocal imaging; NIS Elements AR 5.11 for time lapse experiments; Image lab 2.0.1 software was used for acquisition of western blot images.

Data analysis

- To quantify the nuclear to cytosolic localization of YAP-TAZ, we adapted MATLAB (R2019a) routines, deposited in the public repository github (https://github.com/SZambranoS/RoutinesNucCytoYAP).
- For 3D Imaging cluster data analyses an implementation of the algorithm from Gregoretti et al., 2016 (DOI: 10.1007/978-1-4939-6380-5_16) was adopted.
- For STORM images data analysis STORM images data analysis we followed the routine described in Ricci et al., 2015 (DOI: 10.1016/j.cell.2015.01.054).
- Fiji (1.52) was used for immunofluorescence data analysis.
- NIS elements (AR 5.11) was used for time-lapse experiments analysis
- For Differential Gene expression analysis, Raw reads from fastq files were first checked for their quality using FastQC and trimmed using Trimmomatic
- 3'UTR RNA-seq: FastQC 0.11.8, trimmomatic 0.39, STAR 2.7.0, Clust 1.10.7, Bedtools 2.28.0, HOMER 4.91

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. 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 RNA-seq raw data are deposited in Gene Expression Omnibus (GEO) database under the accession GSE135550. Raw data from RNA-seq were analyzed and described in the following figures and table: Fig. 7, Extended Data Fig. 9, Supplementary Table 1, Supplementary Table 2.

The custom made code for quantifying the cytoplasmic/nuclear ratio of YAP/TAZ is deposited in the public repository github (https://github.com/SZambranoS/ RoutinesNucCytoYAP).

**Field-specific reporting**

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

**Life sciences study design**

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

| Sample size | Two independent clones were derived and analyzed for both WT and KMT2D mutant MSCs. This design is generally accepted for CRISPR-based genome editing as it permits to assess the reproducibility of the data. Each cell line was analyzed in at least three independent experiments. Two independent samples of primary fibroblasts derived from healthy and KS donors were also considered for the analyses. This design permits to determine the data reproducibility independently from the genetic mutations affecting KMT2D. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded |
| Replication | All experiments were performed in at least three biologically independent replicates. All attempts at replication were successful and used for statistical analyses. |
| Randomization | Two independent clones of MSCs for each genotype (WT and KMT2D mutant) were randomly selected and independently analyzed. |
| Blinding | All the experiments were conducted using the same experimental conditions, both in vitro (cell culture) and in vivo (Medaka fish). For this reason data collection and analyses of all studies were conducted not blinding. |

**Reporting for specific materials, systems and methods**

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | n/a |
|---------------------------------|-----|
| Antibodies | Involved in the study |
| Eukaryotic cell lines | Involved in the study |
| Palaeontology | n/a |
| Animals and other organisms | n/a |
| Human research participants | n/a |
| Clinical data | n/a |

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

**Antibodies**

Antibodies used

- Target Company Code Application
  - MLL4 Sigma HPA035977 IF/WB
  - UTX Cell Signaling 33510 IF/WB
  - PA1 Bethyl A301-978A IF/WB
  - WRD5 Bethyl A302-430A WB
  - Histone H3 Cell Signaling 9715 IF/WB
  - BRD4 abcam ab128874 IF/WB
Validation

[MLL4_Sigma_HPA035977]: the antibody has been validated in immunohistochemistry, immunofluorescence and chromatin immunoprecipitation (doi: 10.1038/nm.3940, doi: 10.1007/s00401-012-1070-9). Species Reactivity: Human.

[UTX_Cell Signaling_33510]: the antibody has been validated for western blotting, immunohistochemistry and immunoprecipitation (doi: 10.15252/embj.2019102808). Species Reactivity: Human, Mouse, Rat, Monkey.

[PA1_Bethyl_A301-978A]: the antibody has been validated for western blotting and immunoprecipitation (doi: 10.1371/journal.pone.0225180). Species Reactivity: Human, Mouse, Rat, Monkey.

[KMT2D_Sigma_AMAB91368]: the antibody has been validated for western blotting, immunohistochemistry and immunoprecipitation (doi: 10.1016/j.cellrep.2019.06.065). Species Reactivity: Human, Mouse, Rat, Monkey.

[Lamin A_Santa Cruz_sc-71481]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation (doi: 10.1016/j.cellrep.2019.02.102). Species Reactivity: Human, Mouse, Rat, Monkey.

[phospho-Lamin A/C_Santa Cruz_sc-71481]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation (doi: 10.1016/j.cellrep.2019.12.057; doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.

[BRD4_abcam_ab128874]: the antibody has been validated for western blotting, immunocitochemistry, immunofluorescence and immunoprecipitation (doi: 10.1126/science.aar3958). Species Reactivity: Human, Mouse, Rat.

[BMI1_Millipore_05-637]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation (doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.

[BMI1_Millipore_05-637]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation (doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.

[BRD4_abcam_ab128874]: the antibody has been validated for western blotting, immunocitochemistry, immunofluorescence and immunoprecipitation (doi: 10.1126/science.aar3958). Species Reactivity: Human, Mouse, Rat.

[H3K7me3_Millipore_07-449]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation (doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.

[H3K7me3_Millipore_07-449]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation (doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.

[H3K4me1_Abcam_ab8895]: the antibody has been validated for western blotting, immunoprecipitation and immunocitochemistry. Species Reactivity: Human, Mouse, Rat, Monkey.

[H3K4me1_Abcam_ab8895]: the antibody has been validated for western blotting, immunocomplex formation and immunoprecipitation. Species Reactivity: Human, Mouse, Rat, Monkey.

[YAP_TAZ_Cell Signaling_8418]: the antibody has been validated for western blotting and immunoprecipitation. Species Reactivity: Human, Mouse and Monkey. For immunofluorescence, the antibody was tested for its specificity comparing the signal to noise ratio to the isotype control. (doi: 10.7554/eLife.20982).

[YAP_Santa Cruz_sc-271134]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation ( doi: 10.1371/journal.pone.0225180). Species Reactivity: Human, Mouse and Rat.

[YAP1 (phospho Ser127)_GeneTex_GTX130424]: the antibody has been validated for western blotting. Species Reactivity: Human, Mouse and Rat.

[H4K16ac_Millipore_06-762]: the antibody has been validated for western blotting and immunoprecipitation ( doi: 10.1126/science.aar3958). Species Reactivity: Human, Mouse, Rat.

[H3K27ac_Abcam_ab4729]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation ( doi: 10.1016/j.cellrep.2019.12.057; doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.

[H3K27ac_Abcam_ab4729]: the antibody has been validated for western blotting, immunocitochemistry and immunoprecipitation ( doi: 10.1016/j.cellrep.2019.12.057; doi: 10.1186/s13046-019-1492-5). Species Reactivity: Human, Mouse, Rat, Monkey.
immunohistochemistry. Reactivity: Human. For immunofluorescence, the antibody was tested for its specificity comparing the signal to noise ratio to the isotype control.

[ALR (MLL4)_BETHYL_A300-BL1185]: the antibody has been validated for western blotting (doi: 10.1038/nm.3940).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  hTERT-immortalized human adipose-derived MSCs are a kind gift from Dr. Peter Tatrai. This cell line is not commercially available.
NIH 3T3 and HEK293T are commercially available cell lines from ATCC.

human primary fibroblasts derived from either healthy or Kabuki patients have been bought from Genomic and Genetic Disorders Biobank.

Authentication  None of the cell lines used have been authenticated.

Mycoplasma contamination  All cell lines resulted negative for mycoplasma contamination.

Commonly misidentified lines  No misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals  WT medaka fish (Oryzias latipes), Cab-strain. Medaka fish embryos were all analyzed at the embryonic stage St40. Given the early stage of embryogenesis it was not possible to determine the sex of the examined fishes.

Wild animals  The study did not involve wild animals.

Field-collected samples  The study did not involve field-collected samples.

Ethics oversight  All studies on fish were conducted in strict accordance with the institutional guidelines for animal research and approved by the Italian Ministry of Health; Department of Public Health, Animal Health, Nutrition, and Food Safety in accordance to the law on animal experimentation (D.Lgs. 26/2014). The ethical committee of the TIGEM Institute, (Italy) reviewed and approved all animal treatments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.