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## **KAT2A complexes ATAC and SAGA play unique roles in cell maintenance and identity in hematopoiesis and leukemia**

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### **Abstract:**

Epigenetic histone modifiers are key regulators of cell fate decisions in normal and malignant hematopoiesis. Their enzymatic activities are of particular significance as putative therapeutic targets in leukemia. In contrast, less is known about the contextual role in which those enzymatic activities are exercised, and specifically, how different macromolecular complexes configure the same enzymatic activity with distinct molecular and cellular consequences. We focus on KAT2A, a lysine acetyltransferase responsible for Histone 3 Lysine 9 acetylation, which we recently identified as a dependence in Acute Myeloid Leukemia stem cells, and that participates in 2 distinct macromolecular complexes: Ada Two-A-Containing (ATAC) and Spt-Ada-Gcn5-Acetyltransferase (SAGA). Through analysis of human cord blood hematopoietic stem cells and progenitors, and of myeloid leukemia cells, we identify unique respective contributions of the ATAC complex to regulation of biosynthetic activity in undifferentiated self-renewing cells, and of the SAGA complex to stabilisation or correct progression of cell type-specific programs with putative preservation of cell identity. Cell type and stage-specific dependencies on ATAC and SAGA-regulated programs explain multi-level KAT2A requirements in leukemia and in erythroid lineage specification and development. Importantly, they set a paradigm against which lineage specification and identity can be explored across developmental stem cell systems.

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## **KAT2A complexes ATAC and SAGA play unique roles in cell maintenance and identity in hematopoiesis and leukemia**

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## KEY POINTS

1. KAT2A is required at different stages of human cord blood erythroid development with ATAC and SAGA complex specificity
2. KAT2A regulates leukemia cell maintenance and identity through specific participation in ATAC and SAGA complexes

## ABSTRACT

*Epigenetic histone modifiers are key regulators of cell fate decisions in normal and malignant hematopoiesis. Their enzymatic activities are of particular significance as putative therapeutic targets in leukemia. In contrast, less is known about the contextual role in which those enzymatic activities are exercised, and specifically, how different macromolecular complexes configure the same enzymatic activity with distinct molecular and cellular consequences. We focus on KAT2A, a lysine acetyltransferase responsible for Histone 3 Lysine 9 acetylation, which we recently identified as a dependence in Acute Myeloid Leukemia stem cells, and that participates in 2 distinct macromolecular complexes: Ada Two-A-Containing (ATAC) and Spt-Ada-Gcn5-Acetyltransferase (SAGA). Through analysis of human cord blood hematopoietic stem cells and progenitors, and of myeloid leukemia cells, we identify unique respective contributions of the ATAC complex to regulation of biosynthetic activity in undifferentiated self-renewing cells, and of the SAGA complex to stabilisation or correct progression of cell type-specific programs with putative preservation of cell identity. Cell type and stage-specific dependencies on ATAC and SAGA-regulated programs explain multi-level KAT2A requirements in leukemia and in erythroid lineage specification and development. Importantly, they set a paradigm against which lineage specification and identity can be explored across developmental stem cell systems.*

## INTRODUCTION

KAT2A is a histone acetyltransferase required for correct mesodermal specification in the developing mouse embryo<sup>1</sup>. It stabilises pluripotency of mouse embryonic stem cells<sup>2</sup>, and is required for survival of neural stem and progenitor cells<sup>3</sup>. In hematopoiesis, Kat2a has been shown to regulate proliferation and activation of T cell subsets<sup>4</sup>, and maturation of invariant natural killer T (iNKT) cells<sup>5</sup>, and it restricts terminal differentiation of granulocytic cells<sup>6</sup>. We<sup>7</sup> and others<sup>6</sup> have not found that Kat2a play a central role in hematopoietic stem cells. In contrast, we have identified KAT2A as a requirement in Acute Myeloid Leukemia (AML) cell lines and patient samples<sup>8</sup>. Using a conditional knockout mouse model, we showed that

Kat2a loss results in transcriptional instability of general metabolic regulation programs such as translation and ribosomal protein synthesis, leading to probabilistic loss of functional leukemia stem-like cells<sup>7</sup>. Kat2a exerts transcriptional control through histone or non-histone lysine acetylation, including in the examples cited within the hematopoietic system. Specifically, regulation of leukemia stem-like cells<sup>7</sup> and control of T-cell subset activation<sup>4</sup> are linked to histone 3 lysine 9 acetylation (H3K9ac) at gene promoters, a role that is evolutionary conserved from the original identification of Gcn5 in yeast<sup>9</sup>, and associated with transcriptional activation<sup>10</sup>. Granulocytic and iNKT cell maturation, on the other hand, depend on protein acetylation of key transcriptional regulators, respectively Cebpa<sup>6</sup> and Egr2<sup>5</sup>. More recently, Kat2a has been shown to catalyse histone succinylation, another acyl modification which, like H3K9ac, is associated with transcriptional activation<sup>11</sup>.

KAT2A exerts its activity in the context of 2 macromolecular complexes, Ada-Two-A-Containing (ATAC) and Spt-Ada-Gcn5-Acetyltransferase (SAGA) (Fig. 1A). These complexes share the histone acetyl-transferase (HAT) activity by KAT2A, with a single subunit difference within the HAT module (TADA2A in ATAC and TADA2B in SAGA)<sup>12</sup>. Additionally, the 2 complexes exhibit different chromatin specificities and regulate distinct sets of genes<sup>13</sup>. Integration of KAT2A in either complex is a requirement for its full HAT activity<sup>14</sup>, however analysis of their differential contributions to KAT2A function in a given system has not been performed to date. The yeast KAT2A-containing SAGA was the first multi-modular HAT complex to be isolated<sup>10</sup>. Since then, several studies unveiled its molecular architecture: SAGA comprises 19 subunits organised in 4 functionally distinct modules<sup>15</sup>, a structure highly conserved from yeast to human<sup>12</sup>. In addition to the KAT2A participating HAT module, which also includes TADA2B, TADA3 and CCDC101 (SGF29), SAGA contains: (1) a H2B de-ubiquitination (DUB) module centred on USP22 enzymatic activity; (2) a Core module comprising SPT20, which is specific to SAGA, and 5 TATA-binding protein associated factors (TAFs), 3 of which are shared with TFIID; and (3) the transcription factor interaction module, TRRAP. Two recent reports on the Cryo-EM structure of the yeast SAGA complex described the interactions between the different modules with key implications for gene activation<sup>16,17</sup>. The SAGA Core module contains an octamer-like fold that facilitates TBP loading onto TATA promoters<sup>17</sup>; the two enzymatic HAT and DUB modules connect flexibly to the Core<sup>16,17</sup>, suggesting functional independence between them. The ATAC complex, in turn, is exclusive to multicellular eukaryotes<sup>12</sup>. It was first linked to chromatin remodelling functions in *Drosophila*<sup>18</sup>, where it preferentially targets histone H4<sup>19</sup>. ATAC histone substrates in mammalian cells are less clear, although human

ATAC preferentially modifies H3<sup>20</sup>. ATAC specific elements include DNA-binding subunit ZZZ3, HAT module component TADA2A, and YEATS2, which is required for assembly of the ATAC complex and has acetyl-reading activity<sup>21</sup>. Moreover, ATAC comprises additional HAT activity by KAT14, which is essential for ATAC assembly and required in embryonic development<sup>22</sup>. Additionally, both KAT2A-containing complexes have been implicated in malignant transformation<sup>23</sup>. ATAC-YEATS2 was shown to be highly amplified in non-small cell lung cancer (NSCLC) and required for malignant cell survival<sup>21</sup>. SAGA complex cofactor TRRAP interacts with multiple proteins key to oncogenesis, such as c-Myc and E2F proteins<sup>24</sup>. Expression of SAGA *USP22* associates with an oncogenic signature of poor prognosis<sup>25</sup>. However, recent studies propose a tumor suppressor function of *USP22*<sup>26</sup>, including in AML<sup>27</sup>, suggesting dependency on cell context for functional consequences and fate decisions.

Herein, we attempted to characterize the role of KAT2A-containing ATAC and SAGA complexes in normal and leukemic blood cell function. We identified unique ATAC-specific requirements for specification or propagation of early erythroid-committed progenitors, which were distinct from the participation of the SAGA complex in progression of erythroid differentiation. In AML cells, ATAC and SAGA controlled distinct aspects of biosynthetic maintenance and identity preservation of leukemia cells, thus suggesting a dichotomy between ATAC and SAGA-centred KAT2A functions that has implications for normal and malignant developmental decisions.

## METHODS

### Cell lines

K562, MOLM13 and Kasumi-1 lines (kind gift from Brian Huntly, Cambridge, UK) and KG1a (kind gift from Joanna Baxter, Cambridge Blood and Stem Cell Biobank) were maintained in RPMI supplemented with 20% FBS (Fetal Bovine Serum, Invitrogen), 1% Penicillin/Streptomycin/Amphotericin (P/S/A, Invitrogen) and 2mM L-Gln. HEK 293T cells were grown in DMEM supplemented with 10% FBS, and P/S/A and L-Gln as above. Cultures were kept at 37°C and 5% CO<sub>2</sub>.

### Human CB CD34+ cells and AML patient samples

CB samples and AML patient samples were obtained with informed consent under local ethical approval, REC 07MRE05-44 (Cambridge) and EC code number 94/2016/O/Tess (Bologna). Mononuclear cells (MNC) were isolated by Ficoll-Paque density gradient

(StemCell Technologies) centrifugation. CB MNC were enriched for CD34<sup>+</sup> cells using the RosetteSep™ Human CB CD34 Pre-Enrichment Cocktail (Stem Cell Technologies) as per manufacturer's instructions.

### **Lentiviral packaging and transduction**

Viral constructs containing control shRNA (CTRLsh, non-eukaryotic gene targeting) or shRNA targeting *KAT2A* (KAT2Ash), *SGF29* (CCDC101sh), *SUPT20H* (SUPT20Hsh and SUPT20sh2) *USP22* (USP22sh), *ZZZ3* (ZZZ3sh), *TADA2A* (TADA2Ash and TADA2Ash2), and *TADA2B* (TADA2Bsh) (Supplemental Table 1) were packaged in HEK 293T cells as previously described<sup>28</sup>, using Turbofect (Thermo) or trans-IT (Mirus) as lipofection reagents. Cell lines were transduced overnight with 1-2 T75 packaging flask-equivalents (FE)/10<sup>6</sup> cells and washed the following day as described<sup>28</sup>. GFP<sup>+</sup> cells were sorted and used in downstream assays 4 days later. CD34<sup>+</sup> CB cells were pre-stimulated in serum-free medium (HSC expansion medium XF, Miltenyi Biotec) with SCF, TPO and Flt3L (respectively 200, 20 and 20 ng/mL) for up to 24h and transduced overnight using 2FE/2-3\*10<sup>5</sup> cells. Cells were washed the following day and cultured in half the cytokine concentration for an additional 2-3 days prior to sorting.

### **Colony-forming cell (CFC) assays**

Sorted CB cells were seeded in methyl-cellulose-based semi-solid medium (StemMACS HSC-CFU Media complete with EPO, Miltenyi Biotec) for assessment of multi-lineage colony-forming activity. Sorted cells were counted and resuspended in IMDM 10% FBS supplemented with P/S/A and 2mM L-Gln prior to addition to the semi-solid medium, which was plated in duplicate onto 35mm-dishes, with 200-300 cells/dish. In the case of CFC assays performed in the presence of the KAT2A inhibitor MB-3 (Abcam), this was added to the methylcellulose in a final concentration of 200μM, and dispersed by vortexing, prior to addition of CB cells, as described<sup>8</sup>. Colonies were scored by microscopy at 10-12 days at the point of full colony hemoglobinization.

### **Mixed lineage differentiation cultures**

CB CD34<sup>+</sup> cells were cultured for up to 7 days in serum-free liquid culture (StemSpan, Stem Cell Technologies) in the presence of SCF, Flt3-L, IL-3, and IL-6 (1x CC100, Stem Cell Technologies) and EPO 3U/μl (R&D). Transduced CD34<sup>+</sup> / HSC were seeded as GFP<sup>+</sup> cells

immediately after sorting. In other experiments, enriched CD34<sup>+</sup> cells were resuspended in serum-free medium and cytokines, in the presence on MB-3 (Abcam) 100 $\mu$ M. Fresh cytokines and, where appropriate, inhibitor and vehicle DMSO, were added every 2-3 days. At different time points, cells were tested by flow cytometry for the presence of CD34, CD13, and CD71/CD235a surface markers.

### **Erythroid differentiation and induction cultures**

Transduced HSC were tested in erythroid differentiation conditions in serum-free liquid culture (HSC expansion medium XF, Miltenyi Biotec) in the presence of hydrocortisone ( $10^{-5}$  M), SCF (100 ng/mL), TPO (10 ng/mL) and EPO (3U/mL). Cultures were followed up for 9 days with daily live and dead cell counts and regular flow cytometry analysis for CD34, CD71 and CD235a markers (Supplemental Table 4). K562 cells were induced to the erythroid lineage in the presence of 1.5% DMSO as described<sup>29</sup>. Cells were monitored for differentiation markers (CD71 and CD235a) and/or activation of erythroid-affiliated molecular programs during a 6-day culture period.

### **AML-MS5 co-culture**

MS5 murine bone marrow stromal cells were grown in IMDM supplemented with 10% FBS, 1% P/S/A and 2mM L-Gln. Cells were sub-cultured twice a week and passage 2 was used to carry out experiments in 48-well plates. AML cells were thawed in PBS 2% FBS, 1% P/S/A and pre-stimulated with H5100 (Stem Cell Technologies) supplemented with IL-3 20 ng/mL, G-CSF 20 ng/mL and TPO 20ng/mL (3GT, 1% HEPES, 1% P/S/A for approximately 4h. Cells were then transduced with the desired lentiviral constructs in transduction media: H5100 with SCF (100 ng/mL), Flt-3 (100 ng/mL), IL-3 (60 ng/mL), TPO (10 ng/mL), 1% HEPES, 1% P/S/A. Cells were washed twice in PBS 2% FBS, 1% P/S/A and once in H5100 1% P/S/A and seeded onto MS5 stroma. Cultures were kept at 37°C, 5% CO<sub>2</sub> for up to three weeks, with twice-weekly demi-population and fresh addition of H5100-3GT. For KAT2A inhibition experiments, AML MNC were seeded onto MS-5 stroma in H5100-3GT in the presence of 100 $\mu$ M of MB-3 (Abcam) or DMSO (0.1%), and demi-populated as above with fresh addition of MB-3. Cells were stained for flow cytometry analysis once a week (Supplemental Table 4).



### Chromatin immunoprecipitation (ChIP)

For ChIP-sequencing, chromatin was prepared in duplicate from K562 cells (9 sonication cycles, 30"ON/30"OFF) and immunoprecipitated using anti-SPT20 and anti-ZZZ3 sera prepared in the Tora Lab<sup>13</sup>. All procedures, including library preparation and sequencing were performed as described<sup>7</sup>. For ChIP-qPCR, K562 cells were immunoprecipitated with anti-H3K9ac antibody or rabbit IgG (Supplemental Table 2); eluted DNA was diluted and quantified by SYBR green qPCR using 2 $\mu$ l DNA per triplicate reaction (primers in Supplemental Table 3). Peak enrichments relative to rabbit IgG were determined using the  $2^{\Delta\Delta Ct}$  method with a reference intergenic region or *KRT5*.

### Statistical analysis

Statistical analysis was performed in GraphPad Prism 7 software (GraphPad Software). Data are reported as mean  $\pm$  SEM. Significance calculated by 2-tailed t-test at  $p < 0.05$  (see individual figure legends for details).

## RESULTS

### *KAT2A-containing ATAC and SAGA complexes have unique targets in hematopoietic cells*

In order to identify unique ATAC and SAGA targets and characterize complex-specific roles of KAT2A in the blood system, we performed chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) of ZZZ3 (ATAC-specific) and SPT20 (SAGA-specific) subunits (Fig. 1A) in human K562 cells. K562 are a chronic myelogenous leukemia cell line with multilineage potential: cells display aspects of molecular differentiation into erythroid, megakaryocytic and macrophage identities under defined cytokine conditions<sup>29</sup>; in steady state, K562 cells represent immature leukemia blasts with self-renewal properties. K562 cells have been previously used as a model for understanding molecular mechanisms of normal, particularly erythroid, and malignant blood specification<sup>30-33</sup>. We made use of specific sera against human ZZZ3 and SPT20 and performed ChIP-seq experiments in duplicate in self-renewing K562 cells. As in previous reports<sup>13</sup>, we found limited overlap between ATAC and SAGA targets (Fig. 1B; Supplemental File 1). Bound peaks were preferentially found in the vicinity of the transcriptional start site (TSS) (Fig. 1C) and were robustly enriched for ZZZ3 and SPT20 experimental targets, respectively (Fig. S1A-B), as catalogued in the ENCODE database<sup>34</sup>. ZZZ3 peaks were more proximal than SPT20's, in contrast with previously described enhancer association of ATAC complexes in lymphoblast

and HeLa cells<sup>13</sup>. Our results are nevertheless consistent with a distinct *ZZZ3* ChIP-seq experiment in human NSCLC cells, which revealed strong enrichment of *ZZZ3* peaks in regions  $\pm 1$  kb of TSS<sup>21</sup>. This suggests that ATAC-complex enhancer region occupancy may be cell type or context-dependent. Interrogation of functional associations of *ZZZ3* and SPT20 peaks highlighted complex-specific biologies (Fig. S1C-D). These are reflected in distinct gene ontology categories associated with complex-specific peaks, which encompass RNA and ribosomal metabolism in the case of ATAC (Fig. 1D and S1C) and transcriptional activity for SAGA (Fig. S1D). Moreover, SAGA specifically binds red blood cell-associated genes (Fig. 1D), which may indicate a unique role in erythroid differentiation or identity. Inspection of publicly available K562 ChIP-seq datasets in the ENCODE database confirmed that SPT20 and *ZZZ3*-bound regions coincided with H3K9ac peaks, a hallmark of KAT2A enzymatic activity (Fig. 1E)<sup>9</sup>. By making use of lentiviral-delivered shRNA targeting SPT20 or *ZZZ3*, we verified that selected SAGA and ATAC ChIP-seq target loci, did indeed reduce promoter H3K9ac (Fig. 1F) upon *SUPT20H* and *ZZZ3* knockdown (Fig. 1G, S1E), respectively. The same target loci had reduced gene expression upon *SUPT20H* or *ZZZ3* loss, suggesting that the chromatin binding had activating consequences for gene expression in a complex-specific manner (Fig. 1G). Observed gene expression changes could be partially recapitulated upon *KAT2A* gene expression knockdown (Fig. S1F-G), particularly in respect of *ZZZ3*-bound elements, which could reflect differential dependence and/or redundancy of histone acetyltransferase activity in either complex. Indeed, K562 cells were more clearly dependent on *ZZZ3* than on SPT20 for propagation in culture (Fig. S1H). Loss of *KAT2A* had an intermediate effect (Fig. S1H), in line with independent activities of each complex in hematopoietic cell maintenance and/or identity, which might be balanced by the action of *KAT2A*.

We have recently established a role for *KAT2A*<sup>7,8</sup> in maintenance of AML cells, which could be akin to our observations in K562 cells. In contrast, we and others failed to identify a specific requirement for *Kat2a* in normal mouse hematopoiesis<sup>6,7</sup>, which contrasts with SAGA SPT20 chromatin binding of erythroid lineage loci. We therefore sought to systematically dissect SAGA and ATAC-mediated *KAT2A* contributions to normal and myeloid leukemic hematopoiesis in primary and cultured human cells.

### ***ATAC is selectively required for erythroid specification from cord blood HSC***

We started by inspecting putative differential functional contributions of SAGA and ATAC complexes to normal human hematopoiesis by transducing CD34<sup>+</sup> cord blood (CB) cells with lentiviral-delivered *ZZZ3* or *SUPT20H* *shRNAs* (Fig. 2A). We flow-sorted transduced (GFP<sup>+</sup>) CD34<sup>+</sup> cells as stem and multipotent progenitor cells (HSC) or as lineage-restricted myelo-lymphoid (MLP), megakaryocytic-erythroid (MEP) and granulocytic-monocytic (GMP) progenitors (Fig. 2B), and assessed their cell differentiation and proliferation potential in colony-forming (CFC) progenitor assays (Fig. S2A). We did not observe differences in the stem and progenitor proportions of transduced cells with either construct (Fig. S2B-C). In contrast, CFC assay output revealed a unique defect in erythroid (E) specification from HSC upon *ZZZ3* loss (Fig. 2C), with no changes to generation of mixed-lineage (Mix) or GM colonies. Colony formation from downstream lineage-restricted progenitors was not affected, suggesting a unique requirement for *ZZZ3* in early erythroid commitment, and a dispensable role for the ATAC component post-erythroid commitment, as well as in the myelo-monocytic lineages. *SUPT20H* expression knockdown, on the other hand, did not result in changes in colony-forming efficiency from either HSC or lineage-restricted progenitors (Fig. 2D). However, knockdown of the de-ubiquitinase component of the SAGA complex, *USP22* (Fig. S2D-E), resulted in loss of erythroid colony formation from committed MEP (Fig. S2F), which did not significantly affect pre-commitment HSC and multipotent progenitors (Fig. S2F). In agreement, proportions of CD34<sup>+</sup> progenitors were unaffected (Fig. S2G). Taken together, the data support contrasting roles for the ATAC and SAGA complexes in normal human blood progenitor biology, with early erythroid lineage specification dependent on *ZZZ3*. SAGA elements, in turn, participate in development of the erythroid lineage post-commitment, as suggested by gene regulation in K562 cells, although their requirement may be less absolute.

### ***KAT2A regulates human CB erythroid progenitor specification and survival***

Given the differential role of ATAC and SAGA elements in the erythroid lineage, we asked if *KAT2A* itself was required in human CB progenitor specification or differentiation. To reiterate, chemical inhibition of *KAT2A* activity at the 100 $\mu$ M dose effective in AML cell lines had not resulted in an overall reduction in colony-formation from CB CD34<sup>+</sup> cells<sup>8</sup>, and we and others had not found a requirement for *Kat2a* in mouse bone marrow hematopoiesis<sup>1,2</sup>. Nevertheless, re-inspection of *Kat2a* expression in bone marrow

subpopulations of our conditional knockout model<sup>7</sup> revealed that gene expression ablation in MEP was less extensive than in myelo-monocytic cells (n=4; Mean±SD: 0.39±0.20 in MEP vs 0.13±0.08 in GMP, 2-tailed t-test p-val = 0.054), compatible with selective preservation of unexcised *Kat2a* allele-carrying MEP cells and a possible partial requirement for *Kat2a* expression. In agreement, transduction of CB CD34+ cells with *KAT2A shRNA* resulted in a significant and specific proportional decrease in KAT2A-depleted MEP (Fig. 3A), suggesting a defect in specification of CB progenitors committed to the erythroid lineage. Inspection of progenitor activity of CB CD34+ cells in CFC assays upon *KAT2A* knockdown (Fig. S3A) or chemical inhibition (Fig. S3B) detected a reduction in erythroid colony formation. The same reduction was observed upon depletion of the Tudor-domain protein SGF29 (gene *CCDC101*) (Fig. S3C-D), a KAT2A partner common to SAGA and ATAC complexes. Equally, culture of *KAT2A* knockdown (Fig. S3E) or MB-3 inhibited (Fig. S3F) CD34+ cells in mixed-lineage differentiation conditions resulted in relative loss of differentiated erythroid cells, compatible with hindered specification of the red blood cell lineage. Dissection of HSC and progenitor CD34+ compartments revealed that loss of *KAT2A* affected erythroid lineage output from both HSC and MEP (Fig. 3B) without changes to myelo-monocytic lineage output (Fig. 3B), thus capturing both ATAC and SAGA-associated effects. Quantitative RT-PCR transcriptional analysis of *KAT2A* knockdown HSC shows down-regulation of early erythroid regulatory genes, namely *GATA2* and *EPOR*, (Fig. 3C), a finding compatible with the global reduction of erythroid and megakaryocytic programs apparent upon RNA-seq analysis (Fig. 3D-E and Supplemental File 2). Analysis of effects of *KAT2A* knockdown on cell cycle and apoptosis is suggestive of putative effects on cell survival peri-erythroid commitment (Fig. S3G-H), with specific increases in apoptotic HSC and MEP, which do not extend to other lineages. Taken together, the data support a function for *KAT2A* in specification and/or survival of erythroid-megakaryocytic progenitors.

### ***SAGA facilitates progression of erythroid differentiation***

Colony-forming progenitor assays of CB CD34+ cells associated with loss of KAT2A-containing ATAC complexes with impairment of early erythroid and megakaryocytic specification (Fig. 2C). The data also suggested that KAT2A-containing SAGA complexes may play a role in erythroid differentiation post-commitment (Fig. S2F). However, CFC assays are end-point assays that do not permit a detailed dissection of mechanisms of differentiation, and we attempted to overcome this limitation using a liquid culture system. Differentiation of total CB CD34+ cells in the presence of hydrocortisone, SCF, TPO and

EPO resulted in progressive loss of CD34 and accumulation of glycophorin A (CD235a)-positive cells, denoting effective erythroid differentiation (Fig. 4A), which was not qualitatively impaired upon *KAT2A* knockdown (Fig. 4B). However, erythroid differentiation cultures initiated from *KAT2A*-depleted HSC were quantitatively impaired, being severely restricted in their expansion (Fig. 4C). Reduced *KAT2A* knockdown cell growth was accompanied by a general trend towards increased cell death (Fig. S4A), compatible with the observed *KAT2A* role in HSC and MEP cell survival. To circumvent the limiting cell numbers in transduced primary CB cultures, we inspected erythroid lineage progression and associated molecular programs in K562 cells. Exposure of K562 cells to 1.5% DMSO resulted in accumulation of CD235a<sup>+</sup> cells and progressive loss of the earlier CD71 transferrin receptor (Fig. S4B) and was accompanied by loss of *GATA2* and up-regulation of *EPOR* and *TALI*, denoting molecular erythroid lineage progression<sup>29</sup> (Fig. S4C). DMSO-induced erythroid cultures initiated by *KAT2A* knockdown cells followed a similar molecular progression (Fig. 4D). In contrast, *SUPT20H* depletion significantly perturbed induction of erythroid programs in K562 cells, which failed to down-regulate *GATA2*, and variably up-regulated *EPOR* and *TALI*, suggestive of a defect in lineage progression (Fig. 4D). We confirmed the findings with a second *SUPT20H* shRNA (*SUPT20Hsh2*) (Fig. S4D-F). Accordingly, inspection of the expression pattern of SAGA-specific elements during *in vitro* maturation of committed erythroid progenitors from human CB<sup>35</sup> showed that several elements associate with late differentiation (Fig. S4G), including members of the Core, the SAGA-specific HAT subunit *TADA2B* and the H2B de-ubiquitinase *USP22* (Supplemental File 3), which specifically affected erythroid colony-formation from MEP in human CB. In contrast, inspection of the detailed single-cell profiling of erythroid development by Tusi et al.<sup>36</sup> captured *Kat2a* and *Zzz3* enrichment, but no elements of the SAGA complex, at the transition of multipotent progenitors to the erythroid and megakaryocytic lineages (Fig. S4H; Supplemental File 4), compatible with an early specification role of ATAC that does not extend into terminal erythroid differentiation. Indeed, loss of *ZZZ3* allowed down-regulation of *GATA2* and up-regulation of *TALI* during K562 erythroid induction (Fig. 4D). However, *EPOR* was not consistently up-regulated (Fig. 4D), a trend confirmed upon knockdown of a second ATAC element, *TADA2A* (Fig. S4E), which resulted in minimal *EPOR* up-regulation, which significantly lower than that observed for *CTRLsh* ( $1.52 \pm 0.13$  *TADA2Ash* vs  $3.19 \pm 0.49$  *CTRLsh* fold change relative to undifferentiated cells;  $p=0.0214$ ). Inspection of the K562 *ZZZ3* ChIP-seq data showed binding of the *EPOR* locus in one of the 2 replicate samples (Supplemental File 1 and Fig. S4I), and we checked if *ZZZ3* knockdown resulted in loss of

H3K9ac at the *EPOR* promoter. Indeed, we observed that *ZZZ3*, but not *SUPT20H* loss, decreased *EPOR* H3K9 acetylation (Fig. 4E), suggesting that *KAT2A* requirement for early erythroid specification may be mediated by ATAC control of *EPOR* expression and downstream signalling. However, we could not detect consistent down-regulation of *EPOR* in steady-state K562 cells upon *ZZZ3* or *TADA2A* knockdown (Fig. S4J), suggesting that regulation of *EPOR* by ATAC may be context-dependent, and not an absolute requirement for *KAT2A*-mediated regulation of early erythroid specification or survival.

In conclusion, our analysis of normal erythroid lineage progression indicates that *KAT2A* plays stage-specific roles, which differentially align with each of the two complexes it integrates. On the one hand, *KAT2A* regulates specification and survival of erythroid progenitors through participation in the ATAC complex, which controls biosynthetic activity, and to some extent, *EPOR* expression. On the other hand, *KAT2A* fine-tunes progression of erythroid lineage programs through participation in SAGA, which is only required in fully-committed progenitors and may play a contributory rather than an absolute role. Notably, *SUPT20H* regulation of erythroid molecular progression in K562 cells exceeds the contribution of *KAT2A* itself. It is possible that SAGA complexes also utilise the orthologue histone acetyltransferase *KAT2B* in this context, which can compensate for *KAT2A* loss. Increased *KAT2B* expression in progressed erythroid differentiation, accompanying up-regulation of SAGA elements (Supplemental File 3) is compatible with this view.

### ***KAT2A complexes uniquely maintain proliferation and identity of MOLM13 AML cells***

Having demonstrated that *KAT2A*-containing ATAC and SAGA complexes differentially sustain early lineage establishment, and differentiation of erythroid progenitor cells from human CB, we asked whether they also made specific contributions to AML biology. We started by investigating the model AML cell line MOLM13, which we had previously shown to be dependent on *KAT2A* expression and activity<sup>8</sup>. We knocked down expression of ATAC elements *ZZZ3* and *TADA2A*, and of SAGA components *SUPT20H* and *TADA2B* (Fig. 5A), all of which, like *KAT2A* depletion, impacted expansion of MOLM13 cultures (Fig. S5A-B). At a molecular level, depletion of ATAC, but not SAGA elements, impacted expression of ribosomal protein genes (Fig. 5B), in line with a pervasive control of protein biosynthetic activity by the ATAC complex, which we observed in K562 cells, and others also reported in lung cancer cell lines depleted of *ZZZ3* and *YEATS2* expression<sup>21,37</sup>. Analysis of expression of self-renewal signature genes *HOXA9* and *HOXA10*, showed a more extensive association with ATAC elements (Fig. 5C). Interestingly, despite the selective impact of ATAC elements

on gene expression, loss of both *SUPT20H* and *ZZZ3* resulted in depletion of H3K9ac in the respective ribosomal protein and *HOX* gene promoters (Fig. 5D), suggesting that locus regulation may be more complex than the promoter binding observed in K562 cells. Loss of *KAT2A* itself affects ribosomal protein gene expression, but not *HOXA* genes (Fig. 5B-C). This recapitulates our *MLL-AF9 Kat2a KO* leukemia mouse model<sup>7</sup>, in which progressive depletion of leukemia stem-like cells does not seem dependent on the classical *Hoxa* signature. Despite some ambiguity at a molecular level, ATAC and SAGA complexes do make a distinct cellular impact on the biology of MOLM-13 cells. Loss of ATAC *ZZZ3* and *TADA2A* arrest cell cycle progression in G0/G1 (Fig. 6A; Fig. S5C). The proliferation defect is reflected by a reduced number of *ZZZ3* knockdown cells entering cell division (Fig. 6B-C) over a 3-day culture period (Fig. S5D), as captured by divisional tracking (Fig. S5E). We asked if the proliferation defect consequent to ATAC loss affected MOLM13 cell self-renewal, as measured by *in vitro* re-plating of CFC assays. Indeed, although neither ATAC (*TADA2A*) nor SAGA (*SUPT20H*) knockdowns impacted initial colony-formation, ATAC alone affected re-plating of colony assays (Fig. 6D and S5F), suggesting an effect on self-renewal. In contrast, loss of SAGA *SUPT20H* resulted in extensive differentiation of MOLM13 cells (Fig. 6E-F; Fig. S5G), suggesting a role in preservation of cell identity, which is not unlike SAGA contribution to development of human erythroid cells. Despite a trend towards enhanced apoptosis of *KAT2A* knockdown cells (Fig. 6G; Fig. S5H), which could be an end-point consequence of enhanced differentiation (Fig. 6E-F; Fig. S5G), we did not observe a consistent apoptotic response to knockdown of SAGA (*SUPT20H* and *TADA2B*), or indeed ATAC (*ZZZ3* and *TADA2A*) elements (Fig. 6G; Fig. S5H), suggesting that control of cell survival is not central to *KAT2A* complex-mediated maintenance of MOLM13 AML cells.

Overall, the data support distinct roles for ATAC and SAGA in the model AML cell line MOLM13. ATAC controls biosynthetic activity and impacts self-renewal of MOLM13 cells through molecular control of *HOXA* genes and regulation of cell division. SAGA, on the other hand, preserves cell identity and impedes differentiation. The dichotomy of effects captures different aspects of *KAT2A* regulation of MOLM13 cells and *MLL-AF9*-driven leukemia<sup>7</sup> and broadly aligns with differential participation of *KAT2A* complexes in normal hematopoiesis.

### ***KAT2A-containing SAGA and ATAC complexes regulate cultured and primary CD34+AML cells***

Finally, we sought to extend our analysis of AML cells to include CD34+ lines not originally covered by the screen that established KAT2A as a critical regulator of AML cells<sup>8</sup>. Similarly, we investigated primary CD34+ AML patient samples, for which stem-like compartments have been more clearly established<sup>38</sup>. We initially focused on the CD34+ AML cell lines KG1a and Kasumi1, both of which represent minimally differentiated AML FAB M1 and M2, respectively, and carry distinct molecular abnormalities (KG1a, *FGFR1OP2-FGFR1* fusion; Kasumi1, *RUNX1-RUNX1T1* fusion) representative of human disease<sup>39,40</sup>. In both models, loss of ATAC, but not SAGA elements, restricted cell cycle progression (Fig. 7A; Fig. S6A). Similar to what had been observed in MOLM13 cells, none of the cell lines displayed increased apoptosis in response to loss of KAT2A complexes (Fig. S6B-C). We investigated differentiation consequences of loss of ATAC or SAGA components in KG1a cells, and observed down-regulation of the CD34 marker by flow cytometry upon *ZZZ3* and *SUPT20H* loss (Fig. 7B-C). However, only *SUPT20H* knockdown resulted in morphological differentiation along the monocytic lineage (Fig. 7D-E), supporting the notion that the undifferentiated state of AML cells is sustained through SAGA activity. Finally, we attempted to transduce primary CD34+ AML patient blasts (Supplemental Table 5) with our lentiviral vectors delivering shRNAs against *KAT2A*, *SUPT20H* or *ZZZ3*. We could observe transduced GFP+ cells in less than 50% of the samples transduced, and kept the cells in culture for 2-3 weeks in the presence of MS5 stroma, as described<sup>41</sup>, following up their expansion as well as the preservation of CD34+ and candidate phenotypic leukemia stem-like (L-)GMP (Fig. S7A). Downregulation of *KAT2A* and *ZZZ3*, but, in the sample analysed, not of *SUPT20H*, resulted in reduced expansion of transduced AML cells (Fig. 7F), which specifically affected CD34+ (Fig. 7G) and/or L-GMP (Fig. 7H), suggesting a loss of self-renewal potential. Chemical inhibition of KAT2A activity using MB-3<sup>8</sup> had a similar effect on preservation of CD34+ cells (Fig. S7B), which impacted the proportion of L-GMP (Fig. S7C).

Thus, the data suggest that KAT2A-containing complexes, particularly ATAC, play a role in maintenance of candidate leukemia self-renewing CD34+ cells, with SAGA putatively contributing to the AML differentiation block.



## DISCUSSION

In this study, we dissected the roles of KAT2A in normal and malignant human hematopoiesis through analysis of the macromolecular complexes ATAC and SAGA, in which KAT2A exerts its HAT activity. We have aligned the roles of ATAC with maintenance of biosynthetic molecular activity, namely control of ribosomal protein and translation-associated genes. SAGA, on the other hand, participates in activation or maintenance of molecular programs underlying the characteristics of individual cell types, a property we equate with preservation of cell identity.

In the context of normal hematopoiesis, ATAC control of ribosomal protein genes selectively affects early stages of erythropoiesis, a lineage previously shown to be uniquely dependent on ribosomal assembly and rates of protein synthesis<sup>42,43</sup>, as captured by the selective erythroid defects of congenital ribosomopathies such as Diamond-Blackfan Anemia<sup>44</sup>. Selective loss of individual ribosomal proteins<sup>45</sup> or of global ribosomal protein regulators such as BMI1<sup>46</sup> have been shown to associate with proliferation and survival of early erythroid committed cells, with minimal changes to terminal differentiation. In the case of ATAC, the early nature of the defect may be potentiated by deregulation of the *EPOR* locus, a candidate instructor of erythroid lineage commitment<sup>47</sup>. However, the transcriptional consequences of reduced *EPOR* promoter acetylation upon ATAC loss are inconsistent, suggesting that it may contribute to rather than drive the erythroid defect. In the context of leukemia, ribosomal protein abundance and translational activity have been shown to sustain AML self-renewal<sup>42</sup>. We found that ribosomal protein genes are selectively affected by loss of ATAC elements, which may nevertheless control other self-renewal associated genes, namely the underlying *HOXA* signature associated with *KMT2A/MLL* rearrangements.

In contrast with the universal targeting of ribosomal protein genes by ATAC<sup>21,37</sup>, SAGA may not regulate a constant set of genes, but instead sustain the expression of the unique transcriptomes of individual cell types. This could in part be achieved through control of other transcriptional regulators, as suggested by the functional ChIP enrichments in our data. In the context of normal hematopoiesis, SAGA may exert its putative identity control by fine-tuning normal progression of erythroid programs, a role for which its requirement may be contributory, rather than absolute. The fact that SAGA, as indeed ATAC, requirements seem restricted to the erythroid lineage may reflect the distinctive nature of erythroid/megakaryocytic commitment and differentiation, which segregate from the stem cell root upstream of myeloid and lymphoid lineages<sup>48,49</sup> with *de novo* establishment of transcription programs<sup>50</sup>. In the context of malignant hematopoiesis, SAGA participates in

maintenance of the AML differentiation block, irrespective of the exact stage of cell differentiation, compatible with a general control of cell identity through preservation of existing transcriptional programs<sup>51</sup>. Indeed, like KAT2A itself, we previously captured several SAGA elements (*SUPT20H*, *TRRAP*, *TAF5L*, *TAF6L*, *TAF12*) as candidate genetic vulnerabilities in AML cell lines in a CRISPR drop-out screen<sup>8</sup>. In contrast, we failed to capture ATAC components in the same screen<sup>8</sup>, which nevertheless identified ribosomal proteins as universal vulnerabilities. One possible explanation is that cultured AML cell lines may be permissive to mild or moderate reduction in ribosomal assembly and protein synthesis. Our observed milder consequences to cell expansion of ATAC element loss are compatible with this view, as the screen readout<sup>8</sup> was dependent on acute consequences to proliferation or survival. Also, the specific ATAC-mediated defect of *in vitro* self-renewal of colony-forming cells is progressively displayed, becoming apparent upon colony re-plating, but not affecting initial colony formation. Nevertheless, ATAC biosynthetic regulatory activities are required for supporting proliferation and, at least in some contexts self-renewal, of cells dependent on SAGA-mediated regulation through putative stabilisation of their unique transcriptional programs. The resulting KAT2A activity can be described as preservation of the cellular *status quo* with minimal deviation towards alternative fate choices.

Preservation of the cellular *status quo* as the suggested operating mode of KAT2A-containing complexes is compatible with the role of KAT2A in sustaining, rather than initiating gene transcription<sup>52</sup>, as well as in stabilising promoter activity and minimising variability in transcriptional output as recently described by us in leukemia<sup>7</sup> and embryonic stem cells<sup>2</sup>. Control of transcriptional variability by KAT2A-like acetyltransferases is well established in yeast GCN5<sup>53</sup>, which integrates a SAGA-like complex that was shown to regulate the global transcriptome<sup>54</sup>. Our<sup>7</sup> and other<sup>5</sup> observations in mammalian cells is that Kat2a only acetylates a subset of promoters, which are circumscribed into limited numbers of SAGA-specific and ATAC-specific targets (this paper, and Krebs et al.<sup>13</sup>). Although precise identification of KAT2A complex targets may be limited by the tools available, the number of promoters dependent on KAT2A for H3K9ac is restricted<sup>7</sup>, and functionally aligns with the categories we captured for ATAC and SAGA binding, suggesting a more restricted use in mammalian species as compared to yeast. Furthermore, our data suggest that ATAC and SAGA control of gene transcription may be more specific than control of promoter H3K9 acetylation, indicating that specificity of transcriptional regulation by either complex may rely on unique effects of their additional enzymatic activities, namely H2B de-ubiquitination

by USP22 and H4 acetylation by KAT14<sup>55</sup>. Accordingly, our inspection of *USP22* requirements in erythropoiesis suggest that they align with the stage specificity of SAGA, but may exceed the effects of loss of *SUPT20H* and more directly resemble post-commitment contributions of *KAT2A*. Future studies investigating single and combined requirements of ATAC and SAGA enzymatic subunits in locus and cellular regulation will enhance our understanding of transcriptional control of cell state and fate decisions, including in leukemia and the normal blood system.

In respect of control of transcriptional variability, it is somewhat surprising that *Kat2a*-dependent promoters that respond to *Kat2a* KO with loss of H3K9ac and increased transcriptional variability are enriched in ATAC-dependent translation-associated genes<sup>7</sup>, and indeed enriched for ZZZ3 bound targets (ENCODE ChIP-Seq significance tool;  $p\text{-val}=1.94e^{-4}$ ). Although both complexes regulate H3K9ac, control of transcriptional variability is conserved from yeast, which only contains a SAGA-like complex. One possibility is that ATAC and SAGA have evolved to nucleate distinct molecular functions of KAT2A. A more trivial explanation is that SAGA being preferentially recruited to cell type-specific promoters, comparison with ChIP-seq targets in different cell types may fail to detect enrichment despite a similar molecular regulation. Future analysis of transcriptional variability in response to parallel ATAC and SAGA-specific depletions in the same cell type should clarify this discrepancy. Additional studies will also be needed to understand the discrepancies between the identified requirements for *KAT2A* in human hematopoiesis and the broadly unperturbed hematopoietic development in the mouse. As we pointed out, detailed analysis of our own conditional *Kat2a* knockout model<sup>7</sup> suggests a retention of MEP carrying unexcised *Kat2a* alleles, compatible with a requirement in erythroid specification. Also, we reported reduced mixed colony formation from HSC in *Kat2a* KO cells, which could portray a defect in early erythroid specification. We did not observe defective colony formation from MEP, but cannot exclude these may have been masked by persistence of unexcised cells. On the other hand, a distinct conditional knockout model<sup>6</sup> also failed to identify gross defects in hematopoiesis, although detailed analysis of lineage specification was not performed. To date no other knockouts of ATAC and SAGA subunits have been investigated in the hematopoietic system, at the exception of *Usp22*, which did not affect stem and progenitor compartments or normal myeloid differentiation<sup>27</sup>, but had a surprising tumor suppressor effect in *Kras*-driven transformation. However, a recent screen of deubiquitinating enzymes required for 4-week bone marrow engraftment which identified *Usp15* as a regulator of HSC activity *in vitro* and

*in vivo*<sup>56</sup> also identified Usp22<sup>27</sup> as a possible hit, re-enforcing the notion that additional analysis of ATAC and SAGA-mediated roles in mouse hematopoiesis is necessary.

In conclusion, we have identified unique requirements for ATAC and SAGA complexes in normal and leukemic hematopoiesis that are suggestive of respective pervasive roles in maintenance of biosynthetic activity and cell-type specific programs, through complex-coordinated control of histone modifications and transcriptional stability. Individual cell types may be differentially dependent on the activity of either complex, as suggested by a relatively stronger dependence on ATAC at early stages of erythropoiesis. Indeed, this may pose an opportunity for targeting of KAT2A activity through the SAGA complex in leukemia. In support of the ATAC / SAGA functional dichotomy, a paper contemporary with this report identifies distinct targets and roles of ATAC and SAGA elements in mouse embryonic stem (ES) cells<sup>57</sup>. Similar to our findings, it associates ATAC with regulation of ribosomal protein genes and a pervasive control of pluripotency; SAGA, on the other hand, specifically regulates naïve pluripotency genes, akin to a role in cell identity. However, the authors find that SAGA and ATAC functional roles in mouse ES cells are independent of HAT activity<sup>57</sup>, which is distinct from our observations in the human haematopoietic system and, clearly, in AML. Detailed understanding of distinct chromatin regulatory strategies of SAGA and ATAC complexes for gene transcription, and their usage in different cells, will clarify the discrepancy. This knowledge can be explored for stability or perturbation of cell fate in regeneration and disease.

## DATA SHARING STATEMENT

ChIP-seq and RNA-seq data have been deposited in GEO (accession numbers GSE128902 and GSE128512).

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## AUTHORSHIP

Contribution: CP conceived the study; L.A. and C.P. designed the study; L.A., E.F., S.W., A.F.D., G.G., S.K., M.M-S. H.D., A.Ch., R.A., R.S., and S.G. collected and assembled data; G.G., D.F., A.Cu. E.S., B.J.P.H., and L.T. contributed critical reagents; L.A.; E.F.; S.W., R.K. and C.P. analysed data; L.A. and C.P. interpreted data; L.A. and C.P. wrote the manuscript. All authors approved the final version of the manuscript.

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## FIGURE LEGENDS

### Figure 1. KAT2A-containing ATAC and SAGA complexes have unique targets in hematopoietic cells.

(A) Schematic representation of the human ATAC (left) and SAGA (right) multiprotein complexes. SAGA (right) is organized into distinct structural and functional modules, colored similarly. The KAT2A-containing histone acetyltransferase (HAT) module is depicted in orange and partially shared with ATAC (left), with exception of TADA2B, which is replaced by TADA2A in ATAC. The histone de-ubiquitinase (DUB) module is shown in red; the Core module, which includes SPT20, in blue. TF-binding module, TRRAP, is shown in yellow. TATA-binding protein (TBP), in purple, is not part of the complex architecture but it associates with SUPT3H to recruit SAGA to TATA box and facilitate transcription. The ATAC complex does not have a modular organization. Its main structure is shown in green and includes DNA-binding subunit ZZZ3. Subunits tested in this study are delineated with a dashed line. (B) Venn-diagram of consensus ZZZ3 and SPT20 ChIP-seq binding from 2 independent experiments. (C) Genomic location of ZZZ3 (left) and SPT20 (right) ChIP-seq binding in K562 cells. Summary of consensus peaks from 2 independent ChIP-seq experiments is shown. (D) Representative ChIP-seq peak for ZZZ3 target in K562 cells (*RPS7*) and representative ChIP-seq peak for SPT20 target in K562 cells (*HBB*). (E) Publicly-available ChIP-seq tracks for H3K9ac (ENCFF257CLC) and ZZZ3 (ENCFF856KCV) in K562 cells at the *RPS7* and *HBB* loci retrieved from the ENCODE project portal ([www.encodeproject.org](http://www.encodeproject.org)). The *RPS7* and *HBB* loci in (D) are represented

confirming the presence of H3K9ac peaks and reproducing the selective *ZZZ3* binding at *RPS7* also observed in our data. **(F)** H3K9ac ChIP-qPCR analysis of representative *SPT20* and *ZZZ3* targets upon knockdown in K562 cells.  $N \geq 3$  independent experiments. Mean  $\pm$  SEM of enrichment relative to rabbit-IgG, with normalisation to control intergenic region with no significant H3K9ac enrichment. Two-tailed t-test for significance  $*p < 0.05$ . **(G)** Quantitative RT-PCR analysis of expression of ATAC and SAGA complex targets in K562 cells.  $N \geq 3$  independent experiments, mean  $\pm$  SEM of gene expression relative to *CTRLsh*, normalised to *HPRT1* housekeeping gene. Two-tailed t-test for significance  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ .

**Figure 2. ATAC is selectively required for erythroid specification from CB HSC.**

**(A)** Quantitative RT-PCR validation of *SUPT20H* and *ZZZ3* knockdown in human cord blood HSC. Representative experiment for *SUPT20H*; mean  $\pm$  SEM of 2 individual experiments for *ZZZ3*; gene expression relative to *CTRLsh*, normalised to *HPRT1* housekeeping gene. Paired two-tailed t-test for significance  $*p < 0.05$ . **(B)** Representative sorting plot for transduced HSC and progenitor cells from CB. **(C)** Frequency of colony forming efficiency (CFC) in the HSC/MPP, MEP, GMP and MLP compartments transduced with *ZZZ3sh*. Mean  $\pm$  SEM of 5 individual CB samples (4 for GMP, MLP). Two-tailed paired t-test for significance;  $*p < 0.05$ ,  $**p < 0.01$  **(D)** Frequency of CFC efficiency in the HSC/MPP, MEP, GMP and MLP compartments transduced with *SUPT20Hsh*. Mean  $\pm$  SEM of 4 individual CB samples. Two-tailed paired t-test for significance; no significant differences.

**Figure 3. KAT2A regulates human CB erythroid progenitor specification and survival.**

**(A)** Proportion of *KAT2Ash* transduced CB HSC and progenitors. Mean  $\pm$  SEM of  $>7$  individual sorting experiments. Two-tailed paired t-test for significance;  $*p < 0.05$ ,  $**p < 0.01$ . **(B)** Frequency of CFC efficiency in the HSC/MPP, MEP (top), GMP and MLP (bottom) compartments transduced with *KAT2Ash*. Mean  $\pm$  SEM of 7 individual CB samples. Two tailed paired t-test for significance;  $*p < 0.05$ ,  $**p < 0.01$ . **(C)** Quantitative RT-PCR analysis of expression of erythroid-associated genes in *KAT2Ash* transduced HSC from individual CB samples.  $N \geq 3$  independent experiments, mean  $\pm$  SEM of gene expression relative to *CTRLsh*,

normalised to *HPRT1* housekeeping gene. Two-tailed paired t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . **(D)** MA plot of RNA-seq gene expression analysis of differentially-expressed (DE) genes (red) in *CTRLsh* vs *KAT2Ash* transduced HSC/MPP cells. **(E)** Gene Set Enrichment Analysis (GSEA) plot for erythroid differentiation (right) and platelet signature (left) in the RNA-seq data in (D). Gene signature identifiers are ADDYA Erythroid Differentiation by HEMIN and GNATENKO Platelet signature, respectively, as obtained from the UC San Diego and BROAD Institute Molecular Signatures Database (MSigDB).

**Figure 4. KAT2A SAGA and ATAC complexes differentially affect erythroid lineage progression.**

**(A)** Erythroid (E) differentiation of HSC/MPP sorted from CD34+ cells of individual CB samples transduced with *CTRLsh* (top) and *KAT2Ash* (bottom) in liquid cultures. CD34 marks HSC and progenitors; CD71 and CD235a mark early and late differentiated E cells. Data summarise mean  $\pm$  SEM of 4 independent differentiation experiments. **(B)** Flow cytometry quantitative analysis of *CTRLsh* and *KAT2Ash* HSC in erythroid differentiation cultures in (A). Representative plots. **(C)** Expansion of *CTRLsh* and *KAT2Ash* HSC in erythroid differentiation cultures in (A). **(D)** Quantitative RT-PCR analysis of erythroid gene expression progression in K562 cells transduced with *KAT2Ash*, *SUPT20Hsh*, or *ZZZ3sh* and treated with 1.5% DMSO for erythroid molecular induction. Mean  $\pm$  SD of  $N > 3$  independent experiments; data are represented relative to day 0 normalised to *HPRT1* housekeeping gene. Two-tailed paired t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . *CTRLsh*-transduced cells for the same experiment shown in Fig. S4C. **(E)** H3K9ac ChIP-qPCR analysis of *EPOR* locus in K562 cells upon *SUPT20H* and *ZZZ3* knockdown.  $N \geq 3$  independent experiments. Mean  $\pm$  SEM of enrichment relative to rabbit-IgG, with normalisation to control intergenic region with no significant H3K9ac enrichment. Two-tailed t-test for significance \* $p < 0.05$ .

**Figure 5. ATAC and SAGA differentially impact histone acetylation and gene expression in MOLM13 AML cells.**

**(A)** Quantitative RT-PCR validation of *KAT2A*, *SUPT20H* and *TADA2B* (SAGA-specific subunits) and *ZZZ3* and *TADA2A* (ATAC-specific subunits) knockdown in MOLM-13 cells.  $N \geq 3$  independent experiments, mean  $\pm$  SEM of gene expression relative to *CTRLsh*, normalised to *HPRT1* housekeeping gene. Two-tailed t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . **(B)** Quantitative RT-PCR analysis of ribosomal protein gene

expression in MOLM-13 cells transduced with *KAT2Ash*, SAGA-specific *SUPT20Hsh* and *TADA2Bsh* and ATAC-specific *ZZZ3sh* and *TADA2Ash*.  $N \geq 3$  independent experiments, mean  $\pm$  SEM of gene expression relative to *CTRLsh*, normalised to *HPRT1* housekeeping gene. Two-tailed t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . (C) Quantitative RT-PCR analysis of self-renewal gene signature in MOLM-13 transduced with *KAT2Ash*, SAGA-specific *SUPT20Hsh* and *TADA2Bsh* and ATAC-specific *ZZZ3sh* and *TADA2Ash*.  $N = 2$  biological replicates, each run as 2 or 3 technical repeats; mean  $\pm$  SEM of relative gene expression relative to *CTRLsh*, normalised to *HPRT1* housekeeping gene. Two-tailed nested t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . (D) H3K9ac ChIP-qPCR analysis of ribosomal protein genes and self-renewal genes in MOLM-13 cells upon knockdown of SAGA and ATAC elements, *SUPT20H* and *ZZZ3*, respectively.  $N \geq 3$  independent experiments. Mean  $\pm$  SEM of enrichment relative to rabbit-IgG, with normalisation to control region in *KRT5* locus with no significant H3K9ac enrichment. Two-tailed t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

**Figure 6. ATAC maintains self-propagation and SAGA blocks differentiation of MOLM13 AML cells.**

(A) Quantification of flow cytometry analysis of cell cycle in MOLM-13 cells transduced with *KAT2Ash*, SAGA-specific *SUPT20Hsh* and *TADA2Bsh* and ATAC-specific *ZZZ3sh* and *TADA2Ash*. Corresponding representative plots in Fig. S5C. Mean  $\pm$  SEM of 3 independent experiments. Two-tailed t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ . (B) Representative flow cytometry plots of divisional tracking of MOLM-13 cells transduced with *CTRLsh*, *SUPT20Hsh* and *ZZZ3sh* and loaded with the Tag-IT violet dye (Biolegend) after 3 days of culture. Regions 0, 1 and 2 represent the number of cell divisions relative to initial loading control (also see Fig. S5D-E). (C) Quantification of results in (B) representing the distribution of MOLM-13 cells transduced with *CTRLsh*, *SUPT20Hsh* and *ZZZ3sh* undergone 0-2 cell divisions after 3 days in culture. Mean  $\pm$  SEM of 3 independent experiments. Two-tailed t-test for significance \* $p < 0.05$ . (D) Colony re-plating of MOLM-13 cells transduced with *SUPT20Hsh1*, *SUPT20Hsh2* (SAGA) and *TADA2Ash1* and *TADA2Ash2* (ATAC) as a measure of in vitro self-renewal. Mean  $\pm$  SEM of 2-3 independent experiments. Two-tailed t-test for significance \* $p < 0.05$ . (E) Quantification of blast-like and differentiated cells in MOLM-13 cultures transduced with *CTRLsh*, *KAT2Ash*, *SUPT20Hsh* and *ZZZ3sh*. Scoring of 3 randomly selected fields of  $> 100$  cells; Two-tailed t-test for significance; \* $p < 0.05$ ,

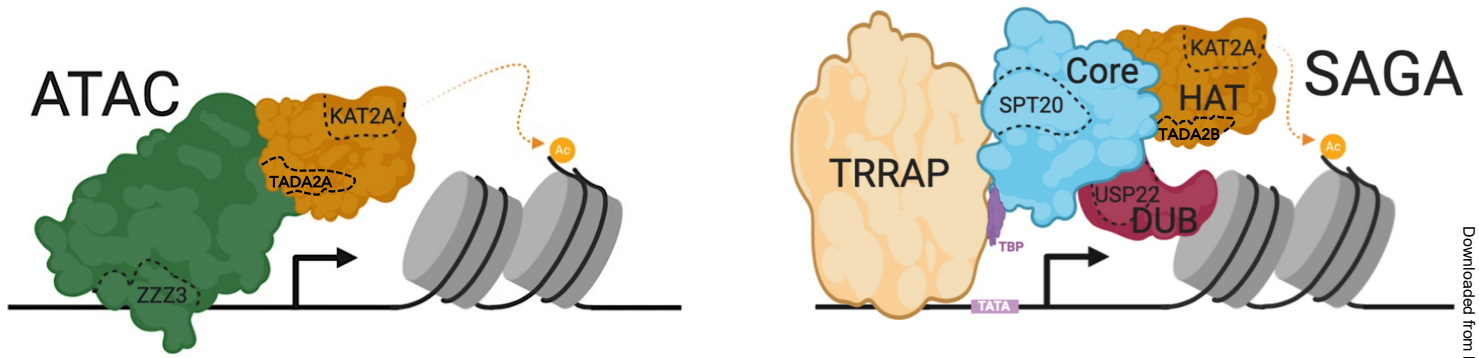
\*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ . **(F)** Representative photographs of MOLM-13 cytopins. White arrow heads denote blast-like cells; black arrow heads denote differentiated cells. Scale 50  $\mu\text{m}$ . **(G)** Quantification of Annexin V+ apoptotic cells in MOLM13 cultures analysed by flow cytometry (representative plots in Fig. S4F). Mean  $\pm$  SEM of  $N > 3$  independent experiments. Two-tailed t-test for significance.

**Figure 7. KAT2A complex activity maintains propagation of undifferentiated cultured and primary CD34+ AML cells.**

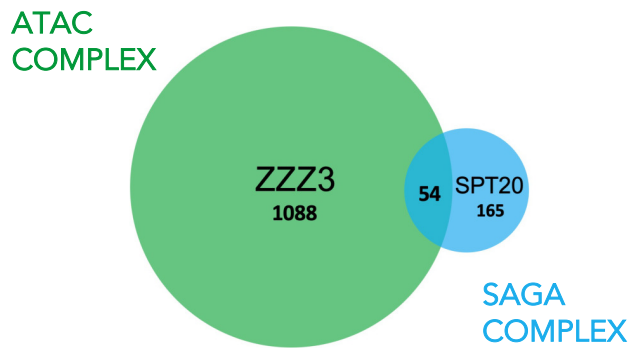
**(A)** Quantification of flow cytometry analysis of cell cycle in Kasumi-1 cells transduced with *KAT2Ash*, *SUPT20Hsh* and *ZZZ3sh*. Mean  $\pm$  SEM of 3 independent experiments. Two-tailed t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ . **(B)** Representative flow cytometry overlay plot of analysis of undifferentiated marker CD34 in KG1a cells transduced with *CTRLsh*, *SUPT20Hsh* and *ZZZ3sh*. **(C)** Quantification of CD34+ cells in transduced KG1a cells in (B).  $N = 3$  independent experiments. Two-tailed t-test for significance \* $p < 0.05$ , \*\* $p < 0.01$ . **(D)** Quantification of blast-like and differentiated cells in KG1a cultures transduced with *CTRLsh*, *SUPT20Hsh* and *ZZZ3sh*. Scoring of 3 randomly selected fields of  $> 100$  cells; Two-tailed t-test for significance; \* $p < 0.05$ . **(E)** Representative photographs of KG1a cytopins. White arrow heads denote blast-like cells; black arrow heads denote differentiated cells. Scale 50  $\mu\text{m}$ . **(F)** Growth of human primary AML cells (CD34+ samples – details in Supplemental File 5) transduced with *CTRLsh*, *KAT2Ash*, *SUPT20Hsh* or *ZZZ3sh* and maintained in the MS5 co-culture system for 2-3 weeks. **(G)** Percentage of GFP+ CD34+ cells in AML samples in (F) analyzed at week 2 of the MS5 co-culture. Analysis gates are presented in Fig. S6a. Data are normalized to global GFP level, to correct for unequal GFP transduction levels. **(H)** Percentage of GFP+ GMP-like (L-GMP) cells at week 2 of the MS5 co-culture system in AML samples in (F). Analysis gates in Fig. S7A. GFP transduction correction as in (G).

# Figure 1

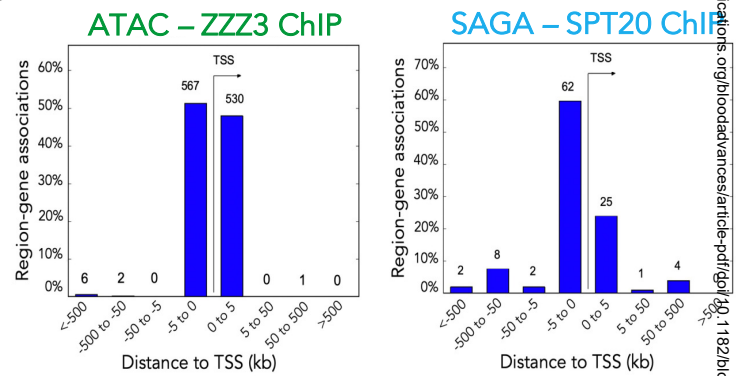
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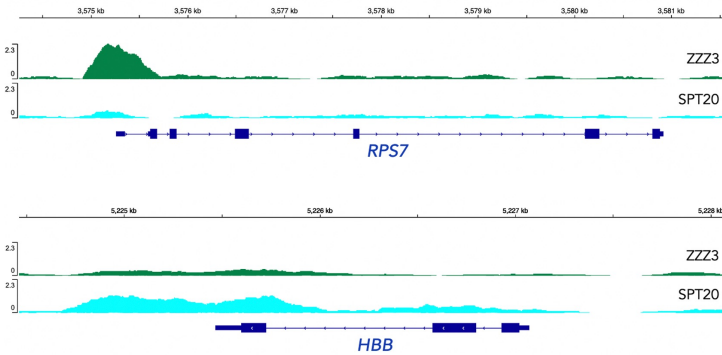
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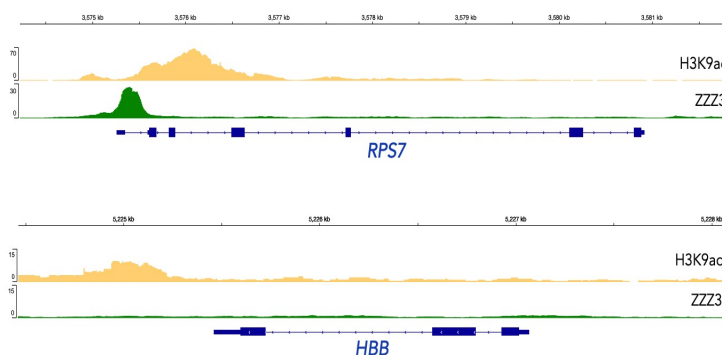
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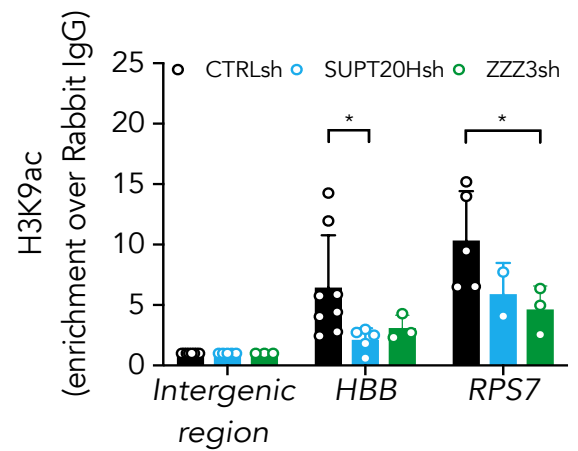
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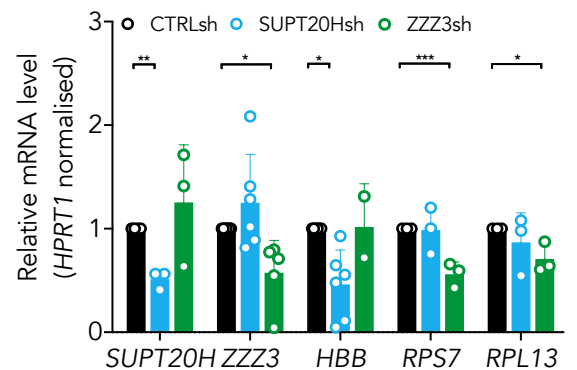
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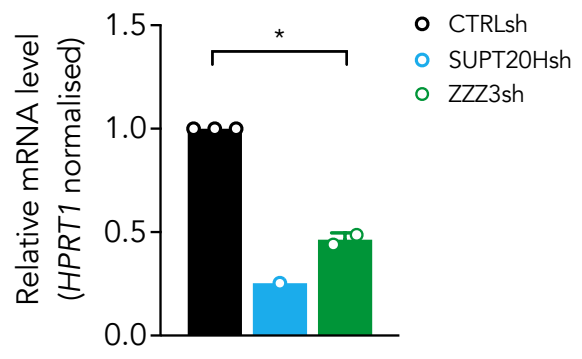


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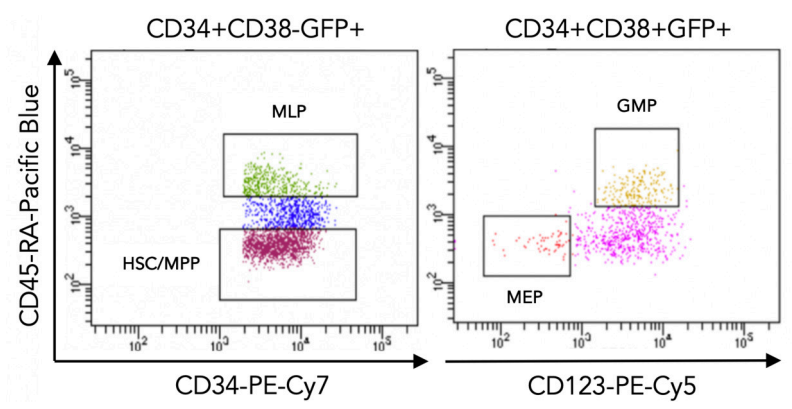


**Figure 2**

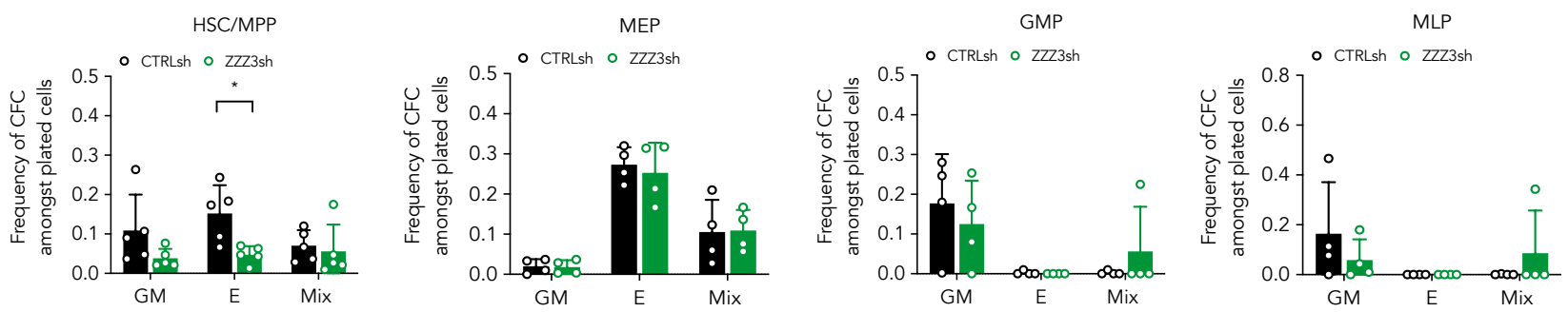
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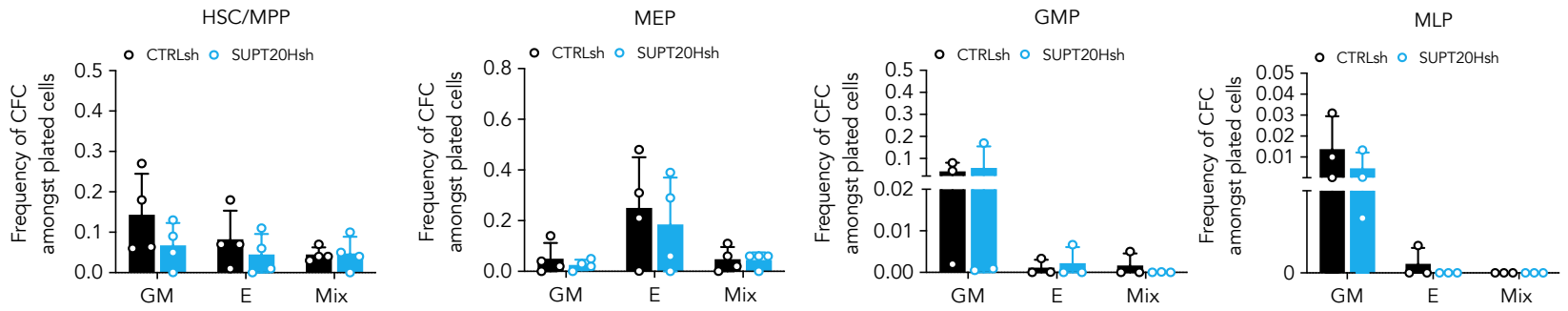
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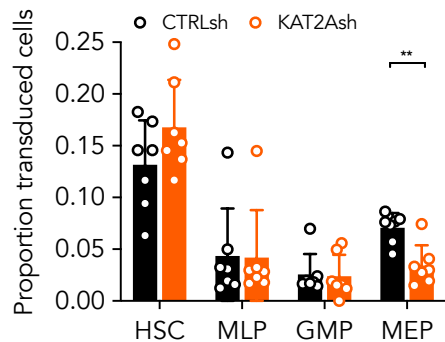
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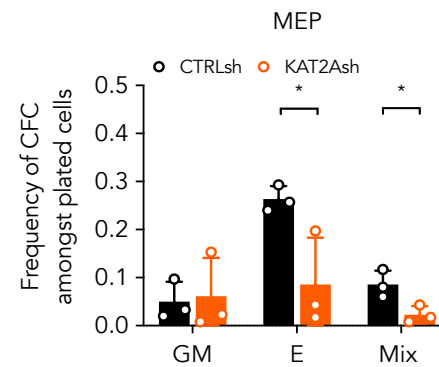
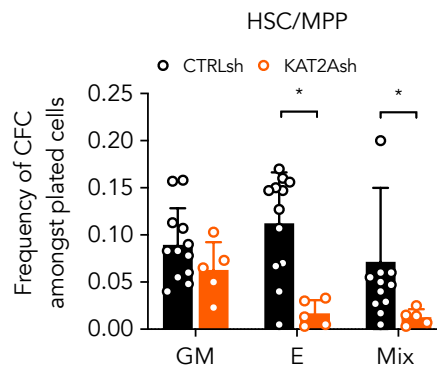


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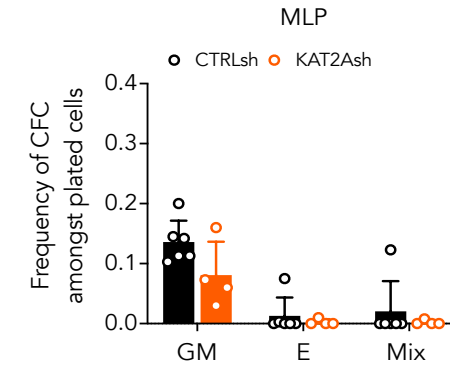
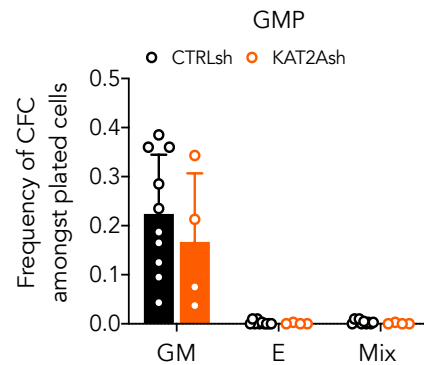
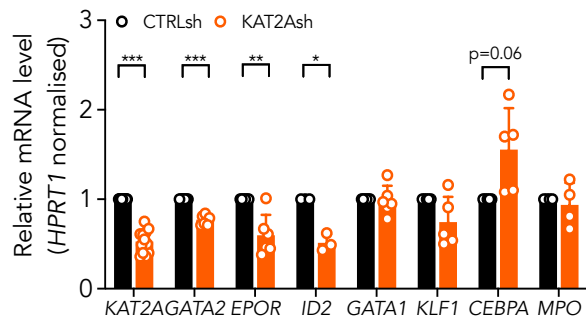
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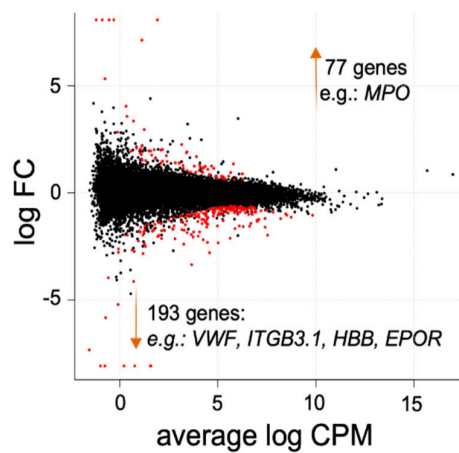
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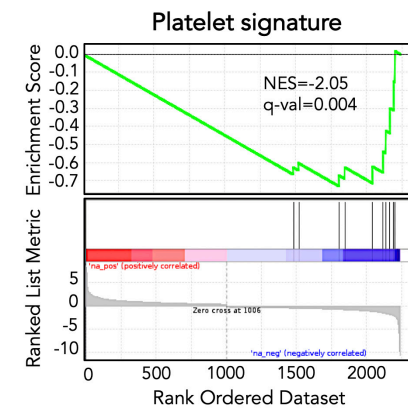
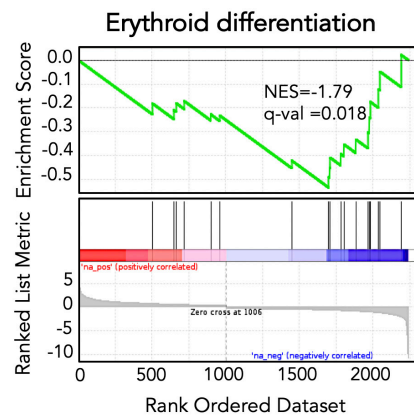
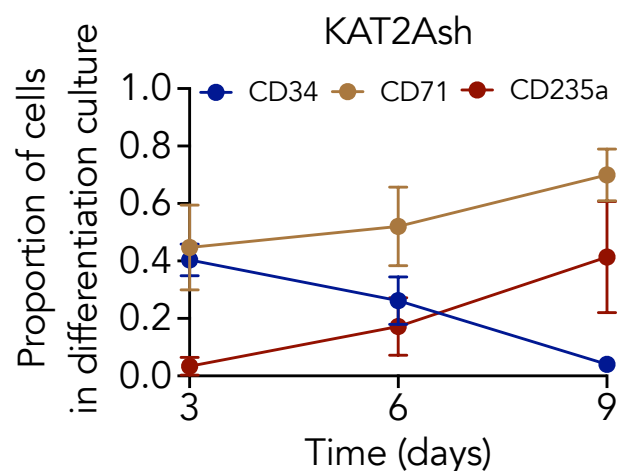
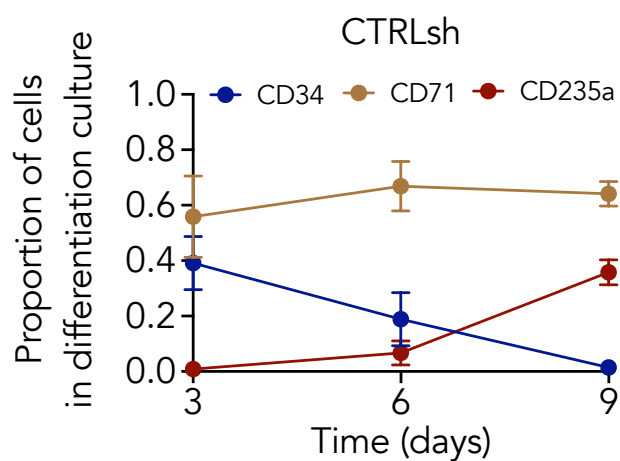
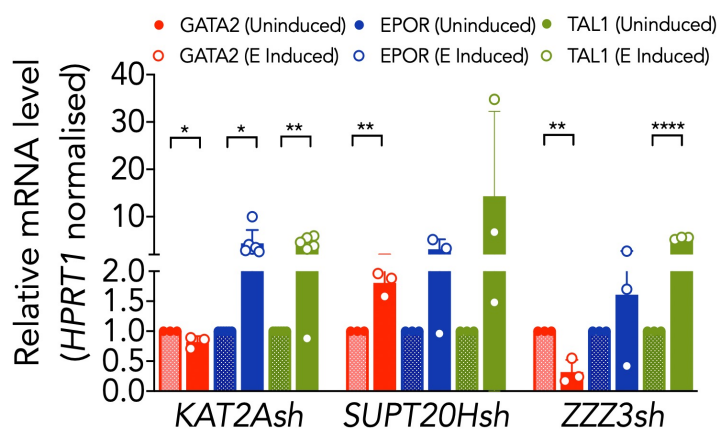


Figure 4

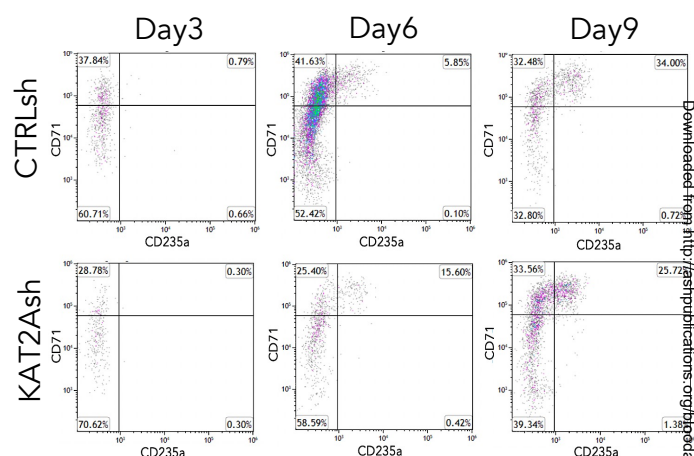
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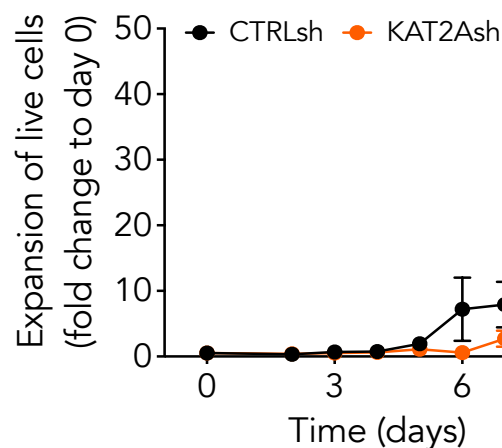
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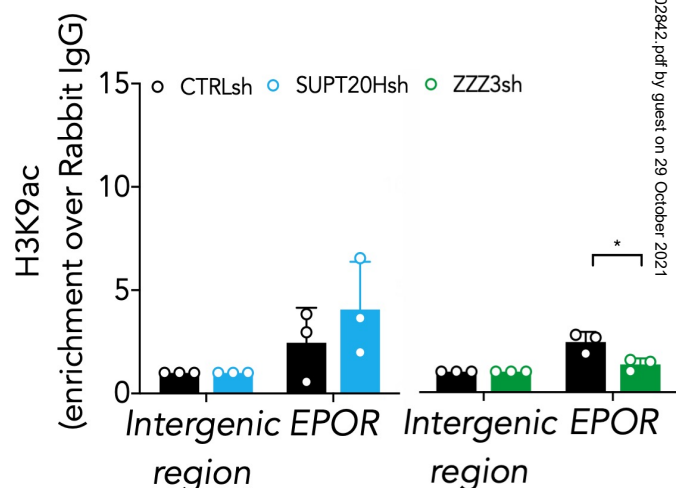
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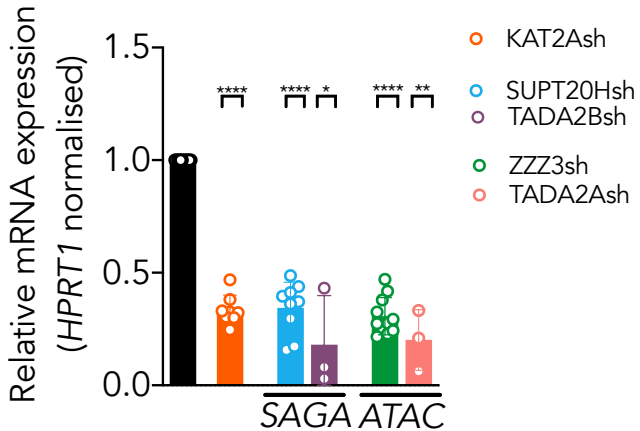


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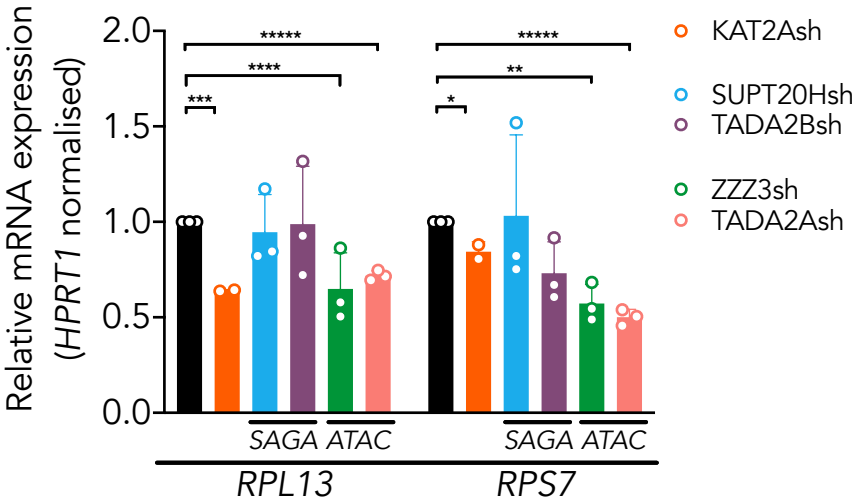


**Figure 5**

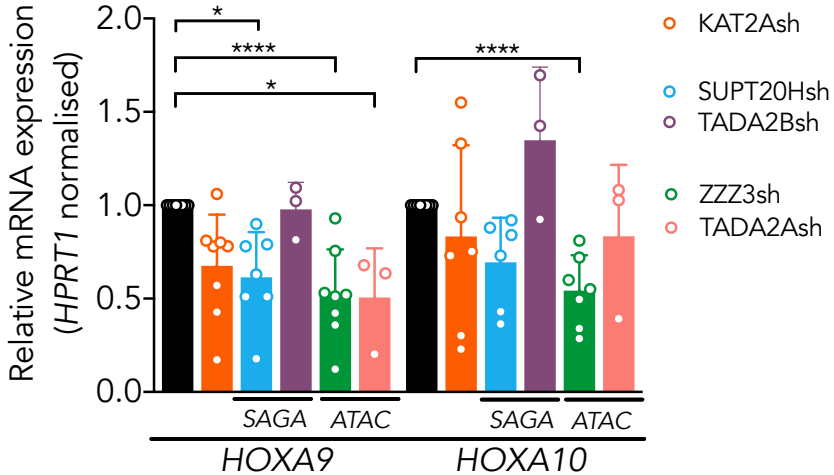
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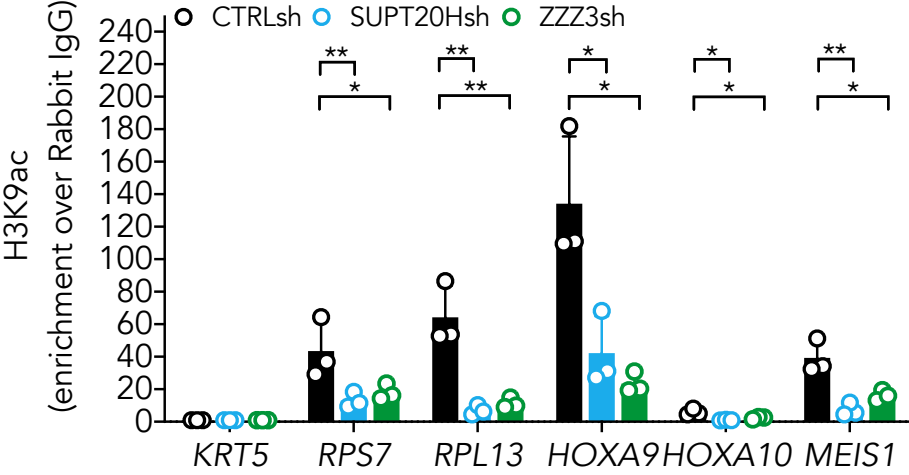
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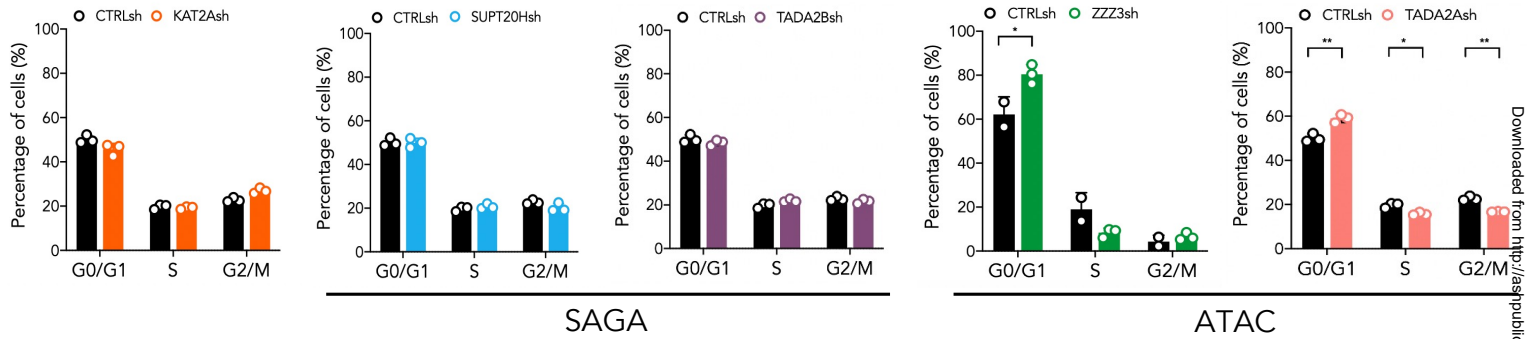


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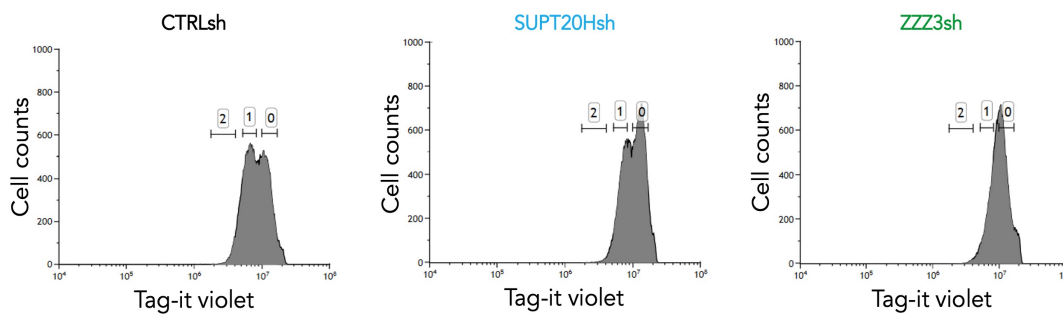


# Figure 6

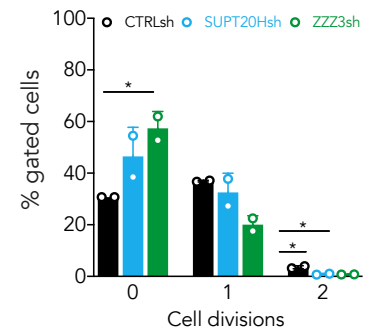
**A**



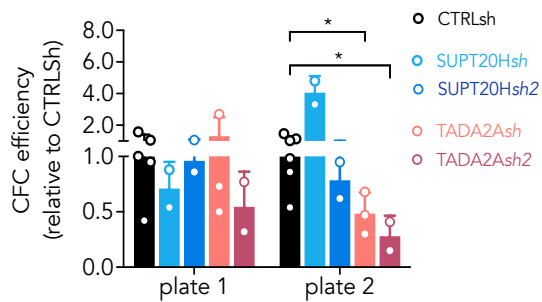
**B**



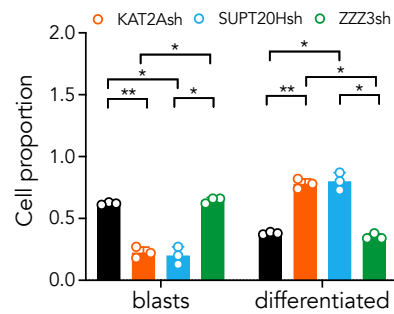
**C**



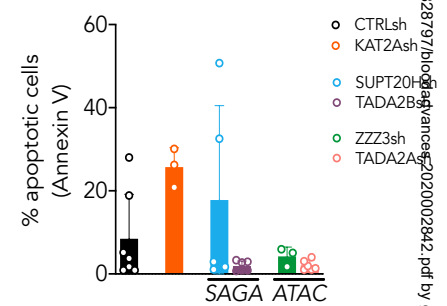
**D**



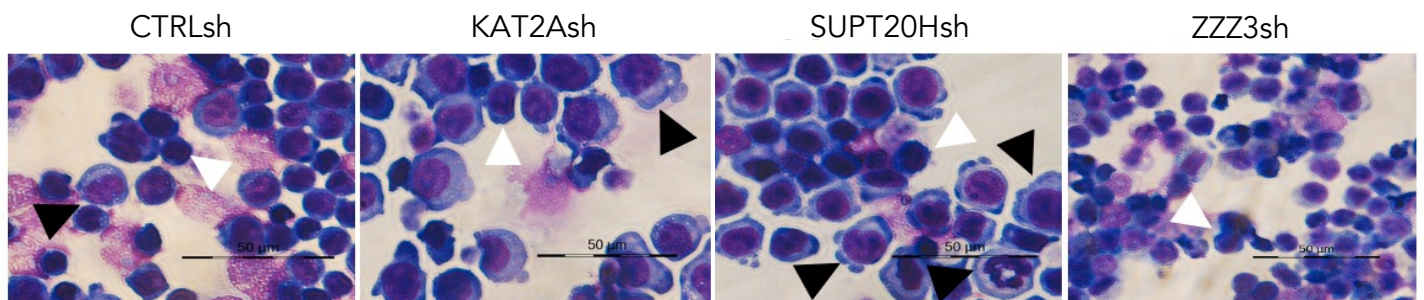
**E**



**G**



**F**



# Figure 7

