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PRINCIPAL INVESTIGATOR: Karina Barbosa Guerra

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Karina Barbosa Guerra

E-Mail: kbarbosa@sbpdiscovery.org

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Sanford Burnham Prebys Medical Discovery Institute
10901 N. Torrey Pines Road
La Jolla, CA 92037-1005

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14. ABSTRACT
Leukemia accounts for ~30% of pediatric cancer diagnoses. Our lab focuses on AML with rearrangements of the AF10 gene (AF10-R), a high-risk subset associated with treatment resistance and disease relapse. A hallmark of 70% of AML cases, including AF10-R, is the dysregulated expression of the HOXA gene cluster and its co-factor MEIS1 (HOX/MEIS). Functionally, HOX/MEIS activation is a critical node in leukemogenesis. It is well established that HOX/MEIS gene expression is sustained by epigenetic regulators that are coopted in leukemogenesis. To comprehensively characterize epigenetic regulators of HOX/MEIS genes, we conducted a phenotypic pooled CRISPR using a custom, high-density domain-focused CRISPR in a MEIS1-GFP leukemia cell line. Our screen uncovered several known and novel candidate regulators of HOX/MEIS expression.

15. SUBJECT TERMS
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1. INTRODUCTION:

Leukemia accounts for ~30% of pediatric cancer diagnoses. The research project focuses on acute myeloid leukemia (AML) with rearrangements of the AF10 gene (AF10-R), a high-risk subset associated with treatment resistance and disease relapse. A hallmark of 70% of AML cases, including AF10-R, is the dysregulated expression of the *HOXA* gene cluster and its co-factor *MEIS1* (HOX/MEIS). Functionally, *HOX/MEIS* activation is a critical node in leukemogenesis. The purpose of this project is to evaluate two top candidate HOX/MEIS regulators in CALM-AF10 driven-leukemogenesis: the KAT7 complex and Casein Kinase enzymes. The proposed studies will provide insights into mechanisms of leukemogenesis and self-renewal in AML, which may also impact therapeutic development in additional subsets of myeloid and lymphoid malignancies.

2. KEYWORDS:

acute myeloid leukemia, self-renewal, blood cancer, pediatric cancer, leukemia stem cells, cancer stem cells, AML, AF10-Rearranged, CALM-AF10

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Our hypothesis, based on evidence from previous studies (A. J. Deshpande et al. 2014; Rau et al. 2016; Kuhn et al. 2016, 2015; Van Vlierberghe et al. 2008), is that aberrant activation of *HOX/MEIS* genes can be reversed by targeting epigenetic regulators important for sustaining their expression. Our objective is to validate the top two candidate HOX/MEIS regulators we found in CALM-AF10 driven-leukemogenesis: the KAT7 complex and Casein Kinase 2. *HOX/MEIS* genes are causal in leukemogenesis and self-renewal in the hematopoietic system. Therefore, our findings may have an impact in a broader range of myeloid and lymphoid malignancies. The table below lists the major goals of the project for the reporting period:

Specific Aim 1. Define mechanistically how the KAT7/JADE3 regulates HOX/MEIS expression.	**Months/ %Completion**
Task 1. Assessment of the KAT7 complex as a pan-regulator of HOX/MEIS genes.	
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Local IACUC Approval	Completed 3/23/20
Task 2. Evaluation of the enzymatic activity of KAT7 and the reader activity of JADE3 for HOX/MEIS expression. We will perform rescue experiments to elucidate which activities of the KAT7 complex are involved in HOX/MEIS regulation and we will functionally corroborate results.	
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Milestone(s) Achieved: potential therapeutic rationale for targeting CKs in KMT2A germline AML.	24
IACUC Approval	Completed 3/23/21

What was accomplished under these goals?

Specific Aim 1. Define mechanistically how the KAT7/JADE3 regulates HOX/MEIS expression.

Task 1. ***Assessment of the KAT7 complex as a pan-regulator of HOX/MEIS genes.*** We will functionally evaluate whether there is a causal relationship between loss of KAT7 and HOX/MEIS expression in the context of leukemia disease self-renewal, initiation, and maintenance, in both *in vivo* and *in vitro* settings.

Subtask 1. Effect of KAT7 and JADE3 loss on HOX/MEIS expression and leukemogenesis in vitro.

In the previous reporting period, we cited recent work concordant with our findings regarding the role of KAT7/JADE3 in the regulation of HOX/MEIS1 genes in AML and their involvement in leukemic self-renewal (MacPherson et al. 2020). Said study described that leukemic stem cells depend on the HBO1 complex, of which the KAT7 acetyltransferase and the plant-homeodomain-containing reader protein JADE3 are a part of. In addition, other recent reports have expanded on the regulation of HSC quiescence and self-renewal by HBO1 (Yang et al. 2022) and the identification of KAT7 as a fitness dependency in MLL-rearranged AML (Au et al. 2021). Chromatin landscape changes upon HBO1 loss have been described in the abovementioned findings, as well as mechanistic insights regarding the interactions between the HBO1 and MLL complexes (Takahashi et al. 2021). Given that the recently published evidence in the context of AML has largely overlapped with our performed and remaining statement of work items (including Tasks 1, 2, and 3 from Aim 1), we considered the mechanistic role of JADE3/KAT7 regulation of HOX-genes and the gaps in knowledge arising from the redundancy of H3K4me3-reading pathways.

In our epigenetics CRISPR screen, described in the Award Project Narrative, we identified the Tudor domain-containing H3K4me3 reader SAGA-associated factor 29 (SGF29) as a candidate regulator of HOX/MEIS gene expression. SGF29 is part of the Spt–Ada–Gcn5 acetyltransferase (SAGA) transcriptional co-activator complex.

The role of SGF29 in AML remains understudied and the mechanism by which it regulates HOX/MEIS expression is yet to be described in the literature. We assessed the impact of SGF29 deletion on human AML cell lines and observed that CRISPR inactivation decreased the growth of the CALM-AF10+ U937 cells over time in a flow cytometry-based competition assay (Fig. 1A, described in the previous reporting period and in A. Deshpande et al. 2019). CRISPR-knockout of SGF29 led to increased retention of the stable CellTrace violet dye in U937 and MOLM13 AML cells, indicating slower proliferation when compared to non-targeting controls (Fig. 1B). In the CRISPR-droplet sequencing experiment described in the FY2021 reporting period (CROP-seq), we tested the transcriptomic effects of genetic loss of SGF29 and identified that AML self-renewal associated genes increase their expression upon SGF29-ko, compared to non-targeting controls, while the differentiation-associated gene LYZ concomitantly increases its expression level (Fig. 1C). SGF29-deleted cells also showed increased uptake of fluorescently labeled heat-inactivated pHrodo™ Red E. coli bioparticles (ThermoFisher Scientific, Cat. A10025), compared to non-targeting controls, indicative of myeloid differentiation in U937 cells (Fig. 2A). In addition, cell cycle analysis by propidium iodide staining showed an accumulation of cells in the G0/G1 phase upon SGF29 knockout, compared to non-targeting controls (Fig. 2B). We obtained similar differentiation-induction results upon loss of SGF29 in MOLM13 and OCI-AML3 AML cell lines, which harbor distinct genetic insults that lead to high expression of HOX/MEIS genes (data not shown).

Using methylcellulose-based colony forming unit (CFU) assays, we tested the effects of Sgf29 loss in established CALM-AF10-, MLL-AF9-, and MLL-AF10- transformed murine AMLs. Upon targeting Sgf29 coding exons with 2 different retrovirally expressed sgRNAs, we observed a decrease in the number of blast-like colonies, compared to the effects of using an Sgf29 intron-targeting sgRNA. There was also a concomitant increase in the number of colonies with a differentiated morphology. Wright-Giemsa stained cytospins showed terminally differentiated or differentiating myeloid lineage cells in colonies with Sgf29 knockout, whereas cells in colonies with intron-targeting Sgf29 sgRNAs showed immature, blast-like morphology.

As proposed in this Subtask, we are currently performing AnnexinV staining assays to assess apoptosis upon loss of Sgf29 in murine AML.

Subtask 2. In vivo effect of Kat7 or Jade3 deletion on leukemia maintenance.

Please see note in Aim 1, Task 1, Subtask 1. In vivo effects of Kat7 and Jade3 loss on leukemia maintenance, as described in the Statement of Work, have been reported (Au et al. 2021; MacPherson et al. 2020).

We tested the *in vivo* effects of SGF29 deletion in cell line xenograft models of Cas9-expressing U937 and MOLM13 AML cells. SGF29 knockout led to significantly increased disease latency in both cell lines, compared to transduction with non-targeting controls. We also tested SGF29 deletion in an MLL-AF10+ AML patient-derived xenograft (PDX) model expressing split-Cas9, generated as previously reported (Liu et al. 2021). Mice injected with NTC-targeting sgRNAs succumbed to AML disease with a median latency of 64 days, while engraftment of samples harboring sgRNAs targeting SGF29 was abolished for up to 116 days of observation (Fig. 4A). AML disease was confirmed by monitoring engraftment of human CD45+ cells by flow-cytometry assessment of peripheral blood, as well as measurements of spleen enlargement (data not shown).

Subtask 3. Testing self-renewal by in vivo stem cell limiting dilution

Please see note in Aim 1, Task 1, Subtask 1. Self-renewal effects of Hbo1/Kat7 loss on stem cell self-renewal using transgenic mice have been recently reported (Yang et al. 2022; Najm and van Galen 2022).

We assessed the effects of Sgf29 loss on the clonogenicity potential of murine Lin- Sca+ Kit+ (LSK) hematopoietic stem and progenitor cells (HSPCs), as a measure of self-renewal of normal HSPCs *in vitro*. We observed that cells did not significantly alter the number of type of colonies formed in CFU assays, indicating that Sgf29 inactivation does not influence the clonogenicity and differentiation of normal HSPCs (Fig. 4B and

4C), unlike AML oncoprotein-transformed HSPCs (Fig. 3). We plan to perform the in vivo limiting dilution experiments in the context of AML-transformed murine cells.

Task 2. Evaluation of the enzymatic activity of KAT7 and the reader activity of JADE3 for HOX/MEIS expression. We will perform rescue experiments to elucidate which activities of the KAT7 complex are involved in HOX/MEIS regulation and we will functionally corroborate the results.

Subtask 1. Identify KAT7/JADE3 activities involved in HOX/MEIS regulation.

Please see note in Aim 1, Task 1, Subtask 1. The acetyltransferase activity of KAT7 and its deposition of H3K14Ac marks at self-renewal associated genes have been recently described (Yang et al. 2022)*, as well as the ancillary scaffolding tole of KAT7 for the recruitment of BRD4 and other MLL-associated proteins to their target genes, including MEIS1* (Au et al. 2021)*. We addressed the role of JADE3 as a substrate of CK2 potentially mediating its HOX/MEIS regulatory activity (Aim 2, Task 2, Subtask 2).*

In our epigenetic CRISPR screen, we identified that top-performing sgRNAs for SGF29 had homology to its Tudor domain. The Tudor domain in SGF29 confers H3K4me2/3 reader activity, thus, we hypothesize it is required for the indexing and transcriptional activation of HOX/MEIS genes. To assess the dependency of the Tudor domain for SGF29 localization in the HOX/MEIS loci, we cloned a FLAG-tagged SGF29^{D196R} mutant cDNA cassette, which completely disrupts binding to H3K4me3 (Bian et al. 2011). We performed ChIP-sequencing using a Flag antibody upon overexpression of SGF29^{D196R} or an empty backbone control in U937 cells. Sequencing read mapping to the HOX/MEIS1 loci, as well as to the self-renewal associated gene BMI1 and MYC was reduced in mutant SGF29-expressing U937 cells, compared to empty backbone controls (Fig. 5A). In addition, we performed RNA-sequencing (RNA-seq) and gene-set enrichment analysis (GSEA) of SGF29 expressing cells, compared to NTC controls, and observed that a previously defined (Chen et al. 2021) gene-set of CALM-AF10 target genes was significantly downregulated in SGF29-deficient cells (Fig. 5B). These results indicate that the reader portion of SGF29 is required for its localization to HOX/MEIS genes, as well as to the expression of the CALM-AF10 oncogenic program.

Subtask 2. Functional validation of enzymatic activities of JADE3 and KAT7 in HOX/MEIS regulation

Please see notes in Aim 1, Task 1, Subtask 1 and Aim 1, Task 2, Subtask 1.

We further tested the functional impact of the human SGF29^{D196R} mutation by CFU assays in a rescue experiment. Murine MLL-AF9 transformed cells were transduced with non-targeting controls or an Sgf29 sgRNA and then with an empty vector control, wild-type SGF29 or SGF29^{D196R} (Fig. 5C). Cells formed tight blast-like colonies in the presence of wild-type SGF29. However, overexpression of a rescue construct harboring the D196R mutation led to a differentiation phenotype in the presence or absence of wild-type SGF29, in a manner similar to the deletion of SGF29 alone. These results indicate that the loss of SGF29 or its ability to interact with H3K4me3 marks in chromatin lead to a differentiation phenotype in CALM-AF10+ U937 AML cells.

Task 3. Evaluation of the role of KAT7/JADE3 regulation of epigenomic lesions at the HOX/MEIS loci. If our studies show that the acetyltransferase activity of KAT7 is important for HOX/MEIS expression, we will determine which of the acetylation marks deposited by this enzyme play a role in this process.

Subtask 1. Mapping of KAT7 acetylation mark deposition.

Please see note in Aim 1, Task 1, Subtask 1.

To evaluate the mechanistic impact of SGF29 loss in SAGA complex recruitment, acetylation and transcriptional inactivation of self-renewal associated genes, we decided to conduct a chromatin enrichment procedure (ChEP, (Kustatscher et al. 2014) and mass spectrometry (Figs. 6A). We characterized the chromatin-associated proteome in U937 cells upon transduction with a non-targeting control or an SGF29-targeting sgRNA. The ChEP procedure

enriched for transcription factors and other annotated chromatin-associated protein categories (Fig. 6B). SGF29 deletion led to a significant loss in the abundance of MEIS1, HOXA13, MYC, and SATB1 AML oncoproteins in the chromatin fraction (Fig. 6C and 7A), compared to non-targeting control-transduced cells. We also observed that KAT2A, a SAGA complex member upstream of SGF29, was significantly reduced in the chromatin fraction upon SGF29 knockout. We probed KAT2A levels in the chromatin and cytoplasmic fractions and confirmed its eviction to the cytoplasm upon SGF29 knockout (Fig. 7C, left). In addition, we performed ChIP-qPCR for H3K9Ac, a mark deposited by the SAGA complex, upon SGF29 deletion and observed a decrease in acetylation levels in HOXA10 and MEIS1 (Fig. 7D). Our combined results point to a novel dependency in HOX-driven AML, whereby the SGF29 Tudor-domain reader of H3K4me3 reader

Subtask 2. Determining JADE3-dependent localization of KAT7 to the HOX/MEIS locus.

Please see note in Aim 1, Task 1, Subtask 1.

Specific Aim 2: Investigate the regulation of HOX/MEIS genes by Casein Kinases (CKs) in AML cells.

Task 1. ***Evaluation of CK regulation of HOX/MEIS genes in KMT2A-germline AML.*** We will assess whether CK modulates HOX/MEIS expression by regulation of wild-type KMT2A in KMT2A-germline cells and address the functional impact.

Subtask 1. Effect of CSNK2A and CSNK2B loss on HOX/MEIS expression and leukemogenesis in vitro.

In the previous reporting period, we provided evidence for the cloning and validation of casein kinase 2 components CSNK2A1(CK2□) and CSNK2B sgRNAs in mouse and human AMLs. We also used the CK2 inhibitor silmitasertib (CX-4945) to treat MEIS1-GFP reporter U937 cells and Meis1-GFP reporter murine AML cells. We observed that MEIS1 reporters respond to genetithe c loss of CSNK2A1 and CSNK2B, and that murine and human aml cells respond to the CK2 inhibitor CX-4945 in proliferation assays, *Meis1* reporter assays and *Hox/Meis* transcript levels by qPCR. As outlined in this subtask, we also tested the colony formation capacity of CALM-AF10- and MLL-AF10- transformed murine AMLs upon CK2 inhibition (Fig. 8A). In CALM-AF10-driven leukemias, we observed a non-significant decrease in blast-like colony counts in the first round of transplantation with CK2 inhibitor treatment, compared to DMSO, while the number of differentiated colonies showed a sustained and statistically significant decrease in colony counts across 3 rounds of transplantation (Fig. 8A). MLL-AF10-driven AML CFUs showed a trend for lower blast-like and differentiated colony counts with CK2 inhibitor treatment, compared to DMSO, while the changes were more pronounced in the second round of transplantation (Fig. 8B). We further tested the effects of CK2 inhibitor treatment on AML differentiation by assessing CD34 and CD38 marker levels in an MLL-AF10 PDX sample by flow cytometry (Fig. 8C). We observed a slight increase of CD38 positive cells (2.5%) upon CK2 inhibitor treatment for 48h, indicative of differentiation induction. CX-4945 treatment also increased the percentage of cells in early and late-stage apoptosis (3- and 2- fold increases, respectively), as measured by propidium iodide and AnnexinV staining by flow cytometry (Fig. 8C, bottom). Taken together, the mild phenotypes observed in the CFU assays could indicate that the effective concentration of the inhibitor decreased over time, as the drug was replenished every 7 days. Our data shows a slight increase in differentiation induction and reports in the literature point to an established role of engagement in apoptotic pathways in hematological malignancies (Tubi et al. 2013; Cozza et al. 2014; Chon et al. 2015).

Task 2. Investigation of the mechanistic basis of CK-mediated HOX MEIS regulation in CALM-AF10 AML. We will address CK regulation of HOX/MEIS via perturbation of KMT2A stability.

Subtask 1. Test CK-mediated KMT2A stability in CALM-AF10 AML.

In the previous reporting period, we conducted experiments that supported our mechanistic understanding of the CK2 regulation of HOX genes. We harnessed our CROP-Seq experiment with combined CRISPR and single-cell RNA-se) readout, CSNK2A1 knockout bulk RNA-seq, total proteomics, and phospho-proteomics to explore the

potential substrates mediating the HOX-regulatory activity of CK2 in AML. We tested and subsequently discarded the role of MEIS1 as a direct CK2 substrate, via the phosphorylation of its Serine 196 residue. Given the evidence described in the previous reporting period from CRISPR validation studies, CROP-seq, phospho-proteomics, and previous reports in the literature, we further inquired about the role of KMT2A phosphorylation as a meaningful mechanism for CK2-mediated regulation HOX/MEIS gene expression.

To explore the differentially expressed genes upon loss of KMT2A in greater depth, we performed bulk RNA-seq on U937 cells targeted with a KMT2A sgRNA or a non-targeting control (NTC). We observed that loss of KMT2A led to the downregulation of self-renewal associated genes activated in CALM-AF10+ AML, in large concordance with the phenotype observed for CK2 deletion (Fig. 9A). In addition, we considered that CK2 deletion led to a transcriptional downregulation of CALM-AF10 target genes, which we had previously defined (Chen et al. 2021). We thus hypothesized that an epigenetic regulator phosphorylated by CK2 could be responsible for the observed CALM-AF10 fusion-level transcriptional response and highlighted those proteins included in our epigenetic CRISPR screen (Fig. 9B). Among the chromatin regulator genes in our pooled library containing CK2 phosphorylation motifs, KMT2A is the top-ranked MEIS1 activator, and its downstream transcriptional targets are also downregulated upon CK2 deletion, as evaluated by GSEA (Fig. 9C). These results indicate that KMT2A regulates a subset of genes that fall under CK2 control.

To test whether CK2 controls MEIS1 transcription via KMT2A phosphorylation, we cloned short cDNA fragments encoding a portion of KMT2A flanking Serine 363, one of the CK2 phosphorylation-motif sites along KMT2A, which we chose given its depletion upon CK2-knockout in our phospho-proteomics dataset described in the previous reporting period. We generated KMT2A-S363 wild-type, alanine (non-phosphorylatable), and glutamate (phosphomimetic) modified cDNA fragments of 120 bp in length, following a recently published short-peptide tiling strategy (Ford et al. 2021). We overexpressed these KMT2A short mutant constructs in our GFP-MEIS1 tagged U937 cell line and assessed GFP fluorescence intensity levels (Fig. 9D). We employed control sgRNAs targeting CSNK2A1 as a positive control for GFP repression, and the cell-essential gene RPS20 as a negative control for GFP signal. In parallel, we performed similar experiments to explore the effects of Serine substitutions in JADE3_S46 and JADE3_S230, KAT7_S345, and HDAC1_S421, chromatin regulators which contain CK2 phosphorylation motifs (Fig. 1B) and decreased peptide levels in our phospho-proteomics dataset. For KMT2A, we observed the expected phenotypes in the control arms, as well as a slight, non-significant increase in GFP expression upon overexpression of the KMT2A wild-type S363 fragment. Given that both alanine and glutamate fragments showed a similar effect, we deemed this result to be inconclusive. In the KAT7 experiment, there were no differences between different peptide-coding fragments. For JADE3, mutant fragments also showed a similar phenotype. In the HDAC1 arm, we observed a decrease in GFP levels in the alanine substitution and a concomitant increase in GFP levels with the glutamate mutant, with the wild-type fragment resembling our negative control. We are currently repeating the HDAC1 experiment in a larger format to assess the expression of HOX/MEIS cluster genes.

Subtask 2. Evaluate the role for KMT2A stability in sustaining HOX/MEIS gene expression in KMT2A-germline AMLs.

To further test the role of KMT2A as a substrate by which CK2 exerts HOX/MEIS control in KMT2A-germline AML, we focused the methyltransferase activity of KMT2A. We performed H3K4me3 ChIP-Seq on CK2 KO or non-targeting control transduced U937 cells. We did not observe changes in H3Kme3 levels on the HOX/MEIS loci, or on self-renewal associated genes, such as SATB1 and MYC (Fig. 9C). We also used the CK2 inhibitor CX-4945 to treat ML2 cells, which harbor a KMT2A-AFDN fusion and have lost the remaining wild-type KMT2A copy. We hypothesized that ML2 cells would not decrease HOX/MEIS expression upon CX-4945 if KMT2A was indeed a required mediator. Upon 48h treatment of U937 and ML2 cells with the inhibitor, we measured the expression of HOXA genes by qPCR and observed that while in U937 cells there was a concentration-dependent decrease in transcript levels, the opposite trend occurred in ML2 cells (Fig. 11A). We then treated a panel of leukemia cell lines with CX-4945 and observed that the IC50 was lower in ML2 cells

compared to the U937 cell line (1.31 µM and 1.99 µM, respectively), indicating that cell viability did not directly correlate to the sensitivity observed to HOXA gene expression (Fig. 11B). In the context of other leukemia cell lines, ML2 has an IC50 similar to other HOX-driven AML subtypes. Taken together, our results cannot discard a role for KMT2A mediating the activity of CK2 in KMT2A-germline AML. However, given a lack of changes in H3K4me3 levels upon CK2 knockout, we concluded that KMT2A is not the main CK2 substrate exerting its action over HOX/MEIS genes.

Task 3. Evaluation of CK pharmacologic inhibition in CALM-AF10-driven leukemogenesis

Subtask 1. Assess whether CK pharmacological treatment reverses HOX/MEIS expression and leukemogenesis CALM-AF10 AML.

A recent study showed the effect of *in vivo* treatment of an AML patient-derived xenograft, the THP1 and CALM-AF10+ U937 AML cell lines with CX-4945 (Klink et al. 2021). The published results overlap with the *in vivo* experiments outlined in our proposed aims in this Subtask. Prior to our assessment of the published results, we had tested the genetic loss of CSNK2A1 *in vivo* using sgRNAs in the U937 cell line. A survival curve for mice injected with U937 cells transduced with non-targeting control (NTC) vs a CK2 sgRNA (CSNK2A1) showed no significant difference in disease latency (Fig. 12A). Given the encouraging results available in the literature, we hypothesize that our observations could be dependent on our genetic deletion approach and decided to continue our experiments on the mechanistic aspect of CK2 activity in our model.

To explore the potential resistance mechanisms to the CK2 inhibitor CX-4945, which could inform its mechanism of action and potential drug synergy interactions, we performed a whole-genome CRISPR resistance screen. To this end, we transduced a whole-genome Cas12a pooled CRISPR library in the NPM1 mutant and HOX-driven AML cell line OCI-AML3 expressing Cas12a. We maintained the cells in culture under 2 µM CX-4945 or DMSO treatment for 20 days to maintain drug selection pressure. We harvested an initial and final timepoint and compared the abundance of sgRNAs in CX-4945-treated vs. DMSO samples to elucidate potential suppressor or synthetic interactor genes (Fig. 12B). Notably, we found that HOXA13 is a potential suppressor interactor of CX-4945 in cell viability. We tested this observation in an arrayed format and a different cell model by treating U937 cells with increasing concentrations of CX-4945 upon deletion of HOXA13 or transduction with a non-targeting control (Fig. 12C). We observed a drop in sensitivity to the inhibitor upon loss of HOXA13, as well as an increase in the IC50 levels, compared to the non-targeting control (HOXA13_sg1 = 10.7 µM, HOXA13_sg4 = 2.63 µM, NTC = 1.7 µM). Future experiments guided towards exploring the contribution of HOXA13 as a CK2-mediating factor are underway in our laboratory.

Harnessing the ChEP technique described in Aim 1, Task 3, Subtask 1, we performed the enrichment procedure for U937 cells upon CK2 knockout or transduction with a non-targeting control (Figs. 6B and 6C). We found that the repressor proteins SUV39H1 and SETDB1 increased their chromatin fraction levels upon CK2 knockout, leading us to explore the potential inclusion of repressive marks to lower HOX/MEIS gene expression levels. Of note, H3K9me3 marks deposited by SETDB1or SUV39H1 are known to antagonize HOX/MEIS gene expression in AML (Müller-Tidow et al. 2010; Ropa et al. 2020; Chu et al. 2020). We treated U937 cells with a range of CX-4945 concentrations upon knockout of SETDB1, SUV39H1, or use of a non-targeting control (Fig. 12D). We observed that targeting SETDB1 decreased sensitivity to the inhibitor, and this indicates a partial rescue effect. We further tested for drug combination effects in U937 cells with CX-4945 treatment and chaetocin, a SUV39H1 inhibitor, or mithramycin A, a SETDB1 inhibitor (Fig. 12E). Chaetocin treatment showed a slightly synergistic effect when used in combination with CX-4945 (ZIP score=0.546), while treatment with mitramycin A was slightly antagonistic to CX-4945 (ZIP score= -3.5647). Our results from the drug treatment experiments are not conclusive and we are currently exploring the mechanism by which CK2 loss leads to the recruitment of repressive complexes at the HOX/MEIS loci.

Figures

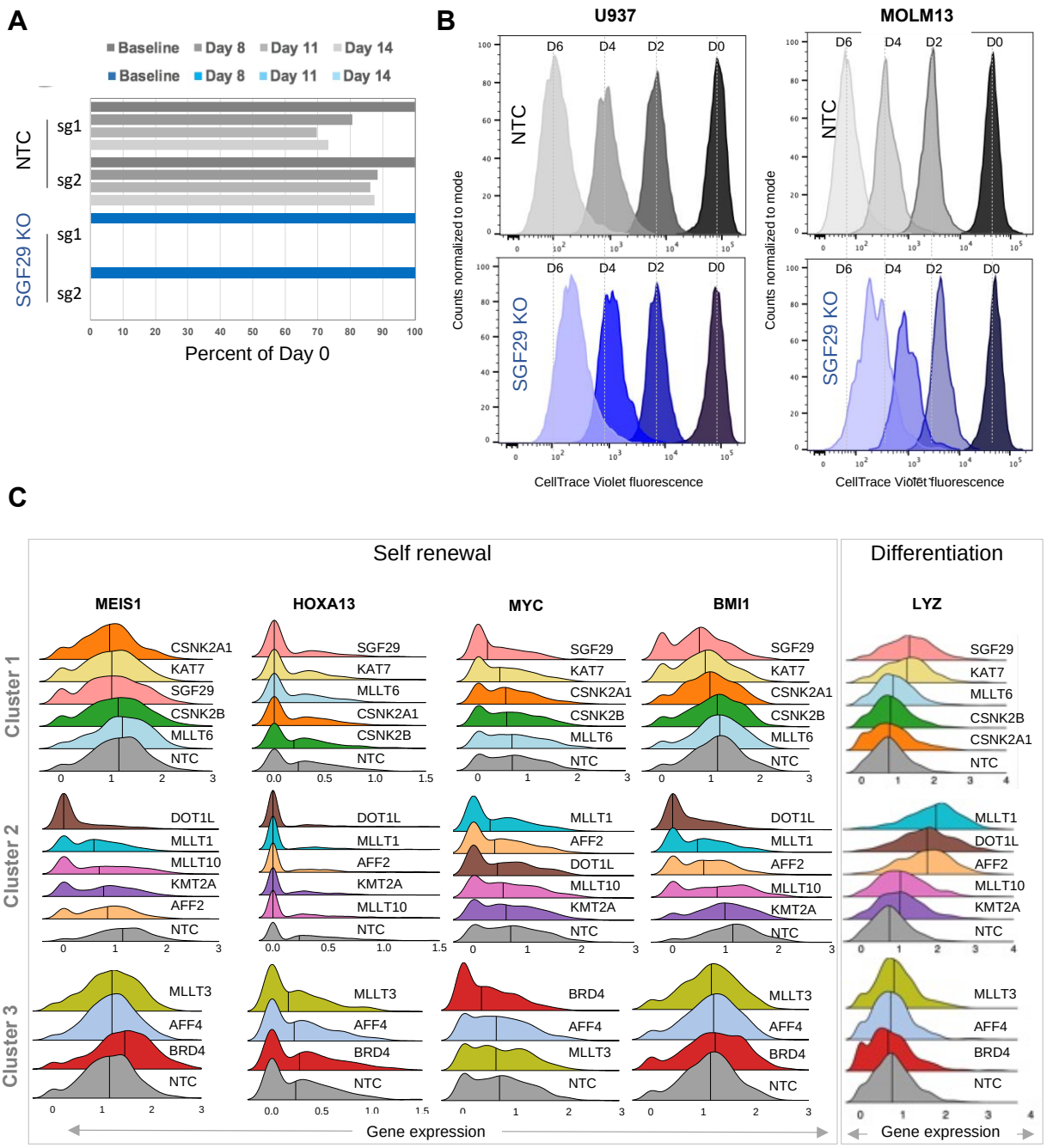

Figure 1. Functional validation studies for the Tudor domain-containing reader SGF29 in AML. (A) Competition assays with percent viability of U937 cells plotted on the X-axis over time, normalized to the baseline measurement; the y-axis indicates the sgRNA used: 2 individual NTC sgRNAs and 2 individual sgRNAs for SGF29 (left). NTC bars are negative controls; n=3 in each condition. (B) Histograms of U937 (left) or MOLM13 (right) cells transduced with an NTC (top) or an SGF29 sgRNA (bottom) show the fluorescence intensity of CellTrace™ Far Red dye measured by flow cytometry over time. (C) Ridge plots for CROP-Seq perturbations. Knockout genes are indicated on the y-axis and RNA expression levels for the gene listed are on the x-axis. The median expression value is indicated.

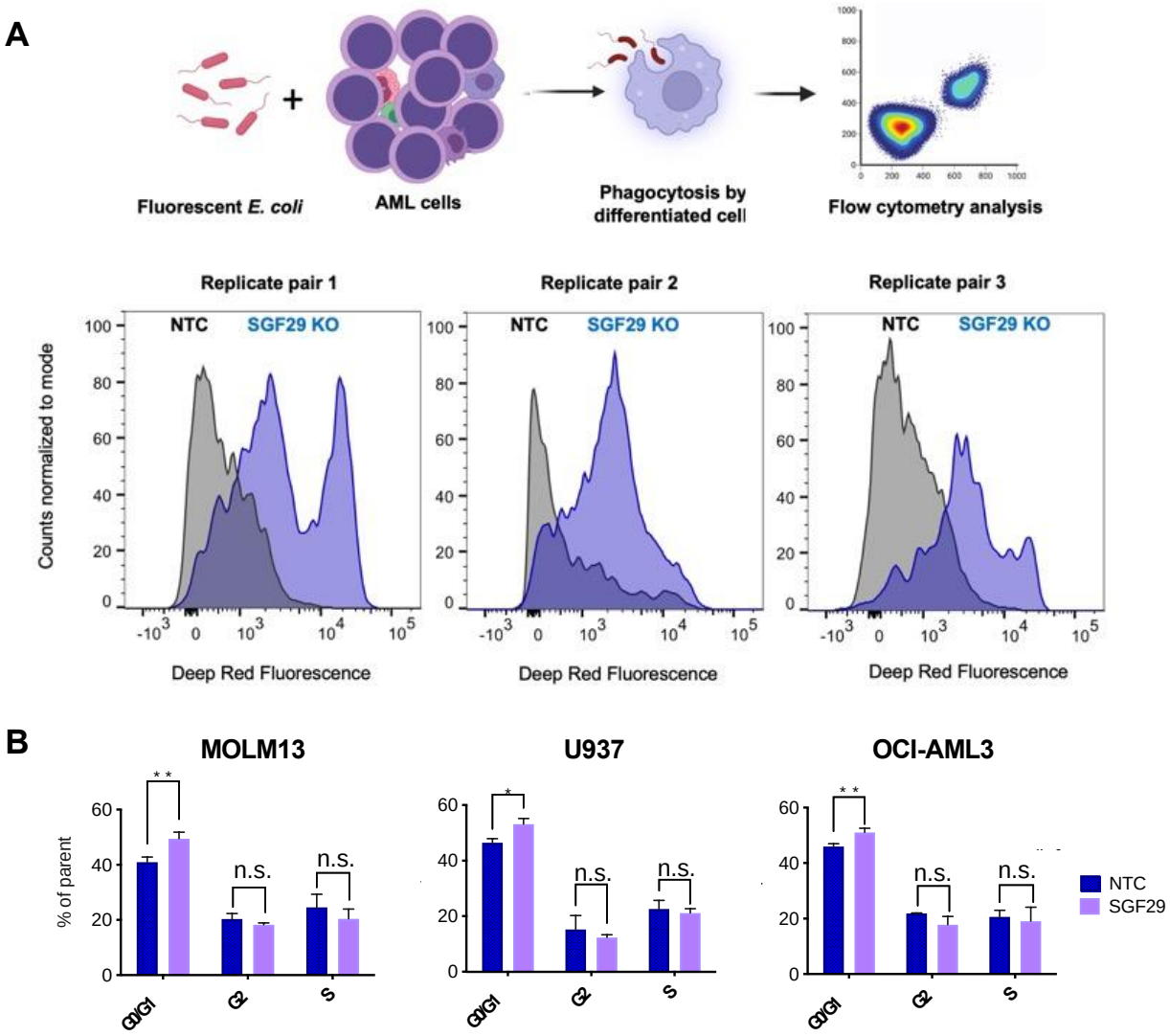

Figure 2. SGF29 deletion induces differentiation and cell cycle arrest in HOX-driven AML cell lines. (A, top) Schematic for differentiation assessment via phagocytosis of fluorescently labeled *E. coli* bioparticles; (bottom) Histograms of U937 cells transduced with an NTC (grey) or an SGF29 sgRNA (violet) show the fluorescence intensity of engulfed pHrodo™ Red E. coli bioparticles measured at 9 days after staining. (B) Cell cycle progression analysis by propidium iodide staining in human AML cells expressing NTC (blue) or SGF29 (light violet) sgRNAs (n=3).

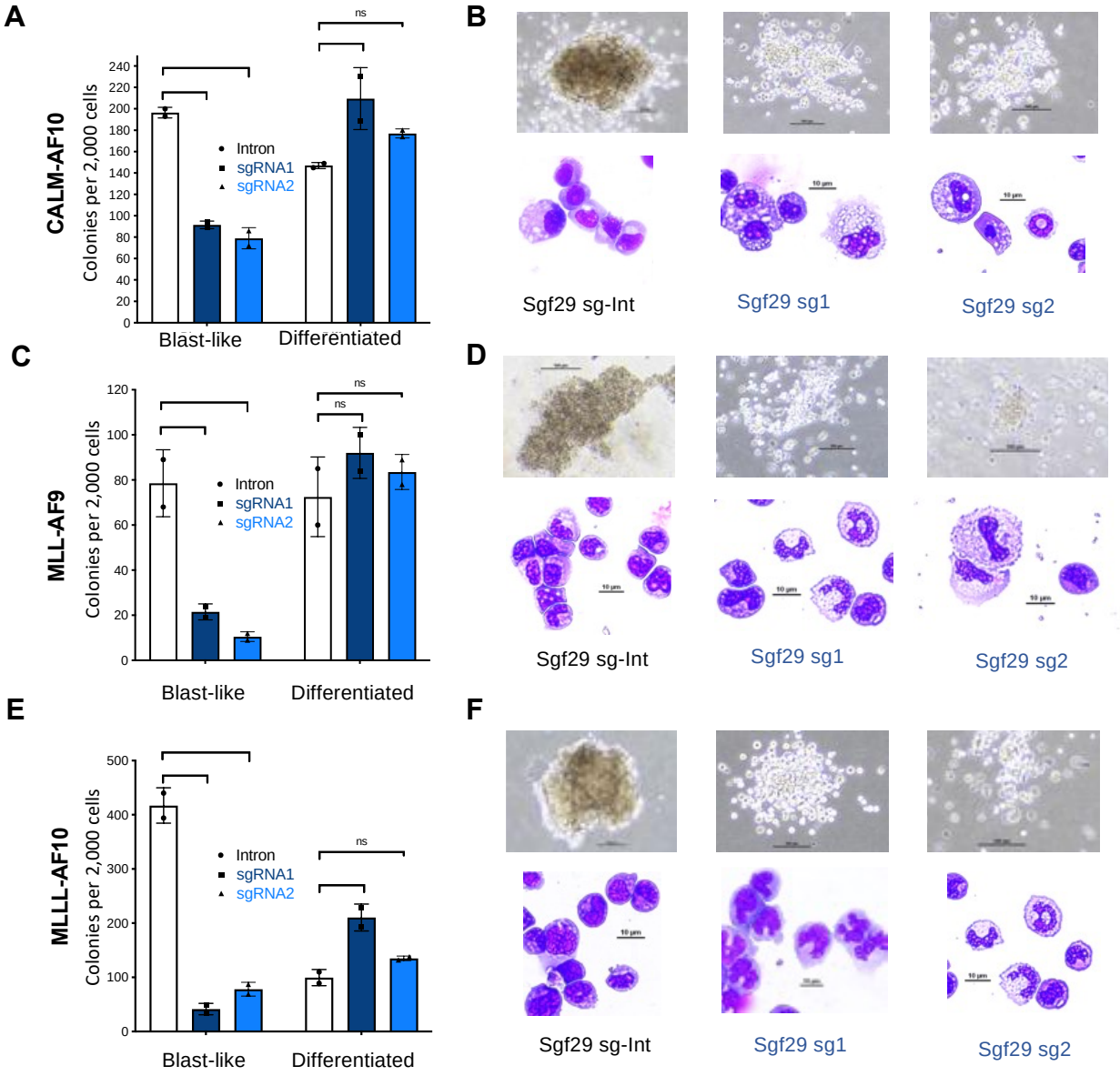

Figure 3. Sgf29 deletion impairs the clonogenicity of transformed hematopoietic cells. Number of colony forming units (CFUs) from (A) CALM-AF10-, (B) MLL-AF9-, and (C) MLL-AF10- transformed murine HSPCs transduced with Sgf29 intron-targeting sgRNA or two independent Sgf29 exon-targeting sgRNAs. CFUs per 2,000 plated cells at 1 week are plotted on the y -axis and categorized as blast-like or differentiated based on colony morphology. Representative bright-field colony images (top, scale bar= 100mm) and Wright-Giemsa-stained cytospins (bottom, scale bar= 10mm) with labeled sgRNAs for (B) CALM-AF10- (D) MLL-AF9- and (F) MLL-AF10- transformed murine AMLs. Measurements and images were obtained after 7 days of methylcellulose culture.

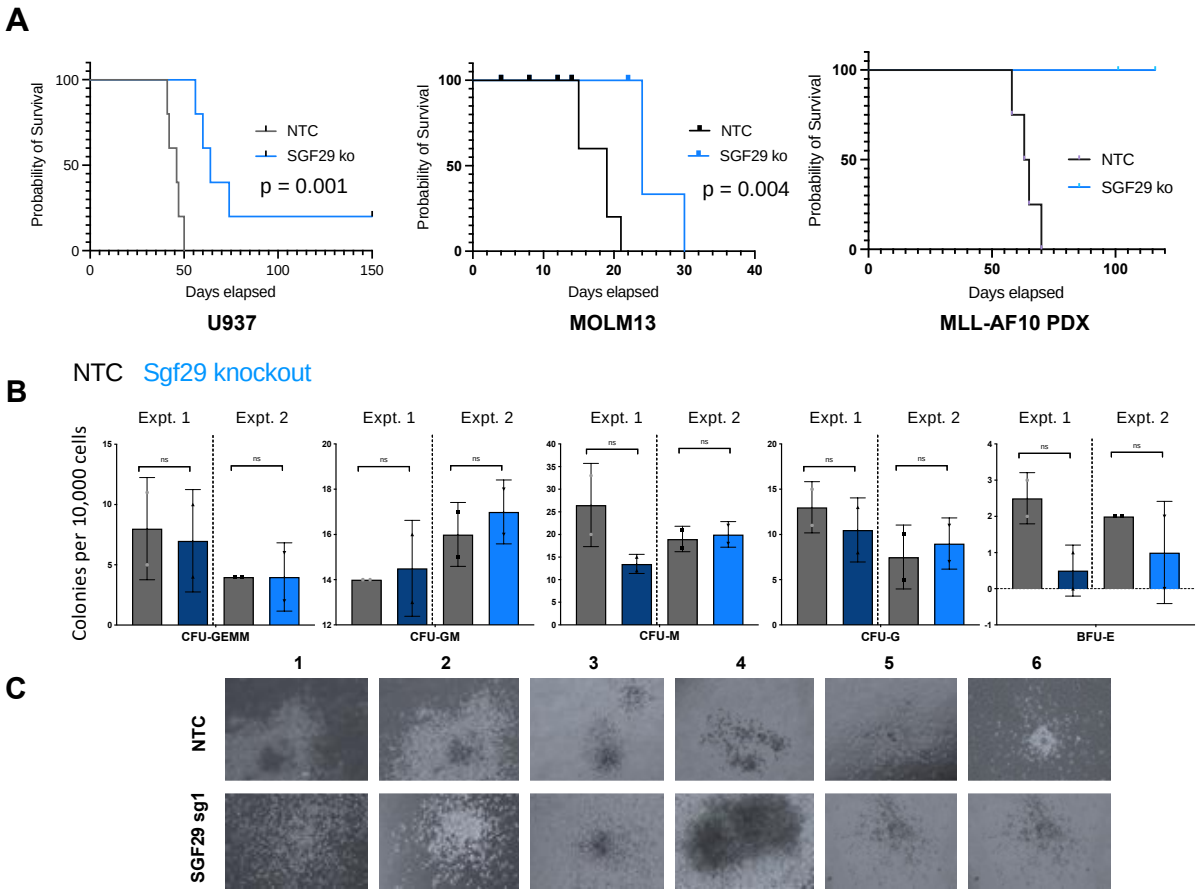

Figure 4. SGF29 deletion increases AML disease latency in vivo and does not impair the clonogenicity of normal hematopoietic cells. (A) Kaplan-Meier curves for NTC versus SGF29 knockout in U937 and MOLM13 AML cell lines and an MLL-AF10 patient-derived xenograft line. (B) Number of CFUs per 10,000 LSK-sorted murine HSPCs expressing Cas9 and NTC (grey) or Sgf29-targeting (blue) sgRNAs, 7 days post-seeding in methylcellulose. The y-axis shows the numbers of different types of colonies from cells expressing the indicated sgRNA, and are categorized by their morphology as CFU-G: colony-forming unit-granulocyte, CFU-M: colony-forming unit-macrophage, CFU-GM: colony-forming unit granulocyte monocyte, CFU-Blast: blast-like colonies. Representative images from each experimental condition are shown in (C).

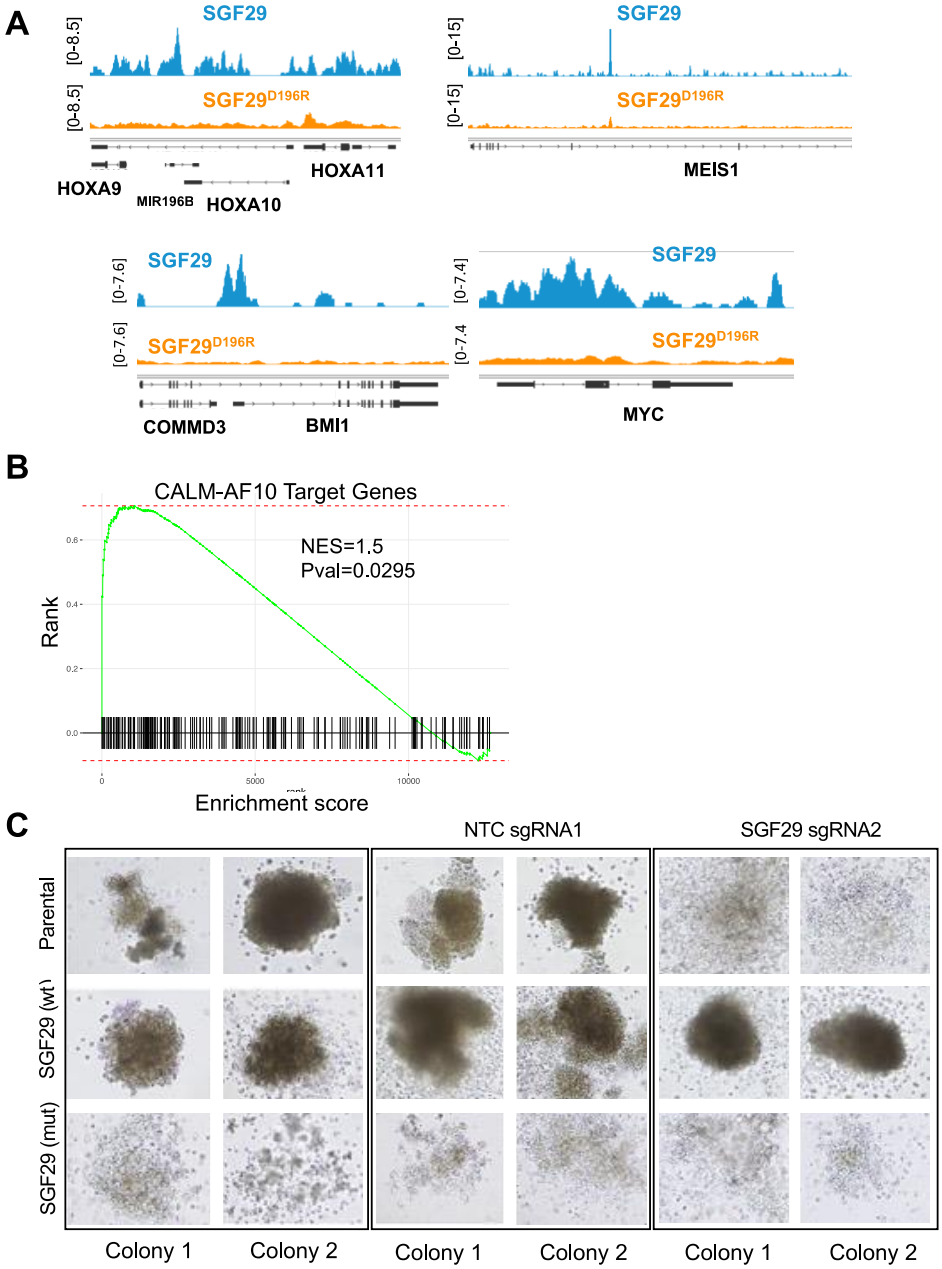

Figure 5. The Tudor domain of SGF29 is essential for its role in myeloid transformation. (A) Genome tracks depicting normalized reads (scale in the y-axis) of *HOX/MEIS* genes, *MYC*, and *BMI1*, in representative Flag ChIP-seq samples of SGF29 wild-type cells compared to SGF29^{D196R}. (B) GSEA plot of CALM-AF10 target gene expression in SGF29 knockout cells. (C) Representative images of CFUs from MLL-AF9 murine leukemias with a mock transduction (left pane), non-targeting control (middle pane) or Sgf29 sgRNA (right pane), rescued with an empty vector (top, parental), human wild-type SGF29 (SGF29 wt, middle) or human SGF29^{D196R} mutant (SGF29 mut, bottom). Representative colonies are shown in bright field.

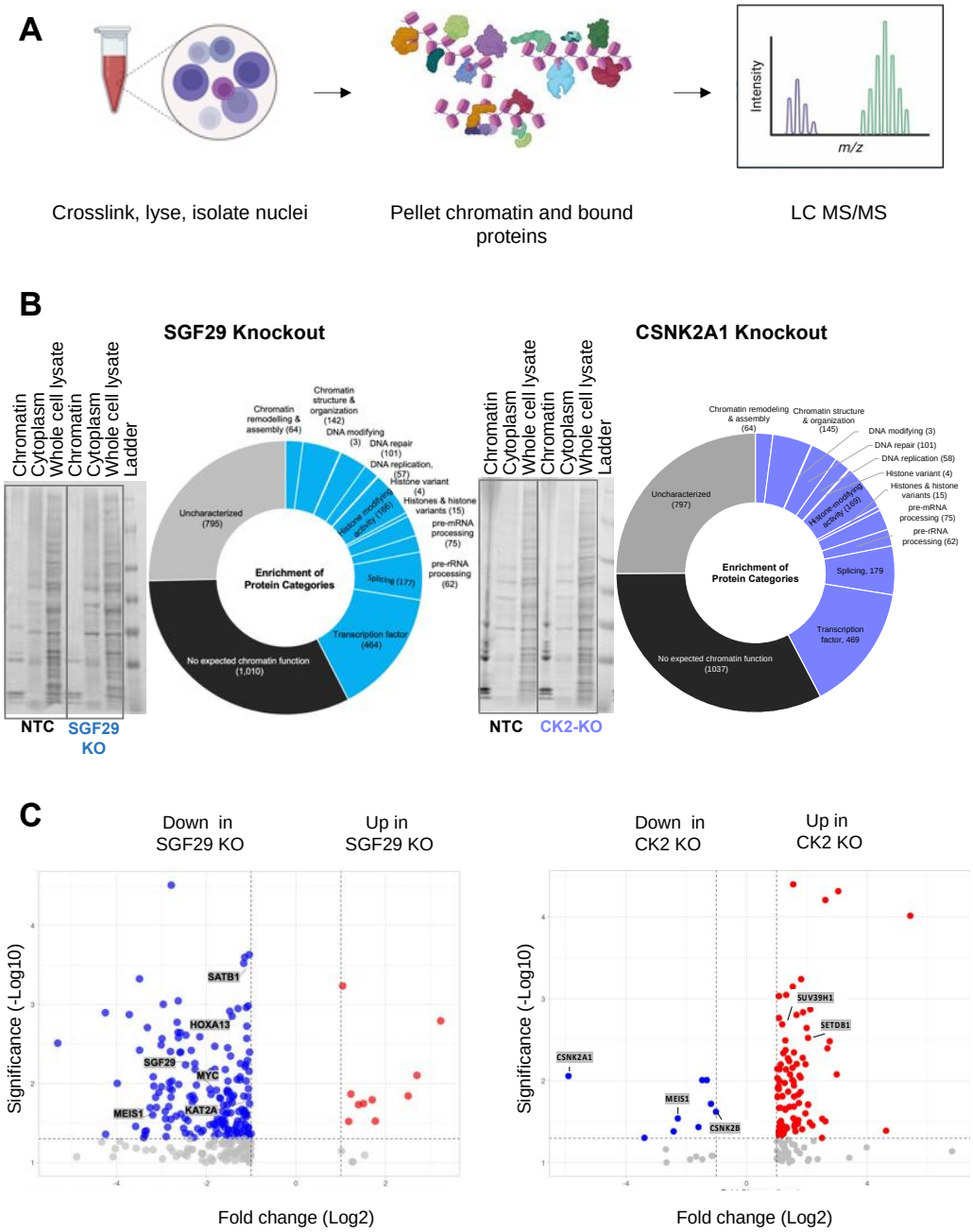

Figure 6. Chromatin enrichment for proteomics (ChEP) procedure and analysis for SGF29- and CSNK2A1- deleted human U937 AML cells. (A) Schematic for the ChEP sample preparation, as previously described (Kustatscher et al. 2014). (B) SDS-PAGE showing whole cell extract, cytoplasmic and ChEP-enriched fractions for NTC vs SGF29 knockout (left) and NTC vs CSNK2A1 knockout (right) experiments. A pie chart next to each gel image shows the proteomic analysis of the ChEP fractions across 4 samples. Proteins were annotated using the categories outlined in Kustatscher et al. (2014). The number of proteins per category is shown in parentheses. (**c**) Volcano plots depicting -log(10) of the adjusted P value on y-axis and log(2) of fold change (LogFC) of all proteins in the ChEP fractions for both SGF29 knockout vs. NTC (left) and CSNK2A1 knockout vs NTC (right). Differentially abundant protein (Absolute FC >2, Adj. P val. < 0.05 are not shown; n=4 for every experimental condition.

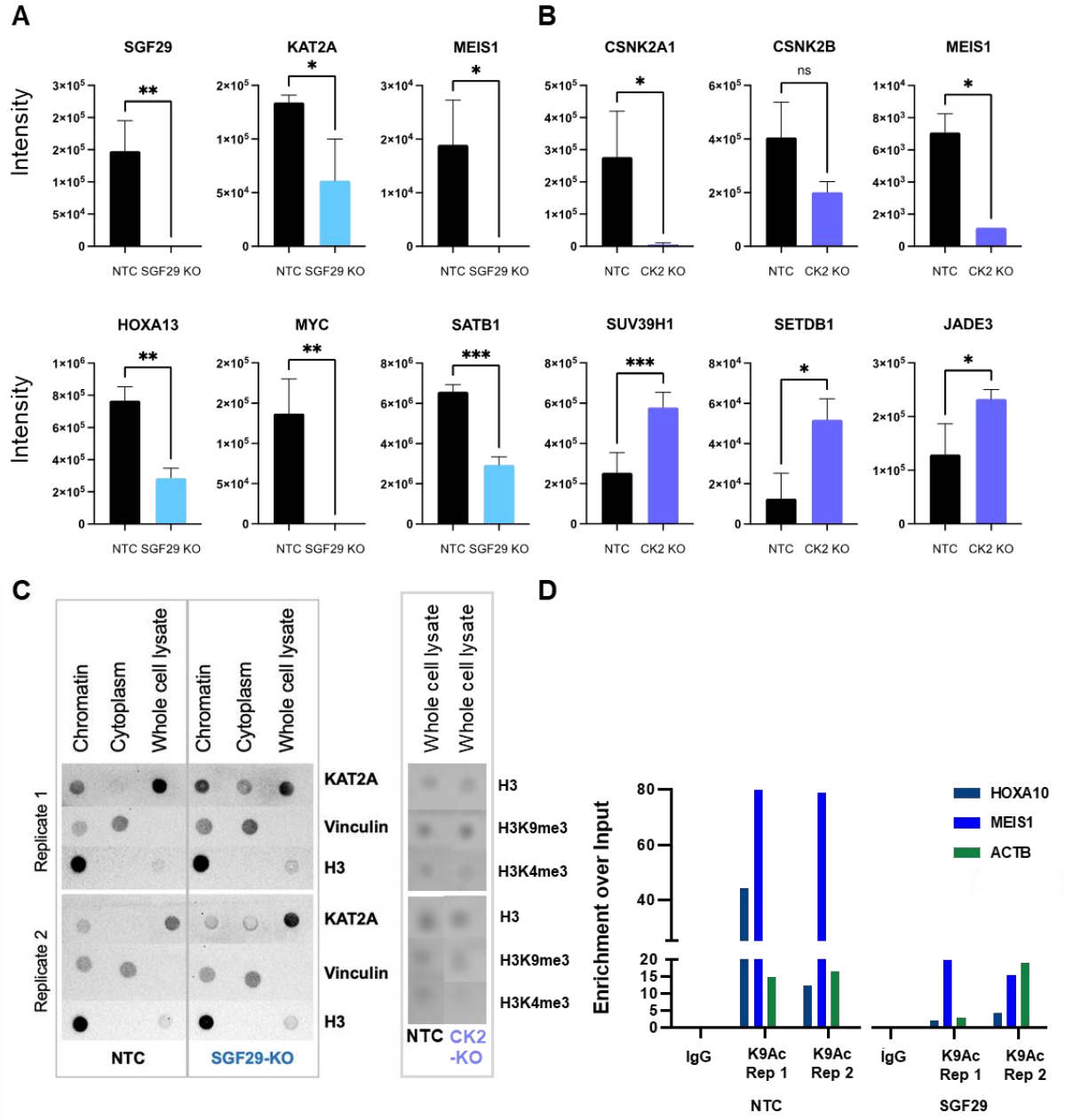

Figure 7. Self-renewal proteins and chromatin modifiers are displaced from chromatin upon loss of SGF29 or CSNK2A1. (A) Bar graphs depicting intensity values for self-renewal-associated proteins enriched by ChEP in the SGF29 knockout vs NTC experiment (B) and CSNK2A1 knockout vs NTC experiment. (C) Dot blots for ChEP-enriched chromatin, cytoplasmic fraction and whole-cell lysate for NTC and SGF29 knockout (left) and NTC and CSNK2A1 knockout (right) U937 cells. (D) cDNA enrichment over input for *HOXA9*, *MEIS1*, and *ACTB* in IgG or H3K9ac ChIP for NTC or SGF29 knockout U937 cells.

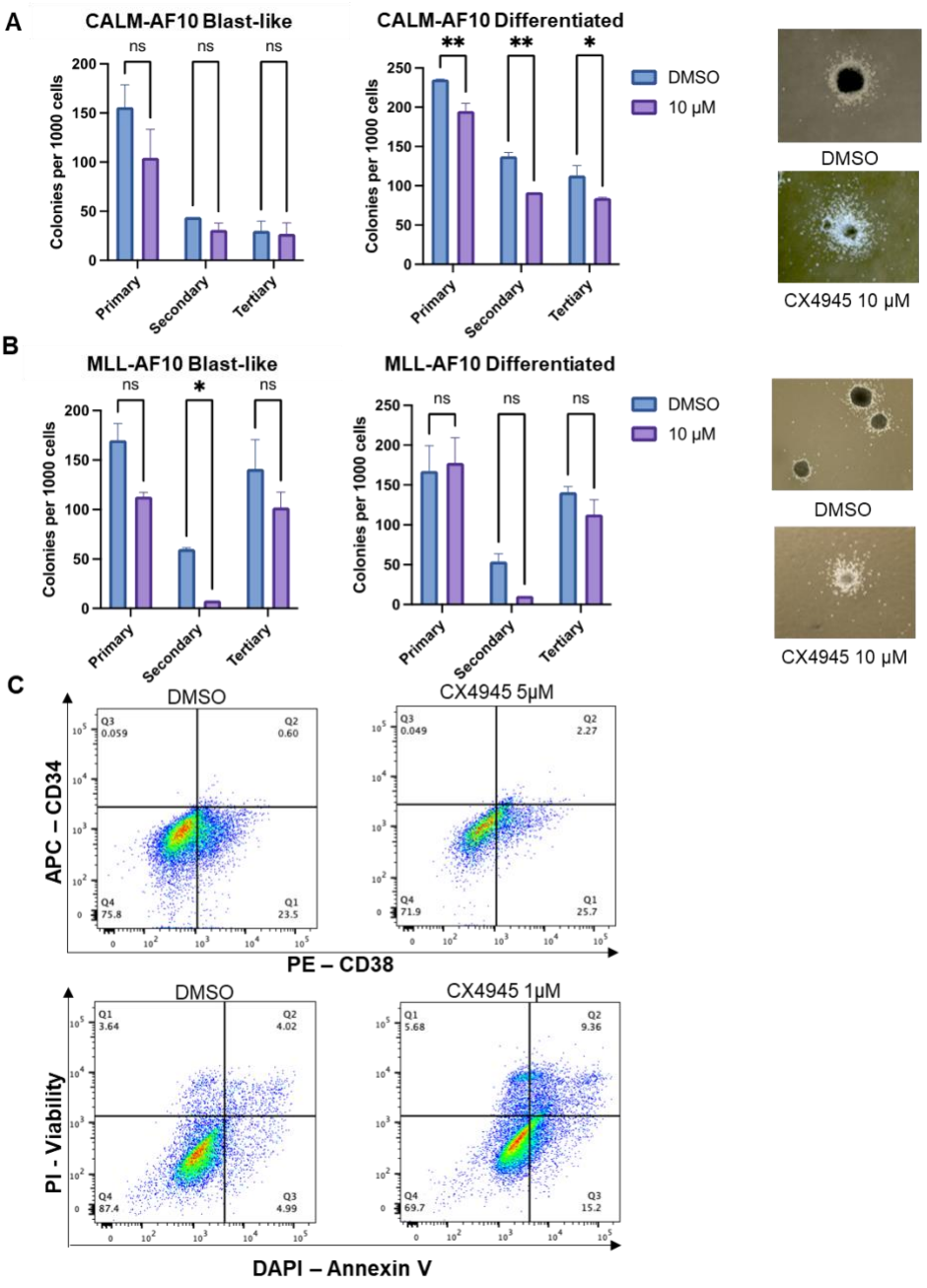

Figure 8. CX-4945 treatment of murine AML cells does not fully impair colony formation and leads to slight increase in differentiation markers in a human MLL-AF10 AML PDX sample. (A, B) CFU counts per 1,000 cells plated for blast-like (left) or differentiated (middle) colonies of (A) CALM-AF10 or (B) MLL-AF10 transformed murine AML cells upon treatment with DMSO or 10 µM CX-4945 over rounds of replating. Representative images of colonies are shown on the right; n=2, * $p < 0.05$. (C, top) Representative flow cytometry profiles for CD34 and CD38 staining of an MLL-AF10 PDX AML sample after 48h of 5 µM CX-4945 treatment. (C, bottom) Representative flow cytometry profile for AnnexinV and propidium iodide (PI) staining in an MLL-AF10 PDX AML sample upon 48h of 1 µM CX-4945 treatment.

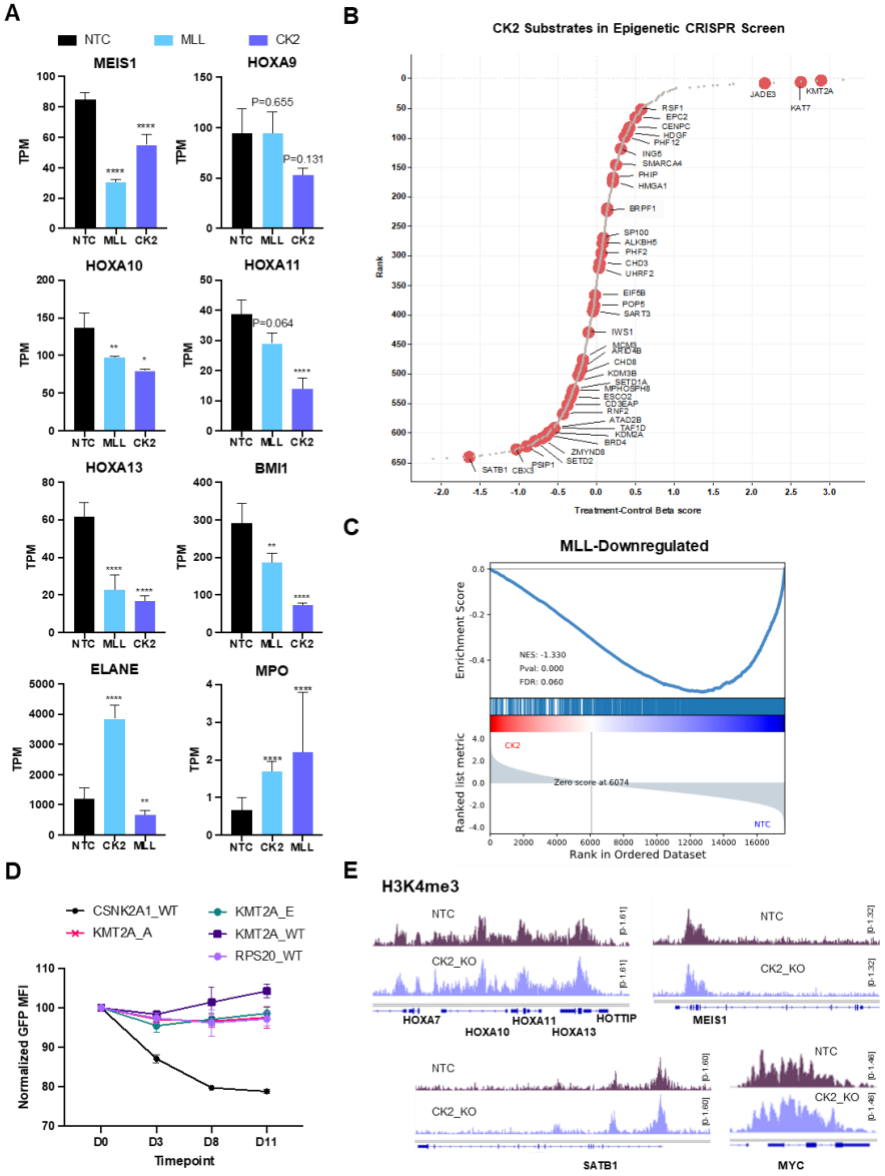

Figure 9. KMT2A shows a partial phenotype as a substrate mediating the CK2 kinase phenotype over *HOX/MEIS* gene expression. (A) Bar graphs depicting normalized gene expression (transcripts per million, TPM) of self-renewal- and differentiation-associated genes in NTC, KMT2A-knockout, and CK2-knockout arms (n=3 for CK2 and KMT2A/MLL arms, n=2 for NTC). (B) Sorted gene-hits based on differential gene-level MAGeCK beta scores for the MEIS1-GFP Low minus MEIS1-GFP High fractions in the Epigenetic CRISPR screen. Those genes containing CK2 phosphorylation motifs are highlighted. (C) GSEA plot of CALM-AF10 target gene expression in CK2 knockout (left) compared to NTC (right) (n=3 for CK2 arms, n=2 for NTC). (D) Baseline- normalized GFP mean fluorescence intensity measurements over time (MFI, y-axis; timepoints on x-axis) of U937 cells expressing short cDNA fragments containing CK2 phosphorylation motifs for KMT2A and control sgRNAs targeting CSNK2A1 or RPS20. (E) Genome tracks depicting normalized reads (scale in the y-axis) of *HOX/MEIS* genes, *MYC*, and *SATB1*, in representative H3K4me3 ChIP-seq samples of CSNK2A1 wild-type cells compared to NTC control.

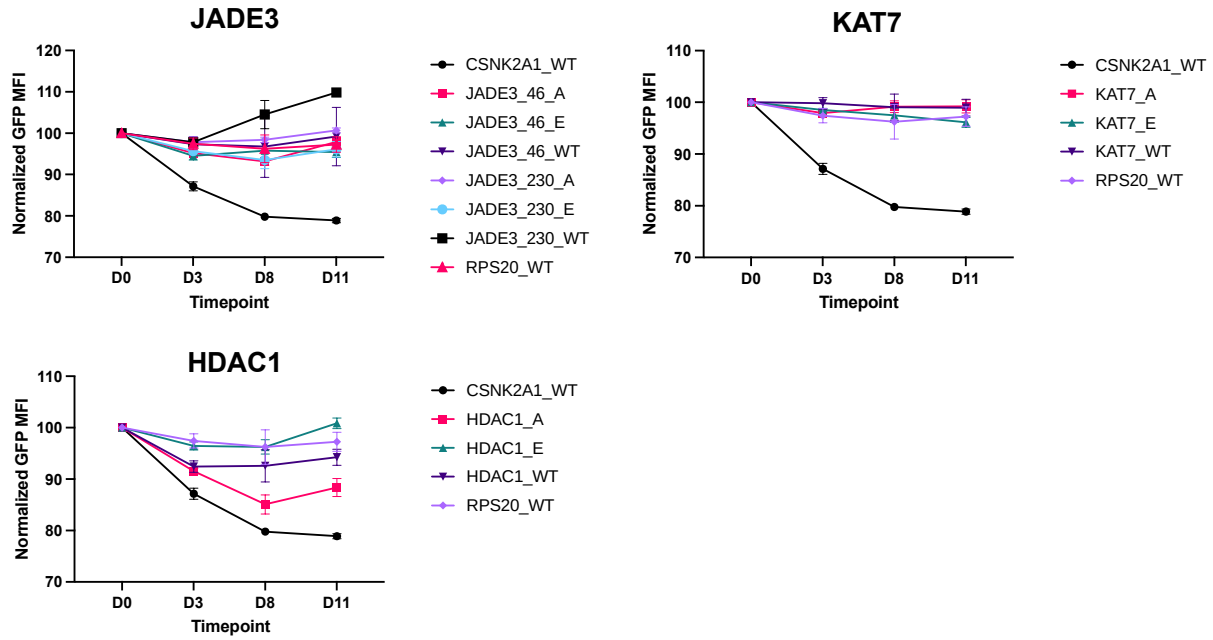

Figure 10. GFP-MEIS1 levels upon CK2-motif containing short peptide fragments overexpression in U937 cells. Baseline-normalized GFP mean fluorescence intensity measurements over time (MFI, y-axis; timepoints on x-axis) of U937 cells expressing short cDNA fragments containing CK2 phosphorylation motifs for JADE3, KAT7 and HDAC1, and control sgRNAs targeting CSNK2A1 or RPS20.

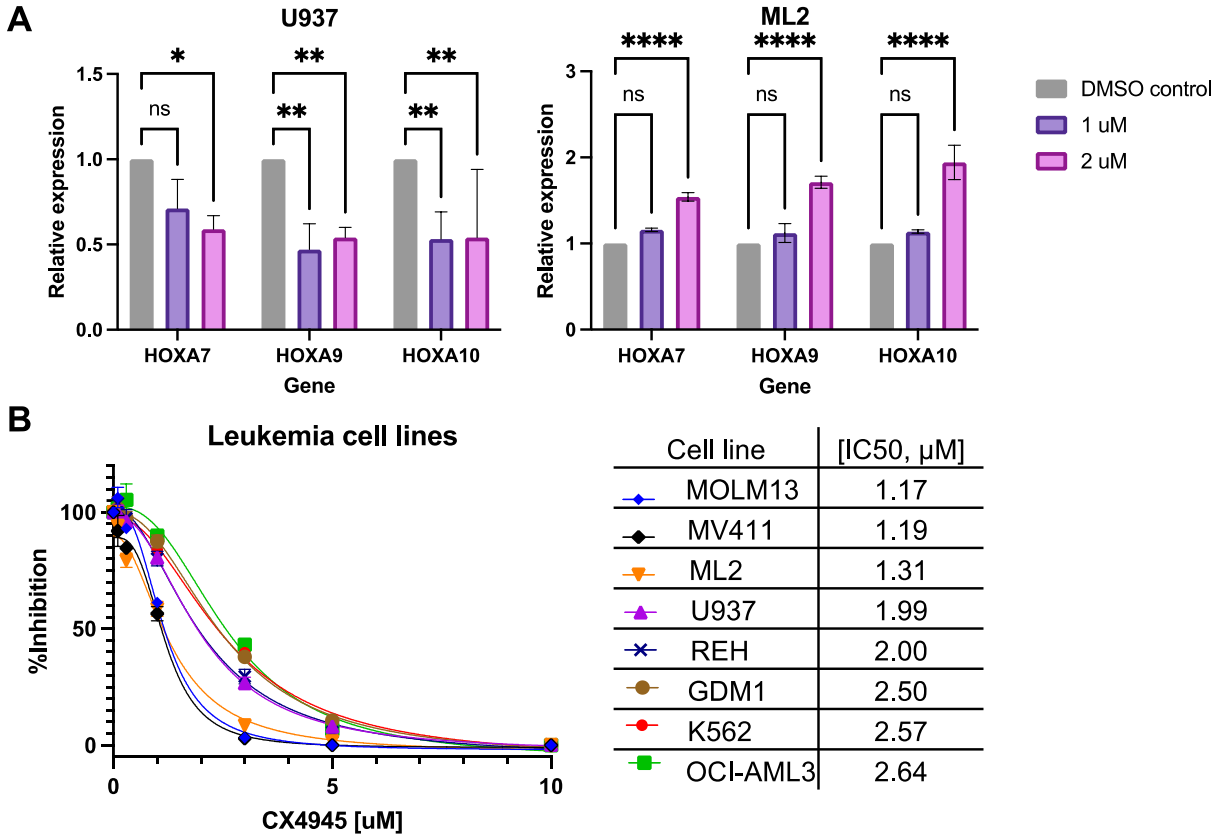

Figure 11. AML cell lines are sensitive to CX-4945 treatment across different subtypes. (B) Left: dose-response curve of a panel of human and mouse AML cell lines treated with CX-4945 in different concentrations, measured at day 3. Right: IC50 calculations for the treated cell lines (n=3). The y axis indicates the normalized counts compared to the untreated control at the same timepoint; the x axis indicates the drug concentration.

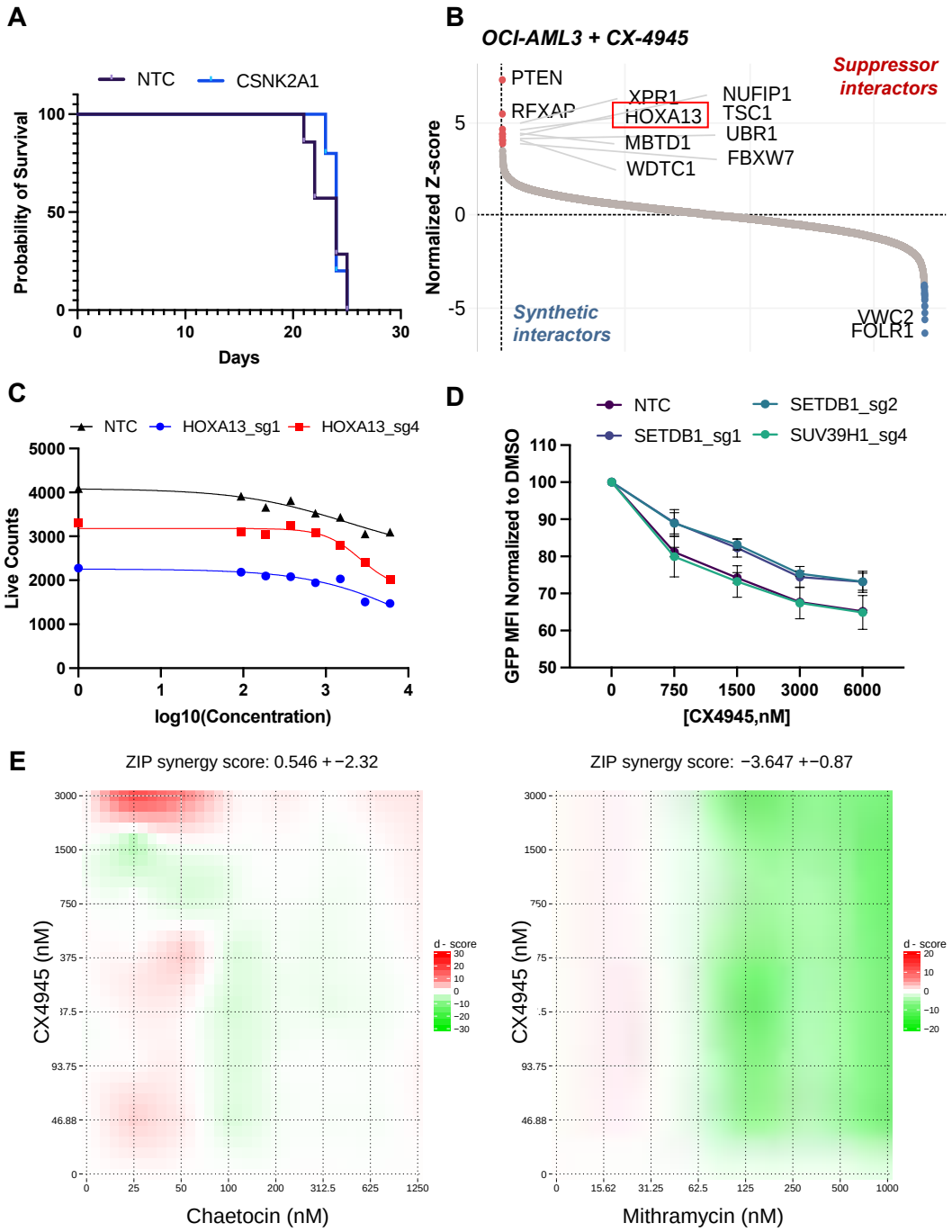

Figure 12. Figure 4. SGF29 deletion increases AML disease latency in vivo and does not impair the clonogenicity of normal hematopoietic cells. (A) Kaplan-Meier curve for NTC versus CSNKA1 knockout in the U937 AML cell line. (B) Sorted Z-scores for CX-4945 drug interactions in a whole-genome CRISPR-Cas12a screen with CX-4945 treatment. (C) Proliferation assay showing live cell counts in the y-axis and the log(10) concentration of CX-4945, measured 72h after the start of treatment in U937 cells expressing 2 independent sgRNAs targeting HOXA13 or a non-targeting control. (D) Baseline-normalized GFP mean fluorescence intensity measurements measured at 72 h of treatment with increasing concentrations of CX-4945 (MFI, y-axis; concentrations on x-axis) of U937 cells expressing sgRNAs targeting SETDB1, SUV39H1 or a non-targeting control. (E) Percentage of live cells across drug combination treatments with (A) CX-4945 and chaetocin and (B) CX-4945 and mithramycin. Drug concentrations are listed on the axes.

References

Au, Yan Zi, Muxin Gu, Etienne De Braekeleer, Malgorzata Gozdecka, Demetrios Aspris, Yusuke Tarumoto, Jonathan Cooper, et al. 2021. "KAT7 Is a Genetic Vulnerability of Acute Myeloid Leukemias Driven by MLL Rearrangements." *Leukemia* 35 (4): 1012–22.

Bian, Chuanbing, Chao Xu, Jianbin Ruan, Kenneth K. Lee, Tara L. Burke, Wolfram Tempel, Dalia Barsyte, et al. 2011. "Sgf29 Binds Histone H3K4me2/3 and Is Required for SAGA Complex Recruitment and Histone H3 Acetylation." *The EMBO Journal* 30 (14): 2829–42.

Chen, Bo-Rui, Anagha Deshpande, Karina Barbosa, Maria Kleppe, Xue Lei, Narayana Yeddula, Pablo Sánchez Vela, et al. 2021. "A JAK/STAT-Mediated Inflammatory Signaling Cascade Drives Oncogenesis in AF10-Rearranged AML." *Blood* 137 (24): 3403–15.

Chon, Hae J., Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon, South Korea, Kyoung J. Bae, Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon, South Korea, Yura Lee, Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon, South Korea, Jiyeon Kim, Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon, South Korea, and Yeon@dankook ac Kr. 2015. "The Casein Kinase 2 Inhibitor, CX-4945, as an Anti-Cancer Drug in Treatment of Human Hematological Malignancies." *Frontiers in Pharmacology* 6. https://doi.org/10.3389/fphar.2015.00070.

Chu, Yajing, Yangpeng Chen, Huidong Guo, Mengke Li, Bichen Wang, Deyang Shi, Xuelian Cheng, et al. 2020. "SUV39H1 Regulates the Progression of MLL-AF9-Induced Acute Myeloid Leukemia." *Oncogene* 39 (50): 7239–52.

Cozza, Giorgio, Cristina Girardi, Alessandro Ranchio, Graziano Lolli, Stefania Sarno, Andrzej Orzeszko, Zygmunt Kazimierczuk, Roberto Battistutta, Maria Ruzzene, and Lorenzo A. Pinna. 2014. "Cell-Permeable Dual Inhibitors of Protein Kinases CK2 and PIM-1: Structural Features and Pharmacological Potential." *Cellular and Molecular Life Sciences: CMLS* 71 (16): 3173–85.

Deshpande, A., B. R. Chen, L. Zhao, K. Saddoris, M. Kerr, N. Zhu, P. Mali, and A. J. Deshpande. 2019. "Investigation of Genetic Dependencies Using CRISPR-Cas9-Based Competition Assays." *Journal of Visualized Experiments: JoVE*, no. 143 (January). https://doi.org/10.3791/58710.

Deshpande, Aniruddha J., Anagha Deshpande, Amit U. Sinha, Liying Chen, Jenny Chang, Ali Cihan, Maurizio Fazio, et al. 2014. "AF10 Regulates Progressive H3K79 Methylation and HOX Gene Expression in Diverse AML Subtypes." *Cancer Cell*. https://doi.org/10.1016/j.ccell.2014.10.009.

Ford, Kyle M., Rebecca Panwala, Dai-Hua Chen, Andrew Portell, Nathan Palmer, and Prashant Mali. 2021. "Peptide-Tiling Screens of Cancer Drivers Reveal Oncogenic Protein Domains and Associated Peptide Inhibitors." *Cell Systems* 12 (7): 716-732.e7.

Klink, M., M. A. Rahman, C. Song, P. K. Dhanyamraju, M. Ehudin, Y. Ding, S. Steffens, et al. 2021. "Mechanistic Basis for In Vivo Therapeutic Efficacy of CK2 Inhibitor CX-4945 in Acute Myeloid Leukemia." *Cancers* 13 (5). https://doi.org/10.3390/cancers13051127.

Kuhn, M. W., M. J. Hadler, S. R. Daigle, R. P. Koche, A. V. Krivtsov, E. J. Olhava, M. A. Caligiuri, et al. 2015. "MLL Partial Tandem Duplication Leukemia Cells Are Sensitive to Small Molecule DOT1L Inhibition." In *Haematologica*, 100:e190-3. Italy.

Kuhn, M. W., E. Song, Z. Feng, A. Sinha, C. W. Chen, A. J. Deshpande, M. Cusan, et al. 2016. "Targeting Chromatin Regulators Inhibits Leukemogenic Gene Expression in NPM1 Mutant Leukemia." *Cancer Discovery* 6 (10): 1166–81.

Kustatscher, Georg, Karen L. H. Wills, Cristina Furlan, and Juri Rappsilber. 2014. "Chromatin Enrichment for Proteomics." *Nature Protocols* 9 (9): 2090–99.

Liu, Wen-Hsin, Kerstin Völse, Daniela Senft, and Irmela Jeremias. 2021. "A Reporter System for Enriching CRISPR/Cas9 Knockout Cells in Technically Challenging Settings like Patient Models." *Scientific Reports* 11 (1): 12649.

MacPherson, Laura, Juliana Anokye, Miriam M. Yeung, Enid Y. N. Lam, Yih-Chih Chan, Chen-Fang Weng, Paul Yeh, et al. 2020. "HBO1 Is Required for the Maintenance of Leukaemia Stem Cells." *Nature* 577 (7789): 266–70.

Müller-Tidow, Carsten, Hans-Ulrich Klein, Antje Hascher, Fabienne Isken, Lara Tickenbrock, Nils Thoennissen, Shuchi Agrawal-Singh, et al. 2010. "Profiling of Histone H3 Lysine 9 Trimethylation

Levels Predicts Transcription Factor Activity and Survival in Acute Myeloid Leukemia." *Blood* 116 (18): 3564–71.
Najm, Fadi J., and Peter van Galen. 2022. "Renewing Your HBO1 Subscription." *Blood*. American Society of Hematology.
Rau, Rachel E., Benjamin A. Rodriguez, Min Luo, Mira Jeong, Allison Rosen, Jason H. Rogers, Carly T. Campbell, et al. 2016. "DOT1L as a Therapeutic Target for the Treatment of DNMT3A-Mutant Acute Myeloid Leukemia." *Blood* 128 (7): 971–81.
Ropa, James, Nirmalya Saha, Hsiangyu Hu, Luke F. Peterson, Moshe Talpaz, and Andrew G. Muntean. 2020. "SETDB1 Mediated Histone H3 Lysine 9 Methylation Suppresses MLL-Fusion Target Expression and Leukemic Transformation." *Haematologica* 105 (9): 2273–85.
Takahashi, S., A. Kanai, H. Okuda, R. Miyamoto, Y. Komata, T. Kawamura, H. Matsui, T. Inaba, A. Takaori-Kondo, and A. Yokoyama. 2021. "HBO1-MLL Interaction Promotes AF4/ENL/P-TEFb-Mediated Leukemogenesis." *ELife* 10 (August). https://doi.org/10.7554/eLife.65872.
Tubi, Laura Quotti, Carmela Gurrieri, Alessandra Brancalion, Laura Bonaldi, Roberta Bertorelle, Sabrina Manni, Laura Pavan, et al. 2013. "Inhibition of Protein Kinase CK2 with the Clinical-Grade Small ATP-Competitive Compound CX-4945 or by RNA Interference Unveils Its Role in Acute Myeloid Leukemia Cell Survival, P53-Dependent Apoptosis and Daunorubicin-Induced Cytotoxicity." *Journal of Hematology & Oncology* 6 (1): 1–15.
Van Vlierberghe, Pieter, Martine van Grotel, Joëlle Tchinda, Charles Lee, H. Berna Beverloo, Peter J. van der Spek, Andrew Stubbs, et al. 2008. "The Recurrent SET-NUP214 Fusion as a New HOXA Activation Mechanism in Pediatric T-Cell Acute Lymphoblastic Leukemia." *Blood* 111 (9): 4668–80.
Yang, Yuqing, Andrew J. Kueh, Zoe L. Grant, Waruni Abeysekera, Alexandra L. Garnham, Stephen Wilcox, Craig D. Hyland, et al. 2022. "The Histone Lysine Acetyltransferase HBO1 (KAT7) Regulates Hematopoietic Stem Cell Quiescence and Self-Renewal." *Blood* 139 (6): 845–58.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

We are currently executing experiments to address the mechanistic details of HOX/MEIS regulation by the epigenetic factors under study in both of our aims. Namely, we have performed a panel of ChIP-seq assays to test the levels and distribution of chromatin marks associated with SGF29 and the SAGA complex, and the repressor proteins SETDB1 and SUV39H1, which take part in the working model by which we hypothesize that CK2 exerts control over HOX/MEIS genes. We anticipate results that will allow us to submit the work contained in the statement of work as one or two research manuscripts for publication before the end of the extension period. In addition, the PI in this grant will submit the results reported so far as a part of her graduate thesis before the end of the extension period.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report.

What was the impact on other disciplines?
Nothing to Report.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to Report.

Changes that had a significant impact on expenditures
Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.

Significant changes in use or care of human subjects
Nothing to Report.

Significant changes in use or care of vertebrate animals
Nothing to Report.

Significant changes in use of biohazards and/or select agents
Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Tiwari, A., Paithane, U., Tashiro, K., Saulnier, O., **Barbosa, K.,** … & Bagchi, A. (2022). The lncRNA PVT1 encodes peptides with oncogenic and tumor suppressive activity. *Manuscript under review*.

  Arnold, O., **Barbosa, K.,** Deshpande, A. J., & Zhu, N. (2022). The Role of DOT1L in Normal and Malignant Hematopoiesis. *Frontiers in Cell and Developmental Biology*, *10*. Published. Acknowledgment of federal support: yes.

  Raveendra-Panickar, D., Finlay, D., Layng, F. I., Lambert, L. J., Celeridad, M., Zhao, M., **Barbosa, K.,** ... & Tautz, L. (2022). Discovery of novel furanylbenzamide inhibitors that target oncogenic tyrosine phosphatase SHP2 in leukemia cells. *Journal of Biological Chemistry*, *298*(1). Published. Acknowledgment of federal support: no.

  Sinha, S.*, **Barbosa, K***., Cheng, K.*, Leiserson, M. D., Jain, P., Deshpande, A., ... & Ruppin, E. (2021). A systematic genome-wide mapping of oncogenic mutation selection during CRISPR-Cas9 genome editing. *Nature communications*, *12*(1), 1-13. Published. Acknowledgment of federal support: no.

Books or other non-periodical, one-time publications.
Nothing to Report.

Other publications, conference papers and presentations.

Barbosa, K. The Epigenetic Regulator Landscape of Stemness Networks in AML. Poster presented at: The International Society for Stem Cell Research Annual Meeting 2022; June 16, 2022; Virtual presentation.

Barbosa, K. High-Density Domain-Focused CRISPR Screens Reveal Epigenetic Regulators of Hox/Meis Gene Expression in Acute Myeloid Leukemia. Poster presented at: The Sanford Burnham Prebys Annual Trainee Research Symposium, 2021; September 23, 2021; La Jolla, CA, United States.

- **Website(s) or other Internet site(s)**
  Nothing to Report.

- **Technologies or techniques**
  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Nothing to Report.

- **Other Products**
  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Name: Karina Barbosa Guerra
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 0000-0002-6233-3332
Nearest person month worked: 12
Contribution to Project: Ms. Barbosa Guerra is responsible for the experimental design and performance of all experimental procedures in this project. She leads all communication endeavors related to the project, under the mentorship of Drs. Deshpande and Reya.
Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Award Chart submitted as an appendix to the Annual Report.

COLLABORATIVE AWARDS:
Nothing to Report.

QUAD CHARTS:
Nothing to Report.

9. APPENDICES:

Award Chart.

W81XWH-20-1-0703: Investigating Mechanisms of Leukemic Self-Renewal in Acute Myeloid Leukemia

PI: Karina Barbosa Guerra, Sanfor Burnham Prebys Medical Research Institution, California

Budget: $273,279.00

Topic Area: Cancer Research Program **Mechanism:** FY19, PRCRP, Horizon Award

Research Area(s): Blood cancers, Cancer in children, adolescents and young adults **Award Status:** 08/01/2020-07/31/2022

Study Goals:

The purpose of this project is to evaluate two top candidate HOX/MEIS regulators in CALM-AF10 driven-leukemogenesis: the KAT7 complex and Casein Kinase (CKs) enzymes. The proposed studies will provide insights into mechanisms of leukemogenesis and self-renewal in AML, which may also impact therapeutic development in additional subsets of myeloid and lymphoid malignancies.

Specific Aims:

Specific Aims: Aim 1: Investigate the function of the KAT7 complex in CALM-AF10 leukemia.
Aim 2: Investigate the regulation of *HOX/MEIS* genes by casein kinases (CKs) in AML cells.

Key Accomplishments and Outcomes:

a) Validation of the chromatin reader SGF29 as a proliferative and functional dependency in AF10-rearranged AMLs *in vitro* and *in vivo.*
b) Transcriptional downregulation of HOX/MEIS-cluster gene expression by a tudor reader domain mutant SGF29.
c) Chromatin enrichment and proteomics for CSNK2A1 knockout reveal regulatory roles for chromatin repressor complex recruitment to HOX/MEIS genes. Enrichment in SGF29 knockout cells reveals eviction of the SAGA transcriptional complex from chromatin.
d) KMT2A has a partial role as a CK2 substrate mediating its control over HOX/MEIS gene expression.

Publications: doi 10.1038/s41467-021-26788-6, 10.1016/j.jbc.2021.101477, 10.3389/fcell.2022.917125
Patents: none to date
Funding Obtained: