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14. ABSTRACT

Deregulation of epigenetic states promotes melanoma progression. MacroH2A, a histone variant associated with transcriptional repression, is downregulated in melanoma vs. benign nevi, where it suppresses proliferation and metastatic potential. However, its role as a barrier to tumorigenesis has not been investigated in vivo. We found that mice constitutively lacking macroH2A variants exhibit accelerated melanoma growth compared to their wild-type counterparts. MacroH2A-deficient tumors display impaired cytotoxic T cell function and increased monocyte infiltration, consistent with a compromised anti-tumor immune response, as well as upregulation of Ccl2, Cxcl1 and Il6, which are myeloid chemo-attractants. Through single-cell transcriptomic profiling of the entire melanoma microenvironment, we identified alterations in the immune cell compartment in macroH2A-deficient tumors. Altogether, our data supports a novel tumor suppressor role for macroH2A through repression of pro-inflammatory signaling within the melanoma microenvironment.

15. SUBJECT TERMS

Melanoma, tumor microenvironment, macroH2A, histone variant, cancer-associated fibroblasts, anti-tumor immunity

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Deregulation of epigenetic states has emerged as a critical driver of aberrant transcriptional programs promoting melanoma development and therapeutic resistance. We previously showed that macroH2A, a histone variant associated with transcriptional repression, is downregulated in melanoma vs. benign nevi and suppresses melanoma cell proliferation and metastatic potential. However, its role as a barrier to tumorigenesis had not been investigated *in vivo*. We assessed macroH2A function during melanoma development in a mouse model where macroH2A is either present (WT) or absent (dKO). We found that melanoma growth was significantly enhanced in dKO animals with concomitant abnormal immune cell function within these macroH2A-deficient tumors. We therefore hypothesized that the absence of macroH2A variants promotes melanoma aggressiveness through a defect in immune cell anti-tumor response. This could stem either from melanoma cells eliciting tolerance by the immune system, from functional deficiencies intrinsic to immune cells, and/or from activation of cancer-associated fibroblasts (CAFs) that can promote an immunosuppressive environment. We proposed to understand the link between macroH2A deficiency and immune tolerance *in vivo* by characterizing how macroH2A loss in melanoma cells promotes their escape from immune surveillance, and how macroH2A regulates the anti-tumor activity of immune cells. This proposal has the potential to highlight macroH2A as a prognostic marker for response to immune checkpoint blockade and/or as a regulator of tractable molecular targets for therapy, and overall, to help refine the clinical benefits of immunotherapy for melanoma patients.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Melanoma, tumor microenvironment, macroH2A, histone variant, cancer-associated fibroblasts, anti-tumor immunity.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. Decipher the immune microenvironment of macroH2A-deficient melanoma at single-cell resolution. Single cell RNA-sequencing (scRNA-seq) provides an unprecedented means to profile the cellular subsets of the melanoma microenvironment. With a focus on the immune cell fraction of the tumor, we will perform scRNA-seq on WT and dKO $BRAF^{V600E}$/PTEN-deficient melanomas. This study will reveal the identity of cells affected by macroH2A loss in the tumor immune microenvironment and provide transcriptional clues to their altered function in anti-tumor activity.

Major Task 1: scRNA-seq of tumor infiltrating immune cells

IACUC approvals; ACURO review and approval
Target project months: 0-3
Status: completed during Year 1

IRB approvals; HRPO review and approval
Target project months: 14-17
Status: completed ahead of schedule during Year 1

Subtask 1: Induce melanomas in macroH2A WT and dKO triallelic murine model (autochthonous, genetically induced model)
Target project months: 6-7
Status: completed during Year 1

Subtask 2: Generate single-cell suspension from three tumors per genotype, isolate populations of interest and process for droplet-based scRNA-seq library generation on Chromium 10X instrument. Sequence libraries on Illumina NextSeq 500 and perform preliminary data processing to generate single cell gene count matrix. Generate cell clusters and annotate them to known cell populations.
Target project months: 8-10
Status: completed during Year 1

Subtask 3: For each identified population, contrast their relative abundance and analyze transcriptional heterogeneity.
Target project months: 10
Status: completed during Year 1

Subtask 4: Run published tools to assess changes in activation state and cell differentiation trajectory upon macroH2A loss.
Target project months: 11
Status: completed during Year 1

Subtask 5: Bioinformatic analysis leveraging known ligand-receptor interactions to identify origin of immunosuppressive signals in dKO tumors.
Target project months: 12
Status: completed during Year 3

Aim 2. Deconvolute macroH2A function in the tumor and immune compartments of the anti-tumor response. Our mouse model lacks macroH2A in all cell types, and thus, impaired anti-tumor immunity could stem from intrinsic changes in tumor cells, or from functional deficiencies of the immune system in these mice. **Aim 2.1**. To clearly delineate the impact of the tumor genotype, we will use macroH2A-deleted melanoma cells (e.g. B16, YUMM, YUMMER) for tumor graft assays in WT immunocompetent hosts and for CD8+ T cell mediated killing assays *in vitro*. Further, we will utilize a novel conditional KO mouse model to ablate macroH2A specifically in melanoma cells. This will allow us to determine whether (and how) melanoma cells deficient for macroH2A induce dysfunction in the immune cell compartment. **Aim 2.2.** To test the functionality of macroH2A-deficient immune cells independently of the tumor genotype, we will assess dKO CD8+ T cell activation *in vitro.* Furthermore, we will generate melanoma grafts in mice with a T cell conditional

macroH2A deletion to test whether macroH2A-deficient T cells fail to control melanoma development. **Aim 2.3**. We will investigate the effects of macroH2A loss on therapeutic outcomes in melanoma by administering BRAF inhibitors (efficacy in BRAF/PTEN murine model is dependent on cytotoxic T cells) or checkpoint blockade therapy.

Major Task 2: Determine if macroH2A deficiency has a tumor-intrinsic effect

Subtask 1: Generate B16-F1, B16-OVA and YUMMER1.7 cell lines harboring macroH2A1 and/or -2 deletion by CRISPR/Cas9 methodology.
Target project months: 1
Status: completed during Year 1

Subtask 2: Characterize the proliferation, migration and invasion characteristics of these cell lines in vitro as a consequence of macroH2A loss.
Target project months: 2-3
Status: completed during Year 1

Subtask 3: Generate subcutaneous allografts of macroH2A-proficient and deficient B16-F1 and YUMMER1.7 cells into C57BL/6 WT hosts, measure growth and immunophenotypic impact of macroH2A loss.
Target project months: 4-7
Status: completed during Year 1

Subtask 4: Generate subcutaneous allografts of most promising cell line from Subtask 3 into C57BL/6 WT hosts and deplete differentially represented immune populations identified in Subtasks 3, then measure impact on tumor growth.
Target project months: 8-12
Status: N/A

Subtask 5: In vitro T cell killing assay using macroH2A CRISPR B16-OVA cells and OT-I CD8+ T cells.
Target project months: 13-14
Status: completed ahead of time during Year 1

Subtask 6: Generate melanoma-specific macroH2A1.2 cKO mouse.
Target project months: 3-12
Status: completed during Year 1

Subtask 7: Induce melanomas with macroH2A1.2 ablation restricted to tumor compartment, analyze growth and immunophenotype.
Target project months: 13-18
Status: completed during Year 3

Major Task 3: Identify genes responsible for macroH2A-mediated immune escape in melanoma cells

Subtask 1: Prepare RNA-seq libraries from macroH2A-deficient melanoma cell lines demonstrating immune escape and sequence on Illumina NextSeq 500
Target project months: 18-22
Status: completed during Year 2

Subtask 2: ChIP for macroH2A, H3K27ac, H3K4me1, H3K4me3, prepare libraries and sequence on Illumina NextSeq 500.
Target project months: 22-26
Status: completed during Year 3

Subtask 3: Integrated analysis of RNA-seq and ChIP-seq from cells +/- macroH2A and overlap with macroH2A-enriched domains.
Target project months: 26-32
Status: completed during Year 3

Major Task 4: Determine if macroH2A deficiency has an immune cell- intrinsic effect

Subtask 1: Stimulate purified WT and dKO CD8+ splenocytes with CD3 and CD28 antibodies in vitro, followed by analysis of activation markers, cytokine production and proliferation.
Target project months: 13
Status: completed ahead of schedule during Year 1

Subtask 2: Generate T-cell specific macroH2A1.2 cKO mouse.
Target project months: 3-9
Status: completed during Year 1

Subtask 3: Analyze growth and immunophenotype of B16 and YUMM1.7 allografts in mice from Subtask 2
Target project months: 9-12
Status: completed during Year 1

Subtask 4: Generate OT-I TCR-transgenic, T-cell specific macroH2A1.2 cKO mouse.
Target project months: 9-15
Status: N/A

Subtask 5: In vitro T cell killing assay using B16-OVA cells and OT-I macroH2A1.2-deleted CD8+ T cells.
Target project months: 16-17
Status: N/A

Subtask 6: Generate Thy1.1/1.2 Pmel-1 TCR-transgenic, T-cell specific macroH2A1.2 cKO mouse; Thy1.1 Pmel-1 TCR-transgenic, macroH2A WT mouse; BRAF/PTEN melanomas on C57BL/6 background (Thy1.2).
Target project months: 16-24
Status: N/A

Subtask 7: Inject congenically marked macroH2A1.2 KO (Thy1.1/1.2) and WT (Thy1.1) Pmel-1 CD8+ T cells into melanoma-bearing Thy1.2 mice. Compare cytotoxic activity and exhaustion of CD8+ T cells in vivo as function of intrinsic macroH2A levels.
One group of 6 B16-bearing Thy1.2 C57BL/6 mice will be injected with a 1:1 mix of macroH2A1.2 KO (Thy1.1/1.2) and WT (Thy1.1) Pmel-1 CD8+ T cells. Each mouse will provide a data point for both WT and macroH2A1.2 KO conditions.
Target project months: 24-26
Status: N/A

Major Task 5: Identify macroH2A-regulated genes responsible for proper T cell function

Subtask 1: Prepare RNA-seq libraries from macroH2A-deficient naïve and activated CD8+ T cells and sequence on Illumina NextSeq 500
Target project months: 18-22
Status: completed during Year 2

Subtask 2: ChIP for macroH2A, H3K27ac, H3K4me1, H3K4me3, prepare libraries and sequence on Illumina NextSeq 500.
Target project months: 22-26
Status: N/A

Subtask 3: Integrated analysis of RNA-seq and ChIP-seq from cells +/- macroH2A and overlap with macroH2A-enriched domains.
Target project months: 26-32
Status: N/A

Major Task 6: Identify therapeutic implications of macroH2A loss in melanoma

Subtask 1: Immunohistochemistry on archival de-identified human melanoma samples; scoring macroH2A levels and tumor infiltrating immune cells.
Target project months: 18-21
Status: completed during Year 3

Subtask 2: Generate WT and dKO BRAF/PTEN melanomas.
Target project months: 25-28
Status: completed ahead of time

Subtask 3: Treat melanoma-bearing mice with BRAF inhibitors and measure response via tumor growth.
To mitigate risk of attrition, 8 instead of 6 mice will be used per genotype. One group of WT and one group of dKO Braf/Pten/Tyr-Cre mice, both will be treated. Growth kinetics will be compared to existing data from non-treated WT and dKO mice.
Target project months: 27-28
Status: completed ahead of time

Subtask 4: Treat melanoma-bearing mice with checkpoint blockade and measure response via tumor growth and recovery of cytotoxic T cell function.
To mitigate risk of attrition, 8 instead of 6 mice will be used per genotype. One group of WT and one group of dKO Braf/Pten/Tyr-Cre mice, both treated. Growth kinetics will be compared to existing data from non-treated WT and dKO mice.
Target project months: 29-30
Status: N/A

Major Task 7: Publication of data from Aims 1&2

Subtask 1: Prepare a manuscript describing our findings, the first to investigate the contribution of histone variants to melanoma progression and immune response in vivo, for a high-impact journal such as Cancer Cell, Nature Medicine, Nature Cancer, or Cancer Discovery.
Target project months: 33-36
Status: completed during Year 3

Filipescu D, Carcamo S, Agarwal A, Tung N, Humblin É, Goldberg MS, Vyas NS, Beaumont KG, Demircioglu D, Sridhar S, Ghiraldini FG, Capparelli C, Aplin AE, Salmon H, Sebra R, Kamphorst AO, Merad M, Hasson D, Bernstein E. MacroH2A restricts inflammatory gene expression in melanoma cancer-associated fibroblasts by coordinating chromatin looping. *Nat Cell Biol*. 2023 Sep;25(9):1332-1345. doi: 10.1038/s41556-023-01208-7. Epub 2023 Aug 21. PMID: 37605008; PMCID: PMC10495263.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1: scRNA-seq of tumor infiltrating immune cells

Subtask 1: Induce melanomas in macroH2A WT and dKO triallelic murine model (autochthonous, genetically induced model)
+
Subtask 2: Generate single-cell suspension from three tumors per genotype, isolate populations of interest and process for droplet-based scRNA-seq library generation on Chromium 10X instrument. Sequence libraries on Illumina NextSeq 500 and perform preliminary data processing to generate single cell gene count matrix. Generate cell clusters and annotate them to known cell populations.
+
Subtask 3: For each identified population, contrast their relative abundance and analyze transcriptional heterogeneity.
+

Subtask 4: Run published tools to assess changes in activation state and cell differentiation trajectory upon macroH2A loss.

The murine melanoma model we created lacks macroH2A in all cell types, and therefore the observed defect in anti-tumor immunity in the dKO could stem from multiple cell types in the TME, e.g., melanoma cells eliciting immunosuppression, dysfunctional immune cells, and/or from the stroma. Hence, we carried out scRNA-seq of $BRAF^{V600E}$/PTEN-deficient melanomas from WT and dKO mice across three independent experiments, generating an integrated dataset of ~24,000 high-quality cells. We identified 33 cell clusters, including melanocytes, immune cells and CAFs, as well as rarer cell populations (Filipescu *et al.*, Fig. 3a). Annotation of clusters was based on specific expression of known lineage markers, similarity, or the most significant cluster-specific genes.

Interestingly, we identified a combination of melanocytes and other neural crest (NC)-derived cells. *Bona fide* melanocytic cells expressing markers associated with pigment production, such as *Mitf*, *Mlana* and *Dct* represented only ~5% of our dataset, while ~25% of cells expressed the NC marker *Sox10* and lacked melanocyte markers (Filipescu *et al.*, Fig. 3d, Extended Data Fig. 3a, b). To dissect the relationship between NC and melanocytes, we performed cell trajectory analysis. One branch incurred a growth suppressive program in the NC arrested cluster, while a second branch dedifferentiated towards a state resembling the migratory and stem-like stages of NC development (NC Aqp1 and Zeb2/Ki-67 clusters), associated with poor prognosis in human melanoma. Relative to total, dKO tumors were enriched in NC Zeb2 to the detriment of NC arrested and Hapln1 cells (Filipescu *et al.*, Fig. 3d-f), suggesting that absence of macroH2A promotes de-differentiation and a more aggressive tumor behavior.

We also identified multiple clusters of mononuclear phagocytes (Filipescu *et al.*, Fig. 3a, Extended Data Fig. 3i-k). In particular, the Mac Mrc1 cluster, which accumulated in the dKO (Filipescu *et al.*, Fig. 3d-f), resembles macrophages enriched in actively growing tumors that become depleted upon immune checkpoint blockade, and by a tumor-enriched state whose human counterpart was associated with poor prognosis in lung adenocarcinoma patients. Based on these similarities, the enrichment of Mac Mrc1 cells in the dKO reinforces the accumulation of immunosuppressive, pro-tumor myeloid cells we previously identified by flow cytometry (Filipescu *et al.*, Fig. 2c). Analysis of lymphoid clusters recapitulated the decrease in cytotoxic T cells (Tc, the sole cluster expressing *Cd8a*; Filipescu *et al.*, Fig. 3d-f, Extended Data Fig. 3l-o), also observed by flow cytometry in dKO tumors (Filipescu *et al.*, Fig. 2c). Importantly, characterization of immune cells by scRNA-seq corroborated both our bulk RNA-seq and immunophenotyping studies, supporting increased immunosuppressive myeloid and decreased cytotoxic T cell infiltration in dKO melanomas, while enabling us to investigate minority cellular composition and pseudotime features otherwise challenging with bulk data.

The scRNA-seq analyses also allowed us to assess the contribution of mesenchymal cells in the tumor stroma to melanoma development. We identified a prototypical CAF cluster expressing the highest levels of *Pdgfra* and *Fap* and CAF-specific lincRNA *Meg3*. A second fibroblast cluster expressed *Wif1*, an emerging marker in papillary dermis. Two clusters with high levels of the myofibroblast marker *Acta2* expressed *Lrrc15* and *Fbln1,* respectively (Filipescu *et al.*, Fig. 3a, Extended Data Fig. 4a), both genes associated with immunoregulatory myofibroblast populations in pancreatic and breast cancer. The CAF Meg3 cluster exhibited a ~3-fold increase in the dKO (Filipescu *et al.*, Fig. 3d-f), raising the possibility that the non-immune stroma contributes to the gene expression changes we identified in the bulk data set. Computational assessment of the contribution of each cluster to the molecular changes within the tumor highlighted this CAF cluster as the top driver of the dKO

transcriptional profile (Filipescu *et al.*, Fig. 4a). Importantly, the cytokines identified as upregulated in bulk tumor RNA-seq (*Ccl2*, *Cxcl1*, *Ccl11*, *Il6*), were also significantly upregulated in the dKO CAF clusters (Filipescu *et al.*, Fig. 4b). The scRNA-seq dataset also indicated CAF-specific genes we could functionalize for cell-sorting experiments, among which we selected the PDGF receptor α (CD140a) for subsequent experiments (Filipescu *et al.*, Extended Data Fig. 4i).

Subtask 5: Bioinformatic analysis leveraging known ligand-receptor interactions to identify origin of immunosuppressive signals in dKO tumors.

This analysis was performed once data on a third WT and dKO tumor in the scRNA-seq study became available. We found CAFs had the most prolific outgoing interactions with other cell types both in the WT and dKO (Filipescu *et al.*, Extended Data Fig. 5a). Differential analysis showed a generalized decrease in the number of interactions in the dKO, but an increase in the strength of communication from CAFs to NC (Filipescu *et al.*, Extended Data Fig. 5b). Importantly, dKO CAFs increased signaling interactions to the mononuclear phagocyte lineage through the CCL2 and IL6 pathways, and to neutrophils and basophils through CXCL1 (Filipescu *et al.*, Fig. 5a). Altogether the scRNA-seq analyses strongly suggested CAFs were the origin of the immunophenotype of macroH2A deficient melanomas, therefore our emphasis shifted on this cell type in subsequent aims, as described below.

Major Task 2: Determine if macroH2A deficiency has a tumor-intrinsic effect

Subtask 1: Generate B16-F1, B16-OVA and YUMMER1.7 cell lines harboring macroH2A1 and/or -2 deletion by CRISPR/Cas9 methodology.

We established macroH2A-deleted B16-F1, B16-GP and YUMMER1.7 melanoma cells, which we used for tumor grafts in mouse hosts with a fully functional immune system, and/or for cytotoxic T cell mediated killing assays in vitro.

Subtask 2: Characterize the proliferation, migration and invasion characteristics of these cell lines in vitro as a consequence of macroH2A loss.
+
Subtask 3: Generate subcutaneous allografts of macroH2A-proficient and deficient B16-F1 and YUMMER1.7 cells into C57BL/6 WT hosts, measure growth and immunophenotypic impact of macroH2A loss.

B16-F1 cells deleted for the macroH2A1 gene (the major form in this cell type) using CRISPR/Cas9 technology did not grow faster than their control counterparts to a statistically significant extent either *in vitro* (Fig. 1a) or when implanted into mice as subcutaneous allografts (Fig. 1b, c). Furthermore, analysis of immune cells in these engrafted tumors did not identify immunophenotypic changes like those we observed in the dKO melanoma model. Each experimental group displayed high variability, and tumor infiltrating immune cell populations mainly correlated with tumor weight (more immunosuppressive cell types such as monocytes and Treg in large tumors, more cytotoxic cell types such as IFNγ-producing CD8+ T cells and NK cells in smaller tumors) (Fig. 1d).

The immunogenic YUMMER1.7 cell line harbors UV-induced mutations that allow the host immune system to reject it in C57BL/6 mice. However, we found that macroH2A1 deletion does not confer the capacity proliferate faster *in vitro* (Fig. 1e) and *in vivo* (Fig. 1f), or to escape tumor rejection

compared to controls (Fig. 1g). Given the 85% rejection rate we observed across CRISPR construct transduced conditions, we did not have sufficient material to perform immunophenotyping.

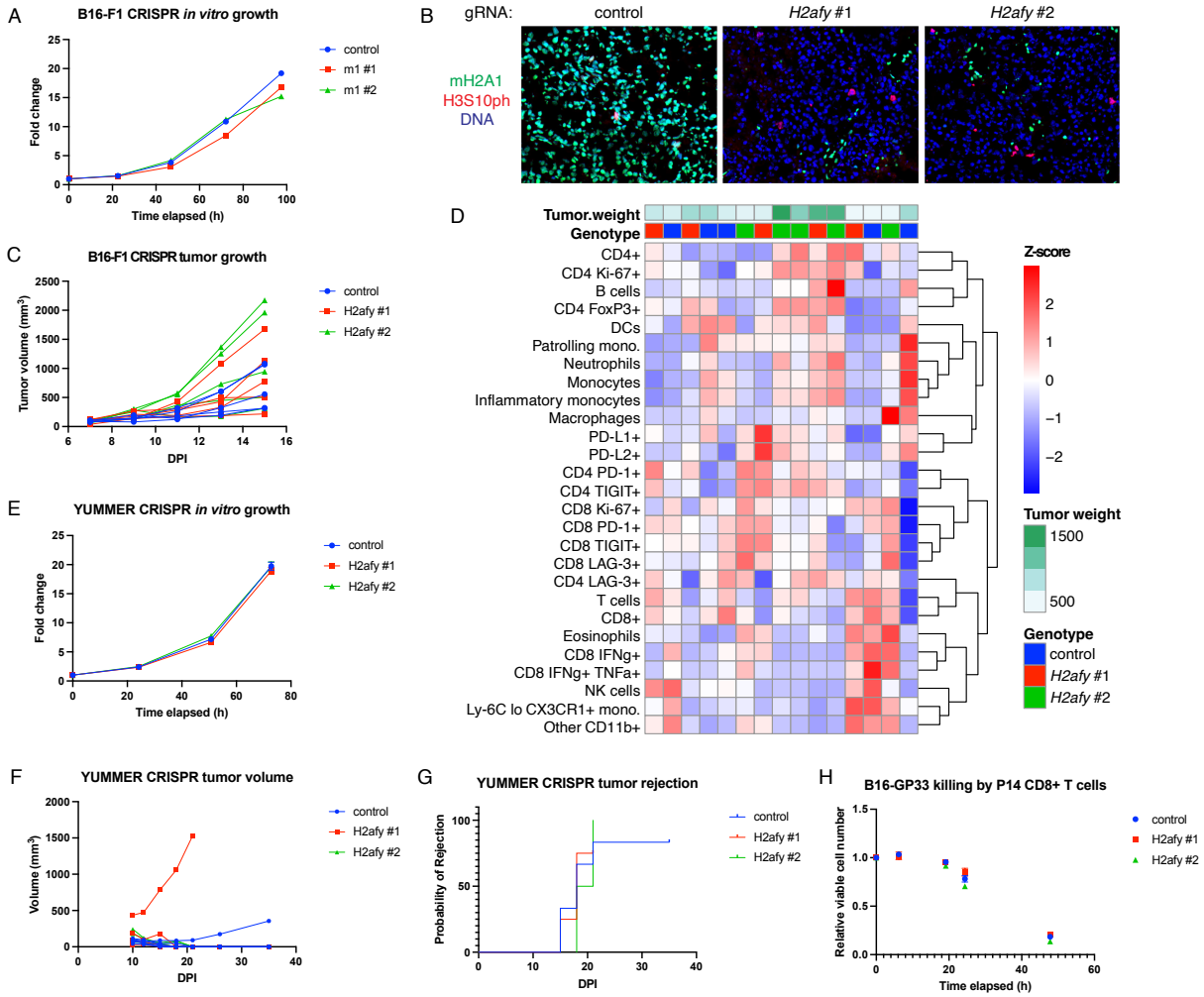

Figure 1. Analysis of tumor-intrinsic effects of macroH2A1 loss using melanoma cell lines. (A) Proliferation analysis of B16-F1 cells transduced with control and macroH2A1-targeting CRISPR/Cas9 constructs. Shown are confluency values relative to initial time point, averaged over triplicate samples. (B) Immunofluorescence of B16-F1 cells from A in allograft tumors demonstrating efficient loss of macroH2A1 protein *in vivo.* (C) Allograft tumor growth using cells from A. No statistically significant differences at any time point were found between groups. (D) Phenotyping by flow cytometry of tumor infiltrating immune cells. Data shown as Z scores for relative population frequencies. Samples and populations are clustered by similarity. (E) Proliferation analysis of YUMMER1.7 cells transduced with control and macroH2A1-targeting CRISPR/Cas9 constructs. (F) Allograft tumor growth using cells from E. No statistically significant differences at any time point were found between groups. (G) Kaplan-Meier curves of tumor rejection (events represent tumor decrease to non-detectable size). (H) T cell killing assay. Target B16-GP33 tumor cells treated with IFNg and P14 cytotoxic T cells activated with Dynabeads were seeded at a 1:3 ratio. Tumor cell numbers were determined via GFP imaging and normalized to initial number of seeded cells to account for plating differences, and to wells devoid of T cells to account for proliferation during the duration of the assay.

Subtask 4: Generate subcutaneous allografts of most promising cell line from Subtask 3 into C57BL/6 WT hosts and deplete differentially represented immune populations identified in Subtasks 3, then measure impact on tumor growth.

We did not pursue this task, as it was dependent on a positive result in Subtask 3. Subtask 3 did not highlight a difference in growth upon individual macroH2A isoform ablation via CRISPR, nor significant immunophenotypic changes (i.e. infiltrating immune cell frequency or tumor rejection) in these allograft models. Therefore, we lacked a functional readout for this subtask and a target cell type to deplete.

Subtask 5: In vitro T cell killing assay using macroH2A CRISPR B16-OVA cells and OT-I CD8+ T cells.

Given our collaboration with Dr. Alice Kamphorst's lab (ISMMS), who leverages lymphocytic choriomeningitis virus (LCMV) infection in mice as a model for T cell exhaustion phenotypes encountered in tumors, we used a model antigen system with similar properties to the proposed B16-OVA / OT-I T cell pair. B16-GP33 cells are derived from B16-F10 and express one of the most widely used antigen in immunology, derived from the LCMV glycoprotein. When induced to express MHC I by IFNγ treatment, they are efficiently killed by activated CD8+ T cells isolated from P14 mice that express a transgenic T cell receptor specific for the GP33 antigen [1]. However, upon macroH2A1 deletion, we observed these cells are destroyed to the same extent as their control counterparts (Fig. 1h).

Subtask 6: Generate melanoma-specific macroH2A1.2 cKO mouse.

We were successful in generating the proposed 4-allele genetically engineered mouse model, despite a major challenge we encountered in the process (see problems or delays section).

Subtask 7: Induce melanomas with macroH2A1.2 ablation restricted to tumor compartment, analyze growth and immunophenotype.

This task was significantly delayed by difficulties with obtaining sufficient numbers of the melanoma-specific macroH2A1.2 cKO mouse strain (see problems or delays section). Furthermore, numerous mice induced for tumor development developed spontaneous tumors beyond the site treated with 4-HT, independently of macroH2A1.2 genotype, which we attribute to leaky transgene expression. These mice had to be removed from our analysis. Using the number of mice we were able to obtain and follow up to experimental input, we did not detect any impact of macroH2A1.2 deletion in melanocytes on tumor growth (Fig. 2a). However, high variability (Fig. 2b) and cohort attrition due to spontaneous tumor development resulted in a difficult model to work with. Due to the lack of a tumor growth phenotype and small tumor size (almost half below 50 mm^2 at 50 DPI), we did not perform any immunophenotypic analysis in this model.

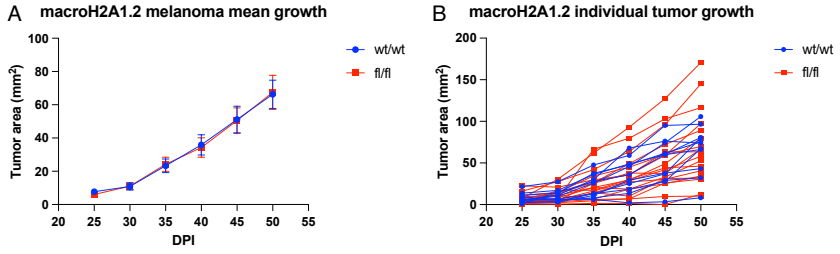

Figure 2. MacroH2A1.2 isoform deletion in the melanoma compartment does not affect tumor growth. (A) Mean of autochthonous tumor growth in mice with indicated genotypes for macroH2A1.2 gene. No statistically significant differences at any time point were found between groups ($n_{wt/wt}$ = 11, $n_{fl/fl}$ = 18). Error bars represent SEM. (B) individual tumors in (A) shown.

Major Task 3: Identify genes responsible for macroH2A-mediated immune escape in melanoma cells

Through multiple approaches defined in previous tasks, we were unable to identify melanoma-intrinsic properties that indicate immune evasion. However, scRNA-seq of the entire TME and transcriptomic analyses of CAFs isolated from WT and dKO melanomas demonstrated that CAFs are the major source of pro-inflammatory signals in dKO melanomas. This oriented our subsequent analyses away from melanoma cells and towards CAFs, where performing the subtasks detailed in the SOW for Major Task 3 appeared most biologically significant.

Subtask 1: Prepare RNA-seq libraries from macroH2A-deficient melanoma cell lines demonstrating immune escape and sequence on Illumina NextSeq 500

We successfully performed RNA-seq on CAFs flow-sorted from 4 WT and 4 dKO melanomas based on CD140a (*Pdgfra*) expression, which we found specific for this cell type (Filipescu *et al.*, Extended Data Fig. 4i). We confirmed the significant upregulation of several proinflammatory cytokines and chemokines such as *Ccl2*, *Cxcl1* and *Il6* cytokines, and the activation of the TNFα-NF-κB inflammatory signaling pathway (Filipescu *et al.*, Fig. 4d, e). Differentially expressed genes assessed in the sorted CAFs overlapped in a statistically significant manner with those identified by scRNA-seq in the combined mesenchymal populations (Filipescu *et al.*, Extended Data Fig. 4j).

Subtask 2: ChIP for macroH2A, H3K27ac, H3K4me1, H3K4me3, prepare libraries and sequence on Illumina NextSeq 500.

We successfully carried out genome-wide macroH2A1 profiling using Cut&Run [2] in primary WT CAF cultures. We profiled H3K27me3 occupancy by Cut&Run in WT and dKO cells but did not detect major changes upon macroH2A loss. Enhancers are emerging regulators of inflammatory gene transcription [3], notably in the context of the NF-κB signaling, a pathway that mediates activation of *CCL2*, *CXCL1* and *IL6*-associated (super)enhancers [4–7] and is upregulated in the absence of macroH2A (Filipescu *et al.*, Fig. 2a). Moreover, macroH2A suppresses a subset of enhancers [8], and FOSL2, whose enrichment at enhancers marks their activation in the context of tumorigenesis [9,10], is upregulated in dKO CAFs. Therefore, we investigated the epigenetic regulatory landscape of inflammatory genes by performing ChIP-seq for H3K27ac, a mark of active enhancers and promoters. We identified active enhancers in serum stimulated, cultured CAFs, and found dKO CAFs had increased H3K27ac levels at 4116 enhancers and decreased H3K27ac at 2652 enhancers (Filipescu *et al.*, Extended Data Fig. 7c).

Subtask 3: Integrated analysis of RNA-seq and ChIP-seq from cells +/- macroH2A and overlap with macroH2A-enriched domains.

As previously shown [11], and consistent with its association with gene repression, genome-wide macroH2A was excluded from the bodies of highly expressed genes and retained at lowly expressed ones, in anti-correlation with H3K27ac levels at promoters. We found macroH2A enriched at significant differentially expressed genes (DEGs), including upregulated inflammatory genes, compared to a control set of static genes with matched expression (Filipescu *et al.*, Fig. 6a-c). Intergenic enrichment of macroH2A suggested it may also regulate traditional enhancers (TEs) and superenhancers (SEs). Like DEGs, differentially activated enhancers (DAEs) were significantly enriched in macroH2A in WT cells (Filipescu *et al.*, Fig. 6d-e).

Emerging evidence suggests that macroH2A impacts 3D chromatin organization at several scales [12–14]. To assess this, and to annotate functional promoter-enhancer pairs, we performed Micro-C [15]

coupled with promoter capture (pcMicro-C) [16] in CAFs. By profiling macroH2A-dependent chromatin looping, we found that gain of H3K27ac in the dKO was more frequently associated with unique dKO loops, specifically within genomic regions highly enriched for macroH2A (Filipescu *et al.*, Fig. 7b). Changes in the total number of loops originating in each gene were similarly associated with DEGs (Filipescu *et al.*, Fig. 7c), altogether suggesting that macroH2A instructs functional looping properties of chromatin fibers it is highly enriched in. Together with changes in enhancer activity, this suggests that macroH2A may enforce the position of enhancers relative to nuclear compartments, or the ability of enhancers to interact with their cognate promoters. In this model, macroH2A-dependent regulatory mechanisms converged on a small set of inflammatory genes (Filipescu *et al.*, Fig. 7d), including *Ccl2* (Filipescu *et al.*, Fig. 7e), *Il6* (Filipescu *et al.*, Fig. 7f). and *Ptgs2* (Filipescu *et al.*, Fig. 7g), underscoring a role for macroH2A in limiting inflammatory signaling *in vivo*. Notably, CAFs hijack these inflammatory genes as a mechanism of tumor immune escape [17–20], and these findings suggest that dKO CAFs promote an immunosuppressive environment that leads to increased melanoma burden.

Major Task 4: Determine if macroH2A deficiency has an immune cell- intrinsic effect

Subtask 1: Stimulate purified WT and dKO CD8+ splenocytes with CD3 and CD28 antibodies in vitro, followed by analysis of activation markers, cytokine production and proliferation.

We observed that macroH2A deficiency rendered cytotoxic T cells more amenable to activation *in vitro* as shown by increased proliferation, CD44 and PD-1 positivity (Fig. 3a). This difference, however, does not explain the reduction of T cells in dKO tumors, and suggests the latter is a consequence of other changes in the microenvironment.

Subtask 2: Generate T-cell specific macroH2A1.2 cKO mouse.
+
Subtask 3: Analyze growth and immunophenotype of B16 and YUMM1.7 allografts in mice from Subtask 2

We generated macroH2A-proficient melanoma grafts in mice where only T cells are deficient for macroH2A1.2, the predominant isoform in this cell type (Fig. 3b), to determine whether macroH2A loss in T cells renders them unable to control melanoma development. Engrafted with the highly immunogenic YUMMER1.7 melanoma cell line, these mice did not display statistically significant differences in tumor growth (Fig. 3c) or rate of rejection (Fig. 3d), suggesting that macroH2A-deficient T cells do not suffer from an intrinsic inability to control tumor growth. Furthermore, phenotyping of lymphoid cells in immune-escaping tumors and remnants of incompletely cleared tumors at endpoint ($n_{WT} = 4$, $n_{dKO} = 6$) did not reveal statistically significant differences in T cell functionality (Fig. 3e).

Subtask 4: Generate OT-I TCR-transgenic, T-cell specific macroH2A1.2 cKO mouse.
+
Subtask 5: In vitro T cell killing assay using B16-OVA cells and OT-I macroH2A1.2-deleted CD8+ T cells.
+

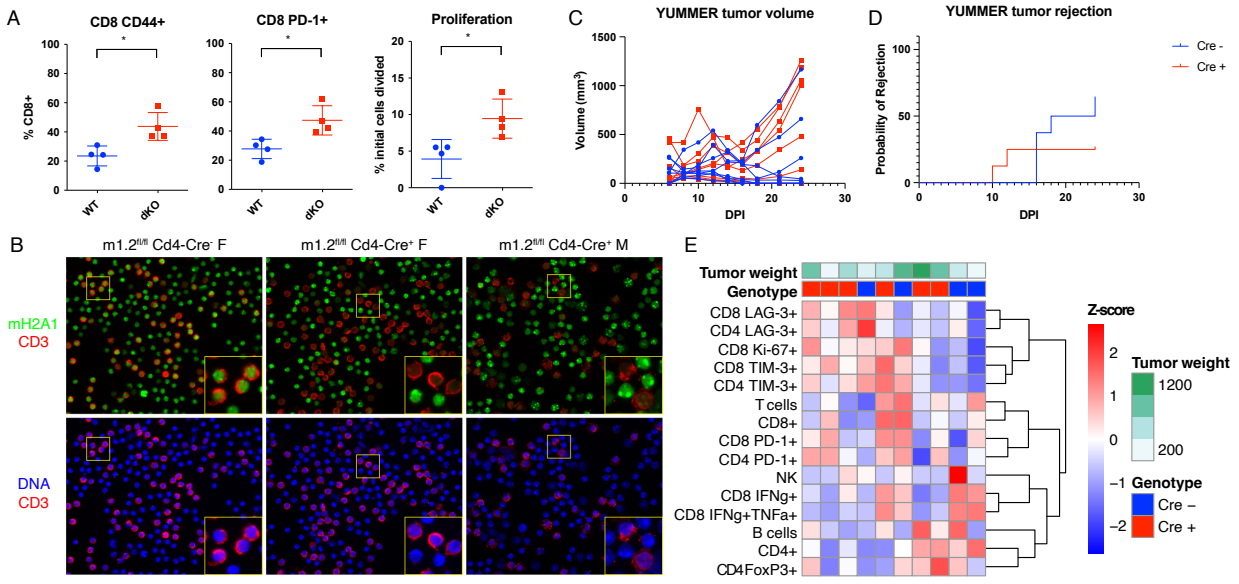

Figure 3. Analysis of T cell-intrinsic effects of macroH2A1 loss. (A) Flow cytometric analysis of in vitro activated CD8+ splenocytes from tumor-naïve WT and dKO mice. Cells were activated with a cocktail of CD3 and CD28 antibodies for 72 hours. Proliferation was determined using the CFSE dilution method. (B) Immunofluorescence in splenocytes from Cre- (macroH2A1.2 not deleted) and Cre+ (macroH2A1.2 deleted) mice reveals specific loss of macroH2A1 signal in the T cell compartment labeled by CD3. (C) Allograft tumor growth of YUMMER1.7 cells in mice lacking macroH2A1 in the T cell compartment. No statistically significant differences at any time point were found between groups. (D) Kaplan-Meier curves of tumor rejection for experiment in C. (E) Phenotyping by flow cytometry of lymphocyte populations infiltrating tumors from C. Data shown as Z scores for relative population frequencies. Samples and populations are clustered by similarity.

Subtask 6: Generate Thy1.1/1.2 Pmel-1 TCR-transgenic, T-cell specific macroH2A1.2 cKO mouse; Thy1.1 Pmel-1 TCR-transgenic, macroH2A WT mouse; BRAF/PTEN melanomas on C57BL/6 background (Thy1.2).

+

Subtask 7: Inject congenically marked macroH2A1.2 KO (Thy1.1/1.2) and WT (Thy1.1) Pmel-1 CD8+ T cells into melanoma-bearing Thy1.2 mice. Compare cytotoxic activity and exhaustion of CD8+ T cells in vivo as function of intrinsic macroH2A levels.

We did not pursue these tasks due to the negative results obtained in the previous subtasks of Major Task 4. Those results did not demonstrate a role for macroH2A1.2 in the immunophenotype or cell killing ability of CD8+ T cells. This could be either due to other macroH2A isoforms compensating for its absence, or due to the predominant effect of other cell types, namely CAFs, in the immune escape of macroH2A-deficient tumors. In addition, experiments in these proposed subtasks are unable to model the role of CAFs. Altogether, under these considerations, we expected a negative result using this complex *in vivo* system.

Major Task 5: Identify macroH2A-regulated genes responsible for proper T cell function

Subtask 1: Prepare RNA-seq libraries from macroH2A-deficient naïve and activated CD8+ T cells and sequence on Illumina NextSeq 500

We observed that macroH2A deficiency caused a limited number of changes in gene expression upon activation of naïve CD8 T cells. Among the 52 genes upregulated in the dKO, several were associated with T cell activation, in line with previous immunophenotypic findings in Major Task 4, Subtask 1.

The direction of this difference, however, does not explain the reduction of T cells in dKO tumors, and suggests the latter is a consequence of other changes in the tumor microenvironment. Therefore, since the hypothesis they were based on was no longer accurate, and since we showed the cell type of origin of the phenotype was represented by CAFs, the rest of the subtasks of Major Task 5 were performed on CAFs instead, as detailed under Major Task 3.

Major Task 6: Identify therapeutic implications of macroH2A loss in melanoma

Subtask 1: Immunohistochemistry on archival de-identified human melanoma samples; scoring macroH2A levels and tumor infiltrating immune cells.

We performed this task on an initial batch of 10 human primary melanomas, where a pathologist manually scored macroH2A1 and macroH2A2 levels, lymphocyte infiltration and frequency of CD8 T cells. This cohort had overall very little variation in macroH2A levels, which did not allow us to identify a correlation with CD8 infiltration. This was likely due to the small number of samples analyzed or the age of the tissue blocks, which may have had poor tissue quality. Furthermore, our data supported a revised hypothesis that macroH2A levels in CAFs specifically (as opposed to the entire tumor) were driving the immunophenotype, therefore scoring macroH2A levels in bulk would likely not be informative. To overcome this issue, we examined human melanoma-derived primary CAF cultures (Filipescu *et al.*, Extended Data Fig. 5g). These revealed homogenous levels of macroH2A1 protein, while macroH2A2 spanned almost an order of magnitude (Filipescu *et al.*, Fig. 5f). MacroH2A2^{low} CAFs secreted more CCL2, CXCL1 and IL-6 when stimulated (Filipescu *et al.*, Fig. 5g, Extended Data Fig. 5h), but this did not reach significance due to low sample size. Therefore, we analyzed a large pan-cancer scRNA-seq dataset [21] comprising over 56,000 CAFs across 98 samples. On a per-sample basis, while *CCL2*, *CXCL1* and *IL6* were positively correlated to each other, the negative correlation between *IL6* and *MACROH2A2* was significant (Filipescu *et al.*, Extended Data Fig. 5i, j). This suggests the relationship between macroH2A2 and inflammatory signaling is conserved in human CAFs and is not exclusive to melanoma. Taken altogether, these data suggest that CAFs with decreased levels of macroH2A are a major source of inflammatory cytokines in the melanoma TME and promote an immunosuppressive environment.

Subtask 2: Generate WT and dKO BRAF/PTEN melanomas.
+
Subtask 3: Treat melanoma-bearing mice with BRAF inhibitors and measure response via tumor growth.

We treated 6 mice per genotype with BRAF inhibitor from day 25 post-induction until tumors escaped therapy to reach sizes comparable to untreated animals (Fig. 4a). Tumor growth was significantly inhibited by BRAFi for a much longer period than anticipated, requiring at least 100 days of continuous drug administration. However, given the high variation in response to drug treatment (CV of 27% in WT, 37% in dKO), the increased average tumor size in the dKO did not reach significance. Power calculation ($\alpha = 0.05$, $1\text{-}\beta = 0.8$) estimates a sample size of ~50 per group would be required to reach significance at day 50. This number of mice under treatment is unachievable given the drug cost and experimental effort involved. Furthermore, dKO tumors under BRAFi were on average 31% larger than WT counterparts during the course of the treatment, an increase on par with the 30% higher size of dKO vs. WT observed in the absence of treatment. This suggested the effect of BRAFi is independent of macroH2A levels. Calculation of tumor growth inhibition (%TGI) as (1 - (mean

volume of treated tumors)/(mean volume of control tumors)) x 100% [22] also showed that BRAFi did not have inferior efficacy in the dKO (Fig. 4b). Altogether, this data suggests loss of macroH2A promotes tumor growth through distinct pathways from those targeted by BRAFi.

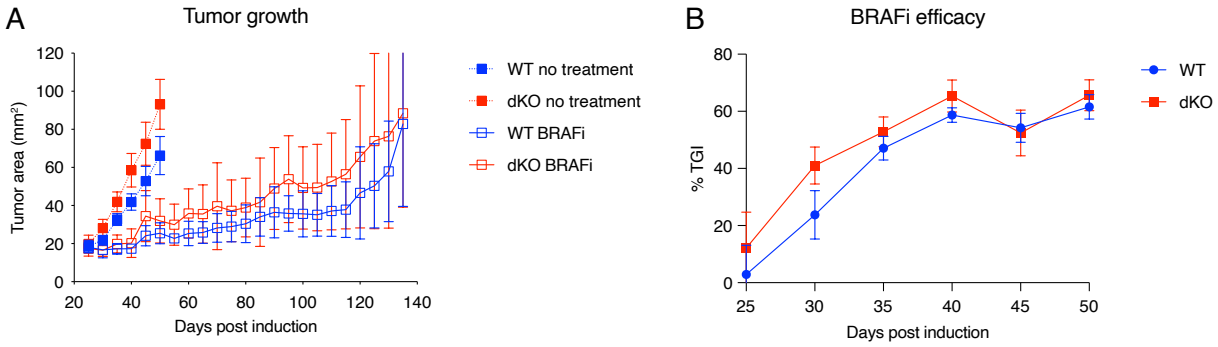

Figure 4. BRAF inhibition delays murine melanoma progression independently of macroH2A. a) Growth kinetics of WT and dKO mouse melanomas under BRAFi, retrospectively compared to untreated mice. Vemurafenib (also known as Zelboraf or PLX4032) was admixed in chow at 1.42 g/kg administered ad libitum from 25 days post tumor induction onwards**.** $n_{WT\ no\ treatment} = 22$, $n_{dKO\ no\ treatment} = 22$, $n_{WT\ BRAFi} = 6$, $n_{dKO\ BRAFi} = 6$. Mean and 95% confidence interval error bars are shown. b) Calculation of BRAFi efficacy as percent tumor growth inhibition (% TGI) at time points shared between no treatment and BRAFi mice. Mean and SEM shown.

Subtask 4: Treat melanoma-bearing mice with checkpoint blockade and measure response via tumor growth and recovery of cytotoxic T cell function.

While we considered performing immune checkpoint blockade, anti PD-1 or PD-L1 monotherapy has been attempted in this melanoma model and shows minimal effects (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4097121/ see Fig. 5). Furthermore, these $BRAF^{V600E}$/$Pten^{null}$ tumors respond only partially to anti-CTLA-4 + anti-PD-L1 combination therapy (https://www.nature.com/articles/nature14404 in Figure 4), which is not surprising given the lack of neoantigens in these tumors. Given these drawbacks, and the considerable expense of the drug regimen for a cohort of 20 mice over a period of 25 days, we did not pursue this approach and instead focused on the mechanistic characterization of the role macroH2A plays in inflammatory gene regulation in CAFs. This ultimately allowed us to identify a novel mode of gene expression regulation involving 3D chromatin structure (Filipescu *et al.*, Fig. 7 and Extended Data Fig. 9), which was a key novel finding of our resulting publication (see below).

Major Task 7: Publication of data from Aims 1&2

Subtask 1: Prepare a manuscript describing our findings, the first to investigate the contribution of histone variants to melanoma progression and immune response in vivo, for a high-impact journal such as Cancer Cell, Nature Medicine, Nature Cancer, or Cancer Discovery.

We published a study entitled "*MacroH2A restricts inflammatory gene expression in melanoma cancer-associated fibroblasts by coordinating chromatin looping*" in *Nature Cell Biology* in August 2023, and is openly accessible through the publisher's website:

https://www.nature.com/articles/s41556-023-01208-7

The impact of this study was highlighted both by a News & Views piece in the same edition of *Nature Cell Biology*, which labeled our findings *"a milestone"* for showing how histone variants influencing cytokine genes have a major impact on immunosuppression (https://www.nature.com/articles/s41556-023-01183-z), and as a Research Watch piece in *Cancer Discovery* (https://aacrjournals.org/cancerdiscovery/article/13/11/2302/729840/MacroH2A-Regulates-Inflammation-and-Chromatin).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Participation in professional development activities for key personnel Dan Filipescu, PhD:
2023 Society for Melanoma Research Congress, Philadelphia, Pennsylvania (invited oral presentation)
2021 EMBO Workshop on Physiology and Function of Histone Variants, virtual (invited oral presentation)

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

N/A, Final report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We have identified an important role for histone variants in regulating inflammation in the host cells of the melanoma tumor microenvironment, a novel finding to our knowledge.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Founders for the macroH2A1.2$^{wt/wt}$ and macroH2A1.2$^{fl/fl}$ mouse sublines were generated but have a limited lifespan due to leaky Cre transgene activation and development of spontaneous melanomas when reaching breeding age in the majority of mice. Of note, JAX laboratories, which provide the melanoma strain used to derive these mice, now highlights this issue in the strain description: https://www.jax.org/strain/013590. This resulted in significant delays in expanding the strain and obtaining cohorts of mice in sufficient numbers for melanoma induction by 4-HT application in order to compare 12 macroH2A1.2$^{wt/wt}$ and 12 macroH2A1.2$^{fl/fl}$ mice as proposed. However, these studies are now laregly completed: no statistically significant differences were found between groups ($n_{wt/wt} = 11$, $n_{fl/fl} = 18$).

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  The manuscript supported by this award was published in *Nature Cell Biology* in August 2023, and is openly accessible through the publisher's website: https://www.nature.com/articles/s41556-023-01208-7
  Acknowledgement of support: YES

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  Seminar presentation by key personnel Dan Filipescu, PhD:
  2023 Society for Melanoma Research Congress, Philadelphia, Pennsylvania (invited oral presentation)
  2023 Institut de Génétique Humaine, Montpellier, France (oral presentation)

2023 ISSMS SBDRC Transition-to-independence grant awardee seminar (oral presentation)
2023 Institut Curie, Paris, France (oral presentation)
2022 ISSMS SBDRC Retreat (oral presentation)
2021 EMBO Workshop on Physiology and Function of Histone Variants, virtual (invited oral presentation)

Seminar presentation by key personnel Emily Bernstein, PhD:
2023 Department of Cell Biology, Albert Einstein College of Medicine, New York, NY.
2023 Molecular Biology Program Research Seminar Series, MSKCC, New York, NY.
2023 SBDRC seminar series, University of Michigan. Virtual Event.
2023 Wistar Institute Distinguished Lectures in Cancer Research Series. Philadelphia, PA.
2023 Fox Chase Annual Cancer Epigenetics Institute Symposium, Philadelphia, PA.
2022 5th Biennial Miami Epigenetics & Cancer Symposium, Miami, FL.
2022 Boston Epigenetics Society, Virtual Event. (~500 attendees)

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report.

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report.

- **Other Products**
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*

- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Emily Bernstein*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-6533-8326*
Person months worked:	*0.72*
Contribution to Project:	*Dr. Bernstein oversaw the execution of this project and the publication of this work in Nature Cell Biology.*

Name:	*Dan Filipescu*
Project Role:	*Assistant Professor*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-6381-2557*
Person months worked:	*5 calendar*
Contribution to Project:	*Dr. Filipescu was responsible for leading the experimental execution and played a senior role in driving this project.*

Name:	*Sina Jostes*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-3212-6727*
Person months worked:	*5.5 calendar*
Contribution to Project:	*Dr. Jostes was responsible for collecting and propagating all melanoma related materials for the project.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Dr. Emily Bernstein

New active support
*Title: Hepatic stellate cell plasticity and maladaptive fibrogenic memory in chronic liver disease
Goals: The objective of this research is to clarify how the epigenome and specifically DNA methylation patterns encode this maladaptive cell memory and amplify HSC's fibrogenic response following re-injury.
Specific Aims:
Aim 1: Define the regulatory elements controlling fibrogenic memory in HSCs.
Aim 2: Determine how UHRF1 and the DNA methylome control fibrogenic memory in HSCs. Aim 3: Uncover novel regulatory nodes driving HSC's maladaptive response in re-injury. Contracts/ Grants Officer: BURGESS-BEUSSE, BONNIE L
Contact Address: 9000 Rockville Pike, Bethesda, Maryland 2089
*Status of Support: Current
Project Number: R01DK136016
Name of PD/PI: Wang, Shuang
Role: Co-Investigator
*Supporting Agency: NIDDK
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 4/1/2023 - 2/29/2028
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	Calendar Months
1. 2024	0.600
2. 2025	0.600
3. 2026	0.600
4. 2027	0.600
5. 2028	0.600

Completed Support

*Title: Deciphering the tumor suppressive functions of macroH2A

Goals: We propose to dissect the role of macroH2A variants in melanoma progression and mammary carcinoma development using novel in vivo mouse models. Our studies will elucidate the epigenetic and transcriptional programs regulated by macroH2A that support its tumor suppressive functions.
Specific Aims
Aim 1: Determine the mechanisms by which macroH2A contributes to melanoma suppression in vivo.
Aim 2: Investigate macroH2A deficiency in mammary epithelial cells and tumor development in vivo.
Contract/Grants Officer: Paul Okano
Contact Address: 9000 Rockville Pike, Bethesda, Maryland 2089
*Status of Support: Completed
Project Number: 5R01CA154683
Name of PD/PI: Bernstein, Emily
Role: PI
*Supporting Agency: NCI
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 6/16/2017 - 5/31/2023
*Total Award Amount (including Indirect Costs):

*Title: Epigenetic and microenvironmental regulation of dormant disseminated cancer
Goals: Metastases can arise from dormant disseminated tumor cells (DTCs) years after primary tumor resection. We will study unexplored areas of HNSCC DTC biology including how target organ niche cues (TGFβ2, IL6), epigenetic regulators (macroH2A), and specific chromatin states, promote dormancy of DTCs.
Specific Aims:
Aim 1: Investigate the role of macroH2A in regulating dormancy of HNSCC DTCs
Aim 2: Decipher how ARID1A affects the tumor cell epigenome to enforce dormancy
Aim 3: Identify chromatin states permissive for dormancy and senescence induction by microenvironmental signals
Contracts/Grants Officer: Elizabeth Snyderwine
Contact Address: 9000 Rockville Pike, Bethesda, Maryland 2089
*Status of Support: Completed
Project Number: 7R01CA218024
Name of PD/PI: Aguirre-Ghiso, Julio
Role: Co-Investigator
*Supporting Agency: AECOM/NIH
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/1/2021 - 5/31/2023
*Total Award Amount (including Indirect Costs):

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or

domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* https://ebrap.org/ *for each unique award.*

N/A

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

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