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Cohesin mutations alter DNA damage repair and chromatin structure and create therapeutic vulnerabilities in MDS/AML

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SUMMARY: We developed models of cohesin-mutant MDS and AML and demonstrated a shift from STAG2- to STAG1-cohesin complexes, increased DNA damage and sensitivity to PARP inhibition.
ABSTRACT

The cohesin complex plays an essential role in chromosome maintenance and transcriptional regulation. Recurrent somatic mutations in the cohesin complex are frequent genetic drivers in cancer including myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Here, using genetic dependency screens of STAG2-mutant AML, we identified DNA damage repair and replication as genetic dependencies in cohesin-mutant cells. We demonstrated increased levels of DNA damage and sensitivity of cohesin-mutant cells to PARP inhibition. We developed a mouse model of MDS in which Stag2 mutations arise as clonal secondary lesions in the background of clonal hematopoiesis driven by Tet2 mutations, and demonstrated selective depletion of cohesin-mutant cells with PARP inhibition in vivo. Finally, we demonstrated a shift from STAG2- to STAG1-containing cohesin complexes in cohesin-mutant cells, which is associated with longer DNA loop extrusion, more intermixing of chromatin compartments, and increased interaction with PARP and RPA proteins. Our findings inform the biology and therapeutic opportunities for cohesin-mutant malignancies.
INTRODUCTION

The cohesin complex is a multimeric protein complex that forms a ring structure around DNA molecules and plays multiple key roles in spatial organization of eukaryotic genomes. Cohesin proteins are involved in several essential cellular functions, including sister chromatid cohesion, chromatin loop organization, transcriptional activation, and DNA replication and damage repair, among others (reviewed in(1)). More recently, the cohesin complex was identified as one of the most frequently mutated protein complexes in cancer, including myeloid malignancies, glioblastoma, breast cancer, bladder cancer and Ewing sarcoma(2, 3). The mechanisms by which cohesin mutations cause cellular transformation are unknown, and currently no therapies are known to exhibit selective efficacy in cohesin-mutant cancers.

Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are clonal diseases of mutated hematopoietic stem and progenitor cells (HSPC) characterized by abnormal differentiation and proliferation caused by somatic mutations in genes encoding transcription factors, epigenetic regulators, chromatin modifiers and splicing factors(4, 5). The core components of the cohesin complex STAG2, SMC1, SMC3, RAD21, as well as its modulators PDS5 and NIPBL, are collectively mutated in 13% of patients with de novo AML, 21% of patients with secondary AML, and 11% of patients with MDS, where they are associated with poor overall survival (5-10). Mutations in the cohesin genes are nearly always mutually exclusive, heterozygous, predicted loss-of-function (LOF) lesions, which are thought to be acquired early during the progression from clonal hematopoiesis of indeterminate prognosis (CHIP) to MDS (4, 11). Targeted inactivation of Smc3 or Stag2, and overexpression of mutant cohesin genes in wild type mouse and human HSPC has been previously studied(12-15) but no cohesin-mutant models currently exist that recapitulate the natural evolution of cohesin-mutant myeloid disease in the context of clonal hematopoiesis.

Two different cohesin complexes are known to co-occur in somatic vertebrate cells, each containing the core components SMC1A, SMC3, and RAD21, and alternatively including STAG2 or the less abundant STAG1(16). STAG2- and STAG1- complexes associate with centromeres and telomeres, respectively, but their sister chromatid cohesion-independent functional differences are not fully understood(1). STAG2 mutations account for over 85% of cohesin mutations in MDS, whereas STAG1 is rarely mutated in MDS or AML(6, 7, 10, 17). The mechanism underlying clonal expansion of these driver mutations is unlikely related to defects in
sister chromatid cohesion given lack of association between STAG2 mutations and complex karyotype and aneuploidy(10, 18). STAG1- versus STAG2- containing complexes have been recently shown to differentially contribute to chromatin organization, facilitating longer loops at topologically associating domain (TAD) boundaries and shorter more transient nested enhancer-promoter contacts, respectively(19-22). Furthermore, Stag2/Runx1 deficiency has been shown to disrupt enhancer-promoter looping and effect transcriptional pausing leading to selective gene dysregulation(22).

We sought to determine the effects of STAG2 mutations observed in patients on the cohesin complex composition and genetic dependencies, with the goal of understanding the mechanisms by which these mutations contribute to cellular transformation and how cohesin-mutant malignancies could be therapeutically targeted.
RESULTS

Genetic synthetic vulnerabilities in STAG2-mutant cells
To study the cellular consequences of cohesin mutations in myeloid malignancies, we used CRISPR-Cas9 to engineer a spectrum of predicted LOF STAG2, SMC3 and RAD21 mutations identified in patients in AML cell lines wild type for all cohesin subunits and modulators. The different mutants largely phenocopy each other and are consistent with loss of function of STAG2 or haploinsufficiency of SMC3 or RAD21 (Supplementary Fig. S1A-G, Methods). We first focused on STAG2, the most frequently mutated subunit of the cohesin complex and hypothesized that different STAG2 mutations would be associated with mutant-specific genetic dependencies.

We performed genome-scale CRISPR-Cas9 screens in 6 wild type and 5 STAG2-mutant U937 cell lines representing different STAG2 mutations using the Avana sgRNA library, which targets a total of 20,000 protein-coding genes with four unique sgRNAs per gene and includes 1000 non-targeting sgRNA controls(23). We observed that STAG2-mutant cells were strongly dependent on STAG1, as has been recently reported in the context of bladder cancer and Ewing sarcoma cell lines(24, 25) (Fig. 1A, Supplementary Fig. S2A). Since both STAG1- and STAG2-containing cohesin complexes participate in sister chromatid cohesion, we examined whether loss of STAG1 in STAG2-deficient cells would lead to aberrant sister chromatid cohesion. We found that loss of both STAG1 and STAG2, but not loss of either one alone, led to sister chromatid cohesion defects as assessed by premature centromere separation and railroad chromosomes (Fig. 1B), providing a mechanistic basis for the synthetic lethality of STAG1 and STAG2. The absence of sister chromatid cohesion defects in cells harboring loss of STAG2 alone is in agreement with lack of aneuploidy or complex karyotype in patients with STAG2-mutant MDS and AML(5, 10, 26), as well as previous studies in yeast suggesting that sister chromatid cohesion is unaffected with up to 87% loss of cohesin levels(27). Therefore, STAG2-mutant cells are dependent on the presence of STAG1 and do not have overt sister chromatid cohesion defects unless accompanied by a simultaneous loss of STAG1.

In addition to STAG1, we identified preferential dependency of STAG2-mutant cells on multiple components of the DNA damage repair and replication machinery (Fig. 1A), as well as lineage-defining transcription factors, and genes involved in mRNA processing (Supplementary Table S1). Of note, we identified multiple members of the base excision repair (PARP1), homologous recombination (BRIP1, RAD51B, RAD51C, RAD54L2, XRCC2, XRCC3, PARP1), mismatch
repair machinery (MSH2, POLD3, EXO1) and DNA replication (RPA2, POLD3) as specific synthetic vulnerabilities of STAG2-mutant cells (Fig. 1A). Therefore, we observed differential dependency of STAG2-mutant cells on DNA damage repair and replication-associated pathways.

**Altered cohesin complex composition and interactome in STAG2-mutant cells**

Having demonstrated that STAG2 mutations lead to genetic dependencies on STAG1 and DNA damage repair and replication, we hypothesized that these cohesin-dependent vulnerabilities could be associated with mutant-specific protein complex alterations. To examine the effect of STAG2 loss on the composition of the cohesin complex, we employed immunoprecipitation using an antibody against the core cohesin ring subunit SMC1A followed by quantitative mass spectrometry (IP-MS) (Supplementary Fig. S2B). In wild type cells, we detected all members of the cohesin complex, including direct binding partners SMC3 and RAD21, as well as indirect binding partners STAG1, STAG2 and PDS5B (Supplementary Fig. S2C and S2D, Supplementary Table S2a). In comparison, examination of STAG2-mutant clones revealed STAG1 to be among the most enriched proteins preferentially incorporated into the cohesin complex in cohesin-mutant cells (hereafter referred to as STAG1-cohesin complex for simplicity) (Fig. 1C, Supplementary Table S2b). We validated this switch to STAG1-containing complexes in STAG2-mutant cells using immunoprecipitation followed by western blotting (IP-WB) (Supplementary Fig. S2E). A switch from STAG2 to STAG1-cohesin complexes was consistent with the dependency of STAG2-mutant cells on STAG1 (Fig. 1A). Unexpectedly, we also observed increased incorporation of STAG1 into cohesin complexes in cells with heterozygous mutation of a non-STAG1/2 paralog cohesin subunit, SMC3 (Supplementary Fig. S2F and S2G, Supplementary Table S2c). These studies provide evidence that mutation of either STAG2 or SMC3 causes a shift to STAG1-containing cohesin complexes.

We next examined whether the shift from STAG2- to STAG1-containing cohesin complexes is associated with additional changes in its interactome that could explain the genetic dependencies that we had observed. We observed a significant increase in the interaction of the STAG1-cohesin complex with proteins involved in DNA replication and DNA damage repair (e.g. PARP1, RPA1-3; p=0.024) (Fig. 1C), transcription factors and splicing proteins (Supplementary Table S2b). Similarly, we observed changes in the interaction of the STAG1-cohesin complex with the DNA damage repair, replication, and splicing machinery in SMC3-mutant cells (Supplementary Fig. S2F), suggesting that mutations affecting different cohesin subunits may affect the cohesin complex structure and interactome concordantly. These findings demonstrate a high concordance
between the cellular processes highlighted by IP-MS experiments and genetic dependency screens in cohesin-mutant cells, especially as it relates to DNA replication and damage repair.

**Stalled replication forks and accumulation of dsDNA breaks in STAG2-mutant cells**

Having found that STAG2 mutations were associated with a genetic dependency on components of the DNA replication and damage repair pathways, and altered interaction of these proteins with the cohesin complex, we examined whether cells bearing STAG2 mutations accumulate DNA damage. Cohesin has been shown to organize chromatin loops at DNA replication factories in order to mediate replication stress tolerance and restart stalled replication forks(28-32). Since aberrant replication forks could serve as a potential source of DNA damage in cohesin-mutant cells, we investigated DNA replication fork processivity using a DNA fiber assay(33). Loss of STAG2 was associated with an increase in the number of stalled replication forks (6% in wild type cells versus 25% in STAG2-mutant cells, p<0.05) and a tendency to lose replication origin firing (Fig. 1D). Furthermore, we observed significant replication fork asymmetry and a 27% decrease in the replication fork rate in STAG2-mutant cells (Supplementary Fig. S3A-B), both of which are consistent with replication fork slowing and stalling. Therefore, STAG2 mutations are associated with abnormal replication fork processivity and stalling, a phenomenon that may be due to aberrant spatial arrangement of replication origins and/or ineffective restarting of replication forks in the absence of a normal cohesin complex.

Stalled replication forks can lead to DNA damage, including dsDNA breaks if inappropriately resolved. The cohesin complex has been previously implicated in dsDNA break repair and intra-S and G2M checkpoint(34). STAG1- versus STAG2- containing cohesin complexes have been shown to affect repair pathway choice, with STAG2-cohesin complex being preferentially involved in sister chromatid homologous recombination repair(35). We therefore examined γ-H2Ax accumulation using immunoblotting as an indicator of dsDNA breaks. We observed accumulation of γ-H2Ax staining under homeostatic conditions across all STAG2-mutant cell lines (Fig. 1E). This was associated with activation of the ATR and ATM DNA damage checkpoints as assessed by phosphorylation of ATM and ATR proteins (Fig. 1F), even in the absence of mitomycin C treatment, which is predicted to induce DNA damage by blocking both replication and transcription. These data therefore indicate that one of the functional consequences of STAG1-cohesin complexes is aberrant DNA damage repair and increased genomic instability.

**STAG2-mutant cells are sensitive to PARP inhibition in vitro and in vivo**
We next addressed whether altered DNA damage response in STAG2-mutant cells creates a vulnerability that can be exploited therapeutically. In our CRISPR screen for genetic dependencies, we found STAG2-mutant cells to have a genetic dependency on PARP1 (Fig. 1A, p=0.006); and in our IP-MS experiments, we found an increased association of PARP1 with the cohesin complex in STAG2-mutant cells (Fig. 1C, log2FC=1.03). Furthermore, genetic screens in S.cerevisiae and C. elegans had previously identified synthetic lethality interactions between replication fork mediators, including PARP (poly (ADP-ribose) polymerase) genes, and mutant cohesin(36). We therefore tested sensitivity of STAG2-mutant cells to PARP inhibition. PARP inhibitors, including talazoparib, inhibit PARP catalytic activity and trap PARP at the sites of DNA damage, rendering cells that are dependent on non-homologous end joining repair of dsDNA breaks particularly sensitive to these agents. Treatment with talazoparib resulted in ~70-fold increased sensitivity of STAG2-mutant cells as compared to wild type cells (Fig. 2A). In addition, STAG2-mutant cells were outcompeted by wild type cells in the presence of talazoparib (Fig. 2B). We also sought to determine whether mutations in other components of the cohesin complex result in a similar dependency on PARP inhibition. We found that heterozygous inactivation of SMC3 or RAD21 was associated with increased sensitivity to PARP inhibition to a similar extent as STAG2 mutations in U937 and K562 cells (Fig. 2C, Supplementary Fig. S3C), suggesting that all core cohesin complex mutations we have tested may act as biomarkers of response to PARP inhibition.

We reproduced this effect in xenograft animals injected with individual or competitive mixtures of wild type and STAG2-mutant AML cells and observed a genotype-specific effect of talazoparib on STAG2-mutant cells (Fig. 2D-E). Finally, in order to examine whether response to PARP inhibition in primary human leukemia cells is STAG2 mutation dependent, we treated STAG2-mutant and wild type primary AML patient samples with talazoparib and noted a dose and genotype-dependent sensitivity to the drug (Supplementary Fig. S3D). Cumulatively, these studies indicate that cohesin complex mutations result in increased association of DNA repair factors with mutant STAG1-containing cohesin complexes, impaired DNA damage repair and increased sensitivity to PARP inhibitors.

Development of cohesin-mutant mouse models of MDS and AML
In order to extend our observations made in AML cell lines to primary models of STAG2-mutant myeloid disease, we developed a syngeneic mouse model in which Stag2 mutations arise as secondary lesions in the background of clonal hematopoiesis driven by Tet2 mutations, as is seen
in the development of human MDS (4) (Fig. 3A). Hematopoietic stem and progenitor cells (Lineage-, Sca1+, c-Kit+ cells) harvested from Mx1-Cre; Cas9 heterozygous C57BL.6 mice were transduced with sgRNA targeting \( \text{Tet2} \) or non-targeting sgRNA (NTG) and transplanted into lethally irradiated SJL recipient mice. Engraftment and clonal expansion of \( \text{Tet2} \)-mutant cells were confirmed by fluorescent protein reporter expression and next-generation sequencing (37), and were not associated with an overt phenotype (Fig. 3B). Next, c-Kit enriched bone marrow cells from mice with \( \text{Tet2} \) mutations (\( \text{Tet2} \) indel fraction 0.62) were transduced with sgRNAs targeting \( \text{Stag2} \) or NTG and transplanted into secondary recipient mice. Cells with \( \text{Tet2/Stag2} \) genetic editing expanded relative to \( \text{Tet2/NTG} \) cells by 2 months post-transplantation (Fig. 3C). In contrast to \( \text{Tet2} \)-only mutant mice, \( \text{Tet2/Stag2} \)-mutant mice developed leukocytosis, absolute monocytosis, anemia, and thrombocytopenia (Fig. 3D).

Morphologic evaluation of \( \text{Tet2/Stag2} \) bone marrow revealed fewer megakaryocytes and increased hemophagocytosis consistent with macrophage activation in comparison to \( \text{Tet2} \)-only mutant mice (Fig. 3E). NGS confirmed predicted loss of function frameshift mutations in \( \text{Tet2} \) and \( \text{Stag2} \) (mean \( \text{Tet2} \) indel fraction 0.80 in \( \text{Tet2/NTG} \) mice; mean \( \text{Tet2} \) and \( \text{Stag2} \) indel fractions 0.64 and 0.63, respectively, in \( \text{Tet2/Stag2} \) mice). In concordance with our AML cell line data, \( \text{Tet2/Stag2} \)-mutant bone marrow cells exhibited higher levels of dsDNA breaks and increased sensitivity to treatment with talazoparib when cultured in vitro (Supplementary Fig. S3E-G). Ex vivo low dose irradiation of bone marrow cells led to an increase in dsDNA breaks in \( \text{Tet2/NTG} \) cells treated with vehicle but did not appreciably increase already elevated levels of dsDNA breaks in \( \text{Tet2/Stag2} \) or any of the talazoparib treated cells. These studies demonstrate that our CRISPR-Cas9 model with sequential acquisition of \( \text{Tet2} \) and \( \text{Stag2} \) mutations results in aberrant hematopoiesis with \( \text{Stag2} \)-mutant-specific alterations in DNA damage response.

**Talazoparib depletes cohesin-mutant clones in *in vivo* models of MDS and AML**

\( \text{Tet2/Stag2} \) and \( \text{Tet2} \)-mutant clones and the associated hematologic phenotypes were serially transplantable, enabling evaluation of genotype-specific response to the PARP1 inhibitor talazoparib *in vivo*. Forty recipient mice transplanted with \( \text{Tet2} \) or \( \text{Tet2/Stag2} \) mutant bone marrow cells were stratified into treatment groups with talazoparib or vehicle (Fig. 4A). Expression of congenic markers and fluorescent reporters linked to \( \text{Tet2} \) and \( \text{Stag2} \) sgRNA expression were used to monitor mice during 4 weeks of treatment. \( \text{Tet2/Stag2} \)-mutant mice but not \( \text{Tet2} \)-only mutant mice treated with talazoparib demonstrated a significant loss of mutant cells as determined by NGS and flow cytometry (Fig. 4B, Supplementary Fig. S4A). In addition, we observed
normalization of leukocytosis, monocytosis and thrombocytopenia in *Tet2/Stag2* mutant mice treated with talazoparib (Fig. 4C), which was associated with increased numbers of megakaryocytes on blinded review (Fig. 4D, Supplementary Fig. S4B, p=0.007).

We next wanted to examine whether response to PARP inhibition is cohesin mutation dependent in primary patient derived leukemia cell xenografts. We developed 2 unique serially transplantable PDX models of *STAG2* and *RAD21*-mutant AML (Fig. 4E, Supplementary Fig. S4C and S4E) and evaluated the efficacy of talazoparib in both models *in vivo*. We noted a decrease in disease burden and increased survival of cohesin-mutant PDX models treated with talazoparib as compared to vehicle (Fig. 4F and 4G, Supplementary Fig. S4D and S4F). Therefore, in both primary mouse HSPC and human AML cells, *STAG2*-mutant cells are selectively sensitive to treatment with talazoparib.

**STAG2 loss alters chromatin compartmentalization and looping**

In order to understand the impact of the aberrant cohesin complex on chromatin compartmentalization and looping which has been previously linked to DNA replication stress and damage(38), we performed Hi-C(39, 40) in a set of *STAG2*-wild type and knockout AML cell lines. This unbiased genome-wide chromosome conformation analysis enables evaluation of chromatin organization at multiple tiers of genome organization, including compartments, topologically associating domains (TADs) and loops. Compartments are apparent by the plaid pattern of interaction in Hi-C interaction maps (Fig. 5A). Analysis of this pattern is routinely performed by principal component analysis, where PC1 typically captures the positions of compartment domains. The strength of compartmentalization can then be visualized and quantified by re-arranging chromatin interaction maps by ordering loci according to their PC1 value to produce compartmentalization “saddle plots”(41). We observed a global weakening of compartmentalization and spatial segregation of active and inactive chromatin domains in *STAG2*-knockout cells (Fig. 5B), consistent with more intermixing between expression-rich “A compartments” and expression-poor “B compartments” as defined previously(39). Also, the number and location of TAD boundaries, determined by insulation score analysis(42), was largely preserved between *STAG2*-wild type and mutant cells (Supplementary Fig. S5A), but the strength of TAD boundary insulation was globally weakened (Fig. 5C and 5D, Supplementary Fig. S5B).

Finally, we assessed the effects of STAG2 loss on the strength and size-distribution of positioned loops that are apparent as dots in Hi-C interaction maps and correspond to enriched CTCF-CTCF
interactions at the bases of the loops(43). Visual inspection of the heatmaps revealed stronger dots further away from the diagonal (arrows in Fig. 5E). Genome-wide average loop size and loop density can be estimated by analysis of the relationship between interaction frequency ($P$) and genomic distance ($s$) (Supplementary Fig. S5C). Specifically, the position of a local maximum in the derivative of $P(s)$ has been previously shown to represent the average loop size(44). In $STAG2$-wild type cells loops are on average 100-200 kb, while in $STAG2$ knockout cells the average loop size is 200-300 kb. In addition, we noted the loop density to be reduced in $STAG2$ knockout cells (Fig. 5F).

Combined, these findings show that in the absence of $STAG2$, $STAG1$-containing cohesin complexes extrude larger and somewhat fewer loops. Possibly, $STAG1$-containing cohesin complexes are blocked less efficiently at CTCF sites (resulting in reduced insulation at CTCF sites and TAD boundaries), allowing the loops to more frequently pass CTCF sites and the formation of larger loops. This longer-range extrusion process is also expected to lead to more intermixing of A and B compartments.

**STAG2 loss leads to increased co-localization of cohesin with DNA replication and damage repair proteins**

A unifying hypothesis for genetic and pharmacologic dependencies on DNA replication and damage response is that altered chromatin insulation and compartment structure in cohesin-mutant cells leads to shifts in the physical co-localization of proteins involved in these cellular processes. We addressed this hypothesis using super-resolution microscopy. Staining for SMC1A protein, we identified wild type and mutant cohesin protein complex aggregates as distinct nuclear puncta (Fig. 5G). We observed increased co-localization of the $STAG1$-cohesin complex with PARP1 and RPA1 (Fig. 5G). Therefore, we observed $STAG2$ mutant-dependent alterations in the physical co-localization of the cohesin complex with DNA damage, concordant with our previously identified changes in the cohesin interactome and genetic dependencies. Put in context with our Hi-C analysis, we hypothesize that these changes may be driven by increased processivity of the $STAG1$-cohesin complex associated with a loss of TAD boundary insulation and longer loop extrusion.

These findings are consistent with a model in which cohesin complexes in wild type and cohesin-mutant cells, defined by their unique composition have differential ability to maintain chromatin organization as it relates to spatial organization of DNA damage repair machinery. Decreased co-
localization and function of these components in turn creates vulnerabilities that have the potential to be exploited therapeutically in patients with cohesin-mutated malignancies.
DISCUSSION

Our studies establish a role for DNA damage, DNA replication and chromatin architecture in the biology and therapeutic targeting of cohesin-mutant myeloid malignancies. Using genetic screens, IP-MS, chromatin conformation studies and super-resolution microscopy, we demonstrate that the introduction of cohesin mutations results in a switch from STAG2 to STAG1-cohesin complexes and differential cohesin dependence on the DNA damage repair and replication. We observed global spatial chromatin reorganization, including longer loop extrusion, loss of insulation at TAD boundaries and intermixing of compartments, associated with changes in cohesin interaction with DNA replication and damage machinery, which may explain the basis for the genetic dependency we observed. We extended these findings to loss-of-function mutations in other members of the cohesin complex, including SMC3 and RAD21, and identify cohesin mutations as potential biomarkers of response to treatment with PARP inhibitors.

There are currently very limited therapeutic options for patients with MDS and no therapies have been identified with selective activity in cohesin-mutant disease. We found that cohesin mutations cause a 70-fold increased sensitivity to PARP inhibition and are a potential biomarker of PARP inhibitor sensitivity in cohesin-mutant myeloid malignancies. PARP inhibitors are currently approved by the FDA for treatment of breast and ovarian cancer in the context of germline BRCA mutations and have been previously tested in a Phase 1 study of unselected patients with advanced hematologic malignancies(45). The effect of talazoparib monotherapy in cohesin-mutated AML or MDS with excess blasts is under investigation in a pilot proof-of-concept study (ClinicalTrials.gov Identifier NCT03974217). STAG2- mutant glioblastoma cells have been previously shown to be sensitive to PARP inhibition in vitro(31, 46), and cohesin mutations may be potential biomarkers of PARP sensitivity in bladder cancer and Ewing sarcoma, where these mutations are common. In addition, combination treatment of hypomethylating agents and PARP inhibition should be considered given sensitivity of cohesin-mutant MDS and genetically engineered CD34+ cells to hypomethylating agents(9, 47), as well as increased sensitivity of PARP inhibition when administered with low dose hypomethylating agents in preclinical studies(48). Finally, the role of PARP inhibition as a therapeutic strategy may be efficacious in a wider range of myeloid malignancies characterized by DNA damage repair defects, including IDH1/2-, FLT3-ITD-, splicing factor mutant and AML1-ETO rearranged AML(49).
A number of studies have previously examined the effects of complete cohesin loss on chromatin organization, both in the context of an inducible loss of the essential cohesin subunit RAD21 in human cells and loss of the cohesin-loading factor Nipbl in mice(50, 51). These studies confirmed the essential function of the cohesin complex in formation of topologically associated domains, which is independent of compartment organization. The models used to establish these findings represent a complete loss of cohesin complex, which is not tolerated in human cells, and as a result is not implicated in human disease. Patients with cohesin-mutant myeloid malignancies never present with complete, biallelic inactivation of any cohesin subunit, with the notable exception of STAG2, which has a paralog, STAG1. Complete loss of STAG1 and STAG2 is synthetically lethal in our studies as well in other recent reports(24, 25) and demonstrated in primary mouse hematopoietic stem cells using Stag2-conditional knockout mice(15). The models used in our study aimed to recapitulate the extent of cohesin dysfunction that would be observed in human disease, using both engineered cell lines as well as primary mouse models of cohesin-mutant MDS and AML. Cohesin mutations are early but usually not initiating lesions in myeloid malignancies. Our in vivo model of cohesin-mutant MDS arising in a setting of Tet2-mutant clones recapitulates the sequential acquisition of cohesin mutations in the context of Tet2-mutant clonal hematopoiesis and the phenotype observed in patients. The approach we developed is highly adaptable and can be used to not only model different stages of disease progression in hematopoietic malignancies, but also address the question of order of mutation acquisition and questions of necessity versus sufficiency for transformation using different genetic combinations.

Cohesin has been previously shown to organize chromatin loops at DNA replication factories into rosette-like structures, which allows for organized firing of multiple origins of replication and is disrupted with lower levels of cohesin leading to longer loop formation, similar to our observations with STAG2- versus STAG1-cohesin complexes (38). How exactly changes in chromatin organization mediated by the switch from STAG2- to STAG1-containing cohesin complexes in cohesin-mutant cancer cells lead to DNA replication stress and DNA damage repair defects cells remain to be elucidated.

Significant progress has been made in the last two decades in our understanding of the spatial organization of eukaryotic genomes (reviewed in (52)). Acquisition of genetic lesions that affect chromatin architecture are common mechanisms of cellular transformation(53, 54). Our study sheds light on how mutations affecting the cohesin complex alter the biology of mutant MDS and leukemia cells in a manner that creates a specific therapeutic vulnerability to PARP inhibitors.
METHODS

Cell Lines
U937 and K562 cells were obtained from the Broad Institute Cancer Cell Line Encyclopedia where they were authenticated by short tandem repeat (original source of U937 and K562 cells was ATCC). Cells were grown in RPMI (Invitrogen) and supplemented with 10% fetal calf serum (Sigma) and 100u/mL penicillin and 100ug/mL streptomycin (Invitrogen).

Mice
8-10 week old female NSGS mice (NOD-SCID; IL2Rγ null; Tg(IL3, CSF2, KITL); strain 013062) and 8-10 week old female SJL mice (B6.SJL-Ptprc; strain 002014) were obtained from The Jackson laboratory. Conditional Cas9 knock-in mice (strain 026175) and Mx1-Cre mice (strain 003556), both obtained from The Jackson laboratory, were bred to generate donors for bone marrow transplantation studies. All mice were housed in a pathogen-free animal facility in microisolator cages and experiments were conducted according to an IACUC approved protocol at the Broad Institute.

Generation of cohesin-mutant single cell clones
U937- and K562-Cas9 expressing cells were first generated by lentiviral transduction with a lentiviral vector pLX-311Cas9 (Addgene #96924). sgRNAs targeting STAG2, SMC3, RAD21 or non-targeting sgRNAs were cloned into a minimal backbone plasmid (Addgene #41824) and transfected into U937-Cas9 or K562-Cas9 cells using nucleofection (Lonza, Nucleofector II). GFP+ cells were single cell sorted into 96 well plates and grown up into single cell clones, which were confirmed by DNA sequencing of the targeted locus, as well as Western blotting. Of note, since STAG2 is an X-linked gene which undergoes normal X-inactivation in females, patients with predicted loss-of-function (LOF) STAG2 mutations are predicted to lack normal STAG2 expression. Therefore, in both cases of U937 cells and K562 cells, which both carry two copies of the X chromosome, we screened for presence of homozygous STAG2 LOF mutations. See Supplemental Methods.

The following sgRNAs targeting human genes were used in this study individually:
- STAG2 (exon 4): TCTGGTCCAAACCGAATGAA
- STAG2 (A1): AATGTCTTACTGCTCTACAA
- STAG2 (A2): CTGAATGTCATCCTCCCGT
- STAG1 (G1): GGAATTAGAGGAGCAGGCCG
- SMC3 (gRNA 1): GATAAAATGAGACGAGCCC
- SMC3 (gRNA 5): GAATATACCATTTACAATC
- RAD21 (gRNA 2): TGTGTTCGAGTGTAATTTAG
- Non-targeting (GC1): GACGGAGGCTAAGCGTCGCAA
- Non-targeting (GC3): GATCGTTTCCGCTTAACGCG

The single cell derived cell lines generated and used in the paper are listed in Supplemental Data Table 3.

CRISPR lentiviral transduction
sgRNAs targeting STAG2 or SMC3 were cloned into lentiCRISPRv2 (Addgene #52961) as described (http://genome-engineering.org/gecko/wp-content/uploads/2013/12/lentiCRISPRv2-and-lentiGuide-oligo-cloning-protocol.pdf).

Genome-wide CRISPR screening and differential dependency analysis
5 STAG2-mutant and 6 STAG2-wild type U937 cells expressing Cas9 were infected with the genome-wide human Avana LentiGuide-Puro CRISPR library (Broad Genetic Perturbations Platform), which contains ~75,000 sgRNAs targeting ~19,000 genes and 1000 controls, in two separate experiments, as previously described (23). See Supplemental Methods.

**Western blotting, immunoprecipitation and mass spectrometry**
Details of Western blotting, immunoprecipitation and immunoprecipitation followed by mass spectrometry performed using iTRAQ and TMT6 labeling are detailed in the Supplemental Methods.

**In vitro talazoparib treatment and competition assays**
Talazoparib was purchased from Selleck (S7048) and dissolved in DMSO. All drug dose response assays were conducted using CellTiter-Glo luminescent cell viability assay (Promega). Competition experiments were carried out with GFP- and mCherry- labeled cohesin-mutant cells mixed at different ratios, and monitored by flow cytometry. See Supplemental Methods.

**DNA fiber assay, Cohesion defect analysis, Super-resolution microscopy**
See Supplemental Methods

**Bone marrow transplantation assays**
C-kit enriched cells isolated from male donors were transduced with the lentiviral constructs containing Tet2, Stag2 or non-targeting (NTG) sgRNA. We specifically used male donors for our experiments since Stag2 is an X-linked gene and we were interested in modeling loss of Stag2 expression similar to what has been observed in patients with STAG2 LOF mutations. Cells were spin-infected with lentivirus in at 37°C for 90 min at 2000rpm, washed twice and injected into animals within 6 hours after transduction.

The following sgRNAs targeting mouse genes were used in the study:
- Tet2 sgRNA: TCAGGGGCGATGATGTACAT
- Stag2 sgRNA: TAACACACAAAGACAGTACG

Please see Supplemental Methods for additional details of transplantation assays and mouse analysis.

**Generation of patient-derived xenograft (PDX) models**
PDX#1 AML model was generated from bone marrow mononuclear cells of a patient with STAG2-mutant AML, confirmed by Dana-Farber Cancer Institute’s Rapid Heme Panel (RHP) sequencing (STAG2 p.31012* VAF 0.92; ASXL1 p.G642fs* VAF 0.513; NRAS p.G13D VAF 0.426; RUNX1 p.320* VAF 0.48). PDX#2 AML was generated from a skin lesion of a patient with RAD21-mutant AML (RAD21 p.R586* VAF 0.287; FLT3 p.D835Y VAF 0.514; RUNX1 p.Q265 VAF=0.295; WT1 p.R301fs16* VAF 0.287; WT1 p.T377fs* VAF 0.278). See Supplemental Methods.

**In vivo drug treatment**
Two donor Tet2/Stag2 and two donorTet2/NTG mice were used to generate a cohort of mice for in vivo talazoparib experiments. 10 mice per arm were dosed with 0.25mg/kg talazoparib or vehicle in 0.5% methylcellulose once daily by oral gavage. For drug treatment of PDX models, the mice were dosed with 0.25mg/kg talazoparib or vehicle in 0.5% methylcellulose (n=9-10 mice/arm for STAG2-mutant PDX, n=4 mice/arm for RAD21-mutant PDX). See Supplemental Methods.
**Indel sequencing analysis.** All sequencing analyses were performed on DNA extracted from 1 million murine bone marrow cells using the QIAamp DNA Micro Kit (Qiagen 56304). A sequential PCR was used to amplify the predicted Cas9 cut sites at both the Tet2 and Stag2 locus, as previously described, and all primers are listed in the table below (Tothova et al., 2017). Next generation sequencing using the MiSeq desktop sequencer (Illumina) was performed, and 300bp single end reads were used to identify indels. The depth of sequencing was above 10,000 reads for each gene. CRISPR-Seq, a publicly available method on Terra (https://terra.bio/), a cloud based genomic analysis platform, was used to detect indels. The pipeline has been previously described (Tothova et al., 2017). Reads were mapped against mouse genome build Mm10.

The following sequencing primers were used:

For mTet2 sgRNA (TCAGGGGCGATGATGTACAT):

**FORWARD 5'- Sequencing Primer - Genomic Primer - 3'**

ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGTCACCCTCAATAGAGAAGACA

**REVERSE 5'- Sequencing Primer - Genomic Primer - 3'**

GTGACTGGAGTCAGACGTTGCTCTTCGCAGATC TGGGCAGCTTCCCATATCCT

For mStag2 sgRNA(TAACACACAAAGACAGTACG):

**FORWARD 5'- Sequencing Primer - Genomic Primer - 3'**

ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGGCTGCATAATAATAGCCTAAAC

**REVERSE 5'- Sequencing Primer - Genomic Primer - 3'**

GTGACTGGAGTCAGACGTTGCTCTTCGCAGATC TGGGCAGCTTCCCATATCCT

**Hi-C methods and data processing**

Hi-C was performed as described previously (40) with some minor modifications. Please see Supplemental Methods for details.

**Statistics**

A P value less than 0.05 or an adjusted P value/FDR of less than 0.05 were considered significant. Statistical significance of differences in cohesion defects between wildtype and single and double cohesin knockout cells was determined using a one-way ANOVA (Fig. 1B). Statistical significance of enrichment of DNA damage repair and replication proteins was determined using a one-tailed Fisher's exact test (Fig. 1C). Statistical significance of differences in replication fork stalling between wildtype and single and double cohesin knockout cells was determined using a two-tailed unpaired t test (Fig. 1D). Statistical significance of differences in leukemia burden between mice treated with talazoparib versus vehicle was determined using a two-tailed unpaired t test (Figs. 2D, 2E, 4F and 4G). Statistical significance of differences in blood counts of Tet2/NTG and Tet2/Stag2 mice in the presence and absence of treatment with talazoparib was determined using two-tailed unpaired t tests (Fig. 3D and 4C). Statistical significance of differences in Stag2 and Tet2 indel fraction in the bone marrow of Tet2/NTG and Tet2/Stag2 mice treated with talazoparib or vehicle was determined using two-tailed unpaired t tests (Fig. 4B). Statistical significance of differences in survival of RAD21-mutant PDX model treated with talazoparib or vehicle was determined using Kaplan Meier survival analysis (Fig. 4G). Statistical significance of differences in colocalizing coefficients of cohesin with PARP or RPA1 protein in STAG2 wild type and mutant cells was determined using two-tailed unpaired t tests (Fig. 5G).

**Study approval**
All mouse experiments were conducted according to an IACUC approved protocol at the Broad Institute.

**AUTHOR CONTRIBUTIONS**


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REFERENCES


Figure 1.
Figure 1: Identification of DNA replication and damage repair as a dependency in STAG2-mutant cells

A, Volcano plot depicting differential dependencies in STAG2-mutant versus WT cells. Composite data for 5 STAG2-mutant cell lines (U937 STAG2-KO2, STAG2-KO3, KOC5, KOD5C, KOG8B) and 6 STAG2-WT cell lines (U937 WT-1, WT-2, NCB1, NCB12, NCB2A, NCC4) is shown. Respective sets of genes representing dependency in STAG2-mutant over WT cells with FDR<5% are shown in color.

B, Metaphase chromosome spreads and cohesion defect analysis in WT, STAG1 KO, STAG2 KO and double STAG1 STAG2 KO cells. Mean +/-SD is shown for two independent biological replicates of STAG2 wild type (U937 WT-1, WT-2) and STAG2 knockout cells (U937 STAG2-KO3, STAG2-KO4) transduced with STAG1 or control sgRNAs. 100 metaphase spreads were scored for each sample in a blinded fashion. * denotes p<0.0001 (one-way ANOVA). PCS = premature centromere separation. Railroad = railroad chromosomes.

C, Log2 fold change (FC) of protein enrichment after SMC1A IP-MS in WT and STAG2-knockout (KO) cells. Rep1 and Rep2 correspond to different mutant clones. Proteins belonging DNA damage repair and replication gene set are highlighted in green. Enrichment p value was determined using a one-tailed Fisher’s exact test (p( DNA damage and replication)=0.024). U937 WT-1, WT-2, STAG2-KO5 and STAG2-KO6 were used in this experiment.

D, Representative images depicting replication structures of single combed DNA molecules labeled with IdU (red) and CIdU (green) in WT and STAG2-knockout cells. Quantification of replication origin firing (Orig), progressing replication forks (Prog) and stalled replication forks (Stall) in WT and STAG2-mutant cells. Data from 3 WT (U937 WT-1, WT-2, WT-3) and 3 STAG2-knockout (U937 STAG2-KO2, STAG2-KO3, STAG2-KO4) cell lines combined. p<0.05 (unpaired Student t-test).

E, Assessment of dsDNA breaks in WT and STAG2-mutant cells using Western blotting for γ-H2Ax. Representative images shown. U937 WT-1, WT-2, STAG2 KO-1, STAG2 KO-2, STAG2 KO-3, STAG2 KO-4, STAG2 KO-5 and STAG2 KO-6 were used in this experiment. β-actin was used as a loading control.

F, Western blotting for DNA damage checkpoint proteins ATM, pATM, ATR, pATR, CHK1, pCHK1, CHK2, and pCHK2 in WT and STAG2-mutant cells in the presence and absence of mitomycin C. Vinculin was used as a loading control.
Figure 2.

A. U937 cells viability assay showing IC50 values for WT-1 (500 nM), WT-2 (7-30 nM), STAG2-KO3, and STAG2-KO5.

B. Graph showing DMSO and 100 nM talazoparib effects on % live cells over time for WT-1 (mCherry) and STAG2-KO2 (GFP).

C. Graph showing IC50 values for K562 cells: WT-1 (360 nM), STAG2 (16 nM), RAD21 (9 nM), and SMC3 (6 nM).

D. Experimental timeline for STAG2-KO2 and WT-1 with 0.25 mg/kg talazoparib or vehicle p.o. daily.

E. Experimental timeline for STAG2-KO2 and WT-1 with 0.25 mg/kg talazoparib or vehicle p.o. daily, followed by analysis of BM.

F. Graph showing viable cells normalized to vehicle for WT and STAG2-KO.

G. Graph showing % live mCherry+ cells in BM for WT and STAG2-KO.

H. Graph showing % live GFP+ cells in BM for WT and STAG2-KO.

p = 0.01 for WT vs. STAG2KO and p = 0.02 for STAG2 KO vs. vehicle.
**Figure 2: STAG2-mutant AML cell lines are more sensitive to PARP inhibition in vitro and in vivo**

A, Drug dose response curves of WT and STAG2-mutant U937 cell lines treated with the PARP inhibitor talazoparib. U937 WT-1, WT-2, STAG2 KO-3, and STAG2 KO-5 cells were used for this experiment. IC\(_{50}\) (WT) = 500nM, IC\(_{50}\) (STAG2 KO) = 7-30nM on Day 12 of treatment. Error bars represent SD of measurements of triplicate technical replicates.

B, Competition assay with WT (U937 WT-1-mCherry) and STAG2-knockout (U937 STAG2-KO2-GFP) cells mixed in 1:10 ratio in the presence of DMSO or talazoparib (100nM) in vitro. % Live GFP+ or mCherry+ cells were determined using flow cytometry. Error bars represent SD of measurements of triplicate technical replicates.

C, Drug dose response curves of WT and STAG2-, SMC3-, and RAD21-mutant K562 clones treated with talazoparib. IC\(_{50}\) (WT) = 360nM, IC\(_{50}\) (STAG2 KO) = 16nM, IC\(_{50}\) (SMC3 het) = 6nM, and IC\(_{50}\) (RAD21 het) = 9nM on Day 12 of treatment. Error bars represent SD of measurements of three technical replicates.

D, Schematic of the in vivo drug treatment of WT and STAG2KO xenografts. WT (U937 WT-1-mCherry) and STAG2-knockout (U937 STAG2-KO2-GFP) cells were mixed 1:1 and transplanted into NSGS recipients and dosed with talazoparib or vehicle by oral gavage at 0.25mg/kg once a day, starting on day 7 after transplantation. Leukemia burden in mice treated with talazoparib or vehicle was assessed in the bone marrow of animals at the time of sacrifice. % Live GFP+ or mCherry+ cells were determined using flow cytometry. Mean +/- SD is shown. p=0.01 (Student t-test). n=10 mice per group.

E, Schematic of the in vivo drug treatment of WT and STAG2 KO xenografts. WT (U937 WT-1 mCherry) or STAG2 KO (U937 STAG2-KO2-GFP) cells were transplanted into NSGS recipients and dosed with talazoparib or vehicle by oral gavage at 0.25mg/kg once a day starting on day 7 after transplantation. Leukemia burden in mice treated with talazoparib or vehicle was assessed in the bone marrow of animals at the time of sacrifice. % Live GFP+ or mCherry+ cells were determined using flow cytometry. Mean +/- SD is shown. p=0.02 (Student t-test). n=5 mice per group.
Figure 3.
Figure 3: Development of primary models of cohesin-mutant MDS

A, Schematic of the sequential bone marrow transplant used to generate Tet2/Stag2-mutant models of myeloid disease.

B, Morphologic evaluation of bone marrow section of mice injected with NTG and Tet2-mutant cells. H&E staining, 10X magnification. No appreciable differences were observed. Scale bar =0.5mm.

C, Flow cytometry analysis of peripheral blood samples of mice sequentially transplanted with Tet2/NTG and Tet2/Stag2 3 months after transplantation. BFP reporter is linked to expression of sgRNA targeting Stag2, and RFP reporter is linked to expression of sgRNA targeting Tet2. Expansion of BFP+ and BFP+RFP+ cells in Tet2/Stag2 animals. n=7 per arm. Mean +/-SD shown.

D, Absolute white blood cell (WBC) count, neutrophil count, lymphocyte count, monocyte count, hematocrit and platelet count were measured in Tet2/NTG and Tet2/Stag2-mutant mice 12 weeks after bone marrow transplantation. Mean +/- SD is shown. p values were determined using the Student t-test. n=20 mice per group.

E, Morphologic evaluation of bone marrow of a representative Tet2/NTG and Tet2/Stag2-mutant mouse shows a decrease in megakaryocytes and increased erythrophagocytosis in Tet2/Stag2-mutant mice. Images were stained using H&E and imaged at 10X (scale bar = 0.5mm) and 40X (scale bar = 0.125mm) magnification.
Figure 4.

**A**

Tet2/NTG or Tet2/Stag2

CD45.2+ → CD45.1+

0.25 mg/kg talazoparib or vehicle p.o. daily

wk0 wk8 drug dosing wk12

**B**

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**C**

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<tr>
<td>Talazoparib</td>
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**D**

H&E, 10X

H&E, 40X

**E**

H&E, 10X

H&E, 40X

CD45

CD34

CD33

MGG

**F**

PDX AML #1

STAG2/RUNX1/ASXL1/NRAS

0.25 mg/kg talazoparib or vehicle p.o. daily

Spleen weight (mg)

CD45+ viable BM cell number

**G**

PDX AML #2

RAD21/RUNX1/WT1/FLT3

0.25 mg/kg talazoparib or vehicle p.o. daily

Spleen weight (mg)

Survival (%)
**Figure 4. Talazoparib treatment preferentially depletes cohesin-mutant clones in primary mouse and human cell *in vivo* models of cohesin-mutant myeloid diseases**

**A**, Schematic of the *in vivo* drug treatment of *Tet2*/NTG and *Tet2*/Stag2-mutant mice with talazoparib. CD45.2+ *Tet2*/NTG or *Tet2*/Stag2-mutant cells were transplanted into CD45.1+ recipients and dosed with talazoparib or vehicle by oral gavage at 0.25mg/kg once a day starting 8 weeks after transplantation.

**B**, Bone marrow analysis of *Stag2* and *Tet2* indel fraction by NGS demonstrates a genotype-specific response to talazoparib treatment in *Tet2*/Stag2 but not *Tet2*/NTG-mutant clones. Mean +/- SD is shown. p values were determined using the Student t-test. n=10 mice per group.

**C**, Complete blood count analysis shows normalization of the white blood cell (WBC), monocyte and platelet counts in talazoparib treated *Tet2*/Stag2 animals. Mean +/- SD is shown. p values were determined using the Student t-test. n=10 mice per group.

**D**, Morphologic evaluation of bone marrow of representative *Tet2*/Stag2-mutant mice treated with talazoparib or DMSO shows an increased megakaryocyte number and persistent erythrophagocytosis in *Tet2*/Stag2-mutant mice treated with talazoparib. Images were stained using H&E and imaged at 10X (scale bar = 0.5mm) and 40X (scale bar = 0.125mm) magnification.

**E**, Generation of a STAG2-mutant AML PDX model model in NSGS mice. Staining with H&E, Modified Giemsa May-Grunwald (MGG) and immunohistochemistry (IHC) show expansion of immature CD45+CD34+CD33+ myeloid blasts in the bone marrow. Images were taken using 10x (scale bar = 0.5mm) and 100X magnification (scale bar = 0.05mm). NGS sequencing confirmed presence of a single STAG2-mutant clone with the following VAFs: *STAG2* p.R1012* (0.92), *RUNX1* p.R320* (0.43), *ASXL1* p. G642fs*(0.48) and *NRAS* p.G13D(0.45).

**F**, Schematic of the *in vivo* drug treatment of STAG2-mutant AML PDX model. *STAG2*/RUNX1/ASXL1/NRAS-mutant PDX cells were transplanted into NSGS recipients and dosed with talazoparib or vehicle by oral gavage at 0.25mg/kg once a day starting 12 weeks after transplantation. Mice were sacrificed after completing 4 weeks of treatment and showed a decrease in spleen size and human CD45+ bone marrow disease burden in the talazoparib-treated arm. Mean +/- SD is shown. p values were determined using the Student t-test. n=9-10 mice per group.

**G**, Schematic of the *in vivo* drug treatment of RAD21-mutant AML PDX model. *RAD21*/RUNX1/WT1/FLT3-mutant PDX cells were transplanted into NSGS recipients and dosed with talazoparib or vehicle by oral gavage at 0.25mg/kg once a day starting 8 weeks after transplantation. Treatment with talazoparib led to a decrease in spleen size and improved overall survival. Mean +/- SD is shown. p values were determined using the Student t-test and Kaplan-Meier survival analysis. n=4 mice per group.
Figure 5.
Figure 5: STAG2 loss disrupts normal chromatin folding and association with DNA replication and damage repair proteins

A, Hi-C interaction maps for chromosome 1 binned at 100kb in WT vs STAG2-KO cells to visualize compartments. Arrow heads depict examples of weakened compartments.

B, Saddle plots of Hi-C data binned at 100kb resolution normalized by genomic distance. Saddle plots were generated using the PC1 from the sample. The numbers at the center of the heatmaps indicate compartment strength calculated as the ratio of \((AA+BB)/(AB+BA)\) using the values from a 10 bin X 10 bin square starting from the corner. The saddle plot demonstrates global weakening of compartments in STAG2-KO cells. Heatmaps were generated using Hi-glass from pooled reads from 2 independent WT (U937 WT-1, WT-2) and STAG2-knockout (U937 STAG2-KO3, STAG2-KO4) cell lines.

C, Hi-C interaction maps for a genomic region in chromosome 5 binned at 25kb in WT vs STAG2-KO cells to visualize TADs. Heatmaps were generated using Hi-glass from pooled reads from 2 independent WT (U937 WT-1, WT-2) and STAG2-knockout (U937 STAG2-KO3, STAG2-KO4) cell lines. Arrow heads depict examples of loss of TAD insulation.

D, Insulation score as defined by (42) as a function of distance from TAD boundaries demonstrates global weakening of insulation at TAD boundaries.

E, Hi-C interaction maps for a genomic region in chromosome 5 binned at 10kb in WT vs STAG2-KO cells to visualize loops. Arrow heads depict examples of gain of longer loops.

F, Relationship between interaction frequency \((P)\) and genomic distance \((s)\) is used to estimate the average loop size and density in STAG2 KO and WT cells. The position of a local maximum in the derivative of \(P(s)\) represents the average loop size, while the difference between the local maximum and the valley directly to the right of it is a measure for the loop density (loops per kb; (44)). Extruded loops are longer in STAG2 KO cells compared to WT cells (represented by dashed lines, U937 WT1, WT2 ~100 kb; U937 STAG2-KO3, KO4 ~ 200kb) and the density of loops is reduced in STAG2 KO cells (represented by dotted lines).

G, Super-resolution structured illumination microscopy of co-localization of SMC1A with PARP1 in WT (U937 WT-1, WT-2) and STAG2-knockout (U937 STAG2-KO-3, STAG2-KO5) cells. Fluorescence signal displayed alone and merged with the nuclear Hoechst stain. Quantification of co-localization of SMC1A with PARP1 or RPA1 in WT (U937 WT-1) and STAG2-knockout (U937 STAG2-KO5) cells using Manders colocalization coefficient. Box and whiskers represent mean +/- Tukey. p<0.0001, unpaired Student t-test.