

Divergent Effects of *Dnmt3a* and *Tet2* Mutations on Hematopoietic Progenitor Cell Fitness

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SUMMARY

The DNA methylation regulators *DNMT3A* and *TET2* are recurrently mutated in hematological disorders. Despite possessing antagonistic biochemical activities, loss-of-function murine models show overlapping phenotypes in terms of increased hematopoietic stem cell (HSC) fitness. Here, we directly compared the effects of these mutations on hematopoietic progenitor function and disease initiation. In contrast to *Dnmt3a*-null HSCs, which possess limitless self-renewal *in vivo*, *Tet2*-null HSCs unexpectedly exhaust at the same rate as control HSCs in serial transplantation assays despite an initial increase in self-renewal. Moreover, loss of *Tet2* more acutely sensitizes hematopoietic cells to the addition of a common co-operating mutation (*Flt3*^{ITD}) than loss of *Dnmt3a*, which is associated with a more rapid expansion of committed progenitor cells. The effect of *Tet2* mutation manifests more profound myeloid lineage skewing in committed hematopoietic progenitor cells rather than long-term HSCs. Molecular characterization revealed divergent transcriptomes and chromatin accessibility underlying these functional differences.

INTRODUCTION

Hematopoiesis is a hierarchy with hematopoietic stem cells (HSCs) at the apex (Orkin and Zon, 2008). Tasked with self-renewal to replenish the stem cell pool, and differentiation to maintain blood production, HSCs must possess functional integrity for the lifetime of an individual. Somatic mutations acquired during aging can adversely affect this balance, resulting in hematologic disorders. Two alleles recurrently mutated in blood diseases are the epigenetic regulators DNA methyltransferase 3 alpha (*DNMT3A*) and tet methylcytosine dioxygenase 2 (*TET2*) (Cancer Genome Atlas Research Network, 2013). Tumor evolution analysis suggests that these mutations are established in HSCs of these patients (Abelson et al., 2018; Shlush et al., 2014). In addition, variants in *DNMT3A* and *TET2* are the most prevalent events associated with clonal hematopoiesis (CH), where pathogenic mutations are found in the blood of elderly people lacking overt disease (Buscarlet et al., 2017; Genovese et al., 2014; Jaiswal et al., 2014; Xie et al., 2014). These data suggest that *DNMT3A* and *TET2* mutations confer fitness advantages to HSCs.

Despite similarities in disease phenotypes, *DNMT3A* and *TET2* possess antagonistic biochemical activity. *DNMT3A* catalyzes addition of methyl groups to DNA forming 5-methylcytosine (Okano et al., 1999), while *TET2* promotes DNA demethylation by oxidizing the methyl group to 5-hydroxymethylcytosine (Koh et al., 2011). Each mutation alters the DNA methylome in a predictable manner. In patients with acute myeloid leukemia (AML), *DNMT3A* mutations yield mild hypomethylation of the genome (Spencer et al.,

2017), while *TET2* mutations result in hypermethylation (Figuroa et al., 2010). However, loss of function of either enzyme in murine hematopoietic progenitors paradoxically results in similar altered function, including a competitive advantage of mutant cells (Celik et al., 2015; Challen et al., 2012, 2014; Li et al., 2011; Moran-Crusio et al., 2011). The mechanisms contributing to increased fitness of *DNMT3A*- and *TET2*-mutant HSCs remain largely undefined. The goal of this study was to directly compare loss-of-function effects of *Dnmt3a* and *Tet2* at the HSC level through functional assays and molecular profiling.

RESULTS

Loss of *Dnmt3a* and *Tet2* Enhances Self-Renewal in HSCs to Different Degrees

To directly compare the effects of *Dnmt3a* and *Tet2* loss of function on HSC fate, we performed parallel competitive HSC transplants. Floxed *Dnmt3a* (Kaneda et al., 2004) or *Tet2* (Moran-Crusio et al., 2011) mice were crossed with *Mx1-Cre* (Kuhn et al., 1995). Treatment with polyinosinic-polycytidylic acid (pI:pC) created conditional knockout mice (*Dnmt3a*-KO^{Mx1} and *Tet2*-KO^{Mx1}). *Mx1-Cre*;*Dnmt3a*^{+/+};*Tet2*^{+/+} mice (Control^{Mx1}) were similarly treated with pI:pC. Eight weeks after pI:pC, 200 HSCs (Lineage⁻ c-Kit⁺ Sca-1⁺ CD48⁻ CD150⁺) were transplanted with 2.5 × 10⁵ whole bone marrow (WBM) competitor into wild-type mice (Figure 1A). No differences were noted in HSC abundance in donor mice (Figure S1A). Blood analysis (Figure S1B) revealed significantly higher contribution to all major



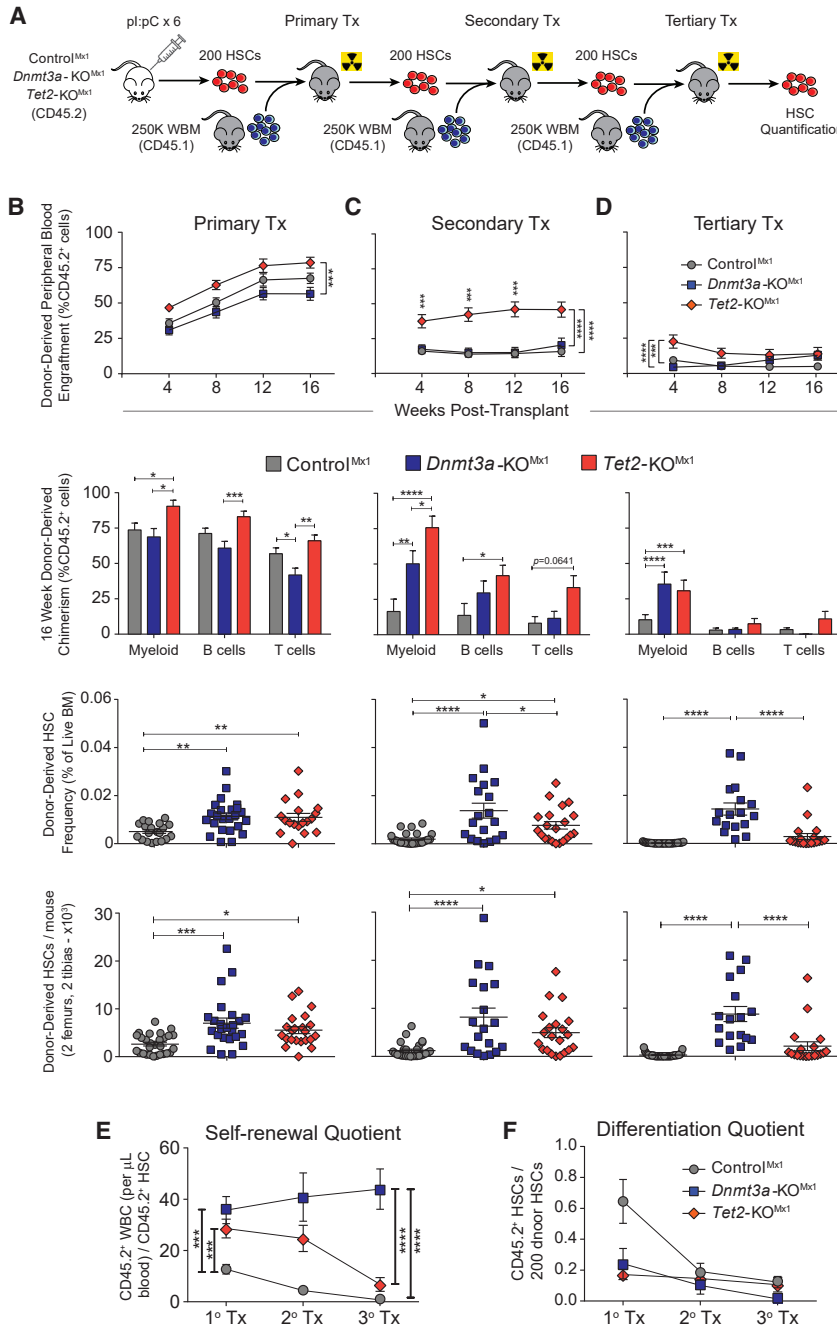


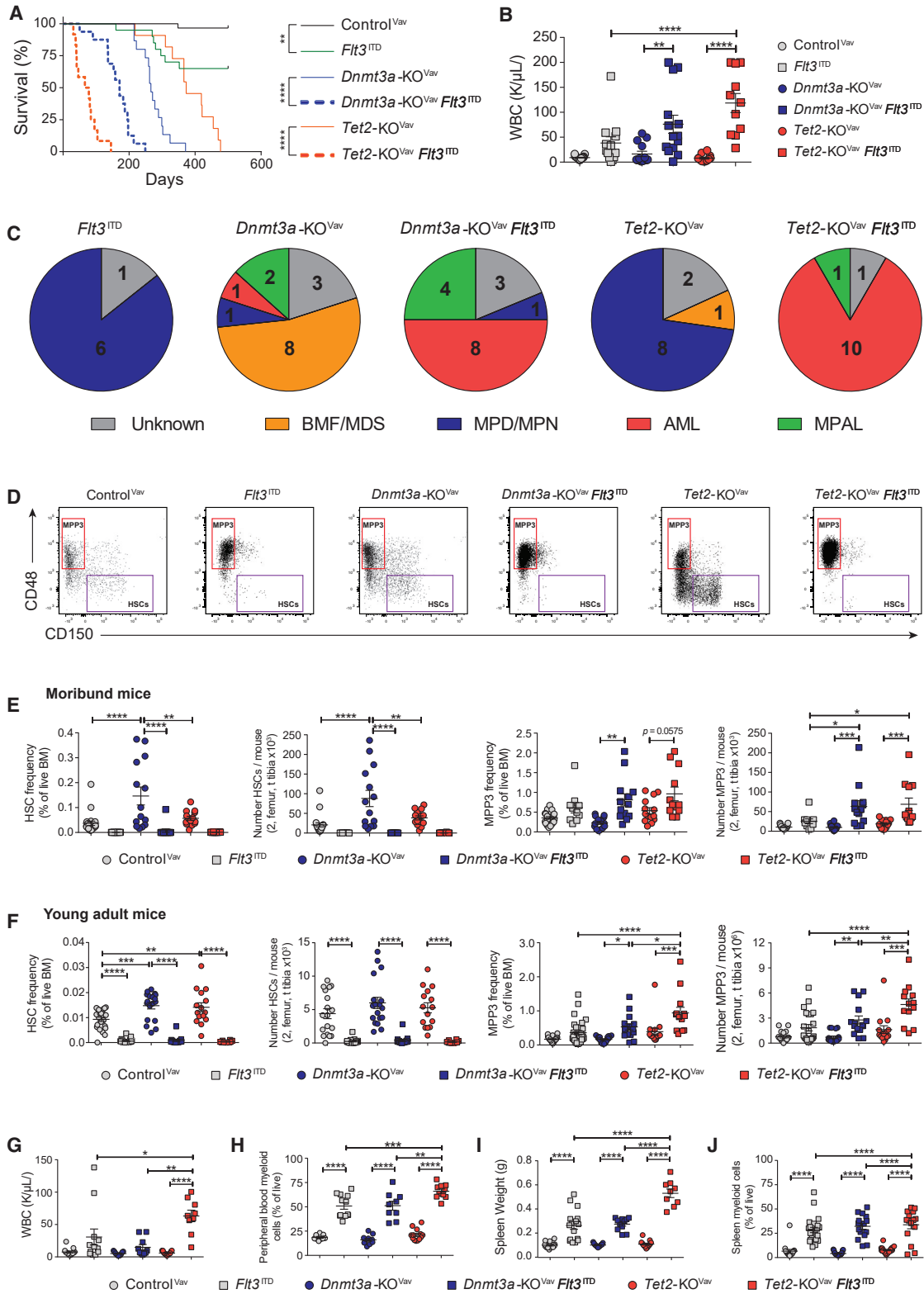
Figure 1. Loss of Dnmt3a and Tet2 Enhance Self-Renewal in HSCs to Different Degrees

(A) HSC serial transplantation schematic. In descending column order—contribution of 200 Control^{Mx1}, *Dnmt3a*-KO^{Mx1} (3aKO), and *Tet2*-KO^{Mx1} (T2KO) HSCs to peripheral blood, lineage chimerism, HSC frequency, and HSC number in (B) primary (CNT n = 28; 3aKO n = 24; T2KO n = 22), (C) secondary (CNT n = 27; 3aKO n = 19; T2KO n = 21), and (D) tertiary (CNT n = 33; 3aKO n = 23; T2KO n = 19) transplants. (E) Self-renewal and (F) differentiation quotients of indicated HSC genotypes after each transplant. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001. Mean \pm SEM is shown.

hematopoietic lineages from *Tet2*-KO^{Mx1} HSCs compared with control and *Dnmt3a*-KO^{Mx1} HSCs in primary recipients (Figure 1B). In the bone marrow (BM) 18 weeks post-transplant (Figure S1C), the abundance of both mutant HSC populations was 2-fold higher than Control^{Mx1} HSCs (Figure 1B).

Loss of *Dnmt3a* confers HSCs with unlimited self-renewal (Jeong et al., 2018). To test if *Tet2*-KO^{Mx1} HSCs possess the same ability, serial competitive HSC transplantation was performed. Two hundred donor-derived (CD45.2⁺) HSCs were

purified from primary recipients and transferred to secondary recipients with 2.5×10^5 fresh (CD45.1⁺) WBM competitor. *Tet2*-KO^{Mx1} HSCs maintained significantly higher blood production (Figure 1C), and donor-derived HSCs were increased elevated in recipients of both mutant HSC genotypes (Figure 1C). When examining other progenitor populations (Figure S1D), donor-derived multipotent progenitor 1 ([MPP1] Lineage⁻ c-Kit⁺ Sca-1⁺ CD48⁻ CD150⁻) and MPP3 (Lineage⁻ c-Kit⁺ Sca-1⁺ CD48⁺



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CD150⁻) populations were significantly increased in *Tet2*-KO^{Mx1} secondary recipients (Figures S1E and S1F). However, after tertiary transplant, *Tet2*-KO^{Mx1} HSCs surprisingly exhausted to similar levels as control HSCs (Figure 1D).

The ratio of donor-derived HSCs to 200 input HSCs (“self-renewal quotient”) quantifies the average self-renewal of a single test HSC (Challen et al., 2012). *Dnmt3a*-KO^{Mx1} and *Tet2*-KO^{Mx1} HSCs both possess greater self-renewal on a per-HSC basis than control HSCs after primary transplant (Figure 1E). But the self-renewal quotient of *Tet2*-KO^{Mx1} HSCs overlaps with Control^{Mx1} HSCs at the end of tertiary transplant, in contrast to *Dnmt3a*-KO^{Mx1} HSCs (Figure 1E). When examining the differentiation capacities of HSCs (16-week WBC count multiplied by percentage of donor-derived peripheral blood cells at 16 weeks divided by the total number of test HSCs, or the “differentiation quotient”) both *Dnmt3a*-KO^{Mx1} and *Tet2*-KO^{Mx1} HSCs display a reduced differentiation output on a per-HSC basis (Figure 1F).

Tet2 and Dnmt3a Loss of Function Divergently Influence Rate of Transformation from Same Co-operating Mutation

To compare functional contribution of *Dnmt3a* and *Tet2* loss of function with leukemogenesis, we crossed *Flt3* internal tandem duplication (*Flt3*^{ITD}) knockin mice (Lee et al., 2007) to *Vav-Cre;Dnmt3a*^{fl/fl} or *Vav-Cre;Tet2*^{fl/fl} mice (*Dnmt3a*-KO^{Vav} and *Tet2*-KO^{Vav}). *Flt3*^{ITD} mutation significantly decreased time to morbidity in both genetic backgrounds, but the magnitude varied. Median survival in a *Tet2*-deficient background decreased ~5.2-fold with expression of *Flt3*^{ITD}, whereas survival of *Dnmt3a*-KO^{Vav} mice only decreased ~1.5-fold (Figure 2A). Both *Dnmt3a*-KO^{Vav}*Flt3*^{ITD/+} and *Tet2*-KO^{Vav}*Flt3*^{ITD/+} mice presented with leukocytosis (Figure 2B), anemia, splenomegaly (Figure S2A), and AML (Figure 2C). A proportion of *Dnmt3a*-KO^{Vav}*Flt3*^{ITD/+} mice developed mixed phenotype acute leukemia, consistent with the role of *Dnmt3a* as a T cell leukemia tumor suppressor (Kramer et al., 2017). *Dnmt3a*-KO^{Vav} mice without *Flt3*^{ITD} predominantly developed BM failure resembling myelodysplastic syndromes,

whereas most *Tet2*-KO^{Vav} mice developed myeloproliferative disorders or myeloproliferative neoplasms.

Analysis of BM progenitors in moribund mice revealed significant expansion of the MPP3 population in *Flt3*^{ITD} mice (Figure 2D). *Flt3*^{ITD} depleted HSCs such that even the enhanced self-renewal of *Dnmt3a*-KO^{Vav} could not rescue (Figure 2E). The synergism between *Tet2* loss of function and *Flt3*^{ITD} alleles in promoting MPP3 expansion was already detectable in the BM of young adult mice lacking overt disease (Figure 2F), associated with leukocytosis (Figure 2G), myeloproliferation (Figures 2H and S2B), splenomegaly (Figures 2I and 2J), and lymphoid depletion (Figure S2C). Even though *FLT3*^{ITD} mutations co-occur in patients with AML with both *DNMT3A* and *TET2* mutations (Cancer Genome Atlas Research Network, 2013), the synergism in *Tet2*-KO^{Vav}*Flt3*^{ITD/+} mice show that founding mutations in *Dnmt3a* and *Tet2* have disparate sensitivities to the same co-operating mutation.

Tet2 Mutation Does Not Impart Ectopic Self-Renewal to Hematopoietic Progenitors, but Loss of Dnmt3a Confers Phenotypic Plasticity

Expansion of the MPP3 population in moribund *Flt3*^{ITD} mice implicated this BM compartment as the disease-initiating cell population. To test this, 250 MPP3 from young mice were transplanted with 2.5 × 10⁵ wild-type WBM competitor cells. Regardless of genotype, donor-derived cells were barely detectable in the blood (Figure S3A) and failed to engraft the BM and generate disease (data not shown). This demonstrates that neither loss of *Dnmt3a* nor *Tet2*, alone or combined with *Flt3*^{ITD}, imparts self-renewal properties to normally non-self-renewing progenitors.

Transplantation of Control^{Vav}, *Tet2*-KO^{Vav}, and *Dnmt3a*-KO^{Vav} WBM against equal numbers of wild-type BM confirmed the competitive advantage of unfractionated *Tet2*-KO^{Vav} BM (Figures S3B and S3C). But as *Tet2*-KO^{Mx1} HSC self-renewal was eventually exhausted (Figure 1D), this suggested that a non-HSC progenitor population may contribute to the observed myeloid dominance from *Tet2*-mutant BM. We performed competitive transplant of

Figure 2. Tet2 and Dnmt3a Loss of Function Divergently Influence Rate of Transformation from Same Co-operating Mutation

- (A) Kaplan-Meier plot comparing time to morbidity between Control^{Vav} (n = 30), *Flt3*^{ITD} (n = 20), *Dnmt3a*-KO^{Vav} (n = 15), *Tet2*-KO^{Vav} (n = 11), *Dnmt3a*-KO^{Vav}*Flt3*^{ITD} (n = 16), and *Tet2*-KO^{Vav}*Flt3*^{ITD} (n = 12) mice.
 (B) White blood cell count of day 600 Control^{Vav} and moribund mice of indicated genotypes.
 (C) Pathological diagnosis of moribund mice.
 (D) Representative flow cytometry plots of moribund mice demonstrating expansion of MPP3 (red box) and depletion of HSCs (purple box) in *Flt3*^{ITD} genotypes.
 (E) Frequency and number of HSCs and MPP3 in moribund mice.
 (F–J) (F) Frequency and number of HSCs and MPP3 in 8-week-old Control^{Vav} (n = 18), *Flt3*^{ITD} (n = 14), *Dnmt3a*-KO^{Vav} (n = 18), *Dnmt3a*-KO^{Vav}*Flt3*^{ITD} (n = 10), *Tet2*-KO^{Vav} (n = 15), and *Tet2*-KO^{Vav}*Flt3*^{ITD} (n = 9) mice. Pathological analysis of young adult mice showing (G) WBC counts, (H) peripheral blood myeloid cells, (I) spleen weights, and (J) spleen myeloid cells. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Mean ± SEM is shown.

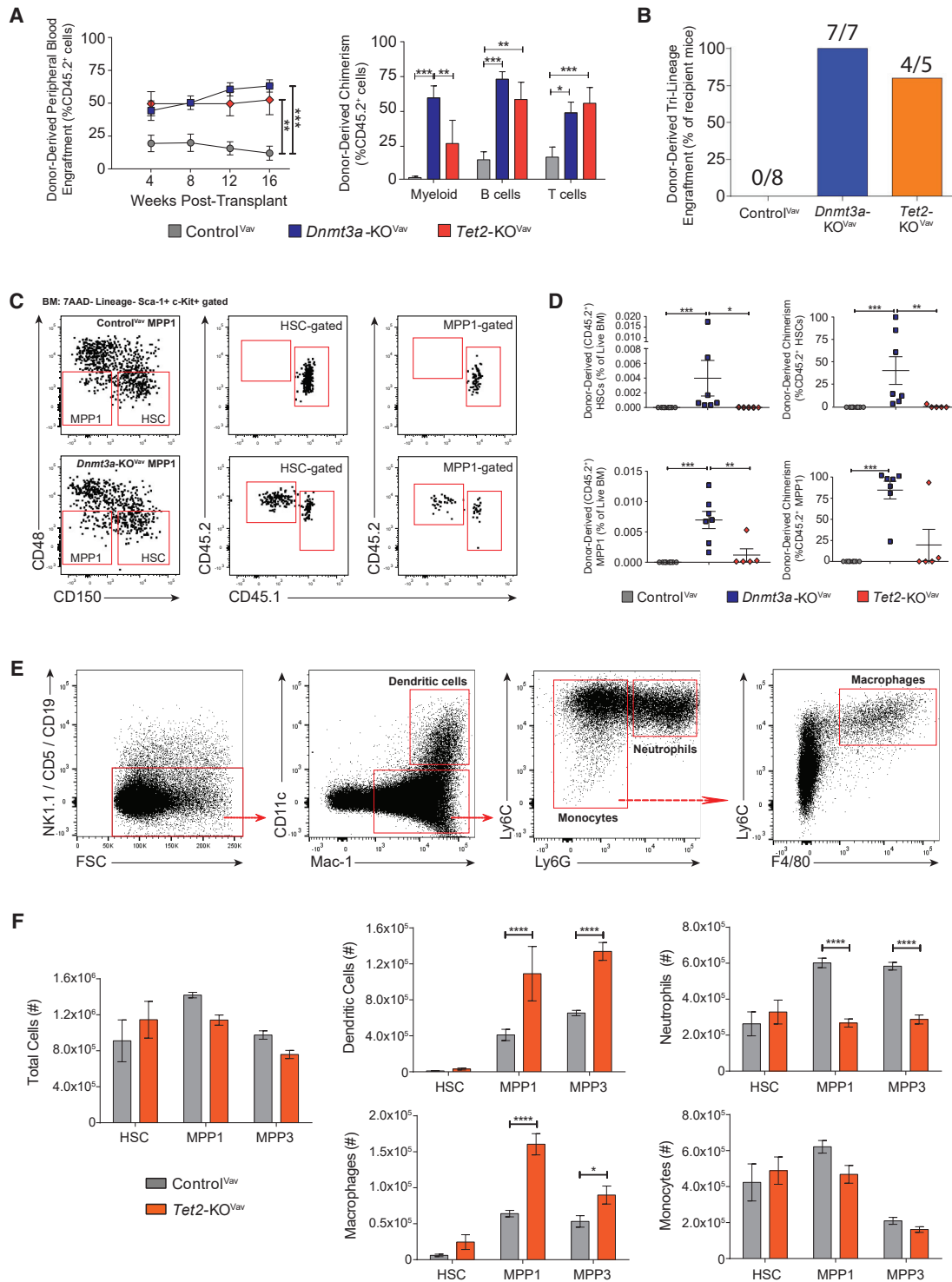


Figure 3. Tet2 Mutation Does Not Impair Ectopic Self-Renewal to Hematopoietic Progenitors, but Skews Myeloid Differentiation of Committed Progenitor Cells

(A) Donor-derived peripheral blood cells and 16-week lineage chimerism in recipients of 200 MPP1 from Control^{Vav} (n = 8), Dnmt3a-KO^{Vav} (n = 7), and Tet2-KO^{Vav} (n = 5) mice.

(B) Frequency of MPP1-transplanted mice with >1% donor-derived engraftment in myeloid, B cell, and T cell lineages.

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200 MPP1 (Figure 3A). Peripheral blood analysis revealed long-term tri-lineage engraftment among recipients of both *Dnmt3a*-KO^{Vav} and *Tet2*-KO^{Vav} MPP1 (Figure 3B), whereas all recipients of Control^{Vav} MPP1 had <1% donor-derived myeloid cells (Celik et al., 2018). From BM analysis (Figure 3C), *Tet2*-KO^{Vav} MPP1 failed to self-renew but *Dnmt3a*-KO^{Vav} MPP1 reconstituted the BM MPP1 compartment (Figure 3D). Surprisingly, we also observed donor-derived HSCs in all *Dnmt3a*-KO^{Vav} MPP1 recipients (Figures 3C and 3D). Loss of *Dnmt3a* may bestow epigenetic plasticity between the HSC and MPP1 compartments, reinforcing the importance of *Dnmt3a* in maintenance of hematopoietic cell type identity.

Loss of Tet2 Skews Myeloid Differentiation of Committed Progenitor Cells

As increased myeloid output from *Tet2*-deficient HSCs could not be attributed to differences in self-renewal, proliferation (Figure S3D), or apoptosis (Figure S3E), we hypothesized that epigenetic dysfunction may skew myeloid differentiation from hematopoietic progenitors. To quantify this, *in vitro* assays were performed with purified HSCs, MPP1, and MPP3. In methylcellulose, all genotypes initially had the same number of colonies (Figure S3F). Both mutant alleles sustained some colony-forming potential in MPP1 and MPP3 in serial plating. A third passage distinguished *Dnmt3a*-KO^{Vav} HSCs with a significant increase in colony number (Figure S3F). The lack of serial replating of *Tet2*-KO^{Vav} MPP3 *in vitro* was consistent with engraftment failure *in vivo*.

Progenitors were then cultured with hematopoietic cytokines in OP9 stroma-coated plates. After 2 weeks, analysis with myeloid markers Gr-1 and CD11b (Mac-1) showed clear differences from *Tet2*-KO^{Vav} progenitors. A Gr-1^{hi} population was lacking while Gr-1^{mid} and Gr-1^{lo} populations were more prevalent (Figure S3G). Further immunophenotyping (Figure 3E) revealed a disproportionate production of myeloid cells. Loss of *Tet2* increased dendritic cell and macrophage production from MPP1 and MPP3 at the expense of neutrophils (Figure 3F). This suggests that the primary function of *Tet2* mutations in hematopoietic progenitors is not to increase self-renewal, but rather skew myeloid output.

Dnmt3a and *Tet2* Loss of Function Alter Hematopoietic Progenitor Function through Distinct Molecular Mechanisms

Transcriptional profiling was performed to elucidate mechanisms underlying the functional differences between

HSCs over serial transplantation. Unfortunately, the diminution of Control^{Mx1} and *Tet2*-KO^{Mx1} HSCs at later transplant stages permitted only two replicates (Table S1). As a supplement, HSCs were purified from Control^{Vav}, *Dnmt3a*-KO^{Vav}, and *Tet2*-KO^{Vav} adult mice (Table S2). The gene expression profiles of Control^{Vav} and *Dnmt3a*-KO^{Vav} HSCs were remarkably similar (Figure S4A), despite their functional differences. *Tet2*-KO^{Vav} HSCs showed a larger dysregulated expression signature (Figure 4A). Of the 24 genes downregulated in *Dnmt3a*-KO^{Vav} HSCs, 11 also showed significant repression in *Tet2*-KO^{Vav} HSCs (Figure 4B). Genes, such as *Mki67* and *Hmgb2* also showed consistent downregulation in the mutant HSCs across serial transplant (Figure S4B) and may contribute to the differentiation block. A total of 62 out of 288 (21.5%) genes upregulated in *Dnmt3a*-KO^{Vav} HSCs were shared with *Tet2*-KO^{Vav} HSCs, including *jun* and *Fos*, which form the AP-1 transcription factor complex important in HSC stress response (Mallaney et al., 2019). Transcriptional analysis was also performed on Control^{Vav} and *Tet2*-KO^{Vav} MPP3 from young adult mice to understand the myeloid lineage skewing arising from loss of *Tet2*. Although there were fewer differentially expressed genes (DEGs) than the HSC comparison of the same genotypes (Figure 4C), gene set enrichment analysis revealed significantly dysregulated gene sets (Figure 4D). The major difference was diminished nuclear factor κ B signaling in *Tet2*-KO^{Vav} MPP3 (Figure 4E).

ATAC sequencing (ATAC-seq) was performed to examine chromatin accessibility in HSCs, MPP1, and MPP3. The most striking difference was global reduction of open chromatin in *Tet2*-KO^{Vav} HSCs and MPP1 (Figure 4F), consistent with the function of *Tet2* in promoting DNA demethylation at enhancers (Sardina et al., 2018; Wang et al., 2018). However, *Tet2*-KO^{Vav} MPP3 recovered chromatin accessibility above Control^{Vav} levels, including addition of new peaks at 800 hematopoietic enhancers and 1,232 promoters enriched for myeloid function (Figure S4C). The chromatin landscape of Control^{Vav} and *Dnmt3a*-KO^{Vav} progenitors revealed fewer differences. A multi-dimensional scaling analysis showed the *Dnmt3a*-KO^{Vav} MPP1 cluster closer to HSCs than Control^{Vav} MPP1 (Figure 4G), supporting the notion of a reduced epigenetic barrier between these cell types in the absence of *Dnmt3a*. Moreover, areas with reduced chromatin accessibility in *Dnmt3a*-KO^{Vav} MPP1 (Table S3) involved genes involved in hematopoietic lineage specification, such as *Fli1* and *Izkf1*, which may contribute to the differentiation block.

(C) Representative plots showing donor-derived MPP1 and HSCs in recipients of *Dnmt3a*-KO^{Vav} MPP1.

(D) Frequency and chimerism of donor-derived HSCs and MPP1 in recipients of 200 MPP1.

(E) Representative immunophenotyping of *in vitro* differentiated progenitor cells.

(F) Immunophenotypic populations produced via *in vitro* differentiation of progenitor cells from Control^{Vav} and *Tet2*-KO^{Vav} mice (n = 4 per population of each genotype). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001. Mean \pm SEM is shown.

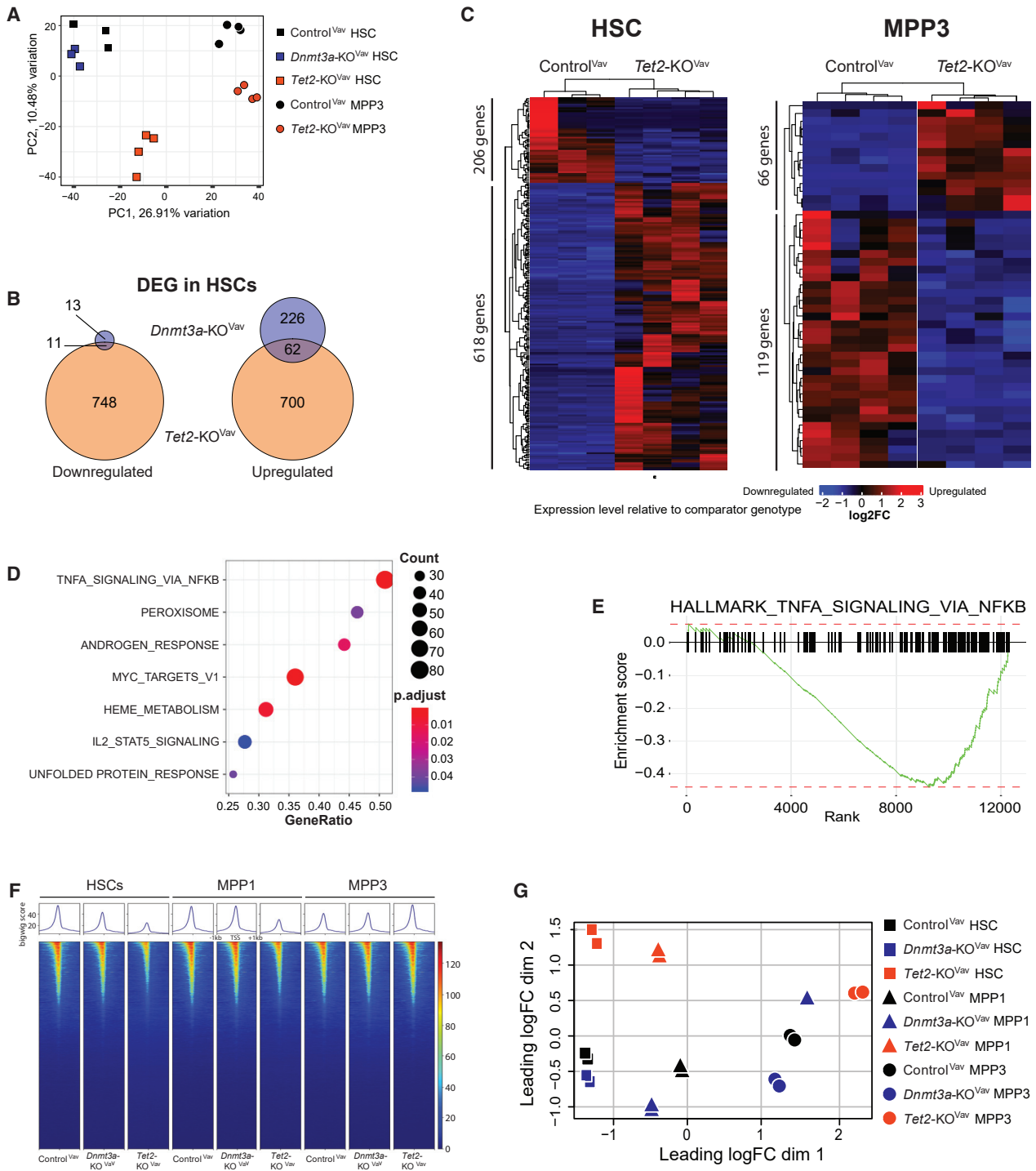


Figure 4. Dnmt3a and Tet2 Loss of Function Alter Hematopoietic Progenitor Function through Distinct Molecular Mechanisms

(A) Principal-component analysis plot of gene expression in Control^{Vav} HSCs (n = 3) and MPP3 (n = 4), *Dnmt3a*-KO^{Vav} HSCs (n = 3), and *Tet2*-KO^{Vav} HSCs (n = 4).

(B) Venn diagrams displaying DEGs overlap in *Dnmt3a*-KO^{Vav} and *Tet2*-KO^{Vav} HSCs compared with Control^{Vav} HSCs.

(C) Heatmaps displaying DEGs ($p < 0.05$, fold-change > 1 or < -1) between Control^{Vav} and *Tet2*-KO^{Vav} HSCs and MPP3.

(D) Gene set enrichment analysis showing differentially regulated pathways between Control^{Vav} and *Tet2*-KO^{Vav} MPP3.

(E) Gene score enrichment plot of "Hallmark TNF α Signaling via NF κ B" gene set in *Tet2*-KO^{Vav} MPP3.

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DISCUSSION

Somatic mutations in *DNMT3A* and *TET2* comprise approximately 70% of all variants in age-related CH. The persistence of these mutations (Young et al., 2016) coupled with their recurrence in blood malignancies of diverse lineages suggests a stem cell origin. Mouse model studies suggest a similar increase in HSC self-renewal after inactivation of both genes. Previous studies demonstrate a competitive advantage for *Tet2*-deficient cells in BM transplantation assays (Li et al., 2011; Moran-Crusio et al., 2011). Although this is assumed to be due to enhanced HSC self-renewal, these experiments have been performed with WBM or less pure populations of progenitors. Our results using highly purified HSCs show that *Tet2* loss of function modestly increased self-renewal on a per-HSC basis during early passages of transplantation, but this effect was transient and *Tet2*-deficient HSCs exhausted to comparable levels as control HSCs after tertiary transplant. This is in stark contrast to *Dnmt3a*-deficient HSCs which show no signs of self-renewal diminution. Moreover, by transplanting defined numbers of HSCs, our quantification shows that, on a per-cell basis, *Tet2*-mutant HSCs show a similar differentiation deficit as *Dnmt3a*-mutant HSCs. This was not anticipated given the high peripheral blood chimerism observed from transplantation of *Tet2*-null WBM, particularly in the myeloid lineage. Rather, our data suggest that increased myeloid output results from skewed differentiation in progenitors lacking *Tet2*. More open chromatin at enhancers of pro-myeloid differentiation genes in *Tet2*-KO^{Vav} MPP3 may contribute to this phenotype.

Our data are instructive for how these mutations are propagated in humans with chromatin immunoprecipitation. *DNMT3A* mutations are highly specific for the HSC compartment, with the increase in self-renewal potential allowing these mutations to be efficiently propagated in humans with age. The fact that the mutations do not cause massive transcriptional changes still allows these mutant HSCs to function as effective stem cells, but with greater self-renewal to withstand external stresses that would normally force HSC depletion. Although *Tet2* loss of function does not induce ectopic self-renewal in either normal or malignant progenitor cells, this mutation alters the myeloid output of progenitors and potentially sensitizes them to secondary mutations that further drive proliferation. This aligns with evidence in humans showing that these mutations do not have equal potential. Analysis of individuals with CH shows *DNMT3A* mutations can be

found in all blood lineages, whereas *TET2* mutations are often absent from T cells (Buscarlet et al., 2018), supportive of differential effects on progenitor lineage differentiation. Our data suggest a model for *TET2*-mutant CH whereby the mutation is acquired in HSCs, but the functional effects of myeloproliferation are realized by more downstream progenitors.

EXPERIMENTAL PROCEDURES

Detailed methods are provided in [Supplemental Information](#).

Mice and Transplantation

The Institutional Animal Care and Use Committee at Washington University approved all animal procedures. All mice were C57Bl/6 background. *Dnmt3a*^{fl/fl} (Kaneda et al., 2004) and *Tet2*^{fl/fl} (Moran-Crusio et al., 2011) mice were crossed to *Flt3*^{ITD} (Lee et al., 2007), *Vav-Cre* (Georgiades et al., 2002), and *Mx1-Cre* (Kuhn et al., 1995) strains. To induce *Mx1-Cre*, six doses (300 µg) of polyinosinic:polycytidylic acid (pI:pC; Sigma, no. p1530) were administered every 48 h via intraperitoneal injection to 8-week-old mice. Mice were allowed to recover for 6 weeks after the last pI:pC injection before sacrifice for HSC purification. Transplant recipients (C57Bl/6 CD45.1, The Jackson Laboratory strain no. 002014), received a split dose of irradiation (10.5 Gy) ~4 h apart. Cells were transplanted via retro-orbital injection.

RNA-Seq Data, Quality Control, and Analysis

Reads were aligned with STAR v.2.5.4b with Gencode release M20 (GRCm38.p6) genome assembly. Unambiguous read counts were calculated by HTSEQ-count. Data were imported into Noiseq v.2.28.0 for differential gene expression analysis with TMM normalization and batch correction. RNA-seq data are available under GEO GSE139911.

ATAC-Seq

Open chromatin was profiled via the Omni-ATAC method (Corces et al., 2017). Reads were aligned to mm10 with BWA mem v.0.7.17. Duplicates were removed with Picard tools MarkDuplicates v.2.0.1 and bam files were processed with snakePipes v.1.3.1. Differential chromatin accessibility was assessed using Rsubread v.1.34.7 and EdgeR v.3.26.8. Peaks were intersected with enhancers using bedtools v.2.25.0. Bigwig tracks and heatmaps were created via deepTools2 v.3.3.1. ATAC-seq data are available under GEO GSE139911.

Statistics

Student's t test, and one- and two-way ANOVA were used for statistical comparisons where appropriate. Kruskal-Wallis test was used for non-normal data. Survival curves were analyzed using a Mantel-Cox log rank test. Significance is indicated using the

(F) ATAC-seq heatmaps from Control^{Vav}, *Dnmt3a*-KO^{Vav}, and *Tet2*-KO^{Vav} mice. Signals displayed are peaks 1 kb up- and downstream of transcription start sites of protein coding genes.

(G) Multi-dimensional scaling plot with distances approximating the largest log₂ fold-changes in the top 500 peaks between ATAC-seq samples.



following convention: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. All graphs represent mean \pm SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.stemcr.2020.02.011>.

AUTHOR CONTRIBUTIONS

Conceptualization, G.A.C.; Data Curation, E.L.O.; Formal Analysis, E.L.O. and G.A.C.; Funding Acquisition, E.L.O. and G.A.C.; Investigation, E.L.O., A.C.K., C.M., H.C., W.K.K., J.F., E.H., C.R.C.Z., and G.A.C.; Project Administration, G.A.C.; Software, E.L.O.; Supervision, G.A.C.; Visualization, E.L.O. and G.A.C.; Writing—Original Draft, E.L.O.; Writing—Review & Editing, G.A.C.

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