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Neutrophil-derived S100A8/A9 is associated with inflammation that supports *Dnmt3a*-mutant hematopoiesis

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Authorship

J.J.M. and J.J.T. designed experiments. J.J.M., N.R.F. and G.J.N. performed experiments. R.Z. and A.V. provided critical reagents and advised on experimental design. J.J.M., N.R.F., G.J.N. and J.J.T. analyzed data. J.J.M. and J.J.T. interpreted data and wrote the manuscript. All authors edited the manuscript.

Conflict of Interest Disclosure

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Data Availability

All single cell and bulk RNA-seq datasets analyzed in this study were previously published.

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Clonal hematopoiesis (CH) is an age-related condition marked by expansion of hematopoietic stem cells (HSCs) and their progeny carrying somatic mutations that confer a selective advantage, increasing risk of hematologic malignancy, cardiovascular disease and inflammatory bone loss.¹ Chronic inflammation is a key driver of this expansion. For example, CH mutations in DNA methyltransferase 3a (*Dnmt3a*) cause overproduction of TNF α and IL-6, and blocking these cytokines reduces the competitive advantage of mutant cells.^{2,3} The upstream signals initiating this inflammation remain unclear, but S100A8/A9 are strong candidates. These calcium-binding proteins form a heterodimer to activate innate immune signaling via TLR4 and its co-receptor CD14.⁴ S100A8/A9 signaling promotes pro-inflammatory cytokine production and inflammasome activation, and elevated levels are linked to poor prognosis in hematologic malignancies.⁵ Here, we investigate S100A8/A9 as a potential driver of *Dnmt3a*-mutant CH. *In vitro* experiments used male and female mice (2-4 months of age), and *in vivo* experiments used female mice, approved by The Jackson Laboratory's Animal Care and Use Committee (protocol #12004-02).

To characterize S100A8/A9, we used a transplant model in which BM from *Dnmt3a*-mutant or Mx1-Cre control mice was transplanted into busulfan-conditioned wild-type recipients (**Figure 1A**). After 12 weeks, *Dnmt3a*-mutant recipients had elevated extracellular BM S100A8 and S100A9 compared to controls (**Figure 1B**). To identify the putative cellular source, we analyzed published scRNA-seq data from mice transplanted with *Dnmt3a*-mutant or Fgd5-CreERT2 control BM cells.⁶ Independent of genotype, *S100a8* and *S100a9* were most abundant in neutrophil populations, particularly mature neutrophils and immature granulocytes (**Supplementary Figure 1A**). Comparing *Dnmt3a*-mutant versus control neutrophils and immature granulocytes, both transcripts were modestly but significantly increased (**Figure 1C**). As S100A8/A9 proteins accumulate intracellularly, a modest but sustained transcriptional increase could result in a more substantial increase in protein level. We assessed intracellular protein in *Dnmt3a*-mutant and Mx1-Cre control mice and observed increased S100A8/A9 in both *Dnmt3a*-mutant neutrophils (CD11b⁺ Gr-1^{hi}) and immature granulocytes (CD11b⁺ Gr-1^{int}) (**Figure 1D-E**, gating strategy shown in **Supplementary Figure 1B**) with no difference in population frequencies (**Supplementary Figure 1C**). These data show that the *Dnmt3a* mutation increases S100A8/A9 expression in neutrophil populations.

We established an *in vitro* culture system to investigate the consequences of S100A8/A9 production by *Dnmt3a*-mutant cells on inflammatory cytokine levels. Hematopoietic progenitor cells (c-Kit⁺) were isolated from steady-state control or *Dnmt3a*-mutant Mx1-Cre mice and cultured in conditions enabling progenitor cell differentiation for 7 days (**Figure 2A**). These cultures produced neutrophils (CD11b⁺ Gr-1^{hi}) and immature granulocytes (CD11b⁺ Gr-1^{int}) at similar proportions across genotypes (**Figure 2B**). Analysis of the conditioned media (CM) from these cultures revealed increased S100A8 and S100A9 produced by *Dnmt3a*-mutant cells (**Figure 2C**), demonstrating that production can be modeled *in vitro*. S100A9 levels in the CM was significantly positively correlated with the levels of TNF α ($r = 0.9229$, $p = 0.0011$), IL-6 ($r = 0.9428$, $p = 0.0005$) and IL-1 α ($r = 0.7068$, $p = 0.0500$) (**Figure 2D**). To evaluate if S100A8/A9 drives inflammatory cytokine production, we cultured hematopoietic progenitor cells (c-Kit⁺) from control or *Dnmt3a*-mutant mice in the presence or absence of the S100A8/A9 inhibitor tasquinimod (**Figure 2E**). Tasquinimod significantly reduced TNF α and IL-6 production by *Dnmt3a*-mutant cells but did not alter IL-1 α (**Figure 2F**), consistent with the weaker association between S100A9 and IL-1 α levels. These data support that S100A8/A9 signaling selectively elevates inflammatory factors like TNF α and IL-6.

To investigate whether S100A8/A9 signals directly to hematopoietic stem and progenitor cells (HSPCs) or indirectly through inflammatory cytokine production, we examined transcript expression of S100A8/A9 receptors (*Tlr4* and *Cd14*) and TNF α receptors (*Tnfrsf1a* and *Tnfrsf1b*) using our scRNA-seq data.⁶ IL-6 ligand/receptor transcripts were too low for robust analysis. *Dnmt3a*-mutant HSC/MPP showed substantially higher expression of *Tnfrsf1a* and *Tnfrsf1b* than *Tlr4* and *Cd14* (**Supplementary Figure 2A**). Additionally, TLR4 surface levels were lower on HSPCs relative to mature myeloid cells (**Supplementary Figure 2B**), suggesting that HSPCs are better equipped to respond to TNF α than to S100A8/A9 directly. As established tools to computationally assess ligand-receptor interactions (CellPhoneDB, CellChat) do not model S100A8/A9-TLR4/CD14 interactions, we instead calculated a descriptive interaction score (the product of ligand- and receptor-detection frequency, validated in wild-type cells (**Supplementary Figure 2C**)). This analysis identified macrophages, mesenchymal stromal cells and monocyte/neutrophil progenitors as the likely receivers of the neutrophil-produced S100A8/A9 (**Supplementary Figure 2D**) and immature neutrophils, neutrophils and monocyte progenitors as plausible sources of TNF α (**Supplementary Figure 2E**). Together, these data support a model in which S100A8/A9 from *Dnmt3a*-mutant neutrophils engages TLR4/CD14 signaling on myeloid populations to stimulate TNF α production, which then confers a selective advantage to *Dnmt3a*-mutant HSPCs. This is consistent with our prior finding that TNF α -TNFR1 signaling directly supports *Dnmt3a*-mutant HSCs.² However, our data cannot exclude a direct contribution of S100A8/A9 signaling to HSPC function, which warrants further study.

We next examined whether inhibition of S100A8/A9-driven inflammatory cytokine production impacts the competitive advantage of *Dnmt3a*-mutant HSPCs. In a competitive *ex vivo* co-culture assay, CD45.2⁺ control or *Dnmt3a*-mutant c-Kit⁺ progenitors were cultured with CD45.1⁺ wild-type competitor progenitors with or without tasquinimod for 7 days (**Figure 3A**, gating and starting HSPC proportions in **Supplementary Figure 2F-G**). *Dnmt3a*-mutant cells showed a competitive advantage over wild-type, with an increased proportion of total CD45.2⁺ cells (**Figure 3B**). This competitive advantage was unaffected by tasquinimod, as were neutrophil or immature granulocyte proportions (**Supplementary Figure 2H**). In contrast, the proportion of *Dnmt3a*-mutant HSPCs, elevated relative to control, was significantly reduced by tasquinimod (**Figure 3C**). To assess effects *in vivo*, we treated competitive transplant recipients with tasquinimod in their drinking water for 2 weeks or 10 weeks (**Figure 3D**, gating in **Supplementary Figure 3A**). With short-term (2-week) treatment, tasquinimod reduced peripheral blood macrophage and monocyte frequencies in both control and *Dnmt3a*-mutant recipients (**Supplementary Figure 3B**), confirming *in vivo* activity.⁷ In the BM, *Dnmt3a*-mutant cells showed higher chimerism than control, and this was significantly reduced by tasquinimod without affecting control cells (**Figure 3E**). *Dnmt3a*-mutant BM also contained a higher proportion and number of HSCs (Lin⁻ Sca-1⁺ c-Kit⁺ Flt3⁻ CD150⁺ CD48⁻), which was significantly reduced by tasquinimod (**Figure 3F-G**), with no effect on wild-type HSC numbers (**Supplementary Figure 3C**). Prolonged (10-week) tasquinimod treatment caused severe dermatitis requiring early sacrifice, with attrition in both tasquinimod-treated groups (2/5 each; **Supplementary Figure 3D**). This toxicity has not been previously reported. Surviving mice at 10 weeks showed similar directional changes with expanded *Dnmt3a*-mutant HSCs trending toward reduction with tasquinimod (**Figure 3H-I**). To corroborate these findings with an independent molecule, we treated competitive transplant recipients with the S100A8/A9-specific antibody AEC7A or IgG2a-LALAPG isotype control^{8,9} monthly for 3 months (**Figure 3J**). While total BM chimerism was unaffected by AEC7A (**Figure 3K**), the elevated HSC proportion and HSC number in *Dnmt3a*-mutant BM were significantly reduced by AEC7A treatment (**Figure 3L-M**). There was no effect of AEC7A on wild-type

HSC numbers (**Supplementary Figure 3E**). Together, these data demonstrate that S100A8/A9 supports the competitive advantage of *Dnmt3a*-mutant HSCs in the BM.

To assess whether elevated S100A8/A9 is specific to the *Dnmt3a*-R878H (human *DNMT3A*-R882H) hotspot or generalizable across CH, we analyzed independent public datasets. *S100a8* was upregulated in neutrophils in *Dnmt3a*-knockout, *Tet2*-knockout, *Asx1*-mutant and *Jak2*-mutant mice¹⁰⁻¹², and *S100a9* was upregulated in neutrophils and/or monocytes in *Dnmt3a*- and *Tet2*-knockout mice. In human CH datasets, *S100A8* and *S100A9* were upregulated in *DNMT3A*-mutant monocytes and in *TET2*-mutant monocytes (**Supplementary Figure 3F**), indicating that S100A8/A9 induction extends beyond the *DNMT3A* hotspot and is conserved across species. Notably, targeting the S100A8/A9 alarmin axis is already advancing clinically in related myeloid diseases. Tasquinimod is in a Phase I/II trial for myelofibrosis (HOVON 172, NCT06605586)¹³, with preclinical support in MDS and AML models.¹⁴ Our data extend this treatment rationale to the pre-malignant setting, suggesting that disrupting S100A8/A9-driven inflammation at the CH stage may suppress mutant HSC expansion before overt disease develops.

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Figure Legends

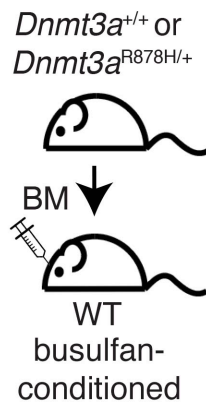
Figure 1. *Dnmt3a*-mutant hematopoiesis is associated with increased neutrophil expression of S100A8/A9. (A) Schematic of busulfan-conditioned transplant experimental design. (B) Concentration of S100A8 and S100A9 in BM extracellular fluid of control or *Dnmt3a*^{R878H/+} transplant mice. **P* < 0.05 by Mann-Whitney test (n = 6 control, n = 8 *Dnmt3a*^{R878H/+} biological replicates). (C) Violin plots of log₂(CPM+1) expression values for *S100a8* (top) and *S100a9* (bottom) in neutrophils (left) and immature granulocytes (right) in control or *Dnmt3a*^{R878H/+} transplanted mice analyzed from published single cell RNA-seq data.⁶ **P* < 0.05 by unpaired *t* test with Welch's correction (cells isolated from n = 4 biological replicate mice per genotype). (D,E) Quantification (D) and representative histograms (E) of intracellular flow cytometric analysis of S100A8/A9 in BM populations in steady-state control and *Dnmt3a*^{R878H/+} mice. Neut; neutrophils (CD11b⁺ Gr1^{hi}), Imm Gran; immature granulocytes (CD11b⁺ Gr1^{int}), Mono; monocytes (CD11b⁺ Gr1^{lo}), B cells (B220⁺ CD11b⁻), T cells (CD3e⁺ B220⁻ CD11b⁻), Mast cells (FCeR1a⁺ B220⁻ CD11b⁻). **P* < 0.05 by two-way ANOVA with Sidak's multiple comparisons test (n = 5 control, n = 4 *Dnmt3a*^{R878H/+} biological replicate mice).

Figure 2. S100A8/A9 promotes inflammatory cytokine production ex vivo. (A) Schematic of conditioned media (CM) experimental design. (B) Proportion of neutrophils (CD11b⁺ Gr1^{hi}) and immature granulocytes (CD11b⁺ Gr1^{int}) after 7-day culture of c-Kit⁺ hematopoietic progenitors from control or *Dnmt3a*^{R878H/+} mice. Not significant by two-way ANOVA with uncorrected Fisher's LSD (n = 4 biological replicate mice per group). (C) Concentration of S100A8 and S100A9 in CM collected from 7-day cultures. **P* < 0.05, ** *P* < 0.01 by unpaired *t* tests (n = 4 biological replicate mice per group). (D) Correlation between concentration of TNFα (left), IL-6 (center) or IL-1α (right) with the concentration of S100A9 in CM collected from 7-day cultures. Significance values calculated by two-tailed Pearson correlation (n = 4 biological replicate mice per group). (E) Schematic of culture experiment in which control or *Dnmt3a*^{R878H/+} c-Kit⁺ hematopoietic cells were cultured for 7 days in the presence or absence of tasquinimod followed by collection of CM. (F) Concentration of TNFα (left), IL-6 (center) or IL-1α (right) in CM. ***P* < 0.01 by Kruskal-Wallis test with uncorrected Dunn's test (n = 6 control vehicle, n = 10 control tasquinimod, n = 6 *Dnmt3a*^{R878H/+} vehicle, n = 10 *Dnmt3a*^{R878H/+} tasquinimod).

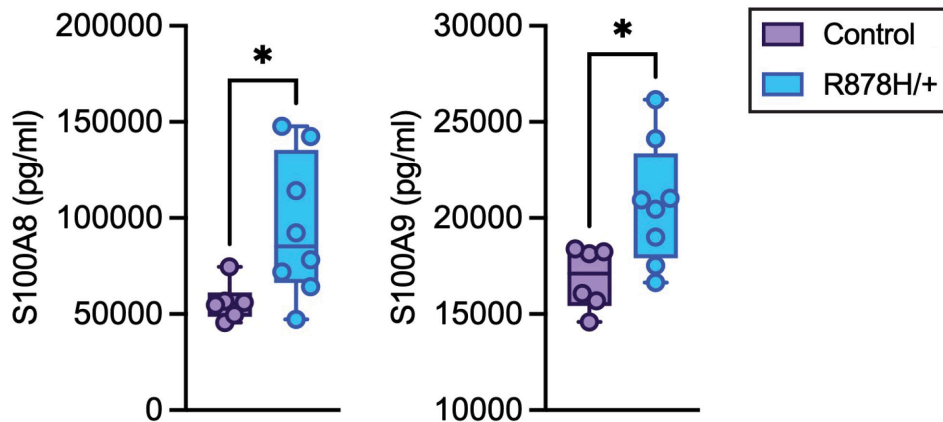
Figure 3. Inhibition of S100A8/A9 signaling reduces *Dnmt3a*-mutant HSC expansion. (A) Schematic of competitive co-culture assay in which CD45.2⁺ control or *Dnmt3a*^{R878H/+} c-Kit⁺ hematopoietic cells were cultured with CD45.1⁺ competitor cells in the presence or absence of tasquinimod for 7 days. (B-C) Frequency of CD45.2⁺ cells (B) and phenotypic HSCs (Lin⁻ Sca-1⁺ c-Kit⁺) in CD45.2⁺ cells (C) after 7-day co-culture with or without tasquinimod. **P* < 0.05, *****P* < 0.0001 by Brown-Forsythe and Welch's ANOVA with unpaired *t* test (n = 7 control vehicle, n = 8 control tasquinimod, n = 8 *Dnmt3a*^{R878H/+} vehicle, n = 7 *Dnmt3a*^{R878H/+} tasquinimod). (D) Schematic of competitive transplant experiment with tasquinimod provided ad libitum in the drinking water (30mg/kg/day). (E-G) Frequency of donor (CD45.2⁺) cells (E), frequency of donor-derived HSCs (Lin⁻ Sca-1⁺ c-Kit⁺ Flt3⁻ CD150⁺ CD48⁻) (F) and total number of donor-derived HSCs (G) in the BM of recipient mice receiving control or tasquinimod-supplemented water for 2 weeks. **P* < 0.05, ***P* < 0.01 by Kruskal-Wallis test with uncorrected Dunn's test (n = 10 control vehicle, n = 10 control tasquinimod, n = 8 *Dnmt3a*^{R878H/+} vehicle, n = 10 *Dnmt3a*^{R878H/+} tasquinimod). (H-I) Frequency of donor (CD45.2⁺) cells (H) and donor-derived HSCs (I) in the BM of recipient mice receiving control or tasquinimod-supplemented water for 10 weeks. **P* < 0.05 by Kruskal-Wallis test with

uncorrected Dunn's test (n = 5 control vehicle, n = 3 control tasquinimod, n = 5 *Dnmt3a*^{R878H/+} vehicle, n = 3 *Dnmt3a*^{R878H/+} tasquinimod). **(J)** Schematic of competitive transplant experiment with AEC7A or IgG2a-LALAPG control administered at 100µg/mouse once per month for 3 months. **(K-M)** Frequency of donor (CD45.2⁺) cells **(K)**, frequency of donor-derived HSCs **(L)** and total number of donor-derived HSCs **(M)** in the BM of recipient mice receiving IgG or AEC7A treatment for 12 weeks. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by Kruskal-Wallis test with uncorrected Dunn's test (n = 4 control +IgG, n = 4 control +AEC7A, n = 4 *Dnmt3a*^{R878H/+} +IgG, n = 5 *Dnmt3a*^{R878H/+} +AEC7A).

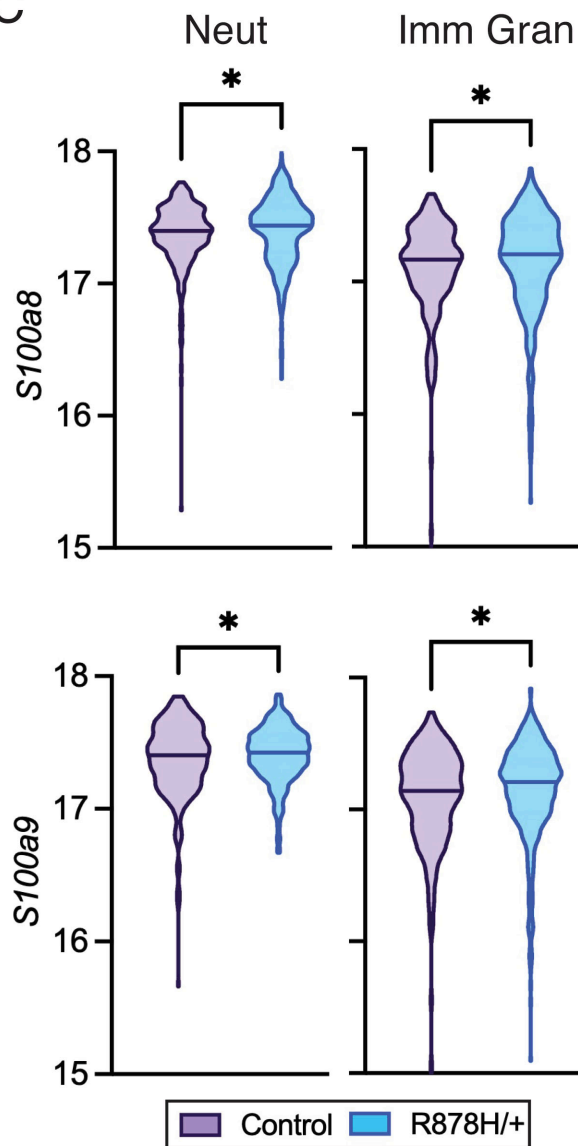
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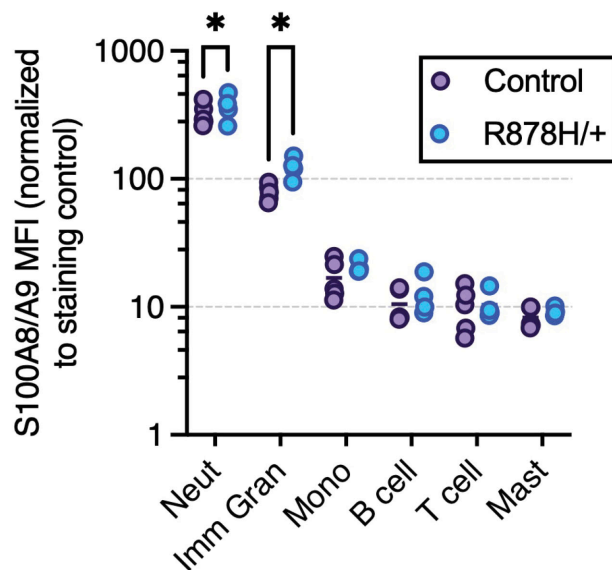
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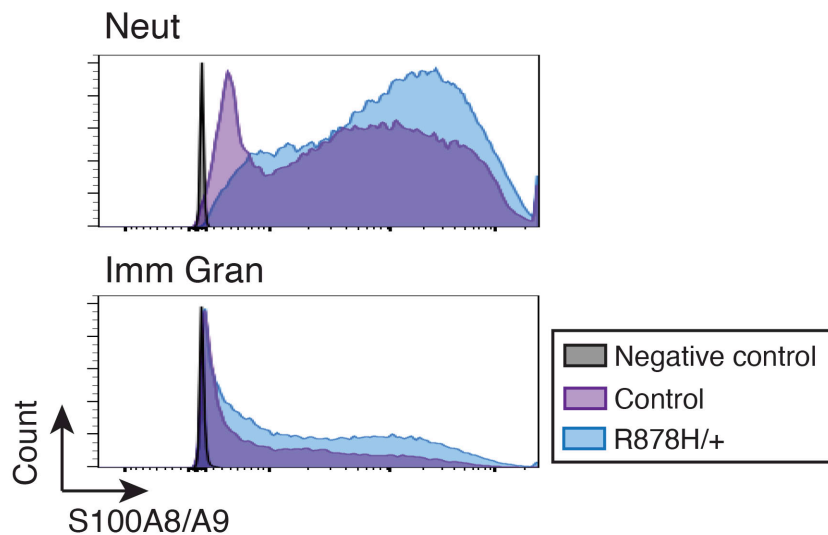
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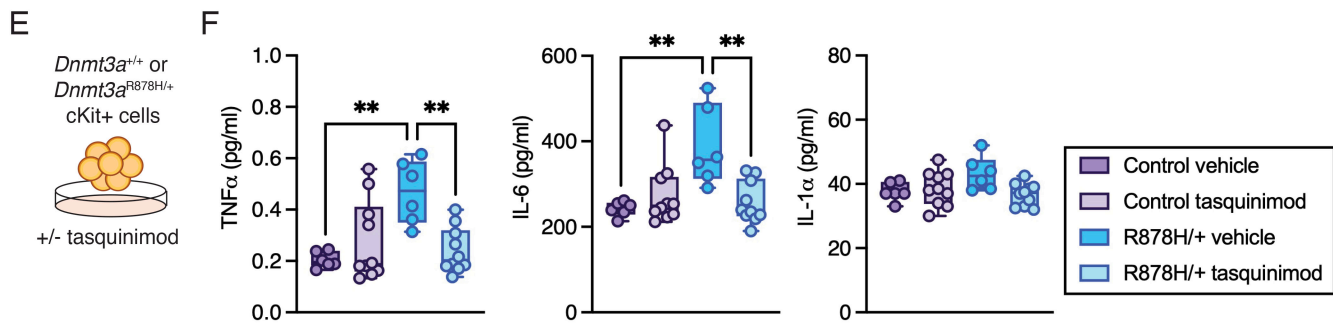
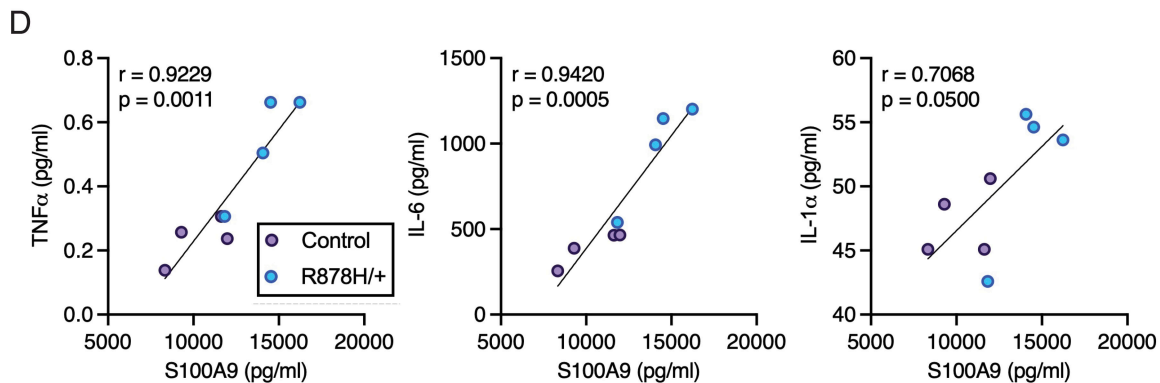
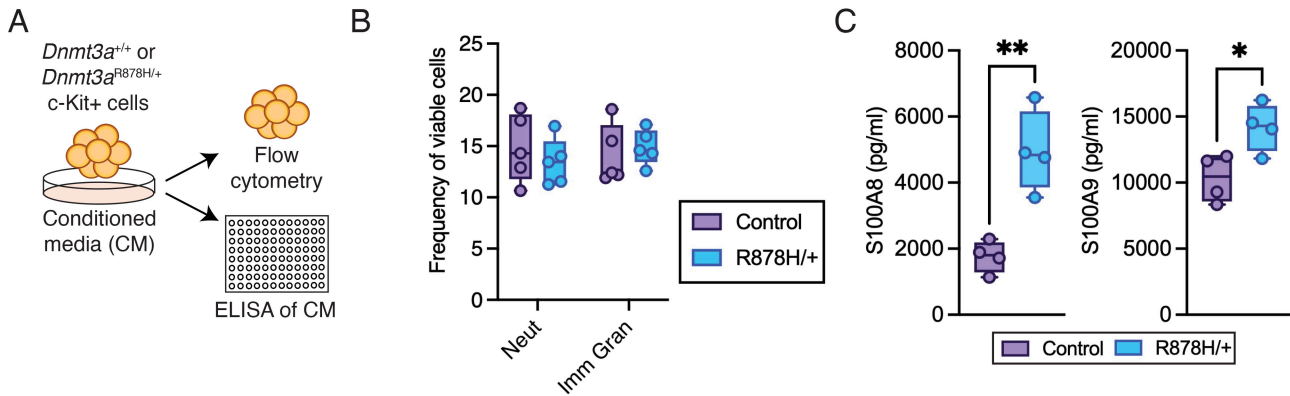
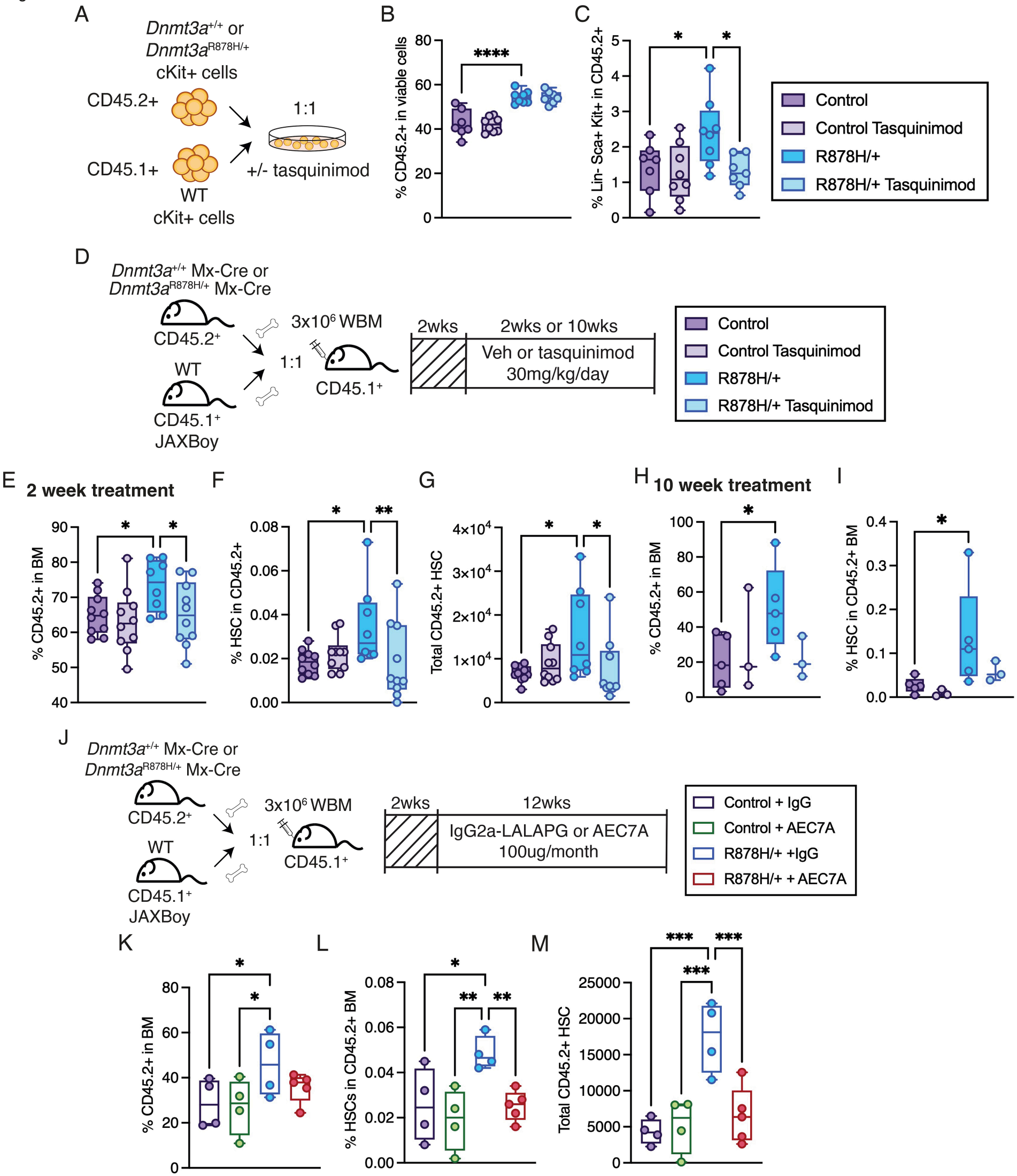
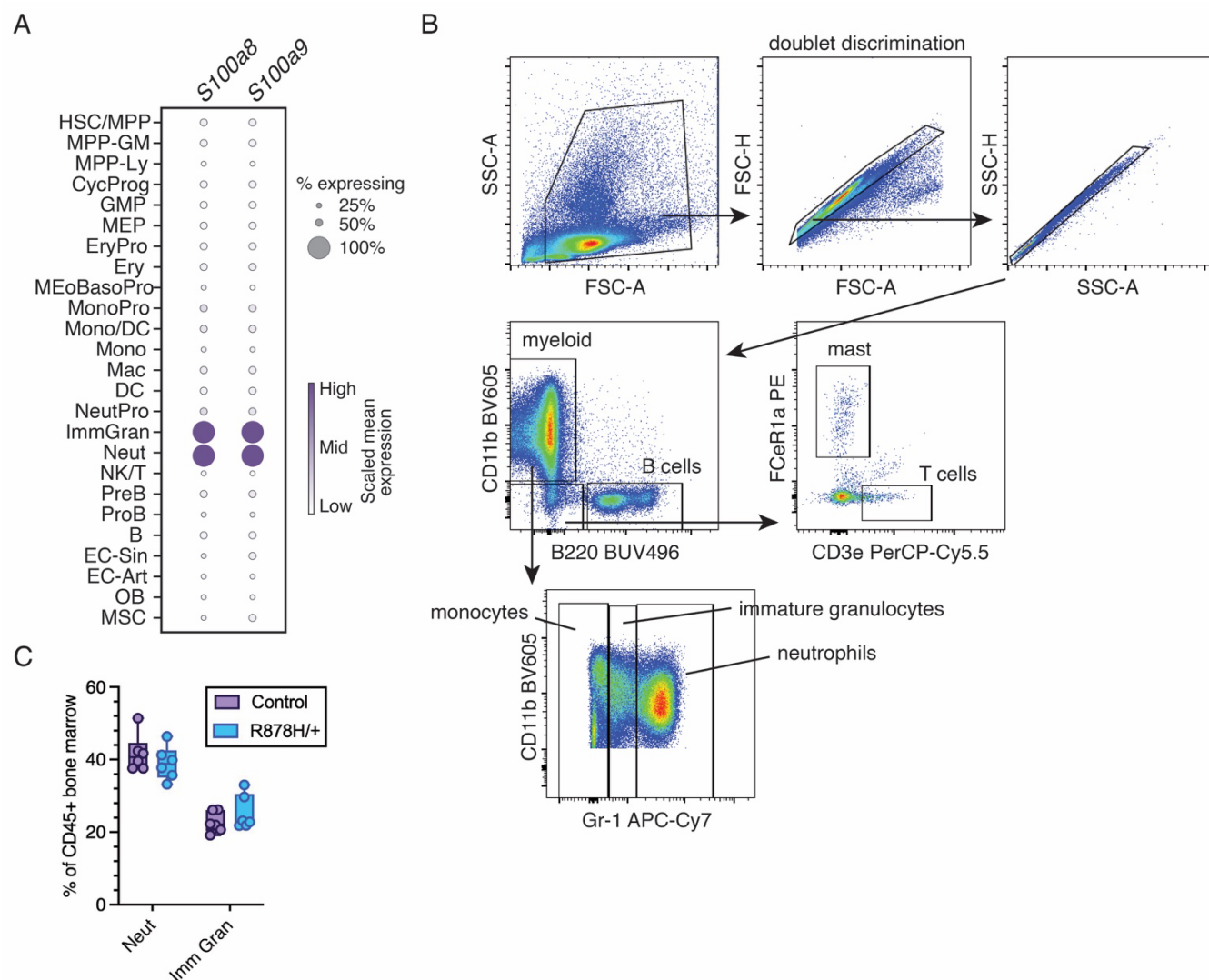
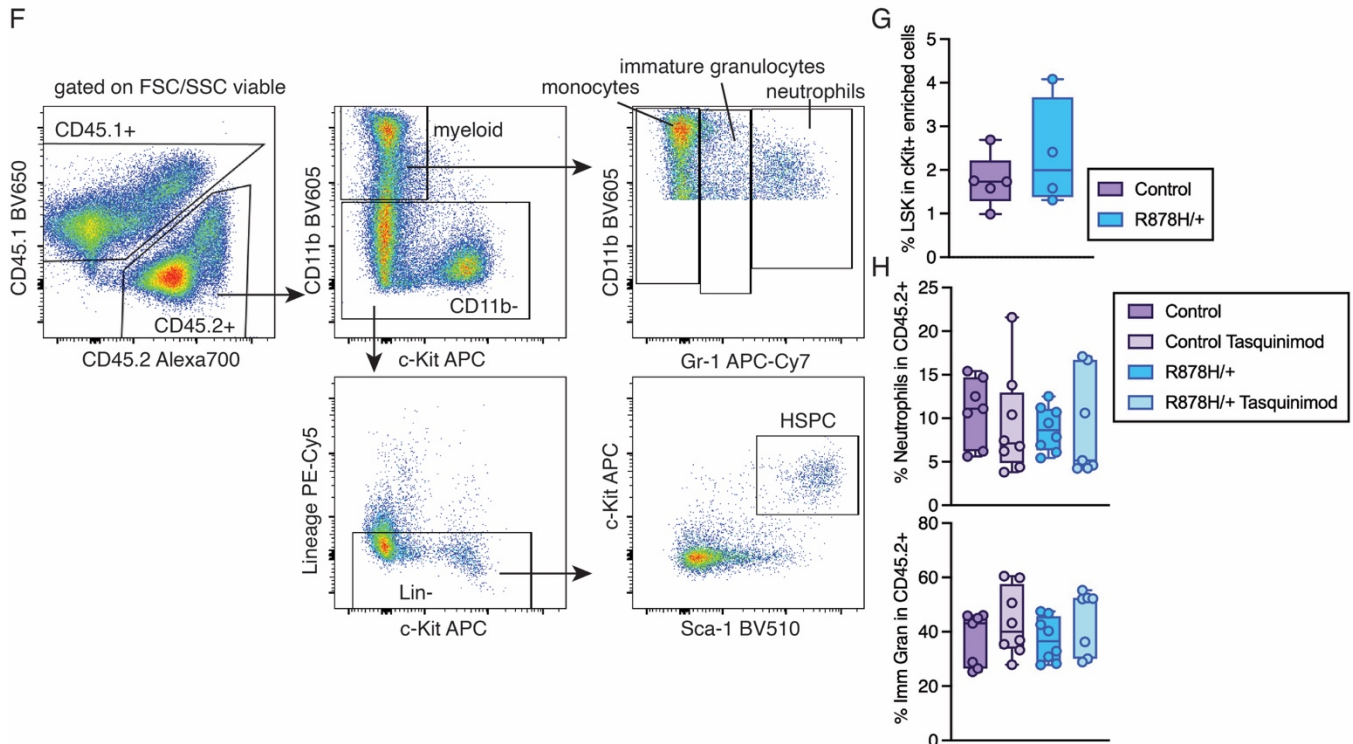
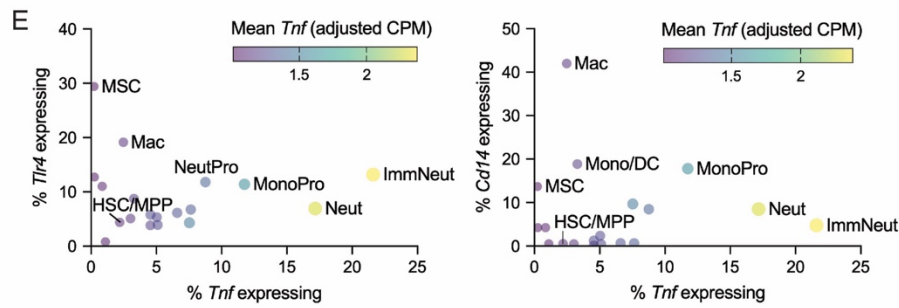
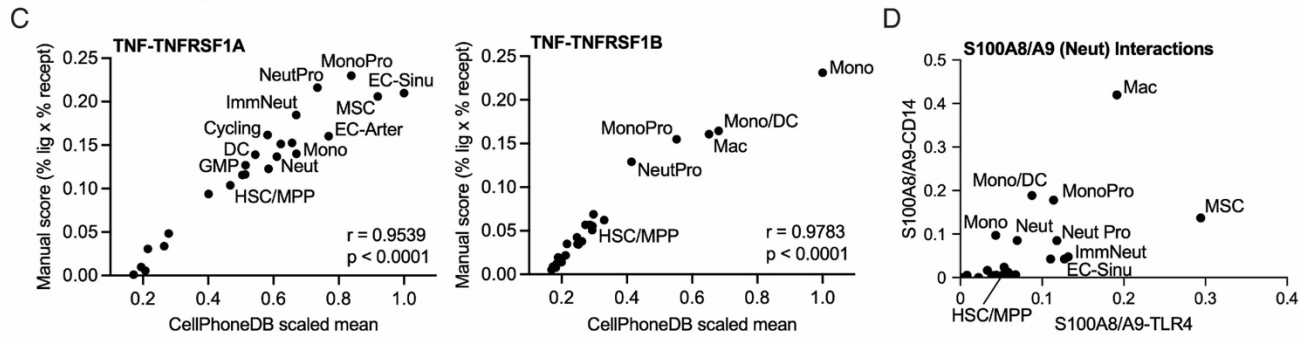
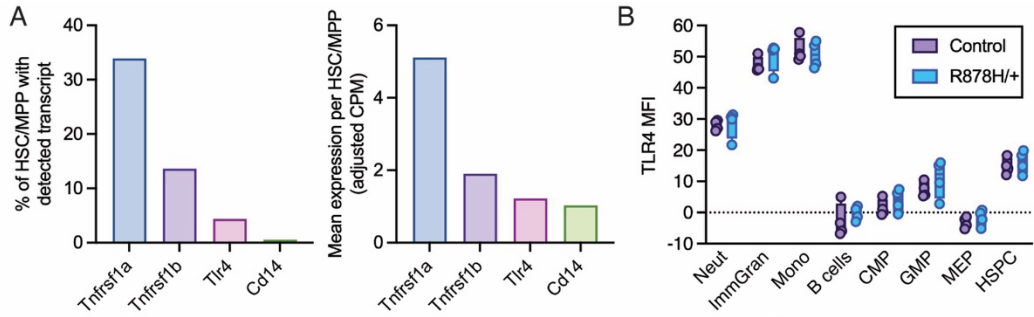


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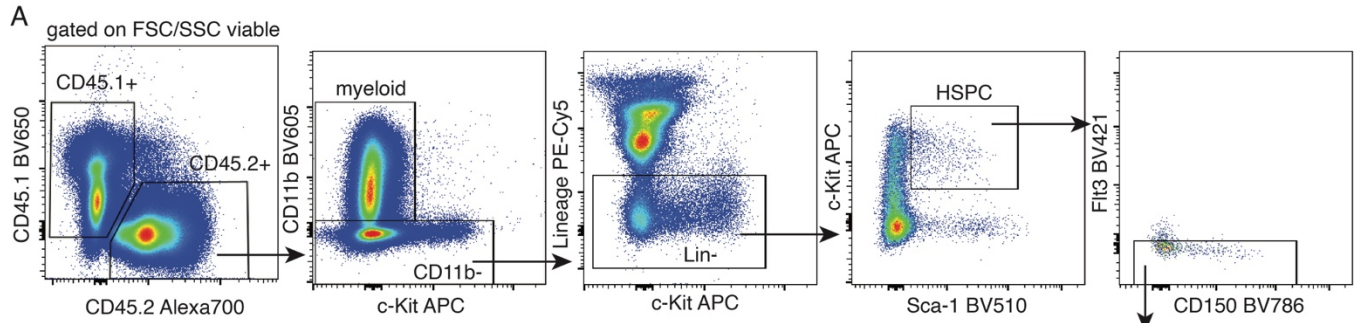




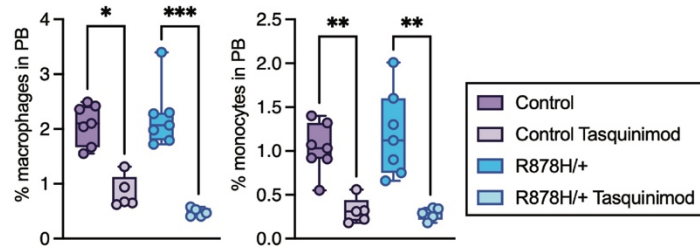
Supplementary Figure 1. Additional analysis of expression data and representative cellular proportions from control mice. (A) Single-cell RNA-seq (scRNA-seq) analysis of *S100a8* and *S100a9* across control (Mx1-Cre) only hematopoietic and non-hematopoietic cell populations derived from busulfan-conditioned transplant (n = 4 individual biological replicate mice per genotype). Reanalysis of data generated in Mistry et al.⁶. Circle size represents frequency of population in which transcript was detected, color intensity represents scaled mean expression. HSC/MPP: hematopoietic stem and multipotent progenitor cells, MPP-GM: granulocyte-macrophage-primed multipotent progenitor cells, MPP-Ly: lymphoid-primed multipotent progenitor cells, CycProg: cycling progenitor cells, GMP: granulocyte-macrophage progenitor cells, MEP: megakaryocyte-erythroid progenitor cells, EryPro: erythroid progenitor cells, Ery: erythroblasts, MEoBasoPro: mast-eosinophil-basophil progenitor cells, MonoPro: monocyte progenitor cells, Mono/DC: monocytes and dendritic cells, Mono: monocytes, Mac: macrophages, DC: dendritic cells, NeutPro: neutrophil progenitor cells, ImmGran: immature granulocytes, Neut: neutrophils, NK/T: natural killer and T cells, PreB: pre-B cells, ProB: pro-B cells, B: B cells, EC-Sin: sinusoidal endothelial cells, EC-Art: arteriolar endothelial cells, OB: osteoblasts, MSC: mesenchymal stromal cells. **(B)** Representative gating strategy for fixed BM cells to evaluate intracellular S100A8/A9 staining. **(C)** Flow cytometric quantification of neutrophil (CD11b⁺ Gr1^{hi}) and immature granulocyte (CD11b⁺ Gr1^{int}) frequencies in the BM of steady-state control or *Dnmt3a*^{R878H/+} mice. Not significant by two-way ANOVA with uncorrected Fisher's LSD (n = 6 mice per genotype).



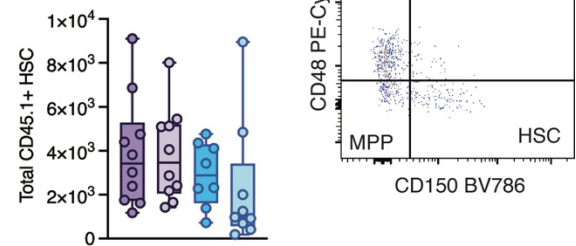
Supplementary Figure 2. Predictive modeling of S100A8/A9 signaling response and ex vivo tasquinimod culture phenotypes. (A) Frequency of transcript detection (left) and mean per-cell expression level (right) of *Tnfrsf1a*, *Tnfrsf1b*, *Tlr4* and *Cd14* in *Dnmt3a*^{R878H/+} HSC/MPP re-analyzed from scRNA-seq data.⁶ (B) Flow cytometric quantification of TLR4 mean fluorescence intensity (MFI) across BM hematopoietic populations in control and *Dnmt3a*^{R878H/+} mice. Not significant by 2-way ANOVA with Sidak's multiple comparisons test (n = 4 per genotype). (C) Scatter plots comparing the manual interaction score (product of the % of ligand-expressing cells and the % of receptor-expressing cells) against the corresponding CellPhoneDB scale mean for TNF-TNFRSF1A (left) and TNF-TNFRSF1B (right) across all control cell populations re-analyzed from scRNA-seq data.⁶ (D) Manual interaction scores for S100A8/A9-TLR4/CD14 from 'sender' neutrophils across all 'receiver' cell populations re-analyzed from scRNA-seq data.⁶ (E) Scatter plots showing the percentage of cells expressing *Tlr4* (left) or *Cd14* (right) versus the percentage of cells expressing *Tnf*. Color indicates mean *Tnf* expression level (adjusted counts per million, CPM). Re-analysis of data described in Mistry et al.⁶ (C-E) Each dot represents one cell population. Labels were excluded for low-scoring populations clustering together due to space constraints. (F) Representative gating strategy for analysis after 7-day ex vivo co-culture. (G) Frequency of HSPCs (Lin⁻ Sca-1⁺ c-Kit⁺; LSK) in cKit⁺-enriched starting BM cells that were seeded in 7-day ex vivo co-culture. Not significant by unpaired t test (n = 5 control, n = 4 *Dnmt3a*^{R878H/+}). (H) Frequency of neutrophils (CD11b⁺ Gr-1^{hi}) and immature granulocytes (CD11b⁺ Gr-1^{int}) in CD45.2⁺ cells after 7-day ex vivo co-culture with or without tasquinimod. Not significant by Brown-Forsythe and Welch's ANOVA with unpaired t test (n = 7 control vehicle, n = 8 control tasquinimod, n = 8 *Dnmt3a*^{R878H/+} vehicle, n = 7 *Dnmt3a*^{R878H/+} tasquinimod).



B 2 week treatment



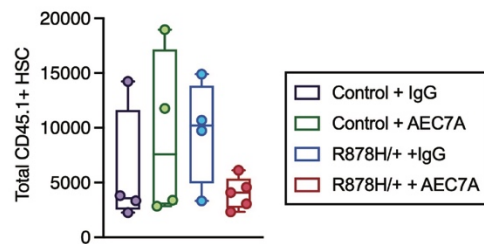
C 2 week treatment



D

Treatment Group	Enrolled	Survived to 10 wk	Attrition (cause)
Control vehicle	5	5 (100%)	None
Control tasquinimod	5	3 (60%)	2/5 (dermatitis)
<i>Dnmt3a</i> -mutant vehicle	5	5 (100%)	None
<i>Dnmt3a</i> -mutant tasquinimod	5	3 (60%)	2/5 (dermatitis)

E 12 week treatment



F

Reference	CH genotype	Cell type / comparison design	S100A8	S100A9	Species/system
Abplanalp et al. ¹	DNMT3A	Classical monocytes; within-patient, mutant vs. WT cells (n=5)	Up DNMT3A (log2FC ~0.5, p<0.001) See Fig. 2f in reference article	Up DNMT3A (log2FC ~0.55, p<0.001) See Fig. 2f in reference article	Human (primary patient samples)
Heimlich et al. ²	TET2, DNMT3A	Classical monocytes; between-group (CH n=17 vs. non-CH n=7)	Up TET2 (log2FC 0.04-0.95, p<0.001) DNMT3A (log2FC 0.16-0.68, p<0.001)	Up TET2 (log2FC 0.17-0.61, p<0.001) DNMT3A (log2FC 0.25-0.53, p<0.001)	Human (primary patient samples)
Huerga Encabo et al. ³	TET2	CD14+ monocytes, paired (n=3), unstimulated	Trend Up TET2 (log2FC 0.79, ns)	Trend Up TET2 (log2FC 0.84, ns)	Human cells (CRISPR-edited HSPC)
Mohammed Ismail et al. ⁴	TET2, DNMT3A	PBMC monocytes; mutation-resolved, TET2-CH (n=3), DNMT3A-CH (n=2) (VAF 6–8%)	Trend Up TET2 (log2FC 1.15, ns) DNMT3A (log2FC 0.15, ns)	Trend Up TET2 (log2FC 1.17, ns) DNMT3A (log2FC 0.35, ns)	Human (primary patient samples)

Supplementary Figure 3. *In vivo* tasquinimod and AEC7A phenotypes and re-analysis of publicly available human CH datasets. (A) Representative gating strategy for BM analysis of transplanted mice. (B) Frequency of macrophages (CD11b⁺ Ly6G⁻ Ly6C⁻ F4/80⁺) (left) and monocytes (CD11b⁺ Ly6G⁻ Ly6C⁻ F4/80⁻) (right) in the PB of mice receiving vehicle or tasquinimod-supplemented water for 2 weeks. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by Kruskal-Wallis test with uncorrected Dunn's test (n = 7 control vehicle, n = 5 control tasquinimod, n = 7 *Dnmt3a*^{R878H/+} vehicle, n = 5 *Dnmt3a*^{R878H/+} tasquinimod). (C) Total number of CD45.1⁺ recipient wild-type HSCs in mice after 2 weeks treatment of tasquinimod or vehicle control. Not significant by Kruskal-Wallis test with uncorrected Dunn's test (n = 10 control vehicle, n = 10 control tasquinimod, n = 8 *Dnmt3a*^{R878H/+} vehicle, n = 10 *Dnmt3a*^{R878H/+} tasquinimod). (D) Full accounting of mice enrolled in 10-week tasquinimod *in vivo* transplant experiment. Twenty mice (5 per arm) were enrolled across four treatment groups. Attrition occurred only in tasquinimod-treated groups. All deaths were attributed to euthanasia for dermatitis. (E) Total number of CD45.1⁺ recipient wild-type HSCs in mice after 12 weeks treatment of AEC7A or IgG control. Not significant by Kruskal-Wallis test with uncorrected Dunn's test (n = 4 control IgG, n = 4 control AEC7A, n = 4 *Dnmt3a*^{R878H/+} IgG, n = 5 *Dnmt3a*^{R878H/+} AEC7A). (F) Reanalysis of published human CH datasets to evaluate S100A8/A9 expression. Datasets analyzed from: ¹Abplanalp WT, Schuhmacher B, Cremer S, et al. Cell-intrinsic effects of clonal hematopoiesis in heart failure. *Nat Cardiovasc Res* 2023;2(9):819-834; ²Heimlich JB, Bhat P, Parker AC, et al. Multiomic profiling of human clonal hematopoiesis reveals genotype and cell-specific inflammatory pathway activation. *Blood Adv* 2024;8(14):3665-3678; ³Huerga Encabo H, D'Agostino G, Sturgess K, et al. Human TET2-mutant clonal hematopoiesis expansion is driven by distinct inflammatory signaling responses in stem cells versus myeloid progeny. *Blood Cancer Discov* 2026;7(2):287-305; ⁴Mohammed Ismail W, Fernandez JA, Binder M, et al. Single-cell multiomics reveal divergent effects of DNMT3A- and TET2-mutant clonal hematopoiesis in inflammatory response. *Blood Adv* 2025;9(2):402-416. ns indicates not significant.