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Mitochondrial genetics cooperate with nuclear genetics to selectively alter immune cell development/trafficking

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ABSTRACT

The nuclear genome drives differences in immune cell populations and differentiation potentials, in part regulated by changes in metabolism. Despite this connection, the role of mitochondrial DNA (mtDNA) polymorphisms (SNP) in this process has not been examined. Using mitochondrial nuclear exchange (MNX) mice, we and others have shown that mtDNA strongly influences varying aspects of cell biology and disease. Based upon an established connection between mitochondria and immune cell polarization, we hypothesized that mtDNA SNP alter immune cell development, trafficking, and/or differentiation. Innate and adaptive immune cell populations were isolated and characterizated from the peritoneum and spleen. While most differences between mouse strains are regulated by nuclear DNA (nDNA), there are selective changes that are mediated by mtDNA differences (e.g., macrophage (CD11c) differentiation), These findings highlight how nuclear-mitochondrial crosstalk may alter pathology and physiology via regulation of specific components of the immune system.

1. Introduction

Therapeutic strategies are becoming increasingly personalized, driving a need for an ever greater knowledge of the underlying biology of the individual. Our previous results using mitochondrial nuclear exchange (MNX) mice identified mitochondrial DNA single nucleotide polymorphisms (mtDNA SNP) as quantitative trait loci (QTL) that, in conjunction with the nDNA, regulate cancer progression and metastatic efficiency and susceptibility [1–3]; atherosclerosis and cardiac failure [4]; and selectively alter nDNA methylation and gene expression patterns [5]. How mtDNA SNP impact the underlying biology surrounding complex disease mechanisms and homeostasis is an important question in defining future therapeutic strategies [6].

The above-referenced metastasis and tumorigenicity data were obtained by crossing transgenic mice with female MNX mice (to ensure transfer of mtDNA to progeny). However, we also recognized that any observed phenotypic changes could be a result of altered mtDNA in stromal cells. To isolate the stroma as an experimental variable, nuclear DNA-matched syngeneic tumor cells were injected into MNX mice (i.e., only the stroma had different mtDNA), and we observed profound changes in lung colonization [54]. Briefly, whenever C57BL/6J mtDNA was present in stromal cells, metastasis efficiency was lower. In contrast, C3H/HeN mtDNA was correlated with increased metastasis. In this study, we therefore tested the hypothesis that mtDNA alters components of the immune system at baseline as an explanation for the observed stroma-correlated changes in cancer metastatic efficiency.

Inbred mouse strains have provided important tools for the identification of genetic influences on cancer progression and metastatic susceptibility [7]. Murine genetic diversity is reflected in overall immune development [8] and functionality [9]. Petkova et al. from Jackson laboratories observed large variability in immune profiles across seven different mouse strain lineages representing different haplogroups [10]. They observed the greatest variation in circulating lymphocytes. Haplogroup 1 (BALB/cJ and C3H/HeJ) had low lymphocyte percentages (36–78%), while Haplogroup 4 (C57BL/6J) had the highest percentages (68–80%). B cells were the predominant driver of these differences, with CD8 and CD4 T cells and NK cell variances being more subtle [10]. B cell percentages in C3H/HeJ mice

Abbreviations: CC, C57BL/6J-mt$^{MNX(C57BL/6J)}$; CH, C57BL/6J-mt$^{MNX(C3H/HeN)}$; HC, C3H/HeN-mt$^{MNX(C57BL/6J)}$; HC, C3H/HeN-mt$^{MNX(C3H/HeN)}$; FBS, Fetal Bovine Serum; LPM, Large Peritoneal Macrophage; mtDNA, Mitochondrial DNA; MNX, Mitochondrial Nuclear Exchange; NK, Natural Killer; nDNA, Nuclear DNA; ANOVA, One Way Analysis of Variance; PBS, Phosphate Buffered Saline; SNP, Polymorphisms; QTL, Quantitative Trait Loci; SPM, Small Peritoneal Macrophage; T_{eff}, T effector; T_{cm}, T central memory; T_{em}, T effector memory

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(35.1%–42.9%) and C57BL/6J (68.1%–82.0%) were at opposite ends of the spectrum, leading us to question potential mechanisms behind these differences, and the underlying genetics at play.

The potential that mitochondrial genetics contribute to immune profiles is supported by observations that metabolism is closely linked to immune differentiation and polarization [11]. T cell activation is largely dependent on the relative ratio of glycolysis and oxidative phosphorylation [12–14]. For example, upon antigen recognition by the T cell receptor, T cells undergo a metabolic switch from oxidative phosphorylation to glycolysis to facilitate the requirements necessary for expansion [15–17]. Furthermore, the ability of $CD8^+$ memory T cells to engage fatty acid oxidation and maintain spare respiratory capacity is critical for their formation, which has important implications for tumor recurrence [18–20]. Likewise, T cell populations infiltrating infections vary by mouse strain [21,22].

Taken together, accumulating data suggest that complex genetic mechanisms regulate baseline immune system composition as well as function. Changes in immune profiles and corresponding differential immune responses could be mediated, in part, by mt-SNP which, in turn, contribute to the phenotypic differences observed in MNX mice. This hypothesis was then translated into three corollaries: (1) mt-SNP regulate broad immune cell populations (e.g., B cells, T cells, macrophages, etc.); (2) mt-SNP alter localization of immune cell populations (e.g., spleen vs. peritoneum); and, (3) mt-SNP alter polarization/differentiation of immune (sub)populations.

The MNX mouse model affords a unique opportunity to test some of these non-mutally exclusive hypotheses, isolating contributions of the mtDNA as an experimental variable. Immune cells were harvested from two rich immune environments – spleen and peritoneum – which differ in prevalence of innate and adaptive immune components.

2. Materials and methods

2.1. Mitochondrial Nuclear Exchange (MNX)

Mitochondrial Nuclear Exchange (MNX) stable mouse lines were created as previously reported [4,23] (Table 1:). Wild-type C57BL/6J and C3H/HeN mice were purchased from Jackson laboratories and Envigo laboratories, respectively, and WT breeding colonies were maintained. MNX counterparts were generated and breeding colonies maintained. All animal studies were approved by the Institutional Animal Care and Use Committee at the university of Kansas Medical Center. Tissues were collected from all of the strains at age 1–2 months. In addition, we attempted to diminish any role for additional factors to further focus on the role of mtDNA. The first being sex. Male and female mice were collected, as sex hormones have previously been demonstrated to impact immune cell development with estrogen supporting immune enhancement, and testosterone appearing to play more of a suppressive role [24,25]. Circadian rhythm [26–28] microbiome [11], and age [29] can greatly affect the immune system. All mice were age-matched (1–2 months), euthanized at the beginning of the light cycle (always within the first three hours after the lights have been turned on (12 h on-off cycle) (ZT0–3; 7–10 am)) to avoid changes in immune localization based on circadian rhythm, and collected and analyzed from multiple litters and multiple parents to normalize potential microbiome fluctuations as well as to mitigate inter-individual heterogeneity. Splenocytes were also collected from 6 month old mice to determine age dependent changes.

Table 1
Wild-type (WT) and MNX models utilized for this study.

Mouse strain	nDNA	mtDNA	Abbreviation
C57BL/6J-$mt^{MNX(C57BL/6J)}$	C57BL/6J	C57BL/6J	CC
C57BL/6J-$mt^{MNX(C3H/HeN)}$	C57BL/6J	C3H/HeN	CH
C3H/HeN-$mt^{MNX(C3H/HeN)}$	C3H/HeN	C3H/HeN	HH
C3H/HeN-$mt^{MNX(C57BL/6J)}$	C3H/HeN	C57BL/6J	HC

2.2. Isolation of splenocytes

Splenocytes were harvested from wild-type and MNX mice and placed in phosphate-buffered saline (GIBCO) containing 3% FBS (Atlanta Biologicals). Spleens were ruptured; red blood cells lysed for 3 min; filtered through a 70 μm filter and then counted.

2.3. Isolation of peritoneal exudate

For isolation of peritoneal exudate, 5 mL of ice cold PBS containing 3% FBS solution was injected into the peritoneum of a euthanized mouse. The peritoneum was then gently massaged to release cells into the buffer. Cells were then extracted using a polyethylene transfer pipet. Cells were passed through a 70 μm filter and counted.

2.4. Flow cytometry

Cells were collected for flow cytometric analysis (Spleen = 2×10^6; Peritoneum = 1×10^6). Cells were stained with a Live/Dead stain to exclude dead cells and Fc receptors blocked with TruStain FcX (anti-mouse CD16/32) (Biolegend). Cells were then stained with antibodies listed in Table S1, fixed with 1% paraformaldehyde overnight, and analyzed using the BD LSR II (Becton Dickinson). Data was analyzed using FlowJo (FlowJo LLC).

Antibody concentrations were titrated to reach optimal signal-to-noise ratio. Antibodies were then used to delineate populations from within the spleen or the peritoneum. Panels used to delineate cell populations within the spleen or peritoneum are outlined in Table S1.

2.5. Statistical analysis

Experiments included at least six mice per group in each of three independent replicates. Both male and female mice were used. Data were analyzed using SigmaPlot (Systat Software Inc.). Results were averaged ± SEM and p-values were generated using one-way analysis of variance (ANOVA) on ranks followed by multiple comparisons analyses to determine significance between MNX and wild-type (WT) groups. A p-value $< .05$ was considered significant.

3. Results and discussion

3.1. The nuclear genome regulates broad immune cell subclasses across mouse strain backgrounds

The immune system plays a large role in protection from diseases as well as being carefully balanced to prevent autoimmunity. Since humans have varying responses and susceptibilities to diseases [30], it is important to understand all of the contributing factors associated with differential pathogenesis. Genetic variation plays an important role in immune cell population percentages and functional activation [10,31]}. Importantly, these differences in disease susceptibilities have also recently been associated with quantitative trait loci across individuals, which further correlates with differences in gene expression [32]. To test whether mitochondrial genetics may impact immune cell localization, function or polarization, spleens and peritoneal fluid were collected from wild-type (CC and HH) and MNX mice (CH and HC). Tissues were collected from 1 to 2 month old mice to identify immune population differences present in mice corresponding to the age when experimental metastasis assays were performed [54]. Throughout this paper, data are represented by white boxes (splenocytes) and grey boxes (peritoneum) to facilitate reader comparisons between figures. Using gating strategies focused on identifying primarily adaptive immunity (lymphoid) within the spleen and innate immunity (myeloid)

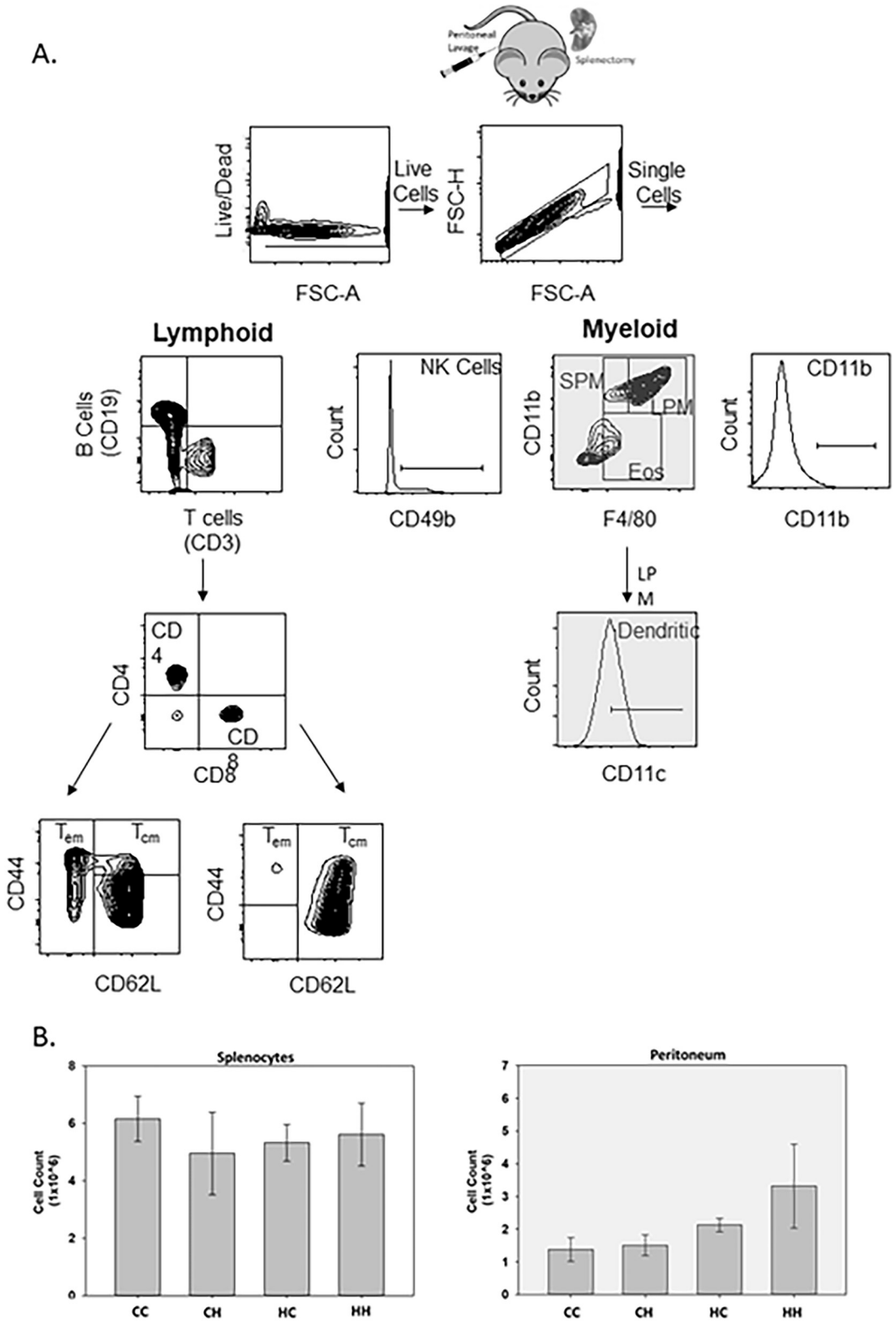


Fig. 1. Splenocyte and peritoneal cell counts were not significantly different in MNX mice. A. Spleen and peritoneal gating strategies. Dead cells were excluded using either ghost red (peritoneum) or zombie violet (spleen) viability dye in conjunction with forward scatter-area (FSC-A) to also exclude debris. Live, single cells (FSC-A/FSC-height (FSC-H)) were then gated on granulocyte and lymphocyte populations based on size and granularity (FSC-A/side scatter-area (SSC-A)). Lymphoid and myeloid populations were next analyzed for selected populations using the indicated differentiation markers. B cells ($CD19^+/CD3^-$), T cells ($CD3^+/CD19^-$), Helper T cells ($CD3^+$,$CD4^+$,$CD8^-$), Cytotoxic T cells ($CD3^+$,$CD4^-$,$CD8^+$). $T_{eff/em}$ = T effector/effector memory cell ($CD44^+/CD62L^-$) and T_{cm} = T central memory cell ($CD44^+/CD62L^+$). SPM = Small Peritoneal Macrophages ($CD11b^+/F4/80^{Low}$), LPM = Large Peritoneal Macrophages ($CD11b^+/F4/80^{High}$), Eos = Eosinophils/Mast Cells ($CD11b^{Low}/F4/80^{Low}$). Throughout this manuscript myeloid populations will be signified by the grayed boxes and lymphoid populations signified by the white boxes. B. Cells were counted after red blood cell lysis and isolation into single cell suspensions. Average cell counts based on two independent experiments. Spleen (n = 12) and peritoneum (n = 10–13). Cells (2×10^6) were stained for splenocyte flow analysis, and 1×10^6 cells were stained for peritoneum flow analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

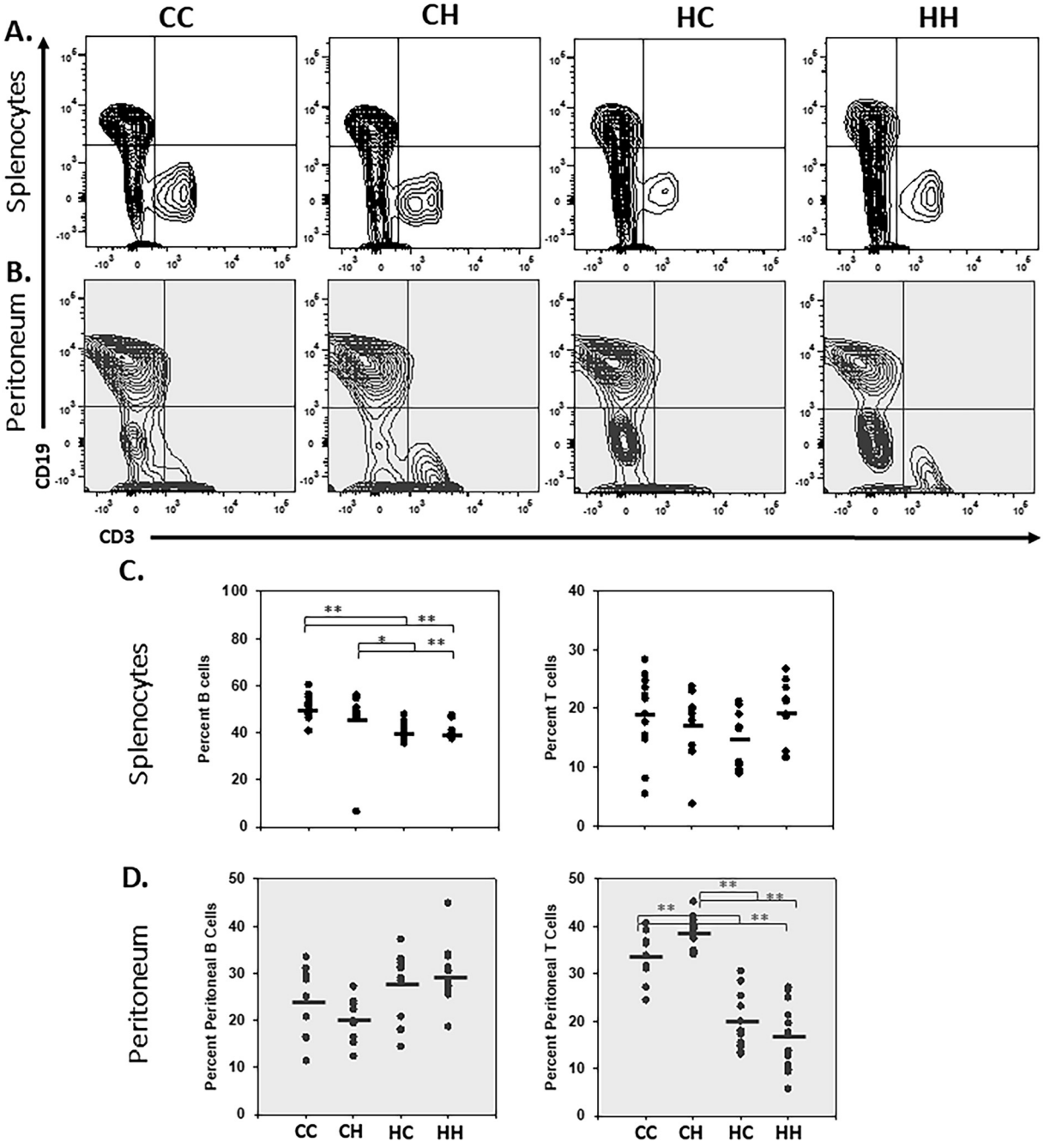


Fig. 2. Nuclear haplotypes alter resident splenic and peritoneal lymphoid populations. A. Cells isolated from the spleen and peritoneum (of 1–2-month-old male and female mice) were analyzed by flow cytometry. Cells gated on the lymphoid population were characterized for expression of CD19 and CD3. CD19 positive populations were gated to identify B cells and CD3 positive populations were gated to identify T cells and representative plots are depicted B. Vertical point scatter plots for T- (CD3 +) and B-cell (CD19 +) populations in MNX and WT mice. Horizontal lines represent the mean (spleen; n = 12, and peritoneum n = 10–13). One-way ANOVA and multiple comparisons *t*-test (Dunn's post-test); *, $P < .05$, **, $P < .005$)). Similar data collected at 6-months are shown in Figs. S3 and S5.

within the peritoneum (Fig. 1A), cell numbers obtained from spleens were similar across all strains (Fig. 1B). However, a slight increase was observed in the number of cells obtained from the peritoneum with C3H nuclear DNA (HC and HH) (Fig. 1B). From these studies we identified nuclear- as well as mitochondria-driven effects (Table 2). As expected, some populations including $CD4^+$, and $CD8^+$ T cells remained unchanged between wild-type and MNX backgrounds (Table 2, Figs. 2 & S1), while others (see below) were different.

To analyze the populations in more detail, we first identified the most prominent populations in the spleen and peritoneum [33,34]. B cells within the spleen are 40–50% of splenocytes. We observed nuclear regulation with B cells accounting for 51% and 47% of CC and CH splenic lymphocytes, but only 40% and 41% for HH and HC splenocytes ($p < .05$) (Fig. 2A & C). In addition $CD11b^+/F4/80^+$ large peritoneal macrophages are the predominant population within the peritoneum along with $CD11b^{low}/F4/80^{low}$ eosinophils, which also exhibit strong nuclear regulation (Table 2). These results suggest that major immune population differences observed across mouse strain backgrounds are mostly driven by the nuclear genome. These findings are expected as many reports have highlighted a role for regulation of immune cells based on the nuclear genome [35]. In addition, based on the smaller size of the mitochondrial genome we predict that the mt-SNP act as

Table 2
Immune populations change based on nuclear and mitochondrial DNA. Immune populations are organized based on regulation patterns observed amongst WT and MNX strains.

No difference		nDNA Mediated		mtDNA Mediated	
Spleen	Peritoneum	Spleen	Peritoneum	Spleen	Peritoneum
$CD3^+$ (T cells)	$CD19^+$ (B cells)	$CD19^+$ (B cells)	$CD11b^{Low}$, $F480^{Low}$ (Eosinophils)	$CD3^+$; $CD8^+$; $CD44^+$; $CD62L^-$ (CD8 Effector Memory/ Effector T cells)	$CD11c^+$ Macrophages
$CD3^+$; $CD8^+$ (Cytotoxic T cells)	CD11b+, $F480^{low}$ (Small Peritoneal Macrophages)	$CD3^+$; $CD4^+$; $CD44^+$; $CD62L^-$ (CD4 Effector Memory)	$CD3^+$ (T cells)	$CD11b^+$	
$CD3^+$; $CD4^+$ (Helper T cells)	$CD11b^+$, $F480^{High}$ (Large Peritoneal Macrophages)	$CD3^+$; $CD4^+$; $CD44^+$; $CD62L^-$ (CD4 Central Memory)			
$CD3^+$; $CD8^+$; $CD44^+$; $CD62L^-$ (CD8 Central Memory)					
$CD49b^+$ (NK Cells)					

quantitative trait loci (QTL). Quantitative traits are phenotypes that are impinged upon by multiple genes. Immune functionality is clearly polygenic. Therefore, we next hypothesized that mt-SNP may act to fine tune polarization differences amongst broader immune cell populations.

3.2. mtDNA alters basal levels of $T_{effector}$/$T_{effector\ memory}$ populations

Mitochondrial metabolism is an important regulator of immune cell polarization. T cells require a shift between oxidative phosphorylation and glycolysis for effector and memory cell polarization [12–14]. Also, mutations that disrupt the electron transport chain result in reduced T memory cell polarization [9]. These findings led us to hypothesize that more subtle changes imparted by mt-SNP would have a greater impact on polarization potential especially in regards to populations that exhibit dynamic fluidity between polarization states [36].

$CD8^+$ $T_{effector}$/ $T_{effector\ memory}$ ($T_{eff/em}$) cells ($CD44^+$ / $CD62L^-$) make up a low percentage of CD8 T cells due to a lack of activating stimuli. Despite this, HH mice still exhibit a higher percentage of $CD44^+$/$CD62L^-$ $CD8^+$ T cells in comparison to the other backgrounds [CC 2%, CH 2%, HC 2%, HH 4%] ($P < .001$; HH vs CC) (Fig. 3A & C). No mitochondrial or nuclear regulation was observed for $CD8^+$ T central memory (T_{cm}) cells ($CD44^+$ / $CD62L^+$) [CC 11%, CH 13%, HC 6%, HH 11%] (Fig. 3A & C).

$CD4^+$ $T_{eff/em}$ cell populations do not appear to exhibit mtDNA-mediated differences, but rather exhibited stronger nuclear regulation [CC 7%, CH 8%, HC 13%, HH 12%] (Fig. 3B & D). $CD4^+$ T_{cm} cells ($CD44^+$ / $CD62L^+$) also exhibited primarily nuclear regulation with higher percentages observed with the C3H nuclear background [CC 7%, CH 7%, HC 10%, HH 9%] ($P < .001$; CC/CH vs HC; $P < .05$; CH vs HH) (Fig. 3B & D).

As T_{cm} and $T_{eff/em}$ populations change and develop over time, we next analyzed splenocyte immune populations at 6 months of age. Analysis of $CD19^+$ B cells and $CD8^+$ T cells exhibited nuclear regulation, whereas no significant differences were observed for total or $CD4^+$ T cells (Fig.S2). We next examined CD4 and CD8 $T_{eff/em}$ and T_{cm} differentiation. Whereas no significant difference was observed for CD4 T cell subsets, significant nuclear regulation was observed for CD8 T_{cm} cells ([CC 16%, CH 19%, HC 9%, HH 10%] ($P < .001$; CH vs HC, CC/CH vs HH; $P < .05$; CC vs CH) (Fig. S3), which is consistent with what we observed in 1–2 month old mice (Fig. 3C). In addition, CD8 $T_{eff/em}$ cells exhibited a conserved trend towards mitochondrial regulation with CH mice exhibiting higher percentages of $T_{eff/em}$ populations compared to HH mice ([CC 3.5%, CH 4%, HC 5%, HH 5%] (Fig. S3); however, no statistically significant difference was observed.

To further verify that these differences were not sex-mediated, we analyzed the naive immune populations (1–2 months) for both males and females. Whereas subtle variations were observed, genomic haplotypes predominated over sex-mediated effects. Both nuclear as well as mitochondrial trends remained conserved in both male and female populations when analyzed individually (Figs. S4 & S5).

As T cell development varies with age [37], all analyses reported here focus on combined mice aged 4–6 weeks since that corresponds to previous reports for changes in metastatic behavior with MNX mice [54]. Additionally, both male and female mice were analyzed and no sexual-dimorphisms were observed. Importantly, our observed differences in $T_{eff/em}$ cell differentiation were not associated with increasing age or sex (Fig. S6).

An important factor for T cell differentiation is the mitochondrial spare respiratory capacity, which has previously been demonstrated to impact T_{cm} differentiation by providing the necessary survival capacities to become a long lived T_{cm} cell [15]. To further support a role for mt-SNP in regulating these differentiation potentials, our lab has previously shown that the spare respiratory capacity of PyMT cancer cells harboring C57BL/6J mtDNA is reduced in comparison to cancer cells harboring FVB/NJ or BALB/cJ mtDNA [1]. However, whereas full

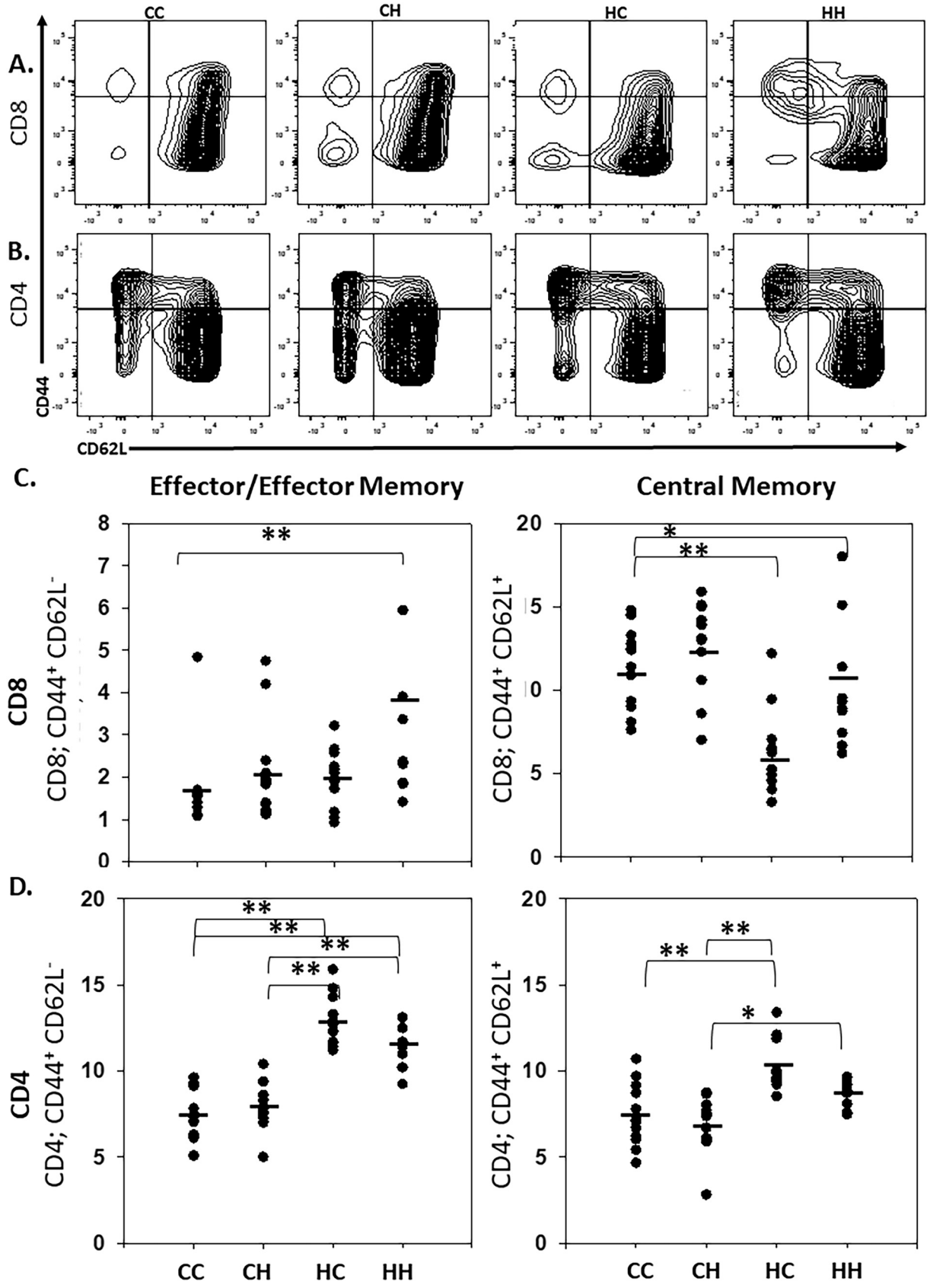


Fig. 3. Mitochondrial haplotypes alter splenic effector/effector memory CD4 and CD8 T cell populations. A/B. Cells isolated from the spleen (of 1–2-month-old male and female mice) were analyzed by flow cytometry. Central memory and effector/effector memory cells were then sub-gated on CD8 (A) and CD4 (B) positivity and analyzed for CD44 and CD62L. $CD44^+$, $CD62L^-$ populations represent T effector/T effector memory cells and $CD44^+$, $CD62L^+$ represent T central memory cells. C/D. Vertical point scatter plots for $CD8^+$ (C) and $CD4^+$ (D) effector/effector-memory and central memory T cell populations in MNX and WT mice. Horizontal lines represent the mean (spleen; n = 12). One-way ANOVA and multiple comparisons t-test (Tukey and Dunn's post-test); *, $P < .05$, **, $P < .005$)). Similar data collected at 6-months are shown in Figs. S4 and S6.

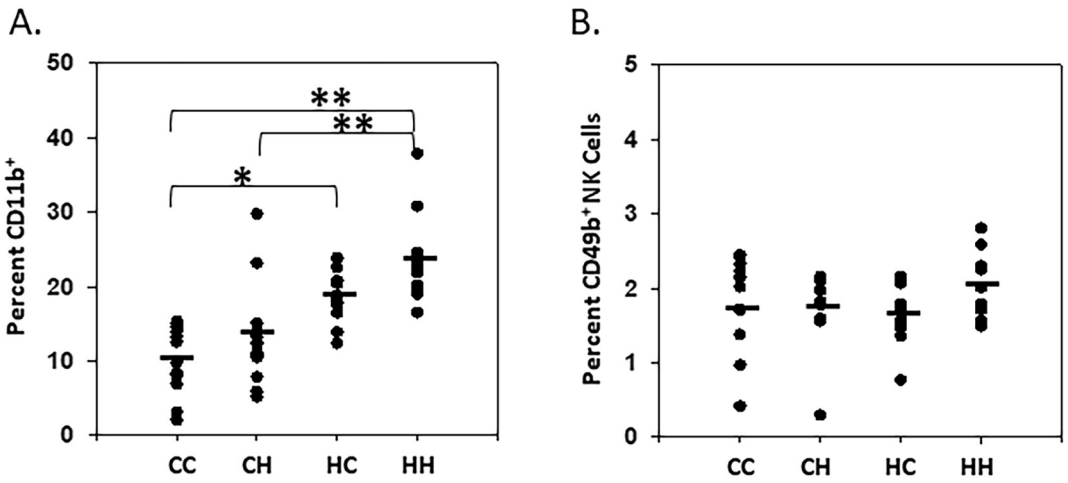


Fig. 4. mtDNA alters CD11b expression in splenocyte populations A/B. Cells isolated from the spleen (of 1–2-month-old male and female mice) were analyzed by flow cytometry. Splenocytes were analyzed for A. $CD11b^+$ myeloid cells and B. $CD49b^+$ Natural Killer cells (spleen; n = 12). One-way ANOVA and multiple comparisons t-test (Dunn's post-test); *, P < .05, **, P < .005)).

metabolomic analysis would be required on each individual cell type (and differentiation state) to further determine the role of mt-SNP in regulating T cell metabolism, it is intriguing to speculate that mt-SNP may impact T cell metabolism through subtle changes in oxidative phosphorylation capacities. Our data, therefore, provides preliminary evidence for a role in mtDNA regulating T cell polarization and/or population skewing. Furthermore, a role for mtDNA in altered polarization towards T_{cm} or $T_{eff/em}$ populations may provide a possible alternative explanation for varying rates of immunotherapy response.

3.3. mtDNA alters CD11b expression in splenocyte populations

We next analyzed myeloid populations within the spleen. CD11b is an integrin commonly associated with innate immune activation [38] and expressed by a large proportion of myeloid populations [39]. Analysis of CD11b expression identified a correlation between CD11b expressing cells and mtDNA [CC 10%, CH 13%, HC 19%, HH 23%] (Fig. 4a). Natural Killer (NK) cells are commonly associated with cancer cell elimination within experimental metastasis models [40–42]; so, we next analyzed splenocyte CD49b expression, but found no significant baseline differences based on strain variation (Fig. 4b). Having observed a trend towards an increase in mtDNA regulation of the $CD11b^+$ populations within the spleen, we next focused on myeloid population differentiation and recruitment in the myeloid rich environment of the peritoneum.

3.4. mtDNA alters CD11c expression in large peritoneal macrophage populations

Similar to T cell activation, activation of other immune cell types requires a switch from a resting state of oxidative phosphorylation to an active state that is more reliant on glycolysis [43]. Macrophages exhibit a high level of plasticity [36]. Previous reports within the peritoneum have highlighted a capacity for resident macrophage differentiation into dendritic cells based on increased levels of CD11c [44]. So-called large (LPM) and small peritoneal macrophages (SPM) are the predominant macrophage populations in the peritoneum. Both traffic from different locations – small from bone marrow and large from fetal macrophages [45]. LPM and SPM were analyzed based upon the gating protocol established by Ghosn et al., where macrophage populations were identified based on F4/80 and CD11b positivity [46]. At baseline, the majority of peritoneal macrophages are LPM based upon high levels of F4/80 (CC, 46%; CH, 46%; HC, 43%; HH, 48%) (Fig. 5). There was also strong nuclear regulation of $CD11b^{low}$ and $F4/80^{low}$ cells [CC 11%; CH 15%; HC 2%; HH 3% ($p \leq .001$)], which have previously been demonstrated to be predominantly eosinophils [22,47,48] (Fig. 5A & C).

Previous work from Ados et al. demonstrated that, at baseline, $CD11c^+$ is predominantly expressed in LPM [45], which led us to focus on CD11c dendritic cell skewing within this population. Analysis for positivity in LPM identified significant differences between wild-type HH and the three other strains CC, CH, and HC [CC 16%; CH 13%; HC 9%; HH 3% ($p \leq .001$)] (Fig. 5B & C). These data highlight a role for C57BL/6J mt-SNP in altering expression of CD11c and potential skewing of macrophage subpopulations.

Tissue resident macrophages exhibit unique epigenetic regulation based on the microenvironment [49]. Our previous work demonstrated that mt-SNP selectively alter nuclear transcription [5], suggesting that responses to microenvironmental signals may vary between strains. Consistent with this, BALB/c mice respond to infection with *Leishmania major* by producing a Th2 response; whereas, most other strains respond via Th1 [8]. These data further highlight how subtle genetic changes can alter how immune systems respond and/or develop.

While the mechanism(s) responsible for the differential recruitment and/or polarization are not yet fully elucidated, numerous possibilities exist. Based on given evidence CD11c expression may be regulated by differential nuclear-mitochondrial signaling which, in turn, alters cytokine/chemokine production within the peritoneal microenvironment. Together the result is population skewing and polarization. Whereas less evidence exists to suggest that the differential expression of CD11c is affecting the complement system, it is nonetheless intriguing to speculate upon this potential role. The complement system represents an ancient form of innate immunity that has evolved from a single cell origin [50]. As mitochondria are thought to have evolved from a single-celled bacterium [51], it is possible that they have retained the capacity to signal and regulate the complement system leading to the differences in CD11c expression observed.

4. Conclusion

This report is significant because it establishes, for the first time to our knowledge, that polymoprhisms in mtDNA are amongst the QTL for immune profiles and/or function. Mitochondrial genomes combine with nuclear genomes to affect the composition of immune cells within each tissue. Moreover, they also influence how the immune cells become activated and/or polarized.

Four polymorphisms have been reported in C57BL/6J and C3H/HeN mtDNA [6]. Briefly, NDIII has an A to C transversion at nucleotide 9461, COXIII has a G to A transition at nucleotide 9348 resulting in a

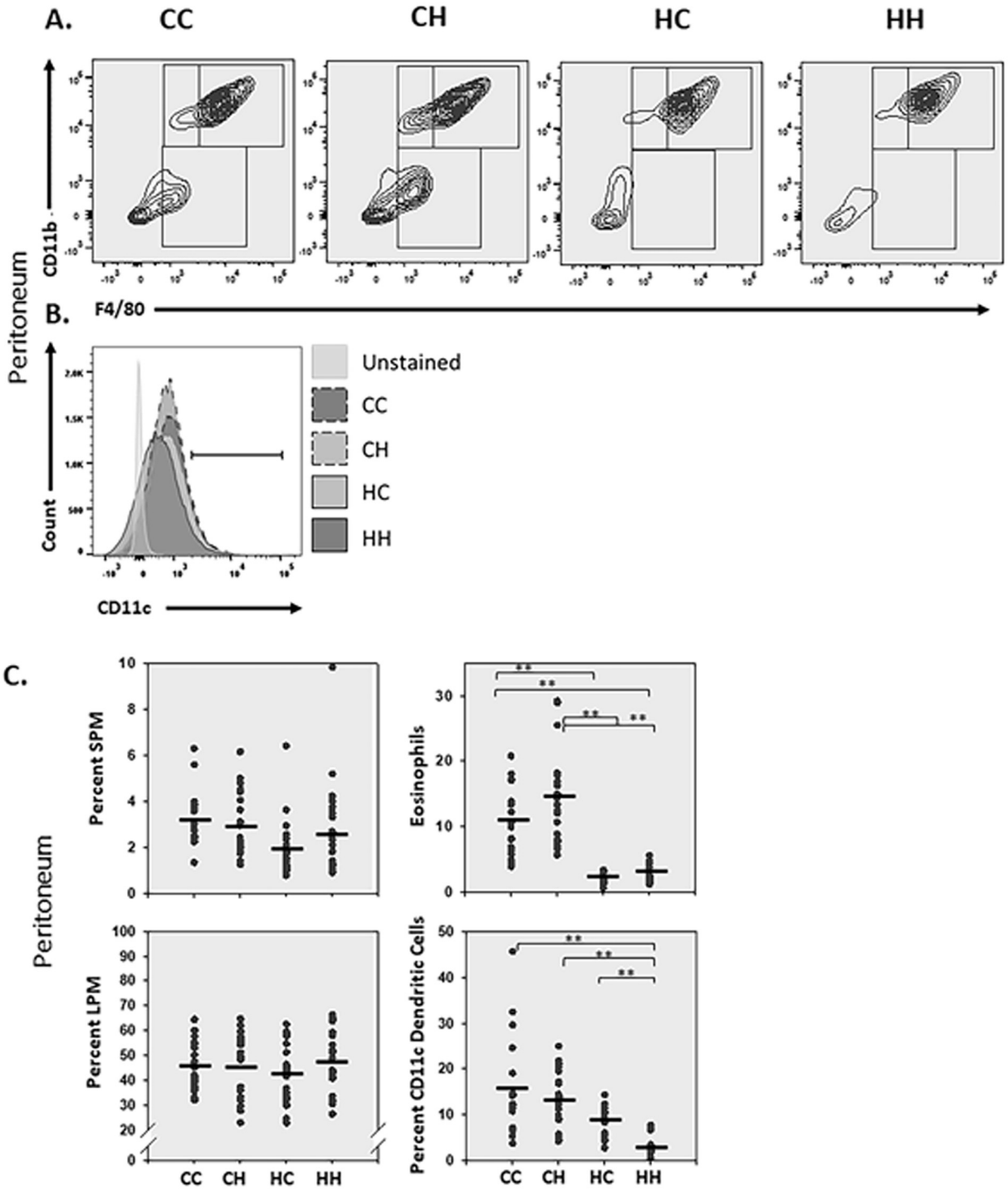


Fig. 5. Mitochondrial haplotypes alter resident peritoneal immune cell populations. A. Cells isolated from the peritoneal cavity (in 1–2-month-old male and female mice) were analyzed by flow cytometry. Cells of the myeloid lineage were gated on CD11b and F4/80. $CD11b^{high}$ and $F4/80^{+}$ cells represent large peritoneal macrophages (LPM), $CD11b^{high}$ and $F4/80^{low}$ represent small peritoneal macrophages (SPM), $CD11b^{low}$ and $F4/80^{low}$ represent eosinophils/mast cells. B. LPM were further analyzed for CD11c expression. C. Vertical point scatter plots for LPM, SPM and eosinophil/mast cell populations in MNX and WT mice. Black horizontal lines represent the mean (peritoneum $n = 18–23$). One-way ANOVA and multiple comparisons t-test ((Dunn's post-test); **, $P < .005$).

isoleucine to valine substitution, and two mutations are present in $tRNA^{ARG}$ in which C57 mitochondria have an adenine deletion at position 9821, while C3H mitochondria have an additional thymine located at 9820. At present, a definitive explanation why these polymorphic mutations would differentially affect immune profiles/function is not self-evident. COXIII mtDNA SNP status has a significant impact on mouse heart mitochondria Complex IV respiration and COX enzyme activity [4]. Interestingly, Tarasenko et al. found that cytochrome C oxidase can serve as a checkpoint following T cell activation regulating cell fate decisions [9], suggesting that COXIII SNP may impact immune activation/differentiation. Attempts to extrapolate the murine findings in this report to human mtDNA using MITOMAP [52,53] have not yet yielded perfect synteny, which precludes definitive cause-effect conclusions to be drawn. Additionally, since > 90% of mitochondrial proteins are nucleus-encoded, one must also take into account how the electron transport proteins interact with other

components.

Why are our findings relevant? First, mt-SNP, along with a handful of nDNA SNP can be used to distinguish races (clades or mouse strains). Changes in immune function could be part of the explanation why there are racial disparities with regard to disease susceptibility and/or progression, especially diseases with strong immune components. Second, response to cancer immunotherapy is heterogeneous as are side effects. Perhaps analysis of mt-QTL may provide a stronger foothold into better prediction of (non)responses or undesirable side effects. Third, mt-QTL affecting immune profiling and function may impinge upon the ability of patients to accept/reject tissue and/or bone marrow transplants. While these possibilities are speculative at this time, the size of the mitochondrial genome makes sequencing and testing of the hypotheses tractable.

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Transparency document

The Transparency document associated with this article can be found, in online version.

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