Sex differences in NK cells mediated by the X-linked epigenetic regulator UTX

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Abstract

Viral infection outcomes are sex-biased, with males generally more susceptible than females.

Paradoxically, the numbers of anti-viral natural killer (NK) cells are increased in males

compared to females. Using samples from mice and humans, we demonstrate that while

numbers of male NK cells are increased compared to females, they display impaired production

of the anti-viral cytokine IFN-γ. These sex differences were not due solely to divergent levels of

gonadal hormones, since these differences persisted in gonadectomized mice. Instead, these

differences can be attributed to lower male expression of X-linked *Kdm6a* (UTX), an epigenetic

regulator which escapes X inactivation in female NK cells. NK cell-specific UTX deletion in

females phenocopied multiple features of male NK cells, which include increased numbers and

reduced IFN-γ production. Integrative ATAC-seq and RNA-seq analysis revealed a critical role

for UTX in the regulation of chromatin accessibility and gene expression at loci important in NK

cell homeostasis and effector function. Consequently, NK cell-intrinsic UTX levels are critical for

optimal anti-viral immunity, since mice with NK cell-intrinsic UTX deficiency show increased

lethality to mouse cytomegalovirus (MCMV) challenge. Taken together, these data implicate

UTX as a critical molecular determinant of NK cell sex differences and suggest enhancing UTX

function as a new strategy to boost endogenous NK cell anti-viral responses.

Introduction

Evolutionarily conserved sex differences exist in both innate and adaptive immune responses[1,2].

While males are less susceptible to autoimmunity, they also mount a limited anti-viral immune

response compared to females[3]. For instance, males have a higher human cytomegalovirus

(HCMV) burden after infection, suggesting increased susceptibility to viral threats[4]. This has

also been recently illustrated with the COVID-19 pandemic, in which the strong male bias for

severe disease has been postulated to reflect sex differences in immune responses[5]. Multiple

studies in humans and mice have recently reported differences in immune cell distribution

and/or function in males vs. females[6-10]. However, the molecular basis for these differences, and

the mechanisms by which these differences influence disease outcomes, remain incompletely

understood.

Sex differences in mammals are defined not only by divergent gonadal hormones, but

also by sex chromosome dosage[1]. Expression of a subset of X-linked genes, for example, is

higher in females (XX) than males (XY). While females undergo random X chromosome

inactivation (XCI) to maintain similar levels of X-linked protein expression between sexes, XCI

is incomplete, with 3-7% of X chromosome genes escaping inactivation in mice and 20-30%

escaping inactivation in humans[11]. As such, differential levels of X-linked gene expression in

females vs. males have been linked to sex differences in a wide range of conditions including

neural tube defects[12] and autoimmune disease[13].

As circulating type 1 innate lymphocytes, NK cells serve as an early line of defense

against herpesvirus family members[14]. The importance of NK cells in anti-viral immunity is

illustrated in patients with defective NK cell numbers or functionality, who are highly susceptible

to infection by herpesviruses, such as HCMV and Epstein-Barr virus (EBV)[15,16]. Similarly, NK

cells are required for the control of mouse cytomegalovirus (MCMV) and other viral infections[17-

19], as mice with either genetic deficiency in NK cell function or loss of NK cell numbers have a

significant increase in viral titers and mortality following MCMV infection[20-25]. Thus, NK cells are

critical in anti-viral immunity across species.

87   Given this role for NK cells, it was therefore unexpected that NK cells are increased in

virus-susceptible males[6-10]. Beyond NK cell numbers, other previously unappreciated sexually

dimorphic NK cell feature(s) may instead account for sex differences with viral infections. Here,

we show that NK cells in males are simultaneously expanded in number and deficient in effector

function across mice and humans. These sex differences in NK cell composition and function

are not completely due to hormonal differences, since these differences persisted in

gonadectomized mice. Through expression screening, we identified the epigenetic regulator

UTX (encoded by gene *Kdm6a*) as an XCI escapee that is expressed at significantly lower

levels in male NK cells across mice and humans. Conditional ablation of UTX in female mouse

NK cells, which mimics lower UTX expression in male NK cells, recapitulated NK cell

phenotypes associated with male sex, such as increased NK cell numbers and lower production

of IFN-γ. Furthermore, parallel Assay for transposase-accessible chromatin using sequencing

(ATAC-seq) and bulk RNA sequencing (RNA-seq) of WT and UTX^{NKD} NK cells revealed a

critical role for UTX in regulating chromatin accessibility and transcription of gene clusters

involved in NK cell fitness (*Bcl2*) and effector response (*Ifng and Csf2).* Notably, UTX^{NKD} mice

had increased mortality in response to MCMV infection, suggesting a critical role for UTX in the

production of optimal anti-viral effector responses. Ultimately, our findings demonstrate that

divergent UTX levels underlie sex differences in NK cell homeostasis and effector function.

Enhancing UTX function may therefore represent a novel strategy for optimizing NK cell-

mediated anti-viral immunity.

Results

NK cells display sexually dimorphic phenotypes independent of gonadal sex hormones.

Due to the critical role of NK cells in anti-viral immunity and increased male susceptibility to cytomegalovirus (CMV) and other herpesvirus family infections[4], it was surprising that multiple studies have reported that human males display increased NK cell numbers[6-10]. A recent investigation examining spleens of C57BL/6 mice also reported increased numbers of NK cells in males vs. females[26]. Consistent with this, our data show that splenic NK cells are increased in frequency **(Fig. 1a,b)** and absolute numbers **(Fig. 1c)** in male C57BL/6 mice compared to females. These findings suggest that other sexually dimorphic features beyond NK cell numbers may account for increased male susceptibility to viral infections. In response to viral infection, NK cells are critical for early production of proinflammatory cytokines, particularly IFN-γ[27]. To test if sex differences exist in NK cell-intrinsic function, we compared effector cytokine production in NK cells from female vs. male mice *ex vivo*. Stimulation with IL-12 and IL-18, cytokines known to induce robust IFN-γ production by NK cells[28], resulted in lower IFN-γ production by male NK cells **(Fig. 1d-f)**. Similarly, stimulation of activated human NK cells (TCRβ⁻ CD3⁻ CD56⁺) isolated from peripheral blood mononuclear cells (PBMCs) with IL-12 and K562 leukemia cells resulted in lower %IFN-γ⁺ **(Fig. 1g)** and IFN-γ MFI **(Fig. 1h)** in male compared to female NK cells**.** Thus, while NK cell numbers are increased, male NK cell effector function is consistently reduced in both mice and humans.

Female or male sex is based on a composite of gonadal hormones (e.g., estrogens or androgens) and sex chromosomes (e.g., 46XX or 46XY)[1]. Previous studies demonstrated direct effects of gonadal hormones in regulation of IFN-γ production by NK cells[29], but it remains possible that NK cell sex differences can also be attributed to sex chromosome complement. To test this possibility, we gonadectomized mice to abolish the effect of sex hormones. Gonadectomy failed to eliminate sex differences in NK cell frequency **(Fig. 1i),** absolute numbers **(Fig. 1j)** and IFN-γ protein production in response to cytokine stimulation **(Fig. 1k)**,

indicating that gonadal hormones are not solely responsible for sex differences in NK cells.

Thus, we hypothesized that chromosomal complement, in particular X chromosome dosage,

may also play an important role.

X-linked UTX escapes X-inactivation and has higher expression in female NK cells.

While 46XX females undergo X chromosome inactivation (XCI) to control dosages of X-linked

genes, a subset of genes escapes XCI (termed XCI escapees), often resulting in higher

expression in females compared to males. Thus, XCI escapees are prime candidates for

mediating phenotypic sex differences in NK cells. Five genes (*XIST, DDX3X, KDM6A, EIF2S3,*

KDM5C) have previously been identified as XCI escapees in both humans and mice[30]. *XIST*

was excluded from further analysis because it is not expressed in male cells due to its known

role in X chromosome inactivation in female cells[1]. All 4 remaining genes were significantly

downregulated in male vs. female NK cells, from humans **(Fig. 2a)** and mice **(Fig. 2b).** The

greatest differential expression in both human and mouse NK cells was seen with *Kdm6a* (also

known as UTX*) **(Fig. 2a,b**). Male NK cells also expressed lower UTX protein levels compared to

female NK cells in mice **(Fig. 2c,d)**. These data indicate that expression levels of *Kdm6a* (UTX)

is sex-biased in NK cells.

In NK cells derived from gonadectomized mice, differences persisted in *Kdm6a*

transcript levels **(Fig. 2e)** and UTX protein levels **(Fig. 2f).** Additionally, using the four core

genotype (FCG) mouse model, which uncouples sex chromosome complement (XX or XY) and

gonadal sex organ (ovaries or testes)[31], *Kdm6a* transcript levels were also lower in mice with

one X chromosome (XY) independent of gonadal composition **(Extended Data Fig. 2a)**.

Together these findings suggest that increased UTX expression in female mice is not due to

hormonal effects and instead point to a primary role for X chromosome dosage.

UTX deletion recapitulates male NK cell phenotypes of frequency and IFN-γ production.

To determine if UTX mediates sex differences in NK cells, we generated mice with a conditional

deletion of UTX in NK cells (*Kdm6a*$^{fl/fl}$ x *Ncr1*$^{Cre+}$, referred to as UTX^{NKD} hereafter) with WT

(*Kdm6a*$^{fl/fl}$ x *Ncr1*$^{Cre-}$) littermates used as controls. To control for gonadal hormone and sex

chromosome effects, comparisons were made only in female mice (female WT vs. female

UTX^{NKD} littermates). We confirmed decreased UTX protein expression in NK cells from UTX^{NKD}

mice using flow cytometry **(Extended Data Fig. 2b,c).** Similar to male mice, female UTX^{NKD}

mice displayed increased splenic NK cell frequency **(Fig. 3a,b)** and absolute numbers **(Fig. 3c)**

in the spleen, blood, lungs, liver and bone marrow, demonstrating that this increase was not

tissue specific. Furthermore, IFN-γ protein production in response to IL-12 and IL-18 stimulation

was decreased in NK cells from UTX^{NKD} vs. WT mice **(Fig. 3d-f)**. These results implicate UTX in

limiting NK cell numbers and promoting IFN-γ production, suggesting divergent UTX levels may

play a causal role in NK cell sex differences.

Global changes in NK cell chromatin accessibility and transcription mediated by UTX

Recent studies have identified NK cell regulatory circuitry (regulomes) that prime innate

lymphoid cells for swift effector responses even prior to NK cell activation[32,33]. As an epigenetic

modifier, UTX can alter transcription by organizing chromatin at regulatory elements of target

gene loci[34]. To investigate the UTX-mediated modifications on chromatin accessibility and gene

expression in NK cells, we performed Assay for Transposase-Accessible Chromatin using

sequencing (ATAC-seq) in tandem with bulk RNA sequencing (RNA-seq) on sort-purified WT

(CD45.1^{+}) and UTX^{NKD} (CD45.2^{+}) NK cells from WT:UTX^{NKD} mixed bone marrow chimeras

(mBMCs) **(Fig. 4a)**. Using mBMCs allowed for an internally controlled experiment to minimize

environmental confounding factors. Principle Component Analysis (PCA) of both ATAC-seq and

RNA-seq data revealed that samples clustered together by genotype **(Fig. 4b)**, indicating that

loss of UTX results in profound changes in both the chromatin landscape and transcriptome of

NK cells. ATAC-seq revealed 3569 peaks decreased and 2113 peaks increased in accessibility

in UTX^{NKD} compared to WT NK cells (Log$_2$ Fold Change > ±0.5, adjusted p-value < 0.05, FDR <

0.05) **(Supplementary Table 1)**. Moreover, RNA-seq identified 577 decreased and 377

increased genes in UTX^{NKD} vs. WT (Log$_2$ Fold Change > ± 0.5, adjusted p-value < 0.05, FDR <

0.05) **(Supplementary Table 2)**. Thus, these data suggest UTX plays an active role in

controlling the NK regulome at baseline.

Integrative analysis of ATAC-seq and RNA-seq identified 400 genes that are both

differentially accessible and expressed with a significant positive correlation (Spearman

correlation: R = 0.62, p < 2.2x10^{-16}) between the mean log$_2$ fold change of ATAC-seq peaks and

log$_2$ fold change of RNA-seq expression **(Extended Data Fig. 3a)**. Fuzzy c-means clustering[35]

of both the ATAC-seq and RNA-seq datasets identified six major clusters which were

significantly decreased (Clusters 1, 2, 3, and 6) or increased (Clusters 4 and 5) in accessibility

(**Fig. 4c)** and expression **(Fig. 4d)** in UTX^{NKD} NK cells. For functional enrichment analysis,

g:Profiler[36] was used to analyze clusters of differentially expressed genes identified by RNA-seq

(Fig. 4e). Major pathways such as immune system process, cytokine production, IFN-γ

production, lymphocyte activation, and immune effector process were associated with

decreased expression in UTX^{NKD} (Clusters 1, 2, 3, and 6) **(Fig. 4e)**. At the same time, pathways

such as developmental process, biosynthetic process, and metabolic process were significantly

associated with increased expression in UTX^{NKD} (Clusters 4 and 5) **(Fig. 4e)**. Collectively, these

findings implicate UTX in the coordinate regulation of genes associated with NK cell

homeostasis and effector function.

Furthermore, UTX is known to interact with transcription factors (TFs) to coordinate

target gene transcription[34]. To identify putative UTX TF partners, we performed HOMER

(Hypergeometric Optimization of Motif Enrichment)[37] TF motif analysis on each cluster of

significant differentially accessible peaks **(Fig. 4f)**. TFs associated with modulating effector

function during viral infection such as Runt (Runx1 and Runx2)[38] and T-box (Eomes, T-bet, Tbr1

and Tbx6)[39] family TFs were more significant and had a higher percentage of target motifs

associated with clusters displaying decreased accessibility in UTX[NKD] (Clusters 1, 2, 3, and 6)

(Fig. 4f). Conversely, TFs associated with proliferation, differentiation, and metabolism in the

zinc finger family TFs (KLF1, KLF5, KLF6, KLF14, Sp2 and Sp5)[40] were more significantly

associated with clusters displaying increased accessibility (Clusters 4 and 5) **(Fig. 4f)**. These

data suggest that UTX poises the chromatin accessibility of several genes at steady state

known to influence NK cell fitness and effector responses, while also controlling genome-wide

accessibility of transcription factor binding sites implicated in these processes.

UTX coordinately regulates chromatin accessibility and expression of apoptosis pathway

genes.

The observed expansion of NK cell numbers in UTX[NKD] mice **(Fig. 3a-c)** could either be due to

higher NK cell proliferation or increased resistance to apoptosis. However, UTX[NKD] NK cells

paradoxically displayed a lower proportion of cells expressing the cell division marker Ki67

(Extended Data Fig. 4a). UTX[NKD] NK cells also showed less CFSE dilution in response to IL-15,

a cytokine known to induce NK cell proliferation **(Extended Data Fig. 4b,c)**. Thus, these results

suggest that higher NK cell numbers observed in UTX[NKD] mice were not due to increased

proliferation. In contrast, increased survival is likely the cause of expanded NK cell numbers in

UTX[NKD] mice.

Among the differentially accessible and expressed genes in NK cells lacking UTX were

those important in controlling apoptosis. An anti-apoptotic gene, *Bcl2,* showed increased

accessibility and expression in UTX[NKD] vs. WT NK cells (**Fig.5a, Extended Data Fig. 3b**).

Previous studies in mice have demonstrated that Bcl-2 can inhibit the pro-apoptotic function of

Bim to promote NK cell survival[41], thus, we interrogated the expression of these proteins in

UTX^NKD NK cells. While naïve UTX^NKD NK cells showed increased intracellular protein

expression of Bcl-2 compared to WT NK cells **(Fig. 5b,d),** UTX-deficient NK cells also displayed

a modest increase in intracellular Bim levels **(Fig. 5c,e)**. Importantly, the Bcl-2:Bim ratio was

significantly higher in UTX^NKD NK cells **(Fig. 5f)**, suggesting UTX-deficiency likely results in a

higher proportion of pro-survival proteins present in NK cells. Notably, male NK cells also

displayed a significant increase in Bcl-2:Bim ratio, which may underlie the expanded NK cell

numbers observed in male mice **(Fig. 5g)**. These results implicate UTX in regulation of NK cell

fitness to restrict numbers at homeostasis.

UTX is critical for NK cell IFN-γ production and effector function.

Since NK cells are early responders to MCMV, rapid effector molecule production is critical for

NK cell mediated anti-viral control. ATAC-seq and RNA-seq of NK cells from WT:UTX^NKD

mBMCs identified various chromatin accessibility and transcript changes at steady state **(Fig.**

4b,c). Specifically, genes involved in the NK effector response (*Ifng* and *Csf2*)[27,42] showed

decreased accessibility and expression in UTX^NKD compared to WT NK cells **(Fig. 6a, Extended**

data fig. 3b-d). Moreover, qRT-PCR verified decreases in *Ifng* transcript levels in UTX^NKD vs.

WT NK cells at rest **(Fig. 6b).** Similarly, male NK cells, which express lower levels of UTX

(Fig.2c,d), also had a similar decrease in *Ifng* transcript levels at rest **(Fig. 6c)**. Thus, through

shaping of the chromatin landscape, UTX controls levels of effector gene transcripts available

prior to immune challenge.

To determine whether UTX deficiency also led to decreased chromatin accessibility and

transcription of effector genes in NK cells during infection, we performed ATAC-seq on NK cells

isolated from MCMV-infected WT:UTX^NKD mBMCs on D1.5 post-infection (PI). Similar to naïve

UTX-deficient NK cells, D1.5 PI UTX^NKD NK cells also showed decreased chromatin accessibility

at the *Ifng* and *Csf2* loci **(Fig. 6d,e, Extended Data Fig. 3e,f)**. qRT-PCR confirmed decreased

Ifng and *Csf2* transcripts in NK cells from UTX^{NKD} mice at D1.5 PI **(Fig. 6f)**. Similarly, UTX^{NKD}

NK cells showed decreased IFN-γ protein expression in UTX^{NKD} NK cells on D1.5 PI indicating

that UTX expression in NK cells is required for optimal IFN-γ production following viral infection

(Fig. 6g,h). To confirm whether dosage of UTX expression in mature NK cells associates with

NK cell production of IFN-γ during viral infection, we generated transgenic mice to achieve

tamoxifen-inducible UTX deletion (*Rosa26*$^{ERT2CRE/+}$ x *Kdm6a*$^{fl/fl}$: iUTX$^{-/-}$). Tamoxifen

administration in WT:iUTX$^{-/-}$ mBMCs resulted in differential degrees of UTX protein loss, and

displayed a positive correlation between intracellular UTX levels and IFN-γ production on D1.5

PI **(Extended Data Fig. 5)**. Since IFN-γ production by NK cells is critical for protection against

MCMV[27], we challenged WT and UTX^{NKD} mice with a sublethal dose of MCMV and monitored

survival. While WT mice controlled MCMV infection (n = 8/8 survived), UTX^{NKD} mice rapidly

succumbed to infection (n = 3/8 survived) **(Fig. 6i)**. These results demonstrate a requirement for

NK cell-intrinsic UTX in the control of effector molecule production and protection against

MCMV infection.

Discussion

Sex is a critical biological variable in determining outcomes to viral infections[3]. This was recently illustrated with COVID-19, in which male sex was identified as a major risk factor for severe disease[5]. Moreover, recent studies have linked NK cell dysfunction within severe COVID-19 disease[43]. Given the importance of NK cells in anti-viral immunity, understanding the root causes of sex differences in NK cell biology will have far-reaching implications in optimizing anti-viral immunity. In this study, we demonstrated that lower expression of UTX in XX UTX[NKD] NK cells mimics levels in XY NK cells, which may contribute to increased NK cell numbers and decreased IFN-γ production in males **(Extended Data Fig. 6a)**. UTX is expressed at lower levels in male NK cells across mice and human and this observation is independent of gonadal hormones in mice. NK cell UTX is required for controlling NK cell fitness, modulating accessibility of transcription factor binding motifs, increasing chromatin accessibility at effector gene loci, and poising NK cells for rapid response to virus infection **(Extended Data Fig. 6b)**. Together, these findings support a model in which divergent UTX expression contributes to sex differences in NK cell numbers and effector function.

Our findings indicate that UTX restricts NK cell numbers at steady state, since NK cells are increased at baseline in UTX[NKD] mice. This is in contrast to UTX deficiency in other immune cell types, which have been reported to result in moderate (CD8^{+} and CD4^{+} T cells) or severe (iNKT) decreases in peripheral cell numbers[44-46]. Interestingly, T cell-specific UTX-deficiency is associated with CD8^{+} T cell accumulation during viral infection. Thus, it is possible that UTX-mediated gene programs that inhibit CD8^{+} T cell numbers during inflammation are shared by NK cells at rest[46]. Indeed, increased Bcl-2 levels were observed in both UTX-deficient NK cells and UTX-deficient CD8^{+} T cells, suggesting that UTX down-regulates this anti-apoptotic factor in both innate and adaptive cytotoxic lymphocytes.

NK cell-mediated effector functions during viral infection include cytokine production (IFN-γ) and cytotoxic molecule expression[47]. Our results from simultaneous ATAC-seq and

RNA-seq suggest that UTX poises the chromatin landscape of NK cells at rest to quickly

respond to viral challenge by increasing accessibility and transcription of effector loci such as

Ifng and *Csf2* prior to viral infection[27,42]. Decreased IFN-γ protein levels were seen at D1.5 post

MCMV-infection in UTX-deficient NK cells, suggesting decreased effector functionality during

inflammation. These results support a previous study that suggests the existence of an NK cell

regulome[27,42], in which NK cell chromatin accessibility is actively maintained at steady-state and

demonstrates a critical role for UTX in its maintenance before and during inflammation.

As a histone demethylase, UTX has intrinsic catalytic ability to demethylate H3K27me3

(a repressive histone mark) to poise chromatin for active gene expression[48]. In addition to its

catalytic activity, UTX functions in multiprotein complexes with other epigenetic regulators (e.g.

SWI/SNF, MLL4/5 and p300) to mediate chromatin remodeling in a demethylase-independent

manner[48,49]. In CD8^{+} T cells, UTX binds to enhancer and TSS of effector genes to promote

effector gene programs in a demethylase-independent manner[46]. In contrast, demethylase

activity in iNKT cells is required for the development and function and H3K27 methylation

correlated with gene programs important for CD4^{+} T follicular helper cell development[44,50]. Thus,

the molecular mechanisms by which UTX functions appears to be immune cell specific. A

previous study treated human NK cells with a small molecule inhibitor of H3K27me3

demethylases (GSK-J4) and found reduced cytokine expression (IFN-γ, TNF-α, GM-CSF, and

IL-10) in response to *in vitro* stimulation[51]. However, GSK-J4 is not a specific tool for studying

UTX-mediated mechanisms because it also inhibits Jmjd3, another H3K27me3 demethylase as

well as having non-specific effects on other histone demethylases[52]. Thus, further studies using

more precise genetic modification strategies are needed to understand the mechanisms by

which UTX functions in NK cells.

UTX has been reported to interact with lineage specific TFs in T cells to target particular

effector loci[34]. Our studies using HOMER motif analysis revealed potential interactions between

UTX and TFs associated with modulating NK cell effector function during viral infection (Runx1,

Runx2, Eomes, T-bet, Tbr1, and Tbx6). Moreover, these analyses also point to UTX interactions

with TFs associated with NK cell proliferation, differentiation, and metabolism (KLF1, KLF5,

KLF6, KLF14, Sp2 and Sp5). In line with a physiologic role for TFs in UTX-mediated control of

chromatin accessibility, TF binding motifs were strongly enriched in ATAC-seq gene clusters.

Further studies will be needed to experimentally verify these interactions in mouse and human

NK cells.

344   NK cells are critical for control of HCMV infection in humans because NK cell-deficient

individuals develop disseminated herpesvirus infections[16,53]. Sex differences in immune

response to multiple viruses have been reported, including immune responses to HCMV[53]. Our

data support a model in which sex differences in anti-viral immunity can partially be explained

by differences in UTX expression in NK cells, given the increased susceptibility of UTX^{NKD} mice

to MCMV challenge. UTX deficiency has also been associated with Kabuki Syndrome and

Turner Syndrome[44,54], two human conditions associated with immune dysregulation and

increased infections. Our findings suggest the possibility that UTX deficiency in human NK cells

may contribute to decreased viral immunosurveillance observed in these patients, although

future work will be needed to support this hypothesis.

354   Weighing factors that define patient subsets with different immune responses will allow

us to move past a "one-size-fits-all" therapeutic approach to a precision medicine paradigm.

Understanding sex differences in NK cell function and their molecular underpinnings is an

important step toward incorporating sex as a biological factor in treatment decisions. In males

with severe viral illness, for instance, enhancing NK cell UTX activity may provide therapeutic

benefit. We expect that these insights will be important not only in the setting of viral infections,

but also in other infections and cancer, where NK cells also play an important role. These

findings may also have important implications for adoptive cellular therapies, in which NK cells

are the subject of intense interest[55,56].

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Author Contributions

M.I.C., L.R., J.H.L., R.Y.T., H.H., A.P.A., T.E.O. and M.A.S. designed the study; M.I.C., J.H.L.,

R.Y.T., L.R., and S.C. performed the experiments; M.I.C., R.T.Y., F.M., and M.P. performed

bioinformatics analysis; M.A.S, M.I.C. and T.E.O. wrote the manuscript.

Competing Interests Statement

T.E.O. is a scientific advisor for Xyphos Inc., a company that has financial interest in human NK

cell-based therapeutics.

Figure 1

Figure 1: Sex differences in IFN-γ production and NK cell numbers in mouse and human NK cells are independent of gonadal hormones. a) Representative dot plots, **b)** percentage, and **c)** absolute numbers of splenic NK cells (CD3⁻ TCRβ⁻ NK1.1⁺) in female and male C57BL/6 mice (n = 15 per group). **d)** Representative contour plots, and bar graphs depicting **e)** percentages IFN-γ⁺ and **f)** relative IFN-γ mean fluorescence intensity (MFI) of total NK cells with

no treatment (NT) or in response to IL-12 (20 ng/ml) and IL-18 (10 ng/ml) stimulation of splenic

NK cells from female vs. male mice for 5 hours *ex vivo* (n = 5 per group, data representative of

2 independent experiments, normalized to female IL-12/18 treatment). **(g-h)** Human NK cells

isolated from peripheral blood of female (n = 6) and male (n = 7) donors were cultured and

stimulated with 10 ng/ml of IL-12 for 16 hours in the presence of K562 cells. **g)** Percentage of

IFN-γ$^+$ NK cells, and **h)** relative MFI of IFN-γ in TCRβ$^-$CD3$^-$CD56$^+$ female and male human NK

cells (normalized to female IL-12 treatment). **i)** Frequency and **j)** absolute numbers of splenic

NK cells identified in gonadectomized mice (n = 6 per group). **k)** IFN-γ MFI of total splenic NK

cells isolated gonadectomized female and male mice stimulated with IL-12 (20 ng/ml) and IL-18

(10 ng/ml) for 5 hours *ex vivo* (n = 6 per group). Samples were compared using two-tailed

Student's t test and data points are presented as individual mice with the mean ± SEM (N.S.,

Not Significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

Figure 2: X-linked UTX displays sexually dimorphic gene expression independent of sex

hormones. a) Relative expression of X-chromosome inactivation escapee genes using DICE

database which performed RNA-seq on sorted NK cells from human females (n = 36) vs. males

(n = 54) normalized to female. **b)** Relative expression of X chromosome inactivation escapee

genes by RT-qPCR in NK cells from female vs. male mice (C57BL/6; 8 week old, n = 5 per

group). Genes are ordered by increasing fold change. **c)** Representative histogram and **b)**

relative MFI of UTX protein expression in splenic NK cells from naïve female vs. male mice by

flow cytometry (C57BL/6; 8 week old). **e)** Relative expression of *Kdm6a* (UTX) by RT-qPCR of

isolated splenic NK cells normalized to female and **f)** UTX MFI of NK cells by flow cytometry

from spleens of gonadectomized female (ovariectomized) and male (castrated) mice (n = 6 per

group). Samples were compared using two-tailed Student's t test and data points are presented

as individual mice with the mean ± SEM (*, p <0.05; **, p <0.01; ***, p<0.001).

Figure 3

Figure 3: UTX regulates NK cell numbers and IFN-γ expression in NK cells. a)

Representative flow cytometry dot plots of splenocytes from WT and UTX[NKD] mice, gated on NK

cells (CD3^{-},TCRβ^{-}NK1.1^{+}). **b)** Percentage and **c)** absolute numbers of NK cells isolated from the

spleen, blood, lungs, liver, and bone marrow of WT and UTX[NKD] mice (n = 6-10 per group).

Numbers represent (spleen, lungs and liver – entire organ), (blood - per 1 ml of blood), and

(Bone Marrow - 2 femurs per mouse). **d)** Representative contour plots, **e)** frequency, and **f)** MFI

of IFN-γ of total NK cells in response to IL-12 (20 ng/ml) and IL-18 (10 ng/ml) stimulation of

splenic NK cells from WT and UTX[NKD] mice for 5 hours *ex vivo* (n = 5 per group). Data are

representative of 2-4 independent experiments. Samples were compared using two-tailed

Student's t test and data points are presented as individual mice with the mean ± SEM (N.S.,

Not Significant; *, p <0.05; **, p <0.01; ***, p<0.001).

Figure 4

Figure 4: Global changes in NK cell chromatin accessibility and transcription mediated

by UTX. a) Schematic of mixed bone marrow chimeras (mBMCs) produced by transferring cells

from WT (CD45.1+) and UTX^NKD (CD45.2+) bone marrow donors into a lymphodepleted host

(CD45^{1x2}) and allowed to reconstitute for 6 weeks. Then NK cells were sorted from spleens of

mBMCs for ATAC-seq and RNA-seq library preparation. **b)** Principal component analysis (PCA)

of chromatin accessibility (ATAC-seq) and transcriptional (RNA-seq) changes in WT and

UTX^NKD NK cells at steady state. **c,d)** Line graphs (left) and heatmap (right) of fuzzy c-means

clustered differentially **c)** accessible peaks identified by ATAC-seq and **d)** expressed genes

identified by RNA-seq of splenic NK cells from WT:UTX^{NKD} mBMCs (adjusted p-value < 0.05

and membership score > 0.5)**.** Line graphs show mean (black line) and standard deviation (red

ribbon) of mean-centered normalized log2 values of significant (FDR and adjusted p-value <

0.05) **c)** peaks of accessibility by ATAC-seq and **d)** gene expression by RNA-seq. **e)** Pathway

analysis of significant fuzzy c-means clustered RNA-seq genes using g:Profiler with point size

indicating -log10(p-value). **f)** HOMER motif analysis of significant fuzzy c-means clustered

ATAC-seq peaks grouped by transcription factor family (top) and transcription factor (bottom).

Point size indicates percentage of target sequences featuring motif and red gradient indicates -

log10(p-value) of enrichment. All genes displayed in heatmaps met the following threshold of

significance: FDR < 0.05, p < 0.05, and Log2FC > 0.5.

Figure 5

Figure 5: UTX regulates genes involved in apoptosis in NK cells through chromatin

remodeling. a) Representative tracks of ATAC-seq signal (WT: green and UTX[NKD]: red) and

RNA-seq transcript signal (WT: blue and UTX[NKD]: purple) of *Bcl2* in NK cells isolated at rest

from WT:UTX[NKD] mBMCs. Representative histogram of b) Bcl-2 and c) Bim protein expression,

MFI of d) Bcl-2, e) Bim, and f) Bcl-2:Bim ratio from flow cytometric analysis of total peripheral

blood NK cells (CD3^{-}TCRβ^{-}NK1.1^{+}) from WT:UTX[NKD] mBMCs (n = 4). g) Bcl-2:Bim MFI ratio of

total splenic NK cells (CD3^{-}TCRβ^{-}NK1.1^{+}) from gonadectomized female and male mice (n = 6

per group). Data represent 2-3 independent experiments. Samples were compared using two-

tailed Student's t test and data points are presented as individual mice with the mean ± SEM (*,

$p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

Figure 6

Figure 6: UTX controls the expression of effector genes critical for NK cell-mediated anti-viral immunity. a) Representative tracks of ATAC-seq signal (WT: green and UTX[NKD]: red) and RNA-seq transcript signal (WT: blue and UTX[NKD]: purple) of genes involved in NK effector function (*Ifng* and *Csf2)* in NK cells isolated at rest (D0) from WT:UTX[NKD] mBMCs. **b)** Relative expression of *Ifng* by RT-qPCR of isolated splenic NK cells normalized to WT from WT:UTX[NKD] mBMCs (n = 3). **c)** Relative expression of *Ifng* transcripts by RT-qPCR in NK cells from female vs. male mice normalized to female mice (C57BL/6; 8 week old, n = 8 per group). **d)**

Representative tracks of ATAC-seq signal (WT: green and UTX^{NKD}: red) of genes involved in NK

effector function (*Ifng* and *Csf2)* in NK cells isolated at from WT:UTX^{NKD} mBMCs at D1.5 post-

MCMV infection. **e)** Normalized read counts of accessibility at a representative peak located in

the *Ifng* and *Csf2* loci at D1.5 post-MCMV infection of WT:UTX^{NKD} mBMCs (n = 3). **f)** Expression

by RT-qPCR of *Ifng* and *Csf2* transcript levels at D1.5 post-MCMV infection of WT:UTX^{NKD}

mBMCs relative to WT (n = 6-8). Flow cytometric analysis of **g)** percent IFN-γ$^+$ and **h)**

normalized IFN-γ MFI relative to WT in splenic NK cells at D1.5 Post MCMV infection of

WT:UTX^{NKD} mBMCs (n = 14).**i)** Kaplan-Meier survival curves of WT and UTX^{NKD} mice infected

with MCMV (n = 8 per genotype). Mantel-Cox test (**, p=0.0093). Data are representative of 2-3

independent experiments. Two-tailed paired Student's t-test was performed, and data points

depict individual mice with the mean ± SEM (*, p <0.05; **, p <0.01; ***, p<0.001; ****,

p<0.0001).

Extended Data Figure 1

Extended Data Figure 1: Male NK cells from mice and humans produce less IFN-γ than females in response to proinflammatory cytokines. a) Gating strategy for identification of NK cells (CD3⁻,TCR-β⁻ NK1.1⁺) in spleen of C57BL/6 mice. MFI of IFN-γ of NK cells from **b)** mouse stimulated with no treatment (NT) or IL-12 (20 ng/ml) and IL-18 (10 ng/ml) and **c)** human NK cells stimulated with no treatment (NT) IL-12 (10ng/ml). Samples were compared using paired two-tailed Student's t test and data points are presented as individual samples with the mean ± SEM (N.S., Not Significant; *, p<0.05; ****, p<0.0001).

Extended Data Figure 2

Extended Data Figure 2: UTX expression in FCG and UTX^NKD mice. a) Relative expression

of *Kdm6a* (UTX) by RT-qPCR from NK cell isolated from spleens of Four Core Genotype (FCG)

mice (gonadal female XX n = 5, gonadal female XY n = 5, gonadal male XX n = 3, and gonadal

male XY n = 5). **b)** Representative histogram plot and **c)** MFI of UTX protein expression in

splenic NK cells (CD3⁻ TCRβ⁻ NK1.1⁺) in WT (*Kdm6a*^fl/fl^ x *Ncr1*^cre-^) and UTX^NKD (*Kdm6a*^fl/fl^ x

Ncr1^cre+^) mice at steady state (n = 7). Samples were compared using paired two-tailed

Student's t test and data points are presented as individual mice with the mean ± SEM

(**,p<0.01; ****, p<0.0001).

Extended Data Figure 3

Extended Data Figure 3: Integrative ATAC and RNA seq analysis reveal concomitant

changes in chromatin accessibility and transcription mediated by UTX. a) Scatter plot

highlighting genes that were differentially accessible and expressed (FDR and adjusted p-value

< 0.05) colored by fuzzy c-means cluster (see **Figure 4**). Y-axis depicts mean $\log_2$ fold change

of ATAC accessibility peaks and x-axis depicts $\log_2$ fold change of RNA-seq transcript levels.

Best fit regression line (red) with standard error (light red ribbon). Positive correlation calculated

by Spearman correlation of dataset (R=0.62, p<2.2x10^{-16}). **b)** Specific genes of interest

528   highlighted in red (*Ifng, Csf2, Casp3, Bcl2*) with known roles in NK cell gene programs. **c-f)**

529   Representative tracks of ATAC-seq signal of genes involved in NK effector function **c)** *Ifng* and

530   **d)** *Csf2* in NK cells isolated at rest (D0) and **e)** *Ifng* and **f)** *Csf2* 1.5 days post MCMV infection

531   from WT:UTX^NKD mBMCs. Red highlighted areas represent significant differentially accessible

532   regions (FDR and p-value < 0.05).

Extended Data Figure 4

534

535   **Extended Data Figure 4: UTX deficient NK cells are less proliferative than WT. a)**

536   Percentage of Ki67$^+$ total NK cells in blood of WT:UTX^{NKD} mBMCs (n=28). **b)** Representative

537   flow cytometry plot and **c)** quantification of CFSE expansion, division and proliferation indexes

538   of CFSE-labeled splenic NK cells from WT:UTX^{NKD} mBMCs stimulated *ex vivo* with IL-15 (50

539   ng/mL) for 4 days. Data represent 2-3 independent experiments. **a)** Samples were compared

540   using paired and **b-c)** unpaired two-tailed Student's t test and data points are presented as

541   individual mice with the mean ± SEM (*, p <0.05; **, p <0.01).

542

Extended Data Figure 5

Extended Data Figure 5: UTX regulates IFN-γ production in a dose-dependent manner.

WT:UTX$^{-/-}$ mBMCs were produced from WT (CD45.1$^+$) and iUTX$^{-/-}$ (CD45.2$^+$) and treated with tamoxifen for 3 days (1mg/day) prior to MCMV infection. **g)** Representative flow cytometry plot and **h)** %IFN-γ$^+$ and **i)** IFN-γ MFI of splenic NK cells at D1.5 post-MCMV infection of WT:iUTX$^{-/-}$ mBMCs (n = 6). **j)** Correlation of IFN-γ vs. UTX MFI of total splenic NK cells (n = 12). Two-tailed correlation of XY data performed. Pearson's r^2 =0.775, and p <0.0001. Data are representative of 2-3 independent experiments. Samples were compared using paired two-tailed Student's t test and data points are presented as individual mice with the mean ± SEM (**, p<0.01; ****, p<0.0001).

Extended Data Figure 6: Schematic of UTX-mediated sex differences in regulation of NK cell homeostasis and effector function. a) Schematic of how differential UTX expression levels may underlie sexual dimorphism in NK cell composition and function. In male mice, lower UTX levels is associated with expansion of NK cell numbers and decreased NK cell IFN-γ production. **b)** Model in which UTX plays a role to control NK cell fitness and effector function. UTX controls 1) NK cell fitness through restricting Bcl-2 expression, 2) accessibility of TF binding motifs (Runx1/2, T-bet, Eomes, KLF/Sp), and 3) direct changes in chromatin accessibility of effector loci (*Ifng, Csf2*). Ultimately, UTX poises NK cells in an optimal epigenetic state to rapidly respond to viral infection by robust effector molecule production (IFN-γ) resulting in protection against viral infection.

 Contact for Reagent and Resource Sharing

 Further information and requests for resources and reagents should be directed and will be

 fulfilled by the Corresponding Authors, Timothy O'Sullivan (tosullivan@mednet.ucla.edu) and

 Maureen Su (masu@mednet.ucla.edu)

 Method Details

 Mice

 Mice were bred at UCLA in accordance with the guidelines of the institutional Animal Care and

 Use Committee (IACUC). The following mouse strains were used in this study: C57BL/6

 (CD45.2) (Jackson Labs, #000664), B6.SJL (CD45.1) (Jackson Labs, #002114), $Rosa26^{ERT2Cre}$,

 $Ncr1^{Cre}$, $Kdm6a^{fl/fl}$ and FCG mice. For experiments with gonadectomy, procedure was performed

 by Jackson Laboratories Surgical Services. For experiments in UTX^{NKD} mice, only female mice

 were used to control for Y chromosome and sex hormone independent effects. Thus,

 experiments were conducted using 6-8 week old age-matched females in accordance with

 approved institutional protocols. For comparisons between male and female WT, we used 6-8

 weeks age-matched littermates. Mixed bone marrow chimeras (mBMCs) were generated by

 depleting host CD45.1 x CD45.2 mice by intraperitoneal (i.p.) injection of busulfan (1mg/mL) at

 20mg/kg for 3 consecutive days, which were then reconstituted 24 hours later with various

 mixtures of bone marrow cells from WT (CD45.1) and knockout (CD45.2) donor mice in the

 presence of an anti-NK1.1 antibody (at 1 mg/ml; clone: PK136) to deplete any remaining mature

 NK cells.

 MCMV infection

 MCMV (Smith) was serially passaged through BALB/c hosts three times, and then salivary

 gland viral stocks were prepared with a dounce homogenizer for dissociating the salivary glands

 of infected mice 3 weeks after infection. Experimental mice in studies were infected with MCMV

by i.p. injection of 7.5 x 10^3 plaque-forming units (PFU) in 0.5 mL of PBS. Mice were monitored

and weighed daily and sacrificed when body weight dropped over 20% from initial weight.

Isolation and enrichment of mouse NK cells

Mouse spleens, livers, lungs, and blood were harvested and prepared into single cell

suspensions as described previously[57]. Splenic single cell suspensions were lysed in red blood

cell lysis buffer and resuspended in EasySep™ buffer (Stemcell). To avoid depleting Ly6C$^+$ NK

cells we developed a custom antibody cocktail as follows: splenocytes were labeled with 10 µg

per spleen of biotin conjugated antibodies against CD3 (17A2), CD19 (6D5), CD8 (53-6.7),

CD88 (20/70), Ly6G (1A8), SiglecF (S17007L), TCRβ (H57-597), CD20 (SA275A11), CD172a

(P84) and magnetically depleted from total splenocyte suspensions with the use of anti-biotin

coupled magnetic beads (Biolegend)[58].

Ex vivo stimulation of lymphocytes

~5 x 10^5 NK cells were stimulated for 5 hours in complete RPMI media (RPMI 1640 + 25 mM

HEPES + 10% FBS, 1% L-glutamine, 1% 200 mM sodium pyruvate, 1% MEM-NEAA, 1%

penicillin-streptomycin, 0.5% sodium bicarbonate, 0.01% 55 mM 2-mercaptoethanol) , Brefeldin

A (1:1000; BioLegend) and Monensin (2uM; BioLegend) with or without recombinant mouse IL-

12 (20 ng/ml; Peprotech) and recombinant mouse IL-18 (10ng/ml; Peprotech). Cells were

cultured in complete RPMI media alone as a negative control (No Treatment or NT).

Human NK cell culture and Stimulation

Human peripheral blood mononuclear cells (PBMCs) from anonymous healthy donors were

obtained from leukoreduction filters after platelet apheresis from the UCLA Virology Core. NK

cells were isolated using the EasySep Human NK Cell Isolation Kit (Stem Cell Technologies)

following manufacturer instructions. Following isolation, cells were maintained in 24-well G-Rex

plates (Wilson Wolf) in NK MACS media (Miltenyi Biotech) supplemented with human IL-2 (100

IU/mL, Peprotech) and human IL-15 (20 ng/mL, Peprotech) at a plating density of $5x10^6$ cells

per well. For cytokine stimulation, 14 d IL-2/IL-15 activated human NK cells were plated with

K562 leukemia cells at an effector:target (E:T) ratio of 2.5:1 in addition to human IL-2 (100

IU/mL, Peprotech), human IL-15 (20 ng/mL, Peprotech), human IL-12 (10 ng/mL, Peprotech),

and/or human IL-18 (100 ng/mL, Peprotech) in complete RPMI media (Thermo Fisher). NK cells

were stimulated with cytokines for 16 h before analysis by flow cytometry.

Proliferation assays

CellTrace™ CFSE (Thermo) stock solution was prepared per the manufacturers' instructions

and diluted at 1:10,000 in 37C PBS. Isolated NK cells were incubated in 0.5mL of diluted CFSE

solution for 5 minutes at 37C. The solution was quenched with 10X the volume of complete

RPMI media. Cells were then washed and plated with 50 ng/ml of recombinant mouse IL-15

(Peprotech) and cultured for 4 days to assess proliferation. Flow cytometry was used to quantify

CFSE dilution 4 days post-stimulation. Samples were compared to levels of CFSE labeled on

Day 0. FlowJo's proliferation tool was used to model and measure number of divisions in

addition to expansion, division, and proliferation indices.

Flow Cytometry and Cell Sorting

Cells were analyzed for cell-surface markers using fluorophore-conjugated antibodies

(BioLegend, eBioscience). Cell surface staining was performed in FACS Buffer (2% FBS and 2

mM EDTA in PBS) and intracellular staining was performed by fixing and permeabilizing using

the eBioscience Foxp3/Transcription Factor kit for intranuclear proteins or BD Cytofix/Cytoperm

kits for cytokines. Flow cytometry was performed using the Attune NxT Acoustic Focusing

cytometer and data were analyzed with FlowJo software (TreeStar). NK cells were identified as

CD3⁻ TCRβ⁻ NK1.1⁺ cells: see gating strategy in Extended Data Fig. 1. Cell surface and

intracellular staining was performed using the following fluorophore-conjugated antibodies:

CD45.1 (A20), CD45.2 (104), NK1.1 (PK136), TCRβ (H57-597), CD3 (17A2), IFN-γ (XMG1.2),

Ly6C (HK1.4), BCL2 (BCL/10C4), UTX (N2C1 - GeneTex), Goat anti-rabbit H&L (Abcam -

ab6717), BIM (c34c5), CD90 (30-H12), Ki-67 (16A8). Isolated splenic NK cells were sorted

using Aria-H Cytometer to > 95% purity.

RNA-seq and ATAC-seq library construction and analysis

RNA was isolated from the cells using RNeasy Mini kit (Qiagen) and used to generate RNA-seq

libraries followed by sequencing using Illumina HighSeq 4000 platform (single end, 50bp). The

reads were mapped with HISAT2 (version 2.2.1) to the mouse genome (mm10). The counts for

each gene were obtained by HtSeq[59], in print, online at doi:10.1093/bioinformatics/btu638).

Differential expression analyses were carried out using DESeq2[60] (version 1.24.0) with default

parameters. Genes with adjusted p value <0.05 were considered significantly differentially

expressed. Sequencing depth normalized counts were used to plot the expression values for

individual genes.

ATAC-seq libraries were produced by the Applied Genomics, Computation, and

Translational Core Facility at Cedars Sinai in the following manner: 50,000 cells per sample

were lysed to collect nuclei and treated with Tn5 transposase (Illumina) for 30 minutes at 37°C

with gentle agitation. The DNA was isolated with DNA Clean & Concentrator Kit (Zymo) and

PCR amplified and barcoded with NEBNext High-Fidelity PCR Mix (New England Biolabs) and

unique dual indexes (Illumina). The ATAC-Seq library amplification was confirmed by real-time

PCR, and additional barcoding PCR cycles were added as necessary while avoiding

overamplification. Amplified ATAC-Seq libraries were purified with DNA Clean & Concentrator

Kit (Zymo). The purified libraries were quantified with Kapa Library Quant Kit (KAPA

Biosystems) and quality assessed on 4200 TapeStation System (Agilent). The libraries were

pooled based on molar concentrations and sequenced on an Illumina HighSeq 4000 platform

(paired end, 100bp).

ATAC-seq fastq files were trimmed to remove low-quality reads and adapters using

Cutadapt[61] (version 2.3). The reads were aligned to the reference mouse genome (mm10) with

bowtie2[62] (version 2.2.9). Peak calling was performed with MACS2[63] (version 2.1.1). The peaks

from all samples were merged into a single bed file, peaks from different samples that were

closer than 10bp were merged into a single peak. HTseq[59] (version 0.9.1) was used to count the

number of reads that overlap each peak per sample. The peak counts were analyzed with

DESeq2[60] (version 1.24.0) to identify differentially accessible genomic regions. Peaks with

adjusted p-value < 0.05 were considered significantly differentially accessible. The peak counts

were visualized with Integrated Genome Browser, (version 9.1.8).

Fuzzy c-means clustering was used for both ATAC-seq and RNA-seq using significant

(p-value and FDR <0.05, Log_2FC +/- 0.5) normalized counts generated from DESeq2. MFuzz

package (version 3.14) within R was used to perform this analysis into 6 clusters with a

membership score of >0.5. The differentially accessible ATAC peaks were analyzed using the

findMotifsGenome.pl function from HOMER[37] (version 4.9.1) of each cluster to identify enriched

cis-regulatory motifs of transcription factors. Pathway analysis of clustered RNA-seq data was

performed using g:Profiler using the g:GOSt function. Top relevant pathways were selected

from KEGG Biological Pathways and Gene Ontology Pathways (Biological Processes and

Molecular Function).

Statistical Analyses

For graphs, data are shown as mean ± SEM, and unless otherwise indicated, statistical

differences were evaluated using a Student's t-test. For Kaplan-Meir survival curve, samples

were compared using the Log-rank (Mantel-Cox) test with correction for testing multiple

hypotheses. A p-value < 0.05 was considered significant. Graphs were produced and statistical

analyses were performed using GraphPad Prism and ggplot2 library in R. Spearman Correlation

on best fit regression line was performed using ggpubr library in R.

Data Availability

Sequencing datasets are accessible from GEO with accession number GSE185065. Data can

be accessed by reviewers using the access token ilqlqswavvqhtir.

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