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The biological and prognostic role of long non-coding RNA NEAT1 in acute myeloid leukemia

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Abstract

Nuclear paraspeckle assembly transcript 1 (NEAT1) is a long non-coding RNA associated with the promotion of several solid cancers. As part of the FANTOM6 project, we aimed to define its role in acute myeloid leukemia (AML) and myelopoiesis, since it remains largely uninvestigated. We show that NEAT1 expression increases during myelopoiesis, especially in monocytes. NEAT1 expression is elevated in AML compared to normal bone marrow (NBM), especially in AML with inv(16)and t(8;21). In addition, NEAT1 expression correlates positively with *ASXL1*, *KRAS* and *NRAS* mutations, but negatively with TP53 mutant AML. Higher NEAT1 expression is associated with better overall survival in AML, independent of other known risk factors. Knockdown of NEAT1 in AML cells induces monocytic differentiation and upregulated gene expression of genes involved in glucose metabolism. By investigating genome-wide RNA and DNA interactions using RADICL-sequencing, we show that NEAT1 binds to the *RUNX2* locus that is associated with differentiation, metabolism, and glucose uptake. The results suggest that the lncRNA NEAT1 has a role in myelopoiesis, AML and is implicated in glycolysis.

Introduction

Improved RNA-sequencing performances of eukaryotic organisms has revealed a disproportionately limited number of protein-coding transcripts compared to the total size of the transcriptome (Taft et al., 2007). Since then, it has been established that the majority of the genome is transcribed in a developmentally-regulated manner and gives rise to a substantial number of non-protein coding RNAs (ncRNAs), which play crucial roles in numerous cellular processes (Carninci et al., 2005; Mattick & Makunin, 2006). According to the length of the transcript, ncRNAs are divided into small ncRNAs (<200nt) and long ncRNAs (≥200nt, lncRNA) (Zhang et al., 2019).

The lncRNA nuclear paraspeckle assembly transcript 1 (*NEAT1*) is transcribed from the familial tumor syndrome multiple endocrine neoplasia (MEN) type 1 locus on chromosome 11q13.1 and encodes two transcriptional variants, NEAT1-1 (3.7 kb) and NEAT1-2 (23 kb) (Bond & Fox, 2009). Both variants are mono-exonic transcripts overlapping each other (Bond & Fox, 2009; Dong et al., 2018). NEAT1 is enriched in the nucleus but is also found in the cytoplasm (van Heesch et al., 2014) and appears to be mostly dispensable for normal embryonic development and adult life as mice lacking NEAT1 expression develop normally (Nakagawa et al., 2011).

NEAT1 deregulation has been reported in various solid tumors, associates with poor prognosis, and has been proposed as a prognostic marker (Peng et al., 2017; Sun et al., 2016; D. Zhao et al., 2017). Studies have shown that NEAT1 often acts as a competing endogenous RNA (ceRNA), resulting in upregulation of oncogenes (S. D. Li et al., 2018; Tu et al., 2018). Conversely, in hematological malignancies it appears to affect different biological processes. In some studies, it has been described as a tumor suppressor, involved in the inhibition of leukemogenesis (Yan et al., 2021; C. Zhao et al., 2019).

Acute myeloid leukemia (AML) is the most common acute leukemia. AML is characterized by a dismal prognosis, predominantly due to high relapse rates (DiNardo & Perl, 2019; Grimwade et al., 2016). Therefore, it is imperative that AML biology needs to be clearly elucidated to develop treatments, evade treatment resistance, and establish new biomarkers of clinical relevance. Increasing numbers of studies associate lncRNA to AML pathology (Feng et al., 2020; Gourvest et al., 2019), yet functional involvement of the relatively well-characterized NEAT1 remains elusive.

In the current study, we aimed to characterize the role of NEAT1 in AML. We show that NEAT1 exerts a specific expression pattern during normal myelopoiesis, as well as in AML, and that its expression associates to certain genetic AML subgroups. We also show that NEAT1 is associated with prognosis in AML independently of other risk variants. By knocking down NEAT1 expression in AML cells, we have further elucidated the biological role of NEAT1 in AML. By using RNA And DNA Interacting Complexes Ligated and sequenced (RADICL-seq), we define the binding pattern of NEAT1 to chromatin in AML patient samples.

Materials & Methods

Cell culture and antisense oligo-mediated knockdown of NEAT1

Kasumi-1 cells were grown in supplemented RPMI-1640 medium. NEAT1 knockdown in Kasumi-1 cells was performed by electroporation using two antisense oligos (ASOs). For detailed information see Supplementary Material.

Quantitative Real-Time PCR

Quantitative PCR was performed using SYBR Green Master mix (Thermo Fisher Scientific) with GAPDH as reference gene. For detailed information see Supplementary Material.

Expression of NEAT1 during normal hematopoiesis, AML patients and leukemic cell lines

In order to study the expression of NEAT1 during normal hematopoiesis, publicly available microarray data was obtained from hematopoietic cell populations using HemaExplorer (http://servers.binf.ku.dk/bloodspot/) and Rapin-cohort (Rapin et al., 2014; Svendsen et al., 2016). Cap Analysis of Gene Expression sequencing (CAGE-seq) data on hematopoietic cell populations and leukemic cell lines was retrieved from publicly available data from the FANTOM5 project (RIKEN, Japan, http://fantom.gsc.riken.jp/5/datafiles/latest/basic/). Data

was auto-scaled by group and represented using Integrative Genomes Viewer version 2.11 (IGV, Broad Institute) (Robinson et al., 2011).

Further, expression data from AML patient and normal bone marrow (NBM) samples was obtained from the Microarray Innovations in LEukemia study (MILE, n=615, microarray) (Haferlach et al., 2010; Kohlmann et al., 2008) and from the Swedish Clinseq AML-patient cohort (n=325, bulk RNA-sequencing) for clinical correlation analysis (Wang et al 2017 Leukemia). Correlation of 93 glycolytic-related genes (Reactome pathway *Glycolysis*) with NEAT1 expression was performed using the Clinseq cohort.

Further, histone modifications as markers for active gene transcription were analyzed between high or low NEAT1-expressing AML patients and NBM samples (n=4 each) using ChIP-sequencing data from both AML patients and NBM. For detailed information see Supplementary Material.

CAGE-sequencing on NEAT1-knockdown Kasumi-1 cells

CAGE-sequencing was performed on NEAT1-depleted (n=4) and scramble-negative-control transfected (NC, n=4) Kasumi-1 cells. CAGE libraries were prepared and sequenced as previously described (Forrest et al., 2014; Shiraki et al., 2003). CAGE-sequencing was done using Illumina HiSeq2500 using 50nt long single-reads and aligned to human reference genome GRChr38 (STAR alignment). CAGE data processing and analysis was done as previously described (Forrest et al., 2014; Shiraki et al., 2003). For detailed information see Supplementary Material.

RADICL-sequencing

We performed RNA And DNA Interacting Complexes Ligated and sequenced (RADICL-seq) on two AML patient bone marrow samples (mononuclear cells). The RADICL-seq was done as previously described (Bonetti et al., 2020). Reactome analysis was performed on identified NEAT1 target genes using ClueGO in CytoScape. For detailed information see Supplementary Material.

Results

NEAT1 is upregulated during normal myeloid differentiation

We first investigated the levels of NEAT1 expression during normal hematopoiesis using publicly available expression data (GSE42519 and HemaExplorer). NEAT1 is highly expressed in all myeloid maturation stages (Figure 1A). Compared to immature hematopoietic stem cells (HSC), NEAT1 is down-regulated during early maturation steps, such as in common-myeloid progenitor (CMP), granulocytic-myeloid progenitor (GMP), megakaryocyte–erythroid progenitor (MEP) and promyelocyte (PM) populations, while it is upregulated compared to HSC in peripheral mononuclear cells (PMC) and monocytes (Fig 1A). The results could be confirmed in a validation cohort using the HemaExplorer data (SupplFig 1A). In line with this, we detected a substantial increase in transcription start site (TSS) signal in monocytes (3074 tags per million (TPM)) and neutrophils (1242 TPM) compared to CD34+ HSPC cells (83 TPM), using CAGE-sequencing data from the FANTOM5 project (Fig 1B). The TPM values are shown after scaling by normalization factors calculated by RLE (Relative Log Expression) method. These findings show that NEAT1 expression is strongly associated with myeloid maturation, and that it could potentially play a role during myeloid differentiation.

NEAT1 is upregulated in normal-karyotype AML and core-binding factor leukemia but downregulated in APL

In order to define NEAT1 expression in AML, we compared the expression in AML to normal bone marrow (NBM) cells from healthy donors (mononuclear cells, MNC) using data from the Microarray Innovations in LEukemia (MILE) study (Labaj et al., 2017) and found NEAT1 to be upregulated in AML ($p<0.01$) (Fig 2A). The levels of H3K4methylation3 and H3K27acetylation, which both are associated with active transcription, are elevated in the NEAT1 locus in AML with high NEAT1 expression compared to AML with low expression and normal hematopoietic CD34+ progenitors (Fig 2B), suggesting that epigenetic deregulation contribute to the elevated NEAT1 expression in AML. AML with normal karyotype (NK) shows an increase in NEAT1 expression both compared to NBM ($p<0.0001$) (SupplFig 2), and AML with karyotype abnormalities (Fig 2C). Further dissecting AML with complex karyotype (CK), we observed increased NEAT1 expression in core-binding factor leukemia with inv(16), or t(8;21),compared to NBM ($p<0.001$; $p<0.01$) (Fig 2D). In contrast, NEAT1 expression is downregulated in APL (t(15;17) compared to NBM, ($p<0.001$) (Fig 2D).

NEAT1 deregulation is associated with *TP53*, *ASXL1*, *KRAS* and *NRAS* mutations

To analyze the NEAT1 expression in NK-AML we correlated NEAT1 expression with known driver mutations in NK-AML using a Swedish ClinSeq AML cohort (Wang et al., 2017). AML patient samples with mutated (mut) *TP53* showed significantly lower NEAT1 expression compared to wild-type (wt) *TP53* AML samples (Fig 3A). Furthermore, NEAT1 was upregulated in AML with *ASXL1*, *KRAS* or *NRAS* mut compared to wt samples (Fig 3B-D). Other recurrently mutated genes in AML such as *NPM1*, *DNMT3A, TET2, IDH1/2, KIT, STAG1, U2AF1*, and *EZH2* were not associated to NEAT1 expression (Data not shown).

NEAT1 expression correlates with AML cell maturation and white blood cell count

As NEAT1 is upregulated in mature hematopoietic cells, especially in monocytes, we investigated whether NEAT1 expression correlated with French-American-British (FAB) classification of AML sub-type classification, as a mean to analyze their differentiation status (Grimwade et al., 2003). The patients were stratified according to either a less differentiated status (FAB M0-M2), or more mature and monocytic AML status (M4-M5), excluding patients with acute promyelocytic leukemia (APL, M3) as well as more mature subtypes of other lineage (acute erythroid and megakaryocytic leukemia, M6 and M7). In line with our observed higher NEAT1 expression in monocytes during normal hematopoiesis, NEAT1 expression was also significantly higher in the group with more mature and monocytic AML cells ($p<0.05$), (Fig 4A). We further correlated NEAT1 expression with various clinical markers using the ClinSeq-AML cohort. NEAT1 expression positively correlated with white blood cell count (Pearson correlation $R=0.27$, $p<0.001$), (Fig 4B). However, no correlation between NEAT1 expression and other clinical variables such as blasts, hemoglobin, neutrophils, and platelets could be established in our cohort data (SupplFig 3A-D).

Elevated NEAT1 expression is associated with increased overall survival in AML patients

Next, we investigated whether NEAT1 expression is associated with survival in intensively-treated AML patients. Indeed, patients with high NEAT1 expression showed increased overall survival (OS) compared to patients with low NEAT1 expression (median OS, 95% CI: 16.3 months (11.6-25.9) vs 8.4 months (1.9-20.5), $p<0.05$), (Fig 4C). Further sub-stratification of NEAT1 expression into high expression (CPM>300), intermediate (80>CPM<300) and low expression (CPM<80), showed that OS was more similar in patients with very high expression or intermediate, while low expression was implicated with poorer outcome ($p=0.06$), (SupplFig 3E). Univariate cox proportional hazards analysis indicated that low NEAT1 expression

(CMP<80) was predictive of worse survival (HR=0.496, p<0.05). In order to determine whether NEAT1 expression is an independent prognostic marker for better overall survival in AML, we performed a multivariate cox proportional hazards analysis including known prognostic markers for AML, such as ELN2017 classification, and karyotype status (NK vs non-NK) as covariates. This showed that low NEAT1 expression indeed has independent prognostic value (HR=0.901, p<0.001) (Fig 4D).

NEAT1 knockdown induces monocytic differentiation and inhibits proliferation in the AML cell line Kasumi-1

Analyzing CAGE data retrieved from FANTOM5, we determined the transcription levels of NEAT1 transcription start sites (TSS) in various leukemic cells, in order to select a suitable cell model for functional studies of NEAT1. A TSS signal could be found in the majority of the cell lines at varying levels (SupplFig 4A). The Kasumi-1 cell line was selected for further functional studies, since it expresses NEAT-1 and is a well-characterized, and differentiation-inducible cell model for AML that carries the chromosomal aberration t(8;21), representing a subgroup with higher NEAT1 expression. In order to investigate the functional role of NEAT1 in AML cells, NEAT1 was knocked down in Kasumi-1 cells (SupplFig 4B), using two antisense oligos (ASOs) (SupplTab 2). NEAT1-depleted Kasumi-1 displayed a significant increase in the monocytic differentiation marker CD14 for both ASOs (Fig 5A). In contrast, CD11b, a granulocytic marker, was not significantly different in NEAT1-depleted cells (SupplFig 4C). In addition, NEAT1 depletion decreased the levels of the cell proliferative marker Ki67 (Fig 5B). Also, the anti-apoptotic factors BCL2 (p<0.01), MCL1 (p<0.01) and BCL-XL (BCL2L1, p=0.01) were downregulated in NEAT1- depleted cells (Fig 5C-5E), whereas there was no significant difference in pro-apoptotic factor BAX (SupplFig 4D). These findings suggest that

NEAT1 knockdown is inhibiting proliferation by inducing monocytic differentiation while further promoting apoptosis in Kasumi-1 cells.

NEAT1 depletion affects genes involved in glucose metabolism

To further investigate the molecular and transcriptional effects of NEAT1, we performed CAGE in NEAT1-depleted Kasumi-1 using the combination of two ASOs (SupplTab 2). Principal component analysis (PCA) showed that NEAT1-depleted samples clustered together distant from the negative control (NC)-transfected samples (SupplFig 5A). Among the differentially expressed genes in NEAT1-depleted samples compared to control, a great majority of genes (94.6%) were significantly up-regulated, and only three genes were significantly down-regulated (Fig 6A, SupplTab 3), indicating direct, or indirect transcriptional repression of genes by NEAT1. Reactome analysis of differentially expressed genes from CAGE analysis showed a significant enrichment of genes involved in the regulation of glucose metabolism and glycolysis (Fig 6B, SupplTab4), indicating a role of NEAT1 in a commonly dysregulated energy-source in malignant cells. To further investigate the impact of NEAT1 on glycolysis, we analyzed the expression of 93 glycolytic pathway-related genes (Reactome pathway *Glycolysis* genes) in correlation to dichotomized NEAT1 expression using the Clinseq-AML cohort. Notably, the majority of the 93 genes (53%) was significantly correlating with NEAT1 expression. Of these, 20 genes, including PPP2R5D, GOT1 and GOT2, were significantly positively correlated with NEAT1 expression (Fig 6C, SupplTab5), and 29 genes, including HK3 and PFKFB4, were significantly negatively correlated with NEAT1 expression (Fig 6D). To elucidate the difference between positive and negative correlating glycolytic genes, we performed an enrichment analysis on both gene lists using GOrilla. While no hits were obtained using the negative correlating genes, the glycolytic genes positively correlating

with NEAT1 expression where enriched-for-process terms associated with positive regulation of glycolytic, catabolic, and metabolic processes and metabolic (SupplFig 5B).

NEAT1 binds to *RUNX2* and several epigenetic regulators

Previous analyses of genome-wide DNA-binding regions of NEAT1 have showed that NEAT1 bind to chromatin in a cell specific pattern, with a preference for binding to the 5′ ends of the target genes (Bonetti et al., 2020; West et al., 2014). Therefore, we aimed to determine the NEAT1 binding genes in AML patients. We applied a newly-established technique RADICL-seq, which identifies interactions between RNA and DNA at a genome wide level (Bonetti et al., 2020), analyzing two AML patients. We identified 128 NEAT1-target genes that were common between both AML samples (Fig 7A, SupplTab 6,). NEAT1 target genes were higher expressed than average genes (Fig 7B). NEAT1 binding was associated with high gene expression and 17 of the NEAT1 target genes were among the 25% highest expressed genes in Kasumi-1 cells (Fig 7C). Hence, NEAT1 binding was associated with high gene expression. However, a small number (11 genes) of the NEAT1 target genes were not expressed. Similar correlation between NEAT1 binding and gene expression has previous been described in human breast adenocarcinoma cell line MCF-7 (West J et al 2014). Among the NEAT1 target genes we found the transcription factors *RUNX2*, *SOX6* and *FOSL2*. In addition, Reactome analysis of the 128 NEAT1 target genes identified RUNX1- and RUNX2-regulated genes among the NEAT1-target genes (Fig 7D). In addition, NEAT1 was found to bind to the loci of several epigenetic regulators such as *KMT2A, KMT5B*, and *CHD7*. Overall, the RADICL-sequencing data suggests that NEAT1 binds to several genes that have been demonstrated to be important for myeloid differentiation and AML, suggesting a role of NEAT1in myeloid differentiation.

Discussion

In this study, we aimed to investigate the role of the lncRNA NEAT1 in AML compared to normal hematopoiesis. Our results suggest that NEAT1 expression is involved in key cellular processes in AML, such as metabolism, and differentiation, and correlates with prognosis.

Recently, there is growing interest in lncRNAs dysregulation in cancer as several lncRNAs have been functionally linked to disease development (Carlevaro-Fita et al., 2020; Sunwoo et al., 2009; Y. Zhang et al., 2013). Although recent studies have indicated that NEAT1 may modulate cell proliferation, cell cycle progression, and apoptosis in AML cell lines, the role of NEAT1 is poorly understood in hematological malignancies. (C. Zhao et al., 2019; D. Zhao et al., 2017). We analyzed the NEAT1 expression patterns in AML patients and observed a significant upregulation of NEAT1 expression in patients with normal karyotype AML (nk-AML) compared to healthy NBM. Besides, the increased NEAT1 expression is associated with mutated *ASXL1*, *KRAS, NRAS*. All three genes can all be functionally linked to NEAT1, which further highlights the need to better understand its role in AML. *ASXL1* is mutated in approximately 11% of *de novo* AML cases and is associated with poor prognosis (Pratcorona et al., 2012). *ASXL1* belongs to the enhancer of trithorax and polycomb genes that are responsible for maintaining activation and silencing of polycomb-group (PcG) proteins and trithorax-group (trxG) proteins (Fisher et al., 2010). Interestingly, NEAT1 is a target of both wild-type and mutant ASXL1 (Yamamoto et al., 2021). Activating mutations of *KRAS* and *NRAS* are common in *de novo* AML, occurring in 11-16% and 4-5%, respectively, and associated with decreased survival (Ball et al., 2019). Previous studies in solid tumors have shown that knockdown of NEAT1 leads to suppression of cancer progression, via suppression of oncogenic pathways that encompassed the RAS proteins (Gong et al., 2016; Zhou et al.,

2019). The upregulated NEAT1 expression in mutant *ASXL1, KRAS*, and *NRAS* AML patients, supports the notion that control of NEAT1 expression could be orchestrated, and depend upon several different factors. Indeed, this can be further demonstrated by our finding that *TP53* mutant patients exhibit significant lower levels of NEAT1 compared to wild-type patients. NEAT1 is also a transcriptional target of p53, and its induction contributes to the tumor suppressor functions of p53 (Idogawa et al., 2017, 2019). In AML, *TP53* mutations occur in around 10% of patients and are associated with dismal prognosis, and a high risk of relapse (Barbosa et al., 2019). Therefore, new treatment options are urgently needed for this patient group. Transcriptional activation of *NEAT1* by p53 could be therapeutically exploited in AML patients with low NEAT1 expression by re-activation of mutant p53. So far, some small molecules, such as Eprenetapopt (APR-246), were investigated for their clinical use in in phase I and II clinical trials (Bykov et al.,2002; Q. Zhang et al., 2018).

Furthermore, we observed significant upregulation of NEAT1 in certain core-binding factor leukemia, carrying chromosomal aberrations, such as t(8;21), or inv(16), and as previously reported (Zeng et al., 2014), a downregulation of NEAT1 in APL. Increased NEAT1 expression was associated with more mature and monocytic AML FAB subtypes (M4-M5) and correlated with better OS of AML patients. In contrast, in solid cancers overexpression is associated with inferior survival (Dong et al., 2018; Ghaforui-Fard & Taheri, 2019; Yu et al., 2017). The discrepancy between solid cancers and AML concerning effect on prognosis may in part be attributed to the expression patterns of the different isoforms of NEAT1. At least two human isoforms of NEAT1 have been described (Clemson et al., 2009; Hutchinson et al., 2007) which have been shown to have distinct roles in regulating phenotypes of cancer cells (Dong et al., 2018). The presence of distinct NEAT1 isoforms has not been considered in this, or previous studies carried out in AML and hence needs to be further elucidated upon.

NEAT1 has been reported to be downregulated in hematopoietic malignancies, such as chronic myeloid leukemia (CML), APL and or Multiple Myeloma (Zeng et al., 2018) (Zeng et al., 2014) (Sedlarikova et al., 2017). Furthermore, knockdown of NEAT1 has been shown to block ATRA-induced differentiation in the APL cell line NB4 (Zeng et al., 2014). Our results showed upregulation of the monocytic marker CD14 and decreased proliferation after NEAT1 knockdown in Kasumi-1 cells. The role of NEAT1 in proliferation was recently supported by Taina and colleges that showed that elevated NEAT1 expression in Multiple Myeloma cells increase proliferation and viability (Taiana E et al, 2022). This suggests, at least partially distinct mechanisms for the regulation of key genes controlling myeloid differentiation.

Taken together, we have demonstrated that NEAT1 is involved in the regulation of several critical pathways in AML. Differential expression analysis of genes in NEAT1-depleted Kasumi-1 cells, revealed an enrichment for several genes involved in the glycolytic pathway. Further, gene expression correlation analysis between NEAT1 and glycolytic genes highlighted pronounced positive and negative glycolytic gene expression dependencies by NEAT1 expression levels. This is in line with a recently published report which describes NEAT1 as a critical modulator of glycolysis, through the binding of the penultimate enzymes, in breast cancer (Park et al., 2021). Moreover, consistent with our finding that NEAT1 expression correlates with better overall survival, glycolytic genes with reported bad prognostic implication in AML, including PPP2R5D, GOT1 and GOT2, correlated negatively with NEAT1 expression (Taweel et al., 2020, Chen et al., 2020), while glycolytic gene HK3 with reported lower expression in high-risk AML, correlated positively with NEAT1 expression. Also, glycolytic gene PFKFB4, associated with AML FAB M4/5, positively correlated with NEAT1 expression in the Clinseq-cohort (Wang et al., 2020) strengthening our finding of

elevated NEAT1 expression in monocytic AML. Our RADICL-sequencing provides evidence for NEAT1 to be involved in several critical pathways. RUNX1 and RUNX2 controlled-pathways were enriched, which have been implicated in metabolism and glucose uptake, further supporting the notion that NEAT1 regulates these processes (Wei et al, 2015; Xing et al, 2021). In addition, RUNX1 and RUNX2 are also important transcription factors regulating differentiation. Hence, our results indicate how NEAT1 may control pathways such as myeloid differentiation. RUNX1 has been previously shown to bind to the locus of NEAT1 and induce its expression in breast cancer (Barutcu et al., 2016; Choudhry et al., 2015). We show that NEAT1 binds to the *RUNX2* loci, suggesting that NEAT1 and RUNX1/2 constitute a regulatory feed-back loop. One could speculate that NEAT1 interacts and gets loaded to RUNX at the RUNX1/2 promoters, since both RUNX1/2 and NEAT1 binds to the *RUNX1/2* loci. In addition, NEAT1 binds to the *CHD7* loci. CHD7 interacts with RUNX1 and enhance the transcriptional activity of RUNX1 and is important for RUNX1 mediated leukemogenesis (Zhen et al., 2017). RUNX1 has also recently been shown to bind to the *FOSL2* loci (Thomsen et al 2021), another of the demonstrated NEAT1 target loci. Those results further suggest that NEAT1 and RUNX1/2 are included in the same regulatory axis in myeloid development and AML.

Overall, our study provides insights how NEAT1 participates in the regulation of myeloid differentiation and metabolisms with implication for AML biology.

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Author contributions

S.M. and A.L. conceived the study, S.M. carried out the experiments, A.N. performed expression analyses, J.W.S. performed CAGE and A.E. and A.B. performed and analyzed

RADICL-seq. P.C supported CAGE and RADICL-seq. H.M. helped with sample preparation. S.M. A.N. SL and AL analyzed the data and wrote the manuscript.

The authors declare that they have no conflict of interest.

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Data availability statements

Available upon request.

Figure Legends

Figure 1. NEAT1 is upregulated during normal hematopoietic differentiation. (A) Distribution of NEAT1 gene expression levels during normal hematopoiesis. Microarray data obtained from the dataset GSE42519 (“Rapin-cohort”, n=44). Gene expression intensity is

presented as robust multiarray average (RMA). Data is shown as box and whisker plot with interquartile range (IQR). Black dots represent outliers; grey data points represent all data. The sample number is indicated in parentheses (n). Global p-value determined by Kruskal–Wallis test and adjusted by FDR (p.adj). P-value of pairwise comparison was determined by Wilcoxon test where cell populations of progenitor, precursor and mature cells were compared to hematopoietic stem cell (HSC) population (●, reference type). Cell populations in GSE42519 comprised of HSC, multi-potent progenitor (MPP, ns), common myeloid progenitor (CMP, p=0.038), granulocytic-myeloid progenitor (GMP, p=0.00117), megakaryocytic-erythrocytic progenitor (MEP, p=0.00925), promyelocytes (PM, p=0.00216), myelocytes (MY, ns), metamyelocytes (MM, p=0.0238), band cells (BC, ns), polymorphonuclear leukocytes (PMN, p=0.0238) and monocytes (Mono, p=0.00952). Abbreviation: ns, not significant (p>0.05).

(B) CAGE peaks indicating transcription start sites (TSSs) for NEAT1 genomic loci (25kb) in primary hematopoietic cells. Data is shown for hematopoietic stem cells (HSC), polymorphonuclear neutrophils (PMN) and $CD14^{+}$ monocytes (Mono). RNA coverage data was obtained from RNA-seq data of the Clinseq-AML cohort and represents depth of aligned reads. Reference Gene (RefSeq Gene) track indicates location of NEAT1_1 and NEAT1_2 gene (GRCh37). CAGE data was obtained from FANTOM5 project (RIKEN), scaled by group [0-6647] and visualized using IGV.

Figure 2. NEAT1 is upregulated in normal karyotype AML and core-binding factor leukemia but downregulated in APL. (A) Distribution of NEAT1 gene expression levels across normal bone marrow samples (NBM, n=73) and compared to all AML (n=399). Microarray data obtained from the Microarray Innovations in LEukemia (MILE) study (n=615). Gene expression intensity is presented as robust multiarray average (RMA) shown as box and

whisker plot with interquartile range (IQR). Black dots represent all data points. Grey dots represent outliers. The sample number is indicated in parentheses (n). Global p-value determined by Kruskal–Wallis test and adjusted by FDR (p.adj). P-value of pairwise comparison was determined by Wilcoxon test where AML (NK, p=0.0098) were compared to NBM (●, reference type). **(B)** Histone modifications in NEAT1 locus (31kb) using ChIP-seq data from AML patients and NBM comparing H3K4me3 (green) and H3K27ac (blue) levels in either high NEAT1 expression (NEAT1-high) or low NEAT1 expression (NEAT1-low). Data tracks of four samples were merged for each track. Reference Gene (RefSeq Gene) track (bottom) displays genomic location of NEAT1_1 and NEAT1_2 isoform (chr 11, GRCh37). Data was scaled by group and visualized using IGV.

(C) Distribution of NEAT1 gene expression levels across AML without (NK, n=351) or with (non-NK, n=191) chromosomal aberrations and **(D)** Distribution of NEAT1 gene expression levels across normal bone marrow samples (NBM) and compared to four different AML subtypes with chromosomal aberrations. Gene expression data obtained from the Microarray Innovations in LEukemia (MILE) study (n=615). Gene expression intensity is presented as robust multiarray average (RMA) shown as box and whisker plot with interquartile range (IQR). Black dots represent all data points. Grey dots represent outliers. The sample number is indicated in parentheses (n). Global p-value determined by Kruskal–Wallis test and adjusted by FDR (p.adj). P-value of pairwise comparison was determined by Wilcoxon test where non-NK AML (non-NK, $p=6.9x10^{-6}$) was compared to NK AML (●, reference type). AML entities include MLL-AML (p=0.24643, ns), inv(16) (p=0.00027), t(8;21) (p= 0.00336) and t(15;17) AML (APL, p=0.00017). Abbreviation: ns, not significant (p>0.05). Global p-value determined by Kruskal–Wallis test and adjusted by FDR (p.adj).

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Figures

Figure 1.

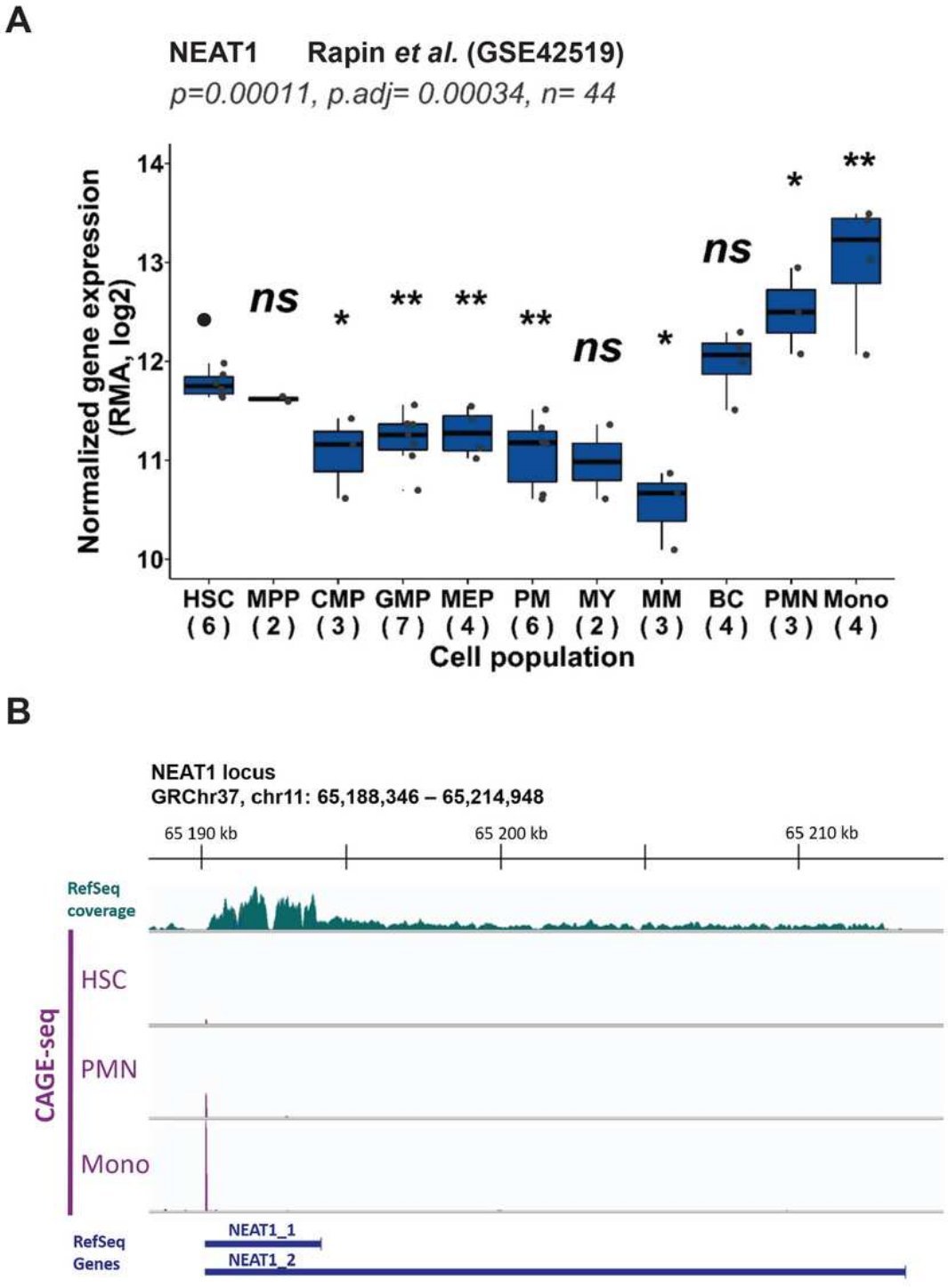

Figure 1

NEAT1 is upregulated during normal hematopoietic differentiation. (A) Distribution of NEAT1 gene expression levels during normal hematopoiesis. Microarray data obtained from the dataset GSE42519 ("Rapin-cohort", n=44). Gene expression intensity is presented as robust multiarray average (RMA). Data

is shown as box and whisker plot with interquartile range (IQR). Black dots represent outliers; grey data points represent all data. The sample number is indicated in parentheses (n). Global p-value determined by Kruskal–Wallis test and adjusted by FDR (p.adj). P-value of pairwise comparison was determined by Wilcoxon test where cell populations of progenitor, precursor and mature cells were compared to hematopoietic stem cell (HSC) population (, reference type). Cell populations in GSE42519 comprised of HSC, multi-potent progenitor (MPP, ns), common myeloid progenitor (CMP, p=0.038), granulocytic-myeloid progenitor (GMP, p=0.00117), megakaryocytic-erythrocytic progenitor (MEP, p=0.00925), promyelocytes (PM, p=0.00216), myelocytes (MY, ns), metamyelocytes (MM, p=0.0238), band cells (BC, ns), polymorphonuclear leukocytes (PMN, p=0.0238) and monocytes (Mono, p=0.00952). Abbreviation: ns, not significant (p>0.05). **(B)** CAGE peaks indicating transcription start sites (TSSs) for NEAT1 genomic loci (25kb) in primary hematopoietic cells. Data is shown for hematopoietic stem cells (HSC), polymorphonuclear neutrophils (PMN) and CD14+ monocytes (Mono). RNA coverage data was obtained from RNA-seq data of the Clinseq-AML cohort and represents depth of aligned reads. Reference Gene (RefSeq Gene) track indicates location of NEAT1_1 and NEAT1_2 gene (GRCh37). CAGE data was obtained from FANTOM5 project (RIKEN), scaled by group [0- 6647] and visualized using IGV.

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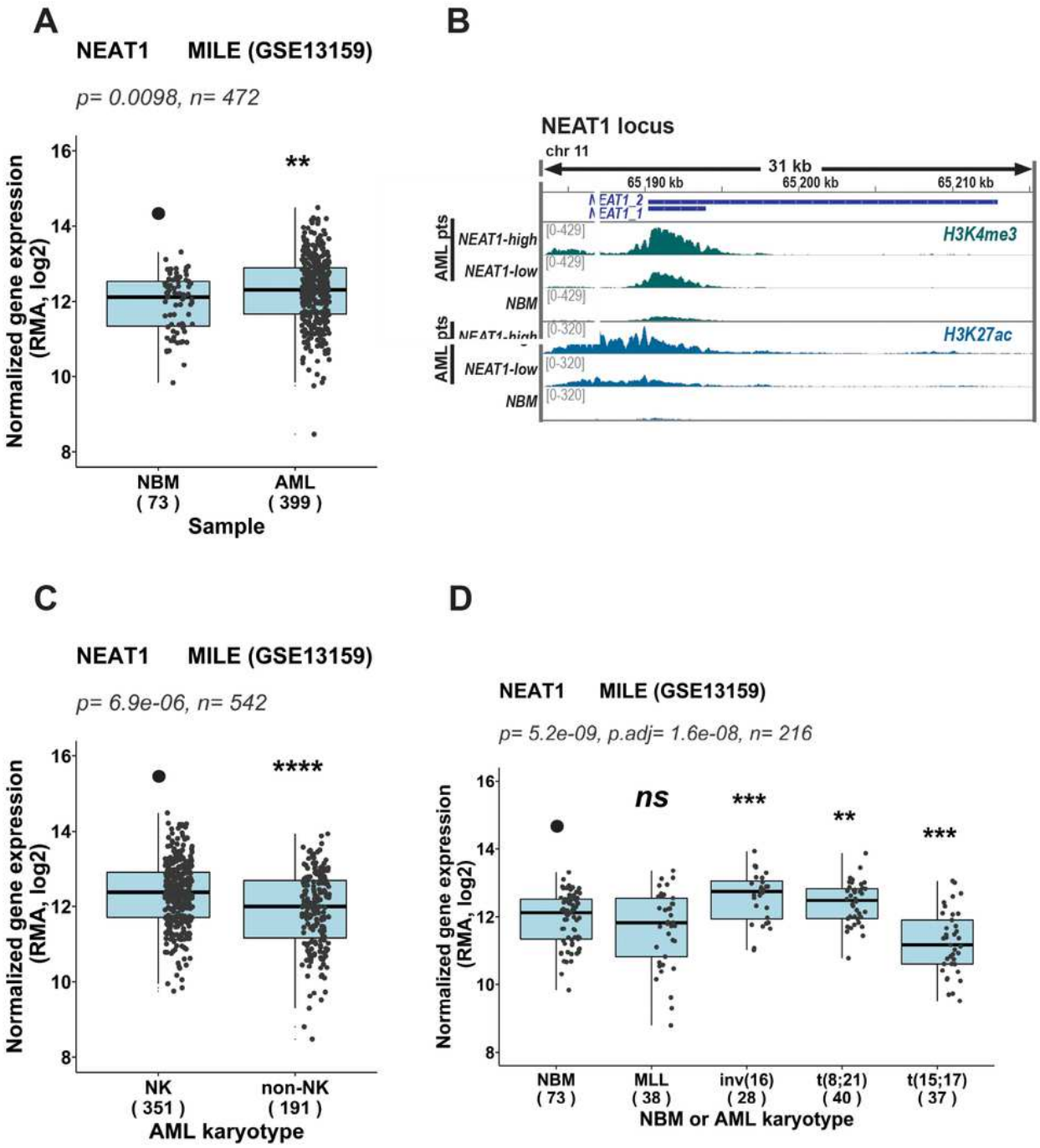

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NEAT1 is upregulated in normal karyotype AML and core-binding factor leukemia but downregulated in APL. (A) Distribution of NEAT1 gene expression levels across normal bone marrow samples (NBM, n=73) and compared to all AML (n=399). Microarray data obtained from the Microarray Innovations in LEukemia (MILE) study (n=615). Gene expression intensity is presented as robust multiarray average (RMA) shown as box and whisker plot with interquartile range (IQR). Black dots represent all data points.

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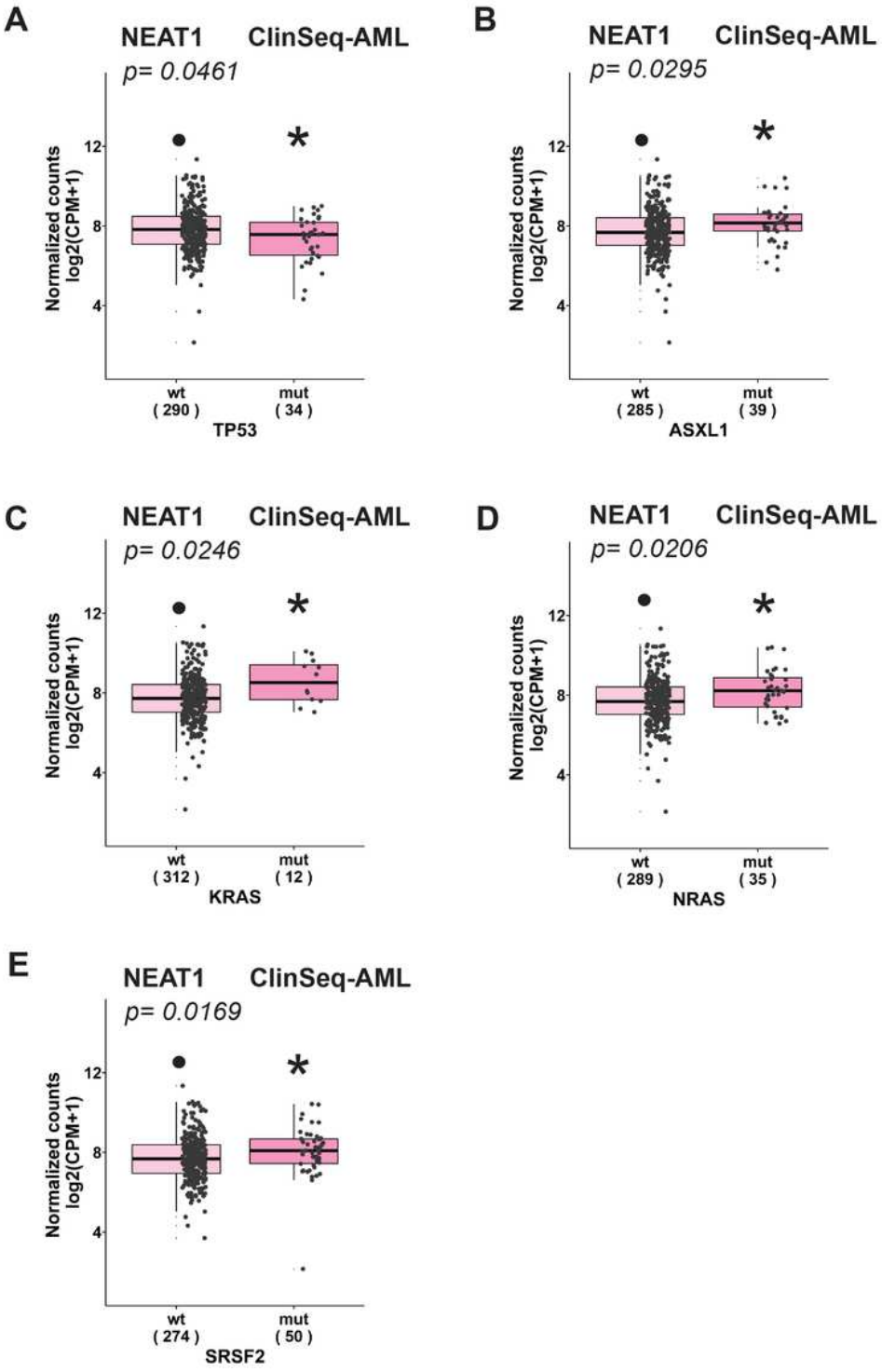

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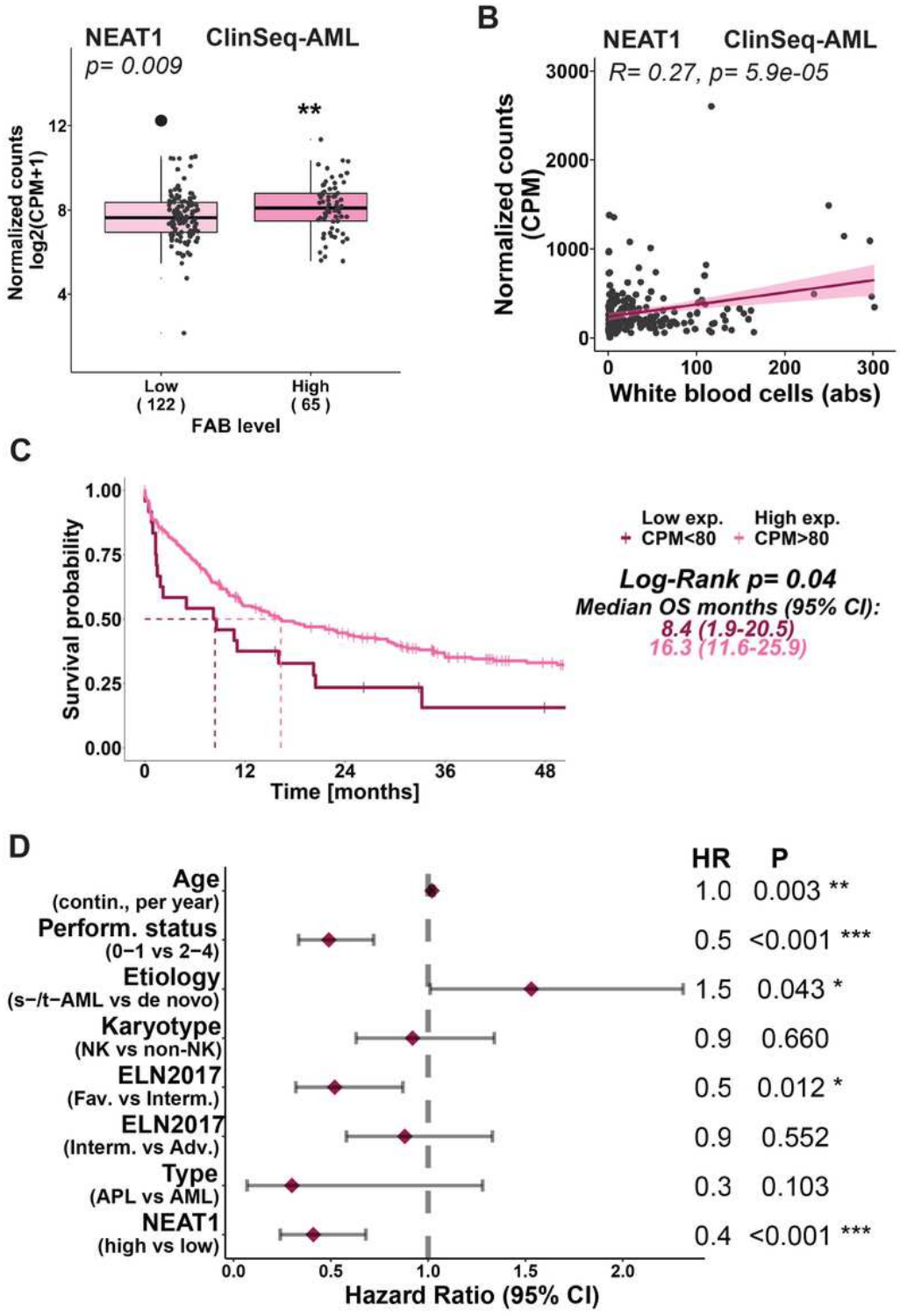

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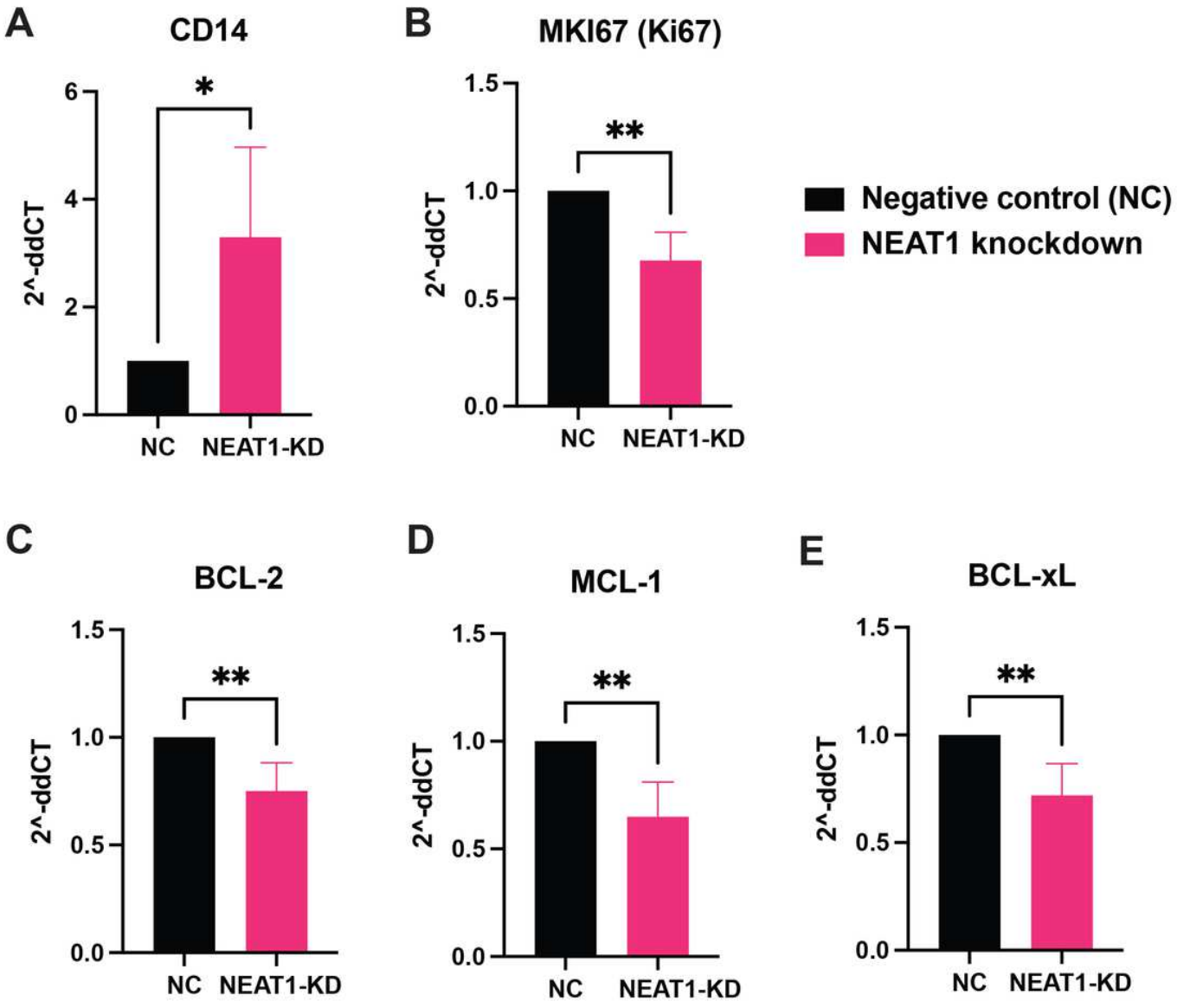

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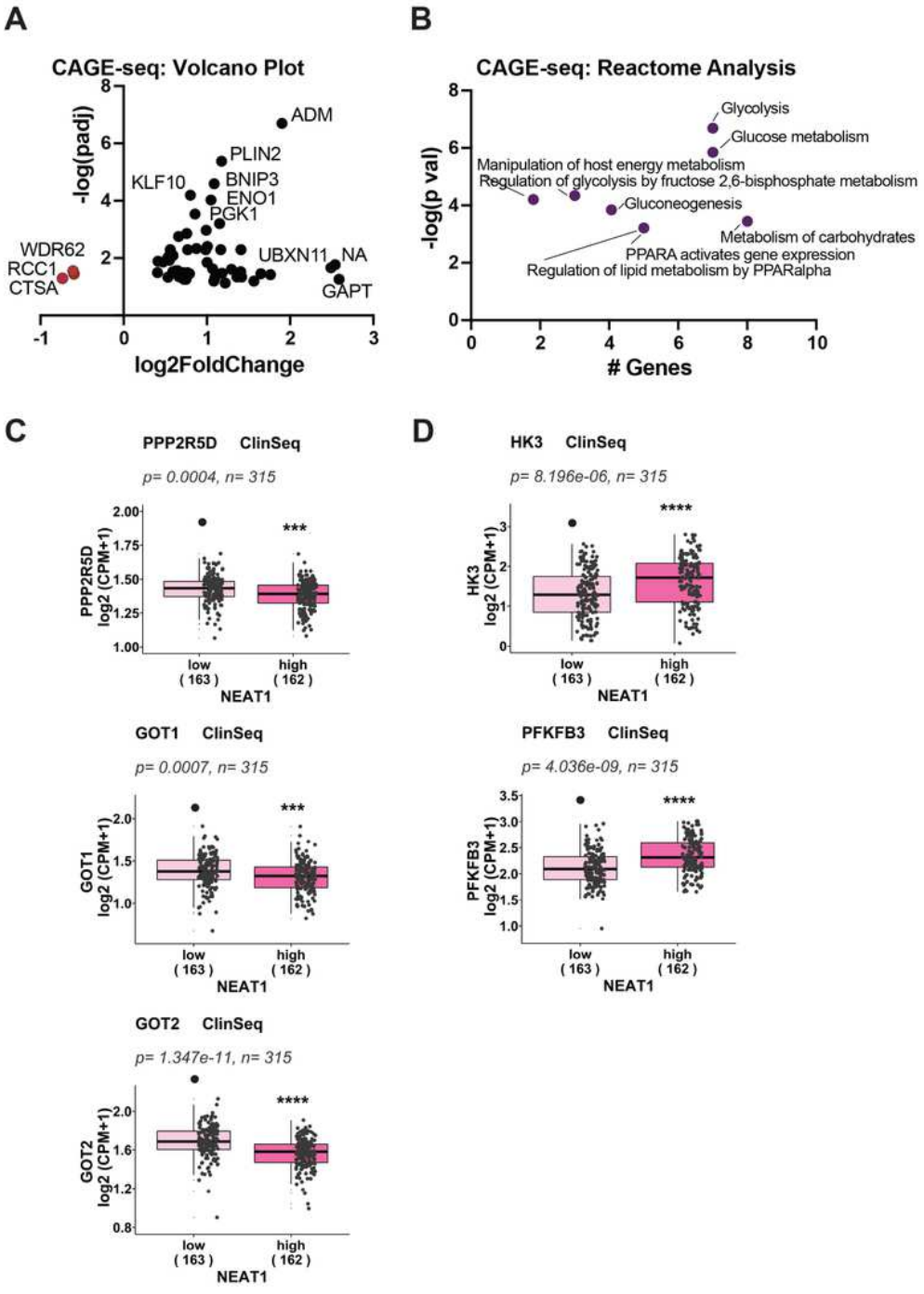

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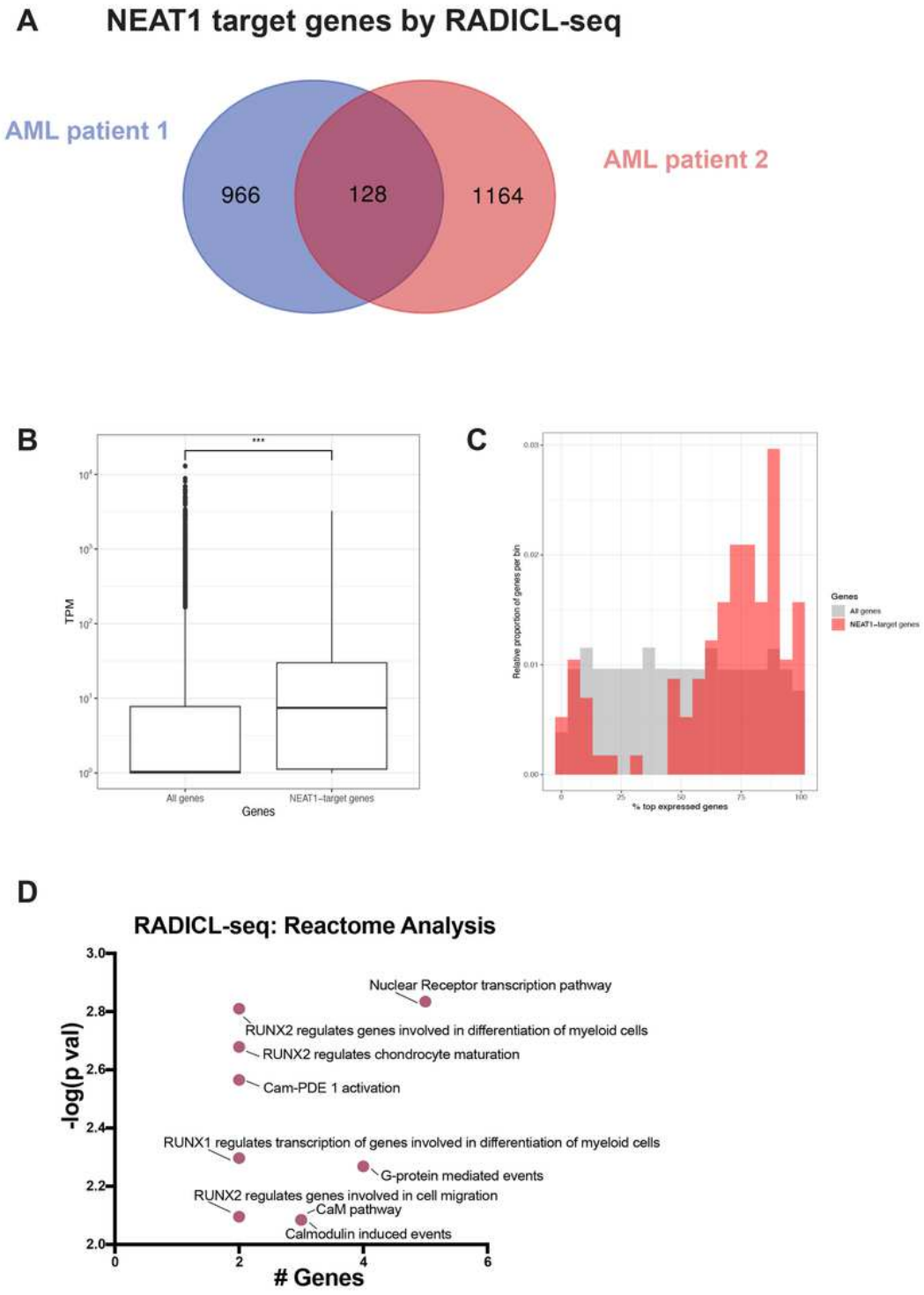

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MiliaraNEATSupMaterial.pdf
- SupTable1Primersequences.xlsx
- SupTable2AOS.xlsx
- SupTable3CAGENEAT1KD.xlsx
- SupTable4GO.xlsx
- SupTable5correlations.xlsx
- SupTable6RADICLseq.xlsx
- SupplFig1.pdf
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- SupplFig3.pdf
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