# MAT2A as Key Regulator and Therapeutic Target in MLLr Leukemogenesis 

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## Supplementary Methods

## Gene expression analyses

Reads were trimmed and cleaned up from contaminating adapters, polyA read through events, and low quality tails with BBDuk of the BBMap [62] tools suite (v38.67) using the following parameters: [k=13 ktrim=r useshortkmers=t mink=5 qtrim=r trimq=10 minlength=20] as well as employing the polyA and truseq_rna k-mer resources. Quality of the cleaned fastq files was assessed using FastQC (v0.11.4) [63, 64] before aligning reads with STAR (v2.7.0a) against the Ensembl H.sapiens genome v95 using the following parameters:[--outFilterType BySJout-outFilterMultimapNmax 20 -alignSJoverhangMin 8 alignSJDBoverhangMin 1 -outFilterMismatchNmax 999 -outFilterMismatchNoverL max 0.6 alignIntronMin 20 --outSAMattributes NH HI NM M]. Read counts for all genes were obtained using the featureCounts function of Rsubread (v2.0.0) with [GTF.featureType="exon" GTF.attrType="gene_id" useMetaFeatures=TRUE strandSpecific=1] and DESeq2 (v1.26) [65]. Transcripts covered with <1 read were excluded from subsequent analyses leaving 23.293 genes for determining differential expression. Significance thresholds were set to $|\log 2 \mathrm{FC}| \geq 0.5$ and BHadjusted $p$-value $\leq 0.1$. Surrogate variable analysis (sva, v3.26.0) [66] was used to minimize unwanted variation between samples. Differentially expressed genes (DEGs) were examined in downstream analyses for overrepresented canonical pathways and disease aspects with the most recent knowledge base of Ingenuity Pathway Analysis (IPA, v01-16). Only terms with at least three genes were considered. Interactions between DEGs were derived from IPA and visualized with Cytoscape [67].

Supplementary Tables

Table S1. Primer sets for RT-qPCR to quantify relative mRNA expression levels.

| Name | Sequence (5'3') |
| :---: | :---: |
| 18S rRNA fwd | CGGCTACCACATCCAAGGAA |
| 18S rRNA rev | GCTGGAATTACCGCGGCT |
| hu MEIS1 fwd | TGGCCACACGTCACACAGT |
| hu MEIS1 rev | TTTGTCCTTATCAGGGTCATCATC |
| hu HOXA9 fwd | ATGAGAGCGGCGGAGACA |
| hu HOXA9 rev | CGCGCATGAAGCCAGTT |
| hu MAT2A fwd | TCTTCCAAAGGTTTTGACTACAAGAC |
| hu MAT2A rev | AGCCAAACATTAAGCCCTGGT |
| huCDKN1A fwd | TGGAGACTCTCAGGGTCGAAA |
| huCDKN1A rev | CCGGCGTTTGGAGTGGTA |
| huLAMP5 fwd | CCAACCTTTTGACATTATCTCAGATTT |
| huLAMP5 rev | TTGCTCCCGCTCATCCA |
| huCXCR4 fwd | TGGAGGGGATCAGTATATACACTTCA |
| huCXCR4 rev | TCATAGTCCCCTGAGCCCATT |
| huCDK1 fwd | CCATTGACTAACTATGGAAGATTATACCA |
| huCDK1 rev | TGTCTACCCTTATACACAACTCCATAGG |

Table 2. Most differentially expressed genes of MLL-AF4 cells treated with PF-9366 compared to DMSO control. MLL-AF4 cells were treated with $15 \mu \mathrm{M}$ of PF-9366 or vehicle (DMSO) for 4 days and two biological replicates ( $\mathrm{n}=2$ ) were used for RNA-seq. The top up- and down-regulated genes ranked by adjusted p-value are shown.

| Gene | $\operatorname{logFC}$ | Adj. p-value |
| :---: | :---: | :---: |
| VCAN | -1.75424241 | $2.3502 \mathrm{E}-17$ |
| MALAT1 | 1.23960918 | $5.5077 \mathrm{E}-15$ |
| ABCG1 | 4.53786533 | $6.8832 \mathrm{E}-12$ |
| NEAT1 | 1.54858668 | $3.6131 \mathrm{E}-11$ |
| CDKN1A | 1.63818129 | $2.5831 \mathrm{E}-10$ |
| TARBP1 | 1.59297755 | $2.7678 \mathrm{E}-09$ |
| IFI6 | 1.54993958 | $8.3779 \mathrm{E}-08$ |
| PLAC8 | -1.31980931 | $8.3779 \mathrm{E}-08$ |
| MT2A | -2.17730764 | $1.1129 \mathrm{E}-07$ |
| SOX4 | -1.15971622 | $1.1281 \mathrm{E}-06$ |
| CXCR4 | -0.94062791 | $2.1118 \mathrm{E}-06$ |
| ACPP | -1.89200163 | $3.9511 \mathrm{E}-06$ |
| PLIN2 | 1.19252527 | $7.5285 \mathrm{E}-06$ |
| CTSC | -1.03995125 | $9.7484 \mathrm{E}-06$ |
| MT-TL1 | 0.96366607 | $4.3491 \mathrm{E}-05$ |
| MAT2A | 1.04809981 | $6.6571 \mathrm{E}-05$ |
| MT1H | -3.58994646 | $9.1747 \mathrm{E}-05$ |
| TYMP | 0.91988146 | 0.00017035 |
| MT-ND6 | 0.93029829 | 0.00017035 |
| GABPB1-AS1 | 1.16341383 | 0.00023592 |
| ACAT2 | -1.02169596 | 0.00039853 |
| ANP32E | -0.92615736 | 0.00105122 |
| OAZ1 | -0.67269662 | 0.00105122 |
| ABCA1 | 3.0159732 | 0.00163224 |
| IRF8 | -0.91266434 | 0.00181206 |
| HMGB2 | -0.73968999 | 0.00215973 |
| C19orf38 | -2.13047762 | 0.00335439 |
| TMEM97 | -1.25392963 | 0.00394383 |
| FGD2 | -2.05658855 | 0.00578347 |
| MAN2A2 | 1.07818151 | 0.00618254 |
| LINC00685 | 2.01729224 | 0.00813506 |
| CD14 | 0.70081371 | 0.00881832 |
| NGFR | -3.0884782 | 0.0089542 |
| MYLIP | 0.87693769 | 0.00903504 |
| VIM | -0.70787279 | 0.01359915 |
| RB1 | -1.27508179 | 0.01359915 |


| PLTP | 1.04604357 | 0.01359915 |
| :---: | :---: | :---: |
| LAPTM5 | 0.67118504 | 0.01690308 |
| MT1X | -1.56656477 | 0.019355 |
| SRRM2 | 0.58245028 | 0.01935625 |
| FASN | 0.66011712 | 0.01935625 |
| BCAT1 | -0.86734093 | 0.0226492 |
| SAPCD2 | -0.82305676 | 0.02379786 |
| FTH1 | 0.61891478 | 0.02473858 |
| ACTA2 | 1.74733019 | 0.02473858 |
| ETV1 | -3.27142883 | 0.02533037 |
| PSAT1 | -0.95821737 | 0.02533037 |
| JUN | 0.94619992 | 0.02547328 |
| MT1G | -2.49105465 | 0.03788124 |
| IRS2 | 0.70454355 | 0.03864554 |
| HTRA3 | 1.13739561 | 0.04771929 |
| TGFBI | -0.76339965 | 0.05345073 |
| CTSB | 0.60951319 | 0.05450355 |
| GOLGA8A | 1.13291029 | 0.05450355 |
| GGT5 | -2.08399121 | 0.05450355 |
| SLC20A1 | 0.71301397 | 0.05616531 |
| STMN1 | -0.55588437 | 0.06043213 |
| TMEM158 | 2.21094617 | 0.06052583 |
| CLEC5A | 1.85066641 | 0.06739138 |
| MDK | -3.43802962 | 0.06739138 |
| MDM2 | 0.59029142 | 0.06739138 |
| WSB1 | 0.6121163 | 0.06739138 |
| LAMP5 | -0.61469936 | 0.06739138 |
| CPNE7 | 1.65893052 | 0.07569192 |
| RNU4ATAC | -1.5679689 | 0.07666438 |
| RPS27L | 0.51840689 | 0.08337264 |
| TFRC | 0.65673972 | 0.0943774 |
| CDK1 | -0.72323939 | 0.0943774 |
| FURIN | 0.69614509 | 0.0943774 |
| KCTD5 | -0.82984697 | 0.0943774 |
| FDPS | -0.59437789 | 0.09754882 |
| DPYSL3 | -1.21154341 | 0.09754882 |
| WASF1 | 0.8929807 | 0.09786513 |
| ALDOC | -1.11734961 | 0.09805786 |

## Supplementary Figures



Figure S1. Expanded data for western blots including molecular weight marker as already shown in Figure 1C. Uncropped data figure for images shown in Figure 1C. Representative western blot analysis shows increased MAT2A expression in MLLr cells compared to culture expanded CD34+ huCB control cells. MAT2A protein is shown in (A). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control (B).


Figure S2. PF-9366 preferentially suppresses proliferation and viability of MLLr cell lines. (A) MLLr cell lines SEM and THP-1 or non-MLLr cell line SKM-1 were treated with increasing concentrations of PF9366 or vehicle (DMSO) for 6 days and relative cell count was determined by staining with Trypan blue. Pooled data of three biological replicates $(\mathrm{n}=3)$ performed in technical triplicates are shown. Calculated $\mathrm{IC}_{50}$ values are indicated. (B) Relative cell count was further confirmed by flow cytometry using counting beads. Pooled data of three biological replicates $(n=3)$ performed in technical triplicates are shown. Calculated $\mathrm{IC}_{50}$ values are indicated. (C) To assess proliferation curves, cell lines were again treated with PF-9366 (10 $\mu \mathrm{M}, 15 \mu \mathrm{M})$ or vehicle (DMSO) and total cell count was determined by Trypan blue staining every second day. The mean of pooled data of three biological replicates $(\mathrm{n}=3)$ performed in technical triplicates is shown. One-way ANOVA was used with Sidak correction: ${ }^{*}, p<0.05$. (D) Cell viability of cell lines upon inhibition treatment with PF-9366 (10 $\mu \mathrm{M}, 15 \mu \mathrm{M}$ ) or control (DMSO) was determined after 6 days via fluorescence-based read of alamarBlue assay. Experiment was performed in three biological replicates $(\mathrm{n}=3)$ and technical triplicates. Bars represent the mean. Error bars indicate SD. Oneway ANOVA was used with Sidak correction: *,$p<0.05$.


Figure S3. siRNA-mediated MAT2A knock-down.(A) Jurkat cells (non-MLLr), THP-1 (MLL-AF9) and CRISPR/Cas9-MLLr cells with $t(4 ; 11)$ and $t(9 ; 11)$ were nucleofected with MAT2A siRNA, non-target control siRNA (ctrl) or no siRNA (no). Fold change of MAT2A expression measured by RT-qPCR is displayed for day 1 and day 3 after nucleofection. Pooled data of two independent experiments ( $\mathrm{n}=2$ ) performed in technical triplicates are shown. Bars represent the mean. Error bars indicate SD. One-way ANOVA was used with Sidak correction: ${ }^{*}, p<0.05$. (B) Total cell amount was microscopically determined 3 days after nucleofection by Trypan blue staining. Pooled data of two independent
experiments $(\mathrm{n}=2)$ performed in technical duplicates are shown. Bars represent the mean. Error bars indicate SD. One-way ANOVA was used with Sidak correction: ${ }^{*}, p<0.05$. (C) Representative flow cytometry analyses of apoptotic cells 3 days after MAT2A siRNA knock-down determined by Annexin V staining are shown.


Figure S4. Expanded data for western blots including molecular weight marker as already shown in Figure 2D. Uncropped data figure for images shown in Figure 2D. Representative western blot analysis shows decreased histone methylation in MLLr cells upon PF-9366 treatment. Histone H3 was used as loading control.


Figure S5. Inhibition of MAT2A diminishes DNA synthesis and enhances apoptosis of MLLr cell lines. MLLr cell lines SEM and THP-1 or non-MLLr cell line SKM-1 were treated with PF-9366 (10 $\mu \mathrm{M}, 15$ $\mu M$ ) or vehicle (DMSO). (A) Pooled data (left) from three biological replicates ( $n=3$ ) performed in technical triplicates and representative (right) cell cycle analysis of single cells after 4 days is shown. Data were acquired using BrdU staining and flow cytometry. (B) Annexin V staining revealed a dosedependent translocation of phosphatidylserine to the outer leaflet of the plasma membrane in apoptotic cells after 6 days of treatment. Experiment was performed in three biological replicates ( $\mathrm{n}=3$ ) and technical triplicates. Pooled (left) and representative (right) data are shown. Bars or dots represent the mean. Error bars indicate SD. One-way ANOVA was used with Dunnett correction: *, $p<0.05$.


Figure S6: Validation of RNA-seq data via RT-qPCR. RNA-seq data displayed in Figure 5 were validated using RT-qPCR in CRISPR/Cas9-MLL-AF4 (M-4) and -AF9 (M-9) cells. Bars represent the mean. Error bars indicate SD. Student's t-test was used: *, $p<0.05$.






Figure S7. Combinational treatment with PF-9366 and chemotherapy or targeted therapy augments anti-leukemic effects. (A) MLL-AF4/-AF9 cells were treated with increasing concentrations of cytarabine (Ara-C) for 6 days and cell count was determined by flow cytometry using counting beads. The mean of two biological replicates $(\mathrm{n}=2)$ performed in technical triplicates is shown. Calculated IC50 value is indicated. (B) A scheme of the MLLr dependent MAT2A pathomechanism with SAM within the metabolic pathway influencing DOT1L and PRMT5 resulting in aberrant histone, DNA or protein methylation is displayed. (C) MLL-AF4/-AF9 cells were treated with increasing concentrations of EPZ004777 (DOT1Li) or (D) EPZ015666 (PRMT5i) and PF-9366 (MAT2Ai) for dose-response curves to determine IC50 values of single treatments. Additionally, dose-response curves of the combinational treatment with MAT2Ai at a constant drug ratio of 1:10 were analyzed. Relative cell count was determined by flow cytometry using counting beads and the mean is displayed. Experiments were performed in three biological replicates $(\mathrm{n}=3)$ and technical triplicates. IC50 values are indicated. Error bars indicate SD. For synergy calculation, IC50 values were used to display isobolograms at $50 \%$ inhibition levels and to calculate combination indices (CI) displayed in (E) and (F). $\mathrm{CI}<1$ indicates synergy.


Figure S8. PF-9366 in combination with chemotherapy or targeted therapies leads to an MLLr-specific additive effect validated with cell lines. Cell lines SKM-1 (A), SEM (B), THP-1 (C) were treated with 10 nM cytarabine (Ara-C), $10 \mu \mathrm{M}$ PF-9366 (MAT2Ai), $1 \mu \mathrm{M}$ DOT1Li (EPZ004777) and $1 \mu \mathrm{M}$ PRMT5i (EPZ015666) either as single agents or simultaneously for 2 or 6 days as indicated. Vehicle (DMSO) was used as control. Relative cell count was determined by flow cytometry using counting beads. Experiment was performed in three biological replicates $(\mathrm{n}=3)$ and technical duplicates. Apoptotic cells were detected using Annexin V staining after 6 days of respective treatments. Pooled data of three biological replicates $(\mathrm{n}=3)$ performed in technical duplicates are shown. Data were acquired using flow cytometry. Bars represent the mean. Error bars indicate SD. One-way ANOVA was used with Sidak correction: * $p<$ 0.05.


Figure S9. PF-9366 alone or in combination with targeted therapies has no negative impact on control cells. CD34+ control cells were treated with 10 nM cytarabine (Ara-C), $10 \mu \mathrm{M}$ PF-9366 (MAT2Ai), $1 \mu \mathrm{M}$ DOT1Li (EPZ004777) and $1 \mu \mathrm{M}$ PRMT5i (EPZ015666) either as single agents or simultaneously for 6 days. Vehicle (DMSO) was used as control. (A) Relative cell count was determined by flow cytometry using counting beads. Experiment was performed in three biological replicates $(\mathrm{n}=3)$ and technical duplicates. (B) Apoptotic cells were detected using Annexin V staining after 6 days of respective treatments. Pooled data of three biological replicates $(\mathrm{n}=3)$ performed in technical duplicates are shown. Data were acquired using flow cytometry. Bars represent the mean. Error bars indicate SD. One-way ANOVA was used with Dunnett correction: ${ }^{*}, p<0.05$.

