**Figure S1, related to Figure 1. Selective AML gene dependencies**

A, A heatmap of 225 selective AML dependencies reflecting probability of dependency inferred from the skewed LRT scores (Methods). Rows and columns are hierarchically clustered on Pearson correlation with complete linkage.

B, Kaplan-Meyer plots of overall survival in TCGA AML patients (Network 2013) with MEF2D and IRF8 expression above the 60\(^{th}\) percentile (red line) and below the 40\(^{th}\) percentile (blue line).

C, Kaplan-Meyer plots of overall survival in BeatAML patients (Tyner et al. 2018) with MEF2D and IRF8 expression above the 60\(^{th}\) percentile (red line) and below the 40\(^{th}\) percentile (blue line).
| Sample type | KMT2A status | KMT2A subtype |
|-------------|--------------|---------------|
| Primary AML | Non-KMT2Ar   |               |
|              | KMT2A point  |               |
|              | KMT2A PTD     |               |
|              | KMT2A translocation (partner unknown) |   |
|              | KMT2A multi  |               |

| Similarity |
|------------|
| 0          |
| 0.25       |
| 0.5        |
| 0.75       |
| 1          |

| Sample type | KMT2A status | KMT2A subtype |
|-------------|--------------|---------------|
| Primary AML | Non-KMT2Ar   |               |
|              | KMT2A point  |               |
|              | KMT2A PTD     |               |
|              | KMT2A translocation (partner unknown) |   |
|              | KMT2A multi  |               |

| Similarity |
|------------|
| 0          |
| 0.25       |
| 0.5        |
| 0.75       |
| 1          |
**Figure S2, related to Figure 2. A superenhancer-based classification of AML: primary samples and PDX models.** The heatmap is a similarity matrix based on AML classification in Figure 2B. Primary and PDX samples are hierarchically clustered with complete linkage using Pearson correlation of the scores of 4798 superenhancers recurrent in at least 2 samples. The bars at the top reflect presence of coding mutations (grey color designates unknown) and sample type.
Figure S3, related to Figure 2. A superenhancer-based classification of AML: primary samples and PDX models. The heatmap is a signal matrix (a full version of the AML classification in Figure 2B). Primary and PDX samples are hierarchically clustered with complete linkage using Pearson correlation of the scores of 4798 superenhancers recurrent in at least 2 samples, forming 6 distinct clusters. The bars at the top reflect presence of coding mutations (grey color designates unknown) and sample type. The side plot reflects $p$-values of non-random distribution of somatic mutations across the 6 clusters, calculated by the one-sided chi-square test, demonstrating that KMT2A rearrangements are the only mutation that shows significant enrichment in any cluster ($p<0.05$).
Figure S4

[Heatmap diagram showing the expression levels of various genes across different cell lines.]

- **Sample type**: KMT2A status
- **KMT2A subtype**: KMT2A PTD
- **KMT2A translocation (partner unknown)**
- **Unknown**
- **Cell line**: F36P, HEL, HEL9217, TF1, UT7, Kasumi1, SKNO1, KG1, U937, NB4, HL60, PL8985
- **KMT2A type**: Non-KMT2Ar, KMT2A point, KMT2A PTD, KMT2A-ARFΔN, KMT2A-ARFΔF1, KMT2A-Δ del, KMT2A-ARFΔΔN, KMT2A-MLT-10, KMT2A-ARFΔΔN, KMT2A-MLT-3, KMT2A-MLT-3 (partner unknown), Unknown
- **Similarity**: 0, 0.5, 1
Figure S4, related to Figure 2. A superenhancer-based classification of AML: cell lines. The heatmap is a similarity matrix of the cell lines clustered with complete linkage using Pearson correlation of the scores of 4798 superenhancers recurrent in at least 2 samples. The heatmap reflects pairwise Pearson correlation of superenhancer scores. The bars at the top reflect presence of coding mutations and sample type.
Figure S5, related to Figures 2 and 3. Mutational landscape and survival profile of KMT2Ar-like AML.

A, Enrichment of somatic mutations in KMT2Ar-like AMLs from the BeatAML cluster 7 (defined by mRNA expression of core regulatory TFs; Figure 3A). The bar plot demonstrates p-values calculated by chi-square test of mutation distribution between KMT2Ar-negative samples in cluster 7 (i.e. KMT2Ar-like) versus KMT2Ar-negative samples in all other clusters. Only FLT3 mutations are significantly over-represented in cluster 7, with an enrichment value of 1.7-fold compared to randomly expected distribution. The 38 most common point mutations and translocations from the BeatAML study were included in this analysis and are visualized in the order of decreasing overall frequency. Patients with KMT2Ar AML were excluded from the analysis.

B, Kaplan-Meyer plot of overall survival of the BeatAML patients (Tyner et al. 2018), demonstrating no significant difference in survival of patients from cluster 7, including KMT2Ar and KMT2Ar-like AML, compared to other clusters.

C, Kaplan-Meyer plot of overall survival in BeatAML patients (Tyner et al. 2018), demonstrating no significant difference in survival of KMT2Ar-like AML patients from cluster 7 compared to other clusters. KMT2Ar AMLs were excluded from the analysis.
Figure S6

A. IRF8 degradation

B. MEF2D degradation

C. TF Knockout
Figure S6, related to Figures 5-7. Application of targeted TF degradation to elucidate direct transcriptional effects of MEF2D and IRF8.

A, Targeted degradation of IRF8 results in loss of cell viability. Cells carrying a fusion-based IRF8 degron were incubated with DMSO vs. dTAG'1 and cell viability was followed by quantification of ATP pools using a luciferin-based assay.

B, Targeted degradation of MEF2D results in loss of cell viability. The experiment was carried out as in (B).

C, CRISPR/Cas9 knockout of MEF2D results in a loss of cell viability that is quantitatively similar to the effects of MEF2D degradation. MYB knockout was used as positive control.
Figure S7

A

B

C

D

E

Hours: 0 2 4 8 24 48 72 0 2 4 8 24 48 72

DMSO dTAG-1

MEF2D

MEF2C

β-actin
Figure S7, related to Figure 5. Volcano plots of SLAM-seq and mRNA-seq following MEF2D degradation, and regulation of MEF2C by MEF2D. Core regulatory TFs are highlighted in blue.

A, SLAM-seq 2 hours after MEF2D degradation.
B, SLAM-seq 24 hours after MEF2D degradation.
C, mRNA-seq 2 hours after MEF2D degradation.
D, mRNA-seq 24 hours after MEF2D degradation.
E, Western blot showing reduced MEF2C levels after prolonged MEF2D degradation.
### A

| Gene ID | 2 h SLAM-seq | 24 h SLAM-seq | 2 h RNA-seq | 24 h RNA-seq |
|---------|---------------|---------------|-------------|-------------|
| FUT4    | 0.009         | 0.936         | 0.087       | 0.087       |
| ZFP96   | 0.008         | 0.012         | 0.129       | 0.129       |
| BHLHE40 | 0.001         | 0.141         | 0.167       | 0.167       |
| PTGER4  | 0.011         | 0.143         | 0.167       | 0.167       |
| KLF10   | 0.002         | 0.101         | 0.129       | 0.129       |
| MYC     | 1.900         | 1.936         | 1.936       | 1.936       |
| F3      | 0.141         | 0.370         | 0.060       | 0.060       |
| TNFAP3  | 0.003         | 0.093         | 0.117       | 0.117       |
| TXNIP   | 0.438         | 0.449         | 0.507       | 0.507       |
| PMAPI   | 0.050         | 0.024         | 0.034       | 0.034       |
| PIK1    | 0.081         | 0.039         | 0.046       | 0.046       |
| KLF2    | 0.124         | 0.209         | 0.047       | 0.047       |
| ID2     | 0.204         | 0.507         | 0.060       | 0.060       |
| TP53R   | 0.908         | 0.843         | 0.060       | 0.060       |
| KLF6    | 5.0E-04       | 0.222         | 0.060       | 0.060       |
| KLF11   | 0.109         | 0.228         | 0.060       | 0.060       |
| CDCA4/1B| 0.354         | 0.375         | 0.060       | 0.060       |
| CDCA2/3P| 0.012         | 0.023         | 0.060       | 0.060       |
| ZBTB33  | 0.032         | 0.103         | 0.060       | 0.060       |
| RBM16   | 0.038         | 0.120         | 0.060       | 0.060       |
| CPEB2   | 0.054         | 0.293         | 0.060       | 0.060       |
| GAT2    | 0.003         | 0.531         | 0.060       | 0.060       |
| DUSP6   | 0.005         | 0.037         | 0.060       | 0.060       |

### B

| Gene ID | 2 h SLAM-seq | 24 h SLAM-seq | 2 h RNA-seq | 24 h RNA-seq |
|---------|---------------|---------------|-------------|-------------|
| MYC     | 1.900         | 1.936         | 1.936       | 1.936       |
| LMO2    | 0.013         | 0.032         | 0.129       | 0.129       |
| ZEB2    | 0.951         | 0.914         | 0.129       | 0.129       |
| HOXA9   | 0.350         | 0.375         | 0.129       | 0.129       |
| MYB     | 0.999         | 1.466         | 0.129       | 0.129       |
| RPS1    | 0.036         | 0.047         | 0.129       | 0.129       |
| RUNX1   | 0.167         | 0.281         | 0.129       | 0.129       |
| E2F3    | 0.376         | 0.414         | 0.129       | 0.129       |
| ETV6    | 0.014         | 0.038         | 0.129       | 0.129       |
| GFI1    | 0.019         | 0.095         | 0.129       | 0.129       |
| ZMYND8  | 0.520         | 0.875         | 0.129       | 0.129       |
| MEF2D   | 0.877         | 0.878         | 0.129       | 0.129       |
| MAX     | 0.385         | 0.425         | 0.129       | 0.129       |
| FLI1    | 0.318         | 0.381         | 0.129       | 0.129       |
| SPI1    | 0.943         | 0.967         | 0.129       | 0.129       |
| STAT5B  | 0.989         | 0.820         | 0.129       | 0.129       |
| SREBF1  | 0.898         | 0.820         | 0.129       | 0.129       |
| TRAF4   | 0.841         | 0.959         | 0.129       | 0.129       |
| ZNF210  | 0.074         | 0.185         | 0.129       | 0.129       |
| LY1     | 0.142         | 0.260         | 0.129       | 0.129       |
| FOSS2   | 0.400         | 0.428         | 0.129       | 0.129       |
| GATA2   | 0.051         | 0.149         | 0.129       | 0.129       |
| HHEX    | 0.127         | 0.253         | 0.129       | 0.129       |
Figure S8, related to Figure 5. A table summary of SLAM-seq and RNA-seq data following MEF2D degradation

A. Genes demonstrating a statistically significant change in transcription rate (SLAM-seq adjusted p-value <0.1) at 2 hours following MEF2D degradation.

B. Changes in core regulatory TF transcription after MEF2D degradation.
Figure S9

A. Gene set enrichment terms ranked by p-val

B. Gene expression data

C. Table: JQ1, µM

|          | 12.5 | 6.25 | 3.125 | 1.563 | 0.781 | 0.391 |
|----------|------|------|-------|-------|-------|-------|
| YKL-05-009, nM |      |      |       |       |       |       |
| 400      | 0.05 | 0.091| 0.022 | -0.05 | 0.05  | 0.031 |
| 200      | 0.08 | 0.136| 0.018 | 0.058 | 0.064 | 0.06  |
| 100      | 0.049| 0.048| 0.059 | -0.0  | 0.14  | 0.076 |
| 50       | 0.029| 0.024| 0.043 | 0.015 | 0.064 | 0.06  |
| 25       | 0.057| 0.045| 0.039 | -0.01 | 0.17  | 0.136 |
| 12.5     | 0.049| 0.068| 0.07  | 0.011 | 0.069 | 0.072 |

D. Table: MYC861, µM

|          | 3.125 | 1.563 | 0.781 | 0.391 | 0.195 | 0.098 |
|----------|-------|-------|-------|-------|-------|-------|
| YKL-05-009, nM |      |      |       |       |       |       |
| 400      | -0.05 | 0.03 | 0.089 | 0.102 | 0.133 | 0.032 |
| 200      | -0.04 | 0.095| 0.091 | 0.145 | 0.095 | 0.034 |
| 100      | 0.033 | 0.016| 0.037 | 0.079 | 0.15  | 0.072 |
| 50       | -0.04 | -0.02 | 0.252 | 0.063 | 0.042 | 0.05  |
| 25       | 0.04  | 0.039| 0.093 | 0.06  | 0.059 | 0.038 |
| 12.5     | 0.034 | 0.005| 0.029 | 0.023 | 0.044 | 0.052 |

G. Relative viability

H. Relative viability
**Figure S9, related to Figure 5. A functional interaction between MYC and MEF2D**

A, Gene set enrichment analysis of the genes showing significant changes in transcription rates after MEF2D degradation using Ehrichr, filtered to display only human-derived data (Kuleshov et al. 2016).

B, Intersection of gene sets demonstrating significant changes in transcription (adjusted p-value <0.05) measured by SLAM-seq after MEF2D degradation and indirect MEF2C/D inhibition by YKL-05-099 treatment.

C, Excess over bliss synergy matrix demonstrating synergy between SIK/MEF2 inhibitor YKL-05-099 and bromodomain inhibitor JQ1. Red color demonstrates synergy.

D, Excess over bliss synergy matrix demonstrating synergy between SIK/MEF2 inhibitor YKL-05-099 and MYC inhibitor MYCi361. Red color demonstrates synergy.

E, Cloning strategy for a doxycycline-inducible lentiviral vector expressing MYC-P2A-TagBFP.

F, Induction of MYC expression demonstrated by TagBFP fluorescence after addition of doxycycline.

G, Forced exogenous expression of MYC fails to rescue the cell viability loss following MEF2D degradation.

H, As a positive control, forced exogenous expression of MYC completely rescues MYC knockout by CRISPR/Cas9. Exogenous MYC expression was induced by doxycycline.
Figure S10

A. SLAM-seq Log2 FC at 2h vs. Log10 p-value plot.

B. RNA-seq Log2 FC at 2h vs. Log10 p-value plot.

C. SLAM-seq Log2 FC at 2h vs. RNA-seq Log2 FC at 2h.

D. RNA-seq Log2 FC at 24h vs. Log10 p-value plot.

E. RNA-seq Log2 FC at 24h vs. RNA-seq Log2 FC at 2h.

F. SLAM-seq Log2 FC at 2h vs. RNA-seq Log2 FC at 2h with correlation coefficient r = 0.11.

G. Number of genes with Log2 FC at 2h vs. Log2 FC at 24h.

H. Western blot analysis of IRF8, MEF2D, MEF2C, and β-actin.

I. Expression of AAAS1 and IRF8 KO conditions.
Figure S10, related to Figure 7. Targeted degradation of IRF8

A, Volcano plot of SLAM-seq 2 hours after IRF8 degradation.

B, Volcano plot of SLAM-seq 24 hours after IRF8 degradation. Core regulatory TFs are highlighted in blue.

C, Volcano plot of RNA-seq 2 hours after IRF8 degradation. Core regulatory TFs are highlighted in blue.

D, Volcano plot of RNA-seq 24 hours after IRF8 degradation. Core regulatory TFs are highlighted in blue.

E, A cross-plot of genome-wide changes in mRNA pools measured after 2 vs. 24 hours of IRF8 degradation demonstrates a poor correlation between early and late transcriptional response.

F, A cross-plot of genome-wide changes in mRNA pools (mRNA-seq) vs. transcription rates (SLAM-seq) measured after 2 hours of IRF8 degradation.

G, A distribution plot of genome-wide changes in nascent transcription rates (SLAM-seq) and mRNA pools (RNA-seq) after 2 and 24 hours of IRF8 degradation.

H, Western blot showing reduced MEF2C and MEF2D levels after IRF8 degradation.

I, Western blot showing reduced MEF2C and MEF2D levels 24 hours after IRF8 knockout.
| Gene ID | MV14147 dep. probability | 2 hrs SLAM-seq | 24 hrs SLAM-seq | 2 hrs RNA-seq | 24 hrs RNA-seq |
|----------|---------------------------|----------------|----------------|---------------|---------------|
| C9orf21  | -0.089                    | -1.672         | 9.9E-28        | 4.4E-24       | -2.795        |
| MEF2D    | 0.139                     | 6.7E-27        | 1.5E-23        | 2.118         | 4.6E-13       |
| FAM107B  | 4.0E-04                   | 2.153          | 3.2E-13        | 1.241         | 0.002         |
| CCR2     | 0.369                     | 2.247          | 6.9E-16        | 3.951         | 0.001         |
| DCAPN1   | 0.014                     | 0.002          | 0.003          | 1.830         |
| KCNMA3   | 0.017                     | -3.755         | 5.2E-11        | 5.965         | 1.8E-06       |
| CBX6     | 0.018                     | -0.989         | 7.7E-11        | -1.325        |
| TIFAB    | 0.006                     | 0.019          | 0.003          | -1.478        |
| LDLR     | 0.021                     | -0.526         | 5.0E-10        | 2.5E-07       |
| MALAT1   | 0.856                     | 1.4E-07        | 0.002          | 0.220         |
| CCR1     | 0.386                     | 1.6E-06        | 3.6E-06        | -1.633        |
| CPM      | 0.017                     | -0.502         | 1.1E-05        | 1.815         |
| KLF1     | 0.012                     | 0.003          | 0.003          | 0.532         |
| SERPINB8 | 0.005                     | -1.678         | 4.9E-05        | -1.501        |
| POUI2F2  | 0.009                     | -0.519         | 0.045          |
| ABHD15   | 0.609                     | -1.336         | 3.6E-10        | -1.321        |
| IL6R     | 0.015                     | -0.526         | 5.0E-10        | 0.002         |
| TRB1     | 0.020                     | 0.004          | 0.003          | 0.004         |
| SCD      | 0.919                     | 0.005          | 0.005          | 0.005         |
| HMACS1   | 0.055                     | 1.003          |
| OPN3     | 0.013                     | 2.046          |
| ADGRC5   | 0.014                     | 0.006          |
| CSF1R    | 0.017                     | -0.519         |
| MSMO1    | 0.022                     | 0.006          |
| TGFBR1   | 0.030                     | 0.007          |
| P2RY2    | 0.006                     | 0.007          |
| DDIT3    | 0.067                     | 2.116          |
| ZBTB33   | 0.031                     | -0.502         |
| B3GNT7   | 0.009                     | 0.004          |
| CALR     | 0.010                     | 0.007          |
| DUSP7    | 0.016                     | 0.004          |
| INHBA    | 0.014                     | 0.007          |
| JUN      | 0.374                     | 1.026          |
| SH2B3    | 0.014                     | 1.070          |
| LIN2     | 0.007                     | 0.007          |
| TIFA     | 0.050                     | 0.007          |
| ZNF616   | 0.005                     | 0.002          |
| RNF41    | 0.010                     | 0.002          |
| EGR1     | 0.008                     | 0.007          |
| HELQ     | 0.010                     | 0.002          |
| BINS-T1  | 1.691                     |
| MBP      | 0.020                     | 0.003          |
| ZNF785   | 3.5E-04                   | 1.544          |
| KLF2     | 0.020                     | 0.002          |
| HES6     | 0.007                     | 0.003          |
| RHOU     | 0.030                     | 0.009          |
| TME1D10  | 0.483                     |
| BCL2     | 0.576                     | 0.064          |
| FLVC2    | 0.030                     | -0.611         |
| CRKL     | 0.802                     |
| HNRNPA3  | 0.138                     | 0.025          |
| LRC38C   | 0.547                     | -0.817         | 6.8E-04        | 0.055         |

Figure S11: MV14147 drop out score for different genes.
| Gene ID | MV411 dep. probability | MV411 drop out score | log$_2$ FC | pvalue | padj | log$_2$ FC | pvalue | padj | log$_2$ FC | pvalue | padj | log$_2$ FC | pvalue | padj |
|---------|------------------------|----------------------|-------------|--------|------|-------------|--------|------|-------------|--------|------|-------------|--------|------|
| LINC00599 | 0.849 | 7.3E-04 | 0.059 | 0.435 | 0.169 | 0.562 | 0.725 | 0.212 | 0.715 | 0.685 | 0.010 | 0.167 |
| NLRP3   | 0.002 | 0.130 | -1.080 | 8.1E-04 | 0.063 | -1.668 | 1.8E-04 | 0.027 | -0.688 | 7.2E-04 | 0.071 | -0.959 | 1.4E-08 | 2.0E-06 |
| ZNF189  | 5E-04 | 0.229 | -0.858 | 8.2E-04 | 0.063 | -1.239 | 0.036 | 0.347 | -0.349 | 0.056 | 0.603 | 0.110 | 0.787 | 0.960 |
| GLUL    | 0.002 | 0.122 | 0.357 | 9.0E-04 | 0.066 | 0.521 | 0.058 | 0.383 | 0.141 | 0.191 | 0.708 | 1.087 | 1.1E-05 | 8.1E-04 |
| FADS1   | 0.008 | 0.034 | 0.710 | 9.1E-04 | 0.066 | -0.743 | 0.145 | 0.531 | -0.093 | 0.419 | 0.809 | 0.232 | 0.022 | 0.261 |
| MIR9-3HG | 0.780 | 9.1E-04 | 0.066 | -0.337 | 0.355 | 0.720 | 0.125 | 0.467 | 0.826 | 0.078 | 0.607 | 0.903 |
| GORASP1 | 0.591 | -0.207 | 1.392 | 8.9E-04 | 0.066 | 0.727 | 0.366 | 0.303 | 0.040 | 0.542 | 0.998 | 2.1E-11 | 4.9E-09 |
| ZNF766  | 0.001 | 0.215 | -0.591 | 9.9E-04 | 0.068 | -0.855 | 0.020 | 0.270 | 0.040 | 0.735 | 0.925 | 0.120 | 0.454 | 0.846 |
| TMEM70  | 0.087 | -0.203 | -0.996 | 0.001 | 0.072 | -1.093 | 0.029 | 0.312 | -0.213 | 0.248 | 0.735 | -0.138 | 0.544 | 0.880 |
| GNA13   | 0.003 | 0.073 | -0.838 | 0.001 | 0.072 | -1.282 | 0.011 | 0.208 | -0.540 | 0.008 | 0.291 | -0.629 | 0.242 | 0.717 |
| KCNQ1OT1 | 0.770 | 0.001 | 0.073 | 0.888 | 0.823 | 0.946 | 0.808 | 0.003 | 0.193 | -0.002 | 0.994 | 0.999 |
| RGS16   | 0.003 | 0.080 | 0.657 | 0.001 | 0.078 | -0.988 | 0.005 | 0.149 | 0.694 | 0.001 | 0.100 | -0.649 | 0.123 | 0.570 |
| NBSG1   | 0.005 | 0.046 | 0.584 | 0.001 | 0.079 | -0.431 | 0.227 | 0.609 | 0.705 | 0.000 | 0.004 | -0.100 | 0.727 | 0.940 |
| RCSD1   | 0.000 | -0.164 | -0.778 | 0.001 | 0.080 | -0.990 | 0.027 | 0.310 | -0.279 | 0.018 | 0.405 | -0.022 | 0.923 | 0.987 |
| HNRRPK2B1 | 0.314 | -0.379 | 0.585 | 0.001 | 0.086 | 0.488 | 0.189 | 0.571 | -0.034 | 0.775 | 0.938 | 0.109 | 0.420 | 0.832 |
| CXCL8   | 0.028 | -0.094 | 0.819 | 0.001 | 0.086 | -0.134 | 0.722 | 0.904 | 0.898 | 1.6E-05 | 0.004 | 0.062 | 0.902 | 0.984 |
| DMXL2   | 0.091 | -0.207 | 1.322 | 0.001 | 0.090 | 0.189 | 0.723 | 0.904 | -0.009 | 0.952 | 0.990 | 0.547 | 0.222 | 0.699 |
| BNIP2   | 0.060 | -0.164 | -0.792 | 0.002 | 0.093 | -0.811 | 0.025 | 0.298 | -0.523 | 0.015 | 0.388 | -0.325 | 0.350 | 0.799 |
| RAB11FIP1 | 3E-04 | 0.279 | -0.560 | 0.002 | 0.097 | -0.832 | 0.002 | 0.113 | -0.352 | 4.7E-04 | 0.052 | -0.210 | 0.221 | 0.697 |
Figure S11, related to Figure 7. A table summary of SLAM-seq and RNA-seq data following IRF8 degradation. Genes demonstrating a statistically significant change in transcription rate (SLAM-seq adjusted p-value <0.1) at 2 hours following IRF8 degradation are presented.
Figure S12

A

H3K27ac HiChIP

SE1

SE2

chr16:85,879,263

chr16:86,042,950

10 rpm/bp

H3K27ac (MV411)

IRF8

E1

E2

E3

E4

E5 Ctrl

MED1

IRF8

MEF2D

MEF2C

RUNX2

MEIS1

Direct KMT2A fusion targets

Distance from TSS, kb

Non-KMT2A

KMT2A

B

IRF8

Relative IRF8 mRNA expression

Ctrl

E1

E2

E3

E4

E5

Distance from TSS, kb

C

% of GFP cells

D

Normalized IRF8 mRNA counts

p = 4.35\times 10^{-6}

p = 0.527

p = 0.029

E

MEIS1 KO

RUNX2 KO

AAVS1 control rep.1

AAVS1 control rep.2

AAVS1 control rep.3

MEIS1 KO rep.1

MEIS1 KO rep.2

MEIS1 KO rep.3

MEIS1

RUNX1

RUNX2

β-actin
**Figure S12**, related to figure 7. Structure of the IRF8 locus and mechanism of its activation in KMT2A-rearranged AML.

A, H3K27 acetylation, TF binding and 2D structure at the IRF8 locus. The top two tracks are H3K27ac metatracks representing primary AMLs. *Red*, tracks from pediatric patients with KMT2Ar AML (*n*=10); *grey*, tracks from the pediatric patients with non-KMT2Ar AML (*n*=9). Each metatrack is a collection of semi-transparent area plots representing individual samples and the average profile is represented by a thick line. The rest of the ChIP-seq tracks represent TF binding and H3K27 acetylation profiles in the MV411 cell line. Orange tracks represent TFs known to be the direct targets of KMT2A fusion oncoproteins and hypothesized to play a role in inducing IRF8 expression. Shaded areas represent 2 superenhancers associated with the IRF8 locus (SE1 and SE2). E1-E5 represent the constituent enhancers selected for the dCas9-KRAB-MeCP2 enhancer interference experiment. Superenhancer-promoter loops are demonstrated by the H3K27ac HiChIP and Micro-C 2D contact maps obtained in MV411 cells.

B, MV411 cells stably expressing dCas9-KRAB-MECP2 were transduced with lentiviral vectors encoding gRNAs targeting the E1-E5 enhancers highlighted in panel A. Expression of IRF8 was measured by RT-qPCR.

C, Viability of MV411 cells stably expressing dCas9-KRAB-MECP2 and transduced with GFP-expressing lentiviral vectors encoding gRNAs targeting the indicated enhancers was measured as fraction of GFP-positive cells.

D, Knockouts of the 3 core regulatory TFs directly activated by KMT2A fusion oncoproteins do not decrease IRF8 expression. Expression of IRF8 was measured by RNA-seq 72 hours following electroporation of MV411 cells with pre-assembled Cas9/sgRNA complexes targeting the indicated TF genes. A guide RNA targeting the AAVS1 “safe harbor” locus was used as a negative control. P-values were calculated by BH-adjusted Wald test.
E, Validation of gene knockouts by Western blotting. MV411 cells were electroporated with pre-assembled Cas9/sgRNA complexes targeting the indicated genes and protein depletion was verified by Western blot 72 hours post electroporation. Paralog-specific knockout of RUNX2 is shown. Western blot visualizing the MEF2C knockout is found in Figure 6H.
Differentiation of Hematopoietic cells in mRNA space
(data from Corces et al., 2016)

B

C

Correlation between Myeloid index and IRF8 mRNA expression log (TPM+1)

$\tau = 0.562$

$p = 6.65 \times 10^{-39}$
Figure S13, related to figure 7. Expression of IRF8 correlates with the myeloid differentiation state.

A. Expression of IRF8 during normal human hematopoiesis. The plot was generated using the Bloodspot database with primary data from GSE42519 (Bagger et al. 2016; Rapin et al. 2014).

B. Normal progenitors and leukemic cells are plotted according to their indices of myeloid and lymphoid development using primary data from (Corces et al. 2016). A myeloid differentiation index, computed from the mRNA expression of 19 cell type-specific markers (Methods), correctly reproduced both the normal myeloid trajectory and the functional AML hierarchy.

C. Expression of IRF8 correlates with the myeloid differentiation index in the BeatAML dataset. KMT2A-rearranged and KMT2A-like leukemia samples are color-coded as shown.
Supplementary Figure References

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