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Discovery of IRF8 as a potential selection biomarker for FHD-609, a degrader of BRD9, in preclinical models of acute myeloid leukemia (AML)

Epicypher 2023

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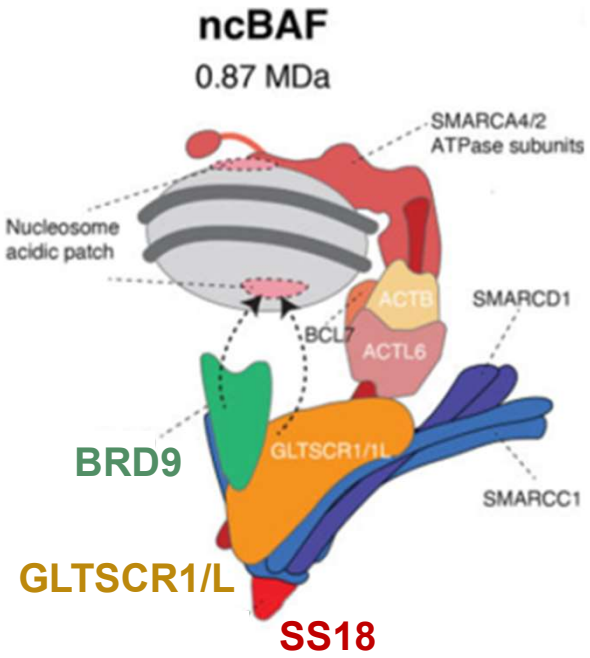
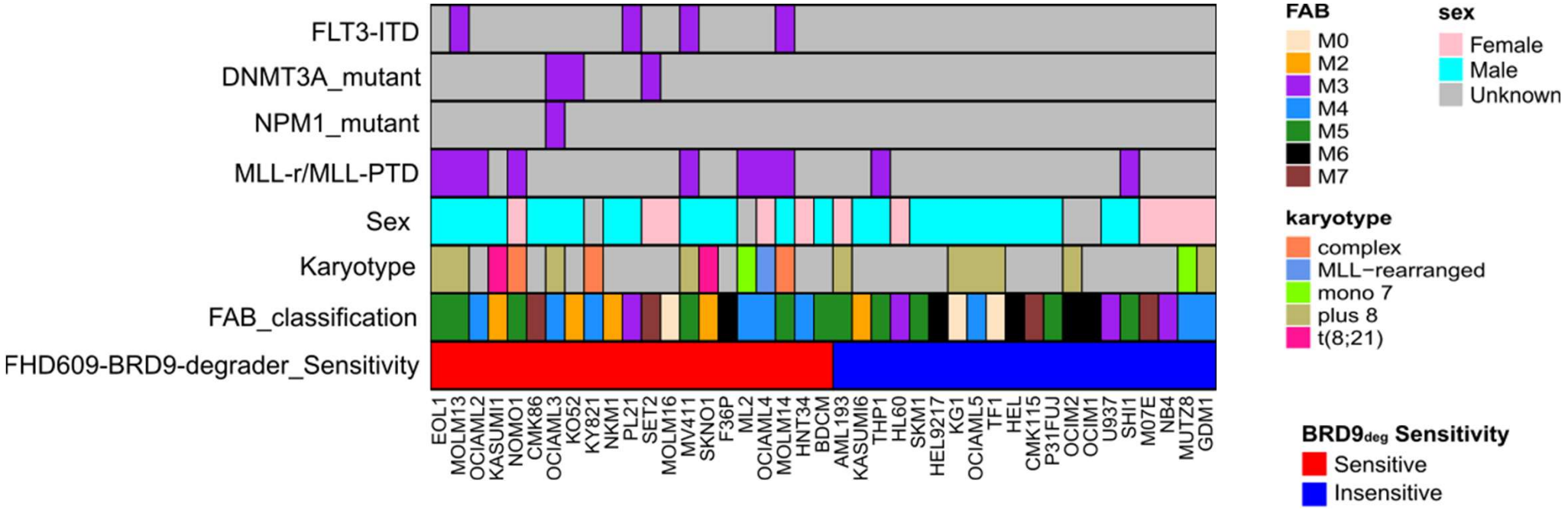
Introduction to BRD9

- Bromodomain-containing protein 9 (BRD9) is a component of the ncBAF chromatin remodeling complex
- BRD9 is a well-established target in Synovial Sarcoma (SS18-SSX fusion) and SMARCB1-loss cancers
- BRD9 inhibitors have recently shown activity in a subset of AML (acute myeloid leukemia) cell lines (Weisberg et al 2022; Hohmann et al 2016; Zhou et al 2021)
- FHD-609 is a clinical stage potent and selective degrader of BRD9
- Identifying subset of AML patients most likely to respond to FHD-609 is critical

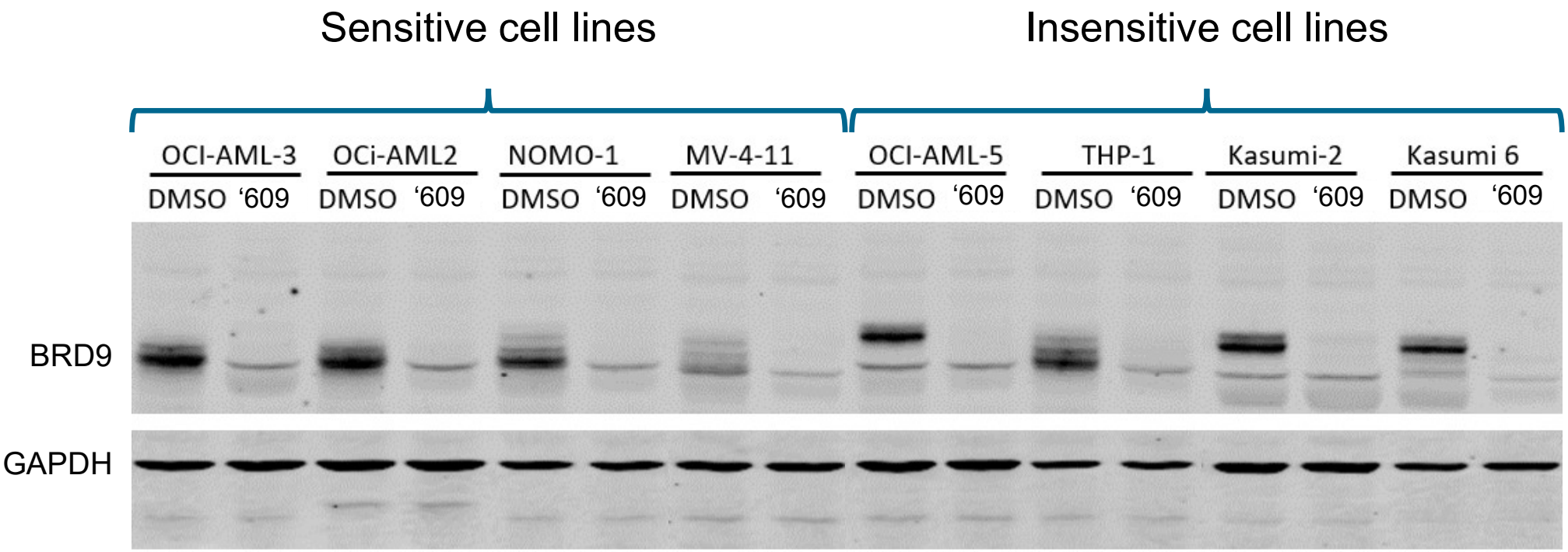


Response of AML cell lines to FHD-609 BDR9 degrader is not predicted by FAB class, karyotype or mutational status

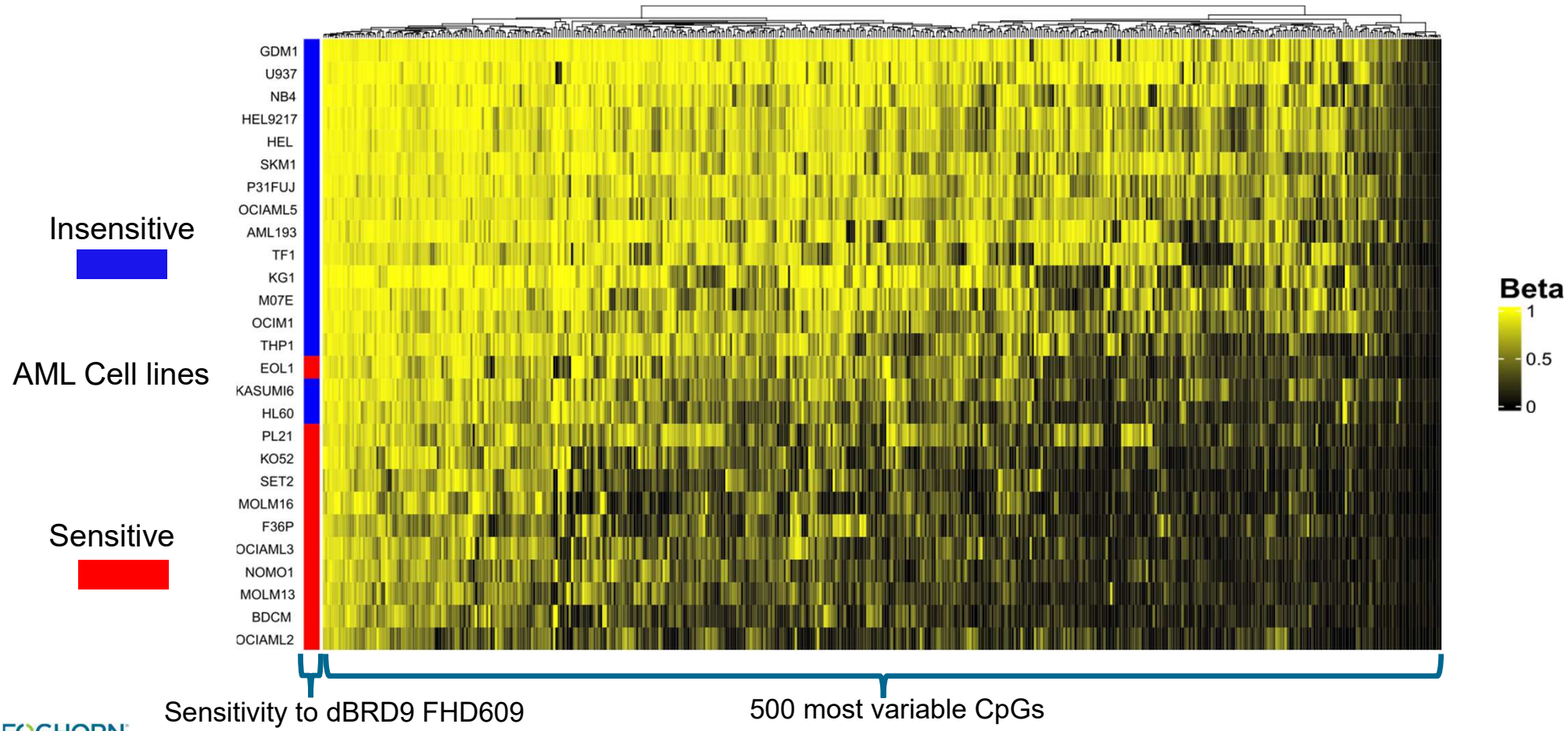


FAB = French American British classification (based on morphology)

FHD-609 degrades BRD9 in all AML cell lines



Strong correlation between CpG sites' DNA methylation status and BRD9-degrader sensitivity



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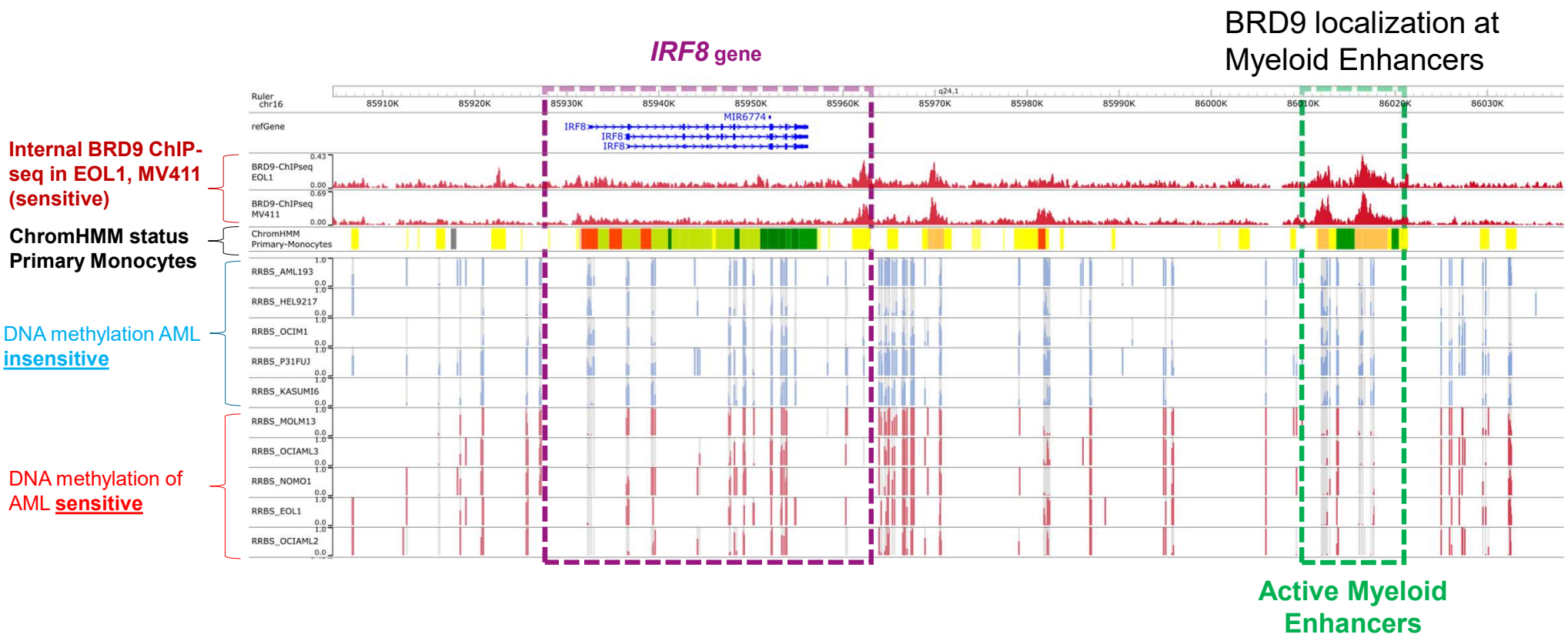
IRF motif is enriched at methylation sites that correlate with sensitivity to BRD9 degrader

The most enriched motifs nearby are also myeloid-relevant TFs:

Rank	Motif	Name
1		T1ISRE(IRF)/ThioMac-Ifnb-Expression/Homer
2		NFAT:AP1(RHD,bZIP)/Jurkat-NFATC1-ChIP-Seq(Jolma_et_al.)/Homer
3		NFIL3(bZIP)/HepG2-NFIL3-ChIP-Seq(Encode)/Homer
4		IRF3(IRF)/BMDM-Irf3-ChIP-Seq(GSE67343)/Homer

BRD9 co-localizes at myeloid enhancers for IRF8

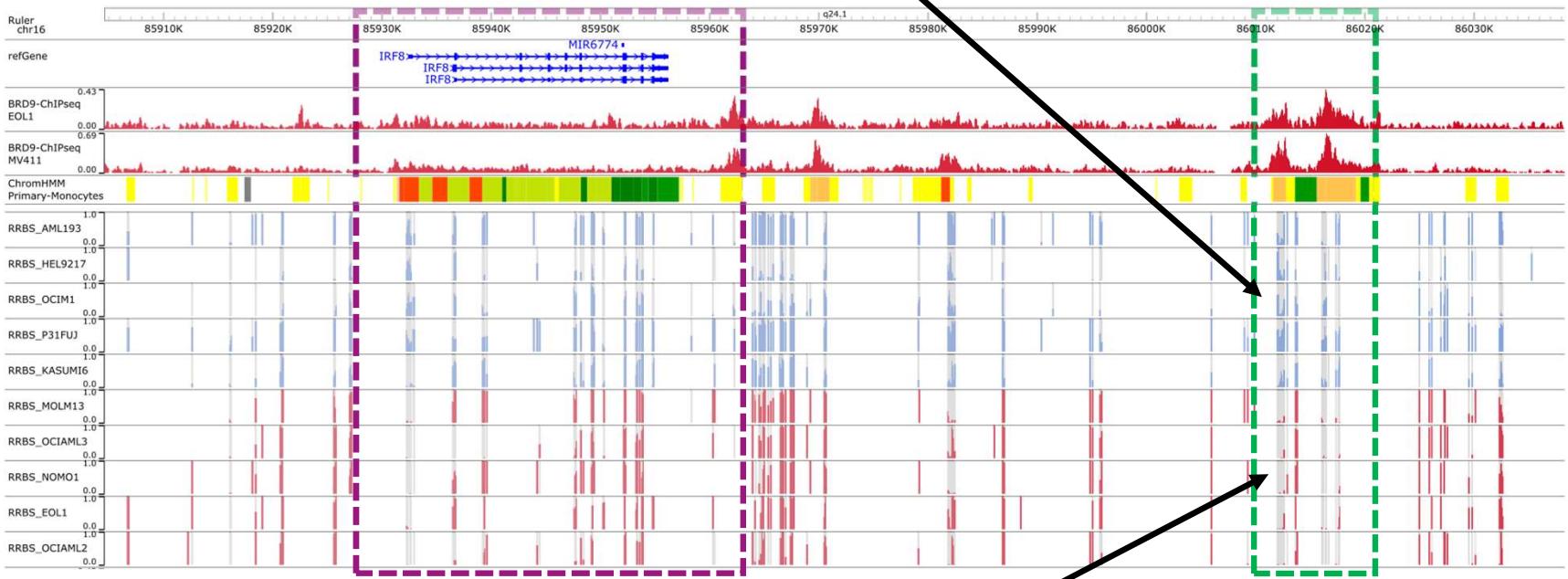


BRD9 co-localizes at myeloid enhancers for IRF8

Insensitive cell lines have higher methylation

BRD9 localization at Myeloid Enhancers

***IRF8* gene**



Internal BRD9 ChIP-seq in EOL1, MV411 (sensitive)

ChromHMM status Primary Monocytes

DNA methylation AML **insensitive**

DNA methylation of AML **sensitive**

Active Myeloid Enhancers

Sensitive cell lines have lower methylation

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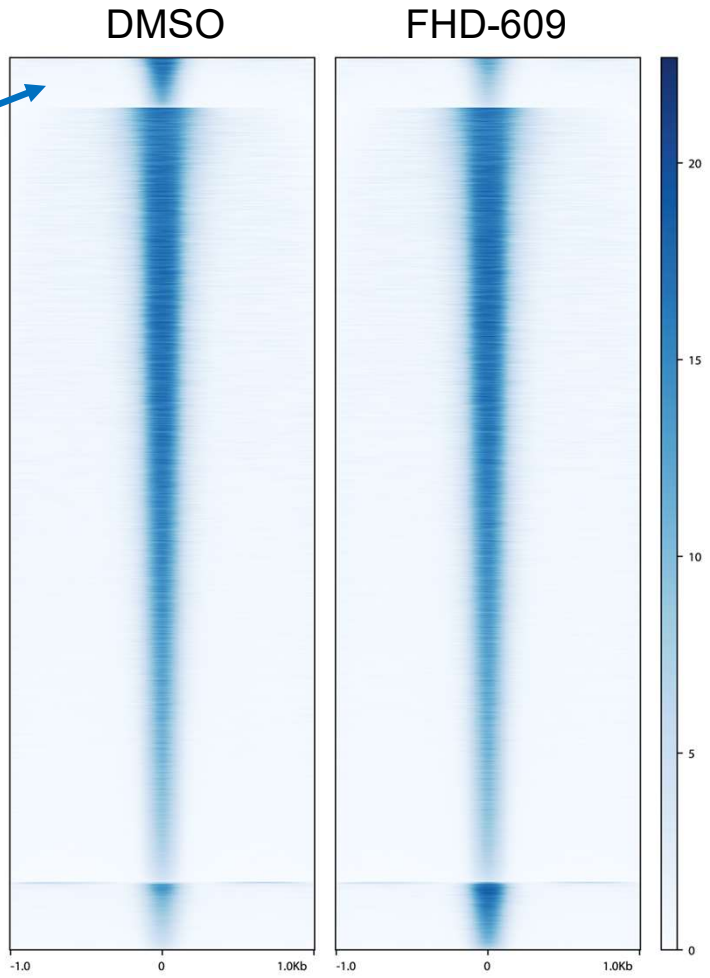
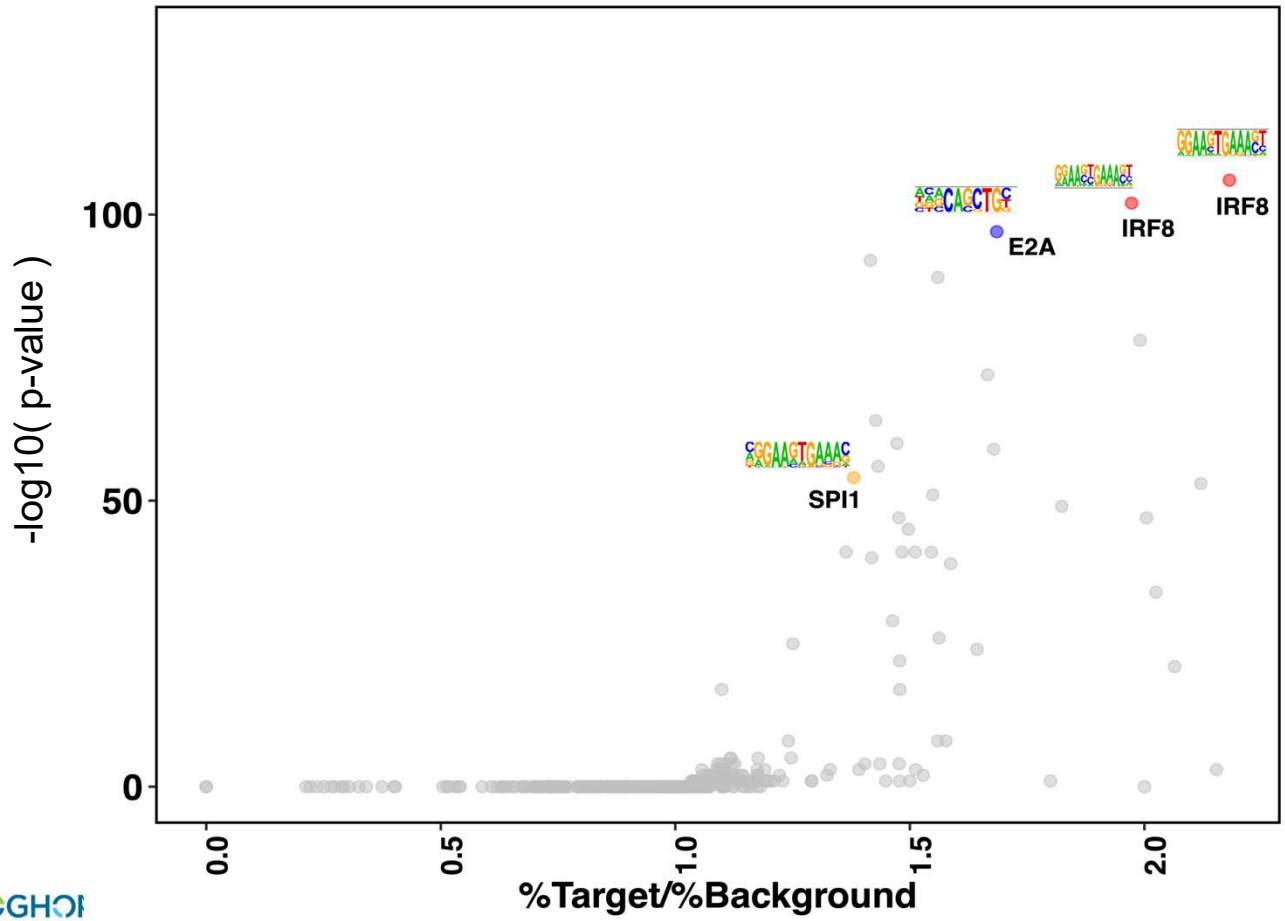
FHD-609 leads to loss in chromatin accessibility in BRD9-sensitive EOL1

N=5575 regions lose accessibility – chromatin closes – when treating with FHD-609 (ATAC-seq)



EOL1 cells treated with FHD-609 at 30nM for 72 h

IRF8 motif is enriched in regions that lose accessibility when EOL1 (sensitive to dBRD9) is treated with FHD-609 BRD9-degrader



IRF8 motif has strongest statistical significance and highest relative percentage of peaks

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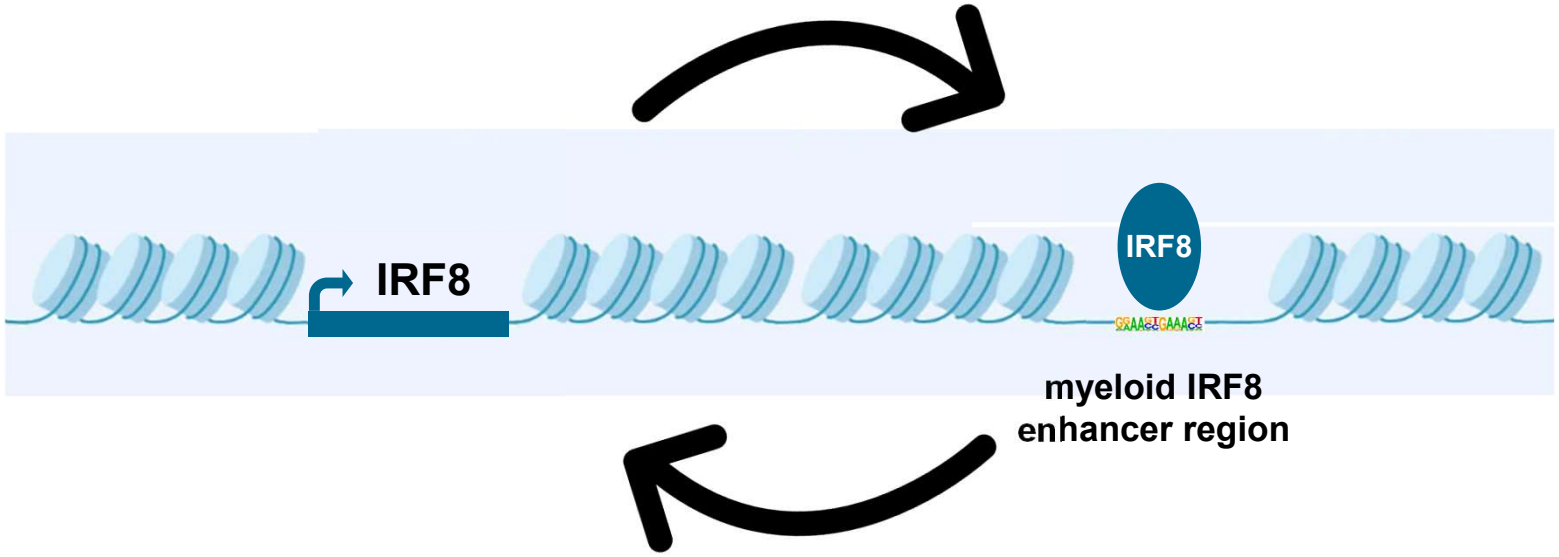
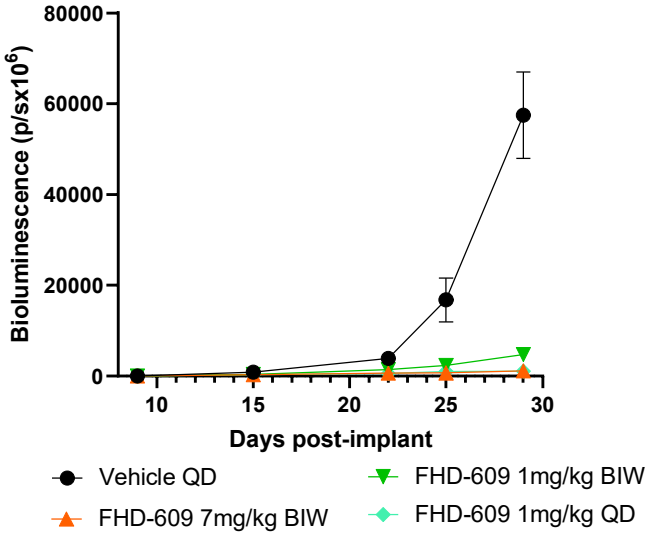
BRD9 is critical for maintaining the IRF8 positive feedback loop



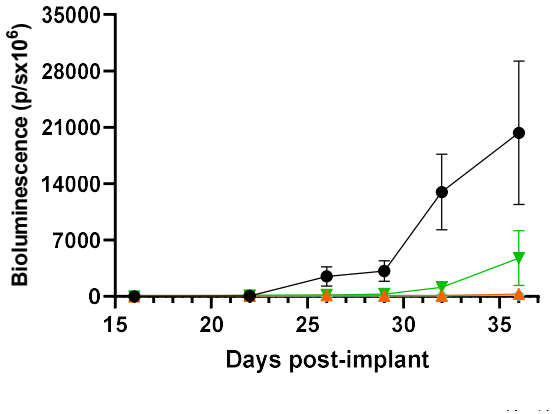
- Based on DepMap, IRF8-high AML cell lines are dependent on IRF8
- Directly drugging IRF8, a transcription factor, is very challenging
- BRD9 regulates access to IRF8 enhancer
- BRD9 represents a cancer vulnerability based on IRF8 lineage addiction

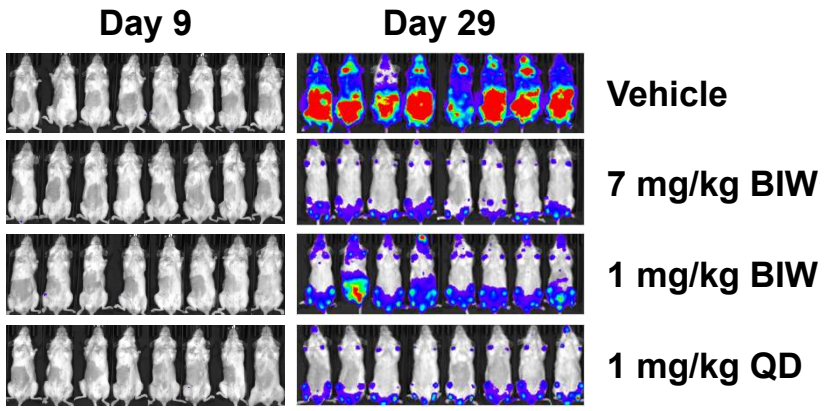
IRF8-high AML cell lines show strong response to FHD-609 in vivo

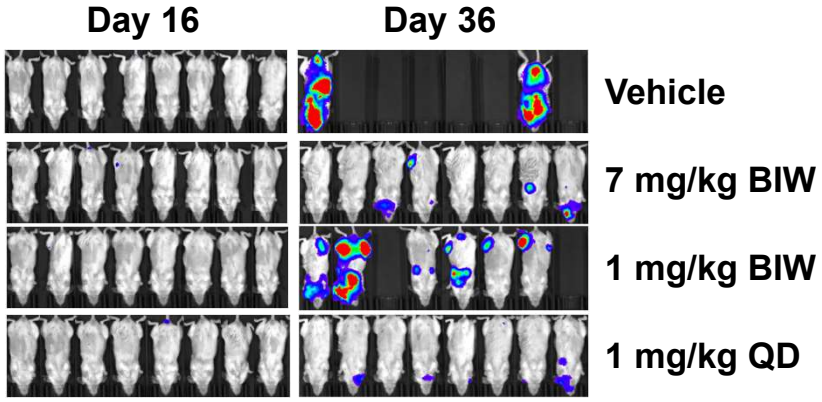
OCIAML2 Luc model



EOL1 Luc model







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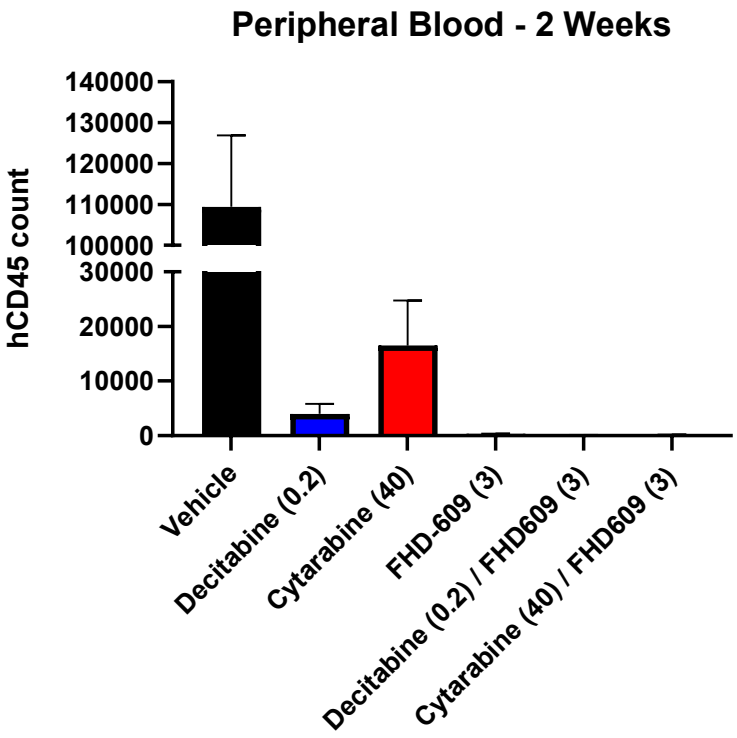
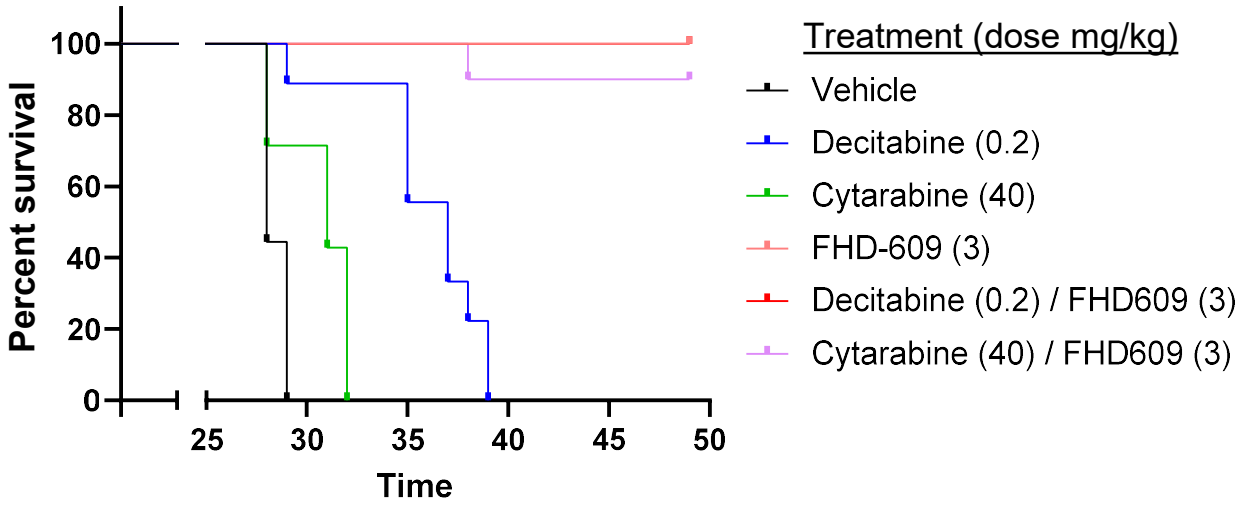
AML PDX with high IRF8 expression shows strong response to FHD-609 in vivo

Model: DFAM68555





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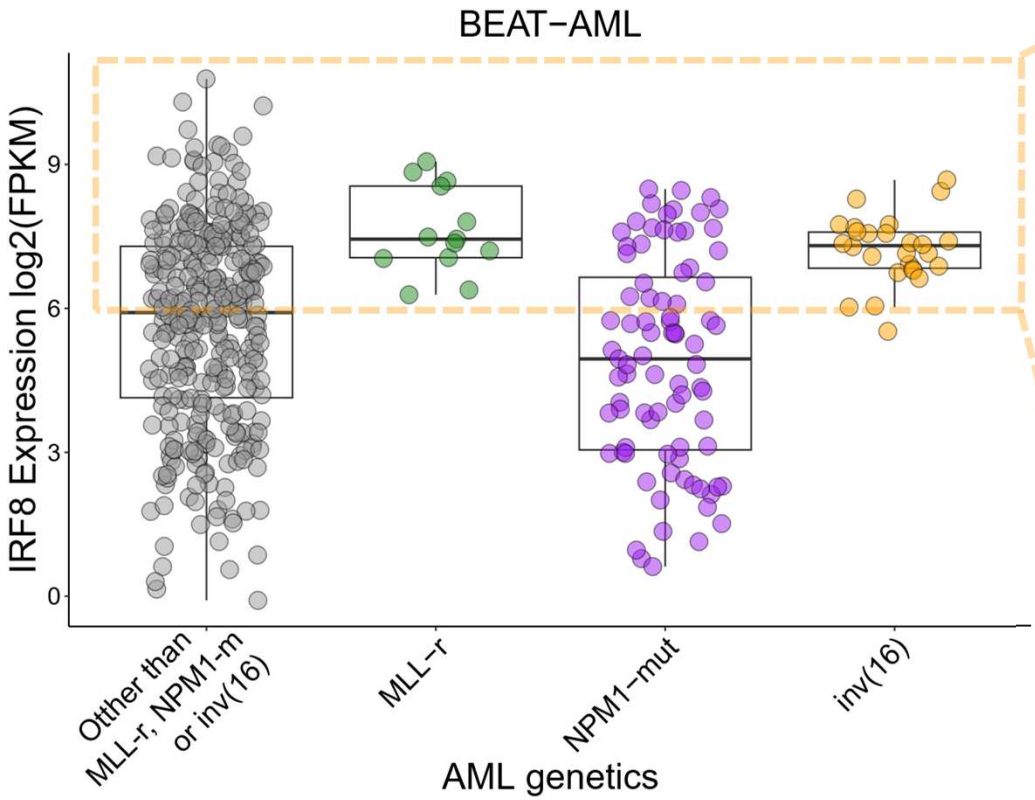
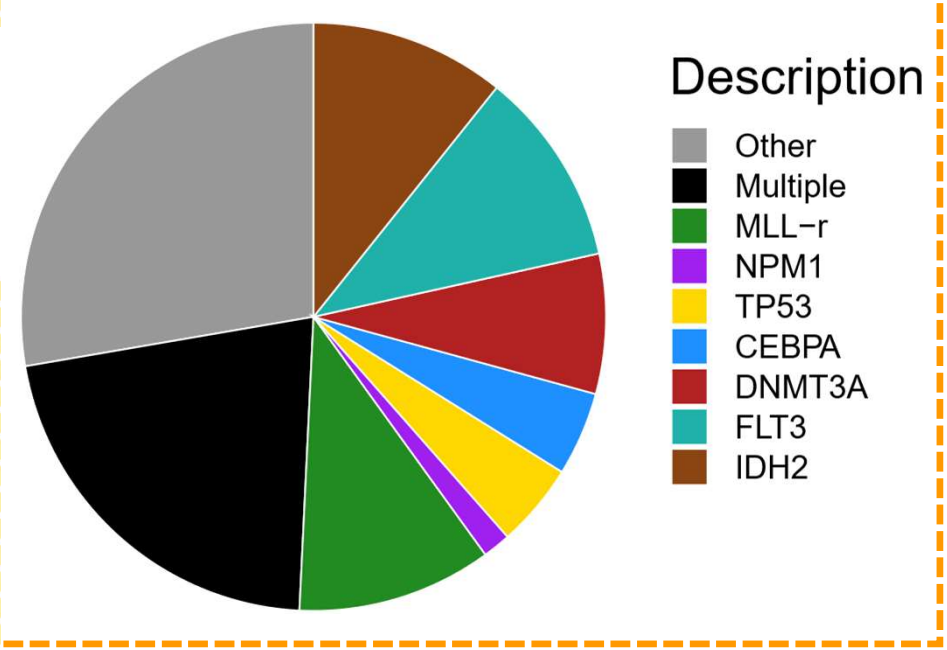
Subset of AML patients with diverse genetic backgrounds have high IRF8 expression



Genetic status of IRF8-high AML's



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Summary

- ~50% of AML cell lines are sensitive to the BRD9 degrader FHD-609
- Cell line response to FHD-609 correlates with IRF8 DNA methylation motifs and BRD9 genome localization
- Direct impact of FHD-609 treatment causes loss of chromatin accessibility at IRF8-related regions in sensitive AML cell lines
- FHD-609 disrupts IRF8 positive feedback loop and reveals a cancer vulnerability based on IRF8 lineage dependence
- IRF8 expression prospectively shown to predict response of AML patient-derived xenograft (PDX)

Acknowledgements

Computational Biology
David Mayhew
Sal Topal
Flore Uzan
GiNell Elliott
Jordana Muwanguzi
Luis M. M. Soares

Pharmacology
Ammar Adam
Victoria Garbitt-Amaral
Oliver Mikse
Brandon Antonakos
Hafiz Ahmad
Mei Yun Lin

Biology
Claudia Dominici
Salonee Parikh
Qianhe Zhou
Christina Xu
Michael Bocker
Huawei Chen
Ho Man Chan
Ryan Kruger

Chemistry
Zhaoxia Yang
Jason T. Lowe
Matt Netherton
Johannes Voigt
Sabine Ruppel
David S. Millan

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