Pre-clinical efficacy of targeting BAF complexes through inhibition of the dual ATPases BRG1 and BRM by FHD-286 in cellular models of AML

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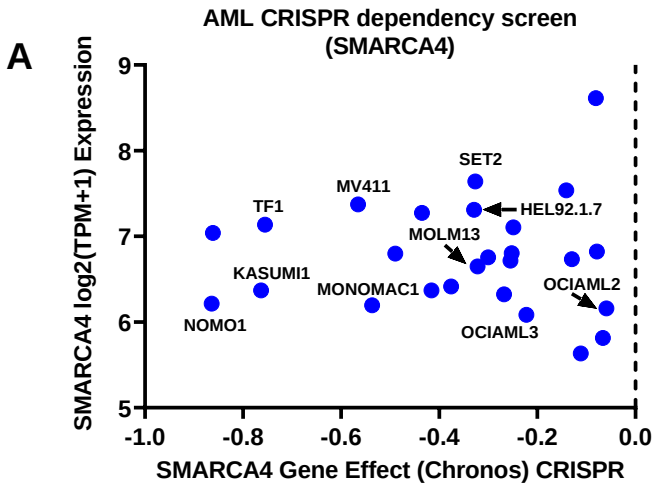
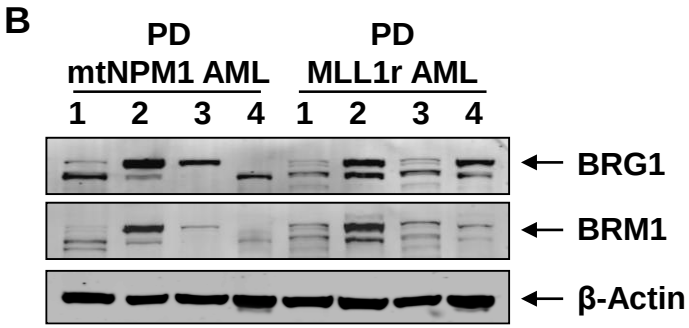
Dual ATPases SMARCA4 (BRG1) and SMARCA2 as therapeutic targets in AML

- **SWI/SNF chromatin remodeling complexes regulate essential cellular processes, e.g., cell cycle progression, differentiation, and genome stability.**
- **The mammalian SWI-SNF complexes are also known as BAF complexes. They are composed of up to 12 subunits, including one of two mutually exclusive catalytic subunits, SMARCA4 (BRG1) or SMARCA2 (BRM). These utilize energy from ATP hydrolysis for nucleosome repositioning or ejection to increase DNA accessibility for transcriptional complexes.**
- **Activity of BAF complexes is essential for lineage specific gene expression by TFs in hematopoiesis. Expression and dependency on BRG1/BRM have been documented for AML maintenance. BRG1 loss in AML cells was shown to induce cell-cycle arrest and apoptosis.**
- **Recently, small molecule inhibitors of dual BRM and BRG ATPase activity have been developed, which repress BRG1/BRM-dependent, enhancer-driven gene-expression of oncogenes, including MYC.**
- **FHD-286 (Foghorn Therapeutics) is a highly potent, selective, small molecule, oral, catalytic inhibitor of BRG1 and BRM.**

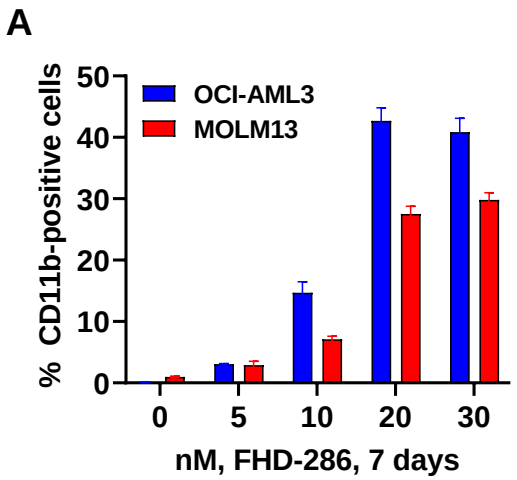
- **In the present studies, we interrogated the in vitro and in vivo efficacy of FHD-286 and its molecular correlates in AML cell models with MLL1 rearrangement, mutant NPM1 or chromosome 3q26 lesions.**

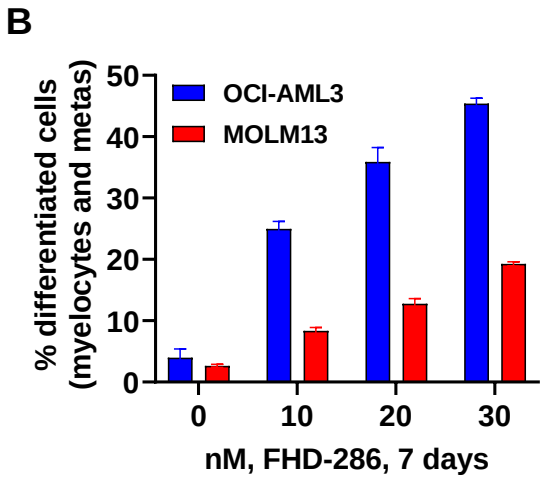
CRISPR screen revealed SMARCA4 to be a dependency in AML cell lines and expressed in patient-derived AML cells with MLL1r or mtNPM1

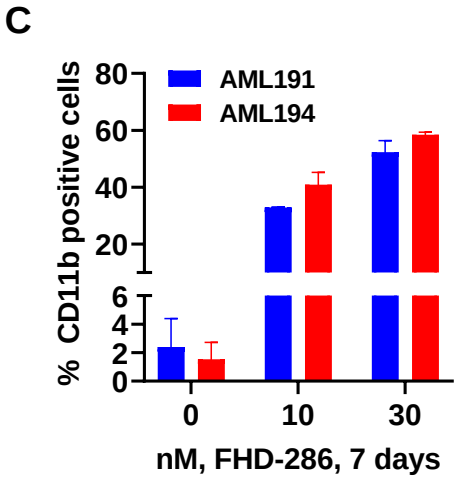


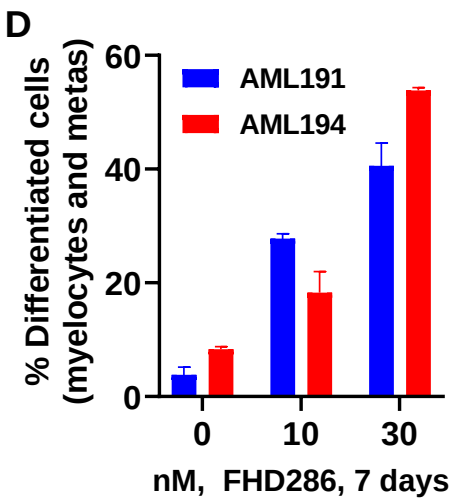


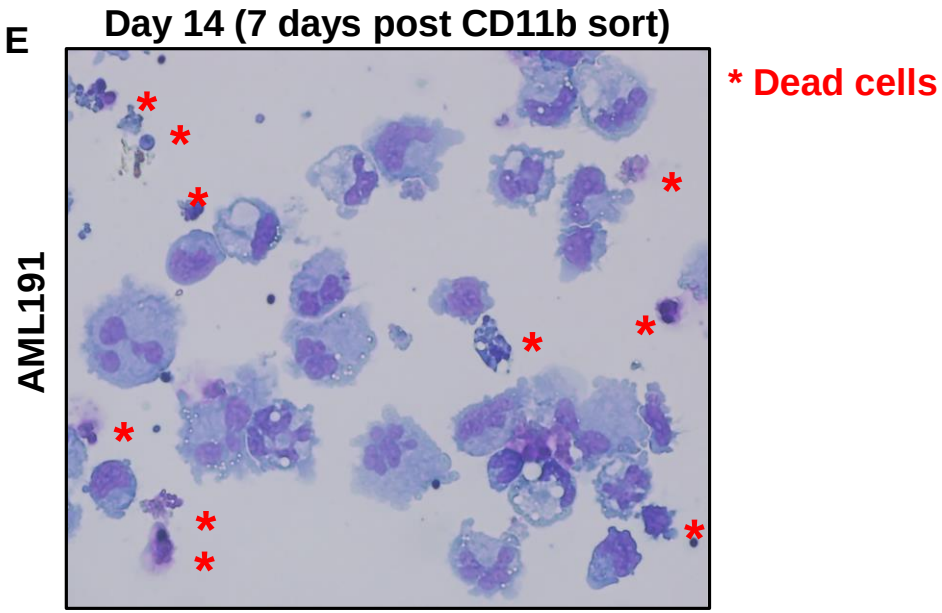
Treatment with FHD-286 induces differentiation followed by loss of viability in AML cells with MLL1r or mtNPM1 or 3q26 MECOM locus lesion

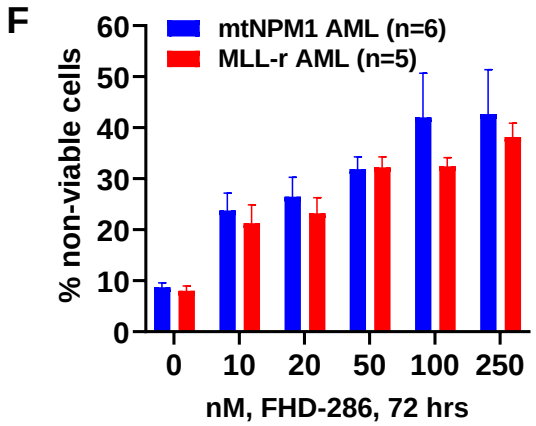




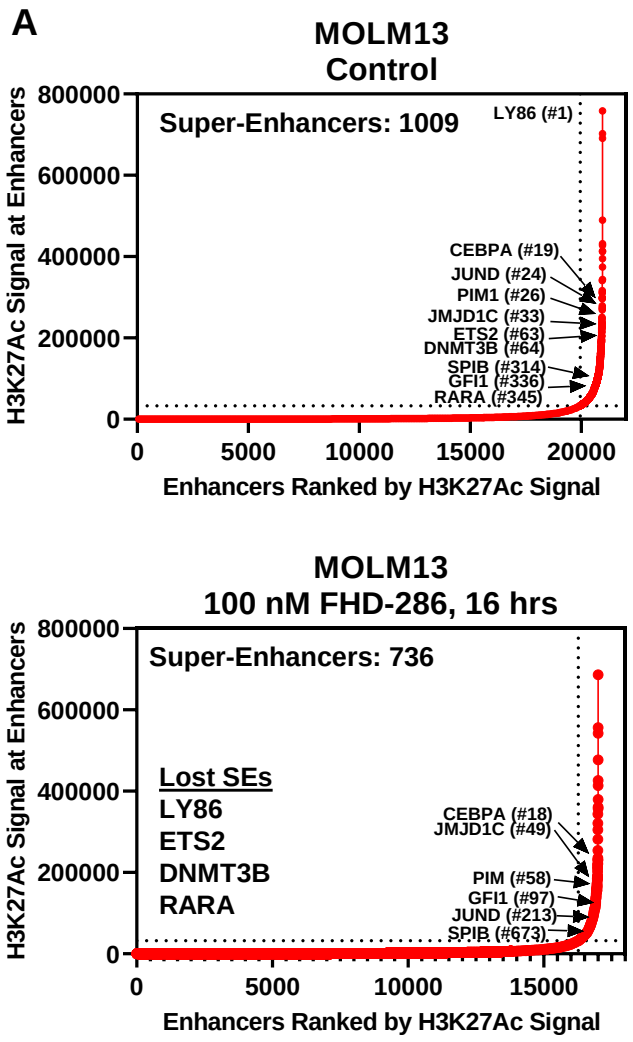


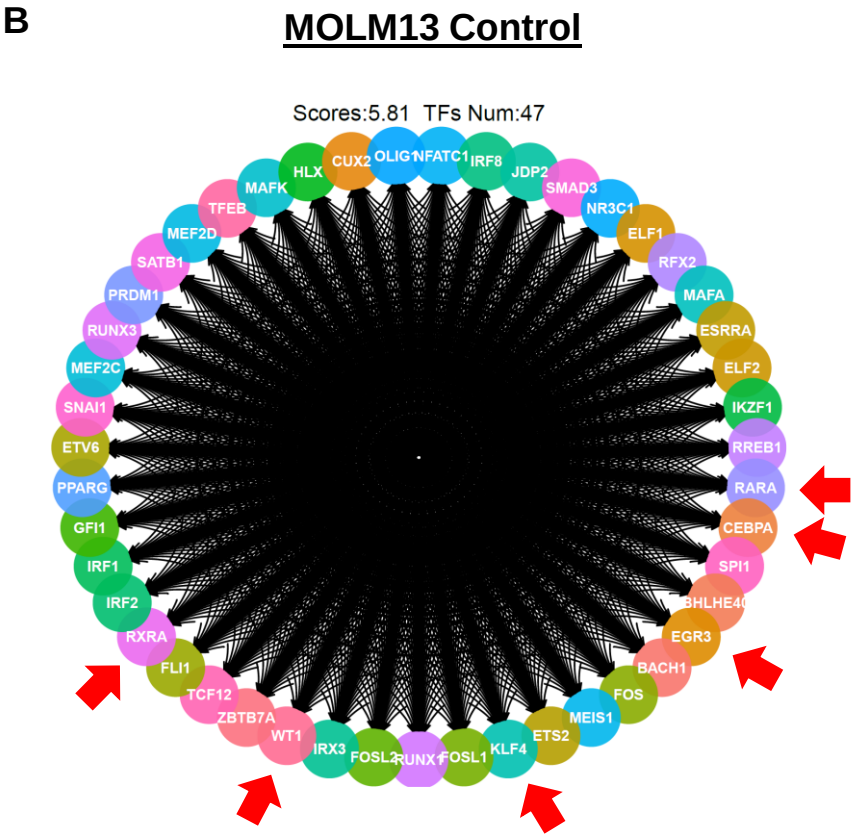


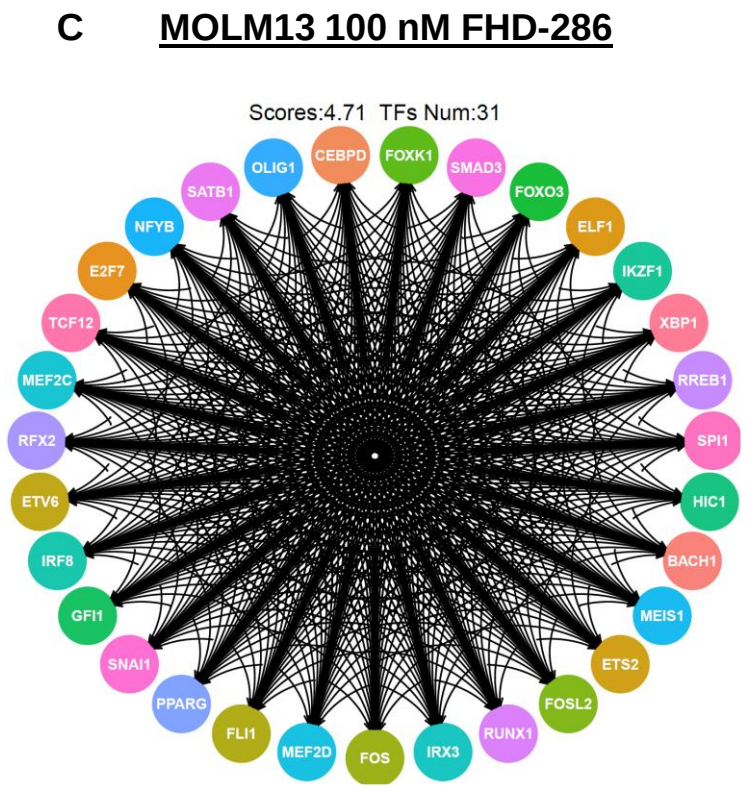




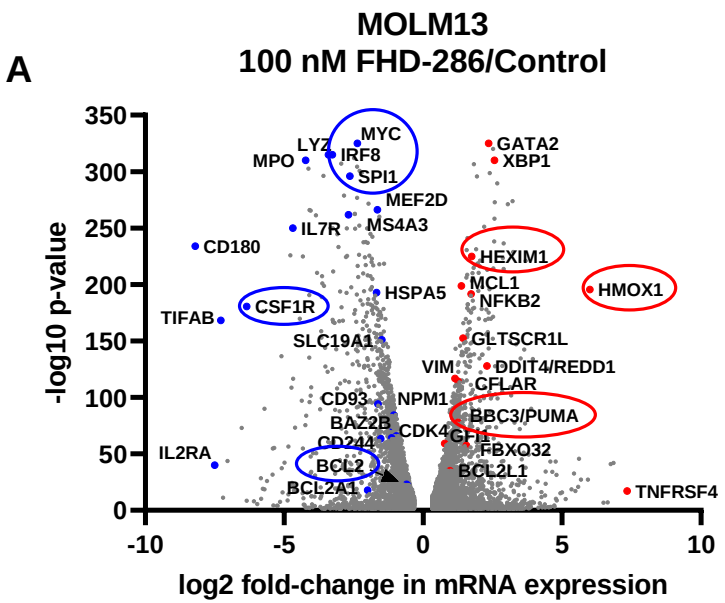
FHD286 treatment reduced ChIP-seq (H3K27Ac) peaks at super-enhancer-driven gene expressions and core transcription factor circuitry in AML cells with MLL1r

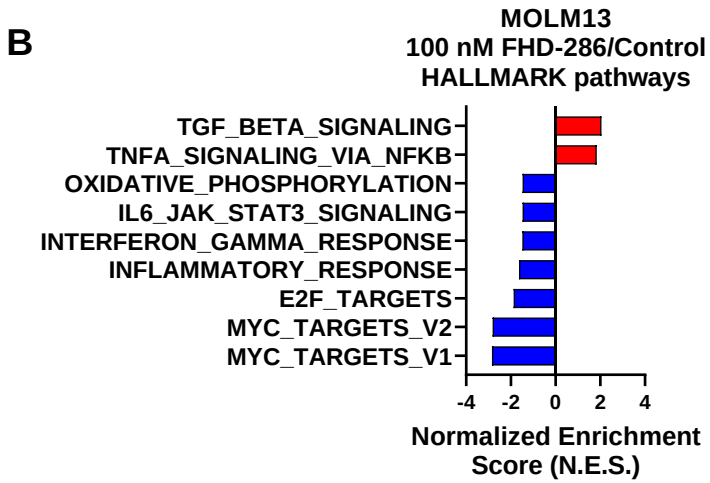


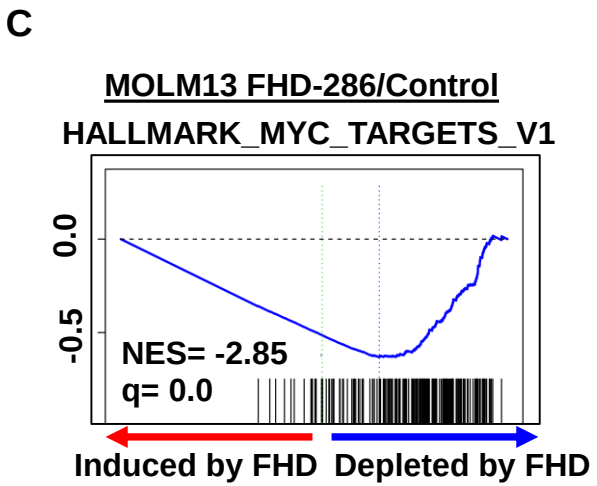


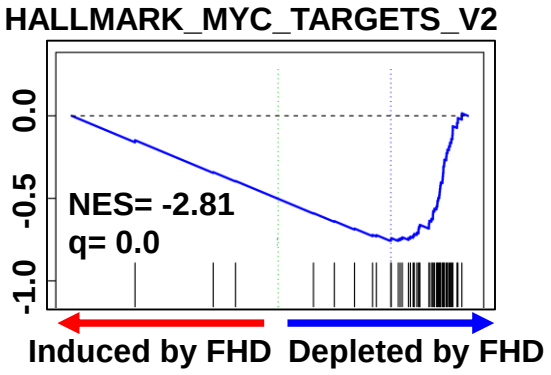


FHD-286 induced mRNA perturbations and negative enrichment of mRNA of gene-sets including c-Myc targets in AML cells with MLL1r



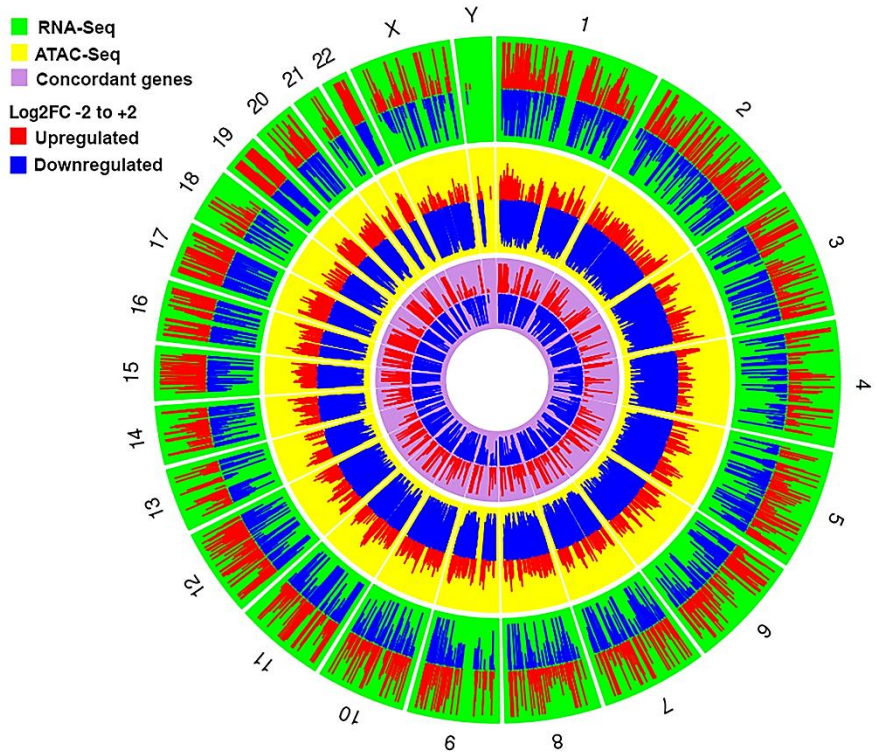




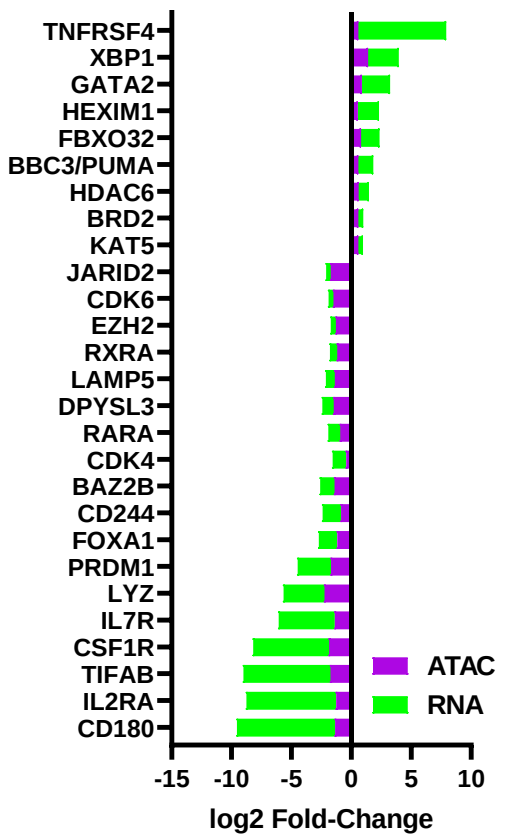


Concordantly perturbed chromatin accessibility (ATAC-Seq) and mRNA expressions (RNA-Seq) due to FHD-286 treatment in AML cells with MLL1r

A

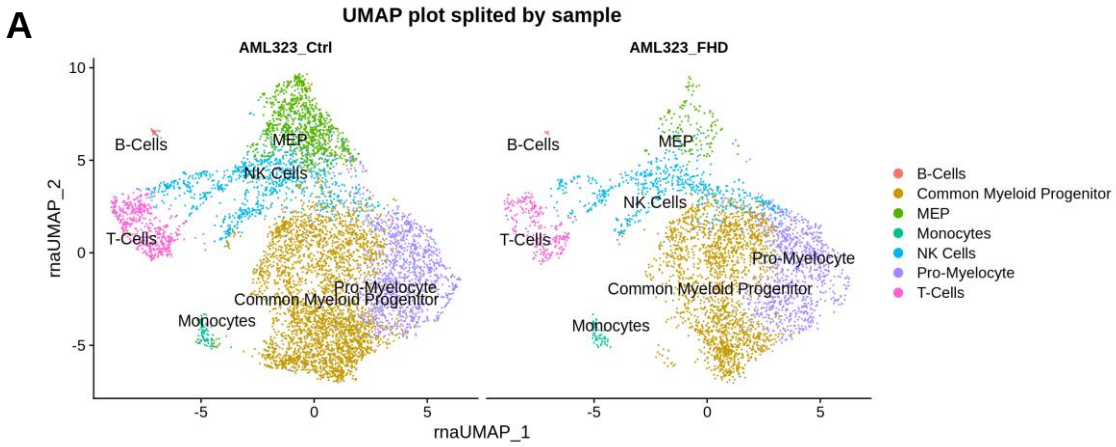


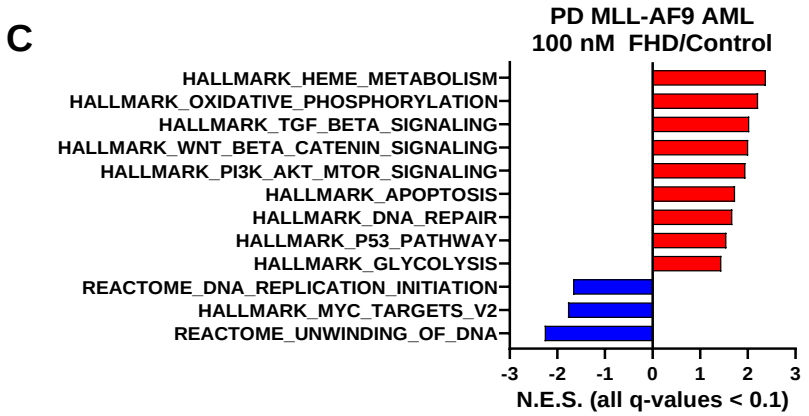
B

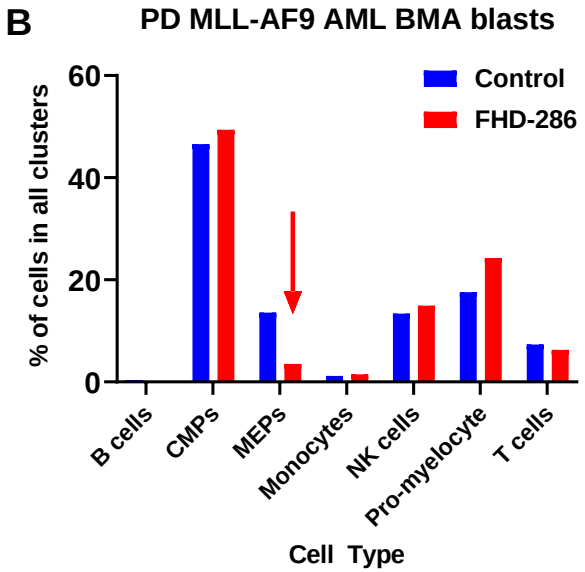


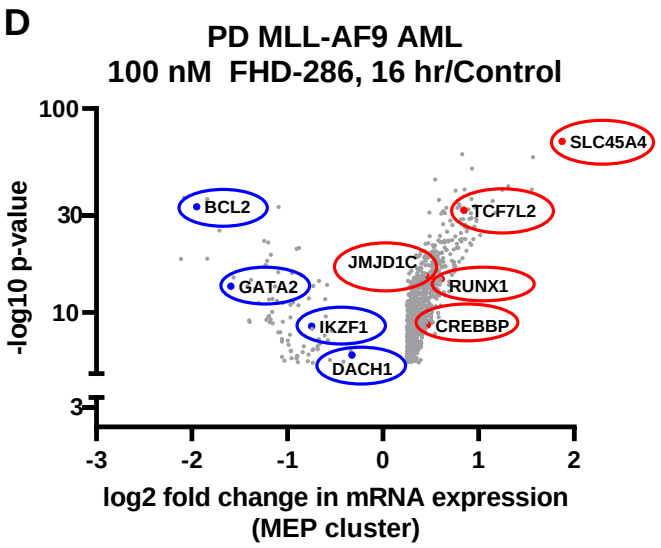
# of concordant genes up in both RNA-Seq and ATAC-Seq	1887
# of concordant genes down in both RNA-Seq and ATAC-Seq	2289

FHD-286 treatment reduced MEPs (sc-RNA-Seq) with perturbations in mRNA gene-sets

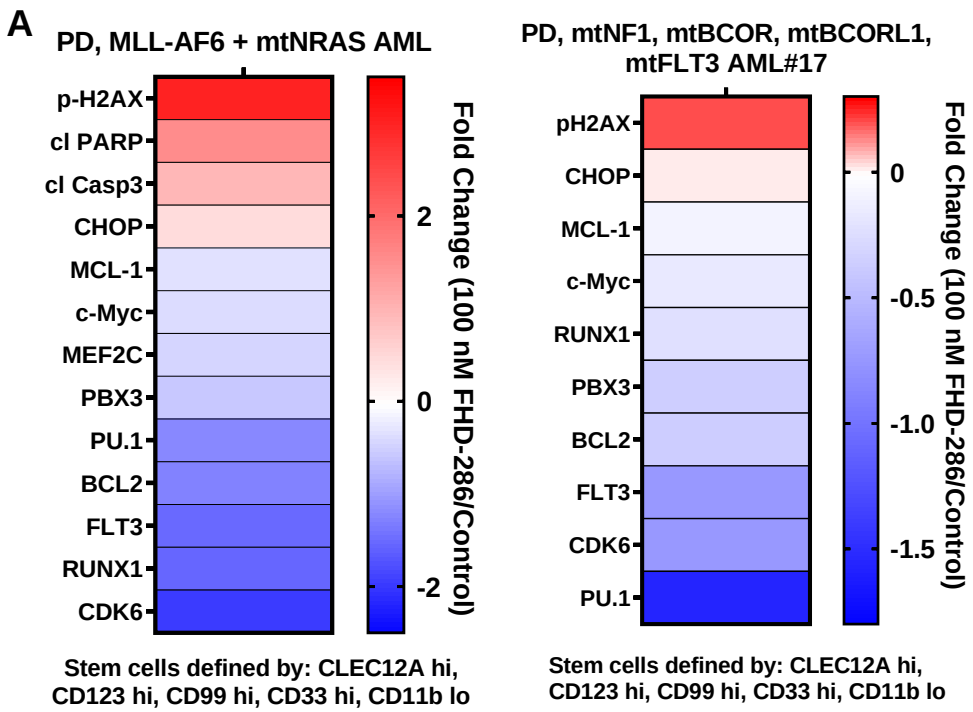


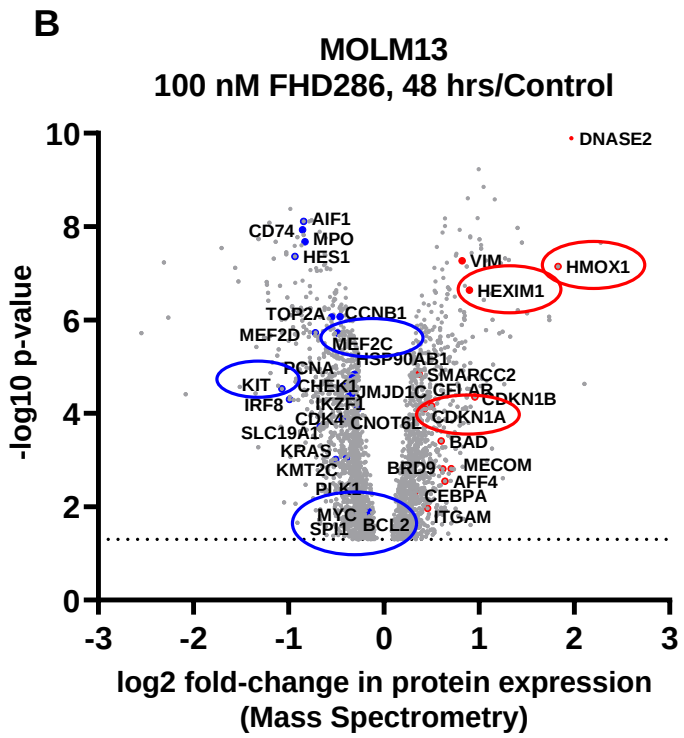


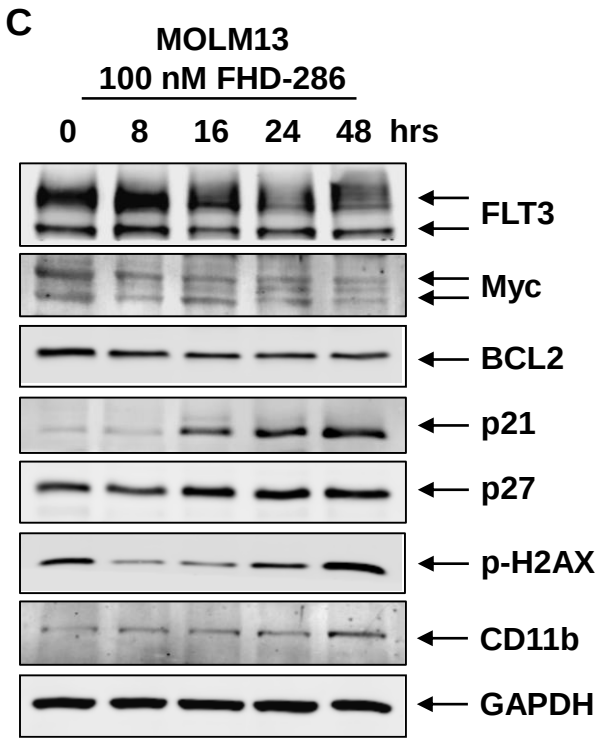




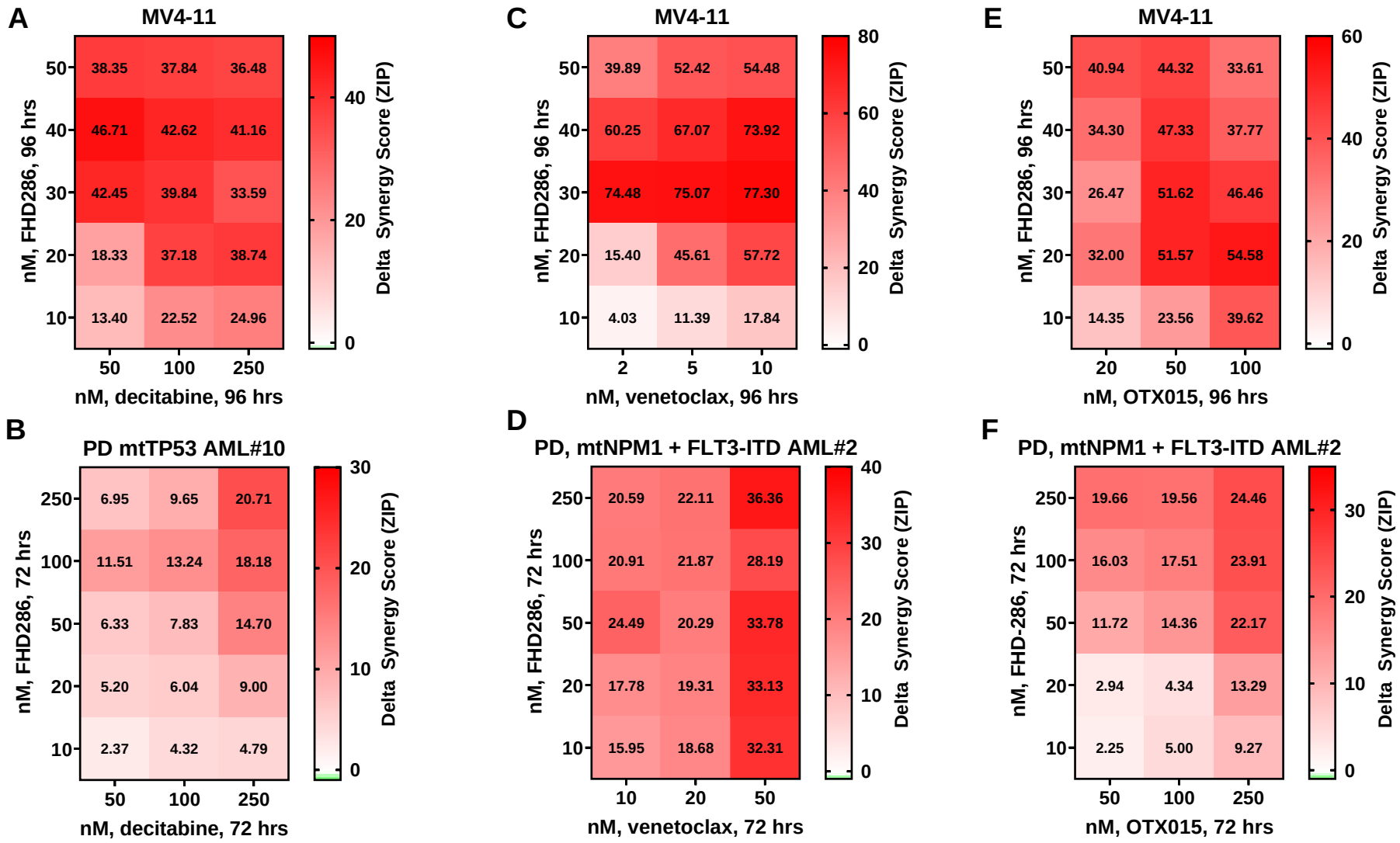
FHD-286 treatment reduced protein expressions of c-Myc, CDK6, BCL2 and PU.1 with upregulation of p-H2AX, cleaved PARP and Caspase-3 in phenotypically defined AML LSCs



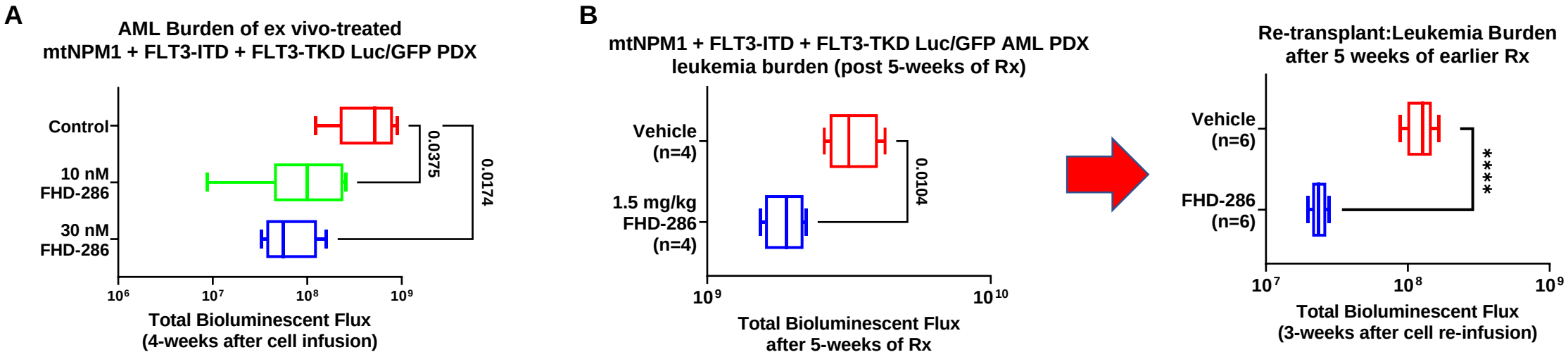




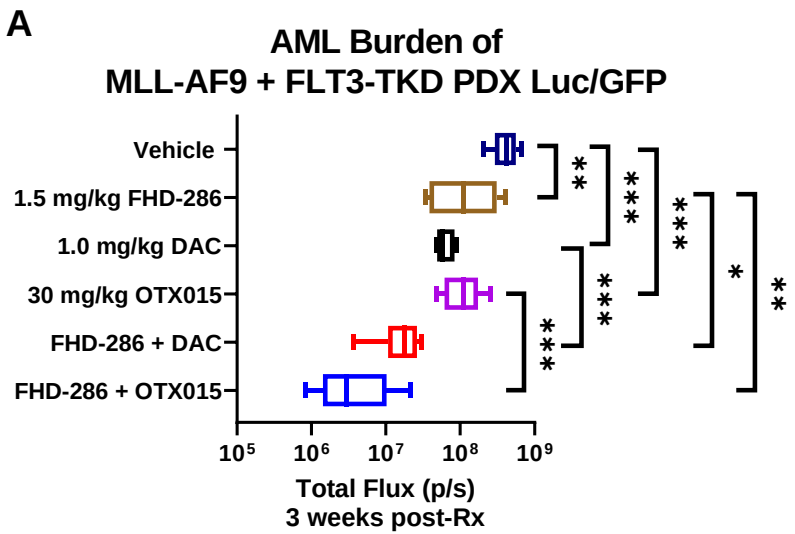
Co-treatment with FHD-286 and decitabine, venetoclax or BETi synergistically induces in vitro loss of viability in AML cells with MLL1r or mtNPM1

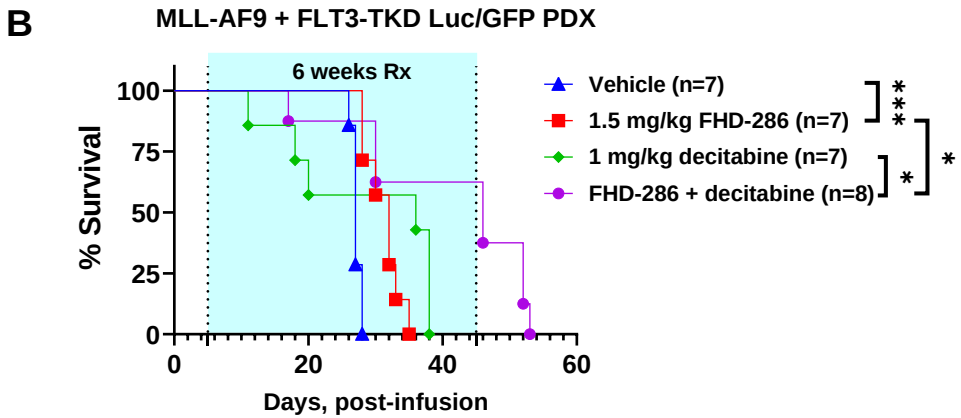


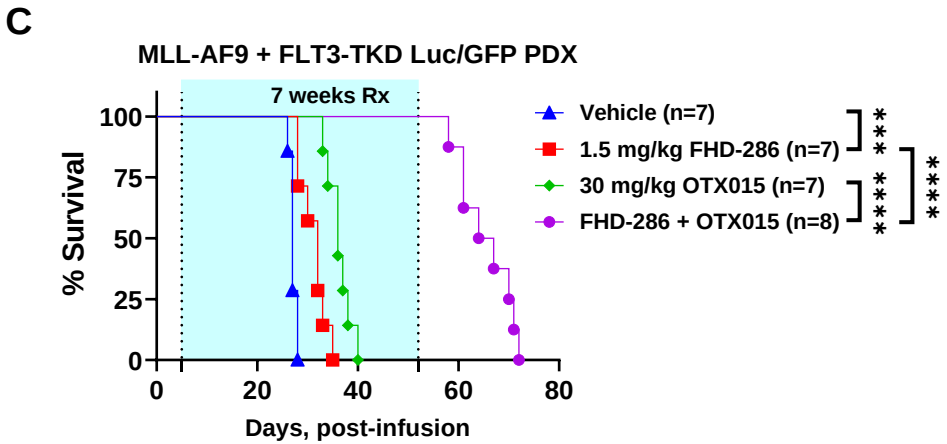
Treatment with FHD-286 significantly reduces in vivo leukemia initiating potential of a mtNPM1 + FLT3-ITD AML PDX

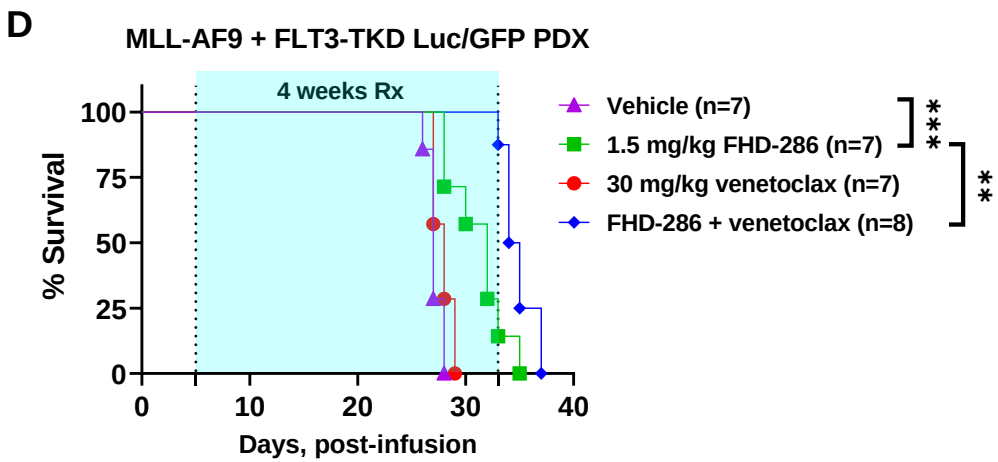


Superior in vivo efficacy following treatment with FHD-286-based combination with decitabine, venetoclax or BETi in PDX model of AML with MLL1r









Conclusions and Future Directions

- **Treatment with FHD-286 overcomes differentiation block and induced differentiation in AML cells with MLL-r, mtNPM1 and chromosome 3q26 lesions.**

- **FHD-286 negatively enriched gene-sets of MYC and E2F targets, inflammatory response and IL6-JAK-STAT3 signaling, while positively enriching gene-sets of TGFβ and TNFα signaling.**

- **In limited number of samples of AML stem progenitor cells, FHD-286 treatment showed a protein-expression perturbation signature with reduction of c-Myc, PBX3, PU.1, BCL2, MCL1, CDK6 and FLT3, but upregulation of p-H2AX and CHOP.**

- **Compared to treatment with each drug alone, co-treatment with FHD-286 and BETi, decitabine or venetoclax exerted synergistic in vitro lethality and superior in vivo efficacy in reducing AML burden and improving survival, without causing host toxicity in a PDX model of MLL-r AML.**

- **The preclinical efficacy of FHD-286 highlights the rationale and promise of interrogating the efficacy of FHD-286 monotherapy and combinations with targeted agents for therapy of AML, especially the high-risk AML subtypes including those with MLL-r, mtNPM1 or chromosome 3q26 lesions and EVI1 overexpression.**

Acknowledgments

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