ARTICLE - Acute Myeloid Leukemia

Potent synergy of DHODH and SREBP inhibition in acute myeloid leukemia via disruption of cholesterol and lipid metabolism

Shanna M. Hogeling,[1] Dominique Sternadt,[1] Nikita La Rose,[1] Marjan Geugien,[1] Diego Pereira-Martins,[1] Fiona A.J. van den Heuvel,[1] Anna Kuchnio,[2] E. Christine Pietsch,[3] Ulrike Philippar,[2] Gerwin Huls[1] and Jan Jacob Schuringa[1]

[1]Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [2]Discovery Oncology, Janssen Research & Development, Beerse, Belgium and [3]Discovery Oncology, Janssen Research & Development, Spring House, PA, USA

Correspondence: J.J. Schuringa
j.j.schuringa@umcg.nl

Received: March 28, 2025.
Accepted: December 1, 2025.
Early view: December 11, 2025.

https://doi.org/10.3324/haematol.2025.287918

Supplementary Materials and Methods and Supplementary Figures

Potent synergy of DHODH and SREBP inhibition in acute myeloid leukemia via disruption of cholesterol and lipid metabolism

Shanna M. Hogeling[1], Dominique Sternadt[1], Nikita La Rose[1], Diego Pereira-Martins[1], Marjan Geugien[1], Fiona A.J. van den Heuvel[1], Anna Kuchnio[2], E. Christine Pietsch[3], Ulrike Philippar[2], Gerwin Huls[1] and Jan Jacob Schuringa[1*]

[1]Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[2]Discovery Oncology, Janssen Research & Development, Beerse, BE
[3]Discovery Oncology, Janssen Research & Development, Spring House, US

[*]correspondence:
Prof.dr. J.J. Schuringa, PhD
Department of Experimental Hematology
University Medical Center Groningen
Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, The Netherlands
Phone: + 31503612354, Fax: +31503615960
E-mail: j.j.schuringa@umcg.nl

Supplementary Materials and Methods

Quantitative real-time PCR

RNA samples were prepared from cell lines and primary AML patient samples treated with DMSO or menin inhibitor for various timepoints. Total RNA was isolated using the RNeasy Mini Kit from Qiagen (Venlo, The Netherlands) according to the manufacturer's protocol and reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad). Subsequently, the cDNA was amplified using SsoAdvanced SYBR Green Supermix (Bio-Rad) on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad). Primer sequences are listed in table 1.

Table 1. Primers used for mRNA expression

Target	**Forward**	**Reverse**
DHCR7	AGGACTTTAGCCGGTTGAGA	AGCCATTGGGCCCTCC
HMGCS1	TCTATCCTTCACACAGCTCTTTC	TCTCAAGGGCAACAATTCCC
ACTB	AGGCCAACCGCAAGAAG	ACAGCCTGGATAGCAACGTACA
RPL30	ACTGCCCAGCTTTGAGGAAAT	TGCCACTGTAGTGATGGACAC

LC-MS/MS analysis

Lipids were analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Shimadzu LC40 UPLC system coupled to a ZenoTOF 7600 mass spectrometer (Sciex). 2 µL lipid extracts were injected and separated on a Waters Acquity CSH C18 1.7 um, 2.1 x 100 mm column, with a gradient of mobile phase A consisting of 40/60/0.1 water/acetonitrile/formic acid + 10 mM ammonium formate and mobile phase B consisting of 10/89/1/0.1 acetonitrile/isopropanol/water/formic acid + 10 mM ammonium formate. The concentration of B was increased from 20 to 50% in 2.1 min, and the to 54% in 9.8 min. It was then increased to 70% in 0.1 min and to 99% in 5.9 min and decreased to 20% in 0.1 min where it was held for 1.9 min. The total run time was 20 min, the flow rate was 0.4 mL/min and the column temperature 55 °C. MS data acquisition was performed in positive mode using data dependent acquisition at a declustering potential of 50 V. An 100 ms MS1 survey scan at m/z 100-2000 was followed by up to 40 data dependent MS2 scans (Top40), of precursor ions exceeding 100 cps. MS2 scans using collision induced dissociation (CID) were acquired at m/z 50-2000 using Zeno trapping, for 5 ms and at a collision energy of 40 eV, with a Zeno threshold of 20000 cps.

Untargeted data analysis with MS-DIAL

Data analysis was performed with MS-DIAL version 5.5.250404 using the lipidomics workflow: 24 data files were processed and aligned, and normalized with SPLASH using peak intensities. Reference-matched lipids were exported as a csv file for further data analysis. For further data analysis, data was log-2 transformed before using the package lipidr[1] to perform differential and enrichment analysis of lipid classes, carbon lengths and unsaturation levels.

LFQ proteome data analysis

The LFQ proteome dataset on primary AML samples that were thawed and sorted for CD34+ or CD117+ was previously published (PXD030487).[7] Pearson correlations with the PFQ proteome were performed using the calculated AUC and only genes with a row max of > 50 were taken along in further analysis. Gene set enrichment analysis were performed using ranked pearson coefficient values.

Statistics

All statistical analyses were performed using the student t test paired or unpaired and were expressed as means ± SEM for all other comparisons. Differences were considered statistically significant at $p \leq 0.05$.

References

1. Mohamed A, Molendijk J, Hill MM. Lipidr: A Software Tool for Data Mining and Analysis of Lipidomics Datasets. J Proteome Res 2020;19(7):2890–2897.

Supplementary Tables – See Excel files

Supplementary table 1. Overview of patient samples included in study. Viability data associated with Supplementary Figure 1C, area under the curve (AUC) and CD11b data associated with Figure 1B, ZIP scores associated with Supplementary Figure 4E and mutation status are shown.

Supplementary table 2. Lipidomics data. LC-MS/MS based lipidome data of HL60 and THP1 cells treated with DHODHi for 24h.

Supplementary Figures

Supplementary Figure 1. Sensitivity to DHODH inhibition of primary AML samples co-cultured on MS5

A. Schematic overview depicting the gating strategy used in the DHODHi drug screen. B-C. Viability of primary AML samples co-cultured for seven days on MS5. D. Comparison of AUC values from primary AML patient samples treated with the DHODH inhibitor versus the proliferation index (PI). E. Boxplots showing DHODHi AUC values of the proliferation of mutant vs wild type subgroups. F-G. Dose-dependent effect on proliferation, normalized Annexin V (%), normalized CD11b MFI and normalized CD14 MFI in cell lines HL60 and THP1 upon treatment with DHODHi (n=6 independent experiments). Statistical analysis by unpaired Student's t test (E), one-way ANOVA (F-G) or Simple Linear Regression (D).

Supplementary Figure 2. ssGSEA analysis of DHODHi-sensitive primary AML samples shows a diverse metabolic program.

Single sample GSEA (ssGSEA) of eight DHODHi-sensitive primary AML samples showing a selection of metabolism signatures. Area under the curve (AUC) values are taken from the drug screen data in figure 1B.

Supplementary Figure 3. LC-MS/MS-based lipidomics on DHODHi-treated THP1 cells reveals a profound impact on lipid metabolism

A. Volcanoplot showing the differentially expressed lipids of THP1 cells treated with 3 nM of DHODHi versus DMSO for 24 hours. Classes are depicted by colors in the legend. B. Enrichment analysis for lipid classes comparing DHODHi versus DMSO treated cells. C. Volcanoplot showing differentially expressed lipids of HL60 cells versus THP1 cells. D. Enrichment analysis for lipid classes comparing HL60 versus THP1 cells. E. Total number and relative saturation levels of up and down regulated differentially expressed lipids in DHODHi-treated cells. F. Triglycerides (TGs) abundances in DMSO and DHODHi-treated THP1 cells divided by saturation class. G. MFI fold change of neutral lipids (BODIPY 493/503) in DMSO and DHODHi-treated THP1 cells. Statistical analysis by paired Student’s t test (F) and unpaired Student’s t test * $P > 0.05$ (G).

Supplementary Figure 4. Combination treatment with DHODHi and dipyridamole results in strong synergism in AML cell lines and primary AML samples

A. Dose-dependent effect on proliferation in THP1 cells upon treatment with dipyridamole cultured in RPMI with FCS and P/S. Representative of three independent experiments. B. MFI fold change in cholesterol (BODIPY-cholesterol) in four-day dose-dependent dipyridamole-treated THP1 cells. Bar graphs represent the mean ± SEM of at least three independent experiments. C. Log2 expression of transcriptome data obtained from CCLE of cell lines HL60 and THP1. D. Treatment of AML3, AML6, AML14 and AML27 with DMSO, DHODHi (0.3, 3.0 and 30 nM) and dipyridamole (1, 5 and 10 µM) for four days co-cultured on MS5 cells. Synergy distribution plots of treated AML patient samples. E. Summary of primary AML samples treated with DHODHi and dipyridamole showing effect on proliferation ($CD45^+/7AAD^-$ viable counts; n=14) and differentiation (MFI of CD11b and CD14; n=10). Per AML values are normalized to the DMSO control. Statistical analysis by unpaired Student's t test or one-way ANOVA. * $P > 0.05$, ** $P > 0.01$, *** $P > 0.001$, **** $P > 0.0001$.

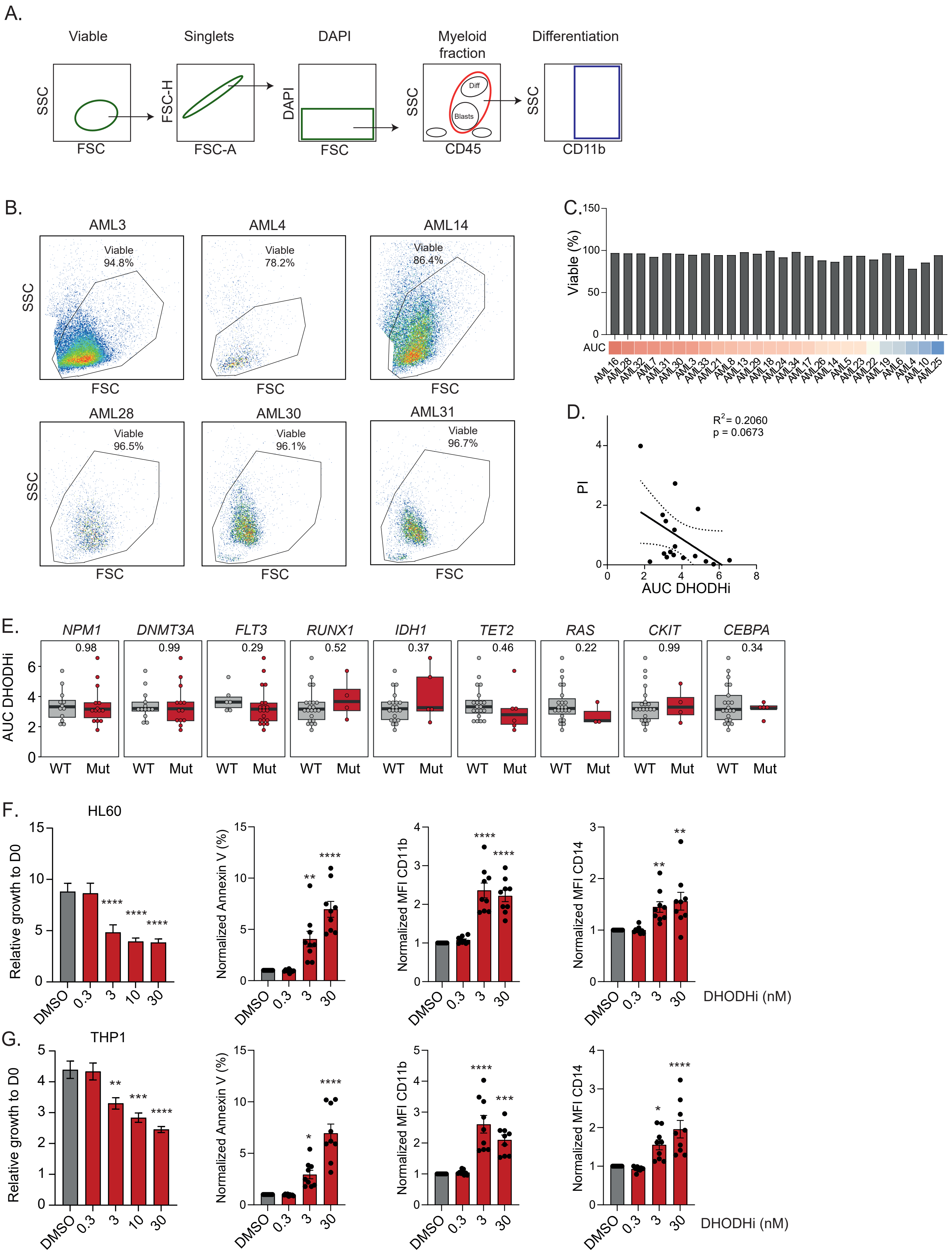
Supplementary Figure 5. Effects of combined DHODHi and dipyridamole on viable cell counts of normal CD34+ cells compared to primary AML samples

A. Normalized viable cell counts ($CD45^+/7AAD^-$ or $CD34^+/7AAD^-$ (CB)) of primary AML samples (n=14) and healthy $CD34^+$ cord blood (CB) cells (n=4) treated with DHODHi and dipyridamole (5 and 10 µM) both co-cultured on MS5 cells. B-C. Effect on differentiation shown by MFI values of CD11b and CD14 from healthy $CD34^+$ CB treated with DHDOHi and dipyridamole. Statistical analysis by one-way ANOVA. * $P > 0.05$, ** $P > 0.01$, *** $P > 0.001$, **** $P > 0.0001$.

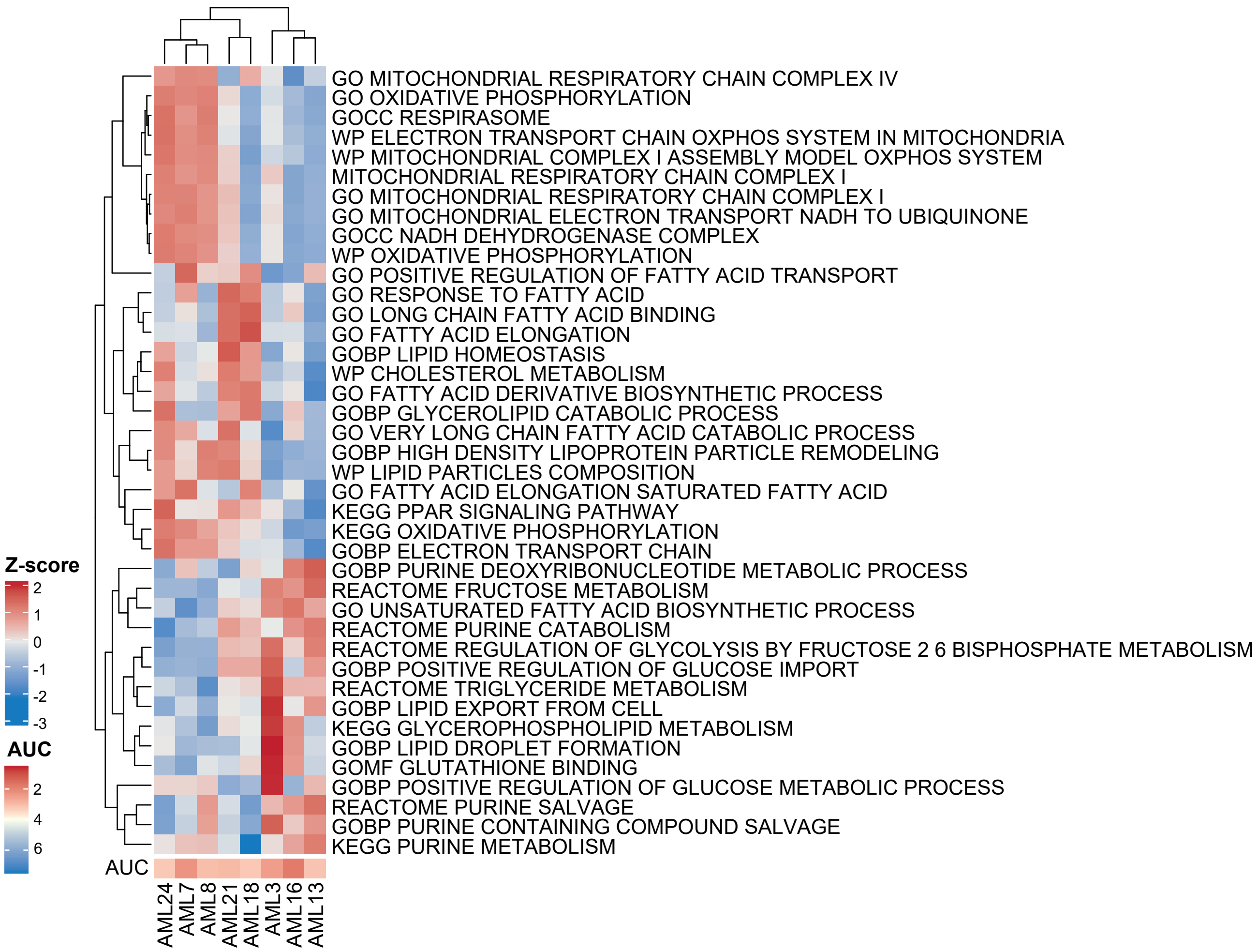
Supplementary Figure 6. Combination treatment of DHODHi with ENT or cholesterol inhibitors reveal heterogeneous responses

A. HL60 and THP1 cells treated with DHODHi and ENT inhibitor Dilazep (0,1, 1 and 10 µM). Representative of three independent experiments. B. Synergy distribution plots of data shown in panel A and of two primary AML samples treated with DHODHi and Dilazep. C. HL60 and THP1 cells treated with DHODHi and cholesterol inhibitor Rosuvastatin (0,3, 3 and 30 µM). Representative of three independent experiments. D. Synergy distribution plots of data shown in panel C and of one primary AML sample treated with DHODHi and Rosuvastatin. E. HL60 and THP1 cells treated with DHODHi and cholesterol inhibitor Atorvastatin (2, 10 and 20 µM). Representative of three independent experiments. F. Synergy distribution plots of data shown in panel E. All data is normalized to DMSO controls. Statistical analysis by one-way ANOVA. * $P > 0.05$, ** $P > 0.01$, *** $P > 0.001$, **** $P > 0.0001$.

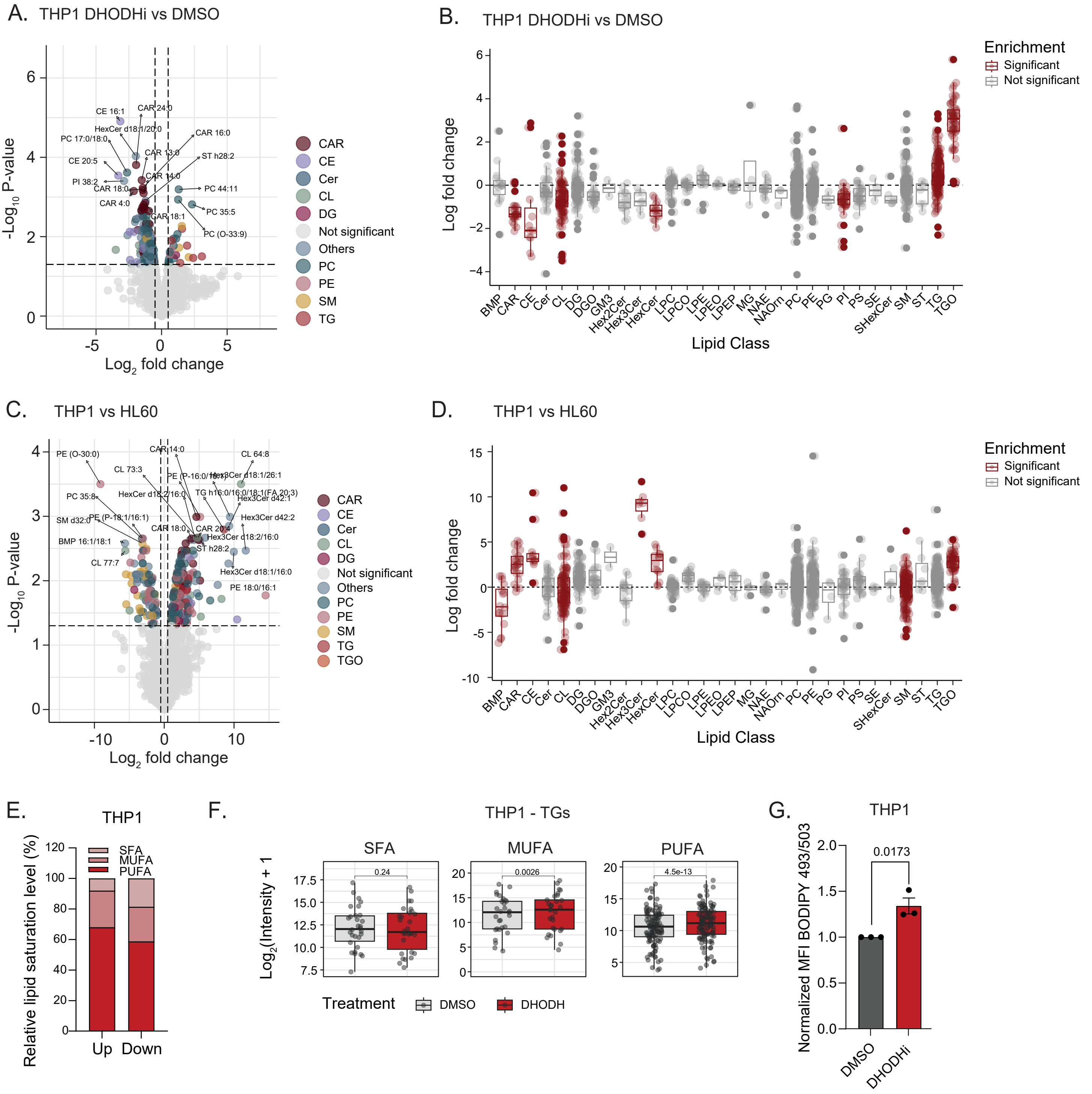
Supplementary Figure 1



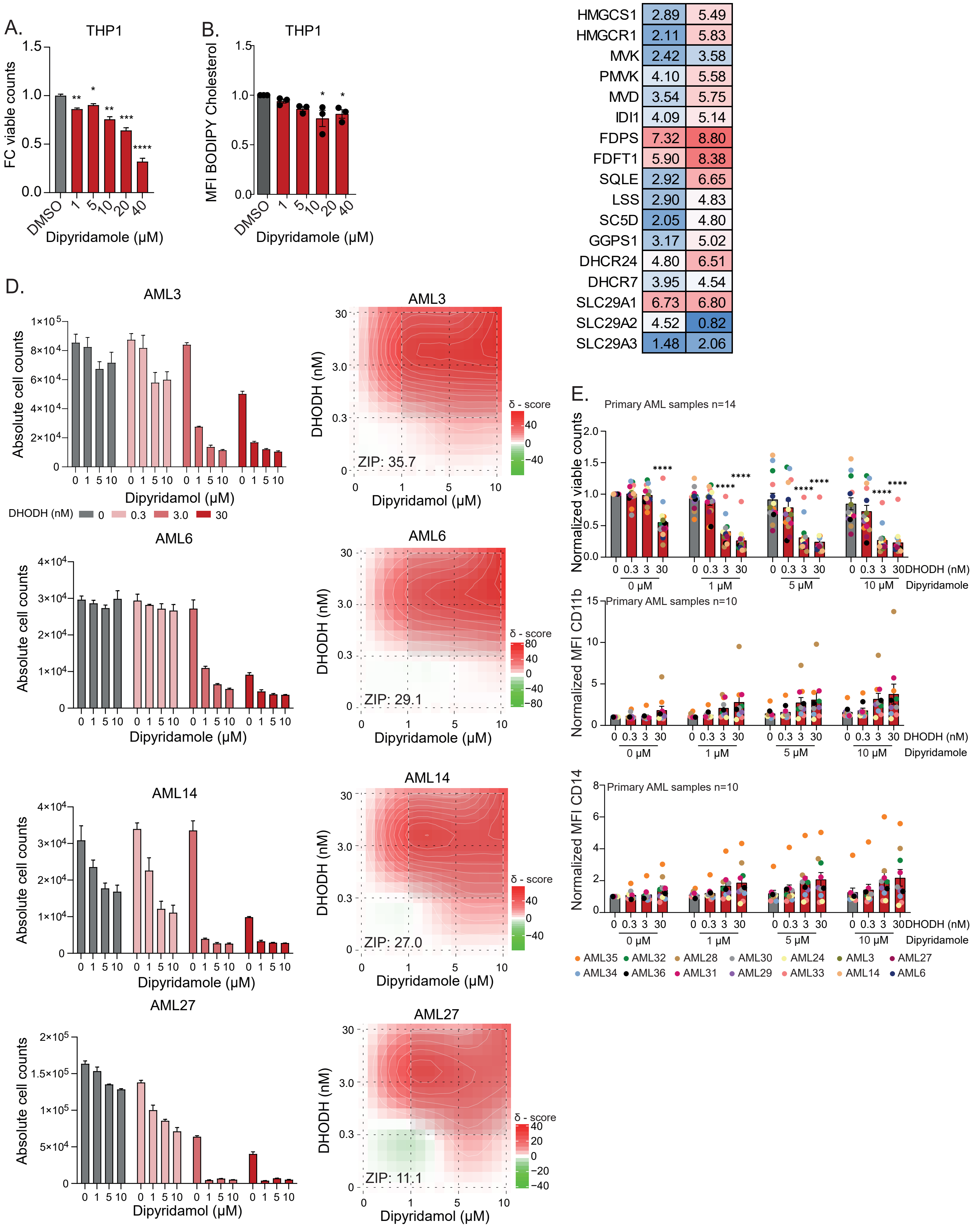
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

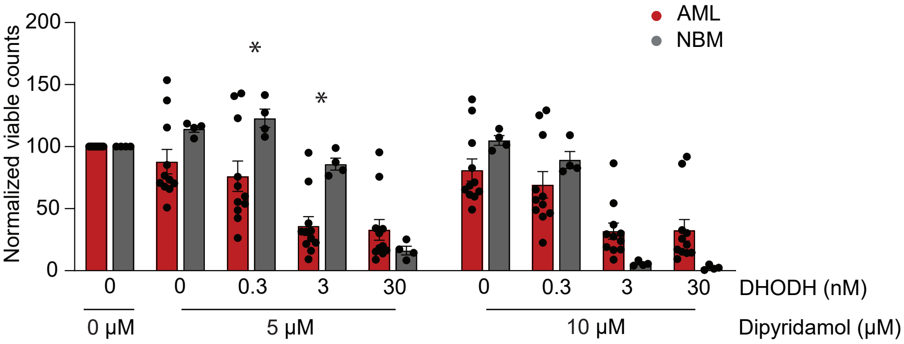


C.

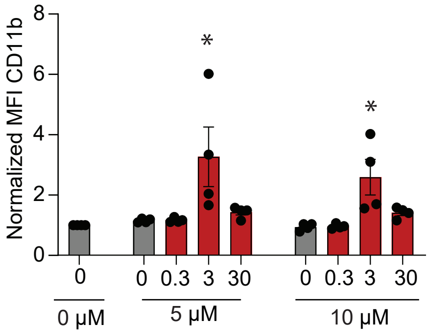
	HL60	THP1
HMGCS1	2.89	5.49
HMGCR1	2.11	5.83
MVK	2.42	3.58
PMVK	4.10	5.58
MVD	3.54	5.75
IDI1	4.09	5.14
FDPS	7.32	8.80
FDFT1	5.90	8.38
SQLE	2.92	6.65
LSS	2.90	4.83
SC5D	2.05	4.80
GGPS1	3.17	5.02
DHCR24	4.80	6.51
DHCR7	3.95	4.54
SLC29A1	6.73	6.80
SLC29A2	4.52	0.82
SLC29A3	1.48	2.06

Supplementary Figure 5

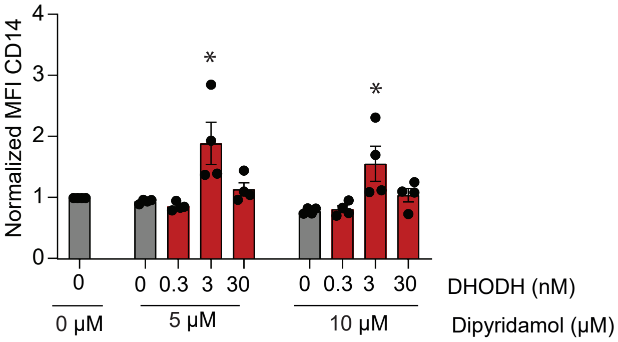
A. AML vs Healthy



B. Healthy



C. Healthy



Supplementary Figure 6